Photo-controllable Toxicity Recovery from Selenium-based Amphiphiles

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1. Materials and instruments

Dichloromethane was distilled over calcium hydride before use. 3, 4, 5-tris(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)benzoate and tetraphenylporphyrin were synthesized according to the previous reported literature. [1,2] Selenium, sodium borohydride and bromododecane were purchased from Aladdin and used as received. All other reagents and solvents were analytical pure and used as received unless otherwise mentioned.

^{1}H and ^{77}Se NMR spectra in $CDCl_3$ were determined by a Bruker AVANCE III HD 400 Spectrometer with tetramethylsilane as the internal standard. For ^{77}Se NMR, diphenyl diselenide was used as the external standard. High resolution mass spectrum was recorded on a Waters LCT Premier XE spectrometer with methanol as the solvent. Fourier Transform Infrared Spectroscopy (FT-IR) measurements were conducted on a Spectrum One FT-IR spectrophotometer and potassium bromide pellet was employed as the matrix. Dynamic light scattering was performed on a BECKMAN COULTER Delasa Nano C particle analyzer at room temperature. TEM samples were prepared by dropping the micellar solution (1 mg/mL) on to a carbon coated copper grid, and the images were observed on a JEOL JEM1400 electron microscope operated at 100 kV. CLSM images were taken from a Nikon A1R confocal microscope.

2. Methods

2.1 Synthesis of DSeMTTG

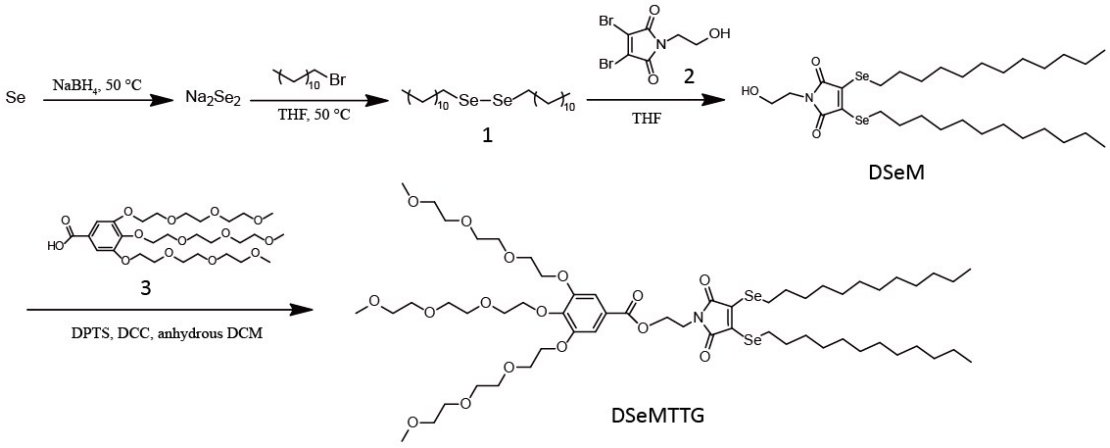


Fig. S1. Detailed synthetic route of selenium-containing compound DSeMTTG.

Synthesis of didodecyl diselenide *1*.

Compound ***1*** was synthesized according to the procedure of previous work.[3] In brief, sodium borohydride (0.227 g, 6 mmol) in 2 mL water was added into the suspended solution of selenium (0.237 g, 3 mmol) in 15 mL water with magnetic stirring at room temperature. After the initial vigorous reaction had subsided, another equivalent of selenium (0.237 g, 3 mmol) was added. The mixture was stirred for 15 min and then heated at 50 °C for 40 min. Na_2Se_2 was obtained as a brownish red solution. The fresh prepared sodium diselenide aqueous solution was added dropwise to a solution of bromododecane (1.5g, 6 mmol) in 10 mL THF, and the reaction was stirred under nitrogen at 50 °C for 2 h. After evaporation of THF, the residue was extracted into dichloromethane. The organic layer was collected and removed by vacuum evaporation to obtain didodecyl diselenide as a yellow liquid. The crude product was used immediately without further purification.

