CHEMPHOTOCHEM

Supporting Information

A Semisynthetic Peptide–Metalloporphyrin Responsive Matrix for Artificial Photosynthesis

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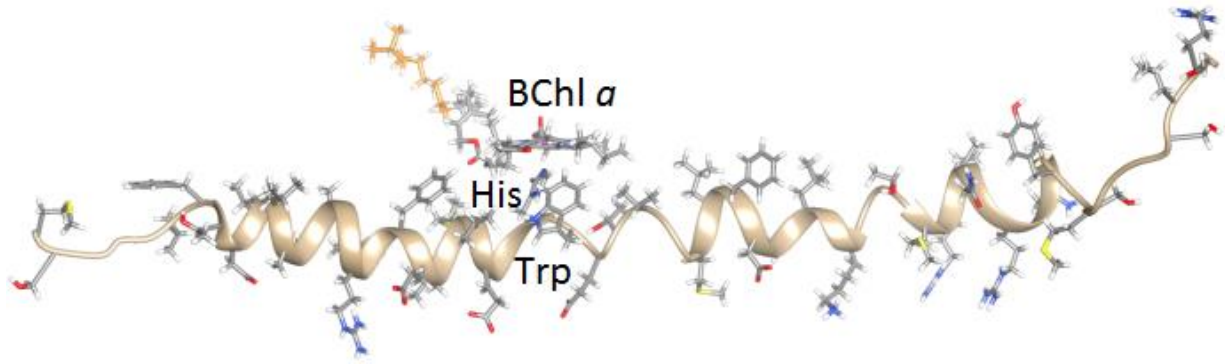


SFigure 1. The structure of the CsmA-BChl a complex from the baseplate (PDB 5LCB).

S1. Complex constitution

The peptide was synthesized using standard solid-phase Fmoc based chemistry and purified by Reverse phase High-performance liquid chromatography (RP-HPLC). To ensure the similar biological activity with natural CsmA, the modification of N-Terminal Acetylation and C-Terminal Amidation were included. The sequence of the peptide is:

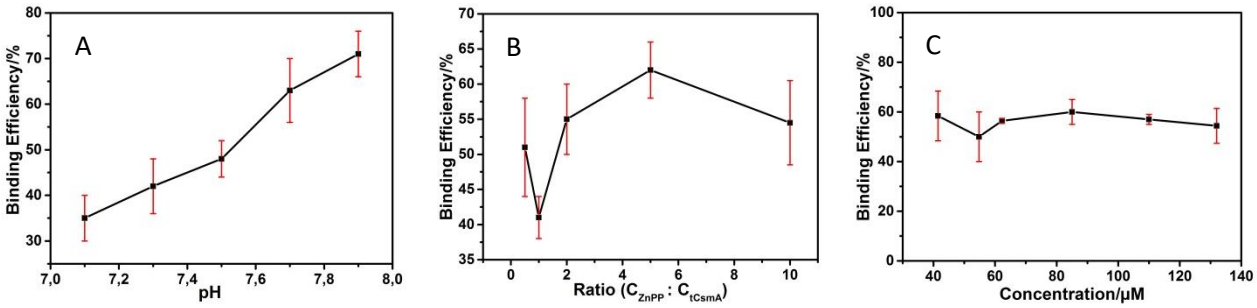
Ac-FTDILAAAGRIFEVMVEGHWETVGMLFDSLGKGTMRINRNAYG-NH_2

The complex formation was optimized by testing different conditions in the dialysis buffer containing 50 mM potassium phosphate (SFigure 1 A-C). CsmA protein was analysed using 17.5 % SDS gels containing Tris-Tricine gel buffers.

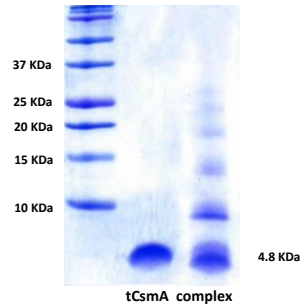
The binding efficiency was calculated by equation 1.

$$E = A_{430} \times V_{final} \ / \ (C_{tCsmA} \times V_{original} \times \epsilon_{430}) \quad (eq.1)$$

A_{430} is the 430 nm UV absorption of complex, V_{final}=130 µL, $V_{original}$=100 µL (after dialysis, the volume increased.), C_{tCsmA} is the concentration of tCsmA in 6M 1-propanol pH 7.9 50 mM Potassium Phosphate buffer, ϵ_{430} is the molar extinction coefficient of complex at 430 nm (see S2).

