Theoretical Methods for the Description of the Solvent Effect in Biomolecular Systems

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1. Introduction

The environment plays a key role in the determination of the properties and reactivity of substances in condensed phases. The complexity of chemical phenomena in solution has made it necessary to develop a variety of models and computational techniques to represent molecules in solution. These techniques differ in the level of detail used to describe the chemical system, the physical rules underlying the process of interest, and the mathematical formulas used to describe these rules. The final goal of all these models is the understanding of the behavior of molecules in important environments.

Similar considerations apply to the more specific context of biomolecules. Modern molecular biology has revealed the intimate connection between life processes and the structure and function of biomolecules. Cells can survive owing to the maintenance of well-organized supramolecular structures and very complex macromolecular machinery that mediates fundamental processes, like replication and translation of the genetic code, enzyme catalysis, or the transfer of information through molecular messen-

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gers. The challenge for theoretical chemistry in this field is to link the available 3-D information about biomolecules with their function, which should be explained in terms of the well-defined physics of molecular interactions.

Our purpose is to present a comprehensive review of the current theoretical methods available for the description of molecules in biologically relevant environments, including not only pure solvents, but also large macromolecular systems which have long been recognized as a special type of highly anisotropic solvent.[1,2]

A wide range of techniques has been developed to model solvent effects in biomolecules, and one of the goals here is to summarize the most important features of these computational approaches, emphasizing the basic formalism and offering a critical discussion of their strengths and weaknesses. We aim to provide sufficient information to enable the reader to choose the best method to be used for the study of a particular solvated system.

Because of the rapid progress in computing technology, the number of computational approaches to the description of solvent effects on chemical and biochemical processes has grown considerably. Therefore, we have been very selective in presenting the computational methods, addressing the reader to key references in each section for more comprehensive reviews on the specific area. Thus, the review focuses only on methods which are systematically applied to the study of solvation in large biomolecular systems. Accordingly, most quantum mechanics self-consistent reaction field methods (QM-SCRF), which have been extensively reviewed by other authors,[3,4] are not discussed in detail here. Finally, we should note that the selection of literature to illustrate the potential applications of theoretical methods is by no means exhaustive and references have mainly been extracted from the articles published in the last two years. Thus, we also address the reader to a limited selection of recent reviews[5−8] focused on methods other than the QM-SCRF ones, which can be valuable to complement the information presented here.

We have tried to cover all the main approaches for the description of solvation in systems of biological importance. After a general introduction on the nature of the solvent effect **(**section 2**),** we present discrete−classical (molecular dynamics and Monte Carlo) methods **(**section 3**)**, mixed QM−classical (QM/MM) methods **(**section 4**)**, empirical methods **(**section 5**)**, classical−continuum approaches **(**section 6**)**, and other types of approaches to the description of solvation **(**section 7**)**.

2. The Solvent

A solvent has traditionally been defined as a substance that is liquid under the conditions of application, in which other substances can be dissolved and from which they can be recovered unchanged on removal of the solvent.[9] Many substances can conform to this classical definition, and in fact, the concept can be expanded to binary or multicomponent liquid mixtures. The concept of a solvent can be even more general in biological systems, where large macromolecules can be considered as a special type of solvent.[1,2]

Water, the most abundant component of living organisms, is undoubtedly the most important solvent in biological processes. Liquid water has a complex structure due to the ability of the water molecule to act as both a hydrogen-bond donor and acceptor. The water−water hydrogen bond is very strong, as demonstrated by its experimental gas-phase dimerization enthalpy of -3.6 ± 0.5 kcal/mol.[10] The structure and properties of liquid water are dominated by these hydrogen-bond interactions, which are responsible for the formation of an extended, dynamic hydrogen-bonded network.[11] Biological processes, nevertheless, encompass a wide array of physical and chemical events, such as the transport of substances through membranes, the binding of ligands to receptors, and enzyme-catalyzed chemical reactions.[12−14] Clearly, the traditional concept of a

solvent needs to be enlarged, since in addition to aqueous solutions, biological processes also occur in highly anisotropic environments such as the interior of proteins or in relatively ordered supramolecular structures such as biological membranes. In fact, the control of metabolic processes depends on the ability of the cell to modify the environment of the metabolites, thus influencing their chemical reactivity. Hence, detailed knowledge of the principles governing biological systems cannot be achieved without considering the influence of the surrounding medium on biochemical molecules.

2.1. The Nature of the Solvent Effect

Qualitative knowledge of the solvent effect can be gained from empirical approaches based on specific properties of the solute and solvents. There are several sets of independent solvent characteristics, which include properties such as the level of structure, polarity or softness, electron-pair and hydrogen-bond donor/acceptor ability, polarizability, acidity/basicity, and hydrophobicity/hydrophilicity.[15−26] Standard comprehensive discussions of these properties can be found in the reviews by Reichardt.[27,28] All this information has been exploited in the context of empirical linear free-energy relationships to gain an understanding of the influence of the solvent on a variety of chemical phenomena, including solubility, phase transfer, and chemical equilibria and kinetics.[29−35]

From a microscopic point of view, solvation involves the formation of a set of interactions between a solute and a solvent as well as a change in the interactions of the solvent molecules in the vicinity of the solute. Thus, a key step in the understanding of solvation is the determination of the structure adopted by solvent molecules around the solute, since the nature and strength of the associated interactions is intimately connected to the macroscopic properties of the solvated system. It is not surprising, then, that a large body of work focuses directly on elucidating the solvent shell structure around the solute.[36−55] Particular attention has been paid to the structure of the hydration shell around ions[56−66] and to the solvation of hydrophobic solutes.[67−71]

For quantitative treatments of solute−solvent interactions, it is convenient to adopt Ben-Naim's definition of a solvation process.[72−74] According to Ben-Naim, the solvation of a solute can be defined as the process in which a particle of the solute is transferred from a fixed position in the gas phase into a fixed position in solution at constant temperature, pressure, and solvent composition. The free energy of solvation (ΔG_{sol}), the key parameter in describing solvation,[75−81] is then defined as the reversible work spent in the transfer of the solute under those conditions at equal numeral densities in the gas phase and in solution. According to this definition, ΔG_{sol} incorporates both the free-energy contributions related to direct solute−solvent interactions and those arising from internal changes in the solute and solvent upon solvation.

Conceptually, the free energy of solvation can be determined through the addition of several contributions, as noted in the seminal works by Huron and Claverie.[82−84] Generally, the solvation process is partitioned into three different steps: cavitation, dispersion−repulsion, and electrostatics (see eq 2.1 and refs 3, 4, 85−87). The first step is the formation of a cavity large enough to accommodate the solute within the solvent. Since this is accomplished by breaking down the cohesive forces between solvent molecules, the free energy of cavitation (ΔG_{cav}) is unfavorable to solvation. In the second step, dispersion−repulsion (also denoted as van der Waals) forces between the solute and solvent molecules are 'switched on'. These forces, which are universal and apply to both solute and solvent molecules, contribute favorably to solvation (ΔG_{vw}), since the solute cavity is created in regions where the dispersion forces are stronger than the repulsive forces. These two terms, cavitation and dispersion−repulsion, are often referred to as steric or nonelectrostatic contributions. Finally, the third step considers the electrostatic contribution (ΔG_{ele}). This later term measures the work spent in building up the charge distribution of the solute in solution. ΔG_{ele} includes two components: (i) the work necessary to create the solute's gas-phase charge distribution in solution and (ii) the work required to polarize the solute charge distribution by the solvent. It is worth noting that the electrostatic contribution includes not only the gain of the electrostatic interaction energy between the solute and solvent molecules, but also the work needed to generate the solvent reaction field induced by the solute charge distribution. Within the framework of the linear free-energy response theory,[88] the electrostatic free-energy contribution to solvation is one-half of the solute−solvent electrostatic interaction energy.

$$\Delta G_{sol} = \Delta G_{ele} + \Delta G_{vw} + \Delta G_{cav} \qquad (2.1)$$

The breakdown of the solvation process into steps has facilitated the development of formalisms that enable an accurate understanding of the solvation features for different solutes and solvents.[3,4,85−87] Thus, for polar solvents such as water, the dispersion contribution is moderate and cannot fully counteract the cavitation term, whereas for apolar solvents, the cavitation work is smaller (in absolute terms) than the dispersion−repulsion contribution due to the weaker interactions between solvent molecules. For apolar solutes, in apolar solvents the steric term can be the main contribution to solvation, since solute−solvent electrostatic interactions are weak, and the dispersion term favoring solvation can compensate for the cavitation work, which is not very large in apolar solvents. On the contrary, for most solutes of interest, in polar solvents such as water the electrostatic term makes the dominant contribution to the free energy of solvation, due to the strength of solute−solvent electrostatic interactions.

A direct application of the free energy of solvation concept can be found in the determination of the partition coefficient (P) of a given solute between two immiscible solvents, typically water and an apolar organic solvent. This parameter is related to the free energy of transfer ($\Delta\Delta G_{transfer}$) of the solute between

the two solvents (eq 2.2). The partition coefficient is very useful for understanding differential solvation effects[89−103] and has been used to explain biological and pharmacological phenomena like adsorption and transport of substances through cell membranes as well as hydrophobic bonding ability.[104−110]

$$\log P = -\frac{\Delta\Delta G_{\text{transfer}}}{2.303RT} = -\frac{\Delta G_{\text{sol,org}} - \Delta G_{\text{sol,wat}}}{2.303RT} \quad (2.2)$$

2.2. Solvent Effects on Electronic Properties

The solute−solvent interactions established upon solvation affect the internal (nuclear and electronic) degrees of freedom of the solute. For a given nuclear configuration, the transfer of the solute from the gas phase to a condensed phase changes the electron distribution of the solute, thus altering its chemical properties. This change is manifested in diverse properties, such as the lengthening in the dipole moment,[111,112] the change in the molecular electrostatic potential,[113−118] the variation in the molecular volume,[119] and even the spin density.[4] The extent of the electronic polarization effect is clearly reflected in the enlargement of the dipole moment of water upon condensation, which changes from 1.855 D for an isolated water molecule[120] to 2.4−2.6 D in the condensed phase.[121,122] In general, the enhancement of the dipole moment upon solvation has been estimated to be 20−30% of the gas-phase values for neutral solutes in aqueous solution.[111,112,123] Significantly larger solvent-induced charge redistributions have been reported for push−pull π-conjugated molecules.[124] Even in less polar solvents such as chloroform, the solvent-induced polarization is not negligible, as indicated by the dipole moment increases of 8−10% which have been determined for neutral molecules.[125] As noted by Cramer and Truhlar,[4] an in-depth knowledge of these effects would be valuable when examining the relationship between cavity definition and electrostatics in QM continuum models. However, the experimental measurement of the solute polarization in high dielectric media is problematic. In lieu of these data, comparing the QM continuum model results with the results provided by discrete theoretical methods can provide insight into the effects of the solvent on the solute charge distribution.[123]

2.3. Solvent Effects on Nuclear Distribution

The solvent also can change the nuclear configuration of the solute as a result of the tendency of polar solvents to stabilize structures with large charge separations.[87,126−129] The solvent-induced changes to local geometrical parameters (i.e., the enlargement of carbonyl bonds upon hydration) are moderate. Of more importance are the changes that the solvent can induce in the general molecular conformation of the solute. Thus, the solvent can change the conformational population of the solute with respect to its gas-phase state. There are numerous, recent examples in the literature that show the relevance of the solvent effect in modulating the conformational space of different solutes, including among others[130−132] small- and medium-size organic molecules,[133−145] carbohydrates,[146−149] and peptides.[150−161]

2.4. Solvent Effects on Spectroscopic Properties

The impact of a solvent on the spectroscopic characteristics of a solute has been the subject of an intense research effort, which is difficult to concisely summarize here for space limitations. We address the reader to specialized references[27,28,162−201] for a more detailed treatment of this important issue.

The effect of solvation on electronic transitions in the ultraviolet or visible range can be explained by considering the differential solvation of the ground and excited states, which depends on the change in the solute charge distribution in these two states.[27,28,162−164] Since electrons are expected to be less tightly held in the excited state, the solute's charge separation should decrease upon its transition from the ground to an excited state, and the solute−solvent electrostatic interactions are thus generally expected to cause a blue shift in the spectra. On the other hand, dispersion effects tend to favor the excited state, since it is usually more polarizable than the ground state, and this tends to produce red shifts upon solvation. According to the Franck−Condon principle, vertical transitions will alter the solute's electron charge distribution but not its internal geometry, so the cavitation component is expected to have little effect on the spectra. Overall, the direction of a solute's spectral shift upon solvation depends on the relative polarity of the solute in both its ground and excited states as well as on the solvent's polarity and polarizability.

The extreme velocity of photoexcitation precludes any large-scale solvent reorganization during this process. Therefore, for an absorption process, the excited state of the solute "fits" into the cage structure which the solvent molecules adopted to accommodate the ground-state charge distribution of the solute. Accordingly, this process corresponds to a nonequilibrium solvation phenomena,[165−172] since only electronic (noninertial) relaxation of the solvent molecules occurs. Similar considerations apply to downward vertical transitions, i.e., fluorescence. Much recent literature is focused on the theoretical description of these processes,[173−186] especially excited-state proton transfers.[187−191]

There are also notable solvent effects on vibrational spectra[192−196] which depend on the magnitude of both nonspecific and specific, i.e., hydrogen bond, solute−solvent interactions. Theoretical representation of these effects is especially difficult, since it is necessary to separate the inertial and noninertial portions of the solvent response,[3] and this requires a careful evaluation of both the nature of the solute vibrations and the properties of the surrounding medium.[197−199] We address the reader to specialized references[197−201] for a more complete explanation on this issue.

2.5. Solvent Effects on Tautomerism and Acidity/Basicity

Tautomerism is another extremely solvent-dependent chemical process that affects the activity of

biomolecules. Two classic examples are the keto/enol equilibrium of (i) β-diketones, in which the enol form is the most populated species in apolar solvents whereas the keto species is the most stable tautomer in aqueous solution,[28] and (ii) 4-pyridone, in which the population ratio between the keto and enol tautomers changes by a factor of 10^4 upon its transfer from the gas phase to an aqueous solution.[202] Several recent studies have focused on the tautomeric equilibria of small organic compounds,[203−208] of heterocycles,[128,209−212] and especially of nucleic acid bases and compounds structurally related to them.[45,46,213−220] Theoretical and experimental data show that polar solvents generally displace the tautomeric equilibrium so as to increase the population of the most polar tautomer, and this effect can be large enough to change the intrinsic tautomeric preference.

Acid/base equilibria also play a key role in most chemical and biological processes. The acid/base properties of a solute in solution depend on a subtle balance between (i) the intrinsic acidity/basicity of the solute in the gas phase and (ii) the ability of the solvent to stabilize neutral and ionized species. Clearly, the former is expected to be predominant in very apolar solvents and the latter in polar solvents. This balance is illustrated by the ionizable side chains of amino acids, whose acidity/basicity is strongly dependent on their exposure to solvent. Thus, a single ionizable residue tends to be in its neutral form when buried in the very apolar environment in the interior of the protein, while its ionized form is expected to predominate when the side chain is exposed to solvent.[221] The importance of ionization in chemical and biochemical processes along with the difficulty of experimentally analyzing it have stimulated the use of theoretical methods to predict solvent effects on the intrinsic acid/base properties of molecules. The prediction of absolute pK_a values is extremely difficult, since the gas-phase proton affinity and the free energies of solvation of neutral and ionic species are difficult to accurately determine.[222−225] The theoretical determination of relative pK_a values for related compounds is more feasible.[97,226−233]

2.6. Solvent Effects on Reactive Processes

A solvent influences chemical reactivity by (i) modulating the intrinsic characteristics of the reactants, (ii) introducing friction, and (iii) differentially stabilizing reactants, products, and transition states. As already noted, changes in the intrinsic reactive properties of a solute are mainly related to polarization of its charge distribution and will not be further examined here.

Friction, including both mechanical and dielectric effects, greatly influences the dynamics of molecular systems.[3] At 300 K, a typical hydrogen bond between a pair of water molecules will separate and form new contacts with other neighbors within a few picoseconds. Introducing solute molecules influences the motion of water in several different ways. A highly charged ion will orient first hydration shell water molecules about itself, and these will have large residence times compared to bulk water. The mobility of the solutes is, in turn, limited by their interactions with water molecules. Thus, over very short periods of time, small solutes will exhibit small-amplitude motions within their solvation cages, whereas at longer times, rearrangement of the hydrogen-bond network permits greater solute displacements. Collisions of the solute with solvent molecules interrupt these displacements, giving rise to frictional effects, which modulate solute diffusion and effective barriers to internal motion in flexible molecules.[234,235] Likewise, solvent friction that occurs when the energy barrier is crossed in a reactive process has a remarkable influence on the rate constant for the reaction.[236−240]

The interaction of solvent molecules with reactants, products, and transition states can affect a chemical reaction, especially if the reaction occurs in a polar solvent that interacts strongly with the reactive species. This can be especially important when there are large differences in the polarities of the reactants, transition state, or products. A classic example that illustrates this solvent effect is the SN_2 reaction, in which water molecules induce large changes in the kinetic and thermodynamic characteristics of the reaction. Another example is provided by the nucleophilic attack of an $R-O^-$ group on a carbonyl center, which is very exothermic and occurs without an activation barrier in the gas phase but is clearly endothermic with a notable activation barrier in aqueous solution.[241−244]

A survey of the recent literature provides numerous examples of the influence of the solvent on the thermodynamics and kinetics of chemical reactions. In particular, attention has been paid to proton-transfer reactions'[245−249] hydrolysis,[250−256] Diels−Alder and cyclization reactions,[257−261] ring opening,[262] decarboxylation,[263−265] and other reactions.[266−279]

2.7. Solvent Effects on Molecular Association

Whether or not molecular association occurs between solute molecules is largely dependent on the solvent. Thus, in polar solvents such as water, polar structures will be well solvated due to nonspecific (dipole−dipole and dipole−induced dipole contacts) and specific (hydrogen-bonding) interactions with the solvent. Under these conditions, the association of two solute molecules will disrupt many favorable solute−solvent electrostatic interactions and thus will make an unfavorable (electrostatic) contribution to the complex formation. On the other hand, since the association reduces the size of the solute cavity, the unfavorable steric contribution will decrease due to the reduction of the cavitation term. Bimolecular association also leads to the loss of translational and rotational freedom and even to the freezing of internal rotations, thus giving rise to an unfavorable entropic contribution, which must be overcome by stabilizing noncovalent interactions. The interactions of the solute with the solvent may affect the entropy of dimerization.[3,280−283] Thus, solute rotation dynamics can be modulated by the disruption of specific interactions of the solute with the solvent molecules.[284] The couplings between large-amplitude motions of the solute can also be altered upon solvation. Overall, while the association of polar

solutes can occur in apolar solvents, many polar solute associations will be disfavored in high-dielectric media.

