

ISCI, Volume 23

Supplemental Information

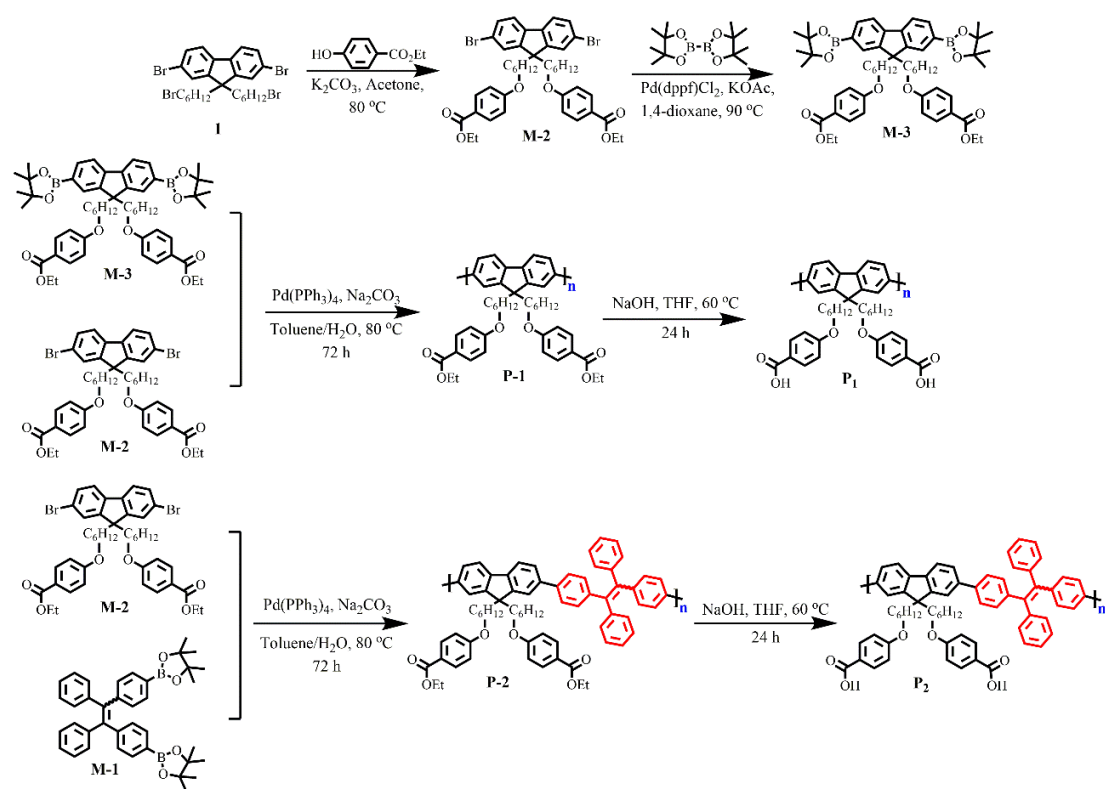
Highly Efficient Aggregation-Induced

Electrochemiluminescence of Polyfluorene Derivative

Nanoparticles Containing Tetraphenylethylene

Si-Yuan Ji, Wei Zhao, Hang Gao, Jian-Bin Pan, Cong-Hui Xu, Yi-Wu Quan, Jing-Juan Xu, and Hong-Yuan Chen

SUPPLEMENTAL FIGURES



Scheme S1. The synthesis route of the polymers (P_1 and P_2). Related to Scheme 1.

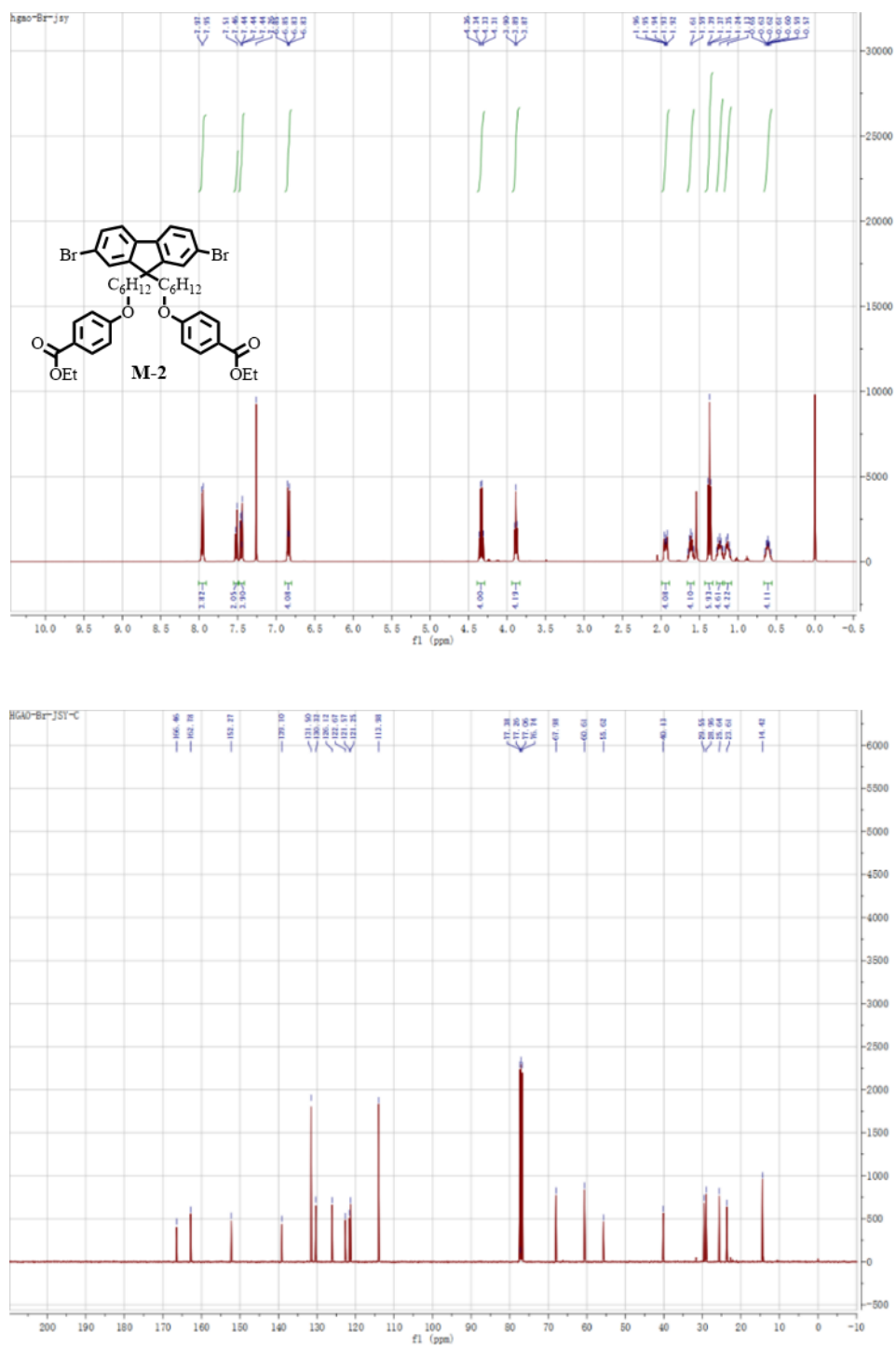


Figure S1. ¹H (400 MHz, CDCl₃, 25°C) and ¹³C (100 MHz, CDCl₃, 25°C) NMR Spectra of M-2. Related to Scheme 1.

