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Conformational Freedom in Tight Binding Enzymatic Transition State Analogues

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Abstract

Transition state analogues of bacterial 5 -methylthioadenosine/S-adenosylhomocysteine nucleosidases (MTANs) disrupt quorum sensing pathways in *Escherichia coli* and *Vibrio cholerae*, demonstrating potential to limit pathogenicity without placing bacteria under intense selective pressure that leads to antibiotic resistance. Despite the similarity of the crystal structures of *E. coli* MTAN (*Ec*MTAN) and *V. cholerae* MTAN (*Vc*MTAN) bound to the transition state (TS) analogue BuT-DADMe-Immucillin-A (BDIA), *Ec*MTAN demonstrates femtomolar affinity for the TS analogue whereas *Vc*MTAN possesses only picomolar affinity. Protein dynamic interactions are therefore implicated in this inhibitor affinity difference. We conducted molecular dynamics (MD) simulations of both *Ec*MTAN and *Vc*MTAN in complex with BDIA to explore differences in protein dynamic architecture. Simulations revealed that electrostatic and hydrophobic interactions with BDIA are similar for both enzymes and thus unlikely to account for the difference in inhibitor affinity. The *Ec*MTAN-BDIA complex reveals a greater flexibility and conformational freedom of catalytically important atoms. We propose that conserved motions related to the *Ec*MTAN transition state correlate with the increased affinity of BDIA for *Ec*MTAN. Transition state analogues permitting protein motion related to formation of the transition state are better mimics of the enzymatic transition state and can bind more tightly than those immobilizing catalytic site dynamics.

Keywords

molecular dynamics; quorum sensing; *Vibrio*; *Escherichia*; MTAN; MTA

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Supporting Information Available

Supporting information includes a schematic of the trajectory generation procedure; a visual comparison of the average structures of *Ec*MTAN-BDIA and *Vc*MTAN-BDIA; an expanded computational methods section; a list of all hydrogen bonds detected in *Ec*MTAN-BDIA and *Vc*MTAN-BDIA simulations; a list of distances in the hydrophobic regions of these two systems, and a table comparing RMSFs when the *Ec*MTAN and *Vc*MTAN are unbound, bound to MDIA, and bound to BDIA; and the CHARMM topology and parameter file for BDIA for reference or use. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Introduction

Enzymatic transition states, determined through a combination of experimental kinetic isotope effects and computational techniques, provide a template for the design of powerful transition state analogue inhibitors.¹ These chemically stable mimics of the enzymatic transition state are among the tightest-binding noncovalent inhibitors known.² Transition state analogues were developed for a class of bacterial enzymes involved in quorum sensing and nucleoside metabolism, the 5-methylthioadenosine/S-adenosyl homocysteine nucleosidases (MTANs).^{3–6} The relative affinity of these transition state analogues for MTANs of various species of bacteria revealed two broad classes of transition states for MTANs. Those with an early, partially dissociative transition state include *Neisseria meningitidis* MTAN, and those with a fully dissociative S_N1 transition state include *Escherichia coli* MTAN (*Ec*MTAN) and *Vibrio cholerae* MTAN (*Vc*MTAN).^{5,7} Transition state analogues that are derivatives of Immucillin-A bind preferentially to MTANs with partially dissociated transition states, while the derivatives of DADMe-Immucillin-A bind preferentially to MTANs with fully dissociated transition states.

The role of MTANs in quorum sensing involves the formation of quorum sensing precursors and the metabolic recycling of 5-methylthioadenosine (MTA) to S-adenosylmethionine. Disrupting quorum sensing pathways has the potential to limit bacterial pathogenicity, making MTANs attractive targets. The DADMe-Immucillin-A derivatives are potent inhibitors of both *Ec*MTAN and *Vc*MTAN and have been shown to block quorum sensing without causing growth inhibition or inducing drug resistance.^{4,5,7}

