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by

Andrew William Ritchie, B.S. Chem

Dissertation

Presented to the Faculty of the Graduate School of
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for the Degree of

Doctorate of Philosophy

The University of Texas at Austin

August 2015

**<Title of dissertation or treatise, double-spaced,
and centered>**

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The University of Texas at Austin, 2015

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<Abstract: May not exceed 350 words. It should be a continuous description, not disconnected notes or an outline.>

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Chapter 1 Introduction

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Chapter 2 Simulation Methods

2.1 LABELING AND MUTATING PROTEINS *IN SILICO*

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2.2 ENHANCED MOLECULAR DYNAMICS IN AMBER03: N-DIMENSIONAL UMBRELLA SAMPLING AND WEIGHTED HISTOGRAM ANALYSIS METHOD

An umbrella sampling strategy was used to obtain a Boltzmann-weighted statistical ensemble of thiocyanate probe orientations for all MD sampling. Through examining simulated protein structures, it became increasingly apparent that a second degree of freedom, χ_1 , was relevant to our probe conformational distributions. Therefore, two different umbrella sampling strategies were tested: one-dimensional sampling about the thiocyanate χ_2 dihedral angle and two-dimensional umbrella sampling about the thiocyanate χ_1 and χ_2 dihedral angles, shown in Figure 2-1. All molecular dynamics were completed using the GROMACS¹ software package at 300 K with the AMBER03² force field and periodic boundary conditions.

Six probe locations on RalGDS were examined: N27C_{SCN}, G28C_{SCN}, N29C_{SCN}, Y31C_{SCN}, K32C_{SCN}, and N54C_{SCN}, in the monomeric state and docked to each GTPase system examined. We have therefore examined all probe locations and mutated constructs for which experimental data are available. Six structures for each system modeled were generated by fixing the thiocyanate χ_2 dihedral angle from 0° to 300° in 60° increments. Each structure was sampled with a dihedral potential that was flat within $\pm 30^\circ$ of the fixed-dihedral position and quadratic with a force constant of 1000 kJ mol⁻¹ rad⁻² otherwise. These restraining potentials were carried through for the duration of the system set-up and simulation. Each structure was energy minimized with cut-off electrostatics, solvated with tip3p water³ in a dodecahedron box, charge balanced by randomly replacing the appropriate number of water molecules with sodium or chloride ions using the genion GROMACS utility, and solvent relaxed by sampling for 20 ps with position restraints on all non-solvent heavy atoms with a force constant of 1000 kJ mol⁻¹ nm⁻² using PME^{4,5} electrostatics with a real-space cut-off of 0.9 nm, spacing of 0.12 nm,

and interpolation order 4. Each rotamer of each system was then sampled using the GROMACS stochastic dynamics integrator, constraints on hydrogen-bonds using the LINCS algorithm⁶, and PME electrostatics for 3 ns, recording snapshots every 5 ps, for a total of 18 ns of simulation and 3606 frames for each system.

Each frame was assigned to one of 72 5° bins from -180° to 175° based on the χ_2 dihedral angle. The weighted histogram analysis method (WHAM)^{7,8} was then used to calculate a torsional potential of mean force (PMF) for each of N bins i , which is related to the torsional probability distribution for each bin i (P_i) described by equation (2-1):

$$P_i = \frac{e^{-\beta \cdot PMF_i}}{\sum_{j=0}^N e^{-\beta \cdot PMF_j}}, \beta = \frac{1}{k_b T} \quad (2-1)$$

which is the typical Boltzmann distribution function for a state i divided by the partition function, where T is the temperature in Kelvin, k_b is the Boltzmann constant, and PMF_j is the PMF for some state j .

The two-dimensional umbrella sampling was done in much the same way as the one-dimensional sampling, with few minor changes. The χ_1 angle was fixed from 0° to 300° in 30° increments, resulting in 12 structures. Each of these structures then had the χ_2 angle fixed from 0° to 300° in 30°, resulting in 144 total structures. To avoid steric clashes in the starting structures, for each χ_2 rotation, the distance between the center of mass coordinate of each rotated atom and every non-rotated atom was calculated. If a distance was found to be under 1.5 Å, the χ_2 angle was rotated $\pm 1.5^\circ$ from the dihedral center and the distances were recalculated. This was done until all non-bonded atoms were at least 1.5 Å from each rotated atom. Next, two harmonic dihedral restraining potentials were generated for each structure, one for the χ_1 dihedral angle and one for the χ_2 dihedral angle. Following the same set-up strategy used in the one-dimensional

sampling, the system was then energy minimized with cut-off electrostatics and dihedral force constants of $1000 \text{ kJ mol}^{-1} \text{ rad}^{-2}$, solvated in tip3p water in a dodecahedron box, and charge balanced as described above. The system underwent solvent relaxation using PME electrostatics for 20 ps with a force constant of $1000 \text{ kJ mol}^{-1} \text{ nm}^{-2}$ on heavy backbone atoms, dihedral force constants of $150 \text{ kJ mol}^{-1} \text{ rad}^{-2}$, and unrestrained side-chain atoms. Each of the 144 χ_1 - χ_2 rotamers were then sampled for 400 ps using the GROMACS stochastic dynamics integrator with PME electrostatics (again, with a real-space cut-off of 0.9 nm, spacing of 0.12 nm, and interpolation order 4) and dihedral restraining potentials of $70 \text{ kJ mol}^{-1} \text{ rad}^{-2}$, for a total of 57.6 ns of simulation and 11664 frames for each system. It is worth reiterating that each step used a progressively smaller dihedral restraining potential. This was done to ensure that the dihedral angles of the final structure before sampling were as close as possible to the umbrella-sampling window while still allowing nearby residues to relax to orientations that accommodate the inclusion of our probe. Starting with a large restraining potential fixes the probe to a specific location orientation and forcibly moves nearby residues to accommodate the probe to minimize interaction energies. Subsequent weakening of the restraining potential allows the probe to respond to its surroundings in a manner more typical of MD, allowing both the probe as well as the residues near the probe to relax to energy minimized orientations. Without this subsequent weakening, many simulations resulted in dihedral forces becoming larger than the integrator can or is expected to handle, which were usually caused by steric clashes between the probe and side-chain atoms.

