# Molecular Simulations of Enzyme Catalysis

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## Abstract

Molecular modelling and simulation techniques have proved **powerful** tools for helping to understand how proteins and other biomacromolecules function at an atomic level. The study of enzyme reactions is a particularly challenging application of these methods because of the variety of processes of differing length and time scales that can contribute to catalysis. Among these are the bond-breaking and forming chemical steps, the diffusion of ligands into and out of the active site and conformational changes in the enzyme's structure.
This contribution gives a synopsis of the range of molecular simulation techniques that are available for studying enzyme reactions with particular emphasis on methods designed for the investigation of the chemical catalytic steps. The capabilities and limitations of current approaches will be described and possible future developments discussed. Special attention is given to the interface between molecular simulation and systems biology modelling and to how the STRENDA guidelines would need to be adapted to allow the reporting of enzyme data determined from simulation.

## Introduction

Numerical modelling and simulation are important tools for the study of biological systems and will undoubtedly become more so as the power and the sophistication of computers and their algorithms increases [1]. Enzyme reactions represent a particularly challenging area for simulation because of the wide range of length and time scales upon which processes that are important to catalysis occur.

The aim of this article is to provide an overview of the state-of-the-art in the simulation of enzyme reactions at an atomic level. It starts with a brief summary of the types of method that exist for simulating different aspects of an enzyme reaction and is followed by a more detailed presentation of the principles behind and an application of one particular approach – the hybrid potential method. The next sections highlight some of the advantages and limitations of current simulation techniques and the article terminates with a discussion of how atomic-level simulation could contribute to systems biology modeling and with various recommendations for how the STRENDA guidelines would need to be modified to report data derived from simulation [2].

## Methods for Simulating Enzyme Catalysis

A number of processes, that span a wide-range of length- and time-scales and the relative importance of which varies with the enzyme, contribute to an enzyme-catalyzed reaction [3]. For an isolated enzyme, these processes include: (i) diffusion and binding of substrates in and out of the enzyme's active site; (ii) conformational structural changes, such as loop movements or domain closure, that may be necessary for substrate binding and release or for catalytic activity; and (iii) chemical catalytic steps that involve the breaking and forming of bonds and the transfer of electrons. In addition, there can be other indirect phenomena which influence a reaction. Thus, for example, the optimum catalytic activities of many enzymes can only be attained if they are in specific states, such as when they are bound to non-substrate ligands, when they are covalently modified in some fashion or when they are oxidized or reduced.

Because of the variety of processes entering into enzyme catalysis, no single theoretical technique suffices for modelling an enzyme reaction and so a diverse series of approaches have been developed [4]. Three of the most important categories of technique illustrated in Fig. 1 and are:

1. Quantum chemistry (QC). These methods are appropriate for studying the chemical steps in catalysis as they can be used to compute the wavefunction and, hence, the electron density of a molecular system. The most accurate methods are the *ab initio* and density functional theory methods but they are also the most expensive. They can be used to treat systems of a few tens of atoms on time-scales of the order of tens of picoseconds. By contrast, the less accurate but also

quicker semi-empirical QC methods can handle systems of a few hundred atoms on time-scales of a few nanoseconds.

2. Molecular mechanics (MM) and classical molecular dynamics (CMD). MM techniques are typically much faster than QC methods as they employ empirically-derived functions to calculate the potential energy of a system. They have the disadvantage, though, that they are less generally applicable than QC methods and are inappropriate for simulating chemical reactions. In conjunction with CMD, they can be used to simulate systems of up to several tens of thousand of atoms for time-scales of a few hundred nanoseconds. These methods are particularly well-adapted to studying processes, such as conformational change and ligand-binding, where atomic-level detail is needed but no chemical reactions occur.

3. Coarse-grained (CG) models and Brownian dynamics (BD). Unlike the QC and MM techniques, CG models of a molecular system do not attempt to represent its full atomic detail – instead, atoms are grouped and modelled as larger particles. As an example, common CG models of proteins use one or two particles to describe each amino acid rather than the ten to twenty that would normally be required [5]. CG models also often treat the solvent with some sort of continuum model and dispense with a particle-based representation altogether. CG models, because of their simplicity, can be used to study very large molecular and macromolecular systems and, in conjunction with BD, to simulate processes on time-scales of up to the order of milliseconds. CG/BD models are well-adapted for calculating the values of ligand–enzyme diffusional-encounter rate constants (see reference [6] and the chapter by Stein and co-workers in this volume) and for studying other mesoscopic dynamical processes where atomic-level detail is not needed.

