Supplementary Information

Enhancement of thermal stability of Bacillus subtilis 168 glycosyltransferase YjiC based on PoPMuSiC algorithm and its catalytic conversion of rare ginsenoside PPD

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Table S1 A list of oligonucleotides used in this study.

Primer	Sequence[a] (5′→3′)
E52V_F	GTGTGGCACTGATTTATCATACCTCTC
E52V_R	CTATTTAGTCACGGTGTGGCGG
K65G_F	CCGGGCCAGATTCGTGAAATG
K65G_R	GTAAAGTGCTTAGACCGGGCC
D75V_F	GATGGAAAAAAATGTGGCACCG
D75V_R	GAGTCGCCACGGTGTAAAAAAAG
L78H_F	GAAAAAAATGATGCACCGCACAG
L78H_R	GTCCGACACGCCACGTAG
P89R_F	GCATTCTGCGTCAGCTGGAAGAAC
P89R_R	CAAGAAGGTCGACTGCGTCTTACGAG
K125I_F	CTGAATGTTCCGGTTATTATTCTGTG
K125I_R	GTGTGTTATTATTGGCCTTGTAAGTC
E141P_F	CAGCTGGGTAATCCGGATATG
E141P_R	GTCGTATAGGCCTAATGGGTC
E159P_F	CATATCTGCCGCAGGAAAAAC
E159P_R	GTCAAAAAGGACGCCGTC
E175P_F	GTTCCGCCGGCACTGAATATTG
E175P_R	GTATTTTTGTTATAAGTCACGGCCGC
N178I_F	CGGCATAAAAACAATAATCAGTGCTTCCG
N178I_R	CCGTCAAGGCCTTCGTGACTAATAAC
S203I_F	GTTTTGTTGGTCCGATCCTG
S203I_R	GTGGGTCCTAGCCTGGTTG
E209P_F	GAACGTAAACCGAAAGAAAGCCTG
E209P_R	GTTAGTCGTCCGAAAGAAAGCC
L214E_F	CTGGAAATTGATAAAGATGATCGTCCGC
L214E_R	GCTAGTAGAAATAGTTAAAGGTCCGAAAG
P313W_F	GGTTGTTATTTGGCAGATGTATGAACAGG
P313W_R	TGTAGACGGTTTATTGTTGGTCGCCTTC
K336P_F	GCCGCCGGAAGAAGTTACC
K336P_R	CCGGCGGCAGATAAACACC
D367M_F	CAGAAAATGGTTAAAGAAGCAGGTGG
D367M_R	GGTGGACGAAGAAATTGGTAAAAGAC

[a] The mutated bases are underlined

Table S2. The selected single-site mutants and their folding free energy (ΔΔG) values based on the PoPMuSiC 2.1 algorithm.

Mutants	ΔΔG	Mutants	ΔΔG
E52V	-0.78	E175P	-0.72
K65G	-0.72	N178I	-1.56
D75V	-0.69	S203I	-0.81
L78H	-0.75	E209P	-0.94
P89R	-0.97	L214E	-0.58
K125I	-1.67	P313W	-1.79
E141P	-0.46	K336P	-0.89
E159P	-0.90	D367W	-0.42

Table S3. Heat-sensitive residues of the WT and variants

Enzyme	Regions of heat-sensitive residues
WT	4, 48-63, 65, 68-89, 91, 113, 118, 119, 123-139, 152, 154-157, 172-174, 177-190, 192, 193-196, 211, 213-215, 272, 318, 321, 347-348, 350-352, 356-367, 369, 372-388
K125I	18-19, 22, 25-26, 56-57, 59-69, 81, 83-95, 97, 113-139, 147-153, 156, 159, 161-164, 168-169, 217, 235, 245-247, 266-277, 341-348, 378-388
K125I/N178I	60-62, 132-133, 135-141, 143-145, 162-166, 168, 186-193, 214, 230-232, 234, 236-251, 257-276, 316-317, 320-321, 324-342, 361-388
M315F	4, 49, 66-68, 71-78, 122, 124-141, 143-160, 171, 175, 181, 186-204, 207, 215, 217, 237, 269-272, 296-297, 299-325, 327-330, 338-367, 373-375, 377-388

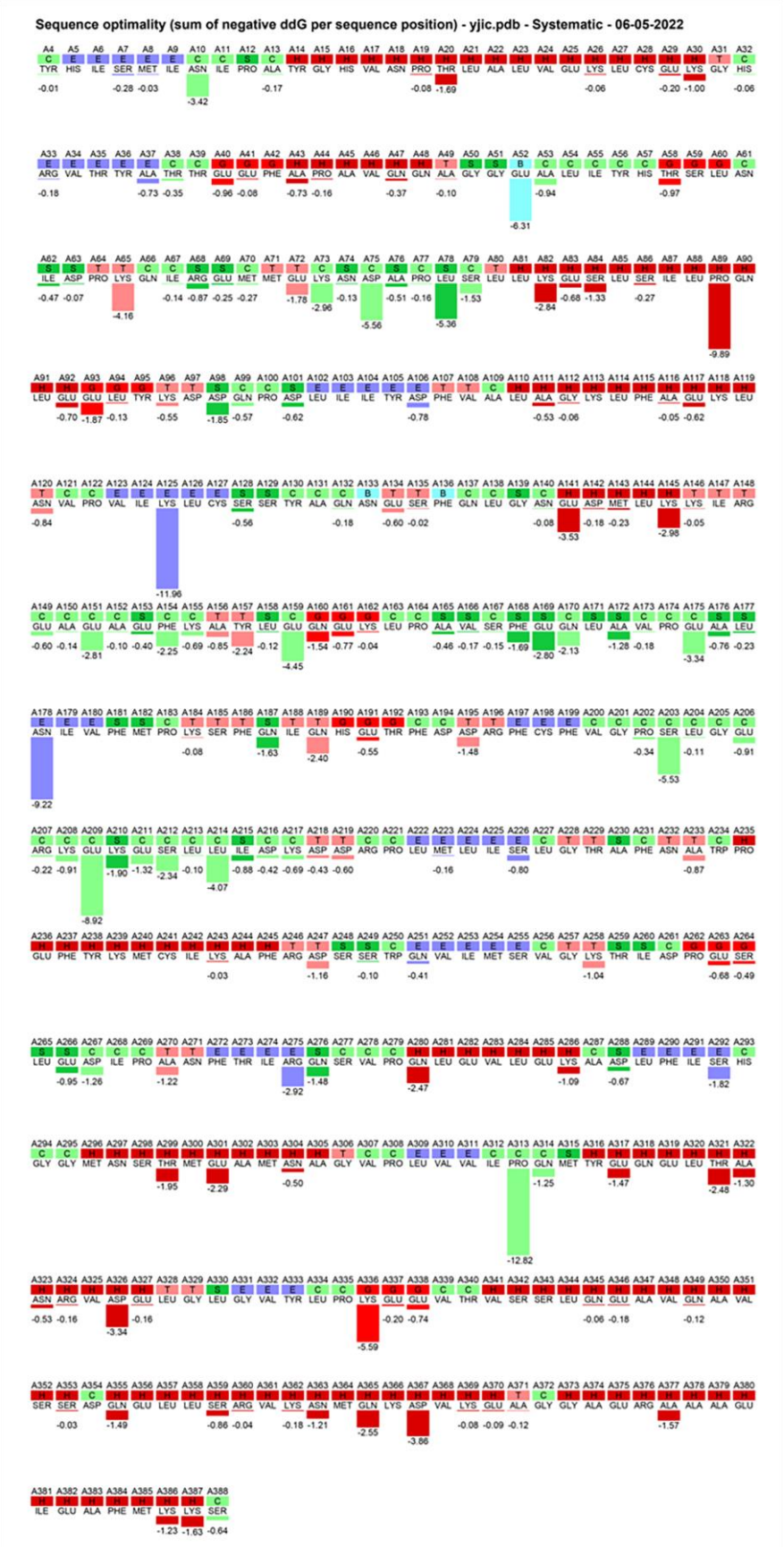


Figure S1. The analysis of the BS-YjiC protein structures by PoPMuSiC.

