Application of Engineered Myoglobins for Biosynthesis of Clofazimine by Integration with Chemical Synthesis

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Supporting Information

Contents

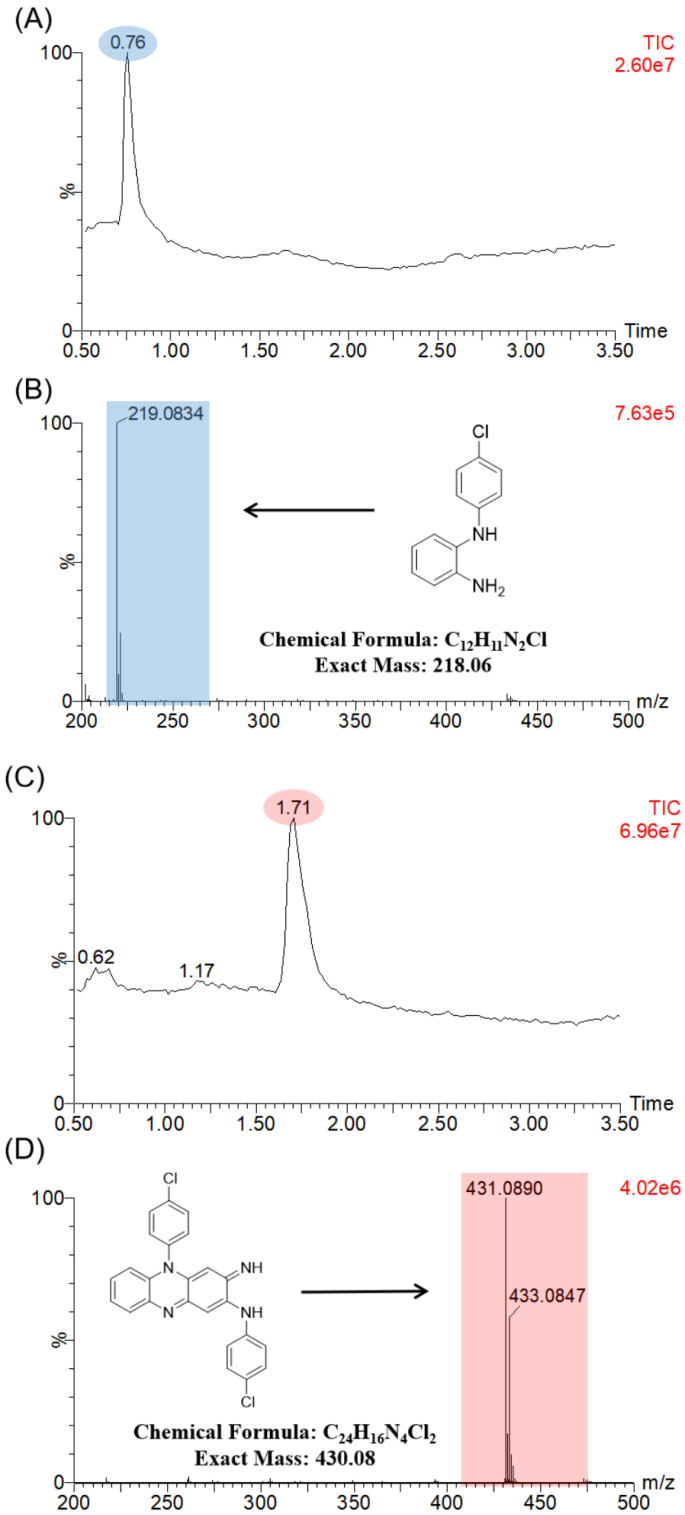

Figure S1. UPLC-ESI-MS traces of (A), (B) substrate (0.25 mM, calculated, 218.06 Da; observed, 219.08 Da, $[M+H]^+$), and (C), (D) the oxidative coupling product catalyzed by F43Y/T67R Mb in the presence of 2 mM H_2O_2 at 25 ℃ (calculated, 430.08 Da; observed, 431.09 Da, $[M+H]^+$).