An even more dramatic effect of solvation on molecular association is the solvent-induced change in the configurational space of noncovalent complexes. Thus, the relative arrangement of interacting molecules depends on the properties of the solvent. This is illustrated, for instance, in the subtle balance between hydrogen-bonded and stacking-type interactions which occur in the association of nucleic acid bases in aqueous solution versus those which occur in the gas phase.[220,285−293] This balance currently constitutes a challenge for theoretical methods. Another example of a solvent effect on molecular association is the stability of salt bridges in peptides and proteins, which depends on the degree of their exposure to solvent but also depends on the microscopic local environment when they are buried in the interior of proteins.[294−298] There are also several recent examples of the impact of the solvent on the dimerization of a variety of neutral polar molecules.[299−303]

2.8. Solvation and Biomacromolecules

Biomacromolecules can be considered to behave as if they were a special anisotropic solvent surrounding a small solute (the ligand). Alternatively, they can be considered in their entirety as a solute immersed in an aqueous solvent or biological membrane. Solvent effects are thus expected to be crucial in determining both the structure and reactivity of biomacromolecules.

Nucleic acids illustrate the impact of the environment on the structure of biomacromolecules. DNA is surrounded by a dense cationic atmosphere,[304−308] which influences its helical conformation[309−311] and the stability of unusual DNA forms.[312−314] Hydration of the DNA is also very specific, as evidenced by the existence of the "spine of hydration" located in the minor groove of the physiological B-DNA.[315,316] Hydration is vital to maintaining the active conformation of DNA, as shown by the change from active (B-) to inactive (A-) conformations induced by partial dehydration[317−320] as well as by the role of hydration in modulating cation binding to bases.[321] The solvent also affects the structure and functions of proteins,[12−14,235] as recognized in the exploitation of nonaqueous enzymology in biotechnology research and development.[322−325]

Biomacromolecules are dynamic structures, and the structural fluctuations occurring in these molecules affect their biological activity.[235] Because proteins and nucleic acids are structurally complex, they undergo a very wide range of internal motions, from fast, localized processes to large-scale collective motions such as protein folding. One example is the involvement of "breathing" events in the approach of substrates to, and the release of products from, the active site of enzymes.[326−328]

The effect of water molecules on the structure of macromolecules is essential to their functioning. In fact, liquid water has been referred to "as the lubricant of life",[329] since water molecules "catalyze" rapid conformational fluctuations by a repertoire of transient hydrated conformations, which in turn provide a low-energy pathway between the conformational states of biomacromolecules.[330−332] Information on the role of the solvent in this process can be gained from theoretical simulations of the flexibility of peptides in solution, which in turn can help us understand the nucleation of secondary structural elements in proteins.[333−339] The effect of the solvent on the configurational space sampling of biomolecules is particularly important in protein folding.[340−351]

Many biological processes are mediated by ligand binding. The binding free energy is determined by a balance between two contributions: (i) the hydration of the ligand−receptor complex relative to the hydration of the separated ligand and receptor and (ii) the change in the free energy related to the interaction between receptor and ligand. Binding can be interpreted as a change in the surrounding environment (water ↔ receptor for the ligand and water ↔ ligand for the receptor), which emphasizes the importance of solvation effects on ligand−receptor binding. In fact, biological receptors can be thought of as highly inhomogeneous, anisotropic "solvents".[1,2,352−358]

3. Classical Discrete Models: Monte Carlo and Molecular Dynamics

The most obvious way to reproduce the solvent effect is to surround the solute with a large number of solvent molecules, which are represented at the same level of atomic detail as the solute. The dynamic nature of the solvated system is then represented by means of Monte Carlo (MC) or molecular dynamics (MD) algorithms.

3.1. Basic Theory

The molecular interactions of solvated systems can be represented at three different levels: (i) pure quantum mechanical (QM), (ii) pure classical (MM), and (iii) mixed (QM/MM) levels. The pure QM treatment is the most rigorous approach a priori, but in practice, it is not useful for systems of biochemical interest because of the tremendous cost of QM simulations for very large systems, even when low levels of QM theory are used. The QM/MM approach defines the solute or a small part of a large solute (i.e., the active site of an enzyme) at the QM level, while the rest of the system (including the solvent) is represented at the classical level. QM/MM methods can be used to study reactivity in macromolecules or to analyze fine details of the solvation of small solutes (see below). However, for most applications in biochemistry the pure classical methods are still the obvious choice.

Classical MC and MD methods rely on force fields, which are parametrized to describe molecular interactions. For biochemical studies, the force fields used are similar to those shown in eqs 3.1−3.7, where the total energy is the sum of "bonded" (eqs 3.1−3.5) and "nonbonded" (eqs 3.6 and 3.7) terms. The bonded terms account for changes in the potential energy resulting from the modification of bond lengths (stretching), angles (bending), and dihedrals (proper

and improper torsions). The nonbonded terms account for electrostatic and van der Waals interactions between atoms which are at least three bonds apart.

$$E_{pot} = E_{str} + E_{bend} + E_{tor} + E_{itor} + E_{vw} + E_{ele} \quad (3.1)$$

$$E_{str} = \sum_{str} K_1(L - L_0)^2 \quad (3.2)$$

$$E_{bend} = \sum_{bend} K_b(\Theta - \Theta_0)^2 \quad (3.3)$$

$$E_{tor} = \sum_{tor} \sum_{n} \frac{V_n}{2}[1 + \cos(n\Phi - \alpha)] \quad (3.4)$$

$$E_{itor} = \sum_{tor} \frac{V_{itor}}{2}[1 - \cos(2\Phi)] \quad (3.5)$$

$$E_{vw} = \zeta_{vw} \sum_{k,l} \left[\left(\frac{A_{kl}}{r_{kl}^{12}}\right) - \left(\frac{B_{kl}}{r_{kl}^{6}}\right)\right] + \sum_{i,j} \left[\left(\frac{A_{ij}}{r_{ij}^{12}}\right) - \left(\frac{B_{ij}}{r_{ij}^{6}}\right)\right] \quad (3.6)$$

$$E_{ele} = \zeta_{ele} \sum_{k,l} \frac{Q_k Q_l}{r_{kl}} + \sum_{i,j} \frac{Q_i Q_j}{r_{ij}} \quad (3.7)$$

In eqs 3.1−3.5, K_l and K_b are stretching and bending force constants, L_0 and Θ_0 are equilibrium lengths and angles, Φ stands for dihedral (proper or improper) angles, α is the phase angle, n is the periodicity of the Fourier term, V_n is the proper torsional barrier for the nth Fourier term, and V_{itor} is the improper torsional barrier. In eqs 3.6 and 3.7, A and B represent van der Waals parameters, Q are charges, r_{ij} are interatomic distances, and ζ is the 1−4 scaling factor.

Once the potential energy is computed using eqs 3.1−3.7, Boltzmann samplings are obtained using Metropolis−Monte Carlo[78,81,359−368] or Newtonian molecular dynamics.[80,369−371] The configurational space of the solvated system is typically defined using internal coordinates in MC simulations, while Cartesian coordinates are used in most MD simulations. Owing to their use of internal coordinates, MC techniques allow fine control of the configurational space, which is ideal for the study of small molecules in solution. Although several authors have reported the use of MC simulations in macromolecular systems represented at the atomic level,[372−375] most studies of macromolecules use MD.

Many force fields have been developed for the study of macromolecules in solution.[376−390] Despite the simplicity of their formalism, the latest force fields[384−390] provide reliable descriptions of biomolecular systems, partly due to the higher accuracy of the reference data used in the force-field parametrization. Force-field parameters for solvents, which are typically obtained by fitting simulation results to the experimental data of pure liquids, provide quite consistent descriptions of most static properties of solvents. However, they do not give reliable representations of some key properties such as the dielectric constant of the pure liquid.[391−395] The models of solvents which are currently used were mostly developed in the 1970s and 1980s,[396−402] and there is a general consensus that not much improvement to them is possible unless the formalism of the force field is modified (see below).

3.2. Information Gained from MD(MC) Simulations

MD (or MC) simulations provide very valuable information about the structural and dynamic behavior of biomolecules. In the following discussion we note some information that can be gained from MD simulations, which is often difficult to obtain using other simulation techniques, and point out areas where future advances are needed.

3.2.a. Average Structural Information

The analysis of trajectories provides the time-averaged configuration of the solute. For nonequilibrium processes such as protein folding and unfolding, the analysis of the structures sampled during the trajectory allows us to examine the time evolution of the molecular system.[79,403−410]

3.2.b. Solute Conformational Flexibility

Large solutes such as proteins or nucleic acids are flexible in solution, and their functional role is often dependent on these structural fluctuations. MD trajectories can be used to examine the dynamics of biomolecules. Principal component analysis (PCA) is applied to the MD trajectories to identify the most important movements in the macromolecules.[327,411] It is also possible to compute the entropy difference between two stable states of a macromolecule using techniques such as the covariance matrix.[412,413] Under some assumptions, this can be used to gain insight into concepts such as "preorganization", "rigidity", and "entropy trapping", whose importance has been described in several works.[414−416]

3.2.c. Solvent Structure

MD (or MC) provides an acceptable picture of the solvent structure around a solute. For small spherical solutes, the solvent structure can be represented by radial distribution functions (eq 3.8). For macromolecules, 3-D distribution functions (df) also can be defined as shown in eqs 3.8 and 3.9,[417−423] where the 3-D space around the macromolecule has been divided into small volume elements (i,j,k). In eqs 3.8 and 3.9, N_y stands for the number of solvent molecules found when sampling in the spherical layer located between the distances r and $r + dr$ from the solute, ρ_y is the density of the pure solvent (y), and i,j,k represent a grid element of dimensions l_i, l_j, and l_k.

$$g(r) = \frac{\langle N_y(r, r + dr)\rangle}{4\pi\rho_y r^2\, dr} \quad (3.8)$$

$$df(i,j,k) = \frac{\langle N_y(i,j,k)\rangle}{l_i l_j l_k \rho_y} \quad (3.9)$$

Distribution functions can be used either to obtain structural details of the solvent around the solute or

to estimate solvation free energies. 3-D distribution functions, which are computed on a grid defined by the MD-averaged structure of a macromolecule, can be integrated to determine the preferential solvation free energy of a particular site on the macromolecule, as shown in eq 3.10, where the sum includes all the grid elements that define the state of interest.

$$\Delta G_{sol} = -RT \ln\left(\sum_{i,j,k}^{state} df(i,j,k)\right) \qquad (3.10)$$

In our experience eq 3.10 provides suitable estimates of the preferential solvation free energies when (i) the state of interest can be defined in terms of the grid elements and (ii) no dramatic conformational change in the macromolecule takes place during the trajectory. Other cases contain too much noise to ensure an acceptable estimate of the free energy.

3.2.d. Energy Analysis

The total energy of a solvated system varies markedly during the trajectory, which limits its quantitative use. However, most of the statistical noise stems from solvent−solvent interactions, and MD-averaged values for solute−solute and solute−solvent interactions often can be determined with only moderate noise by performing long MD simulations. It is then possible to define a "pseudo-energy" function by postprocessing and averaging the MD trajectories, as noted in eq 3.11, where the free energy of solvation (ΔG_{sol}) is computed for the entire trajectory using continuum models (see below). Recent studies have exploited this approach to rationalize the differences in the stabilities of different conformations of a given macromolecule.[424−427] The most serious shortcomings of this method are (i) the nonnegligible noise in the averages and (ii) the difficulty in coupling discrete solute−solute interactions with continuum solvation calculations.

$$E = \langle E^{force-field}_{solute-solute} \rangle + \langle \Delta G_{sol} \rangle \qquad (3.11)$$

The potential energies collected from MD trajectories also can be examined using methods based on extended linear response theory.[88,369,428−432] The steric components of solvation (see eq 3.12) can then be determined empirically from the solvent-accessible surface (cavitation) and from the solute−solvent van der Waals (dispersion−repulsion) interaction energy. In eq 3.12, s stands for the solute and x for the solvent, α and β are empirical factors, and SAS is the solvent-accessible surface of the solute.

$$\Delta G_{sol} = \frac{1}{2}\langle E^{sx}_{ele} \rangle + \alpha SAS^{s} + \beta \langle E^{sx}_{vw} \rangle \qquad (3.12)$$

Although linear response theory accurately represents the free-energy change associated with the generation of a charge in an homogeneous solvent, it might not be suitable for the representation of "solvation" in a macromolecule. Accordingly, the term $^1/_2$ in eq 3.12 should be replaced by another empirical parameter, γ in eq 3.13, which must be optimized in conjunction with α and β.[428,433]

$$\Delta G_{sol} = \gamma \langle E^{sx}_{ele} \rangle + \alpha SAS^{s} + \beta \langle E^{sx}_{vw} \rangle \qquad (3.13)$$

Extended linear free-energy response methods have been combined with standard thermodynamic cycles (see Figure 1) to determine the differences

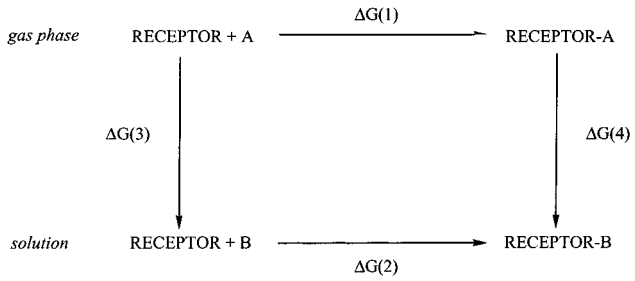


Figure 1. Thermodynamic cycle used to determine the difference in binding free energy.

between the binding free energies of related compounds (eq 3.14).[428−433,434,435] Initial studies in this area were performed by Lee et al.[436] However, the use of the technique is limited since (i) the statistical noise in the averages of the solute−solvent interaction energies is nonnegligible, (ii) intrasolute contributions to binding are not included, and (iii) α, β, and γ have to be parametrized for each protein of interest.

$$\Delta\Delta G^{A-B}_{binding} = \Delta\Delta G^{A-B}_{sol}(protein) - \Delta\Delta G^{A-B}_{sol}(water) \qquad (3.14)$$

3.3. Free-Energy Perturbation and Thermodynamic Integration

The combination of statistical mechanics and MD or MC samplings allows the computation of free-energy differences between two related states by using several algorithms. The most popular methods used in solvation studies are free-energy perturbation (FEP) and thermodynamic integration (TI), which will be very briefly introduced here. However, other related algorithms such as potential of mean force, umbrella sampling, slow growth, weighted histograms, or multiconfigurational thermodynamic integration have also been used to compute free-energy differences in solution.[78,80,368,437−443]

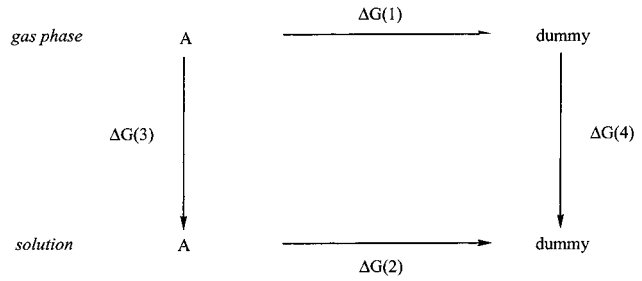
According to FEP, the free-energy difference when a system changes from state P to state Q can be computed using eq 3.15, while TI determines this difference using eq 3.16. In both cases, λ is a coupling parameter which controls a smooth change from state P to state Q (see eq 3.17, where H stands for the Hamiltonian that defines states P or Q). The change from P to Q takes place through an artificial route taking advantage of the state function nature of the free energy. Note that eqs 3.15−3.17 provide the difference in Gibbs free energy under the assumption that the change in the PV term is zero.

$$\Delta G^{P\rightarrow Q} = -\sum_{\lambda=0}^{1-\Delta\lambda} RT \ln \langle \exp -(E_{\lambda+\Delta\lambda} - E_{\lambda})/RT \rangle_{\lambda} \quad (3.15)$$

$$\Delta G^{P\rightarrow Q} = \sum_{\lambda=0}^{1-\Delta\lambda} [\int_{\lambda}^{\lambda+\Delta\lambda} \langle \partial E_{\lambda'}/\partial\lambda' \rangle_{\lambda'} \, d\lambda] \quad (3.16)$$

$$H_{\lambda} = (1-\lambda)H_P + \lambda H_Q \quad (3.17)$$

FEP and TI can be used to estimate the solvation free energy,[444−447] which is defined as the difference between the free energies associated with the annihilation of a molecule in the gas phase and in solution (see eq 3.18 and Figure 2). Likewise FEP and



ΔGsol(A) = ΔG(3)= ΔG(1)+ΔG(4)-ΔG(2)

Figure 2. Thermydynamic cycle used to compute the free energy of solvation.

TI, together with eq 3.14, can be used to determine the difference between the binding free energies of two molecules.[78,81,366−368,448]

$$\Delta G^{A}_{sol} = \Delta G^{\text{gasphase}}_{A\rightarrow\text{dummy}} - \Delta G^{\text{solution}}_{A\rightarrow\text{dummy}} \quad (3.18)$$

FEP and TI are rigorous techniques and do not require additional empirical parameters other than those implicit in the force field. These methods can reproduce the free energies of hydration for small neutral solutes with average errors of around 1 kcal/mol.[444,447] These errors are only slightly higher than those found using the most refined versions of self-consistent reaction field methods[3,4,449,450] or extended linear response theory,[428−433] both of which require much more accurate calibration. The range of application of FEP and TI is limited, however, since states P and Q in eqs 3.15−3.17 have to be similar to reduce the statistical noise in the free-energy estimates. Thus, they are widely used to determine binding free-energy differences for similar molecules but are not used when the two molecules are very different.

3.4. Recent Improvements in MD and MC Simulation

MD and MC simulations are very powerful for representing biomolecular systems, but they have several technical and formal problems. Current research in the field aims to overcome these limitations.

3.4.a. Accuracy of the Force Fields

Force fields are parametrized using a mixture of experimental and theoretical data.[376−390] Owing to obvious computational limitations, the early generations of force fields were parametrized using low-level QM calculations, which sharply limited their accuracy. Several groups[384−390] recently addressed the force-field reparametrization using higher level QM calculations, which has improved the results obtained from the calculations. However, most of the current force fields are limited by (i) the use of a reduced set of charges to represent Coulombic interactions and (ii) the neglect of polarization effects.

