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Bridging quantum mechanics and structure-based drug design

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1. ABSTRACT

The last decade has seen great advances in the use of quantum mechanics (QM) to solve biological problems of pharmaceutical relevance. For instance, enzymatic catalysis is often investigated by means of the so-called QM/MM approach, which uses QM and molecular mechanics (MM) methods to determine the (free) energy landscape of the enzymatic reaction mechanism. Here, I will discuss a few representative examples of QM and QM/MM studies of important metalloenzymes of pharmaceutical interest (i.e. metallophosphatases and metallo-beta-lactamases). This review article aims to show how QM-based methods can be used to elucidate ligand-receptor interactions. The challenge is then to exploit this knowledge for the structure-based design of new and potent inhibitors, such as transition state (TS) analogues that resemble the structure and physicochemical properties of the enzymatic TS. Given the results and potential expressed to date by QM-based methods in studying biological problems, the application of QM in structure-based drug design will likely increase, making of these once-prohibitive computations a routinely used tool for drug design.

2. INTRODUCTION

Quantum mechanics (QM) stands out as a powerful computational approach to characterizing the structure, dynamics, reactivity and energetics of (bio)molecules (1). Nevertheless, applicability of QM is limited by the elevated computational cost (i.e. time) associated with its high accuracy. Indeed, computational tools for drug design mostly employ molecular mechanics (MM)-based approaches, which are much less computationally demanding (and much less accurate)(2). In recent years, however, QM has shown significant advances with respect to its use for solving biological problems. In fact, QM-based methods are nowadays more accessible thanks to several key factors, namely the extraordinary increase of computational power in recent decades, the diminished price of CPU, and the development and implementation of more efficient algorithms for wave function calculations. All this, together with the promise represented by the acceleration of calculations running on GPU, promotes QM methods as an additional tool in the vast computational armamentarium for drug design (3-6).

The cardinal paradigm of drug design is that the

drug exerts its efficacy by inhibiting the function of one or more targets, such as enzymes or channels. That is, a tight interaction of a small molecule (i.e. the drug) with its target blocks the target's function and this in turn provides beneficial pharmacological effects. Thus, the characterization of the ligand-target binding is of paramount importance in the drug design process. In this respect, QM methods can offer major improvements in drug design compared to MM methods, since QM provides insights into the molecules of interest at the atomistic (electronic) level. This costly information is required, for instance, in studying bond forming/breaking reactions such as those catalyzed by enzymes, in describing polarization effects and charge transfer, and, ultimately, in estimating more accurately the interaction energy between the ligand and its receptor.

Computational drug design can be divided into two broad areas, namely (1) structure-based drug design (SBDD), where the structure of both receptor and ligand is known and used for drug design and (2) ligand-based (LB) approaches, in which only the structure of the ligand is known, while the receptor's structure is unknown. Here, the focus will be on representative applications of QM methods applied to therapeutically relevant targets for SBDD. LB drug design is not part of this review article. Briefly, QM in LB has been historically used to investigate the energy, geometry, and electronic features (i.e. orbitals, dipole moment, atomic charges, etc.) of small organic molecules. This information is then used to perform quantitative structure-activity relationship (QSAR) and 3D QSAR studies for ligand design and optimization(7, 8).

In SBDD, QM methods find a broad application since they deal with the ligand, the receptor, and the interaction between the two. The detailed understanding of the ligand-receptor interaction is then used in the rational design of inhibitors. Within SBDD, one can consider two types of QM applications. The first type are those more directly related to the evaluation and design of new inhibitors, such as QM-corrected scoring functions for docking calculations and QM-derived binding affinity estimations (9-12). These calculations serve to indicate promising compounds as good inhibitors for drug design. The second type of QM applications are those related to a detailed characterization of the structure and function of the receptor. These calculations can then be used for drug design after further calculations. This is the case with QM-based electrostatic potential maps used to characterize the binding site of the receptor (13, 14), with QM calculations to determine the protonation states of key residues of the binding pocket (15-17), and with QM-based studies of the reaction mechanism of pharmaceutically relevant enzymes that are targets for drug discovery (4). The latter will be the main subject of this review article, which discusses some of the recent QM studies of pharmaceutically relevant enzymes. In particular, I emphasize QM-based computational investigations of metalloenzymes, with focus on the reaction mechanisms, on the role of metal ions in catalysis, and on the dynamics and flexibility of the enzyme catalytic site.

Two classes of metalloenzymes will be the subject of this article: 1) metallophosphatases, which catalyze the phosphoryl transfer reaction and include several proteins that are targets for SBDD, such as ribonuclease H (RNase H) for antiviral drugs; and 2) beta-lactamases, which hydrolyze all kinds of beta-lactam antibiotics and thus are targets for improving the efficacy of beta-lactam drugs. The connection between the elucidation of enzymatic reaction mechanisms and drug design can be found in transition state (TS) theory, which indicates TS analogues as potent inhibitors (18). That is, a detailed understanding of biocatalysis should enable rational TS-based drug design.

This article is not intended to be an exhaustive review of computational enzymatic catalysis, which has been the subject of several reviews (see refs(19-28)). The intent, here, is to use representative examples to show how QM-based methods can be used to elucidate the ligand-receptor interaction in great detail, to initiate SBDD studies, and to assist the challenging process of drug discovery.

3. METHODS

Here, I briefly introduce the basic concepts and vocabulary of QM methods. This will be sufficient for understanding this review article. However, the interested reader is encouraged to delve into the review articles and books that focus on the theoretical background of QM methods (29-31).

QM methods can be classified as ab initio, density functional theory (DFT), or semiempirical methods. Ab initio QM methods aim to solve the Schrödinger equation. They deal with the wave function of the system. They are the most accurate and computationally expensive methodologies. The term ab initio indicates that the calculations are based on first principles only, and no empirical data are used. However, it is complicated to find the exact solution for the Schrödinger equation, and approximate methods must be used for polyelectronic systems. The simplest approximation is the Hartree-Fock (HF) scheme, in which the many-electron wave function is reduced to a product of antisymmetric one-electron wave functions (Slater determinant). However, each electron only feels the mean-field potential of all the other electrons. HF accounts for exchange effects, while it fails to recognize that electronic motions are correlated, neglecting the correlation effects. More sophisticated methods can improve the HF approximation. One way to include correlation effects is the Møller-Plesset (MP) perturbation theory(32), which applies a perturbation to the HF solution. Second order Møller–Plesset (MP2) calculations are quite standard, while the computational cost increases considerably if higher orders are used. Additional methods to account for correlation effects and to treat a polyelectronic system are the configurational interaction (CI) and coupled cluster (CC) methods. However, these methods further increase the computational cost (i.e. CPU-time) associated with their high accuracy. The high computational cost of ab initio calculations still limits their use for drug design.

