Free Energies of Chemical Reactions in Solution and in Enzymes with Ab Initio Quantum Mechanics/Molecular Mechanics Methods

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enzyme catalysis, solution reaction, enzyme proficiency, multiscale method, potential of mean force, QM/MM

Abstract

Combined quantum mechanics/molecular mechanics (QM/MM) methods provide an accurate and efficient energetic description of complex chemical and biological systems, leading to significant advances in the understanding of chemical reactions in solution and in enzymes. Here we review progress in QM/MM methodology and applications, focusing on ab initio QM-based approaches. Ab initio QM/MM methods capitalize on the accuracy and reliability of the associated quantum-mechanical approaches, however, at a much higher computational cost compared with semiempirical quantum-mechanical approaches. Thus reaction-path and activation free-energy calculations based on ab initio QM/MM methods encounter unique challenges in simulation timescales and phase-space sampling. This review features recent developments overcoming these challenges and enabling accurate free-energy determination for reaction processes in solution and in enzymes, along with applications.

INTRODUCTION

QM: quantum mechanics

MM: molecular mechanics

MD: molecular dynamics

DFT: density-functional theory

Understanding chemical reactions in solution and in enzymes is of ultimate importance. The majority of chemical reactions for synthesis or manufacture take place in solution. Investigating solvent effects on reaction mechanisms has long been and continues to be an area of important and challenging inquiry. In biological systems, most biological functions are accomplished through the binding of ligands with proteins and/or a series of chemical reactions catalyzed by specific enzymes with different efficiency and specificity. The structure-function relationship and the catalytic role of enzymes are thus fundamental subjects in biochemical research. They are also essential for the development of new or better inhibitors and enzymes, which have important practical applications, ranging from drug design to the development of novel catalysts in industrial processes. As an increasing amount of structural information for proteins and enzymes becomes available, the structure-function relationship becomes an even more important link in biological science. Quantitative tools such as simulations will make key contributions to the investigation of this topic.

In the process of chemical reactions, only a small number of atoms directly participate in bond forming or breaking processes. Many other atoms in the system do not undergo changes in electronic structure, but instead serve as a steric and electrostatic environment to influence the properties and reactivity of the active site. The quantum mechanics/molecular mechanics (QM/MM) approach, first developed by Warshel & Levitt (1), is a multiscale/multiresolution simulation approach: The interesting part of the system, such as the active site of an enzyme, is described at the electronic level with QM, whereas the rest of the system is described at the atomistic level with MM. This combined QM/MM methodology allows reliable electronic-structure calculations for enzymatic reactions with a realistic and atomistic description of the enzyme environment. Such an approach takes advantage of the applicability and accuracy of the QM methods for chemical reactions in systems of tens of atoms and the computational efficiency of the MM description for the rest of the enzyme and solvent, which normally consists of many thousands of atoms. The development of combined QM/MM methods has enabled simulations of complex chemical and biological processes, leading to significant advances in our understanding. In particular, simulations have generated considerable insight into chemical reaction mechanisms in solution and in enzymes, as discussed in several recent reviews (2–8).

We can classify the QM/MM approach into two types according to the level of QM theory used. One type involves the use of semiempirical methods such as MNDO, AM1, PM3, empirical valence bond, and the recently developed self-consistent-charge density-functional tight-binding method (9–12). The majority of the work in the field is carried out with these methods. The computational efficiency of semiempirical QM/MM is such that direct molecular dynamics (MD) sampling is readily affordable; thus free-energy and reaction-dynamics calculations can be routinely performed.

The other type of QM/MM calculation (7, 13–20) uses ab initio QM via wavefunction theory or density-functional theory (DFT). DFT is the most popular approach because of the optimal balance of efficiency and accuracy (21–23). Recent

development in approximate functionals has resulted in even better accuracy at a similar computational cost (24). However, because ab initio QM calculations are computationally much more demanding than semiempirical methods, rigorous statistical mechanics sampling and reaction-dynamics calculations with an ab initio QM/MM method are most challenging.

In the present review, we focus on the theoretical development and application of ab initio QM/MM free-energy simulation methods for chemical reactions in solution and in enzymes.

TS: transition state

PMF: potential of mean force

Reaction Free Energy and Rate

The fundamental properties of a chemical reaction process include the equilibrium distribution of states, kinetics, and the reaction mechanism. Thermodynamic laws tell us that equilibrium in a reaction process is determined by the free-energy difference between the reactant and product states. This free-energy difference determines the equilibrium, but not the reaction rate in general.

Reaction rate can be determined rigorously using theory based on classical statistical mechanics or quantum statistical mechanics. The exact classical rate constant $k(T)$ at temperature T is

$$k(T) = \gamma(T)\frac{1}{\beta h}\exp(-\beta\Delta G^{\ddagger}(T)), \quad (1)$$

where $\gamma(T)$ is the transmission coefficient, $\beta = 1/k_B T$, k_B is the Boltzmann constant, and $\Delta G^{\ddagger}(T)$ is the molar activation free energy [i.e., the free-energy difference between the reactant and transition state (TS)] (2, 3, 25). Within the TS approximation, $\gamma(T) = 1$, and $\Delta G^{\ddagger}(T)$ then determines the rate.

TS is a special state of the reacting system whose population controls how rapidly the system can transfer from the reactant valley to the product valley. If the dynamics of a system is projected onto the reaction coordinate that characterizes the reaction process, TS is the point with the highest (free) energy on this one-dimensional potential of mean force (PMF). Identification of TS is crucial not only for rate determination, but also for understanding the reaction mechanism.

Modeling Enzyme Catalysis

For enzymatic reactions, experimental studies provide crucial and indispensable information concerning the reaction mechanism, thermodynamics, and kinetics. However, experimental data are often insufficient for determining the detailed reaction mechanism (26). Most importantly, experimental study cannot directly determine the TS structure, which is crucial for biomedicinal research such as inhibitor or drug design.

Complementary to experimental study, simulations can yield atomistic or even electronic information regarding the effects of site-specific interactions on the reaction process, the reaction path, and the TS structure (2–4, 6, 12, 16, 27). Simulation studies of many enzymatic processes have addressed and also raised important issues in enzyme catalysis, such as covalent catalytic mechanisms (28, 29), the contribution

of the preorganized electrostatic environment of enzymes (30, 31), the effects of strain and conformational dynamics of the enzyme-substrate complex, nonequilibrium dynamical effects, and quantum tunneling effects (32, 33).

