Supporting Information

Dual-Responsive Nanoparticles Constructed Using Light and Redox-Responsive Linkages

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1. Experimental Section

1.1 Materials

The chloroform-d ($CDCl_3$, 99.8% D, Sigma-Aldrich), dimethylsulfoxide-d_6 (DMSO-d_6, 99.9% D, Novachem), acetone-d_6 (99.9% D, Sigma-Aldrich), tetrahydrofuran (THF, HPLC grade, Chem-Supply), butylated hydroxytoluene (BHT, ≥99%, Sigma-Aldrich), N,N-diisopropylethylamine (DIPEA. ≥99%, Sigma-Aldrich), dichloromethane (DCM, AR grade, Chem-Supply), acryloyl chloride (97.0%, Sigma-Aldrich), sodium hydrogen carbonate($NaHCO_3$, AR grade, Chem-Supply), magnesium sulfate dried powder ($MgSO_4$, LR grade, Chem-Supply), 2,2′-(ethylenedioxy)diethanethiol (95%, Sigma-Aldrich), dimethylformamide (DMF, AR grade, Chem-Supply), triethylamine (TEA, Et_3N, ≥99.5%, Sigma-Aldrich), 2,2'-dipyridyldisulfide (DPS, 98%, Sigma-Aldrich), poly(ethylene glycol) methyl ether (PEG-OH, average Mn 2000, Sigma-Aldrich), cholesterol (CHOL, 97%, Oakwood chemical), succinic anhydride (99%, Sigma-Aldrich), 4-(dimethylamino)pyridine (DMAP, 99%, Sigma-Aldrich), N,N'-diisopropylcarbodiimide (DIC, 99%, Sigma-Aldrich), iodoacetamide (≥99% , Sigma-Aldrich), dithiothreitol (DTT, ≥99.0%, Sigma-Aldrich) were used as purchased. 20x Phosphate buffered saline (PBS) was prepared by dissolving 160 g Sodium Chloride (NaCl, ≥99.0%, Sigma-Aldrich), 4.0 g, potassium chloride (KCl, ≥99.0%, Sigma-Aldrich), 28.8 g potassium phosphate monobasic (KH_2PO_4, ≥99.0%, Sigma-Aldrich) and 4.8 g sodium phosphate dibasic (Na_2HPO_4, ≥99.0%, Sigma-Aldrich) into 1.0 L deionized water. The PBS was diluted 20 times and adjusted to a suitable pH before use. 100 kDa MWCO Spectra-Po Float-A-Lyzer dialysis tubes (Sigma-Aldrich), syringe filters with a 0.45 μm pore size polyethersulfone (PES) membrane (Merck Millipore) were used as per instructions.

1.2 Characterization

1.2.1 Nuclear Magnetic Resonance (NMR) Spectroscopy

^{1}H NMR spectroscopy was performed on a Varian 400 MR spectrometer at room temperature. The data was analyzed using the MestReNova software package. Solvent peaks were used as references: deuterated chloroform ($CDCl_3$: δH 7.26 ppm), deuterated DMSO (DMSO-d6: δH 2.50 ppm). Chemical shifts (δH) were reported in parts per million (ppm).

1.2.2 Gel Permeation Chromatography (GPC)

Gel permeation chromatographic (GPC) was conducted with a Shimadzu system equipped with a column matrix consisting of Styragel HT 4 (10000 Å, 4.6 mm × 300 mm) and Styragel HT 3 (1000 Å, 4.6 mm × 300 mm). The solvent used was HPLC grade tetrahydrofuran (THF) containing 0.1 g/L butylated hydroxytoluene (BHT). The temperature was kept at 50 degrees and the flow rate was 0.3 mL/min. Data collection and analysis were carried out on ASTRA 6 from Wyatt Technology. The system is calibrated using low dispersity poly(methyl methacrylate) (PMMA) standards purchased from Polymer Laboratories and the reported relative molecular weights were calculated from PMMA standards.

1.2.3 Dynamic Light Scattering (DLS)

Nanoparticle properties were analyzed using a DLS SZ-100 (Horiba Scientific, Japan). The scattering angle was fixed at 90° or 173°, and the temperature was fix at 37℃ or 25℃.

1.2.4 UV-vis spectroscopy

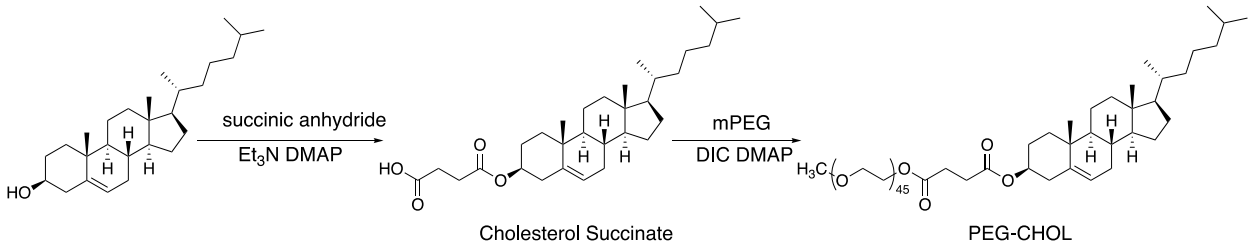
The Cary 60 UV-vis spectroscopy (Agilent Co Ltd, USA) was employed to measure the absorbance of monomers, polymers, and nanoparticles at room temperature. Before measurement, all samples were diluted by deionized water or THF and then transferred into a 1 cm pathlength cuvette to reach an absorbance value within the range 0.1 to 1.0 a.u.

1.2.5 Transmission cryo-electron microscopy (Cryo-EM)

Cu/Carbon, 300 mesh grids were glow discharged in a Pelco EasiGlow at 30 mA for 30 s. A 4 μL droplet of the nanoparticle suspension was applied to the grid in a Vitrobot Mark IV held at 4 °C and 100% relative humidity. Grids were blotted in the Vitrobot with Whatman filter paper, using a blot force of -3 and blot time of 3 s, and vitrified in liquid ethane cooled to around -180 °C by liquid nitrogen. Cryo-EM images were acquired using a Tecnai F30 transmission electron microscope at an acceleration voltage of 300 kV and a cryotomography (FEI) software was used to modify the magnifications of the images.

