Scalable molecular dynamics

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Molecular Modeling of Complex Biological Systems

Molecular dynamics simulations enable the study of the time evolution of molecular systems by taking many small successive time steps under atomic forces that are calculated from a parameterized set of interaction functions. These are simple functions describing bonded and non-bonded atomic interactions, so that large molecular systems can be simulated for many time steps. The simulations provide energetic and kinetic properties in the form of statistical ensemble averages. The resulting trajectories can be analyzed for a variety of geometric and kinetic properties and correlations between them. These simulations have been carried out for many different systems, especially in computational biochemistry.

NWChem is the computational chemistry software suite developed and maintained by the Molecular Sciences Software Group (MSSG) of the Environmental Molecular Sciences Laboratory at the Pacific Northwest National Laboratory. This software has been designed to run efficiently on a variety of massively parallel computer architectures, including distributed memory (e.g. IBM-SP, Linux clusters) and shared memory (e.g. CRAY-T3E) architectures. In NWChem the molecular dynamics simulations module is based on domain decomposition. This allows reduction of memory requirements through the distribution of data across many processors as well as reduction of communication requirements because of the locality of interactions in cut-off based atomic interaction lists. This type of decomposition does require sophisticated load balancing techniques to be implemented, however, because of the heterogeneity of biological systems. In NWChem we have implemented a combination of global and local load balancing techniques, resulting in a significant increase of the parallel scaling that can be achieved. In NWChem we use the AMBER force field with the GLYCAM extensions for simulations of saccharides.

Many of the enzymatic systems of interest involve complex processes such as electron transfer and proton hopping mechanisms. A practical approach that is generally applicable to studies of enzymatic electron transfer processes is based on the semi-classical description derived by Marcus and Sutin. In their formulation the rate of the electron transfer reaction is expressed as:

\[ k_{ET} = \frac{2\pi}{\hbar} |H_{IF}|^2 \frac{1}{\sqrt{4\pi\lambda k_B T}} \exp \left[ -\left( \Delta G_{et}^0 + \lambda \right)^2 / 4\lambda k_B T \right] \]

(1)

The exponential term in Eq.(1) comprises the nuclear factor and involves the change in free energy \( \Delta G_{et}^0 \) and the reorganization energy \( \lambda \) that accompany the electron transfer reaction. \( H_{IF} \) is the electronic factor, the coupling matrix element between the initial state (I) and the final state (F) of the reaction (in the literature, \( H_{IF} \) is also denoted with \( H_{AB} \) or \( V_{AB} \)). For overviews of electron transfer theories, we refer the reader to several reviews and articles. Our research has focused on merging...
state-of-the-art advances in chemical theoretical methodologies (accurate density functional theory (DFT) in a mixed Hamiltonian representation of the protein environment) and computational methodologies (massively parallel computations of electron coupling matrix element $H_{IF}$ for very large systems treated at the level of electron correlation theory).

The first set of challenges involves the accurate quantum chemical characterization of the initial and final states in the series of electron transfer processes. Accuracy refers to the characterization of the geometrical structure of the active center in its oxidized, reduced, and intermediate states, (including accounting for the dynamical effects of the protein conformation), of the charge and spin distributions in these states (these are closely related to the determination of the iron oxidation state), of their relative energies, and of their redox potentials (including the effects of the protein environment). Our and others’ experience lead us to adopt a mixed Hamiltonian quantum mechanical / molecular mechanics (QM/MM) methodology in which the QM level of theory is of the DFT type based on hybrid functionals such as B3LYP or B3PW91 because of their demonstrated ability to reproduce and predict accurately the electronic structure of the active site (including spin states) in several model systems of biological significance. The MM level of theory is expected to adequately emulate the protein environment and its polarization effects onto the quantum representation of the QM active sites. Fully classical models of the protein will guide the simulation of the effects of the long-range domain structure and dynamics of the enzyme on the QM wave function. Except for the enhancements to the QM/MM module in our NWChem suite of codes, this task relies for the most part on the existing capability to perform very efficiently DFT calculations on very large model systems using the NWChem code.

