

**THEORETICAL INVESTIGATION OF THE KINETICS AND  
THERMODYNAMICS OF CONFORMATIONAL EQUILIBRIUM IN CROWDED  
MEDIUM**

**MUHAMMAD SAJID IQBAL**  
**Bachelor of Science, Government College University Faisalabad, 2009**  
**Master of Science, University of Eastern Finland, 2012**

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**MUHAMMAD SAJID IQBAL**

Date of Defence: June 06, 2017

**Dr. Marc R. Roussel**

Supervisor

Professor

Ph.D.

**Dr. H. J. Wieden**

Committee Member

Professor

Ph.D.

**Dr. Kenneth Vos**

Committee Member

Associate Professor

Ph.D.

**Dr. Athan Zovoilis**

Internal Examiner

Assistant Professor

Ph.D.

**Dr. Ramon Grima**

External Examiner,

The University of Edinburgh

Reader

Ph.D.

**Dr. Michael Gerken**

Chair,

Thesis Examination Committee

Professor

Ph.D.

# Dedication

To my lovely parents,

*Muhammad Iqbal and Zeenat Bibi*

# Abstract

Macromolecular crowding alters the thermodynamic activities and kinetics of biochemical processes through an excluded volume effect. A theoretical framework, comprised of the Monte Carlo, extended scaled particle theory, and the transition state theory models is employed to compute the thermodynamic activities of three systems in virtually crowded media filled with polyethylene glycol molecules chosen from appropriately constructed conformational ensembles. The theoretical framework covers all the key steps required to prepare the equilibrated crowded medium and subsequently calculate the thermodynamic activities and kinetics. The thermodynamic activity depends on the geometrical parameters of all fluid particles and the model extracts the geometrical parameters by generating a convex hull from atomic coordinates. Moreover, it considers the nonideal contributions of crowder aggregates. This model predicts low to moderate crowding effects consistently with experimental findings with an enhancement in the stability of folded conformations from  $-0.2$  to  $-2.0kT$  over open conformations due to crowding.

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# List of Abbreviations

<b>AdK</b>	.....	Adenylate kinase
<b>BD</b>	.....	Brownian dynamics
<b>BHS</b>	.....	Bonded hard-sphere
<b>DFT</b>	.....	Density functional theory
<b>ESP</b>	.....	Electrostatic potential
<b>FF</b>	.....	Forcefield
<b>FHS</b>	.....	Fused hard-sphere
<b>FRET</b>	.....	Forster resonance energy transfer
<b>FTIR</b>	.....	Fourier transform infrared spectroscopy
<b>HF</b>	.....	Hartree Fock
<b>HFF</b>	.....	Forcefield parameters for PEG with hydroxyethyl terminal
<b>LJ</b>	.....	Lennard-Jones
<b>MC</b>	.....	Monte Carlo
<b>MD</b>	.....	Molecular dynamics
<b>MEP</b>	.....	Molecular electrostatic potential
<b>MFF</b>	.....	Forcefield parameter for PEG with methyl terminal
<b>MP2</b>	.....	Møller-Plesset perturbation theory of second order
<b>NI</b>	.....	Nonideal
<b>PEG</b>	.....	Polyethylene glycol
<b>PFF</b>	.....	Forcefield parameters for PEG with propyl terminal
<b>PHF</b>	.....	Post-Hartree Fock
<b>PVP</b>	.....	Polyvinylpyrrolidone
<b>QM</b>	.....	Quantum mechanics
<b>RESP</b>	.....	Restrained electrostatic potential
<b>RRMS</b>	.....	Relative root mean square
<b>SANS</b>	.....	Small-angle neutron scattering
<b>SPT</b>	.....	Scaled particle theory
<b>TER</b>	.....	Telomerase RNA
<b>TERT</b>	.....	Telomerase reverse transcriptase
<b>TS</b>	.....	Transition state
<b>TST</b>	.....	Transition state theory

# Chapter 1

## Introduction

### 1.1 Macromolecular crowding

The kinetics and thermodynamics of biochemical reactions have traditionally been studied in experiments using dilute solutions of total concentrations less than about 1 g/l [1–6]. The total concentration of dilute solution includes the total amount of solute proteins, and buffer salts [7]. In contrast to experimental settings, the biochemical reactions occur naturally in highly concentrated and confined cellular environments in the presence of billions of proteins. The cellular environment contains a total concentration from 50 to 400 g/l of extensive, complex and cross-linked structures of proteins, nucleic acids, enzymes, polysaccharides and cytoskeleton fibers (Figure 1.1) [8–11].

The total concentration in the cell depends on the presence of a variety of macromolecules that are present at very high concentrations. In 1981, Minton [12, 13] named such a medium crowded rather than concentrated because no single species is present at high concentration. In the crowded medium, all these macromolecules are called background or crowder species [4, 5, 12, 14, 15]. All these macromolecules occupy a significant fraction of the total volume in the eukaryotic and prokaryotic cells [16] and alter the kinetics and thermodynamics of biochemical reactions significantly by increasing the total free energy of the reaction medium [4, 9, 14] through steric, electrostatic, and hydrophobic interactions [3, 7, 12].

Macromolecular crowding does not always increase the rate of reaction to the same extent [18–22]. The crowding alters the reaction rates based on the reaction mechanism

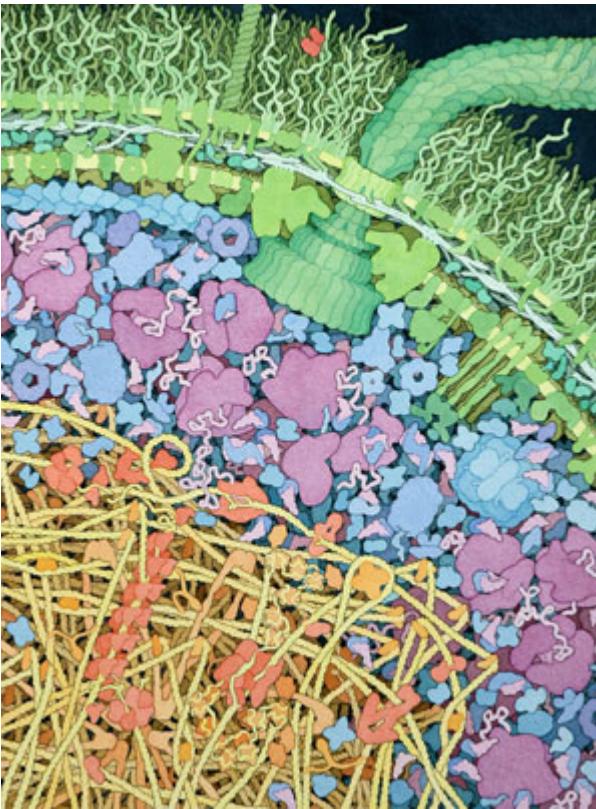


Figure 1.1: The cross section diagram of an *Escherichia coli* cell depicts different regions of the cell in different colors. A large flagellar motor passing through the cell wall is shown in green, the cytoplasmic proteins are blue, and the central nucleus region containing DNA and histones in yellow and orange colors. The cellular organization showed compact packing of different sized and shaped macromolecules at the total concentration [17]. (This illustration is provided by David S. Goodsell, the Scripps Research Institute, and is in the public domain. See <http://mgl.scripps.edu/people/goodsell/illustration/public>)

and type of molecules present in the medium. For example, crowding could reduce the rate constant of diffusion limited reactions by limiting the diffusion of reacting species through additional molecular obstacles [23], whereas the rate of an activation controlled reaction could increase by allowing more time for the formation of a transition complex and subsequently forming a final product [3, 20]. The majority of previous studies have shown that the crowding alters the reaction rate by the excluded volume effect [9, 12, 24, 25] and the magnitude of the excluded volume depends on the type, size and shape of molecules [3, 5, 26]. The excluded volume will be discussed in detail in the next section 1.2.

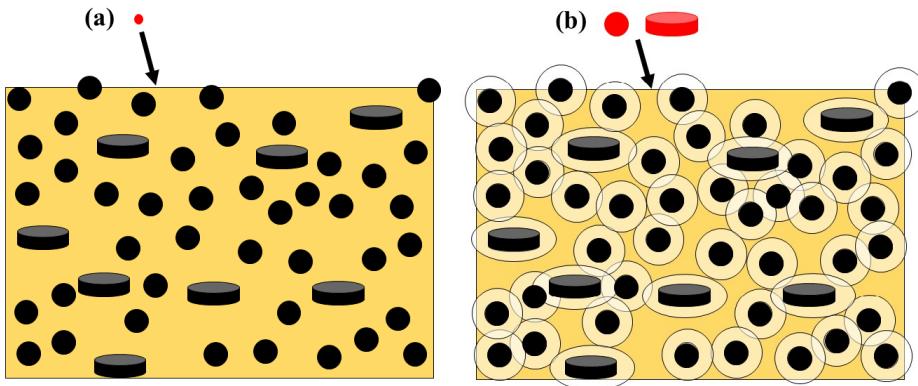


Figure 1.2: The concept of excluded volume is illustrated, using a rectangular shaped cell filled with sphere and disk shaped black molecules occupying approximately 30 percent of total space. Attempts to place three different sized and shaped red colored test molecules in the solution are made. The smallest spherically shaped molecule, in case (a), could be placed in the majority of the space in the yellow region, and more than sixty five percent of the total space is available for the test molecule. However, the percentage of free available volume varies for the other two particles shown in case (b). The sphere and disk shaped test molecules could occupy 10 to 15 percent and less than 5 percent of free space respectively. These amounts of available volume are rough estimations. The excluded volume for the red sphere is illustrated by the light color large circles around the solution particles.

## 1.2 Excluded volume effect

One can understand how macromolecular crowding affects the thermodynamics of a biochemical reaction through an excluded volume concept. The excluded volume is defined as free space that is excluded by the solution molecules to the solute particle, depending on the sizes and shapes of the solution and solute molecule. The extent of this virtually present free space depends on the sizes and shapes of the background molecules and the solute molecule [4, 9, 12, 27, 28]. The concept of the excluded volume can be understood from the illustration in Figure 1.2, which demonstrates the dependence of the magnitude of the excluded volume on the shapes and sizes of the background and solute molecules. The background molecules represent the solution molecules present in the rectangular outline whereas the solute molecules or test molecules represent the additional molecules that are tried to be placed in the solution. The excluded volume is fundamentally produced due to steric repulsion interactions among the fluid particles [3, 5, 7].

The example (Figure 1.2) explains the concept of excluded volume where the rectangu-

lar outline depicts the cell filled with spherical and disk shaped macromolecules occupying approximately 30 percent of the total volume. The percent of free available volume to the additional solute molecule is determined by its size and shape. If a very small test molecule (Figure 1.2 case a) relative to the background molecules is placed in the defined region, it can be placed almost anywhere in the yellow area that is not occupied by the background molecules. However, if a test molecule (Figure 1.2 case b) of the same or larger size (sphere, disk) relative to the background molecules is placed in the medium, there is very limited dark yellow space available to the new molecule. The free volume, the volume where additional test molecule can be placed, is reduced because the centre of a test molecule can only approach the centre of any background molecule to a certain distance, indicated by the black circular and elliptical lines around each spherical and disk background molecule, respectively. The circular or elliptical lines are formed by rotating the spherical test molecule around the background spherical and disk shaped molecules. However, the ellipse is not a true representation of the excluded volume for the disk shaped molecules in a three dimensional space. Due to steric repulsion, two molecules cannot occupy the same place at the same time. The additional volume occupied by the circular or elliptical shell is called excluded volume of the spherical shaped molecule and could not be occupied by any of the background or test molecule. The excluded volume depends on the sizes and shapes of solute and solvent molecules. The example illustrated that each molecular conformation has its own excluded volume. Thus, the excluded volume is defined as the volume which is present in the medium but physically inaccessible to an additional molecule of a particular size and shape [6, 29, 30].

The excluded volume alters the effective concentration of solute species in the crowded medium significantly. According to freshman chemistry, the reactivity of any solute species depends on the concentration or the number of molecules per unit volume, which is strictly true only for very dilute solutions. In the crowded medium where many different species are present at high total concentration, the solute molecules are no longer ideal and their

reactivity is defined by an effective concentration. Thus, the chemical potential ( $\mu$ ) [31] of the solute in dilute solution cannot represent the chemical potential of the same solute in a crowded nonideal (NI) solution truly. The chemical potential is a partial molar Gibbs free energy of a particular chemical specie in the solution at constant temperature, pressure and number of moles of other species in the mixture. The chemical potential can also be defined in more than one ways under different conditions as shown in equation 1.1. Therefore it is necessary to add NI contributions due to the excluded volume, electrostatic, hydrophobic and hydrophilic interactions to the chemical potential of an ideal solute that is present in the crowded medium to represent the real solution (Equation 1.2 [3]).

$$\mu_i = \left( \frac{\partial G}{\partial N_i} \right)_{T,p,N_j \neq i} = \left( \frac{\partial H}{\partial N_i} \right)_{S,p,N_j \neq i} = \left( \frac{\partial A}{\partial N_i} \right)_{V,T,N_j \neq i} = \left( \frac{\partial U}{\partial N_i} \right)_{S,V,N_j \neq i} \quad (1.1)$$

$$\mu_i = \mu_i^0 + kT \ln a_i \quad (1.2)$$

where:

$$a_i = \gamma_i c_i / c^\circ$$

$$\mu_i = \mu_i^{\text{ideal}} + \mu_i^{\text{NI}}$$

$$\mu_i^{\text{ideal}} = \mu_i^0 + kT \ln c_i / c^\circ$$

$$\mu_i^{\text{NI}} = kT \ln \gamma_i$$

where the ideal contribution ( $\mu_i^{\text{ideal}}$ ) is the free energy change in the absence of any type of solute-solute interactions whereas the NI contribution ( $\mu_i^{\text{NI}}$ ) is the free energy change due to the presence of solute-solute interactions.  $k$  is the Boltzmann constant,  $T$  is the temperature,  $\gamma_i$  is the activity coefficient,  $c_i$  is the solute concentration,  $c^\circ$  is the standard

concentration, and  $a_i$  is the thermodynamic activity of solute  $i$ . Under ideal conditions the activity is approximately equal to concentration for each solute species.

The activity is described as an effective concentration of a particular species in a solution [32]. The excluded volume theory hypothesizes that the effect of crowders is to reduce the available volume, so that the contribution to the activity due strictly to the crowding effect can be computed by replacing the concentration by the number of solute particles per available volume. Crowding reduces the available volumes and causes significant NI effects [3, 13, 33, 34]. The reduction in the available volume due to excluded volume can increase the thermodynamic activity of the test particle by several orders of magnitude. Consequently, the activity coefficient is greater than unity and the molecule shows NI behaviour. In a simple way, the activity coefficient of solute  $i$  is expressed as a ratio of the total volume to the available volume in the crowded medium (Equation 1.3) [3, 7, 9, 14]. This expression for  $\gamma_i$  arises from the simplest theory of the effect of excluded volume on thermodynamic activities.

$$\gamma_i = \frac{V_{\text{total}}}{V_{\text{available}}} \quad (1.3)$$

where  $\gamma_i$  is the activity coefficient of solute  $i$ ,  $V_{\text{total}}$  is the total volume of the compartment, and  $V_{\text{available}}$  is the free volume available to the solute particle  $i$  in the solution.

The magnitude of the excluded volume is directly influenced by the number density, shapes, and sizes of the test and background species in *in vitro* experiments [35–37]. In living cells, especially in the eukaryotic cell, the amount of excluded volume depends on the concentration of bulk soluble species in each compartment and spaces confined by the cytoskeletal elements. Generally, these macromolecules occupy 5–40 percent of the total volume, in the range of 50–400 g/l concentration. Therefore, this is the most common concentration range used to study the consequences of crowding [8, 9, 11, 30, 38, 39].

The macromolecular crowding potentially affects the protein behaviour in the crowded milieu, such as conformational equilibrium [4, 19, 21, 24, 25, 28, 35, 38, 40–43], enzyme

activities [14, 44–47], protein binding events [19, 37, 48, 49], phase separation [50–52], pathological protein aggregation [53–56], and association reactions [10, 57–60]. Imagine a macromolecule with two different conformations, A and B, in a conformational equilibrium. There is an equilibrium between A and B in any medium which implies  $\mu_A = \mu_B$ . If this reaction is transferred from dilute solution to a solution with inflexible crowders, then the chemical potentials of both conformations increase, but the chemical potential of the least compact conformation increases more due to decrease in available volume. To restore equilibrium, the reaction will shift towards the more compact conformation until the condition  $\mu_A = \mu_B$  is once again reached [13, 29, 53, 61]. Alternatively, the conformational equilibrium shift, from a dilute to a crowded solution, will initially cause the reaction to proceed in a direction that increases the available volume.

### 1.3 Crowding effects on the conformational equilibrium

Proteins form unique conformations to perform a variety of biological tasks [24, 62, 63]. These conformational changes occur due to genetically encoded information in proteins, protein-solvent interactions, ligand binding and release from active sites, phosphorylation, exposure to light, and macromolecular crowding [24, 47, 64–68]. A better understanding of conformational changes could unveil the reactivity mechanism of different states of a single protein in different biochemical reactions. Therefore, various experimental techniques such as Forster resonance energy transfer (FRET), neutron and X-ray scattering, NMR spectroscopy, X-ray crystallography, hydrogen-deuterium exchange, and mutation studies have been applied to investigate the mechanism of internal movements of proteins. Further, many computer simulations have been conducted to sample alternative conformations of probe proteins to understand the structure and function of each different conformation [24, 67–70].

For example, adenylate kinase (AdK) equilibrates between open and closed conformations. Explicit long time scale molecular dynamics (MD) and bias exchange metadynamics

simulations were conducted to investigate the intermediate conformational states and transition pathways in the presence and absence of ligands. The simulation results indicate no significant energy barrier is crossed to transit between open and closed conformations in the ligand-free AdK form, whereas a closed conformation is energetically favored in the ligand-bound AdK form by 8 kcal mol<sup>-1</sup> and requires a high energy to pass the energy barrier in order to form an open conformation [44].

It is well recognized that small and large scale conformational changes play an essential role in triggering the catalytic activity of biological enzymes [67–69]. In these experimental and theoretical studies, the protein conformational changes have been examined under dilute conditions. However, proteins evolve and function in very heterogeneous and crowded medium, in which macromolecules occupy approximately 5 to 40 percent of the total volume [39, 71]. In this regard, the excluded volume theory explains the crowding effects on the conformational equilibria qualitatively [12, 19, 24, 25, 27, 72]. One of our goals is to explore whether it can be used quantitatively to estimate the macromolecular crowding effects on the conformational equilibrium between two conformations of a single macromolecule.

A vast majority of research on the macromolecular crowding has been dedicated to study its effects on protein folding, and on binding processes in experimental and theoretical studies [15, 25, 26, 29, 37, 42, 43, 51, 52, 55, 71, 73–79]. In *in vitro* experiments, the crowding effect is induced by using synthetic high molecular weight polymers such as polyethylene glycol (PEG) [51, 52, 58, 80], polyvinylpyrrolidone (PVP) [30, 63], Ficoll [58, 80], dextran [30, 58, 80], bovine serum albumin [80, 81], and ovalbumin [63]. These crowding agents have to comply with different criteria to generate the crowding effect in *in vitro* experiments. The crowding agents should have molecular mass in the range from 50 to 200 kDa, be globular shaped to keep the viscosity at a minimum level, neutral with no ability to develop any electrostatic, hydrodynamic or hydrophobic interactions with itself or with solute molecules, highly soluble, similar in size to the probe protein, and raise no difficulties in the spectroscopic testing [3, 10, 15, 29, 37, 82]. Experimental data showed

that low molecular weight crowding agents are not ideal to use due to formation of soft interaction among the crowder molecules whereas the high molecular weight crowding agents are found to be more effective in producing the excluded volume effects. However, none of the crowding agents is perfect [15, 26, 29, 37, 42, 43, 51, 52, 55, 73–79].

Recently, a few studies have been conducted to investigate the effect of macromolecular crowding on the conformational equilibrium. In this regards, a post-processing atomistic modelling approach [21, 37, 59] has been developed to study the conformational equilibria and the transition rates in crowded medium of seven different proteins pairs. In this model, a representative conformation of each protein is sampled by employing explicit MD simulations at room temperature and subsequently the change in the free energy is estimated by taking the difference of the chemical potential for each conformation in dilute and crowded medium. The spherical shaped crowders of 15 and 30 Å radius, occupying up to 35 percent of available space, were used to produce the excluded volume effect [19, 24, 25]. In another study, conformational equilibrium between pseudoknot and hairpin telomerase conformations was investigated by performing coarse grained Langevin dynamics simulations under crowded conditions. The simulated melting temperature curves for each conformations were found to be in agreement with experimental UV melting data [47]. The insertion algorithm based on the scaled particle theory has been applied to reproduce the simulation results in the presence of spherical shaped crowding agents.

Typically, the computer simulations and analytical scaled particle theory model approximates the shapes of solute and crowder molecules as hard spheres or cylinders and estimates the macromolecular crowding effect qualitatively. In this thesis, an attempt is made by using an extended scaled particle theory (SPT) model [83] and a Monte Carlo (MC) simulation model [84–86] to estimate the excluded volume effects on the conformational equilibria of three pair of macromolecules quantitatively through the transition state theory (TST) [87] in the presence of crowders. Conformational ensembles of polyethylene glycol (PEG) were used as a crowding agent in this investigation. The following section briefly describes the

appropriate criterion in light of experimental and theoretical studies to select PEG conformations that could be used as crowders and the details on three systems in conformational equilibrium. Later on, details on three methods, i.e. the SPT, MC and TST models, that will be used to estimate the crowding effects on the conformational equilibrium of three selected systems are discussed.

### 1.3.1 Crowders: Polyethylene glycol

Polyethylene glycol of molecular weight 8 kDa will be used as a crowding agent in this study because it is used in many experimental and theoretical studies of crowding due to its high solubility in water and in other solvents [55, 88, 89]. Experimental studies conducted in aqueous solutions of 8 kDa PEG revealed that PEG structures exist in diverse shapes such as pulled fibers [90, 91], coiled [90, 92–94], helical [89, 92–96], extended hairpin [93, 97] and compact globular conformations [55, 98] with a radius of gyration from 15 to 46 Å [55, 80, 90–92, 94–96, 98–102].

The radius of gyration is an important property with units of length that is used to characterize polymer size in the experiments and computer simulations [6, 103]. The radius of gyration is defined as the root mean square of the distance of all atoms from the centre of mass of the molecule. The radius of gyration is computed with equation 1.4 and has been used to track the structural changes in the conformational sampling simulations that are performed in different simulation programs [104–106]. The folding behaviour of PEG depends on various factors such as molecular weight of polymer, PEG concentration, type of solvent, impurities in water, temperature, and intra- and intermolecular hydrogen bonding [55, 89, 90, 92–96, 98–102].

$$R_g = \sqrt{\frac{1}{N} \sum_{i=1}^N (\vec{r}_i - \vec{r}_{CM})^2} \quad (1.4)$$

where  $R_g$  is radius of gyration,  $N$  is total number of atoms,  $\vec{r}_i$  is the position vector to each atom and  $\vec{r}_{CM}$  is the centre of mass of the molecule. Mathematically,  $\vec{r}_{CM}$  for the atoms of

a molecule with masses  $m_i$  and positions  $x_i$  can be represented by equation 1.5. The centre of mass moves as if the whole mass of the molecule is concentrated there.

$$\overrightarrow{r_{CM}} = \left( \frac{\sum m_i \sum x_i}{\sum m_i} \right) \quad (1.5)$$

Small-angle neutron scattering measurements revealed that different solvents played a crucial role toward the folding behavior of PEG [89, 93, 101]. PEG formed helices and coiled structures in H<sub>2</sub>O and D<sub>2</sub>O solvents respectively [89, 93]. Dynamic light scattering and small angle neutron scattering experiments demonstrated that PEG formed aggregates in water and formed conformations with radii of gyration in the range of 32 to 46 Å in water and deuterated water [107]. The water produced a hydration layer around the PEG and stabilized the helix structures through strong hydrogen bonding [89–92, 95, 96, 108]. It turned out that intra- and intermolecular hydrogen bonding present between terminal hydroxyl groups and ether oxygen atoms is responsible for PEG folding. Fourier transform infrared spectroscopy (FTIR) and X-ray spectroscopy measurements showed that PEG has a tendency to segregate from the aqueous solution containing a mixture of PEG and polypropyl glycol [89, 93, 101]. The segregated PEG stimulates the intermolecular hydrogen bonding between the PEG molecules. This mechanism induced the formation of PEG aggregates. Similarly, other studies demonstrated that PEG formed random coiled structures in purified water and methanol which inhibits aggregation [107, 109]. Moreover, quantum chemistry calculations indicated the presence of intra- and intermolecular hydrogen bonding in the global minimum PEG structures which produced compact conformations in computer simulations that agreed with experimental findings [101].

8 kDa PEG will be used as a crowding agent in this study. As seen above, the conformational analysis confirms that PEG has formed a variety of conformations that differ in sizes and shapes depending on the experimental conditions. It is therefore better to construct ensembles of PEG conformations of different shapes and sizes in order to model these diverse conformations of PEG crowders in the crowded medium than using a single PEG

conformation. To achieve this goal, computer simulations such as Monte Carlo and molecular dynamics simulation approaches were used to construct diverse PEG conformations and to prepare the crowded media by packing these conformations into simulation boxes. Previous experimental and theoretical studies showed that PEG formed conformations with radii of gyration in the range of 15 to 46 Å [55, 63, 90, 91, 107–112]. We sampled PEG conformations from a slightly wider range from 10 to 60 Å. The range is chosen arbitrarily by assuming it would capture enough diverse PEG conformations to mimic the full range of PEG aqueous solution structures. These simulation boxes would mimic the cellular crowded environments and would help us to investigate the macromolecular crowding effects on the conformational equilibrium of the three pairs of macromolecules presented in the following subsections.

### 1.3.2 Telomerase RNA (2K96-1NA2)

Telomerase is a ribonucleoprotein complex that plays an essential role in regulating the length of the telomere in the cells [113, 114]. The telomerase extends the 3' termini of linear chromosomes by adding successive units of telomere such as dTTAGGG in vertebrates [115, 116]. The shortening of telomeres is directly associated with short life time of cells and ultimately leading to death. The activity of telomerase is also essential for proliferation of cancer cells. Therefore, investigation on the telomerase activity mechanism is of tremendous interest due to its vital role in lengthening of telomeres, ageing, cancer, dyskeratosis congenita, and aplastic anemia diseases [117–119].

Two major components, namely telomerase reverse transcriptase (TERT) and telomerase RNA (TER) are required to assemble a functional ribonucleoprotein complex. The activity of the ribonucleoprotein complex is linked to the conformational changes of the TER component. Two conformations namely pseudoknot (2K96 [120]) and hairpin (1NA2 [119]) of TER component are found in equilibrium and work as a conformational switch [47]. The assembly and functionality of the ribonucleoprotein complex is associated with the forma-

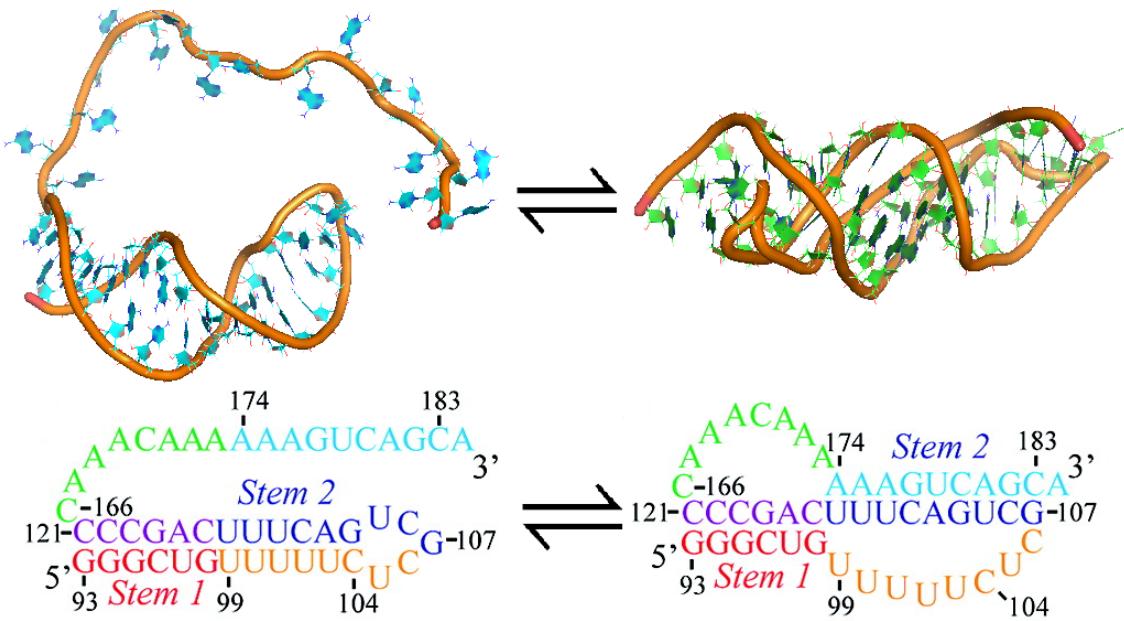


Figure 1.3: Tertiary structures and secondary structures of the telomerase RNA conformational switch. Human telomerase RNA equilibrates between hairpin (left) and pseudoknot (right) structures, acting as a conformational switch and the activity of the conformational switch is linked to the pseudoknot conformation.

tion of the pseudoknot conformation of the TER which is directly linked to the activity of telomerase. The basic secondary structure of the TER conformational switch consists of two stems, S1 and S2, and loops, L1 and L2 respectively [46, 113] (Figure 1.3). *In vitro* experiments and NMR findings showed evidence for the conformational equilibrium between RNA pseudoknot and RNA hairpin structures [120]. Other factors such as the mutations, temperature and crowding may affect the conformational changes of the switch [47].

### 1.3.3 Adenylate kinases (4AKE-1AKE)

Adenylate kinase (AdK) is one of the most important enzymes whose function is to regulate the concentration of adenine nucleotides and facilitate the interconversion reaction of ATP, ADP, and AMP [121–123]. Adenylate kinase is also sensitive to the cellular energy state changes due to fluctuations in the cellular AMP levels. The catalytic activity of adenylate kinase is linked to conformational changes where the enzyme switches from the open conformation (inactive conformation 4AKE [64]) to a rigid closed conformation

(active conformation 1AKE [122]) in the ligand bound form [124, 125] (Figure 1.4). Large conformational changes of an AdK occur by binding of ATP or AMP to the enzyme [126]. In the present studies, we will try to estimate the crowding effects on the free energy landscape of the conformational equilibrium. The ligand is relatively small sized as compared to crowders and both AdK conformations and therefore it is assumed that the crowding will not affect the thermodynamics of the ligand significantly. As a result, the nonideal contributions to the conformational equilibrium due to crowding on a small sized ligand would be negligible.

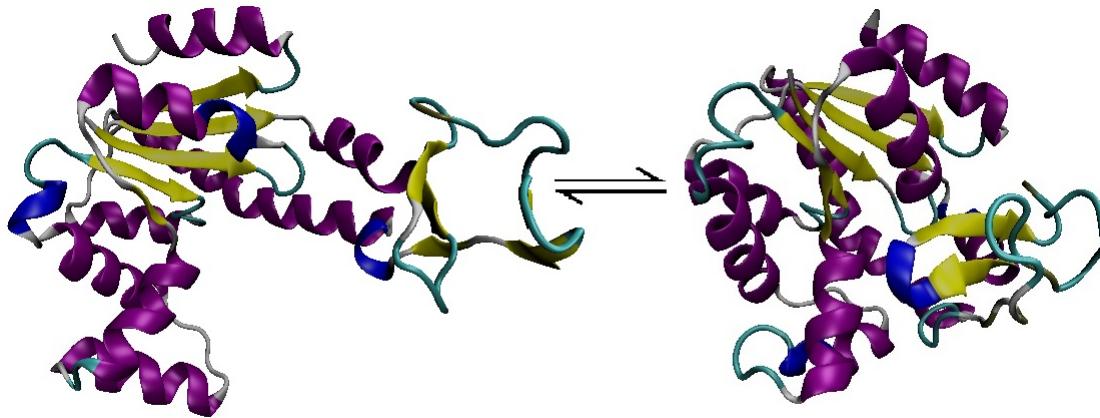


Figure 1.4: Conformational equilibrium between open (4AKE [64]) and closed (1AKE [64]) conformations of adenylate kinase enzyme.

#### 1.3.4 The *lac* repressor (2PE5-2P9H)

Repressor proteins regulate the expression of particular genes by inhibiting their expression in the transcription process. The lactose (*lac*) repressor is one of the common examples of a repressor which limits the availability of proteins that are necessary to metabolise the sugar lactose [127–129]. In the absence of lactose, the *lac* repressor binds to the promoter region on the DNA in a closed conformation (2P9H [129]) (Figure 1.5). The *lac* repressor blocks the RNA polymerase’s way on the DNA to transcribe the *lac* genes, and therefore limits the production of lactose metabolism proteins [130]. The conformational equilibrium of the *lac* repressor is driven by binding to allolactose [131]. These conformational changes

occur in the crowded medium and it is interesting to explore the effect of crowding on the energetic landscape changes.

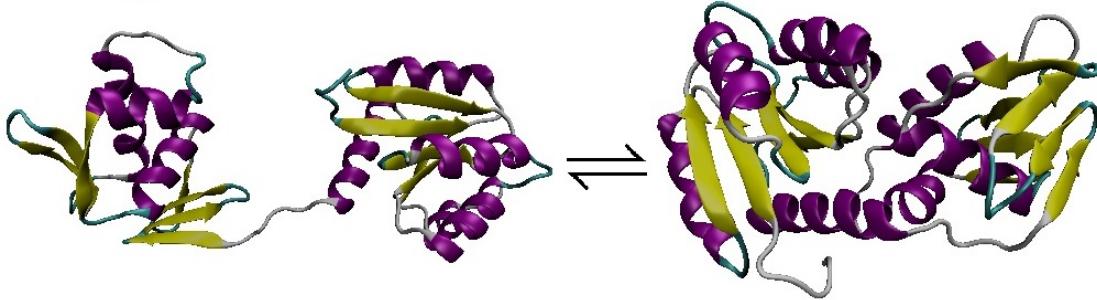


Figure 1.5: Conformational equilibrium between open (2PE5 [129]) and closed conformations (2P9H [129]) of the *lac* repressor. The activity of the repressor is linked to the formation of the closed conformation.

## 1.4 Macromolecular crowding models

Many experimental and computer simulation models have been developed to determine the excluded volume effects on various types of reactions occurring in crowded media [19, 24, 25, 38, 44, 45, 47, 53, 132]. In addition to experimental and computer simulations, other theoretical approaches such as Brownian dynamics (BD) simulations [133, 134], lattice-based models [134], the Smoluchowski theory [135] and the stochastic approaches [136, 137] to investigate the crowding effects by placing the hard sphere crowders in the three dimensional BD simulations which were found to be very expensive computationally. Further improvements were made to BD simulation models by incorporating crowder free method to enhance the computational efficiency of BD simulations and reproduce results quickly [134]. Typically, the protein and crowders are modeled as hard spheres of approximate sizes in these models and the stochastic nature of the biochemical reactions occurring in the cellular interiors is neglected. In this regard, robust analytical models based on the chemical master equation were developed to estimate the crowding effects on the intrinsic noise associated with the biochemical reactions [138].

The spherical representation of probe and crowder molecules is not a realistic repre-

smentation of the cellular interior which is filled with a variety of macromolecules [135]. Experimental investigations made by different techniques such as tandem affinity purification process, matrix-assisted laser desorption, and liquid chromatography tandem mass spectrometry were applied to determine the size distribution of different macromolecules in the cells [139, 140]. These investigations are helpful to explore the heterogeneity of the crowded medium incorporated by the presence of different types of macromolecules. It turns out that the reaction kinetics and thermodynamics could not follow the law of mass action and power-law approximation in the heterogeneous reaction medium [137, 141]. Further studies showed that the biochemical reactions such as enzymatic reactions follow a fractal-like kinetics in the crowded intracellular environments [141, 142].

The previous investigations were performed by using a variety of computer simulation models. In the present study, a statistical extended model of the scaled particle theory for convex shaped particles, Monte Carlo simulations and the transition state theory are employed to investigate the thermodynamics and kinetics of conformational equilibrium of three systems in crowded medium. In these models, the original shapes of molecules are used in computing the excluded volume by taking into account the atomic coordinate details.

#### 1.4.1 Theoretical model: The scaled particle theory

To estimate the magnitude of the excluded volume effect based on the size and shape of molecules, a theoretical model of the scaled particle theory has been developed. This model estimates the amount of work required to place additional molecules in a fluid medium in the presence of other particles. The outcome relies on the virial coefficients and hard core repulsion interactions between the molecules. One of the earliest models of this type was developed by Laurent and Ogston where they examined the effect of high concentration of hyaluronic acid on protein partitioning [143–145]. Different variants of the scaled particle theory have been developed over the time, where the majority of them treated the fluid

particles as hard spheres, cylinders or fused spheres [146–157]. In 1971, Gibbons [83, 158] developed the most generalized model of the scaled particle theory, applicable to compute the thermodynamic properties of convex shaped particles of different sizes in a crowded medium. Subsequently, Minton and Zhou [29, 33, 37, 81] applied this model to compute the protein activity in a crowded solution by approximating the protein shapes as hard spheres and incorporating the additional attractive interactions. So far, the scaled particle theory model has been very helpful to develop theoretical insights on how the macromolecular crowding affects the protein conformational equilibrium, folding and association reactions based on the excluded volume effect.

Regarding the historical development of SPT models, the first statistical model of hard sphere particles was developed by Reiss *et al.* [159] and further extended to fluid mixtures by Helfand *et al.* [146]. Reiss *et al.* [159] developed an equation of state of rigid sphere fluid particles in 1959 by measuring the density of rigid spherical molecules as a function of distance from a rigid sphere solute particle of an arbitrary size. The accuracy of results depends on the accurate calculations of the virial coefficients and the equation of state computes the first three virial coefficients exactly and gives the next two coefficients within three and five percent error respectively. In the equation of state of a general gas (Equation 1.6)  $p$  is the pressure,  $v$  is the volume of each molecule,  $k$  is Boltzmann’s constant,  $T$  is the absolute temperature, and the coefficients  $B, C, \dots$  represent the second, third,  $\dots$  virial coefficients. These coefficients are a function of temperature and indicate the deviation of a real gas from ideal behaviour. In other words, these coefficients calculate the interaction potential between the molecules which, for hard particles, depends on the shape, size and concentration of molecules in the given volume.

$$pv = kT(1 + Bv^{-1} + Cv^{-2} + \dots) \quad (1.6)$$

In 1964, Lebowitz *et al.* [160], derived a new scaled particle theory model in a simple manner to estimate the thermodynamic properties, i.e. partial molar volume change at constant

pressure in the mixtures of hard spheres. In the following year [148], this model was extended to one, two and three dimensional systems containing mixtures of hard spheres by integrating the exact solution of the radial distribution function [146, 159] obtained from the Percus-Yevick integral equation. Analytical results from the Percus-Yevick integral equation were tested against machine computations (molecular dynamics simulation results) and found in good agreement [160].

Later on, the SPT model was improved by including the solvation contributions [149, 153]. Additionally, numerous bonded hard-sphere (BHS) [161–165] and fused hard-sphere (FHS) models [166, 167] were developed and extended by Boulik [168–171] and Nezbeda [172]. The first FHS model approximated the shapes of linear homonuclear diatomic molecules as fused spheres. Later on, the FHS model was extended to diatomic dumbbell shaped heteronuclear molecules [170, 173], non-linear triatomic [172, 174], and four, six, or twelve hard fused spheres [166], and polyatomic fluids [173].

A new version of the scaled particle theory of arbitrarily shaped fluid particles has been introduced in 1969 by Gibbons [158] to capture the description of systems of arbitrary shaped particles more realistically. This new SPT model was an extension to the theory of Lebowitz *et al.* [148] and calculates the chemical potential and excluded volumes as a function of three geometrical parameters. In the following year, the model [83] was further extended to compute the thermodynamics of convex shaped particles of different sizes. Isihara and Hayashida [175, 176], Kihara [177], Labik *et al.* [174], Tjipto *et al.* [178] and Boublík [179] derived the shape dependent second virial coefficient for different convex shaped particles in terms of three geometrical parameters.

To understand the basic logic of the scaled particle theory model, let us consider a convex shaped particle of characteristic linear dimension  $R_j$  placed in a cube of finite dimensions in the presence of background molecules with a characteristic linear dimension  $R_i$ . The average radius of curvature, surface area and the volume of the characteristic convex shaped particles is expressed as  $R$ ,  $S$ , and  $V$ , respectively. Alternatively, the geometrical

Table 1.1: Geometric parameters of different shapes [180]

Shape	Example	Dimensions	R (curvature)	S (area)	V (volume)
Sphere	Ar	Radius $r$	$r$	$4\pi r^2$	$4\pi r^3/3$
Thin Rod	$\text{CO}_2$	Length $l$	$l/4$	0	0
Circular disk	$\text{C}_6\text{H}_6$	Radius $r$	$\pi r/4$	$2\pi r^2$	0
Rectangular parallelepiped	$\text{C}_2\text{H}_6$	sides $l_1, l_2, l_3$	$(l_1 + l_2 + l_3)/4$	$2(l_1 l_2 + l_1 l_3 + l_2 l_3)$	$l_1 l_2 l_3$
Thin hexagon	$\text{C}_6\text{H}_6$	side $l$	$3l/4$	$3l^2\sqrt{3}$	0
Regular tetrahedron	$\text{CH}_4$	side $l$	$3(\tan^{-1}\sqrt{2}l)/2\pi$	$l^2\sqrt{3}$	$l^3\sqrt{2}/12$
Regular octahedron	$\text{SF}_6$	side $l$	$(3/\pi)l \cot^{-1}\sqrt{2}$	$2l^2\sqrt{3}$	$(l^3\sqrt{2})/3$
Cylinder	$\text{C}_2\text{H}_6$	Length $l$ , Radius $r$	$(\pi r + l)/4$	$2\pi r(r + l)$	$\pi r^2 l$
Prolate spherocylinder	$\text{N}_2$	Length $l$ , Radius $r$	$(r + l)/4$	$2\pi r(2r + l)$	$\pi r^2(4r/3 + l)$
Oblate spherocylinder	$\text{C}_6\text{H}_6$	Length $l$ , Radius $r$	$(r + \pi l)/8$	$4\pi r^2 + \pi^2 r l + \pi l^2/2$	$\pi r(4r^2/3 + \pi lr/2 + l^2/2)$

parameters can also be represented in terms of the characteristic shape parameters  $a$ ,  $b$  and  $c$ . Then the average radius of curvature, surface area and the volume can be expressed as  $aR$ ,  $bR^2$ ,  $cR^3$ . For example, these coefficients  $a$ ,  $b$  and  $c$  have values of  $1$ ,  $4\pi$  and  $4\pi/3$  for a sphere respectively. These coefficients give the radius of curvature, surface area and volume after multiplying with  $R$ ,  $R^2$ , and  $R^3$  respectively. However, one can use values of  $R$ ,  $S$  and  $V$  directly for a particle of known shape. The shape coefficients for six different shapes are presented by Gibbons [158] and the radius of curvature, surface area and volume for different shapes are provided in table 1.1. The NI part of the chemical potential of the  $j$ th type of particle depends on the total number density  $d$ , on the geometrical parameters  $R, S, V$  whose formulas are given below and on the fraction of occupied volume  $Y$  (Equation 1.7 [19]).

$$\frac{\mu_j^{\text{NI}}}{kT} = -\ln(1-Y) + \left( \frac{dS}{1-Y} \right) R_j + \left( \frac{dR_i}{(1-Y)} + \frac{(dS_i)^2}{8\pi(1-Y^2)} \right) S_j + \left( \frac{d}{1-Y} + \frac{d^2 R_i S_i}{(1-Y)^2} + \frac{(dS_i)^3}{12\pi(1-Y)^3} \right) V_j \quad (1.7)$$

where:

$$d = \sum_{i=1}^n d_i$$

### 1.4.2 Computer simulation model: Monte Carlo simulations

Both molecular dynamics (MD) and Monte Carlo (MC) simulation methods are used to perform conformational sampling. The motion of molecules in MD simulation is defined in term of forces on the atoms, calculation of acceleration using Newton's second law and then building the trajectories by integrating the acceleration values over time [181]. The MD simulations could be performed in the Amber [105], CHARMM [106] and GROMACS [182] packages. Alternatively, the conformational sampling is performed using the MC algorithms [183]. The MC algorithms generate the new configurations of a molecule

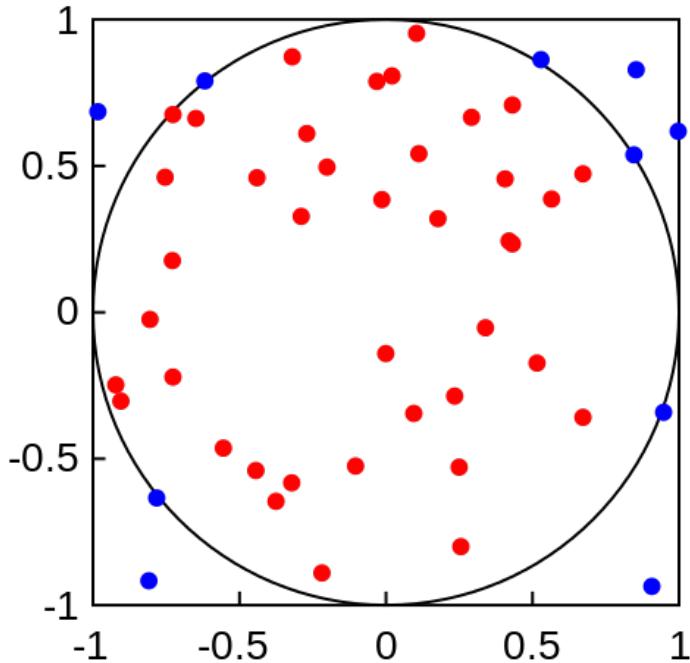


Figure 1.6: An illustration of Monte Carlo integration to estimate the value of  $\pi$ . The area of the circle is computed first by multiplying the area of the square with the ratio of points inside the circle to total number of points, and then the value of  $\pi$  is estimated by dividing the circle area by the square of the radius of the circle. (The figure is taken from <https://commons.wikimedia.org/wiki/File:MonteCarloIntegrationCircle.svg> and made available under the Creative Commons CC0 1.0 Universal Public Domain Dedication licence.)

of interest using stochastic methods. Due to the stochastic nature of MC algorithms, MC models generate a conformational ensemble equilibrated for a particular temperature. The MC algorithms are fast relative to MD methods due to no calculations of forces and accelerations to construct the trajectories. The working principle of MC simulations to perform conformational sampling is to select a random atom or group of atoms and move it randomly. In addition to these internal moves, the whole molecule could also be translated and rotated randomly. After performing each move, the new trial structure is evaluated using the Metropolis criterion based on initial and final energy values after performing an MC move [85, 86].

In addition to the standard MC conformational sampling algorithm, the MC integration technique is used for numerical integration using random numbers. A famous example of

MC integration is the determination of the value of  $\pi$ , where the accuracy of the estimated value is increased by increasing number of sample points (Figure 1.6) [184, 185]. The MC integration technique could also be applied to compute the fraction of available volume in a crowded medium. The working principle of the technique is inserting the protein of interest at random places and orientations in a crowded medium and determining the fractional available volume based on the fraction of successful insertions based on the lack of steric clashes.

### 1.4.3 The transition state theory

The transition state theory (TST) was originally developed by Henry Eyring [87] for gas phase reactions. The basic idea of TST is that the reactants are in equilibrium with the relatively high energy transition state (TS) (Figure 1.7). The high energy activated complex forms the products and the rate of such reaction is estimated using the kinetic theory. Moreover, TST is also used to investigate the various factors affecting rate constants, in particular thermodynamic quantities like activity coefficients, entropies, temperature, pressure and Gibbs free energies [186, 187]. Consider an open conformation A which is in equilibrium with the transition state TS. The TS forms a final compact conformation B. The reaction involving a conformational change proceeds in the forward direction (Equation 1.8) can be written in term of TST as follows (Equation 1.10):



$$\text{rate} = k_1[A] \quad (1.9)$$



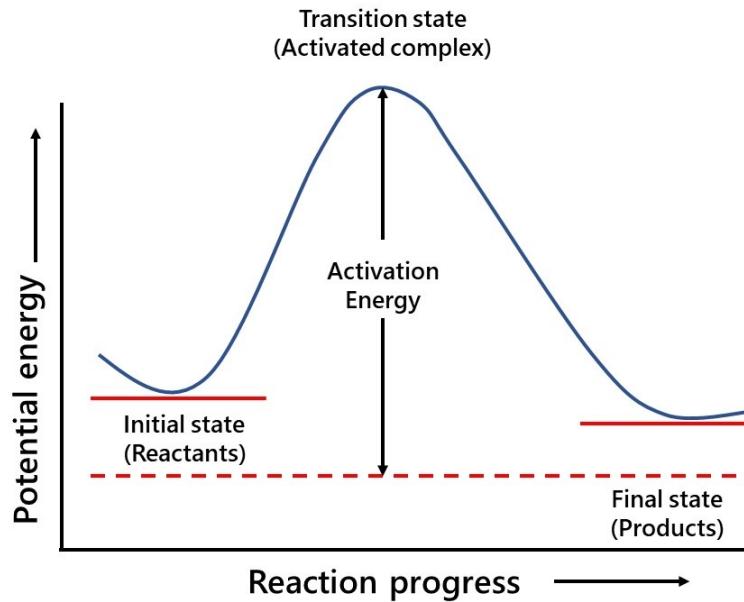


Figure 1.7: Illustration of the formation of high energy transition state of the reactants before converting into final products.

The rate of reaction depends on the concentration of the TS state, therefore

$$\text{rate} = k^\ddagger [TS]$$

The equilibrium constant  $K^\ddagger$  under crowded conditions is defined as

$$K^\ddagger = \frac{\gamma_{TS}[TS]}{\gamma_A[A]}$$

where  $\gamma_{TS}$  and  $\gamma_A$  are the activity coefficients of the transition state and the reactant  $A$  respectively. Rearranging the expression for the  $[TS]$  value,

$$[TS] = \frac{K^\ddagger \gamma_A[A]}{\gamma_{TS}}$$

The rate of reaction using the TST model is therefore;

$$\text{rate} = k^\ddagger [TS] = k^\ddagger \left( \frac{K^\ddagger \gamma_A[A]}{\gamma_{TS}} \right)$$

Comparing the TST rate equation with the reaction rate from Equation 1.9, we observe

$$k_1 = k^\ddagger K^\ddagger \left( \frac{\gamma_A}{\gamma_{TS}} \right)$$

Alternatively, the final rate of reaction can be written as

$$k_1 = k_0 \left( \frac{\gamma_A}{\gamma_{TS}} \right) \quad (1.11)$$

where  $k_0 = k^\ddagger K^\ddagger$  is the rate constant under dilute conditions.

Under ideal solution conditions the activity coefficients are taken as one in the TST expression (Equation 4.2). This approximation underestimates or overestimates the rate constant by ignoring the higher thermodynamic activities in crowded solutions. It depends on the ratio of activity coefficients of a reactant and the transition state. If, for example, the TS is less compact than A, then  $\frac{\gamma_A}{\gamma_{TS}} < 1$ , and the dilute-solution rate constant would overestimate the rate constant in the crowded solution. It is proposed that the accurate reaction rates could be achieved by incorporating the activity coefficient values which are computed by the scaled particle theory and MC simulations.

## 1.5 Objectives of the present study

Macromolecular crowding has a great impact on cellular processes such as the catalytic activities of proteins by favouring different conformations. A successfully developed kinetic theory including macromolecular crowding effects would be helpful to understand the behaviour of biochemical processes such as conformational equilibrium in detail. The objectives of the current study are classified into the following two key sections:

1. Preparation of crowded media by packing diverse and dynamic solution structures of 8 kDa PEG. This section will be composed of the selection of suitable computer simulation models (i.e. MC and MD simulations) to generate 8 kDa PEG conformations, packing and further equilibration of packed crowded media. This section will

also help to evaluate the aggregation behaviour of PEG after packing and equilibration process. The details regarding the selection of the suitable methods to pack and equilibrate the crowded medium and the corresponding results will be covered in the ‘Methods’ (Chapter 2) and ‘Construction of crowded medium’ (Chapter 3) respectively.

2. The development of appropriate models (i.e. SPT and MC models) to compute the thermodynamic activities of macromolecules of interest with minimum geometrical approximations and subsequently estimating the kinetics of conformational equilibrium via the transition state theory by incorporating the thermodynamic activities calculated in crowded solutions. This section will be composed of the extension of the SPT model and the development of the MC method to estimate the thermodynamic activities. The development of these models and the corresponding outcomes will be covered in ‘Methods’ (Chapter 2) and ‘Conformational equilibrium in crowded media’ (Chapter 4) respectively.

# Chapter 2

## Methods

The goal of this study is to investigate the crowding effects on conformational equilibrium by computing thermodynamic activities and fractional available volumes through analytical and computer simulation models. In this chapter, the detailed procedures used to determine the macromolecular crowding effects in each model are presented. In this context, two types of models are discussed, namely the scaled particle theory (SPT) [83, 146] and Monte Carlo (MC) simulations [188–190]. The SPT is a famous analytical model to compute the macromolecular crowding effect by calculating the excluded volume and chemical potential in the crowded medium using simple geometric approximations. The purpose of this chapter is to present the extended SPT and MC methods that will be used to estimate the crowding effects.

Figure 2.1 describes the overall setup applied in this work. The scheme includes all the key steps such as the conformational sampling of crowding agent molecules, packing and equilibration of simulation boxes and the calculation of fraction of available volumes and chemical activities. The process starts from the construction of conformational ensembles of crowders. In this regard, Monte Carlo and Molecular Dynamics sampling techniques are employed, which may require development of forcefield parameters for the crowder prior to performing the MD or MC sampling. Subsequently, the crowders are used to prepare the crowded environments. In the last step, MC and SPT calculations are performed to calculate the effect of crowding on the thermodynamics of conformational equilibrium.

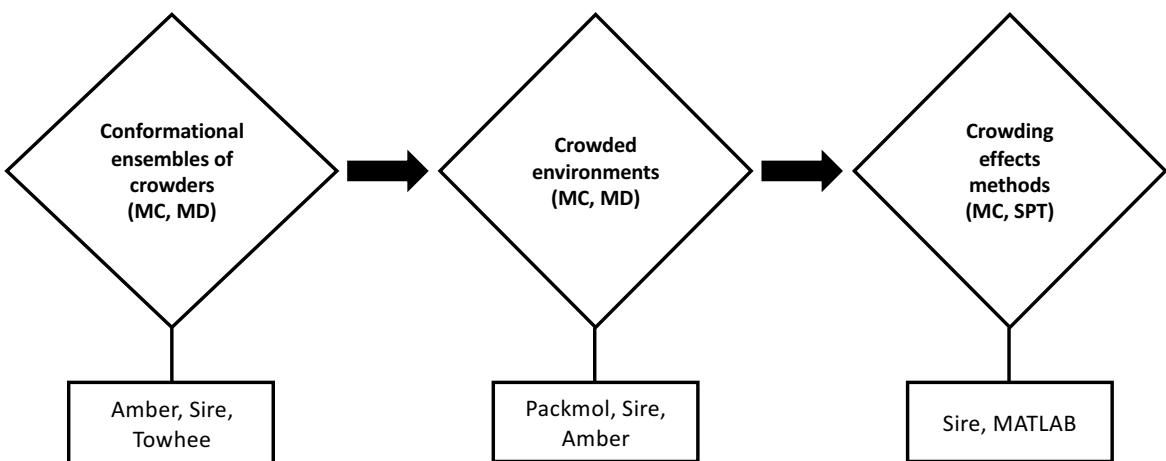


Figure 2.1: The schematic diagram represents the complete work-flow and techniques used in investigating the macromolecular crowding effects on the conformational equilibrium. The diamond shape represents the general step and the corresponding linked rectangle carries the appropriate computer programs to complete the task. Three major steps were performed in this investigation. The first step is the preparation of crowder molecules. This could be achieved through MD and MC conformational sampling with the Amber, Sire and MCCCCS Towhee programs. The next step is the preparation of crowded environments, using these conformational ensembles of crowders. This step requires optimized packing and equilibration of the packed boxes. In the last step, the thermodynamics and kinetics of the conformational equilibrium is computed with the MC or SPT models under the crowded conditions.

## 2.1 Ensemble of PEG conformations

Polyethylene glycol (PEG) is commonly used in computer simulations and experiments to mimic the crowding *in vivo* [80, 98, 111, 191] but there is no representative crystal structure of high molecular weight PEG found in the protein data bank. A dimer of the PEG monomer ethylene oxide (<http://www.rcsb.org/pdb/ligand/ligandsummary.do?hetId=PEG>), is found in many different crystal structures as a ligand but this PEG fragment requires an additional 180 monomers to construct an 8 kDa PEG. Moreover, the crystal structures found in previous reports [55, 89, 93, 101, 109] are not directly relevant to the solution structures. To solve the problem of the missing PEG structure, the conformational sampling simulations are performed in two steps. In the first step, the structure of 8 kDa PEG is constructed and optimized. This single structure is not a representative of solution structures as a PEG solution contains random conformations of PEG [58, 92]. Therefore, conformational en-

sembles of PEG are generated by performing MC and MD simulations.

These computer simulations require forcefield parameters for the PEG if these parameters are not provided in the program of interest. These forcefield parameters are essential to perform simulations and helpful in evaluating the conformational changes by calculating the total energy of the structure. MCCCCS Towhee [104], Sire [192, 193], and Amber [105, 194] packages were employed to construct the ensembles of PEG conformations. MCCCCS Towhee provides general forcefield parameters that facilitates the construction of PEG, whereas Sire and Amber do not. The PEG forcefield parameters are critical to perform simulations in both programs. A number of publications on the development of the PEG forcefield parameters have been reported by different researchers over time [98, 132, 195–197]. However, PEG forcefield parameters did not come by default in Amber. The PyRED server [198] was used to construct the forcefield parameters for PEG which allows us to perform conformational sampling simulations. This section explains the details of forcefield parameters development and subsequently the details of MC and MD sampling methods that are employed to construct an initial PEG and its conformational ensembles.

### 2.1.1 Forcefield parameters for PEG

The automated tools for building forcefields reduce the probability of errors that arise when a forcefield is built manually. Many approaches such as the Mulliken [199], Lowdin population analysis [200], atoms in molecules theory [201], the AMI-BCC method [202, 203] and many other models [204–207] have been developed to derive the atomic charges and subsequent parameters with desired accuracy. However, none of the approaches has been proven to be the best in all respects [208]. To build the missing PEG forcefield parameters in Amber and Sire, the RESP (the restrained electrostatic potential) ESP (electrostatic potential) charge Derive (R.E.D.) toolkit was used [208, 209].

The RESP ESP charge Derive (R.E.D.) toolkit provides a straightforward way to generate the forcefield parameters for molecules that are not included in the standard Amber

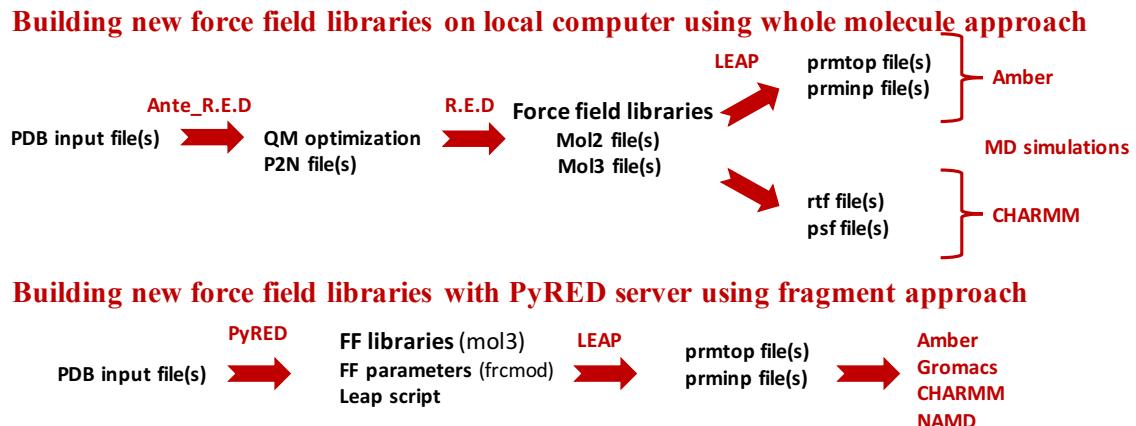


Figure 2.2: The schematic diagram represents the steps to carry out by R.E.D and PyRED server to construct the forcefield libraries of non-standard molecules.

forcefield with a two-step calculation [208, 210]. The first step is to compute the molecular electrostatic potential (MEP) surface around the optimized structure using a quantum mechanics (QM) program while the RESP and ESP charges are determined in the second step by fitting the charge values to reproduce the MEP surface. Geometry optimization and MEP surface calculations are performed using QM programs such as Gaussian09 [211], GAMESS [212, 213] or FIREFLY [214] while charge fitting to reproduce the MEP surface can be done using the RESP [209] or FITCHARGE [215] programs. The RESP, ESP charges and forcefield parameter of PEG can be generated using *antechamber* (Amber) or the R.E.D. program (<http://q4md-forcefieldtools.org/>). There are two ways to construct the PEG forcefield library files in the R.E.D program. The first option is to generate all the files on a local computer with Perl, a quantum mechanics optimization program such as Gaussian09, GAMESS, or FIREFLY, and Ambertools14 (*xleap/tleap*). However the other way is simpler and all the jobs can be executed on the PyRED server [198]. The PyRED server offers two approaches, namely the “whole molecule approach” and the “fragment approach” to construct forcefield parameters. The required input and resultant output files description for both approaches is available at <http://q4md-forcefieldtools.org/>. The global procedure regarding development of forcefield parameters on R.E.D via a local machine and the PyRED server is illustrated in Figure 2.2.

### 2.1.2 Case 1. Whole molecule approach

Four key steps, formation of the optimized structure, determination of the MEP, calculation of atomic charges by reproducing the MEP, and generation of *top/crd* files are performed as follows:

1. The PEG structure was constructed in GaussView 5.0 and the geometry optimization was performed in the Gaussian09 QM program.
2. The first computation of the R.E.D program using a PDB file generates the three new files namely PDB, P2N and the script files necessary for QM optimization calculations in the local directory. The P2N file is important because it contains the detailed information of atom names and their connectivities, which are required in the charge fitting step and for creating the *top/crd* file.
3. The final step involves the generation of parameter files by executing the *leap* script in the presence of parameter files.

### 2.1.3 Case 2. Fragment approach

The forcefield parameters of the PEG polymer can also be developed easily through using a R.E.D.S Dev server at <http://q4md-forcefieldtools.org/>. A two-step fragment approach requires a PDB file, and two input files (*System.config* and *Project.config*) to construct parameters. (Input files are provided in Appendix A.1.1.) In the first step, the molecule is split into three fragments namely, A, B and C (Figure 2.3) by calculating the charges on the three groups. Before proceeding with the first step, the ‘A’ terminal has to be replaced with a methyl group because the program accepts only heavy atoms on a terminal site rather than a single hydrogen as fragments. If the hydrogen at the ‘A’ terminal is not replaced then the program produced only a single fragment rather than the three desired fragments. The second step is similar to the previous ‘whole molecule’ approach that constructs the force-field parameters. These are the simplified key steps to implement the fragment approach for PEG:

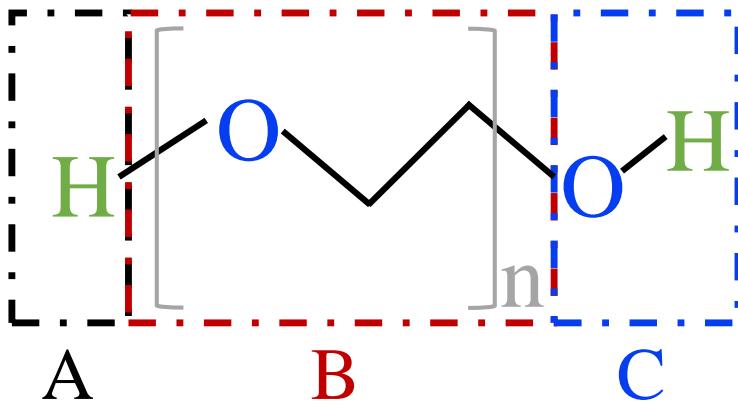


Figure 2.3: Definition of three PEG fragments as ‘A’, ‘B’ and ‘C’ where ‘B’ is the repeating monomer.

1. Generate the structure of a PEG molecule containing one monomer only ( $n = 1$ ) using the GaussView 5.0 package, with unique atomic names.
2. PyRED requires *System.config* and *Project.config* configuration files to run jobs on the server. The *System.config* file contains details about the geometry optimization QM method, whereas the *Project.config* configuration file contains the information on charge constraints. The charge constraints are required to form desired fragments. The QM calculations were performed with Gaussian09 using Hartree Fock (HF)/6-31G (d) [216], density functional theory (DFT) B3LYP/6-31G (d) [217, 218], and Møller-Plesset perturbation theory of second order MP2/cc-pVTZ methods [219, 220].

Two charge constraints are applied to generate and compute charges on three fragments in a two-step process. In the first step, charges on the ‘A’ and ‘C’ terminals (methyl and hydroxyl groups respectively) are calculated by defining single INTRA-MCC2 charge constraint (Equation 2.1).

$$\text{MOLECULE1} - \text{INTRA} - \text{MCC1} = 0.0 \mid 1 \ 2 \ 3 \ 4 \ 12 \ 13 \mid \text{Remove} \quad (2.1)$$

where *MOLECULE1*, *INTRA*, *MCC1* represents the molecule name, intra-molecular charge constraint, and molecular charge constraint on two groups in a given molecule

respectively. The charge constraint is assigned a zero value which represents the overall charge of the molecule. In the next section, the atomic positions of the terminal groups ('A', and 'C' respectively) are defined and the *Remove* specifier cuts the structure.

After completing the job, charges on 'A' and 'C' terminals are calculated by taking the average of the sum of individual atomic charges of 'A' and 'C' terminal. Mathematically, it can be expressed by the following simple relation:

$$\text{Total charge} = |q_{\text{CH}_3}| + |q_{\text{OH}}| \quad (2.2)$$

$$\text{Fragment charge} = \frac{|\text{Total charge}|}{2} \quad (2.3)$$

Both terminal groups get equal and opposite charges. Thus, the methyl group has positive charge and hydroxyl has negative charge in the particular case considered here. In case of identical terminal groups, for example methyl on both sides, one terminal connected with subsequent atom of lower electronegativity will get positive charge and the other terminal attached to a subsequent atom of higher electronegativity will get negative. This charge definition is essential to build neutral fragments. For example, the charge values for the 'A' and 'C' terminals are found to be +0.230 and -0.230 respectively. The charge value for the central fragment is calculated by running a second PyRED job with two INTRA-MCC2 charge constraints on each terminal group.

$$MOLECULE1 - \text{INTRA} - \text{MCC2} = 0.230 \mid 1 \ 2 \ 3 \ 4 \mid \text{Remove} \quad (2.4)$$

$$MOLECULE1 - \text{INTRA} - \text{MCC2} = -0.230 \mid 12 \ 13 \mid \text{Remove} \quad (2.5)$$

3. Finally, forcefield parameter library files for any length of PEG molecule can be constructed by repeating the central fragment in the growing sequence of polymer in

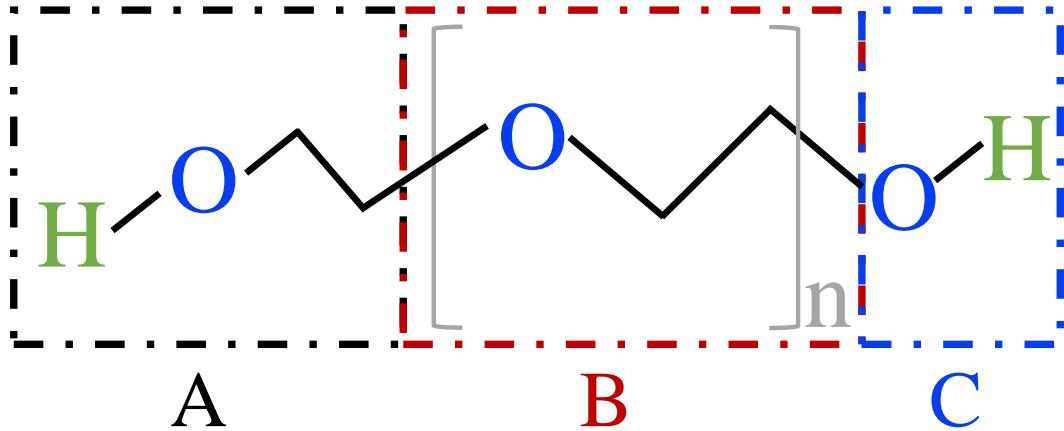


Figure 2.4: Three fragments namely, A, B and C, of a PEG molecule without methyl substitution.

*xtleap/tleap* script file.

$$\text{PEG} = [A - (B)_n - C] \quad (2.6)$$

where ‘A’, ‘B’, ‘C’, and ’n’ represents methyl, central, and hydroxyl terminals and number of central monomers respectively.

The resultant PEG has one methyl terminal instead of the original hydrogen terminal. Winger et al. showed that the terminal methyl groups are responsible for compact structure formation during MD simulations [98]. Therefore, an attempt was made to construct the initial PEG structure with a hydrogen atom as the ‘A’ terminal by attaching a whole PEG residue on terminal ‘A’ (Figure 2.4). The forcefield parameters were built by applying the fragment approach as discussed in the present section. The results of these forcefields will be discussed in chapter 3.

#### 2.1.4 MC sampling in MCCCS Towhee

MCCCS Towhee [104] was used to construct an initial optimized PEG structure at first, and subsequently conformational ensembles by conducting MC simulations. MCCCS Towhee was developed to perform vapour-liquid phase equilibrium simulations for flexible molecules using the Gibbs ensemble. It provides more than seventy built-in forcefields,

which facilitates the construction and optimization of large structures through MC conformational sampling. We experimented with MC simulations under different conditions such as using either NPT [221] or NVT [222–224] ensembles, different random number generators and different temperatures, pressures and box dimensions to maximize diversity of ensemble structures. The MC simulation protocol in MCCCS Towhee could be described in four steps:

1. Define the input parameters in MC protocol such as the type of ensemble, temperature, pressure and length of MC simulations.
2. Define the box dimensions, and the output frequency of the desired results such as how frequently the energy and structure coordinates are written to output files.
3. Select appropriate forcefield parameters and type of algorithm to perform MC moves on the structure.
4. Construct the desired structure by defining atomic names, charges and connectivities.

In the first simulation protocol to construct and optimize the PEG structure, NPT [221] ensemble MC simulations using the Amber96 [225] forcefield at 101 kPa pressure and 300, 600 and 1200 K temperatures were performed. The NPT ensemble was chosen because the calculations were found to be less expensive than NVT ensemble calculations. Since the aim is to enhance the conformational sampling at this point, therefore the MC simulations were performed at three temperatures, i.e. 300, 600 and 1200 K. The initial PEG molecule was built by joining 141 O–CH<sub>2</sub>–CH<sub>2</sub> monomers in a 100 × 100 × 100 Å<sup>3</sup> periodic box and optimized by performing 1.2 million Monte Carlo moves at 1200 K. Each MC move involves volume change to maintain a constant pressure, intra-molecular bond length and angle changes, and translation movement about the center of mass. The frequency at which each move is performed can be adjusted by defining the probability values between zero to one. The performance of any particular move can be suppressed by assigning the probability of zero. After equilibration with 1.2 million MC moves, an additional 180,000 MC

moves were performed to save the structure after every 60,000 moves for a total of three equilibrated structures to be used as starting points for the generation of conformational ensembles. These three PEG conformations differ significantly in radius of gyration from each other. To generate the PEG conformational ensembles in the second step, a total of nine 12-million-step-long MC simulations were performed at 300, 600, and 1200 K using three equilibrated structures obtained in the earlier equilibration step. The input file for MCCCS Towhee is provided in Appendix A.2.

### 2.1.5 MC sampling in Sire

Sire is a free, open source molecular simulation framework that allows the users to write and develop new algorithms to conduct numerical and molecular simulations [193]. Sire is composed of a collection of independent libraries written in C++. These libraries are used as building blocks or modules that contain a set of attributes and operations to perform particular tasks. For example, ‘Sire.Move’ is a module that provides all necessary functions to perform all types of intra- and inter-molecular moves on the structure. By connecting these blocks via a Python interface, one can develop, perform and analyse MC simulations. Further, Sire is compatible with the Amber package and offers a complete implementation of the Amber [194, 226], OPLS, [227] and CHARMM [106] forcefields. There are three ways to accomplish MC sampling with Sire (Figure 2.5). Following are three key steps to construct a structure and conduct MC conformational sampling using Sire (all Sire inputs files are provided in Appendix A.3.2):

1. Load the Sire libraries.

- The libraries contain all the necessary information such as atomic names, charges, and parameters to calculate energies.

2. Load the molecule from a Sire script, PDB file, or Amber topology and coordinate (*top/crd*) files.

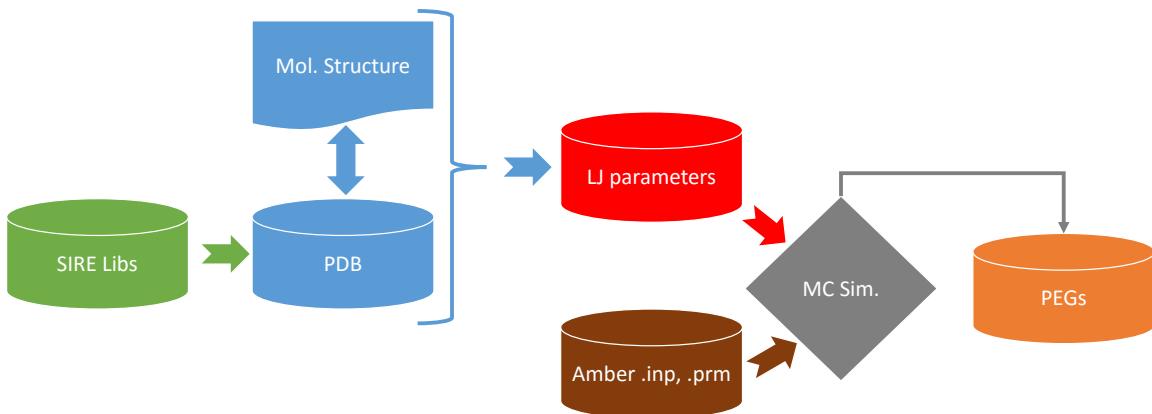


Figure 2.5: The schematic diagram represents the three key steps necessary to perform MC conformational sampling in Sire. In the first step, the Sire libraries are loaded, followed by loading the molecular structure in the form of PDB or Amber coordinate and topology files. The initial structure can also be constructed using a script. The script or PDB formats require Lennard-Jones (LJ) atomic charges and energy parameters, whereas Amber input *.prmtop* and *.inpcrd* files do not require any LJ forcefield parameters to initiate the MC simulations.

- In the Sire script, the new molecule can be constructed using the “Molecule()” constructor. This is the most complicated and tedious choice, requiring a lengthy script in order to populate the constructor with atomic names, connectivities, coordinates and Lennard-Jones (LJ) forcefield parameters. The advantage of this method is that Sire libraries provide all the necessary information regarding construction of the molecule and there is no need to build any forcefield parameter libraries as we do in the majority of simulations. The drawback of this choice is that the intra-molecular energies cannot be calculated in simulations with intra-molecular moves.
- The easiest and most efficient way to construct a molecule is with a structure builder program such as Gaussview [228], Chemdraw [229], or Avogadro [230]. Then the corresponding Amber topology *.top* and coordinate *.crd* files are generated using the *tleap* utility program.

### 3. Perform internal moves to conduct MC conformational sampling.

- Sire performs MC moves on the molecule with the help of a random number generator. The random numbers are used to pick the random bonds, angles and dihedral angles of a molecule and to perturb the selected bonds, angles and dihedrals by a randomly selected amount. After performing each move, the algorithm performs the canonical acceptance test to determine whether the given move is accepted or not. The energy difference is calculated by taking the difference of the energies before and after the move and then the canonical acceptance test uses the computed energy difference to calculate the values of  $x$  according to equation 2.7 [86]. Moves are accepted if they have a negative  $\Delta E$  or if  $x$  is greater than a randomly generated number between 0 to 1.

$$x = \exp(-\Delta E/kT) \geq \text{random}(0, 1) \quad (2.7)$$

where  $\Delta E$  is the energy difference before and after performing an MC move,  $k$  is the Boltzmann constant, and  $T$  is the temperature in Kelvin. It is noted that  $x$  is a function of temperature, and that the number of accepted moves will increase as the temperature rises. Moreover, the number of accepted moves is also increased significantly if bonds and angles are perturbed by a small magnitude. This will take a long time to converge the system. The larger moves will help to explore the conformational space quickly, whereas small moves are useful to optimize the structure.

The equilibrated PEG structure in MCCCS Towhee and a linear PEG structure are used as starting points to generate PEG ensembles. The input Amber *.top* and *.crd* files are prepared using PEG parameters along with Amber96 forcefield parameters. In the MC simulation protocol, 2-million-move-long MC simulations were performed at 101 kPa and using temperature cycles alternating between 1200 and 300 K. The first 1000 internal moves were performed at 1200 K and then a structure is saved after performing an additional 1000

moves at 300 K. This temperature quenching approach was not only applied to enhance the sampling of the energy and conformational spaces but also to save the conformations at a physiological temperature. Each move perturbs the bond lengths and bond and dihedral angles randomly between -0.5 to +0.5 Å and -20 to +20 degrees respectively. The ranges of these moves were selected based on trial and error and these values produced reasonably diverse conformational ensembles in a reasonable amount of time.

### 2.1.6 MD sampling in Amber

Finally, MD simulations have been employed in Amber to construct the library of PEG conformations. In general, the Amber MD simulations are accomplished in two steps, namely minimization and MD simulations (Figure 2.6). The minimization is necessary to remove intermolecular steric clashes/overlapping while MD simulation samples the conformational space. In general, the Amber MD simulations are accomplished in two steps, namely minimization and MD simulations. The minimization is necessary to remove intermolecular steric clashes/overlapping while MD simulation samples the conformational space.

A library of PEG conformations was built by performing a two-step MD simulation on the PEG structure. The initial system containing a PEG molecule was minimized using the implicit solvent model (the standard pairwise generalized Born model [231]) with 5000 steepest descent and 2500 conjugate gradient minimization steps. The cut-off range for long-range non-bonded interactions was set to 10 Å. In the final step, 10 ns long MD simulations were run with a 1 fs time step. The temperature of the system was maintained at 300 K using the Langevin thermostat. All input files are provided in Appendix A.4.

## 2.2 Packing and equilibration

The next step is to pack and equilibrate the simulation boxes from 0.1 to 0.6 g cm<sup>-3</sup> concentration. The Packmol package [232, 233] is used to pack the simulation boxes. Pack-

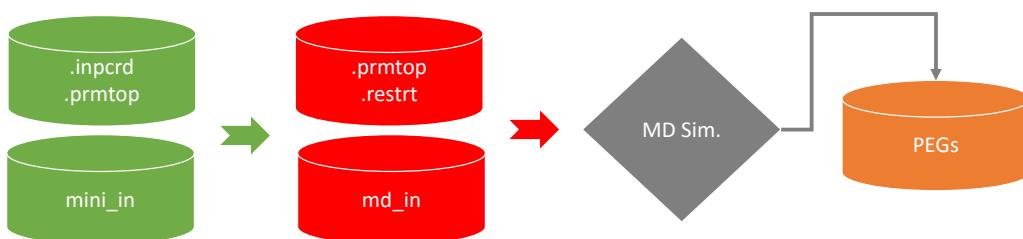


Figure 2.6: This schematic diagram represents the MD simulations performed with Amber. The process starts with PEG coordinate *crd* and topology *top* files. The ‘*mini\_in*’ file contains the input parameters necessary for the minimization step. On completion of minimization, a new *.restrt* coordinate file is created. The resultant *.restrt* coordinate file and ‘*MD.in*’ file are used to conduct the MD simulations. The ‘*MD.in*’ file contains all necessary inputs such as length, step size and output files for MD simulations.

*mol* is a simple program that facilitates the construction of boxes with desired dimension, shape and concentration. Random conformations of PEG were chosen from the conformational libraries and packed in a cubic box of dimension  $300 \times 300 \times 300 \text{ \AA}^3$ . The details on the packing algorithm are described by Martinez [232, 233]. In the packing algorithm, tolerance is an important parameter to enhance the packing quality by keeping the minimum boundary-boundary distance between molecules without steric clashes. The default value of tolerance is 1 Å and we used 3.0 to 5.0 Å in packing of simulation boxes. The default tolerance can also be used to pack the systems. However it can produce systems with a tight packing at higher concentrations and result in very high total energies. A large tolerance facilitates the optimized packing without steric clashes but it takes a long time. Sometimes, it is hard to get a packing configuration satisfying the large tolerance criterion. In this situation, the program keeps the best packed configuration (input file available in Appendix A.5). Figure 2.7 illustrates a simulation box packed with PEG molecules.

The simulation boxes were packed using the PEG conformations prepared under dilute conditions. Therefore, in order to consider the effect of crowding on the distribution of PEG conformations, the six simulation boxes were further equilibrated using the three approaches as explained below.

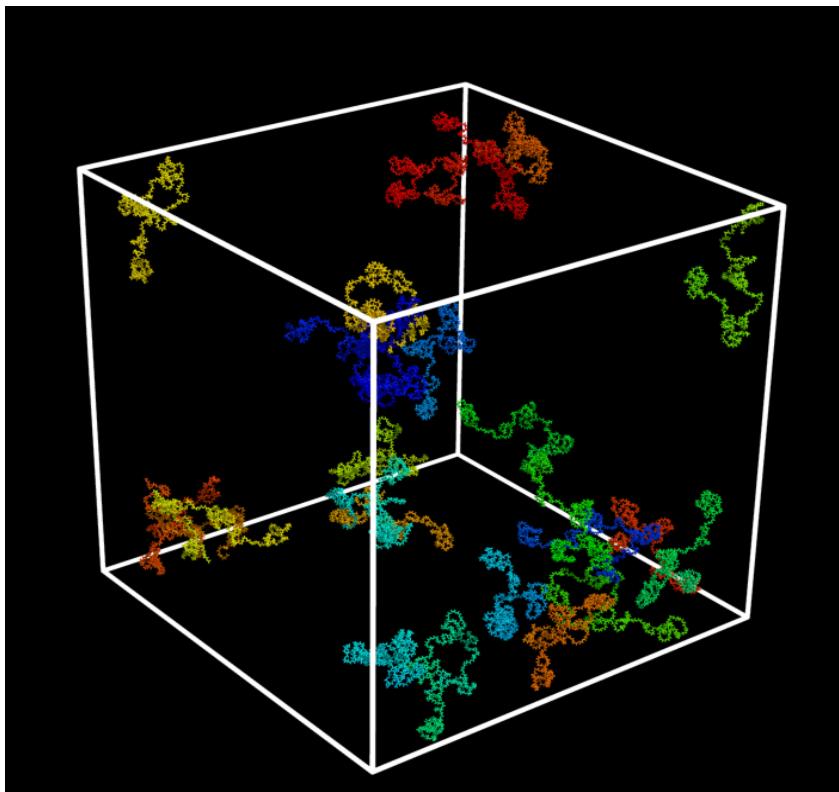


Figure 2.7: The packing of PEG at  $0.1 \text{ g cm}^{-3}$  concentration in a cubic box of  $300 \times 300 \times 300 \text{ \AA}^3$  dimensions with Packmol using  $3.0 \text{ \AA}$  tolerance. The different colours are used for illustration purposes and represent the individual molecules.

### 2.2.1 Minimization with Sire

MC rigid body and internal moves were performed on the molecules in the simulation boxes (input file available in Appendix A.5.1). The rigid body moves are responsible for translating and rotating the molecule while internal moves fold/unfold the structure by moving randomly chosen bonds, angles and dihedral angles. The acceptance criterion for MC moves is the standard canonical MC acceptance test at  $300 \text{ K}$  temperature (Equation 2.7). The algorithm performs 1000 iterations in a series to minimize the system in which each iteration runs 10,000 in total MC rigid body and internal moves. The algorithm saves the restart file and prints out the ratio of the number of accepted to rejected moves after every 100 iterations. After completing the 1000 iterations, the algorithm runs an additional  $2 \times 10$  iterations and populates these two windows with energy values. These energy values are used in a Student's  $t$  test [234, 235] to determine whether the system has equilibrated or not.

This test compares the energy values stored into two windows and checks the condition if the significance level  $s < 0.1$  is satisfied to terminate the simulations, otherwise continue the loop for an additional  $2 \times 10$  iterations until the condition is satisfied.

### 2.2.2 Swapping equilibrium with Sire

The algorithm swaps a randomly chosen molecule in the simulation box with a randomly selected molecule from the PEG library (input file available in Appendix A.5.2). Successful swaps were determined by the standard canonical MC acceptance criterion [86] at 300 K (Equation 2.7). If the molecule insertion is accepted, the algorithm keeps the configuration for the next step; otherwise, it returns the simulation box to its pre-swap configuration and starts the process over, selecting a new molecule for swapping. The algorithm places the molecule selected from the library randomly within the simulation box at a random orientation. It does not generate internal moves in any of the molecules, it is being assumed that the library contains a reasonable sampling of the conformational space. The swapping script performs 0.1 million iterations by default and saves the restart file at every 10,000 steps. The Student's  $t$  test with the same criterion as in method one is applied to stop the calculations. Equilibration by swapping is time consuming given that successful insertions may not occur after a certain number of successful insertions. Therefore, it is good to perform the Student's  $t$  test after completing 10 million iterations to allow the system to run for enough time to see if there are any successful insertions before employing the  $t$  test to terminate the simulations.

### 2.2.3 MD Amber simulations

MD simulations were performed under the same MD protocol as described for a single PEG molecule to equilibrate the simulation boxes (input file available in Appendix A.5.3). However, with this protocol, the pressure fluctuates and results in volume changes. It is important to keep the total volume fixed after equilibration. Therefore, the simulation boxes were equilibrated under periodic boundary conditions. In the first step, the simulation boxes

were minimized by performing 1000 and 500 steps of minimization and steepest descent minimization respectively using constant volume periodic boundary conditions with 10.0 Å cut-off range. In the second step of MD simulations, 20-40 ns long simulations were performed at a constant temperature of 300 K and constant pressure periodic boundary conditions with an average pressure of 1 atm. The MD simulation time increased monotonically with concentration of simulation box.

## 2.3 Fractional available volumes calculations

Fractional available volume in the packed simulation boxes was assessed by performing MC simulations in the Sire molecular simulation framework [16, 17]. Two different algorithms, termed parallel-energy (Appendix A.6.1) and parallel-distance (Appendix A.6.2), were developed. In both algorithms, a randomly oriented probe molecule (protein/RNA) is first placed at a random position inside the simulation box and the successful and failed trials are chosen based on the steric clashes. The fraction of available volume then can be calculated from the ratio ( $p'$ ) of successful insertions to total trials using the following equation.

$$p' = \frac{n_{\text{successful}}}{n_{\text{total}}} \quad (2.8)$$

The threshold energy criterion of 50,000 kcal/mol was set to determine whether there is a steric clash or not. The threshold energy criterion was found by trial and error. One way to predict the threshold energy criterion is to compare the energies of reference and test systems. The reference system contains all the molecules including background (PEGs) and probe molecule (RNA/protein). In contrast, the test system used to insert the probe molecule contains only the PEG background molecules at the same concentration of PEG as in the reference system. The two systems were prepared under the same packing conditions (tolerance and number of steps for packing) in the Packmol package. Reference system energies calculated using Sire ranged from -500 to 500 kcal/mol. The threshold energy at which a steric clash was recognized was set at 100 times the order of magnitude of

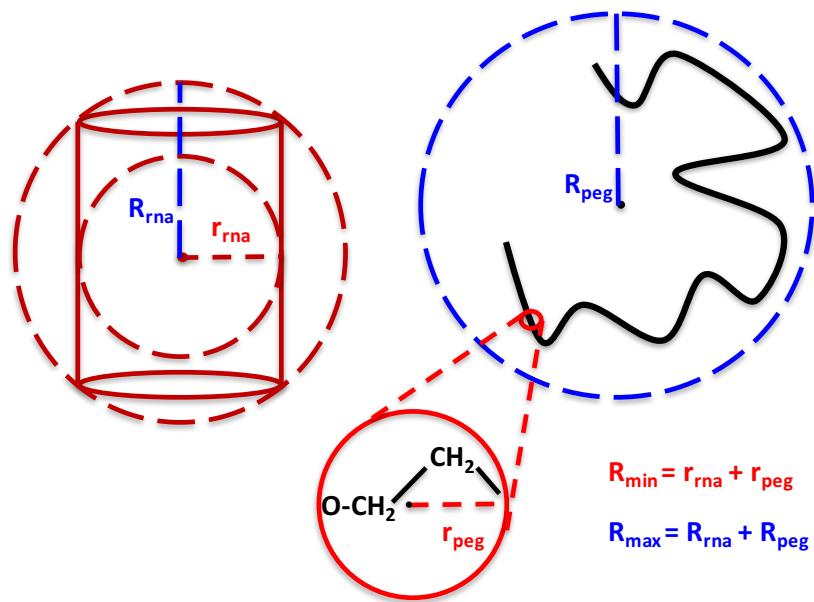


Figure 2.8: Estimation of cutoffs for atom-by-atom checking for steric clashes. The basic concept is described by providing an example where the probe RNA molecule and the PEG crowders are considered roughly cylindrical and spherical respectively. The relevant dimensions of the two molecules are added to generate the bounds  $R_{min}$  and  $R_{max}$  used as cutoffs in our calculations. The RNA studied here has a nearly cylindrical shape, so our estimates of  $r_{RNA}$  and  $R_{RNA}$  give a reasonable representation of the geometry of this molecule. On the other hand, PEG adopts a variety of conformations in solution. For  $r_{PEG}$ , we used the radius of a PEG monomer, yielding a very conservative estimate for  $R_{min}$ , thus avoiding false positives relative to a direct atom-by-atom test for clashes. For  $R_{PEG}$ , we used the radius of a bounding sphere for a typical PEG conformation, again yielding a conservative estimate of  $R_{max}$ , this time avoiding false negatives.

the reference system energies, i.e. 50,000 kcal/mol. The most accurate available volumes can be measured using the parallel distances algorithm. The steric clashes are determined on the basis of the distance between the atoms of probe and background molecules using the van der Waals radii. The minimum threshold distance was set using the sum of the van der Waals radii for each pair of atoms belonging respectively to the probe and a background molecule. The program calculates the distances between the geometric centroids of the probe molecule and all background molecules, and sorts them in ascending order. A simple test on the centroid-to-centroid distances was implemented in order to find the cases for which atom-by-atom checks for clashes were required, as illustrated in Figure 2.8. Molecules whose centroids were closer than  $R_{min}$  (10 Å for our example system

composed of PEG and RNA molecules) were immediately determined to clash, and thus the insertion trial to have failed, while molecules whose centroids were further than  $R_{max}$  (60 Å for our system) were determined to be non-clashing. Molecules whose centroids were between these two extremes were checked atom-by-atom for steric clashes.

The computational cost of both programs depends on the sizes of the molecules and on the concentrations. Energy calculations in Sire are very fast, so the energy threshold algorithm has a significant speed advantage over the distances measuring algorithm. The use of cutoffs to avoid calculating all pairwise atomic distances however greatly improved the speed of the parallel distance algorithm.

The flow chart summarises both algorithms for steric clash determination (Figure 2.9). Script files implementing these methods are provided in supplementary data. In the final step, the fraction of available volume is converted to the non-ideal chemical potential that facilitates the comparison of the scaled particle theory and computer simulation results. Mathematically, the fractional available volume in the crowded solution can be represented with equation 2.9 where  $p'$  represents the ratio of successful insertions to total trials as defined in equation 2.8.

$$\frac{\mu^{\text{NI}}}{kT} = \log\left(\frac{1}{p'}\right) \quad (2.9)$$

This expression is derived by considering a protein molecule (P) in equilibrium in dilute and crowded medium.



The free energy change for the reaction can be written as

$$\Delta G = kT \ln\left(\frac{\alpha_{\text{crowded}}}{\alpha_{\text{ideal}}}\right) \quad (2.11)$$

$\alpha$  is used to represent the activity of the solute instead of  $a$  which is a shape coefficient in

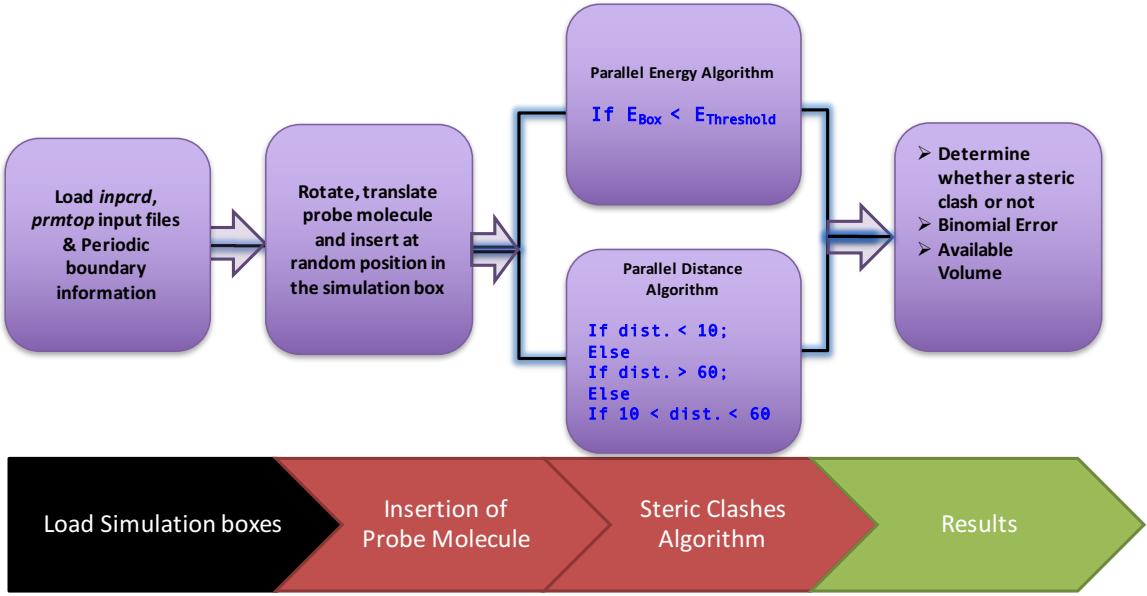


Figure 2.9: Workflow of Monte Carlo simulation algorithms to estimate the fractional available volume.

the scaled particle theory equation. The  $\alpha_{\text{crowded}}$  is

$$\alpha_{\text{crowded}} = \gamma_{\text{crowded}} \left( \frac{C_{\text{crowded}}}{C^\circ} \right) \quad (2.12)$$

where  $C_{\text{crowded}}$  is the concentration under crowded conditions, and  $C^\circ$  is the standard (reference) concentration. In the crowded solution, the occupied volume is an important quantity, so the concentration can be replaced by the volume and number of molecules.

$$\Delta G = kT \ln \left( \frac{n/V_{\text{available}}}{n/V_{\text{total}}} \right) \quad (2.13)$$

on simplifying equation 2.13,

$$\mu^{\text{NI}} = \Delta G = kT \ln \left( \frac{V_{\text{total}}}{V_{\text{available}}} \right) \quad (2.14)$$

which is equivalent to equation 2.9.

## 2.4 The scaled particle theory

Thermodynamic activity is a meaningful quantity in predicting the reaction rates and chemical equilibria [3]. The concentration dependent properties such as thermodynamic activities and nonideal contribution of excluded volume to the chemical potential of a given solute particle can be estimated using a statistical model of hard fluids [4, 12, 82]. This model is named the scaled particle theory and it estimates the concentration dependent properties as a function of shape and size of the particle. The shapes and sizes of given particles are characterized by three geometric parameters, namely; the volume ( $V$ ), surface area ( $S$ ) and radius of curvature ( $R$ ).

An algorithm is presented here that is an extension to the SPT model [83] to estimate the thermodynamic activities of individual macromolecules of an arbitrary shape and size by convexification [236]. A convex shape encapsulates all the given points present in three dimensional space inside it and a line between any two points within the shape will be inside the shape entirely. The construction of the convex hull requires the identification of points on the boundary of the hull. This approximation could represent shapes and sizes more realistically than using a sphere of approximate radius encapsulating the whole molecule. The algorithm has been written in the MATLAB language [237] and is composed of two functions. The first function, *findcurvature.m* (Appendix A.7.1) computes the three geometric parameters while the *findactivity.m* function (Appendix A.7.2) determines the thermodynamic activities of macromolecules of interest at any given concentration. The global procedure for measuring the geometric parameters and predicting thermodynamics of conformational equilibrium involves the following key steps.

1. Extract the 3D coordinates via *pdb2mat.m* function. This file is available at <https://www.mathworks.com/matlabcentral/fileexchange/> with ‘read and write PDB files using matlab’ heading.
2. The 3D coordinates are fed to the convex-hull function [236] to generate the convex hull. This convexification method uses the Delaunay triangulation scheme to form

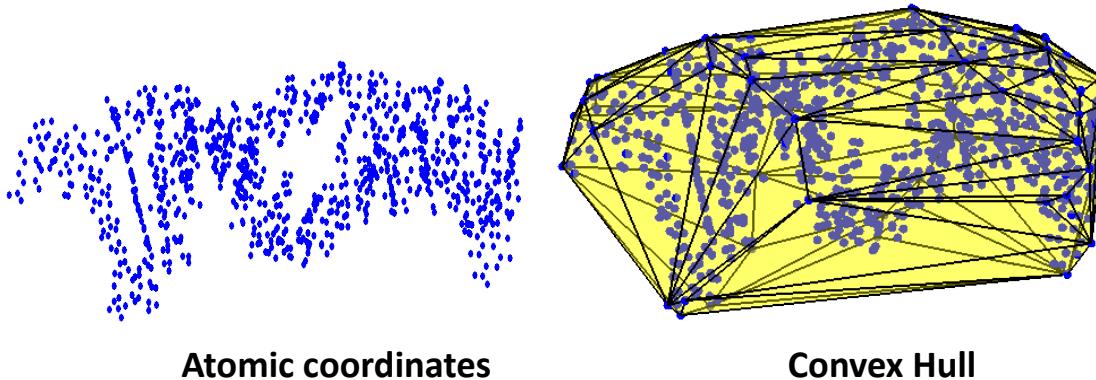


Figure 2.10: Construction of Delaunay triangles by joining the atomic coordinates that lie in the convex hull and to calculate the volume and surface areas.

the convex hull. Delaunay triangulation provides the set of points that are the vertices of the triangles forming a convex hull (Figure 2.10). These points are used in further calculations to estimate the geometric parameters of the molecule.

3. The convexification results in the formation of tetrahedra using the atomic coordinates. The sum of the volumes of all the tetrahedra gives the one possible measure of the volume of the molecule. This is an approximated volume which overestimates the volumes of non-linear molecule if the molecule is not overall convex. The volume of the molecule can also be estimated using different mathematical formulas such as the volume from the molecular weight, radius of curvature, and by rolling a virtual molecule on the surface of the macromolecule [238].
4. Summing the areas of the triangles present on the surface of the molecule gives a measure of the surface area of the molecule. The surface area can also be computed from solvent accessible surface area.
5. The mean radius of curvature  $R$  is computed by solving a least sum of squares sphere fit problem in terms of a least sum of squares plane fit problem using an inverse geometric transformation [239]. The algorithm was described by Coleman *et al.* [240]. In this algorithm a matrix  $M$  is constructed containing the atomic coordinates,  $M = [p_1, p_2, p_3, \dots, p_{N-1}, p_N]$  where  $p$  is the vector of  $x, y, z$  coordinates of

each point. The convex-hull algorithm extracts the  $N$  boundary points on the convex hull and these boundary points are used to solve the least sum of squares plane fit problem through seven iterative steps.

- (a) The matrix  $M$  is split into two sets. Each point of matrix  $M$  is treated as an inversion point  $j_i$  with  $r$ ,  $s$ , and  $t$  values in the first set, representing the  $x$ ,  $y$ , and  $z$  coordinates of the inversion point, while the rest of the points  $[x_i, y_i, z_i]$  in the second set are inverted with respect to inversion point  $j_i$  using the inverse geometric transformation formula. This geometric transformation results in a new set of point represented as  $t_i = [t_{xi}, t_{yi}, t_{zi}]$ , which represent the points on the plane.
- (b) Fit the least sum of squares plane to the set of  $t_i$  points. This step will give a point and a normal unit vector on the plane.
- (c) Find a point named ‘a’ on the plane closest to point  $j_i$  by solving the plane equation using the normal vector and point on the plane from the previous step.
- (d) Invert point ‘a’ by applying the same inversion formula with respect to  $t_a$  to generate  $j_i$  point.
- (e) Estimate the centre of the sphere by taking the average of points  $j_i$  and  $t_a$  points.
- (f) The radius of the first best-fit sphere is calculated by taking half of the distance between the  $j_i$  and  $t_a$
- (g) The algorithm runs a loop over all  $j_i$  points of matrix  $M$  to find the best centre and mean radius of curvature of the fitted sphere through minimizing the mean square error.

## 2.5 The transition state theory

The TST model is applied to investigate the kinetics of conformational equilibrium in a crowded medium. TST computes the rate constant of a given biochemical reaction by in-

corporating the activity coefficients of the transition state and reacting species as explained in section 1.4.3. Under dilute conditions, the activity coefficients of reacting and transition state species are unity and do not contribute to the rate constant. However, in crowded solution, the activity coefficients are no longer unity and cannot be ignored. Theoretically, the scaled particle theory computes the activity coefficients of a given molecule in the solution as a function of fractional occupied volume.

TST computes the rate constant for a conformational equilibrium reaction in a crowded medium using equation 1.1. According to the TST rate constant expression, the rate constant depends on the activity coefficients of the reactant and the transition state. The activity coefficient of existing structures is estimated with the SPT model. However, the structure of the transition state is generally unknown and the SPT model cannot estimate the activity coefficient of the transition state without knowing the corresponding geometrical parameters.

The exact structure of the transition state is hard to determine experimentally due to the transient lifetimes of the transition state. There have been developed transition state spectroscopic methods to characterize the transition state [241–244]. However these are non-routine experiments that cannot be carried out in most laboratories. A variety of theoretical and computation models have been developed to tackle this problem but each method comes with its own shortcomings [245]. For example, molecular mechanics predicts the transition state by locating the crossing point between two energy plots of breaking and new forming bonds. Moreover, structure optimization methods construct a path linking reactants and products. The reactant and product structures are used as endpoints in this process to predict the transition state of reactions [246].

To overcome the difficulty of the transition state structure, an average structure between two conformations is used as an initial guess transition state structure. Amber is used to generate the average structure by feeding the initial and final structures as inputs. Another quick way to construct intermediate structures between two conformations is through using

a morph server (<http://molmovdb.mbb.yale.edu/molmovdb/morph/>) [247]. This server constructs all intermediate structures between the given two conformations. The server moves the coordinates in a rigid body rotation fashion. The number of intermediate structures can vary from 8 to 32 frames. It is better to construct a larger number of intermediate frames because it involves smaller structural changes and minimizes the probability of any large chemical distortions. However, the morph server does not necessarily give structures according to the lowest energy path. The structure with the highest energy is selected as an approximated transition state structure by determining the single point energies for all the intermediate structures including initial and final structures. The program file implementing the transition state theory is available in Appendix A.8.

## 2.6 Summary

We presented two conformational sampling models based on MC and MD frameworks to achieve our first goal regarding the preparation of diverse PEG conformations and finally the preparation and equilibration of crowded media. Afterwards, an extended model of the scaled particle theory with a convexification algorithm and a Monte Carlo method was developed to compute the fractional available volumes and subsequently thermodynamic activities to incorporate the crowding effects on the conformational equilibrium.

# **Chapter 3**

## **Construction of crowded medium**

The aim of the current study was to investigate the macromolecular crowding effects on the conformational equilibrium in a more realistic crowded medium instead of a medium filled with hard spheres. Generally, a statistical model of scaled particle theory (SPT) [83, 158] is used to compute the crowding effects by incorporating the excluded volume effect. However, the SPT model is based on geometric approximations to the molecular shapes. For example, the SPT model treats the macromolecules as hard convex shapes which might either underestimate or overestimate the excluded volumes depending on the size and shape of the molecules, and subsequently predicts the inaccurate thermodynamic activities of molecules of interest [24, 25]. To overcome this difficulty, we introduced a new modified SPT model using a minimum of geometrical approximations. However this new model needs to be tested to see how accurately this extended model could capture the shapes and sizes of macromolecules to compute the crowding effects. To answer these questions, we developed a computer simulation model based on a Monte Carlo approach to test the outcomes of a new SPT model. The combination of these two models i.e. the SPT and computer simulations, offered a general systematic approach which could be used to model and investigate the crowding effects in any other biochemical reaction of interest.

The developed approach is grouped into two sections based on two key objectives. The first objective is to prepare crowded media, in which 5-40% of the total volume is occupied by crowder molecules and the second objective is to apply the SPT and computer simulations models to compute the crowding effects on a biochemical system of interest. We



Figure 3.1: Classification of our approach used to determine the macromolecular crowding effects on the conformational equilibrium. This approach is classified into two sections, namely preparation of crowded media and the study of biochemical systems in crowded media via MC or SPT methods. The highlighted sections belong to the current chapter and the numbers indicate the corresponding results and discussion sections.

split our program recipe into two major sections for simplicity and convenience (Figure 3.1). The first section covers the preparation of crowded systems as highlighted with a blue colour in the figure, while the results that come after the execution of both computer simulation and SPT models will be discussed in the second section.

The first section is comprised of three key steps namely the development of forcefield parameters, construction of libraries of PEG structures, and finally packing and equilibration of crowded systems packed with dynamic solution structures of polyethylene glycol (PEG) of molecular weight 8 kDa (Figure 3.1). We selected PEG as a crowding agent in our model due to its extensive use in crowding studies. Due to the existence of diverse and dynamic PEG structures in solution, we need to develop diverse PEG confor-

mations to populate our crowded systems. PEG conformations of 15 to 40 Å of radius of gyration have been constructed in accordance with experimental studies conducted elsewhere [55, 63, 90, 91, 107–112]. To do so, we performed Molecular Dynamics (MD) or Monte Carlo (MC) conformational sampling simulations. To do that, we need a forcefield which is unavailable sometimes. In a nutshell, three key points of the present part are summarised here:

1. Developing the forcefield parameters for crowders if they are not provided by default with simulation package (section 3.1).
2. Constructing the libraries of crowder conformations by performing conformational sampling simulations (section 3.2).
3. Preparing the crowded systems by packing and equilibrating the crowded systems (section 3.3).

### 3.1 Forcefield parameters for PEG

Forcefield parameters play a critical role in the conformational sampling simulations by providing a means to calculate the total energy of the structure and thus to evaluate the acceptance/rejection of MC moves. The development of forcefield parameters for crowders may not be required if the forcefield parameters come with a simulation program. We used PEG of 8 kDa molecular weight as a crowding agent in the simulations because PEG of this molecular weight was commonly used in experiments and in computer simulations in crowding studies [15, 80, 82]. We used MCCCS Towhee, Sire and Amber to conduct conformational sampling of PEG. MCCCS Towhee provides forcefield parameters for all the atoms needed for PEG, whereas Sire and Amber do not. Amber provides the forcefield parameters for the majority of organic molecules [226] but not for PEG. Similarly, Sire does not come with PEG forcefield parameters and accepts forcefield parameters provided

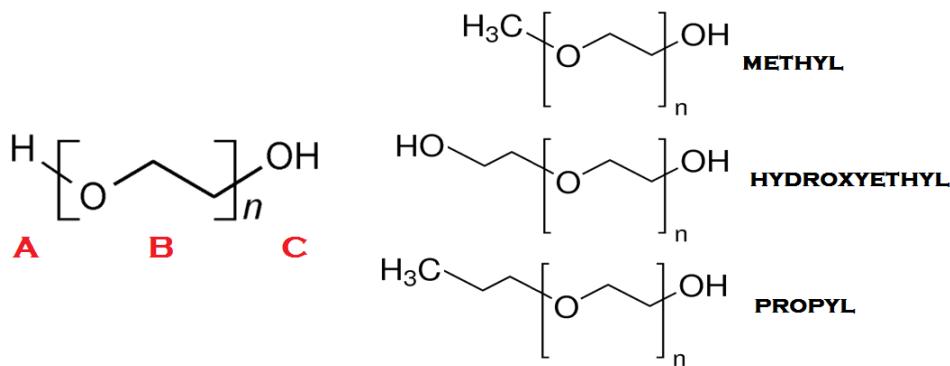


Figure 3.2: Three PEG structures used to develop the forcefield parameters for PEG in the PyRed program. The classification of structures is based on terminal ‘A’. Three PEG analogs were prepared by replacing hydrogen with methyl, hydroxyethyl and propyl groups respectively. The hydroxyethyl variant is an ordinary PEG, in which the last monomer moved into the group ‘A’.

by Amber. The availability of PEG forcefield parameters is critical to run simulations in both programs.

To accomplish the goal of constructing PEG forcefield parameters, the PyRED server was used. PyRED offers two methods for developing forcefield parameters, namely the whole molecule and fragment approaches. The whole molecule approach is only applicable to molecules made of less than 120 atoms, so it cannot be applied to 8 kDa PEG which has 1280 atoms, while the fragment approach is applicable to a polymer. Further, PyRED also provides the capability of generating average forcefield parameters by integrating multiple conformations of the given molecule.

The forcefield parameters of three types of PEGs were constructed as shown in Figure 3.2 through the fragment approach. The initial PEG structures were divided into three fragments namely, ‘A’, ‘B’ and ‘C’. All three structures differ in terminal ‘A’. The aim of choosing three types of PEG was to evaluate the effect of terminal groups on the resultant forcefield parameters and subsequently on the folding behaviour of PEG in the simulations. Previous studies demonstrated that the terminal groups have significant effect in PEG folding in simulation [98]. Moreover, the effects on the forcefield parameters of length of the central fragment by increasing the value of  $n$ , and the type of QM geometry optimization

methods are evaluated.

The results on the development of forcefield parameters are summarized into three major sections:

1. The development of forcefield parameters for PEG with a methyl using a single conformation of PEG that is optimized by different QM methods (subsection 3.1.1).
2. The comparison of forcefield parameters for PEGs with methyl, hydroxyethyl and propyl terminals groups, using a single conformation of PEG optimized by a single QM method (subsection 3.1.2).
3. The effect of increasing number of monomers in the central fragment with  $n = 2, 3$ , and 4 on the forcefield parameters (subsection 3.1.3).
4. The effect of multiple conformations of PEG with hydroxyethyl terminal on the averaged PEG forcefield parameters. All these conformations were optimized by a single QM method (subsection 3.1.4).

### **3.1.1 PEG with methyl terminal group**

The PyRED server constructs the forcefield parameters of a polymer in a two-step procedure. In the first step, PyRED optimized the structure and formed the Molecular Electrostatic Potential (MEP) surface using Quantum Mechanics (QM) calculations. Three QM methods, namely the *ab initio* method with the Hartree Fock (HF) approximation [216], Density Functional Theory (DFT) with the B3LYP approximation [218], and a post-Hartree Fock (PHF) Møller-Plesset perturbation theory [220] of second order (MP2) were selected to optimize the PEG structure. These three methods were selected to find out an appropriate method that could be used to construct the forcefield parameters accurately. In the second step, PyRED determined the atomic charges by reproducing the MEP surface through a charge fitting procedure.

The QM methods determine the wavefunction and energy of the system. HF determines the wavefunction of the system using a single Slater determinant approximation. A Slater determinant satisfies the anti-symmetry requirements of the wavefunction and thus the Pauli exclusion principle. It also represents the many electron wavefunction in terms of single electron wavefunctions and does not incorporate the electron correlation at all. As a result, HF overestimates the system energies. However, PHF methods improve on the HF results by incorporating electron correlation. On the other hand, DFT computes the system energy as a function of electron density without using the HF mean field approximation and considers the contribution of electron correlation. We used the polarized basis set 6-31G(d) with the HF and DFT methods to compute the wavefunction. The basis set choice involves a balance between wavefunction accuracy and computational cost. The small polarized basis set is a good choice for the small PEG molecule that is composed of simple elements such as hydrogen, carbon and oxygen and involves covalent bonding through hybridization. The polarized basis set would be a good choice as it considers atomic orbitals that become distorted in shape by other nuclei. The larger correlation-consistent polarized basis set ‘cc-pVTZ’ including Valence Triple Zeta and polarization on all atoms was also used with the MP2 method for comparison purposes.

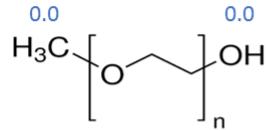
The comparison of all three QM methods was shown in terms of average Electrostatic Surface Potential (ESP) charges obtained by each method (Table 3.1). The HF sometimes gave a charge of opposite sign on a few atoms to the other two methods which turn out larger charge values on the PEG molecule. For example, DFT and MP2/cc-pVTZ calculated the sum of atomic charges of 0.0776 and 0.0974 respectively on the central fragment of PEG and these charges are smaller than 0.6139 estimated by the HF/6-31G(d) method. Consequently, the lower charge values result in lower total energies of 16.24 and 18.08 kcal/mol respectively as compared to 21.81 kcal/mol due to electron correlation treatment.

The fragment approach required two intra-molecular charge constraints in order to define three PEG fragments and to construct forcefield parameters of the PEG polymer prop-

Table 3.1: Predicted atomic charges of PEG with methyl by QM calculations

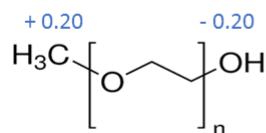
Atoms	HF/6-31G(d)	B3LYP/6-31G(d)	MP2/cc-pVTZ
1C	0.0203	-0.0134	-0.0586
2H	0.0896	0.0924	0.1066
3H	0.0399	0.0397	0.0476
4H	0.0399	0.0397	0.0476
5O	0.0399	-0.4047	-0.388
6C	0.284	0.2645	0.2467
7H	0.0179	0.0135	0.0268
8H	0.0179	0.0135	0.0268
9C	0.273	0.1888	0.1539
10H	-0.0094	0.001	0.0141
11H	-0.0094	0.001	0.0141
12O	-0.748	-0.6543	-0.6454
13H	0.455	0.4182	0.4077

erly (Figure 3.3). The first intra-molecular charge constraint is applied with a total zero charge on PEG. This charge constraint helped to define and determine the atomic charges on methyl ‘A’ and hydroxyl ‘C’ terminal fragments. On completion of the PyRED job with the first charge constraint, the methyl and hydroxyl terminals had total charges of  $\pm 0.47$ ,  $\pm 0.20$  and  $\pm 0.19$  with HF, DFT and MP levels of theory respectively. Both terminals should carry the same amount of charge but of opposite sign due to different electronegativities. These known charge values on both terminal fragments were used in the second intra-molecular charge constraint to construct the forcefield parameters of all three fragments. For example, in the second charge constraint, we used charge values of +0.20 and -0.20 on methyl and hydroxyl respectively obtained from DFT QM method calculations. The charge measurement on the central fragment is necessary step in dividing the PEG into three fragments and constructing the forcefield parameters. The charge measurements do not affect the overall charge on the PEG molecule and formed neutral PEG polymers after joining the three fragments. In a nutshell, the first charge constraint considers zero charges on the whole molecule and provide assistance in finding the charges on the defined terminal groups and the formation of fragments. The second charge constraint treats the ter-

**Step 1. QM optimization**

ATOM	CHARGE
1C	-0.0134
2H	0.0924
3H	0.0397
4H	0.0397
12O	-0.6453
13H	0.4182

Charge constraint-I      0.0 | 1 2 3 4 | 12 13 | Remove  
 Total Terminal Charges = (|CH<sub>3</sub>| + |OH|)/2 = ± 0.20

**Step 2. Fragmentation**

Charge constraint-II      + 0.20 | 1 2 3 4 | Remove  
 - 0.20 | 12 13 | Remove

Figure 3.3: Process of forcefield parameters development by using a fragment approach in the PyRED server. The fragment approach comprises two steps. The PEG structure is optimized with a QM method of choice and atomic charges are determined. In the second step, the three fragments are formed. This process requires two intra-molecular charge constraints to facilitate the fragmentation. A total charge of zero is assigned to terminal groups in the first charge constraint in step 1, whereas the average of the sum of atomic charges on the terminal groups of ±0.20 is used in the second charge constraint. The first charge constraint facilitates the formation of fragments and is used to calculate the charges on the terminal groups. Therefore, an equal amount of the total terminal charge value of 0.20 is assigned to both terminal groups, but opposite in sign depending on the electronegativity of each terminal group. Completion of the PyRED job with a second set of charge constraints results in fragments and forcefield parameters of a PEG polymer.

terminal groups as chemically equivalent groups with respective charges of +0.20 and -0.20 on methyl and hydroxyl terminals and built forcefield parameters and three fragments of the PEG by reproducing the molecular electrostatic surfaces.

The accuracy of the developed forcefield parameters is presented in terms of a relative root mean square (RRMS), a measure of the difference between two data sets, and Pearson correlation coefficient  $r^2$  values. A small value of RRMS approaching zero and a value of  $r^2$  close to one indicates the accuracy of the fitting step and the resultant developed forcefield parameters. In the fragment approach, the accuracy of the fitting procedure is

Table 3.2: RRMS and Pearson correlation coefficient  $r^2$  for three QM methods

Charge Constraints	HF/6-31G(d)		B3LYP/6-31G(d)		MP2/cc-pVTZ	
	RRMS	$r^2$	RRMS	$r^2$	RRMS	$r^2$
No constraints	0.1549	0.9761	0.1595	0.9748	0.1984	0.9613
2 constraints	0.1688	0.9717	0.1692	0.9715	0.2079	0.9574
Difference	0.0139	0.0044	0.0097	0.0033	0.0095	0.0039

the comparison between charge fitting on the single molecule and charge fitting on the fragments of the molecule with charge constraints. A small difference of 0.0097 in the RRMS values between no and two charge constraints using the DFT method indicates the very weak effect of the charge constraints used. The same trend can also be seen in the  $r^2$  values which are 0.97 and 0.97 without and with two charge constraints and indicates a good fit of the MEP. The biggest values of RRMS in Table 3.2 indicated that the electrostatic potential is least well fit to the MP2/cc-pVTZ results. We are not sure about the reason why the MP2/cc-pVTZ method is not producing a good fit. The MP2/cc-pVTZ method with a large basis set and including a correlation correction was used to get reasonably accurate results. It provided us a base to compare the results with what we have obtained from the cheaper DFT and HF methods. The DFT method is found to be more efficient in speed and to produce structures with minimum energies competitive with the MP2/cc-pVTZ outcomes. Based on this analysis, we choose to use DFT in all calculations regarding the development of the forcefield parameters of PEG.

PyRED offers 21 charge fitting models to reproduce the MEP surfaces. The charge models use atomic charges present in different forcefield parameters and computed using different QM levels of theory. For example, the RESP-O1 model uses the atomic charges from the OPLS forcefield parameters while ESP-A1 uses the type of charges from CHARMM FF parameterization. The RESP-A1 is the default charge fitting model and uses the atomic charges from HF/6-31G(d) model. Two charge fitting models, namely RESP-A1 and RESP-Y22 were tested using the B3LYP/6-31G(d) method to regenerate the MEP surface. The RESP-A1 model produced better results by smaller RRMS and larger  $r^2$  values than RESP-

Y22.

### 3.1.2 Comparison of FF parameters with methyl, hydroxyethyl and propyl terminals

The forcefield parameters of PEG with hydroxyethyl (HFF) and propyl (PFF) terminals were also developed to incorporate the effect of the ‘A’ terminal group as compared to PEG with methyl terminal (MFF) (Figure 3.2) and subsequently the effect of resultant forcefield parameters on the folding behaviour of 8 kDa PEG in MD simulations. The B3LYP/6-31G(d) method was used to optimize these initial PEG structures in PyRED during the forcefield parameter development. The resultant forcefield parameters with each terminal were evaluated by performing MD simulations using an 8 kDa PEG.

The total energies of both PEGs with methyl and propyl terminals were converged after completing 5 ns of simulation time (Figure 3.4), while the energy of PEG with hydroxyethyl terminal started from an energy much closer to its final energy. The radius of gyration obtained using these three sets of forcefield parameters showed similar folding behaviour in which all the PEG structures collapsed to very compact conformations and the radius of gyration fluctuated over a very limited range of 1 to 3 Å in the stationary state around a mean value of 12 Å (Figure 3.5). However, PEG with MFF parameters took a long time to collapse to the same structure eventually as the other two. These results suggested the terminal groups did not have a significant effect on the folding behaviour of PEG in MD simulations that were performed in Amber.

It is noted that the starting structures of 8 kDa PEGs with methyl, hydroxyethyl, and propyl terminals differ in radius of gyration from the starting point of simulations. There are two ways to build an 8 kDa PEG structure after developing forcefield parameters. Firstly, we can construct a linear PEG structure by joining monomers in the central fragment and then use any set of forcefield parameters, i.e. MFF, HFF, or PFF to conduct the MD or MC simulations. The advantage of using this method would be the same PEG conformation used in the simulations. However, this method requires to change the terminal groups and

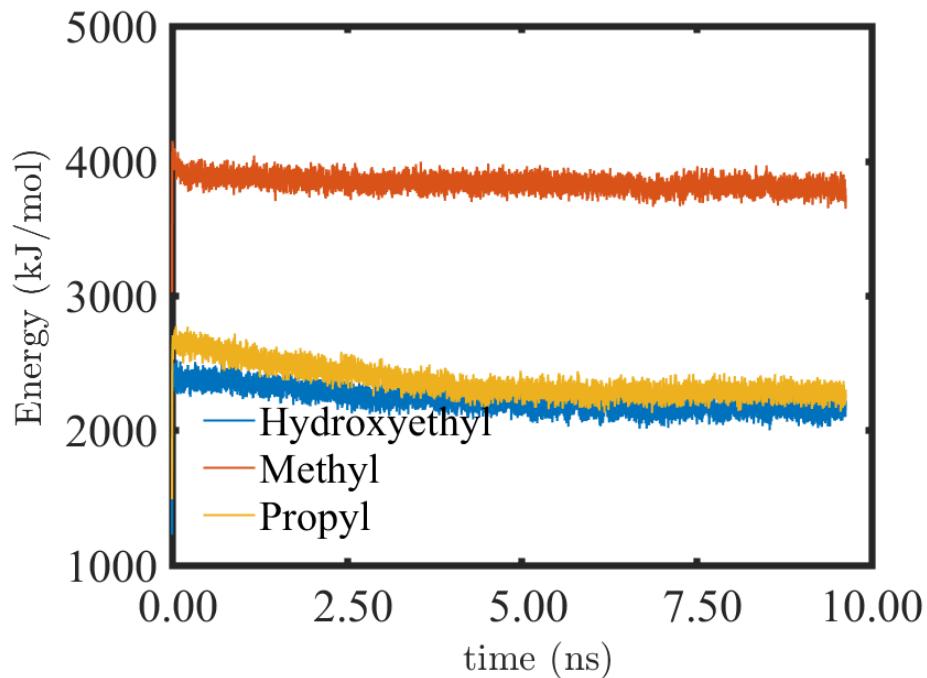


Figure 3.4: Total energy change for three PEG structures in 10 ns long MD simulations performed in Amber using an NPT ensemble at 300 K. All three simulations started using a linear 8 kDa PEG that differ in terminal groups. PEGs with methyl, hydroxyethyl and propyl terminals used the MFF, HFF and PFF forcefield parameters respectively.

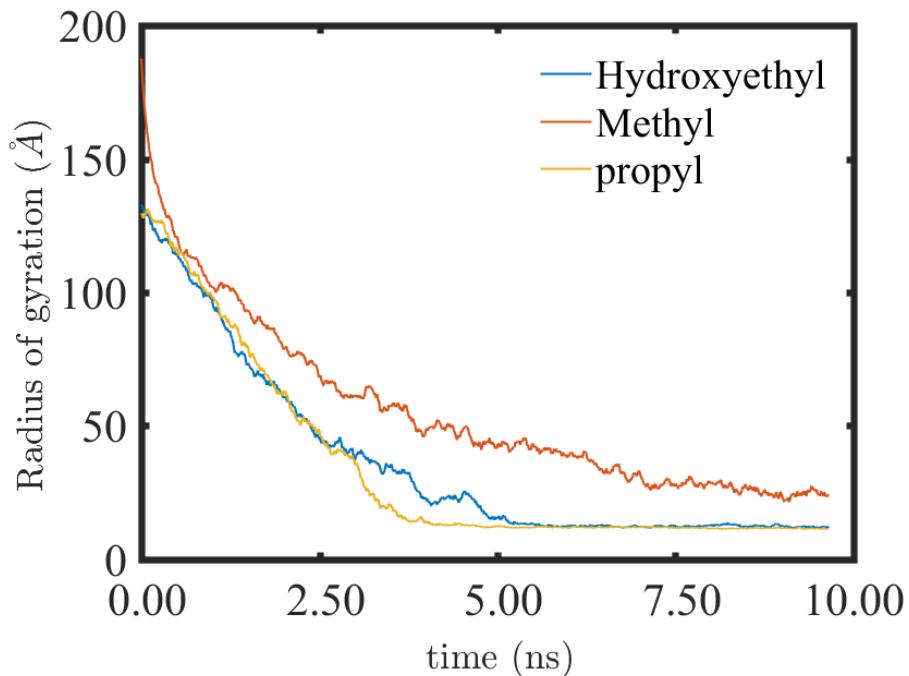


Figure 3.5: Radius of gyration change from start to stationary stage in the MD conformational sampling of three PEG structures with hydroxyethyl, methyl and propyl terminal groups. These 10 ns long simulations were performed at 300 K using an NPT ensemble and implicit solvation model.

rename the atomic names in the PDB file according to the forcefield parameters. The second method would be using the PEG fragments from each forcefield parameter set to construct the 8 kDa PEG structure. This method results in 8 kDa PEGs with different initial conformation depending on the shapes of the fragments. This does not require any additional editing in the PDB file. We used PEGs obtained from the second method and presented corresponding results. We also tested the first method and conducted the simulation with a constant PEG conformation by varying the FF parameters. It is worth noting here that since they all collapse to similar final structures if the simulations are performed for a long enough time, this shows that the results are insensitive to the starting structure. The results demonstrated that the initial conformation of PEG has no effect on the folding behaviour and total energies in MD and MC simulations. To illustrate how different types of conformations are formed in the second method, we construct four types of PEGs by joining ten monomers of different shapes and sizes (Figure 3.6). It showed that the shape of resultant PEG depends on the shapes of monomers of the central fragment.

### 3.1.3 Effect of length of the central fragment

The effect of the length of the central fragment on the resultant forcefield parameters was investigated by using PEGs with methyl and hydroxyethyl terminals. It was achieved by increasing the number of repeating monomer units ( $n = 2, 3, 4$ ) within the central fragment. The B3LYP/6-31G(d) method was used to optimize all eight mini-PEG structures. The increase in length of the central fragment results in smaller RRMS values for PEGs with methyl terminals, whereas larger RRMS values are observed for PEGs with hydroxyethyl terminals (Table 3.3). These results showed that RRMS values are sensitive to the composition and structural orientation of the initial PEG structure.

Afterward, we applied each of the forcefield parameter sets to the same 8 kDa PEG in the MD conformational sampling and found that forcefield parameter sets obtained with an even number of monomers showed approximately a 500-1000 kJ/mol lower energy than

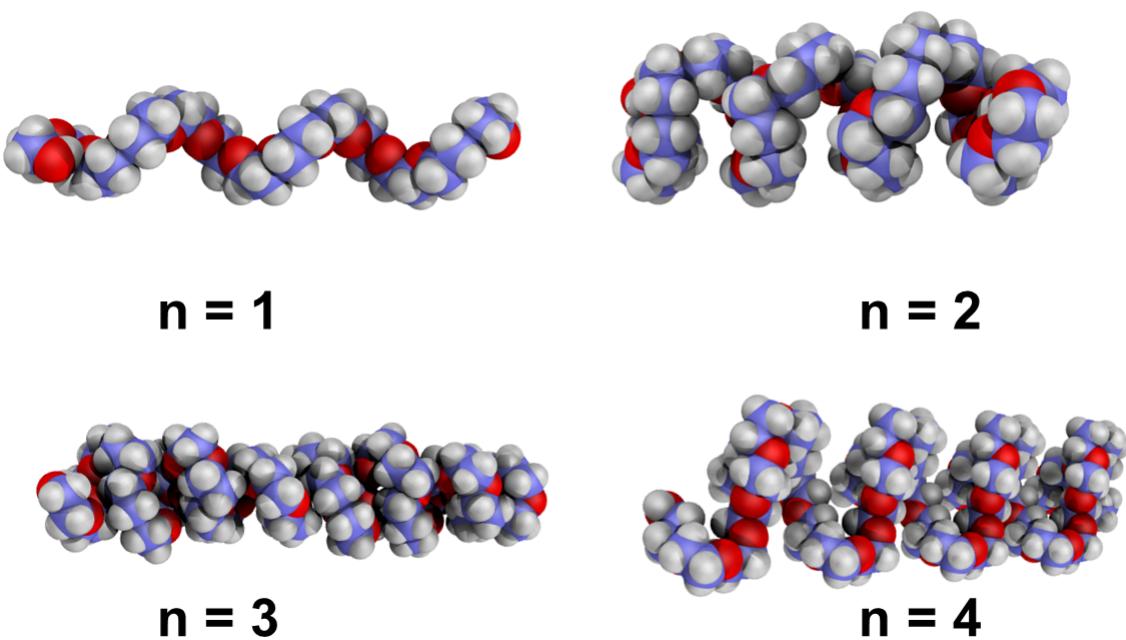


Figure 3.6: Formation of folded and coiled structures of PEG on polymerization of 10 monomers of the central fragment, where the  $n = 1, 2, 3$  or 4 represents the number of monomers in the parental central fragment used in the force field parameter development. The folding trend increased as the length of the central fragment increased.

PEG with a methyl terminal using forcefield parameter sets obtained with an odd number of monomers (Figure 3.7). However, the energies of PEGs using sets of forcefield parameters of PEGs with hydroxyethyl terminals were comparable (Figure 3.8). The lower energies of PEGs utilizing forcefield parameters from a structure with an even number of monomers in the central fragment can be explained in terms of hydrogen bonding. PEG oligomers with an even number of monomers in the central fragment showed a larger number of hydrogen bonds. We assumed maximum of 2.5 to 2.6 Å [248] as a standard length of a hydrogen bond and under this criterion PEG with  $n = 4$  showed four hydrogen bonds with approximate bond lengths of 2.2, 2.4, 2.5 and 2.6 Å (Figure 3.9 (right)) while PEG with  $n = 3$  showed two hydrogen bonds of 2.0, and 1.8 Å (Figure 3.9 (left)). The results showed that the obvious structural differences seen in Figure 3.6 are indicative of the effects of the number of monomers in the central fragment on the types of structures that are favoured by the different forcefields.

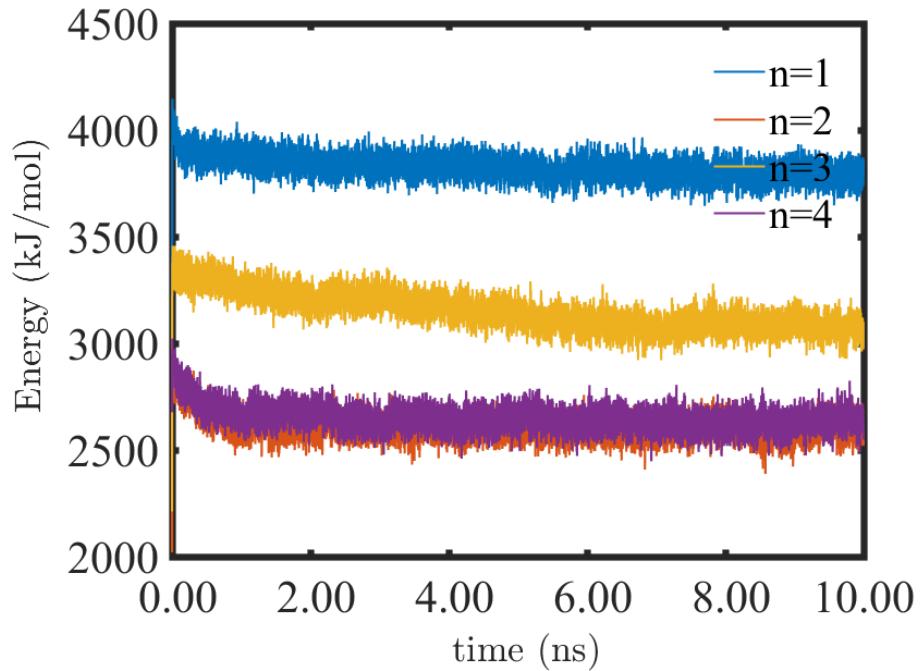


Figure 3.7: Effect of length of central fragments on the total energy of the 8 kDa PEG structure of methyl terminal in the 10 ns long MD conformational sampling simulations performed with an NPT ensemble in an implicit solvent at 300 K using MFF parameters.

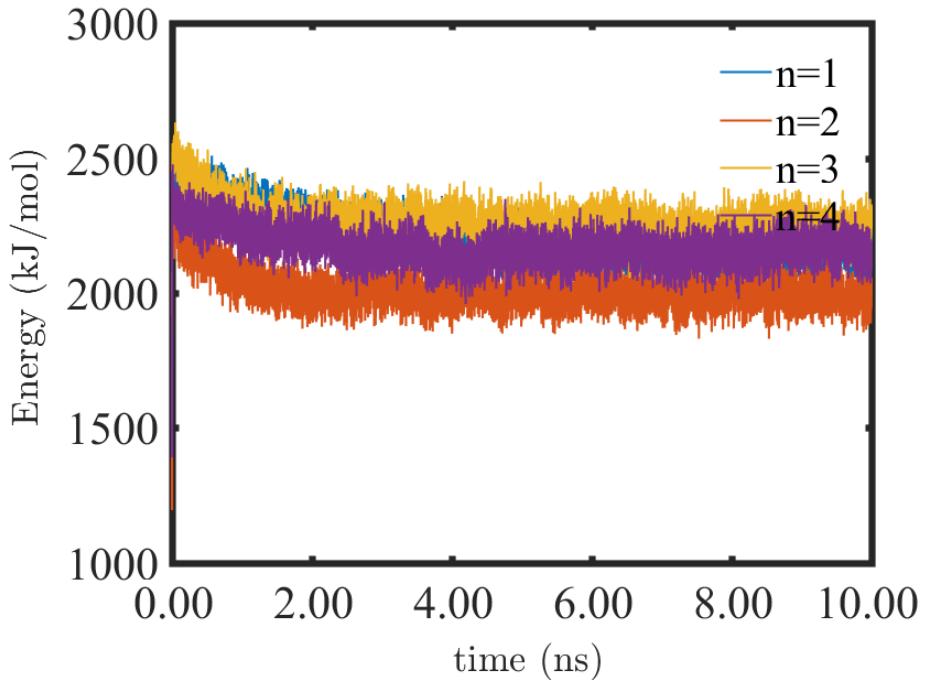


Figure 3.8: Effect of length of central fragments on the total energy of the 8 kDa PEG structure of hydroxyethyl terminal in the 10 ns long MD conformational sampling simulations performed with an NPT ensemble in an implicit solvent at 300 K using HFF parameters.

Table 3.3: Effect of length of the central fragment on the RRMS values for PEGs with methyl and hydroxyethyl terminals

<b>PEG with methyl terminal</b>				
<b>Charge constraints</b>	<b>n1</b>	<b>n2</b>	<b>n3</b>	<b>n4</b>
<b>No constraints</b>	0.15951	0.11475	0.11368	0.098698
<b>Two constraints</b>	0.28579	0.16252	0.14205	0.12250
<b>PEG with hydroxyethyl terminal</b>				
<b>No constraints</b>	0.16318	0.16784	0.19049	0.18355
<b>Two constraints</b>	0.16909	0.19313	0.18912	0.22304

These results demonstrated that the sets of forcefield parameters obtained with hydroxyethyl terminals are better than of PEGs with methyl terminals because it produced more stable structures of PEG with a small difference of energies for different length of central fragments (Figure 3.8). The calculations suggested that the initial conformation of PEG used in the step of developing forcefield parameters played a crucial role. Central fragments with n=2 and 4 monomers tend to form coiled structures through intra-molecular hydrogen bonding and subsequently result in lower total energies of 8 kDa PEGs in MD simulations. However, a central fragment composed of an odd number of monomers formed less coiled structures as compared to fragments with an even number of monomers. These results regarding total energy behaviour in MD simulations (Figures 3.7 & 3.8) suggested that the forcefield parameters that were developed with hydroxyethyl terminals are a good choice to conduct simulations to construct an ensemble of PEG conformations. The folding behaviour of 8 kDa PEG utilizing the MFF and HFF forcefield parameters varying in the length of the central fragment is provided in the Appendix B.3.9.

