Electronic Supplementary Information

A Versatile Synthetic Approach to Tunable Dual-emissive Pdots with Very Small-size Based on Amphiphilic Block Copolymers for Cell Imaging

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Detailed experimental procedures

Materials. N-isopropylacrylamide (NIPAM), acrylic acid (AA), N-vinylpyrrolidone(NVP) and 4,4-azobis(4 cyanovaleric acid) (ACVA) were purchased from Tokyo Chemical Industry (TCI). 4-(dimethylamino) pyridine (DMAP), N, Ndicyclohexylcarbodiimide (DCC), E-caprolactone(ε-CL), Zn powders, stannous octoate ($Sn(Oct)_2$), 4,4'-Dihydroxybenzophenone(BHBP), titanium tetrachloride ($TiCl_4$) and europium oxide (Eu_2O_3) were purchased from J&K Scientific Ltd. Tetrahydrofuran (THF) were purified by distillation. All other reagents were used as received. Doubly distilled water was used during the whole experiment process. 1,1,2,2-tetrakis(4-hydroxyphenyl)-ethylene (TPE-4OH) was synthesized according to our published procedure. [37, 38]

Characterization. All ^{1}H NMR spectra were recorded on a 600 MHz Bruker AM spectrometer using d6-DMSO/$CDCl_3$ as the solvent at room temperature. The Nicolet AVATAR 360FTIR spectrometer was used for recording the FT-IR spectra. The molecular weights and polydispersity index (PDI) of copolymers were characterized by a GPCV2000 gel-permeation chromatography, in which polystyrene was used as the calibration standard and THF was used as the eluent. X-ray photoelectron spectroscopy (XPS) were obtained by Thermo-Fishier Scientific EscaLab 250Xi XPS. TEM images were recorded by a JEM-1200EX microscope at 200 kV. SEM-EDS and EDX elemental mappings analysis measurements were performed on ZEISS ULTRA PLUS scanning electron microscope and the sample was deposited on silicon wafers. The fluorescent spectra were acquired using a F97 Pro spectrometer, with 5.0 nm slit width and 600 nm min^{-1} scan rate. Fluorescence images of the cell were captured under the Zeiss Axio Scope.A1 fluorescence microscope. The time-resolved fluorescence decay was obtained with a high-resolution Edinburgh Instruments FLS920 spectrofluorometer with two 360 nm and 400 nm laser as the excitation sources.

Synthesis of 4s-TPE-PCL-OH. TPE-4OH (0.36 g, 0.91 mmol), ε-CL (5.0 g, 44 mmol) were dissolved in 10.0 mL of anhydrous THF. The reaction mixture had been stirred for 10 min at 120 °C in an oil bath, and then catalyst (stannous octoate, total mass of monomer 5%) was added and the polymerization was carried out for 24 h. The reaction was then quenched by exposure to air and slowly cooled to room temperature. Finally, the crude product was precipitated from methanol, separated by filtration, and purified by the purification method three times. The obtained product was a pale-yellow powder and the isolated yield was 78%. The molecular structure and characteristics 4s-TPE-PCL-OH was confirmed by ^{1}H NMR, IR and GPC (Figure S12, Figure S13 and Table 1). H^1 NMR (600 MHz, $CDCl_3$, δ): 7.03-6.98(d, aromatic backbone), 6.81-6.74 (d, aromatic backbone), 4.04-3.95 (t, -CH_2-CH_2-OH), 2.43-2.14 (t, O=C-CH_2-CH_2-), 1.95-1.48 (m, -CH_2-CH_2-CH_2-), 1.41-1.18 (m, -CH_2-CH_2-CH_2-), 1.11-0.57 (m, -CH_2-CH_2-CH_2-). GPC: M_n (g/mol) = 5.6×10^3, M_w (g/mol) = 7.5×10^3, PDI (M_w /M_n) = 1.329.

Synthesis of 4s-TPE-PCL-AZO. 4s-TPE-PCL-OH (3.23 g, 0.43 mmol), ACVA (0.56 g, 2.0 mmol), and DPTS (0.28 g, 0.24 mmol) were dissolved in 50.0 ml of anhydrous THF. Then, the reaction mixture was stirred at 75 °C for 24 h. The reaction solution then was cooled to room temperature. Thereafter, the reaction mixture was filtered and the filtrate was concentrated by evaporation. The crude product was precipitated with an excess of diethyl ether and

centrifuged. The precipitate was purified three times and dried under vacuum to constant weight. 4s-TPE-PCL-AZO was obtained as a white solid with a yield of 68%. The molecular structure and characteristics of 4s-TPE-PCL-AZO was confirmed by ^{1}H NMR, IR and GPC (Figure S1, Figure S2 and Table 1). H^1 NMR (600 MHz, $CDCl_3$, δ): 7.12-7.05 (d, aromatic backbone), 6.97-6.82 (t, aromatic backbone), 4.02-3.89 (t, O=C-CH_2-CH_2-), 2.83-2.35 (t, O=C-CH_2-CH_2-), 2.29-2.08 (t,-CH_2-CH-C-), 1.82-1.51 (m,-CH_2-CH_2-CH_2-), 1.46-1.27 (m, -CH_2-CH_2-CH_2-), 1.29-1.18 (t,-C-CH_3), 1.17-1.06 (m,-CH_2-CH_2-CH_2-). GPC: M_n (g/mol) = 9.5×10^3, M_w (g/mol) = 1.8×10^4, PDI (M_w /M_n) = 1.91.

