Supporting Information

Dye-Sensitized Lanthanide-Doped Upconversion Nanoprobes for Homocysteine Sensing in Human Serum and Living Cells via a Spatial Optimization Strategy

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1. Experimental Section

1.1 Chemicals and materials

All solvents and chemical reagents were purchased from Shanghai Aladdin Reagent Co., Ltd, and they were used as received. The stock solutions of amino acid were prepared by dissolving them of analytical grade in deionized water, and all were a concentration of 1.0×10^{-3} M. The stock solutions of CyPd (5.0×10^{-2} M) were prepared in ethanol and stored in the refrigerator. UNs encapsulated in amphiphilic polymers (1 mg/mL) were dispersed into water for further dye sensitization experiments.

1.2 Instruments

The ^{1}H NMR and ^{13}C NMR spectra were recorded at 25℃ using a Qone-WNMR-I 400 MHz spectrometer. Mass spectra were determined with a Waters Micromass Quattro Micro ESI mass spectrograph. The size and morphology of UNs were determined using a FEI Talo F200s transmission electron microscope (TEM). Powder X-ray diffraction (XRD) measurements were performed on a Bruker D8 diffractometer in the 2θ range from 10 to 80°. Fourier transform infrared (FTIR) spectra were measured in a Nicolet iS50 spectrometer from samples in KBr pellets. UV-vis absorption spectra were recorded on Shimadzu Uv-2600 spectrophotometer. Fluorescence measurements were carried out with an Edinburgh FLS920 fluorescence spectrometer. The upconversion luminescence spectra were measured on a Fuxiang Optic NOVA-EX spectrometer with external 0-5.0 W adjustable continuous-wave semiconductor lasers at 808 nm (Jiangsu Dowell Optics Technology Co., Ltd, China).

1.3 Practical applications of nanoprobe

(1) Recovery experiments in human serum

Recovery experiments were conducted to determine the concentration of Hcy in human serum. Human serum was obtained from Beijing Solarbio Science & Technology Co., Ltd. Prior to analysis, serum samples were diluted fivefold. Various concentrations of Hcy and Cys were spiked into the serum, and the resulting samples were analyzed using the nanoprobe. Each experiment was repeated five times to

calculate the average value of the detected Hcy concentrations.

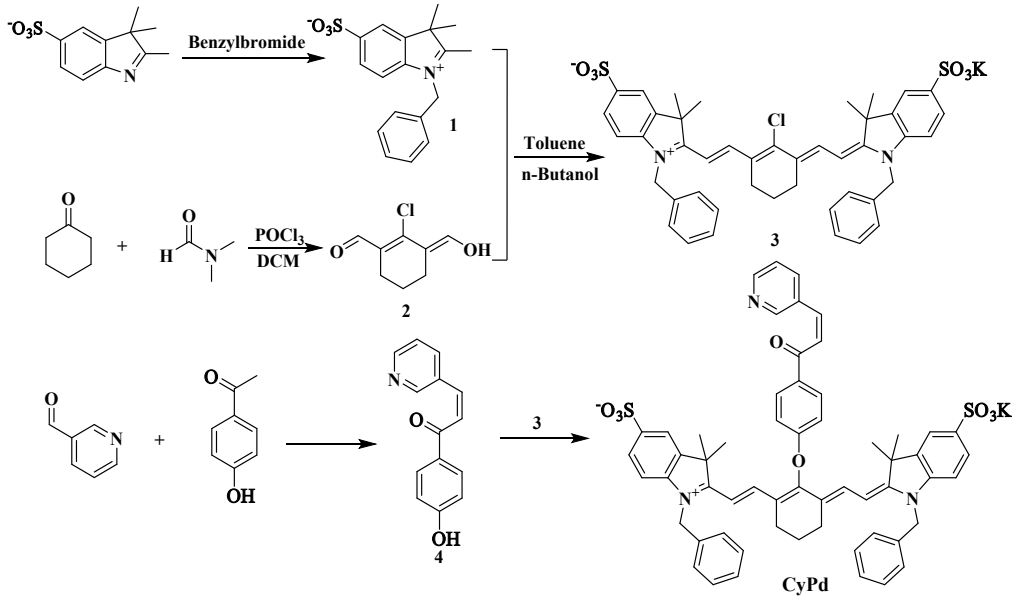
(2) Cell Toxicity

In vitro cytotoxicity was assessed using the CCK-8 assay on HeLa cells, employing 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfonatophenyl)-2H-tetrazolium monosodium salt. HeLa cells (approximately 1×10^4 cells per well) were seeded into a 96-well plate and incubated for 24 h in growth medium at 37°C with 5% CO_2. Different concentrations of CyPd-UNs@PEG (0, 50, 100, 200 and 400 μg/mL) were then added to the cells, followed by an additional 24 h incubation at 37°C. After this, the cells were treated with 5 mg/mL CCK-8 reagent for 2 h at 37°C. Absorbance at 450 nm was measured using a microplate reader (SPECTRA SLT; Labinstruments, Salzburg, Austria). Each treatment was performed in triplicate with six wells per treatment, and the experiment was repeated three times. Cell viability was calculated using the following formula: Cell viability (%)=(mean absorbance value of the treatment group / mean absorbance value of the control group)×100%.

(3) Cell imaging

HeLa cells were cultured in growth medium at 37°C with 5% CO_2 for 24 h before the experiment. The experiment was divided into three groups: (i) cells were incubated with 400 μg/mL CyPd-UNs@PEG at 37°C for 4 h; (ii) cells were pretreated with 0.5 mM N-Ethylmaleimide (NEM) for 30 min, followed by incubation with 400 μg/mL CyPd-UNs@PEG for an additional 4 h; (iii) cells were pretreated with 0.5 mM NEM for 30 min, incubated with 400 μg/mL CyPd-UNs@PEG for 4 h, and then treated with 60 μM Hcy for 30 min before imaging. After incubation, cells were washed five times with PBS buffer and analyzed by confocal upconversion luminescence imaging. Cells were excited with an 808 nm continuous-wave laser at a focused power of approximately 500 mW. Upconversion luminescence emissions were collected in the green (500-560 nm) and red (600-700 nm) regions.

