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Conformational Intermediate That Controls KPC-2 Catalysis and Beta-Lactam Drug Resistance

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Abstract

The KPC-2 carbapenemase enzyme is responsible for drug resistance in the majority of carbapenem-resistant gram-negative bacterial infections in the United States. A better understanding of what permits KPC-2 to hydrolyze carbapenem antibiotics and how this might be inhibited is thus of fundamental interest and great practical importance to development of better anti-infectives. By correlating molecular dynamics simulations with experimental enzyme kinetics, we have identified conformational changes that control KPC-2's ability to hydrolyze carbapenem antibiotics. Related beta-lactamase enzymes can interconvert between catalytically permissive and catalytically nonpermissive forms of an acylenzyme intermediate critical to drug hydrolysis. Using molecular dynamics simulations, we identify a similar equilibrium in KPC-2 and analyze the determinants of this conformational change. Because the conformational dynamics of KPC-2 are complex and sensitive to allosteric changes, we develop an information-theoretic approach to identify key determinants of this change. We measure unbiased estimators of the reaction coordinate between catalytically permissive and nonpermissive states, perform information-theoretic feature selection and, using restrained molecular dynamics simulations, validate the protein conformational changes predicted to control catalytically permissive geometry. We identify two binding-pocket residues that control the conformational transitions between catalytically active and inactive forms of KPC-2. Mutations to one of these residues, Trp105, lower the stability of the catalytically permissive state in simulations and have reduced experimental k_{cat} values that show a strong linear correlation with the simulated catalytically permissive state lifetimes. This understanding can be leveraged to predict the drug resistance of further KPC-2 mutants and help design inhibitors to combat extreme drug resistance.

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ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website. Supporting Methods, Figures, and Tables (PDF)

Keywords

catalytic intermediate; off-pathway; molecular dynamics simulation; beta-lactamase; committor analysis; KPC-2

INTRODUCTION

Antibiotic resistant gram-negative bacteria present a major challenge to global health. In the United States, the class A beta-lactamase KPC-2 is the most common cause of extreme drug resistance, capably hydrolyzing all FDA-approved beta-lactam antibiotics^{1–4}. What distinguishes KPC-2^{5–6} and similar enzymes from less broadly drug-resistant beta-lactamases such as TEM-1⁷ is not the ability to bind substrate, but the ability to deacylate a covalent intermediate in the hydrolysis process. A mechanistic understanding of beta-lactam hydrolysis by KPC-2 in particular thus may yield new opportunities to inhibit this process.

Here, we seek to understand the conformational determinants of catalytic permissivity in the KPC-2 acylenzyme intermediate. This involves integrating two pieces of data: structural and chemical studies suggest that an oxyanion hole is required for efficient deacylation^{1, 8–10} while kinetic studies show the existence of an equilibrium between on-pathway and off-pathway structural forms of the acylenzyme intermediate^{1, 11–15} (Figures 1a, 1b). Our prior work and that of others^{9, 11, 16} suggests that these two may be linked: in studying the related enzyme CTX-M9, we found kinetic evidence of an off-pathway intermediate, while molecular dynamics simulations also showed an equilibrium between conformations forming an oxyanion hole and those that did not. We showed that allosteric mutations that stabilized the oxyanion hole demonstrated increased catalytic activity in CTX-M9. In this work, we hypothesize that these conformational transitions relate to the kinetic relationship between catalytically permissive acylenzyme states and catalytically nonpermissive ones for both CTX-M9 and, critically, KPC-2. We therefore seek to understand the conformational transitions between oxyanion-hole states of KPC-2 and non-oxyanion-hole states, identify what features of the protein potentiate such changes, and to use this information to understand mutations that increase or decrease drug-resistance of this clinically important enzyme.

Understanding transitions in and out of the oxyanion hole and identifying related protein conformational features requires a robust set of order parameters for the free-energy landscape involved. Our initial results indicate that formation of the oxyanion hole state itself, while catalytically required, is not a sufficient determinant of kinetic stability. We therefore employ committor analysis as a means to identify members of the transition state ensemble between catalytically permissive and nonpermissive conformations and to yield an unbiased estimate of the order parameter^{17–19}. Using this analysis, we identify critical changes in the drug-binding pocket that precede and control transitions out of the catalytically permissive state. We demonstrate in molecular dynamics simulations that reversing these transitions drives formation or dissolution of the oxyanion hole. Finally, we validate our predictions by showing that the simulated oxyanion hole lifetimes of mutants at these sites correlates with experimentally measured k_{cat} values for these mutants.