1.3 Synthesis

Scheme S1. Synthesis of PEG-CHOL



1.3.1 Synthesis of Cholesterol Succinate

To a stirred solution of cholesterol (20 mmol, 7.73 g), succinic anhydride (30 mmol, 3.0 g) and DMAP (0.3 mmol, 36.7 mg) in dry DCM (40 mL), was added Et_3N (30 mmol, 4.5 mL) via syringe. The solution was stirred at room temperature for 24 h. The reaction mixture was then concentrated with a rotary evaporator to remove solvent and then 100 ml 2 M HCL was added to neutralize the base. After about 5 min stirring, a white solid formed and was filtered out and washed with water to remove succinic acid. Drying under vacuum overnight afforded cholesterol succinate (9.43g, 97%). ^{1}H NMR (400 MHz, Chloroform-*d*): δ 5.37 (d, J = 5.1 Hz, 1H), 4.63 (m, 1H), 2.67 (d, J = 6.5 Hz, 2H), 2.61 (d, J = 6.5 Hz, 2H), 2.31 (d, J = 8.2 Hz, 2.12 – 0.73 (m, 38H), 0.67 (s, 3H).

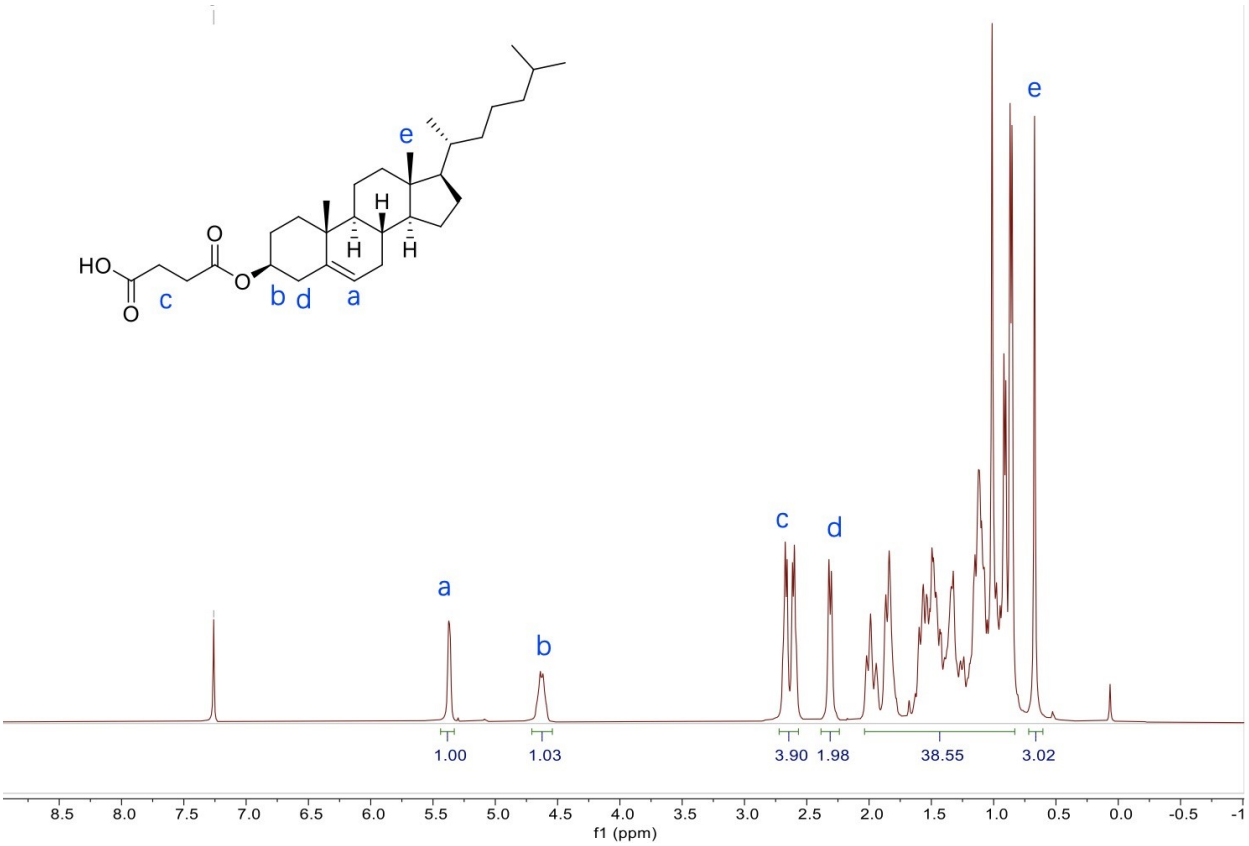


Figure S1. ^{1}H NMR of cholesterol succinate

1.3.2 Synthesis of Methoxy Poly(ethylene glycol) cholesteryl succinate (PEG-CHOL)

A solution containing cholesterol succinate (5 mmol, 2.4 g), poly(ethylene glycol) methyl ether (PEG-OH, 2.5 mmol, 5 g), and DMAP (0.5 mmol, 61 mg) in DCM (40 mL) was stirred and cooled to 0 °C. To this chilled mixture, DIC (5 mmol, 783 µL) was added dropwise, stirred for 24 hours and filtered to remove unwanted solid byproducts. The combined filtrate was washed with water and brine and the organic layer dried over $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed on silica gel eluting with EtOAc and DCM to remove impurities and then 10% MeOH/DCM to obtain the product. Drying under vacuum overnight afforded a yellow solid. ^{1}H NMR (400 MHz, Chloroform-*d*): δ 5.36 (d, J = 5.0 Hz, 1H), 4.70 – 4.52 (m, 1H),

4.27 – 4.21 (m, 2H), 3.64 (m, 178H), 3.38 (s, 3H), 2.69 – 2.55 (m, 4H), 2.31 (d, J = 7.9 Hz, 2H), 2.04 – 0.77 (m, 38H), 0.67 (s, 3H).