### 3.1.4 Effect of multiple PEG conformations

In the last step, average forcefield parameters of twenty three PEGs with hydroxyethyl terminals were developed. PyRED generates an average forcefield that minimizes the error in the PES over all the structures with RRMS value of 0.17307. These multiple conformations were collected in MC sampling conducted at 300 K using the initial structure of

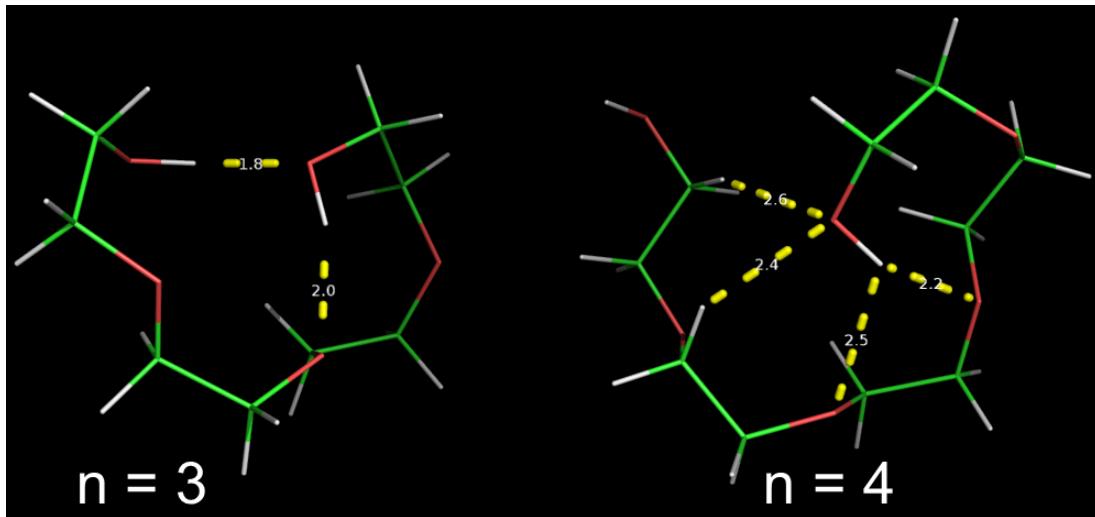


Figure 3.9: Number of hydrogen bonds in initial structures of PEGs with  $n = 3$  and  $4$  used in the forcefield parameter development procedure. These structures were optimized using the B3LYP/6-31G(d) method in the GAUSSIAN program.

PEG with  $n = 1$  and the corresponding set of forcefield parameters. These simulations were performed in Sire and a total of 5000 conformations were saved after every 250 internal moves (program code is available in the Appendix B.3.8). Twenty three conformations were picked with very different radii of gyration. All these conformations were optimized using the B3LYP/6-31G(d) method in the GAUSSIAN QM program. The resultant forcefield parameters such as bond lengths, angles, and dihedrals were found to be similar with the single PEG conformation except for a small variation in the atomic charges on each fragment. QM geometry optimization produced atomic charges on the terminal groups of  $\pm 0.20$ , which is equal to the atomic charges of  $\pm 0.20$  on fragments of PEG with a single conformation.

Sire required forcefield parameters to run MC conformational sampling simulations. These forcefield parameters were constructed at first using a single PEG with hydroxethyl terminal, and then the simulations were performed by incorporating these parameters. These forcefield parameters were further evaluated by running the conformational sampling simulations of 8 kDa PEG and analysing the resultant total energies. The energy plots (Figure 3.10) showed that the total energy of the PEG decreased by 200 kJ/mol while

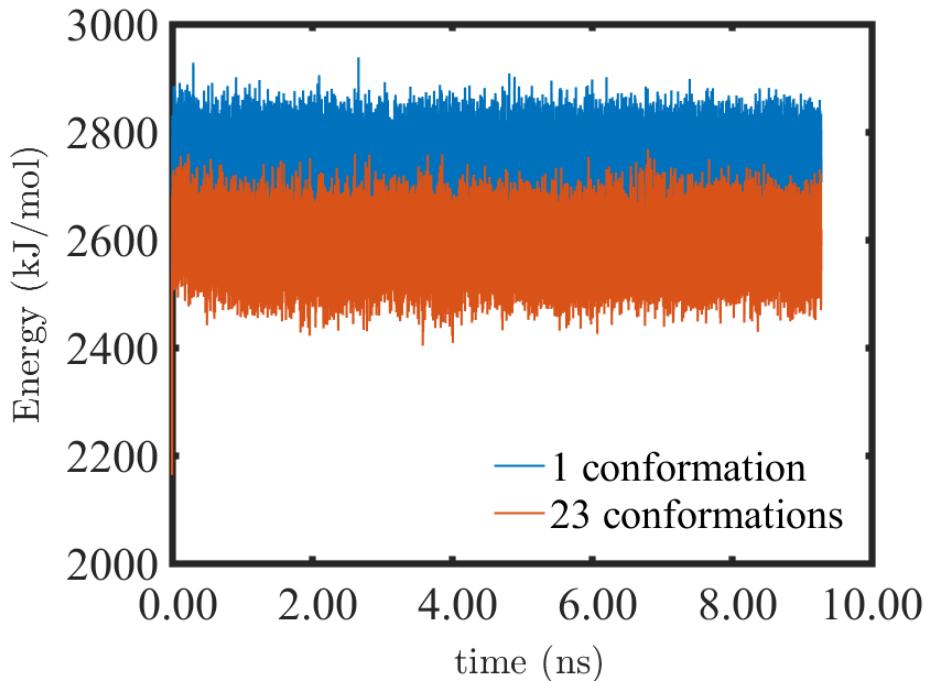


Figure 3.10: Effect of forcefield parameters using a single and multiple PEG conformations on the total energies of MD simulations conducted in Amber. These conformations were optimized with B3LYP/6-31G(d) QM method in the GAUSSIAN programs. 300 K MD simulations were performed using an NPT ensemble in an implicit solvation model with HFF parameters.

using the forcefield generated from multiple conformations. In conclusion, the forcefield parameters developed using multiple conformations and the forcefield parameters of mini-PEG with hydroxyethyl terminal and an even number of monomers in the central fragment result in the most suitable parameters based on the total energies analysis in MD and MC simulation. The complete sets of output forcefield parameter files for a PEG of  $n = 1$  with a methyl terminal, PEG of  $n = 4$  with a hydroxyethyl terminal and PEG of  $n = 1$  with hydroxyethyl terminal with multiple conformations is provided in Appendices B.1, B.2, and B.3 respectively.

Table 3.4: Comparison of the partial atomic charges on the ether oxygen (-OE-) and carbon (-CH<sub>2</sub>-) atoms between PyRED and three different GROMOS forcefield parameter sets, namely 53A6, 53A6v, 53A6o

<b>Atom</b>	<b>methyl-PEG</b>			<b>PEG</b>	<b>GROMOS</b>		
	<b>HF/ 6-31G(d)</b>	<b>B3LYP/6-31G(d)</b>	<b>MP2/ cc-pVTZ</b>		<b>53A6</b>	<b>53A6v</b>	<b>53A6o</b>
-OE-	-0.48	-0.41	-0.40	-0.47	-0.32	-0.42	-0.57
-CH <sub>2</sub> -	0.23	0.23	0.18	0.21	0.16	0.21	0.29

### Concluding remarks - PEG forcefield parameters

The aim of the present section was to develop the appropriate forcefield parameters of PEG to employ in conformational sampling simulations. To achieve this goal, three sets of forcefield parameters of PEG were developed using three different terminal groups for a single and multiple PEG conformations by implementing the fragment approach. In particular, four different cases, namely PEG with methyl, hydroxyethyl, propyl terminals, and PEG with different lengths of central fragment, were studied. The results showed that the terminal groups did not affect the folding behaviour of 8 kDa PEG significantly as shown in the MD conformational sampling in Amber (Figure 3.5). In this regard, other studies showed the terminal methyl groups could be responsible for folding and formation of compact structures of PEG [98]. On the other hand, the length of the central fragment with an even number of monomers played a crucial role in lowering the total energy by 1500 kJ/mol as compared to the central fragment containing an odd number of monomers by incorporating a higher number of hydrogen bonds (Figures 3.7 & 3.9).

In the end, the accuracy of forcefield parameters was tested against the ether parameters present in the GROMOS forcefield. The atomic charges on the oxygen -OE- and methylene -CH<sub>2</sub>-, generated by PyRED are in good agreement with the ether charges from the GROMOS forcefield parameters as shown in Table 3.4. The partial charges of -OE- and -CH<sub>2</sub>- in methyl-PEG and hydrogen-PEG derived by three QM methods (i.e. HF, DFT, MP2) are in good agreement with the GROMOS parameters [197, 249–252]. However, the partial charges of carbon in the original hydrogen terminal PEG showed greater than 50 percent difference with GROMOS parameters. Based on this analysis, all the MC and

MD simulations were carried out using MFF and HFF sets of parameters. The rest of the parameters such as bond lengths, angles, dihedral angles, improper torsional potentials and van der Waals radii are in agreement with the Amber forcefield parameters [227].

In a nutshell, the development of forcefield parameters is the first step towards running the conformational sampling. An attempt was made to construct the missing forcefield parameters of PEG and the effect of different terminal groups, length of central fragments and multiple conformations of mini PEGs on the forcefield parameters was explored to determine if there is any major effect associated with these structural changes. These parameters were further compared with GROMOS parameters, however further experimental validation may be required. Our work on the forcefield parameter development is of an exploratory nature and the final results of crowding effects in the second step don't seem to depend all that strongly on the details of forcefield construction.

## 3.2 Ensemble of PEG conformations

The construction of the diverse conformational ensembles is the most vital component towards building the prerequisite crowded media. So far, we have discussed the general methodology to develop the unavailable forcefield parameters of any polymer and the effect of chain terminals on the folding properties. The objective of the current chapter is to prepare the crowded media where 0 to 40% of total volume is occupied by the crowder molecules of diverse conformations. We choose PEG as a crowding agent as it was commonly used as a crowding agent in many experimental and computer studies [80, 98, 111, 191]. Ensembles of PEG conformations are required in order to prepare these systems. Both Monte Carlo (MC) and Molecular Dynamics (MD) methods were carried out to achieve this goal. MC simulations were carried out in MCCCS Towhee [104] and Sire [193] whereas MD simulations were performed in Amber [105, 194]. These programs were chosen based on different capabilities they offer. For example, MCCCS Towhee runs the MC simulations through a very simple script file, and eliminates the need to develop

forcefield parameters because it provides a set of general forcefield parameters applicable to all elements in PEG. This forcefield is not tuned to PEG type structures and requires proper atomic charges to consider the Coulombic interactions reasonably. On the other hand, it does not provide much flexibility to design and perform simulations according to one's needs. Alternatively, Sire provides a much more flexible and easy approach to perform MC sampling simulations at the cost of requiring programming skills in Python. It accepts Amber [105, 194], CHARMM [106] and Gromacs [182] input files but requires that missing forcefield parameters for non-standard molecules be provided. Lastly, Amber is used to perform MD conformational sampling in addition to two MC simulations.

The conformational sampling simulations were employed using different temperatures, statistical ensembles, initial PEG conformations such as linear and folded, and implicit and explicit solvent models. These different parameters were used to enhance the diversity of the ensemble of PEG conformations to better sample the energetic and conformational space landscapes. The outcomes of these simulations were analysed and discussed by presenting total energy and radius of gyration changes while the efficiency of all methods were evaluated in terms of the distribution of PEG conformations.

### 3.2.1 MC sampling in MCCCS Towhee

A structure of a single PEG and corresponding conformational ensembles were built by performing MC simulations in MCCCS Towhee. The simulations were performed over two steps to achieve these goals.

1. Building the initial optimized 8 kDa PEG structure and constructing ensembles of PEG conformations at 300, 600 and 1200 K.
2. Investigating the effects of different simulation parameters such as the type of ensemble, temperature, initial PEG configurations, and random number generators to improve the conformational sampling.

First of all, 1.2 million-step-long MC simulations using the NPT (constant number of molecules, pressure and temperature) ensemble [221], at 101 kPa pressure, and 300 K temperature were performed to build and optimize the single PEG structure. The Amber forcefield parameters [225] were used to define the composition of PEG and to calculate the structure energies. Afterwards, three 6-million-step-long MC simulations at 101 kPa pressure and three different temperatures: 300, 600, and 1200 K were performed to build the library of PEG conformations using the optimized PEG structure, taken from the previous step. The higher 600 and 1200 K temperatures were chosen to enhance the diversity of ensembles of PEG conformations. In these simulations, the PEG conformations were saved to the library at every 30,000 MC steps up to 6 million total steps. The step size to save the resultant conformations was chosen arbitrarily.

The simulation results were analysed by interpreting the radius of gyration and energy behaviour of PEG. The radius of gyration is an important property that is used to characterize polymer size in the simulations [93]. The radius of gyration  $R_g$  is defined by Equation 1.4, where  $N$  is total number of atoms,  $\vec{r}_i$  is the position vector to each atom and  $\overrightarrow{r_{CM}}$  is the centre of mass of the molecule.  $R_g$  has been used to track the structural changes in the conformational sampling simulations that are performed in different simulation programs [104–106].

MC sampling simulations were performed at three temperatures in order to construct libraries containing diverse PEG conformations. MCCCS Towhee produced PEG conformations in three different ranges in three simulations performed at 300, 600 and 1200 K temperature (Figure 3.11). At 300 K, only small changes occur in the radius of gyration and energy as well. The 300 K data line showed minimal fluctuations in the radius of gyration due to the low number of accepted MC moves. The number of accepted moves at 300 K is quite insufficient for the structure to make significant jumps in the radius of gyration. The situation is slightly better at 600 K where the structure had a higher probability of accepted moves and therefore it helped the PEG to make a relatively larger jump of 2 Å in the begin-

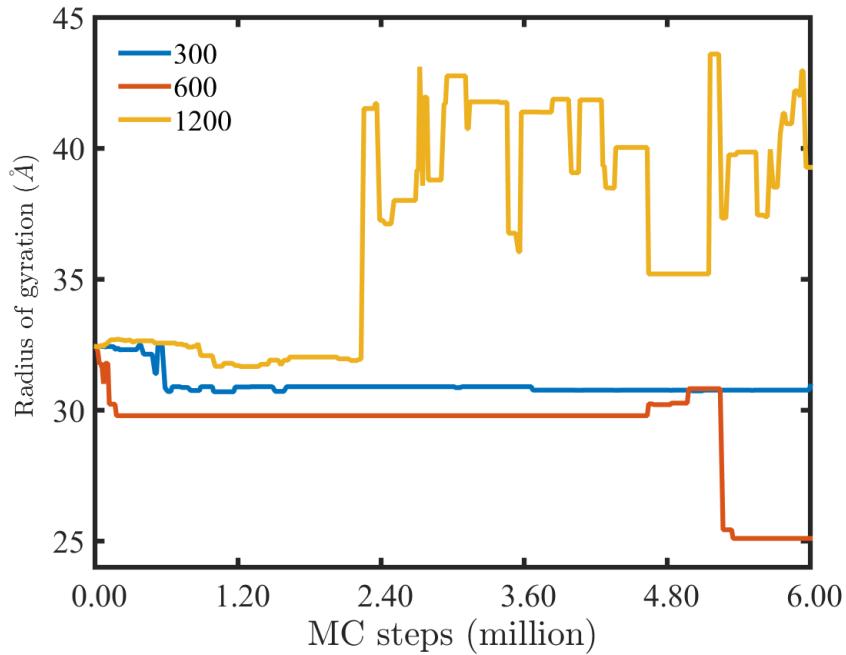


Figure 3.11: Change in gyration radii of the same initial PEG structure in the MC simulations conducted at 300, 600 and 1200 K. The radius of gyration fluctuates at higher amplitude at 1200 K as compared to 300 and 600 K. These simulations were performed using an NPT ensemble up to 6 million MC steps in MCCCS Towhee.

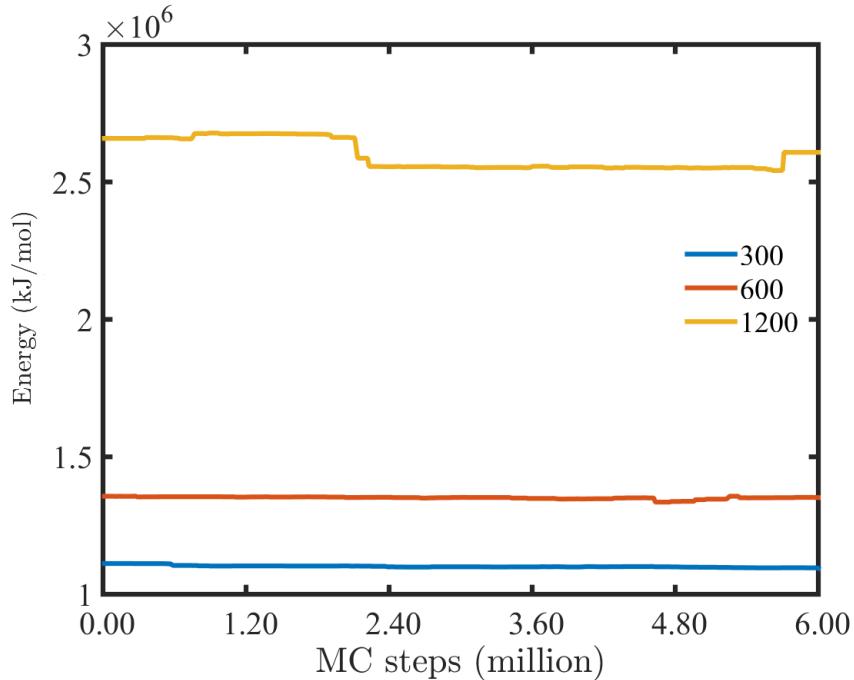


Figure 3.12: Total energy in the conformational sampling simulations conducted at 300, 600 and 1200 K using a same initial PEG structure in MCCCS Towhee. The energy fluctuates at higher magnitude at 1200 K as compared to 300 and 600 K.

ning. Moreover, the interesting behaviour started before 4.80 million steps where the radius of gyration started to edge upward, presumably as the structure explored some kind of low energy pathway out of the local minimum at 30 Å. The small change in the energy at the same point confirmed this behaviour (Figure 3.12) and finally made the structure to jump from 32 Å to 26 Å. In general, these sampling simulations generated conformations in a limited range of radii of gyration at 300 and 600 K. To observe larger structural fluctuations and consequently to build diverse PEG conformations, the simulations were performed at 1200 K. The 1200 K data showed that more diverse conformations were formed at 1200 K and they fell in a wide range from 31 to 44 Å with large fluctuations in energy as expected at higher temperature.

Overall, these results showed that the 300 K simulations did not do much. The 600 K simulations allowed more conformational sampling, eventually finding a very compact conformation and the 1200 K simulations showed large conformational changes. Similarly, the PEG conformation explored a limited range of the energy landscape in all three simulations conducted at 300, 600 and 1200 K (Figure 3.12). The 300 K energy was pretty flat without any significant upward changes. At 600 K, there were some upward changes in the region where the larger changes in the radius of gyration occurred and at 1200 K, we found a greater range of energy fluctuations, including one upward step in energy that was much larger than any seen at the lower temperatures.

Based on these results, it could be concluded that it is hard for MCCCCS Towhee to construct diverse conformations for the large PEG structure at room temperature. Probably, the reason behind the inefficiency is the use of a very general forcefield, and therefore probably that is not very accurate for this kind of work. The other reason could be the limited control over defining the range of magnitude of internal moves, i.e. changes in angles and dihedral angles.

In the second section, different sets of simulation parameters were used to improve the conformational sampling. The simulation parameters includes changing the NPT ensem-

ble to NVT (constant number of molecules, volume and temperature), temperature from 1200 to 12,000K, three initial PEG configuration (Figure 3.13), random number generators ‘DX-1597-2-7’ [253] and ‘RANLUX’ [254] and doubling the length of simulations up to 12 million MC steps. The NPT ensemble was used previously because it is closer to the real experimental conditions and it ran the MC conformational sampling simulations faster than NVT or grand canonical ensembles in MCCCCS Towhee. The NVT ensemble was tested only to see if it can produce diverse conformational ensembles. Three random PEG conformations were picked with very different radii of gyration from the previous MC simulations performed at 1200 K (Figure 3.11) and used to explore the effect of initial configuration on sampling behaviour. The DX-1597-2-7 and RANLUX pseudorandom number generators, with periods of  $10^{14903}$  and  $10^{171}$  respectively, were tested. In total, nine MC simulations were performed using three different initial PEG conformations at three different temperatures (300, 600, and 1200 K) and two different random number generators.

Of the nine simulations described above, only the results regarding total energy and radius of gyration change in a single simulation conducted at 300 K using the NPT ensemble and the ‘RANLUX’ random number generator are presented here. The results showed that the conformational sampling was improved while using the three initial conformations (Figure 3.14). The PEG explored more conformational space with relatively larger jumps from 41 to 29 Å (yellow line with ‘C’ PEG conformation) and 34 to 29 Å (blue line with ‘A’ PEG conformation) as compared to previous MC run performed at 300 K where the radius of gyration changed by 1 Å only (blue line in Figure 3.11). However, the large changes in the radius of gyration were diminished after 8.40 million steps and final conformations formed within a small range from 29 to 31 Å. The radii of gyration of final conformations fell in same range of 30-31 Å as found in the previous 300 K simulations (blue line in Figure 3.11). On the other hand, the energies showed consistent behaviour with small fluctuations after completing 1.20 million steps (Figure 3.14). The rest of the plots of energy and radius of gyration using different sets of parameters are provided in Appendix B.4. These results

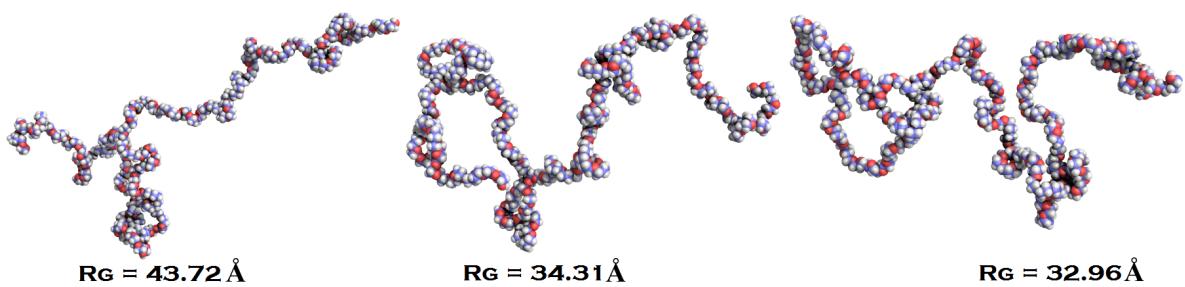


Figure 3.13: Snapshots of three PEG starting structures taken from 6 million steps long production run conducted in MCCCS Towhee using an NPT ensemble at 1200 K. These structures with very different radii of gyration, measured in Å, were picked to be used as an initial configurations in the PEG sampling simulations conducted at 300, 600 and 1200 K.

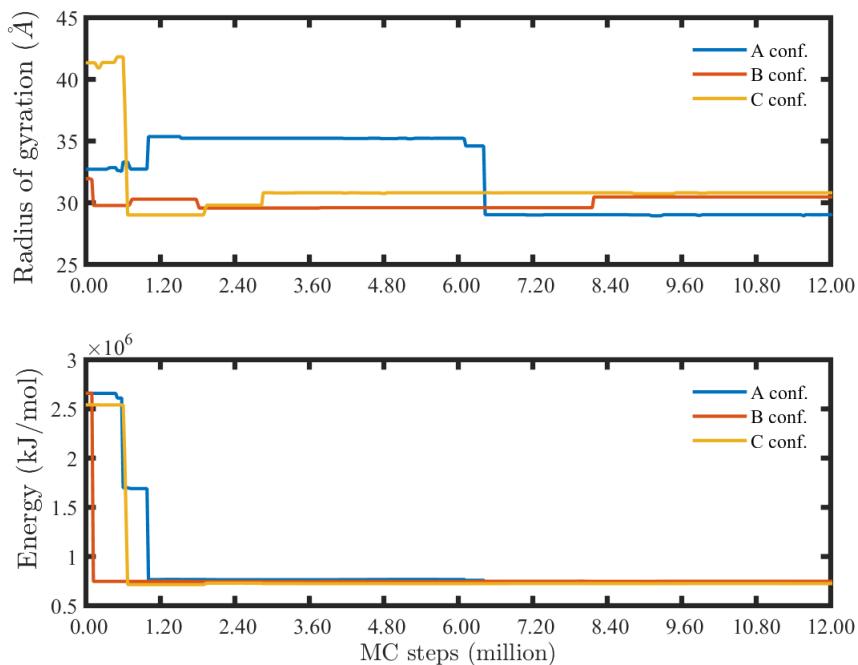


Figure 3.14: Radius of gyration and energy variations in MCCCS Towhee simulations at 300 K for three different starting conformations using the NPT ensemble and ‘RANLUX’ random number generator.

suggest that the change in the random number generators, and NPT to NVT ensemble does not bring any significant improvement in the conformational sampling. However, high temperatures help the PEG to explore the large conformational space and energy quickly by increasing the probability of accepting MC moves that increase energy (Figure 3.12).

### Concluding remarks - MCCCS Towhee simulations

We tried MCCCS Towhee to run conformational sampling simulations because it provides general forcefield parameters to construct 8 kDa PEG and run simulations through a simple script. Mainly, this program is built to investigate the properties of reactions occurring in the gas phase utilizing small sized gas molecules. Probably, MCCCS Towhee was unable to do a great job regarding conformational sampling of large sized polymers due to the lack of a properly tuned forcefield. We have performed numerous simulations by varying temperature, pressure, ensembles and size of simulation box to generate the diverse conformations but the results were not promising. Added to this conclusion, the conformational sampling was improved at a high temperature of 1200 K only.

In a nutshell, MCCCS Towhee is good to build an initial optimized PEG structure quickly through MC simulations without worrying about developing the forcefield parameters and running high level quantum chemistry calculations for geometry optimization.

#### 3.2.2 MC sampling in Sire

MCCCS Towhee produced a library of limited PEG conformations by performing MC simulations. An alternative conformational sampling was performed in Sire to build more diverse PEG conformational ensembles. To initialize the MC simulations in Sire, one can introduce the input structure file through a script, a PDB or Amber *top/crd* files. The most effective way is to use the Amber *top/crd* files because these files provide the necessary forcefield parameters to calculate the total structure energy during the simulation. Further, the total energy is also needed to validate any translational, rotational and vibrational move

that is performed on the structure during the simulations. However, the other input files lack this capability and result in distorted structures in the conformational sampling. In total, a set of three simulations were performed at two different temperatures, using two PEG molecules that differ in their terminal groups and starting linear and folded PEG conformation. These different parameters were used to enhance the diversity of PEG conformations in the library. It is noted that the first two sets of simulations used an original PEG with hydroxyethyl terminals whereas the last set of simulations were performed using PEG with methyl terminal (Figure 3.2). The following simulations were carried out:

1. Two individual MC conformational sampling runs conducted at 300 and 1200 K respectively, using an initial linear PEG structure.
2. One MC conformational sampling run started with an initial linear PEG structure at 1200 K with periodic quenching to 300 K.
3. Two MC simulations using linear PEG structures with methyl and hydroxyethyl terminals to inspect the terminal group effect.

First of all, two separate sampling simulations with one million MC moves were performed at 300 and 1200 K, using a linear PEG structure as a starting configuration. In these simulations, the resultant PEG conformations and the corresponding energies were saved after each one thousand MC moves. The results of simulations conducted at 300 K are presented here only, while the results of simulations performed at 1200 K are provided in Appendix B.5. In both simulations, the total energy increased precipitously in the beginning and then fluctuated about mean values of 1600 and 4800 kJ/mol, respectively, after reaching the equilibrium state (Figure 3.15). The collapse/folding of the initial linear PEG structure is entropy-driven and took the structure towards lower entropy at the expense an increase in the total energy. After reaching an equilibrium state, the energy stabilized and fluctuated about a mean of 1600 kJ/mol.

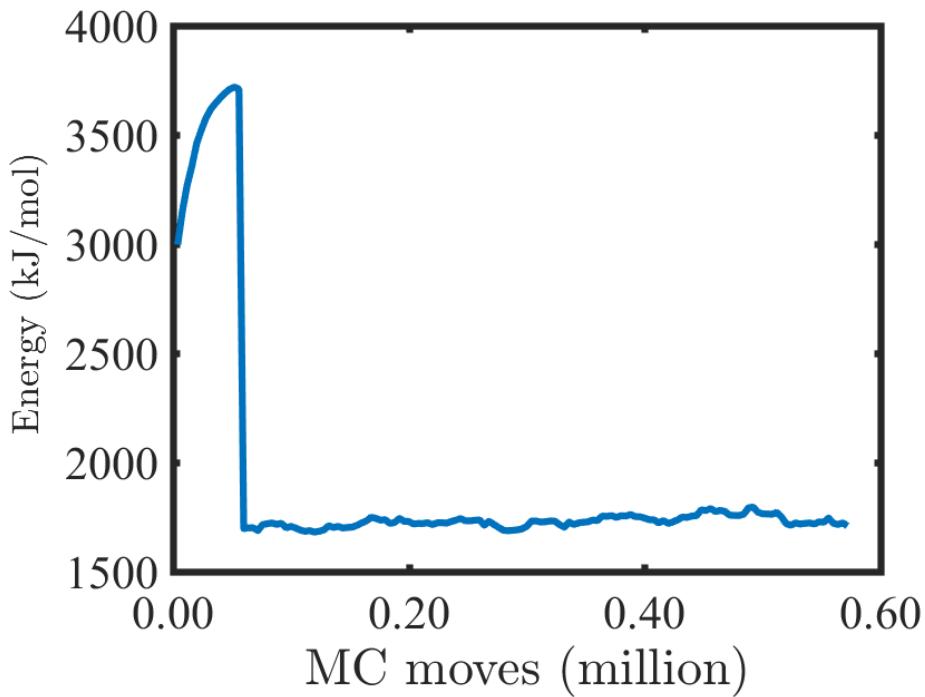


Figure 3.15: Energy fluctuations in the NPT ensemble MC conformational sampling simulations conducted at 300 K. These simulations were started with a linear PEG structure with a hydroxyethyl terminal and used HFF parameters.

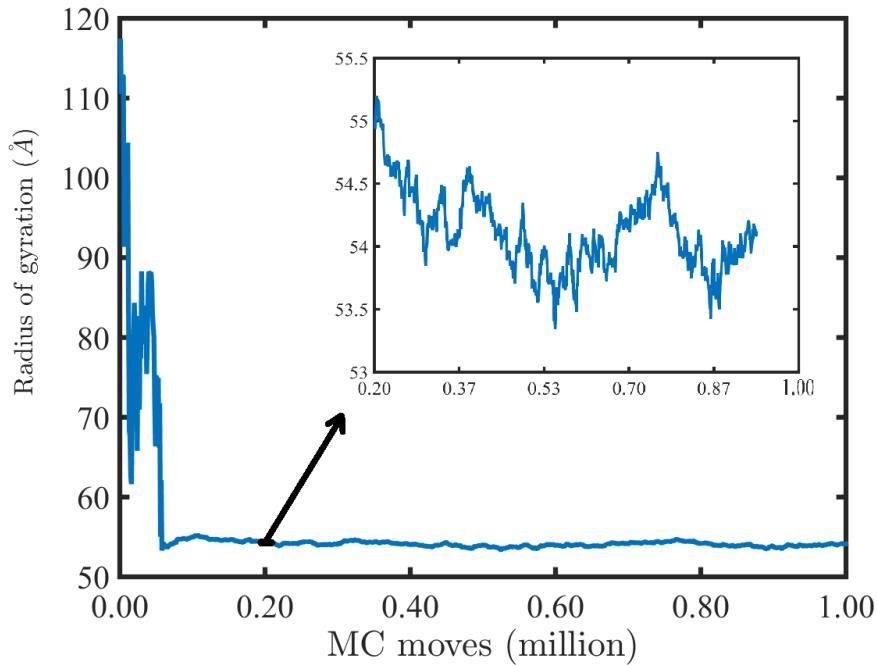


Figure 3.16: Change in the radius of gyration in MC conformational sampling conducted at 300 K, starting with a linear PEG structure. The structure reached an equilibrium state after completing less than 0.2 million steps. The inset diagram shows that large fluctuations that occurred in the beginning diminished in the equilibrium state.

The trend of radius of gyration indicates that the PEG molecule folds quickly in the beginning and remains in a semi-compact structure at the stationary state. There are quick and large fluctuations of a few tens of angstroms in the radius of gyration in the beginning of simulations and finally the structure acquired a stationary state at 54 Å after completing 0.12 million moves approximately (Figure 3.16) and remained in the equilibrated state until the end of simulations. The radius of gyration fluctuates over a small range in the stationary state with a standard deviation of 0.26 Å. These results suggest that PEG is not exploring the conformational space fully at 300 K. A similar trend is observed in sampling performed at 1200 K, in which the total energy and radius of gyration of an equilibrated structure fluctuates about mean values of energy of 4500 kJ/mol and of radius of gyration of 62 Å respectively with standard deviation of 0.16 Å in radius of gyration (Figures B.9 & B.10 in Appendix B.5).

In the second step of this section, a temperature quenching technique was applied to enhance the energetic and conformational sampling. The starting structure was simulated at 1200 K continuously from which a PEG structure was picked out periodically for quenching to 300 K. The quenched PEG structure was simulated further by performing an additional one thousand internal moves. The magnitude of each internal move, such as change in bond lengths, angles, dihedral angles and the number of moves at each step, were chosen using a trial-and-error method. The magnitude of each internal move used in the present simulations is tabulated in Table 3.5. We can change the number of moves and the magnitude of each move if necessary. For example, a larger number of internal moves could converge the structure quickly by changing the radius of gyration at greater rate in the beginning. However, the effect of increasing the number of internal moves diminished after reaching the stationary state. In these simulations, one thousand moves per step were optimal and produced one thousand conformations within four hours of processor time.

It was found that using the quenching approach, the energetic and conformational sampling space landscapes improved significantly in comparison to the simulations performed

Table 3.5: Type and magnitude of MC internal moves implemented in MC simulations in Sire.

Type of Move	Magnitude
Bond	-0.05, +0.05 Å
Angle	-15, +15 °
Dihedral	-15, +15 °

at single temperatures (Figures 3.17 & 3.18). For instance, the equilibrated structure fluctuated over a wide range of radius of gyration between 20 to 45 Å with standard deviation of 12 Å. The results showed the quenching approach enormously enhanced the conformational sampling in comparison to the single temperature simulations in MCCCS Towhee (Figure 3.11). The results showed that the folding rate of a linear PEG structure depends on the number of internal moves per step, the move ranges and the temperature. At higher temperatures, the stationary region includes larger sized open conformations as compared to the conformations at lower temperatures. The higher temperatures allow the execution of internal moves of larger magnitudes which resulted in more diverse and partially folded conformations.

To examine the effect of a larger number of internal moves on the folding rate, another simulation was run by performing 10,000 internal moves per step (Figures 3.19 & 3.20). The resultant energies and radii of gyration agreed with previous simulation results obtained after performing 1000 internal moves per step (Figures 3.17 & 3.18 respectively). The energies fluctuated over a slightly wider range from 4000–6500 kJ/mol as compared to previous energy range from 4200–5200 kJ/mol. However, radii of gyration in both simulations fluctuated in the same range from 20–45 Å. It is found that by increasing the number of internal moves per step vanished the higher energy peaks seen in Figure 3.17 and all the energies fluctuated about a mean value of 6000 kJ/mol (Figure 3.19). These results showed that a higher number of internal moves performed at 300 K results in more stable structures.

It is noted that the final energy fell in the range of 1200 to 1800 kJ/mol in the single temperature simulations conducted at 300 K (Figure 3.15) as compared to a quenching

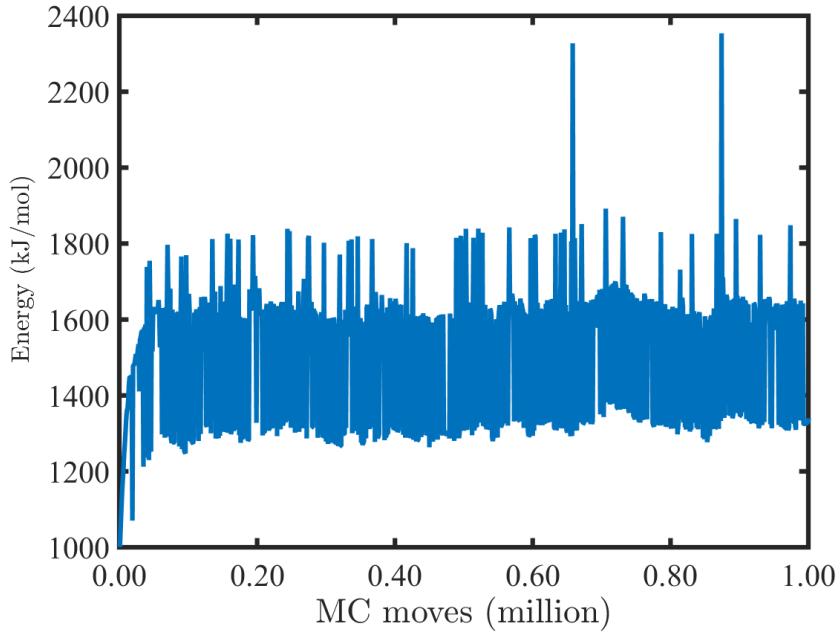


Figure 3.17: Energy change during MC conformational sampling at 300 K, using a temperature quenching approach between 300-1200 K. These simulations used a linear 8 kDa PEG with a hydroxyethyl terminal group and HFF parameters.

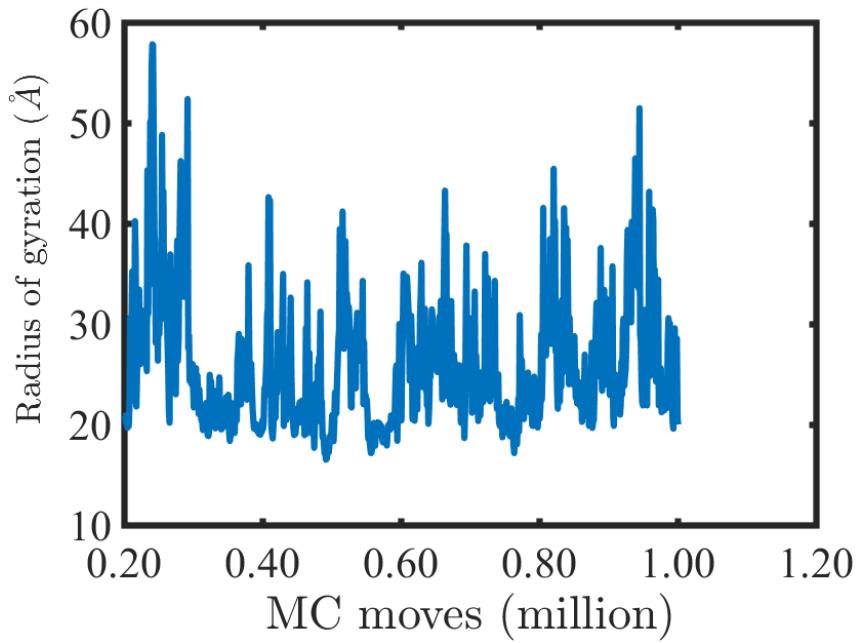


Figure 3.18: Change in the radius of gyration in MC conformational sampling at 300 K, performed with a temperature quenching method. The quenched PEG structures are optimized by performing an additional 1000 internal moves. These simulations started with a linear 8 kDa PEG with methyl terminal group and MFF parameters. Regardless of energy trends, a temperature quenching method is producing radii of gyration in a range that is consistent with the experimental data discussed in the concluding remarks on the Sire MC sampling.

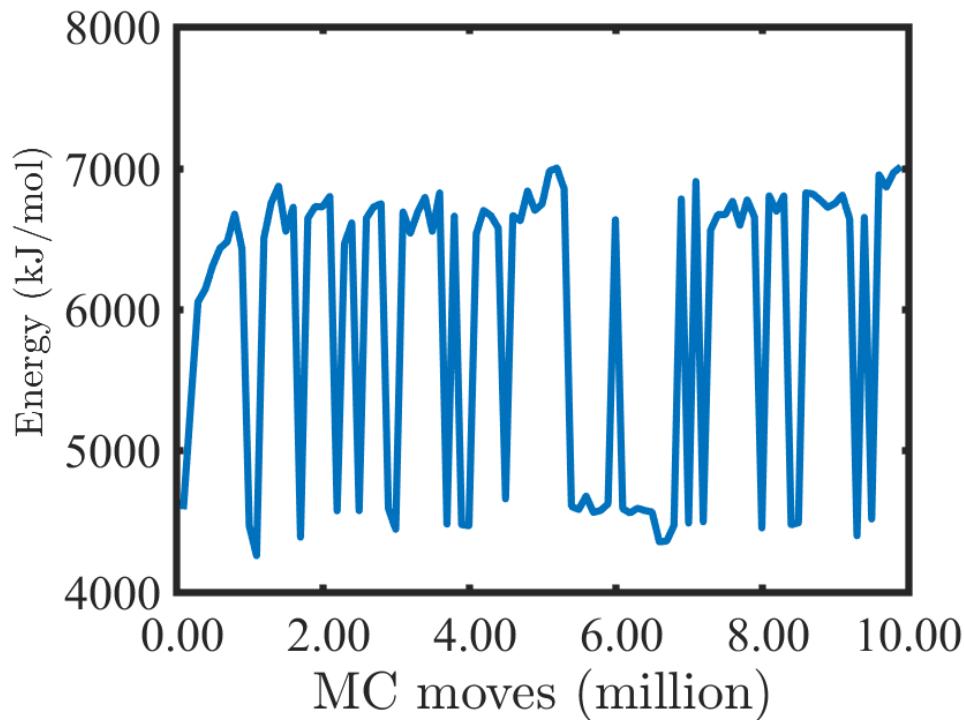


Figure 3.19: Energy change during MC conformational sampling at 300 K, using a temperature quenching approach between 300-1200 K with 10,000 MC moves. These simulations used a linear 8 kDa PEG with methyl terminal group and MFF parameters.

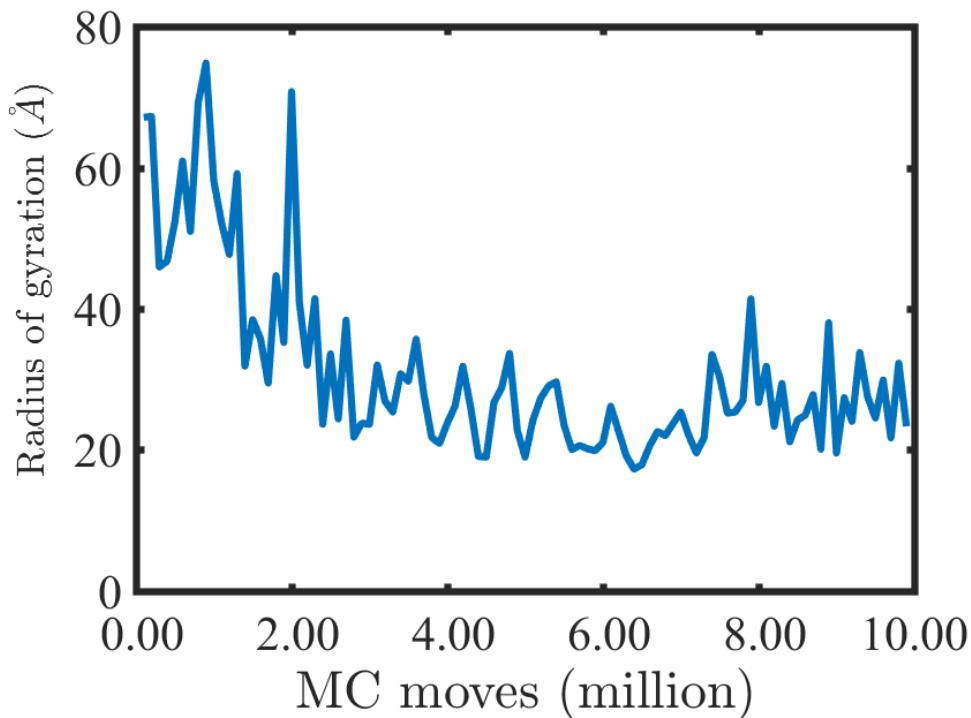


Figure 3.20: Change in the radius of gyration in MC conformational sampling, performed with a temperature quenching method using 10,000 MC moves. These simulations used a linear 8 kDa PEG with methyl terminal group and MFF parameters.

approach where the total energies of the resultant conformations simulated at 300 K fell in the range between 4500 to 7000 kJ/mol. The quenching approach resulted in higher total energies of the resultant structures, even after optimizing the structure at 300 K. To bring the structure energies down to the 1200-1800 kJ/mol range in the quenched simulations, a larger number of internal moves than 1000 were performed at 300 K. We performed an additional 10,000 and 0.5 millions internal moves per step in two individual simulations at 300 K but it was found that the higher number of moves were unable to bring the system energies to 1600-1800 kJ/mol range. However, it spread the energy spectrum over a broad range between 4200 to 6800 kJ/mol (Figures 3.19 and 3.20).

Three typical PEG conformations were chosen from the Sire simulations conducted at 300 K with a temperature quenching approach for illustration purposes (Figure 3.21). These pictures are taken at 0.05, 0.5 and 1.25 million MC moves and showed how the linear PEG folded over time.

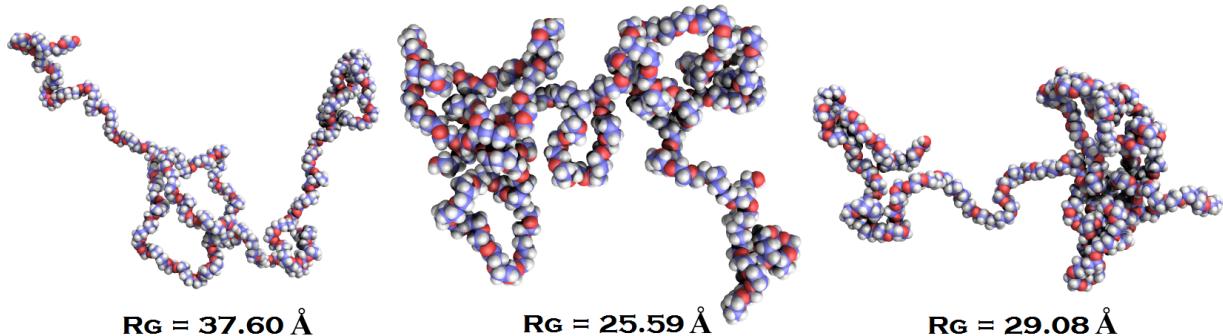


Figure 3.21: PEG snapshots taken at 0.05, 0.5 and 1.25 million MC moves (left to right) to illustrate the PEG conformational sampling. These conformations were picked from the simulation performed in Sire using a quenching approach and started with a linear PEG structure.

In the last step, two simulations using a PEG structure with methyl and hydroxyethyl terminals were performed to investigate the effect of terminal groups on folding behaviour. The results showed that both simulations produced similar energies and radii of gyration trends to the single temperature simulations (Figure 3.15 & 3.16) and the terminal groups did not play a significant role in changing folding behaviour. Since these simulations did

not make any difference in the results, the results are provided in Appendix B.5.

### Concluding remarks - Sire MC sampling

Sire has done a great job in developing the library of diverse PEG conformations as compared to MCCCS Towhee (Figures 3.11 & 3.18 respectively). Sire generated the conformations in a wide range from 18 to 60 Å approximately whereas MCCCS Towhee produced conformations in a very limited range from 30 to 34 Å. It is worth noting that, regardless of large energy fluctuations (Figure 3.17), Sire is giving a spread of radii of gyration (Figure 3.18) that is consistent with the experimental spread. The theoretical and experimental investigations reported 15 [80, 255], 24.5 [63, 110, 112], 25 [90], 27 [55], 28.5 [55], 30 [111], 31 [90, 91], 36 [90], 40 [55], 43 [107], 46 [89, 107], and 40-60 [55] Å radii of gyration of an 8 kDa PEG. In addition, Sire provides a lot of control over simulation design and is also a good tool to optimize the structures locally.

#### 3.2.3 MD sampling in AMBER

So far, two different programs, i.e. MCCCS Towhee and Sire, were used to conduct MC conformational sampling. In addition to MC simulations, conformational sampling simulations were also performed in Amber by using the MD simulation approach. MC simulations, a stochastic approach, are exploring the energy surface by probing the geometry of a given system by changing bond lengths, angles, and dihedral angles randomly [85, 86, 104] whereas MD simulations determine the particle trajectories from Newton's second law. MD simulations require an initial geometry and atomic velocities of the system. The atomic velocities are assigned from the Maxwell distribution and the system is run to achieve the equilibration. The progress of the process is monitored until the total kinetic energy becomes stable [256, 257]. We selected the Amber program [105, 194, 226] among the other MD programs [106, 182] due its vast use in studying biochemical reactions. Amber requires forcefield parameters for all the molecules involved in the simulations to

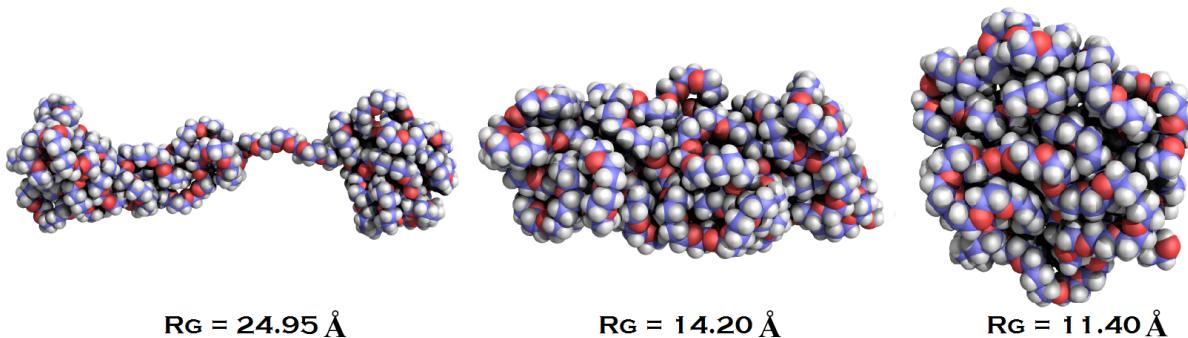


Figure 3.22: PEG snapshots taken at 2.5, 3.0, and 10 ns time steps (left to right) of MD simulations conducted at 300 K in Amber, using PEG of 8 kDa to illustrate the PEG folding.

calculate the total energy. Amber provides these necessary forcefield parameters for the majority of common molecules such as proteins, RNA, DNA, water and many other small organic molecules [226]. However, forcefield parameters for PEG are unavailable in Amber. Therefore, it is necessary to build PEG forcefield parameters before conducting MD conformational sampling. The missing PEG forcefield parameters for Amber MD simulations were generated on the PyRED server [208, 209]. The details on the development of forcefield parameters are covered in section 3.1.

In this section, three individual sets of MD simulations were performed to construct the libraries of PEG conformations. In these simulations, the effects of temperature, of chemical composition of PEG, and of implicit and explicit solvent models on the conformational sampling of PEG were studied. The sole purpose of testing these factors was to enhance the distribution of PEG conformations in the resultant libraries. Eventually, the following two sets of simulations were carried out:

1. MD conformational sampling at 300 K using a PEG with methyl, hydroxyethyl and propyl terminals in an implicit and explicit solvents.
2. Investigate the effect of high temperature by conducting simulations in an implicit solvent at 1200 K.

In the first case, three libraries of PEG conformations was constructed by running 10 ns

long 300 K MD simulations using an NPT ensemble, Langevin thermostat and the implicit solvent Born model [231]. These simulations started with three linear PEG structures. These structures were constructed by joining the three fragments generated during the forcefield parameter step (Figure 3.2). The results showed that PEGs with all three different terminals showed similar folding behaviour, in which the radius of gyration decreased to 12 Å approximately (Figure 3.5). The total energies of both PEGs with methyl and propyl terminals were converged after completing 5 ns of simulation time. However, the total energy of the PEG with hydroxyl terminal dipped over a period of about 5 ns. The only big difference is that it started from an energy much closer to its final energy. To illustrate the folding progress during the MD simulation three snapshots at 2.5, 3.0, and 10.0 ns time steps were taken (Figure 3.22). These snapshots showed that PEG folded to a compact conformation. The structures collapsed due to intra-molecular hydrogen bonding. Previous studies provided evidence of the presence of intra-molecular hydrogen bonding which lead to folded helical and coiled structures of PEG in water [89, 92, 95, 96, 101, 109].

In the second case, two additional 10 ns long MD simulations were performed using an NPT ensemble, Langevin thermostat and the explicit solvation (water) at 300 K to investigate the effect of terminal groups on folding behaviour of PEG. For this purpose, two PEG conformations with methyl and hydroxyethyl terminals were used. The results of these simulations demonstrated the same folding behaviour in which the PEG formed a very compact structure again after reaching the stationary state. Experimental and theoretical investigations showed contradictory results in which a few studies showed that PEG has a self aggregation property which not only leads to compact structures of individual PEG molecules in solution [101] but also forms PEG aggregates [101, 258], while others showed that PEG formed partially folded structures [109, 259]. Moreover, QM calculations also confirmed the presence of intra- and inter-molecular hydrogen bonding in PEG which could lead to such compact conformations [101]. These results demonstrated a folding pattern of coil formation similar to the previous experimental findings [89, 92, 93, 95, 101].

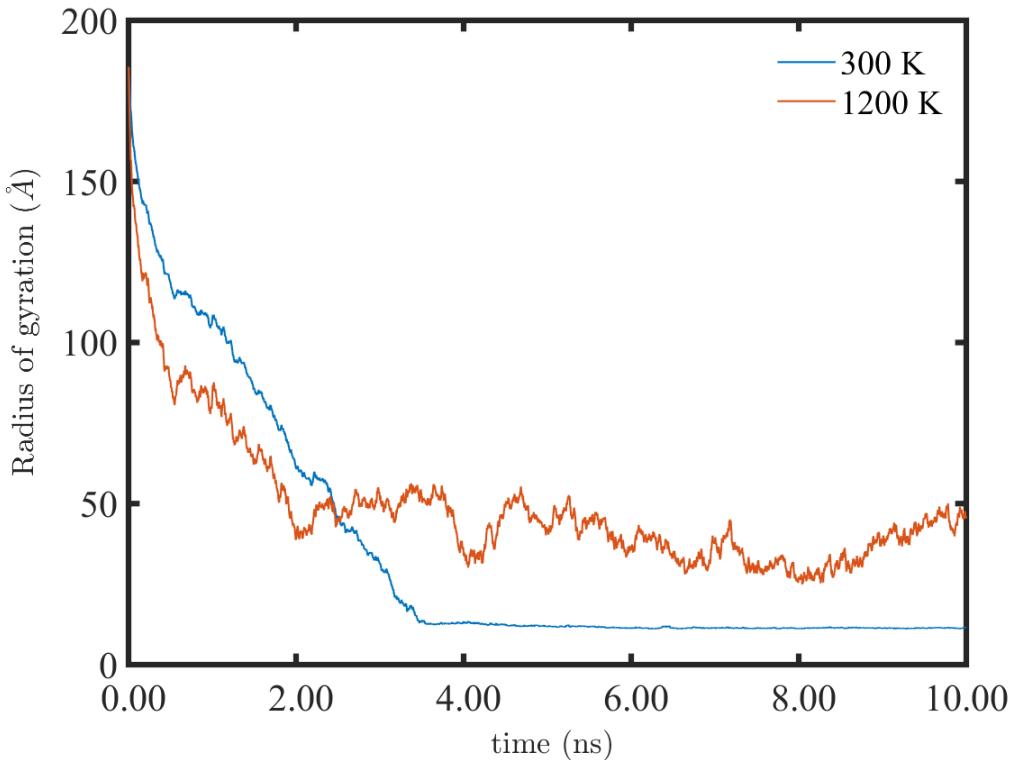


Figure 3.23: Radius of gyration change in the MD conformational sampling of PEG with methyl terminal group, conducted at 300 and 1200 K in Amber using NPT ensemble and implicit solvation model.

In the last step, another 10.0 ns long MD simulation was performed at 1200 K, using a PEG with a methyl terminal. The high temperature in these simulations (Figure 3.23) produced relatively open conformations and the radius of gyration was found fluctuating around a mean of 40 Å.

### Concluding remarks - MD simulations

MD simulations in Amber provided an alternative method to MC simulations in Sire and could be used potentially to construct the ensemble of PEG conformations. MD conformational simulations were conducted on three types of linear PEG structures at 300 and 1200 K in implicit solvent. The folding behaviour of PEG was found to be insensitive to the terminal groups. Moreover, the explicit solvent model has no effect on the folding be-

haviour and eventually PEG with methyl, hydroxyethyl, and propyl terminals collapsed to a compact conformation.

### 3.2.4 Conclusion - Ensemble of PEG conformations

The objective of this section was to develop libraries of PEG conformations that can be used to mimic the crowding environment in computer simulations. In order to populate these libraries with conformations that are as diverse as possible, two approaches, i.e. MC and MD approaches, were chosen and performed under different temperature, PEG composition and explicit and implicit solvents. MC simulations were employed in MCCCS Towhee and Sire while the MD simulations were performed in Amber. In the first category of MC methods, Sire was found to be more productive in terms of utilizing computer resources efficiently, flexibility in running desired types of simulations, and eventually yielding a diverse distribution of PEG conformations as compared to MCCCS Towhee (Figures 3.18 & 3.11 respectively). MCCCS Towhee sampled conformations over a limited energetic and conformational landscape and resulted in PEG conformations falling in a restricted range relative to Sire. Due to this fact, the resultant library of MCCCS Towhee structures was not used for packing simulation boxes. Both MC and MD approaches produced diverse PEG conformations in Sire and Amber respectively. In contrast to MD simulations, MC sampling in Sire was quicker and formed partially folded conformations. It was found that the folding behaviour was insensitive to PEG terminal groups in Sire. However, PEGs with methyl and propyl terminals collapsed in all simulations irrespective of the presence of explicit or implicit solvent models in MD simulations. The diverse distribution is further shown by comparing the radius of gyration of sampled conformations taken from MC in Sire and MD simulations (Figure 3.24). The majority of conformations from MC prevailed in the central region in between 20 to 40 Å while MD simulations formed the majority of conformations from 10 to 20 Å with standard deviations of 7.35 and 5.80 Å

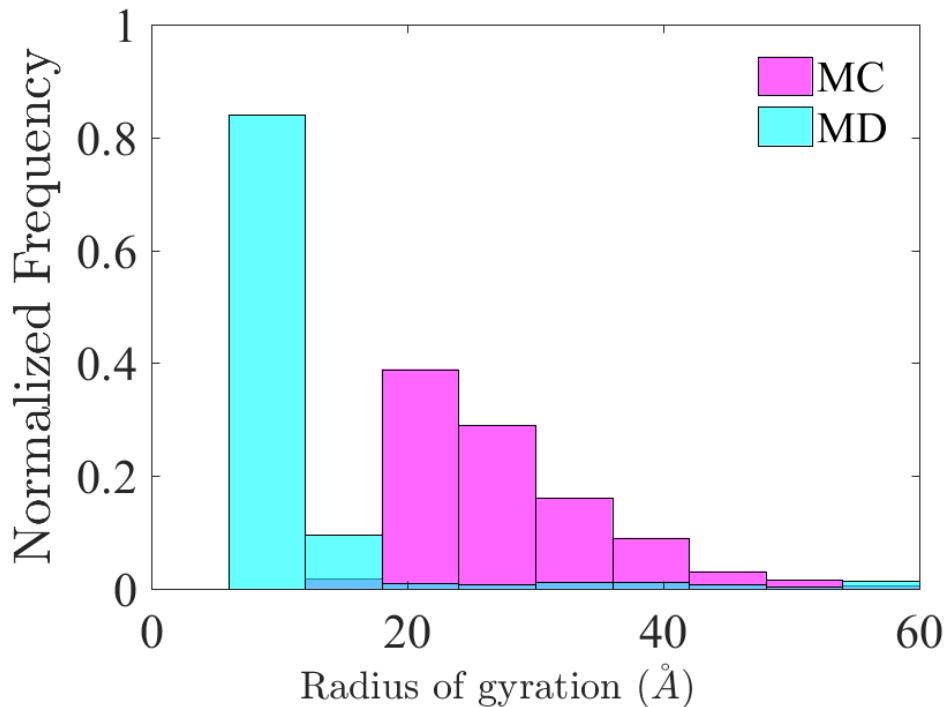


Figure 3.24: Distribution of PEG conformations from start to end in MC Sire and MD Amber simulations performed at 300 K using linear structures of 8 kDa PEG with methyl terminal. PEG conformations of 20-60 radius of gyration were used for packing the crowded systems. The transparency of the bars results in a blue color where the two distributions overlap.

respectively. These results showed that MC sampling simulations produced conformations over a wide range and these values in good agreement with experimental radii of gyration of 24 to 36 Å [55, 63, 90, 91, 107–112].

In a nutshell, we have presented three approaches to develop libraries of PEG conformations. We have used these conformations from both methods to prepare the crowded systems.

### 3.3 Packing and equilibration

MC and MD conformational sampling was performed to build PEG conformational libraries. MD simulations produced diverse conformations with a slightly larger range of radius of gyration as compared to MC simulations in Sire (Figure 3.24). We performed a

variety of MC and MD simulations to achieve our goal of constructing libraries populated with representative diverse and dynamic solution structures of PEG falling in the range of 15 to 60 Å of radius of gyration. Four libraries were populated with PEG conformations from MC and MD simulations performed in Sire and Amber respectively. Two of the libraries contained equilibrated structures only taken from the stationary state region while the other two were populated from the beginning to the stationary state. The last two libraries contained more diverse conformations with radii of gyration from 10 to 125 Å (Figures 3.25 & 3.26).

These conformational ensembles of four libraries were used to prepare the simulation boxes at six different concentrations from 0.1 to 0.6 g cm<sup>-3</sup>. 25 to 150 PEG molecules of 8 kDa molecular weight were packed in boxes of dimensions 300 × 300 × 300 Å<sup>3</sup>. Fifteen copies of the simulation boxes at each concentration were prepared by packing randomly chosen PEG conformations from the library using Packmol [232]. Packmol performed 1,000 trials in total to pack and optimize each simulation box. Each trial tried to place the molecules at random points in space without steric clashes. Packmol saved the final packed configuration on satisfying the tolerance criterion. The tolerance criterion represents the minimum distance between the surface of two molecules. In the preparation of simulation boxes, the tolerance of 3 Å was set. The value of 3 Å was chosen using a trial-and-error method and it results in optimized packing with no steric clashes. A large value greater than 5 Å took a long time for packing the intermediate and high concentration boxes, i.e. 0.3 to 0.6 g cm<sup>-3</sup>, and required more than 1,000 trials to find an optimized configuration satisfying the tolerance criterion. The calculation cost depends on the concentration, type of conformations of the molecules, and the tolerance criterion. At the lowest concentration of 25 molecules of PEG, one trial of packing and optimization took approximately 5 seconds whereas 30 seconds were required at the maximum concentration. The lowest concentration systems were packed and optimized in less than 50 trials and the most concentrated systems completed 800 to 1,000 trials on average. The packed and optimized simulation boxes at

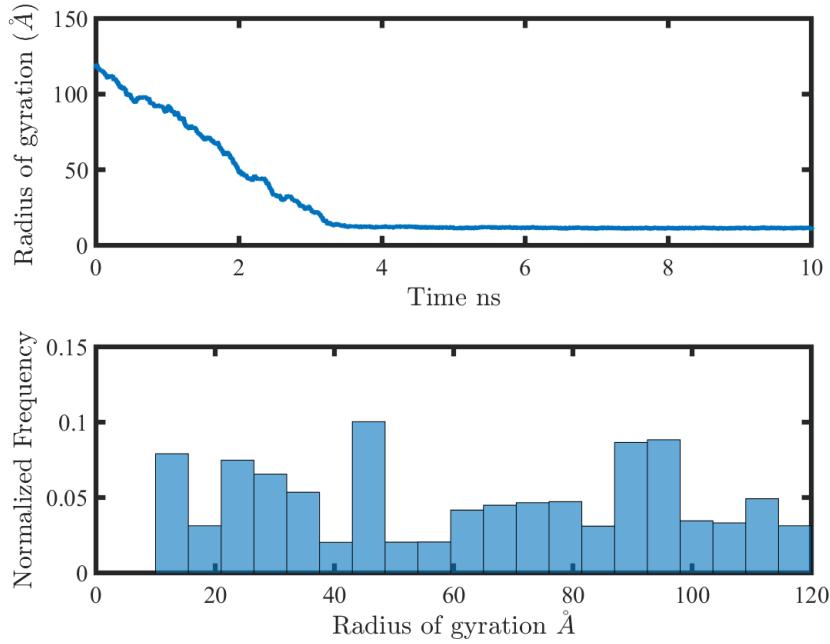


Figure 3.25: Radius of gyration change from start to stationary stage during MD sampling (top) while the distribution of PEG radii is illustrated in the histogram (bottom). The final structures were saved at 300 K up to 5 ns long MD simulations in Amber with a 1 fs time step and using an NPT ensemble, implicit solvation model and a linear PEG structure with a methyl terminal group, using a MFF parameters.

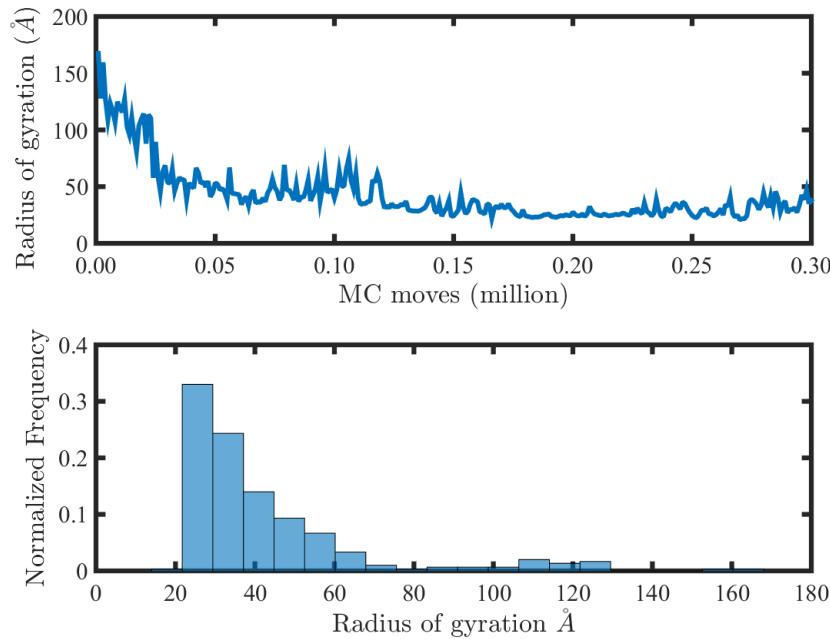


Figure 3.26: Radius of gyration change from start to stationary stage during MC sampling (top) while the distribution of PEG radii is illustrated in the histogram (bottom). The final structures were saved at 300 K up to 0.3 million moves of an MC simulation in Sire started with a linear PEG structure with a methyl terminal, using the MFF parameters.

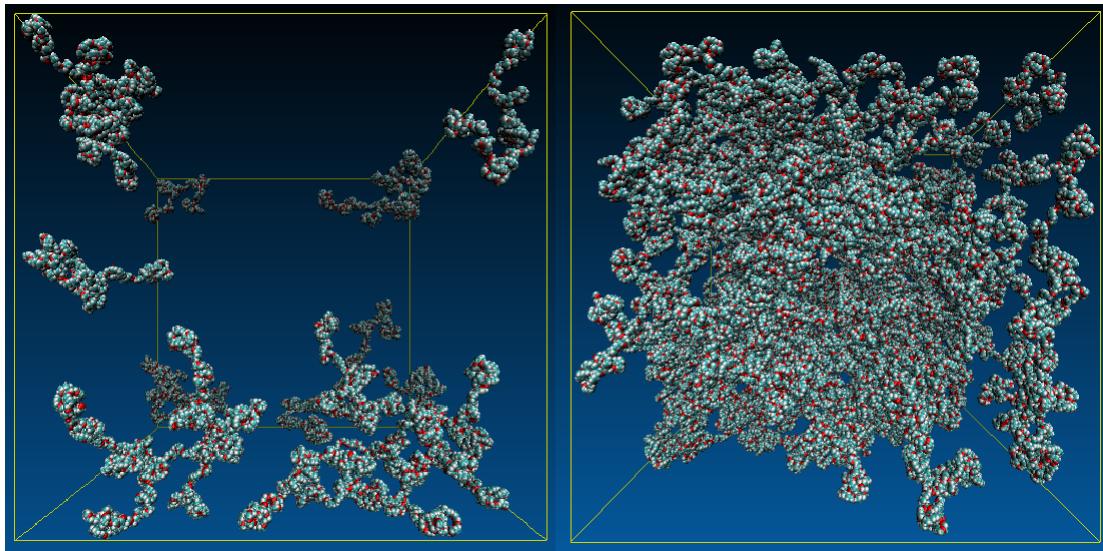


Figure 3.27: Sample packed simulation boxes at the lowest (left) and highest (right) concentration ( $0.1$  and  $0.6 \text{ g cm}^{-3}$  respectively) by Packmol.

the lowest and highest concentrations in a  $300 \times 300 \times 300 \text{ \AA}^3$  box present large spaces at low concentration whereas the box space is almost filled at the highest concentration (Figure 3.27).

It is noted that all of these conformational ensembles of PEG were prepared under dilute conditions. Therefore it was necessary to further equilibrate the simulation boxes to optimize the packing and observe the high concentration effects on the PEG conformations. We developed and applied three approaches, namely MC swapping, MC equilibration (in Sire) and MD simulations in Amber. The following two key points are discussed here to evaluate the efficiency of all three equilibration methods:

1. Equilibration of packed simulation boxes with two Monte Carlo methods (i.e. MC equilibration and MC swapping in Sire) and MD equilibration in Amber.
2. Efficiency of the three equilibration methods in terms of computational cost.

### 3.3.1 MC equilibration

MC equilibration is the first MC method developed to equilibrate the simulation boxes. 5,000 internal folding/unfolding and 5,000 rigid body translational/rotational moves were

performed in each step. Table 3.5 presents the sizes of various types of internal and rigid body moves, implemented in the MC equilibration algorithm. The computation cost depends on the size and concentration of the system. In a case study, the MC equilibration simulations were performed on the lowest concentration system of  $0.1 \text{ g cm}^{-3}$ , where each MC step took 3 minutes of real time on an eight core machine. The calculations run for 400 hours and it can be seen that the system requires more computation in order to reach the equilibrium state (Figure 3.28).

The large energy changes in Figure 3.28 are associated with major conformational changes while performing the internal moves on the PEG molecules. The working speed of the algorithm depends on the concentration directly. After performing 8,000 steps, it was found that PEG molecules folded towards more compact conformations in the presence of other PEG molecules (Figure 3.29). However, there is no evidence found that the PEG molecules formed any clusters or aggregates.

### 3.3.2 MC swapping

MC swapping is an alternative MC approach to the MC equilibration to equilibrate the given system. In this method, the algorithm swapped the PEG molecules between the simulation box and the conformational library. We assumed that the conformational library contains representative and equilibrated PEG solution structures. This was the aim of developing a library populated with diverse PEG conformations. The swapping algorithm performed 1 million steps in total before applying the convergence test. An MC step involves the selection of two random molecules, one in the simulation box and the other in the conformational ensemble library, rotation, translation and swapping the two molecules, and finally the energy calculations. The convergence test determines whether the system is equilibrated energetically or requires additional MC moves using the energy values. The convergence test uses a Student's  $t$  test [234, 235]. This test compared the energy values stored in two windows and checked the condition if the threshold value or significance

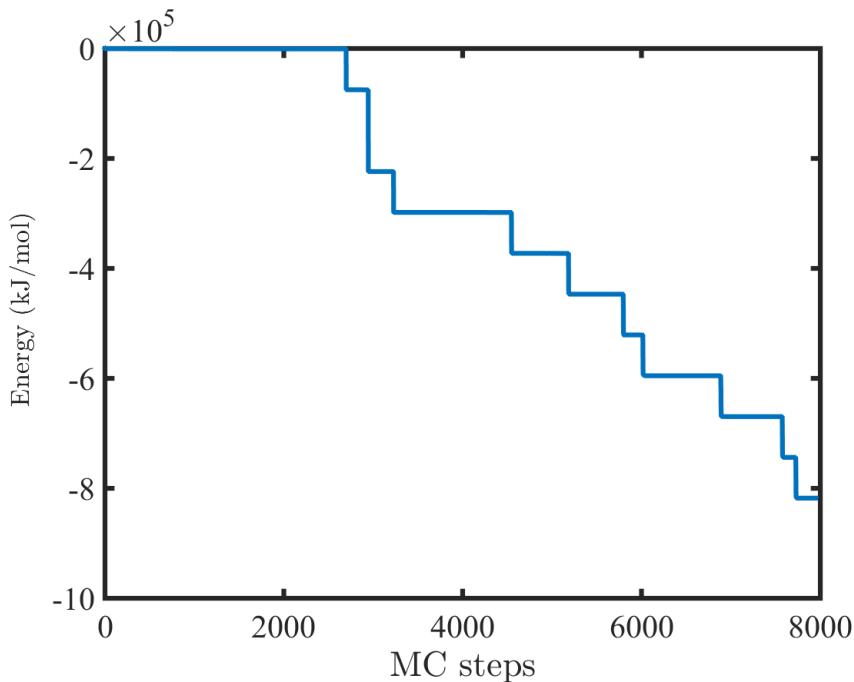


Figure 3.28: The energy change in a 400 hour MC equilibration, performing 5,000 internal and 5,000 rigid body moves in a single step, for a total of 8,000 steps. These MC simulations were performed using a least concentrated system with  $0.1 \text{ g cm}^{-3}$  concentration and used MFF parameters to compute the total energies.

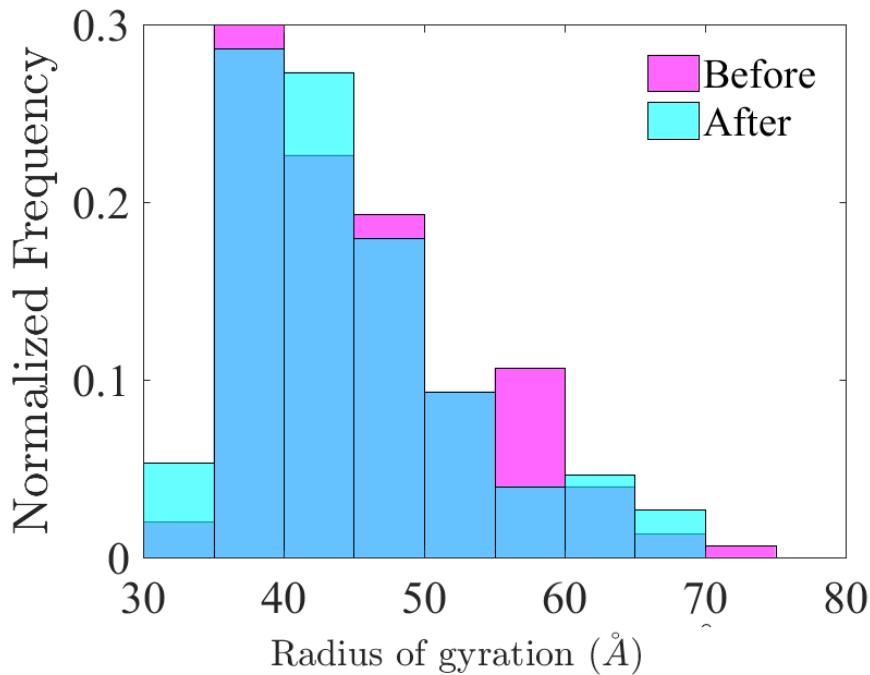


Figure 3.29: Distribution of PEG conformations before and after performing the MC equilibration algorithm at 300 K. These MC simulations were performed using a least concentrated system with  $0.1 \text{ g cm}^{-3}$  concentration. Larger sized conformations generally formed more compact conformation during the equilibration process. The transparency of the bars results in a blue color where the two distributions overlap.

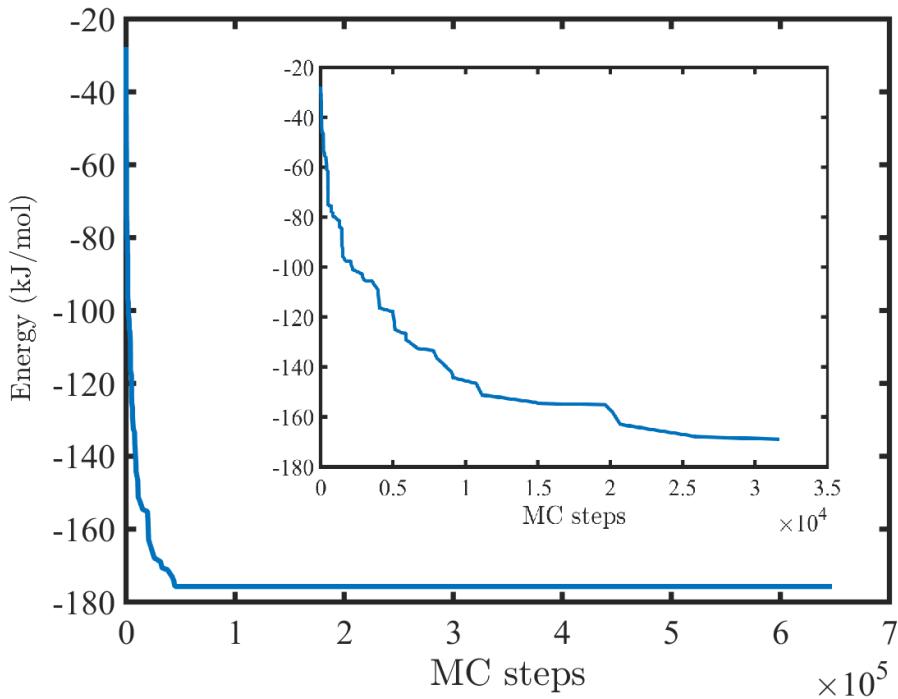


Figure 3.30: The energy change in a million step MC swapping simulation. The system reached the stationary state after completing less than 50 thousand steps. The inset demonstrated the rate of change of energy in the beginning of the simulation. These MC simulations were performed using a least concentrated system with  $0.1 \text{ g cm}^{-3}$  concentration and used MFF parameters to compute the total energies.

level of  $s < 0.1$  is true to terminate the simulations, otherwise continue the loop for an additional  $2 \times 10$  iterations until the condition is satisfied. The lowest concentration system reached an equilibrium state after completing 30 thousand steps (Figure 3.30). Each MC step takes a few seconds to complete.

The MC swapping method converged the system quickly as compared to the MC equilibration but at very high energy. This approach minimized the free energy by increasing the entropy while allowing the energy to increase. The computational cost also depends on the size and concentration of the system. This algorithm used a single core during the swapping process, excluding the energy calculation step where it used all the available cores.

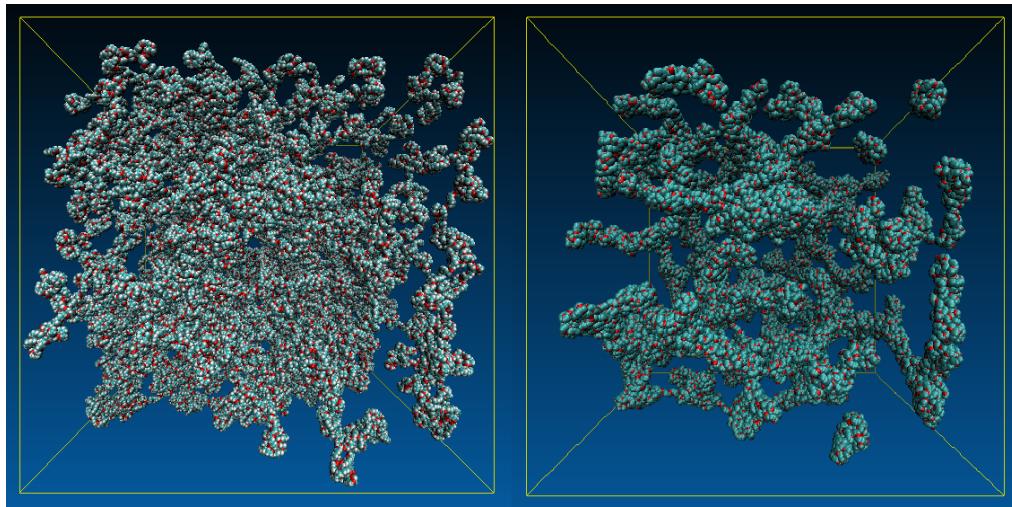


Figure 3.31: The presentation of the simulation box before and after the MD simulations and the formation of PEG aggregates after MD equilibration. These MD simulations were performed on the concentrated system with  $0.6 \text{ g cm}^{-3}$  concentration at 300 K, using an NPT ensemble in an implicit solvent and MFF parameters.

### 3.3.3 MD Amber simulations

MD simulation is the last method used to equilibrate the simulation boxes. The calculations were run on the WestGrid GPU machines using up to 4 nodes and 12 cores per node. It took approximately 60 hours to complete 20 ns long simulations for the lowest concentration (Figure 3.32). However, the computation time increased as a function of system concentration. The system is converged energetically after completing an 800 ps long MD simulation, where afterwards the energy fluctuates about a stable mean value of  $10.5 \times 10^4 \text{ kcal/mol}$ . A comparison of energies of an equilibrated system with an MC and MD approach is provided in the concluding remarks of this section. The PEG formed clusters and aggregates in MD equilibration which is probably due to the forcefield parameters that are responsible for the formation of compact conformations and aggregates as shown in Figure 3.31.

#### Concluding remarks - Equilibration approaches

Three methods of equilibration namely MC equilibration, MC swapping and MD simulations are employed to equilibrate the packed simulation boxes. Among these methods, the

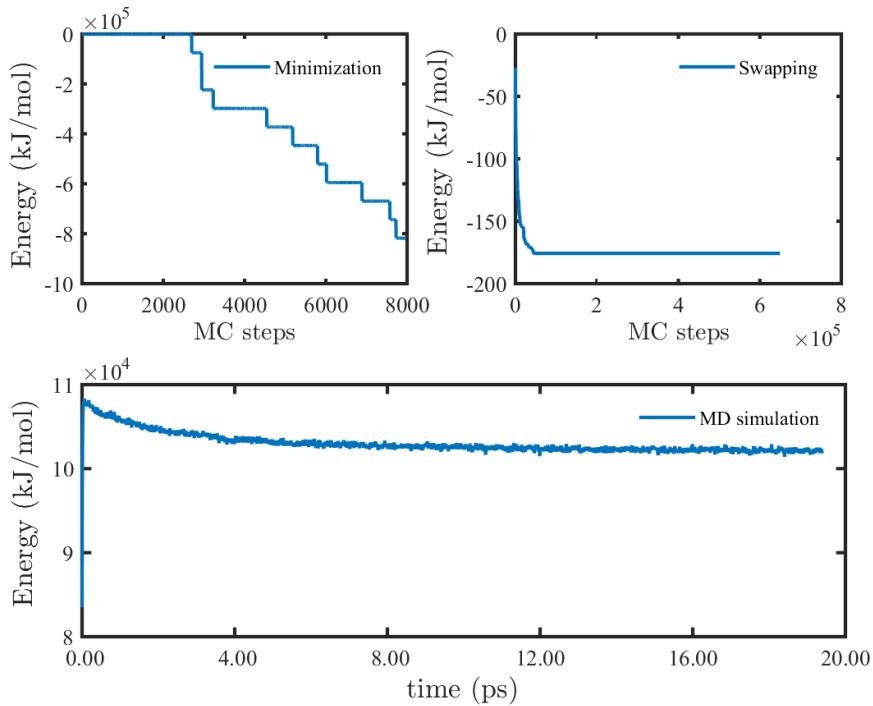


Figure 3.32: The energy change in the MD simulation in addition to MC equilibration and MC swapping. The system is converged after completing 20 ns of simulation time. MC equilibration and MC swapping methods were performed in Sire whereas the MD simulations were performed in Amber. The total energy change scale is different due to the different programs we used. However, all the simulations used the same input files.

MC swapping was found to be the least efficient in terms of using computer resources and taking the system to the lowest energy state. The MC equilibration approach is more efficient in terms of lowering the energy efficiently due to performing internal and rigid body moves. The MC swapping and minimization methods used multiple cores only in energy calculations which is why these approaches are time consuming. On the other hand, Amber MD simulation was the fastest approach using the Compute Canada WestGrid parallel-GPU machine. It equilibrated the simulation box of  $0.1 \text{ g cm}^{-3}$  in approximately 12 hours. However, the computation cost increases with the concentration and it took approximately 60–72 hours for the highest concentration systems.

Moreover, the MC simulation methods did not show any cluster formation during the equilibration process whereas the MD simulations resulted in the formation of aggregates of PEG molecules. The clustering behaviour depends on the number density of the molecules

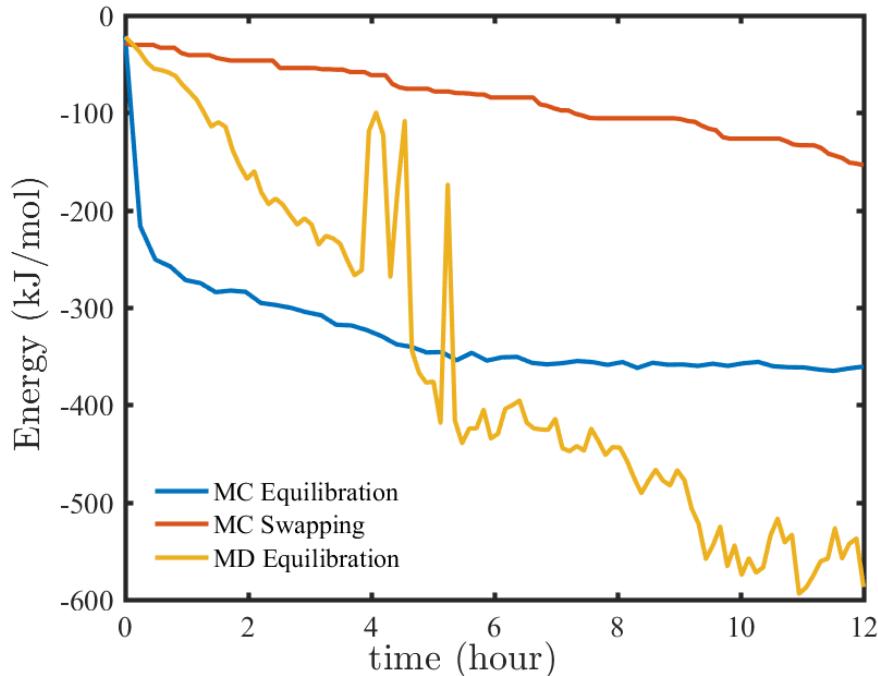


Figure 3.33: Comparison of real time for three methods along with energy decay. The simulations were run for 12 hours. MD simulations were more effective in terms of taking the system to the lowest energy in the given amount of time.

in the given system and a larger number of aggregates formed in the highest concentration of  $0.6 \text{ g cm}^{-3}$ . Previous studies showed that the PEG molecules form clusters in solution [95, 259–261]. Many causes of clustering such as intermolecular hydrogen bonding, impurities in solution, physical cross linking and chain end effects have been reported. The experimental findings of the small-angle neutron scattering (SANS) technique showed that the chain ends of the polymers are responsible for cluster formation [95, 259–261]. In our equilibration MD simulations, we tested the folding and aggregation behaviour of PEGs with methyl, propyl and hydroxyethyl terminal groups and found that PEG molecules showed spontaneous self-assembly behaviour regardless of terminal groups. This self-assembly behaviour leads to the emergence of individual compact structures and subsequently leads to aggregation [101].

The efficiencies of the three approaches were compared based on the real time elapsed using the same computer resources (Figure 3.33). The MD simulations took the system

to the lowest energy level in the given time of approximately 12 hours whereas the MC equilibration is the second best. Considering the real time efficiency, the MD simulations were used to equilibrate all the simulation boxes. Fifteen copies of simulation boxes at each concentration were saved for the calculation of the fraction available volumes and the statistical analysis.

### 3.4 Summary

The aim of the chapter was to prepare the crowded medium filled with diverse PEG conformations. In a nutshell, all the basic steps i.e. construction of forcefield parameters, construction of crowder conformations, packing and equilibration of crowded medium are covered that one needs to perform in order to achieve the goal. Regarding the first step of constructing the ensembles of PEG conformations, the MC simulations in Sire and MD simulations in Amber produced reasonably diverse PEG conformations that are consistent with experimental findings. MD simulations were found to be more efficient among two other MC equilibration methods to equilibrate the packed crowded medium in the final step. Moreover, MD equilibration result in further reduction in size of individual PEGs and also formation of PEG aggregates. Further, the efficiency of equilibration varies with system concentration.

# Chapter 4

## Conformational equilibrium in crowded media

This chapter presents the results of fractional available volumes and kinetics of conformational equilibrium obtained by executing the second part of the program recipe (Figure 4.1 (right)). In the previous chapter, we prepared six crowded systems filled with a variety of PEG conformations. Two methods, namely Monte Carlo (MC) simulations and the extended scaled particle theory (SPT) were used to compute the crowding effects on the conformational equilibrium of three pairs of macromolecules in these crowded systems. The MC and SPT models estimated the crowding effects by computing the fractional available volumes, and subsequently thermodynamic activities for all selected pairs of conformations in the crowded media. The results from both models are further compared with results obtained in other experimental and theoretical studies. Finally, the transition state theory model is applied to estimate the macromolecular crowding effects on the folding kinetics of the GAAA tetraloop receptor.

### 4.1 Fractional available volume calculations

An attempt was made to provide a quantitative description of the effect of macromolecular crowding on the conformational equilibrium of three systems by carrying out MC and SPT simulations. In this regard, various excluded volume theory models [143–157] including the SPT models [29, 33, 37, 81, 83, 158] have been developed to predict the macromolecular crowding effects on the various biochemical reactions. Generally these models

estimated the crowding effects on the equilibria and rates of biochemical reactions in a qualitative sense. However, the SPT formalism is successful in predicting the quantitative effects of crowding in certain cases [3, 12, 82, 262]. The SPT model predicts the crowding effects by estimating the magnitude of an excluded volume effect simply based on the sizes and shapes of molecules present in the medium. We are looking at the effects of crowding due to excluded volume rather than other effects contributed by the electrostatic potentials, enthalpic and entropic intermolecular interactions [37, 262].

It has been found that a mixture of crowders affects the activities more than a single type of crowder [21, 24, 49]. Also, these models present a static picture of the reaction media before and after placing the probe molecule but the cellular interiors are fluids and have characteristic properties of a mobile phase [8]. An addition of a probe molecule could possibly affect the packing of background molecules due to steric repulsion and could lead to a change in packing and size distribution of background molecules in the immediate vicinity of the probe. These factors are totally ignored which could potentially contribute to the thermodynamic activities of reacting species and thus predicting crowding effects quantitatively.

The present work is moving one step towards building a quantitative excluded volume theory model by introducing diverse conformations of PEG crowders to replace the ordinary spherical shaped fluid particles. Moreover multiple copies of crowded systems at each concentration were used to represent the dynamic size distribution of crowders in solution, as well as incorporating effects associated with crowder aggregation in the crowded media. In this regards, an MC simulation and extended SPT models were developed. The computational efficiency and outcomes of both methods is discussed in the following sections.

## 4.2 MC Simulations

MC simulation was our first method to compute the fractional available volumes for three given pairs of probe molecules in the equilibrated simulation boxes in addition to

the SPT model. This MC method calculated the fractional available volumes in a two-step procedure. At first, the algorithm inserted the probe molecule in the crowded medium at a random place with a random orientation and then it evaluated the success or failure of insertion in each trial by determining whether there were steric clashes. Regarding the success or failure of each insertion trial, two MC algorithms i.e. parallel-energy and parallel-distance algorithms were used to find the steric clashes. The name indicated the criterion used in these algorithms to evaluate the steric clashes. For example, the parallel-energy algorithm used the energy threshold to compare with the final energy after placing the probe molecule in the crowded medium to evaluate the success or failure of each insertion trial. We employed both algorithms to find the steric clashes and provided the comparison of resultant fractional available volumes. Moreover, the computational efficiency of both algorithms was also tested. A set of multiple copies of each crowded system with and without PEG aggregates was used to estimate the fractional available volumes for the probe molecules.

#### 4.2.1 Efficiency comparison of two MC algorithms

Two MC methods namely parallel-energy and parallel-distance algorithms were developed to determine whether there is a steric clash or not on placing a molecule in the crowded medium at a random place and orientation. This information was used to determine the fractional available volumes in the crowded medium from the ratio of accepted to total number of trials. In these methods the steric clash is determined based on the atomic distances or energetic threshold criteria. The parallel-distance algorithm used the van der Waals atomic radii [60, 193] to determine the steric clashes whereas the parallel-energy algorithm used an energy threshold of 50,000 kcal/mol ( $\sim$ 200,000 kJ/mol). The energetic criterion was found by trial and error. It was observed that the system with a successful insertion has total energy less than 1000 kcal/mol ( $\sim$ 4200 kJ/mol). However, in certain cases, it was found that the probe molecules are placed in a close vicinity of PEG molecules without steric clashes resulting in higher total energies of 5000–15,000 kcal/mol ( $\sim$ 20,000–62,000 kJ/mol). The

ten times higher threshold energy of 50,000 kcal/mol was chosen to avoid false negatives, i.e. cases where there are not any steric clashes even though the energy is high, but it might be possible the simulations produced false positives with clashes that fall under the energy threshold.

In these two algorithms, computation cost depends on the concentration and type of calculations, i.e. total energies or van der Waals distance calculations. The parallel-energy algorithm was found to be faster than the parallel-distance algorithm. The parallel-energy algorithm estimated the total energy of the whole system at once using a built-in, highly optimized energy function. However, the parallel-distance algorithm performed distance measuring calculations between all atoms of probe and crowder molecules in each insertion trial through many loops. Since the 8 kDa PEG is fairly large, the algorithm spent a lot of time on this stage to determine a steric clash by measuring the atomic distances.

The computational cost of the parallel distance algorithm was reduced by defining additional criteria for determining the steric clashes. One additional criterion eliminated all the background molecules from the steric clash measurement whose centres are separated by more than 60 Å. This radius encapsulated the whole probe molecule and would differ with the size of the probe molecule. Similarly, the additional criterion reported a steric clash instantly if there is any background molecule found within a centre-to-centre distance of 10 Å of the probe molecule. The parallel-distance algorithm with these additional criteria reported a steric clash quickly and eliminates the extensive loop calculations. These threshold values were picked using trial and error considering the shapes and sizes of PEG molecules. Although the parallel-distance algorithm with additional criteria was found to be still expensive but it can give the correct fractional available volume given that it does not involve any approximations.

The parallel-distance algorithm with the additional criteria is computationally expensive but estimated the fractional available volumes more accurately than parallel-energy algorithm. A comparison of the fractional available volumes for the pseudoknot RNA (PDB

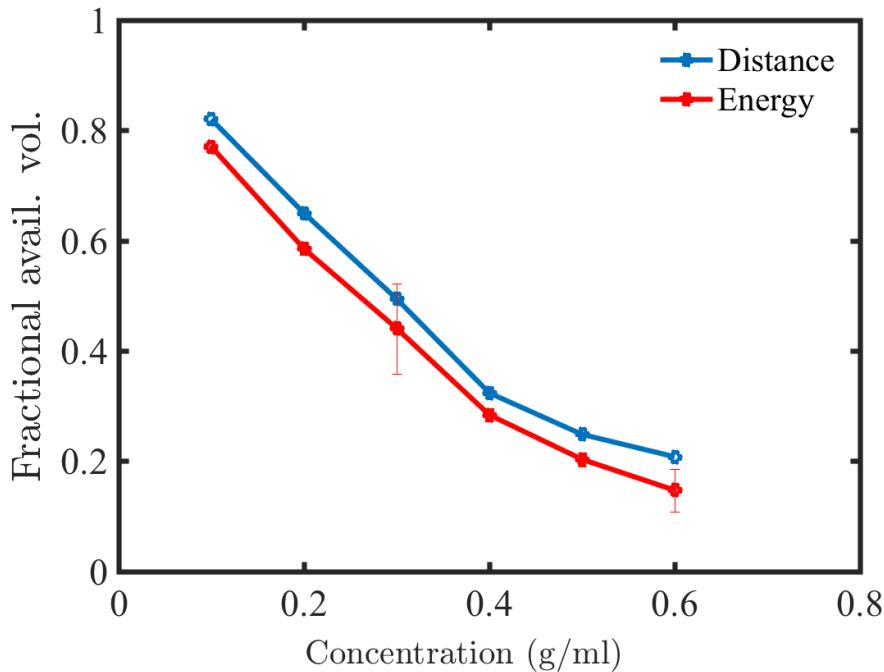


Figure 4.1: The comparison of the parallel distance and energy algorithms in measuring the fractional available volumes for the pseudoknot RNA conformation. The abscissa axis shows the fractional available volume and the ordinate is the concentration of PEG from 0.1 to 0.6 g cm<sup>-3</sup> in the simulation box. Thirty copies of the simulation box at each concentration were used in the parallel-energy algorithm while a single copy was used in the parallel-distance algorithm with the additional criteria to speed up the calculation. The parallel-distance algorithm produced fractional available volumes approximately 5 percent higher than the parallel-energy algorithm.

ID: 2K96) is given in Figure 4.1, using both methods. The results showed a good agreement between both algorithms. The energy-based calculations showed a little less fractional available volumes than the parallel-distance method. The fractional available volumes were lower with the parallel-energy algorithm because these are average values over fifteen boxes while only a single copy was used in the parallel-distance algorithm. The reason to use a single copy of a simulation box was the computational time where the parallel-distance algorithm took an extreme amount of time. On average, the parallel-distance algorithm spends more real time on a single system of the minimum concentration than the parallel-energy algorithm that completes the same number of trials on all fifteen systems of the minimum concentration. The computational cost in both algorithms increased with con-

centration. The parallel-distances algorithm spent almost 80 hours to complete 12,000 insertion trials for the  $0.1 \text{ g cm}^{-3}$  system whereas the parallel-energy algorithm completed the same task in about half an hour using the same computer resources. Hereafter, the calculations of the fractional available volume in the simulation boxes were performed using the parallel-energy algorithm due to the lower computational cost.

#### 4.2.2 Effect of PEG aggregation

The fraction of free volume for three pairs of macromolecules was estimated in the non-equilibrated simulation boxes. The simulation boxes were further equilibrated by MD simulations to observe the high concentration effects on the PEG folding and aggregation behaviour. The fraction of available volume was increased by 20 percent on equilibrating the simulation boxes (Figure 4.2). The significant increase in the fractional available volume was due to clustering and formation of very compact conformations of PEG molecules during the MD simulations. These MD simulations were performed using forcefield parameters of methyl-PEG and hydroxyethyl-PEG (Figure 4.4). We had seen in section 4.2.3 that PEG formed very compact structures during the MD simulations. The QM optimized structures confirmed the formation of intra- and inter-molecular hydrogen bonding which induced such folding and clustering behaviour.

Additional simulations were performed to investigate the effect of aggregation on the fractional available volumes by dissolving the aggregates. The PEG aggregates were dissolved by performing a two-step MC simulation. In the first step, rigid body MC simulations were performed using three systems of  $0.1$ ,  $0.3$ , and  $0.5 \text{ g cm}^{-3}$  concentration. An extreme temperature of  $298,000 \text{ K}$  was set in the simulations to perform rotational and translational moves at large magnitude to dissolve the clusters quickly. These simulations were performed in Sire and the final configurations were saved after every 2500 MC moves. The values for each rotational and translational move were picked randomly between -50 to +50 degrees and -50 to +50 Å respectively. Finally, the resultant systems were further

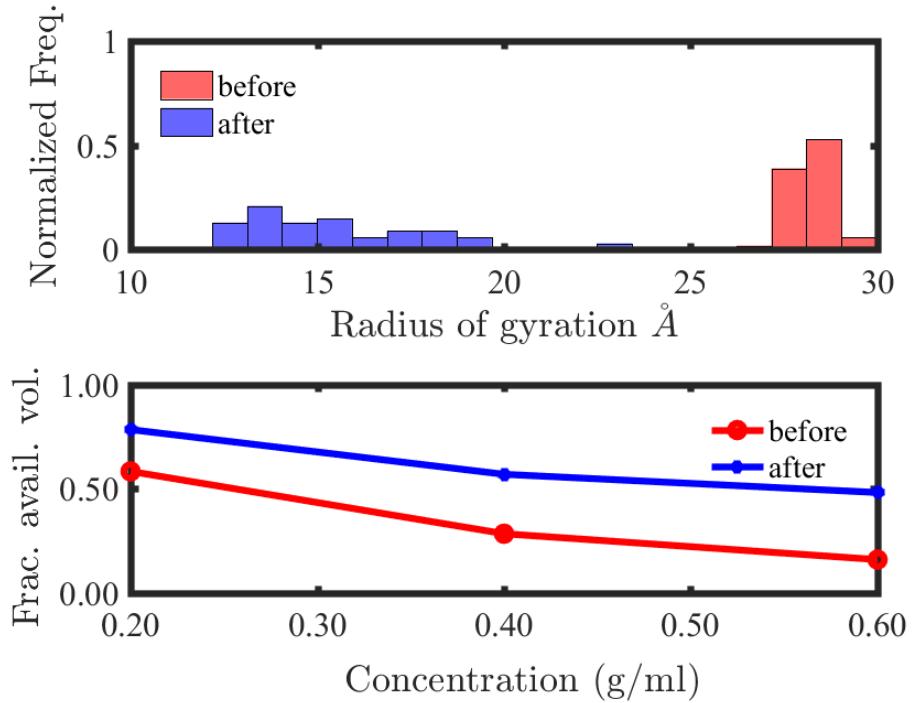


Figure 4.2: The size distribution of PEG conformations, in the system of  $0.1 \text{ g cm}^{-3}$  concentration, before and after the equilibration by performing the MD simulations (top). PEG formed more compact conformations in the range of 12 to 20 Å from 27 to 30 Å, after equilibrating the system. The bottom panel illustrated the significant increase in the fractional available volumes to the probe pseudoknot RNA (PDB:2K96) molecule in the equilibrated systems. The formation of compact PEG conformations along with PEG aggregates induced a significant increase in the fractional available volumes. Further, the clustering trend increases as a function of concentration and consequently the fraction available volume increased by a higher percentage at larger concentrations.

equilibrated by conducting MC simulations at 298 K to refine structures produced at an extreme temperature.

After removing the aggregates, the fractional available volume was estimated using the parallel-energy algorithm. The fraction of available volume decreased with the removal of PEG aggregates as illustrated in Figure 4.3. De-aggregation allows the molecules to disperse well in the given space and, consequently reduced the occurrence of large empty spaces. These results demonstrate that the packing of the background molecules including their sizes and shapes is also crucial in altering the fractional available volumes.

In another set of simulations, additional MD equilibration simulations were performed

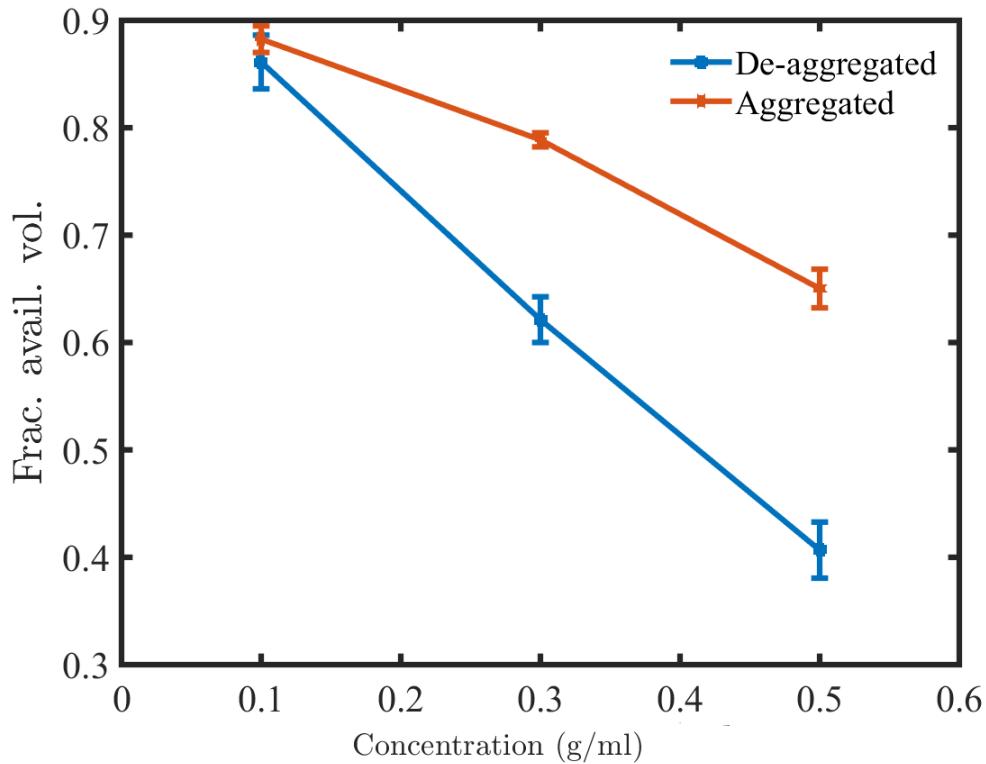


Figure 4.3: Effect of aggregation on the fractional available volume for the pseudoknot RNA (PDB:2K96) at three concentrations. The systems were equilibrated by performing MD simulations and the fractional available volumes were computed by implementing the MC method using the parallel-energy algorithm. In the second step, the aggregates were destroyed by performing MC simulations and then the fractional available volume was measured.

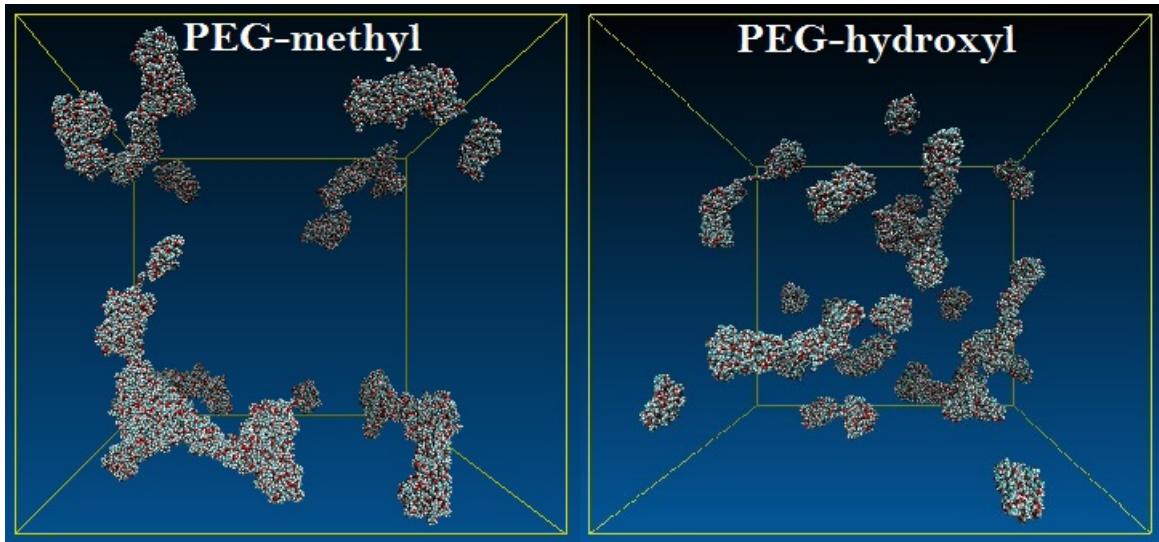


Figure 4.4: Effect of methyl and hydroxyl terminal groups on the folding of PEG and the formation of PEG aggregates in the MD equilibration simulations.

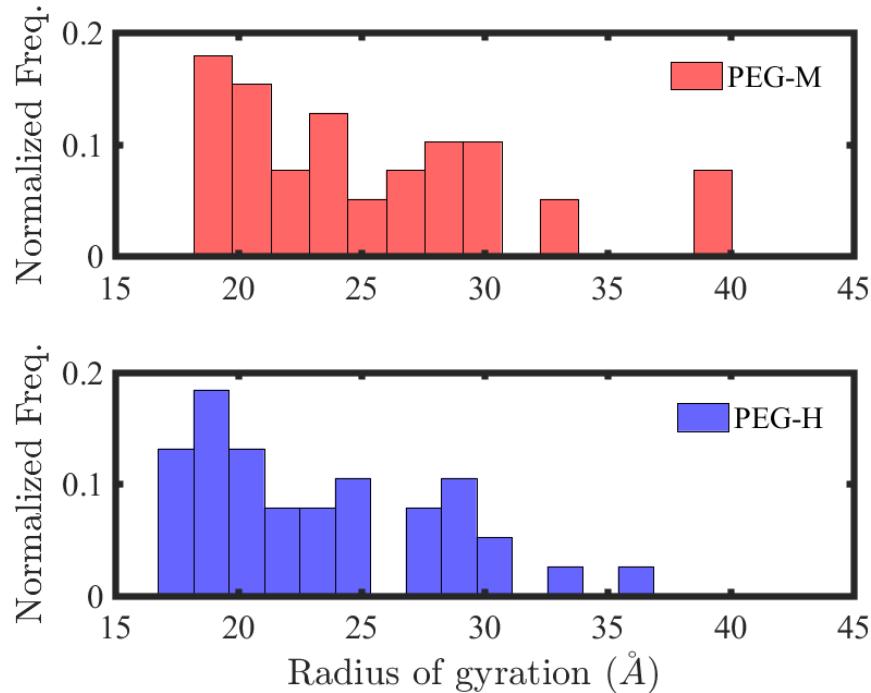


Figure 4.5: Size distribution of PEG aggregates after MD equilibration using forcefield parameters of PEG-methyl (PEG-M) and PEG-hydroxyl (PEG-H) terminals. PEG-H formed smaller sized clusters with a larger amount of individual molecules as compared to PEG-M.

to examine the effect of forcefield parameters on the aggregation behaviour of PEG molecules. It was found that the terminal groups did not play any significant role in affecting the folding behaviour of PEG in the MD sampling simulations and PEGs with methyl, hydroxethyl and propyl terminals formed aggregates in the equilibration step. Here, additional MD equilibration simulations were performed on the three simulation boxes at  $0.2 \text{ g cm}^{-3}$  concentration that were packed with PEGs with hydroxyl terminals. To perform the MD equilibration simulations quickly and to observe any aggregation behaviour, an intermediate concentration of  $0.2 \text{ g cm}^{-3}$  was selected. The simulation results revealed that PEGs with hydroxyl terminals showed similar folding and aggregation behaviour to the PEG with methyl and propyl terminals. Moreover, these results showed that the presence of PEG molecules in the solution affects the folding and aggregation behaviour through intra- and inter-molecular hydrogen bonding interactions. The size of PEG aggregates was found to be similar in both cases (Figure 4.5) and the terminal groups also do not have a large effect

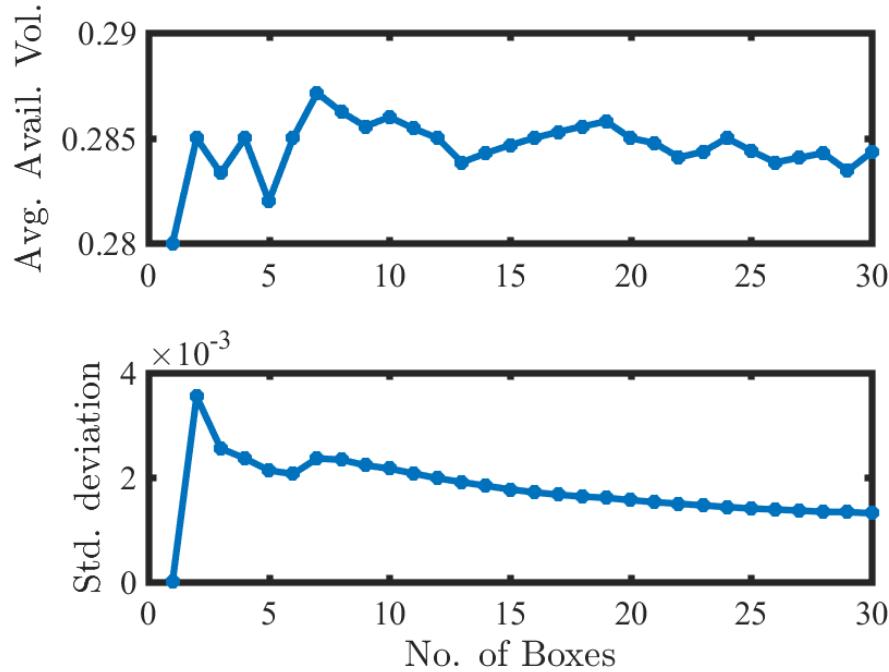


Figure 4.6: The estimated average fractional available volume and the standard deviation with respect to number of copies at  $0.4 \text{ g cm}^{-3}$  concentration. The fraction of available volumes was estimated using the MC simulation method with an energy threshold algorithm.

on the size distribution of PEG aggregates. However, the PEG-H forcefield formed a little bit smaller sized aggregates with a larger fraction of compact individual molecules as compared to the PEG-M. It is found that the PEG-H formed stronger intra-molecular hydrogen bonds as compared to PEG-M which leads to the formation of smaller sized individual molecules.

#### 4.2.3 Statistical analysis

Thirty copies of simulation boxes at each concentration were used to compute the fraction of available volume. These copies of simulation boxes were prepared after equilibrating the packed simulation box using MD simulation. The idea of making thirty copies is to represent the diverse configurations at any given concentration. The change of fractional available volumes and corresponding standard deviation are shown in Figure 4.6 at  $0.4 \text{ g cm}^{-3}$ . The results indicate that thirty copies at each concentrations were sufficient to

converge the average of fractional available volume and standard deviation. The average fractional available volume and corresponding standard deviation stabilized after completing fifteen boxes approximately. Moreover, the size of the standard deviation obtained with fifteen boxes or more showed that sampling of this size range produced three significant figures in the fractional available volume which is really excellent and suggested that it might be reasonable to use fifteen copies to produce statistically accurate results.

The MC method was applied to estimate the fractional available volumes in the non-equilibrated and equilibrated crowded media. Similar calculations were performed using the extended SPT theory model and the results from both models are discussed in section 4.4.

### 4.3 The scaled particle theory

A scaled particle theory (SPT) model has been used to compute the nonideal (NI) chemical potential contribution due to the excluded volume effect of the solute particles in a crowded medium as a function of concentration [4, 7, 27–29, 83, 158, 263]. The NI chemical potential varies as a function of excluded volume which depends on the geometry and number density of molecules in the SPT framework. SPT as it currently exists requires three geometrical parameters namely, radius of curvature  $R$ , surface area  $S$ , and volume  $V$ , but there are certainly shapes, especially shapes of macromolecules, for which these parameters are not a complete description. Moreover, the SPT model currently applies to convex shapes only. Previous SPT models [12, 83, 146–148, 154, 156, 158] approximated the geometry of fluid particles as simple hard spheres or convex shapes. The calculations showed that the geometry played a very crucial role in changing the thermodynamics. Therefore, it is very important to calculate the geometrical parameters of macromolecules correctly in order to estimate the excluded volume effects effectively. One of the key objectives of this study was to build a more reliable model that could extract geometrical parameters of complex-shaped macromolecules effectively rather than using models with

Table 4.1: Comparison of predicted radius of curvature in the original and extended scaled particle theory models. The radius of curvature, measured in Å, is the half of the distance between the atoms present at the maximum distance in the original scaled particle theory while the extended scaled particle theory represents the radius of a least square fit sphere on the convex hull. All the molecules are represented by their respective PDB numbers except the PEG1 and PEG2 conformations. The PEG1 and PEG2 conformations are structures taken from the stationary regions in the MC and MD simulations respectively (Figure 4.7).

Molecule	Original SPT	Extended SPT
2K96 [120]	33.45	28.53
1NA2 [119]	37.52	32.73
1AKE [122]	25.96	22.78
4AKE [64]	30.89	25.41
2PE5 [129]	43.53	30.71
2P9H [129]	27.91	24.72
PEG1	45.78	36.16
PEG2	20.55	17.51
1HHO [264]	28.84	26.13
1FFK [265]	112.21	102.32

simplified geometrical approximation of simple hard spheres or convex shapes. To achieve this, a convexification approach was introduced to estimate the geometrical parameters of the macromolecules using the atomic coordinates. This section presents results from the original and the new extended SPT models.

#### 4.3.1 Geometric parameters by the original and extended SPT models

The excluded volumes and subsequently NI chemical potentials are influenced by the shape, size, and number density of molecules present in the medium. To take into account the size and shape contribution, it is important to extract the geometrical parameters of all solution particles accurately. Two SPT models, i.e. the original and the extended SPT models, were used to measure the geometrical parameters. In the original SPT model, the radius of curvature represents half of the distance between two boundary points that can form a sphere encapsulating the whole molecule. These boundary points are located at maximum distance from each other. The surface area and the volume were calculated from

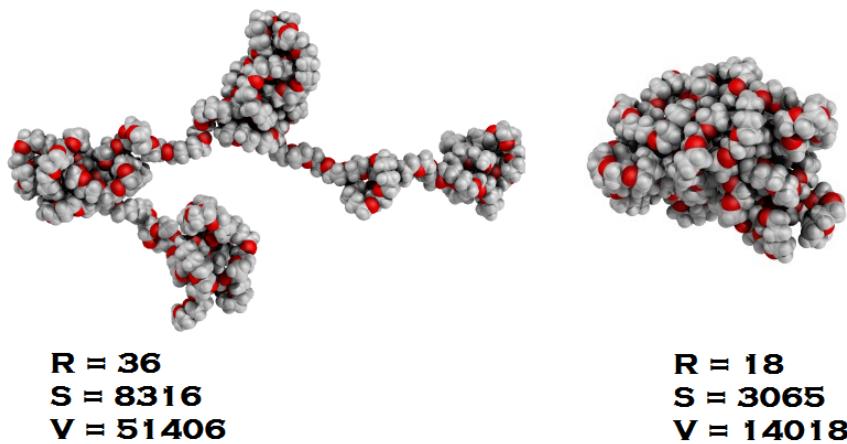


Figure 4.7: Geometrical parameters  $R$ ,  $S$ ,  $V$  of PEG1 (left) and PEG2 (right) measured in Å, Å<sup>2</sup>, and Å<sup>3</sup> units respectively, using the extended SPT model. The red colour illustrated the oxygen atoms while grey colour represented the hydrogen and carbon atoms.

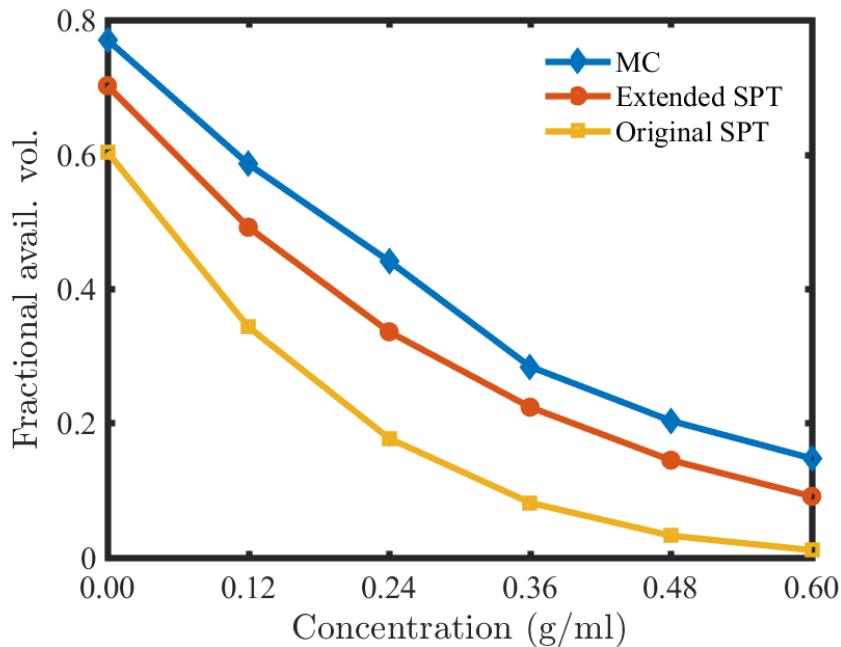


Figure 4.8: Fractional available volume for the pseudoknot RNA closed conformation (PDB:2K96) in the presence of PEG2 crowders. The original SPT model overestimated the sizes of crowders and probe molecules and thus predicted the sharp decrease in the fractional available volume and showed almost zero available volume at 0.6 g cm<sup>-3</sup> concentration. On the other hand, the fraction of available volume decreased slowly in the extended SPT model relative to the original SPT model and produced results in better agreement with MC calculations.

the formulas for a sphere. On the other hand, an extended SPT model measured the radius of curvature by generating the least squares fitted sphere to a convex hull. The surface area and volume were measured by taking the sum of the areas of the triangles present on the surface of the convex hull and taking the sum of the volumes of all the tetrahedra in the convex hull respectively.

The radius of curvature for ten macromolecules is tabulated in Table 4.1 using the original and extended SPT approaches. All the structures were represented using a PDB identification number except PEG1 and PEG2. Both PEG1 and PEG2 structures were taken from the stationary state in the MC and MD simulations respectively (Figure 4.7). The original model predicted a larger radius of curvature than the extended model generally. Consequently, the surface areas and volumes showed larger values in the original model than in the extended model. The radius of curvature for spherical molecules is similar using either method of calculation as we can see in the cases of the 1AKE, 2P9H, PEG2, 1HHO, and 1FFK molecules. However, non-spherical and extended shapes showed a large discrepancy in radius of curvature as given for PEG1.

Both models worked fine for compact and globular shaped molecules. To illustrate the effect of shapes, fractional available volumes for the pseudoknot RNA (closed conformation PDB:2K96) were calculated in the presence of PEG2 using the original and extended SPT models (Figure 4.8). The very compact and spherical shapes of 2K96, and PEG2 conformations made them good candidates to use in both models to investigate the effect of geometrical parameters on the fractional available volume. As expected, lower fractional available volumes were predicted in the original SPT model due to larger geometrical parameters. The fractional available volume decreased quickly and eventually reached to zero after reaching the highest concentration of PEG using the original model.

### 4.3.2 Sensitivity of the SPT calculations

The SPT model estimates the NI contribution to the chemical potential due to crowding as a function of the geometrical parameters of probe and crowder molecules. A sensitivity analysis of the extended SPT model with respect to the geometrical parameters was performed by observing the changes in the chemical potentials by increasing the geometrical parameters of a single conformation of PEG, i.e. radius of curvature, surface area and volume, starting from the minimum to the mean value of each parameter at the different constant concentrations. The radius of curvature and surface area can be increased up to the maximum level, however the increment in the volume results in zero free space in the medium before reaching the maximum value of the volume. There was no available space left after reaching a concentration of  $0.3 \text{ g cm}^{-3}$  while increasing the volume to the maximum level (Figure 4.9). Therefore, the mean values were used for all calculations. The mean values of all three geometrical parameters are estimated by taking the average of geometrical parameters of one thousand conformations in the library. These minimum, average and maximum values are helpful to have a realistic range in which the geometrical parameters could be varied to assess the effect of changes in the geometrical parameters on the chemical potentials (Table 4.2). A series of calculations was performed using PEG2 crowders with the pseudoknot (2K96) probe molecule where the geometrical parameters are increased to average values. Both RNA pseudoknot conformation and crowders differ in size significantly from each other (Table 4.1). The results demonstrated that the radius of curvature and volume are likely to affect the chemical potential more than surface area by increasing the geometrical parameters by 50, 26 and 40% relative to the size of the PEG2 crowder (Figure 4.10).

The type of geometrical parameter affects the chemical potential to a different extent. For example, the chemical potential increased approximately linearly with respect to the radius of curvature with a slope of  $0.12 \text{ \AA}^{-1}$  (Figure 4.11), whereas the chemical potential also increased, but with significant curvature in the plots, as the volume and surface area

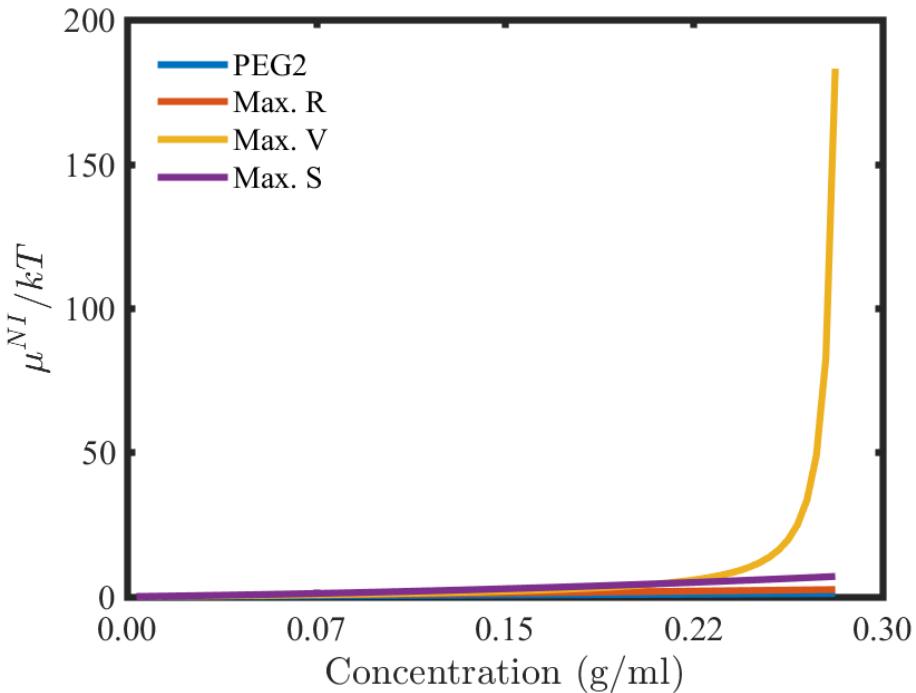


Figure 4.9: Effect of geometrical parameters of PEG2 on the chemical potential of the pseudoknot RNA (2K96) by increasing the  $R$ ,  $S$ , and  $V$  of PEG2 crowders to the maximum magnitude of the original size of PEG2, using the extended SPT model.

Table 4.2: Geometrical parameters  $R$ ,  $S$ ,  $V$  of one thousand conformations of PEG measured in Å, Å<sup>2</sup>, and Å<sup>3</sup> units respectively, using the extended SPT model.

Quantity	Radius	Surface area	Volume
<b>Mean</b>	31.41	7300	49507
<b>Minimum</b>	15.14	2704	11833
<b>Maximum</b>	59.59	30960	353070

increase (Figures 4.12 & 4.13). It was found that the chemical potentials tend to increase approximately linearly at first but then increased sharply in more concentrated solutions, e.g. at 0.2 g cm<sup>-3</sup> (Figures 4.14, 4.15 & 4.16). Again, under higher concentration conditions at 0.2 g cm<sup>-3</sup>, the volume was found to be most effective to influence the rate of change of the chemical potentials as compared to the radius of curvature and the surface area.

The dependence of the chemical potential on the crowder geometry for realistic crowder geometries drawn from six different PEG conformations in the library was also inves-

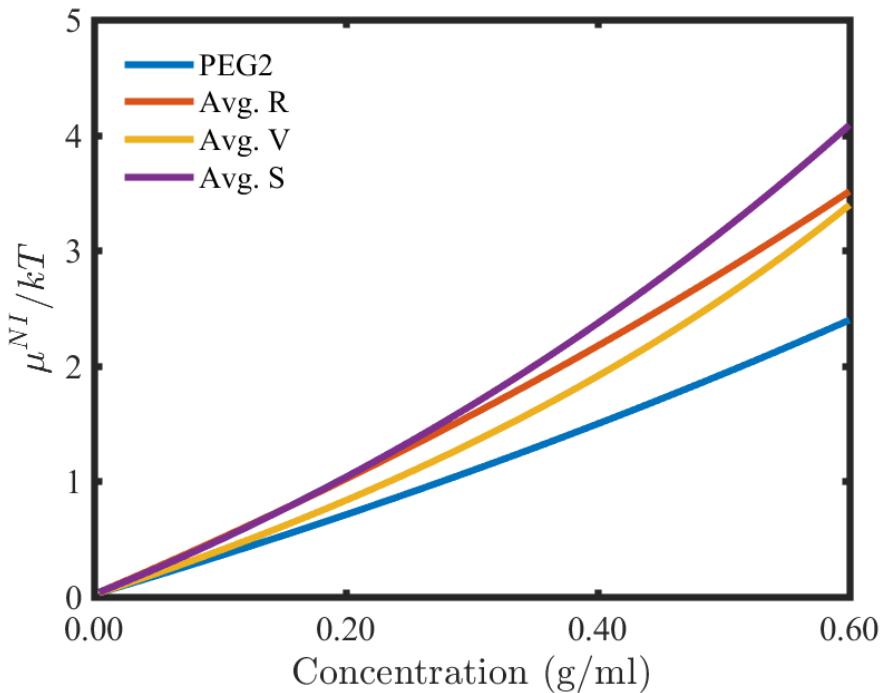


Figure 4.10: Effect of geometrical parameters of PEG2 on the chemical potential of the pseudoknot RNA (2K96) by increasing the  $R$ ,  $S$ , and  $V$  of PEG2 crowders by 50, 26 and 40% of the original size of PEG2, using the extended SPT model.

tigated. A set of six random conformations were chosen from the stationary region of MD simulations and the corresponding geometrical parameters are tabulated in Table 4.3. The calculations showed that chemical potentials vary significantly at higher concentrations depending on the size and shape of the crowder (Figure 4.17). These conformations contribute differently to the chemical potential and therefore it can be concluded that use of a single conformation in the SPT model could be a poor choice to mimic the cellular medium which is populated with biomolecules of different sizes and shapes [3]. The importance of the construction of the PEG conformational ensembles can also be explained by analysing the change in the chemical potentials for different conformations with respect to the radius of curvature (Figure 4.18). The chemical potentials of conformations increase approximately directly with respect to the radius of curvature up to 20 Å at 0.1 g cm<sup>-3</sup> concentration of solution. The chemical potentials varied at small magnitudes for conformations with radius of curvature around 20 Å, whereas the chemical potentials differ significantly for confor-

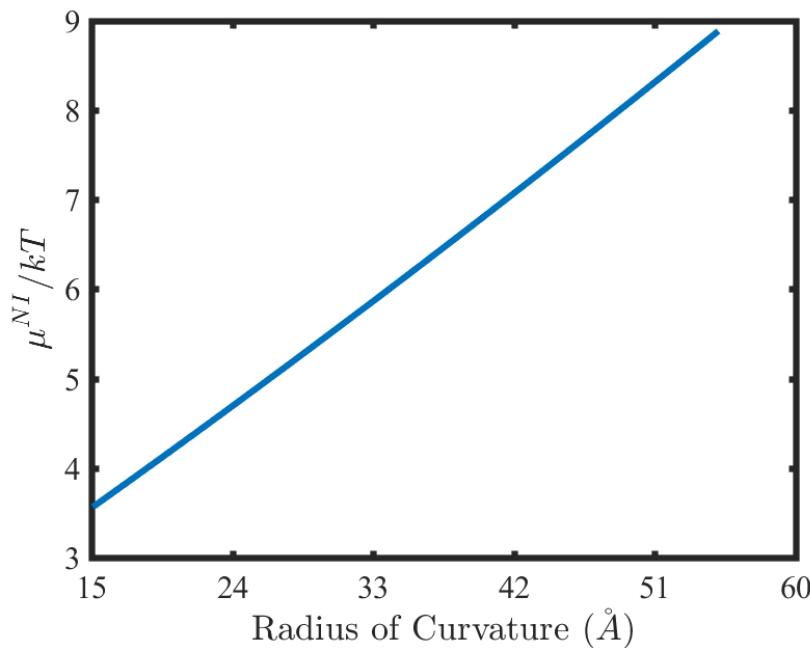


Figure 4.11: The nonideal contribution to the chemical potential of the pseudoknot RNA (2K96) due to increase in the radius of curvature of a single conformation of PEG at constant surface area ( $2904 \text{ \AA}^2$ ) and volume ( $12966 \text{ \AA}^3$ ) at constant concentration of  $0.1 \text{ g cm}^{-3}$ .

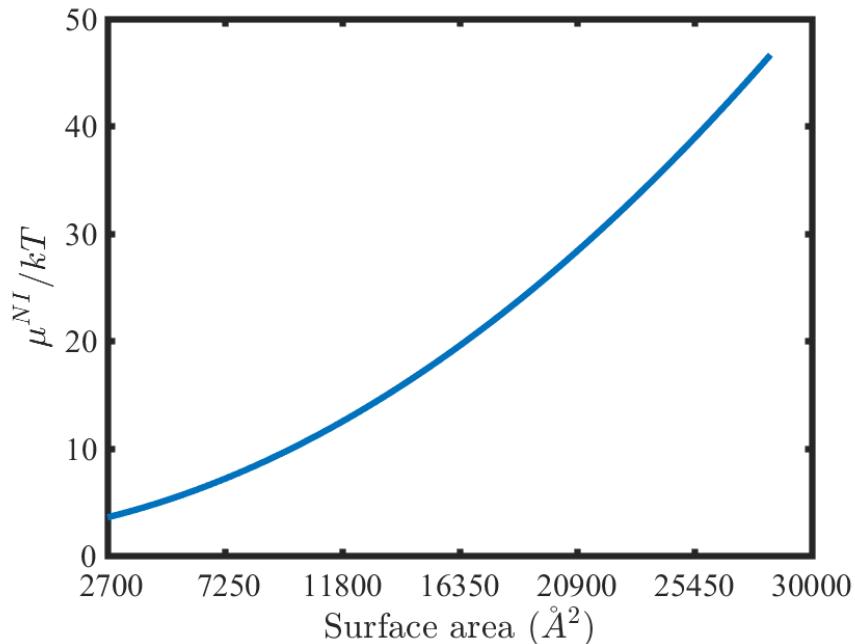


Figure 4.12: The nonideal contribution to the chemical potential of the pseudoknot RNA (2K96) due to increase in the surface area from of a single conformation of PEG at constant radius of curvature ( $16 \text{ \AA}$ ) and volume ( $12966 \text{ \AA}^3$ ) at constant concentration of  $0.1 \text{ g cm}^{-3}$ .

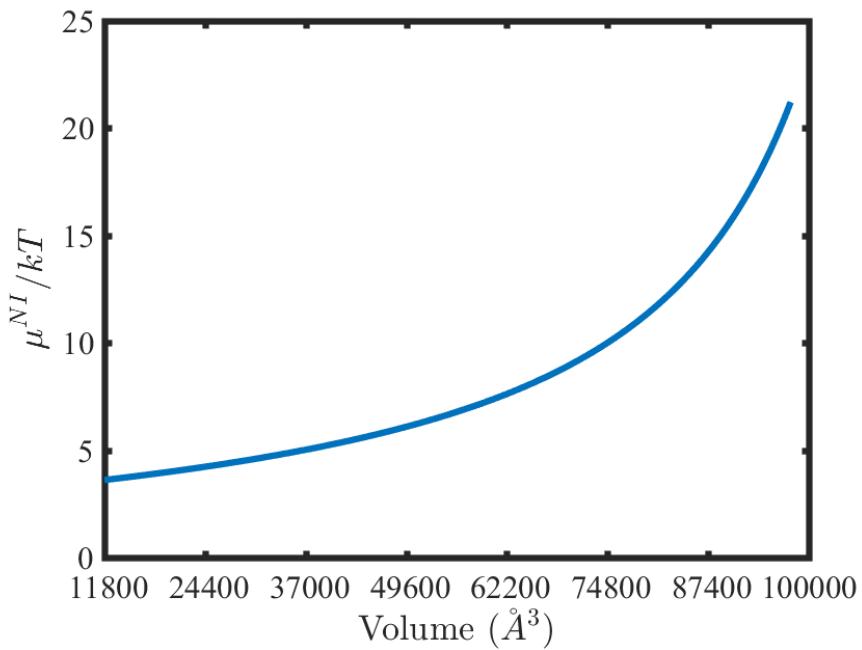


Figure 4.13: The nonideal contribution to the chemical potential of the pseudoknot RNA (2K96) due to increase in the volume of a single conformation of PEG at constant radius of curvature ( $16 \text{ \AA}$ ) and surface area ( $2904 \text{ \AA}^2$ ) at constant concentration of  $0.1 \text{ g cm}^{-3}$ .

