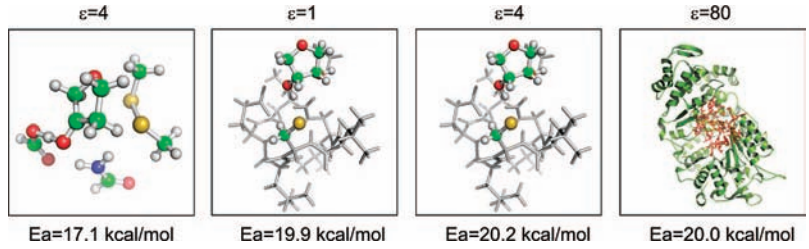
ACCOUNTS
of chemical research

Computational Enzymatic Catalysis

MARIA J. RAMOS* AND PEDRO A. FERNANDES
Requimte, Faculdade de Ciências do Porto, Rua do Campo Alegre 687, 4169-007 Porto, Portugal

RECEIVED ON APRIL 30, 2007

CONSPECTUS



Computational methodologies are playing increasingly important roles in elucidating and presenting the complete and detailed mechanisms of enzymatic reactions because of their capacity to determine and characterize intermediates and transition states from both structural and energetics points of view, independent of their reduced lifetimes and without interfering with the natural reactional flux. These features are turning the field into an active and interesting area of research, involving a diverse range of studies, mostly directed at understanding the ways in which enzymes function under certain circumstances and predicting how they will behave under others.

The accuracy of the computational data obtained for a given mechanistic hypothesis depends essentially on three mutually exclusive factors: the accuracy of the Hamiltonian of the reaction mechanism, consideration of the modulating aspect of the enzyme's structure in the energetics of the active center, and consideration of the enzyme's conformational fluctuations and dynamics. Although, unfortunately, it is impossible at present to optimize these crucial factors simultaneously, the success of any enzymatic mechanistic study depends on the level of equilibrium achieved among them. Different authors adopt different solutions, and this Account summarizes the most favored, with emphasis placed on our own preferences.

Another crucial aspect in computational enzymatic catalysis is the model used in the calculations. Our aim is to build the simplest model that captures the essence of the catalytic power of an enzyme, allowing us to apply the highest possible theoretical level and minimize accidental errors. The choice is, however, far from obvious, ranging from simple models containing tens of atoms up to models of full enzymes plus solvent. Many factors underlie the choice of an appropriate model; here, examples are presented of very different modeling strategies that have been employed to obtain meaningful results.

One particular case study, that of enzyme ribonucleotide reductase (RNR), a radical enzyme that catalyzes the reduction of ribonucleotides into deoxyribonucleotides, is one of the examples illustrating how the successive increase of the system's size does not dramatically change the thermodynamics and kinetics of the reaction. The values obtained and presented speak for themselves in that the only ones that are distinctly different are those calculated using an exceedingly small model, which omitted the amino acids that establish hydrogen bonds with the reactive unit of the substrate.

This Account also describes our computational analysis of the mechanism of farnesyltransferase, a heterodimeric zinc metalloenzyme that is currently one of the most fascinating targets in cancer research. We focus on the present methodologies that we have been using, our models and understanding of the problem, and the accuracy of results and associated problems within this area of research.

Introduction

Enzymes are extremely interesting, complex systems, and their behavior is often tricky and difficult to understand. Experimental methodologies devoted to the study of enzymatic catalysis, in general, typically involve many different areas of expertise such as protein crystallography, NMR, site-directed mutagenesis, kinetics, isotopic label-

Published on the Web 05/09/2008 www.pubs.acs.org/acr
10.1021/ar7001045 CCC: $40.75

ing, and spectroscopy, to name but a few. However, the area of computational enzymatic catalysis involves a range of computational techniques, which in general allow for the measurement of a wider range of observables than experiments with the same level of effort, time, and budget. This fact, in our opinion, turns computational enzymatic catalysis into an amazingly interesting area of research, involving a diversity of studies, mostly directed to understand the way enzymes work in certain circumstances and to predict how they will behave in others.

Many excellent groups have developed research is this field in the past decade (see refs 1−5 and references therein), contributing to great improvement in our understanding on how enzymes work.

Predicting the catalytic or the inhibition mechanism of an enzyme is generally a lengthy and complex task. A complete characterization involves identifying all intermediaries and transition states along the catalytic or the inhibitory cycle, as well as the free energies of activation and reaction for each of the reaction steps. Experimental methodologies typically allow the determination of the initial enzyme/substrate (or substrate analogue) complex, the identification of some albeit not all intermediaries, and the determination of the free energy of activation of the rate-limiting step. The structure and nature of the transition states, as well as their energies, are generally beyond these methodologies. As a result, computational methodologies have taken an increasingly important role, due to their capacity to determine and characterize intermediaries and transition states from a structural and energetics point of view, independently of their reduced lifetime and without interfering with the natural reactional flux.

The simulations of enzymatic systems present some characteristics that make them unique from a computational point of view. They are big systems (typically tens of thousands of atoms) in which only a small region, the active center, participates actively in the reactive cycle. The remaining structure works as a modulator of the active center, either stabilizing or destabilizing the existing stationary states along the potential energy surface (PES). The solvent may or may not play an active role in the catalysis either by participating in the reactive cycle or by modulating indirectly the energetics of the process. The enzymatic structure has a dynamics that encloses several time scales, from side chain rotations, which occur in the nanosecond scale, to conformational rearrangements or domain movements, which typically happen in the microsecond scale or even slower. Solvent dynamics is on the picosecond time scale.

All this diversity in the chemical nature and the dynamics of the systems' participating species causes need for using computational techniques equally diverse. We have chosen two specific, very different examples from the systems that we have been working with lately, in which we have used several different methodologies to be able to achieve our final objectives. They are ribonuclease reductase, a radical enzyme, and farnesyltransferase, a metalloenzyme.

Results and Discussion

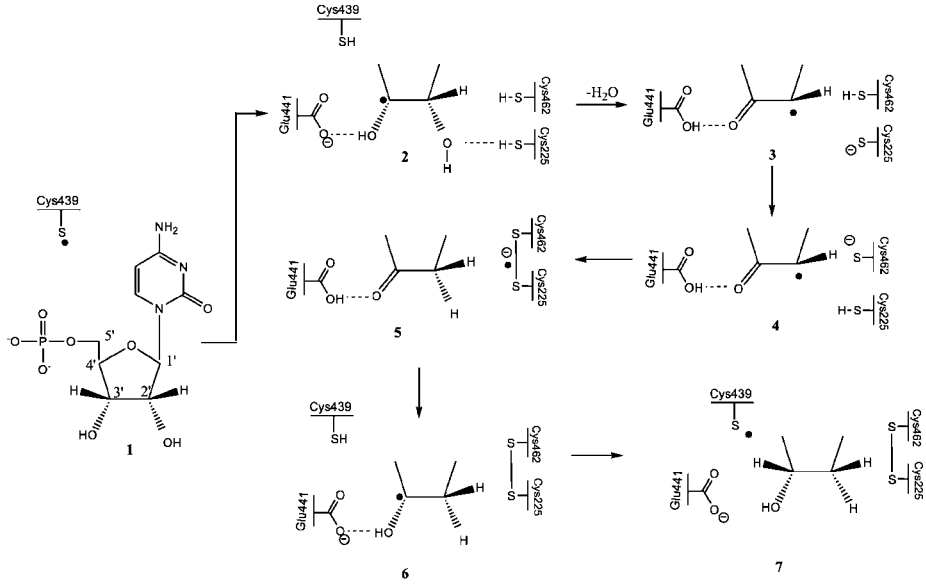
A successful computational scientist should be fully aware of the experimental data available in the literature and related to the system under study. Basically, to put forward the right mechanism for an enzymatic reaction, we have to search for the minimum energy pathway in the PES of our system. However, due to present computing limitations, it is important to make some initial choices on which the mechanistic pathway to determine will surely depend.[6]

The realism of the computational data obtained for a given mechanistic hypothesis depends essentially on three factors of mutually exclusive quality: (i) the system's Hamiltonian accuracy, (ii) the sampling of the reactional space, and (iii) the sampling of the conformational space. The success of a mechanistic study depends on the level of equilibrium achieved among these three factors.

