5 SCIENTIFIC HIGHLIGHT OF THE MONTH

Ab initio methods for biological systems: state of the art and perspectives

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Abstract

Ab initio quantum mechanical simulations of biological systems are expanding their capabilities to approach a variety of fundamental problems in biology. We review the progress on method development in the field achieved in the last years, focusing on emerging techniques that might become more relevant in the near future. A brief survey on recent applications in the field of enzyme catalysis and calculations of protein redox properties is also reported.

1 Introduction

In the last years biology entered the post-genomic era, expanding the research efforts from the single molecule studies to the extensive study of interplay between different biomolecules. A large effort is currently ongoing on protein networks and protein-protein interactions research with the main target to bridge the structural and biochemical properties of individual biological molecules with their collective behavior in cell cycles and signalling [1]. Bridging these scales is an fundamental step in the knowledge of biology and it will bring more quantitative description of biological phenomena, opening new perspectives in drug design, technology and medical research.

At the same time, and from the side of Biophysics and Structural Biology, a mechanistic approach to many biomolecules is made possible thanks to the growing availability of experimentally determined 3D molecular structures refined with atomic resolution. Using structural information, single molecule manipulation, and spectroscopy, is nowadays possible by experiments to unravel movements and electronic properties of living matter with accurate and time resolved details [2].

In this context, molecular modelling is also expanding its role. The understanding of the details at the atomic level of the interactions between proteins, nucleic acids, substrates, cofactors and drugs has acquired a renovated key role in the context of quantitative biology and post-genomics.

A variety of simulation techniques, ranging from electronic structure calculations to classical molecular dynamics (MD) and coarse grain models, has to be used to bridge the gap between spectroscopic data, structural information, and biological significance. The quantitative and detailed understanding of properties and molecular mechanisms of biomolecules has been recently the subject of different ab initio studies in the field of photoreceptors [3–6], electron transfer and redox proteins [7,8], transition metal enzymes [9,10], and computational (bio)spectroscopy (including optical, infrared, Raman, EPR, and NMR spectroscopy) [11–13].

From the point of view of the atomistic computer modelling, two levels of complexity will challenge our community for the next years: the intrinsic chemical complexity of the biomolecules and the necessity to cover the gap, in term of size and time, between what has to be simulated at the quantum level and all the rest of the (necessary) biological environment. The accuracy of ab initio molecular dynamics based on Density Functional Theory (DFT) is sufficient to provide a correct description of the electronic structure for large classes of biomolecules, but not always for specific sets of problems in the field of multi-center transition metal enzymes, photoreceptors and electron transfer proteins. The coupling between DFT-based ab initio MD and classical molecular dynamics, in the framework of Quantum Mechanics / Molecular dynamics simulations [14,15], has successfully contributed to link the quantum properties of an active site to the whole biomolecular environment for a variety of systems [16–19].

Here we will shortly overview the computational methods used in the field of biomolecules mentioning also a selection of emerging methods that may have an increasing relevance in the future for the study of the electronic structure of biological systems. We also report a very limited number of applications, being conscious that it will not be able to cover important contributions to several active groups. Complementary and more complete surveys on ab initio modelling on biological systems can be found in a previous newsletter [20] and in several reviews on the subject [21–25].

2 Methods

2.1 Classical, Quantum and hybrid Quantum/Classical simulations

Dealing with electronic structure calculations of biomolecules cannot abstract from a description of the biomolecules at the level of classical molecular dynamics. The use of force-field based MD is indeed necessary, either to enhance the sampling of the conformational space or to include, using hybrid methods, the environment that cannot be described at the full quantum level because of computational costs. Classical, i.e. force-field based, setups of biological systems have typically $10^4 - 10^5$ atoms and a sampling of the phase space is crucial to overcome conformational and bond breaking/forming barriers. Enhanced sampling techniques can significantly improve the exploration of the phase space of biological macromolecules and reactive systems. Among the others, the recently developed metadynamics [26,27] has been successfully used in biomolecular simulations coupled with classical [28,29] and ab initio molecular dynamics [30,31].