Synthesis of *N*-(2-hydroxyethyl)-3, 4-dibromomaleimide *2*.

Compound ***2*** was synthesized according to the previous literature.[4] A white crystal was obtained. ^{1}H NMR (400 MHz, $CDCl_3$), δ ppm: 3.80 (s, 4H). ^{13}C NMR (90.5 MHz, $CDCl_3$), δ ppm: 164.3, 129.5, 60.2 and 42.1.

Synthesis of *N*-(2-hydroxyethyl)-3, 4-didodecylselanylmaleimide (DSeM).

Sodium borohydride in 10 mL methanol was added with magnetic stirring into the THF solution of didodecyl diselenide fresh prepared above. The mixture was stirred for 10 min with N_2 protection at room temperature. Then ***2*** (1.79 g, 6 mmol) in 10 mL THF was added. The solution was stirred overnight. After evaporation of the solvent, the crude product was purified by column chromatography on silica, eluting with petroleum ether/ethyl acetate (5 : 1, v/v) as the eluent. DSeM was obtained after evaporation of the solvent as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.83–3.69 (m, 4H), 3.32 (t, 4H), 1.81–1.64 (m, 4H), 1.39 (dd, 4H), 1.33–1.21 (m, 32H), 0.88 (t, 6H). HRMS (ESI, *m/z*): $[M + H]^+$ calcd for $C_{30}H_{55}NO_3Se_2$, 638.2591; found, 638.2593.

Synthesis of 3, 4, 5-tris(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)benzoate *3*.

The synthetic procedure was according to the established method.[1] A pale yellow oil was obtained. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.35 (s, 2H), 4.27–4.17 (m, 6H), 3.90–3.84 (m, 4H), 3.83–3.77 (m, 2H), 3.76–3.70 (m, 6H), 3.69–3.61 (m, 12H), 3.57–3.51 (m, 6H), 3.38 (t, 9H).

Synthesise of DSeMTTG.

DSeM (0.222 g, 0.35 mmol), ***3*** (0.212 g, 0.35 mmol) and DPTS (0.103 g, 0.35 mmol) were dissolved in dry DCM. DCC (0.108 g, 0.5 mmol) in 10 mL dry DCM was added dropwise to the solution in an ice-water bath. The reaction mixture was stirred at room temperature overnight. After filtration, the filtrate was concentrated and purified by silica gel column chromatography using DCM/MeOH (50:1-30:1, v/v) as the eluent. A yellow solid was obtained. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.23–7.16 (m, 2H), 4.29 (t, 2H), 4.13 (dd, 6H), 3.88–3.78 (m, 6H), 3.73 (t, 2H), 3.66 (dd, 6H), 3.62–3.52 (m, 12H), 3.51–3.44 (m, 6H), 3.30 (s, 9H), 3.22 (t, 4H), 1.68–1.57 (m, 4H), 1.35–1.12 (m, 34H), 0.81 (t, 6H). HRMS (ESI, *m/z*): $[M + Na]^+$ calcd for $C_{58}H_{101}NO_{16}Se_2Na$, 1250.5348; found, 1250.5342.

2.2 Preparation of DSeMTTG micelles

Nano precipitation method was employed to prepare the nanoparticles. In brief, DSeMTTG (5 mg) was dissolved in ethanol (0.5 mL) and 100 μL of the mixture was injected into 2 mL of water followed by 5 s vortexing and then dialyzed against water to remove ethanol using a dialysis bag (MWCO = 3 500). Dialysis was repeated 2 times to ensure the clearance of organic solvent.

2.3 Preparation of porphyrin-loaded DSeMTTG micelles

TPPC6 (1 mg) was dissolved in 0.5 mL of DMSO and then mixed with the above prepared DSeMTTG micelle solution. After 5 s vortexing, the mixture was dialyzed against water to remove DMSO using a dialysis bag (MWCO = 3500), during which water was renewed 3 times. The final volume of the solution was added to 5 mL with a porphyrin concentration of 0.2 mg mL^{-1} for further experiments.

