Supporting Information

Azoreductase-Triggered Fluorescent Nanoprobe Synthesized by RAFT-Mediated Polymerization-Induced Self-Assembly for Drug Release

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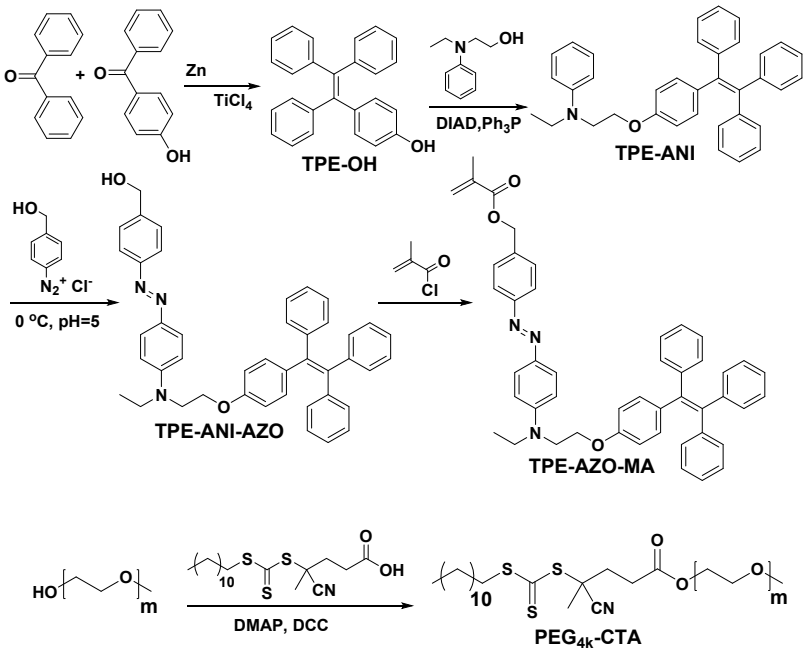
Experimental section

Synthesis



Scheme S1 Synthetic routes of functional monomer TPE-AZO-MA and macromolecular chain transfer agent (PEG_{4K}-CTA).

Synthesis of TPE-OH

Benzophenone (10.00 g, 54.88 mmol), 4-hydroxybenzophenone (13.06 g, 65.86 mmol), zinc powder (15.78 g, 241.46 mmol) and 130 mL THF were added into a 250 mL three-necked round-bottom flask under argon atmosphere and the mixture was stirred at 0 °C. Then $TiCl_4$ (22.9 g, 13.26 mL, 120.74 mmol) was added through a drop funnel. After addition, the mixture was refluxed at 70 °C for 13.3 h. The reaction mixture was treated with 100 mL hydrochloric acid solution (diluted to 100 mL with 50 mL concentrated hydrochloric acid) and extracted with ethyl acetate (200 mL × 4) to obtain the organic layer. The organic phase was washed with water, washed with NaCl solution, dried with anhydrous Na_2SO_4, filtered, and evaporated under vacuum. The crude product was purified by silica column chromatography (petroleum ether/ethyl acetate (V: V = 16:1–8:1). The solution was evaporated to dryness by rotary evaporation and dried in vacuum at 30 °C to obtain white solid (11.43 g, 59.8%). This compound was characterized by ^{1}H NMR. ^{1}H NMR (300 MHz, DMSO-d_6): δ_H (TMS, ppm) = 9.34 (s, 1H), 7.18–6.89 (m, 15H), 6.74 (d, 2H), 6.50 (d, 2H) (Fig. S1).

Synthesis of TPE-ANI

TPE-OH (10.00 g, 28.70 mmol), N-ethyl-N-hydroxyethyl aniline (9.48 g, 9.30 mL, 57.40 mmol), triphenylphosphine (22.58 g, 86.10 mmol) and 100 mL THF were added to a 250 mL three-necked round-bottom flask under argon atmosphere and the mixture was stirred at 0 °C. Then DIAD (17.41 g, 16.95 mL, 86.10 mmol) and 25 mL THF were added to the flask through a drop funnel. Then the mixture was stirred at 0 °C for 15 min and at 50 °C for another 35 h. The reaction mixture was extracted three times with ethyl acetate and washed twice with water to obtain organic phase. Then the organic phase was dried with anhydrous Na_2SO_4, filtered and evaporated under vacuum. The crude product was purified by silica column chromatography (petroleum ether/ethyl acetate (V: V = 40:1–20:1). The solution was concentrated by rotary evaporation and dried in vacuum at 30 °C to obtain light yellow oily liquid (10.85 g, 76.2%). This

compound was characterized by ^{1}H NMR. ^{1}H NMR (300 MHz, DMSO-d_6): δ_H (TMS, ppm) = 7.17–6.82 (m, 19H), 6.74–6.63 (m, 4H), 6.57 (t, 1H), 4.01 (t, 2H), 3.62 (t, 2H), 3.39 (d, 2H), 1.08 (t, 3H) (Fig. S2).

