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Electronic Supplementary Information

Synergistic Integration of Polysulfobetaine Brush-Grafted Porphyrinic Covalent Organic Frameworks with Native Enzymes for Electrolyte-Sensitive Aerobic Photobiocatalysis

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Experiment Section

Materials

All chemicals were obtained from commercial sources and used as received unless otherwise noted. 5,10,15,20-Tetrakis(4-aminophenyl)-21*H*,23*H*-porphyrin (Tp, 98%) and 2,5-dihydroxyterephthalaldehyde (Dh, 98%) were purchased from Jilin Chinese Academy of Sciences Yanshen Technology. Triethylamine (TEA, ≥99.5%), *p*-toluenesulfonic acid (PTSA, 99%), *α*-bromoisobutyryl bromide (BiBB, 98%), [2-(methacryloyloxy)ethyl] dimethyl-(3-sulfopropyl)ammonium hydroxide (SBMA, 95%), 2,2-bipyridyl (BPY, ≥99%), β-nicotinamide adenine dinucleotide reduced disodium salt hydrate (NADH), β-nicotinamide adenine dinucleotide hydrate (NAD^+), glucose (≥99.5%), potassium formate (98%) and L-leucine (99%) were purchased from Sigma Aldrich. Glucose dehydrogenase (GDH, 200 u mg^{-1}), leucine dehydrogenase (LEH, 25 u mg^{-1}) and formate dehydrogenase (FMH, 5.0-15.0 u mg^{-1}) were purchased from Shanghai Yuanye Bio-Technology Co., Ltd. Cuprous bromide (CuBr, 98%, Sigma Aldrich) was purified by stirring in acetic acid for 4 h, followed by washing thoroughly with ethanol and diethyl ether before being stored under an argon atmosphere.

Instrumentation

Nuclear Magnetic Resonance (NMR): ^1H NMR spectra were collected with a Bruker ARX using tetramethylsilane (TMS) as an internal reference. The data obtained were reported as chemical shifts (δ) measured in ppm downfield from TMS. Solid state ^{13}C NMR spectra were recorded on a Bruker AVANCE III 400M spectrometer.

Fourier Transform Infrared Spectroscopy (FTIR): FTIR analysis of the samples was conducted on a NICOLET 5700 FTIR spectrophotometer. Each spectrum was collected by cumulating 64 scans.

Transmission Electron Microscope (TEM): The morphology and size of the samples were analyzed by TEM (JEOL-2010, JEOL Ltd., Japan) operating at an accelerating voltage of 200 kV.

Specific Surface Area Analyzer: The N_2 absorption-desorption isotherms were collected by AUTOSORB-1 Analyzer from Quantachrome Instruments and the specific surface area was acquired by the Brunauer-Emmett-Teller (BET) method, while the pore size distribution was calculated from the desorption branch according to the BJH model.

Thermogravimetric Analysis (TGA): The thermal stability was investigated by TGA. The samples were heated from 30 ºC to around 800 ºC with a heating rate of 10 ºC min^{-1} under a dry nitrogen atmosphere in a thermal analyzer (TGS-II, Perkin-Elmer).

X-Ray Diffraction (XRD): Powder XRD patterns were obtained on a Rigaku Smartlab with Cu Kα line (λ = 1.5418 Å).

X-Ray Photoelectron Spectroscopy (XPS): Surface composition of the samples was investigated by XPS on a Kratos AXIS Ultra DLD spectrometer sourcing with a monochromatized Al Kα X-ray source (1468.71 eV photons).

Zeta Potential: Zeta potential of the samples was measured using a Zetasizer Nano-ZS ZEN3600 from Malvern Instruments Ltd. The concentration of the samples was 0.5 mg mL^{-1}.

Optical Spectrophotometer: Ultraviolet-visible diffuse reflectance (UV-vis DR) spectra were measured by UV-visible spectrophotometer (UV-vis, UV3600, Shimadzu).

Photoluminescence (PL) Spectrophotometer: The PL emission spectra were recorded on a PerkinElmer LS 55 Fluorescence spectrometer and the transient time resolved PL decay measurements were recorded on a Multifunctional Fluorescence Imaging System (FLM300).

Electron Paramagnetic Resonance (EPR): A Bruker ELEXSYS 100G/EMX-8/2.7C spectrometer in conjunction with a split-coil 6T superconducting magnet was used for W-band (95 GHz) electron paramagnetic resonance.

Dynamic Light Scattering (DLS): The DLS experiment was performed using disposable 4 mL plastic cuvettes in the DLS instrument with 1 mL of aqueous solution. Light scattering signals were measured using a Marvin Instrument Ltd. Zetasizer Nano-ZS ZEN3600.

Electrochemical Workstation: Electrochemical measurements were executed on a Metrohm Autolab PGSTAT302N in a three-electrode electrochemical cell equipped with

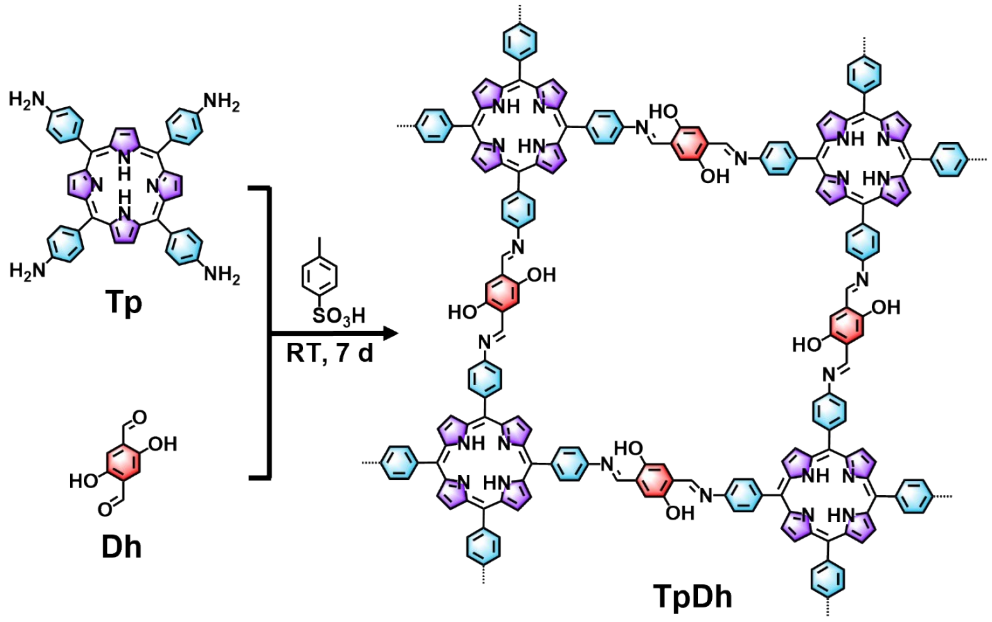
an electrochemical station. First, photocatalysts (6 mg) were dispersed in Nafion (3 mL, 0.2 wt%) by the ultrasonic instrument. Then the samples were dripped on ITO coated glasses which were places on top of a glassy carbon as the working electrode, and the samples were dried under infrared irradiation. With Na_2SO_4 aqueous solution (0.1 M) supplied as the electrode, the Ag/AgCl as the reference electrode and the platinum wire as the counter electrode. Meanwhile, red light (λ_{max} = 680 nm, 1.2 mW cm^{-2}) placed at 2 cm away from the photoelectrochemical cell were employed as the light source.

The photocurrents were tested under red light (λ_{max} = 680 nm, 1.2 mW cm^{-2}) with light on-off cycles at a time interval of 30 s and the scan rate was 100 mV s^{-1}. The Mott-Schottky measurement was performed at frequencies of 500 Hz, 1000 Hz and 1500 Hz in dark conditions. The electrochemical impedance spectroscopy (EIS) was carried out at a bias potential of +0.5 V in the dark.

Circular Dichroism (CD) Spectroscopy: The CD spectra were collected on ChirascanTM CD spectroscopy (Applied Photophysics, Leatherhead, United Kingdom). CD spectra from 190 to 330 nm were collected and scanning speed was 200 nm min^{-1}. The bandwidth was 5 nm. The response time was 2 s. Baseline-corrected was carried out to avoid the signal contributions due to the buffer after scanning. Every sample ran at least two times. All scanning was processed at ambient temperature unless otherwise specified.

Synthesis of TpDh-P

The TpDh-P were synthesized according to the procedures described in the literature with minor modifications.[1-5] Initially, Dh (11.25 mg, 0.068 mmol) was dissolved in dichloromethane (DCM, 30 mL) and added to the beaker. A layer of water (18 mL) was added on top of the aldehyde solution. Afterwards, Tp (22.86 mg, 0.034 mmol) and PTSA (24.0 mg, 0.136 mmol) were dissolved in water (30 mL) and slowly introduced onto the spacer solution over a period of 30 min. The system was left undisturbed at room temperature for 7 days. The resulting thin films at the interface were collected by removing the upper aqueous layer using a dropper and purified through washing with water, *N,N*-dimethylformamide (DMF) and ethanol to obtain the TpDh COFs. The yield of TpDh COFs was 45%.



Scheme S1. The synthetic protocol of TpDh COFs.

Under vigorous stirring, TpDh (50 mg) and TEA (1.13 mL, 8.1 mmol) were sequentially added to DCM (30 mL). The reaction flask was then placed in an ice bath, and BiBB (1.00

mL, 8.1 mmol) in DCM (5 mL) was slowly dosed to the reaction mixture over a period of 30 min. The mixture was stirred at room temperature for 12 h. The crude product was purified through five cycles of centrifugation/redispersion/washing in excessive DCM and methanol. The TpDh-Br was obtained by collecting the centrifuged product and drying it under vacuum at 50 ºC overnight.

In a typical procedure, TpDh-Br (30 mg), SBMA (300 mg, 1.07 mmol), CuBr (20 mg) and BPY (40 mg) were sequentially added to DMSO (5 mL) under vigorous stirring. The reaction mixture was then deoxygenated by sparging with argon for 20 min. The reaction was initiated by immersing the flask in an oil bath at 50 ºC for 6 h after being sealed with a rubber septum. The crude products were purified by repeated extraction with 5 wt% saline thrice to eliminate any leftover reactants. The TpDh-P was then treated with DMF containing AIBN (2 mg mL^{-1}) at 80 ºC under argon atmosphere for 6 h. Finally, the crude product was purified through five cycles of centrifugation/redispersion/washing in excess ethanol. The TpDh-P was obtained by collecting the centrifuged product and drying it under vacuum at 80 ºC overnight. The thickness of PSBMA brushes on TpDh-P was adjusted based on the polymerization times of 3, 6 and 12 h, denoted as TpDh-PS, TpDh-PM and TpDh-PL, respectively.

Photocatalytic Oxidation of NADH by TpDh or TpDh-P

A glass vial was filled with an aqueous dispersion of TpDh (0.30 mg mL^{-1}) or TpDh-PS (0.40 mg mL^{-1}) or TpDh-PM (0.50 mg mL^{-1}) or TpDh-PL (0.90 mg mL^{-1}) and NADH (10 mM) using phosphate buffer solution (PBS, 50 mM, pH 7.4, 10 mL) or deionized water (DI water, 10 mL). The reaction mixture was stirred at a temperature of 25 ºC and left in

the open air. Subsequently, it was subjected to irradiation with red light (λ_{max} = 680 nm, 1.2 mW cm^{-2}) for a duration of 1 h. At different time points, samples were collected from the reaction mixture and subjected to analysis using both ^1H NMR spectroscopy and UV-Vis absorption spectroscopy. The changes in NADH absorption at 340 nm were monitored over different time intervals.

