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Some Practical Approaches to Treating Electrostatic Polarization of Proteins

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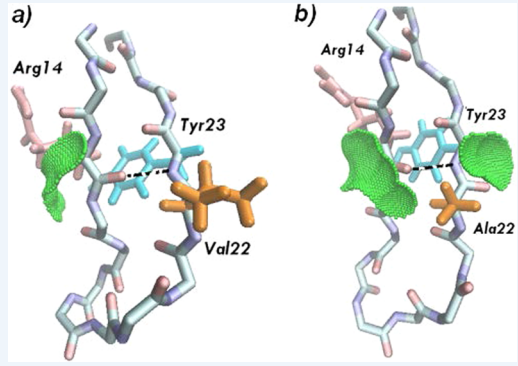
CONSPECTUS: Electrostatic interaction plays a significant role in determining many properties of biomolecules, which exist and function in aqueous solution, a highly polar environment. For example, proteins are composed of amino acids with charged, polar, and nonpolar side chains and their specific electrostatic properties are fundamental to the structure and function of proteins. An important issue that arises in computational study of biomolecular interaction and dynamics based on classical force field is lack of polarization. Polarization is a phenomenon in which the charge distribution of an isolated molecule will be distorted when interacting with another molecule or presented in an external electric field. The distortion of charge distribution is intended to lower the overall energy of the molecular system, which is counter balanced by the increased internal energy of individual molecules due to the distorted charge distributions. The amount of the charge redistribution, which characterizes the polarizability of a molecule, is determined by the level of the charge distortion.



Polarization is inherently quantum mechanical, and therefore classical force fields with fixed atomic charges are incapable of capturing this important effect. As a result, simulation studies based on popular force fields, AMBER, CHARMM, etc., lack the polarization effect, which is a widely known deficiency in most computational studies of biomolecules today. Many efforts have been devoted to remedy this deficiency, such as adding additional movable charge on the atom, allowing atomic charges to fluctuate, or including induced multipoles. Although various successes have been achieved and progress at various levels has been reported over the past decades, the issue of lacking polarization in force field based simulations is far from over. For example, some of these methods do not always give converged results, and other methods require huge computational cost.

This Account reviews recent work on developing polarized and polarizable force fields based on fragment quantum mechanical calculations for proteins. The methods described here are based on quantum mechanical calculations of proteins in solution, but with a different level of rigor and different computational efficiency for the molecular dynamics applications. In the general approach, a fragment quantum mechanical calculation for protein with implicit solvation is carried out to derive a polarized protein-specific charge (PPC) for any given protein structure. The PPC correctly reflects the polarization state of the protein in a given conformation, and it can also be dynamically changed as the protein changes conformation in dynamics simulations. Another approach that is computationally more efficient is the effective polarizable bond method in which only polar bonds or groups can be polarized and their polarizabilities are predetermined from quantum mechanical calculations of these groups in external electric fields. Both methods can be employed for applications in various situations by taking advantage of their unique features.

1. INTRODUCTION

Electrostatic interaction plays a critical role in many biological processes including protein folding,[1,2] protein−ligand binding,[3,4] protein−protein association,[5,6] and a protein's dynamic motion.[7] Current molecular dynamics (MD) simulations of biomolecules are based on classical force fields, and inevitably the accuracy and reliability of the result depend fundamentally on the accuracy of the force field employed in the simulation. An important issue that arises in electrostatic interaction of biomolecules is polarization. Polarization is a phenomenon in which the charge distribution of an isolated molecule will be distorted when interacting with another molecule or in the presence of an external electric field. In the seminal QM/MM work by Warshel and Levitt in 1976, they already included polarization effect for the protein.[8] Since then, the importance of this nonadditive effect has been a subject of many studies,

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and some suggested that a many-body effect could contribute up to 30% of the total interaction energy.[9−12] However, in classical force fields, the electrostatic interaction is represented by a fixed point charge interaction, which lacks the polarization effect. In the past two decades, many attempts have been made to explicitly incorporate polarization effects into molecular modeling.[13] To date, there are several general models that bring polarization effects into force fields such as the fluctuating charge model,[14,15] Drude oscillator,[16,17] induced multipole,[18−22] and quantum mechanical treatment.[23−26] There have been some nice reviews of these methods.[13,27−29] An implicit implementation of polarization effects may also improve the quality of force fields.[30,31]

