Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

QM/MM Modeling of Class A β-Lactamases Reveals Distinct Acylation Pathways for Ampicillin and Cefalexin

– Supporting Information –

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Supporting Methods

Preparing the crystal structures

The AMP and CEX topology files were obtained from CGenFF parameter assignment server (cgenff.umaryland.edu)[25-27]. As stated in the main text, the Toho/AMP and Toho/CEX systems were then created by modifying the substrate topologies of the crystal Toho/benzylpenicillin (PDB entry: 5KMW, 1.10 Å) and Toho/Cephalothin (PDB entry: 2ZQ9, 1.07 Å) structures. We note that the structures of the artificial substrates are very similar to the template ligands: AMP has only one additional amino group on the R^2 side-chain compared to benzylpenicillin. In the case of cephalothin, as the acetate leaving group is cleaved from the crystalline acyl-enzyme complex, the heavy atoms on the cephem ring are identical to those in CEX. We expect CEX to have a very similar ligand bounded conformation as in other ASβLs/CEX complexes (PDB entry: 4ZJ3, 1.7 Å)[S1], therefore the initial conformation of CEX was further corrected by rotating the benzylamine (R^2) group around the carbon-carbonyl bond. In all modeling systems, the experimental Ala166 mutant used to trap the acyl-enzyme intermediates was modified to Glu166 as in the wild-type enzyme. Additionally, both Toho-1 crystal structures used in the current study contain two mutations (Arg274Asn and Arg276Asn). It has been previously reported that both mutants do not greatly impact the catalytic performance of Toho-1 on AMP or CEX[9,13,16].

Protonation, solvation, neutralization and classical minimization

All residues were protonated using the HBUILD module of CHARMM for both systems, yielding the R1 states with a protonated Lys73 and a deprotonated Glu166. The assignment of protonation states on titratable residues was further validated by experimental neutron diffraction data (PDB entry: 5A90, 1.70 Å)[12] and the pKa calculation performed by PropKa[S2] (Table S1). Each simulating system was solvated in an 84Å water box, and neutralized by the addition of sodium and chloride ions. The rest of the system was modeled by classical force fields: CHARMM36 (C36) for proteins[30] and TIP3P water model for solvent. The SHAKE algorithm[S3] was used throughout for constraining bond lengths of water. A distance cutoff of 16 Å was applied for all classical non-bonding interactions. The periodic boundary conditions were employed together with particle mesh Ewald (PME) summation of classical long-range electrostatic interactions[S4]. 200 steepest-decent (SD) followed by 3000

Adopted Basis Newton-Raphson (ABNR) steps of minimization were firstly performed to remove possible bad-contacts in the protonated crystal configuration with the QM region (see below) kept frozen.

DFTB3/3OB/C36 minimization, heating, equilibration, and production dynamics

Based on the classically minimized structures, the semi-empirical QM potential, third-order Density Functional Tight Binding theory (DFTB3/3OB), was used for the β-lactams, the catalytic water, and the reactive residues: Ser70, Lys73, Ser130, and Glu166**.** We note that the classical treatment of the β-lactam bicyclic structures is unreliable due to the lack of similar structures in the CGenFF compound database. The DFTB3 methods has been extensively employed in similar systems due to its accuracy and computational efficiency[S5,S6]. We created the R2 protonation states on Lys73 and Glu166 for both Toho/ligand systems. Practically, the CHARMM RESD restraints were employed to drag one proton from Lys73 to Glu166 during DFTB3/MM minimizations. All systems were again minimized with 500 SD steps followed by 5000 ABNR steps using the DFTB3/C36 potential. The systems were first heated from 10 K to 310 K at a temperature increment of 30 K per 5 ps, and were then equilibrated at 310 K for 100 ps. Lastly, production runs of 100 ps were performed for each system at 310 K regulated by a Langevin thermostat, producing the DFTB3/C36 MD trajectories on four systems: Toho/AMP-R1, Toho/AMP-R2, Toho/CEX-R1, and Toho/CEX-R2 (Fig. S1). All molecular dynamics (MD) simulations were integrated at a 1 fs timestep. All molecular graphics were prepared by UCSF ChimeraX[S7].

Refining the Toho/CEX: R1a to AE1 pathway

The Toho/CEX: R1a to AE1 pathway demonstrated an odd jump between replica 24 to 31 (Fig. 3d). In order to accurately determine the barrier profile on this pathway, we linearly intercepted replica 24 and 31 with 18 replicas and reoptimized this chain-of-18-replicas using the Replica Path Method with holonomic constraints. We note that a kinetic energy potential of 5 kcal mol^{-1} $Å^{-2}$ was applied in to the refining optimization, in order to maintain a continuous transition between the end-point replicas.

Notes on the methodology

Free energy simulations were not employed in the present study for several reasons. First, both substrate molecules (AMP and CEX) are similar in sizes and binding conformations; the configurational entropic contribution upon the ligand binding in both systems are not expected to consequentially differ from each other.

The entropic contribution of the ligands in the bound states are further assessed from their configurational distributions, which is analyzed by the following procedure: First, all the snapshots from the 100 ps AMP and CEX production dynamics are aligned in a pairwise manner. The heavy atom RMSDs are computed for all the AMP-CEX snapshot pairs, and the AMP-CEX snapshot pair with the lowest RMSD were used as the reference configurations. Then, we computed the heavy atom RMSD to the reference configurations along the AMP and CEX trajectories respectively. These RMSD profiles (aligned to the reference AMP/CEX conformations) would show the dynamic distribution of the ligand in the conformational space. As shown in Fig. S2, the RMSD profiles of AMP and CEX show generally overlapped distribution in both R1 and R2 trajectories, suggesting that the explored conformational space of AMP and CEX are generally similar. The conformational entropic contributions from the ligand structures are thus expected to not differ significantly. As mentioned in the main text, due to the absence of a good leaving group at C3', the hydrolysis mechanism of CEX is also believed to be generally identical to that of AMP. Furthermore, our previous study for the acylation in a similar ASβLs system has shown that altering the external MM configuration has only a small impact on the potential energy barriers determined from the CoS optimized MEPs. On the other hand, the computational cost of conducting biased sampling at reasonable *ab initio* QM level on picosecond-timescale is also unfortunately prohibitive, as both systems (Toho/AMP and Toho/CEX) contain more than 120 QM atoms. As previously demonstrated, the RPM optimized MEPs could accurately resemble the catalytic barriers from experimental kinetics studies, we expect that this methodology could properly reflect the energetic barriers on the investigated acylation pathways.

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Supporting Tables

Supporting Table S1. Results of PropKa calculations on titratable residues of the reactant states.

Residue	Toho/AMP: R1	Toho/AMP: R2	Toho/CEX: R1	Toho/CEX: R2	Toho/CEX: R1a
Asp53	2.53	2.74	3.25	1.70	2.36
Asp63	3.87	3.87	4.01	3.94	4.01
Asp87	4.15	3.94	3.12	3.28	3.34
Asp101	2.34	0.04	1.96	2.35	2.55
Asp131	3.72	3.56	3.43	3.29	3.61
Asp146	2.40	2.55	2.38	2.41	2.41
Asp157	2.43	3.12	4.06	4.61	3.62
Asp163	4.77	3.31	4.98	4.95	4.72
Asp176	1.22	1.26	2.84	3.19	2.97
Asp179	2.10	1.64	2.20	2.37	2.04
Asp233	2.38	2.42	4.46	4.19	4.26
Asp240	3.99	3.95	4.00	3.74	3.97
Asp246	**9.00**	**8.11**	**8.60**	**8.32**	**8.74**
Asp277	1.34	1.15	1.44	1.19	1.01
Glu34	3.44	4.29	4.50	4.50	4.50
Glu37	-0.49	-0.47	-0.27	-0.24	-0.32
Glu64	0.33	0.20	0.32	0.19	0.53
Glu85	3.49	3.03	4.40	2.40	1.93
Glu96	4.50	4.50	4.50	4.50	4.50
Glu110	4.57	3.84	4.12	4.12	4.05
Glu121	3.30	3.83	4.57	4.50	4.68
Glu158	4.50	4.50	3.54	4.06	4.50
Glu166	6.00	6.53	4.50	4.50	4.50
Glu201	2.52	2.52	3.23	2.95	3.27
Glu254	4.70	3.84	5.72	6.50	6.01
Glu269	4.50	4.50	4.50	4.50	4.50
Glu273	4.50	4.45	3.76	3.24	3.37
Cys69	12.86	12.54	12.74	12.66	12.35
Tyr60	12.82	13.16	12.94	12.85	12.37
Tyr105	11.12	10.06	11.38	11.19	11.27
Tyr129	10.00	10.14	9.95	10.00	10.00
Tyr241	9.41	10.00	10.07	9.73	10.07
Tyr264	14.94	14.89	14.88	15.05	14.96
Lys38	8.00	8.00	8.00	8.00	8.00
Lys73	7.46	7.47	10.43	10.50	10.29
Lys82	9.97	9.70	10.50	10.50	10.50
Lys87	10.50	10.50	10.15	10.29	10.43
Lys98	10.22	10.36	7.42	7.24	7.25
Lys99	10.50	10.50	9.67	11.82	12.13
Lys111	10.50	10.22	10.15	10.15	10.22
Lys137	9.07	11.66	10.50	10.50	10.50
Lys147	10.08	10.08	10.50	10.50	10.43
Lys191	9.01	9.60	9.52	9.11	9.30
Lys197	10.50	10.50	10.15	10.15	10.15
Lys212	9.94	9.70	9.54	9.86	9.92
Lys227	10.50	10.50	9.90	10.06	9.33

