J Comput Aided Mol Des (2009) 23:837–843
DOI 10.1007/s10822-009-9300-5

Relative solvation free energies calculated using an ab initio QM/MM-based free energy perturbation method: dependence of results on simulation length

M. Rami Reddy · Mark D. Erion

Received: 11 March 2009 / Accepted: 17 August 2009 / Published online: 17 September 2009

Abstract Molecular dynamics (MD) simulations in conjunction with thermodynamic perturbation approach was used to calculate relative solvation free energies of five pairs of small molecules, namely; (1) methanol to ethane, (2) acetone to acetamide, (3) phenol to benzene, (4) 1,1,1 trichloroethane to ethane, and (5) phenylalanine to isoleucine. Two studies were performed to evaluate the dependence of the convergence of these calculations on MD simulation length and starting configuration. In the first study, each transformation started from the same well-equilibrated configuration and the simulation length was varied from 230 to 2,540 ps. The results indicated that for transformations involving small structural changes, a simulation length of 860 ps is sufficient to obtain satisfactory convergence. In contrast, transformations involving relatively large structural changes, such as phenylalanine to isoleucine, require a significantly longer simulation length (>2,540 ps) to obtain satisfactory convergence. In the second study, the transformation was completed starting from three different configurations and using in each case 860 ps of MD simulation. The results from this study suggest that performing one long simulation may be better than averaging results from three different simulations using a shorter simulation length and three different starting configurations.

Keywords Relative solvation free energies · Starting configurations · MD simulations · Quantum mechanical · QM/MM · Thread method

Introduction

Free energy perturbation (FEP) methods [1–3] are well recognized as the most accurate computational approach for calculating relative solvation [4–6] and binding [7–9] free energy differences between two drug candidates and therefore aid in drug design [10, 11, 13; for reviews, see: 12]. The accuracy of FEP calculations, while superior to other less time-consuming methods [for reviews, see: 14, 15], still relies on a molecular mechanics (MM) force field and the accuracy of the equations and parameters that comprise the force field. Force field parameters have been developed for many atom types and molecular structures, but despite these efforts, some commonly found molecular structures such as simple alkyl amines and amides still pose challenges. Moreover, drug candidates often contain new scaffolds that are inadequately represented by the default set of generalized force field parameters or parameterized automatically derived from an extrapolation algorithm. Consequently the user must develop and input the corresponding parameters [16, 17], which is time-consuming and often hampered by the lack of relevant experimental data.

One strategy that eliminates the need for force field parameter development and may enable automation of FEP calculations is to use quantum mechanics (QM) to describe the ligand and molecular mechanics (MM) to describe the surrounding environment (e.g. protein, water). The coupling of QM and MM has been developed and used successfully to determine activation free energies [18–21] and free energy profiles of enzymatic reactions [22–24]. Combined QM/

Electronic supplementary material The online version of this article (doi:10.1007/s10822-009-9300-5) contains supplementary material, which is available to authorized users.

M. R. Reddy (✉) · M. D. Erion
Metabasis Therapeutics, Inc., 11119 North Torrey Pines Road, La Jolla, CA 92037, USA
e-mail: drmrreddy@yahoo.com; reddy@mbasis.com

MM methods have also been used to calculate p*K*a and redox potentials in macromolecular systems [25, 26]. Previously we described the development and validation of a QM/MM-based free energy perturbation methodology both for calculating relative solvation free energies of small molecules and relative binding free energies of enzyme inhibitors [27–29]. In this paper we assess the convergence of relative solvation free energy calculations using a QM/MM based FEP method [27–29] as function of MD simulation length and starting configuration.