Potentials derived from atom-centered point charges are too isotropic and limit the accuracy of the force field by preventing us from detecting all the details of the real molecular charge distribution.[451−454] The introduction of atomic dipoles may improve the charge representation,[452,453] but the force-field calculation is much slower. An alternative is to use multicentric charges,[451,454] which are located not only at the nuclei, but also along chemical bonds or lone pair axes. The use of multicentric charges notably reduces the errors in the electrostatic potential, with only a moderate loss of computational efficiency.[454] However, the use of multicentric charges in force-field calculations is limited by their conformational dependence and by the need to reparametrize the van der Waals parameters when the charges are located outside of nuclei.

The introduction of polarization is one of the major challenges affecting the development of new force fields. Force fields typically use effective pair potentials, which are assumed to account for the effect of the average solvent polarization on the solute charge distribution. However, these potentials are not suitable for representing atoms in different environments or the external and internal parts of proteins.[455] The strategy most widely used to introduce polarization effects is to use the induced dipole theory. According to this approach, the total electrostatic energy of a system is equal to the standard Coulombic potential generated by fixed charges plus a polarization term (E^{ind}) that results from the interaction between the induced dipoles and the permanent field (see eqs 3.19 and 3.20). In eqs 3.19 and 3.20 the index 0 refers to the static charge distribution, E_i^0 is the unpolarized field, and $\Delta\mu_i$ is the induced dipole generated on atom i.

$$E_{ele} = E^0 + E^{ind} \quad (3.19)$$

$$E^{ind} = -{}^1/_2 \sum_i \Delta\mu_i E_i^0 \quad (3.20)$$

The induced dipole $\Delta\mu$ is computed using eq 3.21, where the total field E is obtained by adding the permanent field and the polarization contribution determined from the induced dipoles (eq 3.22). In eqs 3.21 and 3.22, α stands for the atomic polarizability and T is the dipole tensor.

$$\Delta\mu_i = \alpha_i E_i \quad (3.21)$$

$$E_i = E_i^0 + \sum_{j\neq i} T_{ij}\Delta\mu_j \quad (3.22)$$

The use of the induced dipole theory in the study of macromolecules is hindered by its computational cost, which results from both the extra computational effort needed to compute dipole interactions and the interdependence of the induced dipole and the total electric field (see eq 3.19−3.22). Furthermore, the accuracy of the estimated polarization energy which is obtained is limited by several technical problems arising from (i) the use of isotropic polarizabilities, (ii) the transfer of atomic polarizabilities to different molecular environments, (iii) the short-range coupling between induced dipoles, and (iv) the neglect of hyperpolarizability effects. Strategies to solve these problems and increase the efficiency and accuracy of the calculation are explained elsewhere.[402,456−463]

One of the finest alternative approaches for introducing polarization into classical calculations is the fluctuating charge model.[463−466] This approach relies on the principle of electronegativity equalization, which considers that charges are not fixed but instead can flow along the atoms. The optimum set of charges to use is the one that minimizes the total energy functional (E_T) shown in eq 3.23, subject to a charge neutrality constraint. Note that this constraint can be imposed at the level of a fragment, a molecule, or a system, which allows for the charge migration between fragments or molecules.[463]

$$E_T = \sum_i \sum_\alpha \left[E_\alpha(0) + \tilde{\chi}^0_\alpha Q_{i\alpha} + \frac{1}{2} J^0_{\alpha\alpha} Q^2_{i\alpha} \right] + \sum_{i\alpha<j\beta} J_{\alpha\beta}(r_{i\alpha j\beta}) Q_{i\alpha} Q_{j\beta} + E_{vw} \quad (3.23)$$

In eq 3.23, i and j stand for molecules, α and β represent atoms, χ^0 is the Mulliken electronegativity, E_α is the ground-state energy of atom α, $J^0_{\alpha\alpha}$ is twice the hardness of the electronegativity of atom α, and $J_{\alpha\beta}$ is the Coulomb interaction between atoms α and β. $J_{\alpha\beta}$ is calculated as a simple $1/r$ function for intermolecular interactions ($i \neq j$) and as a Slater-based overlap matrix for intramolecular interactions ($i = j$).

For fluctuating charge calculations, the charge elements are treated as "particles" that move from one atom to the other, mimicking the Carr−Parrinello QM molecular dynamics treatment,[467,468] in accordance with Newton's laws, the electroneutrality principle, and the energy functional in eq 3.23. Versions of the method have been adapted to the MC framework[464] and modified to include polarizable dipoles.[469]

The fluctuating charge model has been successfully applied to the study of small solvents. Water models fail in the description of out-of-plane polarization, however, which represents a major limitation of the method.[470] Furthermore, this model has not yet been systematically used for macromolecular systems. This is likely due to (i) the difficulty in parametrizing all the terms in eq 3.23 for large molecules and (ii) the increased computational effort required when a large number of intramolecular Coulombic interactions have to be computed.

3.4.b. Long-Range Effects

Electrostatic interaction decays very slowly with distance. Thus, the use of spherical cutoffs could lead to important errors in MD or MC simulations due to (i) the sharp transition at the cutoff distance and (ii) the neglect of electrostatic interactions beyond the cutoff. Several strategies based on the use of switching and shifting functions[383,471,472] have been developed to smooth the electrostatic function at the cutoff boundary. Other strategies which have been used to capture long-range electrostatic interactions rely on the use of[473−482] (i) continuum models to represent the solvent effect[473−478] and (ii) Ewald strategies.[479−482] Both continuum and Ewald methods include long-range effects quite accurately with only a moderate increase in computational effort. Nevertheless, they are not always useful for treating these effects in FEP and TI calculations. For instance, net charge generation or annihilation (i.e., solvation of ions) cannot be properly computed by this method because of the complexity involved in considering the contribution to the free-energy change due to the solvent molecules beyond the cutoff.

3.4.c. Time Scale

Some biomolecular processes occur on a time scale of seconds, which is far longer than the time scale that can currently be addressed using MD simulations. Recent advances in computer science, along with the advent of more efficient parallel codes, have enabled us to perform MD simulations for proteins on a microsecond time scale,[348] i.e., 10^5-10^6 times longer than what was considered to be "state of the art" just a few years ago.[404,405] However, another increase of 10^5-10^6 is still needed to reach the time scale of biologically relevant phenomena. Current approaches[483−486] to increasing the computational efficiency in MD and MC simulations rely on algorithms designed for massive parallel computers, the use of dual time step integration methods, reducing the complexity of the potential functional, and applying continuum treatments to the solvent (see below).

4. Quantum Mechanics/Molecular Mechanics Methods

4.1. General Principles

Quantum mechanics/molecular mechanics (QM/MM) methods combine quantum mechanical and molecular mechanical approaches to study systems that (i) are too large for full QM treatments and (ii) cannot be properly described by strictly classical methods because they involve large electron density redistributions or the breaking or formation of chemical bonds.[7,8,487−497] To simulate these systems, QM/MM methods treat all the atoms that are directly involved in the chemical process at the QM level while the rest of the system is described using MM force fields. Before discussing their general principles, let us distinguish between combined QM/MM and hybrid QM/MM methods.[498] The former name is generally used to denote those QM/MM methods

where the solute is treated at the QM level and the MM part includes the surrounding solvent molecules. Conversely, the hybrid designation is usually used for those QM/MM methods which partition a single, large molecule into an active part that is treated at the QM level and an assisting MM portion, which implies the "cutting" of one or more covalent bonds. This cutting feature makes it necessary to resort to more complex treatments in hybrid methods than in combined methods (see below).

The Hamiltonian of the whole system can be defined as the sum of three terms (eq 4.1) corresponding to the QM subsystem, the MM subsystem, and the coupling between the QM and MM regions.

$$\hat{H} = \hat{H}_{QM} + \hat{H}_{MM} + \hat{H}_{QM/MM} \tag{4.1}$$

The coupling term in eq 4.1, $H_{QM/MM}$, includes both electrostatic, H_{ele}, and nonelectrostatic, H_{vdW}, contributions (eq 4.2). The nonelectrostatic component accounts for dispersion and repulsion between QM and MM atoms and is represented by a van der Waals expression, as shown in eq 4.3, where S and X are the total interaction sites in the QM and MM systems, A_{sx} and B_{sx} are the van der Waals parameters, and r_{sx} stands for the interatomic distance between the sites s and x, respectively.

$$\hat{H}_{QM/MM} = \hat{H}_{ele} + \hat{H}_{vdW} \tag{4.2}$$

$$\hat{H}^{vdW}_{QM/MM} = \sum_{s=1}^{S}\sum_{x=1}^{X}\left[\left(\frac{A_{sx}}{r_{sx}^{12}}\right) - \left(\frac{B_{sx}}{r_{sx}^{6}}\right)\right] \tag{4.3}$$

The electrostatic interaction energy between the QM and MM subsystems can be expressed at four levels of increasing complexity.[499] These levels consist of (i) the interaction between the unperturbed wave function of the QM subsystem and the nonpolarizable MM charges, (ii) the mechanical embedding of the QM region, (iii) the mechanical embedding plus the polarization of the QM wave function by the permanent field which results from the MM charges, and (iv) the addition of the polarization of the MM part to the embedding and polarization described in iii.

At the simplest level there is no coupling between the QM and MM subsystems. The electrostatic energy is simply the Coulomb interaction between the QM electrostatic potential and the MM charges, which is given by eq 4.4 within the MO-LCAO framework for a closed-shell system. (The index '0' stresses that the QM wave function is not polarized by the surrounding MM charges.) Accordingly, these techniques do not fit into the general notion of QM/MM methods, even though they are still valuable for gaining qualitative insight into the reactive characteristics of molecules.[500−503]

$$E_{ele,0} = \sum_{s=1}^{S}\sum_{x=1}^{X}\frac{Z_s Q_x}{|r_s - r_x|} - \sum_{\mu=1}^{Nocc}\sum_{\nu=1}^{Nocc}\sum_{x=1}^{X} P_{\mu\nu}\left\langle \phi_\mu \left| \frac{Q_x}{|r - r_x|} \right| \phi_\nu \right\rangle \tag{4.4}$$

In eq 4.4, μ,ν denote the basis set of atomic orbitals, N_{occ} is the number of doubly occupied molecular orbitals, $P_{\mu\nu}$ is the $\mu\nu$ element of the first-order density matrix, Z_s is the effective nuclear charge, r_s and r_x are the position vectors for the QM nuclei and the MM particles, respectively, and Q_x stands for the set of charges that represents the charge distribution in the MM region.

The next higher level includes the mechanical embedding of the QM region, as in the IMOMM method reported by Maseras and Morokuma.[504] This method involves an interpolation between independent QM and MM calculations, where the interaction between the QM and MM subsystems is described by a force field. Owing to the lack of electronic embedding of the QM region, these techniques are more appropriate for studying apolar than polar systems. However, the model is simple and robust and can be easily generalized using different layers treated at suitable (QM or MM) levels of theory.[505] These features make this approach very promising for a variety of chemical systems.

Relaxation of the QM wave function by the electric field created from MM charges (the third level of complexity) is achieved by adding the operator shown in eq 4.5 to the gas-phase Hamiltonian of the QM subsystem, H_{QM} (eq 4.6). This leads to a pseudo-Schrödinger equation (eq 4.7), which is solved using the standard self-consistent process,[506] and yields the total energy of the system (E_{tot}) and the polarized wave function of the QM subsystem. In eqs 4.5−4.7, N is the number of electrons, where i,j and s,t denote electrons and nuclei, respectively, and Ψ is the normalized wave function that minimizes the energy of the total Hamiltonian, H, given in eq 4.1.

$$\hat{H}_{ele} = \sum_{s=1}^{S}\sum_{x=1}^{X}\frac{Z_s Q_x}{|r_s - r_x|} - \sum_{n=1}^{N}\sum_{x=1}^{X}\frac{Q_x}{|r - r_x|} \tag{4.5}$$

$$\hat{H}_{QM} = -\frac{1}{2}\sum_{i=1}^{N}\nabla_i^2 - \sum_{s=1}^{S}\sum_{i=1}^{N}\frac{Z_s}{|r_i - r_s|} + \sum_{j<i}^{N}\frac{1}{|r_i - r_j|} + \sum_{s<t}^{S}\frac{Z_s Z_t}{|r_s - r_t|} \tag{4.6}$$

$$E_{tot} = \langle\Psi|\hat{H}|\Psi\rangle = E_{QM} + E_{MM} + E_{QM/MM} \tag{4.7}$$

Finally, the energy of the MM subsystem is determined by means of the molecular mechanical potential energy, which generally contains both bonded and nonbonded terms, as mentioned in section 3.

At the highest level of complexity, relaxation of the MM charge distribution by the electric field created from the QM subsystem and from the rest of MM charges is also included. As pointed out in section 3, this can be achieved by using the induced dipole theory and distributed atomic polarizabilities[487,494,507,508] in the MM region (see eqs 3.19−3.22) or by using fluctuating charge models (see eq 3.23).[463,465,466,470,507−511] Recently, an alternative method to capture many-body polarization effects has been developed for NDDO (neglect of diatomic differential

overlap)-HF wave functions.[512] In this method, each individual molecule in the liquid is represented by an antisymmetric determinant wave function and the interactions between each molecule and its surrounding molecules are determined by a hybrid QM/MM approach. Selected parameters are optimized so that they reproduce the experimental thermodynamic properties of the liquid, which allows for the correction of the electron-correlation effects not explicitly included in the QM calculations.

Because the polarizations of both the MM charges and the QM wave function are mutually dependent, the polarization contribution of the whole system should be determined self-consistently.[513,514] Even though the computational implementation of the necessary equations is quite straightforward, the resulting computational process is very time-consuming and cannot be applied to the study of chemical reactions in biomolecules. Since most of the usual force fields do not contain polarization terms, only the relaxation of the QM subsystem is typically considered. A more reliable approach to QM/MM methods would be to develop classical polarizable force fields for use with them (see section 3).

Classical molecular computations often include a boundary region that simulates the bulk solvent which surrounds the system of interest. The inclusion of this boundary region improves the description of long-range electrostatic effects beyond what can be achieved using the simple truncation scheme. The boundary region can be treated by the Ewald lattice-sum technique, by reaction field approaches, and by fast multipole methods, as mentioned in section 3. When employing QM/MM methods, the usual boundary approximations can be used, even though little attention has yet been devoted to these boundary effects. Gao and Alhambra recently implemented the Ewald−lattice-sum method in QM/MM methods.[515] Warshel utilized the surface-constrained all-atoms model,[516−518] where the simulation system is surrounded by a spherical boundary of explicit solvent molecules, which are constrained to have the same polarization as they would have in an infinite solvent system represented by an all-atom model.

4.2. Implementation of QM/MM Methods

There is a priori no limit to the level of theory that can be utilized for the treatment of the QM subsystem. Thus, implementations of QM/MM hybrid methods within the framework of ab initio[504,519−524] or density functional (DF)[522,525−535] methods have been reported. Although the computational costs of the ab initio and DF calculations are similar, the latter has the advantage of including electron-correlation effects. The application of ab initio methods to the QM/MM formalism requires computation of one-electron integrals involving the set of MM charges in the Fock matrix, as shown in eq 4.8, where μ,ν refer to the basis set of atomic orbitals.

$$F_{\mu\nu}^{sx} = -\sum_{x=1}^{X} Q_x \int \frac{\phi_\mu^*(r)\phi_\nu(r)}{|r - r_x|} dr \qquad (4.8)$$

Likewise, when DF methods are applied, the interactions with the MM charges are introduced via the one-electron Kohn−Sham equations (eq 4.9) through the external potential $\nu(r)$. In eq 4.9, ψ_i is a one-electron Kohn−Sham orbital, e_i is the associated eigenvalue, and the Hamiltonian H_{DF} is given by eq 4.10, where $\rho(r)$ is the electron density, E_{XC} is the exchange-correlation functional, and $\omega(r)$ is the external potential, which adopts the form given by eq 4.11. In eq 4.11, the first and second terms on the right-hand side represent the interaction of electrons with the nuclear charges and with the MM charges, respectively.

$$\hat{H}\Delta G\ \psi_i(r) = e_i\psi_i(r) \qquad (4.9)$$

$$\hat{H}_{DF} = -\frac{1}{2}\sum_{i=1}^{N}\nabla_i^2 + \nu(r) + \int \frac{\rho(r')}{|r - r'|} dr' + \frac{\partial E_{XC}}{\partial(r)} + \sum_{s<t}^{S}\frac{Z_s Z_t}{|r_s - r_t|} + \sum_{s}^{S}\sum_{x}^{X}\frac{Z_s Q_x}{|r_s - r_x|} \qquad (4.10)$$

$$\nu(r) = -\sum_{s=1}^{S}\sum_{i=1}^{N}\frac{Z_s}{|r - r_s|} - \sum_{x=1}^{X}\sum_{i=1}^{N}\frac{Q_x}{|r - r_x|} \qquad (4.11)$$

Even though the treatment of electrostatic interactions is well defined in both ab initio and DF-QM/MM methods, the determination of van der Waals parameters to represent dispersion−repulsion interactions between QM and MM sites (see eq 4.3) is not. Early QM/MM methods directly transferred the MM van der Waals parameters to the QM atoms, but this approach yields erroneous distances between QM and MM atoms. To overcome this limitation, the van der Waals parameters for QM atoms have to be reparametrized, and this is usually achieved by imposing the condition that the QM/MM interaction energies must reproduce the interaction energies of small clusters or bimolecular complexes which have been determined at a high level of theory.[494,519,536−538] The use of these optimized QM van der Waals parameters give better results than does the use of MM values. However, even these parameters do not transfer well between very different interacting systems.[538] An alternative option is to fit the van der Waals parameters to reproduce the free energies of solvation;[539,540] however, these fitted parameters have similar problems with transferability. On the whole, the transferability of the van der Waals formalism to the QM framework appears to be unsatisfactory.

The widespread application of ab initio and DF implementations of QM/MM methods is limited by the cost of the calculations. Thus, relatively small basis sets, typically split-valence sets[519,535] supplemented with polarization functions,[520,526,528,532−534] have to be used. One useful approach is the empirical valence bond (EVB) method developed by Warshel and co-workers.[414,541,542] In this approach the different states in a chemical reaction are described in terms of valence-bond configurations. For practical purposes, the number of resonance structures to be considered can be reduced using an appropriate parametrization that retains an accurate description

of the potential-energy surface for the conversion from reactants to products. In this calibration procedure, the elements of the Hamiltonian are calculated using empirical formulas which depend on the relative position of the nuclei and contain several parameters fitted to reproduce experimental (or high-level theoretical) data either in the gas phase or in solution. The EVB method has proven to be valuable in studying a range of reactive processes in biomolecular systems. One example is provided by the recent work of Bentzien et al.,[521] wherein a hybrid QM(EVB)/MM potential surface is applied to the prediction of activation free energies for the amide hydrolysis in subtilisin.

