Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

Lighting up the PEGylation Agents via the Hantzsch Reaction

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1. Materials

Benzaldehyde (Aladdin, 99%), dimedone (Aladdin, 99%), ammonium acetate, glycine (SCRC, 99%), 4,4'-dithiodipyridine, N,N'-dicyclohexylcarbodiimide (DCC, Aladdin, 99.0%), 4-dimethylamiopryidine (DMAP, Aladdin, 99%), 3-mercaptopropionic acid (Heowns, 98%), 3-bromo-1-propanol (Aladdin, 97%) and *p*-hydroxybenzaldehyde (Aladdin, 98%) were used as purchased. NuPAGE®Novex®4-12% Bis-Tris Protein Gels (1 mm, 10 well), NuPAGE®MOPS SDS Running Buffer (20 ×), NuPAGE®LDS Sample Buffer (4 ×) and NuPAGE®Sample Reducing Agent (10 ×) were purchased from Novex. 4-(3-Hydroxypropoxy)benzaldehyde and 3-(pyridin-2-yldisulfanyl)-propanoic acid were synthesized as literatures[1,2]. mPEG-NH_2 HCl (M_n ~ 5000，~ 95% NH_2 loading degree) and H_2N-PEG-NH_2 (M_n ~ 5000，~ 95% NH_2 loading degree) were purchased from XIAMEN SINOPEG BIOTECH CO., LTD. All solvents were purchased from Sinopharm Chemical Reagent and used directly without further purification.

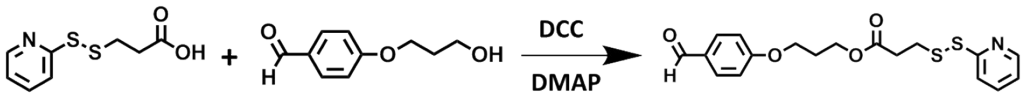
2. Measurements

Gel permeation chromatography (GPC) analyses were performed using N,N-dimethyl formamide (DMF) as the eluent. The GPC system was a Shimadzu LC-20AD pump 45 system consisting of an auto injector, a MZ-Gel SDplus 10.0 μm guard column (50 × 8.0 mm, 10^2 Å) followed by a MZ-Gel SDplus 5.0 μm bead-size column (50-10^6 Å, linear) and a Shimadzu RID-10A refractive index detector. ^{1}H NMR and ^{13}C NMR spectra were obtained using a JEOL JNM-ECA400 (400 MHz) spectrometer for all samples. The ESI-MS data were recorded on a Micro TOF-QII Bruker. The FT-IR spectra were made in a transmission mode on a Perkin-Elmer Spectrum 100 spectrometer (Waltham, MA, USA). The fluorescence measurements were obtained on a Perkin-Elmer LS-55 spectrometer equipped with quartz cuvettes of 1 cm path length.

The HPLC analyses were performed using Reverse phase high performance liquid chromatography (RP-HPLC) which was two Shimadzu LC-6AD pump systems consisting of an auto injector, an Agilent Zorbax 300SB-C18 column, a Shimadzu SPD-M20A diode array detector. The mobile phases were phase A (99.9% H_2O, 0.1% TFA) and phase B (99.9% acetonitrile, 0.1% TFA) respectively. The gradient of the mobile phase is 30%-70% phase B (30 min). Matrix-assisted laser desorption ionization time-of-flight mass (MALDI-TOF MS) spectra were recorded on an AXIMA-PerformanceMA in a linear mode. The absolute quantum yield of fluorescence (Φ_{FL}) values were recorded on an Edinburgh FLSP920.

3. Methods

3.1 Synthesis of 3-(4-formylphenoxy)propy-3-(pyridin-2-yldisulfanyl)propanoate



4-(3-Hydroxypropoxy)benzaldehyde[1] and 3-(pyridin-2-yldisulfanyl)-propanoic acid[2] were synthesized as previous literatures. 3-(Pyridin-2-yldisulfanyl)-propanoic acid (1.07 g, 5.00 mmol), 4-(3-hydroxypropoxy)benzaldehyde (0.90 g, 5.00 mmol) and DMAP (61.00 mg, 0.50 mmol) were dissolved in mixed anhydrous CH_2Cl_2/THF solution (40 mL/10mL). DCC (1.24 g, 6.00 mmol) was then added, and the system was stirred at 25 °C for 24 h. After removing the solvents, cold ethyl acetate (15 mL) was added and the insoluble white solid was removed by filtration. The crude was purified via column chromatography eluting with ethyl acetate/petroleum ether (1:4) to get product as yellow oil (1.2 g, 60% yield).