2.4 Critical micelle concentration (CMC) measurement

Pyrene was used as the fluorescent probe to determine the critical micelle concentration (CMC) of DSeMTTG nanoparticles. A certain amount of pyrene in acetone was added to a series of volumetric flasks. After evaporation of the acetone, DSeMTTG solutions with different concentrations were added to the volumetric flasks. The final concentration of pyrene was 6.0×10^{-7} mol/L. These solutions were gently shaken and equilibrated at 25 °C for 24 h. The fluorescence spectra of pyrene were measured at the excitation wavelength of 335 nm.

2.5 Oxidation of DSeMTTG

After preparation of the porphyrin-loaded DSeMTTG micelle solution, the mixture was irradiated with visible light (420 nm, 400 mW/cm^2) for 20 min, and then freeze dried to powder. FT-IR measurements were conducted to study the absorption changes before and after oxidation.

2.6 Light triggered porphyrin release from DSeMTTG micelles

Porphyrin-loaded DSeMTTG solution (1 mg/mL) was prepared and divided into four aliquots. Each aliquot was irradiated with visible light for 0, 5, 10, and 20 min, respectively. After that, the samples were incubated in 100 mL of PBS (pH = 7.4) with 1% Tween 80 (v/v). The release experiment was performed at 37 °C in a shaking bed. At predetermined time intervals, 2 mL of the supernatants were taken out and replaced with an equal volume of fresh media. The fluorescence of porphyrin released was determined by fluorescence spectroscopy.

2.7 Cell culture

A549 cells (human breast adenocarcinoma cell line) were cultured in Dulbecco's modified Eagle's medium (DMEM) containing antibiotics (50 units per mL penicillin and 50 units per mL streptomycin) and fetal bovine serum (FBS, 10%) under a humidified atmosphere containing 5% CO_2 at 37 °C.

2.8 Cytotoxicity of free DSeMTTG and DSeMTTG micelles to A549 cells

A549 cells were seeded in a 6-well plates (4×10^3 cells/well) and incubated with DMEM at 37 °C for 24 h. Then the cells were separately treated with free DSeMTTG and DSeMTTG micelle solution with different concentrations for another 24 h in the dark. The culture medium was removed and the cells were washed three times with PBS, followed by the addition of fresh DMEM with MTT solution (5 μg/mL). After incubation for 4 h, 150 μL of DMSO was added to each well to extract the formazan products by gentle agitation for several minutes. The absorbance was detected by a spectrophotometric microplate reader at the wavelength of 560 nm.

2.9 Phototoxicity of porphyrin-loaded DSeMTTG micelles against A549 cells

Similar procedure was used to evaluate the phototoxicity of porphyrin-loaded DSeMTTG micelles. A549 cells were seeded in a 6-well plate (4×10^3 cells/well) and incubated with the micelles in the dark for 24 h. Then the cells were exposed to a visible light lamp for various periods of time and incubated at 37 °C for another 24 h. After incubation, the final cell viabilities were measured by using MTT assays.

2.10 Subcellular distribution of porphyrin-loaded DSeMTTG micelles in A549 cells

A549 cells were seeded onto the confocal dish with a density of 1.5×10^5 cells per dish and incubated with DMEM containing 10% FBS for 24 h. Then the culture medium was replaced by DSeMTTG micelle solution (porphyrin concentration: 40 μg/mL) and CellLight Lysosomes-GFP with predetermined amount was added at the same time for the visualization of lysosome. After incubation for 16 h, the cells were washed with PBS solution, followed by irradiation under a visible light LED lamp for 3 min and incubated with fresh full medium for another 4 h. The cells were fixed with paraformaldehyde (4% in PBS solution) and then stained with DAPI to mark the nucleus. CLSM observation was performed on a fluorescence microscope (Nikon AIR).

