Supporting Information

Enhancing Photocatalytic CO_2 Reduction to Formate through One-Pot Self-Assembly of a Semiartificial Cell

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Experimental section

Materials

Formate dehydrogenase from C. boidinii (FDH, EC 1.2.1.2) was purchased from Shanghai Chaoyan Biotechnology Co., Ltd. 2-bromoethylammonium bromide, 4,4'-bipyridine, tetrakis (4-carboxyphenyl) porphyrin, Zinc chloride, – L-Cysteine, 2,3,4,5,6-pentafluorobenzylbromide and 5-Carboxyfluorescein N-succinimidyl ester (FITC) were received from Aladdin (Shanghai, China). Sodium hydroxide, Tris (hydroxymethyl) aminomethan, Sodium dihydrogen phosphate dehydrate, Sodium phosphate dibasic dodecahydrate were purchased from Sinopharm Chemical ReagentCo., Ltd. FITC was provided by KeyGen Biotech. Co., Ltd. Acetone, acetonitrile, triethylamine, hexane, methanol (CH_3OH), concentrated hydrochloric acid (HCl), dichloromethane (CH_2Cl_2), N, N-dimethylformamide (DMF) and triethanolamine (TEOA) were purchased from Ling-Feng (Shanghai, China). Tris-HCl buffer solution (0.05 M) was prepared by adjusting pH to 7 with HCl. All the reagents were used as received without further purification. Deionized water was prepared using a Milli-Q purification system with a resistivity of 18.2 MΩ.

Instrumentation

^{1}H-NMR spectra were collected from a Bruker 600 MHz spectrometer (Bruker, German). Absorbance measurement was performed by a 2450 UV-visible spectrophotometer (Shimadzu, Japan). UV–Vis diffuse reflectance spectroscopy (DRS) was recorded on a Shimadzu UV–vis spectrophotometer (UV-2450) with BaSO4 as the background. Fluorescence (FL) spectra was carried out on a FluoroMax-4 spectrofluorometer with xenon discharge lamp excitation (HORIBA, USA). The ultrafast transient absorption (TA) spectroscopy was carried out on a TA100-1030nm-NIR-MIC spectroscopy (TIME-TECH SPECTRA, Dalian). The morphology of Cys (Zn) and Cys (Zn)/D-TCPP/FDH was characterized using scanning electron microscopy (SEM, Nova Nano SEM450, USA). X-ray diffraction (XRD) patterns of Cys (Zn), (Zn)/DV/TCPP/FDH and (Zn)/D-TCPP/FDH were recorded by a Rigaku Ultima IV X-ray diffractometer (Japan). X-ray photoelectron spectroscopy was carried out with Thermo ESCALAB 250XI (Thermo Scientific, USA), performed with Al Kα

radiation (λ = 0.8339 nm). The secondary protein structure of FDH were monitored by circular dichroism (CD, ABSCIEX-api 3000, Applied Photophysics, UK). For photocurrent-time (I-T) experiments, FTO glass was cut into 1 cm × 3 cm pieces. A 1 mg of Cys(Zn)/TCPP, Cys(Zn)/DV/TCPP, Cys(Zn)/TCPP+DV, Cys(Zn)/D-TCPP or Cys(Zn)/D-TCPP/FDH was mixed with 200 μL of Nafion solution (1% Nafion in distilled water) and drop-casted on the exposed area (1 cm × 1 cm) of the FTO electrode. The resulting electrode was air-dried and used as working electrode for electrochemical measurements.

FITC-labeled FDH for confocal laser scanning microscopy (CLSM)

Fluorescent Labelling of FDH.

First, the solution of 6 mg FDH ($2U \cdot mL^{-1}$) dissolved in PBS buffer (pH 7.0, 10 mL) was prepared. Subsequently, the solution was added with FITC-dimethyl sulfoxide solution ($1\ mg \cdot mL^{-1}$, 1 mL) and stirred for 30 min to be dyed. Then the solution was dialyzed in PBS buffer (pH 7.0) for 1 days to acquire the FITC-labeled FDH solution. All experiments were conducted in the dark condition and the PBS buffer is changed every six hours.

Finally, the FITC-labeled FDH solution was dissolved in the Tris-buffer to form Cys(Zn)/FiTC-FDH microspheres, and then the microspheres was measured to know spatial location of the fluorophore-tagged biomolecules in the microspheres using the confocal laser scanning microscopy (CLSM) technique.