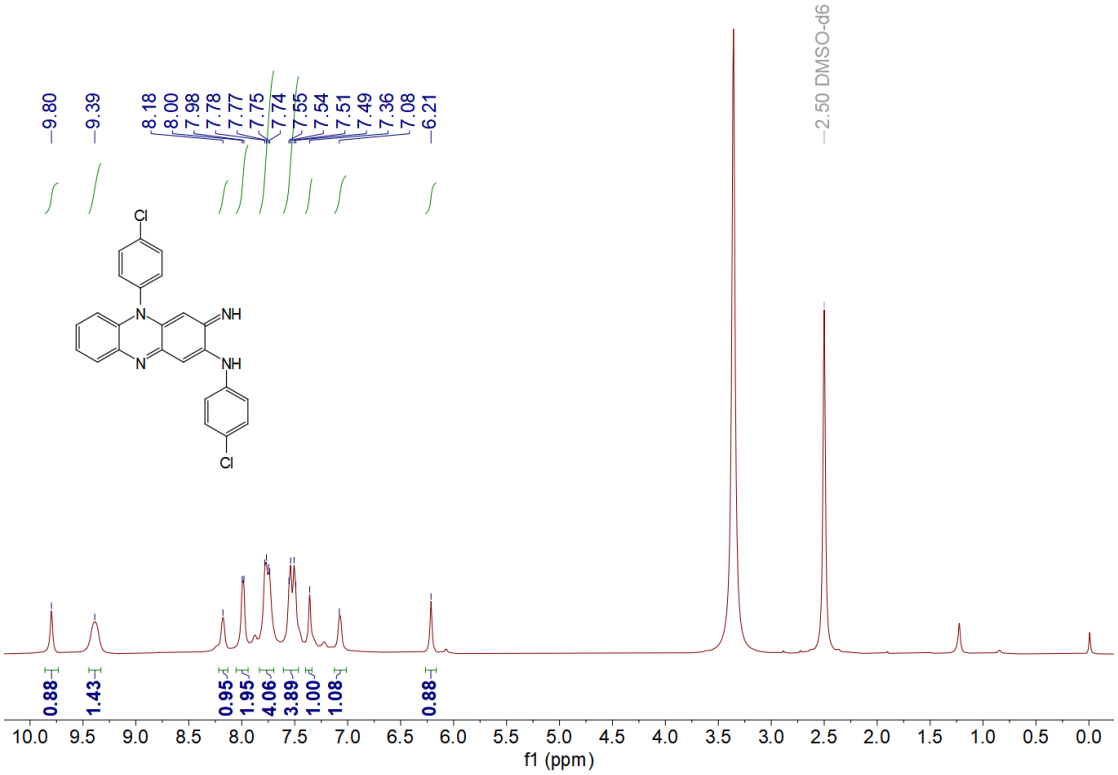

Figure S2. ^{1}H-NMR (500 Hz) spectrum of N-5-CCPIPA in DMSO-d6 at room temperature. δ(ppm) = 9.80 (s, 1H), 9.39 (s, 1H), 8.18 (s, 1H), 7.99 (d, J = 7.7 Hz, 2H), 7.83 – 7.70 (m, 4H), 7.61 – 7.47 (m, 4H), 7.36 (s, 1H), 7.07 (d, J = 7.3 Hz, 1H), 6.21 (s, 1H).

