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Enzyme Homologues Have Distinct Reaction Paths through their Transition States

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Abstract

Recent studies of the bacterial enzymes EcMTAN and VcMTAN showed that they have different binding affinities for the same transition state analogue. This was surprising given the similarity of their active sites. We performed Transition Path Sampling simulations of both enzymes to reveal the atomic details of the catalytic chemical step, which may be the key for explaining the inhibitor affinity differences. Even though all experimental data would suggest the two enzymes are almost identical, subtle dynamic differences manifest in differences of reaction coordinate, transition state structure, and eventually significant differences in inhibitor binding. Unlike EcMTAN, VcMTAN has multiple distinct transition states, which is an indication that multiple sets of coordinated protein motions can reach a transition state. Reaction coordinate information is only accessible from transition path sampling approaches, since all experimental approaches report averages. Detailed knowledge could have a significant impact on pharmaceutical design.

Keywords

enzymes; TPS; reaction coordinate; transition state inhibitor; inhibitor binding strength

1 Introduction

⁵⁰-Methylthioadenosine nucleosidases (MTANs) are important enzymes involved in critical biological processes in many bacteria such as *Escherichia coli* and *Vibrio cholerae*. The key role of the MTA nucleosidases in biological methylation,¹ polyamine biosynthesis,²

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Supporting Information Available

RMSF calculation (Figure S1), structural differences (Figure S2), transition state structures (Figure S3), reaction coordinate identification tests for VcMTAN (Figure S4, S5, S6), reaction coordinate identification tests for EcMTAN (Figure S7, S8, S9), video 1 (representative reaction coordinate for VcMTAN) and video 2 (reaction coordinate for EcMTAN). This material is available free of charge via the Internet at <http://pubs.acs.org>. This material is available free of charge via the Internet at <http://pubs.acs.org/>.

methionine recycling,³ but most importantly in quorum sensing pathways⁴ that induce bacterial pathogenesis factors, has made them important anti-infective drug targets.

Both experimental and computational techniques^{5,6} have been employed to design and analyze analogues that mimic transition state structures. Those mimics are chemically stable and are powerful enzyme inhibitors. Examples of transition state analogues of bacterial MTANs are MT-, EtT-, and BuT-DADMe-ImmA which are highly potent in disrupting quorum sensing molecules in pathogenic strains of *Vibrio cholerae* and *Escherichia coli*. In addition, DADMe-ImmA derivatives present nontoxicity and bioavailability establishing themselves as attractive drug candidates.⁷ However, previous studies found that BuT-DADMe-ImmA (BDIA) binds 1000 times more strongly to the EcMTAN than to the VcMTAN⁸ without any obvious explanation from structure comparisons. The two structures share 60% sequence identity and have almost identical active sites. Crystallographic data and kinetic isotope effect studies showed that these enzymatic complexes have nearly the same transition state structure.⁹ In addition, they act under a common S_N1-type reaction mechanism and have dissociative transition states with ribooxacarbenium ion character, which are both considered to be late with well developed ribocation character.

The nucleosidases catalyze the irreversible hydrolysis of the N9-C1' bond of MTAN to form adenine and thioribose. In particular, the mechanism of action¹⁰ (see Supporting Information Figure S1) involves Asp198/197 (VcMTAN/ EcMTAN) which acts as the catalytic acid and donates a proton to the N7 atom of the bound substrate. The buildup of positive charge on the adenine base draws electrons from the ribosyl group and this causes elongation of the glycosidic bond. Then an oxacarbenium intermediate is formed and stabilized by Glu175/174 (VcMTAN/EcMTAN). The two glutamic acid residues (Glu175/174 and Glu12), and Arg194/193 are responsible for coordinating the catalytic water molecule, which then attacks the anomeric carbon to form the reaction products. MTAN catalyzes reactions similar to nucleoside hydrolases and nucleoside phosphorylases. Nevertheless, MTAN is structurally closer to the nucleoside phosphorylase-1 (NP-1) enzymes so it has been proposed to have a similar catalytic step as the NP-1 enzymes.¹¹ As transition state analogues, such as BDIA, bind more strongly to enzymes with late dissociative transition states⁸ it was also suggested that BDIA resembles EcMTAN's actual transition state structure rather than VcMTAN's.