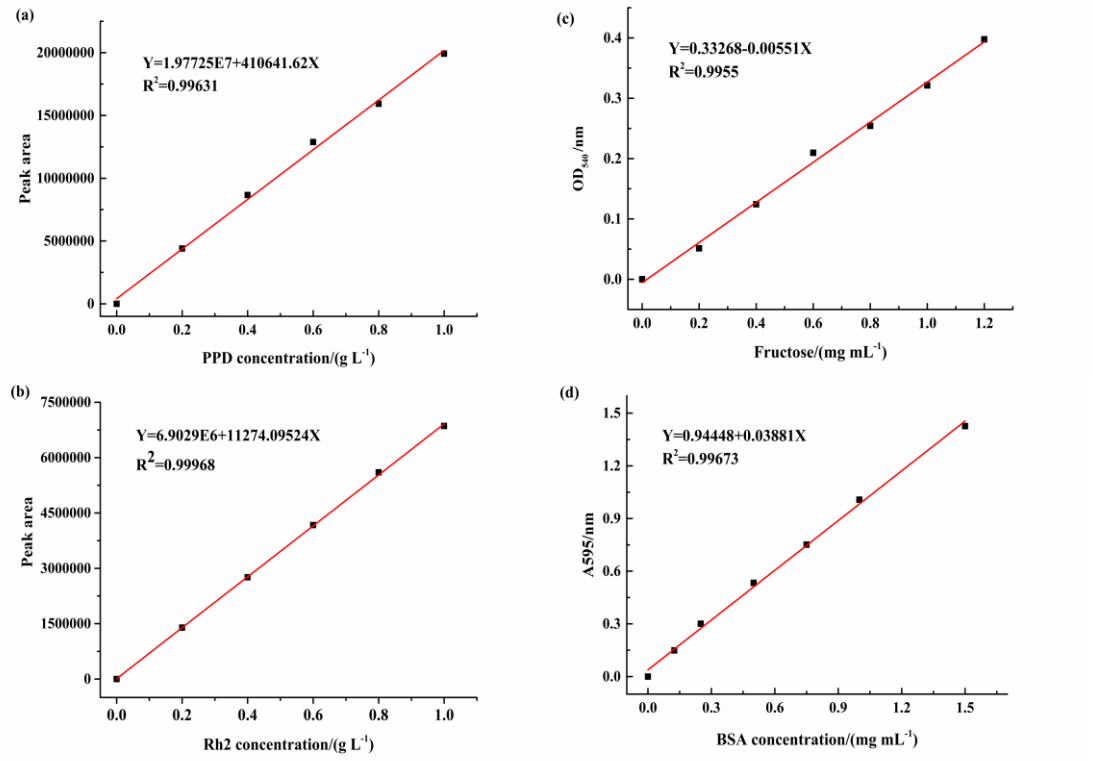


Figure S2. Standard curve. (a) ginsenoside PPD, (b) ginsenoside Rh2, (c) fructose, (d) BSA.

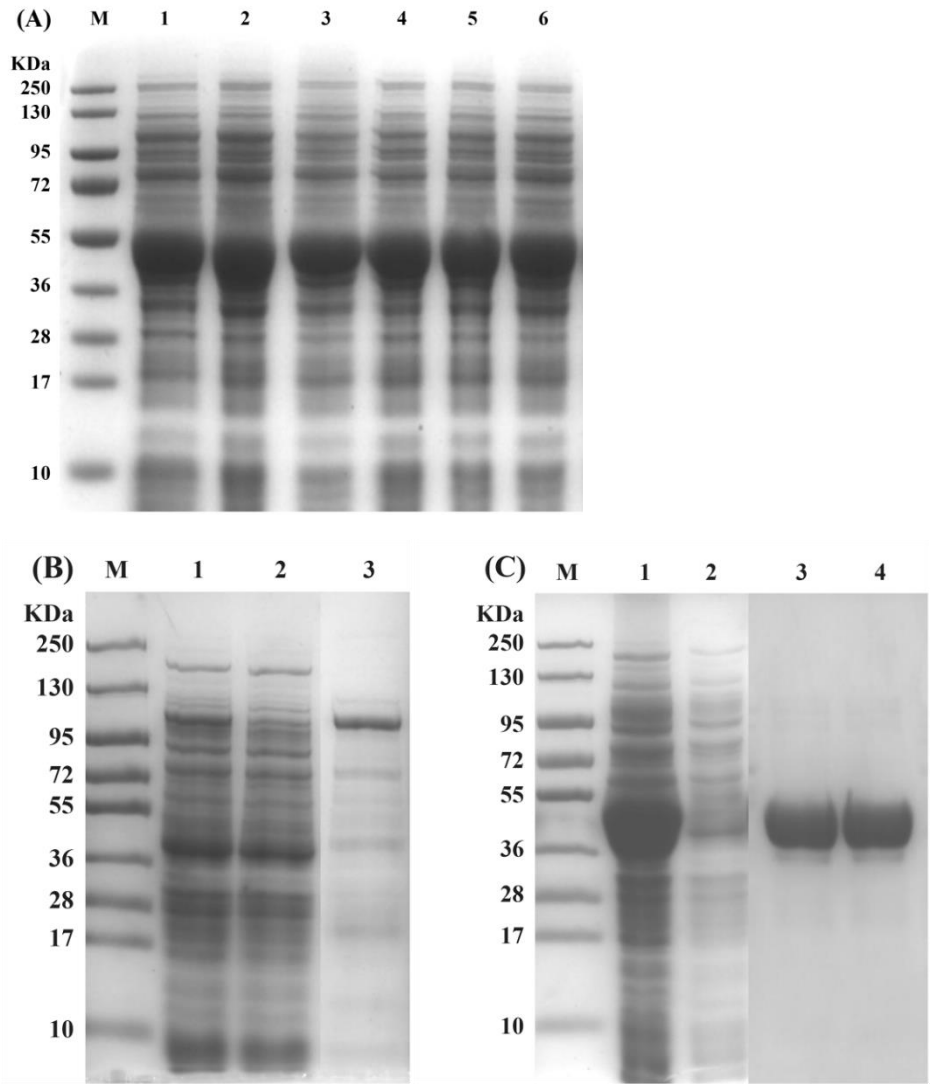


Figure S3. SDS-PAGE (8%) analysis of Bs-YjiC and AtSuSy expressed in E. coli BL21 (DE3). (A) SDS-PAGE analysis of Bs-YjiC and its mutants. M: marker, line 1, WT; line 2, K125I; line 3, N178I; line 4, P313W; line 5, K125I/N178I; and line 6, K125I/P313W. (B) SDS-PAGE analysis of AtSuSy. M: marker, line 1, Crude AtSuSy after cell fragmentation; line 2, Crude AtSuSy which was not induced; line 3, AtSuSy purified by Ni-NTA column. (C) SDS-PAGE analysis of Bs-YjiC and its mutants. M: marker, line 1, Crude Bs-YjiC after cell fragmentation; line 2, Crude Bs-YjiC which was not induced; line 3, WT purified by Ni-NTA column; line 4, K125I/E178I purified by Ni-NTA column.

