Supporting Information for

Novel fluorescent amphiphilic copolymer probes containing azo-tetraphenylethylene bridges for azoreductase-triggered release

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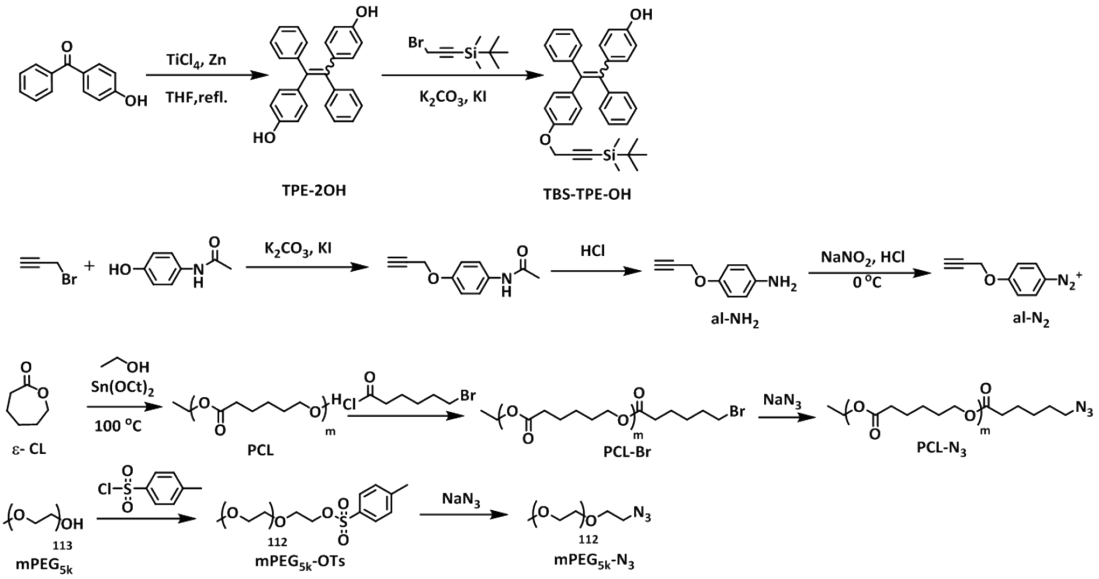
Experimental Section

Synthesis



Scheme S1. Synthetic routes of TBS-TPE-OH, al-N_2, polymers PCL_{3k}-N_3 and $mPEG_{5k}$-N_3

The synthesis of TPE-2OH and TBS-TPE-2OH was conducted according to literatures [1].

Synthesis of al-NH_2

Into a 100 mL round-bottom flask was added a mixture of N-(4-hydroxyphenyl) (15.1g, 100mmol), propargyl bromide (15.46 g, 130 mmol), potassium carbonate (27.8 g, 200 mmol) and a catalytic amount of KI in 200 mL acetone. The mixture was stirred at 80 °C for 18 h. After the reaction, most of the solvent was removed by a rotary vacuum evaporator. Then added 200 mL deionized water into the solution, a lot of white solid precipitated. The mixture was filtered and dried into the vacuum oven drying. After that the white solid was dissolved in 200 mL mixed solvent of acetone and hydrochloric acid and then heated to reflux for 12 h. When the reaction solution was restored to room temperature, sodium hydroxide solution was added to regulate solution to neutrality. Then mixed liquid was extracted by 400 mL ethyl acetate. The organic layer was dried with anhydrous Magnesium Sulfate overnight, filtered and the ethyl acetate evaporated to obtain brown black solution al-NH_2 (13.5 g, 92%). ^{1}H NMR (300

MHz, DMSO): δ_H (ppm) = 6.80-6.60 (m, Ar-H, 2H), 6.59-6.38 (m, Ar-H, 2H), 5.04-4.65 (m, -NH_2, 2H), 4.59(d, -OCH_2-, 2H), 3.46 (t, -C≡CH, 1H).

Synthesis of al-N_2^+

The preparation of diazonium salt of 4-(prop-2-ynyloxy)aniline (al-N_2^+) was as follows: A solution of 4-(prop-2-ynyloxy)aniline (1.10 g, 6.00 mmol), 2 mL deionized water and 1.25 mL chlorane (37%) were prepared into a 10 mL beaker. The mixture was heated to dissolve and then stirred in an ice salt bath to keep the reaction temperature at 0-5^{o}C. Aqueous solution containing $NaNO_2$ (0.46 g, 6.60 mmol) and 30 mL deionized water was added into the resulting mixture drop by drop. During the whole reaction, the temperature of the mixture was controlled at 0-5^{o}C. After the drop was finished, keep stirring for half an hour. Then a solution of al-N_2 was obtained. The crude product was used directly for the coupling reaction.

