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A Hydrophilicity-based Fluorescent Strategy to Differentiate Cysteine/ Homocysteine over Glutathione both *in vivo* and *in vitro*

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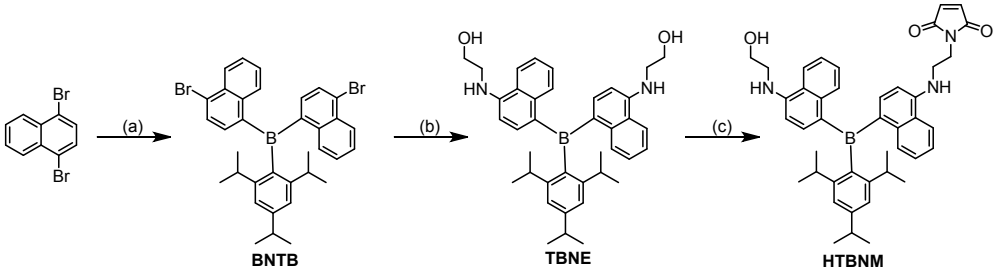
Experimental Procedures and Apparatus

The polyurethane hydrogel (HydroMed D4) was obtained from AdvanSource Biomaterials Corporation.(http://www.advbiomaterials.com/products/hydrophilic/hydromed.html.). Glutathione reduced ethyl ester (GSH-Et) was purchased from Sigma-Aldrich (St. Louis, USA). All other reagents were purchased from J&K CHEM (Beijing, China) and used without further purification. The NIH/3T3 fibroblasts were obtained from China Infrastruture of Cell Line Resources (Beijing Headquarters) for Cell Ordering Service.(http://www.crcpumc.com/). Absorption spectra were recorded on Hitachi UV-3010. Fluorescence spectra were obtained on Hitachi F-7000. Scanning electron microscopy (SEM) was measured on Hitachi S-4800. Dynamic light scattering (DLS) was performed on Zetasizer Nano (ZS90, Malvern). Cells were analyzed using a confocal microscope (OLYMPUS FV 1000-LX81). ^{1}H NMR spectra were obtained on Bruker Avance III 400 HD (400 MHz) spectrometers. MALDI-TOF-MS spectra were measured by Bruker BIFLEX III spectrometer. NIH/3T3 Fibroblasts cells were grown at 37°C under 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM, Gibco; Invitrogen) supplemented with 10% fetal bovine serum.

Experimental Details

1. Synthesis and characterization of the probe



Scheme S1. Synthesis of the Cys/Hcy probe **HTBNM**. Regents and conditions: (a) n-BuLi, TripB(OMe)$_2$, Et$_2$O, -78°C

to room temperature; (b) 2-aminoethanol, $Pd_2(dba)_3$, BINAP, sodium t-butoxide, toluene, reflux; (c) PPh_3, DEAD, maleimide, THF, -78°C to room temperature.

1.1 Synthesis of Compound **BNTB**

bis(4-bromonaphthalen-1-yl)(2,4,6-triisopropylphenyl)borane

2,4,6-triisopropylphenylboronate ($TripB(OMe)_2$) was synthesized according to the reference *Angew. Chem. Int. Ed.* **2011**, 50, 8072-8076.

