Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Using molecular dynamics simulations to evaluate active designs of cephradine hydrolase by molecular mechanics/Poisson Boltzmann surface area and molecular mechanics/generalized Born surface area methods

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Table S1 Catalytic geometrical constraints for TS

Site pair	Type	Atom1	Atom2[a]	Atom3[a]	Atom4[a]	Min[b]	Max[b]
Ser117-TS	Distance	OG	#C15			1.4	1.6
	Angle	CB	OG	#C15		100.0	120.0
	Angle	OG	#C15	#O16		100.0	120.0
	Torsion	OG	#O16	#C15	#N14	100.0	140.0
Tyr118- TS	Distance	N	#O16			2.6	3.0
	Angle	N	HN	#O16		140.0	180.0
	Angle	HN	#O16	#C15		80.0	140.0
Tyr44- TS	Distance	OH	#O16			2.6	3.0
	Angle	OH	HH	#O16		140.0	180.0
	Angle	HH	#O16	#C15		80.0	140.0
His287-Ser117	Distance	NE2	#OG			2.6	3.0
	Angle	NE2	#HG1	#OG		140.0	180.0
	Angle	CE1	NE2	#HG1		90.0	150.0
Asp259-His287	Distance	OD1	#ND1			2.6	3.0
	Angle	CG	OD1	#HD1		90.0	150.0
	Angle	OD1	#HD1	#ND1		140.0	180.0
Asp259-His287	Distance	OD2	#ND1			2.6	3.0
	Angle	CG	OD2	#HD2		90.0	150.0
	Angle	OD2	#HD2	#ND1		140.0	180.0
Phe261-Asp259	Distance	N	#OD2			2.6	3.2
	Angle	HN	#OD2	#CG		90.0	150.0
	Angle	N	HN	#OD2		140.0	180.0
Phe261-Asp259	Distance	N	#OD1			2.6	3.2
	Angle	HN	#OD1	#CG		90.0	150.0
	Angle	N	HN	#OD1		140.0	180.0

[a]Atoms on the latter residue within a site pair are prefixed with '#'

[b]Distance measurements are given in Å. Angle and torsion measurements are given in degrees

Table S2 Variation rules for generating TS library

Type	Atom1[a]	Atom2[a]	Atom3[a]	Atom4	Min[b]	Max[b]	Step[b]
Distance	#OG	C15			1.4	1.6	0.1
Angle	#CB	#OG	C15		105.0	115.0	5.0
Torsion	#CA	#CB	#OG	C15	-180.0	179.0	10.0
Angle	#OG	C15	O16		105.0	115.0	5.0
Torsion	#OG	O16	C15	N14	120.0	120.0	0.0
Torsion	#OG	O16	C15	C17	-120.0	-120.0	0.0
Torsion	#CB	#OG	C15	O16	-180.0	179.0	10.0
Torsion	N14	C15	C17	C18	-180.0	179.0	10.0
Torsion	C15	C17	C18	C19	-180.0	179.0	10.0
Torsion	O16	C15	N14	C7	-180.0	179.0	10.0
Torsion	C15	N14	C7	C8	-180.0	179.0	10.0

[a]Atoms on anchor residue Ser117 are prefixed with '#'
[b]Distance measurements are given in Å. Angle and torsion measurements are given in degrees.

Table S3 Catalytic geometrical constraints for ES

Site pair	Type	Atom1	Atom2[a]	Atom3[a]	Atom4[a]	Min[b]	Max[b]
Ser117-ES	Distance	OG	#C15			2.9	3.1
Tyr118- ES	Distance	N	#O16			2.6	3.0
	Angle	N	HN	#O16		140.0	180.0
	Angle	HN	#O16	#C15		80.0	140.0
Tyr44- ES	Distance	OH	#O16			2.6	3.0
	Angle	OH	HH	#O16		140.0	180.0
	Angle	HH	#O16	#C15		80.0	140.0
His287-Ser117	Distance	NE2	#OG			2.6	3.0
	Angle	NE2	#HG1	#OG		140.0	180.0
	Angle	CE1	NE2	#HG1		90.0	150.0
Asp259-His287	Distance	OD1	#ND1			2.6	3.0
	Angle	CG	OD1	#HD1		90.0	150.0
	Angle	OD1	#HD1	#ND1		140.0	180.0
Asp259-His287	Distance	OD2	#ND1			2.6	3.0
	Angle	CG	OD2	#HD2		90.0	150.0
	Angle	OD2	#HD2	#ND1		140.0	180.0
Phe261-Asp259	Distance	N	#OD2			2.6	3.2
	Angle	HN	#OD2	#CG		90.0	150.0
	Angle	N	HN	#OD2		140.0	180.0
Phe261-Asp259	Distance	N	#OD1			2.6	3.2
	Angle	HN	#OD1	#CG		90.0	150.0
	Angle	N	HN	#OD1		140.0	180.0

