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REVIEW ARTICLE

Poisson-Boltzmann Solvents in Molecular Dynamics Simulations

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Abstract. Recent years have witnessed significant improvement in implicit solvents based on the Poisson-Boltzmann theory, whether in the forms of numerical solution or analytical approximation. Especially worth noting are the improvements and revisions of those implicit solvents for stable dynamics simulations. Given these technical advancements, attentions are now paid to the quality of implicit solvents as compared with the more expensive explicit solvents. The new developments in nonpolar solvents mentioned above and reviewed elsewhere will also result in more accurate simulations of biomolecules. We have also touched the new challenges facing the implicit solvents. That is how to incorporate these solvents in the emerging polarizable force fields. New challenges could also arise from the assumptions underlying all implicit solvents, as recently explored to couple electrostatic and nonelectrostatic components together. In addition, hybrid solvents could eventually become a reality for dynamics simulation even this has been proposed in the early days of computational biochemistry. It is likely that such hybrid solvents will offer the necessary accuracy, as they no longer average out the very degrees of freedom that are of interest in studies where solute/solvent coupling is crucial.

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Contents

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1 Introduction

Molecular dynamic (MD) simulation is one of the important theoretical methods to investigate the structures, dynamics and kinetics of proteins at the atomic level. To describe the interactions between atoms, MD simulations usually adopt a relatively simple potential energy function (U), as follows

$$U=\sum_{\text{bonds}} k_b(b-b_0)^2+\sum_{\text{angles}} k_\theta(\theta-\theta_0)^2+\sum_{\text{torsions}} k_\phi[\cos(n\varphi+\delta)+1] + \sum_{\substack{\text{atom}\\ \text{pairs}}}\left[\frac{Q_iQ_j}{r_{ij}}+\frac{A_{ij}}{r_{ij}^{12}}-\frac{B_{ij}}{r_{ij}^{6}}\right]. \tag{1.1}$$

The first three summations are over deviations of bonds (b) from their equilibrium values (b_0), deviations of angles (θ) from their equilibrium values (θ_0), and rotatable bonds (torsion angles φ with phase n and offset δ). The final summation is over pairs of atoms i and j with charges Q_i and Q_j separated by distance r_{ij}. It describes electrostatic interactions that are represented by a Coulombic potential, and dispersion and exchange repulsion interactions that are represented by a Lennard-Jones 6-12 potential. The parameters in Eq. (1.1) along with the function form of Eq.(1.1) are called force field. Many force fields have been developed for biomolecular simulations, such as Amber [1–6], CHARMM [7,8], and OPLS [9–11]. Use of the potential energy function in Eq. (1.1) allows a rather efficient numerical procedure to be developed to solve the Newtonian equation of motion

$$\frac{d^2\mathbf{r}}{dt^2}=-\nabla U. \tag{1.2}$$

The overwhelming adoption of molecular dynamics in molecular biophysics can be contributed to the often stringent requirement that an atomic-detailed description of biomolecules must be used to elucidate their structures and functions. However even with such a simple functional form of Eq. (1.1), many fundamental biomolecular processes remain largely inaccessible to molecular dynamics simulations when relevant timescales reach microseconds and system sizes exceed more than a few hundred residues. The computational inaccessibility partially comes from the requirement for an accurate description of the aqueous environment that is essential for atomistic biomolecular simulations. To fulfill the requirement even for a medium-sized biomolecule requires thousands of discrete water molecules to be placed around it. The computational cost for

simulating these "extra" thousands of water molecules far exceeds that for simulating the biomolecule alone.

Exploration for alternative treatments of solvation by "implicit or continuum water" balancing simplicity that permits fast calculations without loss of atomic-detailed description of biomolecules has been a constant theme in molecular biophysics throughout most of its still short history. In developing such implicit water models, or implicit solvents to be more general, it is natural to enforce that any implicit solvent approximation retains the proper structural distributions of biomolecules as much as possible from those of the same biomolecules solvated in explicit solvents. This requirement leads to the following formulation of implicit solvents.