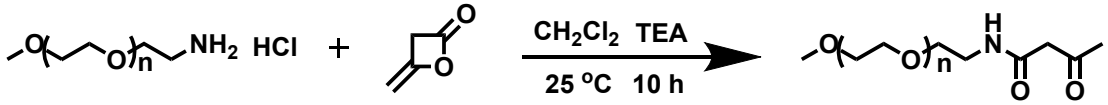
^{1}H NMR (400 MHz, $CDCl_3$, δ/ppm): 9.87 (s, 1H, C<u>H</u>O), 8.45 (d, 1H, *J* = 4.0 Hz, C<u>H</u>N), 7.82 (d, 2H, *J* = 8.0 Hz, C<u>H</u>CCHO), 7.59-7.69 (m, 2H, C<u>H</u>C<u>H</u>CHN), 7.08 (m, 1H, C<u>H</u>CN), 6.99 (m, 2H, C<u>H</u>COC), 4.28 (t, 2H, *J* = 8.0 Hz, C<u>H</u>$_2$OAr), 4.12 (m, 2H, C<u>H</u>$_2$OCO), 3.02(t, 2H, *J* = 8.0 Hz, C<u>H</u>S), 2.79 (t, 2H, *J* = 8.0 Hz, C<u>H</u>CO), 2.15 (m, 2H, ArOCH$_2$C<u>H</u>$_2$CH$_2$).

^{13}C NMR (100 MHz, $CDCl_3$, δ/ppm): 190.9, 171.9, 163.7, 159.7, 149.8, 137.2, 132.1, 130.1, 120.9, 119.8, 114.8, 64.7, 61.5, 33.8, 33.3, 28.5.

ESI-MS: observed (expected): 378.0823 (378.0828) $[M+H]^+$.

IR (ν/cm^{-1}): 3046, 2961, 2739, 1731, 1686, 1598, 1574, 1509, 1470, 1446, 1417, 1348, 1312, 1253, 1214, 1158, 1114, 1082, 1044, 985, 956, 857, 831, 760, 717.

3.2 Synthesis of mPEG-dione



Methoxypolyethylene glycol amine hydrochloride (M_n ~ 5000, 0.5 g, 0.1 mmol) and triethylamine (10.1 mg, 0.1 mmol) were dissolved in anhydrous CH_2Cl_2 (2 mL), then diketene (34.0 mg, 0.4 mmol) was added dropwise at room temperature. The solution was then stirred at 25 °C for 10 h. After removing the solvent, 5.0 mL of toluene was added to re-dissolve the polymer. The final polymer mPEG-dione was obtained by precipitation from toluene to petroleum ether for three times. The ^1H NMR spectrum of obtained polymer has been shown in Figure S2. From the MALDI-TOF MS analysis (Figure S1), the NH_2 group conversion is nearly complete.

The dione-PEG-dione was synthesized according to the same method, the ^1H NMR spectrum of dione-PEG-dione was shown in Figure S5, and the MALDI-TOF MS analysis (Figure S4) suggested the NH_2 group conversion is around complete.

3.3 Synthesis of mPEG-1,4-DHP-PDS

mPEG-dione (M_n ~ 5000, 0.10 g, 0.02 mmol), dimedone (2.8 mg, 0.02 mmol), CHO-PDS (7.5 mg, 0.02 mmol), ammonium acetate (2.3 mg, 0.03 mmol) and glycine (0.2 mg, 0.002 mmol) were dissolved in 0.05 mL of acetonitrile. The mixture was then stirred at 70 °C for 4 hours, and the pure fluorescent protein-reactive PEG derivative could be obtained through simple passing a short neutral alumina column

and precipitation in diethyl ether. From the MALDI-TOF MS spectra (Figure S1), the chain-end functionalization is almost complete.

The PEG-1,4-DHP-PDS (Figure S5: ^{1}H NMR, Figure S4: MALDI-TOF MS) was synthesized according to the same method.