Synthesis of $mPEG_{5k}$- N_3

Into a 50 mL round-bottom flask was added a mixture of $mPEG_{5k}$ (0.5 mmol) and TEA (20 mmol) in 10 mL dry dichloromethane. Paratoluensulfonyl chloride (2 mmol) dissolved in 5 mL dry dichloromethane was then added into the flask dropwise slowly with stirred at 0-5 ^{o}C. After adding the paratoluensulfonyl chloride solution to the flask, the reaction mixture was heated up to 50 °C for an additional 12 h before cooling to room temperature. The mixture was washed with 1 M 1mL dilute hydrochloric acid solution three times to move excessive TEA and the organic phase was stirred with anhydrous Na_2CO_4 and anhydrous $MgSO_4$ to move water and excessive HCl. After that the solution was filtered and evaporated under reduced pressure to afford the concentrate. Then the product as a white powder was precipitated from an excess of cold anhydrous ether, collected using vacuum filtration, and dried in vacuo at 25 °C (2.25 g, yield: 90 %). Then into a 50 mL round-bottom flask was added a mixture of the above-mentioned product (0.2 mmol) and sodium azide (4 mmol) in 20 ml dried DMF. The mixture was stirred at 80 ^{o}C for 24 h. At the end of the reaction, the excess sodium azide was removed by filtration. The filtrate was decompressed and distilled to obtain a pale yellow crude product. The crude product was dissolved in 20 mL

methylene chloride and was washed 3 times with distilled water. The organic phase was dried with anhydrous $MgSO_4$ for 4 h and then was filtered, concentrated. And the concentrated solution was slowly dripped into the cold anhydrous ether. The product $mPEG_{5k}$- N_3 as a white powder was dried in vacuo at 25 °C (885 mg, yield: 89%). ^1H NMR (300 MHz, $CDCl_3$): δ_H (ppm) = 3.74-3.53 (m, -OCH_2CH_2O-, 452H), 3.38 (s, CH_3-, 3H).

Synthesis of PCL_{3k}-N_3.

ε-CL (219 mmol), ethanol (5.48 mmol) and $Sn(Oct)_2$ (0.0365 mmol) were added to a 50 mL Schlenk flask in a glove box. The reaction mixture was stirred at 100 °C for 3 h. Then, the mixture was dissolved in THF. The product was precipitated from an excess of anhydrous ether, collected using vacuum filtration, and dried in vacuo at 25 °C to afford the product PCL_{3k} as a white powder (2g, yield: 80%, $M_{n,SEC}$ = 4500 g mol^{-1}, M_w/M_n =1.10.). Then into a 25 mL round-bottom flask was added a mixture of PCL_{3k} (258 mg, 0.086 mmol) and TEA (240 μL, 0.176 mmol) in 10 mL dry THF. 6-Bromohexanoylchloride (2 mmol) was then added into the flask dropwise slowly with stirred at 0-5^{o}C. After stirred for an additional 12 h at 25 °C, the mixture was filtered and evaporated under reduced pressure to afford the concentrate. Then the product PCL_{3k}-Br as a white powder was precipitated from an excess of cold anhydrous ether, collected using vacuum filtration, and dried in vacuo at 25 °C (230mg, yield:88%).

Into a 25 mL round-bottom flask was added a mixture of 230 mg PCL_{3k}-Br and 123 mg sodium azide in 7 mL dried DMF. The mixture was stirred at 80 ^{o}C for 24 h. At the end of the reaction, the excess sodium azide was removed by filtration. The filtrate was decompressed and distilled to obtain crude product. The crude product was dissolved in 20 mL DCM and was washed 3 times with distilled water. The organic phase was dried with anhydrous $MgSO_4$ for 4 h and then was filtered, concentrated. And the concentrated solution was slowly dripped into the cold methanol. The product PCL- N_3 as a white powder was dried in vacuo at 25 °C (115 mg, yield: 50%). ^1H NMR (300 MHz, $CDCl_3$): δ_H (TMS, ppm) =4.08-4.04(t, 52H, -OCH_2-), 3.27(t, 2H, -CH_2N_3-) 2.33-2.28(t, 52H, -$CH_2\underline{CH_2}CH_2$-), 1.70-1.60(m, 104H, -CH_2-), 1.43-1.33(m, 53H,-CH_2C-), 1.28-1.23(m, 3H, -CH_3).

Synthesis of TBS-TPE-Azo-PEG_{5k}.

