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Perspective: Path Sampling Methods Applied to Enzymatic Catalysis

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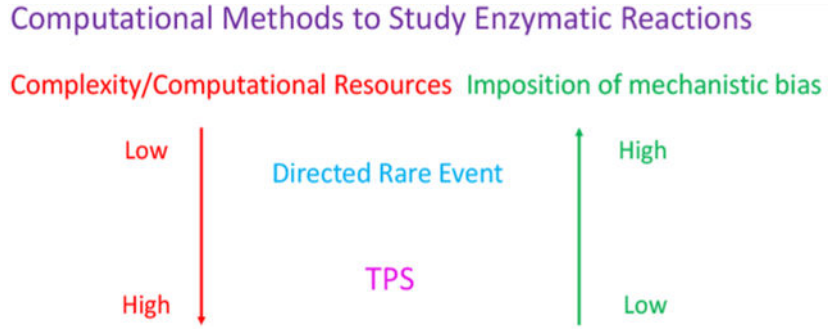
Abstract

This Perspective reviews the use of Transition Path Sampling methods to study enzymatically catalyzed chemical reactions. First applied by our group to an enzymatic reaction over 15 years ago, the method has uncovered basic principles in enzymatic catalysis such as the protein promoting vibration, and it has also helped harmonize such ideas as electrostatic preorganization with dynamic views of enzyme function. It is now being used to help uncover principles of protein design necessary to artificial enzyme creation.

Graphical Abstract



1. INTRODUCTION

One of the amazing success stories of computational chemistry over the past 40 years has been biomolecular simulation. Though perhaps viewed in the early days with some skepticism by a theoretical chemistry community that was focused on highly accurate computations on small molecular systems, the intervening decades have shown the techniques not just to reproduce experiment but to be able, in many cases, to lead experiment. That having been said, simulations that involve the integration of Newton's equation of motion for multiple 10s of thousands of atoms (and now hundreds or millions) are limited to relatively short time explorations of phase space simply because of the

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dimensionality of the systems studied and the limitations of even modern highly parallel supercomputers. This Perspective focuses on the challenge of the simulation of enzymatic catalysis. This adds the additional computational complication of requiring a quantum description for a reacting subsystem. Even when quantum mechanics is restricted to a small subsystem of the overall set of atoms, whatever level of theory is used further slows the exploration of phase space.

The computational complexity inherent in the study of large biological systems is only exacerbated by what might be termed the central challenge of the simulation of reactions in enzymes: reaction is an inherently rare event. Thus, if one were to create a computational system composed of the enzyme with substrate in a Michaelis complex and “push run” on the computer, you would be waiting far longer than any accessible computer simulation time available to observe a reactive event. To graphically demonstrate this, we note that most simple enzymes involve the making and breaking of one or a few chemical bonds—all processes that individually occur on the femtosecond or picosecond time scale. Given that most enzymes turn over on a time scale on the order of milliseconds, the actual chemical event is exceptionally rare. On the other hand, this chemistry is what must be studied if one wants to understand the proximal cause of the catalytic effect.

The challenge of how to overcome this rare event phenomenon has been a major focus of the biomolecular simulation community, and the result has been the development of a rich variety of enhanced sampling techniques. All these techniques have a straightforward goal—the accessibility of rare events on a time scale amenable to computation. There are far too many methods and subtle variations in them to present a full review here, but an exceptionally brief overview is necessary to give perspective to the path sampling methods we herein describe. Apologies to the developers of methods overlooked in this abbreviated description.

In the very broadest of brush, enhanced sampling methods fall into separate categories. In one category, the potential energy surface is altered in some fashion to accelerate transitions from one stable basin to another. This can involve forcing the system to specific regions of configuration space via umbrella sampling,[1] or raising the level of basins via judiciously chosen nonphysical pieces that “fill in the valleys”.[2–4] While extremely effective, these methods require either the assumption of a mechanism (a reaction coordinate or collective variable) or knowledge of the potential surface so that only the valleys are filled in, as opposed to saddle points. While these methods are effective, they contain the difficulty that might be termed “having to know the answer to get the answer”. Another approach involves such tricks as changing the temperature of a system to allow exploration of regions of the potential surface not accessible at the appropriate experimental temperature. In other words, such methods do not alter the potential energy of the system but rather the kinetic energy. There are sophisticated methods that project onto the true experimental temperature, but the dynamics created in such cases are simply not the dynamics in the real system. In addition, when studying such systems as proteins, the range of stability of the protein is highly limited, so the utility of high temperature exploration is not always certain.

A very different approach that involves neither the alteration of potential or kinetic energy is to analyze the potential energy surface to find the minimum potential energy path. The String method and the nudged elastic band method both fall into these categories.[5–12] These can be powerful tools, but at zero temperature, there are no contributions of entropy. There are finite temperature versions of such methods, but they are challenging to implement.[13]

A final method, very different from any of those mentioned, is the milestoning method.[14–16] Here an overall trajectory connecting what might be phase points separated by a very long time dynamical path is separated into swarms of smaller, more manageable pieces. The original version of the algorithm approximates the First Hitting Point Distribution (FHPD) by an equilibrium distribution at the milestone,[17] while more recent versions iterate to a potentially exact result.[18] This method has generated impressive results,[19–23] but finding the proper partition of space to efficiently generate results can be challenging.

A completely different concept, that we would be remiss to not mention, is the construction of specialty purpose computers solely designed to run single force-field molecular dynamics simulations at extraordinary speeds.[24–28] Such computations remain a minority of the applications in the literature for a variety of reasons. First, there are simply limited resources available for such computations. Second, such massively long computations will eventually compete systematic error with physiologic time scale. Third, and perhaps most importantly for the types of problems we study, is the need to employ some level of quantum mechanics to allow the making and breaking of chemical bonds. Because the details of the use of such QM/MM methods varies from problem to problem, it is hard to see how (though one assumes not impossible) such specially designed computers can be used to address the types of problems we consider.

This Perspective describes the use of a technique we and others have employed in the computational study of chemical reactions in enzymes: Transition Path Sampling (TPS.) The question of exactly how enzymes achieve the extraordinary rate accelerations that are manifest is a continuing question in theoretical chemistry and biophysics. To approach this question, we must study the actual chemical event as substrates are transformed to products. The actual transformation is often quite rapid and so not accessible by experiment; therefore, computation and theory have in many ways led in this effort to fully explain the huge rate accelerations. On the other hand, the chemical transformation happens relatively rarely, so study of this problem falls into the challenging subject of rare event simulation. TPS is specifically designed to address such problems. The method has origins in ideas of Lawrence Pratt[29] but was developed in the current form (and its many recent extensions) in David Chandler's group.[30–33] One essential idea behind the development is the recognition that just as one may make a random walk in configuration space form to a rigorously correct statistical distribution via an cceptance criterion, the same may be done in the space of dynamic paths in phase space. This coupled with efficient methods to generate ensembles of trajectories has the potential to be transformative in the study of chemical reactions in complex biological systems.

Since the introduction of TPS by the Chandler group, a large literature base has accumulated with new twists to the algorithm, each with new claimed efficiencies. This specific

Perspective is not designed as a review of these techniques, but in the next section we present a brief review of the method as we implement it. One of the main advantages to TPS is it is both unguided and unbiased. As described in the next section, after designation of a seed initial trajectory, further trajectories are generated by defined and semi-automated algorithms. There is no need to assume a collective variable or a reaction coordinate; in fact the reaction coordinate results from analysis of the TPS ensemble data. The creation of ensembles of reactive trajectories that are dynamically rigorous form a true statistical ensemble, and being unencumbered by mechanistic preconceptions is a major advantage to the methodology. Because the focus of this Perspective is on the results that these techniques have generated over the past 20 years and not on algorithmic development, details of the algorithms will be left to the references.

2. TRANSITION PATH SAMPLING ALGORITHM

The basic transition path sampling algorithm requires 2 components: a trajectory generation algorithm and an acceptance criterion that fulfills the Metropolis criterion and thus generates a proper statistical ensemble. The acceptance criterion has been given many times, and we refer the interested reader to Chandler's original paper. The trajectory generation method is much more of an art form, so we spend a bit of time detailing how we generate trajectories. Pictures such as Figure 1 show the concept but almost none of the practical techniques needed. The first step in a TPS study is generation of an initial trajectory. Such a trajectory need not be dynamically valid; it simply needs to connect a starting stable state with a final stable state. In our studies of catalysis, the initial and final states usually involve the making and breaking of a chemical bond. As we will describe below, our first application of TPS was to the reaction catalyzed by lactate dehydrogenase. The chemistry is shown schematically in Figure 2, and it involves the transfer of a hydride from NADH to pyruvate and a proton from the imidazole group of His-193 to the ketone oxygen of the pyruvate. The initial trajectory needs to cause chemistry to happen, so the method we use is to place a gentle restraint on the hydride and proton (which obviously are in the QM region of a QM/MM model) which causes the equilibrium conditions to shift closer to the C2 carbon of pyruvate and the proton similarly closer to the ketone oxygen of the pyruvate substrate. A schematic picture of how this perturbation works is shown in Figure 3. This results in the biased or seed trajectory, which is obviously not a dynamically rigorous trajectory. This initial trajectory does not explore all regions of configuration space equally (with more points closer to products than reactants,) but it does connect the reactant basin to the product basin.

