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Enzymatic transition states and dynamic motion in barrier crossing

Steven D. Schwartz and

Department of Biochemistry and the Department of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, New York, USA.

Vern L. Schramm

Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York, USA.

Abstract

What are the atomic motions at enzymatic catalytic sites on the timescale of chemical change? Combined experimental and computational chemistry approaches take advantage of transition-state analogs to reveal dynamic motions linked to transition-state formation. QM/MM transition path sampling from reactive complexes provides both temporal and dynamic information for barrier crossing. Fast (femtosecond to picosecond) dynamic motions provide essential links to enzymatic barrier crossing by local or promoting-mode dynamic searches through bond-vibrational space. Transition-state lifetimes are within the femtosecond timescales of bond vibrations and show no manifestations of stabilized, equilibrated complexes. The slow binding and protein conformational changes (microsecond to millisecond) also required for catalysis are temporally decoupled from the fast dynamic motions forming the transition state. According to this view of enzymatic catalysis, transition states are formed by fast, coincident dynamic excursions of catalytic site elements, while the binding of transition-state analogs is the conversion of the dynamic excursions to equilibrated states.

The catalytic power of enzymes to efficiently form transition states has been proposed, since the time of Pauling¹, to involve an equilibrium between the Michaelis complex and a stabilized transition state, providing a rationale for tight binding of transition-state analogs². Recent approaches explore enzymatic transition states from the viewpoints of molecular dynamics, geometry, electron distribution, the chemical lifetime of enzyme-bound transition-state species, and the motion of all atoms in the enzymatic ensemble as barrier crossing occurs^{3–10}. Here, we summarize our perspective on the nature of barrier crossing with an emphasis on combined experimental and computational approaches to the nature of enzymatic transition states and dynamic motions. We conclude that the concept of a stabilized or thermodynamically equilibrated enzymatic transition state should be replaced by a new view in which fast protein dynamics dominates barrier crossing.

Enzymatic catalysis presents daunting challenges of timescales. The catalytic cycle of most enzymes is on the scale of milliseconds, with typical turnovers of 1–100 ms¹¹. Continuous quantum mechanical/molecular mechanics (QM/MM) calculations are typically limited to a few nanoseconds of protein-ligand motions, a small fraction of a single catalytic turnover⁴. Transition-state lifetimes have a lower limit of femtoseconds, corresponding to bond

vern@aecom.yu.edu .

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vibration rates, as transition states are defined by the conversion of a bond-restoring mode to a translational mode. Common language for the action of enzymes uses terms such as ‘tight binding of the transition state’, ‘transition-state stabilization’ and ‘transition-state intermediates’, suggesting that in some way enzymes may be able to form a stabilized or equilibrated form of the transition state (Fig. 1a).

Static models of transition-state species, constructed on the basis of kinetic isotope effects (KIEs), also give the impression of an equilibrated form¹². Quantum chemical interpretations of KIEs give detailed bond order and geometric features at the transition state^{13–16} and have provided transition-state structures for several enzymes^{17–19}. Molecular electrostatic potential surfaces of these transition-state models guide chemical synthesis of transition-state analogs, yielding inhibitors that bind more tightly than substrates by factors of 10^8 or more^{19–22}. Complexes of these inhibitors with their cognate enzymes provide structural models for computational analysis of enzymatic transition states¹⁵.

However, the enzymatic transition state is a state of maximum free energy and is not likely to exist in a thermalized equilibrium between the Michaelis complex, transition state and products. The Eyring formulation of transition-state theory for chemical reactions was cast as an equilibrium thermodynamic problem²³. The equilibrium assumptions of Eyring’s transition-state theory were resolved by Miller and Pechukas with purely dynamic derivations for transition-state theory, but these derivations have not been fully appreciated in understanding enzymatic catalysis^{20,24,25}. Dynamic contributions of protein motion to enzyme-catalyzed reactions have focused on conformational changes with microsecond to millisecond timescales, as these times are correlated with catalysis^{3,26,27}. Slow conformational changes prepare an enzyme to form the transition state, but because these motions are slow they are dynamically uncoupled from passage over the barrier, which occurs on a bond-vibrational timescale. Chemical bonds of interest in chemical biology have vibrational modes in the low femtosecond timescale, and this timescale will be the focus of our viewpoint in investigating transition-state barrier crossing.

Understanding the microscopic mechanism of the transition-state barrier is also pertinent to the generation of designed enzymes and catalytic sites in antibodies^{28,29}. Artificial enzymes have shown a disappointing lack of proficiency, suggesting that they possess incomplete design elements relative to authentic enzymes²⁹. The use of *de novo* design or the immune system to create artificial enzymes tailored to the recognition of transition-state mimics should result in superb catalysts if strong binding of the transition state is sufficient to achieve catalytic rate enhancement. Successful catalytic antibodies or ‘designer’ enzymes typically achieve rate enhancements in the 10^4 to 10^6 range, whereas enzymes commonly achieve enhancements of 10^{12} to 10^{20} relative to solution chemistry^{11,28–30}. Clearly, tight binding of a transition-state mimic is not sufficient to achieve highly proficient catalysis. Native enzymes, unlike evolved or designed enzymes, usually have large protein matrices. The recent use of chimeric enzymes demonstrates that dynamics from the full protein architecture are linked to transition-state formation (see below).

This article addresses atomic motion, reaction coordinate and transition-state lifetime as enzyme-reactant ensembles pass the chemical barrier to become products. Through an interplay of new experimental and computational approaches, we now understand, for several enzymatic reactions, how enzymatic dynamic motion is linked to catalysis. Examples include the relatively simple hydride and proton transfer of lactate dehydrogenase^{6,31} and the complex reaction of purine nucleoside phosphorylase, where the transition state involves bond loss, the possibility of a stabilized intermediate, group migration and bond formation in a single reaction coordinate^{32,33}. These examples combine applications of transition-state analysis, inhibitor design, computational dynamics and

transition-state lifetime to provide information on linked atomic motion of protein and reactants during barrier crossing. To our knowledge, there are no similar *ab initio* measurements of enzymatic transition-state lifetime. This perspective condenses our recent work on transition-state lifetime and attempts to integrate it with slower timescales for enzyme dynamic motion from the literature. This article requires selective referencing since the literature on dynamics in catalysis is vast and that on enzymatic transition-state lifetime is small.