PES: potential energy surface

Despite numerous simulation studies, the origin of enzymatic proficiency remains a current topic of interest because the contributions from different sources may vary in different enzymes (26, 34). Accurate simulation methods will definitely help the understanding of those complex interactions in the enzyme catalysis.

Unique Role of Solution Reactions

Solvent molecules play significant roles in chemical reactions in solution (9, 33, 35, 36). Energetically, the complex electrostatic interactions between the solvent and solute molecules create a reaction environment drastically different from the gas phase, resulting in a different chemical equilibrium and reaction rate. Dynamically, the fluctuation and diffusion of the solvent molecules serve as an energy bath to the motions of the reaction moieties, resulting in different relaxation and barrier-crossing dynamics. Last but not least, direct participation of the solvent molecule in the reaction not only creates a concentration effect, but also may alter the reaction path.

Solution reactions are important references for enzymatic reactions. In most cases, the slow reaction rate of the noncatalyzed solution reaction is the evolutionary driving force for enzymes; the exceptional proficiencies of enzymes are usually determined by the degree of difficulty of the solution reaction (37). From this perspective, understanding enzymatic proficiency requires determining the differences between the enzyme-catalyzed reaction and its corresponding solution reaction.

Solution reactions are often simulated with varying levels of approximation, particularly for simulations employing ab initio approaches. One common practice is to simplify the description of the solvent by using a continuum representation. This reduces the number of degrees of freedom in the simulation, but the isotropic continuum-medium model cannot always correctly reproduce the anisotropic, site-specific interactions between the solute and solvent molecules (**Figure 1**). To make a reliable comparison, one must simulate both the solution and enzymatic reactions at the same level of accuracy.

QUANTUM MECHANICS/MOLECULAR MECHANICS POTENTIAL ENERGY SURFACE

The first requirement for simulating a reaction process is a potential energy surface (PES) that accurately describes the chemical changes with the redistribution of electrons. To account for this effect, in general QM becomes necessary.

With currently available computing resources, a high-level ab initio QM description of the entire system is affordable only for small systems of approximately 100 atoms or less. For reactions in solution or in enzymes, too many electronic degrees of freedom of the system make it difficult, if not impossible, to describe the whole system quantum mechanically. Although semiempirical QM methods using a linear

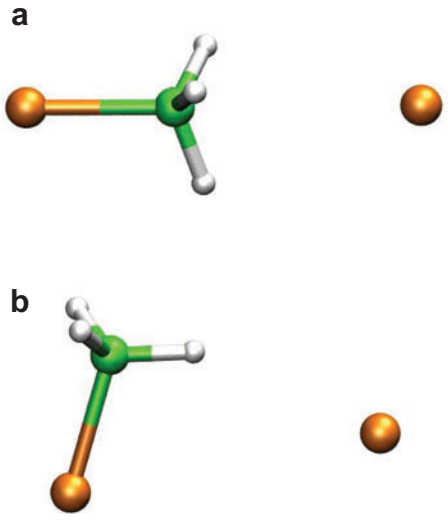

Figure 1

Reactant structure of the $CH_3Cl + Cl^-$ self-exchange reaction. (*a*) Geometry optimized in gas phase. (*b*) Geometry optimized in explicit solvent with the ab initio quantum mechanics/molecular mechanics minimum free-energy path method.

scaling method such as the divide-and-conquer approach have been applied to large proteins and enzymes (38–43), computational speed is still a limiting factor, and the results may be less reliable because of the semiempirical approximations.

An effective and attractive solution to the energetic description of macromolecules is found in the combined QM/MM method (1, 11). By partitioning the entire system into QM and MM subsystems, we can write the total internal energy of the QM/MM system as

$$\begin{aligned} E_{QM/MM}(\mathbf{r}_{QM}, \mathbf{r}_{MM}) = {} & E_{QM}(\mathbf{r}_{QM}, \mathbf{r}_{MM}) + E_{QM/MM,ele}(\mathbf{r}_{QM}, \mathbf{r}_{MM}) \\ & + E_{QM/MM,vdw}(\mathbf{r}_{QM}, \mathbf{r}_{MM}) + E_{QM/MM,cov}(\mathbf{r}_{QM}, \mathbf{r}_{MM}) \\ & + E_{MM}(\mathbf{r}_{MM}). \end{aligned} \quad (2)$$

The first two terms on the right-hand side are the QM internal energy and the electrostatic energy between the QM and MM subsystems, for which the calculation is described in next section. The remaining three terms are the van der Waals energy between the QM and MM subsystems, the covalent interaction energy between the two subsystems, and the purely MM interaction energy of the MM subsystem, respectively, which are described with a given MM force field.

Owing to the diversity in their forms and parameters, simulations performed with different MM force fields may show somewhat large discrepancies in the results (44). Instead of MM force fields, an effective fragment potential method has been developed to derive a simple potential energy function for complex systems from ab initio QM calculations (45).

Quantum Mechanics/Molecular Mechanics Electrostatic Interactions

ESP: electrostatic potential

Computing the first two terms on the right-hand side of Equation 2 is at the core of the QM/MM methods. A straightforward procedure is to compute the two terms together by including the electrostatic potential (ESP) from the MM atoms in the QM calculation; that is,

$$E_{QM} + E_{QM/MM,ele} = \langle \psi | H_{eff} | \psi \rangle, \quad (3)$$

where H_{eff} is the effective QM Hamiltonian including the MM ESP. In this charge-embedding scheme, the QM internal energy and the QM/MM electrostatic energy are computed together in a self-consistent manner. In other words, the polarization of the QM subsystem due to the presence of the MM subsystem is captured at the same level of QM theory.

In the mechanical embedding approach represented by the class of ONIOM methods (46), the systems are hierarchically split into different layers that are described at different levels of theory. The energy function of the outer layer automatically includes the energy of the inner layer, but it is described with a lower-accuracy method.

Quantum Mechanics/Molecular Mechanics Boundary

When one or more covalent bonds connect the QM and MM subsystems, how to handle the boundary between the QM and MM subsystems becomes an important question. Several approaches have been developed (11, 13, 19, 20, 47–50). Traditionally, link hydrogen atoms are added to the MM-bonded QM atoms to saturate the valence orbital of the QM subsystem (11). Using additional hydrogen atoms to cap the QM subsystem is easy to implement, but the resulting system is thermodynamically different from the original one because the total number of atoms is different.