The second set of challenges involves the calculation of the electronic coupling element $H_{IF}$ using the QM/MM, DFT-based, wave functions of the initial and final quantum states involved in various electron transfer elementary steps. The electronic factor reflects the electron transfer distance dependence, the electron transfer directionality, and the role of intervening through-bond and/or through-space bridges between the donor and acceptor sites. To date most calculations of $H_{IF}$ use approximate expressions and/or semi-empirical QM levels of theories. When using ab initio theories, $H_{IF}$ calculations are most often limited to the Hartree-Fock level of theory, albeit it is highly desirable to account for the effects of electron correlation. The uniqueness of the research in this task lies in the development of a capability heretofore not available, to calculate $H_{IF}$ for very large systems while accounting for electron correlation. To this end, the single-determinant form of the DFT/MM wave functions makes it possible to extend the implementation for the calculation of $H_{IF}$ to take advantage of massively parallel computers to treat very large systems, such as the one investigated here. The formulation involves constructing Fock-like matrices using density-like matrices, computational steps that are known to exhibit an extremely high level of parallel computing efficiency.

Marcus’ two-state theory of ET is illustrated simply in Figure 1 below. Curve I shows how the reactant state’s potential energy changes along the reaction coordinate, and curve F describes the product state:

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**Figure 1.** energy surfaces for electron transfer reactants (I) and products (F)
If the system has adequate energy to overcome the activation energy ($\Delta G^0_{et}$), and if the electron transfer coupling energy ($H_{IF}$) is sufficiently large, the system can proceed to electron transfer products, thereby completing the reaction. For organometallic systems, electron transfer is said to be 'adiabatic' (100% crossover, $\kappa=1$), when $H_{IF}$ is greater than about 200 cm\(^{-1}\). The probability for the electron transfer ($\kappa$) is proportional to the square of $H_{IF}$, the strength of the electronic interaction between the electron donor (D) and acceptor (A):

$$\kappa Z = \sqrt{\frac{4\pi^3}{\hbar^2 \lambda k_B T}} |H_{IF}|^2$$

(2)

The $\kappa$ value is unity for an adiabatic process when the interaction energy at the intersection is so large that formation of transition state always leads to the product. In many inter-molecular electron transfer reactions, the $|H_{IF}|^2$ values are large enough that the electron transfer reactions is adiabatic ($\kappa=1$) but small enough that $H_{IF}$ is still negligible compared to $\Delta G^0_{et}$. Such reactions are called adiabatic outer-sphere electron transfers, and the rate of electron transfer is determined solely by the electron transfer driving force (-$\Delta G^0_{et}$) and the nuclear reorganization energy. This balance between nuclear reorganization and reaction driving force determines both the transition-state configuration and the height of the barrier associated with the electron transfer process.

**Molecular Basis for Microbial Adhesion and Geochemical Surface Reactions**

Many important processes in the subsurface, such as oxidation/reduction reactions, mineral dissolution and metal ion precipitation are microbiially mediated, and are believed to take place at the microbial membrane or at the interface between the microbial membrane and mineral surfaces. Because of the complexity and size of these systems, our theoretical understanding of the processes that take place at the interface between biological membranes and geochemical surfaces is very limited. With the advent of modern massively parallel computers and sophisticated, highly efficient software for computational chemistry modeling, the theoretical study of such systems is now feasible. We are focusing our research on the outer membranes of gram-negative bacteria, in particular those that have been found to have the ability to use metal (specifically Fe) reduction in the respiratory cycle, take up solvated metal ions from the environment, show adsorption to mineral surfaces, and, consequently, are potentially important target microbes in the design of bioremediation technologies.

