Supporting information

Energy migration within hexameric hemoprotein reconstituted with Zn porphyrinoid molecules

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Instruments

UV-vis spectral measurements were carried out with a Shimadzu UV-3150 or UV-2550 double-beam spectrophotometer, or a Shimadzu BioSpec-nano spectrometer. Fluorescence spectra were recorded with a JASCO FP-8600 fluorescence spectrometer. Circular dichroism (CD) spectra were recorded at 25 °C on a JASCO spectropolarimeter (Model J-820). ESI-TOF MS analyses were performed with a Bruker Daltonics micrOTOF-II mass spectrometer. ICP-OES (inductively coupled plasma optical emission spectroscopy) was performed on a Shimadzu ICPS-7510 emission spectrometer. The pH measurements were made with an F-52 Horiba pH meter. Size exclusion column chromatography was performed using an ÄKTApurifier system equipped with Superdex 200 10/300 GL (GE Healthcare) at 4 ºC. Air-sensitive manipulations were performed in a UNILab glove box (MBraun, Germany). Fluorescence lifetime measurements were recorded by a HORIBA Fluoromax-4 fluorescence spectrophotometer by excitation at 390 nm. Dynamic light scattering was measured by a Malvern Zetasizer μV light scattering analyzer with 830 nm laser at 25 ºC. The equipment used for flash photolysis experiments is described below.

Materials

Syntheses of Zn protoporphyrin IX, ZnPP,[S1] and Zn chlorin e_6, ZnCe6,[S2] were reported in previous papers. The pUC19 plasmid containing optimized hexameric tyrosine-coordinated heme protein (HTHP) gene was purchased from FASMAC. Distilled water was demineralized using a Millipore Integral 3 apparatus. Other all reagents were of the highest guaranteed grade commercially available and were used as received unless otherwise indicated. A standard zinc solution for ICP-OES was purchased from Wako Pure Chemical Industries.

Amino acid sequence of HTHP (monomer)

SETWLPTLVTATPQEGFDLAVKLSRIAVKKTQPDAQVRDTLRAVYEKDANALIAVSAVVATHF
QTIAAANDYWKD (75 residues)

Experimental procedures

Expression and purification of HTHP. The pUC19 plasmid containing the optimized HTHP gene was used as a template for PCR with oligonucleotide primers (i) 5'-GGGGACAAGTTTGTACAA AAAAGCAGGCTTCGAAG-3' and (ii) 5'-GGGGACCACTTTGTACAAGAAAGCTGGGTC-3'. The PCR product was inserted into a pDONR vector and then a pDEST14 expression vector according to standard protocols for Gateway technology (Invitrogen^{TM}). DNA sequencing was performed to verify the correct insertion of the gene sequence into the expression vector. The resulting expression plasmid was transformed into *E. coli* BL21(DE3). 1 L volumes of a LB medium containing ampicillin (100 mg) were inoculated with 10 mL of the culture (OD = 0.5) of the relevant transformed cells. After the cells were grown aerobically with vigorous shaking at 37 ºC until the OD_{600} reached ~0.5, isopropyl-β-D-1-thiogalactopyranoside (IPTG) was added to a final concentration of 1.0 mM to induce the protein expression. The incubation was continued at 37 ºC for approximately 8 h. The cells were harvested by centrifugation at 4000 x g for 10 min. The harvested cells from 4 L of culture were re-suspended in ca. 50 mL of a 10 mM Tris-HCl buffer (pH 8.0) containing 1mM EDTA and lysed by freeze–thaw cycles with subsequent sonication for 30 sec x 10 times at 4 ºC. The lysate was then centrifuged and the supernatant was collected. The solution was loaded onto a DEAE Fast Flow (GE healthcare) anion-exchange column which was pre-equilibrated in a 10 mM potassium phosphate buffer (pH 6.0). The fraction of the target protein was collected by a 10 mM potassium phosphate buffer (pH 6.0) containing 0.3 M NaCl, and concentrated using an Amicon stirred ultrafiltration cell with a 30-kDa molecular weight cut-off membrane (Millipore). The concentrated solution was passed through a Sephadex G-150 size exclusion column equilibrated with 100 mM potassium phosphate buffer, pH 7.0. The fractions with $R_z > 2.8$ (R_z is the ratio of absorbance values at 402 nm and 280 nm) were collected and concentrated. The obtained HTHP was characterized by SDS-PAGE and ESI-TOF MS (Fig. S1), and stored at –80 ºC.

Analytical SEC measurements. For SEC analysis, 100 mM potassium phosphate buffer at pH 7.0 was used as an eluent. The analysis was performed at 4 ºC at a flow rate of 0.5 mL min^{-1} with monitoring of the absorbance at 280, 402, 418 or 421 nm for detection. The Superdex 200 column was calibrated using the following reagents: Blue Dextran (2000 kDa, used to determine the void volume of the column), ferritin (400 kDa), catalase (232 kDa), albumin (67 kDa), and chymotrypsinogen (25 kDa).

Preparation of apoHTHP. Removal of heme was performed using Teale's method as follows. A solution of HTHP (50 μM as a monomer) in 100 mM of L-histidine solution at 4 ºC was acidified to pH 1.7 by addition of 1 M HCl_{aq}. Unbound heme was extracted with 2-butanone (5 times) and the colorless aqueous solution was neutralized by dialysis with 100 mM potassium phosphate buffer, pH 7.0, (3 times) at 4 ºC. The resulting solution was readily used for experiments.

Reconstitution of HTHP with ZnPP or ZnCe6. rHTHP$^{ZnPP(6/6)}$: A DMSO solution of ZnPP (2 mM, 180 μL) was added to a solution of apoHTHP (30 μM equivalent as a monomer, 10 mL) in 100 mM potassium phosphate buffer, pH 7.0. After standing overnight under an N_2 atmosphere at 4 ºC, the solution was loaded onto a DEAE FF column equilibrated with 100 mM potassium phosphate buffer and the reconstituted protein was eluted with the same buffer containing 500 mM NaCl. The buffer was exchanged to 100 mM potassium phosphate buffer, pH 7.0 using a HiTrap Desalting column. The purified protein was characterized by UV-vis, CD, DLS and SEC measurements. These procedures were performed in the dark. The molar extinction coefficient was determined by ICP-MS analysis: $\varepsilon_{421} = 2.4 \times 10^5\ M^{-1}cm^{-1}$.

rHTHP$^{ZnCe6(6/6)}$: An aqueous solution of ZnCe6 (0.23 mM, 0.65 mL) in glycine-NaOH buffer at pH 10 was added to the apoHTHP solution (15 μM equivalent as a monomer, 10 mL) in 100 mM potassium phosphate buffer, pH 7.0, in the dark. The obtained mixture was dialyzed with 100 mM potassium phosphate buffer, pH 7.0, (3 times) at 4 ºC. The purified protein was characterized by UV-vis, CD, DLS and SEC measurements. These procedures were performed in the dark. The molar extinction coefficient was determined by ICP-MS analysis: $\varepsilon_{418} = 1.8 \times 10^5\ M^{-1}cm^{-1}$.

Titration experiments of photosensitizer molecules to apoHTHP. To a cuvette containing a solution of apoHTHP (17 or 29 μM as a monomer for titration of ZnPP or ZnCe6, respectively) in 100 mM potassium phosphate buffer, pH 7.0, at 4 ºC, various equivalents of ZnPP or ZnCe6 solutions in 0.1 M $NaOH_{aq}$ were added. After 12 h under an N_2 atmosphere in the dark, UV-vis spectra were measured (Fig. S3).

Preparation of rHTHP$^{ZnPP(1/6)}$ or rHTHP$^{ZnCe6(1/6)}$. In the dark, an apoHTHP solution (30 μM equivalent as a monomer, 1.67 mL) in 100 mM potassium phosphate buffer, pH 7.0, was added to rHTHP$^{ZnPP(6/6)}$ or rHTHP$^{ZnCe6(6/6)}$ solution (10 μM equivalent as a monomer, 1 mL) in the same buffer at 25 ºC. After standing for 24 h, equilibration was confirmed by fluorescence measurements.