Our conclusions are that the protein architecture of enzymes has been crafted by evolution to increase the probability of finding relatively rare dynamic interactions that permit rapid barrier crossing. Our perspective focuses on passage over the chemical barrier following the relatively slow events of substrate collision, binding and conformational changes that provide access to transition-state geometry.

We find that the passage over the barrier is rapid compared to the time for an enzymatic cycle (as in Fig. 1b). There is no evidence of a stabilized transition-state species in our examples, and the transition-state lifetime is in the low-femtosecond range— 10^{-10} to 10^{-12} of the time required for a catalytic turnover, even for a complex reaction. The relatively long lifetime of the Michaelis complexes permits a stochastic search through phase space for enzymes to achieve a rare but exact dynamic motion necessary for barrier crossing. In the few femtoseconds of the transition state's existence, the transition-state ensemble cannot equilibrate with surroundings and cannot be described on the basis of thermodynamic parameters. In this interpretation of enzymatic catalysis, the transition-state barrier is probabilistic in nature and is determined by the formation of rare promoting vibrations at the catalytic site that lower the barrier for bond change. This perspective is a departure from the view that the most important enzymatic dynamic motions occur at the same rate as catalysis^{3,10,27,34}. In our opinion, slow conformational changes occurring at a rate similar to that of enzymatic turnover are often linked to steps in the reaction coordinate that are distinct from chemistry (Fig. 1).

Protein architecture and transition-state structure

Transition-state structure directs inhibitor design

Kinetic isotope effects are the change in chemical reaction rate upon nuclear mass substitutions in a reactant^{12,35}. KIE can provide a two-state model of transition-state structure—the bond character of reactants in an experimentally defined reactant state relative to the reactants at the transition state^{36,37}. Structurally related enzymes can have distinct transition-state structures. Thus, human and bovine purine nucleoside phosphorylases (PNPs) share 87% overall amino acid identity and 100% identity of amino acids in contact with the reacting 6-oxypurine riboside, yet the transition-state structures differ substantially (Fig. 2). Bovine PNP is characterized by an early, partially dissociated transition state, while that for human PNP is a fully dissociated, S_N1 transition state^{5,17,18}. Transition-state information has provided access to picomolar inhibitors, with specificity to the cognate PNPs (see Fig. 2 for example)^{18,38}. Different transition states in closely related PNPs suggested involvement of the full protein dynamic architecture in transition-state barrier crossing—a hypothesis that can be tested by transition-state analysis in chimeric PNP.

Chimeric PNP has an altered transition state

Replacement of two residues more than 25 Å from the catalytic site of human PNP with those from the bovine enzyme created a chimeric enzyme (K22E:H104R; E:R-PNP) with largely unchanged catalytic properties (Fig. 2)³⁹. However, KIE analysis and transition-state

analog binding established a transition-state structure that is different from that of either parent (Fig. 2)⁴⁰.

Computational QM/MM analysis comparing the dynamic motions of native PNP and E:R-PNP established that mutation of the remote residues alters dynamic motion throughout the protein³⁹. Altered dynamics between His257 of E:R-PNP and the 5'-hydroxyl group whose motion influences the electronic structure of the transition state were implicated (Fig. 2 and see below).

Bovine PNP has an early transition state with a 1.8-Å partial bond to the leaving group, human PNP forms a later, classic S_N1 transition state, and the chimeric E:R-PNP transition state is late and dissociative (Fig. 2)^{17,18,40}. The reaction coordinate for PNP maintains the purine leaving group and the phosphate nucleophile in relatively stationary positions during the reaction coordinate, while the ribosyl group migrates toward the phosphate, forms a ribocationic transition state and continues migration until captured by bound phosphate⁴¹. This mechanism (nucleophilic displacement by electrophile migration) is now established for several ribosyltransferases and glycosyltransferases^{42,43}. Sugar cations are chemically unstable⁴⁴, but it has been proposed that catalytic sites can stabilize sugar cations to intermediates long enough to permit diffusional exchange of nucleophiles^{45,46}. Below, we discuss the ribocation-intermediate hypothesis for PNP by transition-path sampling and test for stabilization (tight binding) of the ribocation by binding isotope effects (BIEs)^{47,48}.

Tight or loose binding at the transition state?

If enzymes achieve their catalytic potential by binding tightly to and thereby stabilizing their transition states (Fig. 1a), the binding energy of the enzyme–transition state complex is proportional to catalytic rate enhancement^{2,11,49}. If, however, the transition state has a lifetime similar to a single bond vibration (a few femtoseconds), such short-lived transition states cannot energetically equilibrate through an enzyme ensemble during the transition state and cannot be at thermodynamic equilibrium with the system. In contrast, transition-state analog inhibitors are at thermodynamic equilibrium, and a thermodynamic box provides an approximation for the maximum binding energy of transition-state analogs (Fig. 1a). The bond vibrational environment for reactants at the transition state and for complexes of transition-state analogs can be explored in new experiments comparing KIE (transition-state environment) with BIE (transition-state analog environment)⁴⁷.

Bond environments at the transition state versus transition-state analogs

KIE experiments for PNP report on bond vibrational differences between reactants in solution and at the transition state^{18,35}. In contrast, BIEs report on bond vibrational differences between the inhibitor free in solution and in a stable complex with PNP^{47,48}. If the bond environments for the transition state and transition-state analogs are similar, bonds for similar atoms would be expected to experience similar constraints. If the transition-state analogs are bound more tightly than the transition state, more distortion is expected in bound inhibitors than in the transition state.

Kinetic and binding isotope effects for [5'-³H]inosine, a substrate, were compared with binding isotope effects for [5'-³H]ImmH, a 58 pM inhibitor of PNP, and [5'-³H]DADMe-ImmH, an 11 pM inhibitor of the same enzyme (Fig. 3)⁴⁸. The enzyme with inosine at the transition state causes a 3.2% KIE from transition-state interactions. When ImmH and DADMe-ImmH are bound in the catalytic site in isosteric geometry (from X-ray crystallography) with inosine, the 5'-³H BIE values are large at 12.6% for [5'-³H]ImmH and 29.2% for [5'-³H]DADMe-ImmH^{48,50}. These BIEs are unprecedented⁴⁷; they support an

unusually large distortion of the sp^3 geometry at C5' and rigid alignment of the 5'-hydroxyl group to cause a large change in the C5'-H vibrational modes for bound inhibitors⁵⁰.