Both *Ec*MTAN and *Vc*MTAN possess fully dissociative transition states and share virtually identical active site structures (0.4 Å RMSD) when bound to DADMe-Immucillin-A transition state analogues (Figure 1a). Surprisingly, the two enzymes differ in their affinities for the same transition state analogue inhibitor, BuT-DADMe-Immucillin-A (BDIA), by three orders of magnitude. The equilibrium dissociation constant (K_i^*) of BDIA from *Vc*MTAN is 208 picomolar⁷, while the K_i^* of BDIA from *E. coli* MTAN is 296 femtomolar.⁴ Since the affinity of transition state analogues for their cognate enzymes is proportional to their similarity to the transition state, this result suggests that BDIA more closely resembles the transition state of *Ec*MTAN than *Vc*MTAN. Yet, according to transition state structures based on kinetic isotope effects, transition state analogue specificity studies, and crystallographic structural analysis, these enzymes are expected to possess virtually *identical* transition states.^{5,8} Their nearly identical static active site structures when DADMe-Immucillin transition state analogues are bound would be expected to form the same chemical environment for substrate molecules and thus for formation of the transition state. The difference in the binding of this transition state analogue, BDIA, to these enzymes, therefore, must lie beyond the static structures of their active sites.

We explored the mechanism of BDIA binding differences to *Ec*MTAN and *Vc*MTAN by conducting molecular dynamics (MD) simulations of both enzyme-transition state analogue complexes and compared these results with simulations of the unliganded enzymes. MD simulations provide another dimension to the two-state static results from techniques used to probe the structural elements of the transition state and crystal structures. While KIE experiments reveal the character of chemical bonds at the transition state relative to the reactants, they do not provide information about motions of the enzyme at the transition state. Likewise, crystal structures provide equilibrated ground state structures with transition state analogues, but not dynamic information.

Analysis of motions associated with formation of the transition state have provided novel information for several enzymes, especially lactate dehydrogenase and purine nucleoside

phosphorylase.^{9,10} The thermodynamic stability of enzymes in complex with a transition state analogue inhibitor often permits the successful determination of the x-ray crystal structure of these complexes. These crystal structures provide an important starting point for determining hydrogen bonding and ionic interactions based on interatomic distances (see Figure 1b).

However, crystal structures reveal only the lowest energy states averaged over large molecular populations¹¹, without the resolution to provide definitive answers about the presence of particular hydrogen bonding interactions.¹²

Methods

Computational approaches

For each enzyme with and without the transition state analogue—*Ec*MTAN-BDIA, *Vc*MTAN-BDIA, *Ec*MTAN(apo) and *Vc*MTAN(apo)—explicit-solvent constant-number, -temperature, and -volume dynamics simulations (NVT), representing the canonical ensemble, were run using the Chemistry at Harvard Macromolecular Mechanics program (CHARMM) for five nanoseconds following equilibration.¹³ To better sample conformation space at a given computational “cost,” a second five-nanosecond trajectory was run, using different starting velocities for each atom.¹⁴ To further our sampling of conformational space, five additional 1-ns “derivative” trajectories were created by restarting dynamics from the first trajectory by using the position coordinates from that trajectory at 0.5, 1.5, 2.5, 3.5, and 4.5 ns but with new velocities for each atom, chosen from a Boltzman distribution to match the simulation temperature of 300 K. (See Figure S1 for a schematic of the trajectory generation process). That this process led to a greater exploration of conformation space was confirmed by differences in the hydrogen bonding observed in trajectory 1 and trajectory 2 from the same system, for example, as discussed in greater detail below.

An expanded description of the computational methods can be found in the Supporting Information.

Results and Discussion

MD Analysis of Hydrogen-bond Stability

The strength of inhibitor binding is often ascribed chiefly to electrostatic interactions between enzyme and inhibitor. Indeed, hydrogen bonds often play a crucial role in such interactions. A chief aim of our simulations was to observe how H-bond interactions between the active sites and the inhibitors varied through the conformational space of the enzyme-inhibitor complexes. In the CHARMM molecular dynamics software package, hydrogen bonds are handled implicitly: the presence of an H-bond can be inferred from the proximity of a suitable H-bond donor and H-bond acceptor. When the hydrogen of an H-bond donor and an H-bond acceptor were within 2.4 Å of one another (corresponding to a donor-acceptor distance of ~3.4 Å), an H-bond was considered formed. This has been shown to be an accurate criterion for the purpose of comparing relative hydrogen bond stability.¹⁷ Our MD simulations of *Vc*MTAN and *Ec*MTAN bound to the transition state analogue BDIA confirmed many of the hydrogen bonding interactions observed in the crystalline structures of enzyme-transition state analogue complexes, while also revealing H-bond interactions *not* anticipated from these crystal structures. Moreover, differences were observed between the H-bonding of *Vc*MTAN to BDIA and *Ec*MTAN to BDIA (see SI Table 1 for a list of all H-bond interactions between enzymes and inhibitors detected by our simulations). However, these differences involved the specific residues and atoms bound to the transition state analogue at any given moment, not the total amount of hydrogen

