

Supporting Information for:

The Interconnected Roles of Scaffold Hydrophobicity, Drug Loading and Encapsulation Stability in Polymeric Nanocarriers

Sean Bickerton, Siriporn Jiwpanich, and S. Thayumanavan*

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

*To whom correspondence should be addressed. E-mail: thai@chem.umass.edu

Supporting Information figures and text:

Figure S1. UV-vis spectra, 0.5 wt% mixing experiments.

Figure S2. UV-vis spectra, 2 wt% mixing experiments.

Figure S3. UV-vis spectra, 10 wt% mixing experiments.

Figure S4. Fluorescence and UV-vis spectra of pyrene loaded NG samples.

Figure S5. UV-vis spectra of all purified nanogel samples for loading efficiency calculation.

Figure S6. UV-vis spectra of crosslinking reaction byproduct, pyridinethione.

Table S1. I_1 / I_3 values from pyrene spectra of NG samples.

Table S2. Calculated Λ_c values of all NG samples with varying dye amounts.

Table S3. Calculated dye loading amounts for all purified nanogel samples.

Table S4. Calculated crosslinking efficiencies for the nanogel syntheses.

Experimental Sections.

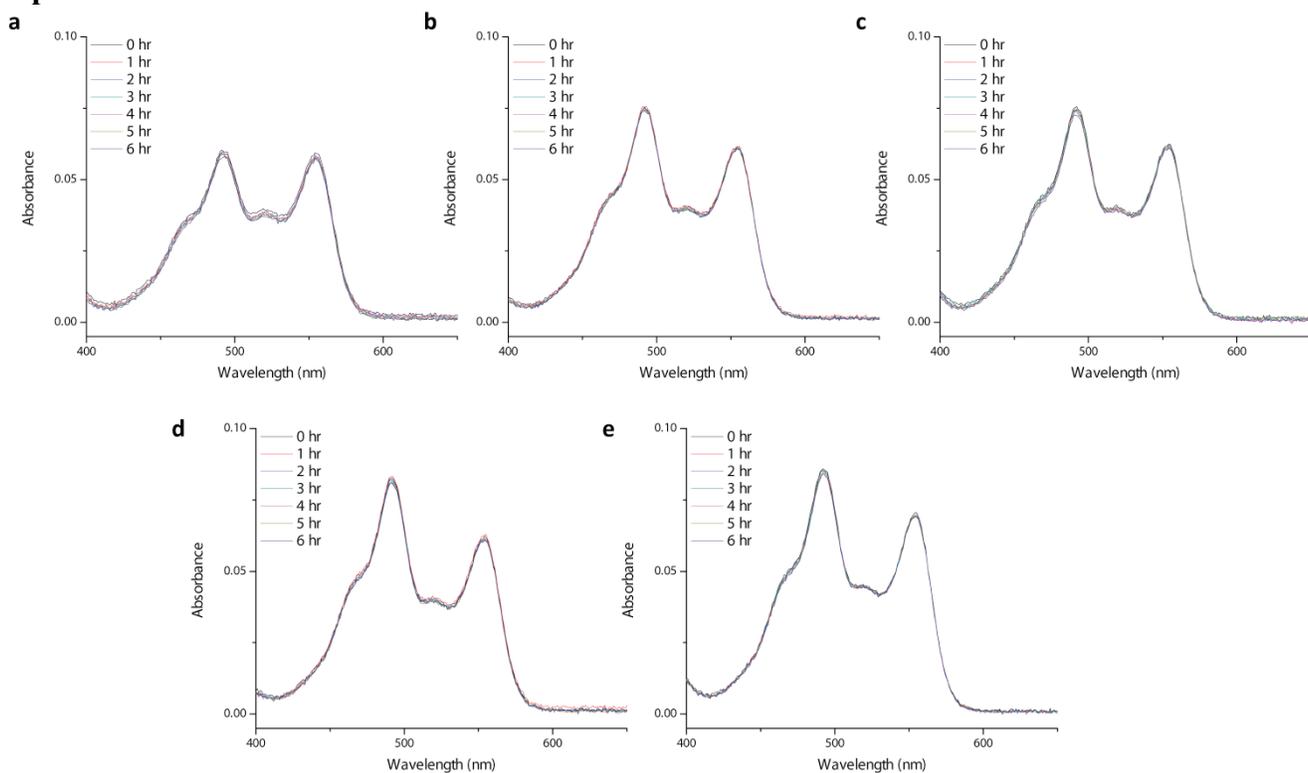


Figure S1. UV-vis spectra of NG2 (a), NG3 (b), NG4 (c), NG5 (d) and NG6 (e) taken during the course of the mixing experiments performed using the nanogel samples prepared with 0.5 wt% DiI/DiO.