Enzymatic Assays

The catalytic activity of Cys(Zn)/FDH microspheres was measured based on its ability to convert NAD^+ to NADH. Sodium formate (200mM) and NAD^+ (10.5 mM) were first mixed with 3 mL of Tris-HCl buffer solution (50 mM, pH 7.0), and then Cys(Zn)/FDH microspheres (equivalent FDH, $0.6\ mg \cdot mL^{-1}$) are added. After incubation at 37°C for some time, the reaction system was centrifuged at 5000 rpm for 10 min. The absorbance

of the supernatant at 340 nm, ascribed to NADH (molar extinction coefficient, 6.22 mM^{-1} cm^{-1})[1], was measured by a spectrophotometer (UV-2600, SHIMADZU). For free FDH, the measuring method is similar without changing reaction conditions. Reusability of the Cys(Zn)/FDH microspheres was examined by measuring the NADH yield during continuous usages. After each usage the Cys(Zn)/FDH microspheres system were washed with buffer solution, fresh substrates were added, and the next reaction measurement was carried out.

[1] U. C. T. Oppermann, C. Filling, K. D. Berndt, B. Persson, J. Benach, R. Ladenstein, H. Jornvall, Biochemistry 1997, 36, 34-40.

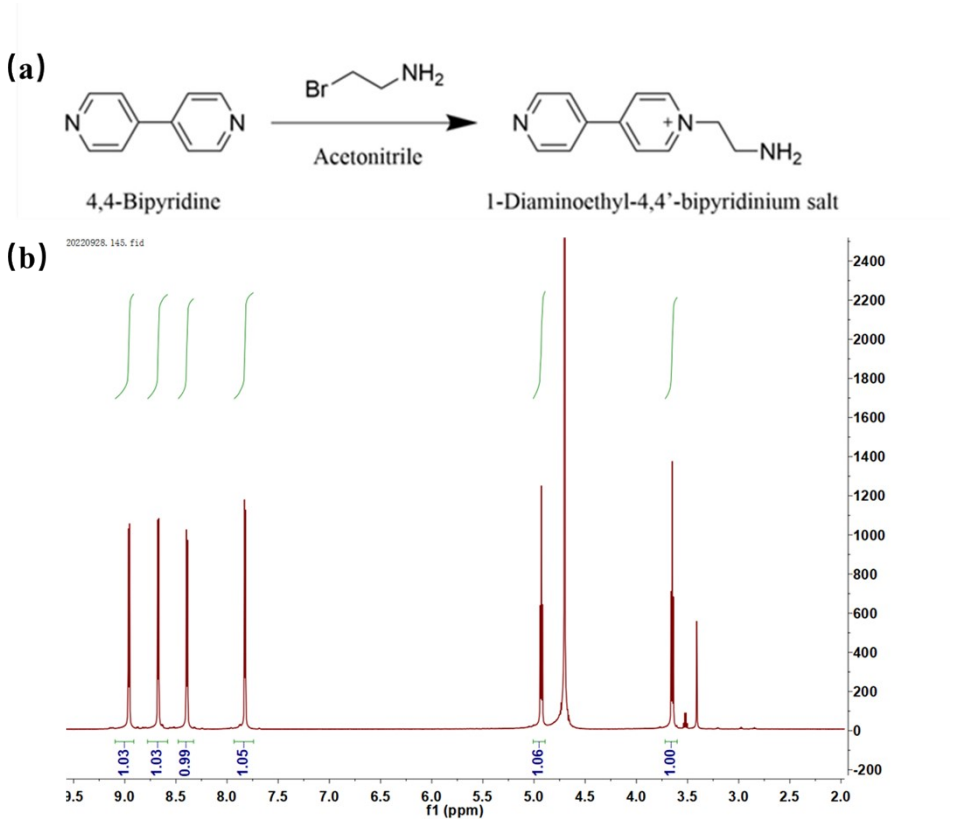

Figure S1. (a) Schematic diagram of DV synthesis, (b) ^{1}H-NMR spectra of DV.