3.4 Conjugation between the polymer and BSA

Freshly prepared BSA solution (0.5 mL, 2.0 mg/mL in PBS buffer, pH 7.0) was added to three small plastic vials, followed by adding different volumes of mPEG-1,4-DHP-PDS solution (11.45 mg/mL in PBS buffer, pH 7.0): 5 μL, 10 μL and 20 μL, respectively. The vials were incubated at 37 °C with gentle shaking for 4 h. After removing salts through centrifugal filtration (MWCO: 30 k, 6 times, 8000 rpm), the concentrated solutions were used for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), MS and HPLC analyses.

The conjugation between PEG-1,4-DHP-PDS and BSA was performed according to the same method.

3.5 Bioactivity evaluation of the fluorescent PEGylated protein

All samples were tested through the same method, typically: 250 μL of BSA/mPEG-1,4-DHP-PDS mixture was placed in a vial, then freshly prepared 4-nitrophenylacetate solution in acetonitrile (1 M, 5μL) was added. The mixture was diluted to 1.0 mL with water, then incubated at 25°C for 3 min prior to the analysis by UV (405 nm) for five times. All data were analyzed using SPSS 16.0 statistical software. Data of the bioactivity of the obtained fluorescent PEGylated proteins were represented as the mean ± SD, and results were analysed by one-way ANOVA. Native BSA was tested for in the same way, and the value was defined as 100%.

The bioactivity of BSA/PEG-1,4-DHP-PDS mixture was tested according to the same method.