3. Characterization of compounds and supplementary figures

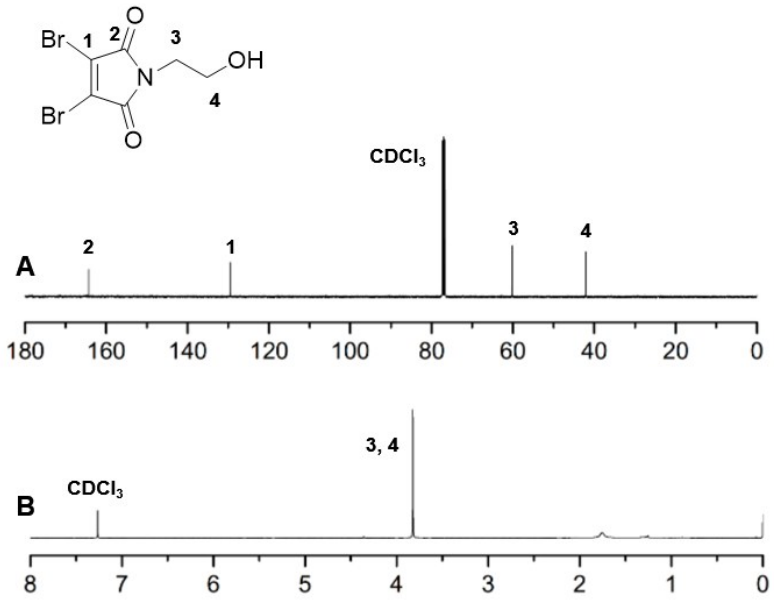


Fig. S2. ^{13}C NMR (A) and ^{1}H NMR (B) spectrum of *N*-hydroxyethyl-3, 4-dibromomaleimide