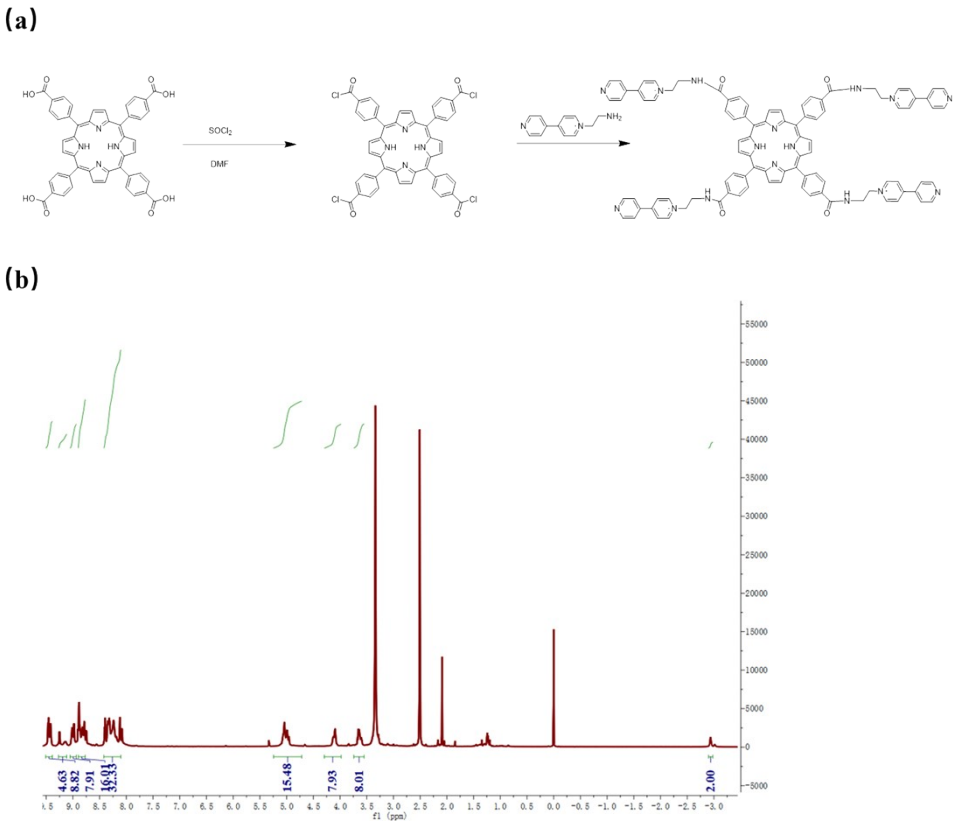

Figure S2. (a) Schematic diagram of D-TCPP synthesis, (b) ^{1}H-NMR spectra of D-TCPP.

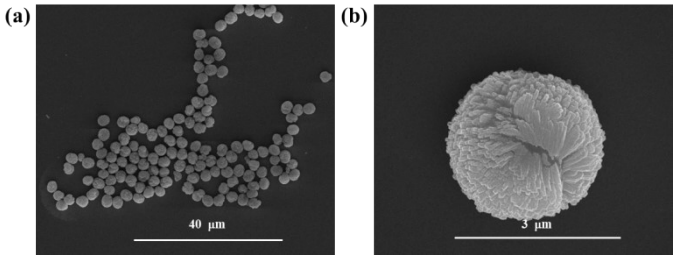

Figure S3. SEM images of Cys (Zn) microspheres.

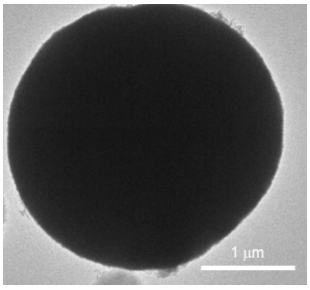

Figure S4. Enlarged TEM image of the edge of a Cys (Zn)/D-TCPP/FDH microsphere.

