Supporting information for:

Surface-Functionalizable Polymer Nanogels with Facile Hydrophobic Guest Encapsulation Capabilities

Ja-Hyoung Ryu, Siriporn Jiwpanich, Reuben Chacko, Sean Bickerton, and S. Thayumanavan*

Department of Chemistry, University of Massachusetts Amherst, Amherst, Massachusetts 01003,

E-mail: thai@chem.umass.edu.

Experimental Section

General. 2,2´-Dithiodipyridine, 2-mercaptoethanol, polyethylene glycol monomethyl ether methacrylate (MW 450), D,L-dithiothreitol (DTT), 1,1'-dioctadecyl-3,3,3',3'-tetramethyl-indocarbocyanine perchlorate (DiI), Nile red, Tat-SH, and other conventional reagents were obtained from commercial sources and were used as received unless otherwise mentioned. Polymer was synthesized with RAFT polymerization and then purified by precipitation with ethyl ether. S-dodecyl-S'-2-(2,2-dimethylacetic acid) trithiocarbonate and pyridyl disulfide ethyl methacrylate (PDSEMA) was prepared using a previously reported procedure.[1] 1H-NMR spectra were recorded on a 400 MHz Bruker NMR spectrometer using the residual proton resonance of the solvent as the internal standard. Molecular weights of the polymers were estimated by gel permeation chromatography (GPC) using PMMA standard with a refractive index detector. Dynamic light scattering (DLS) measurements were performed using a Malvern Nanozetasizer. The fluorescence spectra were obtained from a JASCO FP-6500 spectrofluorimeter. Transmission electron microscopy (TEM) images were taken from JEOL 100CX at 100 KV.

Random copolymer. A mixture of S-dodecyl-S'-2-(2,2-dimethylacetic acid) trithiocarbonate (90 mg, 0.28 mmol), PDSEMA (5 g, 19.6 mmol), polyethylene glycol monomethyl ether methacrylate (4 g, 8.4 mmol) and AIBN (10 mg, 0.056 mmol) was dissolved in DMF (10 ml) and degassed by performing three freeze-pump-thaw cycles. The reaction mixture was sealed and then put into a pre-heated oil bath at 70 ^{o}C for 12 h. The resultant mixture was dissolved in dichloromethane (5 ml) and precipitated in hexane (200 ml). To remove unreactive monomers, the precipitate was further dissolved in dichloromethane (5 ml) and re-precipitated in ethyl ether (200 ml) to yield purified the random copolymer as a waxy liquid. Yield: 78%. GPC (THF) M_n: 24.7 K. PDI: 1.6.

1H NMR (400 MHz, CDCl3) δ: 8.45, 7.68, 7.11, 3.80-3.42, 3.02, 2.04-1.65, 1.24-0.87. The molar ratio between two blocks was determined by integrating the methoxy proton in the polyethylene glycol unit and the aromatic proton in the pyridine and found to be 3:7 (PEO:PDSEMA).

Thiol-modified FITC. A mixture of FITC (10 mg, 0.026 mmol), cystamine dihydrochloride (3.0 mg, 0.013 mmol) and triethylamine (13.0 mg, 0.13 mmol) was dissolved in DMSO (400 μl) and stirred for 4 h. To this reaction mixture was added tris(2-carboxyehtyl)phosphine hydrochloride (8.8 mg, 0.031 mmol) and stirred for 1 h. The resultant mixture was precipitated in ethyl ether and washed with water. The crude product was used for nanogel surface modification without further purification.

Nanogel preparation. The polymer (10 mg) and Nile red (2 mg) were dissolved in 200 μl of acetone and measured amount of DTT (4 μmol, 20 mol% against PDS groups) were added. After stirring for 10 min, 1 ml of deionized water and the mixture solution was stirred overnight at room temperature, open to the atmosphere allowing the organic solvent to evaporate. Excess insoluble Nile red was removed by filtration and pyridothione was removed from the nanogel solution by ultrafiltration (triplicate) using a membrane with a molecular weight cutoff of 10,000 g mol^{-1} (Amicon Ultra cell-10K).

