Supporting Information

Tumor-targeted fluorescent probes for the detection of hNQO1 in cancer cells

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Contents

Measurements

Reagents and instruments

All chemical reagents and solvents were purchased from commercial companies without further purification. Ultrapure water was from a Sartorius Arium 611DI system. ^{1}H-NMR and ^{13}C-NMR were measured with an AV-400 nuclear magnetic resonance spectrometer (Bruker, Switzerland) with tetramethylsilane (TMS) as the internal standard. High resolution mass spectra were performed with a Xevo G2-XS Tof high resolution mass spectrometer (Waters, USA) under standard conditions (ESI, 70 eV). The absorption and emission spectra were recorded on an Evolution 220 UV-Vis spectrophotometer and a Lumina fluorescence spectrometer (Thermo Fisher Scientific, USA), respectively.

Spectral measurements

All stock solutions, including 3 mmol/L of **NNP-Yne**, **NNP-Gal**, **NNP-Bio** and **NNP-Ind** in DMSO, 1 mg/mL of hNQO1 in PBS (100 mmol/L, pH 7.40, containing 100 mmol/L KCl and 0.007% BSA), 30 mmol/L of NADH in PBS, 30 mmol/L of dicumarol in DMSO, 30 mmol/L of amino acids/ions ($NaHSO_3$, Na_2S, DTT, Lys, Gly, L-Arg, L-Glu, GSSG, GSH, Hcy, Cys, glucose, NH_4^+, Mg^{2+}, Cu^{2+}, NO_3^-, SO_4^{2-}, Cl^-) in Tris-HCl or (NAC, His, Met, Trp) in DMSO and 30 mg/mL of proteins (HSA, BSA, OVA, α-chy, *β*-lac, mucin, pepsin, tripsin) in PBS were prepared in advance. The excitation/emission wavelengths were 435 nm/540 nm.

Dynamic experiments: Stock solution of the probe (10 μL), hNQO1 (30 μL) and NADH (10 μL) were added to 3 mL of buffer solution. For **NNP-Ind**, 100 μL of HSA stock solution were added. The absorption and emission spectra of the above solution were measured at different times.

hNQO1 titration: 0-120 μL of hNQO1 solution (10 μg/mL) were added into the probes (**NNP-Yne**, **NNP-Gal** and **NNP-Bio**) solution (10 μmol/L, containing 100 μmol/L NADH) to obtain appropriate concentrations of hNQO1. The emission spectra were recorded 3 min after the addition of hNQO1.

Selectivity: Stock solution of amino acid/ion (50 μL) or protein (100 μL) were added to 3 mL of PBS containing probe (10 μmol/L). For **NNP-Ind**, the solution was containing 1 mg/mL of HSA. The fluorescence spectra were measured after the mixture equilibrium for 3 min (**NNP-Yne**, **NNP-Gal** and **NNP-Bio**)/60 min (**NNP-Ind**).

pH effect: The pH of the solutions containing probe (10 μmol/L) or the reaction product (10 μmol/L) were adjusted with NaOH or HCl aqueous solution. Then the emission spectra of the solutions with different pH values were recorded.

Calculation of fluorescence quantum yield

The fluorescence quantum yield of each probe was calculated according to the following equation with 4-amino-N-(*n*-butyl) naphthalimide as the reference ($\Phi = 0.21$ in DMSO).

$$\Phi^S = \Phi^R \times (S^S/S^R) \times (n^S/n^R)^2 \times (A^R/A^S)$$

Where Φ is the fluorescence quantum yield; A represents the absorbance at the excited wavelength; S is the integral area of the emission peak, and n is the refractive index of the solvent. The superscript "S" and "R" represent the sample and the reference, respectively.

Detection limit

The detection limit (LOD) was calculated according to $3S_b/k$, where "k" is the slope of the fitted line, and "S_b" is the standard deviation of 10 blank measurements.

HPLC traces

High-performance liquid chromatograms (HPLC) were obtained on an iChrom 5100 LC system with a Sinopak C18 reversed phase column (4.6 mm × 25 cm). The mobile phase was degassed with an ultrasonic device for 10 minutes. Mobile phase: A-water, B-acetonitrile; injection volume: 20 μL; flow rate: 1.0 mL/min; detection wavelength: 440 nm; elution condition: gradient elution, 0-30 min 5-95% B.

Theoretical calculations

Following are the details for DFT calculation: 1) The density functional theory (DFT) was employed to optimize the structures of the probes through Gaussian 16; 2) All structure optimizations were performed using DFT-M062X functionals and Def2SVP basis set for the ground states; 3) SMD was selected as the solvation mode and the solvent was water; 4) Frequency calculations were performed to ensure the obtained structures are stable and there is no imaginary frequency; 5) The hole-electron contributions of first excited state were further analyzed on the Multiwfn_3.8 platform.

Confocal fluorescent imaging

L929, MDA-MB-231, HeLa and HepG-2 cells (National Collection of Authenticated Cell Cultures, Shanghai, China) were transferred to confocal dishes and incubated for 24 h followed by washed with PBS (20 mmol/L, pH 7.4) for three times. Then the cells were cultured with serum-free DMEM medium

containing probes (10 μmol/L) for 30 min and washed with PBS another three times. For the control experiments, before incubation with 10 μmol/L of probe, the cells were pre-incubated with 500 μmol/L dicumarol for 6 h or 1 μg/mL hNQO1 (containing 100 μmol/L NADH) for 30 min or a unit *β*-galactosidase for 30 min. For drug induction experiment, before incubation with probe, the cells were pre-treated with 1 μg/mL LPS for 12 h, or 1 mM APAP for 12 h, or 20 μM cisplatin for 30 min, or 1 mM $CoCl_2$ for 24 h. The fluorescence was collected at 425-475 nm for the blue channel and 500-550 nm for the green channel as excited at 405 nm and 488 nm, respectively.

MTT Assay

L929, MDA-MB-231, HeLa and HepG-2 cell lines (National Collection of Authenticated Cell Cultures, Shanghai, China) were cultured in Dulbecco's Modified Eagle Medium (High glucose) containing 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C in an atmosphere of 5% CO_2.

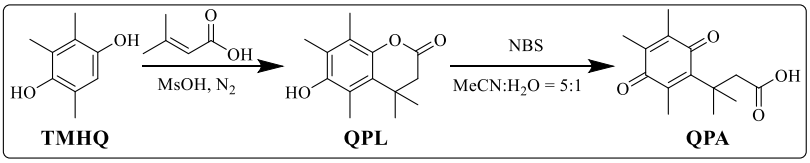
The cells were transferred to a 96-well plate (5000 cells/well) and incubated for 24 h. After removal of the medium, the cells were cultured with different concentrations (0-20 μM) of probes in serum-free DMEM medium for 24 h followed by incubated with 5 mg/mL of MTT for another 4 h. After removal of the medium, 100 μL of DMSO were added to the plate. The absorbance at 570 nm of each solution was measured with a microplate reader. The cell viability was calculated according to the following equation.

$$\text{Cell viability (\%)} = (OD_{sample} - OD_{blank}) / (OD_{control} - OD_{blank}) \times 100\%$$

Where $OD_{control}$ and OD_{sample} are the optical densities of the wells in which the cells incubated without and with (a) certain concentration of the probe, respectively; OD_{blank} represents optical density of the well only containing culture medium and MTT.

Synthesis

Synthesis of QPA [1]



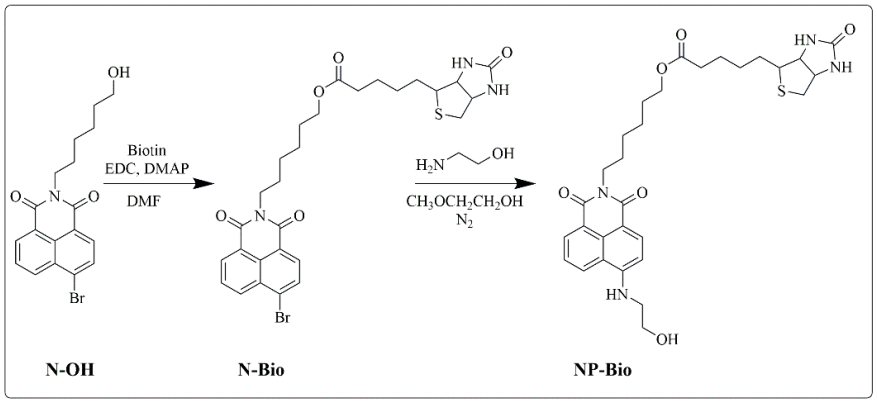
Scheme S1 Synthesis of compound **QPA**.

QPL: TMHQ (2.05 g, 13.5 mmol) and 3,3-dimethylacrylic acid (1.5 g, 15 mmol) were dissolved in 10

mL of methanesulfonic acid (MsOH), and the solution was reacted at 85 ℃ for 3 hours under nitrogen atmosphere. The reaction solution was cooled to room temperature until the point of **TMHQ** in thin-layer chromatography (TLC) disappeared. The resulting product was extracted with ethyl acetate (4 × 100 mL), and the concentrated organic phase was washed with saturated sodium bicarbonate solution and water (2 × 50 mL), dried with anhydrous sodium sulphate, and the solvent was evaporated in vacuo. The crude product was recrystallized with n-hexane and ethyl acetate (2:1, v:v) to obtain a white solid **QPL** (2.8 g, 89%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 4.60 (s, 1H), 2.55 (s, 2H), 2.35 (s, 3H), 2.22 (s, 3H), 2.18 (s, 3H), 1.45 (s, 6H).

QPA: *N*-bromosuccinimide (NBS; 1.87 g, 10.5 mmol) was added in portions to 24 mL of mixture of acetonitrile and water (5:1, v:v) containing **QPL** (2.34 g, 10 mmol). After stirred for 30 min at room temperature, acetonitrile was evaporated under reduced pressure, while the remaining aqueous phase was extracted with dichloromethane (2 × 30 mL). The obtained organic phase was dried with anhydrous sodium sulphate and the solvent was removed to obtain a yellow solid **QPA** (2.25 g, 90%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 3.02 (s, 2H), 2.14 (s, 3H), 1.95 (s, 3H), 1.93 (s, 3H), 1.43 (s, 6H).

Synthesis of NP-Bio



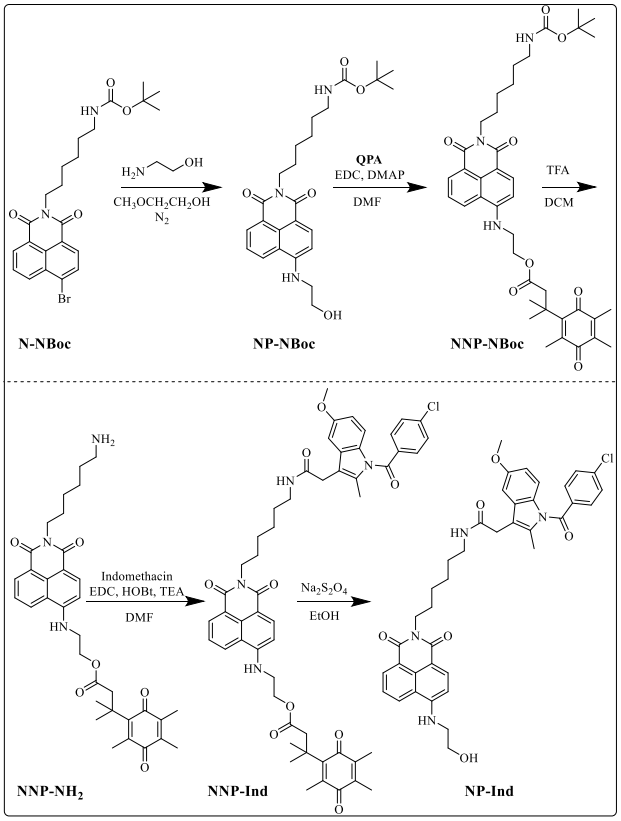
Scheme S2 Synthesis of **NP-Bio**.

N-Bio[2]: A solution of DMF (10 mL) containing D-biotin (310 mg, 1.3 mmol), EDC (740 mg, 3.8 mmol) and DMAP (470 mg, 3.8 mmol) was stirred at room temperature under a nitrogen atmosphere for 30 min. Then 5 mL of DMF containing **N-OH** (1.43 g, 3.8 mmol) was injected into the above solution. After stirred for another 12 h, the solvent was evaporated under reduced pressure and the crude product was purified with column chromatography (DCM:MeOH = 50:1, v:v) to afford a white solid **N-Bio** (705 mg, 90%). ^{1}H-

NMR (600 MHz, $CDCl_3$) δ (ppm): 8.65 (dd, *J* = 7.3, 1.1 Hz, 1H), 8.57 (dd, *J* = 8.4, 1.1 Hz, 1H), 8.41 (d, *J* = 7.9 Hz, 1H), 8.04 (d, *J* = 7.8 Hz, 1H), 7.85 (dd, *J* = 8.5, 7.3 Hz, 1H), 5.29 (s, 1H), 4.95 (s, 1H), 4.54-4.49 (m, 1H), 4.34-4.29 (m, 1H), 4.16 (t, *J* = 7.8 Hz, 2H), 4.06 (t, *J* = 6.6 Hz, 2H), 3.21-3.13 (m, 1H), 2.92 (dd, *J* = 12.8, 5.0 Hz, 1H), 2.73 (d, *J* = 12.8 Hz, 1H), 2.31 (t, *J* = 7.4 Hz, 2H), 1.78-1.71 (m, 3H), 1.66-1.62 (m, 3H), 1.47-1.41 (m, 6H).

NP-Bio: To 20 mL of 2-methoxyethanol containing **N-Bio** (467 mg, 0.8 mmol) dropped 2-aminoethanol (485 μL, 8 mmol). After refluxed(refluxing) for 4 h under nitrogen atmosphere, the solvent was evaporated and the crude product was purified with column chromatography (DCM:MeOH = 50:2, v:v) to afford a yellow solid **NP-Bio** (375 mg, 81%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 8.69 (dd, *J* = 8.6, 1.2 Hz, 1H), 8.43 (dd, *J* = 7.4, 1.1 Hz, 1H), 8.25 (d, *J* = 8.6 Hz, 1H), 7.73 (t, *J* = 5.6 Hz, 1H), 7.68 (dd, *J* = 8.4, 7.3 Hz, 1H), 6.82 (d, *J* = 8.7 Hz, 1H), 6.42 (d, *J* = 1.8 Hz, 1H), 6.35 (s, 1H), 4.91 (t, *J* = 5.6 Hz, 1H), 4.32-4.23 (m, 1H), 4.15-4.08 (m, 1H), 4.04-3.94 (m, 4H), 3.70 (q, *J* = 5.9 Hz, 2H), 3.47 (q, *J* = 5.8 Hz, 2H), 3.11-3.03 (m, 1H), 2.79 (dd, *J* = 12.5, 5.1 Hz, 1H), 2.55 (d, *J* = 12.4 Hz, 1H), 2.26 (t, *J* = 7.4 Hz, 2H), 1.65-1.49 (m, 7H), 1.47-1.39 (m, 1H), 1.39-1.30 (m, 6H).

