Supporting Information

Synthesis of Water-soluble Dinuclear Mn-porphyrin with Multiple Antioxidative Activities

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Experimental section

Chemicals

m-xylylene dibromide was purchased from Sigma-Aldrich Japan. N,N-dimethylformamide (DMF), anhydrous dimethylsulfoxide (DMSO), Mn acetate tetrahydrate $Mn(OAc)_2 \cdot 4H_2O$ and hydrogen peroxide (H_2O_2. 35％) were purchased from Kanto Chemical Co., Inc. *m*-chloroperoxybenzoic acid (*m*-CPBA) was purchased from Tokyo Chemical Industry Co., Ltd. Anion exchange resin, Amberlite IRA-401B was purchased from ORGANO corp. IRA-401B was washed successively with 1M HCL and methanol before use.

Synthesis of $MnM4PyP_3P$

5-(4-pyridyl)-10,15,20-triphenyl-21*H*,23*H*-porphyrin (H_2PyP_3P) was synthesized according to our previous procedure,[1] which was modified for H_2PyP_3P. H_2PyP_3P (50mg, 0.081 mmol) and methyl iodide (0.5mL, 8 mmol) in $CHCl_3$ (5 mL) was refluxed for 6 hours in 10 mL recovery flask equipped with reflux condenser. The reaction mixture was sampled and monitored by TLC (silica gel $CHCl_3$/MeOH=4/1, v/v) at hourly interval. Methyl iodide (0.5 mL, 8 mmol) was added after every sampling. After cooling the reaction mixture, ether (5 mL) was added to it. Purple solids were collected by suction filtration, washed with ether successively, and dried *in vacuo* to give H_2M4PyP_3P as a purple solid. 46mg, 75%. ^{1}H-NMR (DMSO-d_6); δ 9.44 (d, 2H, J=6.5 Hz, py-2,6), 9.02 (d, 2H,J=6.5 Hz, py-3,5), 8.98 (br d, 2H, pyrrole-β), 8.95 (br d, 2H, pyrrole-β), 8.87 (br d, 4H, pyrrole-β), 8.24 (d, 6H, J=6.7 Hz, Ph), 7.91-7.84 (m, 9H, Ph), 4.70 (s, 3H, Me), -2.91 (s, 2H, inner proton).

H_2M4PyP_3P and $Mn(OAc)_2 \cdot 4H_2O$ dissolved in $CHCl_3$/MeOH mixed solvent ($CHCl_3$/MeOH=2/1, v/v) and refluxed and monitored by UV/vis spectrophotometer. The Soret band of $MnM4PyP_3P$ at 464 nm was raised through the reaction while that of H_2M4PyP_3P at 421 nm was fallen. The reaction was continued until no spectral change was observed. After the reaction was finished, the solvent was evaporated *in vacuo*. The resulting solid was dissolved in water and subsequently precipitated as PF_6^- salt with ammonium hexafluorophosphate (NH_4PF_6), and then collected by suction filtration. The PF_6^- salt was dissolved in methanol and subjected to ion exchange resin, Amberlite IRA-401B (Cl^- form). The resulting fraction was concentrated to give greenish solid. Elemental analysis: $MnM4PyP_3P \cdot CH_3OH$ ($C_{45}H_{34}N_5MnCl_2$), Anal (％);C:68.71, H:4.36, N:8.91. Found(％); C:68.46, H:4.61, N:8.55. UV/vis (50mM pH 7.4 phosphate buffer) $\lambda_{max}(\varepsilon)$: 380(41000), 402(43000), 467(84100), 566(10100), 599(7300).