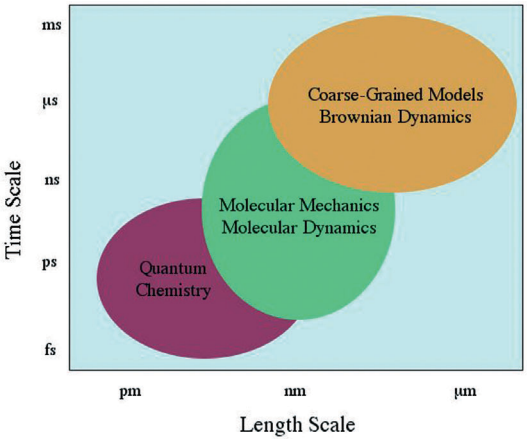


**Figure 1.** A schematic showing the appropriate length- and time-scales for three of the major classes of theoretical techniques that are employed for modelling enzyme reactions.

## Hybrid Potential Approaches

This section and the next focuses on a method, called the hybrid or combined QC/MM potential method, that is one of the primary research interests of the author and which is designed for the study of the chemical catalytic steps in an enzyme reaction [7]. The technique is based upon the following rationale. The modelling of a chemical reaction necessitates the use of QC methods but these are impractical or too expensive to apply to systems of more than about a hundred atoms. This rules out the possibility of studying molecules the size of enzymes. On the other hand, MM methods are very good at being able to handle large systems but are not very good at simulating reactions. Therefore, why not combine the strengths of both methods and use a QC technique to treat the reacting portion of the system and an MM approach to represent the remaining atoms which, although non-reactive, could nevertheless play an important role?

The first hybrid potential was conceived in the 1970 s by Warshel and Levitt for the simulation of the reaction catalysed by lysozyme [8]. It was not until the early-1990 s, however, that they started to be widely used. This lag was due, in part, to a lack of computer power but also because a number of technical issues had to be resolved to have potentials that were sufficiently precise and robust [9]. Hybrid potentials are now employed in all areas of molecular computational science, not just for the simulation of enzymes.

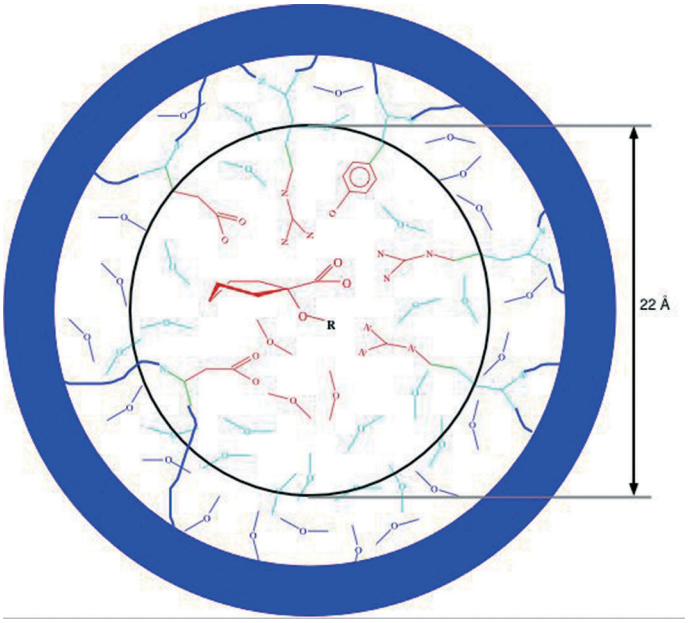


**Figure 2.** An illustration showing an example of how an enzyme, in this case influenza virus neuraminidase, is represented in a QC/MM hybrid potential simulation. The substrates and other enzyme and solvent groups that are implicated in the reaction are in the QC region (red atoms) whereas the remainder of the atoms in the system (light and dark blue atoms) are placed in the MM region.