Modest C5'-H bond vibrational distortion at the transition state and strong distortion for transition-state analogs is consistent with relatively loose catalytic interactions at the transition-state barrier but strong atomic distortion for the tightly bound transition-state analogs.

Dynamics and barrier passage

The difficulty of obtaining dynamic motion as reactions proceed through the transition state can be solved by the *ab initio* computational method known as transition-path sampling (TPS)^{51,52}. The relatively simple transition state of lactate dehydrogenase, where catalytic site chemistry involves hydride and proton transfer^{6,53}, and the more complex mechanism of PNP³³ have both been analyzed by TPS. This approach has also been used to follow the reaction dynamics of chorismate mutase⁵⁴. Despite these limited applications of TPS to enzymatic reactions, it is timely to describe this approach since it provides new, *ab initio* dynamic information for barrier crossing.

TPS for lactate dehydrogenase (LDH)

The first application of TPS to a chemical reaction in an enzyme was for the dehydrogenation of lactate by human heart lactate dehydrogenase (LDH)⁶ (Fig. 4). TPS is a Monte Carlo walk through trajectory space. An initial trajectory is found that connects reactants to products (Fig. 4a). Further trajectories are generated through a shooting algorithm that chooses a random time slice and perturbs the momentum of all atoms by a random amount chosen from a Gaussian distribution. For chemistry to occur, molecular mechanics must be augmented by a quantum treatment of atoms in the catalytic site. We used a QM/MM algorithm, and similar QM/MM techniques have been described extensively^{4,55}. These studies used a new set of code for CHARMM, even though there is now a TPS module included within CHARMM⁵³.

Transition-state properties of LDH

The nature of transition-state passage is revealed by finding the ensemble of transition states in the ensemble of reactive trajectories. A probabilistic definition of the transition state was defined as that position along a trajectory where random assignments of velocities result in equivalent (0.5) probability for progress to products relative to (0.5 probability) return to reactants. A representative commitment probability for the lactate dehydrogenase reaction is shown (Fig. 4c). Each trajectory has a distinct transition state, and it is not an a priori expectation that the members of the transition-state ensemble will show similarity. Comparison of the structures of many transition states for LDH computed in this way has shown that even though many paths are possible through the complex surface, all members of the transition-state ensemble show great similarity (Fig. 4d).

Protein modes linked to the LDH transition state

A final step in our analysis of the LDH reaction was identification of the reaction coordinate⁶. These are the atomic motions that are necessary for reaction to occur. In addition to motion of the hydride and proton, TPS analysis demonstrated that a promoting vibration extends through the body of the protein and is necessary for reaction to occur (Fig. 4e,f).

Clear features of transition-state barrier crossing include the following: (i) the transition state as identified by the committor probability (Fig. 4c) exists only for a fraction of a

femtosecond, and therefore no thermodynamic equilibrium is possible with the Michaelis complex; (ii) the enzyme is not static at the transition state, no stabilized complex exists and small-amplitude vibrations on the picosecond timescale are required for the reaction; and (iii) the reaction coordinate is ~50 fs from reactants to products (Fig. 4c). Thus, TPS analysis reveals a hierarchy of times involved in catalysis. Passage over the barrier occurs within 1 fs. Motions within the protein are necessary to prepare the reactant for this passage and occur on the picosecond timescale. Both of these timescales are many orders of magnitude faster than ligand binding and the conformational changes known to occur in Michaelis complexes before catalysis can occur^{31,56}.

In the next section, we apply TPS analysis to human PNP. This enzyme exhibits more complex dynamic behavior in barrier crossing. Even with more complex chemistry and reaction coordinate motion, its transition state is also characterized by femtosecond chemistry without a stabilized transition-state complex.

PNP

Transition-state analysis, structural biology and transition-state analog analysis have established the catalytic mechanism of PNP to involve a ribocation transition state. TPS was used to provide (i) an *ab initio* analysis of the transition state for comparison with that determined from isotope effects, (ii) the lifetime of the transition state and (iii) dynamic motions at the catalytic site linked to the transition-state barrier passage. Crystal structures of PNP in complex with tight-binding transition-state analog inhibitors showed ‘stacking’ of three oxygen atoms (O5', O4' and a phosphate oxygen atom), an unusual geometry since these electron-rich atoms are mutually repulsive (Fig. 2c)^{41,50}. Electrostatic interactions could serve to polarize the ribosidic bond in response to a dynamic compression mode for these oxygens and thereby create the ribocation transition state. This motion has been dynamically correlated with catalysis^{32,39}. Major questions for PNP are (i) whether the ribocation transition state established from KIE can be reproduced, (ii) whether the ribocation is stabilized, with a lifetime of multiple bond vibrations and (iii) how much time is required for the purine group loss, ribocation migration and capture by the phosphate nucleophile—a complicated reaction coordinate.

TPS for PNP

TPS was applied to trimeric PNP with one catalytic site filled with reactants and treated by QM/MM³³. Recall that the commitment probability for LDH (Fig. 4c) shows a subfemtosecond passage over the transition state with a sharp transition from reactants to products. In PNP the commitment probability shows residence in the transition-state region of approximately 10 fs, allowing time for the complex set of events shown above. But even with this longer transition-state time, the lifetime is far too short to permit thermodynamic equilibrium throughout the enzymatic structure. The interactions at the transition state indicate no features that suggest strong binding of the complex specific to the period of transition-state crossing, which is consistent with comparisons from BIE and KIE (see above).

Features characterizing the PNP transition state

In the Michaelis complex, weak van der Waals and hydrogen bond interactions are formed with distinct parts of the catalytic site ensemble (Fig. 5a). Leaving group hydrogen bond interactions to the purine base are approximately 3 Å away and fluctuate on the femtosecond timescale. Likewise, His257, in hydrogen bond contact with the 5'-hydroxyl oxygen stack, varies from >3 Å to approximately 2.7 Å on the femtosecond timescale. Finally, the phosphate nucleophile positioned under the ribosyl group is located so that the anionic, nucleophilic oxygen is >3 Å from the anomeric carbon. Dynamic fluctuations involving

more than a dozen enzyme-reactant interactions occur simultaneously on the femtosecond timescale. The long life of the Michaelis complex (milliseconds) leading to the 10-fs transition-state lifetime permits extensive dynamic searching to obtain simultaneous minimization of the interactions required for transition-state formation. To reach the transition state, a promoting vibration of His257 causes the oxygen stack to compress at the same time that the leaving group interactions to the purine are minimized, thus assisting departure of the purine (Fig. 5b). The ribocation forms and is attracted toward the phosphate anion to complete the reaction coordinate (Fig. 5c–e). The nine or more hydrogen and ionic bond interactions between enzyme and phosphate also contribute to the overall reaction coordinate since phosphate at the catalytic site is strongly polarized^{57,58}. Although the transition-state lifetime is ~10 fs, the reaction coordinate time is ~60 fs. More than 100 productive barrier crossings have been studied, and overlaid transition-state geometries support the symmetric ribocation transition state earlier established from KIE analysis (Fig. 6 and Supplementary Movie 1).