In addition to the inherent interest on the function of this enzyme, this system is an interesting test case of how very similar enzymes with experimentally determined transition states that are essentially identical seem to have different reaction mechanisms, due to subtle variations in dynamics.

We have investigated the atomic details of the catalytic chemical step for both enzymatic systems. Our lab developed the Transition Path Sampling algorithm for enzyme-catalyzed reactions. We applied this algorithm to interrogate the reaction coordinate pathways to see if differences exist between the enzymes, and if these differences provide explanations for inhibitor specificity. Reaction coordinate information is only accessible from transition path sampling approaches, since all experimental approaches report averages. Examining the atomic details related to the catalytic mechanism, we found that EcMTAN and VcMTAN,

not only have different reaction coordinates but VcMTAN has variable distinct transition states, resulting in more than one reaction path, even though overall the EcMTAN protein structure is more flexible. This may be an indication that several sets of coordinated protein motions can reach related transition states in the *V. cholerae* enzyme. Also, the well defined single reaction path structure of coordinated motions for EcMTAN could explain why transition state analogues bind more tightly.

To identify the reaction coordinate for each system we first obtain the transition state ensemble from the collection of reactive trajectories and then we identify the important residues that participate in the reaction coordinate and explain their contribution.

2 Methods

The starting point of the simulations was crystal structure 1Y6Q (for dimeric *E. coli*) and 3DP9 (for dimeric *V. cholerae*) from the Protein Data Bank. The crystal structure of *E. coli* has 6913 atoms and 219 crystallographic water molecules and the crystal structure of *V. cholerae* has 6941 atoms and 66 crystallographic water molecules. All crystallographic waters were retained. All simulations were performed using the CHARMM^{12,13} molecular dynamics package.

The system was partitioned into quantum and molecular mechanics regions and the generalized hybrid orbital (GHO) method was used to couple them. For both enzymatic systems, the quantum mechanical (QM) region included all of the substrate (MTA), the nucleophilic water, the carboxylate (COO⁻) of equivalent glutamate residues from the active site (Glu174 in the case of EcMTAN and Glu175 in the case of VcMTAN) and the carboxylate (COO⁻) of Glu12. Atom N7 of the purine base was protonated as earlier work has established protonation before reaching the transition state.¹¹ The MM region included all remaining atoms in the protein and solvent. The quantum region was modeled using the AM1 semi-empirical model. The protein was solvated in a sphere of waters with a buffer zone of 12 Å from the edges of the protein, with explicit TIP3 water molecules. During solvation 17433 water molecules were added in VcMTAN and 21310 water molecules in EcMTAN. Then, the system was neutralized by placing 97 potassium and chloride ions for VcMTAN and 108 potassium and chloride ions for EcMTAN. The minimization had three stages. First, each complex was minimized for 100 steps of steepest descent (SD) followed by 1000 steps of adopted basis Newton-Raphson method (ABNR). During this first stage we constrained the sidechain atoms using a harmonic force constant of 10 (kcal/mol)/Å². In the second stage we removed the constraints and minimized for 2000 ABNR steps. In the final stage we switched the QM region on, and minimized for 20000 steps of ABNR. The heating was conducted slowly from 0 to 300 K, for the first 5 ps we applied on the sidechain constraints of harmonic force constant 10 (kcal/mol)/Å², and then removed the constraints, turned on QM/MM, and heated gradually for 250 ps. Finally we equilibrated for 500 ps at 300 K. To analyze the structure of reactive trajectories we used the Transition Path Sampling (TPS) method.¹⁴⁻¹⁷ This method is a Monte Carlo search in reactive trajectory space, where one follows these steps. First, one defines a quantity ("order parameter") that signifies whether the system is in reactants or products. Then, one generates an initial reactive trajectory. One perturbs the momenta of a randomly chosen slice of this reactive

trajectory and propagates from that slice using the new momenta; then one repeats until a new reactive trajectory is generated. Using the new trajectory as a seed, one repeats until a sufficient set of reactive trajectories has been generated. Then for each reactive trajectory, one has to find the time slice that has this property: new trajectories that start from that slice have probability 0.5 to reach reactants and 0.5 to reach products. The set of these iso-probability structures is the Transition State Ensemble (TSE) (or “separatrix”) and is the rigorous definition of a transition state. Note that the separatrix may not necessarily be a narrow region in configuration space and that it contains information about mechanism, because it has been found with trajectories that originated in reactants. TPS finds the separatrix without any bias regarding the chemical nature of the transition state.