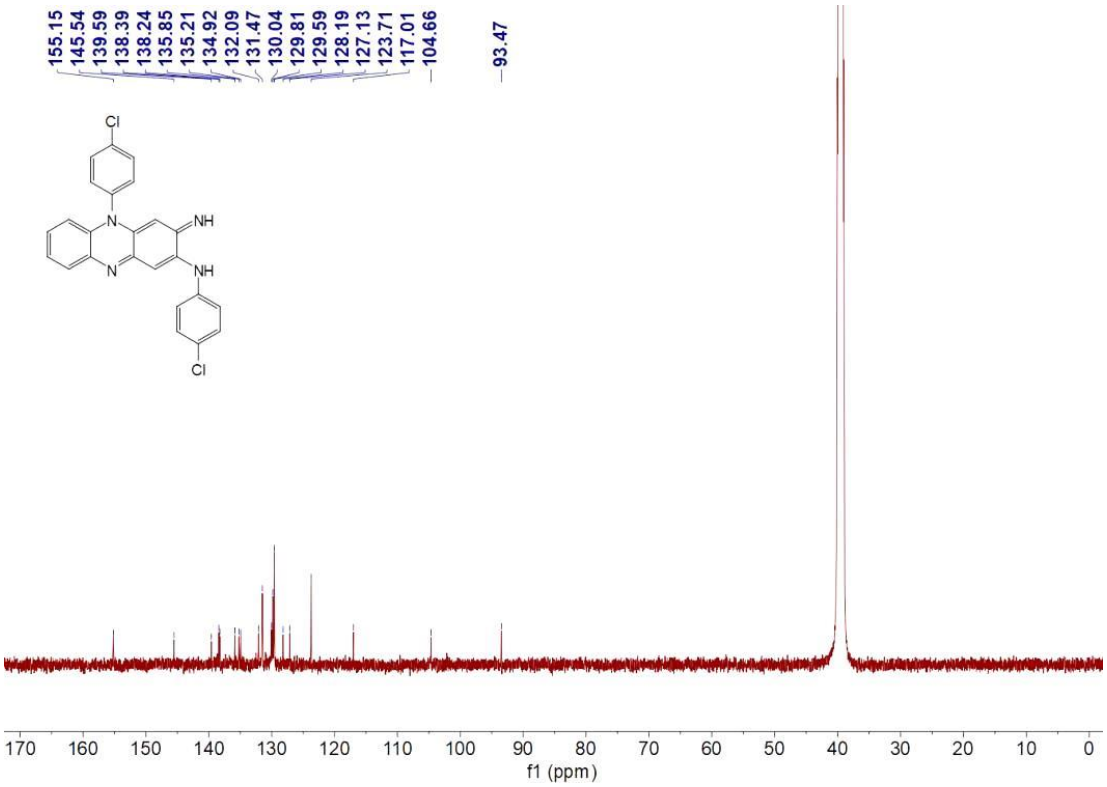

Figure S3. ^{13}C-NMR (126 Hz) spectrum of N-5-CCPIPA in DMSO-d6 at room temperature. δ(ppm) = 155.16, 145.54, 139.59, 138.39, 138.24, 135.85, 135.21, 134.92, 132.09, 131.47, 130.04, 129.81, 129.59, 128.19, 127.13, 123.72, 117.01, 104.66, 93.47.

