Supporting Information

Photochemical properties of myoglobin–CdTe quantum dot conjugates

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Experimental procedures

Instruments.

^{1}H NMR spectra (400 MHz) were recorded on a Bruker DPX400 NMR spectrometer. ^{1}H NMR chemical shift values are reported in ppm relative to the residual solvent resonances. ESI-TOF MS analyses were performed on a Bruker microTOFII mass spectrometer. UV-vis spectra were taken by a SHIMADZU UV-3150 or UV-2550 spectrophotometer equipped with a thermostated cell holder. The pH values were monitored by an F-52 Horiba pH meter. Purification of the proteins was performed using a GE healthcare ÄKTA Purifier system at 4 ºC. The analyses were performed at 4 ºC using a flow rate of 0.5 mL min^{-1} with the detection at 405 nm. Photoluminescence spectra were recorded by using HORIBA Fluoromax-4. The continuous Xe lamp irradiation was carried out by USHIO Optical Modulex SX-U1501XQ (500 W). Distilled water was demineralised by a Barnstead NANOpure DIamond^{TM} system.

Materials.

$NaBH_4$, $CdCl_2$ anhydride, thioglycolic acid and dipotassium hydrogenphosphate were purchased from Wako Pure Chemicals Industries, Tellurium (200 mesh, 99.8%) was purchased from Aldrich, and potassium dihydrogen phosphate was purchased from Kishida Chemical. CdTe QD was prepared according to the literature with a slight modification[1] and the concentration was calculated by the empirical equation in the previous report.[2] Protoporphyrin IX mono *t*-butyl ester (**8**),[3] mono-*N*-Boc protected ethylenediamine (**2**)[4] and 4,4'-dithiodipropanoic acid (**3**)[5] were prepared according to the procedures reported in the literatures.

Preparation of proteins.

The heme dimer **1–1** (0.8 mg, 1 nmol) in 2 mL of pyridine was added to the apoMb (0.7 mmol) solution in 10 mL of 50 mM potassium phosphate (KPi) buffer (pH 7.0). The mixed solution was applied to Sephadex G25 (GE healthcare) gel filtration column to remove pyridine. The collected fraction (1 mL) was concentrated to 100 μL using an Amicon stirred ultrafiltration cell with a 10-kDa molecular weight cutoff membrane and exchanged into a buffer containing 150 mM NaCl. This sample was loaded onto a Superdex 75 column equilibrated with the same buffer. The collected fraction was used after the buffer was exchanged to 50 mM KPi (pH 7.0).

Agarose gel electrophoresis.

A 0.5 % agarose gel was used with 0.5 x Tris/Borate/EDTA buffer (45 mM Tris-borate, 1 mM EDTA). The bands were separated at the applied voltage of 135 V for 15 min. The concentration of CdTe QD in all samples was 3.2 μM and the Mb component in each well was increased by 0.2 eq. After the electrophoresis, the gels were stained by Coomassie Brilliant Blue (CBB) R-250 in 20 % acetic acid aqueous solution for 12 h and destained in the 30 % MeOH, 10 % acetic acid aqueous solution for 24 h.

Stationary photoluminescence measurements.

The samples containing 2.9 μM CdTe QD in a 50 mM KPi buffer solution (pH 7.0) were prepared under dark and incubated at 4 ºC for 2 h before the measurements. The spectra excited at 500 nm were measured under air at 25 °C.

Photoluminescence decay measurements.

The samples containing 1.8 μM CdTe QD in a 50 mM KPi buffer solution (pH 7.0) were degassed and purged with nitrogen before the measurements. Excitation wavelength was 495 nm and the photoluminescence decay at 580 nm was recorded. Decay curves were first fitted to four-exponential equations and then one of the exponential functions with the shortest lifetime derived from light source was removed to be three exponential equation;

$I = A_1\exp(-t/\tau_1) + A_2\exp(-t/\tau_2) + A_3\exp(-t/\tau_3)$.

Photoirradiation experiments.

The samples (1.5 mL) containing 1.8 μM CdTe QD, 3.6 μM protein, and 1 mM triethanolamine as a sacrificial agent in 50 mM KPi buffer were prepared. The solution in 1 cm quartz cell was then irradiated with a 500 W Xe-lamp (410–770 nm) (Ushio Optical Modulex, SX-U1501XQ) equipped with UV and IR cutoff filters at 25 °C. The absorption spectra were measured every 15 s.