One problem that has given rise to much controversy is the explicit and implicit modeling of the enzyme outside the active center. There are basically two methodologies. One intends to represent atomistically the largest possible part of the enzymatic system resorting to molecular mechanics (MM) to deal with the thousands of atoms involved.[7−10] The other tries to reduce as much as possible the atomistic region and introduces the highest possible accuracy in its Hamiltonian, substituting the rest of the enzyme by a dielectric continuum.[1,5,7] Dielectric values between 2 and 4 are appropriate to describe the protein environment, its contribution being usually small, and the difference between values ranging from 2 to 4 is negligible. If a large contribution from the dielectric medium is found, it means that it cannot be accurately accounted for by the dielectric, and the atomistic model must be enlarged.

The main advantage of the first methodology is the better accuracy obtained in the evaluation of the long-range interactions. The main advantage of the second is to be less prone to incidental errors, which occur due to the difficulty in ensuring that a large system does not travel between local conformational minima (unrelated to the reaction coordinate) during the geometry optimization of the stationary states. Even though these minima are an integral part of the PES land-

SCHEME 1. Mechanism for Reduction of Ribonucleotides by RNR[13]



scape, their contribution is only included correctly if they are properly explored and averaged, which is not the case in single-structure geometry-optimized systems. It is only possible to overcome this problem by resorting to extensive sampling of the conformational space or by traveling backward and forward along the reaction coordinate, both computationally demanding processes. In such cases, however, the disadvantage is transferred to the necessary reduction of the accuracy of the Hamiltonian in relation to the small models.

A promising methodology to progress toward an accurate *ab initio* quantum mechanical/molecular mechanical (QM/MM) free energy surface (FES), in solvent and in enzymes, without sacrificing conformational sampling, has been recently presented.[11] The application of the method still demands a lengthy and complicated set of procedures, and a wider set of examples of its accuracy would be beneficial. However, it opens a path toward mastering the nature of the catalytic power of enzymes with *ab initio* accuracy and without investing unreasonable amounts of computer time.

As far as the reactional space is concerned, our experience is that to explore it, an atomistic model of large dimensions is not always needed, since a great deal of the energy involved in reactional transitions involves the breaking and formation of bonds and first sphere interactions, and the long-range interactions are usually less important. However, exceptions are not uncommon, in particular with reactions involving charge transfer, which means that such small models should be employed with caution and after a proper evaluation of the specific system under consideration.

It is absolutely essential that the model includes the substrate or inhibitor and all the residues that we know or suspect to interact with the ligand and to be essential for catalysis or inhibition. The size of models has to vary from system to system. There are many for which the model used can be small, amounting to only tenths of atoms.[12] These cases include metallic centers, radical enzymes, enzymes lacking specific long-range enzyme−ligand interactions, or enzymes with ligands that are very small or have much localized electronic density. It is rewarding when we find out that increasing the model brings no new information to the mechanistic pathway or the energies associated with it.

Ribonucleotide Reductase, a Radical Enzyme. An interesting example is that of the enzyme ribonucleotide reductase (RNR), a radical enzyme that catalyzes the reduction of ribonucleotides into deoxyribonucleotides.

In Scheme 1, it is possible to follow the steps that constitute the currently accepted mechanism of RNR both experimentally[13] and computationally.[14,15]

Figure 1 shows different strategies for modeling the monomer R1 of RNR, as well as the activation and reaction free energies for the first step of the catalytic mechanism of RNR, obtained with different sized models.[7]

The values speak for themselves; the only ones that are predictably different are those calculated with an exceedingly small model in which amino acids that establish hydrogen bonds with the reactive part of the substrate, as well as geometric constraints due to the connections to the backbone were omitted, thus confirming the necessity of including an

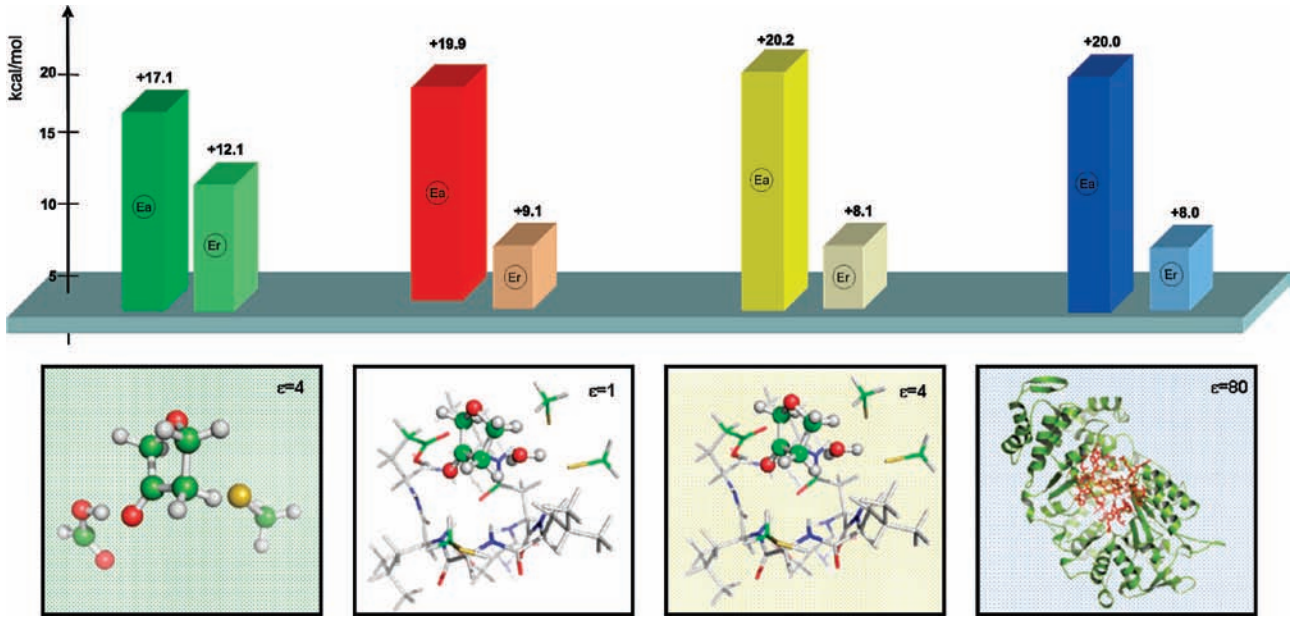


FIGURE 1. Activation (E_a) and reaction energies (E_r) for the first step of the catalytic mechanism of RNR (top) calculated with several enzyme models (bottom). In green is represented a small model in the protein environment ($\epsilon = 4$), in red a bigger model with 140 atoms, in yellow the same model of 140 atoms but in the protein environment ($\epsilon = 4$), and in blue the full R1 monomer in water (implicit solvent, $\epsilon = 80$) in which we have used ONIOM[16] for the energy calculations.[7]

