Supporting Information

A New Strategy to Construct Gold Nanoclusters-Based Optical Probe Using Luminescence Resonance Energy Transfer

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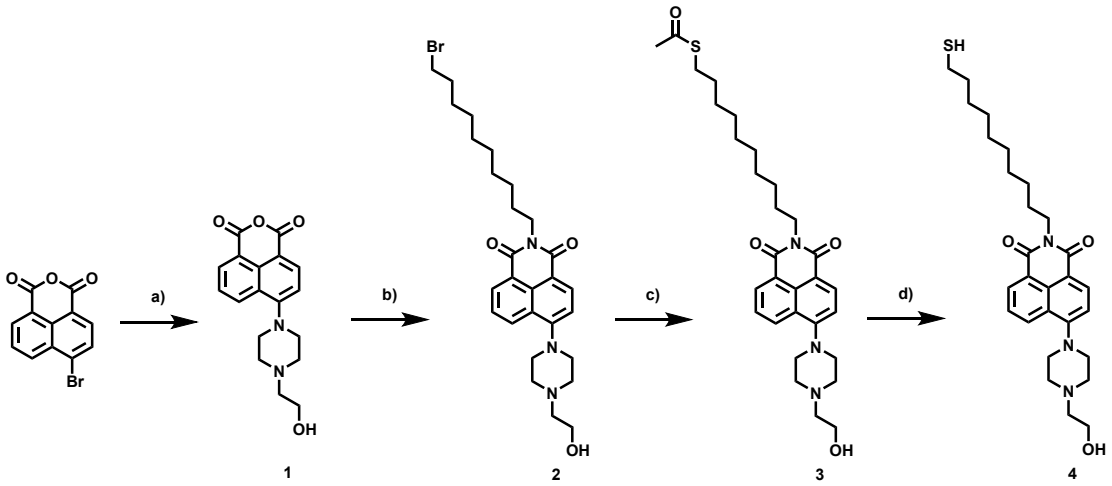
1. Synthesis and characterization of NPA

The naphthalimide derivative NPA has been prepared according to the following scheme:



Scheme S1. Synthesis of the naphthalimide derivative NPA. Conditions: a), N-(2-Hydroxyethyl) piperazine, methylglycol, refluxed, 3 h; b), 10-Bromo-1-decanamine, 1,4-dioxane, reflux, overnight; c), Potassium thioacetate, CH_3CN, 55 ℃, 12 h; d), NaOH, HCl, room temperature, 2 h.

The synthetic route for compound **4** from commercially available materials is depicted in Scheme S1 and compound **1** was prepared according to precious report.[1]

1.1 Synthesis of compound 2.

Compound **1** (2.34 g, 7.2 mmol) was dissolved in 1,4-dioxane (140 mL). 10-Bromo-1-decanamine (2.07 g, 8.8 mmol, 1.2 eq.) was added dropwise and the reaction mixture was heated at reflux overnight. The solution was cooled to room temperature and poured into water (200 mL) giving a yellow precipitate which was altered and washed with water, followed by ether. Air dried to give 2.13 g of compound **2** as a yellow solid (Yield: 54.5 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.51 (dd, J = 8.2, 1.2 Hz, 1H), 8.35 (d, J = 8.0 Hz, 1H), 8.31 – 8.26 (m, 1H), 7.72 (s, 1H), 7.14 (d, J = 8.1 Hz, 1H), 3.62 (dt, J = 6.4, 5.7 Hz, 2H), 3.54 (t, J = 6.1 Hz, 2H), 3.45 (t, J = 4.6 Hz, 2H),

3.23 (ddd, *J* = 10.8, 6.0, 3.2 Hz, 4H), 3.09 (s, 1H), 2.66 (ddd, *J* = 15.4, 5.9, 3.1 Hz, 4H), 2.55 (t, *J* = 5.7 Hz, 2H), 1.77 (tt, *J* = 7.6, 4.6 Hz, 2H), 1.68 (tt, *J* = 7.0, 6.0 Hz, 2H), 1.47 – 1.40 (m, 2H), 1.40 – 1.32 (m, 3H), 1.32 – 1.21 (m, 8H).

