Supporting Information

Catalytic Mechanism of *S*-Acyltransferases: Acylation is Triggered on by a Loose Transition State; Deacylation is Turned off by a Tight Transition State

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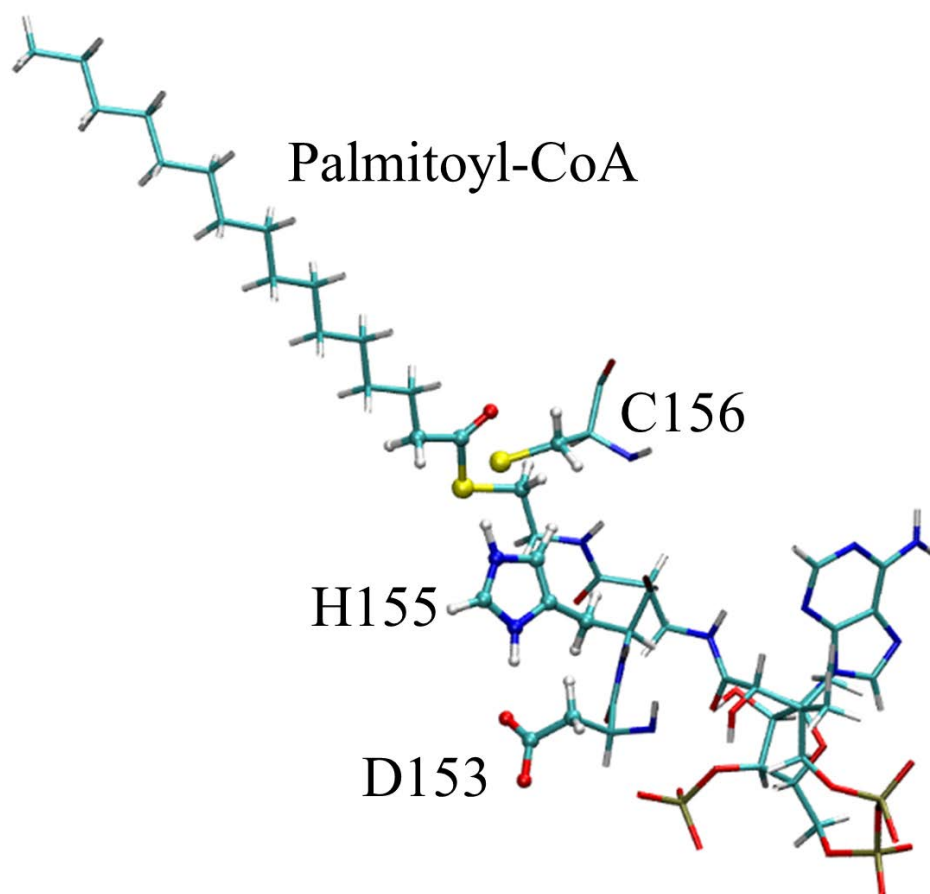
Figure S1. QM atoms. The atoms of QM region are shown as ball and stick; other atoms shown in stick are included in the MM region.