We defined the order parameters to be: the bond breaking C1'-N9 length, $< 1.65 \text{ \AA}$ for reactants and $> 1.65 \text{ \AA}$ for products; the bond forming C1'-O_w length, $> 1.65 \text{ \AA}$ for reactants and $< 1.65 \text{ \AA}$ for products; We generated the first reactive trajectory by applying a harmonic force with constant $85 \text{ (kcal/mol)/\AA}^2$ on both the bond breaking and bond forming distances and propagating for 250 fs, a time scale sufficient for the reaction event to occur. Using this trajectory as seed, we generated 150 reactive trajectories of 250 fs for each enzymatic complex. The reaction may be a rare event, but the actual barrier crossing is very fast: the system spends a long time oscillating in the reactants, until it crosses very quickly over to products – it is this fast barrier crossing that our 250 fs trajectories capture. A large number of reactive trajectories is necessary to ensure decorrelation. From the harvested trajectories we identified a transition state ensemble, that consisted of 21 uncorrelated transition state structures for each enzymatic system. We then performed 2 kinds of analysis on these trajectories.

First, we analyzed the transition state ensemble with the kernel principal component analysis (kPCA).¹⁸ In the ordinary PCA one forms the correlation matrix of the centered data, then its eigenvalues are proportional to the variance along the corresponding eigenvectors. If the first few eigenvectors dominate, then they form a low-dimensional representation of the data. The limitation of ordinary PCA is that it implicitly linearizes the data. Indeed, when we performed PCA on our data, the first few eigenvectors did dominate, but the data on their plane formed a semicircle. To correct the linearization assumption we performed a kPCA analysis. In kPCA one makes a non-linear transformation of the original data to a space where the PCA is valid, performs PCA there and then transforms the result back to the original space. Several forms of this nonlinear transformation have been used in the literature (polynomial, exponential and sigmoidal). Because of the semicircular shape of the data in the preliminary PCA analysis, we used a quadratic polynomial transformation. The first eigenvector of this kPCA analysis had an eigenvalue that dominated the variance, therefore it provided a satisfactory dimensionality reduction of the data.

We also identified the reaction coordinate for these systems. For this, one starts with a structure that lies on the isocommittor surface and guesses some residues or atoms that are candidates for belonging to the reaction coordinate. Then one constrains these residues or atoms and propagates the system. If the guess was correct, the system would stay on the isocommittor surface, which is easy to check by generating several trajectories from the end of the restrained walk and calculate the commitment probabilities. One has to iterate until

one finds a candidate reaction coordinate that leads to the above commitment probabilities to be 0.5 for reactants and products. We used four uncorrelated transition states as starting points for this procedure.

3 Results and Discussion

3.1 Structural and flexibility differences

We calculated the root-mean-square fluctuation (RMSF) of C_α atoms averaged over all reactive trajectories for the two proteins (see Supporting Information Figure S2). Comparing the results residue-by-residue provides significant information about the flexibility of the two enzymes, as well as differences between their active sites and nearby residues. Earlier work from our group¹⁹ had shown that EcMTAN is approximately 8% more flexible than VcMTAN. In the present study we found that while this is true for the whole enzymes, EcMTAN active site residues are less flexible than VcMTAN's, but with some exceptions. Most active site residues seem more flexible in VcMTAN, but residues Val102 and Tyr105 that compose the loop above the 5⁰-alkylthio group show greater flexibility in EcMTAN. Some other residues which are more flexible in VcMTAN are Asn73, Gly149, Asp99 and Ala100. All the above residues were found to participate to varying degrees in the reaction coordinate of the enzymatic systems.

