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Combined Quantum Mechanical/ Molecular Mechanical Methodologies Applied to Biomolecular Systems

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1. Introduction

Most biological transformations are facilited by a special class of proteins, called enzymes, that are able to catalyze biochemical reactions that could not readily occur without them.[1] Their most remarkable properties are their ability to catalyze reactions under mild conditions (room temperature, atmospheric pressure, etc.), their high kinetic rates, and their high substrate specificities. Understanding how enzymes work is one of the central goals of contemporary biochemical research. Ultimately, this will yield a wide range of applications, ranging from the development of new drugs to the design of new protein-based catalysts.

Computational chemistry can play a major role in the elucidation of enzymatic reactivity because it gives molecular-level insights into enzyme catalysis which are difficult to obtain by other means. Verification of a proposed catalytic mechanism requires the determination of the free energy cost of different possible reaction paths through the characterization of the different transition and intermediate states for all possible reaction paths. These types of studies result in the making and breaking of bonds, which requires the use of quantum mechanical methods, either implicitly or explicitly (e.g., via incorporation of a gas-phase potential into a force field method[2−5]).

The most accurate description of an enzymatic system, composed of the enzyme, its subtrate(s), its required cofactor(s), and the solvent surrounding it, in theory, could be achieved by using long-time quantum molecular dynamics simulations, i.e., through the solution of the time-dependent Schrödinger equation on the complete system.[6] However, this goal is far from being realized, due to conceptual and computational bottlenecks. Thus, one is faced with making approximations in order to obtain the most accurate description of the biochemical system of interest. Until recently, theoretical studies of enzymatic reactivity have been carried out by considering the most essential atoms of the active site and substrate and then examining the reactivity of this model system in the gas phase or in a continuum solvent environment.[7,8] These studies can give qualitative insight into the intrinsic reactivity of an enzyme, but they cannot explain, by themselves, enzyme specifity or the role of the microsolvation environment of the enzyme. In light of this, researchers have developed new methodologies that, in principle, can give an accurate description of the influence of an enzyme on a biochemical reaction. Among these, we include the empirical valence bond (EVB) method from Warshel and co-workers[9,10] and the work derived from it by Hwang and co-workers;[11,12] the multiconfigurational molecular dynamics with quantum transitions (MCMDQT) method developed by Hammes-Schiffer and coworkers;[13,14] the quantum-classical molecular dynamics method of McCammon and co-workers;[15,16] the density matrix evolution (DME) method of Berendsen and Mavri;[17,18] the ONIOM method of Morokuma and coworkers;[19,20] and the effective fragment potential (EFP) method of Stevens and co-workers.[21,22]

However, the most widely used method of the past decade has been the combined quantum mechanical/ molecular mechanical (QM/MM) method.[23−27] Its basic strategy is quite simple in concept: it is based on the fact that, in most enzymes, the reactive part is limited to a small number of atoms (an active-site subsystem). This "subsystem", which undergoes most of the electronic changes associated with chemical activity, is described by quantum mechanics (QM), while the rest of the system, which does not require the making or the breaking of bonds, can be represented using a molecular mechanics (MM) force field. The resulting coupling of QM and MM descriptions provides a suitable potential that can model the reactivity of a wide range of complex systems.

Because QM/MM methods are quite easy to implement, they have been widely used to study the reactivity of large systems. One of the first and main application areas has been the study of solvation and reactivity of small molecules in condensed phases (see ref 26 for a review), but other recent application areas include studies of surface reactivity,[28] zeolites,[29] and crystal formation.[30,31]

In this Account, we will exclusively focus on the use of combined QM/MM methods to study the reactivity of

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enzymes. Due to the singular nature of these systems, the enzyme itself must be partitioned into two subsystems, one described by an appropriate QM method, the other by a MM force field. The frontier between these two regions, as a result, requires special attention because covalent bonds form the demarcation between them. In the following sections, we will briefly survey the different QM/MM methodologies that have been developed to properly treat the interactions between QM and MM regions in large biomolecular systems. Then we will present examples of applications of the combined QM/MM approach to the study of enzymatic reactivity.

2. The Quantum Mechanical/Molecular Mechanical Method

2.1. Genesis of the QM/MM Method. In 1976, Warshel and Levitt published the first article that described the basic QM/MM approach.[23] To study the catalytic mechanism of lysozyme, they presented a way in which to represent the complete enzyme−substrate complex together with the surrounding solvent within a combined QM/MM formalism. All of the basic concepts of the QM/MM method were introduced in this publication: the partitioning of the system, the partitioning of the potential energy function, and the evaluation of the interactions between the QM and MM parts. In retrospect, this paper was clearly ahead of its time.

Ten years after this initial article, Singh and Kollman published a paper[24] that described the use of a combined ab initio QM/MM method applied to the $CH_3Cl + Cl^-$ exchange reaction in solution (S_N2) and the gas-phase protonation of polyethers. They introduced the notion of "junction dummy atoms" to saturate the free valencies of the QM atoms linked to MM atoms.

However, current interest in the QM/MM method was largely stimulated by a recent article by Field, Bash, and Karplus.[25] In this study, they examined in detail the strengths and weaknesses of the combined QM/MM approach relative to full quantum mechanical or full molecular mechanical calculations. In this paper, they presented the concept of "link atoms", which are analogous to the junction dummy atoms of Singh and Kollman.

2.2. Common Methodology. Most of the QM/MM papers published since the work of Field et al. share a common methodological approach. The system of interest is partitioned into two subsystems (see Figure 1): one (QM) contains a small number of atoms and is described by quantum mechanics and the other (MM), which represents the rest of the system, is described by a suitable force field.

The Hamiltonian of the whole system can be written as follows:

$$H = H_{QM} + H_{MM} + H_{QM/MM} \quad (1)$$

where H_{QM} is a QM Hamiltonian, H_{MM} is an empirical force field, and $H_{QM/MM}$ is the Hamiltonian that describes the interactions between the QM and MM regions.