METHODS

Molecular dynamics simulations.

Simulations of the KPC-2:meropenem acylenzyme (Figure S1) were performed using structures and parameters we have previously reported¹⁶. Briefly, an initial structure with the beta-lactam carbonyl in an oxyanion hole was constructed by least-squares fitting of a SFC-1:meropenem acylenzyme structure (PDB code 4EV4) onto the KPC-2 *apo* crystal of KPC-2 (PDB code 2OV5) with the carbonyl beta-lactam hydrogen-bonded to backbone amide protons of Ser70 and Thr237^{5, 11}. The protein was placed in an octahedral box with 2 nm minimum periodic separation and solvated with TIP3P water and 150 mM NaCl. This starting state was energy-minimized and equilibrated as previously described prior to production simulations¹⁶. Simulations were run using Gromacs 5.1²⁰ and AMBER99SB-ILDN protein parameters^{21–22}. Meropenem parameters were determined as previously reported¹⁶. Hydrogen bonds were constrained using LINCS and short-range interactions were truncated at 1.2nm. Long-range electrostatics were treated using Particle Mesh Ewald²³. Simulations were run with temperature maintained at 310K using a velocity-rescaling thermostat²⁴ and pressure at 1 bar using a Berendsen barostat. An initial set of 20 simulations each at least 480 ns in length were run from this starting conformation with starting velocities randomly assigned from a Maxwell distribution. Further simulation datasets used in committor analysis and prediction of mutants are described below.

Kinetic map construction.

Conformational states of KPC-2:meropenem were determined via an initial fine structure-based clustering of simulation snapshots taken at 50-ps intervals followed by kinetically driven secondary clustering. A single round of k-centers clustering on RMSD of the drug-binding pocket (see Supporting Information for definition) to a cutoff of 1 Å RMSD was followed by 10 rounds of k-medoids optimization to yield 2402 fine clusters with RMSD of 0.6 Å from each cluster medoid averaged over the dataset. Kinetically driven clustering was then performed using Robust Peron Cluster-Cluster analysis²⁵ on the connectivity graph obtained by mapping the original simulation trajectories onto the fine structural clustering to yield 50 kinetically lumped conformational states. The resulting map was visualized as a directed graph with edge weights between nodes *i* and *j* proportional to the probability of an *i-j* transition in the simulation trajectories. This map was then analyzed for transitions from oxyanion-hole conformational states to non-oxyanion-hole conformational states using a 3.3-Å cutoff definition of a hydrogen bond. Additional details are given in the Supporting Information.

Committor analysis.

Because two metastable free-energy basins were observed in the original set of simulation trajectories, commitment probability¹⁷ between the two was calculated to yield a robust reaction coordinate. The catalytically permissive (EI) basin was defined as hydrogen-bonds according to the Wernet Nilsson criteria²⁶ between: the backbone amides of Thr237 and Ser70 and the beta-lactam carbonyl oxygen, the side chain of Asn132 and meropenem 6α-1R-hydroxyethyl, and the side chains of Glu166 and Asn170. The catalytically nonpermissive (EI*) basin was defined as a loss of the oxyanion hole hydrogen bonds and a

distance greater than 1 nm between Glu166 γ O and Asn170 α C or Asn170 γ C and Glu166 α C. We compute a number of unbiased molecular dynamics trajectories starting from some point X in conformation space and calculate the number of simulations n_{EI} that reach basin EI before basin EI* and the number of simulations n_{EI^*} that reach basin EI* before basin EI. The commitment probability $P_X = n_{EI} / (n_{EI} + n_{EI^*})$ is thus a robust reaction coordinate that depends only on the structural definition of the metastable basins and does not require prior knowledge of any collective variables or order parameters. We performed this analysis on 20 conformational snapshots resampled from an unbiased molecular dynamics simulation trajectory that started in EI and ended in EI* to classify the conformational transition and obtain a member of the transition state ensemble similar to an approach used previously for other complex biomolecular reactions¹⁹. Between 20 and 80 unbiased simulations were used per starting point with a minimum length of 50 ns per simulation. Commitor value uncertainties were estimated via bootstrap resampling. Commitor calculations were rerun in another force-field for the conformational snapshot identified as a transition-state ensemble member, showing a mild perturbation of committor value but overall indicating robustness of results to choice of force-field (see supporting information).

