



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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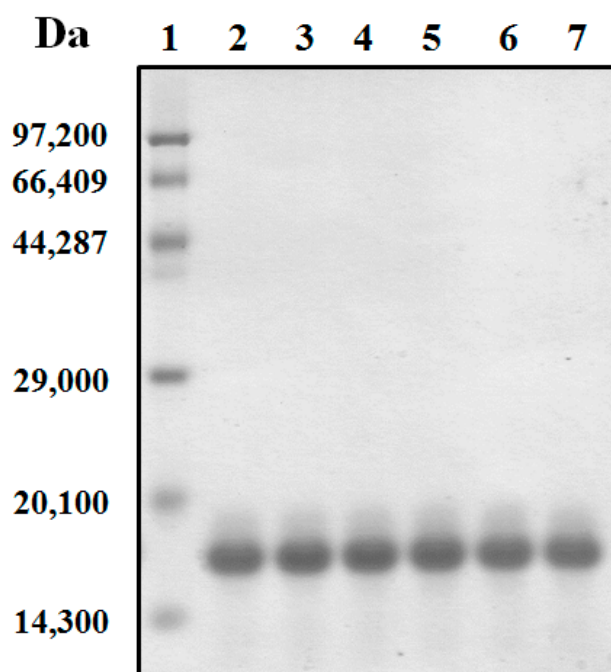


Figure S1. Sodium dodecyl sulfate polyacrylamide gel electrophoresis: Lane 1 marker, lane 2 ACS- α_{15} , lane 3 F598H, lane 4 S594H, lane 5 S594H-GP, lane 6 EFG-F598H, lane 7 YGP-F598H.