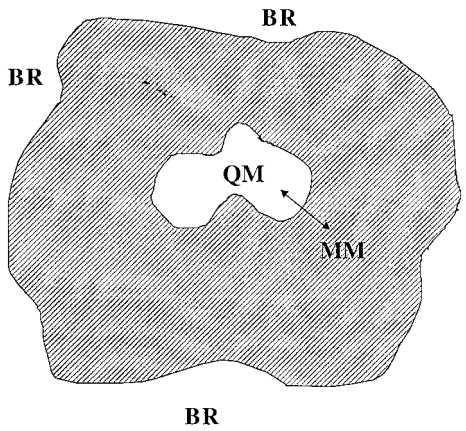

FIGURE 1. Division of the molecular system within a combined quantum mechanical/molecular mechanical calculation. The quantum mechanical (QM) region is surrounded by a molecular mechanical (MM) region and then by a boundary region (BR).

The total energy of the system can likewise be divided into three component parts:

$$E = E_{QM} + E_{MM} + E_{QM/MM} \quad (2)$$

In principle, many levels of accuracy can be used for the QM region; however, only a small number of QM/MM studies of enzymatic systems have made use of either density functional theory (DFT) or ab initio Hartree−Fock (HF) approaches.[32,33] Most have used semiempirical approaches due to the considerable sizes of the QM region. On the other hand, similar restrictions do not apply to the empirical Hamiltonian, so that "typical" force fields (AMBER,[34] CHARMm,[35,36] or GROMOS[37,38]) have been utilized.

One of the key aspects of the QM/MM method is, of course, the interactions between the QM and MM regions. There are two types of interactions: First, when one deals with large biological molecules, the interface between the QM and MM region involves covalent bonds and as a result requires special treatment. Because this aspect of the problem differentiates existing QM/MM approaches, it will be discussed in detail in the following section. Second, to take into account the influence of the enzyme and the surrounding solvent on the QM region, electrostatic and van der Waals interactions between the QM and MM regions must be included. Usually, this is done by adding to the QM Hamiltonian the interactions between the electrons and nuclei of the QM part with the point charges of the MM part.[25] This describes the polarization of the QM wave function by its MM environment but does not incorporate charge transfer between QM and MM regions.[39] In some cases, the MM charges are not directly included into the QM Hamiltonian, but they are taken into account via purely classical electrostatic interactions.[19,40] However, it is our opinion that this approach does not properly account for the polarization of the MM region on the QM region. Consequently, the effect of the enzymatic environment is not fully accounted for when using a purely classical QM/MM interaction model.

We also note that, when using semiempirical methods, electrostatic integrals describing the interactions between the MM charges with the QM electrons must be corrected.[41]

2.3. Different Approaches. Link Atom. When dealing with large systems such as enzymes, it is important to properly treat the covalent bonds that exist at the border between the QM and MM regions. Different solutions to this problem have been described. The most common method is the so-called "link atom" method. Introduced by Singh and Kollman and Field et al. in their initial papers, it consists of adding QM hydrogen atoms in order to fill the free valencies of the QM atoms that are connected to the atoms described by MM. These dummy atoms are treated explicitly during the QM calculations but do not interact with the MM atoms. However, whether these link atoms should or should not interact by means of Coulombic interactions is still open to debate.[41−43] Furthermore, the energy and the gradient are not well-defined because they include dummy atoms whose contributions are not constant. This approach is not ideal, but it does allow for a clear delineation between the QM and MM regions of a biological molecule. Moreover, this is a fairly reliable approach, provided that the frontier bonds are placed sufficiently far away from the reactive atoms. As a result, many application studies have been conducted using the "link atom" QM/MM approach.[44−52]

Local Self-Consistent Field. To avoid the use of "link atoms", Rivail and co-workers have developed the local self-consistent field method (LSCF),[53−55] which uses a combination of hybrid and atomic orbitals to represent the quantum subsystem. It expands the initial idea of Warshel and Levitt to use hybrid orbitals to describe the covalent bonds at the border between the QM and MM regions, but unlike their approach, the LSCF method uses a basis set of atomic orbitals instead of orthogonal valence hybrid orbitals to describe the rest of the QM region.

In the LSCF formalism, the two electrons of the frontier bond are described by a strictly localized bond orbital (SLBO). Provided that this covalent bond is far enough from the chemical reaction center, its electronic properties can be considered as constant along the reaction path. Using model systems, it is possible to define the nature of this SLBO in the QM atomic orbital basis set. By freezing this representation, the rest of the molecular orbitals, which are orthogonal to the SLBO, can then be generated by a *local* self-consistent calculation.[53] This approach has been developed at the semiempirical and ab initio HF levels and has been successfully applied to organic and biochemical systems.[56−60]

Recently, a related approach has been described by Gao et al. in their generalized hybrid orbital (GHO) method.[61] It also uses the concept of hybrid orbitals localized on the frontier atoms, but in this case these orbitals are divided into two sets of auxiliary and active orbitals. The latter set is included in the SCF calculation, while the former generates an effective core potential for the boundary atom. As opposed to the LSCF approach, the GHO method requires a reparametrization of the semiempirical Hamiltonian for the boundary atoms.

Connection Atom. Another way of avoiding the use of link atoms is the new "connection atom" method developed by Thiel and co-workers.[62] In this approach, the MM atoms at the border between the QM and MM regions are described as a QM methyl group with a free sp^3 valence. In other words, a MM atom, which is defined as a "connection atom", enters into the SCF computation as an atom having one electron and one orbital, which mimics the behavior of the free sp^3 orbital of the methyl radical. This elegant approach obviates the need for dummy atoms and, hence, provides a consistent definition of the energy of the system. Connection atoms have been parametrized to be compatible with the popular semiempirical methods such as AM1 and PM3. However, generalization of this method to encompass ab initio HF calculation has not been reported.