Over the past 25 years more than 200 papers have published describing various relative solvation (with explicit solvent) free energy calculations using molecular dynamics (MD) or monte carlo (MC) simulations in conjunction with the thermodynamics cycle perturbation approach (TCP) [4, 9, 30–33]. The results from these studies (which used short MD simulations < 100 ps) suggest that MD simulations using explicit solvent have not fully converged. Nevertheless, satisfactory agreement with experimental results (± 4 kJ/mol) is often achieved. Both McCammon [34] and Kollman [35] examined the behavior of the calculated free energy with the length of the simulation and concluded that simulation times significantly longer than those typically used (200 ps or greater) may be required to yield reliable and accurate results. More recently, Reddy and Erion [40] examined the convergence of relative solvation free energies using conventional free energy methods and its dependence on simulation length. This study indicated that simulation times significantly longer than those typically used are required for reliable and accurate results.

In early days of FEP methods development for calculating relative solvation free energies, the errors about 4 kJ/mol were acceptable because they didn't have enough computer resources and these methods were used mainly for academic interest. Recently, many scientists are using these FEP methods for drug discovery by predicting relative binding affinities between two similar inhibitors to a given protein target for which free energy changes between two similar inhibitors are very small. Therefore, the larger errors in the relative free energies (about 4 kJ/mol) will not be very useful for deciding which inhibitor binds better because very often errors are larger than the free energy changes between two similar inhibitors. So, it is very important to understand the ideal simulation length for calculating relative solvation free energies which provide errors about 2 kJ/mol.

To address the above concerns, we carried out two studies evaluating the effect of the length of simulation and the starting configurations on free energy. In the first study, initially each solvated solute molecule was equilibrated for 20 ps and followed by varied MD simulation lengths from 230 to 2,540 ps (includes 20 ps of MD simulations for initial equilibration) to complete each mutation. In the second study, the relative solvation free energy difference was calculated starting from three different configurations (about 6 kJ/mol energy differences between them) followed by 20 ps of equilibration. The RMSD between these structures at starting point and after 20 ps of equilibration were about 1.2 and 0.7 Å, respectively. For each structure a total of 860 ps (includes 20 ps of equilibration) of MD simulation was required for completing the each mutation.

Theory

Free energy perturbation method

The statistical perturbation theory is due to the classical work of Zwanzig [1] and its detailed implementation in molecular dynamics programs to compute the free energy is available in literature [2–4, 9–13]. For completeness a very brief description of the FEP method is given here. The total Hamiltonian of a system may be written as the sum of the Hamiltonian (H_0) of the unperturbed and the perturbation (H_1) systems:

$$H = H_0 + H_1 \tag{1}$$

The free energy contribution due to the perturbation is given by,

$$G_1 = \frac{1}{\beta}\langle \exp(-\beta H_1)\rangle_0 \tag{2}$$

where $\beta = 1/kT$ and the mean of $\exp(-\beta H_1)$ is computed over the unperturbed ensemble of the system.

In order to compute ΔG, the difference in free energy between the two solute states, the Hamiltonian for states A and B can be linked by the coupling parameter λ in a linear or non-linear manner such that, $H(\lambda)$ represents hypothetical coupled Hamiltonian of the system at a given $\lambda (0 < \lambda \leq 1)$. For simple linear coupling, $H(\lambda)$ can be constructed as,

$$H(\lambda) = \lambda H_A + (1 - \lambda) H_B \tag{3}$$

In the above equation, H_A is the Hamiltonian for the system at state A, and H_B is for state B. For both simple linear and

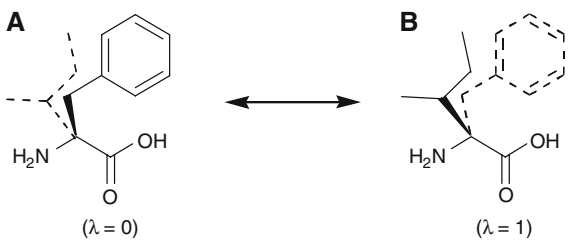

Fig. 1 Molecular threading of phenylalanine (molecule A) and isoleucine (molecule B)

