

Supporting Information for:

**Photo-induced Heterodisulfide Metathesis for Reagent-free
Synthesis of Polymer Nanoparticles**

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Fig. S1 GPC traces for the polymers

Fig. S2 NMR Spectra of the polymers

Fig. S3 Time-dependent ^1H NMR spectra with different concentrations

Fig. S4 Concentration dependent of reaction rate of PDS-OH under photoirradiation

Fig. S5 Time-dependent ^1H NMR spectra of the photo-induced disulfide metathesis of 2-hydroxyethyl 2-pyridyl disulfide (PDS-OH, 1) under anaerobic condition.

Fig. S6 Time-dependent ^1H NMR spectra of the photo-oxidation of DPDS

Fig. S7 Time-dependent ^1H NMR spectra of the photo-oxidation of DPDS without O_2

Fig. S8 DLS sizes in hydrodynamic diameter

Fig. S9 Absorption spectra for the crosslinking density

Fig. S10 Emission spectra for dye release and FRET

Table S1. Calculated crosslinking densities for nanogels

Scheme S1. Photooxidation of 2,2'-dipyridyldisulfide (DPDS) into pyridine-2-sulfonic acid

Scheme S2. Photo-induced reaction of PDS-OH under unaerobic condition.

Experimental Section

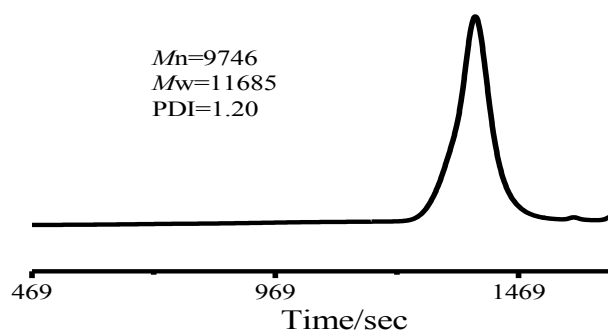


Fig. S1 Evolution of GPC traces for polymer

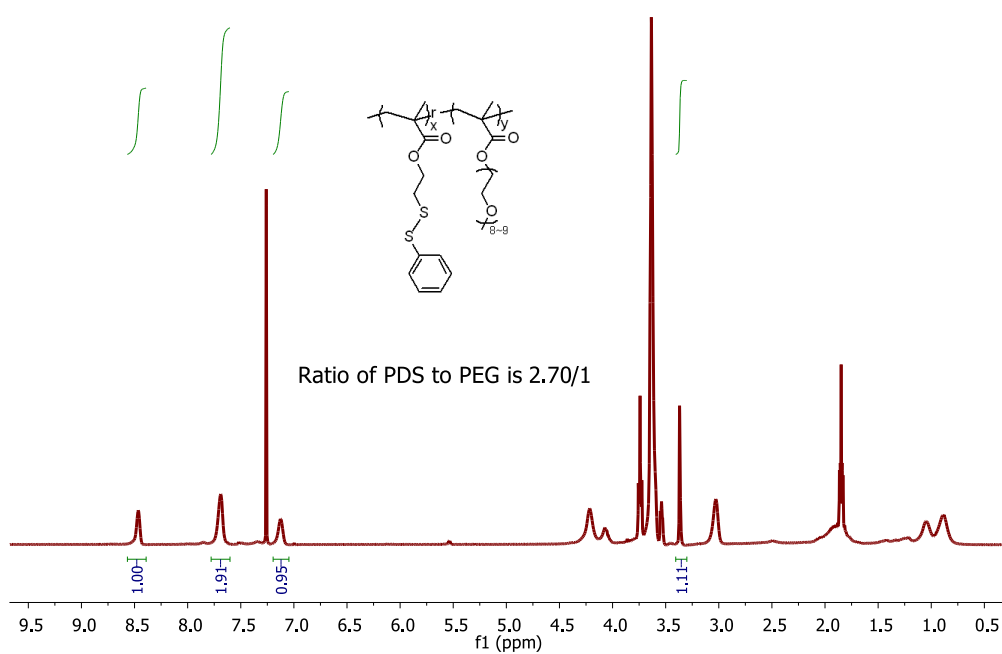
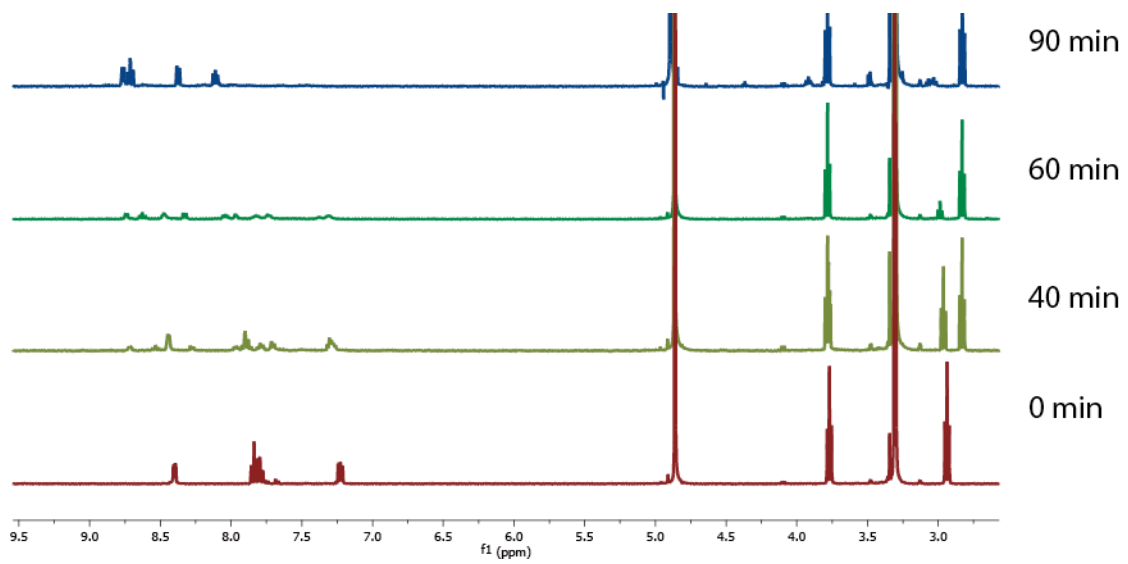
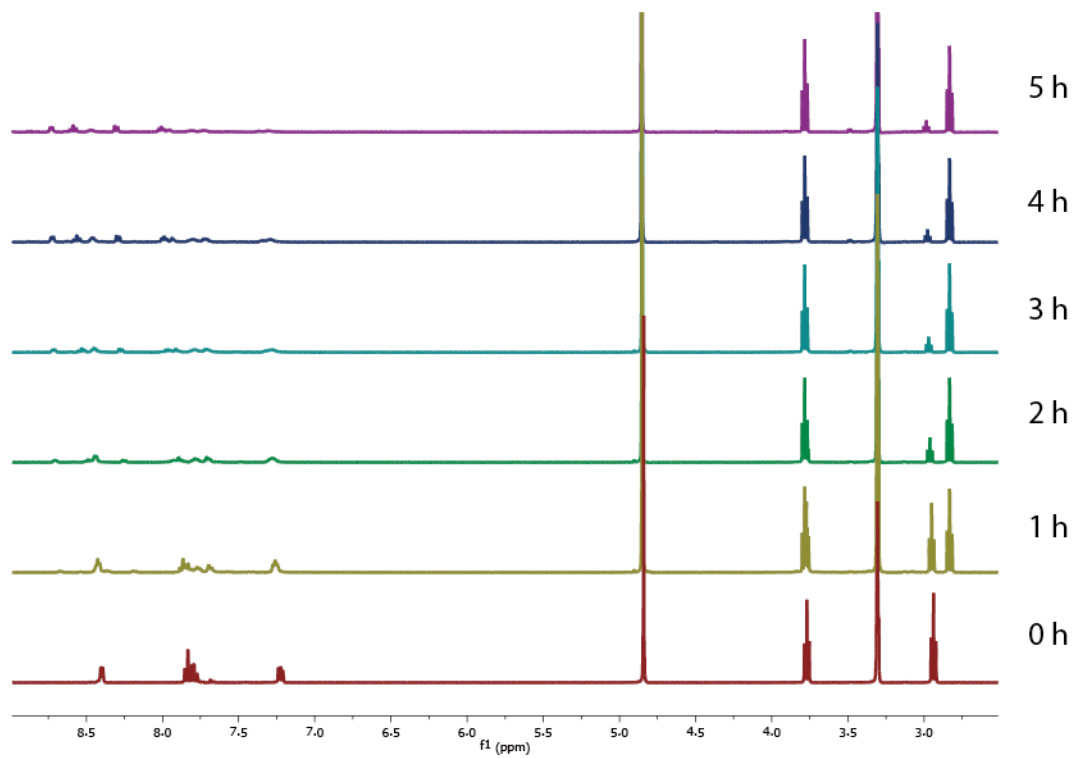
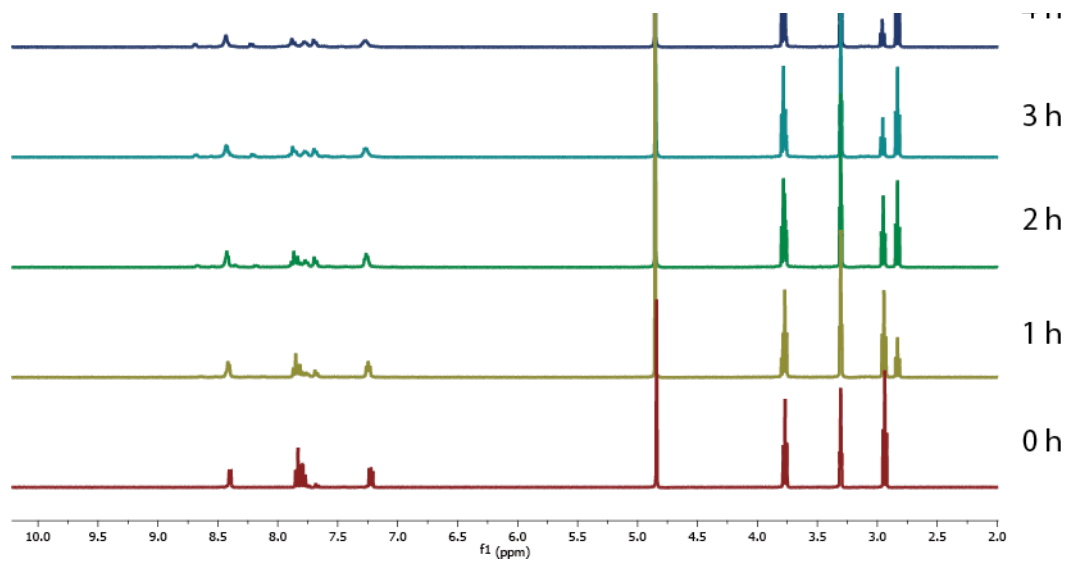