At this point, we use a "shooting" algorithm in which all degrees of freedom are randomly assigned new velocities taken from a Boltzmann distribution with appropriate rescaling of velocities to conserve total energy. A successful trajectory will now fully connect the initial and final stable states. The way in which we generate the ensemble of trajectories is now up to us, if we find an appropriate way to sample phase space. Our approach is to start from a point we infer to be close to the transition state (guessed by examining bond forming and bond breaking distances) and perturb close to that point—say, randomly choosing 20 MD frames on either side of that location. As the computation matures, and we become more decorrelated from the initial bias trajectory, we progressively increase the size of that

possible perturbation window until we cover the entire breath of a trajectory. To do this at the beginning would almost guarantee a success rate of new trajectory generation that was tiny. There are other trajectory generation algorithms, but all boil down to one thing—the most rapid coverage of phase space and most rapid decorrelation from the biased seed trajectory.[34] We have tried other methods and find none obviously more efficient. One point needs to be made for anyone wishing to implement such an algorithm—it is not free of the need for human intervention. Because perturbations in momenta are made randomly, it is important to monitor for movement into nonphysical regions of phase space such as those with local unfolding.

The generation of trajectories provides only the first step. This huge quantity of data needs to be analyzed to gain insight into the mechanistic questions chemists wish to understand. The first step is the computation of a “committor”. One takes each frame on a given TPS trajectory and re-initiates all degrees of freedom with random momenta in each degree of freedom in the same way any shooting move is accomplished (again with total energy held constant). This will generate a probability of transformation into products or back to reactants. In the reactant and product wells we expect to find a probability of zero for transformation into the other, but at intermediate frames nonzero probabilities are found. The point at which there is a 50% probability of going forward to products or backward to reactants is the equicommittor point, and a statistical definition of a transition state for each trajectory. The collection of equicommittor points is a surface which we identify as the transition state ensemble or the stochastic separatrix. We find roughly 50 separate shooting moves at each point along the trajectory sufficient to generate smooth statistics for the committor. Examples of committors are given in Figure 4. Of note, in both cases the rise in committor is rapid compared with the overall turnover of the enzyme. In the case on the left, the reaction is direct; in the right, the committor shows evidence of brief intermediate formation.

Once the committors and stochastic separatrix have been generated, the next question the TPS ensemble can be used to address is the reaction coordinate across the transition state —in other words the set of all atomic motions both necessary and sufficient for reaction to occur. If we poise the system at a point located on the stochastic separatrix and hold what we believe the reaction coordinate degrees of freedom fixed while allowing all others to evolve, we expect to remain on the stochastic separatrix as the reaction coordinate is a surface orthogonal to the separatrix. Re-initiated trajectories should commit to reactants or products peaked about a probability of. 5. Putative reaction coordinate degrees of freedom can be identified and verified in committor distribution analysis by the variability of the separatrix,[35] neural network prediction,[36] likelihood maximization,[37,38] energy flux,[39,40] or for that matter trial and error of amino acid residues near the reacting atoms. In trial and error, the first attempt is always simply the atoms being transferred in any reaction; if this does not result in an appropriately peaked distribution of about 5, more degrees of freedom are added. Examples of both “bad” and “good” committor distributions are given in Figure 5. As we describe in the next section, this basic formalism was first applied in 2005 by our group, and it has now spread to a variety of other groups internationally. Broadening of the methodology was needed to make the method applicable to a broader range of problems and questions. The first advance was the ability to incorporate quantum dynamics into the

TPS methodology. Hydrogen transfer in enzymes has been suspected to involve quantum tunneling, and that is clearly not included in classical TPS simulations. TPS is an inherently trajectory-based method, so path integral methods seemed the logical extension. A variety of approaches had been suggested—we employed the Normal mode centroid method of Cao and Voth.[41] and we were able to implement such an approach in TPS first described by us in 2009[42] and used in an enzyme in 2015.[43,44] The method is technically demanding, so we simply refer the interested reader to the paper of 2009.

More recently,[45] we have applied a method of using both accepted and rejected trajectories from a TPS simulation to allow computation of a free energy barrier. This is of value for chemistry applications because chemists tend to think in terms of free energies and rates. From a free energy, an approximate rate may be calculated using a simple rate law. A similar method was first proposed by Radhakrishnan and Schlick in 2004,[46] but it did not see application to chemistry in complex systems. Details of our approach are given in the reference, but the basic problem faced is direct Boltzmann inversion of a TPS ensemble results in essentially no free energy barrier because the ensemble is defined by successful trajectories. We found this previously when we used a formal definition of free energy as the ensemble average of the reversible work.[44] What this showed was there is essentially no free energy barrier in the enzymatic reaction we were studying or the enthalpic barrier is largely erased due to the function of the enzyme. This in turn means any actual residual free energy barrier is due to the entropic search the enzyme must undergo to allow reaction.

Finally, analysis of the stochastic separatrix only provides information on the reaction coordinate during the rise of the committor from zero to 1—in the cases we have studied, a very tiny time window. We have begun to develop other analysis tools to apply to the ensemble of reactive trajectories to broaden this window of time.[47]

3. ENZYMATIC CATALYSIS STUDIED BY TPS

Early application of TPS were either to “toy” problems such as the gas phase rotation of alanine dipeptide,[48–50] or to conformational rearrangement in biological systems.[51,52] What I might term the “first age” of TPS applications to enzymatic chemistry involved a number of groups and a number of enzymatic reactions. The first application to an actual chemical reaction in an enzyme was published by our group in 2005.[53] This paper studied the hydride and proton transfer in the reaction catalyzed by lactate dehydrogenase. AM1 was used in a QM/MM GHO implementation.[54] It also showed that, in the ensemble of reactive trajectories, each reactive event was accompanied by a dynamic donor–acceptor compression mediated by the enzyme. This was proof of the existence of our previously proposed concept of a Protein Promoting Vibration (PPV) which we had inferred from the computations of spectral densities.[55–57] This work was followed 2 years later by a study of Creheut and Field of chorismite mutase.[58] While they were successful at generating an ensemble of reactive trajectories, they had less success using the trajectories to form detailed conclusions about the reaction coordinate of system—a focus of later work from our group and others. In fact, in 2007, we created a larger collection of reactive trajectories for the lactate dehydrogenase system and, for the first time, used the method of committor distribution analysis to find the true reaction coordinate.[59] The reaction, shown in Figure 2,

would normally have its reaction coordinate described as some set of proton and hydride motion, but committor distribution analysis described in the last section showed that the reaction coordinate must include motion of residues in the protein.

Following these initial successes, our group and others continued to apply the TPS methodology to the study of enzymatic reactions. In particular we generated ensembles of trajectories for the reaction catalyzed by Purine Nucleoside Phosphorylase[60] (Figure 6.) This reaction involves the breaking of heavy atoms bonds between the base and sugar moieties of nucleosides. We again did see a protein motion facilitating compression of highly electronegative oxygen. In solution, this reaction would be described as likely an SN2 reaction with the phosphate group the attacking nucleophile. In the enzyme, the phosphate does not approach in bonding distance until the glycosidic bond is fully broken, and thus the weakening of the bond comes from protein promoting vibrations mediated oxygen compression. More details of this reaction followed.[61,62]

In 2009 we used the method to provide the first comparison of homologous proteins from different species.[63] In the same year, Escobedo and co-workers suggested use of forward flux sampling[64]—a path sampling methodology closely related to transition interface sampling. As stated above, all methods of path sampling share similarities—this method divides path space into smaller configurational regions. In that same year Taraphder and Roy applied TPS methods to the study of carbonic anhydrase.[65] One continuing theme in all of this work had been the importance of protein dynamics as coupled to reaction.[66] One enzymatic reaction which has often been called the “hydrogen atom” of enzymology, dihydrofolate reductase (DHFR) is known to include important millisecond conformational dynamics as part of the overall catalytic cycle, but we found in 2012, through TPS simulations, that the enzyme is just too flexible to support direct coupling of rapid protein dynamics to chemistry.[67]

One of the most widely used techniques in computational enzymology is Transition State Theory (TST.) The method has been reviewed many times, but central to the method is the assumption that the bottleneck to reaction, the Transition State, may be found by studying the dynamics of trajectories—for example the location that minimizes recrossing. An interesting study from Dan Major’s group called these assumptions into question.[68] They found significant differences between transition state structures generated using the machinery of TST and those generated as equicommittor points on TPS ensemble trajectories. Rates are clearly highly averaged quantities, and it is entirely possible a multitude of transition states can yield the same average rate while resulting in very different mechanistic interpretations. In addition, the entire point to TST is to replace dynamics with a statistical assumption—that is that one or more transition states may be identified—and the distribution of phase points on this transition state fully determines rates. Even if this assumption is true, it says nothing about the way chemistry arranges to arrive at that point, so the use of TST for mechanism inference deserves continued study.

