= Electronic Supplementary Information =

Human serum albumin mutants complexed Mn(III) protoporphyrin IX as superoxide dismutase mimics

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Experimental

Materials and apparatus

All reagents were purchased from commercial sources as special grades and used without further purification. Mn(III) protoporphyrin IX (MnPP) was purchased from Frontier Scientific, Inc. Xanthine and catalase (from bovine liver) were purchased from Wako Pure Chemical Industries, Ltd. Ferricytochrome *c* (Cyt. *c*, from bovine heart) was purchased from Sigma-Aldrich Co. Xanthine oxidase (XOD, from butter milk) was purchased from Oriental Yeast. Co., Ltd. UV-Vis absorption spectra were recorded using a UV-visible spectrophotometer 8543 (Agilent Technologies Ltd.) equipped with a temperature control unit 89090A. The water was deionized (18.2 MΩcm) using water purification systems Elix UV and Milli Q Reference (Millipore Corp.).

Preparations of HSA mutants and HSA–MnPP complexes

The designed HSA mutants [HSA(Y161L), HSA(Y161G), HSA(Y161H), HSA(Y161L/L182R), HSA(Y161L/L185R)] were prepared according to our previously reported site-directed mutagenesis and expression techniques.[18b,d] The protein concentration was assayed by Pierce 660-nm Protein Assay Kit (Thermo Fisher Scientific K.K.).

The HSA(wt)–MnPP and HSA(mutant)–MnPP complexes were prepared as described in the literatures for HSA(wt)–FePP.[18b,d] Typically 5 mL of a potassium phosphate buffered (PB) solution (pH 7.8, 50 mM) of HSA (0.1 mM) was mixed with 0.8 mL of 0.688 mM MnPP in DMSO (MnPP/HSA molar ratio of 1.1) and incubated for 16 hr with rotation in the dark at room temperature. The complex was then diluted with PB solution and concentrated to the initial volume using a Microsep advance centrifugal device (10 kDa Mw cut-off; Pall Corp.) at 4000g using a benchtop centrifuge Allegra X-15R (Beckman Coulter, Inc.). These dilution and concentration cycles were repeated to reduce the final concentration of DMSO < 0.1 vol%. The resulting samples were analyzed by a SDS-PAGE to confirm the protein integrity and concentration.

Xanthine–XOD–Cyt. *c* assay

The $O_2^{\bullet-}$ was generated in situ by xanthine–XOD reaction system and the SOD activities of the HSA–MnPP complexes were evaluated using the Cyt. *c* reduction technique.[8,11,12] To the PB solution (pH 7.8, 50 mM, 3.0 mL) containing Cyt. *c* (10 μM), xanthine (50 μM), and catalase (500 U/mL) in a 10-mm path length optical quartz cuvette, an amount of XOD sufficient to give an initial rate of ΔA_{550} = 0.025 min^{-1} (without SOD mimics) (approximately 2.0 mU/mL) was injected at 25 ºC. Immediately after the addition of XOD, increases in the absorption at 550 nm based on the reduced Cyt. *c* was monitored at 25 ºC. The absorbance increases were almost linear for at least 4 min, from which we determined the initial rate constant

(v_i) at various concentration of HSA–MnPP complex. The IC_{50} value is defined as the 50% inhibition concentration of Cyt. *c* reduction.