4. Supporting data

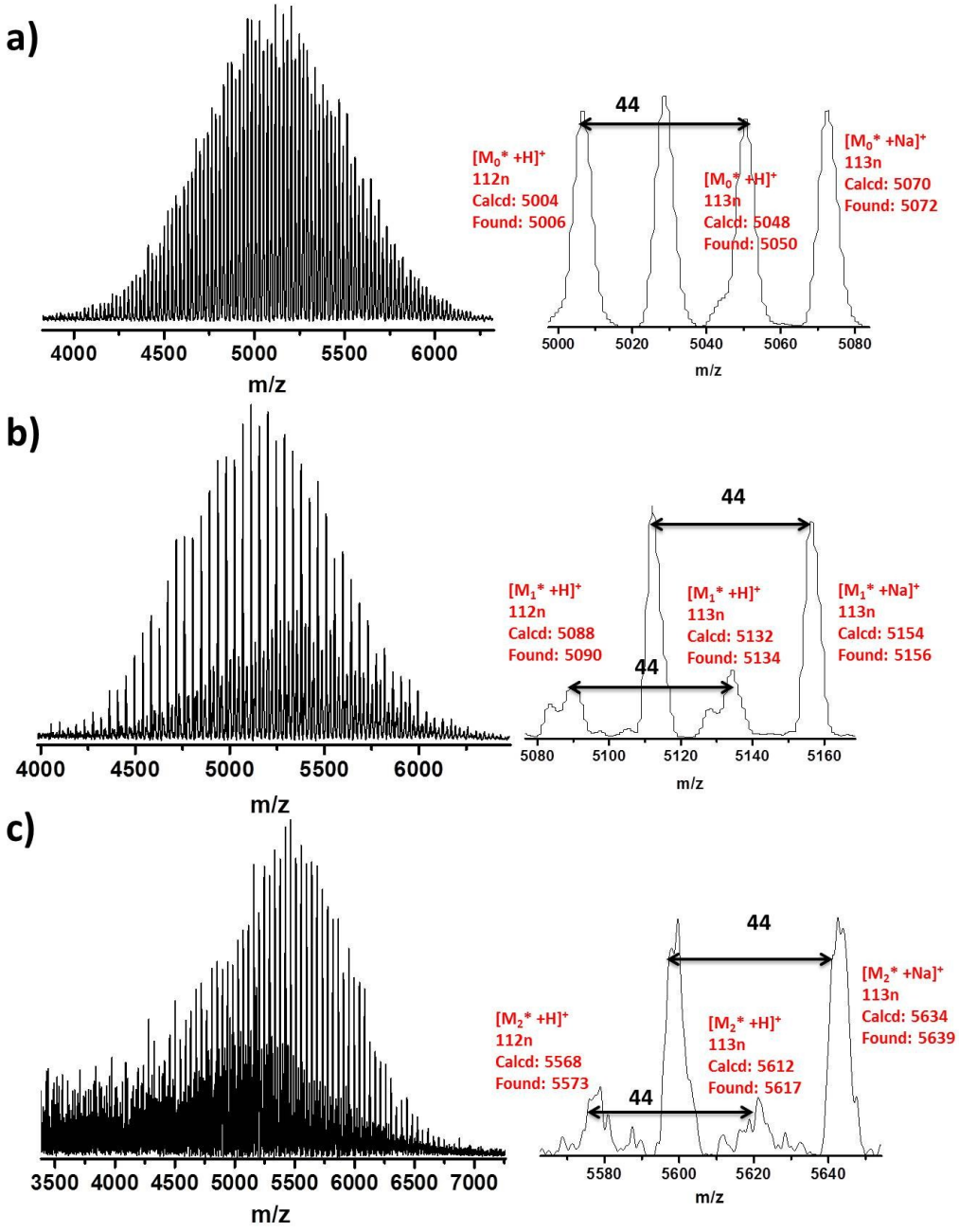


Figure S1. a) MALDI-TOF MS spectra of a) mPEG-NH_2 HCl, b) mPEG-dione, c) mPEG-1,4-DHP-PDS

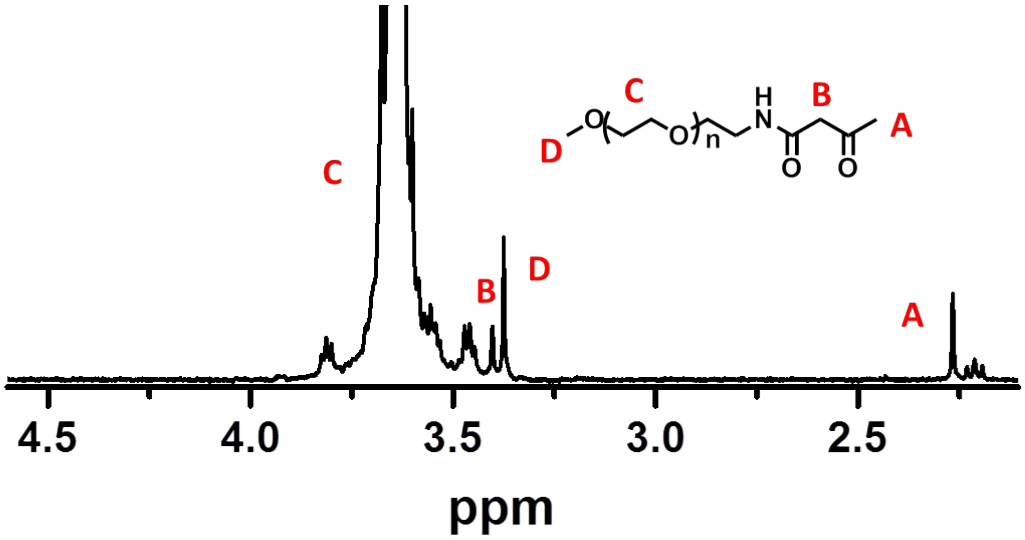


Figure S2. ^{1}H NMR spectrum ($CDCl_3$-d, 400 MHz, portion) of mPEG-dione.

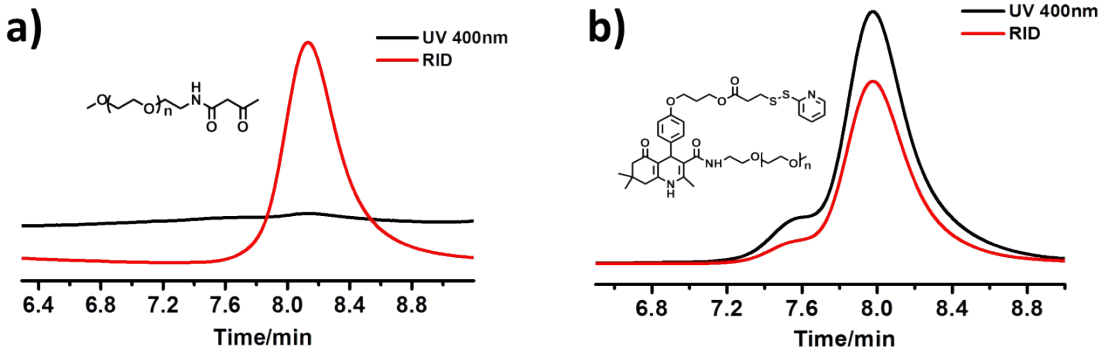


Figure S3. GPC curves of a) mPEG-dione and b) the mPEG-1,4-DHP-PDS using RID and UV detectors.