Flash photolysis measurements. Transient absorption spectroscopy measurements after photoexcitation by femtosecond pulse laser were conducted using an ultrafast source: Integra-C (Quantronix Corp.), an optical parametric amplifier: TOPAS (Light Conversion Ltd.) and a commercially available optical detection system: Helios provided by Ultrafast Systems LLC. The source for the pump and probe pulses were derived from the fundamental output of Integra-C (λ = 786 nm, 2 mJ per pulse and fwhm = 130 fs) at a repetition rate of 1 kHz. 75% of the fundamental output of the laser was introduced into TOPAS for excitation light generation at λ = 420 nm, while the rest of the output was used for white light generation. The laser pulse was focused on a sapphire plate of 3 mm thickness and then a white light continuum covering the visible region from λ = 410 nm to 800 nm was generated via self-phase modulation. A variable neutral density filter, an optical aperture, and a pair of polarizers were inserted in the path in order to generate a stable white light continuum. Prior to generating the probe continuum, the laser pulse was fed to a delay line that provides an experimental time window of 3.2 ns with a maximum step resolution of 7 fs. In our experiments, a wavelength at λ = 420 nm of SHG output was irradiated at the sample cell with a spot size of 1 mm diameter where it was merged with the white probe pulse in a close angle (< 10°). The power of the pump pulse ranging from 12 μJ to 24 μJ was regulated by a variable neutral density filter. The probe beam after passing through the 2 mm sample cell was focused on a fiber optic cable that was connected to a CMOS spectrograph for recording the time-resolved spectra (λ = 450 - 800 nm). Typically, 3000 excitation pulses were averaged for 3 seconds to obtain the transient spectrum at a set delay time. Kinetic traces at appropriate wavelengths were assembled from the time-resolved spectral data. All measurements were conducted at room temperature, 22 ºC.

Figures

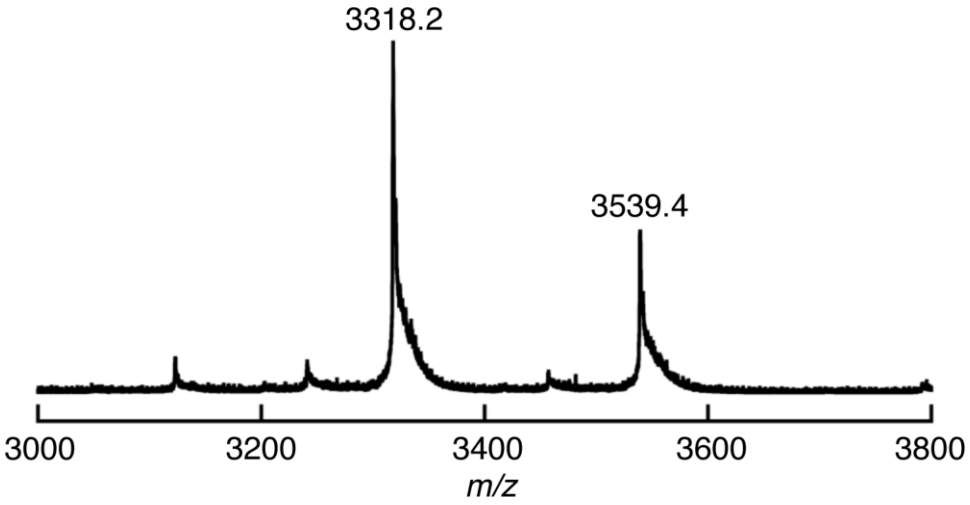

Fig. S1. ESI-TOF mass spectrum of the holo-hexameric form of HTHP. The sample was dissolved in 10 mM NH_4OAc aqueous solution at pH 6.9. Multiply ionized species was observed: found m/z = 3318.2 and 3539.4; calcd m/z = 3318.5 (z = 16+) and 3539.7 (z = 15+).

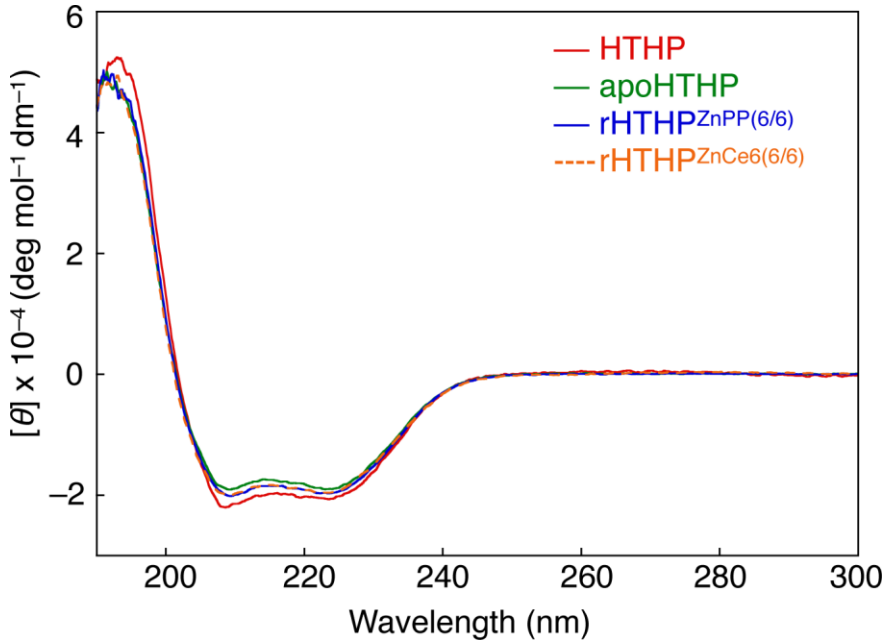

Fig. S2. CD spectra in far-UV region of HTHP, apo-HTHP, rHTHP$^{ZnPP(6/6)}$, and rHTHP$^{ZnCe6(6/6)}$ in 100 mM potassium phosphate buffer, pH 7.0, at 25 ºC.

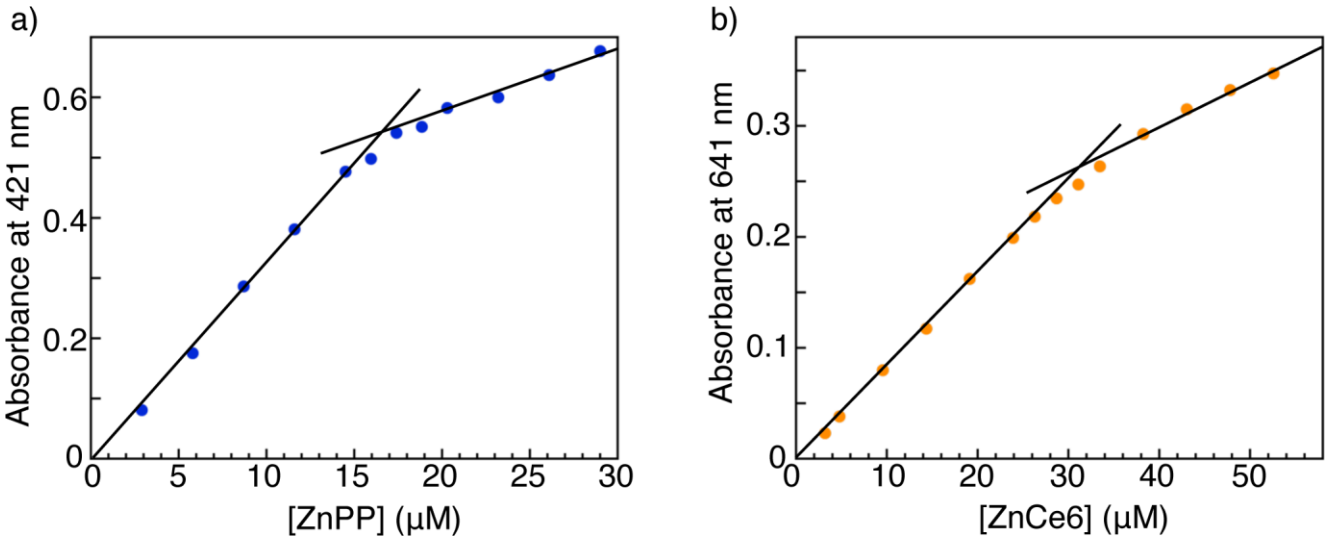

Fig. S3. (a) ZnPP and (b) ZnCe6 titration experiments of apoHTHP (17 μM and 29 μM as monomer concentrations, respectively) in 100 mM potassium phosphate buffer, pH 7.0, at 25 ºC.