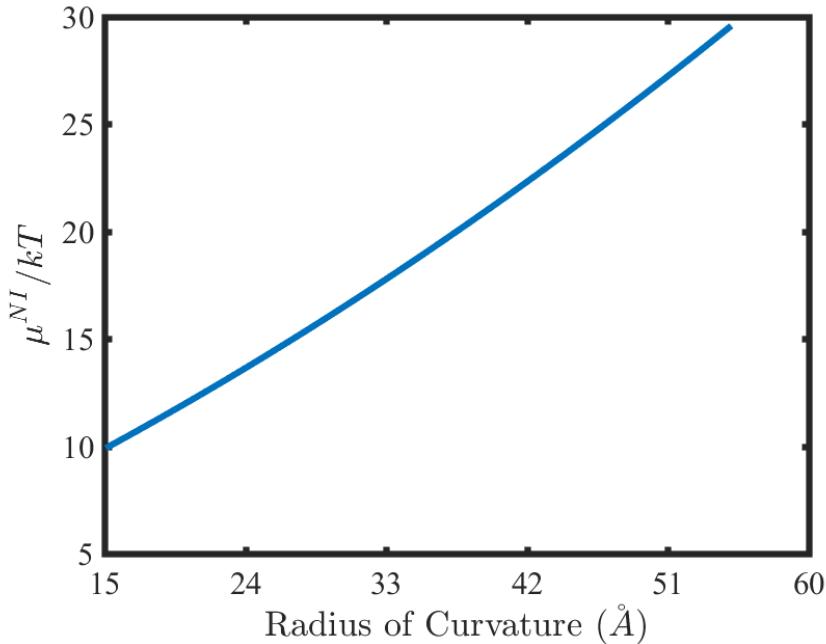


Figure 4.14: The nonideal contribution to the chemical potential of the pseudoknot RNA (2K96) due to increase in the radius of curvature of a single conformation of PEG at constant surface area ( $2904 \text{ \AA}^2$ ) and volume ( $12966 \text{ \AA}^3$ ) at constant concentration of  $0.2 \text{ g cm}^{-3}$ .

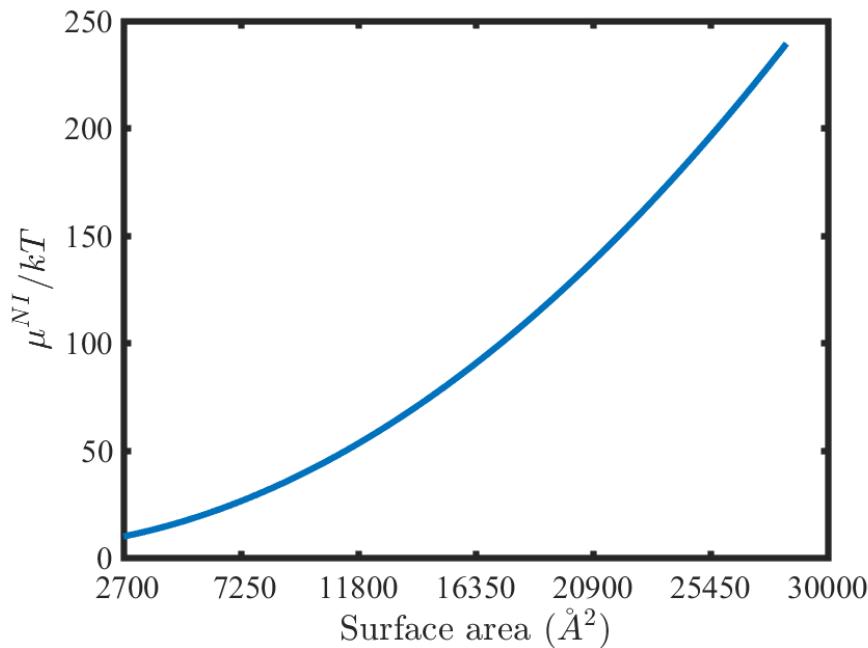


Figure 4.15: The nonideal contribution to the chemical potential of the pseudoknot RNA (2K96) due to increase in the surface area of a single conformation of PEG at constant radius of curvature ( $16 \text{ \AA}$ ) and volume ( $12966 \text{ \AA}^3$ ) at constant concentration of  $0.2 \text{ g cm}^{-3}$ .

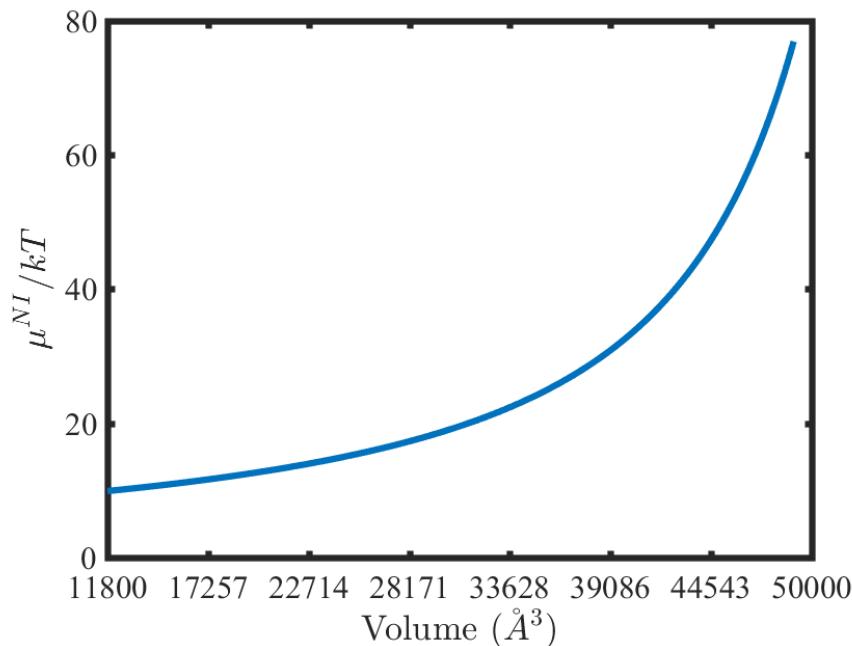


Figure 4.16: The nonideal contribution to the chemical potential of the pseudoknot RNA (2K96) due to increase in the volume from of a single conformation of PEG at constant radius of curvature ( $16 \text{ \AA}$ ) and surface area ( $2904 \text{ \AA}^2$ ) at constant concentration of  $0.2 \text{ g cm}^{-3}$ .

Table 4.3: Geometrical parameters  $R$ ,  $S$ ,  $V$  of six random equilibrated conformations of PEG measured in Å, Å<sup>2</sup>, and Å<sup>3</sup> units respectively, using the extended SPT model.

Conformation	Radius	Surface area	Volume
<b>PEG25</b>	30.04	5679	28975
<b>PEG75</b>	29.65	4613	22649
<b>PEG125</b>	20.35	3458	15462
<b>PEG281</b>	16.32	2955	13166
<b>PEG325</b>	18.16	3054	13368
<b>PEG450</b>	17.51	3065	14018

mations with radius of curvature greater than 25 Å.

This analysis showed that PEG conformations fall into two distinct regions based on their radius of curvature and the corresponding chemical potentials. Figure 4.18 points out an interesting feature that there are conformations in our library with similar radius of curvature that produce significantly different chemical potentials due to different surface areas and volumes. For instance, we chose all thirteen conformations with radius of curvature between 41–42 Å and all produced different chemical potentials ( $\mu^{\text{NI}}/kT$  from 2 to 10) due to different surface areas (from 4776 to 14241 Å<sup>2</sup>) and volumes (from 22780 to 119440 Å<sup>3</sup>). The volume is the most effective quantity to change the chemical potential in these calculations. These results strongly support that it would be appropriate to use an appropriately sampled conformational ensemble of crowders in SPT calculations to compute the nonideal contributions realistically. Further, it may be possible that the cells are populated with particles of similar radii but they could differ in the surface areas and volumes, or particles that have different shapes that differ in radius of curvature and surface area but have similar volumes. These variation in the geometrical parameters of different crowders produce additive effects and increase the chemical potentials significantly as compared to the medium filled with particle of the same sizes and shapes.

Finally, the sensitivity of the SPT model toward the geometrical parameters of a crowder was tested by computing the sensitivity coefficients [266]. The sensitivity coefficients determine the impact of a variable on the uncertainty of the output of a mathematical model. In

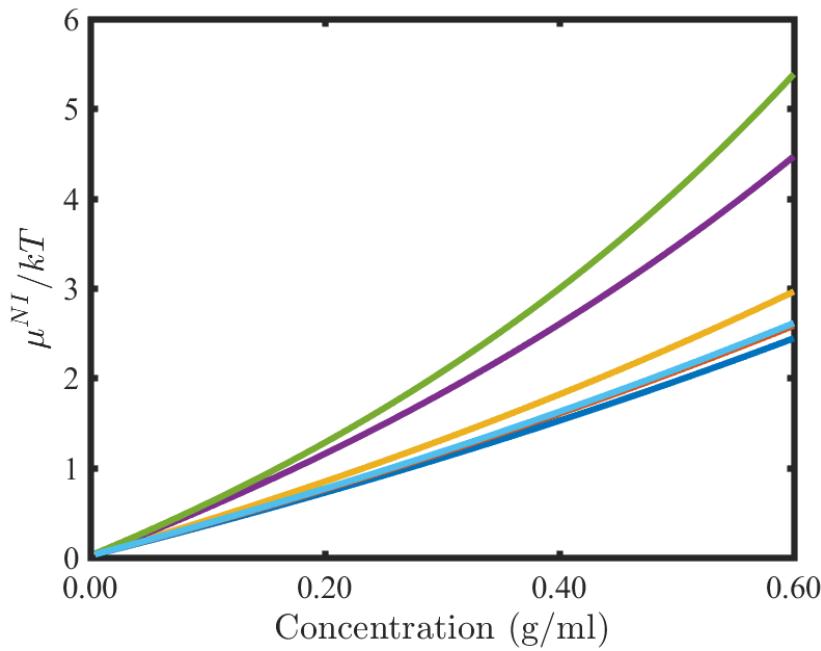


Figure 4.17: Comparisons of NI chemical potential contributions for the pseudoknot RNA (2K96) measured in the crowded boxes filled with 8 kDa PEGs of different conformations. All the six conformations were taken randomly from the stationary state of MD simulations.

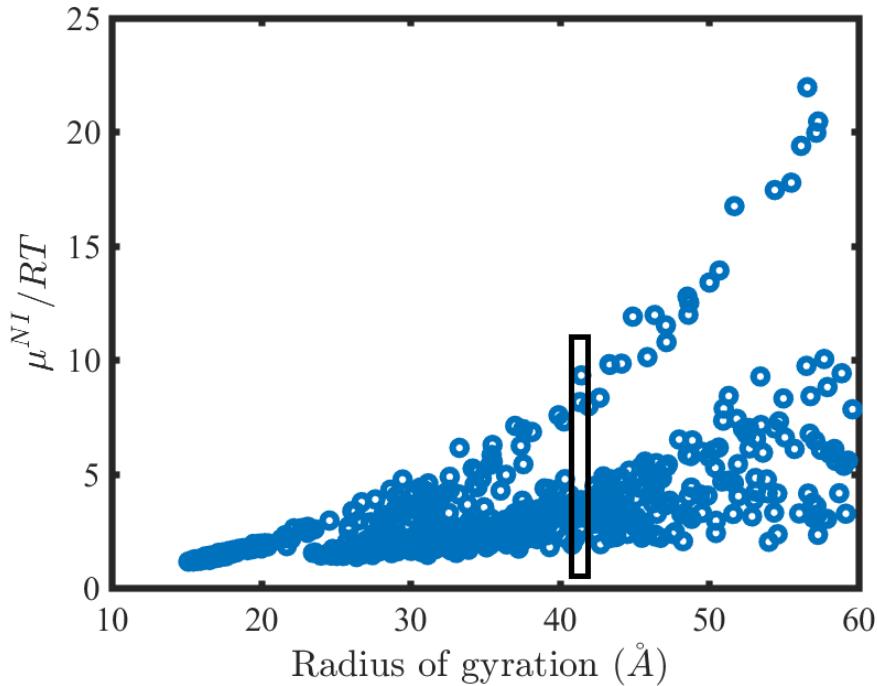


Figure 4.18: The nonideal chemical potential contributions of each conformation of PEG at a fixed concentration of  $0.1 \text{ g cm}^{-3}$  for one thousand PEG conformations. The inset rectangular shape is used to indicate the selected conformations that were used in the case study to evaluate the role of geometrical parameters in affecting the chemical potential of a protein molecule.

our case, the sensitivity coefficient is defined as a partial derivative of the fractional change in the available volume with respect to the fractional change in one of the three geometrical parameters, i.e. radius of curvature, surface area and volume. Mathematically, the sensitivity coefficient can be written in the form of equation 4.1 as a function of radius of curvature, surface area and volume. (All input files are provided in Appendix C.2.) Three sensitivity coefficients were measured by incorporating the small increments of 0.1, 1, 5, and 10% in the radius of curvature, surface area, and volume of the PEG crowder. The results regarding the change in the sensitivity coefficients with respect to radius of curvature, surface area and volume are shown in Figures 4.19– 4.21, and it was found that the sensitivity coefficient values are independent for small increments made in radius of curvature, surface area and volume of the crowder. Further, the sensitivity coefficients are found to be of the order of  $-1$  for the most concentrated systems, which means that fractional available volume is approximately inversely proportional to radius of curvature. In these calculations, volume was found to be the least effective geometrical parameters to affect the sensitivity coefficients at the lowest magnitude of the order of  $-0.3$  for the most concentrated systems. The sensitivity coefficients with respect to radius of curvature and surface area are consistently larger in magnitude than the sensitivity coefficient with respect to volume. It shows that a larger error in volume will not affect the results as badly as errors in the radius of curvature and surface area.

$$C_R^{\text{avail.vol.}} = \frac{\partial \ln(V_{\text{available}})}{\partial \ln(R)} \approx \frac{\ln(V_{\text{available},2}) - \ln(V_{\text{available},1})}{\ln(R_2) - \ln(R_1)} \quad (4.1)$$

$$C_S^{\text{avail.vol.}} = \frac{\partial \ln(V_{\text{available}})}{\partial \ln(S)} \approx \frac{\ln(V_{\text{available},2}) - \ln(V_{\text{available},1})}{\ln(S_2) - \ln(S_1)}$$

$$C_V^{\text{avail.vol.}} = \frac{\partial \ln(V_{\text{available}})}{\partial \ln(V)} \approx \frac{\ln(V_{\text{available},2}) - \ln(V_{\text{available},1})}{\ln(V_2) - \ln(V_1)}$$

This analysis indicates the dependence of the chemical potential on the geometrical parameters. Generally, the chemical potential was found to be more sensitive to the radius of curvature and surface area for smaller and compact shaped crowders but the chemical potential was found to be more influenced by the volume of the larger sized crowders. These calculations indicate that the chemical potential varies with the size and type of folding of crowders and it is crucial to estimate the geometrical parameters correctly in order to calculate the thermodynamic activity accurately.

The simple approximation of the original SPT overestimates the size of crowders as compared to the extended SPT model. Therefore, the fractional available volume decreased significantly as shown in Figure 4.8. Based on the results from the original and extended SPT models, we decided to use the extended SPT model to compute the fractional available volumes and NI chemical potentials at six different concentrations of crowded systems. These physical quantities are interchangeable and we presented our SPT results in terms of the chemical potentials for convenience in this chapter, while the corresponding plots on the fractional available volumes are available in Appendix C.3. This comparison between MC and SPT outcomes will allow us to benchmark the performance of our extended SPT model. Further, the biological significance of these calculations is discussed with reference to experimental results.

#### 4.4 Comparison of SPT and MC simulations

So far, we have discussed the development of MC and SPT models to compute the fractional available volumes of the probe molecule in crowded systems. A single type of crowders of the same size and shape were used to compute the fractional available volumes in the original and extended models in the previous section. The cellular interior is filled with different types of macromolecules that differ in shapes and sizes which affect the chemical potentials of probe molecules at higher magnitude due to an additive effect [24, 25]. To mimic the cellular environment more closely, a mixture of crowders of differ-

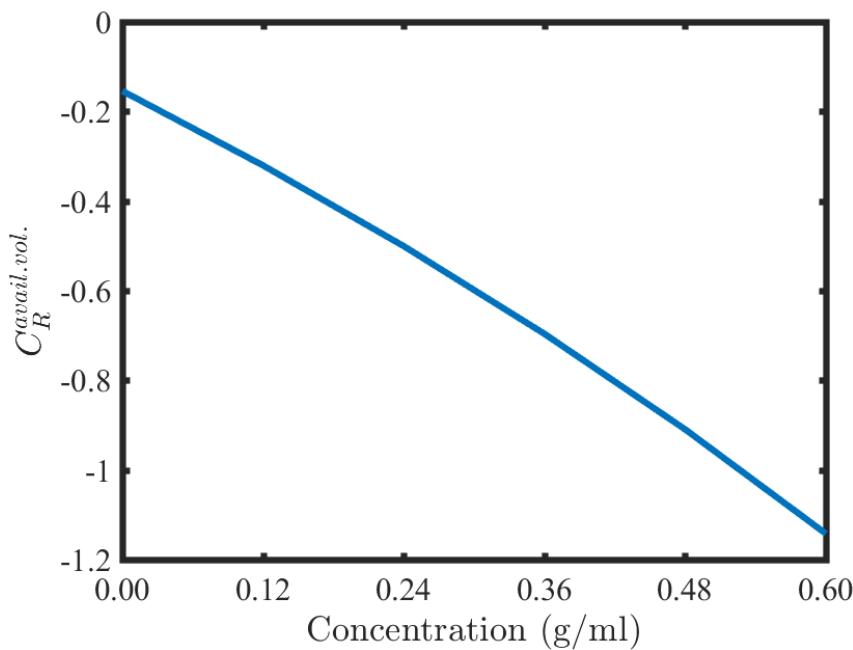


Figure 4.19: The sensivity coefficient for the fractional available volume with respect to radius of curvature of PEG2 crowder. The radius of curvature was increased by 0.1% from the original radius of curvature at six different concentrations from 0.1 to 0.6 g cm<sup>-3</sup>.

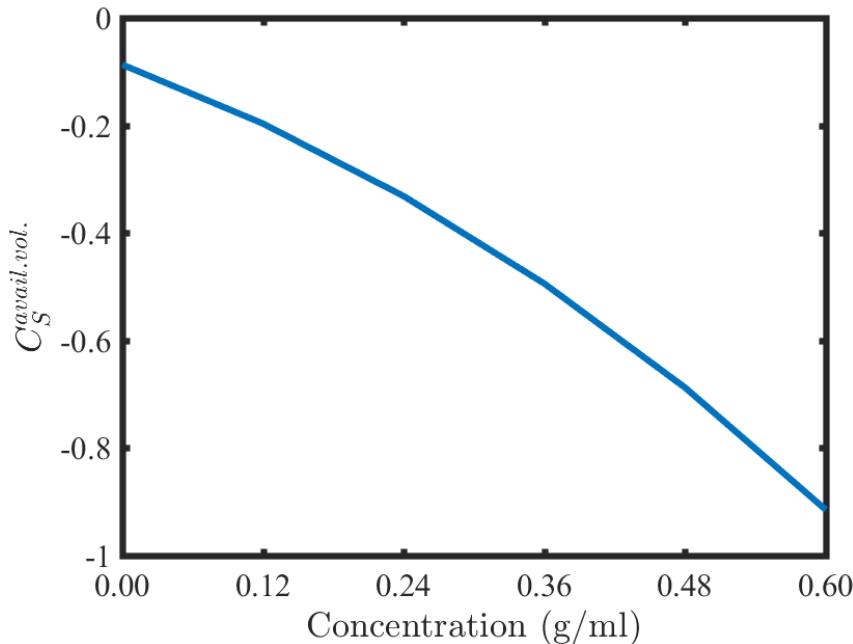


Figure 4.20: The sensivity coefficient for the fractional available volume with respect to surface area of PEG2 crowder. The surface area was increased by 0.1% to the original surface area at six different concentrations from 0.1 to 0.6 g cm<sup>-3</sup>.

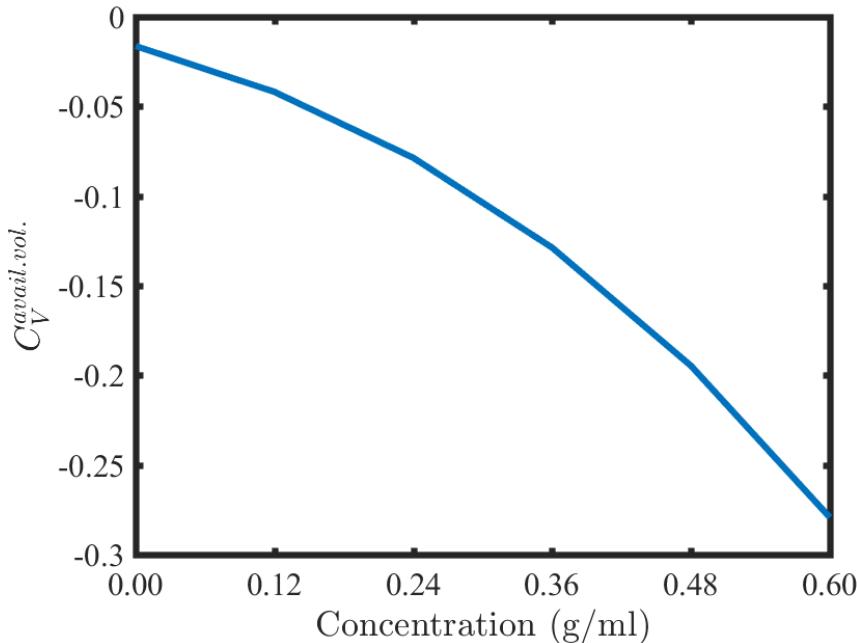


Figure 4.21: The sensivity coefficient for the fractional available volume with respect to volume of PEG2 crowder. The volume was increased by 0.1% to the orignal radius of curvature at six different concentrations from 0.1 to 0.6 g cm<sup>-3</sup>.

ent shapes and sizes has been used to pack the fifteen copies of simulation boxes at each concentration from 0.1 to 0.6 g cm<sup>-3</sup>. These simulation boxes were further equilibrated using MD simulations. Finally, these equilibrated simulation boxes were used to determine the fractional available volumes for six probe molecules by using both the extended SPT and MC methods.

It was found that the aggregation of PEGs increased the fractional available volumes significantly while using the MC method (Figure 4.2). The SPT model computes the fractional available volumes based on the geometries of individual molecules without considering the effect of aggregates. To incorporate the effect of aggregation on the fractional available volume, an algorithm was developed that extracts the aggregates and saves them as a single ‘molecule’ in a PDB format. This algorithm requires a simulation box and the threshold distance between two molecules as input parameters. The threshold distance was used to evaluate whether the surrounding PEGs are a part of a certain cluster or not. The algorithm completes the cluster search in two iterative steps. At first, it picks a molecule randomly

and finds all the surrounding molecules that satisfy the threshold distance criterion and saves them in list ‘A’. In the second step, it chooses each molecule from list ‘A’ and finds any additional surrounding PEGs within the threshold distance, excluding molecules that are already in List ‘A’. This cycle continues until there are no more molecules associated with the particular cluster, and the cluster is saved as a single ‘molecule’. The cluster extraction algorithm is provided in Appendix C.1. The geometric parameters of this molecule are extracted to be used in the extended SPT theory. A threshold of 20 Å was used to extract the PEG clusters. This threshold distance value was picked based on the average of the radius of gyration of all crowders (12 to 15 Å generally), present in the equilibrated boxes. The compact and tightly packed clusters were extracted from the boxes and were saved as a molecule by using this threshold radius of gyration. In this regard, the extended SPT theory is a better fit to the clusters that are at least roughly convex. Otherwise, the convex-hull algorithm overestimates the geometrical parameters of the molecules by encapsulating the large empty spaces inside the convex hull.

An extended SPT model was applied to compute the fractional available volumes and eventually chemical potentials for all six probe conformers with and without PEG aggregates in order to perform a comparison between MC and extended SPT model results (Figure 4.22). These results demonstrated that the MC simulations measured the least NI contribution of crowding towards the chemical potential of pseudoknot RNA (2K96) in the equilibrated systems (red line) as compared to non-equilibrated systems (blue line). The equilibration results in clusters, therefore it opens up lots of free space. The SPT model predicted higher values of chemical potential for pseudoknot RNA (2K96) while using the equilibrated crowded systems (dashed yellow line) as compared to the chemical potential using a single type of crowder (dashed green line). The higher chemical potential values with SPT in crowded systems filled with multiple conformations confirmed the additive contribution of multiple conformations over a single type of conformations. Note that the single crowder (PEG2) was selected from the stationary region in the MD simulations (Fig-

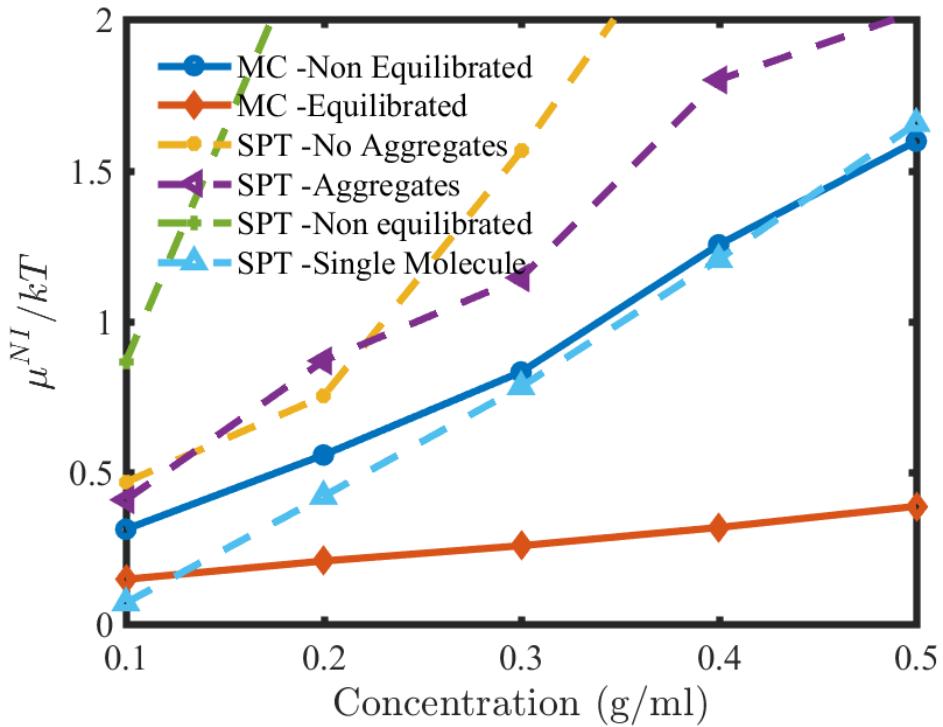


Figure 4.22: Comparisons of NI chemical potential contributions for pseudoknot RNA (2K96) measured in the crowded boxes filled with 8 kDa PEG by using the MC (solid lines) and extended SPT models (dashed lines). Only two types of crowded systems, i.e. non-equilibrated and equilibrated systems, were used to determine the chemical potentials using an MC method. The SPT approach used equilibrated systems only with and without aggregates, whereas ‘SPT Single Molecule’ represents the equilibrated single PEG conformation (PEG2) used to compute the chemical potential. The error bars of these simulations are small and not included for simplicity. For instance, the magnitude of error bars increased with concentration and the size of error bar was found to be less than 0.05 for a system of  $0.1 \text{ g cm}^{-3}$  concentration, whereas the size of error bar increased up to 0.01 for the concentrated systems i.e.  $0.5 \text{ g cm}^{-3}$ .

ure 4.7). The crowded systems were equilibrated with MD simulations resulting in compact and globular shaped crowders. To maintain consistency, the MD equilibrated PEG2 structure was chosen. A number of different MD equilibrated PEG conformations were also used to calculate the chemical potentials as compared to PEG2. It was found that most of the conformations produced different NI contributions to the chemical potentials of the pseudoknot RNA structure, which requires the use of multiple conformations to compute the average effect (Figure 4.17).

The SPT model reproduced the MC results while using a single equilibrated PEG struc-

ture where the chemical potential fit well at intermediate to higher concentrations but deviated at lower concentrations. However, this is not a fair comparison between MC results (solid blue line) and the extended SPT model (dashed green line) because both calculations used different crowded media. In this regard, MC used non-equilibrated crowded systems while the extended SPT used an equilibrated single PEG conformation. The extended SPT model predicted even larger chemical potential values in the non-equilibrated crowded systems. The results of rest of two systems are available in Appendix C.4.

#### 4.4.1 Biological and experimental relevance of results

Conformational changes of macromolecules in the cellular interior are linked to different functions [24]. Here we have investigated how crowding affects the conformational equilibrium of three systems with the MC and SPT models. Figure 4.23 shows plots of the first system, ‘the telomerase RNA’, by presenting the NI contribution to the standard state free energy change ( $\Delta G_{NI}/kT = (\mu_{2K96}^{NI} - \mu_{1NA2}^{NI})/kT$ ) of the conformational equilibrium as a function of concentration of PEG crowders. The change in energy values shows that the higher fractional available volumes are available for the compact shaped pseudoknot RNA conformation (2K96). Thus macromolecular crowding favours the formation of the pseudoknot conformation by shifting the conformational equilibrium ( $1NA2 \rightleftharpoons 2K96$ ) towards the pseudoknot RNA conformation over the hairpin conformation (1NA2).

The MC method as compared to the extended SPT model predicted a fairly small change of the standard state free energy of about  $-0.2kT$  and  $-0.7kT$  relative to zero crowder concentration for the conformational equilibrium ( $1NA2 \rightleftharpoons 2K96$ ) in the equilibrated and non-equilibrated crowded media respectively.

The MC method results showed the crowding exerts more modest effects on the conformational equilibria while the extended SPT predicted somewhat more significant crowding effects. The coarse-grained Langevin dynamics simulations [47] revealed that the pseudoknot conformation is in equilibrium with an extended hairpin conformation and that

crowding enhances the stability of the pseudoknot structure by  $-0.5kT$  in the presence of spherical crowders of 12 Å radius of gyration occupying 30% of the available volume relative to the hairpin structure. The extended SPT model also predicted the same amount of stabilization of about  $-0.5kT$  at 30% occupancy using a single type of crowder (PEG2) (dashed yellow line), however the MC method estimated a slightly smaller stability of  $-0.3kT$  roughly at 30% (solid blue line). The radius of curvature of 17.50 Å, estimated with convex hull, of PEG2 is larger than 12 Å but the volume ( $14018 \text{ \AA}^3$ ), estimated from the convex hull, is smaller than the value obtained using the sphere numerical formula with radius of 17.50 Å ( $22449 \text{ \AA}^3$ ). However, our model, like the coarse-grained Langevin dynamics simulations [47], could not capture the heterogeneity of the cellular environment fully in which the local concentrations and the sizes of crowders can vary from point to point [267]. The local concentration variations may result in minimal to strong crowding effects on the biochemical reactions [47].

The sizes of probe and background molecules played an important role in determining the magnitude of the crowding effect. The conformational changes in the adenylate kinase and the *lac* repressor structures also experienced modest crowding effects similar to the telomerase RNA in general. The closed conformation of adenylate kinase experienced the least crowding effect as compared to the closed conformations of telomerase RNA and the *lac* repressor. The closed states of adenylate kinase and the *lac* repressor are relatively stabilized by  $-0.5$  and  $-2.0kT$  respectively. Our results (Appendix C.5) are in good agreement with other studies which suggest the crowding exerts moderate effects on the conformational changes of proteins [125, 268, 269]. The postprocessing approach, developed by Dong *et al.* [24], predicted the closed conformation of adenylate kinase (1AKE) is stabilized by an amount of approximately  $-0.16kT$  at 35% occupied volume by the spherical crowders of 15 Å radius. The extended SPT model estimated approximately  $-0.2kT$  value in the presence of the PEG2 crowder at 35% which showed almost consistent results with the postprocessing approach outcomes.

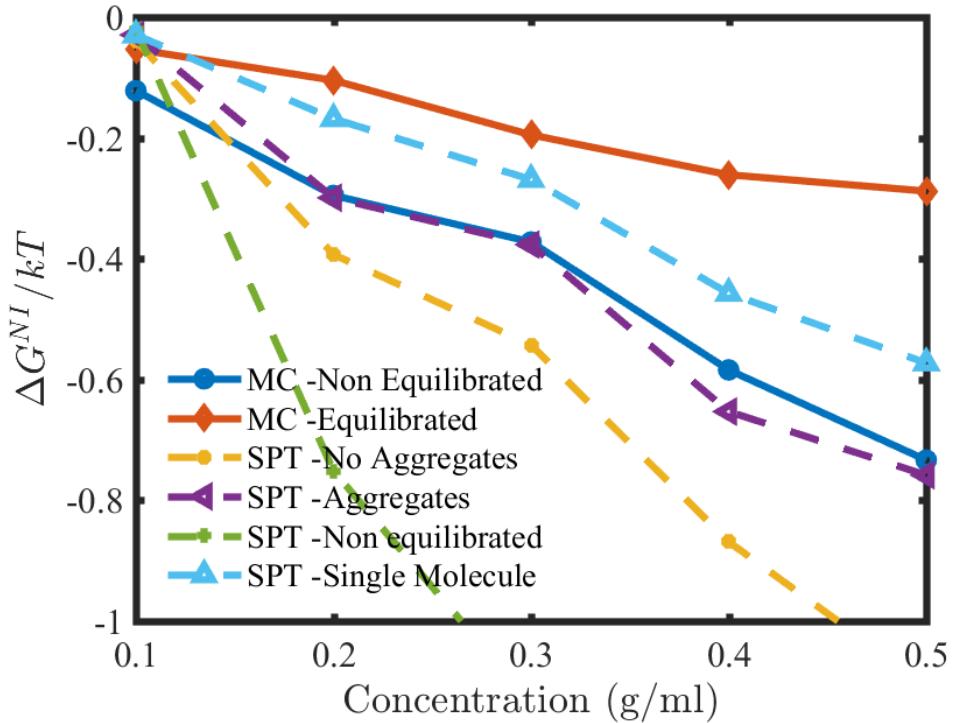


Figure 4.23: NI contribution to the standard state free energy change of conformational equilibrium between pseudoknot and hairpin conformers in the crowded medium (1NA2  $\rightleftharpoons$  2K96). Macromolecular crowding favors the formation of the compact conformation, i.e. pseudoknot conformation, of the telomerase RNA.

The SPT calculations were performed in different ways by using an equilibrated system (Figure 4.22) which differ in PEG aggregates (dashed yellow and violet lines). The results demonstrated the aggregation reduced the number density of crowding bodies while increasing their sizes. This caused a decrease in the chemical potential. Again, the SPT model predicted higher chemical potentials while using a mixture of conformations by incorporating additive effects. These results indicate that the SPT model including the contribution by PEG aggregates would be a good choice to compute the excluded volume effects on the biochemical reactions occurring in the presence of macromolecules of different sizes and shapes, whereas the extended SPT model with a single PEG conformation produced the best results against MC simulations. The extended SPT model with a single PEG conformation is a valid choice when the system is populated with compact shaped crowders of almost same geometrical parameters and the geometrical parameters did not differ sig-

nificantly from each other. For example, if a system is filled with different haemoglobin conformations, assuming all the conformations are of almost same geometrical parameters, the SPT with a single type of crowder could be applied and could produce reliable results. However, use of a single PEG conformation potentially neglects important features of the cellular interiors which are composed of different components. For example Cajal bodies, nuclear speckles and centrosomes in the nucleus, and ribosomes in the cytoplasm could be represented as clusters. The free energy change results (Figure 4.23) obtained from the extended SPT with aggregates predicted free energy changes for the conformational equilibrium reaction ( $1NA2 \rightleftharpoons 2K96$ ) in good agreement with the MC outcomes. These results showed that the extended SPT with aggregates can be applied with confidence but sometime it may require additional trial and error calculations to determine the best SPT model that could reproduce the experimental or MC simulation results.

### **Concluding remarks - Fractional available volumes**

To test the SPT results, the MC method was introduced to estimate the fractional available volumes by integrating one of the two approaches, i.e. the parallel-distance and parallel-energy approaches to determine the steric clashes. The MC parallel-distance algorithm produced more accurate results in comparison to the parallel-energy algorithm but it costs more time and computer resources. The computation cost of the MC parallel-distance algorithm was reduced further by introducing an additional criterion. The computation cost in both MC algorithms increased with concentration.

The extended scaled particle theory model and the MC simulations were performed to estimate the fractional available volumes and subsequently thermodynamic activities of probe molecules at six different concentrations. The aim of these simulations was to improve the SPT model by incorporating a better approximation to measure the geometrical parameters of crowders and probe molecules and to reproduce the MC results. The SPT model illustrated the effect of different shapes and sizes on the fractional available volumes

and chemical potentials. The convex-hull algorithm produced good results with globular shaped crowders but needs to be improved for partially folded and open conformations. The extended SPT model offers many advantages over the original scaled particle theory. The extended model tried to capture the exact shape by using atomic coordinates and subsequently measured the geometrical parameters through forming a convex hull. However, it has a drawback of overestimating the size of molecules of extended and partially folded states. The extended SPT model would be a better choice to estimate the crowding effects in the presence of compact convex shaped molecules over the original SPT model. The extended approach could possibly be used for a nonconvex shape by partitioning the given molecule into smaller convex shapes and the geometrical parameters can be obtained by integrating parameters for all components. This alternative method might work well for getting surface area and volume but might need care for getting the radius of curvature.

## 4.5 Kinetics study by the transition state theory

The transition state theory was used to study the kinetics of conformational equilibrium under crowding conditions. The successful implementation of the transition state theory in a unimolecular reaction requires the activity coefficients of the transition state and a single reactant measured in the crowded medium (Equation 4.2 [87]). Under dilute solution conditions, the activity coefficients of the reactants and the transition states are considered as unity. However, the activity coefficients of the reactant and the transition state are no longer unity in NI solutions. The final rate constant of a unimolecular reaction is derived in the introduction in section 1.4.3 and can be expressed as

$$k_1 = k_0 \left( \frac{\gamma_A}{\gamma_{TS}} \right) \quad (4.2)$$

where  $k_1$  is the rate constant of the reaction,  $k_0$  is the rate constant under dilute conditions, and  $\gamma$  is the activity coefficient of a reactant state A or of a TS state.

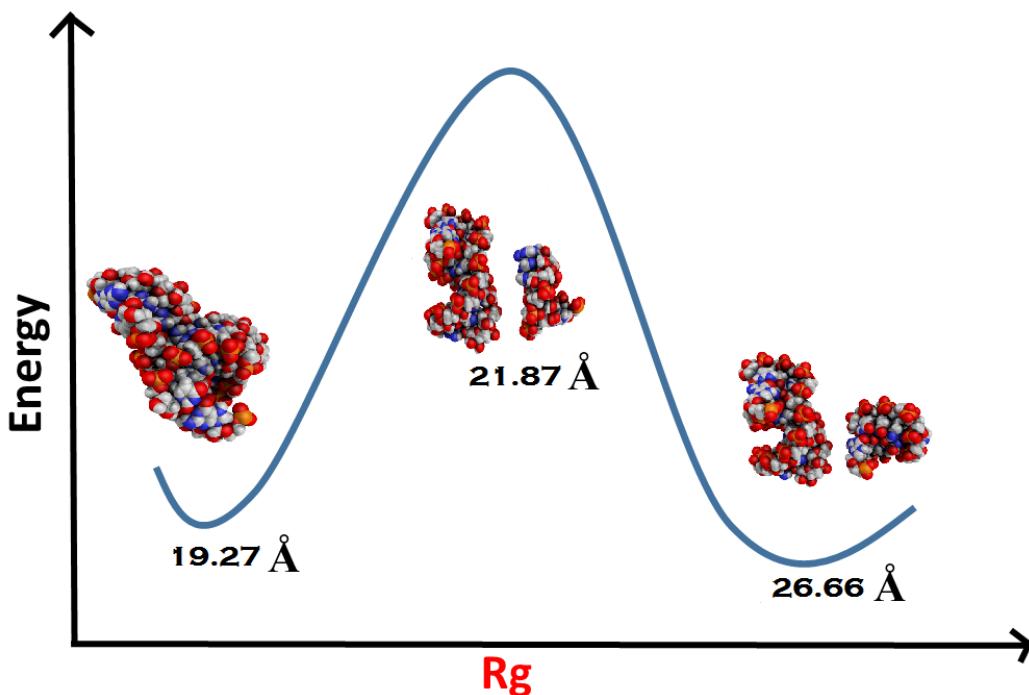


Figure 4.24: Approximation of the transition state between folded and unfolded states of the GAAA tetraloop receptor along with radius of curvature with the morphing approach.

The final rate constant  $k_1$  is a function of the activity coefficients of a reactant and a transition state. These activity coefficients can be estimated by using the MC or extended SPT methods. Both methods can estimate the activity coefficients of the reactant and transition state by using the corresponding structures and the geometrical parameters. However, the structure of the transition state is unavailable and it posed a difficulty to determine the activity coefficient of the transition state in both methods. To overcome this difficulty the transition state structure was approximated by the morphing approach and the approximated structure was used to determine the activity coefficient of the transition state.

The morphing approach produced intermediate structures between two conformations, a reactant and a product. To locate the transition state, single point energy calculations were performed for initial, final and intermediate frames in Sire (Figure 4.24) and the frame with the highest energy was chosen as an approximation to the transition state. In this regard, the transition state of the GAAA tetraloop receptor was approximated between open and closed states. The folded/closed structure of GAAA tetraloop receptor was extracted from

Table 4.4: Geometrical parameters of three structures of GAAA tetraloop receptor estimated by the extended SPT model. The experimental column represents the values of the radii for the three states and PEG crowder approximated by Dupuis *et al.* [80] that were used in the original SPT model calculations. The convexification algorithm estimated the following geometrical parameters  $R$ ,  $S$ ,  $V$  for all structures in Å, Å<sup>2</sup>, and Å<sup>3</sup> units respectively.

State	$R$	$S$	$V$	Experimental ( $R$ )
Folded	19.27	2,937	11,791	12.5
Unfolded	26.66	3,677	16,096	27
TS	21.87	3,417	15,307	16
Crowder	17.51	3,065	14,018	15

the crystal structure 1GID [270] by selecting the following nucleotides 147-156, 220-229, and 245-253, using the Pymol package [271]. These nucleotides represented the folded conformation of the GAAA tetraloop receptor. Similarly, Dupuis *et al.* [80] also extracted the folded structure of GAAA tetraloop receptor. The unfolded state or structure was constructed by editing the folded structure in GaussView 5.0 by following the secondary structure. Moreover, both components of the GAAA tetraloop receptor, i.e. GAAA tetraloop and receptor, moved apart until the radius of curvature by the extended SPT of the unfolded structure becomes almost equal to the approximated value of 27 Å reported by Dupuis *et al.* [80]. Finally these two structures were used to approximate the transition state structure, and subsequently the geometric parameters (Table 4.4) and activity coefficients were estimated with the extended SPT model.

The experimental folding rate constant under dilute conditions ( $k_0$ ) of GAAA tetraloopreceptor was obtained from Dupuis *et al.* [80] where it was measured by using the FRET spectroscopic method and the final rate constant ( $k_1$ ) was measured in the presence of 8 kDa PEG crowders. The measurement of the rate constant  $k_1$  using the transition state theory expression showed a slightly lower rate constant in the presence of the PEG2 crowders by incorporating the activity coefficients for the unfolded and the transition states (Figure 4.25). Despite using the approximated structure of the transition state to predict the activity coefficient, a good estimate of  $k_1$  was obtained. Even at the very highest PEG concentration

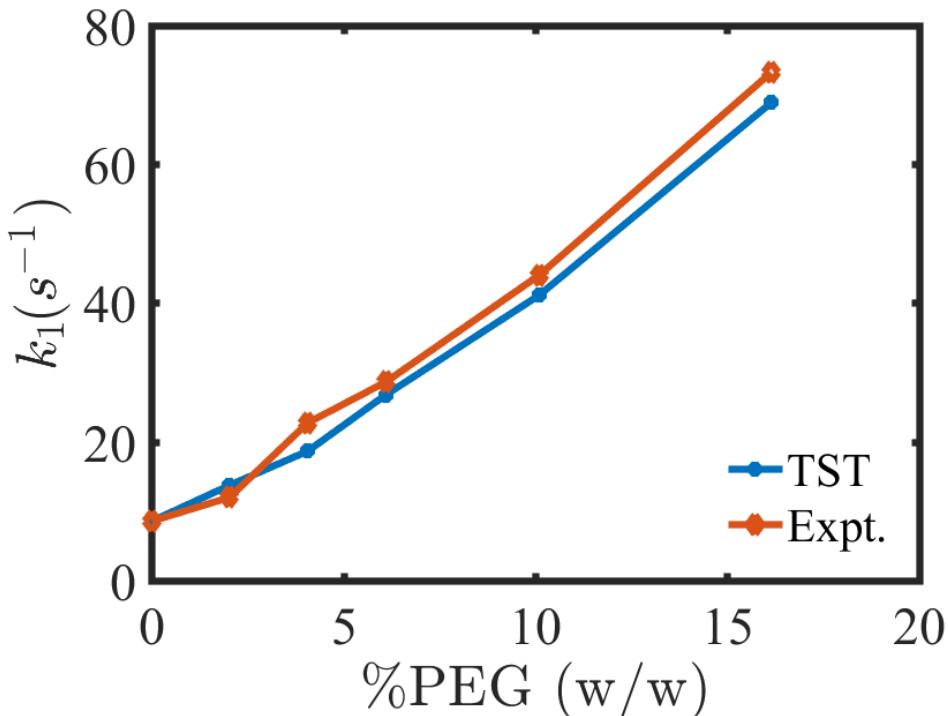


Figure 4.25: Change in the folding rate constant as a function of concentration of 8 kDa PEG. The extended SPT model predicted the slightly lower rate constant by incorporating the activity coefficients of the unfolded and the transition states in the transition state theory expression as compared to the experimental data. PEG2 molecules were used as crowders in these calculations as 15 Å radius of gyration of the PEG was assumed in the experimental measurements.

the estimated rate constant fell within about 5% of the correct rate constant.

### Summary

The crowding effects on the conformational equilibrium of three systems have been investigated by carrying out the MC and SPT simulations. Macromolecular crowding exerts low to moderate effects on the conformational equilibrium and increases stability of folded states by  $-0.2$  to  $-2.0kT$  approximately. The magnitude of the crowding effect is directly influenced by the shapes and sizes of the probe and crowder molecules. Conformational equilibrium involving large conformational changes is expected to experience high crowding effects. The MC and SPT models utilized the real molecular structures to predict the crowding effects, however to get the quantitative effects of crowding these models need additional NI contribution terms such as electrostatic potentials, enthalpic and entropic in-

termolecular interactions. Further, the successful implementation of the transition state theory model along with the MC and extended SPT model might be capable of predicting the rate constants quantitatively for biochemical reactions occurring in crowded media.

# Chapter 5

## Conclusions and future directions

### 5.1 Summary

This research work was dedicated to the development of a new theoretical framework for computing the effects of macromolecular crowding on the activities of macromolecular solutes. This theoretical framework is then applied to study the macromolecular crowding effects on the conformational equilibrium of three systems. The theoretical framework is comprised of two major steps, i.e. the construction of crowded media at different concentrations followed by the study of a biochemical reaction of interest in the crowded media. The first step requires the availability of representative solution structures of the crowder to be used to pack and further to equilibrate the crowded medium. Ensembles of 8 kDa PEG conformations over a wide range of sizes (10–60 Å radius of gyration) were used as crowders. The first step will usually require conformational sampling simulations to generate the ensembles of crowder conformations as it would be unusual for structural libraries to contain solution structures of a large sized crowder. In our case, we constructed ensembles of PEG conformations using MC and MD simulations under different simulation conditions to get as diverse a set of conformations as possible. These conformations are used to pack the crowded systems and are further equilibrated to incorporate the PEG self-crowding effects. The second step comprised a set of methods to be used to estimate the crowding effects on the folding reaction or any other reaction of interest such as association reactions. These methods, i.e. the extended scaled particle theory and Monte Carlo simulations, are applied to estimate the activity coefficients in crowded systems. These can be used to determine the

effect of crowding on an equilibrium or, via TST, on the kinetics of a reaction, provided we can generate a guess for the transition state structure.

## 5.2 Recommended computational methods

The aim of developing the current program is to measure the crowding effects quantitatively. This could be one step towards achieving our goal by using the more realistic crowded medium with the extended scaled particle theory and MC methods. In the text that follows, the best procedures for calculating crowding effects based on the work in this thesis will be presented.

### 5.2.1 Ensembles of crowder conformations

Experimental studies can help to define a biochemical system that can be studied with our program. Our program required solution structures for all the species either of probe molecules or crowders. Different types of crowding agents have been used to mimic the crowding environments in *in vitro* experiments. The availability of the crowder structures may be limited due to lack of solution structures of crowders. For instance, 8 kDa PEG is one of the most commonly used crowders in experimental studies [80, 98, 111, 191], however we do not have detailed information on solution conformations of PEG to the best of my knowledge. Solution structures of PEG were constructed through performing conformational sampling simulations. In this regard, MC simulations in Sire and MD simulations in Amber are found to be equally effective and produced diverse conformational ensembles in a range of radii of gyration of 10 to 60 Å. The folding behaviour of PEG is found to be sensitive to the simulation methods, i.e. whether MC or MD simulations are used and a temperature parameter in these simulations. Therefore a combination of these two methods was used to produce diverse conformational ensembles of a PEG crowder. In this regard, the MD and MC simulations produced equilibrated compact (10–25 Å) and more extended conformations (18–60 Å) of PEG respectively. The recommendation on the selection of an

appropriate MC or MD simulation method is based on the required range of the solution structures of crowders and their folding sensitivity to each simulation method. For crowders insensitive to simulation methods, the MC simulation method would be a good choice for conformational sampling in producing diverse conformations. Moreover, the sensitivity of the crowders towards each method can be tested by conducting MC and MD simulations using an equilibrated structure.

### 5.2.2 Packing and equilibration

The second step involved the preparation of crowded media at different concentrations by packing the random PEG conformations generated in the previous step. Fifteen to thirty instances of crowded systems at each concentration from  $0.1$  to  $0.6\text{ g cm}^{-3}$  were prepared. The packed crowded systems were further equilibrated by conducting MC equilibration, MC swapping and MD equilibration simulations to account for self crowding effects. The multiple instances at each concentration were useful to produce statistically converged results. In our calculations the standard deviation and the average of the fractional available volume were converged after including fifteen instances of crowded media. MD equilibration was found to be the most efficient technique among the other two and showed that PEG experienced large conformational changes in the crowded medium and formed PEG aggregates [89, 92, 93, 95, 101, 109, 258, 259].

The appropriate equilibration method can be selected based on the size of the system. This may also require trial and error calculations to find out the best method for the given system. In general, the MD equilibration method is a good choice for any sized systems filled with crowders of any molecular weight. A system with dimensions of  $50 \times 50 \times 50\text{ \AA}^3$  or greater and filled with crowders of molecular weight greater than 1 kDa can be classified as a larger sized system. The MC equilibration and swapping methods are almost equally effective to the MD equilibration in smaller sized systems of dimension less than  $50 \times 50 \times 50\text{ \AA}^3$  filled with crowders of molecular weight less than 0.5 kDa.

### 5.2.3 Calculations of thermodynamic properties

The third step is the application of the extended scaled particle theory or Monte Carlo method to determine the crowding effects on the biochemical reactions in terms of estimating the fractional available volumes and subsequently chemical potentials. The size and shape distribution of crowders and system molecules played a critical role in affecting the magnitude of the fractional available volume. The SPT model considered the nonideal contribution of individual molecules towards the chemical potentials by extracting the geometrical parameters from atomic coordinates and also taking into account the PEG aggregates contribution. The MC method measured the chemical potential by placing each conformation of the system over and over again in the equilibrated simulation boxes at a random position with a random orientation. The fraction of successful insertions to the total number of insertions gave the estimate of the activity coefficient for each probe molecule. In this regard, the MC method used parallel-energy and parallel-distance algorithms to measure the steric clashes and subsequently activity coefficients for each probe molecule.

The SPT model is very efficient and approximates the crowding effects quickly but depends on the extraction of the correct geometrical parameters of a system and crowder molecules. The MC method does not require extraction of geometrical parameters but uses the atomic coordinates and can estimate the chemical potential accurately. However, the MC method is computationally expensive. In this regard, a parallel-energy algorithm is found to be more efficient for larger systems used in this study as compared to a parallel-distance algorithm. Both algorithms are equally effective and efficient for small sized system as defined above. The SPT model would be a good choice for systems containing convex shaped molecules as it predicts the crowding effects quickly. However it overestimates the crowding effects for systems containing extended or open and non-convex structures due to the convex hull construction (Figure 5.1 (b)).

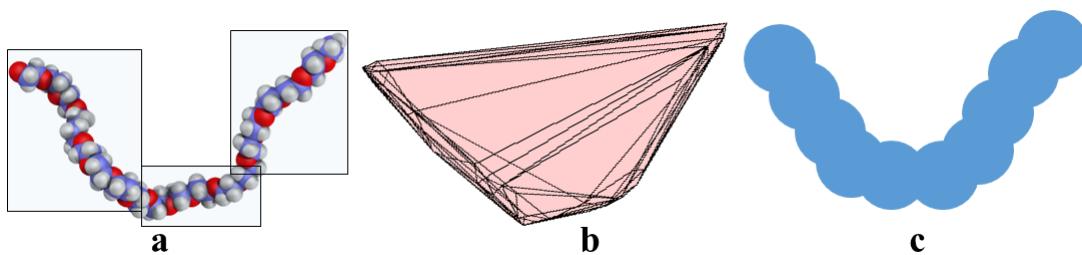


Figure 5.1: Illustration of a small fragment of an 8 kDa PEG to depict the concept of the construction of sub-units (a), convex hull (b) and a rolling method (c). The fragment represents a non-convex conformation where the convex hull algorithm overestimates the geometrical parameters by encapsulating the free space present in the cavity. These two methods could help to approximate more accurate geometrical parameters of a non-convex molecule.

#### 5.2.4 Crowding effects on reaction kinetics

The transition state theory approximated the crowding effects on the folding kinetics. The transition state theory requires activity coefficients of the reactants and a transition state for any chemical reaction. These activity coefficients could be estimated by the extended SPT or MC models which requires the availability of the structures of the reactant(s) and transition state. In this regard, the morphing server provided an alternative solution to construct intermediate conformations between initial and final structures of the system to get an approximated transition state structure. The morphing server takes two input structures to start with and constructs the intermediate structures between these initial and final structures. Therefore, this can be applied to bimolecular association or dissociation reactions if the solution structure of a product in an association reaction or a structure of the reactant in a dissociation reaction is available. Alternatively, the dissociation reaction is the reverse of an association reaction and the approximated TS structure in the dissociation reaction can be used as a TS in the association reaction. It is important to prepare the input files properly and both input files should be equal in terms of number of atoms. For example, two reactants combined in bimolecular association reaction to form a single product. The reactant input file must contain both reactants so the reactant file will have an equal number of atomic coordinates to the product file. Both reactants can be placed at any arbitrarily large distance from each other in the first input file.

This TS model approximated crowding effects well in the unimolecular folding reaction of the GAAA tetraloop receptor (section 4.5). It might be concluded that this model can help one to estimate the crowding effects on the reaction kinetics using an approximated transition state structure with a reasonable accuracy. The MC method would be a good choice for reactions involving denatured and extended states of reactants and TS, while the SPT model is good for reactions containing compact globular shaped reactants and TS structures to approximate the activity coefficient. For example, for a unimolecular unfolding reaction forming an unfolded state from a compact globular folded state or for a bimolecular dissociation reaction occurring between two compact globular shaped proteins, SPT is good to apply to approximate the activity coefficients.

## 5.3 Future directions

The theoretical framework predicted low to moderate crowding effects on the conformational equilibrium correlated with many theoretical and experimental results [24, 47, 125, 268, 269]. The following future improvements can be made to the model to estimate the crowding effects quantitatively. Moreover, the possible steps that can be taken to improve the accuracy of the extraction of geometrical parameters in the extended SPT method and the computational efficiency of the MC method are described in the following section.

### 5.3.1 Accuracy of the geometrical parameters and computational efficiency

The convexification algorithm overestimates the geometrical parameters for non-convex shaped molecules. The accuracy of the geometrical parameters for non-convex shaped molecules can be improved by applying the convexification algorithm on compact shaped sub-units of the molecule. It will be challenging to define the criterion for defining the cut-off boundary points for each sub-unit. Figure 5.1 (a) depicts the idea of defining approximately convex shaped sub-units whose number will depend on the structure of the molecule. The sub-units can differ in size and contain different numbers of atoms. A

combination of three sub-units will represent a coiled cylinder and will give an estimate of geometrical parameters for this coiled cylinder shape. Eventually, this approach will approximate the activity coefficients in the SPT model. Alternatively, the rolling probe methods [238, 272–275] (Figure 5.1(c)) can be used to estimate the volume and the surface area of the molecule by rolling a virtual probe solvent convex shaped molecule of known radius. The virtual probe molecule can be a solvent molecule such as water of 1.5 Å radius [238]. The rolling probe method cannot estimate the radius of curvature but the convex hull algorithm can be used here. These methods may be computationally expensive but the efficiency can be improved by implementing the multiprocessing routines for larger and complexed shaped macromolecules.

In our program, the MC method with a parallel-distance algorithm to compute the fractional available volume is equally effective to the MC method with a parallel-energy algorithm for small sized systems filled with lower molecular weight crowders. Similarly, two equilibration methods i.e. MC equilibration and MC swapping are also equally effective to MD equilibration for small sized systems filled with lower molecular weight crowders. All of these three methods, i.e. MC method with a parallel-distance algorithm, MC equilibration and MC swapping were built in the Sire program. Sire is built in C/C++ and Python frameworks and we have implemented the Python multiprocessing routines to further improve the computational efficiency of an MC method with the parallel-distance algorithm. C/C++ is more powerful and its multiprocessing routines such as OpenMP [276] and MPI [277] would make the computation faster for larger sized systems to approximate the activity coefficients. This would require the rewriting of a single function in the parallel-distance algorithm that executes the iterative loops to find the steric clashes.

### 5.3.2 Heterogeneity of crowded medium

A heterogeneous and complex crowded medium similar to the cellular interior can mimic the cellular interior effectively [37, 262, 278]. For that we may need a set of crow-

ders that can resemble the contents of different types of cells. The heterogeneous crowded medium can be constructed by packing and equilibrating a mixture of routinely used crowders such as PEG, Ficol, and dextran. This method will require to find the appropriate mixture of crowders of different sizes and shapes. Alternatively, solution structures of different macromolecules available in the protein data bank can be used to pack and equilibrate the crowded medium. This approach can provide a library of diverse molecules but it may require to choose macromolecules that can result in an appropriate medium without segregation of some components after packing and equilibration. Moreover, it is important to include the local concentration effects as the concentration varies from point to point inside the cells [47, 267] and these microenvironments can alter the thermodynamic activities of solutes substantially. The microenvironments within, e.g., the nucleus, like a Cajal body or nuclear speckle, may need an ensemble of microenvironments that differ somewhat in dimensions and/or composition. Conformational ensembles of microenvironments are required as the composition and dimensions of these microenvironments fluctuate with time due to flow of cell material such as proteins in between different cell compartments [8, 267, 278]. For example, nuclear speckles are dynamic structures that vary in size and shape and are found in the interchromatin regions of the nucleoplasm. The nuclear speckles are composed of a heterogeneous mixture of proteins and RNA-protein components. The transcriptional state of the cell controls the concentration of these proteins that keep changing continuously due to their exchange between speckles and other nuclear locations [279, 280].

### 5.3.3 Soft interactions contributions

It is worth incorporating the nonideal contributions due to attractive and repulsive electrostatic potentials, enthalpic and entropic intermolecular interactions to quantify the macromolecular crowding effect in the extended scaled particle and Monte Carlo methods. There are numerous ways to estimate the electrostatic contributions to the activity coefficients

such as the Debye-Hückel equations [281], semi-empirical models [281, 282], Poisson-Boltzmann [283–285], and thermodynamic models for solutions [282].

### 5.3.4 Approximate better TS state structure

The approximated transition state from the morphing server is not necessarily a close representative of a true transition state structure. Further development can be made in the theoretical framework to estimate a more realistic transition state structure. There are computational methods [241, 242, 244, 245, 286], as well as experimental transition state spectroscopic methods [243] available to determine the TS structures. However, these methods do not always work for all types of reactions involving complex macromolecules.

Alternatively, the transition state structure can be approximated by using experimental data fitting tools such as the least square error method [239]. The transition state theory equation (Equation 5.1) expresses the relationship between the final rate constant ( $k$ ) of a unimolecular folding reaction, the ideal rate constant ( $k_0$ ) and the activity coefficients of the reactant ( $\gamma_A$ ) and TS ( $\gamma_{TS}$ ) structures. This method requires experimental values of  $k$  under crowded conditions ( $\phi$ ) and  $k_0$  for the reaction and the SPT method can approximate the activity coefficient of the reactant. Running a minimization tool as a function of the sum of squared error can predict the unknown geometric parameters ( $R_{TS}, A_{TS}, V_{TS}$ ) of the transition state in the SPT equation. Mathematically this could be expressed by equation 5.2.

$$k = k_0 \gamma_A \left( \frac{1}{\gamma_{TS}} \right) = k_0 \gamma_A \left( \frac{1}{\gamma_{TS}(R_{TS}, S_{TS}, V_{TS})} \right) \quad (5.1)$$

$$\min || \sum k(\phi) - k_0 \gamma_A \left( \frac{1}{\gamma_{TS}(\phi, k, k_0, R_{TS}, S_{TS}, V_{TS})} \right) ||^2 \quad (5.2)$$

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# Appendix A

## Methods appendix

### A.1 Forcefield parameters for PEG

#### A.1.1 System.config

---

```
1 !!             System.config file
2
3 !! Method & Basis set used in geometry optimization
4 METHOD_OPTCALC = B3LYP
5 BASSET_OPTCALC = 6-31G(d)
6
7 !! Method & Basis set used for MEP computation
8 METHOD_MEPCALC = B3LYP
9 BASSET_MEPCALC = 6-31G(d)
10
11 !! Package used in QM calculations
12 QMSOFT          = GAUSSIAN09
13
14 !! Number of processor(s) used in parallel (in QM calculations)
15 NP              = 8
16
17 !! Maximal amount of memory available in MegaBytes (MB) for the QM jobs
18 MAXMEMVAL      = 12288
```

---

#### A.1.2 Project.config

---

```
1 !!             Project.config file
2
3 !! The title of molecule '$n': Methyl-PEG
4 MOLECULE1-TITLE     = PEG
5
6 !! Request the use of an intra-molecular charge constraint for molecule
7     '$n'
8 MOLECULE1-INTRA-MCC2 = + 0.0176 | 1 2 3 4 | R
9 MOLECULE1-INTRA-MCC2 = - 0.0176 | 13 14 | R
```

---

## A.2 MCCCS Towhee input file

---

```
1 ## Input parameters ensembles, molecules, box dimensions ##
2 inputformat
3 'Towhee'
4 ensemble
5 'npt'
6 temperature
7 300.15d0
8 # ensemble external pressure in kPa
9 pressure
10 101.0d0
11 #types of molecules
12 nmoltypes
13 1
14 #number of molecules of each type
15 nmolctyp
16 1
17 numboxes
18 1
19 stepstyle
20 'cycles'
21 nstep
22 1
23 printfreq
24 1
25 #size of the blocks for computing block averages
26 blocksize
27 2
28 moviefreq
29 10
30 backupfreq
31 50
32 #information about the individual blocks of block averages and max.
   displacement updates.
33 runoutput
34 'full'
35 pdb_output_freq
36 25
37 #output files used with Tramonto
38 loutdft
39 F
40 #output file for lammps
41 loutlammps
42 .false.
```

```
43 #output files for DL_POLY
44 loutdlpoly
45 .false.
46 # output freq. for computing the pressure in each simulation box.
47 pressurefreq
48 10
49 #step freq. for updating max. translational and rotational displacement
50 .
50 trmaxdispfreq
51 10
52 #step freq. for updating the max. volume displacement.
53 volmaxdispfreq
54 50
55 #chemical pot. in ensembles having no insertion/del. move.NPT,NVT.
56 chempotperstep
57 10
58 #Internal style consider the energies between atoms
59 potentialstyle
60 'internal'
61 #number of forcefields
62 ffnumber
63 1
64 ff_filename
65 /usr/local/share/towhee/ForceFields/towhee_ff_Amber96
66 #setting for non-bonded potential types
67 classical_potential
68 'Lennard-Jones'
69 # specifies the manner in which the parameters for unlike atoms are
    determined.
70 classical_mixrule
71 'Lorentz-Berthelot'
72 #shift of nonbonded potential at cutoff
73 lshift
74 .false.
75 #Analytical tail correction
76 ltailc
77 .false.
78 #Hard inner cutoff to avoid the potential hitting infinity for
    repulsion system.0-1
79 rmin
80 1.0d0
81 #The distance beyond which nonbonded potentials are ignored
82 rcut
83 5.0d0
84 #The inner nonbonded cutoff-for noncolumbic and columbic have 5,10.
```

```
85 rcutin
86 4.0d0
87 #electrostatic potential none or computed by Coulomb's law
88 electrostatic_form
89 'none'
90 #T:start simulation by generating positions,box dimensions and max.
   displacement.
91 #F:use towhee_initial for input above parameters
92 linit
93 .false.
94 #initial boxes type
95 initboxtype
96 'dimensions'
97 #input for given molecule: with no input file i.e. coords,template...
   use full cbmc.
98 initstyle
99 'full cbmc'
100 #where to put molecule
101 initlattice
102 'simple cubic'
103 # initial number of each type of molecules place in box
104 initmol
105 1
106 #each number of molecules in each direction in each
107 inix iniy iniz
108 1 1 1
109 # initial box dimensions
110 hmatrix
111 100.0 0.0 0.0
112 0.0 100.0 0.0
113 0.0 0.0 100.0
114 ## types of moves to perform MC simulations ##
115 #pm* variable represents which move is going to perform. input values
   is 0-1
116 #selections of box and molecule for selected move by pm**pr and pm**mt
   respectively. usually input 0 or 1.
117 # Isotropic vol. move
118 pmvol
119 0.01d0
120      pmvlpr
121      1.0d0
122      rmvol
123      0.1d0
124      tavol
125      0.5d0
```

```
126 pm1boxcbswap
127 0.20d0
128          pm1cbswmt
129          1.0d0
130 pmavb1
131 0.0d0
132          pmavb1in
133          0.5d0
134          pmavb1mt
135          1.0d0
136          pmavb1ct
137          1.0d0
138          avb1rad
139          0.05d0
140 pmavb2
141 0.0d0
142          pmavb2in
143          0.5d0
144          pmavb2mt
145          1.0d0
146          pmavb2ct
147          1.0d0
148          avb2rad
149          0.05d0
150 pmavb3
151 0.0d0
152          pmavb3mt
153          1.0d0
154          pmavb3ct
155          1.0d0
156          avb3rad
157          0.05d0
158 pmcb
159 0.60d0
160          pmcbmt
161          1.0d0
162          pmall
163          0.0d0
164 pmback
165 0.0d0
166          pmbkmt
167          1.0d0
168 pmpivot
169 0.0d0
170          pmpivmt
```

```
171      1.0d0
172 pmconrot
173 0.0d0
174      pmcrmt
175 1.0d0
176 pmcrback
177 0.0d0
178      pmcrbmt
179 1.0d0
180 pmplane
181 0.0d0
182      pmplanebox
183 1.0d0
184      planewidth
185 1.0d0
186 pmrow
187 0.0d0
188      pmrowbox
189 1.0d0
190      rowwidth
191 1.0d0
192 pmtraat
193 0.80d0
194      pmtamt
195 1.0d0
196      rmtraa
197 0.5d0
198      tatraa
199 0.5d0
200 pmtracm
201 0.90d0
202      pmtcmt
203 1.0d0
204      rmtrac
205 0.5d0
206      tatrac
207 0.5d0
208 pmrotate
209 1.0d0
210      pmromt
211 1.0d0
212      rmrot
213 0.05d0
214      tarot
215 0.5d0
```

```
216
217 ## cbmc_formulation optional parameter for bond length,bending angles,
218     and dihedral selection. use the default options ##
219
220 #cbmc_formulation
221 #'Martin and Frischknecht 2006'
222 #cbmc_setting_style
223 #'Martin and Frischknecht'
224 ## Final section to construct the forcefield for the molecules in the
225     system. 6 options are available for input_style
226 input_style
227   'basic connectivity map'
228 nunit
229 1277
230 nmaxcbmc
231 1277
232 lpdnames
233 .false.
234 # see each force field for supporting atom name list
235 forcefield
236   'Amber96'
237 #writine a connectivity map based on structure here is H*OCH2CH2n*OH
238     where n is 9.
239 charge_assignment
240   'none'
241 unit ntype
242 1   'HO'
243 vibration
244 1
245 2
246 improper torsion
247 0
248 unit ntype
249 2   'OH'
250 vibration
251 2
252 1 3
253 improper torsion
254 0
255 unit ntype
256 3   'CT'
257 vibration
258 4
259 2 4 5 6
```

```
258 improper torsion
259 0
260 unit ntype
261 4  'H1'
262 vibration
263 1
264 3
265 improper torsion
266 0
267 unit ntype
268 5  'H1'
269 vibration
270 1
271 3
272 improper torsion
273 0
274 unit ntype
275 6  'CT'
276 vibration
277 4
278 3 7 8 9
279 improper torsion
280 0
281 unit ntype
282 7  'H1'
283 vibration
284 1
285 6
286 improper torsion
287 0
288 unit ntype
289 8  'H1'
290 vibration
291 1
292 6
293 improper torsion
294 0
295 unit ntype
296 9  'OS'
297 vibration
298 2
299 6 10
300 improper torsion
301 0
302 unit ntype
```

```
303 10    'CT'
304 vibration
305 4
306 9    11    12    13
307 improper torsion
308 0
309 unit ntype
310 11    'H1'
311 vibration
312 1
313 10
314 improper torsion
315 0
316 unit ntype
317 12    'H1'
318 vibration
319 1
320 10
321 improper torsion
322 0
323 unit ntype
324 13    'CT'
325 vibration
326 4
327 10    14    15    16
328 improper torsion
329 0
330 unit ntype
331 14    'H1'
332 vibration
333 1
334 13
335 improper torsion
336 0
337 unit ntype
338 15    'H1'
339 vibration
340 1
341 13
342 improper torsion
343 0
344 unit ntype
345 16    'OS'
346 vibration
347 2
```

```
348 13 17
349 improper torsion
350 0
351 unit ntype
352 17 'CT'
353 vibration
354 4
355 16 18 19 20
356 improper torsion
357 0
358 unit ntype
359 18 'H1'
360 vibration
361 1
362 17
363 improper torsion
364 0
365 unit ntype
366 19 'H1'
367 vibration
368 1
369 17
370 improper torsion
371 0
372 unit ntype
373 20 'CT'
374 vibration
375 4
376 17 21 22 23
377 improper torsion
378 0
379 unit ntype
380 21 'H1'
381 vibration
382 1
383 20
384 improper torsion
385 0
386 unit ntype
387 22 'H1'
388 vibration
389 1
390 20
391 improper torsion
392 0
```

```
393 unit ntype
394 23  'OS'
395 vibration
396 2
397 20  24
398 improper torsion
399 0
400 unit ntype
401 24  'CT'
402 vibration
403 4
404 23  25  26  27
405 improper torsion
406 0
407 unit ntype
408 25  'H1'
409 vibration
410 1
411 24
412 improper torsion
413 0
414 unit ntype
415 26  'H1'
416 vibration
417 1
418 24
419 improper torsion
420 0
421 unit ntype
422 27  'CT'
423 vibration
424 4
425 24  28  29  30
426 improper torsion
427 0
428 unit ntype
429 28  'H1'
430 vibration
431 1
432 27
433 improper torsion
434 0
435 unit ntype
436 29  'H1'
437 vibration
```

```
438 1
439 27
440 improper torsion
441 0
442 unit ntype
443 30  'OS'
444 vibration
445 2
446 27  31
447 improper torsion
448 0
449 unit ntype
450 31  'CT'
451 vibration
452 4
453 30  32  33  34
454 improper torsion
455 0
456 unit ntype
457 32  'H1'
458 vibration
459 1
460 31
461 improper torsion
462 0
463 unit ntype
464 33  'H1'
465 vibration
466 1
467 31
468 improper torsion
469 0
470 unit ntype
471 34  'CT'
472 vibration
473 4
474 31  35  36  37
475 improper torsion
476 0
477 unit ntype
478 35  'H1'
479 vibration
480 1
481 34
482 improper torsion
```

```
483 0
484 unit ntype
485 36  'H1'
486 vibration
487 1
488 34
489 improper torsion
490 0
491 unit ntype
492 37  'OS'
493 vibration
494 2
495 34  38
496 improper torsion
497 0
498 .
499 .
500 .
501 unit ntype
502 1270  'CT'
503 vibration
504 4
505 1269      1271      1272      1273
506 improper torsion
507 0
508 unit ntype
509 1271  'H1'
510 vibration
511 1
512 1270
513 improper torsion
514 0
515 unit ntype
516 1272  'H1'
517 vibration
518 1
519 1270
520 improper torsion
521 0
522 unit ntype
523 1273  'CT'
524 vibration
525 4
526 1270      1274      1275      1276
527 improper torsion
```

```
528 0
529 unit ntype
530 1274 'H1'
531 vibration
532 1
533 1273
534 improper torsion
535 0
536 unit ntype
537 1275 'H1'
538 vibration
539 1
540 1273
541 improper torsion
542 0
543 unit ntype
544 1276 'OH'
545 vibration
546 2
547 1273 1277
548 improper torsion
549 0
550 unit ntype
551 1277 'HO'
552 vibration
553 1
554 1276
555 improper torsion
556 0
```

---

## A.3 Sire sampling input file

### A.3.1 Input file with all functions

---

```
1 #####  
2 # MyFunctions.py file with all functions  
3 #####  
4 #####  
5 #####  
6 # Import Libraries as listed in Sire sampling input file  
7 #####  
8 #####  
9 from Sire.Mol import *  
10 from Sire.IO import *  
11 from Sire.Vol import *  
12 from Sire.FF import *  
13 from Sire.MM import *  
14 from Sire.CAS import *  
15 from Sire.Maths import *  
16 from Sire.Qt import *  
17 from Sire.Units import *  
18 from Sire.System import *  
19 from Sire.Move import *  
20 from Sire.Stream import *  
21 from Sire.Cluster import *  
22 import sys  
23 from Sire.Vol import *  
24 from Sire.Base import *  
25 import operator  
26 from numpy import *  
27 from scipy import stats  
28 from random import *  
29 import time  
30 import pyprind as prog_bar  
31 import os.path  
32 import re  
33 from Sire.Tools import *  
34 from Sire.Tools.AmberLoader import *  
35 import math  
36 from Sire.Tools import AmberLoader  
37 import sys  
38 #####  
39 #####  
40 # Define functions  
41 #####  
42 #####
```

```
43 def switchTemp(temp1):
44     """
45     Function to quench temperature periodically
46     """
47     global temp
48     if temp1 == 300:
49         temp1 = 1200
50     else:
51         temp1 = 300
52     temp = temp1
53     return temp
54
55 def make_system(GiveMolec):
56     """
57     function to create system and determine energies.
58     """
59     bonds = GiveMolec.property("connectivity").getBonds()
60     angles = GiveMolec.property("connectivity").getAngles()
61     dihedrals = GiveMolec.property("connectivity").getDihedrals()
62     nbonds = len(bonds)
63     nangles = len(angles)
64     ndihedrals = len(dihedrals)
65     intraff = InternalFF("intraff")
66     intraclj = IntraCLJFF("intraclj")
67     intraff.add(GiveMolec)
68     intraclj.add(GiveMolec)
69     system = System()
70     system.add(intraff)
71     system.add(intraclj)
72     return system
73
74 def bondMove(temp,system):
75     """
76     function to perform bond length changing moves.
77     """
78     global naccept
79     global nreject
80
81     ## get the version of the Molecule, currently in the system
82     GiveMolec = system[ MolWithResID("F02") ]
83
84     ## calculate the energy before the move
85     old_energy = system.energy()
86
87     ## randomly choose a bond to move
```



```
130     ## calculate the energy before the move
131     old_energy = system.energy()
132
133     ## randomly choose an angle to move
134     angleid = angles[ rangen.randInt(0,nangles-1) ]
135
136     ## randomly choose an amount to move the angle between -15 and 15
137     degrees
138     delta = rangen.rand(-15, 15) * degrees
139
140     ## change the angle
141     GiveMolec_new = GiveMolec.move().change(angleid, delta, map).commit()
142
143     ## update the system with the moved molecule
144     system.update(GiveMolec_new)
145
146     ## calcualte the new energy after the move
147     new_energy = system.energy()
148
149     ## what is the difference in energy
150     delta_energy = new_energy - old_energy
151
152     ## calculate exp( -dE / kT )
153     x = math.exp( -delta_energy.value() / (k_boltz * temp) )
154
155     ## generate a random number between 0 and 1
156     random_number = rangen.rand(0,1)
157
158     ## Compare exp(-dE/kT) against this random number
159     if x >= random_number:
160         naccept += 1
161     else:
162         nreject += 1
163         # move has been rejected, so we have to move the Molecule back
164         # to its old conformation
165         system.update(GiveMolec)
166
167 def dihedralMove(temp,system):
168     """
169     function to perform dihedral angles changing moves.
170     """
171     global naccept
172     global nreject
```

```

172
173     ## get the version of the Molecule currently in the system
174     GiveMolec = system[ MolWithResID("F02") ]
175
176     ## calculate the energy before the move
177     old_energy = system.energy()
178
179     ## randomly choose an dihedral to move
180     dihedralid = dihedrals[ rangen.randInt(0,ndihedrals-1) ]
181
182     ## randomly choose an amount to move the dihedral between -15 and
183     ## 15 degrees
184     delta = rangen.rand(-15, 15) * degrees
185
186     ## now randomly choose to move either the whole dihedral or just
187     ## rotate around the bond
188     if rangen.randBool():
189         GiveMolec_new = GiveMolec.move().change( BondID(dihedralid.atom1),
190                                                 (),dihedralid.atom2()),
191                                         delta, map).commit()
192     else:
193         GiveMolec_new = GiveMolec.move().change(dihedralid, delta, map)
194                                         .commit()
195
196     ## update the system with the moved molecule
197     system.update(GiveMolec_new)
198
199     ## calcualte the new energy after the move
200     new_energy = system.energy()
201
202     ## what is the difference in energy
203     delta_energy = new_energy - old_energy
204
205     ## calculate exp( -dE / kT )
206     x = math.exp( -delta_energy.value() / (k_boltz * temp) )
207
208     ## generate a random number between 0 and 1
209     random_number = rangen.rand(0,1)
210
211     ## Compare exp(-dE/kT) against this random number
212     if x >= random_number:
213         naccept += 1
214     else:
215         nreject += 1

```

```

212         # move has been rejected, so we have to move the Molecule back
213             to its old conformation
214             system.update(GiveMolec)
215
216     def quenched_conf(system,temp):
217         """
218             function to change temperature periodically.
219             """
220             print("***** 300 start *****")
221             print("Molecule energy Before moves at 300= %s" % system.energy())
222             for i in range(0,1):
223                 move_type = rangen.randInt(1,3)
224
225                 if move_type == 1:
226                     bondMove(temp,system)
227                 elif move_type == 2:
228                     angleMove(temp,system)
229                 elif move_type == 3:
230                     dihedralMove(temp,system)
231
232             print("Molecule energy After moves at 300= %s" % system.energy())
233             mol = system[MolIdx(0)].molecule()
234             radius = radgyr(mol)
235             print("radius of gyration after performing moves at 300 %s" %
236                   radius)
237             PDB().write(system.molecules(), "outputHn300%003d.pdb" % i)
238             print("***** 300 end *****")
239             return
240
241
242     def condition_fn(initial_energy,final_energy):
243         """
244             Condition function for equilibration to give true or false output.
245             The output is used to reverse the system or keep the system to use
246             furhter minimization.
247             """
248             delta_energy = final_energy - initial_energy;
249             #print("delta energy %s"%delta_energy)
250             if delta_energy < 400:
251                 delta_energy
252             else:
253                 delta_energy = 400
254             #print("delta energy after condition %s" %delta_energy)
255             if final_energy <= initial_energy:
256                 condition = "True" #print("insertion successful on first
257                               criteria")

```

```

253     elif randgen.rand() > math.exp(delta_energy/(k_boltz*temperature.
254         to(kelvin))):
255         condition = "True" # print("insertion successful on second
256         condition")
257     else:
258         condition = "False" # print("insertion failed")
259     return condition
260
261 def energy_minimize_system_LJ_CL(box_mols,box_space):
262     """
263     Function to prepare the system containing the molecules, boxSpace
264     Input: Molecules box, boxSpace
265     """
266     # create simulation system to hold box of molecules
267     box_system = System("box")
268     # intermolecule coulomb and LJ energy
269     box_interff = InterFF("box_inter")
270     cljfunc = CLJShiftFunction()
271     cljfunc.setCoulombCutoff(15 * angstrom)
272     cljfunc.setLJCutoff(15 * angstrom)
273     cljfunc.setSpace(box_space)
274     box_interff.setCLJFunction(cljfunc)
275     box_interff.setUseParallelCalculation(True)
276     box_interff.add(box_mols)
277     # add all molecules and forcefields for box to system
278     box_system.add(box_mols)
279     box_system.add(box_interff)
280     # set the periodic box information for each box
281     box_system.setProperty("space", box_space)
282     # system energy (optional)
283     #box_system.energies()
284     box_system.add( SpaceWrapper(Vector(0), box_mols) )
285     return box_system
286
287 def createSystem(outputgroup, probeMolecules_mol, boxSpace):
288     """
289     create a system to hold all of the molecules and forcefields
290     Return the created system and the forcefield energy component
291     that you want to evaluate
292     """
293     system = System()
294     interff = InterFF("int1")
295     cljfunc = CLJShiftFunction()
296     #cljfunc.setCoulombCutoff(0 * angstrom)

```

```

296     #cljfunc.setLJCutoff(15 * angstrom)
297     cljfunc.setSpace(boxSpace)
298     interff.setCLJFunction(cljfunc)
299     #interff.setSwitchingFunction(cljfunc)
300     interff.setUseParallelCalculation(True)
301     system.setProperty("space", boxSpace)
302     outputgroup.add(probeMolecules_mol)
303     interff.add(outputgroup)
304     #interff.add(probeMolecules_mol)
305     system.add(interff)
306     system.add( SpaceWrapper(Vector(0), outputgroup) )
307     system.add(outputgroup)
308     return (system, interff.components().lj())
309
310 def loop_division2(nReplicates,system,residue):
311     """
312         function to determine steric clashes
313     """
314     throws = 0
315     count = 0
316     energyThreshold = 50000
317     for i in range(nReplicates):
318         throws += 1
319         random_translate = rangen.vectorOnSphere( max_translation.value()
320                                         () )
320         random_rotate_axis = rangen.vectorOnSphere()
321         random_rotate_angle = rangen.rand(-max_rotation.value(),
322                                         max_rotation.value()) * degrees
323         random_Molecule = system[MolWithResID(residue)].molecule()
324         moved_Molecule = random_Molecule.move() \
325                         .rotate( \
326                             Quaternion(random_rotate_angle,random_rotate_axis
327                                         ), \
327                                         random_Molecule.evaluate().center() ) \
328                                         .translate(random_translate).commit()
329         system.update(moved_Molecule)
330         output_energy = system.energy(lj_comp).value()
331         #print("energy of testing system=%s and defult system =%r" %(
332             output_energy, energyThreshold))
332         # print("(took %s ms)" % (0.000001*ns))
333         #output_energy = if_function_energy(output_energy, tolerance)
334         montecarlotest = if_function(output_energy,energyThreshold)
335         count += montecarlotest
336         V_free = (count/throws)*output_vol
337         p = count/throws

```

```

338     std_binom = sqrt(throws*p*(1-p))
339     error_binom = (output_vol/throws)*std_binom
340     error_binom_fraction = error_binom/V_free
341     #if i % 100 == 0:
342         #print("STEPS %d: BINOMIAL ERROR T.VOLUME %s: ERROR F.VOLUME
343             %s: ESTIMATED VOLUME %s:" %(i, error_binom,
344                 error_binom_fraction,p))
345     system.clearStatistics()
346     system.mustNowRecalculateFromScratch()
347     SystemExit(system)
348     ratio = count/nReplicates
349     return ratio
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375

```

---

```
376     steps = str(steps)
377     value1 = str(value1)
378     value2 = str(value2)
379     #open log file and combine values and write in it
380     with open (filename, 'w') as f1:
381         f1.write('Steps = '+steps) + (string1 + value1) + (string2 +
382             value2) +'\\n')
383     f1.close()
384     return
385
386 def statistics_test(avg,output_vol,output):
387     """
388     function to determine the statistics of estimated volume.
389     the inputs are ...
390     (avg list of output volumes)steps*dsteps
391     (volume of box)
392     (number of steps)
393     output: print out t-statistics
394     """
395     p = sum(avg) / len(avg)
396     V_free = p*output_vol
397     std_binom = sqrt(output*p*(1-p))
398     error_binom = (output_vol/output)*std_binom
399     error_binom_fraction = error_binom/V_free
400     print("STEPS %s: BINOMIAL ERROR T.VOLUME %s: ERROR F.VOLUME %s:
401           ESTIMATED AVAILABLE VOLUME %s:" %(output, error_binom,
402             error_binom_fraction*100,p))
403     return
404
405 def line_extract(myfile):
406     """
407     utility function for a text file
408     """
409     for line in myfile:
410         print()
411     return line
412
413 def removeCommonElements(a, b):
414     """
415     utility function for a text file
416     """
417     for e in a[:]:
418         if e in b:
419             a.remove(e)
420             b.remove(e)
```

```

418     return a
419
420 def coordinates_check(total_cord):
421     """
422         function to make sure, the box dimensions are greater than minimum
423         required value
424     """
425     total_final1 = []
426     total_final2 = []
427     for i in total_cord[0:3]:
428         if i < -149.99:
429             total_final1.append(i)
430         else:
431             total_final1.append(-150.00)
432
433     for i in total_cord[-3:]:
434         if i > 149.99:
435             total_final2.append(i)
436         else:
437             total_final2.append(150.00)
438     total_final = total_final1+total_final2
439     return total_final
440
441 def radgyr(mol):
442     """
443         function to determine the radius of gyration. the function take a
444         molecule as an input
445     """
446     #step 1. determine centre of mass
447     cmass = mol.evaluate().centerOfMass()
448     #step 2. calculate the radius of gyration
449     vector_collection = []
450     for i in range(mol.nAtoms()):
451         atomicCenter = mol.atom(AtomIdx(i)).evaluate().centerOfMass()
452         difference = ((atomicCenter - cmass).magnitude())**2
453         vector_collection.append(difference)
454     summation = sum(vector_collection)
455     radgyr = sqrt(summation/len(vector_collection))
456     return radgyr
457
458 def collect_radgyr_box(sim_mols):
459     """
460         function to determine and save the radius of gyration and volume in
461         a list. the function take box as an input

```

```

460 """
461 #step 1. collect molecules
462 sim_mols = collect_mc_molecs(sim_mols)
463 rad = []
464 vol = []
465 for molnum in sim_mols.molNums():
466     mol = sim_mols[molnum].molecule()
467     radgyrout = radgyr(mol)
468     volume = (4/3)*pi*(pow(radgyrout,3))
469     vol.append(volume)
470     rad.append(radgyrout)
471 return (rad,vol)
472
473 def list_of_energy_system(simSystem, probMolecule, boxSpace):
474 """
475     Function to generate the systems (molecule groups) to be executed
476         in a parallel fashion.
477     Inputs: Simulation boxes, probe molecule, Box dimensions
478 """
479 residue = probMolecule.residues(ResIdx(0)).residue().name().value()
480 system = System()
481 system.add(simSystem)
482 system.add(probMolecule)
483 system.setProperty("space", boxSpace)
484 random_translate = rangen.vectorOnSphere(max_translation.value())
485 random_rotate_axis = rangen.vectorOnSphere()
486 random_rotate_angle = rangen.rand(-max_rotation.value(),
487                                     max_rotation.value()) * degrees
488 random_water = system[MolWithResID(residue)].molecule()
489 moved_water = random_water.move() \
490             .rotate( \
491                 Quaternion(random_rotate_angle,random_rotate_axis), \
492                 random_water.evaluate().center()) \
493             .translate(random_translate).commit()
494 system.update(moved_water)
495 #PDB().write(system.molecules(),"parallel_random_translate.pdb")
496 new_sysm = collect_mc_molecs(system)
497 return new_sysm
498
499 def is_there_overlap_vwd(n):
500 """
501     Function to determine whether there is overlapping in the given
502         system or not by estimating parallel distances.
503     input : length of input system_list
504 """

```

```

503     count = 0
504     system_from_list = new_systems_list[n]
505     output_vwd = steric_closmol_clashes_vdw3(system_from_list,ResID)
506     count += output_vwd
507     return count
508
509 def multi_processing_files(input_system):
510
511     """
512         Function to run system_lists over multiprocessing using all
513             available cores.
514         Input: system_lists and box dimensions
515     """
516     import multiprocessing
517
518     if __name__=='__main__':
519         np = multiprocessing.cpu_count()
520         print ('You have {0:1d} CPUs'.format(np))
521         n = len(input_system)
522         part_count=[n/np for i in range(np)]
523         print(part_count)
524         pool = multiprocessing.Pool(processes=np)
525         count=pool.map(is_there_overlap_vwd, range(n))
526         accepted_moves = (sum(count))/n
527         return (accepted_moves, pool)
528
529 def steric_closmol_clashes_vdw3(boxMolecs,ResID):
530
531     """
532         Function to determine steric clashes using van der waal radii.
533         Input: simulation system containing probe molecule
534     """
535     boxMolecs.setName("boxMolecs")
536     p = PointRef(boxMolecs[MolWithResID(ResID)].molecule().evaluate().
537                  centerOfGeometry())
538     #p = PointRef(boxMolecs[number].molecule().evaluate().
539     #              centerOfGeometry())
540     c = CloseMols(p,boxMolecs,boxMolecs.nMolecules())
541     g_random = c.closeMolecules()
542     #print(g_random)
543     g = sorted(g_random.items(), key=operator.itemgetter(1)) # sort
544         the dict by value which give tuple
545     #gg = extract_fn(g)
546     print(g)
547     gg = [(mol_num, distance) for mol_num, distance in g if 10 <
548           distance < 70]

```

```

543     print(gg)
544     t = [x[0] for x in gg] # extract sorted with respect to distance
545             molnumbers from tuple
546     #print(t) # sorted molnumbers
547     t_dist = [x[1] for x in gg]
548     print(t_dist) # sorted distances
549     ## select the molecules with condition 10<dist<70
550     selected = boxMolecs[MolWithResID(ResID)].molecule()
551     #selected = boxMolecs[number].molecule()
552     closMol = boxMolecs[t[1]].molecule()
553     if len(t) < 1:
554         return 1
555     else:
556         for i in range(1,len(t)):
557             simSystem_st = boxMolecs[t[i]].molecule()
558             #print(simSystem_st)
559             for j in range(selected.nAtoms()):
560                 icent = selected.atom(AtomIdx(j)).evaluate().center()
561                 vdwprobMolecule = selected.atom(AtomIdx(j)).property("element")
562                 vdwprobMoleculevalue = Element(vdwprobMolecule).vdwRadius().value()
563                 #print(vdwprobMoleculevalue)
564                 #print(vdwprobMolecule)
565                 for k in range(simSystem_st.nAtoms()):
566                     dist = Vector.distance(icent, simSystem_st.atom(
567                         AtomIdx(k)).evaluate().center())
568                     #print("distance of each background atom =%s" % dist)
569                     vdwsimSystem= simSystem_st.atom(AtomIdx(k)).property("element")
570                     vdwsimSystemvalue= Element(vdwsimSystem).vdwRadius().value()
571                     vdwsum= vdwprobMoleculevalue+vdwsimSystemvalue
572                     #print(vdwsum)
573                     if dist<vdwsum:
574                         return 0 #print ("there is a steric clash")
575     return 1 #print ("there is no steric clashes")
576
577 def gen_list(mol):
578     """
579         Function to generate the list of molecules. mol is amber inp/top
580             file
581     """
582     mol_list = []
583     for i in mol.molNums():

```

```

581         j = mol[i].molecule()
582         mol_list.append(j)
583     return mol_list
584
585 def vectorize_distances(mol,mol_list):
586     """
587     function to collect distances between molecules
588     """
589     #dist_list1 = []
590     dist_list2 = []
591     #mol = mol_list[i]
592     #m1 = mol.evaluate().centerOfMass()
593     m2 = mol.evaluate().centerOfGeometry()
594     #del mol_list[i]
595     for i in range(0,len(mol_list)):
596         #dist1 = Vector.distance(m1,mol_list[i].evaluate().centerOfMass
597         ())
597         dist2 = Vector.distance(m2,mol_list[i].evaluate().
598             centerOfGeometry())
598         #dist_list1.append(dist1)
599         dist_list2.append(dist2)
600     return dist_list2
601
602 def list_joiner(parentList,newList):
603     """
604     Function to manipulate lists
605     """
606     totalList = [parentList,newList]
607     return totalList
608
609 def cluster_pdb(mol,remList,indx,set_no_mols):
610     """
611     Function to save a cluster as a pdb
612     """
613     mols = MoleculeGroup()
614     mols.add(mol)
615     for i in indx:
616         mols.add(remList[i])
617     mol_name = mols.nMolecules()
618     name = 'peg_'+str(mol_name)+'_'+str(set_no_mols)+'.pdb'
619     PDB().write(mols.molecules(),name)
620     return mols
621
622 def delete_cluster(mols,mol,remList,indx,set_no_mols):
623     """

```

```

624     function that deletes the cluster from the list and then give the
       new list of remaing molecules.
625     """
626     #print(mols.nMolecules())
627     #print(remList)
628     if int(mols.nMolecules()) > 1:
629         for i in indx:
630             del remList[i]
631     else :
632         remList
633         name = 'peg_1_'+str(set_no_mols)+'.pdb'
634         PDB().write(mol.molecule(),name)
635
636     return remList
637
638 def main_program(mol_list,clstr_tolrce):
639     """
640     Function to iterrate the whole program regarding cluster extraction
       . Not used in this script.
641     """
642     col = []
643     for i in range(0,1): #len(mol_list)
644         mol = mol_list[0]
645         remList = mol_list[0:i:]+mol_list[i+1::]
646         d1 = vectorize_distances(mol,remList)
647         #print(d1)
648         for i in d1:
649             if i < clstr_tolrce:
650                 k = d1.index(i)
651                 col.append(k)
652                 col.sort()
653                 col = col[::-1]
654                 #print(col)
655     return mol,remList,d1,col
656
657 def acc_molecules(presnt_mols,remList,indx,mol_grp,box):
658     """
659     Function to accumulate molecules in a cluster.
660     """
661     futr_mols = []
662     # step 1. remove the childern mols from the list and make it as a
       list of present mol for next iterration
663     if len(indx) > 0:
664         #print('phase 1 start')
665         for ind in indx:

```

```

666         ind.sort()
667         ind = ind[::-1]
668         for i in ind:
669             futr_mols.append(remList[i])
670             mol_grp.add(remList[i])
671             del remList[i]
672             #print(remList)
673             #print('phase 1 ends')
674             # step 2. if futr_mols > 0 then keep the mol_grp
675             if len(indx) == 0:
676                 #print('phase 2 start')
677                 set_no_mols = len(remList)
678                 mol_name = mol_grp.nMolecules()
679                 name = 'peg_'+box+str(mol_name)+'_'+str(set_no_mols)+'.pdb'
680                 PDB().write(mol_grp.molecules(),name)
681                 mol_grp.removeAll()
682                 if len(remList) > 0:
683                     mol = remList[0]
684                     futr_mols.append(mol)
685                     mol_grp.add(mol)
686                     remList = remList[0:0:]+remList[0+1::]
687                     #print('phase 2 ends')
688                     #print(remList)
689             return futr_mols, remList, mol_grp
690
691     def cluster_search(mol,remList,clstr_tolrce):
692         """
693             Function that gives the list of molecules as an index that are part
694             of a cluster.
695         """
696         clust_list = []
697         clust_dist = []
698         clust_list2 = []
699         clust_dist2 = []
700         if len(mol) == 1:
701             #print('running step 1')
702             d1 = vectorize_distances(mol[0],remList)
703             clust_list.append(d1)
704             #print('step 1 done')
705             #print('running step 3')
706             #print(len(clust_list))
707             for i in clust_list[0]:
708                 if i < clstr_tolrce:
709                     k = clust_list[0].index(i)
710                     clust_dist2.append(k)

```

```

710         clust_list2.append(clust_dist2)
711         #print(len(clust_list2))
712         #print('step 3 done')
713     else:
714         #print('running step 2')
715         for i in mol:
716             d1 = vectorize_distances(i, remList)
717             #print(d1)
718             #print(i)
719             clust_dist.append(d1)
720             clust_list.append(d1)
721             #print(clust_list)
722             #print('step 2 done')
723             #print('running step 4')
724             for i in clust_list:
725                 #print('number of molec', len(clust_list))
726                 for q in i:
727                     #print(q)
728                     if q < clstr_tolrce:
729                         l = i.index(q)
730                         #print(l)
731                         clust_dist2.append(l)
732                         #print(clust_dist2)
733                         clust_list2.append(clust_dist2)
734                         #print(clust_list2)
735                         #print('step 4 done')
736             return clust_list2
737
738 def remove_lists(indx):
739 """
740     function to remove same lists from the indx
741 """
742     if len(indx) > 1:
743         indx2 = []
744         indx2.append(indx[0])
745         for i in indx:
746             if indx[0] != i:
747                 indx2.append(i)
748         print(indx2)
749     else:
750         indx2 = indx
751     return indx2
752
753 def average(list):
754 """

```

```
755     function to determine the average of the list:  
756  
757     """  
758     average = float(sum(list))/float(len(list))  
759     return average  
760  
761 def stanDev(values):  
762     """  
763     function to find the standard deviation:  
764     """  
765     length = len(values)  
766     mean = average(values)  
767     total_sum = 0  
768     for i in range(length):  
769         total_sum += (values[i]-mean)**2  
770     std = sqrt(total_sum/length)  
771     return std  
772  
773 def collect_values(inputfile):  
774     """  
775     function to collect fraction available volume from output log file.  
776     example :  
777     system_files = [f for f in os.listdir() if f.endswith('.log')]  
778     with open ('volume_2k96_c2.log','w') as f1:  
779     for file in system_files:  
780         volume = collect_values(file)  
781         f1.write(volume+'\n')  
782     f1.close()  
783  
784     """  
785     file = open(inputfile,'r')  
786     for line in file:  
787         volume = line.split(',') [1].split() [3]  
788     return volume  
789  
790 def remove_charge_RNA_from_swap_sys(box1_mols):  
791     """  
792     function to remove charge, RNA, and exact number of water Molecules  
     from both boxes systems (s3 files)  
793     input: control.s3 and waterbox.s3  
794     """  
795     box1_mols= collect_mc_molecs(box1_mols)  
796     rna_residmolecule = box1_mols[ MolWithResID("RG5") ].molecule()  
797     nwaters = rna_residmolecule.evaluate().mass().value()/18.01528  
798     box1_mols.remove(rna_residmolecule)
```

```

799     charge_mass = 0
800     for molnum in box1_mols.molNums():
801         RNA_Charge = box1_mols[molnum].molecule()
802         if RNA_Charge.nAtoms() ==1:
803             box1_mols.remove(RNA_Charge)
804             charge_mass += RNA_Charge.evaluate().mass().value()/18.01528
805         print("charge ions mass in given system %s" % charge_mass)
806         nwaters = nwaters+charge_mass
807     return (box1_mols,nwaters)
808
809 def remove_nwaters_from_waterbox_sys(box2_mols,nwaters):
810     """
811     function to remove charge, RNA, and exact number of water Molecules
812     from both boxes systems (s3 files)
813     input: control.s3 and waterbox.s3
814     """
815     box2_mols= collect_mc_molecs(box2_mols)
816     nwaters = int(nwaters)
817     for i in range(nwaters):
818         nwater = box2_mols[MolIdx(i)].molecule()
819         box2_mols.remove(nwater)
820     return (box2_mols)
821
822 def add_swap_water_rna_sys(box1_mols,box2_mols,swapWATERS,swapRNA):
823     """
824     function to remove charge, RNA, and exact number of water Molecules
825     from both boxes systems (s3 files) and add swapWATER and
826     swapRNA to these systems (s3). before adding these groups the
827     systems are converted to groups using "collect_mc_molecule"
828     functions
829     input: control.s3 and waterbox.s3
830     ****
831
832     Function use to swap RNA and water molecules between two boxes.
833     where box1_mols is box containing RNA and background molecules
834     while box2_molecules is purely water box where RNA will be
835     placed and from there number of water molecules are moved to
836     box1_mols to keep density fix. It gives two boxes back as output
837     ...
838     Input: box1_mols, box2_mols
839     """
840     box1_mols.add(swapWATERS)
841     box2_mols.add(swapRNA)
842     return (box1_mols,box2_mols)
843

```

```

833 def charge_swap(box1_mols,box2_mols):
834     """
835         function to swap charges between two boxes
836     """
837     charge_mass = 0
838     for molnum in box1_mols.molNums():
839         RNA_Charge = box1_mols[molnum].molecule()
840         if RNA_Charge.nAtoms() ==1:
841             box1_mols.remove(RNA_Charge)
842             charge_mass += RNA_Charge.evaluate().mass().value()
843             box2_mols.add(RNA_Charge)
844     return (box1_mols,box2_mols,charge_mass)
845
846 def steric_closmol_clashes_vdw2(boxMolecs,ResID):
847     boxMolecs.setName("boxMolecs")
848     p = PointRef(boxMolecs[MolWithResID(ResID)].molecule().evaluate().
849                 centerOfGeometry())
849     #p = PointRef(boxMolecs[number].molecule().evaluate().
850                 centerOfGeometry())
850     c = CloseMols(p,boxMolecs,3)
851     g_random = c.closeMolecules()
852     #print(g_random)
853     g = sorted(g_random.items(), key=operator.itemgetter(1)) # sort
854     #print(g)
855     t = [x[0] for x in g] # extract sorted with respct to distance
856     molnumbers from tuple
856     t_dist = [x[1] for x in g]
857     #print(t_dist[1])
858     selected = boxMolecs[MolWithResID(ResID)].molecule()
859     #selected = boxMolecs[number].molecule()
860     closMol = boxMolecs[t[1]].molecule()
861     if t_dist[1] > 70:
862         return 1
863     else:
864         if t_dist[1] < 10:
865             return 0
866         else:
867             for i in range(1,len(t)):
868                 peg50_st = boxMolecs[t[i]].molecule()
869                 #print(peg50_st)
870                 for j in range(selected.nAtoms()):
871                     icent = selected.atom(AtomIdx(j)).evaluate().center()
872                     vdwrna = selected.atom(AtomIdx(j)).property("element"
873 )

```

```

873         vdwrnavalue = Element(vdwrna).vdwRadius().value()
874         #print(vdwrnavalue)
875         #print(vdwrna)
876         for k in range(peg50_st.nAtoms()):
877             dist = Vector.distance(cent, peg50_st.atom(
878                 AtomIdx(k)).evaluate().center())
879             #print("distance of each background atom =%s" %
880                 dist)
881             vdwpeg50=peg50_st.atom(AtomIdx(k)).property("element")
882             vdwpeg50value= Element(vdwpeg50).vdwRadius().value()
883             vdwsum= vdwrnavalue+vdwpeg50value
884             #print(vdwsum)
885             if dist<vdwsum:
886                 return 0 #print ("there is a steric clash")
887             return 1 #print ("there is no steric clashes")
888
889     def Minimization_criteria(dex50,box_space):
890         """
891             Function to determine wether there is need to run rigid body moves
892                 to remove steric clashes in input system.
893             the system is then use for excluded volume measurment based on
894                 energy calculations.
895             the input is system, if the energy of the control system is
896                 greather than 1000, then it will run the 100000
897             moves to stabilizes the system and then perform 800000 steps of
898                 excluded volume. it will save the system after
899             mc for furhter use.
900             """
901
902             #if system_energy_control < 1000:
903             #print("No need to perform MC simulation for minimization")
904             #else:
905             systemdex50=energy_minimize_system_LJ_CL(dex50,box_space)
906             (systemdex50mc,wt_moves)=NVT_ensemble_moves(systemdex50)
907             print("Running Minimization Monte Carlo")
908             for i in range(1,21):
909                 systemdex50mc = wt_moves.move(systemdex50mc,500,True)
910                 if i % 5 ==0:
911                     print("System energy %s after running 5000 moves in 5th
912                         blocks"% systemdex50mc.energy())
913             Sire.Stream.save(systemdex50mc,"minimizedbox.s3")
914             dex50 = collect_mc_molecs(systemdex50mc)
915             return dex50

```

```

909 def list_of_energy_system(peg50, rna_mol, boxSpace):
910     """
911         Function to generate the systems (molecule groups) which are going
912             to append to the list...
913         Inputs: background molecules, RNA molecule, Box space
914         ## New function where systems are appended to a list. and then call
915             then to determine energy
916         """
917         randomvalues = createRandomValuesforRotationTranslation(boxSpace)
918         newMolec = rotateTranslateMolec(randomvalues, rna_mol)
919         new_systm = makemolgroups(peg50, newMolec)
920         return new_systm
921
922
923 def multi_processing_files_distances(input_system):
924
925     Function to run system_lists over multiprocessing using all
926         available cores
927     Input: system_lists and box volume
928
929
930     # Multiprocessing code script
931
932     import multiprocessing
933
934     if __name__=='__main__':
935         np = multiprocessing.cpu_count()
936         print ('You have {0:1d} CPUs'.format(np))
937         n = len(new_systems_list)
938         part_count=[n/np for i in range(np)]
939         print(part_count)
940         pool = multiprocessing.Pool(processes=np)
941         count=pool.map(is_there_overlap_energy, range(n))
942         print ("Number of accepted moves ",(sum(count))/n)
943         pool.close()
944
945     import multiprocessing
946     if __name__=='__main__':
947         np = multiprocessing.cpu_count()
948         print ('You have {0:1d} CPUs'.format(np))
949         n = len(input_system)
950         part_count=[n/np for i in range(np)]
951         print(part_count)
952         pool = multiprocessing.Pool(processes=np)
953         countt=pool.map(is_there_overlap_vwd, range(n))
954         acceptedmoves = (sum(countt))/n

```

```

951     return (acceptedmoves,pool)
952
953 def multi_processing_files_energy(input_system,output_vol):
954
955     Function to run system_lists over multiprocessing using all
956         available cores
957     Input: system_lists and box volume
958
959     import multiprocessing
960
961     if __name__=='__main__':
962         np = multiprocessing.cpu_count()
963         print ('You have {0:1d} CPUs'.format(np))
964         n = len(input_system)
965         part_count=[n/np for i in range(np) ]
966         print(part_count)
967         pool = multiprocessing.Pool(processes=np)
968         count=pool.map(is_there_overlap_energy, range(n) )
969         accepted_moves = (sum(count))/n
970         #pool.close()
971         #V_free = accepted_moves*output_vol
972         #p = accepted_moves
973         #std_binom = sqrt(n*p*(1-p))
974         #error_binom = (output_vol/n)*std_binom
975         #error_binom_fraction = error_binom/V_free
976         #print("SINGLE SYSTEM STEPS %d: BINOMIAL ERROR T.VOLUME %s:
977             ERROR F.VOLUME %s: ESTIMATED VOLUME %s:" %(n, error_binom,
978             error_binom_fraction,p))
979     return (accepted_moves, pool)
980
981 def multi_processing_files_dist(input_system):
982
983     Function to run system_lists over multiprocessing using all
984         available cores
985     Input: system_lists and box volume
986
987     import multiprocessing
988
989     if __name__=='__main__':
990         np = multiprocessing.cpu_count()
991         print ('You have {0:1d} CPUs'.format(np))
992         n = len(input_system)
993         part_count=[n/np for i in range(np) ]
994         print(part_count)
995         pool = multiprocessing.Pool(processes=np)

```

```

992     count=pool.map(is_there_overlap_energy, range(n))
993     accepted_moves = (sum(count))/n
994     #pool.close()
995     #V_free = accepted_moves*output_vol
996     #p = accepted_moves
997     #std_binom = sqrt(n*p*(1-p))
998     #error_binom = (output_vol/n)*std_binom
999     #error_binom_fraction = error_binom/V_free
1000    #print("SINGLE SYSTEM STEPS %d: BINOMIAL ERROR T.VOLUME %s:
1001        ERROR F.VOLUME %s: ESTIMATED VOLUME %s:" %(n, error_binom,
1002          error_binom_fraction,p))
1003    return (accepted_moves, pool)
1004
1005 def list_maker_for_multi(n,peg50, rna_mol, boxSpace):
1006 """
1007 Function to generates lists by appending systems from "list_of_energy_system(peg50, rna_mol, boxSpace)" function
1008 The function uses the "list_of_energy_system(peg50, rna_mol, boxSpace)" function...
1009 Inputs: number of systems in each list, background molecules, RNA molecule, Box space
1010 """
1011 new_systems_lists = []
1012 for i in range(n):
1013     new_systems = list_of_energy_system(peg50, rna_mol, boxSpace)
1014     new_systems_lists.append(new_systems)
1015 return new_systems_lists
1016
1017 def is_there_overlap_energy(n):
1018 """
1019 Function to determine wether there is overlapping in the given system or not by estimating energy.
1020 input : length of input system_list
1021 Note: the system_lists name should be (new_systems_list)
1022 """
1023 count = 0
1024 system_from_list = new_systems_list[n]
1025 output_energy = default_system_energy(system_from_list,boxSpace)
1026 montecarlotest = if_function(output_energy,
1027     percent_increase_tolerance)
1028 count += montecarlotest
1029 return count
1030
1031 def intrasclae_Na(na):
1032 """

```

```

1030     Interscale function to fix the interscale property of ions;
1031     this function is only for sodium, In order to work with other ions
1032     it needs to change the atom name according to inpcrd and prmtop
1033     input files:
1034     Function Input= Molecule
1035     """
1036     sclpairs = CLJNBPairs(na)
1037     sclpairs.set( AtomName("Na+"), AtomName("Na+"), CLJScaleFactor(0,0)
1038                 )
1039     na = na.edit().setProperty("intrascale", sclpairsna) \
1040           .commit()
1041     return na
1042
1043
1044     def intrasclae_Cl(na):
1045         """
1046         Interscale function to fix the interscale property of ions;
1047         this function is only for sodium, In order to work with other ions
1048         it needs to change the atom name according to inpcrd and prmtop
1049         input files:
1050         Function Input= Molecule
1051         """
1052         sclpairs = CLJNBPairs(na)
1053         sclpairs.set( AtomName("Cl-"), AtomName("Cl-"), CLJScaleFactor(0,0)
1054                     )
1055         na = na.edit().setProperty("intrascale", sclpairsna) \
1056               .commit()
1057     return na
1058
1059
1060     def sodium_charge_group(rna):
1061         """
1062             Function to generate the new molecule group used to estimate
1063             the system intraCLJFF force field energetics. This function use
1064             the intrascale_Na function:
1065
1066             Function input: molecule group/direct molecule group from amber
1067             files and make sure the RNA/Protein ResID is similar to
1068             function
1069             """
1070
1071             tt = MoleculeGroup()
1072             tt.add(rna[MolWithResID("RG5")].molecule())
1073             for molnum in rna.molNums():
1074                 t = rna[molnum].molecule()
1075                 if t.nAtoms() == 1:
1076                     t = intrasclae_Na(t)
1077                     tt.add(t)

```

```

1073     return tt
1074
1075 def chloride_charge_group(rna):
1076     """
1077         Function to generate the new molecule group used to estimate
1078         the system intraCLJFF force field energetics. This function use
1079         the intrascale_Na function:
1080
1081         Function input: molecule group/direct molecule group from amber
1082         files and make sure the RNA/Protein ResID is similar to
1083         function
1084         """
1085     tt = MoleculeGroup()
1086     tt.add(rna[MolWithResID("RG5")].molecule())
1087     for molnum in rna.molNums():
1088         t = rna[molnum].molecule()
1089         if t.nAtoms() == 1:
1090             t = intrascale_Cl(t)
1091             tt.add(t)
1092     return tt
1093
1094 def createRandomValuesforRotationTranslation(boxSpace):
1095     """
1096         This function creates a random position, a random orientation
1097         vector and a random angle. It returns a list with these three
1098         components.
1099
1100     Function Input: boxspace
1101     """
1102     v=Vector(0,0,0) # Creates a vector for the random point generation
1103     #rand_seed = 140154
1104     gen=RanGenerator() # Creates a random generator object
1105     insertion_point=boxSpace.getRandomPoint(v,gen) # Creates a random
1106         point within the confinements of the box
1107     orientation_vector=gen.vectorOnSphere() # Creates a random
1108         orientation vector
1109     orientation_angle=gen.rand(-two_pi,two_pi)*radians # Creates a
1110         random orientation angle
1111     output=(insertion_point,orientation_vector,orientation_angle) #
1112         Creates the output list
1113     return output
1114
1115 def createRandomValuesforRotationTranslation2(boxSpace):
1116     """
1117         This function creates a random position, a random orientation

```

```

1114     vector and a random angle. It returns a list with these three
1115     components.
1116
1117     Function Input: boxspace
1118     """
1119     v=Vector(0,0,0) # Creates a vector for the random point generation
1120     #rand_seed = 140154
1121     gen=RanGenerator() # Creates a random generator object
1122     max_translation = 150 * angstrom
1123     max_rotation = 360 * degrees
1124     random_translate = gen.vectorOnSphere( max_translation.value() )
1125     random_rotate_axis = gen.vectorOnSphere()
1126     random_rotate_angle = gen.rand(-max_rotation.value(),
1127                                     max_rotation.value()) * degrees
1128     #insertion_point=boxSpace.getRandomPoint(v,gen) # Creates a random
1129     # orientation_vector=gen.vectorOnSphere() # Creates a random
1130     # orientation vector
1131     #orientation_angle=gen.rand(-two_pi,two_pi)*radians # Creates a
1132     # random orientation angle
1133     output=(random_translate,random_rotate_axis,random_rotate_angle) #
1134     # Creates the output list
1135     return output
1136
1137     def percise_swap_water_rna(box1_mols_pdb,box2_mols_pdb):
1138     """
1139     Function to remove and add water molecules more percisely the
1140     places where the RNA is removed and inserted.
1141     this function removes and adds water molecules simultanously from
1142     both boxes while keeping the RNA as
1143     removed variable. when water molecules addition/removal done, then
1144     the rna molecule is added to to the waterbox.
1145     Inputs: boxgroup of rna+peg_removeRNA,waterbox_removewater,
1146             rna_tobeinserted,waterbox_tobetakentoinsert
1147
1148     this function also care about charges
1149     """
1150
1151     # UPLOAD SYSTEMS
1152     box1_mols_pdb= collect_mc_molecs(box1_mols_pdb)
1153     box2_mols_pdb= collect_mc_molecs(box1_mols_pdb)
1154     RNA_removed = box1_mols_pdb[MolWithResID("RG5")].molecule()
1155     box1_mols_pdb.remove(RNA_removed)
1156     RNA_removed = RNA_removed.edit().renumber().commit()
1157     nwaters = RNA_removed.evaluate().mass().value()

```

```

1151     charge_mass = 0
1152     charge_holder_group = MoleculeGroup()
1153     water_holder_group = MoleculeGroup()
1154     # COLLECT CHARGES
1155     for molnum in box1_mols_pdb.molNums():
1156         RNA_Charge = box1_mols_pdb[molnum].molecule()
1157         if RNA_Charge.nAtoms() ==1:
1158             box1_mols_pdb.remove(RNA_Charge)
1159             charge_holder_group.add(RNA_Charge)
1160             charge_mass += RNA_Charge.evaluate().mass().value()
1161     print("charge ions mass in given system %s" % charge_mass)
1162     nwaters = (nwaters+charge_mass)/18.01528
1163     nwaters = int(nwaters)
1164     print("number of water swap by rna =%s"%nwaters)
1165     # COLLECT WATERS
1166     for j in range(nwaters):
1167         RNA_removed_ref_point = PointRef(RNA_removed.atoms(AtomIdx(j)) .
1168                                         evaluate().center())
1169         #print("center of %s atom =%s"%(j, RNA_removed_ref_point.point())
1170         )
1171         p = RNA_removed_ref_point.point()
1172         c = CloseMols(p,box2_mols_pdb,10)
1173         g_random = c.closeMolecules()
1174         g = sorted(g_random.items(), key=operator.itemgetter(1))
1175         t = [x[0] for x in g]
1176         for i in range(0,len(t)):
1177             removal_water = box2_mols_pdb[t[i]].molecule()
1178             if removal_water.nAtoms() == 3:
1179                 box2_mols_pdb.remove(removal_water)
1180                 center_geometry =
1181                     createRandomValuesforRotationTranslationofSwapwaterMolecs
1182                     (p)
1183                     removal_water = rotateTranslateMolec(center_geometry,
1184                     removal_water)
1185                     water_holder_group.add(removal_water)
1186     print("number of water molecules swapped from waterbox =%s%" %
1187           water_holder_group.nMolecules())
1188     # SWAPPING OF WATER AND RNA MOLECULES NOW
1189     print("swaping now ...")
1190     box2_mols_pdb.add(RNA_removed)
1191     for molnum in charge_holder_group.molNums():
1192         charge_mol = charge_holder_group[molnum].molecule().edit()
1193             renumber().commit()
1194             box2_mols_pdb.add(charge_mol)
1195     for molnum in water_holder_group.molNums():

```

```

1189         water_mol = water_holder_group[molnum].molecule().edit() .
1190             renumber().commit()
1191         box1_mols_pdb.add(water_mol)
1192     print("done")
1193     output = (box1_mols_pdb, box2_mols_pdb)
1194     return output
1195
1196 def wswap_water(s1,s2):
1197     """
1198         Function to remove and add water molecules more percisely the
1199             places where the RNA is removed and inserted.
1200         this function removes and adds water molecules simultanously from
1201             both boxes while keeping the RNA as
1202             removed variable. when water molecules addition/removal done, then
1203                 the rna molecule is added to to the waterbox.
1204         Inputs: boxgroup of rna+peg_removerNA,waterbox_removewater,
1205                 rna_tobeinserted,waterbox_tobetakentoinsert
1206
1207         this function also care about charges
1208     """
1209     box1_mols_pdb= collect_mc_molecs(s1)
1210     box2_mols_pdb= collect_mc_molecs(s2)
1211     RNA_removed = box1_mols_pdb[MolWithResID("RG5")].molecule()
1212     box1_mols_pdb.remove(RNA_removed)
1213     RNA_removed = RNA_removed.edit().renumber().commit()
1214     nwaters = RNA_removed.evaluate().mass().value()
1215     charge_mass = 0
1216     charge_holder_group = MoleculeGroup()
1217     water_holder_group = MoleculeGroup()
1218     # COLLECT CHARGES
1219     for molnum in box1_mols_pdb.molNums():
1220         RNA_Charge = box1_mols_pdb[molnum].molecule()
1221         if RNA_Charge.nAtoms() ==1:
1222             box1_mols_pdb.remove(RNA_Charge)
1223             charge_holder_group.add(RNA_Charge)
1224             charge_mass += RNA_Charge.evaluate().mass().value()
1225     print("charge ions mass in given system %s" % charge_mass)
1226     nwaters = (nwaters+charge_mass)/18.01528
1227     nwaters = int(nwaters)
1228     print("number of water swap by rna =%s"%nwaters)
1229     # COLLECT WATERS
1230     for j in range(nwaters):
1231         RNA_removed_ref_point = PointRef(RNA_removed.atoms(AtomIdx(j)) .
1232             evaluate().center())

```

```

1227     print("center of %s atom =%s"%(j, RNA_removed_ref_point.point())
1228         )
1229     p = RNA_removed_ref_point.point()
1230     c = CloseMols(p, box2_mols_pdb, 10)
1231     g_random = c.closeMolecules()
1232     g = sorted(g_random.items(), key=operator.itemgetter(1))
1233     t = [x[0] for x in g]
1234     for i in range(0, len(t)):
1235         removal_water = box2_mols_pdb[t[i]].molecule()
1236         if removal_water.nAtoms() == 3:
1237             box2_mols_pdb.remove(removal_water)
1238             center_geometry =
1239                 createRandomValuesforRotationTranslationofSwapwaterMolecs
1240                 (p)
1241             removal_water = rotateTranslateMolec(center_geometry,
1242             removal_water)
1243             water_holder_group.add(removal_water)
1244             print("number of water molecules swapped from waterbox =%s"%
1245                 water_holder_group.nMolecules())
1246             # SWAPPING OF WATER AND RNA MOLECULES NOW
1247             print("swaping now ...")
1248             box2_mols_pdb.add(RNA_removed)
1249             for molnum in charge_holder_group.molNums():
1250                 charge_mol = charge_holder_group[molnum].molecule().edit().
1251                     renumber().commit()
1252                 box2_mols_pdb.add(charge_mol)
1253                 for molnum in water_holder_group.molNums():
1254                     water_mol = water_holder_group[molnum].molecule().edit().
1255                         renumber().commit()
1256                     box1_mols_pdb.add(water_mol)
1257                     print("done")
1258                     output = (box1_mols_pdb, box2_mols_pdb)
1259                     return output
1260
1261 def chemicalPotential_widom_insertion(systemi, insertionMolec, boxspace,
1262 nsteps):
1263     """
1264     Function to find the chemical potential by find the free energy
1265         change before and after adding the molecule
1266     to the given system.
1267     Input: systemi (system containing box of molecules), insertionMolec
1268         (insertion molecule), boxspace, nsteps (number of time water to
1269         be inserted)
1270     """
1271     import math

```

```

1261 # two lists to collect energy values as a testing case
1262 avg_delta_energies = 0
1263 avg_delta_accepted_energies = 0
1264 # throws is the number of configurations generated by placing
    additional water molecule in the box. this is used for taking
    average.
1265 throws = 0
1266 temperature = 298.15 * kelvin
1267 # 'collect_mc_molecs' is a function to generate a group which is
    use to add additional molecule. (we cant add additional molecule
    to systemi directly)
1268 outputgroup = collect_mc_molecs(systemi)
1269 insertionMolec = insertionMolec.edit().renumber().commit()
1270 # 'energy_minimize_system_LJ_CL' function makes a system to
    calculate energy. old_energy is the energy of the box without
    additional molecule
1271 old_system = energy_minimize_system_LJ_CL(outputgroup,boxspace)
1272 old_energy = old_system.energy().value()
1273 # 'createSystem_for_chemicalpotential' function adds the water to
    system and gives energy of total system
1274 (system,energy)=createSystem_for_chemicalpotential(outputgroup,
    insertionMolec, boxspace)
1275 # Running a loop over nsteps to generate nsteps configurations with
    different orientation and position of additional water in box.
1276 for i in range(nsteps):
1277     throws += 1
1278     # creat random angle and translation values for additional water
        molecule in box to generate configurations
1279     randomvalues = createRandomValuesforRotationTranslation(boxspace
        )
1280     newMolec = rotateTranslateMolec(randomvalues,insertionMolec)
1281     system.update(newMolec)
1282     new_energy = system.energy().value()
1283     ## what is the difference in energy
1284     delta_energy = (new_energy - old_energy)
1285     ## calculate RT*exp( -dE / RT ) where k_boltz is value of gas
        constant; 1.9872041(18)103      kcalK1mol1
1286     x = -(k_boltz * temperature.value())*(math.exp( -delta_energy /
        (k_boltz * temperature.value()) ))
1287     avg_delta_energies += x/throws
1288     print("Energy before =%s and after adding water =%s; deltaE =%
        and -RT*exp( -dE / RT ) = %s"%(old_energy,new_energy,
        delta_energy,x))
1289     #other test
1290     selection_criterial = 1

```

```

1291     if x <= selection_criterial:
1292         avg_delta_accepted_energies += x/throws
1293     else:
1294         system.update(newMolec)
1295         mu = avg_delta_energies #-(k_boltz * temperature.value())*log1p(
1296             avg_delta_energies)
1297         mu2 = avg_delta_accepted_energies #-(k_boltz * temperature.value())
1298             *log1p(avg_delta_accepted_energies)
1299         print("chemical potential of single water molecule for all
1300             configurations =%s and accepted configuratios =%s"%(mu,mu2))
1301         output = (mu2,mu)
1302     return output
1303 """
1304 def chemicalPotential_widom_insertion(systemi,insertionMolec,boxspace,
1305 nsteps):
1306     #Function to find the chemical potential by find the free energy
1307     # change before and after adding the molecule
1308     #to the given system.
1309     #Input: system, insertion molecule,number of steps for deltaE,
1310     #systemswapwaters(number of waters swap with RNA)
1311     import math
1312     rangen = RanGenerator()
1313     avg_delta_energies = 0
1314     avg_delta_accepted_energies = 0
1315     throws = 0
1316     temperature = 298.15 * kelvin
1317     outputgroup = collect_mc_molecs(systemi)
1318     ingroup = MoleculeGroup()
1319     ingroup.add(insertionMolec)
1320     mol = ingroup.moleculeAt(0).molecule()
1321     insertionMolec = mol.edit().renumber().commit()
1322     old_system = energy_minimize_system_LJ_CL(outputgroup,boxspace)
1323     old_energy = old_system.energy().value()
1324     (system,energy)=createSystem_for_chemicalpotential(outputgroup,
1325             insertionMolec, boxspace)
1326     for i in range(nsteps):
1327         throws += 1
1328         randomvalues = createRandomValuesforRotationTranslation(boxspace
1329             )
1330         newMolec = rotateTranslateMolec(randomvalues,insertionMolec)
1331         system.update(newMolec)
1332         new_energy = system.energy().value()
1333         ## what is the difference in energy

```

```

1328     delta_energy = -(new_energy - old_energy)
1329     ## calculate exp( -dE / kT )
1330     x = math.exp( delta_energy / (k_boltz * temperature.value()) )
1331     avg_delta_energies += x/throws
1332     print("Energy before=%s and after adding water=%s; deltaE=%s
           and exp( -dE / kT ) = %s"%(old_energy,new_energy,
           delta_energy,x))
1333     #other test
1334     selection_criterial = 1
1335     selection_criteia2 = 0
1336     if x <= selection_criterial:
1337         avg_delta_accepted_energies += x/throws
1338     elif x > selection_criteia2:
1339         avg_delta_accepted_energies += x/throws
1340     else:
1341         system.update(newMolec)
1342     mu = avg_delta_energies #-(k_boltz * temperature.value())*log1p(
1343         avg_delta_energies)
1344     mu2 = avg_delta_accepted_energies #-(k_boltz * temperature.value())
1345         *log1p(avg_delta_accepted_energies)
1346     print("chemical potential of single water molecule for all
           configurations=%s and accepted configuratios=%s"%(mu,mu2))
1347     output = (mu2,mu)
1348     return output
1349 """
1350 def createRandomValuesforRotationTranslationofSwapwaterMolecs(
1351     centerofgeometryofRNAatom):
1352 """
1353 This function creates a random orientation
1354 vector and a random angle values for water molecueles to be swaped
1355 with RNA.
1356 The function take the input of RNA atom (center of mass, or centre
1357 of geometry) and
1358 It returns a list with these three
1359 components.
1360
1361 Function Input: centre of mass or centre of geometry
1362 """
1363 v=Vector(0,0,0) # Creates a vector for the random point generation
1364 #rand_seed = 140154
1365 gen=RanGenerator() # Creates a random generator object
1366 insertion_point=centerofgeometryofRNAatom # Creates a random point
1367     within the confinements of the box
1368 orientation_vector=gen.vectorOnSphere() # Creates a random
1369     orientation vector

```

```

1363     orientation_angle=gen.rand(-two_pi,two_pi)*radians # Creates a
1364         random orientation angle
1364     output=(insertion_point,orientation_vector,orientation_angle) #
1364         Creates the output list
1365     return output
1366
1367 def rotateTranslateMolec(randomValues,molec):
1368     """
1369     This function uses a random position, a random orientation vector
1370     and a random angle in 'randomValues' to transform molecule 'molec'
1371     into a new molecule 'newMolec'.
1372
1373     Function Input: random values generated from
1374     createRandomValuesforRotationTranslation(boxSpace) function and the
1375     molecule required to rotate and translate.
1376
1377     """
1378     map=PropertyMap() # Creates a property Map object
1379     molCenter=molec.evaluate().center(map) # Obtains the center of the
1380         molecule using 'map'
1380     newMolec=Mover_Molecule_(molec) # Creates a new molecule 'newMolec'
1381         using a Mover constructor and the molecule 'molec' to be able
1381         to rotate and translate it
1381     newMolec=newMolec.rotate(Quaternion(randomValues[2],randomValues
1381         [1]),molCenter,map)# Rotates the molecule
1382     newMolec=newMolec.translate(randomValues[0]-molCenter,map) #
1382         Translates the molecule to a random position
1383     newMolec=newMolec.commit() # Commits the new molecule
1384     return newMolec
1385
1386 def makemolgroups(newMolec,peg):
1387     """
1388     Function use to make new molecule group by adding the RNA/protein
1389     background molecules. the output is a molecule group used for
1389         steric
1390     clash functions.
1391
1392     Function Input: rna molecule, crowders group
1393     """
1394     newmolgroup = MoleculeGroup()
1395     newmolgroup.add(newMolec)
1396     newmolgroup.add(peg)
1397     return newmolgroup
1398
1399 def steric_closmol_clashes(boxMolecs):

```

```

1400 """
1401     function to estimate the parallel distances to determine wether
1402     there is any steric clash or not in the given molecule group.
1403
1404     Function Input: molecule group
1405     note: make sure the rna/protein ResID match in function and
1406           set the tolerance criteria if desire:
1407 """
1408 boxMolecs.setName("boxMolecs")
1409 p = PointRef(boxMolecs[MolWithResID("FUL")].molecule().evaluate() .
1410               centerOfGeometry())
1411 c = CloseMols(p,boxMolecs,5)
1412 g_random = c.closeMolecules()
1413 #print(g_random)
1414 g = sorted(g_random.items(), key=operator.itemgetter(1)) # sort
1415     the dict by value which give tuple
1416 #print(g)
1417 t = [x[0] for x in g] # extract sorted with respct to distance
1418     molnumbers from tuple
1419 selected = boxMolecs[MolWithResID("FUL")].molecule()
1420 closMol = boxMolecs[t[1]].molecule()
1421 for i in range(1,len(t)):
1422     peg_st = boxMolecs[t[i]].molecule()
1423     #print(peg_st)system.update(newMolec)
1424     for j in selected.atoms(AtomIdx()):
1425         icent = j.evaluate().center()
1426         for j in peg_st.atoms(AtomIdx()):
1427             dist = Vector.distance(icent, j.evaluate().center())
1428             if dist<1.05:
1429                 return 0 #print ("there is a steric clash")
1430             return 1 #print ("there is no steric clashes")
1431
1432 def steric_closmol_clashes_vdw(boxMolecs):
1433 """
1434     function to estimate the parallel distances to determine wether
1435     there is any steric clash or not in the given molecule group using
1436         the vander waal radii as tolerance criteria.
1437     It estimate the sum vander waal radii of 2 elements and compare it
1438         with actual distance between them. if actual
1439     distance is LESS than vander waal radii then it will report steric
1440         clash.
1441     Function Input: molecule group
1442     note: make sure the rna/protein ResID match
1443         in function
1444
1445
1446
1447

```

```

1438 """
1439     boxMolecs.setName("boxMolecs")
1440     p = PointRef(boxMolecs[MolWithResID("WAT")].molecule().evaluate() .
1441                   centerOfGeometry())
1441     #p = PointRef(boxMolecs[number].molecule().evaluate() .
1442                   centerOfGeometry())
1442     c = CloseMols(p,boxMolecs,2)
1443     g_random = c.closeMolecules()
1444     #print(g_random)
1445     g = sorted(g_random.items(), key=operator.itemgetter(1)) # sort
1446     #    the dict by value which give tuple
1446     #print(g)
1447     t = [x[0] for x in g] # extract sorted with respect to distance
1448     molnumbers from tuple
1448     selected = boxMolecs[MolWithResID("WAT")].molecule()
1449     #selected = boxMolecs[number].molecule()
1450     closMol = boxMolecs[t[1]].molecule()
1451     for i in range(1,len(t)):
1452         peg_st = boxMolecs[t[i]].molecule()
1453         #print(peg_st)
1454         for j in range(selected.nAtoms()):
1455             icent = selected.atom(AtomIdx(j)).evaluate().center()
1456             vdwrna = selected.atom(AtomIdx(j)).property("element")
1457             vdwrnavalue = Element(vdwrna).vdwRadius().value()
1458             #print(vdwrna)
1459             for k in range(peg_st.nAtoms()):
1460                 dist = Vector.distance(icent, peg_st.atom(AtomIdx(k)) .
1461                                         evaluate().center())
1461                 #print("distance of each background atom =%s" % dist)
1462                 vdwpeg=peg_st.atom(AtomIdx(k)).property("element")
1463                 vdwpegvalue= Element(vdwpeg).vdwRadius().value()
1464                 vdwsum= vdwrnavalue+vdwpegvalue
1465                 #print(vdwsum)
1466                 if dist<vdwsum:
1467                     return 0 #print ("there is a steric clash")
1468     return 1 #print ("there is no steric clashes")
1469
1470 """
1471 def default_system_energy3(outputgroup,boxSpace):
1472
1473     function to estimate the energy tsystem.update(newMolec)o determine
1474     wether
1474     there is any steric clash or not in the given molecule group.
1475
1476     Function Input: molecule group, boxspace

```

```

1477     note: set the tolerance criteria if desire:
1478
1479     system= System()
1480     int1 = InterCLJFF("int1")
1481     #int2 = InterCoulombFF("int2")
1482     #int3 = InterFF("int3")
1483     int1.add(outputgroup)
1484     #int2.add(outputgroup)
1485     #int3.add(outputgroup)
1486     system.add(outputgroup)
1487     system.add(int1)
1488     #system.add(int2)
1489     #system.add(int3)
1490     system.setProperty("space", boxSpace)
1491     system_energy = system.energy().value()
1492     print("energy of control system =%s" % system_energy)
1493     return system_energy
1494 """
1495
1496 def default_system_energy2(outputgroup,boxSpace):
1497 """
1498     function to estimate the energy to determine wether
1499     there is any steric clash or not in the given molecule group.
1500     Function Input: molecule group, boxspace
1501     note: set the tolerance criteria if desire:
1502 """
1503     #system= System()
1504     interff = InterFF("int1")
1505     cljfunc = CLJShiftFunction()
1506     cljfunc.setCoulombCutoff(0 * angstrom)
1507     cljfunc.setLJCutoff(5 * angstrom)
1508     cljfunc.setSpace(boxSpace)
1509     interff.setCLJFunction(cljfunc)
1510     #interff.setSwitchingFunction(cljfunc)
1511     interff.setUseParallelCalculation(True)
1512     interff.add(outputgroup)
1513     #system.add(outputgroup)
1514     #system.add(interff)
1515     #total_nrg = system.energy()
1516     #system.setProperty("space", boxSpace)
1517     #system_energy = (system.energy().value(), 3)
1518     #coul_nrg = int1.energy( int1.components().coulomb() )
1519     #print("coul energy = %s" % coul_nrg)
1520     #lj_nrg = system.energy( interff.components().lj() )
1521     lj_nrg = interff.energy( interff.components().lj() )

```

```

1522     #print("LJ energy = %s" % lj_nrg)
1523     #print("total energy of system =%s" % system_energy)
1524     #print("all energies = %s " % system.energies())
1525     return lj_nrg.value()
1526
1527 def add_newMolec_to_crowder(newMolec,peg):
1528     """ Add the molecule and crowders to generate the new molecule
1529         group
1530         used for further calculations:
1531
1532         Function Input: rna molecule, crowder molecule group
1533         """
1534     outputgroup= peg.add(newMolec)
1535     return outputgroup
1536
1537 def steric_clash_energy(outputgroup,defaultenergy,boxSpace):
1538     """ Function similar to default_system_energy and used to
1539         determine the steric clashes via energy:
1540
1541         Function Input: moleculegroup containing everything,
1542                         defult energy as tolerance criteria
1543                         boxspace
1544         note: BUGGY ! dont use until fixed!
1545         """
1546     systemc= System()
1547     int1 = InterCLJFF("int1")
1548     int2 = InterCoulombFF("int2")
1549     int3 = InterFF("int3")
1550     int1.add(outputgroup)
1551     int2.add(outputgroup)
1552     int3.add(outputgroup)
1553     systemc.setProperty("space", boxSpace)
1554     systemc.add(outputgroup)
1555     systemc.add(int1)
1556     systemc.add(int2)
1557     systemc.add(int3)
1558     #print(systemc.nMolecules())
1559     system_energy = systemc.energy().value()
1560     print("energy of testing system=%s and defult system =%r" %(system_energy, defaultenergy))
1561     if system_energy > defaultenergy:
1562         return 0
1563     return 1
1564 def initial_configurations(newMolec,tolerance,boxspace):