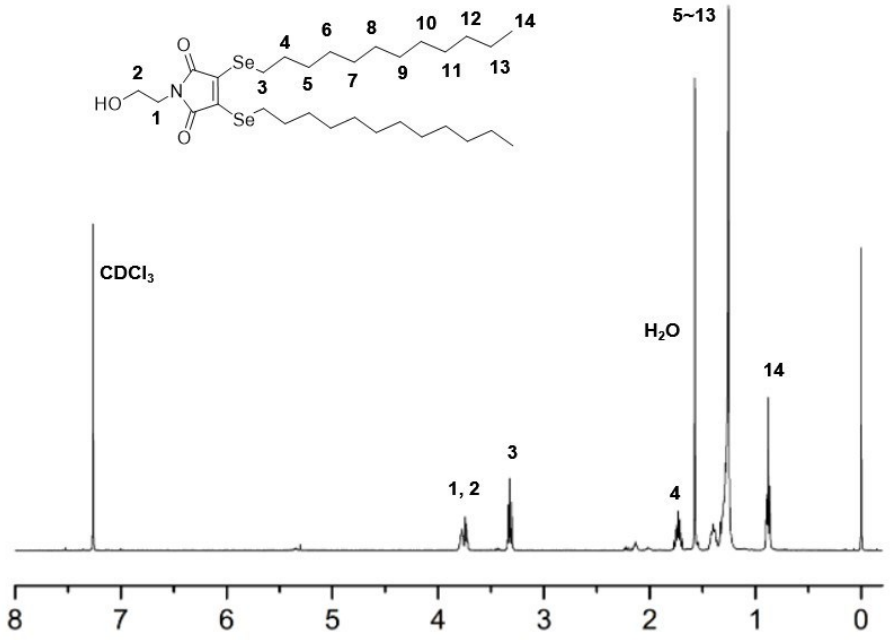


Fig. S3. ^{1}H NMR spectrum of DSeM in $CDCl_3$

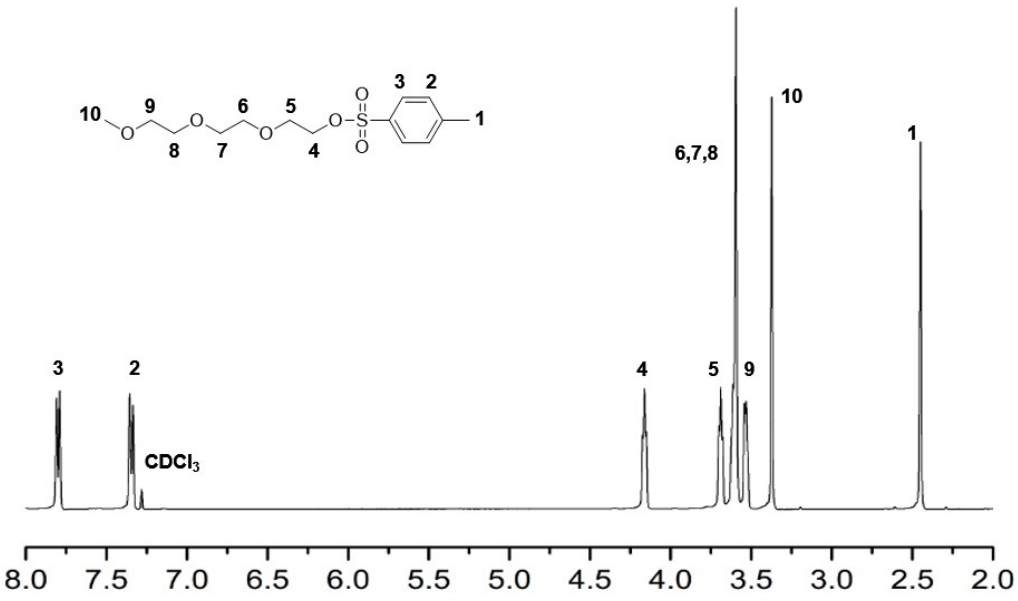


Fig. S4. ^{1}H NMR spectrum of monomethyl triethylene glycol monotosylate in $CDCl_3$