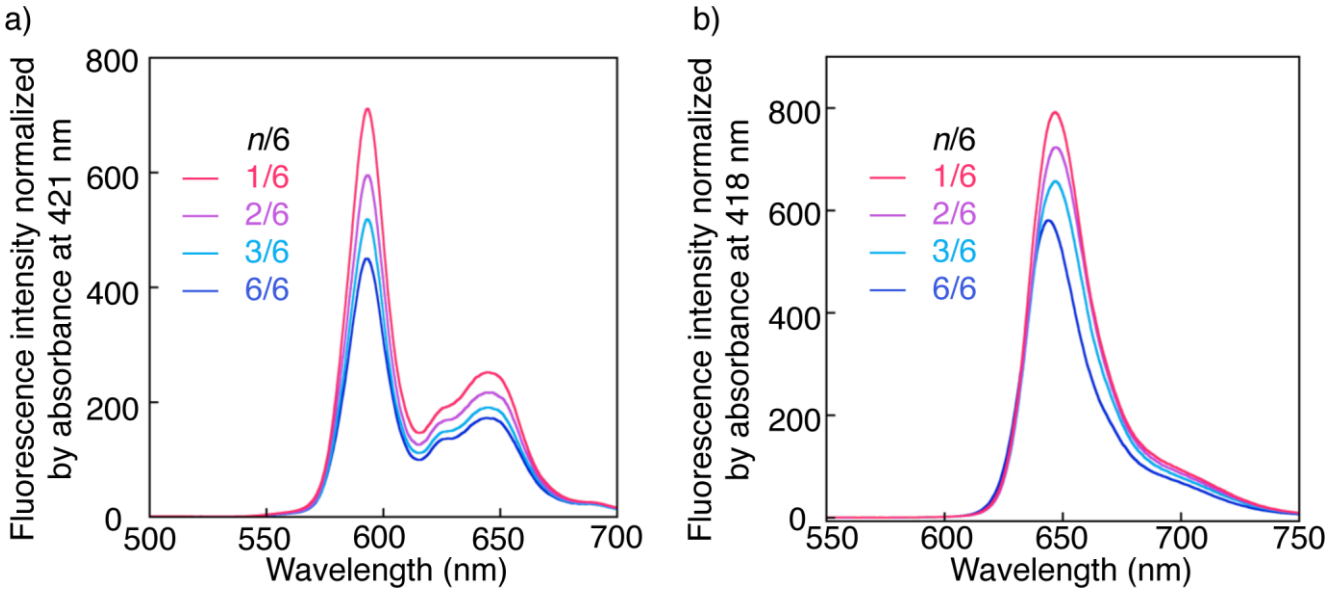

Fig. S4. Fluorescence spectra of (a) rHTHP$^{\mathrm{ZnPP}(n/6)}$ and (b) rHTHP$^{\mathrm{ZnCe6}(n/6)}$ in 100 mM potassium phosphate buffer, pH 7.0, at 25 ºC. The intensities were normalized by absorbance at each Soret peak maximum. Excitation wavelength is 421 nm or 418 nm for rHTHP$^{\mathrm{ZnPP}(n/6)}$ or rHTHP$^{\mathrm{ZnCe6}(n/6)}$, respectively.

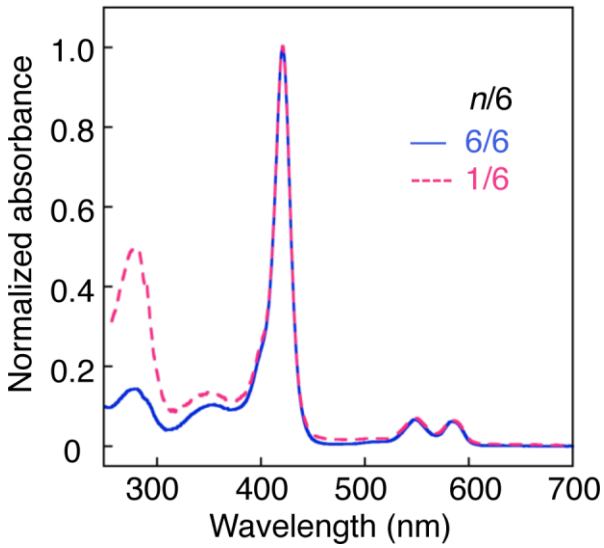

Fig. S5. UV-vis absorption spectra of rHTHP$^{ZnPP(6/6)}$ (solid blue line) and rHTHP$^{ZnPP(1/6)}$ (pink broken line) in 100 mM potassium phosphate buffer, pH 7.0, at 25 ºC.

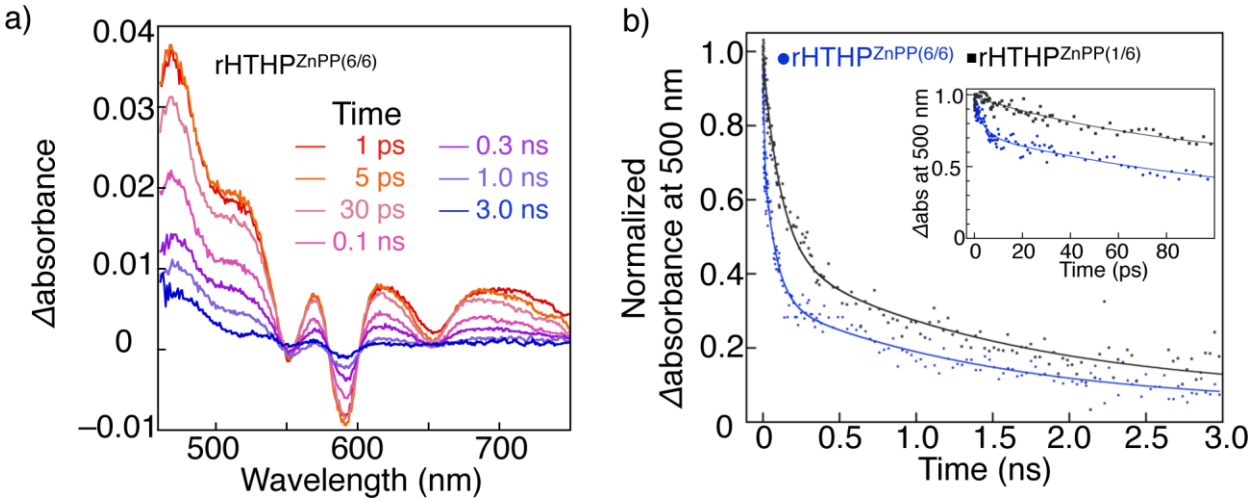

Fig. S6. (a) Transient absorption spectra of rHTHP$^{ZnPP(6/6)}$ after excitation by femtosecond pulse laser at 420 nm (fwhm = 130 fs, laser power = 24 μJ pulse^{-1}). (b) Time courses of the absorption at 500 nm after excitation for rHTHP$^{ZnPP(n/6)}$. Insets show the decay curves within 100 ps. Solid lines are triple- or double-exponentially fitted curves: $\Delta Abs(t) = y_0 + A_1\exp(-t/\tau_1) + A_2\exp(-t/\tau_2) + A_3\exp(-t/\tau_3)$ for rHTHP$^{ZnPP(6/6)}$ and $\Delta Abs(t) = y_0 + A_2\exp(-t/\tau_2) + A_3\exp(-t/\tau_3)$ for rHTHP$^{ZnPP(1/6)}$, where A and τ are population and lifetime of the decay, respectively. For τ_3, each fluorescence lifetime was applied. Conditions: [ZnPP] = 10 μM in 100 mM potassium phosphate buffer, pH 7.0, at 22 ºC under N_2 atmosphere.

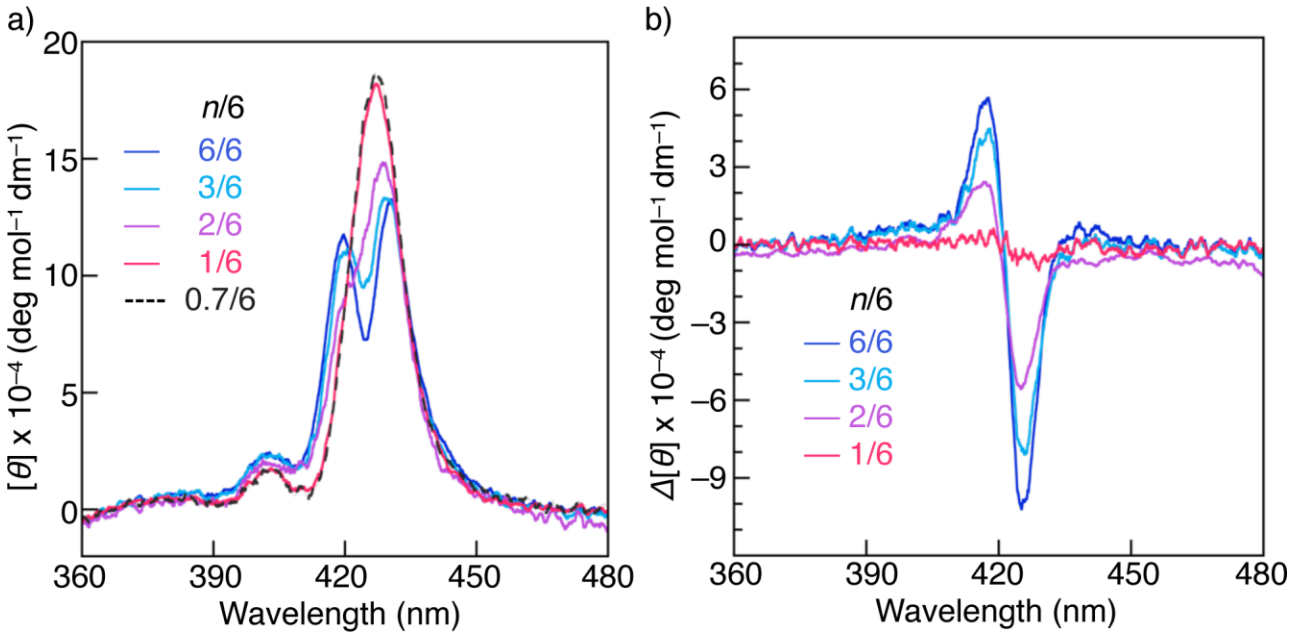

Fig. S7. (a) CD spectra in visible region for rHTHP$^{\mathrm{ZnPP}(n/6)}$. (b) Differential CD spectra generated by subtraction of rHTHP$^{\mathrm{ZnPP}(0.7/6)}$ from rHTHP$^{\mathrm{ZnPP}(n/6)}$. The samples were dissolved in 100 mM potassium phosphate buffer, pH 7.0, at 25 ºC.