In summary, several QM/MM methods have been recently developed in order to model the structure and reactivity of large biological systems. These different methods share common features as well as different ways of solving the border problem between the QM and MM regions. However, at the time we are writing this Account, no study has been yet published to compare these different QM/MM methods, and, although we are aware of some ongoing research,[63,64] it is still unclear whether any of the QM/MM methods mentioned above are more reliable than any of the others.

3. Examples of QM/MM Studies on Enzymes

In this section, we present some recent examples of QM/MM studies on enzymes which have used different QM/MM boundary region representations. These examples are meant to show the reader how powerful the QM/MM approach is when applied to the study of enzymatic reactivity.

All of the enzymes described in the following examples have a zinc atom (Zn^{2+}) at their catalytic sites. Modeling the behavior of a zinc atom and its ligands is a challenging task when using a pure MM force field.[65−67] Indeed, its large positive charge can result in substantial polarization effects, local geometric changes, and coordination number changes.[65] QM/MM methods solve these problems by treating the metal ion and its ligands quantum mechanically while treating the surrounding environment with a force field. In this approach, polarization of the metal ion and its ligands can be effectively dealt with since it is explicitly included in the QM/MM model. Furthermore, the dynamics of the coordination sphere is included as well as the ability to undergo coordination changes.

In the following, we first present a QM/MM molecular dynamics simulation of human carbonic anhydrase II (HCAII), which demonstrates the need for a quantum representation of the active site of this enzyme. In the second example, we present a study of peptide hydrolysis by thermolysin, which demonstrates the applicability of the QM/MM approach to reactivity.

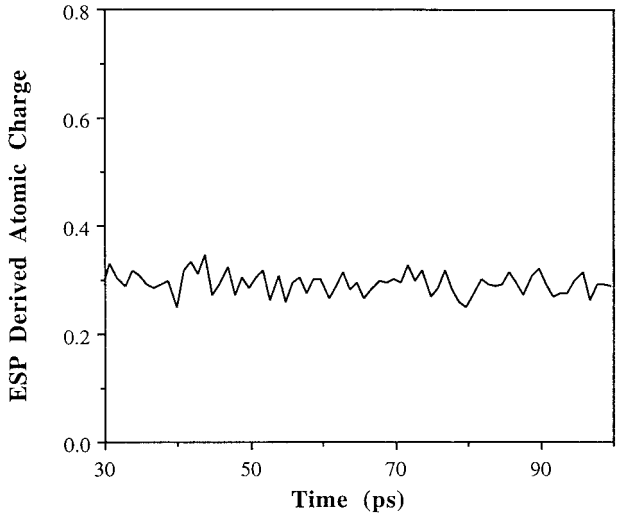

FIGURE 2. Fluctuations of the charge on the hydrogen atom bound to the C-δ_2 atom in His-119.

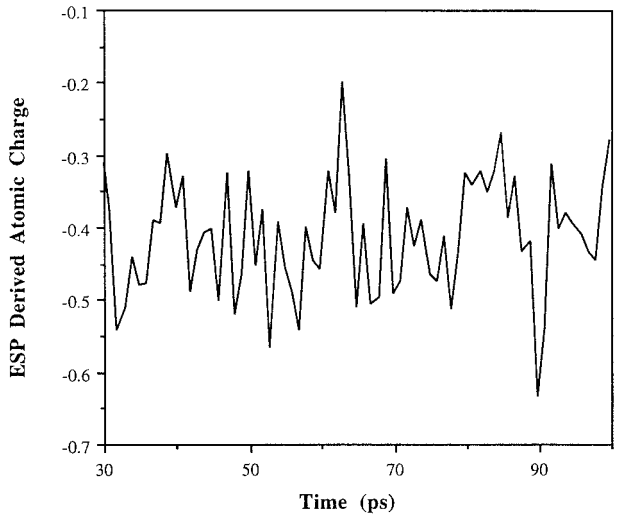

FIGURE 3. Fluctuations of the charge on the C-ϵ_1 atom in His-94.

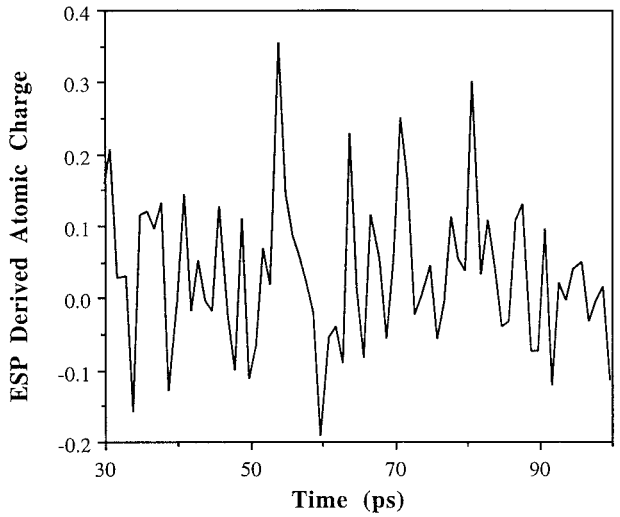

FIGURE 4. Fluctuations of the charge on the N-δ_1 atom in His-119.

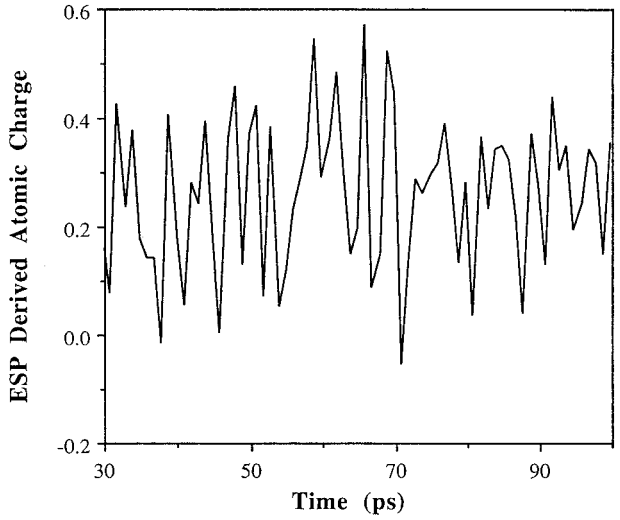

FIGURE 5. Fluctuations of the charge on the Zn atom in the zinc−water forms of HCAII.