The electronic reorganization process can be precisely described by quantum mechanical calculations. In the past several years, the polarized protein specific charges (PPC) model has been developed for protein dynamics based on quantum mechanical calculations.[32−52] Fitting of electron density for a given structure into partial atomic charges is a straightforward way to incorporate polarization effects, and the fitted charge can be employed in MD simulations. The polarization effect in protein structure and dynamics has been explored extensively in MD simulations with PPC. It is found that the polarization effect plays an important role in pK_a shifts for ionizable residues,[32] hydrogen bond stability,[33−38] protein folding and native structure stabilization,[33,39−44] dynamic properties of proteins,[34,45−47] protein−ligand binding affinity,[37,48−51] and protein−protein (DNA) recognition specificity,[52] in agreement with many other studies.[53]

2. POLARIZED PROTEIN-SPECIFIC CHARGE

Atomic charges in existing force fields are amino acid based; that is, they are fitted to the electrostatic potential (ESP) from QM calculations of individual amino acids. These atomic charges do not reflect the polarization state of the protein. To remedy this deficiency, one can fit atomic charges to the ESP that are generated from QM calculations of the protein. It is impractical to calculate the electronic structure of a protein containing thousands of atoms directly. An alternative protocol is to divide the protein molecule into small pieces and assemble the electron density of fragments backward to generate the electron density of the whole protein. This strategy is usually recognized as the "divide-and-conquer" (D&C) method.[29,54] Molecular fractionation with conjugate caps (MFCC) is a representative D&C method for large biological molecules.[55] By combining MFCC method and continuous solvation models, one can solve the electronic structure of proteins in a more realistic environment, especially water.[32,56,57] Partial charges for every amino acid in the protein can be fitted to the polarized electron density of each protein fragment using the restrained electrostatic potential (RESP) fitting method. The fitted atomic charges are no longer amino acid-specific as for the nonpolarized charge model, and they correctly represent the polarized condition of each amino acid embedded in a unique electrostatic environment in the solvated protein.[32] Therefore, this charge model is termed the polarized protein-specific charge (PPC). The basic procedures in fitting PPC can be summarized in Figure 1. The idea of PPC has also been adopted by some other groups.[58,59]

The numerical difficulty of linear dependence in all the electrostatic potential based charge fitting methods has been known for years.[60,61] Recently, we proposed a simple way to mitigate the impact of this numerical difficulty on the fitted

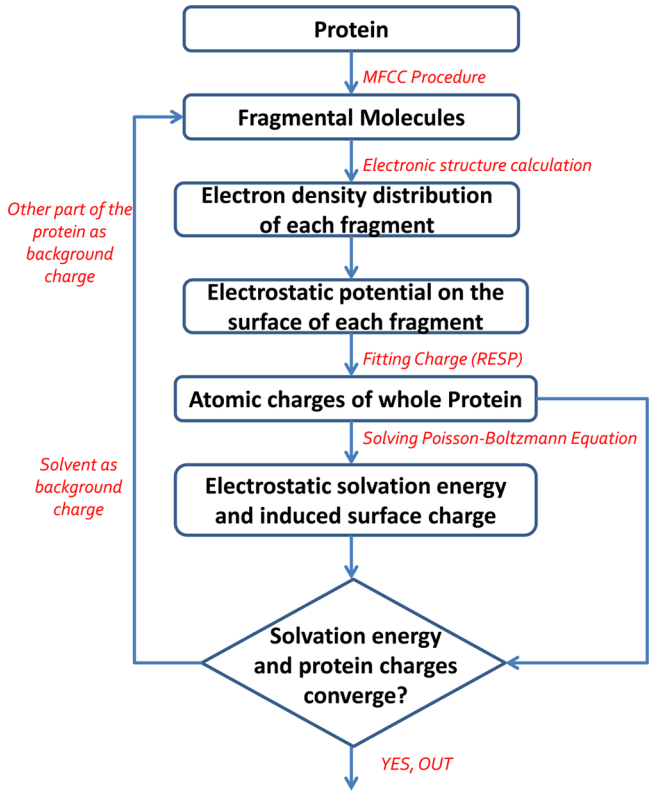

Figure 1. Flowchart for PPC fitting.

charges.[62] In this method, the atomic charge for each atom is divided into two parts. One is the base charge, which is a good mean-field approximation to the charge distribution and can be taken from pairwise AMBER, CHARMM, or OPLS force fields. The other is a system-dependent small perturbation. Instead of directly fitting the total atomic charge, we remove the contribution of the base charge from the "true" ESP and fit the charge perturbation to the residual ESP. A nonuniform weight that is reversely proportional to the square of the base charge is assigned to each atom. Therefore, nonpolar atoms have large weights to keep them less labile, while polar atoms have more freedom to vary with respect to the chemical environment. The atomic charge from this fitting scheme is termed the delta RESP charge, or dRESP charge. It has been shown that the dRESP charges for the polar atoms are very close to the RESP charge and those for the nonpolar atoms are nearly invariable with conformational change.[62] Therefore, dRESP can be as effective as RESP charge in delineating strong Coulomb interactions among polar atoms, and it can suppress the fluctuation in potential energy caused by fake charge separation.