NAD^+/NADH Interconversion by TpDh or TpDh-P

The reaction mixture comprised TpDh (1.2 mg mL^{-1}) or TpDh-PS (1.6 mg mL^{-1}) or TpDh-PM (2.0 mg mL^{-1}) or TpDh-PL (3.6 mg mL^{-1}), GDH (1.5 mg) and NAD^+ (33.2 mg, 2.8 mM) in PBS (50 mM, pH 7.4, 18 mL). To initiate the enzyme reaction, glucose (180.2 mg, 100 mM) was added to the mixture. The progress of the reaction was monitored by measuring the changes in the UV-Vis absorbance intensity at 340 nm, corresponding to NADH. Absorption data was collected at 30 min intervals. Each cycle consisted of a 30 min dark period followed by a 30 min exposure to red light (λ_{max} = 680 nm, 1.2 mW cm^{-2}). This sequence of dark-light cycles was repeated twelve times. The pH value during the circular process was determined to be 7.15 ± 0.05.

The reaction mixture comprised TpDh (1.2 mg mL^{-1}) or TpDh-PS (1.6 mg mL^{-1}) or TpDh-PM (2.0 mg mL^{-1}) or TpDh-PL (3.6 mg mL^{-1}), LMH (5 mg) and NAD^+ (33.2 mg, 2.8 mM) in PBS (50 mM, pH 7.4, 18 mL). To initiate the enzyme reaction, L-leucine (235.8 mg, 100 mM) was added to the mixture. The progress of the reaction was monitored by measuring the changes in the UV-Vis absorbance intensity at 340 nm, corresponding to NADH. Absorption data was collected at 30 min intervals. Each cycle consisted of a 30 min dark period followed by a 30 min exposure to red light (λ_{max} = 680 nm, 1.2 mW cm^{-2}). This

sequence of dark-light cycles was repeated twelve times. The pH value during the circular process was determined to be 7.10 ± 0.05.

The reaction mixture comprised TpDh (1.2 mg mL^{-1}) or TpDh-PS (1.6 mg mL^{-1}) or TpDh-PM (2.0 mg mL^{-1}) or TpDh-PL (3.6 mg mL^{-1}), FMH (5 mg) and NAD^+ (33.2 mg, 2.8 mM) in PBS (50 mM, pH 7.4, 18 mL). To initiate the enzyme reaction, potassium formate (168.2 mg, 100 mM) was added to the mixture. The progress of the reaction was monitored by measuring the changes in the UV-Vis absorbance intensity at 340 nm, corresponding to NADH. Absorption data was collected at 30 min intervals. Each cycle consisted of a 30 min dark period followed by a 30 min exposure to red light (λ_{max} = 680 nm, 1.2 mW cm^{-2}). This sequence of dark-light cycles was repeated twelve times. The pH value during the circular process was determined to be 7.20 ± 0.05.

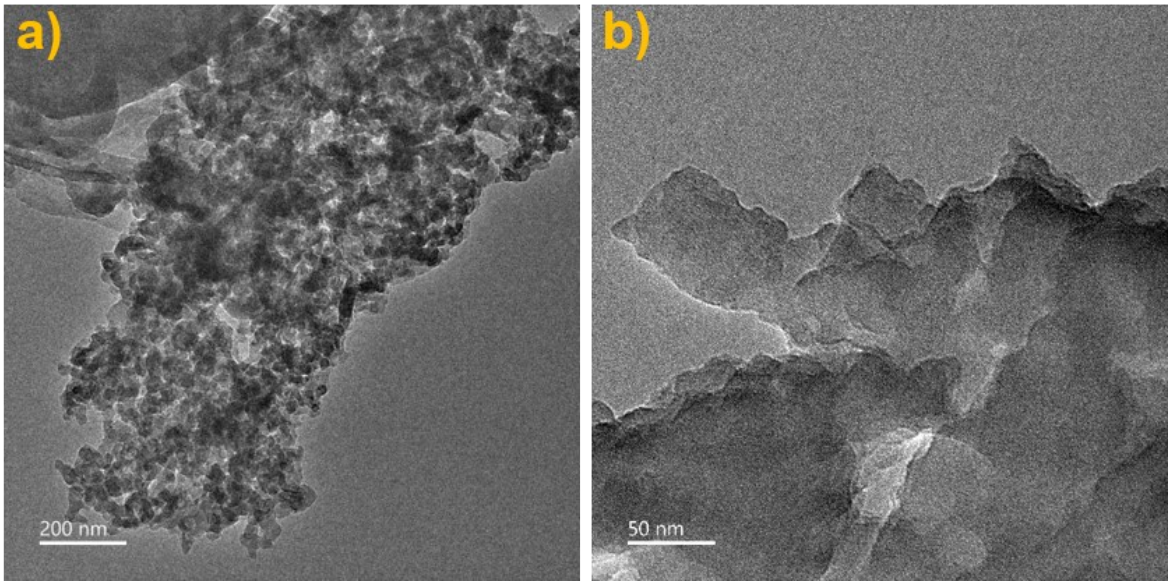


Fig. S1 TEM images of (a) TpDh and (b) TpDh-PM.

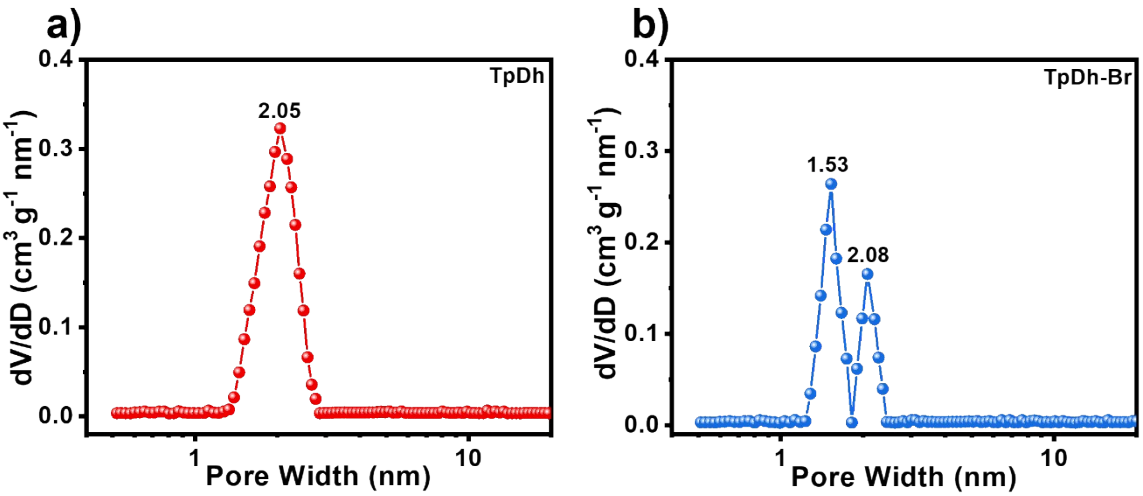


Fig. S2 Pore width distribution of (a) TpDh and (b) TpDh-Br derived from BJH method.

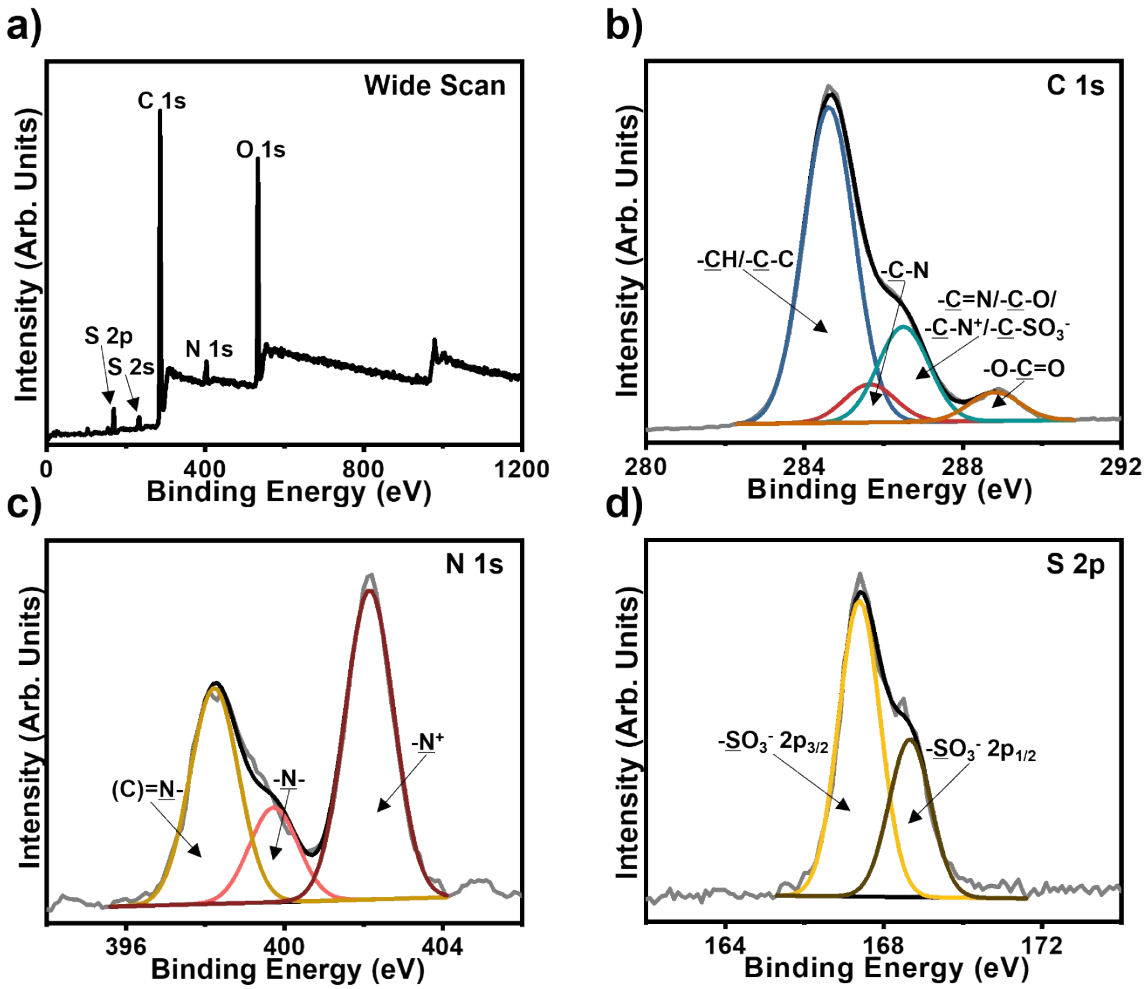


Fig. S3 XPS (a) wide scan, (b) C 1s, (c) N 1s and (d) S 2p core-level spectra of TpDh-PS.