TPS and barrier crossing for other systems

The only other case in which enzymatic catalysis has been studied by TPS is that of chorismate mutase⁵⁴. Although a group of transition paths was gathered, no attempt was made to isolate the reaction coordinate. In addition to this work, there is a growing literature in which TPS is applied to nonchemical steps in biological systems—for example, the initial step in DNA repair⁵⁹, polypeptide folding^{60,61}, activation of a signaling protein⁶² and micelle fusion⁶³.

Other approaches to barrier crossing

Although this perspective focuses on the scant literature of enzymatic transition-state lifetime, we would be remiss to not mention the monumental literature on dynamics in catalysis, both computational and experimental. It is of interest to note that transition-state lifetimes have been estimated for the reaction of $H_2 + H$ in the gas phase, and remarkably, at 5–20 fs, they are similar to those in our enzymatic examples⁶⁴. Thus, once the dynamic search has optimized groups in an enzymatic catalytic site, reaction chemistry occurs on timescales familiar from gas-phase chemistry.

Many computational approaches to study barrier crossing in enzymes have the limitation of assuming starting point models for specific reactions. TPS is a departure from previous methods in that it is free of preconceived reaction parameters. Examples of other approaches include the application of Marcus theories to enzymes⁶⁵; these theories are most frequently applied to proton-coupled hydride transfer^{66–70}. Others working on hydride transfer have applied the Kuznetsov-Ulstrup models to fitting their experimental data⁷¹. Another major area of analysis is the rigorous application of transition-state theory to enzymatic reactions^{4,72–74}. Others have pioneered methods for condensed phases and later applied them to enzymes^{75,76}.

There are, however, caveats with each method. Kuznetsov-Ulstrup models (which are similar to Marcus theory), along with any Marcus model, assume statistical distributions of conformations. The same is true of any transition-state theory. While relatively slow motions are equilibrated and may be treated statistically, the importance of fast motions is likely to be lost in these modeling schemes. Recently, the concepts of “preorganization” and active site compression have been introduced into the views taken from Kuznetsov-Ulstrup modeling⁷⁷. This proposal is fully consistent with our views of conformational stochastic searches combined with rapid promoting vibrations^{6,15,31,33}.

Application of Marcus-like models of proton transfer to enzymatic reactions, the so called Kuznetsov-Ulstrup model (see above), assumes a fixed distribution of transfer distances rather than rapid protein motion that creates coupling of the protein to the transferred particle. These principles have been used to hunt for gating distances, and more recently as signposts for promoting vibrations similar to those described here^{78–80}. In one case, the promoting vibration is caused by substrate held in an unnatural geometry⁸⁰, a situation similar to the ribosyl oxygen interactions at the transition state of PNP. Other hydride transfer reactions (DHFRs) show similar signs of dynamics-modulated tunneling^{81–83}. In this case, loss of dynamic coupling in the promoting vibrations severely restricts the catalytic efficiency.

An evolving consensus is that enzymes accomplish the coupling of dynamics to particle transfer and more complex reactions in a variety of ways, and nature has made repeated use of promoting vibrations to assist catalysis.

While these approaches are consistent with our views of conformational stochastic searches combined with rapid promoting vibrations, it is important to note that there is a significantly different emphasis of physical viewpoint. Each of these theories views chemical reaction as a one-dimensional process influenced by the environment (a bath). The environment can be complex, but the reaction coordinate is inherently transition-state theory with a one-dimensional reaction coordinate with one or more dynamic bottlenecks to reaction.

In our view, enzymatic reactions are multidimensional in physical space and in the vast timescales that operate in enzymes. Motions from fully equilibrated millisecond-long conformational changes as well as femtosecond-long atomic vibrational modes contribute to and are part of complex reaction coordinates. Our TPS work, isotope effect measurements and transition-state experiments interrogate the fastest timescales and provide combined computational and experimental probes of barrier passage for an ensemble of reactions. This timescale view and complex reaction coordinate is compatible with previous theories of enzymatic action, but differs in its focus on transition-state lifetime and motions coupled to that timescale.

Others have proposed that transition-state formation in enzymes is driven primarily by electrostatic forces, with dynamic motion being unimportant⁸⁴. It has also been proposed that most enzymes form covalent or quasi-covalent intermediates⁸⁵. For one member of the hydride transfer family and one ribosyltransferase, the enzymes treated in this perspective, we propose that dynamic action is the driving force that creates variable electronic interactions between enzyme and reactants on the timescale of bond vibrational modes, thereby inducing barrier crossing. We suggest that deeper exploration with other enzymatic systems will yield similar findings.

Conclusions

We summarize recent experimental and computational evidence for a new perspective in understanding how enzymes accomplish transition-state barrier crossing. The standard view of a strongly bound transition state in thermodynamic equilibrium with the Michaelis complex and products is not possible in our examples. It is of course possible that other enzymatic reactions may differ from the present enzymes. Most likely, these would be enzymes with multiple transition states where reactive intermediates exist in deep but metastable wells. Even in the case of PNP, in which there is a short lifetime to the transition state, it is far too brief for there to be any full equilibrium with the enzyme. There is no evidence for equilibrated tight binding during barrier passage. Transition-state formation by

PNP is better described in the language of dynamic probability than by equilibrium chemistry.

How does this analysis of catalysis impact inhibitor design via the transition-state analog paradigm? It does not. Transition-state inhibitors capture the dynamic excursions that exist simultaneously to facilitate barrier crossing and capture them in a thermodynamically stable state. Transition-state analogs are stable molecules, able to lock the dynamic motion of the enzymatic transition state in place. The dynamic potential used for catalysis is transformed into thermodynamic energy, forming enzyme-inhibitor complexes with long lifetimes.