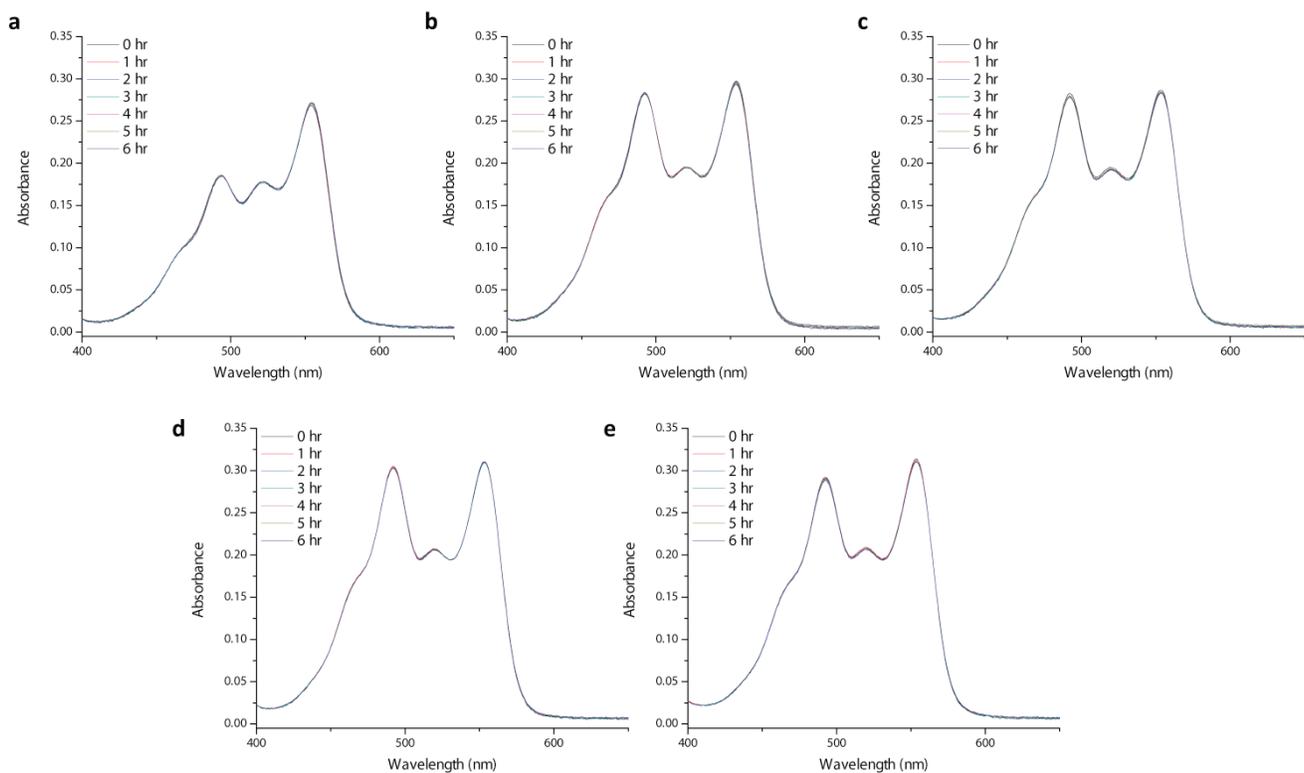


Figure S2. UV-vis spectra of NG2 (a), NG3 (b), NG4 (c), NG5 (d) and NG6 (e) taken during the course of the mixing experiments performed using the nanogel samples prepared with 2 wt% DiI/DiO.

