Supporting information for:

Thiol-reactive amphiphilic block copolymer for coating gold nanoparticles with neutral and functionable surfaces

Hongwei Chen[1,*], Hao Zou[1,2], Hayley J. Paholak[1], Masayuki Ito[3], Wei Qian[3], Yong Che[3], and Duxin Sun[1,*]

[1]Department of Pharmaceutical Sciences, College of Pharmacy, University of Michigan, Ann Arbor, Michigan 48109, [2]Department of Pharmaceutical Science, College of Pharmacy, Second Military Medical University, 325 Guo He Road, Shanghai 200433, PR China, [3]IMRA America, Inc. 1044 Woodridge Avenue, Ann Arbor, Michigan 48105, Email: duxins@umich.edu (D. Sun); hongweic@umich.edu (H. Chen)

Synthesis of Hydroxyethylpyridyl Disulfide (Compound 1)[1]

Aldrithiol-2, (15 g, 0.068 mol) was dissolved in 75 mL of methanol. 1 mL of glacial acetic acid was then added. To this mixture, a solution of mercaptoethanol (2.65 g, 0.034 mol) in 25 mL of methanol was added drop-wise at room temperature over 0.5 h under continuous stirring. Once the addition was complete, the reaction mixture was stirred at room temperature overnight. The stirring was stopped and the solvent was evaporated to obtain the crude product as yellow oil. The crude product was then purified by column chromatography using silica gel as the stationary phase (silica gel 60 Å, 230-400 mesh) and a mixture of ethyl acetate/hexane as the eluent. The purification was monitored by TLC. The excess aldrithiol came out first at 15% ethyl acetate/hexane, then the polarity of the eluent was increased to 40% ethyl acetate/hexane to collect the desired product as pale yellow oil. Yield: 77%. ^{1}H NMR (Fig. S1): ($CDCl_3$, 400 MHz), δ (ppm): 8.50 (m, 1H, aromatic proton *ortho*-N), 7.59 (m, 1H, aromatic proton *meta*-N), 7.42 (m,

1H, aromatic proton *para*-N), 7.15 (m, 1H, aromatic proton, *ortho*-disulfide linkage), 5.61 (b, 1H, $HOCH_2CH_2$-S-S), 3.80 (t, 2H, -S-S-CH_2CH_2OH), 2.95 (t, 2H, -S-S-CH_2CH_2OH).

Synthesis of Pyridyldisulfide Ethymethacrylate (PDSM)

To a solution of Compound 1 (4.88 g, 26.0 mmol) in 20 mL of dry dichloromethane, 3.95 g (39.0 mmol) of triethylamine was added and the mixture was cooled in an ice bath. To this cold mixture, a solution of methacryloyl chloride (4.08 g, 39.0 mmol) in 10 mL of dry dichloromethane was added drop-wise with continuous stirring. After completion of the addition in about 0.5 hour, the mixture was stirred at room temperature for six hours in an ice bath. The stirring was stopped and the solid was removed by filtration. The filtrate was washed with 3×30 mL distilled water and then 30 mL brine. The organic layer was collected, dried over anhydrous $MgSO_4$ and concentrated by rotary evaporation at room temperature to obtain the crude product as pale yellow oil. It was then purified by column chromatography using silica gel as the stationary phase and mixture of ethyl acetate/hexane as the eluent. The purification was monitored by TLC. The pure product was collected at 25% ethyl acetate/hexane. Yield: 82%. ^{1}H NMR (Fig. S2): ($CDCl_3$, 400 MHz), δ (ppm): 8.44 (m, 1H, aromatic proton *ortho*-N), 7.67 (m, 2H, aromatic proton *meta*-N and *para*-N), 7.09 (m, 1H, aromatic proton, orthodisulfide linkage), 6.01 (d, 1H, vinylic proton, *cis*-ester), 5.56 (d, 1H, vinylic proton, *trans*-ester) 4.38 (t, 2H, -S-S-CH_2CH_2O-), 3.08 (t, 2H, -S-S-CH_2CH_2O-), 1.92 (s, 3H, methyl proton of the methacryloyl group).

Synthesis of Dithiobenzoic Acid (DTBA)[2]

To a thoroughly dried 500 mL, three-necked round-bottomed flask equipped with a magnetic stir bar, addition funnel (250.0 mL), thermometer, and rubber septum for liquid transfers, sodium methoxide (25% solution in methanol, 108 g, 0.5 mol) was added. Anhydrous methanol (125 g)

was added to the flask, followed by rapid addition of elemental sulfur (16.0 g, 0.5 mol). Benzyl chloride (31.5 g, 0.25 mol) was then added drop-wise via the addition funnel over a period of 1.0 h, at room temperature under a dry nitrogen atmosphere. The reaction mixture was heated to reflux in an oil bath for 10 h. After this time, the reaction mixture was cooled to 7 °C using an ice bath. The precipitated salt was removed by filtration and the solvent removed *in vacuo*. To the residue, deionized water (250 mL) was added. The solution was then transferred to a 2.0 L separatory funnel. The crude sodium dithiobenzoate solution was washed with diethyl ether (3×100 mL). Diethyl ether (100 mL) and 1.0 N HCl (250 mL) were added, and dithiobenzoic acid was extracted into the ethereal layer. Deionized water (250 mL) and 1.0 N NaOH (300 mL) were added, and sodium dithiobenzoate was extracted to the aqueous layer. This washing process was repeated one more time to finally yield a solution of sodium dithiobenzoate.

Synthesis of Di(thiobenzoyl) Disulfide

Potassium ferricyanide (III) (32.93 g, 0.1 mol) was dissolved in deionized water (500 mL). The sodium dithiobenzoate solution (350 mL) was transferred to a 1.0 L conical flask equipped with a magnetic stir bar. The potassium ferricyanide solution was added drop-wise to the sodium dithiobenzoate via an addition funnel over a period of 1.0 h under vigorous stirring. The red precipitate was filtered and washed with deionized water until the washings became colorless. The solid was dried *in vacuo* at room temperature overnight.