Identification of protein conformational transitions that control catalytic permissivity.

Since beta-lactam drug reorientation occurred relatively late in the transition between catalytically permissive and nonpermissive states, we performed information-theoretic feature selection to determine which protein conformational transitions control catalytic permissivity. In addition to the 20 conformations for which committor values were calculated directly, we imputed committor values for related snapshots via agglomerative clustering, yielding 35,651 conformational snapshots fully committed to EI and 4,448 snapshots fully committed to EI*. To assess the accuracy of this imputation, we selected three snapshots assigned to EI commitment and three assigned to EI* by this procedure that did not yet satisfy the EI or EI* criteria and had an RMSD from the “parent” snapshot for which a committor value was determined equal to the median value of the cluster. We ran 20 unbiased trajectories from each of these six starting structures and found committor values equal to the parent structure in all six cases. This procedure will of course only be accurate for small RMSD clustering thresholds and for committor values not particularly close to the transition state.

These two sets of EI-committed and EI*-committed conformations were reweighted to yield a balanced dataset, and minimum-redundancy, maximum-relevance feature selection²⁷ was then applied to identify the 10 protein-protein interatomic distances that best differentiated EI and EI* states (Table S1; see Supporting Information for details on clustering and feature selection).

To test the effect of these top 10 distances on determining rather than just reporting on catalytic permissivity, we selected KPC-2:meropenem conformations that were strongly committed to either EI or EI* and asked whether biasing the EI-starting conformations to the top-10 distances found in EI* conformations would change overall commitment and vice versa. To test this, we ran an additional set of 20 committor-analysis trajectories with a Hamiltonian bias on the top 10 distances using a minimal-biasing potential formulation²⁸.

These simulations were run until commitment to either EI or EI* to estimate committor values under biasing and compare with unbiased committor values.

Testing of KPC-2 point mutants.

A series of Trp105 point mutants were generated, simulated, and the EI state lifetime compared to experimentally measured k_{cat} values as follows: each point mutant structure was generated via Modeller²⁹ using a KPC-2:meropenem conformational snapshot in the EI state as a template. Energy minimization and equilibration were performed identically to the KPC-2:meropenem wild-type enzyme with the exception that position restraints were applied to each atom in the mutant residue 105 that had a matching atom in the wild-type structure, bringing the mutant residue 105 into alignment with the wild-type Trp105. 20 simulations were run per mutant, each until EI* state commitment or > 100 ns. EI state lifetime for each mutant was estimated by calculating the average probability of satisfying criteria for state EI across all simulations and fitting a double-exponential decay to these data after applying a Gaussian filter to correct for fast time-scale fluctuations. The aggregate decay constant, $\tau = A_1\tau_1 + A_2\tau_2$, where A is amplitude and τ is the decay constant, was then compared to experimental k_{cat} values previously reported³⁰.

RESULTS AND DISCUSSION

Kinetic map of KPC-2 conformational transitions.

We constructed an initial kinetic map of conformational transitions of KPC-2 by starting 20 independent simulation trajectories of the KPC-2:meropenem acylenzyme from the catalytically permissive, oxyanion hole state. 11 of these assumed a non-permissive state with loss of the oxyanion hole within 500 ns of simulation. We then used these simulations to construct a kinetic map of the conformational transitions associated with loss of the oxyanion hole in KPC-2. This map (Figure 1c), generated by fine structural clustering and then analysis of the resulting kinetic network (see Methods), surprisingly showed that loss of the oxyanion hole occurred quite late in the transition from permissive to nonpermissive states. Both oxyanion-hole and non-oxyanion-hole conformations formed kinetic subnetworks with substantial exchange among conformational clusters (Figure S2), but the pathway from oxyanion-tonon-oxyanion conformations included a large number of oxyanion-hole conformations that demonstrated unidirectional flow towards the non-oxyanion-hole state within our initial sampling (Table S2). This finding suggests that the hydrogen bonds supporting an oxyanion hole are alone insufficient to define a metastable free-energy basin for catalytically permissive conformations of KPC-2. We therefore developed a more robust set of criteria to capture this metastable basin as outlined below.

An unbiased reaction coordinate for KPC-2 conformational transitions.