2. Synthesis of cyanine dye



Scheme S1 Synthetic routes to CyPd.

Synthesis of compound 3

Compound 1 (1.0 g, 4.2 mmol), compound 2 (0.29 g, 1.7 mmol), and 20 mL of n-butanol/toluene mixed solvent (7:3 v/v) were added to a single necked flask under a nitrogen atmosphere. The mixture was stirred at 110 ℃ for 4 h, and the solution changed from purple red to black green. After removed the solvent, the remaining solid was washed three times with ether. The filtered solid was recrystallized from water and acetone to obtain a grass green solid (0.77 g, 56.8%). ^{1}H NMR (400 MHz, DMSO-d_6) δ:8.24 (d, *J*=16 Hz, 2H), 7.85 (s, 2H), 7.63(d, *J*=8 Hz, 2H), 7.38-7.26 (m, 12H), 6.38 (d, *J*=16 Hz, 2H), 5.53 (s, 4H), 3.17 (s, 1H), 1.72 (s, 12H). 1.06 (t, *J*=8 Hz, 1H).

Synthesis of compound 4

4-hydroxyacetophenone (0.14 g, 1 mmol) and 40% NaOH (0.5 mL) solution were added to ethanol (5 mL) and mixed for 15 min. Then, 3-pyridineformaldehyde (0.094 mL, 1 mmol) was added to the mixture and stirred at room temperature for 10 h. After that, the solvent of the mixture was evaporated to 50%, and the pH of the solution was adjusted to 5-6. The filtered solid was treated twice with pure water to obtain a light yellow solid (0.18 g, 78.6%). ^{1}H NMR (400 MHz, DMSO-d_6) δ:10.50 (s, 1H), 8.685

(d, *J*=4 Hz, 1H), 8.14 (d, *J*=16 Hz, 1H), 8.03 (d, *J*=8 Hz, 2H), 7.895 (d, *J*=4 Hz, 2H), 7.67 (d, *J*=8 Hz, 1H),7.45-7.42 (m, 1H), 6.92 (d, *J*=8 Hz, 2H).

Synthesis of CyPd

Under a nitrogen atmosphere, sodium hydride (0.10 g, 2.5 mmol, 60%) and compound 4 (45 mg, 0.20 mmol) were initially combined in 5 mL of anhydrous dimethylformamide (DMF) and stirred at room temperature for 30 minutes until no further gas bubbles were produced. Subsequently, a solution containing compound 3 (79 mg, 0.10 mmol) in 2 mL of anhydrous DMF was added to the mixture, which was then stirred in the dark at room temperature for 4 d. After the reaction, the solvent was removed under reduced pressure, and the solid residue was washed twice with diethyl ether and acetone to yield a green solid product. (43 mg, 41.9% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ:8.705 (d, *J*=4 Hz, 1H), 8.11 (t, *J*=8 Hz, 2H), 7.93 (d, *J*=12 Hz 3H), 7.67 (d, *J*=16 Hz, 2H), 7.44 (t, *J*=8 Hz, 1H), 7.34-7.14 (m, 16H), 7.08 (d, *J*=8 Hz, 1H), 6.82 (d, *J*=16 Hz, 1H), 6.63 (d, *J*=8 Hz, 1H), 5.89 (d, *J*=8 Hz, 1H), 5.723 (d, *J*=16 Hz, 1H), 5.37 (d, *J*=16 Hz, 1H), 4.89 (s, 2H), 2.33 (s, 6H), 1.60 (s, 6H), 1.13 (s, 6H), 1.03 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6,) δ:172.60, 148.45, 143.53, 142.58, 141.56, 129.11, 126.79, 125.58, 122.93, 112.11, 102.21, 72.15, 70.24, 61.00, 51.18, 49.43, 44.22, 40.65, 40.44, 40.23, 40.02, 39.82, 39.61, 39.40, 32.20, 28.93, 27.98, 26.53, 26.36, 22.86, 22.31, 22.05, 21.52, 20.93. MS (TOF): calculated M_r =985.2 for $C_{58}H_{53}N_3O_8S_2$, found: m/z = 985.1.

3. Synthesis of oleic acid coated upconversion nanoparticles

Synthesis of β-$NaGdF_4$:Yb20%,Er2%

A solution was prepared by dissolving 1 mmol of rare earth chlorides ($LnCl_3$, comprising Gd^{3+}, Yb^{3+}, and Er^{3+}) in a molar ratio of 78:20:2 in 5 mL of methanol, which was subsequently added to a mixture of 7 mL of 85% oleic acid and 15 mL of 98% octadecene. Under continuous stirring, the solution was heated to 140°C and maintained for 40 min to form a transparent solution. After the reaction mixture had cooled to 50°C, a methanol solution containing NaOH (2.5 mmol) and NH_4F (4.0 mmol) was added. The reaction was kept at 50°C for 30 min, then stirred at 110°C to evaporate methanol and water. Subsequently, the reaction mixture was heated to 325°C

and maintained for 1.5 h under an argon atmosphere. Finally, ethanol was added to the solution and the precipitate was collected by centrifugation. After washing with ethanol and hexane, the purified nanoparticles were redispersed in hexane.