Synthesis of NP-Ind



Scheme S3 Synthesis of **NNP-Ind**.

NP-NBoc[2]: To 50 mL of 2-methoxyethanol containing **N-NBoc** (1.19 g, 2.5 mmol) dropped 2-aminoethanol (1.5 mL, 25 mmol). After refluxed(refluxing) for 4 h under nitrogen atmosphere, the solvent was evaporated and the crude product was purified with column chromatography (DCM:MeOH = 50:2, v:v) to afford a yellow solid **NP-NBoc** (965 mg, 85%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.48 (d, *J* = 7.3 Hz, 1H), 8.36 (d, *J* = 8.4 Hz, 1H), 8.11 (dd, *J* = 8.4, 1.1 Hz, 1H), 7.55 (t, *J* = 7.9 Hz, 1H), 6.66 (d, *J* = 8.4 Hz, 1H), 5.84 (s, 1H), 4.62 (s, 1H), 4.11 (t, *J* = 7.5 Hz, 2H), 4.06 (t, *J* = 5.1 Hz, 2H), 3.56 (t, *J* = 5.1 Hz, 2H), 3.10 (q, *J* = 6.6 Hz, 2H), 1.79-1.60 (m, 4H), 1.53-1.47 (m, 2H), 1.43 (s, 9H), 1.40-1.38 (m, 2H).

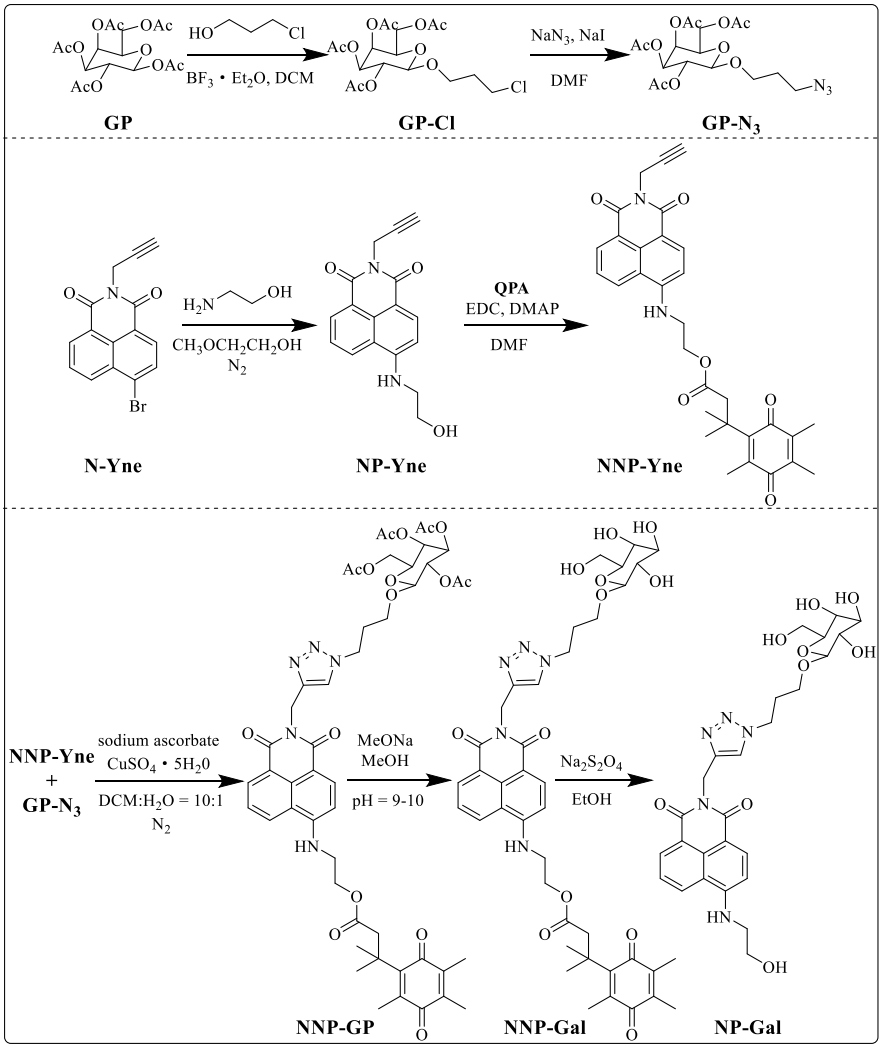
NNP-NBoc: A solution of DMF (10 mL) containing **QPA** (375 mg, 1.5 mmol), EDC (860 mg, 4.5 mmol) and DMAP (549 mg, 4.5 mmol) was stirred at room temperature under a nitrogen atmosphere for 30 min. Then 5 mL of DMF containing **NP-NBoc** (228 mg, 0.5 mmol) was injected into the above solution. After stirred for another 12 h, the solvent was evaporated under reduced pressure and the crude product was purified with column chromatography (DCM:MeOH = 50:1, v:v) to afford a yellow solid **NNP-NBoc** (250 mg, 73%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.59 (dd, *J* = 7.3, 1.0 Hz, 1H), 8.46 (d, *J* = 8.3 Hz, 1H), 8.04 (dd, *J* = 8.5, 1.0 Hz, 1H), 7.57 (dd, *J* = 8.4, 7.3 Hz, 1H), 6.65 (d, *J* = 8.4 Hz, 1H), 5.87 (s, 1H), 4.56 (s, 1H), 4.45 (t, *J* = 5.0 Hz, 2H), 4.21-4.10 (m, 2H), 3.60 (t, *J* = 5.0 Hz, 2H), 3.15-3.08 (m, 2H), 3.06 (s, 2H), 2.11 (s, 3H), 1.87 (s, 3H), 1.83 (s, 3H), 1.73 (p, *J* = 7.2 Hz, 2H), 1.54-1.45 (m, 4H), 1.43 (s, 9H), 1.42 (s, 6H), 1.41-1.37 (m, 2H).

NNP-NH_2: **NNP-NBoc** (250 mg, 0.36 mmol) was dissolved in 5 mL of TFA/DCM (1:1, v:v) and stirred at room temperature for 10 min. The solvent was evaporated and the crude product was purified with column chromatography (DCM:MeOH = 50:3, v:v) to afford a yellow solid **NNP-NH_2** (1800 mg, 85%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.49 (d, *J* = 7.3 Hz, 1H), 8.35 (d, *J* = 8.3 Hz, 1H), 8.07 (s, 2H), 8.00 (d, *J* = 8.4 Hz, 1H), 7.50 (t, *J* = 7.8 Hz, 1H), 6.59 (d, *J* = 8.4 Hz, 1H), 5.97 (s, 1H), 4.44 (t, *J* = 5.0 Hz, 2H), 4.09 (t, *J* = 7.1 Hz, 2H), 3.64-3.53 (m, 2H), 3.05 (s, 2H), 3.00 (s, 2H), 2.10 (s, 3H), 1.86 (s, 3H), 1.83 (s, 3H), 1.77-1.65 (m, 4H), 1.54-1.44 (m, 2H), 1.41 (s, 6H), 1.38-1.32 (m, 2H).

NP-Ind: A solution of ethanol (15 mL) containing **NNP-Ind** (19 mg, 0.02 mmol) and sodium hydrosulfite (70 mg, 0.4 mmol) was stirred at room temperature for 10 min. The insoluble impurities were removed by filtration and the filtrate was evaporated under reduced pressure to remove ethanol. The crude product was separated with silica gel column chromatography (DCM:MeOH = 50:2, v:v) to obtain a yellow solid **NP-Ind** (13 mg, 95%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.47 (d, *J* = 7.3 Hz, 1H), 8.34 (d, *J* = 8.3

Hz, 1H), 8.14 (d, *J* = 8.5 Hz, 1H), 7.66-7.53 (m, 3H), 7.43 (d, *J* = 8.1 Hz, 2H), 6.90 (d, *J* = 2.5 Hz, 1H), 6.82 (d, *J* = 9.0 Hz, 1H), 6.74-6.62 (m, 2H), 5.94 (t, *J* = 5.2 Hz, 1H), 5.80 (t, *J* = 5.3 Hz, 1H), 4.14-3.98 (m, 4H), 3.80 (s, 3H), 3.64 (s, 2H), 3.57 (dd, *J* = 9.5, 4.4 Hz, 2H), 3.19 (q, *J* = 6.5 Hz, 2H), 2.38 (s, 3H), 1.49-1.35 (m, 4H), 1.34-1.29 (m, 4H).

Synthesis of NP-Yne and NP-Gal



Scheme S4 Synthesis of **NNP-Yne** and **NNP-Gal**.

GP-Cl [3]: To a solution of dichloromethane (40 mL) containing 3-chloro-1-propanol (1.90 g, 20 mmol) and **GP** (3.90 g, 10 mmol) slowly added boron fluoride ethyl ether (3.55 g, 25 mmol) at 0 ℃ under nitrogen protection. The above solution was stirred at room temperature and traced with TLC. After the point of **GP** disappeared, the reaction solution was poured into 100 mL of ice water and extracted with dichloromethane (3 × 100 mL). The combined organic phase was washed with saturated sodium bicarbonate solution (2 × 100 mL), then the solvent was evaporated under reduced pressure. The residue was separated by silica gel column

chromatography (PE:EA = 10:1, v:v) to give a colorless oily liquid **GP-Cl** (4.03 g, 95%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 5.40 (dd, *J* = 3.5, 1.2 Hz, 1H), 5.20 (dd, *J* = 10.5, 7.9 Hz, 1H), 5.03 (dd, *J* = 10.5, 3.4 Hz, 1H), 4.48 (d, *J* = 7.9 Hz, 1H), 4.22-4.11 (m, 2H), 4.03-3.97 (m, 1H), 3.92 (td, *J* = 6.6, 1.2 Hz, 1H), 3.73-3.66 (m, 1H), 3.62 (dd, *J* = 7.2, 5.2 Hz, 2H), 2.16 (s, 3H), 2.14-2.09 (m, 1H), 2.08 (s, 3H), 2.06 (s, 3H), 1.98-1.92 (m, 1H).

GP-N_3: A solution of *N*,*N*-dimethylformamide (DMF, 15 mL) containing sodium azide (2.47 g, 38 mmol), **GP-Cl** (4.03 g, 9.5 mmol) and sodium iodide (140 mg, 0.95 mmol) was stirred at 80 ℃ under a nitrogen atmosphere for 18 h. Then the reaction solution was poured into ice-water (150 mL) and extracted with ethyl acetate (3 × 150 mL). The combined organic phase was washed with saturated brine (2 × 150 mL) and removed the solvent. The crude product was separated by silica gel column chromatography (PE:EA = 5:1, v:v) to give **GP-N_3** (3.77 g, 92%) as a colorless oily liquid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 5.39 (dd, *J* = 3.4, 1.2 Hz, 1H), 5.20 (dd, *J* = 10.5, 7.9 Hz, 1H), 5.02 (dd, *J* = 10.5, 3.4 Hz, 1H), 4.47 (d, *J* = 7.9 Hz, 1H), 4.23-4.08 (m, 2H), 4.01-3.87 (m, 2H), 3.60 (ddd, *J* = 9.7, 8.1, 4.9 Hz, 1H), 3.41-3.31 (m, 2H), 2.15 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 1.99 (s, 3H), 1.93-1.76 (m, 2H).

NP-Yne: To 100 mL of 2-methoxyethanol containing **N-Yne** (939 mg, 3 mmol) dropped 2-aminoethanol (1.8 mL, 30 mmol). After refluxed for 4 h under nitrogen atmosphere, the solvent was evaporated and the crude product was purified with column chromatography (DCM:MeOH = 100:1, v:v) to afford 660 mg of yellow solids **NP-Yne** (75%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 8.73 (d, *J* = 8.4 Hz, 1H), 8.46 (d, *J* = 7.3 Hz, 1H), 8.28 (d, *J* = 8.6 Hz, 1H), 7.84 (t, *J* = 5.6 Hz, 1H), 7.70 (dd, *J* = 8.4, 7.3 Hz, 1H), 6.84 (d, *J* = 8.6 Hz, 1H), 4.91 (t, *J* = 5.6 Hz, 1H), 4.74 (d, *J* = 2.4 Hz, 2H), 3.70 (q, *J* = 5.9 Hz, 2H), 3.48 (q, *J* = 5.8 Hz, 2H), 3.09 (d, *J* = 2.3 Hz, 1H).

NP-Gal: A solution of ethanol (15 mL) containing **NNP-Gal** (16 mg, 0.02 mmol) and sodium hydrosulfite (70 mg, 0.4 mmol) was stirred at room temperature for 10 min. The insoluble impurities were removed by filtration and the filtrate was evaporated under reduced pressure to remove ethanol. The crude product was separated with silica gel column chromatography (DCM:MeOH = 50:6, v:v) to obtain a yellow solid **NP-Gal** (10 mg, 90%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 8.75 (d, *J* = 8.4 Hz, 1H), 8.45 (d, *J* = 7.3 Hz, 1H), 8.28 (d, *J* = 8.6 Hz, 1H), 7.96 (s, 1H), 7.81 (t, *J* = 5.5 Hz, 1H), 7.69 (t, *J* = 7.9 Hz, 1H), 6.83 (d, *J* = 8.7 Hz, 1H), 5.26 (s, 2H), 4.92 (t, *J* = 5.7 Hz, 1H), 4.88 (d, *J* = 4.6 Hz, 1H), 4.65 (d, *J* = 5.5 Hz, 1H), 4.53 (t, *J* = 5.6 Hz, 1H), 4.39 (t, *J* = 7.1 Hz, 2H), 4.32 (d, *J* = 4.7 Hz, 1H), 4.02 (d, *J* = 7.5 Hz, 1H), 3.71 (q, *J* = 5.7 Hz, 3H), 3.59 (t, *J* = 4.0 Hz, 1H), 3.53-3.44 (m, 4H), 3.40-3.35 (m, 1H), 3.30-3.24 (m, 2H), 3.23-3.15 (m,

1H), 2.06-1.95 (m, 2H).

