Rational Design of Artificial Dye-decolorizing Peroxidases using Myoglobin by Engineering Tyr/Trp in the Heme Center

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Supporting Information

Contents

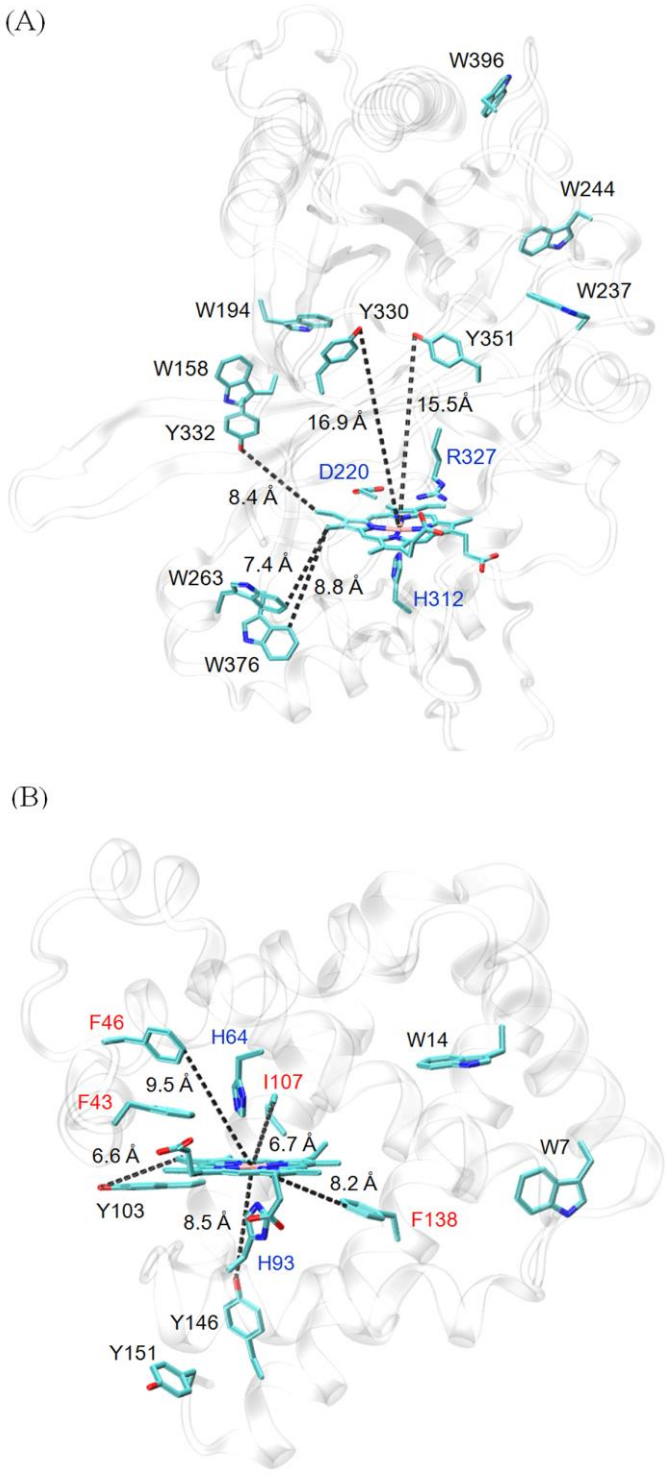

Figure S1. X-ray structure of (A) the bacterial *Tc*DyP from *thermomonospora curvata* (PDB code 5JXU), and (B) sperm whale Mb (PDB code 1JP6), showing the location of all Tyr and Trp residues in the protein scaffold, and some other key residues in the heme pocket.