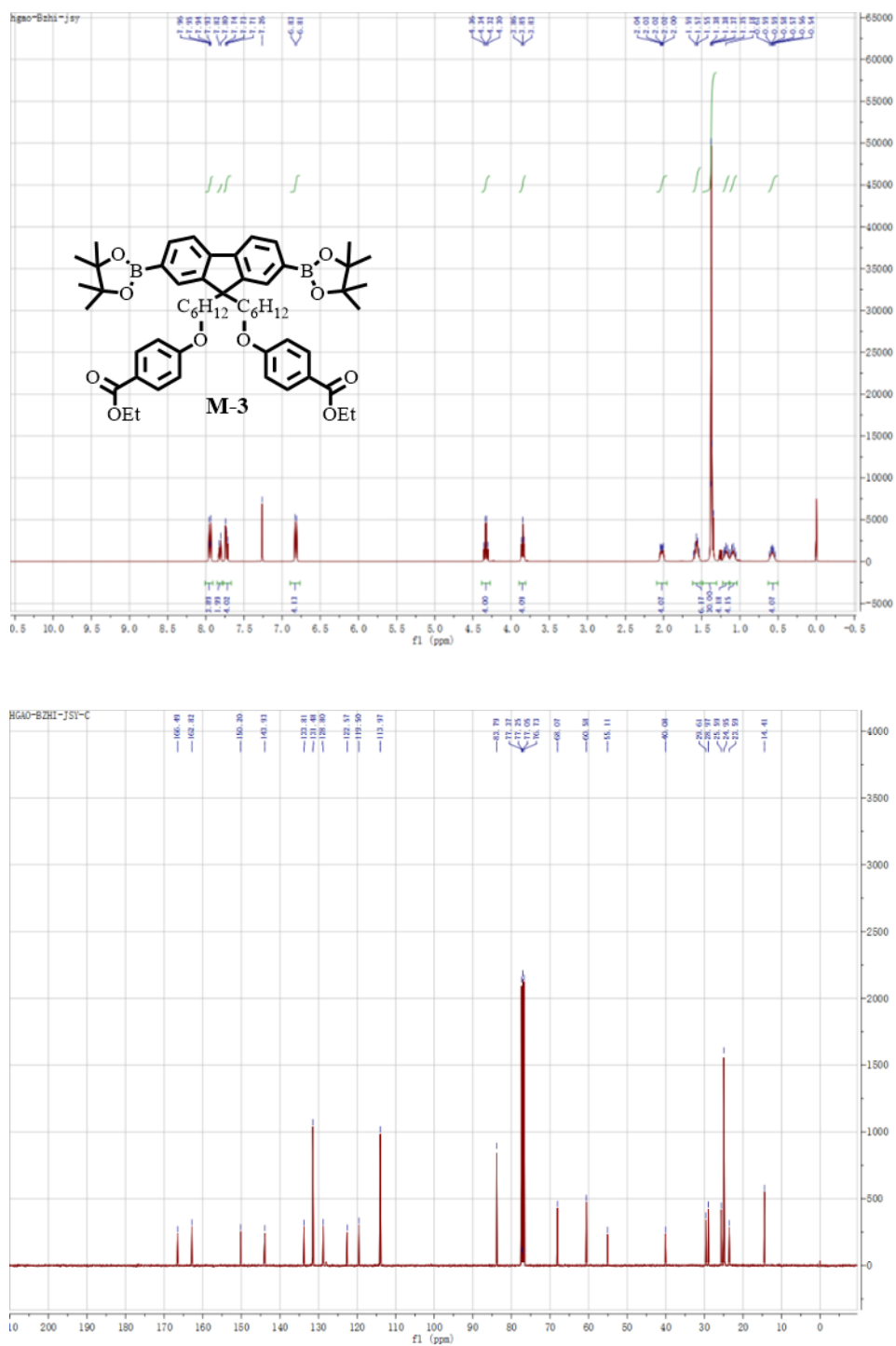


Figure S2. ^1H (400 MHz, CDCl_3 , 25°C) and ^{13}C (100 MHz, CDCl_3 , 25°C) NMR Spectra of M-3. Related to Scheme 1.

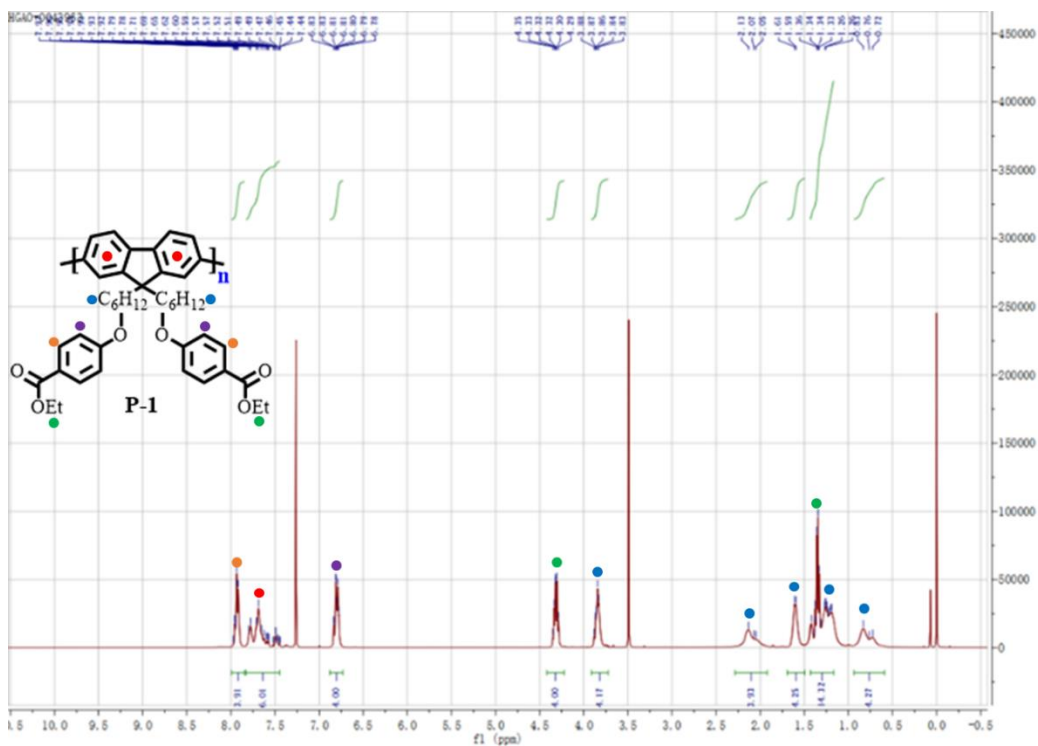


Figure S3. ^1H (400 MHz, CDCl_3 , 25°C) NMR Spectra of P-1. Related to Scheme 1.