2.1. Effect of Polarization on Protein Dynamics

Protein structure and dynamics are determined by the intraprotein and protein−water noncovalent interactions. Protein's native structure is a result of the subtle balance of all the interactions, and it is highly sensitive to local weak interactions. Early mutagenesis studies have found that tiny perturbations to the interaction network in a protein may cause large scale conformational changes. An inaccurate force field could drive the whole system away from its native state. Previous studies have already found many examples of failed simulations due to a force field defect.[63,64]

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A straightforward way to measure the quality of a force field is to compare the protein structure ensembles and dynamics derived from MD simulation and experiment. Nuclear magnetic resonance (NMR) relaxation experiments provide direct information on structural distribution and dynamic behavior of a protein, which can be used for force field validation. The dynamic behavior of proteins can be measured by the N−H bond order parameter. Simulations show that flexibilities of the proteins are overestimated under AMBER charge with much smaller order parameters than those obtained from NMR experiments, indicating that the standard force field may "allow too much motion". PPC performs much better in reproducing the dynamic behavior of proteins as measured by NMR relaxation experiment.[34,45]

Besides the structure prediction for proteins, another grand challenge in the current computational biochemistry is to predict the free energy changes in biochemical processes such as enzyme catalysis and substrate transportation through the membrane at chemical accuracy. A free energy profile can be constructed directly or indirectly from the distributions of conformational ensembles sampled from MD simulations.[65] However, it can be contaminated by an inaccurate conformational ensemble, for instance, caused by an incorrect interaction potential. The pK_a shift of ionizable residues in protein can be calculated from the free energy change associated with the proton−protein binding process. The free energy estimated from the simulation employing PPC accurately reproduced the experimental value of pK_a shift for Asp26 buried inside thioredoxin (see Figure 2),[32] whereas some previous calculations using classical AMBER and CHARMM force fields overestimated pK_a shift by twice as much.[66]

2.2. Effect of Polarization on Protein−Ligand Binding

Protein−protein and protein−ligand interactions are vital to biomolecular recognition, inhibition of enzymes, apoptosis, and so forth. Unveiling the receptor−ligand binding mechanism is also important in computer aided drug design.[3] Many methods have been developed to calculate the binding affinity,[67−69] in which the electrostatic interaction is well characterized, but the polarization effect is rarely accounted for. By performing MD simulation and free energy calculation with PPC, the importance of the polarization effect in protein−protein and protein−ligand binding has been presented.[33,37,48−50,52]

Avidin−biotin is a model system for the study of protein−ligand association due to its strong affinity. Biotin binds with avidin tightly through eight hydrogen bonds. We investigated the origin of the binding affinity difference of avidin to biotin and 2′-iminobiotin through MD simulations using PPC and showed that the difference was almost entirely from the strengthened electrostatic interaction.[48] In contrast, the simulation with the nonpolarized AMBER force field failed to capture the difference. Another study showed that there were significant charge redistributions within the residues in the first binding shell to facilitate the residence of biotin in the active site of streptavidin, and this electrostatic polarization effect was the main cause of a 1000-fold loss of binding affinity after F130L mutation.[37] In a recent linear interaction energy (LIE) study of avidin binding complexes with PPC, a remarkable consistency between the predicted binding affinities and the experimental measurement has been observed.[51]

2.3. Effect of Polarization on Energetics of the Hydrogen Bond

The skeleton of a protein's folded structure is shaped by main chain hydrogen bonds. These polar groups polarize each other and enhance the stability of the hydrogen bond. The polarity of a certain hydrogen bond is determined by its local electrostatic environment. Protein is not a homogeneous entity. Therefore, the polarization states of hydrogen bonds vary greatly. The polarization state of a hydrogen bond buried deep inside the protein should be different from that of a hydrogen bond exposed to solvent molecules. Thus, their energetic contributions in protein folding are different. Accurate calculation of these hydrogen bonds in simulation is imperative in quantifying the energetics of hydrogen bonds in protein folding. We have investigated the role of the local electrostatic environment in determining hydrogen bond strength in the Pin WW domain through computational mutation study.[35] It shows in Figure 3 that this hydrogen bond is accessible to more solvent molecules

