

Electronic Supplementary Information

## Red Light Responsive Diselenide-containing Block Copolymer Micelles

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### Experimental part:

#### Materials.

Meso-tetra(4-sulfonatophenyl)porphyrin (Por), 5,10,15,20-tetrakis(4-(trimethylamino)-phenyl)-21H,23H-porphine tetratosylate (Por<sup>+</sup>) and 9,10-anthracenedipropionic acid (ADPA) were obtained from Sigma–Aldrich. Doxorubicin (Dox) (hydrochloride, 99.5%) was purchased from Zhongshuo Pharmaceutical Co., Ltd. (Beijing, China). And all the other solvents were analytical grade agents purchased from Beijing Chemical Reagent Company (Beijing, China).

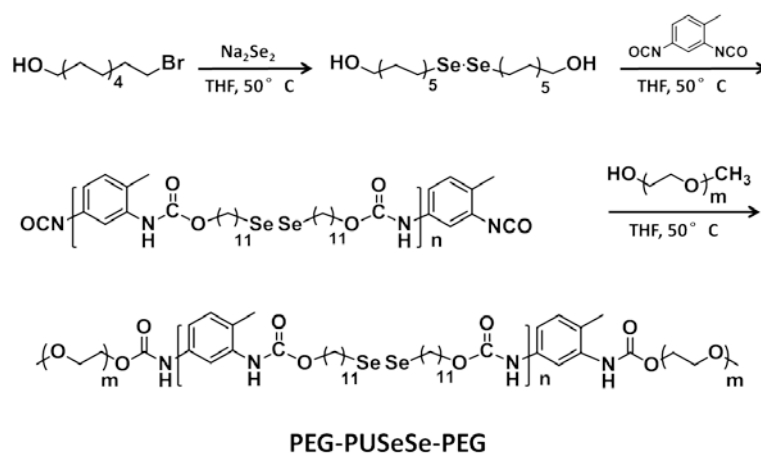
#### Instruments.

The <sup>1</sup>H-NMR spectra were recorded on a JEOL JNM-ECA 600 (600MHz) spectrometer. UV/Vis and fluorescence spectra were recorded on Hitachi U-3010 and Hitachi F-7000 spectrophotometers, respectively. Transmission electron microscopy was performed on JEOL JEM-2010 microscope or Hitachi H-7650B microscope. Dynamic light scattering was performed

on a Malvern ZS90 Zetasizer to analyze the size distribution of the aggregates. Fourier transform infrared (FT-IR) spectra were obtained on a Bruker IFS 66v/s spectrometer using KBr substrates. Red light ranged from 600 nm to 780 nm was produced by xenon light (300 W) which was cut with several optical filters.

### Synthesis of PEG-PUSeSe-PEG block copolymers.

The diselenide-containing polymers were synthesized according to previous procedures.<sup>1</sup> Di-(1-hydroxyundecyl) diselenide was synthesized through the reaction of disodium diselenide and 11-bromoundecanol as the monomer. The monomer was polymerized with a slight excess of toluene diisocyanate (TDI) and then the two active ends were finally terminated by polyethylene glycol (PEG) monomethyl ether of different molecular weight, including 750 g/mol, 1900 g/mol and 5000 g/mol.



**Scheme S1** Synthetic route of PEG-PUSeSe-PEG block copolymers.

### Preparation of PEG-PUSeSe-PEG aggregates.

5.0 mg of PEG-PUSeSe-PEG was dissolved in 1 mL of DMF. The solution was then dropped slowly into 45 mL of deionized water while oscillating with ultrasound. DMF was removed by dialysis against deionized water for 24 hours. The dialysis bag stops molecules weighted more than 7000 g/mol from passing through. After the dialysis, water was added into the solution until

the volume reached 50 mL. The concentration of the final solution was 0.1 mg/mL. The final solution was used for DLS experiments to obtain the size distribution of the aggregates. TEM samples were prepared by drop-coating the solution onto the carbon-coated copper grids and staining with 0.2% phosphotungstic acid hydrate.