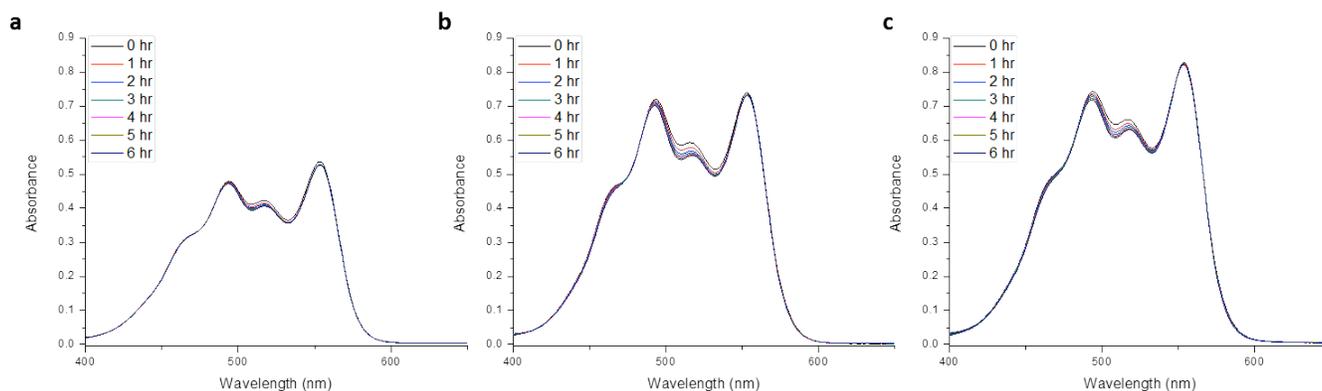


Figure S3. UV-vis spectra of NG4 (a), NG5 (b) and NG6 (c) taken during the course of the mixing experiments performed using the nanogel samples prepared with 10 wt% DiI/DiO.

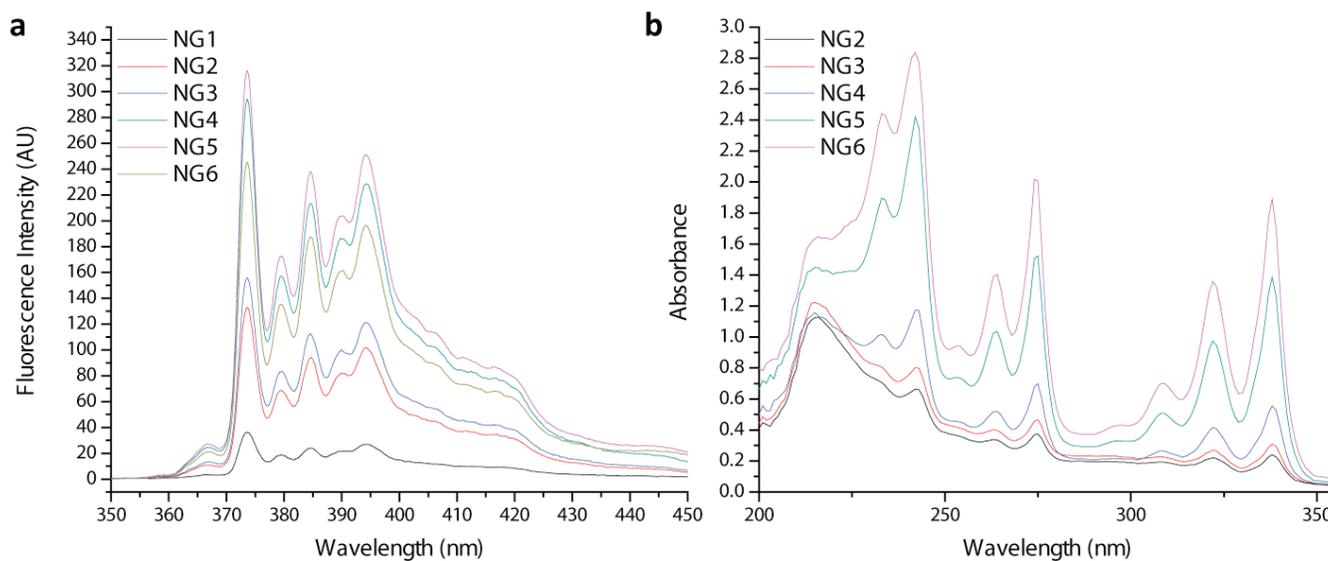


Figure S4. (a) Fluorescence spectra of NG1-NG6 (recorded at $0.05 \text{ mg}\cdot\text{mL}^{-1}$ nanogel) prepared with 10 wt% pyrene from which the reported I_1/I_3 values were calculated. (b) UV-vis spectra of NG2-NG6 (recorded at $1 \text{ mg}\cdot\text{mL}^{-1}$ nanogel) prepared with 10 wt% pyrene reveal a trend of enhanced pyrene loading with increased nanogel hydrophobicity.