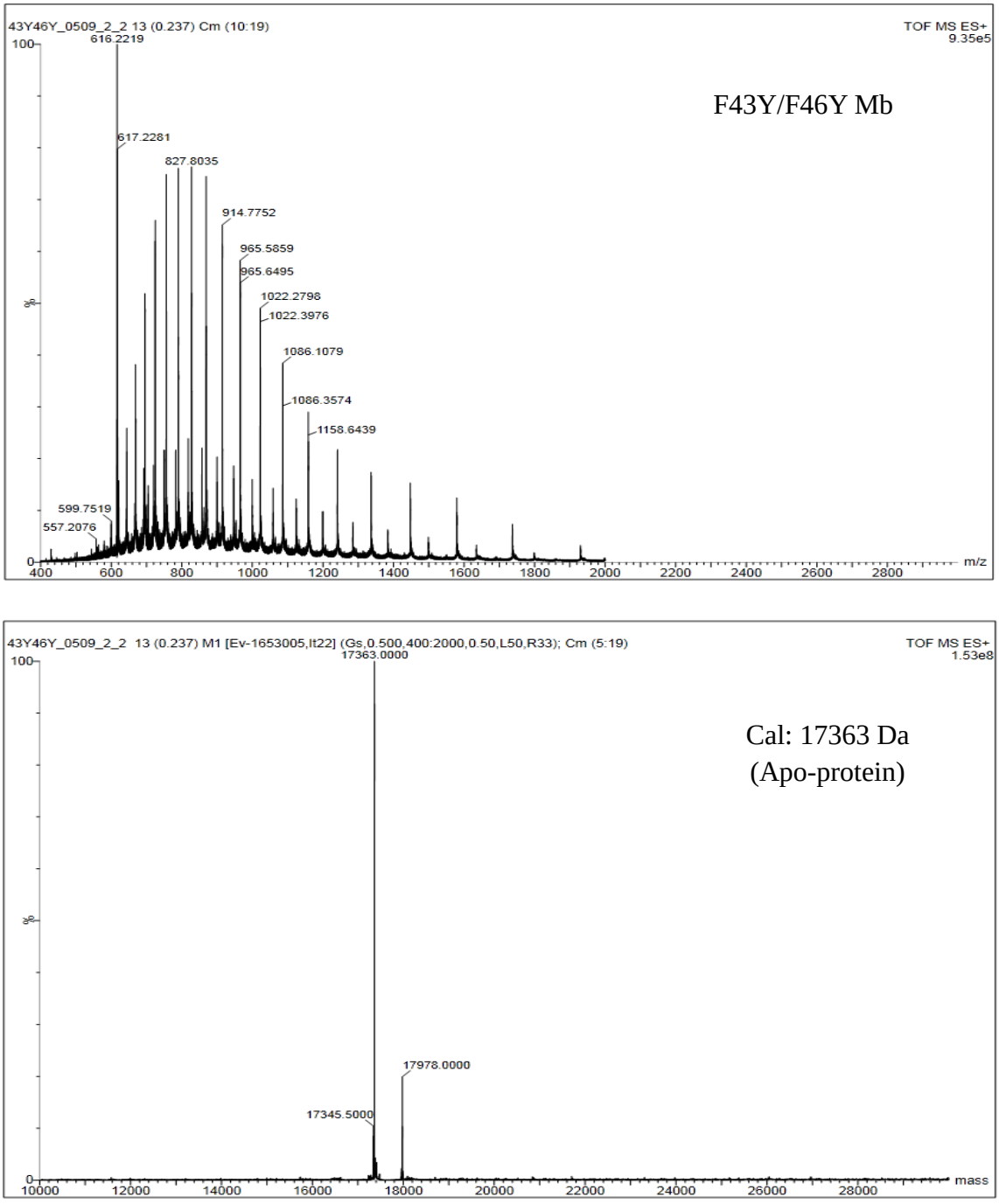

Figure S2A. MS spectra of F43Y/F46Y Mb: calculated molecular weight for the apo-protein, 17363 Da, and the observed, 17363 Da.