To start, we note that Eq. (1.1) is pairwise additive, i.e., it can be decomposed into a summation of terms involving a pair of atoms only. Thus the potential energy of a solvated biomolecular system can always be written as

$$U(\mathbf{r}_u,\mathbf{r}_v)=U(\mathbf{r}_u)+U(\mathbf{r}_v)+U(\mathbf{r}_u:\mathbf{r}_v) \tag{1.3}$$

with $\mathbf{r}_u$ and $\mathbf{r}_v$ representing the degrees of freedom of the solute (biomolecule) and solvent (water) molecules, respectively, and $U(\mathbf{r}_u:\mathbf{r}_v)$ representing solute/solvent coupled interaction potential. In an *NVT* ensemble (with fixed number of atoms for both biomolecule and water molecules at constant volume and temperature), the probability distribution for the solvated system at $(\mathbf{r}_u,\mathbf{r}_v)$ is

$$P(\mathbf{r}_u,\mathbf{r}_v)=\frac{\exp[-\beta U(\mathbf{r}_u,\mathbf{r}_v)]}{\int d\mathbf{r}_u d\mathbf{r}_v \exp[-\beta U(\mathbf{r}_u,\mathbf{r}_v)]}. \tag{1.4}$$

In simulations with implicit solvents, we are not interested in the solvent degrees of freedom, $\mathbf{r}_v$. Therefore only the reduced probability distribution for the solute at $\mathbf{r}_u$ is of interest. Apparently

$$P(\mathbf{r}_u)=\int d\mathbf{r}_v P(\mathbf{r}_u,\mathbf{r}_v).$$

This gives

$$P(\mathbf{r}_u)=\frac{\exp[-\beta W(\mathbf{r}_u)]}{\int d\mathbf{r}_u \exp[-\beta W(\mathbf{r}_u)]}, \tag{1.5}$$

where

$$\exp[-\beta W(\mathbf{r}_u)]\equiv\int d\mathbf{r}_v \exp[-\beta U(\mathbf{r}_u,\mathbf{r}_v)].$$

Here $W(\mathbf{r}_u)$ is defined as a potential of mean force, or reversible work, of a given solute configuration $\mathbf{r}_u$. It can be shown that the gradient of $W(\mathbf{r}_u)$ with respect to a solute atomic coordinate r_i is related to its mean force component F_i:

$$\frac{\partial W(\mathbf{r}_u)}{\partial r_i}=\left\langle\frac{\partial U(\mathbf{r}_u)}{\partial r_i}\right\rangle=\langle F_i\rangle. \tag{1.6}$$

$W(\mathbf{r}_u)$ can be decomposed into two parts, $U(\mathbf{r}_u)$, and a solvent-induced term $W_s(\mathbf{r}_u)$:

$$\begin{aligned} W(\mathbf{r}_u) &= -kT\ln\int d\mathbf{r}_v \exp[-\beta U(\mathbf{r}_u,\mathbf{r}_v)] \\ &= U(\mathbf{r}_u)+W_s(\mathbf{r}_u), \end{aligned} \tag{1.7}$$

with

$$W_s(\mathbf{r}_u) = -kT\int d\mathbf{r}_v \exp[-\beta U(\mathbf{r}_v,\mathbf{r}_u:\mathbf{r}_v)], \tag{1.8}$$

since the term involving $U(\mathbf{r}_u)$ is independent of $\mathbf{r}_v$ and can be moved out of the integral.

By definition, $W(\mathbf{r}_u)$, is the reversible work to assemble solute atoms into their final configuration $\mathbf{r}_u$ in the presence of solvent molecules. Most modern implicit solvation schemes compute $W(\mathbf{r}_u)$ by separating the assembly process into two steps: (1) switching on non-electrostatic interactions of the solute atoms, and (2) switching on electrostatic interactions by charging up solute atomic charges.

In step (1), the assembly reversible work takes into account the solute covalent terms $(U(\mathbf{r}_p)^{\text{cov}})$, i.e. the bond, angle and torsion angle terms in Eq. (1.1), the solute van der Waals term $(U(\mathbf{r}_p)^{\text{vdw}})$ in Eq. (1.1), and the non-electrostatic solvation reversible work $W_s(\mathbf{r}_p)^{\text{nes}}$. Thus, the reversible work of this step can be expressed as

$$W(\mathbf{r}_u)^1 = U(\mathbf{r}_u)^{\text{cov}}+U(\mathbf{r}_u)^{\text{vdw}}+W_s(\mathbf{r}_u)^{\text{nes}}. \tag{1.9}$$

In principle, the non-electrostatic solvation reversible work can be modeled as a repulsive cavity component and an attractive dispersion component, as will be reviewed in Section 5.