Ab initio molecular dynamics at finite temperature is commonly used to study biological systems in the framework of Density Functional Theory [32] because of the good compromise between

chemical accuracy and computational costs. The choice of the exchange-correlation functional is crucial for many biomolecules. Gradient corrections are usually necessaries and different approaches may be preferred according to the kind of system and calculation performed. The popularity of BLYP functional [33,34] comes from its capabilities to give a good description for hydrogen bonding systems [35,36]. The B3LYP hybrid functional [37] can provide a better estimate of reaction barriers [38] but it has not been extensively used so far in ab initio MD because of the high computational cost of its implementation within a pure plane-wave scheme. Becke and Perdew [33, 39] functionals are preferred for transition metal complexes whereas Perdew, Burke and Ernzerhof functional [39] is more used in excited states calculations. Two alternatives are currently used in ab initio molecular dynamics: a pure plane wave (PW) approach and a Gaussian and plane wave (GPW) method [40–42]. In the PW method core electrons can be replaced using norm-conserving (Trouillier-Martins [43], Goedecker, Teter and Hutter [44]) and non-norm-conserving ultrasoft (Vanderbilt [45]) pseudopotentials. The Gaussian and plane wave method offers a convenient alternative, specially suited for the use of hybrid exchange-correlation functionals. Recent simulations of several tens of picoseconds on liquid water recently demonstrated the possibility to perform ab initio MD using B3LYP functional [46]. To circumvent the inability of current exchange-correlation functionals to incorporate dispersion forces, a pseudopotential scheme has been developed by the group of Rothlisberger [47,48]. Higher angular momentum dependent terms of the pseudopotentials are optimized using correlated calculations references, obtaining very good results for weakly bound systems [49,50] and liquid water [51].

Using hybrid Quantum Mechanics / Molecular Mechanics (QM/MM) approaches it is possible to limit the size of the part of the system that is described at the quantum level to the atoms belonging to the reactive part of the system (for instance the active site of the enzyme or the chromophore of a photoreceptor), whereas the rest of the atoms (protein and solvent) are treated at the classical force-field level. Quantum Mechanics and Molecular Mechanics system can be coupled in a fully Hamiltonian way using the scheme from Rothlisberger and coworkers [14,15]. A recent extension of QM/MM techniques in the PWG approach is reported by Laino et al. [52,53]. A more extensive method overview of QM/MM applications to biomolecules has been also reported in several reviews [20–25,54].

It is interesting to note in the literature an expansion of ab initio MD techniques to the study of systems that were previously considered well described by classical mechanics. One example is represented by potassium channel proteins, usually treated at the level of classical mechanics since no bond breaking or forming processes is occurring during ion permeation through the channel pore. On the contrary, ab initio calculations demonstrated that the interaction between the permeating cations and the protein carbonyl ligands cannot be straightforwardly described by standard nonpolarizable force-fields [16,55,56]. In addition, the permeation mechanism is coupled with a proton transfer between surrounding ionizable residues [57] that cannot be modelled classically.

2.2 Calculations of redox properties

Redox properties can be calculated according to a MD method based on Marcus theory [58] as originally proposed by Warshel [59]. This scheme has been developed and implemented for use

in DFT based ab initio molecular dynamics simulations [8, 60–64]. For a large class of proteins the ET activity falls within the Marcus regime, and the oxidation free energy (ΔA) and the reorganization free energy (λ) for the half reaction can be computed from ensemble averages of the vertical ionization energy (ΔE), according to the following equations:

$$\Delta A = \frac{1}{2}(\langle \Delta E \rangle_{red} + \langle \Delta E \rangle_{ox}) \tag{1}$$

$$\lambda = \frac{1}{2}(\langle \Delta E \rangle_{red} - \langle \Delta E \rangle_{ox}) \tag{2}$$

Subscripted angular brackets denote averages over equilibrium trajectories of the system in reduced (*red*) and oxidized (*ox*) state. In the hybrid scheme used for the study of redox properties in rubredoxin and which we review as an example here ΔE is still computed using DFT but the atomic configurations are extracted from classical simulations. The aim of the method is to combine the long time scale accessibility of the classical model with the quantum-mechanical methods to calculate ionization energies. When the size of the system does not permit a full DFT calculation a QM/MM approach can be used to calculate the energy of single configuration [7,65].

