Supporting Information for:

Multi-Stimuli Responsive Amphiphilic Assemblies through Simple Post Polymerization Modification

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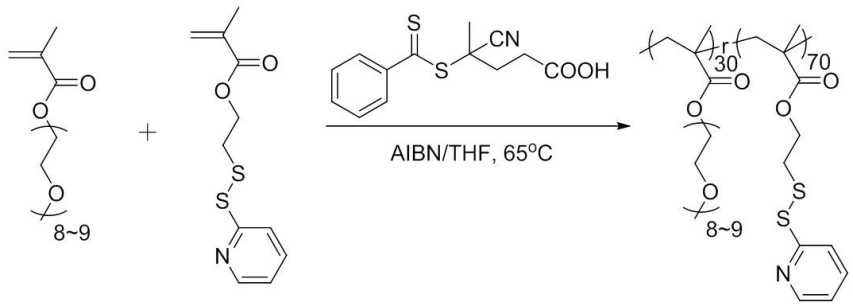
Materials and Methods: All the reagents were from commercial source and used as received unless otherwise mentioned. Pyridyl disulfide ethyl methacrylate (PDSMA) was synthesized according to previous report.[1] ^{1}H NMR spectra were recorded on a Bruker DPX-400 MHz NMR spectrometer using the residual proton resonance of the solvent as the internal standard. Gel permeation chromatography (GPC) was used to estimate the molecular weight of polymers using PMMA standard and THF as the elution. Dynamic light scattering (DLS) results were measured by a Malvern Nanozetasizer. UV-vis absorption spectra were obtained by a Carry 100 Scan spectrometer. Fluorescence spectra were recorded on a JASCO FP-6500 spectrofluorimeter.

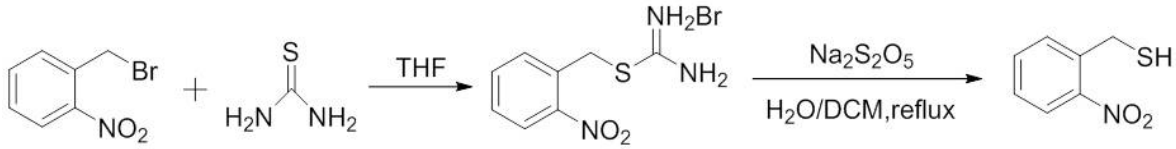
Synthesis of PDS-PEG polymer:



Scheme 1: Synthetic protocol of PDS-PEG polymer

A mixture of 4-cyano-4-(phenylcarbonothioylthio) pentanoic acid (27.9 mg, 0.1 mmol), PDSMA (535.5 mg, 2.1 mmol), polyethylene glycol monomethyl ether methacrylate (Average Mw: 500, 450mg, 0.9 mmol) and AIBN (3.28 mg, 0.02 mmol) was dissolved in 4 mL THF and degassed by three times of freeze-pump-thaw cycles. Then the reaction was transferred to a pre-heated oil bath (65^{o}C) and stirred for 24 hours under argon atmosphere. The polymer was precipitated in cold ethyl ether and washed with ethyl ether for several times to remove unreacted monomers. ^{1}H NMR spectrum of PDS-PEG was shown in Figure S1.

Synthesis of o-nitrobenzyl thiol:



Scheme 2: Synthetic protocol of o-nitrobenzyl thiol

O-nitrobenzyl thiol was prepared according to a literature.[2] A mixture of o-nitrobenzyl bromide (3.0 g, 13.9 mmol) and thiourea (1.2 g, 15.7 mmol) in 50 mL THF was stirred for overnight. The white precipitate was filtered, washed with ethyl acetate and dried under vacuum. Then 2.15 g of the white solid (7.4 mmol) was suspended in a mixture of 40 mL DCM and 30 mL water, and $Na_2S_2O_5$ (5.6 g, 28.9 mmol) was added. The suspension was refluxed for 4 hours under argon atmosphere and then cooled to room temperature. The organic phase was collected, dried with Na_2SO_4, and concentrated to get 1.1 g of product. 1H NMR (400MHz, $CDCl_3$) 8.00 (dd, 2H), 7.58(m, 1H), 7.39-7.49 (m, 2H), 4.00 (d, 2H), 2.14 (t, 1H). Yield: 88%.

