

# Free Energy Simulations: Applications to the Study of Liquid Water, Hydrophobic Interactions and Solvent Effects on Conformational Stability\*

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## Abstract

Free energy simulations using the Metropolis Monte Carlo method and the coupling parameter approach with umbrella sampling are described for several problems of interest in structural biochemistry: the liquid water, the hydrophobic interaction of alkyl and phenyl groups in water, and solvent effects on the conformational stability of the alanine dipeptide and the dimethyl phosphate anion in water. Proximity analysis of the results is employed to identify stabilizing factors. Implications of the result with respect to the structural chemistry of proteins and nucleic acids is considered.

## Introduction

With the present generation of digital computers and now supercomputers, systems of molecules representative of the liquid state have become accessible to study by large scale numerical methods based on statistical thermodynamic and molecular dynamic theories of mixtures, and referred to as "computer simulations." Recent years have seen the emergence of computer simulation studies on liquid water, [1], aqueous solutions [2], and the initial extensions of these procedures to the study of biomolecules in solution [3-5]. In principle, calculation of many useful thermodynamic variables and molecular indices of hydration can be obtained from computer simulations on aqueous solutions. However, research methodologies in this field are still very much under development, and much remains to be learned both about and from computer simulation applied to biomolecular systems. One of the most interesting and urgent areas of current study in computer simulation is the determination of free energy.

Statistical thermodynamics tells us that the basic relationship between the configurational energy and the excess free energy  $A$  of a system is via the partition function  $Z$ ,

$$A = \frac{-kT \ln Z}{V^N} = \frac{-kT \ln \langle \exp(-E/kT) \rangle}{V^N} \quad (1)$$

where  $k$  is the Boltzmann's constant,  $T$  is temperature, and the brackets denote a Boltzmann configurational average or expectation value of the enclosed quantity. For the free energy difference between two different systems with potentials  $E_0$  and  $E_1$ , Eq. (1) gives

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$$\Delta A = -kT \ln \left\langle \exp \left[ \frac{-(E_1 - E_0)}{kT} \right] \right\rangle. \quad (2)$$

Conventional Monte Carlo and molecular dynamic computer simulations, although firmly grounded in Boltzmann statistical mechanics and dynamics, do not involve the computation of a partition function owing to convergence problems. The Metropolis method [6] was in fact cleverly designed to avoid this difficulty in the calculation of mean energy by Markov-chain Monte Carlo simulation. In molecular dynamics, the physical nature of the calculated particle trajectories serves this purpose at least equally well. However, in the absence of a partition function one is unable to compute the free energy directly in a conventional Monte Carlo or molecular dynamics calculation. The determination of the free energy via the expectation value approach using Eq. (2) encounters difficulties of similar origin except when  $E_0$  and  $E_1$  are quite similar [7, 8]. Thus, under ordinary circumstances we lack access in computer simulation to free energy, the fundamental index of thermodynamic stability of the system.

A number of approaches to this problem have been suggested. For several years now we have been working with the "coupling parameter approach," which involves a parameter that carries the system smoothly from a reference state  $\xi = 0$  to the system of interest  $\xi = 1$ . In a modern sense this approach originates in the derivation of an important integral equation in liquid state theory by Kirkwood [56]. The coupling parameter approach used in conjunction with the procedures of umbrella sampling developed more recently by Valleau and coworkers [9, 10] provides a means of calculation of free energy in computer simulations applicable to diverse problems in structural biochemistry. In this account we describe briefly the coupling parameter approach to free energy simulations, and review recent free energy simulation studies carried out in this laboratory on liquid water, prototype systems for the study of hydrophobic interactions, and problems in the area of solvent effects on conformational stability of protein and nucleic acid constituents.