3.1. QM/MM Molecular Dynamics of HCAII. HCAII, one of seven isozymes of the zinc metalloprotein human carbonic anhydrase (HCA), is a 260-residue protein with a mass of ∼29 kDa. A single zinc atom is located in the enzyme active site and is necessary for catalytic activity. The active site itself lies at the bottom of a deep cavity (15 Å deep) in the protein, which is readily accessible to solvent. The active site cavity is divided into hydrophobic and hydrophilic regions, with a network of hydrogen-bonded water molecules connecting the active-site region and the surrounding solvent environment. The catalytically necessary zinc atom lies at the bottom of the active-site cleft and is tetrahedrally coordinated by three histidine residues (His-94, -96, and -119) and a fourth ligand, whose identity is pH dependent. At high pH (>8), the fourth ligand is an hydroxide ion, while at acidic pH the fourth coordination site is occupied by a water molecule.

To evaluate the capabilities of a coupled QM/MM method and to examine the structure and dynamic properties of the HCAII active site, Hartsough and Merz have simulated the dynamics of this system using a combined QM/MM model.[68] They used the PM3 semiempirical Hamiltonian to describe the side chains of His-94, His-96, His-119, the catalytic zinc atom, and the fourth ligand (either water or hydroxide). The AMBER united atom model represented the remainder of the enzyme, and TIP3P water molecules were used to represent the solvent within 15 Å of the zinc atom. Junctions between the QM and MM regions were made between C-β and C-γ of the His residues using the "link atom" approach of Field, Bash, and Karplus. MD simulations were carried out on both forms (water and hydroxide) of HCAII. A 15-Å sphere was defined around the active-site zinc atom, and only residues within this sphere as well as the cap water molecules were allowed to move during the MD simulations. Each MD simulation consisted of 100 ps.

In what we believe to be the first reported QM/MM molecular dynamics simulations on a protein, Hartsough and Merz demonstrated that QM and MM methodologies are able to provide a reasonable depiction of active-site geometry and dynamics. Moreover, they showed that using a full MM description of a zinc protein with fixed point charges can lead to an inaccurate model. In particular, they reported the variation of the atomic charges

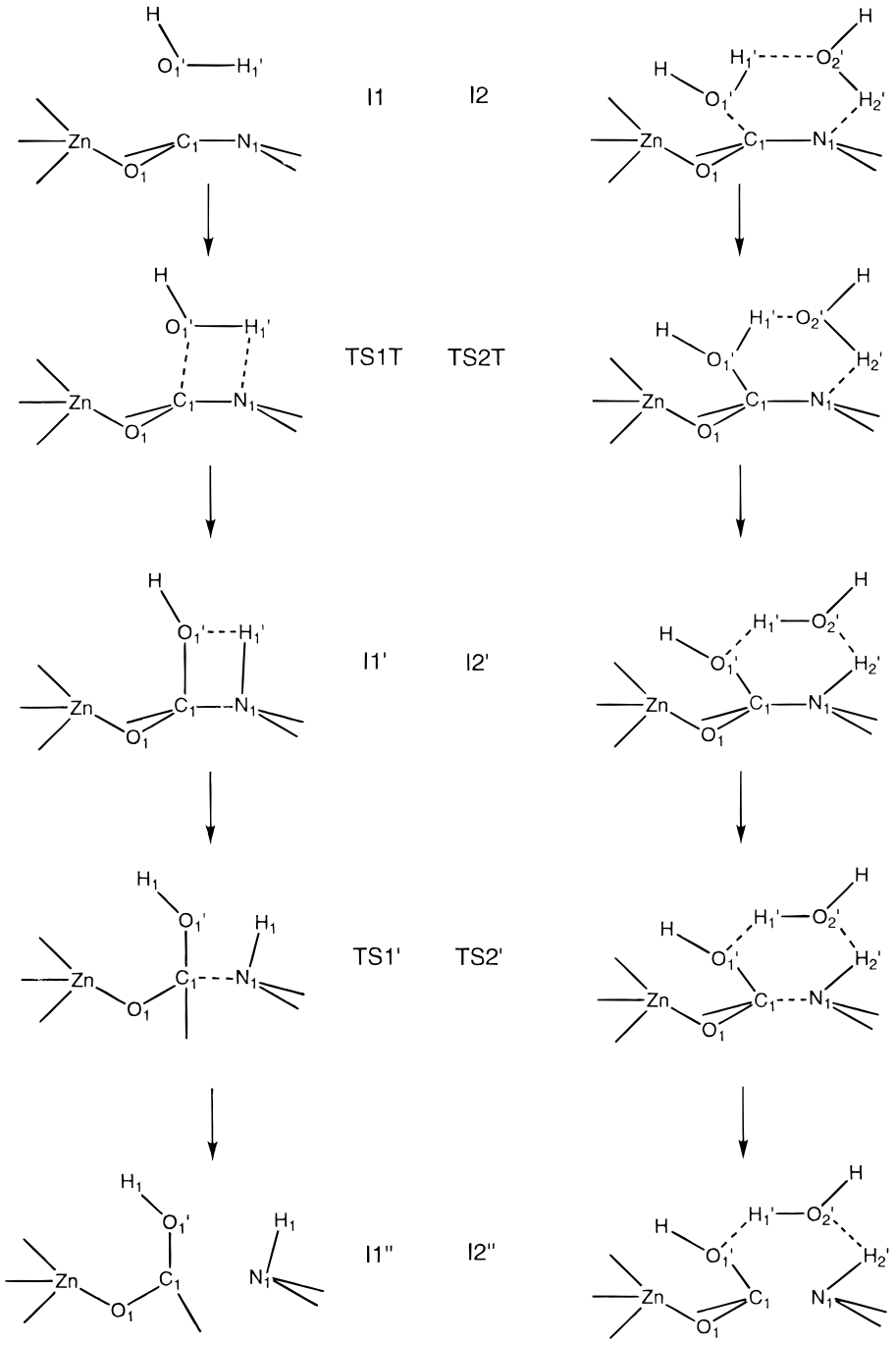

FIGURE 6. Nonassisted and water-assisted hydrolysis mechanisms of formamide by thermolysin.

