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Born-Oppenheimer ab initio QM/MM Molecular Dynamics Simulations of Enzyme Reactions

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Abstract

There are two key requirements for reliably simulating enzyme reactions: one is a reasonably accurate potential energy surface to describe the bond forming/breaking process as well as to adequately model the heterogeneous enzyme environment; the other is to perform extensive sampling since an enzyme system consists of at least thousands of atoms and its energy landscape is very complex. One attractive approach to meet both daunting tasks is Born-Oppenheimer ab initio QM/MM molecular dynamics simulation (aiQM/MM-MD) with umbrella sampling. In this chapter, we describe our recently developed pseudobond Q-Chem–Amber interface, which employs a combined electrostatic-mechanical embedding scheme with periodic boundary condition and the particle mesh Ewald method for long-range electrostatics interactions. In our implementation, Q-Chem and the sander module of Amber are combined at the source code level without using system calls, and all necessary data communications between QM and MM calculations are achieved via computer memory. We demonstrate the applicability of this pseudobond Q-Chem–Amber interface by presenting two examples, one reaction in aqueous solution and one enzyme reaction. Finally, we describe our established aiQM/MM-MD enzyme simulation protocol, which has been successfully applied to study more than a dozen enzymes.

Keywords

QM/MM; Molecular dynamics; Free energy; Simulation; Enzyme reaction

1. INTRODUCTION

The idea of combining quantum mechanical (QM) and molecular mechanical (MM) methods (Warshel & Levitt, 1976) aims to take advantage of the applicability of QM

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methods for modeling chemical reactions and the computational efficiency of MM calculation. Such a combined QM/MM approach allows to study systems significant larger than a pure QM method can do, and has been widely used in modeling chemical reactions in complex system, from the solid and surface catalysis to solution and enzyme reactions (Gao & Truhlar, 2002; Hu & Yang, 2008; Senn & Thiel, 2009; Zhang, 2006). Meanwhile, due to the complexity of the energy landscape of an enzyme system in aqueous solution, which consists of at least thousands of degrees of freedom and is highly flexible, extensive sampling with a reasonably accurate potential energy surface is needed. The free energy changes associated with an enzyme reaction process are not only better defined, but also can be directly compared with corresponding experimental results.

One most attractive approach to meet both above daunting tasks -- a reasonably accurate potential energy surface for describing enzyme reactions and extensive sampling to calculate free energy change -- is Born-Oppenheimer ab initio QM/MM molecular dynamics simulation (aiQM/MM-MD): at each time step, the atomic forces as well as the total energy of the enzyme system are calculated with the aiQM/MM method on the-fly, and Newton equations of motion are integrated. In spite of the conventional wisdom that such simulations would be computationally too expensive to be feasible for simulating enzyme reactions, in last several years we have advanced the ab initio QM/MM-MD approach by interfacing Q-Chem (Shao, Molnar, Jung, Kusmann, Ochsenfeld, Brown et al., 2006) with Tinker (Ponder, 2004) and Amber (Case, Darden, Cheatham III, Simmerling, Wang, Duke et al., 2012) programs at the source code level and substantially enhancing its efficiency. We have demonstrated this state-of-the-art approach to be feasible and successful in elucidating the catalytic power of enzymes (Wang, Hu, & Zhang, 2007) and characterizing complicated multistep reaction mechanisms (Ke, Smith, Zhang, & Guo, 2011; Lior-Hoffmann, Wang, Wang, Geacintov, Broyde, & Zhang, 2012; Rooklin, Lu, & Zhang, 2012; Shi, Zhou, Wang, & Zhang, 2013; Wu, Wang, Zhou, Cao, & Zhang, 2010; Y. Zhou, Wang, & Zhang, 2010). Very recently, we have successfully applied this state-of-the-art approach to simulate covalent inhibition (Lei, Zhou, Xie, & Zhang, 2015) as well as design time-dependent HDAC2 selective inhibitor (J. Zhou, Li, Chen, Wang, Luo, Zhang et al., 2015).

