Electronic Supplementary Information

Why does Tautomycetin Thioesterase Prefer Hydrolysis to Macrocyclization? Theoretical Study on Its Catalytic Mechanism

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Figure S1. Alignment of the most dominant cluster of two 300 ns MD trajectories in TMC-TE (left) and PIK-TE (right) systems.



Figure S2. The root-mean-square deviation (RMSD) values and root-mean-square fluctuation (RMSF) values of each 6×50 ns MD simulations in TMC-TE and PIK-TE systems.



Figure S3. The RMSD values of each 2×300 ns MD simulations in TMC-TE and PIK-TE systems.



Figure S4. Three clusters based on $d(N_{\varepsilon}-O_3)$ and $d(C_1-O_3)$ by K-means in TMC-TE (left) and PIK-TE (right) systems.



Figure S5. The distribution of distances $d(N_{\epsilon}-O_3)$ and $d(C_1-O_3)$ in 2×300 ns MD simulations in TMC-TE system.



Figure S6. The distribution of distances $d(N_{\varepsilon}-O_3)$ and $d(C_1-O_3)$ in 2×300 ns MD simulations in PIK-TE system.



Figure S7. Alignment of the crystal structures of PIK/DEBS/NRPS TEs to TMC TE.



Figure S8. $d(N_{\epsilon}-O_3)$ distances during 6×50 ns MD simulations in Y161A TMC-TE system.



Figure S9. Critical structures along the PESs of the hydrolysis in TMC-TE system.



Figure S10. Energy profile of nucleophilic attack from IM in TMC-TE system.



Figure S11. Two-dimensional PESs of the hydrolysis in TMC-TE system.



Figure S12. Critical structures along the PESs of the macrocyclization in TMC-TE system.



Figure S13. Two-dimensional PESs of the macrocyclization in TMC-TE system.



Figure S14. Critical structures along the PESs of the hydrolysis in PIK-TE system.



Figure S15. Two-dimensional PESs of the hydrolysis in the PIK-TE system.



Figure S16. Critical structures along the PESs of the macrocyclization in PIK-TE system.



Figure S17. Two-dimensional PESs of the macrocyclization in the PIK-TE system.

Table S1. Summary of $d(N_{\epsilon}-O_{wat})$ and $d(C_1-O_{wat})$ in the 300 ns MD simulations of TMC-TE system

TMC-wat	The population of	The population of	The population	
	$d(N_{\epsilon}-O_{wat}) \leq 3.0 \text{ Å}$	$d(C_1-O_{wat}) \le 4.5 \text{ Å}$	of PRS	
wat-5057	2.39%	2.97%	2.32%	
wat-365	1.94%	68.33%	1.31%	
wat-1098	1.29%	1.69%	1.26%	
wat-9048	1.61%	1.86%	1.23%	
wat-5215	1.02%	1.30%	0.99%	
wat-5988	1.02%	1.45%	0.97%	
wat-775	0.97%	1.24%	0.95%	
wat-7663	0.96%	1.17%	0.93%	
wat-8239	0.97%	1.16%	0.89%	
wat-4725	0.78%	0.92%	0.74%	
others	22.98%	30.46%	11.51%	
Total	35.94%	112.54%	23.08%	

PIK-wat	The population of	The population of	The population
	$d(N_{\epsilon}$ -O _{wat}) \leq 3.0 Å	$d(C_1-O_{wat}) \le 4.5 \text{ Å}$	of PRS
wat-6609	3.09%	4.43%	2.85%
wat-7188	1.48%	2.06%	1.29%
wat-10003	1.31%	1.77%	1.19%
wat-10096	1.20%	1.79%	1.08%
wat-6105	1.62%	1.09%	0.90%
wat-6119	0.90%	1.47%	0.80%
wat-2973	0.88%	1.53%	0.79%
wat-9793	0.66%	0.84%	0.60%
wat-4619	0.66%	0.88%	0.58%
wat-1577	0.63%	0.89%	0.56%
others	11.28%	19.18%	8.53%
Total	23.72%	35.94%	19.17%

Table S2. Summary of $d(N_{\epsilon}-O_{wat})$ and $d(C_1-O_{wat})$ in 300 ns MD simulations of PIK-TE system

Table S3. Energetic corrections at different levels with M062X method during the hydrolysis inTMC-TE system