In step (2), the charging process wraps both the solute Coulombic contribution in Eq. (1.1), $U(\mathbf{r}_p)^{\text{es}}$, and the solvent-induced contribution, $W_s(\mathbf{r}_p)^{\text{es}}$, into the charging reversible work:

$$W(\mathbf{r}_u)^2 = U(\mathbf{r}_u)^{\text{es}}+W_s(\mathbf{r}_u)^{\text{es}}. \tag{1.10}$$

The reversible work of charging the solute, $W(\mathbf{r}_u)^2$, may be computed by solving the Poisson equation

$$\nabla\cdot\varepsilon(\mathbf{r})\nabla\phi(\mathbf{r}) = -\rho(\mathbf{r}), \tag{1.11}$$

where ε is the dielectric constant, ϕ is the electrostatic potential, and ρ is the charge density, i.e. all atomic charges within the solute. All three variables are functions of position vector $\mathbf{r}$. A dissolved electrolyte may be accommodated by the use of the Poisson-Boltzmann equation instead of the Poisson equation

$$\nabla\cdot\varepsilon(\mathbf{r})\nabla\phi(\mathbf{r}) = -\rho(\mathbf{r})-\sum n_i^0 q_i \exp[-\beta q_i\phi(\mathbf{r})], \tag{1.12}$$

where n_i^0 is the number density of counterions of type i in the bulk solution, q_i is the charge of the counterions of type i, and $\beta = 1/kT$. Here k is the Boltzmann constant, and T is the temperature. When the electrostatic field is weak, Eq. (1.12) can be linearized as

$$\nabla\cdot\varepsilon(\mathbf{r})\nabla\phi(\mathbf{r}) = -\rho(\mathbf{r})+\sum\beta n_i^0 q_i^2\phi(\mathbf{r}). \tag{1.13}$$

Once the electrostatic and nonelectrostatic reversible works and their derivatives on all atoms are solved, they can be used in Eq. (1.2) to propagate the solvated biomolecular system. When the Langevin heat bath is used, the solute atoms distribution will follow a probability distribution as in Eq. (1.5).

Implicit solvents apparently have several advantages over explicit solvents. For example, in implicit solvent simulations there is no need for lengthy pre-equilibration of systems, mostly the artificially placed water molecules. Artifacts due to periodic boundary conditions are not of concern because implicit solvation corresponds to the infinite dilute solution. Implicit solvent simulations generally give improved sampling due to the absence or reduction of solvent viscosity. Of course, all these benefits of implicit solvents come at a price of making a dramatic simplification of discrete solvent structures. For example, solute-solvent hydrogen bonds are no longer explicitly present; instead, they come in implicitly and contribute to the overall solvation free energy.

Due to these advantages, many efforts have been made to develop or improve various implicit solvents. A class of implicit solvents based on the Poisson-Boltzmann (PB) equation has become widely accepted [12, 13] and has been widely used in free energy calculations [14–16], dynamic simulations [17–19], pKa evaluations [20–23], and constant pH simulations [24–26]. This review focuses on this class of implicit solvents, especially on its applications in dynamics simulations. Thus, it is far from exhaustive. The readers are referred to other recent review articles on solvation for more general reviews [12,13,27–37].

2 Adaptation of Poisson-Boltzmann solvents to dynamics simulations of biomolecules

Adaptation of PB solvents to dynamic simulations requires numerical solution of the 3-D partial differential equation. However, the numerical procedure has been a bottleneck, largely limiting their application to calculations with static structures only. The difficulty lies in the numerical procedure to apply such solvents, which involves discretization of the partial differential equation into a system of linear equations that tends to be rather large: it is not uncommon to have a million unknowns in biochemical applications. In addition, the setup of the linear system before the numerical solution and post-processing to obtain energies and forces are both nontrivial. The most commonly used finite-difference (FD) approach was first introduced into biochemical studies in the early 1980s [38–40]. The programs Delphi [41], UHBD [42], Grasp [43] and others have greatly assisted its adoption by the biochemistry community. The boundary element approach has also been used for molecular mechanics [44–47], though less widely than the finite-difference approach. One advantage in applying the boundary element approach lies in the fact that the dimension of the linear system is much smaller than that from the finite difference scheme due to the different dimensionality (3-D v.s. 2-D). However, the corresponding linear system is much denser, making it more difficult to solve. The finite-element approach was also introduced into chemistry, with the idea that it could

reduce the number of grids when compared with the uniform finite-difference grid with the same grid resolution near solute atoms [48–51]. However, the regular pattern in the matrix from finite-difference discretization is lost, making it harder to write a highly efficient solver. Recently, multi-grid approaches have been introduced into the field of biochemistry to speed up the convergence of various solvers [50–52]. Their performance gain is especially useful for very large systems [53].

Due to the computational expense for solving the PB equation numerically, considerable efforts have been invested in approximating the solution of the PB equation, via methods such as the semi-analytical generalized Born (GB) model [54], the induced multipole model [55], the dielectric screening model [56, 57], and others. The pair-wise GB model, in particular, has been widely accepted as an efficient estimation of the solution of the PB equation as recently reviewed [29, 31–37].