n-BuLi (15.0 mL of 2.22 M solution in n-hexane) was added to a solution of 1,4-dibromonaphthalene (9.44g, 33mmol) in degassed Et_2O (100mL) at -78°C under argon. The mixture was stirred for 2 h at -78°C and 2 h at room temperature (RT). Then $TripB(OMe)_2$ (4.14g, 15mmol) was added by injection at -78°C. The mixture was stirred for 2 h at -78°C, then recovered to RT and stirred overnight. The reaction solvent was removed by a rotary evaporator, washed with saturated NaCl aqueous solution and extracted with methylene chloride. The organic layer was dried over anhydrous $MgSO_4$ and filtered. The solvent was removed by a rotary evaporator, and the crude product was purified by column chromatography (silica gel, hexane as eluent) to afford **BNTB** (6.15g, 65.5%) as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (d, *J* = 8.0 Hz, 2H), 7.77 (d, *J* = 7.5 Hz, 2H), 7.58 (d, *J* = 5.6 Hz, 2H), 7.46 (m, 4H), 7.09 (m, 2H), 7.01 (s, 2H), 2.94 (m, 1H), 2.65 (m, 2H), 1.31 (d, *J* = 6.8 Hz, 6H), 0.94 (d, *J* = 6.0 Hz, 6H), 0.80 (d, *J* = 6.0 Hz, 6H); MALDI-TOF (m/z) : $[M-H]^-$ calcd for $[C_{35}H_{35}BBr_2-H]^-$ 625.1, found 625.1.

1.2 Synthesis of Compound **TBNE**

2,2'-((((2,4,6-triisopropylphenyl)boranediyl)bis(naphthalene-4,1-diyl))bis(azanediyl))diethanol

BNTB (1.26g, 2mmol), $Pd_2(dba)_3$ (0.20g, 0.2mmol), BINAP (0.26g, 0.40mmol)and sodium t-butoxide (1.20g, 12mmol) were dissolved in degassed toluene (20 mL). 2-amino ethanol (1.22g, 20mmol) with 15 mL toluene was added dropwise in the above solution at 80°C. Then the mixture was stirred for 12 h at 80°C. The reaction solvent was removed by a rotary evaporator, washed with saturated NaCl aqueous solution and extracted with CH_2Cl_2. The organic layer was dried over anhydrous $MgSO_4$ and filtered. The solvent was removed by a rotary evaporator, and the crude product was purified by column chromatography (silica gel, eluent: 25% Ethyl acetate in petroleum ether) to afford **TBNE** (0.68g, 58.1%) as a yellow powder. ^{1}H NMR (300 MHz, CD_2Cl_2) δ 7.86 (d, *J* = 8.7, 2H), 7.77 (d, *J* = 3.3, 2H), 7.55 (m, 2H), 7.31 (m, 2H), 7.00 (m, 4H), 6.69 (m, 2H), 3.99 (t, *J* = 5.1, 4H), 3.51 (m, 4H), 2.93 (m, 1H), 2.76 (m, 2H), 1.31 (d, *J* = 6.9, 6H), 1.00 (d, *J* = 6.6, 6H), 0.77 (d, *J* = 6.6, 6H); MALDI-TOF (m/z) : $[M^+]$ calcd for $[C_{39}H_{47}BN_2O_2]^+$ 586.4, found 586.4.

1.3 Synthesis and Characterization of probe **HTBNM**

1-(2-((4-((4-((2-hydroxyethyl)amino)naphthalen-1-yl)(2,4,6-triisopropylphenyl)boryl)naphthalen-1-yl)amino)ethyl)dimaleimide. Diethyl azodicarboxylate (DEAD, 174mg, 1mmol) was added to a solution of Triphenylphosphane (PPh_3, 262mg, 1mmol) in degassed THF (5mL) at -78°C under argon and stirred for 5 min. Then **TBNE** (586mg, 1mmol) with 5 mL THF was added to above solution at -78°C and stirred for another 5 min. Maleimide (97mg, 1mmol) with 2 mL THF was added to the mixture and stirred for 1 h at -78°C, then recovered to RT and stirred overnight. The reaction solvent was removed by a rotary evaporator, washed with saturated NaCl aqueous solution and extracted with CH_2Cl_2. The organic layer was dried over anhydrous $MgSO_4$ and filtered. The solvent was removed by a rotary evaporator, and the crude product was purified by column chromatography (silica gel, eluent: 30% Ethyl acetate in petroleum ether) to afford **HTBNM** (0.35g, 52.6%) as a yellow powder. ^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.88 (m, 1H), 7.78 (d, *J* = 8.4, 1H), 7.73 (d, *J* = 8.4, 2H), 7.53 (d, *J* = 8.0, 2H), 7.31 (t, *J* =