For calculations of redox reactions in proteins an important issue is to have reliable starting structure for the initial configurations of the oxidized and reduced forms. Indeed proteins can undergo some major rearrangement upon oxidation/reduction which can be quite difficult to model.

Another issue regards the quality of our DFT description for the metal centers. In particular GGA functionals have been widely employed for calculations on biological systems, but they are not accurate enough for the description of metal centers where electronic correlation plays an essential role. In the case of rubredoxin, which we will discuss in a following section, the functional accuracy has been tested comparing electron detachment energies of small clusters ($Fe(SCH_3)_4$) with previous calculations and experimental data.

2.3 Emerging techniques

For many chemical and biochemical systems, DFT turned out to be a good compromise between method reliability and computational cost. Albeit this success in static calculations and ab initio molecular dynamics, the current approximations to the exchange-correlation functional are source of well-recognized and serious failures [66]. Systems where these limitations are evident are for examples, free radicals and transition metals with semi-filled $d-$shell (such as Cr, Mo, Fe, Ni, Mn) [67]. The drawbacks also affect the time dependent version of Density Functional Theory (TD-DFT) [68], causing unsatisfying estimates in the calculation of electronic excitations of a variety of molecular systems, one among the simplest being liquid water [69, 70]. The use of correlated quantum chemistry techniques, such as perturbative methods (MP2,MP4), coupled cluster (CC), and configuration interaction (CI) are limited to rather small biomolecules because the required computational resources grow very rapidly with the system size (see for instance ref. [71]). The searching for highly-accurate and size-scalable quantum algorithms will be in the next years a crucial challenge for the community working on ab-initio methods for biomolecules. An incomplete survey on emerging techniques is reported hereafter.

DFT extended schemes were proposed to study transition metal complexes because of the diffi-

culties encountered by current functionals to correctly estimate the electronic correlation effects, especially in multi-center transition metal complexes. In the so called broken symmetry approach the spin coupling constant J between antiferromagnetically coupled metal centers is estimated by carrying out independent calculations for several spin configurations. The energy of all different spin states can be subsequently estimated using a Heisenberg Hamiltonian with coupling J. This technique has been successfully used to study iron-sulfur catalytic centers [72] and it has been recently extended and applied in an ab-initio molecular dynamics context [73]. The dynamical magnetostructural properties of the $2Fe-2S$ iron-sulfur cluster of ferredoxin were studied using QM/MM [74], revealing the time-dependent interplay between the magnetic properties of the di-iron center and the protein environment. Another extension of DFT methods relevant for transition-metal active site chemistry is the DFT+U method, where a generalized-gradient approximation is augmented by a Hubbard U term [75]. A self-consistent DFT+U approach has been recently shown to successfully reproduce the electronic properties of the iron dimer and the spin and energetics of gas-phase iron-based reactions [76, 77]. Although not easily generalized to all transition-metal systems, both the above cited methods have the advantage that they require a computational effort comparable with that of plain DFT.

Green's function based methods, such as GW approximation and Bethe-Salpeter equation are traditionally used to calculate electronic excitations in condensed matter systems [78]. In a combined approach with classical molecular dynamics, these methods have been recently successfully extended to calculations of absorption spectra of liquid water [70, 79]. The excited state properties of the indole molecule, the aromatic component of the tryptophan amino-acid, has been also studied in solution by calculating electronic excitations using many body perturbation theory on snapshots extracted from a QM/MM simulation based on DFT [80]. These encouraging results suggests that Green's function methods can be suitable and affordable techniques to study the electronic excitations of other biomolecules that cannot be properly approached using TDDFT.