Most QM/MM studies of biomolecules are still carried out within the framework of semiempirical methods (especially AM1[543] and PM3[544−546] Hamiltonians).[547−570] These studies address a wide range of concerns, including solvation, conformational flexibility, spectroscopic processes in solution and in macromolecules, and chemical reactivity in solution and for enzymes. However, the use of semiempirical Hamiltonians in QM/MM methods has some potential limitations, and these require that the results be carefully analyzed to validate this computational scheme.

One major drawback of semiempirical QM/MM methods is the lack of parameters to describe particular atoms in the system of interest. Another is that semiempirical methods do not adequately describe some types of bonding arrangements.[543−546] Various strategies have been designed to overcome these limitations. In some studies, a mixture of parameters from different semiempirical QM methods has been used to describe the QM atoms in the system.[570] Another approach is to reoptimize the parameters associated with the semiempirical QM method.[571] A third strategy is to scale the semiempirical energy so that it reproduces the free energy along the reaction path as determined at a high level of theory.[572]

An additional limitation of semiempirical QM/MM methods is that they do not have a clear definition of the electrostatic potential. In ab initio or DFT methods, the electrostatic potential is unequivocally computed from the wave function of the QM system and the Coulomb operator.[573,574] Nevertheless, since the latest versions of semiempirical Hamiltonians rely on the NDDO approximation and simplified functional forms are used to evaluate core−core, one-electron, and two-electron integrals, several options for computing the semiempirical electrostatic potential are available.[575−583]

Field et al.[547] proposed that the electronic contribution to the electrostatic potential, V^{el}_{QM}, can be obtained using an NDDO-based expression (eq 4.12) where the Ohno−Kloppman parameters that account for the damping of classical Coulomb interactions for the MM atom are assigned a value of zero. Furthermore, for the nuclear contribution, V^{nuc}_{QM}, they adopted an expression related to the core−core interaction (eqs 4.13−4.15), where only one parameter was retained per MM atom type, α_x. This parameter was set to 5.0 in order to achieve the highest fit to ab initio data.

$$V^{el}_{QM} = -\sum_{\mu,\nu} P_{\mu\nu}(\mu\nu|s^x s^x) \tag{4.12}$$

$$V^{nuc}_{QM} = \sum_{s=1}^{S} Z_s\left[(s^s s^s|s^x s^x) f(s,x) + \frac{1}{r_{sx}} g(s,x)\right] \tag{4.13}$$

$$f(s,x) = 1 + e^{-\alpha_s r_{sx}} + e^{-\alpha_x r_{sx}} \tag{4.14}$$

$$g(s,x) = \sum_i K_{s,i}\, e^{-L_{s,i}(r_{sx}-M_{s,i})^2} + \sum_j K_{x,j}\, e^{-L_{x,j}(r_{sx}-M_{x,j})^2} \tag{4.15}$$

In eq 4.12, s^x denotes a notional s orbital placed on the MM atom and μ and ν are atomic orbitals centered on the QM atom. In eqs 4.14 and 4.15 α, K, L, and M are parameters that depend on the atom type.

Thompson[513] used a similar approach to that taken by Field et al., with the main difference being the exclusion of Gaussian expansion terms in the function $g(s,x)$ for both QM and MM atoms. Ford and Wang[584] and Bakowies and Thiel[585] also ruled out Ohno−Klopman parameters for the MM atom and the Gaussian expansion terms. They did, however, introduce parameters (ω_s and δ_s) into the exponential function $f(s,x)$ (see eqs 4.16a and 4.16b) which were optimized to reproduce the HF/6-31G(d) electrostatic potential and field in suitably chosen regions of space.

$$f(s,x) = 1 + e^{-\omega_s r_{sx}} \tag{4.16a}$$

$$f(s,x) = 1 + e^{-\omega_s (r_s - \delta_s)} \tag{4.16b}$$

In the approach reported by Vasilyev et al.,[586] a scaling parameter (0.095 for MNDO and AM1, 0.097 for PM3) was introduced to correct the magnitude of the Ohno−Kloppman factor and the extra terms in the core−core energies were omitted. In agreement with the formalisms adopted in several self-consistent reaction field models,[587,588] Cummins and Gready used expressions where the Ohno−Klopman parameters for both QM and MM atoms were set to zero.[539,540] They also omitted the functions $f(s,x)$ and $g(s,x)$, so that the interaction energy followed a pure Coulombic model. Finally, Théry et al.[589] adopted an approach whereby the electronic contribution follows the usual semiempirical expression without any additional simplification, but the nuclear contribution to the interaction energy of a MM site is expressed as the addition of two terms (eq 4.17). These terms represent the interaction between the QM core and the core, Z_x, of a classical pseudo-atom bearing an "implicit electronic population" given by $P_x = Z_x - Q_x$. In eq 4.17, the first term corresponds to the semiempirical core−core repulsion for QM and MM atoms with core charges Z_s and Z_x and the second term represents the interaction between the quantum core Z_s and the implicit electron population of the classical atom, P_x.

$$Q_x V_{QM}^{nuc} = \sum_{s=1}^{S} Z_s Z_x \left[(s^s s^s | s^x s^x) f(s,x) + \frac{1}{r_{sx}} g(s,x) \right] - Z_s P_x (s^s s^s | s^x s^x) \quad (4.17)$$

As can be seen from the preceding discussion, the expression used must be chosen to balance the electronic and nuclear contributions to the QM/MM electrostatic interaction energy. Otherwise, the quality of the results for the semiempirical QM/MM method, which depend on the systems and properties investigated, could be affected.

4.3. Separating QM and MM Regions

Regardless of the level of theory applied to the QM subsystem, QM/MM methods always require separation of the QM and MM subsystems. Clearly, a natural separation exists for processes involving small solutes in solution, since the solute is treated quantum mechanically and the solvent molecules form the MM region. However, the way in which the system should be partitioned for chemical reactions in biomolecules is less obvious. In this case, the division between the QM and MM subsystems necessarily implies partitioning covalent bonds, and this leads to serious methodological problems. Different strategies have been proposed to overcome this difficulty.

The early QM/MM methods followed the "link atom" approach,[488,489,547] which defines an additional "nonphysical" link atom, typically a hydrogen, in the QM portion to satisfy the free valence of the broken covalent bond that separates the QM and MM regions. Unfortunately, the use of this approach in QM/MM calculations presents several drawbacks.[499,590] Since the link atom is included in the self-consistent treatment of the QM subsystem, explicit corrections to the energy must be made to account for any interactions involving the link atom. In addition, there is some controversy over how to treat the QM/MM interactions involving the link atoms. Likewise, the link atom corrections work reasonably well for geometries close to the optimized QM structures, but they are not suitable for geometries far from the minimum, where they can yield artificial results from geometry optimization calculations. Finally, this approach can also lead to unstable simulations, which require consistent total energies and gradients.

An alternative option, which minimizes the artifacts resulting from the separation of the QM and MM regions, was reported by Maseras and Morokuma.[503] In their approach, the QM subsystem is saturated with link atoms and optimized and then the MM subsystem is also optimized in the presence of the frozen QM fragment. This process is repeated until the energy of the entire system converges. However, this multistage approach does not reproduce the coupling between the QM and MM regions in the optimization process. To solve this problem, Bersuker et al.[591] suggested using an additional, intermediate QM region to smooth the transition between the QM and MM subsystems.

A different approach to partitioning the QM and MM regions was adopted by Warshel and Levitt,[487] who included a single hybrid orbital with a single electron for each of the QM atoms at the junction between the QM and MM regions. The remaining interactions for these atoms were treated using MM terms. Similarly, the group at Nancy has proposed using the local self-consistent method[592] to treat frontier bonds. The basic assumptions of this method are (i) that these bonds have constant and well-defined properties and (ii) that each is a simple bond and can be described by a strictly localized bond orbital. This orbital is expressed as a linear combination, with constant coefficients, of two well-defined hybrid orbitals, one on each atom of the bond. The orthogonal hybrid orbitals determined for small model compounds are assumed to be fully transferable, so that they can be kept frozen in the actual QM/MM calculation. This approach is easy to implement in semiempirical QM/MM methods[592,593] but becomes more complex in ab initio methods.[594] A generalization of the local self-consistent approach has recently been reported by Gao et al.[595]

The division between QM and MM regions is obviously a key factor in the application of QM/MM methods to the study of chemical processes in biomolecules. As a general guideline, the QM/MM boundary should be as far away from the reactive center as is computationally feasible in order to minimize results. However, this is not always possible and appropriate procedures that provide well-defined QM/MM potential-energy surfaces for modeling large systems need to be further examined.

4.4. QM/MM in Molecular Dynamics and Monte Carlo

Since analytical expressions are available to handle each of the different contributions to the total energy of the system, the forces acting on the atoms can then be calculated from the derivatives of the energy with respect to the coordinates of QM and MM atoms (eqs 4.18a and 4.18b, where α stands for each of the Cartesian coordinates of QM and MM atoms). This derivation is quite straightforward, especially if nonpolarizable potentials are used. The resulting energy and the associated forces can be applied to geometry optimizations and molecular dynamics simulations.

$$F_{s,\alpha} = -\frac{\partial E_{TOT}}{\partial R_{s,\alpha}} \quad (4.18a)$$

$$F_{x,\alpha} = -\frac{\partial E_{TOT}}{\partial R_{x,\alpha}} \quad (4.18b)$$

QM/MM models are even more easily implemented with Monte Carlo codes. However, this approach can be less efficient because the energy and wave function of the QM subsystem must be recomputed for each new configuration. (Note that for equivalent calculations the number of different configurations generated by MC methods is always severalfold larger than those generated by MD techniques.) Strategies used to reduce this problem include (i) the use of spherical cutoffs, such that solvent movements outside the cutoff radius do not affect the QM wave function,

even though the total energy is affected, and (ii) repetition of the SCF calculations only when the solute moves.[573]

The implementation of QM/MM methods using either molecular dynamics or Monte Carlo approaches allows the potential-energy surface to be sampled in exactly the same way as with purely classical methods, as mentioned in section 3.

4.5. Recent Advances

A series of very recent studies have focused on new procedures to minimize some of the problems of QM/MM methods and to expand their range of applicability.

The accuracy of QM/MM methods largely depends on the characteristics of the coupling Hamiltonian used. As already noted, one of the procedures utilized to improve the QM/MM Hamiltonian is to reparametrize the Lennard−Jones parameters on QM atoms so that they reproduce high-level theoretical results for molecular complexes. A recent study has raised the question of whether the parameters derived from molecular complexes are really suitable for condensed matter simulations.[596] The results show that the choice of the van der Waals parameters for QM atoms has a large effect on the QM/MM results. More surprisingly, the authors show that parameters fitted to small complexes lead to rather weak coupling between the QM and MM molecules. These results stress the need to carefully select the van der Waals parameters for the QM subsystem and suggest that more appropriate procedures should be designed for that purpose.

Better partitioning schemes for the junction between the QM and MM regions might be obtained by using the alternative pseudo-bond method.[597] Instead of using a hydrogen atom to saturate the free valence as in the link−atom approach, the pseudo-bond approach forms a pseudo-bond with the QM atom by replacing the MM atom in the broken covalent bond with a one-free valence boundary atom (X_{ps}) that has a parametrized effective core potential. The X_{ps} atom is included in the QM subsystem and, except for the MM atom of the frontier covalent bond, the rest of the atoms in the environment form the MM subsystem. In designing the effective core potential of X_{ps}, the pseudo-bond QM−X_{ps} is made to mimic the original QM−MM frontier bond, such that it has a similar bond length and strength as well as similar effects in the rest of the QM regions. Likewise, to ensure charge consistency and to avoid false electrostatic interactions, a zero-point charge is assigned to the MM atoms directly bonded to or up to two bonds away from the QM subsystem. Unlike the link−atom approach, this method does not consider any additional atoms and avoids double counting of the interactions. Thus, it provides a consistent definition for the energy and forces of the whole QM/MM system.

The adjusted connection atom method developed by Antes and Thiel[598] is another recent approach to dividing a system into its QM and MM parts. This method is conceptually similar to the preceding pseudo-bond approach. The adjusted connection atom replaces the MM atom in the frontier covalent bond and can be regarded both as an especially parametrized QM atom that is able to interact with the other QM atoms and as a standard MM atom that is able to interact with the other MM atoms. Following the implementation of this approach using semiempirical methods, the parameters for the connection atom are calibrated to reproduce theoretical QM reference data such as energies, geometries, dipole moments, and charges. This parametrization ensures that the adjusted connection atom remains at approximately the same position as the MM atom that it replaced in the frontier bond and that it maintains similar electronic properties in the QM subsystem. As noted for the pseudo-bond method, no additional atoms are incorporated into the original system; thus, any double counting of the interactions is avoided and well-defined potential-energy surfaces are obtained.

Many current studies are aimed at increasing the efficiency of the configurational sampling in QM/MM simulations. This sampling is seriously hindered by the cost of the QM/MM calculations, since relaxation of the QM region by the MM electric field requires millions of SCF calculations. An alternative strategy suggested for QM/MM methods coupled to Monte Carlo techniques is to introduce polarization effects by means of a perturbational treatment, referred to as generalized molecular interaction potential with polarization (GMIPp).[599,600] This treatment, which was developed on the basis of the original work by Francl,[601] avoids recalculating the wave function of the QM subsystem for each configuration of the MM subsystem. This is achieved by using a perturbative expression to evaluate the polarization of the QM subsystem by the MM charges, as noted in eq 4.19, where ϵ denotes the molecular orbital energies and $c_{\mu i}$ is the coefficient of atomic orbital μ in the molecular orbital i. Computer time also can be saved by choosing suitable cutoffs for the energy differences between occupied and virtual orbitals and for the integrals in eq 4.19. This approach is currently used with ab initio QM/MM methods.[602]

$$\sum_{i}^{\text{occ}}\sum_{j}^{\text{vir}} \frac{1}{\epsilon_i - \epsilon_j}\left\{\sum_{\mu}\sum_{\nu} c_{\mu i} c_{\nu j}\left\langle \phi_\mu \left| \sum_x \frac{Q_x}{|r_x - r|} \right| \phi_\nu \right\rangle\right\}^2 \quad (4.19)$$

Finally, the application of QM/MM methods has been extended to study dynamic effects in an enzyme-catalyzed reaction. In this example, the semiclassical variational transition-state theory with multidimensional tunneling contributions[237,238,603] was applied to the QM/MM potential-energy surface for the proton-transfer reaction in yeast enolase.[604] This combined treatment provides an alternative to current approaches and allows the inclusion of quantum effects on enzyme reactions.[605−607]

5. Empirical Approaches

Empirical methods treat the solvent at a very low computational cost by employing simple equations together with parameters fitted to experimental data or to high-level calculations. For the study of biological systems, empirical methods have been developed

at two levels: (i) screening of the electrostatic interactions between the elements of the system and (ii) solvation of each element of the system. Only some of the most common empirical approaches will be outlined here.

5.1. Methods for Electrostatic Screening

The solvent molecules surrounding a solute reorient their charge distribution to react against the solute charge distribution. This generates a reaction field which modulates the interaction of the solute with other molecules (see Figure 3). The magnitude

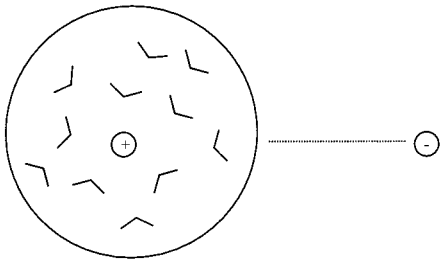


Figure 3. Schematic representation of the solvent screening effect.

of the electrostatic interactions is thus reduced, since the orientation of the solvent molecules opposes the electric field generated by the solute.

For homogeneous solvents and very dilute solutions, the screening effect could be represented using a macroscopic dielectric constant (eq 5.1, where i and j denote two atoms of the solute separated by a distance of r_{ij}). Unfortunately, for biologically relevant systems, the effective dielectric constant depends on the distance between the charged groups.[75,354,608,609] When two atoms are close to each other, solvent molecules cannot fill the space between them and their microenvironment is similar to that of a gas phase. However, when two atoms are far apart, solvent molecules fill the space between them and screen their electrostatic interactions.

$$E_{\mathrm{ele}}^{\,i-j} = \frac{q_i q_j}{\epsilon r_{ij}} \tag{5.1}$$

The solvent screening effect can be modeled by replacing the macroscopic permittivity with a distance-dependent dielectric function, of which linear dependence is the simplest formalism.[610−616] Thus, ϵ is defined as shown in eq 5.2, where EPS is a constant factor that is generally assigned values from 1 to 4.5. This type of dielectric function was widely used in early MD simulations of nucleic acids and proteins when implicit solvent molecules were not used.

$$\epsilon(r_{ij}) = \mathrm{EPS}\ r_{ij} \tag{5.2}$$

Warshel (eq 5.3)[76] along Olson and co-workers (eq 5.4)[617] developed more complex functions in which the permittivity changes exponentially with the distance. The factor (1 ± 0.5) in eq 5.3 was introduced to account for variations in $\epsilon(r_{ij})$ for different systems.

$$\epsilon(r_{ij}) = \{1 + 60[1 - \exp(-0.1 r_{ij})]\}(1 \pm 0.5) \tag{5.3}$$

$$\epsilon(r_{ij}) = 4 \exp(0.1 r_{ij}) \tag{5.4}$$

Hingerty and co-workers[618] developed a sigmoidal function to calculate the dielectric response (eq 5.5) based on Debye's theory of ionic saturation. Their method, which can reproduce interactions between small nucleic acids and ions in aqueous solution, has become the basis of the latest dielectric functions. In eq 5.5, $\beta = \exp(r_{ij}/\alpha)$ and the parameters D and α are 78 and 2.5 for aqueous solutions, respectively.

$$\epsilon(r_{ij}) = D - (D-1)\left(\frac{r_{ij}}{\alpha}\right)^2 \frac{\beta_{ij}}{(\beta_{ij}-1)^2} \tag{5.5}$$

Ramstein and Lavery (eq 5.6)[619] slightly modified Hingerty's function and parametrized it to reproduce the energy profile of conformational movements in DNA. In eq 5.6, D is 78 (aqueous environments) and α is a parametrized constant that ranges from 0.16 to 1.2[619−621] for hydrated macromolecules. Other authors, after additional slight modifications of the expression, derived parameters for it by fitting them to Poisson−Boltzmann or MD simulations with explicit solvent.[620,621] This method yielded reasonable representations of the electrostatic interactions in DNA structures.

$$\epsilon(r_{ij}) = D - \left(\frac{D-1}{2}\right)[(\alpha r_{ij})^2 + 2\alpha r_{ij} + 2] \exp(-\alpha r_{ij}) \tag{5.6}$$

Mehler and Solmajer[622] developed another sigmoidal dielectric function (eq 5.7) to describe the electrostatic interactions in proteins, and this has been successfully used to predict the binding of small molecules to proteins.[623−626]

$$\epsilon(r_{ij}) = A + \frac{B}{1 + k \exp(-\lambda B r_{ij})} \tag{5.7}$$

where

$$B = \epsilon_o - A;\ A = -8.5525,\ \lambda = 0.003627,\ k = 7.7839$$

Distance-dependent dielectric functions are an inexpensive way to qualitatively treat screening effects in macromolecules. Thus, they were widely used in early MD simulations and are still currently used when modeling ligand docking.[623−630] However, these functions cannot always be used to simulate the dielectric response. Indeed, they do not yield any information about the solvation of each individual element of the system, which limits their ability to predict the behavior of molecules in solution.

