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Polarizable Force Fields: History, Test Cases, and Prospects

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Abstract: A consistent treatment of electrostatic energies is arguably the most important requirement for the realistic modeling of biological systems. An important part of electrostatic modeling is the ability to account for the polarizability of the simulated system. This can be done both macroscopically and microscopically, but the use of macroscopic models may lead to conceptual traps, which do not exist in the microscopic treatments. The present work describes the development of microscopic polarizable force fields starting with the introduction of these powerful tools and following some of the subsequent developments in the field. Special effort has been made to review a wide range of applications and emphasize cases when the use of polarizable force fields is important. Finally, a brief perspective is given on the future of this rapidly growing field.

1. The Emergence of Polarizable Force Fields

Electrostatic effects, and solvation effects in particular, play a major role in determining the energetics and dynamics of charge transfer and related processes in solution (e.g. refs 1−3). Such effects also play a crucial role in determining the function of macromolecules (e.g. refs 4−13). Thus, the ability to quantify electrostatic interactions is essential for the quantitative description both of processes in solution and for structure−function correlation studies of proteins (e.g. ref 5). However, accomplishing this task has been quite challenging for both microscopic and macroscopic approaches (for reviews see e.g. refs 6−13).

Here, we will focus on one crucial aspect of the microscopic modeling of electrostatic energies, namely, the treatment of electronic polarizability. We will start by presenting some of the historical background of this rapidly growing field. We will then move to key examples and finally to a discussion of the prospects of the field.

The idea that matter can be represented by induced dipoles goes back to the early literature on electrostatics. However, the rationalization of the proper description of microscopic polarization and the replacement of electronic polarization by classical polarizable induced diploes is more recent. In fact, most textbooks treat the energetics of polarizable matter in a macroscopic way whose relationship to the microscopic world is not clear. For example, according to the well-established macroscopic theory (e.g. refs 14 and 15), one can express the energy of a polarizable volume element by

$$W = -\frac{1}{2}\mathbf{P}\mathbf{E}_0 = -\frac{1}{2}\alpha\mathbf{E}_0^{\,2} \tag{1a}$$

where $\mathbf{P}$ is the induced polarization, $\mathbf{E}_0$ is the macroscopic field, and α is the corresponding polarizability. However, the validity of such a treatment in microscopic systems may look less clear to a chemist who comes from the molecular atomistic background, where it is known that the interaction between a charge and the induced dipole of a single atom in a collection of atoms is given by $W = -\boldsymbol{\mu}\boldsymbol{\xi}_0 = -\alpha\boldsymbol{\xi}_0^2$ (where $\boldsymbol{\xi}$ is the microscopic field on the atom). Thus, the origin of the (1/2) factor is not obvious. This point can be verified by trying to ask a physics or electrical engineering professor how the factor 1/2 in microscopic systems is obtained. The typical answer usually involves the well-known $\int Q\mathrm{d}Q = Q_0^2/2$ macroscopic integral,[15] or arguments about the linear response nature of matter, but it will not satisfy those who

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insist on a molecular explanation. In fact, the microscopic relationship for a collection of charges and induced dipoles is[16]

$$W = -\sum_{i,j} Q_i(\boldsymbol{\mu}_j \cdot \mathbf{r}_{ij})/r_{ij}^3 + \sum_{j>j'} \boldsymbol{\mu}_j[\nabla(\boldsymbol{\mu}_{j'} \cdot \mathbf{r}_{j'j})/r_{j'j}^3] + \frac{1}{2}\sum_j \alpha_j |\boldsymbol{\xi}_j|^2 \quad (1b)$$

where the first term comes from the interaction of the charges i with the dipoles j, the second term from the interaction of the dipoles j and j', and the third term from the energy that must be spent in distorting the electron cloud of the atom to create the induced dipoles. This energy cost can be verified by using a model that views the electron as being attached to the nuclear core by a spring or by actual quantum mechanical calculations which consider an atom in an external field. At any rate, we can rewrite eq 1b as[16]

$$W = -\frac{1}{2}\sum_{i,j} Q_i(\boldsymbol{\mu}_j \cdot \mathbf{r}_{ij})/r_{ij}^3 \quad (1c)$$

or in other words (see also ref 9)

$$W = -\frac{1}{2}\sum_j \boldsymbol{\mu}_j \cdot \boldsymbol{\xi}_j^0 \quad (1d)$$

where $\boldsymbol{\xi}_j^0$ is the field on the jth dipole from the charges in the system. This field does not include the field from the other dipoles; that leads, however, to the actual value of $\boldsymbol{\mu}_j$. The above derivation has not appeared, to the best of our knowledge, in the early macroscopic literature.

Similar problems arise when one tries to consider other features of polarizable matter in a microscopic way by starting from macroscopically based textbooks. Here, one becomes puzzled about the nature of the dielectric constant of small molecular size volume elements, and the problem can only be resolved by microscopic treatments, as was done in section 1 of ref 9.

The problem may become even more profound when one tries to solve time-dependent problems in polarizable matter by starting from a macroscopic perspective (see for example the controversy about nonequilibrium effects,[17,18] which could be easily resolved microscopically by using, for example, a polarizable empirical valence bond (EVB) type model). The conceptual difficulties with the macroscopic picture (and the corresponding dielectric behavior) of the polarizable (nonpolar) medium disappear once one takes a fully microscopic treatment of a collection of induced dipoles into account. Such a microscopic derivation has been presented in refs 9 and 16. Classical treatments of electronic polarizability of isolated molecules emerged in the early 1970s[19] in addition to quantum mechanical treatments of isolated molecules in electric fields.[20,21] As far as classical treatments are concerned, the work of Applequist and co-workers[19] has provided a classical way of evaluating the polarization of an isolated molecule in the gas phase by an external electric field. Although this has been an important advance in the field, it was neither developed into an approach for calculations of the energy of interacting molecules nor for a tool in force field studies.

