



Supporting Information for

Functional Conversion of Acetyl-Coenzyme a Synthase to a Nickel Superoxide Dismutase via Rational Design of Coordination Microenvironment for the Ni_d-Site

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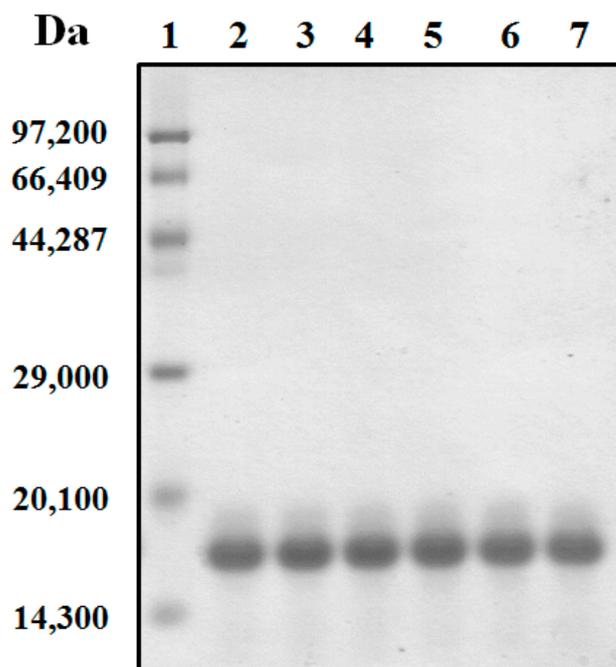


Figure S1. Sodium dodecyl sulfate polyacrylamide gel electrophoresis: Lane 1 marker, lane 2 ACS- α_{15} , lane 3 F598H, lane 4 S594H, lane 5 S594H-GP, lane 6 EFG-F598H, lane 7 YGP-F598H.