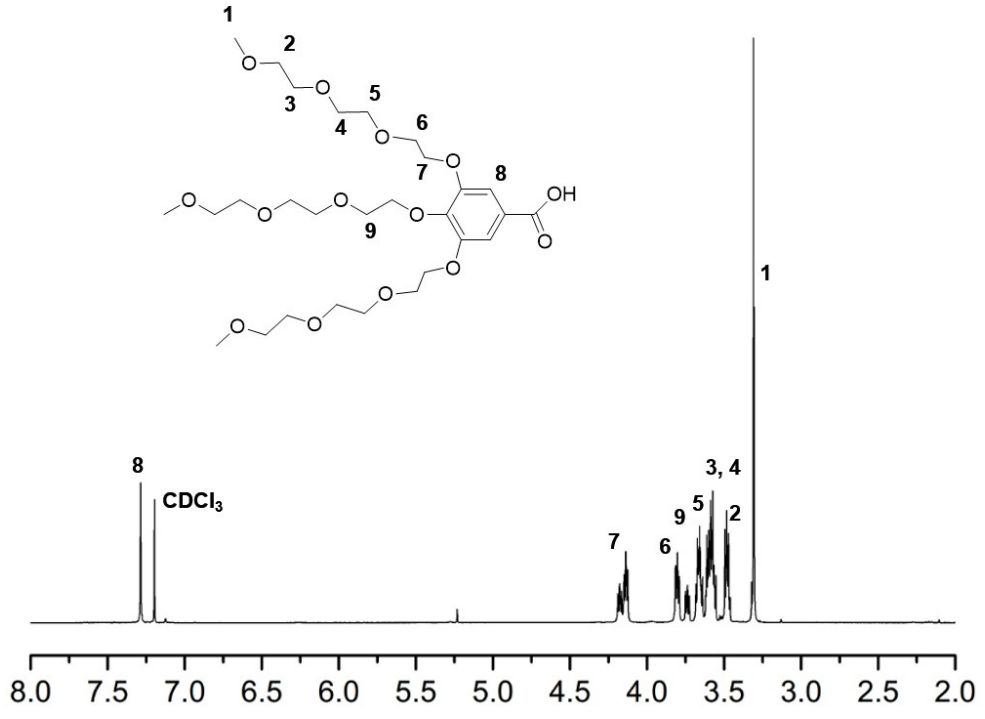


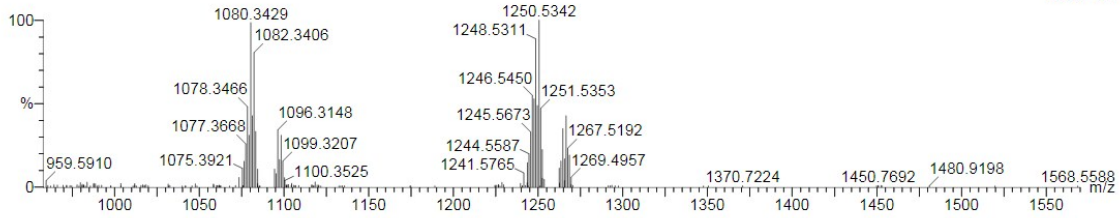
Fig. S5. ^{1}H NMR spectrum of compound ***3*** in $CDCl_3$

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
200 formula(e) evaluated with 1 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-58 H: 0-102 N: 0-1 O: 0-16 Se: 0-2 Na: 0-1

WA-ZHANG ECUST institute of Fine Chem 07-Jan-2016 20:37:46
DSM-4 175 (1.185) Cm (173:177) 1: TOF MS ES+ 3.42e+003



Minimum:				-1.5								
Maximum:		300.0	50.0	100.0								
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula					
1250.5342	1250.5348	-0.6	-0.5	10.5	72.8	0.0	C58	H101	N	O16	Se2	Na

Fig. S6. HRMS spectrum of DSeMTTG

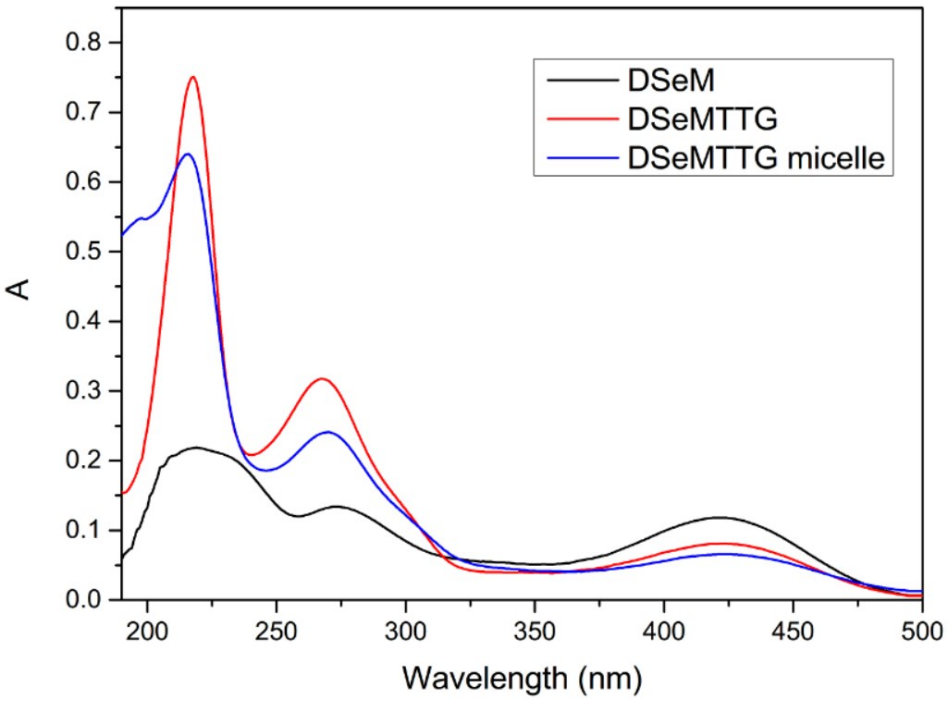


Fig. S7. The absorption spectra of DSeM (black) and DSeMTTG (red) in ethanol, and DSeMTTG micelles (blue) in water