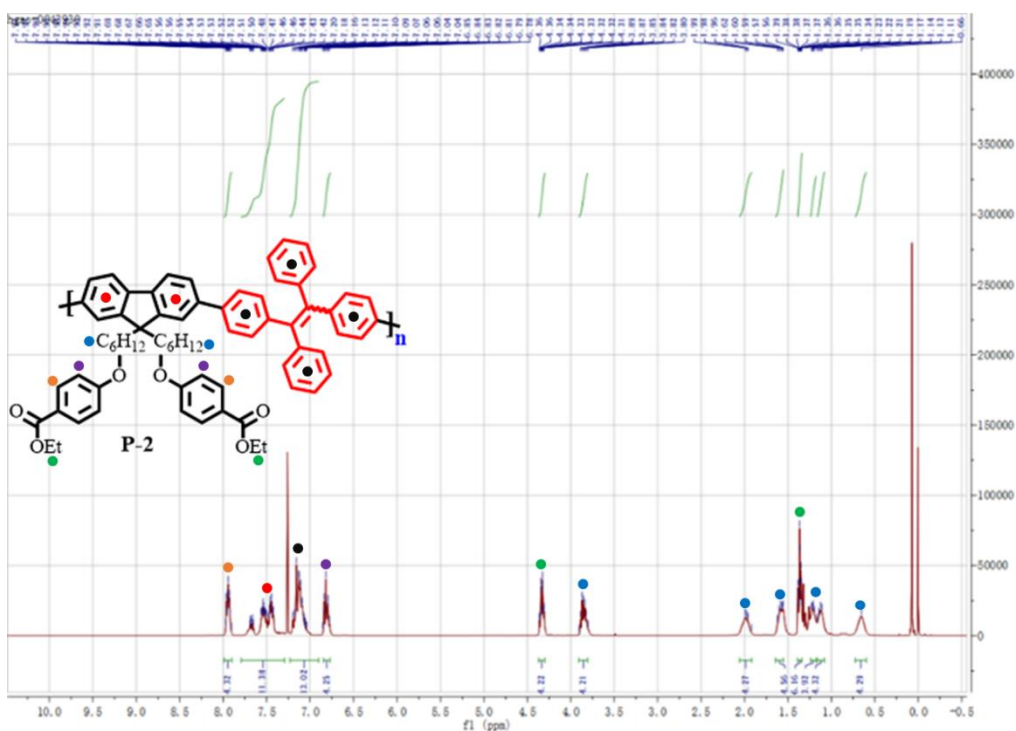


Figure S4. ^1H (400 MHz, CDCl_3 , 25°C) NMR Spectra of P-2. Related to Scheme 1.

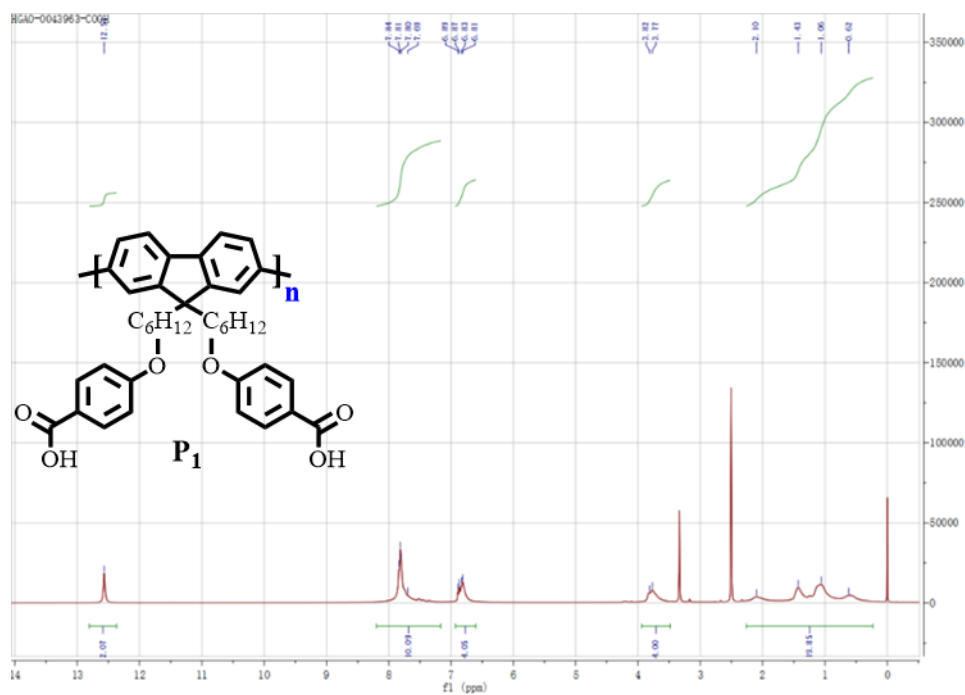


Figure S5. ^1H (400 MHz, DMSO-d_6 , 25°C) NMR Spectra of P_1 . Related to Scheme 1.

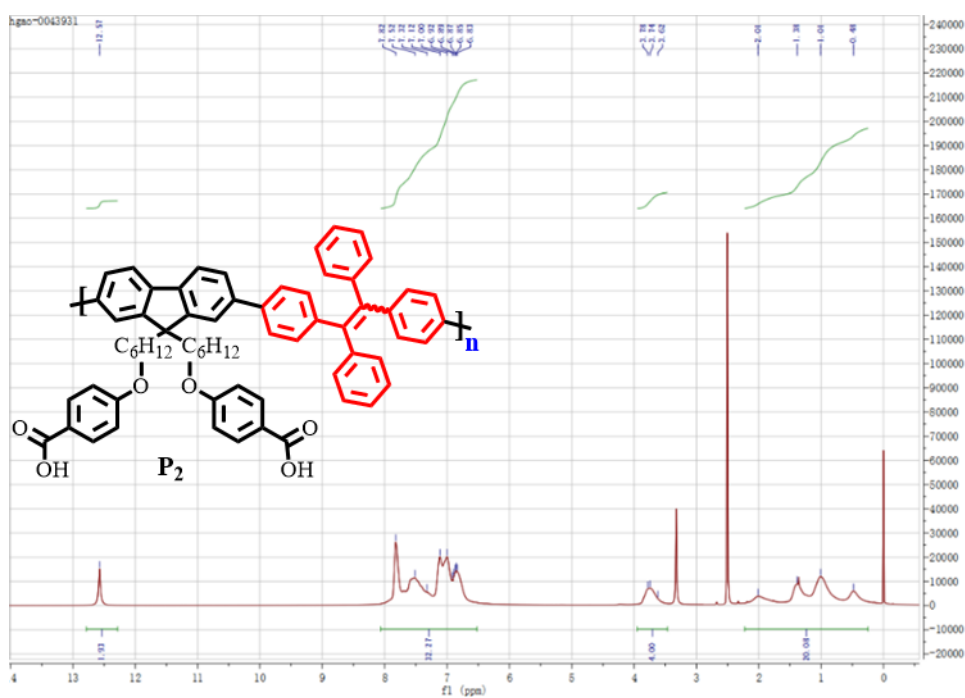


Figure S6. ^1H (400 MHz, DMSO-d_6 , 25°C) NMR Spectra of P_2 . Related to Scheme 1.

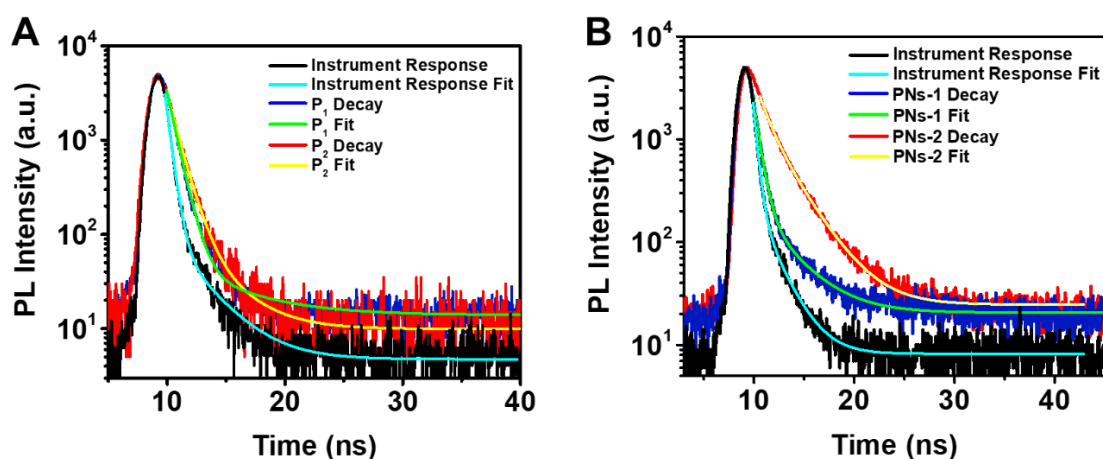


Figure S7. The PL lifetime decay curves of (A) P_1 and P_2 , (B) PNs-1 and PNs-2. The pulse width of the pulsed excitation source is 998.5 ps. Related to Figure 1 and Table 1.