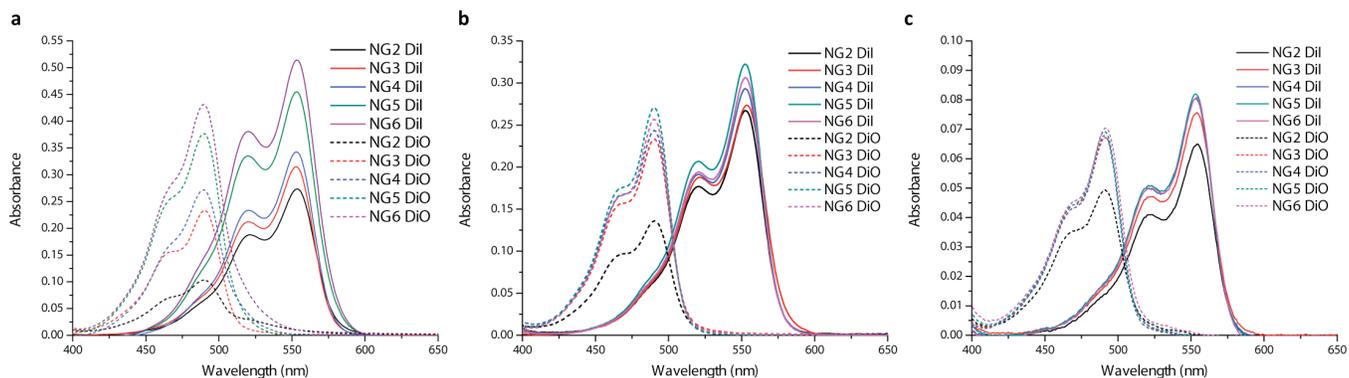


Figure S5. UV-vis spectra of the purified nanogel samples prepared with 10 wt% (a; recorded at 0.05 mg.mL^{-1} nanogel), 2 wt% (b; recorded at 0.1 mg.mL^{-1} nanogel) and 0.5 wt% (c; recorded at 0.1 mg.mL^{-1} nanogel) DiI/DiO that were used to calculate the reported loading efficiencies.

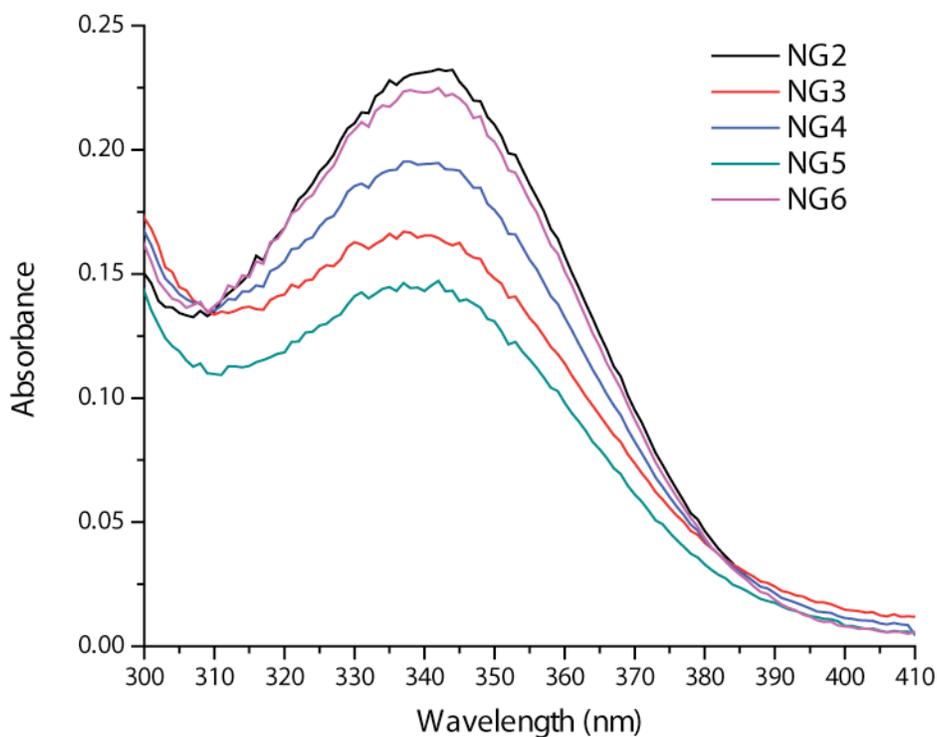


Figure S6. Representative UV-vis traces of the pyridinethione byproduct produced during the nanogel crosslinking reaction (recorded at 0.1 mg.mL^{-1} nanogel). Crosslinking efficiencies were determined using the molar extinction coefficient of $8.08 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ at 343 nm.

Table S1. I_1 , I_3 , and I_1/I_3 values from pyrene fluorescence spectra of 10 wt% loaded **NG1** – **NG6** from two repeated experiment trials.

Sample	I_1 (Trial 1 / Trial 2)	I_3 (Trial 1 / Trial 2)	I_1/I_3
NG1	36.176 / 36.138	23.860 / 23.997	1.52 / 1.51
NG2	132.743 / 135.565	94.081 / 96.099	1.41 / 1.41
NG3	155.886 / 166.179	112.357 / 120.337	1.39 / 1.38
NG4	294.266 / 308.328	213.453 / 226.512	1.38 / 1.36
NG5	316.279 / 328.17	238.187 / 251.324	1.33 / 1.31
NG6	245.211 / 237.468	187.597 / 185.13	1.31 / 1.28

Table S2. Calculated Λ_c values for all nanogel samples prepared with 10 wt%, 2 wt%, 1 wt% and 0.5 wt% Dil/DiO (10 wt% and 1 wt% studies were only performed with **NG4-NG6**; 1 wt% data not shown).