7.2, 2H), 6.99 (m, 4H), 6.75 (s, 2H), 6.70 (m, 1H), 6.53 (d, *J* = 7.6, 1H), 4.00 (t, *J* = 4.4, 4H), 3.51 (t, *J* = 5.2, 4H), 2.93 (m, 1H), 2.76 (m, 2H), 1.31 (d, *J* = 6.8, 6H), 1.00 (m, 6H), 0.76 (m, 6H); MALDI-TOF (m/z) : $[M+Na]^+$calcd for $[C_{43}H_{48}BN_3O_3+Na]^+$ 688.4, found 688.3. HR-MALDI (m/z) : $[M]^+$ 665.3790, found 665.3787.

2. Preparation of the composite nanogel

2.1 Preparation of **TBNE/PU** nanogel

TBNE (0.59 mg, 1μmol) was dissolved in 100mL of a 160 ppm solution of the polyurethane hydrogel (PU) in an ethanol/water (9:1, v/v) mixture. The remaining preparation process was identical as HTBNM/PU.

2.2 Preparation of **HTBNM/PU** nanogel

HTBNM (0.67 mg, 1μmol) was dissolved in 100mL of a 160 ppm solution of the polyurethane hydrogel (PU) in an ethanol/water (9:1, v/v) mixture. The mixture was stirred for 2 h, then dialyzed against distilled water for 24 h, with an interval of 2-3 h to exchange the water. Finally, the aqueous dispersion of nanogel was filtered through a 0.22 μm filter to remove large aggregates. Then the resultant suspension was quantified by measuring the absorption intensity of HTBNM. The weight ratio of HTBNM and PU is 0.13 percent calculated by the absorption method.

3. Appearance and size distribution of HTBNM/PU

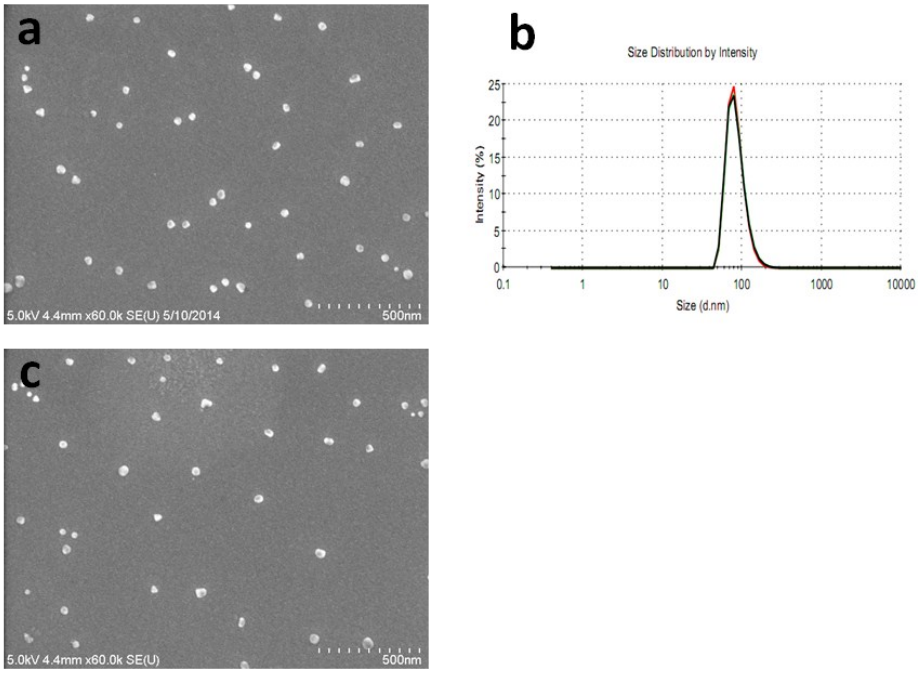


Figure S1. (a) Scan electron microscopy image of HTBNM/PU prepared by freeze-drying method. (b) Size distribution by intensity of fresh HTBNM/PU (black), HTBNM/PU standing after 24h (green) and HTBNM/PU added Cys (1mM) (red) in phosphate buffer (10mM, pH 7.4) measured via dynamic light scattering at 37°C. (c) Scan electron microscopy image of HTBNM/PU upon addition of Cys (1mM).