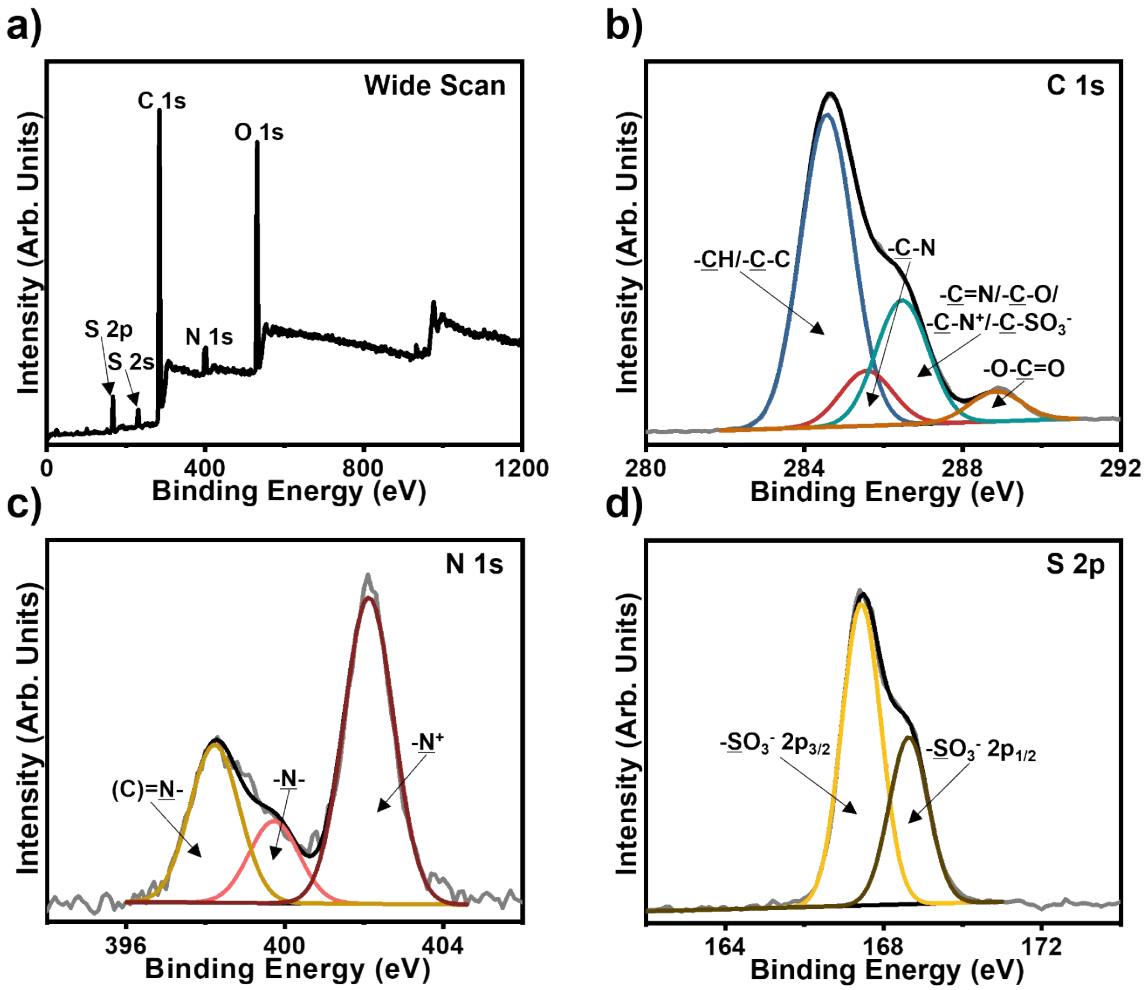


Fig. S4 XPS (a) wide scan, (b) C 1s, (c) N 1s and (d) S 2p core-level spectra of TpDh-PM.

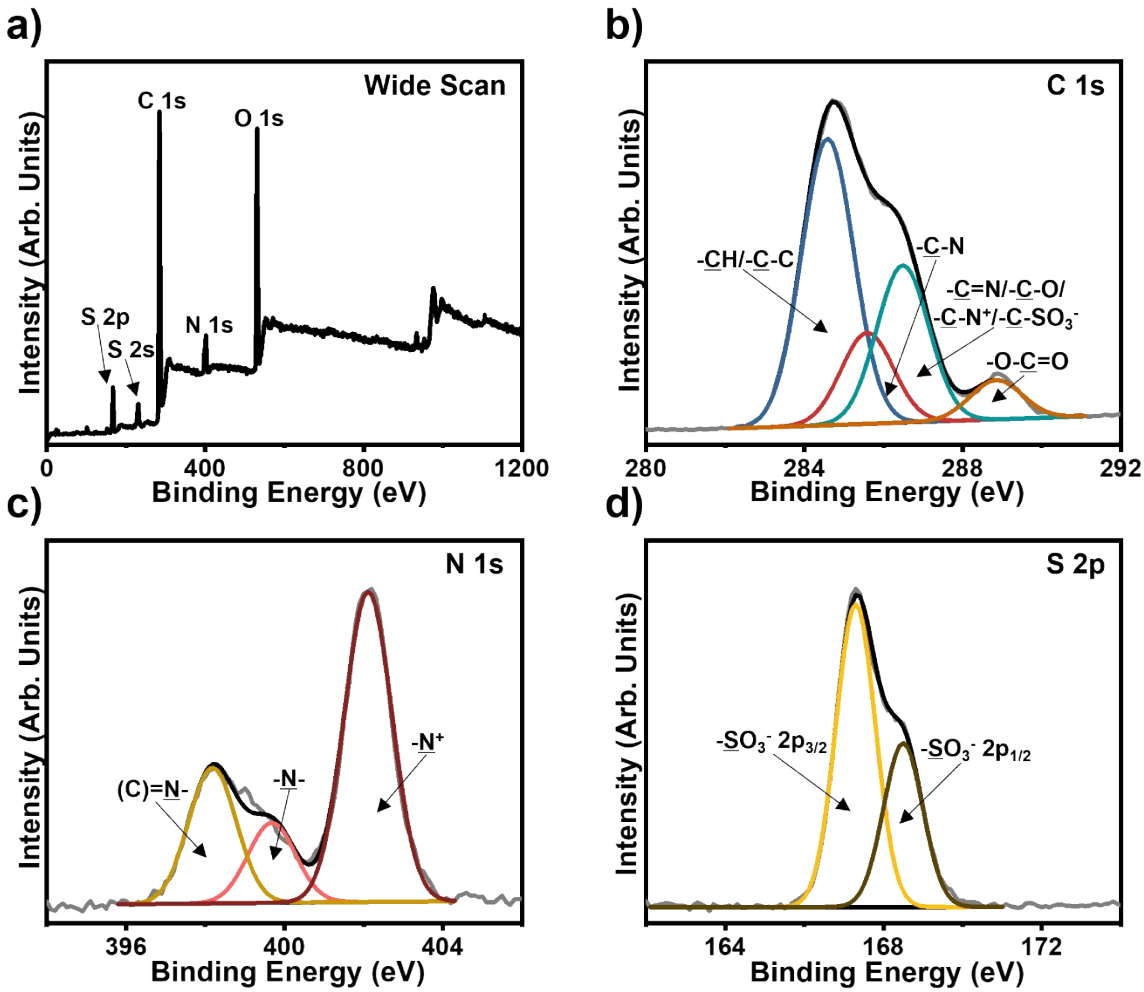


Fig. S5 XPS (a) wide scan, (b) C 1s, (c) N 1s and (d) S 2p core-level spectra of TpDh-PL.

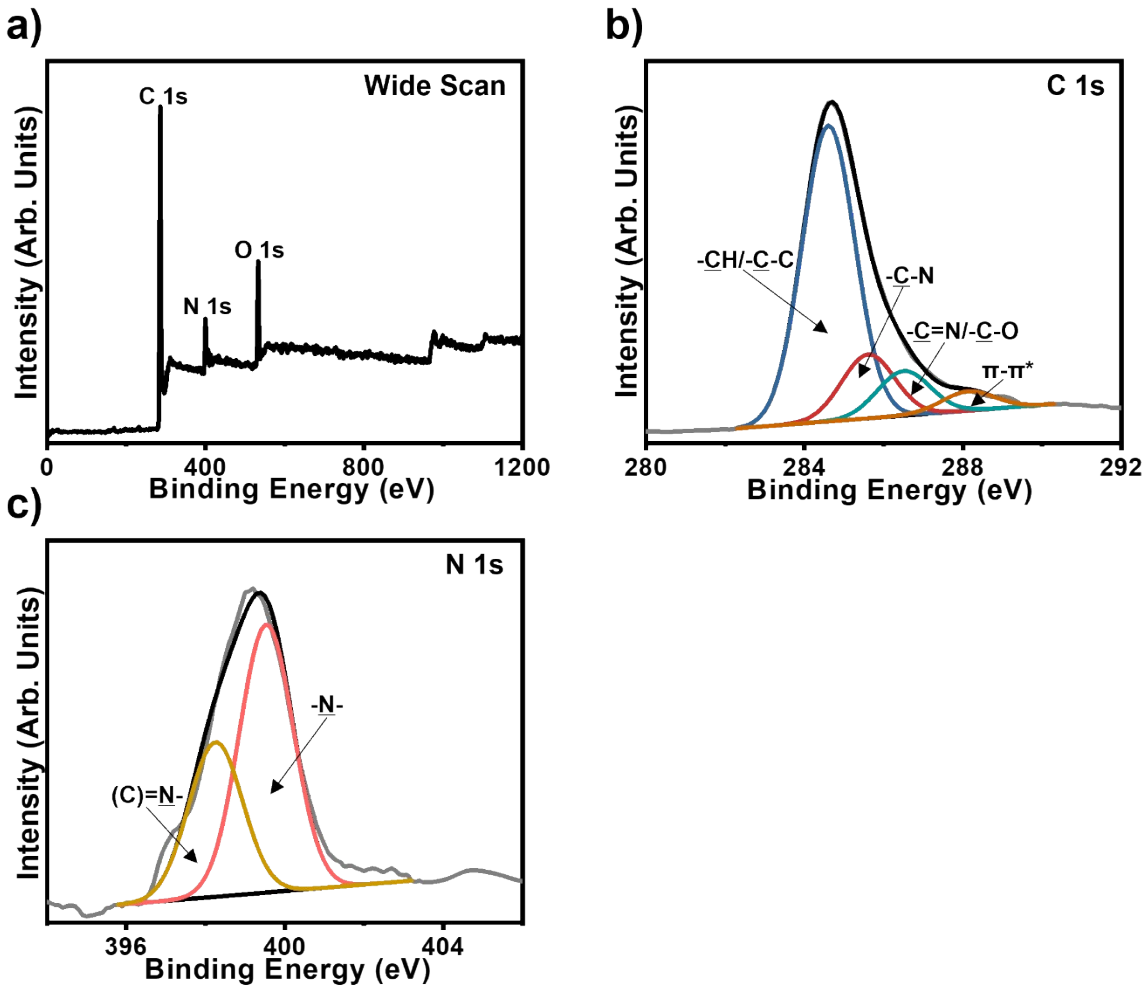


Fig. S6 XPS (a) wide scan, (b) C 1s and (c) N 1s core-level spectra of TpDh.