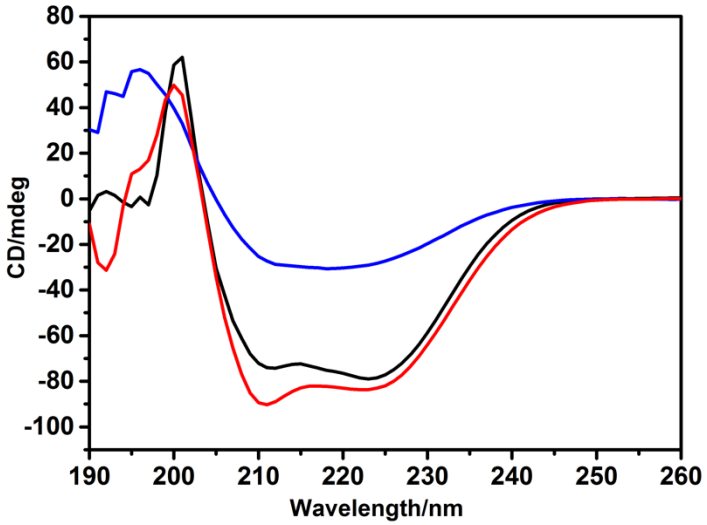


SFigure 2: Optimization of the binding between CsmA and ZnPP during dialysis. A: The influence of the pH of KPi buffer (C_{tCsmA}=70 µM, C_{ZnPP}=350 µM). B: The influence of the ratio of CsmA and ZnPP in KPi pH 7.9 buffer (C_{tCsmA}=50 µM). C: The influence of the tCsmA concentration in KPi pH 7.9 buffer (C_{tCsmA} : C_{ZnPP}=1 : 5).



SFigure 3. Tricine-SDS gel analysis of the complex and tCsmA, prior loading all the samples were boiled for 4 min. Lane 1: Marker, lane 2: 5 µg tCsmA, lane 3: 10 µL 80 µM ZnPP-CsmA complex.

S2. Secondary structure



SFigure 4. CD spectra. black: 50 µM tCsmA-ZnPP complex in pH 7.9 50 mM potassium phosphate buffer; red: 50 µM tCsmA in pH 7.9 6 M 1-propanol 50 mM potassium phosphate buffer. Both CD signals are characteristic for a helical secondary structure. In contrast, while the tCsmA possesses a maximum 50 µM solubility in MQ water, it shows a denatured β-sheet structure, and the binding cannot proceed in this condition (blue line). Samples were measured in quartz cuvettes with a 2 mm path length. Spectra were recorded from 260 to 190 nm at 1 nm intervals with a 1 nm bandwidth at 25 °C.