4. The selectivity of HTBNM/PU for detection Cys/Hcy

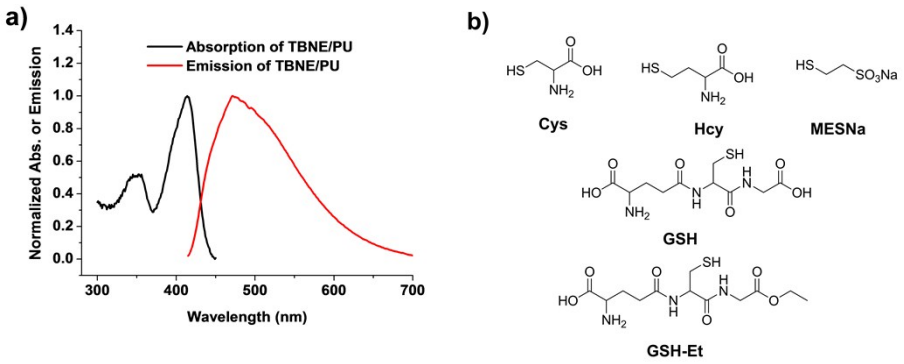


Figure S2. (a) Normalized absorption and emission spectra of TBNE/PU (2.0 μM) in phosphate buffer (10mM, pH 7.4) at 37°C. (b) The structure of tested thiols (Cys, Hcy, MESNa, GSH and GSH-Et).

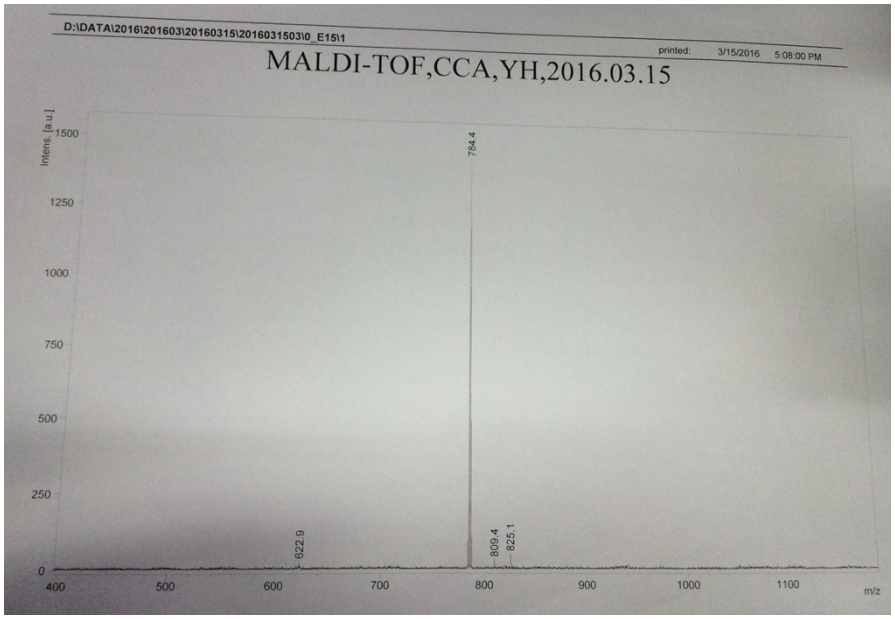


Figure S3. MALDI-TOF MS of HTBNM added Cys.