The interaction of microbes with their environment involves a broad range of scales, including the atomic scale of the interaction involving individual functional groups (Å scale), the molecular scale in the formation of long molecular chains (nm scale), the scale of molecular assemblies leading to the formation of membranes (µm scale), and molecular to macroscopic scale of the interactions of membranes with minerals and other surfaces (µm and greater scales). The key geochemical and biochemical interactions and reactions at the interface of microbial membranes take place across all of these spatial scales. The study of the dynamics and energetics of the interactions between bacterial membranes and mineral surfaces and the process of exchange and uptake by the membrane of charged species from the mineral or from solution is complex. This work involves the integration of modeling and experimental capabilities to study these complex systems at an atomic level of detail. These systems consist of a number of complex interacting components: lipid membranes, polysaccharide membranes, trans-membrane proteins, minerals, and solvated ions. So, not only do these systems involve many spatial and temporal scales, they also involve complex assemblies and exhibit complex behavior. The goal of our research program is to integrate computational and experimental tools required to obtain a detailed understanding of the biomolecular and geochemical interactions that take place in systems consisting of all of these different components.
Gram-negative bacteria are characterized by the existence of a cellular envelope consisting of an inner membrane that encloses the cytoplasm and an outer cell wall providing the structural rigidity required for the protection of the bacterium. The space between the two membranes is referred to as the periplasmic space. The outer membrane is an asymmetric bilayer of phospholipids in the inner leaflet and lipopolysaccharides (LPS’s) in the outer leaflet. The outer membrane is strengthened by peptidoglycan in the periplasm anchored into the outer membrane by lipoproteins. A schematic diagram of the bacterial envelope of gram-negative bacteria is shown in Figure 1. Lipopolysaccharide molecules form the main constituent of the outer leaflet of the cell wall, contributing approximately 40% of the mass of the outer membrane. They can extend 5 to 10 nanometer outward from the cell surface. The abundance of gram-negative bacteria in the environment and their specific interactions with solvated ions and mineral surfaces has prompted many experimental studies of their potential for use in bioremediation. Many metal ion complexes found in contaminated soil and water are potential biohazards because of their toxic and genotoxic effects. For the development of efficient and cost-effective bioremediation strategies, it is of crucial importance to understand the molecular processes that take place at the interface between microbial membranes and their immediate environment. Because LPS forms the main constituent determining the negative charge of the exterior cell wall, it is believed to be a key factor in the processes that control attachment to mineral surfaces, the uptake of metal ions, and the microbially induced precipitation and dissolution reactions. Consequently, environmental microbiology and biogeochemistry research is focusing on the study of LPS’s in order to characterize its structural and functional properties and the role it plays in biogeochemical processes. The complexity of bacterial cell walls requires a combination of experimental studies and theoretical and computational modeling investigations to provide insight in the complex interactions, and in the design of additional experiments. The current theoretical understanding of the reactions and adsorption processes mediated by the bacterial cell surface is very limited, in large part because of the size and complexity of these systems. Only a few, limited molecular modeling studies of LPS molecules or membranes containing LPS have been reported. Our computational research program is focused on the development of the tools required to increase our theoretical understanding of the molecular mechanisms of the LPS that determine metal binding, bacterial attachment to mineral surfaces, and microbially mediated reduction, precipitation and dissolution processes. Such understanding will be fundamental to increasing our knowledge of the bioavailability of reducible soil minerals, microbial transport mechanisms, and the binding, oxidation and reduction and precipitation of solvated metal ions. Such information will be crucial to the design of novel, effective microbial remediation technologies.

Complete and detailed composition information is available for the LPS from a number of bacteria, such as Escherichia coli and Pseudomonas aeruginosa, and partial LPS compositions have been determined for many other gram-negative bacteria, including Rhodobacter sphaeroides. Our studies focusing on P. aeruginosa, a bacterium naturally found in lakes and marine sediments, because of the extensive experimental information that is available for P. aeruginosa, including the complete genome, molecular structural models of trans-membrane proteins, and experimental data on the binding affinities of a variety of metal ions, including Na⁺, Al³⁺, Ca²⁺, Fe³⁺, La³⁺, Eu³⁺, Yb³⁺, and UO₂²⁻. LPS molecules consist of three distinct regions: a lipid A, a core polysaccharide (PS), and an O-specific chain. In P. aeruginosa, the expression of the two identified types of O-specific chain appears to be environmentally controlled, and provides a means of classification of the LPS membrane. Lipid A is formed by two glucosamine-phosphate groups bound to lipid chains that vary in length (on the order of 12 carbon atoms) between the different strains of the bacterium. The polysaccharide core has a conserved saccharide sequence in all strains. The core region is the most highly charged region of the LPS, concentrated primarily on the 4-phosphoglucomaminyl- (1-6) glucosamine-1” phosphate head groups and the carboxylic acid groups of the 2-keto-3-deoxyoctulosonic acid (KDO) moieties. Although a wide range of ions is capable of binding in the outer membrane, including alkali, alkaline earth, transition and rare earth metal ions, the main metal species found are calcium (Ca²⁺), magnesium (Mg²⁺), and sodium (Na⁺). Extraction experiments have shown that Ca²⁺ is predominantly responsible for stabilization of the LPS. The dication
Mg$^{2+}$ is involved in LPS-protein stabilization, and Na$^+$ is involved in the interactions of the phospholipid interior part of the outer membrane. The two types of O-specific chain are labeled A for the common, neutral, homopolymer of α-D-rhamnose, and B for the strain-specific, highly charged heteropolymer of uronic acid derivatives and rare sugars such as pseudaminic acid. The transport of ions across bacterial membranes commonly occurs through protein channels. There is ample experimental evidence, however, that *P. aeruginosa* binds ions such as Cu$^{2+}$, Fe$^{3+}$, La$^{3+}$, Eu$^{3+}$, and UO$_2^{2+}$, in the cell wall, suggesting the availability of binding sites in the LPS membrane for metal ions. Supporting this hypothesis is the experimental finding that the level of binding of these ions appears to depend on the type of O-specific chain. In addition, phosphate and carboxyl groups in the polysaccharide core were also found to be responsible for metal ion binding.