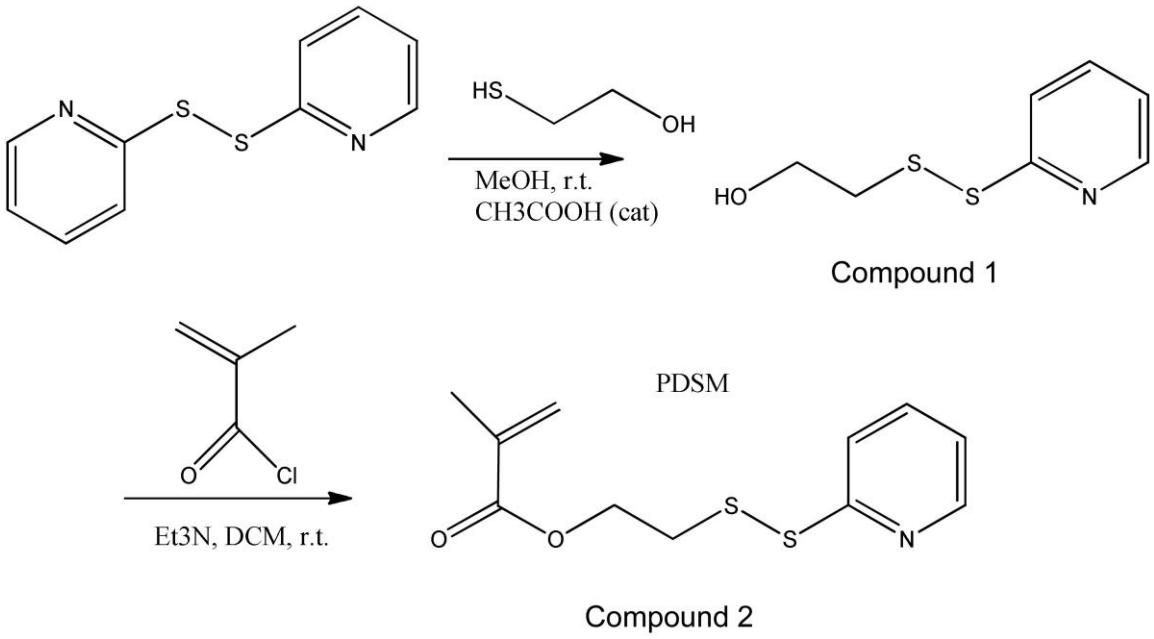
Synthesis of 4-Cyanopentanoic Acid Dithiobenzoate (CPAD)

To a 250 mL round-bottomed flask, anhydrous ethyl acetate (80.0 mL) was added. To the flask dry 4,4′-azobis(4- cyanopentanoic acid) (5.84 g, 21.0 mmol) and di(thiobenzoyl) disulfide (4.25 g, 14.0 mmol) were added. The reaction solution was heated at reflux for 18 h and then the ethyl acetate was removed *in vacuo*. The crude product was isolated by column chromatography using

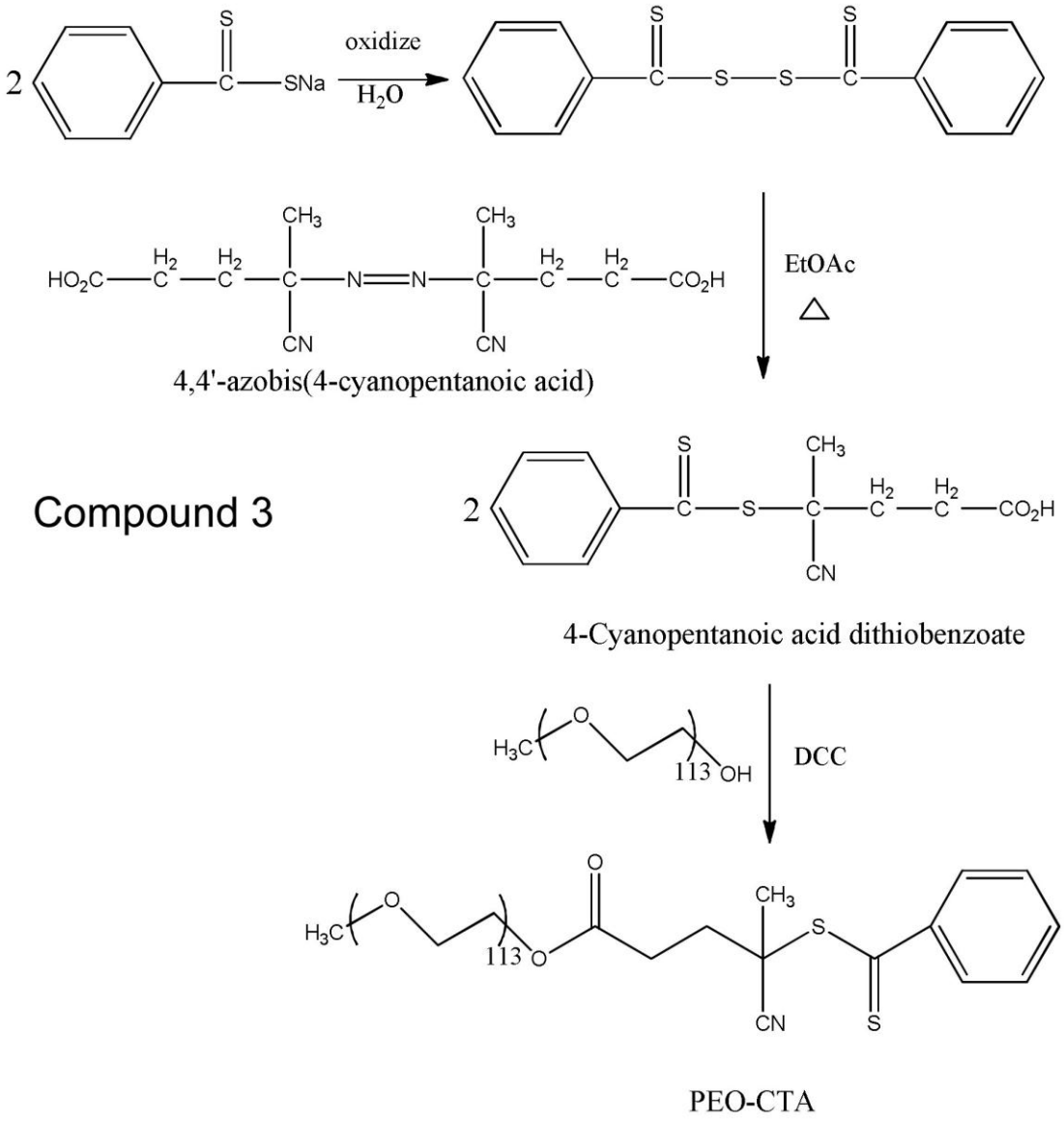
ethyl acetate/hexane (2/3) as eluent. Monitoring by TLC, fractions with only one band and were red in color were combined and dried over anhydrous sodium sulfate overnight. The solvent mixture was removed *in vacuo*, whereupon it crystallized. The target compound was recrystallized from benzene. Yield: 66%. ^{1}H NMR (Fig. S3): ($CDCl_3$, 400 MHz), δ (ppm): 7.4–8.0 (aromatic protons labeled with 1, 2, and 3), 2.5–3.0 (methylene protons labeled with 4, and 5), 2.0 (methyl protons labeled with 6). ^{13}C NMR (Fig. S4): ($CDCl_3$, 400 MHz) further confirmed the structure as the peaks are assigned and labeled in the spectrum.