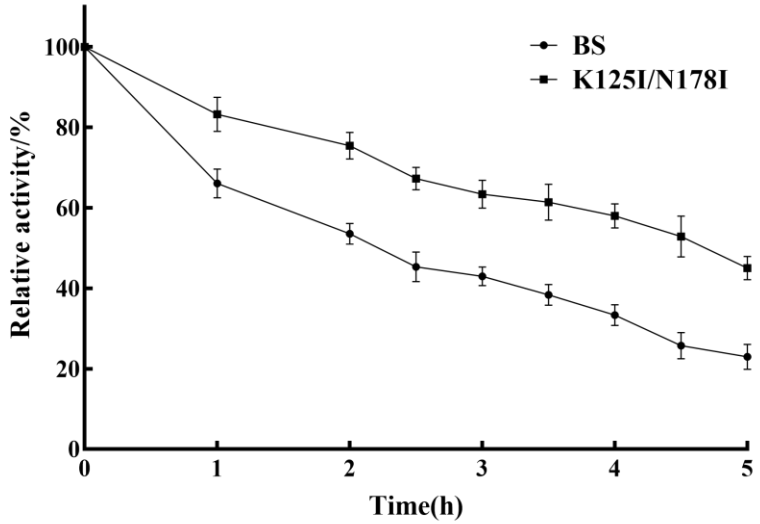


Figure S4. Thermostability of BS-YjiC and its mutants at 55 °C.

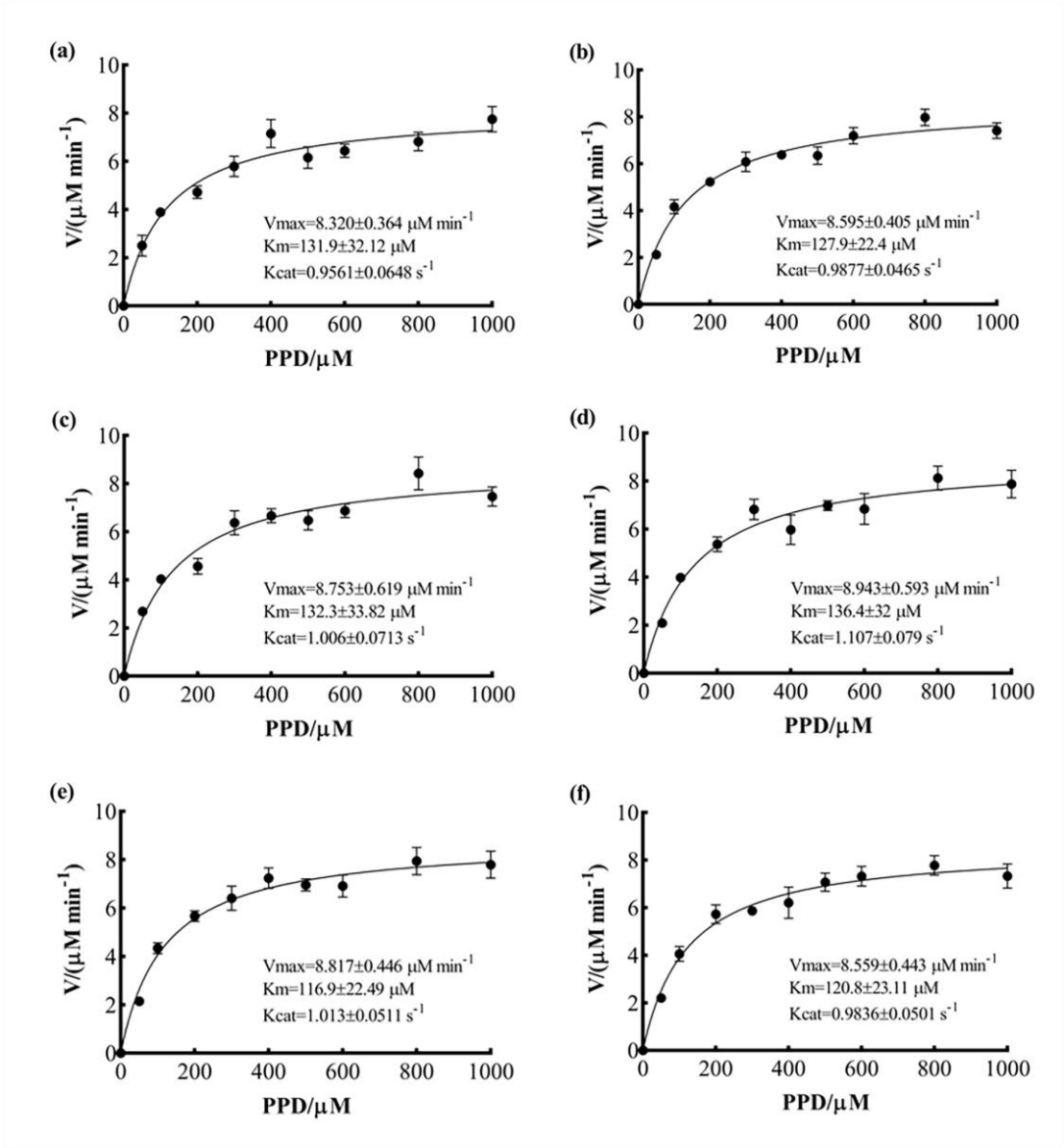


Figure S5. Kinetic plot of the enzymatic reactions to ginsenoside PPD. (a) WT, (b) K125I, (c) N178I, (d) P313W, (e) K125I/N178I, (f) K125I/P313W.

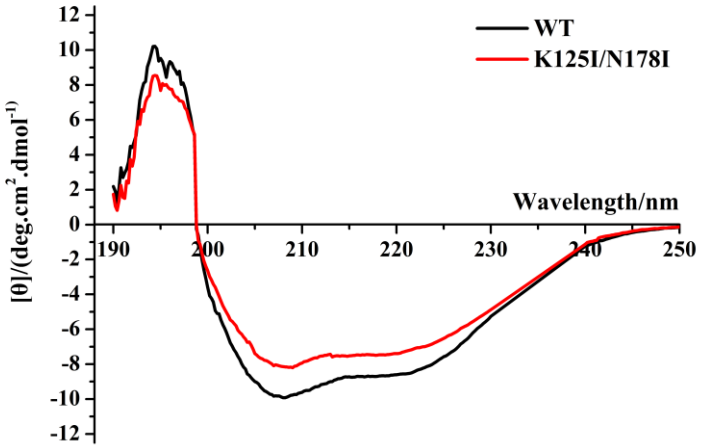


Figure S6. CD spectra of WT and mutant K125I/N178I glycosyltransferase.

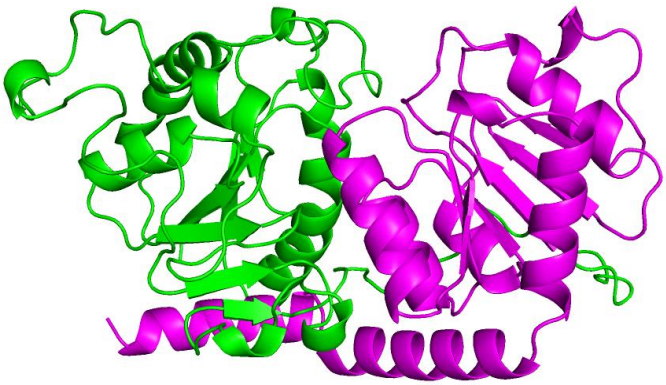


Figure S7. Overall structure of the Bs-YjiC displayed with PYMOL. The N-terminal and C-terminal domains are marked in green and magenta, respectively.