Fig. S2 NMR Spectra of the polymers

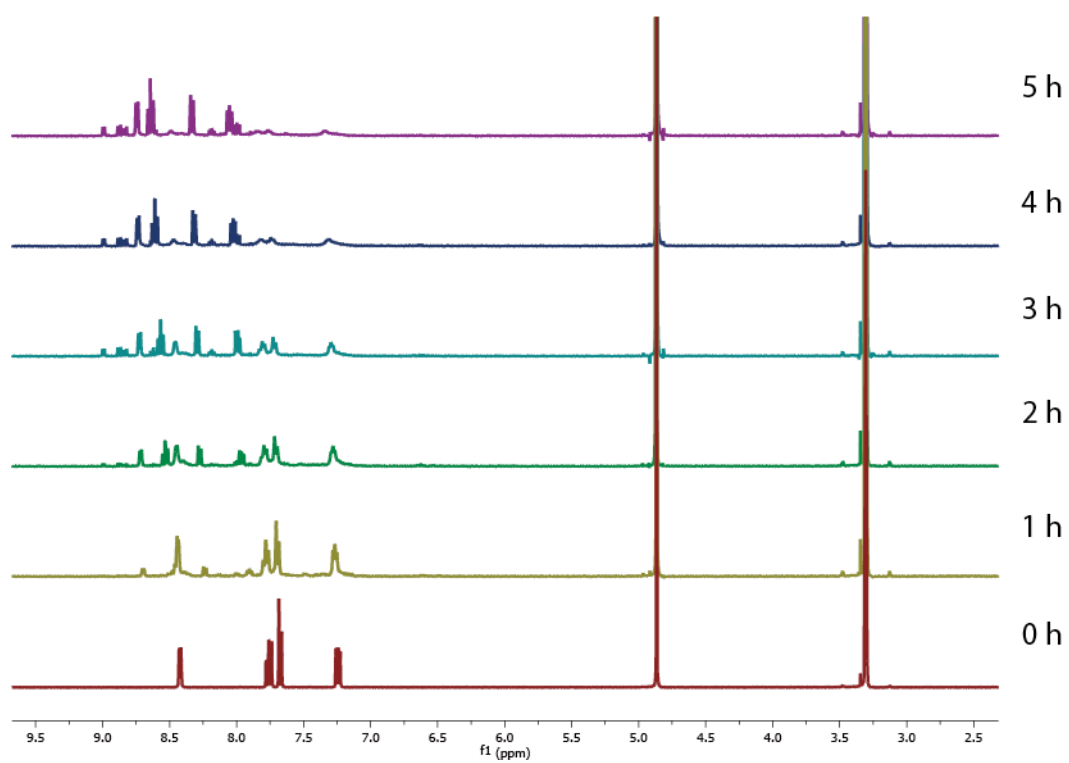


5 mg/mL PDS under photoirradiation





2.5 mg/mL DPDS under photoirradiation



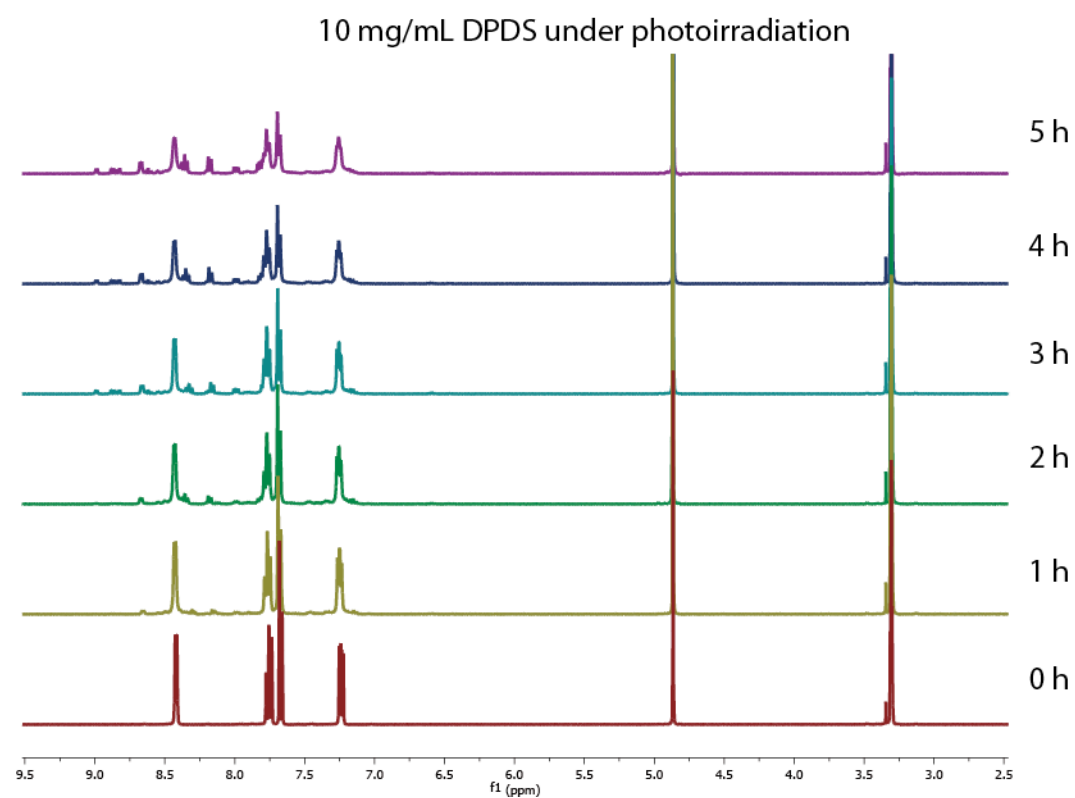
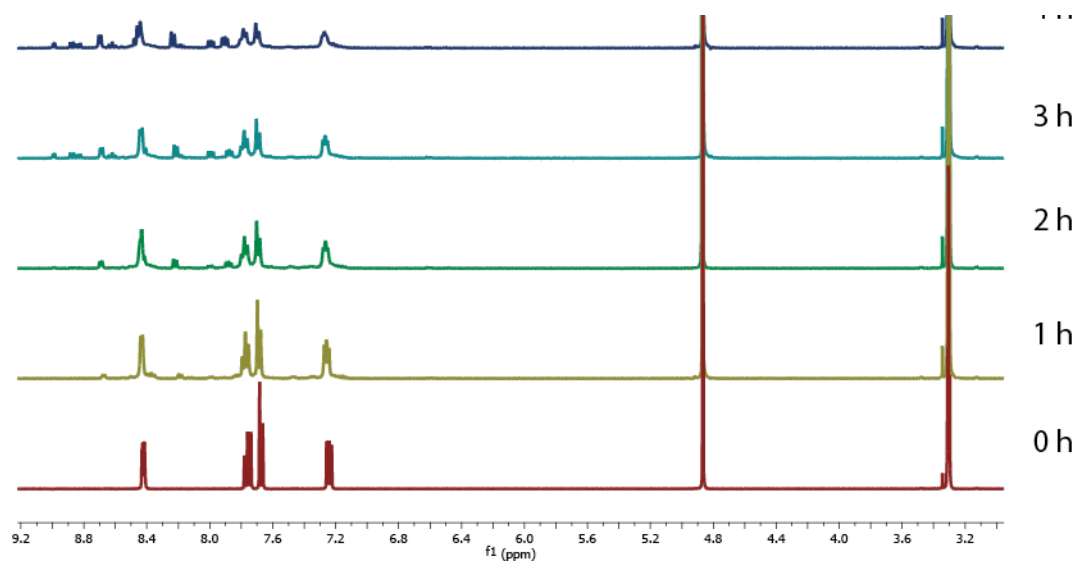


Fig. S3 Time-dependent ^1H NMR spectra of PDS and DPDS with different concentrations.

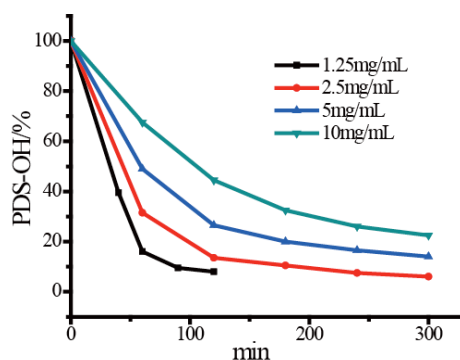