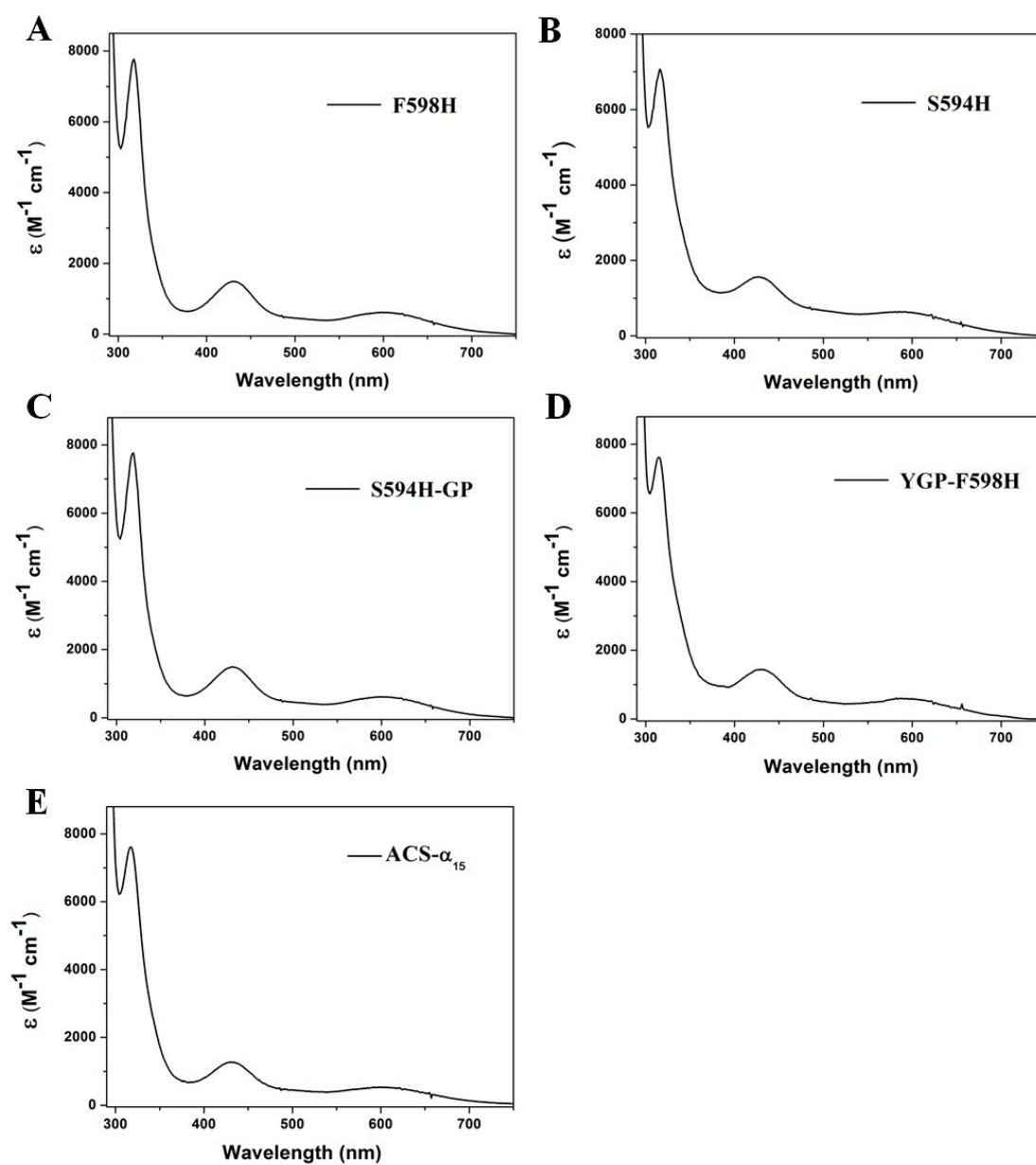


Figure S2. UV/Vis spectra of (A) F598H, (B) S594H, (C) S594H-GP, (D) YGP-F598H and (E) ACS- α_{15} .