```

```

1565 """
1566     Function to add the molecules to the new molecule group of defined
1567         space,
1568     the function take a single molecule as an input (backgroundmols)
1569         along with freshly defined
1570     molecule group(newemptygroup).tolerance distance between molecues
1571         while boxSpace is
1572     box dimensions
1573
1574     Function Input: crowders molecules
1575             tolerance, dist. between crowder molec.
1576             boxspace
1577             note: Not finished yet, Dont try!
1578 """
1579 #     randomvalues = createRandomValuesforRotationTranslation(boxSpace)
1580 #     newMolec = rotateTranslateMolec(randomvalues,mol)
1581 newgroup = MoleculeGroup()
1582 p = PointRef(newMolec.molecule().evaluate().centerOfGeometry())
1583 c = CloseMols(p,newgroup,5)
1584 g_random = c.closeMolecules()
1585 if len(g_random) == 0:
1586     newgroup.add(newMolec)
1587 else:
1588     g = sorted(g_random.items(), key=operator.itemgetter(1)) #
1589     #sort the dict by value which give tuple
1590     #print(g)
1591     t = [x[0] for x in g]
1592     selected = newgroup[t[0]].molecule().evaluate().centerOfGeometry()
1593     for i in range(1,len(t)):
1594         closemols = newgroup[t[i]].molecule().evaluate().centerOfGeometry()
1595         if (selected-closemols).length() > tolerance:
1596             newgroup.add(newMolec)
1597     return newgroup
1598
1599 def if_function(system_energy,tolerance):
1600 """
1601     Function to determine wether the system has steric clash or not
1602         based on energies
1603     Input : system under study, default tolerance energy
1604 """
1605     if system_energy < tolerance:
1606         return 1
1607     return 0

```

```
1603
1604 def if_function_energy(system_energy, tolerance):
1605     """
1606         Function to determine whether the system has normal energy output or
1607         very small values
1608         Input : system energy under study
1609     """
1610     if system_energy < -tolerance:
1611         return abs(system_energy)
1612     return system_energy
1613
1614 def createSystem_for_chemicalpotential(outputgroup, biom_mol, boxSpace):
1615     """
1616         create a system to hold all of the molecules and forcefields
1617         Return the created system and the forcefield energy component
1618         that you want to evaluate
1619     """
1620     system = System()
1621     interf = InterFF("int1")
1622     cljfunc = CLJShiftFunction()
1623     cljfunc.setSpace(boxSpace)
1624     interf.setCLJFunction(cljfunc)
1625     #interff.setSwitchingFunction(cljfunc)
1626     interf.setUseParallelCalculation(True)
1627     system.setProperty("space", boxSpace)
1628     outputgroup.add(biom_mol)
1629     interf.add(outputgroup)
1630     system.add(interff)
1631     system.add( SpaceWrapper(Vector(0), outputgroup) )
1632     system.add(outputgroup)
1633     return (system,system.energy())
1634
1635 def createSystem(outputgroup, biom_mol, boxSpace):
1636     """
1637         create a system to hold all of the molecules and forcefields
1638         Return the created system and the forcefield energy component
1639         that you want to evaluate
1640
1641     """
1642     system = System()
1643     interf = InterFF("int1")
1644     cljfunc = CLJShiftFunction()
1645     #cljfunc.setCoulombCutoff(0 * angstrom)
```

```
1646 #cljfunc.setLJCutoff(15 * angstrom)
1647 cljfunc.setSpace(boxSpace)
1648 interff.setCLJFunction(cljfunc)
1649 #interff.setSwitchingFunction(cljfunc)
1650 interff.setUseParallelCalculation(True)
1651 system.setProperty("space", boxSpace)
1652 outputgroup.add(biom_mol)
1653 interff.add(outputgroup)
1654 #interff.add(biom_mol)
1655 system.add(interff)
1656 system.add( SpaceWrapper(Vector(0), outputgroup) )
1657 system.add(outputgroup)
1658 return (system, interff.components().lj())
1659
1660 def create_single_System(outputgroup,boxSpace):
1661 """
1662     create a system to hold all of the molecules and forcefields
1663     Return the created system and the forcefield energy component
1664     that you want to evaluate
1665
1666 """
1667 system = System()
1668 interff = InterFF("int1")
1669 cljfunc = CLJShiftFunction()
1670 cljfunc.setCoulombCutoff(10 * angstrom)
1671 cljfunc.setLJCutoff(10 * angstrom)
1672 cljfunc.setSpace(boxSpace)
1673 interff.setCLJFunction(cljfunc)
1674 #interff.setSwitchingFunction(cljfunc)
1675 interff.setUseParallelCalculation(True)
1676 system.setProperty("space", boxSpace)
1677 interff.add(outputgroup)
1678 system.add(interff)
1679 system.add( SpaceWrapper(Vector(0), outputgroup) )
1680 system.add(outputgroup)
1681 return (system, interff.components().lj())
1682
1683 def remove_water_to_balance_density(system_input,nwater):
1684 """
1685     function to remove the random extra water molecules from PDB file
1686     before making the top,cord files
1687     and running minimization...
1688     system, moleculegroup and number of nwater to remove.
1689 """
1690 system = collect_mc_molecs(system_input)
```

```

1690     rangen = RanGenerator()
1691     nwaters = system.nMolecules()
1692     for i in range(nwater):
1693         water_id = rangen.randInt(0, nwaters-1)
1694         random_water = system[ MolIdx(water_id) ].molecule()
1695         if random_water.nAtoms() == 3:
1696             system.remove(random_water)
1697     system
1698     PDB().write(system.molecules(), "afterRemovingExWater.pdb")
1699     return system
1700
1701 def density_of_single_box(system, space):
1702     """
1703     Function to determine the total density of system
1704     Input : system or molecule group
1705     """
1706     box_volume = space.volume().value()
1707     grams = 1.6605402e-24 # convert the molecular mass in to grams
1708     cube_centi = 1.0e-24 # cubic angstrom to cubic centimeter --volume
                           conversion
1709     mass = 0
1710     massIdx = 0
1711     for molnum in system.molNums():
1712         mol = system[molnum].molecule()
1713         mass_mol = mol.evaluate().mass().value()
1714         mass += mass_mol
1715     """
1716     for i in range(system.nMolecules()):
1717         mol = system[MolIdx(i)].molecule()
1718         mass_mol = mol.evaluate().mass().value()
1719         massIdx += mass_mol
1720
1721     densityIdx = (massIdx*grams) / (box_volume*cube_centi)
1722     """
1723     density = (mass*grams) / (box_volume*cube_centi)
1724     print("density of given box is %s= g/cc "%density)
1725     return density
1726
1727 def box_density_printer(rna_mass, mass_singlepeg, peg_density, boxspace):
1728     """
1729     Function to estimate the density of box and number of background
           molecules
1730     to generate the intial configurations via PACKMOL package.
1731     Function also gives number of water molecules used to swap rna and
           fill the

```

```

1732     the second box of system to maintain overall density fix. density
1733         units g/cc
1734
1734     Function Input: rna single molecule/moleculegroup
1735             peg single molecule/molecule group of peg only
1736             required peg density in 1g/cc overall concentration
1737             [0.1,0.2,...,0.9]
1738             boxspace
1739
1740
1741     rna_mass = rna[MolIdx(0)].molecule().evaluate().mass().value()
1742     mass_singlepeg = peg[MolIdx(0)].molecule().evaluate().mass().value
1743         ()
1743     box_volume = boxspace.volume().value()
1744     peg_density= 0.2
1745
1746     note: the order of input files is critical!
1747         Desire density [1,2,3...]g/cc can be change in function
1748
1749     """
1750     #rna_mass = rna[MolIdx(0)].molecule().evaluate().mass().value()
1751     #mass_singlepeg = peg[MolIdx(0)].molecule().evaluate().mass().value
1751         ()
1752     box_volume = boxspace.volume().value()
1753     grams = 1.6605402e-24 # convert the molecular mass in to grams
1754     cube_centi = 1.0e-24 # cubic angstrom to cubic centimeter --volume
1754         conversion
1755     desired_density = 1 # desired overall density
1756     mass_peg = peg_density*(box_volume*cube_centi)/(grams)
1757     number_peg = mass_peg/mass_singlepeg
1758     print("number of peg molecules %s at %s g/cc density" % (
1758         number_peg,peg_density))
1759     rna_density = (rna_mass*grams)/(box_volume*cube_centi)
1760     print("density of rna molecule g/cc= ", rna_density)
1761     density_forwater = desired_density-(rna_density+peg_density)
1762     print("density to be filled by water molecule g/cc= ",
1762         density_forwater)
1763     mass_water = density_forwater*(box_volume*cube_centi)/(grams)
1764     number_water = mass_water/18.01528 # mol.wegith of water = 18.01528
1765     print("number of water molecules =%s" % number_water)
1766     mass_water_empty_box = desired_density*(box_volume*cube_centi)/(
1766         grams)
1767     number_warter_empty_box = mass_water_empty_box/18.01528
1768     print("number of water molecules in empty box to make density 1 g/
1768         cc are =%s " % number_warter_empty_box)

```

```

1769     number_water_swap = rna_mass/18.01528
1770     print("number of water molecules displace by RNA = %s" %
1771             number_water_swap)
1772     return
1773
1773 def box_density(rna_mass,mass_singlepeg,peg_density,box_volume):
1774     grams = 1.6605402e-24
1775     cube_centi = 1.0e-24
1776     desired_density = 1
1777     mass_peg = peg_density*(box_volume*cube_centi)/(grams)
1778     number_peg = mass_peg/mass_singlepeg
1779     #print("number of peg molecules %s at %s g/cc density" %
1780             number_peg,peg_density))
1780     rna_density = (rna_mass*grams)/(box_volume*cube_centi)
1781     #print("density of rna molecule g/cc= ", rna_density)
1782     density_forwater = desired_density-(rna_density+peg_density)
1783     #print("density to be filled by water molecule g/cc= ",
1784             density_forwater)
1784     mass_water = density_forwater*(box_volume*cube_centi)/(grams)
1785     number_water = mass_water/18.01528
1786     #print("number of water molecules =%s" % number_water)
1787     mass_water_empty_box = desired_density*(box_volume*cube_centi)/(
1788             grams)
1788     number_warter_empty_box = mass_water_empty_box/18.01528
1789     #print("number of water molecules in empty box to make density 1 g/
1790             cc are =%s " % number_warter_empty_box)
1790     number_water_swap = rna_mass/18.01528
1791     #print("number of water molecules displace by RNA = %s" %
1792             number_water_swap)
1792     return (number_peg, number_water,number_warter_empty_box)
1793
1794 def swap_nwaters_for_density(nwaters, box1_mols, box2_mols):
1795     """
1796         Function to swap water molecules between two boxes to maintain the
1797             density
1798         Input: nwaters, box1_mols, box2_mols
1799     """
1799     nwaters = int(nwaters)
1800     for i in range(nwaters):
1801         nwater = box2_mols[MolIdx(i)].molecule()
1802         box2_mols.remove(nwater)
1803         box1_mols.add(nwater)
1804     return (box1_mols,box2_mols)
1805
1806 def energy_minimize_system(box_mols,box_space):

```

```

1807 """
1808     Function to prepare the system containing the molecules, boxSpace
1809     Input: Molecules box, boxSpace
1810 """
1811     # create simulation system to hold box of molecules
1812     box_system = System("box")
1813     # intermolecule coulomb and LJ energy
1814     box_interff = InterCLJFF("box_inter")
1815     box_interff.add(box_mols)
1816     # intramolecular bond, angle and dihedral energy
1817     box_intraff = InternalFF("box_intra")
1818     box_intraff.add(box_mols)
1819     # intramolecular coulomb and LJ energy
1820     box_intraclj = IntraCLJFF("box_intraclj")
1821     box_intraclj.add(box_mols)
1822     # add all molecules and forcefields for box to system
1823     box_system.add(box_mols)
1824     box_system.add(box_interff)
1825     box_system.add(box_intraff)
1826     box_system.add(box_intraclj)
1827     # set the periodic box information for each box
1828     box_system.setProperty("space", box_space)
1829     # system energy (optional)
1830     box_system.energies()
1831     return box_system
1832
1833 def make_pdb(energy,system,energyltol):
1834     energyltol = - abs(energyltol * 4)
1835     if energy < energyltol:
1836         PDB().write(system.molecules(),"lower_energy.pdb")
1837     return
1838
1839 def default_system_energy(outputgroup,boxSpace):
1840 """
1841     function to estimate the energy to determine whether
1842     there is any steric clash or not in the given molecule group.
1843
1844     Function Input: molecule group, boxspace
1845     note: set the tolerance criteria if desire:
1846 """
1847     #system= System()
1848     interff = InterFF("int1")
1849     #cljfunc = CLJShiftFunction()
1850     cljfunc = CLJShiftFunction() # 10*angstrom
1851     #cljfunc.setCoulombCutoff(0 * angstrom)

```

```

1852     #cljfunc.setLJCutoff(5 * angstrom)
1853     cljfunc.setSpace(boxSpace)
1854     interff.setCLJFunction(cljfunc)
1855     #interff.setSwitchingFunction(cljfunc)
1856     interff.setUseParallelCalculation(True)
1857     interff.add(outputgroup)
1858     #system.add(outputgroup)
1859     #system.add(interff)
1860     #total_nrg = system.energy()
1861     #system.setProperty("space", boxSpace)
1862     #system_energy = (system.energy().value(), 3)
1863     #coul_nrg = int1.energy( int1.components().coulomb() )
1864     #print("coul energy = %s" % coul_nrg)
1865     #lj_nrg = system.energy( interff.components().lj() )
1866     lj_nrg = interff.energy( interff.components().lj() )
1867     #print("LJ energy = %s" % lj_nrg)
1868     #print("total energy of system =%s" % system_energy)
1869     #print("all energies = %s " % system.energies())
1870     return lj_nrg.value()
1871
1872 def swap_water_rna(box1_mols,box2_mols):
1873 """
1874     Function use to swap RNA and water molecules between two boxes.
1875     where box1_mols is box containing RNA and background molecules
1876     while box2_molecules is purely water box where RNA will be
1877     placed and from there number of water molecules are moved to
1878     box1_mols to keep density fix. It gives two boxes back as output
1879     ...
1880     Input: box1_mols, box2_mols
1881 """
1882     rna_residmolecule = box1_mols[ MolWithResID("RG5") ].molecule()
1883     nwaters = rna_residmolecule.evaluate().mass().value()/18.01528
1884     box1_mols.remove(rna_residmolecule)
1885     (box1_mols,box2_mols,charge_mass)=charge_swap(box1_mols,box2_mols)
1886     print("charge ions mass in given system %s" % charge_mass)
1887     nwaters = nwaters+charge_mass
1888     (box1_mols,box2_mols) = swap_nwaters_for_density(nwaters, box1_mols
1889             , box2_mols)
1890     box2_mols.add(rna_residmolecule)
1891     return (box1_mols,box2_mols)
1892
1893 def collect_mc_molecs(sim_mols):
1894 """
1895     Function to collect molecules in a new molecule group so to use in
1896     next MC steps of code

```

```

1890 """
1891     optimized_mols = MoleculeGroup()
1892     for molnum in sim_mols.molNums():
1893         mol = sim_mols[molnum].molecule()
1894         optimized_mols.add(mol)
1895     return optimized_mols
1896
1897 def NPT_ensemble_moves(system):
1898 """
1899     Function to define property of move(temp, pressure, volume)
1900     Input: system output from ("energy_minimize_system") function
1901     this function gives the system over which moves will be performed
1902         while wt_moves represent the collection of moves according to
1903             NPT ensemble.
1904 """
1905     ## create a Rigid Body move that will translate and rotate the
1906     ## molecules in system
1907     list_of_groups = system.groups() [-1]
1908     MGName = system.groupNames() [-1]
1909     system.add( SpaceWrapper(Vector(0), list_of_groups) )
1910     Rigid_move = RigidBodyMC(system[MGName])
1911     Rigid_move.setTemperature( 25*celsius )
1912     Rigid_move.setMaximumTranslation(0.50 * angstrom)
1913     Rigid_move.setMaximumRotation(0.50 * degrees)
1914     Rigid_move.setUseOptimisedMoves
1915     vol_mov = VolumeMove(system[MGName])
1916     vol_mov.setMaximumVolumeChange( 0.1 * system.nMolecules() *
1917         angstrom3 )
1918     vol_mov.setPressure( 1*atm )
1919     wt_moves = WeightedMoves()
1920     wt_moves.add( Rigid_move, system[MGName].nMolecules() )
1921     wt_moves.add( vol_mov, system[MGName].nMolecules() )
1922     system_out = wt_moves.move(system, 5, True)
1923     return (system,wt_moves)
1924
1925 def NPT_ensemble_moves_large_trans_rot(system):
1926 """
1927     Function to define property of move(temp, pressure, volume)
1928     Input: system output from ("energy_minimize_system") function
1929     this function gives the system over which moves will be performed
1930         while wt_moves represent the collection of moves according to
1931             NPT ensemble.
1932 """
1933     ## create a Rigid Body move that will translate and rotate the
1934     ## molecules in system

```

```

1928     list_of_groups = system.groups() [-1]
1929     MGName = system.groupNames() [-1]
1930     system.add( SpaceWrapper(Vector(0), list_of_groups) )
1931     Rigid_move = RigidBodyMC(system[MGName])
1932     Rigid_move.setTemperature( 25*celsius )
1933     Rigid_move.setMaximumTranslation(2.50 * angstrom)
1934     Rigid_move.setMaximumRotation(5.50 * degrees)
1935     Rigid_move.setUseOptimisedMoves
1936     vol_mov = VolumeMove(system[MGName])
1937     vol_mov.setMaximumVolumeChange( 0.1 * system.nMolecules() *
1938         angstrom3 )
1939     vol_mov.setPressure( 1*atm )
1940     wt_moves = WeightedMoves()
1941     wt_moves.add( Rigid_move, system[MGName].nMolecules() )
1942     wt_moves.add( vol_mov, system[MGName].nMolecules() )
1943     system_out = wt_moves.move(system, 5, True)
1944     return (system,wt_moves)
1945
1946 def NVT_ensemble_moves_large_trans_rot(system):
1947     """
1948         Function to define property of move(temp, pressure, volume)
1949         Input: system output from ("energy_minimize_system") function
1950         this function gives the system over which moves will be performed
1951             while wt_moves represent the collection of moves according to
1952                 NPT ensemble.
1953     """
1954     ## create a Rigid Body move that will translate and rotate the
1955     ## molecules in system
1956     list_of_groups = system.groups() [-1]
1957     MGName = system.groupNames() [-1]
1958     system.add( SpaceWrapper(Vector(0), list_of_groups) )
1959     Rigid_move = RigidBodyMC(system[MGName])
1960     Rigid_move.setTemperature( 25*celsius )
1961     Rigid_move.setMaximumTranslation(5.50 * angstrom)
1962     Rigid_move.setMaximumRotation(15.0 * degrees)
1963     Rigid_move.setUseOptimisedMoves
1964     wt_moves = WeightedMoves()
1965     wt_moves.add( Rigid_move, system[MGName].nMolecules() )
1966     system_out = wt_moves.move(system, 5, True)
1967     return (system,wt_moves)
1968
1969 def NVT_ensemble_moves(system):
1970     """
1971         Function to define property of move(temp, pressure, volume)
1972         Input: system output from ("energy_minimize_system") function

```

```

1969     this function gives the system over which moves will be performed
1970         while wt_moves represent the collection of moves according to
1971             NPT ensemble.
1970 """
1971     ## create a Rigid Body move that will translate and rotate the
1972         molecules in system
1972     list_of_groups = system.groups() [-1]
1973     MGName = system.groupNames() [-1]
1974     system.add( SpaceWrapper(Vector(0), list_of_groups) )
1975     Rigid_move = RigidBodyMC(system[MGName])
1976     Rigid_move.setTemperature( 25*celsius )
1977     Rigid_move.setMaximumTranslation(2.50 * angstrom)
1978     Rigid_move.setMaximumRotation(10.50 * degrees)
1979     Rigid_move.setUseOptimisedMoves
1980     wt_moves = WeightedMoves()
1981     wt_moves.add( Rigid_move, system[MGName].nMolecules() )
1982     system_out = wt_moves.move(system, 5, True)
1983     return (system,wt_moves)
1984
1985 def simulation_moves(box_system_mc,box_wt_moves,steps,filename1,
1986     filename2,filename3):
1986 """
1987     Function to perform moves on a system to minimize the energy values
1988     Input: system_box containing all forcefield and molecules,
1989             weight_moves,steps,'file1.txt','file2.txt' names
1989 """
1990     first_energy = box_system_mc.energy().value()
1991     set_p_value = 0.1
1992     test_energy = [1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,
1993         first_energy]
1993     calculated_p_value = float('inf')
1994     # energy_diff between moves while simulation
1995     x = 1
1996     with open (filename1, 'w') as f1:
1997         with open (filename2, 'w') as g1 :
1998             while calculated_p_value > set_p_value:
1999                 for i in range(x,x+20):
2000                     box_system_mc = box_wt_moves.move(box_system_mc, steps,
2001                         True)
2001                     print("%d: %s" % (i, box2_system_mc.energy()))
2002                     energy = box_system_mc.energy().value()
2003                     test_energy.append(energy)
2004                     energy_difference= test_energy[-2]-test_energy[-1]
2005                     f1.write(str(energy_difference)+'\n')
2006                     g1.write(str(energy)+'\n')

```

```

2007     window1 = test_energy[-20:-10]
2008     window2 = test_energy[-10:]
2009     Individual_samples = stats.ttest_ind(window1, window2)
2010     calculated_p_value= Individual_samples[-1]
2011     x = x+1
2012     box_system_mc.update
2013     if i % 1 == 0:
2014         print ("The Individual t-statistic is %s and the p-
2015             value is %s and energy of system is = %s" % (
2016                 Individual_samples[0],Individual_samples[1],energy)
2017             )
2018             #Sire.Stream.save(box_system_mc,filename3)
2019             E = min(test_energy[-20:])
2020             print("Energy of system after Monte Carlo Minimization = %s:" % E)
2021             print("Move information:")
2022             print(box_wt_moves)
2023             # collect the molecules in new molecule group for further MC
2024             box_mols_mc = collect_mc_molecs(box_system_mc)
2025             PDB().write(box_system_mc.molecules(), filename3)
2026             f1.close()
2027             g1.close()
2028             Sire.Stream.save(box_system_mc,filename3)
2029             return (E,box_mols_mc)
2030
2031     def simulation_moves_simple(box_system_mc,box_wt_moves,steps):
2032         """
2033             Function to perform moves on a system to minimize the energy values
2034             Input: system_box containing all forcefield and molecules,
2035                 weight_moves,steps,'file1.txt','file2.txt' names
2036         """
2037         first_energy = box_system_mc.energy().value()
2038         box_system_mc = box_wt_moves.move(box_system_mc, steps, True)
2039         return (box_system_mc)
2040
2041     def pdbmaker(input_filename,output_filename,copy_numberoflines):
2042         """
2043             Function to create pdbs from pdb file containing all frames
2044         """
2045         file = open(input_filename,'r')
2046         lines = file.readlines()
2047         f1 = open(output_filename, 'w')
2048         for i in range(0,copy_numberoflines):
2049             f1.write(lines[i])
2050         f1.close()
2051         open('all.pdb', 'w').writelines(lines[copy_numberoflines:])


```

```

2048     return
2049
2050 def str_maker(filename):
2051     file = str(filename)
2052     return file
2053
2054 def pdbdist(input_filename,output_filename,set_1_start,set_1_end,
2055             set_2_start,set_2_end):
2056     #Function to find the distances between two sets atoms of each pdb
2057     frame.
2058     """
2059     1- pdb file name
2060     2- output file name containing diameters
2061     3- start of first set of atoms
2062     4- end of first set of atoms
2063     5- start second
2064     6- end second
2065     Measure the distance between atoms of each set and write in output
2066     file
2067     """
2068     set1 = []
2069     set2 = []
2070     file = open(input_filename,'r')
2071     lines = file.readlines()
2072     f1 = open(output_filename, 'w')
2073     for i in range(set_1_start,set_1_end):
2074         f1.write(lines[i])
2075     for j in range(set_2_start,set_2_end):
2076         f1.write(lines[j])
2077     f1.close()
2078     return
2079
2080 def radius_from_pdb(inputfilename,set1,set2):
2081     #set1 = 240
2082     #set2 =
2083     #molecule = PDB().read(inputfilename)
2084     molecule= inputfilename[MolIdx(0)].molecule()
2085     list1 = []
2086     list2 = []
2087     dist_list = []
2088     for i in range(set1):
2089         l1 = molecule.atom(AtomIdx(i)).evaluate().center()
2090         #print(l1)

```

```

2090         list1.append(l1)
2091     #print("length of list1 %s" %list1)
2092     for j in range(set1,set2):
2093         l2 = molecule.atom(AtomIdx(j)).evaluate().center()
2094         #print(l2)
2095         list2.append(l2)
2096     #print("length of list2 %s" %list2)
2097     for k in range(len(list1)):
2098         atom1 = list1[k]
2099         for l in range(len(list2)):
2100             dist = Vector.distance(atom1,list2[l])
2101             #print(dist)
2102             dist_list.append(dist)
2103     #print(len(dist_list))
2104     dist = max(dist_list)
2105     return dist
2106
2107 def load_files_to_prepare_system_for_flexibility(inpcrd,prmtop,
2108     dimensions,save_sys_name):
2109     """
2110     function to load files (inpcrd, prmtop, dimensions of box files and
2111     assign flexibility)
2112     the input files added with route directories ....
2113     """
2114
2115     # Load the molecules and the periodic box from the crd/top files
2116     (mols, space) = Amber().readCrdTop(inpcrd,prmtop)
2117     mols.setName("mols")
2118     cord_file = dimensions
2119     cord_file = open(cord_file, 'r')
2120     line = line_extract(cord_file)
2121     total_cord = [float(b) for a in re.findall(r'{.*?}', line) for
2122         b in a.split()]
2123     print("Original Box Dimensions %s" %total_cord)
2124     total_cord = coordinates_check(total_cord)
2125     maxcord = Vector(total_cord[0:3])
2126     mincord = Vector(total_cord[-3:])
2127     space = PeriodicBox(mincord, maxcord)
2128     print("assigning flexibility ... ")
2129     new_group = GenerateFlexibility_all_molecules(mols)
2130     print("flexibility assigned ... ")
2131     return (new_group,space)
2132
2133 def GenerateFlexibility_all_molecules(peg):
2134     """

```

---

```

2132     function to determine flexibility of all molecules in the box.
2133     """
2134     npeg = peg.nMolecules()
2135     new_group = MoleculeGroup("Mols")
2136     for i in range(npeg):
2137         print(i)
2138         random_peg = peg.moleculeAt(i).molecule()
2139         flexibility = AmberLoader.generateFlexibility(random_peg)
2140         random_peg = random_peg.edit() \
2141             .setProperty("flexibility", flexibility) \
2142             .commit()
2143         new_group.add(random_peg)
2144     return new_group

```

---

### A.3.2 Quenching sampling program

---

```

1 """
2 Sire script to perform conformational sampling using a temperature
3 quenching method.
4
5 Input Files: PEG.inpcrd, PEG.prmtop
6 output: pdb files save after every n steps
7 """
8 #####
9 # Import Libraries
10 #####
11 From MyFunctions import *
12
13 #####
14 # Main program
15 #####
16
17 # Get the structural details
18
19 map = { "weight function" : RelFromMass() }
20
21 # Load the molecule & extract structural details
22
23 (GiveMolec, space) = Amber().readCrdTop("peg.inpcrd", "peg.prmtop")
24 GiveMolec = GiveMolec[MolIdx(0)].molecule()
25 bonds = GiveMolec.property("connectivity").getBonds()
26 angles = GiveMolec.property("connectivity").getAngles()
27 dihedrals = GiveMolec.property("connectivity").getDihedrals()
28 nbonds = len(bonds)

```

```
29 nangles = len(angles)
30 ndihedrals = len(dihedrals)
31 nbonds = len(bonds)
32 nangles = len(angles)
33 ndihedrals = len(dihedrals)
34
35 # create a random number generator for the moves
36 rangen = RanGenerator()
37
38 # create a forcefield to calculate the intramolecular (bond, angle,
   dihedral) energy of the Molecule
39 intraff = InternalFF("intraff")
40
41 # create a forcefield to calculate the intramolecular (coulomb and LJ)
   energy of the Molecule
42 intraclj = IntraCLJFF("intraclj")
43 intrasoft = IntraSoftCLJFF("intrasoft")
44 intrabase = IntraCLJFFBase("intrabase")
45
46 # add the Molecule to both forcefields
47 intraff.add(GiveMolec)
48 intraclj.add(GiveMolec)
49
50 # create system to hold everything
51 system = System()
52 system.add(intraff)
53 system.add(intraclj)
54
55 # list two quenching temperatures
56 temp = 1200
57 temp2 = 300
58 naccept = 0
59 nreject = 0
60
61 # perform 200 blocks of 20 moves
62 for i in range(1,2000):
63     print("Molecule energy Initial = %s" % system.energy())
64     print("Performing block %s" % i)
65     print(" MC Temperature %s " %temp)
66
67     for j in range(0,10):
68         # randomly choose which move to run
69         move_type = rangen.randInt(1,3)
70
71         if move_type == 1:
```

```
72         bondMove(temp,system)
73     elif move_type == 2:
74         angleMove(temp,system)
75     elif move_type == 3:
76         dihedralMove(temp,system)
77     print("Block complete: number of accepted moves = %s (acceptance
78           ratio %s %%)" % \
79           (naccept, 100.0*naccept / (naccept+nreject)) )
79     print("Molecule energy Final = %s" % system.energy())
80     radius_list = []
81 #file2 = open('radiusoutput.log','w')
82     mol = system[MolIdx(0)].molecule()
83     radius = radgyr(mol)
84     print("radius of gyration %s" %radius)
85 #radius_list.append(radius)
86     PDB().write(system.molecules(), "peg%0d.pdb" % i)
87     system300 = system
88     PEG = system[MolIdx(0)].molecule()
89     if i % 1 ==0:
90         quenched_conf(system300, temp2)
91     system = make_system(PEG)
92     print("***** The End *****")
93     print("***** The End *****")
```

---

## A.4 MD sampling in Amber

### A.4.1 mini.in

---

```
1 initial minimization prior to MD GB model
2 &cntrl
3   imin    = 1,
4   maxcyc = 500,
5   ncyc   = 250,
6   ntb     = 0,
7   igb     = 1,
8   cut     = 10
9 /
```

---

### A.4.2 md.in

---

```
1 MD Generalized Born, 12 angstrom cut off
2 &cntrl
3   imin = 0, ntb = 0,
4   igb = 1, ntp = 100, ntwx = 100,
5   ntt = 3, gamma_ln = 1.0,
6   temp = 300.0, temp0 = 300.0
7   nstlim = 10000000, dt = 0.001,
8   cut = 12.0
9 /
```

---

## A.5 Packing and Equilibration

---

```
1 # Packmol input file
2 tolerance 3.0
3 discalc 2.0
4 randominitialpoint
5 add_amber_ter
6 nseed 12345
7 nloop 1000
8 filetype pdb
9 output box101.pdb
10 structure peg725.pdb
11   number 1
12   inside box -150. -150. -150. 150. 150. 150.
13 end structure
14 structure peg328.pdb
15   number 1
16   inside box -150. -150. -150. 150. 150. 150.
17 end structure
18 structure peg576.pdb
19   number 1
20   inside box -150. -150. -150. 150. 150. 150.
21 end structure
22 structure peg770.pdb
23   number 1
24   inside box -150. -150. -150. 150. 150. 150.
25 end structure
26 structure peg768.pdb
27   number 1
28   inside box -150. -150. -150. 150. 150. 150.
29 end structure
30 structure peg701.pdb
31   number 1
32   inside box -150. -150. -150. 150. 150. 150.
33 end structure
34 structure peg366.pdb
35   number 1
36   inside box -150. -150. -150. 150. 150. 150.
37 end structure
38 structure peg506.pdb
39   number 1
40   inside box -150. -150. -150. 150. 150. 150.
41 end structure
42 structure peg709.pdb
43   number 1
```

```
44    inside box -150. -150. -150. 150. 150. 150.
45 end structure
46 structure peg540.pdb
47    number 1
48    inside box -150. -150. -150. 150. 150. 150.
49 end structure
50 structure peg816.pdb
51    number 1
52    inside box -150. -150. -150. 150. 150. 150.
53 end structure
54 structure peg37.pdb
55    number 1
56    inside box -150. -150. -150. 150. 150. 150.
57 end structure
58 structure peg396.pdb
59    number 1
60    inside box -150. -150. -150. 150. 150. 150.
61 end structure
62 structure peg24.pdb
63    number 1
64    inside box -150. -150. -150. 150. 150. 150.
65 end structure
66 structure peg513.pdb
67    number 1
68    inside box -150. -150. -150. 150. 150. 150.
69 end structure
70 structure peg677.pdb
71    number 1
72    inside box -150. -150. -150. 150. 150. 150.
73 end structure
74 structure peg306.pdb
75    number 1
76    inside box -150. -150. -150. 150. 150. 150.
77 end structure
78 structure peg724.pdb
79    number 1
80    inside box -150. -150. -150. 150. 150. 150.
81 end structure
82 structure peg318.pdb
83    number 1
84    inside box -150. -150. -150. 150. 150. 150.
85 end structure
86 structure peg192.pdb
87    number 1
88    inside box -150. -150. -150. 150. 150. 150.
```

```
89 end structure
90 structure peg783.pdb
91   number 1
92   inside box -150. -150. -150. 150. 150. 150.
93 end structure
94 structure peg109.pdb
95   number 1
96   inside box -150. -150. -150. 150. 150. 150.
97 end structure
98 structure peg827.pdb
99   number 1
100  inside box -150. -150. -150. 150. 150. 150.
101 end structure
102 structure peg679.pdb
103   number 1
104   inside box -150. -150. -150. 150. 150. 150.
105 end structure
106 structure peg644.pdb
107   number 1
108   inside box -150. -150. -150. 150. 150. 150.
109 end structure
```

---

### A.5.1 Equilibration with Sire

---

```
1 """
2 MC Equilibration with sire
3 Input files: simulation systems, box dimensions
4 output: Equilibrated simulation system
5 """
6 ######
7 # Import Libraries as listed in Sire sampling input file
8 ######
9 from MyFunctions import *
10
11 #####
12 #      Main program
13 #####
14
15 # Load the systems to perform equilibration
16 inpcrd = "box1001.inpcrd"
17 prmtop = "box1001.prmtop"
18 dimensions = 'box1001.dat'
19 save_sys_name = "flex_system.s3"
20
21 # Determine number of systems needs to equilibrate
```

```

22 check = os.path.isfile("flex_system.s3")
23 check = str(check)
24 if check == 'True':
25     print("Minimized or system with flexibility is available and load
          it")
26     new_groups = load("flex_system.s3")
27     new_group = collect_mc_molecs(new_groups)
28     (simSystem,ljcp)=create_single_System(new_group,new_groups.
          property('space'))
29 else:
30     print("Making system with flexibility and defining rigid and
          internal moves...")
31     (new_group,space)=load_files_to_prepare_system_for_flexibility(
          inpcrd,prmtop,dimensions,save_sys_name)
32     (simSystem,ljcp)=create_single_System(new_group,space)
33
34     print("system is done!")
35 ## Definition of parameters for simulations
36
37 # Initialize random generator
38 rangen = RanGenerator()
39 # Set the temperature
40 temperature = 298.15 * kelvin
41 # Specify the maximum amount to move the bonds, angles and dihedrals
42 max_delta_bond = 0.05 * angstrom
43 max_delta_angle = 3.5 * degrees
44 max_delta_dihedral = 10 * degrees
45 ## specify the maximum number of bonds, angles and dihedrals to move
          per move
46 max_num_move = 25
47 # set the maximum delta parameters
48 params = {}
49 params["bond flex"] = max_delta_bond
50 params["angle flex"] = max_delta_angle
51 params["dihedral flex"] = max_delta_dihedral
52 params["h dihedral flex"] = max_delta_dihedral
53 params["maxvar"] = max_num_move
54 Parameter.push(params)
55
56 # Perform Rigid body and internal MC moves
57 (system_mc,wt_moves)=NVT_ensemble_moves(simSystem)
58 MGNName = system_mc.groupNames()[-1]
59 internalmove = InternalMove(system_mc[MGNName])
60 Sire.Stream.save( system_mc, "flex_system.s3" )
61

```

---

```

62 # Save energy values
63 file = open('Energy_peg5.log','w')
64 print("Perform MC simulations")
65
66 # perform 5000 blocks of 2*5000 moves
67 for i in range(1,1001):
68     print("performing step %s"%i)
69     print("system_mc start energy %s"%system_mc.energy())
70     system_mc = simulation_moves_simple(system_mc,wt_moves,5000)
71     print("system energy after RIGID BODY mc % s " % system_mc.energy
72         ())
73     internalmove.move(system_mc, 5000)
74     print("Energy %s, nAccepted = %s, nRejected = %s" % \
75         (system_mc.energy(), internalmove.nAccepted(),
76         internalmove.nRejected()))
77     print("system final energy after internal Moves %s"%system_mc.
78         energy())
79     file.write(str(system_mc.energy())+'\n')
80     print("*****")
81     if i % 100 == 0:
82         #PDB().write(system_mc.molecules(), "peg_%0000000d.pdb" % i)
83         Sire.Stream.save( system_mc, "rstpeg_%000000d.s3" % i )
84 file.close()

```

---

### A.5.2 Swapping equilibrium with Sire

---

```

1 """
2 Script to equilibrate the PEG systems by inserting of a random peg from
    PEG library. If the peg is placed then keep the configuration
    otherwise reverse the configuration by placing the original peg in
    the system.
3
4 Steps to perform equilibrium
5 1- load the initial configuration system of desired concentration and
    determine energy E1
6 2- select random peg molecule and remove it from the system
7 3- pick the random molecule from the library and insert it and
    determine E2
8 4- check if E2 < E1 -> accepted
9 5- if not, then reverse the system.
10 """
11 #####
12 # Import Libraries as listed in Sire sampling input file
13 #####
14 from MyFunctions import *

```

```

15
16 ######
17 #      Main program
18 #####
19
20 # time counter
21 start_time = time.clock()
22 t = QTime()
23 t.start()
24
25 # load simulation system and dimensions and estimate energy
26 (peg, boxSpace)= Amber().readCrdTop("box101.inpcrd", "box101.prmtop")
27 cord_file = 'box1001.dat'
28 cord_file = open(cord_file,'r')
29 line = line_extract(cord_file)
30 total_cord = [float(b) for a in re.findall(r'{(.*)?}', line) for b
   in a.split()]
31 print("Original Box Dimensions %s" %total_cord)
32 total_cord = coordinates_check(total_cord)
33 maxcord = Vector(total_cord[0:3])
34 mincord = Vector(total_cord[-3:])
35 boxSpace = PeriodicBox(mincord, maxcord)
36 initial_energy = default_system_energy(peg,boxSpace)
37
38 # Place a molecule from a library to the simulation box
39 randgen = RanGenerator()
40 npeg = peg.nMolecules()
41 peg_id = randgen.randint(0,npeg-1)
42 random_peg = peg[MolIdx(peg_id)].molecule()
43 peg.remove(random_peg)
44 peg.update(peg)
45 after_remove_energy = default_system_energy(peg,boxSpace)
46 print("after removing peg %s"%peg.nMolecules())
47 peg.update(peg)
48
49 # select random molecule from the library
50 lib_peg_id = randgen.randint(1,887)
51 inpcrd = 'input_files/library/peg'+str(lib_peg_id)+'.inpcrd'
52 prmtop = 'input_files/library/peg'+str(lib_peg_id)+'.prmtop'
53 (new_peg, space)= Amber().readCrdTop(inpcrd,prmtop)
54 new_peg = new_peg[MolIdx(0)].molecule()
55 new_peg = new_peg.edit().renumber().commit()
56 molnum = new_peg.number()
57

```

```
58 # simulation temperature and internal moves performed on the molecule
  and add to system
59 temperature = 298.15 * kelvin
60 max_translation = 150 * angstrom
61 max_rotation = 360 * degrees
62 (system,lj_comp)=createSystem(peg, new_peg, boxSpace)
63 random_translate = randgen.vectorOnSphere( max_translation.value() )
64 random_rotate_axis = randgen.vectorOnSphere()
65 random_rotate_angle = randgen.rand(-max_rotation.value(),
  max_rotation.value()) * degrees
66 random_water = system[molnum].molecule()
67 moved_water = random_water.move() \
  .rotate( \
    Quaternion(random_rotate_angle,random_rotate_axis), \
    random_water.evaluate().center() ) \
  .translate(random_translate).commit()
68 system.update(moved_water)
69 final_energy = system.energy(lj_comp).value()
70 print("intial peg system energy %s "%initial_energy)
71 print("after remove energy %s "%after_remove_energy)
72 print("system energy after adding %s "%final_energy)
73 #PDB().write(system.molecules(),"testing.pdb")
74 condition = condition_fn(initial_energy,final_energy)
75 print("result from condition %s"%condition)
76 if condition == "True":
77   new_system = collect_mc_molecs(system)
78   success_energy = default_system_energy(new_system,boxSpace)
79   print("successful insertion energy %s "% success_energy)
80 else:
81   new_system = collect_mc_molecs(system)
82   new_system.remove(random_water)
83   new_system.add(random_peg)
84   after_all_energy = default_system_energy(new_system,boxSpace)
85   print("reverse system energy %s "% after_all_energy)
86 print(*****)
```

---

### A.5.3 MD Equilibration with Amber

#### A.5.3.1 Execution file

---

```
1 #!/bin/bash
2 #PBS -S /bin/bash
3 #PBS -l walltime=20:00:00
4 #PBS -l nodes=1:ppn=12
5 #PBS -l mem=22gb
```

```
6
7 cd $PBS_O_WORKDIR
8
9 echo "Current working directory is `pwd`"
10
11 INPUT=gbin2
12 OUTPUT=mdout.out
13 PARM=box106.prmtop
14 INPCRD=box106.inpcrd
15
16
17 #export AMBERHOME=/global/software/amber/amber11
18 #PATH=$PATH:$AMBERHOME/exe
19 export AMBERHOME=/global/software/amber/amber14p8_at14p21
20 . $AMBERHOME/amber.sh
21
22 echo "Node file: $PBS_NODEFILE :"
23 echo -----
24 cat $PBS_NODEFILE
25 echo -----
26 NUM_PROCS=`/bin/awk 'END {print NR}' $PBS_NODEFILE`
27 echo "Running on $NUM_PROCS processors."
28
29
30 echo "prog started at: `date`"
31 mpieexec -n $NUM_PROCS pmemd.MPI -O -i $INPUT -o $OUTPUT -p box106.
    prmtop -c box106.inpcrd -r box106_md.rst -x box106_md.mdcrd
32
33 echo "prog finished at: `date`"
34 -----
```

---

### A.5.3.2 Parameter file

---

```
1 equilibration of crowded systems
2 &cntrl
3 imin = 0, irest = 0, ntx = 1,
4 ntb = 2, pres0 = 1.0, ntp = 1,
5 taup = 2.0,
6 cut = 10.0, ntr = 0,
7 ntc = 2, ntf = 2,
8 tempi = 300.0, temp0 = 300.0,
9 ntt = 3, gamma_ln = 1.0,
10 nstlim = 1200000, dt = 0.002,
11 ntp = 100, ntwx = 100, ntwr = 100000
12 /
```



## A.6 Fractional available volumes calculations

### A.6.1 Parallel-energy algorithm

---

```
1 """
2 Parallel-energy algorithm to find the fractional available volume based
3 on finding the system energies.
4
5 Input files: simulation systems, probe molecules, box dimensions
6 output: Txt file containing fractional available volume
7 """
8 ##### Import Libraries as listed in Sire sampling input file
9 #####
10 from MyFunctions import *
11
12 #####
13 # Main program
14 #####
15
16 # Name of RNA/protein molecule to be inserted in a box
17 RNA = "2k96"
18 path2 = "../rna/"
19 inpcrd_prob = RNA+'.inpcrd'
20 inpcrd_prob = path2+inpcrd_prob
21 prmtop_prob = RNA+'.prmtop'
22 prmtop_prob = path2+prmtop_prob
23
24 # Box dimensions are uploaded automatically from vmd.dot files. These
25 # files should be in path1 directory.
26 nReplicates = 10001
27 dsteps = 100
28 irange = int(nReplicates/dstecs)
29 prit = 100
30
31 #Find total simulation systems to be used in Monte Carlo simulations
32 print("Loading the available files to be used in Monte Carlo
33 Simulations")
34 path1 = "../peg1_samples/"
35 system_files1 = [f for f in os.listdir(path1) if f.endswith('.inpcrd'
36
37 system_files2 = [f for f in os.listdir(path1) if f.endswith('.prmtop'
38
39 system_files3 = [f for f in os.listdir() if f.endswith('.log')]
40 print("Number of systems available are %d" % len(system_files1))
41 system_numbers = []
```

```

38 for f in system_files1:
39     system_numbers.append(f[4:6])
40 system_numbers2 = []
41 for f in system_files3:
42     system_numbers2.append(f[-6:-4:])
43 system_numbers = [elem for elem in system_numbers if elem not in
44     system_numbers2]
44 print("Number of systems to run MC %s" % len(system_numbers))
45 print()
46 print("Loading input molecule = %s" % RNA)
47 (probeMolecules, boxSpace1)= Amber().readCrdTop(inpcrd_prob,prmtop_prob
        )
48 probeMolecules.setName("probeMolecules")
49 probeMolecules_mol = probeMolecules[MolIdx(0)].molecule()
50 number = probeMolecules_mol.number()
51 residue = probeMolecules_mol.residues(ResIdx(0)).residue().name().value
        ()
52
53 print("++++++START MC ++++++")
54 print()
55 # probeMolecules/PROTEIN INPUT
56 for i in system_numbers:
57     start_time = time.clock()
58     t = QTime()
59     t.start()
60     inpcrd = 'box1'+i+'.inpcrd'
61     inpcrd = path1+inpcrd
62     prmtop = 'box1'+i+'.prmtop'
63     prmtop = path1+prmtop
64     cord_file = 'box1'+i+'.dat'
65     cord_file = path1+cord_file
66     print("Running System %s" % i)
67     cord_file = open(cord_file,'r')
68     line = line_extract(cord_file)
69     total_cord = [float(b) for a in re.findall(r'{(.*)}', line) for
        b in a.split()]
70     print("Original Box Dimensions %s" %total_cord)
71
72 #total_cord = coordinates_check(total_cord)
73 mincord = Vector(total_cord[0:3])
74 maxcord = Vector(total_cord[-3:])
75 boxSpace = PeriodicBox(mincord, maxcord)
76 print(boxSpace)
77 (simSystem,boxSpace1)= Amber().readCrdTop(inpcrd,prmtop)
78 simSystem.setName("simSystem")

```

```
79
80     #residue = probeMolecules_mol.residues(ResIdx(0)).residue().name() .
81     #             value()
82     irange = int(nReplicates/dsteps)
83     count = 0
84     throws = 0
85     output_vol = boxSpace.volume().value()
86
87     # STERIC CLASHES AND SYSTEM ENERGIES CALCULATIONS
88     system_energy = default_system_energy(simSystem,boxSpace)
89     system_energy = abs(system_energy)
90     print("system energy ( crowders only ) from pdb = %s:"%
91           system_energy)
92     system_energy_probeMolecules = default_system_energy(probeMolecules
93           ,boxSpace)
94
95     # time counter
96     start_time = time.clock()
97     t = QTime()
98     t.start()
99
100    # Specify the temperature of the simulation
101    temperature = 298.15 * kelvin
102
103    # Specify the maximum amount by which to translate each Molecule
104    max_translation = 150 * angstrom
105
106    # Specify the maximum amount by which to rotate each Molecule
107    max_rotation = 360 * degrees
108    rangen = RanGenerator()
109
110    # create a system to hold the molecules and forcefields
111    (system,lj_comp) = createSystem(simSystem, probeMolecules_mol,
112        boxSpace)
113    t2 = QElapsedTimer()
114    t2.start()
115    energy = system.energy(lj_comp)
116    ns = t2.nsecsElapsed()
117    print("Initial system energy = %s kcal mol-1 (took %s ms)" %
118          (energy.value(),0.000001*ns))
119    loop_i = i
120    avg = []
121    for i in range(1,irange):
122        output = loop_division2((i * dsteps)-((i-1)*dsteps),system,
123                               residue)
```

```
119     avg.append(output)
120     t_steps = irange*dsteps-(dsteps)
121     if i % prit == 0:
122         statistics_test(avg,output_vol,i*dsteps)
123     p = sum(avg)/len(avg)
124     print("average of excluded volume %s" % p)
125     V_free = p*output_vol
126     std_binom = sqrt(t_steps*p*(1-p))
127     error_binom = (output_vol/t_steps)*std_binom
128     error_binom_fraction = error_binom/V_free
129     print("COMPLETE STEPS %s: BINOMIAL ERROR in Total Vol. %s: ERROR
130           in Fraction vol. %s: Available VOLUME %s:" %(t_steps,
131           error_binom,error_binom_fraction*100,p))
130     lloopi = str(loop_i)
131     filename = RNA +'box10'+lloopi+'.log'
132     filename2 = RNA +'radgy_box10'+lloopi+'.txt'
133     log_file_writer(filename,t_steps,p,error_binom_fraction*100)
134     v= boxSpace.volume().value()
135     print()
136     (rad_list,vol_list) = collect_radgyr_box(simSystem)
137     vol = sum(vol_list)
138     a = (v-4*vol)/v
139     log_file_writer2(filename2,t_steps,a,vol)
140     print("numerical estimate of available volume (radgyr) = % s" % a)
141     print("done system %s :" % loop_i)
142     elapsed = (time.clock() - start_time)/3600
143     print("(wall time %d h for given system %s)" % (elapsed,loop_i))
144     print("*****")
```

---

## A.6.2 Parallel-distance algorithm

---

```
1 """
2 Parallel-distance algorithm
3
4 Input Files: simulation box, probe molecule
5
6 output: Txt file containing fractional available volume
7 """
8 ######
9 # Import Libraries as listed in Sire sampling input file
10 #####
11 from MyFunctions import *
12
13 #####
14 # Main program
```

```
15 ######
16
17 # time counter
18 start_time = time.clock()
19 t = QTime()
20 t.start()
21 # load simulation system
22 (simSystem,boxSpace)= Amber().readCrdTop("box1001.inpcrd","box1001.
    prmtop")
23 print("Number of Molecules in given system %s" % simSystem.nMolecules
    ())
24 simSystem.setName("peg")
25
26 # define system dimensions
27 mincoords = Vector( -150.0, -150.0, -150.0 )
28 maxcoords = Vector( 150.0, 150.0, 150.0)
29 boxSpace = PeriodicBox(mincoords, maxcoords)
30 output_vol = boxSpace.volume().value()
31
32 # load probe molecule
33 (probMolecule, boxSpace1)= Amber().readCrdTop("2k96.inpcrd","2k96.
    prmtop")
34 probMolecule.setName("probMolecule")
35 probMolecule = probMolecule[MolIdx(0)].molecule()
36 probMolecule=probMolecule.edit().renumber().commit()
37 number = probMolecule.number()
38 ResID = probMolecule.residues(ResIdx(0)).residue().name().value()
39
40 # Perform MC simulations to compute the steric clashes
41 print("Running Monte Carlo Simulations over lists of systems")
42 new_system_count_outputs = 0
43 output_of_lists_ratio = []
44 for k in range(1,3):
45     new_systems_list = list_maker_for_multi(4,simSystem, probMolecule,
        boxSpace)
46     n = (len(new_systems_list))
47     total = n*k
48     (accepted_moves,pool) = multi_processing_files(new_systems_list)
49     pool.close()
50     output_of_lists_ratio.append(accepted_moves)
51     new_system_count_outputs = sum(output_of_lists_ratio)/len(
        output_of_lists_ratio)
52     V_free = new_system_count_outputs*output_vol
53     p = new_system_count_outputs
54     std_binom = sqrt(n*p*(1-p))
```

```
55     error_binom = (output_vol/n)*std_binom
56     error_binom_fraction = error_binom/V_free
57     print("TOTAL SYSTEM STEPS %d: BINOMIAL ERROR T.VOLUME %s: ERROR F.
58             VOLUME %s: ESTIMATED VOLUME %s:" %(total, error_binom,
59                 error_binom_fraction,p))
60 # Compute the fractional available volumes from ratio of successful to
61 # total trials and standard deviations
62 new_system_count_outputs = sum(output_of_lists_ratio)/len(
63     output_of_lists_ratio)
64 V_free = new_system_count_outputs*output_vol
65 p = new_system_count_outputs
66 std_binom = sqrt(n*p*(1-p))
67 error_binom = (output_vol/n)*std_binom
68 error_binom_fraction = error_binom/V_free
69 print("FULL SYSTEM STEPS %d: BINOMIAL ERROR T.VOLUME %s: ERROR F.
70             VOLUME %s: ESTIMATED VOLUME %s:" %(n,error_binom,
71                 error_binom_fraction,p))
72 print(new_system_count_outputs)
73 print("(Done in %d ms)" % t.elapsed())
74 s = t.elapsed()/1000
75 hours, remainder = divmod(s, 3600)
76 minutes, seconds = divmod(remainder, 60)
77 print ('%s h:%s m:%s s' % (hours, minutes, seconds))
78 elapsed = (time.clock() - start_time)/3600
79 print("(wall time %d h)" % elapsed)
```

---

## A.7 The scaled particle theory

### A.7.1 findcurvature.m

```

36      pt1 = pt1(1,:);
37      end
38
39  % compute individual vectors
40 v12 = bsxfun(@minus, pt2, pt1);
41 v13 = bsxfun(@minus, pt3, pt1);
42
43  % compute area from cross product
44 Area = (sum(vectorNorm3d(cross(v12, v13, 2)) / 2)) * (10^-10)^2;
45
46  %% Step 3: %%% Calculation of radius of curvature %%%
47
48  % Reference: Coleman, R. G.; Burr, M. A.; Souvaine, D. L.; Cheng, A.
        C., An intuitive approach to measuring protein surface curvature
        . Proteins 2005, 61 (4), 1068-74. %%%
49  % Step 3 has two major sections , (1,2) while second section has
        eight (a-h) steps.
50
51
52  %% Section 1: Define a set of points to find the least sum of squares
        sphere.
53  % set of all points from convex hull (P = K -> pt1,pt2,pt3)
54  % P = [pt11,pt21,pt31]; % Set of all convexhull points
55  %P = [pt1(:,1),pt2(:,2),pt3(:,3)]; % Previous set of points used
        for calculations
56  P = [pt(:,1),pt(:,2),pt(:,3)]; % points from unique set of K
57
58
59  %% Section 2 : For each point pi belongs to P where pi is one row
        vector from P.
60
61 s = size(P,1); %size of matrix
62 for i=1:s
63     pit = [P(i,:)] ;           %set of pi = p,q,r points from P
        matrix
64     Px = [P(1:i-1,:);P(i+1:s,:)]; % corresponding Rest of points in
        P excluding p,q,r
65
66  %% Sec. 2 : (a) Let pi be the inversion point p,q,r and Px points be
        all other points in P . %%
67  % define the p,q,r points of pi row as Sp,Sq,Sr
68  % define the points xi, yi, zi of Px matrix as SPx, SPy, SPz
69
70  sPx = size(Px,1);          % size of Px matrix
71      Sp = pit(1);    %p point

```

```

72 Sq = pit(2) ; % q point
73 Sr = pit(3); %r point
74 for k = 1:sPx;
75 SPx = Px(k,1); % xi points
76 SPy = Px(k,2); % yi points
77 SPz = Px(k,3); % zi points
78
79 %% Sec. 2 : (b) Invert Px = xi,yi,zi points using the inversion defined
    in the methods to generate ti = Xi,Yi,Zi points %%
80 %Inversive transformation formula given as Xi, Yi, Zi. This will
    give a matrix of ti points with respect to each pi point
81
82 K = 1;
83 Xi = K^2*(SPx-Sp) / ((SPx-Sp)^2+(SPy-Sq)^2+(SPz-Sr)^2)+
    Sp;
84 Yi = K^2*(SPy-Sq) / ((SPx-Sp)^2+(SPy-Sq)^2+(SPz-Sr)^2)+
    Sq;
85 Zi = K^2*(SPz-Sr) / ((SPx-Sp)^2+(SPy-Sq)^2+(SPz-Sr)^2)+
    Sr;
86 ti(k,:) = [Xi Yi Zi];
87 tix = ti(:,1);
88 tiy = ti(:,2);
89 tiz = ti(:,3);
90 end
91 %% Sec. 2 : (c)Find the least sum of squares plane fit to the points ti
92 .
93 % Fit the plane to the ti points by using affine_fit function to get
    normal vector n_plane and point (u) p_plane.
94 Cmtx = cov(ti);
95 [V, LAMBDA] = eig(Cmtx);
96 %the mean of the samples belongs to the plane
97 p_plane = mean(ti,1);
98 %The samples are reduced:
99 R = bsxfun(@minus,ti,p_plane);
100 %Computation of the principal directions if the samples
    cloud
101 [V,D] = eig(R'*R);
102 %Extract the output from the eigenvectors
103 n_plane = V(:,1);
104 nplanex = n_plane(1,1);
105 nplaney = n_plane(2,1);
106 nplanez = n_plane(3,1);
107 V = V(:,2:end);
108

```

```

109 %% Sec. 2 : (d) Find the point on the plane closest to pi . Call this
110   % we hanve already normal vector(gives a,b,c) and point on plane(
111   % gives u -> x0,y0,z0) .
112   %Method 1:
113   %Equation of plane ax+by+cz = ax0 +by0+cz0=Kappa point
114   % on plane close to pi
115   %xi = pix+zeta*a
116   %yi = piy+zeta*b
117   %zi = piz+zeta*c
118   %to calculate zeta=
119   %a(pix+zeta*a)+b(piy+zeta*b)+c(piz+zeta*c)=kappa
120   %plugin the zeta value in xi,yi,zi equation to get
121   %the
122   %coodrinates of point on plane close to pi.
123   Kappa = n_plane(1,1)*p_plane(1,1)+n_plane(2,1)*
124   %p_plane(1,2)+n_plane(3,1)*p_plane(1,3);
125   %n_plane(1,1)*(pi(1,1)+zeta*n_plane(1,1))+n_plane
126   % (2,1)*(pi(1,2)+zeta*n_plane(2,1))+n_plane(3,1)*(
127   % piz+zeta*n_plane(3,1))
128   zeta =Kappa-((n_plane(1, 1)*pit(1, 1)+n_plane(2, 1)*
129   % pit(1, 2)+n_plane(3, 1)*pit(1, 3)*(n_plane(1, 1)
130   % ^2+n_plane(2, 1)^2+n_plane(3, 1)^2));
131   a1 = [pit(1,1)+zeta*n_plane(1, 1),pit(1,2)+zeta*
132   % n_plane(2,1),pit(1,3)+zeta*n_plane(3,1)] ;
133
134
135
136
137 %% Sec. 2 : (e) Transform a using the inversion defined in the methods
138   % to generate a-prime=tia .
139   %Define a as;
140   xia = a(1,1);
141   yia = a(1,2);
142   zia = a(1,3);

```

```
143 % inversive transformation of cls_a which is a points
144 Xia = K^2*(xia-Sp) / ((xia-Sp)^2+(yia-Sq)^2+(zia-Sr)^2)
      +Sp;
145 Yia = K^2*(yia-Sq) / ((xia-Sp)^2+(yia-Sq)^2+(zia-Sr)^2)
      +Sq;
146 Zia = K^2*(zia-Sr) / ((xia-Sp)^2+(yia-Sq)^2+(zia-Sr)^2)
      +Sr;
147 tia = [Xia Yia Zia]; %vector of inversive a vector
148 tiax = tia(1,1);
149 tiay = tia(1,2);
150 tiaz = tia(1,3);
151 %% Sec. 2 : (f) Define the sphere center, ci , as the average of pi and
   tia .
152
153 ci=[(pit+tia)/2];
154 %% Sec. 2 : (g) Define the radius for the sphere given center ci
155 ri=norm(pit-tia)/2;
156
157 %% Sec. 2 : (h) If the least sum of squares is lower than the previous
   best fit, keep ci and the radius.
158 error1=0;
159 for k = 1:sPx
160
161 error1 = error1+sqrt((Px(k,1)-ci(1,1))^2+(Px(
   k,2)-ci(1,2))^2+(Px(k,3)-ci(1,3))^2)-ri)^2;
162
163 end
164 error1;
165 if i==1
166 smallest_error = error1;
167 best_ci=ci;
168 best_ri=ri;
169 elseif error1<smallest_error;
170 smallest_error = error1;
171 best_ci=ci;
172 best_ri=ri;
173 end
174 %% Sec. 3 : Output the best found center and radius.
175 curvrad = best_ri*10^-10; %conversion from Angstram to meters
176
177 end
178
179 end
```

---

### A.7.2 findactivity.m

```
1 function [Y,mu] = findactivity(N,Vi,Rad,area,vcell)
2 % calculation of activity coefficients of given molecules under
   different concentration of crowders of different sizes and shapes
3 %Inputs Geometric parameters (Rs,Rc,As,Ac,vs,vc) are the radius, area,
   volume of under study and crowder molecule
4 %vcell volume of cell compartment containg reagents and crowder
   molecules. these are calculated by findcurvature.m function
5 %Ni(number of crowder molecules; values vary over a wide range,example,
   3000:6000 or 3000:100:6000)
6 %outputs :
7 %Y fraction volume occupancy,
8 %mu chemical potential
9
10 %% 2-calculation of chemical potential
11 % Vi,di,Ai,Bi,Ci,Yi individual molecule componenets,while d,A,B,C,Y are
   the sum of individual components to compute mu(chemical pot.)
12 %N1=1; Input N1 as part of N. It could be any number, not necessarily
   1. This could matter for really bulky solutes like ribosomes.
13
14         di = N/vcell;
15         Ai = Rad.*di;
16         Bi = area.*di;
17         Ci = Rad.^2.*di;
18         Yi = Vi.*di;
19         d = sum(di);
20         A = sum(Ai);
21         B = sum(Bi);
22         C = sum(Ci);
23         Y = sum(Yi);
24         mu= [ (-log(1-Y)+B*Rad(1,1)/(1-Y)+4*pi*A*Rad(1,1)
           ^2/(1-Y)+B^2*Rad(1,1)^2/(2*(1-Y)^2)+(4*pi*(1/3))
           *(d/(1-Y)+B^2*C/(3*(1-Y)^3)+A*B/(1-Y)^2)*Rad
           (1,1)^3)];
25 end
```

---

### A.7.3 The SPT main program

```
1 %% script to apply extended scaled particle theory to determine the
   chemical potential and standard devi. using all MD optimized and
   non-optimized simulation boxes
2 clc;
3 clear;
4
```

```

5 % conformational pair
6 probe1 = 'lake.pdb';
7 probe2 = '4ake.pdb';
8 %-----
9 % Input coordinates of cell compartment containing all species to its
   determine volume 'vcell'.
10 side_length = 3.01*10^-8; % side of box in meters
11 cell_volume= side_length^3 ;%input('Enter volum of cell compartment');
12 %-----
13 % extract coordinates of conformational pair molecules and subsequently
   calculate the geometrical parameters through convex-hull algorithm
14 cor = pdb2mat(probe1);
15 cor2 = pdb2mat(probe2);
16 x1 = (cor.X)';
17 y1 = (cor.Y)';
18 z1 = (cor.Z)';
19 [Rs1 As1 vs1] = findcurvature_cav2(x1,y1,z1);
20 x1 = (cor2.X)';
21 y1 = (cor2.Y)';
22 z1 = (cor2.Z)';
23 [Rs2 As2 vs2] = findcurvature_cav2(x1,y1,z1);
24
25 % dgk represents the number of copies of crowded media at each
   concentration
26 for dgk = 1:4
27 box =dgk;
28 num = num2str(box)
29 filename1 = strcat('box',num,probe1(1:4),'.txt')
30 filename2 = strcat('box',num,probe2(1:4),'.txt')
31 %-----
32
33 %Rs2 = 2*Rs2;
34
35
36 % System 1-- PEG-25 -- 0.1 g/ml
37
38 dirs = sprintf('peg25/box');
39 for b = 1:box
40 list_of_file1 = generate_list(dirs,b) ;
41 [radius, area,vi] = crowderParameters2(list_of_file1,b,dirs);
42 range = length(list_of_file1);
43 [Ycp1 mucp1] = thermodynamics(radius, area,vi,Rs1, As1, vs1,cell_volume
   ,range);
44 fprintf('System %s thermodynamics is done \n',probe1)

```

```

45 [Ycp2 mucp2] = thermodynamics(radius, area,vi,Rs2, As2, vs2,cell_volume
   ,range);
46 fprintf('System %s thermodynamics is done \n',probe2)
47 Ycp1boxes(b) = Ycp1;
48 mucp1boxes(b)= mucp1;
49 Ycp2boxes(b) = Ycp2;
50 mucp2boxes(b)= mucp1;
51 end
52
53 % System 2-- PEG-50 -- 0.2 g/ml
54
55 dirs50 = sprintf('peg50/box');
56 for b = 1:box
57 list_of_file1 = generate_list(dirs50,b) ;
58 [radius50, area50,vi50] = crowderParameters2(list_of_file1,b,dirs50);
59 range50 = length(list_of_file1);
60 [Ycp1_50 mucp1_50] = thermodynamics(radius50, area50,vi50,Rs1, As1, vs1
   ,cell_volume,range50);
61 fprintf('System %s thermodynamics is done \n',probe1)
62 [Ycp2_50 mucp2_50] = thermodynamics(radius50, area50,vi50,Rs2, As2, vs2
   ,cell_volume,range50);
63 fprintf('System %s thermodynamics is done \n',probe2)
64 Ycp1_50boxes(b) = Ycp1_50;
65 mucp1_50boxes(b)= mucp1_50;
66 Ycp2_50boxes(b) = Ycp2_50;
67 mucp2_50boxes(b)= mucp2_50;
68 end
69
70 % System 3-- PEG-75 -- 0.3 g/ml
71
72 dirs75 = sprintf('peg75/box');
73 for b = 1:box
74 list_of_file1 = generate_list(dirs75,b) ;
75 [radius75, area75,vi75] = crowderParameters2(list_of_file1,b,dirs75);
76 range75 = length(list_of_file1);
77 [Ycp1_75 mucp1_75] = thermodynamics(radius75, area75,vi75,Rs1, As1, vs1
   ,cell_volume,range75);
78 fprintf('System %s thermodynamics is done \n',probe1)
79 [Ycp2_75 mucp2_75] = thermodynamics(radius75, area75,vi75,Rs2, As2, vs2
   ,cell_volume,range75);
80 fprintf('System %s thermodynamics is done \n',probe2)
81 Ycp1_75boxes(b) = Ycp1_75;
82 mucp1_75boxes(b)= mucp1_75;
83 Ycp2_75boxes(b) = Ycp2_75;
84 mucp2_75boxes(b)= mucp2_75;

```

```

85 end
86
87 % System 4-- PEG-100 -- 0.4 g/ml
88
89 dirs100 = sprintf('peg100/box');
90 for b = 1:box
91 list_of_file1 = generate_list(dirs100,b);
92 [radius100, area100,vi100] = crowderParameters2(list_of_file1,b,dirs100
    );
93 range100 = length(list_of_file1);
94 [Ycp1_100 mucp1_100] = thermodynamics(radius100, area100,vi100,Rs1, As1
    , vs1,cell_volume,range100);
95 fprintf('System %s thermodynamics is done \n',probe1)
96 [Ycp2_100 mucp2_100] = thermodynamics(radius100, area100,vi100,Rs2, As2
    , vs2,cell_volume,range100);
97 fprintf('System %s thermodynamics is done \n',probe2)
98 Ycp1_100boxes(b) = Ycp1_100;
99 mucp1_100boxes(b)= mucp1_100;
100 Ycp2_100boxes(b) = Ycp2_100;
101 mucp2_100boxes(b)= mucp2_100;
102 end
103
104 % System 5-- PEG-125 -- 0.5 g/ml
105
106 dirs125 = sprintf('sample5/box');
107 for b = 1:box
108 list_of_file1 = generate_list(dirs125,b);
109 [radius125, area125,vi125] = crowderParameters2(list_of_file1,b,dirs125
    );
110 range125 = length(list_of_file1);
111 [Ycp1_125 mucp1_125] = thermodynamics(radius125, area125,vi125,Rs1, As1
    , vs1,cell_volume,range125);
112 fprintf('System %s thermodynamics is done \n',probe1)
113 [Ycp2_125 mucp2_125] = thermodynamics(radius125, area125,vi125,Rs2, As2
    , vs2,cell_volume,range125);
114 fprintf('System %s thermodynamics is done \n',probe2)
115 Ycp1_125boxes(b) = Ycp1_125;
116 mucp1_125boxes(b)= mucp1_125;
117 Ycp2_125boxes(b) = Ycp2_125;
118 mucp2_125boxes(b)= mucp2_125;
119 end
120
121 % System 6-- PEG-150 -- 0.6 g/ml
122
123 dirs150 = sprintf('samples6/box');

```

```

124 for b = 1:box
125 list_of_file1 = generate_list(dirs150,b);
126 [radius150, area150, vi150] = crowderParameters2(list_of_file1,b,dirs150
    );
127 range150 = length(list_of_file1);
128 [Ycp1_150 mucp1_150] = thermodynamics(radius150, area150,vi150,Rs1, As1
    , vs1,cell_volume,range150);
129 fprintf('System %s thermodynamics is done \n',probe1)
130 [Ycp2_150 mucp2_150] = thermodynamics(radius150, area150,vi150,Rs2, As2
    , vs2,cell_volume,range150);
131 fprintf('System %s thermodynamics is done \n',probe2)
132
133 Ycp1_150boxes(b) = Ycp1_150;
134 mucp1_150boxes(b)= mucp1_150;
135 Ycp2_150boxes(b) = Ycp2_150;
136 mucp2_150boxes(b)= mucp2_150;
137 end
138 %-----
139
140 % collect chemical potentials and fraction of occupied volumes
141 %% Figure section
142 std_chem1 = [std(mucp1boxes) std(mucp1_50boxes) std(mucp1_75boxes)
    std(mucp1_100boxes) std(mucp1_125boxes) std(mucp1_150boxes)];
143 std_chem2 = [std(Ycp1boxes) std(Ycp1_50boxes) std(Ycp1_75boxes) std(
    Ycp1_100boxes) std(Ycp1_125boxes) std(Ycp1_150boxes)];
144 chem1_all = [mean(mucp1boxes) mean(mucp1_50boxes) mean(mucp1_75boxes)
    ) mean(mucp1_100boxes) mean(mucp1_125boxes) mean(mucp1_150boxes)
    ];
145 excl_all = [mean(Ycp1boxes) mean(Ycp1_50boxes) mean(Ycp1_75boxes)
    mean(Ycp1_100boxes) mean(Ycp1_125boxes) mean(Ycp1_150boxes)];
146 chem2_all = [mean(mucp2boxes) mean(mucp2_50boxes) mean(mucp2_75boxes)
    ) mean(mucp2_100boxes) mean(mucp2_125boxes) mean(mucp2_150boxes)
    ];
147 exc2_all = [mean(Ycp2boxes) mean(Ycp2_50boxes) mean(Ycp2_75boxes)
    mean(Ycp2_100boxes) mean(Ycp2_125boxes) mean(Ycp2_150boxes)];
148
149 % write the files
150
151 writeFile(filename1,excl_all',std_chem1',chem1_all')
152
153 writeFile(filename2,exc2_all',std_chem2',chem2_all')
154
155 end
156
157 peg = [0.1:0.1:0.6];

```

```

158
159 % chemical potential
160 h= figure;
161 plot(peg(~isnan(peg)),chem1_all(~isnan(peg)),'o-',peg(~isnan(peg)),
162     chem2_all(~isnan(peg)),'ro-','LineWidth',3) ; % 'MarkerSize', 8
163 %hold on
164 %errorbar(peg(~isnan(peg)),vol_r(~isnan(peg)),p2k96std)
165 set(gca,'FontName', 'Times')
166 set(gca,'LineWidth', 3);
167 set(gca,'FontSize', 20);
168 xlabel('Concentration g/cc','interpreter','latex')
169 ylabel(' $\mu$ ','interpreter','latex')
170 %title('Frac. avail. vol. in Single, Random and Random (after equi.)
171             PEG conformations by MC simulations')
172 legend(probe1,probe2,'Location','southeast')
173 legend boxoff
174 set(gca, 'LineWidth', 3);
175 set(gca,'FontSize', 22);
176 %axis ([0.1 0.6 0 0.9])
177 what = 'chem_pot';
178 directory = 'article1_thesis_plots/';
179 name = [ directory probe1(1:4) probe2(1:4) what];
180 print(h, '-dpng',name)
181
182 % excluded volumes
183 h= figure;
184 plot(peg(~isnan(peg)),exc1_all(~isnan(peg)),'o-',peg(~isnan(peg)),
185     exc2_all(~isnan(peg)),'ro-','LineWidth',3) ; % 'MarkerSize', 8
186 %hold on
187 %errorbar(peg(~isnan(peg)),vol_r(~isnan(peg)),p2k96std)
188 set(gca,'FontName', 'Times')
189 set(gca,'LineWidth', 3);
190 set(gca,'FontSize', 20);
191 xlabel('Concentration g/cc','interpreter','latex')
192 ylabel(' $\phi$ ','interpreter','latex')
193 %title('Frac. avail. vol. in Single, Random and Random (after equi.)
194             PEG conformations by MC simulations')
195 legend(probe1,probe2,'Location','southeast')
196 legend boxoff
197 set(gca, 'LineWidth', 3);
198 set(gca,'FontSize', 22);
199 %axis ([0.1 0.6 0 0.9])
200 what = 'exc_vol';

```

```

199 directory = 'article1_thesis_plots/';
200 name = [ directory probe1(1:4) probe2(1:4) what];
201 print(h, '-dpng',name)
202
203
204 % total free energy change and stable conformation
205 con1 = [chem1_all-chem2_all];
206 con2 = [chem2_all-chem1_all];
207 h= figure;
208 plot(peg(~isnan(peg)),con1(~isnan(peg)),'o-','LineWidth',3); %
    MarkerSize', 8
209 %hold on
210 %errorbar(peg(~isnan(peg)),vol_r(~isnan(peg)),p2k96std)
211 set(gca,'FontName', 'Times')
212 set(gca, 'LineWidth', 3);
213 set(gca,'FontSize', 20);
214 xlabel('Concentration g/cc','interpreter','latex')
215 ylabel('\Delta G','interpreter','latex')
216 %title('Frac. avail. vol. in Single, Random and Random (after equi.)
    PEG conformations by MC simulations')
217 legend(probe1,'Location','southeast')
218 legend boxoff
219 set(gca, 'LineWidth', 3);
220 set(gca,'FontSize', 22);
221 %axis ([0.1 0.6 0 0.9])
222 what = 'Free_energy';
223 directory = 'article1_thesis_plots/';
224 name = [ directory probe1(1:4) what];
225 print(h, '-dpng',name)
226
227
228 h= figure;
229 plot(peg(~isnan(peg)),con2(~isnan(peg)),'ro-','LineWidth',3); %
    MarkerSize', 8
230 %hold on
231 %errorbar(peg(~isnan(peg)),vol_r(~isnan(peg)),p2k96std)
232 set(gca,'FontName', 'Times')
233 set(gca, 'LineWidth', 3);
234 set(gca,'FontSize', 20);
235 xlabel('Concentration g/cc','interpreter','latex')
236 ylabel('\Delta G','interpreter','latex')
237 %title('Frac. avail. vol. in Single, Random and Random (after equi.)
    PEG conformations by MC simulations')
238 legend(probe2,'Location','southeast')
239 legend boxoff

```

```
240 set(gca, 'LineWidth', 3);  
241 set(gca,'FontSize', 22);  
242 %axis ([0.1 0.6 0 0.9])  
243 what = 'Free_energy';  
244 directory = 'article1_thesis_plots/';  
245 name = [ directory probe2(1:4) what];  
246 print(h, '-dpng',name)
```

---

## A.8 TST main program

---

```

1  %
% % % % % % % % % % % % % % % % % % % % % % % % % % % % % % % % % % %
%
2  %
3  % Kinetics of conformational equilibrium
4  %
5  %
% % % % % % % % % % % % % % % % % % % % % % % % % % % % % % % % %
%
6
7  clc;
8  clear;
9
10 % load pdb structures of crowder, reactant, product and transition
    state
11 crowder = 'pegmd.pdb';
12 probe1 = 'unfold.pdb'
13 probe2 = 'fold.pdb';
14 tst_state = 'tsRNA.pdb';
15 %peg = 0.1:0.1:0.6;
16
17 % import rate experimental constant
18 kexdata = importdata('kexdata.txt');
19 concent_x = kexdata(:,2)';
20 k_ideal = kexdata(:,1)';
21
22 % call function to estimate the folding rate constant
23 [k_final1,k_final2] = tst_rate(k_ideal,probe1,probe2,crowder,tst_state)
    ;
24
25 % Comparison of experimental and theoretical folding rate constants
26 h= figure;
27 plot(concent_x,k_final1,'-*','LineWidth', 3);
28 hold on
29 plot(concent_x,k_ideal,'-d','LineWidth', 3);
30 set(gca,'FontName', 'Times')
31 set(gca, 'LineWidth', 3);
32 set(gca,'FontSize', 20);
33 xlabel('\%PEG (w/w)', 'interpreter', 'latex')
34 ylabel('$k_{\{1\}} s^{\{-1\}}$', 'interpreter', 'latex')
35 %ylabel('k', 'interpreter', 'latex')
36 %title('Frac. avail. vol. in Single, Random and Random (after equi.)'
      PEG conformations by MC simulations')

```

```
37 legend('TST','Expt.', 'location', 'southeast')
38 legend boxoff
39 set(gca, 'LineWidth', 3);
40 set(gca, 'FontSize', 22);
41 filename = strcat('kinetics_plots/kinetics_', probe1(1:4));
42 print(h, '-dpng', 'kinetics_plots/mu_nonEqui2KNA')

1 function [k_final1,k_final2] = tst_rate(k_ideal,probe1,probe2,crowder
    ,tst_state)
2
3 % function to determine the final kinetic rate
4
5 % step 1. calculate the chemical potentials of conformational pair and
    its
6 % transition state.
7
8 [mu1, m] = spt_org_vs_ext_mu_calculations(probe1, crowder);
9 [mu2, m] = spt_org_vs_ext_mu_calculations(probe2, crowder);
10 [mu3, m] = spt_org_vs_ext_mu_calculations(tst_state, crowder);
11 mu1 = mu1(8:8:50);
12 mu2 = mu2(8:8:50);
13 mu3 = mu3(8:8:50);
14 % step2. convert chemical potential to activity coefficients
15 gamma1 = exp(mu1)
16 gamma2 = exp(mu2)
17 gammatst = exp(mu3)
18
19 % step3. tst final rate calculations k = k_ideal(
    activity_coefficient_probe1/activity_coefficient_tst_state)
20
21 k_final1 = k_ideal(1,1)*(gamma1./gammatst)
22 k_final2 = k_ideal(1,1)*(gamma2./gammatst)
23
24 end
```

---

# Appendix B

## Construction of crowded medium appendix

### B.1 PEG with a methyl terminal

#### B.1.1 FF libraries for ‘A’ fragment

---

```
1 #
2 # Generated by PyRED version SEP-2015
3 #     http://q4md-forcefieldtools.org
4 #
5 @<TRIPOS>MOLECULE
6 F01
7     11    10    1    0    1
8 SMALL
9 USER_CHARGES
10 @<TRIPOS>ATOM
11     1 C1    -2.370830 -0.184098  0.000006 CT 1   F01 -0.4499 0.0000 ****
12     2 H11   -3.164666  0.567675  0.000013 H1 1   F01  0.1575 0.0000 ****
13     3 H12   -2.481280 -0.820832  0.892855 H1 1   F01  0.1575 0.0000 ****
14     4 H13   -2.481284 -0.820824 -0.892854 H1 1   F01  0.1575 0.0000 ****
15     5 O     -1.141292  0.507437  0.000007 OS 1   F01 -0.1875 0.0000 ****
16     6 C2    -0.025639 -0.360464 -0.000008 CT 1   F01  0.2409 0.0000 ****
17     7 H21   -0.027678 -1.012405 -0.889175 H1 1   F01  0.0469 0.0000 ****
18     8 H22   -0.027676 -1.012432  0.889144 H1 1   F01  0.0469 0.0000 ****
19     9 C3    1.229623  0.498292  0.000007 CT 1   F01 -0.5486 0.0000 ****
20    10 H31   1.221070  1.147076  0.889147 H1 1   F01  0.2007 0.0000 ****
21    11 H32   1.221067  1.147105 -0.889118 H1 1   F01  0.2007 0.0000 ****
22 @<TRIPOS>BOND
23     1     1     2 1
24     2     1     3 1
25     3     1     4 1
26     4     1     5 1
27     5     5     6 1
28     6     6     7 1
29     7     6     8 1
```

```
30      8      6      9 1
31      9      9     10 1
32     10      9     11 1
33 @<TRIPOS>SUBSTRUCTURE
34      1 F01           1 ****          0 **** * ****
35 @<TRIPOS>HEADTAIL
36 O 0
37 C3 1
38 @<TRIPOS>RESIDUECONNECT
39 1 0 C3 0 0 0 0
```

---

### B.1.2 FF libraries for ‘B’ fragment

---

```
1 #
2 # Generated by PyRED version SEP-2015
3 #     http://q4md-forcefieldtools.org
4 #
5 @<TRIPOS>MOLECULE
6 F02
7      7      6      1      0      1
8 SMALL
9 USER_CHARGES
10 @<TRIPOS>ATOM
11      1 O      -1.141292  0.507437  0.000007 OS 1   F02 -0.1875 0.0000 ****
12      2 C2     -0.025639 -0.360464 -0.000008 CT 1   F02 0.2409 0.0000 ****
13      3 H21    -0.027678 -1.012405 -0.889175 H1 1   F02 0.0469 0.0000 ****
14      4 H22    -0.027676 -1.012432  0.889144 H1 1   F02 0.0469 0.0000 ****
15      5 C3     1.229623  0.498292  0.000007 CT 1   F02 -0.5486 0.0000 ****
16      6 H31    1.221070  1.147076  0.889147 H1 1   F02 0.2007 0.0000 ****
17      7 H32    1.221067  1.147105 -0.889118 H1 1   F02 0.2007 0.0000 ****
18 @<TRIPOS>BOND
19      1      1      2 1
20      2      2      3 1
21      3      2      4 1
22      4      2      5 1
23      5      5      6 1
24      6      5      7 1
25 @<TRIPOS>SUBSTRUCTURE
26      1 F02           1 ****          0 **** * ****
27 @<TRIPOS>HEADTAIL
28 O 1
29 C3 1
30 @<TRIPOS>RESIDUECONNECT
31 1 0 C3 0 0 0 0
```

---

### B.1.3 FF libraries for ‘C’ fragment

---

```
1 # Generated by PyRED version SEP-2015
2 #     http://q4md-forcefieldtools.org
3 #
4 @<TRIPOS>MOLECULE
5 FOO
6      9     8     1     0     1
7 SMALL
8 USER_CHARGES
9 @<TRIPOS>ATOM
10    1 O    -1.141292  0.507437  0.000007 OS 1   FOO -0.1875 0.0000 ****
11    2 C2   -0.025639 -0.360464 -0.000008 CT 1   FOO 0.2409 0.0000 ****
12    3 H21  -0.027678 -1.012405 -0.889175 H1 1   FOO 0.0469 0.0000 ****
13    4 H22  -0.027676 -1.012432  0.889144 H1 1   FOO 0.0469 0.0000 ****
14    5 C3   1.229623  0.498292  0.000007 CT 1   FOO -0.5486 0.0000 ****
15    6 H31  1.221070  1.147076  0.889147 H1 1   FOO 0.2007 0.0000 ****
16    7 H32  1.221067  1.147105 -0.889118 H1 1   FOO 0.2007 0.0000 ****
17    8 O4   2.340493 -0.390181 -0.000010 OH 1   FOO -0.4160 0.0000 ****
18    9 H41  3.147923  0.144212 -0.000016 HO 1   FOO 0.3934 0.0000 ****
19 @<TRIPOS>BOND
20    1    1    2 1
21    2    2    3 1
22    3    2    4 1
23    4    2    5 1
24    5    5    6 1
25    6    5    7 1
26    7    5    8 1
27    8    8    9 1
28 @<TRIPOS>SUBSTRUCTURE
29      1 FOO           1 ****          0 **** ****
30 @<TRIPOS>HEADTAIL
31 O 1
32 O 0
33 @<TRIPOS>RESIDUECONNECT
34 1 0 0 0 0 0 0
```

---

### B.1.4 FF parameters for PEG-M

---

```
1 FRCMOD file generated by PyRED version SEP-2015 - q4md-forcefieldtools
 .org
2 MASS      mass          pol        Source
3 CT        12.010       0.878      taken from parm10.dat
4 H1        1.008       0.135      taken from parm10.dat
5 HO        1.008       0.135      taken from parm10.dat
```

---

```

6 OH      16.000      0.465      taken from parm10.dat
7 OS      16.000      0.465      taken from parm10.dat
8
9 BOND   K(kcal.mol-1.ang-2) Dist0(ang) Source
10 CT-CT    310.0      1.526      taken from parm10.dat
11 CT-H1    340.0      1.090      taken from parm10.dat
12 CT-OH    320.0      1.410      taken from parm10.dat
13 CT-OS    320.0      1.410      taken from parm10.dat
14 HO-OH    555.0      0.960      adapted from parm10.dat 553.0
15
16 ANGLE   K(kcal.mol-1.rad-2) Theta0(deg) Source
17 CT-CT-H1  50.0      109.50     taken from parm10.dat
18 CT-CT-OH  50.0      109.50     taken from parm10.dat
19 CT-CT-OS  50.0      109.50     taken from parm10.dat
20 H1-CT-H1  35.0      109.50     taken from parm10.dat
21 H1-CT-OH  50.0      109.50     taken from parm10.dat
22 H1-CT-OS  50.0      109.50     taken from parm10.dat
23 CT-OH-HO  55.0      108.50     taken from parm10.dat
24 CT-OS-CT  60.0      109.50     taken from parm10.dat
25
26 DIHEDRAL Path V(kcal.mol-1.rad-1) Phase(deg.) Period Source
27 H1-CT-CT-H1 1  1.55555556e-01  0.0      3.  adapted from parm10.
          dat i.e X-CT-CT-X 1.4/9
28 H1-CT-CT-OH 1  0.00000000e+00  0.0      -3.  taken from parm10.
          dat
29 H1-CT-CT-OH 1  2.50000000e-01  0.0      1.  taken from parm10.
          dat
30 H1-CT-CT-OS 1  0.00000000e+00  0.0      -3.  taken from parm10.
          dat
31 H1-CT-CT-OS 1  2.50000000e-01  0.0      1.  taken from parm10.
          dat
32 OH-CT-CT-OS 1  1.44000000e-01  0.0      -3.  taken from parm10.
          dat
33 OH-CT-CT-OS 1  1.17500000e+00  0.0      2.  taken from parm10.
          dat
34 CT-CT-OH-HO 1  1.60000000e-01  0.0      -3.  taken from parm10.
          dat
35 CT-CT-OH-HO 1  2.50000000e-01  0.0      1.  taken from parm10.
          dat
36 H1-CT-OH-HO 1  1.66666667e-01  0.0      3.  adapted from parm10.
          dat i.e X-CT-OH-X 0.5/3
37 CT-CT-OS-CT 1  3.83000000e-01  0.0      -3.  taken from parm10.
          dat
38 CT-CT-OS-CT 1  1.00000000e-01  180.0     2.  taken from parm10.
          dat

```

---

```

39 H1-CT-OS-CT 1      3.83333333e-01      0.0      3. adapted from parm10.
      dat i.e X-CT-OS-X 1.15/3
40
41 IMPROPER          V(kcal.mol-1.rad-1) Phase(deg.) Period Source
42
43 NONBON    R*(ang)  Eps(kcal.mol-1)   Source
44 CT         1.9080   0.10940000  taken from parm10.dat
45 H1         1.3870   0.01570000  taken from parm10.dat
46 HO         0.0000   0.00000000  taken from parm10.dat
47 OH         1.7210   0.21040000  taken from parm10.dat
48 OS         1.6837   0.17000000  taken from parm10.dat

```

---

### B.1.5 Fitting statistics of a whole molecule approach

---

```

1
2 -----
3     Restrained ESP Fit 2.4 q4md-forcefieldtools
4 -----
5     RESP-A1 - RESP input generated by PyRED version SEP-2015
6 -----
7
8
9     inopt      =      0     ioutopt      =      1
10    nmep       =      2     iqopt       =      2
11    ihfree     =      1     irstrnt     =      1
12    iunits      =      0     qwt        =  0.00100000
13
14    multiple-MEP run of 2 MEP
15
16    Reading input for MEP 1 weight: 1.000
17    PEG nconf=1 norient=1 nmep=1/2
18
19    Total charge (ich): 0
20    Number of centers: 13
21    1   6   0
22    2   1   0
23    3   1   2
24    4   1   2
25    5   8   -1
26    6   6   0
27    7   1   0
28    8   1   7
29    9   6   0
30   10   1   0
31   11   1   10

```

```
32      12     8    -1
33      13     1    -1
34
35  Reading input for MEP 2 weight: 1.000
36  PEG nconf=1 norient=2 nmep=2/2
37
38  Total charge (ich): 0
39  Number of centers: 13
40      14     6     0
41      15     1     0
42      16     1     2
43      17     1     2
44      18     8    -1
45      19     6     0
46      20     1     0
47      21     1     7
48      22     6     0
49      23     1     0
50      24     1    10
51      25     8    -1
52      26     1    -1
53      1     1     1     2     1     3     1     4
54  since IQOPT>1, 26 new q0 values
55  will be read in from file ESP.Q0 (unit 3)
56 -----
57  reading mult_esp constraint info
58 -----
59      1     1     2     1
60      1     2     2     2
61      1     6     2     6
62      1     7     2     7
63      1     9     2     9
64      1    10     2    10
65
66 -----
67  Atom  Ivary
68 -----
69      6     0
70      1     0
71      1     2
72      1     2
73      8    -1
74      6     0
75      1     0
76      1     7
```

```
77      6    0
78      1    0
79      1   10
80      8   -1
81      1   -1
82
83      6    1
84      1    2
85      1    2
86      1    2
87      8   -1
88      6    6
89      1    7
90      1    7
91      6    9
92      1   10
93      1   10
94      8   -1
95      1   -1
96
97 -----
98
99
100 Total number of atoms = 26
101 Weight factor on initial charge restraints= 0.001000
102
103
104 There are 3 charge constraints
105
106 Reading esp"s for MEP 1
107 total number of atoms = 13
108 total number of esp points = 699
109
110 Center      X          Y          Z
111     1  -0.1031513E+01 -0.2458118E+01  0.2078699E-04
112     2  -0.3087856E+01 -0.2257916E+01  0.9448630E-05
113     3  -0.4574554E+00 -0.3535972E+01  0.1687282E+01
114     4  -0.4574346E+00 -0.3536012E+01 -0.1687219E+01
115     5  0.0000000E+00  0.0000000E+00  0.0000000E+00
116     6  0.2671096E+01  0.0000000E+00  0.0000000E+00
117     7  0.3424533E+01 -0.9747906E+00 -0.1680261E+01
118     8  0.3424531E+01 -0.9747830E+00  0.1680274E+01
119     9  0.3546946E+01  0.2737385E+01  0.0000000E+00
120    10  0.2781372E+01  0.3695176E+01  0.1680210E+01
```

```

121      11      0.2781370E+01  0.3695170E+01 -0.1680223E+01
122      12      0.6234777E+01  0.2701150E+01 -0.3779452E-05
123      13      0.6819031E+01  0.4435102E+01 -0.3212534E-04
124
125  Reading esp"s for MEP 2
126  total number of atoms = 13
127  total number of esp points = 682
128
129  Center   X           Y           Z
130    1  0.6343621E+01  0.2777408E+01 -0.2078699E-04
131    2  0.6779594E+01  0.4796950E+01 -0.9448630E-05
132    3  0.7195270E+01  0.1902189E+01 -0.1687282E+01
133    4  0.7195302E+01  0.1902159E+01  0.1687219E+01
134    5  0.3688080E+01  0.2544046E+01  0.0000000E+00
135    6  0.2874090E+01  0.0000000E+00  0.0000000E+00
136    7  0.3572911E+01 -0.1014660E+01  0.1680261E+01
137    8  0.3572903E+01 -0.1014656E+01 -0.1680274E+01
138    9  0.0000000E+00  0.0000000E+00  0.0000000E+00
139   10  -0.6789332E+00  0.1021038E+01 -0.1680210E+01
140   11  -0.6789275E+00  0.1021038E+01  0.1680223E+01
141   12  -0.7845802E+00 -0.2571027E+01  0.3779452E-05
142   13  -0.2614103E+01 -0.2599086E+01  0.3212534E-04
143  Initial ssvpot = 0.200
144
145
146  Number of unique UNfrozen centers= 6
147
148  Non-linear optimization requested.
149  qchnge = 0.5760737946E-01
150  qchnge = 0.1258790760E-02
151  qchnge = 0.3838047843E-04
152  qchnge = 0.1523500291E-05
153  qchnge = 0.7222776353E-07
154
155  Convergence in 4 iterations
156
157  1  PEG nconf=1 norient=1 nmep=1/2
158  2  PEG nconf=1 norient=2 nmep=2/2
159
160          Point Charges Before & After Optimization
161
162  no. At.no.   q(init)      q(opt)     ivary d(rstr)/dq
163  1   6       -0.448503    -0.449958    0     0.002169
164  2   1        0.183169     0.157519    0     0.000000
165  3   1        0.143968     0.157519    2     0.000000

```

---

```

166      4   1      0.143966      0.157519      2      0.000000
167      5   8     -0.187505     -0.187505     -1      0.004706
168      6   6      0.189513      0.240868      0      0.003834
169      7   1      0.072139      0.046930      0      0.000000
170      8   1      0.072139      0.046930      7      0.000000
171      9   6     -0.566528     -0.548535      0      0.001793
172     10   1      0.210122      0.200655      0      0.000000
173     11   1      0.210119      0.200655     10      0.000000
174     12   8     -0.415967     -0.415967     -1      0.002337
175     13   1      0.393367      0.393367     -1      0.000000
176
177     14   6     -0.448503     -0.449958      1      0.002169
178     15   1      0.183169      0.157519      2      0.000000
179     16   1      0.143968      0.157519      2      0.000000
180     17   1      0.143966      0.157519      2      0.000000
181     18   8     -0.187505     -0.187505     -1      0.004706
182     19   6      0.189513      0.240868      6      0.003834
183     20   1      0.072139      0.046930      7      0.000000
184     21   1      0.072139      0.046930      7      0.000000
185     22   6     -0.566528     -0.548535      9      0.001793
186     23   1      0.210122      0.200655     10      0.000000
187     24   1      0.210119      0.200655     10      0.000000
188     25   8     -0.415967     -0.415967     -1      0.002337
189     26   1      0.393367      0.393367     -1      0.000000
190
191 Sum over the calculated charges: -0.000
192
193 Statistics of the fitting:
194 The initial sum of squares (ssvpot)          0.200
195 The residual sum of squares (chipot)          0.016
196 The std err of estimate (sqrt(chipot/N))    0.00344
197 ESP relative RMS (SQRT(chipot/ssvpot))      0.28579
198 The Pearson correlation coefficient (r2)     0.91986
199
200 Center of Mass (a.u.):
201 #MEP      X          Y          Z
202   1      2.33126    0.61925   0.00000
203   2      2.38786    0.51238  -0.00000
204
205 Dipole moments (Debye) computed:
206 -with respect to the origin of coordinates (ooc)
207 -with respect to the center of mass (com)
208 #MEP        D          Dx         Dy         Dz
209   1 ooc    0.40235    0.14725   0.37443  -0.00003
210   1 com    0.40235    0.14725   0.37443  -0.00003

```

```
211
212      2 ooc    0.40235   -0.40150   -0.02613   0.00003
213      2 com    0.40235   -0.40150   -0.02613   0.00003
214
215 Traceless Quadrupole moments (Buckingham) computed:
216 -with respect to the origin of coordinates (ooc)
217 -with respect to the center of mass (com)
218 #MEP      X        Y        Z
219     1 ooc X  -8.08665
220             Y  15.50912  15.92587
221             Z -0.00035 -0.00026 -7.83922
222     1 com X  -8.56785
223             Y 13.97860  15.79837
224             Z -0.00024 -0.00023 -7.23052
225
226     2 ooc X 19.63488
227             Y 3.88446 -13.43319
228             Z -0.00019 -0.00014 -6.20170
229     2 com X 21.65003
230             Y 4.31011 -14.41950
231             Z -0.00030 -0.00016 -7.23053
232
233 Traceless Quadrupole moments (Buckingham) in principal axes computed:
234 -with respect to the origin of coordinates (ooc)
235 -with respect to the center of mass (com)
236 #MEP      X        Y        Z
237     1 ooc X 23.53295
238             Y 0.00000 -7.83922
239             Z 0.00000 -0.00000 -15.69373
240     1 com X 22.15790
241             Y -0.00000 -7.23052
242             Z -0.00000  0.00000 -14.92738
243
244     2 ooc X 20.08506
245             Y 0.00000 -6.20170
246             Z -0.00000  0.00000 -13.88336
247     2 com X 22.15791
248             Y 0.00000 -7.23053
249             Z -0.00000  0.00000 -14.92739
```

---

### B.1.6 Fitting statistics of a fragment approach

---

1

2

3 -----  
3        Restrained ESP Fit 2.4 q4md-forcefieldtools

```
4 -----
5   RESP-A1 - RESP input generated by PyRED version SEP-2015
6 -----
7
8
9   inopt      =    0   ioutopt     =    1
10  nmep       =    2   iqopt       =    2
11  ihfree     =    1   irstrnt    =    1
12  iunits     =    0   qwt        =  0.00100000
13
14 multiple-MEP run of 2 MEP
15
16 Reading input for MEP 1 weight: 1.000
17 PEG nconf=1 norient=1 nmep=1/2
18
19 Total charge (ich): 0
20 Number of centers: 13
21   1   6   0
22   2   1   0
23   3   1   2
24   4   1   2
25   5   8   -1
26   6   6   0
27   7   1   0
28   8   1   7
29   9   6   0
30  10   1   0
31  11   1  10
32  12   8   -1
33  13   1   -1
34
35 Reading input for MEP 2 weight: 1.000
36 PEG nconf=1 norient=2 nmep=2/2
37
38 Total charge (ich): 0
39 Number of centers: 13
40   14   6   0
41   15   1   0
42   16   1   2
43   17   1   2
44   18   8   -1
45   19   6   0
46   20   1   0
47   21   1   7
48   22   6   0
```

```
49      23   1   0
50      24   1   10
51      25   8  -1
52      26   1  -1
53 since IQOPT>1, 26 new q0 values
54 will be read in from file ESP.Q0 (unit 3)
55 -----
56 reading mult_esp constraint info
57 -----
58      1   1   2   1
59      1   2   2   2
60      1   6   2   6
61      1   7   2   7
62      1   9   2   9
63      1  10   2  10
64 -----
65 -----
66      Atom  Ivary
67 -----
68      6   0
69      1   0
70      1   2
71      1   2
72      8  -1
73      6   0
74      1   0
75      1   7
76      6   0
77      1   0
78      1  10
79      8  -1
80      1  -1
81
82      6   1
83      1   2
84      1   2
85      1   2
86      8  -1
87      6   6
88      1   7
89      1   7
90      6   9
91      1  10
92      1  10
93      8  -1
```

```
94      1    -1
95
96  -----
97
98
99  Total number of atoms = 26
100 Weight factor on initial charge restraints= 0.001000
101
102
103 There are 2 charge constraints
104
105 Reading esp"s for MEP 1
106 total number of atoms = 13
107 total number of esp points = 699
108
109 Center   X           Y           Z
110     1   -0.1031513E+01 -0.2458118E+01  0.2078699E-04
111     2   -0.3087856E+01 -0.2257916E+01  0.9448630E-05
112     3   -0.4574554E+00 -0.3535972E+01  0.1687282E+01
113     4   -0.4574346E+00 -0.3536012E+01 -0.1687219E+01
114     5   0.0000000E+00  0.0000000E+00  0.0000000E+00
115     6   0.2671096E+01  0.0000000E+00  0.0000000E+00
116     7   0.3424533E+01 -0.9747906E+00 -0.1680261E+01
117     8   0.3424531E+01 -0.9747830E+00  0.1680274E+01
118     9   0.3546946E+01  0.2737385E+01  0.0000000E+00
119    10   0.2781372E+01  0.3695176E+01  0.1680210E+01
120    11   0.2781370E+01  0.3695170E+01 -0.1680223E+01
121    12   0.6234777E+01  0.2701150E+01 -0.3779452E-05
122    13   0.6819031E+01  0.4435102E+01 -0.3212534E-04
123
124 Reading esp"s for MEP 2
125 total number of atoms = 13
126 total number of esp points = 682
127
128 Center   X           Y           Z
129     1   0.6343621E+01  0.2777408E+01 -0.2078699E-04
130     2   0.6779594E+01  0.4796950E+01 -0.9448630E-05
131     3   0.7195270E+01  0.1902189E+01 -0.1687282E+01
132     4   0.7195302E+01  0.1902159E+01  0.1687219E+01
133     5   0.3688080E+01  0.2544046E+01  0.0000000E+00
134     6   0.2874090E+01  0.0000000E+00  0.0000000E+00
135     7   0.3572911E+01 -0.1014660E+01  0.1680261E+01
136     8   0.3572903E+01 -0.1014656E+01 -0.1680274E+01
137     9   0.0000000E+00  0.0000000E+00  0.0000000E+00
```

```

138      10   -0.6789332E+00  0.1021038E+01 -0.1680210E+01
139      11   -0.6789275E+00  0.1021038E+01  0.1680223E+01
140      12   -0.7845802E+00 -0.2571027E+01  0.3779452E-05
141      13   -0.2614103E+01 -0.2599086E+01  0.3212534E-04
142 Initial ssvpot =  0.200
143
144
145 Number of unique UNfrozen centers= 6
146
147 Non-linear optimization requested.
148 qchnge =  0.4779949113E-01
149 qchnge =  0.2078834013E-02
150 qchnge =  0.3762550571E-03
151 qchnge =  0.8584675882E-04
152 qchnge =  0.2008878879E-04
153 qchnge =  0.4716565444E-05
154 qchnge =  0.1108009325E-05
155 qchnge =  0.2603213499E-06
156
157 Convergence in 7 iterations
158
159      1 PEG nconf=1 norient=1 nmep=1/2
160      2 PEG nconf=1 norient=2 nmep=2/2
161
162          Point Charges Before & After Optimization
163
164      no. At.no.    q(init)      q(opt)      ivary d(rstr)/dq
165      1   6       -0.016076    0.047733     0    0.009025
166      2   1        0.088113    0.041585     0    0.000000
167      3   1        0.040852    0.041585     2    0.000000
168      4   1        0.040851    0.041585     2    0.000000
169      5   8       -0.369797   -0.369797    -1    0.002610
170      6   6        0.163081    0.145121     0    0.005674
171      7   1        0.043831    0.036152     0    0.000000
172      8   1        0.043830    0.036152     7    0.000000
173      9   6        0.141422    0.165939     0    0.005162
174     10   1        0.020794    0.015819     0    0.000000
175     11   1        0.020792    0.015819    10    0.000000
176     12   8       -0.628092   -0.628092    -1    0.001572
177     13   1        0.410400    0.410400    -1    0.000000
178
179     14   6       -0.016076    0.047733     1    0.009025
180     15   1        0.088113    0.041585     2    0.000000
181     16   1        0.040852    0.041585     2    0.000000
182     17   1        0.040851    0.041585     2    0.000000

```

```

183    18   8      -0.369797     -0.369797    -1      0.002610
184    19   6       0.163081      0.145121     6      0.005674
185    20   1       0.043831      0.036152     7      0.000000
186    21   1       0.043830      0.036152     7      0.000000
187    22   6       0.141422      0.165939     9      0.005162
188    23   1       0.020794      0.015819    10      0.000000
189    24   1       0.020792      0.015819    10      0.000000
190    25   8      -0.628092     -0.628092    -1      0.001572
191    26   1       0.410400      0.410400    -1      0.000000
192
193 Sum over the calculated charges: -0.000
194
195 Statistics of the fitting:
196 The initial sum of squares (ssvpot)          0.200
197 The residual sum of squares (chipot)         0.005
198 The std err of estimate (sqrt(chipot/N))    0.00192
199 ESP relative RMS (SQRT(chipot/ssvpot))     0.15951
200 The Pearson correlation coefficient (r2)     0.97480
201
202 Center of Mass (a.u.):
203 #MEP      X          Y          Z
204    1    2.33126    0.61925   0.00000
205    2    2.38786    0.51238  -0.00000
206
207 Dipole moments (Debye) computed:
208 -with respect to the origin of coordinates (ooc)
209 -with respect to the center of mass (com)
210 #MEP      D          Dx         Dy         Dz
211    1 ooc    0.30717   -0.05429   0.30233  -0.00002
212    1 com    0.30717   -0.05429   0.30233  -0.00002
213
214    2 ooc    0.30717   -0.27141   0.14384   0.00002
215    2 com    0.30717   -0.27141   0.14384   0.00002
216
217 Traceless Quadrupole moments (Buckingham) computed:
218 -with respect to the origin of coordinates (ooc)
219 -with respect to the center of mass (com)
220 #MEP      X          Y          Z
221    1 ooc X -11.57828
222           Y  16.44103  18.38709
223           Z -0.00032 -0.00025 -6.80882
224    1 com X -11.11225
225           Y  15.37549  17.85687
226           Z -0.00026 -0.00024 -6.74461
227

```

```
228      2 ooc X 22.64213
229          Y    4.43607 -16.50541
230          Z   -0.00024 -0.00016 -6.13671
231      2 com X 24.09193
232          Y    4.11158 -17.34731
233          Z   -0.00030 -0.00017 -6.74461
234
235 Traceless Quadrupole moments (Buckingham) in principal axes computed:
236 -with respect to the origin of coordinates (ooc)
237 -with respect to the center of mass (com)
238 #MEP       X        Y        Z
239     1 ooc X 25.64825
240         Y   -0.00000 -6.80882
241         Z   -0.00000  0.00000 -18.83943
242     1 com X 24.49594
243         Y   -0.00000 -6.74461
244         Z   -0.00000  0.00000 -17.75133
245
246     2 ooc X 23.13851
247         Y   0.00000 -6.13671
248         Z   -0.00000  0.00000 -17.00180
249     2 com X 24.49594
250         Y   0.00000 -6.74461
251         Z   -0.00000  0.00000 -17.75133
```

---

### B.1.7 Amber Leap script for PEG-M

---

```
1 #
2 # Generated by PyRED version SEP-2015
3 #     http://q4md-forcefieldtools.org
4 #
5 logfile q4md-forcefieldtools.log
6 source /home/sajid/amber14/dat/leap/cmd/oldff/leaprc.ff99SB
7 # Web site: http://ambermd.org/doc6/html/AMBER-sh-5.9.html#sh-5.9.8
8 alias q quit
9 alias e edit
10 alias c charge
11
12 # Web site: http://ambermd.org/doc6/html/AMBER-sh-5.9.html#sh-5.9.73
13 verbosity 2
14
15 # Web site: http://ambermd.org/doc6/html/AMBER-sh-5.9.html#sh-5.9.2
16 addAtomTypes {
17     { "CT" "C" "sp3" }
18     { "H1" "H" "sp3" }
```

```
19          { "HO"  "H"   "sp3"  }
20          { "OH"  "O"   "sp3"  }
21          { "OS"  "O"   "sp3"  }
22      }
23
24 # To force the correspondance between residue names
25 # PDB file versus force field libraries:
26 # Web site: http://ambermd.org/doc6/html/AMBER-sh-5.9.html#sh-5.9.7
27 # addPdbResMap {
28 #           { 0 ALA NALA } { 1 ALA CALA }
29 #           { ADE DADE }
30 #       }
31
32 # Web site: http://ambermd.org/doc6/html/AMBER-sh-5.9.html#sh-5.9.41
33 frcmod1 = loadAmberParams ./frcmod.known
34 frcmod2 = loadAmberParams ./frcmod.correspondence
35 frcmod3 = loadAmberParams ./frcmod.unknown
36
37 # Web site: http://q4md-forcefieldtools.org/Tutorial/leap-mol3.php
38 # Web site: http://q4md-forcefieldtools.org/Tutorial/leap-mol2.php
39 F00 = loadmol3 ../Mol_m1/Mol-ia1_m1-c1.mol2
40 F01 = loadmol3 ../Mol_m1/Mol-ia2_m1-c1.mol2
41 F02 = loadmol3 ../Mol_m1/Mol-ia3_m1-c1.mol2
42 U01 = loadmol3 ../Mol_m1/Mol-sm_m1-c1.mol2
43
44 # To match the residue names found in the PDB file
45 # Web site: http://ambermd.org/doc6/html/AMBER-sh-5.9.html#sh-5.9.19
46 # ZZZ = copy F00
47
48 # If a copy is done, define the molecule and residue
49 # Web site: http://ambermd.org/doc6/html/AMBER-sh-5.9.html#sh-5.9.63
50 # set ZZZ name "ZZZ"
51 # set ZZZ.1 name "ZZZ"
52
53 # Let's load the PDB file
54 # Web site: http://ambermd.org/doc6/html/AMBER-sh-5.9.html#sh-5.9.44
55 # VAR = loadPdb Your-PDB-file.ent
56
57 # Let's save the prmtop and prmcrd file with specific file extensions
58 # (to be automatically recognized by VMD http://www.ks.uiuc.edu/
# Research/vmd/)
59 # Web site: http://ambermd.org/doc6/html/AMBER-sh-5.9.html#sh-5.9.54
60 # saveAmberParm F00 F00.parm7 F00.rst7
61
62 # q
```



## B.2 PEG with a hydroxyethyl terminal, $n = 4$

### B.2.1 FF libraries for ‘A’ fragment

---

```

1  #
2  # Generated by PyRED version SEP-2015
3  #      http://q4md-forcefieldtools.org
4  #
5  @<TRIPOS>MOLECULE
6  F01
7      36    35    1    0    1
8  SMALL
9  USER_CHARGES
10 @<TRIPOS>ATOM
11     1 O      0.580060  0.596993  1.362506 OH  1  F01 -0.5648 0.0000 *****
12     2 H      0.956305 -0.258286  1.077795 HO  1  F01  0.3529 0.0000 *****
13     3 C1    1.618871  1.425664  1.857862 CT  1  F01  0.1140 0.0000 *****
14     4 H11   1.129961  2.292237  2.319691 H1  1  F01  0.0772 0.0000 *****
15     5 H12   2.200939  0.913356  2.638542 H1  1  F01  0.0772 0.0000 *****
16     6 C2    2.575896  1.915881  0.776888 CT  1  F01 -0.3406 0.0000 *****
17     7 H21   2.006000  2.374434 -0.043419 H1  1  F01  0.1496 0.0000 *****
18     8 H22   3.238036  2.685724  1.200975 H1  1  F01  0.1496 0.0000 *****
19     9 O2A   3.391276  0.846837  0.308560 OS  1  F01 -0.2183 0.0000 *****
20    10 C3    3.247128  0.471015 -1.046879 CT  1  F01  0.0320 0.0000 *****
21    11 H31   3.302948  1.346991 -1.714888 H1  1  F01  0.0630 0.0000 *****
22    12 H32   4.106735 -0.173556 -1.257141 H1  1  F01  0.0630 0.0000 *****
23    13 C4    1.966642 -0.299758 -1.357485 CT  1  F01  0.0458 0.0000 *****
24    14 H41   1.082105  0.340491 -1.237110 H1  1  F01  0.0320 0.0000 *****
25    15 H42   2.006303 -0.629912 -2.408737 H1  1  F01  0.0320 0.0000 *****
26    16 O4A   1.901618 -1.419033 -0.482965 OS  1  F01 -0.2479 0.0000 *****
27    17 C5    1.079023 -2.494176 -0.919166 CT  1  F01 -0.0149 0.0000 *****
28    18 H51   1.252522 -2.697451 -1.988036 H1  1  F01  0.0722 0.0000 *****
29    19 H52   1.406404 -3.365310 -0.342685 H1  1  F01  0.0722 0.0000 *****
30    20 C6    -0.407636 -2.277694 -0.681377 CT  1  F01  0.0291 0.0000 *****
31    21 H61   -0.769452 -1.367904 -1.174977 H1  1  F01  0.0565 0.0000 *****
32    22 H62   -0.962978 -3.128999 -1.112583 H1  1  F01  0.0565 0.0000 *****
33    23 O6A   -0.608171 -2.214190  0.722036 OS  1  F01 -0.2638 0.0000 *****
34    24 C7    -1.958554 -2.088639  1.141754 CT  1  F01  0.0414 0.0000 *****
35    25 H71   -2.606855 -2.776515  0.575989 H1  1  F01  0.0526 0.0000 *****
36    26 H72   -1.966234 -2.397915  2.193046 H1  1  F01  0.0526 0.0000 *****
37    27 C8    -2.515411 -0.675057  1.056055 CT  1  F01  0.0388 0.0000 *****
38    28 H81   -3.422234 -0.610989  1.683118 H1  1  F01  0.0579 0.0000 *****
39    29 H82   -1.765362  0.021519  1.450727 H1  1  F01  0.0579 0.0000 *****
40    30 O8A   -2.851627 -0.374587 -0.296695 OS  1  F01 -0.3569 0.0000 *****
41    31 C9    -3.230348  0.977760 -0.511287 CT  1  F01  0.1844 0.0000 *****
42    32 H91   -3.940873  0.987102 -1.345037 H1  1  F01  0.0646 0.0000 *****

```

---

43 33 H92 -3.753836 1.379055 0.373108 H1 1 F01 0.0646 0.0000 \*\*\*\*  
44 34 C1B -2.028597 1.865663 -0.855680 CT 1 F01 -0.7797 0.0000 \*\*\*\*  
45 35 H1 -1.248058 1.771187 -0.088120 H1 1 F01 0.3562 0.0000 \*\*\*\*  
46 36 H1B -1.597999 1.531544 -1.805348 H1 1 F01 0.3562 0.0000 \*\*\*\*  
47 @<TRIPOS>BOND  
48 1 1 2 1  
49 2 1 3 1  
50 3 3 4 1  
51 4 3 5 1  
52 5 3 6 1  
53 6 6 7 1  
54 7 6 8 1  
55 8 6 9 1  
56 9 9 10 1  
57 10 10 11 1  
58 11 10 12 1  
59 12 10 13 1  
60 13 13 14 1  
61 14 13 15 1  
62 15 13 16 1  
63 16 16 17 1  
64 17 17 18 1  
65 18 17 19 1  
66 19 17 20 1  
67 20 20 21 1  
68 21 20 22 1  
69 22 20 23 1  
70 23 23 24 1  
71 24 24 25 1  
72 25 24 26 1  
73 26 24 27 1  
74 27 27 28 1  
75 28 27 29 1  
76 29 27 30 1  
77 30 30 31 1  
78 31 31 32 1  
79 32 31 33 1  
80 33 31 34 1  
81 34 34 35 1  
82 35 34 36 1  
83 @<TRIPOS>SUBSTRUCTURE  
84 1 F01 1 \*\*\*\* 0 \*\*\*\* \*\*\*  
85 @<TRIPOS>HEADTAIL  
86 0 0  
87 C1B 1

---

88 @<TRIPOS>RESIDUECONNECT  
 89 1 0 C1B 0 0 0 0

---

### B.2.2 FF libraries for ‘B’ fragment

---

```

1  #
2  # Generated by PyRED version SEP-2015
3  #      http://q4md-forcefieldtools.org
4  #
5  @<TRIPOS>MOLECULE
6  F02
7      28    27     1     0     1
8  SMALL
9  USER_CHARGES
10 @<TRIPOS>ATOM
11    1 O2A    3.391276  0.846837  0.308560 OS 1   F02 -0.2183 0.0000 ****
12    2 C3     3.247128  0.471015 -1.046879 CT 1   F02  0.0320 0.0000 ****
13    3 H31    3.302948  1.346991 -1.714888 H1 1   F02  0.0630 0.0000 ****
14    4 H32    4.106735 -0.173556 -1.257141 H1 1   F02  0.0630 0.0000 ****
15    5 C4     1.966642 -0.299758 -1.357485 CT 1   F02  0.0458 0.0000 ****
16    6 H41    1.082105  0.340491 -1.237110 H1 1   F02  0.0320 0.0000 ****
17    7 H42    2.006303 -0.629912 -2.408737 H1 1   F02  0.0320 0.0000 ****
18    8 O4A    1.901618 -1.419033 -0.482965 OS 1   F02 -0.2479 0.0000 ****
19    9 C5     1.079023 -2.494176 -0.919166 CT 1   F02 -0.0149 0.0000 ****
20   10 H51    1.252522 -2.697451 -1.988036 H1 1   F02  0.0722 0.0000 ****
21   11 H52    1.406404 -3.365310 -0.342685 H1 1   F02  0.0722 0.0000 ****
22   12 C6    -0.407636 -2.277694 -0.681377 CT 1   F02  0.0291 0.0000 ****
23   13 H61   -0.769452 -1.367904 -1.174977 H1 1   F02  0.0565 0.0000 ****
24   14 H62   -0.962978 -3.128999 -1.112583 H1 1   F02  0.0565 0.0000 ****
25   15 O6A   -0.608171 -2.214190  0.722036 OS 1   F02 -0.2638 0.0000 ****
26   16 C7    -1.958554 -2.088639  1.141754 CT 1   F02  0.0414 0.0000 ****
27   17 H71   -2.606855 -2.776515  0.575989 H1 1   F02  0.0526 0.0000 ****
28   18 H72   -1.966234 -2.397915  2.193046 H1 1   F02  0.0526 0.0000 ****
29   19 C8    -2.515411 -0.675057  1.056055 CT 1   F02  0.0388 0.0000 ****
30   20 H81   -3.422234 -0.610989  1.683118 H1 1   F02  0.0579 0.0000 ****
31   21 H82   -1.765362  0.021519  1.450727 H1 1   F02  0.0579 0.0000 ****
32   22 O8A   -2.851627 -0.374587 -0.296695 OS 1   F02 -0.3569 0.0000 ****
33   23 C9    -3.230348  0.977760 -0.511287 CT 1   F02  0.1844 0.0000 ****
34   24 H91   -3.940873  0.987102 -1.345037 H1 1   F02  0.0646 0.0000 ****
35   25 H92   -3.753836  1.379055  0.373108 H1 1   F02  0.0646 0.0000 ****
36   26 C1B   -2.028597  1.865663 -0.855680 CT 1   F02 -0.7797 0.0000 ****
37   27 H1    -1.248058  1.771187 -0.088120 H1 1   F02  0.3562 0.0000 ****
38   28 H1B   -1.597999  1.531544 -1.805348 H1 1   F02  0.3562 0.0000 ****
39 @<TRIPOS>BOND
40      1      1      2 1

```

```
41      2      2      3 1
42      3      2      4 1
43      4      2      5 1
44      5      5      6 1
45      6      5      7 1
46      7      5      8 1
47      8      8      9 1
48      9      9     10 1
49     10      9     11 1
50     11      9     12 1
51     12     12     13 1
52     13     12     14 1
53     14     12     15 1
54     15     15     16 1
55     16     16     17 1
56     17     16     18 1
57     18     16     19 1
58     19     19     20 1
59     20     19     21 1
60     21     19     22 1
61     22     22     23 1
62     23     23     24 1
63     24     23     25 1
64     25     23     26 1
65     26     26     27 1
66     27     26     28 1
67 @<TRIPOS>SUBSTRUCTURE
68      1 F02           1 ****          0 ****  ****
69 @<TRIPOS>HEADTAIL
70 O2A 1
71 C1B 1
72 @<TRIPOS>RESIDUECONNECT
73 1 O2A C1B 0 0 0 0
```

---

### B.2.3 FF libraries for ‘C’ fragment

---

```
1 #
2 # Generated by PyRED version SEP-2015
3 #     http://q4md-forcefieldtools.org
4 #
5 @<TRIPOS>MOLECULE
6 F00
7      30    29     1     0     1
8 SMALL
9 USER_CHARGES
```

10 @<TRIPOS>ATOM

11    1 O2A    3.391276  0.846837  0.308560 OS 1    F00 -0.2183  0.0000 \*\*\*\*  
 12    2 C3    3.247128  0.471015 -1.046879 CT 1    F00  0.0320  0.0000 \*\*\*\*  
 13    3 H31    3.302948  1.346991 -1.714888 H1 1    F00  0.0630  0.0000 \*\*\*\*  
 14    4 H32    4.106735 -0.173556 -1.257141 H1 1    F00  0.0630  0.0000 \*\*\*\*  
 15    5 C4    1.966642 -0.299758 -1.357485 CT 1    F00  0.0458  0.0000 \*\*\*\*  
 16    6 H41    1.082105  0.340491 -1.237110 H1 1    F00  0.0320  0.0000 \*\*\*\*  
 17    7 H42    2.006303 -0.629912 -2.408737 H1 1    F00  0.0320  0.0000 \*\*\*\*  
 18    8 O4A    1.901618 -1.419033 -0.482965 OS 1    F00 -0.2479  0.0000 \*\*\*\*  
 19    9 C5    1.079023 -2.494176 -0.919166 CT 1    F00 -0.0149  0.0000 \*\*\*\*  
 20    10 H51    1.252522 -2.697451 -1.988036 H1 1    F00  0.0722  0.0000 \*\*\*\*  
 21    11 H52    1.406404 -3.365310 -0.342685 H1 1    F00  0.0722  0.0000 \*\*\*\*  
 22    12 C6    -0.407636 -2.277694 -0.681377 CT 1    F00  0.0291  0.0000 \*\*\*\*  
 23    13 H61    -0.769452 -1.367904 -1.174977 H1 1    F00  0.0565  0.0000 \*\*\*\*  
 24    14 H62    -0.962978 -3.128999 -1.112583 H1 1    F00  0.0565  0.0000 \*\*\*\*  
 25    15 O6A    -0.608171 -2.214190  0.722036 OS 1    F00 -0.2638  0.0000 \*\*\*\*  
 26    16 C7    -1.958554 -2.088639  1.141754 CT 1    F00  0.0414  0.0000 \*\*\*\*  
 27    17 H71    -2.606855 -2.776515  0.575989 H1 1    F00  0.0526  0.0000 \*\*\*\*  
 28    18 H72    -1.966234 -2.397915  2.193046 H1 1    F00  0.0526  0.0000 \*\*\*\*  
 29    19 C8    -2.515411 -0.675057  1.056055 CT 1    F00  0.0388  0.0000 \*\*\*\*  
 30    20 H81    -3.422234 -0.610989  1.683118 H1 1    F00  0.0579  0.0000 \*\*\*\*  
 31    21 H82    -1.765362  0.021519  1.450727 H1 1    F00  0.0579  0.0000 \*\*\*\*  
 32    22 O8A    -2.851627 -0.374587 -0.296695 OS 1    F00 -0.3569  0.0000 \*\*\*\*  
 33    23 C9    -3.230348  0.977760 -0.511287 CT 1    F00  0.1844  0.0000 \*\*\*\*  
 34    24 H91    -3.940873  0.987102 -1.345037 H1 1    F00  0.0646  0.0000 \*\*\*\*  
 35    25 H92    -3.753836  1.379055  0.373108 H1 1    F00  0.0646  0.0000 \*\*\*\*  
 36    26 C1B    -2.028597  1.865663 -0.855680 CT 1    F00 -0.7797  0.0000 \*\*\*\*  
 37    27 H1    -1.248058  1.771187 -0.088120 H1 1    F00  0.3562  0.0000 \*\*\*\*  
 38    28 H1B    -1.597999  1.531544 -1.805348 H1 1    F00  0.3562  0.0000 \*\*\*\*  
 39    29 O1C    -2.423158  3.220232 -1.059148 OH 1    F00 -0.4010  0.0000 \*\*\*\*  
 40    30 H1C    -2.656440  3.589225 -0.193369 HO 1    F00  0.3859  0.0000 \*\*\*\*

41 @<TRIPOS>BOND

42    1    1    2 1  
 43    2    2    3 1  
 44    3    2    4 1  
 45    4    2    5 1  
 46    5    5    6 1  
 47    6    5    7 1  
 48    7    5    8 1  
 49    8    8    9 1  
 50    9    9    10 1  
 51    10  9    11 1  
 52    11  9    12 1  
 53    12 12    13 1  
 54    13 12    14 1

---

```

55      14    12    15 1
56      15    15    16 1
57      16    16    17 1
58      17    16    18 1
59      18    16    19 1
60      19    19    20 1
61      20    19    21 1
62      21    19    22 1
63      22    22    23 1
64      23    23    24 1
65      24    23    25 1
66      25    23    26 1
67      26    26    27 1
68      27    26    28 1
69      28    26    29 1
70      29    29    30 1
71 @<TRIPOS>SUBSTRUCTURE
72      1 F00           1 ****          0 **** *
73 @<TRIPOS>HEADTAIL
74 O2A 1
75 0 0
76 @<TRIPOS>RESIDUECONNECT
77 1 O2A 0 0 0 0 0

```

---

#### B.2.4 FF parameters for PEG-H

1	FRCMOD	<b>file</b>	generated by PyRED version SEP-2015 - q4md-forcefieldtools.org	
2	MASS	mass	pol	Source
3	CT	12.010	0.878	taken <b>from</b> parm10.dat
4	H1	1.008	0.135	taken <b>from</b> parm10.dat
5	HO	1.008	0.135	taken <b>from</b> parm10.dat
6	OH	16.000	0.465	taken <b>from</b> parm10.dat
7	OS	16.000	0.465	taken <b>from</b> parm10.dat
8				
9	BOND	K(kcal.mol <sup>-1</sup> .ang <sup>-2</sup> )	Dist0(ang)	Source
10	CT-CT	310.0	1.526	taken <b>from</b> parm10.dat
11	CT-H1	340.0	1.090	taken <b>from</b> parm10.dat
12	CT-OH	320.0	1.410	taken <b>from</b> parm10.dat
13	CT-OS	320.0	1.410	taken <b>from</b> parm10.dat
14	HO-OH	555.0	0.960	adapted <b>from</b> parm10.dat 553.0
15				
16	ANGLE	K(kcal.mol <sup>-1</sup> .rad <sup>-2</sup> )	Theta0(deg)	Source
17	CT-CT-H1	50.0	109.50	taken <b>from</b> parm10.dat
18	CT-CT-OH	50.0	109.50	taken <b>from</b> parm10.dat

```

19 CT-CT-OS      50.0      109.50      taken from parm10.dat
20 H1-CT-H1      35.0      109.50      taken from parm10.dat
21 H1-CT-OH      50.0      109.50      taken from parm10.dat
22 H1-CT-OS      50.0      109.50      taken from parm10.dat
23 CT-OH-HO      55.0      108.50      taken from parm10.dat
24 CT-OS-CT      60.0      109.50      taken from parm10.dat
25
26 DIHEDRAL      Path V(kcal.mol-1.rad-1) Phase(deg.) Period Source
27 H1-CT-CT-H1  1  1.555555556e-01    0.0      3.  adapted from parm10.
          dat i.e X-CT-CT-X 1.4/9
28 H1-CT-CT-OH  1  0.00000000e+00    0.0      -3.  taken from parm10.
          dat
29 H1-CT-CT-OH  1  2.50000000e-01    0.0      1.  taken from parm10.
          dat
30 H1-CT-CT-OS  1  0.00000000e+00    0.0      -3.  taken from parm10.
          dat
31 H1-CT-CT-OS  1  2.50000000e-01    0.0      1.  taken from parm10.
          dat
32 OH-CT-CT-OS  1  1.44000000e-01    0.0      -3.  taken from parm10.
          dat
33 OH-CT-CT-OS  1  1.17500000e+00    0.0      2.  taken from parm10.
          dat
34 OS-CT-CT-OS  1  1.44000000e-01    0.0      -3.  taken from parm10.
          dat
35 OS-CT-CT-OS  1  1.17500000e+00    0.0      2.  taken from parm10.
          dat
36 CT-CT-OH-HO  1  1.60000000e-01    0.0      -3.  taken from parm10.
          dat
37 CT-CT-OH-HO  1  2.50000000e-01    0.0      1.  taken from parm10.
          dat
38 H1-CT-OH-HO  1  1.66666667e-01    0.0      3.  adapted from parm10.
          dat i.e X-CT-OH-X 0.5/3
39 CT-CT-OS-CT  1  3.83000000e-01    0.0      -3.  taken from parm10.
          dat
40 CT-CT-OS-CT  1  1.00000000e-01   180.0      2.  taken from parm10.
          dat
41 H1-CT-OS-CT  1  3.83333333e-01    0.0      3.  adapted from parm10.
          dat i.e X-CT-OS-X 1.15/3
42
43 IMPROPER      V(kcal.mol-1.rad-1) Phase(deg.) Period Source
44
45 NONBON      R*(ang)  Eps(kcal.mol-1)  Source
46 CT           1.9080  0.10940000  taken from parm10.dat
47 H1           1.3870  0.01570000  taken from parm10.dat
48 HO           0.0000  0.00000000  taken from parm10.dat

```

---

49	OH	1.7210	0.21040000	taken <b>from</b> parm10.dat
50	OS	1.6837	0.17000000	taken <b>from</b> parm10.dat

---

**B.2.5 Fitting statistics of a whole molecule approach**

```

1
2 -----
3   Restrained ESP Fit 2.4 q4md-forcefieldtools
4 -----
5   RESP-A1 - RESP input generated by PyRED version SEP-2015
6 -----
7
8
9   inopt      =      0   ioutopt     =      1
10  nmep       =      2   iqopt       =      2
11  ihfree     =      1   irstrnt    =      1
12  iunits     =      0   qwt        =  0.00100000
13
14 multiple-MEP run of 2 MEP
15
16 Reading input for MEP 1 weight: 1.000
17 PEG nconf=1 norient=1 nmep=1/2
18
19 Total charge (ich): 0
20 Number of centers: 38
21   1   8   -1
22   2   1   -1
23   3   6   0
24   4   1   0
25   5   1   4
26   6   6   0
27   7   1   0
28   8   1   7
29   9   8   -1
30  10   6   0
31  11   1   0
32  12   1   11
33  13   6   0
34  14   1   0
35  15   1   14
36  16   8   -1
37  17   6   0
38  18   1   0
39  19   1   18
40  20   6   17

```

```
41      21    1   18
42      22    1   18
43      23    8   -1
44      24    6   13
45      25    1   14
46      26    1   14
47      27    6   10
48      28    1   11
49      29    1   11
50      30    8   -1
51      31    6    6
52      32    1    7
53      33    1    7
54      34    6    3
55      35    1    4
56      36    1    4
57      37    8   -1
58      38    1   -1
59
60  Reading input for MEP 2 weight: 1.000
61  PEG nconf=1 norient=2 nmep=2/2
62
63  Total charge (ich): 0
64  Number of centers: 38
65      39    8   -1
66      40    1   -1
67      41    6    0
68      42    1    0
69      43    1    4
70      44    6    0
71      45    1    0
72      46    1    7
73      47    8   -1
74      48    6    0
75      49    1    0
76      50    1   11
77      51    6    0
78      52    1    0
79      53    1   14
80      54    8   -1
81      55    6    0
82      56    1    0
83      57    1   18
84      58    6   17
85      59    1   18
```

```
86      60   1   18
87      61   8   -1
88      62   6   13
89      63   1   14
90      64   1   14
91      65   6   10
92      66   1   11
93      67   1   11
94      68   8   -1
95      69   6    6
96      70   1    7
97      71   1    7
98      72   6    3
99      73   1    4
100     74   1    4
101     75   8   -1
102     76   1   -1
103     since IQOPT>1, 76 new q0 values
104     will be read in from file ESP.Q0 (unit 3)
105     -----
106     reading mult_esp constraint info
107     -----
108     1    3    2    3
109     1    4    2    4
110     1    6    2    6
111     1    7    2    7
112     1   10    2   10
113     1   11    2   11
114     1   13    2   13
115     1   14    2   14
116     1   17    2   17
117     1   18    2   18
118
119     -----
120     Atom  Ivary
121     -----
122     8   -1
123     1   -1
124     6    0
125     1    0
126     1    4
127     6    0
128     1    0
129     1    7
130     8   -1
```

131	6	0
132	1	0
133	1	11
134	6	0
135	1	0
136	1	14
137	8	-1
138	6	0
139	1	0
140	1	18
141	6	17
142	1	18
143	1	18
144	8	-1
145	6	13
146	1	14
147	1	14
148	6	10
149	1	11
150	1	11
151	8	-1
152	6	6
153	1	7
154	1	7
155	6	3
156	1	4
157	1	4
158	8	-1
159	1	-1
160		
161	8	-1
162	1	-1
163	6	3
164	1	4
165	1	4
166	6	6
167	1	7
168	1	7
169	8	-1
170	6	10
171	1	11
172	1	11
173	6	13
174	1	14
175	1	14

176        8      -1  
177        6      17  
178        1      18  
179        1      18  
180        6      17  
181        1      18  
182        1      18  
183        8      -1  
184        6      13  
185        1      14  
186        1      14  
187        6      10  
188        1      11  
189        1      11  
190        8      -1  
191        6      6  
192        1      7  
193        1      7  
194        6      3  
195        1      4  
196        1      4  
197        8      -1  
198        1      -1  
199  
200 -----  
201  
202  
203 Total number of atoms = 76  
204 Weight factor on initial charge restraints= 0.001000  
205  
206  
207 There are 2 charge constraints  
208  
209 Reading esp"s for MEP 1  
210 total number of atoms = 38  
211 total number of esp points = 1343  
212  
213 Center    X                  Y                  Z  
214        1    0.6921875E+01    0.0000000E+00    0.0000000E+00  
215        2    0.5544932E+01    0.5228702E+00    -0.1112595E+01  
216        3    0.9202531E+01    0.1152364E+01    -0.8079542E+00  
217        4    0.1072558E+02    0.2609371E+00    0.2795339E+00  
218        5    0.9562533E+01    0.7840227E+00    -0.2822476E+01  
219        6    0.9277383E+01    0.3997800E+01    -0.3611852E+00

```

220    7    0.8802075E+01  0.4410003E+01  0.1618172E+01
221    8    0.1120370E+02  0.4696360E+01  -0.7156279E+00
222    9    0.7613559E+01  0.5264367E+01  -0.2054157E+01
223   10    0.5553478E+01  0.6584469E+01  -0.9804749E+00
224   11    0.6176675E+01  0.7837775E+01  0.5641002E+00
225   12    0.4813837E+01  0.7765209E+01  -0.2509881E+01
226   13    0.3430350E+01  0.4895583E+01  0.0000000E+00
227   14    0.4056095E+01  0.3789568E+01  0.1641580E+01
228   15    0.1852413E+01  0.6112052E+01  0.6096502E+00
229   16    0.2659389E+01  0.3276995E+01  -0.2001433E+01
230   17    0.1857468E+00  0.2249537E+01  -0.1779595E+01
231   18   -0.1154398E+01  0.3722932E+01  -0.1172917E+01
232   19   -0.3315184E+00  0.1637799E+01  -0.3686874E+01
233   20    0.0000000E+00  0.0000000E+00  0.0000000E+00
234   21    0.6444419E+00  0.4778852E+00  0.1910420E+01
235   22   -0.2000224E+01  -0.5758354E+00  0.1444752E+00
236   23    0.1471839E+01  -0.1979148E+01  -0.1052676E+01
237   24    0.1412512E+01  -0.4302171E+01  0.2879905E+00
238   25   -0.5345884E+00  -0.4774070E+01  0.8535023E+00
239   26    0.2058463E+01  -0.5732796E+01  -0.1062803E+01
240   27    0.3134847E+01  -0.4391665E+01  0.2589051E+01
241   28    0.3428997E+01  -0.6385129E+01  0.3132179E+01
242   29    0.4966289E+01  -0.3565571E+01  0.2077797E+01
243   30    0.1982725E+01  -0.3065992E+01  0.4632599E+01
244   31    0.3569103E+01  -0.2776484E+01  0.6779027E+01
245   32    0.2341747E+01  -0.2722364E+01  0.8445220E+01
246   33    0.4830066E+01  -0.4423860E+01  0.6986255E+01
247   34    0.5135153E+01  -0.3415831E+00  0.6656649E+01
248   35    0.6202132E+01  -0.2522557E+00  0.4877610E+01
249   36    0.3857658E+01  0.1285613E+01  0.6697336E+01
250   37    0.6728485E+01  -0.8666283E-01  0.8813608E+01
251   38    0.8069788E+01  -0.1325148E+01  0.8657421E+01
252
253 Reading esp"s for MEP 2
254 total number of atoms = 38
255 total number of esp points = 1345
256
257 Center      X          Y          Z
258   1  0.6013108E+01  0.0000000E+00  0.0000000E+00
259   2  0.4787887E+01  0.8174350E+00  0.1112595E+01
260   3  0.6399177E+01  -0.2525923E+01  0.8079542E+00
261   4  0.8009296E+01  -0.3248305E+01  -0.2795339E+00
262   5  0.6908099E+01  -0.2605140E+01  0.2822476E+01
263   6  0.4126024E+01  -0.4239071E+01  0.3611852E+00
264   7  0.3514439E+01  -0.4091448E+01  -0.1618172E+01