Lys234	**3.62**	**3.36**	**3.46**	**3.52**	**3.17**
Lys271	10.50	10.50	10.50	10.43	10.50
Lys284	10.50	10.50	10.43	10.50	10.50
Arg43	11.39	11.47	18.21	18.51	18.44
Arg61	18.09	18.02	12.50	12.43	12.43
Arg65	11.24	10.95	12.22	12.22	12.08
Arg96	12.50	12.08	10.57	11.50	11.37
Arg153	11.85	11.89	10.71	11.34	11.02
Arg161	11.73	11.73	11.80	11.87	11.80
Arg164	12.25	12.41	12.46	12.33	12.48
Arg178	11.73	11.99	11.73	11.66	11.69
Arg204	11.63	11.23	11.14	11.31	11.67
Arg222	12.29	12.32	11.77	12.18	11.79
Arg275	12.01	11.66	11.94	12.01	12.01

Bold entries: The protonation states assigned for Asp246 and Lys234 refers to the neutron diffraction data from ref. 12: Asp246 is deprotonated while the Lys234 is fully protonated.

Supporting Table S2. Comparison of energies of AE1 and AE2 states. Energies presented are relative energies from the corresponding reactant state.

Pathway	E_{AE1} (kcal mol^{-1})	E_{AE2} (kcal mol^{-1})	ΔE_{AE} (kcal mol^{-1})
Toho/AMP: R1	-2.06	-0.17	-1.89
Toho/AMP: R2	-12.90	-9.33	-3.57
Toho/CEX: R1	9.94	12.33	-2.39
Toho/CEX: R2	-15.30	-11.65	-3.65
Toho/CEX: R1a	10.23	13.74	-3.51

Supporting Table S3. Reaction profile of Toho/AMP:R1 to AE1, energies in kcal mol^{-1}.

No.	E_{ref}	E_{total}	$E_{QM+QM/MM(elec)}$	ChElPG charges				
				Ser70 Oγ	Ser130 Oγ	AMP N4	AMP C7	AMP O7
1 (R1)	0.00	-1874913.39	-1861811.27	-0.86	-0.58	-0.54	0.88	-0.72
2	0.25	-1874913.14	-1861810.85	-0.86	-0.57	-0.55	0.88	-0.72
3	0.05	-1874913.34	-1861811.13	-0.86	-0.58	-0.55	0.88	-0.72
4	0.19	-1874913.20	-1861811.01	-0.86	-0.58	-0.54	0.89	-0.72
5	0.12	-1874913.27	-1861810.93	-0.86	-0.58	-0.55	0.88	-0.72
6	0.25	-1874913.14	-1861810.70	-0.86	-0.58	-0.56	0.88	-0.72
7	0.24	-1874913.15	-1861810.51	-0.86	-0.58	-0.56	0.89	-0.73
8	0.39	-1874913.00	-1861810.26	-0.86	-0.58	-0.58	0.91	-0.73
9	0.44	-1874912.95	-1861810.03	-0.86	-0.58	-0.58	0.92	-0.73
10	0.65	-1874912.74	-1861809.69	-0.86	-0.58	-0.57	0.91	-0.73
11	0.76	-1874912.63	-1861809.43	-0.86	-0.58	-0.57	0.91	-0.73
12	0.98	-1874912.41	-1861809.07	-0.87	-0.58	-0.56	0.91	-0.73
13	1.21	-1874912.18	-1861808.64	-0.88	-0.58	-0.57	0.93	-0.73
14	1.59	-1874911.80	-1861808.15	-0.88	-0.58	-0.58	0.95	-0.74
15	1.89	-1874911.50	-1861807.69	-0.87	-0.58	-0.58	0.95	-0.74
16	2.40	-1874910.99	-1861807.08	-0.87	-0.59	-0.58	0.98	-0.75
17	3.22	-1874910.17	-1861806.31	-0.87	-0.59	-0.61	0.99	-0.75
18	4.69	-1874908.70	-1861804.65	-0.84	-0.59	-0.61	0.97	-0.74
19	7.19	-1874906.20	-1861801.70	-0.84	-0.58	-0.62	1.00	-0.76
20	10.31	-1874903.08	-1861798.30	-0.81	-0.58	-0.64	1.02	-0.78
21	13.14	-1874900.25	-1861795.70	-0.77	-0.57	-0.62	1.03	-0.82
22	14.00	-1874899.39	-1861795.12	-0.72	-0.55	-0.58	0.97	-0.85
23	13.59	-1874899.80	-1861795.58	-0.67	-0.53	-0.51	0.88	-0.85
24	13.18	-1874900.21	-1861795.75	-0.62	-0.53	-0.46	0.81	-0.84
25	13.00	-1874900.39	-1861795.59	-0.62	-0.53	-0.42	0.80	-0.85
26	13.08	-1874900.31	-1861795.13	-0.59	-0.53	-0.41	0.78	-0.85
27	13.90	-1874899.49	-1861793.70	-0.57	-0.51	-0.36	0.73	-0.84
28	12.72	-1874900.67	-1861792.87	-0.49	-0.65	-0.18	0.56	-0.76
29	7.70	-1874905.69	-1861797.33	-0.44	-0.42	-0.18	0.53	-0.73
30	5.32	-1874908.07	-1861799.64	-0.46	-0.45	-0.25	0.54	-0.70
31	3.51	-1874909.88	-1861801.55	-0.47	-0.44	-0.32	0.55	-0.66
32	2.18	-1874911.21	-1861803.04	-0.49	-0.41	-0.37	0.58	-0.65
33	1.02	-1874912.37	-1861804.14	-0.49	-0.43	-0.39	0.56	-0.63
34	0.49	-1874912.90	-1861805.32	-0.48	-0.43	-0.44	0.54	-0.61
35	0.38	-1874913.01	-1861805.82	-0.49	-0.44	-0.45	0.57	-0.61
36	0.19	-1874913.20	-1861806.29	-0.48	-0.45	-0.44	0.56	-0.61
37	0.02	-1874913.37	-1861806.61	-0.48	-0.46	-0.47	0.56	-0.61
38	0.01	-1874913.38	-1861806.85	-0.48	-0.46	-0.47	0.56	-0.61
39	0.09	-1874913.30	-1861806.58	-0.47	-0.46	-0.48	0.56	-0.60
40	0.11	-1874913.28	-1861806.67	-0.47	-0.46	-0.48	0.56	-0.60
41	-0.11	-1874913.50	-1861807.01	-0.47	-0.47	-0.49	0.56	-0.60
42	0.07	-1874913.32	-1861806.66	-0.47	-0.47	-0.51	0.55	-0.60
43	-0.10	-1874913.49	-1861806.91	-0.47	-0.45	-0.50	0.55	-0.60
44	0.00	-1874913.39	-1861806.76	-0.47	-0.47	-0.51	0.56	-0.60
45 (AE2)	-0.17	-1874913.56	-1861806.82	-0.47	-0.47	-0.52	0.56	-0.60
46	-0.02	-1874913.41	-1861806.76	-0.46	-0.45	-0.52	0.55	-0.60

47	0.10	-1874913.29	-1861806.65	-0.45	-0.43	-0.52	0.55	-0.60
48	0.32	-1874913.07	-1861806.46	-0.44	-0.42	-0.52	0.54	-0.60
49	0.61	-1874912.78	-1861806.26	-0.43	-0.40	-0.52	0.54	-0.60
50	0.97	-1874912.42	-1861806.08	-0.42	-0.39	-0.51	0.54	-0.60
51	1.27	-1874912.12	-1861805.99	-0.42	-0.40	-0.51	0.55	-0.60
52	1.42	-1874911.97	-1861806.04	-0.44	-0.41	-0.51	0.57	-0.61
53	1.34	-1874912.05	-1861806.24	-0.46	-0.42	-0.51	0.60	-0.61
54	1.06	-1874912.33	-1861806.51	-0.49	-0.44	-0.51	0.62	-0.62
55	0.66	-1874912.73	-1861806.74	-0.50	-0.46	-0.52	0.63	-0.62
56	0.13	-1874913.26	-1861806.99	-0.52	-0.48	-0.52	0.64	-0.62
57	-0.47	-1874913.86	-1861807.23	-0.52	-0.49	-0.53	0.65	-0.62
58	-0.99	-1874914.38	-1861807.39	-0.53	-0.49	-0.54	0.66	-0.63
59	-1.42	-1874914.81	-1861807.48	-0.53	-0.50	-0.54	0.66	-0.63
60	-1.74	-1874915.13	-1861807.58	-0.53	-0.50	-0.54	0.66	-0.63
61	-1.92	-1874915.31	-1861807.67	-0.52	-0.51	-0.55	0.66	-0.63
62	-2.07	-1874915.46	-1861807.73	-0.52	-0.51	-0.55	0.66	-0.63
63 (AE1)	-2.06	-1874915.45	-1861807.74	-0.52	-0.52	-0.55	0.66	-0.63

Supporting Table S4. Reaction profile of Toho/AMP:R2 to AE1, energies in kcal mol^{-1}.