non-linear coupling of two solute states, when $\lambda = 0$, $H_\lambda = H_B$ i.e., the system is purely in state A and when $\lambda = 1$, $H_\lambda = H_B$ at which point the system is purely in state B. During intermediate value of λ, the solute is a mixture of A and B. This type of coupling ensures a very smooth conversion of two solutes A and B, allowing the system to readjust its configuration smoothly as a function of the state. If we divide the range of λ into N windows, at each window λ_i, the solute state is perturbed between λ_{i+1} and λ_{i-1} states by taking the reference state as $H(\lambda_i)$. The free energy difference between the two solute states A and B is a simple summation over all windows of $(G_1(\lambda_i))$ as given by,

$$\Delta G = \sum_{i=1}^{N} G_1(\lambda_i) \tag{4}$$

The evaluation of G_1 at λ_{i+1} and λ_{i-1} is a check for possible hysteresis in the calculation and is a measure of the statistical error for the free energy change.

Thread method or double topology method

Since A and B are often structurally dissimilar molecules, we used the thread method [13, 41–44] for all the transformations instead of more commonly used atom-to-atom mapping procedures. Accordingly, as exemplified in Fig. 1, each transformation required "threading" together two molecules, referred to herein as molecule A (e.g. phenylalanine) and molecule B (e.g. isoleucine), such that the common atoms (amino acid backbone) were represented by a single topology (Fig. 1). The non-common atoms are the side chain atoms which for $\lambda = 0$ are real atoms for molecule A and dummy atoms for molecule B (dashed structure in Fig. 1). At the completion of the transformation ($\lambda = 1$), the side chain atoms for molecule A are dummy atoms and for molecule B are real atoms and hydrogen atoms are removed for clarity.

The non-common atoms of each molecule, i.e. the portion of the molecules that must be transformed, were represented by two separate topologies each defined using their associated geometries and each capable of interacting only with the environment and not each other. At the start of the simulation, the non-common atoms of molecule B exist entirely as dummy atoms, which are atoms identical to real atoms except that their Lennard-Jones parameters and atomic charges are set to zero. At intermediate points during the transformation, all atoms in both topologies have fractional Lennard-Jones parameters and charges. At the end of the simulation, the topology that started as dummy atoms (molecule B) is entirely real atoms whereas the topology that started as real atoms (molecule A) is entirely dummy atoms.

The thread method in conventional FEP calculations entails scaling the MM parameters according to λ and calculating the corresponding MM energies and forces. In contrast, the QM/MM-based FEP method uses ab initio quantum mechanical methods to calculate the energies and forces for the solute in the system and MM to describe the solvent. In order to calculate the QM energy and forces, we implemented a procedure [13, 43] that separated the threaded molecule into two molecules (A and B) at each dynamic step. The ab initio quantum mechanical energies and forces were then computed and the combined energies and forces recomputed using the lambda coupling method. The interaction energy between the perturbing system and the surroundings (C) at any value of λ is calculated by coupling the charges, radius and well depth of the perturbing system as:

The partial charge on atom i at any value of λ is given by

$$q_{i\lambda} = \lambda q_i^A + (1-\lambda) q_i^B \tag{5}$$

The radius and well depth of atom i at a given value of λ is given by

$$r_{i\lambda} = \lambda r_i^A + (1-\lambda) r_i^B \tag{6}$$

$$\varepsilon_{i\lambda} = \lambda \varepsilon_i^A + (1-\lambda) \varepsilon_i^B \tag{7}$$

Ab initio QM forces and energies are calculated for each molecule, A and B, separately and then scaled based on λ using Eqs. (8) and (9),

$$f_\lambda^i = \lambda f_A^i + (1-\lambda) f_B^i \tag{8}$$

$$E_\lambda^{QM} = \lambda E_A^{QM} + (1-\lambda) E_B^{QM} \tag{9}$$

Accordingly, the energy and force will correspond to molecule B when $\lambda = 0$ and to molecule A when $\lambda = 1$. The total energy for the system is determined using Eq. (10) wherein the term $E_{QM/MM}$ represents the interaction energy involving an atom i in the MM part of the system and an atom j in the QM part of the system. The free energy change (Eq. 11) is decomposed into the free energy contribution from the subsystem treated by ab initio QM and the free energy contribution from the surroundings, i.e. the subsystem not treated by QM (non-QM or NQM).