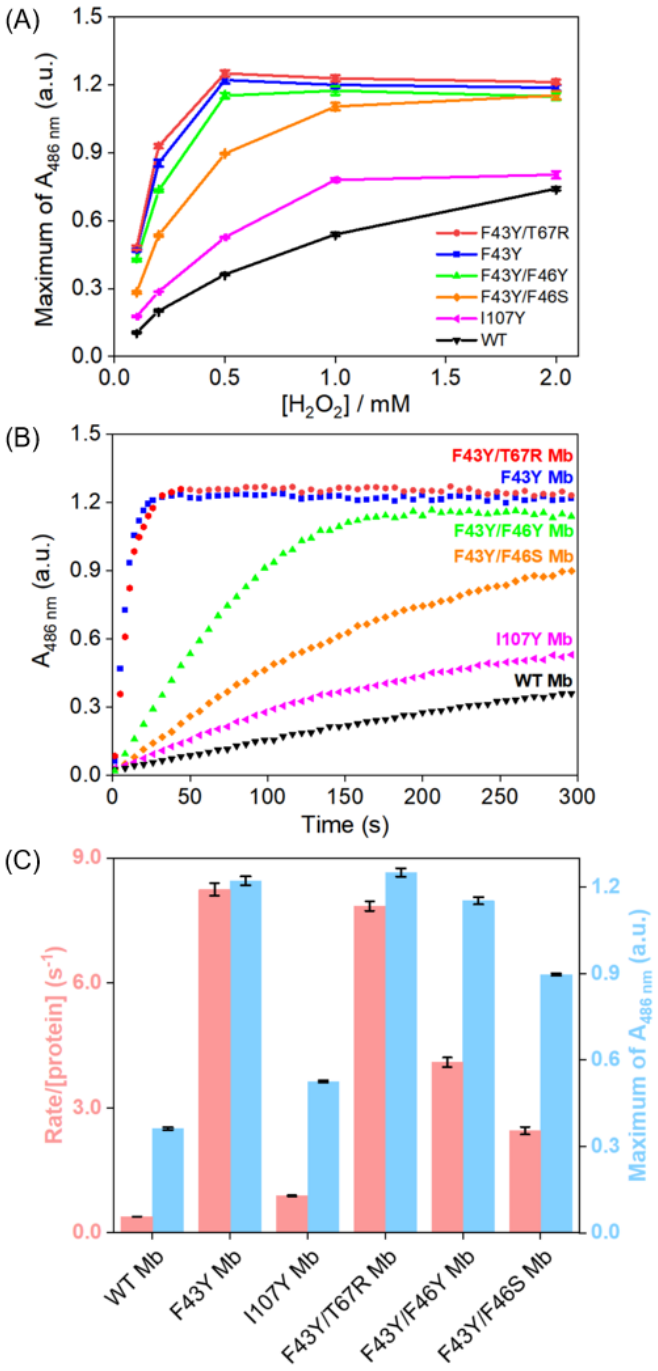

Figure S4. (A) Plots of the maximum absorbance of the oxidative coupling product of N-4-CPBDA catalyzed by WT Mb and its mutants at different concentrations of H_2O_2 within 5 min (pH 6.0, 25 ℃). (B) Representative time-dependent absorbance changes at 486 nm, and (C) Comparison of the initial rates and the maximum absorbance of the reactions catalyzed by WT Mb and its mutants with 0.5 mM H_2O_2. All assays were carried out in triplicate.

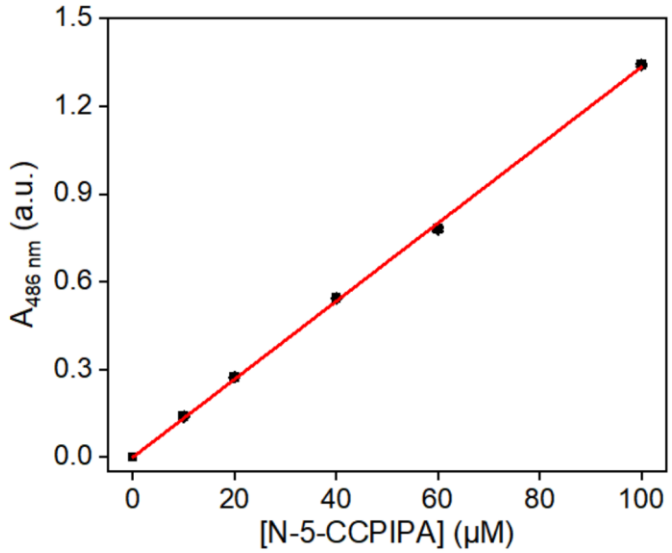

Figure S5. Standard curve of N-5-CCPIPA monitored at 486 nm using Agilent 8453 diode array spectrometer. The extinction coefficient was measured to be $\varepsilon_{486\ nm}$ = 13 mM^{-1} cm^{-1} (100 mM potassium phosphate buffer containing 5% methanol (v/v), pH 6.0). All assays were carried out in triplicate.