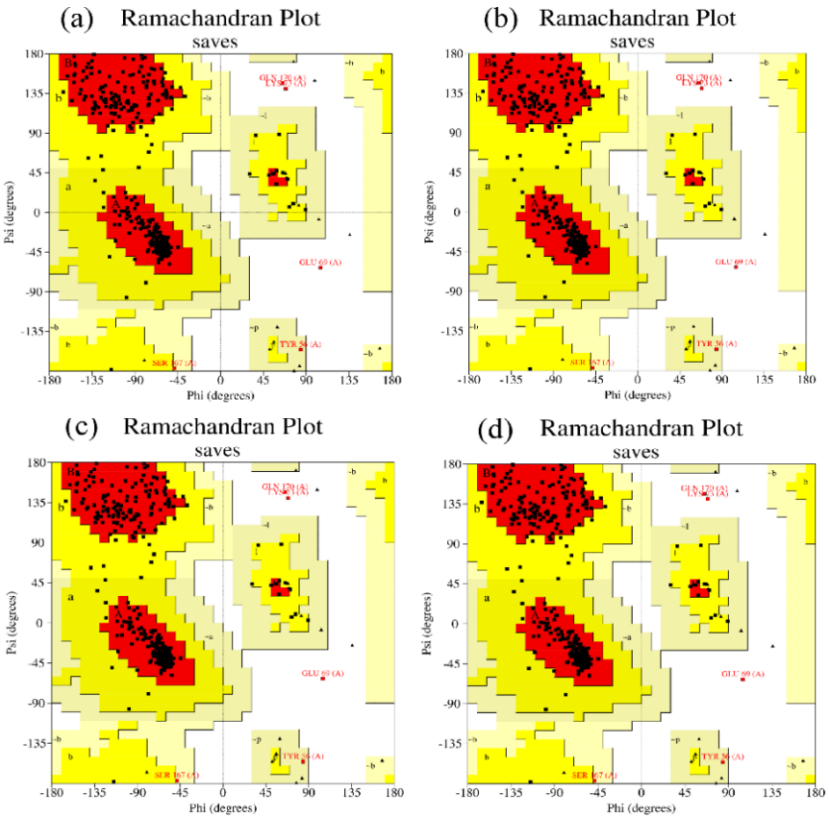


Figure S8. Ramachandran plots of YjiC and its mutants. (a) WT, (b) K125I, (c) N178I, (d) K125I/N178I. Residues in the most favored regions are displayed in red. Residues in additionally allowed regions are presented in yellow.

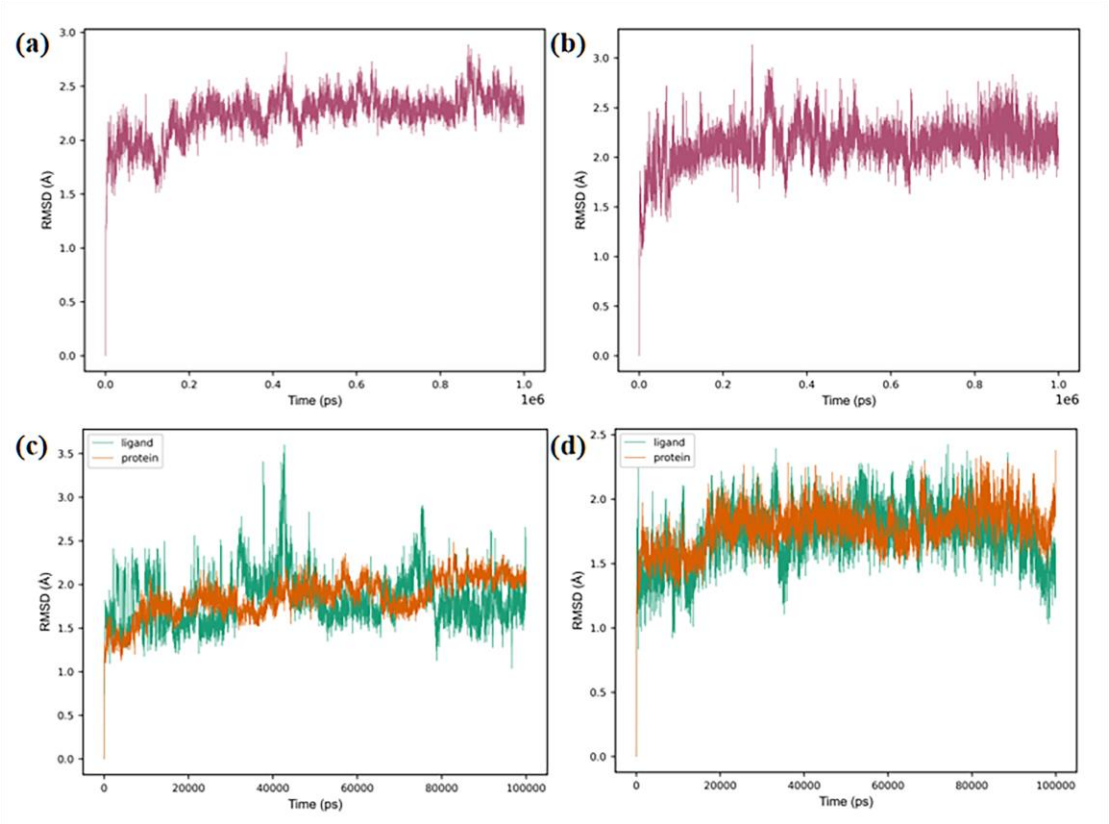


Figure S9. RMSD of the BS-YjiC-ligand complexes. (a) WT, (b) ligand, (c) WT-ligand complex, (d) mutant K125I/N178I-ligand complex.