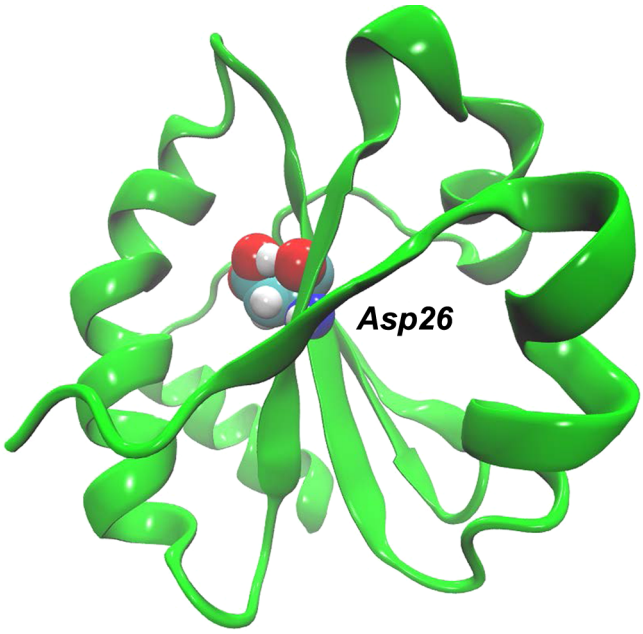

Figure 2. Structure of thioredoxin (PDB ID 1XOA) with buried Asp26.

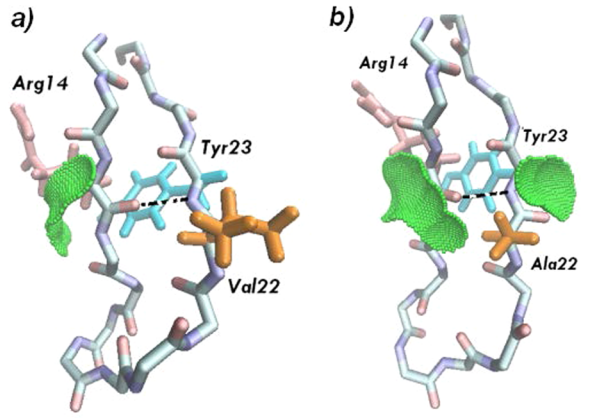

Figure 3. Structure of the Pin WW domain with a hydrogen bond formed between CO in Arg14 and NH in Tyr23. Solvent accessible surface around this hydrogen bond is represented by green surface in (a) wild type and (b) V22A mutant protein. Reprinted with permission from ref 35. Copyright 2011 American Chemical Society.

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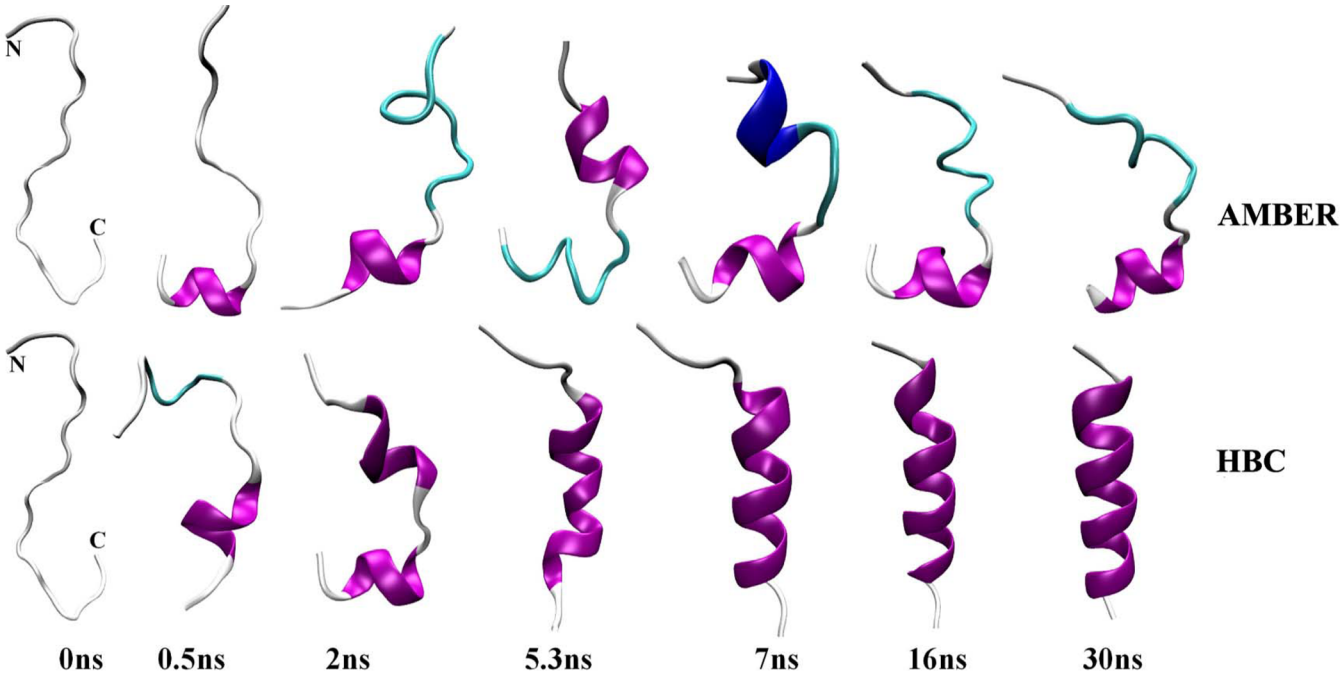