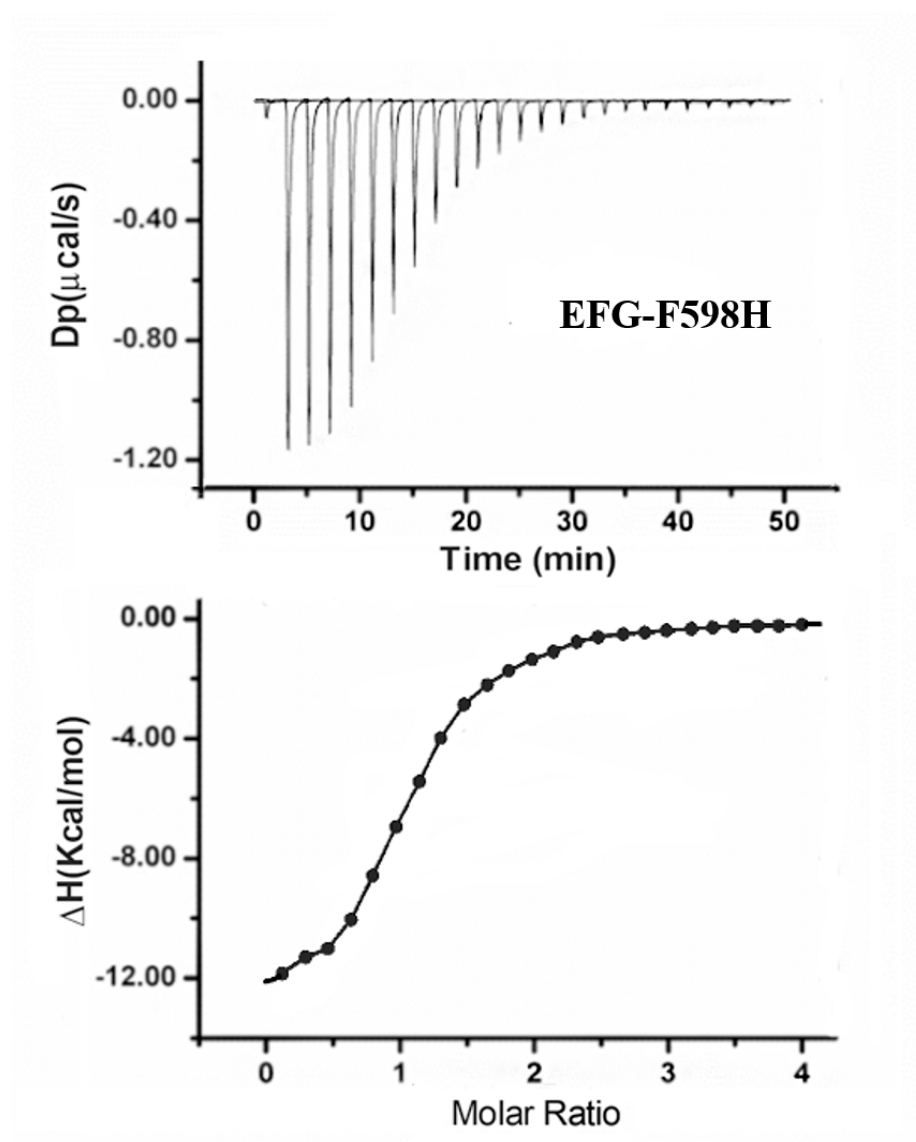


Figure S3. Calorimetric titration result for titrations of 1.0 mM NiCl_2 into a solution of 50 μM protein of apo-EFG-F598H. Curve fitted with a one-site sequential binding model. Protein sample (50 μM) was titrated against 20 mM HEPES buffer (pH 7.4) containing 1 mM NiCl_2 . Titrations were performed 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 $K_d = 6.4 \pm 0.5 \mu\text{M}$.

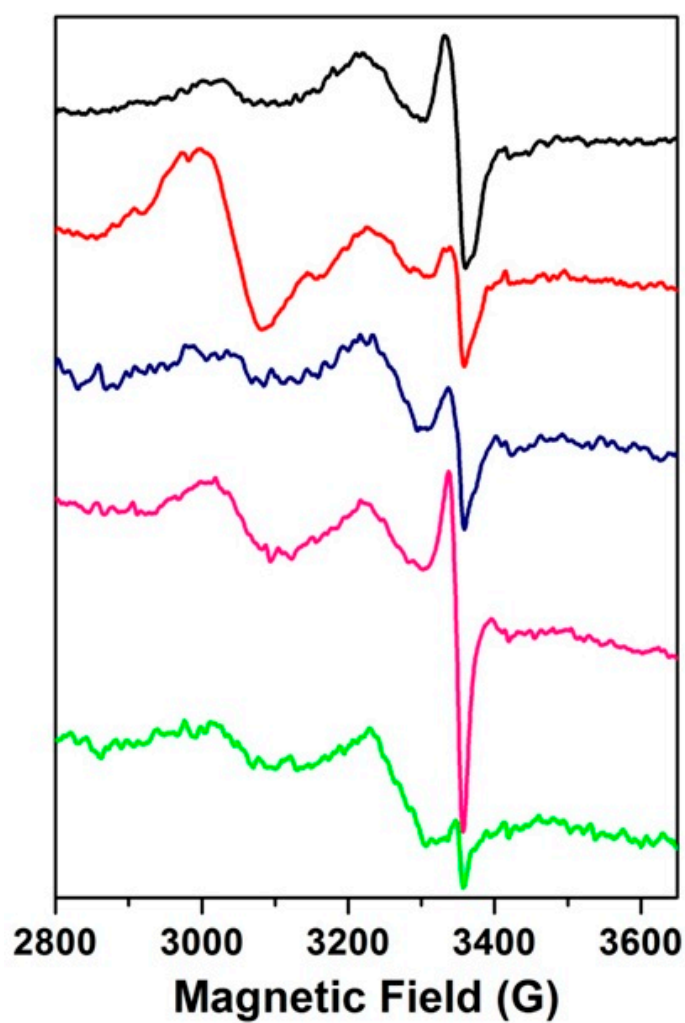


Figure S4. X-band EPR spectra of F598H (black), S594H (red), S594H-GP (blue), EFG-F598H (pink) and YGP-F598H (green) at 2 K in the presence of ~ 1 equiv. of KO_2 (microwave frequency, 9.49 GHz; microwave power, 20mW; modulation amplitude, 5 G).