Another emerging technique in the field of biomolecule is Quantum Monte Carlo. It has been successfully employed in the past to compute ground-state properties of systems where electron correlations play a crucial role (see for instance the review [81]). An extended formulation has expanded its capabilities to investigate electronically excited states [82]. The QMC method, traditionally largely applied in many body physics and in Bose condensates, has been also used to correctly tackle difficult cases in quantum chemistry such as: radicals [83], transition metals [84,85], electronic excited states [82,86], anion-π and π-π interactions in aromatic molecules [87], van der Waals forces [88], and hydrogen bonding interactions [89, 90]. For hydrogen bonding systems, the water dimer dispersion curve was investigated both at the Variational Monte Carlo and at the Diffusion Monte Carlo level [90]. The experimental binding energy and the MP2 energy curve as a function of the distance between the two water molecules for the dimer were fairly reproduced. The physical interpretation of the resonating valence bond variational wave function offered also the possibility to dissect the covalent and dispersion van der Waals contributions to the H-bonding energy, estimated to be about 1.5 and 1.1 kcal/mol, respectively. The energetics of larger water clusters were also investigated by QMC and compared with MP2 and DFT [91]. From the side of electronic excitations, QMC calculations have shown to provide the correct excited state energy surface in one of the most representative example of TDDFT failure, the protonated Schiff-base model [82], which is a small analogue of the retinal protonated Schiff base,

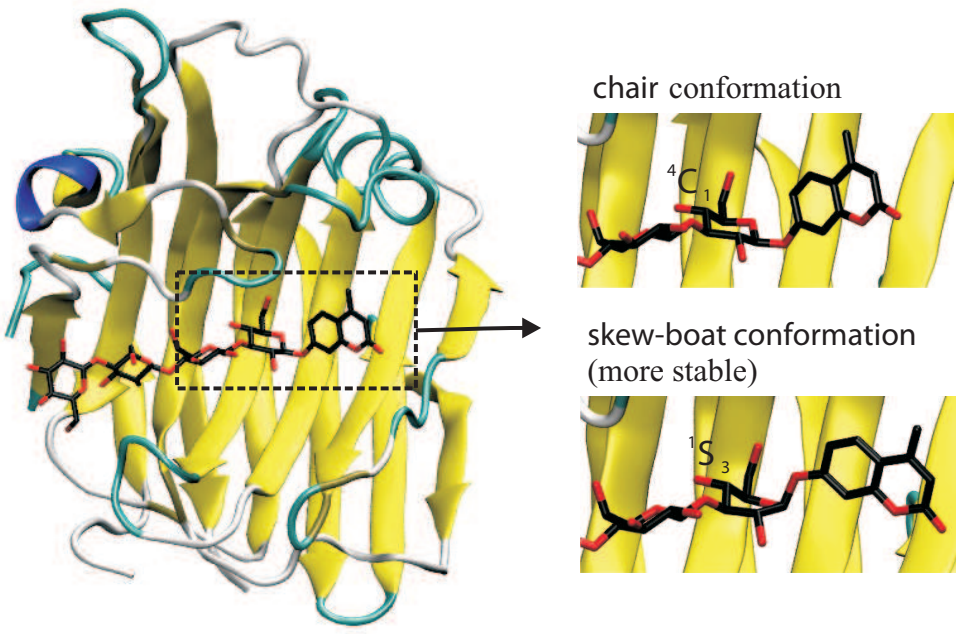

Figure 1: Minimum energy structures obtained from QM/MM simulations of the enzyme-substrate complex of Bacillus 1,3-1,4-β-glucanase, a family 16 glycoside hydrolase (Reference: Biarnes et al. J. Biol. Chem. 2006). The substrate (a 4-methylumbelliferyl tetrasaccharide) is shown in licorice representation.

the chromophore of rhodopsin.