Synthesis of polymer 1 (P1):

100 mg of PDS-PEG polymer was dissolved in 5 mL DCM, and then excess O-nitrobenzyl thiol (72 mg, 0.43 mmol, 2 eq. to PDS units) in 1 mL DCM was added. The solution was stirred for overnight, concentrated, and washed with excess amount of cold ethyl ether (or dialyzed against DCM) to remove undesired small molecules. ^{1}H NMR spectrum of P1 was shown as below:

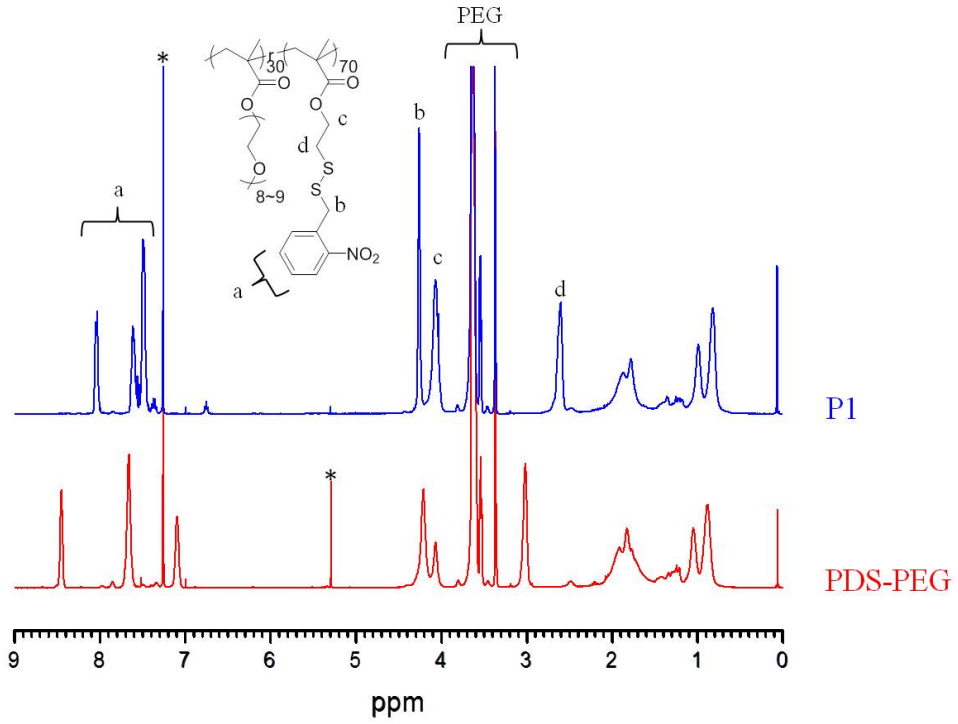
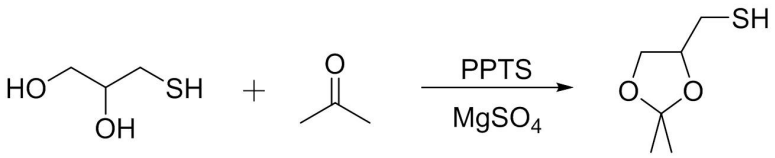


Figure S1: NMR spectra of PDS-PEG and **P1**. The change in peaks between 7~9 ppm indicates the replacement of pyridine group to nitrobenzyl group. The (*) indicates residual solvent peaks, which are $CDCl_3$ and DCM, respectively.

Synthesis of 2,2-dimethyl-1,3-dioxolane-4-methanethiol:



Scheme 3: Synthetic protocol of 2,2-dimethyl-1,3-dioxolane-4-methanethiol

The synthesis of this ketal containing thiol was reported previously.[3] Briefly, 3-Mercapto-1,2-propanediol (1.08 g, 10 mmol), pyridinium p-toluenesulfonate (PPTS, 0.25 g, 1 mmol) and 1 g of $MgSO_4$ were stirred in 10 mL acetone for two days. The insolubility was filtered, and the residual liquid was concentrated and purified by column chromatography (SiO_2) using hexane/ethyl acetate (9:1 v:v) as eluant to give 0.88 g of product. ^{1}H NMR (400MHz, $CDCl_3$) 4.08~4.21 (m, 2H), 3.77 (m, 1H), 2.75 (m, 1H), 2.61(m, 1H), 1.48 (t, 1H), 1.44 (s, 3H), 1.37 (s, 3H). Yield: 57%.

Synthesis of polymer 2 (P2):

100 mg of PDS-PEG polymer was dissolved in 5 mL DCM, and then excess (2,2-dimethyl-1,3-dioxolan-4-yl)methanethiol (64 mg, 0.43 mmol, 2 eq. to PDS units) in 1mL DCM was added. The solution was stirred for overnight, concentrated, and washed with excess amount of cold ethyl ether (or dialyzed against DCM) to remove undesired small molecules.