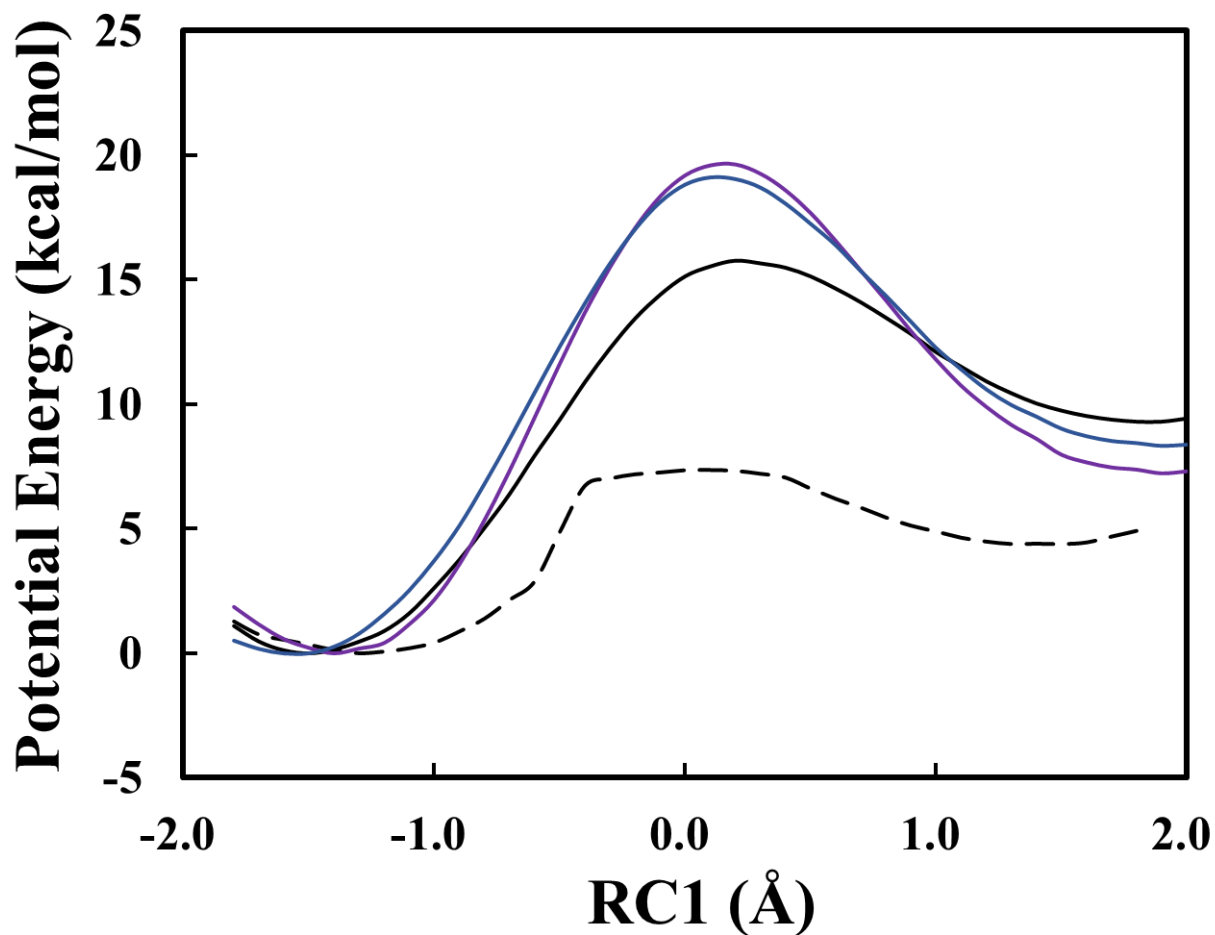
Figure S2. Auto-palmitoylation reaction results of hDHHC20 from reaction coordinate and single point energy calculations. Plot of the potential energy *vs* the reaction coordinate (RC1) at QM/MM(DFTB3:CHARMM36m) level (dash line), QM/MM(B3LYP/6-31G*:CHARMM36m) level (black line), and further single-point energy calculations at the QM/MM(B3LYP/6-311+G**:CHARMM36m) level (blue line) and the QM/MM(ωM06-D3/6-311+G**:CHARMM36m) level (purple line) using the geometries optimized at the QM/MM(B3LYP/6-31G*:CHARMM36m) level.