Cell culture. The cell viability of the nanogels was tested against 293T cells. 293T cells were cultured in T75 cell culture flasks using Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12) with 10% fetal bovine serum (FBS) supplement. The cells were seeded at 10,000 cells/well/200 μl in a 96 well plate and allowed to grow for 24 hours under incubation at 37 ^{o}C and 5% CO_2. These cells were then treated with nanogels of different concentrations and were incubated for another 24 hours. Cell viability was measured using the Alamar Blue assay with each data point measured in triplicate. Fluorescence measurements were made using the plate reader SpectraMax M5 by setting the excitation wavelength at 560 nm and monitoring emission at 590 nm on a black well plate.

Surface modification. 1 mg of FITC or thiol-modified FITC was dissolved in 100 μl of DMF and then 2 ml of nanogel (1 mg ml^{-1}) in water was added. The mixture solution was stirred overnight at room temperature. Non-conjugated or physically absorbed dye molecules were removed by ultrafiltration (thrice) using a membrane with a molecular weight cutoff of 10,000 g mol^{-1}. For Tat peptide modification, 10 μl of nanogel (1 mg ml^{-1}) which contains 1% DiI molecules was added to 90 μl of phosphate pH 5 buffer. To this solution, 10 μl of Tat peptide solution (1 mg ml^{-1}) was added. After stirring overnight, this 100 μl of this solution was added to MCF-7 cells, which were grown in coverslip-bottomed Petri dishes, to make a total of 2 ml of culture

medium. Cells with surface modified nanogels and control nanogels were incubated for 2 h before confocal imaging.

Laser scanning confocal microscopy. The laser confocal experiment was performed using MCF-7 cells. MCF-7 cells were cultured in T75 cell culture flask containing DMEM/F12 with 10% FBS supplement. 50,000 cells in 2 ml of culture medium were seeded in coverslip-bottomed Petri dishes and allowed to grow for 3 days at 37 ^{o}C in a 5% CO_2 incubator. These cells were treated with 100 µl of nanogels and incubated at 37 ^{o}C for 2 h before observing them by confocal microscopy.

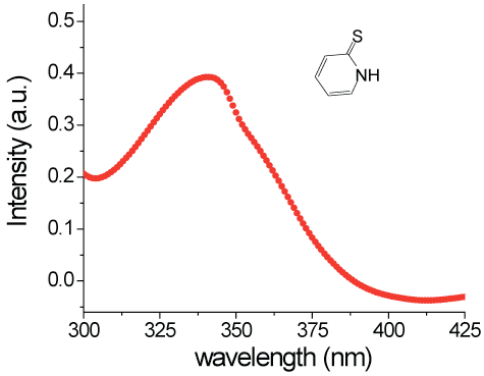

Figure S1. Absorption of pyridothione in UV-vis. To estimate remaining amount of pyridine groups in the nanogel, the amount of pyridothione which is a byproduct during nanogel synthesis by disulfide bond formation (characteristic absorption at 350 nm) was monitored. Based on the calculation of pyridothione molar concentration using the extinction coefficient, the remaining PDS groups were determined to be 62% (38% of PDS groups were used in the crosslinking reaction). Because the total PDS composition of the polymer is 70%, we estimated that the crosslinking density corresponds to 13%.