Synthesis of MnPD

H_2PyP_3P (600mg, 0.976mmol) and *m*-xylylene dibromide (118mg 0.447mmol) were

dissolved in degassed DMF and stirred at 100℃ for 21 hours under N_2. After the reaction, the solvent was evaporated *in vacuo*. The resulting solid was subjected to chromatography on alumina gel eluted by $CHCl_3$ to remove unreacted H_2PyP_3P, and then eluted by $CHCl_3$/MeOH/DMF mixed solvent ($CHCl_3$/MeOH=4:1, DMF was added a few drops per 10-mL mixed solvent) to collect other bands. The collected fractions were combined and re-chromatographed on alumina gel eluted by $CHCl_3$/MeOH(9/1) to give H_2PD as a purple solid (293mg. 69%). ^1H NMR (DMSO-d_6) δ9.72(d, 4H, J=4.6 Hz py-2,6), 9.13(d, 4H, J=4.6 Hz py-3,5), 8.94(s, 4H, pyrrole-β), 8.79(s, 4H, pyrrole-β), 8.54(s, 8H, pyrrole-β), 8.33(s, 1H, xylyl-2), 8.22(d, 4H, $Ph_{trans\ to\ pyridyl}$ -2,6), 8.09(d, 2H, xylyl-4,6), 7.91~7.85(m, 7H, xylyl-5 & $Ph_{trans\ to\ pyridyl}$-3,4,5), 7.62~7.56(m, 12H, $Ph_{cis\ to\ pyridyl}$-2,4,6) 7.37~7.35(m, 8H, $Ph_{cis\ to\ pyridyl}$-3,5), 6.40(s, 4H, benzyl), -2.96(s, 4H, inner proton).

H_2PD and $Mn(OAc)_2 \cdot 4H_2O$ in degassed methanol was refluxed and monitored by UV/vis spectrophotometer. The Soret band of MnPD at 467 nm was raised through the reaction while that of H_2PD at 415 nm was fallen. The reaction was continued until no spectral change was observed. After the reaction was finished, the solvent was evaporated *in vacuo*. The resulting solid was dissolved in water and subsequently precipitated as PF_6^- salt with ammonium hexafluorophosphate (NH_4PF_6), and then collected by suction filtration. The PF_6^- salt was dissolved in methanol and subjected to ion exchange resin, Amberlite IRA-401B (Cl^- form). The resulting fraction was concentrated to give greenish solid. Elemental Analysis: MnPD·5.5CH_3COOH ($C_{105}H_{84}O_{11}N_{10}Mn_2Cl_4$), Anal (%):C:65.48, H 4.42, N 7.31. Found (%): 65.43, H 4.59, N 7.32. UV/vis (50mM pH 7.4 phosphate buffer) $\lambda_{max}(\varepsilon)$: 379(46800), 401(48800), 467(104200),566(12800),601(9600).

Measurement of catalase activity

Catalase activity was measured by monitoring O_2 concentration with Clark-type oxygen electrode. To 900μL of Mn-porphyrin in 50mM pH 7.4 phosphate buffer (1~20μM), was added a 100μL of 10mM H_2O_2 in Milli-Q water. The monitoring was started after the addition of the H_2O_2 solution. Observed rate constant (k_{obs}) at each concentration of Mn-porphyrin was determined as a rate of O_2 production in initial 5 seconds. Catalase activity (k_{CAT}) was determined from the slope of the plot of k_{obs} as a function of the Mn-porphyrin concentration.

In vivo **antioxidative activity**

In vivo antioxidative activity of Mn-porphyrin derivatives was examined with skeletal muscle-specific SOD deficient (HSA-*Sod2*$^{-/-}$) mice. This mouse exhibits short running time on treadmill due to severe oxidative stress in skeletal muscle. First, running time of the mouse

was recorded on treadmill. After 24 hours, Mn-porphyrin derivatives (MnPD and $MnM4Py_4P$) dissolved in PBS were intraperitoneally injected (36 mg/kg) in the mice. The running time of the mouse was recorded another 8 and 24 hours after the injection. The antioxidative activity of Mn-porphyrin derivatives was evaluated from the retrieval of the running time.[2]