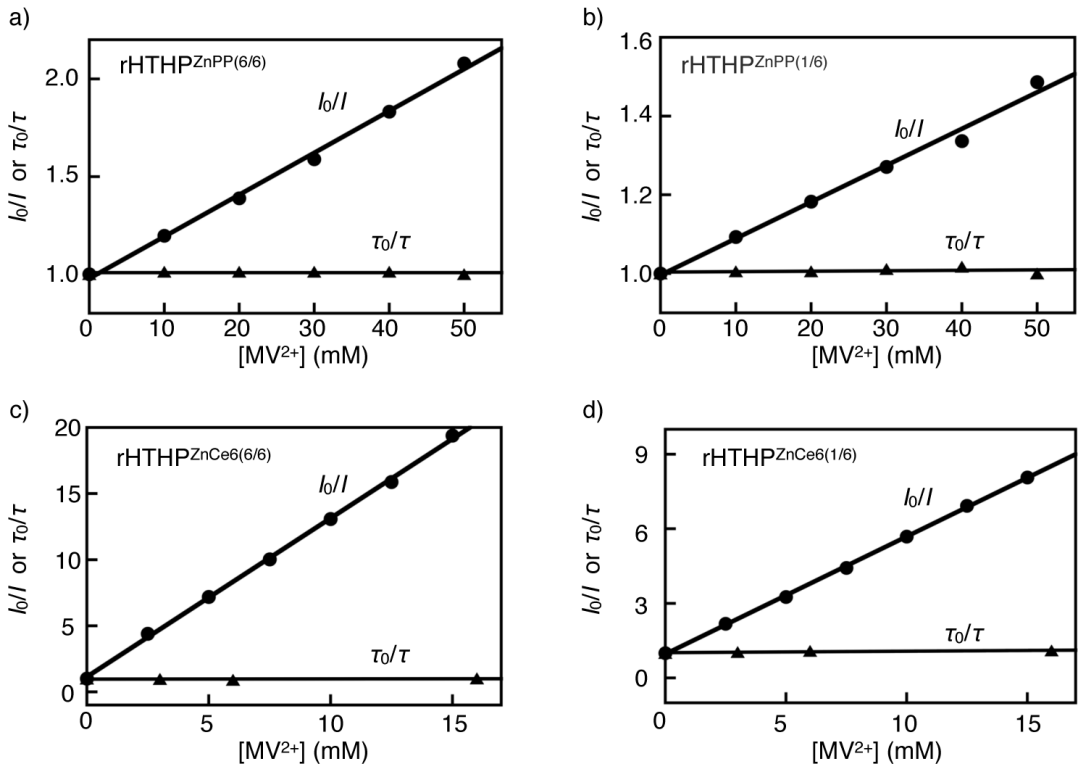

Fig. S8. Stern-Volmer plots of steady-state (closed circle) and time-resolved (closed triangle) fluorescence emissions of (a) rHTHP$^{ZnPP(6/6)}$, (b) rHTHP$^{ZnPP(1/6)}$, (c) rHTHP$^{ZnCe6(6/6)}$ and (d) rHTHP$^{ZnCe6(1/6)}$ by titration of methyl viologen. Conditions (steady-state experiments): [ZnPP] = 4.0 μM or [ZnCe6] = 2.0 μM in 100 mM potassium phosphate buffer, pH 7.0, at 25 ºC under N_2 atmosphere and λ_{ex} = 421 nm (rHTHP$^{ZnPP(n/6)}$) or 418 nm (rHTHP$^{ZnCe6(n/6)}$), λ_{em} = 592 nm (rHTHP$^{ZnPP(n/6)}$) or 645 (rHTHP$^{ZnCe6(n/6)}$). Conditions (time-resolved experiments): [ZnPP] = 3.1 μM or [ZnCe6] = 1.6 μM in 100 mM potassium phosphate buffer, pH 7.0, at 25 ºC under N_2 atmosphere, λ_{ex} = 390 nm and λ_{em} = 592 nm (rHTHP$^{ZnPP(n/6)}$) or 645 (rHTHP$^{ZnCe6(n/6)}$).

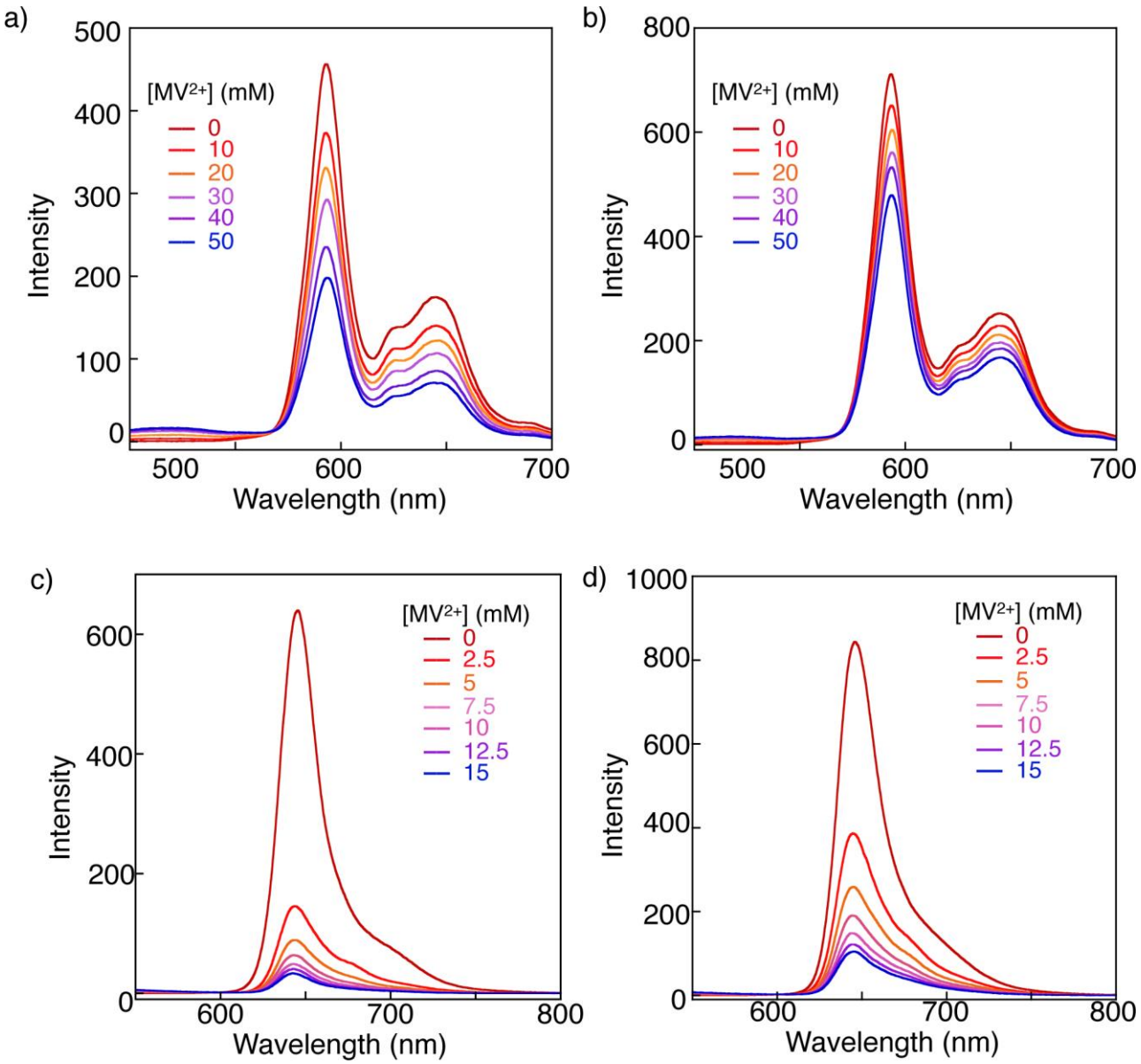

Fig. S9. Fluorescence spectra of (a) rHTHP$^{ZnPP(6/6)}$, (b) rHTHP$^{ZnPP(1/6)}$, (c) rHTHP$^{ZnCe6(6/6)}$ and (d) rHTHP$^{ZnCe6(1/6)}$ with various concentrations of methyl viologen. Conditions: [ZnPP] = 4.0 μM or [ZnCe6] = 2.0 μM in 100 mM potassium phosphate buffer, pH 7.0, at 25 ºC under N_2 atmosphere and λ_{ex} = 421 nm (rHTHP$^{ZnPP(n/6)}$) or 418 nm (rHTHP$^{ZnCe6(n/6)}$).