**Molecular Simulations Studies of Complex Enzymatic Reactions**

This research involves the development of computational methods and implementations in support of ongoing experimental and theoretical multi-project efforts at PNNL aimed at obtaining a detailed characterization and molecular level understanding of microbially-mediated metal reduction processes in the subsurface. This work is critical to advance the potential of subsurface bioremediation technologies. The ability to reduce and stabilize a variety of metals, including radionuclides and other contaminants such as Cr and Tc, into insoluble forms, with enhanced efficiency and selectivity, is a major objective of the Department of Energy (DOE), and its importance is illustrated by the multi-project effort that the DOE supports here and elsewhere, targeting the role of Fe(III)-reducing bacteria in the subsurface.

The focus of our research on complex enzymatic reactions is on obtaining a detailed molecular-level characterization and understanding of the reactivity of enzymes used by these and other bacteria. These efforts lie on the premise that such an understanding can be obtained through computational modeling and simulations. The flavocytochrome c$_3$ of *Shewanella frigidimarina* is the initial target for the application of our theoretical methods to the characterization of enzyme reactivity. This enzyme is involved in the respiratory reduction of Fe(III), and this research will contribute to the information being collected in other major research programs at PNNL that focus on *Shewanella sp.* Furthermore there exist a number of experimental, structural and spectroscopic data from which we can calibrate our theoretical methods, including crystal structures for the soluble enzymes Ifc$_3$ and Fcc$_3$, whose function and reactivity are similar to the other membrane-bound cytochromes that form part of the same respiratory cycle.

From the current knowledge of the structure and reactivity of some of the cytochromes of *Shewanella sp.*, it is apparent that the elucidation of their molecular-level processes, including the electron and proton transfer steps, requires that existing computational tools and techniques be extended beyond present capabilities. Because these enzymatic reactions combine long-range electron transfer, proton transfer, and conformational protein dynamics for their mechanism of action, the outcome of our research is the development of advanced modeling and simulations tools which enable the modeling and simulation of these increasingly complex systems with a reduced level of approximation, an increased level of model sophistication, and an increased level of accuracy compared to what has been available heretofore. This goal is achieved through the extension and the development of new modeling capabilities in the context of massively parallel computations and within the framework of the NWChem computational chemistry software suite. We are building extensively upon the existing massively parallel electronic structure and classical molecular dynamics simulation capabilities of NWChem, and develop enhancements of existing functionalities as well as new ones. This is in particular for the theoretical characterization of proton hopping and electron transfer in protein systems and the calculation of redox properties of specific protein functional groups involved in electron transfer.

One of the biological systems whose reactivity is of particular interest to characterize by means of computer simulations is a soluble fumarate reductase of *Shewanella sp.*, an enzyme that catalyzes the
chemical reduction of fumarate into succinate. For its function, this enzyme depends on long range electron transfer, proton transfer, and conformational transitions in the active site that are controlled by the long-time dynamic behavior of the protein domains. *Shewanella* bacteria are among a class of microbes that are able to use insoluble mineral iron and other metals as final electron acceptor in the respiratory cycle. These ubiquitous bacteria are involved in many geochemical processes, including the redox chemistry of contaminated soils. Consequently, the study of these bacteria is of great importance to the DOE.