Synthesis of PEO Macro-RAFT Agent

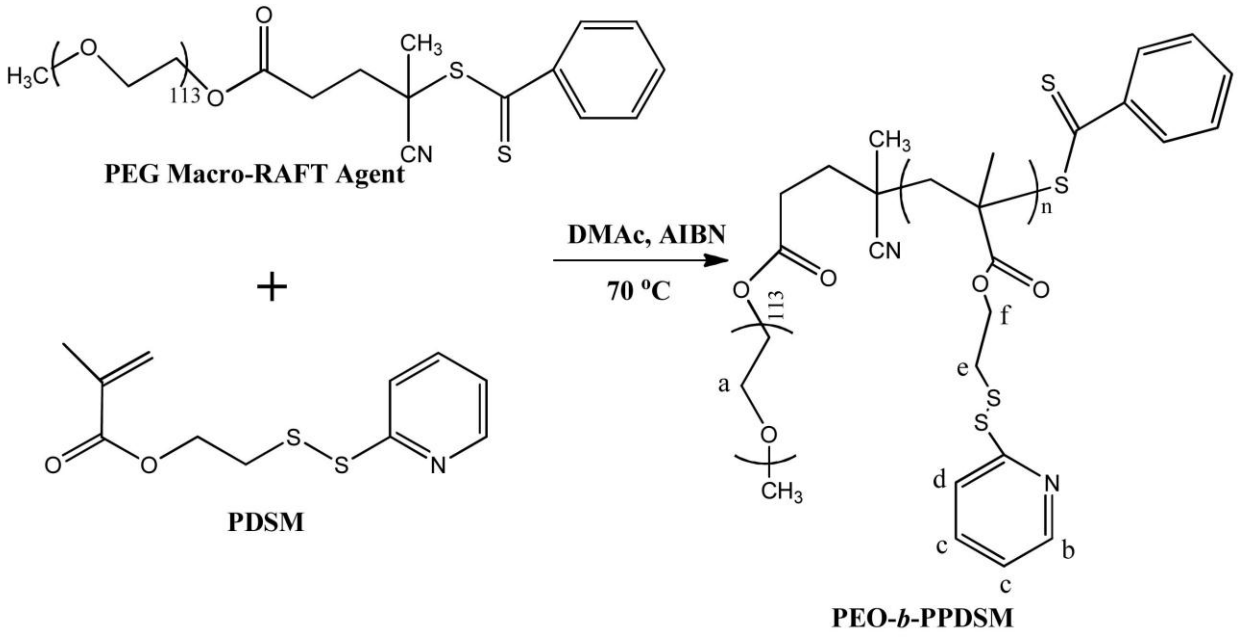
In a 250 mL one-neck round-bottom flask equipped with a magnetic stir bar, PEO-OH (10.0 g) was dissolved in 150 mL of toluene. After azeotropic distillation of 10 mL of toluene at reduced pressure to remove traces of water, 573.5 mg of CPAD and 64.3 mg of 4-dimethylaminopridine (DMAP) were added. When the solution was homogenized by stirring, 1.16 g of 1,3-dicyclohexylcarbodiimide (DCC) was added in portions. The reaction mixture was stirred at room temperature for three days. The precipitated urea was filtered. PEO-based macro-RAFT agent with pink color was obtained by precipitation of the filtrate into excess of diethyl ether three times, and then dried under vacuum at room temperature for two days. Yield: 93%. ^{1}H NMR (Fig. S5): ($CDCl_3$, 400 MHz), δ (ppm): 7.3–7.9 (aromatic protons), 4.2 (methylene protons of newly formed ester groups), 3.42–3.63 (methylene protons of PEG repeat units), 2.32–2.55 (methylene protons of CPAD), 1.9 (methyl protons of CPAD).



Scheme S1. Synthesis of pyridyldisulfide ethymethacrylate (PDSM).



Scheme S2. Synthesis of PEO macro-RAFT agent (PEO-CTA).



Scheme S3. Schematic illustration of polymerization of thiol-reactive block copolymer PEO-*b*-PPDSM using PEO macro-RAFT agent.

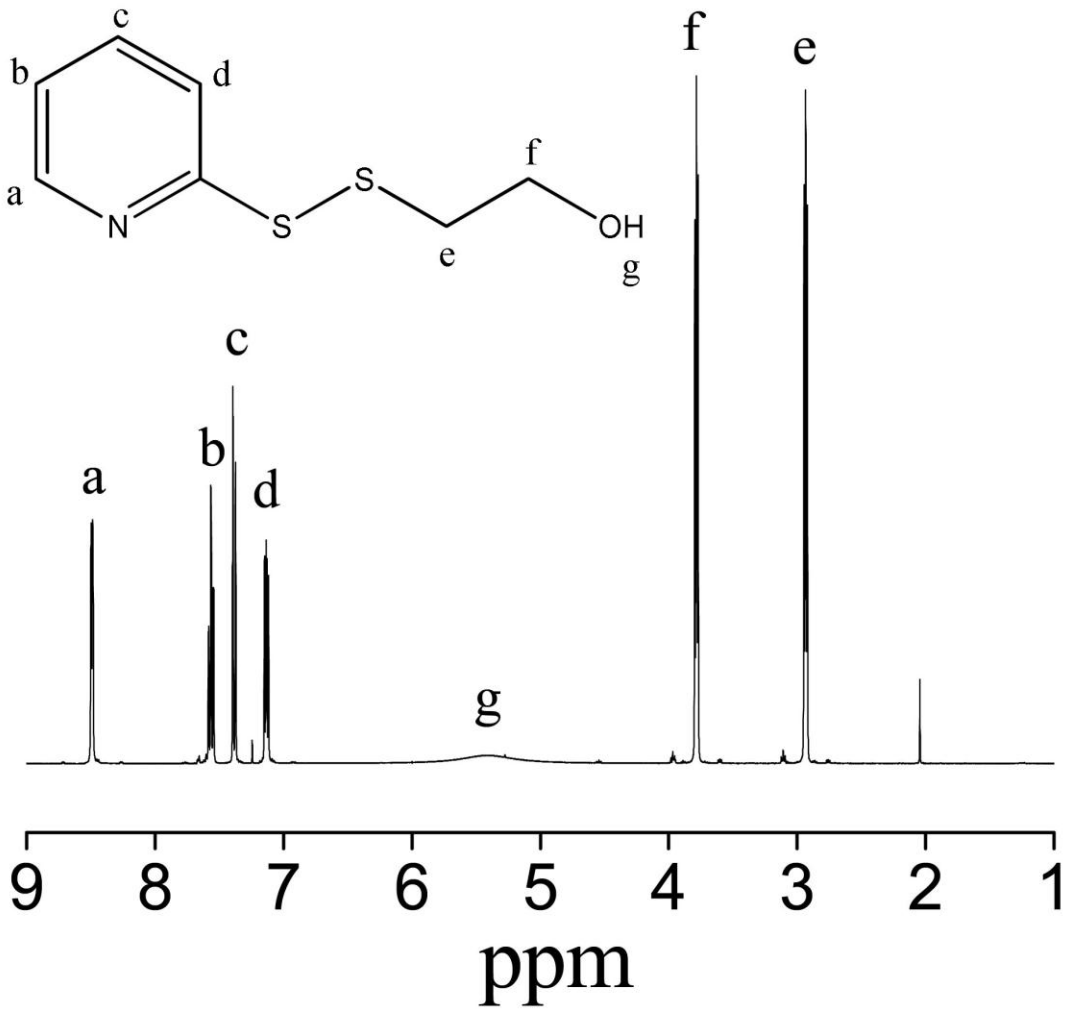


Fig. S1. ^{1}H NMR spectrum of hydroxyethyl pyridyldisulfide (Compound 1) in $CDCl_3$ solvent (400 MHz).