1.2 Synthesis of compound 3.

Potassium thioacetate (2.74 g, 24.00 mmol) and compound **2** (1.30 g, 2.40 mmol) were dissolved in CH_3CN (40 mL). The reaction mixture was stirred at 55 ℃ for 12 h under nitrogen atmosphere. After the solvent was evaporated under reduced pressure, the crude product was purified by silica gel column chromatography using CH_2Cl_2/MeOH (10:1, v/v) as eluent to give 0.85 g of compound **3** as a yellow solid (Yield: 65.9 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.51 (dd, *J* = 8.2, 1.2 Hz, 1H), 8.35 (d, *J* = 8.0 Hz, 1H), 8.31 – 8.26 (m, 1H), 7.71 (s, 1H), 7.14 (d, *J* = 8.1 Hz, 1H), 3.62 (dt, *J* = 6.5, 5.7 Hz, 2H), 3.54 (t, *J* = 6.1 Hz, 2H), 3.23 (ddd, *J* = 10.8, 6.0, 3.2 Hz, 4H), 3.09 (s, 0H), 2.89 (t, *J* = 6.4 Hz, 2H), 2.66 (ddd, *J* = 15.4, 5.9, 3.1 Hz, 4H), 2.55 (t, *J* = 5.7 Hz, 2H), 1.68 (tt, *J* = 7.0, 6.1 Hz, 2H), 1.59 (q, *J* = 6.5 Hz, 2H), 1.44 – 1.36 (m, 2H), 1.36 – 1.22 (m, 11H).

1.3 Synthesis of compound 4.

Compound **3** (107.8 mg 0.2 mmol) was dissolved in water (20 mL). And then 1 mL of NaOH (1 M) and 1 mL of HCl (1 M) were then added dropwise, respectively. The reaction was carried out at room temperature for 2 h. After the reaction extracted with CH_2Cl_2 and brine and dried over sodium sulfate. The organic layer was removed and the crude residue was purified by column chromatography using CH_2Cl_2/MeOH (10:1, v/v) as eluent to give 51.2 mg of compound **4** as an orange-red solid (yield of 51.5 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.51 (dd, *J* = 8.2, 1.2 Hz, 1H), 8.35 (d, *J* = 8.0 Hz, 1H), 8.31 – 8.26 (m, 1H), 7.71 (s, 1H), 7.14 (d, *J* = 8.1 Hz, 1H), 3.62 (dt, *J* = 6.5, 5.7 Hz, 2H), 3.54 (t, *J* = 6.1 Hz, 2H), 3.23 (ddd, *J* = 10.8, 5.9, 3.2 Hz, 4H), 3.09 (s, 1H), 2.70 – 2.60 (m, 6H), 2.55 (t, *J* = 5.7 Hz, 2H), 1.72 – 1.58 (m, 4H), 1.40 (p, *J* = 6.7 Hz, 2H), 1.33 (d, *J* = 6.8 Hz, 1H), 1.32 – 1.22 (m, 10H).