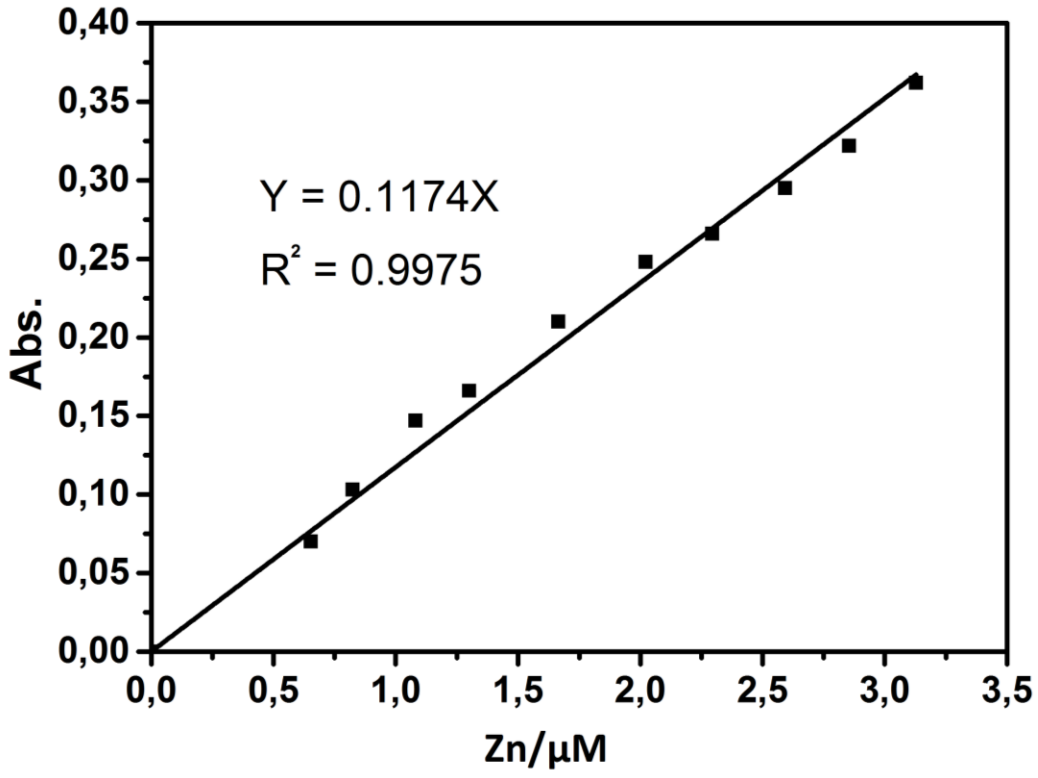
S3. The stoichiometry of tCsmA-ZnPP complex

Different concentrations of the tCsmA-ZnPP complex was prepared for a standard curve representing the referred relationship between the absorption reading of complex at 430 nm and Zn concentration determined by ICP-OES (Stable 1). ICP-OES is a type of emission spectroscopy that uses the inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wavelengths characteristic of a particular element. It is a flame technique with a flame temperature in a range from 6000 to 10000 K. The intensity of this emission is indicative of the concentration of the element within the sample. SFigure 3 represents a standard curve obtained using complex and its Zn concentration determined by ICP-OES. A linear relationship between Zn concentration determined by ICP-OES and the absorbance reading at 430 nm of complex is presented. The complex was dissolved in KPi buffer.

STable 1: The concentration of zinc measured by ICP-OES with corresponding UV absorption at 430 nm

Zn (C,µM) Measured by ICP- OES	Complex Abs. at 430nm
0.06295	0.070
0.82441	0.103
1.08106	0.147
1.30101	0.166
1.66412	0.210
2.02203	0.248
2.29428	0.266
2.59254	0.295
2.85408	0.322
3.12787	0.362

The molar extinction coefficient (ε) can be determined using equation: $\varepsilon = A / c \cdot l$, which was converted based on the Beer-Lambert law, $A = \varepsilon \cdot c \cdot l$, where A represents an actual absorption at the given wavelength, path length l is 1 cm in our study, and the molar concentration *c* was obtained from Zn concentration (C_{Zn}, µM) determined by ICP-OES. The molar extinction coefficient was calculated to be 117400 M^{-1} cm^{-1}.



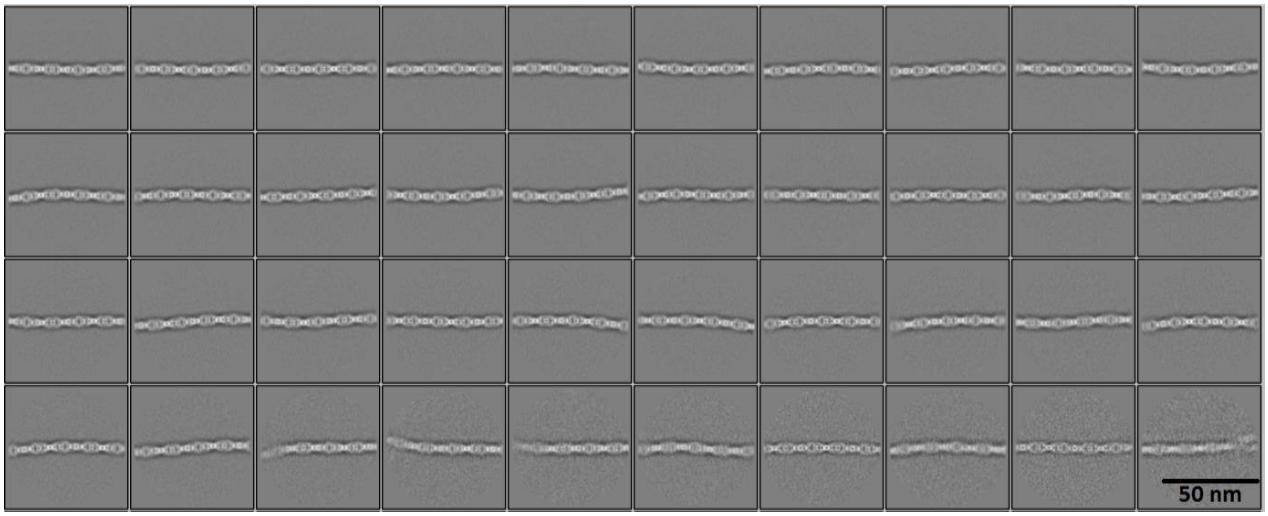
SFigure 4: Standard curve represents the relationship between the Zn concentration in CsmA - ZnPP determined by ICP-OES and its absorbance maximum (λ = 430 nm).