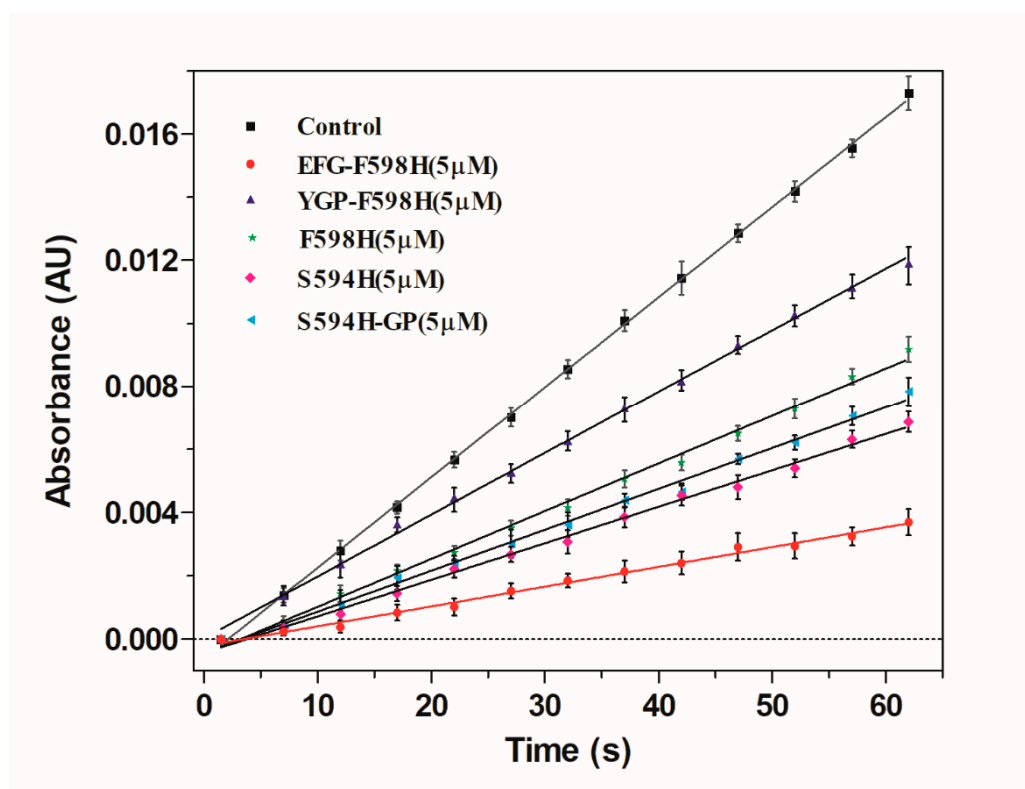


Figure S5. Measurement for SOD activity are using xanthine oxidase/NBT system. Black square: control, red circle: EFG-F598H, blue triangle: YGP-F598H, green star: F598H, magenta square: S594H, cyan triangle: S594H-GP. The results were presented as means \pm SEM.