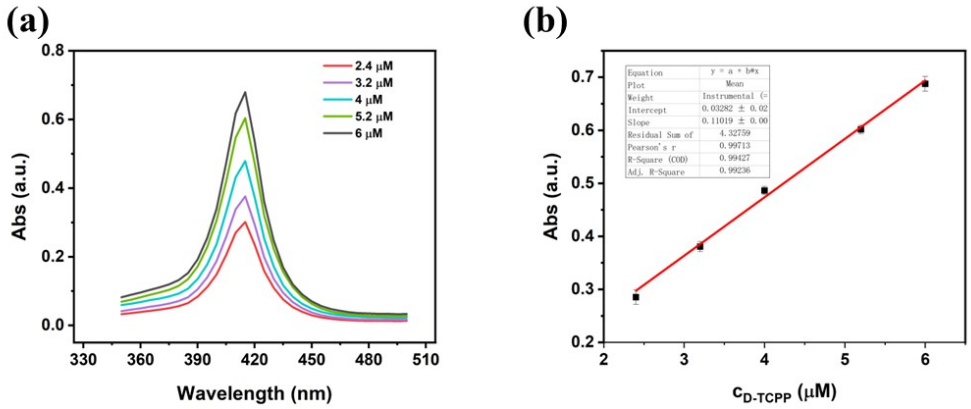

Figure S5. Standard curve of UV-vis absorbance of D-TCPP versus its concentration.

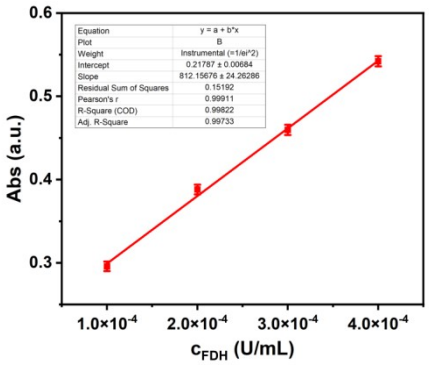

Figure S6. Standard curve of UV-vis absorbance of NADH versus concentration of FDH.

After encapsulation the FDH in the microspheres, the solid sample was obtained by centrifugation and washed repeatedly with Tris-buffer. All the supernatants were collected and combined to regenerate the NADH. The quantity of unencapsulated FDH was determined using the standard curve in Figure S6.

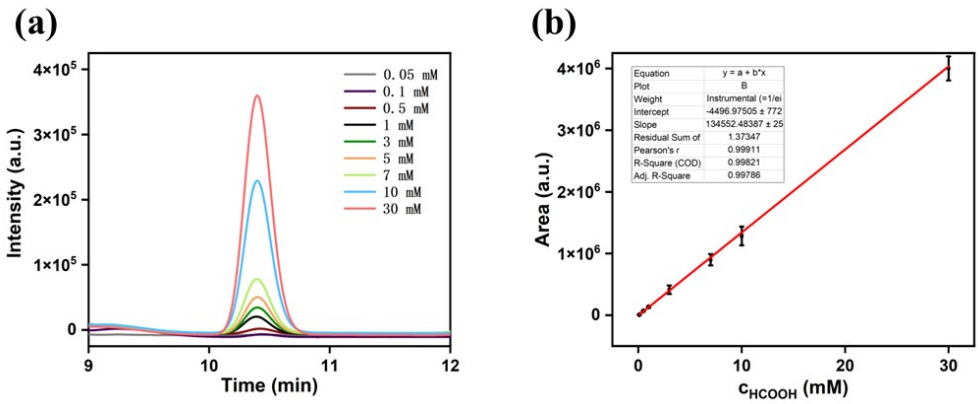

Figure S7. Standard curve of peak area of HCOOH measured by HPLC versus its concentration.

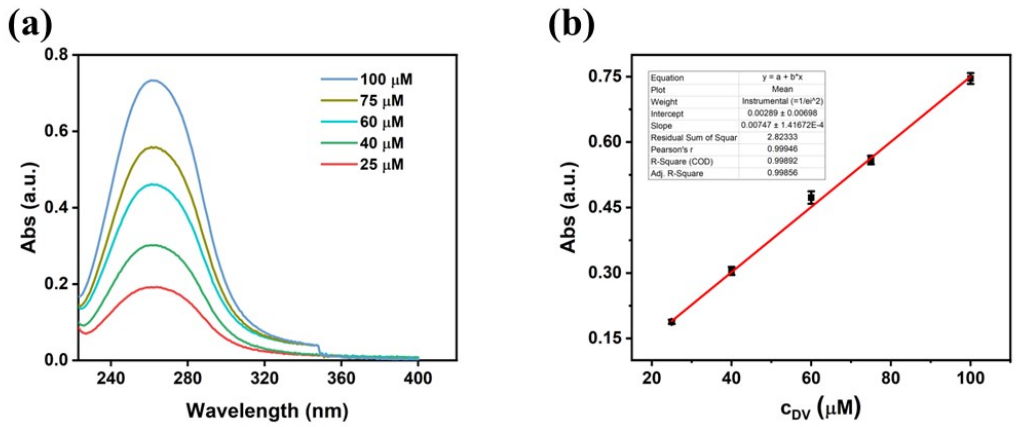

Figure S8. Standard curve of UV-vis absorbance of DV versus its concentration.