Figure 4. Snapshots of intermediate structures of peptide 2I9M in simulations using AMBER (upper) and dynamically polarized charge (lower). α-helix, purple; coil, white; turn, cyan. Reprinted with permission from ref 74. Copyright 2010 American Chemical Society.

in the V22A mutant than in the wild-type protein. Although the geometry of the Arg14−Tyr23 hydrogen bond was not affected by the mutation, this hydrogen bond was about 0.6 kcal/mol stronger in a hydrophobic environment. Our computational result agreed well with the experiment,[70] while the AMBER force field with fixed charge was unable to capture this important feature, that is, the strength modulated by the local electrostatic environment.

3. POLARIZABLE PROTEIN CHARGE

3.1. Dynamic Polarized Protein-Specific Charge

Protein has an ensemble of structures under physiological conditions. Furthermore, its function is highly related to conformational changes, some of which are on large scales. Large scale conformational change is inevitably accompanied by electron redistribution. The important role played by electrostatic polarization and charge transfer in protein folding has been known for many years.[71,72] Therefore, using a single set of PPC is not suitable for all studies. A straightforward idea is to fit the atomic charge at each step of the MD simulation. However, updating atomic charge for the whole protein based on high level quantum mechanical calculations at all the steps is still too demanding. To a good approximation, we assume that charge redistribution in a residue is significant only when it undergoes alternation of strong Coulomb interactions, such as hydrogen bonds and salt bridges. Besides, a hydrogen bond is a good indicator of secondary structure. We proposed a simplified but effective way to implement polarization effects during large scale conformational change, which are termed the dynamically adapted hydrogen bond charges. During the MD simulation, main chain hydrogen bonds are checked periodically. If a main chain hydrogen bond is formed or cleaved, those residues participating in this hydrogen bonding will have their atomic charges refitted. The time interval between two successive checks of a hydrogen bond should be short enough to make sure that the charge is suitable for the trajectory between these two checkpoints.

We also proposed an even less demanding way to include the polarization effect.[43] A pair of alanine dipeptides connected through a main chain hydrogen bond is used for the parametrization. By systematically alternating the bond distance, we can obtain the relationship between charge redistribution and hydrogen bond length through quantum mechanical calculations of all the configurations. We further assume that the charge alternation only takes place within the amide group and within the carbonyl group involved in the hydrogen bond and no inter-residue charge transfer is allowed. The charge flows as a function of bond distance between N and O atoms can be well fitted to single exponential functions as

$$\Delta q_{\mathrm{N}} = -0.493 \times \exp(-0.455 d_{\mathrm{ON}}) \quad (1)$$

and

$$\Delta q_{\mathrm{O}} = -0.334 \times \exp(-0.466 d_{\mathrm{ON}}) \quad (2)$$

for N (the opposite of that for H) and O (the opposite of that for C) atoms.

3.2. Effect of Polarization on Protein Folding

Protein folding is a journey toward the global free energy minimum by burying hydrophobic side chains and forming secondary and higher order structures. Hydrogen bonding is an essential part in the formation of secondary structures. This directional Coulomb interaction causes strong distortion to the electronic structures of both the hydrogen bond donor and acceptor to facilitate the hydrogen bonding.[73] Usually, pairwise force fields underestimate this interaction. Therefore, the enthalpy change may not be enough to compensate for the entropy loss in the formation of secondary structure under these force fields, and the folded state is no longer the global free energy minimum. This limitation of force field has been observed in the folding simulations of a short peptide (PDB