A Boltzmann-weighted statistical ensemble of structures obtained from two-dimensional sampling was then assembled using WHAM. Each frame was assigned to one of 5184 5° by 5° bins. The bins were assigned based on equation (2-2), where b_1 is the one-dimensional χ_1 bin number, b_2 is the one-dimensional χ_2 bin number, B_i is the

total number of bins in degree of freedom i (72 for all two-dimensional sampling done), and i_{index} refers to the subscript on b . Conditional probability was assumed.

$$\text{Bin}(b_1, b_2) = \sum_{i=b_1, b_2} B_i^{2-i_{index}} \cdot i \quad (2-2)$$

To validate our 2D WHAM code, we examined a Ryckaert-Bellemans dihedral potential, shown in equation (2-3), where ϕ is some angle and n and C_n are some example parameters obtained from the GROMACS manual, shown in Table 2-1. This is a very simple potential function with no contributions from any other source, unlike the potential energy calculation in a protein, which will be influenced by various force field parameters such as bond force constants. However in WHAM, the sources of the potentials are not distinguished, and we are able to use the simple Ryckaert-Bellemans model to validate the code. We constructed the PMF from the sum of the potentials for a given pair of coordinates and the unbiased probability distribution, $p^\circ(\chi_1, \chi_2)$, using the ratio of the Boltzmann distribution function to the partition function, shown in equation (2-4), over an array of 2D dihedral angles ranging from -180° to 180° .

$$V_{rb} = \sum_{n=0}^5 C_n (\cos(\phi - \pi))^n \quad (2-3)$$

Next, we constructed the PMF and probability distribution landscapes, shown in Figure 2-1a. We then applied biasing potential windows to each dimension in a manner that mimics the methods used in GROMACS. The biased probability, $p(\chi_1, \chi_2)$, is given by equation (2-5). We then performed a Monte Carlo simulation centered on each of 144 biased windows, each with dimensions of $30^\circ \times 30^\circ$, with a probability $p(\chi_1, \chi_2)$ of sampling a given pair of dihedral angles, which was then analyzed using our 2D WHAM code to return the unbiased PMF and $p^\circ(\chi_1, \chi_2)$.

$$p^\circ(\chi_1, \chi_2) = \frac{e^{-\beta(V_{rb}(\chi_1) + V_{rb}(\chi_2))}}{\iint e^{-\beta(V_{rb}(\chi_1) + V_{rb}(\chi_2))} d\chi_1 d\chi_2} \quad (2-4)$$

$$p(\chi_1, \chi_2) = \frac{e^{-\beta(V_{rb}(\chi_1)+V_1+V_{rb}(\chi_2)+V_2)}}{\iint e^{-\beta(V_{rb}(\chi_1)+V_1+V_{rb}(\chi_2)+V_2)} d\chi_1 d\chi_2} \quad (2-5)$$

Figure 2-1 shows the analytical PMF and probability distributions (a), WHAM PMF and probability distributions on 144 windows each containing 40 frames (b), WHAM PMF and probability distributions on 144 windows each containing 80 frames (c), WHAM PMF and probability distributions on 144 windows each containing 160 frames (d), and WHAM PMF and probability distributions on 144 windows each containing 1000 frames (e). The major features of the probability distribution become clear after only 40 frames; after 80 frames the probability distributions look very similar to the analytical distribution, and after 160 frames very little improvement is seen. We can also see that the high probability regions, representing the staggered orientations, have PMF landscapes that look like the analytical PMF, although the gauche regions appear to be ill characterized still. After 1000 frames the probability distribution is nearly identical to the analytical distribution and the moderate ranges of the PMF (light blue, > 17.92 kJ mol⁻¹) now quantitatively approach the predictions of the analytical expression. Exact analytical PMF matching of low probability regions is very slow, requiring ≥ 10000 frames. From these results we conclude that the PMF converges to the analytical expression slowly for regions of low probability and quickly for regions of high probability.

Table 2-1: Sample Parameters for Ryckaert Bellemans dihedral potential function used for validating 2D WHAM code

	Constraint (kJ mol⁻¹)		Constraint (kJ mol⁻¹)
C₀	9.28	C₃	-3.06
C₁	12.16	C₄	-26.24
C₂	-13.12	C₅	-31.5

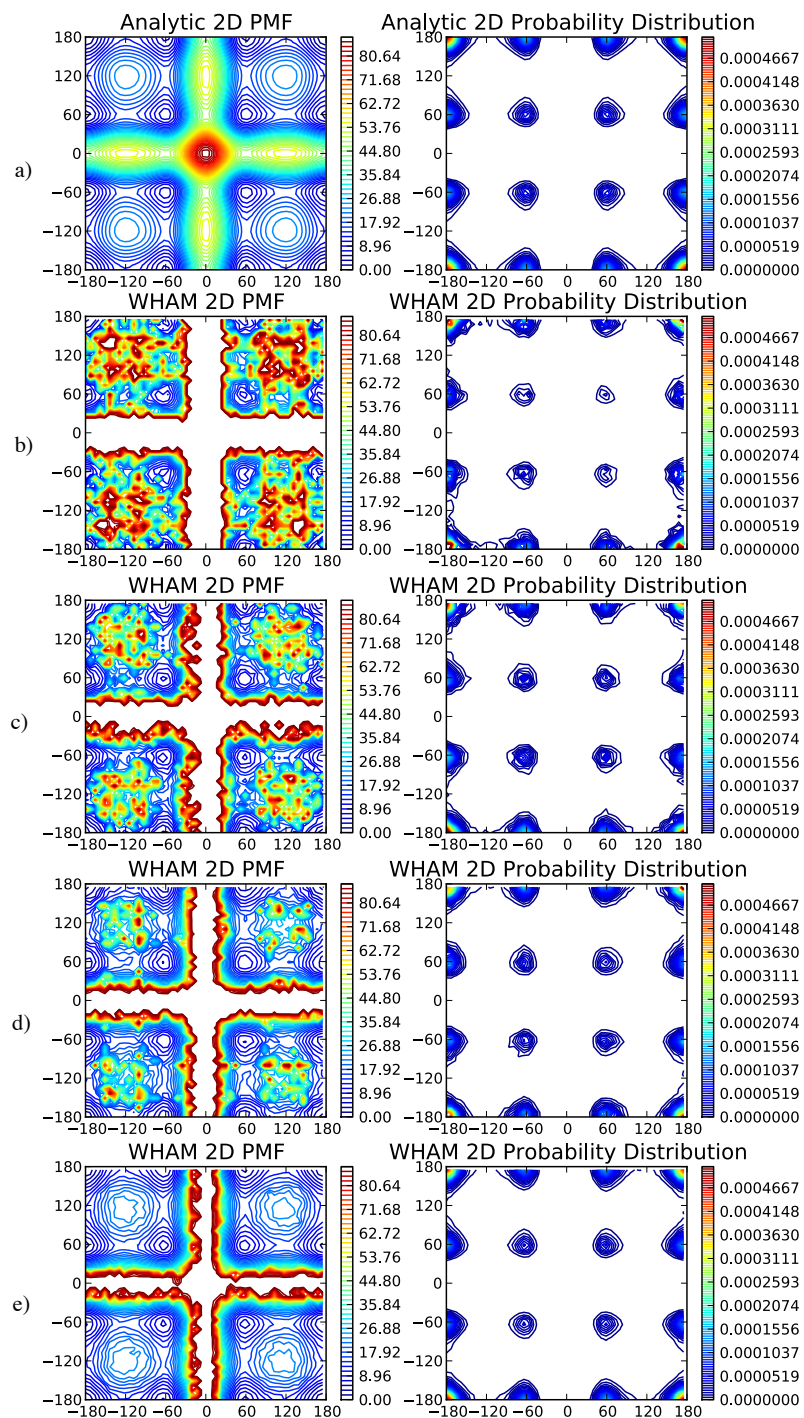


Figure 2-1: 2D WHAM Validation

Comparison between the PMF and probability distributions of a) an analytic Ryckaert-Bellemans dihedral potential and Monte Carlo 2D umbrella sampling for b) 40 frames/biasing window; c) 80 frames/biasing window; d) 160 frames/biasing window; e) 1000 frames/biasing window. Units on the PMF are kJ mol^{-1} .