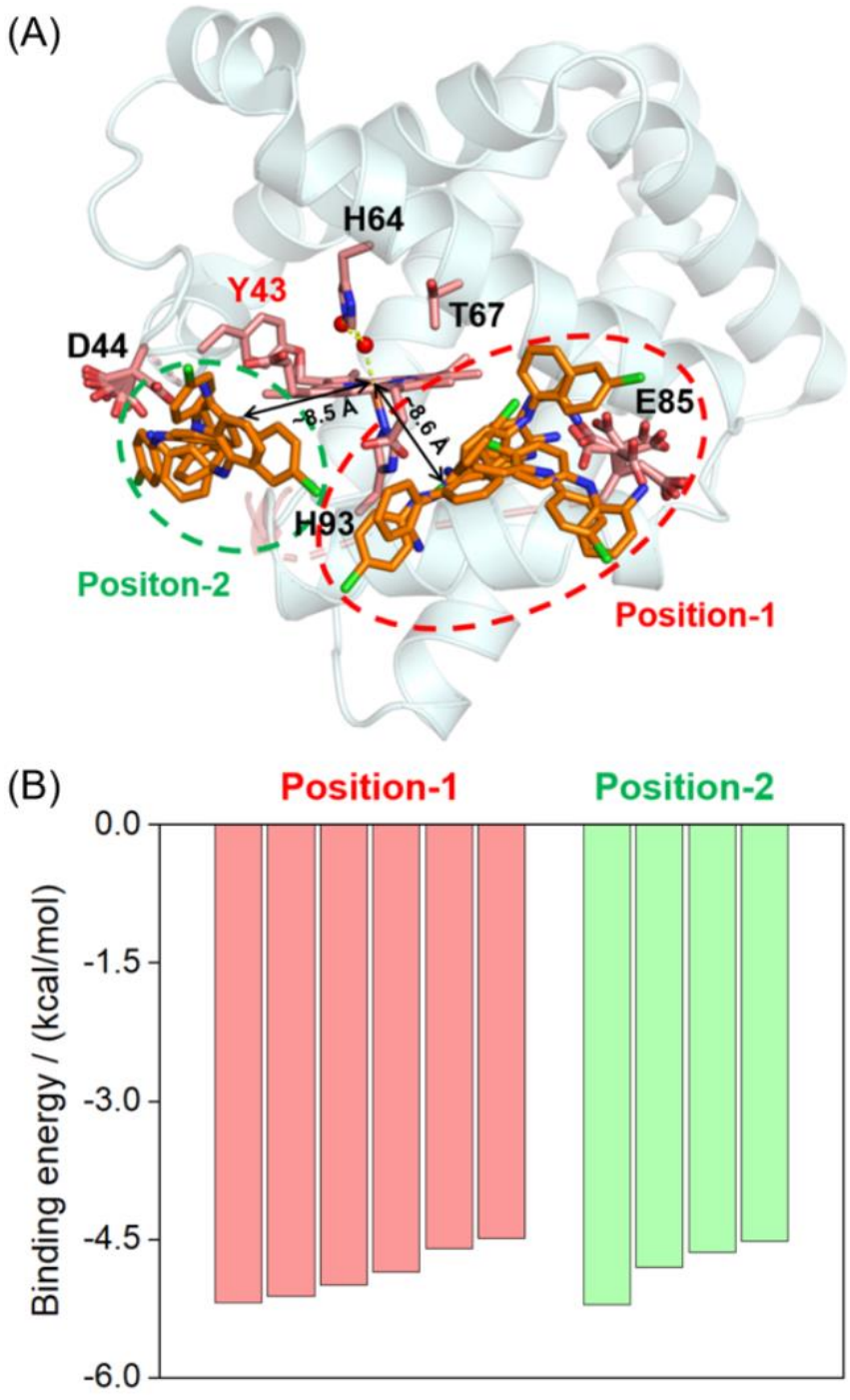

Figure S6. (A) Docking structures of N-4-CPBDA binding to F43Y Mb with the lowest 10 binding energies. Dotted arrows indicate the closest distance between the heme iron and the N-atom of the secondary amine of N-4-CPBDA in both positions. The N-4-CPBDA binding positions are indicated by circle lines. (B) Comparison of the binding energies between those bound to the position-1 and position-2.