Fig. S4 Concentration dependent of reaction rate of PDS under photoirradiation.

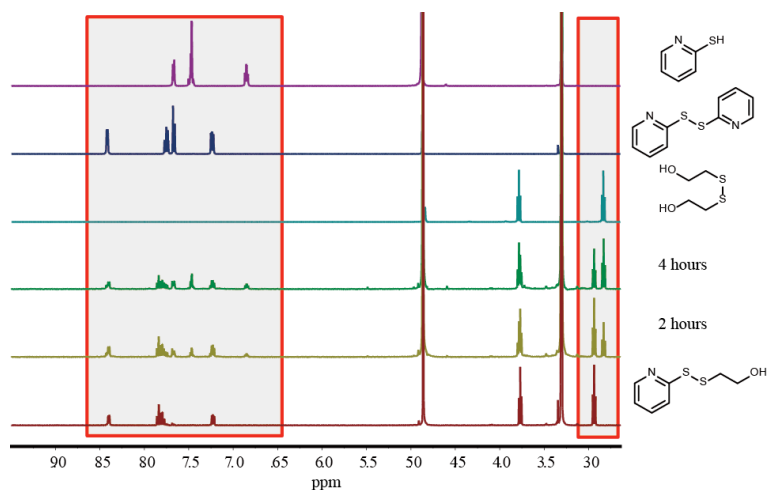


Fig. S5 Time-dependent ^1H NMR spectra of the photo-induced disulfide metathesis of 2-hydroxyethyl 2-pyridyl disulfide (PDS-OH, 1) under anaerobic condition. Control spectra of bis(2-hydroxyethyl) disulfide (2), 2, 2'-dipyridyl disulfide (DPDS, 3) and 2-thiopyridone were also included. The concentration was 2.5 mg/mL.