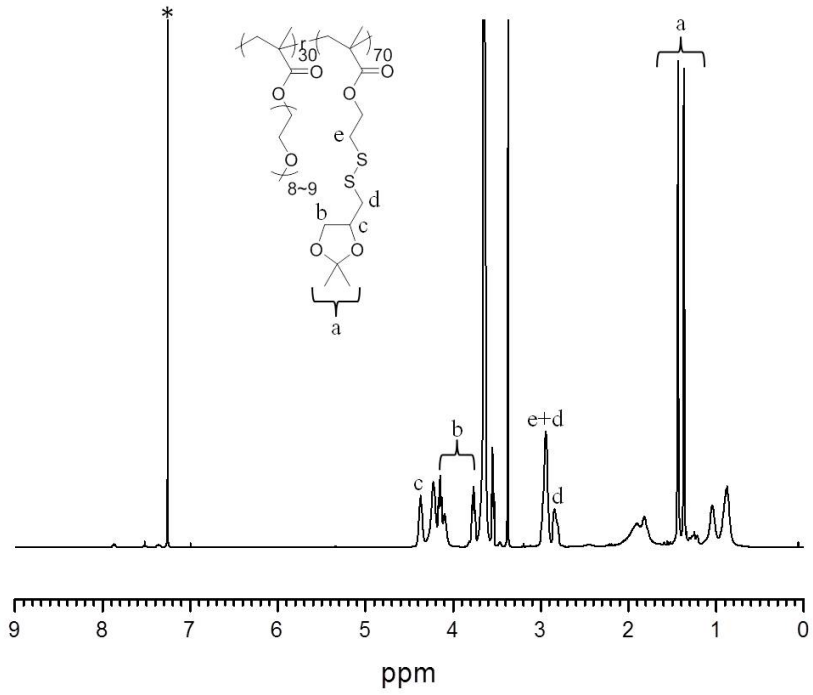


Figure S2: NMR spectrum of **P2**. The disappearances of peaks between 7~9 ppm and the peaks which are assigned as a indicated PDS are changed to ketal group. (*) is the residual solvent $CDCl_3$.

Synthesis of polymer 3 (P3):

P3 was synthesized by a two-step post polymerization modification method. 100 mg of PDS-PEG polymer was dissolved in 5 mL DMF, and a calculated amount of 3-mercaptopropionic acid (6.79 mg, 0.064 mmol, 0.3eq. to PDS units) in 1 mL DMF was added. The solution was stirred for overnight and then dialyzed against DI water to remove organic solvent and by-product. The aqueous media was removed by freeze-drying to obtain carboxyl modified polymer. The obtain polymer was then dissolved in 10 mL DMF, EDC·HCl (2 eq. to carboxyl group), N-hydroxysuccinimide (1.5 eq. to carboxyl group) was

added into the solution and stirred for 4h. 4-(2-aminoethyl) benzenesulfoamide (1.5 eq. to carboxyl group) was then added to the mixture and stirred for overnight. The solution was thoroughly dialyzed against DI water and lyophilized to get **P3**.

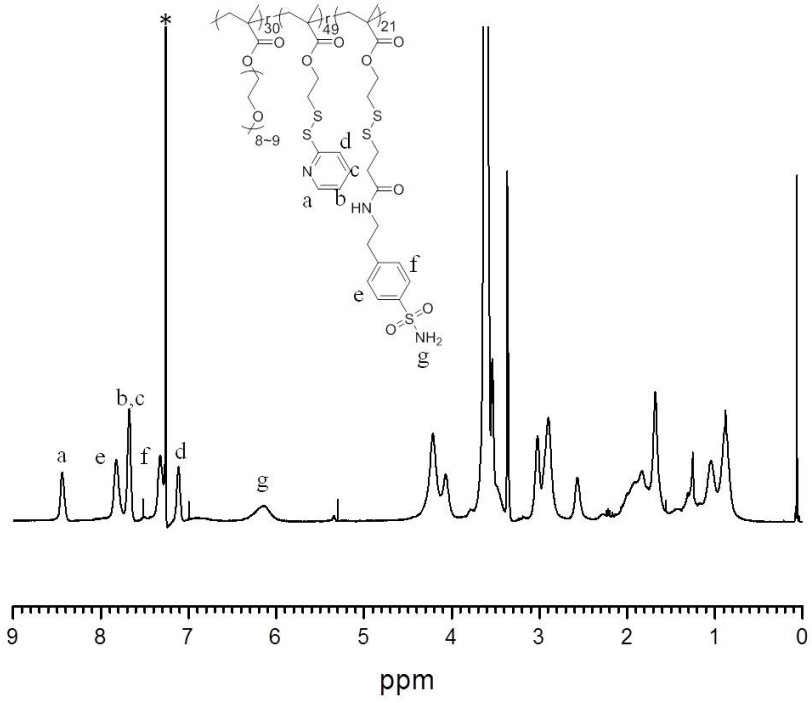


Figure S3: NMR spectrum of **P3**. The peaks which are assigned as a, b, c, d indicated part of PDS are still remained in polymer, which the peaks assigned as e, f, g were attributed to the benzene ring and sulfonamide. (*) is the residual solvent $CDCl_3$.