#### **Critical Aggregate Concentration (CAC) of PEG-PUSeSe-PEG**

The CACs of the three kinds of block copolymers were determined by fluorescence method using pyrene as the probe. A series of PEG-PUSeSe-PEG aqueous solution with different concentrations were prepared. Then a little amount of pyrene acetone solution ( $10^{-4}$  mg/mL) was added into the PEG-PUSeSe-PEG solution. The mixed solution was dealt with ultrasound to remove the acetone. After that, fluorescence spectra were collected with the excited wavelength at 335 nm. The fluorescence intensity at  $I_1$  (372 nm) and  $I_3$  (383 nm) was recorded to calculate the value of  $I_1/I_3$ . The value  $I_1/I_3$  was plotted against the logarithm of the concentration to determine the CAC, as shown in Fig. S2.

#### **Oxidation of PEG-PUSeSe-PEG upon red light irradiation.**

The PEG-PUSeSe-PEG aggregate solution described above was mixed with Por solution. In the final solution, the concentration of PEG-PUSeSe-PEG was 0.1 mg/mL and the concentration of Por was 0.01 mg/mL. The solution was then irradiated with red light ranged from 600 nm to 780 nm for a certain time (1 hour, 2 hour, 5 hour). After that, the solution was froze and dried up. The sample powder was mixed with KBr in a mass ratio of 1:200, followed by extruding into thin substrate for the FT-IR experiments. Control experiments were performed without incorporating Por into the PEG-PUSeSe-PEG aggregates.

#### **Loading of Dox into PEG-PUSeSe-PEG1900 aggregates and its releasing.**

2.0 mg of PEG-PUSeSe-PEG and 2.0 mg of Dox were dissolved in 0.4 mL of DMF respectively. The two solutions were mixed together and dropped into 18 mL of deionized water while oscillating with ultrasound. DMF and the excess amount of Dox that was not incorporated into the aggregates were removed by dialysis against deionized water. The dialysis bag used here stops molecules weighted more than 3500 g/mol from passing through. After dialysis, the solution was filled with water to 20 mL and 0.1 mg/mL of PEG-PUSeSe-PEG aggregates solution incorporated with Dox was obtained afterwards.

The release of Dox in the PEG-PUSeSe-PEG1900 aggregates was monitored by fluorescence spectroscopy. Mix 3 mL of 0.1 mg/mL PEG-PUSeSePEG1900 incorporated with Dox as described above with 0.15 mL of 0.2 mg/mL Por solution, put the solution in the dialysis bag with a 7000 molecular weight cut off. And the dialysis bag was then put in 10 mL of deionized water. Irradiate the solution with red light ranged from 600 nm to 780 nm for 1 hour, take out 1 mL of the solution inside the dialysis bag and do the fluorescence spectroscopy and DLS experiments. After that, the solution was put back to the dialysis bag for further irradiation. Control experiments were done with the same procedure except without irradiation of red light at the same time.