5.2. Methods for Group Solvation

5.2.a. Methods Based on the Molecular Topology

To a first approximation, the free energy of solvation can be determined from empirical parameters that represent the intrinsic solvation properties of the constituent groups on the molecule (eq 5.8, where g^k

is the intrinsic solvation parameter of a given group, *k*). This is called a fractional approach and has seldom been used to compute solvation free energies. However, it is a popular method for calculating transfer free energies and partition coefficients (see section 2.1), which are primarily used in molecular modeling studies to represent the partition of a solute between water and organic environments such as biological membranes.[22,104−106,631−634]

$$\Delta G_{\mathrm{sol}} = \sum_{k=1}^{N} g_{\mathrm{sol}}{}^{k} \tag{5.8}$$

Fractional methods were first developed in the 1960s and 1970s. Early models by Leo and Hansch[22,104,106,631] and Rekker[632,633] were used to predict the partition coefficients for series of aromatic or aliphatic molecules. These methods were improved by introducing corrections for the effect of molecular topology on the hydrophobic characteristics of each group.[105,106,634] Algorithms based on fractional constants have been implemented in many computer programs, where they can be used to estimate partition coefficients.[634−644] These methods give acceptable results for 40−70% of the molecules studied. However, they can only be applied to molecules similar to those used in the parametrization data set. A detailed, critical comparison of the most popular algorithms can be found in ref 634.

5.2.b. Methods Based on the Solvent-Accessible Surface

Equation 5.8 has been improved by assuming that the atom/group contribution to the solvation free energy depends on the exposure of the solute to the solvent as well as on the intrinsic solvation properties of the system (eq 5.9, where σ_k is the solvation parameter of the residue *k* per unit area and A_k is its solvent-accessible area). This implies that geometrical and environmental effects have to be considered when determining the free energy of solvation. Because of their formal simplicity, methods for computing solvation (or transfer) free energies which are based on the calculation of the solvent-accessible surface have been widely used to study solvent effects in proteins.[645,646] Thus, in the early 1970s, Chothia[645] exploited experimental data reported by Nozai and Tanford[646] to suggest that the solvation free energy of a protein could be computed from the solvent-accessible surface of its residues. A general empirical factor, σ (eq 5.9), equal to 24 cal/(mol Å^2)was used to compute the protein hydrophobicity from the solvent-accessible surface of its residues.[645]

$$\Delta G_{\mathrm{sol}} = \sum_{k=1}^{N} \sigma_k A_k \tag{5.9}$$

On the basis of Chothia's ideas, Eisenberg and McLachlan[647] determined the octanol/water transfer free energy of a protein using the solvent-accessible surface of individual atoms in the protein (eq 5.10). In eq 5.10, *z* stands for an atom type, N_z is the number of atoms per atom type, and *k* represents an atom of the protein. Empirical parameters for each atom type (−C, N(O), O^-, N^+, and S−) were developed using Fauchere and Pliska's estimates of each residue's partition coefficient.[648]

$$\Delta G_{\mathrm{transfer}} = \sum_{l=1}^{Z}\sum_{k=1}^{N_Z} \alpha_{kx} A_{kz} \tag{5.10}$$

Scheraga and co-workers[649] developed a model whereby the hydration free energy of a protein was determined from the contributions of its various small functional groups. Empirical parameters were determined from the hydration free energies of small molecules containing the groups of interest.[650,651]

Methods based on the solvent-accessible surface present several shortcomings: (i) the calculation of the solvent-accessible surface is not very efficient, (ii) the suitability of fitting solvation parameters for groups on proteins to experimental data for small molecules is unclear, (iii) the solvent screening of intrasolute interactions is neglected, and (iv) the electrostatic contributions to solvation are not explicitly treated.

Even when analytical methods[652−655] and other efficient algorithms[656−659] are utilized, the calculation of solvent-accessible surfaces is not fast enough to allow its efficient implementation in docking, MC, or MD methods. (For a discussion of this limitation, see ref 659.) Thus, faster hydration shell models have been developed. In these models the solute's exposure to the solvent is represented without an explicit calculation of the solvent-accessible surface, which is permissible since the solvent effect primarily arises from the interaction of the solute with the first hydration shell.[338,660−672] This approach reduces the cost of evaluating the solvation free energy compared to models based on the solvent-accessible surface, with only a moderate loss of numerical accuracy.[660−667]

Hydration shell models assume that the free energy of solvation is a function of the solvation of the different groups in the molecule. The solvation of these different groups in turn depends on (i) the intrinsic hydration potential of the group and (ii) the number of solvent molecules excluded from the hydration shell of a group by other nearby groups. This is noted in eq 5.11, where (*r*) is added to stress that the group contribution depends on the molecular geometry.

$$\Delta G_{\mathrm{sol}} = \sum_{k=1}^{N} \Delta G_{\mathrm{sol}}^{\mathrm{k}}(r) \tag{5.11}$$

The intrinsic contribution of the group (ΔG_{ref}; see eq 5.12) is equal to the solvation free energy of the group when it is fully exposed to the solvent. The effect of the surrounding groups on the solvation of a group *k* depends on the size of vicinal groups and their distance from that group *k*. These factors can be quickly calculated using algorithms such as those recently developed by Lazaridis and Karplus (eq 5.12).[338,660] In eq 5.12, V_j is the volume of group *j* and $f_k(r)$ is a Gaussian function of the distance between the groups, as shown in eq 5.13, where R_k is the van der Waals radius of group *k* and λ_k is taken as the thickness of the first hydration shell. α_k is given by

eq 5.14, where $\Delta G_k'$ is determined by assuming that the solvation contribution of a totally buried group is zero.[660]

$$\Delta G_{\mathrm{sol}}{}^{k}(r) = \Delta G_{\mathrm{ref}}{}^{k} - \sum_{j \neq k} f_k(r_{jk}) V_j \qquad (5.12)$$

$$f_k(r) = \frac{\alpha_k}{4\pi r} \exp -\left[\left(\frac{r - R_k}{\lambda_k}\right)^2\right] \qquad (5.13)$$

$$\alpha_k = 2\Delta G_k'/(\pi \lambda_k)^{1/2} \qquad (5.14)$$

An even simpler formalism that is related to Lazaridis' and Karplus' hydration shell model has been developed by van Gunsteren and co-workers.[367] According to their method, the solvation effect is represented by a mean potential term (eq 5.15), which is added to the force-field potential energy. This function accounts for the work needed to partially desolvate an atom or a group and depends on (i) the intrinsic solvation of the atom or group, (ii) their sizes, (iii) the size of the solvent molecule, and (iv) the distance between the atoms or groups.

$$V^{\mathrm{mean}}(r_{ij}) = V_{\mathrm{des}}{}^{ij} \text{ if } r_{ij} < R_1{}^{ij}$$

$$V^{\mathrm{mean}}(r_{ij}) = V_{\mathrm{des}}{}^{ij}\left\{1 - \left[\frac{(r_{ij} - R_1{}^{ij})}{(R_2{}^{ij} - R_1{}^{ij})}\right]^2\right\}^2 \text{ if } R_1{}^{ij} \leq r_{ij} \leq R_2{}^{ij}$$

$$V^{\mathrm{mean}}(r_{ij}) = 0 \text{ if } r_{ij} > R_2{}^{ij} \qquad (5.15)$$

In eq 5.15, r_{ij} is the distance between the atoms or groups i and j, $R_1{}^{ij}$ is the distance at which desolvation is complete, and $R_2{}^{ij}$ is the distance at which solvation is complete. The parameters $R_1{}^{ij}$ and $R_2{}^{ij}$ depend on the size of both the solvent molecules and the atoms or groups of the solute and are derived from experimental aqueous second virial coefficients for small molecules.

As noted above, one drawback to these methods is that they require a specific parametrization process. Most authors adopt values derived from experimentally determined solvation (or transfer) free energies of small systems.[367,645−667] However, the transferability of these "physically based" parameters to large molecules is not guaranteed, which has led to the adoption of "statistically based" parametrization processes in their place.[668−673] In statistically based processes, the parameters are systematically modified to ensure that specific properties of the macromolecules are reproduced, which allows not only solvation parameters but also other interaction parameters to be fitted. Statistically based parameters have been used to discriminate between the native and nonnative structures of proteins and to examine protein folding.[668−673] However, they are difficult to implement in force-field methods. Furthermore, whether they provide a more accurate description of solvation than do methods that rely on physically based potentials remains unclear.[668,674]

The use of eq 5.11 implies that solvent screening of the solute's Coulombic interactions and its effect on the intramolecular energy is neglected. Thus, intrasolute charge−charge interactions are overestimated, yielding an inaccurate potential energy for the system. This can be solved by using (i) distance-dependent dielectric constants (for example, see refs 367 and 660) or (ii) more detailed expressions for the electrostatic component of the solvation free energy (see below).

Finally, methods based on solvent-accessible surfaces or hydration shells omit the explicit treatment of electrostatic interactions, which are expected to be accounted for during the parametrization process. The intrinsic solvation characteristics of a given atom or group depend on the nature of the neighboring atoms or groups, and the positions of these neighboring atoms or groups can change. Obviously, these effects can be included by explicitly considering their electrostatic contribution to solvation (see below), but many empirical methods introduce them implicitly by defining several different parameters for an atom that depend on what atoms or groups are attached to it (for example, see refs 337, 660, 661, and 664). A more complex strategy has been developed by Cramer and Truhlar in their SM5.0R model.[675] This method assumes that the solvation free energy can be determined using an expression similar to eq 5.10 (k stands for an atom in the molecule) but where the solvation parameter σ_k depends on the position and nature of the remaining atoms (eq 5.16).

$$\sigma_k = \sigma_i{}^{0} + \sum_{j \neq k} f(R_{jk}) \qquad (5.16)$$

In eq 5.16, k is an atom of type i, $\sigma_i{}^0$ stands for the intrinsic solvation parameters of a type i atom, and f is a complex function which depends on the atom type. For example, the σ_k of a hydrogen atom is computed as shown in eq 5.17, where $T(R_{kj})$ is a switching function (eq 5.18) that controls the effect of atoms close to atom k and σ_{Hj} is an empirical coupling constant. Thus, the atomic solvation parameters for each atom depend on their environment, and this allows for fine control of the environmental effects on the solvation characteristics, even though it also requires a complex parametrization process.

$$\sigma_k = \sigma_H{}^{0} + \sum_{j=\mathrm{C,O,N,S}} \sigma_{Hj} T(R_{kj}) \qquad (5.17)$$

$$T(R_{kj}) = \exp\left[-\left(\frac{\Delta R}{\Delta R - R_{kj} + R}\right)\right] \text{ if } R_{kj} \leq R + \Delta R \qquad (5.18)$$

$$T(R_{kj}) = 0 \text{ otherwise}$$

The SM5.0R model was parametrized to reproduce the free energy of solvation in small- and medium-sized organic molecules. Whether it also reliably represents solvation free energies in proteins or nucleic acids remains to be determined.

6. Continuum Electrostatic Methods

When polar solutes are placed in polar solvents, the largest contribution to solvation comes from

solute−solvent electrostatic interactions. These can be treated by classical or QM/MM MC or MD simulations that use a discrete description of the solvent. Alternatively, the electrostatic effects can be accounted for by using the theory of polarizable fluids, where the solvent is treated as a continuum environment.

Continuum electrostatic methods can be divided in two families, depending on the description (QM or classical) of the solute. If a QM formalism is used, the solvation free energy is determined as noted in eq 6.1,[3,4,489,676−684] where Ψ is the solute wave function, $H°$ is the solute Hamiltonian, V_R is a perturbational operator representing the solvent reaction field, and the indexes '0' and 'sol' represent gas-phase and solvent environments, respectively. It should be noted that the solute wave function and the reaction field are interdependent in this formalism, which makes it necessary to treat their mutual coupling using self-consistent procedures.

$$\Delta G_{ele} = \left\langle \Psi^{sol} \middle| \hat{H}^0 + \frac{1}{2}\hat{V}_R(\Psi^{sol}) \middle| \Psi^{sol} \right\rangle - \langle \Psi^0 | \hat{H}^0 | \Psi^0 \rangle \quad (6.1)$$

Many QM continuum (also called self-consistent reaction field; SCRF) methods have been developed. (For key references, see refs 3, 4, 85−87, 489, and 685−688.) The main differences between these methods concern (i) the definition of the solute/solvent boundary, (ii) the representation of the solvent reaction field, and (iii) the description of the solute charge distribution. SCRF methods are valuable for examining the solvent effects on small- and medium-sized molecules, and the latest versions estimate the free energy of solvation for large series of molecules with mean errors of less than 1 kcal/mol for water and even less for apolar solvents. However, their general application is limited by the need to perform a QM calculation on the solute, which is not affordable for large solutes such as biomacromolecules. Since SCRF methods have recently been reviewed by different authors,[3,4,85,86,449,450,677,678,682,686−688] we will omit a detailed discussion of these methods and instead refer the reader to refs 3, 4, 85, 677−679, and 682 for a comprehensive explanation of the basic formalism, to refs 3 and 4 for revisions of recent applications, and to refs 449, 450, and 688 for a comparison of these SCRF methods with other methods.

In the following, we shall limit our attention to classical continuum treatments of the solvent effect in biomolecular systems. However, we first briefly analyze the partitioning of the free energy of solvation into several contributions, typically cavitation, electrostatics, dispersion, and repulsion (see section 2). This partitioning scheme is very convenient from a computational point of view. In fact, it constitutes the strategy (with some degree of flexibility) generally adopted by the latest versions of QM continuum models.[3,4] This strategy was outlined in early works by Huron and Claverie[82−84] and by Bonnaccorsi et al.[689] Nevertheless, alternative computational procedures have recently been devised whereby the electrostatics, dispersion, and repulsion are collectively computed.[690,691]

According to statistical mechanics, the free energy of solvation can be obtained by using a charging process, as given in eq 6.2, where V_{XS} is the full interaction potential between the solute and solvent, g_{XS} is the correlation function, ρ_s is the solvent density, and λ is a charging parameter.[691,692] By varying the value of λ from 0, where the solute−solvent interaction is switched off, to 1, where the interaction is completely active, one can compute the reversible work associated with charging the solute in the solvent.

$$\int_0^1 d\lambda \int dr[\rho_S V_{XS}\, g_{xs}(r,\lambda)] \quad (6.2)$$

Assuming that the interaction potential V_{XS} contains the four contributions mentioned above, eq 6.2 can be solved by resorting to four consecutive integrations, each of which is associated with a different charging parameter. These are (i) a parameter related to a length for cavitation,[693,694] (ii) a parameter associated with the electric charge for electrostatics,[88] (iii) a parameter related to the electron transition densities for dispersion,[695,696] and (iv) a parameter related to the electron overlap for repulsion.[696] The order of these separate charging processes must be chosen so as to reduce any possible couplings between the components of V_{XS} (see refs 691 and 692 for more details). The most important solvent reorganization effects that are due to inclusion of the solute are generally associated with cavitation and electrostatics. The coupling between the electrostatic and dispersion effects appears to be small, at least for neutral solutes.[696] Finally, since cavitation contains the largest portion of the repulsion terms present in the free energy of solvation, the coupling of the repulsion contribution with the other terms is expected to be small. In our opinion, more research is necessary to determine the relative couplings between the different contributions as well as their effect on the solute properties. Indeed, this information could be exploited to refine the theoretical methods by which molecules in biological systems are studied.

6.1. The Classical Electrostatic Problem

Classical continuum methods assume a classical treatment of the solute charge distribution. The solute is placed in the interior of a cavity in a polarizable continuum medium, which is characterized by the solvent dielectric constant (ϵ_s). Permittivities in the interior of the solute cavity (ϵ_{int}) generally range from 1 to 8. The electrostatic component of the free energy of solvation is estimated as one-half of the solute−solvent electrostatic interaction energy, according to linear response theory.[88] The differences between the continuum electrostatic models mainly consist of their definitions of the solute/solvent boundary, whether they include ionic screening, and how they calculate the solute−solvent electrostatic interaction energy.