using electrostatic potential (ESP)-derived charges evaluated as a function of the MD trajectory. Figures 2−5 represent the fluctuations of the charges on atoms H-δ_2 in His-119 (the hydrogen bound to C-δ_2 in His-119), C-ϵ_1 in His-94, N-δ_1 in His-119, and Zn during the last 70 ps of the simulation on the zinc−water form of HCAII. Figure 5 clearly shows that the ESP-derived atomic charges of the zinc ion vary substantially and that a static charge model is inadequate for the modeling of systems with large charge flux (e.g., metalloenzymes). The charges on select atoms of the ligands also vary (see Figures 3 and 4), but when they are not in direct (or "resonant") contact with the metal center (e.g., the hydrogen bound to the C-δ_2 in His-119—see Figure 2), their ESP-derived charges do not vary significantly. The use of a MM description is therefore justified when atoms are far enough from the location where the most important electronic changes occur. The results obtained from this study clearly demonstrated the strengths of using a QM/MM approach to describe the energy, the structure, and the dynamics of a zinc protein.

3.2. Reactivity of Thermolysin. Recently, Antonczak and co-workers focused on the reactive process of another zinc protease: thermoysin.[60] Like HCAII, thermolysin contains only one zinc atom, which is essential for its catalytic activity. This metal ion is bound to the enzyme by three amino acid ligands: His-142, His-146, and Glu-166, the fourth ligand being the enzyme substrate. In this paper, the hydrolysis of a model peptide substrate (formamide)

Table 1. Relative Energetics (ΔE) and Contributions (ΔEC) for the Stationary Points of the Hydrolysis Reaction Mechanisms (in kcal/mol)

Nonassisted Mechanism					
	I1	TS1T	I1′	TS1′	I1″
ΔE	0.0	60.1	16.0	17.4	2.7
ΔEC	0.0	5.0	−3.9	−1.4	−4.3
Water-Assisted Mechanism					
	I2	TS2T	I2′	TS2′	I2″
ΔE	0.0	48.9	0.7	2.9	−0.7
ΔEC	0.0	6.4	−1.9	−1.1	0.6

by thermolysin was studied. Two mechanisms were considered, one involving only one water molecule (i.e., the "nonassisted" mechanism) and the other involving two water molecules (i.e., the "water-assisted" mechanism). The semiempirical LSCF formalism was used by these authors. The QM region was described by the AM1 Hamiltonian and included the zinc atom, the three amino acid ligands, the formamide, and one or two water molecules. The rest of the system, including the remainder of the enzyme and the crystallographic water molecules, was described using the AMBER force field. In contrast to the previous example, the authors did not perform MD simulations but "froze" the MM region while allowing the QM part to move (e.g., $E_{MM} = 0$ in eq 2). This permitted the computation of the second derivatives of the QM/MM energy and allowed the location of transition states using standard algorithms.

The reaction paths for both water-assisted and nonassisted mechanisms are presented in Figure 6, and the corresponding energy fluctuations are given in Table 1. The first transition states (TS1T and TS2T), which correspond to the insertion of water, were the rate-limiting step for these reactions. An intermediate was observed for each mechanism (I1′ and I2′), and these corresponded to a state in which the peptide bond was partially broken. Not surprisingly, Antonczak et al. found that the water-assisted mechanism was preferred over the nonassisted one. However, one of the most interesting parts of this study was the use of the QM/MM approach to evaluate the influence of the enzyme environment on the reactive processes. Using eq 2, the term $E_{QM/MM}$ can be evaluated, and, once evaluated, it can be used to determine whether the enzyme facilitates the reaction.[25] In this case, the results indicated that thermolysin slightly destabilizes the transition states for both reaction pathways. At first, this is unexpected because one is trained to think that enzymes decrease energy barriers in order to catalyze reactions. But in this case, one has to remember that formamide is only a model peptide and not the natural substrate (i.e., thermolysin is not a "formamidase"). In more recent work, Antonczak and co-workers showed that thermolysin does, indeed, stabilize the transition states during the hydrolysis of a natural substrate (e.g., the Gly-Phe-Leu tripeptide).[69]

4. Perspectives

In this Account, we presented the basics of the QM/MM methodology, and we highlighted the different approaches available to solve the boundary problem associated with studying enzymes with QM/MM Hamiltonians. Several examples were given that demonstrate how powerful the QM/MM approach is in describing the structure and energetics of enzymatic systems. Nonetheless, the QM/MM approach still needs to be improved in order to become a highly reliable method that can be applied to a wide range of problems. First, as pointed out earlier, a comparison between all the existing QM/MM approaches is still lacking, and it is not yet clear whether one approach gives more reliable results than another (i.e., "link atom", LSCF, "connection atom", etc.). Second, continued improvements in the QM level used and in the MM representation will be required. For example, most of the applications published so far have used semiempirical methods for the QM region and fixed point-charge models (i.e., nonpolarized) for the MM region. Significant improvements will be realized soon as the use of more reliable ab initio and DFT theories becomes more common. Future use of force fields with polarizability and/or charge-transfer properties will enhance our understanding of the response of the MM region to the QM wave function. Finally, use of new "linear scaling" methods[70−73] should allow us to increase the QM region size without negatively impacting the speed of the simulations.