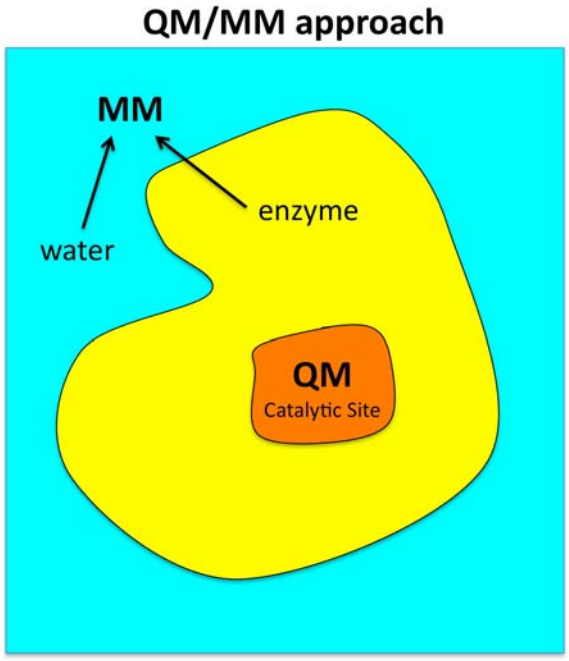

Figure 1. Schematic representation of the hybrid quantum mechanics/ molecular mechanics (QM/MM) approach. The enzyme (in yellow) and the solvent (in blue) are treated at the MM level of theory, while only part of the enzyme is treated at the QM level. The QM part includes the atoms involved in the enzymatic reaction.

The central idea of DFT(33), proposed by the theorem of Hohnberg and Kohn(34) in 1964, is that the ground-state energy of a system of interacting electrons is a unique functional of its electron density. One year later, Kohn and Sham proposed a way to solve the Hohnberg-Kohn theorem(35) for a set of interacting electrons. The KS approach proposed a functional for the energy where the so-called exchange-correlation energy functional needs to be approximated. To overcome this issue, several different approximations are used, of which the most basic is the local density approximation (LDA). Later improved approximations include the generalized gradient approximation (GGA), which has led to several gradient-corrected parameterizations for the exchange-correlation energy functional. Among the most common gradient-corrected functionals, there is the Becke gradient-exchange correction, and the Lee, Yang and Parr correlation functional, commonly abbreviated as the BLYP functional (36, 37). The hybrid B3LYP functional (38) combines HF with the Becke functional (36), the LYP functional (37) , the VWN functional(39) and three empirical parameters. Calculations at the DFT/B3LYP level of theory are rather widespread in quantum chemistry, and are also widely used to study biological systems. In 1985, Car and Parrinello proposed a new method of performing DFT-based molecular dynamics(40) (the CPMD method), which allows one to simulate (biological) systems at their actual temperature, thus including entropic effects. CPMD has been successfully applied to a number of pharmaceutically relevant targets, as reported in these reviews (27, 28). Overall, DFT is the most popular QM method for electronic structure calculations, and its drug design applications are constantly increasing.

Semiempirical methods are simplified versions of ab initio and DFT methods, in which some hard to compute integrals are neglected in order to speed up calculations. This approximation is compensated for by empirical corrections, derived from experimental data. Standard semiempirical methods are MNDO, AM1, and PM3. These are parameterized primarily with respect to ground-state properties, with particular emphasis on the energies and geometries of organic molecules. The low computational cost of these semiempirical methods is counterbalanced by their limited accuracy, which depends on the type of parameterization and empirical corrections.

When dealing with macromolecules such as enzymes, which are the focus of computations in this review article, QM and molecular mechanics (MM) methods can be used together, forming the so-called QM/MM approach (19) (Figure 1). Consider that a model system of a protein immersed in explicit solvent can easily reach a size of 100,000 atoms or more, while only a few hundred atoms, at most, can be described at the QM level. In QM/MM studies of enzymatic catalysis, the region of interest of the model system (the enzyme's binding pocket and the ligand) is treated at a higher level of accuracy (QM level), while the remainder of the system is treated at the MM level of theory. The QM region can be described at several different levels of theory, spanning from semiempirical to ab initio or DFT. Since its first appearance in 1976 (22), the QM/MM approach has mainly been used to study enzymatic catalysis. However, QM/MM calculations have also been used recently for protein-ligand binding affinity calculations (9, 10).

4. QM-BASED STUDIES OF PHARMACEUTICALLY RELEVANT TARGETS

This review article focuses on some representative QM/MM studies of therapeutically relevant enzymes. The selected studies will show how QM-based methods allow researchers to investigate enzymatic mechanisms, including proton transfer events, and metal-aided reactions, and to describe dynamic features of the enzyme's catalytic site. The following sections are not exhaustive. The interested reader is referred to the several reviews that concern computational studies of enzymatic catalysis (19-28).

The connection between the atomic-level understanding of the enzymatic mechanism and the rational design of ligands relies primarily on transition state (TS) theory, which defines TS analogues as potent inhibitors. TS theory explains that the catalytic power of enzymes derives from their ability to lower the TS energy along the enzymatic reaction pathway, thus decreasing the activation barrier for the catalytic reaction. The lowering of TS energy is a direct consequence of the tight (i.e. favorable) interaction between the ligand and the receptor in TS geometry. For this reason, a ligand resembling the

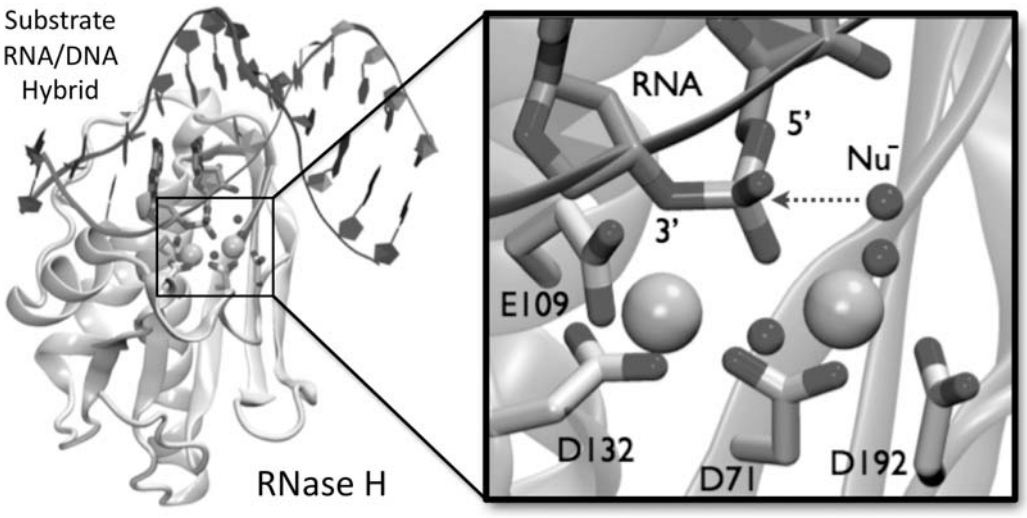

Figure 2. On the left, the crystallographic structure of the complex RNase H / RNA•DNA hybrid is shown. The panel on the right offers a close view of the RNase H catalytic site, where two Mg2+ ions are surrounded by carboxylic groups, the scissile phosphate of the substrate RNA strand and the nucleophilic oxygen atom.

geometry and physicochemical properties of the TS is a potent inhibitor (i.e. a TS analogue). Given the fleeting nature of the TS reaction, which does not allow a direct experimental observation of its geometry or chemical features, it is necessary to employ high-level electronic structure calculations to determine which TS characteristics to use as template for designing TS analogues (18). These potent inhibitors can ultimately be used as a promising starting point for rational drug design.