$$E_{tot} = E_{QM} + E_{MM} + \sum_{i=1}^{M} \sum_{j=1}^{L} E_{QM/MM}^{ij} \tag{10}$$

$$\Delta G_{tot} = \Delta G_{QM} + \Delta G_{NQM} \tag{11}$$

Computational details

Relative differences in solvation free energies for the transformations shown in Table 1 were calculated using

Table 1 Relative solvation free energy results (kj/mol)

System	Length of MD run (ps)[a]	$\Delta\Delta G$ (cal)[b]	$\Delta\Delta G$ (expt)[c]
$CH_3OH \rightarrow CH_3CH_3$	230	31.5 ± 2.8	29.0
	440	30.8 ± 2.2	
	860	29.7 ± 1.7	
	1,700	29.5 ± 1.3	
	2,540	29.4 ± 1.1	
$CH_3CONH_2 \rightarrow CH_3COCH_3$	230	29.9 ± 3.4	24.8
	440	28.7 ± 3.0	
	860	27.9 ± 2.2	
	1,700	27.4 ± 1.9	
	2,540	27.1 ± 1.7	
$C_6H_5OH \rightarrow C_6H_6$	230	20.5 ± 2.9	23.5
	440	21.2 ± 2.3	
	860	22.3 ± 1.8	
	1,700	22.7 ± 1.5	
	2,540	22.9 ± 1.3	
$CH_3CCl_3 \rightarrow CH_3CH_3$	230	10.5 ± 2.8	8.4
	440	9.7 ± 2.5	
	860	9.2 ± 2.1	
	1,700	8.9 ± 1.6	
	2,540	8.7 ± 1.1	
PHE → ILE	230	9.3 ± 4.6	12.1
	440	10.0 ± 3.8	
	860	10.6 ± 3.3	
	1,700	11.1 ± 2.6	
	2,540	11.4 ± 2.2	
	3,380	11.6 ± 1.9	

PHE phenylalanine, *ILE* isoleucine

[a] Each simulation started from the same well-equilibrated configurations; [b] calculated using HF/3-21G* for forces and energies and HF/6-31G*/ESP for partial atomic charges; [c] values obtained from experimental data reported in the literature [52, 53]

the newly implemented ab initio QM/MM based-FEP method with the thermodynamic cycle depicted in Fig. 2 and the corresponding Eq. 12. The ab initio part of the code is originated from the QUEST program developed at UCSF by Singh and Kollman [20, 45, 46].

$$\Delta\Delta G_{sol} = \Delta G_{aq} - \Delta G_{gas} \quad (12)$$

The FEP calculations in the solvent phase were performed starting with one solute which was mapped onto the other solute and solvated in a rectangular box of SPC/E water [47, 48]. All water molecules located greater than 19 Å from the nearest solute atom or less than 2.5 Å from any solute atom were removed. The van der Waals parameters for all the solute atoms were taken from the Galaxy data base (Galaxy Molecular Modeling Software and AM2000 Macromolecular Simulation package, AM

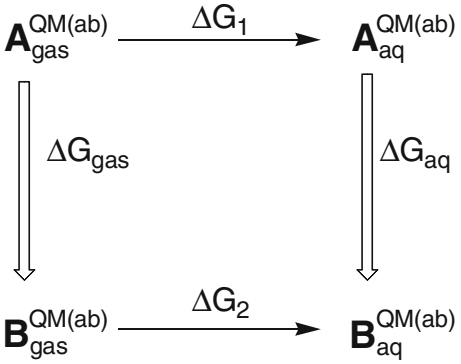

Fig. 2 Thermodynamic cycle for computing solvation free energy differences between two solutes A and B. QM(ab), indicates that the solutes were treated using ab initio quantum mechanics