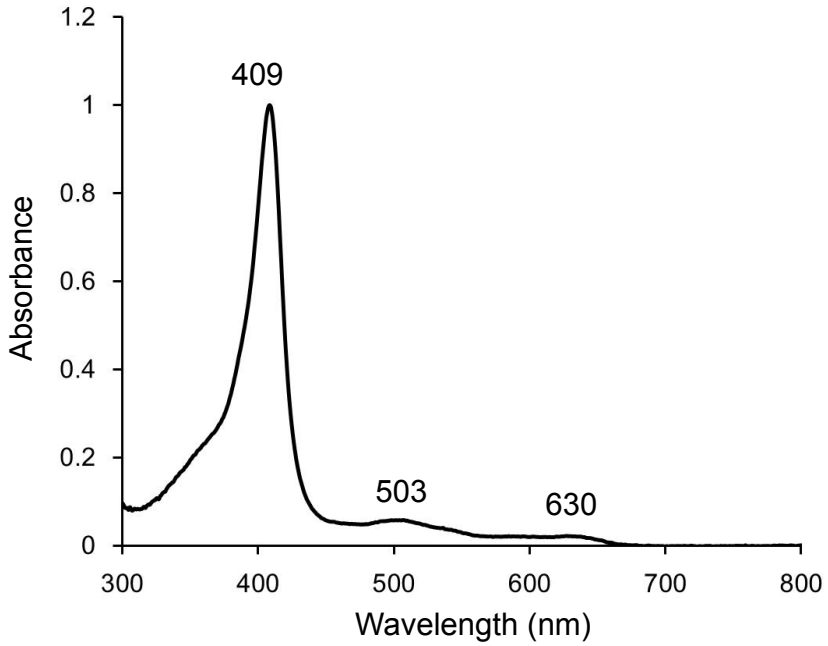

Fig. S1 Absorption spectra of rMb_2(**1-1**) in 50 mM KPi buffer solution (pH 7.0).

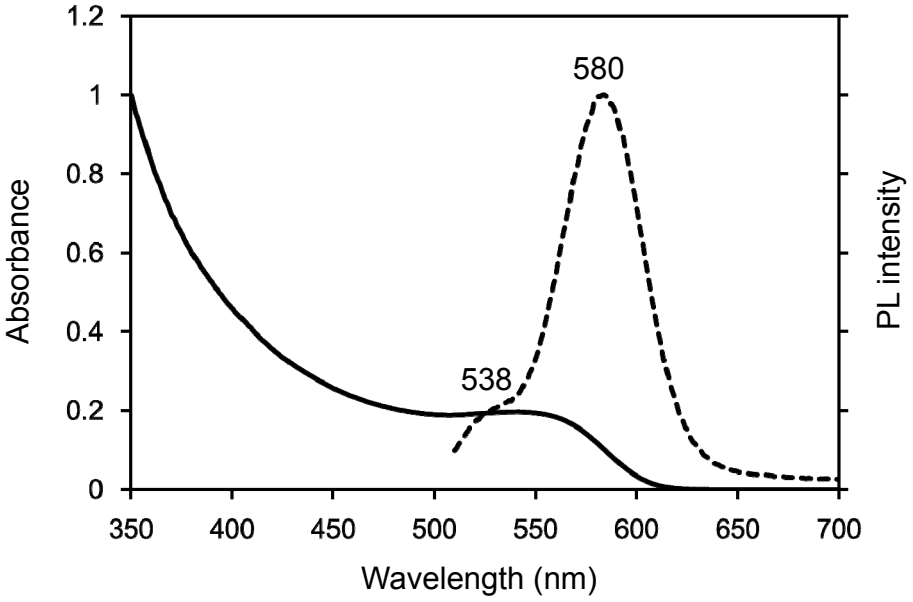

Fig. S2 Absorption (solid line) and photoluminescence (dashed line) spectra of CdTe QD (ca. 3.2 nm) in 50 mM KPi buffer solution (pH 7.0).