Classical microscopic treatments of the energetics of induced dipoles for solutions and large molecules emerged only in the mid 1970s. In particular, a preliminary attempt to study dielectric effects in nonpolar environments was reported by Hopfinger,[22] who placed a methyl group between two charges. However, this study overlooked the fact that most of the dielectric effects come from the molecules around the charges rather than between them. Thus, the first physically consistent microscopic study of dielectric effects in nonpolar environments was reported by Warshel and Levitt (WL),[16] who simulated the electrostatic environment in lysozyme by a classical polarizable force field and represented the effect of the surrounding solvent by a grid of Langevin-type dipoles. Similar approaches were used for other proteins (e.g. ref 23) and for polarizable grids of dipoles (e.g. ref 24). Alder and co-workers[25,26] subsequently used a polarizable model for simulations of charges and dipoles in nonpolar solvents. Thus, the use of polarizable force fields dates back to the work of Warshel and Levitt,[16] who introduced this approach as a general way of capturing the effect of electronic polarization and the corresponding dielectric constant in protein modeling. This was done using both iterative and noniterative approaches. Subsequent early instructive studies include those reported in refs 27 and 28.

The use of polarizable force fields became an integral part of the simulations in our group,[29,30] and we analyzed its effect on electrostatic modeling in many subsequent studies.[9,31,32] The general realization that the effect of induced dipoles is important has been relatively slow (some workers initially argued that this cannot be an important effect[33]), but it is now widely appreciated.

Recent works have advanced the use of polarizable models to many force field programs and also refined the accuracy of such models.[34−43] Furthermore, the use of polarizable models in simulations has progressed significantly, and many studies have implemented polarizable water models.[27,39,40,44−47] The general advances in the development of polarizable force fields will be described by other workers in this issue, including detailed descriptions of specific implementations and their differences and similarities to earlier models.

Although we leave it up to other workers to describe their specific implementations, we would like to comment on the fact that the inclusion of induced dipoles allows one to transfer gas-phase ab initio potentials to condensed phases. That is, Wallqvist and Karlstrom[48] have shown that it is possible to represent the gas-phase potential of a water dimer by a potential surface that includes classical induced dipoles. A further crucial step was done by Kuwajima and Warshel[44] who demonstrated that a polarizable potential that was fitted to an ab initio potential of a water dimer can be directly transferred to condensed phases and reproduces, for example, the many-body effect of water molecules on the dipole moment of each water molecule in condensed phases.

We would also like to clarify that in contrast to the possible implications from a recent study,[46] the KW model is a quite consistent model, and its inability to reproduce the exact gas-phase dimer spectra properties is entirely due to the fact that the MCY gas-phase ab initio potential available at that time[49] was not perfect (the MCY and KW potentials give identical

gas-phase results as verified by Saykally and co-workers[50]). The point of the KW paper was to show how to transfer ab initio potentials to solution and not how to improve ab initio calculations.

To conclude this section, it might be useful to re-emphasize that a general-purpose polarizable force field program has been already available as early as 1975. It was originally implemented in the program used in ref 16 which of course provided all the relevant parameters. Subsequently, it was implemented in the POLARIS and ENZYMIX programs.[29,51] A detailed description of the program, the parameters, and the performance is given in ref 29. Several versions of the polarizable force field have been used both in simplified PDLD studies (e.g. refs 16, 24, and 30) and in MD simulations starting with ref 52 as well as countless subsequent studies by our group. Thus, claims that such programs were only recently developed are not useful.

2. Calibration of Cation Force Fields Using Binding Energies to Valinomycin

The most crucial need for a polarizable force field is probably in the treatment of ions and ionized groups. To demonstrate this point, we will describe a recent calibration study, which was aimed at refining force field parameters for studies of ion channels. We start this section by pointing out that one of the most important factors in any reliable study of the selectivity of biological ion channels is the accuracy of the parameters that describe the solvation of the ions by water and by the protein environment.[53] In view of the challenges of obtaining converging results in ion channels studies, it is obviously important to reduce any errors associated with the accuracy of the force field. The calibration of force field parameters can be done by using results from high level ab initio calculations of simple systems in the gas phase. Unfortunately, those parameters do not always give proper results in a condensed phase. Therefore, it is a reasonable approach to adjust force field parameters to reproduce experimental hydration energies (e.g. refs 54−56). An improved agreement for highly charged ions can be obtained by specialized approaches (e.g. ref 57). At any rate, regardless of the procedures used, it is absolutely crucial to validate and refine the parameters by comparing calculated and observed solvation energies in proteins and solutions. The problem is, however, that convergence errors in the protein active site can be larger than the "errors" in the force field parameters. Moreover, since it is trivial to reproduce the solvation in water by adjusting the force field parameters, it is important to use in the refinement process additional information which reflects the difference between the solvation of the cation in the protein and in water. In our view, the best strategy is to compare the "solvation" energy of the cations in water and in macrocycles. Of course, requiring that the resulting force field will also reproduce ab initio results can augment this type of treatment. At any rate, we describe below a systematic force field calibration by calculations of cation solvation energies in water and in a system that contains the key groups of the cation binding sites. In our view, valinomycin is an excellent system for the validation of cation parameters because it is relatively simple (cyclododecadecipeptide), the solvation of its polar groups is closely related to the corresponding solvation in proteins, and it shows cation binding selectivity.[58] The relative simplicity of valinomycin is crucial since it allows for proper convergence, which is hard to obtain in studies of cations binding to proteins.

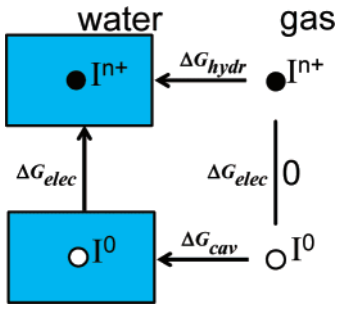

Figure 1. The thermodynamic cycle used for calculations of absolute solvation energies.