[a]Atoms on the latter residue within a site pair are prefixed with '#'

[b]Distance measurements are given in Å. Angle and torsion measurements are given in degrees

Table S4 Variation rules for generating ES library

Type	Atom1[a]	Atom2[a]	Atom3[a]	Atom4	Min[b]	Max[b]	Step[b]
Distance	#OG	C15			2.9	3.1	0.1
Angle	#CB	#OG	C15		0.0	180.0	10.0
Torsion	#CA	#CB	#OG	C15	-180.0	179.0	10.0
Angle	#OG	C15	O16		0.0	180.0	10.0
Torsion	#OG	O16	C15	N14	0.0	180.0	10.0
Torsion	#CB	#OG	C15	O16	-180.0	179.0	10.0
Torsion	N14	C15	C17	C18	-180.0	179.0	10.0
Torsion	C15	C17	C18	C19	-180.0	179.0	10.0
Torsion	O16	C15	N14	C7	-180.0	179.0	10.0
Torsion	C15	N14	C7	C8	-180.0	179.0	10.0

[a]Atoms on anchor residue Ser117 are prefixed with '#'
[b]Distance measurements are given in Å. Angle and torsion measurements are given in degrees.

Table S5 Results of t-test for fit between the experimental kinetic parameters and the calculated free energy changes.

Kinetic parameters	Methods	ε_p	R^2	t values	Critical t value (p=0.05)	Results
K_m~ΔG_{bind}	MM/GBSA	1	0.7053	4.892	2.228	passed
	MM/PBSA	1	0.7531	5.523		passed
	MM/PBSA	4	0.7728	5.831		passed
	MM/PBSA	24	0.6784	4.593		passed
k_{cat}/K_m~$\Delta G^{\ddagger}$	MM/GBSA	1	0.1095	1.109		failed
	MM/PBSA	1	0.1609	1.385		failed
	MM/PBSA	4	0.5001	3.163		passed
	MM/PBSA	24	0.5005	3.166		passed
k_{cat}~$\Delta G^{\ddagger}_{cat}$	MM/GBSA	1	0.2509	1.830		failed
	MM/PBSA	1	0.0914	1.003		failed
	MM/PBSA	4	0.2426	1.789		failed
	MM/PBSA	24	0.4724	2.992		passed

Figure S1 The regression between the experimental turnover numbers and the predicted activation energies of WT and its eleven variants by MM/GBSA and MM/PBSA methods. The ordinate is scaled logarithmically, and the hypothetical coordination systems are shown in dashed lines with the WT as the origin. (A) MM/GBSA method; (B) MM/PBSA method based on ε_p=1; (C) MM/PBSA method based on ε_p=4; (D) MM/PBSA method based on ε_p=24.

Figure S1(A)

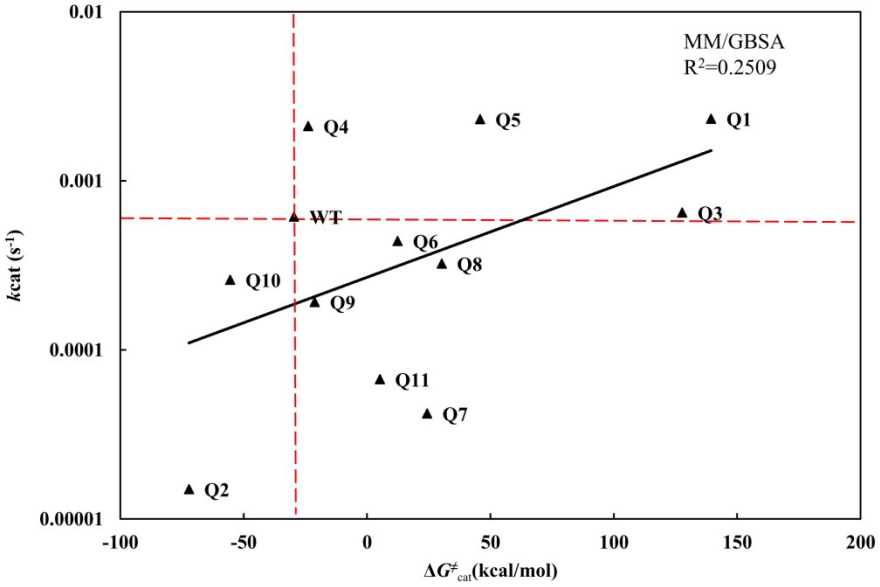

Figure S1(B)