We employed committor analysis to develop a more robust reaction coordinate for conformational transitions of the KPC-2 acylenzyme between catalytically permissive and nonpermissive states. Briefly, committor analysis relies on running a large number of molecular dynamics trajectories starting from the same point in conformation space; given a set of metastable basins $\{A, B, \dots\}$, the committor value of that starting point in phase space is defined as $(a_i, b_i, \dots)$, where a_i is the fraction of trajectories that reach state A before any

other defined basin. This provides a reaction coordinate for an arbitrarily complex free-energy landscape that depends only on the definitions of the metastable basins. Based on prior analysis of KPC-2^{5, 11, 31}, we defined the catalytically permissive basin as conformations fulfilling the following criteria: hydrogen bonds between: (1) the backbone amides of Thr237 and Ser70 and the beta-lactam carbonyl oxygen that form the oxyanion hole, (2) the side chain of Asn132 and meropenem 6 α -1R-hydroxyethyl, and (3) the side chains of Glu166 and Asn170. We defined the nonpermissive basin as conformations showing 1) a loss of the oxyanion hole and (2) distance greater than 1 nm between Glu166 γ O and Asn170 α C or Asn170 γ C and Glu166 α C (Figures 2a, 2b, S3). We then ran “shooting” trajectories from starting conformations resampled from an unbiased permissive-to-nonpermissive molecular dynamics trajectory of the KPC-2 acylenzyme (Figure 2c) and applied committor analysis in order to better understand the free-energy landscape of this transition.

Analysis of the transition state controlling catalytic permissivity.

Committor analysis showed two strongly-committed regions with a relatively broad region of moderate commitment between them (Figure 3a). This is consistent with the probability density projection from our initial simulations rendered in Figure 2c, which suggests a relative “plateau” of the free-energy surface between the two large metastable basins, although both the estimated free-energy surface and the committor values suggest that this intermediate region is far from uniform. As expected from the kinetic map analysis, the member of the transition state ensemble identified via committor analysis still showed the hydrogen bonding pattern that defines an oxyanion hole, confirming that loss of the oxyanion hole occurs after commitment to nonpermissivity in our simulations. Structural comparison of the catalytically permissive basin (EI) and the catalytically nonpermissive basin (EI*) showed movements in the SDN motif loop and the loop containing Trp105 away from the drug (Figure 3b). The member of the transition state ensemble was via gross analysis structurally intermediate between EI and EI*.

Several structural features of this transition were immediately apparent. The meropenem carbonyl group that participates in deacylation is oriented towards Thr237 and the oxyanion-hole hydrogen bonding sites in EI and transition-state conformations but away from Thr237 in EI* conformations. Additionally, the deacylating water is positioned for nucleophilic attack on the carbonyl carbon in EI conformations, but there is no similarly positioned water in the transition-state ensemble member or EI* conformations. Both of these conformational features of the EI state are widely believed to be important for deacylation^{10, 12, 32–33}.

Since all of the key distances controlling catalytic permissivity increased between EI and EI* states, we hypothesized that some of the conformational changes involved in loss of catalytic permissivity might also affect the binding pocket size. Indeed, the solvent-accessible surface area of the binding pocket significantly increased from catalytically permissive states to nonpermissive states (Figure 4b), and the free-energy plateau region showed intermediate values.

The active-site volume also increases progressively from EI conformations to the transition-state ensemble member to EI* conformations. Recent work on stability-activity tradeoffs in

among KPC-2 mutants demonstrated a number of mutations increasing ceftazidime drug-resistance and k_{cat} that are less thermostable³⁴. These mutants however have lower drug resistance and k_{cat} for meropenem substrates. It was proposed that this decreased stability was necessary to accommodate the larger ceftazidime substrate in the active site. One might therefore speculate that the conformational accessibility of the EI* state with its larger active-site volume might be related to the ability of an enzyme to accommodate larger beta-lactam substrates. Quantitative analysis and computational testing of the key protein conformational features controlling the EI to EI* transition of KPC-2 follows.

Protein conformational changes controlling catalytic permissivity.

Because the KPC-2 transition between catalytically permissive and nonpermissive states is relatively complex, we used information-theoretic feature selection to identify a set of key protein conformational changes that control catalytic permissivity. We applied mRMR feature selection (see Methods) to rank independent interatomic distances that best differentiate conformations in the EI basin from those in the EI* basin (see Methods). We selected the top ten distances (Figure 4a; Table S1) and set up the following test to evaluate their effect. If biasing these distances towards EI-committed values reverses commitment to the EI* state and biasing these distances towards EI*-committed values reverses commitment to the EI state, then they can be considered to control catalytically permissive versus nonpermissive conformations. As shown in Figures 4c and 4d, rerunning committor calculations with these biases applied indeed reversed commitment, and since none of the distances involved either the drug or residues used to assess commitment, we conclude that these interatomic distances indeed control oxyanion hole stability and catalytic permissivity. Sequential re-formation of key interactions necessary for catalysis are shown in Figure S4. We thus conclude that loss of catalytic permissivity involves relaxation of the binding-pocket structure in ways that permits the beta-lactam substrate to lose proper orientation for hydrolysis.