Selected examples from two classes of metalloenzymes are reported here. The first class of proteins is represented by the metallophosphatases, which catalyze the transfer of the phosphate group in a large number of proteins, among which are several important drug discovery targets. In particular, we review two proteins that catalyze the phosphoryl transfer reaction and that exemplify QM-based calculations applied to pharmaceutically relevant targets. The first is ribonuclease H (RNase H), which is a target for developing antiviral drugs. This enzyme catalyzes a nucleotidyl transfer reaction in the presence of two Mg^{2+} ions contained in the catalytic site. The second is the phosphatase activity in soluble epoxide hydrolase (sEH), which is a promising target for hypertension and acute respiratory syndrome treatment. The sEH carries out the phosphatase activity in its N-terminal lobe with a single Mg^{2+} ion in the catalytic site. The second class of proteins are the metallo (Zn) beta-lactams, which are important targets for the discovery of new resistant antibiotics.

Phosphoryl transfer reactions are ubiquitous in biology because of their use as regulatory mechanisms in both eukaryotes and prokaryotes. Hydrolysis and subsequent transfer of phosphate is the central chemical process of many metabolic pathways, including kinase cascade activation, membrane transport, gene transcription, and motor mechanisms. A multitude of enzymes are thus involved in the catalysis of such processes, and extensive experimental data have accumulated (41-46). Nevertheless, mechanistic details of enzymatic phosphoryl transfers are still debated. In particular, the nature of the enzymatic TS is of interest in order to define the phosphoryl transfer mechanism, which can be dissociative or associative, according to the metaphosphate-like or phosphorane-like nature of the TS geometry. Also, the possible existence of a stable penta-coordinated phosphate intermediate along the phosphoryl transfer is still debated, while only one crystal structure, the beta-phosphoglucomutase crystal (47), has shown such an intermediate. Finally, many enzymes catalyze such reactions in the presence of positively charged metal ions (e.g., Mg^{2+}, Zn^{2+}, Mn^{2+}, Ca^{2+}), which help stabilize the overall negative charge of the scissile phosphate. In this regard, it is of interest to understand the role of the metal ions, and thus appreciate the often elegant ways in which an enzyme is able to increase the efficiency of the catalytic process. Importantly, the necessary chemical steps in these phosphoryl transfer reactions are the protonation of the leaving group and deprotonation of the attacking nucleophile. These processes often involve water-bridge (WB) proton shuttles, which are facilitated by electrostatic stabilization provided by the metal ion(s). With this in mind, it is useful to compare how the same types of reaction can be carried out and accelerated by a different enzymatic structural environment (i.e. RNase H versus sEH), with particular focus on the role of metal ion(s) in facilitating the enzymatic reaction.

4.1. Ribonuclease H

Ribonuclease H (RNase H) is one of the endonuclease proteins. These proteins typically contain metal ions, which are required for their biological function. Specifically, endonucleases catalyze the metal-ion-dependent phosphodiester hydrolysis of either RNA or DNA strands (Figure 2). Such enzymes are ubiquitous in nature, being vital in nucleic acid synthesis, recombination,

processing and degradation. The large negative charge on the backbone of RNA and DNA strands is a characteristic of the substrate of the metalloendonuclease protein. For this reason, positively charged metal ions are often found in the catalytic pocket of the protein, where one, two or even three ions are coordinated to negatively charged amino acids, water molecules, and the scissile phosphate of the substrate (48). These cations play a variety of key roles, they: i) neutralize the large negative charge of the substrate RNA/DNA strand, helping to maintain the structural integrity of the protein/substrate complex; ii) intensify/regulate the affinity for the substrate, and sometimes induce specificity for a particular sequence; iii) promote nucleophile formation and its attack on the scissile phosphate; and iv) facilitate the reaction mechanism by stabilizing the transition state (TS) and leaving group exit (49). Most metalloendonucleases use Mg^{2+} as a cofactor, though other divalent metals such as Mn^{2+} or Zn^{2+} have been identified (48, 49). Understanding the role of metal ions in endonuclease activity is fundamental for developing potentially innovative strategies to manipulate genes and to design structural probes or novel drugs. Moreover, the design and modeling of artificial proteins that can produce biomimetic hydrolysis of DNA and RNA is an area of current research. Some of the difficulties in emulating the reactivity and specificity of natural metalloendonucleases could likely be solved by clarifying their mechanism of action.

In detail, RNase H belongs to the nucleotidyl-transferase (NT) superfamily and hydrolyzes the phosphodiester linkages, which form the backbone of the RNA strand in RNA·DNA hybrids(50, 51). In the presence of two Mg^{2+} or Mn^{2+} ions, the cofactors necessary for optimal activity, RNase H hydrolyzes the P-O3' bond of the RNA strand, degrading RNA·DNA hybrids. High-resolution crystal structures have revealed the arrangement of the complex formed by RNase H from *Bacillus halodurans* (Bh) and its substrate, the RNA·DNA hybrid(52-55). A divalent bimetal architecture of the catalytic site has been defined where two Mg^{2+} ions are jointly coordinated to a non-bridging oxygen of the scissile phosphate of the substrate RNA strand (Figure 2). Always, the activity changes with the nature of the metal ion and/or its concentration. In fact, RNase H has optimal activity at Mg^{2+}concentration of 10-20 mM, while it is inhibited at 50 mM (the so-called 'attenuation' effect). Moreover, while Mg^{2+} and Mn^{2+} can promote the enzymatic function when in the correct concentration, Ca^{2+} inhibits the RNase H endoribonuclease activity. Importantly, due to RNase H activity in HIV reverse transcriptase (HIV-RT), it represents a promising target for anti-HIV drug design (56, 57).

Recently, Car-Parrinello(40) molecular dynamics (CPMD), in its QM/MM implementation(58), has been used to clarify mechanistic and dynamical details of the bimetal-aided nucleotidyl transfer reaction(59) in RNase H. These QM/MM simulations employ the DFT/BLYP level of theory for the QM part. Important aspects of the catalysis are: 1) the nature of the enzymatic mechanism (concerted one-step or stepwise, with formation of a stable phosphorane intermediate); 2) the energetics and formation mechanism of the nucleophilic hydroxide ion; 3) the role of the pro-R_p oxygen during the catalysis; and finally 4) the role of the two metal cofactors in aiding the catalysis. Two different reagent states have been taken into account in studying the enzymatic reaction (Figure 3). In one, the nucleophilic species is a water molecule (P_{WAT}). In the other, a hydroxide ion (P_{OH-}) is the reactive nucleophile. As expected, due to the high nucleophilicity of the OH^- group, P_{OH-} shows the lowest free energy barrier (~10.5 kcal mol^{-1}). Nevertheless, P_{WAT} shows a competitive mechanism (free energy barrier of ~16 kcal mol^{-1}) if dehydration energy is also taken into account (~3 kcal mol^{-1}). These free energy values are qualitatively consistent with the available kinetic data for substrate analogs for HIV-1 RNase H activity. In fact, Shaw-Reid *et al.* (57) measured the kinetic parameters of 4 substrate analogs for HIV-1 RNase H activity. The experimental k_{cat} values range from 1.01 to 0.11 min^{-1}, which correspond to a free energy range of ~19.8 to ~21.2 kcal mol^{-1}.

