Supporting Information for Mechanism and Kinetics of Aztreonam Hydrolysis Catalyzed by Class–C β–Lactamase: A Temperature-Accelerated Sliced Sampling Study

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1 Simulation Details of Reverse Acylation of Acyl–Enzyme Complex

From the previous studies,[1] it was established that acylation follows a single water molecule assisted mechanism. Thus, while simulating the reverse-acylation, we considered only one water molecule mediated catalytic mechanism. However, during the QM/MM TASS simulation, it was observed that one of the MM water molecules replace the catalytic QM

water molecules in the active site due to the open boundary between QM and MM water molecules. To alleviate this problem, we treated all the three water molecules found in the vicinity of the catalytic water using QM, however, restrained their oxygen atoms loosely to their equilibrium positions.

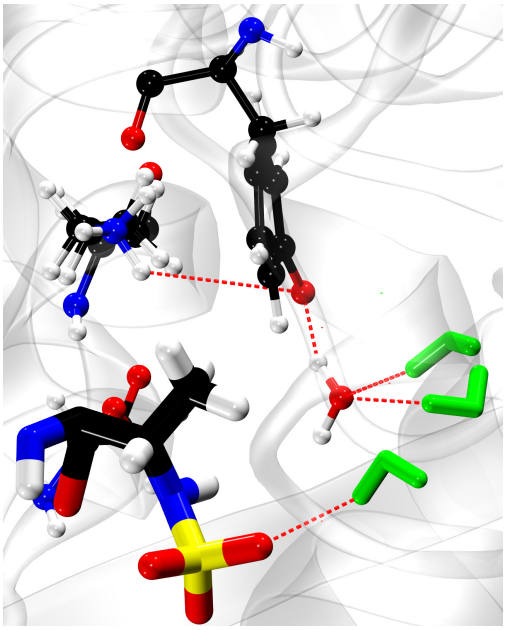

Figure S1: Active site structure of **EI** is shown. Attacking water molecule (W_1) is shown in ball and stick while the neighborhood water molecules are treated by QM are shown in green color.

2 Definition of Coordination Number Type CV

Coordination number of atom A with a group of atoms B, $C[\mathrm{A}-\mathrm{B}]$ is defined by a smooth function depending on the distance d_J between atom A and atom J belonging to group B,

$$C[\mathrm{A}-\mathrm{B}] = \sum_{J \in \mathrm{B}} \frac{1-\left(\frac{d_J}{d_0}\right)^p}{1-\left(\frac{d_J}{d_0}\right)^{p+q}}$$

where d_0 is a parameter which is chosen based on the type of atoms A and B, as given in SI Table S1. We used $p = 6$ and $q = 6$ in the above equation so that the function decays

smoothly from 1 to 0 about $d_J = d_0$.

Table S1

A	B	d_0 (in Å)
N	H	1.3
O	H	1.3

3 Conventional Metadynamics Simulation of EP from EIb

Here two CVs were chosen: a) distance between $Ser_{64}O_\gamma$ and $AztC_2$, $d[Ser_{64}O - AztC_2]$; b) coordination number of $Ser_{64}O_\gamma$ to the terminal protons of $Lys_{67}N_\zeta$, $C[Ser_{64}O_\gamma - Lys_{67}N_\zeta H_\zeta]$. Gaussian height was fixed to 0.6 kcal mol^{-1} and the hill width parameter was fixed to 0.05 (units of CVs). Extended Lagrangian metadynamics simulation was performed where the CV mass was set to 50 a.m.u. and the coupling constants were set to 2 a.u. Temperature of the auxiliary variables was maintained at 300 K by velocity scaling. An adaptive MTD time step was used such that the bias potential was updated only when the CVs make a displacement of least 1.5 times the width of a Gaussian from the previous Gaussian center. Free energy surface obtained from this metadynamics simulation is given in Figure S2. It is noted here that the basin for **EP** was not sampled in this simulation (thus appearing shallow) since the interest here was only to estimate the **EIb** $\rightarrow$ **EP** (forward) free energy barrier.

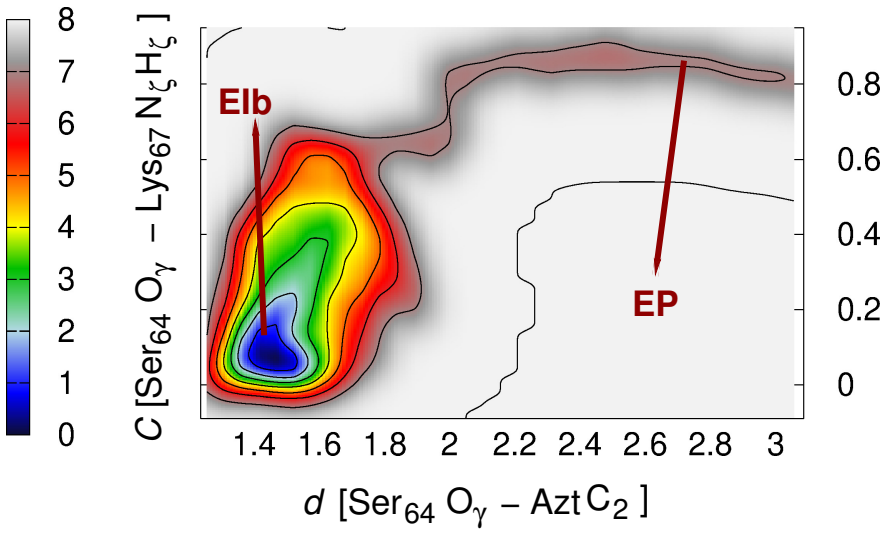

Figure S2: Free energy landscape as obtained from conventional metadynamics simulation of **EIb** $\rightarrow$ **EP**; Contour lines are drawn for every 1 kcal mol^{-1}. Here, d[Ser$_{64}$O $-$ AztC$_2$] is in Å.

References

(1) Tripathi, R.; Nair, N. N. Mechanism of Acyl–enzyme Complex Formation from the Henry–Michaelis Complex of Class C β-Lactamases with β-Lactam Antibiotics. *J. Am. Chem. Soc.* **2013**, *135*, 14679.