Inspection of the active site of each enzymatic complex (see Supporting Information Figure S3) shows that VcMTAN, in addition to an overall more rigid scaffold, also has a more compressed active site where the purine base, catalytic water and glutamates are closer to each other. In EcMTAN we see a looser arrangement. For example, in the active site Glu12 and Glu174 are separated by 7.23 Å in EcMTAN and by 6.15 Å in VcMTAN. The catalytic water in EcMTAN is 4.18 Å from the anomeric carbon, while in VcMTAN this distance is shorter by 0.78 Å. On the other hand, the water is closer to the glutamates in EcMTAN. The distance between the water and Glu12/Glu174 is 3.85 Å and 4.98 Å respectively in EcMTAN, and 3.96 Å and 5.87 Å in VcMTAN. The distance between N6 of the adenine base and the water molecule is 9.46 Å in VcMTAN and 11.87 Å in EcMTAN.

3.2 Transition state identification

From our TPS analysis we generated a transition state ensemble, selected 21 uncorrelated reactive trajectories and calculated the commitment probability along them. This is the probability to reach products if a new trajectory is generated from a time slice with random Boltzmann-distributed momenta. The average over the 21 uncorrelated trajectories for this probability plotted vs time is shown in Figure 1a. In VcMTAN the committor probability rises from 0 to 1 in approximately 21 fs. The rise was much slower, almost 81 fs in EcMTAN, because in this enzyme the water molecule is further away from both the anomeric carbon and from Glu12, so more time is needed for it to be activated by the glutamate and attack the primary carbon. It is worth noting that in VcMTAN the committor vs time-slice curve is approximately symmetric with respect to the transition state, while in EcMTAN it is not.

If we combine these results with geometrical properties of the transition state (Figure 1b) we obtain significant information. EcMTAN has a "late" transition state compared to

VcMTAN, since the distance between the oxygen of the water molecule and the primary carbon is closer to product formation in EcMTAN, in particular, 2.22 Å in EcMTAN and 3.43 Å in VcMTAN. This is also an indication that the transition state analogue mimics the actual EcMTAN transition state better, because it resembles a late transition state.

In Figure 2a we plot projections of reactive trajectories for VcMTAN and EcMTAN on the plane of the bond forming and bond breaking distances. It is clear that the “reaction tube” formed in the transition region by the reactive trajectories is wider in VcMTAN, which allows for the possibility of several geometrically distinct transition states in VcMTAN. So though the overall enzyme is less flexible, the *V. cholerae* enzyme has more paths from reactants to products.

We performed a kPCA analysis of the transition state structures, to obtain a low-dimensional representation of the transition state ensemble. Figure 2b shows plots of the projection of the TSE onto the plane of the first two principal components. For EcMTAN the eigenvalue of the first principal component completely dominates the variance of the data, this is why the projection is a straight line parallel to the horizontal axis. On the other hand, the 2nd principal component makes a contribution in VcMTAN, and we notice 3 different clusters of transition states on the plane of the dominant principal components, grouped by circles in Figure 2b. This result is a strong indication that there are several geometrically distinct transition states in VcMTAN.

When we superimposed the transition state structures for each system, we noticed a difference in the location of the catalytic water. Its position varies among the transition states, relative not only to the ring but also to the glutamates (see Supporting Information Figure S4).

3.3 Reaction coordinate identification

Having obtained the separatrix we now discuss how the reaction coordinate can be extracted from it. As explained earlier, the procedure is to first guess a reaction coordinate; starting from a point on the separatrix to propagate the system while constraining the atoms of the presumed reaction coordinate; at the end of the restrained walk to perform a committor analysis; if the guess for the reaction coordinate is correct, the system will have remained on the separatrix, which has by definition commitment probability 0.5.

We used 4 transition states for each system. First, we tested as a putative reaction coordinate only the bond forming and bond breaking distances. The histogram of the commitment probability after the end of the restrained walk was not a successful guess for the reaction coordinate (see Supporting Information Figure S5).

Then, in addition to the bond forming and bond breaking distances, we applied a force of 2000 (kcal/mol)/Å² on the active site. The committor histogram improved, but again it didn't peak near 0.5 (see Supporting Information Figure S6).

After various tests, constraining different sets of residues, we arrived at a successful reaction coordinate, for a specific transition state (Figure 3a). However, the committor test failed

when we tested the same reaction coordinate on the remaining three transition states (see Supporting Information Figure S7). This shows that VcMTAN has different reaction coordinates corresponding to its distinct transition states.