Force field parameters for the cations were obtained for both polarizable and nonpolarizable force fields and were first adjusted to reproduce experimental hydration free energies.[54−56] These were then validated by comparing calculated relative binding energies (to valinomycin) with the corresponding experimental values.[59,60] The calculations of the hydration energy were based on the thermodynamic cycle described in Figure 1. This cycle divides the hydration energy into two contributions, the electrostatic and the cavitation energy, using

$$\Delta G_{\mathrm{hydr}}(I^{n+}) = \Delta G_{\mathrm{elec}}(I^0 \rightarrow I^{n+}) + \Delta G_{\mathrm{cav}} \tag{2}$$

where I^0 and I^{n+} are the uncharged and ionized state of a cation respectively, and ΔG_{cav} is the free energy of solvation of the uncharged cation. The electrostatic contribution, ΔG_{elec}, was calculated by the adiabatic charging (AC) free energy perturbation (FEP) approach[1,61] using

$$V_m(\lambda_m) = V_0(1 - \lambda_m) + V_1\lambda_m \tag{3}$$

$$\exp\{-\Delta G(\lambda_m \rightarrow \lambda_{m+1})\beta\} = \langle \exp\{-(V_{\lambda_{m+1}} - V_{\lambda_m})\}\beta\rangle_{V_{\lambda_m}} \tag{4}$$

$$\Delta G_{V_0 \rightarrow V_1} = \sum_{m=0}^{n-1} \Delta\Delta G_{\lambda_m \rightarrow \lambda_{m+1}} \tag{5}$$

where V_0 is the potential where the charge of the cation is zero, V_1 is the potential where the charge of the cation is +1 or +2 depending on the cation type and λ_m are mapping windows between V_0 and V_1. Typically, 51 windows were used with a 5 ps simulation time and 1 fs time steps.

The force field potential for the interaction between the cation and other atoms was defined by

$$V_{I-W} = \sum_j (A_I A_j r_{Ij}^{-12} - B_I B_j r_{Ij}^{-6}) + \sum_j C Q_I q_j / r_{Ij} + U_{\mathrm{ind}}(\mathbf{r}) \tag{6}$$

where I represents a cation, j represents other atoms, A_i and B_i are the vdW parameters for the given atom, Q_I and q_j are the charges (or residual charges) of the ion and the jth solvent atom, while C is 332. The charges are given in atomic units,

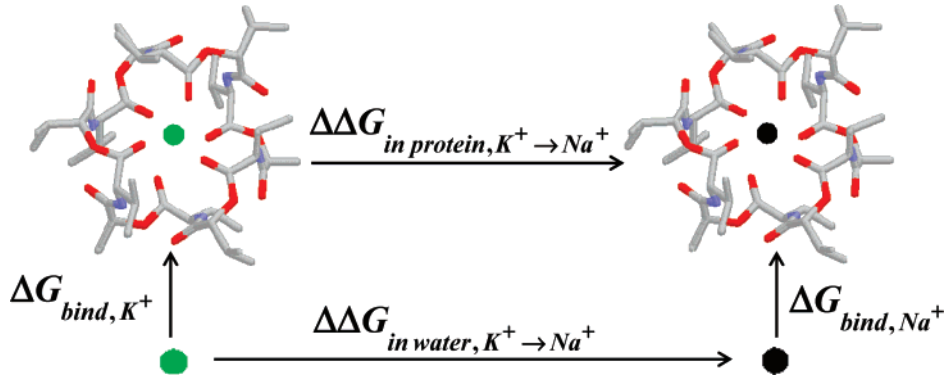

Figure 2. The thermodynamic cycle used for evaluation of the relative binding energy of sodium and potassium to valinomycin.

the distance in Å, and the energy in kcal/mol. The cavitation energy (the nonelectrostatic contribution ΔG_{cav}) was calculated by a FEP treatment, in which V_1 was defined as the potential where the vdW parameters A and B of the cation are at their values in eq 6 and V_0 is the potential where A and B are set to zero.

After calibrating the solvation energy in water, we moved to the next step of evaluating the free energy of binding of the cations to valinomycin (the "protein"). In principle, we could evaluate the energetics of the absolute binding energies using the thermodynamic cycle of Figure 2. However, in the present case, we focus on the relative binding energies. These relative binding energies were obtained by taking the difference of the free energies to transform the cation in valinomycin (surrounded by water) and in bulk water. For example, for the K^+ and Na^+ pair we used

$$\Delta\Delta G_{\text{bind},K^+\rightarrow Na^+} = \Delta G_{\text{bind},Na^+} - \Delta G_{\text{bind},K^+} = \Delta\Delta G_{\text{protein},K^+\rightarrow Na^+} - \Delta\Delta G_{\text{water},K^+\rightarrow Na^+} \quad (7)$$

The mutation of the cations was done by an AC FEP procedure using 51 windows of 5 ps with 1 fs time steps.

The refined parameters and the corresponding hydration energies are summarized in Table 1, and the results for monovalent ions are also given in Figure 3. As seen from the table, we obtained very reasonable results for both the nonpolarizable and polarizable force fields. In fact, a better agreement for the divalent ions can be easily obtained by using six center dummy atom models for the ion (e.g., refs 57 and 62). At any rate, optimized parameters were then used to evaluate the relative binding free energies of cations to valinomycin and the calculated results are summarized in Figure 4. As seen from the figure, we obtained reasonable results for the binding of monovalent ions (Figure 4a) to valinomycin for both the polarizable and nonpolarizable force fields, although the order of the binding selectivity of cations was not always correct. This is clearly satisfactory considering the 1 kcal/mol error range of the parametrization for the hydration energies. However, in the case of the divalent ions (Figure 4b) the polarizable model gives significantly better results than the nonpolarizable model. More specifically, both the polarizable and nonpolarizable force fields give reasonable results in (A), while in (B) only the polarizable force field does (e.g., the deviations in the case of $Sr^{2+} \rightarrow Ca^{2+}$ are around 4 kcal/mol).