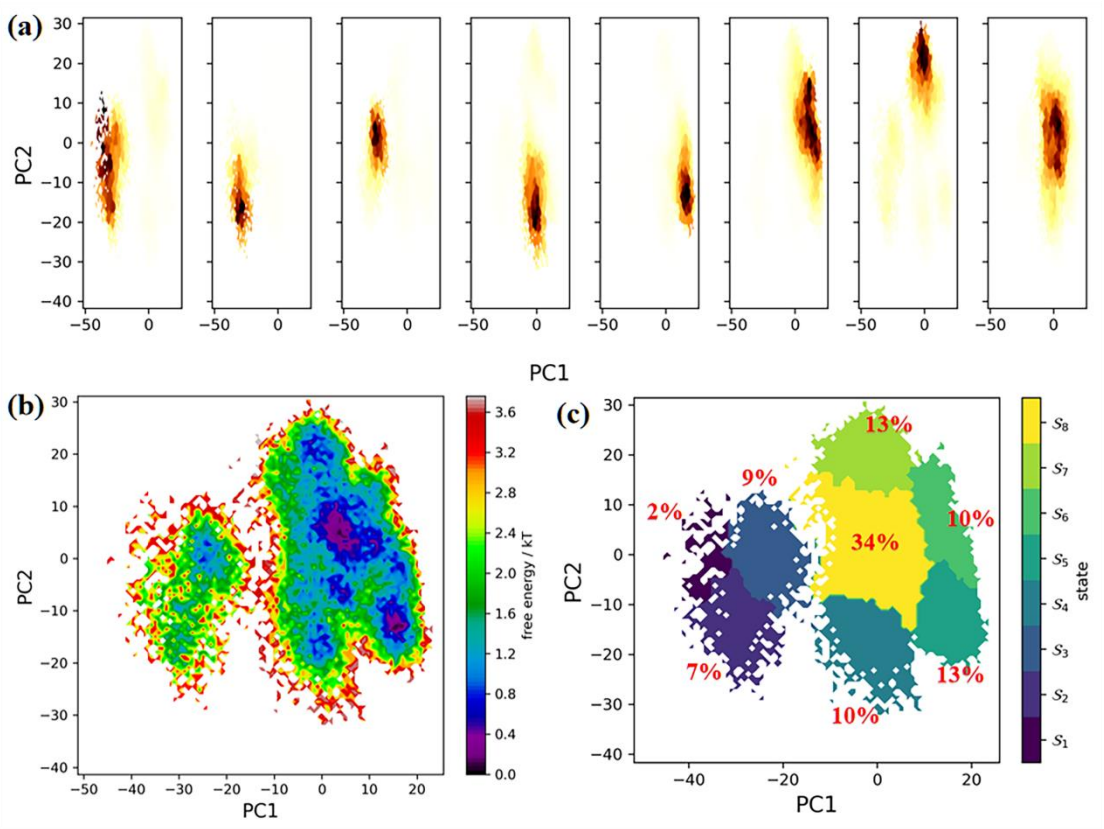
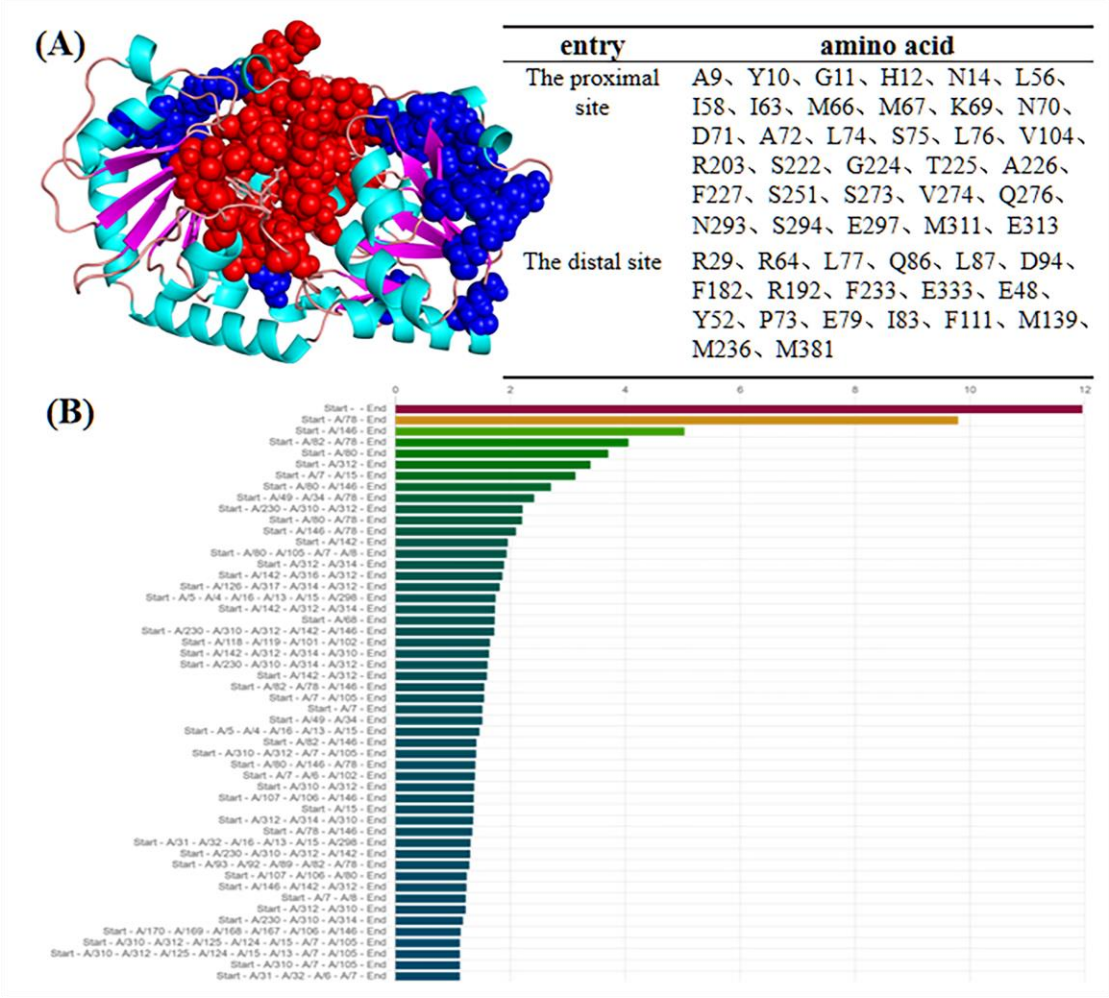


Figure S10. Principal component analysis (PCA) of Markov chain model (MSM). (a) multiple, (b) Free energy surface (FEL), (c) states.



entry	amino acid
The proximal site	A9、Y10、G11、H12、N14、L56、I58、I63、M66、M67、K69、N70、D71、A72、L74、S75、L76、V104、R203、S222、G224、T225、A226、F227、S251、S273、V274、Q276、N293、S294、E297、M311、E313
The distal site	R29、R64、L77、Q86、L87、D94、F182、R192、F233、E333、E48、Y52、P73、E79、I83、F111、M139、M236、M381

Figure S11. Distal effect pathway. (A) Red represents the proximal site, blue represents the distal site. (B) The weighting proportion here refers to the importance of each path in the signal transduction pathway (Due to Pymol counting issues, the actual amino acid ranking should be +1). The higher the weight, the more important the role of this path in the entire regulatory process.