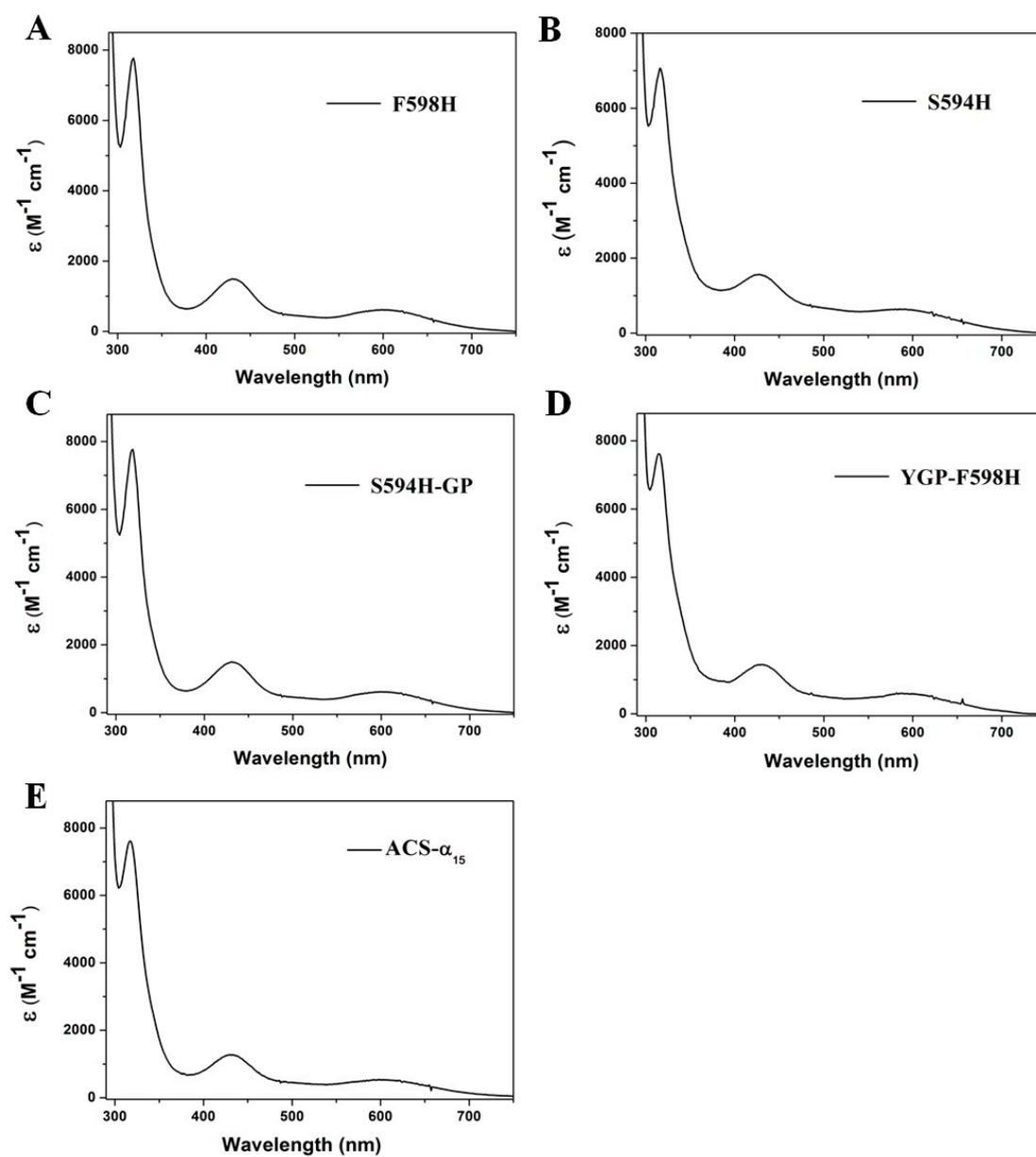


Figure S2. UV/Vis spectra of (A) F598H, (B) S594H, (C) S594H-GP, (D) YGP-F598H and (E) ACS- α_{15} .