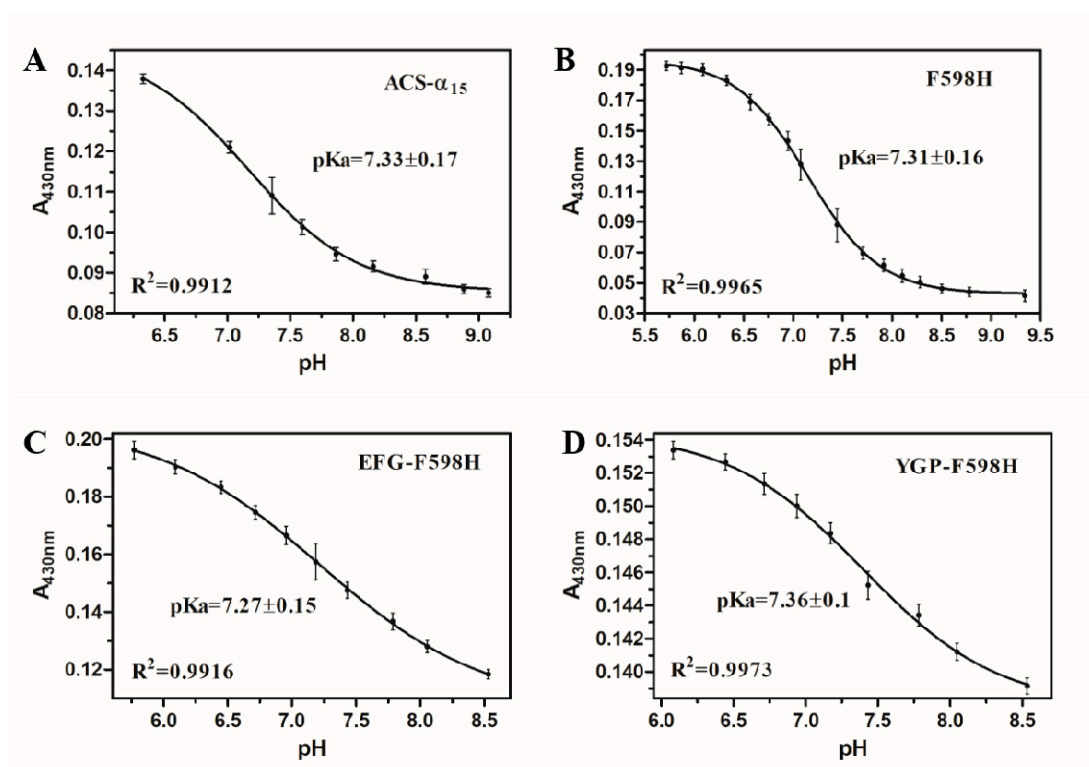


Figure S6. Optical pH titration based on the change in absorbance at 430 nm of ACS- α_{15} , F598H, EFG-F598H, and YGP-F598H. The results were presented as means \pm SEM.