Synthesis of TPE-ANI-AZO

P-aminobenzyl alcohol (3.96 g, 32.14 mmol), 10 mL H_2O, 6.70 mL concentrated hydrochloric acid (80.35 mmol) were added to a 50 mL beaker, stirred to be clear and transparent, and cooled to 0 °C. $NaNO_2$ (2.22 g, 32.14 mmol) was dissolved in 20 mL H_2O, stirred in an ice bath for 45 min, and slowly dripped into the above solution to prepare diazonium salt solution. TPE-ANI (5.31 g, 10.71 mmol), 16 mL NaOAc/HOAc buffer solution (pH = 5), 150 mL THF and 150 mL EtOH were added to a 500 mL round-bottom flask. The mixture was cooled to 0 °C, stirred and added with diazonium salt slowly. The mixture was stirred for 18.5h at 0 °C, adjusted to pH from 6.5 to 7.5 by NaOAc solution, and extracted with dichloromethane (250mL × 3) to obtain organic phase. Then the organic phase was washed with NaCl solution (300 mL×2), dried with anhydrous Na_2SO_4, filtered and evaporated under vacuum. The crude product was purified by column chromatography on silica gel (petroleum ether/ethyl acetate (V: V = 8:1–4:1). The solution was concentrated by rotary evaporation and dried in vacuum at 30 °C to obtain orange red oily liquid (3.52 g, 52.1%). This compound was characterized by ^{1}H NMR. ^{1}H NMR (300 MHz, DMSO-d_6): δ_H (TMS, ppm) = 7.89–7.63 (m, 4H), 7.46 (d, 2H), 7.30–6.78 (m, 19H), 6.70 (d, 2H), 5.33 (dt, 1H), 4.72–4.42 (m, 2H), 4.21–3.95 (m, 2H), 3.81 (d, 2H), 3.52 (d, 2H), 1.15 (t, 3H) (Fig. S3).

Estimating the degree of polymerization (DP) of PEG_m-CTA

The DP of PEG_m-CTA and number-average molecular weight ($M_{n,NMR}$) of PEG_m-CTA determined by ^{1}H NMR studies according to Eq. S1 and S2:

$$m = (I_{3.47\text{-}3.85}/4)/(I_{4.20\text{-}4.31}/2)+1/2 \quad \text{(S1)}$$

$$M_{n,NMR} = m \times M_{EG} + M_0 \quad \text{(S2)}$$

$I_{3.47\text{-}3.85}$: the integrations at 3.47-3.85 ppm in ^{1}H NMR relative to the -OCH_2CH_2O- of EG units in PEG_m-CTA.

$I_{4.20\text{-}4.31}$: the integrations at 4.20-4.31 ppm in ^{1}H NMR relative to the -$COOC\underline{H}_2CH_2O$-

of in PEG_m-CTA.

M_{EG}: the molecular weight of ethylene glycol (EG) monomer.

M_0 : the molecular weight of the end group.

Estimating DPs of PEG_{101}-*b*-P(BMA_y-*co*-TPE-AZO-MA_x) copolymer

The DP of PEG_{101}-*b*-P(BMA_y-*co*-TPE-AZO-MA_x) and $M_{n,NMR}$ of PEG_{101}-*b*-P(BMA_y-*co*-TPE-AZO-MA_x) was determined by ^{1}H NMR studies according to Eqs. S3 – S5:

$$x = (I_{4.65\text{-}5.30}/2)/(I_{3.36\text{-}3.39}/3) \quad \text{(S3)}$$

$$y = (I_{1.68\text{-}2.30}/4)/(I_{3.36\text{-}3.39}/3)\text{-}(I_{4.65\text{-}5.30}/4)/(I_{3.36\text{-}3.39}/3)\text{-}0.5 \quad \text{(S4)}$$

$$M_{n,NMR} = 101\times M_{EG}+x\times M_{TPE\text{-}AZO\text{-}MA}+y\times M_{BMA}+M_0 \quad \text{(S5)}$$

$I_{4.65\text{-}5.30}$: the integrations at 4.65-5.30 ppm in ^{1}H NMR relative to the Ar-C$\underline{H}_2$-O- of TPE-AZO-MA units in PEG_{101}-*b*-P(BMA_y-*co*-TPE-AZO-MA_x).

$I_{3.36\text{-}3.39}$: the integrations at 3.36-3.39 ppm in ^{1}H NMR relative to the C$\underline{H}_3$O- of end group in PEG_{101}-*b*-P(BMA_y-*co*-TPE-AZO-MA_x).

$I_{1.68\text{-}2.30}$: the integrations at 1.68-2.30 ppm in ^{1}H NMR relative to the -COO-C$\underline{H}_2$- of BMA units , the -C$\underline{H}_2$$CH_2$COO- close to PEG, the -CS-S-CH_2C$\underline{H}_2$-$(CH_2)_9$-CH_3 and the hydrophobic main chain except the repeating unit connected with the –SCSS- in PEG_{101}-*b*-P(BMA_y-*co*-TPE-AZO-MA_x).

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

$M_{TPE\text{-}AZO\text{-}MA}$: the molecular weight of TPE-AZO-MA monomer.

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

M_0: the molecular weight of the end group.

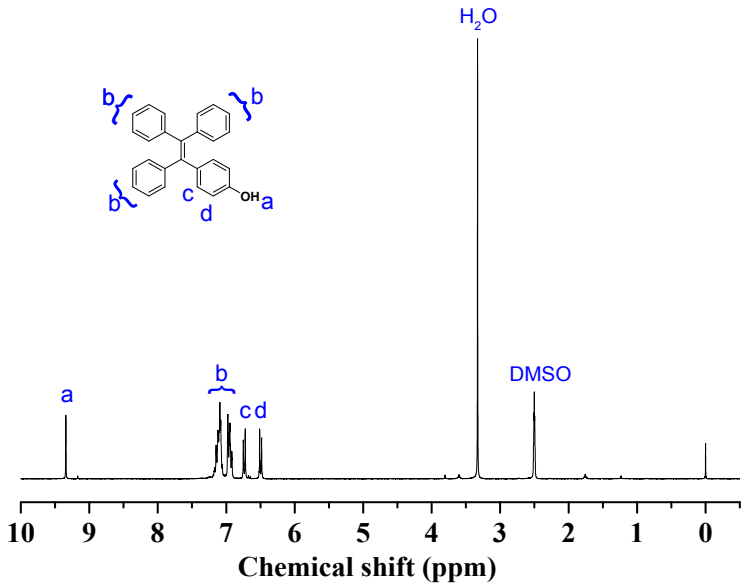


Fig. S1 ^{1}H NMR spectrum of TPE-OH in DMSO-d_6.