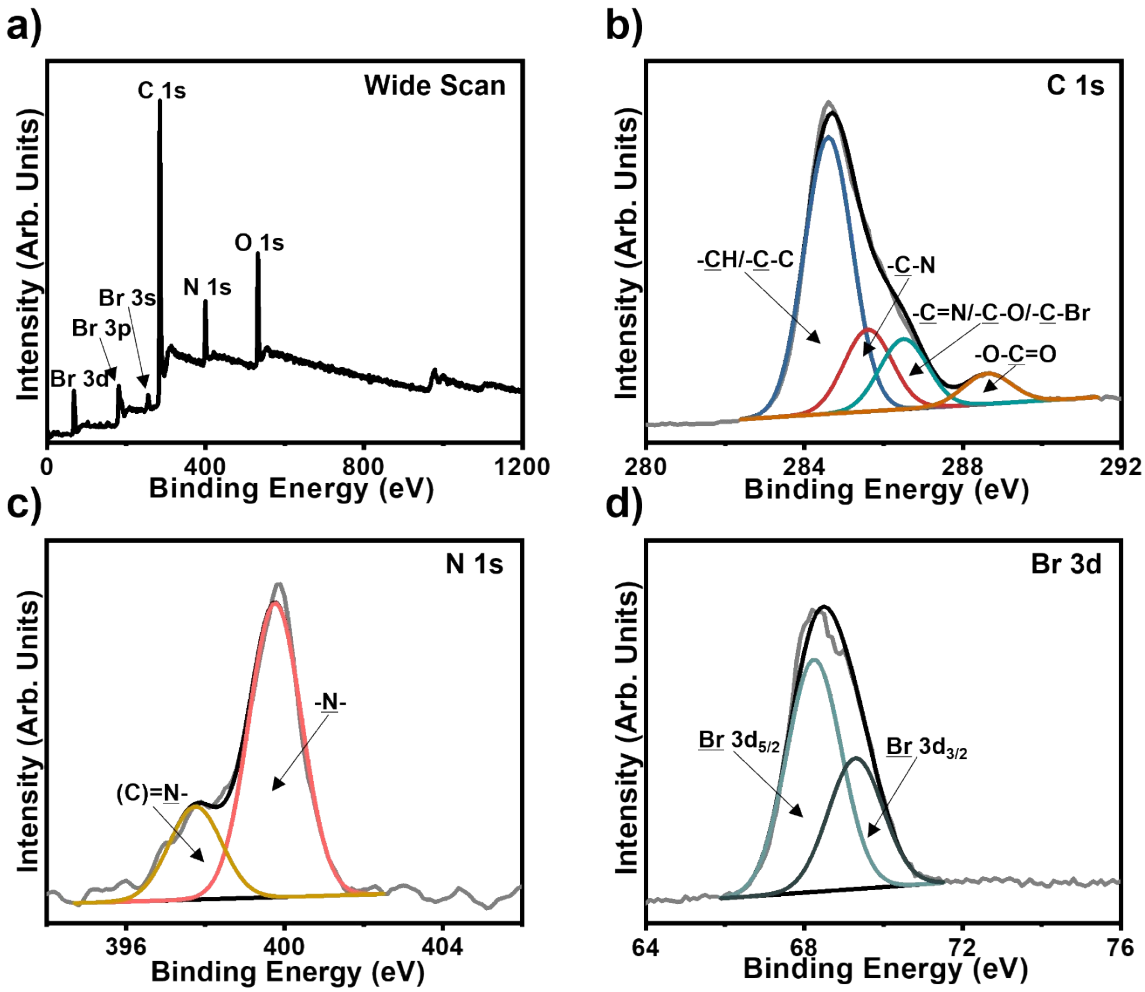


Fig. S7 XPS (a) wide scan, (b) C 1s, (c) N 1s and (d) Br 3d core-level spectra of TpDh-Br.

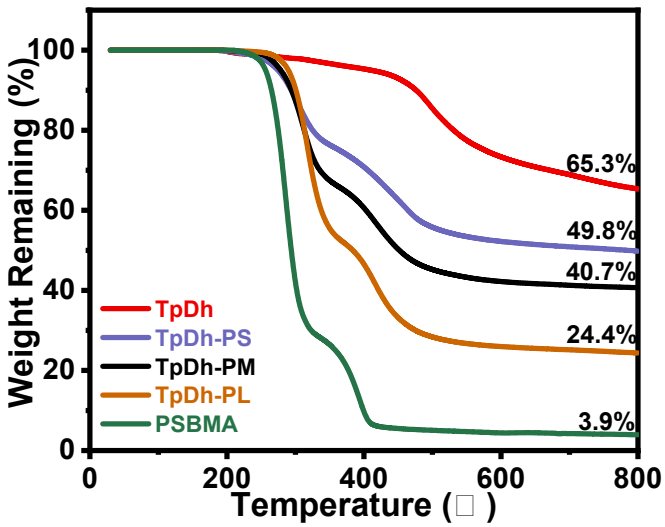


Fig. S8 TGA curves of TpDh, TpDh-PS, TpDh-PM, TpDh-PL and PSBMA.

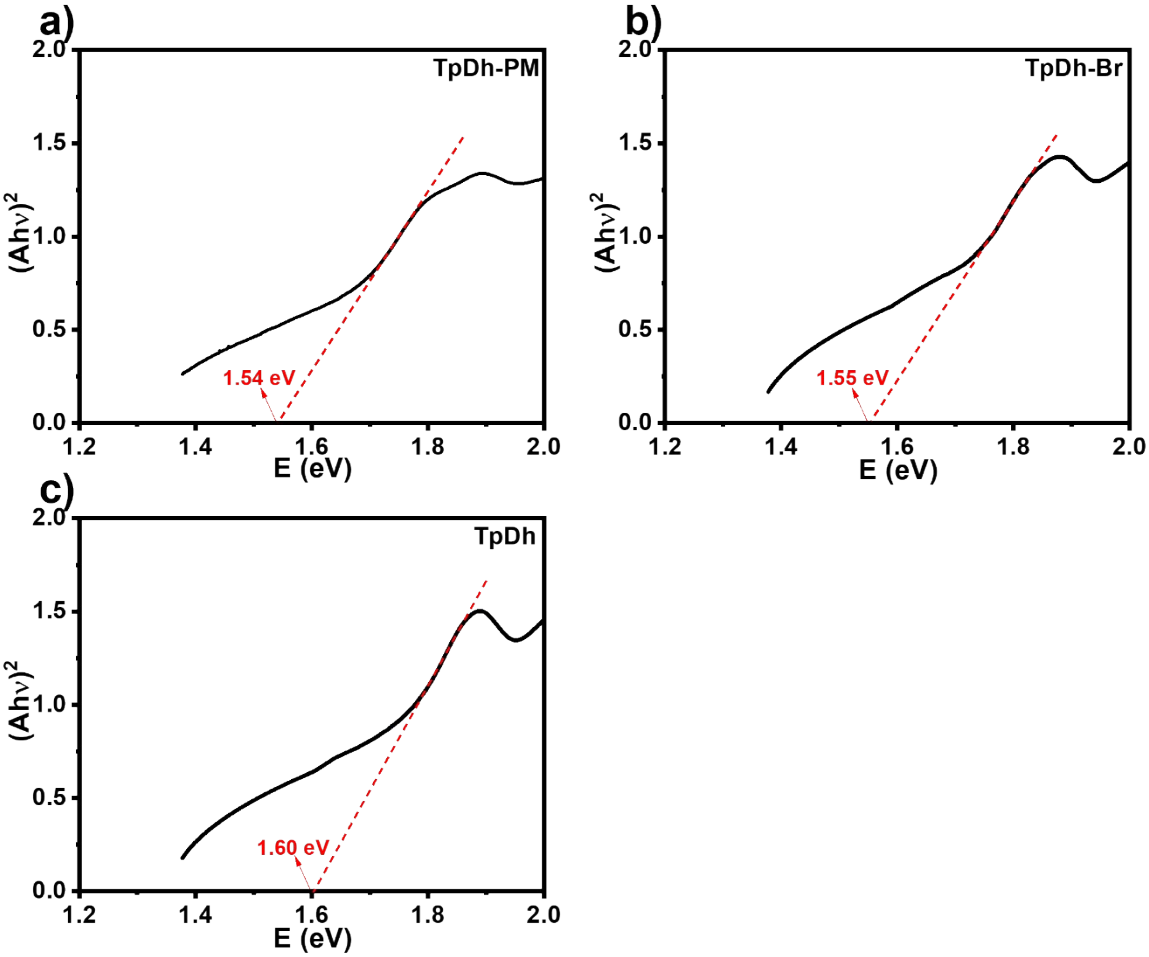


Fig. S9 Kubelka-Munk plots for the calculated bandgap energies of (a) TpDh-PM, (b) TpDh-Br and (c) TpDh.

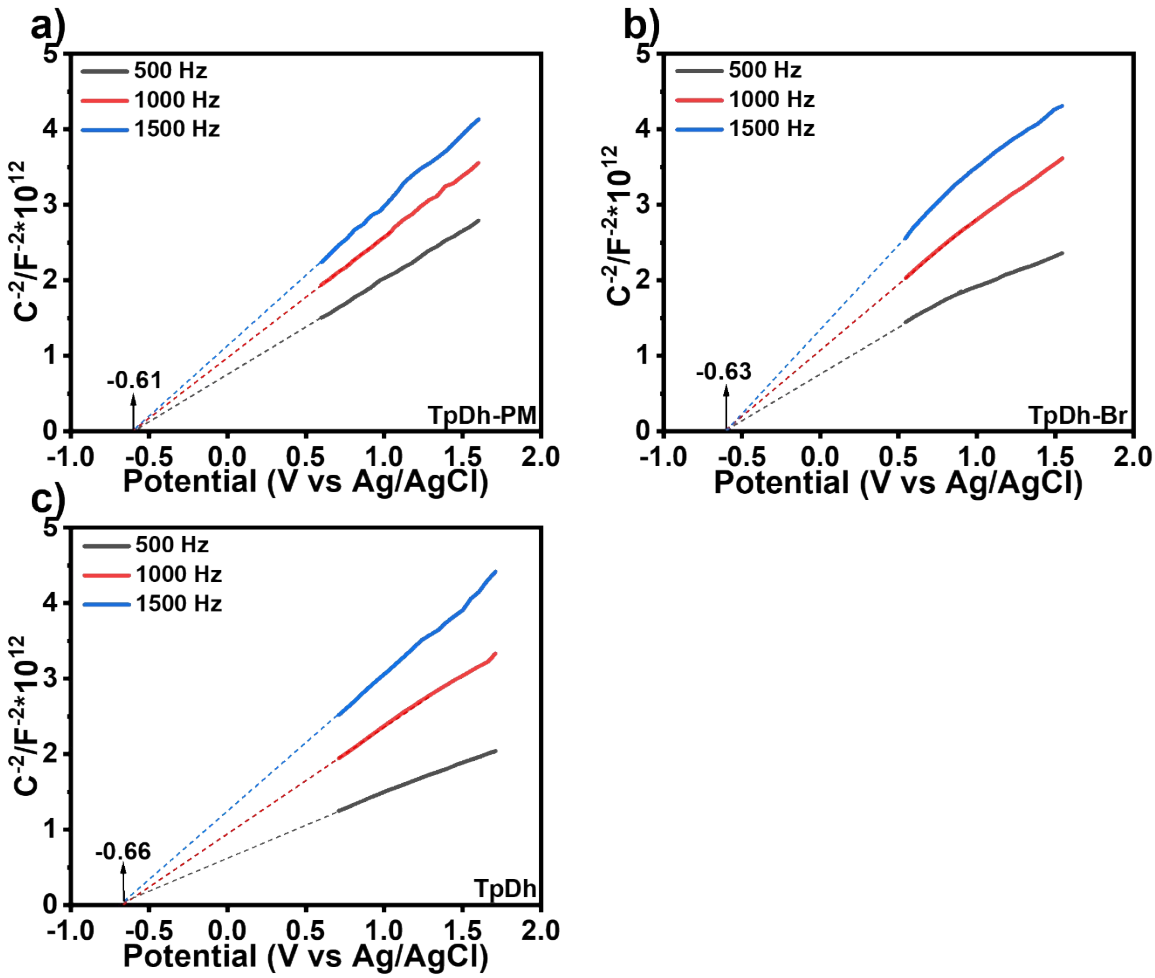


Fig. S10 Mott-Schottky plots of (a) TpDh-PM, (b) TpDh-Br and (c) TpDh obtained from different frequencies.