^{1}H NMR spectrum

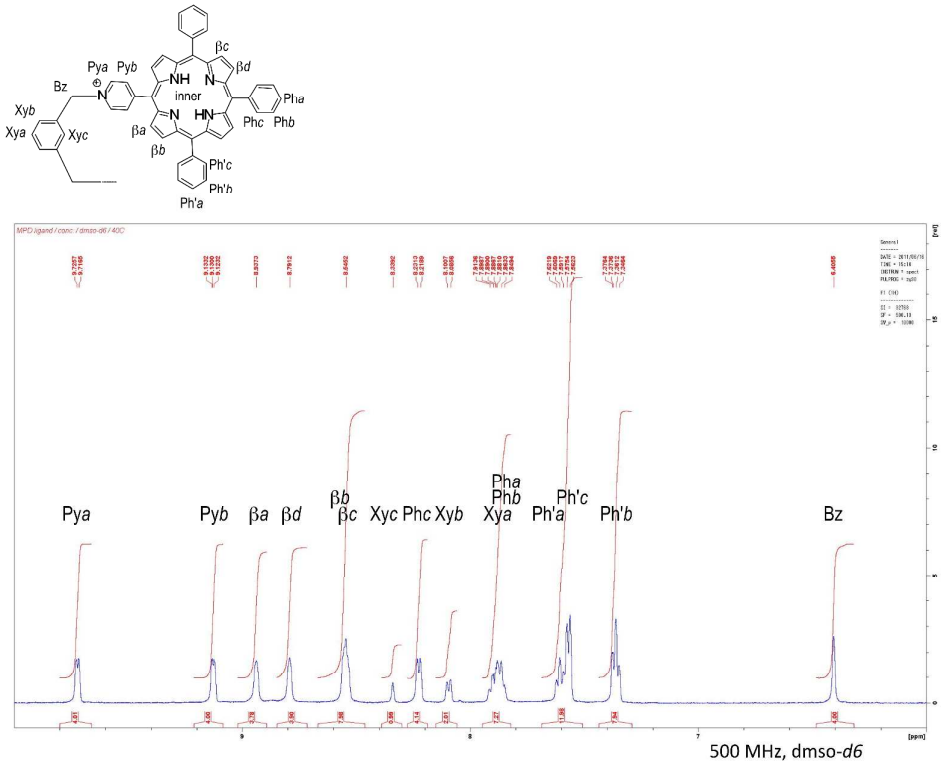

Figure S1. ^{1}H NMR spectrum of H_2PD in DMSO-d_6 (500 MHz).

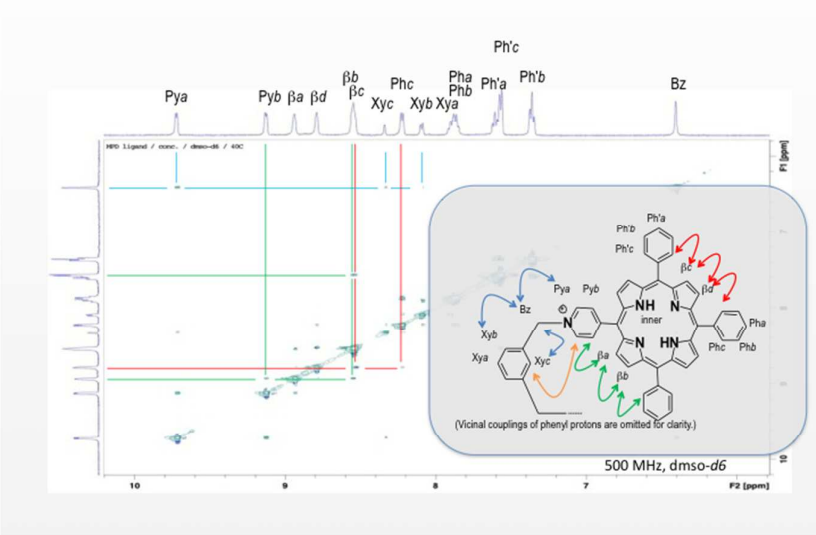

Figure S2. NOESY spectrum of H_2PD in DMSO-d_6 (500 MHz).