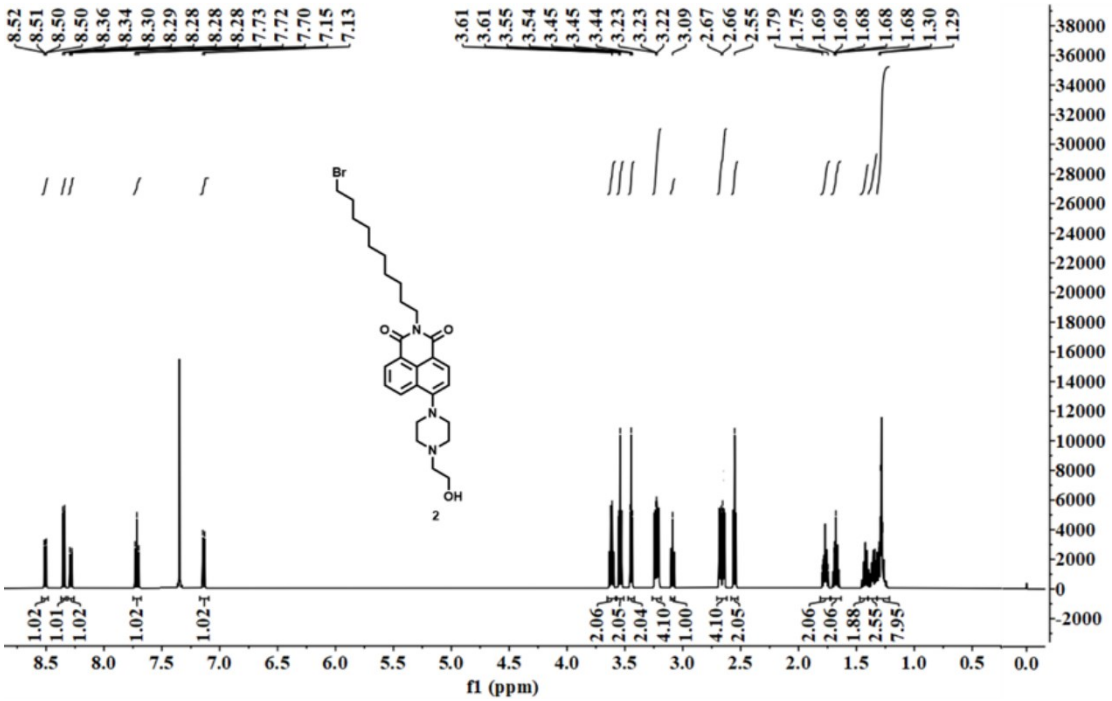


Figure S1. ^{1}H NMR spectrum of compound **2** ($CDCl_3$, 500 MHz).

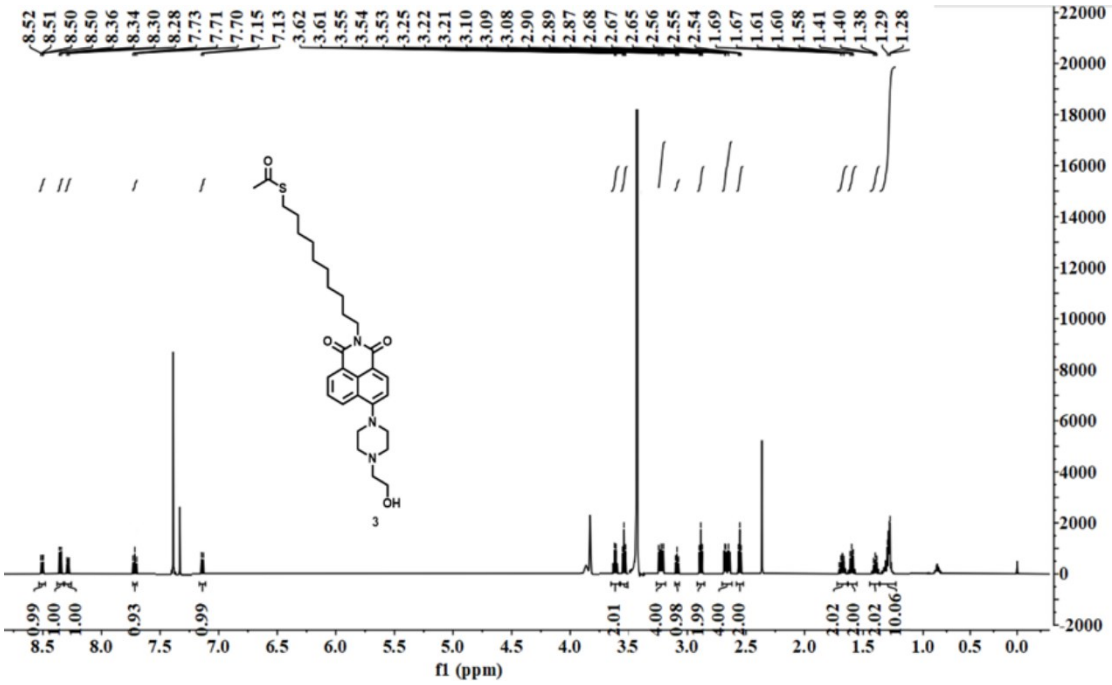


Figure S2. ^{1}H NMR spectrum of compound **3** ($CDCl_3$, 500 MHz).