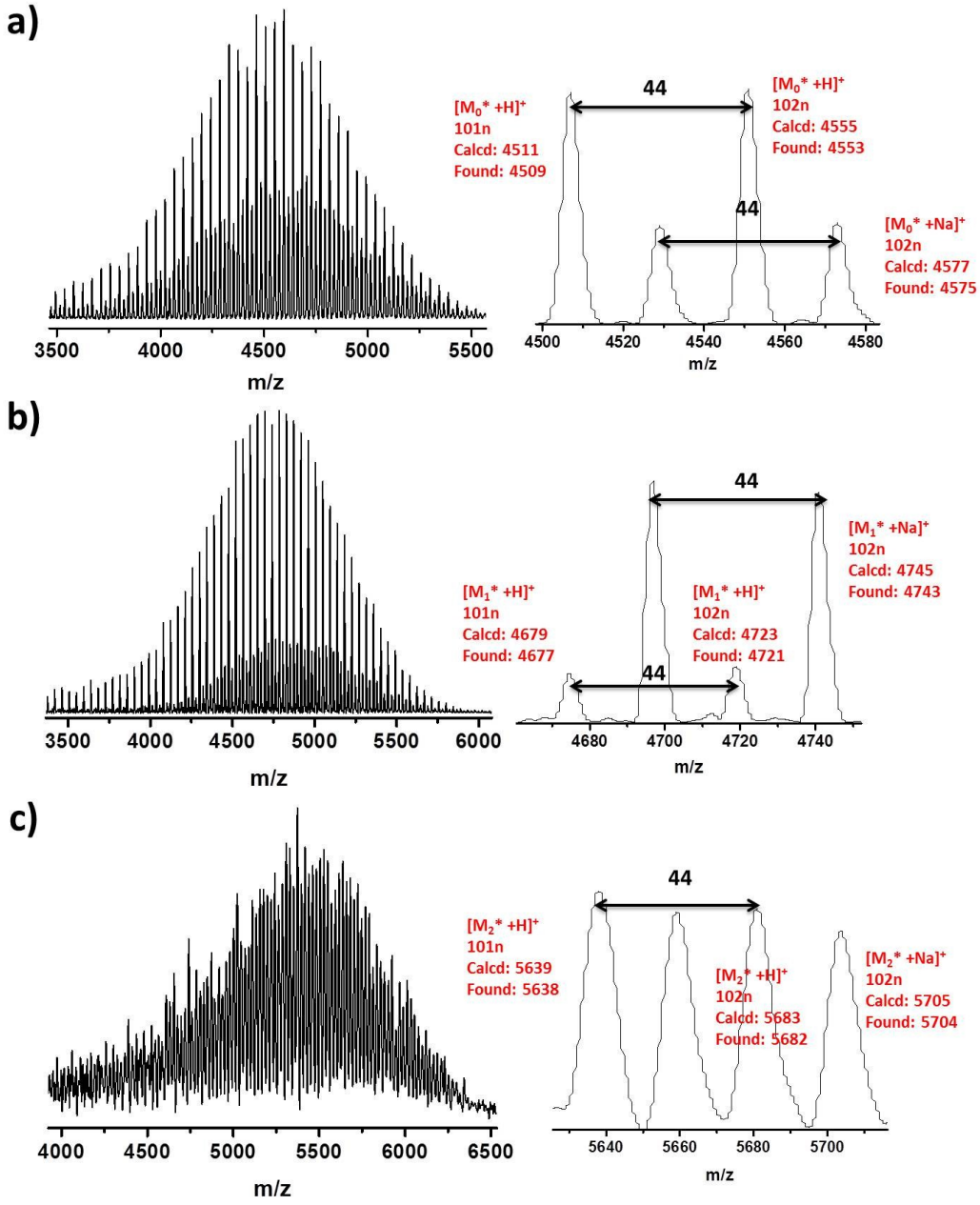


Figure S4. a) MALDI-TOF MS spectra of a) NH_2-PEG-NH_2, b) dione-PEG-dione, c) telechelic PEG-1,4-DHP-PDS.