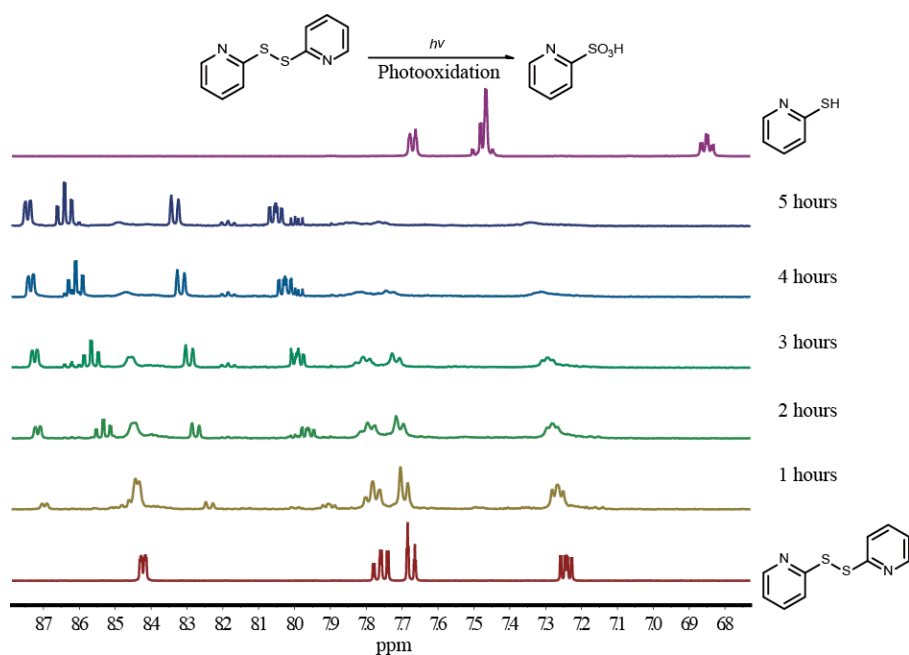


Fig. S6 Time-dependent ^1H NMR spectra of the photooxidation of 2, 2'-dipyridyl disulfide (DPDS).

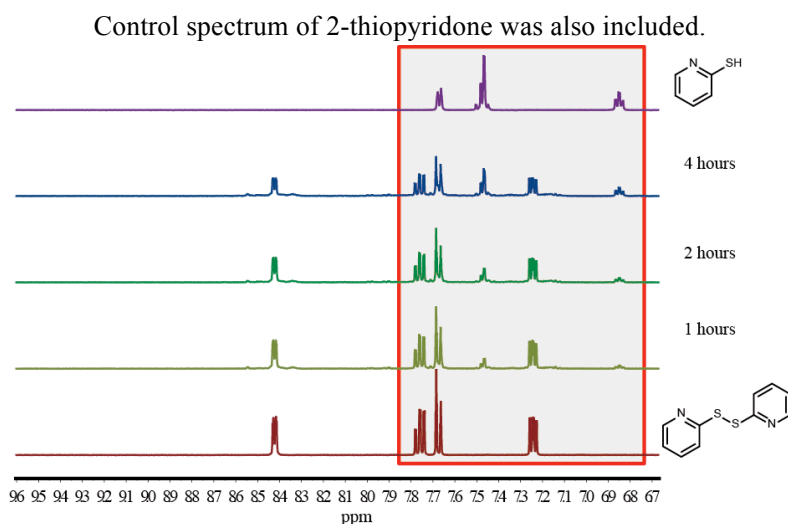


Fig. S7 Time-dependent ^1H NMR spectra of DPDS under the photoirradiation without O_2

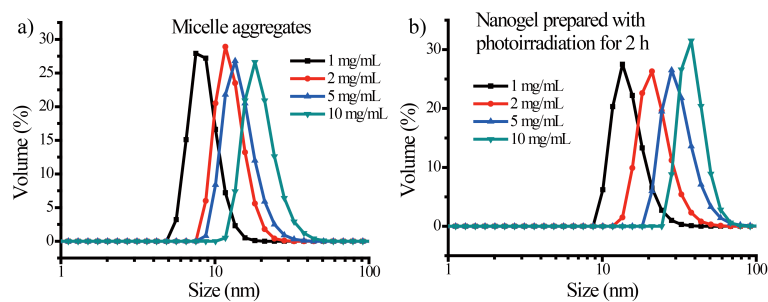


Fig. S8 DLS sizes in hydrodynamic diameter, a) micelle aggregates prepared with polymer solutions with different concentrations, b) nanogels prepared with polymer solutions with different concentrations, the photoirradiation time was 2 h for all samples.