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entry 2I9M), which adopts a helical structure under the experiment conditions. Starting from an extended structure, the folded state has not been reached in a 30 ns MD simulation under AMBER03 in continuous solvent and in 150 ns MD simulations under various AMBER force fields in a TIP3P water box (see Figure 4).[38,74] The melting temperature under the AMBER03 force field is lower than the temperature used in the NMR experiment to determine its structure. When the polarization effect is turned on,[38,43,74] this peptide can fold to its native structure, which is the global free energy minimum. The compensation of enthalpy and entropy limits the length of a helix. Lack of polarization effect lowers this limit falsely. The solution structure of the b30-82 domain of subunit b of *Escherichia coli* F1FO ATP synthase is a long helix, as determined by NMR experiments. With the polarization effect included in the interaction potential, the folded structure can be reached in a direct MD simulation. Comparatively, it adopts several short helical fragments under a pairwise AMBER03 force field (see Figure 5).[44]

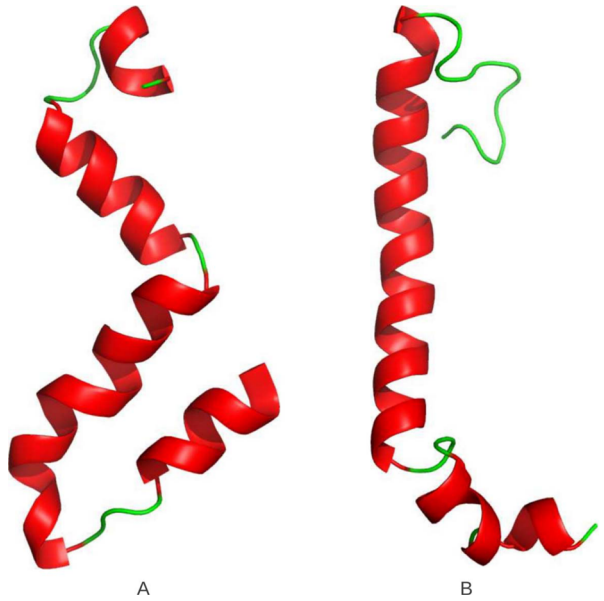

Figure 5. Final structures of peptide 2KHK in simulations using AMBER03 (left) and dynamically polarized charge (right). Reprinted with permission from ref 44. Copyright 2013 AIP Publishing LLC.

3.3. Effective Polarizable Bond Method

There are two opposing energetic effects that occur during the polarization process. On the one hand, electron redistribution will enhance the interaction energy between the molecule and the environment in order to lower the electrostatic energy of the system. On the other hand, the internal energy of the molecule will increase as a result of distortion of the electron charge distribution. These two opposing energetic effects counter balance each other, and the molecule reaches its eventual polarized state under the electric field generated by surrounding molecules. Using this rationale, we have developed a practical polarizable model, termed effective polarizable bond method (EPB), to include polarization effects efficiently in simulation.[75,76] The EPB model keeps the "effective charge" character of the classical force field and provides a good correction to the traditional force field for MD simulation by introducing "fluctuating" character for atomic charges of the polarizable groups.

Consider transferring a polar group, CO, from gas phase to liquid phase; the total electrostatic energy of the system can be written as

$$E = E_{\text{p-cost}} + E_{\text{ele}} = [k(\mu_{\text{liquid}} - \mu_{\text{gas}})^2] + [q_C\Phi_C + q_O\Phi_O] \quad (3)$$

where $E_{\text{p-cost}}$ is the polarization cost energy, $1/k$ represents polarizability of the CO group, q_C and q_O are, respectively, the ESP charges of the C and O atoms, and Φ_C and Φ_O are the electrostatic potential applied to C and O atoms, respectively. The polarization process can be treated as charge transfer along the polarizable bond. Suppose the charge transferred from atom O to atom C is Δq; the final partial charges are

$$q_C = q_C^{\text{gas}} + \Delta q \quad (4)$$

and

$$q_O = q_O^{\text{gas}} - \Delta q \quad (5)$$

The dipole moment change along the CO bond in the polarization process is given by

$$\mu_{\text{liquid}} - \mu_{\text{gas}} = \Delta q d_{CO} \quad (6)$$

where d_{CO} is the length of CO bond. Thus, eq 3 can be rewritten as

$$E = k(\Delta q d_{CO})^2 + (q_C^{\text{gas}} + \Delta q)\Phi_C + (q_O^{\text{gas}} - \Delta q)\Phi_O \quad (7)$$

Minimization of eq 7, charge transfer along CO bond under a given electric field can be calculated as

$$\Delta q = \frac{\Phi_O - \Phi_C}{2{d_{CO}}^2 k} \quad (8)$$

Just as in the traditional force field, the concept of effective charge can also be introduced in the fluctuating charge model.