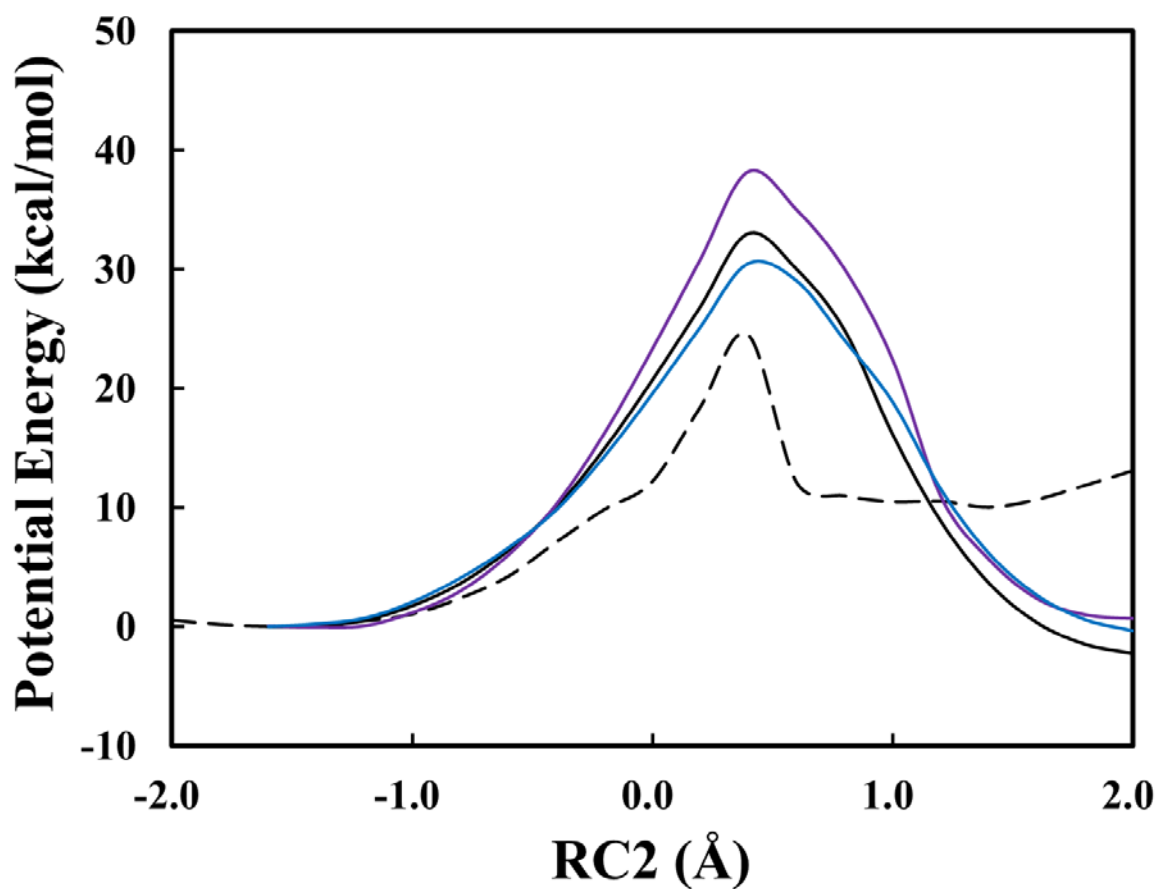
Figure S3. Deacylation reaction results of hDHHC20 from reaction coordinate and single point energy calculations. Plot of the potential energy *vs* the reaction coordinate (RC2) at QM/MM(DFTB3:CHARMM36m) level (dash line), QM/MM(B3LYP/6-31G*:CHARMM36m) level (black line), and further single-point energy calculations at the QM/MM(B3LYP/6-311+G**:CHARMM36m) level (blue line) and the QM/MM(ωM06-D3/6-311+G**:CHARMM36m) level (purple line) using the geometries optimized at the QM/MM(B3LYP/6-31G*:CHARMM36m) level.