Synthesis of β-$NaGdF_4$:Yb20%,Er2%@$NaGdF_4$:Yb10%,Nd10% (OA-UNs)

Core-shell nanoparticles were synthesized via epitaxial growth. A mixture was prepared by combining 1 mmol of $LnCl_3$ (with Ln^{3+} being Gd^{3+}, Yb^{3+}, and Nd^{3+}) in an 80:10:10 molar ratio with 7 mL of oleic acid and 15 mL of octadecene. The solution was heated to 140°C to achieve a transparent solution. Once the reaction mixture had cooled to 50°C, $NaGdF_4$:Yb,Er nanoparticles in 10 mL of hexane were added along with a methanolic solution containing NaOH (2.5 mmol) and NH_4F (4.0 mmol). The reaction was maintained at 50°C for 30 minutes, followed by stirring at 110°C to evaporate methanol and water. Then, the reaction mixture was heated to 325°C and held under an argon atmosphere for 1.5 h. Ultimately, ethanol was introduced at room temperature, and the resultant precipitate was isolated via centrifugation. Following a centrifugal washing process with ethanol and hexane, the nanoparticles were further purified and subsequently redispersed in a hexane solution.

4. Assembly of OA-UNs, DSPE-PEG2000 and CyPd

Assembly of DSPE-PEG2000-functionalized UNs (UNs@PEG)

OA-UNs (10.0 mg**)** and polyethylene glycol monomethyl ether-2000-octacoalkyl phosphatidyl ethanolamine (DSPE-PEG2000) (10.0 mg) was mixed in chloroform, and stirred overnight at room temperature to obtain a homogeneous phase. Chloroform was then slowly evaporated under a nitrogen atmosphere. The uniform thin film was dispersed in ultrapure water through ultrasonic treatment.

UNs@PEG loaded with CyPd (CyPd-UNs@PEG)

CyPd was dissolved in ethanol, and slowly added into the water solution of UNs@PEG. The mixture was stirred for 12 h. Excess CyPd was removed by centrifugation at 12000 rpm/min for 15 min. The precipitate was washed with distilled water by sonication and centrifugation. The prepared CyPd-UNs@PEG were dispersed into deionized water for later use.

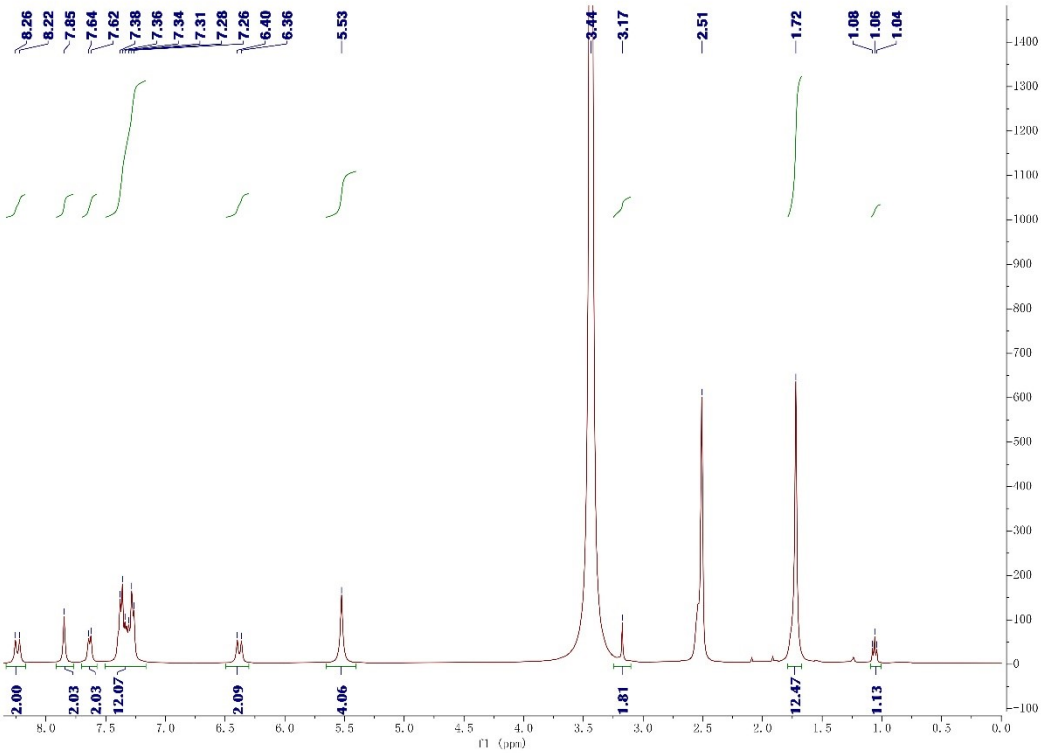


Fig.S1 [1]H NMR spectrum of compound 3.