It is convenient to express the polarization cost energy term in eq 3 in the form of electrostatic interactions. For the CO group, eq 3 can be rewritten as

$$\begin{aligned} E &= E_{\text{self}} + E_{\text{ele}} \\ &= k(\Delta q d_{CO})^2 + [(q_C^{\text{gas}} + \Delta q)\Phi_C + (q_O^{\text{gas}} - \Delta q)\Phi_O] \\ &= \tilde{q}_C\Phi_C + \tilde{q}_O\Phi_O \end{aligned} \quad (9)$$

where $\tilde{q}_C$ and $\tilde{q}_O$ are, respectively, the effective charges of C and O atoms. Combination of eqs 8 and 9 leads to

$$\begin{aligned} E &= k(\Delta q d_{CO})^2 + [(q_C^{\text{gas}} + \Delta q)\Phi_C + (q_O^{\text{gas}} - \Delta q)\Phi_O] \\ &= \Delta q\cdot(\Delta q k {d_{CO}}^2) + [(q_C^{\text{gas}} + \Delta q)\Phi_C + (q_O^{\text{gas}} - \Delta q)\Phi_O] \\ &= \Delta q\cdot\left(\frac{\Phi_O - \Phi_C}{2{d_{CO}}^2 k} k {d_{CO}}^2\right) + [(q_C^{\text{gas}} + \Delta q)\Phi_C \\ &\quad + (q_O^{\text{gas}} - \Delta q)\Phi_O] \\ &= \left(q_C^{\text{gas}} + \frac{1}{2}\Delta q\right)\Phi_C + \left(q_O^{\text{gas}} - \frac{1}{2}\Delta q\right)\Phi_O \\ &= \tilde{q}_C\Phi_C + \tilde{q}_O\Phi_O \end{aligned} \quad (10)$$

The effective fluctuating charges (EFQ) can be defined as

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$$\tilde{q}_C = q_C^{gas} + \frac{1}{2}\Delta q \quad (11)$$

$$\tilde{q}_O = q_O^{gas} - \frac{1}{2}\Delta q \quad (12)$$

The polarization penalty is a negative contributor in the polarization process. The net effect of EFQ is that the amount of charge transferred is reduced when polarization penalty is merged into electrostatic interaction using point charge. This new charge model inherited the effective character of the classic force field and the fluctuating feature of previous polarizable models. Since polarization penalty is treated properly, this model avoids the problem of over polarization and is numerically stable. Figure 6 shows that hydrogen bond structure was well preserved in MD simulations when the effective polarizable bond model was used but it deviates greatly from the crystal structure when AMBER99SB charge was used.

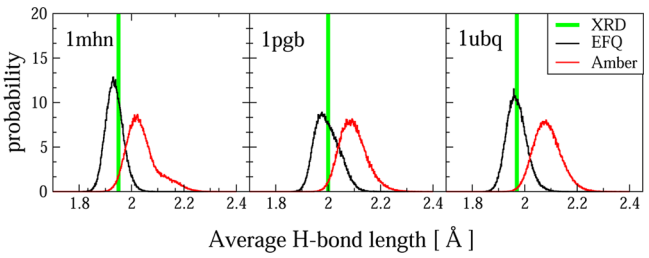

Figure 6. Distribution of H−O bond length in MD simulation under the effective polarizable bond model and AMBER99SB for the SMN Tudor domain, the B1 immunoglobulin-binding domain of protein G, and ubiquitin. Experimental values are indicated by XRD. Reprinted with permission from ref 75. Copyright 2013 American Chemical Society.

4. STATIC AND DYNAMIC POLARIZATION EFFECT

Most of the polarization effect comes from the polarization of hydrogen bonds, which may lead to both enhanced static interaction energy and dynamic stability. The static polarization effect comes from electron redistribution of a given conformation directly, while the dynamic polarization effect arises from conformation redistribution indirectly.[37,48,49] Protein does not bind to ligand with a fixed conformation. Both protein and ligand are flexible. The binding complex exists as a dynamic ensemble in water. Figure 7 shows some representative conformations of biotin in the active site of streptavidin. Biotin binds to streptavidin more tightly when polarization effect is turned on. Thus, the dynamic polarization effect may have a strong influence on the calculated net contribution of hydrogen bonding to protein−ligand association. The static and dynamic polarization effects are coupled, and both of them contribute significantly to the overall stability of protein complexes. The static polarization effect has also been investigated by several early studies in protein−drug binding research.[12,77−79] The importance of the dynamic polarization effect in protein−ligand binding is much less investigated. A recent study found that the binding affinity between biotin and avidin is strongly underestimated when AMBER03 force field is used since the dynamic stability of the hydrogen bond between biotin and Tyr33 is not well maintained in MD simulations under this unpolarized force field.[48] It is also true for intraprotein hydrogen bonds, which can be implied from Figure 6.