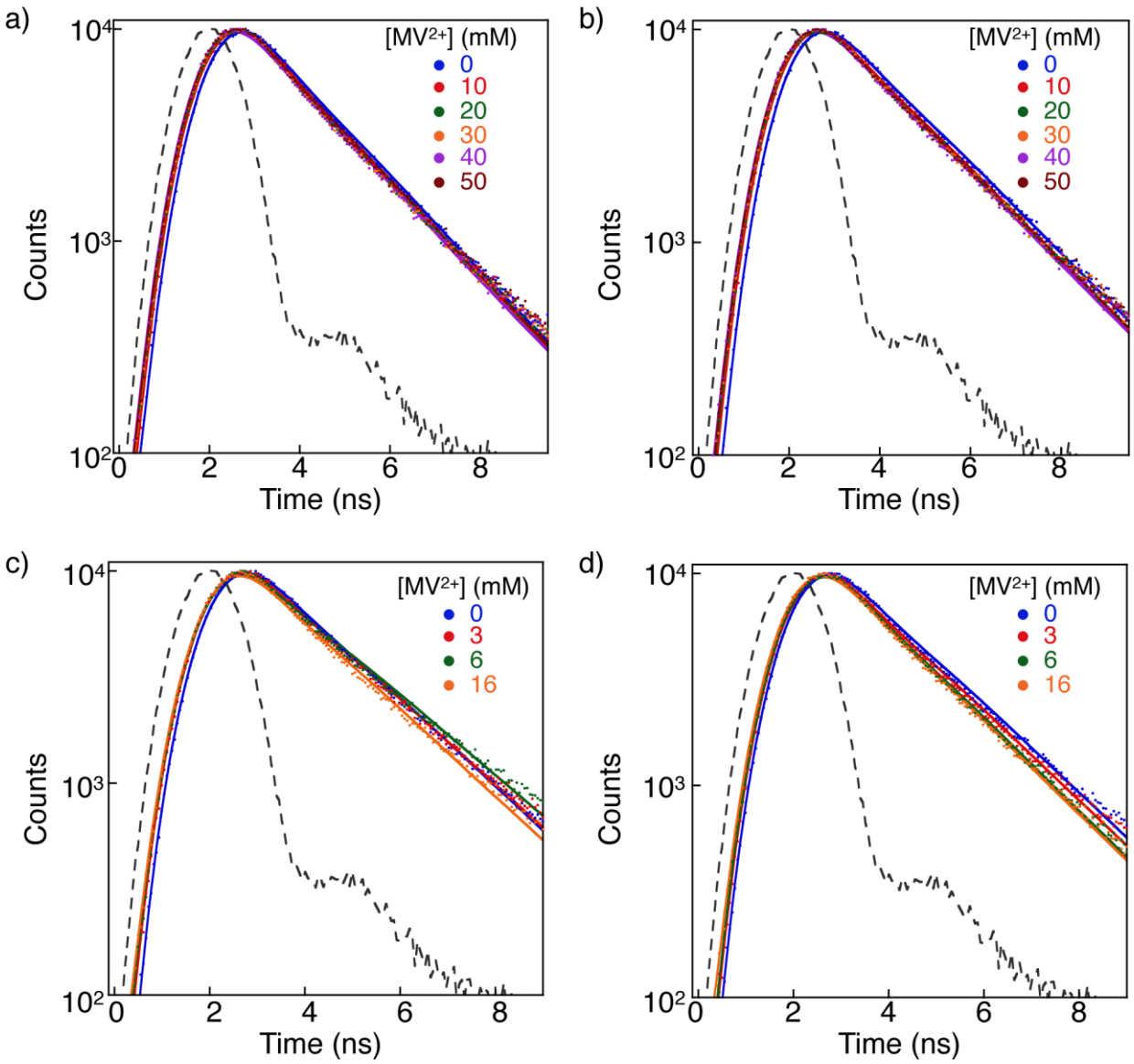

Fig. S10. Time-resolved fluorescence decay profiles of (a) rHTHP$^{ZnPP(6/6)}$, (b) rHTHP$^{ZnPP(1/6)}$, (c) rHTHP$^{ZnCe6(6/6)}$ and (d) rHTHP$^{ZnCe6(1/6)}$ with various concentrations of methyl viologen. The instrument response is shown by the black broken line. Conditions: [ZnPP] = 3.1 μM or [ZnCe6] = 1.6 μM in 100 mM potassium phosphate buffer, pH 7.0, at 25 ºC under N_2 atmosphere, λ_{ex} = 390 nm and λ_{em} = 592 nm (rHTHP$^{ZnPP(n/6)}$) or 645 (rHTHP$^{ZnCe6(n/6)}$).