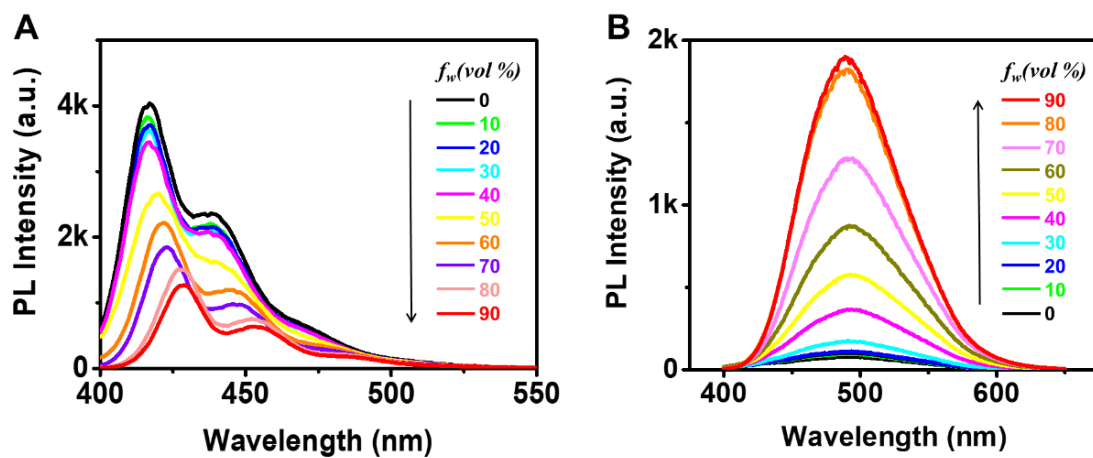


Figure S8. The PL spectra of (A) P_1 and (B) P_2 in THF/water mixtures with different water fractions (f_w). Related to Figure 1.

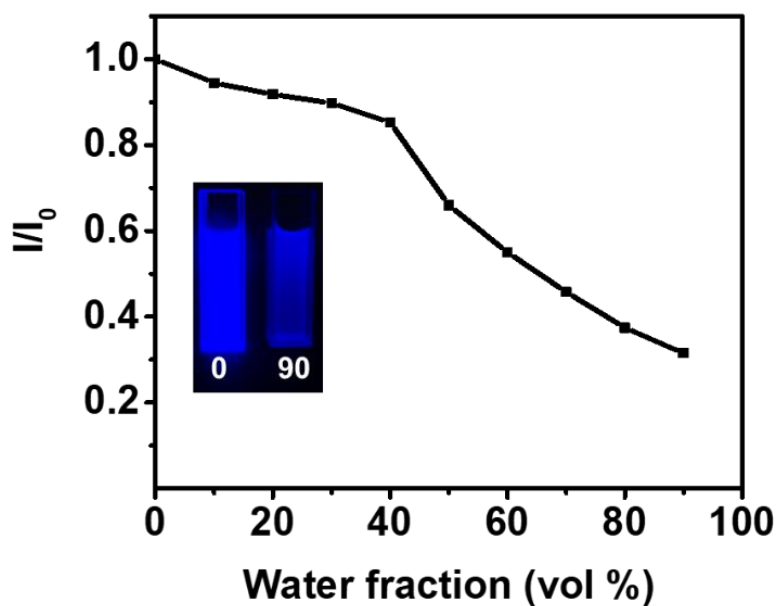


Figure S9. ACQ effect of P_1 . Plots of I/I_0 vs water fractions in THF/water mixtures (10 μM), where I_0 and I were the PL intensities in pure THF and THF/water mixtures, respectively. Inset: Photographs of P_1 in THF/water mixtures (0, 90%) taken under 365 nm UV lamp. Related to Figure 1.

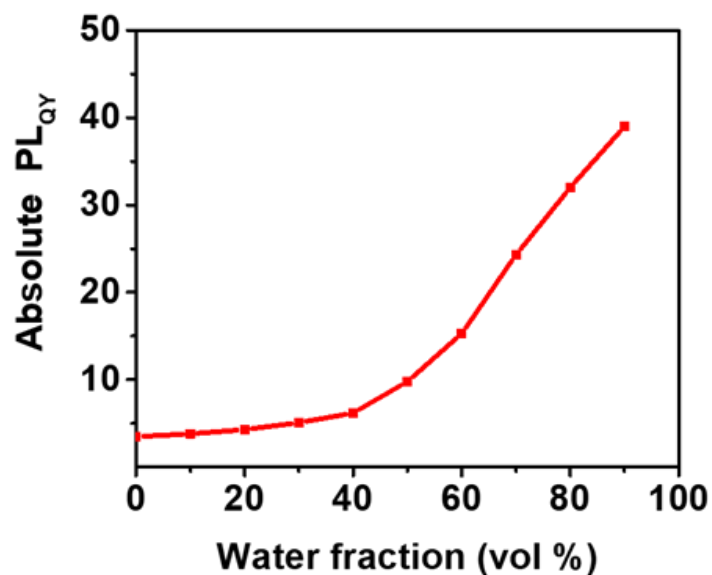


Figure S10. Plot of absolute fluorescence quantum efficiencies (QY_{PL}) of P_2 vs water fractions in THF/ H_2O mixtures (10 μM). Related to Figure 1

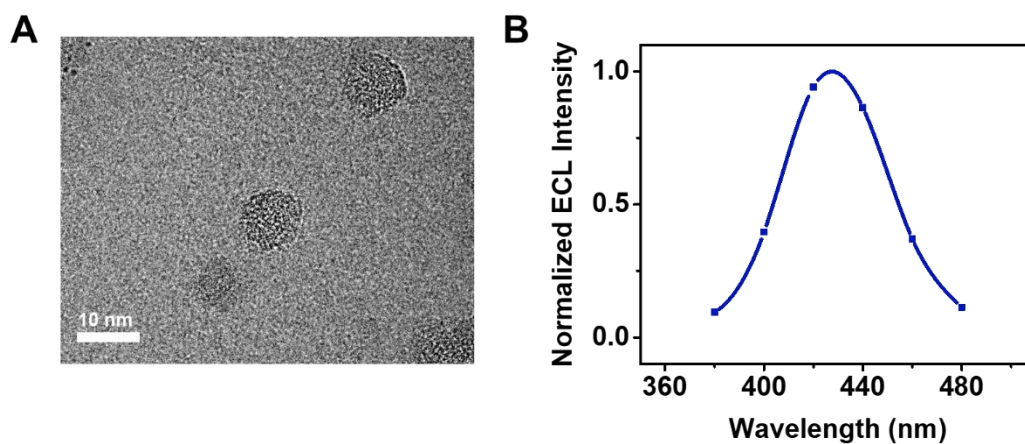


Figure S11. (A) High-resolution TEM image of 9 nm PN-1. Scale bar, 10 nm. Data are represented as mean \pm TEM. (B) Normalized ECL spectrum of PN-1 generated through annihilation route by pulsing potential from approximately 100 mV past the peak potentials. Related to Figure 1 and Figure 3.