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References

(1) Voet, D.; Voet, J. G. *Biochemistry*; John Wiley & Sons: New York, 1995.
(2) Jorgensen, W. L.; Buckner, J. K. Effect of Hydration on the Structure of an S_N2 Transition State. *J. Phys. Chem.* **1986**, *90*, 4651.
(3) Madura, J. D.; Jorgensen, W. L. Ab Initio and Monte Carlo Calculations for a Nucleophilic Addition Reaction in the Gas Phase and in Aqueous Solution. *J. Am. Chem. Soc.* **1986**, *108*, 2517.
(4) Kollman, P. A.; Merz, K. M., Jr. Computer Modeling of the Interactions of Complex Molecules. *Acc. Chem. Res.* **1990**, *23*, 246−252.
(5) Merz, K. M., Jr. Gas-Phase and Solution Phase Potential Energy Surfaces for $CO_2 + nH_2O$ (n=1,2). *J. Am. Chem. Soc.* **1990**, *112*, 7973−7980.
(6) Cohen-Tannoudji, C.; Diu, B.; Laloë, F. *Quantum Mechanics*; John Wiley & Sons: New York, 1977.
(7) Dive, G.; Dehareng, D.; Peeters, D. Proposition for the Acylation Mechanism of Serine Proteases: a One Step Process? *Int. J. Quantum Chem.* **1996**, *58*, 85.
(8) Mulholland, A. J.; Richards, W. G. Modeling Enzyme Reaction Intermediates and Transition States: Citrate Synthase. *J. Phys. Chem. B* **1998**, *102*, 6635−6646.
(9) Warshel, A. *Computer Modeling of Chemical Reactions in Enzymes and Solutions*; John Wiley & Sons: New York, 1991.

(10) Åqvist, J.; Warshel, A. Simulation of Enzyme Reactions Using Valence Bond Force Fields and Other Hybrid Quantum-Classical Approaches. *Chem. Rev.* **1993**, *93*, 2523−2544.
(11) Hwang, J. K.; Pan, J. J. Simulation of Chemical Reactions in Solution by a Combination of Classical and Quantum Mechanical Approach. *Chem. Phys. Lett.* **1995**, *243*, 171−175.
(12) Hwang, J. K.; Pan, J. J. Classical Trajectory Mapping Approach for Simulations of Chemical Reactions in Solution and in Enzymes. *J. Phys. Chem.* **1996**, *100*, 909−912.
(13) Hammes-Schiffer, S. Multiconfigurational Molecular-Dynamics with Quantum Transitions—Multiple Proton-Transfer Reactions. *J. Chem. Phys.* **1996**, *105*, 2236−2246.
(14) Decornez, H.; Drukker, K.; Hurley, M. M.; Hammes-Schiffer, S. Proton Transport Along Water Chains and NADH Hydride Transfer in Solution. *Ber. Bunsen-Ges. Phys. Chem.* **1998**, *102*, 533−543.
(15) Bala, P.; Grochowski, P.; Lesyng, B.; McCammon, J. A. Quantum-Classical Molecular Dynamics Simulations of Proton-Transfer Processes in Molecular-Complexes and in Enzymes. *J. Phys. Chem.* **1996**, *100*, 2535−2545.
(16) Grochowski, P.; Lesyng, B.; Bala, P.; McCammon, J. A. Density-Functional Based Parametrization of a Valence Bond Method and Its Applications in Quantum-Classical Molecular Dynamics Simulations of Enzymatic Reactions. *Int. J. Quantum Chem.* **1996**, *60*, 1143−1164.
(17) Berendsen, H. C.; Mavri, J. Quantum Simulation of Reaction Dynamics by Density Matrix Evolution. *J. Phys. Chem.* **1993**, *97*, 13464.
(18) Mavri, J.; Berendsen, H. C. Calculation of the Proton-Transfer Rate Using Density Matrix Evolution and Molecular Dynamics Simulations: Inclusion of the Proton Excited States. *J. Phys. Chem.* **1995**, *99*, 12711−12717.
(19) Maseras, F.; Morokuma, K. IMOMM−A New Integrated *ab initio* + Molecular Mechanics Geometry Optimization Scheme of Equilibrium Structures and Transition States. *J. Comput. Chem.***1995**, *16*, 1170−1179.
(20) Svensson, M.; Humbel, S.; Froese, R. J.; Matsubara, T.; Sieber, S.; Morokuma, K. ONIOM − A Multilayered Integrated MO+MM Method for Geometry Optimizations and Single Point Energy Predictions: A Test for Diels−Alder Reactions and $Pt(P(t\text{-}Bu)_3)_2 + H_2$ Oxidative Addition. *J. Phys. Chem.* **1996**, *100*, 19357−19363.
(21) Day, P. N.; Jensen, J. H.; Gordon, M. S.; Webb, S. P.; Stevens, W. J.; Krauss, M.; Garmer, D.; Basch, H.; Cohen, D. An Effective Fragment Method for Modeling Solvent Effects in Quantum Mechanical Calculations. *J. Chem. Phys.* **1996**, *105*, 1968−1986.