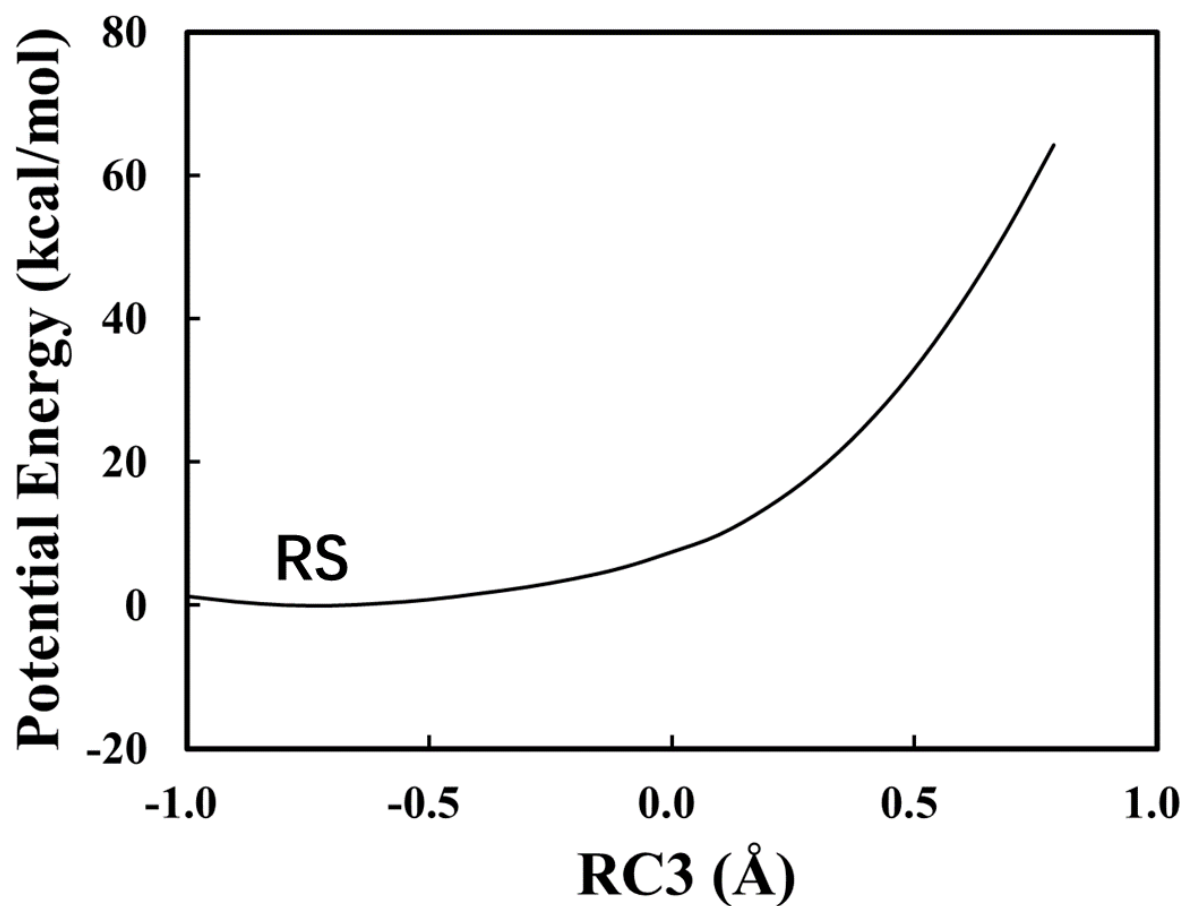
Figure S4. Proton transfer from His154 to Cys156 from reaction coordinate calculation. Plot of the potential energy *vs* the reaction coordinate (RC3) at QM/MM(B3LYP/6-31G*:CHARMM36m) level. Definitions of the reaction coordinate (RC3): RC3 = r(N...H) - r(S$^\gamma$...H).
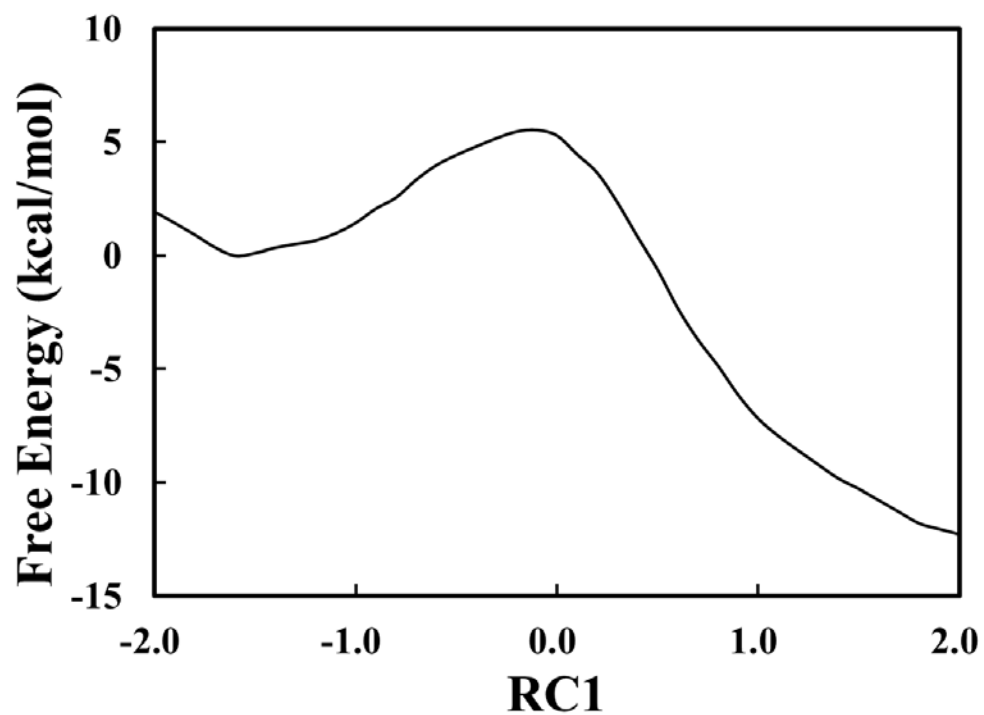
Figure S5. QM/MM(DFTB3:CHARMM36m) PMF calculation results for auto-palmitoylation reaction at 303.15 K.