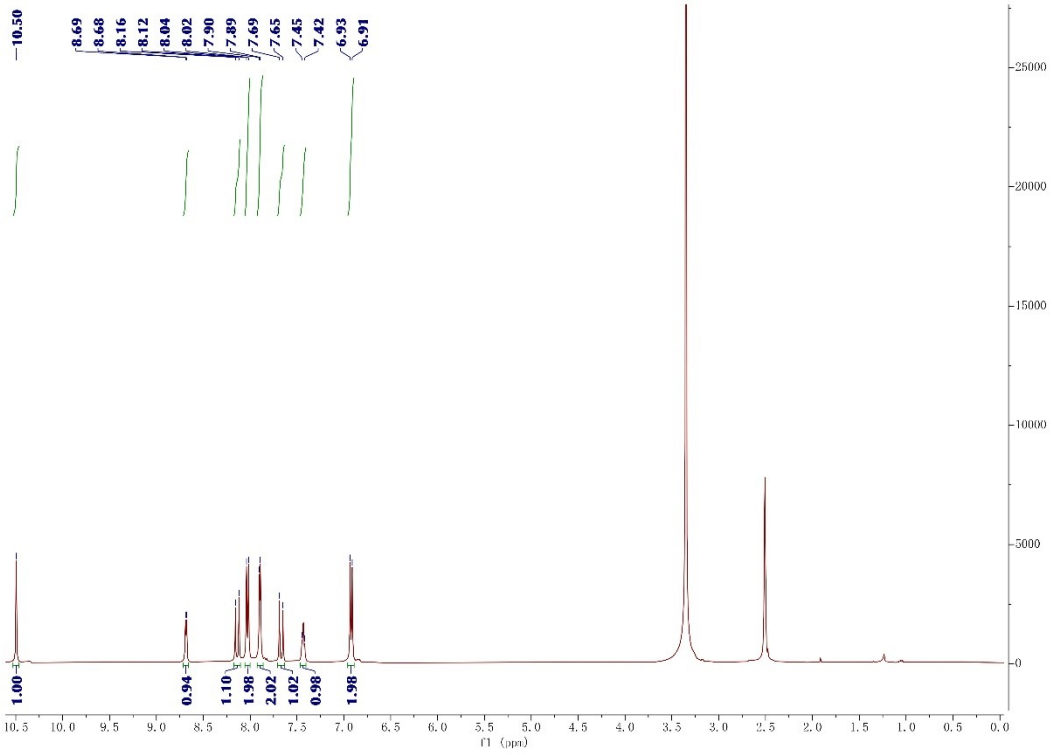


Fig.S2 [1]H NMR spectrum of compound 4.

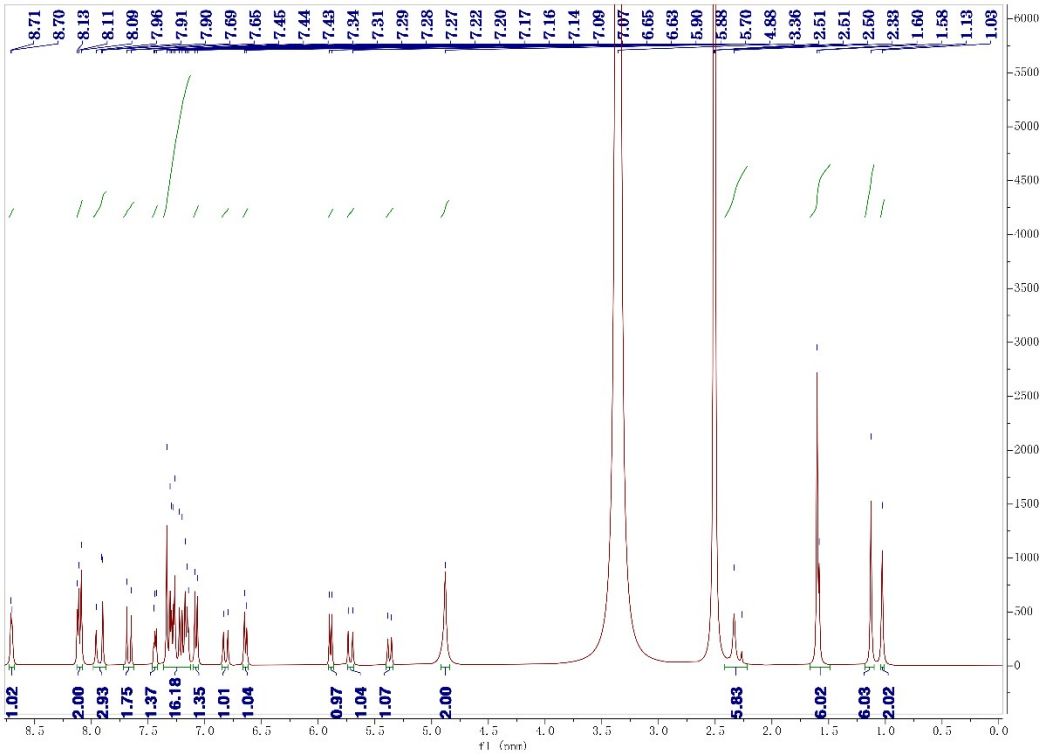


Fig.S3 ^{1}H NMR spectrum of CyPd.

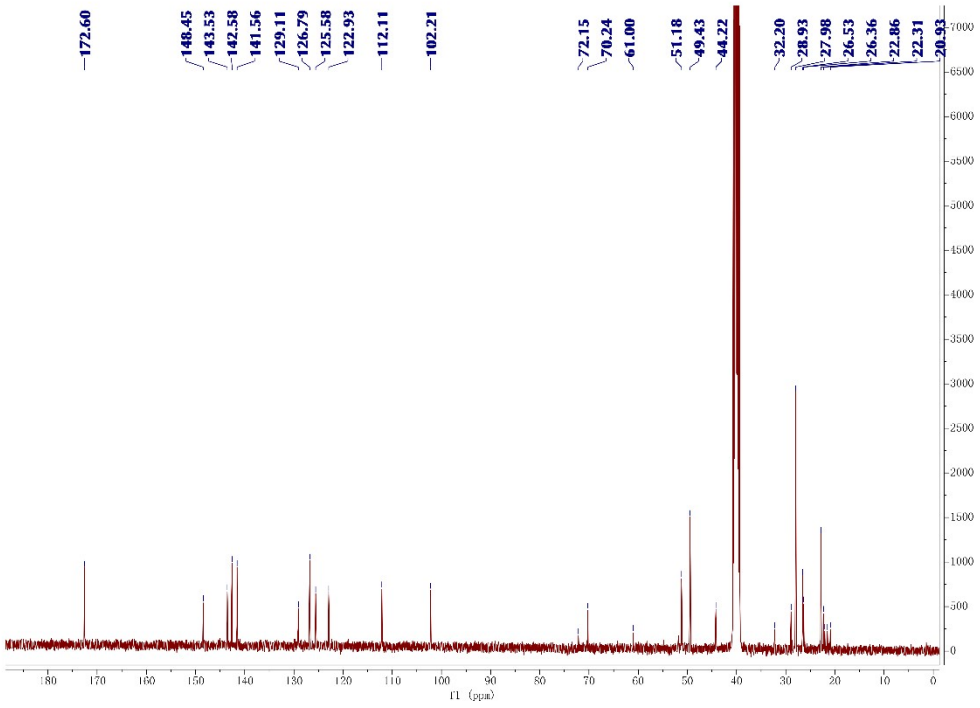


Fig.S4 ^{13}C NMR spectrum of CyPd.