3 Applications

3.1 Enzyme catalysis

The way enzymes perform its catalytic function has long fascinated not only biologists but also chemists and physicists [92–94], because subtle changes in the species involved (e.g. ligand, substrate, enzyme) may lead to serious diseases [95]. Therefore, elucidating how enzymes work at the atomic level is extremely relevant to find better drugs. Scientists have long sought the origin of the lowering of the activation energy barrier by enzymes, i.e. whether they stabilize the TS or raise the relative energy of the substrate (substrate preorganization [96]). Nevertheless, the molecular details of many enzymatic mechanisms still remain a mystery. Very often catalysis depends on the interplay between structure/electronic reorganization and dynamics. For instance, a distortion of one single sugar unit from a chair conformation to a boat-like is crucial for glycoside hydrolases to break the glycosidic bonds in carbohydrates. At the same time, the distortion raises the charge at the anomeric carbon and elongates the glycosidic bond distance, thus favoring catalysis [97] (Figure 1).

Another example is the binding of oxygen to myoglobin (Mb) and hemoglobin (Hb). The heme

active center changes its electronic configuration upon binding, from a high spin (i.e. maximum number of unpaired electrons) to low-spin state, at the same time that the bond between the iron atom and the oxygen ligand develops [98]. The decomposition of the superoxide radical (O_2^-) into hydrogen peroxide and oxygen by superoxide dismutases, for instance, involves changes in the coordination state of the active species as well as in their oxidation states [95]. Deciphering these processes from an electronic point of view is necessary for understanding the mechanisms behind the enzymatic catalysis, as well as designing small molecules able to affect the biological function of the protein.

In the past few years, ab initio methods have contributed important insights into the catalytic mechanisms and structural features of a variety of enzymes (for recent reviews see e.g. [54]; [99]). This progress has been possible also because of the increased computer power, and the continuous development of techniques such as QM/MM and methods to accelerate sampling of free energy surfaces (metadynamics [26], transition path sampling [100] or steered molecular dynamics [101]). Because of the large number of ab initio applications to enzymes that appeared in the literature in the last few years, it is clearly impossible to review all of the work appeared so far. Here we will review only few representative examples, hoping to give a flavor of which problems can be addressed nowadays with ab initio methods. Metal-containing proteins represent almost half of the proteome of living organisms. Very often, the metal is present in the active site and plays a role in catalysis. Zinc metallo β-lactamases (MβLs) hydrolyze the β-lactam N-C bond of β-lactam antibiotics aided by one or two Zn^{2+} ions. Ab initio QM/MM simulations have shown that the flexibility of the Zn^{2+} coordination sphere plays a key role in the enzyme reaction [54, 102]. Mg^{2+} ions are present in several enzymes that hydrolyze chemical bonds such as epoxide hydrolase and ATPases [103]. In both cases, ab initio and QM/MM simulations including solvent water molecules in the QM region found that the water molecules assist the chemical reaction. These calculations were done in moderately large systems (about 50 QM atoms) but required large simulation times (100-200 ps). Similarly, two Mg^{2+} metals support the formation of a metastable intermediate along the reaction in ribonuclease H, but the role of solvent waters in mediating proton transfer events is crucial ([104]). All these works suggest the general importance of explicitly including solvation effects at the catalytic site for the correct description of an enzymatic mechanism at the atomic level.

Hemeproteins (metalloprotein containing a heme prosthetic group) are an important group of proteins and enzymes that carry out a variety of relevant biological functions, including oxygen transport and storage (hemoglobin and myoglobin), electron transfer (cytochromes), disproportionation of toxic hydrogen peroxide (catalases) and oxidation of substrates (peroxidases). This diversity of functions originates from the versatility of the heme group and the variety of interactions with protein scaffolds that generate different heme environments [105]. Because of the large size of the iron-porphyrin (38 atoms), ab initio calculations in hemeproteins are particularly demanding [106] (e.g. the iron-porphyrin, without the substituent groups forming the heme, plus several protein residues in its vicinity easily makes about 150 atoms) [107]. For the hybrid catalase-peroxidase, which contains a Met-Tyr-Trp adduct above the heme (essential for catalysis), the number of QM atoms to be included is about 250 [10]. During the last few years, a large effort has been devoted not only to characterize reaction intermediates with a complex electronic structure (e.g. the main reaction intermediate of the reaction cycle of peroxidases,

catalases, cytochrome P450 and nitric oxide synthase is an oxyferryl-porphyrin cation radical named "compound I", Cpd I [107, 108]) but also in elucidating the mechanism of their formation/disappearance [108, 109]. For peroxidases, in which the active site is solvent-exposed, the mechanism of Cpd I formation was found to rely on the entrance of water molecules to the active site [109]. Again, this reinforces the active role that water molecules play in enzyme catalysis.