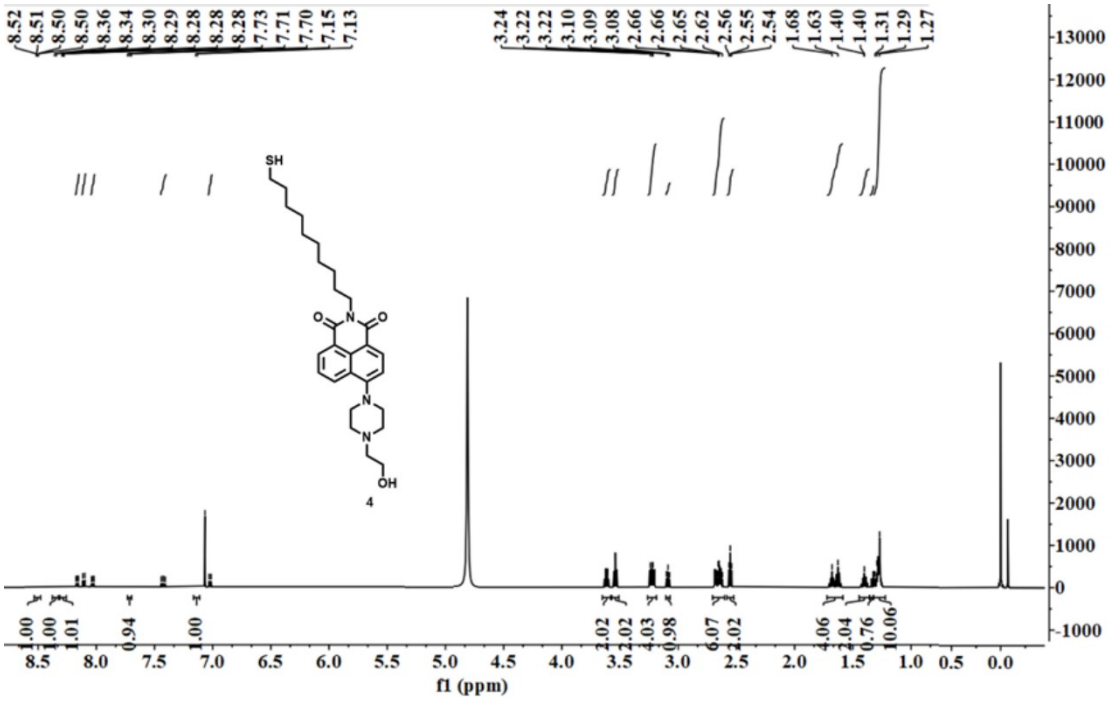


Figure S3. ^{1}H NMR spectrum of compound **4** ($CDCl_3$, 500 MHz).

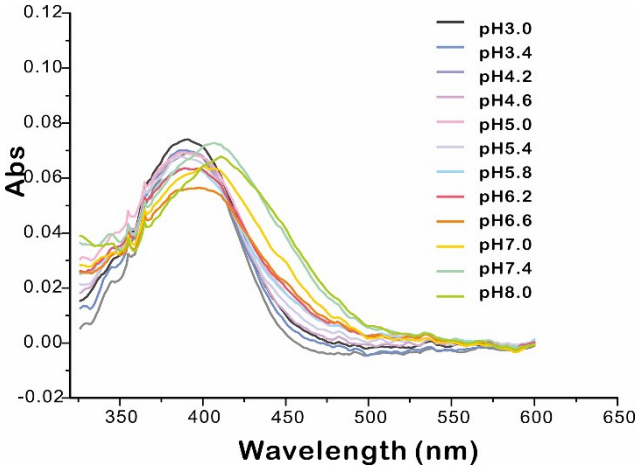


Figure S4. Absorption spectra of NPA in buffers with pH changing from 3.4 to 8.0. The maximum absorption of NPA (~400 nm) was almost unchanged in acidic condition (pH 3-6.6), and had a slight red shift in alkaline condition (pH 6.6-8), which may arise from intramolecular charge transfer (ICT).