NNP-GP: Dropwise added 1 mL of copper (II) sulfate pentahydrate solution (25 mg, 0.1 mmol) to a mixed solution of dichloromethane and water (10 mL, v:v = 10:1) containing NNP-Yne (63 mg, 0.12 mmol), GP-N3 (43 mg, 0.1 mmol) and sodium ascorbate (40 mg, 0.2 mmol). The above solution was stirred overnight at room temperature under a nitrogen atmosphere, and then extracted with dichloromethane (3 × 10 mL). The combined organic phase was washed with saturated brine. After evaporated under reduced pressure, the residue was separated with silica gel column chromatography (DCM:MeOH = 50:2, v:v) to give a yellow solid **NNP-GP** (74 mg, 77%). 1H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.54 (d, J = 7.2 Hz, 1H), 8.44 (d, J = 8.4 Hz, 1H), 8.02 (d, J = 8.4 Hz, 1H), 7.63 (s, 1H), 7.51 (t, J = 7.8 Hz, 1H), 6.63 (d, J = 8.5 Hz, 1H), 5.48 (d, J = 2.4 Hz, 2H), 5.37 (dd, J = 3.5, 1.1 Hz, 1H), 5.19 (dd, J = 10.5, 7.9 Hz, 1H), 4.98 (dd, J = 10.5, 3.5 Hz, 1H), 4.47-4.29 (m, 5H), 4.12 (dd, J = 6.6, 2.1 Hz, 2H), 3.90-3.83 (m, 2H), 3.59 (s, 2H), 3.40 (td, J = 9.1, 4.4 Hz, 1H), 3.06 (s, 2H), 2.16 (s, 3H), 2.15-2.12 (m, 1H), 2.11 (s, 3H), 2.10-2.07 (m, 1H), 2.07 (s, 3H), 2.02 (s, 3H), 1.98 (s, 3H), 1.87 (s, 3H), 1.84 (s, 3H), 1.42 (s, 6H).

Reference

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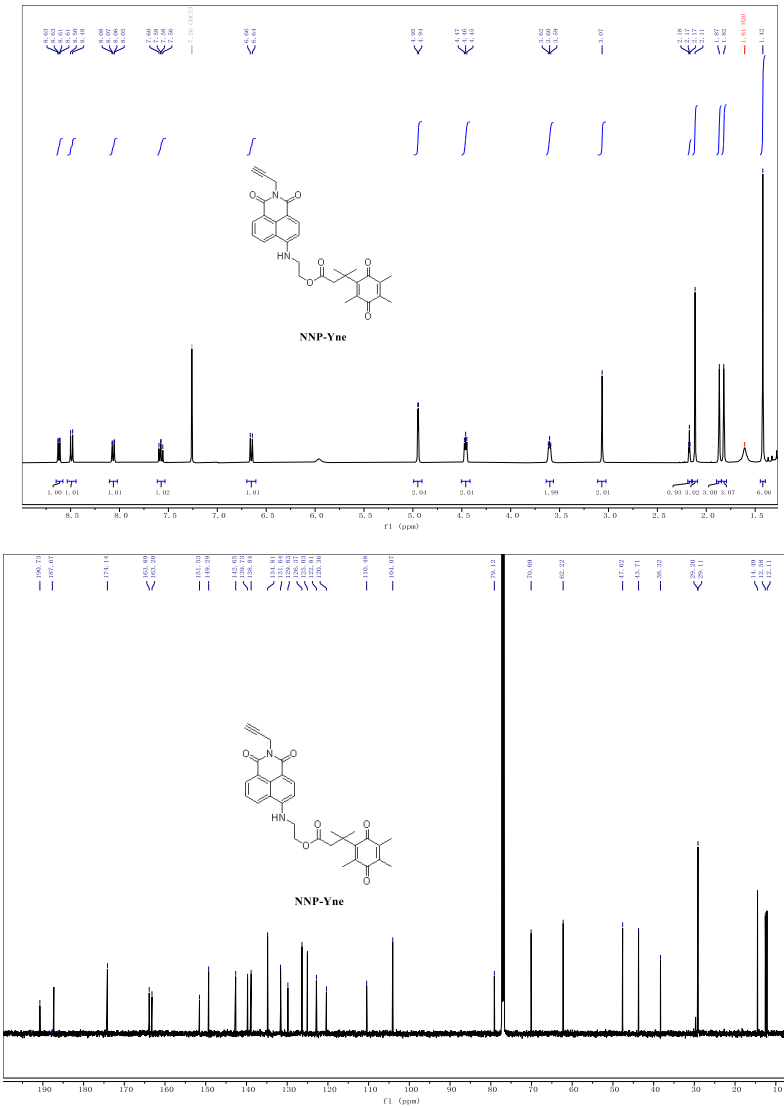
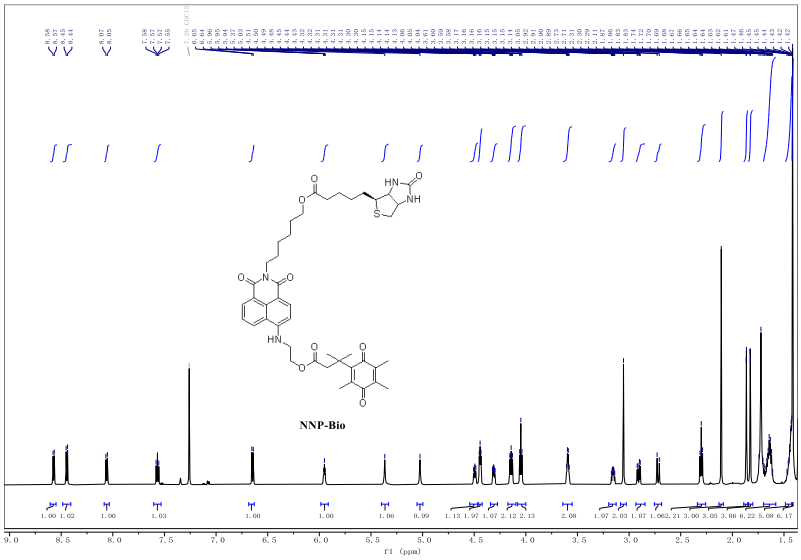
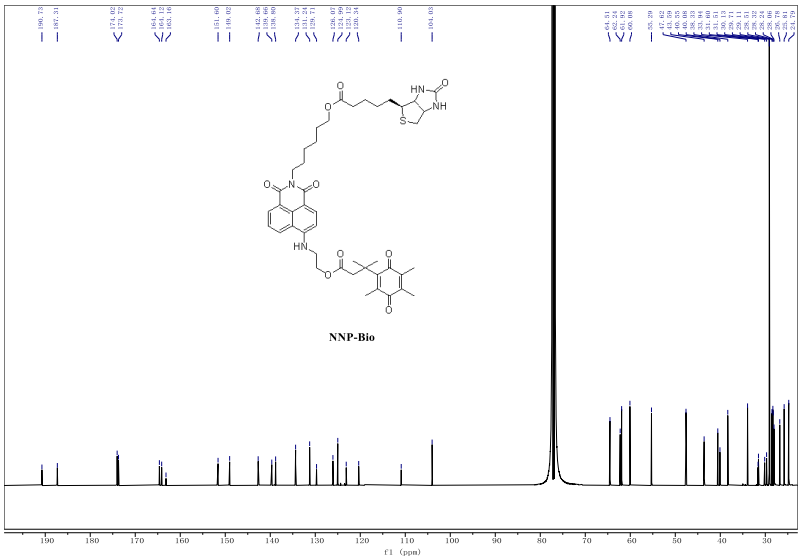


Fig. S1 ^{1}H-NMR, ^{13}C-NMR and ESI-Mass spectra of **NNP-Yne**.





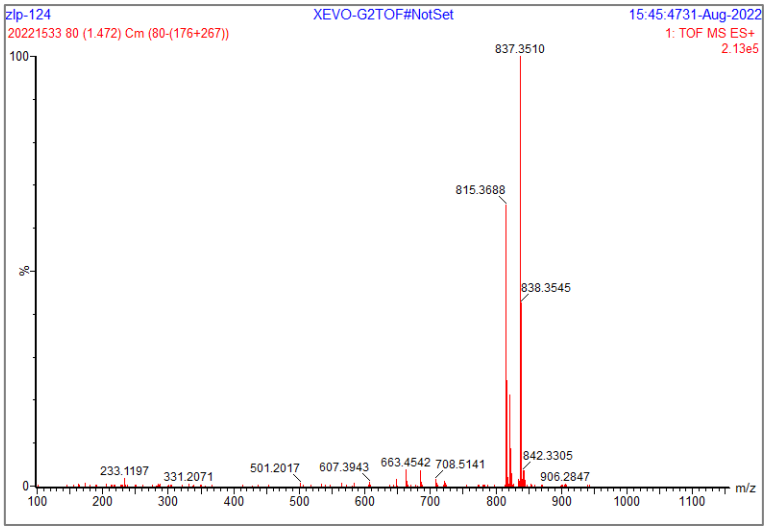


Fig. S2 ^{1}H-NMR, ^{13}C-NMR and ESI-Mass spectra of **NNP-Bio**.

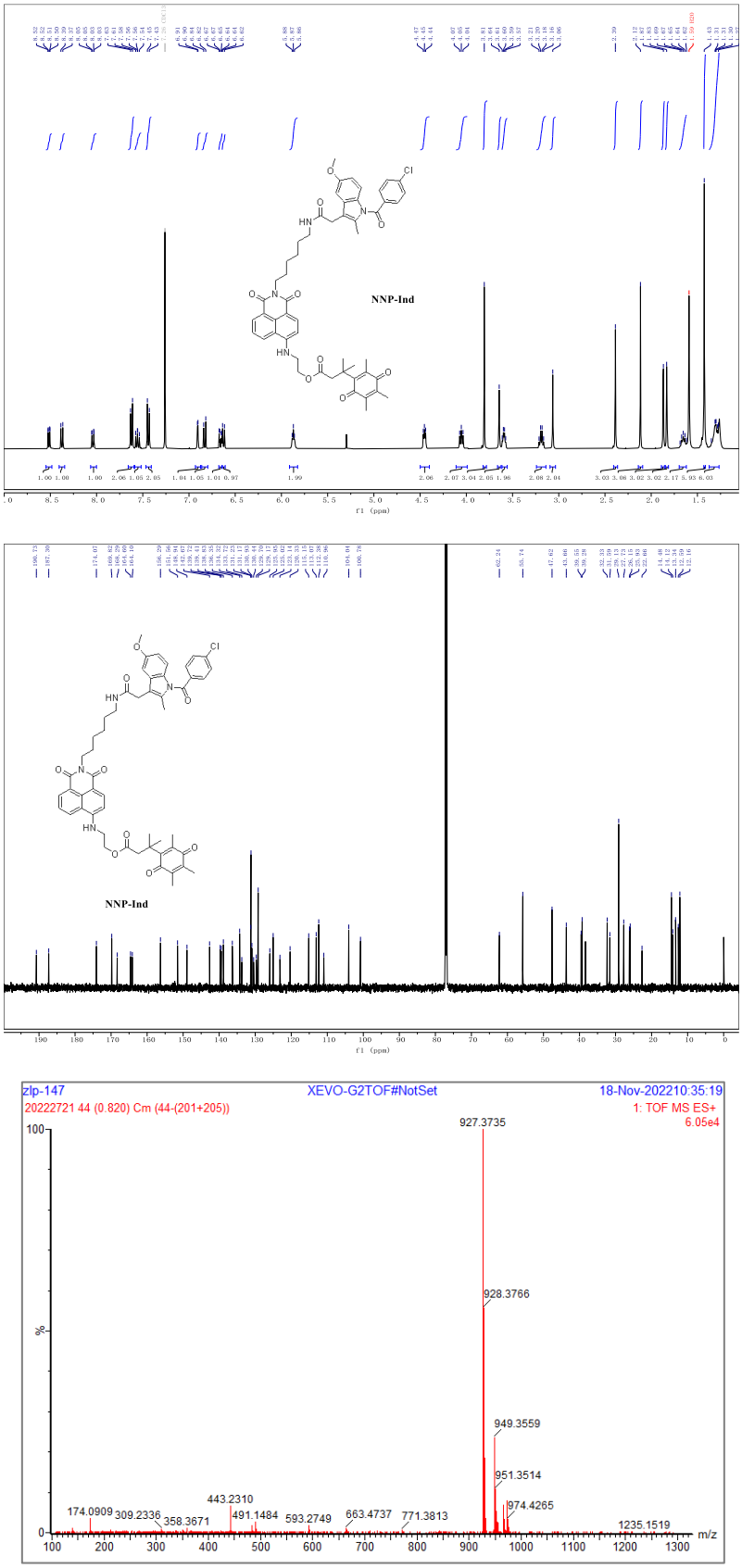
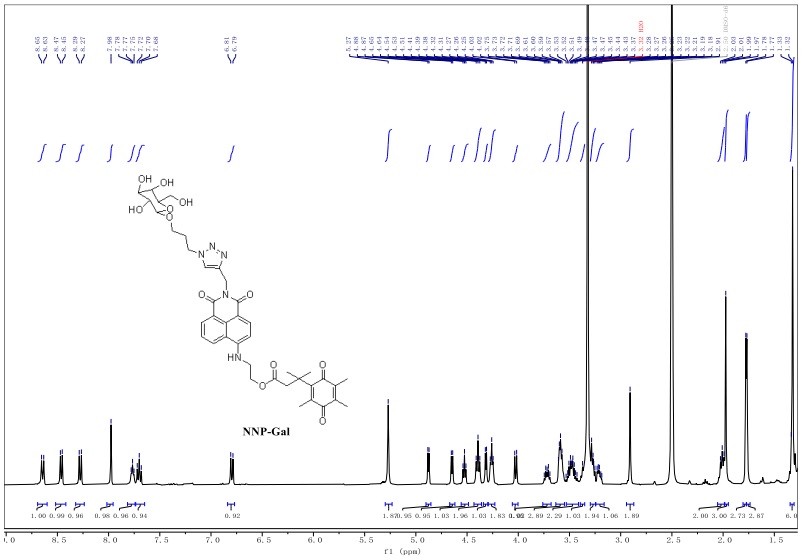
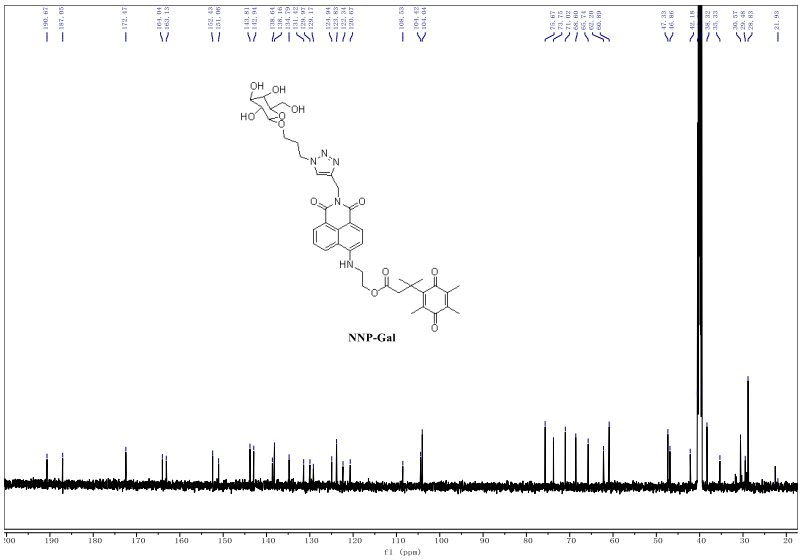


Fig. S3 ^{1}H-NMR, ^{13}C-NMR and ESI-Mass spectra of **NNP-Ind**.