Combining BCA assay with the molar extinction coefficient of complex, we can calculate the stoichiometry of complex (STable 2).

STable 2: The amount of protein in the complex was determined using the colorimetric BCA protein assay. The stoichiometry of complex can be calculated from the ratio of the protein concentration to the concentration of Zn.

ZnPP (C, µM) Measured from A430 nm	tCsmA (C, µM) Measured by BCA assay	Ratio C_{ZnPP} / C_{tCsmA}
13.8	13.6	1.01
8.5	8	1.06
3.3	3.5	0.94

S4 cryo-EM

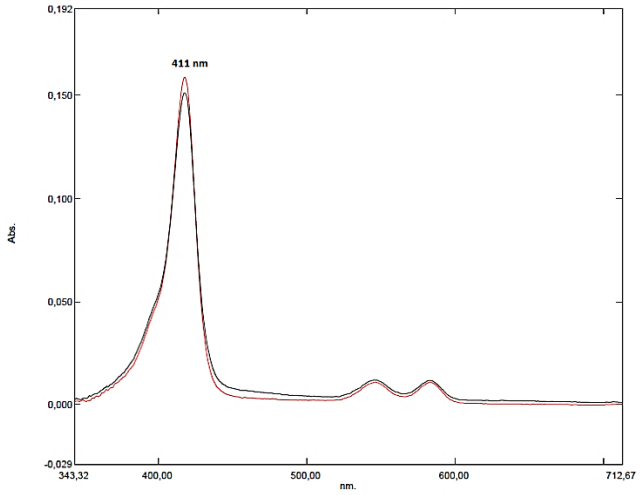


SFigure 6. 50 classes 2D classification from 99142 particles by RELION2.0.

S5. Linear dichroism spectroscopy (LD)

STable 3: Preparation of the squeezing gel

Components	Volume (µL)
Glycerol (100%)	1500
37% (29:1) acrylamide	405
APS (10%)	30
TEMED	4.5
Complex (50 µM)	500
KPi buffer (3M, pH 7.9)	41.5
MQ water	519
Total	3000

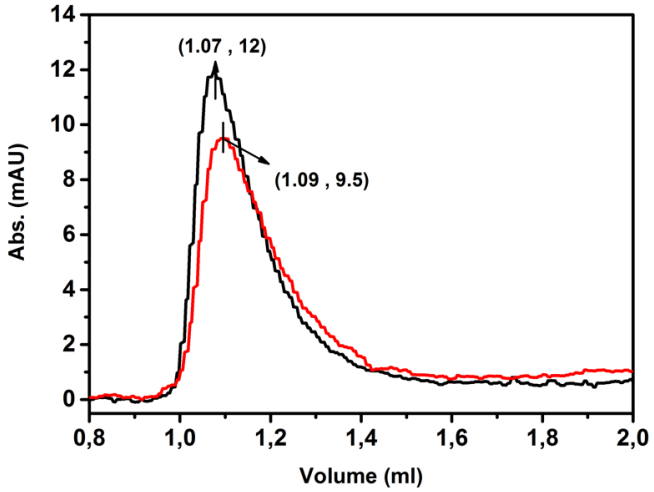


SFigure 7. UV-Vis spectra of the ZnPP-tCsmA complex after two hours in squeezing gel (black trace) and pure ZnPP (red trace) in squeezing gel. For both samples the major response is at 411 nm.