In the below, first we describe our recently developed pseudobond Q-Chem–Amber interface with a combined electrostatic-mechanical embedding scheme, periodic boundary condition and the particle mesh Ewald method for long-range electrostatics interactions; then we present two examples to demonstrate its applicability to simulate chemical reactions in aqueous solution and enzymes; and finally we describe our established aiQM/MM-MD enzyme simulation protocol and discuss several important computational details regarding ab initio QM/MM MD simulations of enzyme reactions.

2. METHODS

2.1 The pseudobond approach to describe the QM/MM boundary across covalent bonds

The treatment of the QM/MM boundary across covalent bonds is a critical problem concerning the accuracy and applicability of the combined QM/MM methods to study enzyme reactions (Field, 2002; Field, Bash, & Karplus, 1990). In the pseudobond approach (Zhang, 2005, 2006; Zhang, Lee, & Yang, 1999), as illustrated in Figure 1, the boundary

atom of the QM sub-system Y is replaced by a one-free-valence boundary Cps atom to form a pseudobond Cps – X. The Cps atom has seven valence electrons, nuclear charge as +7, an angular-momentum-independent effective core potential, and a parameterized STO-2G basis set. The Cps atom is parameterized to make the Cps – X pseudobond mimic the original Y – X bond with similar bond length and strength, and also similar effects on the rest of the active part. The Cps atoms and all atoms in the active part form a well-defined (often closed-shell) QM sub-system, which can be treated by quantum mechanical methods. The rest atoms form the MM sub-system, which will be represented by a molecular mechanical force field. The developed pseudobonds are independent of the molecular mechanical force field and offer a smooth connection at the QM/MM interface with-out introducing additional degrees of freedom into the system. Up to now, the Cps(*sp*3)-C(*sp*3), Cps(*sp*3)-C(*sp*2, carbonyl) and Cps(*sp*3)-N(*sp*3) pseudobonds have been developed for the cutting of protein backbones and nucleic acid bases with the 6-31G* basis set. The parameterization is performed with density functional calculations using hybrid B3LYP exchange-correlation functional, but the same set of parameters is applicable to Hartree–Fock and MP2 methods, as well as DFT calculations with other exchange-correlation functionals (Zhang, 2006).

2.2 A dual-focal ab initio QM/MM-PME potential with the periodic boundary condition

In our implementation of ab initio QM/MM-MD with Q-Chem (Shao et al., 2006) and Tinker (Ponder, 2004), the spherical boundary condition is employed. Although this description is sufficient for many applications, it would not be appropriate to study chemical processes that are strongly influenced by long-range interactions and dynamics. A more reliable and robust description is the periodic boundary condition with the Ewald method for long-range electrostatics interactions (Nam, Gao, & York, 2005; Walker, Crowley, & Case, 2008). As shown in Figure 2, the total potential energy of the QM/MM (PBC) system can be written as:

$$E^{PBC}[\rho+q, \rho+q] = E^{RS}[\rho, \rho] + E^{RS}[\rho, q] + \Delta E^{PBC} + E^{PBC}[q, q],$$

$$\Delta E^{PBC} = (E^{PBC}[\rho, \rho] - E^{RS}[\rho, \rho]) + (E^{PBC}[\rho, q] - E^{RS}[\rho, q]).$$

$E^{RS}[\rho, \rho]$ and $E^{RS}[\rho, q]$ only include interactions within the same cell, and are treated by an effective Hamiltonian H_{eff} as done in non-periodic boundary systems. The $E^{PBC}[q, q]$ is an MM term, and can be treated by the particle mesh Ewald method. One key idea of the QM/MM-Ewald method (Nam et al., 2005; Walker et al., 2008) is to approximate the charge density ρ with atomic charges, Q , in calculating the PBC correction term:

$$\Delta E^{PBC} \approx (E^{PBC}[Q, Q] - E^{RS}[Q, Q]) + (E^{PBC}[Q, q] - E^{RS}[Q, q]) = \Delta E^{PBC}[Q, Q] + \Delta E^{PBC}[Q, q]$$