The earliest attempts to use implicit solvents based on the PB theory in dynamic simulations date back to as early as the 1990s when Davis *et al.* [58], Zauhar [59], Sharp [60], Luty *et al.* [61], and Gilson *et al.* [62, 63] contributed to adapting numerical PB solvents for dynamic simulations. However, those successful attempts could only apply such solvents in simulations to small organic molecules, such as dipeptides and tetra-peptides, since the cost of using implicit solvents on macromolecules was prohibitively high. In fact, the per-step simulation cost is higher with the finite difference approach than that with explicit water even at the 1 Å grid spacing, though it can be argued that the solvent is always equilibrated in implicit solvents, whereas it takes a long time to achieve equilibration in explicit water simulations. Its inefficiency sharply limits the practical applications of implicit solvents in routine dynamics simulations of macromolecules.

Recently, there has been renewed interest in finding ways to apply implicit solvents, both numerical and semi-analytical GB approaches, in dynamic simulations [18, 19, 33, 50, 51, 57, 64–76]. Whether numerical procedures or semi-analytical GB procedures are used to solve the PB equation, the common continuum approximation of electrostatic solvation as dielectric response may pose computational challenges in the applications to biomolecules especially in dynamics simulations.

2.1 Simulation stability and dielectric models

The first challenge is to achieve computational stability in dynamics simulations [18]. In part, the stability in dynamics simulations is determined by the solute dielectric model that has to be smooth both over time and over space. This is the case whether numerical or semi-analytical methods are used [18, 77].

Typically, implicit solvents use a solute molecular surface definition for dielectric assignment. The solvent excluded surface is the most used definition. However, setting up the dielectric map with this surface can be time-consuming [78], though recent developments have dramatically improved its efficiency [41]. In addition, testing of this surface definition in molecular dynamics indicates that it is numerically unstable [18, 77]. In fact, none of the native proteins that were tested is stable with this surface definition [18]. This

is because dynamic variables need to be sufficiently smooth over time for dynamics simulations to be stable. Otherwise, it is difficult to use a reasonable time step, such as 1-2 femtosecond at room temperature. The limitation of the solvent excluded surface is in the reentry portion, as recently analyzed [18]. It is found that in simulations of proteins at room temperature, large reentry volume generated by non-bonded atoms comes and goes as often as every femtosecond when the nearby atoms undergo vibrational motion, resulting in rapidly fluctuating forces [18].

The van der Waals surface, or the hard sphere surface, represents the low-dielectric molecular interior as the union of the atomic van der Waals volumes. This definition is both efficient and smooth over time, i.e. the surface changes rather smoothly in dynamics simulations at room temperature. However, there are too many nonphysical high (solvent) dielectric pockets inside the solute interior in the van der Waals definition, as shown in Fig. 6 of Ref. [79]. These small buried "solvent pockets" result in electrostatic field changing rapidly over space, causing dynamics to be unstable. It has been pointed out that van der Waals surface has an additional artifact of assigning the apparent protein interior dielectric constant to a much higher value, as in the pKa calculations which may or may not be wanted. A more controlled approach on the interior dielectrics is definitely needed.

The Gaussian(-like) density approach has recently been used in the definition of dielectric boundary for implicit solvents [65, 80, 81]. In this type of approach, a distance-dependent density/volume exclusion function is used to define each atomic volume. This is in contrast to the hard sphere definition of atomic volume as in the van der Waals or the solvent excluded surface. The trailing tail outside the cavity radii can be used to smooth out the small cracks and crevices formed by neighboring atoms. This definition is smooth over both time and space. However, the cost of computing the volume exclusion/density function at every grid edge is a major concern when this approach is applied to numerical solvers. In addition, its ability to smooth out the reentry region depends on the length of the trailing tail outside the cavity radii. A long tail makes the surface smoother, but it also makes atomic cavities appear larger, changing the underlining physics of the solvent model. On the other hand, a short tail does not properly cover the reentry region effectively.

Considering these limitations, the modified van der Waals surface was proposed [18]. This surface definition proceeds by first computing the solvent accessible surface of the solute. All solute atoms are then classified as solvent exposed, i.e. with non-zero solvent accessible surface area, or as buried, i.e. with zero solvent accessible surface area. For the exposed atoms, the atomic cavity radii are used directly; for buried atoms, its cavity radii are increased by the solvent probe radius (termed as modified radii). In the second step, the standard van der Waals surface is generated with these modified radii. The harmonic dielectric smoothing [82] is applied at this step to smooth the dielectric transition between solvent and solute. After step two, the dielectric distribution within and around buried atoms is very smooth, i.e. it is all part of the solute low dielectric. However, the dielectric distribution around exposed atoms can still show spatial fluctu-