acceptable first shell of amino acids in the minimum model. All the others seem to converge to the same value of 20 kcal/mol for the activation energy, regardless of the size of model. In the case of RNR and similar ones, the role played by the enzyme seems to be that of preorganizing the geometry of the active center, imposing stereospecificity in the reaction, and changing the mechanism to a fast, radical one, not viable in water.[15] These effects obviously constitute no problem for a computer to mimic. Less often, we encounter enzymatic systems for which the active center is very open to the solvent or involving very flexible or charged species, establishing specific long-range enzyme−ligand interactions, or involving conformational changes or even ligands with highly delocalized electronic density. In these cases, it is generally more difficult to handle small models because the results produced can prove unrealistic. We have come across one of those systems: the related enzymes thioredoxin and DsbA, which present very different pK_a's for a homologous active site cysteine. In order to clarify the long-waited, elusive molecular-level origin of this difference, it has been necessary to use the entire enzymes. With such models, a ΔpK_a of 4.5 was obtained (the experimental value range is 4.0−4.5).[17] The largest contribution came from an α helix adjacent to the active site, whose microdipoles stabilized the thiolates of both enzymes to a different extent.

We have conducted a series of mechanistic studies to elucidate the catalytic and inhibition pathways of RNR. Even

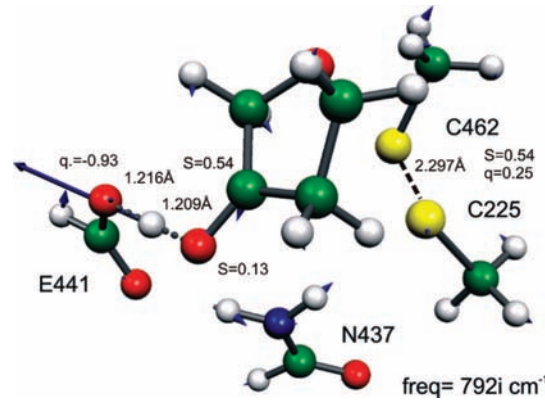
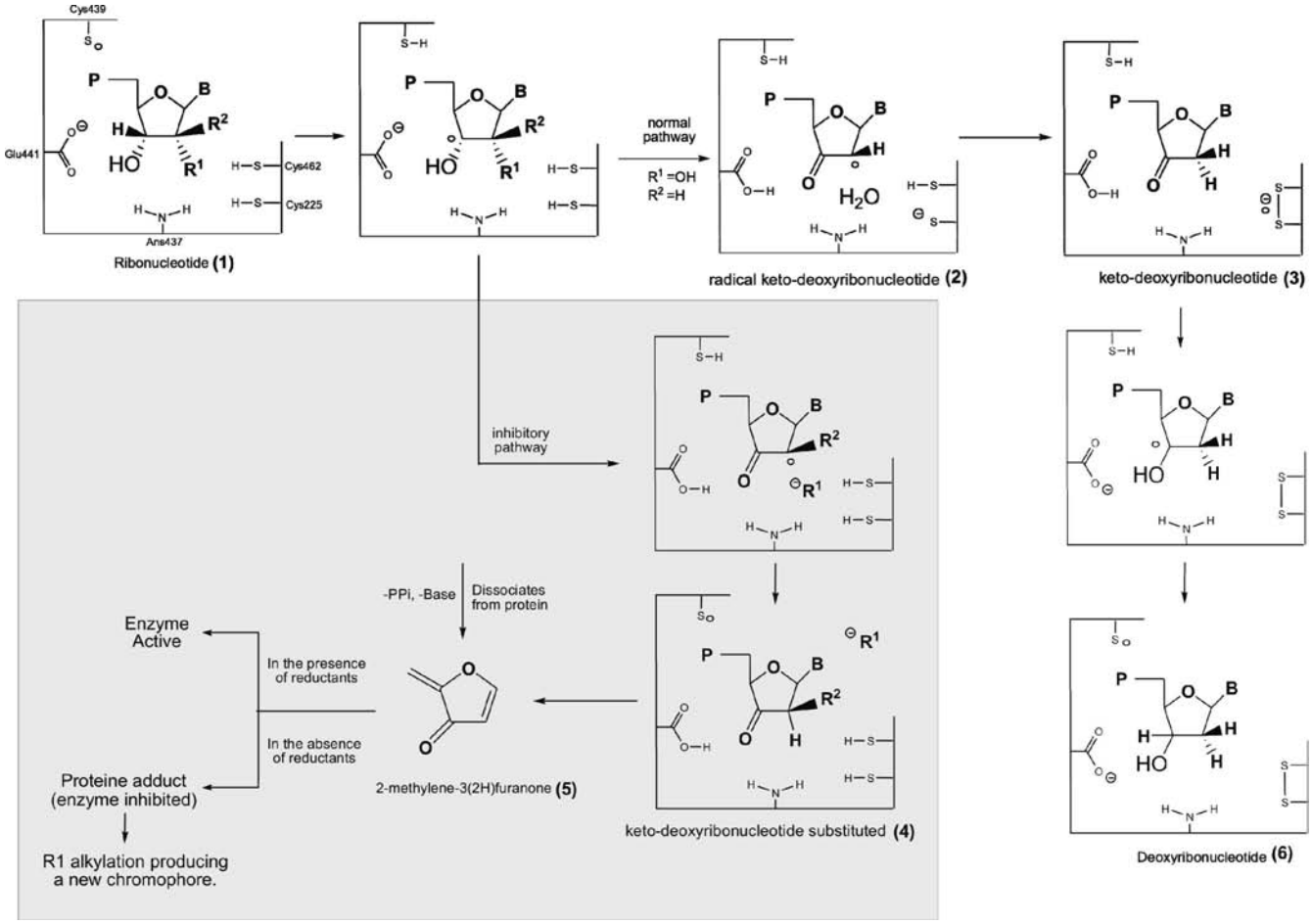


FIGURE 2. Transition state for the rate-limiting step of RNR. Atomic charges (q) and spin densities (S) are depicted.

though the whole substrate mechanism has been studied in detail, and all its stationary points identified, maybe the most important result has been the clarification of the rate-limiting disulfide formation pathway. The radical anionic disulfide was shown to generate the neutral disulfide in a single step, through a very complex transition state, illustrated in Figure 2, involving a coupled proton and electron transfer, with concomitant reduction of the disulfide. The radical transfer was not mediated through Asn437 or Glu441.