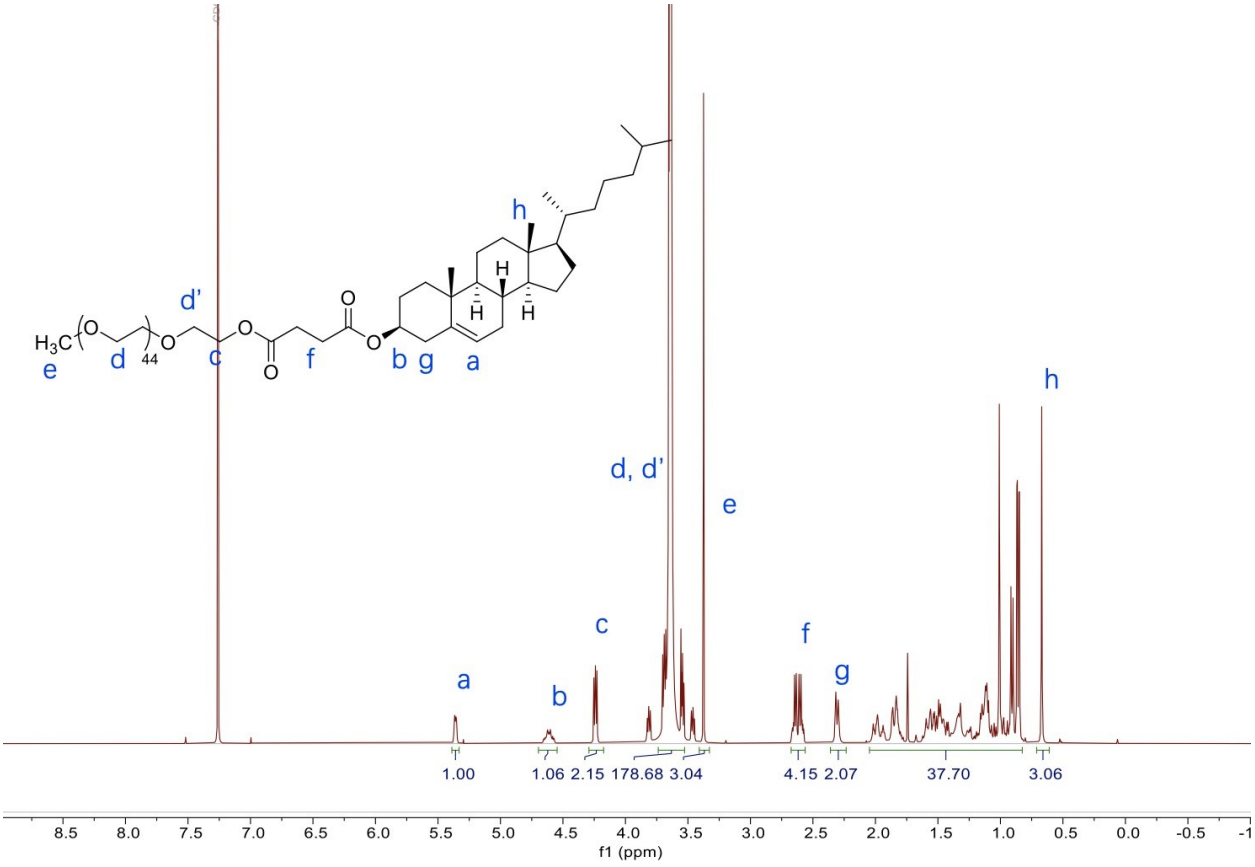
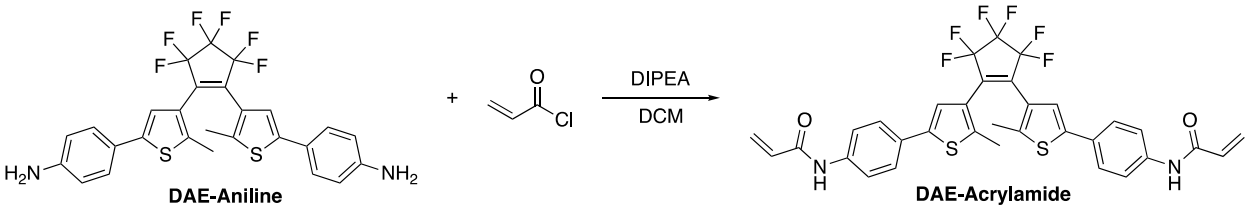


Figure S2. [1]H NMR of methoxy poly(ethylene glycol) cholesteryl succinate (PEG-CHOL)

1.3.3 Synthesis of Diarylethene Crosslinker

Scheme S2. Synthesis of DAE



DAE-Acrylamide: DAE-Aniline was prepared according to the literature.[1] DAE-Aniline (0.3 mmol, 183 mg, 1 eq), DIPEA (1 mmol, 174 μL, 3 eq) and dry DCM (3 mL) were added to an oven-

dried round-bottom flask under nitrogen. Acryloyl chloride (0.3 mmol, 81μL, 3 eq) was added dropwise to the mixture at 0 °C. The mixture was stirred at room temperature overnight. Then, a saturated $NaHCO_3$ solution was added to quench the reaction. The mixture was extracted with DCM three times. The organic layer was dried over $MgSO_4$ followed by reduced pressure. The mixture was purified by flash chromatography, using 20%-30% acetone in hexane, yielding blue powders. ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.47 (s, 2H), 7.80 (d, J = 8.6 Hz, 4H), 7.61 (d, J = 8.7 Hz, 4H), 7.43 (s, 2H), 6.46 (dd, J = 16.9, 9.8 Hz, 2H), 6.36 (dd, J = 16.9, 2.2 Hz, 2H), 5.73 (dd, J = 9.8, 2.3 Hz, 2H), 2.06 (s, 6H). ^{19}F NMR (376 MHz, Acetone) δ -110.76 (t, J = 5.4 Hz), -132.67 (p, J = 5.4 Hz).