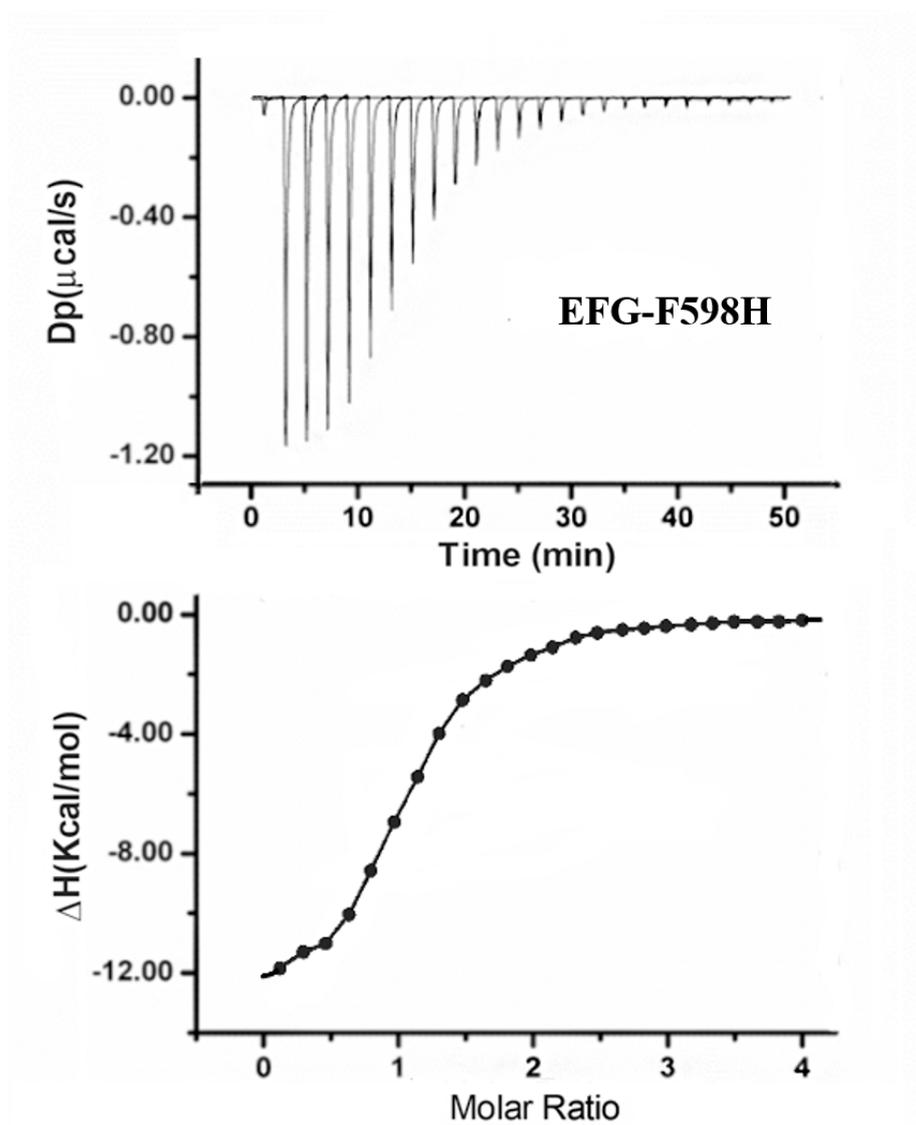


Figure S3. Calorimetric titration result for titrations of 1.0 mM NiCl₂ into a solution of 50 μM protein of apo-EFG-F598H. Curve fitted with a one-site sequential binding model. Protein sample (50 μM) was titrated against 20 mM HEPES buffer (pH 7.4) containing 1 mM NiCl₂. Titrations were performed as 25 injections (1.5 μl each) of a metal stock solution into a protein solution in the sample cell (200 μl) of the calorimeter. Each metal injection required a 120 s relaxation interval between successive injections. The data correction and curve fitting were performed using Origin 7.0. The ITC result for EFG-F598H was shown in Figure S7. Curve fitted with a one-site sequential binding model well and $K_d = 6.4 \pm 0.5 \mu\text{M}$.

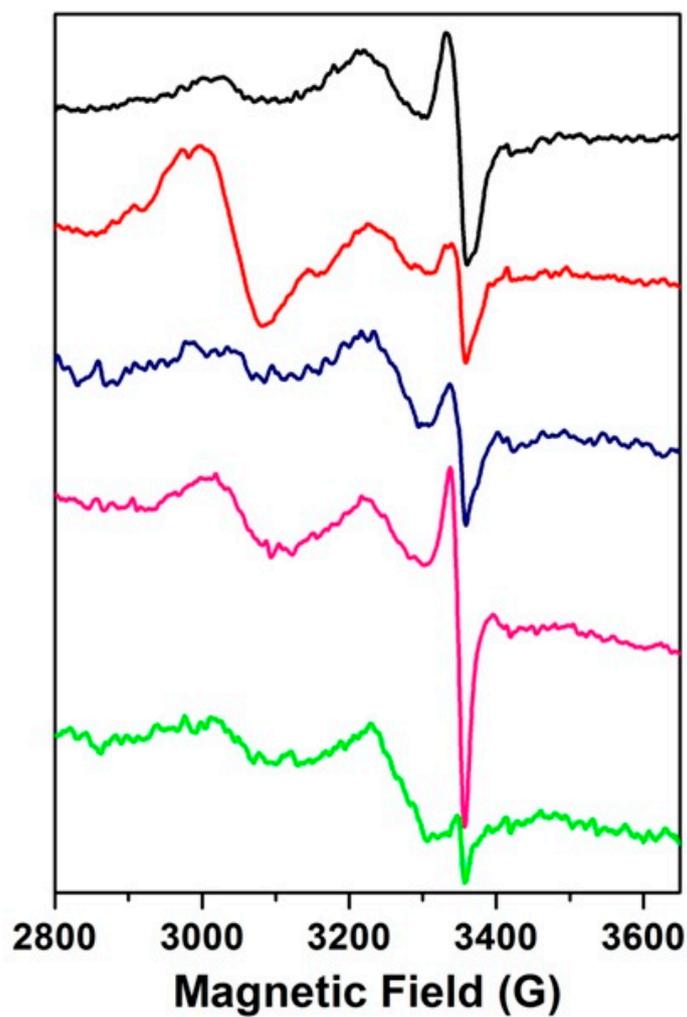


Figure S4. X-band EPR spectra of F598H (black), S594H (red), S594H-GP (blue), EFG-F598H (pink) and YGP-F598H (green) at 2 K in the presence of ~ 1 equiv. of KO_2 (microwave frequency, 9.49 GHz; microwave power, 20mW; modulation amplitude, 5 G).