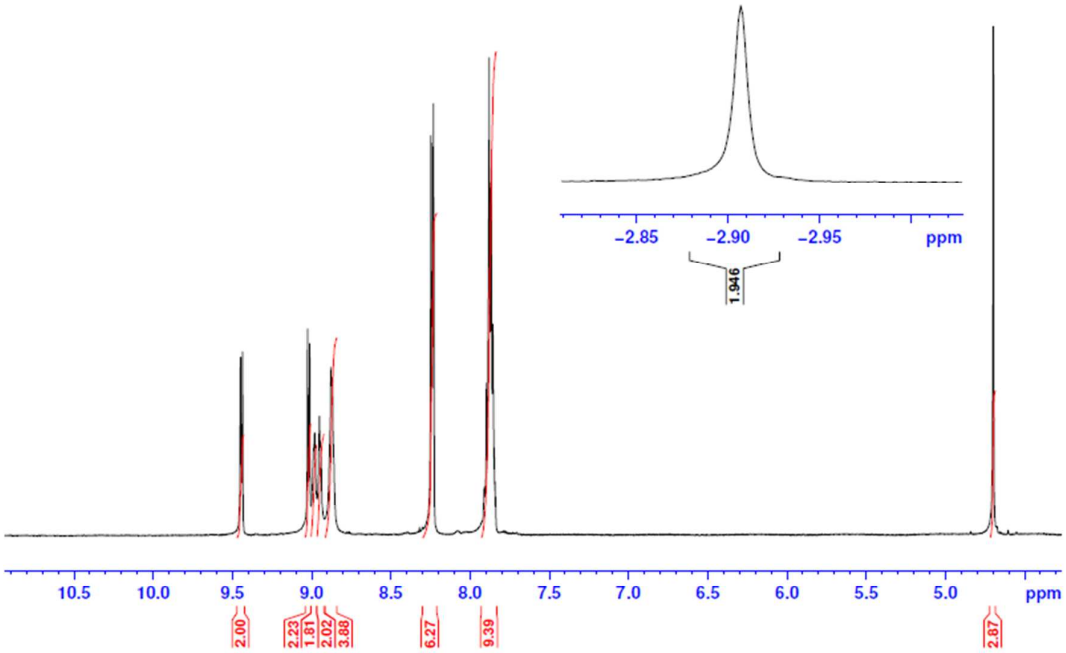

Figure S3. ^{1}H NMR spectrum of H_2M4PyP_3P in DMSO-d_6 (500 MHz). Inset: inner proton (2H).

SOD activity

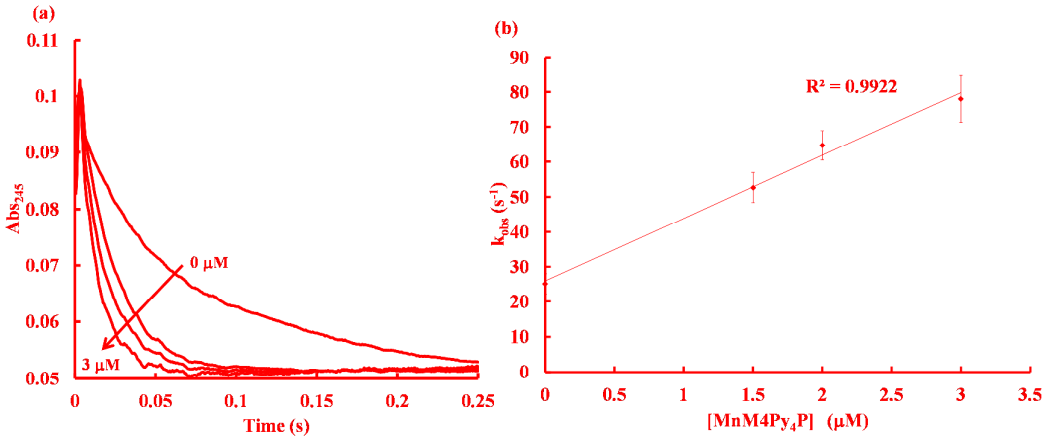

Figure S4. (a) Time decay of superoxide in the presence of $MnM4Py_4P$. (b) Plot of k_{obs} as a function of $MnM4Py_4P$ concentration. k_{obs} was calculated from the decay of superoxide in initial five milliseconds.