Synthesis of 4s-TPE-PCL-b-PNIPAM. 4s-TPE-PCL-b-PNIPAM was synthesized by in-situ initiation of monomeric NIPAM polymerization. First, the initiator 4s-TPE-PCL-AZO (0.39 g, 0.05 mmol) and the monomer NIPAM (2.26 g, 20 mmol) were dissolved in 30.0 mL of anhydrous THF. After the reaction system was vacuumed to maintain an anhydrous oxygen-free environment, the reaction mixture refluxed at 75 °C for 24 h. After the reaction, the crude product was precipitated with an excess of n-hexane. 4s-TPE-PCL-b-PNIPAM was obtained as a white solid with a yield of 56%. The molecular structure and characteristics of 4s-TPE-PCL-b-PNIPAM was confirmed by ^{1}H NMR，IR and GPC (Figure S3, Figure 1 and Table 1). H^1 NMR (600 MHz, DMSO, δ): 7.95-7.80 (s, -NH-C=O), 7.69-7.54 (d, aromatic backbone), 7.23-7.01 (d, aromatic backbone), 4.72-4.68 (s, O=C-CH_2-CH_2-), 4.14-3.74 (q,-NH-CH-CH_3), 3.73-3.44 (d, O=C-CH-CH_2-), 2.15-1.71 (t, O=C-CH_2-CH_2-), 1.49-1.28 (t,-C-CH_2-CH-), 1.25-1.15 (t,-C-CH_3), 1.14-0.74 (q,-CH-CH_3). GPC: M_n (g/mol) =4.6×10^4, M_w (g/mol) = 8.2×10^4, PDI (M_w /M_n) = 1.774.

Synthesis of 4s-TPE-PCL-b-PAA. The preparation procedures of 4s-TPE-PCL-b-PAA was similar to that of 4s-TPE-PCL-b-PNIPAM. It should be noted that acrylic acid was used for the synthesis of 4s-TPE-PCL-b-PAA, which was obtained as a white solid with a yield of 62%. The molecular structure and characteristics of 4s-TPE-PCL-b-PAA was confirmed by ^{1}H NMR, IR and GPC (Figure S4, Figure 1 and Table 1). H^1 NMR (600 MHz, DMSO, δ): 7.36-7.31 (d, aromatic backbone), 7.21-7.16 (d, aromatic backbone), 4.65-4.49 (t,-O=C-CH_2-CH_2-), 3.60-3.51 (d, O=C-CH-CH_2-), 2.42-2.31 (t, O=C-CH_2-CH_2-), 2.19-2.13 (d, t,-C-CH_2-CH_2-), 2.12-2.03 (t, -C-CH_2-CH_2-), 1.98-1.81 (t, O=C-CH_2-CH_2-), 1.79-1.74 (m, -CH_2-CH_2-CH_2-), 1.71-1.61 (m, -CH_2-CH_2-CH_2-), 1.53-1.48 (t, -C-CH_3), 1.45-1.41 (m, -CH_2-CH_2-CH_2-). GPC: M_n (g/mol) = 5.0×10^4, M_w (g/mol) = 6.7×10^4, PDI (M_w /M_n) = 1.340.

Synthesis of 4s-TPE-PCL-b-PVP. The preparation procedures of 4s-TPE-PCL-b-PVP was similar to that for 4s-TPE-PCL-b-PNIPAM. It should be noted that N-vinylpyrrolidone was used for the synthesis of 4s-TPE-PCL-b-PVP, which was obtained as a white solid with a yield of 59%. The molecular structure and characteristics of 4s-TPE-PCL- b-PVP was confirmed by ^{1}H NMR，IR and GPC (Figure S5, Figure 9, Table 1). H^1 NMR (600 MHz, DMSO, δ): 7.07-6.96 (d, aromatic backbone), 6.86-6.79 (d, aromatic backbone), 4.75-4.64 (t, O=C-CH_2-CH_2-), 4.54-4.32 (t,-N-CH_2-CH_2-), 4.09-3.74 (m,-CH_2-CH-CH_2-), 3.65-3.47 (t, O=C-CH_2-CH_2-), 3.45-3.12 (m, -CH_2-CH_2-CH_2-), 3.11-2.83 (t, O=C-CH_2-CH_2-), 2.67-2.38 (t, O=C-CH_2-CH_2-), 2.33-2.29 (t,-C-CH_2-CH_2-), 2.21-2.16 (t, -C-CH_2-CH-), 2.04-1.77 (m, -CH_2-CH_2-CH_2-), 1.68-1.59 (m, -CH_2-CH_2-CH_2-), 1.47-1.36 (t,-C-CH_3), 1.13-0.99 (m, -CH_2-CH_2-CH_2-). GPC: M_n (g/mol) = 7.4×10^4, M_w (g/mol) = 9.2×10^4, PDI (M_w /M_n) = 1.254.