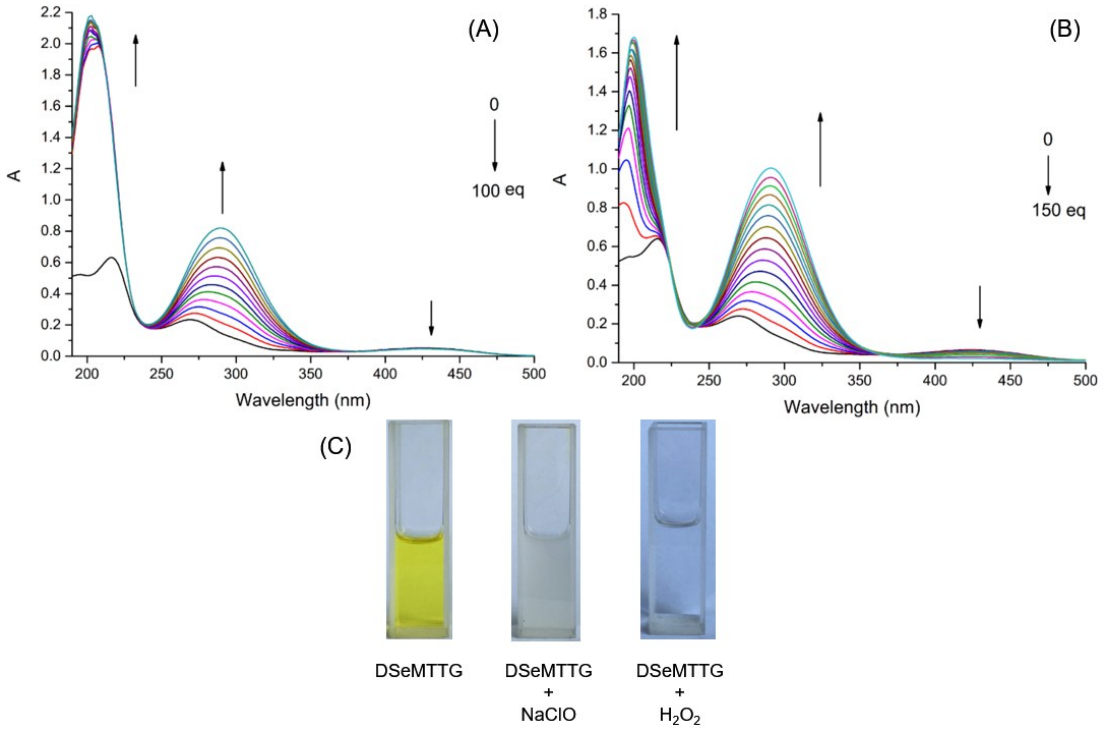


Fig. S8. Absorption spectra of 20 μM DSeMTTG in ethanol upon addition of 100 eq of H_2O_2 (A) , 150 eq of NaClO (B), and their corresponding color changes in naked eye (C).