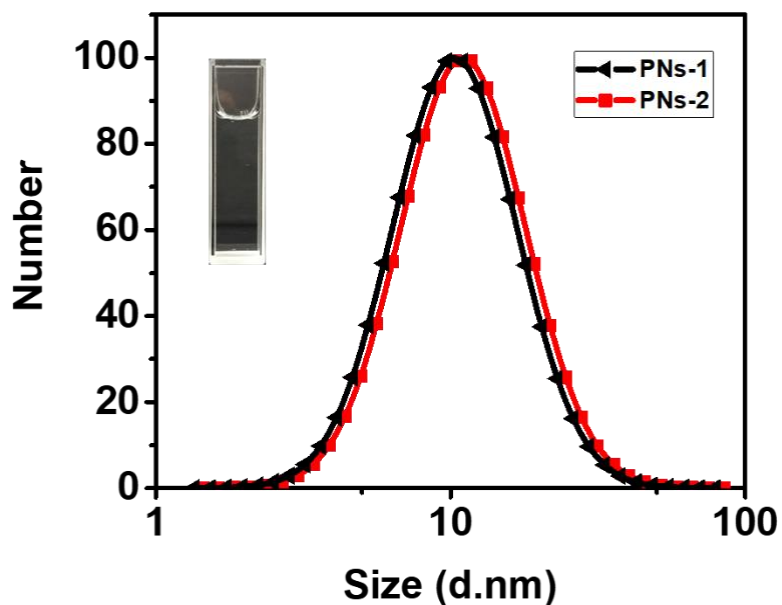


Figure S12. The DLS size distribution of the PN-1 and PN-2. Inset: Photograph of PN-2 aqueous solution. Related to Figure 1.

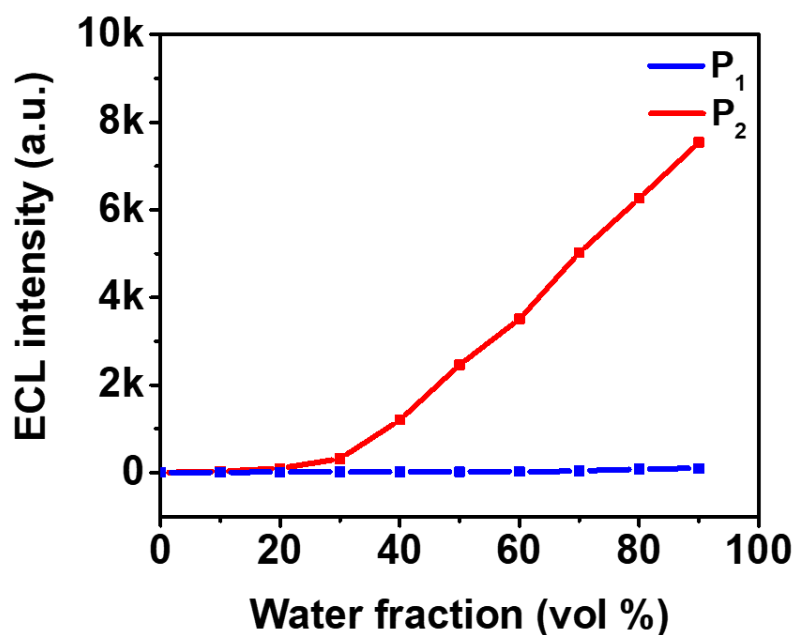


Figure S13. Plot of the ECL intensity changes of P₁ (blue) and P₂ (red) upon variation of the H₂O fraction of the THF/H₂O mixtures, upon addition of 75 mM TPrA as co-reactant. PMT = 500 V. Related to Figure 2.

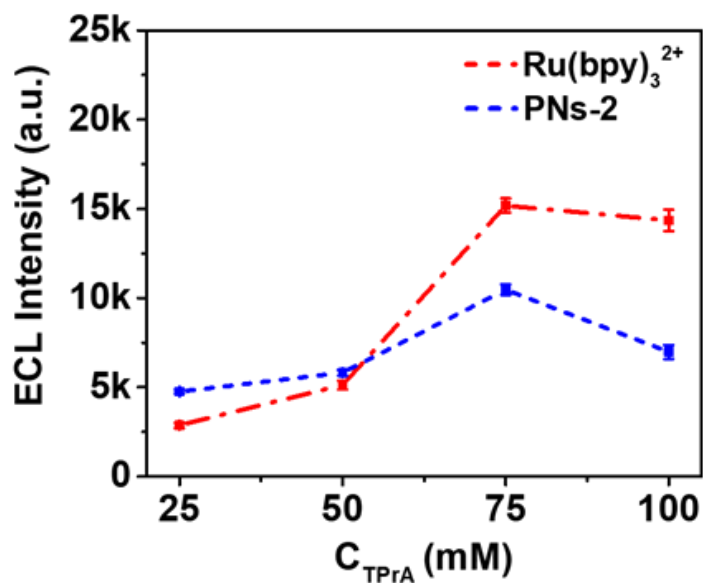


Figure S14. Effect of TPrA concentration on the ECL intensity of 20 μM PNs-2 (blue) or Ru(bpy)₃²⁺ (red) in water containing 0.1 M LiClO₄ as the supporting electrolyte. PMT = 500 V. Related to Figure 2.

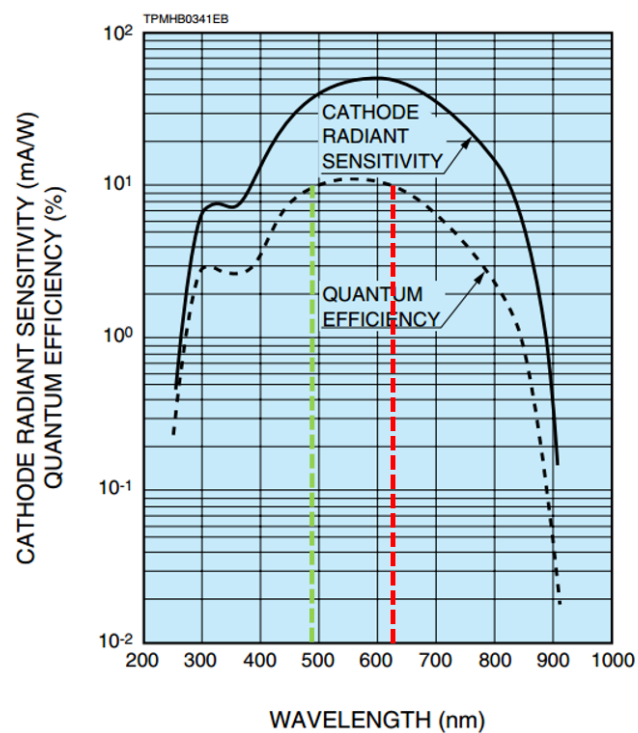


Figure S15. The spectral response curve of Hamamatsu R2257 PMT used in our ECL test. Related to Figure 2 and 3.

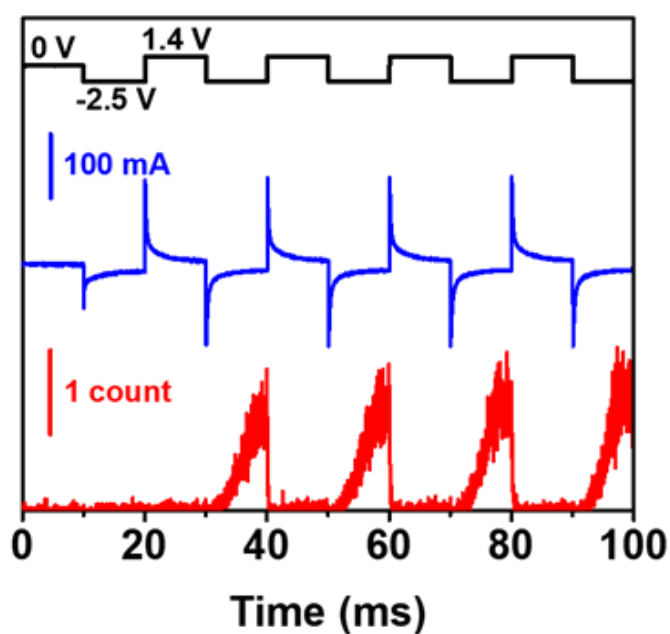


Figure S16. Transient profiles of current (blue), ECL (red) and applied voltage (black) of PN-2 modified GCE in 0.10 M PBS. The applied potential was repetitively stepped from -2.5 V to +1.4 V vs Ag/AgCl and the pulse width is 10 ms. Related to Figure 3.