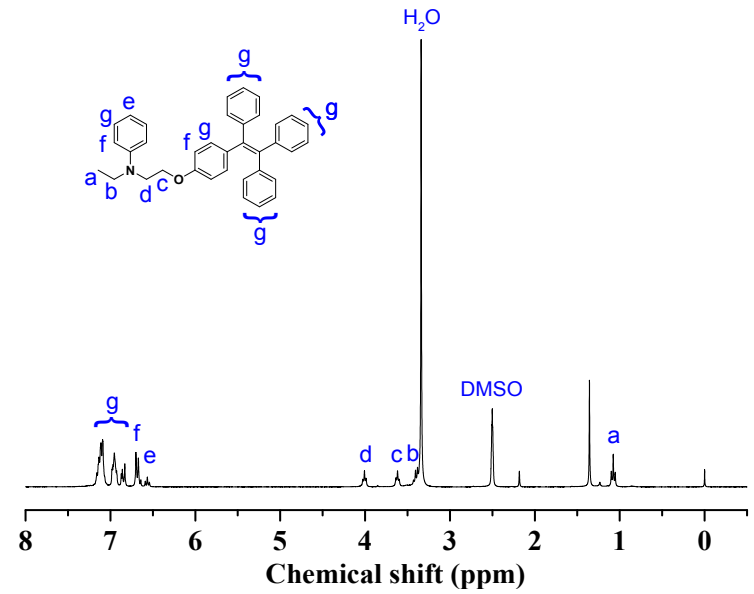


Fig. S2 ^{1}H NMR spectrum of TPE-ANI in DMSO-d_6.

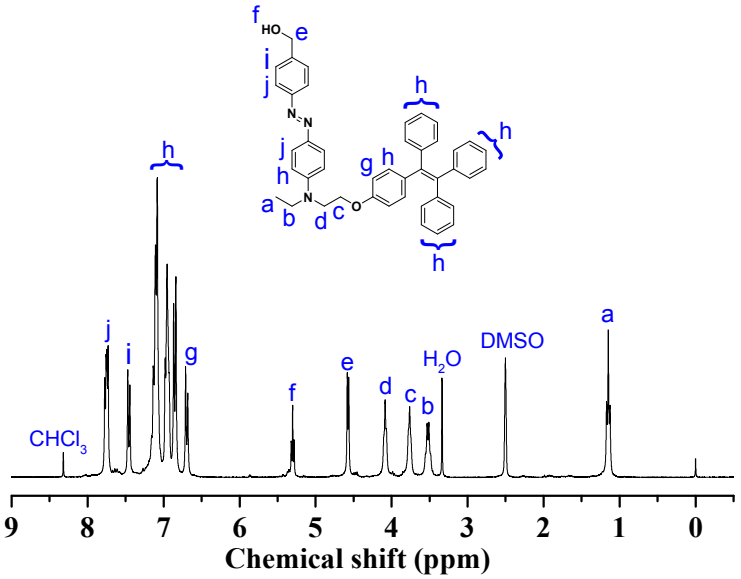


Fig. S3 ^{1}H NMR spectrum of TPE-ANI-AZO in DMSO-d_6.

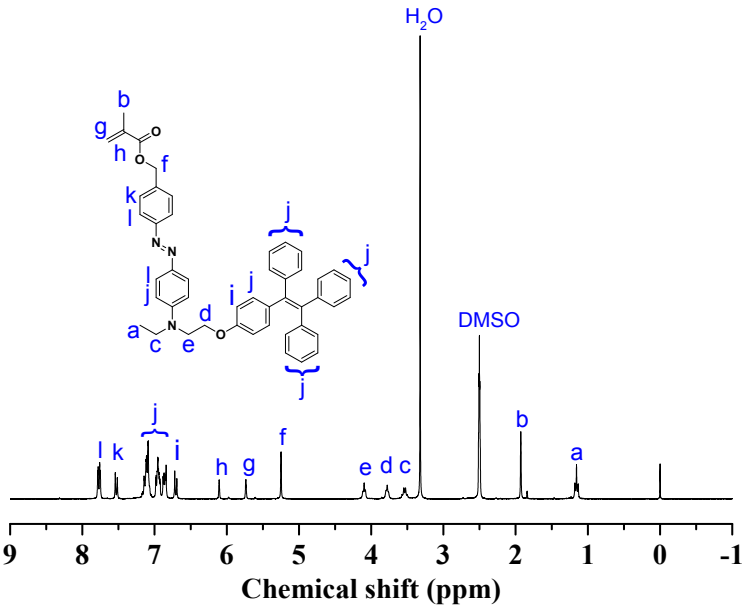


Fig. S4 ^{1}H NMR spectrum of TPE-AZO-MA in DMSO-d_6.