### Theory

In conventional mean energy Monte Carlo or molecular dynamics computer simulations, one treats a system of particles such as the atoms of a protein, the molecules of a liquid, and most recently, both together [11]. In condensed phase problems, the system is usually configured in a cell subject to periodic boundary conditions. The configurational energy of individual complexions of the system and configurational forces on individual molecules are evaluated from analytical energy functions parameterized from experimental data or quantum mechanical calculations. From this point on, computer simulation on molecular systems is fundamentally a numerical integration of the mean energy expression and other ensemble averages on the computer, with the sampling carried out with a probabilistic (Monte Carlo) or deterministic (molecular dynamics) strategy. In a typical study, structural, energetic, and dynamical properties of the system are often of greater interest than mean energy, but the stability of this quantity remains the basic index of convergence in the calculations. The quality of the mean energy is directly dependent upon the quality of intermolecular potentials used in the simulation.

The extension of mean energy simulation to the determination of free energy is best described by considering two states of a chemical system,  $S_0$  and  $S_1$ , which may differ from each other in any conceivable way. Let us define a free energy function  $A(\xi)$ , where  $\xi$  is a coupling parameter that, on the interval from 0 to 1 carries the reference  $S_0$  into the system of interest  $S_1$ . The free energy difference between  $S_0$  and  $S_1$  is then

$$\Delta A = \int_0^1 \left\langle \frac{\partial}{\partial \xi} E(\xi) \right\rangle_{E(\xi)} d\xi \quad (3)$$

where the subscript  $E(\xi)$  denotes a Boltzmann average on the energy function  $E(\xi)$ . The discussion here is carried out based on the Helmholtz free energy and the  $(T, V, N)$  ensemble but can be easily extended to Gibbs free energy and the  $(T, P, N)$  ensemble. The simplest applications of the coupling parameter approach, with  $\xi$  identified with the volume or the inverse temperature of the system, lead to the virial expression for pressure and the van't Hoff equation, respectively, textbook cases in physical chemistry.

The applications of the coupling parameter approach of interest in our current computer simulation studies involve topographical changes in the system. We prefer this to the molecular landscape in the problem under consideration. There are three broad classes of topographical transition coordinates: structural transition coordinates, reaction coordinates, and creation/annihilation coordinates as summarized in Figure 1. The use of the coupling parameter approach on each of these coordinates is illustrated in the applications described below.

Alternative approaches to the free energy problem include simulations in the  $(T, V, \mu)$  ensemble [12, 13], the particle insertion method [14–18], and overlap ratios [19, 20].

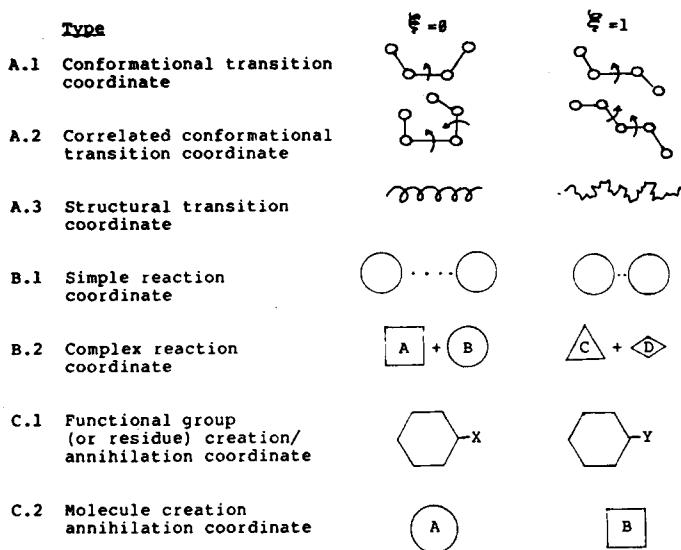


Figure 1. Examples for the various applications of the coupling parameter.

### Liquid Water Studies

For the special case of  $E(\xi)$  linear in  $\xi$ ,

$$E(\xi) = (1 - \xi)E_0 + \xi E_1, \quad (4)$$

$$\Delta A = \int_0^1 \langle E_1 - E_0 \rangle_{E(\xi)} d\xi. \quad (5)$$

Numerical realizations of Eq. (5) involve a series of simulations, carried out developing mean energy as a discrete function of coupling parameter, with the final integration over  $\xi$  carried out numerically.