2. Synthesis and photophysical properties of NPA-MPCs

2.1 Preparation of NPA protected gold nanoclusters (NPA-MPCs).

The synthesis of monolayer protected nanoclusters with NPA ligand (NPA-MPCs) was modified according to a previously reported two-step procedure. All glassware was washed with aqua regia and rinsed with ultrapure water during the synthesis. $HAuCl_4 \cdot 3H_2O$ (100 mg, 0.254 mmol) was first dissolved in 1 mL water, and then extracted into N_2 purifed toluene (25 mL) through phase transfer reaction with tetraoctylammonium bromide (100 mg, 0.1828 mmol). During stirring, the organic phase gradually turned reddish-orange. After $HAuCl_4 \cdot 3H_2O$ was completely extracted into the organic phase, the aqueous phase was removed, and dioctylamine (0.336 g, 1.3923 mmol) was added (the amount of dinoctylamine was calculated to obtain nanoclusters with the size of about 2 nm). The above mixture was stirred vigorously under N_2. After 30 min, when the color of the mixture faded, 100 μL freshly prepared $NaBH_4$ (9.3 mg, 0.246 mmol) aqueous solution was rapidly added into the system. Due to the strong reduction effect of $NaBH_4$, the solution turned black rapidly, indicating the rapid formation of nanoclusters. After 2 hours of stirring, NPA dissolved in isopropanol solution (0.0254 mmol, 300 μL) was added to the above solution. Stirring was continued overnight and crude product was obtained by rotary evaporation. Then the crude product was re-dissolved in dichloromethane and the large particles were filtered out. By vacuum drying, solid NPA-MPCs uniform were obtained.

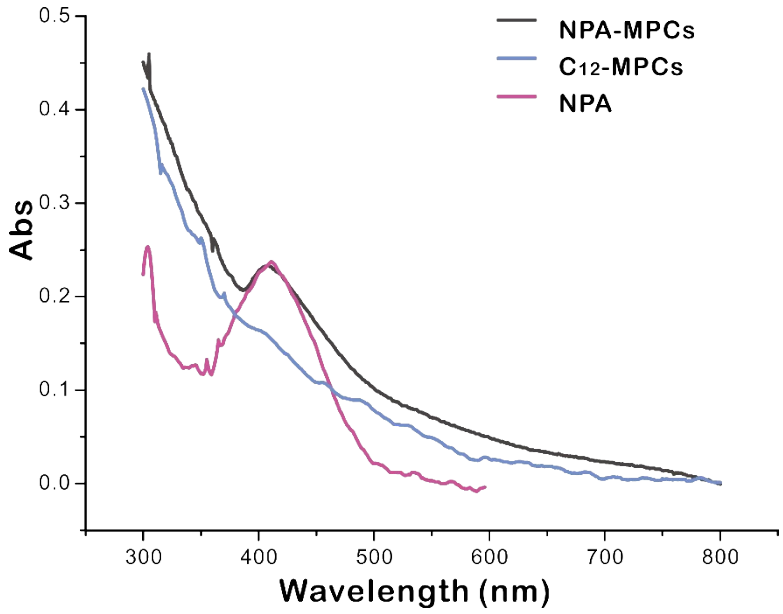


Figure S5. Absorption spectra comparison of NPA-MPCs, C12-MPCs and NPA. The absorption profile of NPA-MPCs is different from that of NPA florophore alone. Both of them have distinct absorption maximum at about 400 nm, corresponding to the absorption of NPA florophore. While in the range of 500-800 nm, the absorption of NPA basically drops to baseline and the absorption of NPA-MPCs appears as a featureless line that gradually weakens, which is similar to the absorption of the reference sample CH3(CH2)11SH stabilized MPCs (abbreviated as C12-MPCs). The absorption profiles further indicate the successful synthesis of nanoclusters. In addition, no surface plasmon resonance (SPR) characteristic peak of large gold nanoparticles was observed at about 520 nm, which further confirmed the size homogeneity of the ultrasmall NPA-MPCs.