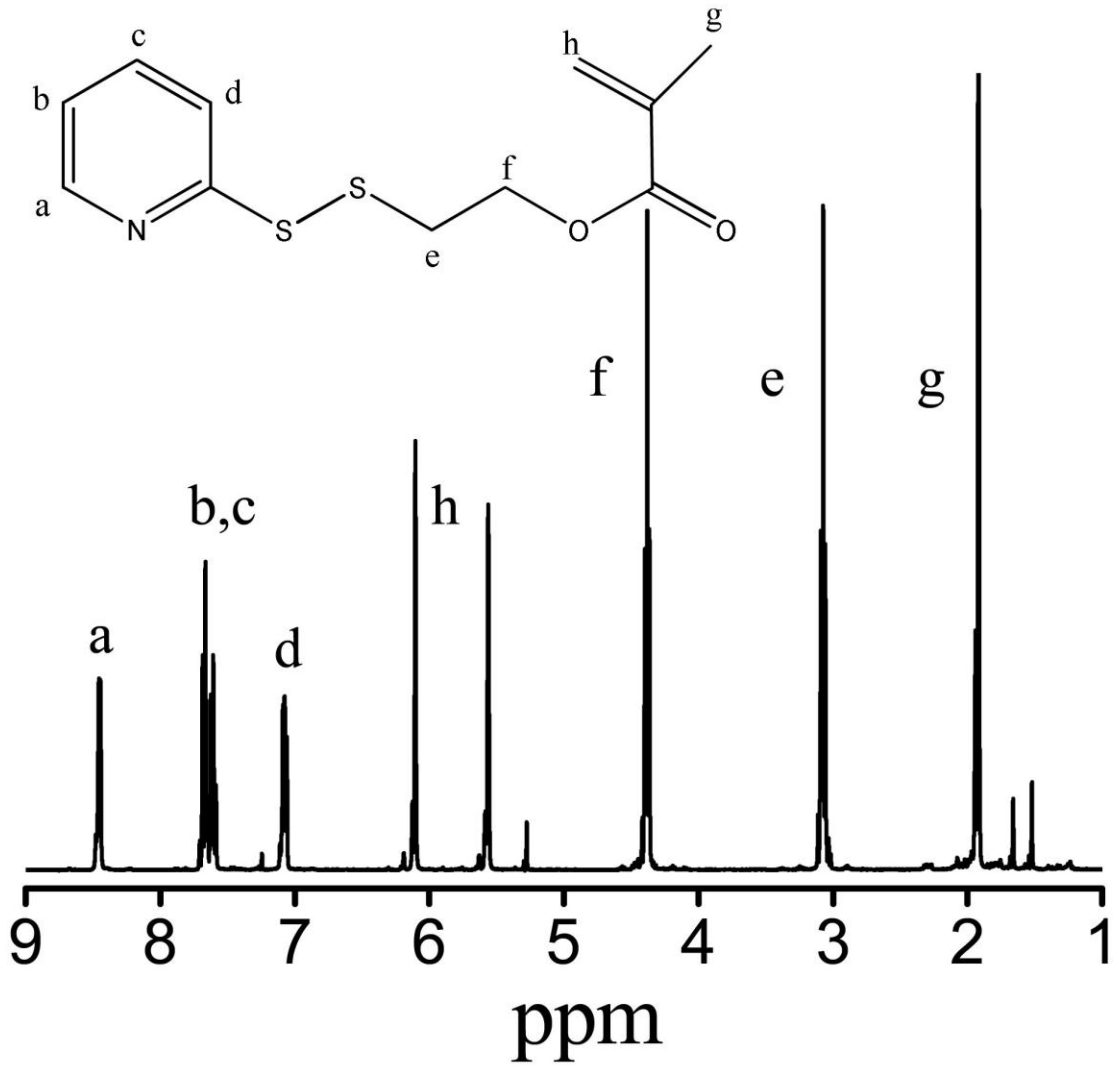


Fig. S2. ^{1}H NMR spectrum of PDSM monomer (Compound 2) in $CDCl_3$ solvent (400 MHz).

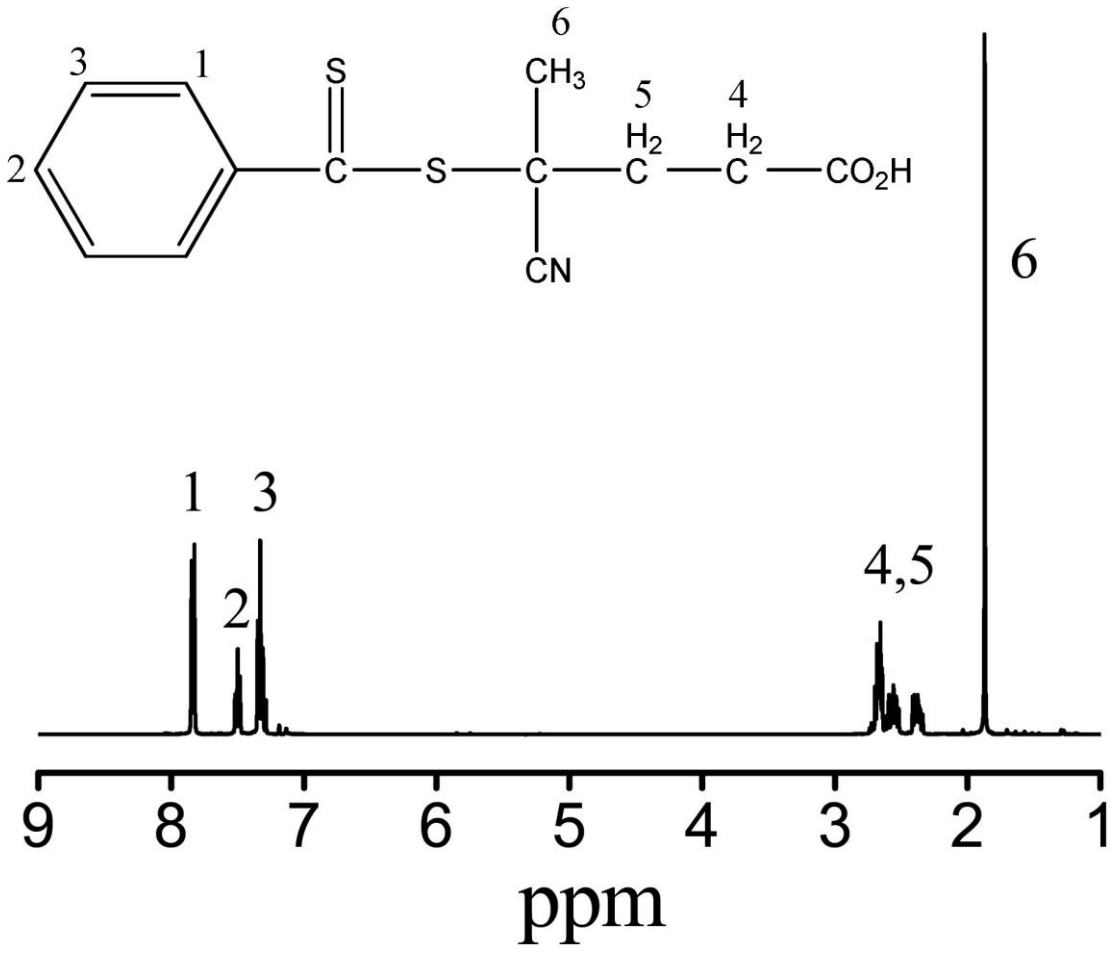


Fig. S3. ^{1}H NMR spectrum of 4-cyanopentanoic acid dithiobenzoate (Compound 3) in $CDCl_3$ solvent (400 MHz).