```

```
265      8      0.4675813E+01 -0.6213011E+01  0.7156279E+00
266      9      0.2128742E+01 -0.3619902E+01  0.2054157E+01
267     10     -0.1422113E+00 -0.2709202E+01  0.9804749E+00
268     11     -0.8007317E+00 -0.3944316E+01 -0.5641002E+00
269     12     -0.1532987E+01 -0.2792624E+01  0.2509881E+01
270     13      0.0000000E+00  0.0000000E+00  0.0000000E+00
271     14      0.1263803E+01  0.1327589E+00 -0.1641580E+01
272     15     -0.1906626E+01  0.5783336E+00 -0.6096502E+00
273     16      0.8701149E+00  0.1567516E+01  0.2001433E+01
274     17      0.2702951E+00  0.4178031E+01  0.1779595E+01
275     18     -0.1707430E+01  0.4413583E+01  0.1172917E+01
276     19      0.4679925E+00  0.4954370E+01  0.3686874E+01
277     20      0.1993907E+01  0.5635456E+01  0.0000000E+00
278     21      0.1979032E+01  0.4833297E+01 -0.1910420E+01
279     22      0.1301290E+01  0.7598303E+01 -0.1444752E+00
280     23      0.4459859E+01  0.5586353E+01  0.1052676E+01
281     24      0.6316706E+01  0.6983522E+01 -0.2879905E+00
282     25      0.5570313E+01  0.8842767E+01 -0.8535023E+00
283     26      0.7856523E+01  0.7288316E+01  0.1062803E+01
284     27      0.7389643E+01  0.5633245E+01 -0.2589051E+01
285     28      0.9183424E+01  0.6551274E+01 -0.3132179E+01
286     29      0.7780508E+01  0.3662501E+01 -0.2077797E+01
287     30      0.5641361E+01  0.5801491E+01 -0.4632599E+01
288     31      0.6326793E+01  0.4341836E+01 -0.6779027E+01
289     32      0.5570064E+01  0.5309665E+01 -0.8445220E+01
290     33      0.8400187E+01  0.4271775E+01 -0.6986255E+01
291     34      0.5253744E+01  0.1653004E+01 -0.6656649E+01
292     35      0.5800561E+01  0.7324521E+00 -0.4877610E+01
293     36      0.3187180E+01  0.1748244E+01 -0.6697336E+01
294     37      0.5971372E+01  0.2077697E+00 -0.8813608E+01
295     38      0.7758518E+01 -0.1651261E+00 -0.8657421E+01
296 Initial ssvpot =  0.442
297
298
299 Number of unique UNfrozen centers= 10
300
301 Non-linear optimization requested.
302 qchnge =  0.2269581368E-01
303 qchnge =  0.1442777487E-02
304 qchnge =  0.1163225555E-03
305 qchnge =  0.9182987111E-05
306 qchnge =  0.7248932661E-06
307
308 Convergence in 4 iterations
309
```

310       1   PEG nconf=1 norient=1 nmep=1/2  
 311       2   PEG nconf=1 norient=2 nmep=2/2  
 312  
 313                  Point Charges Before & After Optimization  
 314  
 315       no.   At.no.   q(init)    q(opt)    ivary   d(rstr)/dq  
 316       1    8      -0.585064   -0.585064   -1      0.001685  
 317       2    1      0.386220    0.386220    -1      0.000000  
 318       3    6      0.128266    0.133478    0      0.005996  
 319       4    1      0.040840    0.050068    0      0.000000  
 320       5    1      0.041780    0.050068    4      0.000000  
 321       6    6      0.066218    0.050631    0      0.008921  
 322       7    1      0.048474    0.041777    0      0.000000  
 323       8    1      0.037129    0.041777    7      0.000000  
 324       9    8      -0.328538   -0.328538   -1      0.002912  
 325      10   6      -0.014615   0.019526    0      0.009815  
 326      11   1      0.064010    0.064468    0      0.000000  
 327      12   1      0.092006    0.064468   11     0.000000  
 328      13   6      0.071593    0.044207    0      0.009146  
 329      14   1      0.006493    0.053366    0      0.000000  
 330      15   1      0.055238    0.053366   14     0.000000  
 331      16   8      -0.284445   -0.284445   -1     0.003317  
 332      17   6      -0.000117   0.008855    0      0.009961  
 333      18   1      0.061387    0.067887    0      0.000000  
 334      19   1      0.084520    0.067887   18     0.000000  
 335      20   6      0.018437    0.008855   17     0.009961  
 336      21   1      0.051198    0.067887   18     0.000000  
 337      22   1      0.063521    0.067887   18     0.000000  
 338      23   8      -0.284445   -0.284445   -1     0.003317  
 339      24   6      -0.010209   0.044207   13     0.009146  
 340      25   1      0.073391    0.053366   14     0.000000  
 341      26   1      0.080815    0.053366   14     0.000000  
 342      27   6      0.006552    0.019526   10     0.009815  
 343      28   1      0.052308    0.064468   11     0.000000  
 344      29   1      0.096069    0.064468   11     0.000000  
 345      30   8      -0.328538   -0.328538   -1     0.002912  
 346      31   6      0.041494    0.050631    6     0.008921  
 347      32   1      0.079468    0.041777    7     0.000000  
 348      33   1      0.037826    0.041777    7     0.000000  
 349      34   6      -0.024871   0.133478    3     0.005996  
 350      35   1      0.137508    0.050068    4     0.000000  
 351      36   1      0.136925    0.050068    4     0.000000  
 352      37   8      -0.585064   -0.585064   -1     0.001685  
 353      38   1      0.386220    0.386220   -1     0.000000

354

355	39	8	-0.585064	-0.585064	-1	0.001685
356	40	1	0.386220	0.386220	-1	0.000000
357	41	6	0.128266	0.133478	3	0.005996
358	42	1	0.040840	0.050068	4	0.000000
359	43	1	0.041780	0.050068	4	0.000000
360	44	6	0.066218	0.050631	6	0.008921
361	45	1	0.048474	0.041777	7	0.000000
362	46	1	0.037129	0.041777	7	0.000000
363	47	8	-0.328538	-0.328538	-1	0.002912
364	48	6	-0.014615	0.019526	10	0.009815
365	49	1	0.064010	0.064468	11	0.000000
366	50	1	0.092006	0.064468	11	0.000000
367	51	6	0.071593	0.044207	13	0.009146
368	52	1	0.006493	0.053366	14	0.000000
369	53	1	0.055238	0.053366	14	0.000000
370	54	8	-0.284445	-0.284445	-1	0.003317
371	55	6	-0.000117	0.008855	17	0.009961
372	56	1	0.061387	0.067887	18	0.000000
373	57	1	0.084520	0.067887	18	0.000000
374	58	6	0.018437	0.008855	17	0.009961
375	59	1	0.051198	0.067887	18	0.000000
376	60	1	0.063521	0.067887	18	0.000000
377	61	8	-0.284445	-0.284445	-1	0.003317
378	62	6	-0.010209	0.044207	13	0.009146
379	63	1	0.073391	0.053366	14	0.000000
380	64	1	0.080815	0.053366	14	0.000000
381	65	6	0.006552	0.019526	10	0.009815
382	66	1	0.052308	0.064468	11	0.000000
383	67	1	0.096069	0.064468	11	0.000000
384	68	8	-0.328538	-0.328538	-1	0.002912
385	69	6	0.041494	0.050631	6	0.008921
386	70	1	0.079468	0.041777	7	0.000000
387	71	1	0.037826	0.041777	7	0.000000
388	72	6	-0.024871	0.133478	3	0.005996
389	73	1	0.137508	0.050068	4	0.000000
390	74	1	0.136925	0.050068	4	0.000000
391	75	8	-0.585064	-0.585064	-1	0.001685
392	76	1	0.386220	0.386220	-1	0.000000
393						
394			Sum over the calculated charges:	-0.000		
395						
396			Statistics of the fitting:			
397			The initial <b>sum</b> of squares (ssvpot)		0.442	
398			The residual <b>sum</b> of squares (chipot)		0.015	
399			The std err of estimate (sqrt(chipot/N))		0.00235	

```

400    ESP relative RMS (SQRT(chipot/ssvpot))          0.18355
401    The Pearson correlation coefficient (r2)        0.96784
402
403 Center of Mass (a.u.):
404 #MEP      X          Y          Z
405   1   4.30234   0.64250   1.33386
406   2   3.96897   1.75963  -1.33386
407
408 Dipole moments (Debye) computed:
409 -with respect to the origin of coordinates (ooc)
410 -with respect to the center of mass (com)
411 #MEP      D          Dx         Dy         Dz
412   1 ooc   0.70969   0.00509  -0.54961   0.44896
413   1 com   0.70969   0.00509  -0.54961   0.44896
414
415   2 ooc   0.70969   0.45043   0.31498  -0.44896
416   2 com   0.70969   0.45043   0.31498  -0.44896
417
418 Traceless Quadrupole moments (Buckingham) computed:
419 -with respect to the origin of coordinates (ooc)
420 -with respect to the center of mass (com)
421 #MEP      X          Y          Z
422   1 ooc X  24.68769
423           Y -20.11401  19.26294
424           Z  16.51201 -4.80233 -43.95063
425   1 com X  24.90135
426           Y -16.36531  20.66739
427           Z  13.43482 -4.09644 -45.56874
428
429   2 ooc X  40.13168
430           Y -4.08858   6.64815
431           Z -14.91870  6.63824 -46.77983
432   2 com X  37.56796
433           Y -7.33151   8.00079
434           Z -11.13609  8.55938 -45.56875
435
436 Traceless Quadrupole moments (Buckingham) in principal axes computed:
437 -with respect to the origin of coordinates (ooc)
438 -with respect to the center of mass (com)
439 #MEP      X          Y          Z
440   1 ooc X  45.04103
441           Y -0.00000   2.67595
442           Z  0.00000   0.00000 -47.71698
443   1 com X  41.19415
444           Y  0.00000   6.86644

```

---

```

445      Z    0.00000  0.00000 -48.06059
446
447      2 ooc X  43.34445
448          Y   -0.00000  6.53850
449          Z   -0.00000  0.00000 -49.88296
450      2 com X  41.19417
451          Y   -0.00000  6.86643
452          Z    0.00000  0.00000 -48.06060

```

---

### B.2.6 Fitting statistics of a fragment approach

---

```

1
2 -----
3     Restrained ESP Fit 2.4 q4md-forcefieldtools
4 -----
5     RESP-A1 - RESP input generated by PyRED version SEP-2015
6 -----
7
8
9     inopt      =      0     ioutopt      =      1
10    nmep       =      2     iqopt       =      2
11    ihfree     =      1     irstrnt     =      1
12    iunits     =      0     qwt        =  0.00100000
13
14    multiple-MEP run of 2 MEP
15
16    Reading input for MEP 1 weight: 1.000
17    PEG nconf=1 norient=1 nmep=1/2
18
19    Total charge (ich): 0
20    Number of centers: 38
21    1    8    -1
22    2    1    -1
23    3    6    0
24    4    1    0
25    5    1    4
26    6    6    0
27    7    1    0
28    8    1    7
29    9    8    -1
30   10    6    0
31   11    1    0
32   12    1   11
33   13    6    0
34   14    1    0

```

```
35      15   1   14
36      16   8  -1
37      17   6   0
38      18   1   0
39      19   1  18
40      20   6   0
41      21   1   0
42      22   1  21
43      23   8  -1
44      24   6   0
45      25   1   0
46      26   1  25
47      27   6   0
48      28   1   0
49      29   1  28
50      30   8  -1
51      31   6   0
52      32   1   0
53      33   1  32
54      34   6   0
55      35   1   0
56      36   1  35
57      37   8  -1
58      38   1  -1
59
60  Reading input for MEP 2 weight: 1.000
61  PEG nconf=1 norient=2 nmep=2/2
62
63  Total charge (ich): 0
64  Number of centers: 38
65      39   8  -1
66      40   1  -1
67      41   6   0
68      42   1   0
69      43   1   4
70      44   6   0
71      45   1   0
72      46   1   7
73      47   8  -1
74      48   6   0
75      49   1   0
76      50   1  11
77      51   6   0
78      52   1   0
79      53   1  14
```

```
80      54     8    -1
81      55     6     0
82      56     1     0
83      57     1    18
84      58     6     0
85      59     1     0
86      60     1    21
87      61     8    -1
88      62     6     0
89      63     1     0
90      64     1    25
91      65     6     0
92      66     1     0
93      67     1    28
94      68     8    -1
95      69     6     0
96      70     1     0
97      71     1    32
98      72     6     0
99      73     1     0
100     74     1    35
101     75     8    -1
102     76     1    -1
103     1     1     1     2     1     3     1     4     1     5     1     6     1     7     1     8
104 since IQOPT>1, 76 new q0 values
105 will be read in from file ESP.Q0 (unit 3)
106 -----
107 reading mult_esp constraint info
108 -----
109     1     3     2     3
110     1     4     2     4
111     1     6     2     6
112     1     7     2     7
113     1    10     2    10
114     1    11     2    11
115     1    13     2   13
116     1    14     2   14
117     1    17     2   17
118     1    18     2   18
119     1    20     2   20
120     1    21     2   21
121     1    24     2   24
122     1    25     2   25
123     1    27     2   27
124     1    28     2   28
```

125        1    31        2    31  
126        1    32        2    32  
127        1    34        2    34  
128        1    35        2    35  
129  
130 -----  
131        Atom    Ivary  
132 -----  
133        8    -1  
134        1    -1  
135        6    0  
136        1    0  
137        1    4  
138        6    0  
139        1    0  
140        1    7  
141        8    -1  
142        6    0  
143        1    0  
144        1    11  
145        6    0  
146        1    0  
147        1    14  
148        8    -1  
149        6    0  
150        1    0  
151        1    18  
152        6    0  
153        1    0  
154        1    21  
155        8    -1  
156        6    0  
157        1    0  
158        1    25  
159        6    0  
160        1    0  
161        1    28  
162        8    -1  
163        6    0  
164        1    0  
165        1    32  
166        6    0  
167        1    0  
168        1    35  
169        8    -1

170      1      -1  
171  
172      8      -1  
173      1      -1  
174      6      3  
175      1      4  
176      1      4  
177      6      6  
178      1      7  
179      1      7  
180      8      -1  
181      6      10  
182      1      11  
183      1      11  
184      6      13  
185      1      14  
186      1      14  
187      8      -1  
188      6      17  
189      1      18  
190      1      18  
191      6      20  
192      1      21  
193      1      21  
194      8      -1  
195      6      24  
196      1      25  
197      1      25  
198      6      27  
199      1      28  
200      1      28  
201      8      -1  
202      6      31  
203      1      32  
204      1      32  
205      6      34  
206      1      35  
207      1      35  
208      8      -1  
209      1      -1  
210  
211

---

212  
213

214 Total number of atoms = 76  
215 Weight factor on initial charge restraints= 0.001000  
216  
217  
218 There are 3 charge constraints  
219  
220 Reading esp"s for MEP 1  
221 total number of atoms = 38  
222 total number of esp points = 1343  
223  
224 Center X Y Z  
225 1 0.6921875E+01 0.0000000E+00 0.0000000E+00  
226 2 0.5544932E+01 0.5228702E+00 -0.1112595E+01  
227 3 0.9202531E+01 0.1152364E+01 -0.8079542E+00  
228 4 0.1072558E+02 0.2609371E+00 0.2795339E+00  
229 5 0.9562533E+01 0.7840227E+00 -0.2822476E+01  
230 6 0.9277383E+01 0.3997800E+01 -0.3611852E+00  
231 7 0.8802075E+01 0.4410003E+01 0.1618172E+01  
232 8 0.1120370E+02 0.4696360E+01 -0.7156279E+00  
233 9 0.7613559E+01 0.5264367E+01 -0.2054157E+01  
234 10 0.5553478E+01 0.6584469E+01 -0.9804749E+00  
235 11 0.6176675E+01 0.7837775E+01 0.5641002E+00  
236 12 0.4813837E+01 0.7765209E+01 -0.2509881E+01  
237 13 0.3430350E+01 0.4895583E+01 0.0000000E+00  
238 14 0.4056095E+01 0.3789568E+01 0.1641580E+01  
239 15 0.1852413E+01 0.6112052E+01 0.6096502E+00  
240 16 0.2659389E+01 0.3276995E+01 -0.2001433E+01  
241 17 0.1857468E+00 0.2249537E+01 -0.1779595E+01  
242 18 -0.1154398E+01 0.3722932E+01 -0.1172917E+01  
243 19 -0.3315184E+00 0.1637799E+01 -0.3686874E+01  
244 20 0.0000000E+00 0.0000000E+00 0.0000000E+00  
245 21 0.6444419E+00 0.4778852E+00 0.1910420E+01  
246 22 -0.2000224E+01 -0.5758354E+00 0.1444752E+00  
247 23 0.1471839E+01 -0.1979148E+01 -0.1052676E+01  
248 24 0.1412512E+01 -0.4302171E+01 0.2879905E+00  
249 25 -0.5345884E+00 -0.4774070E+01 0.8535023E+00  
250 26 0.2058463E+01 -0.5732796E+01 -0.1062803E+01  
251 27 0.3134847E+01 -0.4391665E+01 0.2589051E+01  
252 28 0.3428997E+01 -0.6385129E+01 0.3132179E+01  
253 29 0.4966289E+01 -0.3565571E+01 0.2077797E+01  
254 30 0.1982725E+01 -0.3065992E+01 0.4632599E+01  
255 31 0.3569103E+01 -0.2776484E+01 0.6779027E+01  
256 32 0.2341747E+01 -0.2722364E+01 0.8445220E+01  
257 33 0.4830066E+01 -0.4423860E+01 0.6986255E+01  
258 34 0.5135153E+01 -0.3415831E+00 0.6656649E+01

```

259      35      0.6202132E+01 -0.2522557E+00  0.4877610E+01
260      36      0.3857658E+01  0.1285613E+01  0.6697336E+01
261      37      0.6728485E+01 -0.8666283E-01  0.8813608E+01
262      38      0.8069788E+01 -0.1325148E+01  0.8657421E+01
263
264  Reading esp"s for MEP 2
265  total number of atoms    =     38
266  total number of esp points = 1345
267
268  Center   X           Y           Z
269      1  0.6013108E+01  0.0000000E+00  0.0000000E+00
270      2  0.4787887E+01  0.8174350E+00  0.1112595E+01
271      3  0.6399177E+01 -0.2525923E+01  0.8079542E+00
272      4  0.8009296E+01 -0.3248305E+01 -0.2795339E+00
273      5  0.6908099E+01 -0.2605140E+01  0.2822476E+01
274      6  0.4126024E+01 -0.4239071E+01  0.3611852E+00
275      7  0.3514439E+01 -0.4091448E+01 -0.1618172E+01
276      8  0.4675813E+01 -0.6213011E+01  0.7156279E+00
277      9  0.2128742E+01 -0.3619902E+01  0.2054157E+01
278     10  -0.1422113E+00 -0.2709202E+01  0.9804749E+00
279     11  -0.8007317E+00 -0.3944316E+01 -0.5641002E+00
280     12  -0.1532987E+01 -0.2792624E+01  0.2509881E+01
281     13  0.0000000E+00  0.0000000E+00  0.0000000E+00
282     14  0.1263803E+01  0.1327589E+00 -0.1641580E+01
283     15  -0.1906626E+01  0.5783336E+00 -0.6096502E+00
284     16  0.8701149E+00  0.1567516E+01  0.2001433E+01
285     17  0.2702951E+00  0.4178031E+01  0.1779595E+01
286     18  -0.1707430E+01  0.4413583E+01  0.1172917E+01
287     19  0.4679925E+00  0.4954370E+01  0.3686874E+01
288     20  0.1993907E+01  0.5635456E+01  0.0000000E+00
289     21  0.1979032E+01  0.4833297E+01 -0.1910420E+01
290     22  0.1301290E+01  0.7598303E+01 -0.1444752E+00
291     23  0.4459859E+01  0.5586353E+01  0.1052676E+01
292     24  0.6316706E+01  0.6983522E+01 -0.2879905E+00
293     25  0.5570313E+01  0.8842767E+01 -0.8535023E+00
294     26  0.7856523E+01  0.7288316E+01  0.1062803E+01
295     27  0.7389643E+01  0.5633245E+01 -0.2589051E+01
296     28  0.9183424E+01  0.6551274E+01 -0.3132179E+01
297     29  0.7780508E+01  0.3662501E+01 -0.2077797E+01
298     30  0.5641361E+01  0.5801491E+01 -0.4632599E+01
299     31  0.6326793E+01  0.4341836E+01 -0.6779027E+01
300     32  0.5570064E+01  0.5309665E+01 -0.8445220E+01
301     33  0.8400187E+01  0.4271775E+01 -0.6986255E+01
302     34  0.5253744E+01  0.1653004E+01 -0.6656649E+01
303     35  0.5800561E+01  0.7324521E+00 -0.4877610E+01

```

```

304      36      0.3187180E+01  0.1748244E+01 -0.6697336E+01
305      37      0.5971372E+01  0.2077697E+00 -0.8813608E+01
306      38      0.7758518E+01  -0.1651261E+00 -0.8657421E+01
307 Initial ssvpot =  0.442
308
309
310 Number of unique UNfrozen centers= 20
311
312 Non-linear optimization requested.
313 qchnge =  0.2880537305E-01
314 qchnge =  0.3190330277E-02
315 qchnge =  0.4803236227E-03
316 qchnge =  0.1021344006E-03
317 qchnge =  0.2487031914E-04
318 qchnge =  0.6197372417E-05
319 qchnge =  0.1554030010E-05
320 qchnge =  0.3908564064E-06
321
322 Convergence in 7 iterations
323
324     1 PEG nconf=1 norient=1 nmep=1/2
325     2 PEG nconf=1 norient=2 nmep=2/2
326
327             Point Charges Before & After Optimization
328
329   no. At.no.   q(init)      q(opt)      ivary d(rstr)/dq
330     1   8      -0.564852    -0.564852    -1    0.001743
331     2   1       0.352783     0.352783    -1    0.000000
332     3   6       0.022844     0.113979     0    0.006595
333     4   1       0.086312     0.077240     0    0.000000
334     5   1       0.099246     0.077240     4    0.000000
335     6   6      -0.243550    -0.340580     0    0.002817
336     7   1       0.131838     0.149645     0    0.000000
337     8   1       0.130481     0.149645     7    0.000000
338     9   8      -0.218335    -0.218335    -1    0.004164
339    10   6      -0.033266     0.032006     0    0.009524
340    11   1       0.078702     0.062998     0    0.000000
341    12   1       0.072214     0.062998    11    0.000000
342    13   6       0.089346     0.045808     0    0.009092
343    14   1      -0.008153     0.031955     0    0.000000
344    15   1       0.047697     0.031955    14    0.000000
345    16   8      -0.247887    -0.247887    -1    0.003741
346    17   6      -0.019384    -0.014929     0    0.009890
347    18   1       0.063706     0.072222     0    0.000000
348    19   1       0.079755     0.072222    18    0.000000

```

**B.2. PEG WITH A HYDROXYETHYL TERMINAL,  $N = 4$**

---

349	20	6	0.015047	0.029087	0	0.009602
350	21	1	0.069205	0.056516	0	0.000000
351	22	1	0.060809	0.056516	21	0.000000
352	23	8	-0.263812	-0.263812	-1	0.003544
353	24	6	-0.005199	0.041382	0	0.009240
354	25	1	0.070001	0.052584	0	0.000000
355	26	1	0.072293	0.052584	25	0.000000
356	27	6	0.028340	0.038840	0	0.009322
357	28	1	0.038177	0.057911	0	0.000000
358	29	1	0.110734	0.057911	28	0.000000
359	30	8	-0.356868	-0.356868	-1	0.002698
360	31	6	0.172652	0.184369	0	0.004767
361	32	1	0.104571	0.064579	0	0.000000
362	33	1	0.044342	0.064579	32	0.000000
363	34	6	-0.833795	-0.779558	0	0.001272
364	35	1	0.423482	0.356185	0	0.000000
365	36	1	0.345625	0.356185	35	0.000000
366	37	8	-0.400959	-0.400959	-1	0.002420
367	38	1	0.385859	0.385859	-1	0.000000
368						
369	39	8	-0.564852	-0.564852	-1	0.001743
370	40	1	0.352783	0.352783	-1	0.000000
371	41	6	0.022844	0.113979	3	0.006595
372	42	1	0.086312	0.077240	4	0.000000
373	43	1	0.099246	0.077240	4	0.000000
374	44	6	-0.243550	-0.340580	6	0.002817
375	45	1	0.131838	0.149645	7	0.000000
376	46	1	0.130481	0.149645	7	0.000000
377	47	8	-0.218335	-0.218335	-1	0.004164
378	48	6	-0.033266	0.032006	10	0.009524
379	49	1	0.078702	0.062998	11	0.000000
380	50	1	0.072214	0.062998	11	0.000000
381	51	6	0.089346	0.045808	13	0.009092
382	52	1	-0.008153	0.031955	14	0.000000
383	53	1	0.047697	0.031955	14	0.000000
384	54	8	-0.247887	-0.247887	-1	0.003741
385	55	6	-0.019384	-0.014929	17	0.009890
386	56	1	0.063706	0.072222	18	0.000000
387	57	1	0.079755	0.072222	18	0.000000
388	58	6	0.015047	0.029087	20	0.009602
389	59	1	0.069205	0.056516	21	0.000000
390	60	1	0.060809	0.056516	21	0.000000
391	61	8	-0.263812	-0.263812	-1	0.003544
392	62	6	-0.005199	0.041382	24	0.009240
393	63	1	0.070001	0.052584	25	0.000000

```

394    64   1      0.072293      0.052584      25      0.000000
395    65   6      0.028340      0.038840      27      0.009322
396    66   1      0.038177      0.057911      28      0.000000
397    67   1      0.110734      0.057911      28      0.000000
398    68   8     -0.356868     -0.356868     -1      0.002698
399    69   6      0.172652      0.184369      31      0.004767
400    70   1      0.104571      0.064579      32      0.000000
401    71   1      0.044342      0.064579      32      0.000000
402    72   6     -0.833795     -0.779558      34      0.001272
403    73   1      0.423482      0.356185      35      0.000000
404    74   1      0.345625      0.356185      35      0.000000
405    75   8     -0.400959     -0.400959     -1      0.002420
406    76   1      0.385859      0.385859     -1      0.000000
407
408 Sum over the calculated charges: -0.000
409
410 Statistics of the fitting:
411 The initial sum of squares (ssvpot)          0.442
412 The residual sum of squares (chipot)          0.022
413 The std err of estimate (sqrt(chipot/N))    0.00286
414 ESP relative RMS (SQRT(chipot/ssvpot))     0.22304
415 The Pearson correlation coefficient (r2)    0.95146
416
417 Center of Mass (a.u.):
418 #MEP      X        Y        Z
419    1    4.30234   0.64250   1.33386
420    2    3.96897   1.75963  -1.33386
421
422 Dipole moments (Debye) computed:
423 -with respect to the origin of coordinates (ooc)
424 -with respect to the center of mass (com)
425 #MEP      D        Dx       Dy       Dz
426    1 ooc   0.74627  -0.07127 -0.40243  0.62442
427    1 com   0.74627  -0.07127 -0.40243  0.62442
428
429    2 ooc   0.74627   0.28626   0.29169  -0.62442
430    2 com   0.74627   0.28626   0.29169  -0.62442
431
432 Traceless Quadrupole moments (Buckingham) computed:
433 -with respect to the origin of coordinates (ooc)
434 -with respect to the center of mass (com)
435 #MEP      X        Y        Z
436    1 ooc X  19.33560
437          Y  -18.04555 19.08803
438          Z  18.87377 -4.20002 -38.42363

```

---

```

439      1 com X 20.59248
440          Y -15.22423 20.19230
441          Z 14.75985 -3.98477 -40.78478
442
443      2 ooc X 35.70157
444          Y -2.50992 5.06592
445          Z -16.35508 7.34103 -40.76749
446      2 com X 34.72140
447          Y -5.14749 6.06340
448          Z -11.81455 9.70300 -40.78480
449
450 Traceless Quadrupole moments (Buckingham) in principal axes computed:
451 -with respect to the origin of coordinates (ooc)
452 -with respect to the center of mass (com)
453 #MEP           X           Y           Z
454     1 ooc X 40.75329
455         Y 0.00000 3.31249
456         Z 0.00000 0.00000 -44.06578
457     1 com X 37.91756
458         Y 0.00000 6.23629
459         Z 0.00000 0.00000 -44.15385
460
461     2 ooc X 39.50876
462         Y -0.00000 5.50625
463         Z -0.00000 0.00000 -45.01501
464     2 com X 37.91756
465         Y -0.00000 6.23631
466         Z 0.00000 0.00000 -44.15387

```

---

### B.2.7 Amber Leap script for PEG-H

```

1 #
2 # Generated by PyRED version SEP-2015
3 #     http://q4md-forcefieldtools.org
4 #
5 logfile q4md-forcefieldtools.log
6 source /home/sajid/amber14/dat/leap/cmd/oldff/leaprc.ff99SB
7 # Web site: http://ambermd.org/doc6/html/AMBER-sh-5.9.html#sh-5.9.8
8 alias q quit
9 alias e edit
10 alias c charge
11
12 # Web site: http://ambermd.org/doc6/html/AMBER-sh-5.9.html#sh-5.9.73
13 verbosity 2
14

```

```
15 # Web site: http://ambermd.org/doc6/html/AMBER-sh-5.9.html#sh-5.9.2
16 addAtomTypes {
17     { "CT" "C" "sp3" }
18     { "H1" "H" "sp3" }
19     { "HO" "H" "sp3" }
20     { "OH" "O" "sp3" }
21     { "OS" "O" "sp3" }
22 }
23
24 # To force the correspondance between residue names
25 # PDB file versus force field libraries:
26 # Web site: http://ambermd.org/doc6/html/AMBER-sh-5.9.html#sh-5.9.7
27 # addPdbResMap {
28 #     { 0 ALA NALA } { 1 ALA CALA }
29 #     { ADE DADE }
30 #
31
32 # Web site: http://ambermd.org/doc6/html/AMBER-sh-5.9.html#sh-5.9.41
33 frcmod1 = loadAmberParams ./frcmod.known
34 frcmod2 = loadAmberParams ./frcmod.correspondence
35 frcmod3 = loadAmberParams ./frcmod.unknown
36
37 # Web site: http://q4md-forcefieldtools.org/Tutorial/leap-mol3.php
38 # Web site: http://q4md-forcefieldtools.org/Tutorial/leap-mol2.php
39 F00 = loadmol3 ../Mol_m1/Mol-ia1_m1-c1.mol2
40 F01 = loadmol3 ../Mol_m1/Mol-ia2_m1-c1.mol2
41 F02 = loadmol3 ../Mol_m1/Mol-ia3_m1-c1.mol2
42 U01 = loadmol3 ../Mol_m1/Mol-sm_m1-c1.mol2
43
44 # To match the residue names found in the PDB file
45 # Web site: http://ambermd.org/doc6/html/AMBER-sh-5.9.html#sh-5.9.19
46 # ZZZ = copy F00
47
48 # If a copy is done, define the molecule and residue
49 # Web site: http://ambermd.org/doc6/html/AMBER-sh-5.9.html#sh-5.9.63
50 # set ZZZ name "ZZZ"
51 # set ZZZ.1 name "ZZZ"
52
53 # Let's load the PDB file
54 # Web site: http://ambermd.org/doc6/html/AMBER-sh-5.9.html#sh-5.9.44
55 # VAR = loadPdb Your-PDB-file.ent
56
57 # Let's save the prmtop and prmcrd file with specific file extensions
58 # (to be automatically recognized by VMD http://www.ks.uiuc.edu/
      Research/vmd/)
```

```
59 # Web site: http://ambermd.org/doc6/html/AMBER-sh-5.9.html#sh-5.9.54
60 # saveAmberParm F00 F00.parm7 F00.rst7
61
62 # q
63 PG8000 = sequence { F01 F02 F02
64   F02 F02
65   F02 F02
66   F02 F02
67   F02 F02
68 quit
```

---

## B.3 PEG with hydroxyethyl terminal, multiple conformations

### B.3.1 FF libraries for ‘A’ fragment

---

```
1 #
2 # Generated by PyRED version SEP-2015
3 #     http://q4md-forcefieldtools.org
4 #
5 @<TRIPOS>MOLECULE
6 FOO_0
7      9     8     1     0     1
8 SMALL
9 USER_CHARGES
10 @<TRIPOS>ATOM
11    1 O4    0.056039  0.238964 -0.333802 OS 1   FOO -0.1415 0.0000 ****
12    2 C5    0.862554 -0.062393  0.805661 CT 1   FOO  0.0406 0.0000 ****
13    3 H51   0.362064 -0.773093  1.480781 H1 1   FOO  0.0865 0.0000 ****
14    4 H52   1.072422  0.861795  1.364741 H1 1   FOO  0.0865 0.0000 ****
15    5 C6    2.158639 -0.650715  0.268457 CT 1   FOO -0.4961 0.0000 ****
16    6 H61   1.937152 -1.598539 -0.249741 H1 1   FOO  0.2120 0.0000 ****
17    7 H62   2.836539 -0.872771  1.099235 H1 1   FOO  0.2120 0.0000 ****
18    8 O7    2.825121  0.263058 -0.582429 OH 1   FOO -0.3765 0.0000 ****
19    9 H71   2.154049  0.558849 -1.220066 HO 1   FOO  0.3575 0.0000 ****
20 @<TRIPOS>BOND
21      1     1     2 1
22      2     2     3 1
23      3     2     4 1
24      4     2     5 1
25      5     5     6 1
26      6     5     7 1
27      7     5     8 1
28      8     8     9 1
29 @<TRIPOS>SUBSTRUCTURE
30      1 FOO           1 ****          0 **** ****
31 @<TRIPOS>HEADTAIL
32 O4 1
33 O 0
34 @<TRIPOS>RESIDUECONNECT
35 1 O4 0 0 0 0 0
```

---

### B.3.2 FF libraries for ‘B’ fragment

---

```
1 #
2 # Generated by PyRED version SEP-2015
3 #     http://q4md-forcefieldtools.org
4 #
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
5 @<TRIPOS>MOLECULE
6 FOO_1
7      9     8     1     0     1
8 SMALL
9 USER_CHARGES
10 @<TRIPOS>ATOM
11    1 O4    0.056042  0.239002 -0.333815 OS 1   FOO -0.1415 0.0000 ****
12    2 C5    0.862539 -0.062346  0.805655 CT 1   FOO  0.0406 0.0000 ****
13    3 H51   0.362030 -0.773014  1.480797 H1 1   FOO  0.0865 0.0000 ****
14    4 H52   1.072434  0.861848  1.364716 H1 1   FOO  0.0865 0.0000 ****
15    5 C6    2.158613 -0.650718  0.268480 CT 1   FOO -0.4961 0.0000 ****
16    6 H61   1.937109 -1.598551 -0.249695 H1 1   FOO  0.2120 0.0000 ****
17    7 H62   2.836498 -0.872765  1.099272 H1 1   FOO  0.2120 0.0000 ****
18    8 O7    2.825130  0.263018 -0.582424 OH 1   FOO -0.3765 0.0000 ****
19    9 H71   2.154075  0.558808 -1.220079 HO 1   FOO  0.3575 0.0000 ****
20 @<TRIPOS>BOND
21    1     1     2  1
22    2     2     3  1
23    3     2     4  1
24    4     2     5  1
25    5     5     6  1
26    6     5     7  1
27    7     5     8  1
28    8     8     9  1
29 @<TRIPOS>SUBSTRUCTURE
30    1 FOO           1 ****          0 ***** ****
31 @<TRIPOS>HEADTAIL
32 O4 1
33 0 0
34 @<TRIPOS>RESIDUECONNECT
35 1 O4 0 0 0 0 0
```

---

#### B.3.3 FF libraries for ‘C’ fragment

---

```
1 #
2 # Generated by PyRED version SEP-2015
3 #      http://q4md-forcefieldtools.org
4 #
5 @<TRIPOS>MOLECULE
6 FOO
7      9     8     1     0     1
8 SMALL
9 USER_CHARGES
10 @<TRIPOS>ATOM
11    1 O4    0.120336  0.316293 -0.330261 OS 1   FOO -0.1415 0.0000 ****
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```

12      2 C5      0.852468 -0.409806  0.648487 CT 1   F00  0.0406 0.0000 *****
13      3 H51     0.334290 -1.337227  0.937709 H1 1   F00  0.0865 0.0000 *****
14      4 H52     0.994444  0.207307  1.550713 H1 1   F00  0.0865 0.0000 *****
15      5 C6      2.201878 -0.728258  0.020348 CT 1   F00 -0.4961 0.0000 *****
16      6 H61     2.046413 -1.394144 -0.843957 H1 1   F00  0.2120 0.0000 *****
17      7 H62     2.834973 -1.253815  0.743166 H1 1   F00  0.2120 0.0000 *****
18      8 O7      2.884980  0.454842 -0.353210 OH 1   F00 -0.3765 0.0000 *****
19      9 H71     2.237247  0.967802 -0.865402 HO 1   F00  0.3575 0.0000 *****
20 @<TRIPOS>BOND
21      1      1      2 1
22      2      2      3 1
23      3      2      4 1
24      4      2      5 1
25      5      5      6 1
26      6      5      7 1
27      7      5      8 1
28      8      8      9 1
29 @<TRIPOS>SUBSTRUCTURE
30      1 F00           1 *****
31 @<TRIPOS>HEADTAIL
32 O4 1
33 O O
34 @<TRIPOS>RESIDUECONNECT
35 1 O4 0 0 0 0 0

```

---

#### B.3.4 FF parameters for PEG-H(m)

```

1 FRCMOD file generated by PyRED version SEP-2015 - q4md-forcefieldtools
.org
2 MASS      mass          pol        Source
3 CT        12.010       0.878      taken from parm10.dat
4 H1        1.008        0.135      taken from parm10.dat
5 HO        1.008        0.135      taken from parm10.dat
6 OH        16.000       0.465      taken from parm10.dat
7 OS        16.000       0.465      taken from parm10.dat
8
9 BOND    K(kcal.mol-1.ang-2) Dist0(ang)  Source
10 CT-CT    310.0        1.526      taken from parm10.dat
11 CT-H1    340.0        1.090      taken from parm10.dat
12 CT-OH    320.0        1.410      taken from parm10.dat
13 CT-OS    320.0        1.410      taken from parm10.dat
14 HO-OH    555.0        0.960      adapted from parm10.dat 553.0
15
16 ANGLE   K(kcal.mol-1.rad-2) Theta0(deg)  Source
17 CT-CT-H1 50.0         109.50     taken from parm10.dat

```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```

18 CT-CT-OH      50.0       109.50      taken from parm10.dat
19 CT-CT-OS      50.0       109.50      taken from parm10.dat
20 H1-CT-H1      35.0       109.50      taken from parm10.dat
21 H1-CT-OH      50.0       109.50      taken from parm10.dat
22 H1-CT-OS      50.0       109.50      taken from parm10.dat
23 CT-OH-HO      55.0       108.50      taken from parm10.dat
24 CT-OS-CT      60.0       109.50      taken from parm10.dat
25
26 DIHEDRAL      Path V(kcal.mol-1.rad-1) Phase(deg.) Period Source
27 H1-CT-CT-H1   1   1.55555556e-01    0.0      3.  adapted from parm10.
          dat i.e X-CT-CT-X 1.4/9
28 H1-CT-CT-OH   1   0.00000000e+00    0.0      -3.  taken from parm10.
          dat
29 H1-CT-CT-OH   1   2.50000000e-01    0.0      1.  taken from parm10.
          dat
30 H1-CT-CT-OS   1   0.00000000e+00    0.0      -3.  taken from parm10.
          dat
31 H1-CT-CT-OS   1   2.50000000e-01    0.0      1.  taken from parm10.
          dat
32 OH-CT-CT-OS   1   1.44000000e-01    0.0      -3.  taken from parm10.
          dat
33 OH-CT-CT-OS   1   1.17500000e+00    0.0      2.  taken from parm10.
          dat
34 CT-CT-OH-HO   1   1.60000000e-01    0.0      -3.  taken from parm10.
          dat
35 CT-CT-OH-HO   1   2.50000000e-01    0.0      1.  taken from parm10.
          dat
36 H1-CT-OH-HO   1   1.66666667e-01    0.0      3.  adapted from parm10.
          dat i.e X-CT-OH-X 0.5/3
37 CT-CT-OS-CT   1   3.83000000e-01    0.0      -3.  taken from parm10.
          dat
38 CT-CT-OS-CT   1   1.00000000e-01   180.0     2.  taken from parm10.
          dat
39 H1-CT-OS-CT   1   3.83333333e-01    0.0      3.  adapted from parm10.
          dat i.e X-CT-OS-X 1.15/3
40
41 IMPROPER      V(kcal.mol-1.rad-1) Phase(deg.) Period Source
42
43 NONBON        R*(ang)  Eps(kcal.mol-1)  Source
44 CT            1.9080   0.10940000  taken from parm10.dat
45 H1            1.3870   0.01570000  taken from parm10.dat
46 HO            0.0000   0.00000000  taken from parm10.dat
47 OH            1.7210   0.21040000  taken from parm10.dat
48 OS            1.6837   0.17000000  taken from parm10.dat

```

---

**B.3.5 Fitting statistics of a whole molecule approach**

```
1
2 -----
3   Restrained ESP Fit 2.4 q4md-forcefieldtools
4 -----
5   RESP-A1 - RESP input generated by PyRED version SEP-2015
6 -----
7
8
9   inopt      =      0   ioutopt     =      1
10  nmep       =     46   iqopt       =      2
11  ihfree     =      1   irstrnt    =      1
12  iunits     =      0   qwt        =  0.00100000
13
14 multiple-MEP run of 46 MEP
15
16 Reading input for MEP 1 weight: 1.000
17 PEG2 nconf=1 norient=1 nmep=1/46
18
19 Total charge (ich): 0
20 Number of centers: 17
21   1   8   -1
22   2   1   -1
23   3   6   0
24   4   1   0
25   5   1   4
26   6   6   0
27   7   1   0
28   8   1   7
29   9   8   -1
30  10   6   6
31  11   1   7
32  12   1   7
33  13   6   3
34  14   1   4
35  15   1   4
36  16   8   -1
37  17   1   -1
38
39 Reading input for MEP 2 weight: 1.000
40 PEG2 nconf=1 norient=2 nmep=2/46
41
42 Total charge (ich): 0
43 Number of centers: 17
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
44    18    8   -1
45    19    1   -1
46    20    6    0
47    21    1    0
48    22    1    4
49    23    6    0
50    24    1    0
51    25    1    7
52    26    8   -1
53    27    6    6
54    28    1    7
55    29    1    7
56    30    6    3
57    31    1    4
58    32    1    4
59    33    8   -1
60    34    1   -1
61
62 Reading input for MEP 3 weight: 1.000
63 PEG2 nconf=2 norient=1 nmep=3/46
64
65 Total charge (ich): 0
66 Number of centers: 17
67    35    8   -1
68    36    1   -1
69    37    6    0
70    38    1    0
71    39    1    4
72    40    6    0
73    41    1    0
74    42    1    7
75    43    8   -1
76    44    6    6
77    45    1    7
78    46    1    7
79    47    6    3
80    48    1    4
81    49    1    4
82    50    8   -1
83    51    1   -1
84
85 Reading input for MEP 4 weight: 1.000
86 PEG2 nconf=2 norient=2 nmep=4/46
87
88 Total charge (ich): 0
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
89 Number of centers: 17
90    52    8   -1
91    53    1   -1
92    54    6    0
93    55    1    0
94    56    1    4
95    57    6    0
96    58    1    0
97    59    1    7
98    60    8   -1
99    61    6    6
100   62    1    7
101   63    1    7
102   64    6    3
103   65    1    4
104   66    1    4
105   67    8   -1
106   68    1   -1
107
108  Reading input for MEP 5 weight: 1.000
109  PEG2 nconf=3 norient=1 nmep=5/46
110
111 Total charge (ich): 0
112 Number of centers: 17
113    69    8   -1
114    70    1   -1
115    71    6    0
116    72    1    0
117    73    1    4
118    74    6    0
119    75    1    0
120    76    1    7
121    77    8   -1
122    78    6    6
123    79    1    7
124    80    1    7
125    81    6    3
126    82    1    4
127    83    1    4
128    84    8   -1
129    85    1   -1
130
131  Reading input for MEP 6 weight: 1.000
132  PEG2 nconf=3 norient=2 nmep=6/46
133
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
134 Total charge (ich): 0
135 Number of centers: 17
136    86   8   -1
137    87   1   -1
138    88   6    0
139    89   1    0
140    90   1    4
141    91   6    0
142    92   1    0
143    93   1    7
144    94   8   -1
145    95   6    6
146    96   1    7
147    97   1    7
148    98   6    3
149    99   1    4
150   100   1    4
151   101   8   -1
152   102   1   -1
153
154 Reading input for MEP 7 weight: 1.000
155 PEG2 nconf=4 norient=1 nmep=7/46
156
157 Total charge (ich): 0
158 Number of centers: 17
159    103   8   -1
160    104   1   -1
161    105   6    0
162    106   1    0
163    107   1    4
164    108   6    0
165    109   1    0
166    110   1    7
167    111   8   -1
168    112   6    6
169    113   1    7
170    114   1    7
171    115   6    3
172    116   1    4
173    117   1    4
174    118   8   -1
175    119   1   -1
176
177 Reading input for MEP 8 weight: 1.000
178 PEG2 nconf=4 norient=2 nmep=8/46
```

```
179
180 Total charge (ich): 0
181 Number of centers: 17
182 120 8 -1
183 121 1 -1
184 122 6 0
185 123 1 0
186 124 1 4
187 125 6 0
188 126 1 0
189 127 1 7
190 128 8 -1
191 129 6 6
192 130 1 7
193 131 1 7
194 132 6 3
195 133 1 4
196 134 1 4
197 135 8 -1
198 136 1 -1
199
200 Reading input for MEP 9 weight: 1.000
201 PEG2 nconf=5 norient=1 nmep=9/46
202
203 Total charge (ich): 0
204 Number of centers: 17
205 137 8 -1
206 138 1 -1
207 139 6 0
208 140 1 0
209 141 1 4
210 142 6 0
211 143 1 0
212 144 1 7
213 145 8 -1
214 146 6 6
215 147 1 7
216 148 1 7
217 149 6 3
218 150 1 4
219 151 1 4
220 152 8 -1
221 153 1 -1
222
223 Reading input for MEP 10 weight: 1.000
```

```
224 PEG2 nconf=5 norient=2 nmep=10/46
225
226 Total charge (ich): 0
227 Number of centers: 17
228   154   8   -1
229   155   1   -1
230   156   6    0
231   157   1    0
232   158   1    4
233   159   6    0
234   160   1    0
235   161   1    7
236   162   8   -1
237   163   6    6
238   164   1    7
239   165   1    7
240   166   6    3
241   167   1    4
242   168   1    4
243   169   8   -1
244   170   1   -1
245
246 Reading input for MEP 11 weight: 1.000
247 PEG2 nconf=6 norient=1 nmep=11/46
248
249 Total charge (ich): 0
250 Number of centers: 17
251   171   8   -1
252   172   1   -1
253   173   6    0
254   174   1    0
255   175   1    4
256   176   6    0
257   177   1    0
258   178   1    7
259   179   8   -1
260   180   6    6
261   181   1    7
262   182   1    7
263   183   6    3
264   184   1    4
265   185   1    4
266   186   8   -1
267   187   1   -1
268
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
269  Reading input for MEP 12 weight: 1.000
270  PEG2 nconf=6 norient=2 nmep=12/46
271
272  Total charge (ich): 0
273  Number of centers: 17
274    188   8   -1
275    189   1   -1
276    190   6    0
277    191   1    0
278    192   1    4
279    193   6    0
280    194   1    0
281    195   1    7
282    196   8   -1
283    197   6    6
284    198   1    7
285    199   1    7
286    200   6    3
287    201   1    4
288    202   1    4
289    203   8   -1
290    204   1   -1
291
292  Reading input for MEP 13 weight: 1.000
293  PEG2 nconf=7 norient=1 nmep=13/46
294
295  Total charge (ich): 0
296  Number of centers: 17
297    205   8   -1
298    206   1   -1
299    207   6    0
300    208   1    0
301    209   1    4
302    210   6    0
303    211   1    0
304    212   1    7
305    213   8   -1
306    214   6    6
307    215   1    7
308    216   1    7
309    217   6    3
310    218   1    4
311    219   1    4
312    220   8   -1
313    221   1   -1
```

```
314
315  Reading input for MEP 14 weight: 1.000
316  PEG2 nconf=7 norient=2 nmep=14/46
317
318  Total charge (ich): 0
319  Number of centers: 17
320    222    8   -1
321    223    1   -1
322    224    6    0
323    225    1    0
324    226    1    4
325    227    6    0
326    228    1    0
327    229    1    7
328    230    8   -1
329    231    6    6
330    232    1    7
331    233    1    7
332    234    6    3
333    235    1    4
334    236    1    4
335    237    8   -1
336    238    1   -1
337
338  Reading input for MEP 15 weight: 1.000
339  PEG2 nconf=8 norient=1 nmep=15/46
340
341  Total charge (ich): 0
342  Number of centers: 17
343    239    8   -1
344    240    1   -1
345    241    6    0
346    242    1    0
347    243    1    4
348    244    6    0
349    245    1    0
350    246    1    7
351    247    8   -1
352    248    6    6
353    249    1    7
354    250    1    7
355    251    6    3
356    252    1    4
357    253    1    4
358    254    8   -1
```

```
359 255 1 -1
360
361 Reading input for MEP 16 weight: 1.000
362 PEG2 nconf=8 norient=2 nmep=16/46
363
364 Total charge (ich): 0
365 Number of centers: 17
366 256 8 -1
367 257 1 -1
368 258 6 0
369 259 1 0
370 260 1 4
371 261 6 0
372 262 1 0
373 263 1 7
374 264 8 -1
375 265 6 6
376 266 1 7
377 267 1 7
378 268 6 3
379 269 1 4
380 270 1 4
381 271 8 -1
382 272 1 -1
383
384 Reading input for MEP 17 weight: 1.000
385 PEG2 nconf=9 norient=1 nmep=17/46
386
387 Total charge (ich): 0
388 Number of centers: 17
389 273 8 -1
390 274 1 -1
391 275 6 0
392 276 1 0
393 277 1 4
394 278 6 0
395 279 1 0
396 280 1 7
397 281 8 -1
398 282 6 6
399 283 1 7
400 284 1 7
401 285 6 3
402 286 1 4
403 287 1 4
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
404    288    8   -1
405    289    1   -1
406
407 Reading input for MEP 18 weight: 1.000
408 PEG2 nconf=9 norient=2 nmep=18/46
409
410 Total charge (ich): 0
411 Number of centers: 17
412    290    8   -1
413    291    1   -1
414    292    6    0
415    293    1    0
416    294    1    4
417    295    6    0
418    296    1    0
419    297    1    7
420    298    8   -1
421    299    6    6
422    300    1    7
423    301    1    7
424    302    6    3
425    303    1    4
426    304    1    4
427    305    8   -1
428    306    1   -1
429
430 Reading input for MEP 19 weight: 1.000
431 PEG2 nconf=10 norient=1 nmep=19/46
432
433 Total charge (ich): 0
434 Number of centers: 17
435    307    8   -1
436    308    1   -1
437    309    6    0
438    310    1    0
439    311    1    4
440    312    6    0
441    313    1    0
442    314    1    7
443    315    8   -1
444    316    6    6
445    317    1    7
446    318    1    7
447    319    6    3
448    320    1    4
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
449    321    1    4
450    322    8   -1
451    323    1   -1
452
453 Reading input for MEP 20 weight: 1.000
454 PEG2 nconf=10 norient=2 nmep=20/46
455
456 Total charge (ich): 0
457 Number of centers: 17
458    324    8   -1
459    325    1   -1
460    326    6    0
461    327    1    0
462    328    1    4
463    329    6    0
464    330    1    0
465    331    1    7
466    332    8   -1
467    333    6    6
468    334    1    7
469    335    1    7
470    336    6    3
471    337    1    4
472    338    1    4
473    339    8   -1
474    340    1   -1
475
476 Reading input for MEP 21 weight: 1.000
477 PEG2 nconf=11 norient=1 nmep=21/46
478
479 Total charge (ich): 0
480 Number of centers: 17
481    341    8   -1
482    342    1   -1
483    343    6    0
484    344    1    0
485    345    1    4
486    346    6    0
487    347    1    0
488    348    1    7
489    349    8   -1
490    350    6    6
491    351    1    7
492    352    1    7
493    353    6    3
```