No.	E_{ref}	E_{total}	$E_{QM+QM/MM(elec)}$	ChElPG charges				
				Ser70 Oγ	Ser130 Oγ	AMP N4	AMP C7	AMP O7
1 (R2)	0.00	-1875848.62	-1861814.62	-0.71	-0.50	-0.49	0.82	-0.73
2	-0.29	-1875848.91	-1861815.03	-0.71	-0.50	-0.50	0.84	-0.73
3	-0.01	-1875848.63	-1861814.61	-0.71	-0.50	-0.49	0.82	-0.73
4	-0.08	-1875848.70	-1861814.91	-0.71	-0.50	-0.49	0.83	-0.73
5	-0.13	-1875848.75	-1861814.73	-0.70	-0.49	-0.49	0.82	-0.73
6	-0.03	-1875848.65	-1861814.95	-0.71	-0.50	-0.49	0.82	-0.73
7	-0.10	-1875848.72	-1861814.69	-0.70	-0.49	-0.49	0.82	-0.73
8	-0.23	-1875848.85	-1861815.23	-0.70	-0.49	-0.49	0.83	-0.73
9	0.04	-1875848.58	-1861814.67	-0.69	-0.49	-0.50	0.83	-0.73
10	-0.22	-1875848.84	-1861815.19	-0.70	-0.49	-0.49	0.82	-0.73
11	0.17	-1875848.45	-1861814.76	-0.69	-0.49	-0.50	0.82	-0.73
12	0.16	-1875848.46	-1861814.89	-0.68	-0.49	-0.50	0.82	-0.73
13	0.57	-1875848.05	-1861814.56	-0.68	-0.48	-0.52	0.83	-0.73
14	0.98	-1875847.64	-1861814.18	-0.68	-0.48	-0.53	0.84	-0.74
15	1.45	-1875847.17	-1861813.66	-0.67	-0.48	-0.55	0.85	-0.74
16	2.38	-1875846.24	-1861812.72	-0.66	-0.47	-0.54	0.82	-0.73
17	4.52	-1875844.10	-1861810.93	-0.71	-0.46	-0.57	0.86	-0.74
18	5.90	-1875842.72	-1861809.54	-0.70	-0.47	-0.59	0.88	-0.76
19	7.27	-1875841.35	-1861808.07	-0.66	-0.47	-0.61	0.89	-0.79
20	8.51	-1875840.11	-1861806.83	-0.62	-0.47	-0.62	0.89	-0.82
21	8.52	-1875840.10	-1861806.92	-0.59	-0.46	-0.62	0.88	-0.86
22	8.02	-1875840.60	-1861807.61	-0.55	-0.44	-0.58	0.82	-0.87
23	7.64	-1875840.98	-1861808.03	-0.52	-0.42	-0.53	0.78	-0.87
24	7.47	-1875841.15	-1861808.05	-0.51	-0.41	-0.52	0.77	-0.87
25	7.63	-1875840.99	-1861807.59	-0.50	-0.40	-0.48	0.76	-0.87
26	8.70	-1875839.92	-1861805.89	-0.48	-0.39	-0.44	0.74	-0.86
27	6.99	-1875841.63	-1861806.29	-0.42	-0.55	-0.23	0.60	-0.78
28	3.75	-1875844.87	-1861808.98	-0.42	-0.52	-0.26	0.59	-0.74
29	1.74	-1875846.88	-1861810.75	-0.42	-0.52	-0.33	0.59	-0.71
30	-3.98	-1875852.60	-1861816.29	-0.42	-0.34	-0.36	0.61	-0.68
31	-6.07	-1875854.69	-1861818.22	-0.43	-0.34	-0.42	0.63	-0.66
32	-7.21	-1875855.83	-1861819.33	-0.43	-0.34	-0.44	0.62	-0.65
33	-7.96	-1875856.58	-1861820.25	-0.45	-0.34	-0.45	0.63	-0.64
34	-8.46	-1875857.08	-1861820.96	-0.47	-0.34	-0.46	0.64	-0.64
35	-8.84	-1875857.46	-1861821.55	-0.48	-0.35	-0.47	0.65	-0.64
36	-9.10	-1875857.72	-1861821.91	-0.48	-0.35	-0.49	0.66	-0.64
37	-9.24	-1875857.86	-1861822.10	-0.47	-0.35	-0.51	0.66	-0.64
38	-9.26	-1875857.88	-1861822.11	-0.47	-0.34	-0.53	0.67	-0.64
39	-9.37	-1875857.99	-1861822.48	-0.47	-0.35	-0.51	0.67	-0.64
40	-9.32	-1875857.94	-1861822.46	-0.46	-0.34	-0.53	0.67	-0.65
41	-9.20	-1875857.82	-1861822.19	-0.46	-0.34	-0.54	0.67	-0.64
42	-9.20	-1875857.82	-1861822.38	-0.46	-0.33	-0.56	0.67	-0.65
43	-9.33	-1875857.95	-1861822.58	-0.46	-0.34	-0.53	0.67	-0.65
44	-9.16	-1875857.78	-1861822.30	-0.47	-0.34	-0.54	0.67	-0.65
45 (AE2)	-9.33	-1875857.95	-1861822.70	-0.44	-0.35	-0.57	0.66	-0.64
46	-9.34	-1875857.36	-1861822.91	-0.43	-0.34	-0.56	0.65	-0.64
47	-9.28	-1875857.30	-1861822.77	-0.42	-0.33	-0.55	0.64	-0.64

48	-9.16	-1875857.18	-1861822.66	-0.41	-0.33	-0.55	0.63	-0.64
49	-9.03	-1875857.05	-1861822.64	-0.40	-0.33	-0.54	0.62	-0.64
50	-9.01	-1875857.03	-1861822.78	-0.42	-0.34	-0.53	0.62	-0.64
51	-9.37	-1875857.39	-1861823.32	-0.46	-0.36	-0.53	0.64	-0.64
52	-10.02	-1875858.04	-1861824.13	-0.49	-0.40	-0.53	0.67	-0.65
53	-10.75	-1875858.77	-1861824.91	-0.50	-0.44	-0.54	0.68	-0.65
54	-11.38	-1875859.40	-1861825.51	-0.51	-0.46	-0.54	0.69	-0.65
55	-11.95	-1875859.97	-1861825.95	-0.51	-0.48	-0.55	0.69	-0.65
56	-12.37	-1875860.39	-1861826.22	-0.51	-0.49	-0.55	0.69	-0.65
57	-12.57	-1875860.59	-1861826.26	-0.51	-0.50	-0.56	0.69	-0.66
58	-12.75	-1875860.77	-1861826.28	-0.51	-0.50	-0.55	0.69	-0.66
59	-12.88	-1875860.90	-1861826.31	-0.51	-0.50	-0.55	0.69	-0.66
60	-12.90	-1875860.92	-1861826.36	-0.51	-0.50	-0.55	0.69	-0.66
61	-12.85	-1875860.87	-1861826.38	-0.51	-0.50	-0.55	0.70	-0.66
62	-13.02	-1875861.04	-1861826.59	-0.52	-0.50	-0.54	0.70	-0.66
63 (AE1)	-12.90	-1875860.92	-1861826.54	-0.52	-0.50	-0.54	0.70	-0.66

Supporting Table S5. Reaction profile of Toho/CEX:R1 to AE1, energies in kcal mol^{-1}.