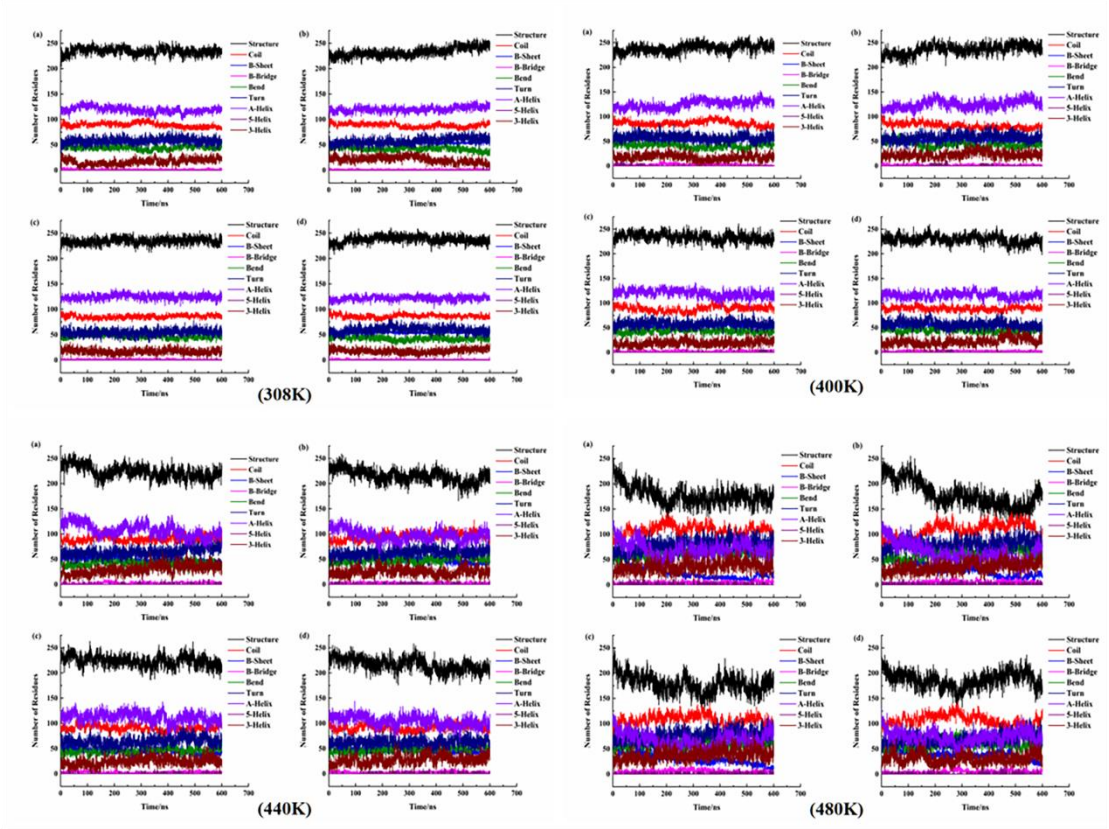


Figure S12. Secondary structure development trajectories of BS-YjiC and its mutants (K125I, K125I/N178I and M315F) at different temperatures. Different secondary structure elements are displayed by colors at the figure's bottom. (a) 308K, (b) 400K, (c) 440K, (d) 480K.

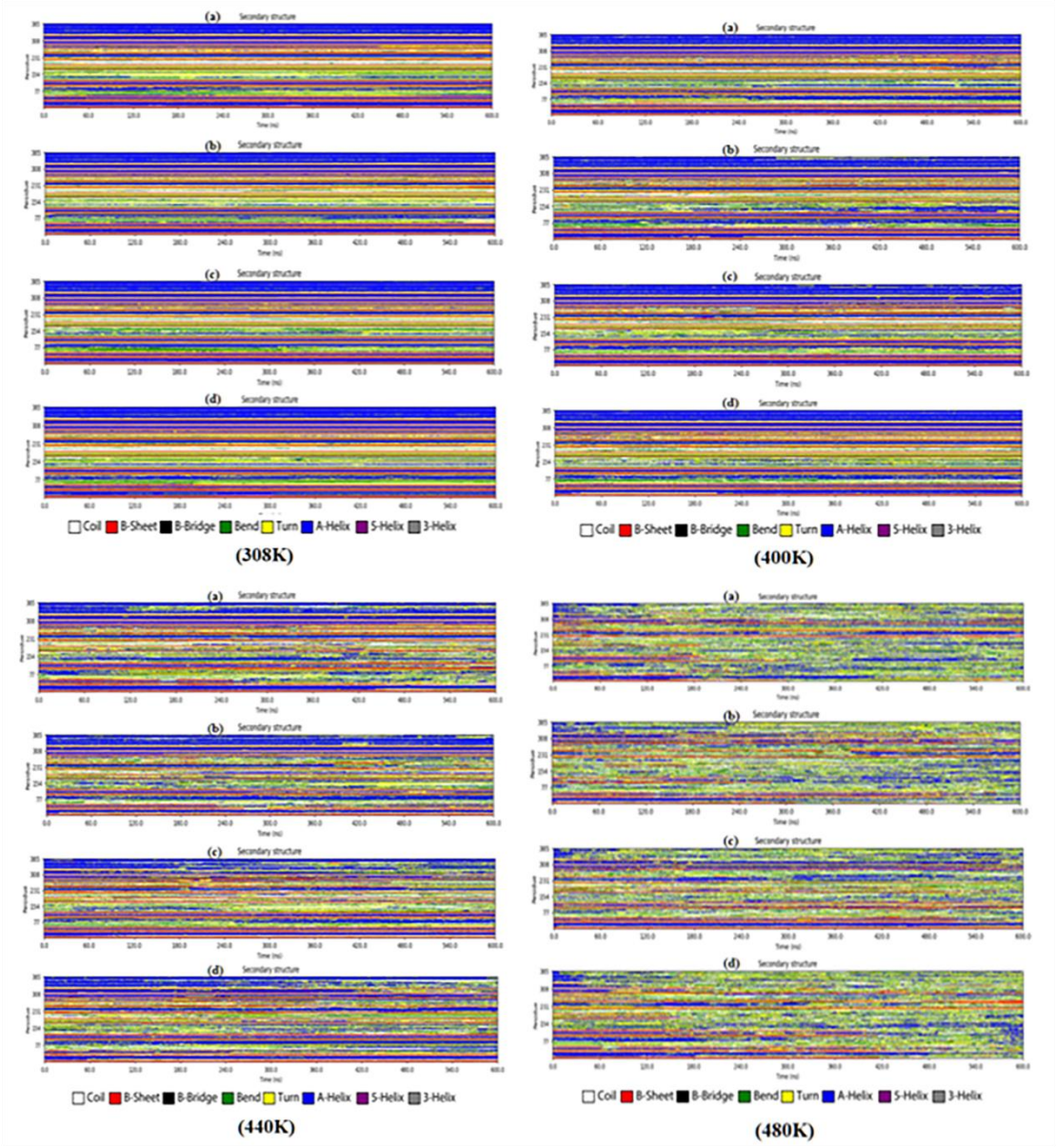


Figure S13. Development trajectories of secondary structure elements of BS-YjiC and its mutants (K125I, K125I/N178I and M315F) at different temperatures. (a) 308K, (b) 400K, (c) 440K, (d) 480K.