Table 1. Cation vdW Parameters and Solvation Energies Calculated with Nonpolarizable (A) and Polarizable (B) Force Fields[a]

	vdW parameters		hydration energy (kcal/mol)		
cation	A	B	$\Delta G_{hydr,calc}$	$\Delta G_{hydr,expt}$	$\Delta\Delta G_{(expt-calc)}$
			(A) Nonpolarizable Force Fields		
Na^+	94	3.89	−98	−98.2	−0.2
K^+	333	4.35	−80.2	−80.6	−0.4
Rb^+	508	4.64	−74.7	−75.5	−0.8
Cs^+	892	5.44	−68.9	−67.8	1.1
Ca^{2+}	205	18.82	−378.4	−380.8	−2.4
Sr^{2+}	470	20.54	−345	−345.9	−0.9
Ba^{2+}	1045	24.13	−312.2	−315.1	−2.9
			(B) Polarizable Force Fields		
Na^+	47	3.89	−97.8	−98.2	−0.4
K^+	205	4.35	−79.7	−80.6	−0.9
Rb^+	318	4.64	−75.9	−75.5	0.4
Cs^+	655	5.44	−68.7	−67.8	0.9
Ca^{2+}	85	18.82	−381.3	−380.8	0.5
Sr^{2+}	242	20.54	−344.8	−345.9	−1.1
Ba^{2+}	668	24.13	−314.4	−315.1	−0.7

[a] The parameters for the solvent and the protein are the standard MOLARIS parameters.[29]

At any rate, the most important conclusion of the present study is that we can easily fit parameters that reproduce the observable solvation energy in water by both polarizable and nonpolarizable models. The advantage of polarizable models only becomes apparent when we move from water to other environments and even then (if we deal with ions that are in contact with water) only in the case of divalent ions.

3. General Applications of Polarizable Force Fields

This section will cover a wide range of examples of the application of polarizable force fields to different systems, focusing mainly on contributions from our lab. In each case, we will emphasize the importance of the use of polarizable force fields relative to the problems associated with other factors (e.g., convergence effects).

3.1. Calibration and Examination by Studies of Solvation Energies of Small Molecules. The modeling of a biological process can be helped enormously by calibrating the calculations or the conceptual considerations relative to the observed (or estimated) solvation free energy of the

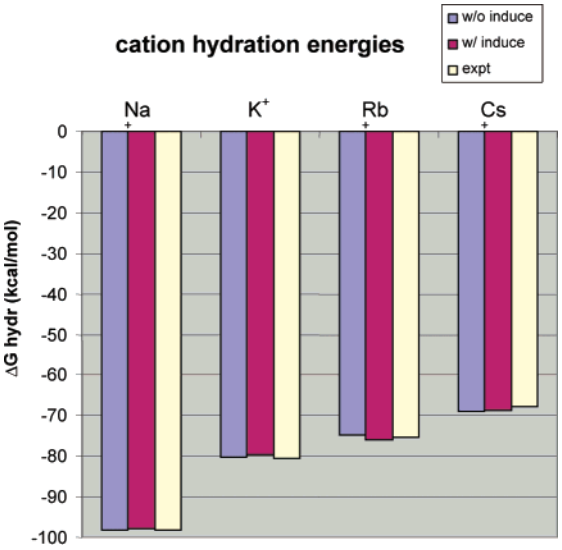

Figure 3. Cation hydration energies obtained after the parametrization. The white bars show the experimental hydration energies, while blue and red bars show the calculated hydration energies with nonpolarizable and polarizable force fields, respectively.

relevant reacting system in aqueous solution (e.g. refs 1 and 9). This is true with regards to enzymatic reactions where the catalytic effect is defined relative to the corresponding solution reaction and, of course, for calculations of ligand binding processes where one has to compare the solvation energy of the ligand in the protein site with the corresponding solvation energy in solution. Early attempts to estimate solvation energies (e.g. refs 63 and 64) were based on the use of the Born or Onsager models with an arbitrary cavity radius. The first attempts to move toward quantitative evaluations of solvation energies can be divided into two branches. One direction involved attempts to examine the interaction between the solute and a single solvent molecule (e.g. ref 65) quantum mechanically. The other direction, which turned out to be more successful, involves the realization that quantitative evaluation of solvation free energies requires parametrization of the solute−solvent van der Waals interaction in a complete solute−solvent system[24] and evaluation of the interaction between the solute and many (rather than one) solvent molecules. Although such an empirical approach was initially considered by the quantum mechanical community as having "too many parameters", it was eventually realized that having an atom-solvent parameter for each type of the solute atoms is the key requirement in any quantitative semiempirical solvation model.

In our view, the successes of calculations of solvation energies of small molecules in solution with a parametrized potential (e.g. refs 24 and 66−69) are very important but, in some respect, obvious. That is, in such cases the environment is uniform, and the solvation free energy is related to the effective atomic radius in a simple way. Thus, reasonable parametrization can usually be accomplished (e.g., see section 3.1 as well as refs 29 and 68). However, the ability to reproduce solvation energies in solution is not a guarantee for reasonable results for the solvation energies of charged ligands in proteins. This issue will be addressed