Another point that has been clarified concerns the 2′-ketoribonucleotide paradox. This species is a very stable intermediate in both the substrate and the inhibition pathways of most 2′-substituted suicide inhibitors. However, in the latter but not in the first, it escapes from the active site and gener-

SCHEME 2. Schematic Representation of the Normal Reduction Pathway and an Inhibitory Mechanism of RNR with Their Common Keto-deoxyribonucleotide Intermediate



ates a species in solution that alkylates the enzyme, promoting RNR inactivation (see Scheme 2).

The reason for such different behavior has been found to lie in the different pattern of hydrogen bonds generated by the anionic substituent of the inhibitors, together with its more favorable solvation free energy when compared with the neutral water molecule eliminated from the substrate.[18]

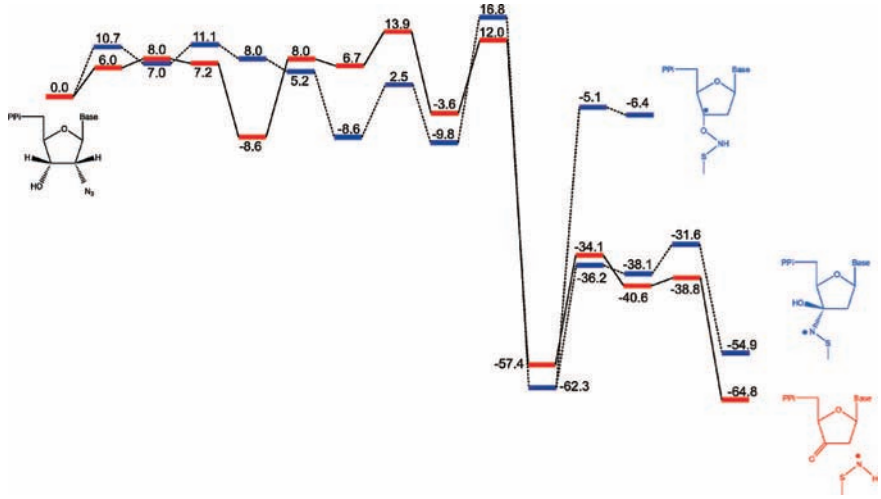
RNR inhibition is clinically very important, due to its well-known anticancer effects.[19] A large set of suicide inhibitors have been studied, namely, 2′-azido-2′-deoxyribonucleoside-5′-diphosphate (N_3NDP),[20,21] 2′-mercapto-2′-deoxyribonucleoside-5′-diphosphate (SHNDP),[22] (*E*)-2′-fluoromethylene-2′-deoxynucleotide-5′-diphosphate (FMNDP),[23] 2′-chloro-2′-deoxyribonucleoside-5′-diphosphate (ClNDP),[21] and 2′,2′-difluoro-2′-deoxyribonucleoside-5′-diphosphate (F_2NDP),[24] their mechanisms being too extensive to review in this Account. Just two of the more interesting cases will be summarized.

The whole pathway of N_3NDP has been mapped on the PES with the help of a large amount of experimental data, and the structure of a well-known radical intermediate, detected experimentally, has been predicted. The predictions turned out to be confirmed by a posterior experimental study, which successfully trapped and characterized the radical intermediate, and the rest of the extremely bizarre mechanism was in remarkable agreement with the theoretical PES illustrated in Scheme 3.[25]

Multiple substrate binding modes is a common problem in enzymes, which is usually due to the lack of specificity of the enzyme for a given substrate. This situation seems to occur when an enzyme binds many different substrates. However, RNR is highly specific for ribonucleotides, and thus it should be expected that substrate binding occurs mostly in a single, well-defined conformation. Just to find out how true this might be, we have conducted a search[15] of the conformational space to look for alternative binding modes besides that of the crystallographic structure. In this particular case, we have used a currently available molecular docking approach.[26]

Subsequently, to make sure that our ligand is in the correct position, we have developed a Multistaged Docking with

SCHEME 3. Pathways for the Suicide Inhibitor N3NDP[a]



[a] The solid line represents the theoretical proposal confirmed later experimentally. The dashed line represents the previous experimental proposals.

an Automated Molecular Modeling protocol, MADAMM,[27] which enables both flexibility of the ligand and flexibility of the receptor to confirm that our initial structure really corresponds to the correct position of the ligand. The results point out that if we do not introduce a certain degree of flexibility in several residues near the active site region, standard docking software will not give reliable results. This is not related to the score function or to the search algorithm but is simply because some residues of the active site occupy some space that is required for the correct binding of the ligand. MADAMM has shown to be very useful to speed up the process, and in the end it provides a collection of structures in which the interaction between the receptor and the ligand is optimized. We observed at this stage that the small changes induced in the protein during the procedure introducing flexibility (such as changes in the side chain rotamers) can lead to backbone movements that are shown to be important for the correct binding of the ligand.

Farnesyltransferase, a Metalloenzyme. One other system that proved challenging and therefore very interesting is that of farnesyltransferase (FTase), shown in Figure 3. Protein FTase is a heterodimeric zinc metalloenzyme,[28] currently one of the most fascinating targets in cancer research. However, despite the thrilling achievements in the development of farnesyltransferase inhibitors (FTIs) over the past few years, the farnesylation mechanism remains, to some degree, a mystery.[29] Detailed knowledge of it would enable the development of more specific inhibitors, rationally designed, with great potential in the treatment of cancer, malaria, and sleeping sickness and even in antiviral activity.

FTase catalyzes the addition of isoprenoid farnesyl from farnesyl diphosphate (FPP) to a cysteine residue of a protein substrate containing a typical −CAAX motif at the carboxyl terminus. In this characteristic motif, C stands for the cysteine residue that is farnesylated, A is an aliphatic amino acid whereas X represents the terminal amino acid residue, usually methionine, serine, alanine, or glutamine.

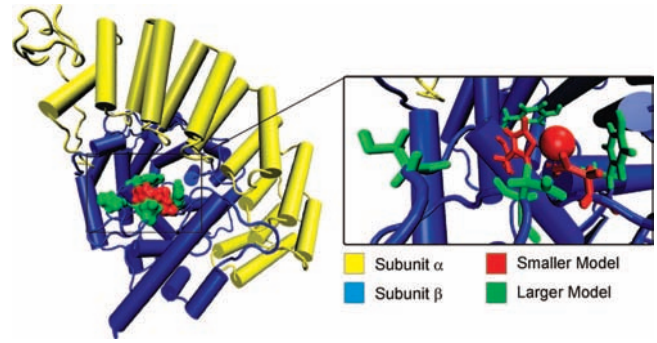


FIGURE 3. Farnesyltransferase and representation of two models used in the elucidation of some of the many doubts remaining in its unsolved catalytic mechanism.