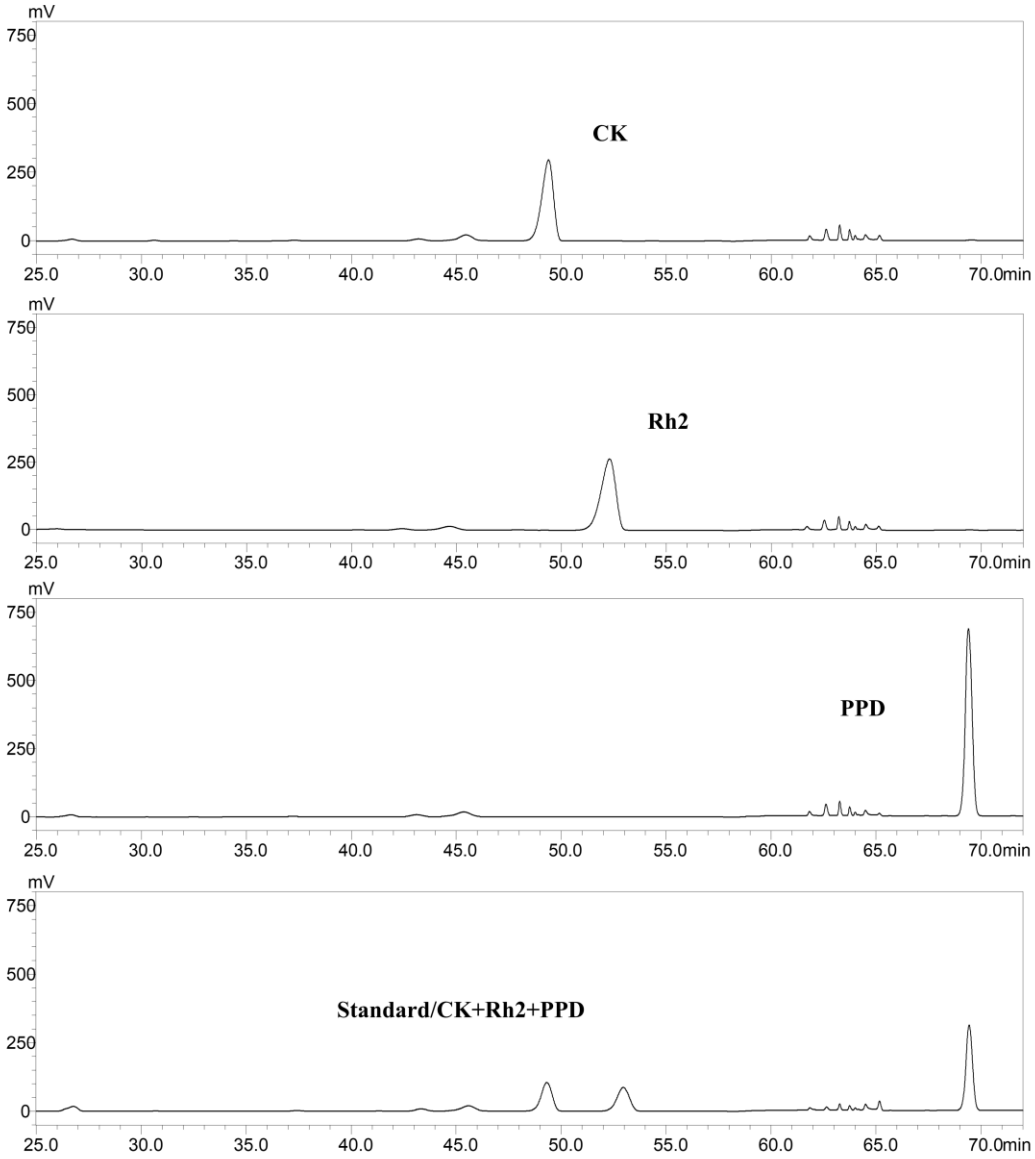


Figure S14. HPLC chromatogram of the Standard ginsenosides.

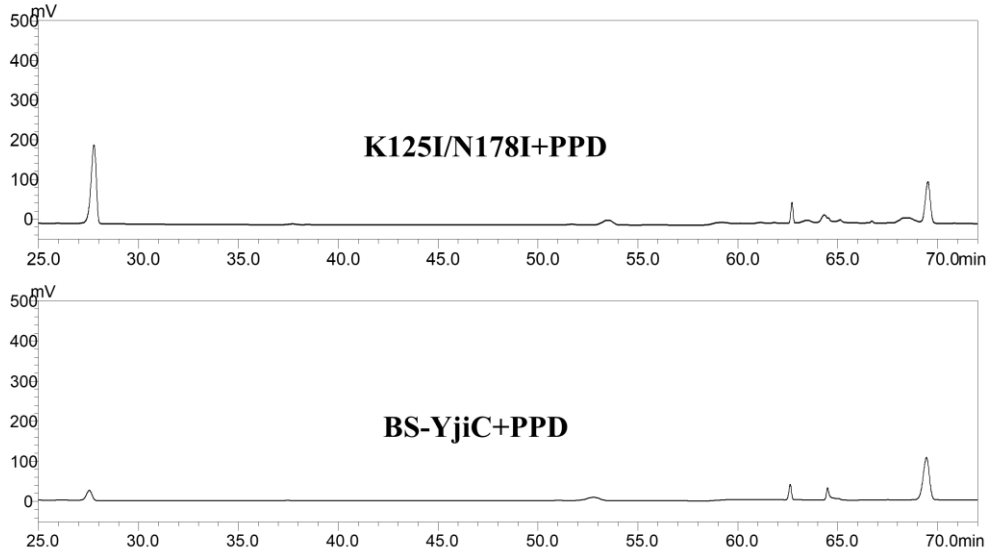


Figure S15. HPLC chromatogram of the BS-YjiC and the mutant K125I/N178I reacted with PPD at 45 ℃, respectively.

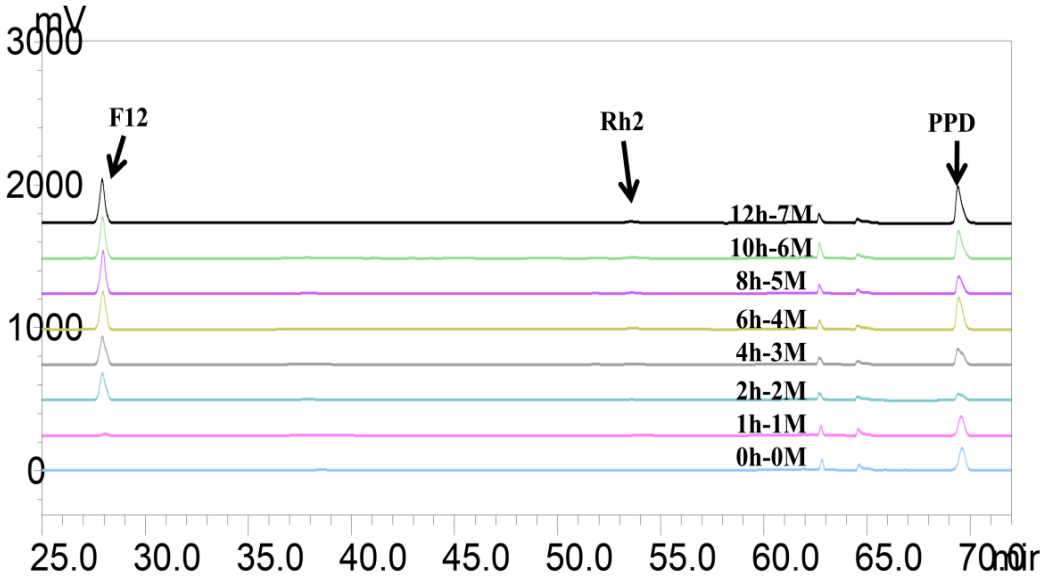


Figure S16. HPLC chromatograms of reaction products of the mutant K125I/N178I-AtSuSy cascade. The reaction system (20 mL) contained 1 mM PPD, 0.25 mM UDP, 50 mM Tris-HCl (pH 8.0), 0.5 M sucrose, 6% DMSO, 100 mU mL^{-1} Bs-YjiC and 240 mU mL^{-1} AtSuSy. The reaction was conducted at 45 ℃ for 14 hours. 100 μL of PPD (200 mM) was added to the reaction mixture at 1, 2, 4, 6, 8, and 10 h, respectively. Fresh enzymes (100 mU mL^{-1} Bs-YjiC and 240 mU mL^{-1} AtSuSy) were added at 4 and 8h.

