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SUPPORTING INFORMATION

An efficient computational assay for β-lactam antibiotic breakdown by Class A β-lactamases

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System set-up

Acylenzyme structures were built using available crystal structures (Table S1). For SHV-1 and BlaC, an acylenzyme structure with meropenem was available, for SFC-1 the Glu166Ala structure was used (mutated back to wild-type enzyme based on SFC-1 Ser70Ala and meropenem structure, PDB: 4EUZ). TEM-1 with meropenem was generated by minimizing the crystal structure with imipenem, and then changing the substrate to meropenem. Acylenzymes for CTX-M-16, NMC-A, KPC-2, and SME-1 were built by aligning the available apoenzyme structures with the SFC-1 meropenem complex, and by combining the coordinates of the antibiotic substrate with the enzyme.

Table S1*. PDB accession codes for acylenzyme structures used in model generation.*

Enzyme	PDB accession code	Acyl-enzyme complex
KPC-2	2OV5	n/a
SFC-1 (E166A)	4EV4	meropenem
NMC-A	1BUE	n/a
SME-1	1DY6	n/a
CTX-M-16	1YLW	n/a
SHV-1	2ZD8	meropenem
BlaC	3DWZ	meropenem
TEM-1	1BT5	imipenem

Protonation states were calculated using the PropKa3.1 program, and hydrogen atoms were added in tLeap. All crystallogprahic water molecules were deleted from the structures, apart from the deacylating water, and the structures were solvated in a 10 Å box of TIP4P-Ew water. Existing charges were neutralized by adding sodium ions (replacing water molecules).

Computational methods

Systems were minimized, heated and equilibrated as described in the ESI of ref. 1. See further "The efficient computational assay protocol" below.

QM/MM free energy calculation

All QM/MM umbrella sampling (US) calculations were done using two reaction coordinates: one for describing the nucleophilic attack (NA, d[DW:O - ACA:C]), and one for describing the proton

transfer (PT, d[Glu:O-DW:H] - d[DW:O-DW:H]).[1] Reaction coordinate values for the standard MFEP (in Å):

PT	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.7	0.6	0.5	0.4	0.3	0.2
NA	3.5	3.4	3.3	3.2	3.1	3.0	2.9	2.8	2.7	2.6	2.5	2.4	2.3	2.2	2.2	2.1

PT, continued	0.2	0.1	0.0	-0.1	-0.2	-0.2	-0.3	-0.4	-0.5	-0.6	-0.7	-0.8	-0.8
NA, continued	2.1	2.1	2.0	1.9	1.8	1.7	1.7	1.7	1.6	1.6	1.5	1.5	1.4

where PT and NA describe the values for the proton transfer and nucleophilic attack reaction coordinates, respectively.

Outputs from US calculations were analysed using the weighted histogram analysis (WHAM)[2] program using 17 bins for PT and 22 bins for NA reaction coordinates. The convergence criterium was set to 0.0000000000001. This analysis was done separately for all three starting structures, and lastly by combining all three results into one WHAM calculation for calculating the overall reaction barrier (as opposed to taking the average of three snapshots). However, especially with shorter sampling times WHAM analysis was prone to give false minima on the (partial) free energy surface, due to an error in the WHAM code used. This was resolved by locating these false minima and deleting the corresponding coordinate lines from the input files. Usually the false minima were due to only few coordinates, and in each case less than 1 % of data was removed. This problem can be overcome by utilizing a different analysis method or by fixing the WHAM code.

The efficient computational assay protocol

The complete efficient computational assay follows these steps:

1. Prepare the starting structures for acylenzymes either directly from crystal structures or based on analogous crystal structures (as described above). Solvate all proteins in a periodic box of TIP4P-Ew water, neutralize charges with counterions.
2. Minimize the structures briefly (100 of steepest descent followed by 900 steps of conjugate gradient).
3. Heat the systems from 50 K to 300 K in 50 ps using Langevin dynamics.
4. Equilibrate the systems in 300 K for 50 ps using unrestrained QM/MM MD (DFTB2/ff12SB), afterwards perform 300 ps of further QM/MM MD to generate starting structures for US.
5. Utilize QM/MM US for calculating deacylation barriers for all β-lactamases. Three different variations are used in the manuscript as described below:
    a. The original assay: Do US in every window for 20 ps, calculate the whole surface (374 windows).
    b. 20 ps along the standard MFEP: Do US in the 29 windows listed above starting from the reactant structure, use the restart file from the previous window as a starting point for the next one. Sample 20 ps in each window.
    c. The efficient assay: 2 ps along the standard MFEP: The same procedure as described in b), except the sampling time is shortened from 20 ps to 2 ps in each US window. Repeat a), b), or c) three times using different starting structures to test the convergence of calculated deacylation barriers.
6. Analyse the results of each individual run using WHAM (and calculate standard deviation). For the overall barrier, the three US calculations were combined into one WHAM run.