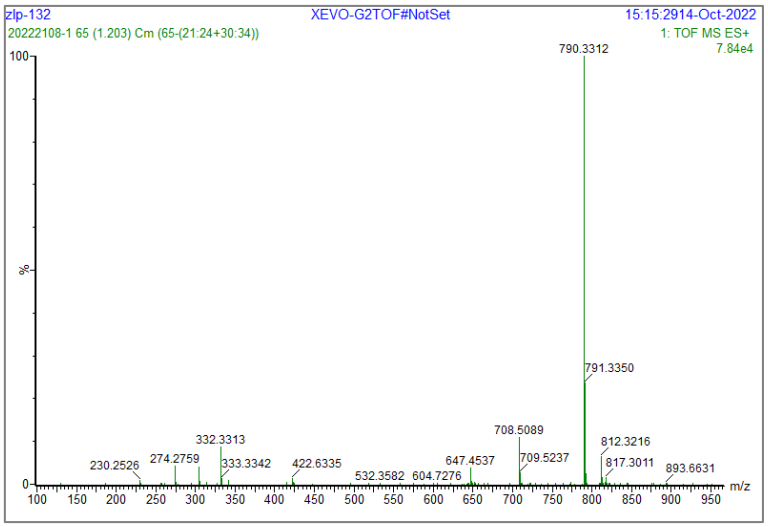
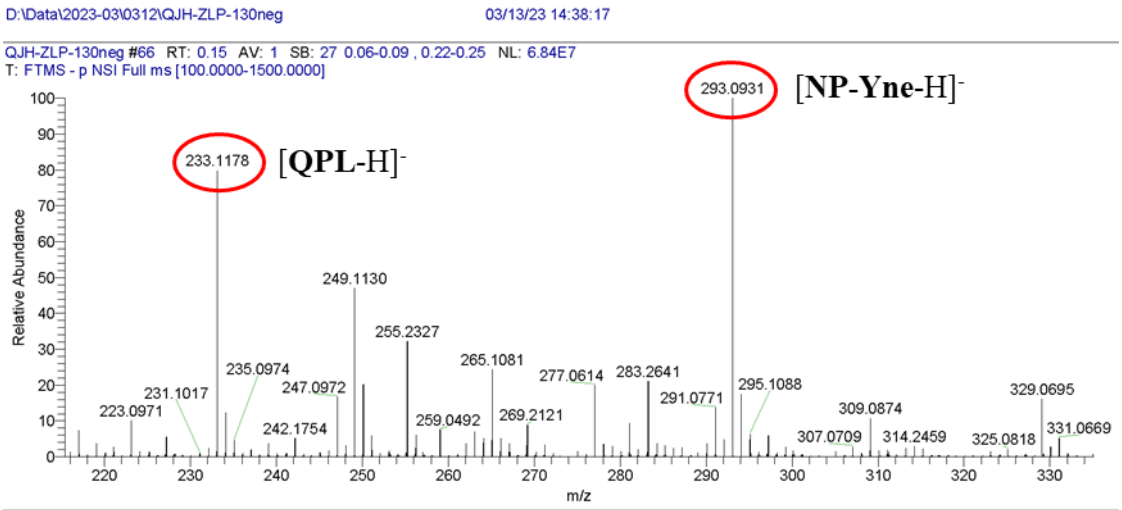


Fig. S4 ^{1}H-NMR, ^{13}C-NMR and ESI-Mass spectra of **NNP-Gal**.



QJH-ZLP-130neg#49-80 RT: 0.11-0.18 AV: 32
T: FTMS - p NSI Full ms [100.0000-1500.0000]
m/z= 232.30-233.70

m/z	Intensity	Relative	Theo. Mass	Delta (ppm)	Composition
233.1178	18242412.0	100.00	233.1172	0.61	$C_{14}H_{17}O_3$

QJH-ZLP-130neg#78 RT: 0.18
T: FTMS - p NSI Full ms [100.0000-1500.0000]
m/z= 292.37-293.84

m/z	Intensity	Relative	Theo. Mass	Delta (ppm)	Composition
293.0931	199365648.0	100.00	293.0921	1.01	$C_{17}H_{13}O_3N_2$

Fig. S5 The ESI-Mass spectrum of **NNP-Yne** in the presence of hNQO1 and NADH.

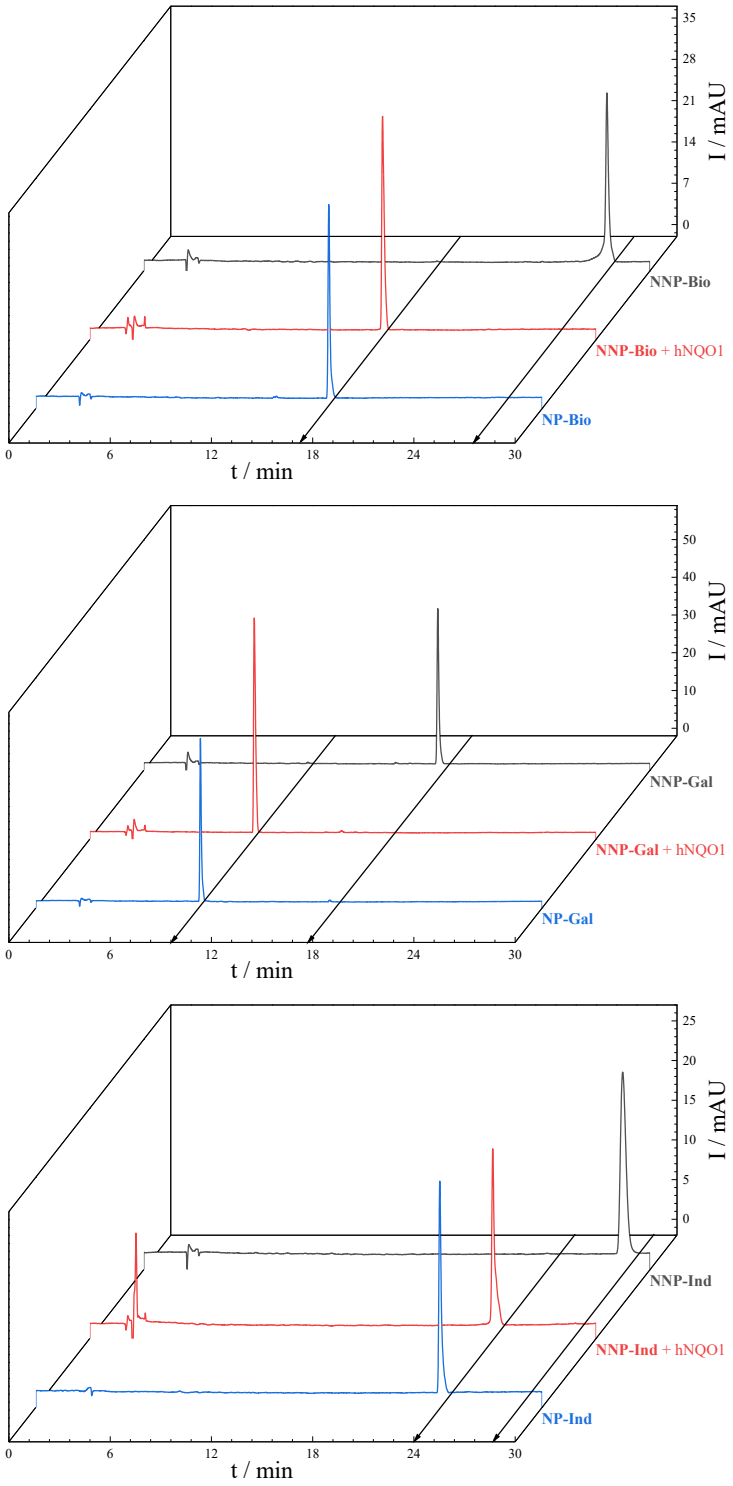


Fig. S6 HPLC chromatograms of hNQO1-probe system in the presence of NADH. [probe] = 10 μM, [hNQO1] = 10 μg/mL, [NADH] = 100 μM, the detection wavelength: 435 nm.

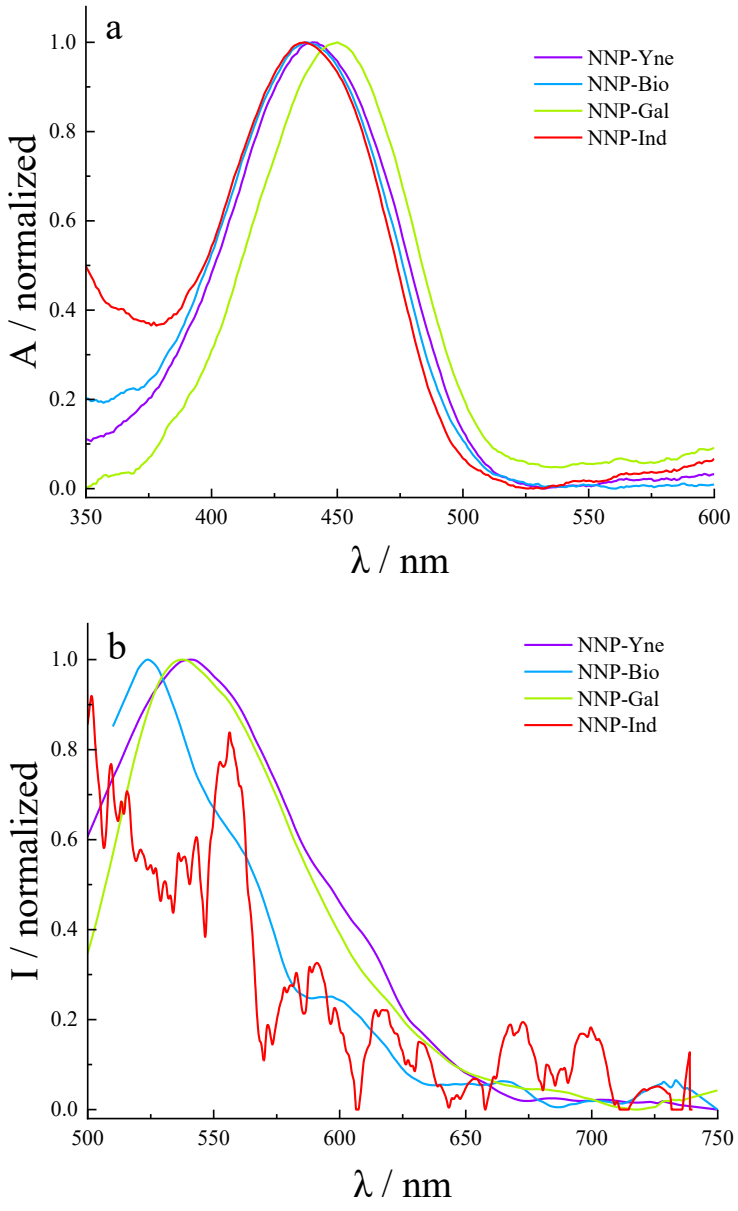


Fig. S7 Normalized absorption (a) and emission (b) spectra of the probes in PBS (100 mM, pH 7.40).

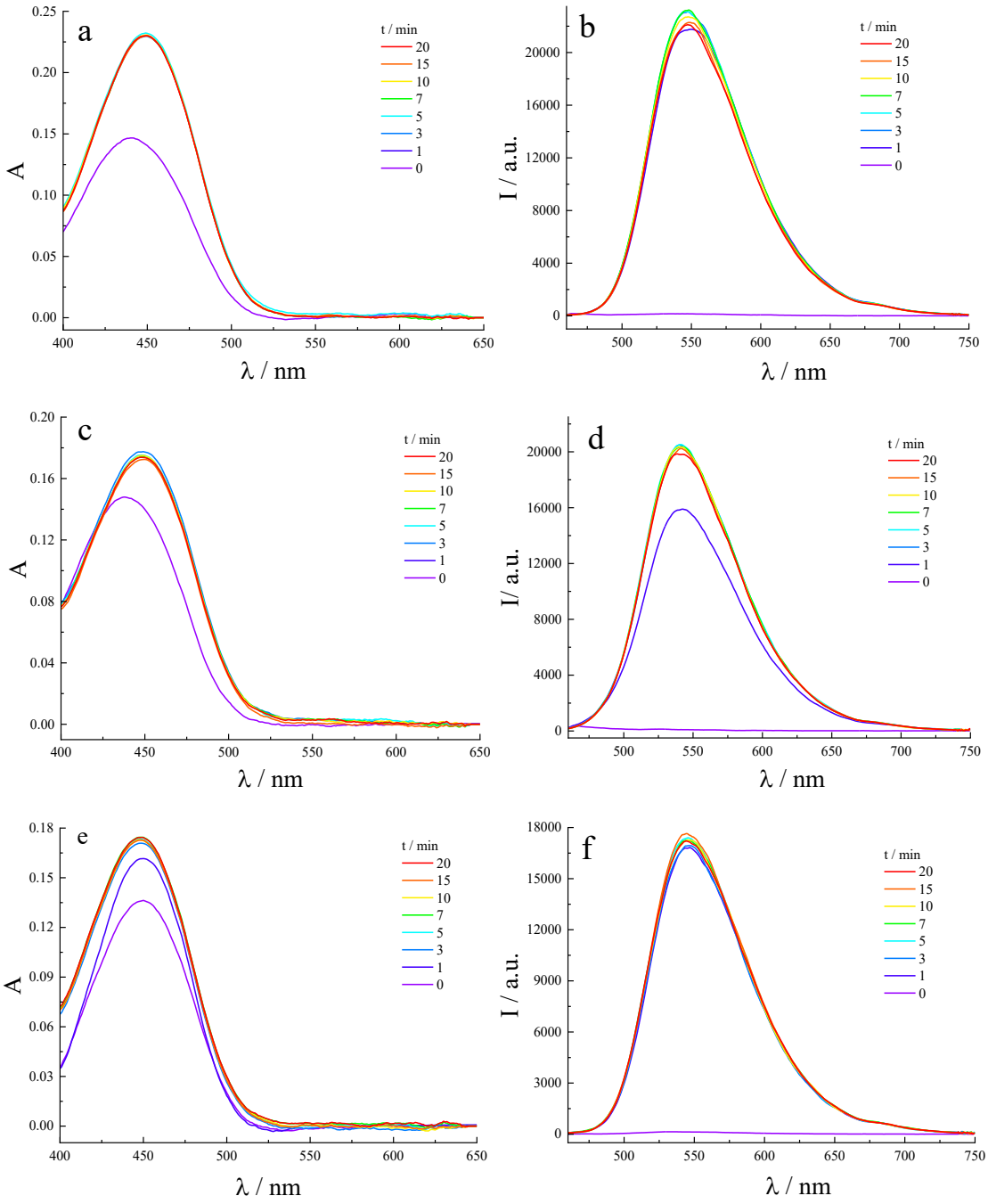


Fig. S8 Time-dependent absorption (a, c, e) and emission (b, d, f) spectra of **NNP-Yne** (a, b), **NNP-Bio** (c, d) and **NNP-Gal** (e, f) mixed with hNQO1 in the presence of NADH. [probes] = 10 μM, [hNQO1] = 10 μg/mL, [NADH] = 100 μM, λ_{ex} = 435 nm.