Into a 5 mL ampoule bottle was added a mixture of 82.2 mg TBS-TPE-Azo and 508 mg $mPEG_{5k}$-N_3 in 6 ml dried anhydrous toluene. Then 37.5 mg $CuSO_4 \cdot 5H_2O$ and 148 mg VcNa were added into the bottle. The mixture was degassed using three freeze/pump/thaw cycles and stirred for 24 h at 60 °C. After that the solution was stirred with ion exchange resin for 24h to remove the copper salts, then filtered and evaporated under reduced pressure to afford the concentrated. And the concentrated solution was also slowly dripped into the cold anhydrous ether. The orange solid was obtained after dried in high vacuum conditions (448 mg, yield: 76%, $M_{n,SEC}$ = 7800 g mol^{-1}, M_w/M_n =1.04.). ^1H NMR (300 MHz, $CDCl_3$): δ_H (TMS, ppm) =8.20(s, 1H, -CHN-), 7.80-7.75(dd, 2H, Ar-H), 7.57-7.54(dd, 1H, Ar-H), 7.12-6.96(m, 15H, Ar-H), 6.78-6.70(m, 3H, Ar-H), 5.42(s, 2H, -OCH_2C-), 4.67(t, 2H, -NCH_2-), 4.61(s, 2H, -O$\underline{CH_2}$C-), 3.93(t, 2H, -OCH_2-), 3.70-3.60(m, 452H, -OCH_2CH_2O-), 3.38(s, 3H, -OCH_3), 0.90-0.87(d, 9H, -$C(CH_3)_3$) ,0.09-0.07(d, 6H, -Si-CH_3).

Synthesis of TPE-Azo-PEG_{5k}.

100 mg TBS-TPE-Azo-PEG_{5k} was dissolved in 3 mL THF with adding 75 μL TBAF/ THF dropwise in 5 mL ampoule bottle. After stilling for 2 h at room temperature , the mixture was diluted with chloroform and then washed with water. After that, the mixture was concentrated by a rotary vacuum evaporator. And the concentrated solution was slowly dripped into the cold anhydrous ether. The orange powder was obtained after dried (96 mg, yield: 96%, $M_{n,SEC}$ = 7400 g mol^{-1}, M_w/M_n =1.02.). ^1H NMR (300 MHz, $CDCl_3$): δ_H (TMS, ppm) =8.20(s, 1H, -CHN-), 7.80-7.75(dd, 2H, Ar-H), 7.57-7.54(dd, 1H, Ar-H), 7.12-6.96(m, 15H, Ar-H), 6.78-6.70(m, 3H, Ar-H), 5.42(s, 2H, -OCH_2C-), 4.67(t, 2H, -NCH_2-), 4.61(s, 2H, -O$\underline{CH_2}$C-), 3.93(t, 2H, -OCH_2-), 3.70-3.60(m, 452H, -OCH_2CH_2O-), 3.38(s, 3H, -OCH_3), 2.55(t , 1H, -C≡CH).

Estimating the number of repeat units of polymer PCL_m -TPE-Azo-PEG_n

The number-average molecular weight $M_{n,NMR}$ of PCL_m -TPE-Azo-PEG_n and number of repeat units of PCL_m -TPE-Azo-PEG_n obtained from ^1H NMR was calculated by

using the following equation S1 and equation S2:

$$M_{n,NMR} = (I_{2.2\text{-}2.4}/2)/(I_{6.7\text{-}6.8}/3)\times M_{CL}+(I_{3.4\text{-}3.4}/4)/(I_{6.7\text{-}6.8}/3)\times M_{EG}+M_0 \quad \text{Equation S1}$$

$$m = (I_{2.2\text{-}2.4}/2)/(I_{6.7\text{-}6.8}/3) \quad \text{Equation S2}$$

$$n = (I_{3.4\text{-}3.8}/4)/(I_{6.7\text{-}6.8}/3) \quad \text{Equation S3}$$

$I_{2.2\text{-}2.4}$: the integrations at 2.2-2.4 ppm in ^{1}H NMR relative to the -CH- (2H) of CL units in PCL.

$I_{3.4\text{-}3.8}$: the integrations at 3.4-3.8 ppm in ^{1}H NMR relative to to the -OCH_2CH_2O- of EG units in PEG