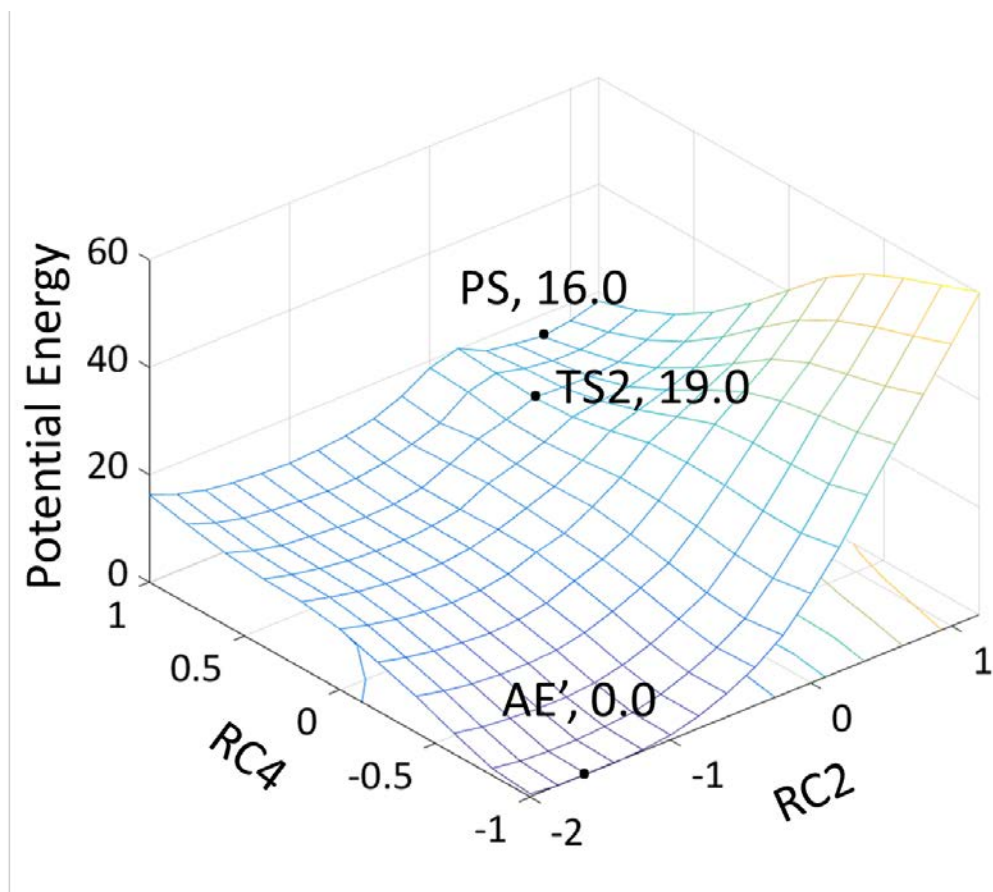
Figure S6. Two-dimensional (2D) potential energy surface (PES) based on RC2 and RC4. As shown in Figure 4C, RC4 define the proton transfer process from water to H154 (RC4 = distance ② - distance ③). Distances are in Å. Energy is in kcal/mol. In TS2, the RC2 = 0.2; RC4 = 0.6. The global mechanism is consistent with one dimensional PES in Figure 4A.