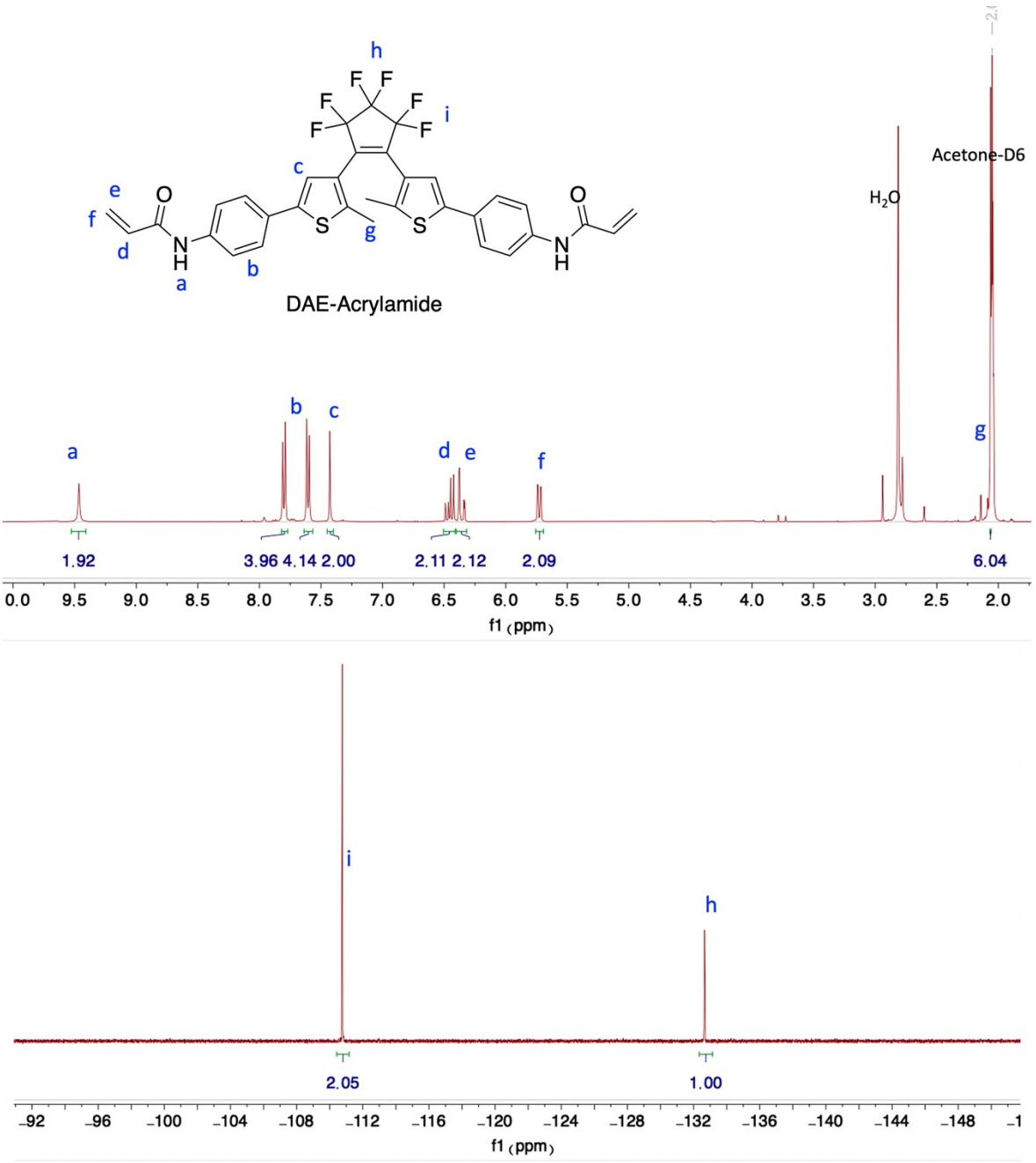


Figure S3. ^{1}H and ^{19}F NMR of DAE-Acrylamide in Acetone-d_6, showing successful acylation on both ends of the DAE.

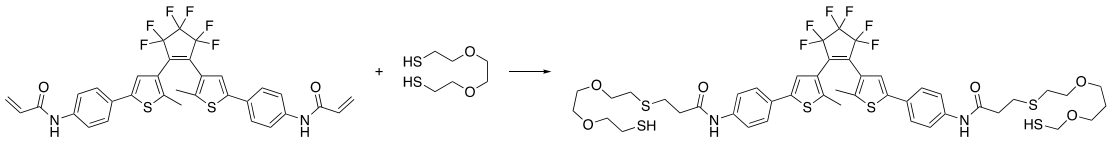
1.3.4 Synthesis of Nanoparticles without Diarylethene

DAE_0THIOL_1 nanoparticle: To a stirred (1000 ppm) PEG-CHOL (1 mg) PBS (10 mM, pH 8.0, and 3 mL) buffer solution, was added dropwise a solution of 2,2′-(ethylenedioxy)diethanethiol (2

mg) in 200 μL DMF. Then, a solution of 2,2'-dipyridyldisulfide (0.8 eq, 1.93 mg) in 19.3 μL DMF was added dropwise. The solution was stirred overnight before transferring to a 100 kDa MWCO dialysis tube for 24 h in PBS (10 mM and pH 8.0) with six buffer changes. Then, the particles were filtered through a 0.45 μm PES syringe filter prior to use.

1.3.5 Synthesis of Diarylethene Nanoparticles

Scheme S3. Thiol-ene reaction of DAE-Acrylamide and thiols.