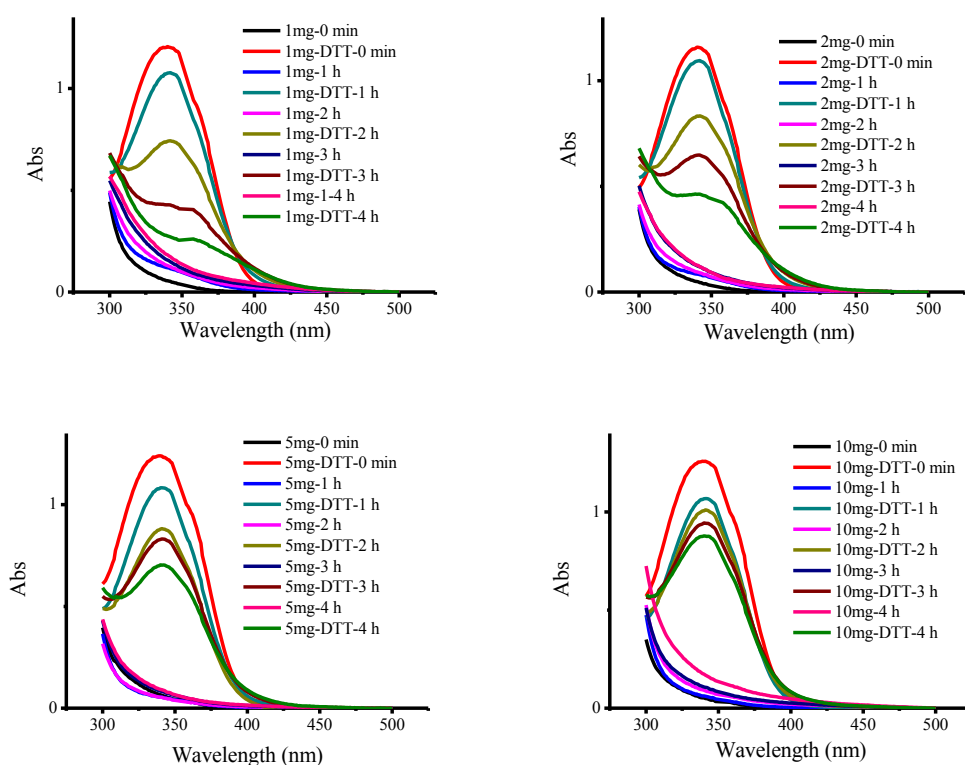


Fig. S9 Absorption spectra for the crosslinking density

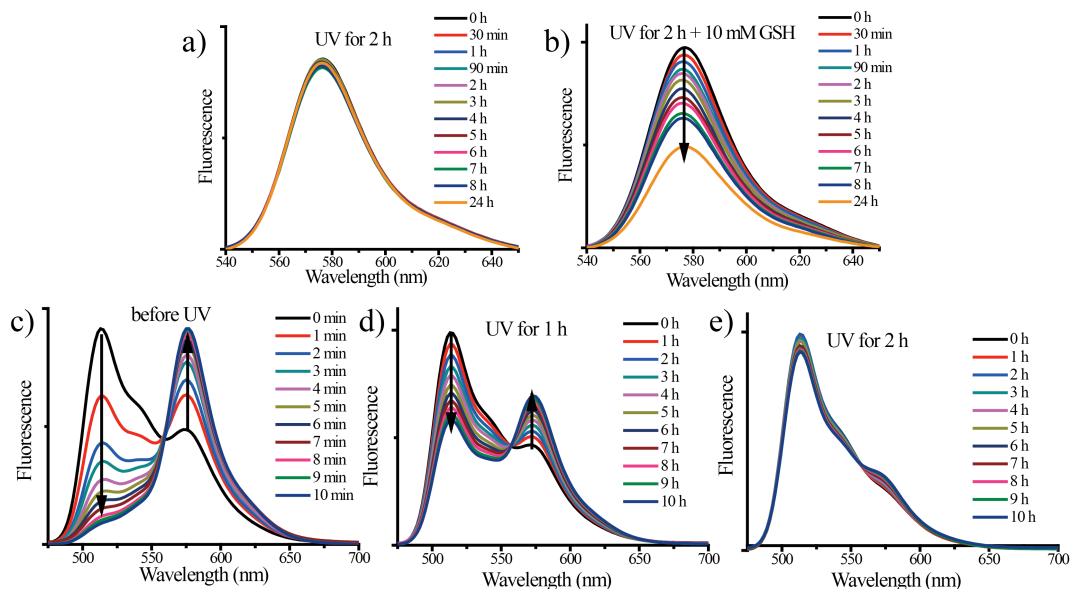
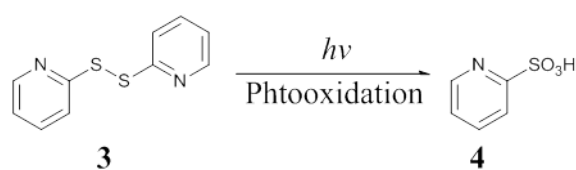


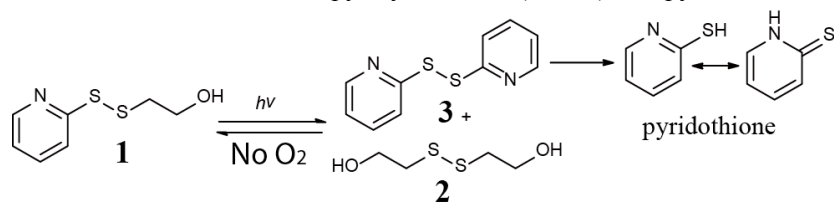
Fig. S10 Dye release from the nanogels prepared via photoirradiation for 2 h in response to varied GSH concentrations, a) 0 mM, b) 10 mM. Sample concentration was 0.1 mg/mL. FRET experiment based DiI/DiO exchange, c) micelle aggregates, d) nanogels prepared via photoirradiation for 1 h, e) nanogels prepared via photoirradiation for 2 h. Sample concentration was 0.1 mg/mL.

Table S1. Calculated crosslinking densities for nanogels

concentration	Crosslinking density/%			
	1 h	2 h	3 h	4 h
1 mg/mL	16	46	76	91
2 mg/mL	13	33	52	69
5 mg/mL	10	29	36	48
10 mg/mL	7	23	30	38



Scheme S1. Photooxidation of 2,2'-dipyridyldisulfide (DPDS) into pyridine-2-sulfonic acid.



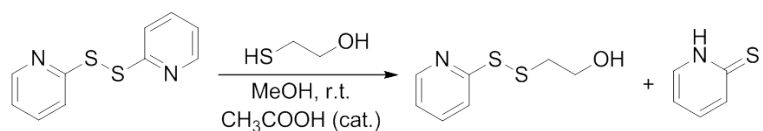
Scheme S2. Photo-induced reaction of PDS-OH under anaerobic condition.

