Supporting Information

for

Real-time Near-infrared Fluorescent Reporting the Azoreductase-triggered Drug Release

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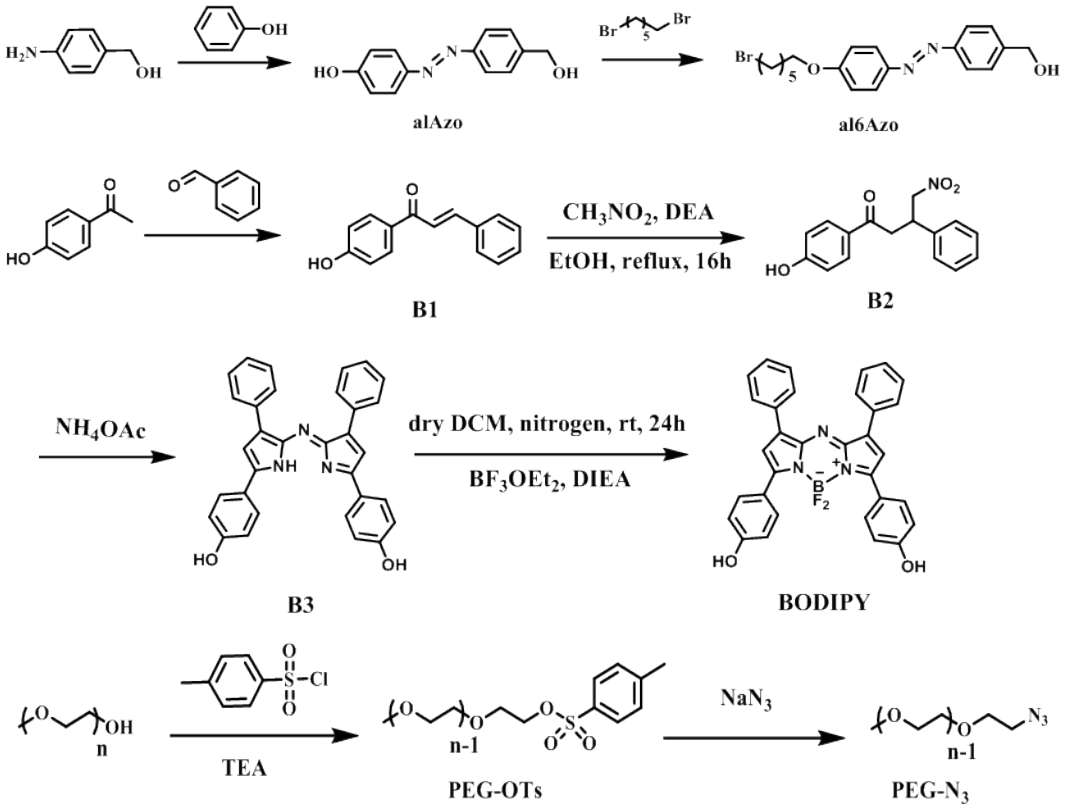
Experimental Section

Synthesis of intermediates



Scheme S1. Synthesis routes of al6Azo, Aza-BODIPY and PEG-N_3.

Synthesis of Aza-BODIPY

The near-infrared fluorescence group Aza-BODIPY was synthesized according to the methods described in the literature. [S1]

Synthesis of al6Azo

P-aminobenzene methanol (2.0 g, 16.2 mmol), 30 mL cool water and 34 mL 37% concentrated hydrochloric acid were mixed in a 250 mL beaker. Sodium nitrite (1.17 g, 17.0 mmol) was dissolved in 8 mL cool water, then added into the p-aminobenzene methanol solution dropwise, and the mixture was stirred for 1 h at 0 ^{o}C. Phenol (1.6 g,17.0 mmol) and potassium carbonate (3.14 g, 22.7 mmol) were dissolved in 25 mL cool water and then added to the above solution dropwise. The mixture was stirred in an ice salt bath to keep the reaction temperature at 0-5 ^{o}C for 2 h. The orange-red precipitate was formed when pH was adjusted to 4-5 with dilute acetic acid solution. The orange soild product was obtained after drying in vacuo at 25 ^{o}C (3.4 g, yield: 92%).