Technologies, San Antonio, TX, 1995) and we have not modified the van der Waals parameters [49] to use in QM/MM method because we got very good agreement with experimental data [27–29] using the existing parameters available in the Galaxy data base. In order to eliminate dependence of the partial atomic charges on the solute molecule's starting geometry, we optimized all the solute molecules using HF/6-31G* basis set and obtained partial atomic charges for all the solute atoms with ESP fitting in the beginning of the simulation (HF/6-31G* OPT ESP). The HF/3-21G* basis set was used for calculating energies and forces of the solute molecules at every step during the molecular dynamics simulations to reduce required computer time for performing QM calculations at each MD step, otherwise it would have taken extra five fold computer time to complete each mutation. In addition, we also tested HF/6-31G* and HF/3-31G* basis sets by calculating energies and forces at each MD step (HF/6-31G* OPT with ESP fitting for calculating partial atomic charges of solute molecules in the beginning of the simulation) for the relative solvation free energies between methanol and ethane and found no significant differences.

Energy minimizations and subsequent FEP calculations used molecular mechanics (MM) and molecular dynamics (MD) simulations, which were carried out using periodic boundary conditions in all directions. In the MD simulations, Newton's equations of motion for all the atoms were solved using the Verlet algorithm [50] with a 1 fs time step. SHAKE was used to constrain all bond lengths [51]. Constant temperature (N, P, T ensemble) was maintained by scaling velocities of all atoms in the system. The non-bonded interaction energies were calculated using a 15 Å residue based cutoff. Initially, each system was minimized using a conjugate gradient method for 2,000 cycles with the solutes frozen and only the water molecules allowed to move. Next, the whole system was energy minimized for

an additional 2,000 cycles followed by 100 cycles of minimization using the SHAKE option to constrain the bond lengths to their equilibrium values. The system was then equilibrated with 20 ps of MD using a time step of 1 fs at a constant temperature (300 K) and pressure (1 atm). The FEP calculations in the gas phase were performed using a similar procedure but without inclusion of the water molecules.

The transformation was achieved using the window method [1–3] as implemented in the Galaxy program and a two-stage procedure previously shown to enhance convergence [27–29]. In the first stage, the atomic charges of molecule A (the starting topology consisting of real atoms) were slowly turned off while the Lennard-Jones parameters of the atoms of molecule B (the starting topology consisting of dummy atoms) were slowly turned on. During the second stage, the Lennard-Jones parameters of molecule A were turned off while the charges of molecule B were turned on. In each stage, the transformation occurred over a total of 21 windows ($\Delta\lambda = 0.05$) with each window consisting of 1.0 and 4.0 ps, 2.0 and 8.0 ps, 4.0 and 16.0 ps, 8.0 and 32.0 ps, 12.0 and 48.0 ps for equilibration and data collection during mutation, respectively using the same starting configurations. The second study used 4.0 ps of equilibration and 16.0 ps of data collection for each mutation with the different starting configurations. Thus, in each mutation MD simulation length was varied from 230 to 2,540 ps for the first study of each system and 860 ps of MD was used for each mutation in the second study. All these total MD simulation lengths includes a 20 ps of MD used for initial equilibration. For all these relative solvation free energy calculations the error bars were estimated for each window by dividing the window statistics into eight groups and computing the standard deviation [27–29]. The reported standard deviation is the root mean square of the window errors.