Table S1. Quantum yield Φ of TBNE/PU and HTBNM/PUwith the addition of thiols

	TBNE/PU	HTBNM/PU	HTBNM/PU+Cys	HTBNM/PU+Hcy	HTBNM/PU+GSH
Φ	0.443	0.001	0.208	0.183	0.007

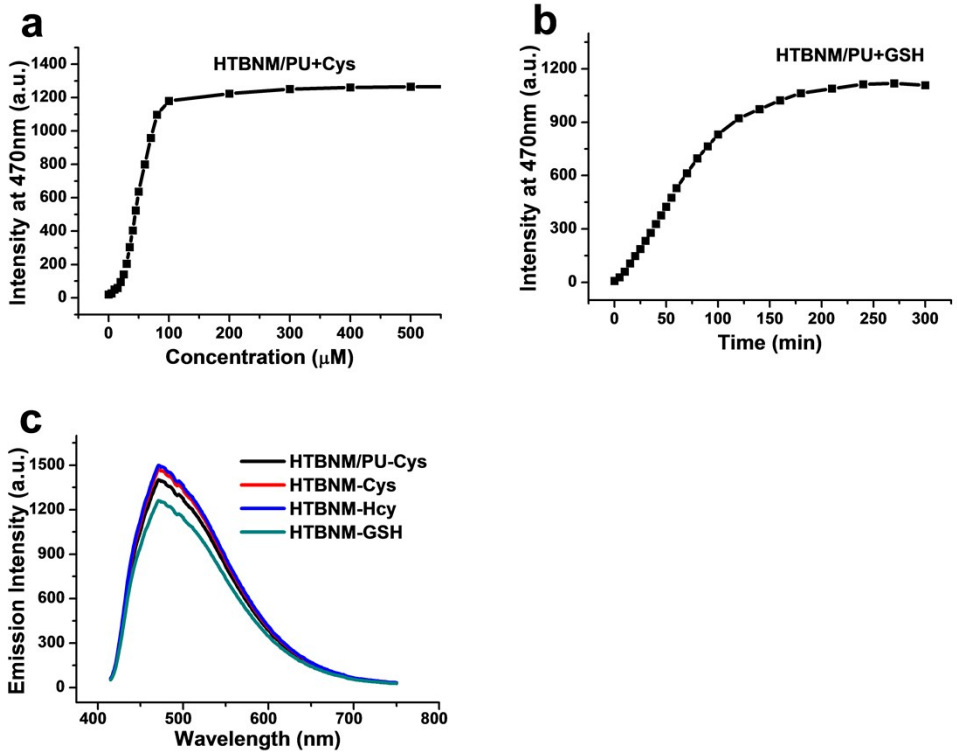


Figure S4. (a) Emission intensity changes at 470 nm of HTBNM/PU(2.0 μM) upon addition of Cys (0 to 500 μM) in phosphate buffer (10mM, pH 7.4) at 37°C. (b) Emission intensity at 470 nm of HTBNM/PU(2.0 μM) towards GSH (1.0 mM) in phosphate buffer (10mM, pH 7.4) at 37°C. (c) Fluorescence emission spectra of probe HTBNM (2.0 μM) before and after addition of biothiols (1.0 mM Cys, Hcy, GSH) in phosphate buffer (10mM, pH 7.4, with 5% DMSO) at 37°C in 10 min.

5. Polarity in PU nanogel

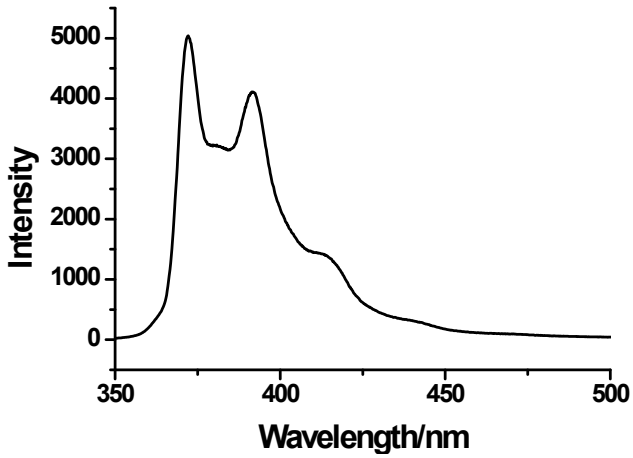


Figure S5. The luminescence spectra of pyrene/PU.