We note that in step 4, QM/MM simulation could be replaced with MM simulation, further decreasing the computational cost. Although not used here, we have tested this for similar systems.

The ‘standard’ MFEP used in steps 5b and 5c was obtained based on the previous simulations,[1] but we note that a sufficiently accurate MFEP can be obtained from one single full-surface 2D umbrella

sampling (one enzyme and substrate that show reasonable efficient hydrolysis) with limited sampling per window (2 ps), thus not significantly adding to the computational cost of the protocol.

WHAM analysis of shorter sampling

To first inspect to possibility of reducing sampling time in each US window, US results for 20 ps sampling along the MFEP were inspected by including only the first 10/5/2/1/0.5 ps in the WHAM analysis. For all three snapshots, the desired amount of sampling was included for each US window, which was followed by the same WHAM analysis as described earlier (where the same US windows for three different snapshots were combined into one). Results for this analysis are presented in Table S2.

Table S2. *$\Delta^{\ddagger}G_{calc}$ values calculated using WHAM, where results from three different calculations are combined into one WHAM analysis.*

Enzyme	$\Delta^{\ddagger}G_{calc}$ (20 ps)	$\Delta^{\ddagger}G_{calc}$ (10 ps)	$\Delta^{\ddagger}G_{calc}$ (5 ps)	$\Delta^{\ddagger}G_{calc}$ (2 ps)	$\Delta^{\ddagger}G_{calc}$ (1 ps)	$\Delta^{\ddagger}G_{calc}$ (0.5 ps)
KPC-2	8.5	8.9	8.9	9.8	10.2	10.5
SFC-1	10.9	11.0	10.3	10.8	11.3	12.8
SME-1	9.5	7.7	7.6	7.6	8.1	8.4
NMC-A	8.8	8.6	8.9	9.0	9.6	10.5
SHV-1	16.1	16.5	16.5	16.8	16.7	17.5
TEM-1	16.3	16.8	16.9	17.2	17.9	18.6
BlaC	15.5	17.0	17.0	17.9	17.9	18.0
CTX-M-16	16.8	17.1	17.2	17.6	17.9	17.9

Alternative proton transfer pathways

The two possible proton transfer pathways were inspected using the efficient protocol. Glu166 acting as the general base has two chemically inequivalent oxygens, which can both act as the proton acceptor. As the DW hydrogen bonds with Glu166:OE2 in MD simulations, it was used as the proton acceptor in all US calculations. However, technically the proton acceptor can also be Glu166: OE1. A comparison of activation barriers for the two different proton transfers are presented in Table S3. Using OE1 as the proton acceptor increases $\Delta^{\ddagger}G_{calc}$ values for all enzymes 2.0-9.7 kcal/mol: new $\Delta^{\ddagger}G_{calc}$ values for carbapenemases are 2.0 – 7.2 kcal/mol higher, and for non-carbapenemases 3.0-9.7 kcal/mol. This suggests that Glu166: OE2 should be used as the proton acceptor when modelling deacylation in class A β-lactamases, and that the hydrogen bonding interaction with Lys73 is important for efficient catalysis.

Table S3. *$\Delta^{\ddagger}G_{calc}$ values for the two possible proton transfer pathways (with 2 ps sampling). All energies in kcal/mol, standard deviations in parenthesis.*

Enzyme	$\Delta^{\ddagger}G_{calc}$ (OE2)	$\Delta^{\ddagger}G_{calc}$ (OE1)	Difference
KPC-2	9.1 (1.1)	14.5 (2.2)	+5.4
SFC-1	9.9 (1.3)	17.1 (1.8)	+7.2
SME-1	9.5 (1.4)	15.2 (2.6)	+5.7
NMC-A	10.4 (0.7)	13.8 (2.1)	+3.4
SHV-1	19.5 (0.5)	29.2 (1.2)	+9.7
TEM-1	23.6 (1.4)	26.6 (1.6)	+3.0
BlaC	24.7 (1.1)	26.7 (2.6)	+2.0
CTX-M-16	17.2 (1.6)	21.1 (0.4)	+3.9

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