How can motions on such disparate timescales be coupled in catalysis? The answer is that enzymes are involved in complex stochastic searches through phase space. Slow configurational motions (microsecond to millisecond) are known to be critical for enzymatic activity. Such motions include loop and domain motions. What is becoming increasingly evident is that as these slow motions are executed, a hunt through far more rapid dynamics proceeds simultaneously. The thermodynamics associated with catalysis is not that of strong binding, but is an inversion of a Boltzmann distribution in an ensemble of enzyme conformations that may be viewed as a probabilistic hunt for that phase space where chemistry is possible.

If one asks what this analysis portends for artificial enzyme design, or even enzyme alteration, the implications are significant. Small and subtle motions of the protein backbone, even those remote from the catalytic site, are central to the function of the catalyst. It is now clear why catalytic antibodies or designed active sites are simply inadequate to accomplish the type of rate enhancement that evolution has selected for enzymes. New design principles incorporating dynamic contributions to catalysis are necessary to achieve the subtle motions with exquisite timing required for high-efficiency catalysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

1. Pauling LC. Molecular architecture and biological reactions. *Chem. Eng. News* 1946;24:1375–1377.
2. Wolfenden R. Transition state analogues for enzyme catalysis. *Nature* 1969;223:704–705. [PubMed: 4979456]
3. Hammes-Schiffer S, Benkovic SJ. Relating protein motion to catalysis. *Annu. Rev. Biochem* 2006;75:519–541. [PubMed: 16756501]
4. Gao J, et al. Mechanisms and free energies of enzymatic reactions. *Chem. Rev* 2006;106:3188–3209. [PubMed: 16895324]
5. Schramm VL. Enzymatic transition state theory and transition state analogue design. *J. Biol. Chem* 2007;282:28297–28300. [PubMed: 17690091]
6. Basner JE, Schwartz SD. How enzyme dynamics helps catalyze a chemical reaction in atomic detail: a transition path sampling study. *J. Am. Chem. Soc* 2005;127:13822–13831. [PubMed: 16201803]
The first report of enzymatic barrier crossing analyzed by transition path sampling.
7. Benkovic SJ, Hammes-Schiffer S. A perspective on enzyme catalysis. *Science* 2003;301:1196–1202. [PubMed: 12947189]
8. Bruice TC. A view at the millennium: the efficiency of enzymatic catalysis. *Acc. Chem. Res* 2002;35:139–148. [PubMed: 11900517]
9. Garcia-Viloca M, Gao J, Karplus M, Truhlar DG. How enzymes work: analysis by modern rate theory and computer simulations. *Science* 2004;303:186–195. [PubMed: 14716003]

10. Henzler-Wildman KA, et al. A hierarchy of timescales in protein dynamics is linked to enzyme catalysis. *Nature* 2007;450:913–916. [PubMed: 18026087]
11. Wolfenden R, Snider MJ. The depth of chemical time and the power of enzymes as catalysts. *Acc. Chem. Res* 2001;34:938–945. [PubMed: 11747411]
12. Biegeleisen J, Wolfsberg M. Theoretical and experimental aspects of isotope effects in chemical kinetics. *Adv. Chem. Phys* 1958;1:15–76.
13. Horenstein BA, Schramm VL. Electronic nature of the transition state for nucleoside hydrolase. A blueprint for inhibitor design. *Biochemistry* 1993;32:20658–20665.
14. Schramm VL. Enzymatic transition states and transition state analogues. *Curr. Opin. Struct. Biol* 2005;15:604–613. [PubMed: 16274984]
15. Schramm VL. Enzymatic transition states: thermodynamics, dynamics and analogue design. *Arch. Biochem. Biophys* 2005;433:13–26. [PubMed: 15581562]
16. Schramm VL. Enzymatic transition states and transition state analog design. *Annu. Rev. Biochem* 1998;67:693–720. [PubMed: 9759501]
17. Kline PC, Schramm VL. Purine nucleoside phosphorylase. Catalytic mechanism and transition-state analysis of the arsenolysis reaction. *Biochemistry* 1993;32:13212–13219. [PubMed: 8241176]
18. Lewandowicz A, Schramm VL. Transition state analysis for human and *Plasmodium falciparum* purine nucleoside phosphorylases. *Biochemistry* 2004;43:1458–1468. [PubMed: 14769022]
Isozymes of purine nucleoside phosphorylase with nearly identical catalytic site structures are found to form different transition states, linking protein architecture to transition state formation.
19. Singh V, Lee JE, Núñez S, Howell PL, Schramm VL. Transition state structure of 5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase from *Escherichia coli* and its similarity to transition state analogues. *Biochemistry* 2005;44:11647–11659. [PubMed: 16128565]
20. Gutierrez JA, et al. Picomolar inhibitors as transition state probes of 5'-methylthioadenosine nucleosidases. *ACS Chem. Biol* 2007;2:725–734. [PubMed: 18030989]
21. Murkin AS, Clinch K, Mason JM, Tyler PC, Schramm VL. Immucillins in custom catalytic-site cavities. *Bioorg. Med. Chem. Lett* 2008;18:5900–5903. [PubMed: 18778937]
22. Gutierrez JA, et al. Transition state analogues of 5'-methylthioadenosine nucleosidase disrupt quorum sensing. *Nat. Chem. Biol* 2009;5:251–257. [PubMed: 19270684]
23. Eyring H. The activated complex in chemical reactions. *J. Chem. Phys* 1935;3:107–115.
24. Miller WH. Quantum mechanical transition state theory and a new semiclassical model for reaction rate constants. *J. Chem. Phys* 1974;61:1823–1834.
25. Pechukas P, McLafferty FJ. On transition state theory and the classical mechanics of collinear collisions. *J. Chem. Phys* 1972;58:1622–1625.
26. Boehr DD, Dyson HJ, Wright PE. An NMR perspective on enzyme dynamics. *Chem. Rev* 2006;106:3055–3079. [PubMed: 16895318]
27. Hammes-Schiffer, S. Kinetic isotope effects for proton-coupled electron transfer reactions. In: Kohen, A.; Limbach, H.-H., editors. *Isotope Effects in Chemistry and Biology*. CRC Press, Taylor & Francis Group; Boca Raton, Florida, USA: 2006. p. 499-519.
28. Xu Y, Yamamoto N, Janda KD. Catalytic antibodies: hapten design strategies and screening methods. *Bioorg. Med. Chem* 2004;12:5247–5268. [PubMed: 15388154]
29. Alexandrova AN, Röthlisberger D, Baker D, Jorgensen WL. Catalytic mechanism and performance of computationally designed enzymes for Kemp elimination. *J. Am. Chem. Soc* 2008;130:15907–15915. [PubMed: 18975945]
30. Lewis CA Jr, Wolfenden R. Uroporphyrinogen decarboxylation as a benchmark for the catalytic proficiency of enzymes. *Proc. Natl. Acad. Sci. USA* 2008;105:17328–17333. [PubMed: 18988736]
31. Quaytman SL, Schwartz SD. The reaction coordinate of an enzymatic reaction: TPS studies of lactate dehydrogenase. *Proc. Natl. Acad. Sci. USA* 2007;104:12253–12258. [PubMed: 17640885]
32. Núñez S, Antoniou D, Schramm VL, Schwartz SD. Promoting vibrations in human purine nucleoside phosphorylase: a molecular dynamics and hybrid quantum mechanical/molecular mechanical study. *J. Am. Chem. Soc* 2004;126:15720–15729. [PubMed: 15571394]