We have been able to solve many of the mechanistic dilemmas,[30] yet the mechanism still remains elusive. However, we draw many conclusions and elucidate the following aspects:

1. The identity of the fourth ligand of the catalytic zinc ion. Contradictory experimental data pointed to a tightly bound water molecule or an almost symmetrical bidentate aspartate. Quantum calculations have shown that both structures, depicted in Figure 4, lie notably close in energy and are connected in the PES through a typical carboxylate-shift transition state[31,32] and thus coexist at room temperature, which explains the ambiguous X-ray distances obtained for this complex.[12]
2. The protonation state of the peptide nucleophilic cysteine. It was known that peptide binding results in the establish-

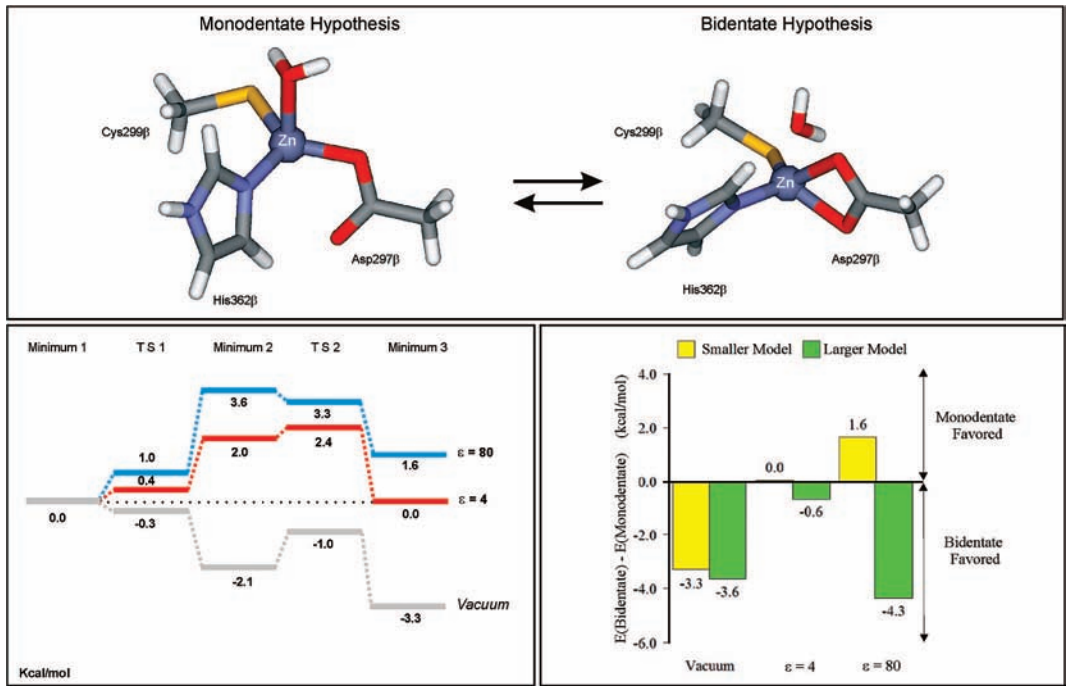


FIGURE 4. Mono- and bidentate equilibrium (top), the PES for the reaction calculated in several different media (bottom left), and the free energy difference between the mono- and bidentate structures (bottom right).

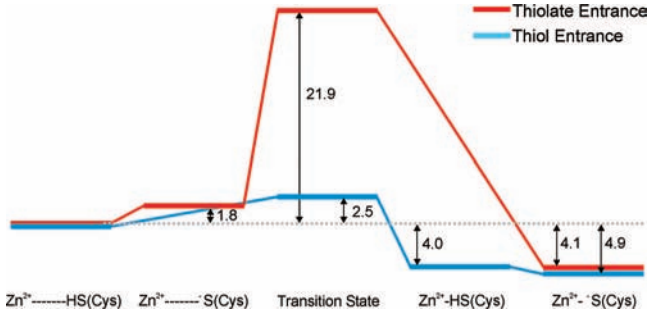


FIGURE 5. The PES for both reactions, i.e. thiolate entrance (red) and thiol entrance (blue).

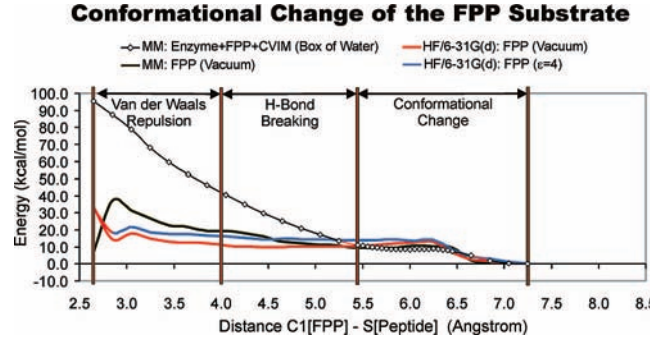


FIGURE 6. PES for FPP rearrangement calculated at several theoretical levels. The energy of the FPP substrate alone in the geometries that it adopts through the conformational change is also depicted.

ment of a zinc−cysteine thiolate bond. However, the protonation state of the nucleophile (thiol/thiolate) was not possible to determine. We have followed the mechanistic path for both hypotheses, and the results shown in Figure 5 left no doubt that a protonated thiol was the responsible for the attack, with a free energy barrier of 2.5 kcal/mol in contrast with the 21.9 kcal/mol barrier for thiolate entrance and binding.[33]

3. The distances paradox, which consists in the existence of a very large distance (more than 8 Å) between the reacting atoms (zinc-bound cysteine sulfur and C1 of farnesyldiphosphate) in all crystallographic structures. The two proposed alternatives, sulfur displacement from the catalytic zinc ion with subsequent attack to C1 or FPP rearrangement, moving C1 toward the nucleophile, were mapped onto the PES. Sulfur displacement presented a very high barrier (up to 22.6 kcal/mol in a DFT model including 119 atoms), higher than the rate-limiting step. FPP rearrangement, through the movement of subunits 1 and 2 of the isoprenyl moiety, led to a plateau in the PES only 10 kcal/mol above the reactants valley, as calculated with a full enzyme model plus solvent with the Amber force field (see Figure 6). Such energy was only due to the conformational change of the substrate. Such conformational energy was recalculated at the Hartree−Fock and DFT levels, with excellent agreement with the MM energies. The conformational rearrangement toward the zinc-bound thiolate places the two reactive atoms in an adequate position for catalysis.[34]
4. The product coordination ambiguity. X-ray crystallographic structures of medium resolution and UV−vis data in a

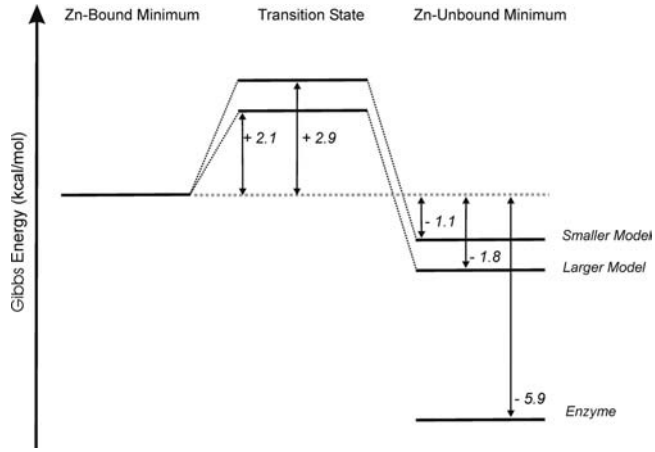


FIGURE 7. The PES for the coordination reaction of the thioether product to the active site zinc ion, using several models, consistently supports the picture of a fast bound/unbound equilibrium, favoring the bound state.