Dye Loading	NG2	NG3	NG4	NG5	NG6
			Λ_c (h^{-1})		
0.5 wt%	0.050	0.018	0.005	0.005	0.000
1 wt%	-----	-----	0.023	0.002	0.000
2 wt%	0.434	0.126	0.051	0.036	0.024
10 wt%	-----	-----	0.100	0.173	0.308

Table S3. Calculated dye loading amounts (mg) of Dil and DiO for each sample based on the recorded absorbance values of the purified nanogels. Dye amounts were calculated using extinction coefficients that were determined in DMF ($\epsilon_{553} = 132,000 \text{ M}^{-1} \text{ cm}^{-1}$ for Dil; $\epsilon_{553} = 137,700 \text{ M}^{-1} \text{ cm}^{-1}$ for DiO) using dye stock solutions of known concentration. These extinction coefficients were used as the UV-vis spectra of the nanogel encapsulated dyes were observed to most closely match those obtained in DMF.

Sample	10 wt% Dye Feeding				2 wt% Dye Feeding				0.5 wt% Dye Feeding			
	A_{553}	Dil(mg)	A_{491}	DiO(mg)	A_{553}	Dil(mg)	A_{491}	DiO(mg)	A_{553}	Dil(mg)	A_{491}	DiO(mg)
NG2	0.273	0.077	0.103	0.026	0.267	0.037	0.136	0.017	0.064	0.009	0.049	0.006
NG3	0.315	0.089	0.233	0.060	0.273	0.038	0.233	0.030	0.075	0.011	0.067	0.009
NG4	0.343	0.097	0.270	0.069	0.293	0.041	0.243	0.031	0.080	0.011	0.068	0.009
NG5	0.455	0.129	0.376	0.096	0.322	0.045	0.270	0.034	0.081	0.012	0.069	0.009
NG6	0.514	0.145	0.429	0.110	0.306	0.043	0.256	0.033	0.080	0.011	0.070	0.009

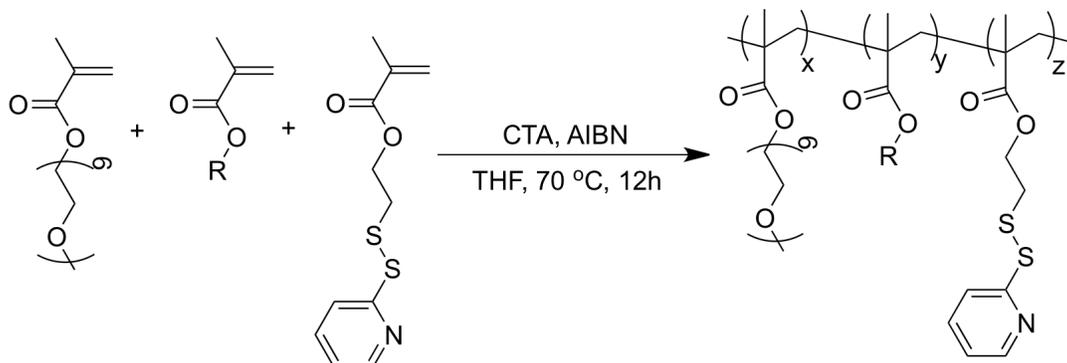
Table S4. Crosslinking efficiencies for the nanogel syntheses calculated using a theoretical yield (based on the %wt of PDS in each polymer and a total polymer amount of 2 mg) and the production of pyridinethione as monitored by its UV-vis spectrum ($\epsilon_{343} = 8.08 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$).

Sample	% wt PDS	Theoretical Yield	A_{343}	Yield	Efficiency
NG2	13.7 %	0.119 mg	0.232	0.064 mg	53%
NG3	11.8%	0.103 mg	0.163	0.045 mg	44%
NG4	12.2 %	0.106 mg	0.192	0.053 mg	50%
NG5	11.4%	0.100 mg	0.143	0.039 mg	40%
NG6	12.9%	0.113 mg	0.222	0.061 mg	54%