In contrast, two other approaches, namely the pseudobond method (13, 47) and the frozen local-orbital method (19, 20, 48–50), do not bear such problems. The pseudobond method assigns the MM-bonded QM atoms a special basis set and an effective core potential designed to mimic the correct covalent bonding involving the boundary QM atoms (**Figure 2**). By making such atoms with a free valence of one, there is no need for additional atoms to saturate the QM/MM covalent bonds. Several methods, following the pseudobond method in using effective core potentials, have subsequently been developed, including the quantum capping potential method (51, 52), the effective group potential technique (53), effective Hamiltonians from a minimum principle (54), variational optimization of effective core potentials for molecular properties (55), and multicentered valence-electron effective potentials (56). A similar approach (57) has also been developed for the semiempirical QM/MM method.

The frozen local-orbital method, first developed by Rivail and coworkers (48), assigns a set of specially designed local orbitals to the boundary QM or MM atoms to maintain closure of the QM subsystem. In different implementation schemes, the magnitude of the neighboring MM charges, the positions of the MM point charges, and the positions of the frozen orbitals can vary.

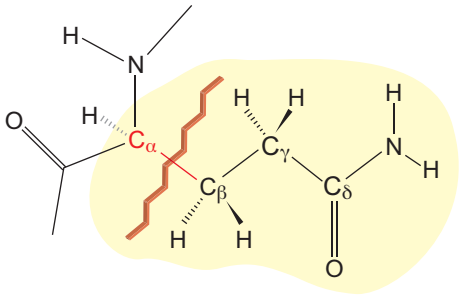

Figure 2

Illustration of the pseudobond method. The side chain of the Gln residue is described with quantum mechanics (*yellow region*) and the C_α atom has a designed effective core potential, one free valence, and a special basis set. The C_α–C_β bond becomes a pseudobond with similar bond length, bond strength, and charge distributions as in a normal C(*sp*3)–C(*sp*3) single bond.

In connection with this QM/MM boundary problem, another important issue is how to compute the electrostatic interactions between the QM subsystem and the nearby MM atomic charges, in particular those MM atoms in covalent contact with the QM atoms. Direct inclusion of those MM point charges in the QM effective Hamiltonian can cause charge penetration and off-balance polarization of the QM electrons. To address this problem, researchers rescaled the MM charges (13) or smeared the MM charges using Gaussian distributions (58).

Long-Range Quantum Mechanics/Molecular Mechanics Electrostatic Interactions

Most QM/MM simulations have not yet addressed the importance of the correct description of long-range QM/MM electrostatic interactions, as many have been performed with stochastic boundary conditions. The inclusion of long-range QM/MM electrostatic interactions is not trivial because the well-established Ewald summation method may not be directly applicable. In fact, several technical concerns must be noted.

The first issue concerns the treatment of a periodic QM subsystem. In biomolecular simulations, usually the simulation box must be large enough so that the interaction between the QM subsystem, which is often charged, and its images is negligible. The second concern is the consideration of the periodically distributed MM charges in the QM calculation with the charge-embedding scheme. Even though Ewald-type methods have been developed for semiempirical methods (59, 60), a similar development for ab initio QM/MM methods is still needed.

The long-range electrostatic interaction is undoubtedly important for stabilizing the enzyme structures, and it may also be important for enzyme functions. However, the key question in both cases might be whether the long-range polarization is significant for the QM subsystem. Even for a charge-transfer reaction, the QM polarization effects are small when the MM point charges are more than $9 \sim 14$ Å from

the QM atoms (61). Beyond this distance, the MM point charges merely contribute by providing a static ESP. Therefore, we have adopted a simple technical scheme in our simulations with the periodic boundary condition (61–63) by using a cutoff of 9 ∼ 14 Å for selecting MM charges for the QM self-consistent field (SCF) calculation. The QM calculation yielded polarized QM ESP charges that in turn were used to represent the QM subsystem during MD simulations with the long-range electrostatic interactions treated by the particle-mesh Ewald method (64).

SCF: self-consistent field

MEP: minimum energy path

MFEP: minimum free-energy path

To avoid the technical difficulties associated with periodic QM/MM systems, researchers have made important progress in approximating long-range QM/MM electrostatic interactions by implementing a stochastic generalized solvent boundary with the self-consistent-charge density-functional tight-binding method (65, 66). Gregersen & York (67) have developed a variational electrostatic projection method that employs a continuum solvent model for long-range electrostatic interactions.

REACTION PATHS

A reaction path provides a clear picture of a chemical reaction mechanism, as well as quantitative energetic information regarding the reactant, TS, and product, which are required for reaction-rate calculations. A reaction path also serves as the structural basis for the detailed analysis of site-specific interactions involved in the reaction process and can be used in the design of new inhibitors.

For reactions of small molecules in the gas phase, one can determine reaction paths as the minimum energy path (MEP) on the total PES. The TS on the PES of the entire system plays the most important role for the rate. One can determine the free energies of the reactant and the TS with the harmonic approximation, based on QM calculations of frequencies.

For reactions in solution and in enzymes, the TS on the PES of the entire system represents only one of many such states because there are many degrees of freedom (e.g., for the solvent and the protein) that are not directly related to the chemical changes but modulate the shape of the PES. In such cases, the progress of a reaction can also often be marked by the structural changes of a reduced set of atoms (e.g., the solute conformation for solution reactions or the conformation of the substrate plus certain active-site residues for enzymatic reactions). Quite conveniently, the reduced set of coordinates can be the same as the QM subsystems. Thus, one can construct the minimum free-energy path (MFEP), a reaction path defined on the PMF of a selected set of coordinates (62, 68–71). The contributions from the rest of the system are ensemble-averaged quantities obtained during MD simulations.

Reaction Coordinate

Usually defined as a set of geometric or energetic parameters, the reaction coordinate characterizes a reaction as the one-dimensional profile connecting reactants to products. The choice of the reaction coordinate is critical. The improper choice of a reaction coordinate can bias the simulation and yield slower convergence (72). The problem of an inappropriately chosen reaction coordinate is more severe in simulations using coordinate-driving techniques in which the choice of the reaction

coordinate not only strongly influences the efficiency of sampling the phase space, but also causes technical difficulties in generating a continuous reaction path. In many cases, the determination of the reaction coordinate is nontrivial, especially for many complicated reactions catalyzed by enzymes, as the changes in specific geometrical quantities (e.g., interatomic distances) might be stepwise or nonlinearly correlated. Furthermore, the environmental degrees of freedom may also contribute to the reaction coordinate as dynamic effects (25).