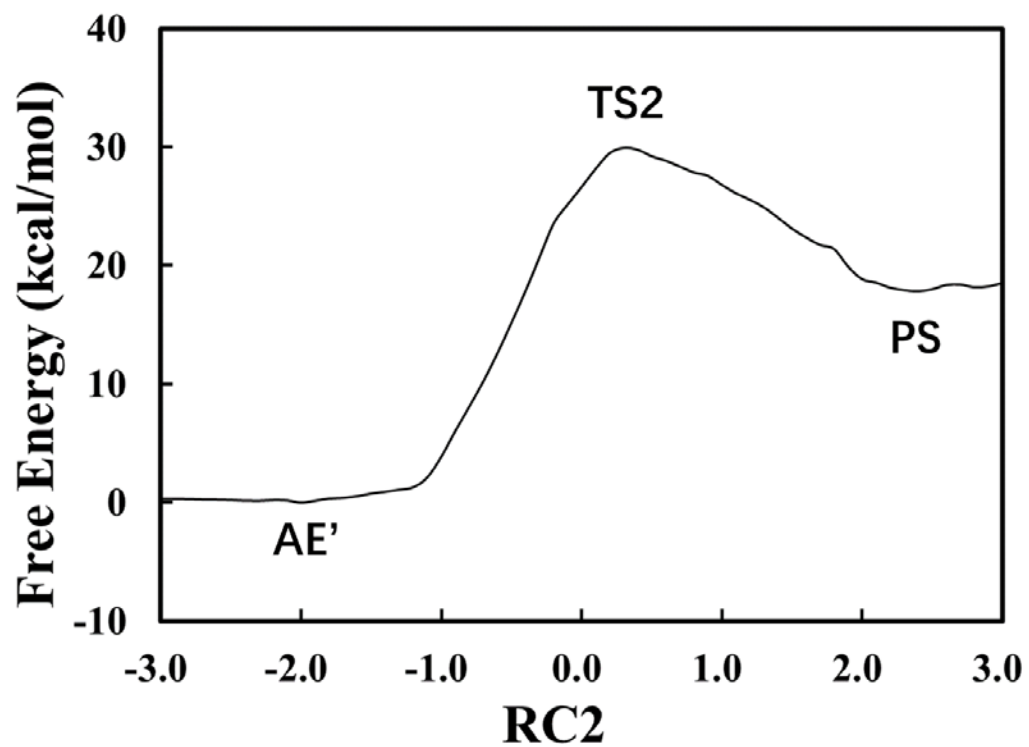
Figure S7. QM/MM(DFTB3:CHARMM36m) PMF calculation results for deacylation reaction with a 0.5-fs integration step.
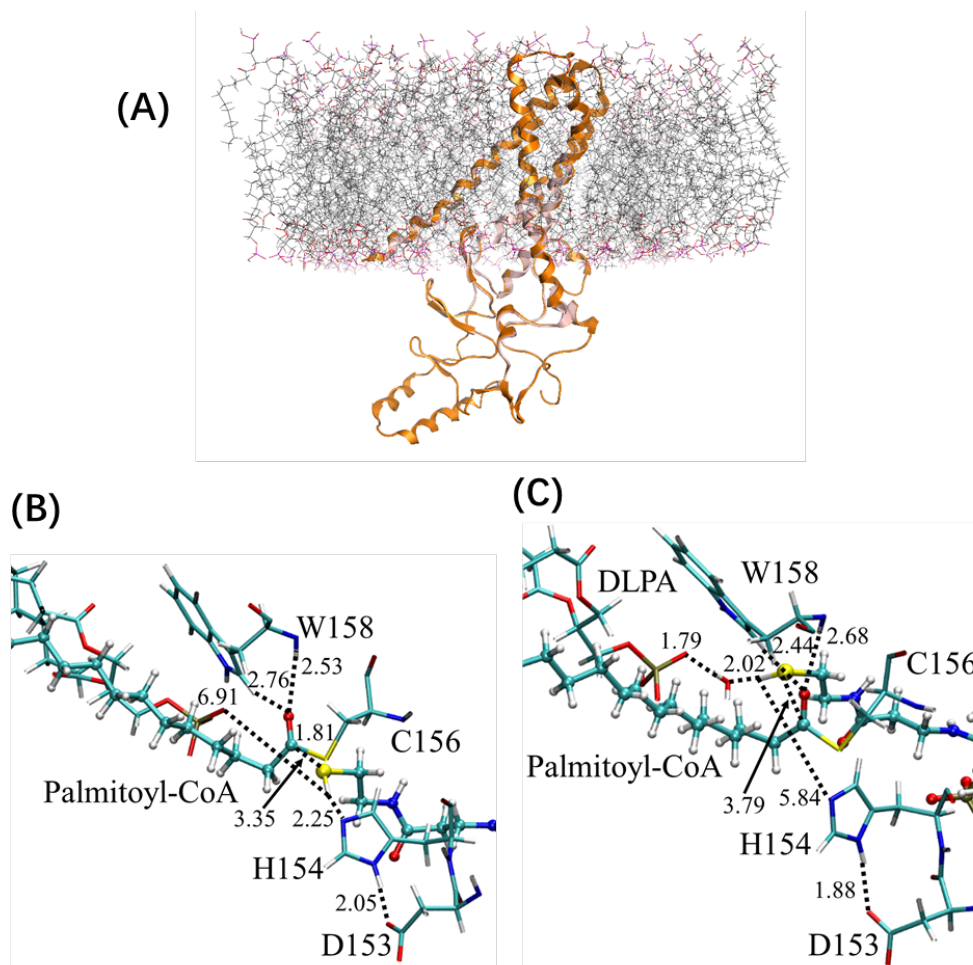
Figure S8. Comparison AE structures from PES and PMF simulations at QM/MM(DFTB3/CHARMM36m) level. (A) Superposition of optimized AE from PES and AE average structure from PMF. The backbone RMSD is 0.244 Å. (B) Optimized AE. The sulfydryl of leaving group forms hydrogen bond with H154. (C) One snapshot close to AE average structure. The sulfydryl of leaving group forms hydrogen bond network with water and 1,2-dilauroyl-D-glycero-1-Phosphatidic acid (DLPA) during 50ps QM/MM(DFTB3/CHARMM36m)   MD simulation. The negative charged phosphate moeity of DLPA is likely a better hydrogen bond acceptor than the sidechain of H154, and this may account for the energy difference of AE state between PES and PMF.