Although such an implementation of ab initio QM/MM-Ewald method (Okamoto, Yamada, Koyano, Asada, Koga, & Nagaoka, 2011; Torras, Seabra, Deumens, Trickey, & Roitberg, 2008) is feasible, its computational cost is higher than that with the spherical boundary condition since a significant more number of point charges are included in the effective Hamiltonian. In addition, the imaging of the QM sub-system is conceptually not appealing, nor is it necessary since the interactions are approximated as charge-charge interactions in

calculations anyway. Here we have developed a dual-focal ai-QM/MM-PME approach: there is only one QM sub-system, whose electron density is polarized by nearby MM atoms while interactions with all remote atoms are represented by classical coulomb interactions, as illustrated in Figure 3.

q^{eff} represents the point charges in the effective Hamiltonian which are chosen by residue, and q represents the charges of surroundings. For a periodic boundary system, only the MM atoms in the central box are considered in the effective Hamiltonian. At distances on the order of unit cell size away, the differences are very small between the potential calculated from fitted point charge Q and charge density ρ (Nam et al., 2005), therefore an approximate quantum mechanics charge distribution Q is used to represent ρ in image boxes. The electrostatic potential fitting (ESP) charges (Cox & Williams, 1981; Momany, 1978) are employed in our interface. This can also be considered as a combined electrostatic-mechanical embedding scheme. The implementation is quite straightforward: the first term would be calculated with the ai-QM/MM method as implemented in Q-Chem using the spherical boundary condition; with the determined charges, the second term would be calculated with the Particle Mesh Ewald (PME) (Darden, York, & Pedersen, 1993) method implemented in Amber; and the third term can also be calculated easily with the MM method.

2.3 Micro-iterative Optimization and Reaction Coordinate Driving

To optimize an enzyme system with an *ab initio* QM/MM method, we employ the micro-iterative minimization approach, in which QM and MM subsystems are minimized separately and iteratively (Zhang, Liu, & Yang, 2000). In each circle, the QM subsystem is optimized one step using *ab initio* QM/MM calculations with frozen MM environment, and then the MM system is fully optimized using MM calculations with a fixed QM sub-system in which the QM charge density represented by ESP charges calculated from the QM/MM method. The iterations will not stop until convergence criterion is reached for both minimizations. To determine the reaction path, we use the reaction coordinate driving (RCD) method: by choosing a proposed reaction coordinate (RC), we carry out a series of restrained minimizations for different points of RC from the initial state to final state to determine a minimal energy path (Zhang et al., 2000). The choice of a proper reaction coordinate is crucial in this approach, which is generally a linear combination of internal coordinates. Sometimes, two-dimensional minimal energy surfaces would be needed for studying complicated reactions.

2.4. Born-Oppenheimer *ab initio* QM/MM Molecular Dynamics Simulations with Umbrella Sampling

In Born Oppenheimer *ab initio* QM/MM MD simulations, in which the atomic forces as well as the total energy of the whole system are calculated at each time step on-the-fly with the hybrid QM/MM potential, and Newton equations of motion are integrated. Thus, it takes accounts of dynamics of reaction center and its environment on an equal footing. In order to make *ab initio* QM/MM molecular dynamics to be time reversible, besides using the time reversible integrator Verlet for atom positions, the lossless time-reversible density matrix propagation (Niklasson, Tymczak, & Challacombe, 2006) has been implemented for the

electronic degrees of freedom. For a new time step, the initial guessed density matrix for SCF is constructed from the converged SCF density matrix of current time step P_n and the initial guessed density matrix for previous time step $\tilde{P}_{n-1}$ in a time-reversible way, $\tilde{P}_{n+1} = 2P_n - \tilde{P}_{n-1}$. In this way, ab initio QM/MM MD would be time-reversible, and would achieve much better energy conservation than employing $\tilde{P}_{n+1} = P_n$. To determine the free energy profile for a given chemical reaction, umbrella sampling (Patey & Valleau, 1975) would be carried out by employing a series of harmonic potentials centered along the chosen reaction coordinate.