Co^{2+}-substituted enzyme suggested the existence of a weak zinc−thioether product coordination, even though extended X-ray absorption fine structure (EXAFS) studies have failed to detect any interaction at all. On the basis of models of several sizes, ranging from small QM to full-enzyme QM/MM models, we have shown the existence of a zinc-coordinated thioether product (Figure 7).[35] An explanation for the contradictory experimental results was also provided.

We have yet to make clear the nature of the chemical step, that is, nucleophilic vs electrophilic hypotheses, and elucidate the location and the role of a magnesium in the catalysis.

The interesting thing about FTase is that to get to all these conclusions for its catalytic mechanism, we had to perform a significant amount of calculations using a plethora of methodologies.

Here, as in RNR, we have used different size models (Figure 2), a smaller model of only 25 atoms at the B3LYP/6-311+G(2d,2p)//B3LYP/SDD level (IEF-PCM, $\epsilon = 1$, 4, and 80) and a larger one with 127 atoms in which we had to use ONIOM[16] with B3LYP/SDD:PM3 for the optimization part and B3LYP/6-311+G(2d,2p):B3LYP/6-31+G(d) (IEF-PCM, $\epsilon = 1$, 4, and 80) for the energy calculations. The preparation of the larger model was carefully planned to capture both the intrinsic characteristics of the zinc complex and the influence of the anisotropic enzyme environment on the zinc coordination sphere.

Some Thoughts on the Methodology That We Have Been Favoring. In a subject where there are no real rules, the ruling principle seems to be that of mastering all the information available in the literature that concerns our system and managing to put it together as in a puzzle to produce a good working mechanistic hypothesis. A creative mind together with adequate computational resources and a talent for "traveling" through a PES can do the trick!

For small models, typically with less than 150 atoms,[7,12,36−38] our preferred choice of methodology has been density functional theory (DFT).[39] We have used it in several catalytic and inhibition mechanisms of various enzymes, having obtained very good values in agreement with experiment.

If DFT has been our choice for geometry optimizations and single-point energy calculations albeit with a larger basis set, presently we have abandoned this as far as the latter cases are concerned, to favor quadratic configuration interaction[40] with single and double excitations and the triple excitation correction added (QCISD(T))[41] for small active site regions (an evaluation of the general performance and limitations of density functionals, compiling hundreds of benchmarks, can be found in ref 42). With technological informatics advances, the choice of QCISD(T) is now feasible, and energy values do improve with this methodology. A recent work has shown that very high theoretical levels (in this case coupled-cluster) might be needed for the high-accuracy calculation of enzymatic barrier heights.

When our system models become bigger or we want to use the whole enzyme, then hybrid quantum mechanics/quantum mechanics (QM/QM) or quantum mechanics/molecular mechanics (QM/MM) are a must. In them, the QM part must describe the part of the system in which the reaction occurs, typically the active site, along with the ligand, that is, the substrate or inhibitor. In fact, in the recent past, we have adopted a QM/QM description of our system by using a DFT//PM3MM hybrid methodology[7,12,15] within the ONIOM scheme.[16] However, nowadays we are favoring the more rewarding DFT//MM methodologies with which we can actually present results that involve the whole enzyme.[17,43] In such systems, it is necessary to freeze atoms beyond a given radius, since geometry optimizations tend to move peripheral regions of the enzyme (unrelated with the reaction coordinate) to different local minima, resulting in spurious contributions for the activation and reaction energies. It must be said that QM/MM methodologies have been introduced long ago,[44] and from then on a plethora of techniques, beyond the one preferred by the authors, have been developed. A more comprehensive approach to these methods can be found in refs 8−10.

The use of pseudopotentials in geometry optimization of metal-containing systems has given excellent results. For zinc, we have found that the SDD potential[45] is in fact very good.[46] Si is another element for which we have used effective core

potentials (ecps), in particular SHC*,[47] and have obtained very accurate results at a fraction of the time of full-electron basis sets.[48]

In our calculations as far as the solvent is concerned, we have adopted a simple strategy that produces good results in the majority of cases. When we calculate the final single point energies we use a polarized continuum model, lately IEF-PCM.[49] However, this strategy will not work in the cases for which optimization with the solvent is a must, in order to account for a very charged system or environment.[50]

Enzymatic systems have a rather unsmooth PES, full of local minima surrounded by high energy barriers. Such is due to the extremely elevated number of degrees of freedom of the system and its own morphology. A protein consists of a semirigid skeleton in which side chains are anchored with a highly correlated orientation, due to the system's high density. Therefore, the variation of a rotamer of a given side chain is only typically possible if reorientation of the neighboring rotamers takes place. Its size and the translational restraint caused by the bond to the backbone result in a dynamics at the nanosecond scale. The reorganization of the protein structure at the level of the backbone itself occurs in time scales that are several times higher. It is thus easy to understand that it is presently not possible to get significant and ergodic sampling of the conformational space, and the results of such an exploration are inevitably conditioned by the starting structure. Therefore, the only possible kind of sampling will be that of a subregion of the conformational space, around the relevant region for the problem under study.

Getting a set of conformations in that subregion has associated however a high computational cost, and as a result, generally we have to settle for a less accurate Hamiltonian.

Up until now, we have opted to favor the Hamiltonian in detriment of the sampling. This option is far from being universally valid and its application essentially depends on the system under study. The evaluation of a reaction mechanism based on optimizations of a single geometry or of a small set of geometries is generally possible, if there is rigor and common sense in the procedure. Possible pitfalls of this methodology have recently been advertised by Warshel and co-workers.[51]

Conclusion

In conclusion, computational enzymatic catalysis is still in its infancy even though particularly rewarding from the point of view that we can actually determine and present a complete and detailed mechanism, which no other single experiment can produce. Additionally, detailed atomic level understanding of enzymatic mechanisms is possible through computationally established mechanisms.

Computational enzymatic catalysis is our favorite field of research, and rather than briefly introducing several of those mechanisms in which we have been involved lately, for example, PFL,[52,53] COX2,[36] uroporphyrinogen III decarboxylase,[37] fumarate reductase,[38] thiol/disulfide exchange,[50] thioredoxin, and DsbA,[17,43] we chose to present in greater detail two cases that we have studied at length, ribonucleotide reductase[7,13,15,18–24] and farnesyltransferase.[12,30,32–35,46] We have chosen them not only because they are very different systems but also for the simple reason that we have had to apply a large spectrum of different methodologies to unravel their respective catalytic and inhibition mechanisms.

Methodology plays a major role in computational enzymatic catalysis, and therefore we presented that which we have favored in the past. With an adequate model, the success of the mechanistic prediction will depend on the domain and the inter-relation of the experimental evidence as well as on the imagination and common sense with which we search the vast reactional space.

Both M.J.R. and P.A.F. would like to acknowledge the Fundação para a Ciência e Tecnologia (FCT, Project POCI/QUI/61563/2004), Portugal, for financial support.

BIOGRAPHICAL INFORMATION

Maria João Ramos obtained her first degree in Chemistry at the University of Porto, Portugal, and her Ph.D. in muon research at The University of Glasgow, U.K., and performed postdoctoral research in molecular modeling at the University of Oxford, U.K. Back in Portugal since 1991, she is now a Professor at the University of Porto, the leader of a group working in computational biochemistry with three main lines of research: computational enzymatic catalysis, protein structure and dynamics, and drug design.