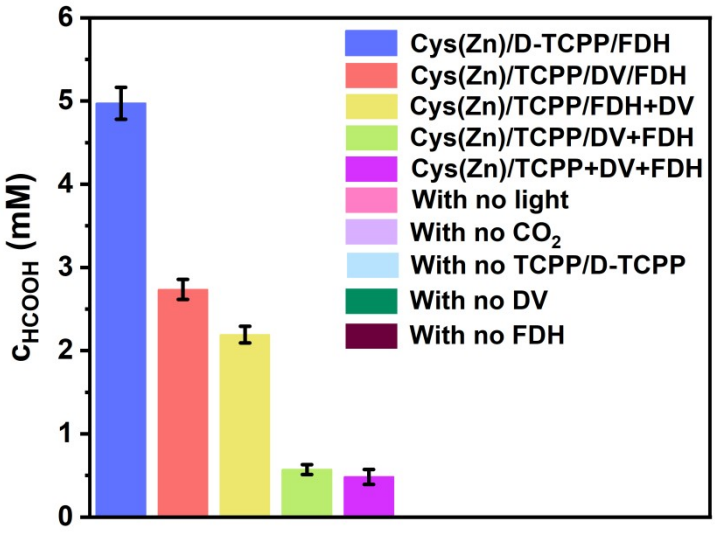

Figure S9. Photocatalytic formate generation via CO_2 reduction with 1mg/mL of Cys (Zn)/D-TCPP/FDH, Cys (Zn)DV/TCPP/FDH, Cys (Zn)/TCPP/FDH+DV, Cys (Zn)/TCPP+DV+FDH and Cys (Zn)/D-TCPP/FDH without light, CO_2, D-TCPP (DV+TCPP) or FDH.

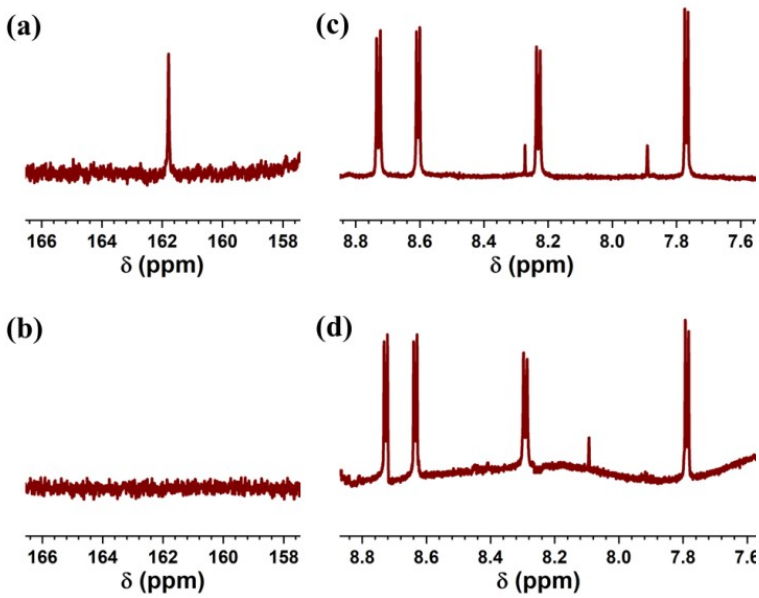

Figure S10. ^{13}C-NMR spectra for the product obtained from reaction with (a) $^{13}CO_2$ and (b) $^{12}CO_2$ as the reactant. ^{1}H-NMR spectra for the product obtained from reaction with (c) $^{13}CO_2$ and (d) $^{12}CO_2$ as the reactant.