Reaction-Path Optimization

The mathematical methods for determining the reaction path are the same for the MEP on the total PES as for the MFEP on the PMF surface of the QM subsystem. The classical method for determining a reaction path is the intrinsic reaction coordinate method in which the steepest descent path is computed from the TS by following the gradient downhill. This method is not efficient for macromolecular systems and also requires knowledge about the TS structure, which is a difficult task without prior knowledge of the reaction path.

Several algorithms have been developed for the determination of reaction paths for large systems in which a chain of conformations along the reaction coordinate is simultaneously optimized. Commonly used methods include the nudged elastic band (NEB) method (73), the Ayala-Schlegel second-order MEP method (74), and string methods (75). Our experience indicates that the NEB method is simple to implement but converges slowly and has difficulty locating TSs. The Ayala-Schlegel method can converge to the correct path, but its application to large molecular systems is troublesome because it requires an initial guess that is close enough to the exact path. As an alternative, we developed a quadratic string method that yields better performance than the NEB method (76). For large systems, it is also important to select a small subset of coordinates as the chemical metric to define the path length for properly positioning the states along the reaction path (77).

One key component of MEP determination is the minimization of the QM/MM total energy, in which the QM calculation of the energy and gradient is the bottleneck. To reduce the number of QM energy and gradient evaluations, we first developed an efficient iterative optimization for such a minimization in which the QM and MM subsystem optimizations are carried out sequentially, rather than concurrently (14). The key is to use a simplified, often QM ESP charge-based, energy function during the optimization of the MM subsystem. Friesner and coworkers (20) introduced a correction term to improve the ESP charge approximation, and Thiel and coworkers (78) proposed a similar method. To improve the ESP charge approximation, Morokuma and coworkers (79) have employed multiple QM calculations in the MM optimization process, as well as ESP multipoles.

Transition-Path Sampling

Instead of using a classical rate theory that depends on the determination of the TS, one can determine the reaction rate using the probability ratio of finding successful

dynamic reaction paths in multidimensional phase space, following the transition-path-sampling method (80). The simulation of a large-scale conformational transition in an enzyme has also been reported (81). The major obstacle for the application of this method to reaction processes is the high computational cost for ab initio QM/MM calculations because a large number of paths must be sampled to ensure a converged ratio of successful and unsuccessful paths.

RPP: reaction-path potential

Reaction-Path Potential Based on Ab Initio QM/MM Methods

The calculation of the QM internal energy and QM/MM electrostatic interactions in Equation 3 is the bottleneck for QM/MM calculations. In the charge-embedding scheme, rigorous treatment of this term requires a SCF calculation for each different QM or MM conformation, which is quite costly for ab initio QM methods. It would be desirable if one could develop an approximate, ab initio–based QM/MM energy function for phase-space sampling and long timescale simulations without the need for SCF calculations.

Lu & Yang (82) developed the ab initio reaction-path potential (RPP) method. The approach involves separating the QM energy into two components: a QM internal energy and an electrostatic interaction energy between the QM and MM subsystems. Each component is then expanded analytically in terms of both the fluctuations of the QM geometry and the MM ESP. The resulting RPP provides a simple, analytic expression for the QM/MM total energy that is valid in the vicinity of the reaction path.

RPP approximates the electrostatic term as the Coulombic interactions between the QM ESP charges (and multipoles) and the MM atomic charges; i.e.,

$$E^{ESP}_{QM/MM}(\mathbf{r}_{QM}, \mathbf{r}_{MM}) = \sum_{j\in MM}\sum_{i\in QM} \frac{q_j Q_i(\mathbf{r}_{QM}, \mathbf{r}_{MM})}{|\mathbf{r}_{QM,i} - \mathbf{r}_{MM,j}|}. \quad (4)$$

Here, $Q_i(\mathbf{r}_{QM}, \mathbf{r}_{MM})$ is the ESP fitted charge of QM atom i, and q_j is the point charge of MM atom j from the MM force field. Whereas the MM atomic charges are constant in common force fields, the QM electrostatic charges are clearly dependent on both $\mathbf{r}_{QM}$ and $\mathbf{r}_{MM}$. The QM internal energy, $E_1(\mathbf{r}_{QM}, \mathbf{r}_{MM})$, is then defined as

$$E_1(\mathbf{r}_{QM}, \mathbf{r}_{MM}) = \langle\Psi|H_{\text{eff}}|\Psi\rangle - E^{ESP}_{QM/MM}(\mathbf{r}_{QM}, \mathbf{r}_{MM}). \quad (5)$$

This QM internal energy is the energy of the QM system in the presence of the ESP of the MM atoms, minus the Coulombic interactions between the QM ESP charges and MM atomic charges. The QM energy also depends on both $\mathbf{r}_{QM}$ and $\mathbf{r}_{MM}$. Obviously, the term $E^{ESP}_{QM/MM}(\mathbf{r}_{QM}, \mathbf{r}_{MM})$ captures the essence of the electrostatic interactions between the QM and MM subsystems. The significance of this QM ESP charge expression is that it provides us great flexibility as the interactions are now expressed in a classical pairwise MM form. To improve the accuracy of ESP fitting, we can add electrostatic multipoles to each atom or bond.

The most important advantage of this separation scheme is that we can now introduce polarization effects into both terms as high-order perturbations. The RPP

model assumes that the QM ESP charges respond linearly to changes in both the external ESPs at the atomic sites and the geometries of the QM system, as

$$Q_i(\mathbf{r}_{QM}, v_{MM}) = Q^0_{QM,i} + \sum_{j\in QM} \chi_{ij}\left[v_{MM}\left(\mathbf{r}^0_{QM,j}\right) - v^0_{MM}\left(\mathbf{r}^0_{QM,j}\right)\right] + \sum_{j\in QM} \kappa_{ij}\left[\mathbf{r}_{QM,j} - \mathbf{r}^0_{QM,j}\right], \quad (6)$$

where $Q^0_{QM,i}$ is a reference ESP charge for QM atom *i*. The QM reference charges are determined at a given QM geometry $\{\mathbf{r}^0_{QM,j}\}$ and with a given external MM ESP $\{v_{MM}(\mathbf{r}^0_{QM,j})\}$ at the position of the QM atoms. After the perturbation of the QM geometry and the external MM ESP, the polarized charges are determined through two response kernels, namely (82, 83),

$$\chi_{ij} = \left(\frac{\partial Q_i}{\partial v_{MM}(\mathbf{r}_{QM,j})}\right)_N \quad (7)$$

and (82)

$$\kappa_{ij} = \left(\frac{\partial Q_i}{\partial \mathbf{r}_{QM,j}}\right)_N. \quad (8)$$

We can now compute the second kernel analytically with a recently introduced ESP fitting method (84). Just as in the polarization effects introduced in the term $E^{ESP}_{QM/MM}(r_{QM}, r_{MM})$, we also expand the term $E_1(r_{QM}, r_{MM})$ around a reference QM geometry and MM ESP to second order in the QM coordinates and first order in the MM ESP fluctuations (63, 82).