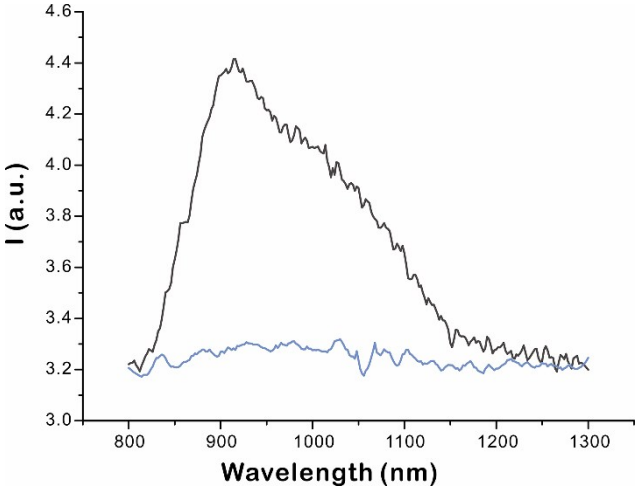


Figure S6. Luminescence of NPA-MPCs under excitation of 405 nm (black) and 550nm (blue). Interestingly, when excited at 405 nm, the luminescence of NPA-MPCs is much stronger than that at 550 nm. That is because 1) the absorption intensity at 405 nm is stronger and 2) 550 nm excites gold core only, and the quantum yield is so low that the luminescence is barely invisible; while 405 nm excites NPA and gold core at the same time, and energy transfer occurs between them.

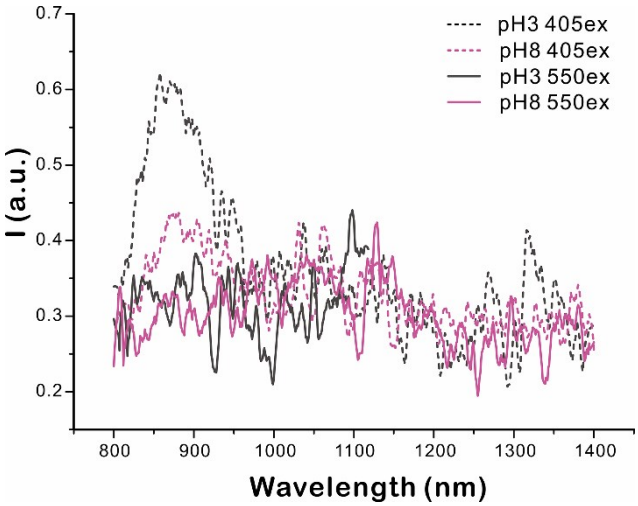


Figure S7. A same sample in buffers of pH 3 and pH 8 and excited under 405 nm and 550 nm, respectively When under 550nm excitation, only gold core can be excited, so there is no pH response. Due to the relatively low power of the excitation source xenon lamp suffers from insufficient power, the NIR luminescence under 550 nm excitation is almost indistinguishable from the baseline.

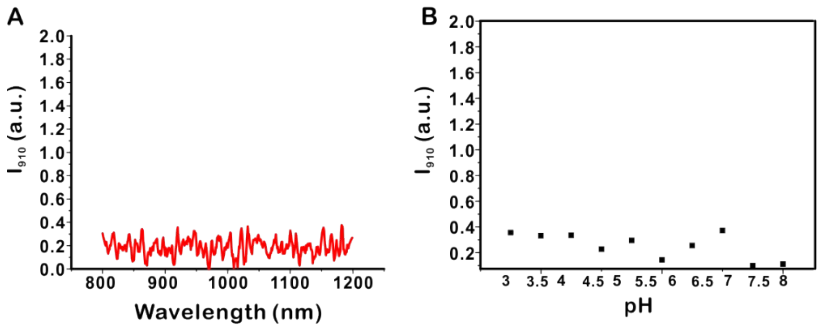


Figure S8 pH titration experiment of C_{12}-MPCs under the same condition of NPA-MPCs. (A) Luminescence measurement of C_{12}-MPCs in pH 6.5 as an example. (B) Plots of I_{910} versus pH for C_{12}-MPCs.