While TPS generates reactive trajectories, and the attendant reaction coordinate committor distribution analysis generates mechanistic information at the barrier crossing that is rigorous within standard computational caveats (QM/MM methods and often semiempirical

QM methods,) experimental support for the conclusions was lacking. The situation was partially addressed with the development of an experimental method to interrogate the importance of protein dynamics as coupled to chemistry in enzymes. The so-called "Born–Oppenheimer" enzyme replaced carbon, nitrogen, and hydrogen with heavy isotopic analogues.[69] When this was done for PNP, no changes in kinetic signatures of slow conformational motion were detected but on-enzyme chemistry was slowed by 30%. Using TPS we were able to show that this effect was caused by a mistiming of the oxygen compression described above, further validating the concept of the protein promoting vibration.[70] We next examined a putative heavy enzyme effect in Lactate Dehydrogenase,[71] and this was also confirmed by experiment.[72]

Beckham and co-workers applied TPS and committor distribution analysis to isolate the chemical mechanism of cellulose hydrolysis by cellobiohydrolase.[73] As the final two papers of this first age of TPS simulations of enzymatic reactions, our group found that enzymatic homologues have at times evolved mechanisms that are very different in atomic detail[74] and we verified our results on hydride transfer in DHFR using heavy enzyme experiments and computations.[75] Other groups have applied the technology to other systems. For example, understanding the function of molecular motors.[76,77] In addition, Taraphder and co-workers used TPS to study the reaction coordinate for proton transfer in carbonic anhydrase,[78] and Mayes and co-workers applied the technique to glycoside hydrolase.[79]

An enduring question in enzymology is the importance of quantum mechanical effects on the dynamics of enzymatically catalyzed reactions. The Klinman group originated many of the ideas and studies, and in certain enzymes, they have found unmistakable signs of quantum tunneling via extreme kinetic isotope effects at physiologic temperatures.[80–82] The question remained as to how important these effects are in some of the earliest enzymes studied for these effects, the alcohol dehydrogenases.[83–89] In order to study this effect with TPS, a quantum dynamics method is needed, and as we have described, we employed Centroid Molecular Dynamics as an approximation to the full path integral in quantum degrees of freedom. Applied to alcohol dehydrogenase along with reversible work free energy calculations, we found a very modest effect, but this is not at variance with the experimental results.[44] Again, the vast majority of the catalytic effect seems to originate from dynamics compression of donor–acceptor distances via a protein promoting vibration that virtually abrogates the barrier. It must be stressed that this is a very different picture of the catalytic effect in an enzyme. Transition state stabilization is simply a picture, but not a mechanism. The actual mechanism of the catalytic effect is provided by analysis of the TPS ensemble of trajectories.

At this point the focus of my group's work shifted from analysis of single enzymes to two closely related questions. First, can we design artificial enzymes, or at least modulate activity positively, and second, how did evolution build the coupling of protein promoting vibrations into enzymes? The questions are obviously coupled because understanding how nature accomplished this feat should inform our attempts to do it artificially. This work was motivated by the recognition that though theoretical chemists/protein designers had been attempting to design artificial enzymes for decades, there had been what can only be termed moderate success.[90] In fact, one of the most prominent protein designers directly stated that

not only were ab initio designs barely active,[91] the designers could not even distinguish why certain designs had some activity and others essentially none.[92] We took the view that given that these designs were based on principles on transition state complementarity and so stabilization, there must be a design principle that had been overlooked.

For our first attempt, we studied the heavy isotopic substituted protein of PNP and attempted to correct the timing of the compression we noted as a cause of the loss of catalytic efficiency. In our first attempt we were able to correct the timing[93] but the overall compression was not strong enough. In a second attempt at design, we were able to make a mutant heavy enzyme that was faster than the light enzyme (an inverse heavy enzyme isotope effect.[94]) We made a similar proposal for a mutant form or aromatic amine dehydrogenase, a more complex PCET chemical reaction.[95]

Attempting to address the design question from a different direction, we employed TPS studies of families of artificial catalytic proteins “designed” by directed evolution. The advantage to using this approach is the evolutionary process is completely agnostic to any specific mechanism of catalytic efficiency. Mutations are chosen purely because they (in these cases) speed chemistry, and it may be accomplished by transition state stabilization, electrostatic preorganization, or any other method. In fact, for a variety of laboratory evolved species we have found that initial designs based on static transition state complementarity are crafted by the optimization of rate to introduce specific coupling of protein dynamics to chemistry. We began the studies on the completely a-biologic Kemp eliminase reaction.[96] We did see evidence of the development of protein dynamics, but the starting structure is so rigid that the dynamics was quite modest. The next system we studied was the retro-aldolase reaction.[97–100] In this case optimization of chemistry results in much more significant inclusion of protein dynamics as a part of the reaction coordinate.[101] Knowing that protein dynamics is altered and in fact coupled to chemistry is only a first step, however. If one is to use this knowledge in an eventual design methodology, it is necessary to understand how the mutations that evolution employed caused this to happen. We were able to show it happens as regions of greater rigidity introduced by increased hydrogen bonding and greater flexibility created by reduced hydrogen bonding cause directed thermal energy transmission in the body of the protein.[102] We note similar effects have been posited to occur via hydrophobic packing.[80,103]

As a final addition to the use of TPS as a way to gain insight into the catalytic effect, we examined another well-known proposed mechanism of catalysis—electrostatic preorganization.[104,105] We followed dipole fields in the active site of the enzyme catechol *O*-methyltransferase and other enzymes using a theoretical analogy of Boxer’s Vibrational Stark effect measurements,[106–111] and we found that there is a change in electric field as the chemical reaction approaches the transition state, and in fact this change is caused by the motion of charged and polar groups that are part of a protein promoting vibration, tying the protein dynamics approach to the electrostatic approach.[112,113]

4. CHALLENGES AND FUTURE DIRECTIONS

The past 3 years has seen a significant expansion in the application of TPS to understanding enzymatic chemistry. We count 9 separate systems from 6 separate groups[47,114–121] being studied in this fashion. The technique is clearly gaining in importance, but there remain basic hurdles to more general application. The most important is simply time scale yet again. While TPS overcomes the extraordinary disparity in time scales in enzymatic catalysis between substrate binding and turnover as compared to chemical transformation, the length of trajectories is simply limited. One can always extend the needed QM/MM trajectories that are produced as part of a TPS ensemble, but it is clear they will never in general reach the millisecond time scale of turnover. Many critical conformational transitions are needed upon binding and before product release.

One approach is to combine Transition path sampling with other methods of rare event sampling—metadynamics is one appealing choice. There have been a few papers combining the methods. For example one approach uses metadynamics to help generate a seed TPS trajectory,[122] and a second mixes both approaches to create a method to efficiently compute free energies.[123] Given the success we describe above for initial trajectory generation in enzymatic reactions, the first is of questionable value, and given the success of the method we described to augment successful TPS trajectories with unsuccessful to find free energies, the second is also of limited value. A more practical and approach may be to simply stitch the methods together. In other words, use TPS to create barrier crossing trajectories for the chemical transition and then take the end configuration of a subset of the reactive ensemble trajectories to initiate a metadynamics investigation of the transition from say chemical product to product release. This is obviously a highly challenging problem. One question is whether the QM/MM potential is maintained as no more chemistry is going to be done. That having been said, changing potentials in the middle of a simulation may introduce unforeseen errors. Such questions will no doubt be a topic for future work.

Another reality of TPS simulations that deserves attention is they are extremely labor intensive. As we mentioned above, the algorithm cannot just be started and allowed to run unmonitored. One could imagine implementation of automated checks, such as RMSD measurements; but this will only account for a portion of the potential problems. Other issues are the appearance of “stray” water molecules between reacting species, etc. At present the only way to locate such problems is to find clues in central distance measurements or visually.

A final critical challenge for the field will be our ability to extend the identification of reaction coordinates (however we define them) beyond the separatrix region just as we extend trajectories. The committor has been called the “perfect reaction coordinate”, but a reaction coordinate that is zero everywhere except for a few 10s or 100s of femtoseconds certainly needs augmentation. We have begun this effort,[47] but it is far from complete.

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REFERENCES

(1). Kästner J Umbrella Integration in Two or More Reaction Coordinates. J. Chem. Phys 2009, 131 (3), 034109. [PubMed: 19624183]

(2). Plimpton SJ; Perez D; Voter AF Parallel Algorithms for Hyperdynamics and Local Hyperdynamics. J. Chem. Phys 2020, 153 (5), 054116. [PubMed: 32770915]

(3). Huang C; Perez D; Voter AF Hyperdynamics Boost Factor Achievable with an Ideal Bias Potential. J. Chem. Phys 2015, 143 (7), 074113. [PubMed: 26298121]

(4). Kim SY; Perez D; Voter AF Local hyperdynamics. J. Chem. Phys 2013, 139 (14), 144110. [PubMed: 24116606]

(5). Pineda JR; Antoniou D; Schwartz SD Slow Conformational Motions that Favor Sub-picosecond Motions Important for Catalysis. J. Phys. Chem. B 2010, 114 (48), 15985–90. [PubMed: 21077591]

(6). Asgeirsson V; Birgisson BO; Bjornsson R; Becker U; Neese F; Riplinger C; Jonsson H Nudged Elastic Band Method for Molecular Reactions Using Energy-Weighted Springs Combined with Eigenvector Following. J. Chem. Theory Comput 2021, 17 (8), 4929–45. [PubMed: 34275279]

(7). Koistinen OP; Asgeirsson V; Vehtari A; Jonsson H Nudged Elastic Band Calculations Accelerated with Gaussian Process Regression Based on Inverse Interatomic Distances. J. Chem. Theory Comput 2019, 15 (12), 6738–51. [PubMed: 31638795]

(8). Mathiesen NR; Jonsson H; Vegge T; Garcia Lastra JM R-NEB: Accelerated Nudged Elastic Band Calculations by Use of Reflection Symmetry. J. Chem. Theory Comput 2019, 15 (5), 3215–22. [PubMed: 30892887]

(9). Chen H; Ogden D; Pant S; Cai W; Tajkhorshid E; Moradi M; Roux B; Chipot C A Companion Guide to the String Method with Swarms of Trajectories: Characterization, Performance, and Pitfalls. J. Chem. Theory Comput 2022, 18 (3), 1406–22. [PubMed: 35138832]

(10). Roux B String Method with Swarms-of-Trajectories, Mean Drifts, Lag Time, and Committor. J. Phys. Chem. A 2021, 125 (34), 7558–71. [PubMed: 34406010]

(11). Suh D; Jo S; Jiang W; Chipot C; Roux B String Method for Protein-Protein Binding Free-Energy Calculations. J. Chem. Theory Comput 2019, 15 (11), 5829–44. [PubMed: 31593627]

(12). Schwartz SD, Ed. Theoretical Methods in Condensed Phase Chemistry; Springer: Dordrecht, The Netherlands, 2002. DOI: 10.1007/0-306-46949-9.