In summary, both P_{OH-} and P_{WAT} show an in-line SN_2-like nucleophilic attack on the scissile phosphorus (Figure 3). This leads to an associative mechanism with phosphorane-like transition states. Importantly, P_{WAT} includes a meta-stable pentavalent phosphorane intermediate, observed so far only in the debated beta-phosphoglucomutase crystal (47). The presence of such an intermediate has also been suggested by the recent study of Elsässer *et al*(60), which applies high level QM/MM calculations to the study of RNase A. Importantly, as in the phosphatase activity of sEH (61, 62) discussed hereafter, water molecules solvating the metal center in RNase H facilitate the migration of protons involved in the phosphoryl transfer reaction. Understanding this aspect of the mechanism is crucial to appreciating the catalytic strategy used by the enzyme to create better attacking and leaving groups. It shows the critical role played by water molecules in enzymatic mechanisms (e.g., phosphoryl transfers). Another essential aspect of the reaction mechanism is that the two Mg^{2+} ions act in a cooperative fashion. In fact, they act simultaneously in order to promote and facilitate both nucleophile formation and leaving group stabilization. As a result, both P_{OH-} and P_{WAT} show a phosphorane-like transition state in which the associative character of the TS is supported by the two ions, which get closer to each other in the TS geometry. Rosta *et al*(63) have focused, more recently, on the first step of the reaction mechanism of RNase H (i.e. the attack of the phosphate diester by water). They have used a different flavor of the QM/MM approach, which implies the DFT/B3LYP level of theory for the QM part. This study confirms the finding of Ref.(59), reported above, both in terms of possible mechanisms and the associated free energy.

Other informative computational studies have been performed recently on phosphoryl transfer reactions in metalloenzymes that cut RNA and/or DNA strands: McCammon *et al*(64) have used CPMD QM/MM calculations and pointed out the active site rearrangements and the (three) metal ion movements in the endonuclease IV, an attractive target for antibacterial, antifungal and

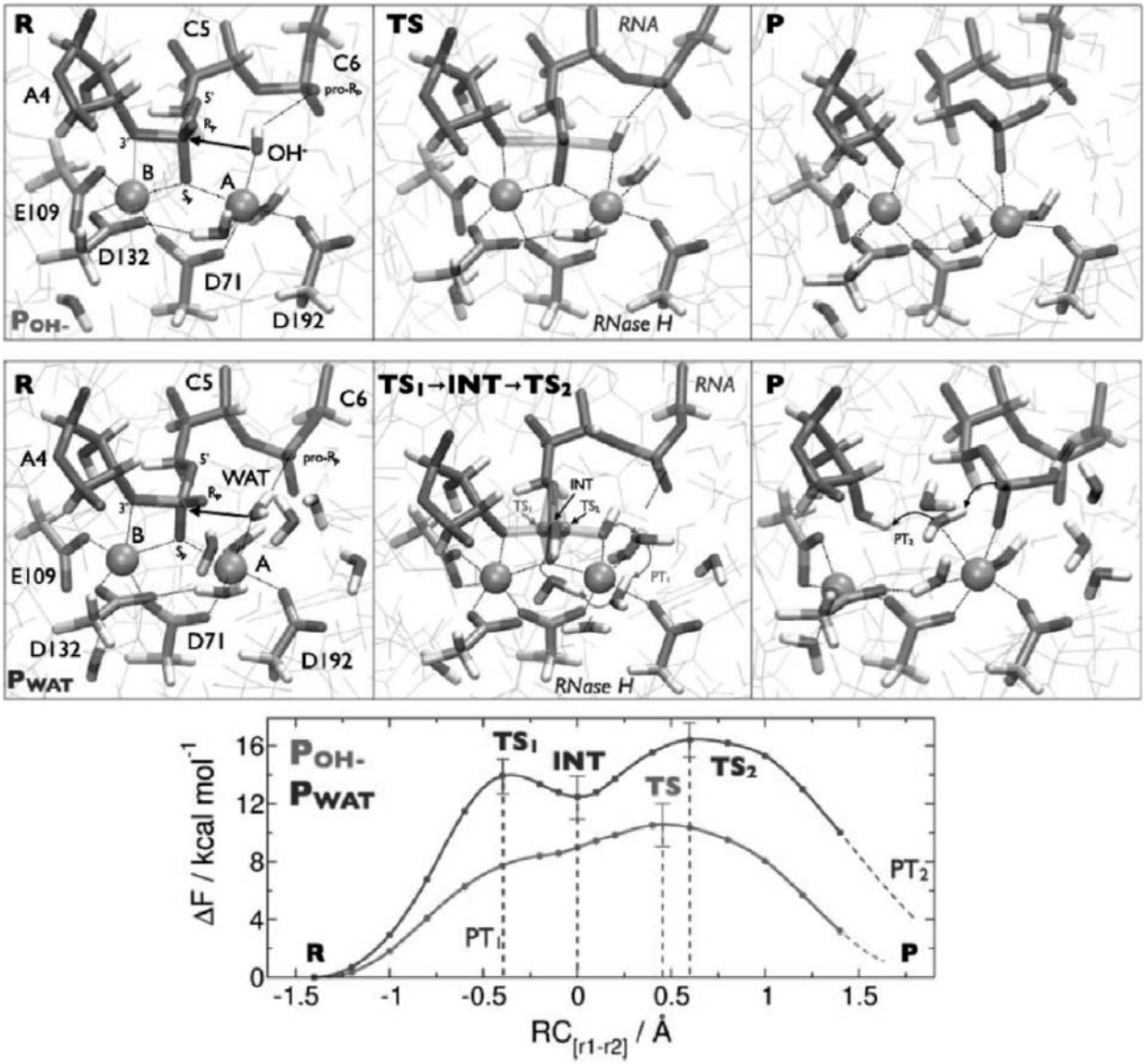

Figure 3. On the top, the structural evolution of the reaction is shown. Representative snapshots from the QM/MM dynamics of the RNase H catalysis are reported. Only QM atoms are highlighted. First row: OH- pathway, where the nucleophilic group is one hydroxide ion. The phosphorane-like TS is shown in the middle. Second row: WAT pathway, where the nucleophilic group is a water molecule. On the bottom, the free-energy profiles of the two reaction pathways are shown.

antimalarial drugs. Karplus *et al*(65) have used ab initio and DFT QM/MM calculations to describe the changes in the internuclear distance of the two Mg^{2+} metal ions in the ribozyme, which is able to tailor defined RNA sequences. For this reason, understanding how the ribozyme works may help the rational design of therapeutic agents such as biosensors for application in functional genomics and gene discovery. The ribozyme was studied by Boero *et al*(66), who used ab initio MD to probe how each atom contributes to the catalysis in a two-metal-aided enzymatic mechanism. It is worth mentioning that the activity of metalloendonucleases has been reported in the presence of different metals. This is the case with the metal-aided activity carried out by the EcoRV protein, a type II restriction endonuclease (67) often used to cut a plasmid vector and insert a gene of interest, during gene cloning. Specifically, two metal ions have been experimentally observed in EcoRV, positioned in two of the three available metal-binding sites. The activity of this enzyme has been measured in the presence of Mg^{2+}, Mn^{2+} and Co^{2+}, while Ca^{2+} blocks the catalysis. Recently, Fischer *et al* have used DFT and QM/MM calculations (where, in this case, the QM part is treated at the semiempirical AM1 level of theory) to shed some light on the reaction mechanism for DNA cleavage. However, how the two metals coordinate their position in three binding sites is still a matter of investigation. In fact, these metal ions must move and reorganize the catalytic geometry in an efficient way to