Prior studies have shown evidence for conformational heterogeneity of the acylenzyme states of beta-lactamases, with some of the data indicating increased solvent accessibility⁸, which agrees well with our simulation findings, as well as conformational flexibility of the substrate³⁵ and potentially tautomerization of carbapenem substrates⁸. All of these would contribute either to the observed conformational heterogeneity of EI* states in our simulations or to further heterogeneity beyond what we have sampled and would support the hypothesis of an EI* ensemble that converts back to catalytically permissive EI states only slowly.

Since all of the key distances controlling catalytic permissivity increased between EI and EI* states, we hypothesized that some of the conformational changes involved in loss of catalytic permissivity might also affect the binding pocket size. Indeed, the solvent-accessible surface area of the binding pocket significantly increased from catalytically permissive states to nonpermissive states (Figure 4b), and the free-energy plateau region showed intermediate values. Sequential re-formation of key interactions necessary for catalysis are shown in Figure S4. We thus conclude that loss of catalytic permissivity

involves relaxation of the binding-pocket structure in ways that permits the beta-lactam substrate to lose proper orientation for hydrolysis.

Testing mutants of key residues controlling permissivity.

More specifically, all ten distances controlling the KPC-2 acylenzyme conformational change involved either Ser130 or Trp105. Ser130 hydrogen-bonds with the meropenem thiazolidine ring, while Trp105 extensively contacts the ring in catalytically permissive conformations (Figure S5). To test the importance of these residues and their interactions with the rest of the binding pocket in controlling carbapenem hydrolysis by KPC-2, we simulated wild-type KPC-2 and four Trp105 mutants that had been characterized experimentally: W105F, W105N, W105V, and W105L³⁰. Since occupancy of the catalytically permissive state should correlate with k_{cat} , we measured the time-autocorrelation function of the catalytically permissive state in our simulations and compared it with the imipenem k_{cat} values measured experimentally (Figures 5, S6). We also simulated the S130A mutant; this mutant does not confer measurable carbapenem resistance in bacteria and a k_{cat} value is not available³⁶, but the EI-state lifetime is comparable to the least stable W105 mutant. We observe a Pearson correlation coefficient of 0.95 between calculated EI-state lifetimes and experimental k_{cat} values. Because k_{cat} is a steady-state measurement, it will reflect the equilibrium coefficient $K_{off-pathway} = [EI]/[EI^*]$. Our finding that k_{inact} , the EI-to-EI* forward rate, correlates strongly with k_{cat} therefore leads us to conclude that the EI-to-EI* forward rates vary between mutants more than the reverse rates, and thus the EI-state lifetime controls the equilibrium constant between these two states. It is thus fortunate that the relatively slow EI-to-EI* equilibration process is controlled by a fast rate that can be readily sampled on molecular dynamics timescales. The strong linear correlation with k_{cat} values further suggests that Trp105 may indeed control transitions between catalytically permissive and nonpermissive states and thence k_{cat} values and carbapenem drug resistance.

CONCLUSIONS

The acylenzyme intermediate of KPC-2 is critically important in differentiating this carbapenemase from less-resistant beta lactamases. Prior work has suggested that the conformational equilibria of this acylenzyme state may determine k_{cat} but a detailed molecular explanation for this has thus far been lacking. Here, we have used classical molecular dynamics simulations of the acylenzyme intermediate and committor analysis to analyze slow conformational changes between a catalytically poised substate of the acylenzyme intermediate and one that lacks key features for catalysis. The changes we identify to the ligand-binding pocket, specifically interactions of Ser130 and Trp105, appear critical in positioning the carbapenem drug for hydrolysis. We study the acylenzyme intermediate because deacylation is rate-limiting for KPC-2 hydrolysis of meropenem; however, since the acylation and deacylation transition states are believed to be conformationally similar, one might speculate that similar effects would apply for beta-lactamase:substrate pairs where acylation is rate-limiting.