Materials and Methods

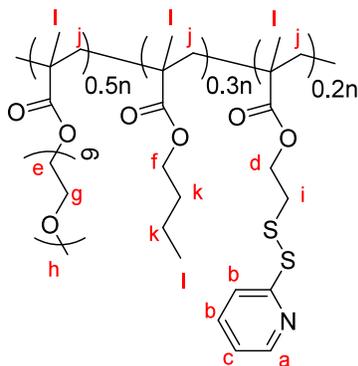
2,2'-Dithiodipyridine, 2-mercaptoethanol, polyethylene glycol monomethyl ether methacrylate (MW 450), butyl methacrylate, hexyl methacrylate, octyl methacrylate, decyl methacrylate, D,L-dithiothreitol (DTT), 1,1'-dioctadecyl-3,3',3'-tetramethylindocarbocyanine perchlorate (DiI), 3,3'-dioctadecyloxacarbocyanine perchlorate (DiO), pyrene, and other conventional reagents and solvents were obtained from commercial sources and were used as received unless otherwise mentioned. Polymers **P1-P6** were synthesized by RAFT polymerization and purified by precipitation. S-dodecyl-S'-2-(2,2-dimethylacetic acid) trithiocarbonate¹ and pyridyldisulfide ethylmethacrylate² (PDSEMA) were prepared using previously reported procedures. ¹H-NMR spectra were recorded on a 400 MHz Bruker NMR spectrometer using the residual proton resonance of the solvent as the internal standard. Chemical shifts are reported in parts per million (ppm). Molecular weights of the polymers were estimated by gel permeation chromatography (GPC) using a PMMA standard with a refractive index detector. Dynamic light scattering (DLS) measurements were performed using a Malvern Nanozetasizer. UV-visible absorption spectra were recorded on a Varian (model EL 01125047) spectrophotometer. Fluorescence spectra were recorded from a JASCO FP-6500 spectrofluorimeter. Transmission electron microscopy (TEM) images were taken from JEOL 100CX at 100 KV.

General Procedure for Random Copolymer Synthesis:



Briefly, a mixture of S-dodecyl-S'-2-(2,2-dimethylacetic acid) trithiocarbonate (CTA), PDSEMA, poly(ethylene glycol) methyl ether methacrylate (PEGMA, Mw 475), the alkyl chain derived methacrylate monomer and AIBN were dissolved in THF and degassed by performing three freeze-pump-thaw cycles. The reaction mixture was sealed, transferred to a pre-heated oil bath at 70 °C and stirred for 12 h. The resultant mixture was dissolved in dichloromethane (0.1 mL) and precipitated from hexanes (5 mL). To remove unreacted monomer, the precipitate was further dissolved in dichloromethane (0.1 mL) and precipitated in ether (5 mL). This precipitation procedure was repeated thrice to yield the random copolymer as a pale yellow, waxy substance.

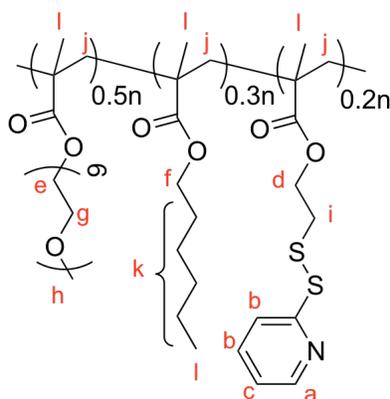
Synthesis of P1:



According to the general procedure for random copolymer synthesis, a mixture of CTA (14.0 mg, 0.04 mmol), PDSEMA (200 mg, 0.78 mmol), PEGMA (930 mg, 1.9 mmol), butylmethacrylate (170 mg, 1.2 mmol) and AIBN (1.3 mg, 7.8 μ mol) were polymerized in THF (2.6 mL). GPC (THF) *Mn*: 55K. PDI: 2.2. ^1H NMR (400 MHz, CDCl_3) δ 8.44 (a), 7.66 (b), 7.10 (c), 4.20 (d), 4.05 (e), 3.89 (f), 3.60 (g), 3.34 (h), 3.00 (i), 2.04-1.80 (j), 1.80-1.22 (k), 1.01-0.78 (l). The molar ratio between the three comonomers was determined by integrating the methoxy proton in the polyethylene glycol unit, the

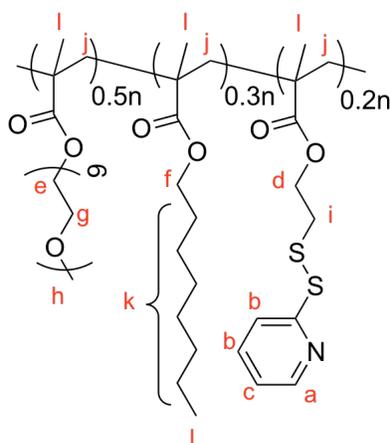
methoxy proton in alkyl derived methacrylate, and the aromatic proton in the pyridine and found to be 5.0 : 3.3 : 1.7 (PEO:ButylMA:PDSEMA).