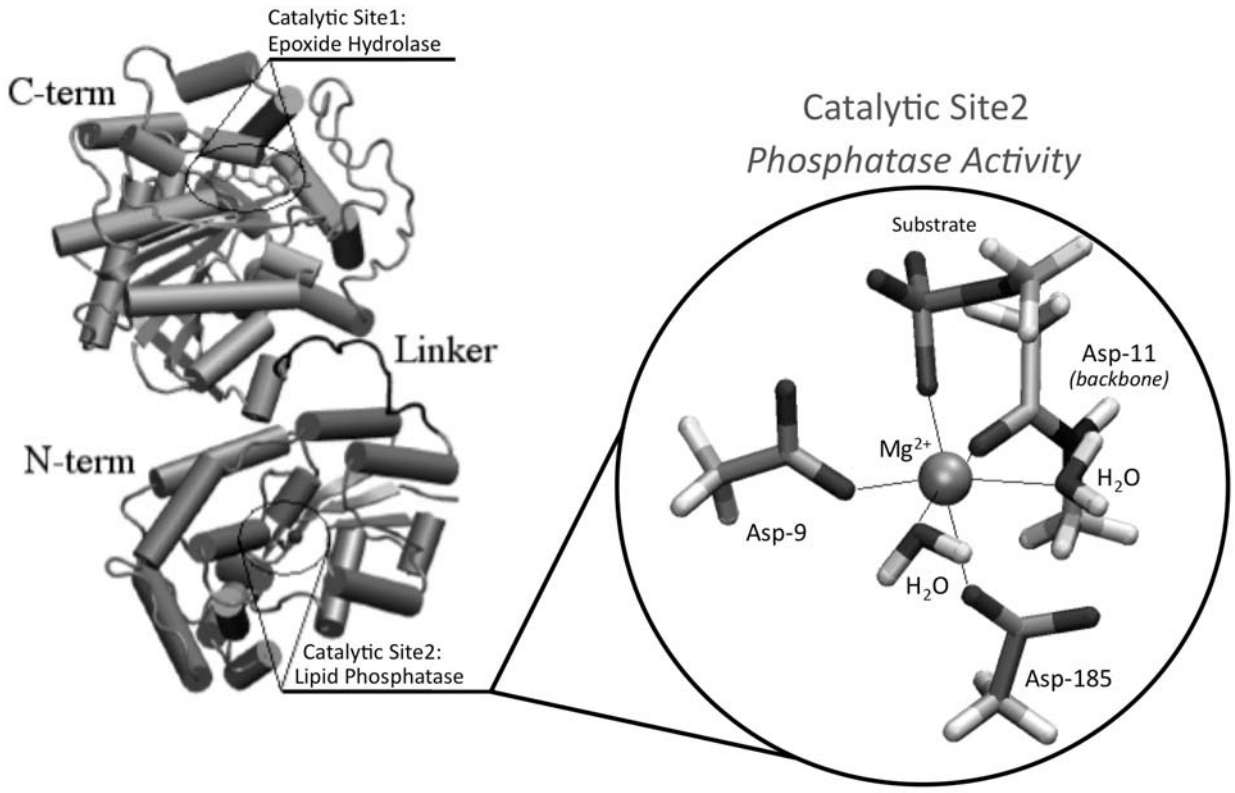

Figure 4. On the left, the crystallographic structure of the soluble epoxide hydrolase (sEH) is shown. On the right, a close view of the catalytic site in the N-terminal domain of the sEH is shown. The Mg2+ ion is surrounded by the ligands, which includes the phosphate group of the substrate, located on top of the Mg2+ ion.

promote the reaction. A further point is how metal ions behave along the reaction coordinate of the enzymatic reaction. This exciting field of research is in its infancy, and much remains to be clarified. One question, among many of interest, is how different ions are able to affect the same catalysis.

4.2. Phosphatase activity in soluble epoxide hydrolase

Recently, a novel metal (Mg^{2+})-dependent phosphatase activity was discovered in the N-terminal domain of the dual-domain protein soluble epoxide hydrolase (sEH), opening a new branch of fatty acid metabolism and providing an additional site for drug targeting(68-70). The initially observed catalytic activity of sEH, namely the hydrolysis of epoxy fatty acids, occurs in the large C-terminal domain, while the novel metal (Mg^{2+})-dependent phosphatase activity of sEH has been discovered in the smaller N-terminal domain. Importantly, structural data of sEH enzymes reveal that the N-terminal domain adopts an alpha/beta fold homologous to that of the haloacid dehalogenase (HAD) superfamily, the majority of which comprises phosphotrasferases. Based on crystallographic findings (71-73), a two-step reaction scheme has been proposed, which describes two phosphoryl transfers taking place in the sEH phosphatase: Step 1) nucleophilic attack on the phosphate group of the phosphoester substrate by Asp9, and protonation of the leaving group by either an intervening water molecule or Asp11; Step 2) hydrolysis of the phosphoenzyme intermediate via a nucleophilic attack at the scissile phosphorus atom by a water molecule (Figure 4). Two CP QM/MM computational studies(61, 62) have provided first-principles-based interpretations of the experimental findings and reveal a catalytic mechanism that agrees with the proposed one, while offering great detail for the two necessary steps (Step 1 and Step 2, Figure 5). In fact, together with a detailed description of phosphoryl transfer reactions catalyzed by the sEH phosphatase, these studies also explain how the Mg^{2+} ion helps the reaction and its efficiency. In particular, they show how metal-substrate connecting water-bridges (WBs) allow for efficient transfer of the protons necessary for nucleophile formation (water deprotonation) and leaving group stabilization during the two phosphoryl transfers that constitute the catalytic cycle. Both steps show an in-line nucleophilic substitution presenting a rather dissociative character, particularly pronounced in Step 2. No evidence of a phospharane species in the TS regions is found; instead, a planar metaphosphate-like transition state that nicely resembles crystal structures of TS analogues is observed. The computed free-energy barriers are in good agreement with experimental values, suggesting Step1 (~19 kcal mol^{-1}) as the rate-determining step of the catalytic cycle (Figure 5). Overall, an important contribution to enhancing catalytic efficiency is made by the nucleophile and leaving group stabilization via WB-mediated proton shuttles, mostly