33. Saen-Oon S, Quaytman-Machleder S, Schramm VL, Schwartz SD. Atomic detail of chemical transformation at the transition state of an enzymatic reaction. *Proc. Natl. Acad. Sci. USA* 2008;105:16543–16548. [PubMed: 18946041]
34. Henzler-Wildman KA, et al. Intrinsic motions along an enzymatic reaction trajectory. *Nature* 2007;450:838–844. [PubMed: 18026086]
35. Cleland WW. The use of isotope effects to determine enzyme mechanisms. *Arch. Biochem. Biophys* 2005;433:2–12. [PubMed: 15581561]
36. Schowen, RL. Hydrogen bonds, transition-state stabilization and enzyme catalysis. In: Kohen, A.; Limbach, H-H., editors. *Isotope Effects in Chemistry and Biology*. CRC Press, Taylor & Francis Group; Boca Raton, Florida, USA: 2006. p. 765-792.
37. Karsten, WE.; Cook, PF. Substrate and pH dependence of isotope effects in enzyme catalyzed reactions. In: Kohen, A.; Limbach, H-H., editors. *Isotope Effects in Chemistry and Biology*. CRC Press, Taylor & Francis Group; Boca Raton, Florida, USA: 2006. p. 793-810.
38. Taylor Ringia EA, et al. L. Transition state analogue discrimination by related purine nucleoside phosphorylases. *J. Am. Chem. Soc* 2006;128:7126–7127. [PubMed: 16734442]
39. Saen-Oon S, Ghanem M, Schramm VL, Schwartz SD. Remote mutations and active site dynamics correlate with catalytic properties of purine nucleoside phosphorylase. *Biophys. J* 2008;94:4078–4088. [PubMed: 18234834]
40. Luo M, Li L, Schramm VL. Remote mutations alter transition-state structure of human purine nucleoside phosphorylase. *Biochemistry* 2008;47:2565–2576. [PubMed: 18281957] Mutations 25–40 Å from the catalytic site are shown to alter transition state structure as established by kinetic isotope effects and binding of transition state analogs.
41. Fedorov A, et al. Transition state structure of purine nucleoside phosphorylase and principles of atomic motion in enzymatic catalysis. *Biochemistry* 2001;40:853–860. [PubMed: 11170405]
42. Vocadlo DJ, Davies GJ, Laine R, Withers SG. Catalysis by hen egg-white lysozyme proceeds via a covalent intermediate. *Nature* 2001;412:835–838. [PubMed: 11518970]
43. Bianchet MA, et al. Electrostatic guidance of glucosyl cation migration along the reaction coordinate of uracil DNA glycosylase. *Biochemistry* 2003;42:12455–12460. [PubMed: 14580190]
44. Jencks WP. When is an intermediate not an intermediate? Enforced mechanisms of general acid-base, catalyzed, carbocation, carbanion, and ligand exchange reaction. *Acc. Chem. Res* 1980;13:161–169.
45. Liu Q, et al. Covalent and noncovalent intermediates of an NAD utilizing enzyme, human CD38. *Chem. Biol* 2008;15:1068–1078. [PubMed: 18940667]
46. Parikh SS, Putnam CD, Tainer JA. Lessons learned from structural results on uracil-DNA glycosylase. *Mutat. Res* 2000;460:183–199. [PubMed: 10946228]
47. Lewis, BE.; Schramm, VL. Enzymatic binding isotope effects and the interaction of glucose with hexokinase. In: Kohen, A.; Limbach, H-H., editors. *Isotope Effects in Chemistry and Biology*. CRC Taylor & Francis; Boca Raton, Florida, USA: 2006. p. 1019-1053.
48. Murkin AS, Tyler PC, Schramm VL. Transition-state interactions revealed in purine nucleoside phosphorylase by binding isotope effects. *J. Am. Chem. Soc* 2008;130:2166–2167. [PubMed: 18229929]
49. Miller BG, Wolfenden R. Catalytic proficiency: the unusual case of OMP decarboxylase. *Annu. Rev. Biochem* 2002;71:847–885. [PubMed: 12045113]
50. Murkin AS, et al. Neighboring group participation in the transition state of human purine nucleoside phosphorylase. *Biochemistry* 2007;46:5038–5049. [PubMed: 17407325]
51. Bolhuis PG, Chandler D, Dellago C, Geissler PL. Transition path sampling: throwing ropes over rough mountain passes in the dark. *Annu. Rev. Phys. Chem* 2002;53:291–318. [PubMed: 11972010] Transition path sampling is described as a way to probe, in an unbiased fashion, the nature of barrier crossing in condensed-phase reactions.
52. Dellago C, Bolhuis PG, Chandler D. Efficient transition path sampling: application to Lennard-Jones cluster rearrangements. *J. Chem. Phys* 1998;108:9236–9245.
53. Caratzoulas S, Schwartz SD. A computational method to discover the existence of promoting vibrations for chemical reactions in condensed phases. *J. Chem. Phys* 2001;114:2910–2918.