Experimental Section

General Methods.

2,2-Dithiodipyridine, 2-mercaptoethanol, polyethyleneglycol monomethylether methacrylate (PEGMA, MW 450), D,L-dithiothreitol (DTT), 1,1'-dioctadecyl-3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) and 3,3'-dioctadecyloxacarbocyanine perchlorate (DiO), 4-Cyano-4-(phenylcarbonothioylthio)-pentanoic acid and other conventional reagents were obtained from commercial sources and were used as received without further purification. The 2,2'-azobisisobutyronitrile (AIBN) was purified by recrystallization from ethanol. 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 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. The fluorescence spectra were obtained from a JASCO FP-6500 spectrofluorimeter.

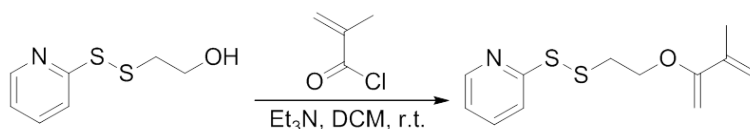
Synthesis of 2-hydroxyethyl 2-pyridyl disulfide (PDS-OH) and 2-thiopyridone



PDS-OH was synthesized according to the previous report.^[1] 2, 2'-dipyridyl disulfide (DPDS) (10.3 g, 0.047 mol) was dissolved in 100 mL of methanol. Then 1.2 mL of glacial acetic acid, used as catalyst, was added. To this mixture, a solution of mercaptoethanol (4.4 g, 0.056 mol, 4 mL) in 100 mL methanol was added drop-wise at room temperature with continuous stirring. Once the addition was over, the reaction mixture was stirred at room temperature overnight. The stirring was stopped; solvent was evaporated to get the crude product as yellow oil which was purified by flash column chromatography using silica gel as stationary phase and mixture of ethyl acetate/hexane as eluent. When the polarity of the eluent was increased to 40% ethyl acetate/hexane, the desired product came out as colorless oil. Yield : 80 % ¹H NMR: (CD₃OD, 400 MHz), δ (ppm): 8.40 (m, 1H, aromatic proton ortho-N), 7.84 (m, 1H, aromatic proton meta-N), 7.80 (m, 1H, aromatic proton para-N), 7.22 (m, 1H, aromatic proton, ortho-disulfide linkage), 3.77(t, 2H, -CH₂OH), 2.94 (t, 2H, -SS-CH₂-). ¹³C-NMR (CD₃OD, 100MHz) δ (ppm): 160.01, 148.93, 137.69, 121.03, 120.11, 59.19, 41.20.

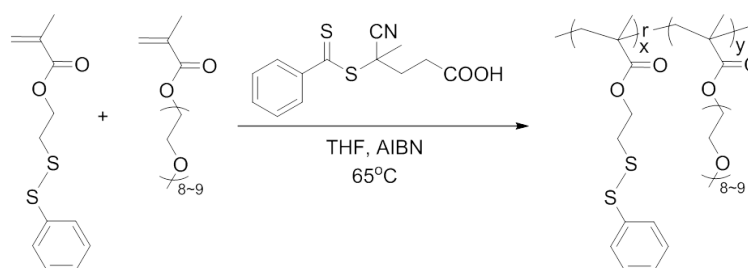
2-thiopyridone can also be prepared at the same time by continually increasing the polarity of the eluent up to 55% ethyl acetate/hexane to get the desired product. After concentrating the solution, light yellow Needle-shaped crystals were got. ¹H NMR: (CD₃OD, 400 MHz), δ (ppm): 7.67 (m, 1H), 7.47 (m, 2H), 6.85 (m, 1H). ¹³C-NMR (CD₃OD, 100MHz) δ (ppm): 177.40, 138.00, 137.25, 133.08, 113.57.

Synthesis of PDS monomer



PDS monomer was also synthesized according to the previous report.^[1] To a solution of PDS-OH (12 g, 64.1 mmol) in 50 mL of dry dichloromethane was added 7.8 g (76.9 mmol) of triethylamine. The mixture was firstly cooled in an ice-bath. To this cold mixture, a solution of methacryloyl chloride (6.7 g, 64.1 mmol) in 25 mL dichloromethane was then added drop-wise with continuous stirring. After the addition was over the reaction mixture was stirred at room temperature overnight. The stirring was stopped and the reaction mixture was washed with 3x100 mL distilled water and then with 100 mL of brine. The organic layer was collected, dried over anhydrous Na₂SO₄ and concentrated to get the crude product as yellow oil. It was purified by column chromatography using silica gel as stationary phase and mixture of ethyl acetate/hexane as eluent. The pure product was collected at 30 % ethylacetate /hexane. Yield: 80 % ¹H NMR: (CDCl₃, 400 MHz), δ (ppm): 8.52 (m, 1H, aromatic proton ortho-N), 7.62-7.83 (m, 2H, aromatic proton meta-N and para-N), 7.15 (m, 1H, aromatic proton, orthodisulfide linkage), 6.23 (d, 1H, vinylic proton, cis-ester), 5.65(d, 1H, vinylic proton, trans-ester) 4.41 (t, 2H, -S-S-CH₂CH₂O-), 3.13 (t, 2H, -S-S-CH₂CH₂O-), 1.91(s, 3H, methyl proton of the methacryloyl group). ¹³C-NMR (CD₃OD, 100MHz) δ(ppm): 167.06, 159.76, 149.73, 137.05, 135.96, , 126.05, 120.85, 119.77, 62.41, 37.44, 18.28.