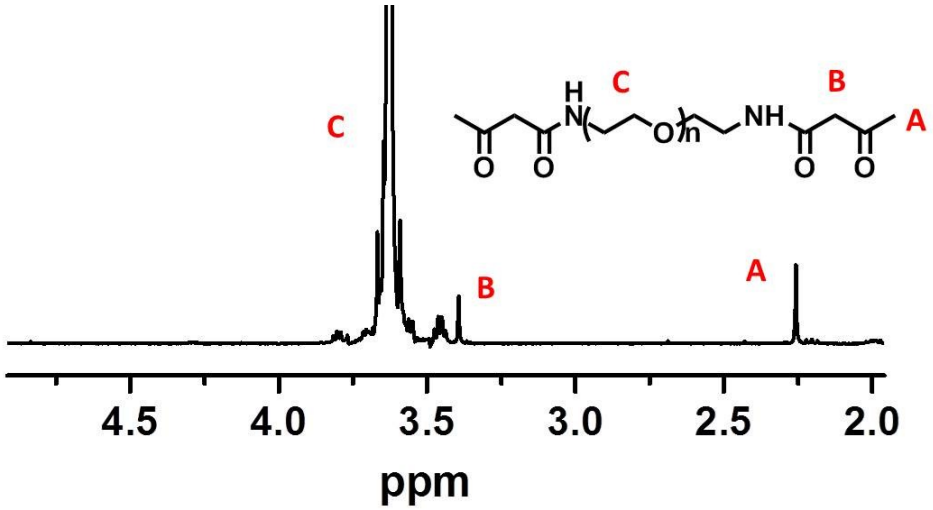


Figure S5. ^{1}H NMR spectrum ($CDCl_3$-d, 400 MHz, portion) of dione-PEG-dione.

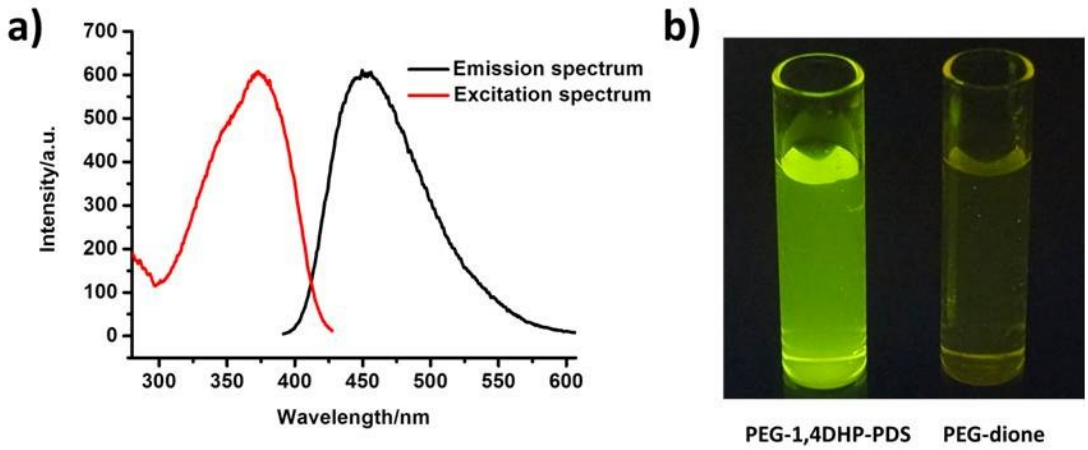


Figure S6. a) Excitation and Emission spectra of PEG-1,4-DHP-PDS; b) The imaging of PEG-1,4-DHP-PDS and PEG-dione solutions in water (2 mg/mL) under UV ~ 312 nm.

