Supporting Information

Fluoresent PEGylation Agent by Thiolactone-based One-pot Reaction: A New Strategy for Theranostic Combines

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Experimental Section

1. Materials

Methoxypolyethylene glycol amine (mPEG-NH_2, Mn ~5000, Sinopeg), dansyl chloride (Heowns, 99%), DL-homocysteinethiolactone hydrochloride (Aladdin, ≥ 99%), 2,2'-dithiodipyridine (Heowns, 99%), 5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB, Heowns, 99%), 4-nitrophenylacetate (Aladdin, 99%), cysteine (Aladdin, ≥ 99%), NuPAGE® Novex® 4-12% Bis-Tris Protein Gels, 1 mm, 10 well, NuPAGE® MOPS SDS Running Buffer (20 ×), NuPAGE® LDS Sample Buffer (4 ×), NuPAGE® Sample Reducing Agent (10 ×) were used as purchased.

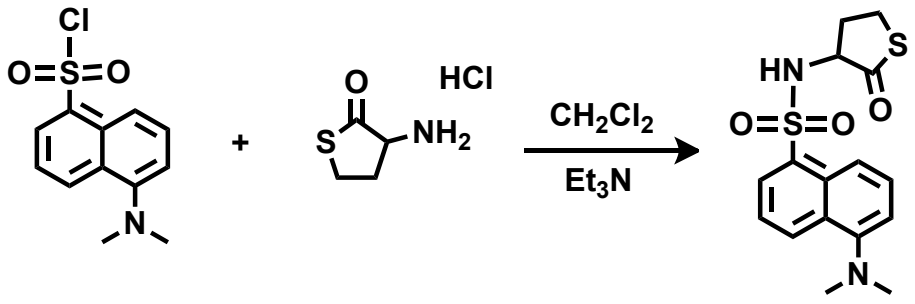
2. Instrumental Analysis

Gel permeation chromatography (GPC) analyses of polymers were performed using N,N-dimethyl formamide (DMF) containing 50 mM LiBr as the eluent. The GPC system was a Shimadzu LC-20AD pump 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), a Shimadzu RID-10A refractive index detector and a Shimadzu SPD-10A UV detector. The system was calibrated with narrow molecular weight distribution polystyrene standards ranging from 200 to 10^6 g mol^{-1}. Reverse phase high performance liquid chromatography (RP-HPLC) 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% H2O, 0.1% TFA) and phase B (99.9% acetonitrile, 0.1% TFA) respectively. The gradient of the mobile phase is 80%-30% phase A (30 min). ^{1}H NMR and ^{13}C NMR spectra were obtained using a JEOL JNM-ECA400 (400MHz) spectrometer for all samples. The ESI-MS data were collected using 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). UV-Visible absorption spectra were recorded on UV/Vis/NIR Perkin-Elmer lambda750 spectrometer (Waltham, MA, USA) using quartz cuvettes of 1 cm path length. The fluorescence measurements were obtained on a Perkin-Elmer LS-55 spectrometer equipped with quartz cuvettes of 1 cm path length. XCell SureLock® Mini-Cell. MALDI-TOF mass spectrometry was performed on an Axima Perfomance and operated in linear mode with an external calibration. Protein samples were prepared by mixing 1:1 v/v ratios of SA matrix (10 mg/mL in acetonitrile)

3. Method

3.1. Synthesis of 5-(dimethylamino)-N-(2-oxotetrahydrothiophen-3-yl) naphthalene-1-sulfonamide (dansyl thiolacton):



Dansyl chloride (0.54 g, 2 mmol) was dissolved with DL-homocysteinethiolactone hydrochloride (0.31 g, 2 mmol) in 15 mL of dry CH_2Cl_2. Triethylamine (0.6 g, 6 mmol) was added to the mixture. The system was stirred at 25 °C for 24 h. After removing the solvents, the solid was washed by water three times and extracted by CH_2Cl_2. The organic layer was dried over $MgSO_4$ and then evaporated to get a yellow solid (0.65 g, 93%).