Synthesis of P2:



According to the general procedure random copolymer synthesis, a mixture of CTA (14.0 mg, 0.04 mmol), PDSEMA (200 mg, 0.78 mmol), PEGMA (930 mg, 1.9 mmol), hexylmethacrylate (200 mg, 1.2 mmol) and AIBN (1.3 mg, 7.8 μ mol) were polymerized in THF (2.7 mL). GPC (THF) M_n : 38K. PDI: 1.5. ^1H NMR (400 MHz, CDCl_3) δ 8.48 (a), 7.70 (b), 7.13 (c), 4.22 (d), 4.08 (e), 3.91 (f), 3.64 (g), 3.37 (h), 3.04 (i), 2.10-1.60 (j), 1.40-1.20 (k), 1.10-0.78 (l). The molar ratio between the three comonomers was determined by integrating the methoxy proton in the polyethylene glycol unit, the methoxy proton in alkyl derived methacrylate, and the aromatic proton in the pyridine and found to be 4.9 : 3.3 : 1.8 (PEO:HexylMA:PDSEMA).

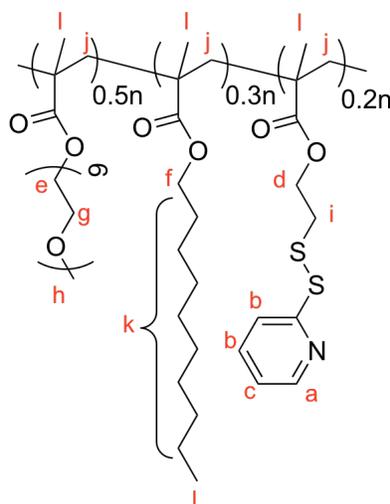
Synthesis of P3:



According to the general procedure for random copolymer synthesis, a mixture of CTA (14.0 mg, 0.04 mmol), PDSEMA (200 mg, 0.78 mmol), PEGMA (930 mg, 1.9 mmol), octylmethacrylate (230 mg, 1.2 mmol) and AIBN (1.3 mg, 7.8 μ mol) were polymerized in THF (2.7 mL). GPC (THF) M_n : 41K. PDI: 1.4. ^1H NMR (400 MHz, CDCl_3) δ 8.48 (a), 7.70 (b), 7.13 (c), 4.22 (d), 4.08 (e), 3.91 (f), 3.64 (g), 3.37 (h), 3.04 (i), 2.10-1.60 (j), 1.40-1.20 (k), 1.20-0.78 (l). The molar ratio between the three

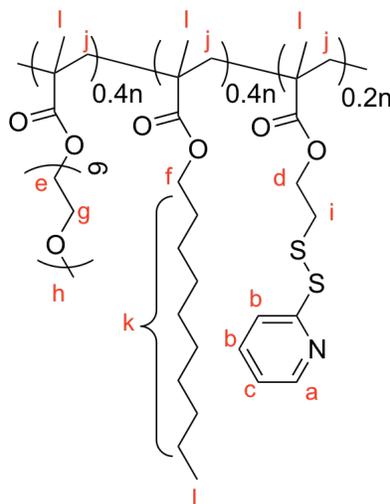
comonomers was determined by integrating the methoxy proton in the polyethylene glycol unit, the methoxy proton in alkyl derived methacrylate, and the aromatic proton in the pyridine and found to be 5.0 : 3.4 : 1.6 (PEO:OctylMA:PDSEMA).

Synthesis of P4:



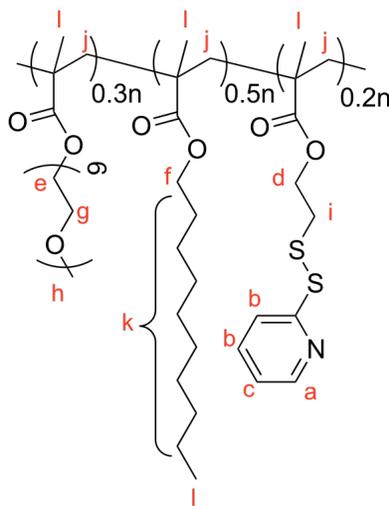
According to the general procedure for random copolymer synthesis, a mixture of CTA (14.0 mg, 0.04 mmol), PDSEMA (200 mg, 0.78 mmol), PEGMA (930 mg, 1.9 mmol), decylmethacrylate (270 mg, 1.2 mmol) and AIBN (1.3 mg, 7.8 μ mol) were polymerized in THF (2.8 mL). GPC (THF) M_n : 49K. PDI: 2.1. ^1H NMR (400 MHz, CDCl_3) δ 8.45 (a), 7.66 (b), 7.10 (c), 4.19 (d), 4.05 (e), 3.88 (f), 3.61 (g), 3.35 (h), 3.04 (i), 2.10-1.60 (j), 1.50-1.20 (k), 1.10-0.70 (l). The molar ratio between the three comonomers was determined by integrating the methoxy proton in the polyethylene glycol unit, the methoxy proton in alkyl derived methacrylate, and the aromatic proton in the pyridine and found to be 5.0 : 3.3 : 1.7 (PEO : DecylMA : PDSEMA).