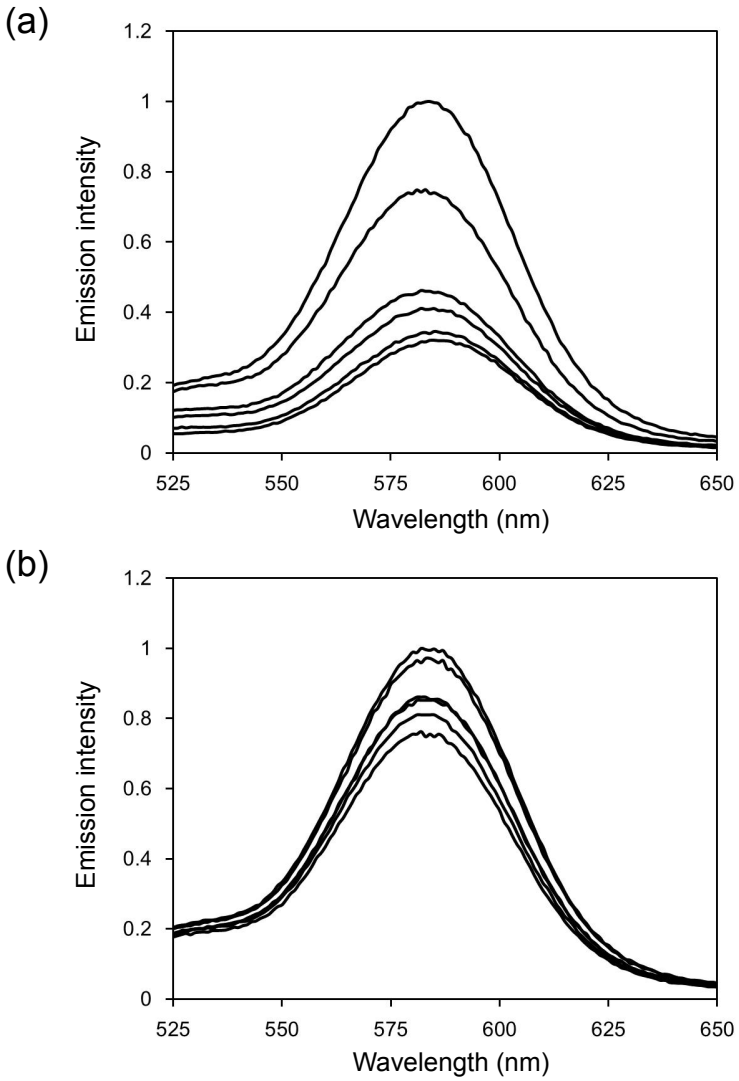

Fig. S3 Photoluminescence spectra excited at 500 nm of (a) rMb(**1**)@CdTe and (b) native Mb (nMb) + CdTe QD in a 50 mM KPi buffer solution (pH 7.0) at 25 ºC. The concentration of CdTe QD was 2.9 μM and that of the protein component was as follows: 0, 0.55, 1.03, 1.46, 1.85, and 2.19 μM for rMb(**1**)@CdTe and 0, 1.12, 2.14, 3.08, 3.94, and 4.73 for nMb + CdTe QD.

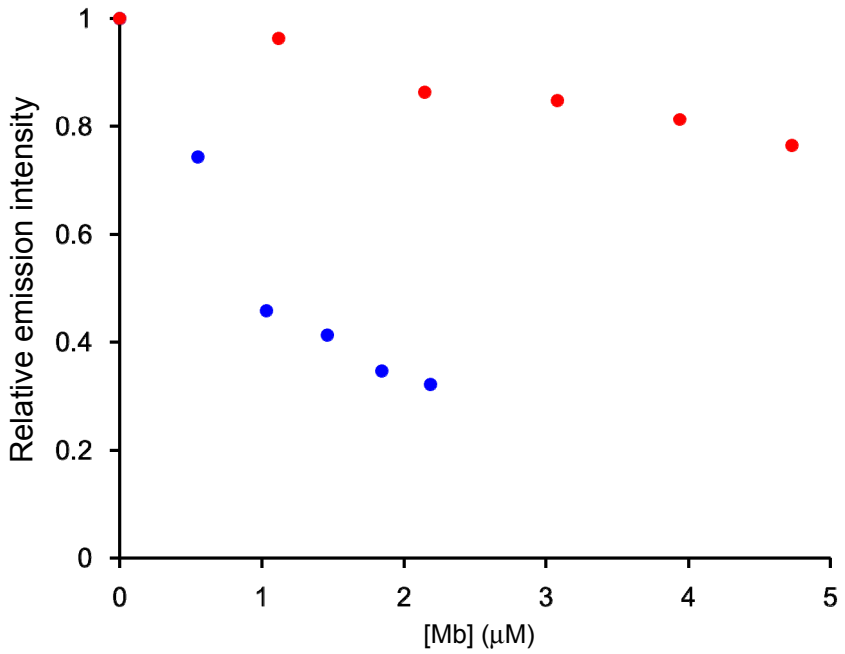

Fig. S4 Normalized photoluminescence intensity as a function of the myoglobin concentration. rMb(**1**)@CdTe (blue) and nMb + CdTe QD (red). (λex = 500 nm).