The resulting approximate RRP QM/MM total energy becomes

$$\widetilde{E}(\mathbf{r}_{QM}, \mathbf{r}_{MM}) = E_1(\mathbf{r}_{QM}, \mathbf{r}_{MM}) + \sum_{j\in MM}\sum_{i\in QM} \frac{q_j\, Q_i(\mathbf{r}_{QM}, \mathbf{r}_{MM})}{|\mathbf{r}_{QM,i} - \mathbf{r}_{MM,j}|} + E_{QM/MM,\,vdw}(\mathbf{r}_{QM}, \mathbf{r}_{MM}) + E_{QM/MM,\,cov}(\mathbf{r}_{QM}, \mathbf{r}_{MM}) + E_{MM}(\mathbf{r}_{MM}). \quad (9)$$

The linear-polarization QM ESP charge model yields quite accurate energetics for reaction systems, given a good initial reference state (62, 82). It is even possible to further simplify this QM ESP charge model by truncating the polarization effects of the QM ESP charges (62, 63). Applications with these simplified QM ESP charge models in the ab initio QM/MM simulation of reaction processes have been successful.

FREE ENERGIES

Classical Alchemical Free-Energy Simulation

Many methods have been developed for computing the free energies of important chemical processes on the basis of classical statistical simulations. The most popular methods include free-energy perturbation (FEP), thermodynamic integration (TI),

umbrella sampling, slow-growth, and a recently developed fast-growth method (85). If one is interested only in equilibrium states, a classical free-energy simulation method might be employed together with a so-called alchemical molecular transformation technique to provide the free-energy difference between stationary states of a reaction process (86).

TI: thermodynamic integration

FEP: free-energy perturbation

The direct application of TI and FEP with a QM/MM energy function requires special care for handling the end states (87). In classical MM force fields, the energy functions are continuous and (almost always) infinitely differentiable with respect to the variables. As a result, without any difficulty one can create nonphysical states of the molecule (e.g., partial existence of an atom or a group) in the simulation process. Nonetheless, QM methods in general do not easily allow a direct smooth process of creating or annihilating atoms. To overcome this difficulty, investigators have developed many methods, including the mixing of energy functions (9, 87), the utilization of a reference state (88–91), and dual transformation (92).

Compared with their critical roles in the MM simulations, classical alchemical free-energy simulations are less popular in the QM/MM simulations of the condensed-phase reactions. Two exceptions are the calculations of residue pK_a of protein molecules (93) and redox potentials (61, 87, 90).

Potential of Mean Force Calculation Along a Reaction Coordinate

When seeking information regarding the reaction rate or the TS structure, explicit modeling of the reaction process becomes necessary. Given an appropriate reaction coordinate, one can apply sampling-based free-energy calculation methods to the study of reaction processes if the QM calculations are fast enough, as in semiempirical QM methods. In such cases, the system is driven from one state to another along the reaction coordinate, and the PMF is computed during this driving process.

In the umbrella sampling method, one computes the PMF by improving the sampling of high-energy, low-probability regions of phase space. Usually the results of umbrella sampling are processed with the weighted histogram analysis method, which provides reduced statistical error. Recently, Kastner & Thiel (94) developed an umbrella integration method that combines the advantages of umbrella sampling and TI.

Instead of the often predefined, fixed-strength, external potentials applied in the umbrella sampling method, the meta-dynamics method developed by Laio & Parrinello (95) allows enhanced sampling of rare conformational states by gradually modulating the PES of the target system.

The slow-growth method is in fact a variant of TI. Instead of sampling a few fixed points on the reaction/transformation path and computing a converged free-energy gradient for each point, the slow-growth method slowly drives the system from one state to the other using a very small step size. The work exerted during this process is summed up to yield an upper-bound estimate of the free-energy difference between the two states. The reverse process yields a lower-bound estimate. Together, the results of multiple forward and backward processes yield a good estimate of the free-energy difference (96). When the slow-growth method is combined with the

coordinate-driving technique, one may obtain the free-energy change with respect to the reaction coordinate.

In analogy to the slow-growth method, a fast-growth method (85) has been developed and used to simulate chemical reactions (97, 98). Unlike the slow-growth method in which each simulated work is an estimate of the true free energy, in the fast-growth method the ensemble-averaged work is the upper-bound (or lower-bound for the reverse process) estimate of the true free energy. This estimate slowly converges to the true free energy upon the convergence of the ensemble. Because each individual simulation is often only in the picosecond timescale range, direct ab initio QM/MM simulations at this timescale might be possible. However, the main drawback of the fast-growth method is that it requires many simulations to yield a good estimate of the free energy, which always possesses a large amount of uncertainty (99).

It is important to realize that the phase-space-sampling methods based on PMF calculation along a reaction coordinate usually require converged sampling and, of course, prior knowledge about the reaction coordinate. Typically, this method is affordable for semiempirical QM/MM methods. As for ab initio QM/MM, several simulations have been reported (63, 100–102). With the steady improvement of computer speed, direct sampling methods may play more important roles in the reaction simulations.

Reducing the costs of ab initio QM calculations in QM/MM free energy calculations has been a major challenge. Jorgensen (35) developed a QM-free energy method, which uses the reaction path optimized for gas-phase reactions to carry out free-energy simulation in the condensed phase. Warshel and coworkers (88, 91) developed a QM(ai)/MM method, the key of which is that the sampling and free energies are first computed with a simplified empirical valence bond potential and then corrected to the ab initio QM level using FEP combined with the linear-response approximation.

QM/MM-FE

Realizing the importance of the reaction path, Yang and coworkers (14, 62, 63) devoted much effort to developing reaction-path optimization and free-energy calculation techniques based on ab initio QM/MM methods. The first method, termed QM/MM-FE (or QM/MM-FEP) (14), is carried out in two stages: optimization of the reaction path and calculation of the free energies for the path.