Synthesis of TPNE. TPNE was prepared by the coordination reaction of 4s-TPE-PCL-b-PNIPAM with Eu(III). The polymer 4s-TPE-PCL-b-PNIPAM was dissolved in 20.0 mL of absolute ethanol. Then $EuCl_3$ ($m_{Polymer}$: m_{EuCl3}=1:1) was added. The mixture was stirred at room temperature for 24 h. After completion of the reaction, it was precipitated with an excess of n-hexane to obtain a white precipitate. It was centrifuged, purified and dried in vacuo. 4s-TPE-PCL-b-PNIPAM-Eu(III) was obtained as a white solid with a yield of 78%. The molecular structure of 4s-TPE-PCL-b-PNIPAM-Eu(III) could be confirmed by IR and XPS (Figure 1 and Figure 2).

Synthesis of TPAE. TPAE was prepared by the coordination reaction of 4s-TPE-PCL-b-PAA with Eu(III). The polymer 4s-TPE-PCL-b-PAA was dissolved in 20.0 mL of absolute ethanol. After $EuCl_3$ ($m_{Polymer}$: m_{EuCl3}=1:2) was added, and the mixture was stirred at room temperature for 24 h. It was precipitated with an excess of n-hexane to obtain a white precipitate, which was centrifuged, purified and dried in vacuo. 4s-TPE-PCL-b-PAA-Eu(III) was obtained as a white solid with a yield of 65%. The molecular structure of 4s-TPE-PCL-b-PAA-Eu(III) could be confirmed by IR and XPS (Figure 1 and Figure 2).

Synthesis of TPVE. TPVE was prepared by the coordination reaction of 4s-TPE-PCL-b-PVP with Eu(III). The polymer 4s-TPE-PCL-b-PVP was dissolved in 20.0 mL of absolute ethanol, $EuCl_3$ ($m_{Polymer}$: m_{EuCl3}=1:2) was added, and the mixture was stirred at room temperature for 24 h. It was precipitated with an excess of n-hexane to obtain a white precipitate, which was centrifuged, purified and dried in vacuo. 4s-TPE-PCL-b-PVP-Eu(III) was obtained as a white solid with a yield of 67%. The molecular structure of 4s-TPE-PCL-b-PVP-Eu(III) could be confirmed by IR and XPS (Figure 1 and Figure 2).

Synthesis of AIE Pdots. AIE Pdots are prepared by nanoprecipitation. Firstly, TPNE, TPAE, TPVE were prepared at a concentration of 1 mg/ml, and then respectively diluted the original solution to 50 μg/mL. The above solution was injected into a colorimetric tube containing 10 ml of ultrapure water. After it was sonicated for 5 min, and removed

the colorimetric tube. Then it placed in a fume hood at room temperature overnight to remove the solvent THF. Finally, the polymer solution was filtered with a 0.22 μm water filter to obtain stable AIE Pdots.

Cytotoxicity by the MTT Assay. Cytotoxicities of three AIE Pdots on live HeLa, A549 and HepG2 cells were assayed by the MTT assay. First, the cells were plated in a 96-well plates at a concentration of 10^5 cells/mL, 200 μl/well, and the edge wells were filled with sterile PBS, gently shaken to adhere to the wall, and placed in a 37 °C, 5% CO_2 incubator for culture. After culturing for 48 h in a series of three AIE Pdots (50, 100, 200, 300 and 400 μg/mL) with different concentrations, the medium of three AIE Pdots was decanted and 10 μL of fresh MTT solution was added to the cells in each well. After the AIE Pdots incubated at 37 °C for 4 h. Subsequently, the MTT/medium was removed carefully and DMSO (100 μL) was added to lyse the cells. Then, the percentage of cell viability was estimated by using an RT-6100 microplate reader to measure the absorbance of the solution at 492 nm. Meanwhile, IC_{50} values were calculated to determine the minimum concentration required to kill 50% of the cells.

Cellular Imaging Experiments. The log phase HeLa, HepG2, and A549 cells were taken and digested with trypsin and counted by a cell counting plate under a microscope by centrifugation. Then we inoculated on a cell slide of a 24-well plate. After adding the medium DMEM and placed in a 37 °C, 5% CO_2 incubator for 24 h. Thereafter, several Pdots of 100 μg/mL were added to each well and continued for 24 h. The cell slides were removed and washed three times with PBS buffer. Finally, cell observation and photo shooting were performed under the Zeiss Axio Scope.A1 fluorescence microscope.

References

1 X. L. Guan, L. Meng, Q. J. Jin, B. C. Lu, Y. B. Chen, Z. F. Li, S. J. Lai and Z. Q. Lei, A New Thermo-, pH-, and CO_2-Responsive Fluorescent 4-Arm Star Polymer with Aggregation-Induced Emission for Long-Term Cellular Tracing, *Macromol. Mater. Eng.,* 2018, **303**(6). 1700553.

2 X. L. Guan, D. H. Zhang, L. Meng, Y. Zhang, T. M. Jia, Q. J. Jin, Q. B. Wei, D. D. Lu and H. C. Ma, Various Tetraphenylethene-Based AIEgens with Four Functional Polymer Arms: Versatile Synthetic Approach and Photophysical Properties, *J. Ind. Eng. Chem.,* 2017, **56**(3), 680.