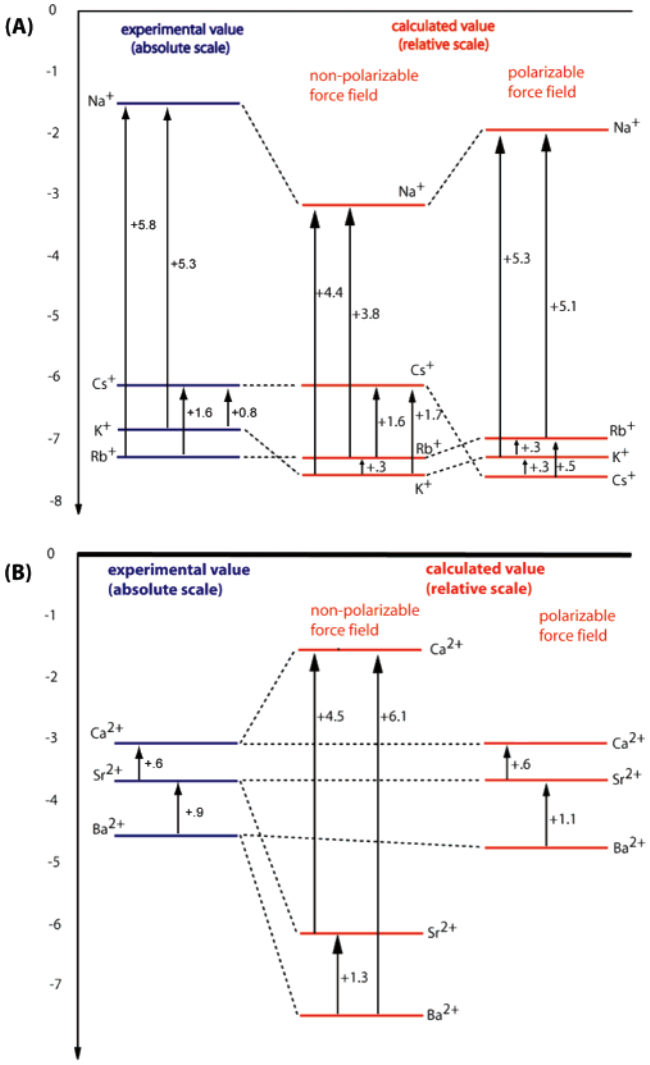

Figure 4. The relative free energies (in kcal/mol) for the binding of cations to valinomycin. The experimental values are shown in blue, while the calculated values are shown in red. The figure gives the results for monovalent (A) and divalent (B) ions. The experimental binding energies are given by reporting the corresponding absolute values, while the calculated values are given as relative energies (e.g., K^+ relative to Na^+). As seen from the figure, both the polarizable and nonpolarizable force fields give reasonable results in (A), while in (B) only the polarizable force field does (e.g., the deviations in the case of $Sr^{2+} \rightarrow Ca^{2+}$ are around 4 kcal/mol).

in subsequent sections. At any rate, since it is always possible to fit parameters that reproduce the solvation of a given molecule, the issue here is whether the use of a polarizable model improves the agreement between the calculated and observed solvation energies in a series of related molecules (where we cannot freely adjust the van der Waals parameters). Some interesting studies along this line were done with the amine series,[69−71] although it is not clear whether the actual agreement was improved by the use of a polarizable model. It is possible that the difficulties in fitting reflect charge transfer to the solvent that has not been accounted for in the models used. Here, the best strategy should probably involve calculations of solvation in small clusters by both ab initio and force field models followed by

adjustment of the force field parameters to reproduce both the solvation in the cluster and in the bulk (e.g. ref 62). Such an approach should allow separation of the charge transfer and inductive effects. At any rate, the parameters obtained by calibration on solvation in solution should be validated when moving to the protein site as was done in the studies described in section 2.

3.2. Evaluation of pK_as of Ionizable Residues in Proteins. Ionizable residues in proteins play a major role in most biological processes including enzymatic reactions, proton pumps, and protein stability. This role involves both the interaction between the ionizable groups and the energetics of the ionization process. Thus, the ability to calculate pK_as of ionizable groups in proteins is crucial in attempts to correlate the structure and function of proteins and to validate different models for electrostatic energies in proteins.[9]

Calculations of pK_as by all-atom FEP approaches have been reported in a surprisingly small number of cases (e.g. refs 52, 72, and 73). Recent works include studies of the pK_a of metal-bound water molecules[74] and proton transfer in proteins[75] as well as functionally important groups (e.g. refs 76−78). All-atom LRA calculations were also reported.[79,80] In only a few cases was any attempt made to actually estimate the error range in these calculations (e.g. ref 81). It appears that the error range of the all-atom models is still somewhat disappointing, although the inclusion of proper long-range treatments and induced dipoles leads to some improvement.[72,79] As far as the effect of induced dipoles is concerned, we would like to clarify that all of the early PDLD studies of pK_as in proteins included explicitly induced dipoles and explored the role of the induced energy (e.g. ref 9). Similarly, most all-atom studies of pK_as in proteins by our group included the use of a polarizable force field.[79] The effect of induced dipoles appeared to be important mainly in the case of ionizable groups in protein interiors (e.g. ref 82).

3.3. Redox Potential of Proteins and Electron Transport Processes. Electron transport processes are involved in key energy transduction processes in living systems (most notably, photosynthesis). Such processes involve changes in the charges of the donor and acceptor involved and are thus controlled by the electrostatic energies of the corresponding charges and the reorganization energies involved in the charge-transfer process. Here, the challenge is to evaluate the redox energies and the reorganization energies using the relevant protein structure. Probably the first attempt to address this problem was reported by Kassner,[83] who represented the protein as a nonpolar sphere. The idea that such a model can be used for analyzing redox properties held on for a long time (see discussion in refs 84−90 and in ref 91). However, the use of the microscopic PDLD model,[92,93] with its self-consistent polarizability treatment, has shown that the evaluation of redox potentials must take the protein permanent dipoles and the penetration of water molecules into account. The role of the protein permanent dipoles has been most clearly established in subsequent studies of iron−sulfur proteins.[94,95] Another interesting factor is the effect of ionized groups on redox potentials. PB studies of redox proteins have progressed significantly since the early studies that considered the protein as a nonpolar sphere (see above). These studies (e.g. refs 84, 87, and 96−98) started to reflect a gradual recognition of the importance of the protein permanent dipoles, although some confusion still exists (see discussion in refs 84 and 90). The realization of the importance of the protein permanent dipoles could not be accomplished in a convincing way without accounting for the effect of the induced dipoles, which has been done in many of the above studies. Microscopic estimates of protein reorganization energies have been reported[31,99,100] and were used very effectively in studies of the rate constants of biological electron transport. This also includes studies of the nuclear quantum mechanical effect associated with the fluctuations of the protein polar groups (for review see ref 101). As far as the role of induced dipoles is concerned, probably the most systematic study to date has been reported by Muegge et al.[99] who explored the dielectric effect in cytochrome *c* for microscopic, semimacroscopic, and macroscopic models. The inclusion of induced dipoles has also been shown to be crucial in studies of photosynthetic systems,[31,101,102] where the correct mechanism was first elucidated theoretically[102] rather than experimentally.