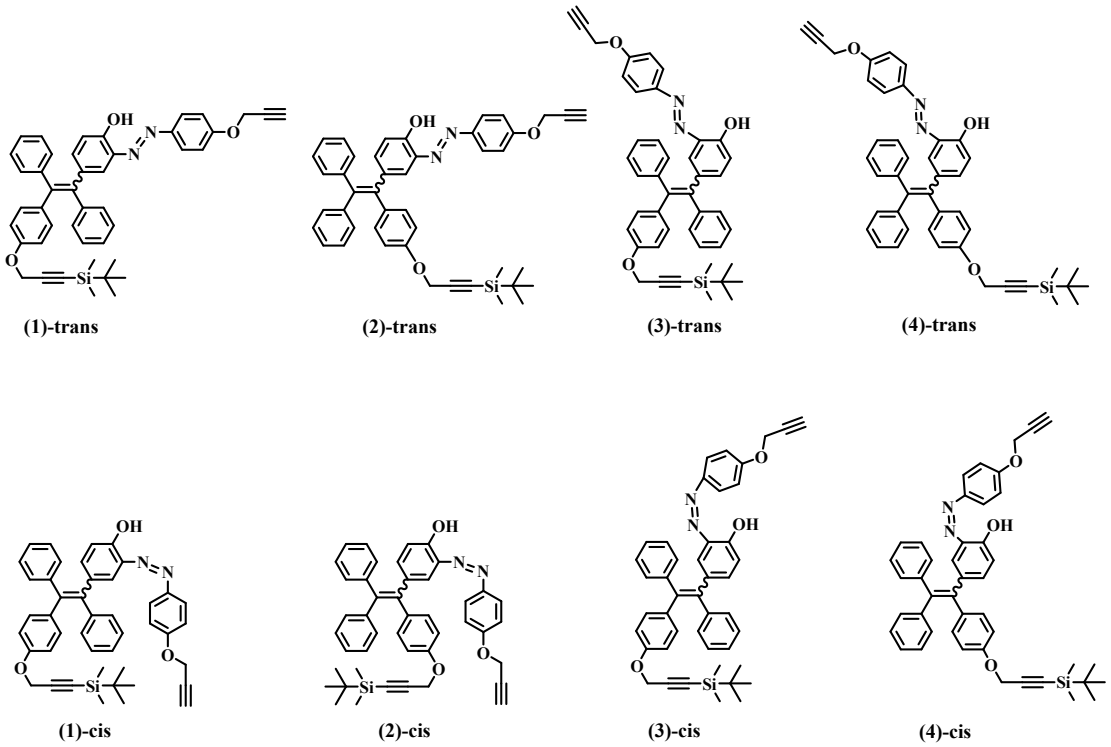
$I_{6.7\text{-}6.8}$: the integrations at 6.7-6.8 ppm in ^{1}H NMR relative to the moieties of TPE in PCL_m -TPE-Azo-PEG_n.

M_{CL}: the molecular weight of CL monomer.

M_{EG}: the molecular weight of EG monomer.

M_0 : the molecular weight of the end group.

Calculation



Scheme S2. *Cis*/*trans* isomers of TBS-TPE-Azo

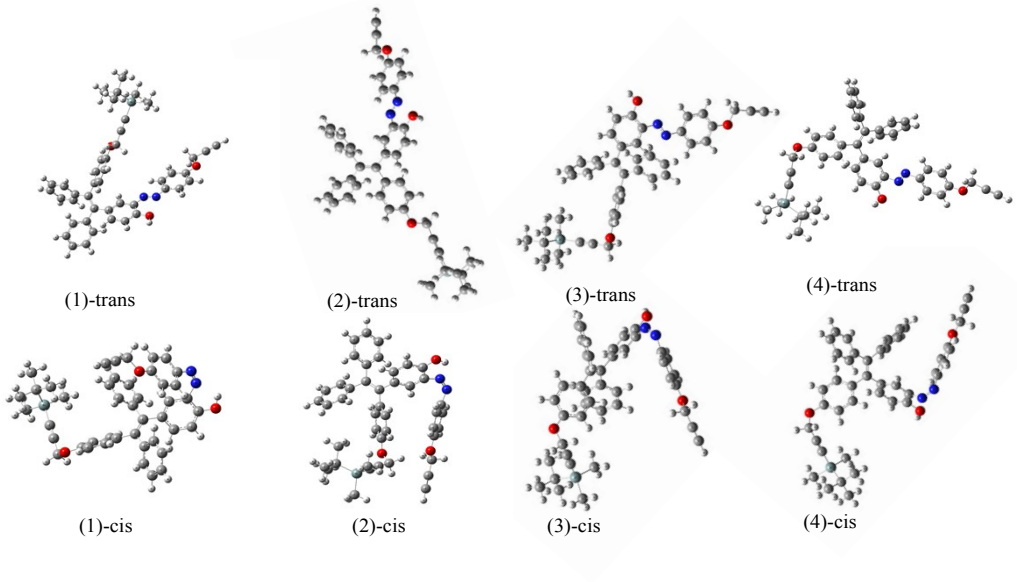


Fig. S1* Optimized structures of *cis* /*trans* isomers of TBS-TPE-Azo

Table S1* Energy (E.) of *cis*/*trans* isomers of TBS-TPE-Azo

E	*Trans*(a.u.)	*Cis* (a.u.)	Delta E(kcal/mol)
1	-2326.61553	-2326.60448	6.935738675
2	-2326.615244	-2326.60483	6.532130675
3	-2326.619739	-2326.59519	15.40174278
4	-2326.619883	-2326.5926	17.12128103

Table S2* Dipole moment (D.M) of *cis*/*trans* isomers of TBS-TPE-Azo

D.M.	*Trans*(Debye)	*Cis*(Debye)
1	1.2584	4.196
2	1.5818	3.9231
3	1.9415	4.261
4	1.5279	1.8932

*The molecular geometry optimized using the DFT method of the three parameter Becke-style hybrid functional (B3LYP) with the basis set of 6-311G(d). All calculations were performed using GAUSSIAN 2009 package.