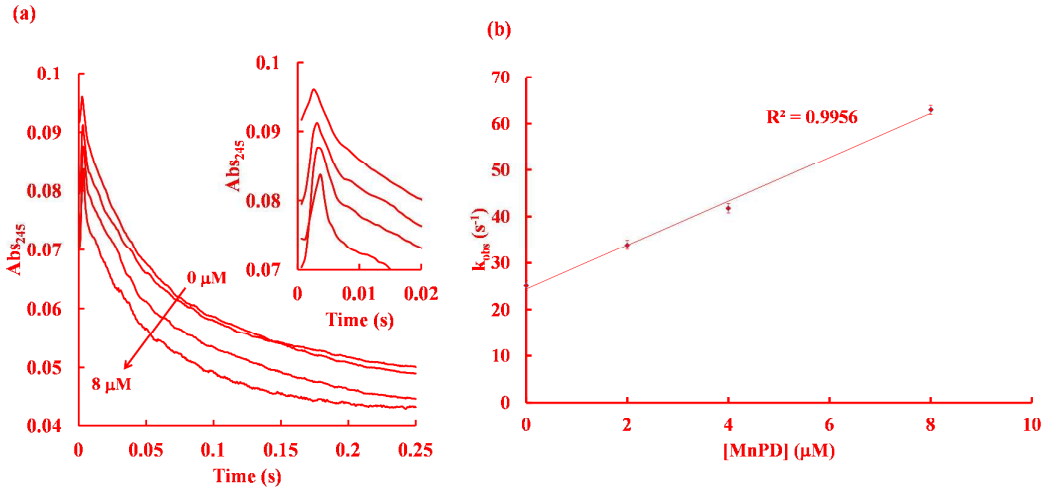

Figure S5. (a) Time decay of superoxide in the presence of MnPD. Inset: Zoom in initial 20 milliseconds. (b) Plot of k_{obs} as a function of MnPD concentration. k_{obs} was calculated from the decay of superoxide in initial milliseconds.

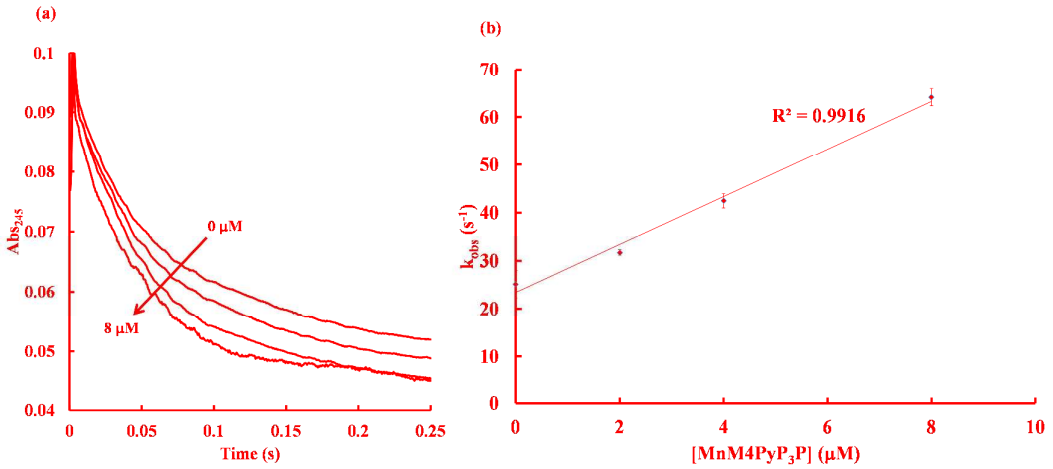

Figure S6. (a) Time decay of superoxide in the presence of $MnM4PyP_3P$. (b) Plot of k_{obs} as a function of $MnM4PyP_3P$ concentration. k_{obs} was calculated from the decay of superoxide in initial five milliseconds.

UV/vis spectra

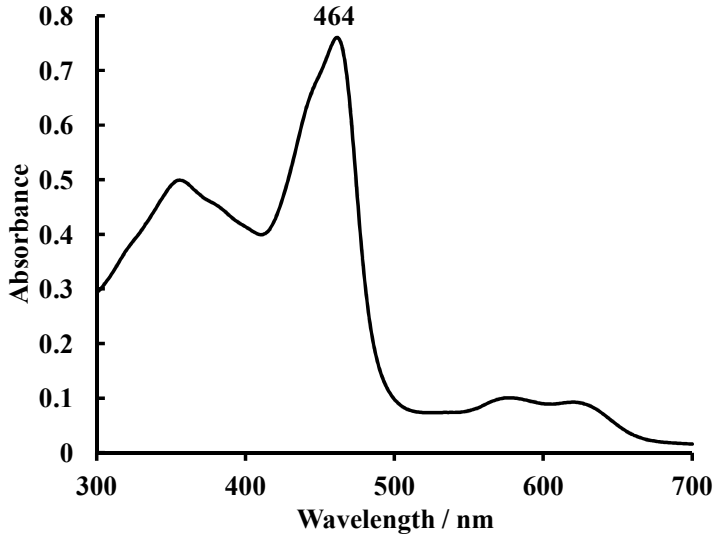

Figure S7. Ground-state UV/vis spectrum of 12 μM MnPD in 50 mM borate buffer (pH 9.4). $\lambda_{max}(\varepsilon)$: 357(39200), 464(63800), 571(7540).