Knowledge of the key changes that determine the equilibrium between catalytically permissive and catalytically non-permissive conformations of KPC-2 will now permit better prediction of additional drug-resistance mutants of KPC-2 and potentially other highly resistant beta lactamases. It also provides a starting point for the computational evaluation of new small-molecule inhibitors for this beta lactamase that is the most common cause of carbapenem-resistant infections in the United States. A number of KPC variants have been reported that either have increased drug resistance and k_{cat} against carbapenem antibiotics³⁷ or that appear to trade off some extent of carbapenem resistance for increased resistance to cephalosporin antibiotics³⁴. While the equilibrium between catalytically permissive and non-permissive states of KPC acylenzyme complexes is certainly not the only factor in determining catalytic rate or substrate range, an increased predictive understanding of this equilibrium will assist in evaluating how such variant carbapenemases function and what the implications might be for future inhibitors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Hydrolysis of beta-lactam drugs by KPC-2 involves an off-pathway intermediate that correlates with conformational changes in the acyl intermediate state. (A) Reaction diagram for beta-lactam hydrolysis by KPC-2 with off-pathway intermediate demonstrated for CTX-M9 and hypothesized for KPC-2. (B) Rendering of hydrogen bonds stabilizing an oxyanion hole in the KPC-2 acyl-enzyme (left) and alternate conformational state that does not stabilize an oxyanion hole (right). (C) Kinetic clustering of KPC-2 simulations shows a subnetwork of oxyanion-hole conformations, a subnetwork of non-oxyanion-hole conformations, and a flux pathway between these that retains an oxyanion-hole hydrogen bonding pattern until relatively late. (D) One-dimensional free-energy schema showing that the transition state for conversion between oxyanion-hole and non-oxyanion-hole conformations occurs before hydrogen bonds are actually lost, as confirmed by subsequent committor analysis.

Figure 2. Metastable basins of catalytically permissive and non-permissive state in KPC-2. Rendered in (A) and (B) are the intra-molecular distances (dashed lines) used to classify metastable basins for catalytically permissive (EI) and catalytically nonpermissive (EI*) states. The EI basin was defined as hydrogen bonds between the displayed atom pairs (yellow dashes). The EI* basin was defined as a lack of oxyanion hole and a distance $> 8\text{\AA}$ between 166 CD and 170 CG (orange dashes). Rendered in (C) is a projection of relative free energy onto a 2D plane defined by PCA of all pairwise heavy-atom distances within the binding pocket. A representative trajectory between EI and EI* is plotted in black.

Figure 3.

Committer values yield a reaction coordinate for catalytic permissivity and member of the transition-state ensemble. (A) Rendering of committer values through the transition region between EI and EI*, identifying a member of the transition state ensemble at 50% commitment. Shaded areas indicate bootstrapped 95% confidence intervals (B) Rendering of the transition state ensemble member and two structures that were EI-committed and EI*-committed (drug not shown). Protein conformational changes from EI to EI* include alterations in the loop containing W105 and the SDN loop. These residues then interact differently with the substrate, and the binding pocket size increases (Figure 4b).

Figure 4.

Identification and validation of conformational changes between permissive and non-permissive states. (A) Molecular rendering of key distances that change between EI and EI* states identified by information-theoretic feature selection. (B) Solvent accessible surface area for the ligand-binding pocket plotted for conformations in the EI permissive state, the EI* nonpermissive state, and states on the free-energy plateau between. Pocket surface area increases significantly from EI to EI* conformations ($p < .0001$ via 2-sample Kolmogorov-Smirnov test). (C,D) Biases on key distances control commitment to EI vs. EI* states. Control of catalytic permissivity by these key distances was validated by selecting starting conformations on either side of the free-energy barrier between EI and EI* and then running MD simulations biased towards the opposite state via restraints on the identified distances. In each case, the applied bias significantly altered commitment compared to an unbiased set of trajectories. Error bars in all plots indicate 95% confidence intervals calculated via bootstrap.

Figure 5.

Trp105 mutants with reduced k_{cat} values show corresponding reduced lifetime in the catalytically permissive acyl intermediate state. (A) Probability of remaining in catalytically permissive state calculated from simulations of Trp105 mutants and the S130A mutant. (B) Lifetime of catalytically permissive state (EI) in each set of mutant simulations is plotted against the corresponding imipenem k_{cat} value. Dashed lines indicate fits (double-exponential for lifetimes, linear for lifetime- k_{cat} relationships). The Pearson correlation coefficient is 0.95.