No.	E_{ref}	E_{total}	$E_{QM+QM/MM(elec)}$	ChElPG charges				
				Ser70 Oγ	Ser130 Oγ	CEX N5	CEX C8	CEX O8
1 (R1)	0.00	-1873821.19	-1861054.90	-0.75	-0.60	-0.31	0.75	-0.67
2	-0.05	-1873821.24	-1861054.73	-0.75	-0.59	-0.31	0.76	-0.68
3	0.10	-1873821.09	-1861054.64	-0.75	-0.60	-0.32	0.76	-0.67
4	-0.12	-1873821.31	-1861054.74	-0.75	-0.59	-0.31	0.76	-0.67
5	0.17	-1873821.02	-1861054.34	-0.75	-0.59	-0.32	0.77	-0.68
6	-0.11	-1873821.30	-1861054.59	-0.75	-0.59	-0.31	0.77	-0.68
7	0.20	-1873820.99	-1861054.11	-0.75	-0.59	-0.32	0.77	-0.68
8	0.21	-1873820.98	-1861053.86	-0.76	-0.57	-0.33	0.78	-0.68
9	0.44	-1873820.75	-1861053.53	-0.77	-0.57	-0.32	0.78	-0.68
10	0.75	-1873820.44	-1861053.01	-0.76	-0.57	-0.32	0.78	-0.68
11	0.77	-1873820.42	-1861052.91	-0.78	-0.57	-0.32	0.79	-0.68
12	1.32	-1873819.87	-1861052.16	-0.78	-0.57	-0.32	0.79	-0.69
13	1.41	-1873819.78	-1861051.98	-0.79	-0.57	-0.30	0.77	-0.69
14	2.08	-1873819.11	-1861051.07	-0.78	-0.57	-0.31	0.76	-0.69
15	2.37	-1873818.82	-1861050.69	-0.79	-0.56	-0.31	0.78	-0.69
16	3.36	-1873817.83	-1861049.46	-0.78	-0.54	-0.31	0.77	-0.69
17	3.80	-1873817.39	-1861048.77	-0.78	-0.53	-0.31	0.78	-0.69
18	4.38	-1873816.81	-1861047.88	-0.78	-0.51	-0.33	0.81	-0.70
19	5.45	-1873815.74	-1861046.64	-0.78	-0.51	-0.36	0.81	-0.71
20	6.54	-1873814.65	-1861045.54	-0.78	-0.52	-0.34	0.81	-0.71
21	8.07	-1873813.12	-1861044.16	-0.79	-0.51	-0.33	0.81	-0.71
22	9.84	-1873811.35	-1861042.49	-0.80	-0.50	-0.31	0.80	-0.71
23	13.16	-1873808.03	-1861038.40	-0.78	-0.48	-0.31	0.80	-0.71
24	16.66	-1873804.53	-1861034.22	-0.73	-0.46	-0.27	0.80	-0.73
25	20.37	-1873800.82	-1861030.20	-0.66	-0.43	-0.19	0.72	-0.74
26	22.82	-1873798.37	-1861027.54	-0.60	-0.42	-0.16	0.68	-0.76
27	23.16	-1873798.03	-1861026.96	-0.55	-0.41	-0.07	0.64	-0.79
28	23.50	-1873797.69	-1861026.33	-0.55	-0.41	0.04	0.59	-0.80
29	24.79	-1873796.40	-1861024.55	-0.53	-0.39	0.10	0.54	-0.79
30	26.49	-1873794.70	-1861021.32	-0.44	-0.52	0.41	0.37	-0.72
31	24.54	-1873796.65	-1861022.92	-0.44	-0.51	0.38	0.37	-0.68
32	19.92	-1873801.27	-1861027.51	-0.44	-0.34	0.34	0.43	-0.67
33	17.80	-1873803.39	-1861029.09	-0.47	-0.35	0.24	0.48	-0.65
34	16.21	-1873804.98	-1861030.47	-0.49	-0.36	0.17	0.50	-0.62
35	15.02	-1873806.17	-1861032.02	-0.50	-0.38	0.10	0.53	-0.62
36	14.15	-1873807.04	-1861033.20	-0.50	-0.41	0.03	0.54	-0.61
37	13.86	-1873807.33	-1861033.71	-0.52	-0.43	-0.02	0.51	-0.60
38	13.34	-1873807.85	-1861034.63	-0.54	-0.45	-0.04	0.51	-0.60
39	12.90	-1873808.29	-1861035.65	-0.54	-0.46	-0.05	0.51	-0.60
40	12.52	-1873808.67	-1861036.33	-0.53	-0.46	-0.03	0.52	-0.60
41	12.38	-1873808.81	-1861036.62	-0.53	-0.47	-0.04	0.52	-0.60
42	12.26	-1873808.93	-1861036.84	-0.53	-0.48	-0.05	0.53	-0.60
43	12.30	-1873808.89	-1861036.95	-0.52	-0.48	-0.05	0.52	-0.60
44	12.24	-1873808.95	-1861036.96	-0.52	-0.48	-0.05	0.53	-0.60
45 (AE2)	12.33	-1873808.86	-1861036.93	-0.52	-0.48	-0.05	0.52	-0.60
46	12.34	-1873808.85	-1861036.82	-0.52	-0.48	-0.04	0.51	-0.60
47	12.51	-1873808.68	-1861036.46	-0.51	-0.47	-0.04	0.50	-0.59

48	12.75	-1873808.44	-1861036.02	-0.49	-0.46	-0.03	0.48	-0.59
49	13.17	-1873808.02	-1861035.41	-0.47	-0.44	-0.02	0.45	-0.58
50	13.66	-1873807.53	-1861034.77	-0.43	-0.42	-0.02	0.43	-0.58
51	14.18	-1873807.01	-1861034.15	-0.38	-0.38	-0.01	0.40	-0.57
52	14.75	-1873806.44	-1861033.69	-0.30	-0.35	-0.01	0.37	-0.57
53	14.99	-1873806.20	-1861033.85	-0.26	-0.34	-0.01	0.36	-0.57
54	14.73	-1873806.46	-1861034.77	-0.29	-0.36	-0.03	0.41	-0.58
55	13.70	-1873807.49	-1861036.51	-0.39	-0.42	-0.06	0.47	-0.59
56	12.44	-1873808.75	-1861038.32	-0.44	-0.47	-0.08	0.51	-0.60
57	11.38	-1873809.81	-1861039.69	-0.46	-0.50	-0.09	0.52	-0.60
58	10.66	-1873810.53	-1861040.55	-0.46	-0.52	-0.10	0.53	-0.60
59	10.30	-1873810.89	-1861040.87	-0.45	-0.52	-0.10	0.53	-0.60
60	10.14	-1873811.05	-1861040.92	-0.45	-0.52	-0.10	0.53	-0.60
61	10.02	-1873811.17	-1861040.98	-0.45	-0.52	-0.10	0.53	-0.60
62	9.98	-1873811.21	-1861040.96	-0.45	-0.52	-0.10	0.53	-0.60
63 (AE1)	9.94	-1873811.25	-1861040.88	-0.45	-0.52	-0.10	0.53	-0.60

Supporting Table S6. Reaction profile of Toho/CEX:R2 to AE1, energies in kcal mol^{-1}.