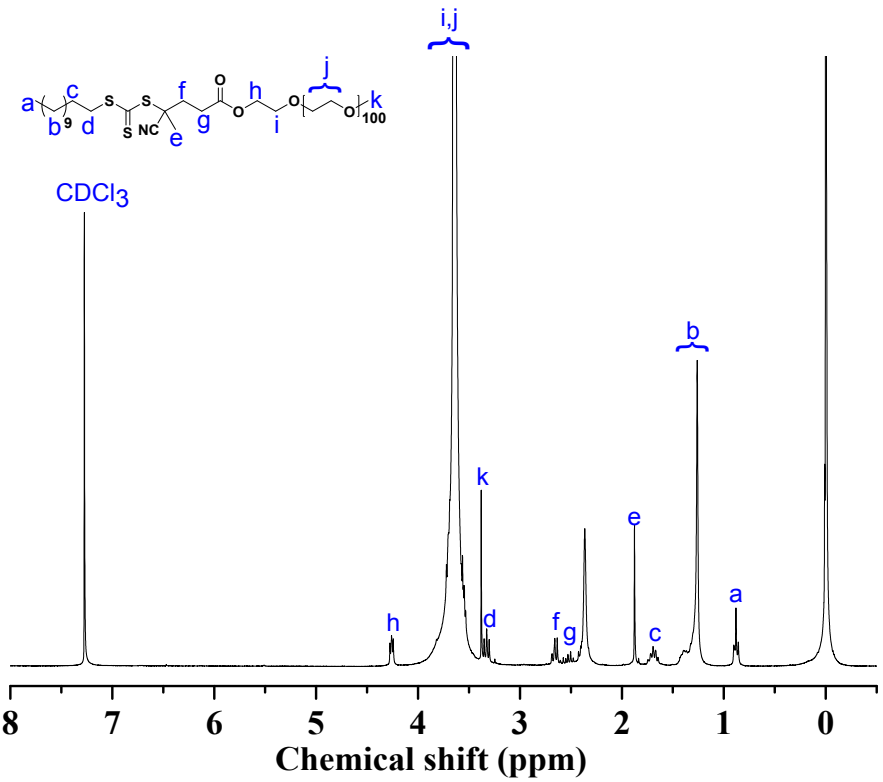


Fig. S5 ^{1}H NMR spectrum of PEG_{4K}-CTA in $CDCl_3$.

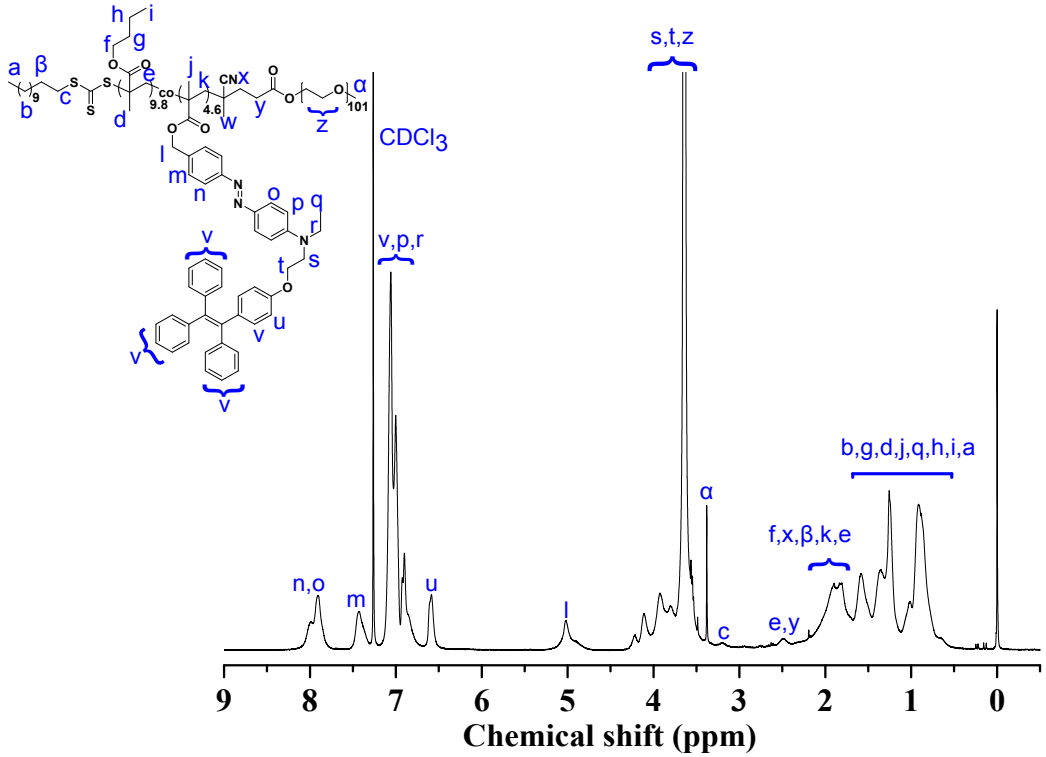


Fig. S6 ^{1}H NMR spectrum of PEG_{101}-*b*-P($BMA_{9.8}$-*co*-TPE-AZO-$MA_{4.6}$) in $CDCl_3$.

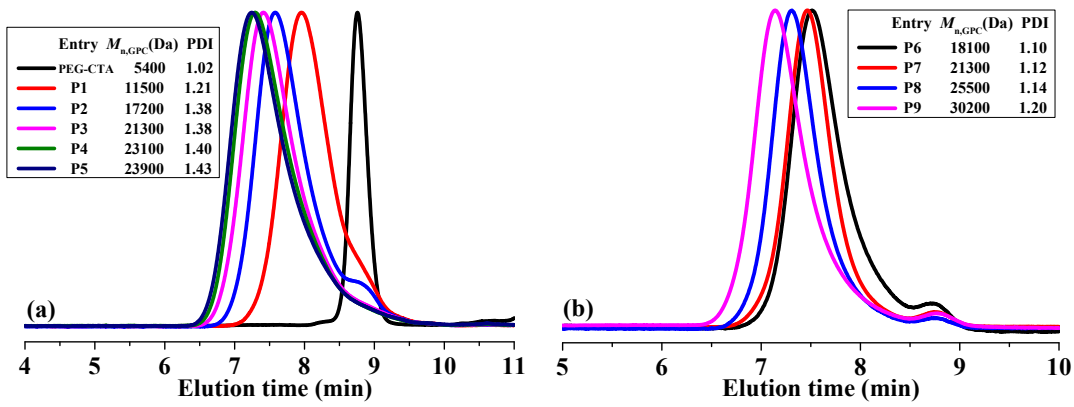


Fig. S7 GPC traces of PEG_{101}-*b*-P(BMA_x-*co*-TPE-AZO-MA_y) copolymers (P1– P9) synthesized by RAFT dispersion copolymerization of BMA/TPE-AZO-MA monomers at PEG_{4k}-CTA/ACVA = 5/1, and BMA/TPE-AZO-MA = 2/1 (P1–P5) and 10/1 (P6–P9) in dioxane/water (9/1, w/w) at 70 °C.