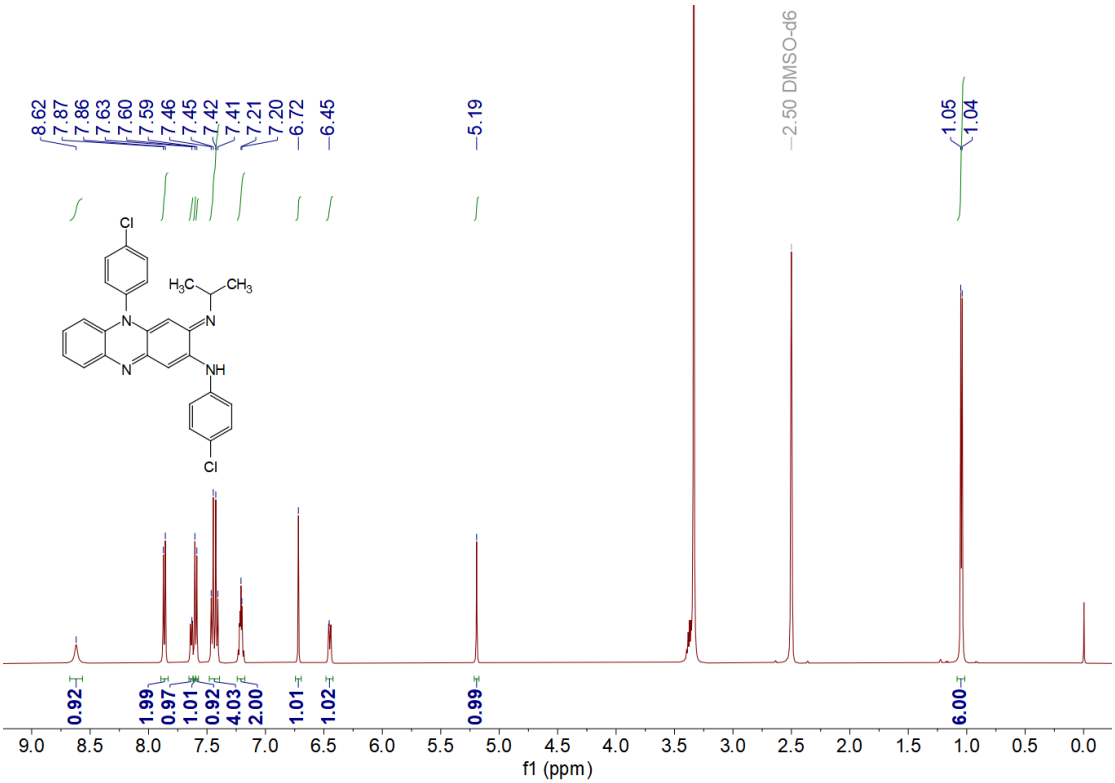

Figure S7. ^{1}H-NMR (500 Hz) spectrum of CFZ in DMSO-d6 at room temperature. δ(ppm) = 8.62 (s, 1H), 7.86 (d, J = 8.6 Hz, 2H), 7.63 (s, 1H), 7.60 (s, 1H), 7.59 (s, 1H), 7.48 – 7.39 (m, 4H), 7.21 (d, J = 3.6 Hz, 2H), 6.72 (s, 1H), 6.45 (s, 1H), 5.19 (s, 1H), 1.04 (d, J = 6.2 Hz, 6H).

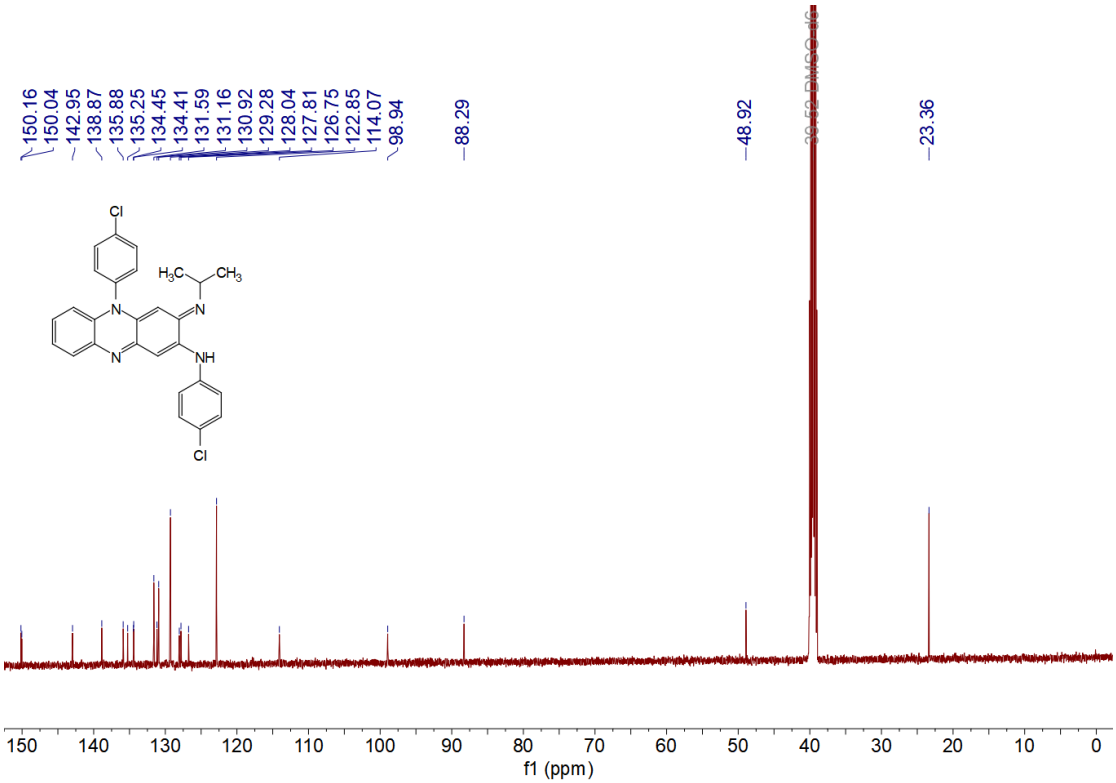

Figure S8. ^{13}C-NMR (126 MHz) spectrum of CFZ in DMSO-d6 at room temperature. δ(ppm) = 150.16, 150.04, 142.95, 138.87, 135.88, 135.25, 134.45, 134.41, 131.59, 131.16, 130.92, 129.28, 128.04, 127.81, 126.75, 122.85, 114.07, 98.94, 88.29, 48.92, 23.36.