Table S1. Distance from calculations.

Reaction Stage	Structure	Method[a]	Distance (Å)								
			1	2	3	4	5	6	7	8	9
Acylation	Reactant State	RC(DFTB3)	3.11	2.14	1.03	1.90	1.81	3.15	3.46	4.92	1.22
		RC(B3LYP)	3.27	1.99	1.10	2.06	1.87	3.96	3.00	5.05	1.20
		PMF(DFTB3)	3.17	1.73	1.09	1.92	1.78	3.27	2.74	4.72	NA[b]
	Transition State 1	RC(DFTB3)	2.18	2.45	1.02	1.85	2.28	3.13	2.58	4.96	1.22
		RC(B3LYP)	2.23	2.43	1.06	1.99	2.43	3.30	2.72	5.01	1.19
		PMF(DFTB3)	2.28	2.49	1.01	1.86	2.09	2.81	2.59	4.61	NA
Deacylation	Acyl-Enzyme State	RC(DFTB3)	3.41	0.98	1.97	2.53	1.81	2.99	2.66	4.53	1.22
		RC(B3LYP)	3.43	0.97	2.41	2.38	1.83	2.82	2.55	4.43	1.20
		PMF(DFTB3)	3.99	1.00	2.39	3.05	1.61	3.81	3.28	4.50	NA
	Transition State 2	RC(DFTB3)	1.60	2.30	1.02	1.90	2.00	2.44	2.59	4.58	1.28
		RC(B3LYP)	1.62	2.45	1.11	1.97	2.02	1.88	2.56	4.70	1.25
		PMF(DFTB3)	1.74	1.95	1.02	1.92	2.02	2.80	3.18	4.52	NA

[a]Reaction coordinate calculation; [b]None Available.