Several calculations of free energy via Eq. (5), or the equivalent, are now to be found in the literature [21–23] and are reviewed [24]. In this laboratory [25] we carried out a determination of the free energy of liquid water using this approach. Monte Carlo simulations were carried out on a system of 64 water molecules under periodic boundary conditions at a density of 1 gm/cm<sup>3</sup> and a temperature 25°C. The reference state for this calculation was chosen as ideal gas, thus  $E_0$  is zero and Eq. (4) reduces to

$$E(\xi) = \xi E_1, \quad (6)$$

where  $E_1$  is the configurational energy of the assembly of water molecules. The parameter  $\xi$  in this application couples in the intermolecular interactions, and  $\xi$  may thus be considered a creation/annihilation coordinate where only the creation branch is active. In the context of thermodynamic integration,  $\xi$  can be considered as a temperature weighting factor and the  $\xi$  coordinate a transcritical tie-line.

The calculated  $\langle E \rangle_{E(\xi)}$ , denoted  $U(\xi)$ , for liquid water is shown in Figure 2. The free energy was determined by an eight-point Gaussian quadrature over  $\xi$ . The calculated free energy turned out to be  $-4.31 \pm 0.7$  kcal/mol versus an observed value of  $-5.74$  kcal/mol. The discrepancy is due primarily to deficiencies in the intermolecular potential function [1].

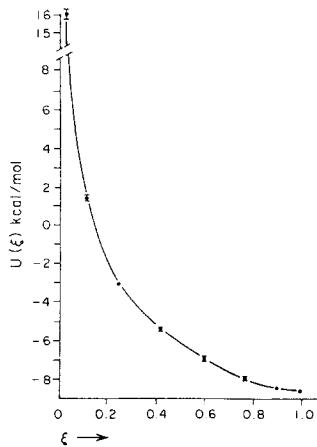


Figure 2.  $U(\xi)$  as a function of the coupling parameter for the computation of the free energy of the MCY water [25].

More recently, Mezei described calculations of the relative free energy of three different water models: MCY, ST2, and SPC, using the coupling parameter approach [26]. A soft sphere reference state was established for calculation of the free energy of the MCY water and then MCY water was used as a reference state for the ST2 and SPC determination. Here the coupling carries the liquid water smoothly from one model to another, an application of  $\xi$  as a creation/annihilation coordinate. Mezei also explored the umbrella sampling method developed by Valleau, Patey, and Torrie [9, 10] in conjunction with Eq. (2) and found it unsuitable for this problem since the two states  $S_0$  and  $S_1$  were too different. This suggested the use of coupling parameter in combination with umbrella sampling to achieve a multistage umbrella sampling where the similitude condition is established by a succession of intermediate states. However, even when three stages were tried, no consistent results could be obtained. Several other applications of multistage sampling and variations are found in the current literature [10, 27, 28].

### *Hydrophobic Interactions*

A different line of approach to free energy simulations involving the coupling parameter, particularly useful but not restricted to conformational changes, is to determine  $A(\xi)$ . However, the  $\xi$  coordinate usually cannot be considered as simply another degree of freedom in an otherwise conventional mean energy simulation. In this case the realization of the system, seeking to describe the equilibrium state dictated by the Boltzmann equation, would end up sampling only a narrow range of  $\xi$  rather than the full interval from 0 to 1. Thus sampling of  $\xi$  requires special, non-Boltzmann sampling techniques beyond those typically used in mean energy simulations.