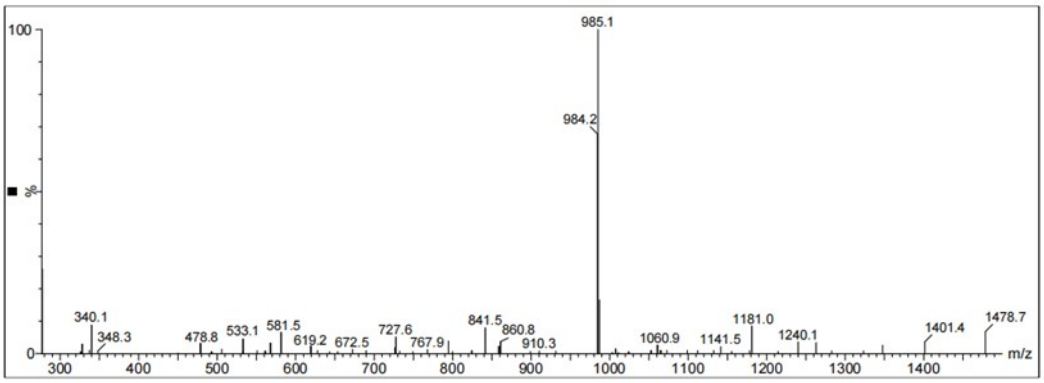


Fig. S5 Mass spectrum of CyPd.

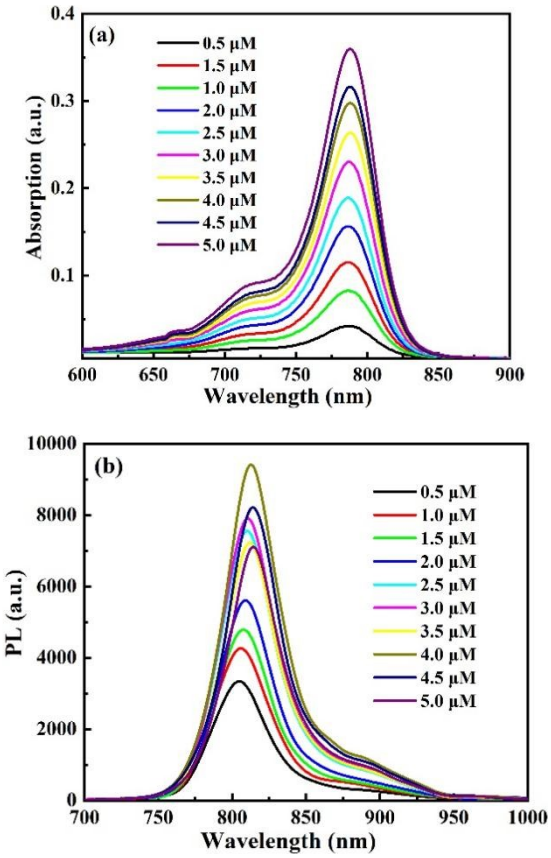


Fig.S6 Absorption (a) and PL (b) spectra of CyPd in the presence of UNs (1 mg mL^{-1}).

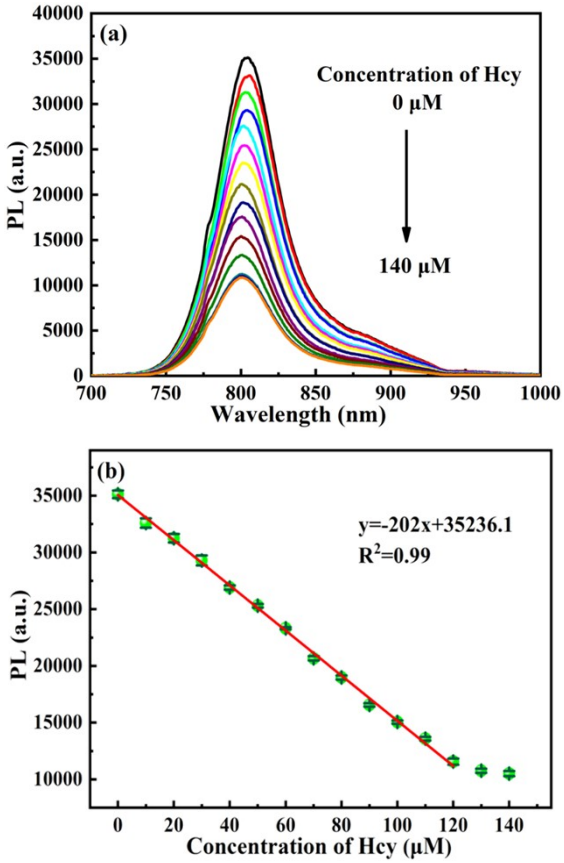


Fig. S7 (a) PL response of CyPd (15 μM) with the increase of Hcy concentration in ethanol under excitation of 785 nm; (b) Relationship between PL intensity and Hcy concentrations.