2.5 Implementation

We have implemented a pseudobond Q-Chem–Amber interface with a combined electrostatic-mechanical embedding scheme, periodic boundary condition and the particle mesh Ewald method for long-range electrostatics interactions. In our implementation, the quantum chemistry package Q-Chem4.0.1 (Krylov & Gill, 2013) and the sander module of molecular mechanics package Amber12 (D.A. Case, T.A. Darden, T.E. Cheatham, C.L. Simmerling, J. Wang, R.E. Duke et al., 2012) are combined at the source code level without using system calls. There is only one executable file for Q-Chem–Amber, and all the necessary data communication between Q-Chem and Amber are via Q-Chem internal files and in memory, that makes the user interface clear and friendly. In this way, QM/MM iterative optimization and QM/MM MD can be run continuously without restarting Q-Chem or Amber executable programs. Apart from the Q-Chem and Amber standard input files, only a few parameters need to be added to specify QM/MM interface. Besides the output of Q-Chem restart files, the interface generates Amber restart and trajectory files by default which can be visualized and analyzed by MD analysis tools. The micro-iterative approach has been implemented for QM/MM geometry minimizations and reaction path explorations. In Born-Oppenheimer molecular dynamics simulations, a lossless time-reversible density matrix propagation scheme has been used for the electronic degrees of freedom. Accuracy, efficiency and applicability of this pseudobond Q-Chem–Amber interface have been extensively examined by simulating chemical reactions in aqueous solution and enzymes.

3. EXAMPLES

3.1 Dissociation of *tert*-butyl chloride in water

To examine the applicability of the Q-Chem–Amber interface, we simulated the *SM* fragmentation of *tert*-butyl chloride in water (Hartsough & Merz, 1995; Peters, 2007), $\text{t-Bu-Cl} \rightarrow \text{t-Bu}^+ + \text{Cl}^-$, in which water plays an indispensable role. For such a charge separation reaction, the proper treatment of long range electrostatic interactions and boundary condition is expected to be very important and spherical boundary condition would not sufficient in this case. Here the reactant was solvated in a box with a 35 Å buffer of water molecules, and equilibrated with 1ns NPT classical MD simulations. By choosing the C-Cl distance as the reaction coordinate, ab initio QM/MM-MD simulations with umbrella sampling have been used to determine free energy profiles with different *qmcut* values. *qmcut* is the distance cutoff for MM charges to be treated as q_{eff} . For each window, 110 ps B3LYP/6-31+G(d) QM/MM-MD simulation was performed, and the last 60 ps data were used for analysis. As shown in Figure 4, all free energy profiles have converged reasonably well in spite of

different *qmcut* values, and the calculated free energy barriers for dissociation are between 18.7 ± 0.2 kcal/mol and 19.0 ± 0.2 kcal/mol, in good agreement with the experimental derived value of 19.5 kcal/mol (Grunwald & Effio, 1974; Winstein & Fainberg, 1957).

3.2 First step of acylation reaction for a serine protease

We have employed the Q-Chem–Amber interface to simulate the first step of acylation reaction catalyzed by porcine-trypsin using periodic boundary condition, which has been previously simulated with the Q-Chem–Tinker interface under spherical boundary condition (Y. Zhou & Zhang, 2011). The simulations were carried out at the B3LYP/6-31+G* QM/MM level with the pseudobond approach, which is the same as in the previous work (Y. Zhou et al., 2011). We tested 20 Å *qmcut* and full box for q_{eff} . The computational time for full box q_{eff} is about 1.5 times of that for the 20 Å *qmcut*, which is mainly caused by the increasing number of one-electron terms in the effective Hamiltonian. The free energy profiles, as shown in Figure 5, are very similar between two *qmcut* values. By considering both efficiency and accuracy, a reasonable default *qmcut* value would be 20 Å. Thus we recommend using 20 Å *qmcut* as the default value to minimize the computational cost. The free energy barrier for the first step is about 15 kcal/mol, in good agreement with the experimental value for activation energy of 15~20 kcal/mol (Fersht, 1999). The free energy barrier is 16.4kcal/mol from our previous study with spherical boundary condition, and the shape of two free energy curves is almost the same for the two boundary conditions. This indicates that spherical boundary condition is reasonably accurate for enzyme reactions that are not strongly influenced by long range electrostatics interactions and dynamics.

4. ENZYME SIMULATION PROTOCOL

In this section, we present our established aiQM/MM-MD enzyme simulation protocol, as shown in Figure 6, which has been successfully applied to study more than a dozen enzymes. For initial preparation and classical MD simulations, the protocol is the same as typical classical simulations of biomolecular systems. Thus here we would focus on the QM/MM modeling part, and mainly address a few essential details that are important for ab initio QM/MM MD simulations.

