Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Electronic Supplementary Information : Molecular Insights into Avibactam Mediated Class C β–Lactamase Inhibition: Competition Between Reverse Acylation and Hydrolysis Through Desulfation

Chandan Kumar Das and Nisanth N. Nair*

Department of Chemistry, Indian Institute Of Technology Kanpur, Kanpur, 208016, India

E-mail: nnair@iitk.ac.in

S1 Details of Enhanced Sampling Simulation

We employed the extended Lagrangian variant of metadynamics[1] approach in this study. In this formulation, collective variables (CVs) are harmonically restrained with the corresponding auxiliary variables. We used a spring constant of 2.0 a. u. for the restraints and the masses of the auxiliary variables were set to 50 a.m.u. Bias potentials were constructed by sum of spherical Gaussian functions with their heights varied between 1.2 to 0.6 kcal mol^{-1} and fixed the Gaussian width parameter of 0.05. Gaussian bias was updated only when displacement of CV in the CV–space is greater than 0.075 (that is 1.5 times the Gaussian width parameter). The velocity rescaling scheme was used for maintaining the temperature of auxiliary variables to 300±200 K. CVs used in metadynamics simulations are listed in Table S1.

While modeling the recyclization of avibactam (**EI** $\rightarrow$ **ES**), we used the well–sliced metadynamics[2] method. In this approach, well–tempered metadynamics bias[3] is added along one CV and umbrella bias potential is added along the other. The restraint umbrella bias potential was applied along the $N_6 \cdots C_7$ bond distance (CV4) chosen for accelerating the ring closure, whereas CV3 (chosen for the deprotonation of N_6) was sampled using well–tempered metadynamics;[3] see Table S1. Umbrella windows were set with a harmonic force constant of 88 kcal mol^{-1} Å^{-2}, placed from 3.7Å to 1.3Å at an interval of 0.05Å. The initial Gaussian height, hill width and ΔT (needed for the well-tempered metadynamics simulation[3]) parameters were set to 0.6 kcal mol^{-1}, 0.05 and 8000 K, respectively. Langevian thermostat was used to maintain the temperature of CVs to 300 K.

In our metadynamics runs, we have employed coordination number CVs (Table S1). Coordination number of an atom A with a group of atoms B (termed as C[A...B]) is defined as,

$$C[\mathrm{A}-\mathrm{B}] = \sum_{J \in \mathrm{B}} \frac{1}{1 + \left(\frac{d_{\mathrm{A}J}}{d^0_{\mathrm{AB}}}\right)^6} \quad . \tag{1}$$

Here, $d_{\mathrm{A}J}$ is the distance between atoms A and J and d^0_{AB} is the cutoff distance between those two atoms.

Table S1: Description of CVs for reactions. Definition of coordination number CV (C[A-B] are as per Eq. (1), and the distance between atom A and B is denoted as d[A-B]. Here, d^0_{AB} (see Eq. (1)) is in Å, total simulation time (τ) is in ps and free energy barrier ($\Delta F^{\ddagger}$) is given in kcal mol^{-1}.

Reactions	CVs	d^0_{AB}	τ	$\Delta F^{\ddagger}$
ES $\rightarrow$ **EI**	CV1 = C[Ser64:$O_\gamma \cdots C_7$]	2.11	8	25
	CV2 = C[$N_6 \cdots C_7$]	2.11		
EI $\rightarrow$ **ES**	CV3 = C[Tyr150:$O_\eta \cdots H_{15}$]	1.33	393[a]	36
	CV4 = d[$N_6 \cdots C_7$]			
EI $\rightarrow$ **EI–SO$_4$**	CV5 = C[$C_5 \cdots C_8$]	2.11	26	29
	CV6 = C[$N_6 \cdots O_{10}$]	2.11		
EI–SO$_4$ $\rightarrow$ **EP–SO$_4$**	CV7 = C[$O_{\mathrm{Wat}} \cdots C_7$]	2.11	22	40
	CV1 = C[Ser64:$O_\gamma \cdots C_7$]	2.11		

[a] Here the total simulation time is the total simulation time over all the umbrella windows.