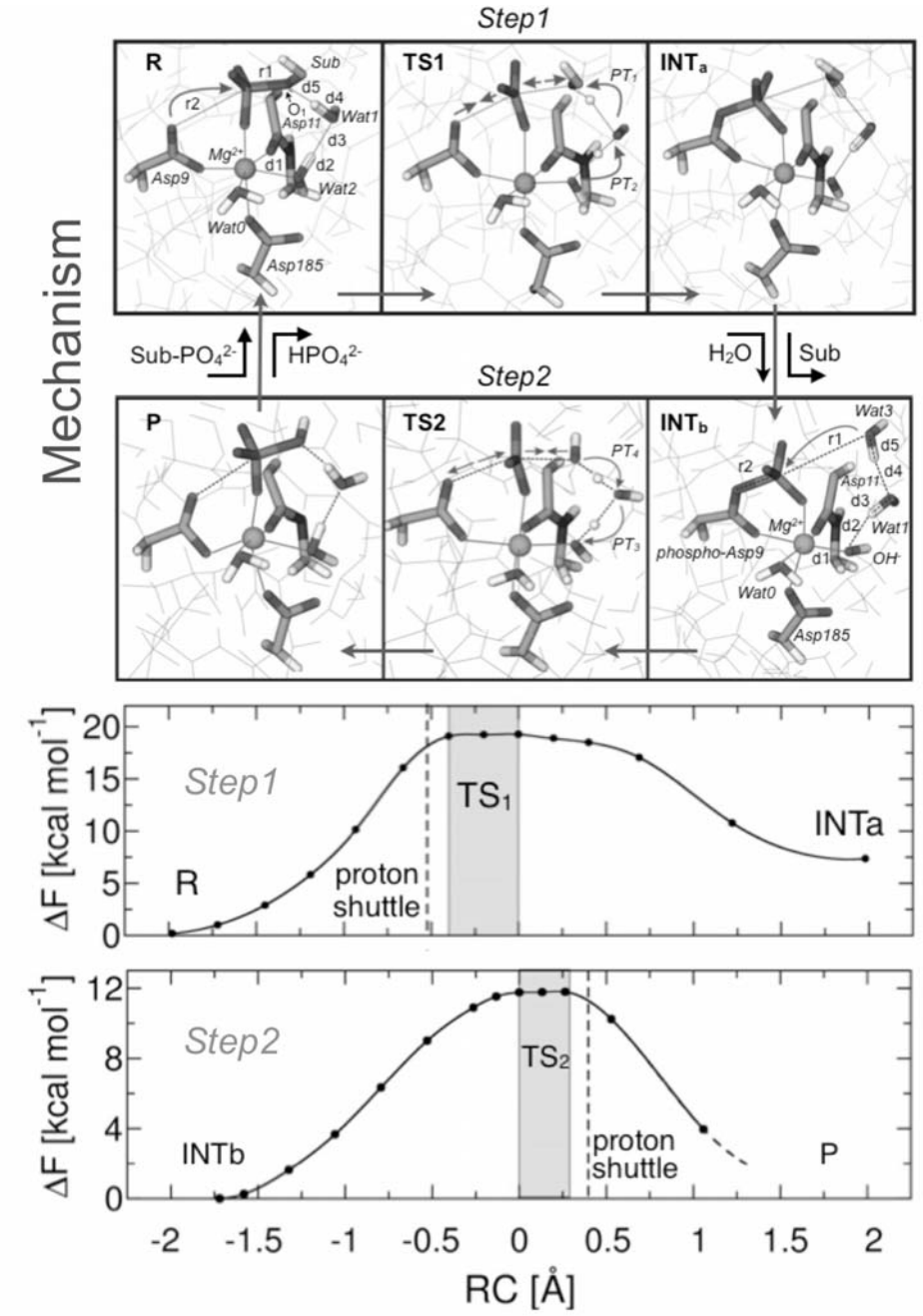

Figure 5. On the top, representative snapshots from the QM/MM dynamics of the sEH catalysis are reported. Only QM atoms are highlighted. First row: nucleophilic attack of Asp9 at the Mg2+-coordinated phosphoryl group, with leads to the phosphoenzyme intermediate formation (INTa). Second row: phosphoryl transfer from the phospho-Asp9 to one attacking solvent water, leading to the product. On the bottom, the two free energy profiles for step1 and step 2 are reported. The proton shuttles are indicated by a vertical dashed line.

induced by the electrostatic effects of the metal ion. In fact, repressing these proton shuttles in MD simulations leads to inhibition of the phosphatase activity.

It is interesting to observe the metal dependence in phosphoryl transfer reactions, by comparing the QM/MM studies concerning sEH (one Mg^{2+} cation) versus RNase H (two Mg^{2+} cations). In fact, based on these QM/MM studies (59, 61, 62, 74), different mechanisms (associative *vs.* dissociative) for phosphoryl-transfers seem to be induced according to the metal(s) geometry and stoichiometry during catalysis. Indeed, during the sEH catalysis, the metaphosphate group that is transferred is stabilized by its apical coordination to the only Mg^{2+} ion

present in the catalytic site. On the other hand, in the RNase H catalysis, the two Mg^{2+} cations stabilize the attacking and leaving groups, while the metaphosphate group is accommodated between the two metals, forming a phosphorane-like TS geometry. In other words, two ions would facilitate the formation of a meta-stable intermediate, as in RNase H. This hypothesis, reported for the first time in ref 59, is still a speculation and needs to be supported by further investigations.

Several other QM/MM studies have clarified the reaction mechanism for phosphoryl transfers in metallophosphatases of pharmaceutical interest. For instance, protein kinases represent important targets for developing new therapies(75). As such, they have invigorated discussions on using phosphatases as drug targets. Indeed, a large number of computational investigations of protein kinases have appeared in the literature in recent years, providing detailed information about the phosphoryl transfer mechanism. My contribution is the study of the phosphoryl transfer catalyzed by cyclin-dependent-kinase 2 (CDK2), by means of full QM and CP QM/MM simulations(74). Recently, Sugita *et al* have investigated the initial phosphoryl transfer catalyzed by phosphoserine phosphatase (PSP), a member of the HAD family. PSP represents an attractive target for SBDD since PSP's aberrant activity has been linked with several pathological conditions, including diabetes, cardiovascular disorders, cancer, and Alzheimer's disease. Interestingly, the enzymatic mechanism for phosphoryl transfer found by Sugita *et al*(76) agrees well with that described for the first phosphoryl transfer catalyzed by the sEH. Other recent examples of QM/MM calculations used to investigate phosphoryl transfers in metalloenzymes include the study of Tunon *et al*(77), who used a semiempirical QM/MM approach to study the enzymatic reaction catalyzed by *Escherichia coli* alkaline phosphatase (EcAP). Their study highlights some of the potential shortcomings of the QM/MM methodology. Sticht *et al*(78) have investigated the phosphoryl transfer catalyzed by the phosphoenolpyruvate:sugar phosphotransferase system (PTS), which does not require a metal ion for catalysis and which represents an integral part of the bacterial sugar metabolism. The protein farnesyltransferase (FTase), a promising target for anticancer drug design, has been the subject of a recent CPMD QM/MM study by Klein *et al*(79), who offered a detailed picture of the metal-aided phosphoryl transfer included in the FTase catalytic pathway. A final example comes from the studies of Warshel *et al*, who have characterized the GTP hydrolysis of the RasGAP system, which regulates a crucial switch in cellular signal transduction(80). This important enzyme has been the subject of several computational studies carried out by others, aimed at clarifying the reaction mechanism(81, 82).