3.4. Electrostatic Effects in Ligand Binding to Proteins. A reliable evaluation of the free energy of ligand binding can potentially play a major role in designing effective drugs against various diseases (e.g. ref 103). Here, there is an interplay between electrostatic, hydrophobic, and steric effects, but accurate estimates of the relevant electrostatic contributions are still crucial. In principle, it is possible to evaluate binding free energies by performing FEP calculations and 'mutating' the ligand to 'nothing' in water and in the protein active site. This approach, however, encounters major convergence problems, and, at present, the reported results are disappointing with the exception of cases of very small ligands. Alternatively, in simple cases one could study the effect of small 'mutations' of the given ligand,[104] for example, a replacement of NH_2 by OH. However, when one is interested in the absolute binding of medium-size ligands, it is essential to use simpler approaches. Perhaps the most useful alternative is offered by the LRA approach augmented by estimates of the nonelectrostatic effects. That is, the LRA approach is particularly effective in calculating the electrostatic contribution to the binding energy.[105,106] With this approximation one can express the binding energy as

$$\Delta G_{\text{bind}} = \frac{1}{2}[\langle U^{\text{p}}_{\text{elec,l}}\rangle_{\text{l}} + \langle U^{\text{p}}_{\text{elec,l}}\rangle_{\text{l}'} - \langle U^{\text{w}}_{\text{elec,l}}\rangle_{\text{l}} - \langle U^{\text{w}}_{\text{elec,l}}\rangle_{\text{l}'}] + \Delta G^{\text{nonelec}}_{\text{bind}} \quad (8)$$

where $U^{\text{p}}_{\text{elec,l}}$ is the electrostatic contribution for the interaction between the ligand and its surroundings, p and w designate the protein and water, respectively, and l and l′ designate the ligand in its actual charged form and the 'nonpolar' ligand (where all the residual charges are set to zero), respectively. In this expression, the terms $\langle U_{\text{elec,l}} - U_{\text{elec,l}'}\rangle$ are replaced by $\langle U_{\text{elec,l}}\rangle$ since $U_{\text{elec,l}'} = 0$. Now, the evaluation of the nonelectrostatic contribution $\Delta G^{\text{nonelec}}_{\text{bind}}$ is still very challenging, since these contributions might not follow the LRA. A useful option, which was used in refs 105 and 106, is to estimate the contributions to the binding

free energy from hydrophobic effects, van der Waals, and water penetration by the PDLD approach. Another powerful option is the so-called linear interaction energy (LIE) approach.[67] This approach starts from the LRA approximation for the electrostatic contribution but neglects the $\langle U_{\mathrm{elec,l}}\rangle_{\mathrm{l}}$ terms. The binding energy is then expressed as

$$\Delta G_{\mathrm{bind}} \approx \alpha[\langle U^{\mathrm{p}}_{\mathrm{elec,l}}\rangle_{\mathrm{l}} - \langle U^{\mathrm{w}}_{\mathrm{elec,l}}\rangle_{\mathrm{l}}] + \beta[\langle U^{\mathrm{p}}_{\mathrm{vdW,l}}\rangle_{\mathrm{l}} - \langle U^{\mathrm{w}}_{\mathrm{vdW,l}}\rangle_{\mathrm{l}}] \quad (9)$$

where α is a constant that is around 1/2 in many cases, and β is an empirical parameter that scales the vdW component of the protein−ligand interaction. A careful analysis of the relationship between the LRA and LIE approaches as well as the origin of the α and β parameters is given in refs 106 and 107.

As far as the effect of induced dipoles is concerned, it seems to us that we are probably not yet at a stage where the inclusion of induced dipoles makes a major difference in binding calculations of neutral molecules, since the convergence problems are still larger than the errors associated with the implicit inclusion of the induced dipoles in the parametrization procedure. However, some of our binding studies did include polarizable force field.[108]

3.5. Enzyme Catalysis. The elucidation of the origin of the catalytic power of enzymes is a subject of big practical and fundamental importance.[1,109−111] The introduction of combined quantum mechanical/molecular mechanics (QM/MM) computational models (e.g. refs 16, 109, and 111−117) provided a way to quantify the main factors that allow enzymes to reduce the activation free energies of the corresponding reactions. QM/MM studies, including those conducted by the empirical valence bond (EVB) method,[1] provided compelling support to the proposal[118] that the electrostatic effects of preorganized active sites play a major role in stabilizing the transition states of enzymatic reactions.[119] In fact, there is now a growing appreciation of this view (e.g. refs 120 and 121). Simulation approaches that focused on the electrostatic aspects of enzyme catalysis (i.e., the difference between the stabilization in the enzyme and in solution) appear to give much more quantitative results than those which focused on the quantum mechanical aspects of the problem but overlooked the proper treatment of long-range effects (see discussion in ref 122). Apparently, some problems can be effectively treated even by PB approaches (see, e.g., ref 123) without considering quantum mechanical issues. Interestingly, evaluation of the activation free energies of enzymatic reactions appeared to be simpler, in terms of the stability of the corresponding results, than other types of electrostatic calculations such as binding free energies (see discussion in ref 124). This advantage has been exploited for a long time in EVB studies (see, e.g., ref 109) and is now being reflected in molecular orbital QM/MM studies (e.g. refs 111, 114, and 125).

Our studies of enzymatic reactions have included explicit treatments of induced dipoles since the initial QM/MM study.[16] In some cases it appeared that one can capture the entire catalytic effect without the use of induced dipoles as long as the focus is on the difference between the reaction in water and the protein active site. However, the inclusion of induced dipoles in simulations of enzymatic reactions has clearly been important in terms of gaining confidence about the importance of electrostatic effects in enzyme catalysis.

3.6. Ion Channels. The control of ion permeation by transmembrane channels underlies many important biological functions (e.g. ref 126). Quantifying the factors that determine ion selectivity by ion channels is a basic problem in protein electrostatics that turns out to be a truly challenging task (e.g. refs 58 and 127). The primary problem is the evaluation of the free energy profile for transferring the given ion from water to the given position in the channel. It is also essential to evaluate the interaction between the conducted ions in the channel if the ion current involves a multi-ion process.[77] Early studies of ion channels focused on the energetics of ions in the gramicidine channel.[32,128] The first microscopic study of this system (or for that matter of any other ion channel) that included all the electrostatic elements of the system (including channel residual charges, channel induced dipoles, solvent, and membrane) explicitly was reported by Åqvist and Warshel.[32] The "solvation" free energy of the system was explored by both the PDLD model and by FEP calculations. The inclusion of the induced dipoles was criticized in ref 33 although the same authors later argued that inclusion of induced dipoles is very important (e.g. ref 129).