(22) Merill, G. N.; Gordon, M. S. Study of small water clusters using the effective fragment potential model. *J. Phys. Chem. A* **1998**, *102*, 2650−2657.
(23) Warshel, A.; Levitt, M. Theoretical studies of enzymatic reactions: Dielectric, electrostatic and steric stabilization of carbonium ion in the reaction of Lysozyme. *J. Mol. Biol.* **1976**, *103*, 227−249.
(24) Singh, U. C.; Kollman, P. A. A Combined *ab initio* Quantum Mechanical and Molecular Mechanical Method for Carrying out Simulations on Complex Molecular Systems: Applications to the $CH_3Cl + Cl^-$ Exchange Reaction and Gas-Phase Protonation of Polyethers. *J. Comput. Chem.* **1986**, *7*, 718−730.
(25) Field, M. J.; Bash, P. A.; Karplus, M. A Combined Quantum Mechanical and Molecular Mechanical Potential for Molecular Dynamics Simulations. *J. Comput. Chem.* **1990**, *11*, 700−733.
(26) Gao, J. Methods and applications of combined quantum mechanical and molecular mechanical potentials. In *Reviews in Computational Chemistry*; Lipkowitz, K. B., Boyd, D. B., Eds.; VCH Publishers: New York, 1996; Vol. 7, pp 119−185.
(27) Mordasini, T. Z.; Thiel, W. Combined Quantum Mechanical and Molecular Mechanical Approaches. *Chimia* **1998**, *58*, 288−291.
(28) Carmer, C. S.; Weiner, B.; Frenklach, M. Molecular Dynamics with Combined Quantum and Empirical Potentials: C_2H_2 Adsorption on Si(100). *J. Chem. Phys.* **1993**, *99*, 1356−1372.
(29) Sherwood, P.; de Vries, A. H.; Collins, S. J.; Greatbanks, S. P.; Burton, N. A.; Vincent, M. A.; Hillier, I. H. Computer Simulation of Zeolite Structure and Reactivity Using Embedded Cluster Methods. *Faraday Discuss.* **1997**, *106*, 79−92.
(30) Ferenczy, G. G.; Csonka, G. I.; Náray-Szabó, G.; Ángyán, J. G. Quantum Mechanical Molecular Mechanical Self-Consistent Madelung Potential Method for Treatment of Polar Molecular Crystals. *J. Comput. Chem.* **1998**, *19*, 38−50.
(31) Eichler, U.; Kölmel, C. M.; Sauer, J. Combining *ab initio* Techniques with Analytical Potential Functions for Structure Predictions of Large Systems: Method and Application to Crystalline Silica Polymorphs. *J. Comput. Chem.* **1997**, *18*, 463−477.
(32) Waszkowycz, B.; Hillier, I. H.; Gensmantel, N.; Payling, D. W. A Theoretical Study of Hydrolysis by Phospholipase A2: The Catalytic Role of the Active Site and Substrate Specificity. *J. Chem. Soc., Perkin Trans. 2* **1990**, *7*, 1259−1268.
(33) Bentzien, J.; Muller, R. P.; Florian, J.; Warshel, A. Hybrid *ab initio* Quantum Mechanics Molecular Mechanics Calculations of Free Energy Surfaces for Enzymatic Reactions: The Nucleophilic Attack in Subtilisin. *J. Phys. Chem. B* **1998**, *102*, 2293−2301.
(34) Cummins, P. L.; Gready, J. E. Molecular Dynamics and Free Energy Perturbation Study of Hydride Ion Transfer Step in Dihydrofolate Reductase Using Combined Quantum and Molecular Mechanical Model. *J. Comput. Chem.* **1998**, *19*, 977−988.
(35) Mulholland, A. J.; Richards, W. G. Acetyl-CoA Enolization in Citrate Synthase: A Quantum Mechanical Molecular Mechanical (QMMM) Study. *Proteins* **1997**, *27*, 9−25.
(36) Bash, P. A.; Field, M. J.; Davenport, R. C.; Petsko, G. A.; Ringe, D.; Karplus, M. Computer Simulation and Analysis of the Reaction Pathway of Triosephosphate Isomerase. *Biochemistry* **1991**, *30*, 5826−5832.
(37) Hu, H.; Liu, H.; Shi, Y. The Reaction Pathway of the Isomerization of D-Xylose Catalyzed by the Enzyme D-Xylose Isomerase: A Theoretical Study. *Proteins* **1997**, *27*, 545−555.
(38) Liu, H.; Müller-Plathe, F.; van Gunsteren, W. F. A Combined Quantum Classical Molecular Dynamics Study of the Catalytic Mechanism of HIV Protease. *J. Mol. Biol.* **1996**, *261*, 454−469.
(39) Nadig, G.; Van Zant, L. C.; Dixon, S. L.; Merz, K. M., Jr. Charge-Transfer Interactions in Macromolecular Systems: A New View of the Protein Water Interface. *J. Am. Chem. Soc.* **1998**, *120*, 5593−5594.