S6. Photostability

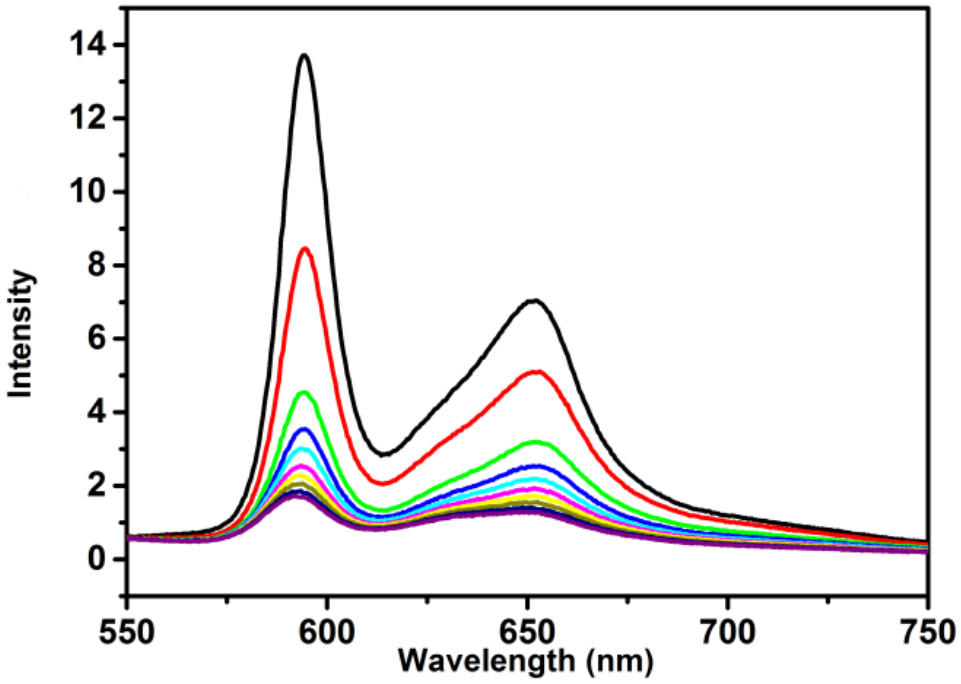
S6.1. Size-exclusion chromatography

The absorbance at 280 nm (in mAU) is plotted against the elution volume (Ve / mL). The column was calibrated using reference markers: Blue Dextran (BD: Ve= 1.1 mL, MW= 2000 KDa), Bovine Serum Albumin (BSA: Ve= 1.8 mL, MW= 66.5 KDa), Carbonic Anhydrase (CAH, Ve= 2.1 mL, MW= 29 KDa, Cytochrome C: Ve= 2.3 mL, MW= 12.4 KDa), β-Mercaptoethanol (Me: Ve= 2.6 mL, MW= 78 D).



SFigure 8. SEC elution spectra collected from the 50 µM tCsmA-ZnPP complex in pH 7.9 50 mM potassium phosphate buffer (black trace) and the same sample after 30 minutes of light exposure (red trace). The data indicates a small shift, which can be attributed to a decrease of the size upon exposure to light, while the signal decrease is consistent with the degradation upon prolonged exposure to light observed with fluorescence spectroscopy (SFigure 6)