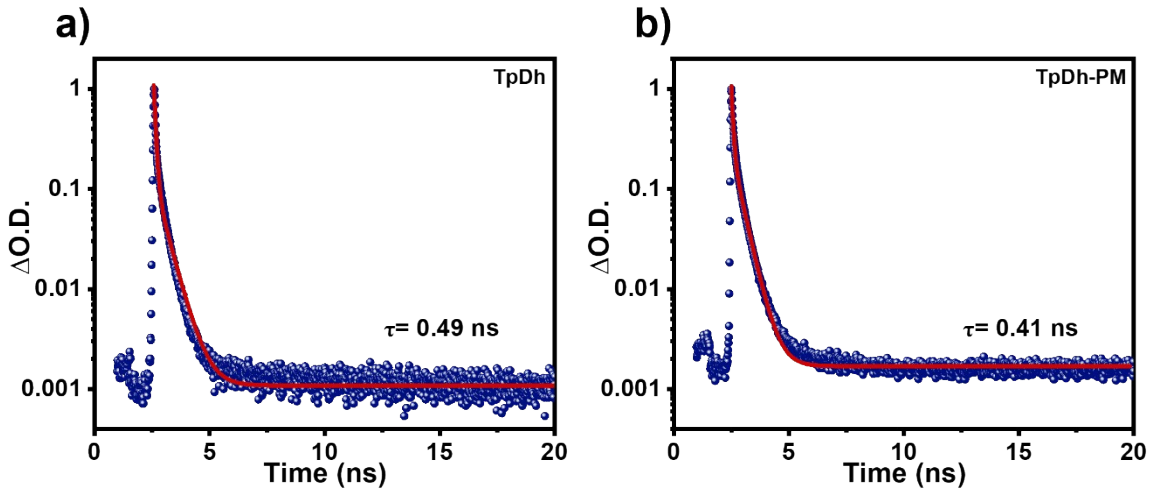


Fig. S11 Time-correlated single-photon counting experiments for (a) TpDh and (b) TpDh-PM in solid state. Samples were excited with a λ_{exc} = 570 nm laser and emission was measured at λ_{em} = 680 nm.

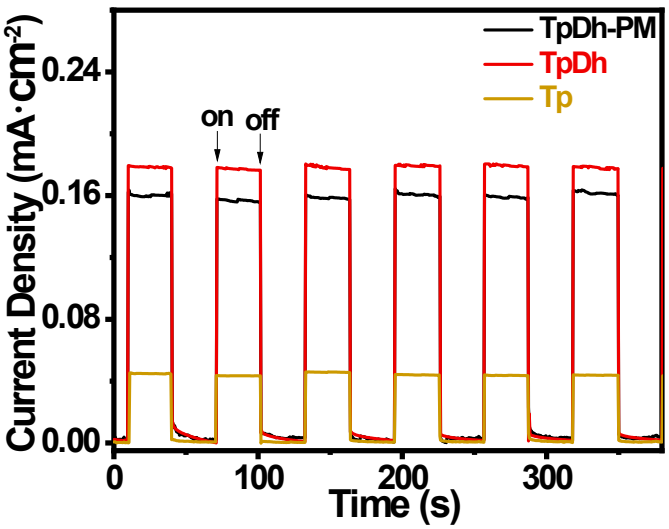


Fig. S12 Transient photocurrent (TPC) measurements of TpDh-PM, TpDh and Tp.

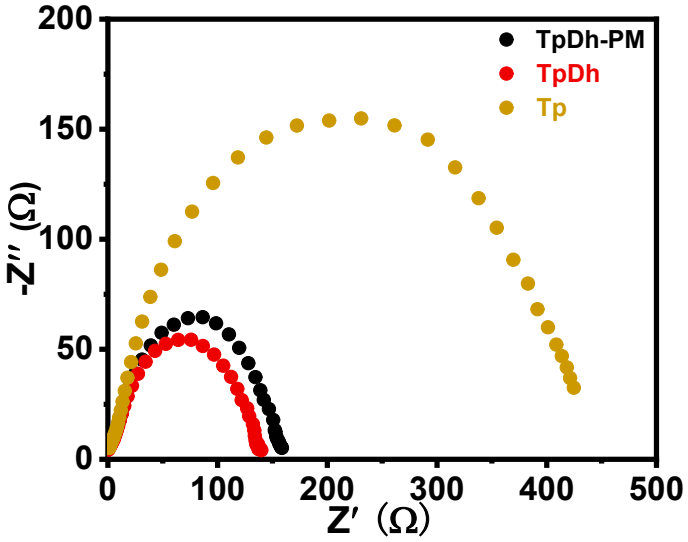


Fig. S13 EIS Nyquist plots of TpDh-PM, TpDh and Tp.

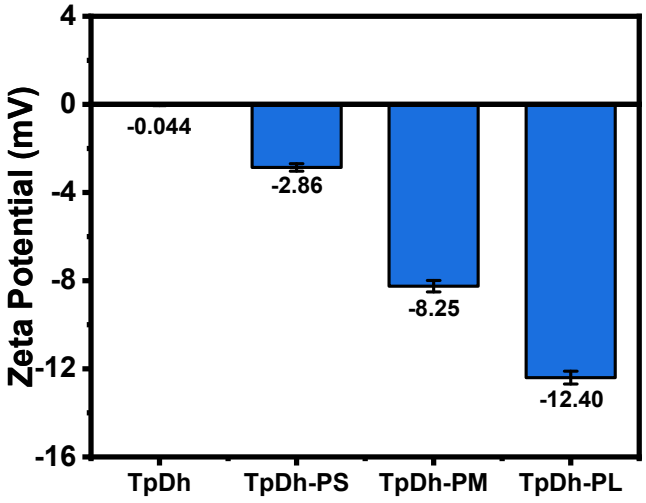


Fig. S14 Zeta potentials of TpDh, TpDh-PS, TpDh-PM and TpDh-PL.

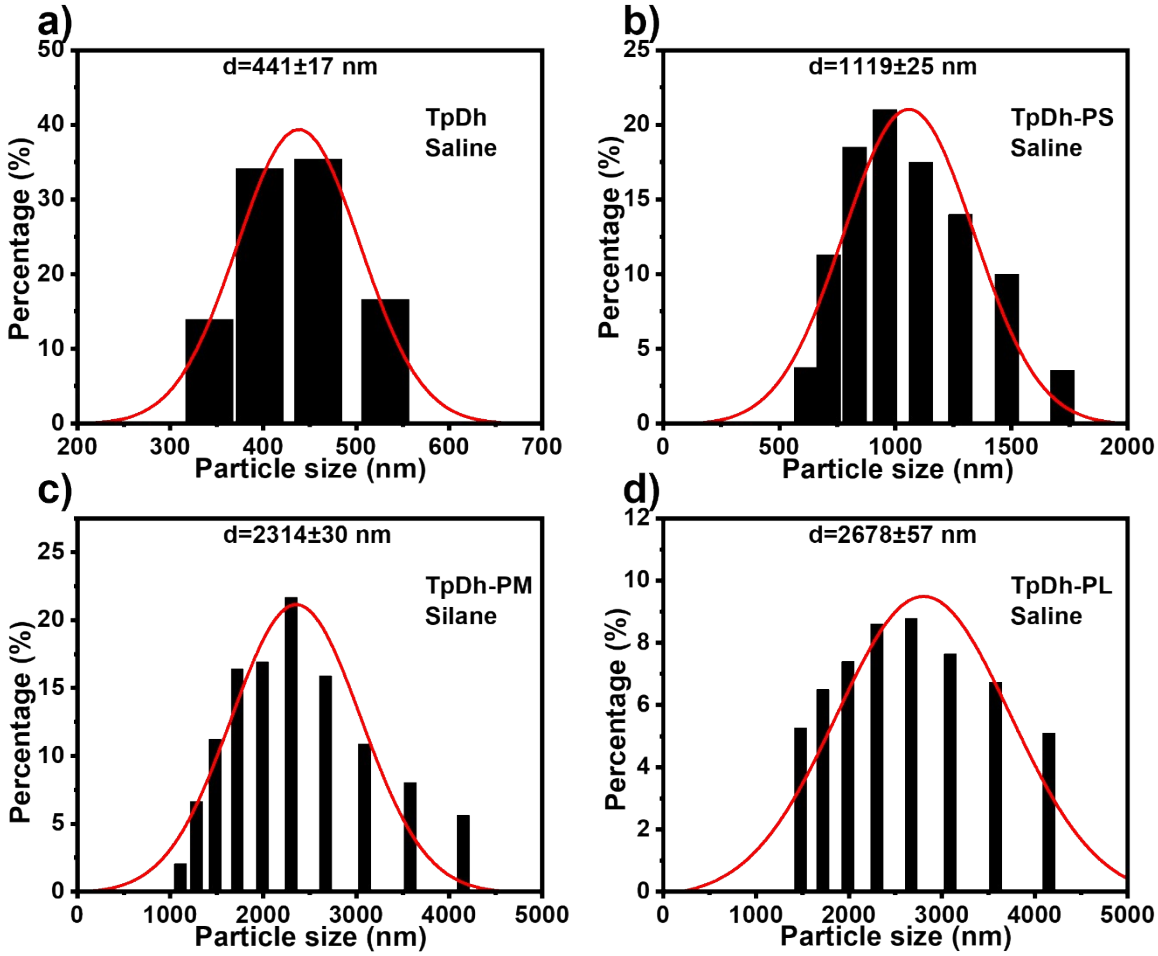


Fig. S15 DLS traces for (a) TpDh, (b) TpDh-PS, (c) TpDh-PM and (d) TpDh-PL in 5 wt% saline.