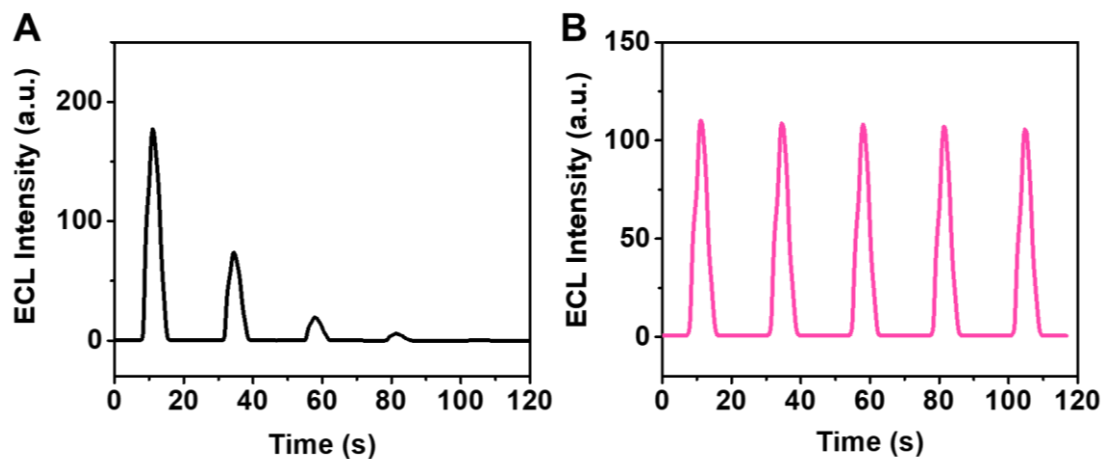


Figure S17. ECL intensity–time curves of 20 μM $\text{Ru}(\text{bpy})_3^{2+}$ modified GCE (A) without Nafion and (B) with Nafion in 0.1 M PBS solution containing 75 mM TPrA as co-reactant. PMT = 200 V. Related to Figure 3.

Table S1. Calculated ECL Efficiency for PNs-2 Relative to That of 20 μM $\text{Ru}(\text{bpy})_3^{2+}$ (Nafion) Under Different Concentrations of TPrA.

C_{TPrA} (mM)	Relative ECL Efficiency (%)
25	286
50	242
75	163
100	145

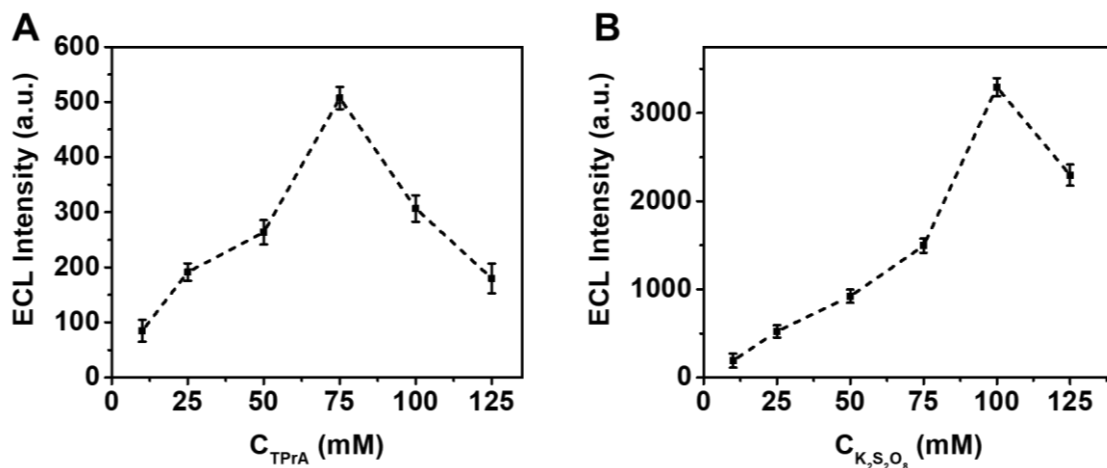


Figure S18. The optimization of experiment conditions. Effects of (A) TPrA and (B) $\text{K}_2\text{S}_2\text{O}_8$ concentration on the ECL intensity of the PN-2 modified GCE. PMT = 200 V. Related to Figure 3.

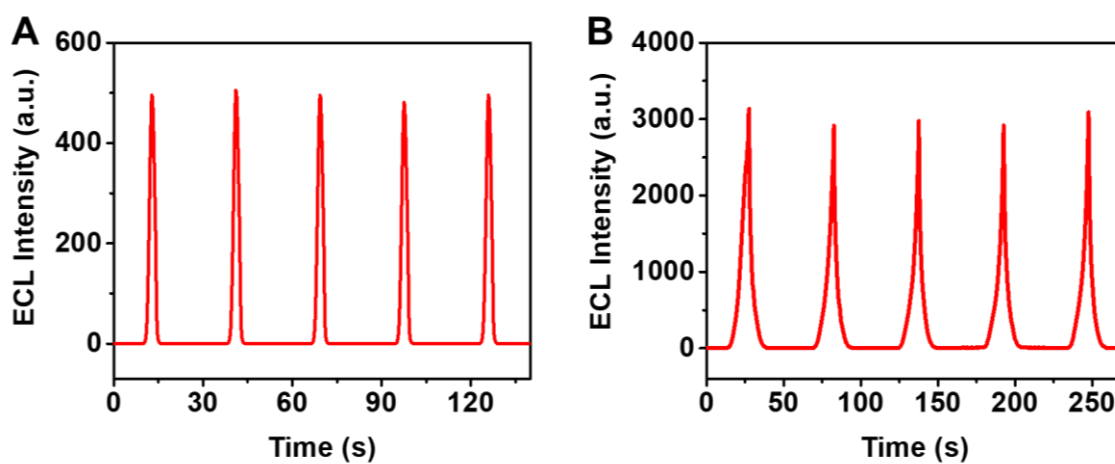


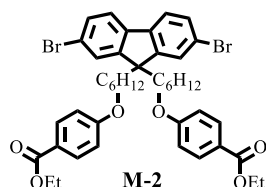
Figure S19. ECL intensity-time curves of PN-2 modified GCE in 0.1 M PBS solution containing (A) 75 mM TPrA and (B) 100 mM $\text{K}_2\text{S}_2\text{O}_8$ as co-reactant. PMT = 200 V. Related to Figure 3.

Transparent Methods

Reagents and materials.