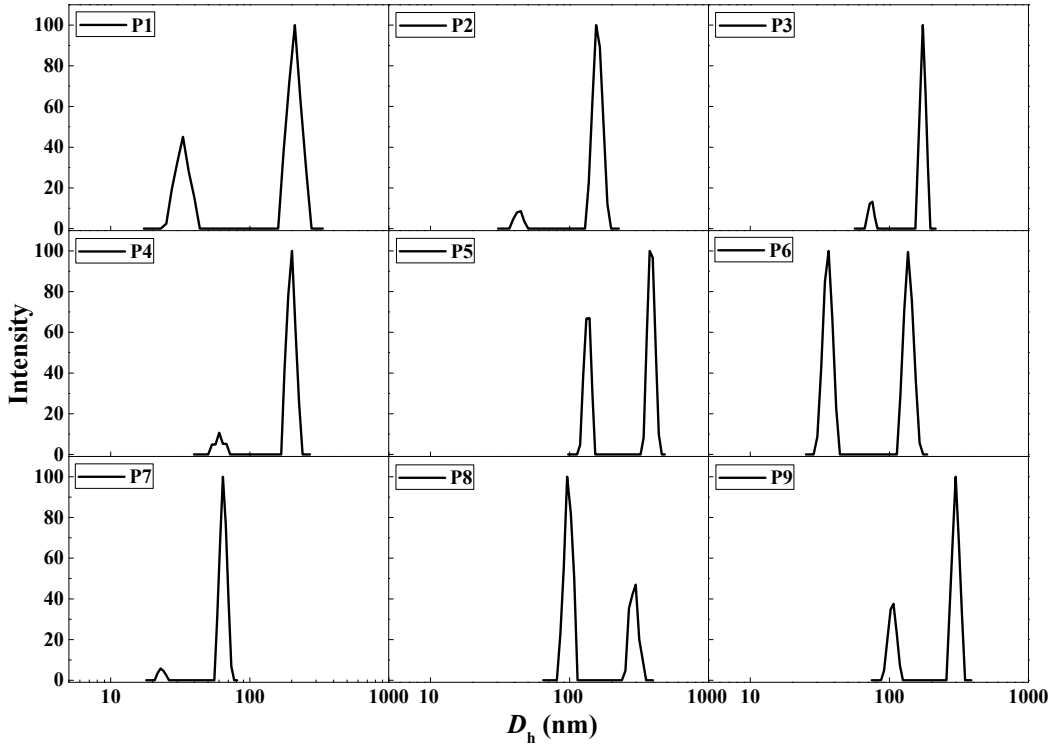


Fig. S8 Hydrodynamic diameter ($D_{h,\ DLS}$) profiles of P1–P9 particles (0.5 mg/mL in PBS solution), as determined by DLS analysis.

P1 ($D_{h,\ DLS}$, 95nm; PDI, 0.345); P2($D_{h,\ DLS}$, 143nm; PDI, 0.161); P3($D_{h,\ DLS}$, 153nm; PDI, 0.161); P4($D_{h,\ DLS}$, 168nm; PDI, 0.127); P5($D_{h,\ DLS}$, 230nm; PDI, 0.193); P6($D_{h,\ DLS}$, 59nm; PDI, 0.249); P7($D_{h,\ DLS}$, 60nm; PDI, 0.057); P8($D_{h,\ DLS}$, 126nm; PDI, 0.169); P9($D_{h,\ DLS}$, 199nm; PDI, 0.194).