Many flavours of hybrid potential have been developed but the simplest and also the most common involve the partitioning of a system between a single QC region and a single MM region. An example of such a partitioning is shown in Fig. 2. The proper formulation of a hybrid potential is quite intricate and depends upon the types of QC and MM potentials that are being combined. The crucial aspect of all formulations though is how the two potentials are coupled or, in other words, how the atoms of the QC and MM regions interact. In the hybrid potentials developed in the author's group, there are two classes of interaction [9,10]. The first class are the non-bonding interactions which occur in all hybrid potential studies. They comprise electrostatic terms between the electrons and nuclei of the atoms in the QC region and the charges of the atoms in the MM region and Lennard-Jones terms which account for the van der Waals and exchange–repulsion interactions between the two sets of atoms. The second class of interaction are the covalent QC/MM interactions which arise whenever a single molecule is split between different regions. Interactions of this type are nearly always present when studying enzyme systems as amino acid groups in the enzyme are almost always catalytically active. The treatment of these interactions is more complicated than that of the non-bonding ones but a number of competing algorithms have been developed that appear to be of roughly equivalent accuracy [7].

Although their formulation is distinct, hybrid potentials can be employed in much the same way as pure QC and pure MM potentials. Thus, for example, it is possible to perform geometry optimizations to locate the stable structures of an enzyme–ligand complex and to run molecular dynamics simulations to investigate the system's dynamics and to calculate its thermodynamics properties. Examples of the application of hybrid potentials in this way will be given in the next section and a full list of quantities accessible by simulation in the section after that.

## An Example of a Hybrid-Potential Study

The author's group has studied approximately fifteen enzymes with hybrid potential methods. These include the nickel–iron hydrogenase from *Desulfovibrio gigas* [11], the influenza virus neuraminidase [12], spinach acetohydroxyacid isomeroreductase [13], chorismate mutase from *Bacillus subtilis* [14], rat aldehyde dehydrogenase [15], various class A beta-lactamases and penicillin-binding proteins (PBPs) [16] and cAMP-dependent protein kinase [17]. The goal in most of these studies was a better understanding of the reaction mechanism catalysed by the enzyme although not exclusively. Thus, for example, in the hydrogenase work the aim was to characterize the active-site structures of various different redox intermediates in the catalytic cycle [11], whereas the beta-lactamase and PBP work was undertaken to determine the binding modes of different classes of antibiotics in the active sites [16]. Almost all the hybrid-potential simulations performed in the author's group have been performed with the group's own simulation package, called Dynamo [4,10], which was conceived specifically for studies with hybrid potentials.

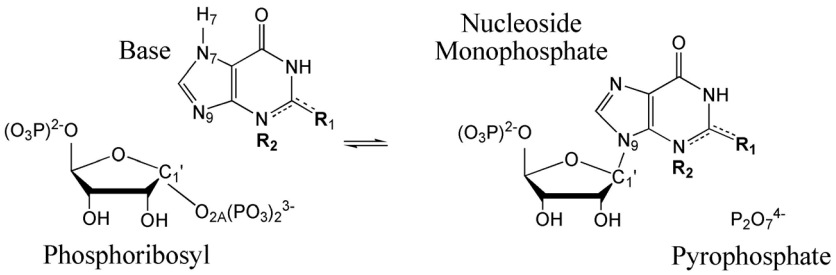


**Figure 3.** A scheme illustrating the reaction catalysed by the enzymes HGXPRTase and HGPRTase. ( R1, R2 ) are ( H, nothing ), ( NH2, nothing ) and ( O, H ) for hypoxanthine, guanine and xanthine, respectively. The nucleoside monophosphates corresponding to these bases are inosine monophosphate, guanosine monophosphate and xanthosine monophosphate. Specially labelled atoms which will be mentioned in the text are C1', H7, N7, N9 and O2A.

This section describes briefly a study of the enzyme hypoxanthine-guanine-xanthine-phosphoribosyltransferase (HGXPRTase) from *Plasmodium falciparum* (Pf) and its human homologue, hypoxanthine-guanine-phosphoribosyltransferase (HGPRTase). This work is representative of the other applications of the Dynamo program [11 – 17] and illustrates nicely what can and cannot be achieved with hybrid-potential techniques. Only a brief discussion of the work will be given here as full technical details of the simulations and a discussion of the results may be found in references [18 – 20].