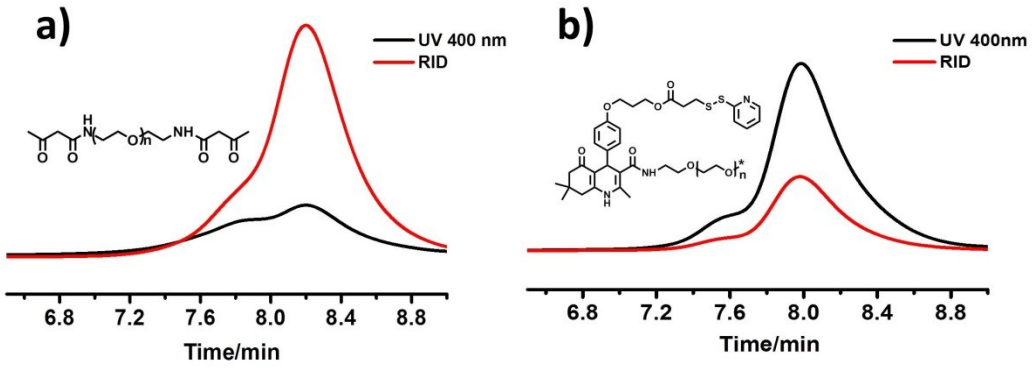


Figure S7. GPC curves of a) Telechelic dione-PEG-dione and b) the telechelic PEG-1,4-DHP-PDS using RID and UV detectors.

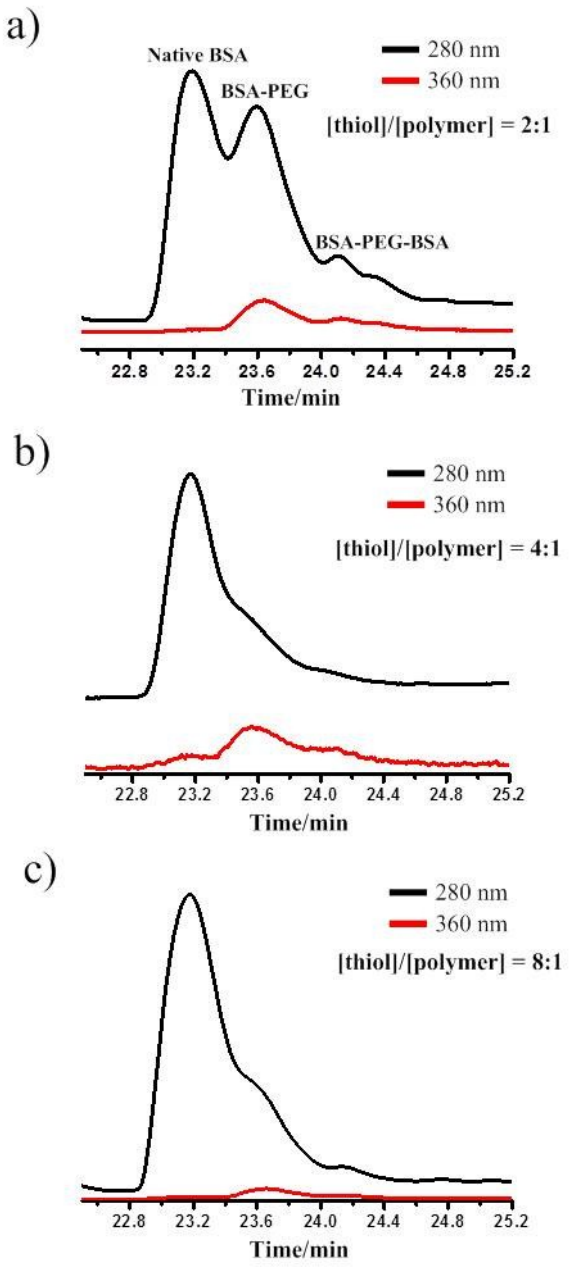


Figure S8. HPLC results of the BSA- telechelic PEG-1,4-DHP-PDS conjugates at different [thiol]/[polymer] ratios. a) 2:1; b) 4:1; c) 8:1.

Reference

(1) Dublanchet, A.-C.; Lusinchi, M.; Zard, S. Z. *Tetrahedron* **2002,** 58, 5715-5721.

(2) Tan, S. Y.; Ang, C. Y.; Li, P.; Yap, Q. M.; Zhao, Y. *Chem. - Eur. J.* **2014**, 20, 11276-11282.