```
494    354    1    4
495    355    1    4
496    356    8   -1
497    357    1   -1
498
499 Reading input for MEP 22 weight: 1.000
500 PEG2 nconf=11 norient=2 nmep=22/46
501
502 Total charge (ich): 0
503 Number of centers: 17
504    358    8   -1
505    359    1   -1
506    360    6    0
507    361    1    0
508    362    1    4
509    363    6    0
510    364    1    0
511    365    1    7
512    366    8   -1
513    367    6    6
514    368    1    7
515    369    1    7
516    370    6    3
517    371    1    4
518    372    1    4
519    373    8   -1
520    374    1   -1
521
522 Reading input for MEP 23 weight: 1.000
523 PEG2 nconf=12 norient=1 nmep=23/46
524
525 Total charge (ich): 0
526 Number of centers: 17
527    375    8   -1
528    376    1   -1
529    377    6    0
530    378    1    0
531    379    1    4
532    380    6    0
533    381    1    0
534    382    1    7
535    383    8   -1
536    384    6    6
537    385    1    7
538    386    1    7
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
539    387    6    3
540    388    1    4
541    389    1    4
542    390    8   -1
543    391    1   -1
544
545 Reading input for MEP 24 weight: 1.000
546 PEG2 nconf=12 norient=2 nmep=24/46
547
548 Total charge (ich): 0
549 Number of centers: 17
550    392    8   -1
551    393    1   -1
552    394    6    0
553    395    1    0
554    396    1    4
555    397    6    0
556    398    1    0
557    399    1    7
558    400    8   -1
559    401    6    6
560    402    1    7
561    403    1    7
562    404    6    3
563    405    1    4
564    406    1    4
565    407    8   -1
566    408    1   -1
567
568 Reading input for MEP 25 weight: 1.000
569 PEG2 nconf=13 norient=1 nmep=25/46
570
571 Total charge (ich): 0
572 Number of centers: 17
573    409    8   -1
574    410    1   -1
575    411    6    0
576    412    1    0
577    413    1    4
578    414    6    0
579    415    1    0
580    416    1    7
581    417    8   -1
582    418    6    6
583    419    1    7
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
584    420    1    7
585    421    6    3
586    422    1    4
587    423    1    4
588    424    8   -1
589    425    1   -1
590
591 Reading input for MEP 26 weight: 1.000
592 PEG2 nconf=13 norient=2 nmep=26/46
593
594 Total charge (ich): 0
595 Number of centers: 17
596    426    8   -1
597    427    1   -1
598    428    6    0
599    429    1    0
600    430    1    4
601    431    6    0
602    432    1    0
603    433    1    7
604    434    8   -1
605    435    6    6
606    436    1    7
607    437    1    7
608    438    6    3
609    439    1    4
610    440    1    4
611    441    8   -1
612    442    1   -1
613
614 Reading input for MEP 27 weight: 1.000
615 PEG2 nconf=14 norient=1 nmep=27/46
616
617 Total charge (ich): 0
618 Number of centers: 17
619    443    8   -1
620    444    1   -1
621    445    6    0
622    446    1    0
623    447    1    4
624    448    6    0
625    449    1    0
626    450    1    7
627    451    8   -1
628    452    6    6
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
629    453    1    7
630    454    1    7
631    455    6    3
632    456    1    4
633    457    1    4
634    458    8   -1
635    459    1   -1
636
637 Reading input for MEP 28 weight: 1.000
638 PEG2 nconf=14 norient=2 nmep=28/46
639
640 Total charge (ich): 0
641 Number of centers: 17
642    460    8   -1
643    461    1   -1
644    462    6    0
645    463    1    0
646    464    1    4
647    465    6    0
648    466    1    0
649    467    1    7
650    468    8   -1
651    469    6    6
652    470    1    7
653    471    1    7
654    472    6    3
655    473    1    4
656    474    1    4
657    475    8   -1
658    476    1   -1
659
660 Reading input for MEP 29 weight: 1.000
661 PEG2 nconf=15 norient=1 nmep=29/46
662
663 Total charge (ich): 0
664 Number of centers: 17
665    477    8   -1
666    478    1   -1
667    479    6    0
668    480    1    0
669    481    1    4
670    482    6    0
671    483    1    0
672    484    1    7
673    485    8   -1
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
674    486    6    6
675    487    1    7
676    488    1    7
677    489    6    3
678    490    1    4
679    491    1    4
680    492    8   -1
681    493    1   -1
682
683 Reading input for MEP 30 weight: 1.000
684 PEG2 nconf=15 norient=2 nmep=30/46
685
686 Total charge (ich): 0
687 Number of centers: 17
688    494    8   -1
689    495    1   -1
690    496    6    0
691    497    1    0
692    498    1    4
693    499    6    0
694    500    1    0
695    501    1    7
696    502    8   -1
697    503    6    6
698    504    1    7
699    505    1    7
700    506    6    3
701    507    1    4
702    508    1    4
703    509    8   -1
704    510    1   -1
705
706 Reading input for MEP 31 weight: 1.000
707 PEG2 nconf=16 norient=1 nmep=31/46
708
709 Total charge (ich): 0
710 Number of centers: 17
711    511    8   -1
712    512    1   -1
713    513    6    0
714    514    1    0
715    515    1    4
716    516    6    0
717    517    1    0
718    518    1    7
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
719 519 8 -1
720 520 6 6
721 521 1 7
722 522 1 7
723 523 6 3
724 524 1 4
725 525 1 4
726 526 8 -1
727 527 1 -1
728
729 Reading input for MEP 32 weight: 1.000
730 PEG2 nconf=16 norient=2 nmep=32/46
731
732 Total charge (ich): 0
733 Number of centers: 17
734 528 8 -1
735 529 1 -1
736 530 6 0
737 531 1 0
738 532 1 4
739 533 6 0
740 534 1 0
741 535 1 7
742 536 8 -1
743 537 6 6
744 538 1 7
745 539 1 7
746 540 6 3
747 541 1 4
748 542 1 4
749 543 8 -1
750 544 1 -1
751
752 Reading input for MEP 33 weight: 1.000
753 PEG2 nconf=17 norient=1 nmep=33/46
754
755 Total charge (ich): 0
756 Number of centers: 17
757 545 8 -1
758 546 1 -1
759 547 6 0
760 548 1 0
761 549 1 4
762 550 6 0
763 551 1 0
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
764 552 1 7
765 553 8 -1
766 554 6 6
767 555 1 7
768 556 1 7
769 557 6 3
770 558 1 4
771 559 1 4
772 560 8 -1
773 561 1 -1
774
775 Reading input for MEP 34 weight: 1.000
776 PEG2 nconf=17 norient=2 nmep=34/46
777
778 Total charge (ich): 0
779 Number of centers: 17
780 562 8 -1
781 563 1 -1
782 564 6 0
783 565 1 0
784 566 1 4
785 567 6 0
786 568 1 0
787 569 1 7
788 570 8 -1
789 571 6 6
790 572 1 7
791 573 1 7
792 574 6 3
793 575 1 4
794 576 1 4
795 577 8 -1
796 578 1 -1
797
798 Reading input for MEP 35 weight: 1.000
799 PEG2 nconf=18 norient=1 nmep=35/46
800
801 Total charge (ich): 0
802 Number of centers: 17
803 579 8 -1
804 580 1 -1
805 581 6 0
806 582 1 0
807 583 1 4
808 584 6 0
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
809      585    1    0
810      586    1    7
811      587    8   -1
812      588    6    6
813      589    1    7
814      590    1    7
815      591    6    3
816      592    1    4
817      593    1    4
818      594    8   -1
819      595    1   -1
820
821 Reading input for MEP 36 weight: 1.000
822 PEG2 nconf=18 norient=2 nmep=36/46
823
824 Total charge (ich): 0
825 Number of centers: 17
826      596    8   -1
827      597    1   -1
828      598    6    0
829      599    1    0
830      600    1    4
831      601    6    0
832      602    1    0
833      603    1    7
834      604    8   -1
835      605    6    6
836      606    1    7
837      607    1    7
838      608    6    3
839      609    1    4
840      610    1    4
841      611    8   -1
842      612    1   -1
843
844 Reading input for MEP 37 weight: 1.000
845 PEG2 nconf=19 norient=1 nmep=37/46
846
847 Total charge (ich): 0
848 Number of centers: 17
849      613    8   -1
850      614    1   -1
851      615    6    0
852      616    1    0
853      617    1    4
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
854    618    6    0
855    619    1    0
856    620    1    7
857    621    8   -1
858    622    6    6
859    623    1    7
860    624    1    7
861    625    6    3
862    626    1    4
863    627    1    4
864    628    8   -1
865    629    1   -1
866
867 Reading input for MEP 38 weight: 1.000
868 PEG2 nconf=19 norient=2 nmep=38/46
869
870 Total charge (ich): 0
871 Number of centers: 17
872    630    8   -1
873    631    1   -1
874    632    6    0
875    633    1    0
876    634    1    4
877    635    6    0
878    636    1    0
879    637    1    7
880    638    8   -1
881    639    6    6
882    640    1    7
883    641    1    7
884    642    6    3
885    643    1    4
886    644    1    4
887    645    8   -1
888    646    1   -1
889
890 Reading input for MEP 39 weight: 1.000
891 PEG2 nconf=20 norient=1 nmep=39/46
892
893 Total charge (ich): 0
894 Number of centers: 17
895    647    8   -1
896    648    1   -1
897    649    6    0
898    650    1    0
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
899    651    1    4
900    652    6    0
901    653    1    0
902    654    1    7
903    655    8   -1
904    656    6    6
905    657    1    7
906    658    1    7
907    659    6    3
908    660    1    4
909    661    1    4
910    662    8   -1
911    663    1   -1
912
913 Reading input for MEP 40 weight: 1.000
914 PEG2 nconf=20 norient=2 nmep=40/46
915
916 Total charge (ich): 0
917 Number of centers: 17
918    664    8   -1
919    665    1   -1
920    666    6    0
921    667    1    0
922    668    1    4
923    669    6    0
924    670    1    0
925    671    1    7
926    672    8   -1
927    673    6    6
928    674    1    7
929    675    1    7
930    676    6    3
931    677    1    4
932    678    1    4
933    679    8   -1
934    680    1   -1
935
936 Reading input for MEP 41 weight: 1.000
937 PEG2 nconf=21 norient=1 nmep=41/46
938
939 Total charge (ich): 0
940 Number of centers: 17
941    681    8   -1
942    682    1   -1
943    683    6    0
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
944    684    1    0
945    685    1    4
946    686    6    0
947    687    1    0
948    688    1    7
949    689    8   -1
950    690    6    6
951    691    1    7
952    692    1    7
953    693    6    3
954    694    1    4
955    695    1    4
956    696    8   -1
957    697    1   -1
958
959 Reading input for MEP 42 weight: 1.000
960 PEG2 nconf=21 norient=2 nmep=42/46
961
962 Total charge (ich): 0
963 Number of centers: 17
964    698    8   -1
965    699    1   -1
966    700    6    0
967    701    1    0
968    702    1    4
969    703    6    0
970    704    1    0
971    705    1    7
972    706    8   -1
973    707    6    6
974    708    1    7
975    709    1    7
976    710    6    3
977    711    1    4
978    712    1    4
979    713    8   -1
980    714    1   -1
981
982 Reading input for MEP 43 weight: 1.000
983 PEG2 nconf=22 norient=1 nmep=43/46
984
985 Total charge (ich): 0
986 Number of centers: 17
987    715    8   -1
988    716    1   -1
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
989    717    6    0
990    718    1    0
991    719    1    4
992    720    6    0
993    721    1    0
994    722    1    7
995    723    8   -1
996    724    6    6
997    725    1    7
998    726    1    7
999    727    6    3
1000   728    1    4
1001   729    1    4
1002   730    8   -1
1003   731    1   -1
1004
1005  Reading input for MEP 44 weight: 1.000
1006  PEG2 nconf=22 norient=2 nmep=44/46
1007
1008 Total charge (ich): 0
1009 Number of centers: 17
1010   732    8   -1
1011   733    1   -1
1012   734    6    0
1013   735    1    0
1014   736    1    4
1015   737    6    0
1016   738    1    0
1017   739    1    7
1018   740    8   -1
1019   741    6    6
1020   742    1    7
1021   743    1    7
1022   744    6    3
1023   745    1    4
1024   746    1    4
1025   747    8   -1
1026   748    1   -1
1027
1028  Reading input for MEP 45 weight: 1.000
1029  PEG2 nconf=23 norient=1 nmep=45/46
1030
1031 Total charge (ich): 0
1032 Number of centers: 17
1033   749    8   -1
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
1034    750    1   -1
1035    751    6    0
1036    752    1    0
1037    753    1    4
1038    754    6    0
1039    755    1    0
1040    756    1    7
1041    757    8   -1
1042    758    6    6
1043    759    1    7
1044    760    1    7
1045    761    6    3
1046    762    1    4
1047    763    1    4
1048    764    8   -1
1049    765    1   -1
1050
1051    Reading input for MEP 46 weight: 1.000
1052    PEG2 nconf=23 norient=2 nmep=46/46
1053
1054    Total charge (ich): 0
1055    Number of centers: 17
1056    766    8   -1
1057    767    1   -1
1058    768    6    0
1059    769    1    0
1060    770    1    4
1061    771    6    0
1062    772    1    0
1063    773    1    7
1064    774    8   -1
1065    775    6    6
1066    776    1    7
1067    777    1    7
1068    778    6    3
1069    779    1    4
1070    780    1    4
1071    781    8   -1
1072    782    1   -1
1073    since IQOPT>1, 782 new q0 values
1074    will be read in from file ESP.Q0 (unit 3)
1075    -----
1076    reading mult_esp constraint info
1077    -----
1078    1    3    2    3    3    3    4    3    5    3    6    3    7    3    8    3
```

B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1079	9	3	10	3	11	3	12	3	13	3	14	3	15	3	16	3
1080	17	3	18	3	19	3	20	3	21	3	22	3	23	3	24	3
1081	25	3	26	3	27	3	28	3	29	3	30	3	31	3	32	3
1082	33	3	34	3	35	3	36	3	37	3	38	3	39	3	40	3
1083	41	3	42	3	43	3	44	3	45	3	46	3				
1084	1	4	2	4	3	4	4	4	5	4	6	4	7	4	8	4
1085	9	4	10	4	11	4	12	4	13	4	14	4	15	4	16	4
1086	17	4	18	4	19	4	20	4	21	4	22	4	23	4	24	4
1087	25	4	26	4	27	4	28	4	29	4	30	4	31	4	32	4
1088	33	4	34	4	35	4	36	4	37	4	38	4	39	4	40	4
1089	41	4	42	4	43	4	44	4	45	4	46	4				
1090	1	6	2	6	3	6	4	6	5	6	6	6	7	6	8	6
1091	9	6	10	6	11	6	12	6	13	6	14	6	15	6	16	6
1092	17	6	18	6	19	6	20	6	21	6	22	6	23	6	24	6
1093	25	6	26	6	27	6	28	6	29	6	30	6	31	6	32	6
1094	33	6	34	6	35	6	36	6	37	6	38	6	39	6	40	6
1095	41	6	42	6	43	6	44	6	45	6	46	6				
1096	1	7	2	7	3	7	4	7	5	7	6	7	7	7	8	7
1097	9	7	10	7	11	7	12	7	13	7	14	7	15	7	16	7
1098	17	7	18	7	19	7	20	7	21	7	22	7	23	7	24	7
1099	25	7	26	7	27	7	28	7	29	7	30	7	31	7	32	7
1100	33	7	34	7	35	7	36	7	37	7	38	7	39	7	40	7
1101	41	7	42	7	43	7	44	7	45	7	46	7				
1102																
1103	-----															
1104	Atom	Ivary														
1105	-----															
1106	8	-1														
1107	1	-1														
1108	6	0														
1109	1	0														
1110	1	4														
1111	6	0														
1112	1	0														
1113	1	7														
1114	8	-1														
1115	6	6														
1116	1	7														
1117	1	7														
1118	6	3														
1119	1	4														
1120	1	4														
1121	8	-1														
1122	1	-1														
1123																

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1124	8	-1
1125	1	-1
1126	6	3
1127	1	4
1128	1	4
1129	6	6
1130	1	7
1131	1	7
1132	8	-1
1133	6	6
1134	1	7
1135	1	7
1136	6	3
1137	1	4
1138	1	4
1139	8	-1
1140	1	-1
1141		
1142	8	-1
1143	1	-1
1144	6	3
1145	1	4
1146	1	4
1147	6	6
1148	1	7
1149	1	7
1150	8	-1
1151	6	6
1152	1	7
1153	1	7
1154	6	3
1155	1	4
1156	1	4
1157	8	-1
1158	1	-1
1159		
1160	8	-1
1161	1	-1
1162	6	3
1163	1	4
1164	1	4
1165	6	6
1166	1	7
1167	1	7
1168	8	-1

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1169        6        6  
1170        1        7  
1171        1        7  
1172        6        3  
1173        1        4  
1174        1        4  
1175        8      -1  
1176        1      -1  
1177  
1178        8      -1  
1179        1      -1  
1180        6        3  
1181        1        4  
1182        1        4  
1183        6        6  
1184        1        7  
1185        1        7  
1186        8      -1  
1187        6        6  
1188        1        7  
1189        1        7  
1190        6        3  
1191        1        4  
1192        1        4  
1193        8      -1  
1194        1      -1  
1195  
1196        8      -1  
1197        1      -1  
1198        6        3  
1199        1        4  
1200        1        4  
1201        6        6  
1202        1        7  
1203        1        7  
1204        8      -1  
1205        6        6  
1206        1        7  
1207        1        7  
1208        6        3  
1209        1        4  
1210        1        4  
1211        8      -1  
1212        1      -1  
1213

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1214	8	-1
1215	1	-1
1216	6	3
1217	1	4
1218	1	4
1219	6	6
1220	1	7
1221	1	7
1222	8	-1
1223	6	6
1224	1	7
1225	1	7
1226	6	3
1227	1	4
1228	1	4
1229	8	-1
1230	1	-1
1231		
1232	8	-1
1233	1	-1
1234	6	3
1235	1	4
1236	1	4
1237	6	6
1238	1	7
1239	1	7
1240	8	-1
1241	6	6
1242	1	7
1243	1	7
1244	6	3
1245	1	4
1246	1	4
1247	8	-1
1248	1	-1
1249		
1250	8	-1
1251	1	-1
1252	6	3
1253	1	4
1254	1	4
1255	6	6
1256	1	7
1257	1	7
1258	8	-1

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1259        6        6  
1260        1        7  
1261        1        7  
1262        6        3  
1263        1        4  
1264        1        4  
1265        8      -1  
1266        1      -1  
1267  
1268        8      -1  
1269        1      -1  
1270        6        3  
1271        1        4  
1272        1        4  
1273        6        6  
1274        1        7  
1275        1        7  
1276        8      -1  
1277        6        6  
1278        1        7  
1279        1        7  
1280        6        3  
1281        1        4  
1282        1        4  
1283        8      -1  
1284        1      -1  
1285  
1286        8      -1  
1287        1      -1  
1288        6        3  
1289        1        4  
1290        1        4  
1291        6        6  
1292        1        7  
1293        1        7  
1294        8      -1  
1295        6        6  
1296        1        7  
1297        1        7  
1298        6        3  
1299        1        4  
1300        1        4  
1301        8      -1  
1302        1      -1  
1303

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1304	8	-1
1305	1	-1
1306	6	3
1307	1	4
1308	1	4
1309	6	6
1310	1	7
1311	1	7
1312	8	-1
1313	6	6
1314	1	7
1315	1	7
1316	6	3
1317	1	4
1318	1	4
1319	8	-1
1320	1	-1
1321		
1322	8	-1
1323	1	-1
1324	6	3
1325	1	4
1326	1	4
1327	6	6
1328	1	7
1329	1	7
1330	8	-1
1331	6	6
1332	1	7
1333	1	7
1334	6	3
1335	1	4
1336	1	4
1337	8	-1
1338	1	-1
1339		
1340	8	-1
1341	1	-1
1342	6	3
1343	1	4
1344	1	4
1345	6	6
1346	1	7
1347	1	7
1348	8	-1

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1349	6	6
1350	1	7
1351	1	7
1352	6	3
1353	1	4
1354	1	4
1355	8	-1
1356	1	-1
1357		
1358	8	-1
1359	1	-1
1360	6	3
1361	1	4
1362	1	4
1363	6	6
1364	1	7
1365	1	7
1366	8	-1
1367	6	6
1368	1	7
1369	1	7
1370	6	3
1371	1	4
1372	1	4
1373	8	-1
1374	1	-1
1375		
1376	8	-1
1377	1	-1
1378	6	3
1379	1	4
1380	1	4
1381	6	6
1382	1	7
1383	1	7
1384	8	-1
1385	6	6
1386	1	7
1387	1	7
1388	6	3
1389	1	4
1390	1	4
1391	8	-1
1392	1	-1
1393		

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1394	8	-1
1395	1	-1
1396	6	3
1397	1	4
1398	1	4
1399	6	6
1400	1	7
1401	1	7
1402	8	-1
1403	6	6
1404	1	7
1405	1	7
1406	6	3
1407	1	4
1408	1	4
1409	8	-1
1410	1	-1
1411		
1412	8	-1
1413	1	-1
1414	6	3
1415	1	4
1416	1	4
1417	6	6
1418	1	7
1419	1	7
1420	8	-1
1421	6	6
1422	1	7
1423	1	7
1424	6	3
1425	1	4
1426	1	4
1427	8	-1
1428	1	-1
1429		
1430	8	-1
1431	1	-1
1432	6	3
1433	1	4
1434	1	4
1435	6	6
1436	1	7
1437	1	7
1438	8	-1

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1439        6        6  
1440        1        7  
1441        1        7  
1442        6        3  
1443        1        4  
1444        1        4  
1445        8        -1  
1446        1        -1  
1447  
1448        8        -1  
1449        1        -1  
1450        6        3  
1451        1        4  
1452        1        4  
1453        6        6  
1454        1        7  
1455        1        7  
1456        8        -1  
1457        6        6  
1458        1        7  
1459        1        7  
1460        6        3  
1461        1        4  
1462        1        4  
1463        8        -1  
1464        1        -1  
1465  
1466        8        -1  
1467        1        -1  
1468        6        3  
1469        1        4  
1470        1        4  
1471        6        6  
1472        1        7  
1473        1        7  
1474        8        -1  
1475        6        6  
1476        1        7  
1477        1        7  
1478        6        3  
1479        1        4  
1480        1        4  
1481        8        -1  
1482        1        -1  
1483

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1484	8	-1
1485	1	-1
1486	6	3
1487	1	4
1488	1	4
1489	6	6
1490	1	7
1491	1	7
1492	8	-1
1493	6	6
1494	1	7
1495	1	7
1496	6	3
1497	1	4
1498	1	4
1499	8	-1
1500	1	-1
1501		
1502	8	-1
1503	1	-1
1504	6	3
1505	1	4
1506	1	4
1507	6	6
1508	1	7
1509	1	7
1510	8	-1
1511	6	6
1512	1	7
1513	1	7
1514	6	3
1515	1	4
1516	1	4
1517	8	-1
1518	1	-1
1519		
1520	8	-1
1521	1	-1
1522	6	3
1523	1	4
1524	1	4
1525	6	6
1526	1	7
1527	1	7
1528	8	-1

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1529	6	6
1530	1	7
1531	1	7
1532	6	3
1533	1	4
1534	1	4
1535	8	-1
1536	1	-1
1537		
1538	8	-1
1539	1	-1
1540	6	3
1541	1	4
1542	1	4
1543	6	6
1544	1	7
1545	1	7
1546	8	-1
1547	6	6
1548	1	7
1549	1	7
1550	6	3
1551	1	4
1552	1	4
1553	8	-1
1554	1	-1
1555		
1556	8	-1
1557	1	-1
1558	6	3
1559	1	4
1560	1	4
1561	6	6
1562	1	7
1563	1	7
1564	8	-1
1565	6	6
1566	1	7
1567	1	7
1568	6	3
1569	1	4
1570	1	4
1571	8	-1
1572	1	-1
1573		

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1574	8	-1
1575	1	-1
1576	6	3
1577	1	4
1578	1	4
1579	6	6
1580	1	7
1581	1	7
1582	8	-1
1583	6	6
1584	1	7
1585	1	7
1586	6	3
1587	1	4
1588	1	4
1589	8	-1
1590	1	-1
1591		
1592	8	-1
1593	1	-1
1594	6	3
1595	1	4
1596	1	4
1597	6	6
1598	1	7
1599	1	7
1600	8	-1
1601	6	6
1602	1	7
1603	1	7
1604	6	3
1605	1	4
1606	1	4
1607	8	-1
1608	1	-1
1609		
1610	8	-1
1611	1	-1
1612	6	3
1613	1	4
1614	1	4
1615	6	6
1616	1	7
1617	1	7
1618	8	-1

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1619	6	6
1620	1	7
1621	1	7
1622	6	3
1623	1	4
1624	1	4
1625	8	-1
1626	1	-1
1627		
1628	8	-1
1629	1	-1
1630	6	3
1631	1	4
1632	1	4
1633	6	6
1634	1	7
1635	1	7
1636	8	-1
1637	6	6
1638	1	7
1639	1	7
1640	6	3
1641	1	4
1642	1	4
1643	8	-1
1644	1	-1
1645		
1646	8	-1
1647	1	-1
1648	6	3
1649	1	4
1650	1	4
1651	6	6
1652	1	7
1653	1	7
1654	8	-1
1655	6	6
1656	1	7
1657	1	7
1658	6	3
1659	1	4
1660	1	4
1661	8	-1
1662	1	-1
1663		

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1664	8	-1
1665	1	-1
1666	6	3
1667	1	4
1668	1	4
1669	6	6
1670	1	7
1671	1	7
1672	8	-1
1673	6	6
1674	1	7
1675	1	7
1676	6	3
1677	1	4
1678	1	4
1679	8	-1
1680	1	-1
1681		
1682	8	-1
1683	1	-1
1684	6	3
1685	1	4
1686	1	4
1687	6	6
1688	1	7
1689	1	7
1690	8	-1
1691	6	6
1692	1	7
1693	1	7
1694	6	3
1695	1	4
1696	1	4
1697	8	-1
1698	1	-1
1699		
1700	8	-1
1701	1	-1
1702	6	3
1703	1	4
1704	1	4
1705	6	6
1706	1	7
1707	1	7
1708	8	-1

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1709	6	6
1710	1	7
1711	1	7
1712	6	3
1713	1	4
1714	1	4
1715	8	-1
1716	1	-1
1717		
1718	8	-1
1719	1	-1
1720	6	3
1721	1	4
1722	1	4
1723	6	6
1724	1	7
1725	1	7
1726	8	-1
1727	6	6
1728	1	7
1729	1	7
1730	6	3
1731	1	4
1732	1	4
1733	8	-1
1734	1	-1
1735		
1736	8	-1
1737	1	-1
1738	6	3
1739	1	4
1740	1	4
1741	6	6
1742	1	7
1743	1	7
1744	8	-1
1745	6	6
1746	1	7
1747	1	7
1748	6	3
1749	1	4
1750	1	4
1751	8	-1
1752	1	-1
1753		

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1754	8	-1
1755	1	-1
1756	6	3
1757	1	4
1758	1	4
1759	6	6
1760	1	7
1761	1	7
1762	8	-1
1763	6	6
1764	1	7
1765	1	7
1766	6	3
1767	1	4
1768	1	4
1769	8	-1
1770	1	-1
1771		
1772	8	-1
1773	1	-1
1774	6	3
1775	1	4
1776	1	4
1777	6	6
1778	1	7
1779	1	7
1780	8	-1
1781	6	6
1782	1	7
1783	1	7
1784	6	3
1785	1	4
1786	1	4
1787	8	-1
1788	1	-1
1789		
1790	8	-1
1791	1	-1
1792	6	3
1793	1	4
1794	1	4
1795	6	6
1796	1	7
1797	1	7
1798	8	-1

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1799	6	6
1800	1	7
1801	1	7
1802	6	3
1803	1	4
1804	1	4
1805	8	-1
1806	1	-1
1807		
1808	8	-1
1809	1	-1
1810	6	3
1811	1	4
1812	1	4
1813	6	6
1814	1	7
1815	1	7
1816	8	-1
1817	6	6
1818	1	7
1819	1	7
1820	6	3
1821	1	4
1822	1	4
1823	8	-1
1824	1	-1
1825		
1826	8	-1
1827	1	-1
1828	6	3
1829	1	4
1830	1	4
1831	6	6
1832	1	7
1833	1	7
1834	8	-1
1835	6	6
1836	1	7
1837	1	7
1838	6	3
1839	1	4
1840	1	4
1841	8	-1
1842	1	-1
1843		

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1844	8	-1
1845	1	-1
1846	6	3
1847	1	4
1848	1	4
1849	6	6
1850	1	7
1851	1	7
1852	8	-1
1853	6	6
1854	1	7
1855	1	7
1856	6	3
1857	1	4
1858	1	4
1859	8	-1
1860	1	-1
1861		
1862	8	-1
1863	1	-1
1864	6	3
1865	1	4
1866	1	4
1867	6	6
1868	1	7
1869	1	7
1870	8	-1
1871	6	6
1872	1	7
1873	1	7
1874	6	3
1875	1	4
1876	1	4
1877	8	-1
1878	1	-1
1879		
1880	8	-1
1881	1	-1
1882	6	3
1883	1	4
1884	1	4
1885	6	6
1886	1	7
1887	1	7
1888	8	-1

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1889	6	6
1890	1	7
1891	1	7
1892	6	3
1893	1	4
1894	1	4
1895	8	-1
1896	1	-1
1897		
1898	8	-1
1899	1	-1
1900	6	3
1901	1	4
1902	1	4
1903	6	6
1904	1	7
1905	1	7
1906	8	-1
1907	6	6
1908	1	7
1909	1	7
1910	6	3
1911	1	4
1912	1	4
1913	8	-1
1914	1	-1
1915		
1916	8	-1
1917	1	-1
1918	6	3
1919	1	4
1920	1	4
1921	6	6
1922	1	7
1923	1	7
1924	8	-1
1925	6	6
1926	1	7
1927	1	7
1928	6	3
1929	1	4
1930	1	4
1931	8	-1
1932	1	-1
1933		

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1934 -----  
1935  
1936  
1937 Total number of atoms = 782  
1938 Weight factor on initial charge restraints= 0.001000  
1939  
1940  
1941 There are 46 charge constraints  
1942  
1943 Reading esp"s for MEP 1  
1944 total number of atoms = 17  
1945 total number of esp points = 809  
1946  
1947 Center X Y Z  
1948 1 0.4978215E+01 0.2437140E+01 -0.4366318E+01  
1949 2 0.4793776E+01 0.3312593E+01 -0.5963597E+01  
1950 3 0.3655614E+01 0.3833419E+01 -0.2498507E+01  
1951 4 0.1659618E+01 0.4112601E+01 -0.3022352E+01  
1952 5 0.4485629E+01 0.5725104E+01 -0.2210686E+01  
1953 6 0.3839901E+01 0.2415871E+01 0.0000000E+00  
1954 7 0.3033619E+01 0.3609201E+01 0.1507497E+01  
1955 8 0.5836249E+01 0.2080700E+01 0.4263770E+00  
1956 9 0.2686593E+01 0.0000000E+00 0.0000000E+00  
1957 10 0.0000000E+00 0.0000000E+00 0.0000000E+00  
1958 11 -0.7670908E+00 0.5504753E+00 -0.1854086E+01  
1959 12 -0.7163309E+00 0.1331210E+01 0.1433034E+01  
1960 13 -0.8033584E+00 -0.2680877E+01 0.6642557E+00  
1961 14 -0.1703059E+00 -0.3970396E+01 -0.8436512E+00  
1962 15 -0.2867062E+01 -0.2790946E+01 0.7758157E+00  
1963 16 0.1597215E+00 -0.3416785E+01 0.3050490E+01  
1964 17 0.1951496E+01 -0.3017114E+01 0.2982980E+01  
1965  
1966 Reading esp"s for MEP 2  
1967 total number of atoms = 17  
1968 total number of esp points = 805  
1969  
1970 Center X Y Z  
1971 1 -0.5095967E+00 -0.1018099E+01 0.4366318E+01  
1972 2 -0.1220183E+01 -0.4744951E+00 0.5963597E+01  
1973 3 -0.1199859E+01 0.7770062E+00 0.2498507E+01  
1974 4 -0.5919019E+00 0.2698551E+01 0.3022352E+01  
1975 5 -0.3264575E+01 0.8429331E+00 0.2210686E+01  
1976 6 0.0000000E+00 0.0000000E+00 0.0000000E+00  
1977 7 -0.7295514E+00 0.1241726E+01 -0.1507497E+01

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1978 8 -0.5575826E+00 -0.1945981E+01 -0.4263770E+00  
1979 9 0.2677041E+01 0.0000000E+00 0.0000000E+00  
1980 10 0.3834464E+01 0.2424490E+01 0.0000000E+00  
1981 11 0.3668166E+01 0.3353897E+01 0.1854086E+01  
1982 12 0.2941733E+01 0.3644440E+01 -0.1433034E+01  
1983 13 0.6599893E+01 0.1994513E+01 -0.6642557E+00  
1984 14 0.7490881E+01 0.8676790E+00 0.8436512E+00  
1985 15 0.7588297E+01 0.3809465E+01 -0.7758157E+00  
1986 16 0.6849098E+01 0.8083511E+00 -0.3050490E+01  
1987 17 0.5716497E+01 -0.6364351E+00 -0.2982980E+01  
1988  
1989 Reading esp"s for MEP 3  
1990 total number of atoms = 17  
1991 total number of esp points = 803  
1992  
1993 Center X Y Z  
1994 1 0.5313660E+01 0.1803796E+01 -0.4290976E+01  
1995 2 0.4633126E+01 0.1401969E+00 -0.3909044E+01  
1996 3 0.4010528E+01 0.3496456E+01 -0.2678163E+01  
1997 4 0.2098730E+01 0.3912011E+01 -0.3399611E+01  
1998 5 0.5067053E+01 0.5277245E+01 -0.2670132E+01  
1999 6 0.3854349E+01 0.2440630E+01 0.0000000E+00  
2000 7 0.2834370E+01 0.3733664E+01 0.1270123E+01  
2001 8 0.5765082E+01 0.2149541E+01 0.7380873E+00  
2002 9 0.2698842E+01 0.0000000E+00 0.0000000E+00  
2003 10 0.0000000E+00 0.0000000E+00 0.0000000E+00  
2004 11 -0.7671758E+00 0.6775083E+00 -0.1810611E+01  
2005 12 -0.6983785E+00 0.1238266E+01 0.1517429E+01  
2006 13 -0.8077804E+00 -0.2714064E+01 0.4967315E+00  
2007 14 -0.1680666E+00 -0.3915510E+01 -0.1081099E+01  
2008 15 -0.2872338E+01 -0.2824749E+01 0.5817862E+00  
2009 16 0.1282349E+00 -0.3593970E+01 0.2842611E+01  
2010 17 0.1923705E+01 -0.3208479E+01 0.2827321E+01  
2011  
2012 Reading esp"s for MEP 4  
2013 total number of atoms = 17  
2014 total number of esp points = 813  
2015  
2016 Center X Y Z  
2017 1 -0.4887020E-01 -0.1591463E+01 0.4290976E+01  
2018 2 0.1745933E+01 -0.1688255E+01 0.3909044E+01  
2019 3 -0.1021108E+01 0.3106426E+00 0.2678163E+01  
2020 4 -0.5786171E+00 0.2216384E+01 0.3399611E+01  
2021 5 -0.3082720E+01 0.1177488E+00 0.2670132E+01  
2022 6 0.0000000E+00 0.0000000E+00 0.0000000E+00

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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2023      7      -0.7322121E+00    0.1475181E+01   -0.1270123E+01  
2024      8      -0.5545288E+00    -0.1851521E+01   -0.7380873E+00  
2025      9      0.2700347E+01    0.0000000E+00    0.0000000E+00  
2026      10     0.3855209E+01    0.2439271E+01    0.0000000E+00  
2027      11     0.3571147E+01    0.3422573E+01    0.1810611E+01  
2028      12     0.3034883E+01    0.3600347E+01    -0.1517429E+01  
2029      13     0.6653895E+01    0.2007985E+01    -0.4967315E+00  
2030      14     0.7466047E+01    0.9156856E+00    0.1081099E+01  
2031      15     0.7637380E+01    0.3826612E+01    -0.5817862E+00  
2032      16     0.7048642E+01    0.7854722E+00    -0.2842611E+01  
2033      17     0.5931926E+01    -0.6723569E+00   -0.2827321E+01  
2034  
2035     Reading esp"s for MEP 5  
2036     total number of atoms = 17  
2037     total number of esp points = 803  
2038  
2039     Center    X               Y               Z  
2040        1    0.5313484E+01    0.1803862E+01   -0.4291056E+01  
2041        2    0.4632977E+01    0.1402592E+00   -0.3909108E+01  
2042        3    0.4010431E+01    0.3496507E+01   -0.2678139E+01  
2043        4    0.2098624E+01    0.3912114E+01   -0.3399521E+01  
2044        5    0.5066992E+01    0.5277272E+01   -0.2670083E+01  
2045        6    0.3854336E+01    0.2440615E+01   0.0000000E+00  
2046        7    0.2834410E+01    0.3733636E+01   0.1270185E+01  
2047        8    0.5765091E+01    0.2149509E+01   0.7380136E+00  
2048        9    0.2698831E+01    0.0000000E+00   0.0000000E+00  
2049        10   0.0000000E+00    0.0000000E+00   0.0000000E+00  
2050        11   -0.7671891E+00    0.6776179E+00   -0.1810567E+01  
2051        12   -0.6983917E+00    0.1238171E+01   0.1517501E+01  
2052        13   -0.8077936E+00    -0.2714089E+01   0.4965690E+00  
2053        14   -0.1680855E+00    -0.3915450E+01   -0.1081330E+01  
2054        15   -0.2872351E+01    -0.2824762E+01   0.5816218E+00  
2055        16   0.1282141E+00    -0.3594138E+01   0.2842405E+01  
2056        17   0.1923688E+01    -0.3208660E+01   0.2827144E+01  
2057  
2058     Reading esp"s for MEP 6  
2059     total number of atoms = 17  
2060     total number of esp points = 813  
2061  
2062     Center    X               Y               Z  
2063        1    -0.4887776E-01   -0.1591282E+01   0.4291056E+01  
2064        2    0.1745920E+01    -0.1688102E+01   0.3909108E+01  
2065        3    -0.1021130E+01    0.3107465E+00   0.2678139E+01  
2066        4    -0.5786813E+00    0.2216520E+01   0.3399521E+01  
2067        5    -0.3082738E+01    0.1178150E+00   0.2670083E+01

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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```
2068      6      0.0000000E+00  0.0000000E+00  0.0000000E+00
2069      7     -0.7322197E+00  0.1475128E+01 -0.1270185E+01
2070      8     -0.5545269E+00 -0.1851550E+01 -0.7380136E+00
2071      9      0.2700331E+01  0.0000000E+00  0.0000000E+00
2072     10      0.3855194E+01  0.2439258E+01  0.0000000E+00
2073     11      0.3571040E+01  0.3422621E+01  0.1810567E+01
2074     12      0.3034960E+01  0.3600306E+01 -0.1517501E+01
2075     13      0.6653909E+01  0.2007966E+01 -0.4965690E+00
2076     14      0.7465984E+01  0.9157083E+00  0.1081330E+01
2077     15      0.7637386E+01  0.3826597E+01 -0.5816218E+00
2078     16      0.7048784E+01  0.7854004E+00 -0.2842405E+01
2079     17      0.5932077E+01 -0.6724363E+00 -0.2827144E+01
2080
2081 Reading esp"s for MEP 7
2082 total number of atoms = 17
2083 total number of esp points = 803
2084
2085 Center      X          Y          Z
2086    1  0.5313641E+01  0.1803776E+01 -0.4290986E+01
2087    2  0.4633130E+01  0.1401799E+00 -0.3908998E+01
2088    3  0.4010535E+01  0.3496454E+01 -0.2678167E+01
2089    4  0.2098745E+01  0.3912030E+01 -0.3399617E+01
2090    5  0.5067081E+01  0.5277230E+01 -0.2670128E+01
2091    6  0.3854347E+01  0.2440628E+01  0.0000000E+00
2092    7  0.2834375E+01  0.3733668E+01  0.1270117E+01
2093    8  0.5765080E+01  0.2149539E+01  0.7380854E+00
2094    9  0.2698846E+01  0.0000000E+00  0.0000000E+00
2095   10  0.0000000E+00  0.0000000E+00  0.0000000E+00
2096   11 -0.7671758E+00  0.6775915E+00 -0.1810577E+01
2097   12 -0.6983747E+00  0.1238192E+01  0.1517488E+01
2098   13 -0.8077861E+00 -0.2714085E+01  0.4966068E+00
2099   14 -0.1680836E+00 -0.3915461E+01 -0.1081284E+01
2100   15 -0.2872344E+01 -0.2824759E+01  0.5816633E+00
2101   16  0.1282406E+00 -0.3594108E+01  0.2842437E+01
2102   17  0.1923701E+01 -0.3208579E+01  0.2827181E+01
2103
2104 Reading esp"s for MEP 8
2105 total number of atoms = 17
2106 total number of esp points = 813
2107
2108 Center      X          Y          Z
2109    1 -0.4884375E-01 -0.1591457E+01  0.4290986E+01
2110    2  0.1745948E+01 -0.1688264E+01  0.3908998E+01
2111    3 -0.1021113E+01  0.3106313E+00  0.2678167E+01
2112    4 -0.5786454E+00  0.2216378E+01  0.3399617E+01
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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```
2113      5   -0.3082723E+01   0.1177110E+00   0.2670128E+01
2114      6    0.0000000E+00   0.0000000E+00   0.0000000E+00
2115      7   -0.7322235E+00   0.1475175E+01  -0.1270117E+01
2116      8   -0.5545288E+00  -0.1851521E+01  -0.7380854E+00
2117      9    0.2700341E+01   0.0000000E+00   0.0000000E+00
2118     10    0.3855202E+01   0.2439275E+01   0.0000000E+00
2119     11    0.3571061E+01   0.3422613E+01   0.1810577E+01
2120     12    0.3034938E+01   0.3600317E+01  -0.1517488E+01
2121     13    0.6653910E+01   0.2007989E+01  -0.4966068E+00
2122     14    0.7466007E+01   0.9157329E+00   0.1081284E+01
2123     15    0.7637382E+01   0.3826623E+01  -0.5816633E+00
2124     16    0.7048759E+01   0.7854193E+00  -0.2842437E+01
2125     17    0.5932016E+01  -0.6723872E+00  -0.2827181E+01
2126
2127 Reading esp"s for MEP 9
2128 total number of atoms = 17
2129 total number of esp points = 803
2130
2131 Center      X          Y          Z
2132     1  0.5313681E+01  0.1803785E+01  -0.4290971E+01
2133     2  0.4633147E+01  0.1401874E+00  -0.3909038E+01
2134     3  0.4010543E+01  0.3496449E+01  -0.2678163E+01
2135     4  0.2098749E+01  0.3912003E+01  -0.3399619E+01
2136     5  0.5067068E+01  0.5277236E+01  -0.2670126E+01
2137     6  0.3854346E+01  0.2440628E+01   0.0000000E+00
2138     7  0.2834362E+01  0.3733668E+01   0.1270111E+01
2139     8  0.5765072E+01  0.2149541E+01   0.7381024E+00
2140     9  0.2698841E+01  0.0000000E+00   0.0000000E+00
2141    10  0.0000000E+00  0.0000000E+00   0.0000000E+00
2142    11  -0.7671796E+00  0.6774857E+00  -0.1810616E+01
2143    12  -0.6983860E+00  0.1238279E+01   0.1517414E+01
2144    13  -0.8077861E+00  -0.2714059E+01   0.4967598E+00
2145    14  -0.1680703E+00  -0.3915526E+01  -0.1081055E+01
2146    15  -0.2872344E+01  -0.2824734E+01   0.5818126E+00
2147    16  0.1282274E+00  -0.3593938E+01   0.2842651E+01
2148    17  0.1923699E+01  -0.3208452E+01   0.2827359E+01
2149
2150 Reading esp"s for MEP 10
2151 total number of atoms = 17
2152 total number of esp points = 813
2153
2154 Center      X          Y          Z
2155     1  -0.4887209E-01  -0.1591489E+01   0.4290971E+01
2156     2   0.1745931E+01  -0.1688281E+01   0.3909038E+01
2157     3  -0.1021111E+01   0.3106218E+00   0.2678163E+01
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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```
2158      4      -0.5786209E+00   0.2216361E+01   0.3399619E+01
2159      5      -0.3082723E+01   0.1177299E+00   0.2670126E+01
2160      6      0.0000000E+00   0.0000000E+00   0.0000000E+00
2161      7      -0.7322159E+00   0.1475188E+01   -0.1270111E+01
2162      8      -0.5545269E+00   -0.1851514E+01   -0.7381024E+00
2163      9      0.2700345E+01   0.0000000E+00   0.0000000E+00
2164     10      0.3855205E+01   0.2439268E+01   0.0000000E+00
2165     11      0.3571165E+01   0.3422566E+01   0.1810616E+01
2166     12      0.3034870E+01   0.3600357E+01   -0.1517414E+01
2167     13      0.6653890E+01   0.2007989E+01   -0.4967598E+00
2168     14      0.7466062E+01   0.9156800E+00   0.1081055E+01
2169     15      0.7637367E+01   0.3826621E+01   -0.5818126E+00
2170     16      0.7048614E+01   0.7854911E+00   -0.2842651E+01
2171     17      0.5931903E+01   -0.6723418E+00   -0.2827359E+01
2172
2173 Reading esp"s for MEP 11
2174 total number of atoms = 17
2175 total number of esp points = 810
2176
2177 Center      X          Y          Z
2178      1      0.5213693E+01   0.1800017E+01   -0.4306839E+01
2179      2      0.4580599E+01   0.1385831E+00   -0.3838846E+01
2180      3      0.3953456E+01   0.3511400E+01   -0.2677564E+01
2181      4      0.2038020E+01   0.3948235E+01   -0.3378240E+01
2182      5      0.5028559E+01   0.5281616E+01   -0.2681015E+01
2183      6      0.3816995E+01   0.2445183E+01   0.0000000E+00
2184      7      0.2799860E+01   0.3732642E+01   0.1279161E+01
2185      8      0.5733349E+01   0.2165333E+01   0.7283306E+00
2186      9      0.2684290E+01   0.0000000E+00   0.0000000E+00
2187     10      0.0000000E+00   0.0000000E+00   0.0000000E+00
2188     11      -0.7626745E+00   0.6819190E+00   -0.1813307E+01
2189     12      -0.7198722E+00   0.1240242E+01   0.1506990E+01
2190     13      -0.9314081E+00   -0.2670854E+01   0.4558983E+00
2191     14      0.3880930E-01   -0.3951037E+01   -0.8665717E+00
2192     15      -0.2969837E+01   -0.2732765E+01   0.2377086E-01
2193     16      -0.4466235E+00   -0.3322922E+01   0.3011643E+01
2194     17      -0.7822010E+00   -0.5110904E+01   0.3213243E+01
2195
2196 Reading esp"s for MEP 12
2197 total number of atoms = 17
2198 total number of esp points = 817
2199
2200 Center      X          Y          Z
2201      1      -0.1668628E-02   -0.1538507E+01   0.4306839E+01
2202      2      0.1771975E+01   -0.1662405E+01   0.3838846E+01
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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```
2203      3   -0.1024814E+01   0.3243431E+00   0.2677564E+01
2204      4   -0.6160677E+00   0.2245970E+01   0.3378240E+01
2205      5   -0.3082956E+01   0.9290271E-01   0.2681015E+01
2206      6   0.0000000E+00   0.0000000E+00   0.0000000E+00
2207      7   -0.7406687E+00   0.1464078E+01   -0.1279161E+01
2208      8   -0.5515751E+00   -0.1856474E+01   -0.7283306E+00
2209      9   0.2694798E+01   0.0000000E+00   0.0000000E+00
2210     10   0.3823088E+01   0.2435647E+01   0.0000000E+00
2211     11   0.3524910E+01   0.3414308E+01   0.1813307E+01
2212     12   0.3000312E+01   0.3610149E+01   -0.1506990E+01
2213     13   0.6638040E+01   0.2158139E+01   -0.4558983E+00
2214     14   0.7391831E+01   0.7396917E+00   0.8665717E+00
2215     15   0.7551031E+01   0.3981726E+01   -0.2377086E-01
2216     16   0.7025941E+01   0.1444176E+01   -0.3011643E+01
2217     17   0.8789356E+01   0.9971253E+00   -0.3213243E+01
2218
2219 Reading esp"s for MEP 13
2220 total number of atoms = 17
2221 total number of esp points = 814
2222
2223 Center      X          Y          Z
2224    1  0.5368704E+01  0.1752536E+01 -0.4247125E+01
2225    2  0.4708965E+01  0.9276665E-01 -0.3813066E+01
2226    3  0.4033437E+01  0.3471111E+01 -0.2687899E+01
2227    4  0.2137439E+01  0.3874297E+01 -0.3457539E+01
2228    5  0.5088244E+01  0.5253185E+01 -0.2683530E+01
2229    6  0.3819728E+01  0.2448437E+01  0.0000000E+00
2230    7  0.2763919E+01  0.3751828E+01  0.1229864E+01
2231    8  0.5714745E+01  0.2180613E+01  0.7864227E+00
2232    9  0.2683721E+01  0.0000000E+00  0.0000000E+00
2233   10  0.0000000E+00  0.0000000E+00  0.0000000E+00
2234   11  -0.7664313E+00  0.8737224E+00 -0.1731220E+01
2235   12  -0.7338392E+00  0.1077582E+01  0.1620357E+01
2236   13  -0.8845675E+00 -0.2741533E+01  0.1807693E+00
2237   14  -0.2055965E+00 -0.3567750E+01  0.1949046E+01
2238   15  -0.6428848E-01 -0.3843788E+01 -0.1380626E+01
2239   16  -0.3562708E+01 -0.2909146E+01  0.2415467E+00
2240   17  -0.4191777E+01 -0.2471939E+01 -0.1422947E+01
2241
2242 Reading esp"s for MEP 14
2243 total number of atoms = 17
2244 total number of esp points = 825
2245
2246 Center      X          Y          Z
2247    1  -0.2066604E-01 -0.1697993E+01  0.4247125E+01
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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```
2248      2      0.1762610E+01 -0.1798091E+01  0.3813066E+01
2249      3     -0.1017631E+01  0.2365616E+00  0.2687899E+01
2250      4     -0.5853842E+00  0.2126148E+01  0.3457539E+01
2251      5     -0.3078126E+01  0.2976318E-01  0.2683530E+01
2252      6      0.0000000E+00  0.0000000E+00  0.0000000E+00
2253      7     -0.7379607E+00  0.1506312E+01 -0.1229864E+01
2254      8     -0.5546232E+00 -0.1831723E+01 -0.7864227E+00
2255      9      0.2699141E+01  0.0000000E+00  0.0000000E+00
2256     10      0.3828659E+01  0.2434449E+01  0.0000000E+00
2257     11      0.3358663E+01  0.3497422E+01  0.1731220E+01
2258     12      0.3160023E+01  0.3553658E+01 -0.1620357E+01
2259     13      0.6687846E+01  0.2083005E+01 -0.1807693E+00
2260     14      0.7151558E+01  0.1119362E+01 -0.1949046E+01
2261     15      0.7342483E+01  0.8750017E+00  0.1380626E+01
2262     16      0.7967060E+01  0.4441848E+01 -0.2415467E+00
2263     17      0.7835223E+01  0.5196499E+01  0.1422947E+01
2264
2265 Reading esp"s for MEP 15
2266 total number of atoms = 17
2267 total number of esp points = 811
2268
2269 Center      X          Y          Z
2270    1  0.5391275E+01  0.1760108E+01 -0.4235290E+01
2271    2  0.4713606E+01  0.1008585E+00 -0.3828165E+01
2272    3  0.4032129E+01  0.3472045E+01 -0.2688642E+01
2273    4  0.2138898E+01  0.3859136E+01 -0.3471468E+01
2274    5  0.5075598E+01  0.5260799E+01 -0.2681068E+01
2275    6  0.3809039E+01  0.2452828E+01  0.0000000E+00
2276    7  0.2741359E+01  0.3754474E+01  0.1221797E+01
2277    8  0.5701825E+01  0.2196976E+01  0.7962266E+00
2278    9  0.2685199E+01  0.0000000E+00  0.0000000E+00
2279   10  0.0000000E+00  0.0000000E+00  0.0000000E+00
2280   11 -0.7820915E+00  0.9058250E+00 -0.1699030E+01
2281   12 -0.7293265E+00  0.1023655E+01  0.1657121E+01
2282   13 -0.8630889E+00 -0.2740318E+01  0.9894416E-01
2283   14 -0.9331467E-01 -0.3634077E+01  0.1811703E+01
2284   15 -0.9511936E-01 -0.3757404E+01 -0.1546053E+01
2285   16 -0.3548703E+01 -0.2707424E+01  0.9553510E-01
2286   17 -0.4134275E+01 -0.4439316E+01  0.1729988E+00
2287
2288 Reading esp"s for MEP 16
2289 total number of atoms = 17
2290 total number of esp points = 824
2291
2292 Center      X          Y          Z
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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```
2293    1    -0.2930020E-01 -0.1726985E+01  0.4235290E+01
2294    2     0.1761429E+01 -0.1802047E+01  0.3828165E+01
2295    3    -0.1019513E+01  0.2217291E+00  0.2688642E+01
2296    4    -0.5828180E+00  0.2104138E+01  0.3471468E+01
2297    5    -0.3080348E+01  0.1818105E-01  0.2681068E+01
2298    6     0.0000000E+00  0.0000000E+00  0.0000000E+00
2299    7    -0.7386164E+00  0.1512833E+01 -0.1221797E+01
2300    8    -0.5558232E+00 -0.1827337E+01 -0.7962266E+00
2301    9     0.2698034E+01  0.0000000E+00  0.0000000E+00
2302   10     0.3816527E+01  0.2441161E+01  0.0000000E+00
2303   11     0.3318797E+01  0.3529487E+01  0.1699030E+01
2304   12     0.3189697E+01  0.3530600E+01 -0.1657121E+01
2305   13     0.6667309E+01  0.2084356E+01 -0.9894416E-01
2306   14     0.7159199E+01  0.1012256E+01 -0.1811703E+01
2307   15     0.7272068E+01  0.9625244E+00  0.1546053E+01
2308   16     0.7756070E+01  0.4539596E+01 -0.9553510E-01
2309   17     0.9574478E+01  0.4350546E+01 -0.1729988E+00
2310
2311 Reading esp"s for MEP 17
2312 total number of atoms = 17
2313 total number of esp points = 811
2314
2315 Center      X          Y          Z
2316    1  0.5391248E+01  0.1760140E+01 -0.4235301E+01
2317    2  0.4713564E+01  0.1008944E+00 -0.3828186E+01
2318    3  0.4032101E+01  0.3472069E+01 -0.2688638E+01
2319    4  0.2138871E+01  0.3859166E+01 -0.3471457E+01
2320    5  0.5075570E+01  0.5260825E+01 -0.2681062E+01
2321    6  0.3809032E+01  0.2452840E+01  0.0000000E+00
2322    7  0.2741348E+01  0.3754466E+01  0.1221810E+01
2323    8  0.5701819E+01  0.2196988E+01  0.7962096E+00
2324    9  0.2685199E+01  0.0000000E+00  0.0000000E+00
2325   10  0.0000000E+00  0.0000000E+00  0.0000000E+00
2326   11  -0.7820971E+00  0.9057759E+00 -0.1699054E+01
2327   12  -0.7293265E+00  0.1023706E+01  0.1657093E+01
2328   13  -0.8630851E+00 -0.2740316E+01  0.9900841E-01
2329   14  -0.9334302E-01 -0.3634034E+01  0.1811797E+01
2330   15  -0.9508534E-01 -0.3757433E+01 -0.1545956E+01
2331   16  -0.3548703E+01 -0.2707442E+01  0.9555400E-01
2332   17  -0.4134254E+01 -0.4439331E+01  0.1731991E+00
2333
2334 Reading esp"s for MEP 18
2335 total number of atoms = 17
2336 total number of esp points = 824
2337
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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```
2338 Center      X          Y          Z
2339     1 -0.2930398E-01 -0.1726956E+01 0.4235301E+01
2340     2  0.1761429E+01 -0.1801999E+01 0.3828186E+01
2341     3 -0.1019517E+01  0.2217499E+00 0.2688638E+01
2342     4 -0.5828350E+00  0.2104161E+01 0.3471457E+01
2343     5 -0.3080352E+01  0.1819428E-01 0.2681062E+01
2344     6  0.0000000E+00  0.0000000E+00 0.0000000E+00
2345     7 -0.7386032E+00  0.1512826E+01 -0.1221810E+01
2346     8 -0.5558157E+00 -0.1827341E+01 -0.7962096E+00
2347     9  0.2698041E+01  0.0000000E+00 0.0000000E+00
2348    10  0.3816525E+01  0.2441165E+01 0.0000000E+00
2349    11  0.3318837E+01  0.3529473E+01 0.1699054E+01
2350    12  0.3189644E+01  0.3530622E+01 -0.1657093E+01
2351    13  0.6667303E+01  0.2084372E+01 -0.9900841E-01
2352    14  0.7159174E+01  0.1012317E+01 -0.1811797E+01
2353    15  0.7272083E+01  0.9625017E+00 0.1545956E+01
2354    16  0.7756076E+01  0.4539609E+01 -0.9555400E-01
2355    17  0.9574473E+01  0.4350550E+01 -0.1731991E+00
2356
2357 Reading esp"s for MEP 19
2358 total number of atoms = 17
2359 total number of esp points = 814
2360
2361 Center      X          Y          Z
2362     1  0.5435935E+01  0.1755421E+01 -0.4221058E+01
2363     2  0.4749316E+01  0.9443528E-01 -0.3837622E+01
2364     3  0.4055717E+01  0.3462615E+01 -0.2689348E+01
2365     4  0.2167132E+01  0.3840159E+01 -0.3488351E+01
2366     5  0.5091305E+01  0.5255876E+01 -0.2677383E+01
2367     6  0.3814975E+01  0.2450627E+01 0.0000000E+00
2368     7  0.2743876E+01  0.3758162E+01 0.1213070E+01
2369     8  0.5702573E+01  0.2192243E+01 0.8074308E+00
2370     9  0.2683207E+01  0.0000000E+00 0.0000000E+00
2371    10  0.0000000E+00  0.0000000E+00 0.0000000E+00
2372    11 -0.7863490E+00  0.9644897E+00 -0.1664416E+01
2373    12 -0.7133716E+00  0.9908608E+00 0.1692529E+01
2374    13 -0.8857032E+00 -0.2746981E+01 0.1661447E-01
2375    14 -0.4428384E-01 -0.3737398E+01 0.1639075E+01
2376    15 -0.2306335E+00 -0.3695480E+01 -0.1699648E+01
2377    16 -0.3564569E+01 -0.2917342E+01 -0.3140725E-02
2378    17 -0.4173494E+01 -0.2375879E+01 0.1637933E+01
2379
2380 Reading esp"s for MEP 20
2381 total number of atoms = 17
2382 total number of esp points = 821
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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2383  
2384   Center   X                   Y                   Z  
2385       1   -0.4847903E-01 -0.1763086E+01 0.4221058E+01  
2386       2   0.1747345E+01 -0.1836143E+01 0.3837622E+01  
2387       3   -0.1019681E+01 0.2057401E+00 0.2689348E+01  
2388       4   -0.5706009E+00 0.2078604E+01 0.3488351E+01  
2389       5   -0.3081905E+01 0.1744028E-01 0.2677383E+01  
2390       6   0.0000000E+00 0.0000000E+00 0.0000000E+00  
2391       7   -0.7379739E+00 0.1520623E+01 -0.1213070E+01  
2392       8   -0.5568456E+00 -0.1822010E+01 -0.8074308E+00  
2393       9   0.2699347E+01 0.0000000E+00 0.0000000E+00  
2394      10   0.3824346E+01 0.2435976E+01 0.0000000E+00  
2395      11   0.3278421E+01 0.3554255E+01 0.1664416E+01  
2396      12   0.3223882E+01 0.3499058E+01 -0.1692529E+01  
2397      13   0.6689573E+01 0.2088331E+01 -0.1661447E-01  
2398      14   0.7235946E+01 0.9091831E+00 -0.1639075E+01  
2399      15   0.7276023E+01 0.1095937E+01 0.1699648E+01  
2400      16   0.7967416E+01 0.4448937E+01 0.3140725E-02  
2401      17   0.7731149E+01 0.5228777E+01 -0.1637933E+01  
2402  
2403    Reading esp"s for MEP 21  
2404    total number of atoms = 17  
2405    total number of esp points = 756  
2406  
2407    Center   X                   Y                   Z  
2408       1   0.4363459E+01 0.2381799E+01 -0.4597811E+01  
2409       2   0.3217119E+01 0.9348871E+00 -0.4665097E+01  
2410       3   0.3596697E+01 0.3826106E+01 -0.2497702E+01  
2411       4   0.1627139E+01 0.4494158E+01 -0.2703790E+01  
2412       5   0.4787770E+01 0.5521375E+01 -0.2437121E+01  
2413       6   0.3897915E+01 0.2398873E+01 0.0000000E+00  
2414       7   0.3238015E+01 0.3576208E+01 0.1588919E+01  
2415       8   0.5896913E+01 0.1964329E+01 0.2996765E+00  
2416       9   0.2676963E+01 0.0000000E+00 0.0000000E+00  
2417      10   0.0000000E+00 0.0000000E+00 0.0000000E+00  
2418      11   -0.7703071E+00 0.1927447E+01 -0.1283275E+00  
2419      12   -0.6936579E+00 -0.8221234E+00 0.1782293E+01  
2420      13   -0.9503734E+00 -0.1595479E+01 -0.2204277E+01  
2421      14   -0.3012212E+01 -0.1834395E+01 -0.2074919E+01  
2422      15   -0.7977856E-01 -0.3466751E+01 -0.2105680E+01  
2423      16   -0.2407738E+00 -0.6022840E+00 -0.4613777E+01  
2424      17   -0.1236741E+01 0.9027788E+00 -0.4937739E+01  
2425  
2426    Reading esp"s for MEP 22  
2427    total number of atoms = 17

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

2428 total number of esp points = 762  
2429  
2430 Center X Y Z  
2431 1 -0.1959514E+00 -0.4226410E+00 0.4597811E+01  
2432 2 0.1613524E+01 -0.5732862E-01 0.4665097E+01  
2433 3 -0.1135328E+01 0.9158349E+00 0.2497702E+01  
2434 4 -0.8373168E+00 0.2974145E+01 0.2703790E+01  
2435 5 -0.3186430E+01 0.6233110E+00 0.2437121E+01  
2436 6 0.0000000E+00 0.0000000E+00 0.0000000E+00  
2437 7 -0.7499208E+00 0.1122142E+01 -0.1588919E+01  
2438 8 -0.5194687E+00 -0.1978630E+01 -0.2996765E+00  
2439 9 0.2691712E+01 0.0000000E+00 0.0000000E+00  
2440 10 0.3905975E+01 0.2385728E+01 0.0000000E+00  
2441 11 0.2537628E+01 0.3946515E+01 0.1283275E+00  
2442 12 0.4953299E+01 0.2631009E+01 -0.1782293E+01  
2443 13 0.5758963E+01 0.2509004E+01 0.2204277E+01  
2444 14 0.6907128E+01 0.4238160E+01 0.2074919E+01  
2445 15 0.7031755E+01 0.8843218E+00 0.2105680E+01  
2446 16 0.4551949E+01 0.2327114E+01 0.4613777E+01  
2447 17 0.3662393E+01 0.3897416E+01 0.4937739E+01  
2448  
2449 Reading esp"s for MEP 23  
2450 total number of atoms = 17  
2451 total number of esp points = 756  
2452  
2453 Center X Y Z  
2454 1 0.4363392E+01 0.2381892E+01 -0.4597811E+01  
2455 2 0.3217122E+01 0.9349211E+00 -0.4665070E+01  
2456 3 0.3596632E+01 0.3826155E+01 -0.2497658E+01  
2457 4 0.1627067E+01 0.4494184E+01 -0.2703710E+01  
2458 5 0.4787689E+01 0.5521430E+01 -0.2437068E+01  
2459 6 0.3897909E+01 0.2398881E+01 0.0000000E+00  
2460 7 0.3238064E+01 0.3576172E+01 0.1588974E+01  
2461 8 0.5896922E+01 0.1964336E+01 0.2996047E+00  
2462 9 0.2676967E+01 0.0000000E+00 0.0000000E+00  
2463 10 0.0000000E+00 0.0000000E+00 0.0000000E+00  
2464 11 -0.7703147E+00 0.1927441E+01 -0.1283389E+00  
2465 12 -0.6936598E+00 -0.8221177E+00 0.1782295E+01  
2466 13 -0.9503715E+00 -0.1595496E+01 -0.2204273E+01  
2467 14 -0.3012216E+01 -0.1834395E+01 -0.2074930E+01  
2468 15 -0.7978990E-01 -0.3466770E+01 -0.2105654E+01  
2469 16 -0.2407416E+00 -0.6023256E+00 -0.4613777E+01  
2470 17 -0.1236682E+01 0.9027504E+00 -0.4937750E+01  
2471  
2472 Reading esp"s for MEP 24

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

2473 total number of atoms = 17  
2474 total number of esp points = 762  
2475  
2476 Center X Y Z  
2477 1 -0.1959986E+00 -0.4225484E+00 0.4597811E+01  
2478 2 0.1613495E+01 -0.5731728E-01 0.4665070E+01  
2479 3 -0.1135342E+01 0.9159029E+00 0.2497658E+01  
2480 4 -0.8373149E+00 0.2974210E+01 0.2703710E+01  
2481 5 -0.3186441E+01 0.6233885E+00 0.2437068E+01  
2482 6 0.0000000E+00 0.0000000E+00 0.0000000E+00  
2483 7 -0.7499113E+00 0.1122072E+01 -0.1588974E+01  
2484 8 -0.5194687E+00 -0.1978643E+01 -0.2996047E+00  
2485 9 0.2691716E+01 0.0000000E+00 0.0000000E+00  
2486 10 0.3905969E+01 0.2385736E+01 0.0000000E+00  
2487 11 0.2537626E+01 0.3946521E+01 0.1283389E+00  
2488 12 0.4953287E+01 0.2631024E+01 -0.1782295E+01  
2489 13 0.5758970E+01 0.2509010E+01 0.2204273E+01  
2490 14 0.6907119E+01 0.4238181E+01 0.2074930E+01  
2491 15 0.7031778E+01 0.8843426E+00 0.2105654E+01  
2492 16 0.4551966E+01 0.2327077E+01 0.4613777E+01  
2493 17 0.3662382E+01 0.3897359E+01 0.4937750E+01  
2494  
2495 Reading esp"s for MEP 25  
2496 total number of atoms = 17  
2497 total number of esp points = 756  
2498  
2499 Center X Y Z  
2500 1 0.4363421E+01 0.2381892E+01 -0.4597813E+01  
2501 2 0.3217121E+01 0.9349457E+00 -0.4665104E+01  
2502 3 0.3596647E+01 0.3826153E+01 -0.2497658E+01  
2503 4 0.1627075E+01 0.4494161E+01 -0.2703714E+01  
2504 5 0.4787689E+01 0.5521435E+01 -0.2437072E+01  
2505 6 0.3897919E+01 0.2398879E+01 0.0000000E+00  
2506 7 0.3238064E+01 0.3576174E+01 0.1588965E+01  
2507 8 0.5896928E+01 0.1964334E+01 0.2996161E+00  
2508 9 0.2676965E+01 0.0000000E+00 0.0000000E+00  
2509 10 0.0000000E+00 0.0000000E+00 0.0000000E+00  
2510 11 -0.7703147E+00 0.1927445E+01 -0.1282973E+00  
2511 12 -0.6936579E+00 -0.8221517E+00 0.1782280E+01  
2512 13 -0.9503772E+00 -0.1595460E+01 -0.2204299E+01  
2513 14 -0.3012216E+01 -0.1834368E+01 -0.2074951E+01  
2514 15 -0.7977856E-01 -0.3466729E+01 -0.2105712E+01  
2515 16 -0.2407719E+00 -0.6022443E+00 -0.4613794E+01  
2516 17 -0.1236695E+01 0.9028525E+00 -0.4937722E+01  
2517

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
2518  Reading esp"s for MEP 26
2519  total number of atoms = 17
2520  total number of esp points = 762
2521
2522  Center      X          Y          Z
2523    1 -0.1960118E+00 -0.4225635E+00 0.4597813E+01
2524    2  0.1613476E+01 -0.5730216E-01 0.4665104E+01
2525    3 -0.1135340E+01  0.9159029E+00 0.2497658E+01
2526    4 -0.8372847E+00  0.2974206E+01 0.2703714E+01
2527    5 -0.3186441E+01  0.6234112E+00 0.2437072E+01
2528    6  0.0000000E+00  0.0000000E+00 0.0000000E+00
2529    7 -0.7499056E+00  0.1122083E+01 -0.1588965E+01
2530    8 -0.5194743E+00 -0.1978638E+01 -0.2996161E+00
2531    9  0.2691718E+01  0.0000000E+00 0.0000000E+00
2532   10  0.3905981E+01  0.2385730E+01 0.0000000E+00
2533   11  0.2537639E+01  0.3946523E+01 0.1282973E+00
2534   12  0.4953329E+01  0.2630997E+01 -0.1782280E+01
2535   13  0.5758953E+01  0.2509018E+01 0.2204299E+01
2536   14  0.6907113E+01  0.4238175E+01 0.2074951E+01
2537   15  0.7031742E+01  0.8843351E+00 0.2105712E+01
2538   16  0.4551917E+01  0.2327133E+01 0.4613794E+01
2539   17  0.3662314E+01  0.3897414E+01 0.4937722E+01
2540
2541  Reading esp"s for MEP 27
2542  total number of atoms = 17
2543  total number of esp points = 756
2544
2545  Center      X          Y          Z
2546    1  0.4363402E+01  0.2381860E+01 -0.4597811E+01
2547    2  0.3217151E+01  0.9348701E+00 -0.4665046E+01
2548    3  0.3596630E+01  0.3826136E+01 -0.2497675E+01
2549    4  0.1627058E+01  0.4494143E+01 -0.2703729E+01
2550    5  0.4787672E+01  0.5521420E+01 -0.2437104E+01
2551    6  0.3897909E+01  0.2398882E+01 0.0000000E+00
2552    7  0.3238044E+01  0.3576180E+01 0.1588957E+01
2553    8  0.5896918E+01  0.1964346E+01 0.2996217E+00
2554    9  0.2676965E+01  0.0000000E+00 0.0000000E+00
2555   10  0.0000000E+00  0.0000000E+00 0.0000000E+00
2556   11 -0.7703147E+00  0.1927441E+01 -0.1283880E+00
2557   12 -0.6936636E+00 -0.8220743E+00 0.1782314E+01
2558   13 -0.9503602E+00 -0.1595543E+01 -0.2204241E+01
2559   14 -0.3012206E+01 -0.1834433E+01 -0.2074912E+01
2560   15 -0.7978423E-01 -0.3466820E+01 -0.2105584E+01
2561   16 -0.2407076E+00 -0.6024182E+00 -0.4613760E+01
2562   17 -0.1236665E+01  0.9026371E+00 -0.4937782E+01
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

2563  
2564   Reading esp"s for MEP 28  
2565   total number of atoms = 17  
2566   total number of esp points = 762  
2567  
2568   Center   X                   Y                   Z  
2569    1   -0.1959721E+00 -0.4225730E+00 0.4597811E+01  
2570    2   0.1613527E+01 -0.5736830E-01 0.4665046E+01  
2571    3   -0.1135323E+01 0.9158935E+00 0.2497675E+01  
2572    4   -0.8372715E+00 0.2974196E+01 0.2703729E+01  
2573    5   -0.3186422E+01 0.6233960E+00 0.2437104E+01  
2574    6   0.0000000E+00 0.0000000E+00 0.0000000E+00  
2575    7   -0.7499075E+00 0.1122091E+01 -0.1588957E+01  
2576    8   -0.5194724E+00 -0.1978638E+01 -0.2996217E+00  
2577    9   0.2691718E+01 0.0000000E+00 0.0000000E+00  
2578   10   0.3905971E+01 0.2385736E+01 0.0000000E+00  
2579   11   0.2537628E+01 0.3946519E+01 0.1283880E+00  
2580   12   0.4953252E+01 0.2631048E+01 -0.1782314E+01  
2581   13   0.5759010E+01 0.2508978E+01 0.2204241E+01  
2582   14   0.6907149E+01 0.4238153E+01 0.2074912E+01  
2583   15   0.7031820E+01 0.8843143E+00 0.2105584E+01  
2584   16   0.4552034E+01 0.2327003E+01 0.4613760E+01  
2585   17   0.3662474E+01 0.3897292E+01 0.4937782E+01  
2586  
2587   Reading esp"s for MEP 29  
2588   total number of atoms = 17  
2589   total number of esp points = 840  
2590  
2591   Center   X                   Y                   Z  
2592    1   0.7218638E+01 0.7890230E+00 0.2501100E+01  
2593    2   0.6168396E+01 -0.7174137E+00 0.2497503E+01  
2594    3   0.6598109E+01 0.2089525E+01 0.2451636E+00  
2595    4   0.7300837E+01 0.1079326E+01 -0.1434884E+01  
2596    5   0.7539066E+01 0.3930908E+01 0.3254429E+00  
2597    6   0.3756970E+01 0.2453446E+01 0.0000000E+00  
2598    7   0.3284125E+01 0.3474250E+01 -0.1755125E+01  
2599    8   0.3039316E+01 0.3559607E+01 0.1612254E+01  
2600    9   0.2668463E+01 0.0000000E+00 0.0000000E+00  
2601   10   0.0000000E+00 0.0000000E+00 0.0000000E+00  
2602   11   -0.7233852E+00 0.9762684E+00 -0.1700736E+01  
2603   12   -0.7403002E+00 0.1032308E+01 0.1655750E+01  
2604   13   -0.9643612E+00 -0.2709848E+01 0.6500657E-01  
2605   14   -0.3030523E+01 -0.2658631E+01 0.3410029E+00  
2606   15   -0.1391462E+00 -0.3685653E+01 0.1691305E+01  
2607   16   -0.2802123E+00 -0.4162908E+01 -0.2077294E+01

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

2608      17      -0.9913937E+00 -0.3350934E+01 -0.3558923E+01  
2609  
2610   Reading esp"s **for** MEP 30  
2611   total number of atoms = 17  
2612   total number of esp points = 835  
2613  
2614   Center   X               Y               Z  
2615    1    0.1175561E+00 -0.3839222E+01 -0.2501100E+01  
2616    2    0.1920472E+01 -0.3490146E+01 -0.2497503E+01  
2617    3    -0.8195496E+00 -0.2744604E+01 -0.2451636E+00  
2618    4    -0.1811378E+00 -0.3796630E+01  0.1434884E+01  
2619    5    -0.2884313E+01 -0.2857950E+01 -0.3254429E+00  
2620    6    0.0000000E+00  0.0000000E+00  0.0000000E+00  
2621    7    -0.7413319E+00  0.8461947E+00  0.1755125E+01  
2622    8    -0.7200763E+00  0.1104586E+01 -0.1612254E+01  
2623    9    0.2684072E+01  0.0000000E+00  0.0000000E+00  
2624   10   0.3766249E+01  0.2439179E+01  0.0000000E+00  
2625   11   0.3167228E+01  0.3496328E+01  0.1700736E+01  
2626   12   0.3122863E+01  0.3534513E+01 -0.1655750E+01  
2627   13   0.6634346E+01  0.2221721E+01 -0.6500657E-01  
2628   14   0.7425446E+01  0.4131120E+01 -0.3410029E+00  
2629   15   0.7191645E+01  0.1071681E+01 -0.1691305E+01  
2630   16   0.7685100E+01  0.1007078E+01  0.2077294E+01  
2631   17   0.7231309E+01  0.1986442E+01  0.3558923E+01  
2632  
2633   Reading esp"s **for** MEP 31  
2634   total number of atoms = 17  
2635   total number of esp points = 838  
2636  
2637   Center   X               Y               Z  
2638    1    0.7181499E+01  0.8565334E+00  0.2647765E+01  
2639    2    0.6101241E+01 -0.6273493E+00  0.2689758E+01  
2640    3    0.6625364E+01  0.2083272E+01  0.3343360E+00  
2641    4    0.7370646E+01  0.1017729E+01 -0.1293684E+01  
2642    5    0.7572716E+01  0.3922223E+01  0.3781512E+00  
2643    6    0.3795462E+01  0.2452014E+01  0.0000000E+00  
2644    7    0.3369897E+01  0.3448561E+01 -0.1778557E+01  
2645    8    0.3036931E+01  0.3572468E+01  0.1580225E+01  
2646    9    0.2689777E+01  0.0000000E+00  0.0000000E+00  
2647   10   0.0000000E+00  0.0000000E+00  0.0000000E+00  
2648   11   -0.7095959E+00  0.1152153E+01 -0.1580164E+01  
2649   12   -0.7335255E+00  0.7975022E+00  0.1778595E+01  
2650   13   -0.8271387E+00 -0.2731310E+01 -0.3344947E+00  
2651   14   -0.2892961E+01 -0.2838986E+01 -0.3783099E+00  
2652   15   -0.1621366E+00 -0.3848816E+01  0.1293455E+01

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
2653      16      0.6254804E-01 -0.3742425E+01 -0.2647986E+01
2654      17      0.1859322E+01 -0.3367611E+01 -0.2689964E+01
2655
2656 Reading esp"s for MEP 32
2657 total number of atoms = 17
2658 total number of esp points = 838
2659
2660 Center     X           Y           Z
2661      1  0.6255182E-01 -0.3742578E+01 -0.2647765E+01
2662      2  0.1859326E+01 -0.3367788E+01 -0.2689758E+01
2663      3 -0.8271387E+00 -0.2731329E+01 -0.3343360E+00
2664      4 -0.1621461E+00 -0.3848741E+01  0.1293684E+01
2665      5 -0.2892959E+01 -0.2839003E+01 -0.3781512E+00
2666      6  0.0000000E+00  0.0000000E+00  0.0000000E+00
2667      7 -0.7335198E+00  0.7975967E+00  0.1778557E+01
2668      8 -0.7096016E+00  0.1152064E+01 -0.1580225E+01
2669      9  0.2689779E+01  0.0000000E+00  0.0000000E+00
2670     10  0.3795464E+01  0.2452014E+01  0.0000000E+00
2671     11  0.3036848E+01  0.3572499E+01  0.1580164E+01
2672     12  0.3369986E+01  0.3448527E+01 -0.1778595E+01
2673     13  0.6625347E+01  0.2083279E+01  0.3344947E+00
2674     14  0.7572701E+01  0.3922230E+01  0.3783099E+00
2675     15  0.7370710E+01  0.1017689E+01 -0.1293455E+01
2676     16  0.7181361E+01  0.8565996E+00  0.2647986E+01
2677     17  0.6101082E+01 -0.6272719E+00  0.2689964E+01
2678
2679 Reading esp"s for MEP 33
2680 total number of atoms = 17
2681 total number of esp points = 827
2682
2683 Center     X           Y           Z
2684      1  0.7407913E+01  0.9460138E+00  0.2367834E+01
2685      2  0.6446606E+01 -0.6127153E+00  0.2462479E+01
2686      3  0.6620644E+01  0.2155376E+01  0.1110573E+00
2687      4  0.7258851E+01  0.1113553E+01 -0.1575387E+01
2688      5  0.7519755E+01  0.4018693E+01  0.8754156E-01
2689      6  0.3766579E+01  0.2462880E+01  0.0000000E+00
2690      7  0.3208311E+01  0.3497630E+01 -0.1720185E+01
2691      8  0.3100995E+01  0.3536429E+01  0.1653865E+01
2692      9  0.2688481E+01  0.0000000E+00  0.0000000E+00
2693     10  0.0000000E+00  0.0000000E+00  0.0000000E+00
2694     11 -0.7240598E+00  0.9262794E+00 -0.1720223E+01
2695     12 -0.7165765E+00  0.1040288E+01  0.1653816E+01
2696     13 -0.8628205E+00 -0.2737845E+01  0.1111726E+00
2697     14 -0.1643022E+00 -0.3740363E+01 -0.1575190E+01
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
2698      15    -0.2930305E+01 -0.2814297E+01  0.8760581E-01
2699      16    -0.7073244E-01 -0.3943894E+01  0.2368048E+01
2700      17     0.1742700E+01 -0.3688518E+01  0.2462625E+01
2701
2702  Reading esp"s for MEP 34
2703  total number of atoms = 17
2704  total number of esp points = 827
2705
2706  Center   X           Y           Z
2707      1    -0.7061528E-01 -0.3944009E+01 -0.2367834E+01
2708      2     0.1742787E+01 -0.3688431E+01 -0.2462479E+01
2709      3    -0.8627884E+00 -0.2737854E+01 -0.1110573E+00
2710      4    -0.1643192E+00 -0.3740274E+01  0.1575387E+01
2711      5    -0.2930275E+01 -0.2814314E+01 -0.8754156E-01
2712      6     0.0000000E+00  0.0000000E+00  0.0000000E+00
2713      7    -0.7240447E+00  0.9263531E+00  0.1720185E+01
2714      8    -0.7165520E+00  0.1040222E+01 -0.1653865E+01
2715      9     0.2688508E+01  0.0000000E+00  0.0000000E+00
2716     10    0.3766594E+01  0.2462857E+01  0.0000000E+00
2717     11    0.3208400E+01  0.3497592E+01  0.1720223E+01
2718     12    0.3100959E+01  0.3536454E+01 -0.1653816E+01
2719     13    0.6620664E+01  0.2155387E+01 -0.1111726E+00
2720     14    0.7258942E+01  0.1113478E+01  0.1575190E+01
2721     15    0.7519768E+01  0.4018705E+01 -0.8760581E-01
2722     16    0.7407870E+01  0.9461423E+00 -0.2368048E+01
2723     17    0.6446736E+01 -0.6126945E+00 -0.2462625E+01
2724
2725  Reading esp"s for MEP 35
2726  total number of atoms = 17
2727  total number of esp points = 762
2728
2729  Center   X           Y           Z
2730      1    0.3191796E+01  0.3130546E+01  0.4488982E+01
2731      2    0.2383649E+01  0.1476269E+01  0.4645417E+01
2732      3    0.5069003E+01  0.2912782E+01  0.2599176E+01
2733      4    0.6446298E+01  0.1419007E+01  0.3056868E+01
2734      5    0.6094313E+01  0.4714042E+01  0.2543828E+01
2735      6    0.3943388E+01  0.2378299E+01  0.0000000E+00
2736      7    0.5445293E+01  0.2291565E+01 -0.1431082E+01
2737      8    0.2636510E+01  0.3903361E+01 -0.5275189E+00
2738      9    0.2674527E+01  0.0000000E+00  0.0000000E+00
2739     10     0.0000000E+00  0.0000000E+00  0.0000000E+00
2740     11    -0.6995237E+00 -0.7193110E+00 -0.1826080E+01
2741     12    -0.7514854E+00  0.1924032E+01  0.2387215E+00
2742     13    -0.9977678E+00 -0.1704393E+01  0.2098444E+01
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

2743 14 -0.2995783E-01 -0.3527943E+01 0.2012902E+01  
2744 15 -0.3030414E+01 -0.2039774E+01 0.1813092E+01  
2745 16 -0.5295182E+00 -0.7491233E+00 0.4582914E+01  
2746 17 -0.1594451E+01 0.7247364E+00 0.4831232E+01  
2747  
2748 Reading esp"s for MEP 36  
2749 total number of atoms = 17  
2750 total number of esp points = 772  
2751  
2752 Center X Y Z  
2753 1 -0.3099113E+00 0.1017213E+01 -0.4488982E+01  
2754 2 0.1530039E+01 0.9515375E+00 -0.4645417E+01  
2755 3 -0.1001409E+01 -0.7415247E+00 -0.2599176E+01  
2756 4 -0.3317849E+00 -0.2659835E+01 -0.3056868E+01  
2757 5 -0.3073265E+01 -0.7982637E+00 -0.2543828E+01  
2758 6 0.0000000E+00 0.0000000E+00 0.0000000E+00  
2759 7 -0.6304428E+00 -0.1365935E+01 0.1431082E+01  
2760 8 -0.7303753E+00 0.1870910E+01 0.5275189E+00  
2761 9 0.2695611E+01 0.0000000E+00 0.0000000E+00  
2762 10 0.3954548E+01 0.2359697E+01 0.0000000E+00  
2763 11 0.4918460E+01 0.2638288E+01 0.1826080E+01  
2764 12 0.2610736E+01 0.3928391E+01 -0.2387215E+00  
2765 13 0.5927974E+01 0.2437731E+01 -0.2098444E+01  
2766 14 0.7081302E+01 0.7254753E+00 -0.2012902E+01  
2767 15 0.7180670E+01 0.4073238E+01 -0.1813092E+01  
2768 16 0.4864740E+01 0.2474260E+01 -0.4582914E+01  
2769 17 0.4065653E+01 0.4107601E+01 -0.4831232E+01  
2770  
2771 Reading esp"s for MEP 37  
2772 total number of atoms = 17  
2773 total number of esp points = 827  
2774  
2775 Center X Y Z  
2776 1 0.7407902E+01 0.9461253E+00 0.2367976E+01  
2777 2 0.6446730E+01 -0.6126888E+00 0.2462598E+01  
2778 3 0.6620655E+01 0.2155363E+01 0.1111197E+00  
2779 4 0.7258885E+01 0.1113447E+01 -0.1575259E+01  
2780 5 0.7519762E+01 0.4018678E+01 0.8752455E-01  
2781 6 0.3766585E+01 0.2462853E+01 0.0000000E+00  
2782 7 0.3208373E+01 0.3497599E+01 -0.1720212E+01  
2783 8 0.3100976E+01 0.3536439E+01 0.1653835E+01  
2784 9 0.2688485E+01 0.0000000E+00 0.0000000E+00  
2785 10 0.0000000E+00 0.0000000E+00 0.0000000E+00  
2786 11 -0.7240561E+00 0.9262889E+00 -0.1720220E+01  
2787 12 -0.7165746E+00 0.1040279E+01 0.1653820E+01

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
2788    13    -0.8628092E+00 -0.2737848E+01  0.1111461E+00
2789    14    -0.1642739E+00 -0.3740344E+01 -0.1575227E+01
2790    15    -0.2930292E+01 -0.2814307E+01  0.8756234E-01
2791    16    -0.7073055E-01 -0.3943913E+01  0.2368012E+01
2792    17     0.1742700E+01 -0.3688530E+01  0.2462610E+01
2793
2794 Reading esp"s for MEP 38
2795 total number of atoms = 17
2796 total number of esp points = 827
2797
2798 Center      X          Y          Z
2799    1   -0.7075323E-01 -0.3943938E+01 -0.2367976E+01
2800    2     0.1742673E+01 -0.3688528E+01 -0.2462598E+01
2801    3    -0.8628149E+00 -0.2737848E+01 -0.1111197E+00
2802    4    -0.1642777E+00 -0.3740331E+01  0.1575259E+01
2803    5    -0.2930298E+01 -0.2814297E+01 -0.8752455E-01
2804    6     0.0000000E+00  0.0000000E+00  0.0000000E+00
2805    7    -0.7240580E+00  0.9263040E+00  0.1720212E+01
2806    8    -0.7165709E+00  0.1040262E+01 -0.1653835E+01
2807    9     0.2688485E+01  0.0000000E+00  0.0000000E+00
2808   10     0.3766585E+01  0.2462855E+01  0.0000000E+00
2809   11     0.3208384E+01  0.3497592E+01  0.1720220E+01
2810   12     0.3100961E+01  0.3536448E+01 -0.1653820E+01
2811   13     0.6620651E+01  0.2155359E+01 -0.1111461E+00
2812   14     0.7258895E+01  0.1113440E+01  0.1575227E+01
2813   15     0.7519768E+01  0.4018667E+01 -0.8756234E-01
2814   16     0.7407870E+01  0.9461140E+00 -0.2368012E+01
2815   17     0.6446721E+01 -0.6127134E+00 -0.2462610E+01
2816
2817 Reading esp"s for MEP 39
2818 total number of atoms = 17
2819 total number of esp points = 827
2820
2821 Center      X          Y          Z
2822    1     0.7407887E+01  0.9460649E+00  0.2367942E+01
2823    2     0.6446642E+01 -0.6127040E+00  0.2462574E+01
2824    3     0.6620655E+01  0.2155357E+01  0.1111083E+00
2825    4     0.7258876E+01  0.1113470E+01 -0.1575293E+01
2826    5     0.7519777E+01  0.4018665E+01  0.8754911E-01
2827    6     0.3766587E+01  0.2462861E+01  0.0000000E+00
2828    7     0.3208358E+01  0.3497607E+01 -0.1720204E+01
2829    8     0.3100986E+01  0.3536437E+01  0.1653841E+01
2830    9     0.2688489E+01  0.0000000E+00  0.0000000E+00
2831   10     0.0000000E+00  0.0000000E+00  0.0000000E+00
2832   11    -0.7240561E+00  0.9263361E+00 -0.1720197E+01
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

2833    12    -0.7165652E+00    0.1040243E+01    0.1653847E+01  
2834    13    -0.8628149E+00    -0.2737848E+01    0.1110611E+00  
2835    14    -0.1643230E+00    -0.3740282E+01    -0.1575368E+01  
2836    15    -0.2930300E+01    -0.2814299E+01    0.8751132E-01  
2837    16    -0.7070032E-01    -0.3944019E+01    0.2367857E+01  
2838    17    0.1742722E+01    -0.3688592E+01    0.2462468E+01  
2839  
2840    Reading esp"s **for** MEP 40  
2841    total number of atoms = 17  
2842    total number of esp points = 827  
2843  
2844    Center    X               Y               Z  
2845    1    -0.7067953E-01    -0.3943949E+01    -0.2367942E+01  
2846    2    0.1742735E+01    -0.3688450E+01    -0.2462574E+01  
2847    3    -0.8627979E+00    -0.2737854E+01    -0.1111083E+00  
2848    4    -0.1642795E+00    -0.3740314E+01    0.1575293E+01  
2849    5    -0.2930281E+01    -0.2814322E+01    -0.8754911E-01  
2850    6    0.0000000E+00    0.0000000E+00    0.0000000E+00  
2851    7    -0.7240523E+00    0.9263191E+00    0.1720204E+01  
2852    8    -0.7165671E+00    0.1040251E+01    -0.1653841E+01  
2853    9    0.2688490E+01    0.0000000E+00    0.0000000E+00  
2854    10    0.3766589E+01    0.2462859E+01    0.0000000E+00  
2855    11    0.3208345E+01    0.3497615E+01    0.1720197E+01  
2856    12    0.3100995E+01    0.3536429E+01    -0.1653847E+01  
2857    13    0.6620659E+01    0.2155372E+01    -0.1110611E+00  
2858    14    0.7258865E+01    0.1113519E+01    0.1575368E+01  
2859    15    0.7519766E+01    0.4018688E+01    -0.8751132E-01  
2860    16    0.7407960E+01    0.9460535E+00    -0.2367857E+01  
2861    17    0.6446777E+01    -0.6127512E+00    -0.2462468E+01  
2862  
2863    Reading esp"s **for** MEP 41  
2864    total number of atoms = 17  
2865    total number of esp points = 827  
2866  
2867    Center    X               Y               Z  
2868    1    0.7407830E+01    0.9461234E+00    0.2368086E+01  
2869    2    0.6446621E+01    -0.6126700E+00    0.2462700E+01  
2870    3    0.6620655E+01    0.2155370E+01    0.1112141E+00  
2871    4    0.7258942E+01    0.1113464E+01    -0.1575149E+01  
2872    5    0.7519756E+01    0.4018688E+01    0.8765683E-01  
2873    6    0.3766589E+01    0.2462855E+01    0.0000000E+00  
2874    7    0.3208433E+01    0.3497575E+01    -0.1720244E+01  
2875    8    0.3100917E+01    0.3536465E+01    0.1653794E+01  
2876    9    0.2688490E+01    0.0000000E+00    0.0000000E+00  
2877    10    0.0000000E+00    0.0000000E+00    0.0000000E+00

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

2878    11    -0.7240523E+00    0.9261982E+00    -0.1720269E+01  
2879    12    -0.7165652E+00    0.1040379E+01    0.1653763E+01  
2880    13    -0.8628168E+00    -0.2737837E+01    0.1112992E+00  
2881    14    -0.1643136E+00    -0.3740427E+01    -0.1575030E+01  
2882    15    -0.2930302E+01    -0.2814282E+01    0.8774376E-01  
2883    16    -0.7071544E-01    -0.3943788E+01    0.2368214E+01  
2884    17    0.1742696E+01    -0.3688278E+01    0.2462840E+01  
2885  
2886    Reading esp"s for MEP 42  
2887    total number of atoms = 17  
2888    total number of esp points = 827  
2889  
2890    Center    X               Y               Z  
2891    1    -0.7071733E-01    -0.3943871E+01    -0.2368086E+01  
2892    2    0.1742705E+01    -0.3688414E+01    -0.2462700E+01  
2893    3    -0.8628187E+00    -0.2737845E+01    -0.1112141E+00  
2894    4    -0.1643098E+00    -0.3740372E+01    0.1575149E+01  
2895    5    -0.2930302E+01    -0.2814288E+01    -0.8765683E-01  
2896    6    0.0000000E+00    0.0000000E+00    0.0000000E+00  
2897    7    -0.7240580E+00    0.9262416E+00    0.1720244E+01  
2898    8    -0.7165709E+00    0.1040328E+01    -0.1653794E+01  
2899    9    0.2688485E+01    0.0000000E+00    0.0000000E+00  
2900    10    0.3766587E+01    0.2462859E+01    0.0000000E+00  
2901    11    0.3208467E+01    0.3497558E+01    0.1720269E+01  
2902    12    0.3100868E+01    0.3536484E+01    -0.1653763E+01  
2903    13    0.6620646E+01    0.2155376E+01    -0.1112992E+00  
2904    14    0.7258991E+01    0.1113451E+01    0.1575030E+01  
2905    15    0.7519749E+01    0.4018693E+01    -0.8774376E-01  
2906    16    0.7407752E+01    0.9461575E+00    -0.2368214E+01  
2907    17    0.6446496E+01    -0.6126019E+00    -0.2462840E+01  
2908  
2909    Reading esp"s for MEP 43  
2910    total number of atoms = 17  
2911    total number of esp points = 827  
2912  
2913    Center    X               Y               Z  
2914    1    0.7407856E+01    0.9461045E+00    0.2368019E+01  
2915    2    0.6446649E+01    -0.6126870E+00    0.2462625E+01  
2916    3    0.6620647E+01    0.2155376E+01    0.1111631E+00  
2917    4    0.7258921E+01    0.1113491E+01    -0.1575217E+01  
2918    5    0.7519747E+01    0.4018693E+01    0.8761714E-01  
2919    6    0.3766583E+01    0.2462857E+01    0.0000000E+00  
2920    7    0.3208390E+01    0.3497592E+01    -0.1720223E+01  
2921    8    0.3100944E+01    0.3536448E+01    0.1653816E+01  
2922    9    0.2688487E+01    0.0000000E+00    0.0000000E+00

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

2923    10    0.0000000E+00    0.0000000E+00    0.0000000E+00  
2924    11    -0.7240561E+00    0.9262832E+00    -0.1720221E+01  
2925    12    -0.7165671E+00    0.1040281E+01    0.1653822E+01  
2926    13    -0.8628016E+00    -0.2737854E+01    0.1111423E+00  
2927    14    -0.1643192E+00    -0.3740321E+01    -0.1575270E+01  
2928    15    -0.2930289E+01    -0.2814308E+01    0.8761526E-01  
2929    16    -0.7065686E-01    -0.3943949E+01    0.2367957E+01  
2930    17    0.1742764E+01    -0.3688505E+01    0.2462544E+01  
2931  
2932    Reading esp"s for MEP 44  
2933    total number of atoms = 17  
2934    total number of esp points = 827  
2935  
2936    Center    X               Y               Z  
2937    1    -0.7070599E-01    -0.3943909E+01    -0.2368019E+01  
2938    2    0.1742715E+01    -0.3688452E+01    -0.2462625E+01  
2939    3    -0.8628149E+00    -0.2737843E+01    -0.1111631E+00  
2940    4    -0.1643211E+00    -0.3740350E+01    0.1575217E+01  
2941    5    -0.2930300E+01    -0.2814288E+01    -0.8761714E-01  
2942    6    0.0000000E+00    0.0000000E+00    0.0000000E+00  
2943    7    -0.7240580E+00    0.9262794E+00    0.1720223E+01  
2944    8    -0.7165671E+00    0.1040290E+01    -0.1653816E+01  
2945    9    0.2688487E+01    0.0000000E+00    0.0000000E+00  
2946    10    0.3766581E+01    0.2462859E+01    0.0000000E+00  
2947    11    0.3208386E+01    0.3497594E+01    0.1720221E+01  
2948    12    0.3100951E+01    0.3536446E+01    -0.1653822E+01  
2949    13    0.6620653E+01    0.2155359E+01    -0.1111423E+00  
2950    14    0.7258893E+01    0.1113502E+01    0.1575270E+01  
2951    15    0.7519762E+01    0.4018674E+01    -0.8761526E-01  
2952    16    0.7407873E+01    0.9460441E+00    -0.2367957E+01  
2953    17    0.6446677E+01    -0.6127550E+00    -0.2462544E+01  
2954  
2955    Reading esp"s for MEP 45  
2956    total number of atoms = 17  
2957    total number of esp points = 827  
2958  
2959    Center    X               Y               Z  
2960    1    0.7407887E+01    0.9460460E+00    0.2367942E+01  
2961    2    0.6446657E+01    -0.6127304E+00    0.2462551E+01  
2962    3    0.6620653E+01    0.2155359E+01    0.1111216E+00  
2963    4    0.7258893E+01    0.1113496E+01    -0.1575287E+01  
2964    5    0.7519762E+01    0.4018672E+01    0.8758502E-01  
2965    6    0.3766585E+01    0.2462855E+01    0.0000000E+00  
2966    7    0.3208373E+01    0.3497599E+01    -0.1720212E+01  
2967    8    0.3100969E+01    0.3536441E+01    0.1653831E+01

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
2968      9      0.2688483E+01  0.0000000E+00  0.0000000E+00
2969     10      0.0000000E+00  0.0000000E+00  0.0000000E+00
2970     11     -0.7240580E+00  0.9262757E+00  -0.1720225E+01
2971     12     -0.7165728E+00  0.1040294E+01   0.1653814E+01
2972     13     -0.8628187E+00  -0.2737846E+01   0.1111631E+00
2973     14     -0.1643173E+00  -0.3740344E+01  -0.1575221E+01
2974     15     -0.2930304E+01  -0.2814290E+01   0.8760770E-01
2975     16     -0.7071355E-01  -0.3943915E+01   0.2368012E+01
2976     17      0.1742711E+01  -0.3688481E+01   0.2462621E+01
2977
2978  Reading esp"s for MEP 46
2979  total number of atoms = 17
2980  total number of esp points = 827
2981
2982  Center    X           Y           Z
2983    1  -0.7067764E-01 -0.3943956E+01 -0.2367942E+01
2984    2   0.1742739E+01 -0.3688479E+01 -0.2462551E+01
2985    3  -0.8628130E+00 -0.2737848E+01 -0.1111216E+00
2986    4  -0.1643268E+00 -0.3740318E+01   0.1575287E+01
2987    5  -0.2930296E+01 -0.2814299E+01  -0.8758502E-01
2988    6   0.0000000E+00  0.0000000E+00  0.0000000E+00
2989    7  -0.7240561E+00  0.9263021E+00   0.1720212E+01
2990    8  -0.7165690E+00  0.1040270E+01  -0.1653831E+01
2991    9   0.2688487E+01  0.0000000E+00  0.0000000E+00
2992   10   0.3766587E+01  0.2462852E+01  0.0000000E+00
2993   11   0.3208401E+01  0.3497586E+01   0.1720225E+01
2994   12   0.3100951E+01  0.3536450E+01  -0.1653814E+01
2995   13   0.6620657E+01  0.2155361E+01  -0.1111631E+00
2996   14   0.7258916E+01  0.1113474E+01   0.1575221E+01
2997   15   0.7519760E+01  0.4018678E+01  -0.8760770E-01
2998   16   0.7407866E+01  0.9460913E+00  -0.2368012E+01
2999   17   0.6446672E+01  -0.6127096E+00 -0.2462621E+01
3000 Initial ssvpot = 9.136
3001
3002
3003 Number of unique UNfrozen centers= 4
3004
3005 Non-linear optimization requested.
3006 qchnge = 0.8642012652E-02
3007 qchnge = 0.2080586726E-03
3008 qchnge = 0.9660723586E-05
3009 qchnge = 0.4841979518E-06
3010
3011 Convergence in 3 iterations
3012
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

3013 1 PEG2 nconf=1 norient=1 nmepl=1/46  
3014 2 PEG2 nconf=1 norient=2 nmepl=2/46  
3015 3 PEG2 nconf=2 norient=1 nmepl=3/46  
3016 4 PEG2 nconf=2 norient=2 nmepl=4/46  
3017 5 PEG2 nconf=3 norient=1 nmepl=5/46  
3018 6 PEG2 nconf=3 norient=2 nmepl=6/46  
3019 7 PEG2 nconf=4 norient=1 nmepl=7/46  
3020 8 PEG2 nconf=4 norient=2 nmepl=8/46  
3021 9 PEG2 nconf=5 norient=1 nmepl=9/46  
3022 10 PEG2 nconf=5 norient=2 nmepl=10/46  
3023 11 PEG2 nconf=6 norient=1 nmepl=11/46  
3024 12 PEG2 nconf=6 norient=2 nmepl=12/46  
3025 13 PEG2 nconf=7 norient=1 nmepl=13/46  
3026 14 PEG2 nconf=7 norient=2 nmepl=14/46  
3027 15 PEG2 nconf=8 norient=1 nmepl=15/46  
3028 16 PEG2 nconf=8 norient=2 nmepl=16/46  
3029 17 PEG2 nconf=9 norient=1 nmepl=17/46  
3030 18 PEG2 nconf=9 norient=2 nmepl=18/46  
3031 19 PEG2 nconf=10 norient=1 nmepl=19/46  
3032 20 PEG2 nconf=10 norient=2 nmepl=20/46  
3033 21 PEG2 nconf=11 norient=1 nmepl=21/46  
3034 22 PEG2 nconf=11 norient=2 nmepl=22/46  
3035 23 PEG2 nconf=12 norient=1 nmepl=23/46  
3036 24 PEG2 nconf=12 norient=2 nmepl=24/46  
3037 25 PEG2 nconf=13 norient=1 nmepl=25/46  
3038 26 PEG2 nconf=13 norient=2 nmepl=26/46  
3039 27 PEG2 nconf=14 norient=1 nmepl=27/46  
3040 28 PEG2 nconf=14 norient=2 nmepl=28/46  
3041 29 PEG2 nconf=15 norient=1 nmepl=29/46  
3042 30 PEG2 nconf=15 norient=2 nmepl=30/46  
3043 31 PEG2 nconf=16 norient=1 nmepl=31/46  
3044 32 PEG2 nconf=16 norient=2 nmepl=32/46  
3045 33 PEG2 nconf=17 norient=1 nmepl=33/46  
3046 34 PEG2 nconf=17 norient=2 nmepl=34/46  
3047 35 PEG2 nconf=18 norient=1 nmepl=35/46  
3048 36 PEG2 nconf=18 norient=2 nmepl=36/46  
3049 37 PEG2 nconf=19 norient=1 nmepl=37/46  
3050 38 PEG2 nconf=19 norient=2 nmepl=38/46  
3051 39 PEG2 nconf=20 norient=1 nmepl=39/46  
3052 40 PEG2 nconf=20 norient=2 nmepl=40/46  
3053 41 PEG2 nconf=21 norient=1 nmepl=41/46  
3054 42 PEG2 nconf=21 norient=2 nmepl=42/46  
3055 43 PEG2 nconf=22 norient=1 nmepl=43/46  
3056 44 PEG2 nconf=22 norient=2 nmepl=44/46  
3057 45 PEG2 nconf=23 norient=1 nmepl=45/46

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

3058        46    PEG2 nconf=23 norient=2 nmep=46/46  
 3059  
 3060              Point Charges Before & After Optimization  
 3061  
 3062        no. At.no.    q(init)    q(opt)    ivary    d(rstr)/dq  
 3063        1    8    -0.540571    -0.540571    -1    0.001819  
 3064        2    1    0.359859    0.359859    -1    0.000000  
 3065        3    6    0.090819    0.139118    0    0.005836  
 3066        4    1    0.035601    0.036348    0    0.000000  
 3067        5    1    0.068479    0.036348    4    0.000000  
 3068        6    6    0.011388    -0.010513    0    0.009945  
 3069        7    1    0.043297    0.061234    0    0.000000  
 3070        8    1    0.069417    0.061234    7    0.000000  
 3071        9    8    -0.286114    -0.286114    -1    0.003299  
 3072        10    6    0.000727    -0.010513    6    0.009945  
 3073        11    1    0.047995    0.061234    7    0.000000  
 3074        12    1    0.063336    0.061234    7    0.000000  
 3075        13    6    0.132235    0.139118    3    0.005836  
 3076        14    1    0.029096    0.036348    4    0.000000  
 3077        15    1    0.055147    0.036348    4    0.000000  
 3078        16    8    -0.540571    -0.540571    -1    0.001819  
 3079        17    1    0.359859    0.359859    -1    0.000000  
 3080  
 3081        18    8    -0.540571    -0.540571    -1    0.001819  
 3082        19    1    0.359859    0.359859    -1    0.000000  
 3083        20    6    0.090819    0.139118    3    0.005836  
 3084        21    1    0.035601    0.036348    4    0.000000  
 3085        22    1    0.068479    0.036348    4    0.000000  
 3086        23    6    0.011388    -0.010513    6    0.009945  
 3087        24    1    0.043297    0.061234    7    0.000000  
 3088        25    1    0.069417    0.061234    7    0.000000  
 3089        26    8    -0.286114    -0.286114    -1    0.003299  
 3090        27    6    0.000727    -0.010513    6    0.009945  
 3091        28    1    0.047995    0.061234    7    0.000000  
 3092        29    1    0.063336    0.061234    7    0.000000  
 3093        30    6    0.132235    0.139118    3    0.005836  
 3094        31    1    0.029096    0.036348    4    0.000000  
 3095        32    1    0.055147    0.036348    4    0.000000  
 3096        33    8    -0.540571    -0.540571    -1    0.001819  
 3097        34    1    0.359859    0.359859    -1    0.000000  
 3098  
 3099        35    8    -0.540571    -0.540571    -1    0.001819  
 3100        36    1    0.359859    0.359859    -1    0.000000  
 3101        37    6    0.090819    0.139118    3    0.005836  
 3102        38    1    0.035601    0.036348    4    0.000000

**B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS**

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3103	39	1	0.068479	0.036348	4	0.000000
3104	40	6	0.011388	-0.010513	6	0.009945
3105	41	1	0.043297	0.061234	7	0.000000
3106	42	1	0.069417	0.061234	7	0.000000
3107	43	8	-0.286114	-0.286114	-1	0.003299
3108	44	6	0.000727	-0.010513	6	0.009945
3109	45	1	0.047995	0.061234	7	0.000000
3110	46	1	0.063336	0.061234	7	0.000000
3111	47	6	0.132235	0.139118	3	0.005836
3112	48	1	0.029096	0.036348	4	0.000000
3113	49	1	0.055147	0.036348	4	0.000000
3114	50	8	-0.540571	-0.540571	-1	0.001819
3115	51	1	0.359859	0.359859	-1	0.000000
3116						
3117	52	8	-0.540571	-0.540571	-1	0.001819
3118	53	1	0.359859	0.359859	-1	0.000000
3119	54	6	0.090819	0.139118	3	0.005836
3120	55	1	0.035601	0.036348	4	0.000000
3121	56	1	0.068479	0.036348	4	0.000000
3122	57	6	0.011388	-0.010513	6	0.009945
3123	58	1	0.043297	0.061234	7	0.000000
3124	59	1	0.069417	0.061234	7	0.000000
3125	60	8	-0.286114	-0.286114	-1	0.003299
3126	61	6	0.000727	-0.010513	6	0.009945
3127	62	1	0.047995	0.061234	7	0.000000
3128	63	1	0.063336	0.061234	7	0.000000
3129	64	6	0.132235	0.139118	3	0.005836
3130	65	1	0.029096	0.036348	4	0.000000
3131	66	1	0.055147	0.036348	4	0.000000
3132	67	8	-0.540571	-0.540571	-1	0.001819
3133	68	1	0.359859	0.359859	-1	0.000000
3134						
3135	69	8	-0.540571	-0.540571	-1	0.001819
3136	70	1	0.359859	0.359859	-1	0.000000
3137	71	6	0.090819	0.139118	3	0.005836
3138	72	1	0.035601	0.036348	4	0.000000
3139	73	1	0.068479	0.036348	4	0.000000
3140	74	6	0.011388	-0.010513	6	0.009945
3141	75	1	0.043297	0.061234	7	0.000000
3142	76	1	0.069417	0.061234	7	0.000000
3143	77	8	-0.286114	-0.286114	-1	0.003299
3144	78	6	0.000727	-0.010513	6	0.009945
3145	79	1	0.047995	0.061234	7	0.000000
3146	80	1	0.063336	0.061234	7	0.000000
3147	81	6	0.132235	0.139118	3	0.005836

**B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS**

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3148	82	1	0.029096	0.036348	4	0.000000
3149	83	1	0.055147	0.036348	4	0.000000
3150	84	8	-0.540571	-0.540571	-1	0.001819
3151	85	1	0.359859	0.359859	-1	0.000000
3152						
3153	86	8	-0.540571	-0.540571	-1	0.001819
3154	87	1	0.359859	0.359859	-1	0.000000
3155	88	6	0.090819	0.139118	3	0.005836
3156	89	1	0.035601	0.036348	4	0.000000
3157	90	1	0.068479	0.036348	4	0.000000
3158	91	6	0.011388	-0.010513	6	0.009945
3159	92	1	0.043297	0.061234	7	0.000000
3160	93	1	0.069417	0.061234	7	0.000000
3161	94	8	-0.286114	-0.286114	-1	0.003299
3162	95	6	0.000727	-0.010513	6	0.009945
3163	96	1	0.047995	0.061234	7	0.000000
3164	97	1	0.063336	0.061234	7	0.000000
3165	98	6	0.132235	0.139118	3	0.005836
3166	99	1	0.029096	0.036348	4	0.000000
3167	100	1	0.055147	0.036348	4	0.000000
3168	101	8	-0.540571	-0.540571	-1	0.001819
3169	102	1	0.359859	0.359859	-1	0.000000
3170						
3171	103	8	-0.540571	-0.540571	-1	0.001819
3172	104	1	0.359859	0.359859	-1	0.000000
3173	105	6	0.090819	0.139118	3	0.005836
3174	106	1	0.035601	0.036348	4	0.000000
3175	107	1	0.068479	0.036348	4	0.000000
3176	108	6	0.011388	-0.010513	6	0.009945
3177	109	1	0.043297	0.061234	7	0.000000
3178	110	1	0.069417	0.061234	7	0.000000
3179	111	8	-0.286114	-0.286114	-1	0.003299
3180	112	6	0.000727	-0.010513	6	0.009945
3181	113	1	0.047995	0.061234	7	0.000000
3182	114	1	0.063336	0.061234	7	0.000000
3183	115	6	0.132235	0.139118	3	0.005836
3184	116	1	0.029096	0.036348	4	0.000000
3185	117	1	0.055147	0.036348	4	0.000000
3186	118	8	-0.540571	-0.540571	-1	0.001819
3187	119	1	0.359859	0.359859	-1	0.000000
3188						
3189	120	8	-0.540571	-0.540571	-1	0.001819
3190	121	1	0.359859	0.359859	-1	0.000000
3191	122	6	0.090819	0.139118	3	0.005836
3192	123	1	0.035601	0.036348	4	0.000000

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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3193	124	1	0.068479	0.036348	4	0.000000
3194	125	6	0.011388	-0.010513	6	0.009945
3195	126	1	0.043297	0.061234	7	0.000000
3196	127	1	0.069417	0.061234	7	0.000000
3197	128	8	-0.286114	-0.286114	-1	0.003299
3198	129	6	0.000727	-0.010513	6	0.009945
3199	130	1	0.047995	0.061234	7	0.000000
3200	131	1	0.063336	0.061234	7	0.000000
3201	132	6	0.132235	0.139118	3	0.005836
3202	133	1	0.029096	0.036348	4	0.000000
3203	134	1	0.055147	0.036348	4	0.000000
3204	135	8	-0.540571	-0.540571	-1	0.001819
3205	136	1	0.359859	0.359859	-1	0.000000
3206						
3207	137	8	-0.540571	-0.540571	-1	0.001819
3208	138	1	0.359859	0.359859	-1	0.000000
3209	139	6	0.090819	0.139118	3	0.005836
3210	140	1	0.035601	0.036348	4	0.000000
3211	141	1	0.068479	0.036348	4	0.000000
3212	142	6	0.011388	-0.010513	6	0.009945
3213	143	1	0.043297	0.061234	7	0.000000
3214	144	1	0.069417	0.061234	7	0.000000
3215	145	8	-0.286114	-0.286114	-1	0.003299
3216	146	6	0.000727	-0.010513	6	0.009945
3217	147	1	0.047995	0.061234	7	0.000000
3218	148	1	0.063336	0.061234	7	0.000000
3219	149	6	0.132235	0.139118	3	0.005836
3220	150	1	0.029096	0.036348	4	0.000000
3221	151	1	0.055147	0.036348	4	0.000000
3222	152	8	-0.540571	-0.540571	-1	0.001819
3223	153	1	0.359859	0.359859	-1	0.000000
3224						
3225	154	8	-0.540571	-0.540571	-1	0.001819
3226	155	1	0.359859	0.359859	-1	0.000000
3227	156	6	0.090819	0.139118	3	0.005836
3228	157	1	0.035601	0.036348	4	0.000000
3229	158	1	0.068479	0.036348	4	0.000000
3230	159	6	0.011388	-0.010513	6	0.009945
3231	160	1	0.043297	0.061234	7	0.000000
3232	161	1	0.069417	0.061234	7	0.000000
3233	162	8	-0.286114	-0.286114	-1	0.003299
3234	163	6	0.000727	-0.010513	6	0.009945
3235	164	1	0.047995	0.061234	7	0.000000
3236	165	1	0.063336	0.061234	7	0.000000
3237	166	6	0.132235	0.139118	3	0.005836

**B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS**

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3238	167	1	0.029096	0.036348	4	0.000000
3239	168	1	0.055147	0.036348	4	0.000000
3240	169	8	-0.540571	-0.540571	-1	0.001819
3241	170	1	0.359859	0.359859	-1	0.000000
3242						
3243	171	8	-0.540571	-0.540571	-1	0.001819
3244	172	1	0.359859	0.359859	-1	0.000000
3245	173	6	0.090819	0.139118	3	0.005836
3246	174	1	0.035601	0.036348	4	0.000000
3247	175	1	0.068479	0.036348	4	0.000000
3248	176	6	0.011388	-0.010513	6	0.009945
3249	177	1	0.043297	0.061234	7	0.000000
3250	178	1	0.069417	0.061234	7	0.000000
3251	179	8	-0.286114	-0.286114	-1	0.003299
3252	180	6	0.000727	-0.010513	6	0.009945
3253	181	1	0.047995	0.061234	7	0.000000
3254	182	1	0.063336	0.061234	7	0.000000
3255	183	6	0.132235	0.139118	3	0.005836
3256	184	1	0.029096	0.036348	4	0.000000
3257	185	1	0.055147	0.036348	4	0.000000
3258	186	8	-0.540571	-0.540571	-1	0.001819
3259	187	1	0.359859	0.359859	-1	0.000000
3260						
3261	188	8	-0.540571	-0.540571	-1	0.001819
3262	189	1	0.359859	0.359859	-1	0.000000
3263	190	6	0.090819	0.139118	3	0.005836
3264	191	1	0.035601	0.036348	4	0.000000
3265	192	1	0.068479	0.036348	4	0.000000
3266	193	6	0.011388	-0.010513	6	0.009945
3267	194	1	0.043297	0.061234	7	0.000000
3268	195	1	0.069417	0.061234	7	0.000000
3269	196	8	-0.286114	-0.286114	-1	0.003299
3270	197	6	0.000727	-0.010513	6	0.009945
3271	198	1	0.047995	0.061234	7	0.000000
3272	199	1	0.063336	0.061234	7	0.000000
3273	200	6	0.132235	0.139118	3	0.005836
3274	201	1	0.029096	0.036348	4	0.000000
3275	202	1	0.055147	0.036348	4	0.000000
3276	203	8	-0.540571	-0.540571	-1	0.001819
3277	204	1	0.359859	0.359859	-1	0.000000
3278						
3279	205	8	-0.540571	-0.540571	-1	0.001819
3280	206	1	0.359859	0.359859	-1	0.000000
3281	207	6	0.090819	0.139118	3	0.005836
3282	208	1	0.035601	0.036348	4	0.000000

**B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS**

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3283	209	1	0.068479	0.036348	4	0.000000
3284	210	6	0.011388	-0.010513	6	0.009945
3285	211	1	0.043297	0.061234	7	0.000000
3286	212	1	0.069417	0.061234	7	0.000000
3287	213	8	-0.286114	-0.286114	-1	0.003299
3288	214	6	0.000727	-0.010513	6	0.009945
3289	215	1	0.047995	0.061234	7	0.000000
3290	216	1	0.063336	0.061234	7	0.000000
3291	217	6	0.132235	0.139118	3	0.005836
3292	218	1	0.029096	0.036348	4	0.000000
3293	219	1	0.055147	0.036348	4	0.000000
3294	220	8	-0.540571	-0.540571	-1	0.001819
3295	221	1	0.359859	0.359859	-1	0.000000
3296						
3297	222	8	-0.540571	-0.540571	-1	0.001819
3298	223	1	0.359859	0.359859	-1	0.000000
3299	224	6	0.090819	0.139118	3	0.005836
3300	225	1	0.035601	0.036348	4	0.000000
3301	226	1	0.068479	0.036348	4	0.000000
3302	227	6	0.011388	-0.010513	6	0.009945
3303	228	1	0.043297	0.061234	7	0.000000
3304	229	1	0.069417	0.061234	7	0.000000
3305	230	8	-0.286114	-0.286114	-1	0.003299
3306	231	6	0.000727	-0.010513	6	0.009945
3307	232	1	0.047995	0.061234	7	0.000000
3308	233	1	0.063336	0.061234	7	0.000000
3309	234	6	0.132235	0.139118	3	0.005836
3310	235	1	0.029096	0.036348	4	0.000000
3311	236	1	0.055147	0.036348	4	0.000000
3312	237	8	-0.540571	-0.540571	-1	0.001819
3313	238	1	0.359859	0.359859	-1	0.000000
3314						
3315	239	8	-0.540571	-0.540571	-1	0.001819
3316	240	1	0.359859	0.359859	-1	0.000000
3317	241	6	0.090819	0.139118	3	0.005836
3318	242	1	0.035601	0.036348	4	0.000000
3319	243	1	0.068479	0.036348	4	0.000000
3320	244	6	0.011388	-0.010513	6	0.009945
3321	245	1	0.043297	0.061234	7	0.000000
3322	246	1	0.069417	0.061234	7	0.000000
3323	247	8	-0.286114	-0.286114	-1	0.003299
3324	248	6	0.000727	-0.010513	6	0.009945
3325	249	1	0.047995	0.061234	7	0.000000
3326	250	1	0.063336	0.061234	7	0.000000
3327	251	6	0.132235	0.139118	3	0.005836

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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3328	252	1	0.029096	0.036348	4	0.000000
3329	253	1	0.055147	0.036348	4	0.000000
3330	254	8	-0.540571	-0.540571	-1	0.001819
3331	255	1	0.359859	0.359859	-1	0.000000
3332						
3333	256	8	-0.540571	-0.540571	-1	0.001819
3334	257	1	0.359859	0.359859	-1	0.000000
3335	258	6	0.090819	0.139118	3	0.005836
3336	259	1	0.035601	0.036348	4	0.000000
3337	260	1	0.068479	0.036348	4	0.000000
3338	261	6	0.011388	-0.010513	6	0.009945
3339	262	1	0.043297	0.061234	7	0.000000
3340	263	1	0.069417	0.061234	7	0.000000
3341	264	8	-0.286114	-0.286114	-1	0.003299
3342	265	6	0.000727	-0.010513	6	0.009945
3343	266	1	0.047995	0.061234	7	0.000000
3344	267	1	0.063336	0.061234	7	0.000000
3345	268	6	0.132235	0.139118	3	0.005836
3346	269	1	0.029096	0.036348	4	0.000000
3347	270	1	0.055147	0.036348	4	0.000000
3348	271	8	-0.540571	-0.540571	-1	0.001819
3349	272	1	0.359859	0.359859	-1	0.000000
3350						
3351	273	8	-0.540571	-0.540571	-1	0.001819
3352	274	1	0.359859	0.359859	-1	0.000000
3353	275	6	0.090819	0.139118	3	0.005836
3354	276	1	0.035601	0.036348	4	0.000000
3355	277	1	0.068479	0.036348	4	0.000000
3356	278	6	0.011388	-0.010513	6	0.009945
3357	279	1	0.043297	0.061234	7	0.000000
3358	280	1	0.069417	0.061234	7	0.000000
3359	281	8	-0.286114	-0.286114	-1	0.003299
3360	282	6	0.000727	-0.010513	6	0.009945
3361	283	1	0.047995	0.061234	7	0.000000
3362	284	1	0.063336	0.061234	7	0.000000
3363	285	6	0.132235	0.139118	3	0.005836
3364	286	1	0.029096	0.036348	4	0.000000
3365	287	1	0.055147	0.036348	4	0.000000
3366	288	8	-0.540571	-0.540571	-1	0.001819
3367	289	1	0.359859	0.359859	-1	0.000000
3368						
3369	290	8	-0.540571	-0.540571	-1	0.001819
3370	291	1	0.359859	0.359859	-1	0.000000
3371	292	6	0.090819	0.139118	3	0.005836
3372	293	1	0.035601	0.036348	4	0.000000

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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3373	294	1	0.068479	0.036348	4	0.000000
3374	295	6	0.011388	-0.010513	6	0.009945
3375	296	1	0.043297	0.061234	7	0.000000
3376	297	1	0.069417	0.061234	7	0.000000
3377	298	8	-0.286114	-0.286114	-1	0.003299
3378	299	6	0.000727	-0.010513	6	0.009945
3379	300	1	0.047995	0.061234	7	0.000000
3380	301	1	0.063336	0.061234	7	0.000000
3381	302	6	0.132235	0.139118	3	0.005836
3382	303	1	0.029096	0.036348	4	0.000000
3383	304	1	0.055147	0.036348	4	0.000000
3384	305	8	-0.540571	-0.540571	-1	0.001819
3385	306	1	0.359859	0.359859	-1	0.000000
3386						
3387	307	8	-0.540571	-0.540571	-1	0.001819
3388	308	1	0.359859	0.359859	-1	0.000000
3389	309	6	0.090819	0.139118	3	0.005836
3390	310	1	0.035601	0.036348	4	0.000000
3391	311	1	0.068479	0.036348	4	0.000000
3392	312	6	0.011388	-0.010513	6	0.009945
3393	313	1	0.043297	0.061234	7	0.000000
3394	314	1	0.069417	0.061234	7	0.000000
3395	315	8	-0.286114	-0.286114	-1	0.003299
3396	316	6	0.000727	-0.010513	6	0.009945
3397	317	1	0.047995	0.061234	7	0.000000
3398	318	1	0.063336	0.061234	7	0.000000
3399	319	6	0.132235	0.139118	3	0.005836
3400	320	1	0.029096	0.036348	4	0.000000
3401	321	1	0.055147	0.036348	4	0.000000
3402	322	8	-0.540571	-0.540571	-1	0.001819
3403	323	1	0.359859	0.359859	-1	0.000000
3404						
3405	324	8	-0.540571	-0.540571	-1	0.001819
3406	325	1	0.359859	0.359859	-1	0.000000
3407	326	6	0.090819	0.139118	3	0.005836
3408	327	1	0.035601	0.036348	4	0.000000
3409	328	1	0.068479	0.036348	4	0.000000
3410	329	6	0.011388	-0.010513	6	0.009945
3411	330	1	0.043297	0.061234	7	0.000000
3412	331	1	0.069417	0.061234	7	0.000000
3413	332	8	-0.286114	-0.286114	-1	0.003299
3414	333	6	0.000727	-0.010513	6	0.009945
3415	334	1	0.047995	0.061234	7	0.000000
3416	335	1	0.063336	0.061234	7	0.000000
3417	336	6	0.132235	0.139118	3	0.005836

**B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS**

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3418	337	1	0.029096	0.036348	4	0.000000
3419	338	1	0.055147	0.036348	4	0.000000
3420	339	8	-0.540571	-0.540571	-1	0.001819
3421	340	1	0.359859	0.359859	-1	0.000000
3422						
3423	341	8	-0.540571	-0.540571	-1	0.001819
3424	342	1	0.359859	0.359859	-1	0.000000
3425	343	6	0.090819	0.139118	3	0.005836
3426	344	1	0.035601	0.036348	4	0.000000
3427	345	1	0.068479	0.036348	4	0.000000
3428	346	6	0.011388	-0.010513	6	0.009945
3429	347	1	0.043297	0.061234	7	0.000000
3430	348	1	0.069417	0.061234	7	0.000000
3431	349	8	-0.286114	-0.286114	-1	0.003299
3432	350	6	0.000727	-0.010513	6	0.009945
3433	351	1	0.047995	0.061234	7	0.000000
3434	352	1	0.063336	0.061234	7	0.000000
3435	353	6	0.132235	0.139118	3	0.005836
3436	354	1	0.029096	0.036348	4	0.000000
3437	355	1	0.055147	0.036348	4	0.000000
3438	356	8	-0.540571	-0.540571	-1	0.001819
3439	357	1	0.359859	0.359859	-1	0.000000
3440						
3441	358	8	-0.540571	-0.540571	-1	0.001819
3442	359	1	0.359859	0.359859	-1	0.000000
3443	360	6	0.090819	0.139118	3	0.005836
3444	361	1	0.035601	0.036348	4	0.000000
3445	362	1	0.068479	0.036348	4	0.000000
3446	363	6	0.011388	-0.010513	6	0.009945
3447	364	1	0.043297	0.061234	7	0.000000
3448	365	1	0.069417	0.061234	7	0.000000
3449	366	8	-0.286114	-0.286114	-1	0.003299
3450	367	6	0.000727	-0.010513	6	0.009945
3451	368	1	0.047995	0.061234	7	0.000000
3452	369	1	0.063336	0.061234	7	0.000000
3453	370	6	0.132235	0.139118	3	0.005836
3454	371	1	0.029096	0.036348	4	0.000000
3455	372	1	0.055147	0.036348	4	0.000000
3456	373	8	-0.540571	-0.540571	-1	0.001819
3457	374	1	0.359859	0.359859	-1	0.000000
3458						
3459	375	8	-0.540571	-0.540571	-1	0.001819
3460	376	1	0.359859	0.359859	-1	0.000000
3461	377	6	0.090819	0.139118	3	0.005836
3462	378	1	0.035601	0.036348	4	0.000000

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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3463	379	1	0.068479	0.036348	4	0.000000
3464	380	6	0.011388	-0.010513	6	0.009945
3465	381	1	0.043297	0.061234	7	0.000000
3466	382	1	0.069417	0.061234	7	0.000000
3467	383	8	-0.286114	-0.286114	-1	0.003299
3468	384	6	0.000727	-0.010513	6	0.009945
3469	385	1	0.047995	0.061234	7	0.000000
3470	386	1	0.063336	0.061234	7	0.000000
3471	387	6	0.132235	0.139118	3	0.005836
3472	388	1	0.029096	0.036348	4	0.000000
3473	389	1	0.055147	0.036348	4	0.000000
3474	390	8	-0.540571	-0.540571	-1	0.001819
3475	391	1	0.359859	0.359859	-1	0.000000
3476						
3477	392	8	-0.540571	-0.540571	-1	0.001819
3478	393	1	0.359859	0.359859	-1	0.000000
3479	394	6	0.090819	0.139118	3	0.005836
3480	395	1	0.035601	0.036348	4	0.000000
3481	396	1	0.068479	0.036348	4	0.000000
3482	397	6	0.011388	-0.010513	6	0.009945
3483	398	1	0.043297	0.061234	7	0.000000
3484	399	1	0.069417	0.061234	7	0.000000
3485	400	8	-0.286114	-0.286114	-1	0.003299
3486	401	6	0.000727	-0.010513	6	0.009945
3487	402	1	0.047995	0.061234	7	0.000000
3488	403	1	0.063336	0.061234	7	0.000000
3489	404	6	0.132235	0.139118	3	0.005836
3490	405	1	0.029096	0.036348	4	0.000000
3491	406	1	0.055147	0.036348	4	0.000000
3492	407	8	-0.540571	-0.540571	-1	0.001819
3493	408	1	0.359859	0.359859	-1	0.000000
3494						
3495	409	8	-0.540571	-0.540571	-1	0.001819
3496	410	1	0.359859	0.359859	-1	0.000000
3497	411	6	0.090819	0.139118	3	0.005836
3498	412	1	0.035601	0.036348	4	0.000000
3499	413	1	0.068479	0.036348	4	0.000000
3500	414	6	0.011388	-0.010513	6	0.009945
3501	415	1	0.043297	0.061234	7	0.000000
3502	416	1	0.069417	0.061234	7	0.000000
3503	417	8	-0.286114	-0.286114	-1	0.003299
3504	418	6	0.000727	-0.010513	6	0.009945
3505	419	1	0.047995	0.061234	7	0.000000
3506	420	1	0.063336	0.061234	7	0.000000
3507	421	6	0.132235	0.139118	3	0.005836

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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3508	422	1	0.029096	0.036348	4	0.000000
3509	423	1	0.055147	0.036348	4	0.000000
3510	424	8	-0.540571	-0.540571	-1	0.001819
3511	425	1	0.359859	0.359859	-1	0.000000
3512						
3513	426	8	-0.540571	-0.540571	-1	0.001819
3514	427	1	0.359859	0.359859	-1	0.000000
3515	428	6	0.090819	0.139118	3	0.005836
3516	429	1	0.035601	0.036348	4	0.000000
3517	430	1	0.068479	0.036348	4	0.000000
3518	431	6	0.011388	-0.010513	6	0.009945
3519	432	1	0.043297	0.061234	7	0.000000
3520	433	1	0.069417	0.061234	7	0.000000
3521	434	8	-0.286114	-0.286114	-1	0.003299
3522	435	6	0.000727	-0.010513	6	0.009945
3523	436	1	0.047995	0.061234	7	0.000000
3524	437	1	0.063336	0.061234	7	0.000000
3525	438	6	0.132235	0.139118	3	0.005836
3526	439	1	0.029096	0.036348	4	0.000000
3527	440	1	0.055147	0.036348	4	0.000000
3528	441	8	-0.540571	-0.540571	-1	0.001819
3529	442	1	0.359859	0.359859	-1	0.000000
3530						
3531	443	8	-0.540571	-0.540571	-1	0.001819
3532	444	1	0.359859	0.359859	-1	0.000000
3533	445	6	0.090819	0.139118	3	0.005836
3534	446	1	0.035601	0.036348	4	0.000000
3535	447	1	0.068479	0.036348	4	0.000000
3536	448	6	0.011388	-0.010513	6	0.009945
3537	449	1	0.043297	0.061234	7	0.000000
3538	450	1	0.069417	0.061234	7	0.000000
3539	451	8	-0.286114	-0.286114	-1	0.003299
3540	452	6	0.000727	-0.010513	6	0.009945
3541	453	1	0.047995	0.061234	7	0.000000
3542	454	1	0.063336	0.061234	7	0.000000
3543	455	6	0.132235	0.139118	3	0.005836
3544	456	1	0.029096	0.036348	4	0.000000
3545	457	1	0.055147	0.036348	4	0.000000
3546	458	8	-0.540571	-0.540571	-1	0.001819
3547	459	1	0.359859	0.359859	-1	0.000000
3548						
3549	460	8	-0.540571	-0.540571	-1	0.001819
3550	461	1	0.359859	0.359859	-1	0.000000
3551	462	6	0.090819	0.139118	3	0.005836
3552	463	1	0.035601	0.036348	4	0.000000

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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3553	464	1	0.068479	0.036348	4	0.000000
3554	465	6	0.011388	-0.010513	6	0.009945
3555	466	1	0.043297	0.061234	7	0.000000
3556	467	1	0.069417	0.061234	7	0.000000
3557	468	8	-0.286114	-0.286114	-1	0.003299
3558	469	6	0.000727	-0.010513	6	0.009945
3559	470	1	0.047995	0.061234	7	0.000000
3560	471	1	0.063336	0.061234	7	0.000000
3561	472	6	0.132235	0.139118	3	0.005836
3562	473	1	0.029096	0.036348	4	0.000000
3563	474	1	0.055147	0.036348	4	0.000000
3564	475	8	-0.540571	-0.540571	-1	0.001819
3565	476	1	0.359859	0.359859	-1	0.000000
3566						
3567	477	8	-0.540571	-0.540571	-1	0.001819
3568	478	1	0.359859	0.359859	-1	0.000000
3569	479	6	0.090819	0.139118	3	0.005836
3570	480	1	0.035601	0.036348	4	0.000000
3571	481	1	0.068479	0.036348	4	0.000000
3572	482	6	0.011388	-0.010513	6	0.009945
3573	483	1	0.043297	0.061234	7	0.000000
3574	484	1	0.069417	0.061234	7	0.000000
3575	485	8	-0.286114	-0.286114	-1	0.003299
3576	486	6	0.000727	-0.010513	6	0.009945
3577	487	1	0.047995	0.061234	7	0.000000
3578	488	1	0.063336	0.061234	7	0.000000
3579	489	6	0.132235	0.139118	3	0.005836
3580	490	1	0.029096	0.036348	4	0.000000
3581	491	1	0.055147	0.036348	4	0.000000
3582	492	8	-0.540571	-0.540571	-1	0.001819
3583	493	1	0.359859	0.359859	-1	0.000000
3584						
3585	494	8	-0.540571	-0.540571	-1	0.001819
3586	495	1	0.359859	0.359859	-1	0.000000
3587	496	6	0.090819	0.139118	3	0.005836
3588	497	1	0.035601	0.036348	4	0.000000
3589	498	1	0.068479	0.036348	4	0.000000
3590	499	6	0.011388	-0.010513	6	0.009945
3591	500	1	0.043297	0.061234	7	0.000000
3592	501	1	0.069417	0.061234	7	0.000000
3593	502	8	-0.286114	-0.286114	-1	0.003299
3594	503	6	0.000727	-0.010513	6	0.009945
3595	504	1	0.047995	0.061234	7	0.000000
3596	505	1	0.063336	0.061234	7	0.000000
3597	506	6	0.132235	0.139118	3	0.005836

**B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS**

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3598	507	1	0.029096	0.036348	4	0.000000
3599	508	1	0.055147	0.036348	4	0.000000
3600	509	8	-0.540571	-0.540571	-1	0.001819
3601	510	1	0.359859	0.359859	-1	0.000000
3602						
3603	511	8	-0.540571	-0.540571	-1	0.001819
3604	512	1	0.359859	0.359859	-1	0.000000
3605	513	6	0.090819	0.139118	3	0.005836
3606	514	1	0.035601	0.036348	4	0.000000
3607	515	1	0.068479	0.036348	4	0.000000
3608	516	6	0.011388	-0.010513	6	0.009945
3609	517	1	0.043297	0.061234	7	0.000000
3610	518	1	0.069417	0.061234	7	0.000000
3611	519	8	-0.286114	-0.286114	-1	0.003299
3612	520	6	0.000727	-0.010513	6	0.009945
3613	521	1	0.047995	0.061234	7	0.000000
3614	522	1	0.063336	0.061234	7	0.000000
3615	523	6	0.132235	0.139118	3	0.005836
3616	524	1	0.029096	0.036348	4	0.000000
3617	525	1	0.055147	0.036348	4	0.000000
3618	526	8	-0.540571	-0.540571	-1	0.001819
3619	527	1	0.359859	0.359859	-1	0.000000
3620						
3621	528	8	-0.540571	-0.540571	-1	0.001819
3622	529	1	0.359859	0.359859	-1	0.000000
3623	530	6	0.090819	0.139118	3	0.005836
3624	531	1	0.035601	0.036348	4	0.000000
3625	532	1	0.068479	0.036348	4	0.000000
3626	533	6	0.011388	-0.010513	6	0.009945
3627	534	1	0.043297	0.061234	7	0.000000
3628	535	1	0.069417	0.061234	7	0.000000
3629	536	8	-0.286114	-0.286114	-1	0.003299
3630	537	6	0.000727	-0.010513	6	0.009945
3631	538	1	0.047995	0.061234	7	0.000000
3632	539	1	0.063336	0.061234	7	0.000000
3633	540	6	0.132235	0.139118	3	0.005836
3634	541	1	0.029096	0.036348	4	0.000000
3635	542	1	0.055147	0.036348	4	0.000000
3636	543	8	-0.540571	-0.540571	-1	0.001819
3637	544	1	0.359859	0.359859	-1	0.000000
3638						
3639	545	8	-0.540571	-0.540571	-1	0.001819
3640	546	1	0.359859	0.359859	-1	0.000000
3641	547	6	0.090819	0.139118	3	0.005836
3642	548	1	0.035601	0.036348	4	0.000000

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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3643	549	1	0.068479	0.036348	4	0.000000
3644	550	6	0.011388	-0.010513	6	0.009945
3645	551	1	0.043297	0.061234	7	0.000000
3646	552	1	0.069417	0.061234	7	0.000000
3647	553	8	-0.286114	-0.286114	-1	0.003299
3648	554	6	0.000727	-0.010513	6	0.009945
3649	555	1	0.047995	0.061234	7	0.000000
3650	556	1	0.063336	0.061234	7	0.000000
3651	557	6	0.132235	0.139118	3	0.005836
3652	558	1	0.029096	0.036348	4	0.000000
3653	559	1	0.055147	0.036348	4	0.000000
3654	560	8	-0.540571	-0.540571	-1	0.001819
3655	561	1	0.359859	0.359859	-1	0.000000
3656						
3657	562	8	-0.540571	-0.540571	-1	0.001819
3658	563	1	0.359859	0.359859	-1	0.000000
3659	564	6	0.090819	0.139118	3	0.005836
3660	565	1	0.035601	0.036348	4	0.000000
3661	566	1	0.068479	0.036348	4	0.000000
3662	567	6	0.011388	-0.010513	6	0.009945
3663	568	1	0.043297	0.061234	7	0.000000
3664	569	1	0.069417	0.061234	7	0.000000
3665	570	8	-0.286114	-0.286114	-1	0.003299
3666	571	6	0.000727	-0.010513	6	0.009945
3667	572	1	0.047995	0.061234	7	0.000000
3668	573	1	0.063336	0.061234	7	0.000000
3669	574	6	0.132235	0.139118	3	0.005836
3670	575	1	0.029096	0.036348	4	0.000000
3671	576	1	0.055147	0.036348	4	0.000000
3672	577	8	-0.540571	-0.540571	-1	0.001819
3673	578	1	0.359859	0.359859	-1	0.000000
3674						
3675	579	8	-0.540571	-0.540571	-1	0.001819
3676	580	1	0.359859	0.359859	-1	0.000000
3677	581	6	0.090819	0.139118	3	0.005836
3678	582	1	0.035601	0.036348	4	0.000000
3679	583	1	0.068479	0.036348	4	0.000000
3680	584	6	0.011388	-0.010513	6	0.009945
3681	585	1	0.043297	0.061234	7	0.000000
3682	586	1	0.069417	0.061234	7	0.000000
3683	587	8	-0.286114	-0.286114	-1	0.003299
3684	588	6	0.000727	-0.010513	6	0.009945
3685	589	1	0.047995	0.061234	7	0.000000
3686	590	1	0.063336	0.061234	7	0.000000
3687	591	6	0.132235	0.139118	3	0.005836

**B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS**

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3688	592	1	0.029096	0.036348	4	0.000000
3689	593	1	0.055147	0.036348	4	0.000000
3690	594	8	-0.540571	-0.540571	-1	0.001819
3691	595	1	0.359859	0.359859	-1	0.000000
3692						
3693	596	8	-0.540571	-0.540571	-1	0.001819
3694	597	1	0.359859	0.359859	-1	0.000000
3695	598	6	0.090819	0.139118	3	0.005836
3696	599	1	0.035601	0.036348	4	0.000000
3697	600	1	0.068479	0.036348	4	0.000000
3698	601	6	0.011388	-0.010513	6	0.009945
3699	602	1	0.043297	0.061234	7	0.000000
3700	603	1	0.069417	0.061234	7	0.000000
3701	604	8	-0.286114	-0.286114	-1	0.003299
3702	605	6	0.000727	-0.010513	6	0.009945
3703	606	1	0.047995	0.061234	7	0.000000
3704	607	1	0.063336	0.061234	7	0.000000
3705	608	6	0.132235	0.139118	3	0.005836
3706	609	1	0.029096	0.036348	4	0.000000
3707	610	1	0.055147	0.036348	4	0.000000
3708	611	8	-0.540571	-0.540571	-1	0.001819
3709	612	1	0.359859	0.359859	-1	0.000000
3710						
3711	613	8	-0.540571	-0.540571	-1	0.001819
3712	614	1	0.359859	0.359859	-1	0.000000
3713	615	6	0.090819	0.139118	3	0.005836
3714	616	1	0.035601	0.036348	4	0.000000
3715	617	1	0.068479	0.036348	4	0.000000
3716	618	6	0.011388	-0.010513	6	0.009945
3717	619	1	0.043297	0.061234	7	0.000000
3718	620	1	0.069417	0.061234	7	0.000000
3719	621	8	-0.286114	-0.286114	-1	0.003299
3720	622	6	0.000727	-0.010513	6	0.009945
3721	623	1	0.047995	0.061234	7	0.000000
3722	624	1	0.063336	0.061234	7	0.000000
3723	625	6	0.132235	0.139118	3	0.005836
3724	626	1	0.029096	0.036348	4	0.000000
3725	627	1	0.055147	0.036348	4	0.000000
3726	628	8	-0.540571	-0.540571	-1	0.001819
3727	629	1	0.359859	0.359859	-1	0.000000
3728						
3729	630	8	-0.540571	-0.540571	-1	0.001819
3730	631	1	0.359859	0.359859	-1	0.000000
3731	632	6	0.090819	0.139118	3	0.005836
3732	633	1	0.035601	0.036348	4	0.000000

**B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS**

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3733	634	1	0.068479	0.036348	4	0.000000
3734	635	6	0.011388	-0.010513	6	0.009945
3735	636	1	0.043297	0.061234	7	0.000000
3736	637	1	0.069417	0.061234	7	0.000000
3737	638	8	-0.286114	-0.286114	-1	0.003299
3738	639	6	0.000727	-0.010513	6	0.009945
3739	640	1	0.047995	0.061234	7	0.000000
3740	641	1	0.063336	0.061234	7	0.000000
3741	642	6	0.132235	0.139118	3	0.005836
3742	643	1	0.029096	0.036348	4	0.000000
3743	644	1	0.055147	0.036348	4	0.000000
3744	645	8	-0.540571	-0.540571	-1	0.001819
3745	646	1	0.359859	0.359859	-1	0.000000
3746						
3747	647	8	-0.540571	-0.540571	-1	0.001819
3748	648	1	0.359859	0.359859	-1	0.000000
3749	649	6	0.090819	0.139118	3	0.005836
3750	650	1	0.035601	0.036348	4	0.000000
3751	651	1	0.068479	0.036348	4	0.000000
3752	652	6	0.011388	-0.010513	6	0.009945
3753	653	1	0.043297	0.061234	7	0.000000
3754	654	1	0.069417	0.061234	7	0.000000
3755	655	8	-0.286114	-0.286114	-1	0.003299
3756	656	6	0.000727	-0.010513	6	0.009945
3757	657	1	0.047995	0.061234	7	0.000000
3758	658	1	0.063336	0.061234	7	0.000000
3759	659	6	0.132235	0.139118	3	0.005836
3760	660	1	0.029096	0.036348	4	0.000000
3761	661	1	0.055147	0.036348	4	0.000000
3762	662	8	-0.540571	-0.540571	-1	0.001819
3763	663	1	0.359859	0.359859	-1	0.000000
3764						
3765	664	8	-0.540571	-0.540571	-1	0.001819
3766	665	1	0.359859	0.359859	-1	0.000000
3767	666	6	0.090819	0.139118	3	0.005836
3768	667	1	0.035601	0.036348	4	0.000000
3769	668	1	0.068479	0.036348	4	0.000000
3770	669	6	0.011388	-0.010513	6	0.009945
3771	670	1	0.043297	0.061234	7	0.000000
3772	671	1	0.069417	0.061234	7	0.000000
3773	672	8	-0.286114	-0.286114	-1	0.003299
3774	673	6	0.000727	-0.010513	6	0.009945
3775	674	1	0.047995	0.061234	7	0.000000
3776	675	1	0.063336	0.061234	7	0.000000
3777	676	6	0.132235	0.139118	3	0.005836

**B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS**

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3778	677	1	0.029096	0.036348	4	0.000000
3779	678	1	0.055147	0.036348	4	0.000000
3780	679	8	-0.540571	-0.540571	-1	0.001819
3781	680	1	0.359859	0.359859	-1	0.000000
3782						
3783	681	8	-0.540571	-0.540571	-1	0.001819
3784	682	1	0.359859	0.359859	-1	0.000000
3785	683	6	0.090819	0.139118	3	0.005836
3786	684	1	0.035601	0.036348	4	0.000000
3787	685	1	0.068479	0.036348	4	0.000000
3788	686	6	0.011388	-0.010513	6	0.009945
3789	687	1	0.043297	0.061234	7	0.000000
3790	688	1	0.069417	0.061234	7	0.000000
3791	689	8	-0.286114	-0.286114	-1	0.003299
3792	690	6	0.000727	-0.010513	6	0.009945
3793	691	1	0.047995	0.061234	7	0.000000
3794	692	1	0.063336	0.061234	7	0.000000
3795	693	6	0.132235	0.139118	3	0.005836
3796	694	1	0.029096	0.036348	4	0.000000
3797	695	1	0.055147	0.036348	4	0.000000
3798	696	8	-0.540571	-0.540571	-1	0.001819
3799	697	1	0.359859	0.359859	-1	0.000000
3800						
3801	698	8	-0.540571	-0.540571	-1	0.001819
3802	699	1	0.359859	0.359859	-1	0.000000
3803	700	6	0.090819	0.139118	3	0.005836
3804	701	1	0.035601	0.036348	4	0.000000
3805	702	1	0.068479	0.036348	4	0.000000
3806	703	6	0.011388	-0.010513	6	0.009945
3807	704	1	0.043297	0.061234	7	0.000000
3808	705	1	0.069417	0.061234	7	0.000000
3809	706	8	-0.286114	-0.286114	-1	0.003299
3810	707	6	0.000727	-0.010513	6	0.009945
3811	708	1	0.047995	0.061234	7	0.000000
3812	709	1	0.063336	0.061234	7	0.000000
3813	710	6	0.132235	0.139118	3	0.005836
3814	711	1	0.029096	0.036348	4	0.000000
3815	712	1	0.055147	0.036348	4	0.000000
3816	713	8	-0.540571	-0.540571	-1	0.001819
3817	714	1	0.359859	0.359859	-1	0.000000
3818						
3819	715	8	-0.540571	-0.540571	-1	0.001819
3820	716	1	0.359859	0.359859	-1	0.000000
3821	717	6	0.090819	0.139118	3	0.005836
3822	718	1	0.035601	0.036348	4	0.000000

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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3823	719	1	0.068479	0.036348	4	0.000000
3824	720	6	0.011388	-0.010513	6	0.009945
3825	721	1	0.043297	0.061234	7	0.000000
3826	722	1	0.069417	0.061234	7	0.000000
3827	723	8	-0.286114	-0.286114	-1	0.003299
3828	724	6	0.000727	-0.010513	6	0.009945
3829	725	1	0.047995	0.061234	7	0.000000
3830	726	1	0.063336	0.061234	7	0.000000
3831	727	6	0.132235	0.139118	3	0.005836
3832	728	1	0.029096	0.036348	4	0.000000
3833	729	1	0.055147	0.036348	4	0.000000
3834	730	8	-0.540571	-0.540571	-1	0.001819
3835	731	1	0.359859	0.359859	-1	0.000000
3836						
3837	732	8	-0.540571	-0.540571	-1	0.001819
3838	733	1	0.359859	0.359859	-1	0.000000
3839	734	6	0.090819	0.139118	3	0.005836
3840	735	1	0.035601	0.036348	4	0.000000
3841	736	1	0.068479	0.036348	4	0.000000
3842	737	6	0.011388	-0.010513	6	0.009945
3843	738	1	0.043297	0.061234	7	0.000000
3844	739	1	0.069417	0.061234	7	0.000000
3845	740	8	-0.286114	-0.286114	-1	0.003299
3846	741	6	0.000727	-0.010513	6	0.009945
3847	742	1	0.047995	0.061234	7	0.000000
3848	743	1	0.063336	0.061234	7	0.000000
3849	744	6	0.132235	0.139118	3	0.005836
3850	745	1	0.029096	0.036348	4	0.000000
3851	746	1	0.055147	0.036348	4	0.000000
3852	747	8	-0.540571	-0.540571	-1	0.001819
3853	748	1	0.359859	0.359859	-1	0.000000
3854						
3855	749	8	-0.540571	-0.540571	-1	0.001819
3856	750	1	0.359859	0.359859	-1	0.000000
3857	751	6	0.090819	0.139118	3	0.005836
3858	752	1	0.035601	0.036348	4	0.000000
3859	753	1	0.068479	0.036348	4	0.000000
3860	754	6	0.011388	-0.010513	6	0.009945
3861	755	1	0.043297	0.061234	7	0.000000
3862	756	1	0.069417	0.061234	7	0.000000
3863	757	8	-0.286114	-0.286114	-1	0.003299
3864	758	6	0.000727	-0.010513	6	0.009945
3865	759	1	0.047995	0.061234	7	0.000000
3866	760	1	0.063336	0.061234	7	0.000000
3867	761	6	0.132235	0.139118	3	0.005836

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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3868 762 1 0.029096 0.036348 4 0.000000  
3869 763 1 0.055147 0.036348 4 0.000000  
3870 764 8 -0.540571 -0.540571 -1 0.001819  
3871 765 1 0.359859 0.359859 -1 0.000000  
3872  
3873 766 8 -0.540571 -0.540571 -1 0.001819  
3874 767 1 0.359859 0.359859 -1 0.000000  
3875 768 6 0.090819 0.139118 3 0.005836  
3876 769 1 0.035601 0.036348 4 0.000000  
3877 770 1 0.068479 0.036348 4 0.000000  
3878 771 6 0.011388 -0.010513 6 0.009945  
3879 772 1 0.043297 0.061234 7 0.000000  
3880 773 1 0.069417 0.061234 7 0.000000  
3881 774 8 -0.286114 -0.286114 -1 0.003299  
3882 775 6 0.000727 -0.010513 6 0.009945  
3883 776 1 0.047995 0.061234 7 0.000000  
3884 777 1 0.063336 0.061234 7 0.000000  
3885 778 6 0.132235 0.139118 3 0.005836  
3886 779 1 0.029096 0.036348 4 0.000000  
3887 780 1 0.055147 0.036348 4 0.000000  
3888 781 8 -0.540571 -0.540571 -1 0.001819  
3889 782 1 0.359859 0.359859 -1 0.000000  
3890  
3891 Sum over the calculated charges: -0.000  
3892  
3893 Statistics of the fitting:  
3894 The initial **sum** of squares (ssvpot) 9.136  
3895 The residual **sum** of squares (chipot) 0.319  
3896 The std err of estimate (sqrt(chipot/N)) 0.00293  
3897 ESP relative RMS (SQRT(chipot/ssvpot)) 0.18693  
3898 The Pearson correlation coefficient (r2) 0.96548  
3899  
3900 Center of Mass (a.u.):  
3901 #MEP X Y Z  
3902 1 2.10084 0.36011 -0.47027  
3903 2 2.60441 0.68375 0.47027  
3904 3 2.19529 0.16307 -0.52164  
3905 4 2.76843 0.52490 0.52164  
3906 5 2.19524 0.16306 -0.52170  
3907 6 2.76845 0.52493 0.52170  
3908 7 2.19529 0.16305 -0.52168  
3909 8 2.76845 0.52489 0.52168  
3910 9 2.19529 0.16308 -0.52163  
3911 10 2.76842 0.52490 0.52163  
3912 11 2.03978 0.19351 -0.50206

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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3913 12 2.79012 0.66614 0.50206  
3914 13 1.60287 0.25389 -0.97223  
3915 14 2.92374 1.08732 0.97223  
3916 15 1.61049 0.26767 -0.98910  
3917 16 2.90235 1.08853 0.98910  
3918 17 1.61048 0.26767 -0.98909  
3919 18 2.90235 1.08854 0.98909  
3920 19 1.61502 0.25239 -0.99994  
3921 20 2.91807 1.07559 0.99994  
3922 21 1.88907 0.91761 -2.06707  
3923 22 2.23132 1.11840 2.06707  
3924 23 1.88906 0.91762 -2.06706  
3925 24 2.23131 1.11842 2.06706  
3926 25 1.88906 0.91764 -2.06707  
3927 26 2.23130 1.11843 2.06707  
3928 27 1.88906 0.91760 -2.06706  
3929 28 2.23134 1.11840 2.06706  
3930 29 2.71741 -0.26663 0.09590  
3931 30 2.90794 -0.15287 -0.09590  
3932 31 2.81917 -0.20027 -0.00006  
3933 32 2.81915 -0.20028 0.00006  
3934 33 2.82451 -0.20803 0.75642  
3935 34 2.82453 -0.20803 -0.75642  
3936 35 1.87223 0.86154 2.04528  
3937 36 2.31314 1.11340 -2.04528  
3938 37 2.82451 -0.20802 0.75644  
3939 38 2.82450 -0.20803 -0.75644  
3940 39 2.82451 -0.20805 0.75640  
3941 40 2.82453 -0.20804 -0.75640  
3942 41 2.82450 -0.20800 0.75653  
3943 42 2.82449 -0.20801 -0.75653  
3944 43 2.82452 -0.20803 0.75645  
3945 44 2.82451 -0.20803 -0.75645  
3946 45 2.82451 -0.20804 0.75644  
3947 46 2.82451 -0.20804 -0.75644  
3948  
3949 Dipole moments (Debye) computed:  
3950 -with respect to the origin of coordinates (ooc)  
3951 -with respect to the center of mass (com)  
3952 #MEP D Dx Dy Dz  
3953 1 ooc 3.90866 -0.50085 3.42197 -1.82124  
3954 1 com 3.90866 -0.50085 3.42197 -1.82124  
3955  
3956 2 ooc 3.90866 -2.87235 1.92622 1.82124  
3957 2 com 3.90866 -2.87235 1.92622 1.82124

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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3958					
3959	3 ooc	1.60120	-0.92151	1.30476	-0.11071
3960	3 com	1.60120	-0.92151	1.30476	-0.11071
3961					
3962	4 ooc	1.60120	-0.78495	1.39120	0.11071
3963	4 com	1.60120	-0.78495	1.39120	0.11071
3964					
3965	5 ooc	1.60119	-0.92144	1.30481	-0.11060
3966	5 com	1.60119	-0.92144	1.30481	-0.11060
3967					
3968	6 ooc	1.60119	-0.78502	1.39116	0.11060
3969	6 com	1.60119	-0.78502	1.39116	0.11060
3970					
3971	7 ooc	1.60127	-0.92149	1.30487	-0.11061
3972	7 com	1.60127	-0.92149	1.30487	-0.11061
3973					
3974	8 ooc	1.60127	-0.78505	1.39123	0.11061
3975	8 com	1.60127	-0.78505	1.39123	0.11061
3976					
3977	9 ooc	1.60119	-0.92151	1.30475	-0.11072
3978	9 com	1.60119	-0.92151	1.30475	-0.11072
3979					
3980	10 ooc	1.60119	-0.78493	1.39120	0.11072
3981	10 com	1.60119	-0.78493	1.39120	0.11072
3982					
3983	11 ooc	2.69491	-2.58171	-0.77134	0.04888
3984	11 com	2.69491	-2.58171	-0.77134	0.04888
3985					
3986	12 ooc	2.69491	1.78506	2.01835	-0.04888
3987	12 com	2.69491	1.78506	2.01835	-0.04888
3988					
3989	13 ooc	1.61957	-1.21993	0.99114	-0.39040
3990	13 com	1.61957	-1.21993	0.99114	-0.39040
3991					
3992	14 ooc	1.61957	-0.38564	1.52377	0.39040
3993	14 com	1.61957	-0.38564	1.52377	0.39040
3994					
3995	15 ooc	2.01573	-1.20776	-1.08718	1.19269
3996	15 com	2.01573	-1.20776	-1.08718	1.19269
3997					
3998	16 ooc	2.01573	1.49146	0.64515	-1.19269
3999	16 com	2.01573	1.49146	0.64515	-1.19269
4000					
4001	17 ooc	2.01583	-1.20776	-1.08717	1.19288
4002	17 com	2.01583	-1.20776	-1.08717	1.19288

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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4003					
4004	18 ooc	2.01583	1.49144	0.64515	-1.19288
4005	18 com	2.01583	1.49144	0.64515	-1.19288
4006					
4007	19 ooc	3.07315	-1.25050	1.08139	2.59058
4008	19 com	3.07315	-1.25050	1.08139	2.59058
4009					
4010	20 ooc	3.07315	-0.45744	1.58868	-2.59058
4011	20 com	3.07315	-0.45744	1.58868	-2.59058
4012					
4013	21 ooc	4.20502	-3.46745	1.43040	1.90079
4014	21 com	4.20502	-3.46745	1.43040	1.90079
4015					
4016	22 ooc	4.20502	0.29804	3.73903	-1.90079
4017	22 com	4.20502	0.29804	3.73903	-1.90079
4018					
4019	23 ooc	4.20496	-3.46737	1.43035	1.90083
4020	23 com	4.20496	-3.46737	1.43035	1.90083
4021					
4022	24 ooc	4.20496	0.29804	3.73895	-1.90083
4023	24 com	4.20496	0.29804	3.73895	-1.90083
4024					
4025	25 ooc	4.20497	-3.46738	1.43036	1.90083
4026	25 com	4.20497	-3.46738	1.43036	1.90083
4027					
4028	26 ooc	4.20497	0.29805	3.73896	-1.90083
4029	26 com	4.20497	0.29805	3.73896	-1.90083
4030					
4031	27 ooc	4.20496	-3.46739	1.43034	1.90080
4032	27 com	4.20496	-3.46739	1.43034	1.90080
4033					
4034	28 ooc	4.20496	0.29805	3.73896	-1.90080
4035	28 com	4.20496	0.29805	3.73896	-1.90080
4036					
4037	29 ooc	3.82911	-3.01243	1.91372	-1.38746
4038	29 com	3.82911	-3.01243	1.91372	-1.38746
4039					
4040	30 ooc	3.82911	-0.52762	3.52968	1.38746
4041	30 com	3.82911	-0.52762	3.52968	1.38746
4042					
4043	31 ooc	1.48959	-0.80834	1.25118	0.00004
4044	31 com	1.48959	-0.80834	1.25118	0.00004
4045					
4046	32 ooc	1.48959	-0.80830	1.25121	-0.00004
4047	32 com	1.48959	-0.80830	1.25121	-0.00004

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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4048  
4049 33 ooc 2.63084 -0.77332 1.18254 -2.21922  
4050 33 com 2.63084 -0.77332 1.18254 -2.21922  
4051  
4052 34 ooc 2.63084 -0.77319 1.18262 2.21922  
4053 34 com 2.63084 -0.77319 1.18262 2.21922  
4054  
4055 35 ooc 2.46612 -1.63900 0.30879 -1.81662  
4056 35 com 2.46612 -1.63900 0.30879 -1.81662  
4057  
4058 36 ooc 2.46612 0.49906 1.59141 1.81662  
4059 36 com 2.46612 0.49906 1.59141 1.81662  
4060  
4061 37 ooc 2.63078 -0.77317 1.18240 -2.21926  
4062 37 com 2.63078 -0.77317 1.18240 -2.21926  
4063  
4064 38 ooc 2.63078 -0.77313 1.18243 2.21926  
4065 38 com 2.63078 -0.77313 1.18243 2.21926  
4066  
4067 39 ooc 2.63082 -0.77326 1.18257 -2.21919  
4068 39 com 2.63082 -0.77326 1.18257 -2.21919  
4069  
4070 40 ooc 2.63082 -0.77324 1.18258 2.21919  
4071 40 com 2.63082 -0.77324 1.18258 2.21919  
4072  
4073 41 ooc 2.63083 -0.77320 1.18249 -2.21927  
4074 41 com 2.63083 -0.77320 1.18249 -2.21927  
4075  
4076 42 ooc 2.63083 -0.77319 1.18250 2.21927  
4077 42 com 2.63083 -0.77319 1.18250 2.21927  
4078  
4079 43 ooc 2.63084 -0.77323 1.18252 -2.21926  
4080 43 com 2.63084 -0.77323 1.18252 -2.21926  
4081  
4082 44 ooc 2.63084 -0.77321 1.18253 2.21926  
4083 44 com 2.63084 -0.77321 1.18253 2.21926  
4084  
4085 45 ooc 2.63083 -0.77324 1.18253 -2.21923  
4086 45 com 2.63083 -0.77324 1.18253 -2.21923  
4087  
4088 46 ooc 2.63083 -0.77321 1.18254 2.21923  
4089 46 com 2.63083 -0.77321 1.18254 2.21923  
4090  
4091 Traceless Quadrupole moments (Buckingham) computed:  
4092 -with respect to the origin of coordinates (ooc)

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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4093 -with respect to the center of mass (com)  
4094 #MEP X Y Z  
4095 1 ooc X -10.17280  
4096 Y 9.32337 8.91971  
4097 Z 7.21166 -3.90688 1.25309  
4098 1 com X -5.73492  
4099 Y -1.80307 6.10414  
4100 Z 12.91184 -0.31095 -0.36922  
4101  
4102 2 ooc X -15.63020  
4103 Y -0.89035 7.66311  
4104 Z 10.66763 15.20111 7.96709  
4105 2 com X 2.50479  
4106 Y -5.73662 -2.13556  
4107 Z 5.28199 11.78614 -0.36922  
4108  
4109 3 ooc X -2.53130  
4110 Y -7.47020 11.38053  
4111 Z 23.05689 17.27343 -8.84923  
4112 3 com X 2.03710  
4113 Y -11.77886 8.85023  
4114 Z 22.67962 18.38259 -10.88732  
4115  
4116 4 ooc X -6.94212  
4117 Y -4.64006 16.18017  
4118 Z 26.15598 13.87655 -9.23805  
4119 4 com X -1.50837  
4120 Y -10.10026 12.39569  
4121 Z 26.31942 12.63221 -10.88732  
4122  
4123 5 ooc X -2.52989  
4124 Y -7.47054 11.37918  
4125 Z 23.05662 17.27389 -8.84929  
4126 5 com X 2.03800  
4127 Y -11.77931 8.84906  
4128 Z 22.67891 18.38318 -10.88706  
4129  
4130 6 ooc X -6.94366  
4131 Y -4.63979 16.18137  
4132 Z 26.15560 13.87563 -9.23771  
4133 6 com X -1.50953  
4134 Y -10.09974 12.39660  
4135 Z 26.31968 12.63128 -10.88707  
4136  
4137 7 ooc X -2.53068

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

4138 Y -7.47011 11.37952  
4139 Z 23.05677 17.27339 -8.84884  
4140 7 com X 2.03755  
4141 Y -11.77918 8.84926  
4142 Z 22.67909 18.38270 -10.88681  
4143  
4144 8 ooc X -6.94364  
4145 Y -4.63967 16.18096  
4146 Z 26.15523 13.87611 -9.23732  
4147 8 com X -1.50928  
4148 Y -10.09997 12.39609  
4149 Z 26.31927 12.63174 -10.88681  
4150  
4151 9 ooc X -2.53144  
4152 Y -7.47016 11.38081  
4153 Z 23.05701 17.27336 -8.84937  
4154 9 com X 2.03697  
4155 Y -11.77876 8.85051  
4156 Z 22.67978 18.38248 -10.88748  
4157  
4158 10 ooc X -6.94171  
4159 Y -4.64019 16.17996  
4160 Z 26.15602 13.87672 -9.23825  
4161 10 com X -1.50808  
4162 Y -10.10037 12.39557  
4163 Z 26.31940 12.63240 -10.88748  
4164  
4165 11 ooc X -13.86098  
4166 Y 3.35645 30.58090  
4167 Z 15.53735 6.34346 -16.71993  
4168 11 com X -2.89807  
4169 Y 6.64732 25.29744  
4170 Z 13.32134 5.71365 -22.39938  
4171  
4172 12 ooc X 34.53180  
4173 Y 4.37262 -5.38635  
4174 Z 11.99000 11.24280 -29.14545  
4175 12 com X 25.38644  
4176 Y -6.45520 -2.98708  
4177 Z 10.78376 9.68580 -22.39937  
4178  
4179 13 ooc X -13.02510  
4180 Y -8.06725 14.33749  
4181 Z 26.87810 14.19440 -1.31239  
4182 13 com X -8.21810

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

4183 Y -10.09760 12.13706  
4184 Z 25.98863 15.88152 -3.91897  
4185  
4186 14 ooc X -3.72068  
4187 Y -7.88463 7.39642  
4188 Z 26.56131 19.91630 -3.67575  
4189 14 com X 0.82115  
4190 Y -14.29157 3.09782  
4191 Z 25.34445 16.89056 -3.91897  
4192  
4193 15 ooc X -20.31001  
4194 Y 4.98306 30.25000  
4195 Z 16.06261 7.21350 -9.93998  
4196 15 com X -17.74934  
4197 Y 8.27589 27.55883  
4198 Z 11.11679 4.99956 -9.80949  
4199  
4200 16 ooc X 35.63349  
4201 Y -6.20351 -18.00234  
4202 Z 6.02229 6.97591 -17.63115  
4203 16 com X 25.96551  
4204 Y -11.75343 -16.15601  
4205 Z 9.17578 8.02395 -9.80950  
4206  
4207 17 ooc X -20.31029  
4208 Y 4.98281 30.25002  
4209 Z 16.06157 7.21226 -9.93974  
4210 17 com X -17.74982  
4211 Y 8.27559 27.55870  
4212 Z 11.11530 4.99828 -9.80888  
4213  
4214 18 ooc X 35.63320  
4215 Y -6.20361 -18.00233  
4216 Z 6.01955 6.97476 -17.63087  
4217 18 com X 25.96516  
4218 Y -11.75356 -16.15627  
4219 Z 9.17396 8.02315 -9.80889  
4220  
4221 19 ooc X -14.17167  
4222 Y -9.02599 13.58768  
4223 Z 6.42660 2.28809 0.58400  
4224 19 com X -12.34952  
4225 Y -11.29749 8.13093  
4226 Z -2.20045 2.96673 4.21860  
4227

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

4228 20 ooc X -5.96236  
4229 Y -8.54270 7.62267  
4230 Z -10.95630 -5.14314 -1.66032  
4231 20 com X -4.06997  
4232 Y -15.12121 -0.14862  
4233 Z 1.77078 -3.24159 4.21859  
4234  
4235 21 ooc X -5.66039  
4236 Y -0.76221 16.94400  
4237 Z 24.55729 0.62901 -11.28361  
4238 21 com X 5.43538  
4239 Y -0.00075 3.07491  
4240 Z 7.47837 2.55397 -8.51029  
4241  
4242 22 ooc X 4.70019  
4243 Y 14.72767 17.25635  
4244 Z -0.08683 14.40123 -21.95654  
4245 22 com X 3.55997  
4246 Y 0.95377 4.95031  
4247 Z 5.66827 5.50630 -8.51028  
4248  
4249 23 ooc X -5.65948  
4250 Y -0.76250 16.94337  
4251 Z 24.55690 0.62961 -11.28390  
4252 23 com X 5.43581  
4253 Y -0.00089 3.07446  
4254 Z 7.47818 2.55429 -8.51027  
4255  
4256 24 ooc X 4.69990  
4257 Y 14.72762 17.25675  
4258 Z -0.08677 14.40051 -21.95665  
4259 24 com X 3.55958  
4260 Y 0.95404 4.95069  
4261 Z 5.66846 5.50602 -8.51027  
4262  
4263 25 ooc X -5.65984  
4264 Y -0.76273 16.94388  
4265 Z 24.55680 0.62952 -11.28404  
4266 25 com X 5.43549  
4267 Y -0.00107 3.07484  
4268 Z 7.47799 2.55419 -8.51032  
4269  
4270 26 ooc X 4.70001  
4271 Y 14.72723 17.25680  
4272 Z -0.08688 14.40039 -21.95681

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

4273 26 com X 3.55967  
4274 Y 0.95365 4.95065  
4275 Z 5.66832 5.50587 -8.51032  
4276  
4277 27 ooc X -5.65953  
4278 Y -0.76228 16.94301  
4279 Z 24.55712 0.62950 -11.28349  
4280 27 com X 5.43590  
4281 Y -0.00080 3.07420  
4282 Z 7.47841 2.55427 -8.51011  
4283  
4284 28 ooc X 4.69986  
4285 Y 14.72802 17.25646  
4286 Z -0.08663 14.40084 -21.95632  
4287 28 com X 3.55947  
4288 Y 0.95424 4.95064  
4289 Z 5.66853 5.50622 -8.51011  
4290  
4291 29 ooc X -13.62223  
4292 Y 4.13358 0.96711  
4293 Z -13.86029 -9.42668 12.65511  
4294 29 com X 3.02434  
4295 Y -5.39726 -6.75737  
4296 Z -7.41617 -10.30531 3.73304  
4297  
4298 30 ooc X -11.68585  
4299 Y 16.42673 6.03958  
4300 Z -5.94194 -3.47380 5.64627  
4301 30 com X -9.15012  
4302 Y 0.00411 5.41710  
4303 Z -12.42741 -2.59970 3.73303  
4304  
4305 31 ooc X -8.77148  
4306 Y -6.97707 9.24399  
4307 Z -24.33867 -15.72368 -0.47252  
4308 31 com X -4.21301  
4309 Y -12.83376 7.36254  
4310 Z -24.33892 -15.72356 -3.14954  
4311  
4312 32 ooc X -8.77008  
4313 Y -6.97747 9.24271  
4314 Z -24.33888 -15.72386 -0.47263  
4315 32 com X -4.21191  
4316 Y -12.83425 7.36145  
4317 Z -24.33863 -15.72399 -3.14954

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

4318  
4319 33 ooc X -10.95985  
4320 Y -9.97125 8.79373  
4321 Z -9.91576 0.68055 2.16612  
4322 33 com X -8.37345  
4323 Y -15.52914 5.22613  
4324 Z 0.96383 -1.47241 3.14731  
4325  
4326 34 ooc X -10.95564  
4327 Y -9.97239 8.78986  
4328 Z 9.91720 -0.67967 2.16578  
4329 34 com X -8.36994  
4330 Y -15.53065 5.22263  
4331 Z -0.96234 1.47338 3.14730  
4332  
4333 35 ooc X 16.20878  
4334 Y 1.48682 -1.58369  
4335 Z -12.49419 -5.95001 -14.62509  
4336 35 com X 19.05329  
4337 Y 2.81072 -9.32674  
4338 Z -1.77307 -4.46800 -9.72654  
4339  
4340 36 ooc X 3.79665  
4341 Y 20.07759 16.89155  
4342 Z 0.27389 -1.41747 -20.68820  
4343 36 com X -0.70392  
4344 Y 13.35152 10.43046  
4345 Z -4.77666 0.53880 -9.72655  
4346  
4347 37 ooc X -10.95751  
4348 Y -9.97307 8.79234  
4349 Z -9.91619 0.67965 2.16517  
4350 37 com X -8.37203  
4351 Y -15.53031 5.22502  
4352 Z 0.96345 -1.47318 3.14701  
4353  
4354 38 ooc X -10.95689  
4355 Y -9.97312 8.79184  
4356 Z 9.91638 -0.67953 2.16506  
4357 38 com X -8.37167  
4358 Y -15.53047 5.22466  
4359 Z -0.96319 1.47335 3.14701  
4360  
4361 39 ooc X -10.95818  
4362 Y -9.97213 8.79178

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

4363 Z -9.91653 0.67994 2.16640  
4364 39 com X -8.37206  
4365 Y -15.53018 5.22447  
4366 Z 0.96289 -1.47307 3.14759  
4367  
4368 40 ooc X -10.95806  
4369 Y -9.97210 8.79170  
4370 Z 9.91614 -0.68019 2.16636  
4371 40 com X -8.37200  
4372 Y -15.53021 5.22440  
4373 Z -0.96332 1.47279 3.14760  
4374  
4375 41 ooc X -10.95680  
4376 Y -9.97172 8.79222  
4377 Z -9.91614 0.67999 2.16458  
4378 41 com X -8.37134  
4379 Y -15.52931 5.22460  
4380 Z 0.96364 -1.47301 3.14674  
4381  
4382 42 ooc X -10.95655  
4383 Y -9.97179 8.79197  
4384 Z 9.91676 -0.67959 2.16458  
4385 42 com X -8.37114  
4386 Y -15.52939 5.22439  
4387 Z -0.96296 1.47345 3.14676  
4388  
4389 43 ooc X -10.95706  
4390 Y -9.97210 8.79183  
4391 Z -9.91678 0.67990 2.16523  
4392 43 com X -8.37125  
4393 Y -15.52988 5.22438  
4394 Z 0.96294 -1.47309 3.14687  
4395  
4396 44 ooc X -10.95735  
4397 Y -9.97189 8.79218  
4398 Z 9.91636 -0.68017 2.16517  
4399 44 com X -8.37169  
4400 Y -15.52971 5.22481  
4401 Z -0.96332 1.47285 3.14687  
4402  
4403 45 ooc X -10.95803  
4404 Y -9.97181 8.79246  
4405 Z -9.91627 0.68013 2.16557  
4406 45 com X -8.37216  
4407 Y -15.52964 5.22505

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
4408      Z   0.96333 -1.47287  3.14711
4409
4410 46 ooc X -10.95693
4411      Y  -9.97216  8.79142
4412      Z   9.91662 -0.67991  2.16551
4413 46 com X -8.37119
4414      Y  -15.53006  5.22408
4415      Z  -0.96296  1.47311  3.14711
4416
4417 Traceless Quadrupole moments (Buckingham) in principal axes computed:
4418 -with respect to the origin of coordinates (ooc)
4419 -with respect to the center of mass (com)
4420 #MEP      X       Y       Z
4421 1 ooc X 12.80610
4422      Y  0.00000  4.71039
4423      Z  0.00000 -0.00000 -17.51649
4424 1 com X 10.56694
4425      Y -0.00000  5.73747
4426      Z  0.00000  0.00000 -16.30441
4427
4428 2 ooc X 24.29523
4429      Y -0.00000 -2.86205
4430      Z -0.00000  0.00000 -21.43318
4431 2 com X 10.56695
4432      Y -0.00000  5.73747
4433      Z  0.00000  0.00000 -16.30443
4434
4435 3 ooc X 23.28277
4436      Y  0.00000 12.57320
4437      Z -0.00000  0.00000 -35.85598
4438 3 com X 20.57720
4439      Y  0.00000 17.53163
4440      Z -0.00000  0.00000 -38.10883
4441
4442 4 ooc X 24.88040
4443      Y  0.00000 12.69174
4444      Z  0.00000  0.00000 -37.57214
4445 4 com X 20.57720
4446      Y  0.00000 17.53163
4447      Z  0.00000  0.00000 -38.10883
4448
4449 5 ooc X 23.28240
4450      Y  0.00000 12.57345
4451      Z -0.00000 -0.00000 -35.85585
4452 5 com X 20.57689
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

4453 Y 0.00000 17.53177  
4454 Z 0.00000 0.00000 -38.10865  
4455  
4456 6 ooc X 24.88014  
4457 Y 0.00000 12.69164  
4458 Z 0.00000 0.00000 -37.57179  
4459 6 com X 20.57689  
4460 Y 0.00000 17.53177  
4461 Z 0.00000 0.00000 -38.10866  
4462  
4463 7 ooc X 23.28249  
4464 Y 0.00000 12.57303  
4465 Z -0.00000 0.00000 -35.85553  
4466 7 com X 20.57676  
4467 Y 0.00000 17.53163  
4468 Z -0.00000 0.00000 -38.10839  
4469  
4470 8 ooc X 24.88037  
4471 Y 0.00000 12.69101  
4472 Z 0.00000 0.00000 -37.57138  
4473 8 com X 20.57676  
4474 Y 0.00000 17.53164  
4475 Z 0.00000 0.00000 -38.10840  
4476  
4477 9 ooc X 23.28284  
4478 Y 0.00000 12.57326  
4479 Z -0.00000 -0.00000 -35.85611  
4480 9 com X 20.57727  
4481 Y 0.00000 17.53165  
4482 Z 0.00000 0.00000 -38.10892  
4483  
4484 10 ooc X 24.88037  
4485 Y 0.00000 12.69185  
4486 Z 0.00000 0.00000 -37.57222  
4487 10 com X 20.57727  
4488 Y -0.00000 17.53165  
4489 Z 0.00000 0.00000 -38.10892  
4490  
4491 11 ooc X 32.10917  
4492 Y 0.00000 -1.11797  
4493 Z -0.00000 0.00000 -30.99120  
4494 11 com X 28.33442  
4495 Y 0.00000 0.90618  
4496 Z 0.00000 0.00000 -29.24060  
4497

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

4498 12 ooc X 37.67503  
4499 Y 0.00000 -2.70736  
4500 Z -0.00000 0.00000 -34.96768  
4501 12 com X 28.33441  
4502 Y -0.00000 0.90619  
4503 Z 0.00000 0.00000 -29.24061  
4504  
4505 13 ooc X 25.34940  
4506 Y -0.00000 13.69800  
4507 Z 0.00000 -0.00000 -39.04740  
4508 13 com X 23.96405  
4509 Y -0.00000 14.73855  
4510 Z -0.00000 -0.00000 -38.70259  
4511  
4512 14 ooc X 28.03776  
4513 Y -0.00000 10.79590  
4514 Z 0.00000 -0.00000 -38.83367  
4515 14 com X 23.96405  
4516 Y -0.00000 14.73855  
4517 Z 0.00000 0.00000 -38.70260  
4518  
4519 15 ooc X 32.73059  
4520 Y 0.00000 -0.72606  
4521 Z 0.00000 -0.00000 -32.00453  
4522 15 com X 30.26097  
4523 Y -0.00000 -4.37635  
4524 Z 0.00000 0.00000 -25.88462  
4525  
4526 16 ooc X 36.84618  
4527 Y -0.00000 -10.83837  
4528 Z 0.00000 -0.00000 -26.00780  
4529 16 com X 30.26098  
4530 Y -0.00000 -4.37635  
4531 Z 0.00000 0.00000 -25.88463  
4532  
4533 17 ooc X 32.72994  
4534 Y 0.00000 -0.72633  
4535 Z 0.00000 0.00000 -32.00362  
4536 17 com X 30.26018  
4537 Y -0.00000 -4.37670  
4538 Z 0.00000 0.00000 -25.88348  
4539  
4540 18 ooc X 36.84541  
4541 Y -0.00000 -10.83937  
4542 Z -0.00000 -0.00000 -26.00604

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

4543 18 com X 30.26017  
4544 Y -0.00000 -4.37672  
4545 Z 0.00000 0.00000 -25.88345  
4546  
4547 19 ooc X 16.27378  
4548 Y 0.00000 2.92110  
4549 Z 0.00000 0.00000 -19.19488  
4550 19 com X 14.41490  
4551 Y 0.00000 2.97314  
4552 Z -0.00000 0.00000 -17.38804  
4553  
4554 20 ooc X 11.74666  
4555 Y -0.00000 6.94026  
4556 Z -0.00000 0.00000 -18.68692  
4557 20 com X 14.41490  
4558 Y -0.00000 2.97313  
4559 Z -0.00000 -0.00000 -17.38803  
4560  
4561 21 ooc X 16.99314  
4562 Y -0.00000 16.21560  
4563 Z -0.00000 0.00000 -33.20874  
4564 21 com X 8.87453  
4565 Y 0.00000 3.24990  
4566 Z -0.00000 0.00000 -12.12443  
4567  
4568 22 ooc X 29.85417  
4569 Y 0.00000 -2.40370  
4570 Z -0.00000 0.00000 -27.45047  
4571 22 com X 8.87452  
4572 Y 0.00000 3.24990  
4573 Z -0.00000 0.00000 -12.12442  
4574  
4575 23 ooc X 16.99250  
4576 Y -0.00000 16.21564  
4577 Z -0.00000 -0.00000 -33.20814  
4578 23 com X 8.87472  
4579 Y 0.00000 3.24961  
4580 Z 0.00000 0.00000 -12.12433  
4581  
4582 24 ooc X 29.85406  
4583 Y -0.00000 -2.40403  
4584 Z -0.00000 0.00000 -27.45003  
4585 24 com X 8.87473  
4586 Y 0.00000 3.24961  
4587 Z -0.00000 0.00000 -12.12433

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

4588  
4589 25 ooc X 16.99306  
4590 Y -0.00000 16.21521  
4591 Z -0.00000 0.00000 -33.20828  
4592 25 com X 8.87426  
4593 Y 0.00000 3.25002  
4594 Z 0.00000 0.00000 -12.12428  
4595  
4596 26 ooc X 29.85372  
4597 Y -0.00000 -2.40365  
4598 Z -0.00000 0.00000 -27.45007  
4599 26 com X 8.87426  
4600 Y 0.00000 3.25002  
4601 Z -0.00000 -0.00000 -12.12429  
4602  
4603 27 ooc X 16.99213  
4604 Y -0.00000 16.21602  
4605 Z -0.00000 0.00000 -33.20814  
4606 27 com X 8.87502  
4607 Y 0.00000 3.24932  
4608 Z -0.00000 0.00000 -12.12434  
4609  
4610 28 ooc X 29.85436  
4611 Y -0.00000 -2.40435  
4612 Z -0.00000 -0.00000 -27.45001  
4613 28 com X 8.87501  
4614 Y 0.00000 3.24931  
4615 Z -0.00000 0.00000 -12.12433  
4616  
4617 29 ooc X 23.34807  
4618 Y -0.00000 -3.76624  
4619 Z 0.00000 0.00000 -19.58183  
4620 29 com X 12.04248  
4621 Y -0.00000 4.74610  
4622 Z 0.00000 -0.00000 -16.78858  
4623  
4624 30 ooc X 18.68799  
4625 Y 0.00000 3.21541  
4626 Z -0.00000 -0.00000 -21.90340  
4627 30 com X 12.04248  
4628 Y -0.00000 4.74610  
4629 Z -0.00000 -0.00000 -16.78858  
4630  
4631 31 ooc X 25.03159  
4632 Y 0.00000 9.83719

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

4633 Z -0.00000 -0.00000 -34.86878  
4634 31 com X 21.52450  
4635 Y -0.00000 15.65325  
4636 Z -0.00000 -0.00000 -37.17775  
4637  
4638 32 ooc X 25.03147  
4639 Y -0.00000 9.83729  
4640 Z -0.00000 -0.00000 -34.86876  
4641 32 com X 21.52449  
4642 Y -0.00000 15.65325  
4643 Z 0.00000 -0.00000 -37.17774  
4644  
4645 33 ooc X 14.90611  
4646 Y 0.00000 4.06378  
4647 Z -0.00000 0.00000 -18.96988  
4648 33 com X 15.62712  
4649 Y 0.00000 2.89916  
4650 Z -0.00000 -0.00000 -18.52627  
4651  
4652 34 ooc X 14.90530  
4653 Y 0.00000 4.06362  
4654 Z -0.00000 -0.00000 -18.96892  
4655 34 com X 15.62710  
4656 Y 0.00000 2.89915  
4657 Z -0.00000 0.00000 -18.52625  
4658  
4659 35 ooc X 21.15575  
4660 Y 0.00000 -0.69826  
4661 Z 0.00000 -0.00000 -20.45749  
4662 35 com X 19.49834  
4663 Y 0.00000 -5.48552  
4664 Z 0.00000 0.00000 -14.01282  
4665  
4666 36 ooc X 31.48097  
4667 Y -0.00000 -10.66350  
4668 Z 0.00000 -0.00000 -20.81747  
4669 36 com X 19.49834  
4670 Y 0.00000 -5.48552  
4671 Z 0.00000 -0.00000 -14.01282  
4672  
4673 37 ooc X 14.90648  
4674 Y 0.00000 4.06338  
4675 Z -0.00000 0.00000 -18.96987  
4676 37 com X 15.62794  
4677 Y 0.00000 2.89875

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

4678 Z -0.00000 -0.00000 -18.52669  
4679  
4680 38 ooc X 14.90631  
4681 Y 0.00000 4.06334  
4682 Z 0.00000 -0.00000 -18.96965  
4683 38 com X 15.62794  
4684 Y -0.00000 2.89875  
4685 Z -0.00000 0.00000 -18.52669  
4686  
4687 39 ooc X 14.90581  
4688 Y 0.00000 4.06409  
4689 Z -0.00000 0.00000 -18.96989  
4690 39 com X 15.62733  
4691 Y -0.00000 2.89942  
4692 Z 0.00000 -0.00000 -18.52676  
4693  
4694 40 ooc X 14.90578  
4695 Y 0.00000 4.06370  
4696 Z 0.00000 -0.00000 -18.96948  
4697 40 com X 15.62733  
4698 Y 0.00000 2.89943  
4699 Z 0.00000 0.00000 -18.52676  
4700  
4701 41 ooc X 14.90568  
4702 Y 0.00000 4.06294  
4703 Z -0.00000 0.00000 -18.96862  
4704 41 com X 15.62693  
4705 Y 0.00000 2.89848  
4706 Z -0.00000 -0.00000 -18.52541  
4707  
4708 42 ooc X 14.90560  
4709 Y 0.00000 4.06341  
4710 Z 0.00000 -0.00000 -18.96901  
4711 42 com X 15.62692  
4712 Y 0.00000 2.89849  
4713 Z -0.00000 0.00000 -18.52541  
4714  
4715 43 ooc X 14.90593  
4716 Y 0.00000 4.06347  
4717 Z -0.00000 0.00000 -18.96940  
4718 43 com X 15.62724  
4719 Y -0.00000 2.89870  
4720 Z 0.00000 -0.00000 -18.52594  
4721  
4722 44 ooc X 14.90594

4723 Y 0.00000 4.06319  
4724 Z 0.00000 -0.00000 -18.96913  
4725 44 com X 15.62726  
4726 Y 0.00000 2.89870  
4727 Z 0.00000 0.00000 -18.52596  
4728  
4729 45 ooc X 14.90593  
4730 Y 0.00000 4.06353  
4731 Z -0.00000 0.00000 -18.96946  
4732 45 com X 15.62723  
4733 Y -0.00000 2.89892  
4734 Z -0.00000 -0.00000 -18.52616  
4735  
4736 46 ooc X 14.90574  
4737 Y 0.00000 4.06348  
4738 Z -0.00000 -0.00000 -18.96922  
4739 46 com X 15.62723  
4740 Y 0.00000 2.89893  
4741 Z -0.00000 0.00000 -18.52616

---

### B.3.6 Fitting statistics of a fragment approach

---

1  
2 -----  
3 Restrained ESP Fit 2.4 q4md-forcefieldtools  
4 -----  
5 RESP-A1 - RESP **input** generated by PyRED version SEP-2015  
6 -----  
7  
8  
9 inopt = 0 ioutopt = 1  
10 nmep = 46 iqopt = 2  
11 ihfree = 1 irstrnt = 1  
12 iunits = 0 qwt = 0.00100000  
13  
14 multiple-MEP run of 46 MEP  
15  
16 Reading **input for** MEP 1 weight: 1.000  
17 PEG2 nconf=1 norient=1 nmep=1/46  
18  
19 Total charge (ich): 0  
20 Number of centers: 17  
21 1 8 -1  
22 2 1 -1  
23 3 6 0

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
24      4   1   0
25      5   1   4
26      6   6   0
27      7   1   0
28      8   1   7
29      9   8  -1
30     10   6   0
31     11   1   0
32     12   1  11
33     13   6   0
34     14   1   0
35     15   1  14
36     16   8  -1
37     17   1  -1
38
39 Reading input for MEP 2 weight: 1.000
40 PEG2 nconf=1 norient=2 nmep=2/46
41
42 Total charge (ich): 0
43 Number of centers: 17
44     18   8  -1
45     19   1  -1
46     20   6   0
47     21   1   0
48     22   1   4
49     23   6   0
50     24   1   0
51     25   1   7
52     26   8  -1
53     27   6   0
54     28   1   0
55     29   1  11
56     30   6   0
57     31   1   0
58     32   1  14
59     33   8  -1
60     34   1  -1
61
62 Reading input for MEP 3 weight: 1.000
63 PEG2 nconf=2 norient=1 nmep=3/46
64
65 Total charge (ich): 0
66 Number of centers: 17
67     35   8  -1
68     36   1  -1
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
69      37   6   0
70      38   1   0
71      39   1   4
72      40   6   0
73      41   1   0
74      42   1   7
75      43   8  -1
76      44   6   0
77      45   1   0
78      46   1  11
79      47   6   0
80      48   1   0
81      49   1  14
82      50   8  -1
83      51   1  -1
84
85      Reading input for MEP 4 weight: 1.000
86      PEG2 nconf=2 norient=2 nmep=4/46
87
88      Total charge (ich): 0
89      Number of centers: 17
90      52   8  -1
91      53   1  -1
92      54   6   0
93      55   1   0
94      56   1   4
95      57   6   0
96      58   1   0
97      59   1   7
98      60   8  -1
99      61   6   0
100     62   1   0
101     63   1  11
102     64   6   0
103     65   1   0
104     66   1  14
105     67   8  -1
106     68   1  -1
107
108     Reading input for MEP 5 weight: 1.000
109     PEG2 nconf=3 norient=1 nmep=5/46
110
111     Total charge (ich): 0
112     Number of centers: 17
113     69   8  -1
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
114      70   1   -1
115      71   6    0
116      72   1    0
117      73   1    4
118      74   6    0
119      75   1    0
120      76   1    7
121      77   8   -1
122      78   6    0
123      79   1    0
124      80   1   11
125      81   6    0
126      82   1    0
127      83   1   14
128      84   8   -1
129      85   1   -1
130
131      Reading input for MEP 6 weight: 1.000
132      PEG2 nconf=3 norient=2 nmep=6/46
133
134      Total charge (ich): 0
135      Number of centers: 17
136      86   8   -1
137      87   1   -1
138      88   6    0
139      89   1    0
140      90   1    4
141      91   6    0
142      92   1    0
143      93   1    7
144      94   8   -1
145      95   6    0
146      96   1    0
147      97   1   11
148      98   6    0
149      99   1    0
150      100  1   14
151      101  8   -1
152      102  1   -1
153
154      Reading input for MEP 7 weight: 1.000
155      PEG2 nconf=4 norient=1 nmep=7/46
156
157      Total charge (ich): 0
158      Number of centers: 17
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
159 103 8 -1
160 104 1 -1
161 105 6 0
162 106 1 0
163 107 1 4
164 108 6 0
165 109 1 0
166 110 1 7
167 111 8 -1
168 112 6 0
169 113 1 0
170 114 1 11
171 115 6 0
172 116 1 0
173 117 1 14
174 118 8 -1
175 119 1 -1
176
177 Reading input for MEP 8 weight: 1.000
178 PEG2 nconf=4 norient=2 nmep=8/46
179
180 Total charge (ich): 0
181 Number of centers: 17
182 120 8 -1
183 121 1 -1
184 122 6 0
185 123 1 0
186 124 1 4
187 125 6 0
188 126 1 0
189 127 1 7
190 128 8 -1
191 129 6 0
192 130 1 0
193 131 1 11
194 132 6 0
195 133 1 0
196 134 1 14
197 135 8 -1
198 136 1 -1
199
200 Reading input for MEP 9 weight: 1.000
201 PEG2 nconf=5 norient=1 nmep=9/46
202
203 Total charge (ich): 0
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
204 Number of centers: 17
205 137 8 -1
206 138 1 -1
207 139 6 0
208 140 1 0
209 141 1 4
210 142 6 0
211 143 1 0
212 144 1 7
213 145 8 -1
214 146 6 0
215 147 1 0
216 148 1 11
217 149 6 0
218 150 1 0
219 151 1 14
220 152 8 -1
221 153 1 -1
222
223 Reading input for MEP 10 weight: 1.000
224 PEG2 nconf=5 norient=2 nmep=10/46
225
226 Total charge (ich): 0
227 Number of centers: 17
228 154 8 -1
229 155 1 -1
230 156 6 0
231 157 1 0
232 158 1 4
233 159 6 0
234 160 1 0
235 161 1 7
236 162 8 -1
237 163 6 0
238 164 1 0
239 165 1 11
240 166 6 0
241 167 1 0
242 168 1 14
243 169 8 -1
244 170 1 -1
245
246 Reading input for MEP 11 weight: 1.000
247 PEG2 nconf=6 norient=1 nmep=11/46
248
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
249 Total charge (ich): 0
250 Number of centers: 17
251 171 8 -1
252 172 1 -1
253 173 6 0
254 174 1 0
255 175 1 4
256 176 6 0
257 177 1 0
258 178 1 7
259 179 8 -1
260 180 6 0
261 181 1 0
262 182 1 11
263 183 6 0
264 184 1 0
265 185 1 14
266 186 8 -1
267 187 1 -1
268
269 Reading input for MEP 12 weight: 1.000
270 PEG2 nconf=6 norient=2 nmep=12/46
271
272 Total charge (ich): 0
273 Number of centers: 17
274 188 8 -1
275 189 1 -1
276 190 6 0
277 191 1 0
278 192 1 4
279 193 6 0
280 194 1 0
281 195 1 7
282 196 8 -1
283 197 6 0
284 198 1 0
285 199 1 11
286 200 6 0
287 201 1 0
288 202 1 14
289 203 8 -1
290 204 1 -1
291
292 Reading input for MEP 13 weight: 1.000
293 PEG2 nconf=7 norient=1 nmep=13/46
```

```
294
295 Total charge (ich): 0
296 Number of centers: 17
297 205 8 -1
298 206 1 -1
299 207 6 0
300 208 1 0
301 209 1 4
302 210 6 0
303 211 1 0
304 212 1 7
305 213 8 -1
306 214 6 0
307 215 1 0
308 216 1 11
309 217 6 0
310 218 1 0
311 219 1 14
312 220 8 -1
313 221 1 -1
314
315 Reading input for MEP 14 weight: 1.000
316 PEG2 nconf=7 norient=2 nmep=14/46
317
318 Total charge (ich): 0
319 Number of centers: 17
320 222 8 -1
321 223 1 -1
322 224 6 0
323 225 1 0
324 226 1 4
325 227 6 0
326 228 1 0
327 229 1 7
328 230 8 -1
329 231 6 0
330 232 1 0
331 233 1 11
332 234 6 0
333 235 1 0
334 236 1 14
335 237 8 -1
336 238 1 -1
337
338 Reading input for MEP 15 weight: 1.000
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
339 PEG2 nconf=8 norient=1 nmep=15/46
340
341 Total charge (ich): 0
342 Number of centers: 17
343 239 8 -1
344 240 1 -1
345 241 6 0
346 242 1 0
347 243 1 4
348 244 6 0
349 245 1 0
350 246 1 7
351 247 8 -1
352 248 6 0
353 249 1 0
354 250 1 11
355 251 6 0
356 252 1 0
357 253 1 14
358 254 8 -1
359 255 1 -1
360
361 Reading input for MEP 16 weight: 1.000
362 PEG2 nconf=8 norient=2 nmep=16/46
363
364 Total charge (ich): 0
365 Number of centers: 17
366 256 8 -1
367 257 1 -1
368 258 6 0
369 259 1 0
370 260 1 4
371 261 6 0
372 262 1 0
373 263 1 7
374 264 8 -1
375 265 6 0
376 266 1 0
377 267 1 11
378 268 6 0
379 269 1 0
380 270 1 14
381 271 8 -1
382 272 1 -1
383
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
384  Reading input for MEP 17 weight: 1.000
385  PEG2 nconf=9 norient=1 nmep=17/46
386
387  Total charge (ich): 0
388  Number of centers: 17
389    273   8   -1
390    274   1   -1
391    275   6    0
392    276   1    0
393    277   1    4
394    278   6    0
395    279   1    0
396    280   1    7
397    281   8   -1
398    282   6    0
399    283   1    0
400    284   1   11
401    285   6    0
402    286   1    0
403    287   1   14
404    288   8   -1
405    289   1   -1
406
407  Reading input for MEP 18 weight: 1.000
408  PEG2 nconf=9 norient=2 nmep=18/46
409
410  Total charge (ich): 0
411  Number of centers: 17
412    290   8   -1
413    291   1   -1
414    292   6    0
415    293   1    0
416    294   1    4
417    295   6    0
418    296   1    0
419    297   1    7
420    298   8   -1
421    299   6    0
422    300   1    0
423    301   1   11
424    302   6    0
425    303   1    0
426    304   1   14
427    305   8   -1
428    306   1   -1
```

```
429
430  Reading input for MEP 19 weight: 1.000
431  PEG2 nconf=10 norient=1 nmep=19/46
432
433  Total charge (ich): 0
434  Number of centers: 17
435    307   8   -1
436    308   1   -1
437    309   6    0
438    310   1    0
439    311   1    4
440    312   6    0
441    313   1    0
442    314   1    7
443    315   8   -1
444    316   6    0
445    317   1    0
446    318   1   11
447    319   6    0
448    320   1    0
449    321   1   14
450    322   8   -1
451    323   1   -1
452
453  Reading input for MEP 20 weight: 1.000
454  PEG2 nconf=10 norient=2 nmep=20/46
455
456  Total charge (ich): 0
457  Number of centers: 17
458    324   8   -1
459    325   1   -1
460    326   6    0
461    327   1    0
462    328   1    4
463    329   6    0
464    330   1    0
465    331   1    7
466    332   8   -1
467    333   6    0
468    334   1    0
469    335   1   11
470    336   6    0
471    337   1    0
472    338   1   14
473    339   8   -1
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
474    340    1   -1
475
476 Reading input for MEP 21 weight: 1.000
477 PEG2 nconf=11 norient=1 nmep=21/46
478
479 Total charge (ich): 0
480 Number of centers: 17
481    341    8   -1
482    342    1   -1
483    343    6    0
484    344    1    0
485    345    1    4
486    346    6    0
487    347    1    0
488    348    1    7
489    349    8   -1
490    350    6    0
491    351    1    0
492    352    1   11
493    353    6    0
494    354    1    0
495    355    1   14
496    356    8   -1
497    357    1   -1
498
499 Reading input for MEP 22 weight: 1.000
500 PEG2 nconf=11 norient=2 nmep=22/46
501
502 Total charge (ich): 0
503 Number of centers: 17
504    358    8   -1
505    359    1   -1
506    360    6    0
507    361    1    0
508    362    1    4
509    363    6    0
510    364    1    0
511    365    1    7
512    366    8   -1
513    367    6    0
514    368    1    0
515    369    1   11
516    370    6    0
517    371    1    0
518    372    1   14
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
519 373 8 -1
520 374 1 -1
521
522 Reading input for MEP 23 weight: 1.000
523 PEG2 nconf=12 norient=1 nmep=23/46
524
525 Total charge (ich): 0
526 Number of centers: 17
527 375 8 -1
528 376 1 -1
529 377 6 0
530 378 1 0
531 379 1 4
532 380 6 0
533 381 1 0
534 382 1 7
535 383 8 -1
536 384 6 0
537 385 1 0
538 386 1 11
539 387 6 0
540 388 1 0
541 389 1 14
542 390 8 -1
543 391 1 -1
544
545 Reading input for MEP 24 weight: 1.000
546 PEG2 nconf=12 norient=2 nmep=24/46
547
548 Total charge (ich): 0
549 Number of centers: 17
550 392 8 -1
551 393 1 -1
552 394 6 0
553 395 1 0
554 396 1 4
555 397 6 0
556 398 1 0
557 399 1 7
558 400 8 -1
559 401 6 0
560 402 1 0
561 403 1 11
562 404 6 0
563 405 1 0
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
564    406    1    14
565    407    8    -1
566    408    1    -1
567
568 Reading input for MEP 25 weight: 1.000
569 PEG2 nconf=13 norient=1 nmep=25/46
570
571 Total charge (ich): 0
572 Number of centers: 17
573    409    8    -1
574    410    1    -1
575    411    6     0
576    412    1     0
577    413    1     4
578    414    6     0
579    415    1     0
580    416    1     7
581    417    8    -1
582    418    6     0
583    419    1     0
584    420    1    11
585    421    6     0
586    422    1     0
587    423    1    14
588    424    8    -1
589    425    1    -1
590
591 Reading input for MEP 26 weight: 1.000
592 PEG2 nconf=13 norient=2 nmep=26/46
593
594 Total charge (ich): 0
595 Number of centers: 17
596    426    8    -1
597    427    1    -1
598    428    6     0
599    429    1     0
600    430    1     4
601    431    6     0
602    432    1     0
603    433    1     7
604    434    8    -1
605    435    6     0
606    436    1     0
607    437    1    11
608    438    6     0
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
609    439    1    0
610    440    1   14
611    441    8   -1
612    442    1   -1
613
614 Reading input for MEP 27 weight: 1.000
615 PEG2 nconf=14 norient=1 nmep=27/46
616
617 Total charge (ich): 0
618 Number of centers: 17
619    443    8   -1
620    444    1   -1
621    445    6    0
622    446    1    0
623    447    1    4
624    448    6    0
625    449    1    0
626    450    1    7
627    451    8   -1
628    452    6    0
629    453    1    0
630    454    1   11
631    455    6    0
632    456    1    0
633    457    1   14
634    458    8   -1
635    459    1   -1
636
637 Reading input for MEP 28 weight: 1.000
638 PEG2 nconf=14 norient=2 nmep=28/46
639
640 Total charge (ich): 0
641 Number of centers: 17
642    460    8   -1
643    461    1   -1
644    462    6    0
645    463    1    0
646    464    1    4
647    465    6    0
648    466    1    0
649    467    1    7
650    468    8   -1
651    469    6    0
652    470    1    0
653    471    1   11
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
654    472    6    0
655    473    1    0
656    474    1   14
657    475    8   -1
658    476    1   -1
659
660  Reading input for MEP 29 weight: 1.000
661  PEG2 nconf=15 norient=1 nmep=29/46
662
663  Total charge (ich): 0
664  Number of centers: 17
665    477    8   -1
666    478    1   -1
667    479    6    0
668    480    1    0
669    481    1    4
670    482    6    0
671    483    1    0
672    484    1    7
673    485    8   -1
674    486    6    0
675    487    1    0
676    488    1   11
677    489    6    0
678    490    1    0
679    491    1   14
680    492    8   -1
681    493    1   -1
682
683  Reading input for MEP 30 weight: 1.000
684  PEG2 nconf=15 norient=2 nmep=30/46
685
686  Total charge (ich): 0
687  Number of centers: 17
688    494    8   -1
689    495    1   -1
690    496    6    0
691    497    1    0
692    498    1    4
693    499    6    0
694    500    1    0
695    501    1    7
696    502    8   -1
697    503    6    0
698    504    1    0
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
699 505 1 11
700 506 6 0
701 507 1 0
702 508 1 14
703 509 8 -1
704 510 1 -1
705
706 Reading input for MEP 31 weight: 1.000
707 PEG2 nconf=16 norient=1 nmep=31/46
708
709 Total charge (ich): 0
710 Number of centers: 17
711 511 8 -1
712 512 1 -1
713 513 6 0
714 514 1 0
715 515 1 4
716 516 6 0
717 517 1 0
718 518 1 7
719 519 8 -1
720 520 6 0
721 521 1 0
722 522 1 11
723 523 6 0
724 524 1 0
725 525 1 14
726 526 8 -1
727 527 1 -1
728
729 Reading input for MEP 32 weight: 1.000
730 PEG2 nconf=16 norient=2 nmep=32/46
731
732 Total charge (ich): 0
733 Number of centers: 17
734 528 8 -1
735 529 1 -1
736 530 6 0
737 531 1 0
738 532 1 4
739 533 6 0
740 534 1 0
741 535 1 7
742 536 8 -1
743 537 6 0
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
744      538    1    0
745      539    1   11
746      540    6    0
747      541    1    0
748      542    1   14
749      543    8   -1
750      544    1   -1
751
752      Reading input for MEP 33 weight: 1.000
753      PEG2 nconf=17 norient=1 nmep=33/46
754
755      Total charge (ich): 0
756      Number of centers: 17
757      545    8   -1
758      546    1   -1
759      547    6    0
760      548    1    0
761      549    1    4
762      550    6    0
763      551    1    0
764      552    1    7
765      553    8   -1
766      554    6    0
767      555    1    0
768      556    1   11
769      557    6    0
770      558    1    0
771      559    1   14
772      560    8   -1
773      561    1   -1
774
775      Reading input for MEP 34 weight: 1.000
776      PEG2 nconf=17 norient=2 nmep=34/46
777
778      Total charge (ich): 0
779      Number of centers: 17
780      562    8   -1
781      563    1   -1
782      564    6    0
783      565    1    0
784      566    1    4
785      567    6    0
786      568    1    0
787      569    1    7
788      570    8   -1
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
789      571   6   0
790      572   1   0
791      573   1  11
792      574   6   0
793      575   1   0
794      576   1  14
795      577   8  -1
796      578   1  -1
797
798 Reading input for MEP 35 weight: 1.000
799 PEG2 nconf=18 norient=1 nmep=35/46
800
801 Total charge (ich): 0
802 Number of centers: 17
803      579   8  -1
804      580   1  -1
805      581   6   0
806      582   1   0
807      583   1   4
808      584   6   0
809      585   1   0
810      586   1   7
811      587   8  -1
812      588   6   0
813      589   1   0
814      590   1  11
815      591   6   0
816      592   1   0
817      593   1  14
818      594   8  -1
819      595   1  -1
820
821 Reading input for MEP 36 weight: 1.000
822 PEG2 nconf=18 norient=2 nmep=36/46
823
824 Total charge (ich): 0
825 Number of centers: 17
826      596   8  -1
827      597   1  -1
828      598   6   0
829      599   1   0
830      600   1   4
831      601   6   0
832      602   1   0
833      603   1   7
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
834    604    8    -1
835    605    6     0
836    606    1     0
837    607    1    11
838    608    6     0
839    609    1     0
840    610    1    14
841    611    8    -1
842    612    1    -1
843
844 Reading input for MEP 37 weight: 1.000
845 PEG2 nconf=19 norient=1 nmep=37/46
846
847 Total charge (ich): 0
848 Number of centers: 17
849    613    8    -1
850    614    1    -1
851    615    6     0
852    616    1     0
853    617    1     4
854    618    6     0
855    619    1     0
856    620    1     7
857    621    8    -1
858    622    6     0
859    623    1     0
860    624    1    11
861    625    6     0
862    626    1     0
863    627    1    14
864    628    8    -1
865    629    1    -1
866
867 Reading input for MEP 38 weight: 1.000
868 PEG2 nconf=19 norient=2 nmep=38/46
869
870 Total charge (ich): 0
871 Number of centers: 17
872    630    8    -1
873    631    1    -1
874    632    6     0
875    633    1     0
876    634    1     4
877    635    6     0
878    636    1     0
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
879    637    1    7
880    638    8   -1
881    639    6    0
882    640    1    0
883    641    1   11
884    642    6    0
885    643    1    0
886    644    1   14
887    645    8   -1
888    646    1   -1
889
890  Reading input for MEP 39 weight: 1.000
891  PEG2 nconf=20 norient=1 nmep=39/46
892
893  Total charge (ich): 0
894  Number of centers: 17
895    647    8   -1
896    648    1   -1
897    649    6    0
898    650    1    0
899    651    1    4
900    652    6    0
901    653    1    0
902    654    1    7
903    655    8   -1
904    656    6    0
905    657    1    0
906    658    1   11
907    659    6    0
908    660    1    0
909    661    1   14
910    662    8   -1
911    663    1   -1
912
913  Reading input for MEP 40 weight: 1.000
914  PEG2 nconf=20 norient=2 nmep=40/46
915
916  Total charge (ich): 0
917  Number of centers: 17
918    664    8   -1
919    665    1   -1
920    666    6    0
921    667    1    0
922    668    1    4
923    669    6    0
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
924    670    1    0
925    671    1    7
926    672    8   -1
927    673    6    0
928    674    1    0
929    675    1   11
930    676    6    0
931    677    1    0
932    678    1   14
933    679    8   -1
934    680    1   -1
935
936 Reading input for MEP 41 weight: 1.000
937 PEG2 nconf=21 norient=1 nmep=41/46
938
939 Total charge (ich): 0
940 Number of centers: 17
941    681    8   -1
942    682    1   -1
943    683    6    0
944    684    1    0
945    685    1    4
946    686    6    0
947    687    1    0
948    688    1    7
949    689    8   -1
950    690    6    0
951    691    1    0
952    692    1   11
953    693    6    0
954    694    1    0
955    695    1   14
956    696    8   -1
957    697    1   -1
958
959 Reading input for MEP 42 weight: 1.000
960 PEG2 nconf=21 norient=2 nmep=42/46
961
962 Total charge (ich): 0
963 Number of centers: 17
964    698    8   -1
965    699    1   -1
966    700    6    0
967    701    1    0
968    702    1    4
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
969    703    6    0
970    704    1    0
971    705    1    7
972    706    8   -1
973    707    6    0
974    708    1    0
975    709    1   11
976    710    6    0
977    711    1    0
978    712    1   14
979    713    8   -1
980    714    1   -1
981
982 Reading input for MEP 43 weight: 1.000
983 PEG2 nconf=22 norient=1 nmep=43/46
984
985 Total charge (ich): 0
986 Number of centers: 17
987    715    8   -1
988    716    1   -1
989    717    6    0
990    718    1    0
991    719    1    4
992    720    6    0
993    721    1    0
994    722    1    7
995    723    8   -1
996    724    6    0
997    725    1    0
998    726    1   11
999    727    6    0
1000   728    1    0
1001   729    1   14
1002   730    8   -1
1003   731    1   -1
1004
1005 Reading input for MEP 44 weight: 1.000
1006 PEG2 nconf=22 norient=2 nmep=44/46
1007
1008 Total charge (ich): 0
1009 Number of centers: 17
1010   732    8   -1
1011   733    1   -1
1012   734    6    0
1013   735    1    0
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
1014    736    1    4
1015    737    6    0
1016    738    1    0
1017    739    1    7
1018    740    8   -1
1019    741    6    0
1020    742    1    0
1021    743    1   11
1022    744    6    0
1023    745    1    0
1024    746    1   14
1025    747    8   -1
1026    748    1   -1
1027
1028 Reading input for MEP 45 weight: 1.000
1029 PEG2 nconf=23 norient=1 nmep=45/46
1030
1031 Total charge (ich): 0
1032 Number of centers: 17
1033    749    8   -1
1034    750    1   -1
1035    751    6    0
1036    752    1    0
1037    753    1    4
1038    754    6    0
1039    755    1    0
1040    756    1    7
1041    757    8   -1
1042    758    6    0
1043    759    1    0
1044    760    1   11
1045    761    6    0
1046    762    1    0
1047    763    1   14
1048    764    8   -1
1049    765    1   -1
1050
1051 Reading input for MEP 46 weight: 1.000
1052 PEG2 nconf=23 norient=2 nmep=46/46
1053
1054 Total charge (ich): 0
1055 Number of centers: 17
1056    766    8   -1
1057    767    1   -1
1058    768    6    0
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```

1059    769    1    0
1060    770    1    4
1061    771    6    0
1062    772    1    0
1063    773    1    7
1064    774    8   -1
1065    775    6    0
1066    776    1    0
1067    777    1   11
1068    778    6    0
1069    779    1    0
1070    780    1   14
1071    781    8   -1
1072    782    1   -1
1073    1    1    1    2    1    3    1    4    1    5    1    6    1    7    1    8
1074 since IQOPT>1, 782 new q0 values
1075 will be read in from file ESP.Q0 (unit 3)
1076 -----
1077 reading mult_esp constraint info
1078 -----
1079    1    3    2    3    3    3    4    3    5    3    6    3    7    3    8    3
1080    9    3   10    3   11    3   12    3   13    3   14    3   15    3   16    3
1081   17    3   18    3   19    3   20    3   21    3   22    3   23    3   24    3
1082   25    3   26    3   27    3   28    3   29    3   30    3   31    3   32    3
1083   33    3   34    3   35    3   36    3   37    3   38    3   39    3   40    3
1084   41    3   42    3   43    3   44    3   45    3   46    3
1085   1    4    2    4    3    4    4    4    5    4    6    4    7    4    8    4
1086   9    4   10    4   11    4   12    4   13    4   14    4   15    4   16    4
1087   17    4   18    4   19    4   20    4   21    4   22    4   23    4   24    4
1088   25    4   26    4   27    4   28    4   29    4   30    4   31    4   32    4
1089   33    4   34    4   35    4   36    4   37    4   38    4   39    4   40    4
1090   41    4   42    4   43    4   44    4   45    4   46    4
1091   1    6    2    6    3    6    4    6    5    6    6    6    7    6    8    6
1092   9    6   10    6   11    6   12    6   13    6   14    6   15    6   16    6
1093   17    6   18    6   19    6   20    6   21    6   22    6   23    6   24    6
1094   25    6   26    6   27    6   28    6   29    6   30    6   31    6   32    6
1095   33    6   34    6   35    6   36    6   37    6   38    6   39    6   40    6
1096   41    6   42    6   43    6   44    6   45    6   46    6
1097   1    7    2    7    3    7    4    7    5    7    6    7    7    7    8    7
1098   9    7   10    7   11    7   12    7   13    7   14    7   15    7   16    7
1099   17    7   18    7   19    7   20    7   21    7   22    7   23    7   24    7
1100   25    7   26    7   27    7   28    7   29    7   30    7   31    7   32    7
1101   33    7   34    7   35    7   36    7   37    7   38    7   39    7   40    7
1102   41    7   42    7   43    7   44    7   45    7   46    7
1103   1   10    2   10    3   10    4   10    5   10    6   10    7   10    8   10

```

**B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS**

---

1104	9	10	10	10	11	10	12	10	13	10	14	10	15	10	16	10
1105	17	10	18	10	19	10	20	10	21	10	22	10	23	10	24	10
1106	25	10	26	10	27	10	28	10	29	10	30	10	31	10	32	10
1107	33	10	34	10	35	10	36	10	37	10	38	10	39	10	40	10
1108	41	10	42	10	43	10	44	10	45	10	46	10				
1109	1	11	2	11	3	11	4	11	5	11	6	11	7	11	8	11
1110	9	11	10	11	11	11	12	11	13	11	14	11	15	11	16	11
1111	17	11	18	11	19	11	20	11	21	11	22	11	23	11	24	11
1112	25	11	26	11	27	11	28	11	29	11	30	11	31	11	32	11
1113	33	11	34	11	35	11	36	11	37	11	38	11	39	11	40	11
1114	41	11	42	11	43	11	44	11	45	11	46	11				
1115	1	13	2	13	3	13	4	13	5	13	6	13	7	13	8	13
1116	9	13	10	13	11	13	12	13	13	13	14	13	15	13	16	13
1117	17	13	18	13	19	13	20	13	21	13	22	13	23	13	24	13
1118	25	13	26	13	27	13	28	13	29	13	30	13	31	13	32	13
1119	33	13	34	13	35	13	36	13	37	13	38	13	39	13	40	13
1120	41	13	42	13	43	13	44	13	45	13	46	13				
1121	1	14	2	14	3	14	4	14	5	14	6	14	7	14	8	14
1122	9	14	10	14	11	14	12	14	13	14	14	14	15	14	16	14
1123	17	14	18	14	19	14	20	14	21	14	22	14	23	14	24	14
1124	25	14	26	14	27	14	28	14	29	14	30	14	31	14	32	14
1125	33	14	34	14	35	14	36	14	37	14	38	14	39	14	40	14
1126	41	14	42	14	43	14	44	14	45	14	46	14				
1127																
1128	-----															
1129	Atom	Ivary														
1130	-----															
1131	8	-1														
1132	1	-1														
1133	6	0														
1134	1	0														
1135	1	4														
1136	6	0														
1137	1	0														
1138	1	7														
1139	8	-1														
1140	6	0														
1141	1	0														
1142	1	11														
1143	6	0														
1144	1	0														
1145	1	14														
1146	8	-1														
1147	1	-1														
1148																

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1149	8	-1
1150	1	-1
1151	6	3
1152	1	4
1153	1	4
1154	6	6
1155	1	7
1156	1	7
1157	8	-1
1158	6	10
1159	1	11
1160	1	11
1161	6	13
1162	1	14
1163	1	14
1164	8	-1
1165	1	-1
1166		
1167	8	-1
1168	1	-1
1169	6	3
1170	1	4
1171	1	4
1172	6	6
1173	1	7
1174	1	7
1175	8	-1
1176	6	10
1177	1	11
1178	1	11
1179	6	13
1180	1	14
1181	1	14
1182	8	-1
1183	1	-1
1184		
1185	8	-1
1186	1	-1
1187	6	3
1188	1	4
1189	1	4
1190	6	6
1191	1	7
1192	1	7
1193	8	-1

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1194	6	10
1195	1	11
1196	1	11
1197	6	13
1198	1	14
1199	1	14
1200	8	-1
1201	1	-1
1202		
1203	8	-1
1204	1	-1
1205	6	3
1206	1	4
1207	1	4
1208	6	6
1209	1	7
1210	1	7
1211	8	-1
1212	6	10
1213	1	11
1214	1	11
1215	6	13
1216	1	14
1217	1	14
1218	8	-1
1219	1	-1
1220		
1221	8	-1
1222	1	-1
1223	6	3
1224	1	4
1225	1	4
1226	6	6
1227	1	7
1228	1	7
1229	8	-1
1230	6	10
1231	1	11
1232	1	11
1233	6	13
1234	1	14
1235	1	14
1236	8	-1
1237	1	-1
1238		

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1239	8	-1
1240	1	-1
1241	6	3
1242	1	4
1243	1	4
1244	6	6
1245	1	7
1246	1	7
1247	8	-1
1248	6	10
1249	1	11
1250	1	11
1251	6	13
1252	1	14
1253	1	14
1254	8	-1
1255	1	-1
1256		
1257	8	-1
1258	1	-1
1259	6	3
1260	1	4
1261	1	4
1262	6	6
1263	1	7
1264	1	7
1265	8	-1
1266	6	10
1267	1	11
1268	1	11
1269	6	13
1270	1	14
1271	1	14
1272	8	-1
1273	1	-1
1274		
1275	8	-1
1276	1	-1
1277	6	3
1278	1	4
1279	1	4
1280	6	6
1281	1	7
1282	1	7
1283	8	-1

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1284	6	10
1285	1	11
1286	1	11
1287	6	13
1288	1	14
1289	1	14
1290	8	-1
1291	1	-1
1292		
1293	8	-1
1294	1	-1
1295	6	3
1296	1	4
1297	1	4
1298	6	6
1299	1	7
1300	1	7
1301	8	-1
1302	6	10
1303	1	11
1304	1	11
1305	6	13
1306	1	14
1307	1	14
1308	8	-1
1309	1	-1
1310		
1311	8	-1
1312	1	-1
1313	6	3
1314	1	4
1315	1	4
1316	6	6
1317	1	7
1318	1	7
1319	8	-1
1320	6	10
1321	1	11
1322	1	11
1323	6	13
1324	1	14
1325	1	14
1326	8	-1
1327	1	-1
1328		

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1329	8	-1
1330	1	-1
1331	6	3
1332	1	4
1333	1	4
1334	6	6
1335	1	7
1336	1	7
1337	8	-1
1338	6	10
1339	1	11
1340	1	11
1341	6	13
1342	1	14
1343	1	14
1344	8	-1
1345	1	-1
1346		
1347	8	-1
1348	1	-1
1349	6	3
1350	1	4
1351	1	4
1352	6	6
1353	1	7
1354	1	7
1355	8	-1
1356	6	10
1357	1	11
1358	1	11
1359	6	13
1360	1	14
1361	1	14
1362	8	-1
1363	1	-1
1364		
1365	8	-1
1366	1	-1
1367	6	3
1368	1	4
1369	1	4
1370	6	6
1371	1	7
1372	1	7
1373	8	-1

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1374	6	10
1375	1	11
1376	1	11
1377	6	13
1378	1	14
1379	1	14
1380	8	-1
1381	1	-1
1382		
1383	8	-1
1384	1	-1
1385	6	3
1386	1	4
1387	1	4
1388	6	6
1389	1	7
1390	1	7
1391	8	-1
1392	6	10
1393	1	11
1394	1	11
1395	6	13
1396	1	14
1397	1	14
1398	8	-1
1399	1	-1
1400		
1401	8	-1
1402	1	-1
1403	6	3
1404	1	4
1405	1	4
1406	6	6
1407	1	7
1408	1	7
1409	8	-1
1410	6	10
1411	1	11
1412	1	11
1413	6	13
1414	1	14
1415	1	14
1416	8	-1
1417	1	-1
1418		