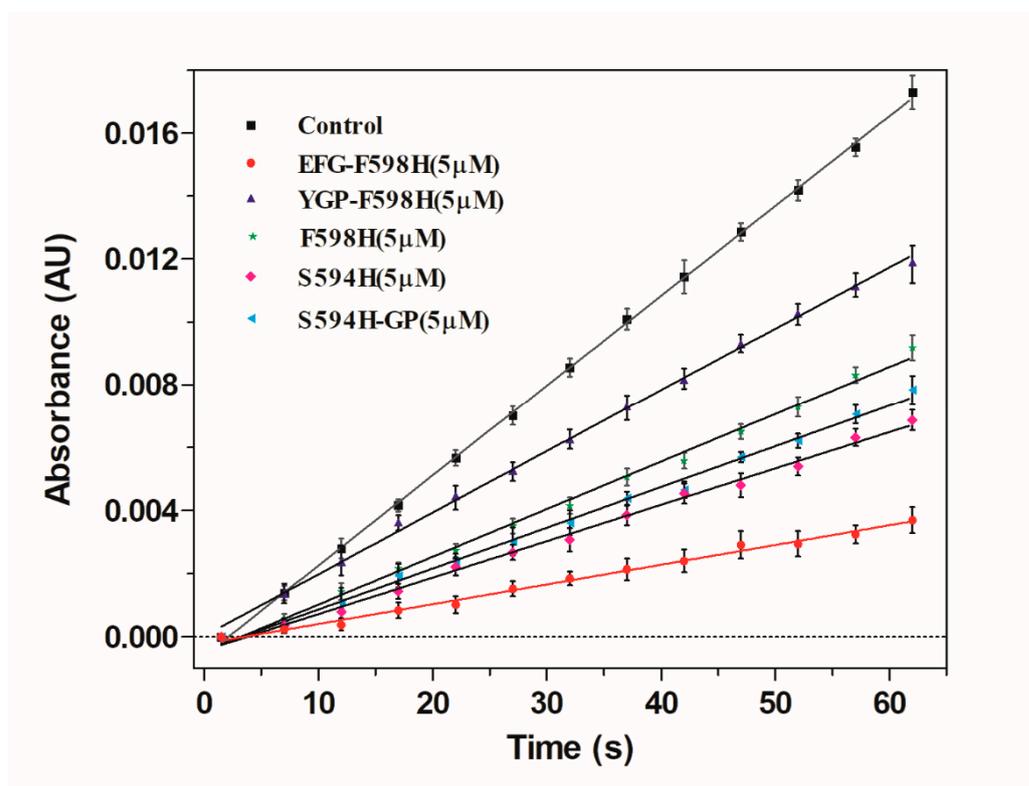


Figure S5. Measurement for SOD activity are using xanthine oxidase/NBT system. Black square: control, red circle: EFG-F598H, blue triangle: YGP-F598H, green star: F598H, magenta square: S594H, cyan triangle: S594H-GP. The results were presented as means \pm SEM.