The nature of the processes involved in the respiratory reactivity of *Shewanella sp.* require that the simulation theories be based on quantum mechanical methods in contrast to many other proteins whose characterization can be achieved by means of classical simulations only. For single protein structures for example, such as crystallographic structures, selected aspects of such studies are certainly feasible, and many have been reported. Of course the function of a biomolecular system may also depend on conformational fluctuations, which can be slow compared to the time scales of molecular computer simulations, even with classical interaction potentials. In some of these cases the required simulation length may be a severe challenge. If, further, quantum-mechanical processes play a role, the study of such dynamical protein systems for the understanding or prediction of their biological functions can only be accomplished with high performance modeling software and high performance computer architectures.

Microbial iron-reduction as a factor in biogeochemical transformations receives increasing attention as possible geochemical evidence for the evolution of Earth, life on Mars, and the presence of deep sub-surface biospheres. In the control of redox balance and carbon cycle in sediments, iron and manganese often play an important role. Because of the wide distribution of iron-reducing bacteria, these microbial processes have significant implications for natural geochemical reaction, as well as the transport of metals and radionuclides.

A variety of phylogenetically diverse microorganisms have the ability to couple the oxidation of organic compounds to metal ion reduction, including species from *Geobacter*, *Desulfiromonas*, *Desulfovibrio*, *Shewanella* and *Pelobacter* genera. Among these, strains of *Shewanella putrefaciens* and *Shewanella frigidimarina* are able to reduce insoluble oxides of Mn(IV) and Fe(III). *S. putrefaciens* is able to reduce Al(III), as well as the soluble ions U(VI) and Tc(VII), and *S. alga* is able to reduce solvated Sr$^{2+}$, depositing these metals as insoluble lower-valency forms. Consequently, these species are attractive bioremediation agents for the removal of heavy metals from contaminated waters.

*S. putrefaciens* and *S. frigidimarina* are Gram-negative, facultative anaerobes that are commonly found in marine and freshwater sediments. In order to support anaerobic growth using insoluble Fe(III) as terminal electron acceptor, these iron-reducing Gram-negative bacteria require a complex electron-transfer pathway that links primary dehydrogenases in the cell interior with the insoluble, polymeric Fe(III) oxyhydroxides at the surface of the outer membrane. When *Shewanella sp.* is cultured under anaerobic or micro-aerobic conditions, a high content of c-type cytochromes is found in both the periplasmic and membrane fractions. CymA, a 21-kDa tetra-heme c-type cytochrome of the NapC/NirT redox family, is a quinol dehydrogenase anchored to the cytoplasmic membrane that oxidizes insoluble quinols, and reduces soluble periplasmic enzymes, such as nitrate, nitrite and fumarate reductases. In most bacteria, c-type cytochromes are anchored to the periplasmic side of the cytoplasmic membrane or are free periplasmic enzymes. However, a number of c-type cytochromes have been identified in the outer membrane of *Shewanella sp.* grown under anaerobic conditions. Reduced forms of these cytochromes are readily oxidized by addition of Fe(III), suggesting that these enzymes form the essential components of electron transfer through the outer membrane of living cells to insoluble metal oxyhydroxides. OmcA, an 85-kDa cytochrome c located in the outer membrane of *S. frigidimarina*
NCIMB400 (previously classified as *S. putrefaciens* NCIMB400) was found to contain 10 low-spin bis-His ligated c-type hemes. A number of soluble c-type cytochromes are found in the periplasmic space of anaerobically grown *S. frigidimarina* NCIMB400, including a 64-kDa tetra-heme flavocytochrome c, fumarate reductase (Fcc3) and a 64-kDa iron-induced tetra-heme flavocytochrome c, fumarate reductase (Ifc3). Fcc3 is the only fumarate reductase present in *S. frigidimarina* under normal physiological conditions. Ifc3 is not expressed in cells that were grown aerobically or anaerobically with available terminal electron acceptors other than Fe(III).

Much is unknown of the electron transfer pathway from the redox centers in the cytoplasmic membrane to the electron acceptors outside the outer membrane. Redox enzymes generally contain multiple redox cofactors mediating electron transfer through the enzyme or to the active site, regulating the catalytic activity of the enzyme through oxidation state changes, or coupling reactions such as binding and pumping of ions. The soluble flavocytochrome c, fumarate reductases of *Shewanella* sp. consist of a single polypeptide organized into three distinct domains. The N-terminal cytochrome domain contains four covalently bound c-type hemes in which the Fe is liganded by histidine side chains. The flavin adenine dinucleotide (FAD) is non-covalently bound to the flavin domain. The C-terminal clamping domain consists of a four-stranded anti-parallel sheet surrounded by seven short helices, and is likely involved in controlling access to the active site and to be inducing conformational changes of the substrate fumarate that initiate reduction.