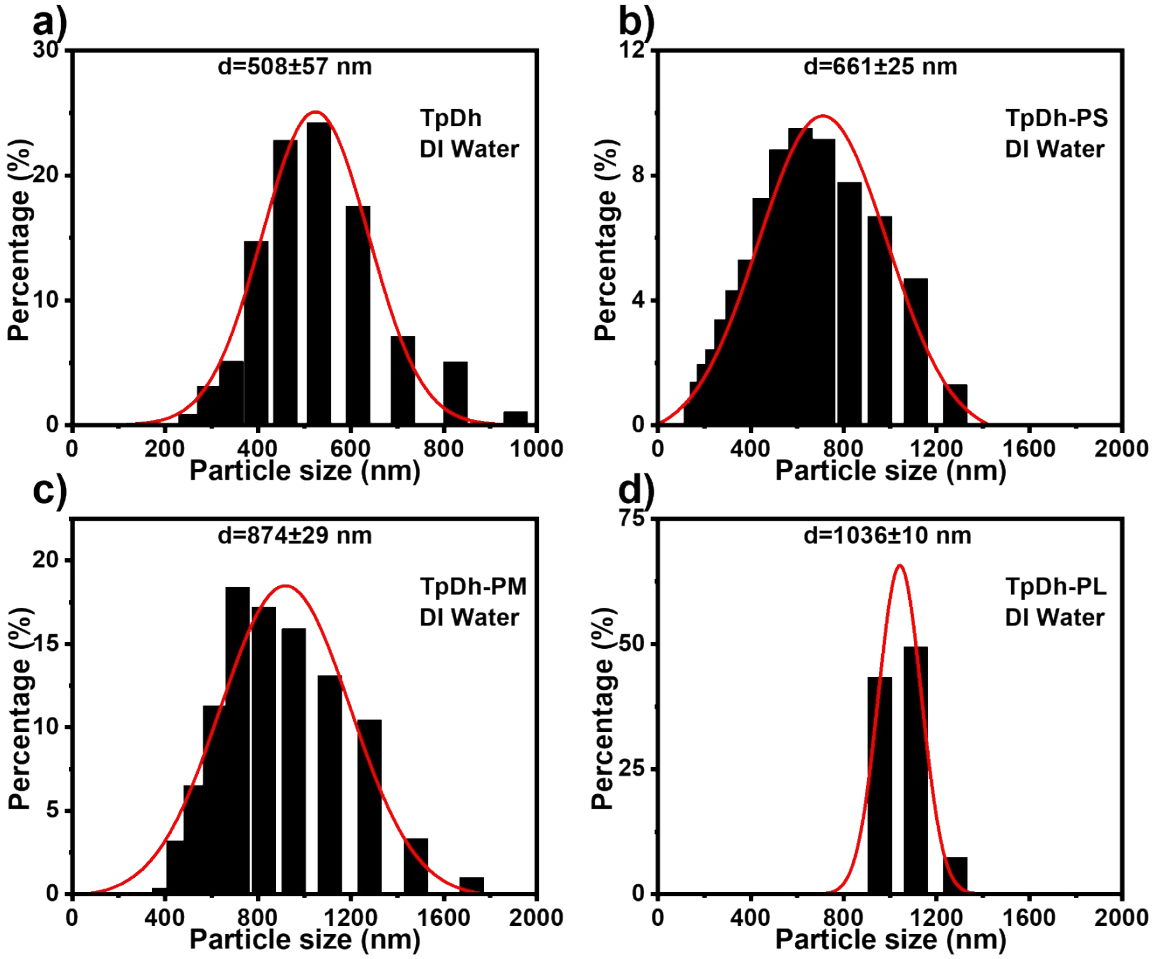


Fig. S16 DLS traces of (a) TpDh, (b) TpDh-PS, (c) TpDh-PM and (d) TpDh-PL in DI water.

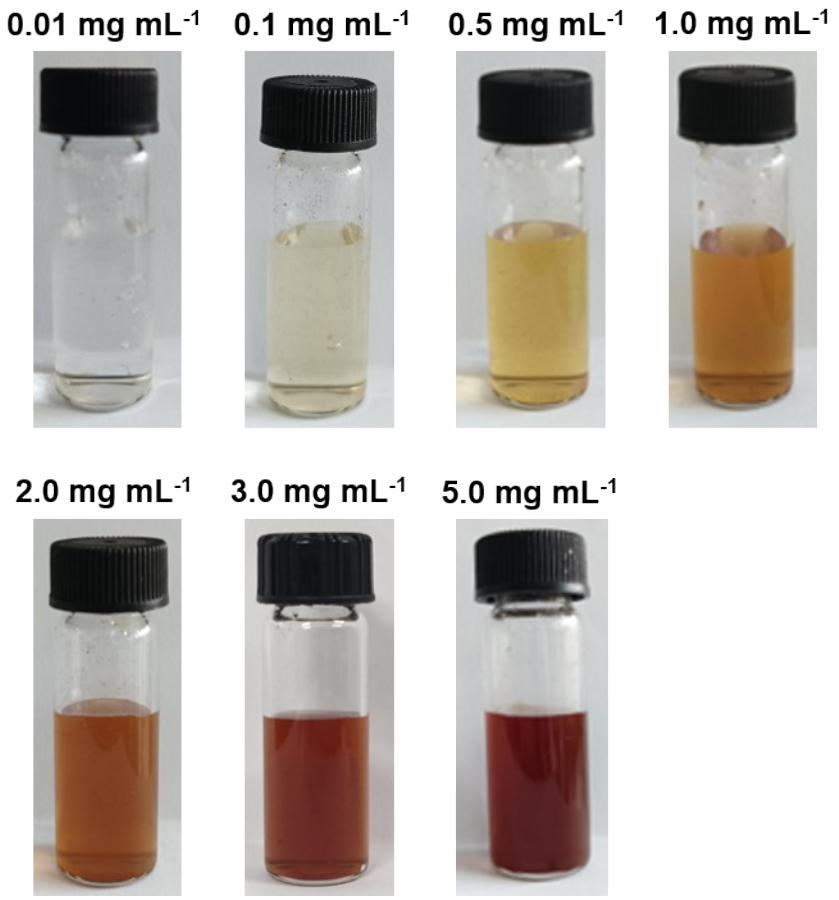


Fig. S17 Snapshots of various concentrations of TpDh-PM dispersed in 5 wt% saline.

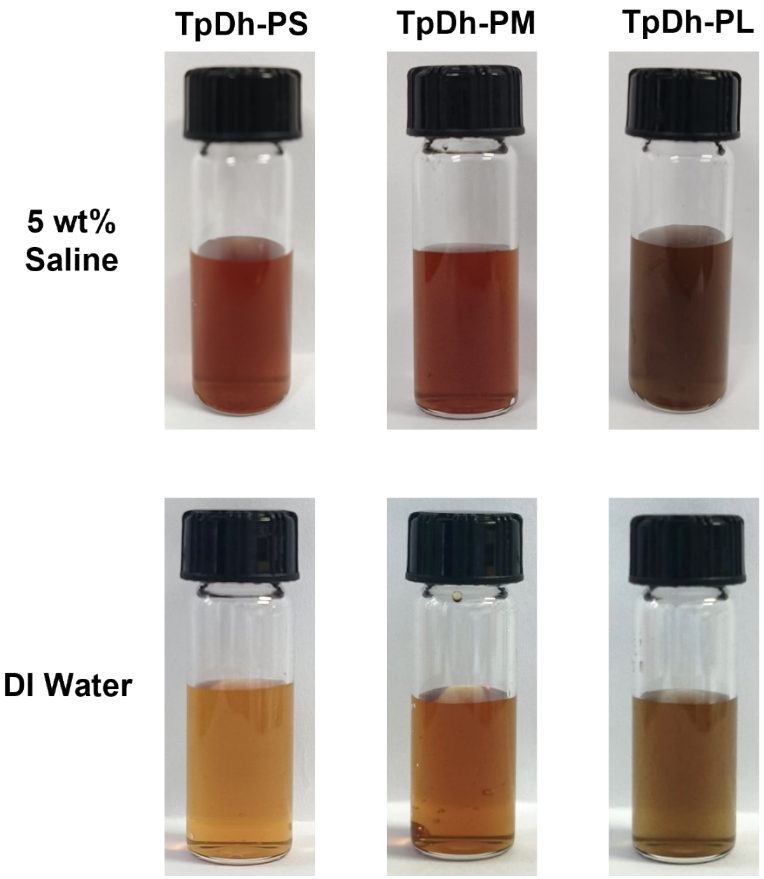
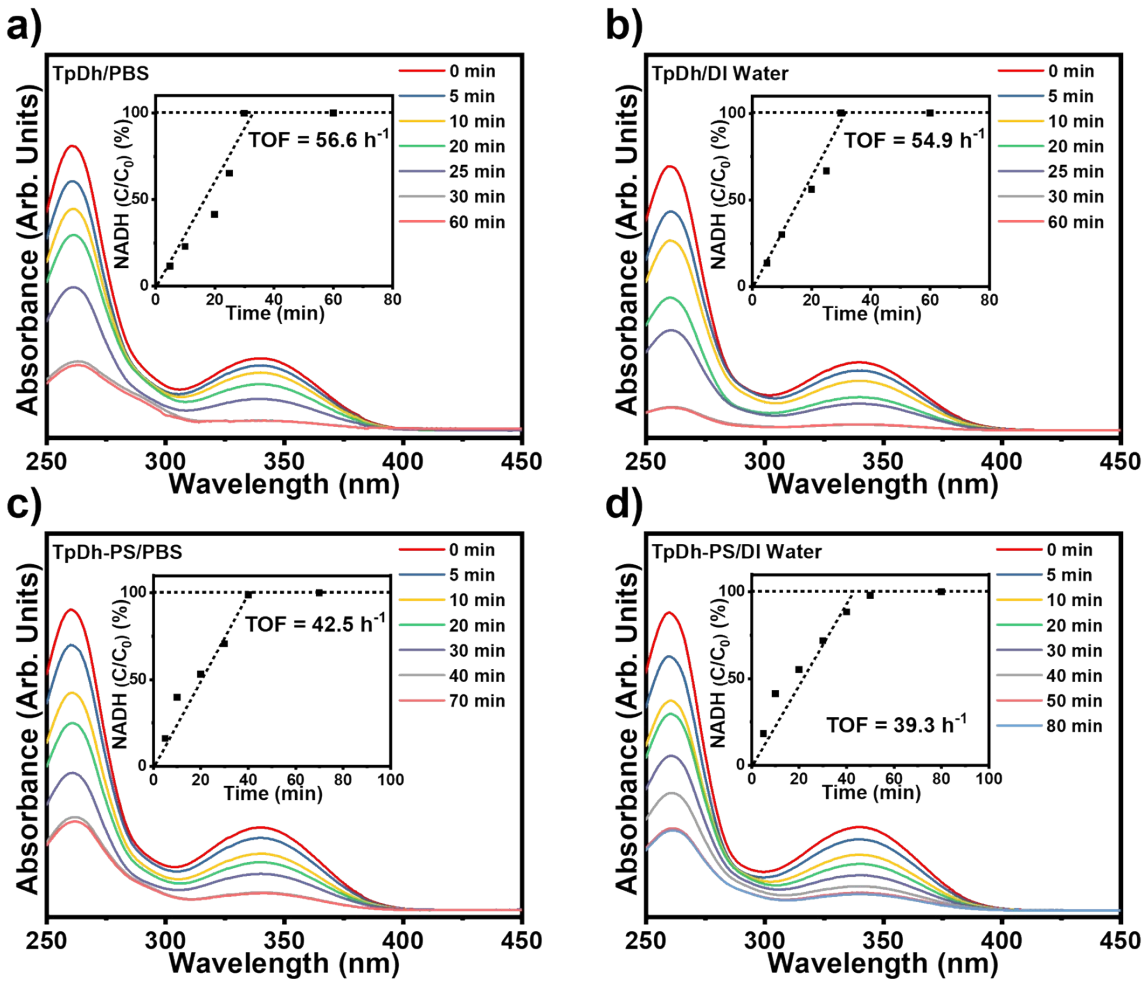