Pf is a protozoan and is one of the Plasmodium species responsible for malaria. The reaction catalysed by HGXPRTase is shown in Fig. 3 and involves transfer of a phosphoribosyl group between a base and a pyrophosphate group. The enzyme is active with hypoxanthine, guanine or xanthine as the base. One of the interests of HGXPRTase is that there exists a human equivalent, called HGPRTase, that catalyses the same reaction except that it has a much reduced activity with xanthine. Despite this, the proteins share an 80% sequence homology in the vicinity of the active site although this drops to about 40% overall. This raises two questions; first, what causes this difference in specificity and, second, could this difference be used to design inhibitors that selectively target the Pf enzyme and, hence, act as potential antimalarial drugs.

The hybrid potential simulations that were performed were designed to partially answer both of these questions. First, because the simulations were carried out to investigate the chemical steps of the reaction only and so could not differentiate specificity due to other processes, such as substrate binding, and, second, because detailed information about the structures along the pathway of the reaction resulting from the simulations could help in identifying features that are important for inhibitor design. A summary of the salient features of the simulations and their results are as follows.

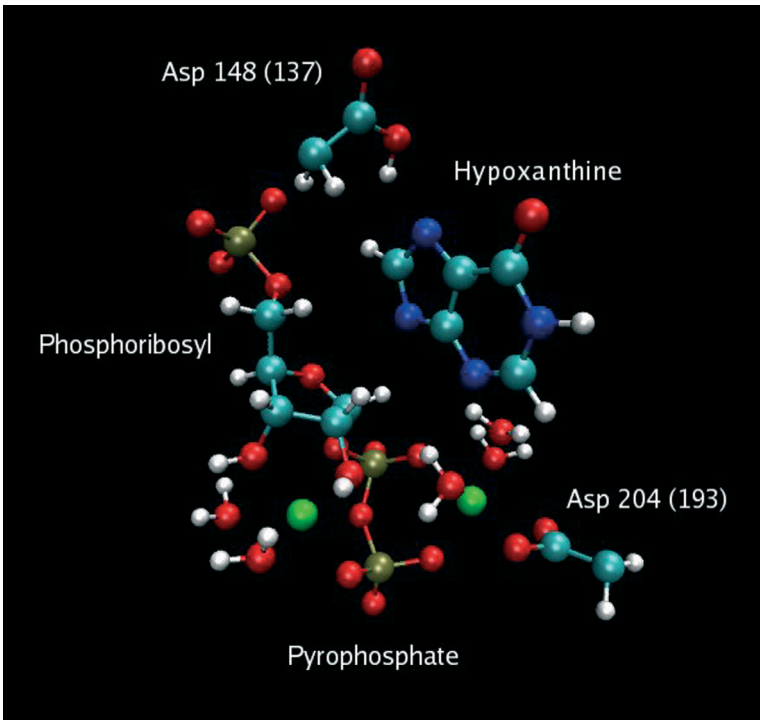


**Figure 4.** An image showing the atoms in the QC region of one of the HGXPRTase simulation models. Where appropriate the HGPRTase residue numbers are in brackets. The hypoxanthine is in its anionic form as its H7 proton has been transferred to Asp148. The magnesium cations are in green.

1. The reactions of HGXPRTase and HGPRTase were studied with both hypoxanthine and xanthine as substrates.

2. The techniques used to study reactions in systems as complicated as enzymes are currently such that it is not possible to expect the preferred mechanism to come out of the calculations directly. Instead, distinct hypotheses have to be made about how the reaction occurs and each hypothesis tested separately. The results of these tests may then be used to exclude particular mechanisms, if, for example, there is disagreement with experimental data, or, alternatively, to indicate that a mechanism is plausible. In the case of the HG(X)PRTases, the hypotheses tested included: (i) passage by a dissociative (SN1-like) mechanism with a stable dissociated intermediate; (ii) passage by various associative (SN2-like) mechanisms without stable intermediates; (iii) investigation of these mechanisms with different ribose sugar conformations; and (iv) investigation of these mechanisms with different protonation states for critical groups in the active site region.

3. Each hypothesis requires a separate simulation and simulation system. These typically comprised about 23,000 atoms including the enzyme, substrates, sur-

rounding water and counterions. All these atoms were treated in the MM region except for about 80 atoms which were put in the QC region (see Fig. 4). These included the substrates, the two magnesiums in the active site, their coordinating water and amino acid residues, and one or two catalytically active amino acid residue side chains.