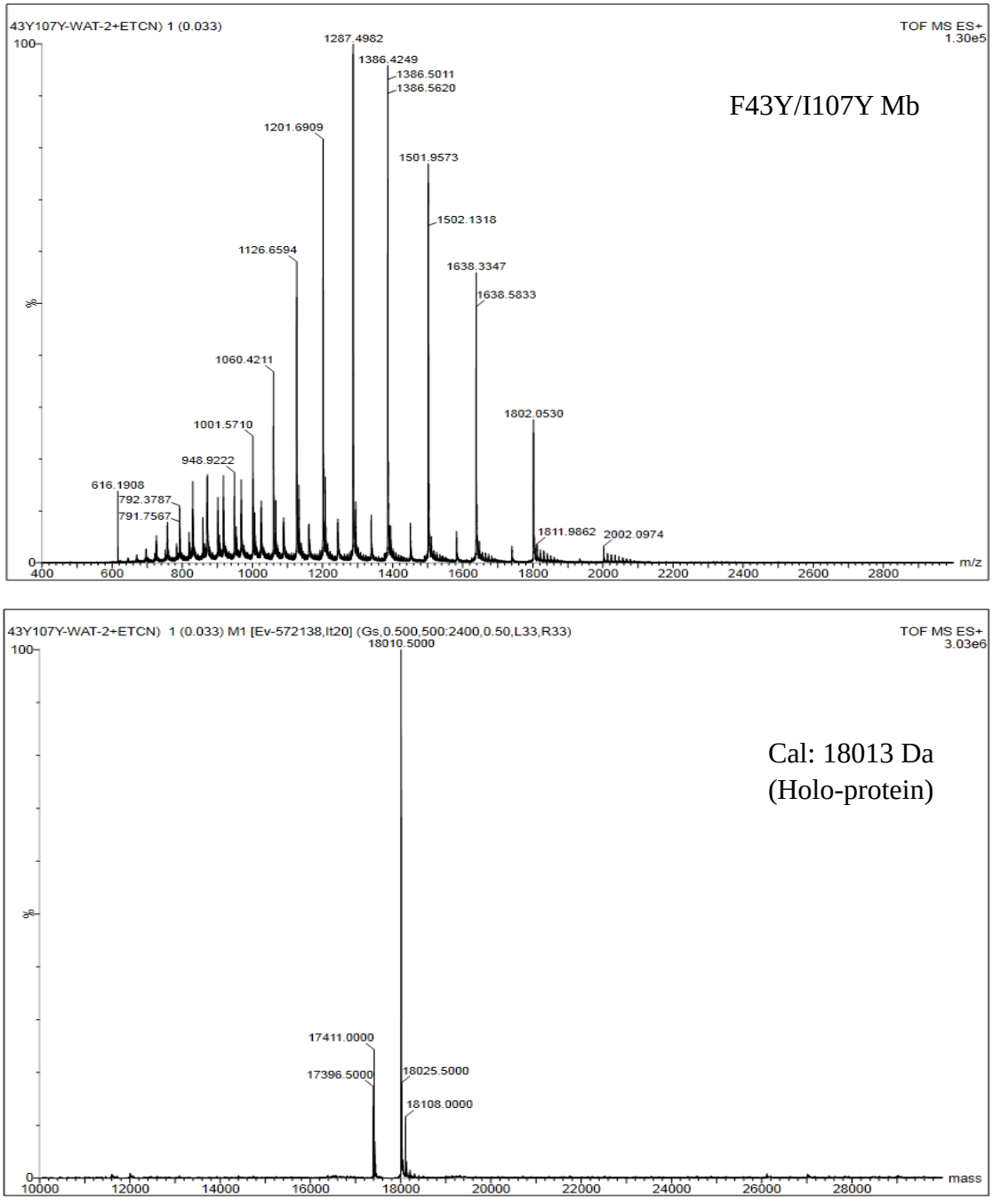

Figure S2B. MS spectra of F43Y/I107Y Mb: calculated molecular weight for the holo-protein, 18013 Da, and the observed, 18010.5 Da.

