Supporting Information for:

Multi-Stimuli Responsive Amphiphilic Assemblies through Simple Post Polymerization Modifications

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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 1H 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 spectrofluorometer.

Synthesis of PDS-PEG polymer:

Scheme 1: Synthetic scheme 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 °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 scheme of o-nitrobenzyl thiol](image)

\(\text{o-Nitrobenzyl thiol was prepared according to a reported literature.}^2 \text{ 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 obtain 1.1 g product. \(^1\)H NMR (400MHz, CDCl_3) \(\delta : 8.00 \) (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:
The synthesis of this ketal containing thiol was reported previously. Briefly, 3-mercaptop-1,2-propanediol (1.08 g, 10 mmol), pyridinium p-toluenesulfonate (PPTS, 0.25 g, 1 mmol) and 1 g MgSO₄ were stirred in 10 mL acetone for two days. The insolubility was filtered, and the residual liquid was concentrated and purified by column chromatography (SiO₂) using hexane/ethyl acetate (v:v = 9:1) as eluant to give 0.88 g product. \(^1\)H NMR (400MHz, CDCl₃) δ: 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-dioxol-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 CDCl₃.

**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, 0.3 eq. 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. Since the feed ratio of 3-mercaptopropionic acid to PDS units is 0.3 eq., while there is 70% of PDS repeating unit on PDS-PEG polymer, the
expected ratio of carboxyl unit on the polymer after thiol-disulfide exchange reaction is 70%*30% = 21%. 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) were added into the solution and stirred for 4 hours. 4-(2-Aminoethyl) benzenesulfoamide (1.5 eq. to carboxyl group) was then added into the mixture and stirred for overnight. The solution was thoroughly dialyzed against DI water and lyophilized to get \textbf{P3}.

\textbf{Figure S3:} NMR spectrum of \textbf{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$_3$.

\textbf{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, 0.7 eq. to PDS units) in 1 mL DCM was added. The solution was stirred for overnight and dialyzed against DCM. Since the feed ratio of 3 o-nitrobenzyl thiol to PDS unit is 0.7 eq., while there is 70% of PDS repeating unit on PDS-PEG polymer, the expected ratio of carboxyl unit on the polymer after thiol-disulfide exchange reaction are 70%*70% = 49%. 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 hours. 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 aromatic protons and sulfoamide. (*) is the residual solvent CDCl$_3$.

**DLS measurements:**

10 mg of P1, P2, P3 or P4 were dissolved in 1mL DI water, stirred at 4 °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 1 hour. Sample 3 was added 850 µL DI water and 100 µL 100 mM GSH stock solution and sample 4 was added 850 µL DI water and 100 µL 100 µM GSH stock solution. Sample 3 and 4 was incubated in room temperature for 24 hours. 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 bCA-II stock solution (120 µM). The sizes were then measured after 30 hours.

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 °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 (120 µM) or BSA solution (120 µM). 50 µL PDS-PEG polymer solution with dye encapsulated was mixed with 450 µL DI water and 500 µL bCA-II solution (120 µM)
μM). 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:

\[
\% \text{ Release of Nile Red} = \frac{(I_t - I_0)}{I_0} \times 100
\]

Where \( I_0 \) = Initial fluorescence intensity of Nile Red

\( I_t \) = fluorescence intensity of Nile Red at each time point

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**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: Emission spectra of Nile Red encapsulated in P2 micelle (0.5 mg/mL) in presence of 10 mM tris(2-carboxyethyl)phosphine (TCEP) in (a) PBS buffer (pH 7.4), (b) 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 similar.
in different pH environment.

Figure S9: Emission spectra of Nile Red encapsulated in P3 micelle (0.5 mg/mL) (a) in 10 mM GSH, (b) in 10 µM GSH, (c) in the presence of 60 µM bCA-II, and (d) in the presence of 60 µM BSA; (e) Emission spectra of Nile Red encapsulated in PDS-PEG polymer micelle (0.5 mg/mL) in the presence of 60 µM bCA-II.
Figure S10: (a) Emission spectra of Nile Red encapsulated in P4 micelle (0.5 mg/mL) in the presence of 60 µM bCA-II, (b) under UV irradiation at 365 nm, (c) in 10 mM GSH, and (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 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.

Figure S12. Mechanism of thiol-pyridyl disulfide exchange reaction. Briefly, free thiol (R₁-SH) attacks pyridyl disulfide to generate unsymmetric disulfide bonds (R₁-S-S-R₂) and...
pyridine-2-thiol. This reaction (Reaction 1) is a reversible process. Meanwhile, the generated pyridine-2-thiol subsequently transforms to pyridine-2-thione because the thione-form is much more stable in solution (Reaction 2). With the generation of pyridine-2-thione, the concentration of pyridine-2-thiol decreases, resulting in the right shift of Reaction 1 equilibrium. Pyridine-2-thiol is a good leaving group. Such feature has improved the reactivity of pyridyl disulfide with free thiols even under mild conditions.\(^4\)