Further experimenting with constraining chemically important residues, we finally obtained reaction coordinates that pass the committor test in all 4 transition states (Figure 3b to 3d). We found that several amino acids were involved in each of the four reaction coordinates linked to the transition state, while others were involved in only one or more of the TSs. (Table 1) Note that they have several residues in common. However, the fact they have differences, shows that there are more than one path to approach a transition state in VcMTAN.

We now present the results for EcMTAN. Testing the results from VcMTAN to EcMTAN, none of the four VcMTAN reaction coordinates were successful for EcMTAN (see Supporting Information Figure S8).

Then we followed the same procedure as for VcMTAN. First we tested a reaction coordinate that consists only of the bond forming and bond breaking distances, then we added constraints on the active site. Neither attempt led to satisfactory commitment histograms (see Supporting Information Figure S9).

After further trials, we found a set of residues that when assumed to be part of the reaction coordinate led to a histogram peaked at 0.5. Unlike VcMTAN, a single reaction coordinate was satisfactory for all 4 transition states in EcMTAN, so in Figure 4 we present the averaged of the four histograms. The residues that we found to be part of the reaction coordinate in EcMTAN are Ile152, Phe151, Arg193, Ser196, Met173, Asp197, Met9, Ser76 and Ile50. Although this set of residues is similar to VcMTAN's set for TS3 and TS4, applying it to TS3-4 of VcMTAN did not give a successful distribution (see Supporting Information Figure S10).

In Table 1 we summarize the reaction coordinate for EcMTAN and for the 4 transition states of VcMTAN. Those residues contribute in different ways to the reaction coordinate, interacting with the three different regions of the active site. More specifically, residues Phe151/152 and Asp 197/198 determine the purine binding, Ser196/197 and Phe208 orient the asparagine towards the adenine base. Asp197/198 then acts as the catalytic acid and donates a proton to the adenine. The buildup of positive charge on the adenine base pulls electrons from the ribosyl group and this causes elongation of the bond. After the bond elongation the oxacarbenium intermediate is formed and stabilized by Glu175. As soon as the bond breaks the adenine base is pulled away and stabilized by Val153/Ile152. In the mean time, Arg194/193 orients the catalytic water molecule which is activated by Glu12 and attacks the primary carbon. There are also other residues, such as Met174/173 which has been proposed to interact with the departing ribose moiety, similarly to an equivalent residue in PNP,²⁰ and Met9 and Ile50 interacting on both sides of the ribosyl region. The catalytic site also includes residues from the adjacent subunit.

The analysis of the previous subsections provided previously unavailable information about the catalytic mechanism. Even though EcMTAN is overall more flexible, specific residues

in the active site are more flexible in VcMTAN. This leads to distinct transition states and reaction paths in VcMTAN. VcMTAN may better utilize protein motions but an enzyme with several transition states would make it harder for a single transition state analogue to mimic a TS and bind strongly. Some other details about the mechanism involve the role of Asp in MTAN which is different than in PNP, where it is an anion in the Michaelis complex and then stabilizes the N7 brought in from solvent as the TS is approached.²¹ Another interesting fact is the nucleophilic water's activation and further attack to the anomeric carbon. This again suggests that MTANs differ from most ribosyl transferases, where the C1' of the ribose migrates to a nearly immobile nucleophile (see Supplementary videos for more information about the differences in the reaction coordinates as well as differences in water and ribocation motion).

In Figure 5 we show the conformational changes of the active site as indicated by the dihedral angles, O4'-C1'-C2'-H1', C8-N9-C1'-O4', C4'-C5'-S5'-CS. At the beginning of the reaction the ring has a high energy C4 *endo* conformation. The buildup of positive charge on the adenine base, causing the elongation of the glycosidic bond and the formation of an oxacarbenium-like intermediate, leads the sugar pucker to adopt a weak C3 *endo* conformation. The product state ribose finally relaxes in a stable C2 *endo* conformation.

The residues mentioned above are those we found in common in every VcMTAN's TS and in EcMTAN. However, there are several additional residues that participate only in some transition states of VcMTAN.

In Figure 6a we distinguish among the participating residues, to those involved in adenine orientation (orange) and to those interacting with the rest of the active site (green). Figure 6a corresponds to TS3 of VcMTAN and we can see the residues embracing the adenine and residues pushing the water molecule. In Figure 6b we depict the residues of the reaction coordinate in EcMTAN, where there is a breathing motion of residues close to adenine (orange) and residues interacting with the 5'-alkylthio group, while other residues (green) are moving towards the catalytic water molecule aiming to set it in position for catalysis.