No.	E_{ref}	E_{total}	$E_{QM+QM/MM(elec)}$	ChElPG charges				
				Ser70 Oγ	Ser130 Oγ	CEX N5	CEX C8	CEX O8
1 (R2)	0.00	-1874102.64	-1861032.64	-0.62	-0.58	-0.41	0.86	-0.72
2	-0.25	-1874102.89	-1861032.79	-0.61	-0.57	-0.41	0.86	-0.72
3	0.08	-1874102.56	-1861032.46	-0.61	-0.58	-0.40	0.85	-0.72
4	-0.04	-1874102.68	-1861032.54	-0.62	-0.57	-0.41	0.86	-0.72
5	-0.04	-1874102.68	-1861032.52	-0.61	-0.57	-0.39	0.84	-0.71
6	0.00	-1874102.64	-1861032.25	-0.61	-0.57	-0.39	0.84	-0.71
7	0.04	-1874102.60	-1861032.35	-0.60	-0.57	-0.39	0.84	-0.71
8	0.02	-1874102.62	-1861032.15	-0.60	-0.56	-0.39	0.83	-0.71
9	0.30	-1874102.34	-1861031.91	-0.59	-0.56	-0.40	0.84	-0.71
10	0.28	-1874102.36	-1861031.92	-0.59	-0.56	-0.40	0.84	-0.71
11	0.60	-1874102.04	-1861031.52	-0.59	-0.56	-0.41	0.84	-0.72
12	0.74	-1874101.90	-1861031.56	-0.57	-0.56	-0.41	0.85	-0.72
13	1.36	-1874101.28	-1861031.15	-0.57	-0.55	-0.39	0.84	-0.73
14	2.42	-1874100.22	-1861030.50	-0.56	-0.54	-0.38	0.84	-0.73
15	4.61	-1874098.03	-1861028.68	-0.55	-0.53	-0.37	0.85	-0.74
16	9.19	-1874093.45	-1861024.51	-0.53	-0.54	-0.41	0.92	-0.78
17	12.43	-1874090.21	-1861022.76	-0.59	-0.58	-0.49	1.08	-0.88
18	11.46	-1874091.18	-1861024.45	-0.58	-0.60	-0.51	1.11	-0.92
19	11.44	-1874091.20	-1861024.49	-0.54	-0.59	-0.46	1.06	-0.92
20	11.49	-1874091.15	-1861024.20	-0.50	-0.52	-0.36	0.95	-0.89
21	11.63	-1874091.01	-1861023.69	-0.47	-0.50	-0.28	0.89	-0.88
22	12.13	-1874090.51	-1861022.71	-0.42	-0.47	-0.14	0.77	-0.85
23	13.70	-1874088.94	-1861020.41	-0.40	-0.46	-0.14	0.73	-0.81
24	10.01	-1874092.63	-1861022.67	-0.31	-0.64	0.03	0.60	-0.72
25	4.33	-1874098.31	-1861028.09	-0.28	-0.62	-0.02	0.60	-0.67
26	0.17	-1874102.47	-1861031.95	-0.26	-0.64	-0.07	0.60	-0.65
27	-2.63	-1874105.27	-1861034.71	-0.26	-0.63	-0.14	0.61	-0.64
28	-4.11	-1874106.75	-1861036.24	-0.27	-0.64	-0.17	0.62	-0.64
29	-8.25	-1874110.89	-1861040.17	-0.28	-0.46	-0.21	0.65	-0.66
30	-9.00	-1874111.64	-1861041.29	-0.30	-0.45	-0.23	0.68	-0.66
31	-9.64	-1874112.28	-1861041.75	-0.33	-0.45	-0.26	0.70	-0.67
32	-9.88	-1874112.52	-1861042.00	-0.36	-0.47	-0.29	0.75	-0.67
33	-10.15	-1874112.79	-1861041.99	-0.39	-0.47	-0.30	0.77	-0.68
34	-10.41	-1874113.05	-1861042.60	-0.41	-0.48	-0.32	0.80	-0.69
35	-10.70	-1874113.34	-1861042.63	-0.43	-0.48	-0.33	0.81	-0.69
36	-10.97	-1874113.61	-1861043.17	-0.44	-0.49	-0.33	0.82	-0.69
37	-11.16	-1874113.80	-1861043.32	-0.44	-0.50	-0.33	0.82	-0.69
38	-11.32	-1874113.96	-1861043.88	-0.46	-0.51	-0.34	0.84	-0.70
39	-11.27	-1874113.91	-1861043.44	-0.45	-0.50	-0.33	0.83	-0.69
40	-11.45	-1874114.09	-1861043.90	-0.45	-0.51	-0.33	0.84	-0.70
41	-11.52	-1874114.16	-1861044.23	-0.46	-0.51	-0.34	0.84	-0.70
42	-11.50	-1874114.14	-1861043.85	-0.45	-0.51	-0.33	0.84	-0.70
43	-11.60	-1874114.24	-1861044.30	-0.46	-0.51	-0.34	0.84	-0.70
44	-11.55	-1874114.19	-1861044.12	-0.46	-0.51	-0.34	0.84	-0.70
45 (AE2)	-11.65	-1874114.29	-1861044.33	-0.45	-0.50	-0.31	0.80	-0.69
46	-11.79	-1874114.00	-1861043.91	-0.45	-0.50	-0.31	0.80	-0.69
47	-11.46	-1874113.67	-1861043.34	-0.42	-0.49	-0.30	0.77	-0.68

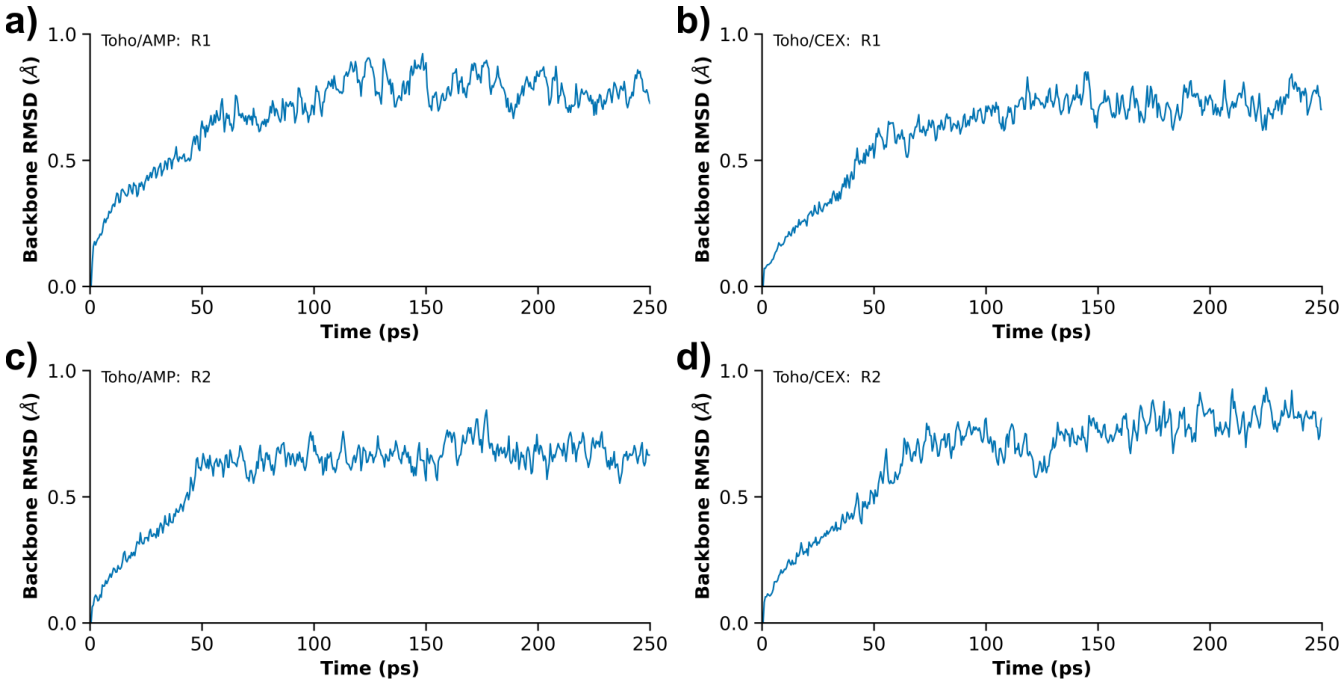
48	-11.15	-1874113.36	-1861042.86	-0.38	-0.47	-0.29	0.74	-0.67
49	-10.73	-1874112.94	-1861042.48	-0.31	-0.46	-0.27	0.68	-0.66
50	-10.63	-1874112.84	-1861042.51	-0.27	-0.45	-0.27	0.65	-0.65
51	-10.59	-1874112.80	-1861042.55	-0.21	-0.42	-0.26	0.60	-0.64
52	-10.52	-1874112.73	-1861042.62	-0.17	-0.41	-0.26	0.58	-0.64
53	-10.61	-1874112.82	-1861043.03	-0.21	-0.43	-0.28	0.61	-0.65
54	-11.39	-1874113.60	-1861044.18	-0.30	-0.47	-0.32	0.69	-0.67
55	-12.82	-1874115.03	-1861045.93	-0.38	-0.51	-0.36	0.76	-0.68
56	-14.03	-1874116.24	-1861047.27	-0.41	-0.55	-0.38	0.79	-0.69
57	-14.67	-1874116.88	-1861048.13	-0.41	-0.58	-0.39	0.79	-0.69
58	-14.91	-1874117.12	-1861048.38	-0.41	-0.59	-0.39	0.79	-0.69
59	-15.01	-1874117.22	-1861048.76	-0.41	-0.58	-0.38	0.80	-0.69
60	-15.08	-1874117.29	-1861048.98	-0.41	-0.58	-0.37	0.80	-0.69
61	-15.14	-1874117.35	-1861048.99	-0.41	-0.58	-0.37	0.81	-0.69
62	-15.21	-1874117.42	-1861049.47	-0.41	-0.57	-0.37	0.81	-0.69
63 (AE1)	-15.30	-1874117.51	-1861049.24	-0.41	-0.58	-0.37	0.80	-0.69

Supporting Table S7. Reaction profile of Toho/CEX:R1a to AE1, the refined profile that intercepts between replica 24 and 31 (No. 1R to 18R) is appended, energies in kcal mol^{-1}.