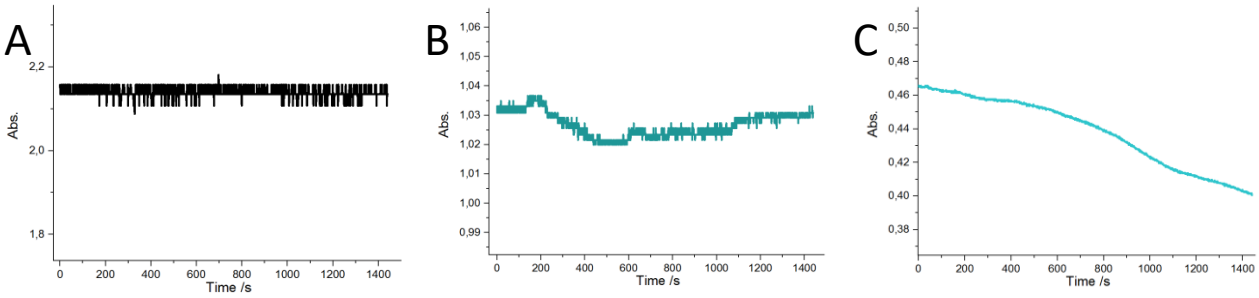
S6.2. Fluorescence spectroscopy



SFigure 9. Fluorescence spectroscopy of the tCsmA-ZnPP complex. The complex was excited at 430 nm for 5 minutes and followed 15 minutes incubation time. The concentration of the complex is 1.6 µM in pH 7.9 50 mM potassium phosphate buffer.

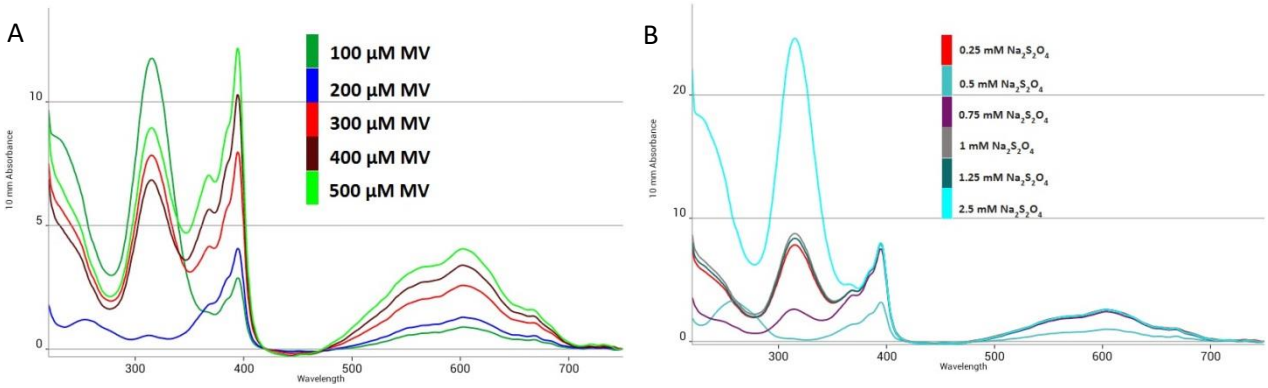
S6.3. UV-Vis spectroscopy

The tCsmA-ZnPP complex was excited at 430 nm with a 1 s cycle time. The experiments were perform by UV-1700 Spectrophotometer (Shimadzu) under the kinetics mode.



SFigure 10. UV-Vis spectra of the ZnPP-tCsmA complex with different concentration. A : 18 µM; B : 9 µM; C : 4.5 µM.

S7. Redox reactions

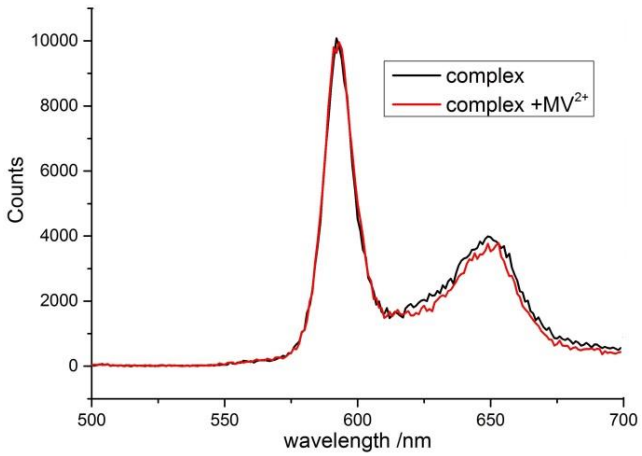


SFigure 11. A : the UV spectra of MV with different concentration with 1.25 mM sodium dithionite in the solution. The ratio of A396 nm/A606 nm is about 3 which is same with the ratio in spectra of MV^+ in SFigure 11B. The peak 606 nm only comes from MV^+ (see SFigure 11B), whereas the peak 396 nm can come from MV^+ and MV^0. That means the sodium dithionite only converts MV to MV^+. If there is MV^0, the MV^0 will contribute the UV absorption at 396 nm, in this case, the ratio A396 nm/A606 nm should be larger than 3. The reaction was also reported in literature.
B: the UV spectra of 300 µM MV with different amounts of sodium dithionite. Adding more excess sodium dithionite doesn't improve the conversion rate. The MV can be saturated at about 60% conversion rate.