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1419	8	-1
1420	1	-1
1421	6	3
1422	1	4
1423	1	4
1424	6	6
1425	1	7
1426	1	7
1427	8	-1
1428	6	10
1429	1	11
1430	1	11
1431	6	13
1432	1	14
1433	1	14
1434	8	-1
1435	1	-1
1436		
1437	8	-1
1438	1	-1
1439	6	3
1440	1	4
1441	1	4
1442	6	6
1443	1	7
1444	1	7
1445	8	-1
1446	6	10
1447	1	11
1448	1	11
1449	6	13
1450	1	14
1451	1	14
1452	8	-1
1453	1	-1
1454		
1455	8	-1
1456	1	-1
1457	6	3
1458	1	4
1459	1	4
1460	6	6
1461	1	7
1462	1	7
1463	8	-1

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1464	6	10
1465	1	11
1466	1	11
1467	6	13
1468	1	14
1469	1	14
1470	8	-1
1471	1	-1
1472		
1473	8	-1
1474	1	-1
1475	6	3
1476	1	4
1477	1	4
1478	6	6
1479	1	7
1480	1	7
1481	8	-1
1482	6	10
1483	1	11
1484	1	11
1485	6	13
1486	1	14
1487	1	14
1488	8	-1
1489	1	-1
1490		
1491	8	-1
1492	1	-1
1493	6	3
1494	1	4
1495	1	4
1496	6	6
1497	1	7
1498	1	7
1499	8	-1
1500	6	10
1501	1	11
1502	1	11
1503	6	13
1504	1	14
1505	1	14
1506	8	-1
1507	1	-1
1508		

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1509	8	-1
1510	1	-1
1511	6	3
1512	1	4
1513	1	4
1514	6	6
1515	1	7
1516	1	7
1517	8	-1
1518	6	10
1519	1	11
1520	1	11
1521	6	13
1522	1	14
1523	1	14
1524	8	-1
1525	1	-1
1526		
1527	8	-1
1528	1	-1
1529	6	3
1530	1	4
1531	1	4
1532	6	6
1533	1	7
1534	1	7
1535	8	-1
1536	6	10
1537	1	11
1538	1	11
1539	6	13
1540	1	14
1541	1	14
1542	8	-1
1543	1	-1
1544		
1545	8	-1
1546	1	-1
1547	6	3
1548	1	4
1549	1	4
1550	6	6
1551	1	7
1552	1	7
1553	8	-1

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1554	6	10
1555	1	11
1556	1	11
1557	6	13
1558	1	14
1559	1	14
1560	8	-1
1561	1	-1
1562		
1563	8	-1
1564	1	-1
1565	6	3
1566	1	4
1567	1	4
1568	6	6
1569	1	7
1570	1	7
1571	8	-1
1572	6	10
1573	1	11
1574	1	11
1575	6	13
1576	1	14
1577	1	14
1578	8	-1
1579	1	-1
1580		
1581	8	-1
1582	1	-1
1583	6	3
1584	1	4
1585	1	4
1586	6	6
1587	1	7
1588	1	7
1589	8	-1
1590	6	10
1591	1	11
1592	1	11
1593	6	13
1594	1	14
1595	1	14
1596	8	-1
1597	1	-1
1598		

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1599	8	-1
1600	1	-1
1601	6	3
1602	1	4
1603	1	4
1604	6	6
1605	1	7
1606	1	7
1607	8	-1
1608	6	10
1609	1	11
1610	1	11
1611	6	13
1612	1	14
1613	1	14
1614	8	-1
1615	1	-1
1616		
1617	8	-1
1618	1	-1
1619	6	3
1620	1	4
1621	1	4
1622	6	6
1623	1	7
1624	1	7
1625	8	-1
1626	6	10
1627	1	11
1628	1	11
1629	6	13
1630	1	14
1631	1	14
1632	8	-1
1633	1	-1
1634		
1635	8	-1
1636	1	-1
1637	6	3
1638	1	4
1639	1	4
1640	6	6
1641	1	7
1642	1	7
1643	8	-1

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1644	6	10
1645	1	11
1646	1	11
1647	6	13
1648	1	14
1649	1	14
1650	8	-1
1651	1	-1
1652		
1653	8	-1
1654	1	-1
1655	6	3
1656	1	4
1657	1	4
1658	6	6
1659	1	7
1660	1	7
1661	8	-1
1662	6	10
1663	1	11
1664	1	11
1665	6	13
1666	1	14
1667	1	14
1668	8	-1
1669	1	-1
1670		
1671	8	-1
1672	1	-1
1673	6	3
1674	1	4
1675	1	4
1676	6	6
1677	1	7
1678	1	7
1679	8	-1
1680	6	10
1681	1	11
1682	1	11
1683	6	13
1684	1	14
1685	1	14
1686	8	-1
1687	1	-1
1688		

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1689	8	-1
1690	1	-1
1691	6	3
1692	1	4
1693	1	4
1694	6	6
1695	1	7
1696	1	7
1697	8	-1
1698	6	10
1699	1	11
1700	1	11
1701	6	13
1702	1	14
1703	1	14
1704	8	-1
1705	1	-1
1706		
1707	8	-1
1708	1	-1
1709	6	3
1710	1	4
1711	1	4
1712	6	6
1713	1	7
1714	1	7
1715	8	-1
1716	6	10
1717	1	11
1718	1	11
1719	6	13
1720	1	14
1721	1	14
1722	8	-1
1723	1	-1
1724		
1725	8	-1
1726	1	-1
1727	6	3
1728	1	4
1729	1	4
1730	6	6
1731	1	7
1732	1	7
1733	8	-1

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1734	6	10
1735	1	11
1736	1	11
1737	6	13
1738	1	14
1739	1	14
1740	8	-1
1741	1	-1
1742		
1743	8	-1
1744	1	-1
1745	6	3
1746	1	4
1747	1	4
1748	6	6
1749	1	7
1750	1	7
1751	8	-1
1752	6	10
1753	1	11
1754	1	11
1755	6	13
1756	1	14
1757	1	14
1758	8	-1
1759	1	-1
1760		
1761	8	-1
1762	1	-1
1763	6	3
1764	1	4
1765	1	4
1766	6	6
1767	1	7
1768	1	7
1769	8	-1
1770	6	10
1771	1	11
1772	1	11
1773	6	13
1774	1	14
1775	1	14
1776	8	-1
1777	1	-1
1778		

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1779	8	-1
1780	1	-1
1781	6	3
1782	1	4
1783	1	4
1784	6	6
1785	1	7
1786	1	7
1787	8	-1
1788	6	10
1789	1	11
1790	1	11
1791	6	13
1792	1	14
1793	1	14
1794	8	-1
1795	1	-1
1796		
1797	8	-1
1798	1	-1
1799	6	3
1800	1	4
1801	1	4
1802	6	6
1803	1	7
1804	1	7
1805	8	-1
1806	6	10
1807	1	11
1808	1	11
1809	6	13
1810	1	14
1811	1	14
1812	8	-1
1813	1	-1
1814		
1815	8	-1
1816	1	-1
1817	6	3
1818	1	4
1819	1	4
1820	6	6
1821	1	7
1822	1	7
1823	8	-1

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1824	6	10
1825	1	11
1826	1	11
1827	6	13
1828	1	14
1829	1	14
1830	8	-1
1831	1	-1
1832		
1833	8	-1
1834	1	-1
1835	6	3
1836	1	4
1837	1	4
1838	6	6
1839	1	7
1840	1	7
1841	8	-1
1842	6	10
1843	1	11
1844	1	11
1845	6	13
1846	1	14
1847	1	14
1848	8	-1
1849	1	-1
1850		
1851	8	-1
1852	1	-1
1853	6	3
1854	1	4
1855	1	4
1856	6	6
1857	1	7
1858	1	7
1859	8	-1
1860	6	10
1861	1	11
1862	1	11
1863	6	13
1864	1	14
1865	1	14
1866	8	-1
1867	1	-1
1868		

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1869	8	-1
1870	1	-1
1871	6	3
1872	1	4
1873	1	4
1874	6	6
1875	1	7
1876	1	7
1877	8	-1
1878	6	10
1879	1	11
1880	1	11
1881	6	13
1882	1	14
1883	1	14
1884	8	-1
1885	1	-1
1886		
1887	8	-1
1888	1	-1
1889	6	3
1890	1	4
1891	1	4
1892	6	6
1893	1	7
1894	1	7
1895	8	-1
1896	6	10
1897	1	11
1898	1	11
1899	6	13
1900	1	14
1901	1	14
1902	8	-1
1903	1	-1
1904		
1905	8	-1
1906	1	-1
1907	6	3
1908	1	4
1909	1	4
1910	6	6
1911	1	7
1912	1	7
1913	8	-1

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

1914	6	10
1915	1	11
1916	1	11
1917	6	13
1918	1	14
1919	1	14
1920	8	-1
1921	1	-1
1922		
1923	8	-1
1924	1	-1
1925	6	3
1926	1	4
1927	1	4
1928	6	6
1929	1	7
1930	1	7
1931	8	-1
1932	6	10
1933	1	11
1934	1	11
1935	6	13
1936	1	14
1937	1	14
1938	8	-1
1939	1	-1
1940		
1941	8	-1
1942	1	-1
1943	6	3
1944	1	4
1945	1	4
1946	6	6
1947	1	7
1948	1	7
1949	8	-1
1950	6	10
1951	1	11
1952	1	11
1953	6	13
1954	1	14
1955	1	14
1956	8	-1
1957	1	-1
1958		

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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1959 -----  
1960  
1961  
1962 Total number of atoms = 782  
1963 Weight factor on initial charge restraints= 0.001000  
1964  
1965  
1966 There are 47 charge constraints  
1967  
1968 Reading esp"s for MEP 1  
1969 total number of atoms = 17  
1970 total number of esp points = 809  
1971  
1972 Center X Y Z  
1973 1 0.4978215E+01 0.2437140E+01 -0.4366318E+01  
1974 2 0.4793776E+01 0.3312593E+01 -0.5963597E+01  
1975 3 0.3655614E+01 0.3833419E+01 -0.2498507E+01  
1976 4 0.1659618E+01 0.4112601E+01 -0.3022352E+01  
1977 5 0.4485629E+01 0.5725104E+01 -0.2210686E+01  
1978 6 0.3839901E+01 0.2415871E+01 0.0000000E+00  
1979 7 0.3033619E+01 0.3609201E+01 0.1507497E+01  
1980 8 0.5836249E+01 0.2080700E+01 0.4263770E+00  
1981 9 0.2686593E+01 0.0000000E+00 0.0000000E+00  
1982 10 0.0000000E+00 0.0000000E+00 0.0000000E+00  
1983 11 -0.7670908E+00 0.5504753E+00 -0.1854086E+01  
1984 12 -0.7163309E+00 0.1331210E+01 0.1433034E+01  
1985 13 -0.8033584E+00 -0.2680877E+01 0.6642557E+00  
1986 14 -0.1703059E+00 -0.3970396E+01 -0.8436512E+00  
1987 15 -0.2867062E+01 -0.2790946E+01 0.7758157E+00  
1988 16 0.1597215E+00 -0.3416785E+01 0.3050490E+01  
1989 17 0.1951496E+01 -0.3017114E+01 0.2982980E+01  
1990  
1991 Reading esp"s for MEP 2  
1992 total number of atoms = 17  
1993 total number of esp points = 805  
1994  
1995 Center X Y Z  
1996 1 -0.5095967E+00 -0.1018099E+01 0.4366318E+01  
1997 2 -0.1220183E+01 -0.4744951E+00 0.5963597E+01  
1998 3 -0.1199859E+01 0.7770062E+00 0.2498507E+01  
1999 4 -0.5919019E+00 0.2698551E+01 0.3022352E+01  
2000 5 -0.3264575E+01 0.8429331E+00 0.2210686E+01  
2001 6 0.0000000E+00 0.0000000E+00 0.0000000E+00  
2002 7 -0.7295514E+00 0.1241726E+01 -0.1507497E+01

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

2003 8 -0.5575826E+00 -0.1945981E+01 -0.4263770E+00  
2004 9 0.2677041E+01 0.0000000E+00 0.0000000E+00  
2005 10 0.3834464E+01 0.2424490E+01 0.0000000E+00  
2006 11 0.3668166E+01 0.3353897E+01 0.1854086E+01  
2007 12 0.2941733E+01 0.3644440E+01 -0.1433034E+01  
2008 13 0.6599893E+01 0.1994513E+01 -0.6642557E+00  
2009 14 0.7490881E+01 0.8676790E+00 0.8436512E+00  
2010 15 0.7588297E+01 0.3809465E+01 -0.7758157E+00  
2011 16 0.6849098E+01 0.8083511E+00 -0.3050490E+01  
2012 17 0.5716497E+01 -0.6364351E+00 -0.2982980E+01  
2013  
2014 Reading esp"s for MEP 3  
2015 total number of atoms = 17  
2016 total number of esp points = 803  
2017  
2018 Center X Y Z  
2019 1 0.5313660E+01 0.1803796E+01 -0.4290976E+01  
2020 2 0.4633126E+01 0.1401969E+00 -0.3909044E+01  
2021 3 0.4010528E+01 0.3496456E+01 -0.2678163E+01  
2022 4 0.2098730E+01 0.3912011E+01 -0.3399611E+01  
2023 5 0.5067053E+01 0.5277245E+01 -0.2670132E+01  
2024 6 0.3854349E+01 0.2440630E+01 0.0000000E+00  
2025 7 0.2834370E+01 0.3733664E+01 0.1270123E+01  
2026 8 0.5765082E+01 0.2149541E+01 0.7380873E+00  
2027 9 0.2698842E+01 0.0000000E+00 0.0000000E+00  
2028 10 0.0000000E+00 0.0000000E+00 0.0000000E+00  
2029 11 -0.7671758E+00 0.6775083E+00 -0.1810611E+01  
2030 12 -0.6983785E+00 0.1238266E+01 0.1517429E+01  
2031 13 -0.8077804E+00 -0.2714064E+01 0.4967315E+00  
2032 14 -0.1680666E+00 -0.3915510E+01 -0.1081099E+01  
2033 15 -0.2872338E+01 -0.2824749E+01 0.5817862E+00  
2034 16 0.1282349E+00 -0.3593970E+01 0.2842611E+01  
2035 17 0.1923705E+01 -0.3208479E+01 0.2827321E+01  
2036  
2037 Reading esp"s for MEP 4  
2038 total number of atoms = 17  
2039 total number of esp points = 813  
2040  
2041 Center X Y Z  
2042 1 -0.4887020E-01 -0.1591463E+01 0.4290976E+01  
2043 2 0.1745933E+01 -0.1688255E+01 0.3909044E+01  
2044 3 -0.1021108E+01 0.3106426E+00 0.2678163E+01  
2045 4 -0.5786171E+00 0.2216384E+01 0.3399611E+01  
2046 5 -0.3082720E+01 0.1177488E+00 0.2670132E+01  
2047 6 0.0000000E+00 0.0000000E+00 0.0000000E+00

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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2048      7      -0.7322121E+00    0.1475181E+01   -0.1270123E+01  
2049      8      -0.5545288E+00    -0.1851521E+01   -0.7380873E+00  
2050      9      0.2700347E+01    0.0000000E+00    0.0000000E+00  
2051      10     0.3855209E+01    0.2439271E+01    0.0000000E+00  
2052      11     0.3571147E+01    0.3422573E+01    0.1810611E+01  
2053      12     0.3034883E+01    0.3600347E+01    -0.1517429E+01  
2054      13     0.6653895E+01    0.2007985E+01    -0.4967315E+00  
2055      14     0.7466047E+01    0.9156856E+00    0.1081099E+01  
2056      15     0.7637380E+01    0.3826612E+01    -0.5817862E+00  
2057      16     0.7048642E+01    0.7854722E+00    -0.2842611E+01  
2058      17     0.5931926E+01    -0.6723569E+00   -0.2827321E+01  
2059  
2060     Reading esp"s for MEP 5  
2061     total number of atoms = 17  
2062     total number of esp points = 803  
2063  
2064     Center    X               Y               Z  
2065     1      0.5313484E+01    0.1803862E+01   -0.4291056E+01  
2066     2      0.4632977E+01    0.1402592E+00   -0.3909108E+01  
2067     3      0.4010431E+01    0.3496507E+01   -0.2678139E+01  
2068     4      0.2098624E+01    0.3912114E+01   -0.3399521E+01  
2069     5      0.5066992E+01    0.5277272E+01   -0.2670083E+01  
2070     6      0.3854336E+01    0.2440615E+01   0.0000000E+00  
2071     7      0.2834410E+01    0.3733636E+01   0.1270185E+01  
2072     8      0.5765091E+01    0.2149509E+01   0.7380136E+00  
2073     9      0.2698831E+01    0.0000000E+00   0.0000000E+00  
2074     10     0.0000000E+00    0.0000000E+00   0.0000000E+00  
2075     11     -0.7671891E+00    0.6776179E+00   -0.1810567E+01  
2076     12     -0.6983917E+00    0.1238171E+01   0.1517501E+01  
2077     13     -0.8077936E+00    -0.2714089E+01   0.4965690E+00  
2078     14     -0.1680855E+00    -0.3915450E+01   -0.1081330E+01  
2079     15     -0.2872351E+01    -0.2824762E+01   0.5816218E+00  
2080     16     0.1282141E+00    -0.3594138E+01   0.2842405E+01  
2081     17     0.1923688E+01    -0.3208660E+01   0.2827144E+01  
2082  
2083     Reading esp"s for MEP 6  
2084     total number of atoms = 17  
2085     total number of esp points = 813  
2086  
2087     Center    X               Y               Z  
2088     1      -0.4887776E-01   -0.1591282E+01   0.4291056E+01  
2089     2      0.1745920E+01    -0.1688102E+01   0.3909108E+01  
2090     3      -0.1021130E+01    0.3107465E+00   0.2678139E+01  
2091     4      -0.5786813E+00    0.2216520E+01   0.3399521E+01  
2092     5      -0.3082738E+01    0.1178150E+00   0.2670083E+01

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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```
2093      6      0.0000000E+00  0.0000000E+00  0.0000000E+00
2094      7     -0.7322197E+00  0.1475128E+01 -0.1270185E+01
2095      8     -0.5545269E+00 -0.1851550E+01 -0.7380136E+00
2096      9      0.2700331E+01  0.0000000E+00  0.0000000E+00
2097     10      0.3855194E+01  0.2439258E+01  0.0000000E+00
2098     11      0.3571040E+01  0.3422621E+01  0.1810567E+01
2099     12      0.3034960E+01  0.3600306E+01 -0.1517501E+01
2100     13      0.6653909E+01  0.2007966E+01 -0.4965690E+00
2101     14      0.7465984E+01  0.9157083E+00  0.1081330E+01
2102     15      0.7637386E+01  0.3826597E+01 -0.5816218E+00
2103     16      0.7048784E+01  0.7854004E+00 -0.2842405E+01
2104     17      0.5932077E+01 -0.6724363E+00 -0.2827144E+01
2105
2106 Reading esp"s for MEP 7
2107 total number of atoms = 17
2108 total number of esp points = 803
2109
2110 Center   X           Y           Z
2111    1  0.5313641E+01  0.1803776E+01 -0.4290986E+01
2112    2  0.4633130E+01  0.1401799E+00 -0.3908998E+01
2113    3  0.4010535E+01  0.3496454E+01 -0.2678167E+01
2114    4  0.2098745E+01  0.3912030E+01 -0.3399617E+01
2115    5  0.5067081E+01  0.5277230E+01 -0.2670128E+01
2116    6  0.3854347E+01  0.2440628E+01  0.0000000E+00
2117    7  0.2834375E+01  0.3733668E+01  0.1270117E+01
2118    8  0.5765080E+01  0.2149539E+01  0.7380854E+00
2119    9  0.2698846E+01  0.0000000E+00  0.0000000E+00
2120   10  0.0000000E+00  0.0000000E+00  0.0000000E+00
2121   11 -0.7671758E+00  0.6775915E+00 -0.1810577E+01
2122   12 -0.6983747E+00  0.1238192E+01  0.1517488E+01
2123   13 -0.8077861E+00 -0.2714085E+01  0.4966068E+00
2124   14 -0.1680836E+00 -0.3915461E+01 -0.1081284E+01
2125   15 -0.2872344E+01 -0.2824759E+01  0.5816633E+00
2126   16  0.1282406E+00 -0.3594108E+01  0.2842437E+01
2127   17  0.1923701E+01 -0.3208579E+01  0.2827181E+01
2128
2129 Reading esp"s for MEP 8
2130 total number of atoms = 17
2131 total number of esp points = 813
2132
2133 Center   X           Y           Z
2134    1 -0.4884375E-01 -0.1591457E+01  0.4290986E+01
2135    2  0.1745948E+01 -0.1688264E+01  0.3908998E+01
2136    3 -0.1021113E+01  0.3106313E+00  0.2678167E+01
2137    4 -0.5786454E+00  0.2216378E+01  0.3399617E+01
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
2138      5   -0.3082723E+01   0.1177110E+00   0.2670128E+01
2139      6    0.0000000E+00   0.0000000E+00   0.0000000E+00
2140      7   -0.7322235E+00   0.1475175E+01  -0.1270117E+01
2141      8   -0.5545288E+00  -0.1851521E+01  -0.7380854E+00
2142      9    0.2700341E+01   0.0000000E+00   0.0000000E+00
2143     10    0.3855202E+01   0.2439275E+01   0.0000000E+00
2144     11    0.3571061E+01   0.3422613E+01   0.1810577E+01
2145     12    0.3034938E+01   0.3600317E+01  -0.1517488E+01
2146     13    0.6653910E+01   0.2007989E+01  -0.4966068E+00
2147     14    0.7466007E+01   0.9157329E+00   0.1081284E+01
2148     15    0.7637382E+01   0.3826623E+01  -0.5816633E+00
2149     16    0.7048759E+01   0.7854193E+00  -0.2842437E+01
2150     17    0.5932016E+01  -0.6723872E+00  -0.2827181E+01
2151
2152 Reading esp"s for MEP 9
2153 total number of atoms = 17
2154 total number of esp points = 803
2155
2156 Center      X          Y          Z
2157     1  0.5313681E+01  0.1803785E+01  -0.4290971E+01
2158     2  0.4633147E+01  0.1401874E+00  -0.3909038E+01
2159     3  0.4010543E+01  0.3496449E+01  -0.2678163E+01
2160     4  0.2098749E+01  0.3912003E+01  -0.3399619E+01
2161     5  0.5067068E+01  0.5277236E+01  -0.2670126E+01
2162     6  0.3854346E+01  0.2440628E+01   0.0000000E+00
2163     7  0.2834362E+01  0.3733668E+01   0.1270111E+01
2164     8  0.5765072E+01  0.2149541E+01   0.7381024E+00
2165     9  0.2698841E+01  0.0000000E+00   0.0000000E+00
2166    10  0.0000000E+00  0.0000000E+00   0.0000000E+00
2167    11  -0.7671796E+00  0.6774857E+00  -0.1810616E+01
2168    12  -0.6983860E+00  0.1238279E+01   0.1517414E+01
2169    13  -0.8077861E+00  -0.2714059E+01   0.4967598E+00
2170    14  -0.1680703E+00  -0.3915526E+01  -0.1081055E+01
2171    15  -0.2872344E+01  -0.2824734E+01   0.5818126E+00
2172    16  0.1282274E+00  -0.3593938E+01   0.2842651E+01
2173    17  0.1923699E+01  -0.3208452E+01   0.2827359E+01
2174
2175 Reading esp"s for MEP 10
2176 total number of atoms = 17
2177 total number of esp points = 813
2178
2179 Center      X          Y          Z
2180     1  -0.4887209E-01  -0.1591489E+01   0.4290971E+01
2181     2   0.1745931E+01  -0.1688281E+01   0.3909038E+01
2182     3  -0.1021111E+01   0.3106218E+00   0.2678163E+01
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

2183       4     -0.5786209E+00   0.2216361E+01   0.3399619E+01  
2184       5     -0.3082723E+01   0.1177299E+00   0.2670126E+01  
2185       6     0.0000000E+00   0.0000000E+00   0.0000000E+00  
2186       7     -0.7322159E+00   0.1475188E+01   -0.1270111E+01  
2187       8     -0.5545269E+00   -0.1851514E+01   -0.7381024E+00  
2188       9     0.2700345E+01   0.0000000E+00   0.0000000E+00  
2189      10     0.3855205E+01   0.2439268E+01   0.0000000E+00  
2190      11     0.3571165E+01   0.3422566E+01   0.1810616E+01  
2191      12     0.3034870E+01   0.3600357E+01   -0.1517414E+01  
2192      13     0.6653890E+01   0.2007989E+01   -0.4967598E+00  
2193      14     0.7466062E+01   0.9156800E+00   0.1081055E+01  
2194      15     0.7637367E+01   0.3826621E+01   -0.5818126E+00  
2195      16     0.7048614E+01   0.7854911E+00   -0.2842651E+01  
2196      17     0.5931903E+01   -0.6723418E+00   -0.2827359E+01  
2197  
2198   Reading esp"s for MEP 11  
2199   total number of atoms = 17  
2200   total number of esp points = 810  
2201  
2202   Center   X                  Y                  Z  
2203    1     0.5213693E+01   0.1800017E+01   -0.4306839E+01  
2204    2     0.4580599E+01   0.1385831E+00   -0.3838846E+01  
2205    3     0.3953456E+01   0.3511400E+01   -0.2677564E+01  
2206    4     0.2038020E+01   0.3948235E+01   -0.3378240E+01  
2207    5     0.5028559E+01   0.5281616E+01   -0.2681015E+01  
2208    6     0.3816995E+01   0.2445183E+01   0.0000000E+00  
2209    7     0.2799860E+01   0.3732642E+01   0.1279161E+01  
2210    8     0.5733349E+01   0.2165333E+01   0.7283306E+00  
2211    9     0.2684290E+01   0.0000000E+00   0.0000000E+00  
2212   10    0.0000000E+00   0.0000000E+00   0.0000000E+00  
2213   11   -0.7626745E+00   0.6819190E+00   -0.1813307E+01  
2214   12   -0.7198722E+00   0.1240242E+01   0.1506990E+01  
2215   13   -0.9314081E+00   -0.2670854E+01   0.4558983E+00  
2216   14   0.3880930E-01   -0.3951037E+01   -0.8665717E+00  
2217   15   -0.2969837E+01   -0.2732765E+01   0.2377086E-01  
2218   16   -0.4466235E+00   -0.3322922E+01   0.3011643E+01  
2219   17   -0.7822010E+00   -0.5110904E+01   0.3213243E+01  
2220  
2221   Reading esp"s for MEP 12  
2222   total number of atoms = 17  
2223   total number of esp points = 817  
2224  
2225   Center   X                  Y                  Z  
2226    1   -0.1668628E-02   -0.1538507E+01   0.4306839E+01  
2227    2   0.1771975E+01   -0.1662405E+01   0.3838846E+01

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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```
2228      3   -0.1024814E+01   0.3243431E+00   0.2677564E+01
2229      4   -0.6160677E+00   0.2245970E+01   0.3378240E+01
2230      5   -0.3082956E+01   0.9290271E-01   0.2681015E+01
2231      6   0.0000000E+00   0.0000000E+00   0.0000000E+00
2232      7   -0.7406687E+00   0.1464078E+01   -0.1279161E+01
2233      8   -0.5515751E+00   -0.1856474E+01   -0.7283306E+00
2234      9   0.2694798E+01   0.0000000E+00   0.0000000E+00
2235     10   0.3823088E+01   0.2435647E+01   0.0000000E+00
2236     11   0.3524910E+01   0.3414308E+01   0.1813307E+01
2237     12   0.3000312E+01   0.3610149E+01   -0.1506990E+01
2238     13   0.6638040E+01   0.2158139E+01   -0.4558983E+00
2239     14   0.7391831E+01   0.7396917E+00   0.8665717E+00
2240     15   0.7551031E+01   0.3981726E+01   -0.2377086E-01
2241     16   0.7025941E+01   0.1444176E+01   -0.3011643E+01
2242     17   0.8789356E+01   0.9971253E+00   -0.3213243E+01
2243
2244 Reading esp"s for MEP 13
2245 total number of atoms = 17
2246 total number of esp points = 814
2247
2248 Center      X          Y          Z
2249    1  0.5368704E+01  0.1752536E+01 -0.4247125E+01
2250    2  0.4708965E+01  0.9276665E-01 -0.3813066E+01
2251    3  0.4033437E+01  0.3471111E+01 -0.2687899E+01
2252    4  0.2137439E+01  0.3874297E+01 -0.3457539E+01
2253    5  0.5088244E+01  0.5253185E+01 -0.2683530E+01
2254    6  0.3819728E+01  0.2448437E+01  0.0000000E+00
2255    7  0.2763919E+01  0.3751828E+01  0.1229864E+01
2256    8  0.5714745E+01  0.2180613E+01  0.7864227E+00
2257    9  0.2683721E+01  0.0000000E+00  0.0000000E+00
2258   10  0.0000000E+00  0.0000000E+00  0.0000000E+00
2259   11  -0.7664313E+00  0.8737224E+00 -0.1731220E+01
2260   12  -0.7338392E+00  0.1077582E+01  0.1620357E+01
2261   13  -0.8845675E+00 -0.2741533E+01  0.1807693E+00
2262   14  -0.2055965E+00 -0.3567750E+01  0.1949046E+01
2263   15  -0.6428848E-01 -0.3843788E+01 -0.1380626E+01
2264   16  -0.3562708E+01 -0.2909146E+01  0.2415467E+00
2265   17  -0.4191777E+01 -0.2471939E+01 -0.1422947E+01
2266
2267 Reading esp"s for MEP 14
2268 total number of atoms = 17
2269 total number of esp points = 825
2270
2271 Center      X          Y          Z
2272    1  -0.2066604E-01 -0.1697993E+01  0.4247125E+01
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
2273      2      0.1762610E+01 -0.1798091E+01  0.3813066E+01
2274      3     -0.1017631E+01  0.2365616E+00  0.2687899E+01
2275      4     -0.5853842E+00  0.2126148E+01  0.3457539E+01
2276      5     -0.3078126E+01  0.2976318E-01  0.2683530E+01
2277      6      0.0000000E+00  0.0000000E+00  0.0000000E+00
2278      7     -0.7379607E+00  0.1506312E+01 -0.1229864E+01
2279      8     -0.5546232E+00 -0.1831723E+01 -0.7864227E+00
2280      9      0.2699141E+01  0.0000000E+00  0.0000000E+00
2281     10      0.3828659E+01  0.2434449E+01  0.0000000E+00
2282     11      0.3358663E+01  0.3497422E+01  0.1731220E+01
2283     12      0.3160023E+01  0.3553658E+01 -0.1620357E+01
2284     13      0.6687846E+01  0.2083005E+01 -0.1807693E+00
2285     14      0.7151558E+01  0.1119362E+01 -0.1949046E+01
2286     15      0.7342483E+01  0.8750017E+00  0.1380626E+01
2287     16      0.7967060E+01  0.4441848E+01 -0.2415467E+00
2288     17      0.7835223E+01  0.5196499E+01  0.1422947E+01
2289
2290 Reading esp"s for MEP 15
2291 total number of atoms = 17
2292 total number of esp points = 811
2293
2294 Center      X          Y          Z
2295    1  0.5391275E+01  0.1760108E+01 -0.4235290E+01
2296    2  0.4713606E+01  0.1008585E+00 -0.3828165E+01
2297    3  0.4032129E+01  0.3472045E+01 -0.2688642E+01
2298    4  0.2138898E+01  0.3859136E+01 -0.3471468E+01
2299    5  0.5075598E+01  0.5260799E+01 -0.2681068E+01
2300    6  0.3809039E+01  0.2452828E+01  0.0000000E+00
2301    7  0.2741359E+01  0.3754474E+01  0.1221797E+01
2302    8  0.5701825E+01  0.2196976E+01  0.7962266E+00
2303    9  0.2685199E+01  0.0000000E+00  0.0000000E+00
2304   10  0.0000000E+00  0.0000000E+00  0.0000000E+00
2305   11 -0.7820915E+00  0.9058250E+00 -0.1699030E+01
2306   12 -0.7293265E+00  0.1023655E+01  0.1657121E+01
2307   13 -0.8630889E+00 -0.2740318E+01  0.9894416E-01
2308   14 -0.9331467E-01 -0.3634077E+01  0.1811703E+01
2309   15 -0.9511936E-01 -0.3757404E+01 -0.1546053E+01
2310   16 -0.3548703E+01 -0.2707424E+01  0.9553510E-01
2311   17 -0.4134275E+01 -0.4439316E+01  0.1729988E+00
2312
2313 Reading esp"s for MEP 16
2314 total number of atoms = 17
2315 total number of esp points = 824
2316
2317 Center      X          Y          Z
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
2318      1      -0.2930020E-01   -0.1726985E+01    0.4235290E+01
2319      2       0.1761429E+01   -0.1802047E+01    0.3828165E+01
2320      3      -0.1019513E+01    0.2217291E+00    0.2688642E+01
2321      4      -0.5828180E+00    0.2104138E+01    0.3471468E+01
2322      5      -0.3080348E+01    0.1818105E-01    0.2681068E+01
2323      6       0.0000000E+00    0.0000000E+00    0.0000000E+00
2324      7      -0.7386164E+00    0.1512833E+01   -0.1221797E+01
2325      8      -0.5558232E+00   -0.1827337E+01   -0.7962266E+00
2326      9       0.2698034E+01    0.0000000E+00    0.0000000E+00
2327     10      0.3816527E+01    0.2441161E+01    0.0000000E+00
2328     11      0.3318797E+01    0.3529487E+01    0.1699030E+01
2329     12      0.3189697E+01    0.3530600E+01   -0.1657121E+01
2330     13      0.6667309E+01    0.2084356E+01   -0.9894416E-01
2331     14      0.7159199E+01    0.1012256E+01   -0.1811703E+01
2332     15      0.7272068E+01    0.9625244E+00    0.1546053E+01
2333     16      0.7756070E+01    0.4539596E+01   -0.9553510E-01
2334     17      0.9574478E+01    0.4350546E+01   -0.1729988E+00
2335
2336 Reading esp"s for MEP 17
2337 total number of atoms = 17
2338 total number of esp points = 811
2339
2340 Center      X          Y          Z
2341      1      0.5391248E+01   0.1760140E+01   -0.4235301E+01
2342      2      0.4713564E+01   0.1008944E+00   -0.3828186E+01
2343      3      0.4032101E+01   0.3472069E+01   -0.2688638E+01
2344      4      0.2138871E+01   0.3859166E+01   -0.3471457E+01
2345      5      0.5075570E+01   0.5260825E+01   -0.2681062E+01
2346      6      0.3809032E+01   0.2452840E+01    0.0000000E+00
2347      7      0.2741348E+01   0.3754466E+01    0.1221810E+01
2348      8      0.5701819E+01   0.2196988E+01    0.7962096E+00
2349      9      0.2685199E+01    0.0000000E+00    0.0000000E+00
2350     10       0.0000000E+00    0.0000000E+00    0.0000000E+00
2351     11      -0.7820971E+00   0.9057759E+00   -0.1699054E+01
2352     12      -0.7293265E+00   0.1023706E+01    0.1657093E+01
2353     13      -0.8630851E+00   -0.2740316E+01    0.9900841E-01
2354     14      -0.9334302E-01   -0.3634034E+01    0.1811797E+01
2355     15      -0.9508534E-01   -0.3757433E+01   -0.1545956E+01
2356     16      -0.3548703E+01   -0.2707442E+01    0.9555400E-01
2357     17      -0.4134254E+01   -0.4439331E+01    0.1731991E+00
2358
2359 Reading esp"s for MEP 18
2360 total number of atoms = 17
2361 total number of esp points = 824
2362
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

2363 Center X Y Z  
2364 1 -0.2930398E-01 -0.1726956E+01 0.4235301E+01  
2365 2 0.1761429E+01 -0.1801999E+01 0.3828186E+01  
2366 3 -0.1019517E+01 0.2217499E+00 0.2688638E+01  
2367 4 -0.5828350E+00 0.2104161E+01 0.3471457E+01  
2368 5 -0.3080352E+01 0.1819428E-01 0.2681062E+01  
2369 6 0.0000000E+00 0.0000000E+00 0.0000000E+00  
2370 7 -0.7386032E+00 0.1512826E+01 -0.1221810E+01  
2371 8 -0.5558157E+00 -0.1827341E+01 -0.7962096E+00  
2372 9 0.2698041E+01 0.0000000E+00 0.0000000E+00  
2373 10 0.3816525E+01 0.2441165E+01 0.0000000E+00  
2374 11 0.3318837E+01 0.3529473E+01 0.1699054E+01  
2375 12 0.3189644E+01 0.3530622E+01 -0.1657093E+01  
2376 13 0.6667303E+01 0.2084372E+01 -0.9900841E-01  
2377 14 0.7159174E+01 0.1012317E+01 -0.1811797E+01  
2378 15 0.7272083E+01 0.9625017E+00 0.1545956E+01  
2379 16 0.7756076E+01 0.4539609E+01 -0.9555400E-01  
2380 17 0.9574473E+01 0.4350550E+01 -0.1731991E+00  
2381  
2382 Reading esp"s for MEP 19  
2383 total number of atoms = 17  
2384 total number of esp points = 814  
2385  
2386 Center X Y Z  
2387 1 0.5435935E+01 0.1755421E+01 -0.4221058E+01  
2388 2 0.4749316E+01 0.9443528E-01 -0.3837622E+01  
2389 3 0.4055717E+01 0.3462615E+01 -0.2689348E+01  
2390 4 0.2167132E+01 0.3840159E+01 -0.3488351E+01  
2391 5 0.5091305E+01 0.5255876E+01 -0.2677383E+01  
2392 6 0.3814975E+01 0.2450627E+01 0.0000000E+00  
2393 7 0.2743876E+01 0.3758162E+01 0.1213070E+01  
2394 8 0.5702573E+01 0.2192243E+01 0.8074308E+00  
2395 9 0.2683207E+01 0.0000000E+00 0.0000000E+00  
2396 10 0.0000000E+00 0.0000000E+00 0.0000000E+00  
2397 11 -0.7863490E+00 0.9644897E+00 -0.1664416E+01  
2398 12 -0.7133716E+00 0.9908608E+00 0.1692529E+01  
2399 13 -0.8857032E+00 -0.2746981E+01 0.1661447E-01  
2400 14 -0.4428384E-01 -0.3737398E+01 0.1639075E+01  
2401 15 -0.2306335E+00 -0.3695480E+01 -0.1699648E+01  
2402 16 -0.3564569E+01 -0.2917342E+01 -0.3140725E-02  
2403 17 -0.4173494E+01 -0.2375879E+01 0.1637933E+01  
2404  
2405 Reading esp"s for MEP 20  
2406 total number of atoms = 17  
2407 total number of esp points = 821

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

2408  
2409   Center   X                   Y                   Z  
2410    1   -0.4847903E-01 -0.1763086E+01 0.4221058E+01  
2411    2   0.1747345E+01 -0.1836143E+01 0.3837622E+01  
2412    3   -0.1019681E+01 0.2057401E+00 0.2689348E+01  
2413    4   -0.5706009E+00 0.2078604E+01 0.3488351E+01  
2414    5   -0.3081905E+01 0.1744028E-01 0.2677383E+01  
2415    6   0.0000000E+00 0.0000000E+00 0.0000000E+00  
2416    7   -0.7379739E+00 0.1520623E+01 -0.1213070E+01  
2417    8   -0.5568456E+00 -0.1822010E+01 -0.8074308E+00  
2418    9   0.2699347E+01 0.0000000E+00 0.0000000E+00  
2419   10   0.3824346E+01 0.2435976E+01 0.0000000E+00  
2420   11   0.3278421E+01 0.3554255E+01 0.1664416E+01  
2421   12   0.3223882E+01 0.3499058E+01 -0.1692529E+01  
2422   13   0.6689573E+01 0.2088331E+01 -0.1661447E-01  
2423   14   0.7235946E+01 0.9091831E+00 -0.1639075E+01  
2424   15   0.7276023E+01 0.1095937E+01 0.1699648E+01  
2425   16   0.7967416E+01 0.4448937E+01 0.3140725E-02  
2426   17   0.7731149E+01 0.5228777E+01 -0.1637933E+01  
2427  
2428   Reading esp"s for MEP 21  
2429   total number of atoms = 17  
2430   total number of esp points = 756  
2431  
2432   Center   X                   Y                   Z  
2433    1   0.4363459E+01 0.2381799E+01 -0.4597811E+01  
2434    2   0.3217119E+01 0.9348871E+00 -0.4665097E+01  
2435    3   0.3596697E+01 0.3826106E+01 -0.2497702E+01  
2436    4   0.1627139E+01 0.4494158E+01 -0.2703790E+01  
2437    5   0.4787770E+01 0.5521375E+01 -0.2437121E+01  
2438    6   0.3897915E+01 0.2398873E+01 0.0000000E+00  
2439    7   0.3238015E+01 0.3576208E+01 0.1588919E+01  
2440    8   0.5896913E+01 0.1964329E+01 0.2996765E+00  
2441    9   0.2676963E+01 0.0000000E+00 0.0000000E+00  
2442   10   0.0000000E+00 0.0000000E+00 0.0000000E+00  
2443   11   -0.7703071E+00 0.1927447E+01 -0.1283275E+00  
2444   12   -0.6936579E+00 -0.8221234E+00 0.1782293E+01  
2445   13   -0.9503734E+00 -0.1595479E+01 -0.2204277E+01  
2446   14   -0.3012212E+01 -0.1834395E+01 -0.2074919E+01  
2447   15   -0.7977856E-01 -0.3466751E+01 -0.2105680E+01  
2448   16   -0.2407738E+00 -0.6022840E+00 -0.4613777E+01  
2449   17   -0.1236741E+01 0.9027788E+00 -0.4937739E+01  
2450  
2451   Reading esp"s for MEP 22  
2452   total number of atoms = 17

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
2453 total number of esp points = 762
2454
2455 Center      X          Y          Z
2456    1 -0.1959514E+00 -0.4226410E+00 0.4597811E+01
2457    2  0.1613524E+01 -0.5732862E-01 0.4665097E+01
2458    3 -0.1135328E+01  0.9158349E+00 0.2497702E+01
2459    4 -0.8373168E+00  0.2974145E+01 0.2703790E+01
2460    5 -0.3186430E+01  0.6233110E+00 0.2437121E+01
2461    6  0.0000000E+00  0.0000000E+00 0.0000000E+00
2462    7 -0.7499208E+00  0.1122142E+01 -0.1588919E+01
2463    8 -0.5194687E+00 -0.1978630E+01 -0.2996765E+00
2464    9  0.2691712E+01  0.0000000E+00 0.0000000E+00
2465   10  0.3905975E+01  0.2385728E+01 0.0000000E+00
2466   11  0.2537628E+01  0.3946515E+01 0.1283275E+00
2467   12  0.4953299E+01  0.2631009E+01 -0.1782293E+01
2468   13  0.5758963E+01  0.2509004E+01 0.2204277E+01
2469   14  0.6907128E+01  0.4238160E+01 0.2074919E+01
2470   15  0.7031755E+01  0.8843218E+00 0.2105680E+01
2471   16  0.4551949E+01  0.2327114E+01 0.4613777E+01
2472   17  0.3662393E+01  0.3897416E+01 0.4937739E+01
2473
2474 Reading esp"s for MEP 23
2475 total number of atoms = 17
2476 total number of esp points = 756
2477
2478 Center      X          Y          Z
2479    1  0.4363392E+01  0.2381892E+01 -0.4597811E+01
2480    2  0.3217122E+01  0.9349211E+00 -0.4665070E+01
2481    3  0.3596632E+01  0.3826155E+01 -0.2497658E+01
2482    4  0.1627067E+01  0.4494184E+01 -0.2703710E+01
2483    5  0.4787689E+01  0.5521430E+01 -0.2437068E+01
2484    6  0.3897909E+01  0.2398881E+01 0.0000000E+00
2485    7  0.3238064E+01  0.3576172E+01 0.1588974E+01
2486    8  0.5896922E+01  0.1964336E+01 0.2996047E+00
2487    9  0.2676967E+01  0.0000000E+00 0.0000000E+00
2488   10  0.0000000E+00  0.0000000E+00 0.0000000E+00
2489   11 -0.7703147E+00  0.1927441E+01 -0.1283389E+00
2490   12 -0.6936598E+00 -0.8221177E+00 0.1782295E+01
2491   13 -0.9503715E+00 -0.1595496E+01 -0.2204273E+01
2492   14 -0.3012216E+01 -0.1834395E+01 -0.2074930E+01
2493   15 -0.7978990E-01 -0.3466770E+01 -0.2105654E+01
2494   16 -0.2407416E+00 -0.6023256E+00 -0.4613777E+01
2495   17 -0.1236682E+01  0.9027504E+00 -0.4937750E+01
2496
2497 Reading esp"s for MEP 24
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

2498 total number of atoms = 17  
2499 total number of esp points = 762  
2500  
2501 Center X Y Z  
2502 1 -0.1959986E+00 -0.4225484E+00 0.4597811E+01  
2503 2 0.1613495E+01 -0.5731728E-01 0.4665070E+01  
2504 3 -0.1135342E+01 0.9159029E+00 0.2497658E+01  
2505 4 -0.8373149E+00 0.2974210E+01 0.2703710E+01  
2506 5 -0.3186441E+01 0.6233885E+00 0.2437068E+01  
2507 6 0.0000000E+00 0.0000000E+00 0.0000000E+00  
2508 7 -0.7499113E+00 0.1122072E+01 -0.1588974E+01  
2509 8 -0.5194687E+00 -0.1978643E+01 -0.2996047E+00  
2510 9 0.2691716E+01 0.0000000E+00 0.0000000E+00  
2511 10 0.3905969E+01 0.2385736E+01 0.0000000E+00  
2512 11 0.2537626E+01 0.3946521E+01 0.1283389E+00  
2513 12 0.4953287E+01 0.2631024E+01 -0.1782295E+01  
2514 13 0.5758970E+01 0.2509010E+01 0.2204273E+01  
2515 14 0.6907119E+01 0.4238181E+01 0.2074930E+01  
2516 15 0.7031778E+01 0.8843426E+00 0.2105654E+01  
2517 16 0.4551966E+01 0.2327077E+01 0.4613777E+01  
2518 17 0.3662382E+01 0.3897359E+01 0.4937750E+01  
2519  
2520 Reading esp"s for MEP 25  
2521 total number of atoms = 17  
2522 total number of esp points = 756  
2523  
2524 Center X Y Z  
2525 1 0.4363421E+01 0.2381892E+01 -0.4597813E+01  
2526 2 0.3217121E+01 0.9349457E+00 -0.4665104E+01  
2527 3 0.3596647E+01 0.3826153E+01 -0.2497658E+01  
2528 4 0.1627075E+01 0.4494161E+01 -0.2703714E+01  
2529 5 0.4787689E+01 0.5521435E+01 -0.2437072E+01  
2530 6 0.3897919E+01 0.2398879E+01 0.0000000E+00  
2531 7 0.3238064E+01 0.3576174E+01 0.1588965E+01  
2532 8 0.5896928E+01 0.1964334E+01 0.2996161E+00  
2533 9 0.2676965E+01 0.0000000E+00 0.0000000E+00  
2534 10 0.0000000E+00 0.0000000E+00 0.0000000E+00  
2535 11 -0.7703147E+00 0.1927445E+01 -0.1282973E+00  
2536 12 -0.6936579E+00 -0.8221517E+00 0.1782280E+01  
2537 13 -0.9503772E+00 -0.1595460E+01 -0.2204299E+01  
2538 14 -0.3012216E+01 -0.1834368E+01 -0.2074951E+01  
2539 15 -0.7977856E-01 -0.3466729E+01 -0.2105712E+01  
2540 16 -0.2407719E+00 -0.6022443E+00 -0.4613794E+01  
2541 17 -0.1236695E+01 0.9028525E+00 -0.4937722E+01  
2542

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
2543  Reading esp"s for MEP 26
2544  total number of atoms = 17
2545  total number of esp points = 762
2546
2547  Center      X          Y          Z
2548    1 -0.1960118E+00 -0.4225635E+00 0.4597813E+01
2549    2  0.1613476E+01 -0.5730216E-01 0.4665104E+01
2550    3 -0.1135340E+01  0.9159029E+00 0.2497658E+01
2551    4 -0.8372847E+00  0.2974206E+01 0.2703714E+01
2552    5 -0.3186441E+01  0.6234112E+00 0.2437072E+01
2553    6  0.0000000E+00  0.0000000E+00 0.0000000E+00
2554    7 -0.7499056E+00  0.1122083E+01 -0.1588965E+01
2555    8 -0.5194743E+00 -0.1978638E+01 -0.2996161E+00
2556    9  0.2691718E+01  0.0000000E+00 0.0000000E+00
2557   10  0.3905981E+01  0.2385730E+01 0.0000000E+00
2558   11  0.2537639E+01  0.3946523E+01 0.1282973E+00
2559   12  0.4953329E+01  0.2630997E+01 -0.1782280E+01
2560   13  0.5758953E+01  0.2509018E+01 0.2204299E+01
2561   14  0.6907113E+01  0.4238175E+01 0.2074951E+01
2562   15  0.7031742E+01  0.8843351E+00 0.2105712E+01
2563   16  0.4551917E+01  0.2327133E+01 0.4613794E+01
2564   17  0.3662314E+01  0.3897414E+01 0.4937722E+01
2565
2566  Reading esp"s for MEP 27
2567  total number of atoms = 17
2568  total number of esp points = 756
2569
2570  Center      X          Y          Z
2571    1  0.4363402E+01  0.2381860E+01 -0.4597811E+01
2572    2  0.3217151E+01  0.9348701E+00 -0.4665046E+01
2573    3  0.3596630E+01  0.3826136E+01 -0.2497675E+01
2574    4  0.1627058E+01  0.4494143E+01 -0.2703729E+01
2575    5  0.4787672E+01  0.5521420E+01 -0.2437104E+01
2576    6  0.3897909E+01  0.2398882E+01 0.0000000E+00
2577    7  0.3238044E+01  0.3576180E+01 0.1588957E+01
2578    8  0.5896918E+01  0.1964346E+01 0.2996217E+00
2579    9  0.2676965E+01  0.0000000E+00 0.0000000E+00
2580   10  0.0000000E+00  0.0000000E+00 0.0000000E+00
2581   11 -0.7703147E+00  0.1927441E+01 -0.1283880E+00
2582   12 -0.6936636E+00 -0.8220743E+00 0.1782314E+01
2583   13 -0.9503602E+00 -0.1595543E+01 -0.2204241E+01
2584   14 -0.3012206E+01 -0.1834433E+01 -0.2074912E+01
2585   15 -0.7978423E-01 -0.3466820E+01 -0.2105584E+01
2586   16 -0.2407076E+00 -0.6024182E+00 -0.4613760E+01
2587   17 -0.1236665E+01  0.9026371E+00 -0.4937782E+01
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

2588  
2589   Reading esp"s for MEP 28  
2590   total number of atoms = 17  
2591   total number of esp points = 762  
2592  
2593   Center   X                   Y                   Z  
2594    1   -0.1959721E+00 -0.4225730E+00 0.4597811E+01  
2595    2   0.1613527E+01 -0.5736830E-01 0.4665046E+01  
2596    3   -0.1135323E+01 0.9158935E+00 0.2497675E+01  
2597    4   -0.8372715E+00 0.2974196E+01 0.2703729E+01  
2598    5   -0.3186422E+01 0.6233960E+00 0.2437104E+01  
2599    6   0.0000000E+00 0.0000000E+00 0.0000000E+00  
2600    7   -0.7499075E+00 0.1122091E+01 -0.1588957E+01  
2601    8   -0.5194724E+00 -0.1978638E+01 -0.2996217E+00  
2602    9   0.2691718E+01 0.0000000E+00 0.0000000E+00  
2603   10   0.3905971E+01 0.2385736E+01 0.0000000E+00  
2604   11   0.2537628E+01 0.3946519E+01 0.1283880E+00  
2605   12   0.4953252E+01 0.2631048E+01 -0.1782314E+01  
2606   13   0.5759010E+01 0.2508978E+01 0.2204241E+01  
2607   14   0.6907149E+01 0.4238153E+01 0.2074912E+01  
2608   15   0.7031820E+01 0.8843143E+00 0.2105584E+01  
2609   16   0.4552034E+01 0.2327003E+01 0.4613760E+01  
2610   17   0.3662474E+01 0.3897292E+01 0.4937782E+01  
2611  
2612   Reading esp"s for MEP 29  
2613   total number of atoms = 17  
2614   total number of esp points = 840  
2615  
2616   Center   X                   Y                   Z  
2617    1   0.7218638E+01 0.7890230E+00 0.2501100E+01  
2618    2   0.6168396E+01 -0.7174137E+00 0.2497503E+01  
2619    3   0.6598109E+01 0.2089525E+01 0.2451636E+00  
2620    4   0.7300837E+01 0.1079326E+01 -0.1434884E+01  
2621    5   0.7539066E+01 0.3930908E+01 0.3254429E+00  
2622    6   0.3756970E+01 0.2453446E+01 0.0000000E+00  
2623    7   0.3284125E+01 0.3474250E+01 -0.1755125E+01  
2624    8   0.3039316E+01 0.3559607E+01 0.1612254E+01  
2625    9   0.2668463E+01 0.0000000E+00 0.0000000E+00  
2626   10   0.0000000E+00 0.0000000E+00 0.0000000E+00  
2627   11   -0.7233852E+00 0.9762684E+00 -0.1700736E+01  
2628   12   -0.7403002E+00 0.1032308E+01 0.1655750E+01  
2629   13   -0.9643612E+00 -0.2709848E+01 0.6500657E-01  
2630   14   -0.3030523E+01 -0.2658631E+01 0.3410029E+00  
2631   15   -0.1391462E+00 -0.3685653E+01 0.1691305E+01  
2632   16   -0.2802123E+00 -0.4162908E+01 -0.2077294E+01

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

2633      17      -0.9913937E+00 -0.3350934E+01 -0.3558923E+01  
2634  
2635      Reading esp"s **for** MEP 30  
2636      total number of atoms = 17  
2637      total number of esp points = 835  
2638  
2639      Center      X                  Y                  Z  
2640      1      0.1175561E+00 -0.3839222E+01 -0.2501100E+01  
2641      2      0.1920472E+01 -0.3490146E+01 -0.2497503E+01  
2642      3      -0.8195496E+00 -0.2744604E+01 -0.2451636E+00  
2643      4      -0.1811378E+00 -0.3796630E+01 0.1434884E+01  
2644      5      -0.2884313E+01 -0.2857950E+01 -0.3254429E+00  
2645      6      0.0000000E+00 0.0000000E+00 0.0000000E+00  
2646      7      -0.7413319E+00 0.8461947E+00 0.1755125E+01  
2647      8      -0.7200763E+00 0.1104586E+01 -0.1612254E+01  
2648      9      0.2684072E+01 0.0000000E+00 0.0000000E+00  
2649      10     0.3766249E+01 0.2439179E+01 0.0000000E+00  
2650      11     0.3167228E+01 0.3496328E+01 0.1700736E+01  
2651      12     0.3122863E+01 0.3534513E+01 -0.1655750E+01  
2652      13     0.6634346E+01 0.2221721E+01 -0.6500657E-01  
2653      14     0.7425446E+01 0.4131120E+01 -0.3410029E+00  
2654      15     0.7191645E+01 0.1071681E+01 -0.1691305E+01  
2655      16     0.7685100E+01 0.1007078E+01 0.2077294E+01  
2656      17     0.7231309E+01 0.1986442E+01 0.3558923E+01  
2657  
2658      Reading esp"s **for** MEP 31  
2659      total number of atoms = 17  
2660      total number of esp points = 838  
2661  
2662      Center      X                  Y                  Z  
2663      1      0.7181499E+01 0.8565334E+00 0.2647765E+01  
2664      2      0.6101241E+01 -0.6273493E+00 0.2689758E+01  
2665      3      0.6625364E+01 0.2083272E+01 0.3343360E+00  
2666      4      0.7370646E+01 0.1017729E+01 -0.1293684E+01  
2667      5      0.7572716E+01 0.3922223E+01 0.3781512E+00  
2668      6      0.3795462E+01 0.2452014E+01 0.0000000E+00  
2669      7      0.3369897E+01 0.3448561E+01 -0.1778557E+01  
2670      8      0.3036931E+01 0.3572468E+01 0.1580225E+01  
2671      9      0.2689777E+01 0.0000000E+00 0.0000000E+00  
2672      10     0.0000000E+00 0.0000000E+00 0.0000000E+00  
2673      11     -0.7095959E+00 0.1152153E+01 -0.1580164E+01  
2674      12     -0.7335255E+00 0.7975022E+00 0.1778595E+01  
2675      13     -0.8271387E+00 -0.2731310E+01 -0.3344947E+00  
2676      14     -0.2892961E+01 -0.2838986E+01 -0.3783099E+00  
2677      15     -0.1621366E+00 -0.3848816E+01 0.1293455E+01

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
2678      16      0.6254804E-01 -0.3742425E+01 -0.2647986E+01
2679      17      0.1859322E+01 -0.3367611E+01 -0.2689964E+01
2680
2681 Reading esp"s for MEP 32
2682 total number of atoms = 17
2683 total number of esp points = 838
2684
2685 Center      X          Y          Z
2686      1  0.6255182E-01 -0.3742578E+01 -0.2647765E+01
2687      2  0.1859326E+01 -0.3367788E+01 -0.2689758E+01
2688      3 -0.8271387E+00 -0.2731329E+01 -0.3343360E+00
2689      4 -0.1621461E+00 -0.3848741E+01  0.1293684E+01
2690      5 -0.2892959E+01 -0.2839003E+01 -0.3781512E+00
2691      6  0.0000000E+00  0.0000000E+00  0.0000000E+00
2692      7 -0.7335198E+00  0.7975967E+00  0.1778557E+01
2693      8 -0.7096016E+00  0.1152064E+01 -0.1580225E+01
2694      9  0.2689779E+01  0.0000000E+00  0.0000000E+00
2695     10  0.3795464E+01  0.2452014E+01  0.0000000E+00
2696     11  0.3036848E+01  0.3572499E+01  0.1580164E+01
2697     12  0.3369986E+01  0.3448527E+01 -0.1778595E+01
2698     13  0.6625347E+01  0.2083279E+01  0.3344947E+00
2699     14  0.7572701E+01  0.3922230E+01  0.3783099E+00
2700     15  0.7370710E+01  0.1017689E+01 -0.1293455E+01
2701     16  0.7181361E+01  0.8565996E+00  0.2647986E+01
2702     17  0.6101082E+01 -0.6272719E+00  0.2689964E+01
2703
2704 Reading esp"s for MEP 33
2705 total number of atoms = 17
2706 total number of esp points = 827
2707
2708 Center      X          Y          Z
2709      1  0.7407913E+01  0.9460138E+00  0.2367834E+01
2710      2  0.6446606E+01 -0.6127153E+00  0.2462479E+01
2711      3  0.6620644E+01  0.2155376E+01  0.1110573E+00
2712      4  0.7258851E+01  0.1113553E+01 -0.1575387E+01
2713      5  0.7519755E+01  0.4018693E+01  0.8754156E-01
2714      6  0.3766579E+01  0.2462880E+01  0.0000000E+00
2715      7  0.3208311E+01  0.3497630E+01 -0.1720185E+01
2716      8  0.3100995E+01  0.3536429E+01  0.1653865E+01
2717      9  0.2688481E+01  0.0000000E+00  0.0000000E+00
2718     10  0.0000000E+00  0.0000000E+00  0.0000000E+00
2719     11 -0.7240598E+00  0.9262794E+00 -0.1720223E+01
2720     12 -0.7165765E+00  0.1040288E+01  0.1653816E+01
2721     13 -0.8628205E+00 -0.2737845E+01  0.1111726E+00
2722     14 -0.1643022E+00 -0.3740363E+01 -0.1575190E+01
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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```
2723    15    -0.2930305E+01 -0.2814297E+01  0.8760581E-01
2724    16    -0.7073244E-01 -0.3943894E+01  0.2368048E+01
2725    17     0.1742700E+01 -0.3688518E+01  0.2462625E+01
2726
2727 Reading esp"s for MEP 34
2728 total number of atoms = 17
2729 total number of esp points = 827
2730
2731 Center      X          Y          Z
2732    1   -0.7061528E-01 -0.3944009E+01 -0.2367834E+01
2733    2     0.1742787E+01 -0.3688431E+01 -0.2462479E+01
2734    3   -0.8627884E+00 -0.2737854E+01 -0.1110573E+00
2735    4   -0.1643192E+00 -0.3740274E+01  0.1575387E+01
2736    5   -0.2930275E+01 -0.2814314E+01 -0.8754156E-01
2737    6     0.0000000E+00  0.0000000E+00  0.0000000E+00
2738    7   -0.7240447E+00  0.9263531E+00  0.1720185E+01
2739    8   -0.7165520E+00  0.1040222E+01 -0.1653865E+01
2740    9     0.2688508E+01  0.0000000E+00  0.0000000E+00
2741   10     0.3766594E+01  0.2462857E+01  0.0000000E+00
2742   11     0.3208400E+01  0.3497592E+01  0.1720223E+01
2743   12     0.3100959E+01  0.3536454E+01 -0.1653816E+01
2744   13     0.6620664E+01  0.2155387E+01 -0.1111726E+00
2745   14     0.7258942E+01  0.1113478E+01  0.1575190E+01
2746   15     0.7519768E+01  0.4018705E+01 -0.8760581E-01
2747   16     0.7407870E+01  0.9461423E+00 -0.2368048E+01
2748   17     0.6446736E+01 -0.6126945E+00 -0.2462625E+01
2749
2750 Reading esp"s for MEP 35
2751 total number of atoms = 17
2752 total number of esp points = 762
2753
2754 Center      X          Y          Z
2755    1   0.3191796E+01  0.3130546E+01  0.4488982E+01
2756    2   0.2383649E+01  0.1476269E+01  0.4645417E+01
2757    3   0.5069003E+01  0.2912782E+01  0.2599176E+01
2758    4   0.6446298E+01  0.1419007E+01  0.3056868E+01
2759    5   0.6094313E+01  0.4714042E+01  0.2543828E+01
2760    6   0.3943388E+01  0.2378299E+01  0.0000000E+00
2761    7   0.5445293E+01  0.2291565E+01 -0.1431082E+01
2762    8   0.2636510E+01  0.3903361E+01 -0.5275189E+00
2763    9   0.2674527E+01  0.0000000E+00  0.0000000E+00
2764   10     0.0000000E+00  0.0000000E+00  0.0000000E+00
2765   11   -0.6995237E+00 -0.7193110E+00 -0.1826080E+01
2766   12   -0.7514854E+00  0.1924032E+01  0.2387215E+00
2767   13   -0.9977678E+00 -0.1704393E+01  0.2098444E+01
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

2768    14    -0.2995783E-01 -0.3527943E+01  0.2012902E+01  
2769    15    -0.3030414E+01 -0.2039774E+01  0.1813092E+01  
2770    16    -0.5295182E+00 -0.7491233E+00  0.4582914E+01  
2771    17    -0.1594451E+01  0.7247364E+00  0.4831232E+01  
2772  
2773    Reading esp"s **for** MEP 36  
2774    total number of atoms = 17  
2775    total number of esp points = 772  
2776  
2777    Center    X                Y                Z  
2778    1    -0.3099113E+00  0.1017213E+01 -0.4488982E+01  
2779    2    0.1530039E+01  0.9515375E+00 -0.4645417E+01  
2780    3    -0.1001409E+01 -0.7415247E+00 -0.2599176E+01  
2781    4    -0.3317849E+00 -0.2659835E+01 -0.3056868E+01  
2782    5    -0.3073265E+01 -0.7982637E+00 -0.2543828E+01  
2783    6    0.0000000E+00  0.0000000E+00  0.0000000E+00  
2784    7    -0.6304428E+00 -0.1365935E+01  0.1431082E+01  
2785    8    -0.7303753E+00  0.1870910E+01  0.5275189E+00  
2786    9    0.2695611E+01  0.0000000E+00  0.0000000E+00  
2787    10    0.3954548E+01  0.2359697E+01  0.0000000E+00  
2788    11    0.4918460E+01  0.2638288E+01  0.1826080E+01  
2789    12    0.2610736E+01  0.3928391E+01 -0.2387215E+00  
2790    13    0.5927974E+01  0.2437731E+01 -0.2098444E+01  
2791    14    0.7081302E+01  0.7254753E+00 -0.2012902E+01  
2792    15    0.7180670E+01  0.4073238E+01 -0.1813092E+01  
2793    16    0.4864740E+01  0.2474260E+01 -0.4582914E+01  
2794    17    0.4065653E+01  0.4107601E+01 -0.4831232E+01  
2795  
2796    Reading esp"s **for** MEP 37  
2797    total number of atoms = 17  
2798    total number of esp points = 827  
2799  
2800    Center    X                Y                Z  
2801    1    0.7407902E+01  0.9461253E+00  0.2367976E+01  
2802    2    0.6446730E+01 -0.6126888E+00  0.2462598E+01  
2803    3    0.6620655E+01  0.2155363E+01  0.1111197E+00  
2804    4    0.7258885E+01  0.1113447E+01 -0.1575259E+01  
2805    5    0.7519762E+01  0.4018678E+01  0.8752455E-01  
2806    6    0.3766585E+01  0.2462853E+01  0.0000000E+00  
2807    7    0.3208373E+01  0.3497599E+01 -0.1720212E+01  
2808    8    0.3100976E+01  0.3536439E+01  0.1653835E+01  
2809    9    0.2688485E+01  0.0000000E+00  0.0000000E+00  
2810    10    0.0000000E+00  0.0000000E+00  0.0000000E+00  
2811    11    -0.7240561E+00  0.9262889E+00 -0.1720220E+01  
2812    12    -0.7165746E+00  0.1040279E+01  0.1653820E+01

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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```
2813    13    -0.8628092E+00 -0.2737848E+01  0.1111461E+00
2814    14    -0.1642739E+00 -0.3740344E+01 -0.1575227E+01
2815    15    -0.2930292E+01 -0.2814307E+01  0.8756234E-01
2816    16    -0.7073055E-01 -0.3943913E+01  0.2368012E+01
2817    17     0.1742700E+01 -0.3688530E+01  0.2462610E+01
2818
2819 Reading esp"s for MEP 38
2820 total number of atoms = 17
2821 total number of esp points = 827
2822
2823 Center      X          Y          Z
2824    1   -0.7075323E-01 -0.3943938E+01 -0.2367976E+01
2825    2     0.1742673E+01 -0.3688528E+01 -0.2462598E+01
2826    3   -0.8628149E+00 -0.2737848E+01 -0.1111197E+00
2827    4   -0.1642777E+00 -0.3740331E+01  0.1575259E+01
2828    5   -0.2930298E+01 -0.2814297E+01 -0.8752455E-01
2829    6     0.0000000E+00  0.0000000E+00  0.0000000E+00
2830    7   -0.7240580E+00  0.9263040E+00  0.1720212E+01
2831    8   -0.7165709E+00  0.1040262E+01 -0.1653835E+01
2832    9     0.2688485E+01  0.0000000E+00  0.0000000E+00
2833   10     0.3766585E+01  0.2462855E+01  0.0000000E+00
2834   11     0.3208384E+01  0.3497592E+01  0.1720220E+01
2835   12     0.3100961E+01  0.3536448E+01 -0.1653820E+01
2836   13     0.6620651E+01  0.2155359E+01 -0.1111461E+00
2837   14     0.7258895E+01  0.1113440E+01  0.1575227E+01
2838   15     0.7519768E+01  0.4018667E+01 -0.8756234E-01
2839   16     0.7407870E+01  0.9461140E+00 -0.2368012E+01
2840   17     0.6446721E+01 -0.6127134E+00 -0.2462610E+01
2841
2842 Reading esp"s for MEP 39
2843 total number of atoms = 17
2844 total number of esp points = 827
2845
2846 Center      X          Y          Z
2847    1     0.7407887E+01  0.9460649E+00  0.2367942E+01
2848    2     0.6446642E+01 -0.6127040E+00  0.2462574E+01
2849    3     0.6620655E+01  0.2155357E+01  0.1111083E+00
2850    4     0.7258876E+01  0.1113470E+01 -0.1575293E+01
2851    5     0.7519777E+01  0.4018665E+01  0.8754911E-01
2852    6     0.3766587E+01  0.2462861E+01  0.0000000E+00
2853    7     0.3208358E+01  0.3497607E+01 -0.1720204E+01
2854    8     0.3100986E+01  0.3536437E+01  0.1653841E+01
2855    9     0.2688489E+01  0.0000000E+00  0.0000000E+00
2856   10     0.0000000E+00  0.0000000E+00  0.0000000E+00
2857   11    -0.7240561E+00  0.9263361E+00 -0.1720197E+01
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

2858    12    -0.7165652E+00    0.1040243E+01    0.1653847E+01  
2859    13    -0.8628149E+00    -0.2737848E+01    0.1110611E+00  
2860    14    -0.1643230E+00    -0.3740282E+01    -0.1575368E+01  
2861    15    -0.2930300E+01    -0.2814299E+01    0.8751132E-01  
2862    16    -0.7070032E-01    -0.3944019E+01    0.2367857E+01  
2863    17    0.1742722E+01    -0.3688592E+01    0.2462468E+01  
2864  
2865    Reading esp"s for MEP 40  
2866    total number of atoms = 17  
2867    total number of esp points = 827  
2868  
2869    Center    X               Y               Z  
2870    1    -0.7067953E-01    -0.3943949E+01    -0.2367942E+01  
2871    2    0.1742735E+01    -0.3688450E+01    -0.2462574E+01  
2872    3    -0.8627979E+00    -0.2737854E+01    -0.1111083E+00  
2873    4    -0.1642795E+00    -0.3740314E+01    0.1575293E+01  
2874    5    -0.2930281E+01    -0.2814322E+01    -0.8754911E-01  
2875    6    0.0000000E+00    0.0000000E+00    0.0000000E+00  
2876    7    -0.7240523E+00    0.9263191E+00    0.1720204E+01  
2877    8    -0.7165671E+00    0.1040251E+01    -0.1653841E+01  
2878    9    0.2688490E+01    0.0000000E+00    0.0000000E+00  
2879    10    0.3766589E+01    0.2462859E+01    0.0000000E+00  
2880    11    0.3208345E+01    0.3497615E+01    0.1720197E+01  
2881    12    0.3100995E+01    0.3536429E+01    -0.1653847E+01  
2882    13    0.6620659E+01    0.2155372E+01    -0.1110611E+00  
2883    14    0.7258865E+01    0.1113519E+01    0.1575368E+01  
2884    15    0.7519766E+01    0.4018688E+01    -0.8751132E-01  
2885    16    0.7407960E+01    0.9460535E+00    -0.2367857E+01  
2886    17    0.6446777E+01    -0.6127512E+00    -0.2462468E+01  
2887  
2888    Reading esp"s for MEP 41  
2889    total number of atoms = 17  
2890    total number of esp points = 827  
2891  
2892    Center    X               Y               Z  
2893    1    0.7407830E+01    0.9461234E+00    0.2368086E+01  
2894    2    0.6446621E+01    -0.6126700E+00    0.2462700E+01  
2895    3    0.6620655E+01    0.2155370E+01    0.1112141E+00  
2896    4    0.7258942E+01    0.1113464E+01    -0.1575149E+01  
2897    5    0.7519756E+01    0.4018688E+01    0.8765683E-01  
2898    6    0.3766589E+01    0.2462855E+01    0.0000000E+00  
2899    7    0.3208433E+01    0.3497575E+01    -0.1720244E+01  
2900    8    0.3100917E+01    0.3536465E+01    0.1653794E+01  
2901    9    0.2688490E+01    0.0000000E+00    0.0000000E+00  
2902    10    0.0000000E+00    0.0000000E+00    0.0000000E+00

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

2903    11    -0.7240523E+00    0.9261982E+00    -0.1720269E+01  
2904    12    -0.7165652E+00    0.1040379E+01    0.1653763E+01  
2905    13    -0.8628168E+00    -0.2737837E+01    0.1112992E+00  
2906    14    -0.1643136E+00    -0.3740427E+01    -0.1575030E+01  
2907    15    -0.2930302E+01    -0.2814282E+01    0.8774376E-01  
2908    16    -0.7071544E-01    -0.3943788E+01    0.2368214E+01  
2909    17    0.1742696E+01    -0.3688278E+01    0.2462840E+01  
2910  
2911    Reading esp"s **for** MEP 42  
2912    total number of atoms = 17  
2913    total number of esp points = 827  
2914  
2915    Center    X               Y               Z  
2916    1    -0.7071733E-01    -0.3943871E+01    -0.2368086E+01  
2917    2    0.1742705E+01    -0.3688414E+01    -0.2462700E+01  
2918    3    -0.8628187E+00    -0.2737845E+01    -0.1112141E+00  
2919    4    -0.1643098E+00    -0.3740372E+01    0.1575149E+01  
2920    5    -0.2930302E+01    -0.2814288E+01    -0.8765683E-01  
2921    6    0.0000000E+00    0.0000000E+00    0.0000000E+00  
2922    7    -0.7240580E+00    0.9262416E+00    0.1720244E+01  
2923    8    -0.7165709E+00    0.1040328E+01    -0.1653794E+01  
2924    9    0.2688485E+01    0.0000000E+00    0.0000000E+00  
2925    10    0.3766587E+01    0.2462859E+01    0.0000000E+00  
2926    11    0.3208467E+01    0.3497558E+01    0.1720269E+01  
2927    12    0.3100868E+01    0.3536484E+01    -0.1653763E+01  
2928    13    0.6620646E+01    0.2155376E+01    -0.1112992E+00  
2929    14    0.7258991E+01    0.1113451E+01    0.1575030E+01  
2930    15    0.7519749E+01    0.4018693E+01    -0.8774376E-01  
2931    16    0.7407752E+01    0.9461575E+00    -0.2368214E+01  
2932    17    0.6446496E+01    -0.6126019E+00    -0.2462840E+01  
2933  
2934    Reading esp"s **for** MEP 43  
2935    total number of atoms = 17  
2936    total number of esp points = 827  
2937  
2938    Center    X               Y               Z  
2939    1    0.7407856E+01    0.9461045E+00    0.2368019E+01  
2940    2    0.6446649E+01    -0.6126870E+00    0.2462625E+01  
2941    3    0.6620647E+01    0.2155376E+01    0.1111631E+00  
2942    4    0.7258921E+01    0.1113491E+01    -0.1575217E+01  
2943    5    0.7519747E+01    0.4018693E+01    0.8761714E-01  
2944    6    0.3766583E+01    0.2462857E+01    0.0000000E+00  
2945    7    0.3208390E+01    0.3497592E+01    -0.1720223E+01  
2946    8    0.3100944E+01    0.3536448E+01    0.1653816E+01  
2947    9    0.2688487E+01    0.0000000E+00    0.0000000E+00

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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2948    10    0.0000000E+00    0.0000000E+00    0.0000000E+00  
2949    11    -0.7240561E+00    0.9262832E+00    -0.1720221E+01  
2950    12    -0.7165671E+00    0.1040281E+01    0.1653822E+01  
2951    13    -0.8628016E+00    -0.2737854E+01    0.1111423E+00  
2952    14    -0.1643192E+00    -0.3740321E+01    -0.1575270E+01  
2953    15    -0.2930289E+01    -0.2814308E+01    0.8761526E-01  
2954    16    -0.7065686E-01    -0.3943949E+01    0.2367957E+01  
2955    17    0.1742764E+01    -0.3688505E+01    0.2462544E+01  
2956  
2957    Reading esp"s for MEP 44  
2958    total number of atoms = 17  
2959    total number of esp points = 827  
2960  
2961    Center    X               Y               Z  
2962    1    -0.7070599E-01    -0.3943909E+01    -0.2368019E+01  
2963    2    0.1742715E+01    -0.3688452E+01    -0.2462625E+01  
2964    3    -0.8628149E+00    -0.2737843E+01    -0.1111631E+00  
2965    4    -0.1643211E+00    -0.3740350E+01    0.1575217E+01  
2966    5    -0.2930300E+01    -0.2814288E+01    -0.8761714E-01  
2967    6    0.0000000E+00    0.0000000E+00    0.0000000E+00  
2968    7    -0.7240580E+00    0.9262794E+00    0.1720223E+01  
2969    8    -0.7165671E+00    0.1040290E+01    -0.1653816E+01  
2970    9    0.2688487E+01    0.0000000E+00    0.0000000E+00  
2971    10    0.3766581E+01    0.2462859E+01    0.0000000E+00  
2972    11    0.3208386E+01    0.3497594E+01    0.1720221E+01  
2973    12    0.3100951E+01    0.3536446E+01    -0.1653822E+01  
2974    13    0.6620653E+01    0.2155359E+01    -0.1111423E+00  
2975    14    0.7258893E+01    0.1113502E+01    0.1575270E+01  
2976    15    0.7519762E+01    0.4018674E+01    -0.8761526E-01  
2977    16    0.7407873E+01    0.9460441E+00    -0.2367957E+01  
2978    17    0.6446677E+01    -0.6127550E+00    -0.2462544E+01  
2979  
2980    Reading esp"s for MEP 45  
2981    total number of atoms = 17  
2982    total number of esp points = 827  
2983  
2984    Center    X               Y               Z  
2985    1    0.7407887E+01    0.9460460E+00    0.2367942E+01  
2986    2    0.6446657E+01    -0.6127304E+00    0.2462551E+01  
2987    3    0.6620653E+01    0.2155359E+01    0.1111216E+00  
2988    4    0.7258893E+01    0.1113496E+01    -0.1575287E+01  
2989    5    0.7519762E+01    0.4018672E+01    0.8758502E-01  
2990    6    0.3766585E+01    0.2462855E+01    0.0000000E+00  
2991    7    0.3208373E+01    0.3497599E+01    -0.1720212E+01  
2992    8    0.3100969E+01    0.3536441E+01    0.1653831E+01

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
2993    9    0.2688483E+01  0.0000000E+00  0.0000000E+00
2994   10    0.0000000E+00  0.0000000E+00  0.0000000E+00
2995   11   -0.7240580E+00  0.9262757E+00 -0.1720225E+01
2996   12   -0.7165728E+00  0.1040294E+01  0.1653814E+01
2997   13   -0.8628187E+00 -0.2737846E+01  0.1111631E+00
2998   14   -0.1643173E+00 -0.3740344E+01 -0.1575221E+01
2999   15   -0.2930304E+01 -0.2814290E+01  0.8760770E-01
3000   16   -0.7071355E-01 -0.3943915E+01  0.2368012E+01
3001   17    0.1742711E+01 -0.3688481E+01  0.2462621E+01
3002
3003 Reading esp"s for MEP 46
3004 total number of atoms = 17
3005 total number of esp points = 827
3006
3007 Center      X          Y          Z
3008    1   -0.7067764E-01 -0.3943956E+01 -0.2367942E+01
3009    2    0.1742739E+01 -0.3688479E+01 -0.2462551E+01
3010    3   -0.8628130E+00 -0.2737848E+01 -0.1111216E+00
3011    4   -0.1643268E+00 -0.3740318E+01  0.1575287E+01
3012    5   -0.2930296E+01 -0.2814299E+01 -0.8758502E-01
3013    6    0.0000000E+00  0.0000000E+00  0.0000000E+00
3014    7   -0.7240561E+00  0.9263021E+00  0.1720212E+01
3015    8   -0.7165690E+00  0.1040270E+01 -0.1653831E+01
3016    9    0.2688487E+01  0.0000000E+00  0.0000000E+00
3017   10    0.3766587E+01  0.2462852E+01  0.0000000E+00
3018   11    0.3208401E+01  0.3497586E+01  0.1720225E+01
3019   12    0.3100951E+01  0.3536450E+01 -0.1653814E+01
3020   13    0.6620657E+01  0.2155361E+01 -0.1111631E+00
3021   14    0.7258916E+01  0.1113474E+01  0.1575221E+01
3022   15    0.7519760E+01  0.4018678E+01 -0.8760770E-01
3023   16    0.7407866E+01  0.9460913E+00 -0.2368012E+01
3024   17    0.6446672E+01 -0.6127096E+00 -0.2462621E+01
3025 Initial ssvpot = 9.136
3026
3027
3028 Number of unique UNfrozen centers= 8
3029
3030 Non-linear optimization requested.
3031 qchnge = 0.1082214511E-01
3032 qchnge = 0.3505139925E-03
3033 qchnge = 0.1954413601E-04
3034 qchnge = 0.1271240293E-05
3035 qchnge = 0.8533682420E-07
3036
3037 Convergence in 4 iterations
```

3038  
3039 1 PEG2 nconf=1 norient=1 nmepl=1/46  
3040 2 PEG2 nconf=1 norient=2 nmepl=2/46  
3041 3 PEG2 nconf=2 norient=1 nmepl=3/46  
3042 4 PEG2 nconf=2 norient=2 nmepl=4/46  
3043 5 PEG2 nconf=3 norient=1 nmepl=5/46  
3044 6 PEG2 nconf=3 norient=2 nmepl=6/46  
3045 7 PEG2 nconf=4 norient=1 nmepl=7/46  
3046 8 PEG2 nconf=4 norient=2 nmepl=8/46  
3047 9 PEG2 nconf=5 norient=1 nmepl=9/46  
3048 10 PEG2 nconf=5 norient=2 nmepl=10/46  
3049 11 PEG2 nconf=6 norient=1 nmepl=11/46  
3050 12 PEG2 nconf=6 norient=2 nmepl=12/46  
3051 13 PEG2 nconf=7 norient=1 nmepl=13/46  
3052 14 PEG2 nconf=7 norient=2 nmepl=14/46  
3053 15 PEG2 nconf=8 norient=1 nmepl=15/46  
3054 16 PEG2 nconf=8 norient=2 nmepl=16/46  
3055 17 PEG2 nconf=9 norient=1 nmepl=17/46  
3056 18 PEG2 nconf=9 norient=2 nmepl=18/46  
3057 19 PEG2 nconf=10 norient=1 nmepl=19/46  
3058 20 PEG2 nconf=10 norient=2 nmepl=20/46  
3059 21 PEG2 nconf=11 norient=1 nmepl=21/46  
3060 22 PEG2 nconf=11 norient=2 nmepl=22/46  
3061 23 PEG2 nconf=12 norient=1 nmepl=23/46  
3062 24 PEG2 nconf=12 norient=2 nmepl=24/46  
3063 25 PEG2 nconf=13 norient=1 nmepl=25/46  
3064 26 PEG2 nconf=13 norient=2 nmepl=26/46  
3065 27 PEG2 nconf=14 norient=1 nmepl=27/46  
3066 28 PEG2 nconf=14 norient=2 nmepl=28/46  
3067 29 PEG2 nconf=15 norient=1 nmepl=29/46  
3068 30 PEG2 nconf=15 norient=2 nmepl=30/46  
3069 31 PEG2 nconf=16 norient=1 nmepl=31/46  
3070 32 PEG2 nconf=16 norient=2 nmepl=32/46  
3071 33 PEG2 nconf=17 norient=1 nmepl=33/46  
3072 34 PEG2 nconf=17 norient=2 nmepl=34/46  
3073 35 PEG2 nconf=18 norient=1 nmepl=35/46  
3074 36 PEG2 nconf=18 norient=2 nmepl=36/46  
3075 37 PEG2 nconf=19 norient=1 nmepl=37/46  
3076 38 PEG2 nconf=19 norient=2 nmepl=38/46  
3077 39 PEG2 nconf=20 norient=1 nmepl=39/46  
3078 40 PEG2 nconf=20 norient=2 nmepl=40/46  
3079 41 PEG2 nconf=21 norient=1 nmepl=41/46  
3080 42 PEG2 nconf=21 norient=2 nmepl=42/46  
3081 43 PEG2 nconf=22 norient=1 nmepl=43/46  
3082 44 PEG2 nconf=22 norient=2 nmepl=44/46

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

3083 45 PEG2 nconf=23 norient=1 nmep=45/46  
 3084 46 PEG2 nconf=23 norient=2 nmep=46/46  
 3085  
 3086 Point Charges Before & After Optimization  
 3087  
 3088 no. At.no. q(init) q(opt) ivary d(rstr)/dq  
 3089 1 8 -0.576790 -0.576790 -1 0.001708  
 3090 2 1 0.360115 0.360115 -1 0.000000  
 3091 3 6 0.256038 0.335117 0 0.002859  
 3092 4 1 -0.000496 0.004532 0 0.000000  
 3093 5 1 0.057037 0.004532 4 0.000000  
 3094 6 6 -0.346939 -0.459651 0 0.002126  
 3095 7 1 0.118234 0.175573 0 0.000000  
 3096 8 1 0.151800 0.175573 7 0.000000  
 3097 9 8 -0.141500 -0.141500 -1 0.005771  
 3098 10 6 0.030353 0.040572 0 0.009266  
 3099 11 1 0.092801 0.086505 0 0.000000  
 3100 12 1 0.096057 0.086505 11 0.000000  
 3101 13 6 -0.507366 -0.496118 0 0.001976  
 3102 14 1 0.203097 0.212018 0 0.000000  
 3103 15 1 0.226558 0.212018 14 0.000000  
 3104 16 8 -0.376457 -0.376457 -1 0.002567  
 3105 17 1 0.357457 0.357457 -1 0.000000  
 3106  
 3107 18 8 -0.576790 -0.576790 -1 0.001708  
 3108 19 1 0.360115 0.360115 -1 0.000000  
 3109 20 6 0.256038 0.335117 3 0.002859  
 3110 21 1 -0.000496 0.004532 4 0.000000  
 3111 22 1 0.057037 0.004532 4 0.000000  
 3112 23 6 -0.346939 -0.459651 6 0.002126  
 3113 24 1 0.118234 0.175573 7 0.000000  
 3114 25 1 0.151800 0.175573 7 0.000000  
 3115 26 8 -0.141500 -0.141500 -1 0.005771  
 3116 27 6 0.030353 0.040572 10 0.009266  
 3117 28 1 0.092801 0.086505 11 0.000000  
 3118 29 1 0.096057 0.086505 11 0.000000  
 3119 30 6 -0.507366 -0.496118 13 0.001976  
 3120 31 1 0.203097 0.212018 14 0.000000  
 3121 32 1 0.226558 0.212018 14 0.000000  
 3122 33 8 -0.376457 -0.376457 -1 0.002567  
 3123 34 1 0.357457 0.357457 -1 0.000000  
 3124  
 3125 35 8 -0.576790 -0.576790 -1 0.001708  
 3126 36 1 0.360115 0.360115 -1 0.000000  
 3127 37 6 0.256038 0.335117 3 0.002859

**B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS**

---

3128	38	1	-0.000496	0.004532	4	0.000000
3129	39	1	0.057037	0.004532	4	0.000000
3130	40	6	-0.346939	-0.459651	6	0.002126
3131	41	1	0.118234	0.175573	7	0.000000
3132	42	1	0.151800	0.175573	7	0.000000
3133	43	8	-0.141500	-0.141500	-1	0.005771
3134	44	6	0.030353	0.040572	10	0.009266
3135	45	1	0.092801	0.086505	11	0.000000
3136	46	1	0.096057	0.086505	11	0.000000
3137	47	6	-0.507366	-0.496118	13	0.001976
3138	48	1	0.203097	0.212018	14	0.000000
3139	49	1	0.226558	0.212018	14	0.000000
3140	50	8	-0.376457	-0.376457	-1	0.002567
3141	51	1	0.357457	0.357457	-1	0.000000
3142						
3143	52	8	-0.576790	-0.576790	-1	0.001708
3144	53	1	0.360115	0.360115	-1	0.000000
3145	54	6	0.256038	0.335117	3	0.002859
3146	55	1	-0.000496	0.004532	4	0.000000
3147	56	1	0.057037	0.004532	4	0.000000
3148	57	6	-0.346939	-0.459651	6	0.002126
3149	58	1	0.118234	0.175573	7	0.000000
3150	59	1	0.151800	0.175573	7	0.000000
3151	60	8	-0.141500	-0.141500	-1	0.005771
3152	61	6	0.030353	0.040572	10	0.009266
3153	62	1	0.092801	0.086505	11	0.000000
3154	63	1	0.096057	0.086505	11	0.000000
3155	64	6	-0.507366	-0.496118	13	0.001976
3156	65	1	0.203097	0.212018	14	0.000000
3157	66	1	0.226558	0.212018	14	0.000000
3158	67	8	-0.376457	-0.376457	-1	0.002567
3159	68	1	0.357457	0.357457	-1	0.000000
3160						
3161	69	8	-0.576790	-0.576790	-1	0.001708
3162	70	1	0.360115	0.360115	-1	0.000000
3163	71	6	0.256038	0.335117	3	0.002859
3164	72	1	-0.000496	0.004532	4	0.000000
3165	73	1	0.057037	0.004532	4	0.000000
3166	74	6	-0.346939	-0.459651	6	0.002126
3167	75	1	0.118234	0.175573	7	0.000000
3168	76	1	0.151800	0.175573	7	0.000000
3169	77	8	-0.141500	-0.141500	-1	0.005771
3170	78	6	0.030353	0.040572	10	0.009266
3171	79	1	0.092801	0.086505	11	0.000000
3172	80	1	0.096057	0.086505	11	0.000000

**B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS**

---

3173	81	6	-0.507366	-0.496118	13	0.001976
3174	82	1	0.203097	0.212018	14	0.000000
3175	83	1	0.226558	0.212018	14	0.000000
3176	84	8	-0.376457	-0.376457	-1	0.002567
3177	85	1	0.357457	0.357457	-1	0.000000
3178						
3179	86	8	-0.576790	-0.576790	-1	0.001708
3180	87	1	0.360115	0.360115	-1	0.000000
3181	88	6	0.256038	0.335117	3	0.002859
3182	89	1	-0.000496	0.004532	4	0.000000
3183	90	1	0.057037	0.004532	4	0.000000
3184	91	6	-0.346939	-0.459651	6	0.002126
3185	92	1	0.118234	0.175573	7	0.000000
3186	93	1	0.151800	0.175573	7	0.000000
3187	94	8	-0.141500	-0.141500	-1	0.005771
3188	95	6	0.030353	0.040572	10	0.009266
3189	96	1	0.092801	0.086505	11	0.000000
3190	97	1	0.096057	0.086505	11	0.000000
3191	98	6	-0.507366	-0.496118	13	0.001976
3192	99	1	0.203097	0.212018	14	0.000000
3193	100	1	0.226558	0.212018	14	0.000000
3194	101	8	-0.376457	-0.376457	-1	0.002567
3195	102	1	0.357457	0.357457	-1	0.000000
3196						
3197	103	8	-0.576790	-0.576790	-1	0.001708
3198	104	1	0.360115	0.360115	-1	0.000000
3199	105	6	0.256038	0.335117	3	0.002859
3200	106	1	-0.000496	0.004532	4	0.000000
3201	107	1	0.057037	0.004532	4	0.000000
3202	108	6	-0.346939	-0.459651	6	0.002126
3203	109	1	0.118234	0.175573	7	0.000000
3204	110	1	0.151800	0.175573	7	0.000000
3205	111	8	-0.141500	-0.141500	-1	0.005771
3206	112	6	0.030353	0.040572	10	0.009266
3207	113	1	0.092801	0.086505	11	0.000000
3208	114	1	0.096057	0.086505	11	0.000000
3209	115	6	-0.507366	-0.496118	13	0.001976
3210	116	1	0.203097	0.212018	14	0.000000
3211	117	1	0.226558	0.212018	14	0.000000
3212	118	8	-0.376457	-0.376457	-1	0.002567
3213	119	1	0.357457	0.357457	-1	0.000000
3214						
3215	120	8	-0.576790	-0.576790	-1	0.001708
3216	121	1	0.360115	0.360115	-1	0.000000
3217	122	6	0.256038	0.335117	3	0.002859

**B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS**

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3218	123	1	-0.000496	0.004532	4	0.000000
3219	124	1	0.057037	0.004532	4	0.000000
3220	125	6	-0.346939	-0.459651	6	0.002126
3221	126	1	0.118234	0.175573	7	0.000000
3222	127	1	0.151800	0.175573	7	0.000000
3223	128	8	-0.141500	-0.141500	-1	0.005771
3224	129	6	0.030353	0.040572	10	0.009266
3225	130	1	0.092801	0.086505	11	0.000000
3226	131	1	0.096057	0.086505	11	0.000000
3227	132	6	-0.507366	-0.496118	13	0.001976
3228	133	1	0.203097	0.212018	14	0.000000
3229	134	1	0.226558	0.212018	14	0.000000
3230	135	8	-0.376457	-0.376457	-1	0.002567
3231	136	1	0.357457	0.357457	-1	0.000000
3232						
3233	137	8	-0.576790	-0.576790	-1	0.001708
3234	138	1	0.360115	0.360115	-1	0.000000
3235	139	6	0.256038	0.335117	3	0.002859
3236	140	1	-0.000496	0.004532	4	0.000000
3237	141	1	0.057037	0.004532	4	0.000000
3238	142	6	-0.346939	-0.459651	6	0.002126
3239	143	1	0.118234	0.175573	7	0.000000
3240	144	1	0.151800	0.175573	7	0.000000
3241	145	8	-0.141500	-0.141500	-1	0.005771
3242	146	6	0.030353	0.040572	10	0.009266
3243	147	1	0.092801	0.086505	11	0.000000
3244	148	1	0.096057	0.086505	11	0.000000
3245	149	6	-0.507366	-0.496118	13	0.001976
3246	150	1	0.203097	0.212018	14	0.000000
3247	151	1	0.226558	0.212018	14	0.000000
3248	152	8	-0.376457	-0.376457	-1	0.002567
3249	153	1	0.357457	0.357457	-1	0.000000
3250						
3251	154	8	-0.576790	-0.576790	-1	0.001708
3252	155	1	0.360115	0.360115	-1	0.000000
3253	156	6	0.256038	0.335117	3	0.002859
3254	157	1	-0.000496	0.004532	4	0.000000
3255	158	1	0.057037	0.004532	4	0.000000
3256	159	6	-0.346939	-0.459651	6	0.002126
3257	160	1	0.118234	0.175573	7	0.000000
3258	161	1	0.151800	0.175573	7	0.000000
3259	162	8	-0.141500	-0.141500	-1	0.005771
3260	163	6	0.030353	0.040572	10	0.009266
3261	164	1	0.092801	0.086505	11	0.000000
3262	165	1	0.096057	0.086505	11	0.000000

**B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS**

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3263	166	6	-0.507366	-0.496118	13	0.001976
3264	167	1	0.203097	0.212018	14	0.000000
3265	168	1	0.226558	0.212018	14	0.000000
3266	169	8	-0.376457	-0.376457	-1	0.002567
3267	170	1	0.357457	0.357457	-1	0.000000
3268						
3269	171	8	-0.576790	-0.576790	-1	0.001708
3270	172	1	0.360115	0.360115	-1	0.000000
3271	173	6	0.256038	0.335117	3	0.002859
3272	174	1	-0.000496	0.004532	4	0.000000
3273	175	1	0.057037	0.004532	4	0.000000
3274	176	6	-0.346939	-0.459651	6	0.002126
3275	177	1	0.118234	0.175573	7	0.000000
3276	178	1	0.151800	0.175573	7	0.000000
3277	179	8	-0.141500	-0.141500	-1	0.005771
3278	180	6	0.030353	0.040572	10	0.009266
3279	181	1	0.092801	0.086505	11	0.000000
3280	182	1	0.096057	0.086505	11	0.000000
3281	183	6	-0.507366	-0.496118	13	0.001976
3282	184	1	0.203097	0.212018	14	0.000000
3283	185	1	0.226558	0.212018	14	0.000000
3284	186	8	-0.376457	-0.376457	-1	0.002567
3285	187	1	0.357457	0.357457	-1	0.000000
3286						
3287	188	8	-0.576790	-0.576790	-1	0.001708
3288	189	1	0.360115	0.360115	-1	0.000000
3289	190	6	0.256038	0.335117	3	0.002859
3290	191	1	-0.000496	0.004532	4	0.000000
3291	192	1	0.057037	0.004532	4	0.000000
3292	193	6	-0.346939	-0.459651	6	0.002126
3293	194	1	0.118234	0.175573	7	0.000000
3294	195	1	0.151800	0.175573	7	0.000000
3295	196	8	-0.141500	-0.141500	-1	0.005771
3296	197	6	0.030353	0.040572	10	0.009266
3297	198	1	0.092801	0.086505	11	0.000000
3298	199	1	0.096057	0.086505	11	0.000000
3299	200	6	-0.507366	-0.496118	13	0.001976
3300	201	1	0.203097	0.212018	14	0.000000
3301	202	1	0.226558	0.212018	14	0.000000
3302	203	8	-0.376457	-0.376457	-1	0.002567
3303	204	1	0.357457	0.357457	-1	0.000000
3304						
3305	205	8	-0.576790	-0.576790	-1	0.001708
3306	206	1	0.360115	0.360115	-1	0.000000
3307	207	6	0.256038	0.335117	3	0.002859

**B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS**

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3308	208	1	-0.000496	0.004532	4	0.000000
3309	209	1	0.057037	0.004532	4	0.000000
3310	210	6	-0.346939	-0.459651	6	0.002126
3311	211	1	0.118234	0.175573	7	0.000000
3312	212	1	0.151800	0.175573	7	0.000000
3313	213	8	-0.141500	-0.141500	-1	0.005771
3314	214	6	0.030353	0.040572	10	0.009266
3315	215	1	0.092801	0.086505	11	0.000000
3316	216	1	0.096057	0.086505	11	0.000000
3317	217	6	-0.507366	-0.496118	13	0.001976
3318	218	1	0.203097	0.212018	14	0.000000
3319	219	1	0.226558	0.212018	14	0.000000
3320	220	8	-0.376457	-0.376457	-1	0.002567
3321	221	1	0.357457	0.357457	-1	0.000000
3322						
3323	222	8	-0.576790	-0.576790	-1	0.001708
3324	223	1	0.360115	0.360115	-1	0.000000
3325	224	6	0.256038	0.335117	3	0.002859
3326	225	1	-0.000496	0.004532	4	0.000000
3327	226	1	0.057037	0.004532	4	0.000000
3328	227	6	-0.346939	-0.459651	6	0.002126
3329	228	1	0.118234	0.175573	7	0.000000
3330	229	1	0.151800	0.175573	7	0.000000
3331	230	8	-0.141500	-0.141500	-1	0.005771
3332	231	6	0.030353	0.040572	10	0.009266
3333	232	1	0.092801	0.086505	11	0.000000
3334	233	1	0.096057	0.086505	11	0.000000
3335	234	6	-0.507366	-0.496118	13	0.001976
3336	235	1	0.203097	0.212018	14	0.000000
3337	236	1	0.226558	0.212018	14	0.000000
3338	237	8	-0.376457	-0.376457	-1	0.002567
3339	238	1	0.357457	0.357457	-1	0.000000
3340						
3341	239	8	-0.576790	-0.576790	-1	0.001708
3342	240	1	0.360115	0.360115	-1	0.000000
3343	241	6	0.256038	0.335117	3	0.002859
3344	242	1	-0.000496	0.004532	4	0.000000
3345	243	1	0.057037	0.004532	4	0.000000
3346	244	6	-0.346939	-0.459651	6	0.002126
3347	245	1	0.118234	0.175573	7	0.000000
3348	246	1	0.151800	0.175573	7	0.000000
3349	247	8	-0.141500	-0.141500	-1	0.005771
3350	248	6	0.030353	0.040572	10	0.009266
3351	249	1	0.092801	0.086505	11	0.000000
3352	250	1	0.096057	0.086505	11	0.000000

**B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS**

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3353	251	6	-0.507366	-0.496118	13	0.001976
3354	252	1	0.203097	0.212018	14	0.000000
3355	253	1	0.226558	0.212018	14	0.000000
3356	254	8	-0.376457	-0.376457	-1	0.002567
3357	255	1	0.357457	0.357457	-1	0.000000
3358						
3359	256	8	-0.576790	-0.576790	-1	0.001708
3360	257	1	0.360115	0.360115	-1	0.000000
3361	258	6	0.256038	0.335117	3	0.002859
3362	259	1	-0.000496	0.004532	4	0.000000
3363	260	1	0.057037	0.004532	4	0.000000
3364	261	6	-0.346939	-0.459651	6	0.002126
3365	262	1	0.118234	0.175573	7	0.000000
3366	263	1	0.151800	0.175573	7	0.000000
3367	264	8	-0.141500	-0.141500	-1	0.005771
3368	265	6	0.030353	0.040572	10	0.009266
3369	266	1	0.092801	0.086505	11	0.000000
3370	267	1	0.096057	0.086505	11	0.000000
3371	268	6	-0.507366	-0.496118	13	0.001976
3372	269	1	0.203097	0.212018	14	0.000000
3373	270	1	0.226558	0.212018	14	0.000000
3374	271	8	-0.376457	-0.376457	-1	0.002567
3375	272	1	0.357457	0.357457	-1	0.000000
3376						
3377	273	8	-0.576790	-0.576790	-1	0.001708
3378	274	1	0.360115	0.360115	-1	0.000000
3379	275	6	0.256038	0.335117	3	0.002859
3380	276	1	-0.000496	0.004532	4	0.000000
3381	277	1	0.057037	0.004532	4	0.000000
3382	278	6	-0.346939	-0.459651	6	0.002126
3383	279	1	0.118234	0.175573	7	0.000000
3384	280	1	0.151800	0.175573	7	0.000000
3385	281	8	-0.141500	-0.141500	-1	0.005771
3386	282	6	0.030353	0.040572	10	0.009266
3387	283	1	0.092801	0.086505	11	0.000000
3388	284	1	0.096057	0.086505	11	0.000000
3389	285	6	-0.507366	-0.496118	13	0.001976
3390	286	1	0.203097	0.212018	14	0.000000
3391	287	1	0.226558	0.212018	14	0.000000
3392	288	8	-0.376457	-0.376457	-1	0.002567
3393	289	1	0.357457	0.357457	-1	0.000000
3394						
3395	290	8	-0.576790	-0.576790	-1	0.001708
3396	291	1	0.360115	0.360115	-1	0.000000
3397	292	6	0.256038	0.335117	3	0.002859

**B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS**

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3398	293	1	-0.000496	0.004532	4	0.000000
3399	294	1	0.057037	0.004532	4	0.000000
3400	295	6	-0.346939	-0.459651	6	0.002126
3401	296	1	0.118234	0.175573	7	0.000000
3402	297	1	0.151800	0.175573	7	0.000000
3403	298	8	-0.141500	-0.141500	-1	0.005771
3404	299	6	0.030353	0.040572	10	0.009266
3405	300	1	0.092801	0.086505	11	0.000000
3406	301	1	0.096057	0.086505	11	0.000000
3407	302	6	-0.507366	-0.496118	13	0.001976
3408	303	1	0.203097	0.212018	14	0.000000
3409	304	1	0.226558	0.212018	14	0.000000
3410	305	8	-0.376457	-0.376457	-1	0.002567
3411	306	1	0.357457	0.357457	-1	0.000000
3412						
3413	307	8	-0.576790	-0.576790	-1	0.001708
3414	308	1	0.360115	0.360115	-1	0.000000
3415	309	6	0.256038	0.335117	3	0.002859
3416	310	1	-0.000496	0.004532	4	0.000000
3417	311	1	0.057037	0.004532	4	0.000000
3418	312	6	-0.346939	-0.459651	6	0.002126
3419	313	1	0.118234	0.175573	7	0.000000
3420	314	1	0.151800	0.175573	7	0.000000
3421	315	8	-0.141500	-0.141500	-1	0.005771
3422	316	6	0.030353	0.040572	10	0.009266
3423	317	1	0.092801	0.086505	11	0.000000
3424	318	1	0.096057	0.086505	11	0.000000
3425	319	6	-0.507366	-0.496118	13	0.001976
3426	320	1	0.203097	0.212018	14	0.000000
3427	321	1	0.226558	0.212018	14	0.000000
3428	322	8	-0.376457	-0.376457	-1	0.002567
3429	323	1	0.357457	0.357457	-1	0.000000
3430						
3431	324	8	-0.576790	-0.576790	-1	0.001708
3432	325	1	0.360115	0.360115	-1	0.000000
3433	326	6	0.256038	0.335117	3	0.002859
3434	327	1	-0.000496	0.004532	4	0.000000
3435	328	1	0.057037	0.004532	4	0.000000
3436	329	6	-0.346939	-0.459651	6	0.002126
3437	330	1	0.118234	0.175573	7	0.000000
3438	331	1	0.151800	0.175573	7	0.000000
3439	332	8	-0.141500	-0.141500	-1	0.005771
3440	333	6	0.030353	0.040572	10	0.009266
3441	334	1	0.092801	0.086505	11	0.000000
3442	335	1	0.096057	0.086505	11	0.000000

**B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS**

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3443	336	6	-0.507366	-0.496118	13	0.001976
3444	337	1	0.203097	0.212018	14	0.000000
3445	338	1	0.226558	0.212018	14	0.000000
3446	339	8	-0.376457	-0.376457	-1	0.002567
3447	340	1	0.357457	0.357457	-1	0.000000
3448						
3449	341	8	-0.576790	-0.576790	-1	0.001708
3450	342	1	0.360115	0.360115	-1	0.000000
3451	343	6	0.256038	0.335117	3	0.002859
3452	344	1	-0.000496	0.004532	4	0.000000
3453	345	1	0.057037	0.004532	4	0.000000
3454	346	6	-0.346939	-0.459651	6	0.002126
3455	347	1	0.118234	0.175573	7	0.000000
3456	348	1	0.151800	0.175573	7	0.000000
3457	349	8	-0.141500	-0.141500	-1	0.005771
3458	350	6	0.030353	0.040572	10	0.009266
3459	351	1	0.092801	0.086505	11	0.000000
3460	352	1	0.096057	0.086505	11	0.000000
3461	353	6	-0.507366	-0.496118	13	0.001976
3462	354	1	0.203097	0.212018	14	0.000000
3463	355	1	0.226558	0.212018	14	0.000000
3464	356	8	-0.376457	-0.376457	-1	0.002567
3465	357	1	0.357457	0.357457	-1	0.000000
3466						
3467	358	8	-0.576790	-0.576790	-1	0.001708
3468	359	1	0.360115	0.360115	-1	0.000000
3469	360	6	0.256038	0.335117	3	0.002859
3470	361	1	-0.000496	0.004532	4	0.000000
3471	362	1	0.057037	0.004532	4	0.000000
3472	363	6	-0.346939	-0.459651	6	0.002126
3473	364	1	0.118234	0.175573	7	0.000000
3474	365	1	0.151800	0.175573	7	0.000000
3475	366	8	-0.141500	-0.141500	-1	0.005771
3476	367	6	0.030353	0.040572	10	0.009266
3477	368	1	0.092801	0.086505	11	0.000000
3478	369	1	0.096057	0.086505	11	0.000000
3479	370	6	-0.507366	-0.496118	13	0.001976
3480	371	1	0.203097	0.212018	14	0.000000
3481	372	1	0.226558	0.212018	14	0.000000
3482	373	8	-0.376457	-0.376457	-1	0.002567
3483	374	1	0.357457	0.357457	-1	0.000000
3484						
3485	375	8	-0.576790	-0.576790	-1	0.001708
3486	376	1	0.360115	0.360115	-1	0.000000
3487	377	6	0.256038	0.335117	3	0.002859

**B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS**

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3488	378	1	-0.000496	0.004532	4	0.000000
3489	379	1	0.057037	0.004532	4	0.000000
3490	380	6	-0.346939	-0.459651	6	0.002126
3491	381	1	0.118234	0.175573	7	0.000000
3492	382	1	0.151800	0.175573	7	0.000000
3493	383	8	-0.141500	-0.141500	-1	0.005771
3494	384	6	0.030353	0.040572	10	0.009266
3495	385	1	0.092801	0.086505	11	0.000000
3496	386	1	0.096057	0.086505	11	0.000000
3497	387	6	-0.507366	-0.496118	13	0.001976
3498	388	1	0.203097	0.212018	14	0.000000
3499	389	1	0.226558	0.212018	14	0.000000
3500	390	8	-0.376457	-0.376457	-1	0.002567
3501	391	1	0.357457	0.357457	-1	0.000000
3502						
3503	392	8	-0.576790	-0.576790	-1	0.001708
3504	393	1	0.360115	0.360115	-1	0.000000
3505	394	6	0.256038	0.335117	3	0.002859
3506	395	1	-0.000496	0.004532	4	0.000000
3507	396	1	0.057037	0.004532	4	0.000000
3508	397	6	-0.346939	-0.459651	6	0.002126
3509	398	1	0.118234	0.175573	7	0.000000
3510	399	1	0.151800	0.175573	7	0.000000
3511	400	8	-0.141500	-0.141500	-1	0.005771
3512	401	6	0.030353	0.040572	10	0.009266
3513	402	1	0.092801	0.086505	11	0.000000
3514	403	1	0.096057	0.086505	11	0.000000
3515	404	6	-0.507366	-0.496118	13	0.001976
3516	405	1	0.203097	0.212018	14	0.000000
3517	406	1	0.226558	0.212018	14	0.000000
3518	407	8	-0.376457	-0.376457	-1	0.002567
3519	408	1	0.357457	0.357457	-1	0.000000
3520						
3521	409	8	-0.576790	-0.576790	-1	0.001708
3522	410	1	0.360115	0.360115	-1	0.000000
3523	411	6	0.256038	0.335117	3	0.002859
3524	412	1	-0.000496	0.004532	4	0.000000
3525	413	1	0.057037	0.004532	4	0.000000
3526	414	6	-0.346939	-0.459651	6	0.002126
3527	415	1	0.118234	0.175573	7	0.000000
3528	416	1	0.151800	0.175573	7	0.000000
3529	417	8	-0.141500	-0.141500	-1	0.005771
3530	418	6	0.030353	0.040572	10	0.009266
3531	419	1	0.092801	0.086505	11	0.000000
3532	420	1	0.096057	0.086505	11	0.000000

**B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS**

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3533	421	6	-0.507366	-0.496118	13	0.001976
3534	422	1	0.203097	0.212018	14	0.000000
3535	423	1	0.226558	0.212018	14	0.000000
3536	424	8	-0.376457	-0.376457	-1	0.002567
3537	425	1	0.357457	0.357457	-1	0.000000
3538						
3539	426	8	-0.576790	-0.576790	-1	0.001708
3540	427	1	0.360115	0.360115	-1	0.000000
3541	428	6	0.256038	0.335117	3	0.002859
3542	429	1	-0.000496	0.004532	4	0.000000
3543	430	1	0.057037	0.004532	4	0.000000
3544	431	6	-0.346939	-0.459651	6	0.002126
3545	432	1	0.118234	0.175573	7	0.000000
3546	433	1	0.151800	0.175573	7	0.000000
3547	434	8	-0.141500	-0.141500	-1	0.005771
3548	435	6	0.030353	0.040572	10	0.009266
3549	436	1	0.092801	0.086505	11	0.000000
3550	437	1	0.096057	0.086505	11	0.000000
3551	438	6	-0.507366	-0.496118	13	0.001976
3552	439	1	0.203097	0.212018	14	0.000000
3553	440	1	0.226558	0.212018	14	0.000000
3554	441	8	-0.376457	-0.376457	-1	0.002567
3555	442	1	0.357457	0.357457	-1	0.000000
3556						
3557	443	8	-0.576790	-0.576790	-1	0.001708
3558	444	1	0.360115	0.360115	-1	0.000000
3559	445	6	0.256038	0.335117	3	0.002859
3560	446	1	-0.000496	0.004532	4	0.000000
3561	447	1	0.057037	0.004532	4	0.000000
3562	448	6	-0.346939	-0.459651	6	0.002126
3563	449	1	0.118234	0.175573	7	0.000000
3564	450	1	0.151800	0.175573	7	0.000000
3565	451	8	-0.141500	-0.141500	-1	0.005771
3566	452	6	0.030353	0.040572	10	0.009266
3567	453	1	0.092801	0.086505	11	0.000000
3568	454	1	0.096057	0.086505	11	0.000000
3569	455	6	-0.507366	-0.496118	13	0.001976
3570	456	1	0.203097	0.212018	14	0.000000
3571	457	1	0.226558	0.212018	14	0.000000
3572	458	8	-0.376457	-0.376457	-1	0.002567
3573	459	1	0.357457	0.357457	-1	0.000000
3574						
3575	460	8	-0.576790	-0.576790	-1	0.001708
3576	461	1	0.360115	0.360115	-1	0.000000
3577	462	6	0.256038	0.335117	3	0.002859

**B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS**

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3578	463	1	-0.000496	0.004532	4	0.000000
3579	464	1	0.057037	0.004532	4	0.000000
3580	465	6	-0.346939	-0.459651	6	0.002126
3581	466	1	0.118234	0.175573	7	0.000000
3582	467	1	0.151800	0.175573	7	0.000000
3583	468	8	-0.141500	-0.141500	-1	0.005771
3584	469	6	0.030353	0.040572	10	0.009266
3585	470	1	0.092801	0.086505	11	0.000000
3586	471	1	0.096057	0.086505	11	0.000000
3587	472	6	-0.507366	-0.496118	13	0.001976
3588	473	1	0.203097	0.212018	14	0.000000
3589	474	1	0.226558	0.212018	14	0.000000
3590	475	8	-0.376457	-0.376457	-1	0.002567
3591	476	1	0.357457	0.357457	-1	0.000000
3592						
3593	477	8	-0.576790	-0.576790	-1	0.001708
3594	478	1	0.360115	0.360115	-1	0.000000
3595	479	6	0.256038	0.335117	3	0.002859
3596	480	1	-0.000496	0.004532	4	0.000000
3597	481	1	0.057037	0.004532	4	0.000000
3598	482	6	-0.346939	-0.459651	6	0.002126
3599	483	1	0.118234	0.175573	7	0.000000
3600	484	1	0.151800	0.175573	7	0.000000
3601	485	8	-0.141500	-0.141500	-1	0.005771
3602	486	6	0.030353	0.040572	10	0.009266
3603	487	1	0.092801	0.086505	11	0.000000
3604	488	1	0.096057	0.086505	11	0.000000
3605	489	6	-0.507366	-0.496118	13	0.001976
3606	490	1	0.203097	0.212018	14	0.000000
3607	491	1	0.226558	0.212018	14	0.000000
3608	492	8	-0.376457	-0.376457	-1	0.002567
3609	493	1	0.357457	0.357457	-1	0.000000
3610						
3611	494	8	-0.576790	-0.576790	-1	0.001708
3612	495	1	0.360115	0.360115	-1	0.000000
3613	496	6	0.256038	0.335117	3	0.002859
3614	497	1	-0.000496	0.004532	4	0.000000
3615	498	1	0.057037	0.004532	4	0.000000
3616	499	6	-0.346939	-0.459651	6	0.002126
3617	500	1	0.118234	0.175573	7	0.000000
3618	501	1	0.151800	0.175573	7	0.000000
3619	502	8	-0.141500	-0.141500	-1	0.005771
3620	503	6	0.030353	0.040572	10	0.009266
3621	504	1	0.092801	0.086505	11	0.000000
3622	505	1	0.096057	0.086505	11	0.000000

**B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS**

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3623	506	6	-0.507366	-0.496118	13	0.001976
3624	507	1	0.203097	0.212018	14	0.000000
3625	508	1	0.226558	0.212018	14	0.000000
3626	509	8	-0.376457	-0.376457	-1	0.002567
3627	510	1	0.357457	0.357457	-1	0.000000
3628						
3629	511	8	-0.576790	-0.576790	-1	0.001708
3630	512	1	0.360115	0.360115	-1	0.000000
3631	513	6	0.256038	0.335117	3	0.002859
3632	514	1	-0.000496	0.004532	4	0.000000
3633	515	1	0.057037	0.004532	4	0.000000
3634	516	6	-0.346939	-0.459651	6	0.002126
3635	517	1	0.118234	0.175573	7	0.000000
3636	518	1	0.151800	0.175573	7	0.000000
3637	519	8	-0.141500	-0.141500	-1	0.005771
3638	520	6	0.030353	0.040572	10	0.009266
3639	521	1	0.092801	0.086505	11	0.000000
3640	522	1	0.096057	0.086505	11	0.000000
3641	523	6	-0.507366	-0.496118	13	0.001976
3642	524	1	0.203097	0.212018	14	0.000000
3643	525	1	0.226558	0.212018	14	0.000000
3644	526	8	-0.376457	-0.376457	-1	0.002567
3645	527	1	0.357457	0.357457	-1	0.000000
3646						
3647	528	8	-0.576790	-0.576790	-1	0.001708
3648	529	1	0.360115	0.360115	-1	0.000000
3649	530	6	0.256038	0.335117	3	0.002859
3650	531	1	-0.000496	0.004532	4	0.000000
3651	532	1	0.057037	0.004532	4	0.000000
3652	533	6	-0.346939	-0.459651	6	0.002126
3653	534	1	0.118234	0.175573	7	0.000000
3654	535	1	0.151800	0.175573	7	0.000000
3655	536	8	-0.141500	-0.141500	-1	0.005771
3656	537	6	0.030353	0.040572	10	0.009266
3657	538	1	0.092801	0.086505	11	0.000000
3658	539	1	0.096057	0.086505	11	0.000000
3659	540	6	-0.507366	-0.496118	13	0.001976
3660	541	1	0.203097	0.212018	14	0.000000
3661	542	1	0.226558	0.212018	14	0.000000
3662	543	8	-0.376457	-0.376457	-1	0.002567
3663	544	1	0.357457	0.357457	-1	0.000000
3664						
3665	545	8	-0.576790	-0.576790	-1	0.001708
3666	546	1	0.360115	0.360115	-1	0.000000
3667	547	6	0.256038	0.335117	3	0.002859

**B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS**

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3668	548	1	-0.000496	0.004532	4	0.000000
3669	549	1	0.057037	0.004532	4	0.000000
3670	550	6	-0.346939	-0.459651	6	0.002126
3671	551	1	0.118234	0.175573	7	0.000000
3672	552	1	0.151800	0.175573	7	0.000000
3673	553	8	-0.141500	-0.141500	-1	0.005771
3674	554	6	0.030353	0.040572	10	0.009266
3675	555	1	0.092801	0.086505	11	0.000000
3676	556	1	0.096057	0.086505	11	0.000000
3677	557	6	-0.507366	-0.496118	13	0.001976
3678	558	1	0.203097	0.212018	14	0.000000
3679	559	1	0.226558	0.212018	14	0.000000
3680	560	8	-0.376457	-0.376457	-1	0.002567
3681	561	1	0.357457	0.357457	-1	0.000000
3682						
3683	562	8	-0.576790	-0.576790	-1	0.001708
3684	563	1	0.360115	0.360115	-1	0.000000
3685	564	6	0.256038	0.335117	3	0.002859
3686	565	1	-0.000496	0.004532	4	0.000000
3687	566	1	0.057037	0.004532	4	0.000000
3688	567	6	-0.346939	-0.459651	6	0.002126
3689	568	1	0.118234	0.175573	7	0.000000
3690	569	1	0.151800	0.175573	7	0.000000
3691	570	8	-0.141500	-0.141500	-1	0.005771
3692	571	6	0.030353	0.040572	10	0.009266
3693	572	1	0.092801	0.086505	11	0.000000
3694	573	1	0.096057	0.086505	11	0.000000
3695	574	6	-0.507366	-0.496118	13	0.001976
3696	575	1	0.203097	0.212018	14	0.000000
3697	576	1	0.226558	0.212018	14	0.000000
3698	577	8	-0.376457	-0.376457	-1	0.002567
3699	578	1	0.357457	0.357457	-1	0.000000
3700						
3701	579	8	-0.576790	-0.576790	-1	0.001708
3702	580	1	0.360115	0.360115	-1	0.000000
3703	581	6	0.256038	0.335117	3	0.002859
3704	582	1	-0.000496	0.004532	4	0.000000
3705	583	1	0.057037	0.004532	4	0.000000
3706	584	6	-0.346939	-0.459651	6	0.002126
3707	585	1	0.118234	0.175573	7	0.000000
3708	586	1	0.151800	0.175573	7	0.000000
3709	587	8	-0.141500	-0.141500	-1	0.005771
3710	588	6	0.030353	0.040572	10	0.009266
3711	589	1	0.092801	0.086505	11	0.000000
3712	590	1	0.096057	0.086505	11	0.000000

**B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS**

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3713	591	6	-0.507366	-0.496118	13	0.001976
3714	592	1	0.203097	0.212018	14	0.000000
3715	593	1	0.226558	0.212018	14	0.000000
3716	594	8	-0.376457	-0.376457	-1	0.002567
3717	595	1	0.357457	0.357457	-1	0.000000
3718						
3719	596	8	-0.576790	-0.576790	-1	0.001708
3720	597	1	0.360115	0.360115	-1	0.000000
3721	598	6	0.256038	0.335117	3	0.002859
3722	599	1	-0.000496	0.004532	4	0.000000
3723	600	1	0.057037	0.004532	4	0.000000
3724	601	6	-0.346939	-0.459651	6	0.002126
3725	602	1	0.118234	0.175573	7	0.000000
3726	603	1	0.151800	0.175573	7	0.000000
3727	604	8	-0.141500	-0.141500	-1	0.005771
3728	605	6	0.030353	0.040572	10	0.009266
3729	606	1	0.092801	0.086505	11	0.000000
3730	607	1	0.096057	0.086505	11	0.000000
3731	608	6	-0.507366	-0.496118	13	0.001976
3732	609	1	0.203097	0.212018	14	0.000000
3733	610	1	0.226558	0.212018	14	0.000000
3734	611	8	-0.376457	-0.376457	-1	0.002567
3735	612	1	0.357457	0.357457	-1	0.000000
3736						
3737	613	8	-0.576790	-0.576790	-1	0.001708
3738	614	1	0.360115	0.360115	-1	0.000000
3739	615	6	0.256038	0.335117	3	0.002859
3740	616	1	-0.000496	0.004532	4	0.000000
3741	617	1	0.057037	0.004532	4	0.000000
3742	618	6	-0.346939	-0.459651	6	0.002126
3743	619	1	0.118234	0.175573	7	0.000000
3744	620	1	0.151800	0.175573	7	0.000000
3745	621	8	-0.141500	-0.141500	-1	0.005771
3746	622	6	0.030353	0.040572	10	0.009266
3747	623	1	0.092801	0.086505	11	0.000000
3748	624	1	0.096057	0.086505	11	0.000000
3749	625	6	-0.507366	-0.496118	13	0.001976
3750	626	1	0.203097	0.212018	14	0.000000
3751	627	1	0.226558	0.212018	14	0.000000
3752	628	8	-0.376457	-0.376457	-1	0.002567
3753	629	1	0.357457	0.357457	-1	0.000000
3754						
3755	630	8	-0.576790	-0.576790	-1	0.001708
3756	631	1	0.360115	0.360115	-1	0.000000
3757	632	6	0.256038	0.335117	3	0.002859

**B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS**

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3758	633	1	-0.000496	0.004532	4	0.000000
3759	634	1	0.057037	0.004532	4	0.000000
3760	635	6	-0.346939	-0.459651	6	0.002126
3761	636	1	0.118234	0.175573	7	0.000000
3762	637	1	0.151800	0.175573	7	0.000000
3763	638	8	-0.141500	-0.141500	-1	0.005771
3764	639	6	0.030353	0.040572	10	0.009266
3765	640	1	0.092801	0.086505	11	0.000000
3766	641	1	0.096057	0.086505	11	0.000000
3767	642	6	-0.507366	-0.496118	13	0.001976
3768	643	1	0.203097	0.212018	14	0.000000
3769	644	1	0.226558	0.212018	14	0.000000
3770	645	8	-0.376457	-0.376457	-1	0.002567
3771	646	1	0.357457	0.357457	-1	0.000000
3772						
3773	647	8	-0.576790	-0.576790	-1	0.001708
3774	648	1	0.360115	0.360115	-1	0.000000
3775	649	6	0.256038	0.335117	3	0.002859
3776	650	1	-0.000496	0.004532	4	0.000000
3777	651	1	0.057037	0.004532	4	0.000000
3778	652	6	-0.346939	-0.459651	6	0.002126
3779	653	1	0.118234	0.175573	7	0.000000
3780	654	1	0.151800	0.175573	7	0.000000
3781	655	8	-0.141500	-0.141500	-1	0.005771
3782	656	6	0.030353	0.040572	10	0.009266
3783	657	1	0.092801	0.086505	11	0.000000
3784	658	1	0.096057	0.086505	11	0.000000
3785	659	6	-0.507366	-0.496118	13	0.001976
3786	660	1	0.203097	0.212018	14	0.000000
3787	661	1	0.226558	0.212018	14	0.000000
3788	662	8	-0.376457	-0.376457	-1	0.002567
3789	663	1	0.357457	0.357457	-1	0.000000
3790						
3791	664	8	-0.576790	-0.576790	-1	0.001708
3792	665	1	0.360115	0.360115	-1	0.000000
3793	666	6	0.256038	0.335117	3	0.002859
3794	667	1	-0.000496	0.004532	4	0.000000
3795	668	1	0.057037	0.004532	4	0.000000
3796	669	6	-0.346939	-0.459651	6	0.002126
3797	670	1	0.118234	0.175573	7	0.000000
3798	671	1	0.151800	0.175573	7	0.000000
3799	672	8	-0.141500	-0.141500	-1	0.005771
3800	673	6	0.030353	0.040572	10	0.009266
3801	674	1	0.092801	0.086505	11	0.000000
3802	675	1	0.096057	0.086505	11	0.000000

**B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS**

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3803	676	6	-0.507366	-0.496118	13	0.001976
3804	677	1	0.203097	0.212018	14	0.000000
3805	678	1	0.226558	0.212018	14	0.000000
3806	679	8	-0.376457	-0.376457	-1	0.002567
3807	680	1	0.357457	0.357457	-1	0.000000
3808						
3809	681	8	-0.576790	-0.576790	-1	0.001708
3810	682	1	0.360115	0.360115	-1	0.000000
3811	683	6	0.256038	0.335117	3	0.002859
3812	684	1	-0.000496	0.004532	4	0.000000
3813	685	1	0.057037	0.004532	4	0.000000
3814	686	6	-0.346939	-0.459651	6	0.002126
3815	687	1	0.118234	0.175573	7	0.000000
3816	688	1	0.151800	0.175573	7	0.000000
3817	689	8	-0.141500	-0.141500	-1	0.005771
3818	690	6	0.030353	0.040572	10	0.009266
3819	691	1	0.092801	0.086505	11	0.000000
3820	692	1	0.096057	0.086505	11	0.000000
3821	693	6	-0.507366	-0.496118	13	0.001976
3822	694	1	0.203097	0.212018	14	0.000000
3823	695	1	0.226558	0.212018	14	0.000000
3824	696	8	-0.376457	-0.376457	-1	0.002567
3825	697	1	0.357457	0.357457	-1	0.000000
3826						
3827	698	8	-0.576790	-0.576790	-1	0.001708
3828	699	1	0.360115	0.360115	-1	0.000000
3829	700	6	0.256038	0.335117	3	0.002859
3830	701	1	-0.000496	0.004532	4	0.000000
3831	702	1	0.057037	0.004532	4	0.000000
3832	703	6	-0.346939	-0.459651	6	0.002126
3833	704	1	0.118234	0.175573	7	0.000000
3834	705	1	0.151800	0.175573	7	0.000000
3835	706	8	-0.141500	-0.141500	-1	0.005771
3836	707	6	0.030353	0.040572	10	0.009266
3837	708	1	0.092801	0.086505	11	0.000000
3838	709	1	0.096057	0.086505	11	0.000000
3839	710	6	-0.507366	-0.496118	13	0.001976
3840	711	1	0.203097	0.212018	14	0.000000
3841	712	1	0.226558	0.212018	14	0.000000
3842	713	8	-0.376457	-0.376457	-1	0.002567
3843	714	1	0.357457	0.357457	-1	0.000000
3844						
3845	715	8	-0.576790	-0.576790	-1	0.001708
3846	716	1	0.360115	0.360115	-1	0.000000
3847	717	6	0.256038	0.335117	3	0.002859

**B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS**

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3848	718	1	-0.000496	0.004532	4	0.000000
3849	719	1	0.057037	0.004532	4	0.000000
3850	720	6	-0.346939	-0.459651	6	0.002126
3851	721	1	0.118234	0.175573	7	0.000000
3852	722	1	0.151800	0.175573	7	0.000000
3853	723	8	-0.141500	-0.141500	-1	0.005771
3854	724	6	0.030353	0.040572	10	0.009266
3855	725	1	0.092801	0.086505	11	0.000000
3856	726	1	0.096057	0.086505	11	0.000000
3857	727	6	-0.507366	-0.496118	13	0.001976
3858	728	1	0.203097	0.212018	14	0.000000
3859	729	1	0.226558	0.212018	14	0.000000
3860	730	8	-0.376457	-0.376457	-1	0.002567
3861	731	1	0.357457	0.357457	-1	0.000000
3862						
3863	732	8	-0.576790	-0.576790	-1	0.001708
3864	733	1	0.360115	0.360115	-1	0.000000
3865	734	6	0.256038	0.335117	3	0.002859
3866	735	1	-0.000496	0.004532	4	0.000000
3867	736	1	0.057037	0.004532	4	0.000000
3868	737	6	-0.346939	-0.459651	6	0.002126
3869	738	1	0.118234	0.175573	7	0.000000
3870	739	1	0.151800	0.175573	7	0.000000
3871	740	8	-0.141500	-0.141500	-1	0.005771
3872	741	6	0.030353	0.040572	10	0.009266
3873	742	1	0.092801	0.086505	11	0.000000
3874	743	1	0.096057	0.086505	11	0.000000
3875	744	6	-0.507366	-0.496118	13	0.001976
3876	745	1	0.203097	0.212018	14	0.000000
3877	746	1	0.226558	0.212018	14	0.000000
3878	747	8	-0.376457	-0.376457	-1	0.002567
3879	748	1	0.357457	0.357457	-1	0.000000
3880						
3881	749	8	-0.576790	-0.576790	-1	0.001708
3882	750	1	0.360115	0.360115	-1	0.000000
3883	751	6	0.256038	0.335117	3	0.002859
3884	752	1	-0.000496	0.004532	4	0.000000
3885	753	1	0.057037	0.004532	4	0.000000
3886	754	6	-0.346939	-0.459651	6	0.002126
3887	755	1	0.118234	0.175573	7	0.000000
3888	756	1	0.151800	0.175573	7	0.000000
3889	757	8	-0.141500	-0.141500	-1	0.005771
3890	758	6	0.030353	0.040572	10	0.009266
3891	759	1	0.092801	0.086505	11	0.000000
3892	760	1	0.096057	0.086505	11	0.000000

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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3893 761 6 -0.507366 -0.496118 13 0.001976  
3894 762 1 0.203097 0.212018 14 0.000000  
3895 763 1 0.226558 0.212018 14 0.000000  
3896 764 8 -0.376457 -0.376457 -1 0.002567  
3897 765 1 0.357457 0.357457 -1 0.000000  
3898  
3899 766 8 -0.576790 -0.576790 -1 0.001708  
3900 767 1 0.360115 0.360115 -1 0.000000  
3901 768 6 0.256038 0.335117 3 0.002859  
3902 769 1 -0.000496 0.004532 4 0.000000  
3903 770 1 0.057037 0.004532 4 0.000000  
3904 771 6 -0.346939 -0.459651 6 0.002126  
3905 772 1 0.118234 0.175573 7 0.000000  
3906 773 1 0.151800 0.175573 7 0.000000  
3907 774 8 -0.141500 -0.141500 -1 0.005771  
3908 775 6 0.030353 0.040572 10 0.009266  
3909 776 1 0.092801 0.086505 11 0.000000  
3910 777 1 0.096057 0.086505 11 0.000000  
3911 778 6 -0.507366 -0.496118 13 0.001976  
3912 779 1 0.203097 0.212018 14 0.000000  
3913 780 1 0.226558 0.212018 14 0.000000  
3914 781 8 -0.376457 -0.376457 -1 0.002567  
3915 782 1 0.357457 0.357457 -1 0.000000  
3916  
3917 Sum over the calculated charges: -0.000  
3918  
3919 Statistics of the fitting:  
3920 The initial **sum** of squares (ssvpot) 9.136  
3921 The residual **sum** of squares (chipot) 0.492  
3922 The std err of estimate (sqrt(chipot/N)) 0.00364  
3923 ESP relative RMS (SQRT(chipot/ssvpot)) 0.23212  
3924 The Pearson correlation coefficient (r2) 0.94698  
3925  
3926 Center of Mass (a.u.):  
3927 #MEP X Y Z  
3928 1 2.10084 0.36011 -0.47027  
3929 2 2.60441 0.68375 0.47027  
3930 3 2.19529 0.16307 -0.52164  
3931 4 2.76843 0.52490 0.52164  
3932 5 2.19524 0.16306 -0.52170  
3933 6 2.76845 0.52493 0.52170  
3934 7 2.19529 0.16305 -0.52168  
3935 8 2.76845 0.52489 0.52168  
3936 9 2.19529 0.16308 -0.52163  
3937 10 2.76842 0.52490 0.52163

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

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3938 11 2.03978 0.19351 -0.50206  
3939 12 2.79012 0.66614 0.50206  
3940 13 1.60287 0.25389 -0.97223  
3941 14 2.92374 1.08732 0.97223  
3942 15 1.61049 0.26767 -0.98910  
3943 16 2.90235 1.08853 0.98910  
3944 17 1.61048 0.26767 -0.98909  
3945 18 2.90235 1.08854 0.98909  
3946 19 1.61502 0.25239 -0.99994  
3947 20 2.91807 1.07559 0.99994  
3948 21 1.88907 0.91761 -2.06707  
3949 22 2.23132 1.11840 2.06707  
3950 23 1.88906 0.91762 -2.06706  
3951 24 2.23131 1.11842 2.06706  
3952 25 1.88906 0.91764 -2.06707  
3953 26 2.23130 1.11843 2.06707  
3954 27 1.88906 0.91760 -2.06706  
3955 28 2.23134 1.11840 2.06706  
3956 29 2.71741 -0.26663 0.09590  
3957 30 2.90794 -0.15287 -0.09590  
3958 31 2.81917 -0.20027 -0.00006  
3959 32 2.81915 -0.20028 0.00006  
3960 33 2.82451 -0.20803 0.75642  
3961 34 2.82453 -0.20803 -0.75642  
3962 35 1.87223 0.86154 2.04528  
3963 36 2.31314 1.11340 -2.04528  
3964 37 2.82451 -0.20802 0.75644  
3965 38 2.82450 -0.20803 -0.75644  
3966 39 2.82451 -0.20805 0.75640  
3967 40 2.82453 -0.20804 -0.75640  
3968 41 2.82450 -0.20800 0.75653  
3969 42 2.82449 -0.20801 -0.75653  
3970 43 2.82452 -0.20803 0.75645  
3971 44 2.82451 -0.20803 -0.75645  
3972 45 2.82451 -0.20804 0.75644  
3973 46 2.82451 -0.20804 -0.75644  
3974  
3975 Dipole moments (Debye) computed:  
3976 -with respect to the origin of coordinates (ooc)  
3977 -with respect to the center of mass (com)  
3978 #MEP D Dx Dy Dz  
3979 1 ooc 3.63096 -0.54983 3.23319 -1.55823  
3980 1 com 3.63096 -0.54983 3.23319 -1.55823  
3981  
3982 2 ooc 3.63096 -2.68088 1.88909 1.55823

**B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS**

---

3983	2 com	3.63096	-2.68088	1.88909	1.55823
3984					
3985	3 ooc	1.47967	-1.00381	1.07715	0.14674
3986	3 com	1.47967	-1.00381	1.07715	0.14674
3987					
3988	4 ooc	1.47967	-0.54401	1.36819	-0.14674
3989	4 com	1.47967	-0.54401	1.36819	-0.14674
3990					
3991	5 ooc	1.47967	-1.00374	1.07720	0.14687
3992	5 com	1.47967	-1.00374	1.07720	0.14687
3993					
3994	6 ooc	1.47966	-0.54408	1.36814	-0.14687
3995	6 com	1.47966	-0.54408	1.36814	-0.14687
3996					
3997	7 ooc	1.47974	-1.00378	1.07726	0.14684
3998	7 com	1.47974	-1.00378	1.07726	0.14684
3999					
4000	8 ooc	1.47974	-0.54412	1.36821	-0.14684
4001	8 com	1.47974	-0.54412	1.36821	-0.14684
4002					
4003	9 ooc	1.47966	-1.00381	1.07714	0.14673
4004	9 com	1.47966	-1.00381	1.07714	0.14673
4005					
4006	10 ooc	1.47966	-0.54400	1.36818	-0.14673
4007	10 com	1.47966	-0.54400	1.36818	-0.14673
4008					
4009	11 ooc	2.80224	-2.63312	-0.91482	0.28695
4010	11 com	2.80224	-2.63312	-0.91482	0.28695
4011					
4012	12 ooc	2.80224	1.93686	2.00469	-0.28695
4013	12 com	2.80224	1.93686	2.00469	-0.28695
4014					
4015	13 ooc	1.66045	-1.44530	0.79406	-0.19409
4016	13 com	1.66045	-1.44530	0.79406	-0.19409
4017					
4018	14 ooc	1.66045	-0.11201	1.64526	0.19409
4019	14 com	1.66045	-0.11201	1.64526	0.19409
4020					
4021	15 ooc	2.27407	-1.42606	-1.18162	1.31967
4022	15 com	2.27407	-1.42606	-1.18162	1.31967
4023					
4024	16 ooc	2.27407	1.66824	0.80426	-1.31967
4025	16 com	2.27407	1.66824	0.80426	-1.31967
4026					
4027	17 ooc	2.27418	-1.42606	-1.18162	1.31985

**B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS**

---

4028	17	com	2.27418	-1.42606	-1.18162	1.31985
4029						
4030	18	ooc	2.27418	1.66824	0.80427	-1.31985
4031	18	com	2.27418	1.66824	0.80427	-1.31985
4032						
4033	19	ooc	3.16779	-1.47898	0.88059	2.65934
4034	19	com	3.16779	-1.47898	0.88059	2.65934
4035						
4036	20	ooc	3.16779	-0.17936	1.71191	-2.65934
4037	20	com	3.16779	-0.17936	1.71191	-2.65934
4038						
4039	21	ooc	4.12947	-3.43812	1.20286	1.94552
4040	21	com	4.12947	-3.43812	1.20286	1.94552
4041						
4042	22	ooc	4.12947	0.48752	3.60968	-1.94552
4043	22	com	4.12947	0.48752	3.60968	-1.94552
4044						
4045	23	ooc	4.12941	-3.43803	1.20280	1.94557
4046	23	com	4.12941	-3.43803	1.20280	1.94557
4047						
4048	24	ooc	4.12941	0.48752	3.60959	-1.94557
4049	24	com	4.12941	0.48752	3.60959	-1.94557
4050						
4051	25	ooc	4.12943	-3.43804	1.20282	1.94558
4052	25	com	4.12943	-3.43804	1.20282	1.94558
4053						
4054	26	ooc	4.12943	0.48753	3.60960	-1.94558
4055	26	com	4.12943	0.48753	3.60960	-1.94558
4056						
4057	27	ooc	4.12941	-3.43805	1.20280	1.94553
4058	27	com	4.12941	-3.43805	1.20280	1.94553
4059						
4060	28	ooc	4.12941	0.48753	3.60960	-1.94553
4061	28	com	4.12941	0.48753	3.60960	-1.94553
4062						
4063	29	ooc	3.84194	-3.11944	1.67530	-1.49095
4064	29	com	3.84194	-3.11944	1.67530	-1.49095
4065						
4066	30	ooc	3.84194	-0.26629	3.53081	1.49095
4067	30	com	3.84194	-0.26629	3.53081	1.49095
4068						
4069	31	ooc	1.44359	-0.96215	1.06007	-0.18571
4070	31	com	1.44359	-0.96215	1.06007	-0.18571
4071						
4072	32	ooc	1.44360	-0.57085	1.31286	0.18571

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

4073	32	com	1.44360	-0.57085	1.31286	0.18571
4074						
4075	33	ooc	2.55094	-0.94737	0.98395	-2.15444
4076	33	com	2.55094	-0.94737	0.98395	-2.15444
4077						
4078	34	ooc	2.55094	-0.52148	1.26243	2.15444
4079	34	com	2.55094	-0.52148	1.26243	2.15444
4080						
4081	35	ooc	2.48321	-1.64991	0.08836	-1.85373
4082	35	com	2.48321	-1.64991	0.08836	-1.85373
4083						
4084	36	ooc	2.48321	0.69867	1.49729	1.85373
4085	36	com	2.48321	0.69867	1.49729	1.85373
4086						
4087	37	ooc	2.55088	-0.94721	0.98384	-2.15450
4088	37	com	2.55088	-0.94721	0.98384	-2.15450
4089						
4090	38	ooc	2.55088	-0.52143	1.26224	2.15450
4091	38	com	2.55088	-0.52143	1.26224	2.15450
4092						
4093	39	ooc	2.55093	-0.94731	0.98399	-2.15444
4094	39	com	2.55093	-0.94731	0.98399	-2.15444
4095						
4096	40	ooc	2.55093	-0.52153	1.26239	2.15444
4097	40	com	2.55093	-0.52153	1.26239	2.15444
4098						
4099	41	ooc	2.55094	-0.94724	0.98393	-2.15451
4100	41	com	2.55094	-0.94724	0.98393	-2.15451
4101						
4102	42	ooc	2.55094	-0.52151	1.26231	2.15451
4103	42	com	2.55094	-0.52151	1.26231	2.15451
4104						
4105	43	ooc	2.55096	-0.94727	0.98395	-2.15451
4106	43	com	2.55096	-0.94727	0.98395	-2.15451
4107						
4108	44	ooc	2.55096	-0.52152	1.26234	2.15451
4109	44	com	2.55096	-0.52152	1.26234	2.15451
4110						
4111	45	ooc	2.55094	-0.94729	0.98397	-2.15448
4112	45	com	2.55094	-0.94729	0.98397	-2.15448
4113						
4114	46	ooc	2.55094	-0.52152	1.26236	2.15448
4115	46	com	2.55094	-0.52152	1.26236	2.15448
4116						
4117			Traceless Quadrupole moments (Buckingham) computed:			

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

4118 -with respect to the origin of coordinates (ooc)  
4119 -with respect to the center of mass (com)  
4120 #MEP X Y Z  
4121 1 ooc X -11.62614  
4122 Y 8.50438 9.64021  
4123 Z 8.51000 -5.12714 1.98593  
4124 1 com X -7.17330  
4125 Y -1.96444 6.72874  
4126 Z 13.29644 -1.82251 0.44457  
4127  
4128 2 ooc X -14.30067  
4129 Y -1.73954 6.28249  
4130 Z 8.52475 15.88618 8.01818  
4131 2 com X 2.62103  
4132 Y -6.64013 -3.06559  
4133 Z 4.08359 12.78442 0.44456  
4134  
4135 3 ooc X -4.12737  
4136 Y -8.50421 12.10238  
4137 Z 24.34793 16.11497 -7.97501  
4138 3 com X 0.64203  
4139 Y -11.99832 9.31730  
4140 Z 23.00525 16.96899 -9.95933  
4141  
4142 4 ooc X -5.41898  
4143 Y -5.39973 14.70646  
4144 Z 24.08569 14.54217 -9.28748  
4145 4 com X -1.55203  
4146 Y -10.95955 11.51136  
4147 Z 25.18112 13.53143 -9.95933  
4148  
4149 5 ooc X -4.12590  
4150 Y -8.50450 12.10122  
4151 Z 24.34767 16.11539 -7.97531  
4152 5 com X 0.64295  
4153 Y -11.99874 9.31630  
4154 Z 23.00452 16.96953 -9.95925  
4155  
4156 6 ooc X -5.42037  
4157 Y -5.39949 14.70773  
4158 Z 24.08523 14.54122 -9.28735  
4159 6 com X -1.55305  
4160 Y -10.95906 11.51230  
4161 Z 25.18134 13.53049 -9.95925  
4162

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

4163 7 ooc X -4.12670  
4164 Y -8.50411 12.10149  
4165 Z 24.34781 16.11492 -7.97480  
4166 7 com X 0.64250  
4167 Y -11.99864 9.31644  
4168 Z 23.00473 16.96908 -9.95894  
4169  
4170 8 ooc X -5.42038  
4171 Y -5.39939 14.70727  
4172 Z 24.08496 14.54174 -9.28689  
4173 8 com X -1.55282  
4174 Y -10.95927 11.51177  
4175 Z 25.18096 13.53096 -9.95896  
4176  
4177 9 ooc X -4.12750  
4178 Y -8.50416 12.10262  
4179 Z 24.34805 16.11490 -7.97513  
4180 9 com X 0.64191  
4181 Y -11.99823 9.31755  
4182 Z 23.00542 16.96889 -9.95946  
4183  
4184 10 ooc X -5.41862  
4185 Y -5.39984 14.70627  
4186 Z 24.08573 14.54233 -9.28764  
4187 10 com X -1.55175  
4188 Y -10.95964 11.51123  
4189 Z 25.18110 13.53161 -9.95947  
4190  
4191 11 ooc X -15.15616  
4192 Y 2.22416 30.59315  
4193 Z 16.80122 5.59949 -15.43699  
4194 11 com X -4.12716  
4195 Y 5.99544 25.13096  
4196 Z 13.77331 4.78220 -21.00381  
4197  
4198 12 ooc X 34.71299  
4199 Y 3.64586 -6.27147  
4200 Z 10.40127 11.78177 -28.44152  
4201 12 com X 24.53499  
4202 Y -7.28201 -3.53118  
4203 Z 10.12856 10.48742 -21.00381  
4204  
4205 13 ooc X -12.55244  
4206 Y -8.18650 14.71941  
4207 Z 27.53640 13.97995 -2.16698

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

4208 13 com X -7.23576  
4209 Y -9.62452 12.04059  
4210 Z 25.79955 15.28376 -4.80484  
4211  
4212 14 ooc X -1.50920  
4213 Y -6.13106 7.46132  
4214 Z 25.45063 19.84501 -5.95212  
4215 14 com X 1.27700  
4216 Y -13.57422 3.52783  
4217 Z 24.72263 16.97062 -4.80483  
4218  
4219 15 ooc X -19.50725  
4220 Y 4.24424 29.95831  
4221 Z 17.14541 7.37192 -10.45105  
4222 15 com X -16.36211  
4223 Y 7.87128 26.81565  
4224 Z 11.53216 4.95573 -10.45354  
4225  
4226 16 ooc X 35.98919  
4227 Y -4.62232 -16.72180  
4228 Z 5.84798 7.40221 -19.26738  
4229 16 com X 25.28553  
4230 Y -11.21087 -14.83198  
4231 Z 9.30895 8.41983 -10.45354  
4232  
4233 17 ooc X -19.50753  
4234 Y 4.24399 29.95833  
4235 Z 17.14439 7.37070 -10.45080  
4236 17 com X -16.36256  
4237 Y 7.87101 26.81553  
4238 Z 11.53073 4.95445 -10.45297  
4239  
4240 18 ooc X 35.98901  
4241 Y -4.62238 -16.72185  
4242 Z 5.84536 7.40114 -19.26716  
4243 18 com X 25.28522  
4244 Y -11.21100 -14.83224  
4245 Z 9.30716 8.41908 -10.45298  
4246  
4247 19 ooc X -13.71825  
4248 Y -9.10954 14.02563  
4249 Z 7.86369 2.73034 -0.30738  
4250 19 com X -11.24147  
4251 Y -10.77468 8.21287  
4252 Z -1.30235 3.06268 3.02860

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

4253  
4254 20 ooc X -3.65189  
4255 Y -6.76741 7.64685  
4256 Z -10.36973 -4.28980 -3.99496  
4257 20 com X -3.40964  
4258 Y -14.39163 0.38105  
4259 Z 2.23445 -2.46646 3.02859  
4260  
4261 21 ooc X -6.97311  
4262 Y -1.74677 16.22822  
4263 Z 25.36111 1.41443 -9.25511  
4264 21 com X 3.68652  
4265 Y -0.34566 2.76185  
4266 Z 8.24427 2.52753 -6.44838  
4267  
4268 22 ooc X 4.95877  
4269 Y 13.82251 15.42597  
4270 Z 0.70033 14.59190 -20.38474  
4271 22 com X 2.67264  
4272 Y 0.17038 3.77574  
4273 Z 5.99212 6.20087 -6.44838  
4274  
4275 23 ooc X -6.97206  
4276 Y -1.74708 16.22752  
4277 Z 25.36073 1.41509 -9.25545  
4278 23 com X 3.68701  
4279 Y -0.34582 2.76136  
4280 Z 8.24410 2.52789 -6.44836  
4281  
4282 24 ooc X 4.95850  
4283 Y 13.82245 15.42636  
4284 Z 0.70042 14.59112 -20.38486  
4285 24 com X 2.67223  
4286 Y 0.17068 3.77614  
4287 Z 5.99235 6.20059 -6.44837  
4288  
4289 25 ooc X -6.97248  
4290 Y -1.74732 16.22803  
4291 Z 25.36066 1.41493 -9.25555  
4292 25 com X 3.68664  
4293 Y -0.34603 2.76173  
4294 Z 8.24394 2.52774 -6.44837  
4295  
4296 26 ooc X 4.95852  
4297 Y 13.82205 15.42646

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

4298 Z 0.70024 14.59107 -20.38498  
4299 26 com X 2.67226  
4300 Y 0.17026 3.77611  
4301 Z 5.99217 6.20049 -6.44837  
4302  
4303 27 ooc X -6.97218  
4304 Y -1.74690 16.22719  
4305 Z 25.36090 1.41503 -9.25501  
4306 27 com X 3.68709  
4307 Y -0.34576 2.76113  
4308 Z 8.24431 2.52794 -6.44822  
4309  
4310 28 ooc X 4.95839  
4311 Y 13.82279 15.42610  
4312 Z 0.70062 14.59143 -20.38450  
4313 28 com X 2.67209  
4314 Y 0.17083 3.77612  
4315 Z 5.99247 6.20074 -6.44821  
4316  
4317 29 ooc X -15.74834  
4318 Y 2.90112 3.14715  
4319 Z -14.95925 -8.43647 12.60119  
4320 29 com X 1.57053  
4321 Y -5.64649 -5.03016  
4322 Z -8.05239 -9.32261 3.45963  
4323  
4324 30 ooc X -9.04736  
4325 Y 15.02207 4.49959  
4326 Z -4.86372 -4.47917 4.54777  
4327 30 com X -8.13088  
4328 Y -1.34234 4.67125  
4329 Z -11.78716 -3.57978 3.45963  
4330  
4331 31 ooc X -10.84824  
4332 Y -8.00340 10.55866  
4333 Z -25.20539 -14.42824 0.28958  
4334 31 com X -5.33141  
4335 Y -13.05366 8.13729  
4336 Z -24.37432 -14.48719 -2.80587  
4337  
4338 32 ooc X -7.05005  
4339 Y -7.63203 7.87440  
4340 Z -22.39501 -16.32342 -0.82435  
4341 32 com X -3.92187  
4342 Y -13.68924 6.72775

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

4343 Z -23.22611 -16.26449 -2.80588  
4344  
4345 33 ooc X -13.16672  
4346 Y -10.87771 10.36918  
4347 Z -10.57682 -0.65833 2.79754  
4348 33 com X -9.44411  
4349 Y -15.60263 6.24568  
4350 Z 0.22133 -2.55143 3.19843  
4351  
4352 34 ooc X -8.85553  
4353 Y -10.51534 7.26981  
4354 Z 8.03825 -1.00163 1.58571  
4355 34 com X -7.74048  
4356 Y -16.34836 4.54206  
4357 Z -2.24855 1.22588 3.19842  
4358  
4359 35 ooc X 14.80345  
4360 Y 0.62816 -2.33807  
4361 Z -12.90494 -7.18294 -12.46539  
4362 35 com X 17.40991  
4363 Y 2.62215 -9.78111  
4364 Z -2.03807 -4.93446 -7.62880  
4365  
4366 36 ooc X 4.09080  
4367 Y 19.48596 15.03808  
4368 Z -0.77427 -1.06049 -19.12887  
4369 36 com X -1.57838  
4370 Y 12.75270 9.20717  
4371 Z -5.31295 0.52457 -7.62879  
4372  
4373 37 ooc X -13.16433  
4374 Y -10.87942 10.36786  
4375 Z -10.57732 -0.65924 2.79646  
4376 37 com X -9.44272  
4377 Y -15.60377 6.24467  
4378 Z 0.22094 -2.55223 3.19805  
4379  
4380 38 ooc X -8.85683  
4381 Y -10.51612 7.27185  
4382 Z 8.03749 -1.00147 1.58498  
4383 38 com X -7.74212  
4384 Y -16.34821 4.54407  
4385 Z -2.24943 1.22586 3.19806  
4386  
4387 39 ooc X -13.16500

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

4388 Y -10.87851 10.36743  
4389 Z -10.57758 -0.65890 2.79757  
4390 39 com X -9.44274  
4391 Y -15.60360 6.24421  
4392 Z 0.22048 -2.55206 3.19852  
4393  
4394 40 ooc X -8.85776  
4395 Y -10.51510 7.27168  
4396 Z 8.03738 -1.00206 1.58608  
4397 40 com X -7.74235  
4398 Y -16.34794 4.54382  
4399 Z -2.24947 1.22537 3.19853  
4400  
4401 41 ooc X -13.16357  
4402 Y -10.87807 10.36761  
4403 Z -10.57728 -0.65894 2.79596  
4404 41 com X -9.44198  
4405 Y -15.60279 6.24413  
4406 Z 0.22118 -2.55209 3.19786  
4407  
4408 42 ooc X -8.85663  
4409 Y -10.51468 7.27205  
4410 Z 8.03787 -1.00150 1.58457  
4411 42 com X -7.74170  
4412 Y -16.34706 4.54384  
4413 Z -2.24921 1.22601 3.19786  
4414  
4415 43 ooc X -13.16385  
4416 Y -10.87849 10.36737  
4417 Z -10.57786 -0.65896 2.79648  
4418 43 com X -9.44191  
4419 Y -15.60339 6.24404  
4420 Z 0.22055 -2.55211 3.19788  
4421  
4422 44 ooc X -8.85735  
4423 Y -10.51489 7.27230  
4424 Z 8.03761 -1.00203 1.58505  
4425 44 com X -7.74219  
4426 Y -16.34747 4.54430  
4427 Z -2.24949 1.22545 3.19789  
4428  
4429 45 ooc X -13.16484  
4430 Y -10.87816 10.36796  
4431 Z -10.57736 -0.65874 2.79687  
4432 45 com X -9.44281

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