DAE_1THIOL_5 nanoparticle: DAE-Acrylamide (0.84 mg), 2,2′-(ethylenedioxy)diethanethiol (1.16 mg), and TEA (2 μL) were dissolved in DMF (20 μL). After incubating for 2 hours, the solution was diluted with an additional 180 μL of DMF and then introduced into a PEG-CHOL (1 mg) PBS (10 mM, pH 8.0, 3 mL) buffer solution, which was being stirred at 1000 rpm. Subsequently, a solution of 2,2'-dipyridyldisulfide (0.8 equivalents, 1.12 mg) in DMF (11.2 μL) was added gradually. The resulting solution was stirred overnight. The next step involved transferring the solution into a dialysis tube with a 100 kDa MWCO and dialyzing for 24 hours against PBS (10 mM, pH 8.0), with the buffer being changed six times. Finally, the particles were passed through a 0.45 μm PES syringe filter before use.

DAE_1THIOL_{10} nanoparticle: DAE-Acrylamide (0.53 mg), 2,2′-(ethylenedioxy)diethanethiol (1.47 mg), and TEA (2 μL) were dissolved in DMF (20 μL). After incubating for 2 hours, the solution was diluted with an additional 180 μL of DMF and then introduced into a PEG-CHOL (1 mg) PBS (10 mM, pH 8.0, 3 mL) buffer solution, which was being stirred at 1000 rpm. Subsequently, a solution of 2,2'-dipyridyldisulfide (0.8 equivalents, 1.42 mg) in DMF (14.2 μL)

was added gradually. The resulting solution was stirred overnight. The next step involved transferring the solution into a dialysis tube with a 100 kDa MWCO and dialyzing for 24 hours against PBS (10 mM, pH 8.0), with the buffer being changed six times. Finally, the particles were passed through a 0.45 μm PES syringe filter before use.

DAE_1THIOL_{30} nanoparticle: DAE-Acrylamide (0.22 mg), 2,2′-(ethylenedioxy)diethanethiol (1.78 mg), and TEA (2 μL) were dissolved in DMF (20 μL). After incubating for 2 hours, the solution was diluted with an additional 180 μL of DMF and then introduced into a PEG-CHOL (1 mg) PBS (10 mM, pH 8.0, 3 mL) buffer solution, which was being stirred at 1000 rpm. Subsequently, a solution of 2,2'-dipyridyldisulfide (0.8 equivalents, 1.82 mg) in DMF (18.2 μL) was added gradually. The resulting solution was stirred overnight. The next step involved transferring the solution into a dialysis tube with a 100 kDa MWCO and dialyzing for 24 hours against PBS (10 mM, pH 8.0), with the buffer being changed six times. Finally, the particles were passed through a 0.45 μm PES syringe filter before use.

1.4 Determination of the critical micelle concentration (CMC) of PEG-CHOL

A stock solution of Nile Red in DCM (15 μL at 0.1 mg/mL) was added to a series of empty vials, which were then placed under vacuum for at least 2 hours to ensure complete solvent removal. To the vials, a PEG-CHOL stock solution (0.1 mg/mL) was added in varied volumes (0.001 – 1.0 mL). Water was added to each vial to bring the total volume of each solution up to 1.0 mL. These solutions were shaken vigorously and then allowed to equilibrate at room temperature for at least 2 hours. Fluorescence measurements were taken at the excitation wavelength of 560 nm, and the emission was monitored at 635 nm. The fluorescent intensity was plotted against the concentration of PEG-CHOL on a logarithmic scale. The intersection point of the fit lines was indicated as the CMC.

2. Supporting schemes and figures

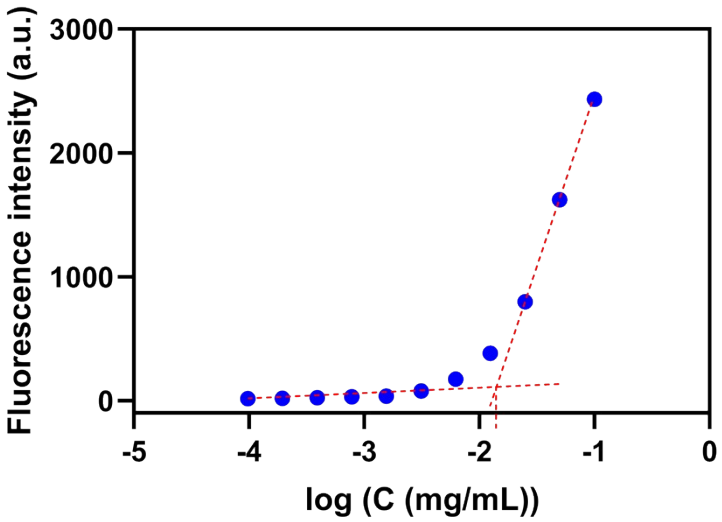
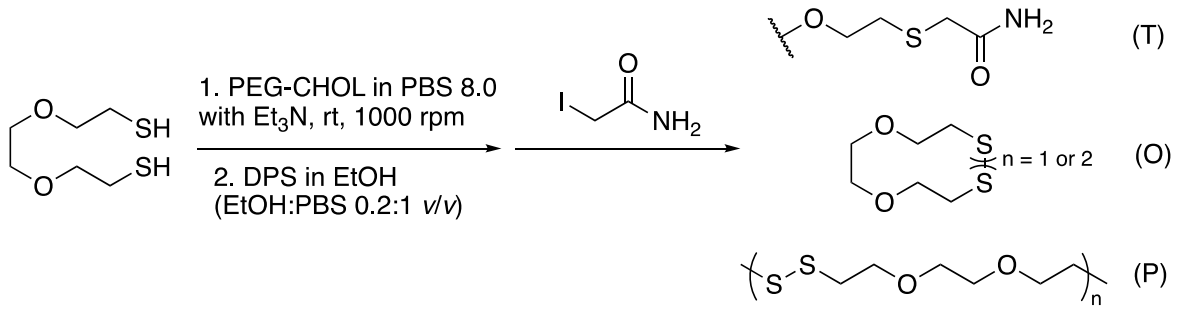


Figure S4. Measurement of the critical micelle concentration of PEG-CHOL in H_2O with the Nile Red encapsulation assay.