2.3 ELECTROSTATIC CLUSTERING IN VIBRATIONAL CHROMOPHORE DIHEDRAL SPACE

The largest bottleneck for these sorts of calculations we do are the electrostatics. A single node on Stampede can generate >10 ns of simulation per day. That number can be increased (logarithmically) by using additional nodes. However, the continuum solvent electrostatics calculations take anywhere from 45-60 seconds (APBS) to \approx 20 minutes (AMOEBA) per frame. If we keep every 4 ps and collect 250 frames per nanosecond, then the electrostatics require 5-8 ns/day for APBS calculations and approximately 0.3 ns/day for AMOEBA calculations. This can be decreased further by running the serial calculations in parallel. Regardless, it would be convenient to find some method of pruning the total number of frames for continuum electrostatics calculations while ensuring that the average field does not differ significantly from the average using every frame.

For convenience, the vacuum electrostatic field at the nitrile due to solute only was chosen as an indicator of total electrostatic field. In the absence of solvent, this is trivial to calculate for both point charge force fields (Amber03) as well as multipole force fields (AMOEBA). This was chosen because it was 1) intuitive and 2) there is consistently a good correlation between the solute Coulomb field and the PB solvent reaction field, as seen in Figure 2-2,git suggesting that frames which well represent the Coulomb field also well represent the reaction field.

We then took advantage of the weighted averaging over binned data. The Boltzmann weighted average is calculated as in equation (2-6),

$$\langle x \rangle = \sum_{i=1}^{nbins} \rho_i \sum_{j=1}^{c_i} \frac{x_{ij}}{c_i} \quad (2-6)$$

where the probability of being in each bin i is ρ_i , the number of times bin i is visited is c_i , and each value in bin i is x_{ij} for $j = 1$ to $j = c_i$. There exists some subarray of

values in bin i that has k_i values, where $k_i \geq c_i$ entries and $\left| \sum_{j=1}^{k_i} \frac{x_{ij}}{k_i} - \sum_{j=1}^{c_i} \frac{x_{ij}}{c_i} \right| \leq \chi$, where χ is some threshold. As χ approaches 0, k_i approaches c_i , and the subarray is the full array and the averages are identical. Using the a set of test data where umbrella windows were centered every 120 degrees (at 60°, 180°, and 300°, the expected alkane maximum probability torsions) with a flat biasing potential $\pm 60^\circ$ of the window center and a force constant of ****XXX**, the clustered average field for each Ral probe in the monomeric state, docked to each of the four Rap1a mutants and each of the four Ras mutants is plotted against their average field for various values of χ , indicated in the upper-left corner of each subplot, from the full data sets in Figure 2-3. From this, it's clear that the clustered averages are linearly correlated to the full averages. The correlation coefficients and best-fit slopes have also been plotted as a function of the cutoff, χ , in Figure 2-4. Even for a relatively large $\chi = 1$, the clustered correlation coefficient and slope is *very* close to 1.0. In general, as χ approaches zero, the correlation coefficient and slope also approach zero. Moreover, at a $\chi = 0.01$, only approximately 20% of all frames are used, which is a significant decrease in computation requirement. This method also has the advantage of guaranteeing that the property the cluster is based on always has a clustered average nearly identical to the full average, which is a useful sanity check.

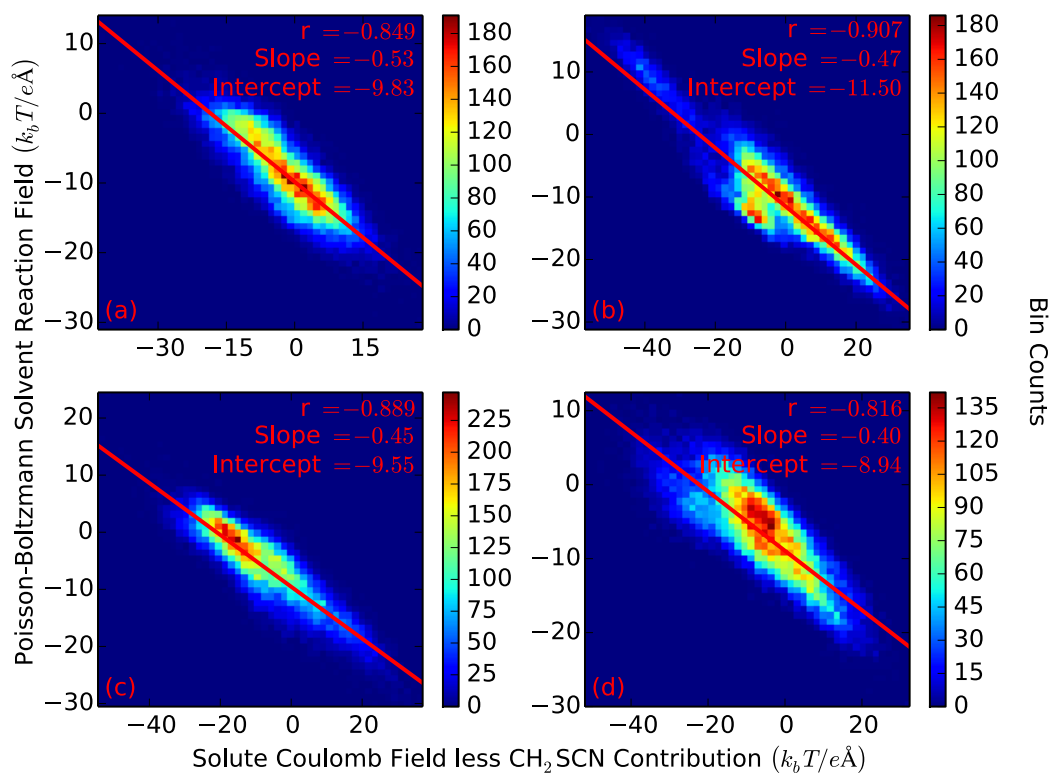


Figure 2-2: PB Solvent Reaction Field vs. Solute Analytic Coulomb Field

Comparison between the analytic Coulomb field at the nitrile bond midpoint due to solute (less the contributions due to the probe itself) (x-axis) and the solvent reaction field at the nitrile bond midpoint (y-axis) using Amber03 point charges for a) Ral G28C_{SCN} monomer; b) Ral N54C_{SCN} monomer; c) Ral G28C_{SCN} docked to wild type Rap; d) Ral N54C_{SCN} docked to wild type Rap. Correlation coefficients (r), slopes (m), and y-intercepts (int), are indicated in the upper-right corner of each figure.