(13). E W; Ren W; Vanden-Eijnden E Finite Temperature String Method for the Study of Rare Events. J. Phys. Chem. B 2005, 109 (14), 6688–93. [PubMed: 16851751]

(14). Elber R Milestoning: An Efficient Approach for Atomically Detailed Simulations of Kinetics in Biophysics. Annu. Rev. Biophys 2020, 49, 69–85. [PubMed: 32375019]

(15). Narayan B; Yuan Y; Fathizadeh A; Elber R; Buchete NV Long-time Methods for Molecular Dynamics Simulations: Markov State Models and Milestoning. Prog. Mol. Biol. Transl Sci 2020, 170, 215–37. [PubMed: 32145946]

(16). Elber R A New Paradigm for Atomically Detailed Simulations of Kinetics in Biophysical Systems. Q. Rev. Biophys 2017, 50, e8. [PubMed: 29233220]

(17). Faradjian AK; Elber R Computing Time Scales from Reaction Coordinates by Milestoning. J. Chem. Phys 2004, 120 (23), 10880–9. [PubMed: 15268118]

(18). Bello-Rivas JM; Elber R Exact Milestoning. J. Chem. Phys 2015, 142 (9), 094102. [PubMed: 25747056]

(19). Cardenas AE; Drexler CI; Nechushtai R; Mittler R; Friedler A; Webb LJ; Elber R Peptide Permeation across a Phosphocholine Membrane: An Atomically Detailed Mechanism Determined through Simulations and Supported by Experimentation. J. Phys. Chem. B 2022, 126 (15), 2834–49. [PubMed: 35388695]

(20). Narayan B; Buchete NV; Elber R Computer Simulations of the Dissociation Mechanism of Gleevec from Abl Kinase with Milestoning. J. Phys. Chem. B 2021, 125 (22), 5706–15. [PubMed: 33930271]

(21). Narayan B; Fathizadeh A; Templeton C; He P; Arasteh S; Elber R; Buchete NV; Levy RM The Transition Between Active and Inactive Conformations of Abl Kinase Studied by

Rock Climbing and Milestoning. Biochim. Biophys. Acta, Gen. Subj 2020, 1864 (4), 129508. [PubMed: 31884066]

(22). Templeton C; Elber R Why Does RNA Collapse? The Importance of Water in a Simulation Study of Helix-Junction-Helix Systems. J. Am. Chem. Soc 2018, 140 (49), 16948–51. [PubMed: 30465606]

(23). Elber R; West A Atomically Detailed Simulation of the Recovery Stroke in Myosin by Milestoning. Proc. Natl. Acad. Sci. U. S. A 2010, 107 (11), 5001–5. [PubMed: 20194770]

(24). Piana S; Robustelli P; Tan D; Chen S; Shaw DE Development of a Force Field for the Simulation of Single-Chain Proteins and Protein-Protein Complexes. J. Chem. Theory Comput 2020, 16 (4), 2494–507. [PubMed: 31914313]

(25). Piana S; Lindorff-Larsen K; Shaw DE Protein Folding Kinetics and Thermodynamics from Atomistic Simulation. Proc. Natl. Acad. Sci. U. S. A 2012, 109 (44), 17845–50. [PubMed: 22822217]

(26). Dror RO; Dirks RM; Grossman JP; Xu H; Shaw DE Biomolecular Simulation: a Computational Microscope for Molecular Biology. Annu. Rev. Biophys 2012, 41, 429–52. [PubMed: 22577825]

(27). Dror RO; Jensen MO; Shaw DE Elucidating Membrane Protein Function through Long-Timescale Molecular Dynamics Simulation. Annu. Int. Conf. IEEE Eng. Med. Biol. Soc 2009, 2340–2. [PubMed: 19965181]

(28). Maragakis P; Lindorff-Larsen K; Eastwood MP; Dror RO; Klepeis JL; Arkin IT; Jensen MO; Xu H; Trbovic N; Friesner RA; Palmer AG III; Shaw DE Microsecond Molecular Dynamics Simulation Shows Effect of Slow Loop Dynamics on Backbone Amide Order Parameters of Proteins. J. Phys. Chem. B 2008, 112 (19), 6155–8. [PubMed: 18311962]

(29). Pratt LR A Statistical Method for Identifying Transition States in High Dimensional Problems. J. Chem. Phys 1986, 85, 5045.

(30). Bolhuis P; Chandler D Transition Path Sampling of Cavitation Between Molecular Scale Solvophobic Surfaces. J. Chem. Phys 2000, 113, 8154–60.

(31). Bolhuis P; Chandler D; Dellago C; Geissler P Transition Path Sampling: Throwing Ropes over Mountain Passes, in the Dark. Annu. Rev. Phys. Chem 2002, 53, 291–318. [PubMed: 11972010]

(32). Bolhuis PG; Dellago C; Chandler D Sampling Ensembles of Deterministic Transition Pathways. Faraday Discuss 1998, 110, 421–36.

(33). Dellago C, Chandler D Bridging the Time Scale Gap with Transition Path Sampling. In Nielaba P, Mareschal M, Ciccotti G, Eds. Bridging the time scales: Molecular simulations for the next decade; Lecture Notes in Physics, Vol. 605; Springer Verlag: New York, 2003. DOI: 10.1007/3-540-45837-9.

(34). Mullen RG; Shea JE; Peters B Easy Transition Path Sampling Methods: Flexible-Length Aimless Shooting and Permutation Shooting. J. Chem. Theory Comput 2015, 11 (6), 2421–8. [PubMed: 26575542]

(35). Antoniou D; Schwartz SD The Stochastic Separatrix and the Reaction Coordinate for Complex Systems. J. Chem. Phys 2009, 130 (15), 151103. [PubMed: 19388729]

(36). Ma A; Nag A; Dinner AR Dynamic Coupling Between Coordinates in a Model for Biomolecular Isomerization. J. Chem. Phys 2006, 124 (14), 144911. [PubMed: 16626249]

(37). Peters B; Trout BL Obtaining Reaction Coordinates by Likelihood Maximization. J. Chem. Phys 2006, 125 (5), 054108. [PubMed: 16942204]

(38). Peters B; Beckham GT; Trout BL Extensions to the Likelihood Maximization Approach for Finding Reaction Coordinates. J. Chem. Phys 2007, 127 (3), 034109. [PubMed: 17655433]

(39). Cheng T; Ma W; Luo H; Ye Y; Yan K Manipulating Reaction Energy Coordinate Landscape of Mechanochemical Diaza-Cope Rearrangement. Molecules 2022, 27 (8), 2570. [PubMed: 35458767]

(40). Wu S; Li H; Ma A A Rigorous Method for Identifying a One-Dimensional Reaction Coordinate in Complex Molecules. J. Chem. Theory Comput 2022, 18 (5), 2836–44. [PubMed: 35427129]

(41). Voth GA; Cao J The Formulation of Quantum Statistical Mechanics Based on the Feynman Path Centroid Density. IV. Algorithms for Centroid Molecular Dynamics. J. Chem. Phys 1994, 101, 6168–83.

(42). Antoniou D; Schwartz SD Approximate Inclusion of Quantum Effects in Transition Path Sampling. J. Chem. Phys 2009, 131 (22), 224111. [PubMed: 20001028]

(43). Dzierlenga MW; Varga MJ; Schwartz SD Path Sampling Methods for Enzymatic Quantum Particle Transfer Reactions. Methods Enzymol 2016, 578, 21–43. [PubMed: 27497161]

(44). Dzierlenga MW; Antoniou D; Schwartz SD Another Look at the Mechanisms of Hydride Transfer Enzymes with Quantum and Classical Transition Path Sampling. J. Phys. Chem. *Lett.* 2015, 6 (7), 1177–81.