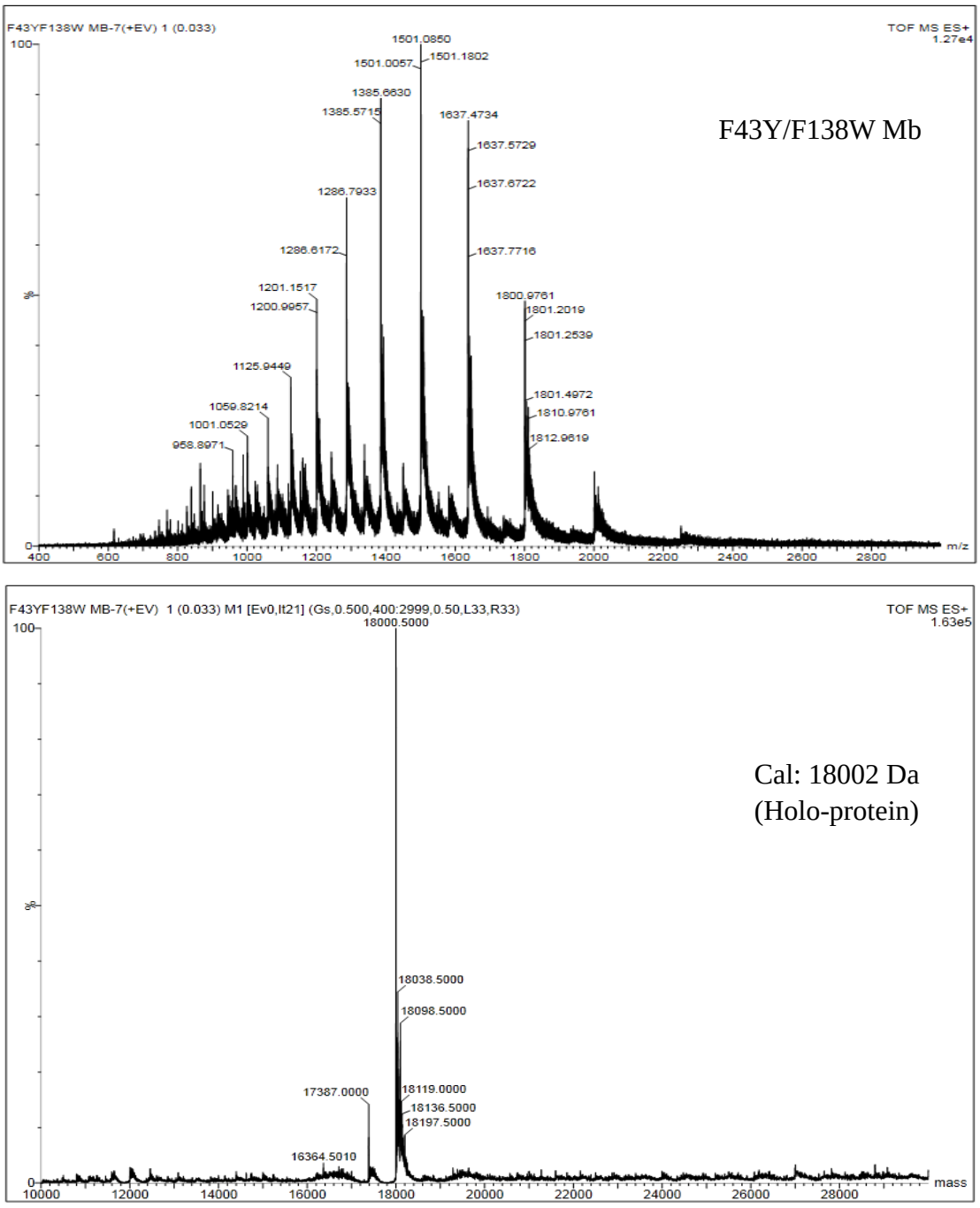

Figure S2C. MS spectra of F43Y/F138W Mb: calculated molecular weight for the holo-protein, 18002 Da, and the observed, 18000.5 Da.