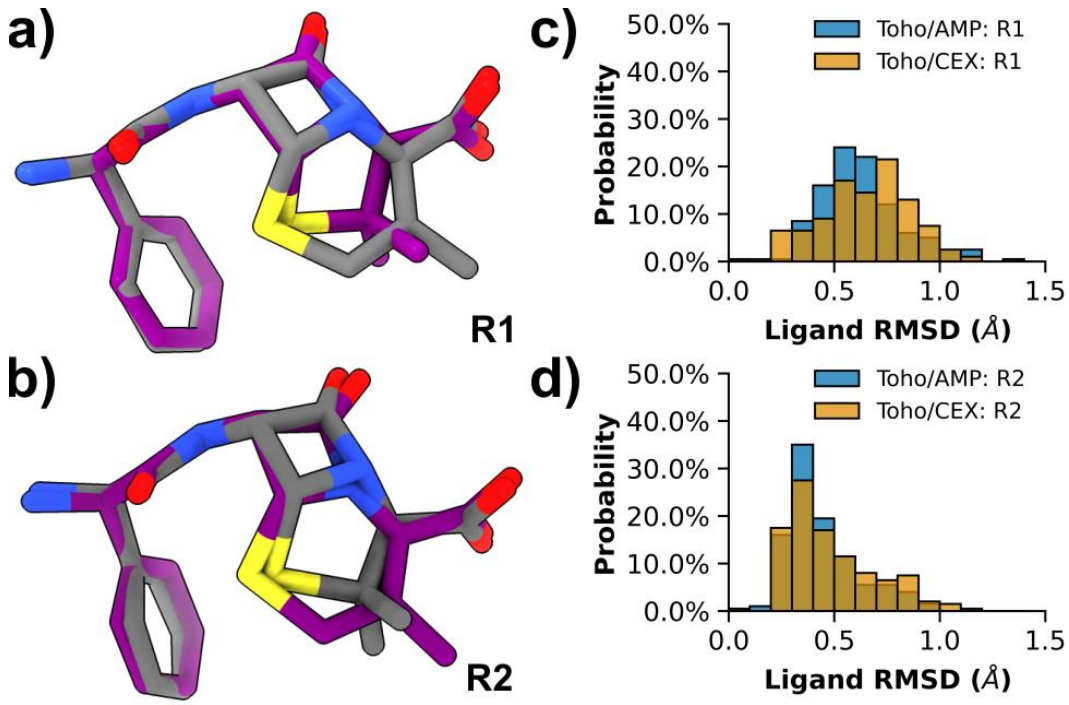
No.	E_{ref}	E_{total}	$E_{QM+QM/MM(elec)}$	ChElPG charges				
				Ser70 Oγ	Ser130 Oγ	CEX N5	CEX C8	CEX O8
1 (R1a)	0.00	-1873557.66	-1861017.88	-0.81	-0.48	-0.19	0.64	-0.64
2	0.19	-1873557.47	-1861017.11	-0.82	-0.48	-0.20	0.65	-0.65
3	-0.11	-1873557.77	-1861017.57	-0.82	-0.48	-0.20	0.66	-0.65
4	0.27	-1873557.39	-1861017.17	-0.82	-0.48	-0.20	0.65	-0.65
5	0.08	-1873557.58	-1861017.15	-0.83	-0.48	-0.20	0.66	-0.65
6	0.35	-1873557.31	-1861016.87	-0.83	-0.48	-0.21	0.66	-0.65
7	0.24	-1873557.42	-1861016.76	-0.83	-0.47	-0.20	0.66	-0.65
8	0.34	-1873557.32	-1861017.06	-0.83	-0.48	-0.20	0.66	-0.65
9	0.54	-1873557.12	-1861016.36	-0.84	-0.48	-0.21	0.67	-0.65
10	1.04	-1873556.62	-1861015.79	-0.85	-0.49	-0.21	0.68	-0.65
11	1.41	-1873556.25	-1861015.22	-0.86	-0.49	-0.21	0.68	-0.66
12	1.95	-1873555.71	-1861014.64	-0.87	-0.49	-0.20	0.68	-0.66
13	2.07	-1873555.59	-1861014.58	-0.88	-0.49	-0.20	0.69	-0.66
14	2.94	-1873554.72	-1861013.45	-0.89	-0.49	-0.20	0.68	-0.66
15	3.86	-1873553.80	-1861012.30	-0.90	-0.49	-0.20	0.68	-0.66
16	4.80	-1873552.86	-1861010.83	-0.89	-0.49	-0.21	0.67	-0.66
17	5.42	-1873552.24	-1861010.25	-0.90	-0.50	-0.21	0.67	-0.66
18	6.73	-1873550.93	-1861008.65	-0.89	-0.51	-0.23	0.64	-0.65
19	8.27	-1873549.39	-1861007.03	-0.90	-0.52	-0.22	0.61	-0.64
20	9.71	-1873547.95	-1861005.64	-0.89	-0.53	-0.23	0.60	-0.63
21	11.27	-1873546.39	-1861004.13	-0.88	-0.53	-0.25	0.59	-0.63
22	12.96	-1873544.70	-1861002.26	-0.88	-0.53	-0.27	0.62	-0.64
23	15.76	-1873541.90	-1860999.55	-0.88	-0.54	-0.27	0.63	-0.63
24	19.95	-1873537.71	-1860995.15	-0.89	-0.53	-0.23	0.63	-0.63
25	28.24	-1873529.42	-1860986.05	-0.85	-0.49	-0.23	0.64	-0.65
26	34.41	-1873523.25	-1860979.23	-0.76	-0.45	-0.17	0.60	-0.69
27	36.87	-1873520.79	-1860976.24	-0.68	-0.42	-0.11	0.55	-0.72
28	39.44	-1873518.22	-1860972.58	-0.65	-0.42	-0.07	0.53	-0.72
29	26.80	-1873530.86	-1860983.58	-0.56	-0.33	0.22	0.47	-0.64
30	21.19	-1873536.47	-1860988.52	-0.54	-0.33	0.07	0.51	-0.59
31	18.12	-1873539.54	-1860991.28	-0.52	-0.38	-0.11	0.57	-0.57
32	16.23	-1873541.43	-1860993.09	-0.50	-0.42	-0.22	0.59	-0.57
33	15.57	-1873542.09	-1860993.85	-0.50	-0.43	-0.27	0.62	-0.58
34	15.07	-1873542.59	-1860994.63	-0.51	-0.42	-0.27	0.63	-0.58
35	14.71	-1873542.95	-1860995.21	-0.52	-0.43	-0.29	0.64	-0.59
36	14.65	-1873543.01	-1860995.61	-0.52	-0.43	-0.27	0.66	-0.59
37	14.57	-1873543.09	-1860995.42	-0.52	-0.43	-0.29	0.64	-0.59
38	14.35	-1873543.31	-1860995.82	-0.52	-0.43	-0.27	0.66	-0.59
39	14.17	-1873543.49	-1860995.72	-0.51	-0.43	-0.29	0.63	-0.58
40	13.93	-1873543.73	-1860996.00	-0.51	-0.42	-0.27	0.63	-0.58
41	13.91	-1873543.75	-1860995.79	-0.51	-0.42	-0.30	0.62	-0.58
42	13.73	-1873543.93	-1860996.05	-0.51	-0.41	-0.27	0.62	-0.58
43	13.87	-1873543.79	-1860995.79	-0.51	-0.42	-0.30	0.61	-0.58
44	13.61	-1873544.05	-1860996.15	-0.51	-0.41	-0.27	0.62	-0.58
45 (AE2)	13.74	-1873543.92	-1860996.02	-0.51	-0.42	-0.30	0.61	-0.58