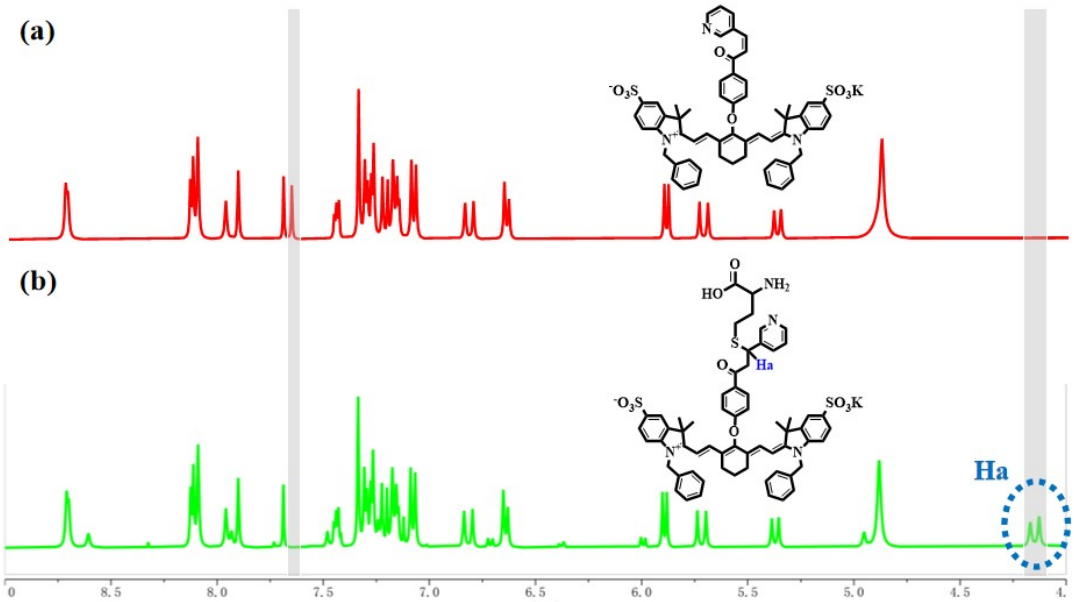


Fig. S8 ^{1}H NMR spectra of (a) CyPd and (b) CyPd with Hcy in DMSO-d_6.

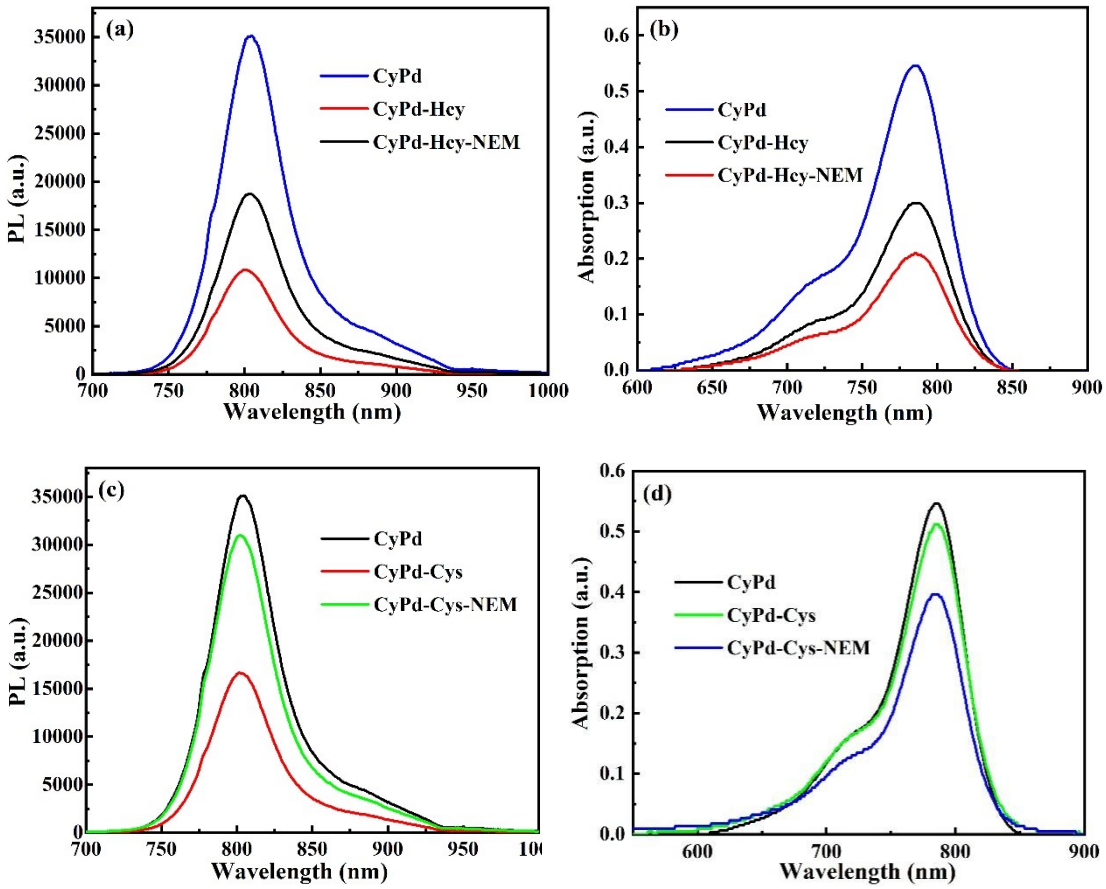


Fig. S9 PL (a) and absorption (b) spectra of CyPd (15 μM) after sequential addition of Hcy (140 μM) and NEM (140 μM), and the PL (c) and absorption (d) spectra of CyPd (15 μM) after sequential addition of Cys (140 μM) and NEM (140 μM).