```
4433      Y -15.60313  6.24466
4434      Z  0.22088 -2.55190  3.19815
4435
4436 46 ooc X -8.85693
4437      Y -10.51509  7.27151
4438      Z  8.03781 -1.00181  1.58542
4439 46 com X -7.74170
4440      Y -16.34778  4.54355
4441      Z -2.24916  1.22568  3.19815
4442
4443 Traceless Quadrupole moments (Buckingham) in principal axes computed:
4444 -with respect to the origin of coordinates (ooc)
4445 -with respect to the center of mass (com)
4446 #MEP      X        Y        Z
4447 1 ooc X 13.14990
4448      Y  0.00000  5.96955
4449      Z -0.00000  0.00000 -19.11945
4450 1 com X 11.83230
4451      Y -0.00000  5.37271
4452      Z -0.00000 -0.00000 -17.20501
4453
4454 2 ooc X 23.74384
4455      Y -0.00000 -4.16636
4456      Z  0.00000 -0.00000 -19.57748
4457 2 com X 11.83230
4458      Y -0.00000  5.37271
4459      Z -0.00000 -0.00000 -17.20502
4460
4461 3 ooc X 22.84154
4462      Y  0.00000 14.01710
4463      Z -0.00000  0.00000 -36.85864
4464 3 com X 19.83475
4465      Y  0.00000 17.63786
4466      Z -0.00000  0.00000 -37.47261
4467
4468 4 ooc X 23.57767
4469      Y  0.00000 12.08110
4470      Z -0.00000  0.00000 -35.65877
4471 4 com X 19.83475
4472      Y -0.00000 17.63787
4473      Z  0.00000  0.00000 -37.47261
4474
4475 5 ooc X 22.84119
4476      Y  0.00000 14.01728
4477      Z -0.00000  0.00000 -36.85847
```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

4478 5 com X 19.83440  
4479 Y 0.00000 17.63797  
4480 Z 0.00000 0.00000 -37.47237  
4481  
4482 6 ooc X 23.57737  
4483 Y 0.00000 12.08090  
4484 Z -0.00000 -0.00000 -35.65826  
4485 6 com X 19.83440  
4486 Y 0.00000 17.63798  
4487 Z 0.00000 0.00000 -37.47238  
4488  
4489 7 ooc X 22.84127  
4490 Y 0.00000 14.01694  
4491 Z -0.00000 0.00000 -36.85820  
4492 7 com X 19.83432  
4493 Y 0.00000 17.63786  
4494 Z -0.00000 0.00000 -37.47218  
4495  
4496 8 ooc X 23.57766  
4497 Y 0.00000 12.08032  
4498 Z -0.00000 0.00000 -35.65799  
4499 8 com X 19.83433  
4500 Y 0.00000 17.63785  
4501 Z 0.00000 0.00000 -37.47219  
4502  
4503 9 ooc X 22.84160  
4504 Y 0.00000 14.01716  
4505 Z -0.00000 0.00000 -36.85877  
4506 9 com X 19.83482  
4507 Y 0.00000 17.63789  
4508 Z 0.00000 0.00000 -37.47271  
4509  
4510 10 ooc X 23.57765  
4511 Y 0.00000 12.08121  
4512 Z -0.00000 0.00000 -35.65886  
4513 10 com X 19.83482  
4514 Y -0.00000 17.63789  
4515 Z 0.00000 0.00000 -37.47271  
4516  
4517 11 ooc X 31.69429  
4518 Y 0.00000 0.49794  
4519 Z -0.00000 0.00000 -32.19223  
4520 11 com X 27.54854  
4521 Y 0.00000 1.19927  
4522 Z -0.00000 -0.00000 -28.74780

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

4523  
4524 12 ooc X 37.10102  
4525 Y 0.00000 -2.59602  
4526 Z -0.00000 0.00000 -34.50500  
4527 12 com X 27.54854  
4528 Y -0.00000 1.19927  
4529 Z 0.00000 0.00000 -28.74781  
4530  
4531 13 ooc X 25.24040  
4532 Y -0.00000 14.44200  
4533 Z 0.00000 -0.00000 -39.68240  
4534 13 com X 23.25755  
4535 Y -0.00000 14.75997  
4536 Z 0.00000 -0.00000 -38.01752  
4537  
4538 14 ooc X 27.51976  
4539 Y 0.00000 9.85864  
4540 Z -0.00000 -0.00000 -37.37840  
4541 14 com X 23.25755  
4542 Y 0.00000 14.75997  
4543 Z -0.00000 0.00000 -38.01752  
4544  
4545 15 ooc X 32.37722  
4546 Y 0.00000 0.35645  
4547 Z 0.00000 0.00000 -32.73367  
4548 15 com X 29.46726  
4549 Y -0.00000 -3.94917  
4550 Z 0.00000 0.00000 -25.51809  
4551  
4552 16 ooc X 36.88013  
4553 Y -0.00000 -10.48485  
4554 Z 0.00000 0.00000 -26.39529  
4555 16 com X 29.46727  
4556 Y -0.00000 -3.94917  
4557 Z 0.00000 -0.00000 -25.51810  
4558  
4559 17 ooc X 32.37657  
4560 Y 0.00000 0.35616  
4561 Z 0.00000 0.00000 -32.73274  
4562 17 com X 29.46649  
4563 Y -0.00000 -3.94960  
4564 Z 0.00000 0.00000 -25.51688  
4565  
4566 18 ooc X 36.87949  
4567 Y -0.00000 -10.48582

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

4568 Z -0.00000 0.00000 -26.39367  
4569 18 com X 29.46649  
4570 Y -0.00000 -3.94962  
4571 Z 0.00000 0.00000 -25.51687  
4572  
4573 19 ooc X 16.75811  
4574 Y 0.00000 3.20334  
4575 Z 0.00000 0.00000 -19.96145  
4576 19 com X 14.00998  
4577 Y -0.00000 2.02038  
4578 Z -0.00000 -0.00000 -16.03036  
4579  
4580 20 ooc X 10.84649  
4581 Y 0.00000 5.86312  
4582 Z 0.00000 0.00000 -16.70961  
4583 20 com X 14.00998  
4584 Y 0.00000 2.02037  
4585 Z -0.00000 0.00000 -16.03035  
4586  
4587 21 ooc X 17.35193  
4588 Y -0.00000 16.24870  
4589 Z -0.00000 0.00000 -33.60063  
4590 21 com X 8.45683  
4591 Y 0.00000 2.99913  
4592 Z -0.00000 0.00000 -11.45596  
4593  
4594 22 ooc X 28.25830  
4595 Y -0.00000 -2.10331  
4596 Z -0.00000 -0.00000 -26.15499  
4597 22 com X 8.45683  
4598 Y 0.00000 2.99912  
4599 Z -0.00000 -0.00000 -11.45595  
4600  
4601 23 ooc X 17.35177  
4602 Y 0.00000 16.24821  
4603 Z -0.00000 0.00000 -33.59999  
4604 23 com X 8.45704  
4605 Y 0.00000 2.99880  
4606 Z -0.00000 0.00000 -11.45584  
4607  
4608 24 ooc X 28.25813  
4609 Y -0.00000 -2.10364  
4610 Z -0.00000 -0.00000 -26.15449  
4611 24 com X 8.45704  
4612 Y 0.00000 2.99881

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

4613 Z -0.00000 -0.00000 -11.45585  
4614  
4615 25 ooc X 17.35165  
4616 Y -0.00000 16.24852  
4617 Z -0.00000 0.00000 -33.60017  
4618 25 com X 8.45656  
4619 Y 0.00000 2.99921  
4620 Z -0.00000 0.00000 -11.45577  
4621  
4622 26 ooc X 28.25780  
4623 Y -0.00000 -2.10325  
4624 Z -0.00000 0.00000 -26.15455  
4625 26 com X 8.45657  
4626 Y 0.00000 2.99921  
4627 Z -0.00000 0.00000 -11.45578  
4628  
4629 27 ooc X 17.35200  
4630 Y -0.00000 16.24797  
4631 Z -0.00000 0.00000 -33.59997  
4632 27 com X 8.45733  
4633 Y 0.00000 2.99857  
4634 Z -0.00000 0.00000 -11.45590  
4635  
4636 28 ooc X 28.25842  
4637 Y -0.00000 -2.10401  
4638 Z -0.00000 -0.00000 -26.15442  
4639 28 com X 8.45732  
4640 Y 0.00000 2.99856  
4641 Z -0.00000 0.00000 -11.45588  
4642  
4643 29 ooc X 23.01939  
4644 Y -0.00000 -0.81843  
4645 Z -0.00000 -0.00000 -22.20095  
4646 29 com X 11.62003  
4647 Y 0.00000 4.30112  
4648 Z -0.00000 -0.00000 -15.92115  
4649  
4650 30 ooc X 17.40973  
4651 Y 0.00000 1.46611  
4652 Z -0.00000 -0.00000 -18.87584  
4653 30 com X 11.62003  
4654 Y -0.00000 4.30114  
4655 Z 0.00000 -0.00000 -15.92117  
4656  
4657 31 ooc X 24.51839

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

4658 Y -0.00000 11.64275  
4659 Z -0.00000 -0.00000 -36.16115  
4660 31 com X 20.79956  
4661 Y 0.00000 16.07991  
4662 Z -0.00000 0.00000 -36.87947  
4663  
4664 32 ooc X 23.75533  
4665 Y -0.00000 9.55120  
4666 Z -0.00000 0.00000 -33.30653  
4667 32 com X 20.79956  
4668 Y -0.00000 16.07990  
4669 Z 0.00000 -0.00000 -36.87945  
4670  
4671 33 ooc X 15.69613  
4672 Y -0.00000 5.94343  
4673 Z 0.00000 0.00000 -21.63956  
4674 33 com X 16.26634  
4675 Y 0.00000 2.85638  
4676 Z -0.00000 -0.00000 -19.12272  
4677  
4678 34 ooc X 14.25364  
4679 Y 0.00000 2.39720  
4680 Z -0.00000 -0.00000 -16.65084  
4681 34 com X 16.26633  
4682 Y -0.00000 2.85637  
4683 Z -0.00000 -0.00000 -19.12270  
4684  
4685 35 ooc X 20.42690  
4686 Y 0.00000 -0.43694  
4687 Z -0.00000 -0.00000 -19.98997  
4688 35 com X 17.91268  
4689 Y 0.00000 -4.13794  
4690 Z -0.00000 0.00000 -13.77475  
4691  
4692 36 ooc X 29.83978  
4693 Y 0.00000 -10.67564  
4694 Z 0.00000 -0.00000 -19.16414  
4695 36 com X 17.91267  
4696 Y -0.00000 -4.13792  
4697 Z 0.00000 0.00000 -13.77474  
4698  
4699 37 ooc X 15.69642  
4700 Y 0.00000 5.94305  
4701 Z 0.00000 0.00000 -21.63947  
4702 37 com X 16.26713

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

---

4703 Y 0.00000 2.85597  
4704 Z -0.00000 -0.00000 -19.12310  
4705  
4706 38 ooc X 14.25473  
4707 Y 0.00000 2.39696  
4708 Z -0.00000 -0.00000 -16.65168  
4709 38 com X 16.26713  
4710 Y -0.00000 2.85598  
4711 Z -0.00000 -0.00000 -19.12311  
4712  
4713 39 ooc X 15.69568  
4714 Y 0.00000 5.94379  
4715 Z 0.00000 0.00000 -21.63947  
4716 39 com X 16.26654  
4717 Y 0.00000 2.85658  
4718 Z -0.00000 -0.00000 -19.12312  
4719  
4720 40 ooc X 14.25407  
4721 Y 0.00000 2.39732  
4722 Z -0.00000 -0.00000 -16.65140  
4723 40 com X 16.26654  
4724 Y -0.00000 2.85659  
4725 Z -0.00000 -0.00000 -19.12313  
4726  
4727 41 ooc X 15.69550  
4728 Y -0.00000 5.94268  
4729 Z 0.00000 0.00000 -21.63818  
4730 41 com X 16.26609  
4731 Y 0.00000 2.85573  
4732 Z -0.00000 -0.00000 -19.12182  
4733  
4734 42 ooc X 14.25389  
4735 Y 0.00000 2.39700  
4736 Z 0.00000 -0.00000 -16.65089  
4737 42 com X 16.26607  
4738 Y -0.00000 2.85573  
4739 Z -0.00000 0.00000 -19.12180  
4740  
4741 43 ooc X 15.69578  
4742 Y 0.00000 5.94317  
4743 Z 0.00000 0.00000 -21.63895  
4744 43 com X 16.26646  
4745 Y 0.00000 2.85593  
4746 Z -0.00000 -0.00000 -19.12239  
4747

```
4748    44 ooc X 14.25431
4749          Y 0.00000 2.39685
4750          Z 0.00000 -0.00000 -16.65116
4751    44 com X 16.26648
4752          Y -0.00000 2.85595
4753          Z -0.00000 0.00000 -19.12243
4754
4755    45 ooc X 15.69580
4756          Y -0.00000 5.94323
4757          Z 0.00000 0.00000 -21.63903
4758    45 com X 16.26644
4759          Y 0.00000 2.85614
4760          Z -0.00000 -0.00000 -19.12258
4761
4762    46 ooc X 14.25407
4763          Y 0.00000 2.39714
4764          Z -0.00000 -0.00000 -16.65121
4765    46 com X 16.26644
4766          Y -0.00000 2.85614
4767          Z -0.00000 0.00000 -19.12258
```

---

### B.3.7 Amber Leap script for PEG-H(m)

---

```
1 #
2 # Generated by PyRED version SEP-2015
3 #     http://q4md-forcefieldtools.org
4 #
5 logfile q4md-forcefieldtools.log
6 source /home/sajid/amber14/dat/leap/cmd/oldff/leaprc.ff99SB
7 # Web site: http://ambermd.org/doc6/html/AMBER-sh-5.9.html#sh-5.9.8
8 alias q quit
9 alias e edit
10 alias c charge
11
12 # Web site: http://ambermd.org/doc6/html/AMBER-sh-5.9.html#sh-5.9.73
13 verbosity 2
14
15 # Web site: http://ambermd.org/doc6/html/AMBER-sh-5.9.html#sh-5.9.2
16 addAtomTypes {
17     { "CT" "C" "sp3" }
18     { "H1" "H" "sp3" }
19     { "HO" "H" "sp3" }
20     { "OH" "O" "sp3" }
21     { "OS" "O" "sp3" }
22 }
```

```
23
24 # To force the correspondance between residue names
25 # PDB file versus force field libraries:
26 # Web site: http://ambermd.org/doc6/html/AMBER-sh-5.9.html#sh-5.9.7
27 # addPdbResMap {
28 #           { 0 ALA NALA } { 1 ALA CALA }
29 #           { ADE DADE }
30 #
31
32 # Web site: http://ambermd.org/doc6/html/AMBER-sh-5.9.html#sh-5.9.41
33 frcmod1 = loadAmberParams ./frcmod.known
34 frcmod2 = loadAmberParams ./frcmod.correspondence
35 frcmod3 = loadAmberParams ./frcmod.unknown
36
37 # Web site: http://q4md-forcefieldtools.org/Tutorial/leap-mol3.php
38 # Web site: http://q4md-forcefieldtools.org/Tutorial/leap-mol2.php
39 F00 = loadmol3 ../Mol_m1/Mol-ia1_m1-c1.mol2
40 F01 = loadmol3 ../Mol_m1/Mol-ia2_m1-c1.mol2
41 F02 = loadmol3 ../Mol_m1/Mol-ia3_m1-c1.mol2
42 U01 = loadmol3 ../Mol_m1/Mol-sm_m1-c1.mol2
43 # F00_8 = loadmol3 ../Mol_m1/Mol-ia1_m1-c10.mol2
44 # F00_9 = loadmol3 ../Mol_m1/Mol-ia1_m1-c11.mol2
45 # F00_10 = loadmol3 ../Mol_m1/Mol-ia1_m1-c12.mol2
46 # F00_11 = loadmol3 ../Mol_m1/Mol-ia1_m1-c13.mol2
47 # F00_12 = loadmol3 ../Mol_m1/Mol-ia1_m1-c14.mol2
48 # F00_13 = loadmol3 ../Mol_m1/Mol-ia1_m1-c15.mol2
49 # F00_14 = loadmol3 ../Mol_m1/Mol-ia1_m1-c16.mol2
50 # F00_15 = loadmol3 ../Mol_m1/Mol-ia1_m1-c17.mol2
51 # F00_16 = loadmol3 ../Mol_m1/Mol-ia1_m1-c18.mol2
52 # F00_17 = loadmol3 ../Mol_m1/Mol-ia1_m1-c19.mol2
53 # F00_0 = loadmol3 ../Mol_m1/Mol-ia1_m1-c2.mol2
54 # F00_18 = loadmol3 ../Mol_m1/Mol-ia1_m1-c20.mol2
55 # F00_19 = loadmol3 ../Mol_m1/Mol-ia1_m1-c21.mol2
56 # F00_20 = loadmol3 ../Mol_m1/Mol-ia1_m1-c22.mol2
57 # F00_21 = loadmol3 ../Mol_m1/Mol-ia1_m1-c23.mol2
58 # F00_1 = loadmol3 ../Mol_m1/Mol-ia1_m1-c3.mol2
59 # F00_2 = loadmol3 ../Mol_m1/Mol-ia1_m1-c4.mol2
60 # F00_3 = loadmol3 ../Mol_m1/Mol-ia1_m1-c5.mol2
61 # F00_4 = loadmol3 ../Mol_m1/Mol-ia1_m1-c6.mol2
62 # F00_5 = loadmol3 ../Mol_m1/Mol-ia1_m1-c7.mol2
63 # F00_6 = loadmol3 ../Mol_m1/Mol-ia1_m1-c8.mol2
64 # F00_7 = loadmol3 ../Mol_m1/Mol-ia1_m1-c9.mol2
65 # F01_8 = loadmol3 ../Mol_m1/Mol-ia2_m1-c10.mol2
66 # F01_9 = loadmol3 ../Mol_m1/Mol-ia2_m1-c11.mol2
67 # F01_10 = loadmol3 ../Mol_m1/Mol-ia2_m1-c12.mol2
```

```

68 # F01_11 = loadmol3 ../Mol_m1/Mol-ia2_m1-c13.mol2
69 # F01_12 = loadmol3 ../Mol_m1/Mol-ia2_m1-c14.mol2
70 # F01_13 = loadmol3 ../Mol_m1/Mol-ia2_m1-c15.mol2
71 # F01_14 = loadmol3 ../Mol_m1/Mol-ia2_m1-c16.mol2
72 # F01_15 = loadmol3 ../Mol_m1/Mol-ia2_m1-c17.mol2
73 # F01_16 = loadmol3 ../Mol_m1/Mol-ia2_m1-c18.mol2
74 # F01_17 = loadmol3 ../Mol_m1/Mol-ia2_m1-c19.mol2
75 # F01_0 = loadmol3 ../Mol_m1/Mol-ia2_m1-c2.mol2
76 # F01_18 = loadmol3 ../Mol_m1/Mol-ia2_m1-c20.mol2
77 # F01_19 = loadmol3 ../Mol_m1/Mol-ia2_m1-c21.mol2
78 # F01_20 = loadmol3 ../Mol_m1/Mol-ia2_m1-c22.mol2
79 # F01_21 = loadmol3 ../Mol_m1/Mol-ia2_m1-c23.mol2
80 # F01_1 = loadmol3 ../Mol_m1/Mol-ia2_m1-c3.mol2
81 # F01_2 = loadmol3 ../Mol_m1/Mol-ia2_m1-c4.mol2
82 # F01_3 = loadmol3 ../Mol_m1/Mol-ia2_m1-c5.mol2
83 # F01_4 = loadmol3 ../Mol_m1/Mol-ia2_m1-c6.mol2
84 # F01_5 = loadmol3 ../Mol_m1/Mol-ia2_m1-c7.mol2
85 # F01_6 = loadmol3 ../Mol_m1/Mol-ia2_m1-c8.mol2
86 # F01_7 = loadmol3 ../Mol_m1/Mol-ia2_m1-c9.mol2
87 # F02_8 = loadmol3 ../Mol_m1/Mol-ia3_m1-c10.mol2
88 # F02_9 = loadmol3 ../Mol_m1/Mol-ia3_m1-c11.mol2
89 # F02_10 = loadmol3 ../Mol_m1/Mol-ia3_m1-c12.mol2
90 # F02_11 = loadmol3 ../Mol_m1/Mol-ia3_m1-c13.mol2
91 # F02_12 = loadmol3 ../Mol_m1/Mol-ia3_m1-c14.mol2
92 # F02_13 = loadmol3 ../Mol_m1/Mol-ia3_m1-c15.mol2
93 # F02_14 = loadmol3 ../Mol_m1/Mol-ia3_m1-c16.mol2
94 # F02_15 = loadmol3 ../Mol_m1/Mol-ia3_m1-c17.mol2
95 # F02_16 = loadmol3 ../Mol_m1/Mol-ia3_m1-c18.mol2
96 # F02_17 = loadmol3 ../Mol_m1/Mol-ia3_m1-c19.mol2
97 # F02_0 = loadmol3 ../Mol_m1/Mol-ia3_m1-c2.mol2
98 # F02_18 = loadmol3 ../Mol_m1/Mol-ia3_m1-c20.mol2
99 # F02_19 = loadmol3 ../Mol_m1/Mol-ia3_m1-c21.mol2
100 # F02_20 = loadmol3 ../Mol_m1/Mol-ia3_m1-c22.mol2
101 # F02_21 = loadmol3 ../Mol_m1/Mol-ia3_m1-c23.mol2
102 # F02_1 = loadmol3 ../Mol_m1/Mol-ia3_m1-c3.mol2
103 # F02_2 = loadmol3 ../Mol_m1/Mol-ia3_m1-c4.mol2
104 # F02_3 = loadmol3 ../Mol_m1/Mol-ia3_m1-c5.mol2
105 # F02_4 = loadmol3 ../Mol_m1/Mol-ia3_m1-c6.mol2
106 # F02_5 = loadmol3 ../Mol_m1/Mol-ia3_m1-c7.mol2
107 # F02_6 = loadmol3 ../Mol_m1/Mol-ia3_m1-c8.mol2
108 # F02_7 = loadmol3 ../Mol_m1/Mol-ia3_m1-c9.mol2
109 # U01 = loadmol3 ../Mol_m1/Mol-sm_m1-c10.mol2
110 # U01 = loadmol3 ../Mol_m1/Mol-sm_m1-c11.mol2
111 # U01 = loadmol3 ../Mol_m1/Mol-sm_m1-c12.mol2
112 # U01 = loadmol3 ../Mol_m1/Mol-sm_m1-c13.mol2

```

```

113 # U01 = loadmol3 ../Mol_m1/Mol-sm_m1-c14.mol2
114 # U01 = loadmol3 ../Mol_m1/Mol-sm_m1-c15.mol2
115 # U01 = loadmol3 ../Mol_m1/Mol-sm_m1-c16.mol2
116 # U01 = loadmol3 ../Mol_m1/Mol-sm_m1-c17.mol2
117 # U01 = loadmol3 ../Mol_m1/Mol-sm_m1-c18.mol2
118 # U01 = loadmol3 ../Mol_m1/Mol-sm_m1-c19.mol2
119 # U01 = loadmol3 ../Mol_m1/Mol-sm_m1-c2.mol2
120 # U01 = loadmol3 ../Mol_m1/Mol-sm_m1-c20.mol2
121 # U01 = loadmol3 ../Mol_m1/Mol-sm_m1-c21.mol2
122 # U01 = loadmol3 ../Mol_m1/Mol-sm_m1-c22.mol2
123 # U01 = loadmol3 ../Mol_m1/Mol-sm_m1-c23.mol2
124 # U01 = loadmol3 ../Mol_m1/Mol-sm_m1-c3.mol2
125 # U01 = loadmol3 ../Mol_m1/Mol-sm_m1-c4.mol2
126 # U01 = loadmol3 ../Mol_m1/Mol-sm_m1-c5.mol2
127 # U01 = loadmol3 ../Mol_m1/Mol-sm_m1-c6.mol2
128 # U01 = loadmol3 ../Mol_m1/Mol-sm_m1-c7.mol2
129 # U01 = loadmol3 ../Mol_m1/Mol-sm_m1-c8.mol2
130 # U01 = loadmol3 ../Mol_m1/Mol-sm_m1-c9.mol2
131
132 # To match the residue names found in the PDB file
133 # Web site: http://ambermd.org/doc6/html/AMBER-sh-5.9.html#sh-5.9.19
134 # ZZZ = copy F00
135
136 # If a copy is done, define the molecule and residue
137 # Web site: http://ambermd.org/doc6/html/AMBER-sh-5.9.html#sh-5.9.63
138 # set ZZZ name "ZZZ"
139 # set ZZZ.1 name "ZZZ"
140
141 # Let's load the PDB file
142 # Web site: http://ambermd.org/doc6/html/AMBER-sh-5.9.html#sh-5.9.44
143 # VAR = loadPdb Your-PDB-file.ent
144
145 # Let's save the prmtop and prmcrd file with specific file extensions
146 # (to be automatically recognized by VMD http://www.ks.uiuc.edu/Research/vmd/)
147 # Web site: http://ambermd.org/doc6/html/AMBER-sh-5.9.html#sh-5.9.54
148 # saveAmberParm F00 F00.parm7 F00.rst7
149
150 # q
151
152 saveoff F00 F00pghlgauss.off
153 saveoff F01 F01pghlgauss.off
154 saveoff F02 F02pghlgauss.off
155
156

```

### B.3. PEG WITH HYDROXYETHYL TERMINAL, MULTIPLE CONFORMATIONS

### B.3.8 Multiple mini-PEG conformations

---

```
1 """
2 Generate multiple miniPEG conformations for FF development
3
4 Input Files: one miniPEG molecule
5
6 output: Txt file containing radgyr and pdb files
7 """
8 ######
9 # Import Libraries as listed in Sire sampling input file
10 #####
11 from MyFunctions import *
12
13 #####
14 # Main program
15 #####
16 # Load initial system
17 inpcrd = "pyred.inpcrd"
18 prmtop = "pyred.prmtop"
19 dimensions = 'box101.dat'
20 save_sys_name = "pyredsampling.s3"
21 input_system = "restart_pyred.s3"
22
23 # Assign flexibility parameters necessary to evaluate internal MC moves
24 import os.path
25 check = os.path.isfile(input_system)
26 check = str(check)
27 if check == 'True':
28     print("Minimized or system with flexibility is available and load
          it")
29     new_groups = load(input_system)
30     new_group = collect_mc_molecs(new_groups)
31     (system1,ljcp)=create_single_System(new_group,new_groups.property(
          'space'))
32 else:
33     print("Making system with flexibility and defining rigid and
          internal moves...")
34     (new_group,space)=load_files_to_prepare_system_for_flexibility(
          inpcrd,prmtop,dimensions,save_sys_name)
35     (system1,ljcp)=create_single_System(new_group,space)
36
37     print("system is done!")
38 ## Define input simulation parameters
39
```

```

40 # Initiate random generator
41 rangen = RanGenerator()
42 ## Set the temperature
43 temperature = 298.15 * kelvin
44 ## Specify the maximum amount to move the
45 ## bonds, angles and dihedrals
46 max_delta_bond = 0.00 * angstrom
47 max_delta_angle = 3.5 * degrees
48 max_delta_dihedral = 10 * degrees
49 ## specify the maximum number of bonds,
50 ## angles and dihedrals to move per move
51 max_num_move = 250
52 # set the maximum delta parameters
53 params = {}
54 params["bond flex"] = max_delta_bond
55 params["angle flex"] = max_delta_angle
56 params["dihedral flex"] = max_delta_dihedral
57 params["h dihedral flex"] = max_delta_dihedral
58 params["maxvar"] = max_num_move
59 Parameter.push(params)
60
61 # Perform Rigid body and Internal MC simulations
62 #(system1,ljcp)=create_single_System(new_group,space)
63 (system_mc,wt_moves)=NVT_ensemble_moves(system1)
64 MGName = system_mc.groupNames()[-1]
65 internalmove = InternalMove(system_mc[MGName])
66 Sire.Stream.save( system_mc, "flex_system_peg1.s3" )
67 start_time = time.clock()
68 t = QTime()
69 t.start()
70 file = open('equi_pyred.log','w')
71 print("Perform MC simulations")
72 # perform 5000 blocks of 2*5000 moves
73 for i in range(1,2):
74     print("performing step %s%i"
75     print("system_mc start energy %s"%system_mc.energy())
76     system_mc = simulation_moves_simple(system_mc,wt_moves,0)
77     print("system energy after RIGID BODY mc % s " % system_mc.energy
78     ())
79     internalmove.move(system_mc, 0)
80     print("Energy %s, nAccepted = %s, nRejected = %s" %
81           (system_mc.energy(), internalmove.nAccepted(),
82            internalmove.nRejected()))
82     print("system final energy after internal Moves %s"%system_mc.
83           energy())

```

```
83     file.write(str(system_mc.energy())+' \n')
84     print("*****
85         ")
86     if i % 1 == 0:
87         PDB().write(system_mc.molecules(), "output/Mol_red%00d.pdb" % i)
88         Sire.Stream.save( system_mc, "output/Mol_red%00d.s3" % i )
89     file.close()
90 elapsed = (time.clock() - start_time)
91 print("time taken %d"%elapsed)
```

---

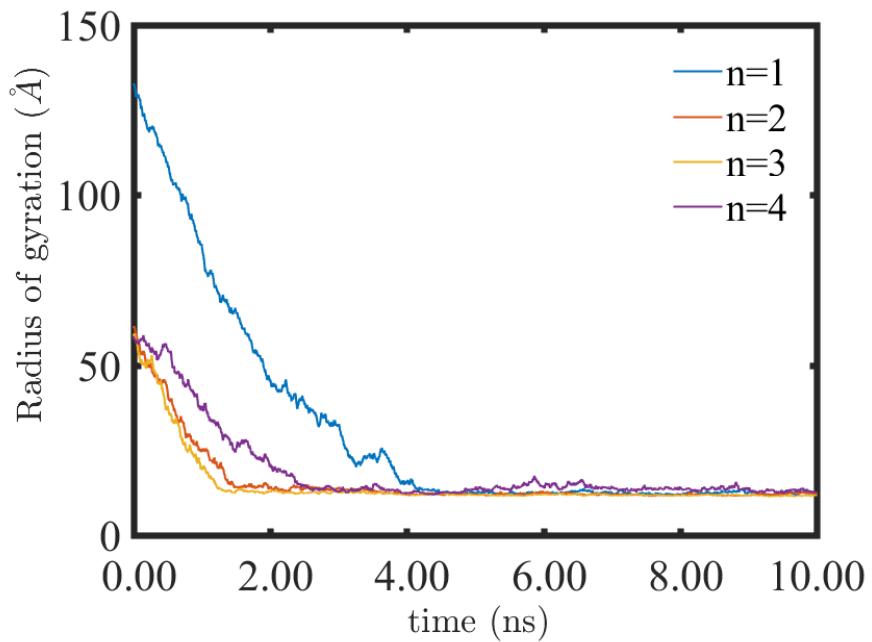
**B.3.9 FF effect on the PEG folding**

Figure B.1: Effect of length of central fragments on the radii of gyration of the 8 kDa PEG structures with hydroxyethyl terminals in the 10 ns long MD conformational sampling simulations performed with an NPT ensemble in an implicit solvent at 300 K using HFF parameters.

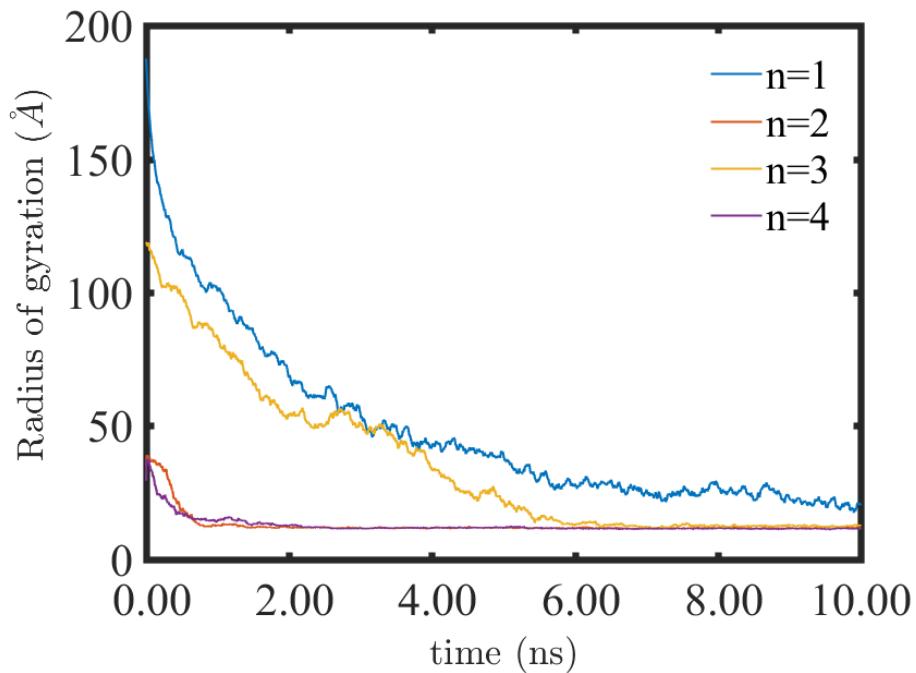


Figure B.2: Effect of length of central fragments on the radii of gyration of the 8 kDa PEG structures with methyl terminals in the 10 ns long MD conformational sampling simulations performed with an NPT ensemble in an implicit solvent at 300 K using MFF parameters.

## B.4 MCCCS Towhee plots

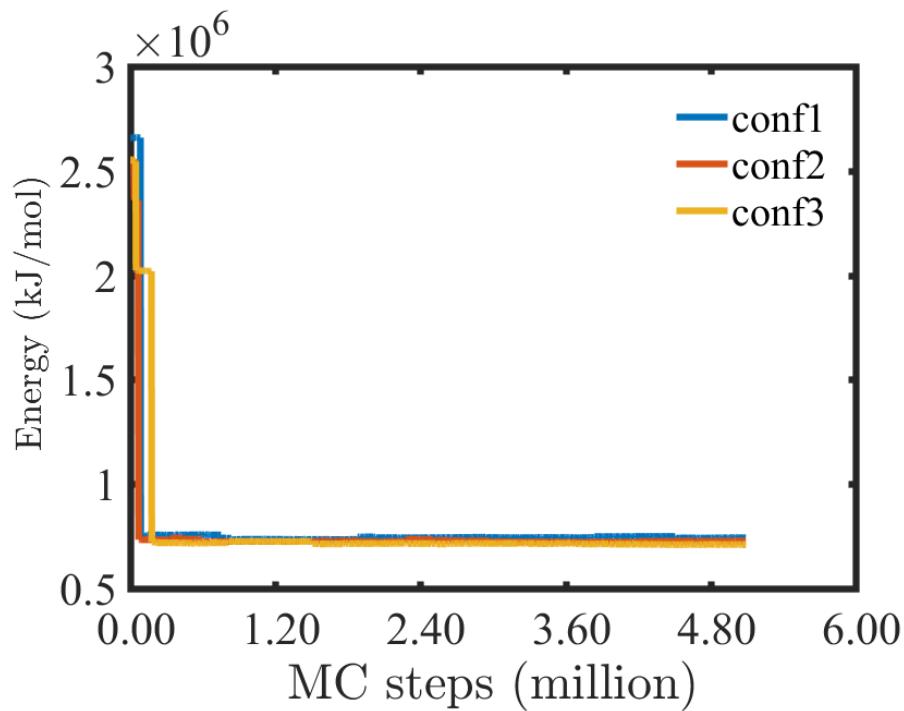


Figure B.3: Total energy variations in MCCCS Towhee simulations conducted at 300 K for three different starting conformations using the NPT ensemble and ‘RANLUX’ random number generator.

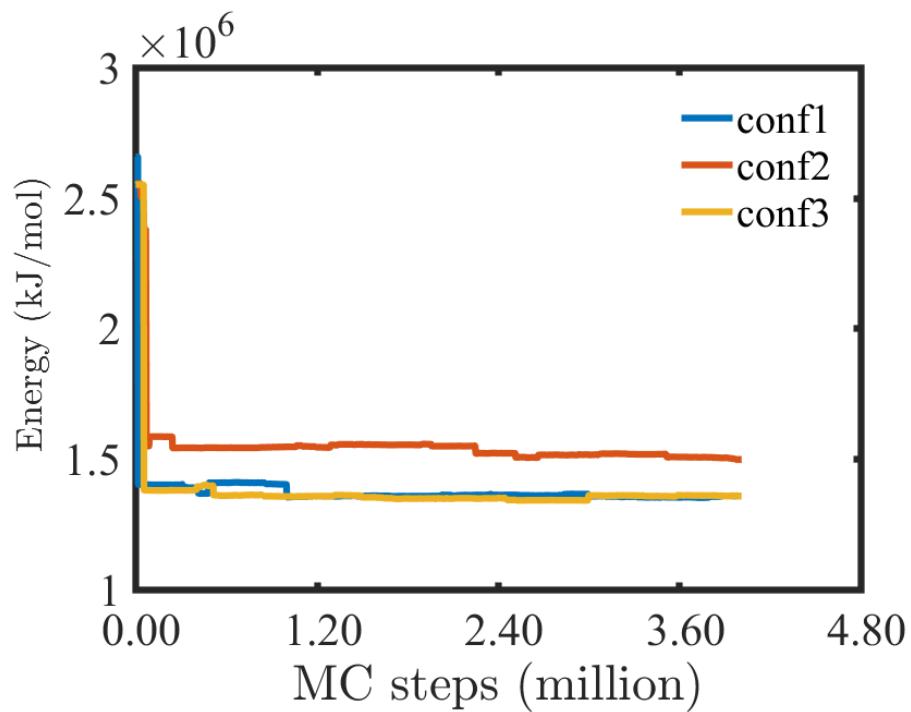


Figure B.4: Total energy variations in MCCCS Towhee simulations conducted at 600 K for three different starting conformations using the NPT ensemble and ‘RANLUX’ random number generator.

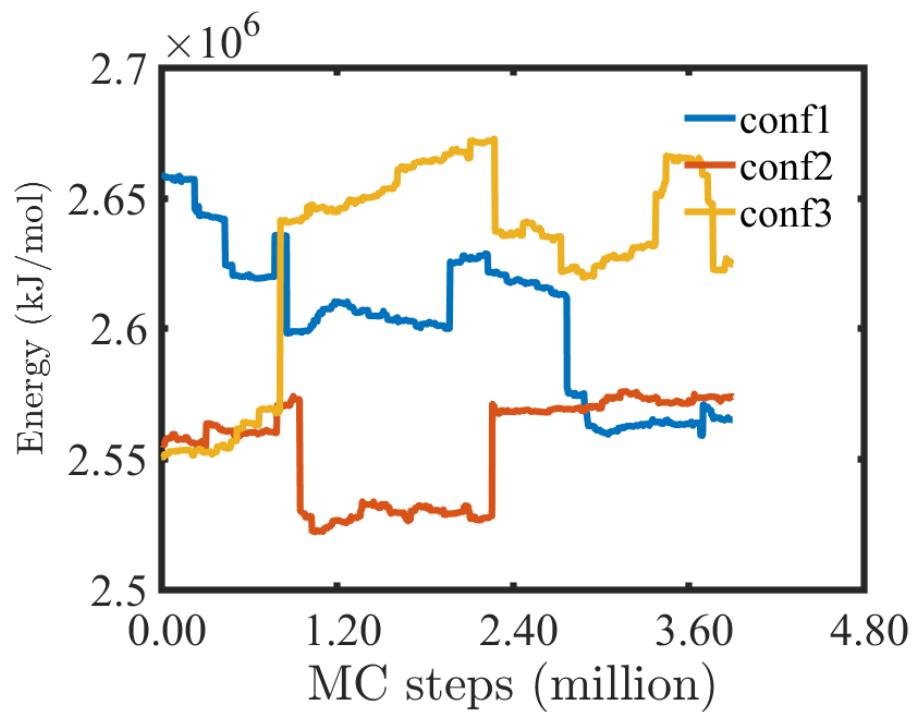


Figure B.5: Total energy variations in MCCCS Towhee simulations conducted at 1200 K for three different starting conformations using the NPT ensemble and ‘RANLUX’ random number generator.

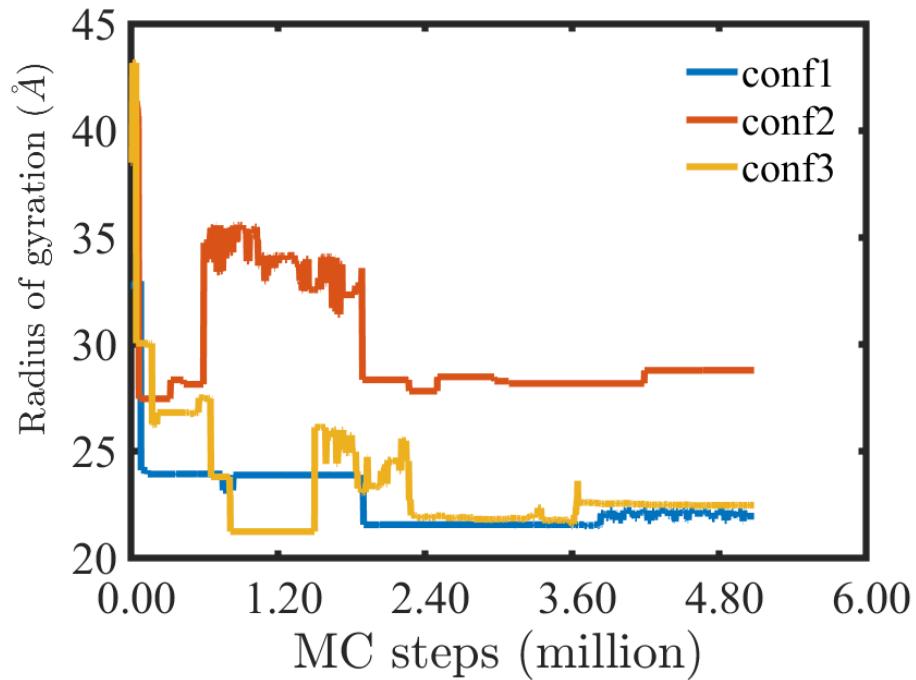


Figure B.6: Radii of gyration variations in MCCCS Towhee simulations conducted at 300 K for three different starting conformations using the NPT ensemble and ‘RANLUX’ random number generator.

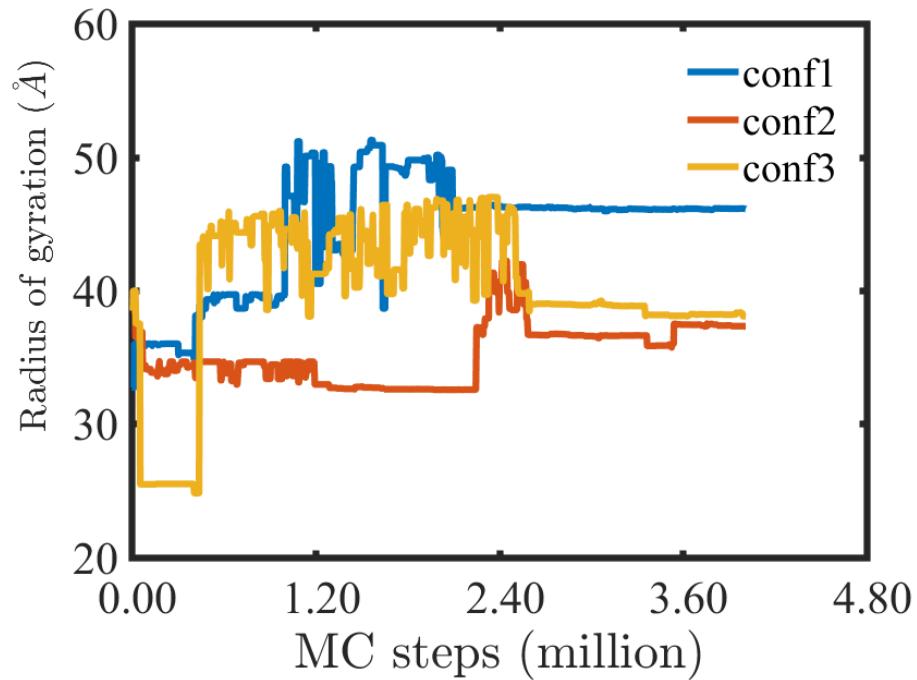


Figure B.7: Radii of gyration variations in MCCCS Towhee simulations conducted at 600 K for three different starting conformations using the NPT ensemble and ‘RANLUX’ random number generator.

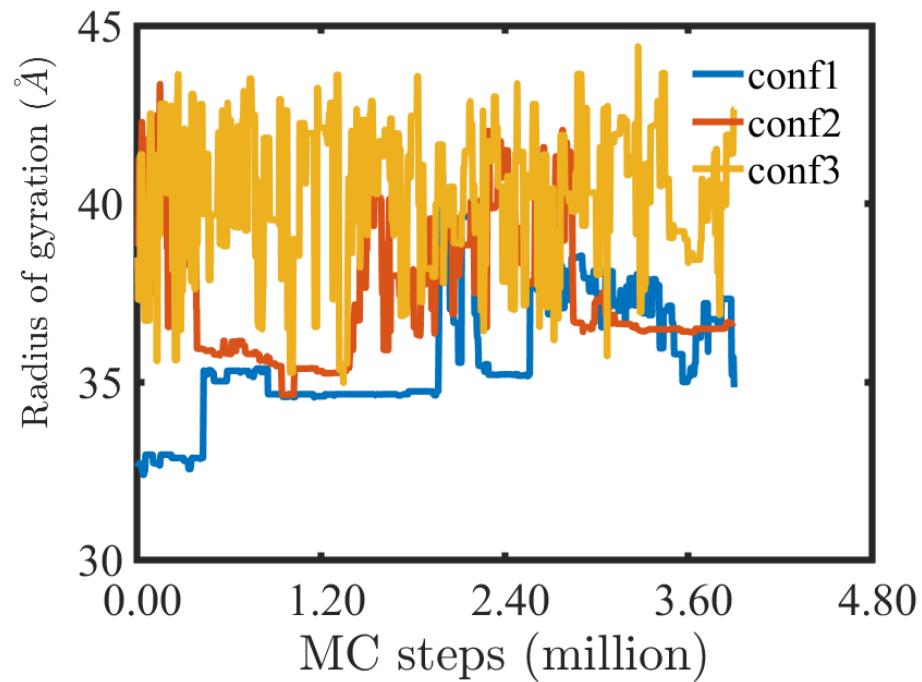


Figure B.8: Radii of gyration variations in MCCCS Towhee simulations conducted at 1200 K for three different starting conformations using the NPT ensemble and ‘RANLUX’ random number generator.

## B.5 Sire plots

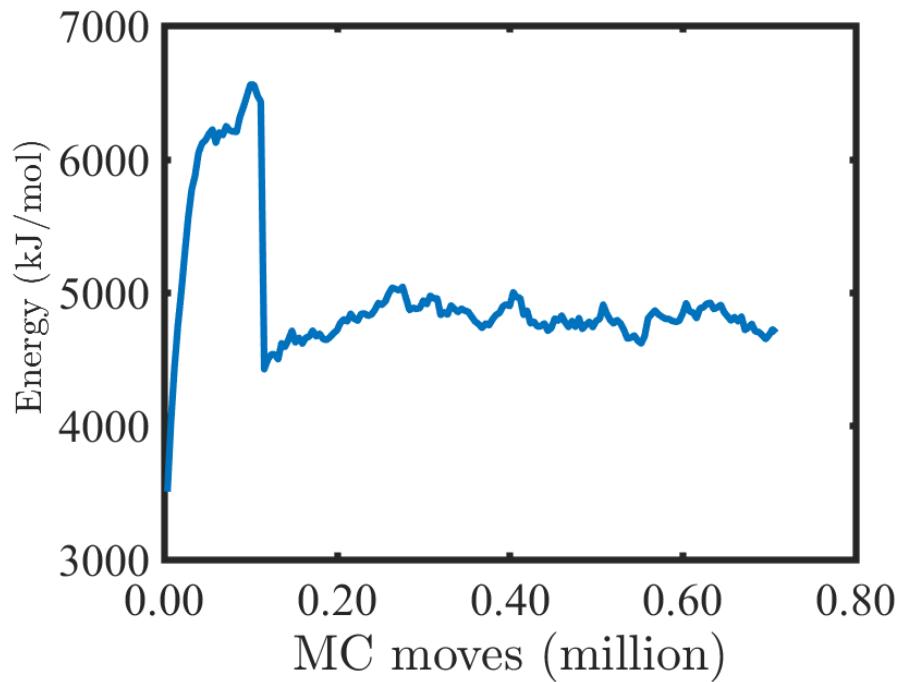


Figure B.9: Energy fluctuations in the NPT ensemble MC conformational sampling simulations conducted at 1200 K. These simulations were started with a linear PEG structure with a hydroxyethyl terminal and used HFF parameters.

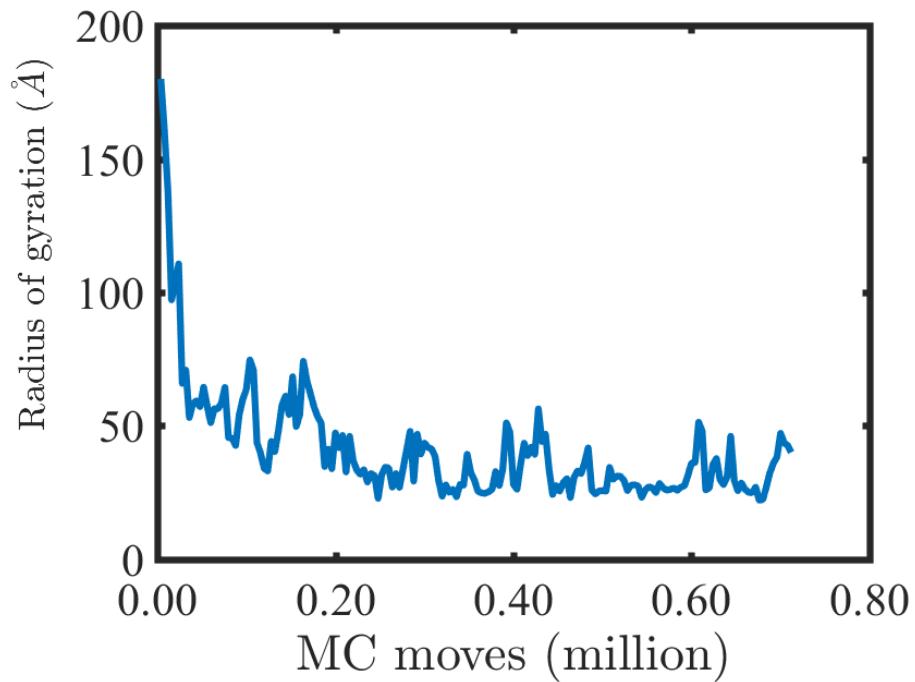


Figure B.10: Change in the radius of gyration in MC conformational sampling conducted at 1200 K, starting with a linear PEG structure. The structure reached an equilibrium state after completing less than 0.05 million steps.

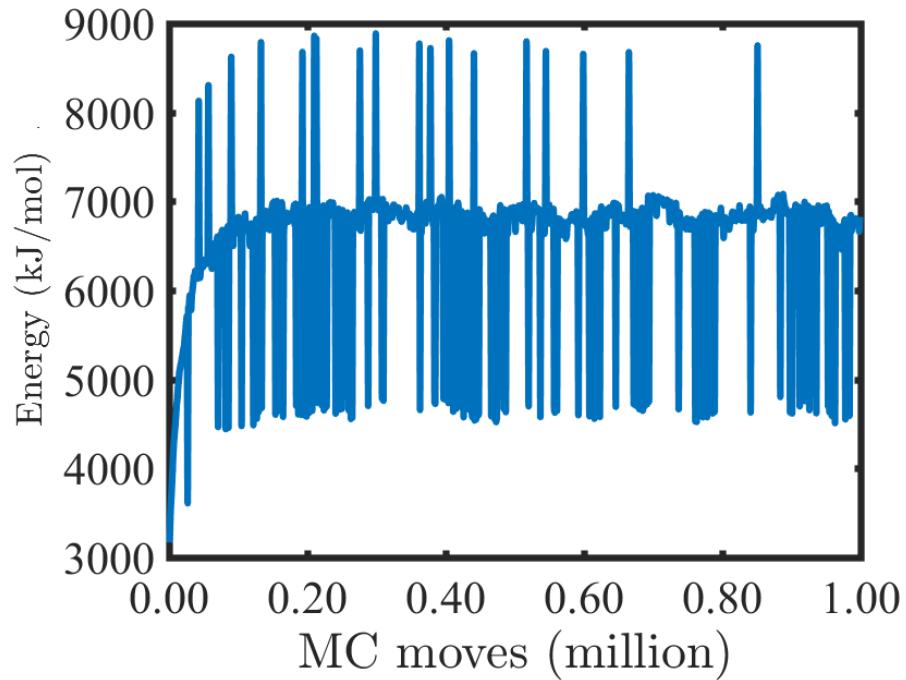


Figure B.11: Energy change during MC conformational sampling at 300 K, using a temperature quenching approach between 300-1200 K. These simulations used a linear 8 kDa PEG with a methyl terminal group and MFF parameters.

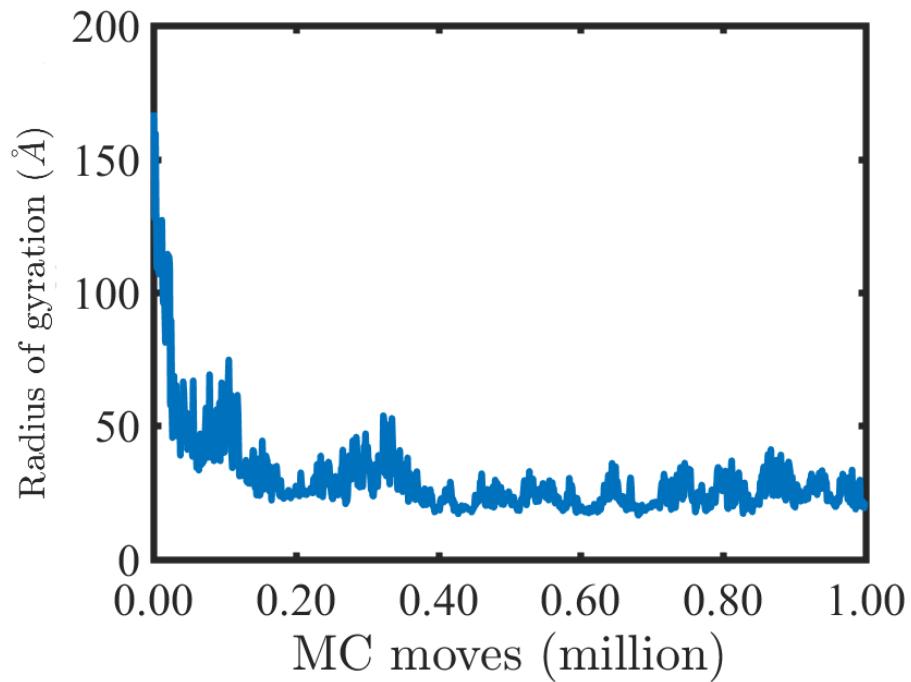


Figure B.12: Change in the radius of gyration in MC conformational sampling at 300 K, performed with a temperature quenching method. The quenched PEG structures are optimized by performing an additional 1000 internal moves. These simulations started with a linear 8 kDa PEG with methyl terminal group and MFF parameters.

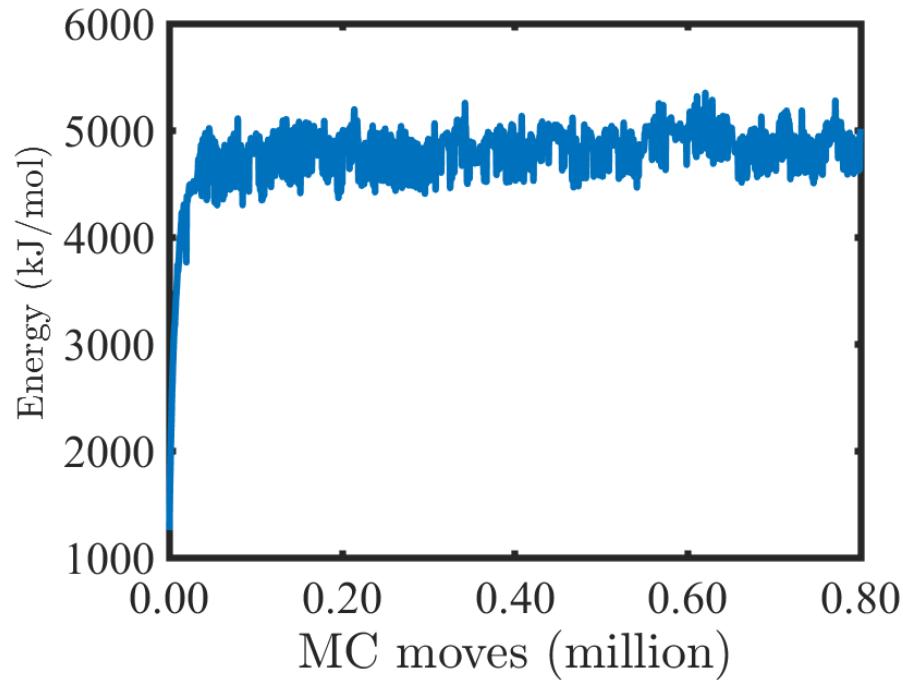


Figure B.13: Energy change during MC conformational sampling at 300 K, using a temperature quenching approach between 300-1200 K. These simulations used a linear 8 kDa PEG with a methyl terminal group and HFF parameters.

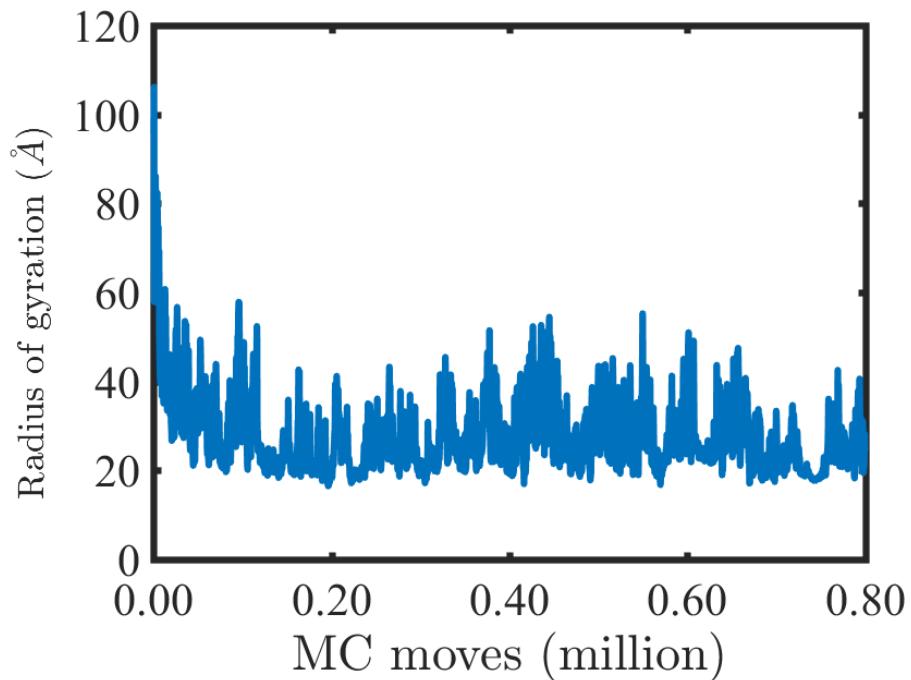


Figure B.14: Change in the radius of gyration in MC conformational sampling at 300 K, performed with a temperature quenching method. The quenched PEG structures are optimized by performing an additional 1000 internal moves. These simulations started with a linear 8 kDa PEG with methyl terminal group and HFF parameters.

# Appendix C

## Conformational equilibrium in crowded media appendix

### C.1 Extract clusters

---

```
1
2 """
3 Program to extract clusters
4 this algorithm pick first molecule randomly and check whether if forms
   cluster with any other molecules around via checking distance
   threshold. It stores all the molecules in a list around the first
   molecule. In the second step, it selects a molecule from the list
   one by one and detects the additional molecules as a part of
   aggregate. This cycle continue till there is no more molecules
   participating in an aggregate. It saves the cluster as a pdb with
   total number of molecules in it and then output the remaining list
   of molecules. The 'newList' will go through the loop again. in any
   case the algorith did not find any cluster then it will save the
   single molecule as a pdb and result in newList with number of
   molecules n-1. For example: if cluster is composed of 3 molecules
   then new list will be n-3 molecules.
5
6 INPUT: .inp/crd files
7
8 OUTPUT: The script saves the output files as a pdb files as '
   peg_box1_3_36.pdb', inwhich 'box1' represents the number of system,
   '3' is number of molecules participating in aggregate, and '36'
   remaining number of molecules in the system.
9 """
10 #####
11 # Import Libraries as listed in Sire sampling input file
12 #####
13 from MyFunctions import *
14 #####
15 #####
```

```

16 #           Main program
17 ######
18 # define tolerance
19 clstr_tolrce = 10
20
21 # determine number of input systems
22 directory = os.path.abspath(os.getcwd())
23 list_of_files = []
24 extension = '.inpcrd'
25
26 for file_name in os.listdir(directory):
27     if file_name.endswith(extension):
28         list_of_files.append(file_name)
29
30 num_systems = len(list_of_files)
31
32 print('Total available systems %d' %num_systems)
33
34 # Loop to extract cluster based on the tolerance criteria
35 for i in range (1,num_systems+1):
36     inpcrd = 'box10'+str(i)+'.inpcrd'
37     prmtop = 'box10'+str(i)+'.prmtop'
38     box = 'box'+str(i)+'_'
39     print('Running system %d' %i)
40     (mols,space) = Amber().readCrdTop(inpcrd,prmtop)
41     mol_list = gen_list(mols)
42     #print('-----Total molecules -----')
43     mol_grp = MoleculeGroup()
44     presnt_mols = []
45     mol = mol_list[0]
46     presnt_mols.append(mol)
47     mol_grp.add(mol)
48     remList = mol_list[0::]+mol_list[0+1::]
49     count = 0
50     while mol_grp.nMolecules() > 0:
51         count = count +1
52         indx = cluster_search(presnt_mols,remList,clstr_tolrce)
53         #print(indx)
54         indx = remove_lists(indx)
55         (presnt_mols, remList, mol_grp) = acc_molecules(presnt_mols,
56                                         remList,indx,mol_grp,box)
56         print('System %d is complete' % i)
57         print('-----')
58 print('All clustering is complete')
59

```

```
60
61 # now make all molecules in a cluster as a single molecule by removing
   'ter' and connect words
62 print('Now finalizing PDB files')
63 directory = os.path.abspath(os.getcwd())
64 list_of_files = []
65 extension = '.pdb'
66
67 for file_name in os.listdir(directory):
68     if file_name.endswith(extension):
69         list_of_files.append(file_name)
70 for fl in list_of_files:
71     gl = open(fl, 'r')
72     lines = gl.readlines()
73     gl.close()
74     nf = open(fl, 'w')
75     for line in lines:
76         if 'CONNECT' in line or 'TER' in line:
77             del line
78         else:
79             nf.writelines(line)
80     nf.close()
81 print('All complete')
```

---

## C.2 Sensitivity coefficient calculations

### C.2.1 Main program (Sensitivity coefficients) file

```

1 %% Sensitivity of SPT plots
2
3 % script to make plot of size distribution vs chemical potential at
4 % constant concentration.
5
6 clc
7 clear
8
9 % Input parameters
10
11 % Cube size and concentration
12 side_length = 0.000000301; % units: meters
13 cell_volume = side_length^3;
14 conc = 0.1; % concentration in the simulation box units: g/ml
15 probe = '2K96.pdb';
16 [R,S,V] = geom_parameters(probe);
17 crowder = 'pegmd2.pdb';
18 [Rc,Sc,Vc] = geom_parameters(crowder);
19 probeParm = [R,S,V];
20 crowderParm = [Rc,Sc,Vc];
21 rangee = 0:(0.6/(6-1)):0.6; % define the concentration range for axis
      on plots
22
23
24 increment_percent = 10;
25 % compute the sensitivity coeff. by changing the geometrical parameters
      by
26 % small increments in percentage.
27 % third integer input in sensitivityCoefficient(probeParm, crowderParm
      ,1,side_length) function represent the 1 percent increment in all
      the geometrical parameters of crowder.
28 [ccr, ccs, ccv] = sensitivityCoefficient(probeParm, crowderParm,
      increment_percent,side_length);
29
30 %% OUTPUT FIGURES BY SOLVING ACTIVITY COEFFICIENT
31 h = figure;
32 plot(ccr,'LineWidth',3)
33 hold on
34 set(gca,'LineWidth',3,'FontSize',15,'FontName','Times');
35 ylabel('$C_{(R)}^{avail. vol.}$','interpreter','latex');
36 xlabel('Concentration (g/ml)','interpreter','latex');
37 set(gca,'XTickLabel',num2str(rangee,'%.2f'));

```

```

38 set(gca,'box','on')
39 filename = strcat('senstivityCCR',int2str(increment_percent));
40 print(h, '-dpng',filename)
41
42 h = figure;
43 plot(ccs,'LineWidth',3)
44 hold on
45 set(gca,'LineWidth',3,'FontSize',15,'FontName','Times');
46 ylabel('$C_{\{S\}}^{avail. vol.}$','interpreter','latex');
47 xlabel('Concentration (g/ml)','interpreter','latex');
48 set(gca,'XTickLabel',num2str(rangee.','.2f'));
49 set(gca,'box','on')
50 filename = strcat('senstivityCCS',int2str(increment_percent));
51 print(h, '-dpng',filename)
52
53
54 h = figure;
55 plot(ccv,'LineWidth',3)
56 hold on
57 set(gca,'LineWidth',3,'FontSize',15,'FontName','Times');
58 ylabel('$C_{\{V\}}^{avail. vol.}$','interpreter','latex');
59 xlabel('Concentration (g/ml)','interpreter','latex');
60 set(gca,'XTickLabel',num2str(rangee.','.2f'));
61 set(gca,'box','on')
62 filename = strcat('senstivityCCV',int2str(increment_percent));
63 print(h, '-dpng',filename)
64
65 STOP
66
67 %% MEASUREMENT OF EFFECT OF GEOMETRICAL PARAMETERS OF 1000
       CONFORMATIONS OF PEG ON THE CHEMICAL POTENTIAL
68
69 % Extract geometrical parameters and write the results in text files
70
71 % for i=1:999;
72 %
73 %     filename = sprintf('peg%d.pdb',i);
74 %     [curvrad,Area,vi] = geom_parameters(filename);
75 %     curvrad1 = curvrad;
76 %         if (curvrad > 6.0000e-09)
77 %             else
78 %
79 %     [i,curvrad1,curvrad]
80 %     number = number_of_molecs(cell_volume,vi,conc);

```

```

81 % [Y,mu] = findactivity2(curvrad,curvrad,Area,Area,vi,vi,
82 % cell_volume,number);
83 % Muex(i)= mu;
84 % size(i) = curvrad*10^10;
85 % rad(i) = curvrad;
86 % vol(i) = vi;
87 % area(i) = Area;
88 % fileID1 = fopen('raDiustxt','w');
89 % fileID2 = fopen('areA.txt','w');
90 % fileID3 = fopen('volUme.txt','w');
91 % fprintf(fileID1,'%d\n',rad);
92 % fprintf(fileID2,'%d\n',area);
93 % fprintf(fileID3,'%d\n',vol);
94 % fclose(fileID1);
95 % fclose(fileID2);
96 % fclose(fileID3);
97 % end
98 %
99 % Upload resultant files from the above loop. No need to run the
100 % expensive
101 % loop again and again
102 radiusfile = importdata('raDiustxt');
103 areafile = importdata('areA.txt');
104 volfile = importdata('volUme.txt');
105 range = length(radiusfile);
106 %
107 % Compute the chemical potentials by the extended SPT model
108 radiusrange = [radiusfile(593),radiusfile(399),radiusfile(444),
109 radiusfile(683),radiusfile(408),radiusfile(36),radiusfile(635),
110 radiusfile(784),radiusfile(64),radiusfile(758),radiusfile(668),
111 radiusfile(612),radiusfile(9)];
112 arearange = [areafile(593),areafile(399),areafile(444),areafile(683),
113 areafile(408),areafile(36),areafile(635),areafile(784),areafile(64),
114 ,areafile(758),areafile(668),areafile(612),areafile(9)];
115 volrange = [volfile(593),volfile(399),volfile(444),volfile(683),volfile
116 (408),volfile(36),volfile(635),volfile(784),volfile(64),volfile
117 (758),volfile(668),volfile(612),volfile(9)];
118 areadifference = min(arearange)*10^20, max(arearange)*10^20
119 voldifference = min(volrange)*10^30, max(volrange)*10^30
120 %
121 for i = 1:range;
122 number = number_of_molecs(cell_volume,volfile(i),conc);
123 [Y,mu] = findactivity2(radiusfile(i),radiusfile(i),areafile(i),
124 areafile(i),volfile(i),volfile(i),cell_volume,number);

```

```

116 Muex(i)= mu;
117 size(i) = radiusfile(i)*10^10;
118 rad(i) = radiusfile(i);
119 vol(i) = volfile(i);
120 size2(i) = volfile(i)*10^30;
121 area(i) = areofile(i);
122 size3(i) = areofile(i)*10^20;
123 end
124
125 % Figures: Change of chemical potentials with respect of geoemtrical
126 % parameters of 1000 conformations
127
128 h=figure;
129 scatter(size,Muex,'LineWidth',3)
130 set(gca,'LineWidth',3,'FontSize',15,'FontName','Times');
131 ylabel('$$\mu^{NI}/kT$$','interpreter','latex');
132 xlabel('Radius of curvature $$AA$$','interpreter','latex');
133 set(gca,'box','on')
134 print(h, '-dpng','senstivity1')
135
136 h=figure;
137 scatter(size2,Muex,'LineWidth',3)
138 set(gca,'LineWidth',3,'FontSize',15,'FontName','Times');
139 ylabel('$$\mu^{NI}/kT$$','interpreter','latex');
140 xlabel('Volume $$AA^3$$','interpreter','latex');
141 set(gca,'box','on')
142 print(h, '-dpng','senstivity2')
143
144 h=figure;
145 scatter(size3,Muex,'LineWidth',3)
146 set(gca,'LineWidth',3,'FontSize',15,'FontName','Times');
147 ylabel('$$\mu^{NI}/kT$$','interpreter','latex');
148 xlabel('Surface area $$AA^2$$','interpreter','latex');
149 set(gca,'box','on')
150 print(h, '-dpng','senstivity3')
151
152 %% CHEMICAL POTENTIAL WITH MINIMUM, AVERAGE, AND MAXIMUM GEOMETRICAL
153 % PARAMETERS
154 % Avg. values
155 mean_rad = mean(rad);
156 mean_vol = mean(vol);
157 mean_area = mean(area);
158
159 % Max values

```

```

160 max_rad = max(rad);
161 max_vol = max(vol);
162 max_area = max(area);
163
164 % Min values
165 min_rad = min(rad);
166 min_vol = min(vol);
167 min_area = min(area);
168
169 % Compute chemical potentials
170 for i = 1:150;
171
172 [Y,mu0] = findactivity2(R,Rc,S,Sc,V,Vc,cell_volume,i);
173 [Y,mu1] = findactivity2(R,max_rad,S,Sc,V,Vc,cell_volume,i);
174 [Y,mu2] = findactivity2(R,Rc,S,Sc,V,max_vol,cell_volume,i);
175 [Y,mu3] = findactivity2(R,Rc,S,max_area,V,Vc,cell_volume,i);
176 mur0(i)= mu0;
177 mur(i)= mu1;
178 muv(i)= mu2;
179 mus(i)= mu3;
180
181 end
182
183 rangee = 0:(0.6/(4-1)):0.6;
184
185 h = figure;
186 plot(mur0,'LineWidth',3)
187 hold on
188 plot(mur,'LineWidth',3)
189 plot(muV,'LineWidth',3)
190 plot(mus,'LineWidth',3)
191 set(gca,'LineWidth',3,'FontSize',15,'FontName','Times');
192 ylabel('$$\mu^{\{NI\}}/kT$$','interpreter','latex');
193 xlabel('Concentration (g/ml)','interpreter','latex');
194 set(gca,'XTickLabel',num2str(rangee.','.2f'));
195 legend('PEG2','Avg. R','Avg. V','Avg. S','Location','northwest');
196 set(gca,'box','on')
197 legend('boxoff')
198 print(h, '-dpng','senstivityMAX')
199
200
201
202 % small increments in radius, volume, and surface area from minimum to
     maximum at
203 % constant concentration

```

```

204
205 stepR = (mean_rad - min_rad)/162;
206 radii = [min_rad:stepR:mean_rad];
207 range2 = length(radii);
208 stepsize = radii(2)*10^10-radii(1)*10^10;
209 maxvalueR = radii(end)*10^10;
210 rangee = 0:(maxvalueR/(5-1)) :maxvalueR;
211
212 for i = 1:range2
213     number = number_of_molecs(cell_volume,Vc,conc);
214     [Y,mu0] = findactivity2(R,radii(i),S,Sc,V,Vc,cell_volume,number);
215     mur0(i)= mu0;
216 end
217 chemcontR = mur0(end)-mur0(end-1)
218
219 h = figure;
220 plot(mur0,'LineWidth',3)
221 set(gca,'LineWidth',3,'FontSize',15,'FontName','Times');
222 ylabel('$$\mu^{NI}/kT$','interpreter','latex');
223 xlabel('Radius of Curvature ($$AA$')','interpreter','latex');
224 set(gca,'XTickLabel',num2str(rangee.','.2f'));
225 set(gca,'box','on')
226 print(h, '-dpng','senstivityRad')
227
228 stepV = (mean_vol - min_vol)/373000;
229 vols = [min_vol:stepV:mean_vol];
230 range3 = length(vols);
231 stepsize3 = vols(2)*10^30-vols(1)*10^30;
232 maxvalueV = vols(end)*10^30;
233 rangee = 0:(maxvalueV/(5-1)) :maxvalueV;
234
235 for i = 1:range3
236     number = number_of_molecs(cell_volume,Vc,conc);
237     [Y,mu3] = findactivity2(R,Rc,S,Sc,V,vols(i),cell_volume,number);
238     mur3(i)= mu3;
239 end
240 chemcontV = mur3(end)-mur3(end-1)
241
242 h = figure;
243 plot(mur3,'LineWidth',3)
244 set(gca,'LineWidth',3,'FontSize',15,'FontName','Times');
245 ylabel('$$\mu^{NI}/kT$','interpreter','latex');
246 xlabel('Volume ($$AA^3$')','interpreter','latex');
247 set(gca,'XTickLabel',num2str(rangee.','.2f'));
248 set(gca,'box','on')

```

```
249 print(h, '-dpng', 'senstivityVol')
250
251 stepS = (mean_area - min_area)/45500;
252 areas = [min_area:stepS:mean_area];
253 range4 = length(areas);
254 stepsize4 = areas(2)*10^20-areas(1)*10^20;
255 maxvalueS = areas(end)*10^20;
256 rangee = 0:(maxvalueS/(6-1)):maxvalueS;
257
258 for i = 1:range4
259     number = number_of_molecs(cell_volume,Vc,conc);
260     [Y,mu4] = findactivity2(R,Rc,S,areas(i),V,Vc,cell_volume,number);
261     mur4(i)= mu4;
262 end
263 chemcontS = mur4(end)-mur4(end-1)
264
265 h = figure;
266 plot(mur4,'LineWidth',3)
267 set(gca,'LineWidth',3,'FontSize',15,'FontName','Times');
268 ylabel('$$\mu^{\{NI\}}/kT$$','interpreter','latex');
269 xlabel('Surface area ($$\AA^2$$)','interpreter','latex');
270 set(gca,'XTickLabel',num2str(rangee.','.2f'));
271 %legend('before','after','Location','northwest');
272 set(gca,'box','on')
273 %legend('boxoff')
274 print(h, '-dpng', 'senstivityArea')
```

---

## C.2.2 Sensitivity coefficients function

---

```
1 function [ccr, ccs, ccv] = senstivityCoefficient(probe, crowder,incr,
           side_length);
2
3 % Function to estimate the senstivity coefficient as follows
4
5 % Method1: c = [ln(v)2 - ln(v)1]/[ln(gem)2- ln(gem)1]
6
7
8 % Cube size and concentration
9 cell_volume = side_length^3; % cubic meters
10
11 % compute percent increment
12 convertIncr1 = (crowder(1,1))*incr/100;
13 convertIncr2 = (crowder(1,2))*incr/100;
14 convertIncr3 = (crowder(1,3))*incr/100;
15 %ratio = (crowder(1,1)+convertIncr1)/crowder(1,1)
```

```
16 %ratios = (crowder(1,2)+convertIncr2)/crowder(1,2)
17 %ratiov = (crowder(1,3)+convertIncr3)/crowder(1,3)
18 for i = 1:6
19 %conc = 0.1;
20 %number = number_of_molecs(cell_volume,crowder(1,3),i)
21
22 % These calculations are used to compute the chemical potentials using
23 % the
24 % extended SPT and then these mu values are converted to the fractional
25 % available
26 % volumes that are used in the sensitivity analysis.
27
28 number = 25*i; % concentration of the box in terms of number of PEG
29
30 % calculations of mu
31 [y,mu1] = findactivity2(probe(1,1),crowder(1,1),probe(1,2),crowder(1,2),
32 ,probe(1,3),crowder(1,3),cell_volume,number);
33 [y,mu2] = findactivity2(probe(1,1),crowder(1,1)+convertIncr1,probe(1,2),
34 ,crowder(1,2),probe(1,3),crowder(1,3),cell_volume,number);
35 [y,mu3] = findactivity2(probe(1,1),crowder(1,1),probe(1,2),crowder(1,2)
36 +convertIncr2,probe(1,3),crowder(1,3),cell_volume,number);
37 [y,mu4] = findactivity2(probe(1,1),crowder(1,1),probe(1,2),crowder(1,2),
38 ,probe(1,3),crowder(1,3)+convertIncr3,cell_volume,number);
39 mu = [mu1 mu2 mu3 mu4];
40 availvol = chem_to_vol(mu);
41
42 % sensitivity coeff. calculations
43 cr = (log(availvol(1,2))-log(availvol(1,1)))/(log(crowder(1,1) +
44 convertIncr1)-log(crowder(1,1)));
45 cs = (log(availvol(1,3))-log(availvol(1,1)))/(log(crowder(1,2) +
46 convertIncr2)-log(crowder(1,2)));
47 cv = (log(availvol(1,4))-log(availvol(1,1)))/(log(crowder(1,3) +
48 convertIncr3)-log(crowder(1,3)));
49
50 % collect sensitivity coeff. in vectors
51 ccr(i) = cr;
52 ccs(i) = cs;
53 ccv(i) = cv;
54 end
55 end
```

---

### C.3 Fractional available volume plots

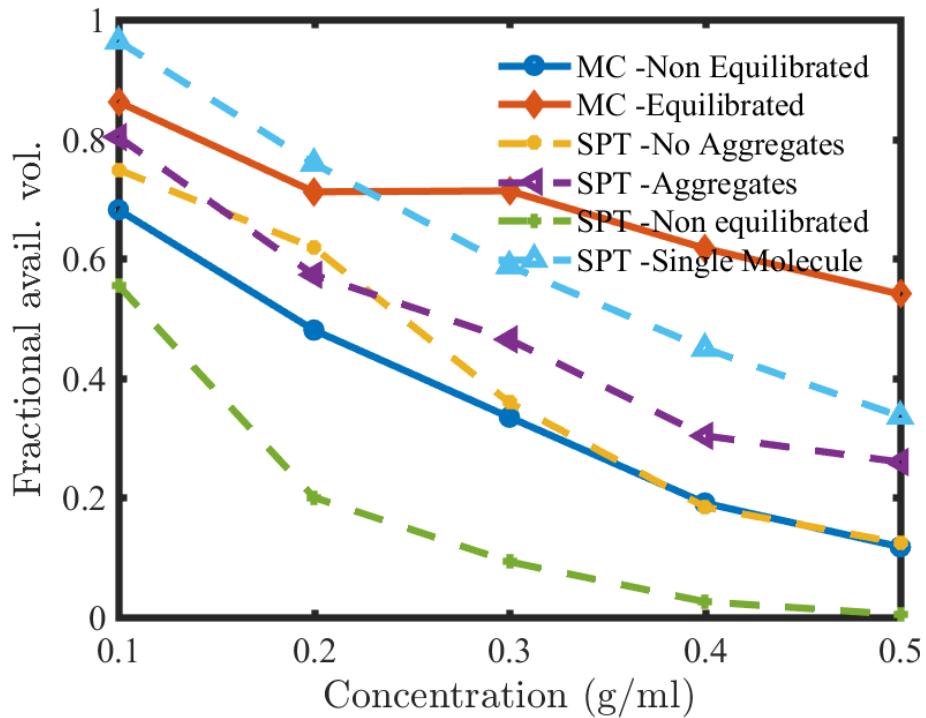


Figure C.1: Comparisons of fractional available volumes for an open conformation of adenylate kinase enzyme (1AKE) measured in the crowded boxes filled with 8 kDa PEG by using the MC (solid lines) and extended SPT models (dashed lines). Only two types of crowded systems, i.e. non-equilibrated and equilibrated systems, were used to determine the chemical potentials using an MC method. The SPT approach used equilibrated systems only with and without aggregates, whereas ‘SPT Single Molecule’ represents the equilibrated single PEG conformation (PEG2) used to compute the fractional available volumes.

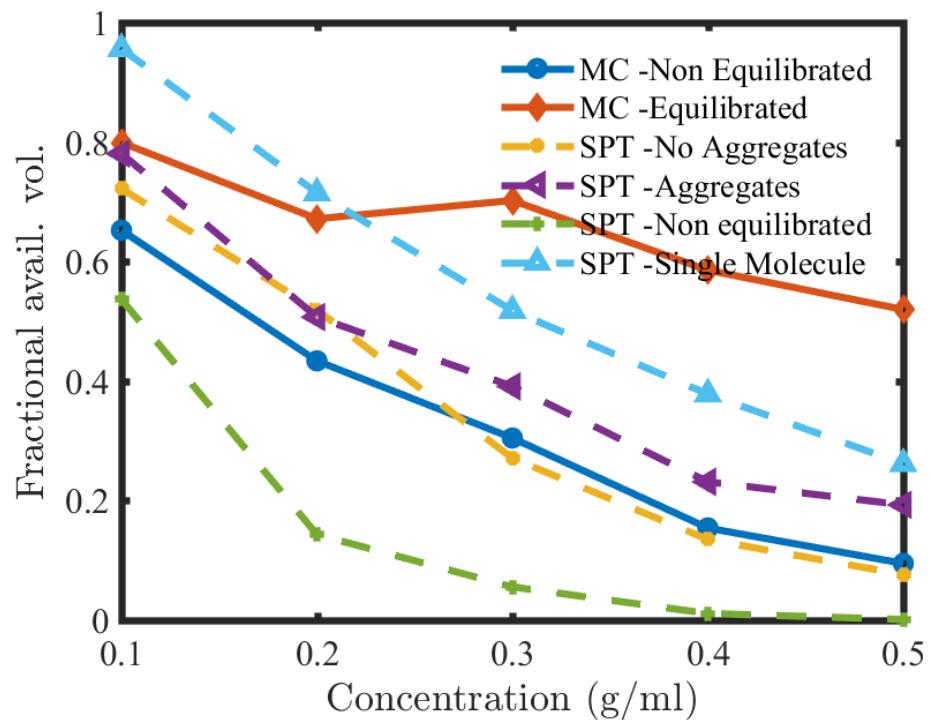


Figure C.2: Comparisons of fractional available volumes for closed conformation of adenylate kinase enzyme (4AKE) measured in the crowded boxes filled with 8 kDa PEG by using the MC (solid lines) and extended SPT models (dashed lines). Only two types of crowded systems, i.e. non-equilibrated and equilibrated systems, were used to determine the chemical potentials using an MC method. The SPT approach used equilibrated systems only with and without aggregates, whereas ‘SPT Single Molecule’ represents the equilibrated single PEG conformation (PEG2) used to compute the fractional available volumes.

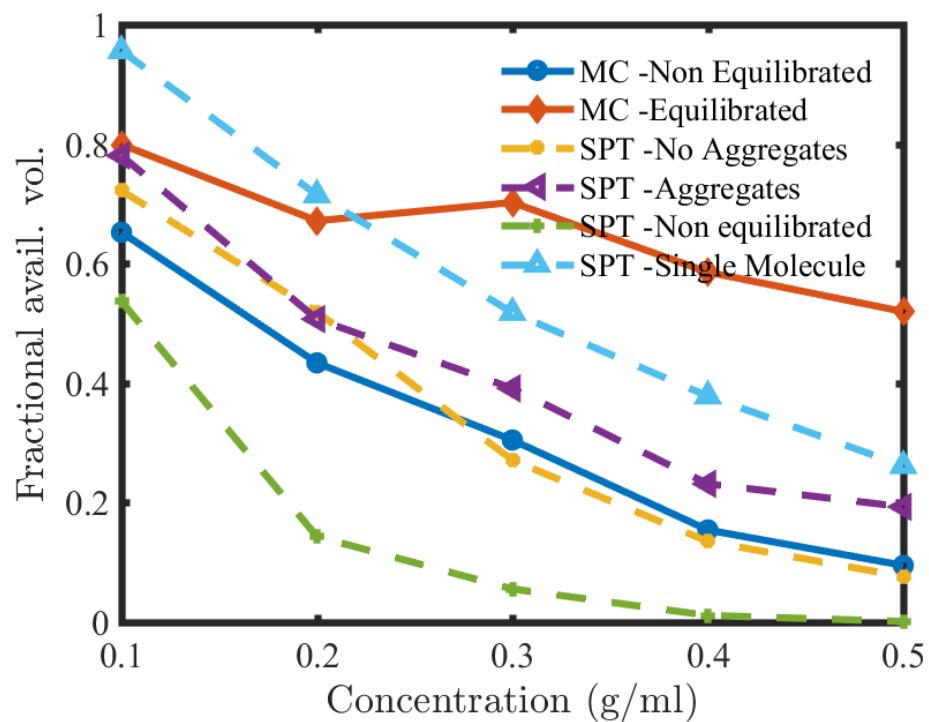


Figure C.3: Comparisons of fractional available volumes for closed conformation of adenylate kinase enzyme (4AKE) measured in the crowded boxes filled with 8 kDa PEG by using the MC (solid lines) and extended SPT models (dashed lines). Only two types of crowded systems, i.e. non-equilibrated and equilibrated systems, were used to determine the chemical potentials using an MC method. The SPT approach used equilibrated systems only with and without aggregates, whereas ‘SPT Single Molecule’ represents the equilibrated single PEG conformation (PEG2) used to compute the fractional available volumes.

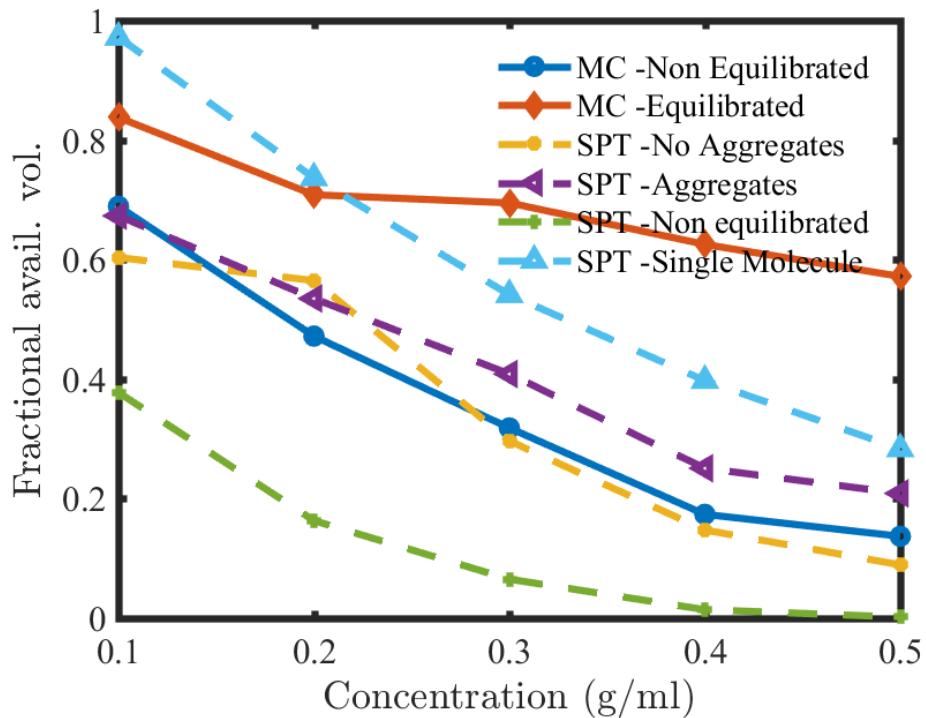


Figure C.4: Comparisons of fractional available volumes for closed conformation of *lac* repressor (2P9H) measured in the crowded boxes filled with 8 kDa PEG by using the MC (solid lines) and extended SPT models (dashed lines). Only two types of crowded systems, i.e. non-equilibrated and equilibrated systems, were used to determine the chemical potentials using an MC method. The SPT approach used equilibrated systems only with and without aggregates, whereas ‘SPT Single Molecule’ represents the equilibrated single PEG conformation (PEG2) used to compute the fractional available volumes.

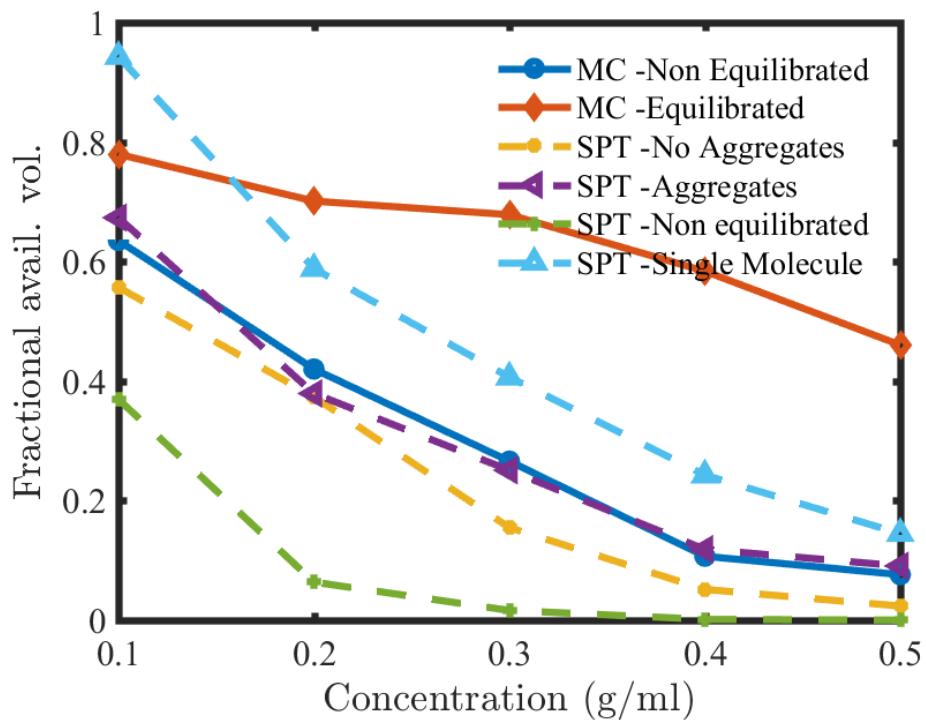


Figure C.5: Comparisons of fractional available volumes for open conformation of *lac* repressor (2PE5) measured in the crowded boxes filled with 8 kDa PEG by using the MC (solid lines) and extended SPT models (dashed lines). Only two types of crowded systems, i.e. non-equilibrated and equilibrated systems, were used to determine the chemical potentials using an MC method. The SPT approach used equilibrated systems only with and without aggregates, whereas ‘SPT Single Molecule’ represents the equilibrated single PEG conformation (PEG2) used to compute the fractional available volumes.

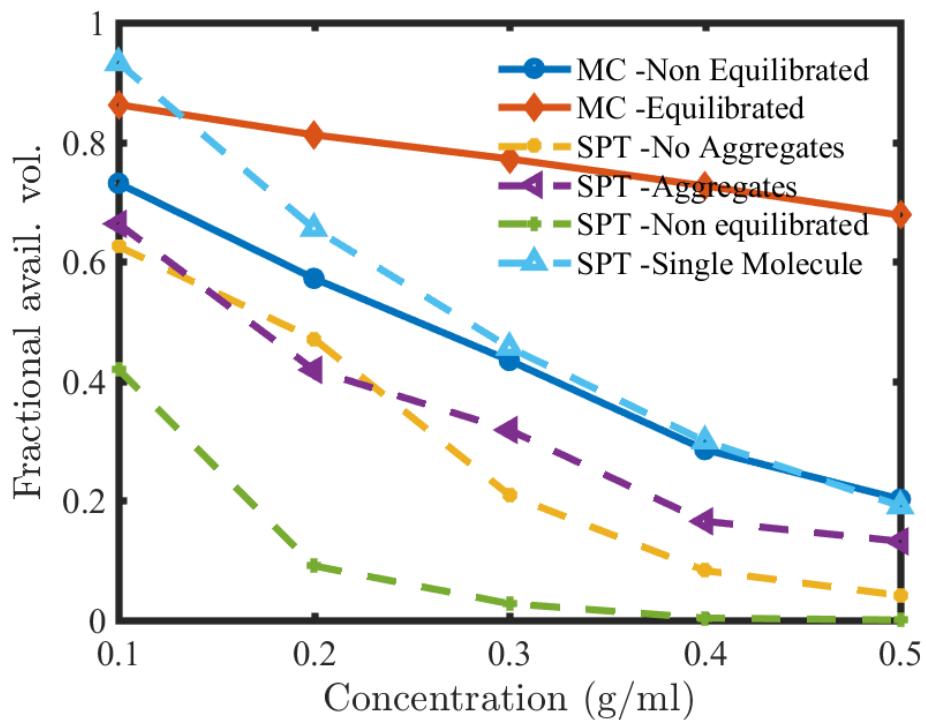


Figure C.6: Comparisons of fractional available volumes for a pseudoknot RNA (2K96) measured in the crowded boxes filled with 8 kDa PEG by using the MC (solid lines) and extended SPT models (dashed lines). Only two types of crowded systems, i.e. non-equilibrated and equilibrated systems, were used to determine the chemical potentials using an MC method. The SPT approach used equilibrated systems only with and without aggregates, whereas ‘SPT Single Molecule’ represents the equilibrated single PEG conformation (PEG2) used to compute the chemical potential.

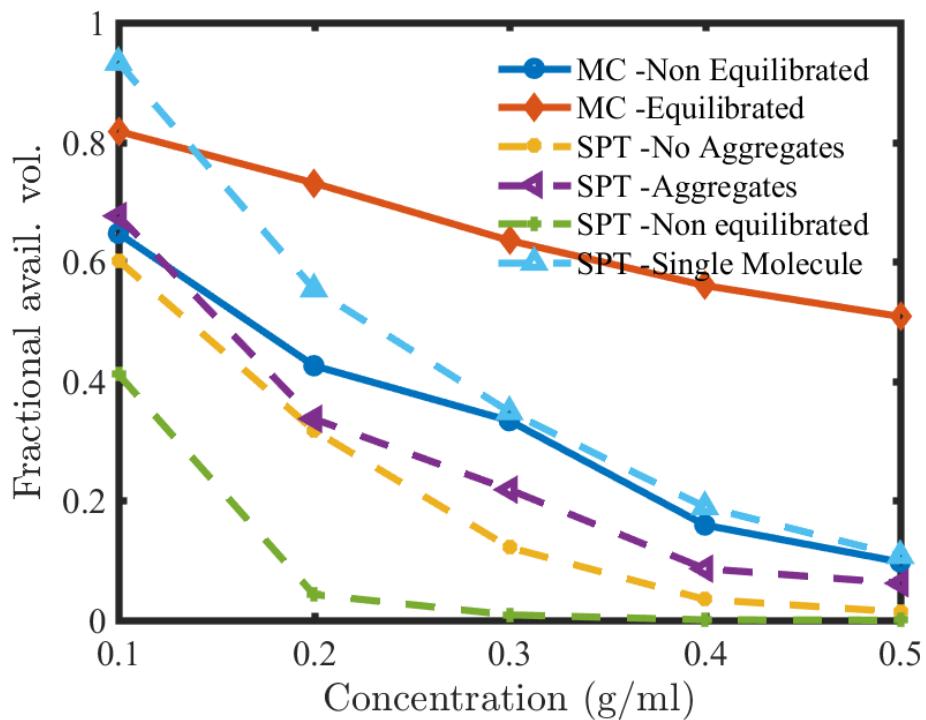


Figure C.7: Comparisons of fractional available volumes for hairpin RNA (1NA2) measured in the crowded boxes filled with 8 kDa PEG by using the MC (solid lines) and extended SPT models (dashed lines). Only two types of crowded systems, i.e. non-equilibrated and equilibrated systems, were used to determine the chemical potentials using an MC method. The SPT approach used equilibrated systems only with and without aggregates, whereas ‘SPT Single Molecule’ represents the equilibrated single PEG conformation (PEG2) used to compute the chemical potential.

## C.4 Nonideal chemical potential plots

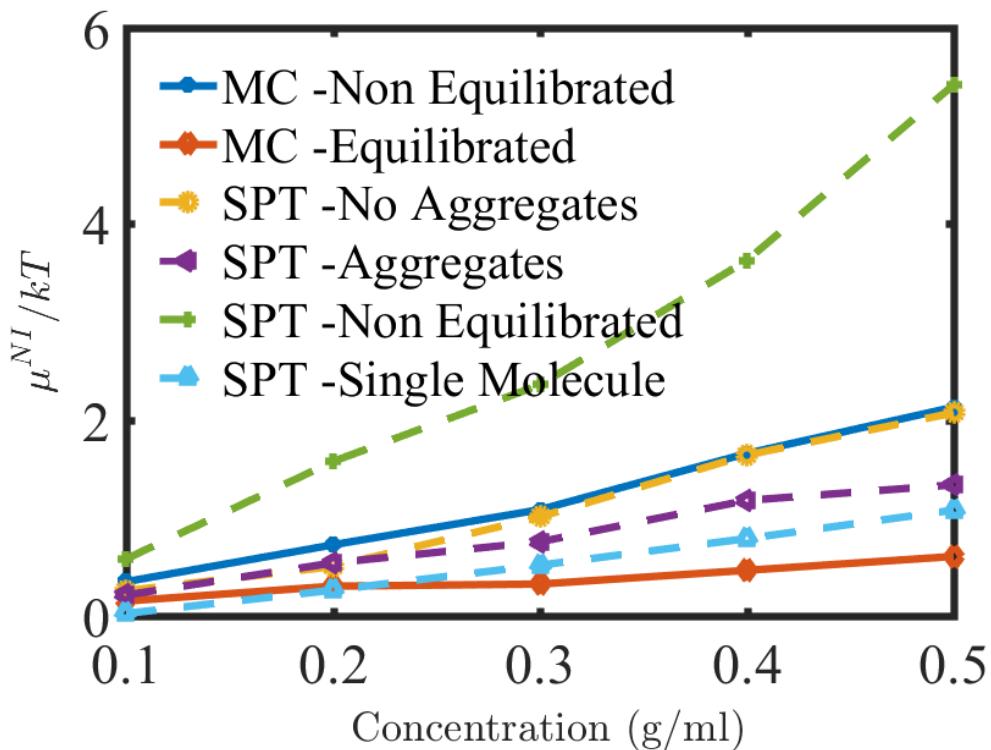


Figure C.8: Comparisons of nonideal chemical potential contributions for an open conformation of adenylate kinase enzyme (1AKE) measured in the crowded boxes filled with 8 kDa PEG by using the MC (solid lines) and extended SPT models (dashed lines). Only two types of crowded systems, i.e. non-equilibrated and equilibrated systems, were used to determine the chemical potentials using an MC method. The SPT approach used equilibrated systems only with and without aggregates, whereas ‘SPT Single Molecule’ represents the equilibrated single PEG conformation (PEG2) used to compute the chemical potential.

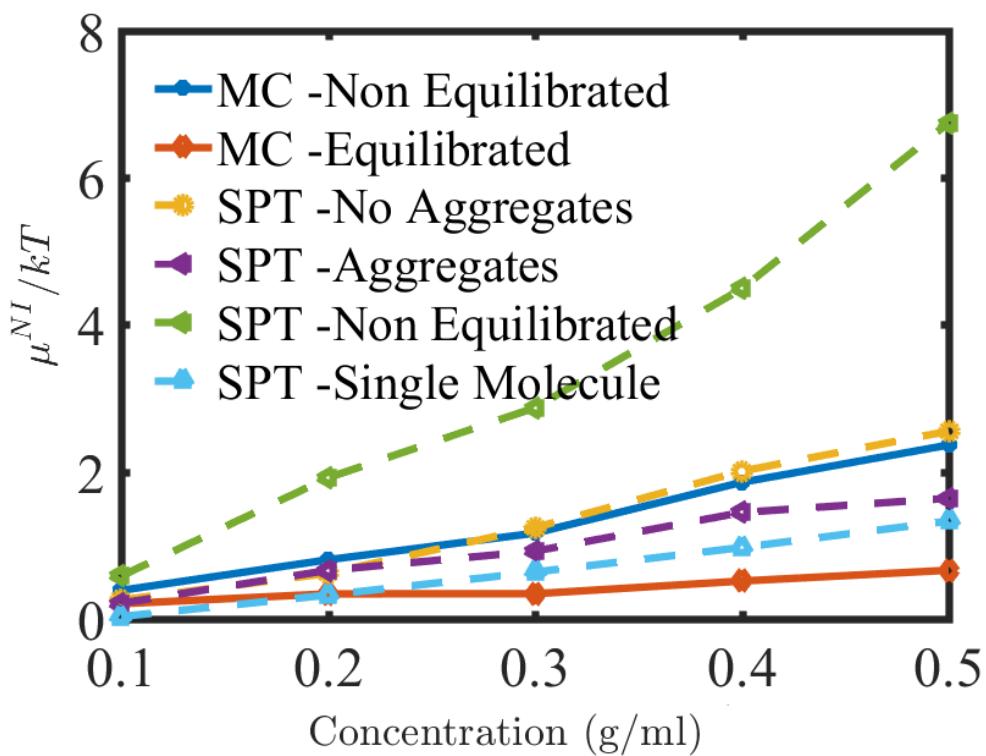


Figure C.9: Comparisons of nonideal chemical potential contributions for closed conformation of adenylate kinase enzyme (4AKE) measured in the crowded boxes filled with 8 kDa PEG by using the MC (solid lines) and extended SPT models (dashed lines). Only two types of crowded systems, i.e. non-equilibrated and equilibrated systems, were used to determine the chemical potentials using an MC method. The SPT approach used equilibrated systems only with and without aggregates, whereas ‘SPT Single Molecule’ represents the equilibrated single PEG conformation (PEG2) used to compute the chemical potential.

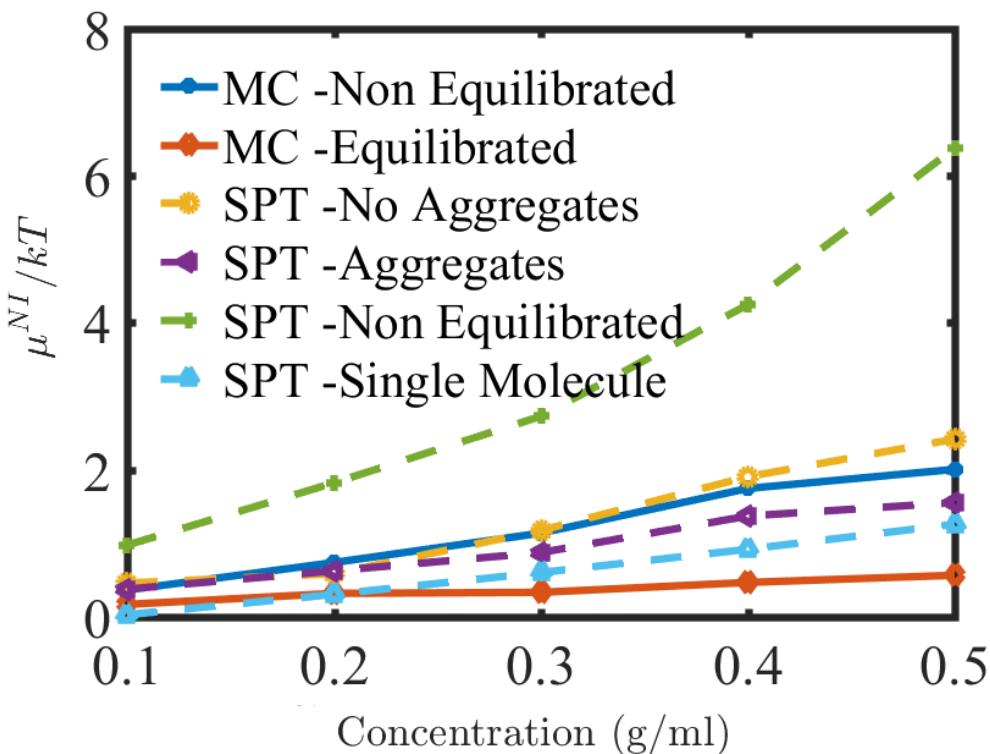


Figure C.10: Comparisons of nonideal chemical potential contributions for closed conformation of *lac* repressor (2P9H) measured in the crowded boxes filled with 8 kDa PEG by using the MC (solid lines) and extended SPT models (dashed lines). Only two types of crowded systems, i.e. non-equilibrated and equilibrated systems, were used to determine the chemical potentials using an MC method. The SPT approach used equilibrated systems only with and without aggregates, whereas ‘SPT Single Molecule’ represents the equilibrated single PEG conformation (PEG2) used to compute the chemical potential.

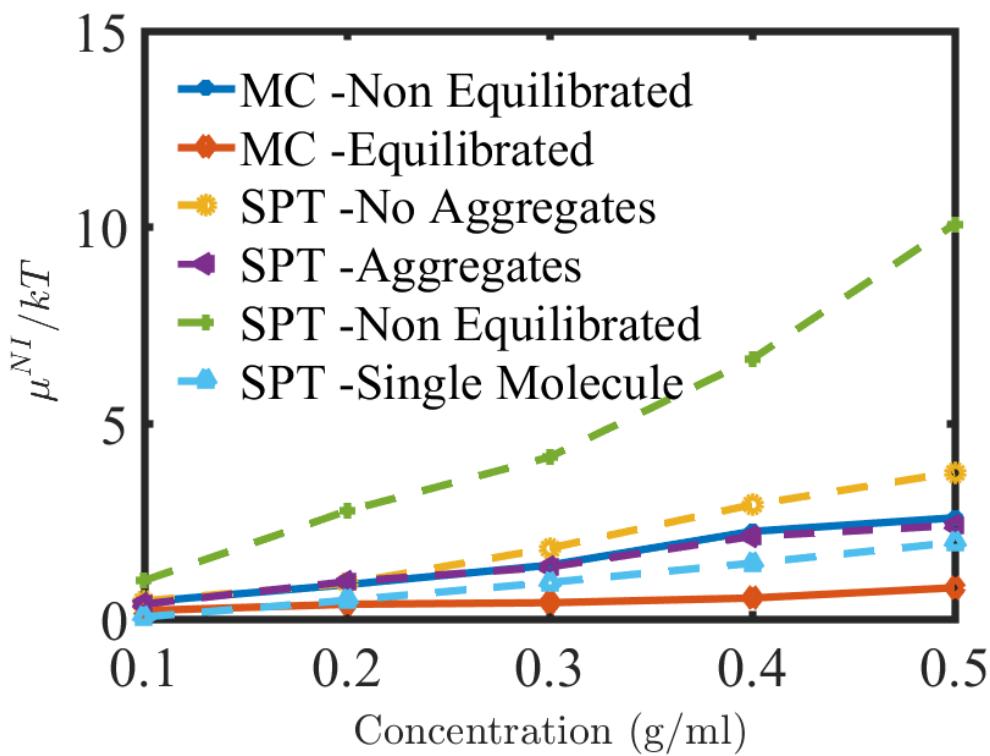


Figure C.11: Comparisons of nonideal chemical potential contributions for open conformation of *lac* repressor (2PE5) measured in the crowded boxes filled with 8 kDa PEG by using the MC (solid lines) and extended SPT models (dashed lines). Only two types of crowded systems, i.e. non-equilibrated and equilibrated systems, were used to determine the chemical potentials using an MC method. The SPT approach used equilibrated systems only with and without aggregates, whereas ‘SPT Single Molecule’ represents the equilibrated single PEG conformation (PEG2) used to compute the chemical potential.

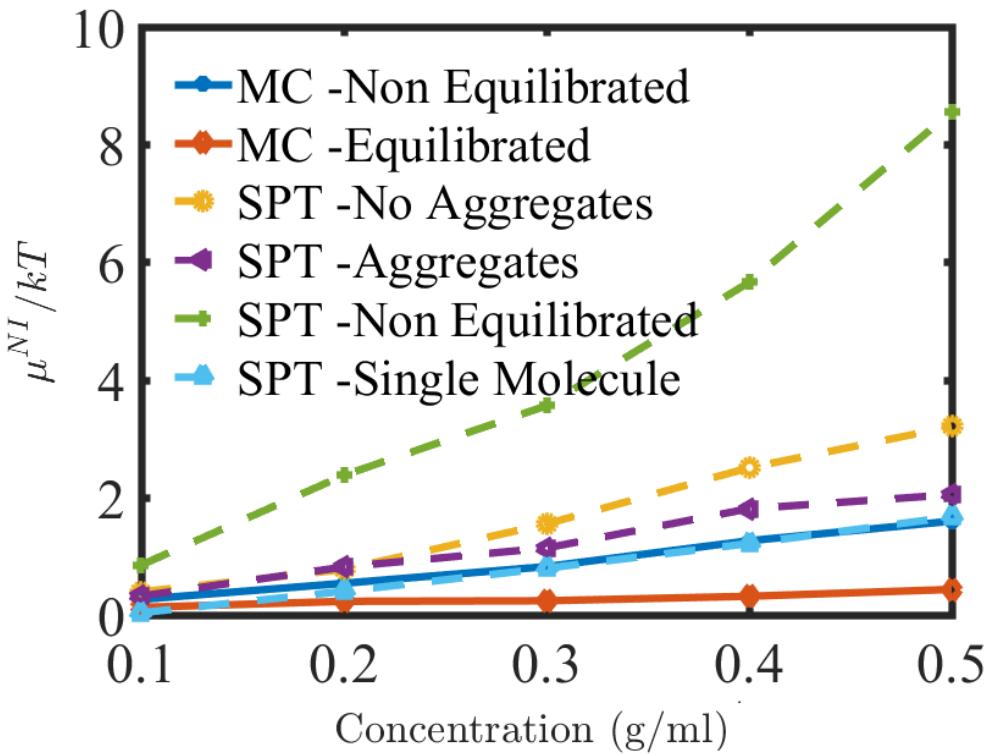


Figure C.12: Comparisons of nonideal chemical potential contributions for pseudoknot RNA (2K96) measured in the crowded boxes filled with 8 kDa PEG by using the MC (solid lines) and extended SPT models (dashed lines). Only two types of crowded systems, i.e. non-equilibrated and equilibrated systems, were used to determine the chemical potentials using an MC method. The SPT approach used equilibrated systems only with and without aggregates, whereas ‘SPT Single Molecule’ represents the equilibrated single PEG conformation (PEG2) used to compute the chemical potential.

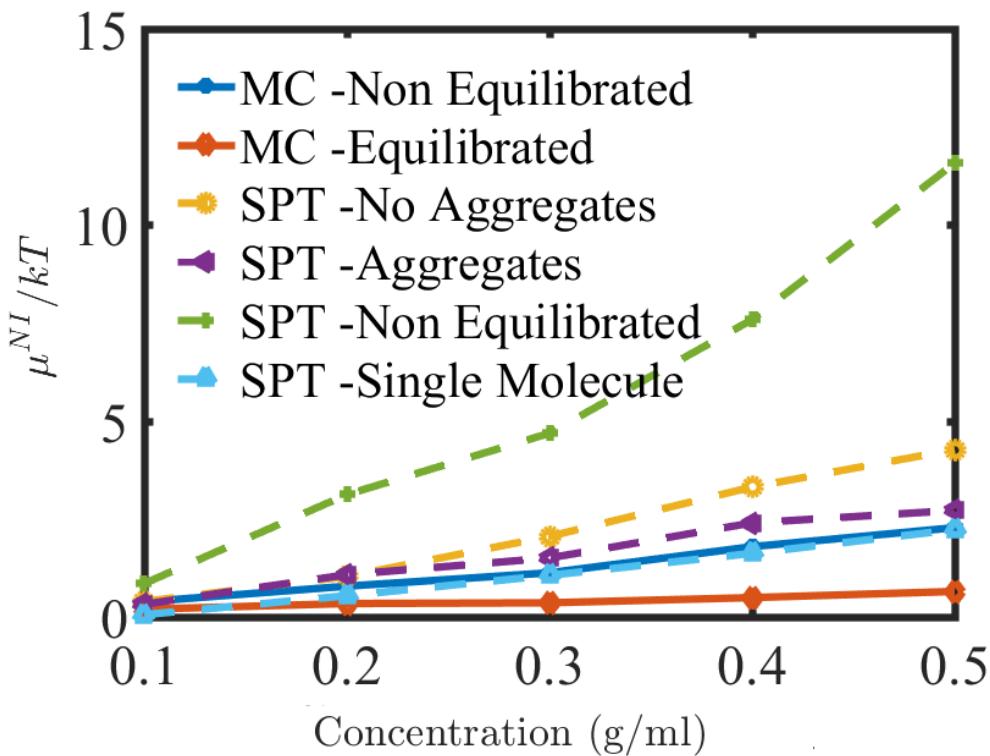


Figure C.13: Comparisons of nonideal chemical potential contributions for hairpin RNA (1NA2) measured in the crowded boxes filled with 8 kDa PEG by using the MC (solid lines) and extended SPT models (dashed lines). Only two types of crowded systems, i.e. non-equilibrated and equilibrated systems, were used to determine the chemical potentials using an MC method. The SPT approach used equilibrated systems only with and without aggregates, whereas ‘SPT Single Molecule’ represents the equilibrated single PEG conformation (PEG2) used to compute the chemical potential.

## C.5 Standard free energy change plots

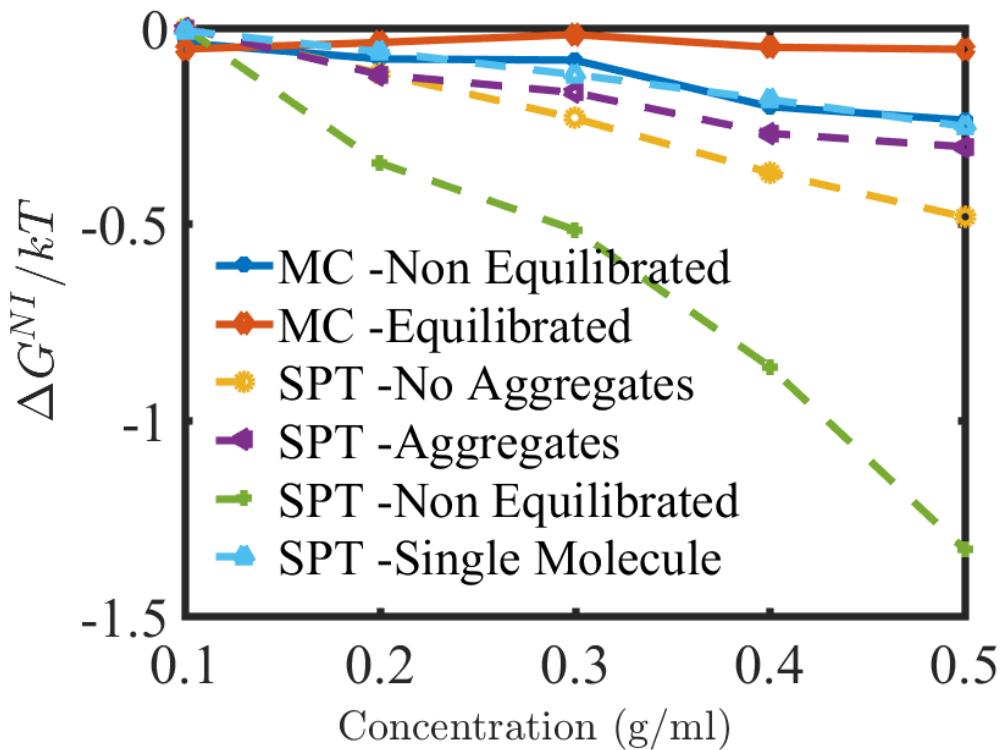


Figure C.14: Nonideal contribution to the standard state free energy change of conformational equilibrium between open and closed conformers in the crowded medium ( $4\text{AKE} \rightleftharpoons 1\text{AKE}$ ). The macromolecular crowding favors the formation of compact conformation of the kinase enzyme.

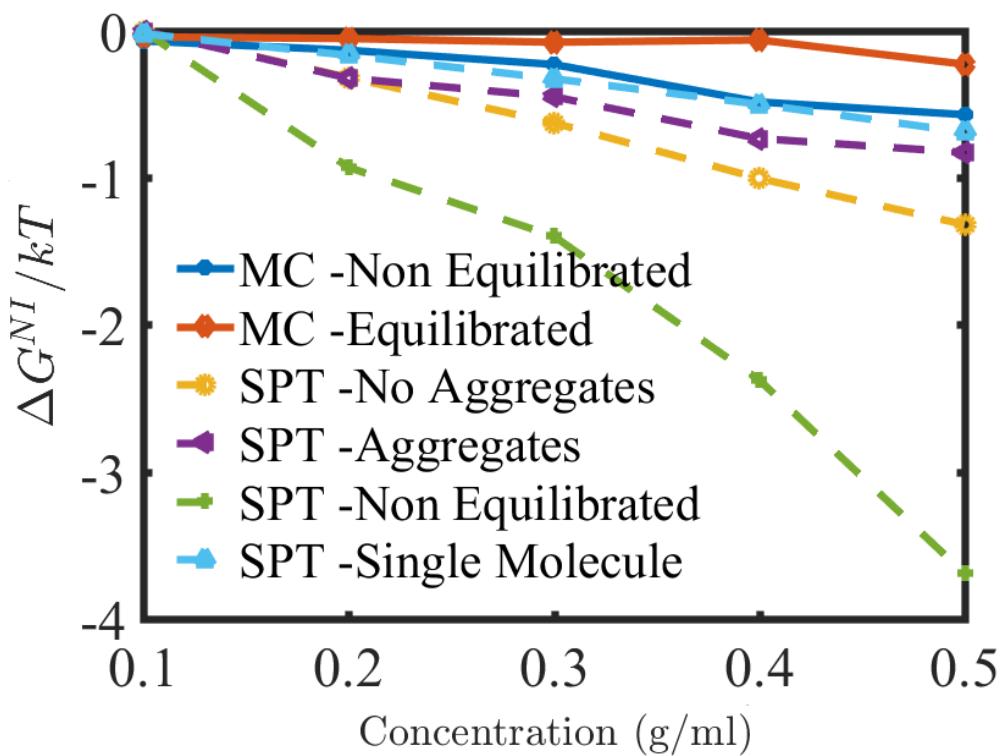


Figure C.15: Nonideal contribution to the standard state free energy change of conformational equilibrium between open and closed conformers in the crowded medium ( $2\text{PE5} \rightleftharpoons 2\text{P9H}$ ). The macromolecular crowding favors the formation of compact conformation of the lac repressor.