Characterization

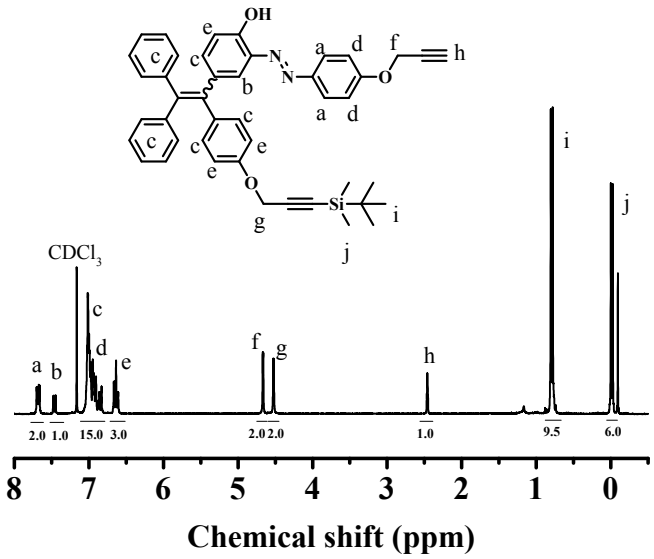


Fig. S2 ^{1}H NMR spectrum of TBS-TPE-Azo in $CDCl_3$.

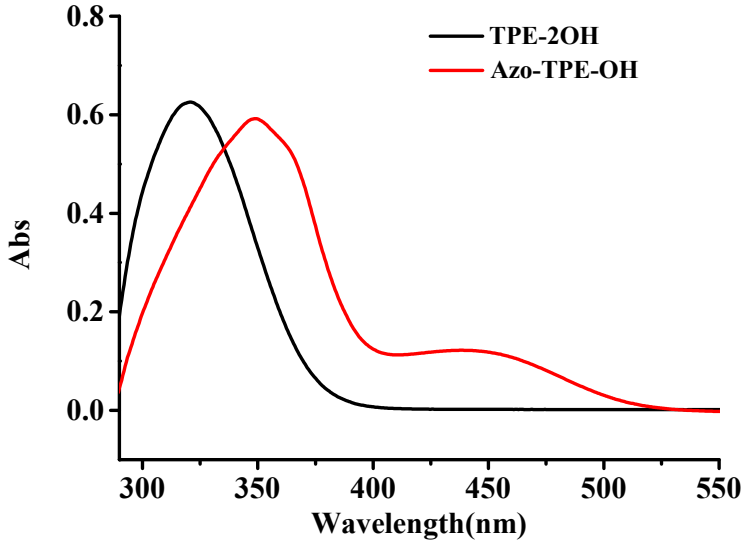


Fig. S3 UV-vis spectra of TBS-TPE-Azo and TPE-2OH in THF.

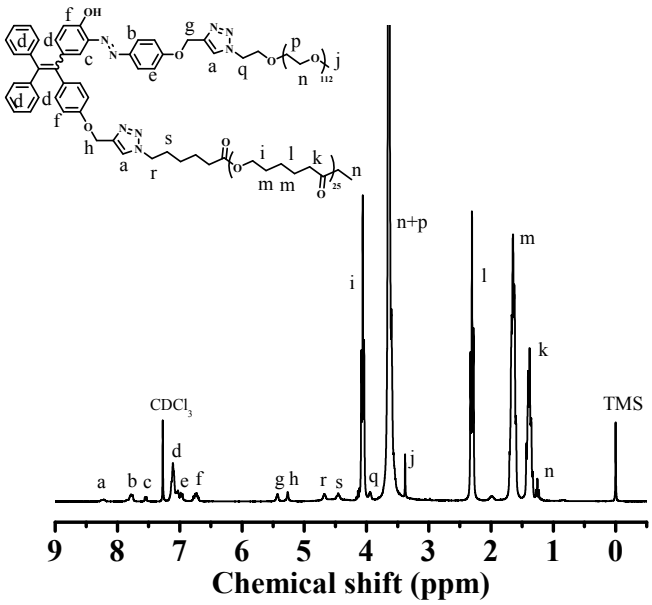


Fig. S4 ^{1}H NMR spectrum of PCL_{3k}-TPE-Azo-PEG_{5k} in $CDCl_3$.