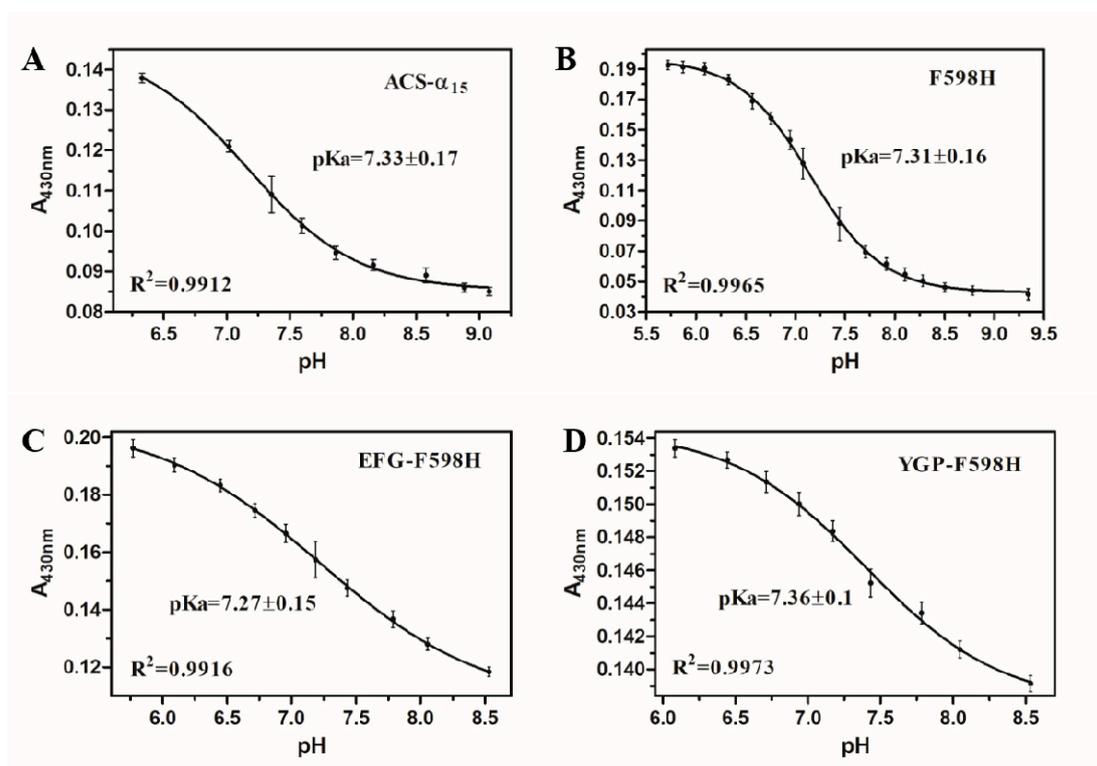


Figure S6. Optical pH titration based on the change in absorbance at 430 nm of ACS- α_{15} , F598H, EFG-F598H, and YGP-F598H. The results were presented as means \pm SEM.

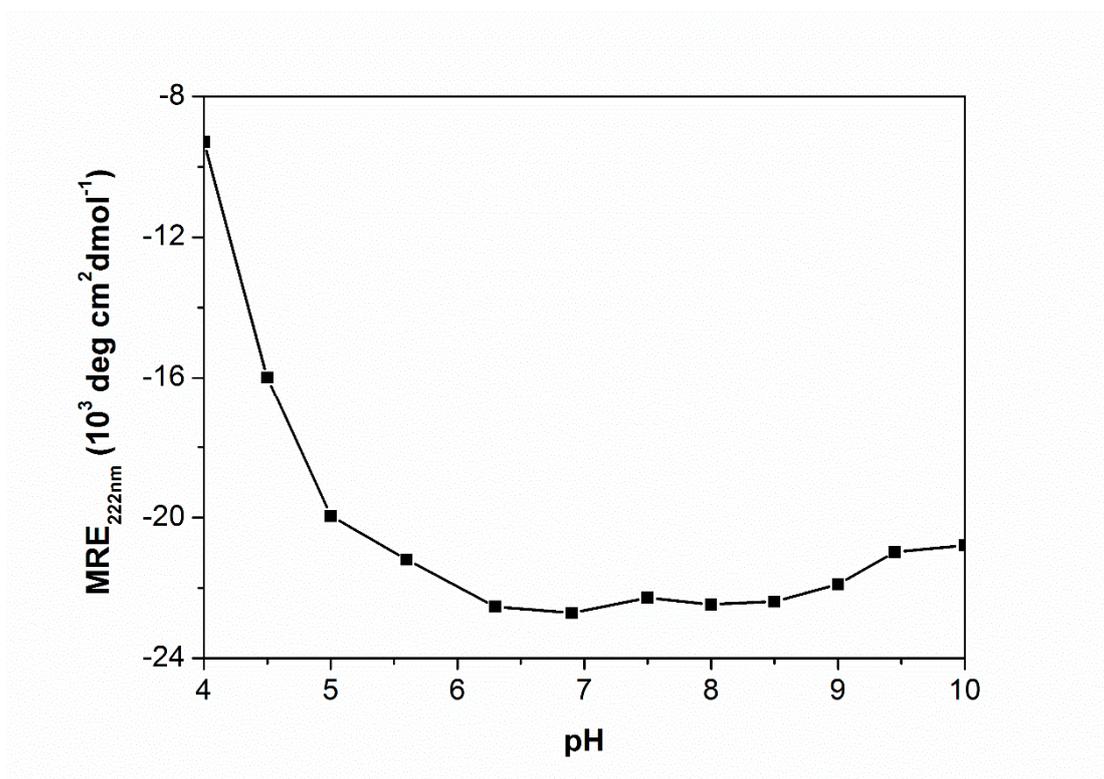


Figure S7. pH dependent changes of EFG-F598H in mean residual ellipticity (MRE) at 222 nm.