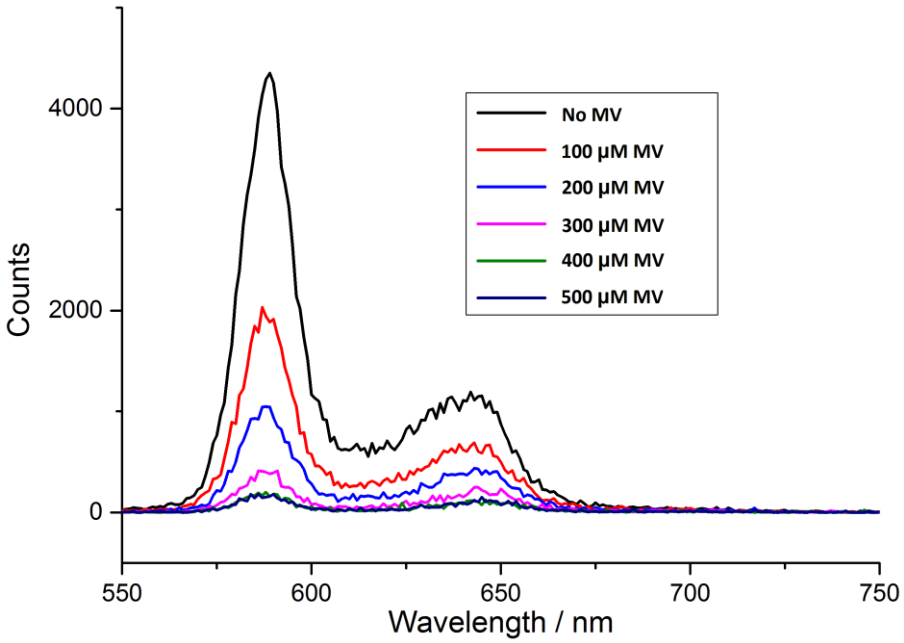
STable 4: The conversion rate of MV^+ (The concentration of sodium dithionite is 1.25 mM, ε606 nm of MV^+ is 13700 M^{-1} cm^{-1}), the data is from the results of SFigure 12B.

MV (µM)	Abs. 606 nm	MV^+ (µM)	MV^+/MV (%)	A396 nm/A 606 nm
100	0.88	64	64	3.1
200	1.27	93	47	3.09
300	2.56	187	62	3.07
400	3.35	245	61	3.03
500	4.02	293	59	3.02

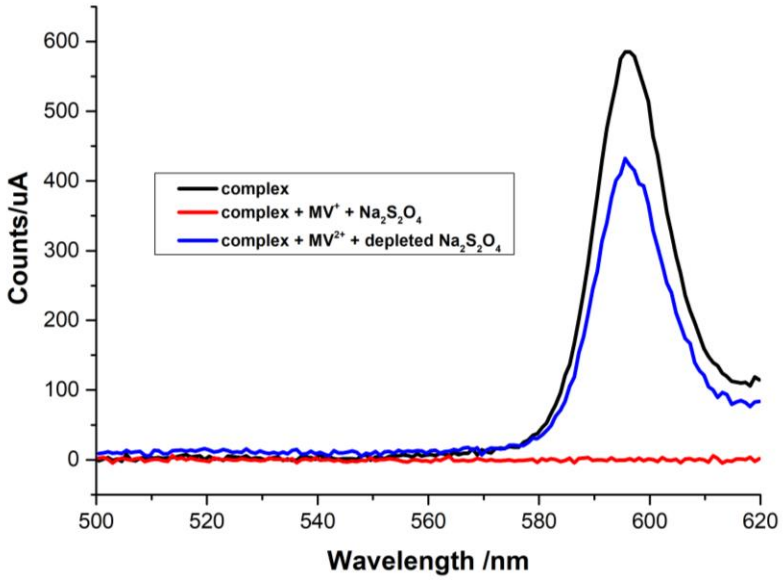
S8. Fluorescence spectroscopy



SFigure 12. Steady state fluorescence spectra of 4.5 µM tCsmA-ZnPP complex and 4.5 µM tCsmA-ZnPP complex with 500 µM MV in KPi buffer with 430 nm excitation light.