Synthesis of polymer 4 (P4):

100 mg of PDS-PEG polymer was dissolved in 5 mL DCM, and a calculated amount of o-nitrobenzyl thiol (25.75 mg, 0.152 mmol, 0.7eq. to PDS units) in 1 mL DCM was added. The solution was stirred for overnight and dialyzed against DCM. Then 3-mercaptopropionic acid (7.1 mg, 0.067 mmol, 0.315 eq. to PDS units) in 1 mL DCM was

added and stirred for overnight. The mixture was dialyzed against DCM, concentrated, and then redissolved in 10 mL DMF, and stirred with EDC·HCl (2 eq. to carboxyl group), N-hydroxysuccinimide (1.5 eq. to carboxyl group) for 4 h. Finally 4-(2-aminoethyl) benzenesulfoamide (1.5 eq. to carboxyl group) was added to the mixture and stirred for overnight. The solution was thoroughly dialyzed against DI water and lyophilized to get **P4**.

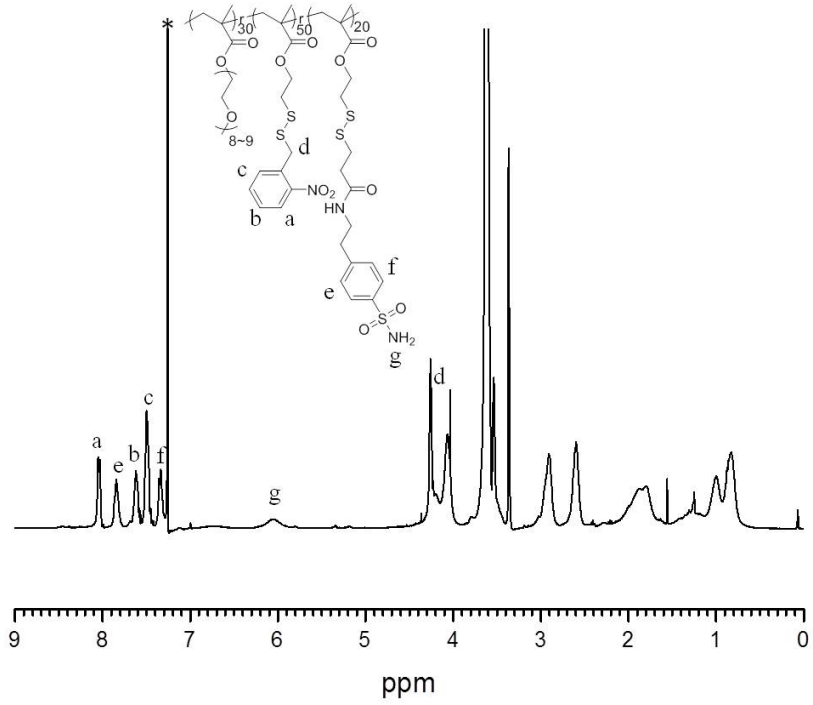


Figure S4: NMR spectrum of **P4**. The peaks which are assigned as a, b, c, d were attributed to o-nitrobenzyl groups, and the peaks which are assigned as e, f, g were attributed to the benzene ring and sulfoamide. (*) is the residual solvent $CDCl_3$.

DLS measurements:

10mg of **P1**, **P2**, **P3** or **P4** were dissolved in 1mL DI water, stirred at 4^{o}C for overnight and then stored in room temperature as stock solution.

Four vials were marked as sample 1, 2, 3, and 4, and each vial was added 50 μL **P1** stock solution. Sample 1 was added 950 μL DI water. Sample 2 was added 950 μL DI water and put into a UV chamber for 1h. Sample 3 was added 850 μL DI water and 100 μL of 100 mM GSH stock solution and sample 4 was added 850 μL DI water and 100 μL of 100 μM GSH stock solution. Sample 3 and 4 was incubated in room temperature for 24 h. Then the samples were transferred to cuvettes and the sizes of 4 samples were then monitored.

50 μL **P2** stock solutions were placed into two vials and then added 950 μL buffers with different pH (7.4 and 5). The samples were incubated in room temperature and the sizes were monitored over time.