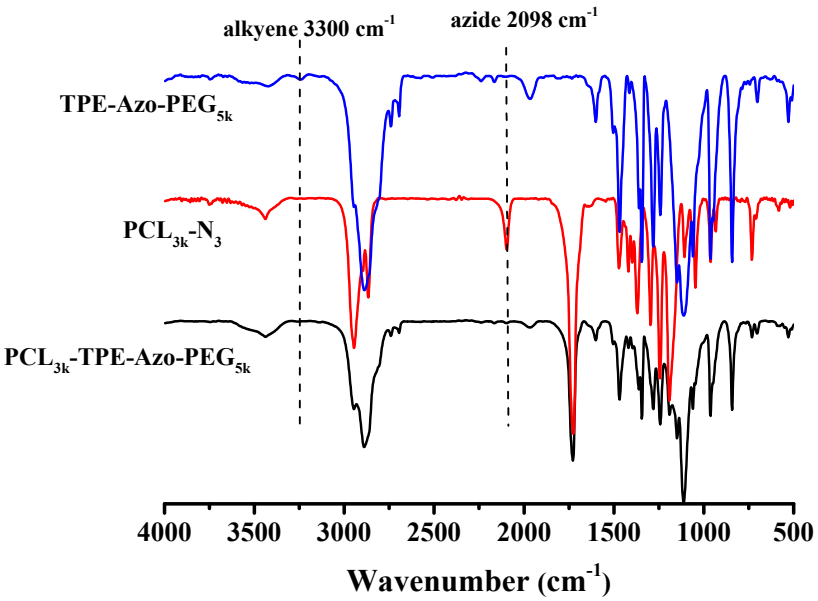


Fig. S5 FT-IR spectra of PCL_{3k}-TPE-Azo-PEG_{5k}, PCL_{3k}-N_3 and TPE-Azo-PEG_{5k}

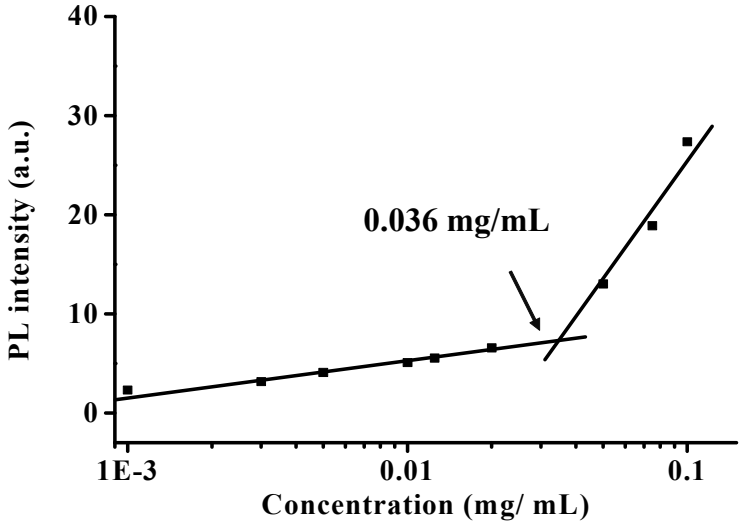


Fig. S6 Fluorescence intensity of Nile Red at 628 nm (λ_{ex}= 550 nm) versus PCL_{3k}-TPE-Azo-PEG_{5k} concentration (mg mL^{-1})

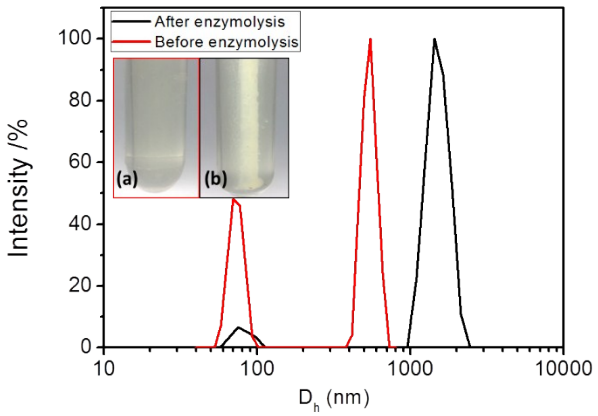


Fig. S7 DLS results for hydrodynamic diameter (D_h, DLS) distributions of PCL_{3k}-TPE-Azo-PEG_{5k} self-assembled aggregates in PB solutions before and after enzymolysis for 48h (scattering angle = 90°, the average diameter was 218nm for aggregates before enzymolysis and then turned into 1318 nm after enzymolysis). The insets show the self-assembled aggregates solutions in PB before and after enzymolysis for 48h.