ation. Thus, the third step is used to smooth out the spatial fluctuation around exposed atoms. In the third step, all dielectric edges that are within a rectangular box with its surfaces one solvent-probe-diameter away from the solute surface are checked. This step is designed to find any buried high dielectric edges and/or intermediate dielectric edges (due to the harmonic smoothing of dielectrics) within the solute interior. If these edges are buried, their dielectric values are changed to the solute dielectric value, i.e. they are changed to low dielectric edges [18]. A different strategy is also possible with the GBMV method, which was originally designed to reproduce solvent excluded surface. Interestingly, the sharp interface between solvent and solute in this method can be made softer by adjusting the β parameter to make more stable dynamics possible [77].

In these attempts to achieve stable dynamics simulations, attention should also be paid to the quality of the solvents. It has been pointed out that solvent excluded surface probably gives the best agreement with explicit solvents and experiment [41,83,84]. Thus, revision of dielectric models for implicit solvents has to balance both dynamic stability and quality [77]. The later point is addressed in Section 3.

2.2 Is discrete water structure important?

The second challenge, more of a nature in the limitation of continuum approximation of solvent dielectric response, is the lack of discrete solvent structure. Experiments and simulations have revealed that buried and bound water molecules may contribute to protein structure, dynamics and function [85–89]. Without explicit water representation, implicit solvent can not study the effect of these water molecules. A recent work reported that the unique water structure in the second solvation shell, not so much by that of the first shell, results in the so-called "water-bridging" phenomenon [90]. This effect causes the existence of second minimum in the potential of mean force in hydrogen-bonded or salt-bridged two-body systems. The second free energy minimum cannot be reproduced by implicit solvents though overall potential of mean force can be obtained [90].

What is troublesome is the observation of the water-bridging effect in energetics of secondary structures: the deviations of implicit solvents from explicit solvents are mostly smaller in anti-parallel conformations than in parallel conformations [90]. Further evidences are from experimentally observed structural water molecules in crystal structures. Many hydrogen-bonded water molecules are observed to be at the water bridging distance, indicating that water bridged minima are very common in biomolecules [91]. Nevertheless, more direct dynamics simulations of biomolecules, especially short peptides that can demonstrate the role of water-bridging effect, are still needed to elucidate its influences in structural and energetic properties of biomolecules.

It is possible to use a complex position-dependent dielectric constant near solute to partially alleviate the above deficiency because the local water density due to the water-bridging effect essentially produces a different dielectric response from that in the bulk water. It is also possible to develop more complex solvation treatments to incorporate a few explicit solvent molecules to overcome the limitations observed here, for example, as

suggest by Yu *et al.* [92], or by hybrid explicit/implicit solvents as recently reviewed by Okur and Simmerling [93]. Both directions would increase the computational complexity and cost of the implicit solvents.

A similar question is whether explicit representation of ions is important. In PB solvents, ions are implicitly treated in the PB equation. Thus the correlation between ions and the solute or solvents can not be studied. Recently, Sharp *et al.* presented a hybrid method, which represents ions explicitly but still leaves solvents implicit, to overcome this question successfully [94]. An additional advantage of their method is that highly charged solutes can be treated as neutral so that it is less challenging to maintain their structural stability in dynamics simulations.

3 The force field aspect of Poisson-Boltzmann solvents

A major advantage of implicit solvents over explicit solvents is their high computational efficiency. However, it is worth asking whether the quality of the implicit solvents is comparable to that of widely used explicit solvents. Ideally, we should directly compare implicit solvent simulations with experimental observables. However, such comparisons are often limited by several factors: the inability to generate convergent ensembles of conformations; the coupling between electrostatic solvation and nonelectrostatic solvation treatments so that it is hard to interpret the disagreement with experiment if any; finally the limited accuracy in the classic molecular mechanics force fields again makes it hard to interpret the disagreement with experiment if any. Thus a more straightforward question to ask is how well implicit solvents agree with explicit solvents under identical simulation conditions.

Doubts in implicit solvents based on the Poisson-Boltzmann theory were raised when these implicit solvents were compared with explicit solvents in protein folding simulations of peptides [95, 96], though it was later reported that adjustment of atomic cavity radii was found to be able to alleviate many of the previously observed deficiencies [97, 98]. Thus a set of more careful comparisons are still needed. In these comparisons, the first issue that has to be paid attention to is atomic cavity radii as discussed in Section 3.1. Once the atomic cavity radii are determined, quantitative comparisons can be performed as discussed in Section 3.2.