The current way to proceed on potential of mean force calculations is to apply the umbrella sampling method. In this approach a series of simulations are carried out, each constrained to sample the local region about points  $\xi_1, \xi_2, \xi_3, \dots$ , respectively, on the interval from 0 to 1. In an individual simulation, a distribution function  $g(\xi)$  is obtained. A particular  $\xi$  simulation is constrained to sample a region about  $\xi$  by adding a harmonic constraint to the configurational energy, namely

$$V(\xi) = E(\xi) + E_H(\xi), \quad (7)$$

$$E_H(\xi) = k_H(\xi - \xi_0)^2, \quad (8)$$

as first employed in this form by Pangali, Rao, and Berne [29, 30]. The non-Boltzmann bias in the results due to the presence of the harmonic term can be removed by means of Valleau's equation,

$$g(\xi') = \frac{\langle \delta(\xi' - \xi) \exp[E_H(\xi)/kT] \rangle_{V(\xi)}}{\langle \exp[E_H(\xi)/kT] \rangle_{V(\xi)}} \quad (9)$$

where  $\delta(\xi' - \xi)$  is the dirac delta counting function for configurations with coupling parameter  $\xi$ . Successive points are chosen on  $\xi$  such that  $g(\xi)$  are overlapping. Overlapping points in the distribution correspond in principle to the same absolute value but in practice differ by a normalization constant. Thus the various computed  $g(\xi)$

can be shifted up or down, and ultimately matched up to produce a  $g(\xi)$  for the entire range of  $\xi$ . The matching can in principle be carried out for any overlapping points, but in practice one chooses those points with relatively low statistical noise levels. An alternative procedure [31] where the harmonic potential  $E_H$  is replaced by a tabulated function determined from the simulation itself, is currently under testing. This biasing potential appears to be capable to cover a much larger range of the coupling parameter than  $E_H$ . Once  $g(\xi)$  has been obtained, the corresponding free energy  $A(\xi)$  can be evaluated as

$$A(\xi) = -kT \ln g(\xi) + C, \quad (10)$$

known as the potential of mean force, commonly denoted  $W(R)$ . Details of the methodology are described further by Pangali et al. [29, 30], particularly, the influence of the boundary condition on the results is potentially greater at larger separations. Several potential mean force determinations using computer simulation are now to be found in the recent literature [29, 30, 32, 33]. Umbrella sampling is also used in conjunction with Eq. (2), making it viable for calculations between less similar systems [10, 27, 28, 34, 35]. A notable free energy simulation has recently been reported by Jorgensen and his group, who determined the potential of mean force on the complex reaction coordinate of the organic SN2 reaction of  $\text{CH}_3\text{Cl}$  and  $\text{Cl}^-$  in water [36] and DMF [37]. The reaction is predicted to be concerted in water but to proceed via a reaction intermediated in DMF, a previously unanticipated result.

Potential of mean force calculations from this laboratory have been directed to the study of the interactions of apolar molecules in water, prototype systems for the theoretical study of the hydrophobic effect. Ravishanker, Mezei, and Beveridge reported a potential of mean force for the interaction of two methane molecules in water obtained from Monte Carlo computer simulation [32]. The behavior of  $W(R)$  as a function of intersolute coordinate, as shown in Figure 3, turned out to be oscillatory, with successive free energy minima corresponding to contact and solvent-separated hydrophobic interactions. This intriguing result, anticipated by F. Franks [38–41] and noted in the integral equation studies of the hydrophobic effect by Pratt and Chandler [41] and the simulations on Neon atoms in ST2 water by Pangali et al. [29, 30] indicates that the hydrophobic effect may act over a longer range of distance than previously anticipated via these solvent-mediated structures. The structure of the solvent

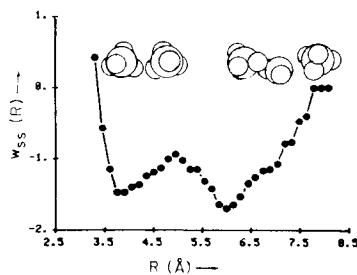


Figure 3. Methane–methane  $W(R)$  computed in Ref. 32 and spacefilling drawings of a randomly chosen contact and solvent-separated structure.

separated forms was studied particularly by Ravishanker et al. [32], who found that the intervening water molecule in the structure is positioned essentially right between the associated methane molecules.

This class of studies has recently been extended to the interaction of phenyl rings in water [57], with the result shown in Figure 4. Here as well an oscillatory character is indicated for the calculated potential of mean force. However, the solvent separated minimum does not in this case involve interstitial water molecules, but rather an impingement. Further details on this phenomenon and the relationship to experimental results and the implications thereof promises to be an interesting area for future research studies.