5. CONCLUDING REMARKS

Electronic polarization effect plays an important role in protein structure, dynamics, and thermodynamic properties through MD simulation and free energy calculation. Inclusion of polarization effect in free energy calculations from MD simulations is indispensable to obtain reliable numerical results at chemical accuracy since the sampled conformation ensemble of a protein is strongly influenced by the quality of the force field utilized. The polarized protein-specific charge and the effective polarizable bond model have been shown to be very effective in incorporating the polarization effect in a series of studies. Strength of the polar interactions is strongly affected by the local electrostatic environment in which the interacting groups are accommodated. Since protein is an inhomogeneous

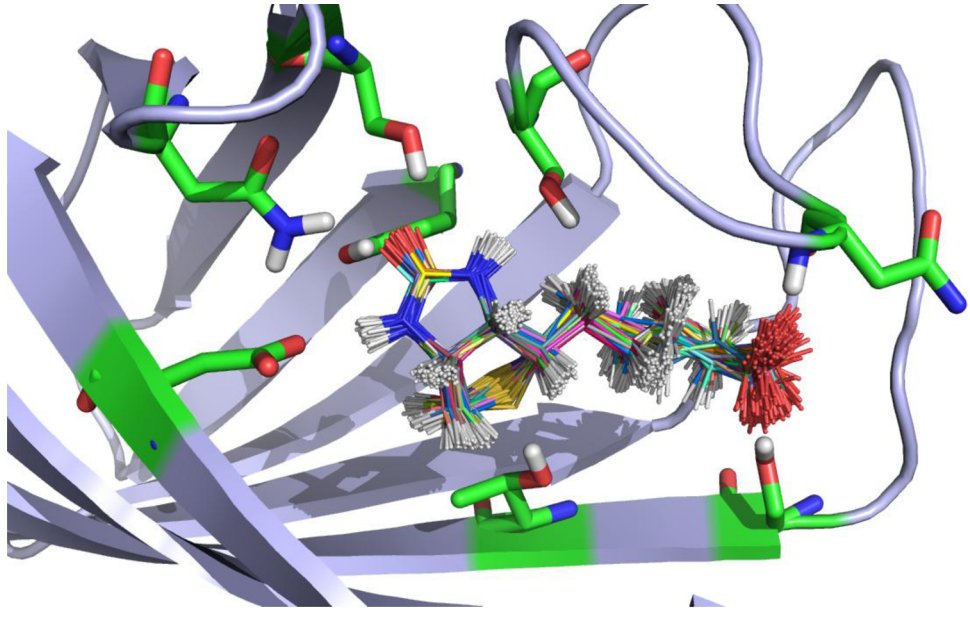

Figure 7. Superposition two hundred structures of the ligand, extracted from MD simulation of streptavidin−biotin complex.

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system, energetic contributions of hydrogen bonds may vary at different parts of the protein. Thus, an accurate description of polarization effect is critical in quantifying the role of hydrogen bonds in protein folding. Polarization effect in protein−protein and protein−ligand binding can be decomposed into the static and the dynamic effect. The static polarization effect is easier to capture, since it originates from strengthened electrostatic interactions between receptor and ligand at a given configuration. The dynamic polarization effect originates from the dynamic stability of polar interaction partners such as the boosted occupancy of inter- and intraprotein hydrogen bonds.

Although great success has been made in simulating protein structure and function with PPC, there are some limitations in this framework. Electrostatic embedding is a good approximation only when the environment is far from the system. Short range interaction cannot be fully described by electrostatic interactions. Repulsion (and probably dispersion) interactions should be included to perturb the electronic structure of each fragment. The first approach coming to mind is the effective fragment potenial, which is capable of implementing the short-range effect.[23] Subtle balance among protein−protein protein−water, and water−water interactions is very important to correctly simulate thermodynamic properties of biochemical processes in water. Adjustment of van der Waals parameters with a special water model is needed in the future to improve the interaction balance of protein in solvent. In the adaptive PPC charge model, electronic structure calculations need to be carried out frequently in the simulation; computational overhead is very demanding. We will solve this problem through developing geometry and environment dependent charge models in the future.

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Notes

The authors declare no competing financial interest.

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Changge Ji studied chemistry and obtained his Ph.D. (2009) at Nanjing University (China). In 2010, he became Associate Professor at East China Normal University. His research focuses on polarizable force fields, protein−ligand interactions, and large scale correlated motions of proteins.

Ye Mei received his B.S. in Chemistry and Ph.D. in Physical Chemistry from Nanjing University in 2002 and 2007, respectively. After two years of training as postdoctoral research fellow in the same University, he joined East China Normal University as Associate Professor. He was promoted to Full Professor in 2012. His research interest is the development of force fields.

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