3.1 Atomic cavity radii

A common limitation in many previous investigations of implicit solvents is the lack of optimized atomic cavity radii for tested implicit solvents. In these studies, van der Waals radii in a force field were directly used to define the solute cavity boundary without optimization. It is well known that PB reaction field energies depend sensitively on the solute cavity boundary, where the solvent induced surface charge density is located. As mentioned above, the solute cavity is defined by solute atomic centers, atomic cavity radii,

and solvent probe, so that different reaction field energies computed with different cavity radii may have different performances. Thus, a set of accurate cavity radii is the basis for quantitative analysis. To date, several sets of systemically optimized cavity radii have been presented [99–102]. In Sitkoff *et al.* [99], atomic charges and radii (PARSE) were obtained by modifying existing force-field or quantum-mechanically-derived values, by fitting to experimental solvation energies of small organic molecules. Roux and coworkers [100, 101] presented their optimized cavity radii, based on the molecular dynamics free energy simulations in explicit solvents.

An important issue that has to be addressed is the transferability of optimized cavity radii from small molecules (usually in the training set) to large molecules (usually biomolecules out of the training set). The cavity radii are usually optimized based on the studies of small molecules. It is not guaranteed that they work well when applied to large and complex biomolecules. However, transferability tests, i.e. tests of cavity radii with biomolecules outside the training set, were only mentioned in Swanson *et al.* [102]. In their study, Swanson *et al.* presented optimized cavity radii for amino acid templates in the Amber force fields. Then the cavity radii were tested on four peptides. Electrostatic solvation free energies and forces calculated with both optimized and non-optimized cavity radii were compared with those in the TIP3P explicit solvent, and a higher accuracy was observed when the optimized cavity radii were used. In the study of Tan *et al.*, a good transferability of empirically optimized cavity radii from small training molecules to large testing molecules was observed [90]. It is found that transferability of cavity radii cannot be taken for granted, as shown for two sets of radii with the NMA dimer as a test case [90]. However, deviations between the tested implicit and explicit solvents are also apparent in hydrogen-bonding and salt-bridging dimers [90].

3.2 Quality of Poisson-Boltzmann solvents

Many comparisons between implicit and explicit solvents were made in the past. In the study of Jeancharles *et al.* [103], electrostatic solvation free energies of twenty small molecules calculated by the finite-difference Poisson-Boltzmann method were compared to those calculated by the free energy perturbation method in the TIP4P explicit solvents. In the study of Marrone *et al.* [104], solvent forces for alanine dipeptide computed with both implicit and explicit solvents were compared. Recently, a comparison of atomic solvation forces was also reported with the hope to apply the implicit solvents in molecular dynamics simulations [105]. Lee and Olson used a hybrid explicit/implicit solvents to evaluate the accuracy of several Poisson-based implicit solvents [83]. They showed that, among various dielectric boundary definitions, the solvent excluded surface has the best agreement with hybrid solvent results. Furthermore, certain modifications of cavity radii and water probe radius of the molecular surface provide varied results [83]. Whether an agreement or a disagreement was observed, a limitation in many previous comparisons was the lack of optimized atomic cavity radii for tested PB solvents.

With optimized cavity radii, Tan *et al.* studied the quality of a PB solvent in hydrogen-

bonding/salt-bridging dimers and peptides of different conformations and different lengths with respect to the TIP3P explicit solvent in the PME treatment of electrostatics [106]. They found an overall agreement between the implicit and explicit solvents for the tested systems [90]. Interestingly, the same mechanism underlining the discrepancy in hydrogen-bonding dimers is also responsible for the larger deviations of certain peptide conformations, such as parallel β-strand dimers [90]. Chocholousova and Feig investigated the performance of the GBMV methods [71,107] in the conformational sampling of DNA with two sets of cavity radii, the one is the set optimized by Roux and co-workers [101, 108], the other is the set of van der Waals radii in the CHARMM force field. They observed differences in conformational sampling of DNA with the two different sets of cavity radii. Their results suggest that depending on the choice of cavity radii the agreement is either closer to experimental data or to explicit solvent simulations [76].

4 Coupling between Poisson-Boltzmann solvents and polarizable force fields

One research area not reviewed above is the applications of implicit solvents with polarizable force fields. It is widely known that electronic polarization plays an important role in many fundamental biochemical processes. As we approach biochemical time scales in simulations, longer time scales lead to large-scale conformational changes and substantial variations in dielectric environment that require proper treatment. Otherwise, it is difficult to represent accurately the energy landscapes and thus the dynamics of interested biomolecules. The advent of polarizable force fields, such as AMBER ff02 force field [109], was timely in addressing these issues. While we anticipate that additive model will continue to play important roles, polarizable force fields are expected to extend our ability to study more complex biochemical processes.