4 Conclusion

In conclusion, we revealed the atomic details of the catalytic chemical step of two bacterial enzymes. Although VcMTAN and EcMTAN share similarities in their sequence identity and their active site structure, they have different binding affinities when bound to the same transition state analogue. We found that EcMTAN is overall more flexible, however VcMTAN has greater flexibility for important active site residues that participate in the reaction coordinate. This demonstrates the importance of understanding the nature of the reaction coordinate in atomic detail. In addition VcMTAN has a more compressed active site geometry. Our study revealed that VcMTAN has several geometrically distinct transition states which are accessed by multiple reaction coordinates. We emphasize that these subtle dynamic differences result in a 3 order of magnitude difference in inhibitor binding strength.

The basic difference among transition state structures lies in the water movement towards the anomeric carbon and Glu12. Water involvement is critical and it certainly affects the possibility of reaching a transition state. It points to the difference in the way the enzyme achieves catalysis. The different position of the water molecule in each transition state suggests that the residues involved in its orientation, such as Arg 193/194, have different dynamic behavior among transition states. Another key point is that the water mobility differentiates MTANs from other N-ribosyl transferases. We found that the reaction coordinates of the two enzymes share some residues, but they are different. From both geometrical properties of the transition states of both enzymes and our commitment probability calculations, we showed that EcMTAN resembles a late transition state. These results are consistent with previous studies which indicated that the BDIA transition state analogue better mimics the transition state of EcMTAN, and provide a reasonable explanation for the difference in binding affinity between the two enzymes. EcMTAN shows a simpler structure of coordinated motions and its transition state has characteristics that makes it easier for a transition state analogue to emulate. Such detailed knowledge could have a significant impact on pharmaceutical design.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Keller, B.; Borchardt, R. Biological methylation and drug design. Humana Press; Clifton, NJ: 1986. p. 385-396.
2. Raina A, Tuomi K, Pajula R. Inhibition of the synthesis of polyamines and macromolecules by 5'-methylthioadenosine and 5'-alkylthiotubercidins in BHK21 cells. Biochem. J. 1982; 204:697-703. [PubMed: 6896990]
3. Surfin J, Meshnick S, Spiess AJ, Garofalo-Hannan J, Pan XQ, Bacchi CJ. Methionine recycling pathways and antimalarial drug design. Antimicrob. Agents Ch. 1995; 39:25112515.
4. Chen X, Schauder S, Potier N, van Dorsselaer A, Pelczar I, Bassler B, Hughson F. Structural identification of a bacterial quorum-sensing signal containing boron. Nature. 2002; 415:545-9. [PubMed: 11823863]
5. 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]
6. Shurki A, Warshel A. Structure/function correlations of proteins using MM, QM/MM, and related approaches: methods, concepts, pitfalls, and current progress. Adv. Protein Chem. 2003; 66:249-313. [PubMed: 14631821]
7. Basu I, Cordovano G, Das I, Belbin T, Guha C, Schramm VL. A transition state analogue of 5'-methylthioadenosine phosphorylase induces apoptosis in head and neck cancers. J. Biol. Chem. 2007; 282:21477-86. [PubMed: 17548352]
8. Gutierrez A, Crowder T, Rinaldo-Matthis A, Ho M, Almo S, Schramm VL. Transition state analogs of 5'-methylthioadenosine nucleosidase disrupt quorum sensing. Nat. Chem. Bio. 2009; 4:251-257. [PubMed: 19270684]