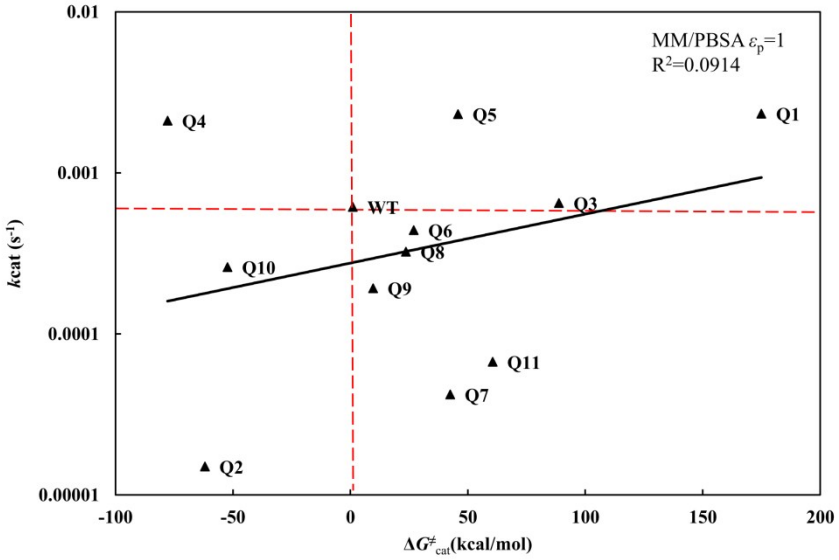

Figure S1(C)

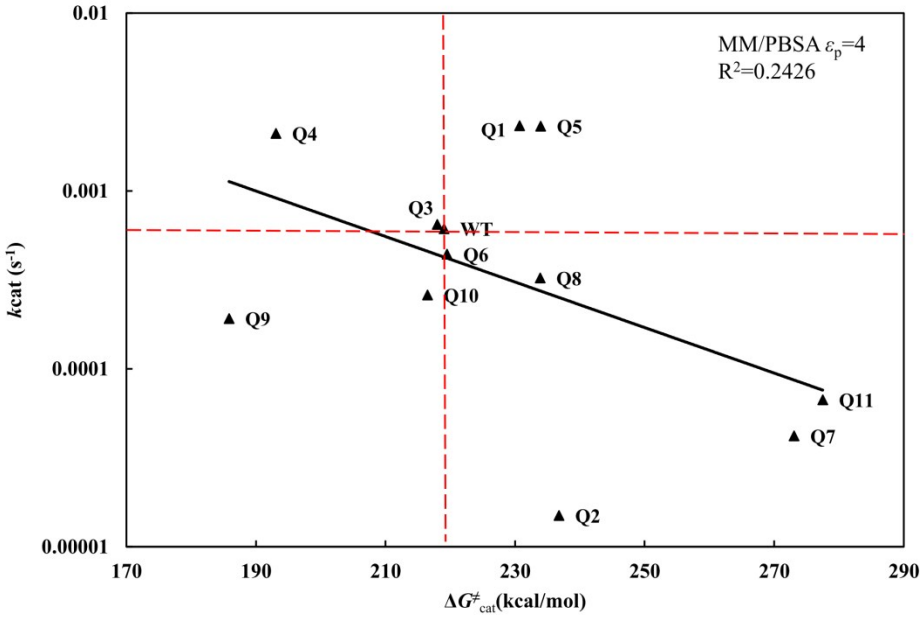

Figure S1(D)

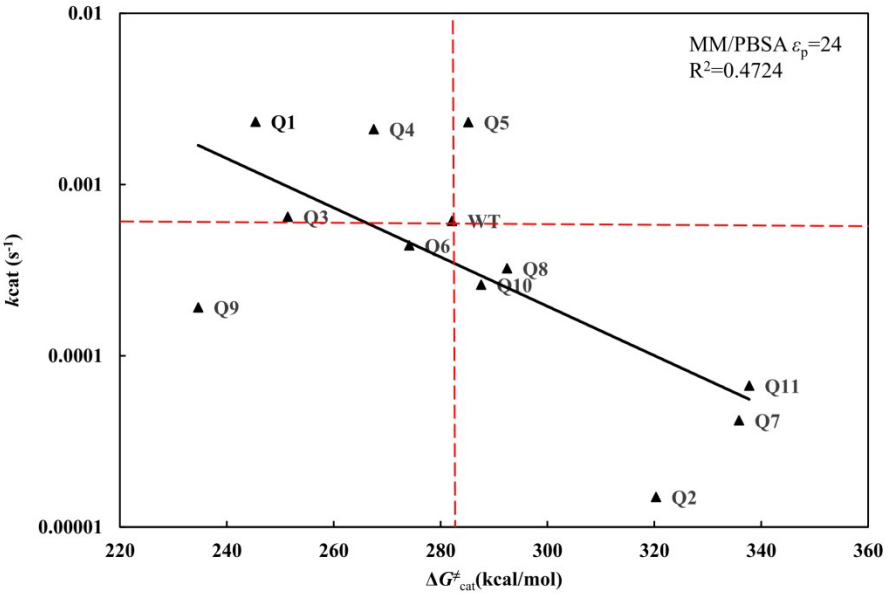

Figure S2 System volume change for the wild type during 20 ns MD simulations.