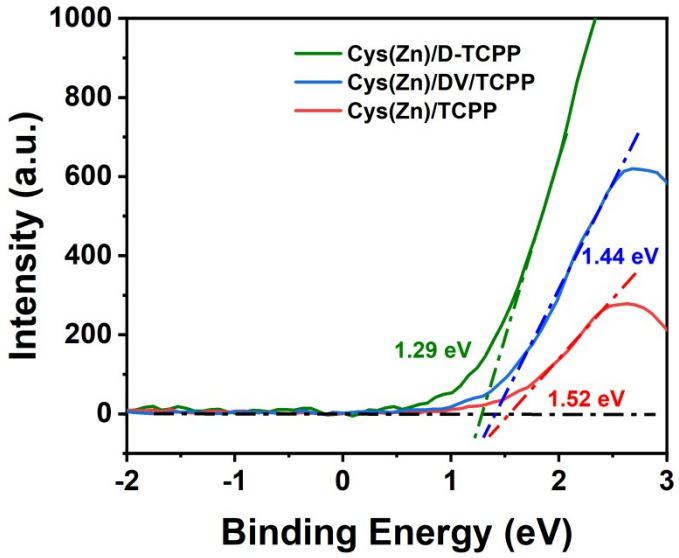

Figure S11. Valence band obtained from XPS.

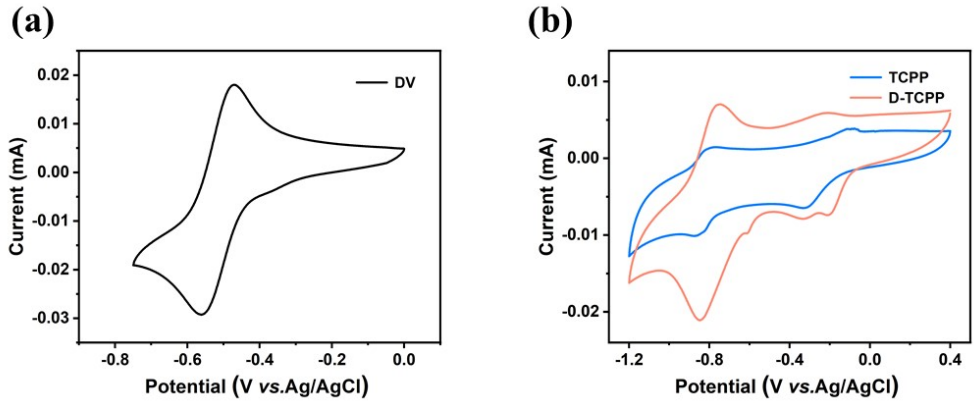

Figure S12. Cyclic voltammogram of DV(a), TCPP and D-TCPP (b) in aqueous solution containing 0.1 M KCl at 100 mV/s using a glassy carbon electrode, a Ag|AgCl reference electrode, and a Pt wire counter electrode at 25 ℃.

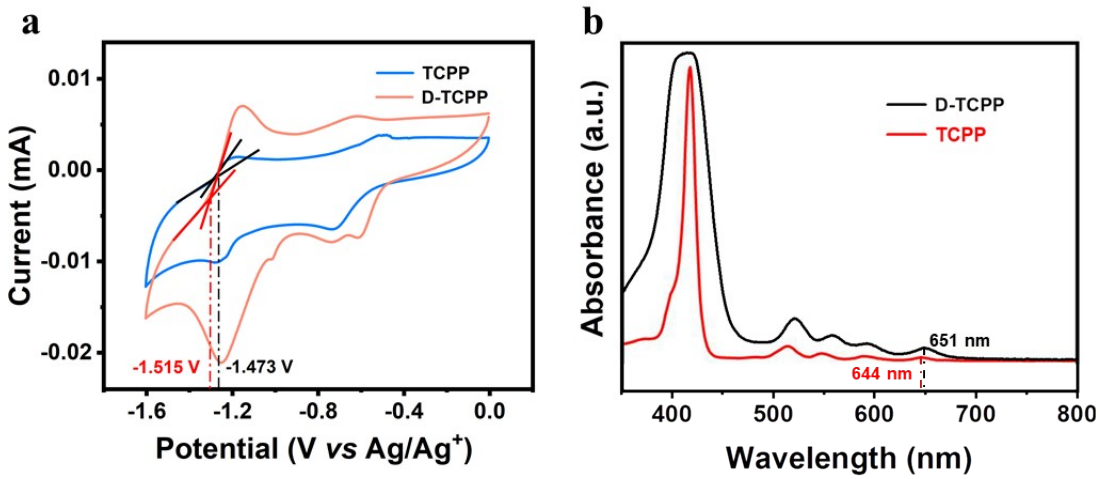

Fig. S13 (a) Cyclic voltammogram of TCPP and D-TCPP in aqueous solution containing 0.1 M KCl at 100 mV/s using a glassy carbon electrode, an Ag/AgCl reference electrode, and a Pt wire counter electrode at 25 ℃. (b) UV-vis absorbance of TCPP and D-TCPP.