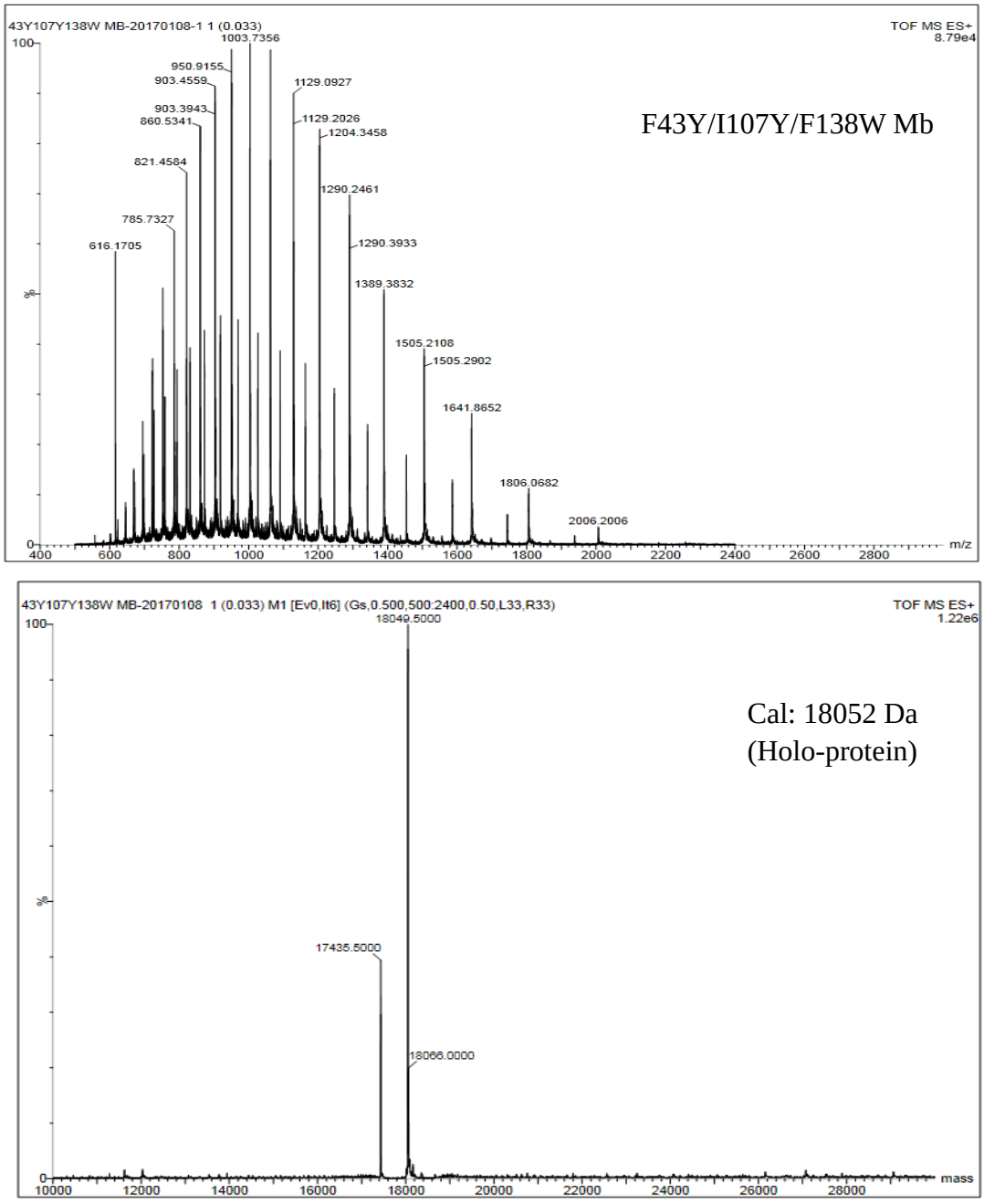

Figure S2D. MS spectra of F43Y/I107Y/F138W Mb: calculated molecular weight for the holo-protein, 18052 Da, and the observed, 18049.5 Da. An apo-form was also observed, 17435.5 Da, calculated, 17436 Da.