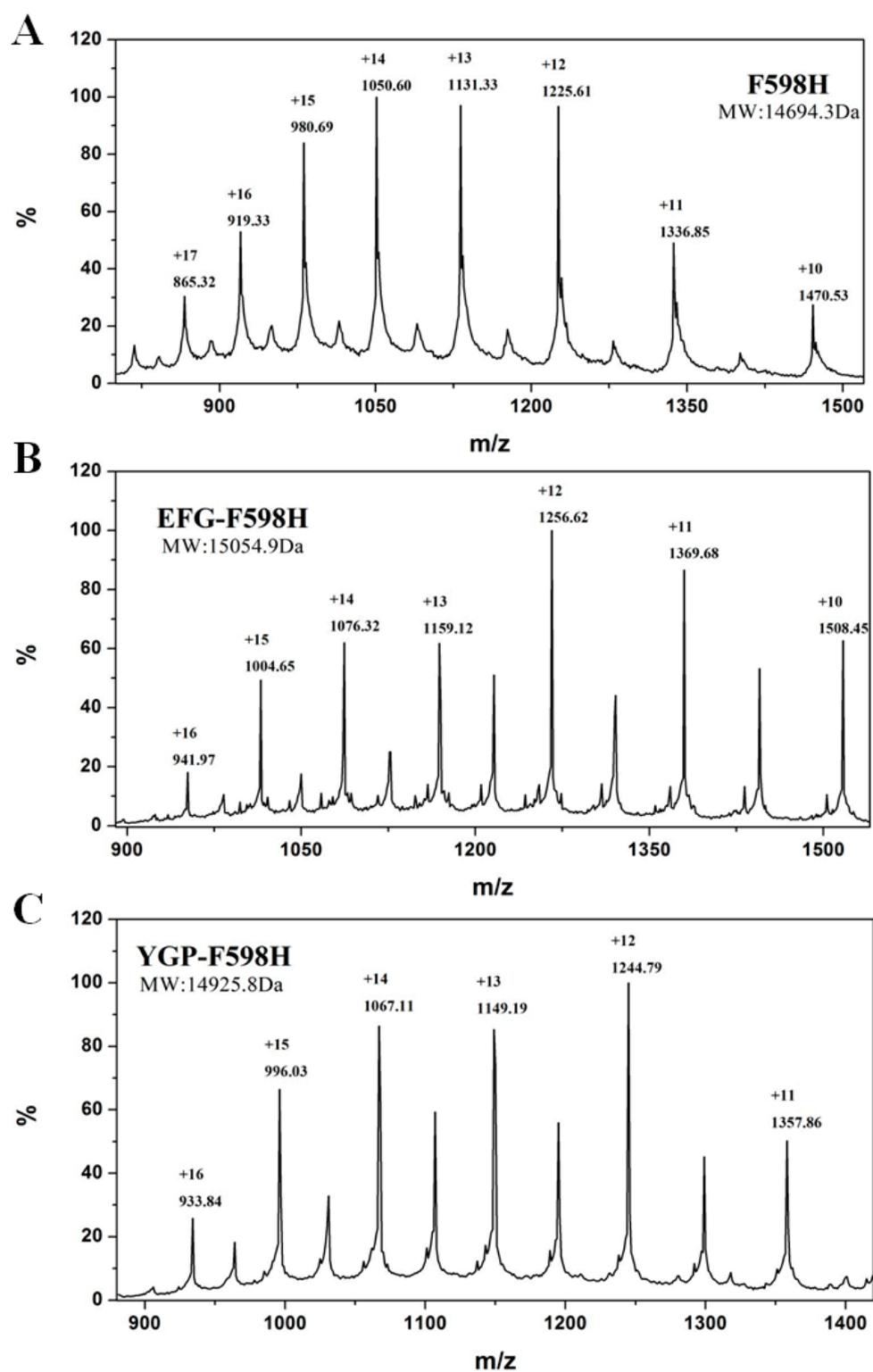


Figure S8. ESI-MS (positive ion mode) of solutions of F598H (A), EFG-F598H (B) and YGP-F598H (C) purified under aerobic conditions.