As shown in Fig. 13a, according to the results of cyclic voltammetry, the HOMO and LUMO orbits are approximated by the following formulas:

$$E_{HOMO}=-e(E_{onset\text{-}ox}+4.4eV)$$

$$E_g(optical)=1240/\lambda_{max}$$

$$E_{LUMO}= E_{HOMO} -E_g(optical)$$

$E_{onset\text{-}ox}$is the onset oxidation reduction potential and E_g is the optical band gap obtained from the absorption spectrum of the dye. The value of E_{red}(DV) is -0.364 V (*vs* SHE). In order to enable the electrons of TCPP to flow to DV, the value of E_{red}(TCPP) has to be negative than that of DV. Therefore, the more negative redox electric pair is mainly used to provide electrons. For TCPP, the value of HOMO is estimated to be -2.927 eV when the value of $E_{onset\text{-}ox}$(TCPP)is -1.473 V (*vs* Ag/Ag^+). For D-TCPP, the value of HOMO is estimated to be -2.485 eV when the value of $E_{onset\text{-}ox}$(D-TCPP) is -1.515 V (*vs* Ag/Ag^+). The maximum absorption wavelength of D-TCPP is 651 nm, and the maximum absorption wavelength of TCPP is 644 nm, so the E_g value of E_g(D-TCPP) is estimated to be 1.90 eV, while that of TCPP is estimated to be 1.93 ev (Fig. S13b). Based on the data above, E_{LUMO} of TCPP and D-TCPP can be calculated to be -4.857 eV and -4.385 eV, respectively.

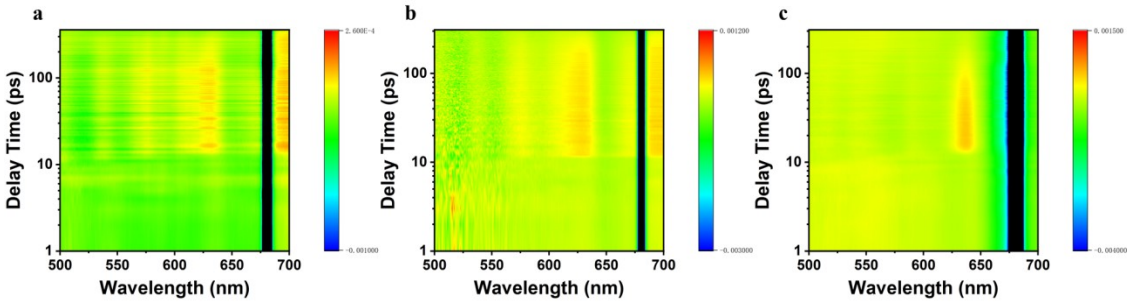

Figure S14. 2D pseudo-color maps of TA spectra of Cys(Zn)/TCPP/FDH (a), Cys(Zn)/DV/TCPP/FDH (b) and Cys(Zn)/D-TCPP/FDH (c) at the excitation of 343 nm.

Table S1 Fitting parameters of PL decay curves.

sample	t_1/ns (rel.%)	t_2/ns (rel.%)	t/ns	k_{ET}(10^7 s^{-1})
Cys(Zn)/TCPP/FDH	**10.4 (2.88)**	**268.4 (97.12)**	**11.3**	
Cys(Zn)/DV/TCPP/FDH	**11.4 (6.20)**	**40.5 (93.80)**	**10.7**	**0.5**
Cys(Zn)/D-TCPP/FDH	**146.4 (16.33)**	**10.2 (83.68)**	**7.9**	**3.8**

Table S2 Fitting parameters of TA decay curves(pump at 343nm; probe at 630nm).

sample	t_1/ps (rel.%)	t_2/ps (rel.%)	t/ps
Cys(Zn)/TCPP/FDH	**124.38 (5.34)**	**5240.90 (94.66)**	**5233.16**
Cys(Zn)/DV/TCPP/FDH	**246.95 (13.09)**	**4208.19 (86.93)**	**4173.76**
Cys(Zn)/D-TCPP/FDH	**29.6 (45.19)**	**159.46 (44.81)**	**159.46**