Synthesis of P5:



According to the general procedure for random copolymer synthesis, a mixture of CTA (14.0 mg, 0.04 mmol), PDSEMA (200 mg, 0.78 mmol), PEGMA (740 mg, 1.6 mmol), decylmethacrylate (350 mg, 1.6 mmol) and AIBN (1.3 mg, 7.8 μ mol) were polymerized in THF (2.6 mL). GPC (THF) M_n : 42K. PDI: 2.0. $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 8.43 (a), 7.65 (b), 7.09 (c), 4.18 (d), 4.04 (e), 3.72 (f), 3.70 (g), 3.33 (h), 3.04 (i), 2.10-1.55 (j,k), 1.48-1.20 (k), 1.10-0.70 (l). The molar ratio between the three comonomers was determined by integrating the methoxy proton in the polyethylene glycol unit, the methoxy proton in alkyl derived methacrylate, and the aromatic proton in the pyridine and found to be 4.2 : 4.3 : 1.5 (PEO:DecylMA:PDSEMA).

Synthesis of P6:



According to the general procedure for random copolymer synthesis, a mixture of CTA (14.0 mg, 0.04 mmol), PDSEMA (200 mg, 0.78 mmol), PEGMA (560 mg, 1.2 mmol), decylmethacrylate (440 mg, 2.0 mmol) and AIBN (1.3 mg, 7.8 μ mol) were polymerized in THF (2.4 mL). GPC (THF) M_n : 52K. PDI: 2.2. $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 8.45 (a), 7.65 (b), 7.09 (c), 4.19 (d), 4.05 (e), 3.88 (f), 3.61 (g), 3.34 (h), 3.00 (i), 2.10-1.50 (j,k), 1.40-1.20 (k), 1.10-0.80 (l). The molar ratio between the three comonomers was determined by integrating the methoxy proton in the polyethylene glycol unit, the methoxy proton in alkyl derived methacrylate, and the aromatic proton in the pyridine and found to be 3.4 : 5.0 : 1.6 (PEO:DecylMA:PDSEMA).

Synthesis of dye-containing nanogels:

Polymers **P1-P6** (2 mg) and DiI, DiO or pyrene (0.01 mg for 0.5 wt%, 0.02 mg for 1 wt%, 0.04 mg for 2 wt% and 0.2 mg for 10 wt%) were dissolved in acetone and a calculated amount (50 mol% to PDS) of DTT was added. After stirring for 10 min, 1.75 mL of MilliQ water was added and the solution was stirred for 24 hours at room temperature, open to the atmosphere to allow for acetone removal. Unencapsulated DiI/DiO was removed by filtration through a 0.45 μm disposable filter. Remaining solution was washed with 0.25 mL of MilliQ water which was also passed through the filter to dilute the final nanogel solution to 1 mg mL^{-1} . Pyridothione and other soluble impurities were removed from the

nanogel solution by dialysis against deionized water for 48 hours using a membrane with a MW cutoff of 7,500 g/mol. After dialysis, all samples were transferred to 5 mL glass vials, which were capped, wrapped in foil and stored at 4° C until mixing experiments were performed. All samples used for these studies were stored for at least one week before mixing studies were performed.

DLS measurement:

Dynamic light scattering experiments were performed using a Malvern Nanozetalyzer. The light source used was a 4 mW He-Ne laser operating at 632.8 nm. The nanogels in deionized water (1mg/mL) were kept at a constant 25 °C throughout the measurement. Dust was eliminated by filtering the solutions through 0.45 µm filters. All the measurements were done at a correlation time of 30 seconds.

Nanogel encapsulated dye mixing:

A solution of nanogel encapsulating DiO (100 µL of 1 mg mL⁻¹ stock) was diluted with MilliQ water (800 µL) in a 1.5 mL fluorescence cuvette. To this solution, nanogel encapsulating DiI (100 µL of 1 mg mL⁻¹ stock) was added. The fluorescence intensities were recorded using an excitation wavelength of 450 nm. Excitation and emission slit widths were varied between the different %wt loading series to obtain in range fluorescence spectra.

References

1. Lai, J. T.; Filla, D.; Shea, R. Functional Polymer from Novel Carboxyl-Terminated Trithiocarbonates as Highly Efficient RAFT Agents. *Macromolecules* **2002**, *35*, 6754-6756.
2. Ghosh, S.; Basu, S.; Thayumanavan, S. Simultaneous and Reversible Functionalization of Copolymers for Biological Applications. *Macromolecules* **2006**, *39*, 5595-5597.