^{1}H NMR (400 MHz, $CDCl_3$, δ/ppm): 8.58 (d, 1H, *J* = 8.5 Hz, C<u>H</u>CS), 8.31-8.17 (m, 2H, SCCHC<u>H</u>C<u>H</u>C), 7.61 (dd, 1H, *J* = 8.5 Hz, 7.8 Hz, NCCHC<u>H</u>CH), 7.53 (dd, 1H, *J* = 8.3 Hz, 7.4 Hz, NCCHCHC<u>H</u>), 7.21 (d, 1H, *J* = 7.5 Hz, NCC<u>H</u>CHCH), 3.71 (dd, 1H, *J* = 12.7 Hz, 6.8 Hz, NC<u>H</u>CO), 3.22-3.14 (m, 2H, $SCH_2C\underline{H_2}$), 2,90 (s, 6H, $NC\underline{H_3}$) 2.81-2.72 (m, 1H, $SC\underline{H_2}CH_2$), 2.02-1.93 (m, 1H, $SC\underline{H_2}CH_2$).

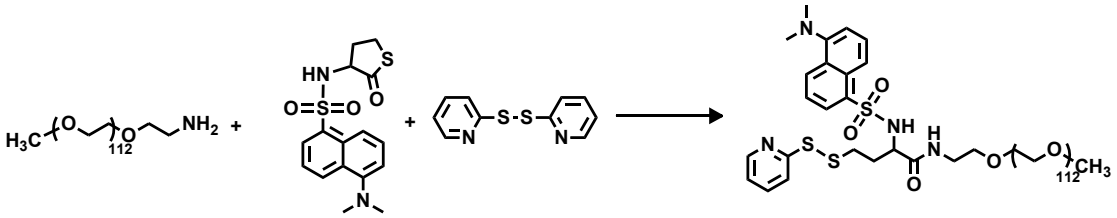
^{13}C NMR (100 MHz, $CDCl_3$, δ/ppm): 203.81, 152.21, 133.92, 131.26, 130.16,129.75, 129.73,128.98, 123.12,118.74, 115.71, 62.17, 45.57, 33.03, 27.66.

IR (ν/cm^{-1}): 2941, 1740, 1322, 1143, 982, 937, 883, 790.

ESI-MS: observed (expected): 351.0836 (351.0832) [M+H$^+$].

PL: λex max = 340 nm, λem max = 514 nm

3.2. Synthesis of the multifunctional PEG derivative (mPEG-dansyl-PD):



The amino terminated methoxypolyethylene glycol (mPEG-NH_2, M_{nNMR} ~ 5000, 100 mg 0.02 mmol), dansyl thiolacton (70 mg, 0.2 mmol), and 2,2'-dithiodipyridine (132 mg, 0.6 mmol) were charged into a dry EP tube along with CH_2Cl_2 (1 mL). The EP tube was put into a thermo-shaker at 40 °C for 18 hours. The crude was then precipitated from CH_2Cl_2 to cold diethyl ether for 3 times, and then dried under vacuum to obtain the pure polymer for further use and characterizations.

^{1}H NMR (400 MHz, $CDCl_3$, δ/ppm): 8.74-8.42 (m, 2H, C<u>H</u>CS, NC<u>H</u>), 8.32 (d, 1H, *J* = 8.4 Hz, SCCHCHC<u>H</u>C), 8.23 (d, 1H, *J* = 8.4 Hz, SCCHC<u>H</u>CHC),7.68-7.57 (m, 1H,

NCCHCHCH), 7.57-7.43 (m, 2H, NCCHCHCH, NCHCHCH), 7.23-7.09 (m, 2H, NCCHCHCH, NCHCHCH), 6.99 (d, 1H, *J* = 6.8 Hz, NCHCHCHCH), 3.39 (s, 3H, OCH_3), 2,89 (s, 6H, NCH_3).

PL: λex max = 331 nm, λem max = 544 nm.