The starting point for the development of continuum models is Poisson's equation (eq 6.3), which relates the electric displacement to the charge density. In eq 6.3, ρ is the charge density and $D(r)$ is the

electric displacement as defined in eq 6.4, where $\Phi(r)$ is the electrostatic potential and $\epsilon(r)$ is the dielectric constant.

$$\nabla \cdot D(r) = 4\pi\rho(r) \tag{6.3}$$

$$D(r) = -\epsilon(r) \cdot \nabla \cdot \Phi(r) \tag{6.4}$$

Some authors have developed versions of this method whereby two different dielectric constants are used to define different parts of the solute.[697,698] Likewise, procedures for treating inhomogeneities and anisotropies in the solvent have also been reported.[3,699−705]

The total charge density includes the solute charge distribution inside the cavity (ρ_{int}) and the charge density generated by the ion atmosphere outside the cavity (ρ_{ext}) in solutions where the ionic strength is not zero (eq 6.5). It should be noted that it is easy to fulfill the requirement that the cavity include all the solute charge distribution using classical methods whereas it is quite complex at the QM level.[3]

$$\rho = \rho_{int} + \rho_{ext} \tag{6.5}$$

The charge density due to the ionic atmosphere can be approximated at equilibrium by a Boltzmann distribution, as shown in eq 6.6, where κ is the inverse Debye−Hückel length and is defined in Gauss units, as shown in eq 6.7. In eq 6.7, N_a is Avogadro's number, e is the charge of the electron, I is the ionic strength, k is Boltzmann's constant, and T is the temperature.

$$\rho_{ext} = -\epsilon(\kappa^2) \sinh[\Phi(r)] \tag{6.6}$$

$$\kappa = \sqrt{\frac{8\pi N_a e^2 I}{10^3 \epsilon_s k T}} \tag{6.7}$$

The Poisson−Boltzmann equation can be expressed in three different forms depending on the ionic strength: (i) for no ionic atmosphere (eq 6.8), (ii) for a low ionic strength (i.e., the $\sinh(\Phi(r))$ function is replaced by the first term of a Fourier expansion, eq 6.9), and (iii) for ionic strengths requiring a general nonlinear expression (eq 6.10).

$$\nabla \cdot [\epsilon(r) \nabla \Phi(r)] = -4\pi\rho_{int}(r) \tag{6.8}$$

$$\nabla \cdot [\epsilon(r) \nabla \Phi(r)] = \kappa'_0{}^2 \Phi(r) = -4\pi\rho_{int}(r) \tag{6.9}$$

$$\nabla \cdot [\epsilon(r) \nabla \Phi(r)] = \kappa'_0{}^2 \sinh[\Phi(r)] = -4\pi\rho_{int}(r) \tag{6.10}$$

$\kappa'_0{}^2$ is 0 inside the cavity and κ^2 outside the cavity

Solving these equations yields the total electrostatic potential at any point (Φ_i) as well as the electrostatic free energy of solvation given by eq 6.11, where the indexes 'sol' and '0' refer to the solution and the gas phase, respectively, and q_i stands for the classical point charges used at the classical level of computation to represent the solute charge distribution. The electrostatic free energy of solvation can be obtained from two independent calculations, one with a dielectric constant of 1 inside and outside the cavity (Φ^0) and the other with a dielectric constant of 1 inside the cavity and ϵ_s outside the cavity.

$$\Delta G_{ele} = \frac{1}{2} \sum_i q_i (\Phi_i^{sol} - \Phi_i^0) \tag{6.11}$$

Unfortunately, the solution of Poisson (or Poisson−Boltzmann) equations is difficult for systems of interest, which has prompted the development of the numerical treatments that are briefly reviewed in the following section.

6.2. Numerical Methods for Solving the Poisson Equation

One numerical approach to the differential Poisson−Boltzmann equations (eqs 6.8−6.10) is to divide the system into small elements and then apply finite elements theory to them.[697] This procedure was adapted by Orttung to solve eq 6.8 for a general molecule[698,706] and was then used to determine the relative pK_a values of small molecules. The method is simple and robust, but only a few applications of it have been reported in the literature.[698,706−708]

The finite-difference approach,[352,353,356,709−716] which involves mapping the molecule on a 3-D cubical grid, is a more popular method. According to this treatment, the Poisson−Boltzmann equation is satisfied at each grid point and all the derivatives are numerically computed. By integrating the Poisson−Boltzmann equation over a small box centered on each grid point, and applying the divergence theorem, the potential at each given grid point, k, can be computed as shown in eq 6.12.[352,353]

$$\Phi_k = \frac{\sum_i \epsilon_i \Phi_i + 4\pi \frac{q_k}{L}}{\sum_i \epsilon_i + N\kappa'_0{}^2 L^2} \tag{6.12}$$

The sum in eq 6.12 encompasses the six grid points ($i = 1, .., 6$) surrounding the grid point k. q_k is the charge assigned to grid point k ($q_k = \rho_k L^3$), and L is the spacing of the cubic grid. N is equal to 0 when the ionic strength is zero (eq 6.8), to 1 when the ionic strength is small (eq 6.9), and to $\sinh(\Phi_k)$ in all other cases (eq 6.10).

According to eq 6.12, the potential at each grid point depends on the potential at the surrounding grid points, which means that an iterative solution must adopted. This self-consistent process can be difficult to achieve due to several different technical problems. Thus, the assignment of charges to cubic grids is not trivial, and smoothing algorithms such as the trilinear weighting function should be used to avoid discontinuities in the charge distribution.[717,718] Another problem with this method is the discontinuity of the dielectric constant which occurs at grid points located close to the cavity boundary.[719] The definition of an electrostatic potential first guess is also delicate, especially at the external faces of the grid,[718,719,721] and there are numerical problems re-

lated to the grid size which can be alleviated using the focusing strategy reported by Honig's group.[718,721] Finally, much work has been devoted to the development of efficient algorithms for reaching convergence in the calculations.[722,723]

Finite difference methods have been implemented in several computer programs, including MEAD,[709,710] DELPHI,[352,720] UHBD,[724] and others,[725,726] and these methods are widely used to study solvation in macromolecules. (For comprehensive reviews, see refs 352, 356, 711, and 716.) The applications for which these methods have been used include calculation of electrostatic potential,[727] ligand binding,[728−730] redox potentials,[731] solvation,[732−735] solvent-induced conformational shifts,[736] and protein structure and flexibility.[294,295,737] Finite difference Poisson−Boltzmann techniques have been very successful in determining the pK_a of protein residues,[714,738−746] which is complicated by the interdependence of the ionization states of the titrable groups on the protein.

Currently, Poisson−Boltzmann calculations are complemented by approximate methods for the evaluation of steric contributions to solvation. These approximate methods are based on empirical linear expressions related to the solvent-accessible surface (see section 5), which permits the total free energy of solvation to be estimated with a similar accuracy to those obtained from discrete calculations.[732,735,747] These results suggest that finite-difference Poisson−Boltzmann calculations could be used in dynamics calculations, thus avoiding the need for explicit treatments of the solvent molecules (see above). In fact, Poisson−Boltzmann methods already have been used in conjunction with Brownian dynamics algorithms.[748−751] However, the implementation of Poisson−Boltzmann methods in MD protocols has been hampered by the need for fast and accurate calculations of the solvation forces at each step of the trajectory, even though efficient methods for the calculation of these forces have been recently reported.[752,753]

Despite the success of finite-difference Poisson−Boltzmann calculations, a few words of caution concerning the accuracy of ΔG_{ele} are in order. First of all, numerical uncertainties occur when large molecules and sparse grids are used. Furthermore, the intrinsic shortcomings of the Poisson−Boltzmann equation should be emphasized. One problem with these methods is that they assume that the solvent is a continuum, an approach that can be too crude in cases where not all the solvent molecules have the same relaxation properties. This problem may affect proteins, since the structural solvent molecules are frequently tightly bound to the macromolecule, and accordingly do not have the same ability as bulk solvent molecules to reorient in response to an external field.

A second problem with these methods involves the definition of the dielectric constant for the solute. A dielectric constant of 1 can be used for small solutes treated at the QM level, but it is not suitable for a classical description of macromolecules since it neglects (i) electronic polarization effects and (ii) polarization effects related to field-induced nuclear reorientations in the macromolecule. Dielectric constant values ranging from 2 to 8 are typically used to represent the dielectric responses of macromolecules, which introduces some degree of arbitrariness into the calculations. Furthermore, it is unclear whether these isotropically averaged values can capture the complex, largely anisotropic dielectric response of a macromolecule. This uncertainty might be alleviated by using Boltzmann samplings of structures, which are obtained from MD or MC simulations that consider explicit solvent as inputs for the classical continuum electrostatic calculations. This would allow at least part of the dielectric response to be taken into account in the structures collected during the sampling.

A third source of uncertainty in these methods involves the definition of the solute cavity, both in terms of its shape and size. A molecular-shaped cavity (such as is used in finite-difference Poisson−Boltzmann methods) guarantees a better representation of solvation than a simple spherical cavity. However, there are no clear rules for determining the proper size of the cavity. In practice, the solute cavity is defined by empirical atomic radii corrected using empirical scaling factors or by an empirical dielectric offset distance. The size of the cavity should then be parametrized for each solvent by fitting it to experimental data. However, in most cases the same cavity definition, which is typically optimized for water, is used for all solvents.

A final problem with finite-difference Poisson−Boltzmann methods, as well as with many other continuum electrostatic calculations, is that they assume that there are no changes in the geometry of the solute upon solvation. This is a reasonable assumption for small solutes, but it is incorrect for large macromolecules, whose conformation should change dramatically upon solvation. In summary, methods based on the numerical solution of the Poisson−Boltzmann equation are extremely useful but caution must be taken in using the results obtained with these techniques.

6.3. Methods Based on Multipole Expansion in Simple Cavities

Poisson's equation can be analytically solved for simple solutes. The electrostatic component of the free energy of solvation is obtained using a slightly different version of eq 6.11 (see eq 6.13, where Φ_i^σ is the electrostatic potential generated at point i by the solvent reaction field). The electrostatic potential Φ^σ is determined by assuming very simple cavities for the solute and by using limited multipole expansions to represent the solute charge distribution.

$$\Delta G_{ele} = \frac{1}{2}\sum_i q_i \Phi_i^\sigma \qquad (6.13)$$

Thus, for a spherical cavity, the electrostatic potential can be written as a series of spherical harmonics[88] of increasing complexity. If the series is limited to the first term (a monopole), the well-known Born equation[754] is obtained (eq 6.14). In eq 6.14, a is the radius of the solute cavity and ϵ is the dielectric

constant, relative to vacuum, in suitable units. The 4π factor (arising from the traditional use of Gauss units in continuum formalisms) is skipped for the sake of simplicity.

$$\Delta G_{ele} = -\frac{1}{2}\left(1 - \frac{1}{\epsilon}\right)\frac{q^2}{a} \qquad (6.14)$$

Equation 6.14 can be modified to account for ionic-strength-dependent terms (see eq 6.9) by using Kirkwood's model,[755] as shown in eq 6.15, where b is the minimum distance between the central point charge and the surrounding ions.

$$\Delta G_{ele} = -\frac{1}{2}\left(1 - \frac{1}{\epsilon}\right)\frac{q^2}{a} - \frac{q^2\,\kappa}{2\epsilon(1+\kappa b)} \qquad (6.15)$$

When a nonpolarizable point dipole is used, Bell's equation (eq 6.16, where μ is the dipole moment) is obtained.[756] Onsager's equation (eq 6.17) is obtained when an isotropic dipolar polarizability (α) is also considered.[757] Related equations for calculating the ΔG_{ele} of a quadrupole placed in the center of a sphere were developed by Abraham and Cooper.[758]

$$\Delta G_{ele} = -\frac{\epsilon - 1}{2\epsilon + 1}\frac{\mu^2}{a^3} \qquad (6.16)$$

$$\Delta G_{ele} = -\frac{\epsilon - 1}{2\epsilon + 1}\frac{\mu^2}{a^3}\left(1 - \frac{\epsilon - 1}{2\epsilon + 1}\frac{2\alpha}{a^3}\right)^{-1} \qquad (6.17)$$

These models are able to reproduce the electrostatic contribution to the free energy of solvation for very simple solutes. However, they provide poor results for real molecules mainly due to errors resulting from (i) the truncation of the multipole expansion and (ii) the use of a spherical cavity to define the solute/solvent boundary.

The multipole expansion converges very slowly,[759−762] which makes it necessary to introduce a large number of spherical harmonics in order to reproduce the real electrostatic potential. Traditional strategies used to reduce this problem are based on the use of limited multipole expansions centered at different points inside a spherical cavity. Models such as those developed by Kirkwood and co-workers[763−765] have been used to qualitatively study protonation in globular proteins.[766−769] Nevertheless, the suitability of these models for use with proteins is severely limited by the simplicity of the basic formalism.[358,770]

Different authors have tried to more realistically describe the solute/solvent boundary (see ref 3 for a review). Models for ellipsoidal cavities have been devised by many authors (see refs 3 and 683). Cylindrical cavities have also been developed and used to study solvent effects in DNA,[771−773] and few models of molecular-shaped cavities recently have been developed.[3,774] However, the most popular multipole method used in biochemical studies is the generalized Born model (GBM), which is explained in detail below.

6.4. Generalized Born Model

In the generalized Born model (GBM),[76,450,775−781] the charge distribution is represented by a limited set of point charges typically centered at nuclei. ΔG_{ele} is then determined from the individual Born solvation of each atom (charge), corrected by the perturbing effect of the other atoms (charges). The most popular algorithm was developed by Still and co-workers[780] and is shown in eqs 6.18 and 6.19, where q stands for the solute charges and ΔG_B is a screening function defined as shown in eqs 6.19 and 6.20, where α is the effective Born radius and d is a constant usually, but not always, equal to 4. Note that we have used our standard nomenclature instead of Still's, which denotes ΔG_{ele} as ΔG_{pol},[780] to emphasize that eq 6.18 does not account explicitly for the electronic polarization contribution to solvation (see below).

$$\Delta G_{ele} = -\frac{1}{2}\left(1 - \frac{1}{\epsilon}\right)\sum_{i=1}^{N}\sum_{j=1}^{N}\frac{q_i q_j}{f_{GB}} \qquad (6.18)$$

where

$$f_{GB} = (r_{ij}^{\,2} + \alpha_i\alpha_j e^{-D_{ij}})^{1/2} \qquad (6.19)$$

$$D_{ij} = \frac{r_{ij}^{\,2}}{d\alpha_i\alpha_j} \qquad (6.20)$$

Equation 6.18 converges to the standard Born equation (eq 6.14) at $r_{ij} = 0$ and to the Born + Coulombic expression at large r_{ij} distances.[734] At short distances ($r_{ij} < 0.1(\alpha_i\alpha_j)^{1/2}$), eq 6.18 provides a good approximation of Bell's equation (eq 6.16). Finally, eq 6.18 includes both the self ($i = j$) and shielding ($j \neq i$) contributions to solvation. Balance between these conditions is critical to correctly representing the solvent effects (see below).

The key parameters in GBM are the effective Born radii (α_i) for the different atoms of the solute. Effective Born radii are not intrinsic atomic properties (such as the van der Waals radii are) but instead depend on the molecular geometry. They are usually computed using eq 6.21,[780] which converges to the intrinsic Coulomb radii (ρ_i) only for an isolated atom. Larger values are obtained if the sphere centered on atom i intersects the spheres centered on other atoms.

$$\alpha_i^{-1} = \int_{\rho_i}^{\infty} F_i(r, \{r_{ij}, \rho_j\}_{\text{all } j})\,\frac{dr}{r^2} \qquad (6.21)$$

In eq 6.21, F_i is a fraction representing the exposed surface area of a sphere of radius r centered at atom i, when it is surrounded by spheres of radius ρ_j centered on the other atoms, j, of the molecule in a given conformation (noted by r_{ij}).

The calculation of effective Born radii from eq 6.21 requires a knowledge of the intrinsic Coulomb radii (ρ), which are obtained from van der Waals radii in force fields[780,782,783] or are fitted to reproduce experimental data.[784−792] In several QM versions of the GBM model developed by Cramer and Truhlar,[785−792] the intrinsic Coulomb radii are replaced by flexible

radii whose values are modulated as a function of the atomic charge.

Equation 6.21 has been used in most versions of the GBM method,[785−792] including the GBM-based QM algorithms. However, solving eq 6.21 is very time-consuming and it cannot be routinely used for macromolecules. This has led to the development of approximate methods for faster evaluation of the effective Born radii,[780,784,793−795] which can then be applied to large systems.[795−799]

One approach for quickly calculating the effective Born radii is based on the pairwise model developed by Schaefer and Froemmel[793] and translated to the GBM framework by Hawkins et al.[794] The basic idea of this method is to consider only the pairwise contributions to the atomic sphere overlap. Using this approach, which is expected to be quite reliable for most small and medium-sized molecules of interest, one can find an analytical expression for the Born radii (eqs 6.22−6.24) and, after a suitable parametrization, obtain results similar to those derived using standard effective Born radii.[784]

$$\alpha_i^{-1} = \rho_i^{-1} - \frac{1}{2}\sum_j \left(\frac{1}{L_{ij}} - \frac{1}{U_{ij}} + A_{ij} + B_{ij}\right) \quad (6.22)$$

$$A_{ij} = \frac{r_{ij}}{4}\left(\frac{1}{U_{ij}^2} - \frac{1}{L_{ij}^2}\right) \quad (6.23)$$

$$B_{ij} = \frac{1}{2r_{ij}} \ln \frac{L_{ij}}{U_{ij}} + \frac{\rho_j^2}{4r_{ij}}\left(\frac{1}{L_{ij}^2} - \frac{1}{U_{ij}^2}\right) \quad (6.24)$$

where

$$L_{ij} = U_{ij} = 1 \text{ if } r_{ij} + \rho_j \le \rho_i$$

$$L_{ij} = \rho_i \text{ if } r_{ij} - \rho_j \le \rho_i < r_{ij} + \rho_j$$

$$L_{ij} = r_{ij} - \rho_j \text{ if } \rho_i \le r_{ij} - \rho_j$$

and

$$U_{ij} = r_{ij} - \rho_j \text{ if } \rho_i \le r_{ij} + \rho_j$$

Another popular method for rapidly determining effective Born radii has been proposed by Still's group.[782] This method is based on the premise[800] that the reduction in ΔG_{ele} of an atom, i, due to the presence of the atomic sphere of other atom, j, is proportional to the volume of this sphere and inversely proportional to the distance between the two atoms raised to the fourth power. This approach is valid only for very large interatomic distances; thus, a series of scaling parameters, which are dependent on molecular topology, must be introduced. The electrostatic free energy of solvation of a single atom with a unit charge (ΔG^i_{ele}) therefore is defined using formulas similar to those displayed in eqs 6.25 and 6.26. A modification of this method, which includes extra fitted parameters, has been suggested by Dominy and Brooks.[795]

$$\Delta G_{ele}{}^i = \left(1 - \frac{1}{\epsilon}\right)\left[-\frac{1}{2(\rho_i + P_1)} + \sum_j^{stretch} \frac{P_2 V_j}{r_{ij}^4} + \sum_j^{bend} \frac{P_3 V_j}{r_{ij}^4} + \sum_j^{nb} \frac{P_4 V_j \mathrm{CCF}}{r_{ij}^4}\right] \quad (6.25)$$

$$\mathrm{CCG} = 1.0 \text{ if } \frac{r_{ij}}{R_i^{vw} + R_j^{vw}} > \frac{1}{P_5}$$

otherwise

$$\mathrm{CCF} = \left\{\frac{1}{2}\left[1.0 - \cos\left\{\left(\frac{r_{ij}}{R_i^{vw} + R_j^{vw}}\right)^2 P_5\right\}\right]\right\}^2 \quad (6.26)$$

In eqs 6.25 and 6.26, r_{ij} is the interatomic distance, V_j is the volume of atom j, and P_{1-5} are fitted parameters. CCF (close contact function) is a function used to correct for deviations arising from strong overlap between the atoms i and j. R_i and R_j are the van der Waals radii of atoms i and j, respectively. (Note that these values are usually different from the Coulomb radii ρ_i and ρ_j.)