4. The reaction mechanism for each simulation system was mapped out using a mixture of three different techniques: (i) saddle-point methods for locating the critical transition-state (TS) structures; (ii) reaction-path methods that generate intermediate structures for the mechanism by interpolating between the reactant and product structures; and (iii) free-energy calculations that determine the free energies for a mechanism as a function of specific reaction-coordinate variables. To give an idea of the computational expense required to test a single hypothesis, a complete study using all three techniques consumed approximately 20,000 hours (or 2.25 years) of computer time. Fortunately this was only necessary in a few instances as most hypotheses could be rejected with much less effort.
5. The preferred mechanism (of the hypotheses that were tested) was found to be identical with similar energetics for both enzymes and both substrates. The mechanism had two steps with an initial transfer of the proton H7 from the base to the adjacent aspartate side-chain followed by phosphoribosyl transfer. The second step was rate-limiting with a barrier of between 70 and 80 kJ per mole. This is in reasonable agreement with the experimental value of 65 kJ per mole and is the type of accuracy that can be expected from simulations of this type. These results indicated that the chemical steps are not responsible for the difference in specificity between HGXPRTase and HGPRTase but are due to other causes, such as binding.
6. Despite the identical mechanisms, an analysis of the TS structures for the rate-limiting phosphoribosyl transfer step in the two enzymes revealed some significant structural differences which could be important in inhibitor design. The most striking of these were the C1'-N9 distances which had average values of 2.56 and 1.82 Å in the Pf and human TS structures, respectively, and the C1'-O2A distances whose average values were 1.80 and 2.29 Å.

## Quantities Accessible by Computation

Simulations are not a replacement for experiment but a complement, the results of which serve to test particular hypotheses about an enzyme reaction and to spur the design of new experiments. Quantities that can be determined more or less routinely by computation include:

1. Structures of stable enzyme–ligand complexes at various stages of the catalytic cycle.
2. Mechanisms. These can be elucidated by finding paths that link reactants and products and pass via known intermediate structures. The structures of unstable

species along the reaction path, such as saddle points or TSs, are inaccessible experimentally but they are of both fundamental and applied interest. First, they are necessary for calculation of the additional quantities occurring later on this list and, second, they are useful in processes, such as inhibitor design, for giving insight into the properties of a ligand that are necessary for tight-binding to an active site.

3. Free energies and associated thermodynamic quantities such as enthalpy and entropy. Free energies are determined as differences between stable or unstable structures. In the former case, the energies may be related to the equilibrium constants between species whereas, in the latter, they can provide an estimate of the rate of a process via transition state theory (TST).
4. Rate constants. For some processes, such as the diffusional encounter of an enzyme with a ligand, rates are calculated directly from simulation. In other cases, such as when investigating the chemical steps in the reaction, it is more usual to first obtain the TST estimate of the rate constant via free-energy calculations and then to correct the TST value for dynamical and quantum effects.
5. Kinetic isotope effects. These may be calculated if the TS structures for a mechanism have been calculated.

Simulation studies of enzyme reactions can require much effort, testing and trial and error. They have the advantage, though, that once a set of simulation systems has been obtained that encapsulate the process being studied, it is straightforward to rerun them under different physical conditions (ionic strength, pH value, pressure, temperature, etc.) or after (limited) mutations have been made in the enzyme or substrate structures.

## Limitations of Current Approaches

Current modelling and simulation methods can provide much useful information about specific aspects of enzyme catalysis when properly applied. They do, however, have some fundamental limitations, among the most important of which are:

1. Almost all simulations require as input protein structures that are determined experimentally, usually by X-ray crystallography, but sometimes by other techniques, such as NMR. The requirements on the precision of these structures depends upon the type of simulation being performed. The results of hybrid potential calculations of the catalytic chemical steps in an enzyme reaction are often very sensitive to the arrangement and orientation of groups in the active site and so it is normal to start with high-resolution protein structures (of 2 Å resolution or better) that have been obtained in the presence of substrates, inhibitors or TS analogues. By contrast, calculations of the rates of diffusional encounter between an enzyme and its substrates are less stringent but, even so, structures need to be of sufficient resolution that accurate representations of the enzymes' charge distributions can be created.