- **QM/MM system setup.** Given a structural snapshot from MM modeling, the QM/MM partition needs to be carried out, and the covalent boundary would be treated with the improved pseudobond approach. Since pseudobonds are basis set dependent, it is important that the QM atoms directly bonded to pseudo atoms are treated with 6-31G* basis set. Meanwhile, for hydrogen atoms of water molecules and hydroxyl hydrogen that have been partitioned into the QM sub-system, their van der Waals (vdW) parameters need to be changed from zero to a tiny value, such as 0.001. Otherwise, in later ab initio QM/MM MD simulations, sometimes a hydrogen atom with zero vdW parameters can jump to collide with an MM atom, which is not physical and would lead to the simulation failure.

- Reactant minimization. This step is carried out by the iterative micro-iterative optimization without employing any restraints. Typically, this step would be straightforward if all previous steps have been done appropriately.
- Reaction path scan. In this step, it is important to check whether there are significant discontinuities in some geometry elements along the reaction path. This information would be useful to help you select more appropriate reaction coordinates. In some cases, two-dimensional minimum energy surfaces would need to be mapped out.
- MM equilibration. This step is to employ nano-second classical molecular dynamics simulations to equilibrate the MM sub-system with QM sub-system fixed at previously QM/MM minimized structures. Since the length of ab initio QM/MM MD simulations is quite short, this step is important that the MM sub-system can be relaxed given the change of QM sub-system.
- Umbrella sampling. In this step, ab initio QM/MM molecular dynamics simulations with restraint will be carried out. For each window along the reaction coordinate, an initial force constant is chosen based on the estimated slope of the determined minimum energy curve, i.e., larger restraint is needed for regions of steeper slope or for allowing direct sampling of the transition state and a smaller restraint is added at flatter regions. Histogram between neighboring windows will be checked to make sure that there are sufficient overlaps. In some challenging cases, two-dimensional umbrella sampling needs to be carried out.
- PMF construction. After adequate sampling by umbrella sampling, the free energy profile or free energy surface can be obtained with the weighted histogram analysis method (WHAM) method (Ferrenberg & Swendsen, 1988; Kumar, Bouzida, Swendsen, Kollman, & Rosenberg, 1992; Souaille & Roux, 2001). One prerequisite is that the individual biased simulations in each window must be sampled along the proper reaction coordinate and overlapped well with each other. The convergence of umbrella sampling can be tested by the PMF calculated from different time spans.

5. CONCLUSION

In this chapter, we have described a Born-Oppenheimer ab initio QM/MM molecular dynamics (aiQM/MM-MD) approach to simulate enzyme reactions. In comparison with many other computational methods that can be used to simulate enzyme reactions, one unique strength of the aiQM/MM-MD approach is to study biological reactions whose mechanism has not been fully elucidated, such as for novel enzymes and new covalent modifiers. With the continuing advances in computer technology and algorithm development, the aiQM/MM-MD approach is expected to become the method of choice to simulate novel biochemical reactions in the very near future.

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Figure 1.
Illustration of the pseudobond approach in the treatment of the QM/MM boundary problem.

Figure 2.
Illustration of a conventional QM/MM periodical boundary system: yellow circles indicate the QM sub-systems while the shaded area q is the MM sub-system.

Figure 3.

Illustration of the dual-focal ai-QM/MM-PME approach, q_{eff} refers to the nearby MM atom charges, while Q are point charges that mimic the QM density which would be a good approximation at long distance.

Figure 4.

Free energy profile for dissociation of tert-butyl chloride in water determined by B3LYP/6-31+G(d) QM/MM molecular dynamics simulations and umbrella sampling with different qmcut values, which indicates the distance for MM charges to be included in electrostatic embedding. Full box indicates all MM charges in the primary box are treated as q_{eff} in Figure 3.

Figure 5. Free energy profile for the first step of acylation reaction for porcine-trypsin determined by B3LYP/6-31+G(d) QM/MM molecular dynamics simulations and umbrella sampling.

QM/MM Enzyme Simulation Protocol

Figure 6.
Illustration of our enzyme simulation protocol.