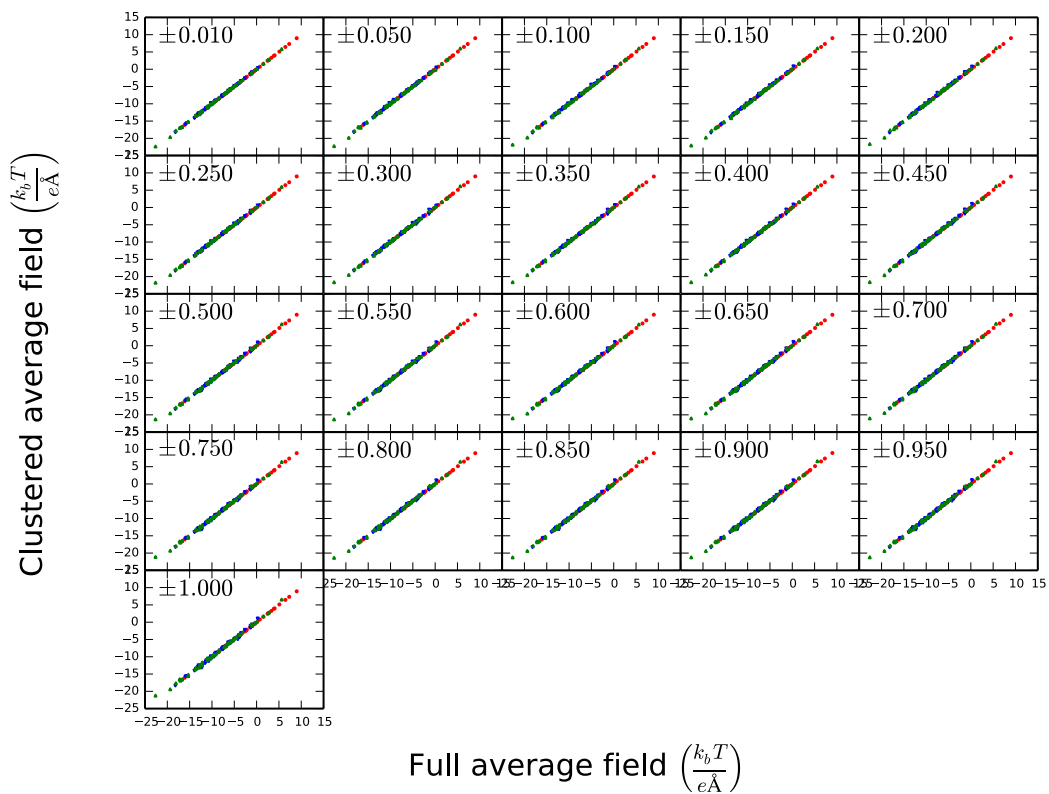


Figure 2-3: Field Values using Clustering Vs. Field Values using All Frames

Average Coulomb electrostatic field (red), solvent reaction field (blue), and the electrostatic field calculated using the AMOEBA force field (green) from clustered frames versus the respective full averages for various cutoff values, χ , indicated in the top left corner of each box.

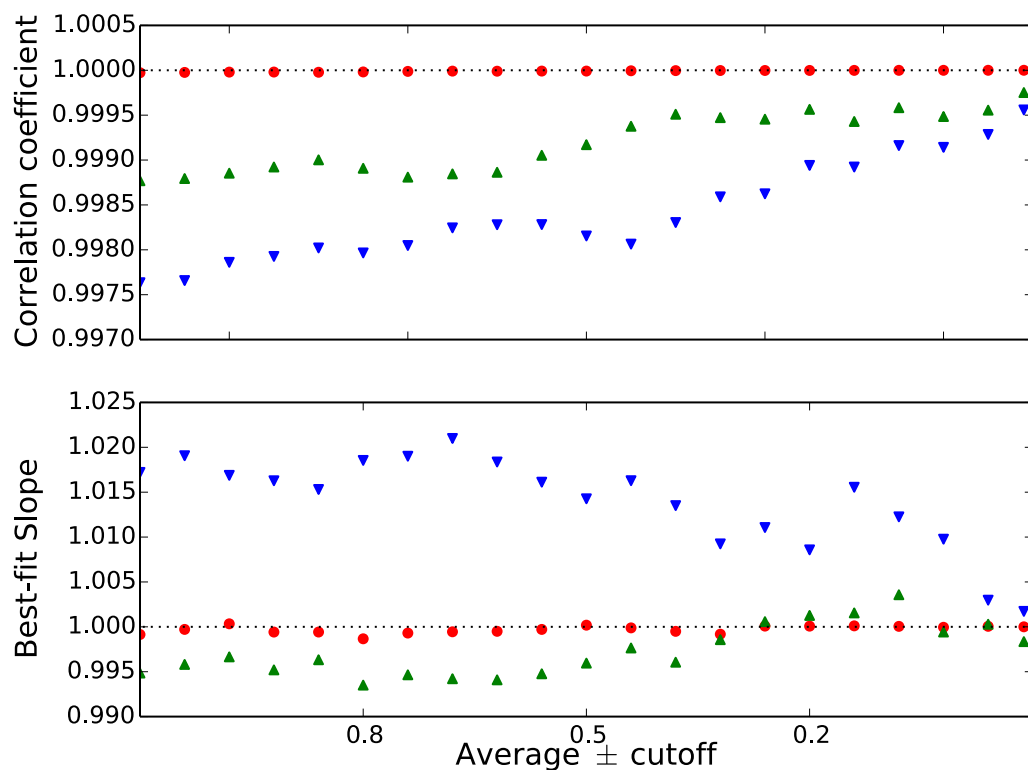


Figure 2-4: Correlations and Slopes at Various Cutoff Values

Correlation coefficients of the Coulomb electrostatic field (red), solvent reaction field (blue), and the electrostatic field calculated using the AMOEBA force field (green) as a function of the cutoff, χ . (Bottom) Best-fit slopes of the Coulomb electrostatic field (red), solvent reaction field (blue), and the electrostatic field calculated using the AMOEBA force field (green) as a function of the cutoff, χ .