The additional figures

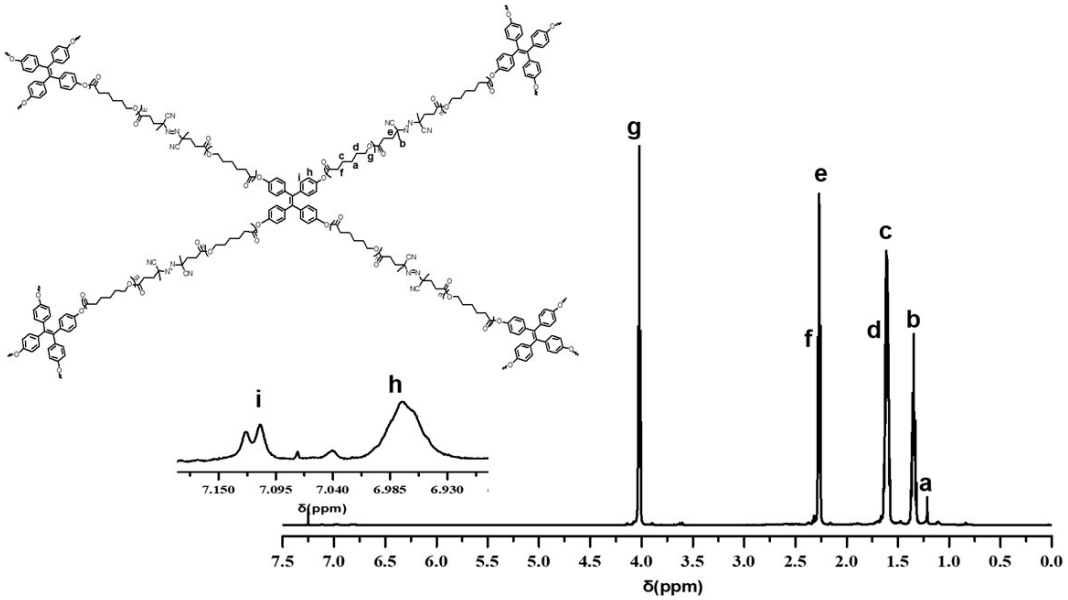

Figure S1 The ^{1}H NMR of 4s-TPE-PCL-AZO

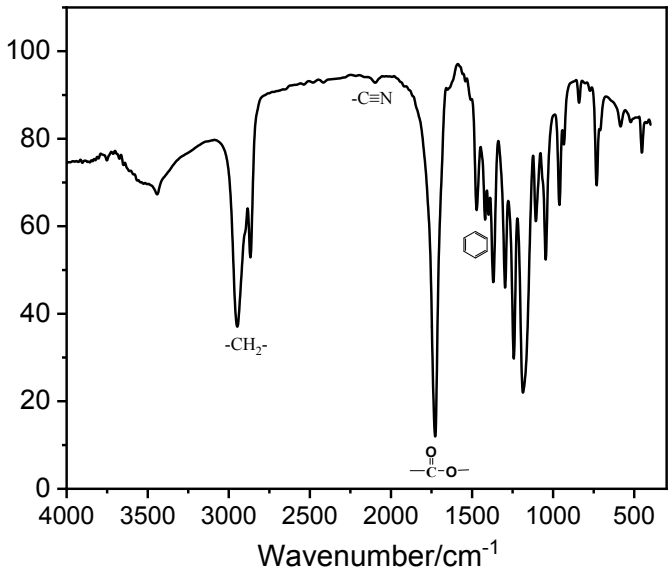

Figure S2 The IR spectra of 4s-TPE-PCL-AZO

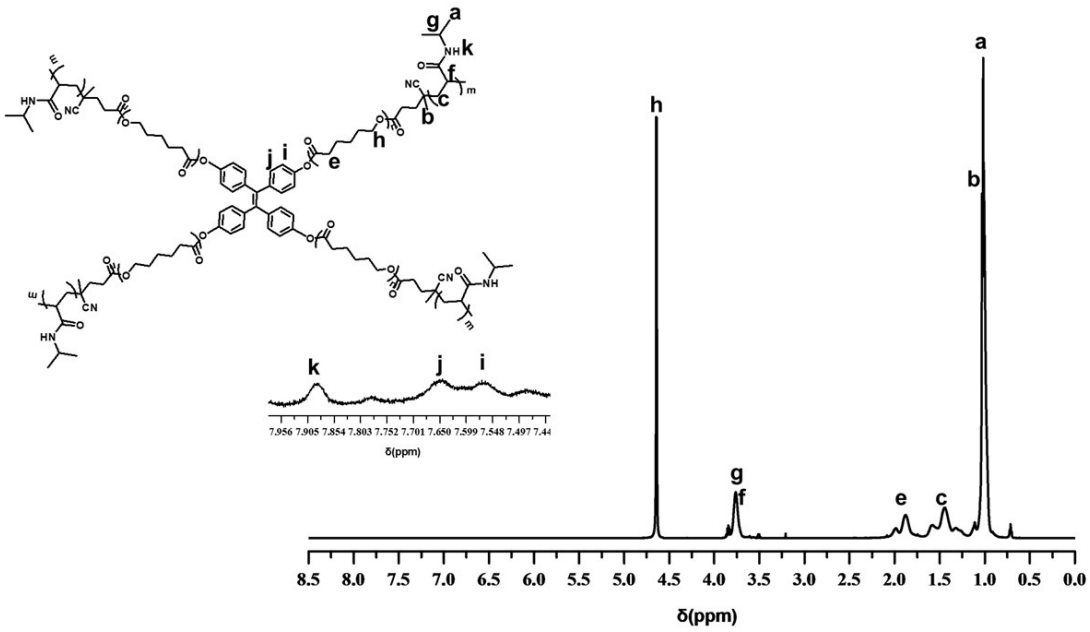

Figure S3 The ^{1}H NMR of 4s-TPE-PCL-b-PNIPAM (TPN)