(45). Balasubramani SG; Schwartz SD Transition Path Sampling Based Calculations of Free Energies for Enzymatic Reactions: The Case of Human Methionine Adenosyl Transferase and Plasmodium vivax Adenosine Deaminase. J. Phys. Chem. B 2022, 126, 5413. [PubMed: 35830574]

(46). Radhakrishnan R; Schlick T Biomolecular Free Energy Profiles By a Shooting/umbrella Sampling Protocol, "BOLAS". J. Chem. Phys 2004, 121 (5), 2436–44. [PubMed: 15260799]

(47). Antoniou D; Schwartz SD Role of Protein Motions in Catalysis by Formate Dehydrogenase. J. Phys. Chem. B 2020, 124 (43), 9483–9. [PubMed: 33064490]

(48). Singh AN; Limmer DT Peptide Isomerization is Suppressed at the Air-Water Interface. J. Phys. Chem. Lett 2022, 13 (2), 574–9. [PubMed: 35014845]

(49). Du WN; Bolhuis PG Adaptive Single Replica Multiple State Transition Interface Sampling. J. Chem. Phys 2013, 139 (4), 044105. [PubMed: 23901958]

(50). Bolhuis PG; Dellago C; Chandler D Reaction Coordinates of Biomolecular Isomerization. Proc. Natl. Acad. Sci. U. S. A 2000, 97 (11), 5877–82. [PubMed: 10801977]

(51). Arora K; Schlick T Conformational Transition Pathway of Polymerase Beta/DNA upon Binding Correct Incoming Substrate. J. Phys. Chem. B 2005, 109 (11), 5358–67. [PubMed: 16863202]

(52). Radhakrishnan R; Schlick T Fidelity Discrimination in DNA Polymerase Beta: Differing Closing Profiles for a Mismatched (G:A) Versus Matched (G:C) Base Pair. J. Am. Chem. Soc 2005, 127 (38), 13245–52. [PubMed: 16173754]

(53). Basner JE; Schwartz SD How Enzyme Dynamics Helps Catalyze a Reaction in Atomic Detail: a Transition Path Sampling Study. J. Am. Chem. Soc 2005, 127 (40), 13822–31. [PubMed: 16201803]

(54). Gao J; Amara P; Alhambra C; Field MJ A Generalized Hybrid Orbital (GHO) Method for the Treatment of Boundary Atoms in Combined QM/MM Calculations. J. Phys. Chem. A 1998, 102 (24), 4714–21.

(55). Antoniou D; Caratzoulas S; Kalyanaraman C; Mincer JS; Schwartz SD Barrier Passage and Protein Dynamics in Enzymatically Catalyzed Reactions. Eur. J. Biochem 2002, 269 (13), 3103–12. [PubMed: 12084050]

(56). Caratzoulas S; Mincer JS; Schwartz SD Identification of a Protein-Promoting Vibration in the Reaction Catalyzed by Horse Liver Alcohol Dehydrogenase. J. Am. Chem. Soc 2002, 124 (13), 3270–6. [PubMed: 11916410]

(57). 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 (7), 2910–8.

(58). Crehuet R; Field MJ A Transition Path Sampling Study of the Reaction Catalyzed by the Enzyme Chorismate Mutase. J. Phys. Chem. B 2007, 111 (20), 5708–18. [PubMed: 17474768]

(59). Quaytman SL; Schwartz SD Reaction Coordinate of an Enzymatic Reaction Revealed by Transition Path Sampling. Proc. Natl. Acad. Sci. U. S. A 2007, 104 (30), 12253–8. [PubMed: 17640885]

(60). Saen-Oon S; Schramm VL; Schwartz SD Transition Path Sampling Study of the Reaction Catalyzed by Purine Nucleoside Phosphorylase. Z. Phys. Chem. (N. F) 2008, 222 (8–9), 1359–74. [PubMed: 20664707]

(61). 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. U. S. A 2008, 105 (43), 16543–8. [PubMed: 18946041]

(62). Nunez S; Wing C; Antoniou D; Schramm VL; Schwartz SD Insight into Catalytically Relevant Correlated Motions in Human Purine Nucleoside Phosphorylase. J. Phys. Chem. A 2006, 110 (2), 463–72. [PubMed: 16405318]

(63). Quaytman SL; Schwartz SD Comparison Studies of the Human Heart and Bacillus stearothermophilus Lactate Dehydrogreanse by Transition Path Sampling. J. Phys. Chem. A 2009, 113 (10), 1892–7. [PubMed: 19053545]

(64). Escobedo FA; Borrero EE; Araque JC Transition Path Sampling and Forward Flux Sampling. Applications to Biological Systems. J. Phys.: Condens. Matter 2009, 21 (33), 333101. [PubMed: 21828593]

(65). Roy A; Taraphder S Transition path sampling study of the conformational fluctuation of His-64 in human carbonic anhydrase II. J. Phys. Chem. B 2009, 113 (37), 12555–64. [PubMed: 19685901]

(66). Schwartz SD Protein Dynamics and the Enzymatic Reaction Coordinate. In Dynamics in Enzyme Catalysis; Klinman J, Hammes-Schiffer S, Eds.; Springer: Berlin, 2013. DOI: 10.1007/128_2012_412.

(67). Dametto M; Antoniou D; Schwartz SD Barrier Crossing in Dihydrofolate Reductasedoes not involve a rate-promoting vibration. Mol. Phys 2012, 110 (9–10), 531–6. [PubMed: 22942460]

(68). Doron D; Kohen A; Nam K; Major DT How Accurate Are Transition States from Simulations of Enzymatic Reactions? J. Chem. Theory Comput 2014, 10 (5), 1863–71. [PubMed: 24860275]

(69). Silva RG; Murkin AS; Schramm VL Femtosecond dynamics coupled to chemical barrier crossing in a Born-Oppenheimer enzyme. Proc. Natl. Acad. Sci. U. S. A 2011, 108 (46), 18661–5. [PubMed: 22065757]

(70). Antoniou D; Ge X; Schramm VL; Schwartz SD Mass Modulation of Protein Dynamics Associated with Barrier Crossing in Purine Nucleoside Phosphorylase. J. Phys. Chem. *Lett.* 2012, 3 (23), 3538–44.

(71). Masterson JE; Schwartz SD Changes in Protein Architecture and Subpicosecond Protein Dynamics Impact the Reaction Catalyzed by Lactate Dehydrogenase. J. Phys. Chem. A 2013, 117 (32), 7107–13. [PubMed: 23441954]

(72). Wang Z; Chang EP; Schramm VL Triple Isotope Effects Support Concerted Hydride and Proton Transfer and Promoting Vibrations in Human Heart Lactate Dehydrogenase. J. Am. Chem. Soc 2016, 138 (45), 15004–10. [PubMed: 27766841]

(73). Knott BC; Haddad Momeni M; Crowley MF; Mackenzie LF; Gotz AW; Sandgren M; Withers SG; Stahlberg J; Beckham GT The Mechanism of Cellulose Hydrolysis by a Two-Step, Retaining Cellobiohydrolase Elucidated by Structural and Transition Path Sampling Studies. J. Am. Chem. Soc 2014, 136 (1), 321–9. [PubMed: 24341799]

(74). Zoi I; Motley MW; Antoniou D; Schramm VL; Schwartz SD Enzyme Homologues have Distinct Reaction Paths Through Their Transition States. J. Phys. Chem. B 2015, 119 (9), 3662–8. [PubMed: 25650981]

(75). Wang Z; Antoniou D; Schwartz SD; Schramm VL Hydride Transfer in DHFR by Transition Path Sampling, Kinetic Isotope Effects, and Heavy Enzyme Studies. Biochemistry 2016, 55 (1), 157–66. [PubMed: 26652185]

(76). Singharoy A; Chipot C Methodology for the Simulation of Molecular Motors at Different Scales. J. Phys. Chem. B 2017, 121 (15), 3502–14. [PubMed: 28423900]

(77). Singharoy A; Chipot C; Moradi M; Schulten K Chemo-mechanical Coupling in Hexameric Protein-Protein Interfaces Harnesses Energy within V-Type ATPases. J. Am. Chem. Soc 2017, 139 (1), 293–310. [PubMed: 27936329]

(78). Paul S; Paul TK; Taraphder S Reaction Coordinate, Free Energy, and Rate of Intramolecular Proton Transfer in Human Carbonic Anhydrase II. J. Phys. Chem. B 2018, 122 (11), 2851–66. [PubMed: 29466004]

(79). Burgin T; Stahlberg J; Mayes HB Advantages of a Distant Cellulase Catalytic Base. J. Biol. Chem 2018, 293 (13), 4680–7. [PubMed: 29321205]

(80). Klinman JP; Offenbacher AR Understanding Biological Hydrogen Transfer Through the Lens of Temperature Dependent Kinetic Isotope Effects. Acc. Chem. Res 2018, 51 (9), 1966–74. [PubMed: 30152685]

(81). Hu S; Sharma SC; Scouras AD; Soudackov AV; Carr CA; Hammes-Schiffer S; Alber T; Klinman JP Extremely Elevated Room-Temperature Kinetic Isotope Effects Quantify the Critical Role

of Barrier Width in Enzymatic C-H Activation. J. Am. Chem. Soc 2014, 136 (23), 8157–60. [PubMed: 24884374]

(82). Meyer MP; Klinman JP Investigating Inner-Sphere Reorganization via Secondary Kinetic Isotope Effects in the C-H Cleavage Reaction Catalyzed by Soybean Lipoxygenase: Tunneling in the Substrate Backbone as well as the Transferred Hydrogen. J. Am. Chem. Soc 2011, 133 (3), 430–9. [PubMed: 21192631]

(83). Nagel ZD; Klinman JP Tunneling and Dynamics in Enzymatic Hydride Transfer. Chem. Rev 2006, 106 (8), 3095–118. [PubMed: 16895320]

(84). Klinman JP Linking Protein Structure and Dynamics to Catalysis: the Role of Hydrogen Tunnelling. Philos. Trans R Soc. Lond B Biol. Sci 2006, 361 (1472), 1323–31. [PubMed: 16873120]

(85). Liang ZX; Tsigos I; Bouriotis V; Klinman JP Impact of Protein Flexibility on Hydride-Transfer Parameters in Thermophilic and Psychrophilic Alcohol Dehydrogenases. J. Am. Chem. Soc 2004, 126 (31), 9500–1. [PubMed: 15291528]

(86). Tsai S; Klinman JP Probes of Hydrogen Tunneling with Horse Liver Alcohol Dehydrogenase at Subzero Temperatures. Biochemistry 2001, 40 (7), 2303–11. [PubMed: 11329300]