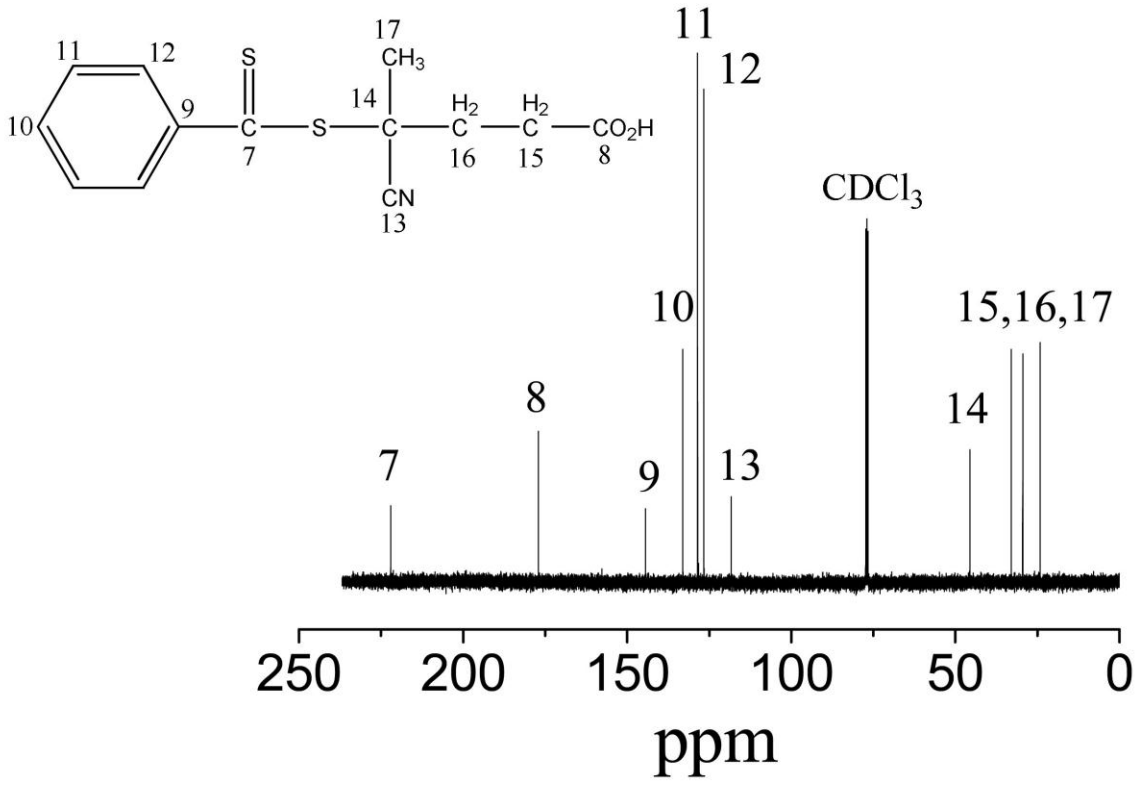


Fig. S4. ^{13}C NMR spectrum of 4-cyanopentanoic acid dithiobenzoate (Compound 3) in $CDCl_3$ solvent (400 MHz).

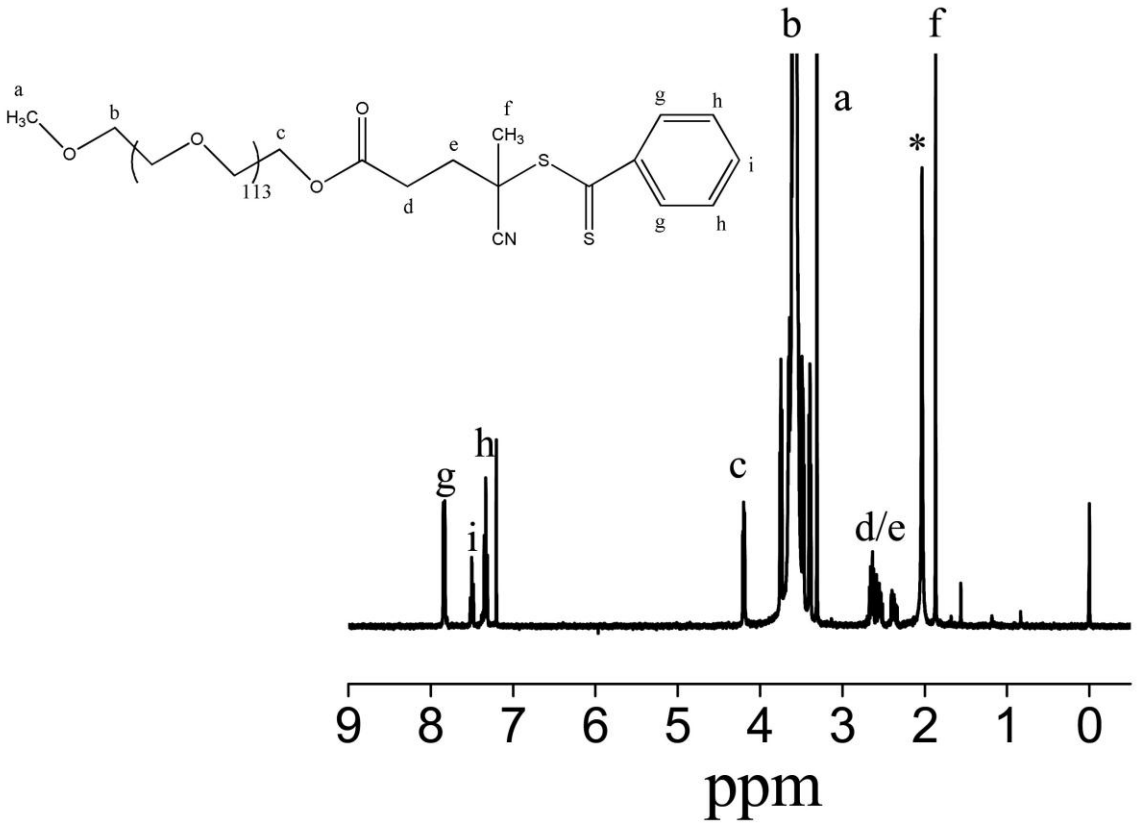


Fig. S5. ^{1}H NMR spectrum of PEO macro-RAFT agent (PEO-CTA) in $CDCl_3$ solvent (400 MHz).