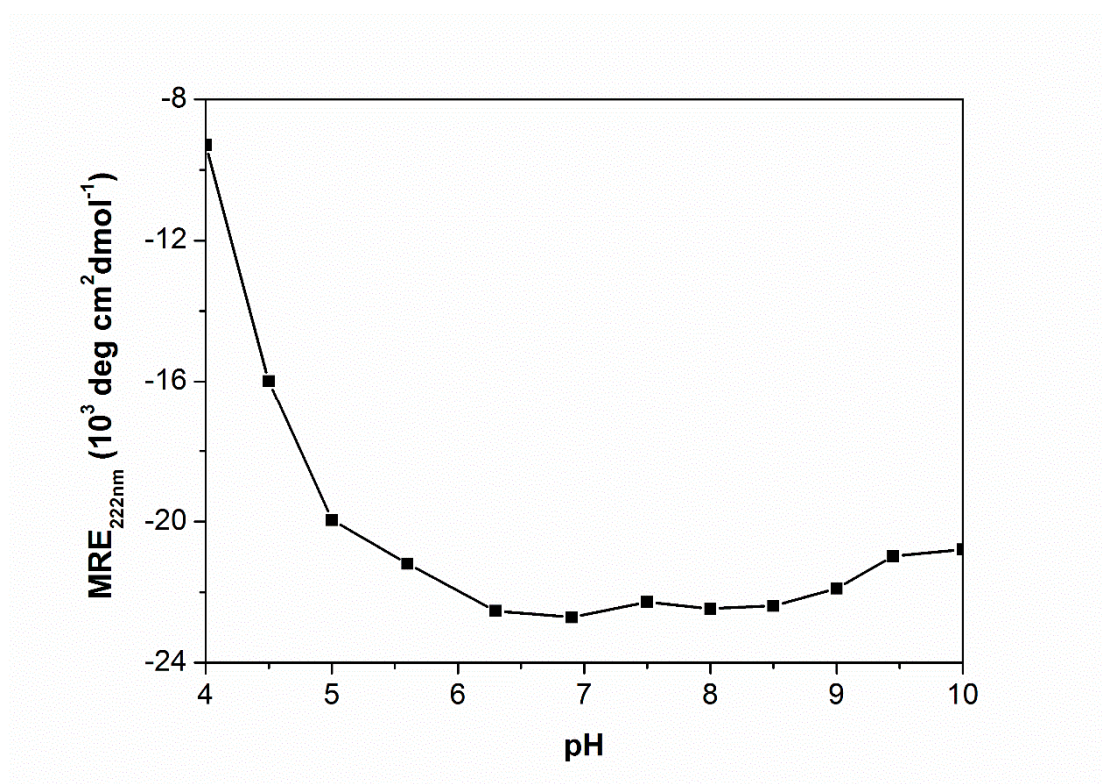


Figure S7. pH dependent changes of EFG-F598H in mean residual ellipticity (MRE) at 222 nm.