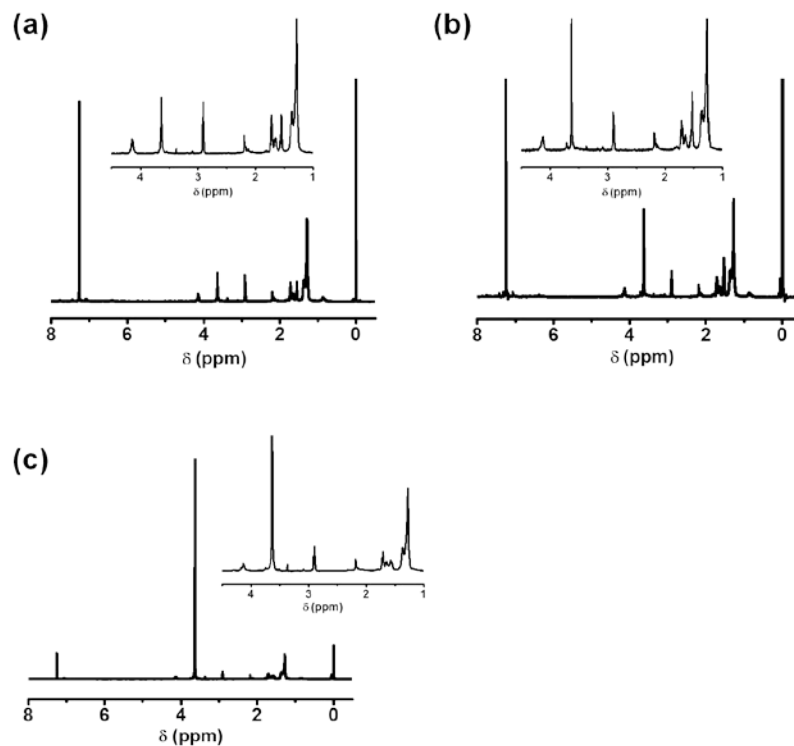
#### **Biocompatibility study of PEG-PUSeSe-PEG.**

The normal human hepatic cell line L-02 was purchased from Cell Resource Center, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. Cells were maintained in RPMI 1640 culture medium (Gibco, USA) supplemented with 10% (v/v) fetal bovine serum (Gibco, USA), 100 U/mL penicillin and 100 µg/mL streptomycin, and incubated at 37 °C under a humidified atmosphere containing 5% CO<sub>2</sub>.

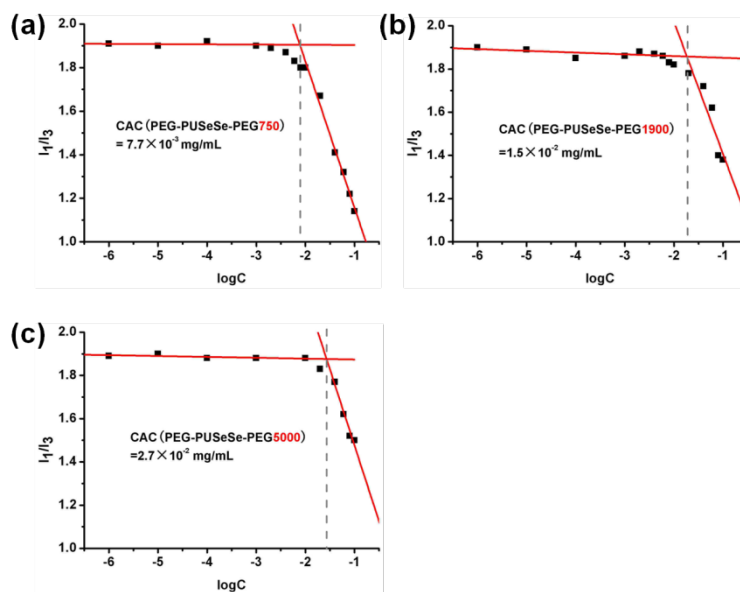
For experiments, L-02 cells were seeded in 96-well culture plate at a density of  $1 \times 10^5$  cell/mL and

allowed to attach for 24 h, then exposed to three kinds of block copolymers with concentrations of 0.001, 0.01 and 0.1 mg/mL for another 24 h. The same procedure was done with the mixed solution of PEG-PUSese-PEG1900 and Por that was irradiated upon red light for 2 h and 5 h. Cells maintained in RPMI without block copolymers were used as control. Viabilities of cells were determined by MTT reduction method. The cells were stained with MTT solution for 4 h and then dissolved in dimethylsulphoxide (DMSO). Optical density at 490 nm was detected by microplate reader (Thermo, USA).