3.3. Measurement of the active thiol group of BSA (Ellman's assay):

The activity of thiol group on BSA surface was evaluated through Ellman's assay. Typically, cysteine solution (1 mM) was prepared in pure water (100 mL) with 1 mL formic acid inside. Then the cysteine solution was diluted with Tris-HCl buffer (pH = 8.3) into cysteine standard solutions (0.025mM, 0.05 mM, 0.1 mM, 0.15 mM, 0.2 mM). 5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB) was prepared to a solution (10 mM) in PBS buffer (pH = 7.0) and then diluted with Tris-HCl buffer (pH = 8.3) into DTNB standard solution (0.1 mM). The cysteine standard solution (0.25 mL) and DTNB standard solution (1.25 mL) were mixed in EP tube and kept for 10 min, then the UV absorption at 412 nm of the mixture was measured for 5 times to get a standard curve (**Figure S3**). In the same way, the UV absorption at 412 nm of the BSA solution (2 mg/mL) and DTNB standard solution was measured to calculate the active thiol group of BSA as 57.8%.

Supporting Data

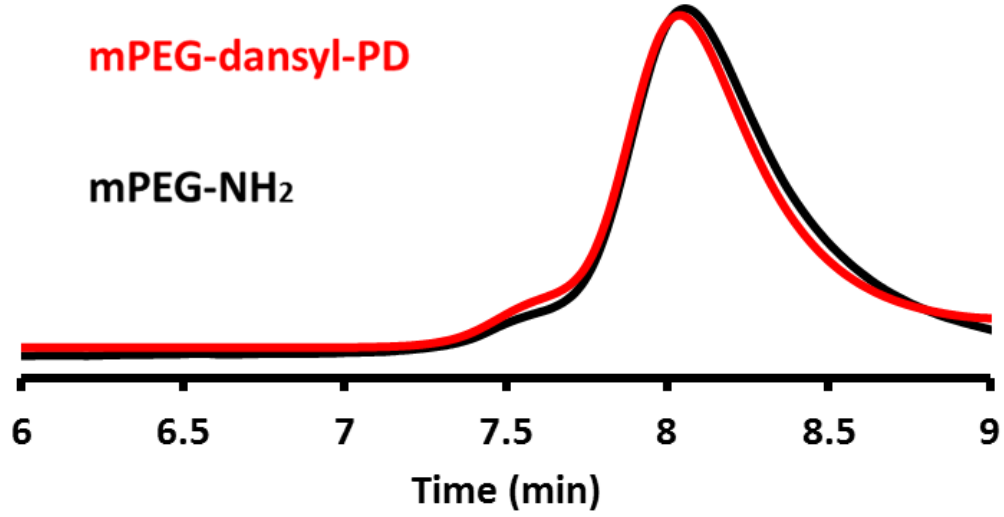


Figure S1. GPC results *via* RID detector of mPEG-NH_2 and mPEG-dansyl-PD (M_{nGPC}~28000, PDI~1.05).