50 μL **P3** stock solutions were placed into two vials, one was added 950μL DI water and the other was added 450 μL DI water and 500 μL of bCA-II stock solution (120 μM). The sizes were then measured after 30 h.

50 μL **P4** stock solutions were diluted with 950 μL DI water and the size was measured.

Nile Red encapsulation:

10 mg of PDS-PEG polymer, **P1**, **P2**, **P3** or **P4** were dissolved in 1 mL DI water, respectively. The polymer solutions were stirred in 4 ^{o}C for overnight. Then 40 μL Nile Red stock solution (5 mg/mL in acetone) was added in each polymer solution. The mixed solutions were stirred for 8 h in room temperature, open to the atmosphere allowing the

organic solvent to evaporate. The solutions were filtered through hydrophilic membranes with pore size of 0.45 μm to remove unencapsulated Nile Red before any experiment was performed.

Guest release study:

To test the redox sensitive properties of each polymer, 50 μL **P1**, **P2**, **P3** or **P4** solution with dye encapsulated were mixed with 850 μL DI water and 100 μL GSH stock solutions (100 mM or 100 μM) and the fluorescence spectra of Nile Red were recorded overtime.

50 μL **P1** solution with dye encapsulated was mixed with 950 μL DI water and placed inside photoreactor and irradiated with UV light at 365 nm (or without UV irradiation) for a certain time period and the fluorescence spectra of Nile Red were recorded overtime.

50 μL **P2** solution with dye encapsulated was mixed with 950 μL buffers with different pH (7.4, 5, and 4) and the fluorescence spectra of Nile Red for each sample were recorded overtime.

50 μL **P3** solution with dye encapsulated was mixed with 450 μL DI water and 500 μL bCA-II solution (120μM) or BSA solution (120μM). 50 μL PDS-PEG polymer solution with dye encapsulated was mixed with 450 μL DI water and 500 μL bCA-II solution (120μM). The fluorescence spectra of Nile Red were recorded overtime.

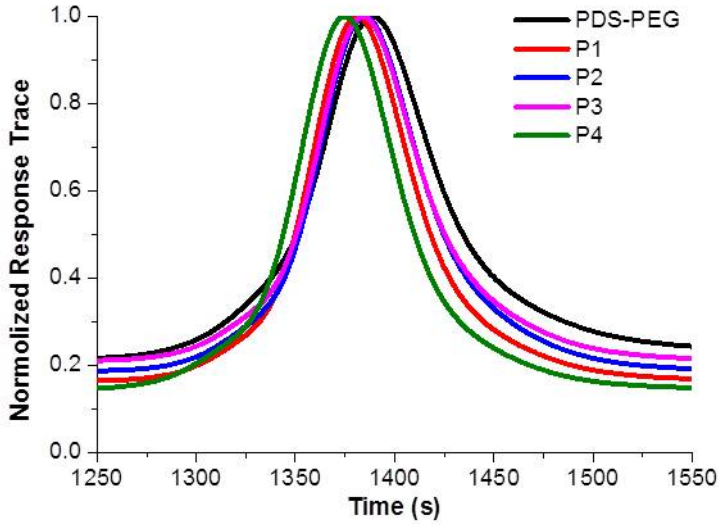
The fluorescence spectra of **P4** solution with dye encapsulated in different stimuli conditions was recorded overtime. The concentration of polymer was also 0.5 mg/mL.

The % release of Nile Red was calculated by using the following equations:

% Release of Nile Red = $(I_t-I_0)/I_t*100$

Where I_0 = Initial fluorescence intensity of Nile Red

I_t = fluorescence intensity of Nile Red at each time point



	PDS-PEG	**P1**	**P2**	**P3**	**P4**
Mw	8500	9500	9100	9200	10500
PDI	1.23	1.17	1.18	1.18	1.17

Figure S5: GPC traces of PDS-PEG polymer, **P1**, **P2**, **P3** and **P4**.