The reaction-path determination has been designed as a sequential optimization process. To exemplify the advantages of this sequential approach, we first examine the situation in which the QM and MM degrees of freedom are optimized concurrently. In this case, each change of the QM and/or MM geometries requires a new QM evaluation of the energy and gradient. Although the energy and gradient calculations are computationally inexpensive for semiempirical QM methods, they are expensive for ab initio QM methods. As it usually takes hundreds to thousands of minimization steps to obtain an adequately converged structure for the whole QM/MM system, the cost for ab initio QM calculations is prohibitive. Inspired by the fact that in certain types of optimization problems it may be desirable to break up a process

into an iterative sequence of two or more steps, we perform the optimization as follows: (*a*) We optimize a subset of the system, ***A***, with the rest of the system, ***B***, held constant; (*b*) we optimize ***B*** with ***A*** fixed according to the results obtained in step (*a*). Thus in the QM/MM-FE method, instead of a concurrent optimization of the QM and MM degrees of freedom, an iterative, sequential optimization protocol was developed that has proven to be effective in reducing the number of QM energy and gradient evaluations. The main idea is that, starting from a given structure of the QM/MM molecular system, the optimization is separated into two processes. We first optimize $\mathbf{r}_{QM}$ with fixed $\mathbf{r}_{MM}$, which is at an approximate minimum in the MM degrees of freedom. Afterward, the conformation of the MM subsystem, $\mathbf{r}_{MM}$, is optimized with fixed $\mathbf{r}_{QM}$. To reduce the number of QM evaluations, we use an approximate QM/MM total energy, $\tilde{E}(\mathbf{r}_{QM}, \mathbf{r}_{MM})$ of Equation 9, for the MM optimization. In this approximate QM/MM total energy, the electrostatic interactions between the QM and MM atoms are approximated by the Coulombic interactions between the point charges of the MM atoms and the ESP fitted charges of the QM atoms. This process is then iterated until convergence, which is normally achieved within a few iterations (often less than 10). Expressed as an algorithm, the ab initio QM/MM-FE optimization procedure is as follows:

1. Initiate a structure of the QM subsystem $\mathbf{r}_{QM}^{(0)}$ and set the cycle number $n = 0$.
2. Increase the cycle number $n = n + 1$.
   a. Carry out an MM minimization with QM atoms fixed at $\mathbf{r}_{QM}^{(n-1)}$:
   $$\mathbf{r}_{MM}^{(n)} = \arg\min_{\mathbf{r}_{MM}} \tilde{E}\left(\mathbf{r}_{QM}^{(n-1)}, \mathbf{r}_{MM}\right). \tag{10}$$
   b. Carry out a QM optimization with MM atoms fixed at $\mathbf{r}_{MM}^{(n)}$:
   $$\mathbf{r}_{QM}^{(n)} = \arg\min_{\mathbf{r}_{QM}} E\left(\mathbf{r}_{QM}, \mathbf{r}_{MM}^{(n)}\right). \tag{11}$$
3. Return to step 2 until converged.

This process can be carried out individually for a single point on the reaction path (e.g., the reactant and product states) or simultaneously for a chain of conformations along the reaction coordinate with a chain-of-states optimization algorithm such as the NEB method, the Ayala-Schlegel method, or the superlinearly convergent quadratic string method.

Once the reaction path is determined, one can carry out a FEP simulation with the approximate QM/MM energy function $\tilde{E}(\mathbf{r}_{QM}, \mathbf{r}_{MM})$ for the optimized QM conformations on the reaction path (14), similar to the QM-FE method (35). The validity and efficiency of QM/MM-FE have also been confirmed by other laboratories (103–105).

QM/MM-MFEP: Path Optimization on a Quantum Mechanics Potential of Mean Force Surface

The development of the QM/MM-FE method has provided a viable way for computing accurate free energies of reactions in enzymes. However, one limitation has hampered the application of this method to the simulation of solution reactions. In

the QM/MM-FE method, the reaction path is optimized on the QM/MM PES, starting from a given initial structure. As a result, the optimized path is influenced by the choice of the initial conformation. In many enzyme-substrate complexes, the dependence of the initial conformation may not cause problems because the active site of the enzyme is usually protected from the bulk solvent. However, when the reaction occurs in solution, or the enzyme active site is exposed to solvent, this dependence becomes significant. The rapid exchange of solvent molecules can also cause difficulty for the convergence of the path optimization process. Similar observations have been made for the enzymatic reactions in which the enzyme undergoes significant conformational changes during the reaction process.

To overcome this problem, we developed the QM/MM-MFEP method in which the reaction path is optimized on the PMF surface of the QM degrees of freedom, instead of the total energy surface (62). Within the QM/MM context, the thermodynamics of the entire system is simplified by defining the PMF of a QM/MM system in terms of the QM conformation as

$$A(\mathbf{r}_{QM}) = -\frac{1}{\beta} \ln \left[\int d\mathbf{r}_{MM} \exp(-\beta E(\mathbf{r}_{QM}, \mathbf{r}_{MM}) \right], \quad (12)$$

where $E(\mathbf{r}_{QM}, \mathbf{r}_{MM})$ is the total energy of the entire system expressed as a function of the coordinates of the QM and MM subsystems, $\mathbf{r}_{QM}$ and $\mathbf{r}_{MM}$, respectively. The gradient of the PMF, also known as the free-energy gradient, is then

$$\frac{\partial A(\mathbf{r}_{QM})}{\partial \mathbf{r}_{QM}} = \left\langle \frac{\partial E(\mathbf{r}_{QM}, \mathbf{r}_{MM})}{\partial \mathbf{r}_{QM}} \right\rangle_{E(\mathbf{r}_{QM}, \mathbf{r}_{MM})}, \quad (13)$$

which appears conveniently as the ensemble average of the gradient of the QM atoms, obtained from MD simulations of the MM atoms. In practice, we use the free-energy perturbation method and its associated gradient instead of Equations 12 and 13 (62, 63).

Our construction of the PMF and PMF gradient is different from other work in terms of the variables of the PMF. For reasons discussed above, we allow all the QM degrees of freedom to contribute to the reaction coordinate, whereas others often use one or a few predefined geometric terms. In the latter case, a Jacobian term may be required to correctly include the effects of geometrical constraints on the reaction coordinates.