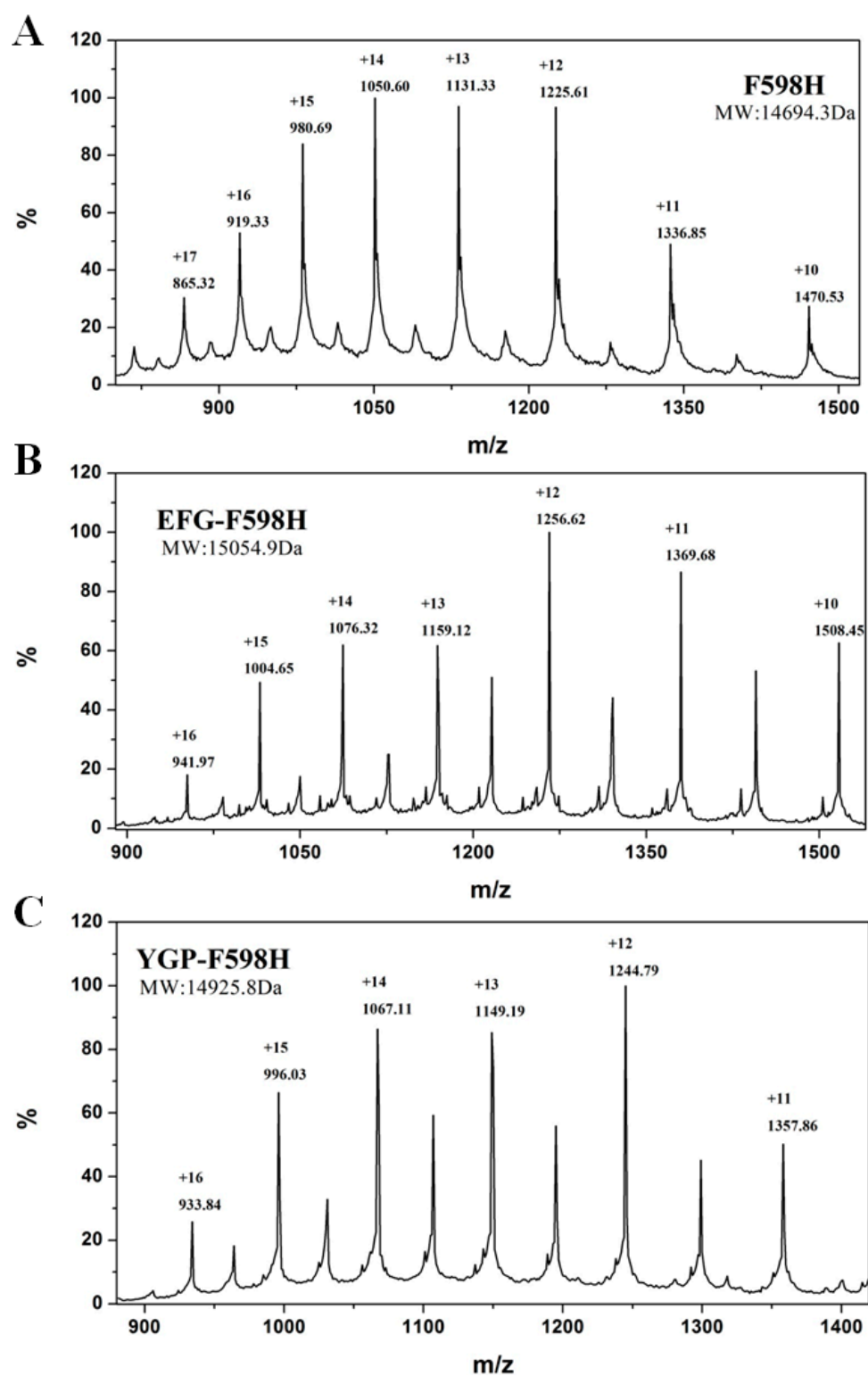


Figure S8. ESI-MS (positive ion mode) of solutions of F598H (A), EFG-F598H (B) and YGP-F598H (C) purified under aerobic conditions.

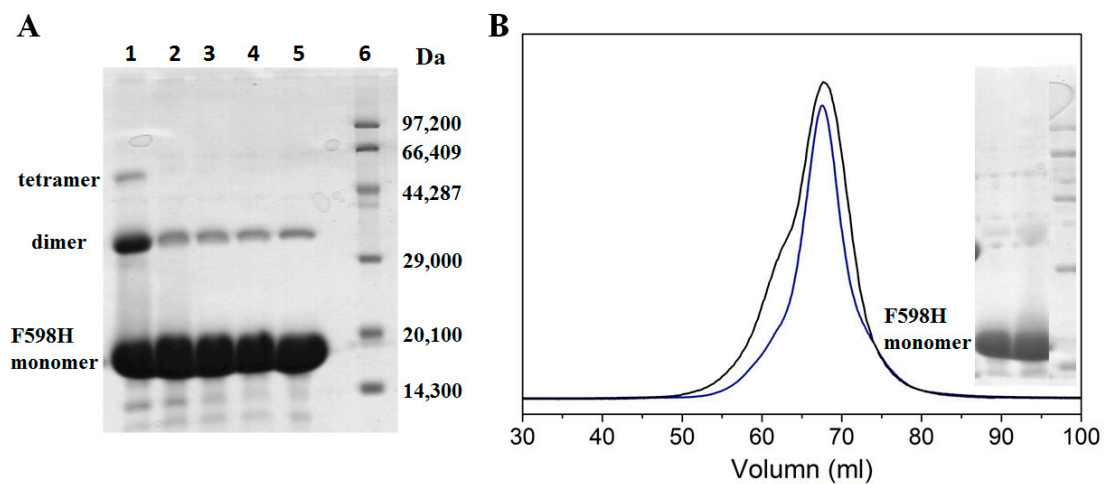


Figure S9. A. Non-reduced SDS-PAGE results of F598H purified under aerobic condition without reductant (lane 1), lane 2-5 show the results after TCEP added as a reductant with concentrations of 2, 10, 20, 50 mM. B. Gel filtration chromatography Superdex™ 75 (HiLoad 10/160) of F598H purified under aerobic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