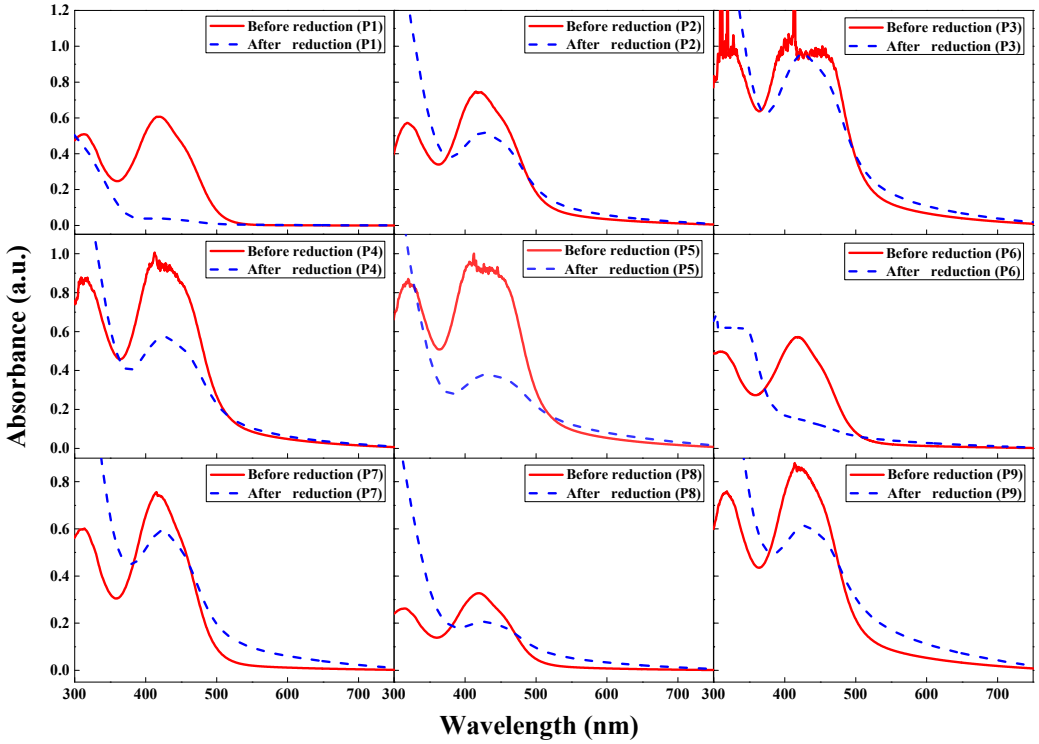


Fig. S9 UV-Vis spectra of P1–P9 particles (0.10 mg/mL in PBS solution) before and after $Na_2S_2O_4$ reduction reaction at 37 °C for 24 h.

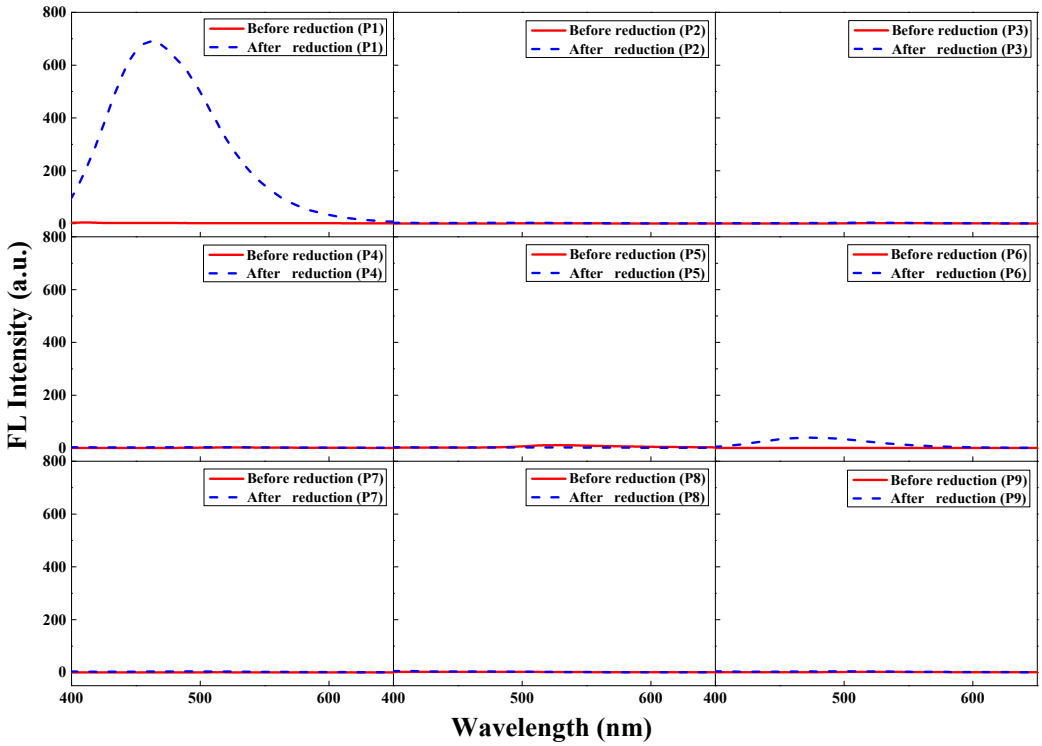


Fig. S10 Fluorescence intensity variation (λ_{ex} = 360 nm) of P1–P9 particles (0.1 mg/mL in PBS solution) before and after $Na_2S_2O_4$ reduction at 37 °C for 24 h.

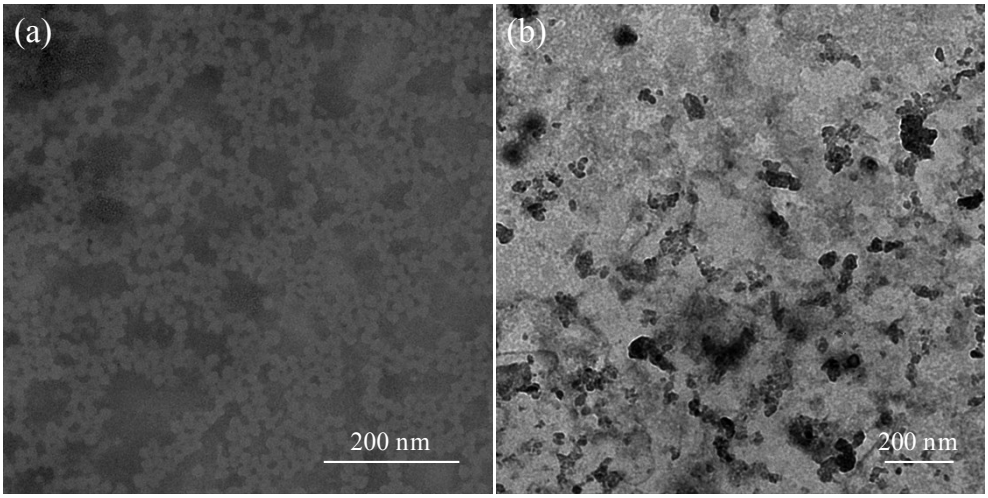


Fig. S11 TEM images of P1 micelles before (a) and after (b) $Na_2S_2O_4$ reduction reaction for 24 h at 37 °C. Herein sample was prepared by drying the dispersion of P1 micelles (0.10 mg/mL in PBS solution).