Synthesis of random copolymer containing PDS groups



Random copolymers were prepared by reversible addition–fragmentation chain transfer (RAFT) polymerization.^[2] A mixture of 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (24 mg, 0.086 mmol), poly(ethylene glycol) monomethyl ether methacrylate (0.4 g, 0.84 mmol), PDS (0.537 g, 2.1 mmol), and 2,2'-azobis(isobutyronitrile) (AIBN; 2.8 mg, 0.017 mmol) was dissolved in tetrahydrofuran (THF) (2 mL) and degassed by performing three freeze–pump–thaw cycles. The reaction mixture was sealed and then transferred into a preheated oil bath at 65 °C for 10 h. To remove unreactive monomers, the resultant mixture was precipitated in cold ethyl ether (20 mL) to yield the random copolymer as a waxy liquid. GPC (THF) *M_w*, 12 kDa; polydispersity, 1.2. ¹H NMR (400 MHz, CDCl₃) δ 8.46, 7.68, 7.11, 4.35–4.09, 3.94–3.37, 3.03, 2.04–1.64, 1.43–0.87. The molar ratio between two blocks was determined by integrating the methoxy proton in the poly-(ethylene glycol) unit and the aromatic proton in the pyridine and was found to be 1:2.70 (PEO:PDS).

Time-dependent ¹H NMR measurement

In 5mm NMR tubes, 1 mL of samples with different concentrations was added. ¹H-NMR spectra were recorded on a 400 MHz Bruker NMR spectrometer using the residual proton resonance of the solvent as the internal standard. These tubes were put inside UV chamber (SYLVANIA F15T8/350/BL, 15w). After each 1 hour's photoirradiation, another NMR spectrum for each sample was taken with the same parameters. The integrations of peak at 2.94 ppm and peak at 2.83 ppm were used to determine the percent of remaining PDS-OH.

Nanoparticles preparation

1 mL of polymer solution of different concentrations was added to a 7 mL vial. These vials were then put inside an UV chamber (SYLVANIA F15T8/350/BL, 15w). After photoirradiation for certain times, nanoparticles with different sizes were formed in solutions. The crosslinking density could be determined by taking some volume of polymer solution from the above solutions into cuvettes for UV-vis measurement. The concentration of these samples was all 0.1 mg/mL. Absorption spectra were taken first. Then, excess DTT were added into these samples and then the solution was observed using absorption spectroscopy again after 3 hours, during which all PDS functionalities could be changed to 2-thiopyridine by DTT.

Determination of crosslinking density

In 7mL small glass vial, 1 mL of polymer solution with different concentrations was added. From them, certain volume of polymer solution was taken and then added into cuvettes for UV-vis measurement. The volume was made to 1 mL by adding water. The concentration of these samples was all 0.1 mg/mL. Absorption spectra were taken. Excess DTT were added into these samples, one more absorption spectra were taken after 3 hours, during which all PDS functionalities could be changed to 2-thiopyridine by DTT.

Then these vials were put inside UV chamber (SYLVANIA F15T8/350/BL, 15w). After each 1 hour's photoirradiation, certain volume of polymer solution was taken from the above solutions and then added into cuvettes for UV-vis measurement. The volume was made to 1 mL by adding water. The concentration of these samples was all 0.1 mg/mL. Absorption spectra were taken. Excess DTT were added into these samples, one more absorption spectra were taken after 3 hours, during which all PDS functionalities could be changed to 2-thiopyridine by DTT.

Preparation of nanogels containing DiI/DiO



In 7mL small glass vial, polymer was dissolved in 1 mL water. Then calculated volume of DiI in acetone stock solution (5 mg/mL) or DiO in acetone stock solution (2.5 mg/mL) was added into the polymer solution. The final dye concentration was about 2 wt%. The mixed solution was stirred overnight at room temperature, open to the atmosphere allowing the organic solvent to evaporate. Then these vials were put inside UV chamber (SYLVANIA F15T8/350/BL, 15w). After 2 hour's photoirradiation, insoluble DiI/DiO was removed by filtration and the byproduct was removed from the nanogel solution by extensive dialysis using a membrane with a molecular weight cut-off of 7,000 g/mol. Finally, nanogels stock solutions with concentration of 1 mg/mL were prepared for further studies.

Redox-responsive guest release experiment

A solution of nanogel containing DiI (100 μ L) was mixed with water (800 μ L) in a cuvette, and then GSH stock solution (100 μ L) was added. The fluorescence spectra were recorded using the excitation wavelength of 530 nm.

For control experiment, A solution of nanogel containing DiI (100 μ L) was mixed with water (900 μ L) in a cuvette. The fluorescence spectra were recorded using the excitation wavelength of 530 nm.

FRET experiment

A solution of nanogel containing DiI (100 μ L) was mixed with a solution of nanogel containing DiO (100 μ L) in a cuvette, and then milliQ water (800 μ L) was added to adjust the volume. The fluorescence spectra were recorded using the excitation wavelength of 450 nm.

Reference

- [1] S. Ghosh, S. Basu, S. Thayumanavan, *Macromolecules* 2006, **39**, 5595-5597.
- [2] L. Li, J.-H. Ryu, S. Thayumanavan, *Langmuir* 2013, **29**, 50-55.