Because the QM degrees of freedom are coupled with the MM degrees of freedom, a straightforward minimization algorithm requires each step in the optimization of the QM conformations to be associated with converged sampling of the MM ensemble. In this optimization scheme, every QM optimization step to a new conformation on the PMF surface is followed by a course of MD sampling of the MM conformations, usually with a simulation time of $100 \sim 1000$ ps. Such extensive MM sampling is required so that the QM PMF and gradient obtained are sufficiently accurate for successful structure and reaction-path optimization of the QM subsystem. Thus, this method is less efficient in practical simulations. First, $100 \sim 1000$ ps of MD simulations on a system of $\sim$10,000 atoms are expensive. Second, such MD simulations must be repeated for each step in the QM optimization process. The intrinsic

fluctuations and limited simulation times for the MD sampling may also contribute to slow convergence in the path optimization if a new MD simulation is always begun immediately after every QM geometry-optimization step.

Sequential Sampling and Optimization for Quantum Mechanics/Molecular Mechanics–Minimum Free-Energy Path Method

As with the QM/MM-FE method, we can improve the efficiency of the QM/MM-MFEP method by reformulating the concurrent optimization of the QM subsystem and the statistical sampling of the MM subsystem into iterative steps of sequential MD sampling of the MM system at a fixed QM structure and the subsequent optimization of the QM subsystem within the fixed MM conformational ensemble (63). The approximate energy function $\tilde{E}(\mathbf{r}_{QM}, \mathbf{r}_{MM})$, introduced in the QM/MM-FE method and described in the RPP model, plays a crucial role in reducing the number of QM energy and gradient evaluations required. In the MD sampling of the MM conformations, $\tilde{E}(\mathbf{r}_{QM}, \mathbf{r}_{MM})$ acts as a reference sampling energy function, $E_{ref}(\mathbf{r}_{MM})$, to drive the motion of the MM atoms without performing a QM calculation at every MD step. In the optimization of the QM subsystem within the fixed-size MM conformational ensemble, it can be used to avoid QM calculations associated with each new conformation in the MM ensemble.

The algorithm of the QM/MM-MFEP method can be described as follows:

1. Initiate a structure of the QM subsystem, $\mathbf{r}_{QM}^{(0)}$, and set cycle number $n = 0$.
2. Increase cycle number $n = n + 1$.
   a. Carry out MD sampling of the MM ensemble with QM atoms fixed at $\mathbf{r}_{QM}^{(n-1)}$:

$$\left\{\mathbf{r}_{MM}^{(n)}(\tau),\ \tau = 1, \ldots, N\right\} \Leftarrow \text{MD sampling based on } E_{ref}(\mathbf{r}_{MM}), \tag{14}$$

   where τ is the step of the MD simulation; N is the number of MD steps; and the reference QM structure is derived from $\mathbf{r}_{QM}^{(n-1)}$, the QM geometry from the previous iteration.

   b. Carry out a QM optimization with the MM ensemble fixed at $\{\mathbf{r}_{MM}^{(n)}(\tau)\}$, where the object of minimization is the QM PMF (or QM free energy) in the n-th iteration given by a finite-sum representation of free-energy perturbation as

$$A^{(n)}(\mathbf{r}_{QM}) = A_{ref} - \frac{1}{\beta} \ln \left\{ \frac{1}{N} \sum_{\tau=1}^{N} \exp\{-\beta \Delta E\} \right\}, \tag{15}$$

   and the corresponding gradient with respect to the i-th QM coordinate is also given by the finite sum

$$\frac{\partial A^{(n)}(\mathbf{r}_{QM})}{\partial \mathbf{r}_{QM,i}} = \frac{\sum_{\tau=1}^{N} \partial \tilde{E}\left(\mathbf{r}_{QM}, \mathbf{r}_{MM}^{(n)}(\tau)\right) \Big/ \partial \mathbf{r}_{QM,i} \exp\{-\beta \Delta E\}}{\sum_{\tau=1}^{N} \exp\{-\beta \Delta E\}}, \tag{16}$$

where

$$\Delta E = \tilde{E}\left(\mathbf{r}_{QM}, \mathbf{r}_{MM}^{(n)}(\tau)\right) - E_{ref}\left(\mathbf{r}_{MM}^{(n)}(\tau)\right), \quad (17)$$

which accounts for the fact that the samples were obtained from a fixed MD simulation of a reference state. E_{ref} and A_{ref} are the reference energy function and reference free energy in the free-energy perturbation expression, respectively.

c. Update the reference structure based on the minimized QM structure $\mathbf{r}_{QM}^{(n)}$.

3. Return to step 2 until converged.

The key feature of our new QM/MM-MFEP algorithm is the iterative QM optimization in a fixed MM ensemble (Equation 14). Because the MM ensemble, $\{\mathbf{r}_{MM}^{(n)}(\tau), \tau = 1, \ldots, N\}$, is finite and remains fixed throughout the course of the QM optimization for $\mathbf{r}_{QM}^{(n)}$, one can obtain the precise PMF (Equation 15) and its gradient (Equation 16) defined within this ensemble. This circumvents the difficult and costly convergence problems associated with MM sampling. The optimization of the PMF can be carried out efficiently using classical numerical optimization tools. Each optimized QM structure $\mathbf{r}_{QM}^{(n)}$ in turn provides the next reference QM structure and its energy function, $\mathbf{r}_{QM}^{ref(n)}$, for the next round of MD sampling of the MM conformations. Each optimized QM structure should improve on the previous one by providing a better QM geometry and corresponding ESP charges for the MM simulation in the next cycle.

The use of a finite, fixed-size ensemble of MM conformations improves the utilization of the MM conformations and avoids repetitive MD sampling at each step of the QM structure optimization. Thus, instead of performing excessive MD samplings that likely have significant overlap with each other, a few cycles of MD simulation are sufficient to yield converged results in the current method. Applications have shown that our QM/MM-MFEP method converges as efficiently as the QM/MM-FE method (63) (**Figure 3**).

With the QM/MM-MFEP method, we describe the complex problem of reactions in solution and in enzymes with reduced degrees of freedom on the QM PMF. Our sequential sampling and optimization implementations make the ab initio QM/MM-MFEP efficient and thus feasible.