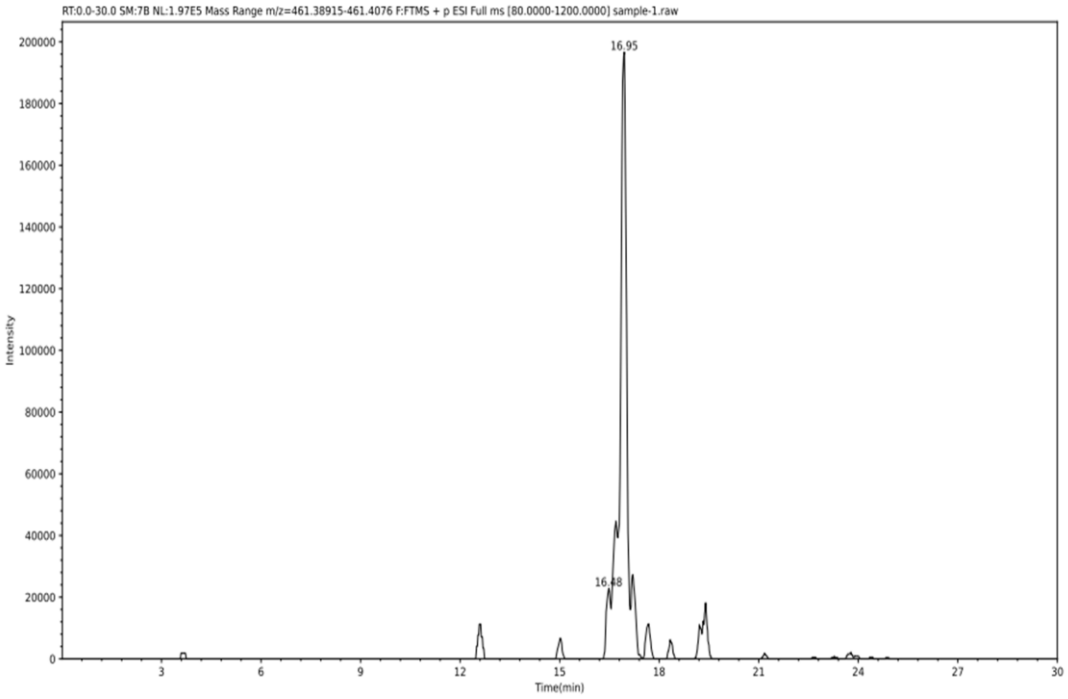


Figure S17. LC-MS spectrum of PPD ($C_{30}H_{52}O_3$; calculated molecular weight: [M+H]+ = 461.39837, DMSO-d6).

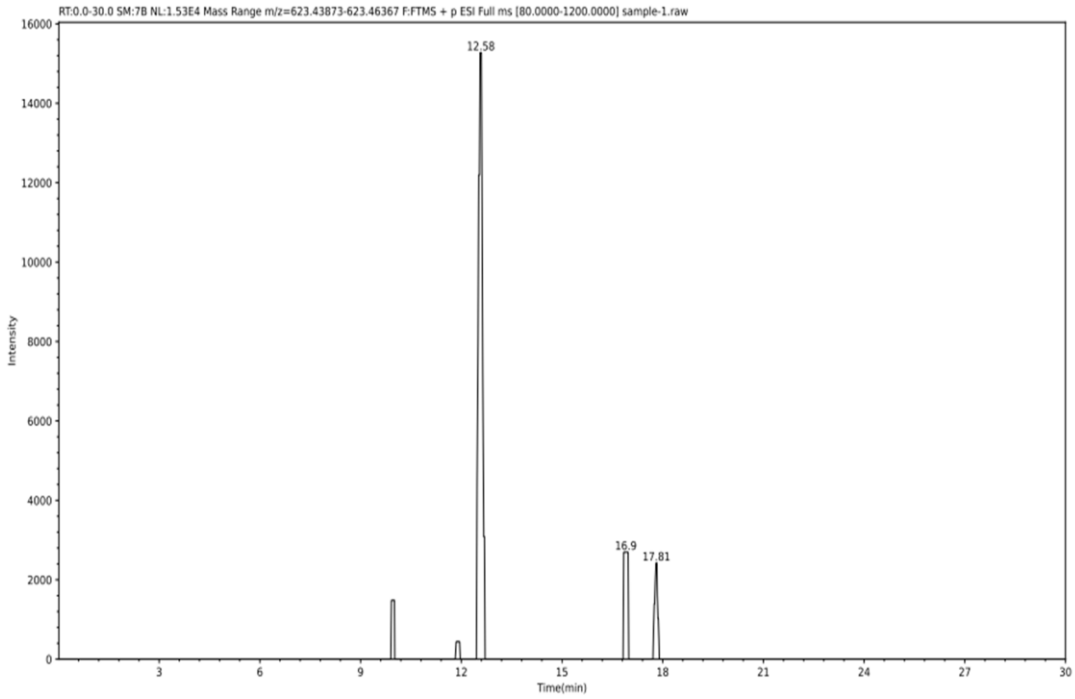


Figure S18. LC-MS spectrum of Rh2 ($C_{36}H_{62}O_8$; calculated molecular weight: [M+H]+ = 623.45120, DMSO-d6).

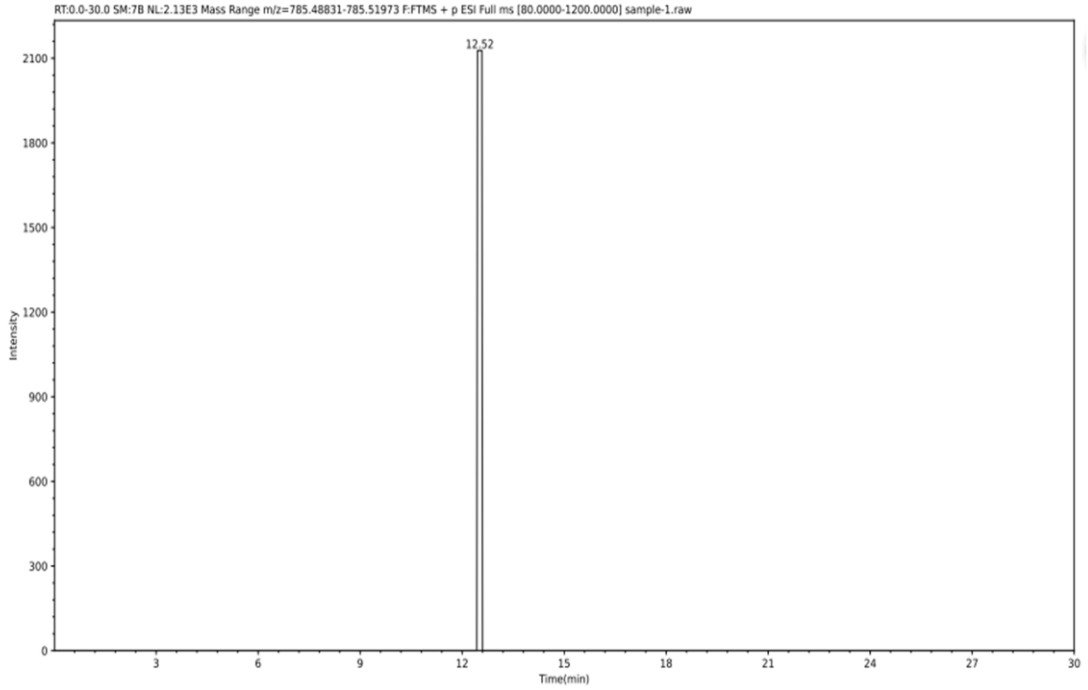


Figure S19. LC-MS spectrum of F12 ($C_{42}H_{72}O_{13}$; calculated molecular weight: [M+H]+ =785.50402, DMSO-d6).