In contrast to peroxidases, the active site of catalases is buried in the protein. Catalase Cpd I reacts with hydrogen peroxide (H_2O_2) and decomposes it to H_2O and O_2 (Figure 2).

QM/MM metadynamics simulations have shown that there are two competing mechanisms to decompose hydrogen peroxide [9]. One of them is consistent with previous proposals based on structural information, whereas the other one explains the results of kinetic investigations on enzyme mutants.

Another problem in this field that has been investigated concerns the long-standing question of how myoglobin discriminates between poisonous CO and O_2. During the past decade, it was demonstrated that CO distortion is not responsible for CO discrimination [110–112]. De Angelis et al. recently quantified the relevance of other factors, mainly hydrogen bonding of the bound oxygen [113]. A large effort has been also devoted to investigate how hemoglobins from different species bind oxygen [114]. Ab initio calculations have also contributed to understand the sometimes ambiguous iron-oxygen distances found in X-ray structures of heme proteins [107, 115, 116].

It should be taken into account that ab initio modeling of enzymes needs accurate structures as input. As the number of high resolution structures of complex systems increases, more accurate analysis can be performed. This is the case, for instance, of recent studies on membrane proteins [117–119], photoactive proteins [17, 120–122] or DNA-protein complexes. The recently solved X-ray structure of a complex between photolyase and a double-stranded DNA oligomer provided a suitable starting structure for performing computational studies to elucidate the mechanistic nature of the photochemical repair. QM/MM molecular dynamics simulations [123] elucidated the role of the various amino acids in the active site of the damaged DNA-enzyme complex.

3.2 Redox properties in proteins

Bio-inorganic oxidation/reduction enzymes and metalloproteins represent more than 40% of IUBMB classified proteins and are not only vital to biological energy conversion in photosynthesis and respiration, but are also critical to a growing number of signalling processes governing gene regulation and expression [124]. Understanding the mechanism of electron transfer (ET) between two metal sites or metal site and organic substrate is therefore of both theoretical and practical importance. The key question is how metalloproteins control which processes are thermodynamically feasible (i.e., reduction potentials) and how fast they occur (i.e., rate constants). Some of the issues that have been raised in this context concern the competition between short range effects, in essence the coordination chemistry of the metal ion, and long range effects, for example the reorganization of the protein, the placement of charged and polar residues, the access to the solvent. Related to this is the question of the relative importance of electronic relaxation effects, such as the difference between hard and soft ligands and electronic polarization of the