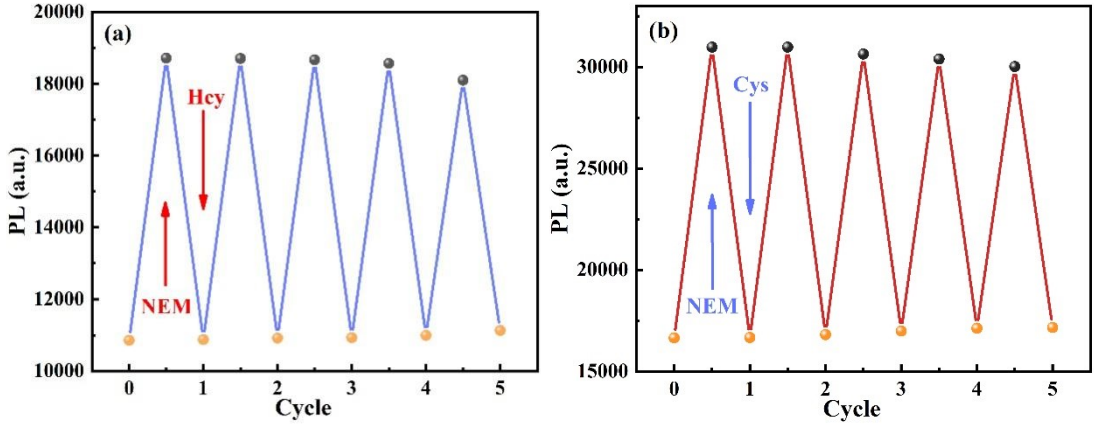
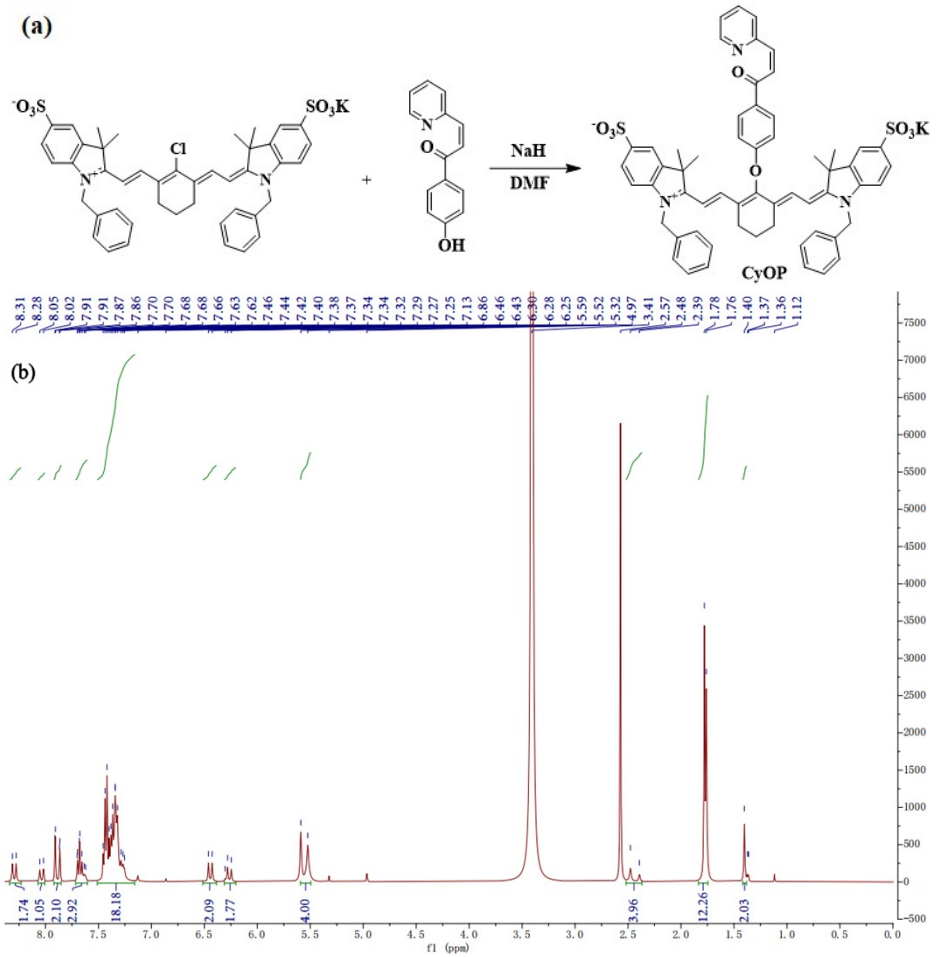


Fig. S10 Reversible study of CyPd (15 μM) by adding (a) Hcy/NEM (140 μM) and (b) Cys/NEM (90 μM) cyclically.



^{1}H NMR (400 MHz, DMSO-d_6) δ:8.295 (d, J=12 Hz, 2H), 8.035 (d, J=12 Hz, 1H), 7.88 (d, J=12 Hz 2H), 7.68 (t, J=8 Hz, 3H), 7.46-7.25 (m, 18H), 6.445 (d, J=12 Hz, 2H), 6.265 (d, J=12 Hz, 2H), 5.55 (d, J=28 Hz, 4H), 2.43 (d, J=12 Hz, 4H), 1.78 (s, 12H), 1.40 (s, 2H).