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	M062X/6-31g(d)	M062X/6-311+g(d)	M062X/6-311+g(d,p)	M062X/6-311+g(2df,2p)
R	-2582.315388 hartree	-2583.042375 hartree	-2583.157421 hartree	-2583.297467 hartree
R'	-2582.323805 hartree	-2583.048629 hartree	-2583.162515 hartree	-2583.302312 hartree
TS1	-2582.302376 hartree	-2583.026911 hartree	-2583.139092 hartree	-2583.278898 hartree
EB1 ^a	13.4 kcal/mol	13.6 kcal/mol	14.7 kcal/mol	14.7 kcal/mol
IM2	-2582.333310 hartree	-2583.053581 hartree	-2583.166022 hartree	-2583.306183 hartree
TS2	-2582.318823 hartree	-2583.040753 hartree	-2583.153940 hartree	-2583.292777 hartree
EB2 ^b	9.1 kcal/mol	8.0 kcal/mol	7.6 kcal/mol	8.4 kcal/mol
EBc	13.5 kcal/mol	13.6 kcal/mol	14.7 kcal/mol	14.7 kcal/mol

EB1^a, EB2^b and EB^c denote energy barriers for step1, step2 and the whole reaction respectively, (kcal/mol).

in TMC-TE system M062X/6-31g(d) M062X/6-311+g(d) M062X/6-311+g(d,p) M062X/6-311+g(2df,2p) R -2429.428306 hartree -2430.095708 hartree -2430.189077 hartree -2430.324855 hartree TS1 -2429.389189 hartree -2430.050039 hartree -2430.140205 hartree -2430.275400 hartree EB1^a 24.5 kcal/mol 28.7 kcal/mol 30.7 kcal/mol 31.0 kcal/mol IM -2429.391938 hartree -2430.053124 hartree -2430.277990 hartree -2430.143253 hartree TS2 -2429.365264 hartree -2430.028608 hartree -2430.120236 hartree -2430.255174 hartree EB2^b 16.7 kcal/mol 15.4 kcal/mol 14.4 kcal/mol 14.3 kcal/mol EBc 43.2 kcal/mol 39.6 kcal/mol 43.7 kcal/mol 42.1 kcal/mol

Table S4. Energetic corrections at different levels with M062X method during the macrocyclization

EB1^a, EB2^b and EB^c denote energy barriers for step1, step2 and the whole reaction respectively, (kcal/mol).

Table S	S5.	Energetic	corrections	at different	levels	with M062X	method	during th	ne hydrol	lysis	in
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P	IK-	ΓЕ	svs	tem
•			0,0	

	M062X/6-31g(d)	M062X/6-311+g(d)	M062X/6-311+g(d,p)	M062X/6-311+g(2df,2p)
R	-2582.225703 hartree	-2582.959066 hartree	-2583.075237 hartree	-2583.215692 hartree
R'	-2582.237019 hartree	-2582.968048 hartree	-2583.082998 hartree	-2583.223368 hartree
TS1	-2582.220213 hartree	-2582.949071 hartree	-2583.062136 hartree	-2583.202542 hartree
EB1 ^a	10.5 kcal/mol	11.9 kcal/mol	13.1 kcal/mol	13.1 kcal/mol
IM	-2582.227467 hartree	-2582.956611 hartree	-2583.068636 hartree	-2583.208755 hartree
TS2	-2582.219552 hartree	-2582.948047 hartree	-2583.061902 hartree	-2583.201819 hartree
EB2 ^b	5.0 kcal/mol	5.4 kcal/mol	4.2 kcal/mol	4.4 kcal/mol
EBc	11.0 kcal/mol	12.6 kcal/mol	13.2 kcal/mol	13.5 kcal/mol

EB1^a, EB2^b and EB^c denote energy barriers for step1, step2 and the whole reaction respectively, (kcal/mol).

Table S6. Energetic corrections at different levels with M062X method during the macrocyclization

in PIK-TE system

	M062X/6-31g(d)	M062X/6-311+g(d)	M062X/6-311+g(d,p)	M062X/6-311+g(2df,2p)
R	-2429.405174 hartree	-2430.07361 hartree	-2430.16712 hartree	-2430.303414 hartree
TS1	-2429.376105 hartree	-2430.041239 hartree	-2430.133234 hartree	-2430.269293 hartree
EB1 ^a	18.2 kcal/mol	20.3 kcal/mol	21.3 kcal/mol	21.4 kcal/mol
IM	-2429.391465 hartree	-2430.052757 hartree	-2430.144034 hartree	-2430.281047 hartree
TS2	-2429.366352 hartree	-2430.029176 hartree	-2430.121924 hartree	-2430.259912 hartree
EB2 ^b	15.8 kcal/mol	14.8 kcal/mol	13.9 kcal/mol	13.3 kcal/mol
EBc	24.4 kcal/mol	27.9 kcal/mol	28.4 kcal/mol	27.3 kcal/mol

EB1^a, EB2^b and EB^c denote energy barriers for step1, step2 and the whole reaction respectively, (kcal/mol).