S2 Supplementary Figures

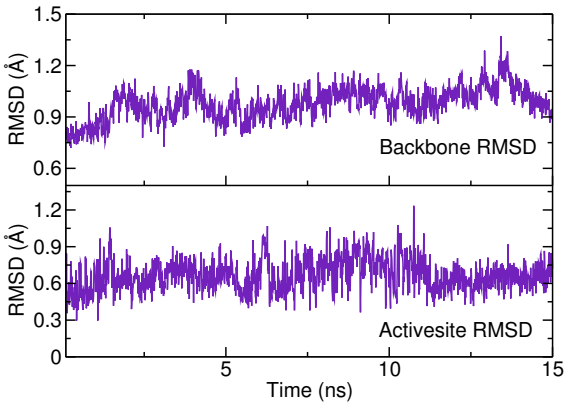

Figure S1: RMSD of the heavy atoms of the protein backbone (upper panel) and the active site (including Ser64, Lys67, Tyr150, Lys315) for CBL:avibactam Michaelis complex structures with respect to X-ray structure (PDB ID: 4OOY[4]). This data shows that the structure of **ES** is equilibrated after 15 ns of NVT ensemble run.

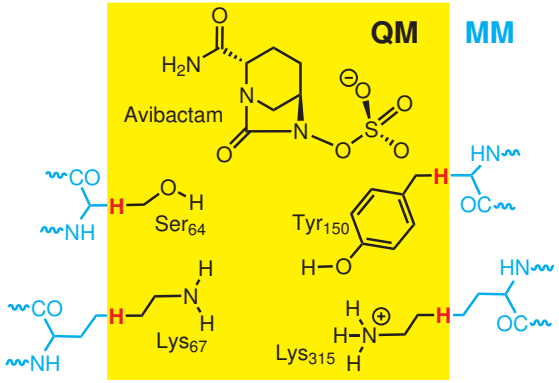

Figure S2: Active site of CBL:avibactam Michaelis complex (**ES**). The highlighted atoms were treated by QM, while the rest of the system was treated by MM. Capping hydrogens are shown in red color. These hydrogen atoms are considered to be bonded only to the QM part of the system during the QM calculation.

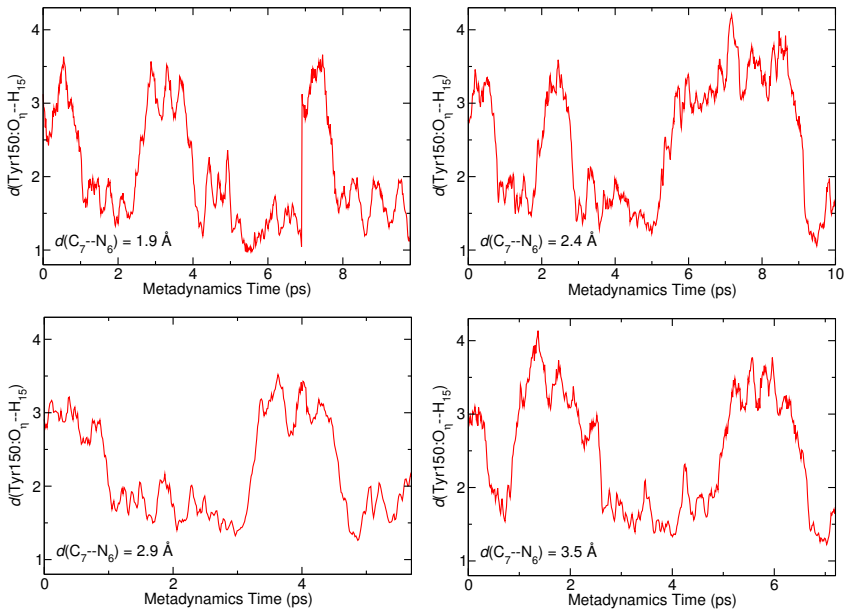

Figure S3: Time evaluation of the Tyr150:$O_\eta \cdots H_{15}$ distance for four representative umbrella windows from the well–sliced metadynamics simulation of **EI** $\rightarrow$ **ES** transformation.

References

(1) Iannuzzi, M.; Laio, A.; Parrinello, M. Efficient exploration of reactive potential energy surfaces using Car-Parrinello molecular dynamics. *Phys. Rev. Lett.* **2003**, *90*, 238302.

(2) Awasthi, S.; Kapil, V.; Nair, N. N. Sampling free energy surfaces as slices by combining umbrella sampling and metadynamics. *J. Comp. Chem.* **2016**, *37*, 1413–1424.

(3) Barducci, A.; Bussi, G.; Parrinello, M. Well-Tempered Metadynamics: A Smoothly Converging and Tunable Free-Energy Method. *Phys. Rev. Lett.* **2008**, *100*, 020603.

(4) Lahiri, S. D.; Johnstone, M. R.; Ross, P. L.; McLaughlin, R. E.; Olivier, N. B.; Alm, R. A. Avibactam and Class–C β–Lactamases: Mechanism of Inhibition, Conservation of the Binding Pocket, and Implications for Resistance. *Antimicrob. Agents Chemother.* **2014**, *58*, 5704–5713.