bonding. Consequently, differences in hydrogen bonding alone cannot account for the thousand-fold difference in affinity for BDIA between *Ec*MTAN and *Vc*MTAN.

MD simulations confirmed that H-bond interactions are maintained from *Vc*MTAN to the purine base of BDIA during the simulation. As seen in Figure 1b, the crystal structure of *Vc*MTAN bound to BDIA (Protein Data Bank ID: 3DP9) indicates that the 9-deazaadenine base forms H-bonds with the main chain atoms of Val153 and the side-chain atoms of Asp198.⁷ Similarly, the crystal structure shows an oxygen of Glu175 forming a hydrogen bond with the 3' oxygen of BDIA while the other glutamate oxygen interacts with the proposed catalytic water molecule (labeled W in Figure 1b), which is further stabilized by H-bonds with Glu12 and Arg194. The hydrogen bond between N7 and O₂ of Asp198 was present for the entirety of all of our simulations, at both active sites (see Figure 2a), while that between N6 and O₁ of Asp 198 was present for 99 percent of the simulation time, also at both active sites.

In the great majority of the sampled configurations, an H-bond was observed between N1 of the base and the backbone nitrogen of Val153. A hydrogen bond between the carbonyl oxygen of Val153 and the N6 of the purine base was less consistently present, with hydrogen bonds forming for between 31 and 81 percent of the time depending on the specific trajectory (corresponding to an average occupancy of 47 percent for active site A and 65 percent for active site B over all trajectories).

The H-bond interactions between the proposed catalytic water with nearby enzyme side-chains were generally less stable than those linking the enzyme to the nucleobase. Notably, as depicted by a dashed orange line in Figure 2a, our simulations support the existence of an additional hydrogen bond not anticipated from the crystal structure.⁷ The side-chain of Ser76 is within H-bonding distance to the pyrrolidine cationic nitrogen, and was present in the majority of sampled conformations. This interaction represents an interaction not apparent in the average of conformers represented by the crystal structure, and also may have been missed by a single long trajectory, as this interaction is absent from active site A throughout trajectory 1.

No crystal structure for *Ec*MTAN bound to BDIA has been reported, but the catalytic site similarity when the enzyme is bound to the related transition state analogue methylthio-DADMe-Immucillin-A (MDIA) (Protein Data Bank ID: 1Y6Q) permitted the replacement of MDIA with BDIA, which differs from MTA only in the length of its 5'-thioalkyl group, as the starting point for our simulations. While the difference in the length of the inhibitor hydrophobic group affects its affinity for the enzyme⁵, the crystal structure of *Ec*MTAN bound to MT-DADMe-ImmA (1Y6Q) indicates an identical pattern of hydrogen bonding between the purine and pyrrolidine rings of inhibitor and the enzyme's catalytic site to those seen in the *Vc*MTAN-BDIA complex.¹⁶ Nonetheless, our simulations detected differences in the interactions at those two locations between the respective enzymes and the transition state analogue that were not evident from comparing their respective crystal structures.