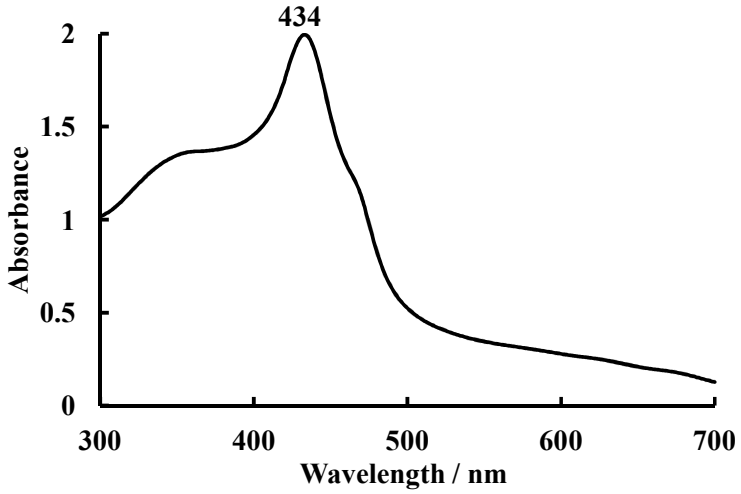

Figure S8. UV/vis spectrum of 30μM MnPD in 50 mM borate buffer (pH 9.4) in the presence of 1 mM H_2O_2. The spectrum was rapidly measured after the mixing of H_2O_2.

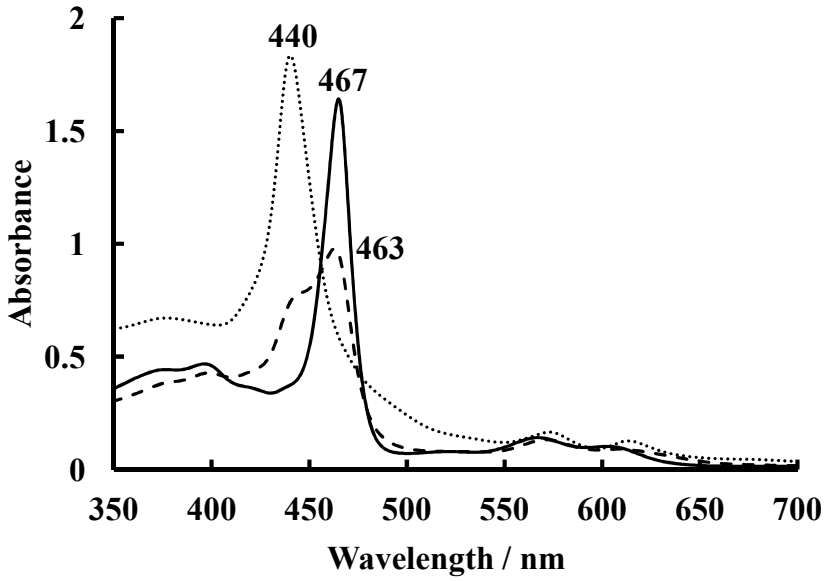

Figure S9. UV/vis spectra of $Mn^{III}(OH_2)PD$ (solid line), $Mn^{IV}(=O)(OH)PD$ (dashed line) and $Mn^{V}(=O)(OH)PD$ (dotted line). MnPD was oxidized by *m*-chloroperbenzoic acid (*m*-CPBA) to form the high-valent oxo-Mn species. The spectra were measured in DMSO-CH_2Cl_2 mixed solvent (1:1, v/v).