The solution of the structure of the KcsA potassium channel[130] provided a model for real biological channels and a major challenge for simulation approaches. Some early studies majorly overestimated the barriers for ion transport (e.g. refs 131 and 132), and the first reasonable results were obtained by the FEP calculations of Åqvist and Luzhkov.[133] These calculations involved the LRF long-range treatment and the SCSSA boundary conditions that probably helped in obtaining reliable results. Microscopic attempts to obtain the selectivity difference between K^+ and Na^+ were also reported.[134] However, these attempts did not evaluate the activation barriers for the two different ions and thus could not be used in evaluating the difference in the corresponding currents. Furthermore, attempts to evaluate the so-called potential of mean force (PMF) for ion penetration, that have the appearance of truly rigorous approaches, have not succeeded in reproducing the actual PMF for moving the ions from water to the channel (see discussion in ref 77).

Our studies of the KcsA potassium channel[53,77] have focused on the evaluation of the selectivity of the ion channel while at the same time using a realistic protein model. It was found that the convergence problems can be overcome in calculations of the energies of the ion binding but become too serious in studies of the activation barriers. Thus, we focused on the use of the semimacroscopic PDLD/S-LRA model combined with Brownian dynamics. However, our studies also involved FEP all-atom calculations of the ion binding using the parameters refined in the procedure described in section 2. These studies also explored the effect of induced dipoles but concluded that in the case of monovalent ions it is reasonable to use nonpolarizable models in view of the fact that the convergence errors are probably larger (at present) than the errors associated with neglect of

the induced effects (considering the fact that the parameters are adjusted accordingly).

3.7. Proton Transport. The discovery of aquaporins and their remarkable role in conducting water molecules through cell membranes has attracted major interest in recent years (e.g. refs 135−137). One of the important questions that has been raised is the origin of the blockage of protons by the aquaporin channels. This issue has been[138] and is continuing to be a major field of interest in the biophysical community.[139−148] Early studies (e.g. refs 139 and 143) suggested that this blockage is due to water orientational effects that disrupt the Grotthuss mechanism.[149−151] However, recent works[140,142,144,145,148,152] came to the conclusion that this is due to the electrostatic barrier, in agreement with our general proposal[153,154] which argued that PTR in proteins is controlled by electrostatic barriers.[155]

Assuming that the above point is generally accepted, we can move to our main subject (which remains quite controversial), namely, the origin of the electrostatic barrier and its magnitude. The controversy reflects significant misunderstanding as well as the diverse background of workers in the field and in some cases even unfamiliarity with the progress in electrostatic calculations. Some authors have attributed the barrier to special structural elements[140,142] and, in particular, to the so-called NPA motif,[138,142,148,152] to the ionized residues,[148] and /or to the helix dipoles.[139,144] On the other hand, Burykin and Warshel (BW) concluded that although the electrostatic barrier reflects all the electrostatic contributions of the channel (polar and nonpolar groups), the barrier will remain very high even when these contributions are removed. The different views can be summarized by a schematic drawing of Figure 2 in ref 155, which presents crucial modifications and clarifications (see below) of a similar illustration that was presented before in ref 144.

At any rate, a recent study[155] examined the origin of the barrier for PTR in aquaporin by semimacroscopic and microscopic calculations and explored the effect of different factors. This study confirmed the BW conclusion and clarified the problems with some of the alternative approaches (e.g., not allowing the protein to relax in Poisson−Boltzmann studies).

Overall, it has been demonstrated that the barrier for PTR in proteins, in general, and in aquaporin, in particular, is determined by the overwhelming reduction in solvation energy upon moving from water to the protein, and this can be modulated by specific electrostatic interactions. The barrier can be eliminated only when the sum of the electrostatic contributions from the protein permanent dipoles, the induced dipoles, and the charges is as large as the solvation in water.

Since the reduction in solvation plays such an important role in PTR in proteins, it is quite obvious that proper microscopic studies of such processes should involve the use of polarizable force fields. In fact, the EVB method[1,156] (that is arguably the most effective current model for treating PTR in a full atomistic way) has included induced dipoles in many of our studies of PT in proteins.[157] Similarly, the adaptation of the EVB by Voth and co-workers has also recently emphasized the need for using polarizable models.[158]

3.8. Helix Macrodipoles versus Localized Molecular Dipoles. The idea that the macroscopic dipoles of alpha helices provide critical electrostatic contribution[159,160] has gained significant popularity and appeared in many proposals. The general acceptance of this idea and the corresponding estimates (see below) are, in fact, a reflection of a superficial attitude. That is, we have here a case where the idea that microscopic dipoles (e.g., hydrogen bonds and carbonyls) play a major role in protein electrostatics[9,118] is replaced by a problematic idea that the source of large electrostatic effects is macrodipoles. The main reason for the acceptance of the helix dipole idea (except the structural appeal of this proposal) is the use of incorrect dielectric concepts. That is, estimates of large helix dipole effects[160−164] involve a major underestimation of the corresponding dielectric constant and the customary tendency to avoid proper validation studies. In more detail, almost none of the attempts to estimate the magnitude of the helix dipole effect have tried to verify this estimate by using the same model in calculations of relevant observables (e.g., pK_a shift and enzyme catalysis). The first quantitative estimate of the effect of the helix dipole[165] established that the actual effect is due to the first few microscopic dipoles at the end of the helix and not to the helix macrodipole. It was also predicted that neutralizing the end of the helix by an opposing charge would have a very small effect. This prediction was confirmed experimentally.[166]

One of the most dramatic recent examples of the need for proper consideration of the helix dipole effect has been provided by the KcsA K^+ channel. The study of ref 167 used PB calculations with $\epsilon_p = 2$ and obtained an extremely large effect from the helix dipoles on the stabilization of the K^+ ion in the central cavity (~ -20 kcal/mol). However, a recent study[53] that used a proper LRA procedure in the framework of the PDLD/S-LRA approach gave a much smaller effect of the helix macrodipole (see Figure 12). Basically, the use of $\epsilon_p = 2$ overestimates the effect of the helix dipole by a factor of 3, and the effect is rather localized on the first few residues. A similar problem occurred with the analysis of the helix dipole in aquaporin where, as stated in section 3.7, it has been suggested that the barrier for PTR is due to the helix dipole.[144,145] However, the careful analysis of ref 155 indicated that the helix macrodipole (or more precisely, its end) only contributes about 4 kcal/mol to the overall barrier. Finally, it is important to note that recent experimental attempts to "neutralize" the effect of the macrodipole in KcsA[168] has confirmed our earlier predictions, as summarized in Figure 5.