Results and discussion

To assess convergence of the relative solvation free energies of small molecules, five test cases were chosen since they constitute relatively large structural changes (e.g. phenylalanine to isoleucine versus methanol to ethane), as well as changes in hydrogen bonding potential (e.g. acetone to acetamide), aromaticity (e.g. benzene to phenol) and electron density (1,1,1-trichloroethane to ethane). Initially the relative solvation free energy difference for the transformation of methanol to ethane is calculated as a function of simulation length. The computed relative solvation free energy differences between methanol and ethane are 31.5 ± 2.8 kJ/mol (230 ps), 30.8 ± 2.2 kJ/mol (440 ps), 29.7 ± 1.7 kJ/mol (860 ps), 29.5 ± 1.3 kJ/mol (1,700 ps), and 29.4 ± 1.1 kJ/mol (2,540 ps), and these results show (Table 1) that a simulation length of 860 ps or longer is sufficient to achieve good agreement with the experimental value of 29.0 kJ/mol [52, 53]. In addition and as expected, the longer the simulation length greater the reduction in standard deviation as illustrated for results obtained using simulation lengths of 230 ps (±2.8) vs. 2,540 ps (±1.1).

Table 2 Relative solvation free energy results (kJ/mol)

System	Length of MD run (ps)[a]	$\Delta\Delta G$ (cal)[b]	$\Delta\Delta G$ (expt)[c]
$CH_3OH \rightarrow CH_3CH_3$	RUN1_860	29.7 ± 1.7	29.0
	RUN2_860	29.4 ± 1.9	
	RUN3_860	29.5 ± 1.7	
$CH_3CONH_2 \rightarrow CH_3COCH_3$	RUN1_860	27.9 ± 2.2	24.8
	RUN2_860	27.6 ± 2.4	
	RUN3_860	27.4 ± 2.3	
$C_6H_5OH \rightarrow C_6H_6$	RUN1_860	22.3 ± 1.8	23.5
	RUN2_860	22.5 ± 1.9	
	RUN3_860	22.1 ± 1.8	
$CH_3CCl_3 \rightarrow CH_3CH_3$	RUN1_860	9.2 ± 2.1	8.4
	RUN2_860	9.4 ± 2.3	
	RUN3_860	9.0 ± 2.1	
PHE → ILE	RUN1_860	10.6 ± 3.3	12.1
	RUN2_860	10.8 ± 3.5	
	RUN3_860	10.4 ± 3.4	

PHE phenylalanine, *ILE* isoleucine

[a] Each simulation started with completely different configurations; [b] calculated using HF/3-21G* for forces and energies and HF/6-31G*/ESP for partial atomic charges; [c] values obtained from experimental data reported in the literature [52, 53]

Transformations were also conducted on the following molecular pairs: (1) acetamide to acetone, (2) phenol to benzene, (3) 1,1,1 trichloroethane to ethane, and (4) phenylalanine to isoleucine. As in the methanol to ethane transformation, each transformation started from the same well-equilibrated configurations and the simulation length was varied from 230 to 2,540 ps. The calculated relative solvation free energy results (Table 1) for these systems, again, clearly show that the standard deviation goes down as MD simulation length increases from 230 ps (29.9 ± 3.4 kcal/mol) to 25,640 ps (27.1 ± 1.7 kcal/mol) for acetone to acetamide, 230 ps (20.5 ± 2.9 kcal/mol) to 2,560 ps (22.9 ± 1.30 kcal/mol) for phenol to benzene, 230 ps (10.5 ± 2.8 kcal/mol) to 2,540 ps (8.7 ± 1.1 kcal/mol) for 1,1,1 trichloroethane to ethane, and 230 ps (9.3 ± 4.6 kcal/mol) to 2,540 ps (11.4 ± 2.2 kcal/mol) and 3,380 ps (11.6 ± 1.9 kcal/mol) for phenylalanine to isoleucine, which are good in agreement with experimental results [52, 53]. The calculated relative solvation free

energies (Table 1) for all these system agree closely for all the molecular dynamics simulations length of 860 ps or longer. The error for the mutation between Phe and Ile is larger as compared to other mutations because it requires a larger structural changes and required 3,380 ps (16 ps of equilibration and 64 ps of data collection in each window) of MD to reduce the error below 2 kcal/mol.