Fig. S18 Snapshots of 3 mg mL^{-1} TpDh-PS, TpDh-PM and TpDh-PL dispersed in 5 wt% saline and DI water.


a)
TpDh/PBS
0 min
5 min
10 min
20 min
25 min
30 min
60 min
TOF = 56.6 h-1
NADH (C/C0) (%)
Time (min)
Absorbance (Arb. Units)
Wavelength (nm)
b)
TpDh/DI Water
0 min
5 min
10 min
20 min
25 min
30 min
60 min
TOF = 54.9 h-1
NADH (C/C0) (%)
Time (min)
Absorbance (Arb. Units)
Wavelength (nm)
c)
TpDh-PS/PBS
0 min
5 min
10 min
20 min
30 min
40 min
70 min
TOF = 42.5 h-1
NADH (C/C0) (%)
Time (min)
Absorbance (Arb. Units)
Wavelength (nm)
d)
TpDh-PS/DI Water
0 min
5 min
10 min
20 min
30 min
40 min
50 min
80 min
TOF = 39.3 h-1
NADH (C/C0) (%)
Time (min)
Absorbance (Arb. Units)
Wavelength (nm)

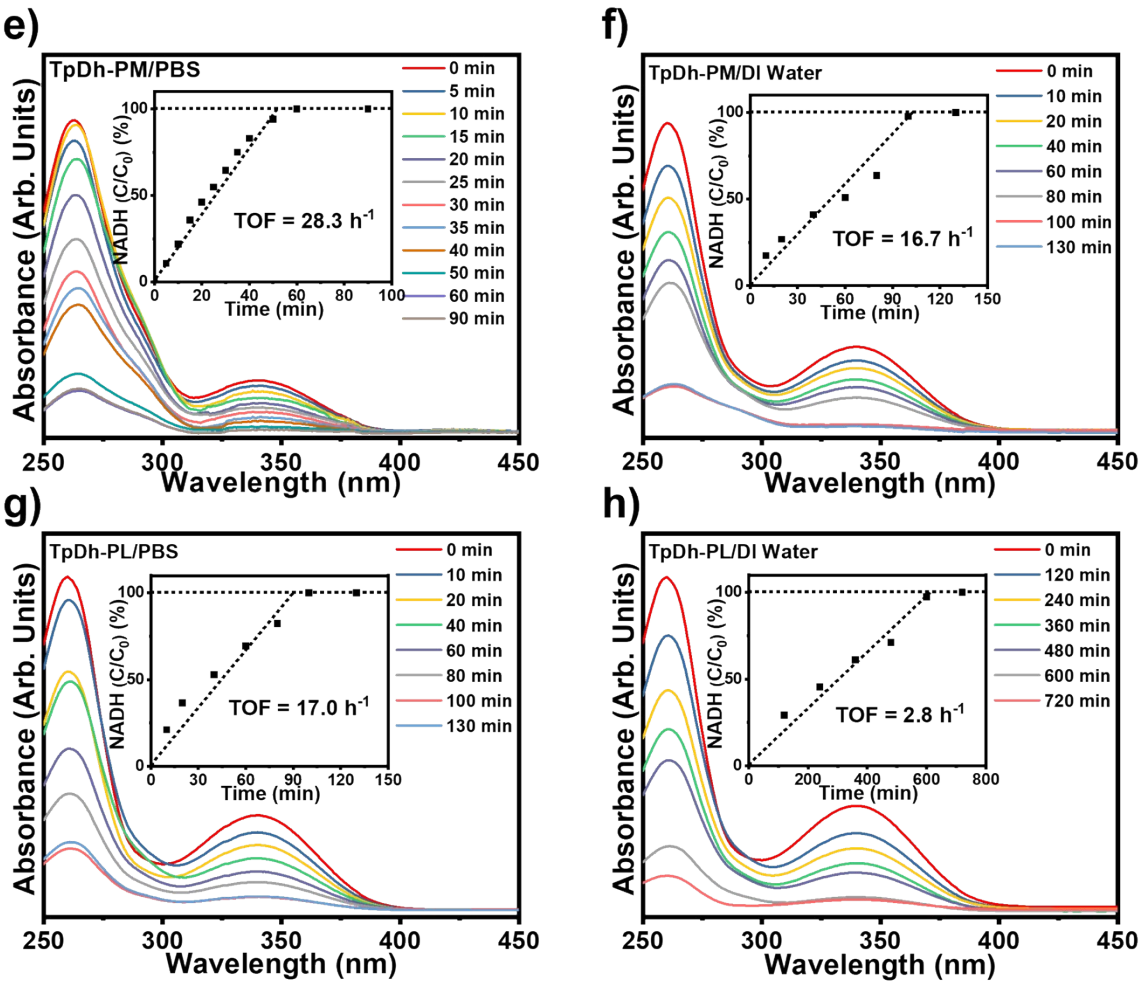


Fig. S19 UV-Vis absorbance spectra of NADH oxidation containing (a,b) TpDh, (c,d) TpDh-PS, (e,f) TpDh-PM and (g,h) TpDh-PL in PBS or DI water.

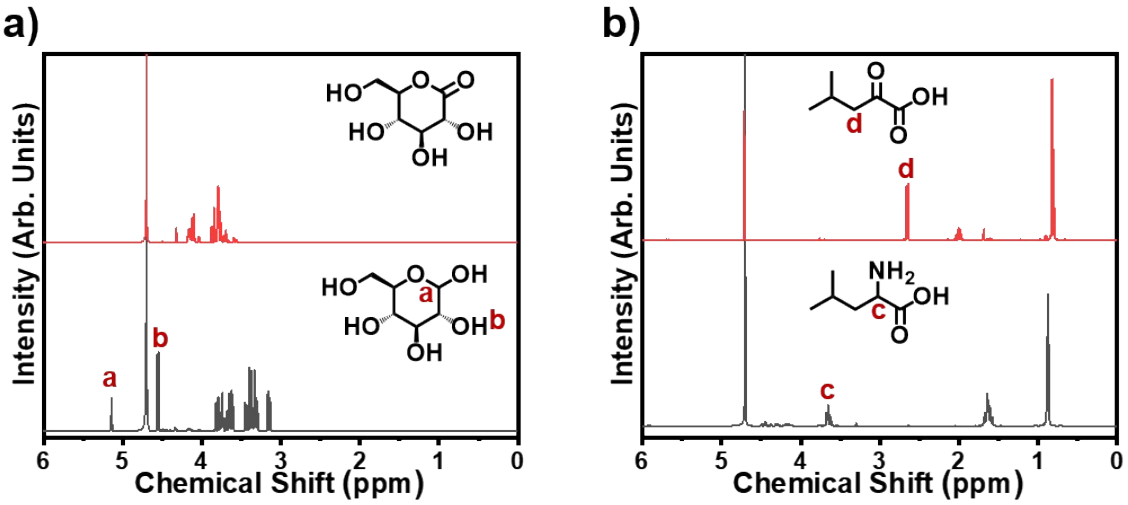


Fig. S20 Analysis of a) TpDh-PM paired with glucose dehydrogenase catalyzed the conversion of glucose into gluconolactone and b) TpDh-PM paired with leucine dehydrogenase catalyzed the conversion of L-leucine into *α*-ketoisocaproate by ^{1}H NMR spectroscopy.

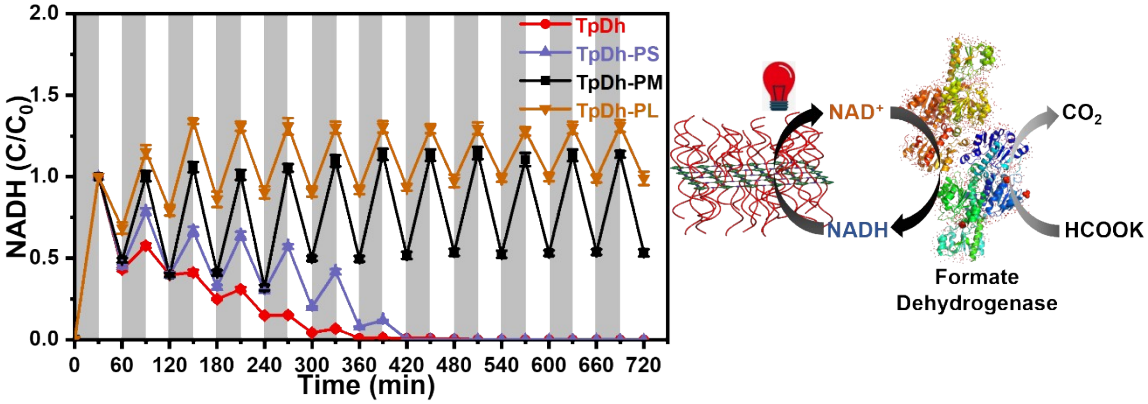


Fig. S21 The TpDh-P or TpDh in conjunction with formate dehydrogenase to regenerate NAD^+ and NADH through alternative addition of reagents (potassium formate) and red light (λ_{max} = 680 nm, 1.2 mW cm^{-2}) in darkness.

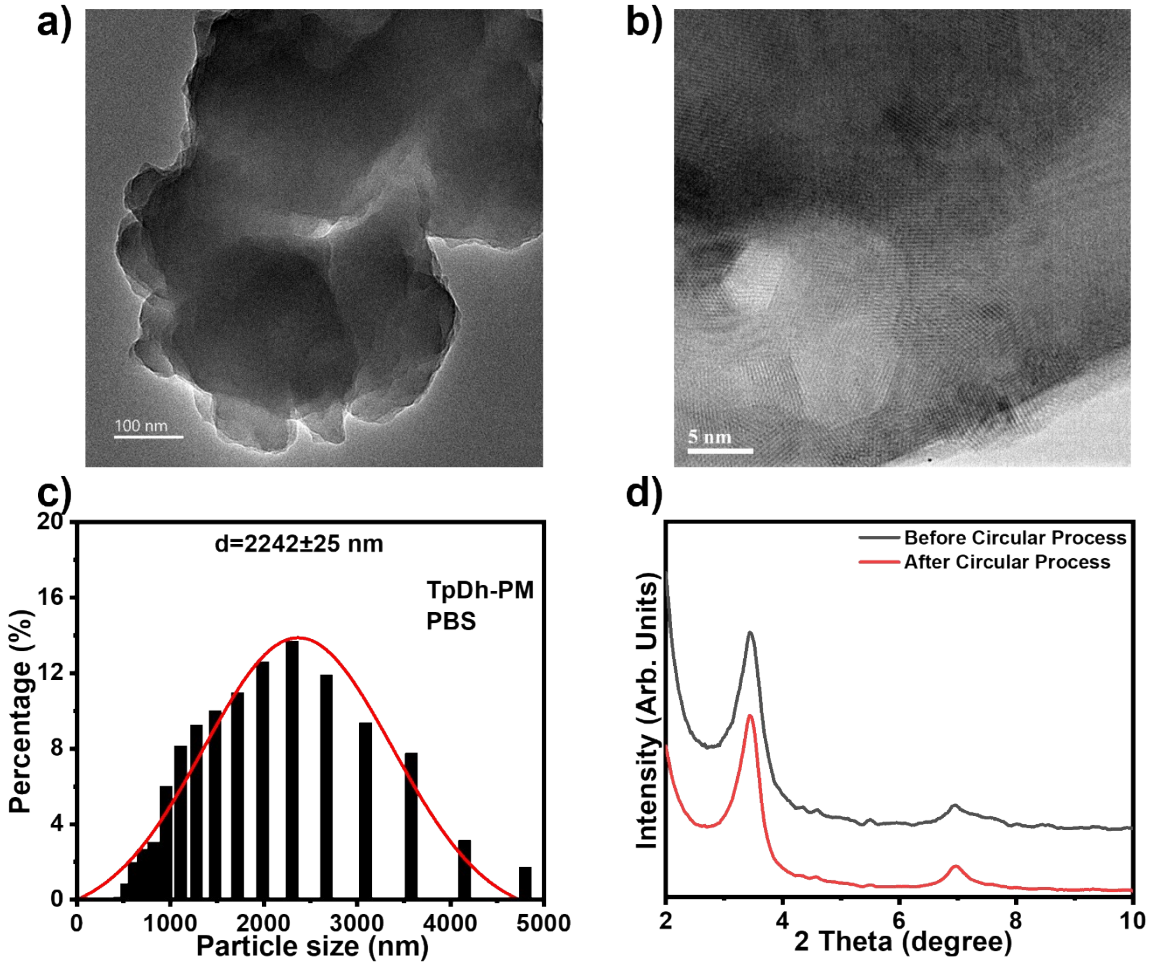


Fig. S22 (a) TEM image, (b) HR-TEM image, (c) DLS traces and (d) XRD patterns of TpDh-PM after twelve reaction cycles.