The active site regions of fumarate reductases of a number of bacteria, including *Escherichia coli* and *Wolinella succinogenes*, are conserved in the *Shewanella* sp. flavocytochrome c. Available crystal structures are listed in Table I. The overall structure of these enzymes is significantly different, however. The fumarate reductase of *E. coli*, for example, consists of four subunits, and is a membrane-bound bi-directional enzyme. The fumarate reductases Fcc3 and Ifc3 of *Shewanella* are unidirectional, soluble enzymes.

The principal function of Fcc3 and Ifc3 is the reduction of fumarate to succinate. The proposed mechanism of the reaction is based primarily on inspection of the crystal structures. Fumarate is held firmly in place on top of the isoalloxazine rings of FAD by hydrogen bonding interactions between the oxygen atoms of one carboxylate group with conserved residue Arg544 (*Shewanella frigidimarina* NCIMB400 Ifc3 numbering), residue Gly547, and conserved residue His503. The oxygen atoms of the other carboxylate form hydrogen bonds with conserved residues His364, Glu377 and Thr376. Upon binding of fumarate in the active site, the second carboxylate group is twisted out of the plane of the molecule as a result of closure of the active site by movement of the capping domain. This twisting results in the formation of a net dipole in the central carbon-carbon bond, which facilitates the transfer of a hydride from the N5 atom of the activated FAD to the positive carbon atom closest to the twisted carboxylate group. In a subsequent step, the carbanion intermediate is protonated.

The transfer process by which the soluble fumarate reductases of *Shewanella* sp. acquire electrons from the cytoplasmic membrane-bound quinols is not well understood. CymA, a membrane-bound tetra-heme cytochrome c, has been suggested to be involved in this electron transfer. The fact that three of the four hemes in the soluble fumarate reductases are easily accessible to solvent, suggests that a direct transfer of electrons from cymA is possible.

The relative position of the hemes in the fumarate reductases is such that the internal electron transfer to the FAD is fast. The distances between the hemes in Fcc3 of *S. frigidimarina* range from 3.9 to 8.0 Å, and the distance from the last heme to FAD is 7.4 Å. Potentiometric measurements and protein film
voltammetry experiments have shown that the reduction potentials of the hemes (see table II, and figure 4) are low compared to other fumarate reductases. This result is consistent with the unidirectional catalysis of the soluble fumarate reductases and bidirectional catalysis of the membrane-bound enzymes.

Recent results of voltammetry experiments\(^8^2\) have raised the possibility of dual electron transfer pathways in Ifc\(_3\) of \(S.\ frigidimarina\) NCIMB400. This would be consistent with the fact that at least three of the four heme-groups are near the surface of the protein.

The origin of the proton is subject to speculation. Several residues have been proposed as the proton donor, primarily based on their proximity to the substrate in the crystal structure. His365 has been proposed as the acid/base catalyst based on reduced activity of the \(E.\ coli\) fumarate reductase after substitution of the equivalent His232 in that enzyme by serine.\(^8^3\)\(^8^4\) It has been argued,\(^8^5\) however, that this reduced activity identifies His365 as a significant residue for enzymatic activity, but not as essential. Also, a recent high-resolution crystal structure suggests that atom N3 of this His365 is involved in a hydrogen bond to a backbone amide group and, therefore, is neutral. An alternative histidine, His504, has been suggested as the proton donor based on its proximity to FAD in a crystal structure of \(S.\ frigidimarina\) NCIMB400 Ifc.\(^8^6\) Although histidines are commonly involved in proton transfer, and mutation experiments identified histidines to play an important role in fumarate reductases, Arg402 through atom NH2 has been suggested as the most likely source of the proton. This is based both on its distance to the fumarate, and the experimental observation that mutation of this residue to alanine completely eliminates fumarate reduction activity.\(^8^7\) The proposed proton transfer occurs through the conserved residues Arg381, Glu378 and Arg402.

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