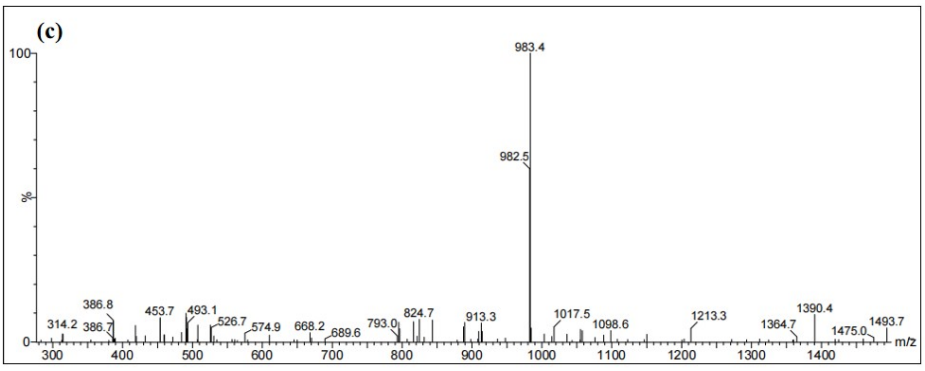


Fig. S11 Synthesis (a), ^{1}H NMR spectrum (b) and mass spectrum (c) of CyOP.

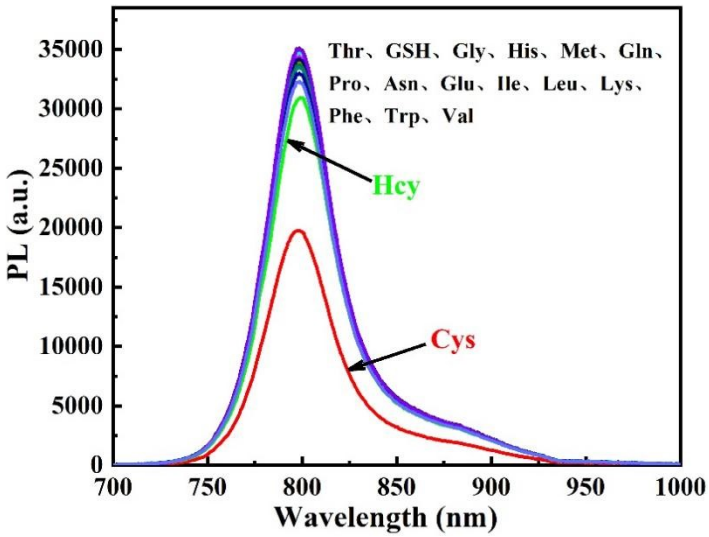


Fig. S12 PL spectra of CyOP (15 μM) upon the addition of various amino acids (100 μM) in ethanol.

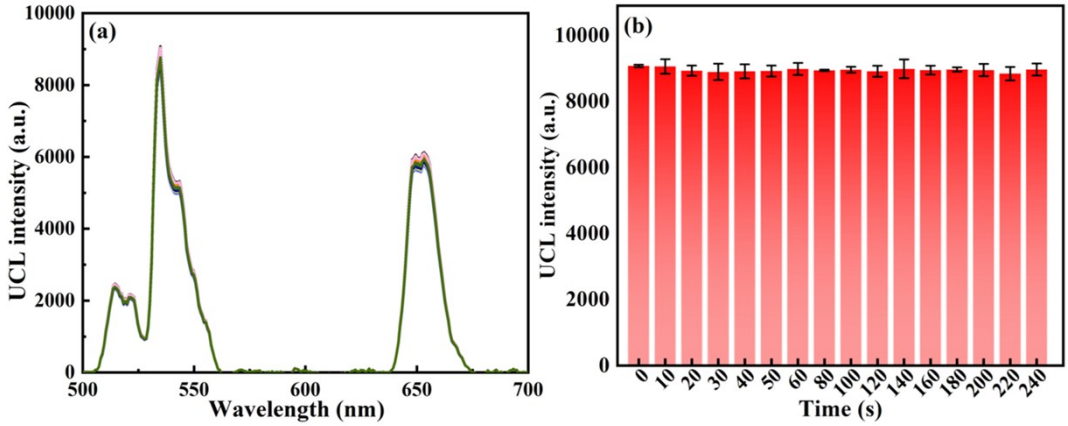


Fig. S13 (a) Photostability experiment of CyPd-UNs@PEG in serum under 808 nm excitation with a power density of 5 W cm^{-2}; (b) Bar chart of upconversion intensity (monitored at 541 nm) over 240 s.

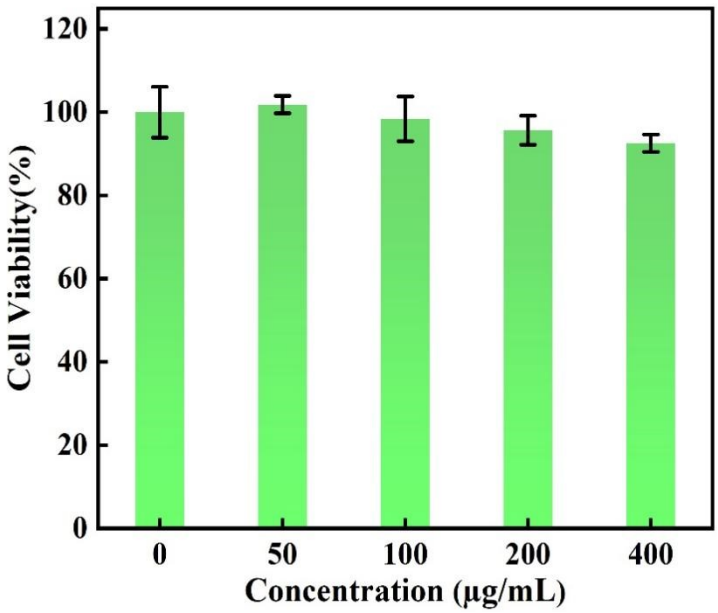


Fig. S14 Cell viability of HeLa cells treated with different concentrations of CyPd-UNs@PEG (0-400 μg/mL) for 24 h.