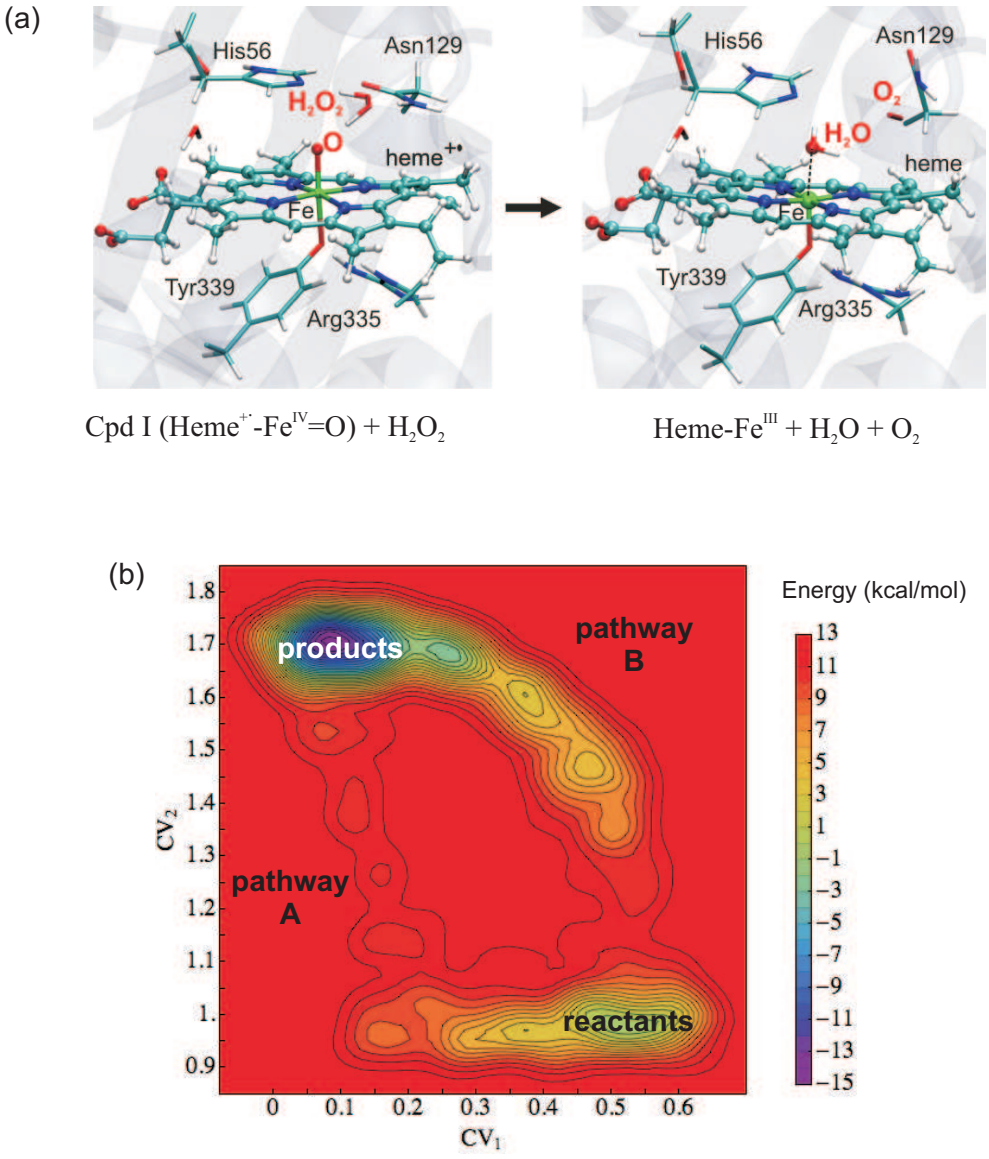

Figure 2: (a) The molecular mechanism of the catalase reaction (optimized structures of the reactants and products). (b) The corresponding reaction free energy surface obtained from QM/MM metadynamics simulations using two collective variables. Reference: Alfonso-Prieto et. al. J. Am. Chem. Soc. 2009.

protein, versus the reorganization due to atomic motion.

In this highlight we discuss as an example the calculations of redox properties of Rubredoxin(Rd) [8], a small and comparatively simple iron-sulfur protein. This is a particularly interesting case, since it is possible to combine a full ab initio description of the electronic structure of the protein in explicit solvent with sampling of the relevant time scale of the protein dynamics using a hybrid method based on a force field molecular dynamics / density functional theory scheme. Applying this scheme within the framework of Marcus theory [58] we are able to reproduce the experimental redox potential difference of 60 mV [125] between a mesophilic and thermophilic rubredoxin within an accuracy of 20 mV. The redox potential is modulated by the hydrogen bond interactions of the ligand cysteines with the NH groups of nearby residues with a stronger network of hydrogen bonds leading to more positive reduction potentials. We also compute the reorganization free energy for oxidation of the protein obtaining 720 meV for the mesophilic and 590 meV for thermophilic variant. Decomposition of the reorganization energy using the classical force field shows that this is largely determined by the solvent, with both short range (an oxidation induced change of coordination number) and long range (dielectric) contributions. The 130 meV higher value for the mesophilic form can be attributed to the different dielectric response of the solvent in the surrounding of the active site. These results underline the importance of a molecular description of the solvent and of a correct inclusion of polarization effects.