#### *Solvent Effects on Conformational Stability*

The coupling parameter approach can also be used to calculate conformational free energy of hydration as a function of structural changes in a system, an area of considerable importance in structural biochemistry and biology. The potential of mean torsion for *n*-butane has been studied by Rebertus, Berne, and Chandler [43] and Jorgensen [44]; this sequence of articles has recently been reviewed by Jorgensen [45]. Our studies in this area focus on the conformational preferences of the alanine dipeptide in water and the dimethyl phosphate anion in water. The alanine dipeptide study involved specific structures chosen from among the possibilities suggested in previous theoretical and experimental work:  $C_7$ ,  $\alpha_R$ , and  $P_{II}$  [46, 47]; c.f. Figure 5. Here the various conformations of interest differ in values of the Ramachandran torsion angles  $\psi$  and  $\phi$ . We mapped the structural change involving the conformational coordinates  $\psi$  and  $\phi$  onto a single  $\xi$  by means of the equation

$$(\psi, \phi) = (1 - \xi)(\psi_0, \phi_0) + \xi(\psi_1, \phi_1), \quad (11)$$

where  $\xi = 0$  selects the reference state  $(\psi_0, \phi_0)$  and  $\xi = 1$  selects the state  $(\psi_1, \phi_1)$ ; here is a correlated conformational transition coordinate. To access computationally

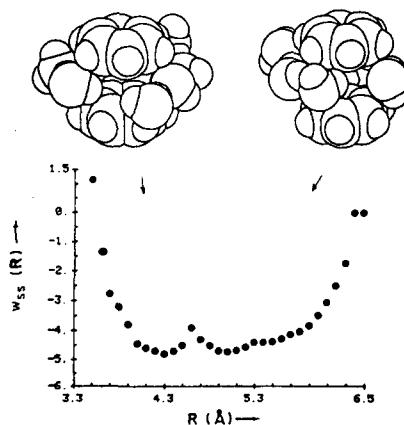


Figure 4. Benzene-benzene  $W(R)$  computed [42] and spacefilling drawings of a randomly chosen contact and solvent-separated structure.

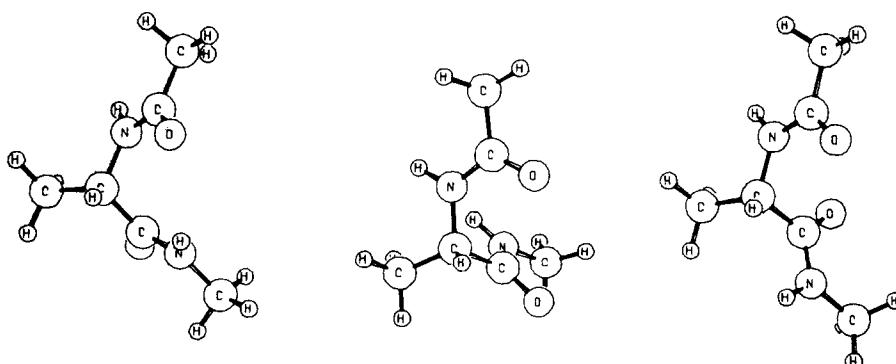


Figure 5. Conformations considered in free energy simulations on the alanine dipeptide:  
(a)  $C_7$ , (b)  $\alpha_R$ , (c)  $P_{11}$  (from left to right).

tractable sampling procedures in computer simulations it is frequently desirable to map changes in the structure involving many internal coordinates onto a single  $\xi$  if possible. In this research, we determined the thermodynamics of hydration for the  $C_7$ ,  $\alpha_R$ , and  $P_{11}$  conformations of the molecule using conventional mean energy simulations and also free energy simulations using the coupling parameter approach. In this study we mapped the two-dimensional  $(\psi, \phi)$  change into one coordinate. One could proceed in a similar manner to map changes in many dimensions onto one  $\xi$ . Since the shape of the free energy minimum is not completely determined, this procedure precludes consideration of the full statistical weight problem, but the free energy difference