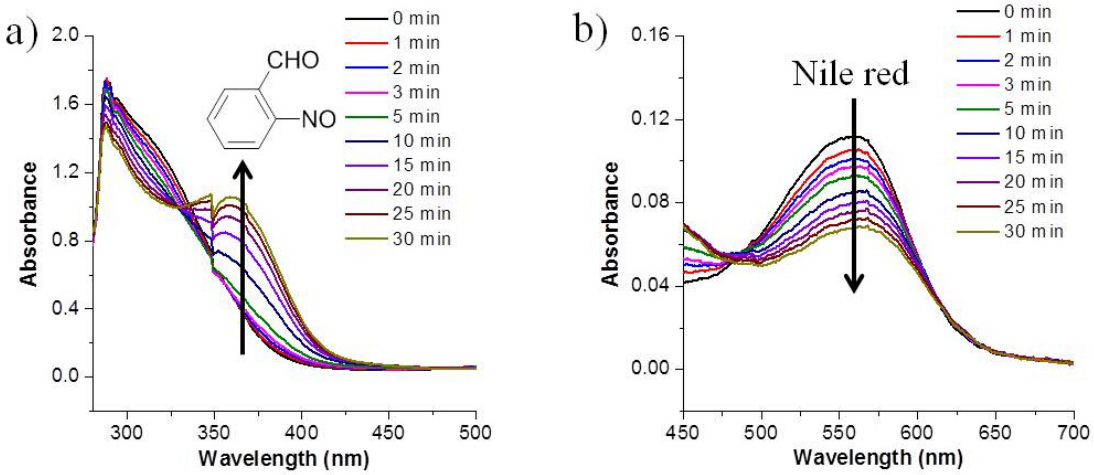


Figure S6: UV-vis spectra of Nile Red encapsulated **P1** (0.5 mg/mL) aqueous solution under UV irradiation at 365 nm for different time; (a) the increase of absorbance at 360 nm is due to the release of 2-nitrosobenzaldehyde; (b) the decrease of absorbance at 560 nm indicates the release of Nile Red.

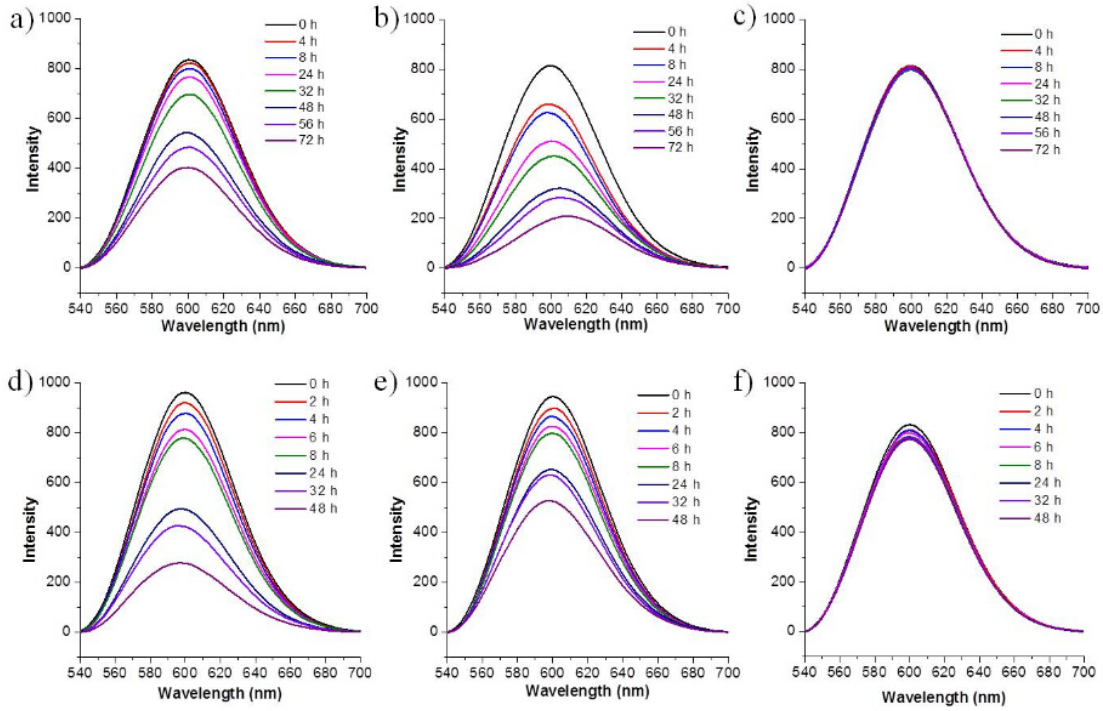


Figure S7: Emission spectra of Nile Red encapsulated in **P2** micelle (0.5mg/mL) in (a)

buffer at pH of 5; (b) buffer at pH of 4; (c) buffer at pH of 7.4; (d) buffer at pH of 7.4 in the presence of 10 mM GSH; (e) buffer at pH of 5 in the presence of 10 mM GSH; (f) buffer at pH of 7.4 in the presence of 10 μM GSH.