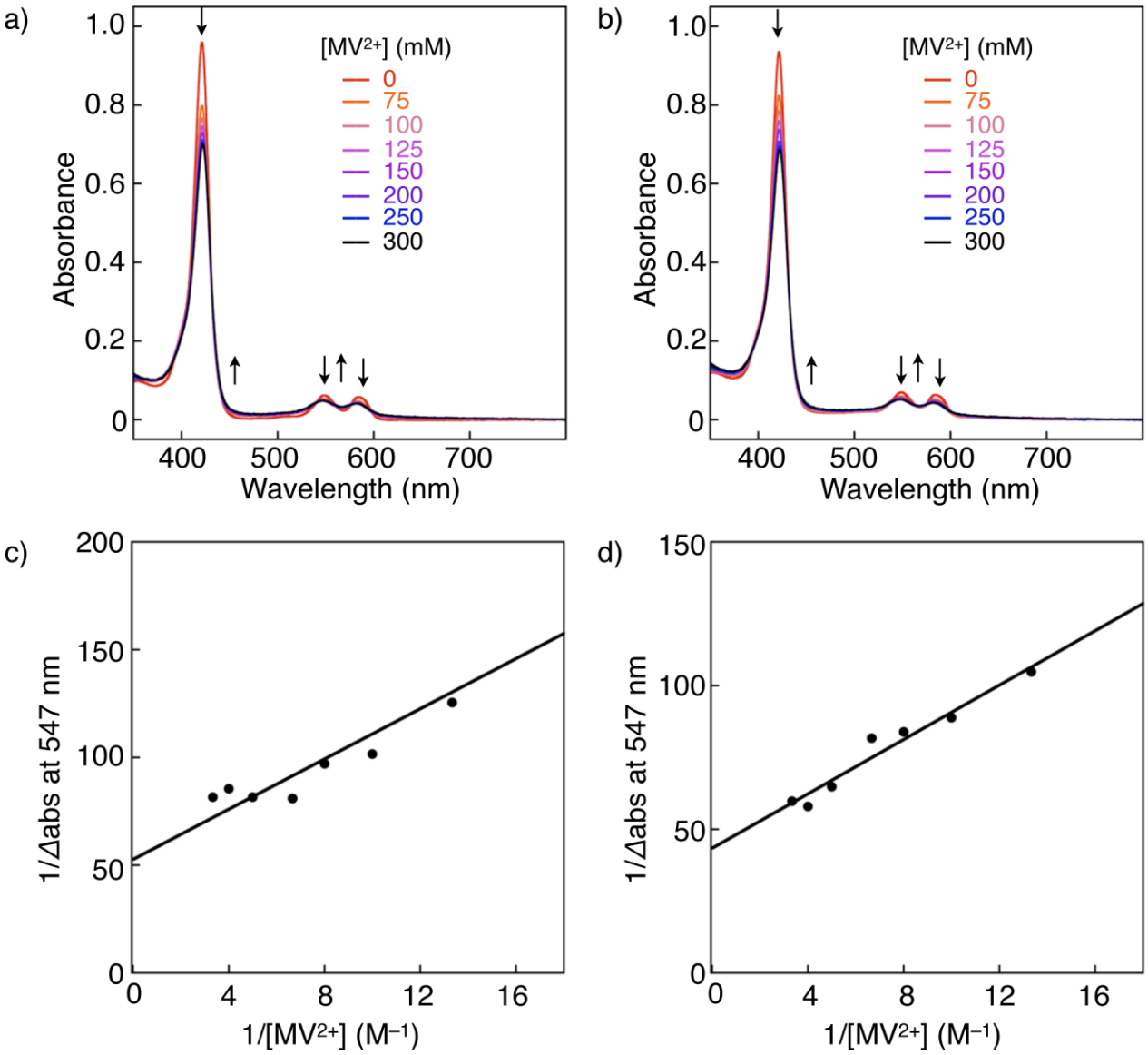

Fig. S11. UV-vis spectral changes of (a) rHTHP$^{ZnPP(6/6)}$ and (b) rHTHP$^{ZnPP(1/6)}$ upon the addition of MV^{2+} and the Benesi–Hildebrand plots of (c) rHTHP$^{ZnPP(6/6)}$ and (d) rHTHP$^{ZnPP(1/6)}$ at 547-nm absorption against various MV^{2+} concentrations. The plots were analyzed by the following equation: $(\Delta Abs)^{-1} = (K_a(\Delta\varepsilon)[ZnPP]_0[MV^{2+}])^{-1} + ((\Delta\varepsilon)[ZnPP]_0)^{-1}$, where K_a and $\Delta\varepsilon$ is the binding constant and differential extinction coefficient by subtraction of that of the MV^{2+}-free photosensitizer from that of the MV^{2+}-bound photosensitizer, respectively. Conditions: $[ZnPP]_0$ = 4.0 μM in 100 mM potassium phosphate buffer, pH 7.0, at 25 ºC.

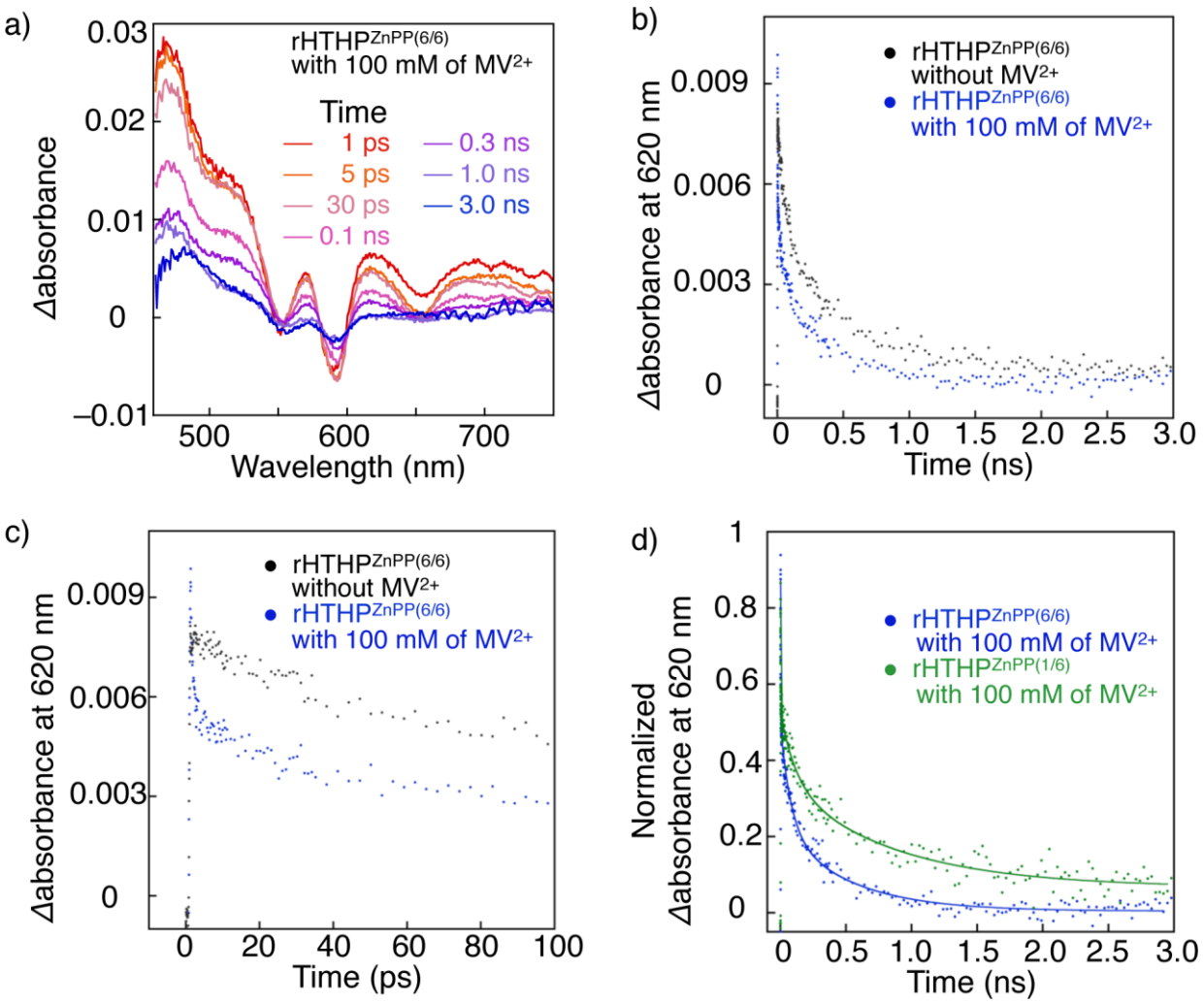

Fig. S12. (a) Transient absorption spectra of rHTHP$^{ZnPP(6/6)}$ with 100 mM of MV^{2+} after excitation by femtosecond pulse laser at 420 nm (fwhm = 130 fs, laser power = 24 μJ pulse^{-1}). (b,c) Differential absorption changes of rHTHP$^{ZnPP(6/6)}$ with 100 mM of MV^{2+} and without MV^{2+} at 620 nm. (d) Normalized absorption changes at 620 nm for rHTHP$^{ZnPP(6/6)}$ and rHTHP$^{ZnPP(1/6)}$ with 100 mM of MV^{2+}. In (d), decay plots were analyzed by triple-exponential curves: $\Delta Abs(t) = y_0 + A_1\exp(-t/\tau_1) + A_2\exp(-t/\tau_2) + A_3\exp(-t/\tau_3)$, where τ_2 for rHTHP$^{ZnPP(6/6)}$ and τ_2 and τ_3 for rHTHP$^{ZnPP(1/6)}$ were fixed in the corresponding values in Table 1. The detailed parameters are summarized in Table 2. The lifetime τ_1 is within 1 ps, which is caused by the charge transfer complex. The lifetime τ_3 for rHTHP$^{ZnPP(6/6)}$ is 0.69 ns, which is regarded as decay time for electron transfer through energy migration. Conditions: [ZnPP] = 10 μM in 100 mM potassium phosphate buffer, pH 7.0, at 22 ºC under N_2 atmosphere.

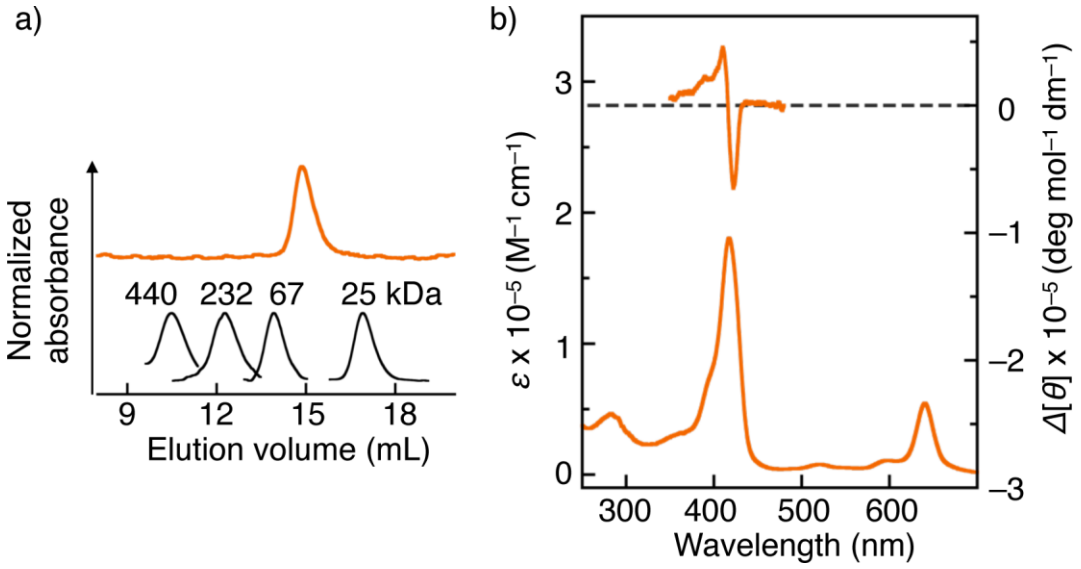

Fig. S13. (a) Analytical SEC trace and (b) UV-vis absorption and spectra of rHTHP$^{ZnCe6(6/6)}$ (orange). In (a), the black traces show various protein standards and orange and black traces were detected by absorptions at 418 and 280 nm, respectively. In (b), the upper orange line is a differential CD spectrum generated by subtraction of the CD spectrum of rHTHP$^{ZnCe6(1/6)}$ from that of rHTHP$^{ZnCe6(6/6)}$.