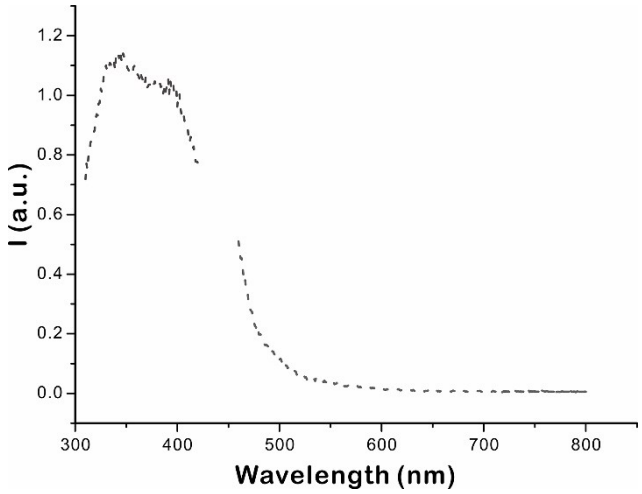


Figure S9. Photoluminescence excitation (PLE) spectra of NPA-MPCs.

3. Cell viability and cell uptake

3.1 Cell Culture Protocols and Cell Viability Assays of HepG2 Cell Lines.

The HepG2 cell lines purchased from Procell Life Science & Technology Co., Ltd. were cultured in Dulbecco's Modified Eagle Medium (DMEM) medium supplemented with 10% fetal bovine serum (Gibco), 1% penicillin, and 1% streptomycin at 37 °C in a 5% CO_2 and 95% air incubator. Cell viability was assessed by a standard Cell Counting Kit-8 (CCK-8), following the manufacturer's protocol.

3.2 Confocal Fluorescence Microscopy for NPA-MPCs.

HepG2 cells were seeded onto glass-bottomed dishes one day before imaging and incubated in DMEM medium and treated with NPA-MPCs for 2h. The medium was then changed into pH gradient high K^+ buffers (125 mM KCl, 20 mM NaCl, 0.5 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 μM nigericin, 5 μM monensin and 25 mM buffer) and further incubated at 37 °C under 5% CO_2 for 15min. Then the fluorescence images were observed by confocal fluorescence microscopy PerkinElmer UltraVIEW VoX.

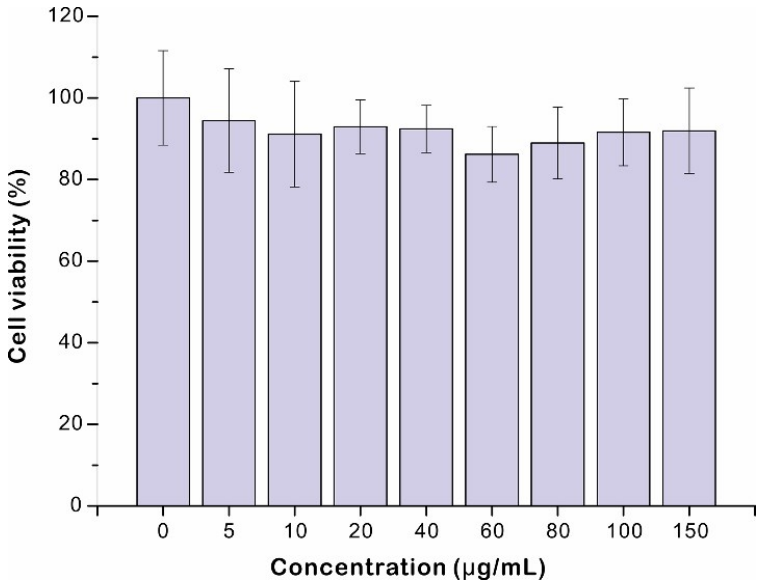


Figure S10. Cell viability of HepG2 cells treated with NPA-MPCs. The cytotoxicity of NPA-MPCs to HepG2 cells was evaluated by CCK-8 cell viability assay. After treating with NPA-MPCs (0-150 μg/mL) for 8 hours, the survival rate of HepG2 cells was still more than 90%. It shows that NPA-MPCs have good biocompatibility and can be further used in biological applications.