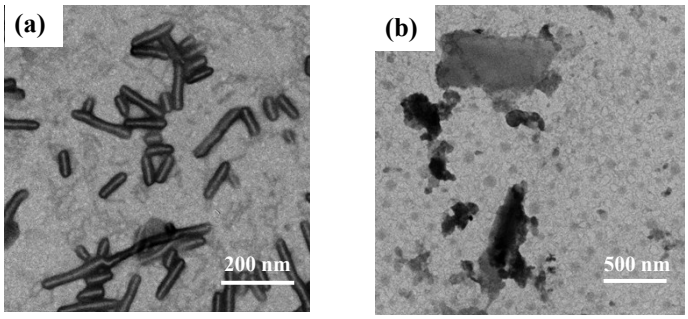


Fig. S8 TEM images of: (a) PCL_{3k}-TPE-Azo-PEG_{5k} micelles before treatment and (b) PCL_{3k}-TPE-Azo-PEG_{5k} micelles after addition of $Na_2S_2O_4$ (0.5 mg) at 37 °C for 24 h.

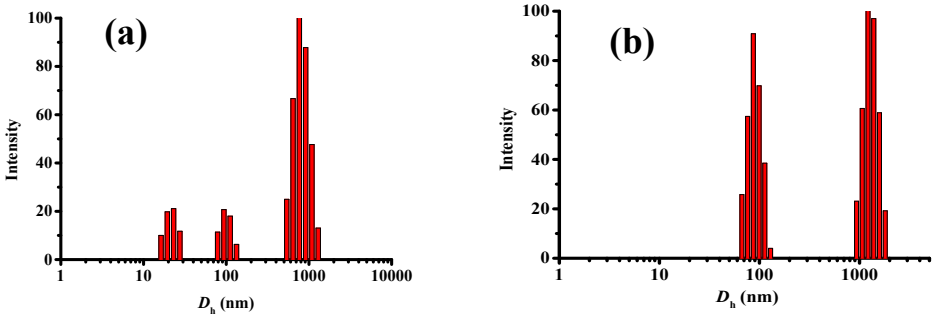


Fig. S9 (a) DLS results for the hydrodynamic diameter ($D_{h\ ,DLS}$) distributions in PB solutions (D_h = 225nm, PDI = 0.446) of untreated PEG_{5k}-TPE-Azo-PEG_{3k} micelles; (b) DLS results for the hydrodynamic diameter ($D_{h\ ,DLS}$) distributions in PB solutions (D_h = 1637 nm, PDI = 0.385) of PEG_{5k}-TPE-Azo-PEG_{3k} micelles after cultivation in 0.5 mg $Na_2S_2O_4$ (PB, pH = 7.4) at 37 °C for 24 h.

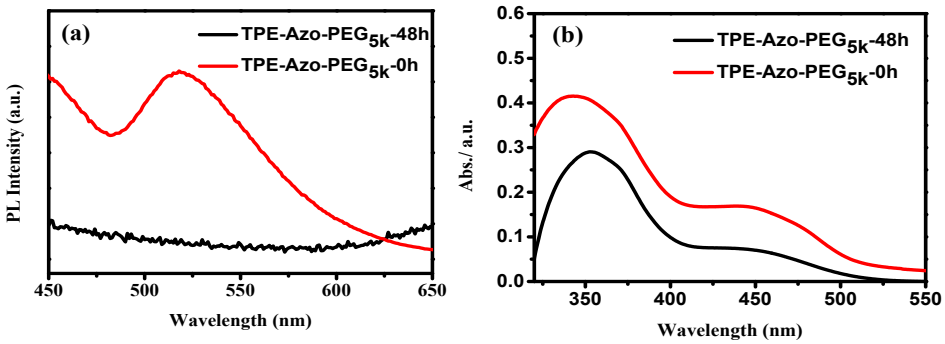


Fig. S10 (a) Fluorescence intensity variation (λ_{ex}=370nm) and (b) UV-vis spectra for the aqueous solution of TPE-Azo-PEG_{5k} in PBS buffer without azoreductase (black

line) and in the presence of azoreductase (red line) for 48h under argon atmosphere at 37 °C (0.075 mg. mL^{-1} TPE-Azo-PEG_{5k} PBS aqueous solution).