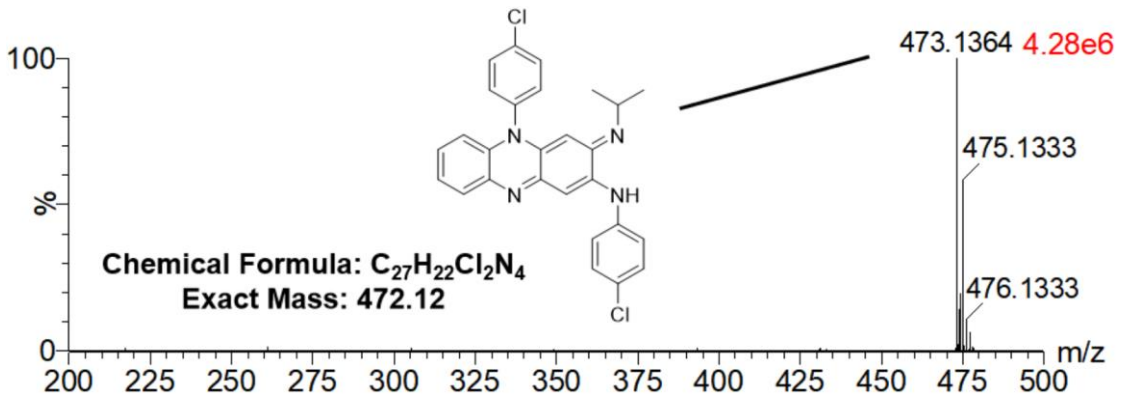

Figure S9. ESI-MS spectrum of the synthesized CFZ (calculated, 472.12 Da; observed, 473.14 Da, $[M+H]^+$).

Table S1. Docking results of N-4-CPBDA binding to F43Y/T67R Mb.

Model	$E_{binding}$ [a]	$E_{inter-mol}$ [b]	E_{vdw} [c]	E_{elec} [d]	E_{total} [e]	$E_{torsional}$ [f]
1	−5.49	−6.39	−2.11	−0.21	−11.24	0.89
2	−4.99	−5.88	−2.57	−0.14	−13.68	0.89
3	−4.88	−5.78	−3.68	−0.18	−13.12	0.89
4	−4.61	−5.5	−2.4	−0.03	−13.62	0.89
5	−4.58	−5.47	−1.82	−0.05	−14.62	0.89
6	−4.18	−5.07	−0.72	−0.02	−13.93	0.89
7	−4.15	−5.04	−1.63	−0.04	−13.85	0.89
8	−4.09	−4.98	−1.69	−0.01	−13.01	0.89
9	−4.08	−4.97	−0.39	−0.01	−13.4	0.89
10	−4.06	−4.96	−2.64	−0.06	−15.38	0.89

[a] Binding energy. [b] Intermolecular energy. [c] van der Waals energies. [d] Electrostatic interaction. [e] Total energy of the complex. [f] Torsional free energy. Unit: kcal/mol.

Table S2. Docking results of N-4-CPBDA binding to F43Y Mb.

Model	$E_{binding}$ [a]	$E_{inter\text{-}mol}$ [b]	E_{vdw} [c]	E_{elec} [d]	E_{total} [e]	$E_{torsional}$ [f]
1	−5.21	−6.1	−3.46	−0.1	−11.73	0.89
2	−5.19	−6.09	−2.36	−0.3	−12.73	0.89
3	−5.12	−6.01	−2.99	−0.25	−12.04	0.89
4	−4.99	−5.88	−2.3	−0.16	−11.65	0.89
5	−4.85	−5.74	−1.9	−0.01	−13.49	0.89
6	−4.8	−5.7	−2.12	−0.06	−12.74	0.89
7	−4.64	−5.54	−3.43	−0.13	−12.5	0.89
8	−4.6	−5.5	−1.98	−0.08	−12.43	0.89
9	−4.52	−5.41	−1.86	−0.02	−11.85	0.89
10	−4.49	−5.39	−1.81	−0.01	−12.56	0.89

[a] Binding energy. [b] Intermolecular energy. [c] van der Waals energies. [d] Electrostatic interaction. [e] Total energy of the complex. [f] Torsional free energy. Unit: kcal/mol.