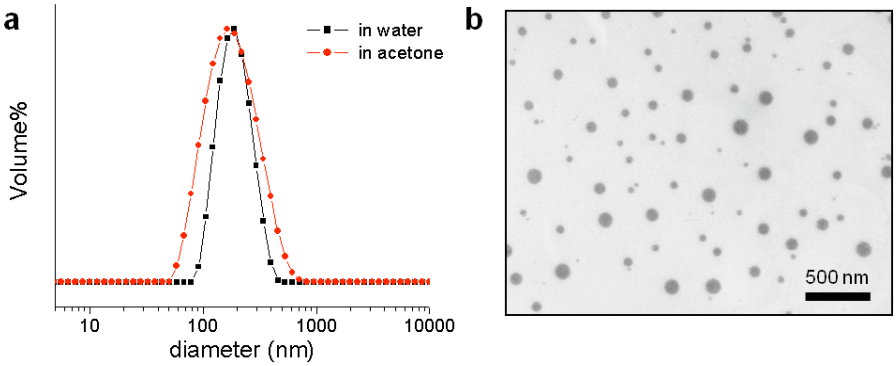

Figure S2. (a) DLS of nanogel (1 mg ml^{-1}) in water and acetone showed almost same size distribution. (b) TEM image of the nanogel. We were interested in identifying whether the obtained particle is indeed a stable crosslinked structure. After the formation of the polymer nanoparticle using the DTT reaction, the size of the assembly in both acetone and water were identical. This illustrates the stability of the assembly and confirms that they are formed by chemical crosslinking of the functionalities. We calculated the water content of the nanogels based on the difference in size of swollen state and dried state nanogels. The average size of swollen gels in water measured by DLS and dried gels measured by TEM is 190 nm and 110 nm, respectively. Therefore from the difference of volume between dry state and swollen state in one particle (6.97×10^5 nm^3 and 3.59×10^6 nm^3 for dried state and swelled state, respectively), we can estimate that a 2.89×10^6 nm^3 volume is filled by water, corresponding to 9.68×10^7 water molecules.

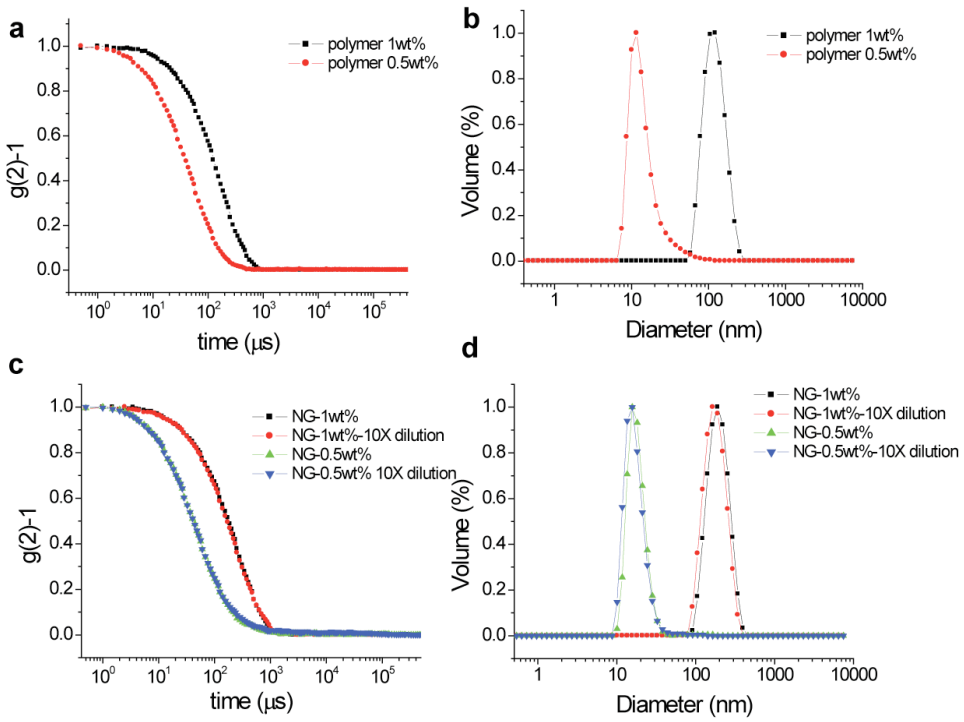

Figure S3. (a) Time correlation function and (b) size distribution of co-polymer **1** at different concentration (0.5 wt% and 1.0 wt%). (c) Time correlation function and (d) size distribution of nanogels which were prepared at different concentration (0.5 wt% and 1.0 wt%).