(87). Chin JK; Klinman JP Probes of a Role for Remote Binding Interactions on Hydrogen Tunneling in the Horse Liver Alcohol Dehydrogenase Reaction. Biochemistry 2000, 39 (6), 1278–84. [PubMed: 10684607]

(88). Kohen A; Cannio R; Bartolucci S; Klinman JP Enzyme Dynamics and Hydrogen Tunnelling in a Thermophilic Alcohol Dehydrogenase. Nature 1999, 399 (6735), 496–9. [PubMed: 10365965]

(89). Bahnson BJ; Klinman JP Hydrogen Tunneling in Enzyme Catalysis. Methods Enzymol 1995, 249, 373–97. [PubMed: 7791619]

(90). Kiss G; Rothlisberger D; Baker D; Houk KN Evaluation and Ranking of Enzyme Designs. Protein Sci 2010, 19 (9), 1760–73. [PubMed: 20665693]

(91). Baker D An Exciting but Challenging Road Ahead for Computational Enzyme Design. Protein Sci 2010, 19 (10), 1817–9. [PubMed: 20717908]

(92). Kipnis Y; Baker D Comparison of Designed and Randomly Generated Catalysts for Simple Chemical Reactions. Protein Sci 2012, 21 (9), 1388–95. [PubMed: 22811380]

(93). Zoi I; Suarez J; Antoniou D; Cameron SA; Schramm VL; Schwartz SD Modulating Enzyme Catalysis through Mutations Designed to Alter Rapid Protein Dynamics. J. Am. Chem. Soc 2016, 138 (10), 3403–9. [PubMed: 26927977]

(94). Harijan RK; Zoi I; Antoniou D; Schwartz SD; Schramm VL Catalytic-Site Design for Inverse Heavy-Enzyme Isotope Effects in Human Purine Nucleoside Phosphorylase. Proc. Natl. Acad. Sci. U. S. A 2017, 114 (25), 6456–61. [PubMed: 28584087]

(95). Zoi I; Antoniou D; Schwartz SD Incorporating Fast Protein Dynamics into Enzyme Design: A Proposed Mutant Aromatic Amine Dehydrogenase. J. Phys. Chem. B 2017, 121 (30), 7290–8. [PubMed: 28696108]

(96). Chen X; Schwartz SD Directed Evolution as a Probe of Rate Promoting Vibrations Introduced via Mutational Change. Biochemistry 2018, 57 (23), 3289–98. [PubMed: 29553716]

(97). Bjelic S; Kipnis Y; Wang L; Pianowski Z; Vorobiev S; Su M; Seetharaman J; Xiao R; Kornhaber G; Hunt JF; Tong L; Hilvert D; Baker D Exploration of Alternate Catalytic Mechanisms and Optimization Strategies for Retroaldolase Design. J. Mol. Biol 2014, 426 (1), 256–71. [PubMed: 24161950]

(98). Giger L; Caner S; Obexer R; Kast P; Baker D; Ban N; Hilvert D Evolution of a Designed Retro-Aldolase Leads to Complete Active Site Remodeling. Nat. Chem. Biol 2013, 9 (8), 494–8. [PubMed: 23748672]

(99). Althoff EA; Wang L; Jiang L; Giger L; Lassila JK; Wang Z; Smith M; Hari S; Kast P; Herschlag D; Hilvert D; Baker D Robust Design and Optimization of Retroaldol Enzymes. Protein Sci 2012, 21 (5), 717–26. [PubMed: 22407837]

(100). Jiang L; Althoff EA; Clemente FR; Doyle L; Rothlisberger D; Zanghellini A; Gallaher JL; Betker JL; Tanaka F; Barbas CF 3rd; Hilvert D; Houk KN; Stoddard BL; Baker D De novo Computational Design of Retro-Aldol Enzymes. Science 2008, 319 (5868), 1387–91. [PubMed: 18323453]

(101). Schafer JW; Zoi I; Antoniou D; Schwartz SD Optimization of the Turnover in Artificial Enzymes via Directed Evolution Results in the Coupling of Protein Dynamics to Chemistry. J. Am. Chem. Soc 2019, 141 (26), 10431–9. [PubMed: 31199129]

(102). Schafer JW; Schwartz SD Directed Evolution's Influence on Rapid Density Fluctuations Illustrates How Protein Dynamics Can Become Coupled to Chemistry. ACS Catal 2020, 10 (15), 8476–84. [PubMed: 33163256]

(103). Offenbacher AR; Sharma A; Doan PE; Klinman JP; Hoffman BM The Soybean Lipoxygenase-Substrate Complex: Correlation between the Properties of Tunneling-Ready States and ENDOR-Detected Structures of Ground States. Biochemistry 2020, 59 (7), 901–10. [PubMed: 32022556]

(104). Jindal G; Warshel A Misunderstanding the Preorganization Concept can Lead to Confusions about the Origin of Enzyme Catalysis. Proteins 2017, 85 (12), 2157–61. [PubMed: 28905418]

(105). Lameira J; Bora RP; Chu ZT; Warshel A Methyltransferases Do Not Work by Compression, Cratic, or Desolvation Effects, but by Electrostatic Preorganization. Proteins 2015, 83 (2), 318–30. [PubMed: 25388538]

(106). Zheng C; Mao Y; Kozuch J; Atsango AO; Ji Z; Markland TE; Boxer SG A Two-Directional Vibrational Probe Reveals Different Electric Field Orientations in Solution and an Enzyme Active Site. Nat. Chem 2022, 14, 891. [PubMed: 35513508]

(107). Wu Y; Fried SD; Boxer SG A Preorganized Electric Field Leads to Minimal Geometrical Reorientation in the Catalytic Reaction of Ketosteroid Isomerase. J. Am. Chem. Soc 2020, 142 (22), 9993–8. [PubMed: 32378409]

(108). Saggu M; Fried SD; Boxer SG Local and Global Electric Field Asymmetry in Photosynthetic Reaction Centers. J. Phys. Chem. B 2019, 123 (7), 1527–36. [PubMed: 30668130]

(109). Fried SD; Boxer SG Electric Fields and Enzyme Catalysis. Annu. Rev. Biochem 2017, 86, 387–415. [PubMed: 28375745]

(110). Wu Y; Boxer SG A Critical Test of the Electrostatic Contribution to Catalysis with Noncanonical Amino Acids in Ketosteroid Isomerase. J. Am. Chem. Soc 2016, 138 (36), 11890–5. [PubMed: 27545569]

(111). Fried SD; Bagchi S; Boxer SG Extreme Electric Fields Power Catalysis in the Active Site of Ketosteroid Isomerase. Science 2014, 346 (6216), 1510–4. [PubMed: 25525245]

(112). Chen X; Schwartz SD Examining the Origin of Catalytic Power of Catechol O-Methyltransferase. ACS Catal 2019, 9 (11), 9870–9. [PubMed: 31750009]

(113). Zoi I; Antoniou D; Schwartz SD Electric Fields and Fast Protein Dynamics in Enzymes. J. Phys. Chem. Lett 2017, 8 (24), 6165–70. [PubMed: 29220191]

(114). Bharadwaj VS; Knott BC; Stahlberg J; Beckham GT; Crowley MF The Hydrolysis Mechanism of a GH45 Cellulase and its Potential Relation to Lytic Transglycosylase and Expansin Function. J. Biol. Chem 2020, 295 (14), 4477–87. [PubMed: 32054684]

(115). Paul TK; Taraphder S Coordination Dynamics of Zinc Triggers the Rate Determining Proton Transfer in Human Carbonic Anhydrase II. ChemPhysChem 2020, 21 (13), 1455–73. [PubMed: 32329944]

(116). Janos P; Tvaroska I; Dellago C; Koca J Catalytic Mechanism of Processive GlfT2: Transition Path Sampling Investigation of Substrate Translocation. ACS Omega 2020, 5 (34), 21374–84. [PubMed: 32905330]

(117). Chen X; Schwartz SD Multiple Reaction Pathways in the Morphinone Reductase-Catalyzed Hydride Transfer Reaction. ACS Omega 2020, 5 (36), 23468–80. [PubMed: 32954200]

(118). Silveira RL; Knott BC; Pereira CS; Crowley MF; Skaf MS; Beckham GT Transition Path Sampling Study of the Feruloyl Esterase Mechanism. J. Phys. Chem. B 2021, 125 (8), 2018–30. [PubMed: 33616402]

(119). Levintov L; Paul S; Vashisth H Reaction Coordinate and Thermodynamics of Base Flipping in RNA. J. Chem. Theory Comput 2021, 17 (3), 1914–21. [PubMed: 33594886]

(120). Chakraborti A; Baldo AP; Tardiff JC; Schwartz SD Investigation of the Recovery Stroke and ATP Hydrolysis and Changes Caused Due to the Cardiomyopathic Point Mutations in Human Cardiac beta Myosin. J. Phys. Chem. B 2021, 125 (24), 6513–21. [PubMed: 34105970]

(121). Brown M; Zoi I; Antoniou D; Namanja-Magliano HA; Schwartz SD; Schramm VL Inverse Heavy Enzyme Isotope Effects in Methylthioadenosine Nucleosidases. Proc. Natl. Acad. Sci. U. S. A 2021, 118 (40), e2109118118. [PubMed: 34580228]

(122). Park JM; Laio A; Iannuzzi M; Parrinello M Dissociation Mechanism of Acetic Acid in Water. J. Am. Chem. Soc 2006, 128 (35), 11318–9. [PubMed: 16939231]