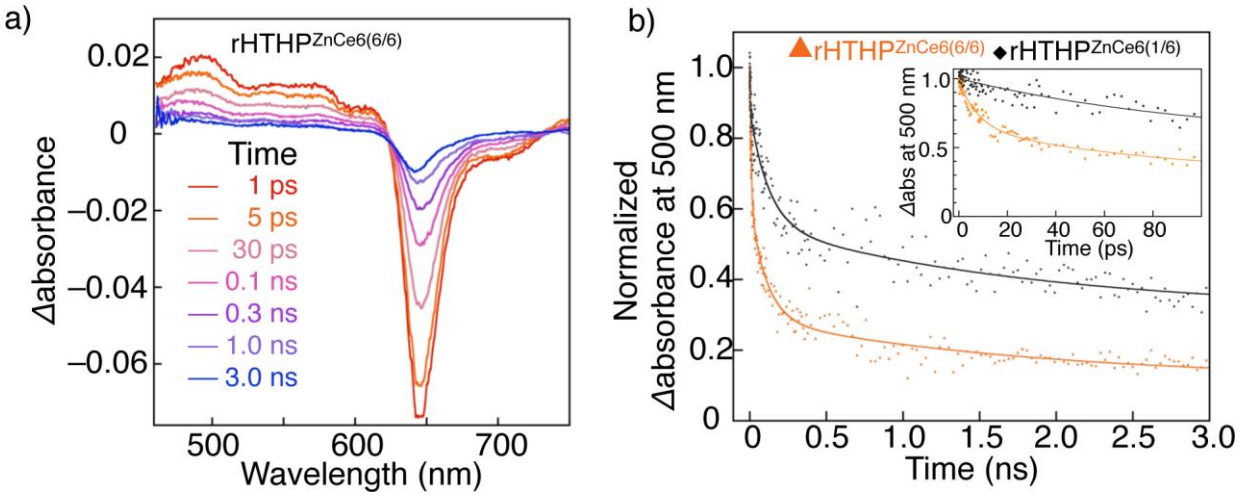

Fig. S14. (a) Transient absorption spectra of rHTHP$^{ZnCe6(6/6)}$ after excitation by femtosecond pulse laser at 420 nm (fwhm = 130 fs, laser power = 24 μJ pulse^{-1}). (b) Time courses of the absorption at 500 nm after excitation for rHTHP$^{ZnCe6(n/6)}$. Insets show the decay curves within 100 ps. Solid lines are triple- or double-exponentially fitted curves: $\Delta Abs(t) = y_0 + A_1\exp(-t/\tau_1) + A_2\exp(-t/\tau_2) + A_3\exp(-t/\tau_3)$ for rHTHP$^{ZnCe6(6/6)}$ and $\Delta Abs(t) = y_0 + A_2\exp(-t/\tau_2) + A_3\exp(-t/\tau_3)$ for rHTHP$^{ZnCe6(1/6)}$, where A and τ are population and lifetime of the decay, respectively. For τ_3, each fluorescence lifetime was applied. Conditions: [ZnCe6] = 14 μM in 100 mM potassium phosphate buffer, pH 7.0, at 22 ºC under N_2 atmosphere.

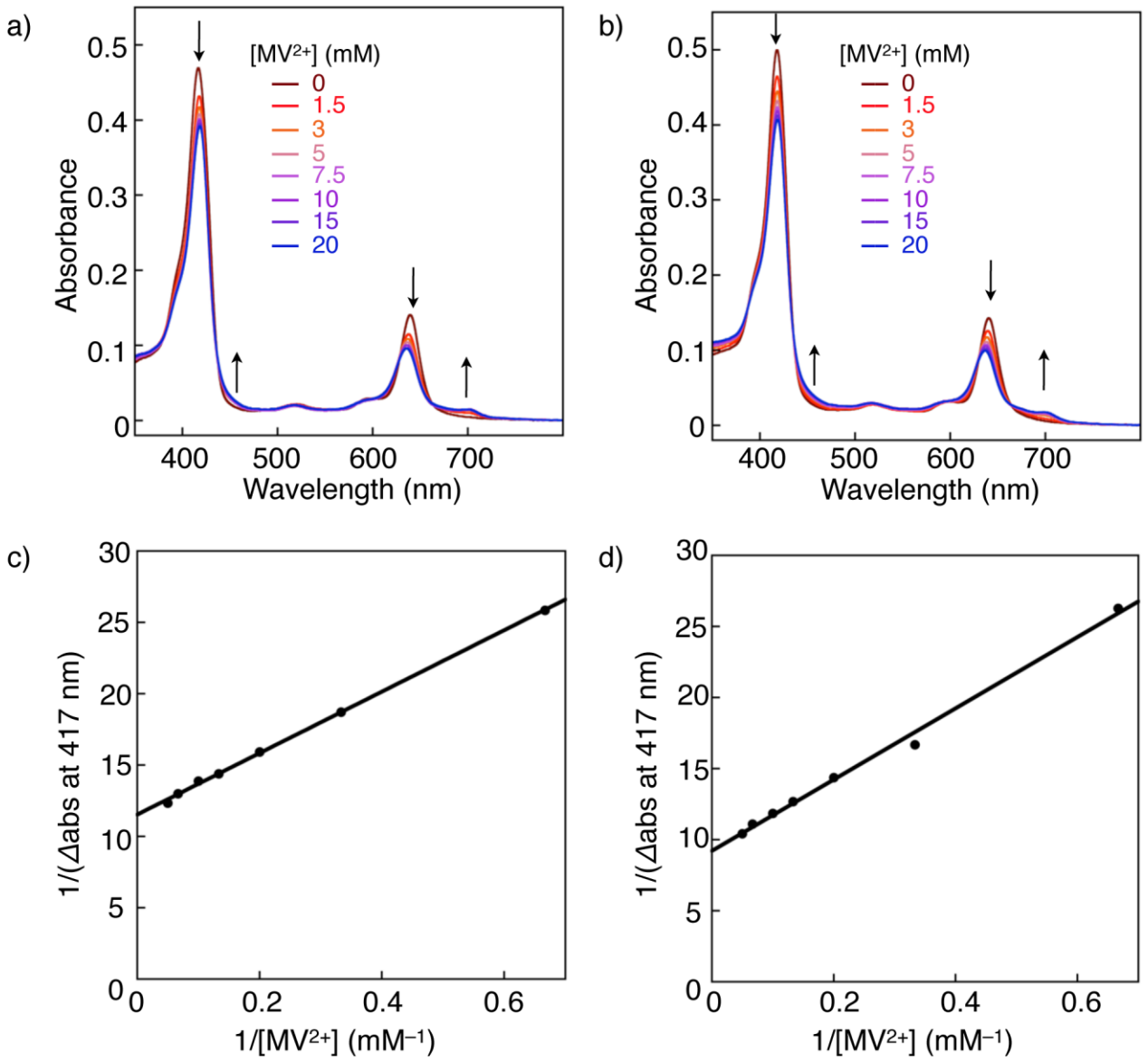

Fig. S15. UV-vis spectral changes of (a) rHTHP$^{ZnCe6(6/6)}$ and (b) rHTHP$^{ZnCe6(1/6)}$ upon the addition of MV^{2+} and the Benesi–Hildebrand plots of (c) rHTHP$^{ZnCe6(6/6)}$ and (d) rHTHP$^{ZnCe6(1/6)}$ at 417-nm absorption against various MV^{2+} concentrations. The plots were analyzed by the following equation: $(\Delta Abs)^{-1} = (K_a(\Delta\varepsilon)[ZnCe6]_0[MV^{2+}])^{-1} + ((\Delta\varepsilon)[ZnCe6]_0)^{-1}$, where K_a and $\Delta\varepsilon$ is the binding constant and differential extinction coefficient by subtraction of that of the MV^{2+}-free photosensitizer from that of the MV^{2+}-bound photosensitizer, respectively. Conditions: $[ZnCe6]_0$ = 2.7 and 2.8 μM for rHTHP$^{ZnCe6(6/6)}$ and (b) rHTHP$^{ZnCe6(1/6)}$, respectively, in 100 mM potassium phosphate buffer, pH 7.0, at 25 ºC.