We were interested in identifying whether there is a correlation between polymer concentration and particle size. For this purpose, we analyzed the size of polymer assemblies that would be obtained prior to the DTT reaction. DLS studies revealed aggregates of about 120 nm at 10 mg ml^{-1} in water, and 12 nm below 5 mg ml^{-1}. Between these two concentrations the two sizes coexist. Subsequent crosslinking reaction by DTT leads to nanoparticles with two different sizes (16 nm and 190 nm by 5 mg ml^{-1} and 10 mg ml^{-1}, respectively). The resulting nanoparticles showed same size (190 nm) when it was diluted ten times, while the polymer **1** at this concentration showed 12 nm size. This indicates that the obtained particle is indeed a stable crosslinked structure. This suggests that we can control the size of nanoparticles by reaction condition.

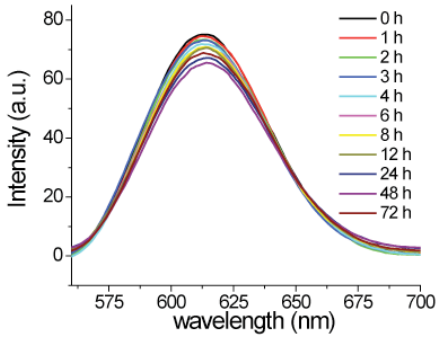

Figure S4. Dye release from the nanogel in response to GSH concentrations (10 μM) at pH 7.4. The release was only occurred at high GSH concentration. To examine the GSH concentration dependent dye release, Nile red containing nanogel solutions (0.05 wt%) in pH 7.4 sodium acetate buffer solution were treated with different concentrations of GSH (10 μM and 10 mM) and the intensity of Nile red emission at 610 nm was monitored for 4 days.

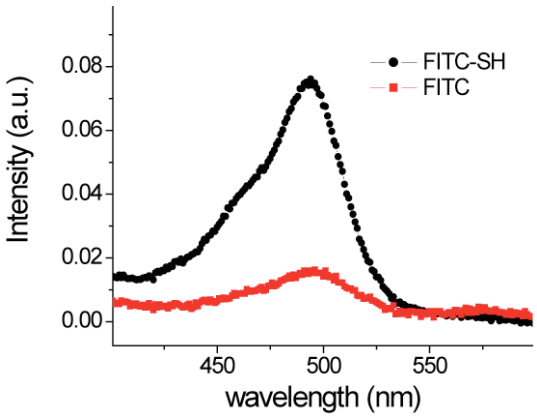

Figure S5. The absorption spectra of nanogel (0.5 mg ml^{-1}) treated with thiol-modified FITC (FITC-SH) and with FITC. To estimate the number of FITC molecules on the nanogel surface, the absorption of FITC at 492 nm wavelength was compared in each nanogel treated with FITC-SH and FITC. Based on the calculation of FITC molar concentration by extinction coefficient and assuming that the nanogel density is around 1.00, the number of FITC molecules on the nanogel surface was determined to be approximately three thousand molecules per particle.

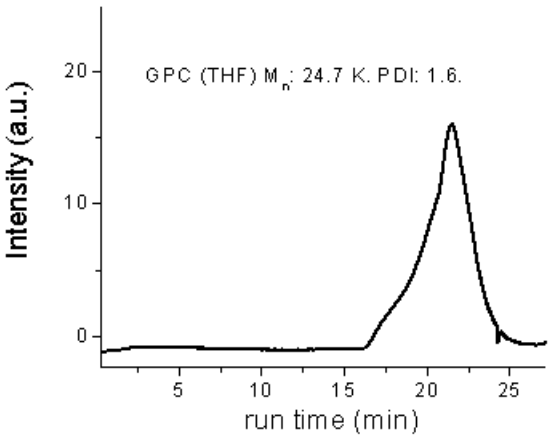

Figure S6. GPC trace of the polymer **1**.