$$\Delta A = kT \ln \left( \frac{g(\xi)_{\xi=0}}{g(\xi)_{\xi=1}} \right), \quad (12)$$

is available. Use of Eq. (12) has the advantage over calculating the mean energy differences from two different simulations in that the statistical uncertainties, resulting from taking a small difference of two large quantities, is eliminated. Note that the  $\xi$  used here can be chosen for convenience, since the free energy difference is a state function independent of path. The results indicate that both the  $\alpha_R$  and  $P_{11}$  conformers are stabilized relative to the  $C_7$  form by hydration, and are both thermally accessible at  $T = 25^\circ\text{C}$ . Proximity analysis [48] of the results reveals that the origin of the stabilization lies in the differential hydration of the carbonyl group of the amide linkage in the alanine dipeptide [46]. Experiments based on IR spectroscopic studies reveal the  $C_7$  conformation to be preferred in the nonpolar solvent  $\text{CCl}_4$  [49, 50]. Nuclear magnetic resonance and circular dichroism spectroscopic evidence for the presence of both  $\alpha_R$  and  $P_{11}$  conformations of the alanine dipeptide in water at room temperature has been presented [51], and thus our simulation results appear to be in general accord with the experiment.

The conformational preferences of dimethyl phosphate can be considered in terms of the phosphodiester torsion angles  $\omega$  and  $\omega'$  as  $gg$ ,  $gt$ , and  $tt$  [52] as shown in Fig-

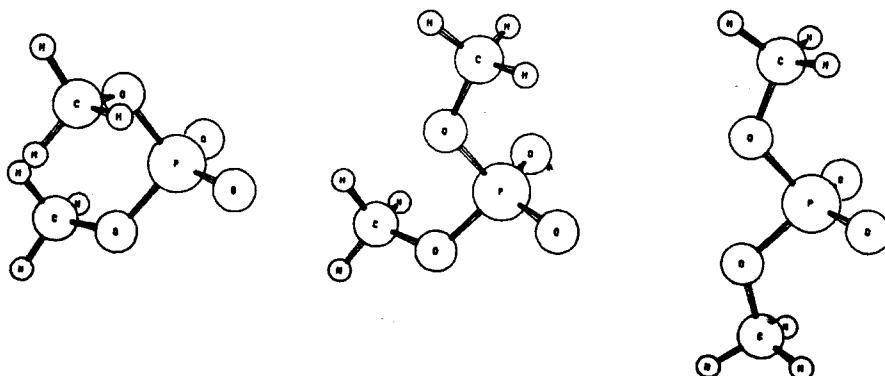


Figure 6. Conformations considered in free energy simulations on the dimethyl phosphate anion *gg*, *gt*, and *tt* (from left to right).

ure 6. The adiabatic potential energy surface for the isolated dimethylphosphate anion is indicated to be relatively flat [53]. Nevertheless oligonucleotides show a marked preference for the *gg* and *gt* forms as shown from crystal hydrate data [54]. We have carried out mean energy simulations on dimethylphosphate anion in 215 water molecules under periodic boundary conditions [55]. The results indicate that the *trans* extended form is markedly destabilized by hydration. Free energy simulations using the coupling parameter approach in a manner analogous to that described for the alanine dipeptide are now underway. Preliminary results indicate that the *trans*-extended form is destabilized with respect to free energy as well as mean energy of hydration and thus suggests a possible explanation of why the *trans* extended form of phosphodiester torsion angles are not found in oligonucleotide crystal hydrates.

### Conclusion

We have described here the coupling parameter approach to free energy simulations provided leading literature references, and reviewed recent studies in the area carried out at Hunter College. The initial results of free energy simulations using the coupling parameter as a topological transition coordinate are generally promising, and we expect that the coupling parameter method will be a useful approach for free energy simulations applied to diverse problems in structural biochemistry. We are currently pursuing free energy simulations applied to the calculation of relative hydration potentials of homologous series of biological molecules, using the coupling parameter as a molecule creation/annihilation coordinate.

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