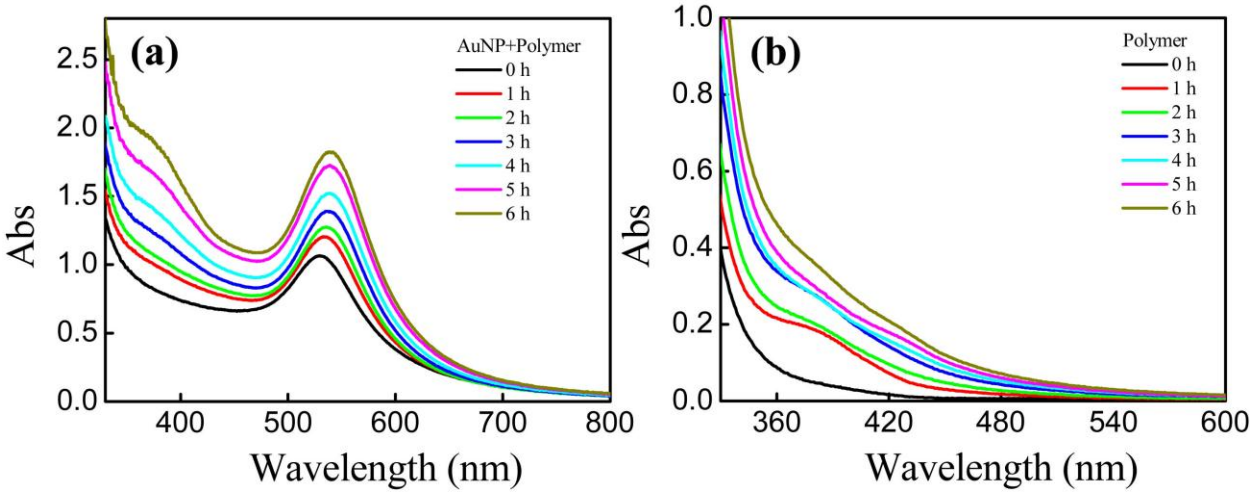


Fig. S6. The absorption spectra of (a) mixture of polymer and AuNPs and (b) polymer only in DMF, after a 10-fold dilution, at different time points upon heat treatment at 130 ºC.

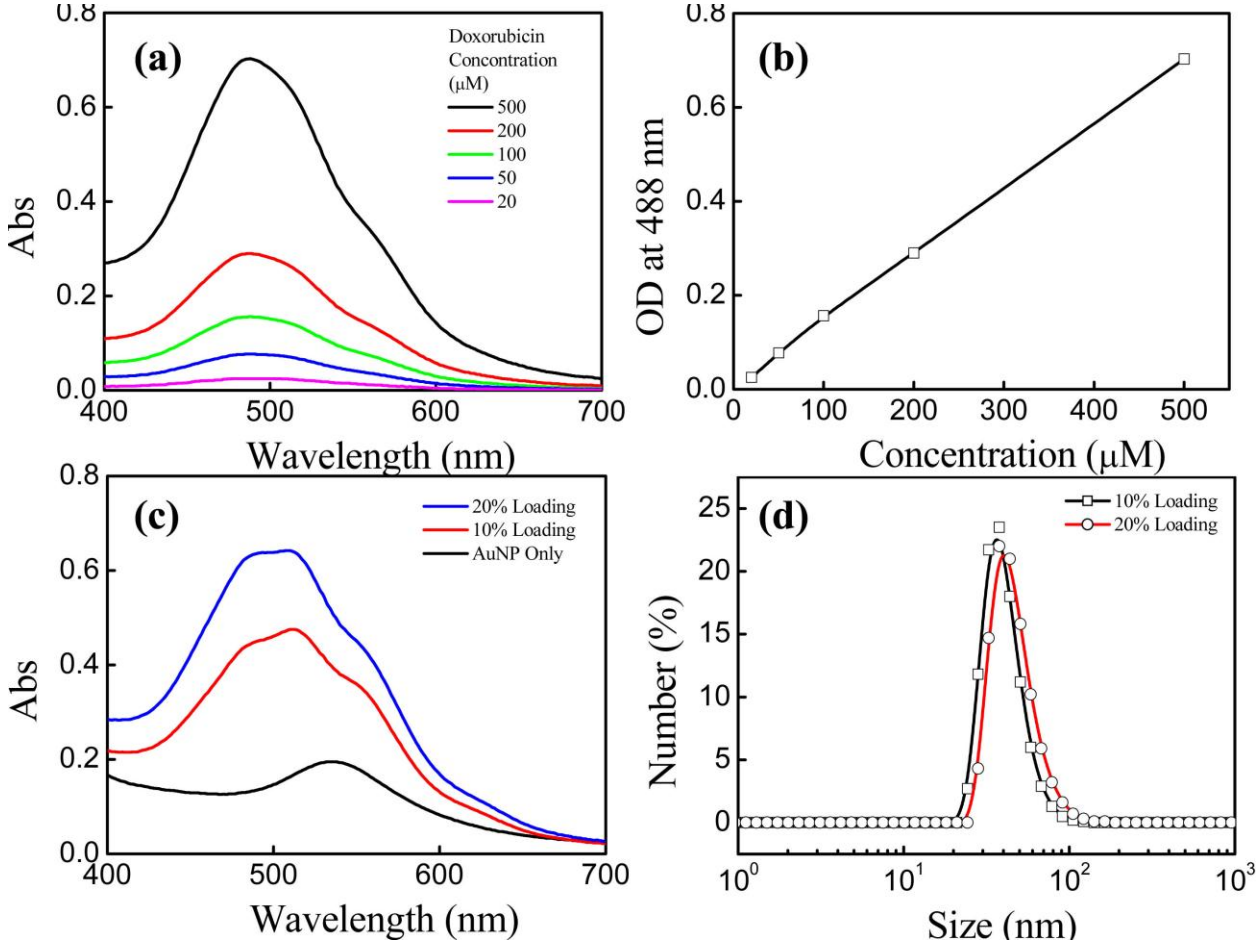


Fig. S7. (a) Optical spectra of free doxorubicin (Dox) at different concentrations in acetate buffer (pH 5.0) and (b) the corresponding calibration curve. (c) Optical spectra of composite nanoparticles co-encapsulating AuNPs and Dox (neutral) in PBS (pH 7.4). (d) Hydrodynamic size distribution of composite nanoparticles loaded with Dox. To load Dox, 1.0 or 2.0 mL of Dox solution (5.0 mg/mL in DMSO treated with TEA, 2 molar eq. to Dox·HCl) was added to the mixture of polymer and gold nanoparticles (2.0 mL) after cooling to room temperature. The organic solution was then transferred to PBS (10 times the volume of the organic solution) and dialysis against 2.0 L of PBS occurred overnight.