Pedro Alexandrino Fernandes obtained his degree in Chemistry at the University of Porto, Portugal. Afterward he has obtained his Ph.D. (1999) in Molecular Dynamics Simulations of Liquid Interfaces and Ion Transfer, under the supervision of Prof. José Ferreira Gomes, at the same University. He joined the University of Porto as an Assistant Professor in 1999 and the research group of Prof. Maria João Ramos in 2000. Since then, he has been dedicated to the field of computational biochemistry, in the areas of protein structure and dynamics, enzymatic catalysis and inhibition, and drug design.

REFERENCES

1 Siegbahn, P. E. M.; Borowski, T. Modeling Enzymatic Reactions Involving Transition Metals. *Acc. Chem. Res.* **2006**, *39*, 729–738.

2 Claeyssens, F.; Harvey, J. N.; Manby, F. R.; Mata, R. A.; Mulholland, A. J.; Ranaghan, K. E.; Schutz, M.; Thiel, S.; Thiel, W.; Werner, H. J. High-Accuracy Computation of Reaction Barriers in Enzymes. *Angew. Chem., Int. Ed.* **2006**, *45*, 6856–6859.

3 Leopoldini, M.; Marino, T.; Michelini, M.; del, C.; Rivalta, I.; Russo, N.; Sicília, E.; Toscano, M. The Role of Quantum Chemistry in the Elucidation of the Elementary Mechanisms of Catalytic Processes: From Atoms, to Surfaces, to Enzymes. *Theor. Chem. Acc.* **2007**, *117*, 765–779.

4 Warshel, A.; Sharma, P. K.; Kato, M.; Xiang, Y.; Lui, H.; Olsson, M. H. M. Electrostatic Basis for Enzyme Catalysis. *Chem. Rev.* **2006**, *106*, 3210–3235.

5 Himo, F. Quantum Chemical Modeling of Enzyme Active Sites and Reaction Mechanisms. *Theor. Chem. Acc.* **2006**, *116*, 232–240.

6 Ramos, M. J.; Fernandes, P. A. Atomic-Level Rational Drug Design. *Curr. Comput.-Aided Drug Des.* **2006**, *2*, 57–81.

7 Cerqueira, N. M. F. S. A.; Fernandes, P. A.; Eriksson, L. A.; Ramos, M. J. Dehydration of Ribonucleotides Catalysed by Ribonucleotide Reductase: The Role of the Enzyme. *Biophys. J.* **2006**, *90*, 2109–2119.

8 Friesner, R. A.; Guallar, V. Ab Initio Quantum Chemical and Mixed Quantum Mechanics and Molecular Mechanics (QM/MM) Methods for Studying Enzyme Catalysis. *Annu. Rev. Phys. Chem.* **2005**, *56*, 389–427.

9 Gao, J.; Truhlar, D. G. Quantum Mechanical Methods for Enzyme Kinetics. *Annu. Rev. Phys. Chem.* **2002**, *53*, 467–505.

10 Warshel, A. Computer Simulations of Enzyme Catalysis: Methods, Progress, and Insights. *Annu. Rev. Biophys. Biomol. Struct.* **2003**, *32*, 425–443.

11 Rosta, E.; Klahn, M.; Warshel, A. Towards Accurate ab Initio QM/MM Calculation of Free-Energy Profiles of Enzymatic Reaction. *J. Phys. Chem. B* **2006**, *110*, 2934–2941.

12 Sousa, S. F.; Fernandes, P. A.; Ramos, M. J. Protein Farnesyltransferase - New Insights into the Zinc-Coordination Sphere Paradigm: Evidence for a Carboxylate-Shift Mechanism. *Biophys. J.* **2005**, *88*, 483–494.

13 Stubbe, J.; Van Der Donk, W. A. Protein Radicals in Enzyme Catalysis. *Chem. Rev.* **1998**, *98*, 705–762.

14 Pelmenschikov, V.; Cho, K.-B; Siegbahn, P. E. M. Class I Ribonucleotide Reductase Revisited: The Effect of Removing a Proton on Glu441. *J. Comput. Chem.* **2004**, *25*, 311–321.

15 Cerqueira, N. M. F. S. A.; Fernandes, P. A.; Eriksson, L. A.; Ramos, M. J. Ribonucleotide Activation by Enzyme Ribonucleotide Reductase: Understanding the Role of the Enzyme. *J. Comput. Chem.* **2004**, *25*, 2031–2037.

16 Dapprich, S.; Komáromi, I.; Byun, K. S.; Morokuma, K.; Frisch, M. J. A new ONIOM implementation in Gaussian98. Part I. The Calculation of Energies, Gradients, Vibrational Frequencies and Electric Field Derivatives. *THEOCHEM* **1999**, *461*, 1–21.

17 Carvalho, A. T. P.; Fernandes, P. A.; Ramos, M. J. Determination of the ΔpKa between the Active Site Cysteines of Thioredoxin and DsbA. *J. Comput. Chem.* **2006**, *27*, 966–975.

18 Cerqueira, N. M. F. S. A.; Fernandes, P. A.; Ramos, M. J. Enzyme Ribonucleotide Reductase: Unraveling an Enigmatic Paradigm of Enzyme Inhibition by Furanone Derivatives. *J. Phys. Chem. B* **2006**, *110*, 21272–21281.

19 Cerqueira, N. M. F. S. A.; Pereira, S.; Fernandes, P. A.; Ramos, M. J. Overview of Ribonucleotide Reductase Inhibitors: An Appealing Target in Anti-Tumour Therapy. *Curr. Med. Chem.* **2005**, *12*, 1283–1294.

20 Pereira, S.; Fernandes, P. A.; Ramos, M. J. Theoretical Study of Ribonucleotide Reductase Mechanism-Based Inhibition by 2′-Azido-2′-deoxyribonucleoside 5′-diphosphates. *J. Comput. Chem.* **2004**, *25*, 227–237.

21 Fernandes, P. A.; Ramos, M. J. Theoretical Studies on the Mode of Inhibition of Ribonucleotide Reductase by 2 ′-Substituted Substrate Analogues. *Chemistry* **2003**, *9*, 5916–5925.

22 Pereira, S.; Fernandes, P. A.; Ramos, M. J. Theoretical Study on the Inhibition of Ribonucleotide Reductase by 2′-Mercapto-2′-deoxyribonucleoside-5′-diphosphates. *J. Am. Chem. Soc.* **2005**, *127*, 5174–5179.

23 Fernandes, P. A.; Ramos, M. J. Theoretical Studies on the Mechanism of Inhibition of Ribonucleotide Reductase by (*E*)-2′-Fluoromethylene-2′-deoxycitidine-5′-diphosphate. *J. Am. Chem. Soc.* **2003**, *125*, 6311–6322.

24 Cerqueira, N. M. F. S. A.; Fernandes, P. A.; Ramos, M. J. Understanding Ribonucleotide Reductase Inactivation by Gemcitabine. *Chemistry*, DOI: 10.1002/chem.200700260.