In the second study, the relative solvation free energy difference between methanol and ethane was calculated starting from three different configurations and using 860 ps of MD simulation for each transformation. The resulting solvation free energies (Table 2) are, 29.7 ± 1.7, 29.4 ± 1.9 and 29.5 ± 1.7 kJ/mol, for three different simulations, which are again in good agreement with the experimental results (29.0 kJ/mol) [52, 53]. These results show that MD simulation length of 860 ps or greater is required to obtain satisfactory convergence even for small changes in the mutation such as methanol to ethane. We have also carried out mutations for other four molecular pairs and calculated the relative solvation free energies using three different starting configurations and 860 ps of MD simulations for each system. The calculated results (Table 2) for these systems indicate that the calculated relative solvation free energies do not change significantly depending upon the starting configurations of MD simulations and moreover that 860 ps of MD simulation achieves satisfactory convergence. These results further suggest that a simulation length of 860 ps is likely sufficient to adequately sample all microstates for the molecules under study except for the mutation Phe to Ile. In addition, the comparison of the calculated relative solvation free energy results between QM/MM based methods and conventional FEP methods [40] indicate that QM/MM based methods require longer simulations to obtain satisfactory errors (about 2 kJ/mol) and this could be due to the accuracy of QM/MM based FEP method or the way the errors were estimated in the published literature using conventional (MM/MM) FEP methods because there are differences in the methods used for estimating errors in the solvation free energies.

Conclusions

The calculated relative solvation free energies using a QM/MM Based-Free Energy Perturbation method for small changes in the mutations such as methanol to ethane a MD simulation length of 860 ps (errors about 2 kJ/mol) is sufficient to obtain satisfactory convergence. In contrast, transformations involving relatively large structural changes, such as phenylalanine to isoleucine, require a significantly longer simulation length (>2,540 ps) to obtain satisfactory convergence. Calculations using longer simulation lengths were associated with smaller standard deviations suggesting that calculations performed previously using short MD simulation lengths (e.g. 50–200 ps) may be subject to significant inaccuracies. In addition, a comparison of calculated relative solvation free energy results between one long MD simulation length of 2,540 ps and averaging three shorter simulations of the length 860 ps each show that performing one long simulation may be better than averaging results from several different shorter length simulations with different starting conformations.

References

1. Zwanzig RJ (1954) J Chem Phys 22:1420–1426
2. Tembe BL, McCammon JA (1984) Comput Chem 8:281–286
3. Pearlman DA (2001) In: Reddy MR, Erion MD (eds) Free energy calculations in rational drug design. Plenum Press, New York, pp 9–35
4. Jorgensen WL, Ravimohan C (1985) J Chem Phys 83:3050–3054
5. Rao BG, Singh UC (1990) J Am Chem Soc 112:3803–3810
6. Agarwal A, Brown FB, Reddy MR (2001) In: Reddy MR, Erion MD (eds) Free energy calculations in rational drug design. Plenum Press, New York, pp 97–117
7. Rizzo RC, Tirado-Rives J, Jorgensen WL (2001) J Med Chem 44:145
8. Rao BG, Tilton RF, Singh UC (1992) J Am Chem Soc 114:4447
9. Reddy MR, Erion MD, Agarwal A (2000) In: Lipkowitz KB, Boyd DB (eds) Reviews in computational chemistry, vol 16. Wiley-VCH Inc., New York, pp 217–304
10. Jorgensen WL (2004) Science 303:1813–1818
11. Simonson T, Archontis G, Karplus M (2002) Acc Chem Res 35:430–437
12. Reddy MR, Erion MD (eds) (2001) Free energy calculations in rational drug design. Plenum Press, New York
13. Reddy MR, Erion MD (2001) J Am Chem Soc 123:6246–6252
14. Alvarej J, Shoichest B (eds) (2005) Virtual screening in drug discovery. CRC Press, Boca Raton, pp 1–453
15. Doman TN, McGovern SL, Witherbee BJ, Kasten TP, Kurumbail R, Stallings WC, Connolly DT, Shoichet BK (2002) J Med Chem 45:2213–2221
16. Bowen JP, Allinger NL (1991) In: Lipkowitz KB, Boyd DB (eds) Reviews in computational chemistry, vol 2. Wiley-VCH, New York, pp 81–97
17. Todebush PM, Bowen JP (2001) In: Reddy MR, Erion MD (eds) Free energy calculations in rational drug design. Plenum Press, New York, pp 37–59
18. Warshel A, Levitt M (1976) J Mol Biol 103:227–249
19. Aqvist J, Warshel A (1993) Chem Rev 93:2523–2544
20. Singh UC, Kollman PA (1986) J Comput Chem 7:718–730
21. Harrison MJ, Burton NA, Hillier IH (1997) J Am Chem Soc 119:12285–12291
22. Rosta E, Klahn M, Warshel A (2006) J Phys Chem B 110:2934–2941
23. Mo Y, Gao J (2006) J Phy Chem B 110:2976–2980
24. Devi-Kesavan LS, Gao J (2003) J Am Chem Soc 125:1532–1540
25. Li G, Cui Q (2003) J Phys Chem B 107:14521–14528
26. Riccardi D, Schaefer P, Yang Y, Yu H, Ghosh N, Prat-Resina X, Konig P, Li G, Xu D, Guo H, Elstner M, Cui Q (2006) J Phys Chem B 110:6458
27. Reddy MR, Singh UC, Erion MD (2004) J Am Chem Soc 126:6224–6225