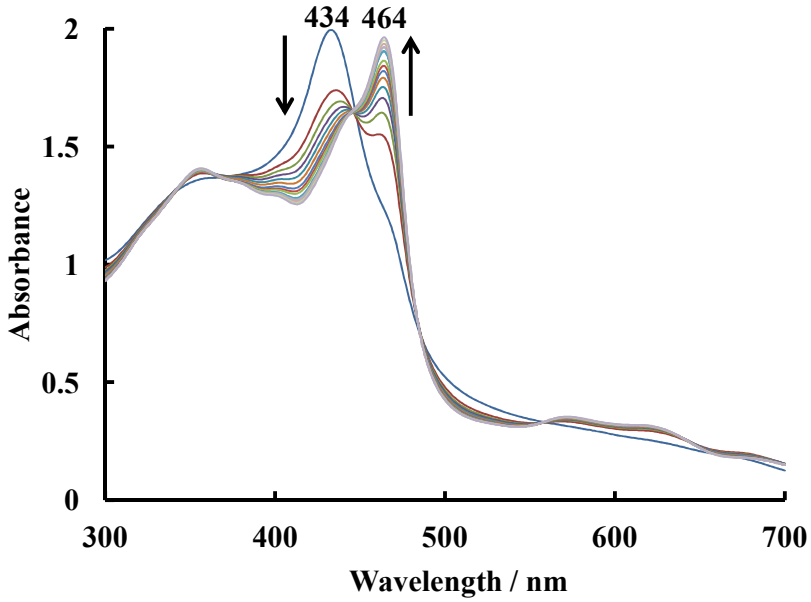

Figure S10. Time-course of UV/vis spectral changes of 30μM MnPD in 50 mM borate buffer (pH 9.4) in the presence of 1mM H_2O_2. The spectra were measured for 70 minutes at 5 minute intervals.

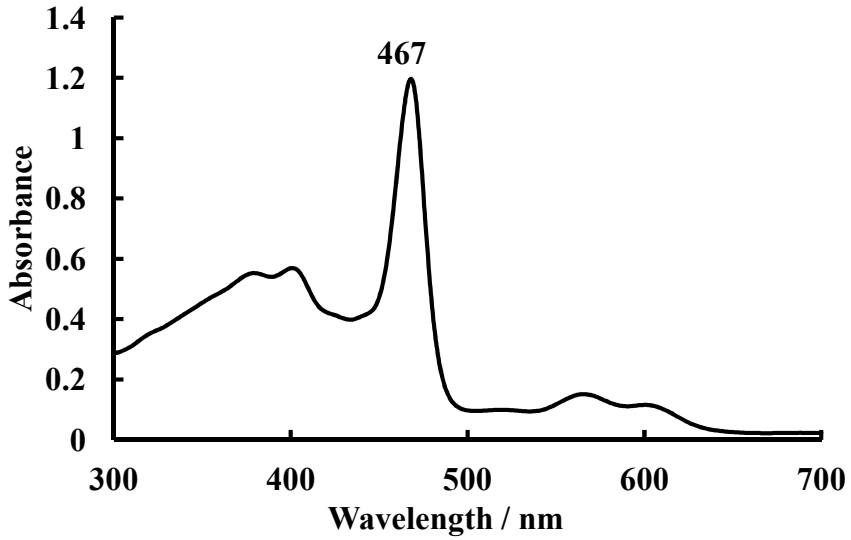

Figure S11. Ground-state UV/vis spectrum of 12μM MnPD in 50mM phosphate buffer (pH 7.4).

References

1 T. Haruyama, S. Asayama, H. Kawakami. Highly Amphiphilic Manganese Porphyrin for Mitochondrial Targeting Antioxidant. *J. Biochem.* **2010**, 147, 153~156.

2 H. Kuwahara, T. Horie, S. Ishikawa, C. Tsuda, S. Kawakami, Y. Noda, T. Kaneko, S. Tahara, T. Tachibana, M. Okabe, J. Melki, R. Takano, T. Toda, D. Morikawa, H. Nojiri, H. Kurosawa, T. Shirasawa, T. Shimizu. Oxidative stress in skeletal muscle causes severe disturbance of exercise activity without muscle atrophy. *Free Radical Biol. Med.* **2010**, 48, 1252~1262.