A major advantage of a DFT scheme here is that it accounts for electronic relaxation effects in response to oxidation/reduction. Such effects include ligand-metal charge transfer and the adjustment of hydrogen bond strength of coordinated protein residues and solvent. Within a DFT description of the vertical ionization energy for the entire solvated system, as applied here, also the instantaneous equilibration of electronic polarization to the new solute charge distribution is included. This can lead to a significantly lower estimate of the reorganization energy compared to a classical model with fixed charges.

We have shown that with modern computational methods calculations on a full small size protein are within reach and can offer a powerful predictive instrument to quantify properties, such as the reorganization energies, which are not easily measured by experiments.

4 Conclusions

In the last years electronic structure techniques and ab initio molecular dynamics have further expanded their capabilities to understand structure/function relationships of biomolecules to an increasing class of systems. In this brief review on the state-of-the art of methods and applications we have tried to make a survey on the last progresses in the field that might be representative but far from being complete. Two main problems are currently preventing ab initio calculations to tackle with success a wider class of challenging problems in biology: the size of the systems and the quality of the electronic structure methods. QM/MM methods are a first step to try to fill this gap and further development of multiscale methods is desirable in the future. We have also seen how different emerging quantum techniques seem promising starting points to go beyond standard DFT calculations. Thanks to this double effort, we may hope to tackle in the next years open questions in electron transfer, electronic excitations of photoreceptors, and

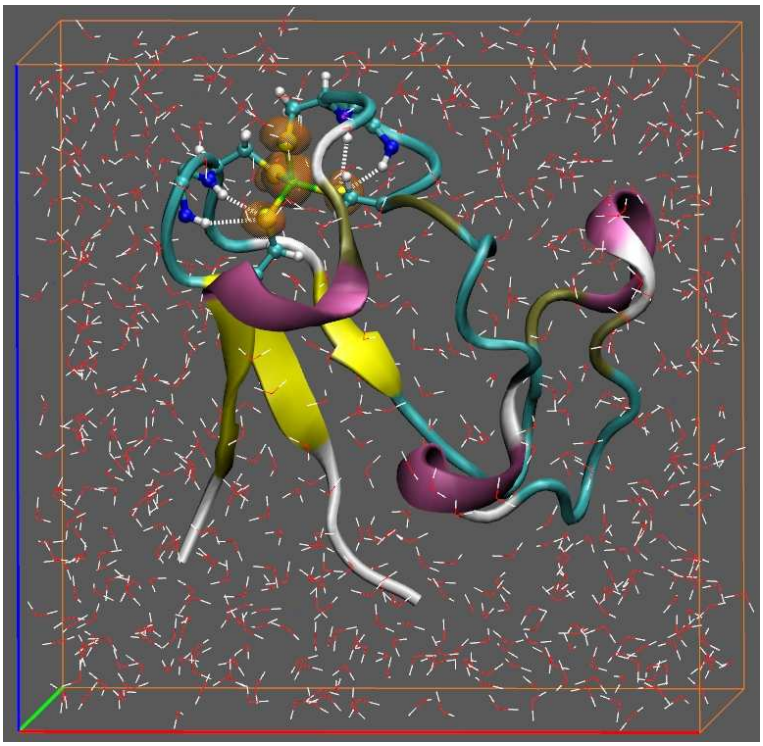

Figure 3: Representative MD configuration of rubredoxin generated from a 1IRO crystal structure [126] as employed in the DFT calculations. The periodically repeated simulation cell, with edges 31.136, 28.095, 30.502 Å contains the protein, 678 water molecules and 9 Na^+ counterions. The orange isosurface represents the spin density for the oxidized state (charge 0, spin 5/2) at 0.005 a.u.. Hydrogen bonds between sulfur atoms in the active center and nearby backbone NH groups are also highlighted.

multi-center transition metal proteins.

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