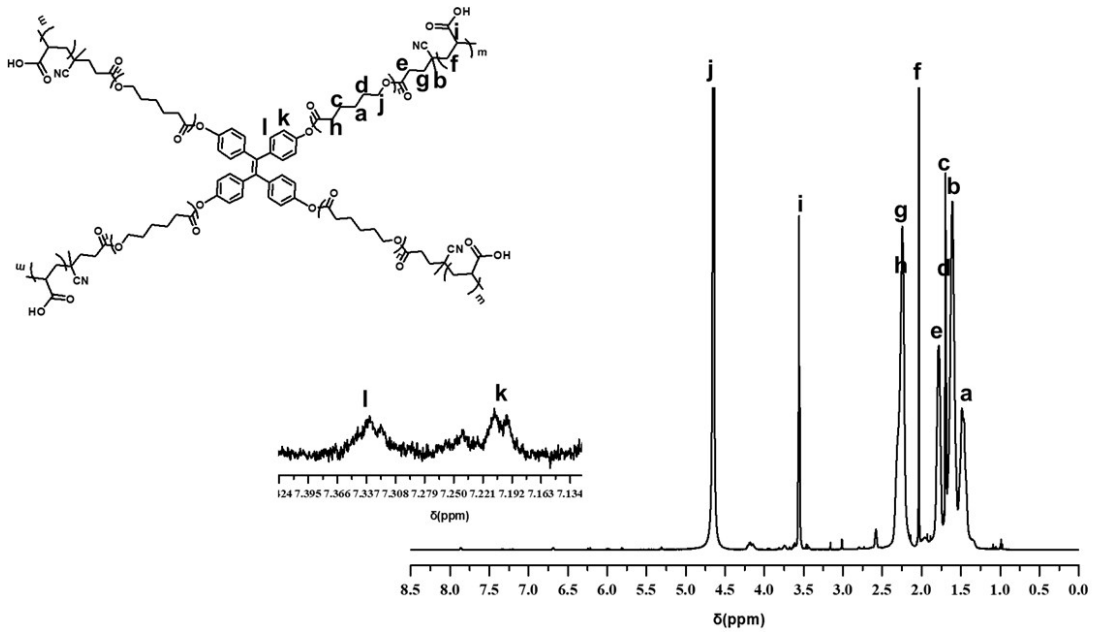

Figure S4 The ^{1}H NMR of 4s-TPE-PCL-b-PAA (TPA)

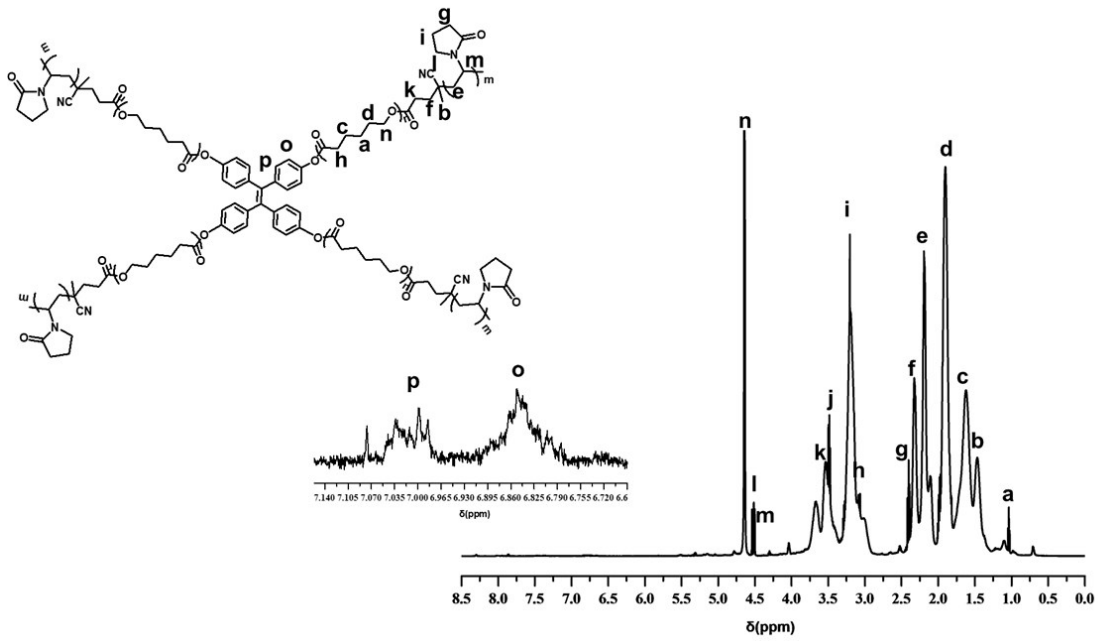

Figure S5 The ^{1}H NMR of 4s-TPE-PCL-b-PVP (TPV)