Two notable differences were observed in the H-bonding interactions between the pyrrolidine moiety of BDIA and nearby residues of the respective enzymes. In *Ec*MTAN, one of the catalytic sites never formed a hydrogen bond with the N1 cation via Ser76 (see Figure 2b) whereas both did in *Vc*MTAN. In *Ec*MTAN there was direct H-bonding between Glu12 and the 3'-hydroxyl of the inhibitor in one of the catalytic sites, an interaction absent from the *Vc*MTAN-BDIA simulations. Moreover, in *Ec*MTAN there are greater differences between the two active sites of the same enzyme complex. Take, for example, the H-bonding between the active site residues and the pyrrolidine moiety. In our simulations of *Ec*MTAN, certain H-bonds between the O3' of the pyrrolidine moiety and nearby active site

side chains were present in one sub unit but not the other. There are a greater number of total interactions between the O3 and nearby sidechains in the *Ec*MTAN-BDIA system, but they are shorter-lived than the single direct H-bond between the O3 of BDIA and the active site of *Vc*MTAN. (See Table S1 for a complete list of hydrogen bonds detected in our simulations.)

The average total number of hydrogen bonds present between the two molecules of BDIA filling the active sites and *Vc*MTAN (12.85, for both subunits) was extremely close to the average number of hydrogen bonds between the two BDIA molecules and *Ec*MTAN (12.45). The slight differences in total hydrogen bonding observed between the transition state analogue BDIA and the two enzymes, respectively, is unlikely to account for the 1000-fold difference in affinity BDIA has for these enzymes.

Hydrophobic Interactions

We also compared the hydrophobic interactions between the butyl tail of BDIA and the hydrophobic regions of the two enzymatic active sites. As hydrophobic “forces” are an entropic phenomenon at room temperature, their influence must be deduced by comparing the actual distances observed between hydrophobic entities. A comparison of the average distances between the four atoms of BDIA’s butyl tail (C20–C23) and the closest atoms of the nearest enzymatic residues shows no significant difference between the two systems, *Vc*MTAN-BDIA (4.61 Å) and *Ec*MTAN-BDIA (4.64 Å). (See Table S2 for a list of the component distances making up the average enzyme-inhibitor atom distance in the hydrophobic region.) Such a small difference in hydrophobic interactions is unlikely to account for the 1000-fold difference in inhibitor affinity for the two enzymes.

Differences between *Vc*MTAN and *Ec*MTAN

Given the similarities in their static catalytic site structures and the overall structures of the enzyme when bound to BDIA (see Figure S2 in the Supporting Information for an overlay of the average structures of the two enzymes), it is not surprising that the two enzyme catalytic site bonding interactions with BDIA are so similar. Many of the equivalent residues in *Ec*MTAN and *Vc*MTAN are in the same position with respect to each other and with respect to the inhibitor molecule. Nonetheless, small differences exist. Specifically, there are more, shorter-lived H-bond interactions in the *Ec*MTAN-BDIA complex than in the *Vc*MTAN-BDIA complex, hinting at a possible explanation for the dramatic difference in affinity: BDIA may be better matched to the flexibility and dynamics of the *Ec*MTAN in its configuration near the transition state.

Differences in enzyme flexibility

To further evaluate the apparent difference in BDIA’s restraint of *Ec*MTAN’s active site and *Vc*MTAN’s active site, we compared root-mean-square fluctuations (RMSF) of C atoms in both *Ec*MTAN and *Vc*MTAN as well as all heavy (non-hydrogen) atoms of the inhibitor molecules. Calculating the RMSF of each C atom allows fluctuations to be spatially mapped by residue. RMSFs were calculated versus a given atom’s average position over the course of that particular simulation. These individual values were averaged (weighted according to simulation length) over all trajectories.

Overall, *Ec*MTAN is approximately eight percent more flexible than *Vc*MTAN. When comparing active site residues, that difference increases to ten percent. Of the twenty-six active site residues, only one, *Vc*MTAN Phe208, is consistently more flexible (i.e., more flexible in both subunits of the enzyme, which suggests an effect that is more robust to different starting conformations) than its equivalent in *Ec*MTAN, Phe207. The C RMSF of *Ec*MTAN Phe208 is nine percent greater than that of *Ec*MTAN Phe207. In contrast, fifteen