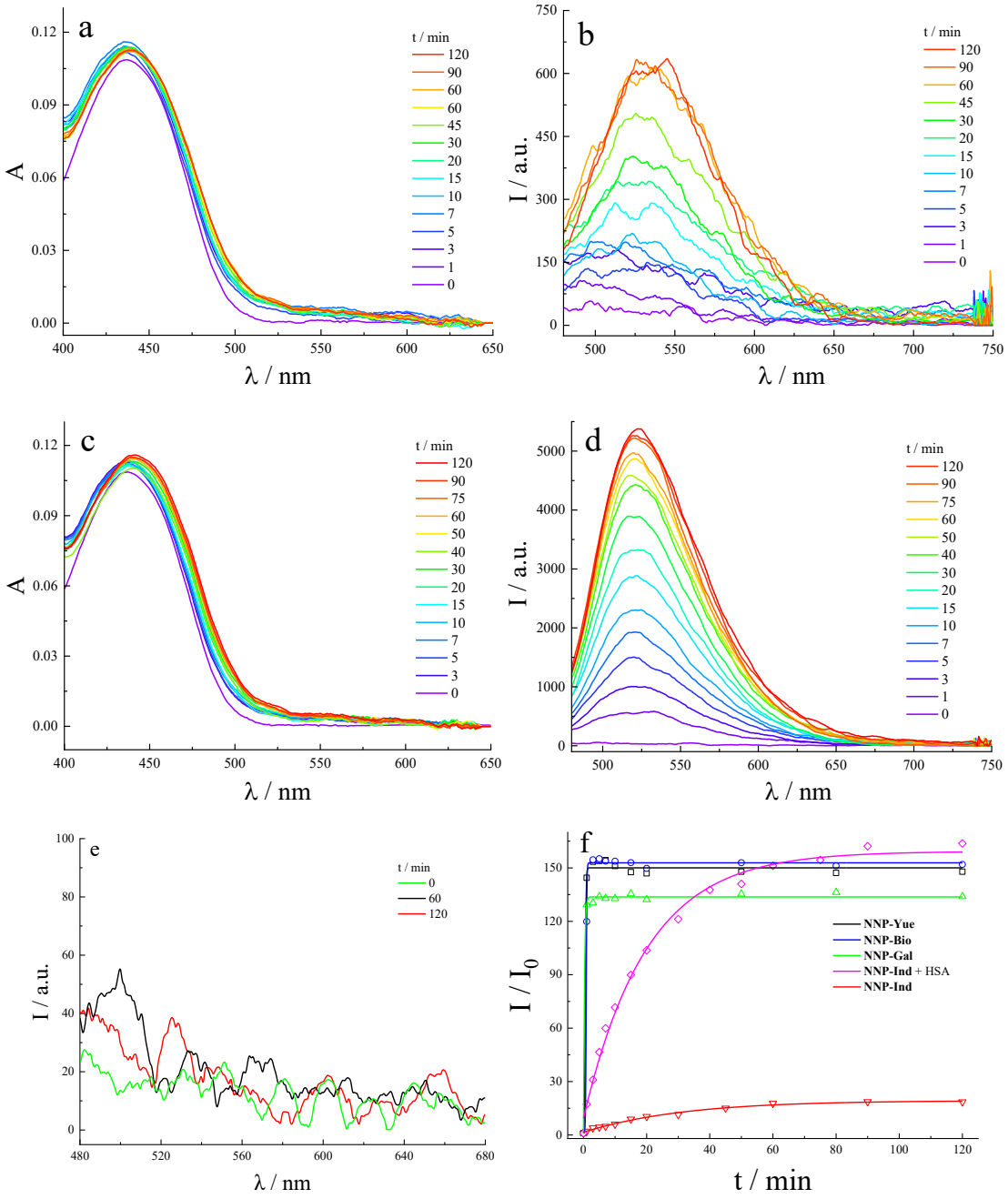


Fig. S9 Time-dependent absorption (a, c) and emission (b, d) spectra of **NNP-Ind** mixed with hNQO1 and NADH in the absence (a, b) or presence (c, d) of HSA; (e) time-dependent emission spectra of **NNP-Ind** mixed with HSA; (f) the fluorescence intensity ratios of different systems as a function of time. [**NNP-Ind**] = 10 μM, [hNQO1] = 10 μg/mL, [NADH] = 100 μM, [HSA] = 1 mg/mL, λ_{ex} = 435 nm, λ_{em} = 540 nm.

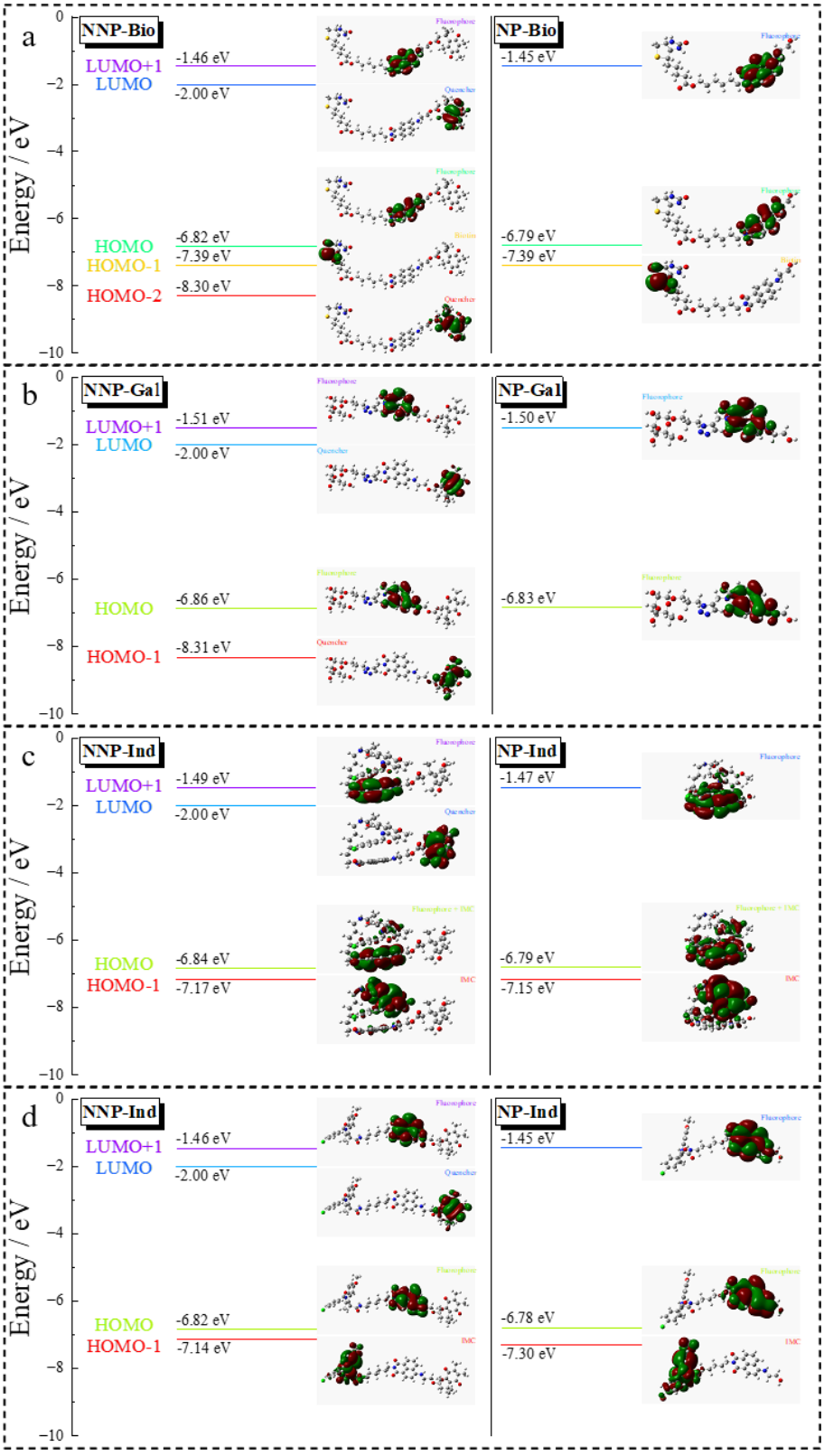


Fig. S10 Frontier molecular orbitals of **NNP-Bio**/**NP-Bio** (a), **NNP-Gal**/**NP-Gal** (b) and **NNP-Ind**/**NP-Ind** (c-d, c: folded conformation and d: unfolded conformation).

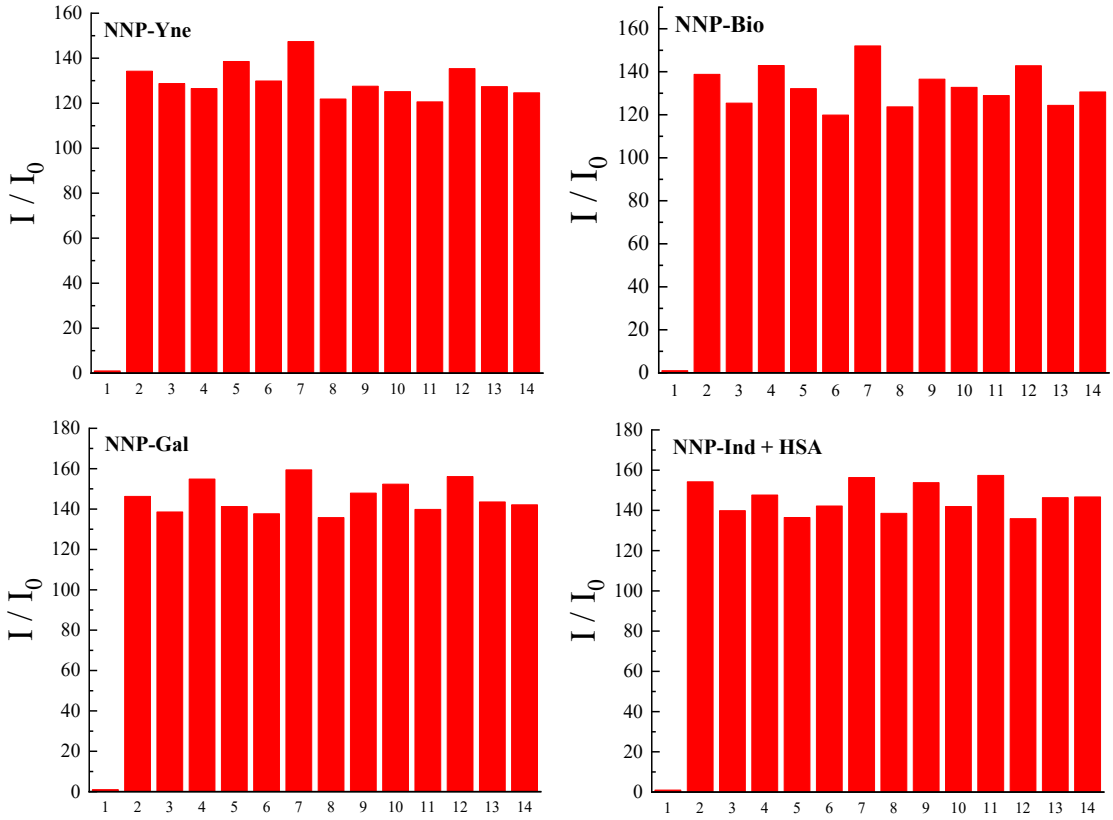


Fig. S11 Effects of some ROSs and common biomolecules on the spectral response of the probes toward hNQO1 in the presence of NADH. (1) none, (2) NaClO + hNQO1, (3) H_2O_2 + hNQO1, (4) $ONOO^-$ + hNQO1, (5) ·OH + hNQO1, (6) $^1O_2$ + hNQO1, (7) carboxylesterase + hNQO1, (8) Cys + hNQO1, (9) Hcy + hNQO1, (10) GSH + hNQO1, (11) OVA + hNQO1, (12) BSA + hNQO1, (13) Pepsin + hNQO1, (14) hNQO1. [probe] = 10 μM, [hNQO1] = 10 μg/mL, [NADH] = 100 μM, [ROS] = 100 μmol/L, [carboxylesterase] = 1 U/mL, [thiol] = 100 μmol/L, [protein] = 1 mg/mL; λ_{ex} = 435 nm, λ_{em} = 540 nm.