2. Simulation studies are computationally intensive. The calculation of diffusional-encounter rates is one of the cheaper types of simulation and takes on the order of a day (or few) on a modern single processor computer for an average size protein. At the other extreme, the computation of free-energy profiles for the chemical steps in a reaction using hybrid potentials typically require several thousand hours of computer time and so special multiprocessor parallel computers must be employed.

3. There are still some aspects of enzyme catalysis that cannot reliably be studied with existing simulation techniques. Two specific examples are: (i) the investigation of reactions in which the enzyme undergoes a significant, but unknown, conformational change.
   Interpolative methods of reasonable precision are available for predicting how a conformational change occurs if structures of the two end states are available but extrapolative prediction is much more difficult; (ii) current QC techniques may not be accurate enough when studying systems with complicated electronic structures, such as radicals and transition metal complexes.

4. Probably the great majority of simulation studies published to date are irreproducible. There are two reasons for this: (i) there is a very large diversity of simulation methods and there has been little attempt at standardizing them. Even with techniques that are nominally the same, comparison can be impractical due to differences in the way that the techniques are programmed or in the parameter sets that are used for the simulations; (ii) much information is needed to reproduce a simulation with a particular program. This includes the simulation conditions, miscellaneous parameter sets and other data, such as atomic coordinates. Many journals do not require that this data is made available as a condition of publication, and, for those that do, there is little enforcement. It should be emphasized that the situation is generally better for calculations performed with purely QC techniques as these are easier to standardize [21]. Therefore, it is, in principle, possible to obtain ”identical” results for a particular system when using different QC programs as long as one has a detailed enough description of the methods employed and access to the starting data for the simulations.

## Connections to Systems Biology

Modelling the behaviour of metabolic, signalling and other pathways has been an area of active research since at least the 1970 s but it has received increased attention in the last few years. This has been due to the emergence of systems biology as a discipline which seeks to provide a multiscale description of how particular aspects of a cell or organism function by integrating diverse experimental and theoretical approaches [22]. One of the principal limitations in systems biology modelling is the lack of experimental data that characterizes how parts of the system behave. This is especially true for the simulation of enzymatic pathways as only for a small number of pathways are the parameters and the laws that govern the kinetics of all the constituent enzymes known.

Modelling and simulation will not be able to resolve this impasse by themselves but they clearly have a role to play given the difficultly in obtaining much of this data experimentally. A two-fold strategy would be appropriate:

1. The use of fast, approximate methods to estimate parameters, such as binding and rate constants, for the enzymes in a pathway. The precision required of these estimates will depend upon the enzyme and the pathway but it seems possible that, for many enzymes, relatively crude estimates will suffice as the simulated behaviour of a pathway will be robust to parameter changes within reasonable bounds. Of the methods described in this chapter, only the CG/BD approach for computing diffusional-encounter rate constants can be considered fast in the sense envisaged here and so new methods will need to be developed.
2. The application of computationally intensive methods, such as hybrid potentials, only for the investigation of the ”critical” enzymes in a pathway.

## Reporting Enzyme Data Obtained Via Simulation

The STRENDA guidelines for reporting enzymology data refer exclusively to data obtained experimentally and need adapting if they are to cover theoretically-derived values as well. A partial list of recommendations includes:

1. A way of distinguishing experimental and theoretical data. One model is employed by the Protein Data Bank (PDB) which separates data from the different sources [23].
2. Most modelling approaches require structures so the identity of the enzyme should be supplemented by the origin of the structures used in the simulation (e. g. the PDB codes).
3. An overview of the theoretical methodology employed. A literature reference would probably be enough for a standard method or a published result but in other cases a more detailed exposition would be necessary.
4. Details of the software and the machines used for the simulations.
5. A summary of the simulation protocol that includes, among other things, descriptions of the parameter sets, physical conditions and methods of data analysis.
6. A minimum set of data files that would allow other workers to reproduce the calculations given equivalent software and computing facilities. The type of data required would vary according to the simulation methodology but for the more complicated approaches it would mean providing starting coordinate and velocity sets for the enzyme system, parameter sets for the MM and QC potentials and sample program input files.

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