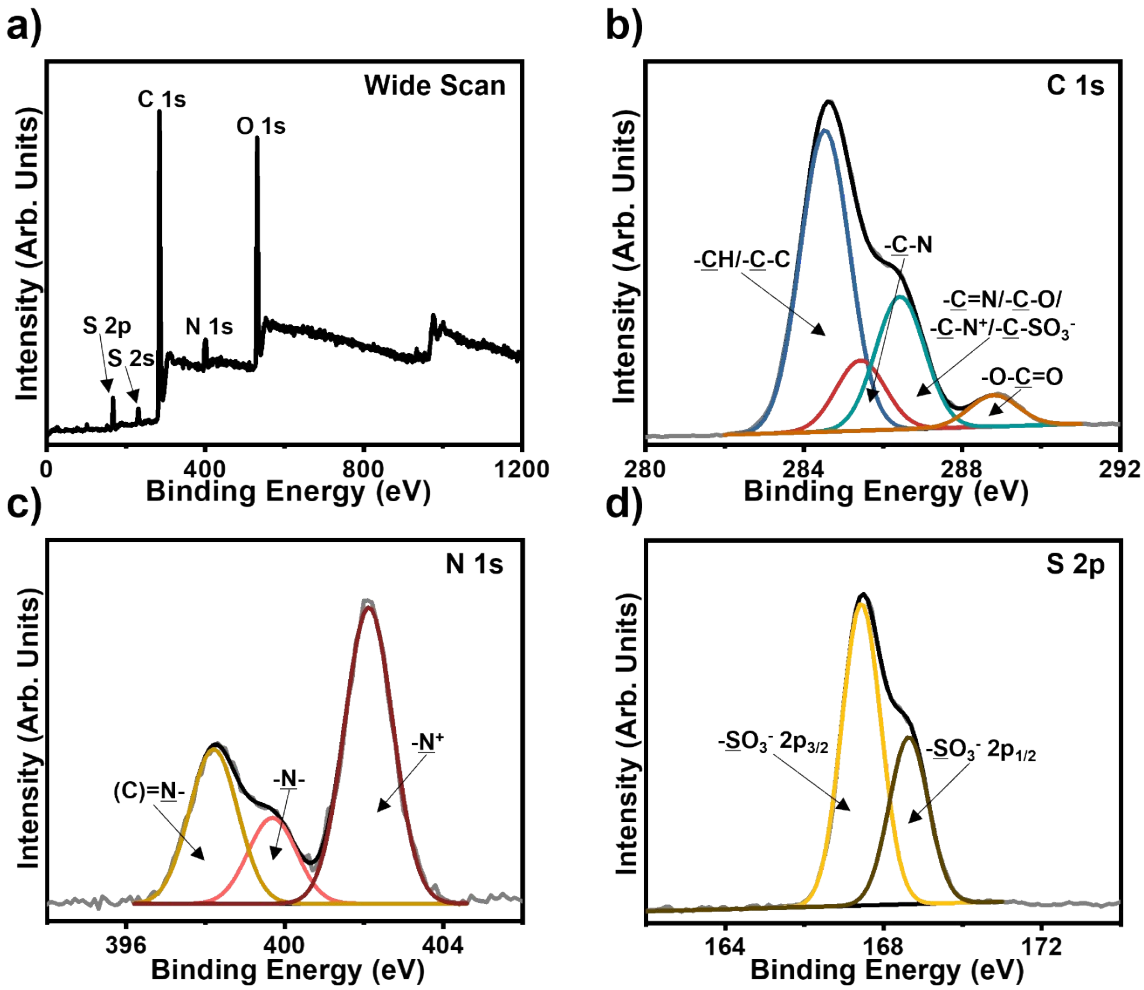


Fig. S23 XPS (a) wide scan, (b) C 1s, (c) N 1s and (d) S 2p core-level spectra of TpDh-PM after twelve reaction cycles.

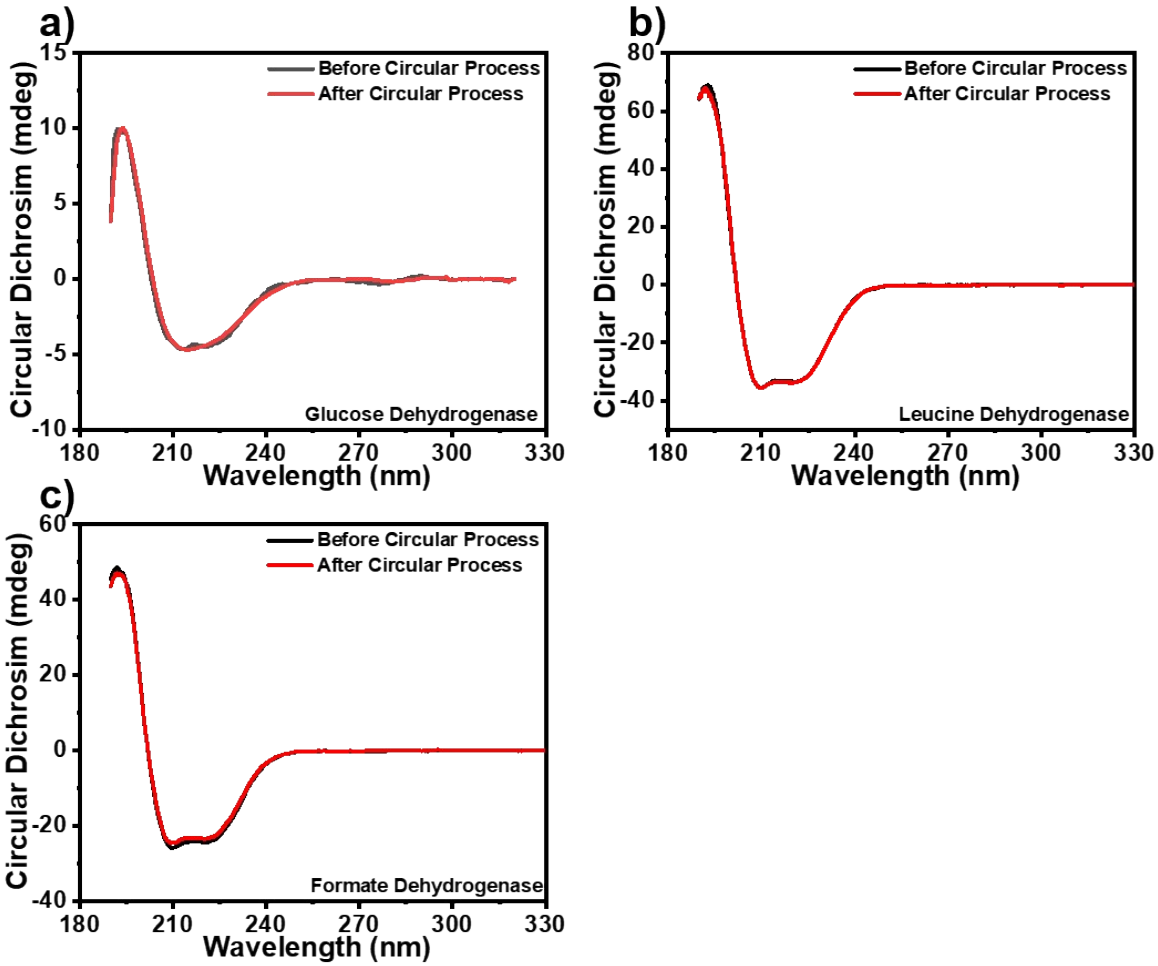


Fig. S24 CD spectra of (a) glucose dehydrogenase, (b) leucine dehydrogenase and (c) formate dehydrogenase before and after twelve reaction cycles.

Table S1. XPS Results of COFs in This Study

Sample	C(%)	O(%)	N(%)	S(%)	Br(%)	N/C
TpDh	85.66	3.12	11.22	-	-	0.131
TpDh-Br	73.24	12.84	9.94	-	3.97	0.136
TpDh-PS	68.91	20.23	7.22	3.53	0.11	0.105
TpDh-PM	68.13	20.94	7.04	3.77	0.12	0.103
TpDh-PL	67.44	21.51	6.81	4.14	0.10	0.101
TpDh-PM (After twelve reaction cycles)	68.19	21.04	7.06	3.64	0.07	0.104

Table S2. Elemental Analysis of COFs in This Study

Sample	C(%)	O(%)	N(%)	S(%)	N/C
TpDh	74.38	7.65	10.78	-	0.144
TpDh-Br	75.50	10.60	9.27	-	0.123
TpDh-PS	64.13	14.33	7.50	2.87	0.117
TpDh-PM	60.77	17.19	7.01	4.59	0.115
TpDh-PL	54.79	22.29	6.11	7.65	0.112
TpDh-PM (After twelve reaction cycles)	60.89	17.06	7.04	4.52	0.116

Table S3. Comparison of Different Metal-Free Photocatalysts Mediated Aerobic Photobiocatalysis

#	Photocatalyst	Catalyst Category	State	Concentration	Light Source	Enzymes	Reaction Cycles	Ref.
1	TpDh-PM	Covalent Organic Frameworks	Crystalline	2.0 mg mL^{-1}	680 nm 1.2 mW cm^{-2}	Glucose Dehydrogenase; Leucine Dehydrogenase; Formate Dehydrogenase; Glucose Dehydrogenase; Glycerol Dehydrogenase	12	This Work
2	NP-CS	Nanoparticles	Amorphous	2.4 mg mL^{-1}	460 nm 0.1 mW cm^{-2}		10	[6]
3	PEG-*b*-PTTMNMA	Polymer Micelles and Vesicles	Amorphous	20 μM, 40 μM	White Light, 30 mW cm^{-2}	Glucose Dehydrogenase	10	[7]
4	S-*h*PrTZ-P	Conjugated Microporous Polymers	Amorphous	1.6 mg mL^{-1}	740 nm 3.0 mW cm^{-2}	Glucose Dehydrogenase; Glycerol Dehydrogenase	9	[5]

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