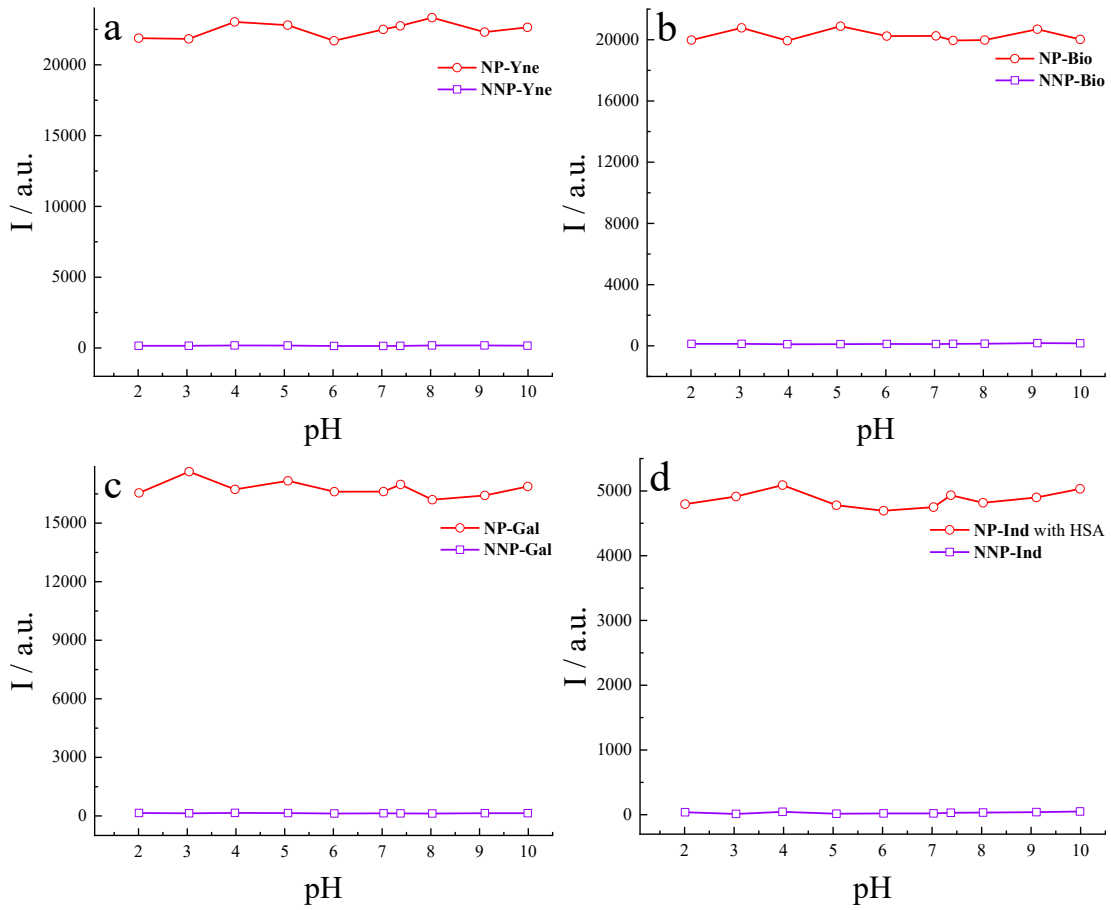


Fig. S12 The fluorescence intensity of **NNP-Yne**, **NP-Yne**, **NNP-Bio**, **NP-Bio**, **NNP-Gal**, **NP-Gal**, **NNP-Ind** and **NP-Ind** (in the presence of 1 mg/mL HSA) as a function of pH. [compound] = 10 μM, λ_{ex} = 435 nm, λ_{em} = 540 nm.

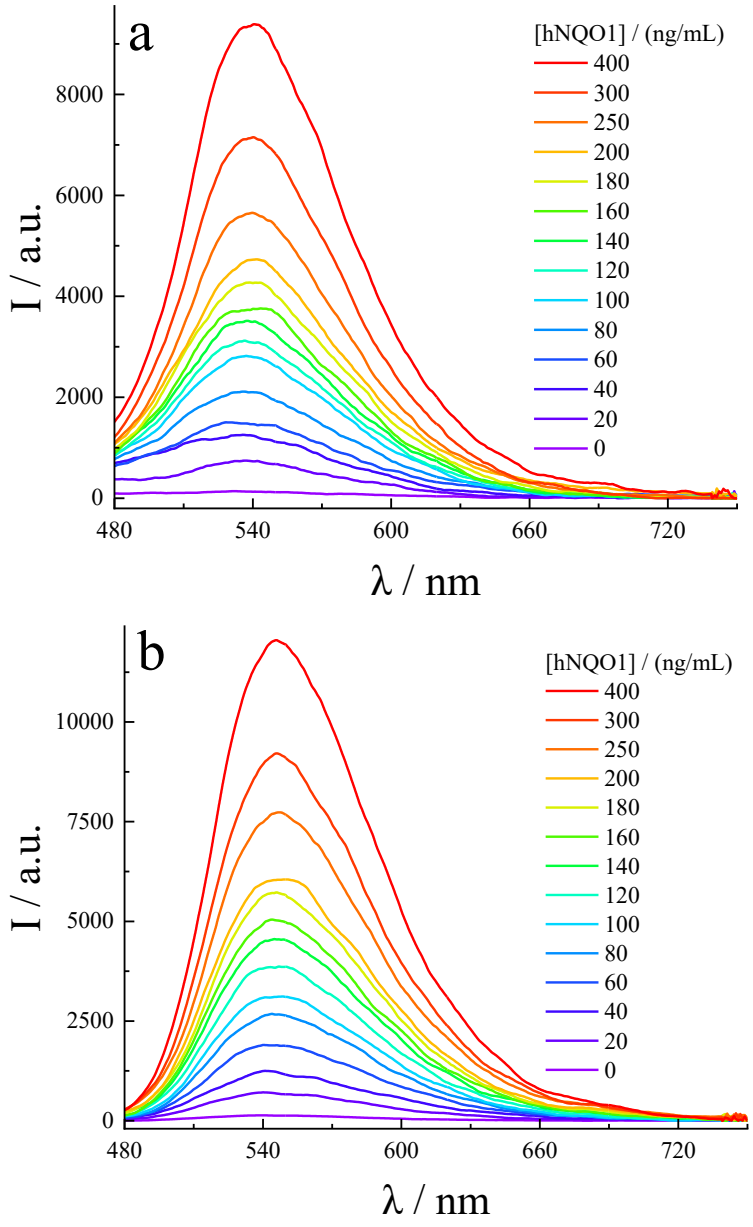


Fig. S13 Effect of hNQO1 concentration on the emission spectra of **NNP-Bio** (a) and **NNP-Gal** (b) in the presence of NADH. [probe] = 10 μM, [NADH] = 100 μM, λ_{ex} = 435 nm.

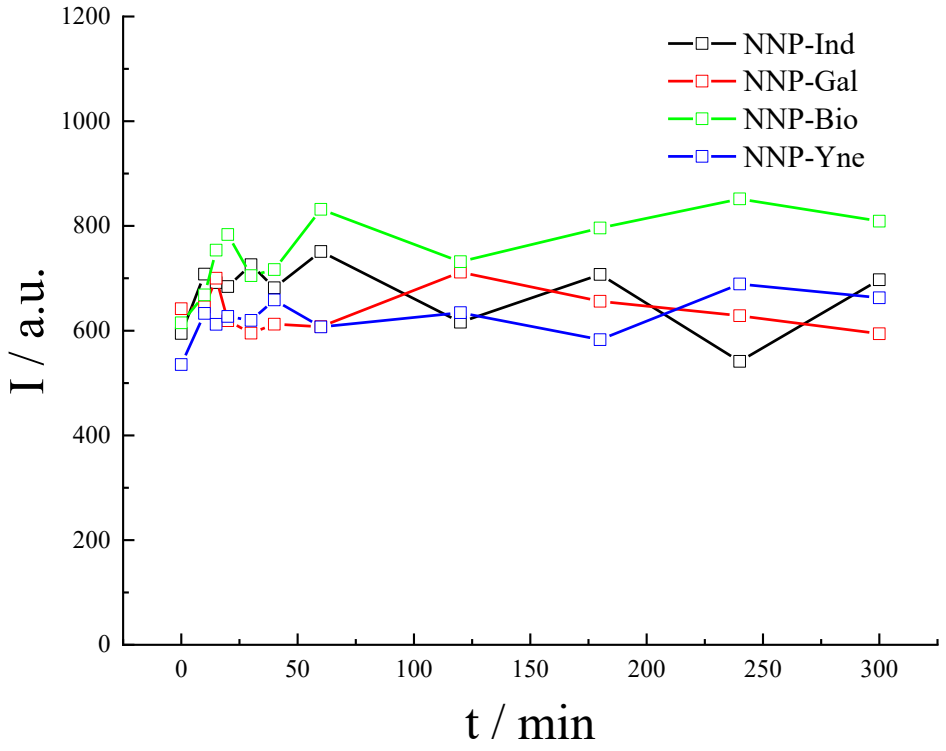


Fig. S14 The fluorescence intensity of **NNP-Yne**, **NNP-Bio**, **NNP-Gal** and **NNP-Ind** in Dulbecco's Modified Eagle Medium as a function of time. [probe] = 10 μM, λ_{ex} = 435 nm, λ_{em} = 540 nm.

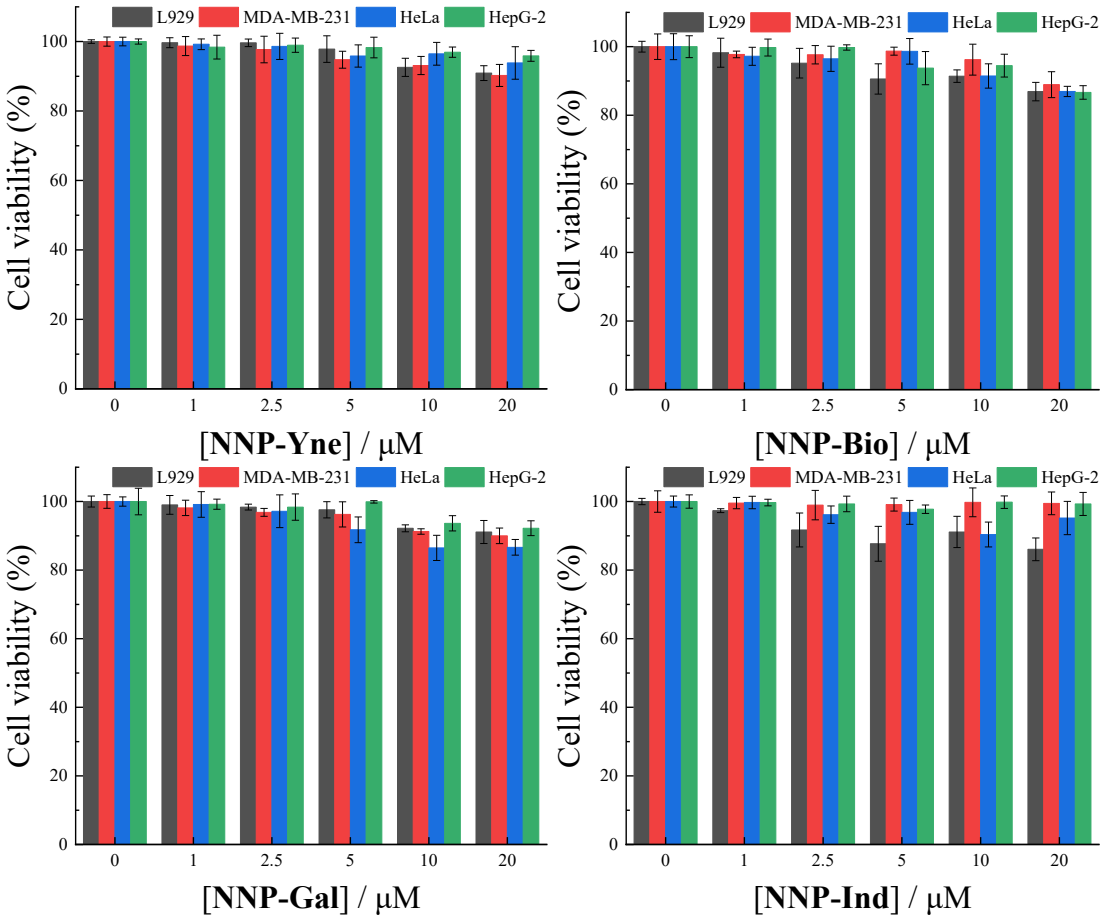


Fig. S15 The cell viability of various cell lines varies with the concentration of **NNP-Yne**, **NNP-Bio**, **NNP-Gal** and **NNP-Ind**.

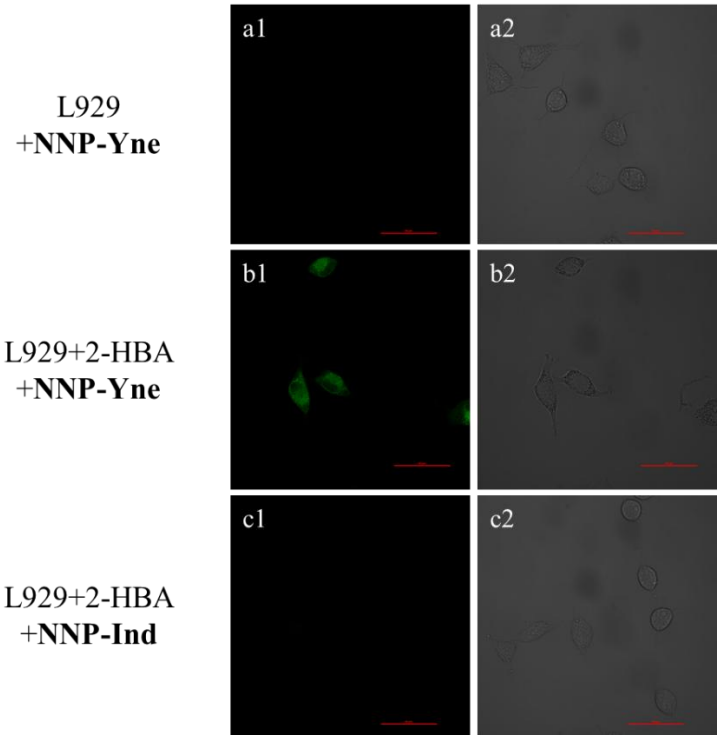


Fig. S16 Confocal fluorescence imaging of L929 cells stained with 10 μM NNP-Yne (a, b)/**NNP-Ind** (c); pre-treated with 10 μM bis (2-hydroxybenzylidene) acetone for 24 h (b-c). (1) green channel; (2) bright field. The fluorescence was collected at 500-550 nm under excitation with a 488 nm laser.

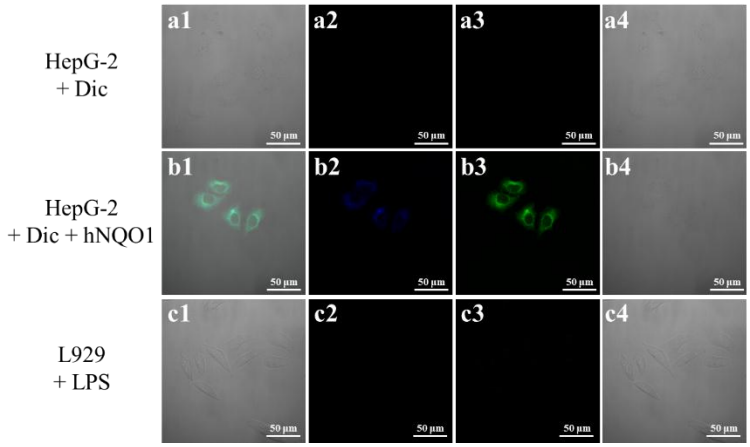


Fig. S17 Confocal fluorescence imaging of HepG-2 (a, b) and L929 (c) cells stained with 10 μM **NNP-Ind**; pre-treated with 500 μM Dic (a), 500 μM Dic and then 1 μg/mL hNQO1 (b), 1 μg/mL LPS (c). (1) Merged of (2), (3) and (4); (2) blue channel; (3) green channel; (4) bright field. For the blue and green channels, fluorescence was collected at 425-475 nm when excited with a 405 nm laser and collected at 500-550 nm under excitation with a 488 nm laser, respectively.