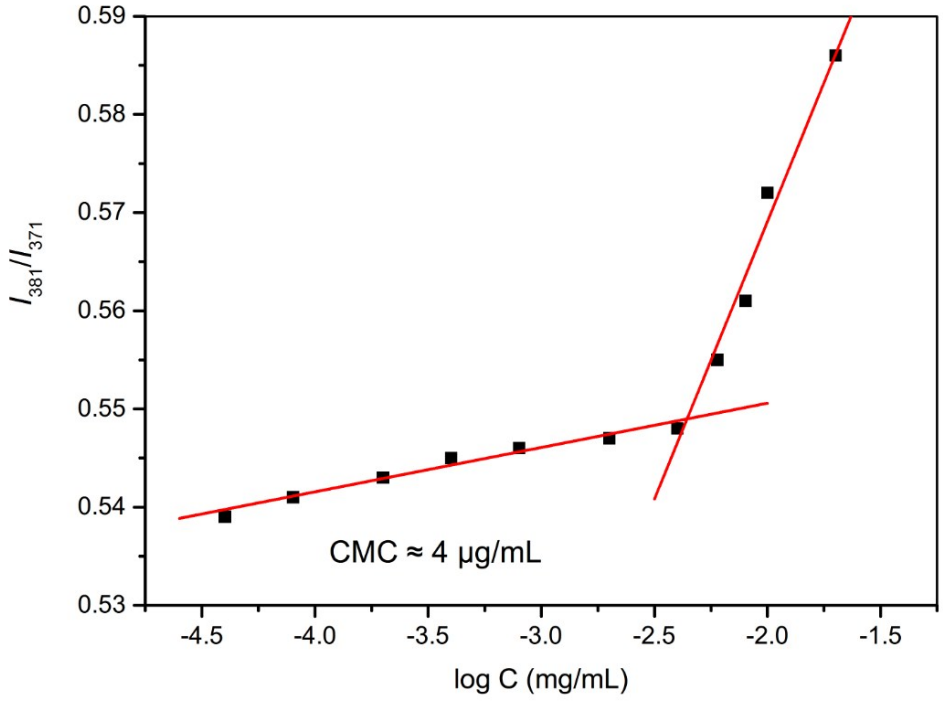


Fig. S9. Ratio change of I_{381}/I_{371} to the logarithm of the micelle concentration. The CMC value of DSeMTTG micelles is about 4 μg/mL.

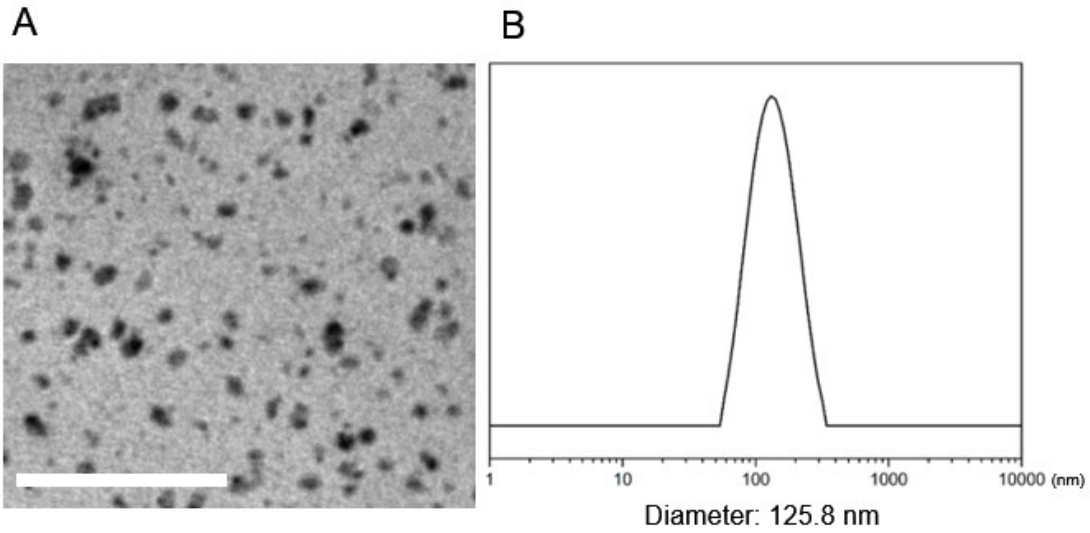


Fig. S10. TEM image (A) and DLS (B) of DSeMTTG micelles. Scale bar = 1 μm.

References

1 H. Wang, Y. Wu, P. Tao, X. Fan and G.-C. Kuang, *Chem. – Eur. J.*, 2014, **20**, 16634–16643.

2 L. Xu, L. Liu, F. Liu, H. Cai and W. Zhang, *Polym. Chem.*, 2015, **6**, 2945–2954.

3 H. Ren, Y. Wu, N. Ma, H. Xu and X. Zhang, *Soft Matter*, 2012, **8**, 1460–1466.

4 L. Castañeda, Z. V. F. Wright, C. Marculescu, T. M. Tran, V. Chudasama, A. Maruani, E. A. Hull, J. P. M. Nunes, R. J. Fitzmaurice, M. E. B. Smith, L. H. Jones, S. Caddick and J. R. Baker, *Tetrahedron Lett.*, 2013, **54**, 3493–3495.