28. Reddy MR, Singh UC, Erion MD (2007) J Comput Chem 28: 491–494
29. Reddy MR, Erion MD (2007) J Am Chem Soc 129:7296–7297
30. Bash PA, Singh UC, Brown FK, Langridge R, Kollman PA (1987) Science 235:574–575
31. Reddy MR, Viswanadhan VN, Weinstein JN (1991) Proc Natl Acad Sci USA 88:10287–10291
32. Beveridge DL, DiCapua FM (1989) Annu Rev Biophys Biophys Chem 18:431–492
33. Rao BG, Singh UC (1989) J Am Chem Soc 111:3125–3131
34. Mitchell MJ, McCammon JA (1991) J Comput Chem 12:271–275
35. Pearlman DA, Kollman PA (1991) J Chem Phys 94:4532–4545
36. Guarnieri F, Still WC (1994) J Comput Chem 15:1302–1310
37. Pearlman DA (1994) J Comput Chem 15:105–123
38. Chipot C, Millot C, Maigret B, Kollman PA (1994) J Phys Chem 98:11362
39. Chipot C, Kollman PA, Pearlman DA (1996) J Comput Chem 17:1112–1131
40. Reddy MR, Erion MD (1999) J Comput Chem 20:1018–1022
41. Singh UC, Benkovic SJ (1988) Proc Natl Acad Sci USA 85: 9519–9523
42. Singh UC (1988) Proc Natl Acad Sci USA 85:4280–4284
43. Erion MD, van Poelje PD, Reddy MR (2000) J Am Chem Soc 122:6114–6115
44. Erion MD, Dang Q, Reddy MR, Kasibhatla S, Jingwei Huang SJ, Lipscomb WN, van Poelje PD (2007) 133:7296–7297
45. Singh UC, Kollman PA (1984) J Comput Chem 5:129–145
46. Singh UC, Kollman PA (1986) QUEST (Version 1.1). University of California, San Francisco
47. Berendsen HJC, Grigera JR, Straatsma TP (1987) J Phys Chem 91:6269–6271
48. Reddy MR, Berkowitz M (1989) Chem Phys Lett 155:173–176
49. Martin ME, Aguilar MA, Chalmet S, Ruiz-Lopez MF (2002) Chem Phys 284:607–614
50. Verlet L (1967) Phys Rev 159:98–103
51. Ryckaert JP, Ciccotti G, Berendsen HJC (1997) J Comput Phys 23:327–341
52. Hine J, Mookerjee PK (1975) J Org Chem 40:292–298
53. Cabani S, Gianni P, Mollica V, Lepori L (1981) J Solut Chem 10:563–595