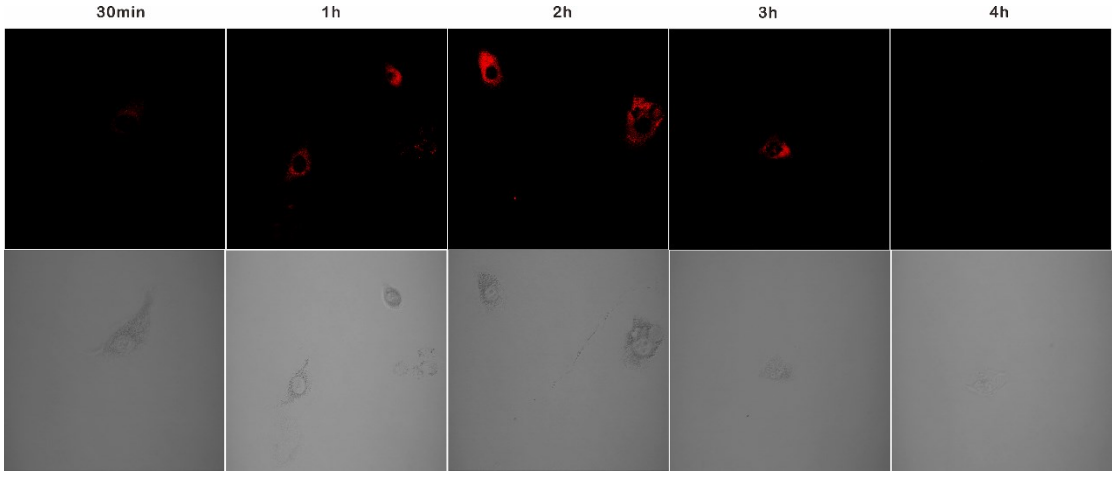


Figure S11. Cell uptake of NPA-MPCs. The first row was collected in the range of 775 ± 70 nm with the excitation wavelength of 405 nm. The second row exhibits the corresponding bright field images.

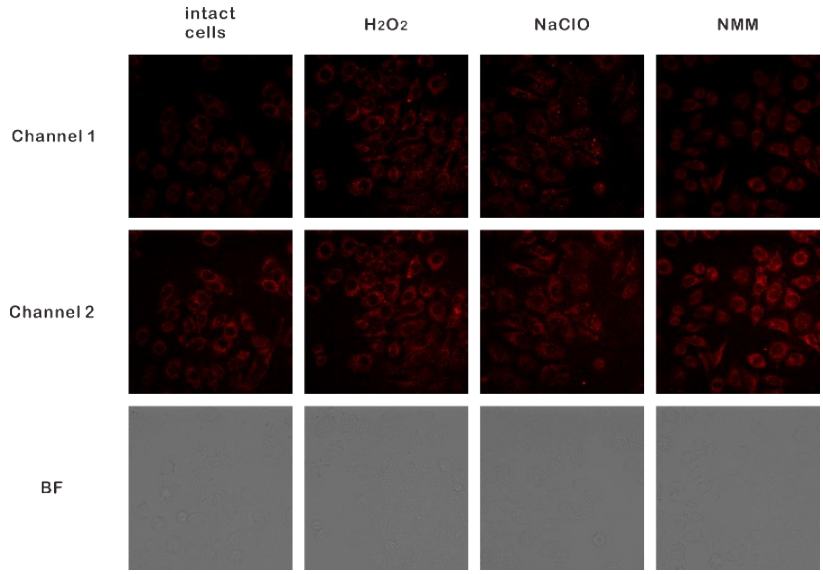


Figure S12. Fluorescent images of HepG2 cells treated with 0.1 mM H_2O_2, 0.1 mM NaClO and 1.0 mM NMM, respectively.

4. References

S1. F. Hu, B. Zheng, D. Wang, Mao. Liu, J. Du and D. Xiao, *Analyst*, 2014, **139**, 3607-3613.