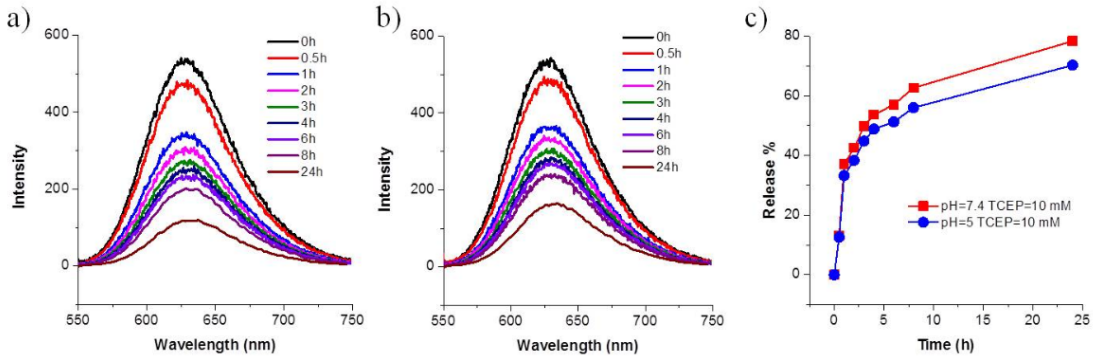


Figure S8: (a) Emission spectra of Nile Red encapsulated in **P2** micelle (0.5mg/mL) in 10 mM tris(2-carboxyethyl)phosphine (TCEP) in PBS buffer (pH 7.4); (b) in 10 mM TCEP in PBS buffer (pH 5); (c) Nile Red release profiles of **P2** in 10 mM TCEP at different pH. Since TCEP is more stable in acidic environment than GSH, the release profiles are much similar in different pH environment.

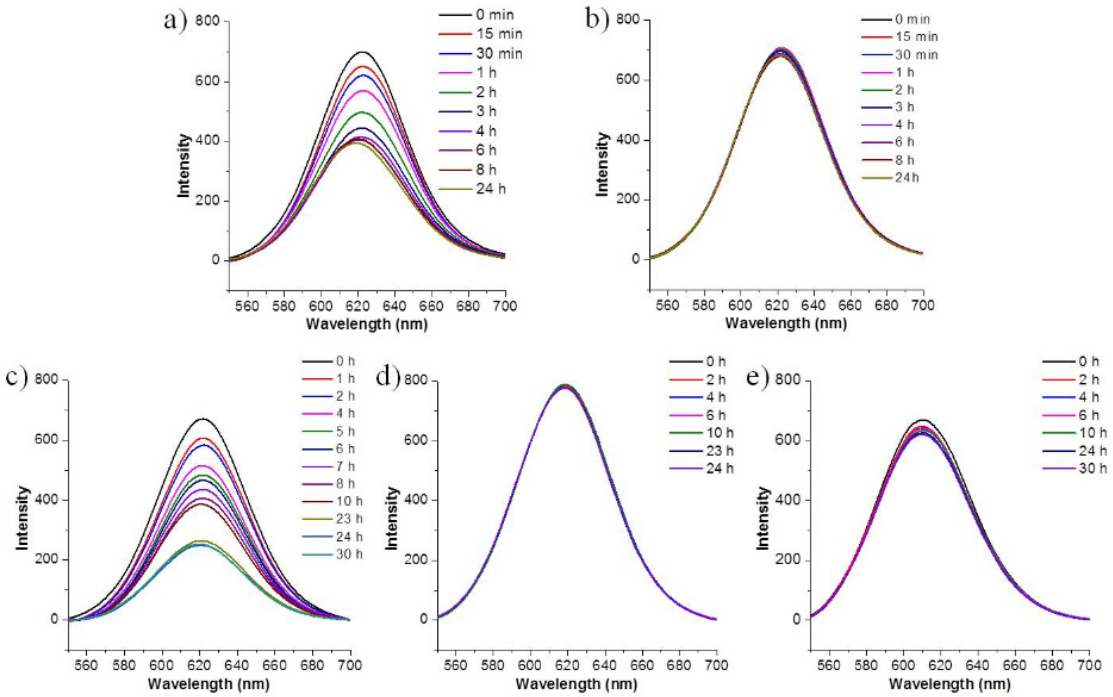


Figure S9: (a) Emission spectra of Nile Red encapsulated in **P3** micelle (0.5mg/mL) in 10 mM GSH; (b) in 10 μM GSH; (c) in the presence of 60 μM bCA-II; (d) in the presence of 60 μM BSA; (e) emission spectra of Nile Red encapsulated in PDS-PEG polymer micelle (0.5mg/mL) in the presence of 60 μM bCA-II.