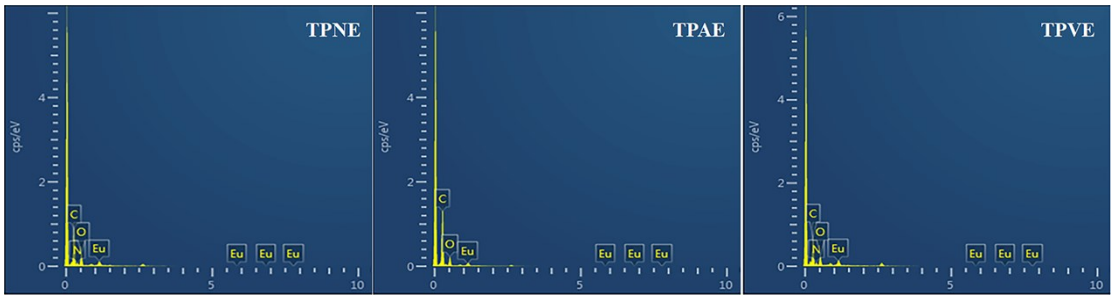

Figure S6. The SEM-EDS analysis of TPNE, TPAE and TPVE.

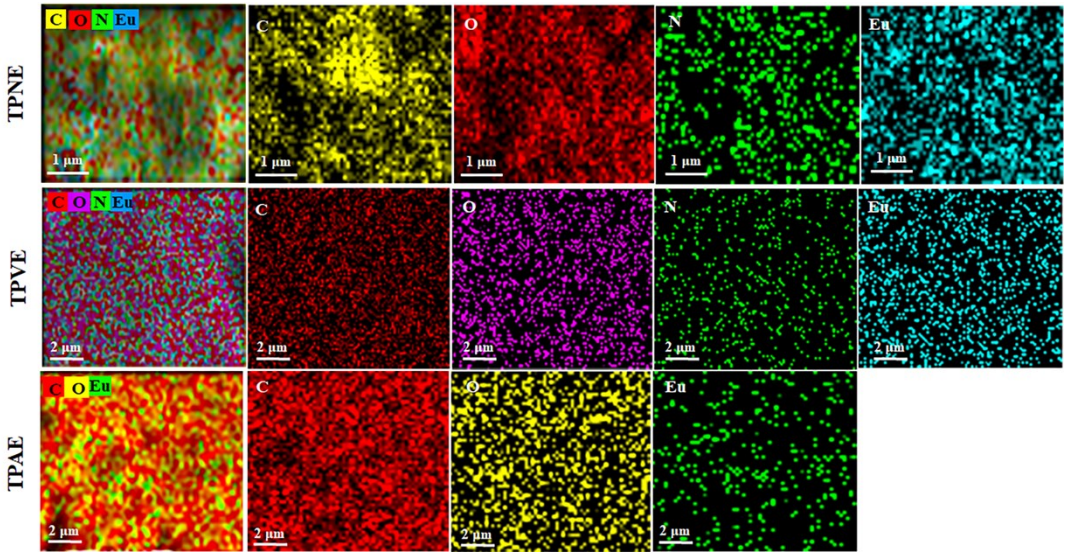

Figure S7 EDX elemental mapping of TPNE, TPAE and TPVE.

Table S1. The weigh percentage of the corresponding elements in TPNE, TPAE and TPVE.

Condition	C / wt%	O / wt%	N / wt%	Eu / wt%
TPNE	62.9	18.6	6.8	11.7
TPAE	60.8	29.8	/	9.4
TPVE	60.1	14.4	10.2	15.3

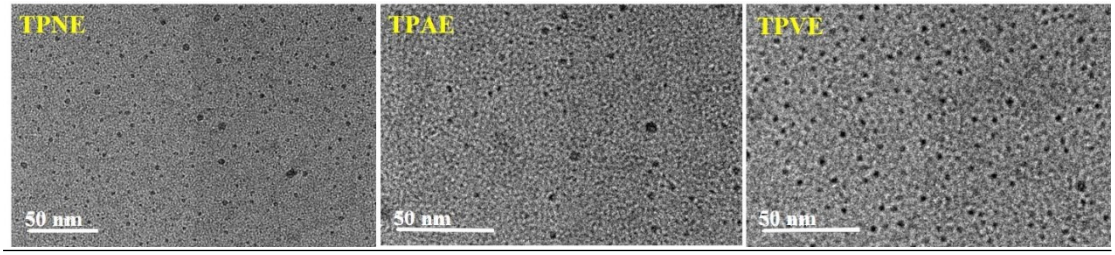

Figure S8 The TEM image of TPNE, TPAE and TPVE Pdots at the thirtieth days.