These examples of QM/MM studies of phosphoryl transfer in enzymes are just a few of the many in the literature. These representative ones have been included in this review to show how useful QM/MM calculations are in achieving a detailed description and understanding of this important enzymatic reaction.

4.3. Metallo beta-lactamases

Metallo beta-lactamases (MBLs) are characterized by one or two Zn ions bound to their active sites. Despite not being as ubiquitous as serine beta-lactamases, MBLs hydrolyze all kinds of beta-lactam antibiotics, including the latest generation of carbapenems. MBLs are increasingly spreading among pathogenic bacteria in the clinical setting and are resistant to all the current clinical inhibitors on the market. Thus, MBLs are important targets for designing effective drugs(83, 84).

The MBL folding frame is a compact alpha-beta/beta-alpha sandwich, which accommodates an active site with one or two Zn ions that are essential for hydrolysis. At the active pocket, the first metal site (Zn1) is coordinated tetrahedrally by three histidines (His116, His118 and His196), and the nucleophilic hydroxide (Figure 6). The coordination of the second metal site (Zn2) is provided by the nucleophile, one water molecule, and a ligand triad of protein residues, which includes Asp120, His263 and Cys221. The characterization of catalytic mechanism in Zn-enzymes by means of experimental techniques is difficult since these metals are silent to most spectroscopic techniques. Thus, computational methods represent a valuable tool for investigating enzymatic catalysis and for providing structural and energetics details of the reactive mechanism.

Dal Peraro *et al* have been very active on MBLs, performing classical MD simulations, full QM and hybrid CP (DFT/BLYP) QM/MM calculations (15, 16, 85-89). First, they used full QM calculations and classical MD simulations of the mono-Zn MBL from *Bacillus cereus* (BcII) and di-Zn from *Bacteroides fragilis* (CcrA) in complex with different types of beta-lactams (e.g. benzylpenicillin, imipenem, and cefotaxime) to produce productive conformations for the study of the enzymatic reaction in all complexes. These studies have also pointed out a few crucial interactions for binding recognition, regardless of the metal content. In particular, one water molecule bridges the beta-lactam carboxylate group and the metal center. When two Zn metals are bound at the active site (as in CcrA, see Figure 6 and 7), WAT is bound to the second Zn (Zn2), completing its coordination shell. Thus, a water-mediated salt-bridge is maintained between the beta-lactam carboxylate moiety and Lys224, which is a conserved residue in most MbL enzymes. It is striking that these common minimal features are sufficient to accommodate different beta-lactams.

Subsequently, Dal Peraro *et al*(87, 89) used hybrid CP (DFT/BLYP) QM/MM calculations to investigate the hydrolysis of a commonly used cephalosporin (cefotaxime), which is actively degraded either by mono-Zn and di-Zn species. It emerged from these studies that both BcII and CcrA enzymes can promote the nucleophilic attack by a metal-bound hydroxide. They can also catalytically activate the nucleophile water (WAT), which eventually leads to the C-N bond cleavage of the beta-lactam ring. Nonetheless, the chemistry and kinetics of the two reactions strongly depend on the Zn architecture and content. Interestingly, in CcrA, the

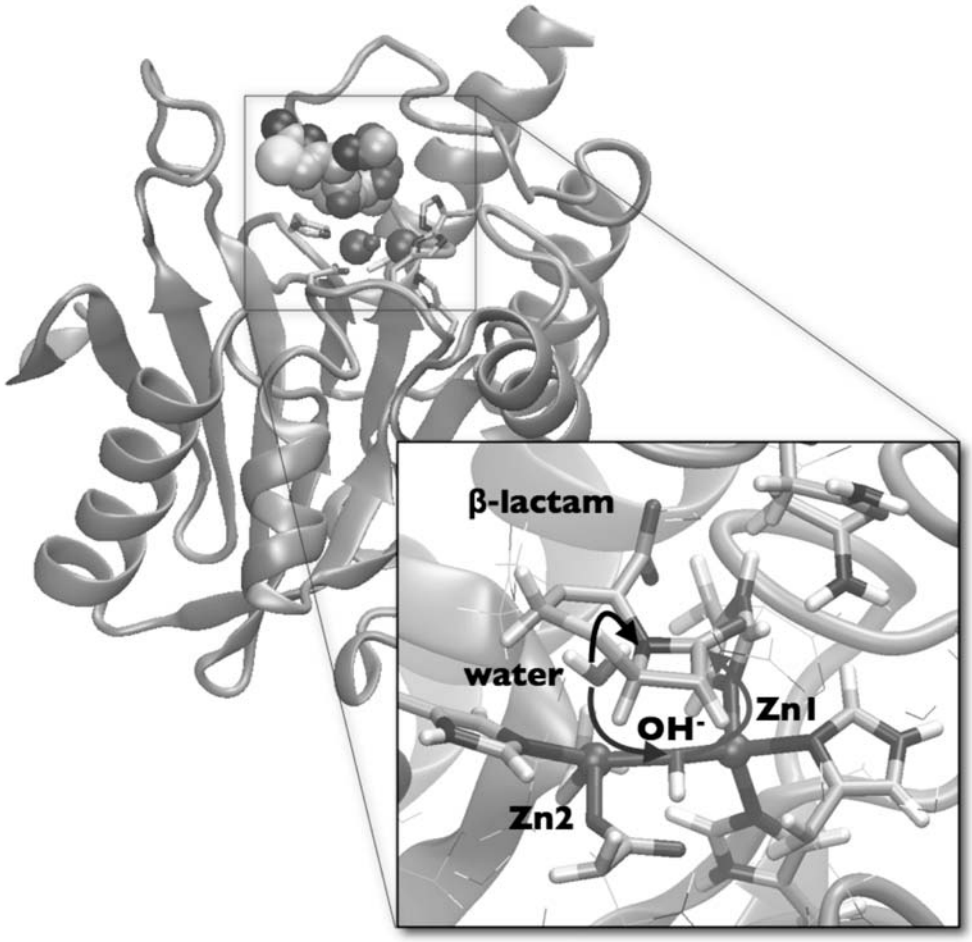

Figure 6. Structure of the dizinc beta-lactamase from *Bacteroides fragilis* (CcrA). The beta-lactam ligand is indicated in space-filled balls. On the right, a close view is shown of the catalytic site, which includes two Zn^{2+} ions.

presence of Zn2 merges leads to a concerted single-step mechanism, explaining the improved catalytic efficiency of di-Zn versus mono-Zn variants, which emerged from the comparison of the calculated free energy of activation for di-Zn (ΔF=18 ± 2 kcal mol-1) and mono-Zn (ΔF=21 ± 3 kcal mol^{-1}) species, respectively.

Overall, the comparison of mono-Zn versus di-Zn explained the great efficiency of the di-Zn one, where a highly concerted single-step mechanism is found (Figures 7). Because the zinc ligands and most active site residues are highly conserved among subclass B1 MBLs, Dal Peraro *et al* proposed that the binding and mechanism found for CcrA could be true for the entire B1 subclass, where beta-lactams might follow similar catalytic pathways. Thus, these common structural elements are flexible enough to accommodate different substrates and to support a broad-spectrum activity in MBLs, based on a similar water-assisted hydrolysis mechanism generally plausible for penicillins, cephalosporins, and carbapenems. Importantly, this might provide a rationale for understanding why mono-bactams such as aztreonam, lacking the common beta-lactam bicyclic core and carboxylate, are not efficiently hydrolyzed by MBLs. Thus, these QM/MM studies of MBL catalysis suggest that Zn-bound water is a common and crucial chemical feature across B1 MBLs. Overall, the carboxylate group present in all beta-lactam antibiotics stabilizes WAT at the active site upon binding, so that the water/beta-lactam entity should be considered as a preferred template for the design of new inhibitors.