Scheme S4. Sample preparation for ^1H NMR study at various time points with 2-iodoacetamide as the quencher to quench any remaining thiol (T) group in the mixture while disulfide bonds (O and P) were not affected.



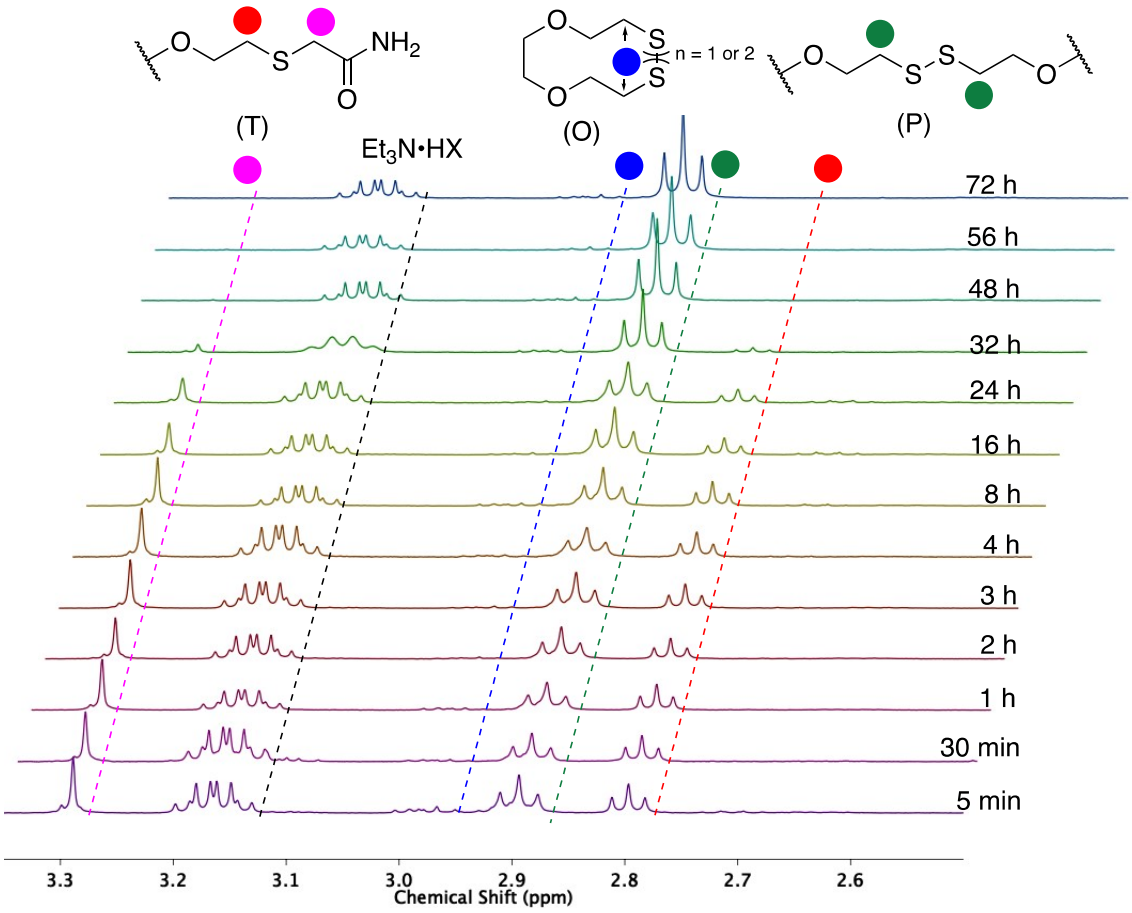


Figure S5. ^{1}H NMR studies of the DPS-mediated disulfide polymerization over time, with 0.5 equivalents of DPS.

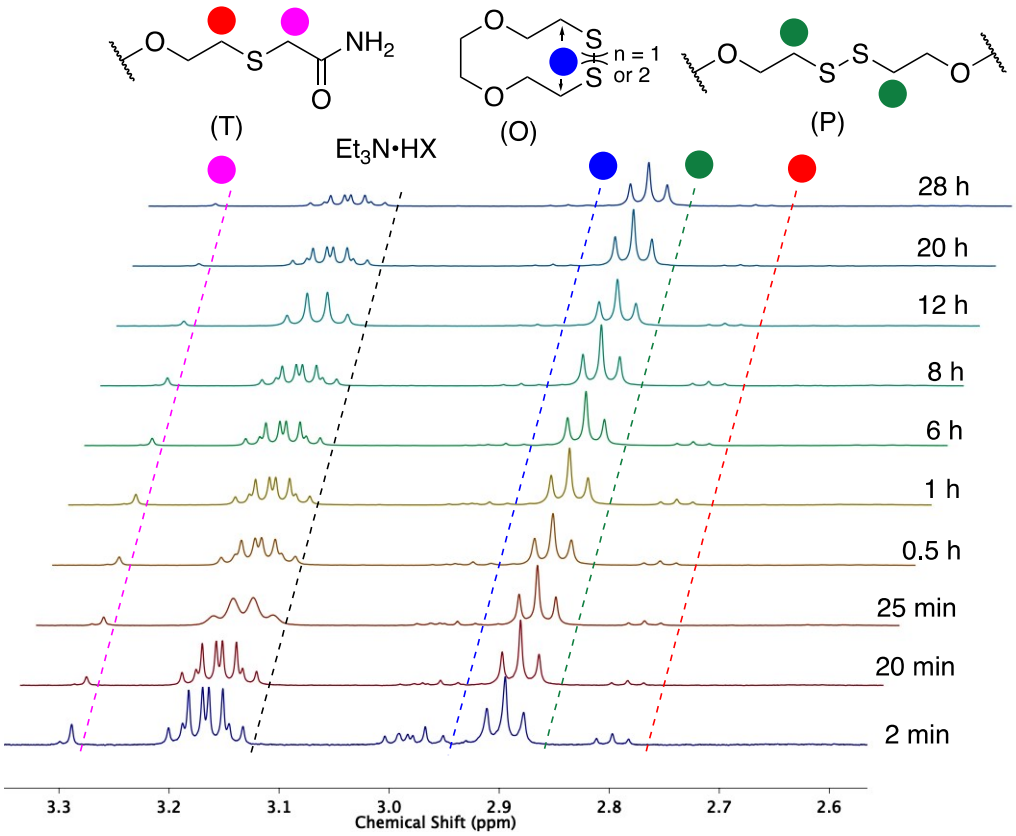


Figure S6. ^{1}H NMR studies of the DPS-mediated disulfide polymerization over time, with 0.8 equivalents of DPS.