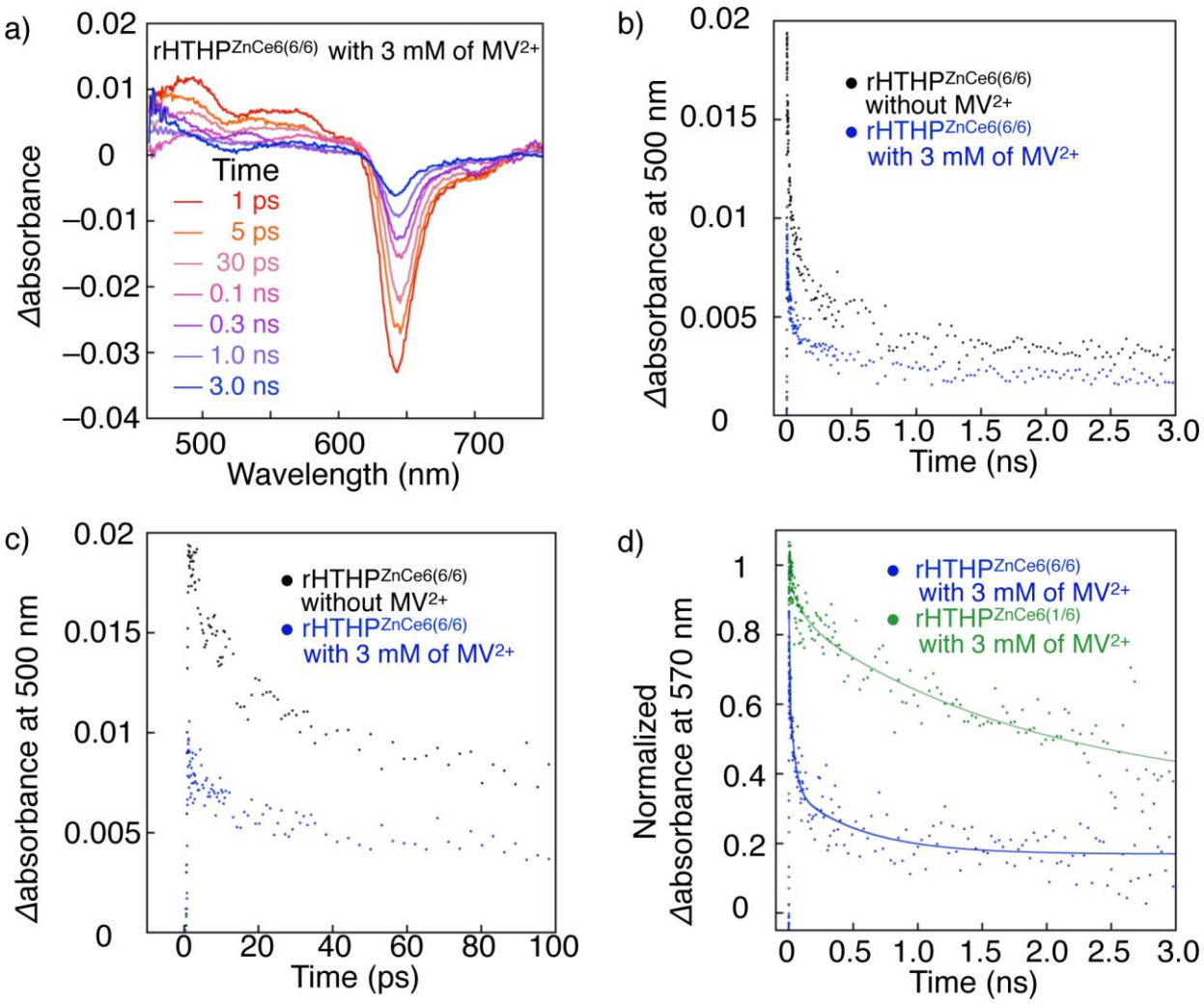

Fig. S16. (a) Transient absorption spectra of rHTHP$^{ZnCe6(6/6)}$ with 3 mM of MV^{2+} after excitation by femtosecond pulse laser at 420 nm (fwhm = 130 fs, laser power = 24 μJ pulse^{-1}). (b,c) Differential absorption changes of rHTHP$^{ZnCe6(6/6)}$ with 3 mM of MV^{2+} and without MV^{2+} at 570 nm. (d) Normalized absorption changes at 570 nm for rHTHP$^{ZnCe6(6/6)}$ and rHTHP$^{ZnCe6(1/6)}$ with 3 mM of MV^{2+}. In (d), decay plots were analyzed by triple-exponential curves: $\Delta Abs(t) = y_0 + A_1\exp(-t/\tau_1) + A_2\exp(-t/\tau_2) + A_3\exp(-t/\tau_3)$, where τ_2 for rHTHP$^{ZnCe6(6/6)}$ and τ_2 and τ_3 for rHTHP$^{ZnCe6(1/6)}$ were fixed in the corresponding values in Table 1. The detailed parameters are summarized in Table 2. The lifetime τ_1 is within 1 ps, which is caused by the charge transfer complex. The lifetime τ_3 for rHTHP$^{ZnCe6(6/6)}$ is 0.54 ns, which is regarded as decay time for electron transfer through energy migration. Conditions: [ZnCe6] = 14 μM in 100 mM potassium phosphate buffer, pH 7.0, at 22 ºC under N_2 atmosphere.

Table S1. Hydrodynamic diameters estimated from dynamic light scattering measurements[a]

	HTHP	apoHTHP	rHTHP$^{ZnPP(6/6)}$	rHTHP$^{ZnCe6(6/6)}$
Hydrodynamic radius (nm)	5.4	6.2	5.6	5.6

[a]Protein samples were dissolved in 100 mM potassium phosphate buffer, pH 7.0, at 25 ºC.

Table S2. Transient absorption decay parameters for evaluation of annihilation[a,b]

Protein	τ_1 (ps)	τ_2 (ps)[c]	τ_3 (ns)[d]
rHTHP$^{ZnPP(6/6)}$	4 [23%]	79 [46%]	1.4 [31%]
rHTHP$^{ZnPP(1/6)}$	n.d.	117 [58%]	1.6 [42%]
rHTHP$^{ZnCe6(6/6)}$*e*	9 [36%]	106 [33%]	1.9 [17%]
rHTHP$^{ZnCe6(1/6)}$*e*	n.d.	122 [35%]	1.9 [27%]

[a]Parameters are derived from the fitting curves in Fig. S6 and S14. Populations are shown as the percentage in square brackets. [b]Excitation laser power = 24 μJ pulse^{-1}. [c]It is a challenging effort to assign the decays relative to τ_2. However, the decays may be caused by water-induced vibrational relaxation[S3] or electron transfer between the photosensitizer molecule and the ligated Tyr residue in the heme binding site.[S4] [d]Fluorescence lifetime. [e]The residual population is assigned as the slow decay over the experimental range.

Table S3. Transient absorption decay parameters under the conditions with methyl viologen[a,b]

Protein	τ_1 (ps)	τ_2 (ps)[d]	τ_3 (ns)
rHTHP$^{ZnPP(6/6)}$	<1 [32%]	79 [22%]	0.69 [46%]
rHTHP$^{ZnPP(1/6)}$	<1 [30%]	117 [29%]	1.6 [e] [39%]
rHTHP$^{ZnCe6(6/6)}$*f*	<1 [28%]	106 [33%]	0.54 [24%]
rHTHP$^{ZnCe6(1/6)}$*f*	<1 [8%]	122 [10%]	1.9 [e] [28%]

[a]Parameters are derived from the fitting curves in Fig. S12 and S16. Populations are shown as the percentage in square brackets. [b]Excitation laser power = 24 μJ pulse^{-1}. The concentrations of MV^{2+} were 100 mM and 3 mM for rHTHP$^{ZnPP(n/6)}$ and rHTHP$^{ZnCe6(n/6)}$, respectively. [d]τ_2 was restricted by each value under the condition without MV^{2+}. [e]Fluorescence lifetime. [f]The residual population is assigned as the slow decay over the experimental range.

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