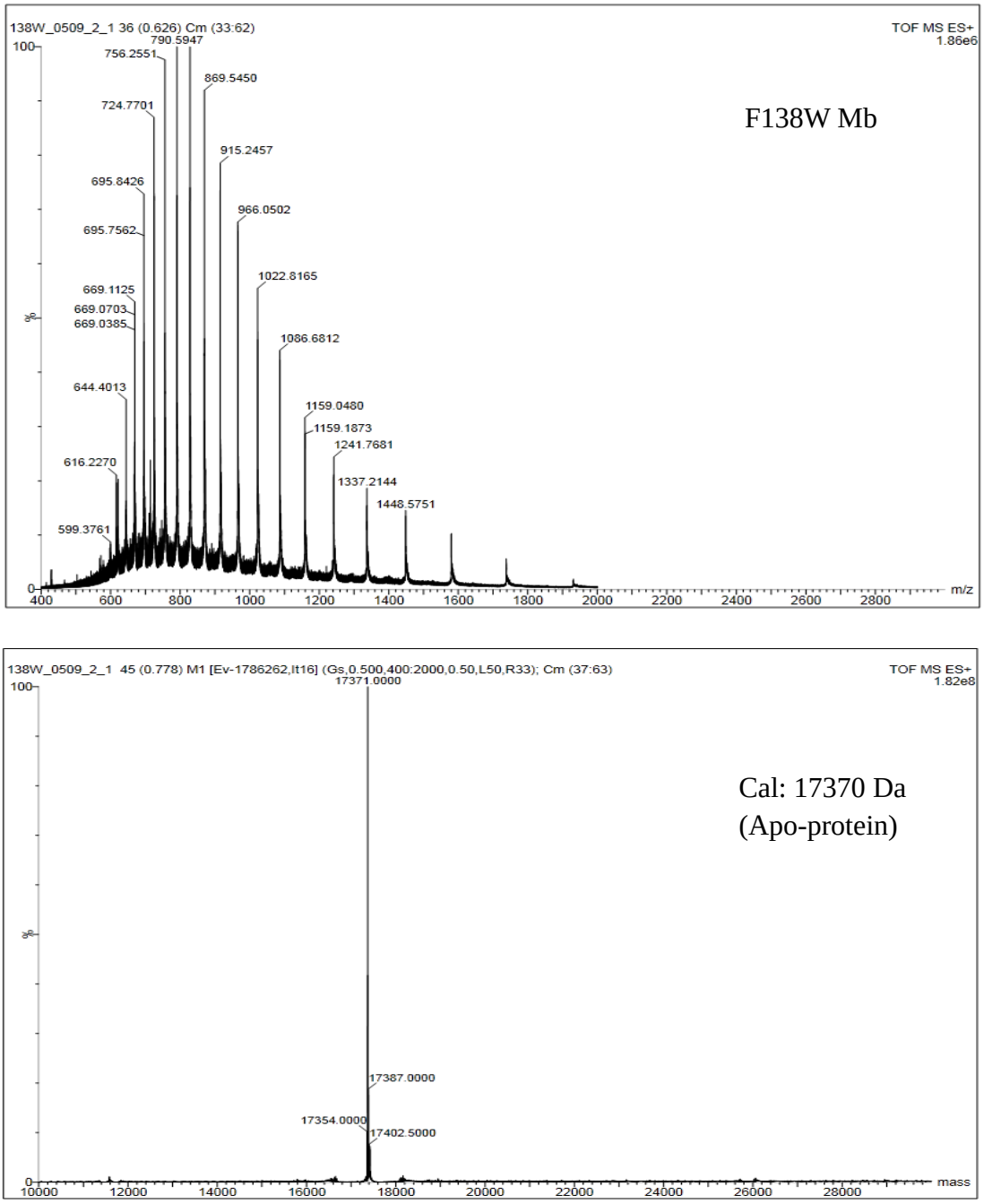

Figure S2E. MS spectra of F138W Mb: calculated molecular weight for the apo-protein, 17370 Da, and the observed, 17371 Da.

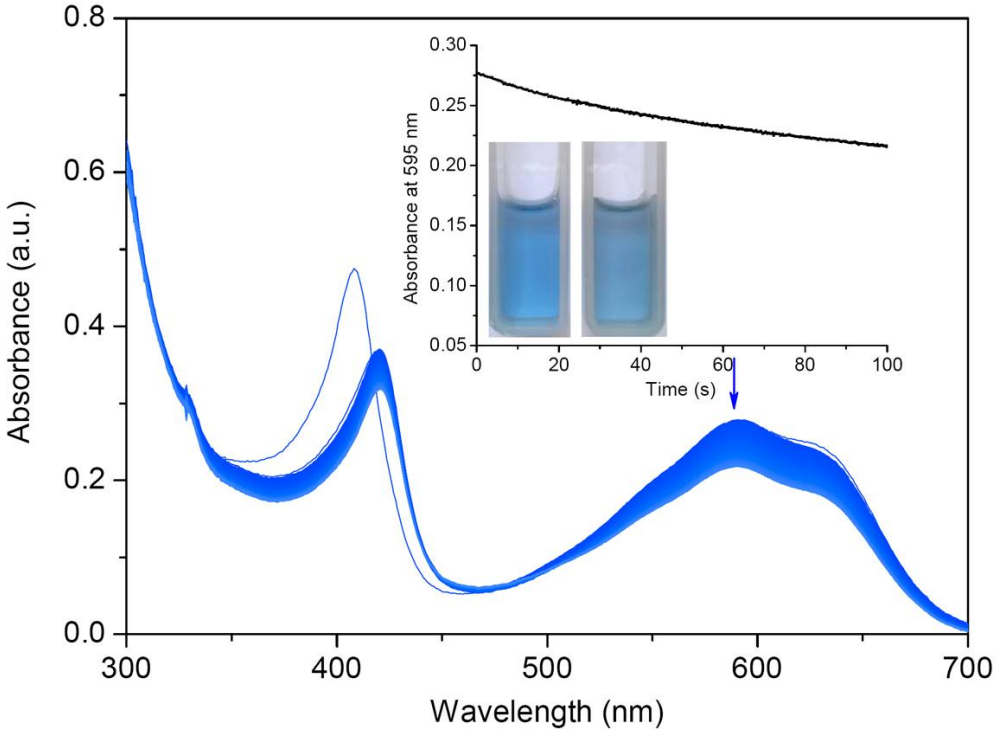

Figure S3. Stopped-flow spectra upon mixing WT Mb (7 μM) in presence of RB19 (100 μM) with H_2O_2 (50 mM) in potassium phosphate buffer (100 mM, pH 7.0) at 25 ^{o}C, for 100 s. Inset: The kinetic change of absorption at 595 nm. The inset photo show the solution before and after RB19 oxidation under the same conditions