of the twenty-six active site residues are consistently more flexible in *Ec*MTAN. Of those fifteen, two are more than forty percent more flexible; four are at least thirty percent more flexible; six more than twenty percent more flexible; and ten are more than seventeen percent more flexible, on average, in *Ec*MTAN than their equivalent active site residues in *Vc*MTAN. (See Figure S3 for a residue-by-residue comparison of C RMSF in *Ec*MTAN and *Vc*MTAN.) Moreover, *Vc*MTAN suffers a larger drop in C RMSF when BDIA binds to it (13.4% less than the unbound enzyme) than *Ec*MTAN does (11.8% less than *Ec*MTAN(apo)); this difference is greater at the active site (22.2% for *Vc*MTAN versus 17.2% for *Ec*MTAN). (See Table S3 for a comparison of average RMSF between enzyme(apo), enzyme-MDIA, and enzyme-BDIA complexes in *Vc*MTAN and *Ec*MTAN.) These differences are not likely to be an artifact of starting conformation as simulations with MDIA, the ligand used to determine the 1Y6Q crystal structure, reveal virtually identical RMSFs (see Table S3); if the greater flexibility of *Ec*MTAN-BDIA were due to modifying the inhibitor of the crystal structure we would expect that *Vc*MTAN-MDIA would be more flexible than *Ec*MTAN-MDIA.

Visualizing differences inflexibility

A “heat map” helps visualize the residues with the greatest absolute difference in RMSF between *Ec*MTAN and *Vc*MTAN enzymes (Figure 3).

Deeper shades of red indicate a larger RMSF (C RMSF of a given residue in *Ec*MTAN minus the C RMSF of the equivalent residue in *Vc*MTAN) whereas deeper shades of blue indicate a more negative RMSF. The inset, focusing on active site A, depicts the active site residues with positive RMSFs that are consistent across both subunits. The deepest red residues in Figure 3—those with the largest RMSF—Val102, Phe105, Tyr107, and Pro113 (corresponding to *Vc*MTAN Ala113), compose the loop that closes over the hydrophobic tail of the inhibitor as it occupies the active site.

This greater RMSF in the hydrophobic region of *Ec*MTAN surrounding the hydrophobic tail of BDIA is reflected by the increased flexibility in the hydrophobic tail of BDIA itself in complex with *Ec*MTAN than when in complex with *Vc*MTAN (see Figure 4).

Among the active site residues that are involved in reaction chemistry, *Ec*MTAN Glu174 is consistently more flexible (on average, 18 percent) than its equivalent in *Vc*MTAN, Glu175. This increased flexibility matches the hydrogen bonding patterns discussed above. In *Vc*MTAN, Glu175 spends the vast majority of the simulation time, 97 percent at active site A and 88 percent at active site B, H-bonded to the 3' hydroxyl of BDIA. In contrast, *Ec*MTAN Glu174 varies in which O atom is bound to BDIA's 3' hydroxyl, when it is bound at all. As Glu174 is one of the residues that binds the nucleophilic water molecule, this movement may be crucial to the reaction itself. As with the hydrophobic tail, the increased flexibility of the enzyme at this point is reflected by an increased flexibility of adjacent inhibitor atoms (see Figure 4). The dynamic simulations indicate that *Ec*MTAN Glu174 alternately forms a hydrogen bond with O3'. As a consequence, inhibitor atoms also show increased flexibility when bound to *Ec*MTAN compared to those bound to *Vc*MTAN. The C2' atom of BDIA is also more flexible in the *Ec*MTAN active site, and suggests that the increased flexibility of Glu174 may also be having an effect on the inhibitor via movement of the catalytic water located near this position.

These potentially crucial differences in flexibility at the active site of *Ec*MTAN versus their homologous residues in *Vc*MTAN may be due differences in the protein sequences of surrounding residues between the two enzymes. Both the series of residues with the largest DRMSF—102, 105, 107, and 113—and the differently behaving Glu174 (with respect to its homolog *Vc*MTAN Glu175) are surrounded by a number of different residues in the two

proteins: *VcMTAN* Iso109 to *EcMTAN* Tyr109; *VcMTAN* Met112 to *EcMTAN* Leu112; *VcMTAN* Gln115 to *EcMTAN* Cys115; *VcMTAN* Val169 to *EcMTAN* Ala168; *VcMTAN* Val170 to *EcMTAN* Iso169; and *VcMTAN* Ser177 to *EcMTAN* Thr176.