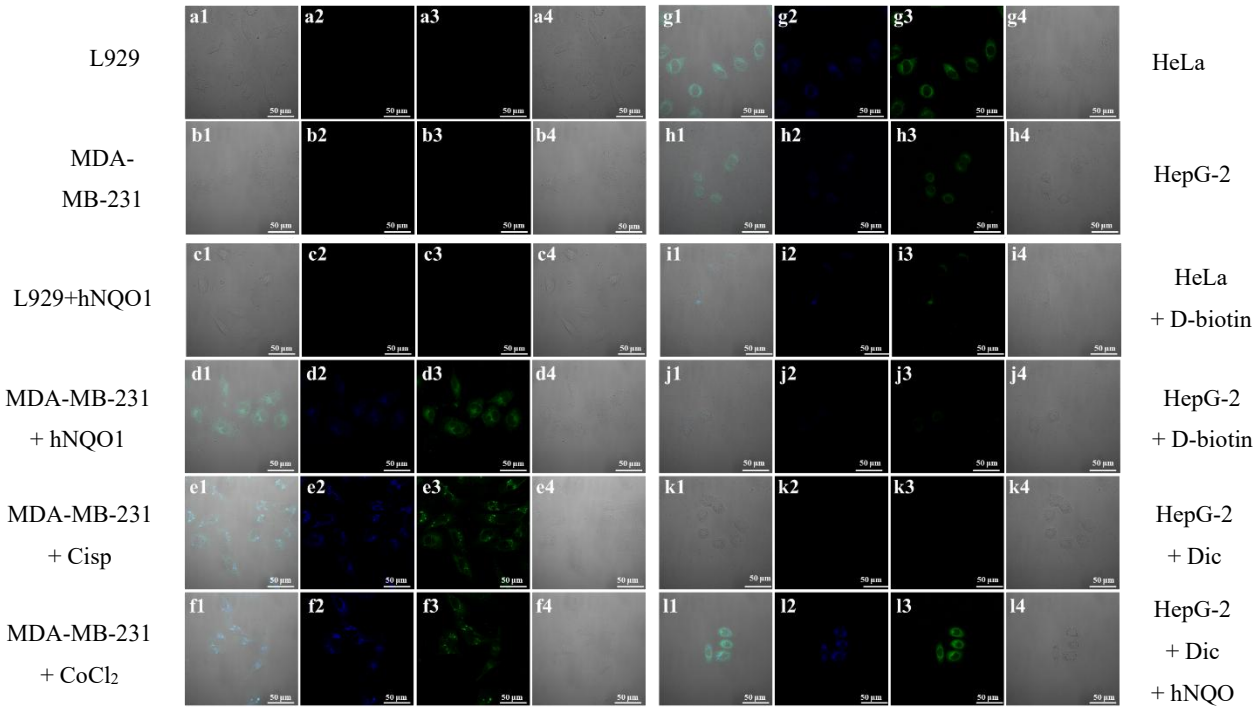


Fig. S18 Confocal fluorescence imaging of L929 (a, c), MDA-MB-231 (b, d-f), HeLa (g, i) and HepG-2 (h, j-l) cells stained with 10 μM **NNP-Bio**; pre-treated with 1 μg/mL hNQO1 (c-d), 20 μM Cisp (e), 1 mM $CoCl_2$ (f), 1 mM D-biotin (i-j), 500 μM Dic (k), 500 μM Dic and then 1 μg/mL hNQO1 (l). (1) Merged of (2), (3) and (4); (2) blue channel; (3) green channel; (4) bright field. For the blue and green channels, fluorescence was collected at 425-475 nm when excited with a 405 nm laser and collected at 500-550 nm under excitation with a 488 nm laser, respectively.

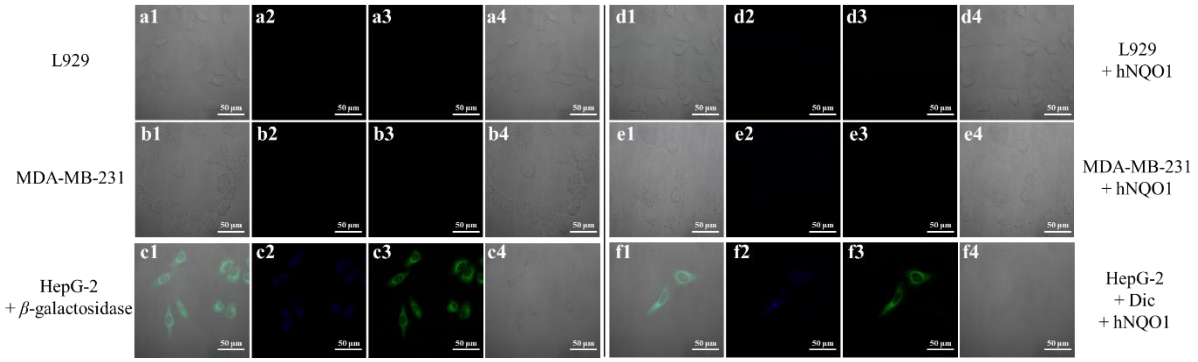


Fig. S19 Confocal fluorescence imaging of L929 (a, d), MDA-MB-231 (b, e) and HepG-2 (b, f) cells stained with 10 μM **NNP-Gal**; pre-treated with 1.0 U *β*-galactosidase (c), 1 μg/mL hNQO1 (d, e), 500 μM dicoumarol and then 1 μg/mL hNQO1 (f). (1) Merged of (2), (3) and (4); (2) blue channel; (3) green channel; (4) bright field. For the blue and green channels, fluorescence was collected at 425-475 nm when excited with a 405 nm laser and collected at 500-550 nm under excitation with a 488 nm laser, respectively.

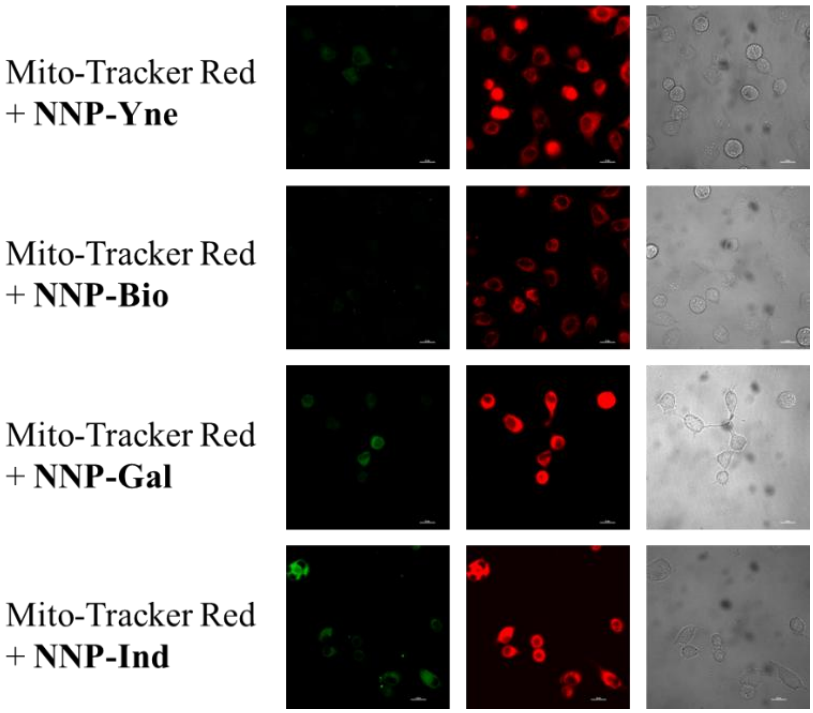


Fig. S20 Confocal fluorescence imaging of the mixed culture cells of normal (L929) and cancer (HepG-2) cell lines co-cultured with Mito-Tracker Red and the probes (green fluorescence). [probe] = 10 μM. For the green and red channels, fluorescence was collected at 500-550 nm when excited with a 488 nm laser and collected at 600-650 nm under excitation with a 561 nm laser, respectively.

Table S1 Comparison of **NNP-Bio**, **NNP-Gal** and **NNP-Ind** with some reported probes

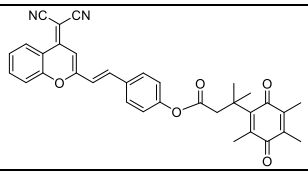
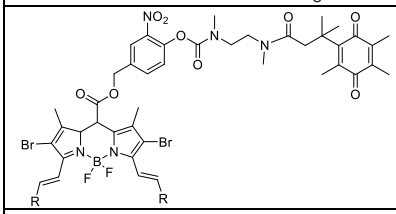
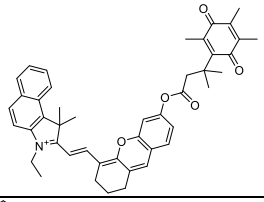
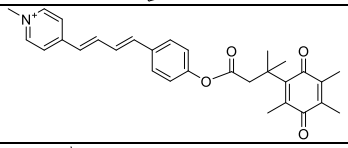
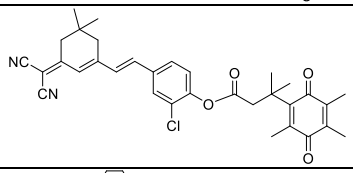
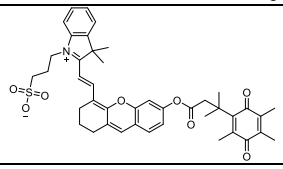
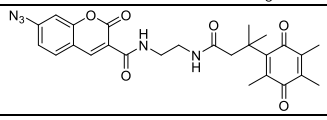
Chemical structure	LOD	Response time	Literature
	nd	2.5 h	[5]
	nd	26 h	[6]
	0.21 μg/mL	6 min	[12]
	54 ng/mL	4 min	[13]
	5.6 ng/mL	80 min	[16]
	4.9 ng/mL	15 min	[31]
	2.49 pg/mL	60 min	[37]
NNP-Gal	5.94 ng/mL	3 min	This work
NNP-Bio	7.88 ng/mL	3 min	
NNP-Ind	nd	60 min	

Table S2 Expressions of the receptor and hNQO1 in different cell lines

		L929	MDA-MB-231	HeLa	HepG-2
receptor	SMVT	-	+	+	+
	ASGP-R	-	-	-	+
	COX-2	-	+	+	+
	hNQO1	-	-	+	+

-: negative; +: positive.

Table S3 Confocal fluorescence imaging results of different cells after incubation with the probes

Cells	Conditions	**NNP-Yne**	**NNP-Bio**	**NNP-Gal**	**NNP-Ind**
L929	/	w	w	w	w
	+ hNQO1	s	w	w	w
	+ LPS	/	/	/	w
MDA-MB-231	/	w	w	w	w
	+ hNQO1	s	s	w	s
	+ Cisp	/	s	/	/
	+ $CoCl_2$	/	s	/	/
HeLa	/	s	s	w	s
	+ β-galactosidase	/	/	s	/
	+ D-biotin	/	w	/	/
	+ celecoxib	/	/	/	w
HepG-2	/	s	s	s	s
	+ β-galactosidase	/	/	s	/
	+ D-biotin	/	w	/	/
	+ celecoxib	/	/	/	w
	+ galactose	/	/	w	/
	+ Dic	w	w	w	w
	+ Dic + hNQO1	s	s	s	s
	+ Dic + APAP	/	/	s	/

“w” and “s” indicate weak and strong fluorescence in living cells, respectively.

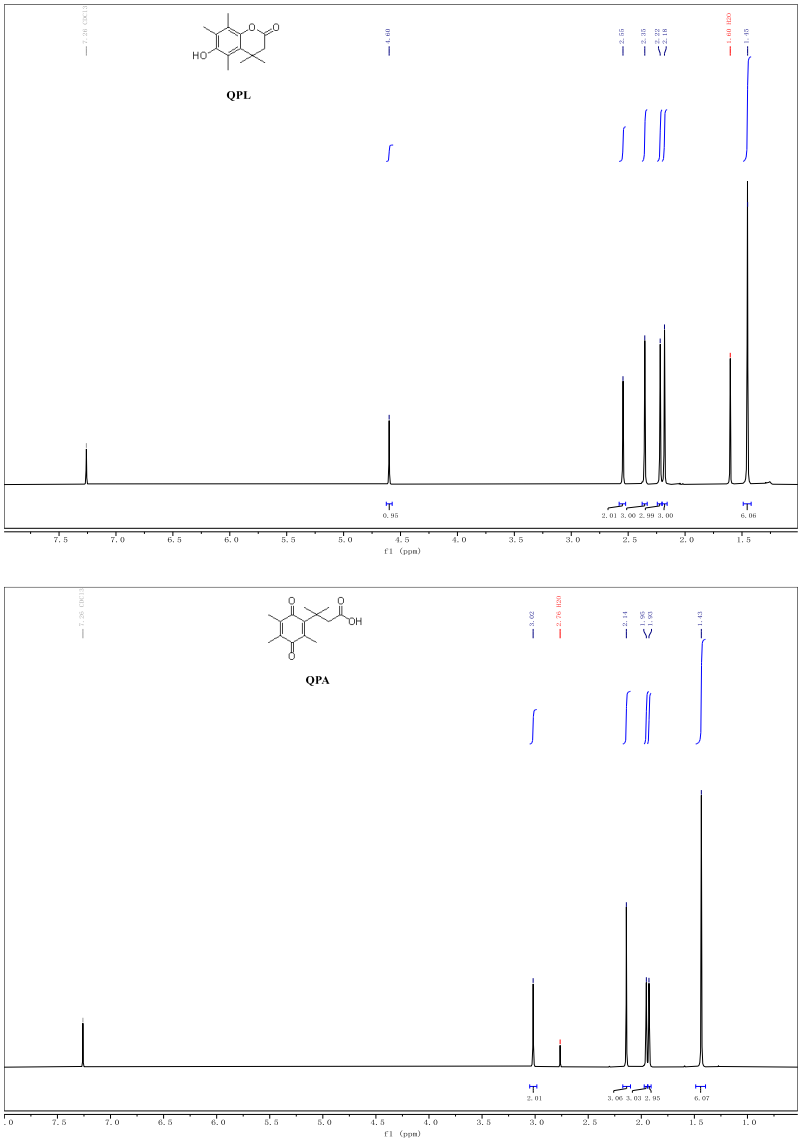


Fig. S18 ^{1}H-NMR spectra of **QPL** and **QPA**.