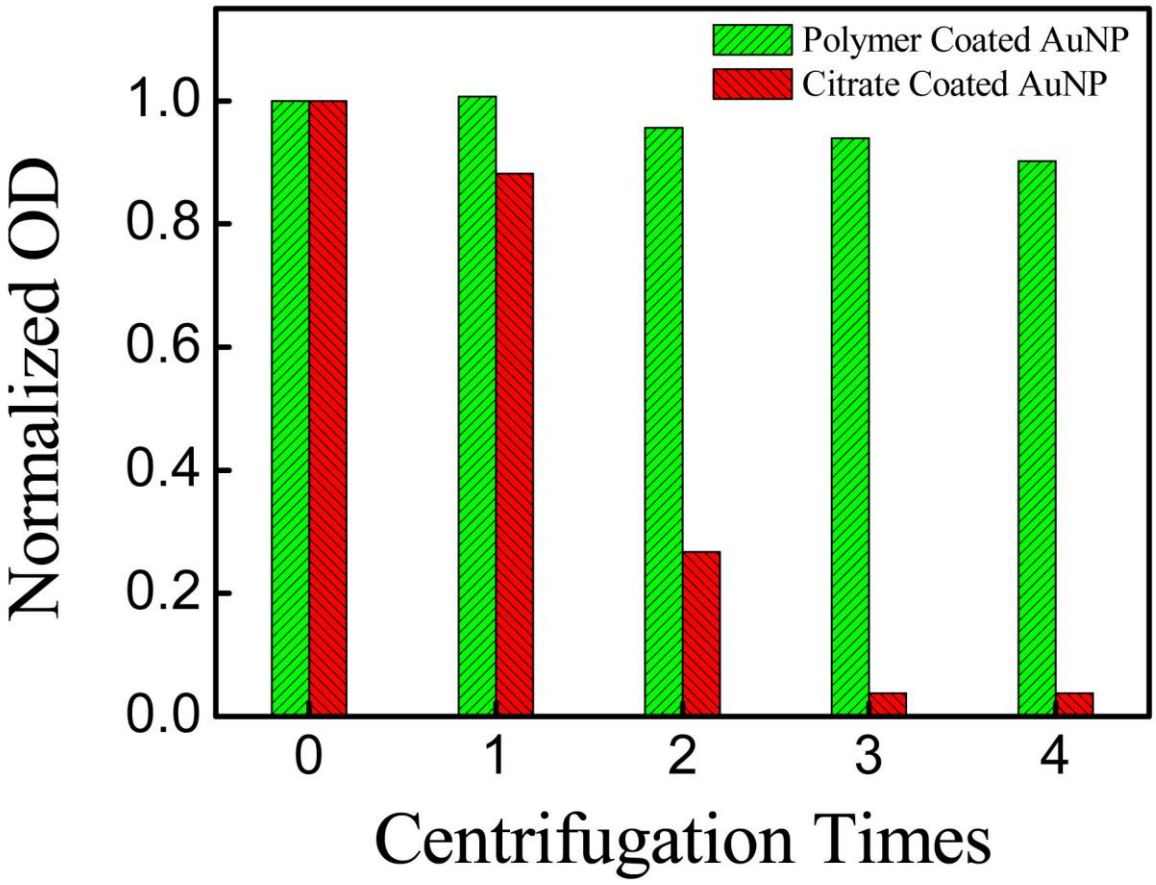


Fig. S8. Normalized optical density (OD) of gold nanoparticles coated with PEO-*b*-PPDSM or citrate after repeated centrifugation. Citrate coated gold nanoparticles (15 nm) were purchased from BBINTERNATIONAL (UK).

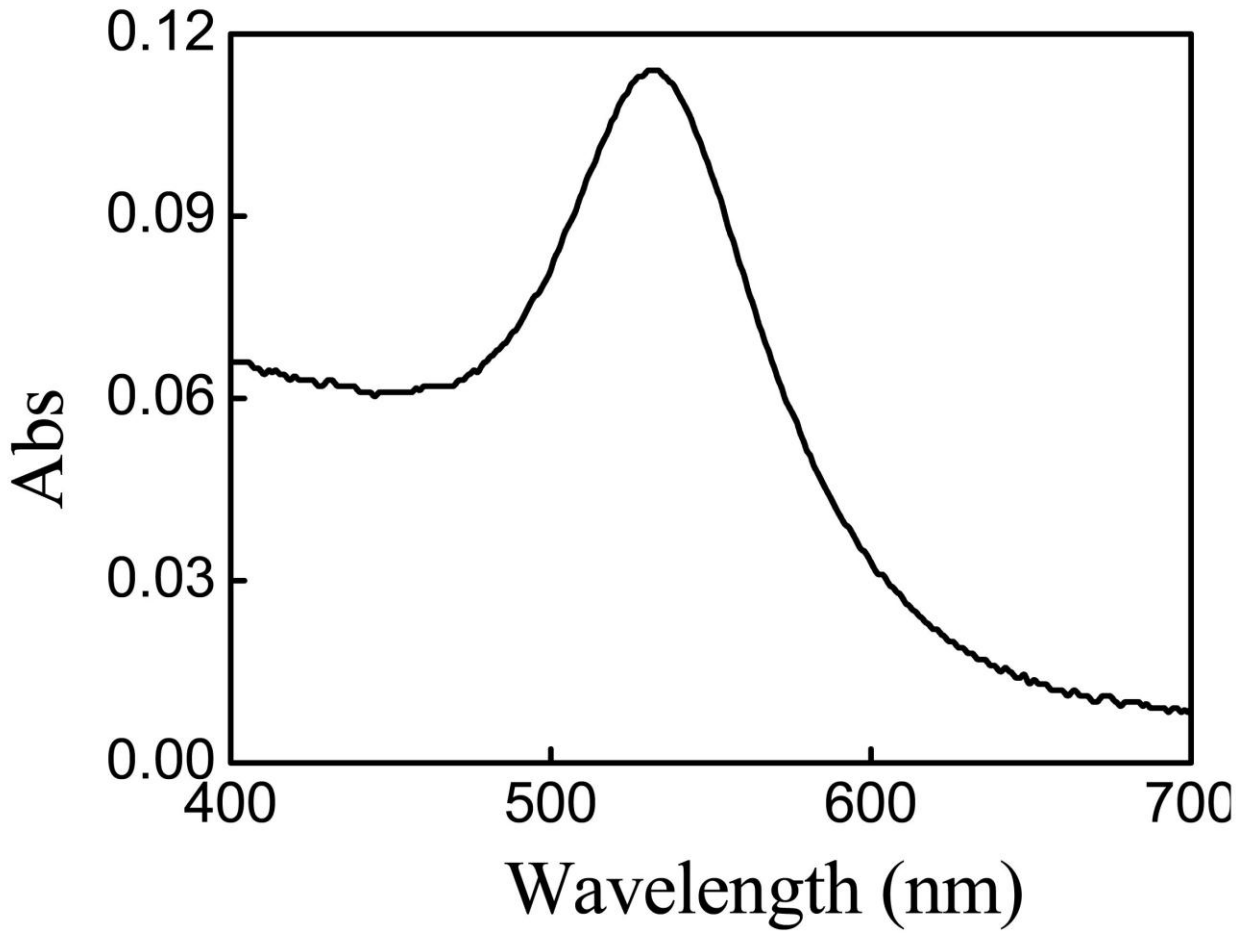


Fig. S9. The UV-vis spectrum of gold nanoparticles treated with FITC lacking a thiol modification, after washing with a 30K Nanosep filter for five times.