46	13.60	-1873544.06	-1860995.95	-0.50	-0.41	-0.30	0.61	-0.58
47	13.61	-1873544.05	-1860995.83	-0.49	-0.40	-0.30	0.60	-0.58
48	13.69	-1873543.97	-1860995.63	-0.49	-0.38	-0.30	0.59	-0.58
49	13.87	-1873543.79	-1860995.39	-0.47	-0.36	-0.31	0.58	-0.58
50	14.13	-1873543.53	-1860995.17	-0.45	-0.34	-0.30	0.57	-0.57
51	14.46	-1873543.20	-1860995.05	-0.43	-0.31	-0.30	0.55	-0.57
52	14.81	-1873542.85	-1860995.27	-0.42	-0.31	-0.30	0.55	-0.58
53	14.75	-1873542.91	-1860995.94	-0.44	-0.35	-0.32	0.57	-0.58
54	14.22	-1873543.44	-1860996.83	-0.47	-0.39	-0.33	0.59	-0.59
55	13.66	-1873544.00	-1860997.32	-0.48	-0.42	-0.33	0.60	-0.59
56	12.94	-1873544.72	-1860997.72	-0.48	-0.44	-0.33	0.61	-0.59
57	12.48	-1873545.18	-1860997.58	-0.48	-0.45	-0.33	0.61	-0.59
58	11.68	-1873545.98	-1860997.80	-0.47	-0.44	-0.32	0.61	-0.59
59	11.27	-1873546.39	-1860997.93	-0.47	-0.45	-0.32	0.60	-0.59
60	10.85	-1873546.81	-1860998.33	-0.47	-0.46	-0.33	0.60	-0.59
61	10.61	-1873547.05	-1860998.66	-0.47	-0.46	-0.33	0.60	-0.59
62	10.35	-1873547.31	-1860998.99	-0.47	-0.47	-0.34	0.60	-0.59
63 (AE1)	10.23	-1873547.43	-1860999.25	-0.47	-0.47	-0.34	0.60	-0.59
1R (24)	19.95	-1873537.71	-1860995.15	-0.89	-0.53	-0.23	0.63	-0.63
2R	22.05	-1873535.61	-1860992.44	-0.89	-0.52	-0.23	0.63	-0.63
3R	25.06	-1873532.60	-1860989.04	-0.89	-0.51	-0.25	0.65	-0.64
4R	28.20	-1873529.46	-1860985.45	-0.87	-0.50	-0.26	0.67	-0.65
5R	31.80	-1873525.86	-1860981.40	-0.86	-0.49	-0.26	0.68	-0.66
6R	34.95	-1873522.71	-1860977.81	-0.83	-0.47	-0.26	0.68	-0.67
7R	37.72	-1873519.94	-1860974.62	-0.81	-0.45	-0.26	0.67	-0.68
8R	40.12	-1873517.54	-1860971.80	-0.77	-0.43	-0.24	0.64	-0.69
9R	43.12	-1873514.54	-1860968.36	-0.74	-0.41	-0.20	0.61	-0.69
10R	50.89	-1873506.77	-1860960.15	-0.71	-0.41	-0.11	0.57	-0.68
11R	52.38	-1873505.28	-1860958.30	-0.66	-0.45	0.04	0.49	-0.65
12R	41.17	-1873516.49	-1860969.16	-0.62	-0.42	0.15	0.46	-0.63
13R	31.07	-1873526.59	-1860978.93	-0.60	-0.35	0.17	0.47	-0.62
14R	25.56	-1873532.10	-1860984.19	-0.58	-0.33	0.14	0.50	-0.61
15R	22.58	-1873535.08	-1860987.00	-0.57	-0.33	0.09	0.53	-0.60
16R	20.57	-1873537.09	-1860988.91	-0.55	-0.34	0.04	0.55	-0.59
17R	19.14	-1873538.52	-1860990.29	-0.53	-0.36	-0.04	0.57	-0.58
18R (31)	18.12	-1873539.54	-1860991.28	-0.52	-0.38	-0.11	0.57	-0.57

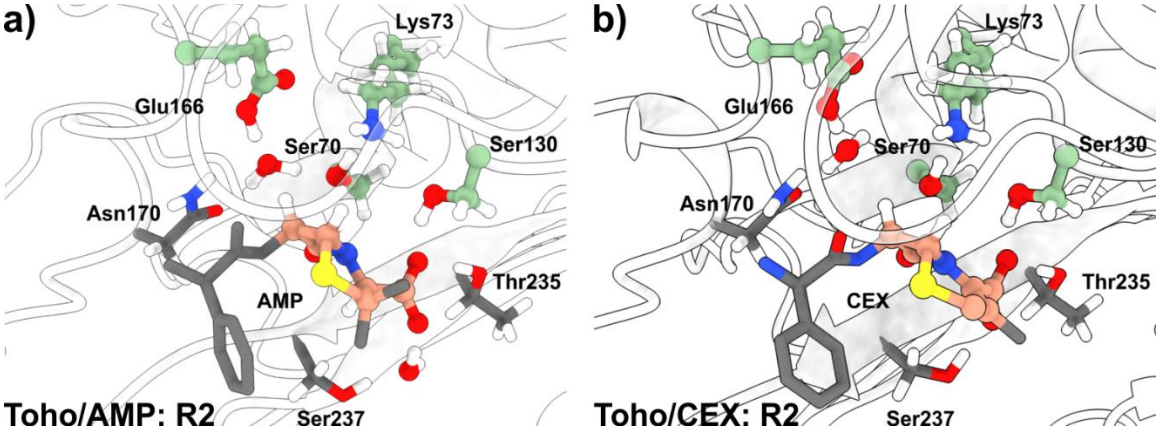
Supporting Figures



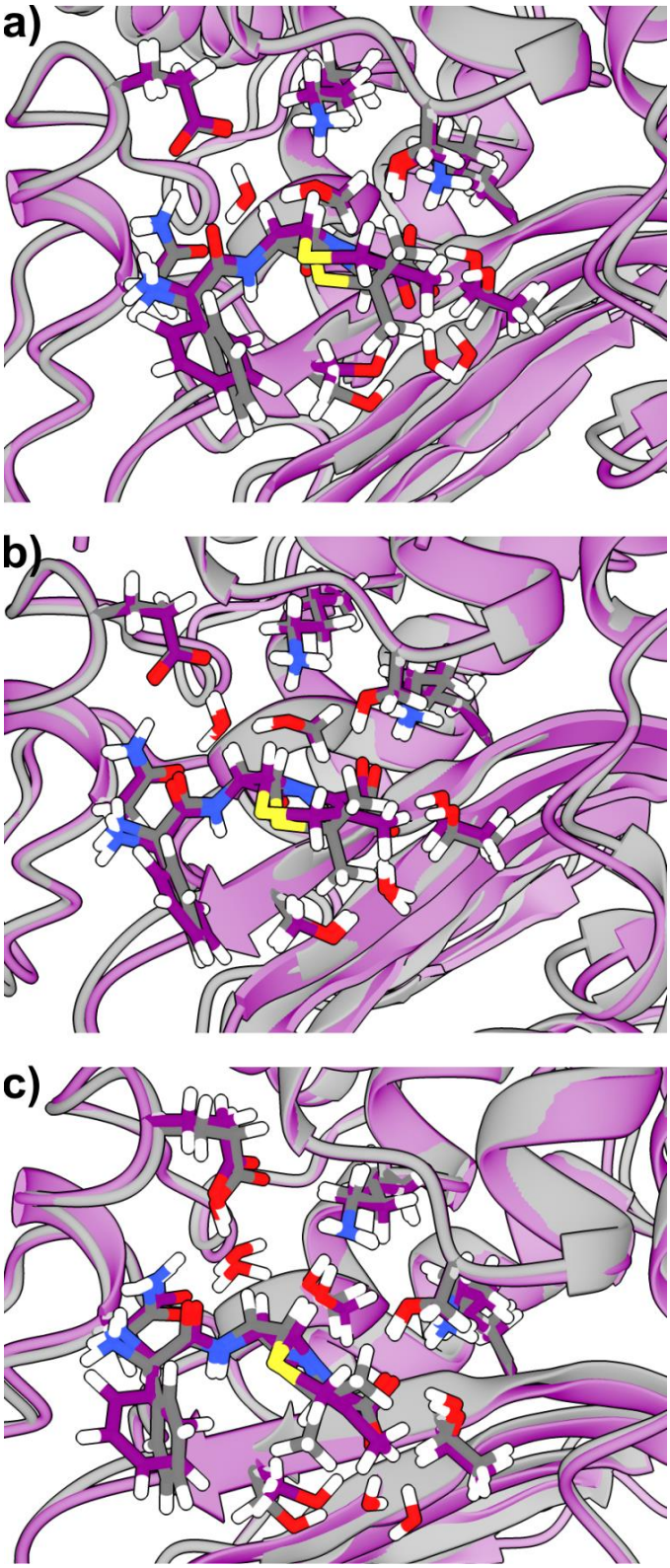
Supporting Figure S1. The backbone RMSD along the DFTB3/3OB/C36 heating, equilibration, and production dynamics of (a) Toho/AMP: R1, (b) Toho/CEX: R1, (c) Toho/AMP: R2, and (d) Toho/CEX: R2.