The parameters P_{1-5} are typically fitted to Poisson−Boltzmann calculations of ΔG^i_{ele} for a large series of molecules. After this parametrization process, the total electrostatic free energy of solvation (ΔG_{ele}) is computed using the standard equations (eqs 6.18−6.20). The quality of the results yielded by this method are similar to those obtained using standard effective Born radii, at least for systems involving small molecules.

The use of approximate Born radii and suitable parameters permits the rapid and accurate calculation of ΔG_{ele}, which should allow GBM to be used in studying macromolecules. However, before this occurs, the method needs additional parametrization. This parametrization must be consistent with the force field used to represent intrasolute interactions, with the Coulomb radii used to define the solute cavity, and with the empirical parameters used to define the steric contribution to solvation. Without this additional parametrization, the current GBM methods provide good results for small molecules but are not as reliable for macromolecules. Adding the extra fitted parameters should yield good approximations to the finite-difference Poisson−Boltzmann results for macromolecules.[425,426,795,796,801]

As is typically done for continuum electrostatic methods, GBM is combined with empirical methods when evaluating the steric contributions to solvation. These combined methods, which are typically referred to as GB−SA models,[425−427,778−802] represent the steric contributions by means of empirical relationships with the solvent-accessible surface (eq 6.27). In eq 6.27, γ_i and SAS_i are the empirical surface tension parameter and the solvent-accessible surface for atom i, respectively. The most elaborated version utilizes surface tension parameters fitted for a large number of different atom types.[4,778,785−792]

$$\Delta G_{ster} = \Delta G_{cav} + \Delta G_{vw} = \sum_i \gamma_i \mathrm{SAS}_i \quad (6.27)$$

The use of GB−SA to dynamically study macromolecules is limited by the required calculation of solvent-accessible surfaces. However, recent studies suggest that GB−SA methods might be valuable for performing long time scale MD simulations using implicit solvent.[795,797] However, even these methods are susceptible to the intrinsic shortcomings of continuum electrostatic models (see above) and, in addition, have a few other shortcomings which are directly related to the semiempirical character of eqs 6.18−6.20.

One problem which arises in GBM methods is attributable to the lack of balance between the "self" and "shielding" contributions to solvation (see eq 6.28). This problem, which could lead to errors in the calculation of solvent-mediated intrasolute interactions, has been recently discussed by Jayaram, Liu, and Beveridge.[801] They compared GBM and finite-difference Poisson−Boltzmann results for model systems and found a good agreement between the ΔG_{ele}'s obtained but poor agreement between their components. The authors suggested that these errors can be alleviated by modifying the constant d, which appears in the screening function f_{GB} (see eq 6.18−6.20), from the standard value of 4.0 to a value of 1.64 and by simultaneously modifying the Coulombic radii to guarantee the accuracy of ΔG_{ele}.

$$\Delta G_{ele} = -\frac{1}{2}\left(1 - \frac{1}{\epsilon}\right)\sum_{i=1}^{N}\sum_{j=1}^{N}\frac{q_i q_j}{f_{GB}} = \Delta G_{shielding} + \Delta G_{self} \tag{6.28}$$

where

$$\Delta G_{shielding} = -\frac{1}{2}\left(1 - \frac{1}{\epsilon}\right)\sum_{i=1}^{N}\sum_{j\neq 1}^{N}\frac{q_i q_j}{f_{GB}} \tag{6.29}$$

$$\Delta G_{self} = -\frac{1}{2}\left(1 - \frac{1}{\epsilon}\right)\sum_{i=1}^{N}\frac{q_i^2}{\alpha_i} \tag{6.30}$$

A second problem, which is common to all electrostatic continuum methods based on the use of eq 6.8, arises due to the neglect of ionic effects. This neglect limits the suitability of GB−SA methods for studying strongly charged polymers such as nucleic acids, where salt effects are critical to explaining their structure and dynamics. One possibility for alleviating this problem has been recently suggested by Case and co-workers.[796] These authors modified the GBM equation (eq 6.18) by substituting the dielectric-dependent factor (eq 6.31). This adequately reproduced the results obtained using finite-difference solutions of the linear Poisson−Boltzmann equation (eq 6.9). However, there is still a need to correct for systematic deviations by multiplying the Debye−Hückel screening parameter (κ) by an empirical parameter (ξ in eq 6.32) that should take values below the unity (0.7 is recommended in ref 131).

$$\left(1 - \frac{1}{\epsilon}\right) \rightarrow \left(1 - \frac{e^{-\lambda}}{\epsilon}\right) \tag{6.31}$$

where

$$\lambda = \xi\kappa f_{GB} \tag{6.32}$$

6.5. Methods Based on the Image Charge Approximation

Image charge approximation methods rely on the fact that the potential generated at the position of a charge q which is at a distance d from a grounded plane is equal to that generated by an opposite charge ($-q$) placed at the same distance inside the conductor.[803] If the conductor is replaced by a dielectric continuum, the image charge can then be determined as $-q(\epsilon - 1)/(\epsilon + 1)$. It is not obvious how to extend the image charge model to nonplanar interfaces. For a simple spherical dielectric,[804] each charge leads to the generation of an infinite number of image charges, each mirroring itself. This series is typically restricted to the first term. This yields simplified expressions for the solvent electrostatic potential (Φ^{σ}), which for high dielectric solvents can accurately reproduce the real ΔG_{ele}. (For a discussion, see refs 3 and 353.) The approximate solution for a set of point charges consists of the real charges plus their individual image charges.

Image charge approximation methods have not been widely used for macromolecules but are clearly applicable to the introduction of long-range effects in discrete MD or MC simulations of condensed phases.

6.6. Boundary Element Methods

The popular boundary element method (BEM; also named apparent surface charge, ASC) is the origin of many of the most recent classical continuum electrostatics algorithms. This method is based on the fact that the reaction potential generated in the solvent by the presence of the solute charge distribution (Φ^{σ}) may be described, at any point of the space, in terms of an apparent charge distribution spread over the solute cavity surface. The apparent charge density (σ) can be determined from the component of the field normal to the surface, as shown in eq 6.33,[3,680−682,805−812] where Φ_{TOT} contains the contribution of both the solute charge distribution and that of the surface apparent charge distribution (eq 6.34) and n is an unit vector normal to the surface.

$$\sigma = \frac{\epsilon - 1}{\epsilon}\frac{\partial \Phi_{TOT}}{\partial n} \tag{6.33}$$

$$\Phi_{TOT} = \Phi^{\rho} + \Phi^{\sigma} \tag{6.34}$$

The solute potential can be computed from Coulomb's law if point charges are used to describe the solute charge distribution, and the solvent potential can be determined by integration over the solute cavity (eq 6.35, where Σ stands for the cavity surface and s is the vector defining a point on Σ).

$$\Phi^{\sigma}(r) = \int_{\Sigma}\frac{\sigma(s)}{|r - s|}\,d^2 s \tag{6.35}$$

If the surface, Σ, is divided into small elements, where the charge density can be assumed to be constant, eq 6.35 can be rewritten in a simplified form (eq 6.36). The apparent charges q_k are determined as shown in eq 6.37, where ΔS_k is the area of the surface element k whose center (where q_k is located) is determined by s_k.

$$\Phi^{\sigma}(r) = \sum_k \frac{q_k}{|r - s_k|} \tag{6.36}$$

$$q_k = \Delta S_k \sigma(s_k) \tag{6.37}$$

Equations 6.34−6.37 can be solved by using one of three procedures: (i) the iterative solution,[680,681] (ii) the direct matrix inversion approach,[805−812] or (iii) the approximate closure method.[813] (For detailed description of these approaches, see ref 3.) Methods based on boundary elements are easy to implement into QM algorithms and, when combined with empirical corrections for the introduction of cavitation and dispersion−repulsion terms, provide excellent descriptions of the solvation of small- and medium-sized molecules. Methods such as the polarizable continuum model (PCM,[680,681] also called MST) and COSMO[814−821] are among the most popular and accurate of these SCRF algorithms. Detailed explanations of these QM methods can be found in specific reviews.[3,4,449,682,687] In the following discussion we limit ourselves to the analysis of a few classical versions which can be used for biologically relevant systems.

Zauhar and co-workers[805−809] developed different algorithms based on the inverse matrix approach to determine electrostatic and hydration effects. Their method has been successfully applied to the determination of hydration free energies and electrostatic potentials for few small proteins.[809] Zauhar's approach has been recently extended by Purisima and co-workers for the efficient computation of the free energy of solvation of rather large molecules.[822,823] Likewise, the PCM method has also been used to study proteins.[824]

The use of Zauhar's method (as with any other BEM method) to study solvation in proteins is ultimately limited by the shortcomings of continuum electrostatic models, specifically the uncertainties associated with the definition of a macroscopic dielectric constant for the protein. One way to partially solve this problem might be to perform the BEM calculation in conjunction with MD algorithms that are able to capture the fluctuation of the protein and thus part of its dielectric response. This solution is hampered by two practical problems: (i) the determination of the solvent-accessible surface can be very slow for large molecules and (ii) it might be necessary to define a very large number of surface elements in order to discretize the molecular surface, which can dramatically slow the calculation of the solute−solvent interaction energy.

6.7. The Semiclassical MST Model

The most popular BEM algorithm was developed by Pisa's group,[3,680−682,687] based on a formalism developed in the early 1980s by Miertus, Scrocco, and Tomasi.[680,681] It was initially formulated within the iterative framework and developed for a QM or classical description of the solute. Due to the popularity of this method, we will briefly discuss its most relevant features. There are several different versions of the PCM, and we will concentrate on the specific details of the version developed at Barcelona.

6.7.a. Theoretical Background of the Classical MST Method

As previously mentioned, the free energy of solvation can be expressed as the addition of three different contributions: (i) cavitation, (ii) van der Waals, and (iii) electrostatic (eq 2.1). In the QM framework, the electrostatic contribution is determined by adding the perturbation operator to the solute Hamiltonian (eq 6.1) and self-consistently solving the corresponding nonlinear Schrödinger equation. In a classical framework, the electrostatic component adopts the expression given by eq 6.38, where $\{Q_i\}$ and $\{q_k\}$ denote the sets of point charges that represent the charge distribution of the solute and the solvent reaction field, r_i and r_k stand for the position vectors of the solute and solvent charges, respectively, M is the number of surface elements, and N is the number of charges that represent the solute charge distribution (which does not necessarily coincide with the number of atoms).

$$\Delta G_{ele} = \frac{1}{2}\sum_{i=1}^{N}\sum_{k=1}^{M}\frac{Q_i q_k}{|r_i - r_k|} \tag{6.38}$$

Most of the applications based on eq 6.38 neglect the contribution arising from the solute polarization. This allows eq 6.38 to be rewritten as eq 6.39, where the index "0" emphasizes that the gas-phase charge distribution of the solute remains unaltered upon solvation and that the solvent's apparent surface charges are generated in response to the gas-phase charge distribution of the solute.

$$\Delta G^0_{ele} = \frac{1}{2}\sum_{i=1}^{N}\sum_{k=1}^{M}\frac{Q_i^0\, q_k(Q_i^0)}{|r_i - r_k|} \tag{6.39}$$

At this level of approximation, the reliability of the (first-order) electrostatic free energy largely depends on the accuracy of the point charges that describe the solute charge distribution. Numerical fitting to the QM electrostatic potential is a natural procedure to derive these charges.[576−583,825−829] This approach has been used by different authors to compute $\Delta G_{ele}{}^0$. In some cases the potential-derived point charges are scaled to improve the results.[830−832]

Other partitionings of the molecular charge distribution are also feasible. For example, one approach consists of assigning prototypical charge distribution functions to chemical subunits (see refs 3, 833, and 834 for detailed explanations). This approach begins by describing the subunits in terms of localized orbitals, called prototypes, which are replaced by their associated nuclear charges plus either one −2 charge or two −1 charges for each electron pair. The

locations of the negative charges are determined by the localized orbitals' charge centers and by the conservation of the dipole and quadrupole moments of the prototypes. The charge distribution of the molecule is then given by a set of positive point charges representing the nuclei and negative point charges in an amount usually less than the number of electrons. This approach has been used to examine solvent effects on DNA double helices.[701]

As for the nonelectrostatic terms, the cavitation component is computed using Pierotti's scaled particle theory,[835] which has been adapted to molecular-shaped cavities according to the procedure proposed by Claverie.[759] The cavitation free energy is expanded in a series of powers of R_{SX} (eq 6.40), which is the radius of a sphere that encloses the solute and excludes the centers of the surrounding solvent molecules. (eq 6.41, where R_X and R_S denote the radii of the solute and solvent molecules, respectively).

$$\Delta G_{cav} = K_0 + K_1 R_{SX} + K_2 R_{SX}^2 + K_3 R_{SX}^3 \quad (6.40)$$

$$R_{SX} = R_S + R_X \quad (6.41)$$

The coefficients, K_k, in the power expansion depend on the pressure, P, the temperature, T, and microscopic properties (the molecular radius, R_X, and the numeral density, n_X) of the solvent (eqs 6.42a−d).

$$K_0 = RT\left[-\ln(1-y) + \frac{9}{2}\left(\frac{y}{1-y}\right)^2\right] - \frac{4\pi R_X^3 P}{3} \quad (6.42a)$$

$$K_1 = -\frac{3RT}{R_X^2}\left[\frac{y}{1-y} + 3\left(\frac{y}{1-y}\right)^2\right] - 4\pi R_X^2 P \quad (6.42b)$$

$$6qK_2 = -\frac{3RT}{R_X}\left[\frac{y}{1-y} + \frac{3}{2}\left(\frac{y}{1-y}\right)^2\right] - 4\pi R_X P \quad (6.42c)$$

$$K_3 = \frac{4\pi P}{3} \quad (6.42d)$$

where $y = 4\pi R_X^3 n_X/3$.

For practical purposes, an effective radius for the solute can be defined from the molecular surface, S_X (eq 6.43), or volume, V_X (eq 6.44), of the solute's molecular-shaped cavity.

$$R_S = \left(\frac{S_X}{4\pi}\right)^{1/2} \quad (6.43)$$

$$R_S = \left(\frac{3V_X}{4\pi}\right)^{1/3} \quad (6.44)$$

Alternatively, a more rigorous description can be obtained using Claverie's generalization[759] of Pierotti's equation (eq 6.45). In this case, the solute's free energy of cavitation is expressed as the sum of the contributions from the Z spheres that define the molecular-shaped solute cavity, weighted by their contribution to the total cavity surface. This approach is preferred over the spherical solution because (i) the use of an effective sphere can lead to artificial results for nonspherical solutes and (ii) it ensures the size-consistency of the nonelectrostatic terms.[836]

$$\Delta G_{cav} = \sum_{z=1}^{Z} \frac{S_Z}{4\pi R_Z^2} \Delta G_{cav}(R_Z) \quad (6.45)$$

In eq 6.45, Z denotes the total number of spheres necessary to enclose the solute, which does not necessarily coincide with the number of atoms in the solute. R_Z and S_Z are the radius of sphere z and its contribution to the solute/solvent interface, respectively, and $\Delta G_{cav}(R_Z)$ is the cavitation work required to generate a sphere of radius R_Z in the solvent, which is determined from eq 6.40.

Finally, the van der Waals contribution to the free energy of solvation is computed using a linear relationship that depends on the solvent-exposed surface of the atoms in the solute, as noted in eq 6.46, where ξ_i denotes the hardness of atom i, which is determined from the experimental free energies of solvation for neutral molecules. Other versions of the PCM method compute this term using a combination of pairwise additive terms that relate to the atoms or groups of atoms on the solute and the solvent[837−839] or at the QM level by including an additional operator in the Hamiltonian.[690]

$$\Delta G_{vw} = \sum_{i=1}^{N} \xi_i S_i \quad (6.46)$$

Early MST versions computed the three contributions to the free energy of solvation based on a unique solute/solvent interface. That interface was defined as the solvent-excluded surface generated by suitably scaling the van der Waals radii of the atoms. Scaling factors of 1.25, 1.60, and 1.80 were optimized for calculations in water,[840,841] chloroform,[125] and carbon tetrachloride,[842] respectively, by using the HF/6-31G(d) version of the MST model. (Slightly different scaling factors were optimized for water in semiempirical versions of the MST model; see refs 793−794 for details).

Recently, the suitability of using a dual-cavity definition, as opposed to a single-cavity approach, for determining the electrostatic and nonelectrostatic contributions to the free energy of solvation was examined.[836] According to this method, the electrostatic term is determined using the cavity that results from scaling the van der Waals radii, while the nonelectrostatic component is determined from the cavity generated by unscaled van der Waals radii. The results obtained from this dual-cavity treatment were found to be in better agreement with chemical intuition, and this approach has been consequently introduced into the latest versions of the MST approach.

6.7.b. Treatment of the Polarization Effect: The Semiclassical MST Model

To account for the polarization of the solute by the solvent reaction field, we[845,846] along with others[847] recently developed a simple expression derived from a perturbation treatment of the linear response approximation of the solvent effect. Such an expres-

sion very accurately reproduces the polarization contribution to the free energy of solvation.