(123). Jobbins SA; Boulfelfel SE; Leoni S Metashooting: a Novel Tool for Free Energy Reconstruction from Polymorphic Phase Transition Mechanisms. Faraday Discuss 2018, 211 (0), 235–51. [PubMed: 30039129]

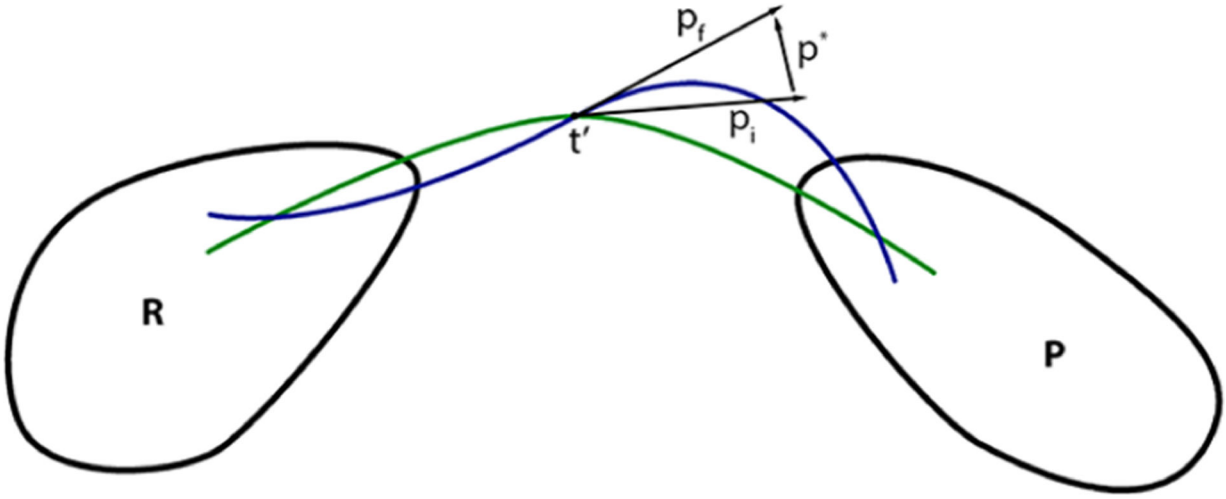

Figure 1.
A schematic of TPS trajectory generation. An initial seed trajectory ($x^{(o)}(t)$) is used to generate further trajectories. We use shooting, a random change in velocity.

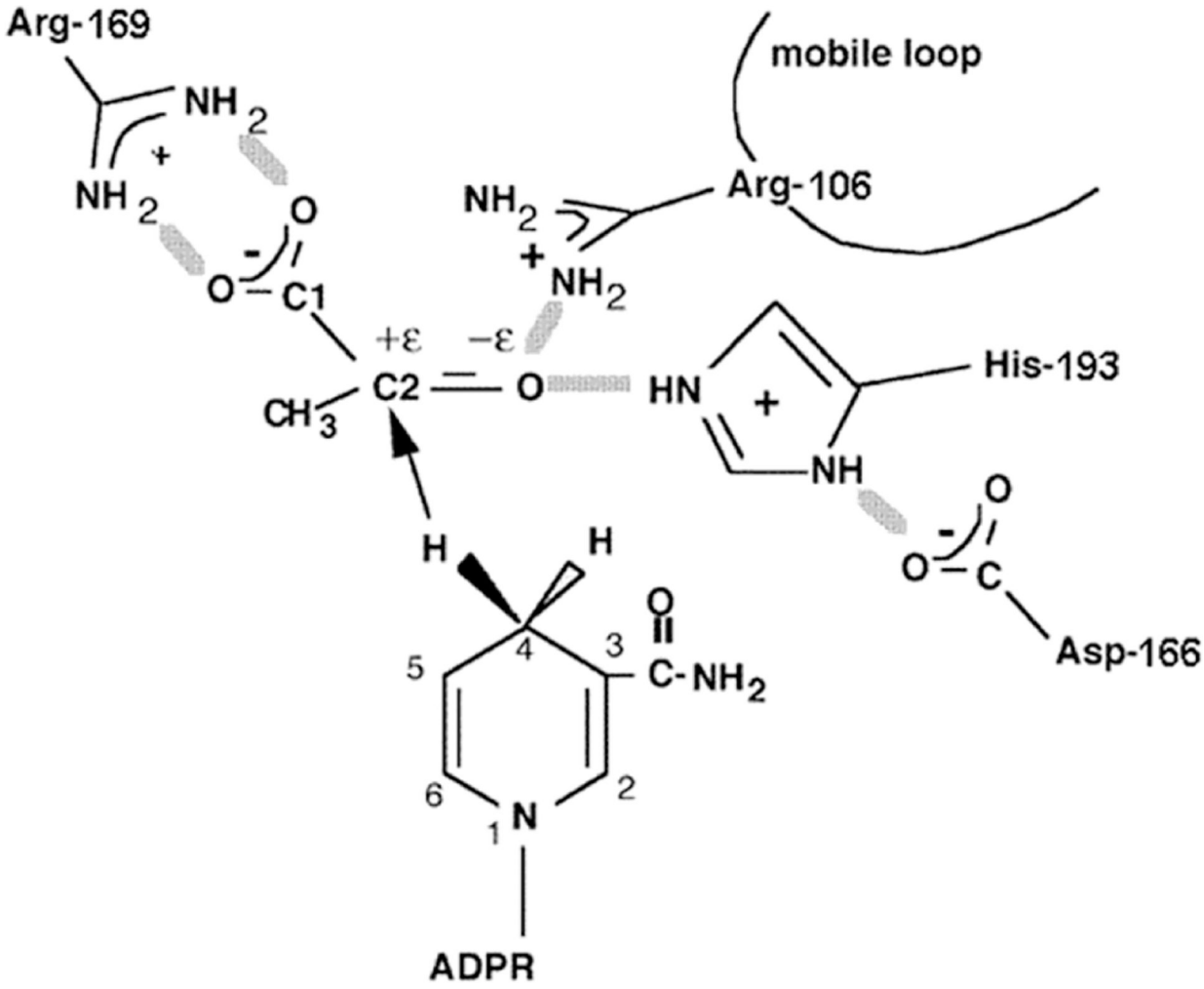

Figure 2.

Chemical reaction catalyzed by lactate dehydrogenase. The reaction involves the hydride transfer of the NC4 hydrogen of NADH from the pro-R side of the reduced nicotinamide ring to the C2 carbon of pyruvate and protein transfer from the imidozole group of His-193 to pyruvate's keto oxygen.

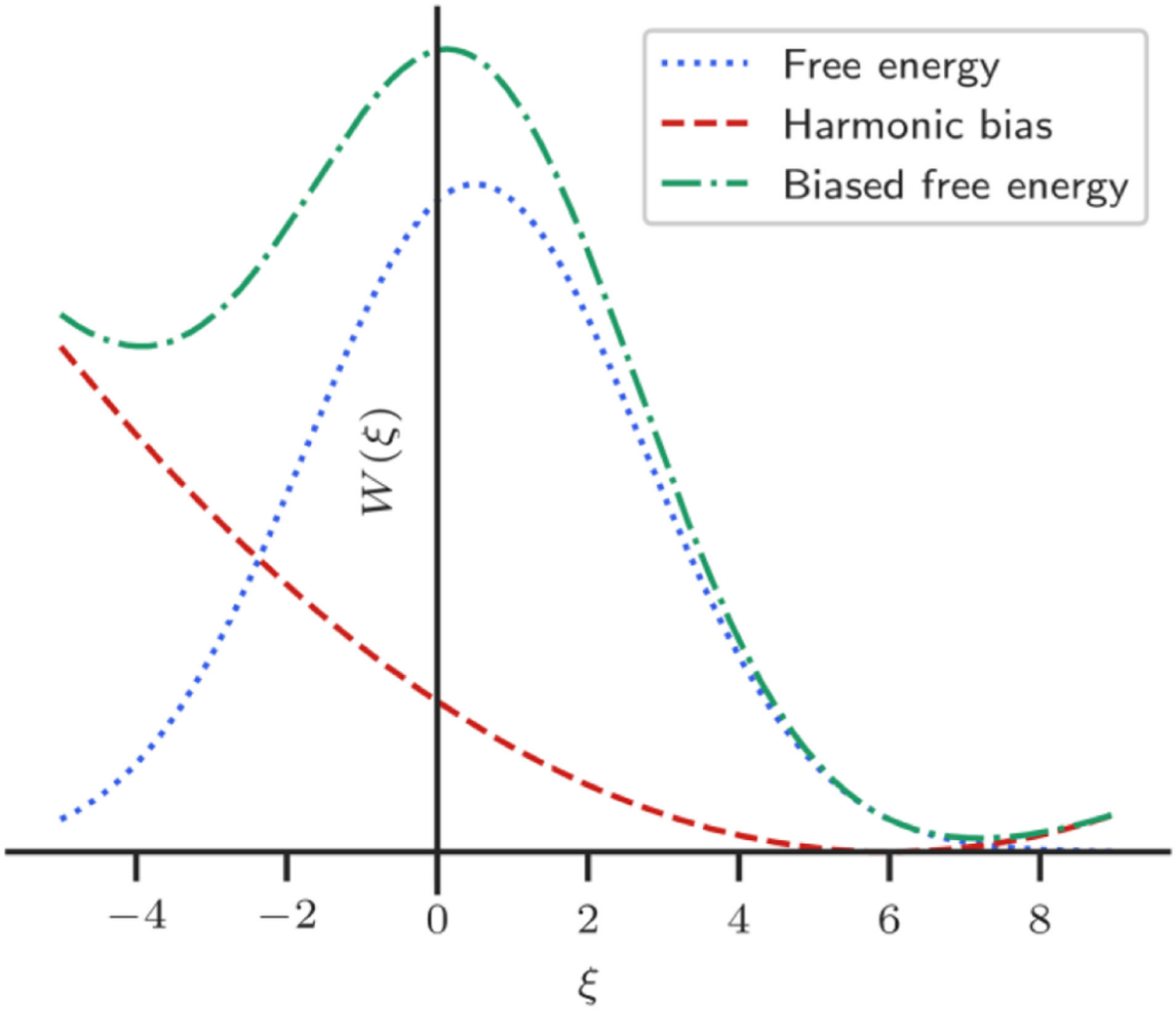

Figure 3.