2.4 PROBE PARAMETERIZATION FOR AMOEBA

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1.	atom	259	55	CB	"CNC CB"	6	12.011	4
2.	atom	260	56	SG	"CNC SG"	16	32.066	2
3.	atom	261	57	CD	"CNC CD"	6	12.011	2
4.	atom	262	58	NE	"CNC NE"	7	14.007	1
5.	atom	263	59	HB	"CNC HB"	1	1.008	1
6.								
7.	multipole	259	8	260	-0.15228			
8.					0.07407	0.00000	0.31740	
9.					-0.15117			
10.					0.00000	-0.21945		
11.					-0.19282	0.00000	0.37062	
12.	multipole	260	259	261	0.33074			
13.					0.44389	0.00000	0.28736	
14.					1.22369			
15.					0.00000	-2.16613		
16.					-0.31636	0.00000	0.94244	
17.	multipole	261	260	262	0.24556			
18.					0.06457	0.00000	-0.41800	
19.					0.15740			
20.					0.00000	0.22633		
21.					0.26601	0.00000	-0.38373	
22.	multipole	12	8	259	0.12898			
23.					0.02551	0.00000	0.07014	
24.					0.19051			
25.					0.00000	0.17012		
26.					-0.01582	0.00000	-0.36063	
27.	multipole	263	259	8	0.09179			
28.					-0.07114	0.00000	-0.02080	
29.					-0.16061			
30.					0.00000	-0.02258		
31.					-0.03267	0.00000	0.18319	
32.	multipole	262	261	260	-0.58843			
33.					-0.00512	0.00000	-0.24238	
34.					0.32332			
35.					0.00000	0.16836		
36.					-0.08202	0.00000	-0.49168	
37.								
38.	polarize	259		1.3340	0.3900	263		
39.	polarize	260		3.3000	0.3900	261		
40.	polarize	261		1.3340	0.3900	260	262	
41.	polarize	262		1.0730	0.3900	261		
42.	polarize	263		0.4960	0.3900	259		
43.								
44.	vdw	58		3.7100	0.1050			
45.	vdw	55		3.7800	0.1060			
46.	vdw	56		4.0050	0.3550			
47.	vdw	57		3.7800	0.1060			
48.	vdw	59		2.8700	0.0330	0.900		
49.	bond	55	7	323.0000	1.5317			
50.	bond	55	56	235.8000	1.8353			
51.	bond	55	59	341.0000	1.0817			
52.	bond	56	57	235.8000	1.7060			
53.	bond	57	58	450.0000	1.1374			
54.	angle	7	55	56	53.2000	109.6948		
55.	angle	7	55	59	42.4400	110.8048		
56.	angle	56	55	59	60.2400	108.4887		
57.	angle	59	55	59	39.5700	108.8231		

58. angle	55	7	3	60.0000	108.7411										
59. angle	55	7	1	80.0000	112.9036										
60. angle	55	7	6	38.0000	109.5706										
61. angle	55	56	57	60.4300	98.5327										
62. angle	56	57	58	60.0000	178.6763										
63. strbnd	7	55	56	18.7000	18.7000										
64. strbnd	7	55	59	11.5000	11.5000										
65. strbnd	56	55	59	11.5000	11.5000										
66. strbnd	55	7	3	18.7000	18.7000										
67. strbnd	55	7	1	18.7000	18.7000										
68. strbnd	55	7	6	11.5000	18.7000										
69. strbnd	55	56	57	-5.7500	-5.7500										
70. torsion	56	55	7	3	-										
	1.010	0.0	1	1.230	180.0	2	1.000	0.0	3	# CYS	3	7	8	12	
71. torsion	56	55	7	1	-0.160	0.0	1	1.080	180.0	2	-				
	1.520	0.0	3	# CYS	1	7	8	12							
72. torsion	56	55	7	6	0.000	0.0	1	0.000	180.0	2	0.475	0.0	3	#	
	CYS	6	7	8	12										
73. torsion	59	55	7	3	0.000	0.0	1	0.000	180.0	2	0.180	0.0	3	#	
	CYS	3	7	8	9										
74. torsion	59	55	7	1	0.000	0.0	1	0.000	180.0	2	0.500	0.0	3	#	
	CYS	1	7	8	9										
75. torsion	59	55	7	6	0.000	0.0	1	0.000	180.0	2	0.299	0.0	3	#	
	CYS	6	7	8	9										
76. torsion	7	55	56	57	-0.4400	0.0	1	-							
	0.2600	180.0	2	0.6000	0.0	3	# EtSCN	C1	C2	S	C				
77. torsion	59	55	56	57	0.0000	0.0	1	0.0000	180.0	2	0.6600	0.0	3	#	
	EtSCN	H2	C2	S	C										
78. torsion	55	7	3	1	0.929	0.0	1	0.328	180.0	2	0.000	0.0	3	#	
	CYS	1	3	7	8										
79. torsion	55	7	3	5	0.000	0.0	1	0.000	180.0	2	0.000	0.0	3	#	
	CYS	5	3	7	8										
80. torsion	55	7	1	3	2.576	0.0	1	1.011	180.0	2	0.825	0.0	3	#	
	CYS	3	1	7	8										
81. torsion	55	7	1	4	0.000	0.0	1	0.000	180.0	2	0.000	0.0	3	#	
	CYS	4	1	7	8										
82. torsion	55	56	57	58	0.0000	0.0	1	0.0000	180.0	2	0.5000	0.0	3	#	
	EtSCN	C2	S	C	N										