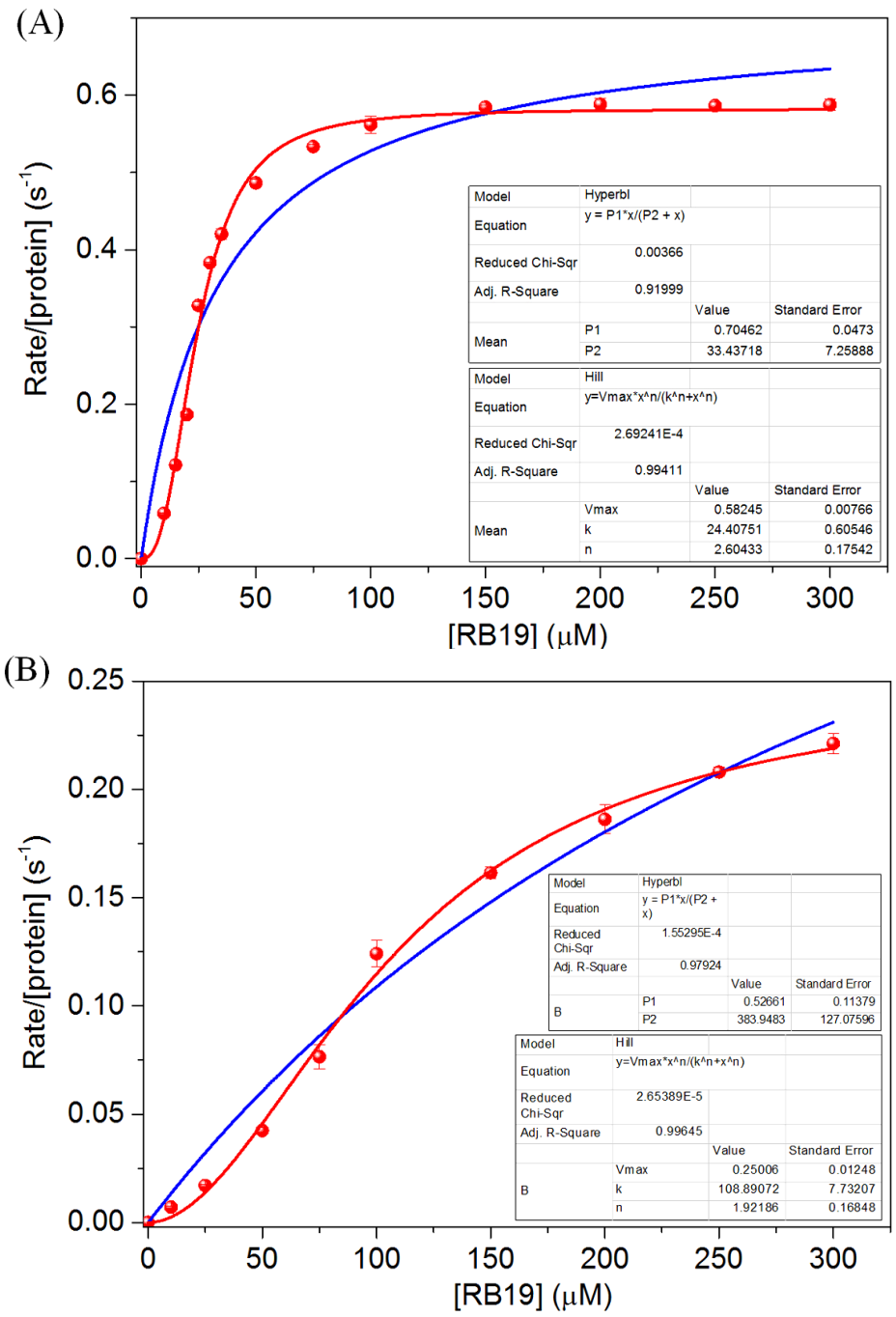

Figure S4. Steady-state rates of the peroxidation as a function of RB19 concentrations, as catalyzed by (A) F43Y/I107Y/F138W Mb and (B) F138W Mb. The data were fitted to Michaelis-Menten equation (blue) and Hill equation (red line), respectively.