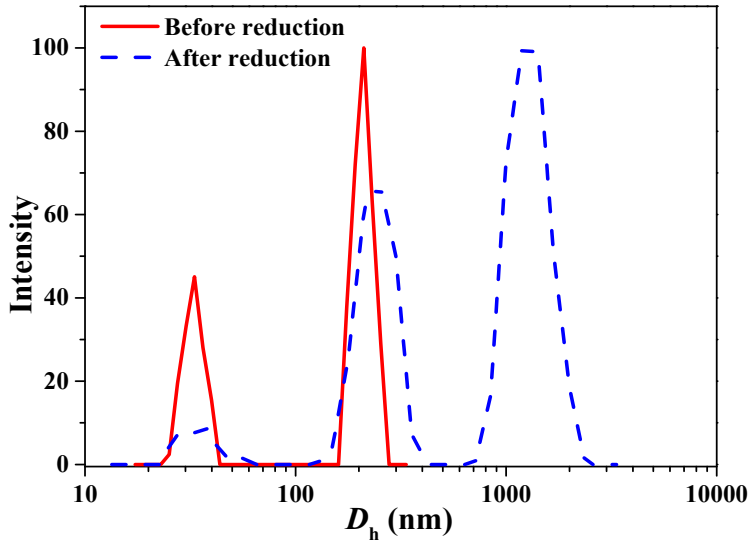


Fig. S12 Change of hydrodynamic diameter (D_h) profile of P1 particles (0.10 mg/mL in PBS buffer) before and after $Na_2S_2O_4$ reduction at 37 °C for 24 h, as determined by DLS analysis.

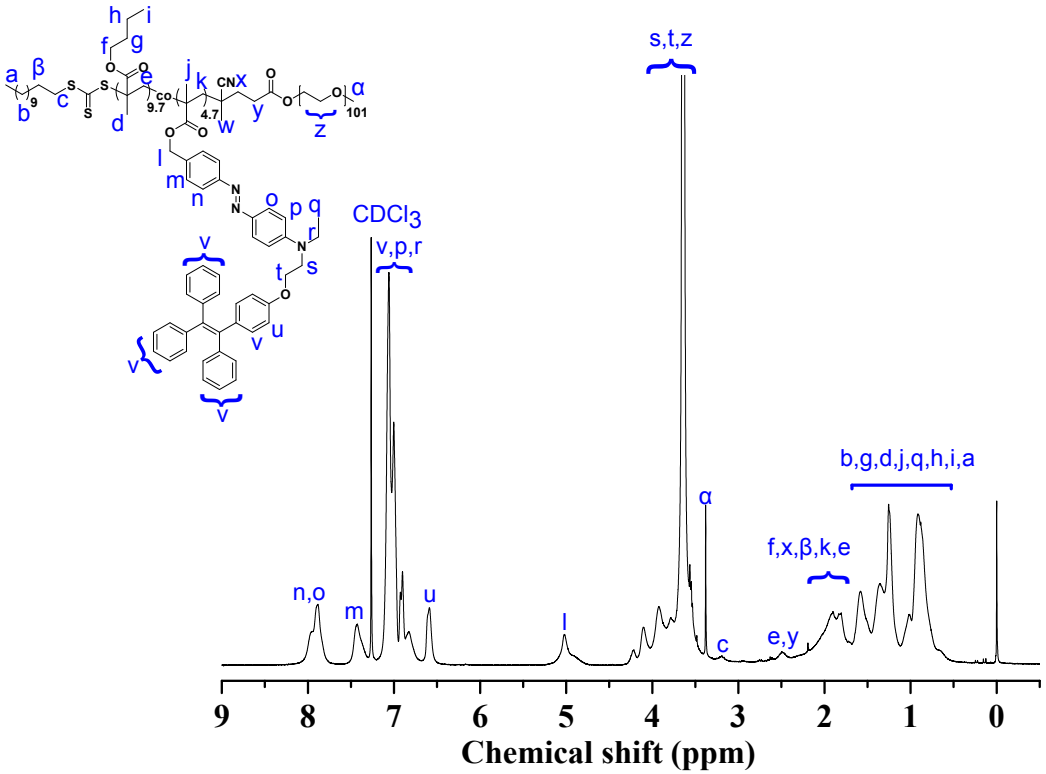


Fig. S13 ^{1}H NMR spectrum of PEG_{101}-*b*-P($BMA_{9.7}$-*co*-TPE-AZO-$MA_{4.7}$) copolymer synthesized by DOX-loading PISA, as recorded in $CDCl_3$。

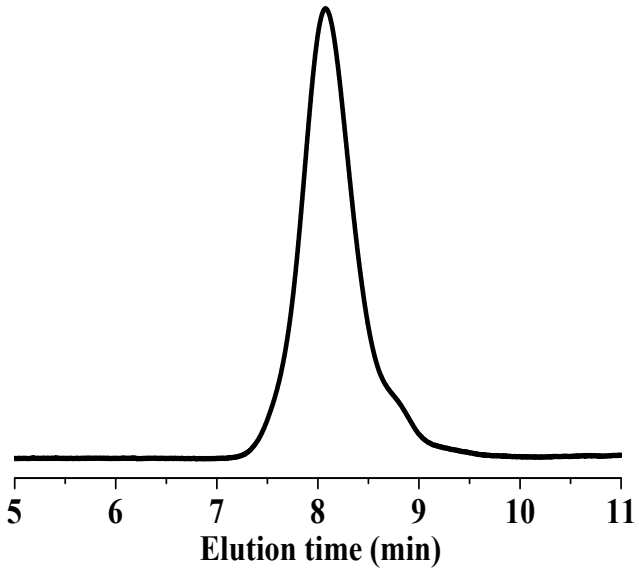


Fig. S14 GPC trace of PEG_{101}-*b*-P($BMA_{9.7}$-*co*-TPE-AZO-$MA_{4.7}$) synthesized by DOX-loading PISA ($M_{n,GPC}$ = 12.6 kg/mol, M_w/M_n = 1.18).