Conclusions

The tight binding of transition state analogues represents the conversion of dynamic modes that are crucial to catalysis into a thermodynamic free energy well.² These structures are far more stable and longer-lived than the Michaelis complex. In the cases of BDIA binding to *VcMTAN* and *EcMTAN*, their dissociation constants are in the pico- and femtomolar range, respectively. A counterintuitive mechanism for the tighter binding of the *EcMTAN*-BDIA complex is suggested by the greater flexibility and increased conformational freedom of the enzyme-transition state analogue complex. Dynamic motion is required to achieve the transition state, and we suggest that the types of motions conserved in the interaction of the *EcMTAN*-BDIA complex are those reflecting formation of the chemically competent transition state. The *VcMTAN* complex is more constrained in these motions, as seen in hydrogen bonding and RMSF studies. The comparative differences in dynamic regions of these proteins are directly involved in catalysis—the 2 and 3 positions, involved in stabilizing the catalytic water, and Glu12 and Glu174, which both stabilize the catalytic water and are in position to accept one of its protons.¹⁶ Moreover, substrate and inhibitor specificity studies, together with transition state analysis, recognize the required motion of the ribosyl hydroxyls as the ribosyl group migrates from the purine leaving group to the enzyme-stabilized nucleophile in these reaction mechanisms.^{7,16}

We conclude that the specific areas of dynamic motion conserved in the *EcMTAN* catalytic sites are important in both inhibitor binding and in transition state formation. Dynamic interactions of protein and substrate that normally carry the substrate along the reaction coordinate toward the transition state instead result in inhibitor motions that are compatible with substrate motions during catalysis. Therefore, the increased flexibility of the transition state inhibitor BDIA when in catalytic site A of *EcMTAN*, versus *VcMTAN*, reflects motions compatible with transition state formation in *EcMTAN*, and these must be slightly different in *VcMTAN* such that the matched flexibility of *EcMTAN* catalytic site and inhibitor represents a *greater* preservation of the protein's native dynamic modes, results in a better dynamic mimic of the transition state and better binding.