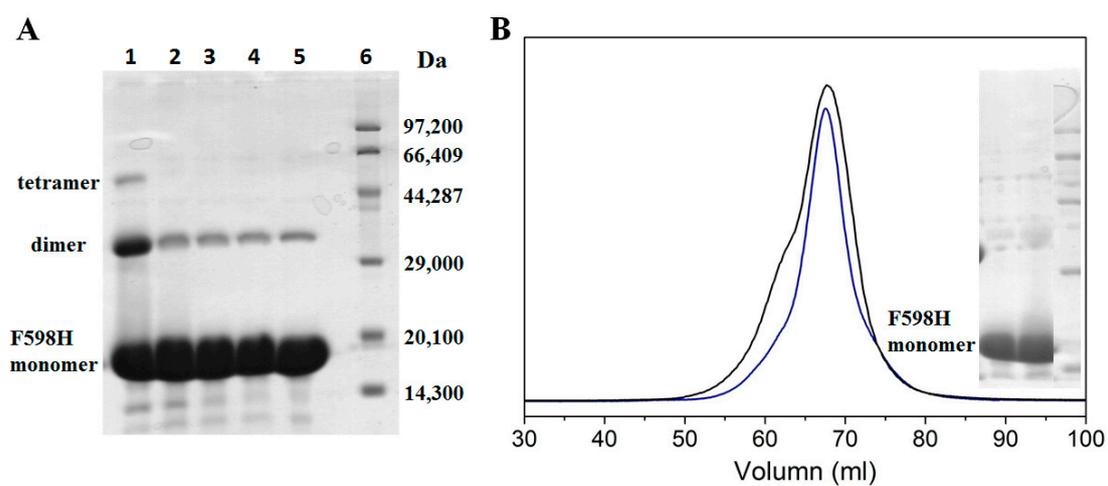


Figure S9. A. Non-reduced SDS-PAGE results of F598H purified under aerobic condition without reductant (lane 1), lane 2-5 show the results after TCEP added as a reductant with concentrations of 2, 10, 20, 50 mM. B. Gel filtration chromatography Superdex™ 75 (HiLoad 10/160) of F598H purified under aerobic condition without reductant (black) and with reductant (blue). The insert show the non-reduced SDS-PAGE results of F598H purified with reductant.

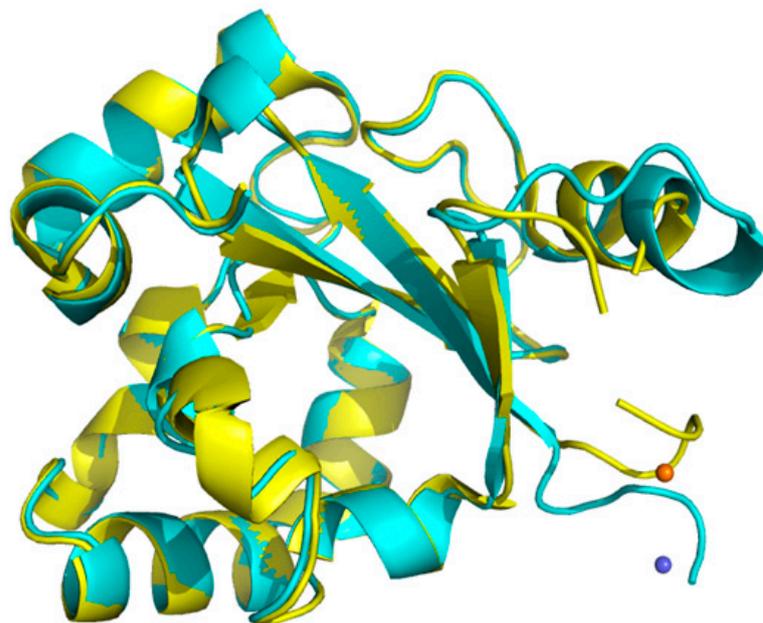


Figure S10. Superposition of F598H (cyan) and α_{15} (yellow).