BEYOND THE CLASSICAL TRANSITION-STATE THEORY

The classical dynamic effects beyond the classical TS theory are described in the transmission coefficient $\gamma(T)$, the determination of which requires an MD simulation starting from the TS (25). Main quantum-mechanical effects can be included in the quantum PMF calculations. There are several ab initio QM/MM studies reported so far. Cui & Karplus (106) calculated $\gamma(T)$ for the proton-transfer reaction catalyzed in triosephosphate isomerase using variational TS theory. We used the RPP, which enables rapid evaluation of the potential energy around the reaction path, to calculate the classical $\gamma(T)$ (107) and also the quantum PMF with a centroid path integral approach (108). When the reaction path and its TS capture the correct physics, TS

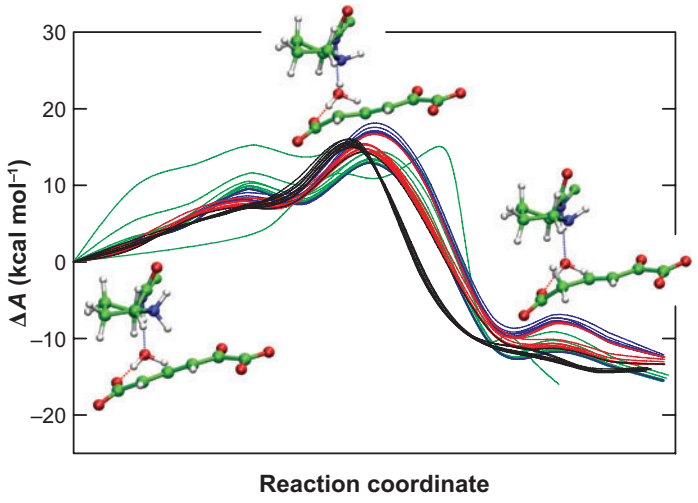

Figure 3

Potential of mean force of the proton transfer reaction catalyzed by 4-oxalocrotonate tautomerase. Colored curves show quantum mechanics/molecular mechanics (QM/MM) minimum free-energy path optimization cycles in the green-blue-red-black sequence. Each color represents a new MM ensemble, and multiple lines of the same color indicate the process of QM optimization within the MM ensemble.

theory is a good approximation, and $\gamma(T)$ is nearly 1 (3, 106–108). Our calculations on triosephosphate isomerase resulted in kinetic isotope effects in excellent agreement with experiments and revealed that the main QM effect is in the zero-point energy (108).

APPLICATIONS OF AB INITIO QUANTUM MECHANICS/ MOLECULAR MECHANICS METHODS

Many enzymatic reaction processes have been modeled by QM/MM methods, most investigated with semiempirical QM/MM methods. As only a small number of cases have been studied using ab initio QM/MM calculations, the number of applications of ab initio QM/MM free-energy simulations is even fewer because of the difficulty in combining ab initio QM with statistical sampling. A comprehensive list can be found in Reference 8. In this section, we briefly review several enzyme systems whose mechanisms have been scrutinized by ab initio QM/MM, some in combination with free-energy simulation.

The enzyme orotidine 5′-monophosphate decarboxylase is one of the most proficient enzymes known and catalyzes the decarboxylation of orotidine 5′-monophosphate without any cofactor or metal ions. The solution reaction has been modeled with a continuum solvent model (109), which supported a carbene intermediate state. Semiempirical simulations have supported the direct decarboxylation mechanism (110, 111). However, the reaction barriers computed in later DFT-based simulations were still higher than the experimental measurement (98, 112, 113).

The enzyme 4-oxalocrotonate tautomerase catalyzes the conversion between 2-oxo-4-hexenedioate and 2-oxo-3-hexenedioate. Cisneros et al. (16, 114–116) have carried out extensive QM/MM-FE simulations on the reaction mechanism and identified the absence of a catalytic acid and the role of an ordered water and the protein backbone in the catalysis. Thiel and coworkers (117) have also studied the mechanism of the enzyme and its mutant.

One enzyme that has undergone extensive characterization is triosephosphate isomerase, which catalyzes the reversible isomerization of dihydroxyacetone phosphate to D-glyceraldehyde-3-phosphate. The mechanism has been examined by semiempirical QM/MM methods (118, 119). Later, investigators carried out several DFT-based QM/MM studies (14, 17, 120, 121). With a higher-resolution structure of the enzyme, Friesner's group (122) modeled the reaction process and obtained results in good agreement with experimental data. The correlation between the reaction and the loop motion has been explored in an interesting way by employing the QM/MM simulation in combination with a loop-structure prediction algorithm.

Kollman and coworkers (123–125) have simulated the reaction of catechol O-methyltransferase with ab initio QM/MM and QM-FE methods. Rod & Ryde (104) also simulated the reaction mechanism with a recently developed quantum-mechanical thermodynamic cycle perturbation method.

Enolase has been simulated both by semiempirical and ab initio QM/MM methods (15, 126). Chorismate mutase catalyzes the Claisen rearrangement from chorismate to prephenate. In addition to being modeled by many semiempirical QM/MM simulations, recently it was modeled by an ab initio QM/MM method with optimization of the reaction path (127–129). For its medicinal significance, β-lactamase has been explored by many groups. Different reaction steps have been examined in detail (130–132). Acetylcholinesterase has been simulated by QM/MM-FE with an emphasis on the effects of conformational dynamics on the reaction process (133, 134). Using the QM/MM-FE method, Zhang and coworkers have studied cAMP-dependent protein kinase (135, 136), histone lysine methyltransferase SET7/9 (137–139), and bacterial peptide deformylase (140, 141). Friesner and coworkers have explored in detail the reaction mechanism of methane monooxygenase (142, 143), as well as the P450cam pathway (144).

A large class of protein and enzymes has been investigated with the Car-Parrinello molecular dynamics/MM method, including ion channels (145), aspartic protease (101), HIV-1 protease (102), and caspases (146).

FUTURE PERSPECTIVES

An important issue in modeling enzyme catalysis is the role of conformational dynamics. Special interest has been raised recently for the contribution of conformational dynamics to enzyme catalysis (27). One needs to consider two issues: the timescale of the conformational dynamics and the coupling scheme between the enzyme dynamics and the reaction process. As the transmission coefficient in the TS rate equation is affected by the stochastic dynamics of the enzyme but its variation seems to have small effect on the reaction rate in general (3, 106, 107), the challenge is to understand

if/how the collective conformational dynamics of the enzyme has an impact on the reaction rate (27).

In the current popular form of the QM/MM approach, MM force fields are not polarizable. As efforts are underway to develop polarizable MM force fields, schemes have been proposed to combine QM with polarizable MM force fields (147–151), but more tests might be needed before a specific scheme is employed. In two scenarios, this issue becomes more complicated. One is the mutual polarization at the QM/MM boundary; the other is the charge-transfer effect between the QM and MM atoms. Generally, the implementation and testing of polarizable MM force fields are important topics in the QM/MM methodology development.

In summary, with the recent development of ab initio QM/MM free-energy simulation methods, realistic and accurate simulations of reactions in solution and in enzymes become feasible. The application of such methods will provide a comprehensive understanding of reactions in solution and in enzymes.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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