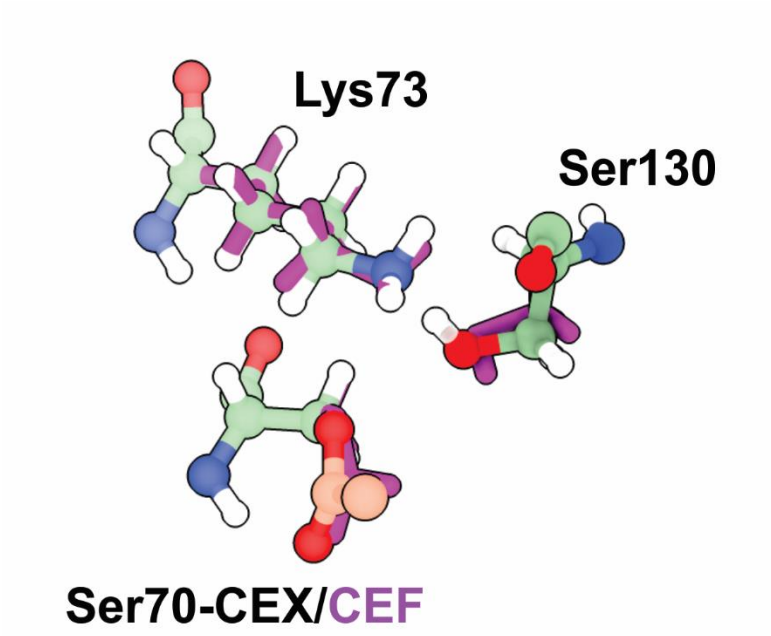
Supporting Figure S2. Superimposed ligand snapshots (the "best-fit" configurations used as the reference) from the (a) R1 and (b) R2 dynamic trajectories. Carbon atoms of AMP and CEX are colored in dark grey and purple, respectively; The distribution of ligand RMSD to the reference snapshots in (c) R1 and (d) R2 dynamic trajectories.



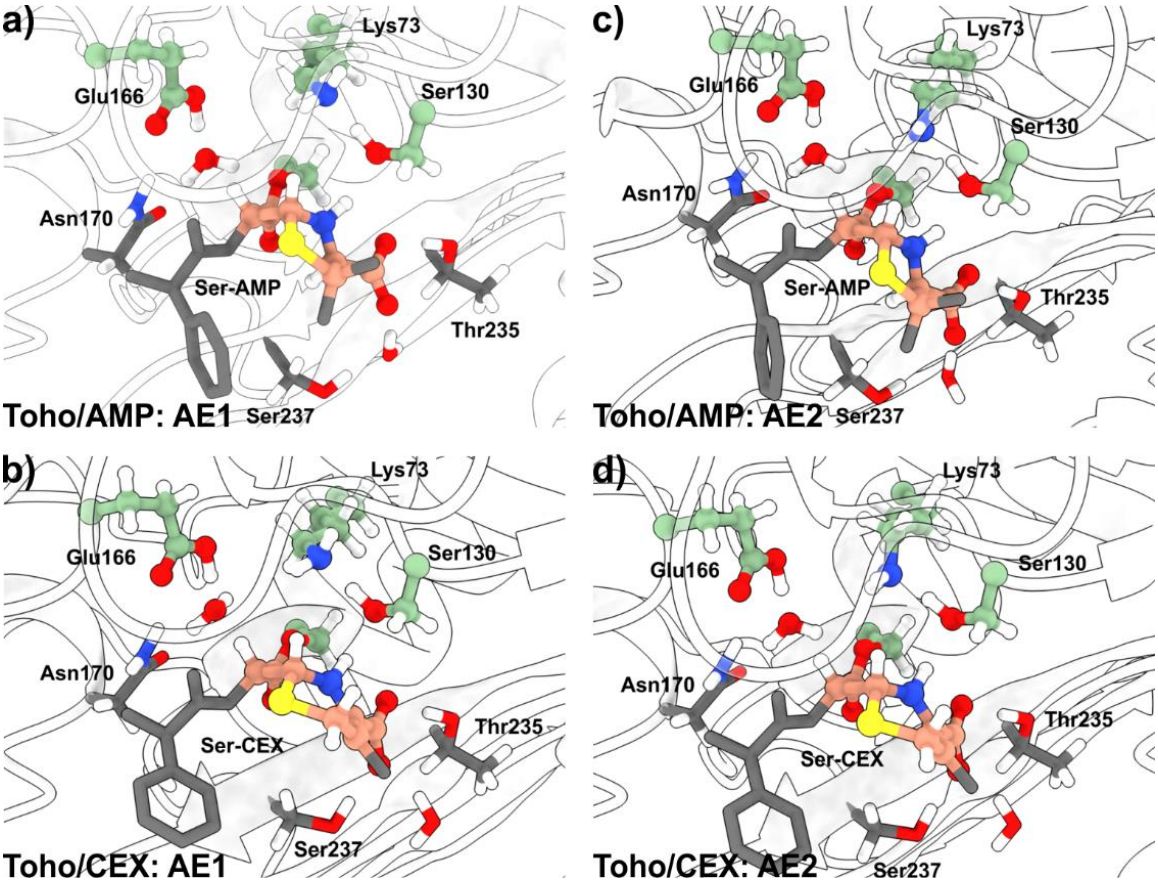
Supporting Figure S3. The conformation of (a) Toho/AMP: R2; and (b) Toho/CEX: R2 states.



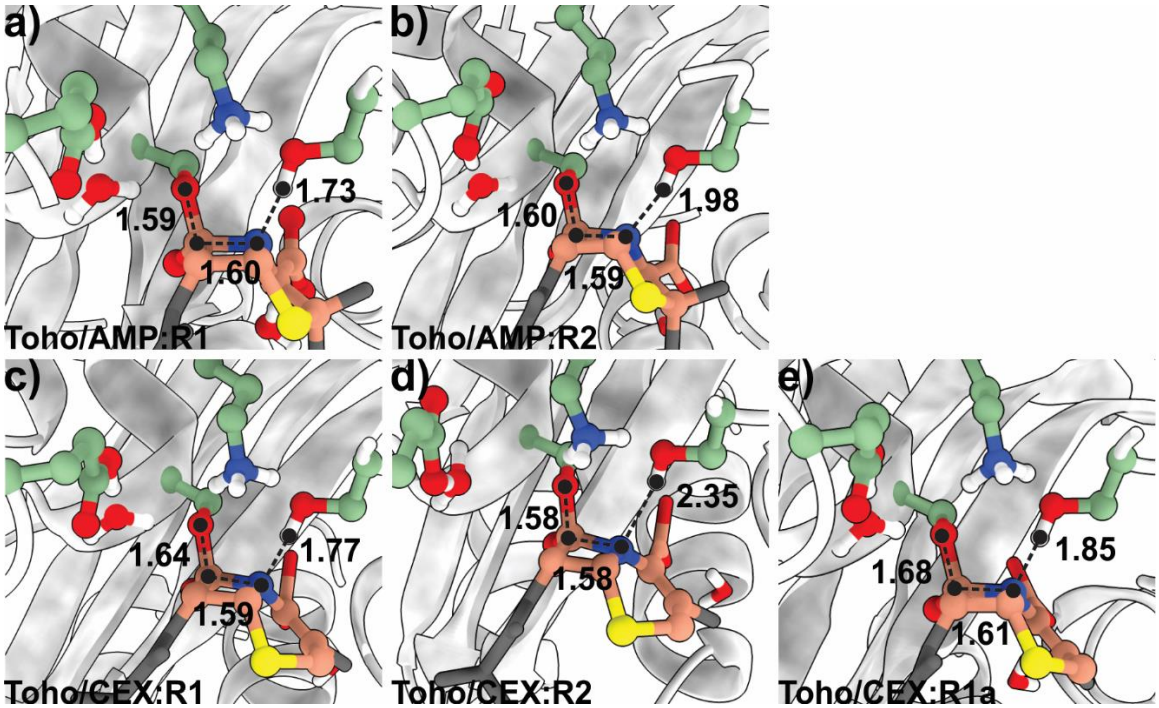
Supporting Figure S4. The superimposed B3LYP/6-31G/C36 optimized conformations of (a) Toho/AMP: R1 and Toho/CEX: R1; (b) Toho/AMP: R1 and Toho/CEX: R1a; (c) Toho/AMP: R2 and Toho/CEX: R2. Cartoon and carbon atoms in dark grey and purple denotes the Toho/AMP and Toho/CEX models, respectively.



Supporting Figure S5. The structural alignment of the AE1 state of Toho/CEX to crystal Toho/cefotaxime acyl-enzyme complex. The crystal structure (PDB 5A93, 1.60 Å)[12] was colored in purple and the calculated structure is colored in: white, blue, red, green, orange for hydrogen, nitrogen, oxygens, carbon atoms on amino acids, carbon atoms on ligand. Note that major part of the ligand is omitted for clarity.



Supporting Figure S6. The conformation of (a) Toho/AMP: AE1; (b) Toho/CEX: AE1; (c) Toho/AMP: AE2; (d) Toho/CEX: AE2 states on the R1 to AE1 pathways.



Supporting Figure S7. The conformations of tetrahedral intermediates of all acylation pathways (noted by labels lower left) and key interatomic distances.

Additional Supporting Data

All RPM optimized pathway geometries (as PSF topologies and DCD trajectories) are attached as an individual supporting data. Only the protein, the substrate, and the solvent water within 15 Å of QM region are included.