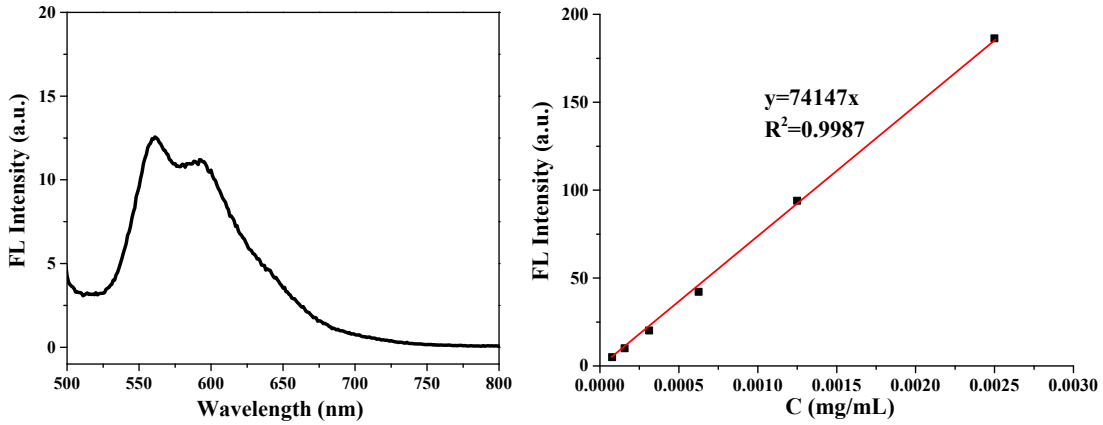


Fig. S15 Fluorescence emission spectrum (λ_{ex} = 480 nm) of the micelles-encapsulated DOX in DMSO (left), and the calibration curve of DOX in DMSO (R^2 stands for the coefficient of determination) (right).

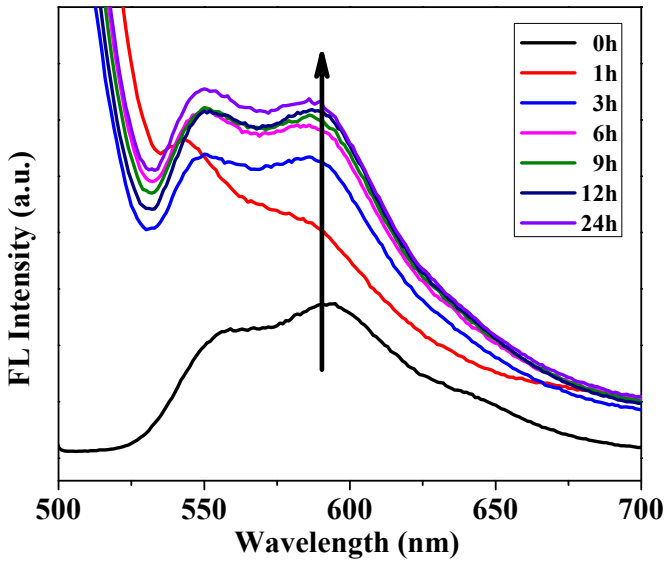


Fig. S16 Evolution of fluorescence emission spectrum (λ_{ex} = 480 nm) of DOX-loaded micelles (0.12 mg/mL PEG_{101}-*b*-P($BMA_{9.7}$-*co*-TPE-AZO-$MA_{4.7}$ in PBS solution) upon azoreductase reduction at 37 °C for different time periods.

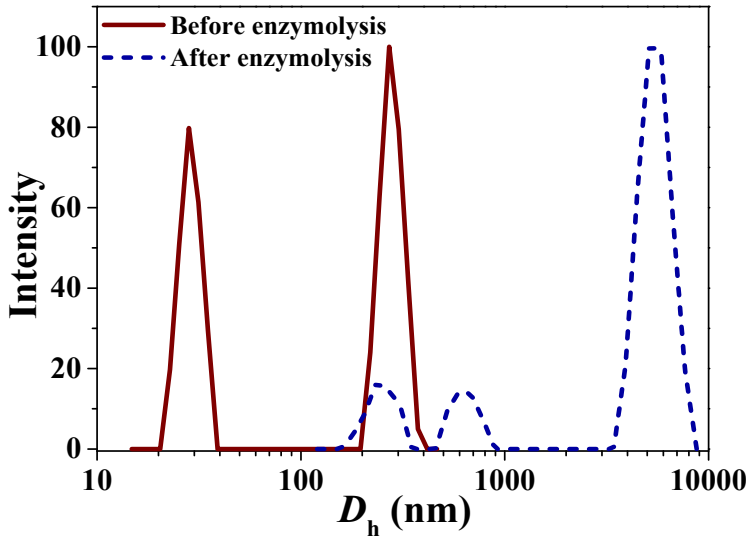


Fig. S17 Changes of DLS hydrodynamic diameter (D_h) profiles of DOX-loaded micelles (0.12 mg/mL in PBS solution) upon enzymolysis reduction for 24 h.