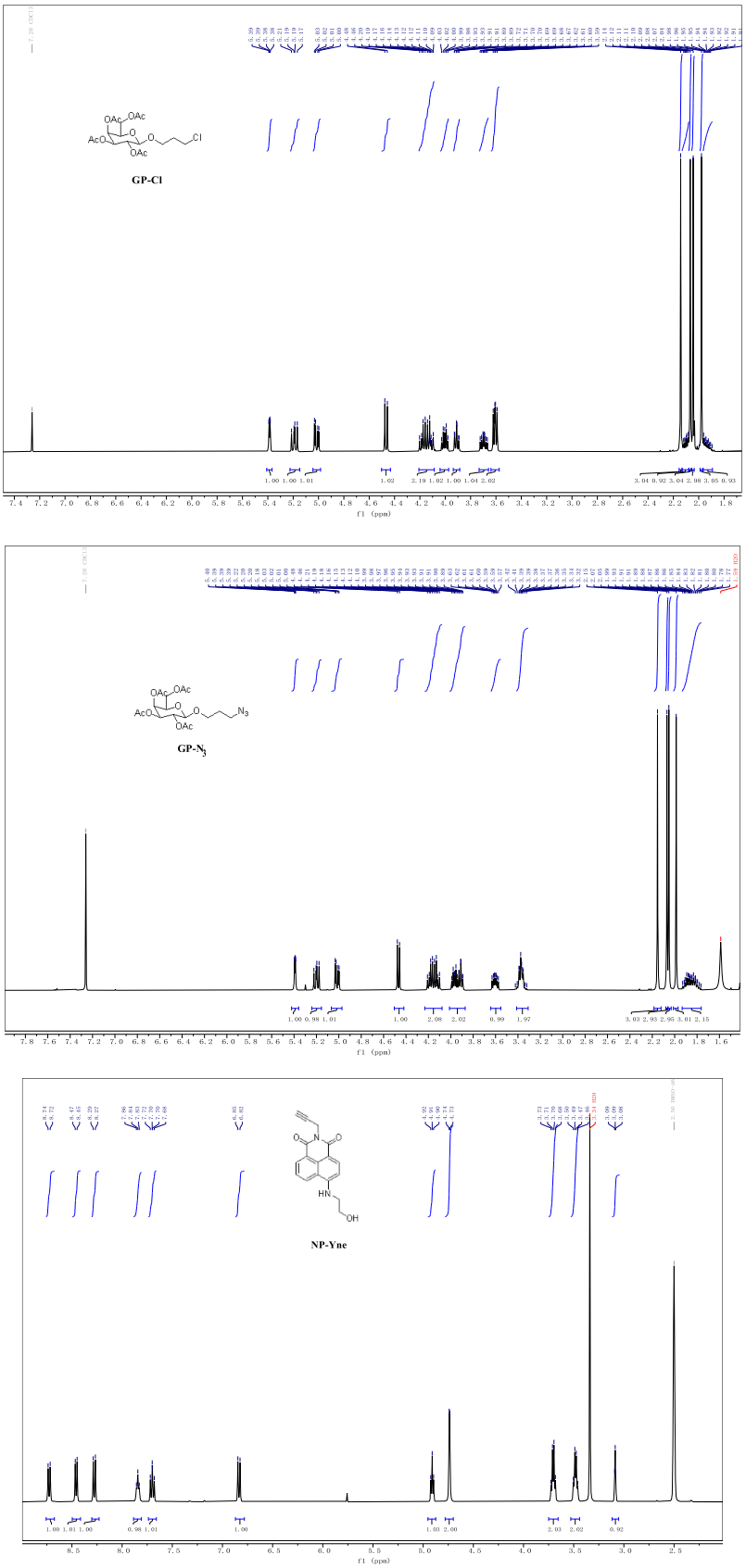


Fig. S19 ^{1}H-NMR spectra of **GP-Cl**, **GP-N$_3$** and **NP-Yne**.

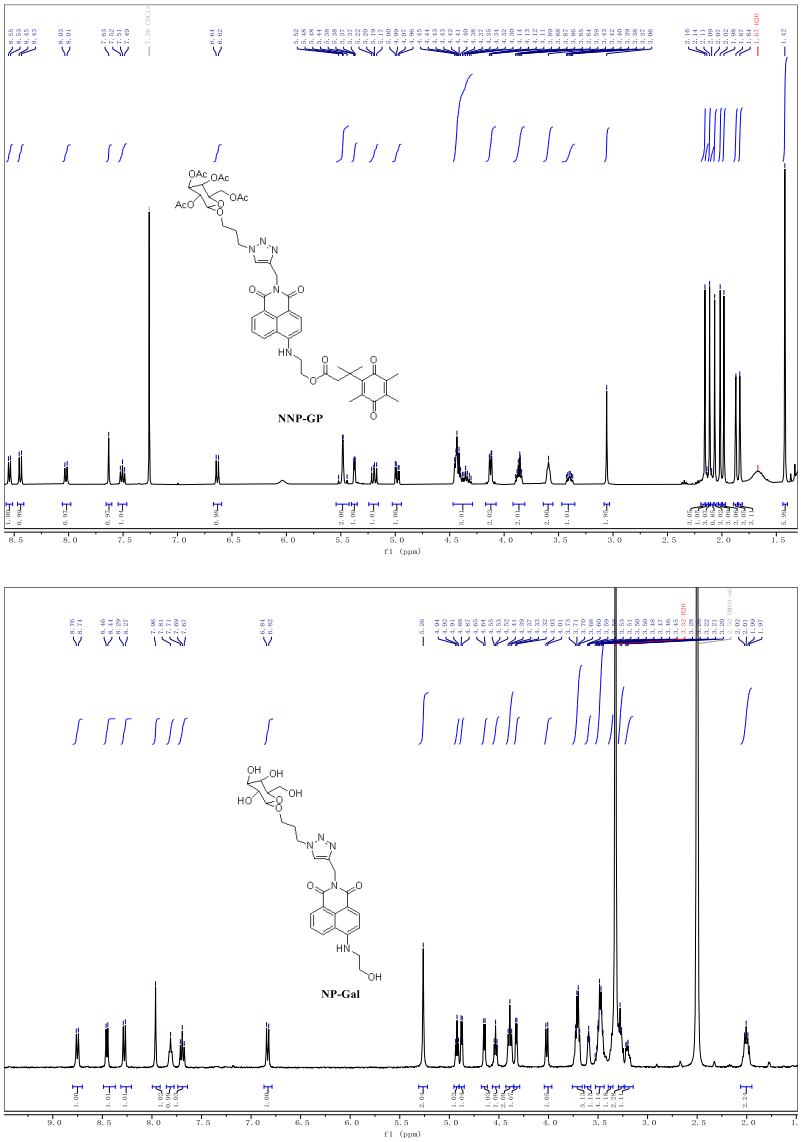


Fig. S20 ^{1}H-NMR spectra of **NNP-GP** and **NP-Gal**.

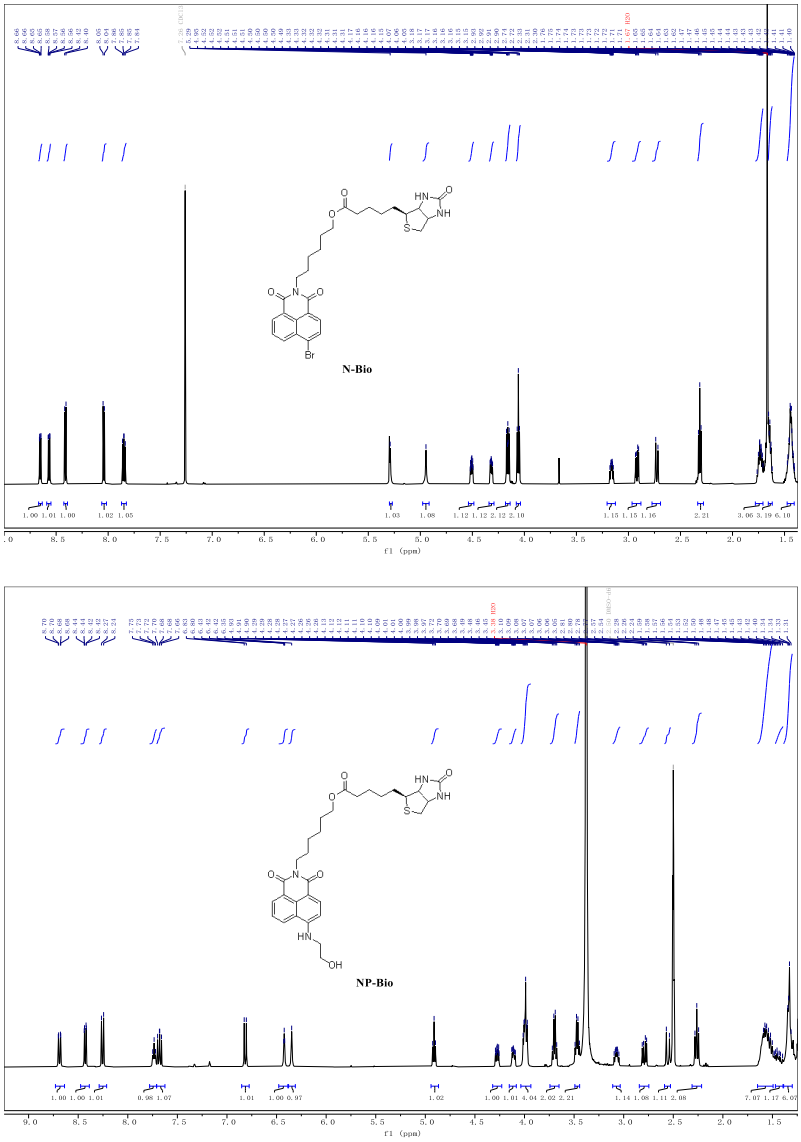


Fig. S21 ^{1}H-NMR spectra of **N-Bio** and **NP-Bio**.

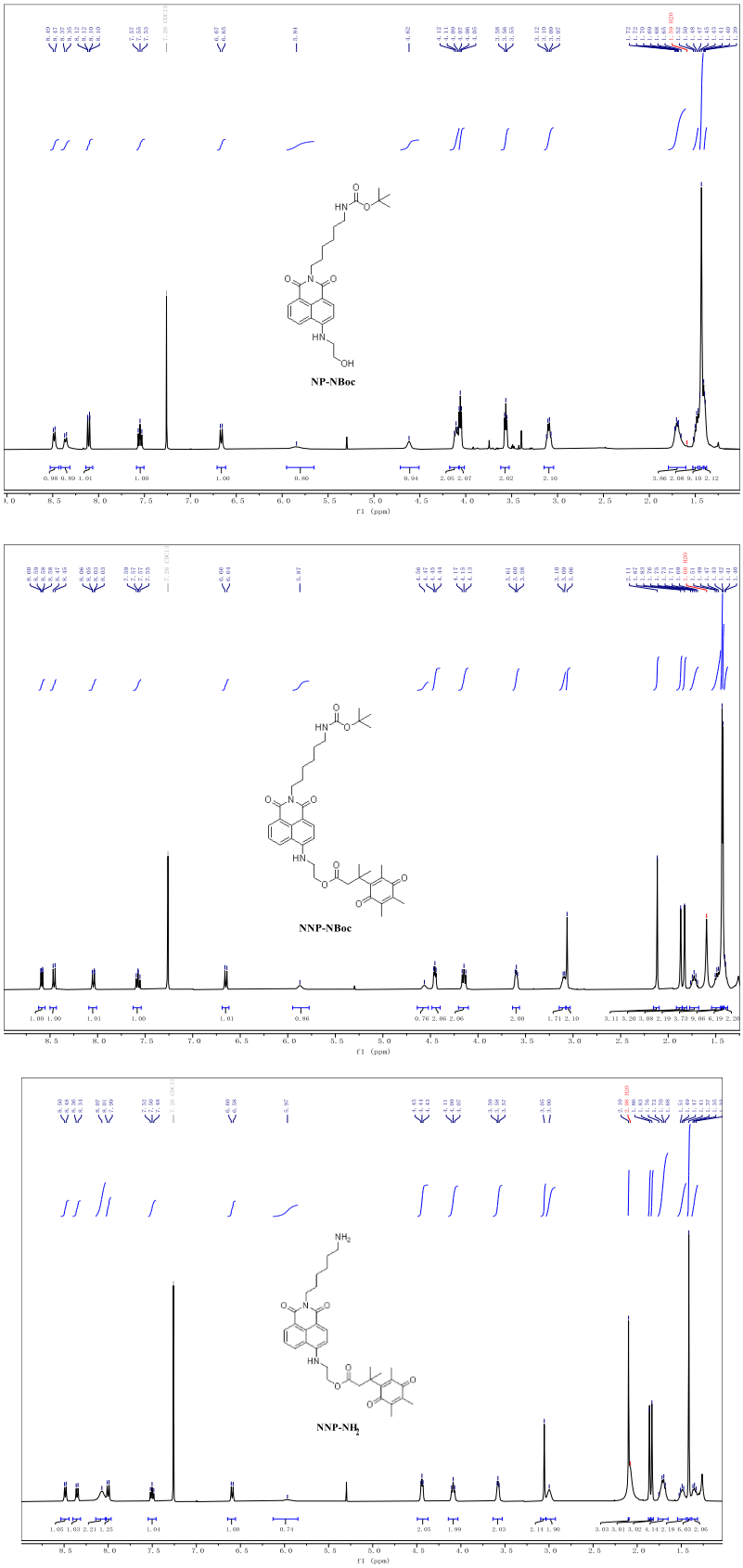


Fig. S22 ^{1}H-NMR spectra of **NP-NBoc**, **NNP-NBoc** and **NNP-NH$_2$**.

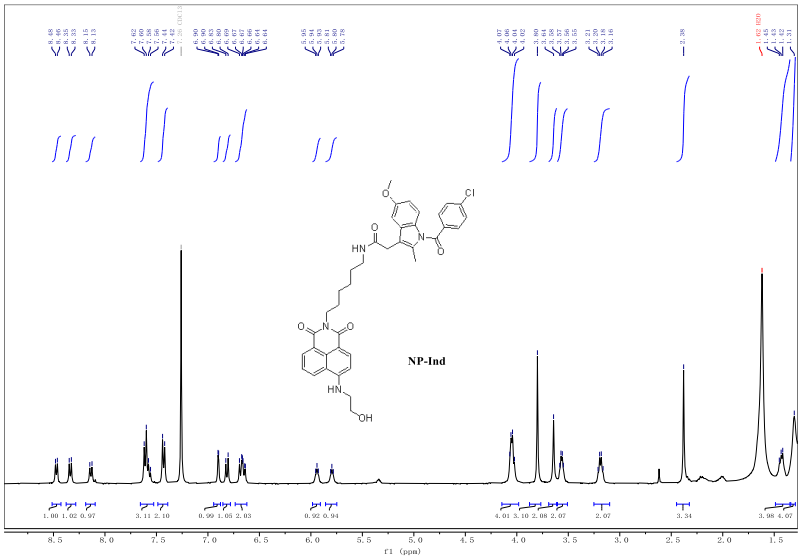


Fig. S23 ^{1}H-NMR spectrum of **NP-Ind**.