54. Crehuet R, Field MJ. A transition path sampling study of the reaction catalyzed by chorismate mutase. *J. Phys. Chem. B* 2007;111:5708–5718. [PubMed: 17474768]
55. Brooks BR, et al. CHARMM: a program for macromolecular energy, minimization, and dynamics calculations. *J. Comput. Chem* 1983;4:187–217.
56. Deng H, et al. On the pathway of forming enzymatically productive ligand-protein complexes in lactate dehydrogenase. *Biophys. J* 2008;95:804–813. [PubMed: 18390601]
57. Deng H, Lewandowicz A, Schramm VL, Callender R. Activating the phosphate nucleophile at the catalytic site of purine nucleoside phosphorylase: a vibrational spectroscopic study. *J. Am. Chem. Soc* 2004;126:9516–9517. [PubMed: 15291536]
58. Deng H, Murkin AS, Schramm VL. Phosphate activation in the ground state of purine nucleoside phosphorylase. *J. Am. Chem. Soc* 2006;128:7765–7771. [PubMed: 16771490]
59. Hu J, Ma A, Dinner AR. A two-step nucleotide-flipping mechanism enables kinetic discrimination of DNA lesions by AGT. *Proc. Natl. Acad. Sci. USA* 2008;105:4615–4620. [PubMed: 18353991]
60. Chopra M, Reddy AS, Abbott NL, de Pablo JJ. Folding of polyglutamine chains. *J. Chem. Phys* 2008;129:135102. [PubMed: 19045125]
61. Juraszek J, Bolhuis PG. Rate constant and reaction coordinate of Trp-cage folding in explicit water. *Biophys. J* 2008;95:4246–4257. [PubMed: 18676648]
62. Ma L, Cui Q. Activation mechanism of a signaling protein at atomic resolution from advanced computations. *J. Am. Chem. Soc* 2007;129:10261–10268. [PubMed: 17655236]
63. Pool R, Bolhuis PG. Sampling the kinetic pathways of a micelle fusion and fission transition. *J. Chem. Phys* 2007;126:244703. [PubMed: 17614573]
64. Chatfield DC, Friedman RS, Schwenke DW, Truhlar DG. Control of chemical reactivity by quantized transition states. *J. Phys. Chem* 1992;96:2414–2421.
65. Marcus RA. Enzymatic catalysis and transfers in solution. I. Theory and computations, a unified view. *J. Chem. Phys* 2006;125:194504. [PubMed: 17129120]
66. Watney JB, Hammes-Schiffer S. Comparison of coupled motions in *Escherichia coli* and *Bacillus subtilis* dihydrofolate reductase. *J. Phys. Chem. B* 2006;110:10130–10138. [PubMed: 16706474]
67. Wong KF, Selzer T, Benkovic SJ, Hammes-Schiffer S. Impact of distal mutations on the network of coupled motions correlated to hydride transfer in dihydrofolate reductase. *Proc. Natl. Acad. Sci. USA* 2005;102:6807–6812. [PubMed: 15811945]
68. Hammes-Schiffer S. Impact of enzyme motion on activity. *Biochemistry* 2002;41:13335–13343. [PubMed: 12416977]
69. Agarwal PK, Billeter SR, Rajagopalan PT, Benkovic SJ, Hammes-Schiffer S. Network of coupled promoting motions in enzyme catalysis. *Proc. Natl. Acad. Sci. USA* 2002;99:2794–2799. [PubMed: 11867722]
70. Hatcher E, Soudackov AV, Hammes-Schiffer S. Proton-coupled electron transfer in soybean lipoxygenase. *J. Am. Chem. Soc* 2004;126:5763–5775. [PubMed: 15125669]
71. Knapp MJ, Klinman JP. Environmentally coupled hydrogen tunneling. Linking catalysis to dynamics. *Eur. J. Biochem* 2002;269:3113–3121. [PubMed: 12084051]
72. Garcia-Viloca M, Truhlar DG, Gao J. Reaction-path energetics and kinetics of the hydride transfer reaction catalyzed by DHFR. *Biochemistry* 2003;42:13558–13575. [PubMed: 14622003]
73. Liu H, Warshel A. The catalytic effect of dihydrofolate reductase and its mutants is determined by reorganization energies. *Biochemistry* 2007;46:6011–6025. [PubMed: 17469852]
74. Warshel A, et al. Electrostatic basis for enzyme catalysis. *Chem. Rev* 2006;106:3210–3235. [PubMed: 16895325]
75. Ruiz-Pernía JJ, Tuñón I, Moliner V, Hynes JT, Roca M. Dynamic effects on reaction rates in a Michael addition catalyzed by chalcone isomerase. Beyond the frozen environment approach. *J. Am. Chem. Soc* 2008;130:7477–7488. [PubMed: 18479090]
76. Roca M, Moliner V, Tuñón I, Hynes JT. Coupling between protein and reaction dynamics in enzymatic processes: application of Grote-Hynes Theory to catechol *O*-methyltransferase. *J. Am. Chem. Soc* 2006;128:6186–6193. [PubMed: 16669689]
77. Klinman JP. An integrated model for enzyme catalysis emerges from studies of hydrogen tunneling. *Chem. Phys. Lett* 2009;471:179–193. [PubMed: 20354595]

78. Hay S, Pudney CR, Sutcliffe MJ, Scrutton NS. Solvent as a probe of active site motion and chemistry during the hydrogen tunnelling reaction in morphinone reductase. *ChemPhysChem* 2008;15:1875–1881. [PubMed: 18668493]
79. Hay S, et al. Barrier compression enhances an enzymatic hydrogen-transfer reaction. *Angew. Chem. Int. Edn Engl* 2009;48:1452–1454.
80. Johannissen LO, Scrutton NS, Sutcliffe MJ. The enzyme aromatic amine dehydrogenase induces a substrate conformation crucial for promoting vibration that significantly reduces the effective potential energy barrier to proton transfer. *J. R. Soc. Interface* 2008;5(Suppl. 3):S225–S232. [PubMed: 18495615]
81. Kanaan N, Martí S, Moliner V, Kohen A. QM/MM study of thymidylate synthase: enzymatic motions and the temperature dependence of the rate limiting step. *J. Phys. Chem. A* 2009;113:2176–2182.
82. Bandaria JN, Dutta S, Hill SE, Kohen A, Cheatum CM. Fast enzyme dynamics at the active site of formate dehydrogenase. *J. Am. Chem. Soc* 2008;130:22–23. [PubMed: 18067303]
83. Wang L, Goodey NM, Benkovic SJ, Kohen A. Coordinated effects of distal mutations on environmentally coupled tunneling in dihydrofolate reductase. *Proc. Natl. Acad. Sci. USA* 2006;103:15753–15758. [PubMed: 17032759]
84. Warshel A, et al. Electrostatic basis for enzyme catalysis. *Chem. Rev* 2006;106:3210–3235. [PubMed: 16895325]
85. Zhang X, Houk KN. Why enzymes are proficient catalysts: beyond the Pauling paradigm. *Acc. Chem. Res* 2005;38:379–385. [PubMed: 15895975]

Figure 1.