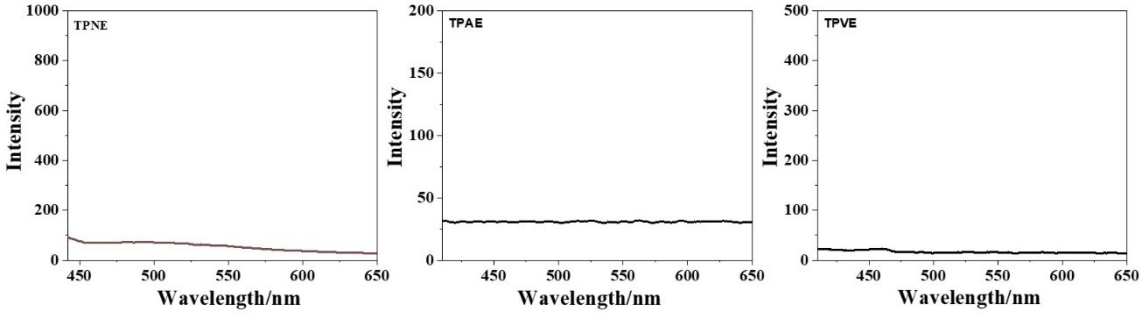

Figure S9 The fluorescence spectra of three kinds of dialysates after TPNE, TPAE and TPVE Pdots were dialyzed against distilled water with membranes made of regenerated cellulose for 7 days

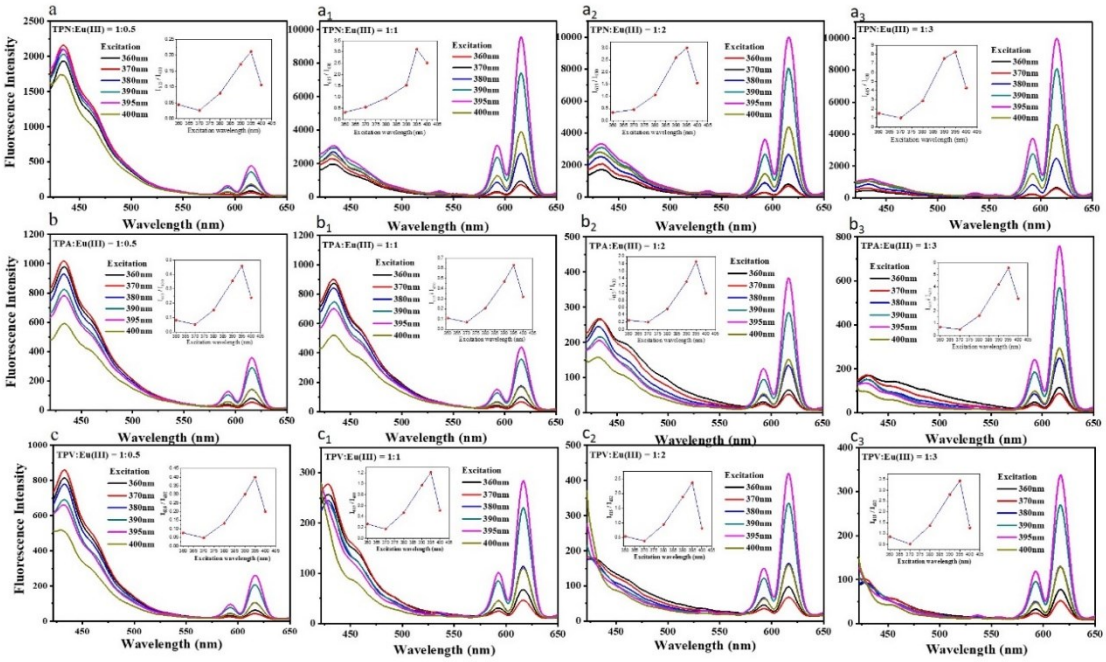

Figure S10 (a, a_1, a_2, a_3) Fluorescence spectra of TPN and Eu(III) at a mass ratio of 1: 0.5, 1: 1, 1: 2, and 1: 3 after complexation at different excitation wavelengths of 360 nm, 370 nm, 380 nm, 390 nm, 395 nm, and 400 nm; (Inset: I_{615} / I_{430} ratio curve with excitation wavelength); (b, b_1, b_2, b_3) Fluorescence spectra of TPA and Eu(III) at a mass ratio of 1: 0.5, 1: 1, 1: 2, and 1: 3 after complexation at different excitation wavelengths of 360 nm, 370 nm, 380 nm, 390 nm, 395 nm, and 400 nm; (Inset: I_{625} / I_{430} ratio curve with excitation wavelength); (c, c_1, c_2, c_3) Fluorescence spectra of TPV and Eu(III) at a mass ratio of 1: 0.5, 1: 1, 1: 2, and 1: 3 after complexation at different excitation wavelengths of 360 nm, 370 nm, 380 nm, 390 nm, 395 nm, and 400 nm; (Inset: I_{625} / I_{432} ratio curve with excitation wavelength).

Table S2. The photophysical properties of TPNE, TPAE and TPVE Pdots.

Condition	λ_{ex} / nm	λ_{em} / nm	Φ_f / %	τ
TPNE (blue)	360	430	18.4	5.109 ns
TPNE (red)	395	615	45.5	1.668 ms
TPAE (blue)	360	430	14.1	3.619 ns
TPAE (red)	395	615	36.9	0.693 ms
TPVE (blue)	360	430	15.5	3.949 ns
TPVE (red)	395	615	42.8	1.386 ms

Φ_f= fluorescence quantum yields in aqueous solution; τ = the fluorescence lifetime in aqueous solution.