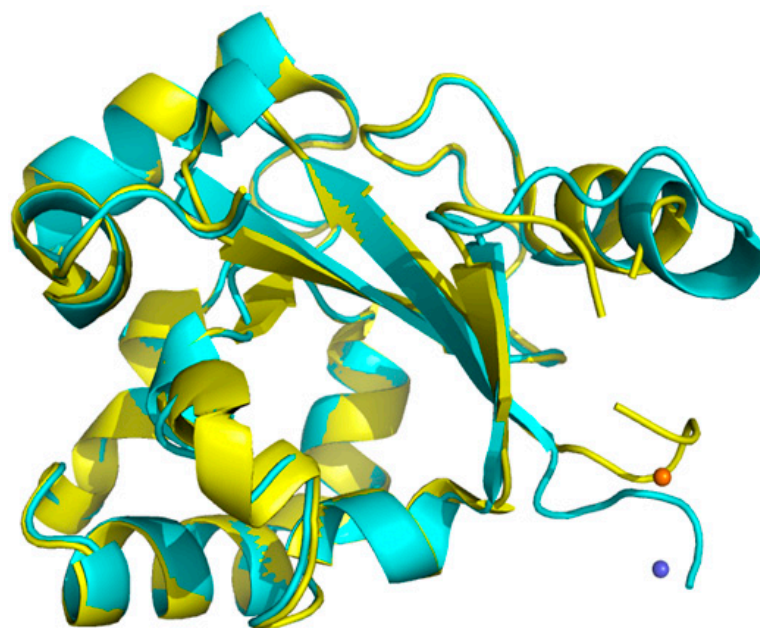


Figure S10. Superposition of F598H (cyan) and α_{15} (yellow).

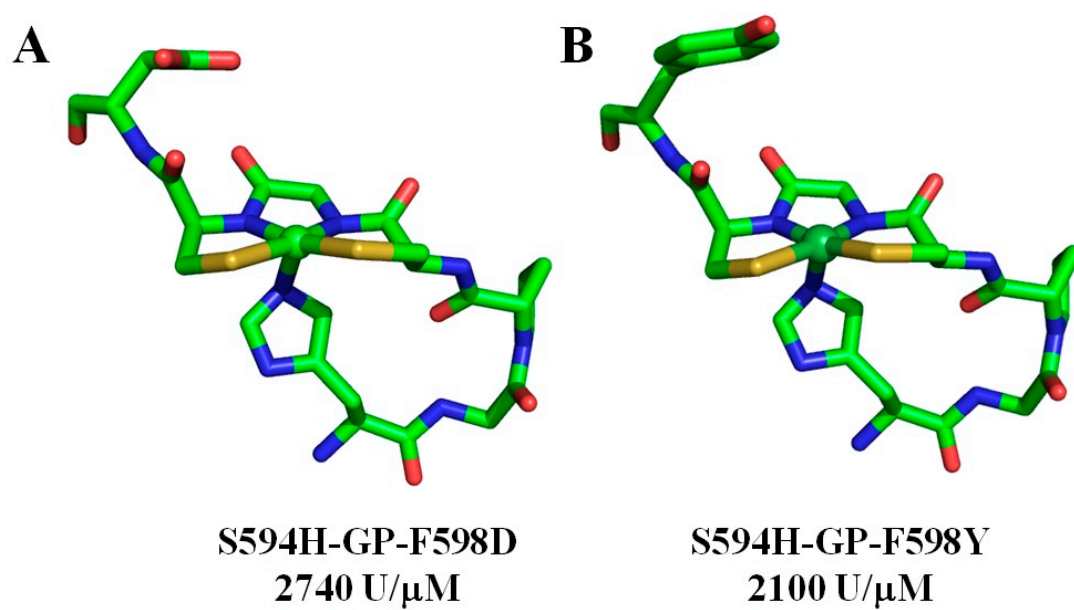


Figure S11. Structures of the molecular model of (A) S594H-GP-F598D and (B) S594H-GP-F598Y.

Table S1. PCR Primers.

| PCR Primers | |
|-------------|---|
| P1 | F: 5'-CGCCATATGATGGCTAGCATGTCGGACTCAG-3' R: 5'-GCCTCATGGCAACCGCAGCTACCACCAATCTGTTCTCTGT-3' |
| P2 | F: 5'-ACAGAGAACAGATTGGTGGTAGCTGCGGTTGCCATGAGGC-3' R: 5'-CCGCTCGAGTCACATAATGGGATCCATGG-3' |
| P3 | F:5'-GAATTCGGATGCGGTTGCCATGAGGCCATTATGG-3' R:5'-ACTTCCACCACCAATCTGTTCTCTG-3' |
| P4 | F:5'-TACGGACCTTGCGGTTGCCATGAGGCCATTATGG-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'-CACGGACCATGCGGTTGCTTCGAGG-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.