Code and Parameters 2-1: Cyanocysteine AMOEBA Parameters

1.	atom	271	67	H1	"MeSCN H1"	1	1.008	1
2.	atom	272	68	C1	"MeSCN C1"	6	12.011	4
3.	atom	273	69	S	"MeSCN S"	16	32.066	2
4.	atom	274	70	C	"MeSCN C"	6	12.011	2
5.	atom	275	71	N	"MeSCN N"	7	14.007	1
6.								
7.	multipole	272	273	271	-0.22754			
8.					0.00000	0.00000	-0.02449	
9.					0.72145			
10.					0.00000	0.72145		
11.					0.00000	0.00000	-1.44290	
12.	multipole	273	272	274	0.33074			
13.					0.44389	0.00000	0.28736	
14.					1.22369			
15.					0.00000	-2.16613		
16.					-0.31636	0.00000	0.94244	
17.	multipole	274	273	275	0.24556			
18.					0.06457	0.00000	-0.41800	
19.					0.15740			
20.					0.00000	0.22633		
21.					0.26601	0.00000	-0.38373	
22.	multipole	271	272	273	0.07989			
23.					0.02678	0.00000	-0.31473	
24.					0.66454			
25.					0.00000	-0.00998		
26.					0.17231	0.00000	-0.65456	
27.	multipole	275	274	273	-0.58843			
28.					-0.00512	0.00000	-0.24238	
29.					0.32332			
30.					0.00000	0.16836		
31.					-0.08202	0.00000	-0.49168	
32.								
33.	polarize	271			0.4960	0.3900	272	
34.	polarize	272			1.3340	0.3900	271 273	
35.	polarize	273			3.3000	0.3900	272 274	
36.	polarize	274			1.3340	0.3900	273 275	
37.	polarize	275			1.0730	0.3900	274	
38.								
39.	vdw	68			3.7800	0.1060		
40.	vdw	69			4.0050	0.3550		
41.	vdw	70			3.7800	0.1060		
42.	vdw	67			2.8700	0.0330	0.900	
43.	vdw	71			3.7100	0.1050		
44.	bond	68	69		235.8000	1.8209		
45.	bond	68	67		341.0000	1.0794		
46.	bond	69	70		235.8000	1.7068		
47.	bond	70	71		450.0000	1.1373		
48.	angle	69	68	67	60.2400	110.5747		
49.	angle	67	68	67	39.5700	110.6263		
50.	angle	68	69	70	60.4300	99.4461		
51.	angle	69	70	71	60.0000	178.9042		
52.	strbnd	69	68	67	11.5000	11.5000		
53.	strbnd	68	69	70	-5.7500	-5.7500		
54.	torsion	67	68	69 70	0.0000 0.0 1	0.0000 180.0 2	0.6600 0.0 3	
55.	torsion	68	69	70 71	0.0000 0.0 1	0.0000 180.0 2	0.5000 0.0 3	

Code and Parameters 2-2: Methyl Thiocyanate AMOEBA Parameters

1.	atom	264	60	C1	"EtSCN C1"	6	12.011	4
2.	atom	265	61	H1	"EtSCN H1"	1	1.008	1
3.	atom	266	62	C2	"EtSCN C2"	6	12.011	4
4.	atom	267	63	H2	"EtSCN H2"	1	1.008	1
5.	atom	268	64	S	"EtSCN S"	16	32.066	2
6.	atom	269	65	C	"EtSCN C"	6	12.011	2
7.	atom	270	66	N	"EtSCN N"	7	14.007	1
8.								
9.	multipole	264	266	265	-0.18333			
10.					0.00000	0.00000	0.32541	
11.					-0.25019			
12.					0.00000	-0.25019		
13.					0.00000	0.00000	0.50038	
14.	multipole	266	264	268	-0.23503			
15.					0.06819	0.00000	0.15097	
16.					-0.26732			
17.					0.00000	-0.08487		
18.					-0.08743	0.00000	0.35219	
19.	multipole	267	266	264	0.09244			
20.					-0.00077	0.00000	-0.03413	
21.					0.02305			
22.					0.00000	0.01348		
23.					-0.00070	0.00000	-0.03653	
24.	multipole	265	264	266	0.08187			
25.					0.01969	0.00000	-0.08459	
26.					0.06966			
27.					0.00000	0.02253		
28.					-0.00032	0.00000	-0.09219	
29.	multipole	268	266	269	0.33074			
30.					0.44389	0.00000	0.28736	
31.					1.22369			
32.					0.00000	-2.16613		
33.					-0.31636	0.00000	0.94244	
34.	multipole	269	268	270	0.24556			
35.					0.06457	0.00000	-0.41800	
36.					0.15740			
37.					0.00000	0.22633		
38.					0.26601	0.00000	-0.38373	
39.	multipole	270	269	268	-0.58843			
40.					-0.00512	0.00000	-0.24238	
41.					0.32332			
42.					0.00000	0.16836		
43.					-0.08202	0.00000	-0.49168	
44.								
45.	polarize	264		1.3340	0.3900	265	266	
46.	polarize	265		0.4960	0.3900	264		
47.	polarize	266		1.3340	0.3900	264	267	
48.	polarize	267		0.4960	0.3900	266		
49.	polarize	268		3.3000	0.3900	269		
50.	polarize	269		1.3340	0.3900	268	270	
51.	polarize	270		1.0730	0.3900	269		
52.								
53.	vdw	60		3.8200	0.1040			
54.	vdw	62		3.7800	0.1060			

55. vdw	64			4.0050	0.3550														
56. vdw	65			3.7800	0.1060														
57. vdw	63			2.8700	0.0330			0.900											
58. vdw	61			2.9800	0.0240			0.920											
59. vdw	66			3.7100	0.1050														
60. bond	60	62		345.3000	1.5227														
61. bond	60	61		341.0000	1.0855														
62. bond	62	64		323.0000	1.8329														
63. bond	62	63		341.0000	1.0809														
64. bond	64	65		235.8000	1.7068														
65. bond	65	66		450.0000	1.1376														
66. angle	62	60	61	42.4400	111.1890														
67. angle	61	60	61	39.5700	108.4478														
68. angle	60	62	64	53.2000	114.3035														
69. angle	60	62	63	42.4400	111.8003														
70. angle	64	62	63	60.2400	108.0491														
71. angle	63	62	63	45.5700	107.8321														
72. angle	62	64	65	60.4300	99.8280														
73. angle	64	65	66	60.0000	179.1383														
74. strbnd	62	60	61	11.5000	11.5000														
75. strbnd	60	62	64	18.7000	18.7000														
76. strbnd	60	62	63	11.5000	11.5000														
77. strbnd	64	62	63	11.5000	11.5000														
78. strbnd	62	64	65	-5.7500	-5.7500														
79. torsion	61	60	62	64	0.0000	0.0	1	0.0000	180.0	2	0.4750	0.0	3						
80. torsion	61	60	62	63	0.0000	0.0	1	0.0000	180.0	2	0.2990	0.0	3						
81. torsion	60	62	64	65	-0.4400	0.0	1	-0.2600	180.0	2	0.6000	0.0	3						
82. torsion	63	62	64	65	0.0000	0.0	1	0.0000	180.0	2	0.6600	0.0	3						
83. torsion	62	64	65	66	0.0000	0.0	1	0.0000	180.0	2	0.5000	0.0	3						