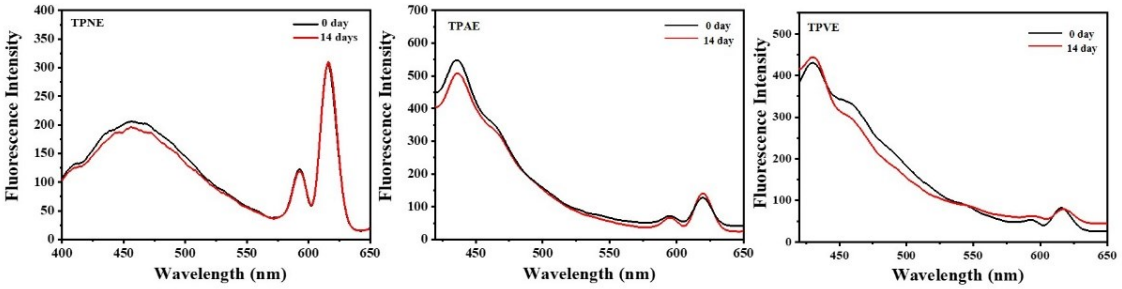

Figure S11 Fluorescence stability of TPNE, TPAE and TPVE in aqueous solutions. The red line refers to the fluorescence spectra of TPNE, TPAE and TPVE after 14 days.

Table S3. The relative quantum yields (Φ_f) of TPNE, TPAE and TPVE Pdots at f_w 0% and f_w 90% (or 98%).

Condition	λ_{em} =430 nm	λ_{em} =615 nm
TPNE (0%)	17.59%	37.59%
TPNE (90%)	49.46%	37.87%
TPAE (0%)	12.85%	25.15%
TPAE (98%)	39.02%	27.85%
TPVE (0%)	18.43%	36.98%
TPVE (90%)	47.30%	37.03%

Table S4 IC_{50} of HepG2, A549 and Hela cells after 24 h incubation with TPNE, TPAE and TPVE Pdots.

Cell	IC_{50} / μg·mL^{-1}		
	TPNE	TPAE	TPVE
HepG2	635.7	731.8	640.7
A549	583.2	551.8	503.0
Hela	609.1	640.8	624.9

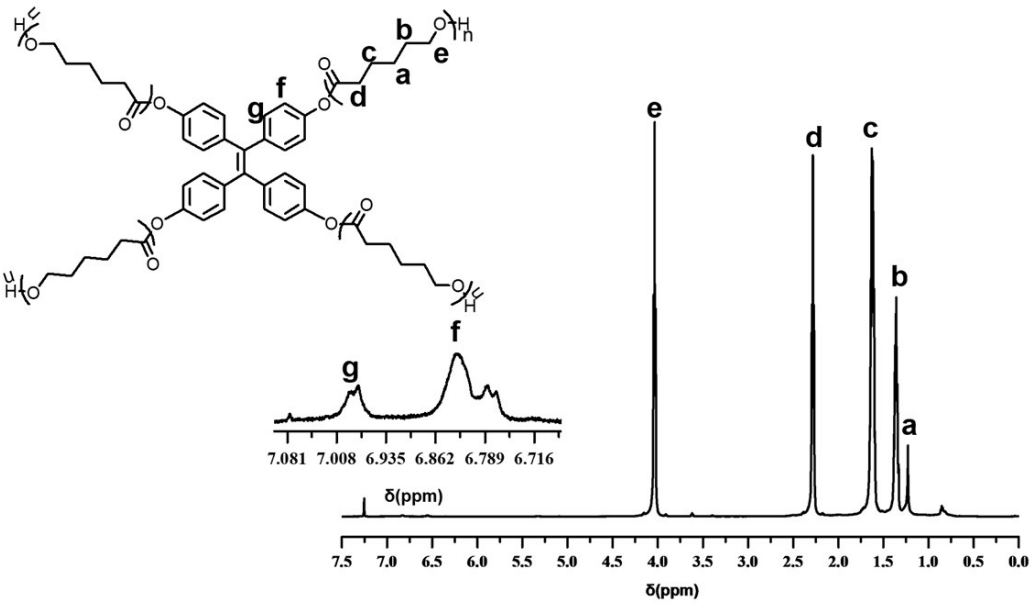

Figure S12 The ^{1}H NMR of 4s-TPE-PCL

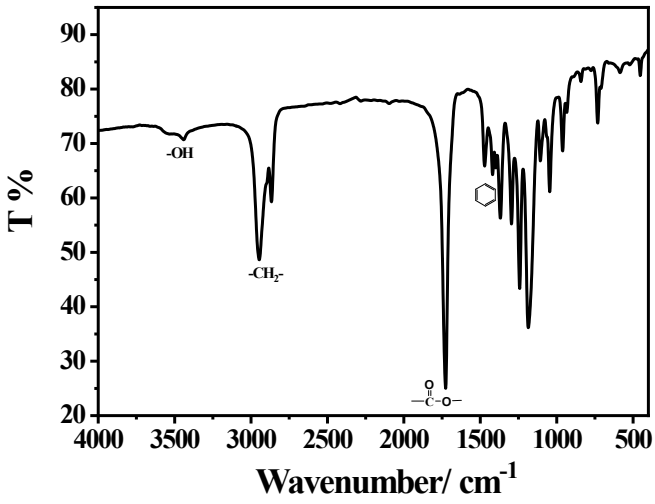

Figure S13 The IR spectra of 4s-TPE-PCL