A more conformationally free enzyme-inhibitor complex being more tightly bound seems counterintuitive. But there is at least one similar documented example. In the case of transition state analogue binding to human purine nucleoside phosphorylase (PNP), transition state analogues that have chemical flexibility to permit similar range of atomic motion of catalytic site amino acid residues and inhibitor groups bind more tightly and with less entropic penalty than more rigid inhibitors.¹⁸ These findings are consistent with the view that the strength of enzyme-transition state inhibitor interaction is proportional to the degree that the transition state inhibitor reflects the actual transition state—which we know to be a dynamic entity.^{10,19} Therefore, the transition state inhibitor that channels but does not eliminate the dynamic excursions of the enzymatic transition state will be the one that binds most tightly. By preserving the conformational freedom and flexibility of these moieties, the *EcMTAN*-BDIA represents a system closer to the enzyme's native transition state than the *VcMTAN*-BDIA system is to *VcMTAN*'s native transition state. The *tightest*-binding transition state inhibitors reduce the probability of ligand release through the preservation of the natural protein fluctuations crucial to catalysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Wolfenden R. Transition State Analogues for Enzyme Catalysis. *Nature*. 1969; 223:704–705. [PubMed: 4979456]
2. Schramm VL. Enzymatic transition states: thermodynamics, dynamics and analogue design. *Archives of Biochemistry and Biophysics*. 2005; 433:13–26. [PubMed: 15581562]
3. Singh V, Shi W, Evans GB, Tyler PC, Furneaux RH, Almo SC, Schramm VL. Picomolar Transition State Analogue Inhibitors of Human 5'-Methylthioadenosine Phosphorylase and X-ray Structure with MT-Immucillin-A†. *Biochemistry*. 2004; 43:9–18. [PubMed: 14705926]
4. Singh V, Evans GB, Lenz DH, Mason JM, Clinch K, Mee S, Painter GF, Tyler PC, Furneaux RH, Lee JE, Howell PL, Schramm VL. Femtomolar Transition State Analogue Inhibitors of 5'-Methylthioadenosine/S-Adenosylhomocysteine Nucleosidase from *Escherichia coli*. *Journal of Biological Chemistry*. 2005; 280:18265–18273. [PubMed: 15749708]
5. Gutierrez JA, Luo M, Singh V, Li L, Brown RL, Norris GE, Evans GB, Furneaux RH, Tyler PC, Painter GF, Lenz DH, Schramm VL. Picomolar Inhibitors as Transition-State Probes of 5'-Methylthioadenosine Nucleosidases. *ACS Chemical Biology*. 2007; 2:725–734. [PubMed: 18030989]
6. Wang S, Haapalainen AM, Yan F, Du Q, Tyler PC, Evans GB, Rinaldo-Matthis A, Brown RL, Norris GE, Almo SC, Schramm VL. A Picomolar Transition State Analogue Inhibitor of MTAN as a Specific Antibiotic for *Helicobacter pylori*. *Biochemistry*. 2012; 51:6892–6894. [PubMed: 22891633]
7. Gutierrez JA, Crowder T, Rinaldo-Matthis A, Ho M-C, Almo SC, Schramm VL. Transition state analogs of 5'-methylthioadenosine nucleosidase disrupt quorum sensing. *Nat Chem Biol*. 2009; 5:251–257. [PubMed: 19270684]
8. Singh V, Lee JE, Núñez S, Howell PL, Schramm VL. Transition State Structure of 5'-Methylthioadenosine/S-Adenosylhomocysteine Nucleosidase from *Escherichia coli* and Its Similarity to Transition State Analogues†. *Biochemistry*. 2005; 44:11647–11659. [PubMed: 16128565]
9. Núñez S, Antoniou D, Schramm VL, Schwartz SD. Promoting vibrations in human purine nucleoside phosphorylase. A molecular dynamics and hybrid quantum mechanical/molecular mechanical study. *Journal of the American Chemical Society*. 2004; 126:15720–15729. [PubMed: 15571394]
10. Quaytman SL, Schwartz SD. Reaction coordinate of an enzymatic reaction revealed by transition path sampling. *Proceedings of the National Academy of Sciences*. 2007; 104:12253.
11. Nagel ZD, Klinman JP. A 21st century revisionist's view at a turning point in enzymology. *Nat Chem Biol*. 2009; 5:543–550. [PubMed: 19620995]
12. Wlodawer A, Minor W, Dauter Z, Jaskolski M. Protein crystallography for non-crystallographers, or how to get the best (but not more) from published macromolecular structures. *FEBS Journal*. 2007; 275:1–21. [PubMed: 18034855]
13. Brooks BR, Brooks CL, MacKerell AD, Nilsson L, Petrella RJ, Roux B, Won Y, Archontis G, Bartels C, Boresch S, Caflisch A, Caves L, Cui Q, Dinner AR, Feig M, Fischer S, Gao J, Hodoseck M, Im W, Kuczera K, Lazaridis T, Ma J, Ovchinnikov V, Paci E, Pastor RW, Post CB, Pu JZ, Schaefer M, Tidor B, Venable RM, Woodcock HL, Wu X, Yang W, York DM, Karplus M. CHARMM: The Biomolecular Simulation Program. *J Comput Chem*. 2009; 30:1545–1614. [PubMed: 19444816]

14. Evanseck JD, Caves LSD, Karplus M. Locally accessible conformations of proteins: Multiple molecular dynamics simulations of crambin. *Protein Science*. 1998; 7:649–666. [PubMed: 9541397]
15. Vanommeslaeghe K, Hatcher E, Acharya C, Kundu S, Zhong S, Shim J, Darian E, Guvench O, Lopes P, Vorobyov I, Mackerell AD. CHARMM general force field: A force field for drug-like molecules compatible with the CHARMM all-atom additive biological force fields. *J Comput Chem*. 2010; 31:671–690. [PubMed: 19575467]
16. Lee JE, Singh V, Evans GB, Tyler PC, Furneaux RH, Cornell KA, Riscoe MK, Schramm VL, Howell PL. Structural Rationale for the Affinity of Pico- and Femtomolar Transition State Analogues of Escherichia coli 5'-Methylthioadenosine/S-Adenosylhomocysteine Nucleosidase. *Journal of Biological Chemistry*. 2005; 280:18274–18282. [PubMed: 15746096]
17. De Loof H, Nilsson L, Rigler R. Molecular dynamics simulation of galanin in aqueous and nonaqueous solution. *J Am Chem Soc*. 1992; 114:4028–4035.
18. Hirschi JS, Arora K, Brooks CL, Schramm VL. Conformational Dynamics in Human Purine Nucleoside Phosphorylase with Reactants and Transition-State Analogues†. *J Phys Chem B*. 2010; 114:16263–16272. [PubMed: 20936808]
19. Saen-oon S, Quaytman-Machleder S, Schramm VL, Schwartz SD. Atomic detail of chemical transformation at the transition state of an enzymatic reaction. *PNAS*. 2008; 105:16543–16548. [PubMed: 18946041]