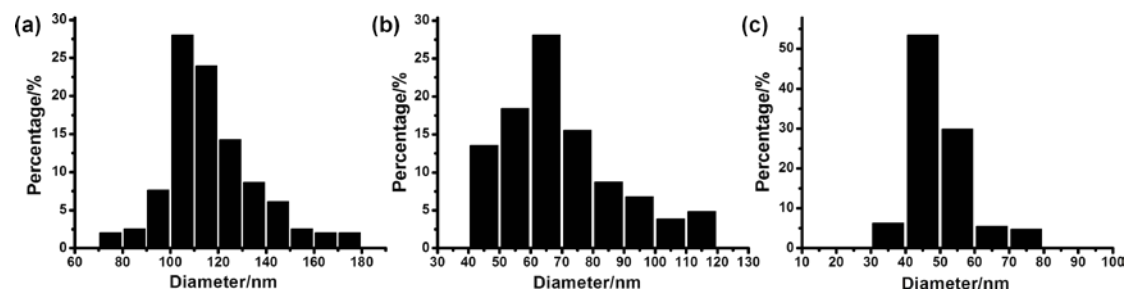
### Results:



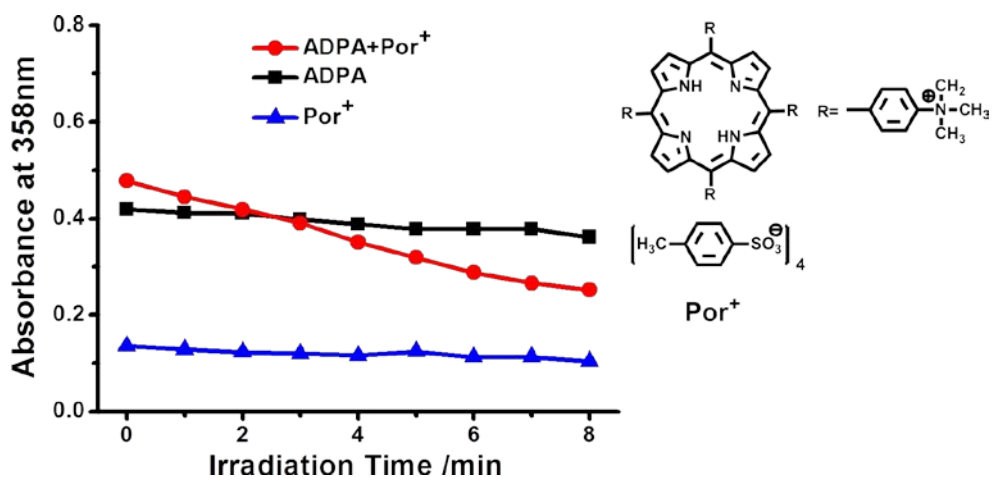
**Fig. S1** <sup>1</sup>H-NMR results of the three kinds of block copolymers (a) PEG-PUSese-PEG750, (b) PEG-PUSese-PEG1900 and (c) PEG-PUSese-PEG5000. Insets of each figure are zoomed in at the range from 1.0 ppm to 4.5 ppm.



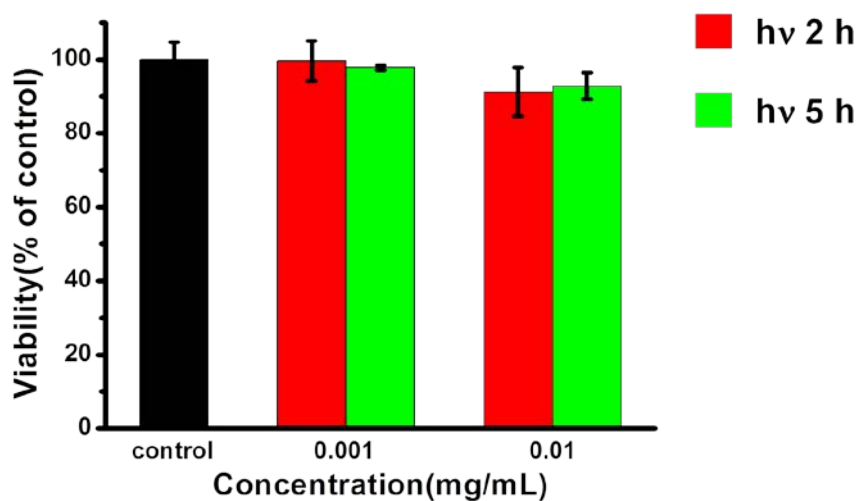
**Fig. S2** The CAC measurement of the three kinds of block copolymers (a) PEG-PUSese-PEG750, (b) PEG-PUSese-PEG1900 and (c) PEG-PUSese-PEG5000.