A schematic representation of using a biased potential to create an initial trajectory. In this coordinate system, we imagine the starting value of the coordinate as −4; MD is then run with the bias on. The hope is the significantly lower potential allows transformation to products. All subsequent trajectories are run with no bias.

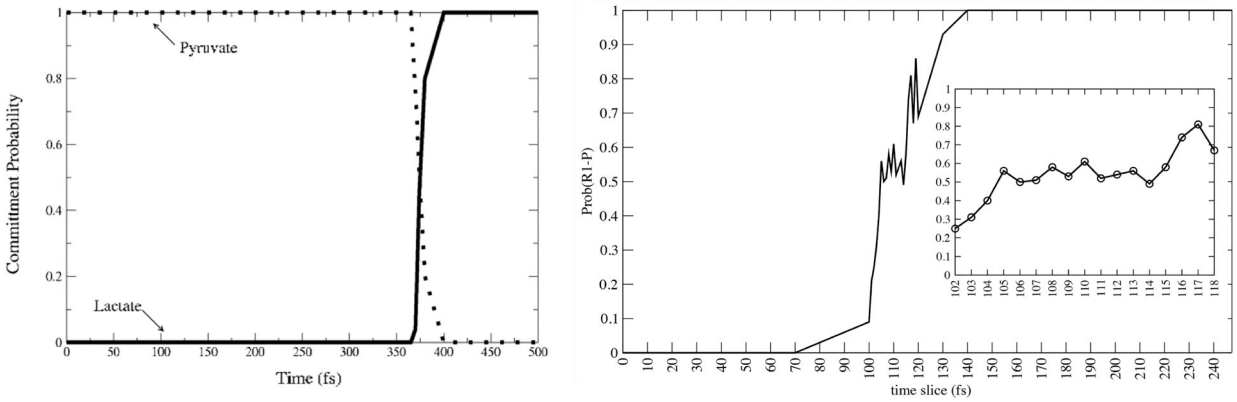

Figure 4.

Committors for the reactions catalyzed by lactate dehydrogenase (LDH, left) and purine nucleoside phosphorylase (PNP, right). Both committors show a rapid rise of probability and so are direct reaction, but in the case of PNP there is a brief formation of an intermediate. Left image (LDH) reprinted with permission from ref 59. Copyright 2007 National Academy of Sciences. Right image (PNP) reprinted with permission from ref 61. Copyright 2008 National Academy of Sciences.

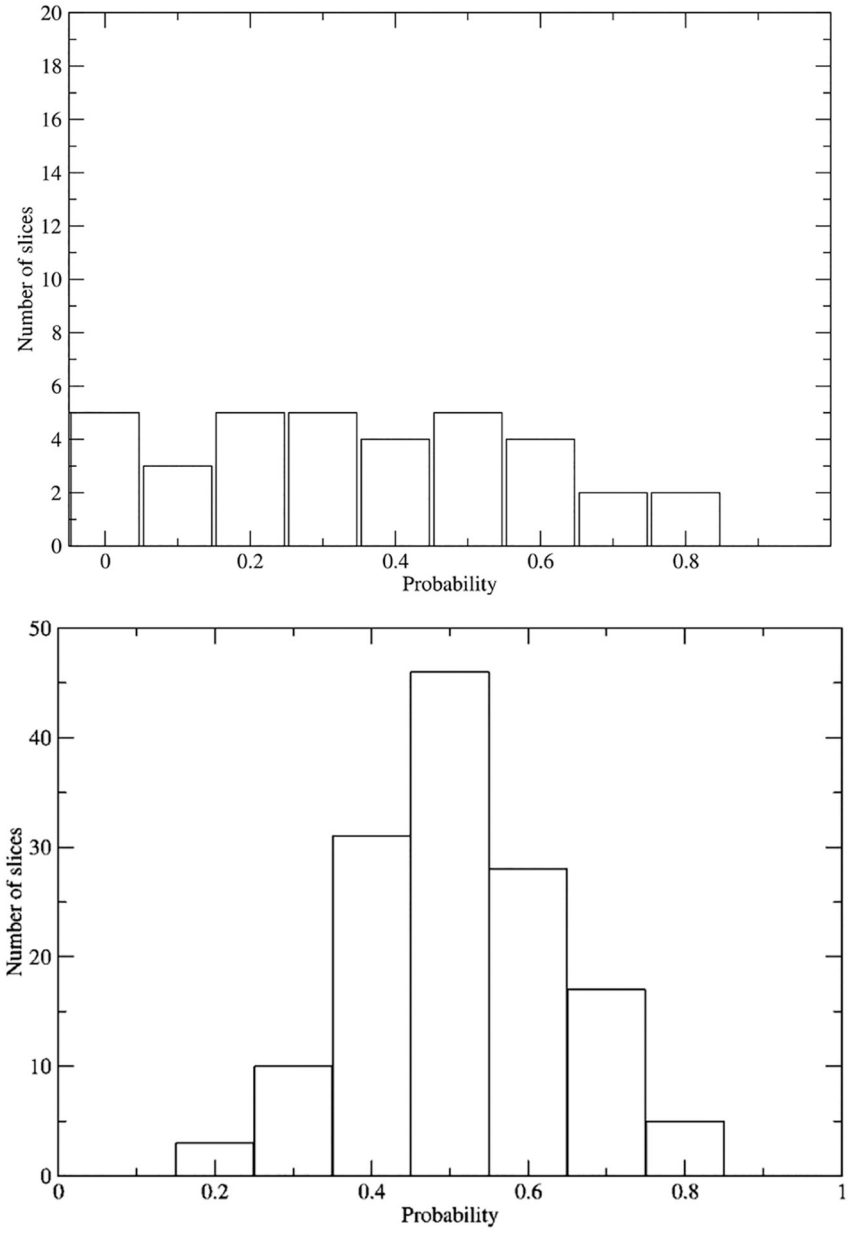

Figure 5.

Committor distributions for putative reaction coordinates for the reaction catalyzed by methylthioadenosine nucleosidase (EcM-TAN). The top distribution includes the bond making and breaking distances and the residues of the active site, the bottom Ile152, Phe151, Arg193, Ser196, Met173, Asp197, Met9, Ser76, and Ile50. This presents both a stark example of the necessary inclusion of protein degrees of freedom and a clear example of both a successful and unsuccessful reaction coordinate. Adapted with permission from ref 74. Copyright 2015 American Chemical Society.

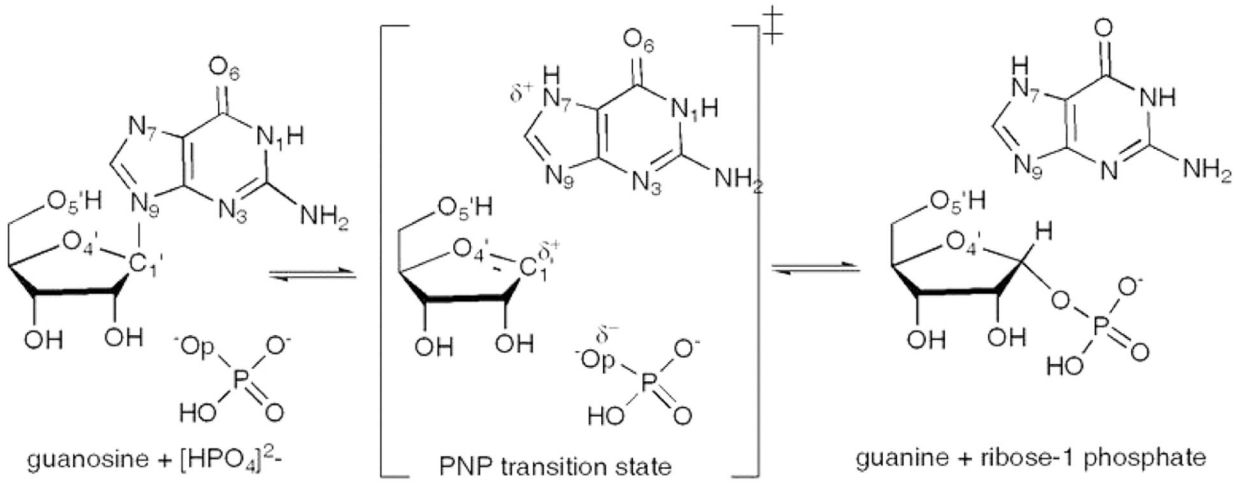

Figure 6.

Chemical reaction catalyzed by human purine nucleoside phosphorylase. One promoting vibration compresses O_4' and O_5'. Another stabilizes the guanine leaving group. Reprinted with permission from ref 61. Copyright 2008 National Academy of Sciences.