Code and Parameters 2-3: Ethyl Thiocyanate AMOEBA Parameters

1.	atom	276	72	C1	"HxSCN C1"	6	12.011	4
2.	atom	277	73	C2	"HxSCN C2"	6	12.011	4
3.	atom	278	74	H1	"HxSCN H1"	1	1.008	1
4.	atom	279	75	C3	"HxSCN C3"	6	12.011	4
5.	atom	280	76	H2	"HxSCN H2"	1	1.008	1
6.	atom	281	77	H3	"HxSCN H3"	1	1.008	1
7.	atom	282	78	C4	"HxSCN C4"	6	12.011	4
8.	atom	283	79	C5	"HxSCN C5"	6	12.011	4
9.	atom	284	80	H4	"HxSCN H4"	1	1.008	1
10.	atom	285	81	H5	"HxSCN H5"	1	1.008	1
11.	atom	286	82	C6	"HxSCN C6"	6	12.011	4
12.	atom	287	83	H6	"HxSCN H6"	1	1.008	1
13.	atom	288	84	S	"HxSCN S"	16	32.066	2
14.	atom	289	85	C	"HxSCN C"	6	12.011	2
15.	atom	290	86	N	"HxSCN N"	7	14.007	1
16.								
17.	multipole	279	282	277	-0.12665			
18.					0.16365	0.00000	0.14302	
19.					0.06092			
20.					0.00000	-0.43628		
21.					-0.27661	0.00000	0.37536	
22.	multipole	276	277	278	-0.15938			
23.					0.00000	0.00000	0.26734	
24.					-0.20136			
25.					0.00000	-0.20136		
26.					0.00000	0.00000	0.40272	
27.	multipole	283	282	286	-0.11656			
28.					0.24230	0.00000	0.09285	
29.					0.15205			
30.					0.00000	-0.43470		
31.					-0.43635	0.00000	0.28265	
32.	multipole	282	279	283	-0.11327			
33.					0.22022	0.00000	0.08479	
34.					0.15602			
35.					0.00000	-0.44910		
36.					-0.36114	0.00000	0.29308	
37.	multipole	277	279	276	-0.12195			
38.					0.19367	0.00000	0.15795	
39.					-0.01138			
40.					0.00000	-0.31282		
41.					-0.22362	0.00000	0.32420	
42.	multipole	286	283	288	-0.18161			
43.					0.04843	0.00000	0.18545	
44.					-0.01034			
45.					0.00000	-0.10804		
46.					-0.18618	0.00000	0.11838	
47.	multipole	280	277	279	0.05998			
48.					0.00441	0.00000	-0.07636	
49.					0.04595			
50.					0.00000	0.03338		
51.					-0.00973	0.00000	-0.07933	

52.	multipole	284	282	279	0.06214		
53.					0.02649	0.00000	-0.05387
54.					0.01977		
55.					0.00000	0.03305	
56.					-0.00324	0.00000	-0.05282
57.	multipole	281	279	282	0.05805		
58.					0.00916	0.00000	-0.05550
59.					0.03877		
60.					0.00000	0.02937	
61.					-0.06123	0.00000	-0.06814
62.	multipole	285	283	282	0.07555		
63.					0.01936	0.00000	-0.04776
64.					0.05942		
65.					0.00000	0.03376	
66.					-0.01589	0.00000	-0.09318
67.	multipole	278	276	277	0.05847		
68.					0.01125	0.00000	-0.10159
69.					0.06569		
70.					0.00000	0.03435	
71.					-0.03952	0.00000	-0.10004
72.	multipole	287	286	283	0.07235		
73.					0.02117	0.00000	-0.04358
74.					0.04575		
75.					0.00000	0.03869	
76.					-0.01006	0.00000	-0.08444
77.	multipole	288	286	289	0.33074		
78.					0.44389	0.00000	0.28736
79.					1.22369		
80.					0.00000	-2.16613	
81.					-0.31636	0.00000	0.94244
82.	multipole	289	288	290	0.24556		
83.					0.06457	0.00000	-0.41800
84.					0.15740		
85.					0.00000	0.22633	
86.					0.26601	0.00000	-0.38373
87.	multipole	290	289	288	-0.58843		
88.					-0.00512	0.00000	-0.24238
89.					0.32332		
90.					0.00000	0.16836	
91.					-0.08202	0.00000	-0.49168
92.							
93.	polarize	276		1.3340	0.3900	277	278
94.	polarize	277		1.3340	0.3900	276	280
95.	polarize	278		0.4960	0.3900	276	
96.	polarize	279		1.3340	0.3900	281	
97.	polarize	280		0.4960	0.3900	277	
98.	polarize	281		0.4960	0.3900	279	
99.	polarize	282		1.3340	0.3900	284	
100.	polarize	283		1.3340	0.3900	285	
101.	polarize	284		0.4960	0.3900	282	
102.	polarize	285		0.4960	0.3900	283	
103.	polarize	286		1.3340	0.3900	287	
104.	polarize	287		0.4960	0.3900	286	
105.	polarize	288		3.3000	0.3900	289	
106.	polarize	289		1.3340	0.3900	288	290
107.	polarize	290		1.0730	0.3900	289	
108.							

109.	vdw	75			3.8200	0.1010	
110.	vdw	72			3.8200	0.1010	
111.	vdw	79			3.8200	0.1010	
112.	vdw	78			3.8200	0.1010	
113.	vdw	73			3.8200	0.1010	
114.	vdw	82			3.7800	0.1060	
115.	vdw	84			4.0050	0.3550	
116.	vdw	85			3.7800	0.1060	
117.	vdw	76			2.9800	0.0240	0.940
118.	vdw	80			2.9800	0.0240	0.940
119.	vdw	77			2.9800	0.0240	0.940
120.	vdw	81			2.9800	0.0240	0.940
121.	vdw	74			2.9600	0.0240	0.920
122.	vdw	83			2.8700	0.0330	0.900
123.	vdw	86			3.7100	0.1050	
124.	bond	75	78		453.0000	1.5299	
125.	bond	75	73		453.0000	1.5298	
126.	bond	75	77		341.0000	1.0885	
127.	bond	72	73		323.0000	1.5282	
128.	bond	72	74		341.0000	1.0863	
129.	bond	79	78		453.0000	1.5312	
130.	bond	79	82		345.3000	1.5276	
131.	bond	79	81		341.0000	1.0866	
132.	bond	78	80		341.0000	1.0879	
133.	bond	73	76		341.0000	1.0878	
134.	bond	82	84		235.8000	1.8351	
135.	bond	82	83		341.0000	1.0810	
136.	bond	84	85		235.8000	1.7063	
137.	bond	85	86		450.0000	1.1375	
138.	angle	78	75	73	48.2000	113.1072	
139.	angle	78	75	77	38.0000	109.3223	
140.	angle	73	75	77	38.0000	109.2787	
141.	angle	77	75	77	39.5700	106.3036	
142.	angle	73	72	74	42.4400	111.1869	
143.	angle	74	72	74	39.5700	107.7267	
144.	angle	78	79	82	48.2000	111.5264	
145.	angle	78	79	81	38.0000	109.6162	
146.	angle	82	79	81	38.0000	109.5120	
147.	angle	81	79	81	39.5700	106.9493	
148.	angle	75	78	79	48.2000	112.7564	
149.	angle	75	78	80	38.0000	109.3923	
150.	angle	79	78	80	38.0000	109.3217	
151.	angle	80	78	80	39.5700	106.4572	
152.	angle	75	73	72	48.2000	112.9179	
153.	angle	75	73	76	38.0000	109.3170	
154.	angle	72	73	76	38.0000	109.3845	
155.	angle	76	73	76	45.5700	106.3035	
156.	angle	79	82	84	53.2000	109.0812	
157.	angle	79	82	83	42.4400	111.1744	
158.	angle	84	82	83	60.2400	108.2095	
159.	angle	83	82	83	39.5700	108.8985	
160.	angle	82	84	85	60.4300	99.5693	
161.	angle	84	85	86	60.0000	179.0730	
162.	strbnd	78	75	73	18.7000	18.7000	
163.	strbnd	78	75	77	11.5000	18.7000	
164.	strbnd	73	75	77	11.5000	18.7000	
165.	strbnd	73	72	74	11.5000	11.5000	