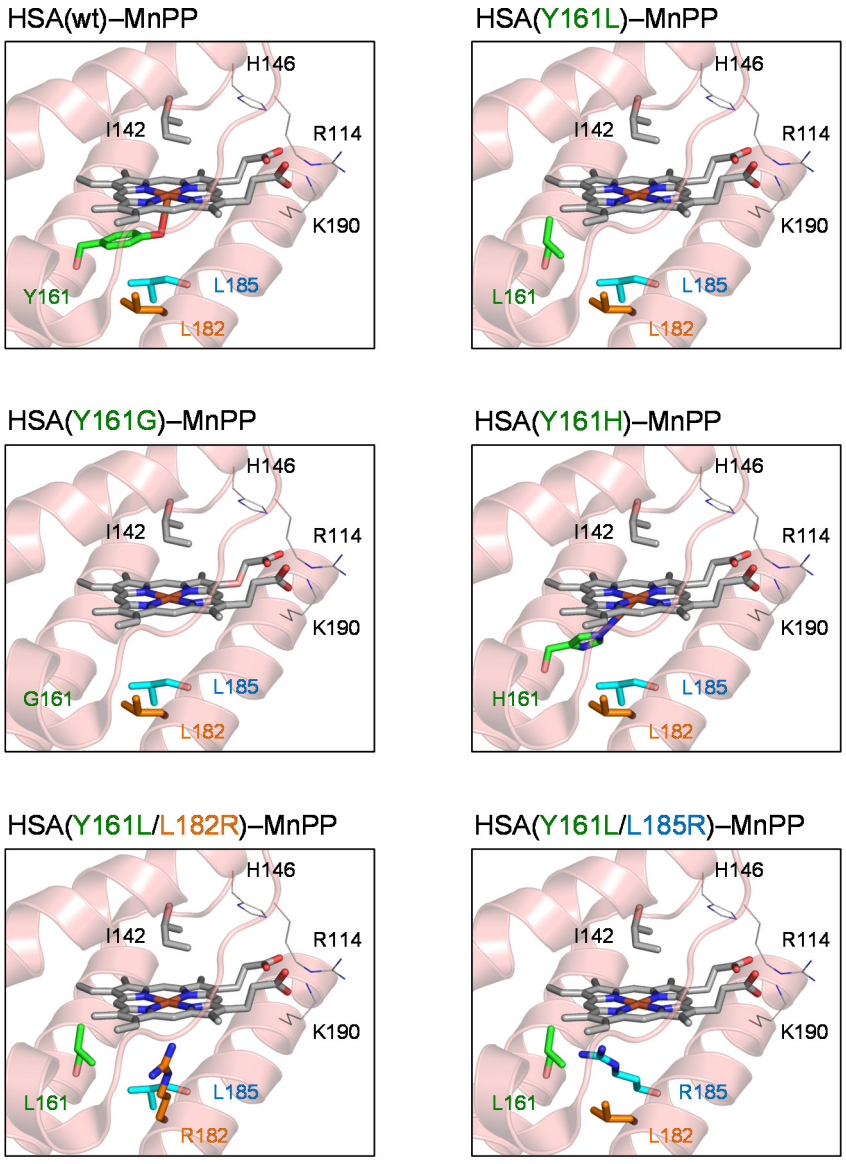

Fig. S1 Structural models of the genetic engineered haem pockets in HSA−MnPP complexes.[22]

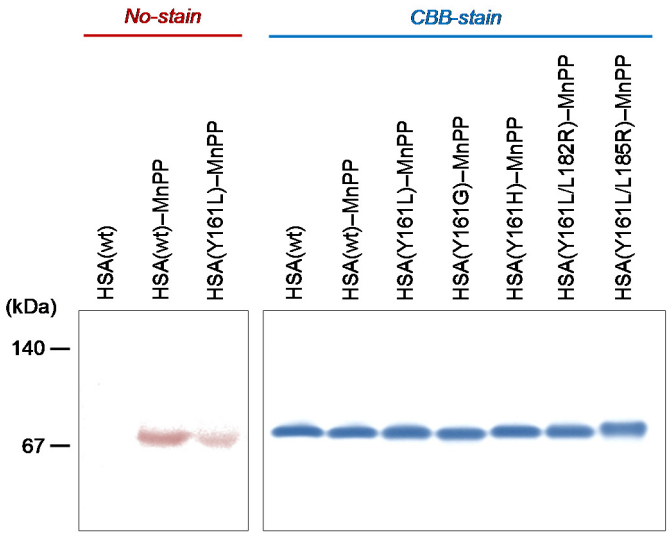

Fig. S2 Native PAGE of HSA−MnPP complexes.

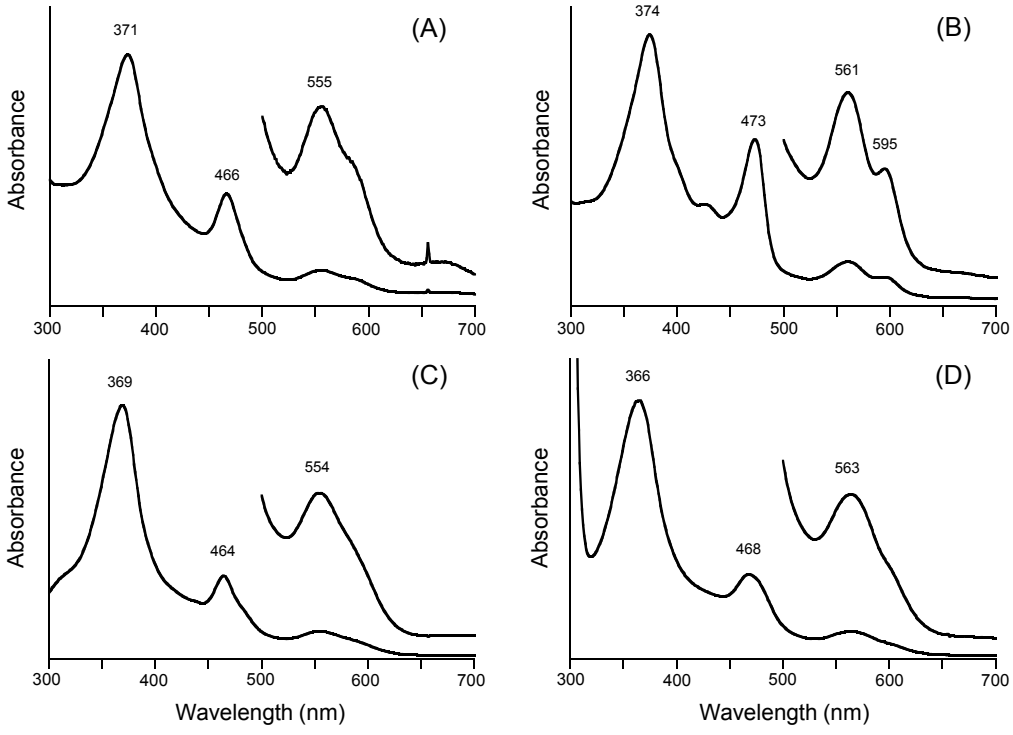

Fig. S3 UV-Vis absorption spectra of (A) HSA(Y161G)–MnPP, (B) HSA(Y161H)–MnPP, (C) MnPP in 50 mM PB solution (pH 7.8), and (D) MnPP in basic aqueous solution (pH 10) with 3 vol% phenol at 25 °C.

Table S1 UV-Vis absorption spectral data of HSA–MnPP complexes in 50 mM PB solution (pH 7.8) at 25 °C.

Compounds	λ_{max} (nm)
HSA(wt)–MnPP	368, 465, 563
HSA(Y161L)–MnPP	371, 466, 554
HSA(Y161G)–MnPP	371, 466, 555
HSA(Y161H)–MnPP	374, 473, 561, 595
HSA(Y161L/L182R)–MnPP	370, 466, 555
HSA(Y161L/L185R)–MnPP	370, 466, 555
MnPP	369, 464, 554
MnPP[a]	370, 465, 555
MnPP + phenol[b]	366, 468, 563

[a]From ref. 26, 27. [b]In basic aqueous solution (pH 10), [phenol] = 3 vol%.