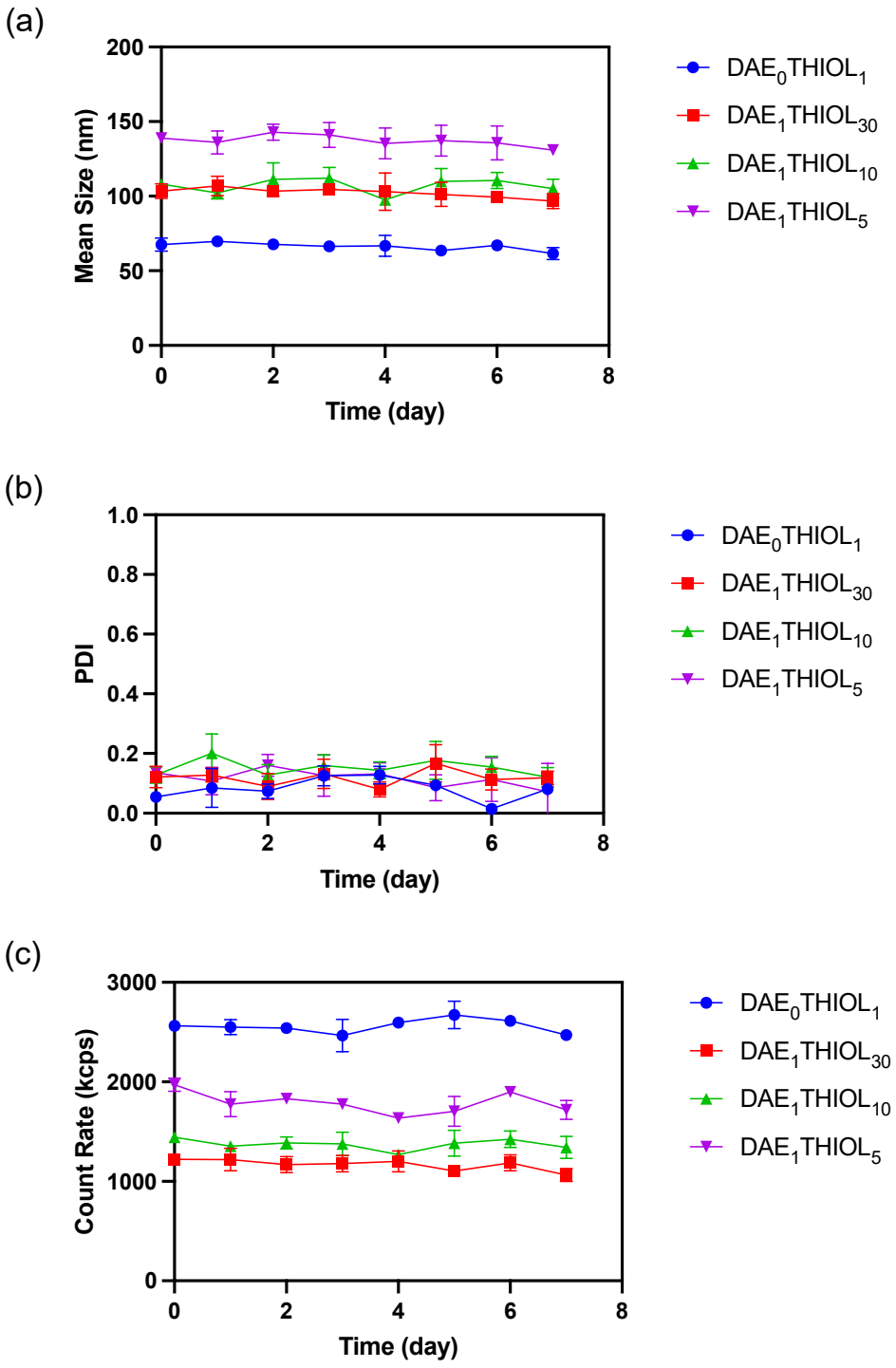


Figure S7. Stability of all nanoparticles over 7 days. (a) Mean size; (b) polydispersity (PDI); (c) count rate (KCPS, kilo counts per second).