The polarization free energy, ΔG_{pol}, which is defined as the change in the free energy of solvation due to relaxation of the solute wave function upon solvation, is given by eq 6.47. $V_R(\Psi^{sol})$ and $V_R(\Psi^0)$ denote the solvent reaction field induced by the solute charge distribution when it is fully relaxed in solution and when in the gas phase, respectively. The first and second terms on the right-hand side of eq 6.47 correspond to the electrostatic free energy of the fully polarized solute in solution and of the solute with a gas-phase charge distribution.

$$\Delta G_{pol} = \left\langle \Psi^{sol} \middle| \hat{H}^0 + \frac{1}{2}\hat{V}_R(\Psi^{sol}) \middle| \Psi^{sol} \right\rangle - \left\langle \Psi^0 \middle| \hat{H}^0 + \frac{1}{2}\hat{V}_R(\Psi^0) \middle| \Psi^0 \right\rangle \quad (6.47)$$

ΔG_{pol} also can be expressed as the addition of (i) the distortion free energy, ΔG_{dis} (eq 6.48), which accounts for the solute's loss of internal stability when its electronic distribution is perturbed by solvation, and (ii) the stabilization free energy, ΔG_{sta} (eq 6.49), which reflects the increased intermolecular stability of the solute/solvent system. Since this latter contribution is expressed in terms of solute−solvent interactions, it can be easily translated to a classical framework. However, QM methods are required to accurately describe the distortion term since it involves electron density changes.

$$\Delta G_{dis} = \langle \Psi^{sol} | \hat{H}^0 | \Psi^{sol} \rangle - \langle \Psi^0 | \hat{H}^0 | \Psi^0 \rangle \quad (6.48)$$

$$\Delta G_{sta} = \left\langle \Psi^{sol} \middle| \frac{1}{2}\hat{V}_R(\Psi^{sol}) \middle| \Psi^{sol} \right\rangle - \left\langle \Psi^0 \middle| \frac{1}{2}\hat{V}_R(\Psi^0) \middle| \Psi^0 \right\rangle \quad (6.49)$$

As noted above, the perturbative treatment of the solvation process[845,846] allows the polarization contribution to be rewritten as eq 6.50, which, quite remarkably, permits ΔG_{pol} to be computed in terms of solute−solvent interactions. More importantly, the addition of eq 6.50 to the electrostatic free energy of the solute when it has the gas-phase charge distribution allows the total electrostatic component of the free energy of solvation to be estimated, as shown in eq 6.51. In contrast to eq 6.1, the simple expression given by eq 6.51 only involves interactions between the solute and solvent molecules, thus facilitating its implementation in classical calculations.

$$\Delta G_{pol} = \left\langle \Psi^0 \middle| \frac{1}{2}\hat{V}_R(\Psi^{sol}) \middle| \Psi^0 \right\rangle - \left\langle \Psi^0 \middle| \frac{1}{2}\hat{V}_R(\Psi^0) \middle| \Psi^0 \right\rangle \quad (6.50)$$

$$\Delta G_{ele} = \Delta G^0_{ele} + \Delta G_{pol} = \left\langle \Psi^0 \middle| \frac{1}{2}\hat{V}_R(\Psi^{sol}) \middle| \Psi^0 \right\rangle \quad (6.51)$$

It is worth noting that eqs 6.50 and 6.51 very accurately reproduce the exact values obtained from a full QM treatment of the solvation of a large variety of solutes.[845,846] Indeed, when eq 6.51 is expressed in a classical framework, the polarization effects can be directly included in the calculation of the electrostatic free energy by using an inexpensive procedure. This procedure requires the definition of two sets of charges for the solute, one which describes the charge distribution of the solute in the gas phase $\{Q_i^0\}$ and the other which describes its charge distribution in solution $\{Q_i^{sol}\}$. The electrostatic free energy then is simply given by eq 6.52, where the solvent reaction field is computed from the charges that represent the fully relaxed charge distribution of the solute in solution. This is in contrast to the usual expression, as given by eq 6.39, where only the gas-phase solute charge distribution is considered.

$$\Delta G_{ele} = \frac{1}{2}\sum_{i=1}^{N}\sum_{k=1}^{M}\frac{Q_i^0 q_k(Q_i^{sol})}{|r_i - r_k|} \quad (.52)$$

As previously noted, the accuracy of the electrostatic free energy of solvation greatly depends on the quality of the charges that simulate the solute charge distribution (Q). Suitable charges can be derived by fitting the QM electrostatic potential determined from the solute wave function in the gas phase and in solution, but better results are obtained when the charges are simultaneously fitted to both the electrostatic potential and field computed at selected points on the cavity surface of the solute.[848]

Combining eq 6.52 with expressions for cavitation (eq 6.45) and van der Waals (eq 6.46) contributions constitutes the essence of the semiclassical MST model. This model has been implemented in classical MC and MD discrete methods[849] and in classical continuum models.[845,846,850] Recently, it also has been combined with standard Monte Carlo techniques to explore the configurational space of solutes in solution, and has been used to study the influence of solvent on the dimerization of pairs of interacting solutes, including both neutral molecules and salt bridges.[220,300,302]

6.8. Other Continuum Electrostatic Methods

Electrostatic continuum methods exist which cannot be easily classified as belonging to any of the categories listed above. Among these the field energy method merits special attention. This approach was developed by Schaefer and co-workers[793,851] and has been utilized by a few other groups[852,853] that are interested in the solvation of macromolecules. The field energy method has much in common with strategies such as the image charge method and the generalized Born model. Its main feature is that the electrostatic free energy of solvation is not determined from eqs 6.13 or 6.11 but instead is computed by integrating the energy density of the electrostatic field (eq 6.53), thus avoiding calculation of the solvent-screened electrostatic potential. In eq 6.38, E_{ele} is the total electrostatic energy of the system and the index R^3 indicates that the integration is carried out over the entire space. The energy density $u(r)$ is determined from the electrostatics displacement vector $D(r)$, as shown in eq 6.54.

$$E_{ele} = \int_{R^3} u(r)\, dr \quad (6.53) \qquad (6.53)$$

$$u(r) = \frac{1}{2} D^2(r)/\epsilon(r) \qquad (6.54)$$

If point charges are used,[793,852,853] the total displacement vector can be determined as the sum of atomic displacements (eq 6.55, where i stands for a center of charge on the solute) and the total electrostatic energy can be determined as shown in eq 6.56, where the terms E^{self} and E^{int} resemble the "self" and "screened" terms found in the GBM method and can be computed as shown in eqs 6.57 and 6.58.

$$D(r) = \sum_i D_i(r) \qquad (6.55)$$

$$E_{ele} = \sum_i E_i^{self} + \sum_{i>j} E_{ij}^{int} \qquad (6.56)$$

$$E_i^{self} = \frac{1}{2} \int_{R^3} \frac{D_i^2(r)}{\epsilon(r)} dr \qquad (6.57)$$

$$E_{ij}^{int} = \int_{R^3} \frac{D_i(r) \cdot D_j(r)}{\epsilon(r)}\, dr \qquad (6.58)$$

Several simplifications are introduced in order to solve eqs 6.57 and 6.58. First, the space is divided as usual into solute and solvent regions, which allows $\epsilon(r)$ to be replaced by the dielectric constants for the solute (inside the cavity) and the solvent (outside the cavity). This allows the two integrals to be partitioned into solute and solvent regions, which facilitates their computation. A second simplification is the assumption that the contribution of the reaction field to the total electric displacement is negligible outside of the solute cavity (the Coulomb field approximation) and that the electric displacement can be determined from the image charge approximation for a "pseudo-planar" solute/solvent boundary.[793] The algorithms that were developed using these approximations appear to be more efficient at calculating the self-energy term than the interaction term. Thus, in recent implementations[793,852,853] the interaction term has been obtained from GBM calculations. Schaefer and Karplus[851] further improved the method by using Gaussian charge distributions to represent the solute charge atmosphere, and Caflisch and co-workers implemented it in a docking program.[852] This method has not yet been extensively used, which makes it difficult to quantitatively compare its results with those from related methods. However, preliminary studies suggest that this method can be a good alternative to GBM (with similar advantages and shortcomings) for analyzing solvation in macromolecules.

7. Other Methods

There are other methods for describing solvation in biological systems which cannot be classified in any of the categories already reviewed in the preceding sections. We will summarize two of the most popular: (i) Langevin dipoles and (ii) the RISM integral equation.

7.1. Langevin Dipoles

The description of environmental effects by means of Langevin dipoles (LD) was pioneered by Warshel's group[354,541,769,854−861] and was later adopted by other authors (for example, see refs 864 and 865). Warshel and co-workers developed different LD models of increasing complexity for the study of processes in solution or in proteins and also extended the LD model to the QM framework.[486,518,854,859] Their most recent version of the LD model is actually a hybrid PCM/LD method, where PCM calculations are carried out to describe the polarization of the solute charge distribution while LD calculations are used to evaluate the solvation free energy.[861−863] Recently, this method has been used to calculate hydration entropies.[861,863] This treatment lies between continuum and discrete methods, since it avoids the use of the dielectric constant but omits a detailed description of solvent molecules.

The simplest system studied by LD models is that of a small polar solute in water.[486,541,769] The solute is placed in the center of a cubic grid, whose spacing (~3 Å) mimics the density of pure water. All grid points beyond a given spherical cutoff or inside the van der Waals sphere of the solute are removed (Figure 4). The Langevin dipoles (eq 7.1) are then

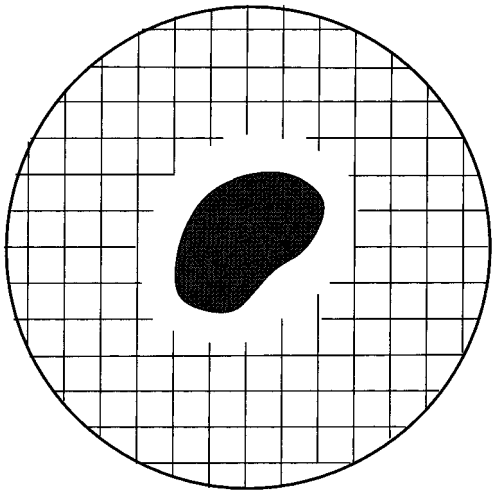


Figure 4. Schematic representation of the grid used to center dipoles in Warshel's Langevin dipole model.

centered at each of the remaining grid points, and the polarization of the solvent by the solute is accounted for by reorienting the solvent dipoles, which generates the reaction field.

$$\mu_i = \mu_0 \left(\coth\left(X_i = \frac{1}{X_i}\right) e_i \right. \qquad (7.1)$$

In eq 7.1, μ_0 represents the water dipole[858] but can be considered to be an adjustable parameter.[354,486,541] e_i is a unit vector indicating the direction of the electric field (ξ_i in Warshel's nomenclature, which is mostly retained here), and X_i is determined as shown in eq 7.2, where the resistance to reorientation by a water molecule is accounted for through the param-

eter C, which adopts values of 1[541] or 0.5,[486] as determined by fitting the LD calculations to all-atom MD simulations.

$$X_i = C\frac{\mu_0\,\xi_i}{k_B T} \tag{7.2}$$

We should note the interdependence between the electric field and the Langevin dipoles. This arises from eqs 7.1 and 7.2 along with the fact that the total electric field at the grid point i is the sum of the electric fields created by the fixed charges of the solute and by the other Langevin dipoles (eq 7.3). This interdependence makes it necessary to use an iterative process in solving eqs 7.1−7.3.[354,541,769]

$$\xi_i = \xi_i^0 + \xi_i^\mu \tag{7.3}$$

Once the Langevin dipoles are defined, the electrostatic contribution to solvation can be determined from eq 7.4. Steric contributions to the solvation can be treated as in standard force-field calculations.

$$\Delta G_{ele} = -\frac{1}{2}\sum_i \mu_i \xi_i^0 \tag{7.4}$$

The LD method requires parametrization of the terms C and μ_0 (eqs 7.1 and 7.2), as well as of the van der Waals parameters that define the exclusion radii for the placement of Langevin dipoles. In fact, the parametrization of this exclusion surface is conceptually similar to the optimization of the cavity size in continuum models (see above).

The LD model is an interesting approach since it combines the speed of continuum methods with a semimicroscopic representation of the solvent. However, it has some limitations due to its simplicity. These simplifications are as follows: (i) the solvent electrostatic potential is expected to be well represented by a dipole term, (ii) the density of water around the solute is assumed to be "nearly" constant, (iii) the dielectric response related to the electron relaxation is omitted, and (iv) the results can depend on the grid spacing and might not be rotationally invariant. Warshel and co-workers improved their method to correct for some of these shortcomings. They reduced the rotational variance of the results by modifying the grid so that the centers of the dipoles are regularly spaced (according to the water density) on the molecular surface.[486] They also refined their use of a finite spherical grid by adding a continuum correction to account for long-range interactions.[541,854] Finally, they have attempted to introduce electronic polarization into the model by means of the induced dipole theory (see the MD-MC section above).

The protein dipole−Langevin dipole (PD−LD) method is a LD model that combines an all-atom treatment of nonaqueous solvents with induced dipoles to represent the electronic polarization. It has been widely used to study processes in proteins. The system is divided into four components: (i) the site of interest inside the protein, (ii) the rest of the protein residues, (iii) the solvent molecules near the protein, and (iv) the bulk solvent. These components are treated at different levels of complexity. The site of interest is treated at the QM level or using classical force fields with or without the inclusion of induced dipoles. The remaining atoms of the protein are represented by point charges, induced dipoles, and suitable van der Waals parameters. The water molecules near the protein are treated as Langevin dipoles, and the remaining solvent is simulated by a continuum electrostatic model.

7.2. RISM Integral Equation

An alternative approach to describing solvent effects in biomolecules is found in the reference interaction site model (RISM), which relies on statistical mechanical integral equation theories of liquids.[866] This model provides detailed information about solute−solvent interactions in terms of statistically averaged site−site distribution functions. The formalism was originally developed by Chandler and coworkers[867−872] and was later improved by others for application to polar liquids and ionic solutions.[873−876]

The RISM approach is derived from pair-correlation theory. Accordingly, both solute and solvent are considered to have a set of interaction sites which interact as described by suitable effective pair potential functions. The total pair-correlation function between interaction sites x and s in the respective molecular species X and S depends on the direct correlation function for finding site x at a given distance from s, along with the probability of site x being correlated with another site s', which is in turn correlated with site s integrated over all possible positions of s', plus triple pairs and so on. For molecular fluids, these relationships can be written in terms of the so-called RISM integral equation (eq 7.5), which is usually written in the more compact matrix form given by eq 7.6.

$$\rho_x\rho_s h_{x\,s}(r) = \sum_{y\in X}\sum_{t\in S}\omega_{xy} * c_{yt} * \omega_{ts} + \sum_{S'}\sum_{y\in X}\sum_{s'\in S'} \quad (.5)$$

$$\rho\mathbf{h}\rho = \rho\omega * \mathbf{c} * \rho\omega + \rho\omega * \mathbf{c} * \rho\mathbf{h}\rho \tag{7.6}$$

In eq 7.5, ρ_x and ρ_s denote the number density of x- and s-type sites in species X and S, respectively, while ρ_S and $\rho_{s'}$ are the respective number densities of species S and S'. ω denotes the intramolecular correlation function, c represents the site−site intermolecular direct correlation function, and h is the site−site total intermolecular correlation function.

A closure relationship is required in order to solve the system of integral equations represented by eq 7.6, and this is generally accomplished by using either the hypernetted chain technique (see ref 873) or slightly modified expressions such as given by eq 7.7,[877−879] where β is the inverse of the Boltzmann constant multiplied by the absolute temperature and U^{eff} is an effective site−site interaction potential function.

$$h_{x\,s}(r) = \exp\{-\beta U_{x\,s}^{eff}(r) = h_{x\,s}(r) - c_{x\,s}(r)\} - 1 \tag{7.7}$$

The interaction potential function is generally expressed as the sum of a short-range Lennard−Jones energy term and a Coulombic interaction energy. In some cases, however, the Coulombic term is corrected by parameters chosen to ensure that the calculated total solvent correlation functions are consistent with the macroscopic dielectric constant of the solvent.[876,877,880−882]

Solving eqs 7.6 and 7.7 makes it possible to compute the free energy of solvation by using the hypernetted chain closure technique (eq 7.8).[879,883,884] An alternative solvation functional is given by eq 7.9,[885] which differs from eq 7.8 in that it does not have the term $^1/_2 h^2_{xs}(r)$. It also assumes that the solvent fluctuations follow a Gaussian statistical distribution and that the solute couples linearly to the solvent with a strength determined by the direct correlation function.

$$\Delta G_{\mathrm{sol}} = 4\pi\rho_S RT \sum_{x\in X}\sum_{s\in S}\int_0^\infty r^2 \left[\frac{1}{2}h_{xs}{}^2(r) - c_{xs}(r) - \frac{1}{2}h_{xs}(r)c_{xs}(r)\right] \mathrm{d}r \quad (7.8)$$

$$\Delta G_{\mathrm{sol}} = 4\pi\rho_S RT \sum_{x\in X}\sum_{s\in S}\int_0^\infty r^2 \left[c_{xs}(r) + \frac{1}{2}h_{xs}(r)c_{xs}(r)\right] \mathrm{d}r \quad (7.9)$$

Most recent applications of the RISM integral equation have focused on the conformational equilibrium of biopolymers in solution and especially on the role of salt effects in these equilibria.[886−891]

Hirata et al.[892,893] coupled the electronic structure of the solute as determined from quantum chemical calculations with the solvent distribution described by the extended RISM method. In their implementation, a set of partial charges for the solute atoms is determined from the previous step in SCF and then plugged into a RISM calculation to obtain a solvent atom distribution around the solute. Subsequently, the electrostatic potential (eq 7.10) produced by the solvent charge distribution at each solute atom is incorporated into the solvated Fock operator in order to perform the next step in the SCF cycle. In eq 7.10, q_s is the partial charge at solvent site s and $g_{xs}(r)$ is the radial distribution function of the solvent molecules' interaction site s around the solute's site x (this latter term is related to the total correlation function by $g_{xs}(r) = h_{xs}(r) + 1$). This process is then repeated until consistency is obtained, which allows both the electronic structure of the solute and the solvent distribution to be simultaneously optimized during the RISM-SCF cycle.

$$V_x = 4\pi\int_0^\infty \sum_{s\in S} q_s\rho_s r g_{xs}(r)\,\mathrm{d}r \quad (7.10)$$

This RISM-SCF method has been applied to the study of solvent-induced shifts in absorption spectra,[892,893] the acidity/basicity of organic compounds,[894−896] and conformation[897] and tautomerism.[205]

An alternative implementation of the RISM theory in semiempirical AM1 and PM3 Hamiltonians, denoted XSOL, has been reported by Gao et al.[898] The main difference between this approach and the RISM-SCF approach lies in the nature of the solute charges employed to solve the extended RISM equations. Thus, whereas Hirata et al. used electrostatic potential-fitted charges, Gao et al. used the charge model 1 (CM1) algorithm developed by Cramer and Truhlar[899] to derive atomic charges for the solute. Furthermore, Gao and co-workers optimized the van der Waals parameters by fitting XSOL estimates of the solvation free energy to their experimental values for a series of small organic solutes.[898]

8. Concluding Remarks

Within the past few years, theoretical chemists have realized that a detailed understanding of chemical or biochemical systems is impossible without an accurate description of their solvent effects. Consequently, a tremendous effort has been made to develop methodological approaches to treat these solvent effects, and at this time, the beginning of the 21st century, many methods for representing solvation in biomolecular systems are available. In this review we have tried to summarize the most important characteristics of the different approaches, emphasizing their strengths and weaknesses. We hope that this will help the reader choose the most suitable approach for dealing with solvent effects in a given biomolecular system.

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