Figure 1.

Crystal structures of *VcMTAN* and *EcMTAN* in complex with DADMe-Immucillin transition state analogues. (a) Overlay of the active site residues of *VcMTAN* (blue) and *EcMTAN* (red) when bound to the transition state analogues BDIA (green) and MDIA (orange), respectively (Protein Data Bank (PDB) entries 3DP9 and 1Y6Q). The oxygen atoms of the proposed catalytic water molecules are depicted in light blue for *VcMTAN* and pink for *EcMTAN*. The backbone RMSD of these active site crystal structures is 0.4 Å. (b) Comparison of inter-atomic distances, in Ångströms, between enzyme and transition state analogue from their respective crystal structures. The distances enumerated in red are from the crystal structure of *EcMTAN* in complex with MDIA (PDB: 1Y6Q) while those in blue are from the crystal structure of *VcMTAN* in complex with BDIA (PDB: 3DP9). The residue names follow the same convention, and purple represents common elements between the two enzymes. BDIA, which is in complex with *VcMTAN*, has a longer alkane tail than MDIA. The additional carbons of BDIA are also depicted in blue for consistency.

Figure 2.

Hydrogen bonding in MD simulations, reported as percent occupancy—the proportion of total time that the hydrogen bond (dashed line) is detected—averaged over all trajectories. The values shown in green represent bonding at the catalytic site formed predominately from subunit A of the homodimer, while those shown in magenta represent bonding at the catalytic site formed predominately from subunit B. (a) Schematic of hydrogen bonding measured between *Vc*MTAN (blue) and BDIA (solid black). The dashed black bonds represent hydrogen bonds predicted from the crystal structure, including those formed with the water molecule, W (pale blue). The dashed orange line represents a hydrogen bond that was not anticipated from the crystal structure (PDB: 3DP9). (b) Schematic of hydrogen bonding measured between *Ec*MTAN (red) and BDIA (solid black). The dashed black bonds represent hydrogen bonds predicted from the crystal structure, including those formed with

the water molecule, W (pale blue). The dashed orange lines represent hydrogen bonds that were not anticipated from the crystal structure (PDB: 1Y6Q).

Figure 3.

The homodimer complex of *Ec*MTAN bound to BDIA is shown with color-map overlay to compare relative fluctuations in *Ec*MTAN and *Vc*MTAN. The *Ec*MTAN dimer is depicted as a cartoon, with active site A on the right and B on the left. The inhibitor heavy atoms are shown as “licorice” segments. By subtracting the Ca RMSF of a residue from the *Vc*MTAN-BDIA system from the corresponding Ca RMSF from the *Ec*MTAN-BDIA system, their relative flexibilities *in situ* can be directly compared. The equivalent calculation was performed for the individual inhibitor heavy atoms, which is also shown using the same color scale. A positive difference (or DRMSF), colored red, corresponds to the *Ec*MTAN-BDIA residue’s Ca (or inhibitor atom) exhibits greater fluctuation in its position through the course of a simulation than its *Vc*MTAN-BDIA counterpart. A negative DRMSF, colored blue, indicates that the *Vc*MTAN BDIA atom exhibits greater fluctuation than its *Ec*MTAN-BDIA counterpart. Inset: A close-up of active site A, with residues Val102, Phe105, Tyr107, Pro113, and Glu174 depicted as thin “licorice” segments.

Figure 4.

Root-mean-squared fluctuation (RMSF) of individual BDIA heavy atoms when occupying *Ec*MTAN active site (red) and *Vc*MTAN active site (blue). Each value represents the average over all trajectories