6. MTT cell viability assay

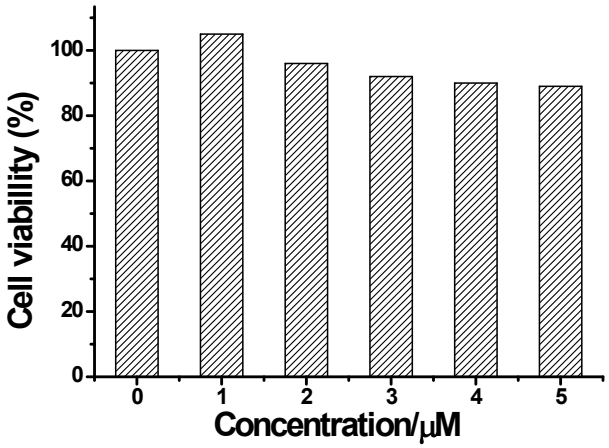


Figure S6. Effects of HTBNM/PU at varied concentrations on the viability of NIH/3T3 fibroblasts cells.

7. Photostability of the probe HTBNM/PU

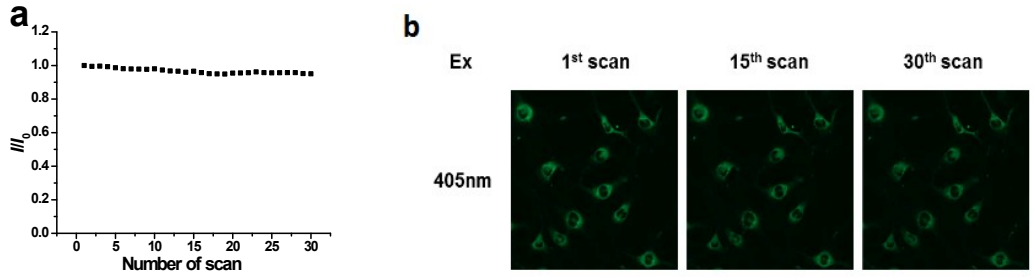


Figure S7. (a) Signal loss of fluorescence emission of HTBNM/PU with increasing number of scans: intensity loss with excitation wavelength at 405 nm; The emission intensity was collected at 480-530 nm, and the time interval was 10s. (b) Fluorescent images of living NIH/3T3 cells stained with HTBNM/ at 1st, 15th, and 30th scans upon excitation wavelength at 405 nm. The emission images were collected at 480-530 nm.

8. The responses of HTBNM/PU for NaSH

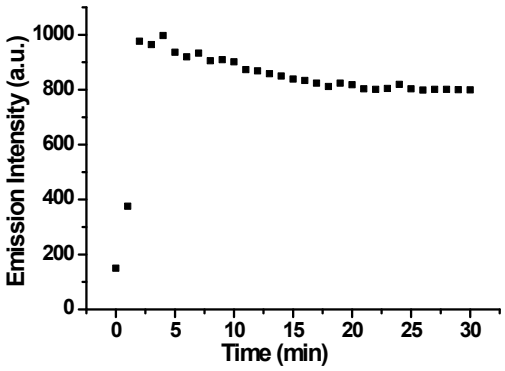


Figure S8. Emission intensity at 470 nm of HTBNM/PU (2.0 μM) towards NaSH (0.5 mM) in phosphate buffer (10mM, pH 7.4) at 37°C.