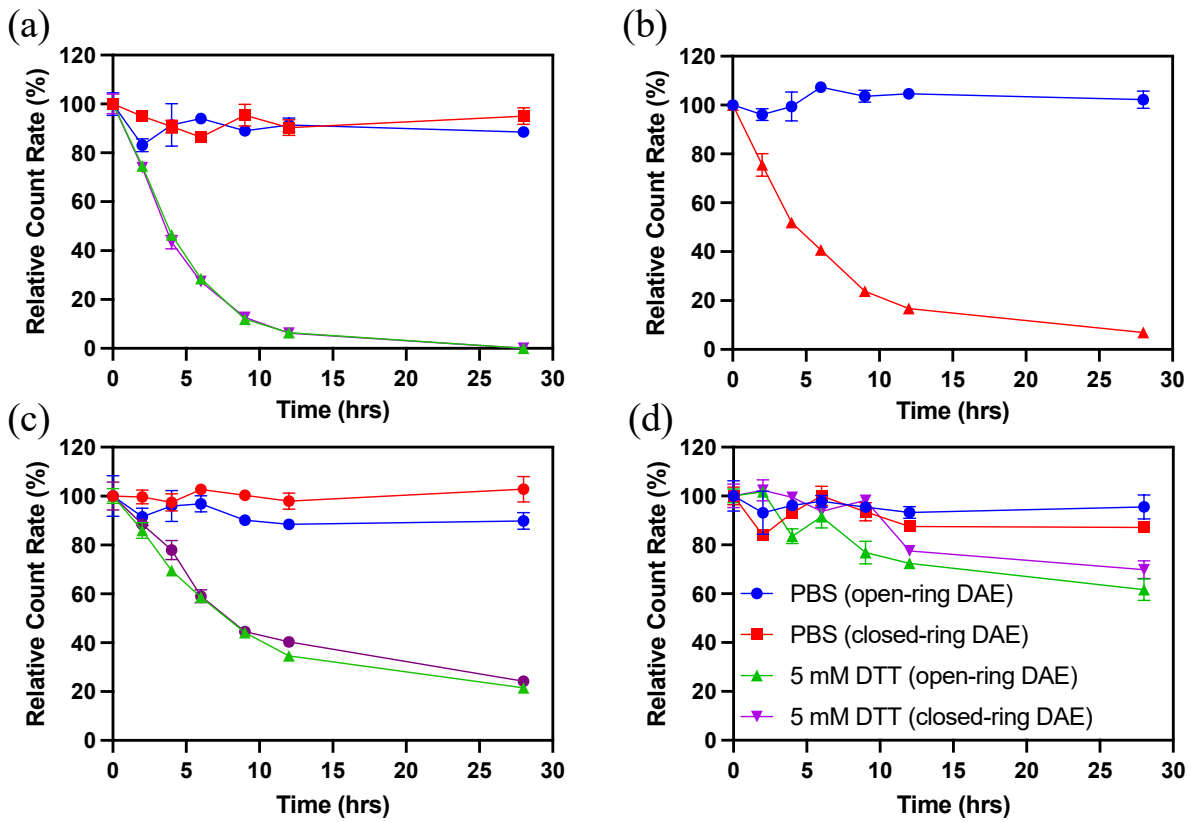


Figure S8. Disassembly profiles of (a) DAE_0THIOL_1 (b) DAE_1THIOL_{30} (c) DAE_1THIOL_{10} (d) DAE_1THIOL_5, with open- or closed-ring forms of DAE, and with or without DTT.

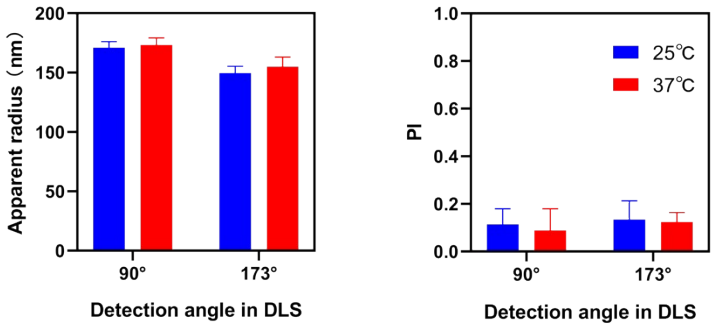


Figure S9. DLS characterization of one sample of nanoparticles at different angles and temperatures, showing that the testing angle and temperature do not strongly influence the particle size.

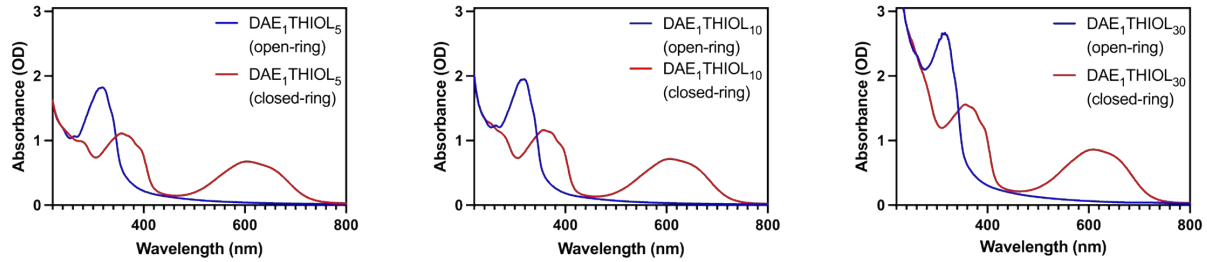


Figure S10. Absorption spectra of DAE_1THIOL_5, DAE_1THIOL_{10}, DAE_1THIOL_{30} in water. The absorption spectra of the open-ring (blue line) and closed-ring (red line) isomers DAE are shown.

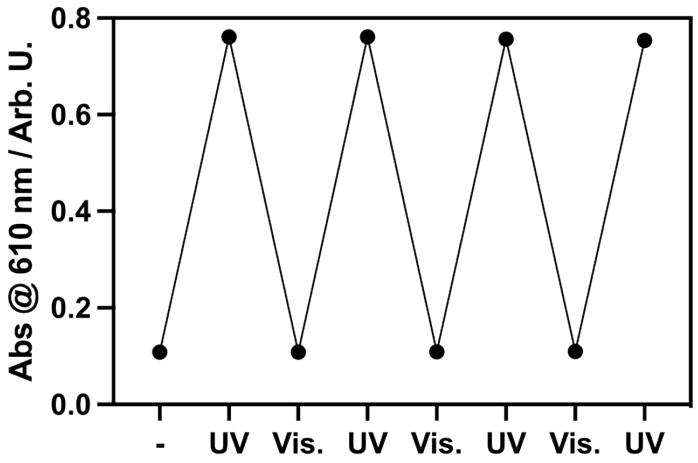


Figure S11. Reversibility of DAE-loaded nanoparticles. The samples were subjected to alternating irradiation with 365 nm UV light and 600 nm visible light. Each irradiation was continued until no further changes were observed in the UV-vis absorption spectrum.

(1) Yagai, S.; Iwai, K.; Karatsu, T.; Kitamura, A. Photoswitchable exciton coupling in merocyanine-diarylethene multi-chromophore hydrogen-bonded complexes. *Angew. Chem., Int. Ed.* **2012**, *51* (38), 9679-9683.