25 Fritscher, J.; Artin, E.; Wnuk, S.; Bar, G.; Robblee, J. H.; Kacprzak, S.; Kaupp, M.; Griffin, R. G.; Bennati, M.; Stubbe, J. Structure of the Nitrogen-Centered Radical Formed during Inactivation of *E. coli* Ribonucleotide Reductase by 2′-Azido-2′-deoxyuridine-5′-diphosphate: Trapping of the 3′-Ketonucleotide. *J. Am. Chem. Soc.* **2005**, *127*, 7729–7738.

26 Sousa, S. F.; Fernandes, P. A.; Ramos, M. J. Protein-Ligand Docking: Current Status and Future Challenges. *Proteins* **2006**, *65*, 15–26.

27 http://www.fc.up.pt/pessoas/nscerque/MADAMM.html, 2007.

28 Reiss, Y.; Goldstein, J. L.; Seabra, M. C.; Casey, P. J.; Brown, M. S. Inhibition of Purified p21 ras Farnesyl:Protein Transferase by Cys-AAX Tetrapeptides. *Cell* **1990**, *62*, 81–88.

29 Long, S. B.; Casey, P. J.; Beese, L. S. Reaction Path of Protein Farnesyltransferase at Atomic Resolution. *Nature* **2002**, *419*, 645–650.

30 Sousa, S. F.; Fernandes, P. A.; Ramos, M. J. Unraveling the Mechanism of the Farnesyltransferase Enzyme. *J. Biol. Inorg. Chem.* **2005**, *10*, 3–10.

31 Rardin, R. L.; Tolman, W. B.; Lippard, S. J. Monodentate Carboxylate Complexes and the Carboxylate Shift: Implications for Polymetalloprotein Structure and Function. *New J. Chem.* **1991**, *15*, 417–430.

32 Sousa, S. F.; Fernandes, P. A.; Ramos, M. J. The Carboxylate Shift in Zinc Enzymes: A Computational Study. *J. Am. Chem. Soc.* **2007**, *129*, 1378–1385.

33 Sousa, S. F.; Fernandes, P. A.; Ramos, M. J. Farnesyltransferase: Theoretical Studies on Peptide Substrate Entrance Thiol or Thiolate Coordination. *THEOCHEM* **2005**, *729*, 125–129.

34 Sousa, S. F.; Fernandes, P. A.; Ramos, M. J. Theoretical Studies on Farnesyltransferase: The Distances Paradox Explained. *Proteins* **2007**, *66*, 205–218.

35 Sousa, S. F.; Fernandes, P. A.; Ramos, M. J. Theoretical Studies on Farnesyltransferase: Evidence for Thioether Product Coordination to the Active-Site Zinc Sphere. *J. Comput. Chem.* **2007**, *28*, 1160–1168.

36 Silva, P. J.; Fernandes, P. A.; Ramos, M. J. A Theoretical Study of Radical-Only and Combined Radical/Carbocationic Mechanisms of Arachidonic Acid Cyclooxygenation by Prostaglandin H Synthase. *Theor. Chem. Acc.* **2003**, *110*, 345–351.

37 Silva, P. J.; Ramos, M. J. Density-Functional Study of Mechanisms for the Cofactor-Free Decarboxylation Performed by Uroporphyrinogen III Decarboxylase. *J. Phys. Chem. B* **2005**, *109*, 18195–18200.

38 Lucas, M. F.; Ramos, M. J. Mechanism of a Soluble Fumarate Reductase from *Shewanella frigidimarina*: A Theoretical Study. *J. Phys. Chem. B* **2006**, *110*, 10550–10556.

39 Hohenberg, P.; Kohn, W. Inhomogeneous Electron Gas. *Phys. Rev.* **1964**, *136*, B864−B871.

40 Pople, J. A.; Head-Gordon, M.; Raghavachari, K. Quadratic Configuration-Interaction - A General Technique for Determining Electron Correlation Energies. *J. Chem. Phys.* **1987**, *87*, 5968–5975.

41 Gauss, J.; Cremer, C. Analytical Evaluation of Energy Gradients in Quadratic Configuration-Interaction Theory. *Chem. Phys. Lett.* **1988**, *150*, 280–286.

42 Sousa, S. F.; Fernandes, P. A.; Ramos, M. J. General Performance of Density Functionals. *J. Phys. Chem. A* **2007**, *111*, 10439–10452.

43 Carvalho, A. T. P.; Fernandes, P. A.; Ramos, M. J. Theoretical Study of the Unusual Protonation Properties of the Active Site Cysteines in Thioredoxin. *J. Phys. Chem. B* **2006**, *110*, 5758–5761.

44 Warshel, A.; Levit, M. Theoretical Studies of Enzymatic Reactions: Dielectric Electrostatic and Steric Stabilization of the Carbonium Ion in the Reaction of Lysozyme. *J. Mol. Biol.* **1976**, *103*, 227–249.

45 Dolg, M.; Wedig, U.; Stoll, H.; Preuss, H. Energy-Adjusted ab Initio Pseudopotentials for the First Row Transition Elements. *J. Chem. Phys.* **1987**, *86*, 866–872.

46 Sousa, S. F.; Fernandes, P. A.; Ramos, M. J. Comparative Assessment of Theoretical Methods for the Determination of Geometrical Properties in Biological Zinc Complexes. *J. Phys. Chem. B* **2007**, *111*, 9146–9152.

47 Rappe′, A. K.; Smedley, T. A.; Goddard, W. A., III. The Shape and Hamiltonian Consistent (SHC) Effective Potentials. *J. Phys. Chem.* **1981**, *85*, 1662–1666.

48 Santos, H. R. R.; Ujaque, G.; Ramos, M. J.; Gomes, J. A. N. F. Effect of the tert-Butiloxycarbonyl Protecting Group on the Adsorption of Protected Amino-Cyclopentene on the Si(100) Surface. *Phys. Rev. B* **2007**, *75*, 125413.

49 Cancès, M. T.; Mennucci, B.; Tomasi, J. A New Integral Equation Formalism for the Polarizable Continuum Model: Theoretical Background and Applications to Isotropic and Anisotropic Dielectrics. *J. Chem. Phys.* **1997**, *107*, 3032–3041.

50 Fernandes, P. A.; Ramos, M. J. Theoretical Insights into the Mechanism for Thiol/Disulfide Exchange. *Chemistry* **2004**, *10*, 257–266.

51 Kla1hn, M.; Braun-Sand, S.; Rosta, E.; Warshel, A. On Possible Pitfalls in ab Initio Quantum Mechanics/Molecular Mechanics Minimization Approaches for Studies of Enzymatic Reactions. *J. Phys. Chem. B.* **2005**, *109*, 15645–15650.

52 Lucas, M.; Ramos, M. J. Theoretical Study of the Suicide Inhibition Mechanism of the Enzyme Pyruvate Formate Lyase by Methacrylate. *J. Am. Chem. Soc.* **2005**, *127* (18), 6902–6909.

53 Lucas, M. F.; Fernandes, P. A.; Eriksson, L. A.; Ramos, M. J. Pyruvate Formate Lyase: A New Perspective. *J. Phys. Chem. B* **2003**, *107*, 5751–5757.