Other informative QM/MM studies have been performed on MBLs to investigate their reactivity. Merz *et al* have been very active in this field, and have investigated, by means of semiempirical QM/MM and DFT calculations, the mechanism of inhibition of important MBLs. Recently, they have studied the mechanism of action of nitrocefin bound to the dizinc metallo-lactamase CcrA from *Bacteroides fragilis*(90). These calculations show that the substrate beta-lactam group interacts with active site zinc ions, replacing the apical water molecule upon formation of the Michaelis complex. Interestingly, this study also explains how a thiazolidinecarboxylic acid inhibitor deactivates the dinuclear zinc center through a two-step reaction, suggesting a rationale for the design of new MBL inhibitors. Guo and Cui have also performed QM/MM

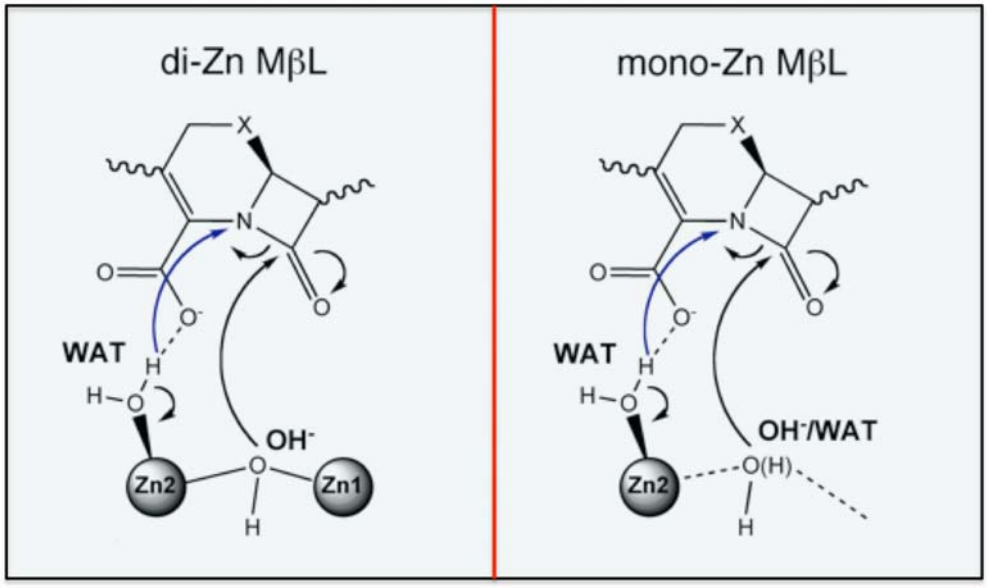

Figure 7. Scheme indicating possible differences in the reaction mechanisms of di-Zn and mono-Zn based MBL hydrolysis.

computations on MBLs (91). In this case, they investigated the initial ring-opening step in the hydrolysis of moxalactam catalyzed by the dizinc L1 beta-lactamase from *Stenotrophomonas maltophilia*, with emphasis on the different role of the Zn ions.

Overall, this review article shows some recent QM/MM computational investigations of important metalloenzymes of pharmaceutical relevance, such as RNase H and MBLs. The literature contains QM/MM studies of other enzymes that could concern the therapeutic area of interest to the reader. It is hoped, however, that these few representative examples have shown the connection between understanding the enzymatic machinery through computations, and the more practical application of these findings in SBDD. Ultimately, SBDD can learn from these QM/MM studies to design potent inhibitors such as TS analogues.

5. CONCLUSIONS

This review has focused on the application of QM-based methods to studying the enzymatic reaction mechanism of therapeutically relevant enzymes. An in-depth understanding of how enzymes work remains one of the most fascinating areas of research, where the scientist is often surprised by the elegant ways in which Nature carries out specific chemical reactions. The challenge is then how to use this knowledge in designing new enzymatic inhibitors. Here, a few representative enzymes belonging to metalloprotein classes have been reviewed. Two enzymes from the metallophosphatases have been discussed. First, the endonuclease activity carried out by the ribonuclease H (RNase H) protein, which represents a promising target for anti-HIV drug design and, secondly, the phosphatase activity in the soluble epoxide hydrolase (sEH), which is a promising target for hypertension and inflammatory conditions. Then, metallo beta-lactamases (MBLs) have been discussed. These enzymes are able to hydrolyze all kinds of beta-lactam antibiotics and represent an important target in developing more potent and resistant antibacterial drugs.

These selected examples have been used to demonstrate how QM-based methods can help in understanding the ligand-receptor interaction in great detail. The resulting information could inspire more practical structure-based drug design (SBDD) efforts, which aim to design potent inhibitors such as transition state (TS) analogues. Although the gap between costly QM-based studies and practical drug design applications has only just started to close, these examples hopefully allow the reader to appreciate the potential offered by a deep understanding of the ligand-receptor interaction, an understanding that is greatly improved by the application of QM-based calculations. In other words, understanding how a machine (the enzyme) works should help us design blockers (drugs) of such machines.

Given the continuous growth of computer power and the improvement of algorithms for computations, QM-based methods are destined to become a powerful tool for SBDD in the near future. Indeed, QM/MM will allow bigger and bigger QM subsystems and/or a better sampling of the chemical space. This will, consequently, lead to a very sophisticated and reliable level of accuracy in studying chemical processes such as enzymatic reactions. In a few years, SBDD will fully integrate QM-based methods as a feasible and effective approach to designing new and potent inhibitors. The accuracy of QM-based calculations will improve our understanding of the ligand-receptor interaction, and this should facilitate the design of better inhibitors. Although a new inhibitor is just the first step toward discovering a new drug, it is undoubtedly crucial. It

is therefore helpful to improve the design of new chemical entities as potent inhibitors and so initiate, in a rational manner, the challenging process of drug discovery.

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Abbreviations: MM: Molecular mechanics, QM/MM: Quantum Mechanics/Molecular Mechanics, TS: Transition State, SBDD: Structure-based drug design, GPU: Graphics processing unit , QSAR: Quantitative structure activity relationship, RNase H: Ribonuclease H , DFT: Density functional theory , HF: Hartree-Fock , MP: Møller-Plesset , CPMD: Car-Parrinello molecular dynamics , MßLs: Metallo ß-lactamases , sEH: soluble epoxide hydrolase

Key Words: Soluble epoxide hydrolase, Ribonuclease H, Metallo beta-lactamases, QM, MM, Quantum Mechanics, Molecular mechanics, SBDD, Structure-Based Drug Design, Modeling, Computational Chemistry, Drug Discovery, Phosphoryl Transfer, Metalloenzymes, QM, Quantum Mechanics, Enzymatic Catalysis, Review

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