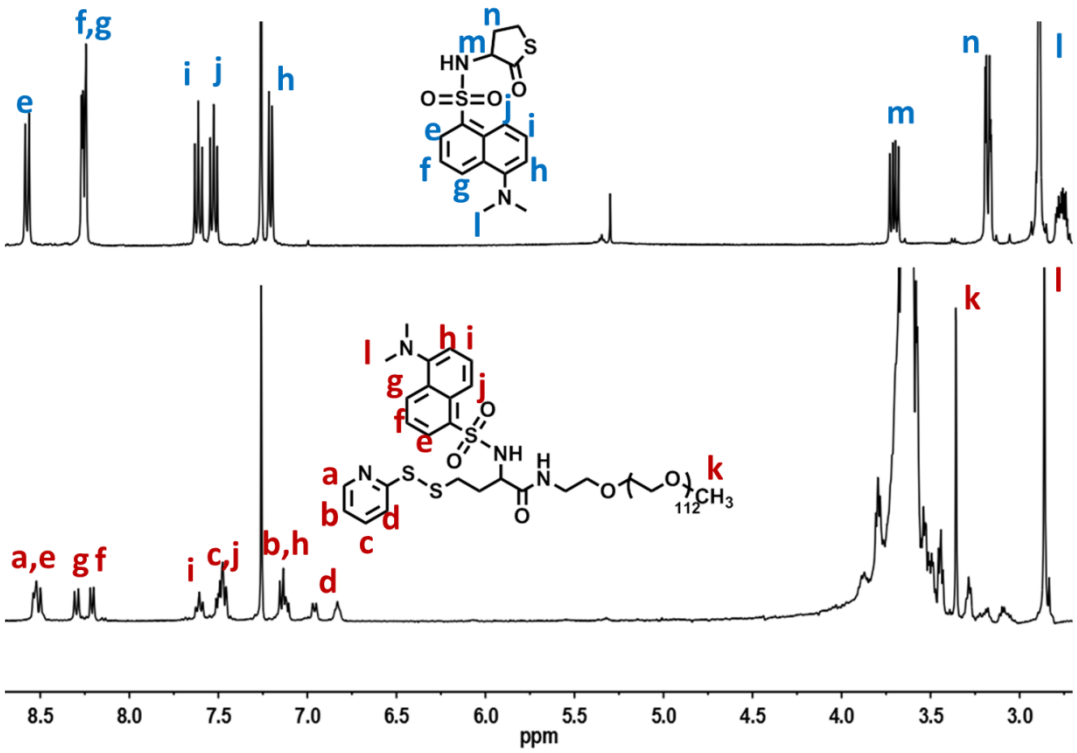


Figure S2. ^{1}H NMR spectrum ($CDCl_3$, 400 MHz, portion) of the dansyl thiolactone and the mPEG-dansyl-PD.

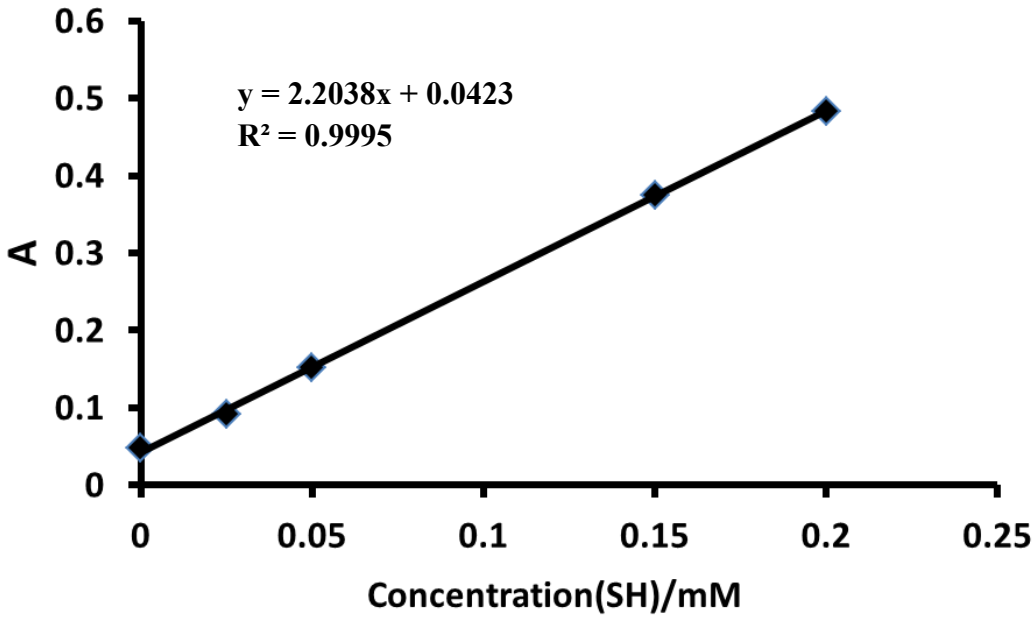


Figure S3. Standard curve of the measurement of the quantity of thiol group.