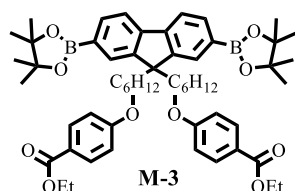
All the chemicals were used as received without further purification. Anhydrous benzene (Bz, 99.8%), tetra-*n*-butylammonium hexafluorophosphate (TBAPF₆), tetrahydrofuran (THF, 99.9%), electrochemical-grade anhydrous acetonitrile (MeCN, 99.8%), Nafion® perfluorinated resin solution (5.0 wt%), lithium perchlorate (LiClO₄) and tris(2,2'-bipyridyl) dichlororuthenium (II) hexahydrate (Ru(bpy)₃²⁺) were purchased from Sigma-Aldrich (USA) and stored into a dry-box. Tripropylamine (TPrA, 98%) and K₂S₂O₈ were purchased from Aladdin Reagent Corporation (Shanghai, China). Phosphate buffer saline (PBS, 0.1 M, pH 7.4) was prepared by mixing stock solution of NaH₂PO₄ and Na₂HPO₄ that contained 0.1 M NaCl. Ultrapure fresh water was obtained from the Millipore water purification system (resistivity of 18.2 MΩ·cm at 25 °C) and used for preparation of all aqueous solutions.

M-2



Compound **1** (2.75 g, 4.23 mmol), 4-hydroxybenzoic acid ethyl ester (2.11 g, 12.7 mmol) and K₂CO₃ (2.9 g, 21.15 mmol) were dissolved in 50 mL of acetone, and the reaction mixture was heated at 80 °C for overnight. After the reaction mixture was cooled to room temperature, the solution was filtered. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (eluent: petroleum ether/ethyl acetate, v/v, 15:1) to give **M-2** as white solid (2.9 g, 84%). ¹H NMR (400 MHz, CDCl₃) δ (ppm): 7.97–7.95 (m, 4H), 7.53–7.51 (m, 2H), 7.47–7.44 (m, 4H), 6.85–6.83 (m, 4H), 4.34 (q, *J* = 7.1 Hz, 4H), 3.89 (t, *J* = 6.5 Hz, 4H), 1.96–1.92 (m, 4H), 1.65–1.58 (m, 4H), 1.37 (t, *J* = 7.1 Hz, 6H), 1.27–1.20 (m, 4H), 1.178–1.10 (m, 4H), 0.65–0.57 (m, 4H); ¹³C NMR (100 MHz, CDCl₃) δ (ppm): 166.46, 162.78, 152.27, 139.10, 131.50, 130.32, 126.12, 122.67, 121.57, 121.25, 113.98, 67.98, 60.61, 55.62, 40.13, 29.55, 28.96, 25.64, 23.61, 14.42.

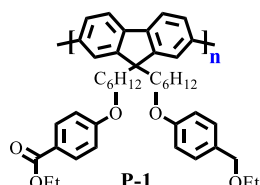
M-3



M-2 (2.5 g, 4.56 mmol), bis(pinacolato)diboron (1.5 g, 5.93 mmol), Pd(dppf)Cl₂ (186.2 mg, 0.228 mmol) and KOAc (1.12 g, 11.4 mmol) were added into 20 mL dioxane solvent. The mixture was stirred at 100 °C under N₂ atmosphere. After reaction for 24 h, the solvent was removed under reduced pressure, and residue was purified by silica gel column chromatography (eluent: petroleum ether/ethyl acetate, v/v, 80:1) to afford

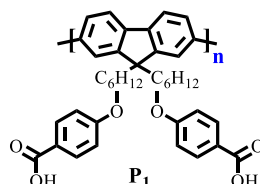
M-3 as white solid (2.4 g, 58%). $^1\text{H NMR}$ (400 MHz, CDCl_3) δ (ppm): 7.96–7.93 (m, 4H), 7.82–7.80 (m, 2H), 7.74–7.71 (m, 4H), 6.83–6.81 (m, 4H), 4.33 (q, $J = 7.1$ Hz, 4H), 3.85 (t, $J = 6.6$ Hz, 4H), 2.04–2.00 (m, 4H), 1.61–1.54 (m, 4H), 1.38–1.35 (m, 30H), 1.22–1.15 (m, 4H), 1.12–1.05 (m, 4H), 0.61–0.54 (m, 4H); $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ (ppm): 166.49, 162.82, 150.20, 143.93, 133.81, 131.48, 128.80, 122.57, 119.50, 113.97, 83.79, 68.07, 60.58, 55.11, 40.08, 29.61, 28.97, 25.59, 24.95, 23.59, 14.41.

P-1



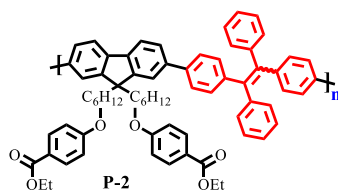
The mixture of **M-2** (158.8 mg, 0.193 mmol), **M-3** (177.0 mg, 0.193 mmol), $\text{Pd}(\text{PPh}_3)_4$ (22 mg, 0.0193 mmol), and K_2CO_3 (533 mg, 3.9 mmol) in Toluene/ H_2O (8 mL: 2 mL) in a Schlenk tube was stirred at 100 °C for 72 h under the argon atmosphere. After reaction, the resulting polymers were purified by being precipitated in methanol twice. Then the solids were dried in vacuum to afford **P-1** as pale yellow solid (227 mg, 85%). $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 7.97–7.92 (m, 4H), 7.79–7.44 (m, 6H), 6.83–6.78 (m, 4H), 4.35–4.29 (m, 4H), 3.88–3.83 (m, 4H), 2.13–2.05 (m, 4H), 1.61–1.59 (m, 4H), 1.42–1.19 (m, 14H), 0.83–0.72 (m, 4H).

P₁



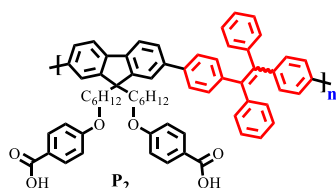
P-1 (227 mg, 0.329 mmol) and NaOH (262.8 mg, 6.57 mmol) were dissolved in 50 mL THF, and the reaction mixture was heated at 80 °C overnight. After the reaction mixture was cooled to room temperature, the solution was adjusted pH to ~2 followed by filtration. The solvent was removed under reduced pressure, and the residue was purified by being precipitated in methanol twice. Then the solids were dried in vacuum to afford **P₁** as pale yellow solid (184 mg, 75%). The M_w and M_n of **P₁** were 2894 and 2504 (PDI = 1.16). $^1\text{H NMR}$ (400 MHz, $\text{DMSO}-d_6$) δ 12.57 (s, 2H), 7.84–6.69 (m, 10H), 6.89–6.81 (m, 4H), 3.82–3.77 (m, 4H), 2.1–0.62 (m, 20H).

P-2



The mixture of **M-1** (86 mg, 0.146 mmol), **M-2** (120 mg, 0.146 mmol), Pd(PPh₃)₄ (19 mg, 0.016 mmol), and Na₂CO₃ (312 mg, 2.9 mmol) in Toluene/H₂O (8 mL: 2 mL) in a Schlenk tube was stirred at 100 °C for 72 h under the argon atmosphere. After reaction, the resulting polymers were purified by being precipitated in methanol twice. Then the solids were dried in vacuum to afford **P-2** as green solid (118 mg, 77%). ¹H NMR (400 MHz, CDCl₃) δ (ppm): 7.97–7.91 (m, 4H), 7.89–7.42 (m, 11H), 7.20–7.04 (m, 13H), 6.85–6.78 (m, 4H), 4.36–4.31 (m, 4H), 3.90–3.80 (m, 4H), 1.99–1.92 (m, 4H), 1.62–1.56 (m, 4H), 1.39–1.34 (m, 6H), 1.23–1.17 (m, 4H), 1.14–1.11 (m, 4H), 0.70–0.58 (m, 4H).