9. Gutierrez A, Luo M, Singh V, Li L, Brown L, Norris E, Evans G, Furneux R, Tyler P, Painter G, et al. Picomolar inhibitors as transition-state probes of 5'-methylthioadenosine nucleosidases. *Chem. Biol.* 2007; 2:725–734.
10. Singh V, Lee J, Nunez S, Howell P, Schramm VL. Transition state structure of 5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase from *Escherichia coli* and its similarity to transition state analogues. *Biochemistry.* 2005; 44:11647–11649. [PubMed: 16128565]
11. Lewandowicz A, Schramm VL. Transition state analysis for human and plasmodium falciparum purine nucleoside phosphorylases. *Biochemistry.* 2004; 43:1458–1468. [PubMed: 14769022]
12. Brooks BR, Brooks CL, Mackerell AD, Nilsson L, Petrella RJ, Roux B, Won Y, Archontis G, Bartels C, Boresch S, et al. CHARMM: the biomolecular simulation program. *J. Comp. Chem.* 2009; 30:1545–1614. [PubMed: 19444816]
13. Brooks B, Bruccoleri R, Olafson B, States D, Swaminathan S, Karplus M. CHARMM: A program for macromolecular energy, minimization, and dynamics calculations. *J. Comput. Chem.* 1983; 4:187–217.
14. 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]
15. Dellago C, Bolhuis P. Transition path sampling simulations of biological Systems. *Top. Curr. Chem.* 2007; 268:291–317.
16. Basner JE, Schwartz SD. How enzyme dynamics helps catalyze a reaction, in atomic detail: a transition path sampling study. *J. Am. Chem. Soc.* 2005; 127:13822–13831. [PubMed: 16201803]
17. Quaytman S, Schwartz S. Comparison studies of the human heart and bacillus stearothermophilus LDH by transition path sampling. *J. Phys. Chem. A.* 2009; 113:1892–1897. [PubMed: 19053545]
18. Schoolkopf, B.; Smola, A. *Learning with kernels: support vector machines, regularization, optimization and beyond.* MIT Press; Cambridge, MA: 2002.
19. Motley M, Schramm VL, Schwartz SD. Conformational freedom in tight binding enzymatic transition state analogues. *J. Phys. Chem. B.* 2013; 117:9591–9600. [PubMed: 23895500]
20. Lee J, Cornell K, Riscoe M, Howell PL. Structure of *Escherichia coli* 5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase inhibitor complexes provide insight into the conformational changes required for substrate binding and catalysis. *J. Biol. Chem.* 2003; 278:8761–8770. [PubMed: 12496243]
21. Kline P, Schramm V. Purine nucleoside phosphorylase. Catalytic mechanism and transition-state analysis of the arsenolysis reaction. *Biochemistry.* 1993; 32:13212–13219.

(a)

(b)

Figure 1.

(a) Commitment probability for VcMTAN (top) and EcMTAN (bottom) (b) Representation of the QM region where the distances refer to bond breaking (C1'-N9) and bond forming (C1'-Ow) in the transition state for VcMTAN (left) and EcMTAN (right).

Figure 2.

(a) Reactive trajectories projected on the plane of bond breaking and bond forming distances for VcMTAN (top) and EcMTAN (bottom). Note that the accessible transition region is wider for VcMTAN. The difference in the width of the “reaction tube” in the transition region is highlighted by the black arrows. (b) Projection of the transition state ensemble on the plane of first and second principal components for VcMTAN (left) and EcMTAN (right).

Figure 3.

(a) Successful committor distribution for TS3 (b) to (d) Successful committor distribution for TS1, TS2, TS4.

Figure 4.
Successful committor distribution for EcMTAN.

Figure 5. Dihedral angles O4'-C1'-C2'-H1'(black), C8-N9-C1'-O4'(red), C4'-C5'-S5'-CS (blue) along a reactive trajectory in VcMTAN.

Figure 6.

(a) Participating residues in VcMTAN (b) Participating residues in EcMTAN. The QM region is colored purple, residues interacting with Adenine base are colored orange and residues interacting with the 5'-alkylthio group, ribose and catalytic water are colored green.

Table 1

Residues that participate in the reaction coordinate for EcMTAN and for 4 transition states of VcMTAN. The numbering of residues that is different in EcMTAN is indicated after the slash.

	Met9	Ile50	Asn73	Ser76	Asp99	Ala100	Val102	Phe105	Gly149	Phe152/151	Val153/Ile152	Met174/173	Arg194/193	Ser197/196	Asp198/197	Phe208
EcMTAN	X	X		X						X	X	X	X	X	X	
VcMTAN 1	X	X		X	X		X		X	X	X	X	X	X	X	
VcMTAN 2	X	X		X			X	X		X	X	X	X	X	X	
VcMTAN 3	X			X						X		X	X	X	X	X
VcMTAN 4	X	X	X	X						X	X	X	X	X	X	