Transition-state stabilization compared to free energy during an enzymatic reaction. **(a)** The transition-state stabilization proposal suggests a thermodynamic equilibrium in which tight binding of reactants (S) to the enzyme (E) at the transition state ($\ddagger$) is proportional to enzymatic rate enhancement ($k_{\text{enz}}/k_{\text{chem}}$). **(b)** Distinct timescales define protein organization leading to transition-state formation, barrier crossing and product release. Reaction barrier crossing is typically shown as taking 50–100 fs, whereas the yellow bar shows a transition-state lifetime of ~5 fs. There is no evidence for a high-energy intermediate, as indicated by the shallow barrier with the question mark.

Figure 2.

Transition-state structures of PNP and the effect of remote mutations. **(a)** The molecular electrostatic potentials are shown at the van der Waals surface as obtained from KIE and computational analysis of human PNP. The stick figures shown above correspond to the potential surfaces. The Michaelis constant for the substrate inosine is contrasted to the dissociation constant for the transition-state mimic DADMe-ImmH. Adapted with permission from ref. ¹⁸. Copyright 2004 American Chemical Society. **(b)** Comparison of the reaction coordinates for inosine arsenolysis catalyzed by human (HsPNP), bovine (BtPNP) and K22E:H104R chimeric human (Bt-HsPNP) PNPs. The highest energetic points are the chemical transition states. The first barrier represents dissociation of the *N*-ribosidic bond, and the second barrier corresponds to the attack of the nucleophile. A ribocation species is between these transition states (dashed blue line). BtPNP has an early dissociative transition

state (black). The HsPNP transition state features full ribocation character. The transition state for Bt-HsPNP involves some bond formation to the oxygen nucleophile. Reprinted with permission from ref. ⁴⁰. Copyright 2008 American Chemical Society. (c) The human PNP trimer, with Lys22 and His104 residues highlighted and the catalytic sites filled with Immucillin-H, a transition-state analog. The Phe159 loop interacts with the catalytic site on the neighboring subunit. The catalytic sites are located near the subunit interface. Lys22 and His104 are located at 45 Å and 25 Å away from the enzyme active site, respectively. The structure is taken from Protein Data Bank accession number 1PF7. (d) The active site residues near the bound transition-state analog Immucillin-G and the phosphate nucleophile. The ImmG:O59, ImmG:N49, H257:Nd and HPO₄:Op atoms are highlighted in red. (e) Active site residues in contact with Immucillin-G and phosphate. The dashed red bonds represent oxygens in close proximity in the transition state and constitute the three-oxygen stack.

Figure 3.

BIEs for $[5'\text{-}^3\text{H}]$ inosine, Immucillin-H and DADMe-Immucillin-H to human PNP. The KIE for inosine was measured for the arsenolysis reaction, and the BIE was measured with sulfate as an unreactive phosphate analog. Reprinted with permission from ref. ⁴⁸. Copyright 2008 American Chemical Society.

Figure 4.

Transition path sampling studies of catalysis by lactate dehydrogenase. **(a)** Schematic drawing of the shooting algorithm. Starting with a single reactive trajectory, an ensemble of reactive paths are generated by randomly choosing a time slice on which a perturbation of each momentum, chosen from a Boltzmann distribution, is applied. **(b)** A correlation function analysis of the resultant trajectories demonstrates that trajectories decorrelate after approximately 30 shooting moves. Panels a, b reprinted with permission from ref. ⁶. Copyright 2005 American Chemical Society. **(c)** A representative commitment probability for a reactive trajectory for the reaction catalyzed by lactate dehydrogenase. If one views the time in the transition-state region as the time spent with a probability of barrier crossing near 0.5, only a fraction of a femtosecond is spent in the transition-state region. **(d)** Overlays of atomic coordinates from a variety of paths through phase space to the transition state reveal that although many nonidentical geometries are sampled, the transition-state features are almost superimposable. **(e,f)** The monomeric unit of LDH and the promoting vibration that is found to be an integral part of the reaction coordinate. The promoting vibration is formed from amino acids extending from one edge of the protein backbone through the active site and to the opposite edge of the enzyme **(f)**. Panels c–f reprinted from ref. ³¹. Copyright (2007) National Academy of Sciences U.S.A.

Figure 5.

Steps in the phosphorolysis of guanosine by PNP. **(a)** PNP compresses a three-oxygen stack to polarize the ribosidic bond and create a ribocation. **(b)** Creation of the ribocation, with full separation of the guanine leaving group. **(c)** Migration of the ribose ring toward the phosphate nucleophile. **(d)** Formation of the product with a new bond to form α -D-ribose 1-phosphate. **(e)** The complex nature of this transition-state barrier process is shown through the committor distribution. The process involves a substantially longer lifetime than the reaction catalyzed by lactate dehydrogenase. The transition-state lifetime (0.5 committor probability) is approximately 10 fs. This time is too short for equilibration through the

protein. The snapshots shown here can be viewed as a movie (see also Fig. 6). Reprinted from ref. ³³. Copyright (2008) National Academy of Sciences USA.

Figure 6.

The starting point snapshot from a movie demonstrating protein and reactant motion for barrier crossing in human PNP by transition-path sampling. The full movie is available as Supplementary Movie 1. The starting point shows guanosine and phosphate as reactants, with the distances (in Å) to protein groups involved in barrier crossing. Reactants are treated by QM and the protein by MM. The ribosyl group is centered in the figure with the 4' ring oxygen in red. The cluster of blue atoms at 2 o'clock is the edge-on view of the guanine leaving group. Multiple interactions in phosphate activation are not shown here, but all protein and reactant atoms were included in the QM/MM calculations. Reprinted from ref. ³³. Copyright (2008) National Academy of Sciences USA.