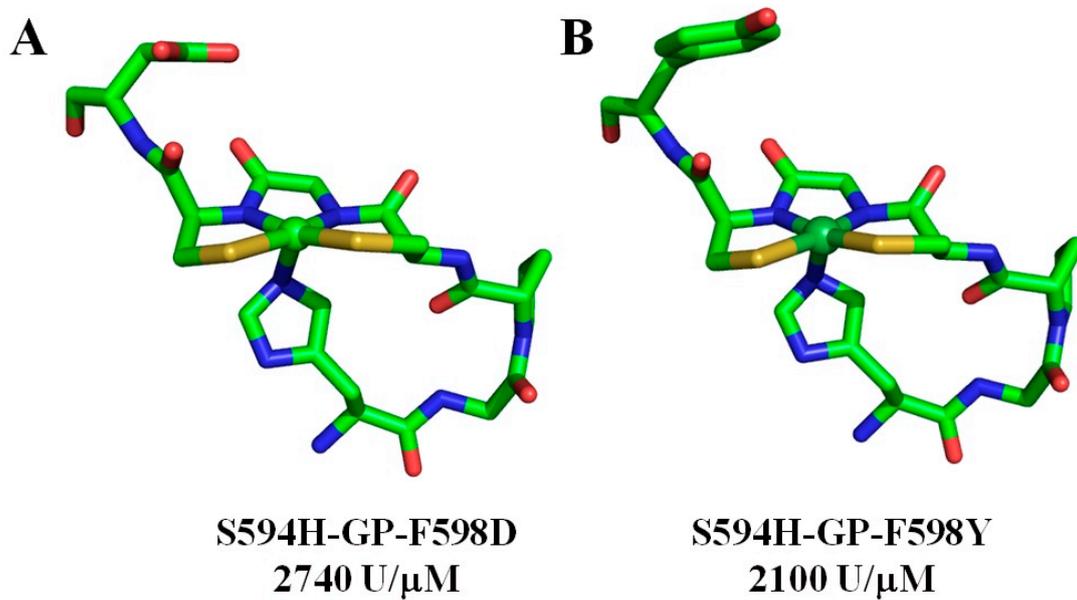


Figure S11. Structures of the molecular model of (A) S594H-GP-F598D and (B) S594H-GP-F598Y.

Table S1. PCR Primers.

PCR Primers	
P1	F: 5'-CGCCATATGATGGCTAGCATGTCCGACTCAG-3' R: 5'-GCCTCATGGCAACCGCAGCTACCACCAATCTGTTCTCTGT-3'
P2	F: 5'-ACAGAGAACAGATTGGTGGTAGCTGCCGTTGCCATGAGGC-3' R: 5'-CCGCTCGAGTCACATAATGGGATCCATGG-3'
P3	F:5'-GAATTCGGATGCCGTTGCCATGAGGCCATTATGG-3' R:5'-ACTTCCACCACCAATCTGTTCTCTG-3'
P4	F:5'-TACGGACCTTGCGTTGCCATGAGGCCATTATGG-3' R:5'-ACCACCAATCTGTTCTCTGTGAGCC-3'
P5	F: 5'-TTCGAGGCCATTATGGCCATCCTGC-3' R: 5'-GCAACCGCAGGAACCACCAATCTGT-3'
P6	F: 5'-CACTGCGGTTGCTTCGAGGCCATTA-3' R: 5'-ACCACCAATCTGTTCTCTGTGAGCC-3'
P7	F: 5'-CACGGACCATGCCGTTGCTTCGAGG-3' R: 5'-ACCACCAATCTGTTCTCTGTGAGCC-3'

Table S2. Data Collection and Refinement Statistics.

Protein name	F598H mutant PDB: 5GOL
Wavelength	1.486 Å Ni peak
Resolution range	31.24 - 2.11 (2.185 - 2.11)
Space group	C1 2 1
Unit cell(a, b, c;α,β, γ)	86.839, 58.569, 120.729; 90, 102.83, 90
Number of molecules per asymmetric unit Z	4
Unique reflections	36472
Multiplicity	8.4 (7.9)
Completeness (%)	98.59 (98.74)
Mean I/sigma(I)	20.1 (4.5)
Wilson B-factor	31.43
R-merge	0.124 (0.896)
R-pim	0.045 (0.327)
Reflections used in refinement	33783 (3366)
Reflections used for R-free	1981 (198)
R-work	0.2029 (0.2514)
R-free	0.2370 (0.2844)
Number of non-hydrogen atoms	4396
macromolecules	4069
ligands	24
solvent	303
Protein residues	540
total number of observations	294561
RMS(bonds)	0.016
RMS(angles)	1.67
Ramachandran favored (%)	97.74
Ramachandran allowed (%)	1.50
Ramachandran outliers (%)	0.75
Rotamer outliers (%)	3.17
Clash score	5.53
Average B-factor	34.56
macromolecules	34.28
ligands	42.76
solvent	37.57

Statistics for the highest-resolution shell are shown in parentheses.