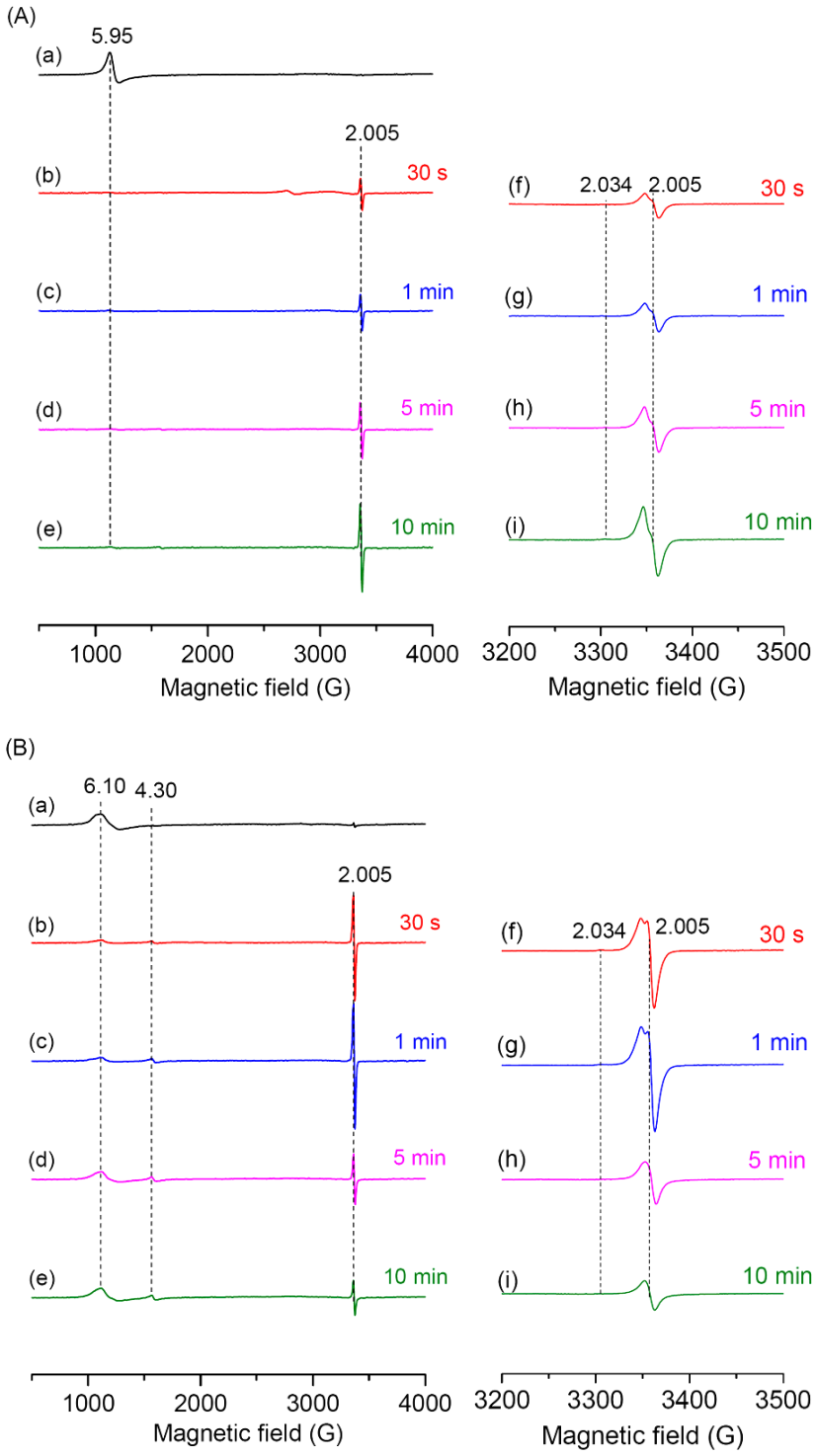

Figure S5. EPR spectra of (A) WT Mb and (B) F43Y Mb (0.5 mM) before (a) after addition of 5 eq. H_2O_2 for 30 s (b), 1 min (c), 5 min (d) and 10 min (e). Detailed EPR spectra at g~2 region at different time points are shown in (f-i). The spectra were collected at 100 K, 0.595 mW power and 9.43 GHz.

Table S1. X-ray crystallography data collection and refinement statistics.

	F43Y/F46Y Mb	**F138W Mb**
Wavelength	0.9795	0.9795
Space group	$P2_12_12_1$	$P2_12_12_1$
Unit-cell dimensions (Å, °)	a = 39.856, b = 48.495, c = 78.669; $\alpha = \beta = \gamma = 90$	a =39.881, b = 48.679, c =77.821; $\alpha = \beta = \gamma = 90$
Resolution (Å)	50-1.70 (1.73-1.70)	50-1.85 (1.88-1.85)
No. of observations	638747	184084
No. of unique reflections[a]	17156 (858)	30559 (1312)
Completeness (%)	98.5 (100.0)	98.9 (85.0)
<I>/ (I)	26.0 (14.9)	23.9 (8.2)
Redundancy	13.9 (14.3)	15.6 (18.4)
R_{sym}[b]	0.11 (0.287)	0.061 (0.559)
R_{cryst}[c] (%)/R_{free}[d] (%)	0.165 / 0.190	0.176 / 0.225
Ramachandran plot, residues in:		
Most favored regions (%)	95.36	95.4
Allowed regions (%)	4.64	4.6
Disallowed regions (%)	0.0	0.0
PDB code	5XKW	5XKV

[a] Numbers in parentheses represent values in the highest resolution shell (Å).

[b] $R_{sym} = \Sigma|I_j - \langle I \rangle| / \Sigma I_j$, where I_j is the observed integrated intensity, <I> is the average integrated intensity obtained from multiple measurements, and the summation is over all observed reflections. [c] $R_{cryst} = \Sigma||F_{obs}| - |F_{calc}|| / \Sigma|F_{obs}|$, F_{obs} and F_{calc} are observed and calculated structure factor amplitudes, respectively.

[d] R_{free} calculated with randomly selected reflections (5%).