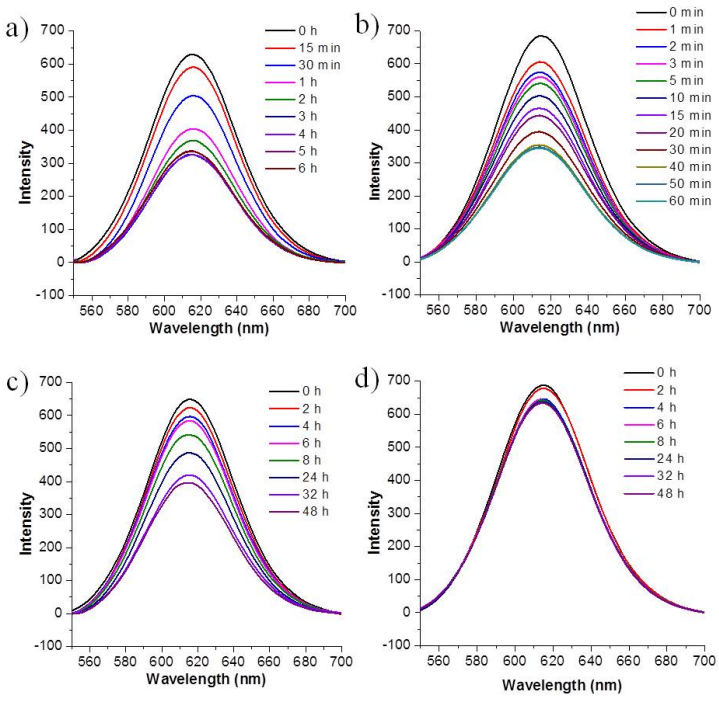


Figure S10: (a) Emission spectra of Nile Red encapsulated in **P4** micelle (0.5mg/mL) in the presence of 60 μM bCA-II; (b) under UV irradiation at 365 nm; (c) in 10 mM GSH; (d) in 10 μM GSH.

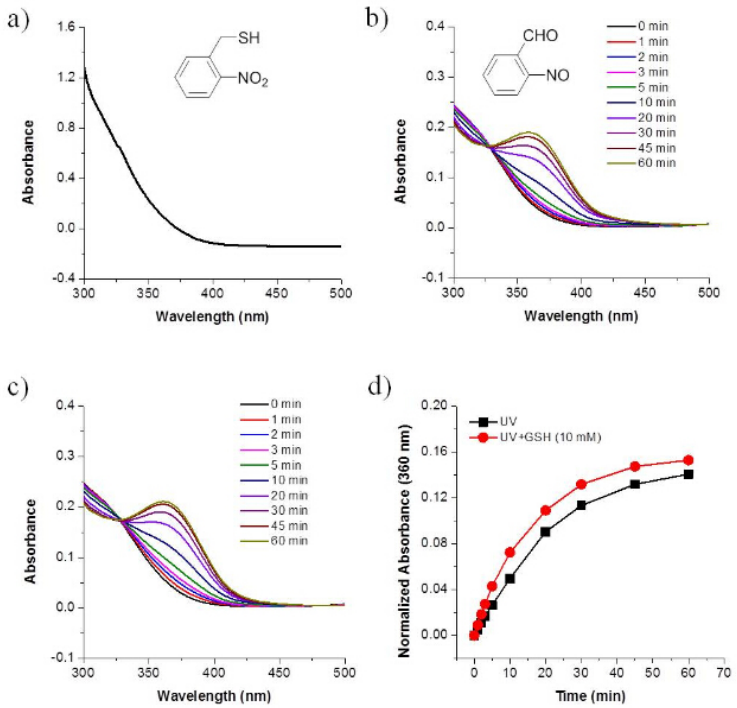


Figure S11: (a) UV-vis spectra of o-nitrobenzyl thiol in aqueous solution; (b) UV-vis spectra of **P4** in aqueous solution under UV light for different time; (c) UV-vis spectra of **P4** in aqueous solution in the presence of 10 mM GSH under UV light for different time. (d) Normalized absorbance increase at 360 nm. O-nitrobenzyl thiol has no absorption peak at 360 nm, while after UV treatment, a distinct peak generates at 360 nm, indicating the byproduct after photocleavage is 2-nitrosobenzaldehyde instead of o-nitrobenzyl thiol. In the presence of 10 mM GSH, the by-product generation is also faster.

(1) Ghosh, S.; Basu, S.; Thayumanavan, S.; *Macromolecules* **2006**, *39*, 5595-5597.

(2) Devarie-Baez, N.O., Bagdon, P.E., Peng, B., Zhao, Y., Park, C. M., and Xian, M.; *Org. Lett.*, **2013**, *15*, 2786-2789

(3) Li, A.H., Moro, S., Forsyth, N., Melman, N., Ji, X, and Jacobson K.A., *J. Med. Chem.*, **1999**, *42*, 706-721