Towards the goal of including polarization, a great deal of efforts has been invested on developing explicit polarizable force fields. A variety of methods have been explored, including the fluctuating charge models in the polarizable OPLS force field [110]; the induced dipole models in the polarizable Amber force field [109]; the detailed multi-pole expansions and more complicated molecular mechanics potentials in the AMOEBA force field [111]; the fluctuating charge and Drude oscillator models in the CHARMM force field [112–114].

There is no conceptual difficulty in applying implicit solvents in the explicit polarizable force fields though detailed procedures have to be worked out. Recently Maple *et al.* reported their success to incorporate a GB solvent into an explicit polarizable force field [115]. Schneiders *et al.* also combined a PB solvent and multi-pole technology in polarizable AMOEBA force field successfully [116].

Given the continuum approximation of solvation polarization by implicit solvents, it is also worth exploring a continuum approximation of electronic polarization [117]. Indeed, continuum treatment of electronic polarization has been often used in many cal-

culations related to solvation free energies [99,118]. Typical implicit solvents, such as numerical Poisson-Boltzmann approaches [12,13] and semi-analytical generalized Born approaches [119–121], have been developed to deal with non-vacuum solute interior. However, a molecular mechanics force field that is designed to be consistent with such a treatment of electronic polarization is yet to be developed. Indeed, it is still unclear whether a continuum treatment of electronic polarization is accurate enough for biomolecular simulation purposes.

Interestingly, a recent study [117] has shown that a continuum dipole moment density can be used to treat electronic polarization for molecular mechanics simulations in implicit solvents with accuracy comparable to an explicit polarizable model [109]. Nevertheless, it should be pointed out that the continuum polarization scheme cannot describe the atomic-detailed polarization within a molecular environment. However, it does give us an efficient and self-consistent approach in treating polar interactions in biomolecular simulations more satisfactory than existing additive force fields with implicit solvents [117].

5 Nonpolar component in Poisson-Boltzmann solvents

Successful application of any implicit solvents in molecular mechanics also requires careful treatment of nonpolar/nonelectrostatic solvation. In the widely used surface area (SA) model, the nonpolar solvation free energy is estimated from the solvent accessible surface area (SAS) of the molecule with a uniform surface tension coefficient:

$$W_{nes} = \gamma \cdot SAS + c, \tag{5.1}$$

where the surface tension coefficient γ represents the contribution to the nonpolar solvation free energy (W_{nes}) per unit surface area. The constant offset is the solvation free energy for a point solute ($SAS = 0$). The use of a single γ is based on the observation that for linear alkanes, solvation free energy approximately increase linearly with SAS [99, 122–124]. However, the correlation has been found to be poor for more general organic molecules (see Fig. 1). In addition, Levy and coworkers [125, 126] and Luo and coworkers [98] have pointed out that inclusion of the SA model tends to reduce the agreement between simulation and experiment in their tested systems.

It has been recognized that in order to obtain a more accurate nonpolar solvation treatment, W_{nes} should be further decomposed into two terms: W_{rep}, repulsive free energy, and W_{att}, attractive free energy [70, 74, 125, 127–131]

$$W_{nes} = W_{rep} + W_{att}, \tag{5.2}$$

where W_{rep} is the solvation free energy from the solute-solvent repulsive interactions and the formation of solute cavity (the excluded volume effect). W_{att} is the free energy for establishing the solute-solvent attractive interactions, but may also include solvent-solvent reorganization component. When decomposed in this way, W_{rep} was found to

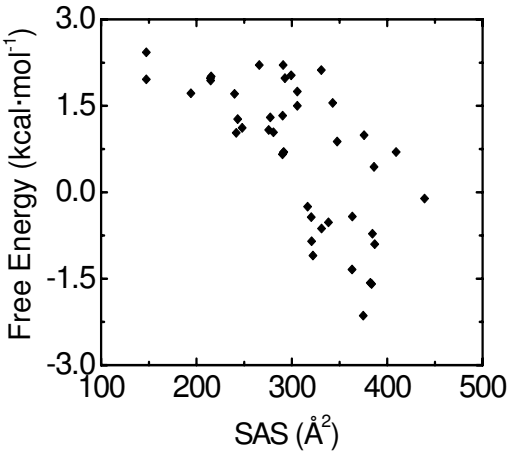

Figure 1: Correlation between SAS and nonpolar solvation free energies of 42 small molecule templates from the Amber force field database [90, 143]. Note that the correlation is negative between the two variables.