166.	strbnd	78	79	82	18.7000	18.7000							
167.	strbnd	78	79	81	11.5000	18.7000							
168.	strbnd	82	79	81	11.5000	18.7000							
169.	strbnd	75	78	79	18.7000	18.7000							
170.	strbnd	75	78	80	11.5000	18.7000							
171.	strbnd	79	78	80	11.5000	18.7000							
172.	strbnd	75	73	72	18.7000	18.7000							
173.	strbnd	75	73	76	11.5000	18.7000							
174.	strbnd	72	73	76	11.5000	18.7000							
175.	strbnd	79	82	84	18.7000	18.7000							
176.	strbnd	79	82	83	11.5000	11.5000							
177.	strbnd	84	82	83	11.5000	11.5000							
178.	strbnd	82	84	85	-5.7500	-5.7500							
179.	torsion	73	75	78	79	0.576	0.0	1	-				
	0.017	180.0	2	2.031	0.0	3							
180.	torsion	73	75	78	80	0.000	0.0	1	0.000	180.0	2	0.000	
	0.0	3											
181.	torsion	77	75	78	79	0.000	0.0	1	0.000	180.0	2	0.000	
	0.0	3											
182.	torsion	77	75	78	80	0.000	0.0	1	0.000	180.0	2	0.000	
	0.0	3											
183.	torsion	78	75	73	72	0.484	0.0	1	0.014	180.0	2	2.221	
	0.0	3											
184.	torsion	78	75	73	76	0.000	0.0	1	0.000	180.0	2	0.000	
	0.0	3											
185.	torsion	77	75	73	72	0.000	0.0	1	0.000	180.0	2	0.000	
	0.0	3											
186.	torsion	77	75	73	76	0.000	0.0	1	0.000	180.0	2	0.000	
	0.0	3											
187.	torsion	74	72	73	75	0.0000	0.0	1	0.0000	180.0	2	0.3410	
	0.0	3											
188.	torsion	74	72	73	76	0.0000	0.0	1	0.0000	180.0	2	0.2990	
	0.0	3											
189.	torsion	82	79	78	75	0.364	0.0	1	-				
	0.024	180.0	2	1.958	0.0	3							
190.	torsion	82	79	78	80	0.000	0.0	1	0.000	180.0	2	0.000	
	0.0	3											
191.	torsion	81	79	78	75	0.000	0.0	1	0.000	180.0	2	0.000	
	0.0	3											
192.	torsion	81	79	78	80	0.000	0.0	1	0.000	180.0	2	0.000	
	0.0	3											
193.	torsion	78	79	82	84	-							
	0.688	0.0	1	0.489	180.0	2	1.957	0.0	3				
194.	torsion	78	79	82	83	0.000	0.0	1	0.000	180.0	2	0.000	
	0.0	3											
195.	torsion	81	79	82	84	0.000	0.0	1	0.000	180.0	2	0.000	
	0.0	3											
196.	torsion	81	79	82	83	0.000	0.0	1	0.000	180.0	2	0.000	
	0.0	3											
197.	torsion	79	82	84	85	-							
	2.643	0.0	1	0.709	180.0	2	1.072	0.0	3				
198.	torsion	83	82	84	85	0.000	0.0	1	0.000	180.0	2	0.000	
	0.0	3											
199.	torsion	82	84	85	86	0.0000	0.0	1	0.0000	180.0	2	0.5000	
	0.0	3											

Code and Parameters 2-4: Hexyl Thiocyanate AMOEBA Parameters

2.5 SMALL MOLECULE SIMULATIONS IN AMOEBA

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Chapter 3 Electrostatic Field Methods

3.1 AMBER03 WITH EXPLICIT TIP3P WATER

3.1.1 Reaction Field Electrostatics

3.1.2 Hybrid Solvent Reaction Field Electrostatics and Solute Coulomb Field

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3.2 AMBER03 WITH POISSON-BOLTZMANN CONTINUUM SOLVENT

3.2.1 Reaction Field Method

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3.2.2 Grid spacing and size

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3.2.3 Box Location

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3.3 AMBER03 WITH POISSON-BOLTZMANN CONTINUUM SOLVENT AND SELECT EXPLICIT TIP3P WATER MOLECULES

3.3.1 5 Å Water Sphere Around the Vibrational Chromophore

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3.3.2 Single Water Molecule Nearest the Vibrational Chromophore

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3.3.3 Water Molecular Hydrogen Bonding to the Vibrational Chromophore

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3.4 AMOEBA

3.4.1 Poisson-Boltzmann Continuum Solvent

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3.4.2 Explicit AMOEBA Water

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3.4.3 Charge Penetration Field Corrections

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Chapter 4 The Role of Electrostatics in Differential Binding of RalGDS to Rap Mutations E30D and K31E Investigated by Vibrational Spectroscopy of Thiocyanate Probes

4.1 INTRODUCTION

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4.2 RESULTS

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4.3 DISCUSSION

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Chapter 5 Optimizing Electrostatic Field Calculations with the Adaptive Poisson-Boltzmann Solver to Predict Electric Fields at Protein-Protein Interfaces I: Sampling and Focusing

5.1 INTRODUCTION

<Body text to begin here.>

5.2 RESULTS

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5.3 DISCUSSION

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Chapter 6 Optimizing Electrostatic Field Calculations with the Adaptive Poisson-Boltzmann Solver to Predict Electric Fields at Protein-Protein Interfaces II: Explicit Near-Probe and Hydrogen Bonding Water Molecules

6.1 INTRODUCTION

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6.2 RESULTS

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6.3 DISCUSSION

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Chapter 7 Electrostatic Fields at Protein-Protein Interfaces: Increased Sampling Time and Various Electrostatic Methods: A Case for Simulating in Polarizable Force Fields

7.1 INTRODUCTION

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7.2 RESULTS

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7.3 DISCUSSION

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Chapter 8 Electrostatic Fields in Small Thiocyanate Molecules with Ensembles Generated using the AMOEBA Force Field

8.1 INTRODUCTION

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8.2 RESULTS

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8.3 DISCUSSION

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Appendix

Glossary

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