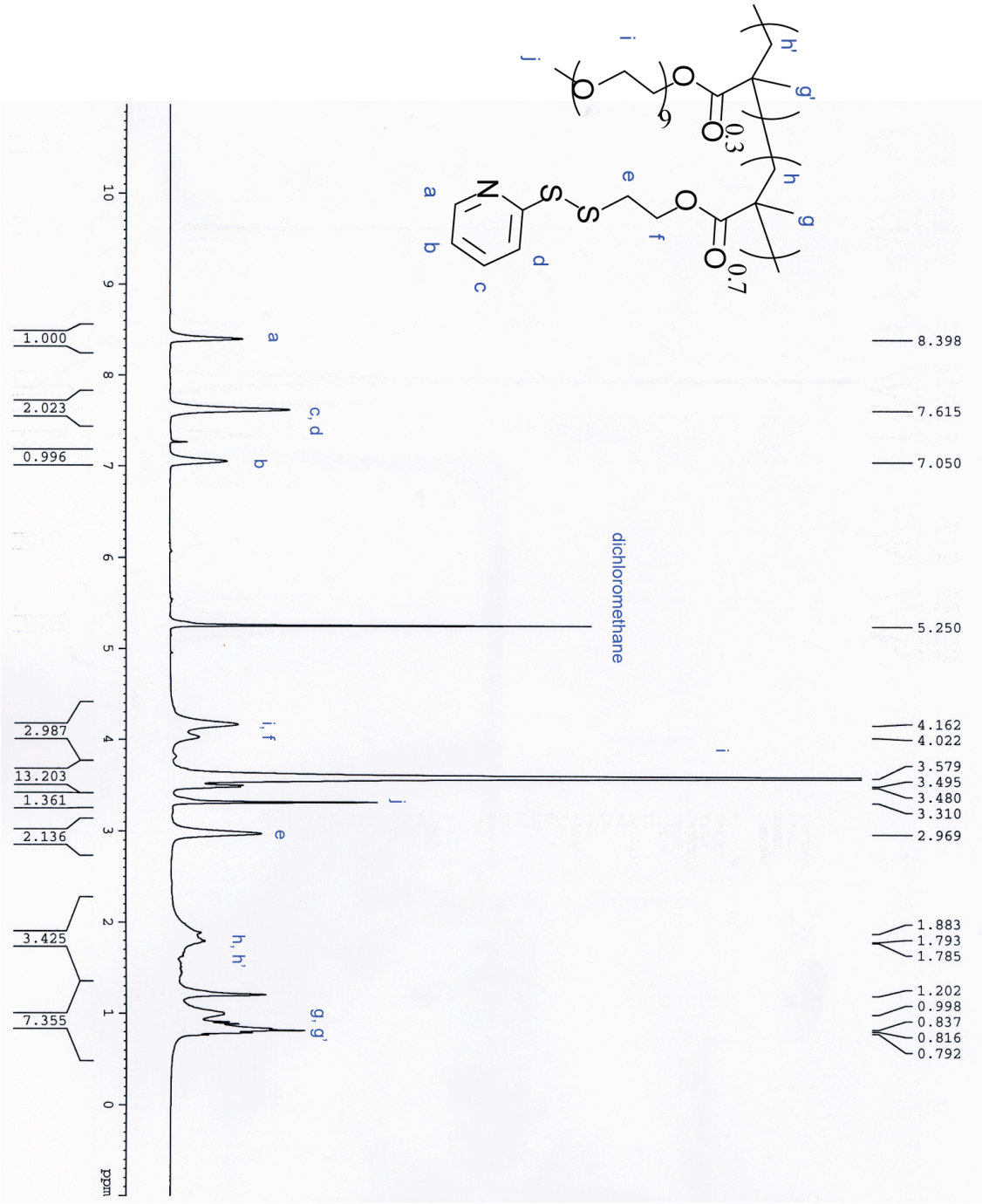

Figure S7. ^{1}H-NMR of the polymer **1**.

References

[1] Ghosh, S.; Basu, S.; Thayumanavan, S. *Macromolecules* **2006**, *39*, 5595-5597.

[2] J. T. Lai, D. Filla and R. Shea, *Macromolecules* **2002**, *35*, 6754.