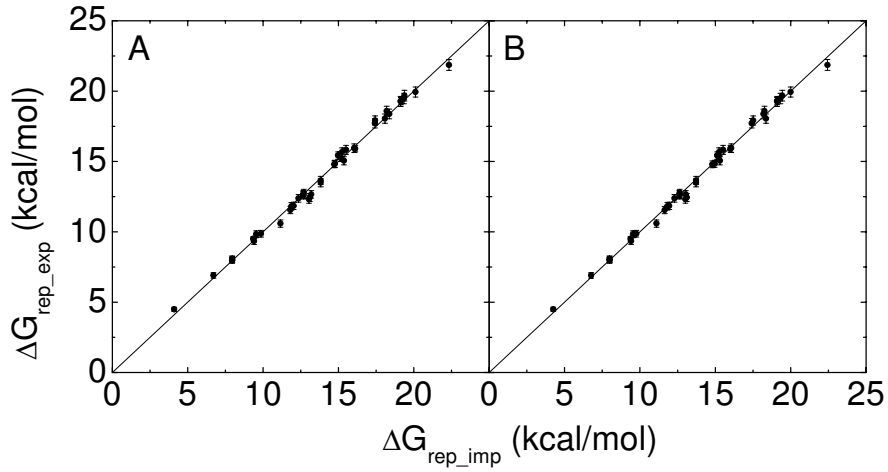

Figure 2: Correlation between SAS or SAV and nonpolar repulsion free energies of the 42 small molecules from the Amber force field database [143, 90]. (A) SAS. Correlation Coefficient: 0.997. RMS Deviation: 0.30kcal/mol. RMS Relative Deviation: 0.026. (B) SAV. Correlation Coefficient: 0.998. RMS Deviation: 0.27kcal/mol. RMS Relative Deviation: 0.022.

have a very good correlation with SAS [132]

$$W_{rep} \approx \gamma \cdot SAS + c. \tag{5.3}$$

Use of molecular volumes (SAV, solvent accessible volume) has also been proposed to correlate with W_{rep} [133–136]. It has been found that correlation with W_{rep} is excellent whether SAS or SAV is used [137] (see Fig. 2).

Efficient computation of W_{att} then becomes a new challenge. Fortunately, according

to Chandler and co-workers [138, 139] and subsequently confirmed in simulations by Levy and co-workers [132], W_{att} can be approximated by the van der Waals attractive interaction potential energy between solute (a) and solvent (w).

$$W_{att} \approx < U_{att,aw} > . \tag{5.4}$$

Based on this approximation, the solute-solvent van der Waals interaction energy can be analytically expressed as the following volume integral

$$U_{att} = \sum_{n=1}^{N_s} \int \rho_{aw}(\mathbf{r}_{aw}) V_{att}(\mathbf{r}_{aw}) d\mathbf{r}_{aw}. \tag{5.5}$$

Here the sum is over all solute atoms (N_s) and the integration is over the solvent occupied volume. $\rho_{aw}(\mathbf{r}_{aw})$ is a solvent distribution function around solute atom a at a given solute-solvent distance $\mathbf{r}_{aw}$. $V_{att}(\mathbf{r}_{aw})$ is the attractive van der Waals potential in a decomposition scheme, for example the WCA scheme [127, 140] and the σ scheme [137]. In implicit solvents, Eq. (5.5) has to be further approximated because $\rho_{aw}(\mathbf{r}_{aw})$ cannot be known *a priori* without equilibrium simulations in explicit solvents. As a first approximation, a uniform distribution (i.e. constant density) can be used. It was reported that the nonpolar solvent can reproduce solvation free energies in explicit solvents very well for monomer systems. But for dimer systems, neither the association energetics nor the nonpolar free energy differences between fully separated conformations and complex conformations can be reproduced consistently.

6 Conclusions

Recent years have witnessed significant improvement in implicit solvents based on the PB theory, whether in the numerical approaches or in the semi-analytical GB approaches. Especially worth noting are the improvements and revisions of those implicit solvents for stable dynamics simulations. Given these technical advancements, attentions have also been paid to the quality of implicit solvents as compared with the more expensive explicit solvents. The new developments in nonpolar solvents mentioned above and reviewed elsewhere will also result in more accurate simulations of biomolecules. We have also touched the new challenges facing the implicit solvents. That is how to incorporate these solvents in the emerging polarizable force fields. New challenges could also arise from the assumptions underlying all implicit solvents, as recently explored to couple electrostatic and nonelectrostatic components together [141, 142]. In addition, hybrid solvents could also become a reality for dynamics simulation even this has been proposed in the early days of computational chemistry. It is likely that such hybrid solvents will offer the necessary accuracy, as they no longer average out the very degrees of freedom that are of interest in studies where solute/solvent coupling is crucial.

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