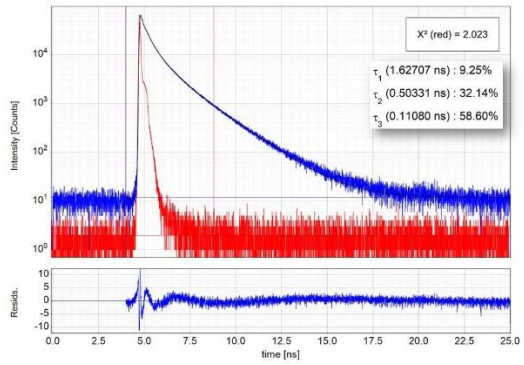
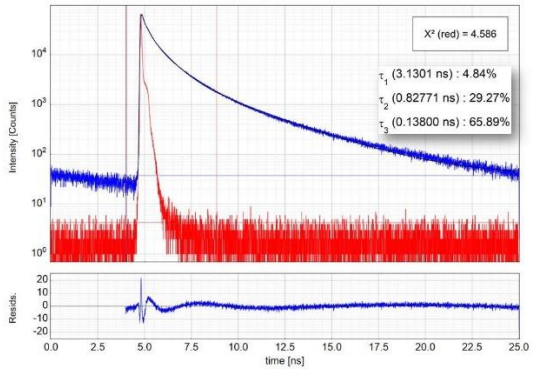
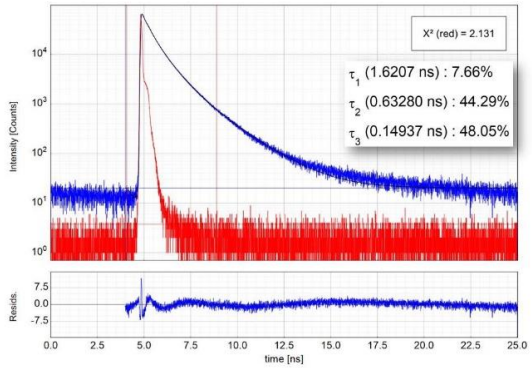
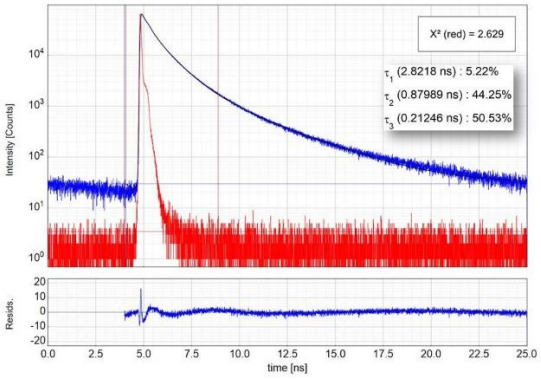


SFigure 13. Steady state fluorescence spectra of 3.5 µM tCsmA-ZnPP complex with different concentration of MV and 1.25 mM sodium dithionite in KPi buffer with 430 nm excitation light.



SFigure 14. Steady state fluorescence spectra of 3.5 µM tCsmA-ZnPP complex (black line), 3.5 µM tCsmA-ZnPP complex with MV and the depleted 1.25 mM sodium dithionite (red line) and 3.5 µM tCsmA-ZnPP complex with MV and the depleted 1.25 mM sodium dithionite (blue line) in KPi buffer with 440 nm excitation light. The decreased intensity of recovered sample (blue line) may be caused by photodissociation or photodegradation due to the continued photo-excitation.

STable 5: Raw data, the corresponded time resolved data and kinetic fits for Table 1 in the article.

Additives	594 nm	650 nm
No MV and $Na_2S_2O_4$		
MV^{2+} (no $Na_2S_2O_4$)		
MV^{+} (excess $Na_2S_2O_4$)