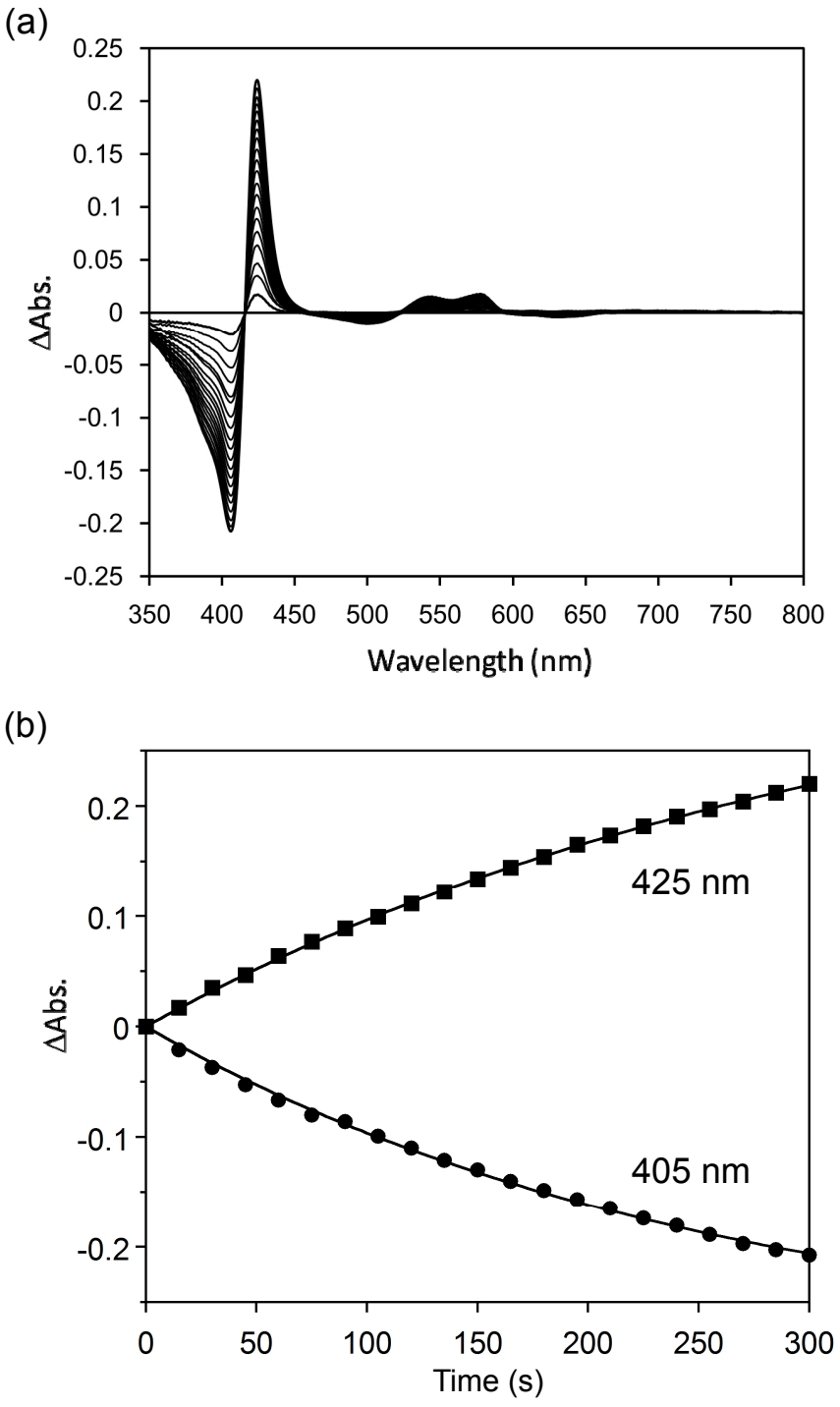

Fig. S5 (a) Differential absorption spectra of nMb + CdTe QD upon the illumination of a light. CdTe QD (1.8 μM), $rMb_2(\mathbf{1}_2)$ (3.6 μM), triethanolamine (1 mM). (b) Profiles at 405 nm (filled circle) and 425 nm (filled square) are plotted.

References

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