The inclusion of induced dipoles either explicitly[165] or implicitly[155] has been a crucial part of the examination of the helix dipole idea, because, in this case, the dielectric effect reduces the helix dipole effect. However, in this respect it is important to point out a misunderstanding that repeatedly appears in some incomplete quantum mechanical studies. There were ab initio attempts to describe the cooperative electrostatic effects, namely, the interaction between charges and collection of amino acids (e.g. refs 169 and 170). These studies concluded that nonadditive effects increase the contribution of the helix dipole and may thus be crucial in enzyme action. Unfortunately, these findings reflect the

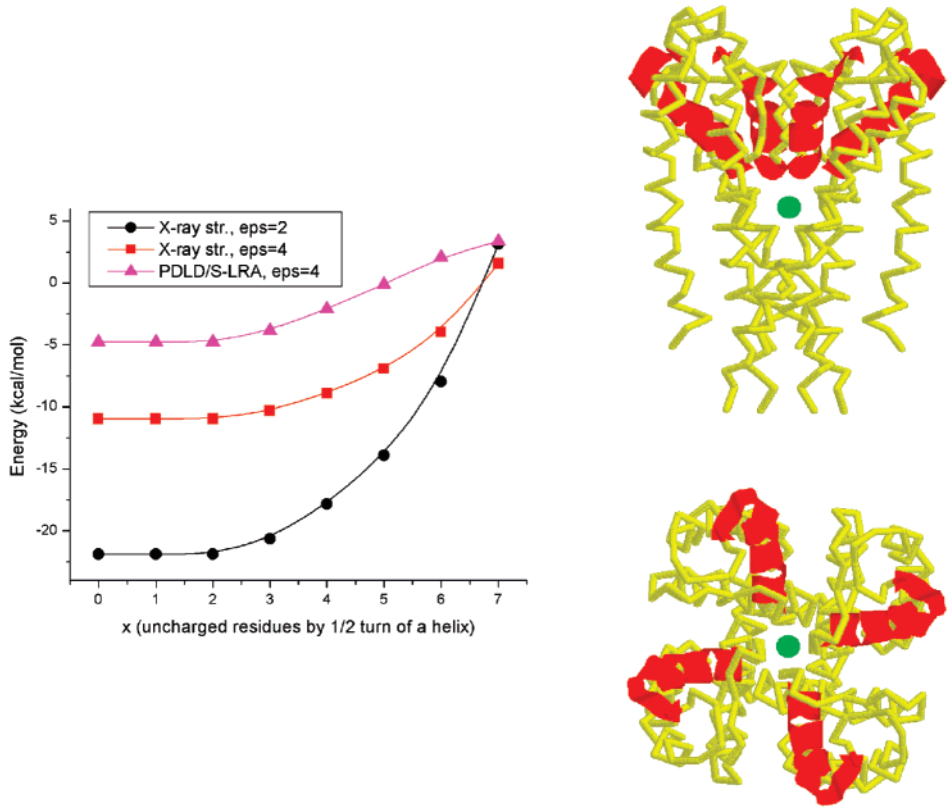

Figure 5. Examination of the effect of the helix dipoles of the KcsA ion channel (upper panel) on a K^+ ion on the central cavity. The lower panel presents the contribution of the residues in the four helices as a function of the dielectric treatment used. It is shown that the use of $\epsilon_p = 2$ drastically overestimates the contribution of the macrodipoles, which is evaluated more quantitatively with the PDLD/S-LRA treatment.

artifact of considering an isolated helix without its surroundings. In this case, the use of a polarizable model (there is no need for any quantum mechanical treatment) demonstrates that the inductive effect *enhances* the interaction. The problem is, however, that most of the dielectric effect comes for the medium around the helix and not from the polarizable matter within the helix (the same is true for the interaction between charges). Thus the effect of the helix dipole is reduced by about one-half due to nonadditive inductive effects when the surrounding is properly included. This fact can be easily verified even in the ab initio studies by embedding the charge and the helix in a polarizable medium.

4. Concluding Remarks

Almost all biological processes are controlled or modulated by electrostatic effects. Thus, the key for quantitative structure−function correlation is the ability to perform accurate electrostatic calculations. Apparently, despite a clear increase in the recognition of the importance of electrostatic effects, there are still significant problems with accepting the need for discriminative validation studies and understanding the relationship between microscopic and macroscopic calculations (see discussion in ref 6).

Nevertheless, one of the issues that is now widely appreciated is the need for polarizable models. This realization is demonstrated by the recent development of many polarizable force fields. However, in some cases we might be overemphasizing the importance of induced dipoles and unjustified in the belief that the reliability problems will disappear once we improve our force field (overlooking convergence issues and other problems).

Despite the advances of polarizable models, there is still a lack of appreciation of simple models that can capture most of the effect of the induced dipoles. For example, in the case of induced dipoles (where the dielectric is small), the noniterative model of WL[16] is very effective, but such models have not been used by the most research groups, with the exception of its adaptation by refs 171 and 172. Similarly, as far as interaction between charges is concerned, it has not been widely realized that the use of Coulomb's law with a dielectric of two is an extremely good approximation even at very close distances (see Figure 13 in ref 9).

Quantum mechanical examinations of the nonadditive effect of induced dipoles are very useful. However, some of these studies have reached incorrect physical conclusions by overlooking hints from simpler approaches. An example is the idea that induced dipoles increase the effect of the helix dipole (see section 3.8). Nevertheless, consistent quantum mechanical studies with QM/MM inclusion of the rest of the environment should be extremely useful in separating the effect of the induced dipoles from the charge-transfer effects.

In conclusion, polarizable force fields offer a practical and effective way of capturing the nonadditive effect of induced dipoles. It is strongly recommended to use such force fields in studies of the charge energetics of protein interiors and in any case where permanent polarization does not account for most of the simulated effect.

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