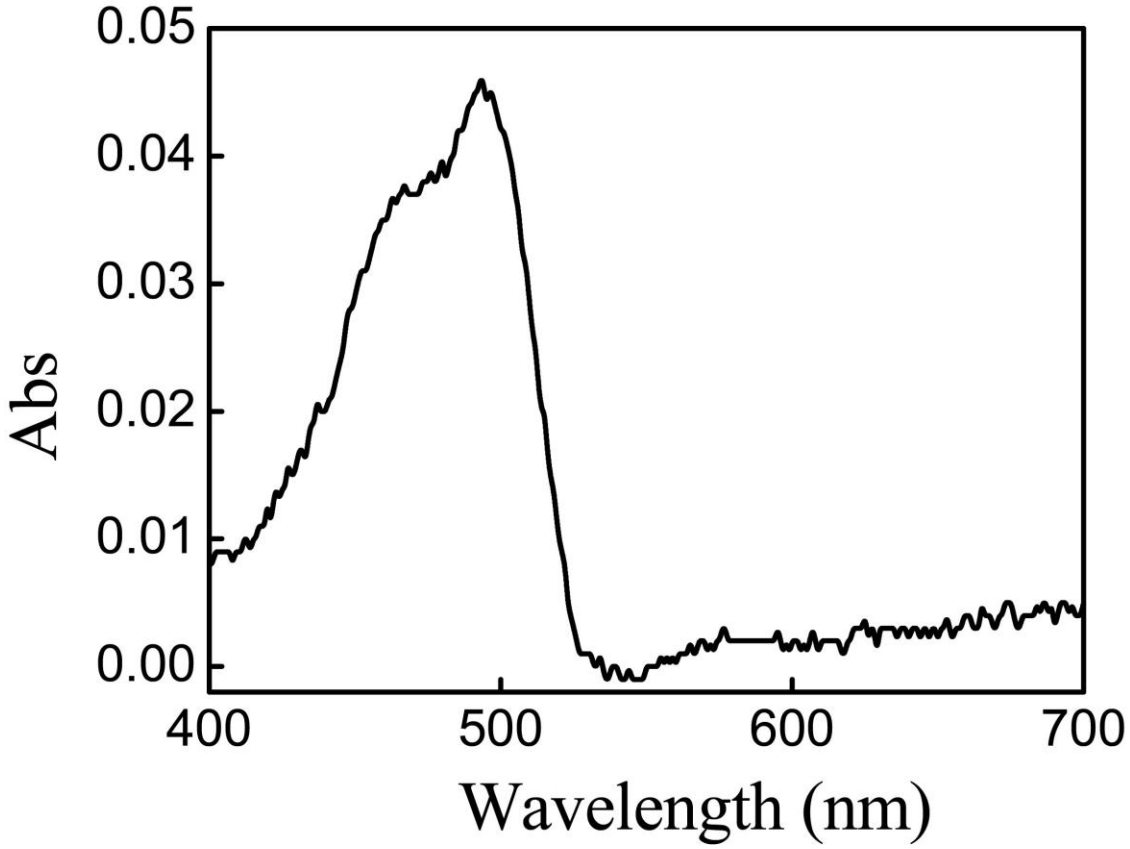


Fig. S10. UV-vis spectrum of gold nanoparticles treated with FITC after subtracting spectrum of gold nanoparticles without treatment.

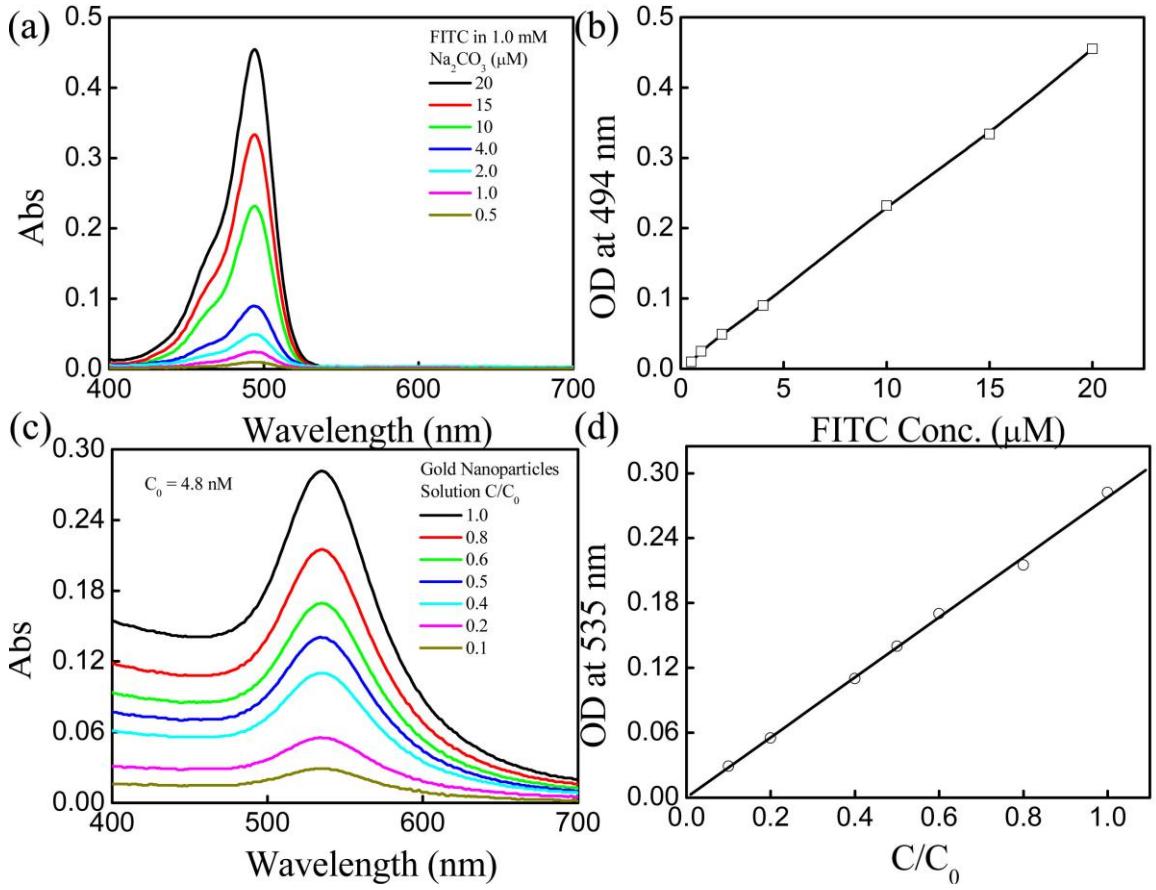


Fig. S11. Absorption spectra and calibration curves of FITC (a, b) and gold nanoparticles coated with PEO-*b*-PPDSM (c, d).

References:

(1) Wong, L. J.; Boyer, C.; Jia, Z. F.; Zareie, H. M.; Davis, T. P.; Bulmus, V. *Biomacromolecules* **2008**, *9*, 1934.

(2) Mitsukami, Y.; Donovan, M. S.; Lowe, A. B.; McCormick, C. L. *Macromolecules* **2001**, *34*, 2248.