P₂



P-2 (118 mg, 0.116 mmol) and NaOH (92.4 mg, 2.31 mmol) were dissolved in 50 mL THF, and the reaction mixture was heated at 80 °C overnight. After the reaction mixture was cooled to room temperature, the solution was adjusted pH to ~2 followed by filtration. The solvent was removed under reduced pressure, and the residue was purified by being precipitated in methanol twice. Then the solids were dried in vacuum to afford **P₂** as green solid (88 mg, 60%). The Mw and Mn of **P₂** were 4817 and 3706 (PDI = 1.30). ¹H NMR (400 MHz, DMSO-d₆) δ 12.57 (s, 2H), 7.82–6.83 (m, 32H), 3.78–3.62 (m, 4H), 2.01–0.48 (m, 20H).

In the ¹H NMR of **P₁** and **P₂**, the peak of 12.57 ppm (the H of carboxylic acid) was appeared, which indicated the successful polymerization of the target polymers. Additionally, **P₁** and **P₂** showed good solubility in polar solvents, such as THF, DMSO, and DMF, due to the presence of carboxyl group.

Apparatus and characterization.

High-resolution transmission electron microscopy was performed with a JEM-2100 transmission electron microscope (JEOL Ltd., Japan). The UV-vis absorption spectra was obtained on a Shimadzu UV-3600 UV-vis-NIR photospectrometer (Shimadzu Co., Japan). The fluorescence spectra was recorded with a Hitachi F-7000 fluorescence spectrophotometer (Hitachi Ltd., Japan) equipped with a xenon lamp. The fluorescence lifetime measurement was performed on a FLS 980 spectrophotometer (Edinburgh Instruments., U.K.). The instrument is based on time-correlated single photon counting (TCSPC) with a 365 nm pulsed LED light source. For the absolute quantum yields (QY_{PL}) measurement at room temperature, the samples were placed in an optical barium sulfate coated integrating sphere (Edinburgh Instruments., U.K.) fitted in the fluorimeter sample chamber and coupled to the FLS980 spectrometer (Edinburgh Instruments., U.K.). The dynamic light scattering (DLS) and Zeta potential analysis were performed on a 90 Plus/BI-MAS equipment (Brookhaven., U.S.A.). The molecular weight was determined by gel permeation chromatography (GPC) analysis,

which was performed on a PL-GPC 50 integrated GPC (Agilent Technologies Inc., U.S.A.) equipped with refractive index detector. The THF was used as the eluent relative to polystyrene standards. And NMR spectra was recorded on a Bruker Advance 400 spectrometer (Bruker Corporation., German) at 400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR reported as parts per million (ppm) from the internal standard tetramethylsilane (TMS).

The entire transient ECL experiment was tested by our self-built instrument. The CHI 660E electrochemical workstation was used as voltage output device. The transient ECL test was done in a self-contained blackbox and three-electrode system was placed in it and a few millimeters from the PMT (Hamamatsu, R9420, Japan) at the bottom of the cassette. The PMT received ECL signal to generate photocurrent, which was directly converted into a voltage signal through the amplifier unit (Hamamatsu, C12419, Japan) and fed into the external input channel of the oscilloscope along with the voltage and current signals recorded by the electrochemical workstation. The oscilloscope as receiving device recorded the entire transient process.

Preparation of the PNPs.

The well-dispersed PNPs were prepared in aqueous solution by reprecipitation method. In short, 1 mL of P_1 (130 $\mu\text{g}/\text{mL}$) or P_2 (190 $\mu\text{g}/\text{mL}$) dissolved in THF was quickly injected into 10 mL of Millipore water and under sonication for 3 min. After that, THF was removed by high purity argon stripping and then rotary evaporation under vacuum. The resulting PNPs solution was filtered through a 0.22 μm pore-size filter (Millex GP, PES membrane). The as-synthesized PNPs were colorless (inset of Figure S12) and transparent, and stable for months when stored at 4 $^\circ\text{C}$.

It is known that the molar mass of the repeating unit of the polymer is 635 g/mol (P_1) and 965 g/mol (P_2), respectively. We could calculate the concentration of the prepared nanoparticle as:

$$\text{PNPs-1: } \frac{130 \mu\text{g}/\text{mL} \times 1 \text{ mL}}{10 \text{ mL} \times 635 \text{ g}/\text{mol}} = 20 \mu\text{M}$$

$$\text{PNPs-2: } \frac{190 \mu\text{g}/\text{mL} \times 1 \text{ mL}}{10 \text{ mL} \times 965 \text{ g}/\text{mol}} = 20 \mu\text{M}$$

Electrochemical and ECL Measurements.

Electrochemical experiments were performed on a CHI 660E electrochemical workstation (CH Instruments Inc., U.S.A.). The cyclic voltammetry (CV) for 0.5 mM polymers were studied in MeCN/Bz (1:1, v/v) mixed solvent and 0.1 M TBAPF₆ as the supporting electrolyte in a homemade glass cell under argon atmosphere. A conventional three-electrode system: a glassy carbon electrode (GCE from CHI Instruments Inc., Shanghai) with 3 mm diameter as the working electrode, a platinum wire as the counter electrode and a silver wire as quasi-reference electrode. Before each experiment, the working electrode was polished with 0.05 μm alumina (CHI Instruments Inc., Shanghai) for several minutes and alternating ultrasound in ethanol and aqueous solution. The counter and reference electrodes were also cleaned by rinsing in water and ethanol solution. Finally, all the electrodes were blown dry with a

stream of nitrogen. The quasi-reference electrode was separated from the electrolyte by glass tube and was calibrated against a saturated calomel electrode (SCE) by the addition of ferrocene as an internal standard at the end of each experiment, taking $E^{\circ}_{Fc/Fc^+} = 0.446$ V vs SCE.

The ECL emission signals were recorded on MPI-E multifunctional electrochemical and chemiluminescent analytical system (Xi'an Remax Analytical Instrument Co. Ltd., China). And we used PNs modified glassy carbon electrode (PNs/GCE) as the working electrode and an Ag/AgCl electrode as the reference electrode, while the counter electrode remained unchanged. Three-electrode system were positioned a few millimeters from the PMT in a homemade blackbox. The ECL spectrum were measured with a series of different optical filters (20 nm spaced), which were placed in front of the PMT window of blackbox and the ECL intensity of corresponding wavelength filter were recorded. The ECL photographs were recorded with an Olympus DP71 cooled CCD camera (2 s exposure) and analyzed by Image-Pro Plus (IPP) 6.0 software. Apart from this, we mixed 1 mL $Ru(bpy)_3^{2+}$ (20 μ M, the same concentration as the PNs-2) with 40 μ L Nafion solution. The mixtures were ultrasonicated for 30 min, and then fixed on the GCE surface and measured ECL intensity under the same experimental conditions. $Ru(bpy)_3^{2+}$ is a molecule with excellent water solubility. Without surface protection, it could dissolve rapidly with dramatically decreased ECL signal intensity (Figure S17A). In order to solve this problem, we usually choose to mix it with Nafion to achieve stable emission with slight compromise of the ECL intensity (Figure S17B). And the ECL efficiency were calculated by the formula reported in previously literature and compared with the standard reference $Ru(bpy)_3^{2+}/TPrA$.

The relative ECL efficiency formula:

$$\frac{\Phi_{ECL}}{\Phi^{\circ}_{ECL}} = \left(\frac{I}{Q}\right) / \left(\frac{I^{\circ}}{Q^{\circ}}\right)$$

where Q and Q° are the values of consumed charges and calculated by integrating current vs time, I and I° are the values of total ECL intensity and calculated by integrating ECL spectrum vs wavelength, and ECL and $^{\circ}ECL$ are the ECL efficiency of the sample and standard ($Ru(bpy)_3^{2+}$), respectively. In this work, the $^{\circ}ECL$ value of $Ru(bpy)_3^{2+}$ was recognized as 1.