(40) Kaminski, G. A.; Jorgensen, W. L. A Quantum Mechanical and Molecular Mechanical Method Based on CM1A Charges: Applications to Solvent Effects on Organic Equilibria and Reactions. *J. Phys. Chem. B* **1998**, *102*, 1787−1796.
(41) Bakowies, D.; Thiel, W. Semiempirical Treatment of Electrostatic Potentials and Partial Charges in Combined Quantum Mechanical and Molecular Mechanical Approaches. *J. Comput. Chem.* **1996**, *17*, 87−108.
(42) Eurenius, K. P.; Chatfield, D. C.; Brooks, B. R.; Hodoscek, M. Enzyme Mechanisms with Hybrid Quantum and Molecular Mechanical Potentials. I. Theoretical Considerations. *Int. J. Quantum Chem.* **1996**, *60*, 1189−1200.
(43) Bakowies, D.; Thiel, W. Hybrid Models for Combined Quantum Mechanical and Molecular Mechanical Approaches. *J. Phys. Chem.* **1996**, *100*, 10580−10594.
(44) Karplus, M.; Evanseck, J. D.; Joseph, D.; Bash, P. A.; Field, M. J. Simulation Analysis of Triose Phosphate Isomerase: Conformational Transition and Catalysis. *Faraday Discuss.* **1992**, *93*, 239−248.
(45) Vasilyev, V. V. Tetrahedral Intermediate Formation in the Acylation Step of Acetylcholinesterases: A Combined Quantum Chemical and Molecular Mechanical Model. *Theochem* **1994**, *110*, 129−141.
(46) Elcock, A. H.; Lyne, P. D.; Mulholland, A. J.; Nandra, A.; Richards, W. G. Combined Quantum and Molecular Mechanical Study of DNA Cross-Linking by Nitrous Acid. *J. Am. Chem. Soc.* **1995**, *117*, 4706−4707.
(47) Harrison, M. J.; Burton, N. A.; Hillier, I. H. Catalytic Mechanism of the Enzyme Papain: Predictions with a Hybrid Quantum MechanicalMolecular Mechanical Potential. *J. Am. Chem. Soc.* **1997**, *119*, 12285−12291.
(48) Cunningham, M. A.; Ho, L. L.; Nguyen, D. T.; Gillilan, R. E.; Bash, P. A. Simulation of the Enzyme Reaction Mechanism of Malate Dehydrogenase. *Biochemistry* **1997**, *36*, 4800−4816.
(49) Ranganathan, S.; Gready, J. E. Hybrid Quantum and Molecular Mechanical (QM/MM) Studies on the Pyruvate to L-Lactate Interconversion in L- Lactate Dehydrogenase. *J. Phys. Chem. B* **1997**, *101*, 5614−5618.
(50) Lee, Y. S.; Hodoscek, M.; Brooks, B. R.; Kador, P. F. Catalytic Mechanism of Aldose Reductase Studied by the Combined Potentials of Quantum Mechanics and Molecular Mechanics. *Biophys. Chem.* **1998**, *70*, 203−216.
(51) Alhambra, C.; Wu, L.; Zhang, Z. Y.; Gao, J. L. Walden-Inversion-Enforced Transition State Stabilization in a Protein Tyrosine Phosphatase. *J. Am. Chem. Soc.* **1998**, *120*, 3858−3866.
(52) Stanton, R. V.; Perakyla, M.; Bakowies, D.; Kollman, P. A. Combined *ab initio* and Free Energy Calculations to Study Reactions in Enzymes and Solution: Amide Hydrolysis in Trypsin and Aqueous Solution. *J. Am. Chem. Soc.* **1998**, *120*, 3448−3457.
(53) Thery, V.; Rinaldi, D.; Rivail, J.-L.; Maigret, B.; Ferenczy, G. G. Quantum Mechanical Computations on Very Large Molecular Systems: The Local Self Consistent Field Method. *J. Comput. Chem.* **1994**, *15*, 269−282.
(54) Monard, G.; Loos, M.; Thery, V.; Baka, K.; Rivail, J.-L. Hybrid Classical Quantum Force Field for Modeling Very Large Molecules. *Int. J. Quantum Chem.* **1996**, *58*, 153−159.
(55) Assfeld, X.; Rivail, J.-L. Quantum-Chemical Computations on Parts of Large Molecules: The *ab initio* Local Self Consistent Field Method. *Chem. Phys. Lett.* **1996**, *263*, 100−106.
(56) Gorb, L. G.; Rivail, J.-L.; Thery, V.; Rinaldi, D. Modification of the Local Self-Consistent Field Method for Modeling Surface Reactivity of Covalent Solids. *Int. J. Quantum Chem.* **1996**, *60*, 313−324.
(57) Reuter, N.; Loos, M.; Monard, G.; Cartier, A.; Maigret, B.; Rivail, J.-L. A Quantum Chemical Approach to Reactions in Biomolecules. *Mol. Eng.* **1997**, *7*, 349−365.
(58) Salvatella, L.; Mokrane, A.; Cartier, A.; Ruiz-López, M. F. The Role of Menthyl Group in Catalyzed Asymmetric Diels−Alder Reactions. A Combined Quantum Mechanics Molecular Mechanics Study. *J. Org. Chem.* **1998**, *63*, 4464−4670.
(59) Salvatella, L.; Mokrane, A.; Cartier, A.; Ruiz-López, M. F. Combined AM1/MM3 computations on organic systems: the Diels−Alder reaction as a test case. *Chem. Phys. Lett.* **1998**, *296*, 239−244.
(60) Antonczak, S.; Monard, G.; Ruiz-López, M. F.; Rivail, J.-L. Modeling of Peptide Hydrolysis by Thermolysin. A Semiempirical and QM/MM Study. *J. Am. Chem. Soc.* **1998**, *120*, 8825−8833.
(61) Gao, J.; Amara, P.; Alhambra, C.; Field, M. J. A Generalized Hybrid Orbital (GHO) Method for the Treatment of Boundary Atoms in Combined QM/MM Calculations. *J. Phys. Chem. A* **1998**, *102*, 4714−4721.
(62) Antes, I. Ph.D. Thesis, University of Zurich, 1998.
(63) Reuter, N.; Karplus, M. Oral communication.
(64) Hodoscek, M. Oral communication.
(65) Hoops, S. C.; Anderson, K. W.; Merz, K. M., Jr. Force Field Design for Metalloproteins. *J. Am. Chem. Soc.* **1991**, *113*, 8262−8270.
(66) Rappé, A. K.; Casewit, C. J.; Colwell, K.; Goddard, W. UFF, a Full Periodic Table Force Field for Molecular Mechanics and Molecular Dynamics Simulations. *J. Am. Chem. Soc.* **1992**, *114*, 10024−10035.
(67) Stote, R. H.; Karplus, M. Zinc Binding in Proteins and Solution: A Simple but Accurate Nonbonded Representation. *Proteins* **1995**, *23*, 12−31.
(68) Hartsough, D. S.; Merz, K. M., Jr. Dynamic Force Field Models: Molecular Dynamics Simulations of Human Carbonic Anhydrase II Using a Quantum MechanicalMolecular Mechanical Coupled Potential. *J. Phys. Chem.* **1995**, *99*, 11266−11275.
(69) Antonzcak, S.; Monard, G.; Ruiz-López, M. F.; Rivail, J.-L. Submitted article.
(70) Dixon, S. L.; Merz, K. M., Jr. Semiempirical Molecular-Orbital Calculations with Linear System Size Scaling. *J. Chem. Phys.* **1996**, *104*, 6643−6649.
(71) Yang, W.; Lee, T.-S. A Density Matrix Divide-and-Conquer Approach for Electronic Structure Calculations of Large Molecules. *J. Chem. Phys.* **1995**, *103*, 5674.
(72) Stewart, J. P. Application of Localized Molecular Orbitals to the Solution of Semiempirical Self-Consistent Field Equations. *Int. J. Quantum Chem.* **1996**, *58*, 133−146.
(73) Daniels, A. D.; Millam, J. M.; Scuseria, G. E. Semiempirical Methods with Conjugate Gradient Density Matrix Search to Replace Diagonalization for Molecular Systems Containing Thousand of Atoms. *J. Chem. Phys.* **1997**, *107*, 425−431.

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