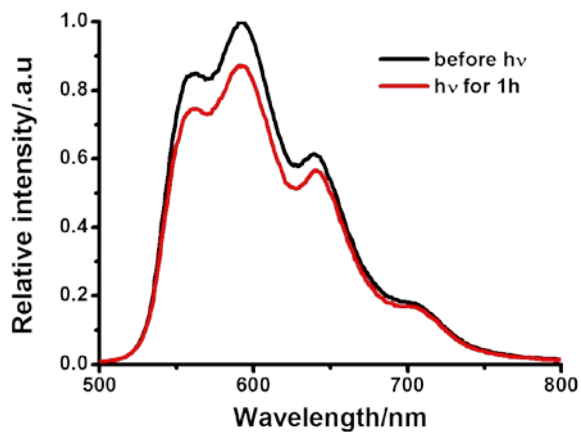
**Fig. S3** Statistical analysis on the size of the micelles formed by three kinds of block copolymers by counting around a hundred micelles from the TEM images, (a) for PEG-PUSese-PEG750, (b) for PEG-PUSese-PEG1900 and (c) for PEG-PUSese-PEG5000. The mean diameter was 118 nm, 73 nm and 50 nm, respectively.



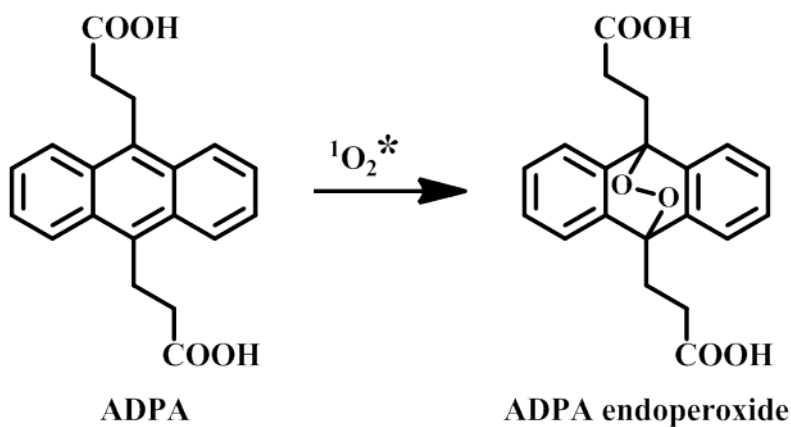
**Fig. S4** UV-Vis spectroscopy characterization of the endoperoxidation of ADPA by singlet oxygen which is produced by Por<sup>+</sup> under red light. Compared with Por, less decrease of the absorbance at 358 nm for ADPA was observed, which means Por<sup>+</sup> shows weaker ability of producing singlet oxygen.



**Fig. S5** The viability of L-02 cells after 24 h exposure to the mixed solution of PEG-PUSeSe-PEG1900 and Por that was irradiated with red light for 2 h and 5 h before usage. Control experiments were done without adding the mixed solution. The concentration of Por in the mixed solution was 0.01 mg/mL.



**Fig. S6** Fluorescence of Dox in the presence of Por before and after red light irradiation. The concentration of Dox was 0.01 mg/mL. The concentration of Por was 0.005 mg/mL. The fluorescence of Dox before and after red light irradiation in the presence of Por was almost the same. The result showed that Dox is quite stable to singlet oxygen.



**Scheme S1** The endoperoxidation of ADPA in the presence of singlet oxygen.

#### References:

- 1 N. Ma, Y. Li, H. Xu, Z. Wang, X. Zhang, *J. Am. Chem. Soc.*, 2010, **132**, 442.