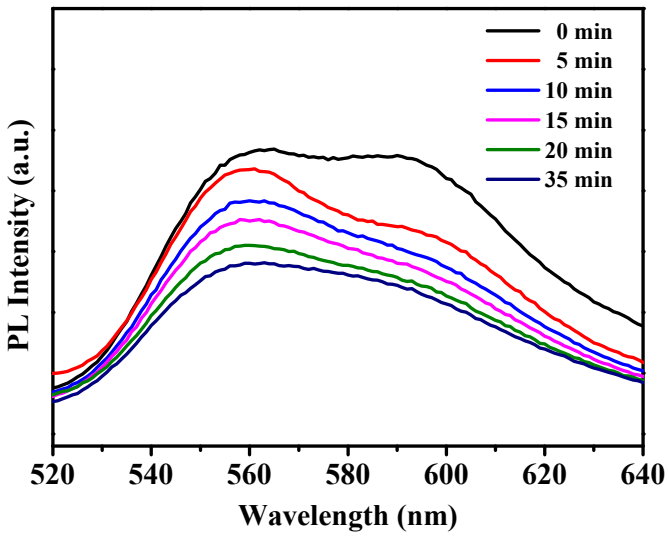


Fig. S11 Fluorescence change of DOX encapsulated in PCL_{3k}-TPE-Azo-PEG_{5k} micelles in PB (50 mM, pH 7.4) at 37 °C after $Na_2S_2O_4$ treatment for different time (micelle concentration: 0.3 mg/mL). λ_{ex} = 480 nm.

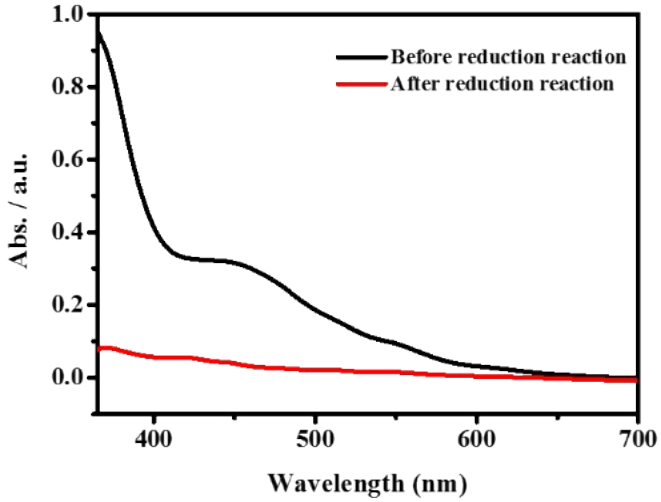


Fig. S12 UV-vis spectra of DOX-loaded PCL_{3k}-TPE-Azo-PEG_{5k} micelles before and after reduction reaction in the presence of $Na_2S_2O_4$ at 37°C.

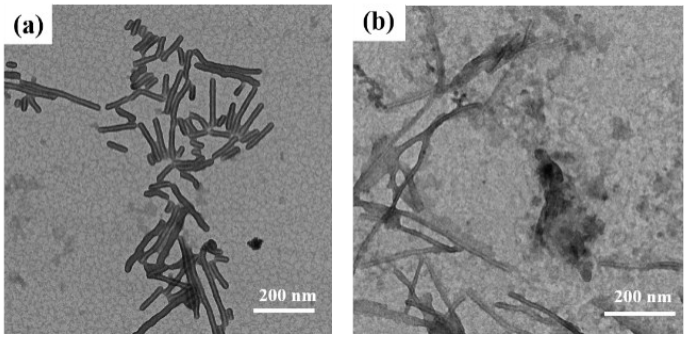


Fig. S13 TEM images of DOX-loaded PCL_{3k}-TPE-Azo-PEG_{5k} micelles before and after reduction reaction. (a) before reduction reaction; (b) after reduction reaction in the presence of $Na_2S_2O_4$ at 37 °C.

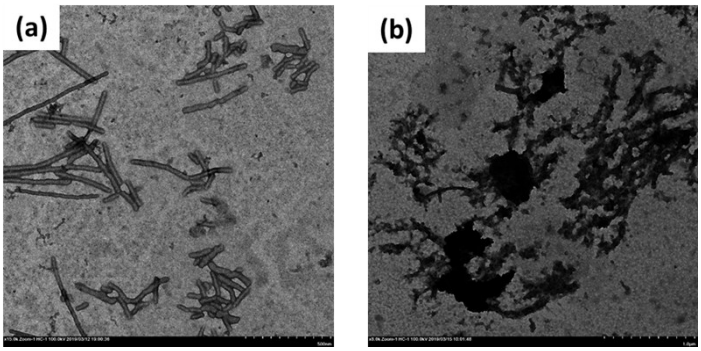


Fig. S14 TEM images of DOX-loaded micelles of PCL_{3k}-TPE-Azo-PEG_{5k} in PB solution (0.1 $mg \cdot mL^{-1}$) before and after reduction reaction. (a) before reduction reaction; (b) after reduction reaction for 48h in the presence of azoreductase at 37 °C.

1. Li, K.; Jiang, G.; Zhou, F.; Li, L.; Zhang, Z.; Hu, Z.; Zhou, N.; Zhu, X., Impact of cyclic topology: odd-even glass transition temperatures and fluorescence quantum yields in molecularly-defined macrocycles. *Polym. Chem.* **2017,** *8* (17), 2686-2692.