The above-mentioned product (912.4 mg, 4 mmol), 6-bromohexane (1.94 g, 8 mmol), potassium carbonate (552 mg, 4 mmol) were added into a 50 mL round-bottom flask and dissolved in 8 mL dried acetone, stirring at 65 ^{o}C for 16 h with the reflux condensed. After cooling to room temperature, ethyl acetate was extracted and washed three times, the organic layer was dried with anhydrous sodium sulfate. After all, column chromatography (petroleum ether/ethyl acetate, V: V = 1:20-1:25) was used to purify product. The orange-yellow product al6Azo was obtained in a yield of 77%. ^{1}H NMR (DMSO-d_6, 300 MHz): δH (ppm) = 7.88-7.80,7.52-7.49,7.13-7.10 (m, Ar-H, 8H), 5.35 (s, OH, 1H), 4.58-4.61 (d, OCH_2, 2H), 4.05-4.10 (t, OCH_2, 2H), 3.52-3.57 (t, $BrCH_2$, 2H), 1.90-1.69, 1.62-1.39 (m, CH_2, 8H).

Synthesis of PEG- N_3

Into a 50 mL round-bottom flask was added a mixture of $mPEG_{20000}$ (2.5 g, 0.125 mmol) and TEA (1.01 g, 10 mmol) in 10 mL dry dichloromethane. Paratoluensulfonyl chloride (0.19 g, 1 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 55 ^{o}C for an additional 24 h before cooling to room temperature. The mixture was washed with 1 M 1 mL dilute hydrochloric acid solution three times to move excessive TEA and the organic phase was stirred with anhydrous Na_2CO_3 and anhydrous Na_2SO_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 ^{o}C (2.4 g, yield: 95 %). Then into a 50 mL round-bottom flask was added a mixture of the above-mentioned product (1.015 g, 0.05 mmol) and sodium azide (65 mg, 4 mmol) in 15 mL dried DMF. The mixture was stirred at 85 ^{o}C for 24 h. At the end of the reaction, the excess sodium azide was removed by filtration. The filtrate was dissolved in 20 mL methylene chloride and was washed with saturated ammonium chloride solution to remove DMF and residual NaN_3. The organic phase was dried with anhydrous Na_2SO_4 and then filtered, concentrated. After that, the

concentrated solution was slowly dripped into the cold anhydrous ether. The product PEG_{20000}- N_3 as a white powder was dried in vacuo at 40 °C (936.3mg, yield: 93%). ^1H NMR (300 MHz, $CDCl_3$): δH (ppm) = 3.74-3.56 (m, -OCH_2CH_2O-, 1596H), 3.38 (s, -CH_3, 3H).

Estimating the number of repeat units of polymer PEG_n-AzaBODIPY-Azo-PLA_m

The number-average molecular weight M_n, NMR obtained from ^1H NMR and number of repeat units (m and n) of PEG_n-AzaBODIPY-AZO-PLA_m were calculated by using the following equation S1, S2 and S3 based on ^1H NMR (Fig. S4):

$M_{n,NMR} = (I_{5.06\text{-}5.35}/2)/(I_{7.38\text{-}7.51}/8)\times M_{LA}+(I_{3.47\text{-}3.85}/4)/(I_{7.38\text{-}7.51}/8)\times M_{EG}+M_0$ Equation S1

$m = (I_{5.06\text{-}5.35}/2)/(I_{7.38\text{-}7.51}/8)$ Equation S2

$n = (I_{3.47\text{-}3.85}/4)/(I_{7.38\text{-}7.51}/8)$ Equation S3

$I_{5.06\text{-}5.35}$: the integrations at 5.06-5.35 ppm in ^1H NMR relative to the -CH- of LA units in PLA.

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

$I_{7.38\text{-}7.51}$: the integrations at 7.38-7.51 ppm in ^1H NMR relative to the moieties of AzaBODIPY in PEG_n-AzaBODIPY-AZO-PLA_m.

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

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

M_0 : the molecular weight of the Alkynyl-AzaBODIPY-AZO-Hydroxyl.

Characterization

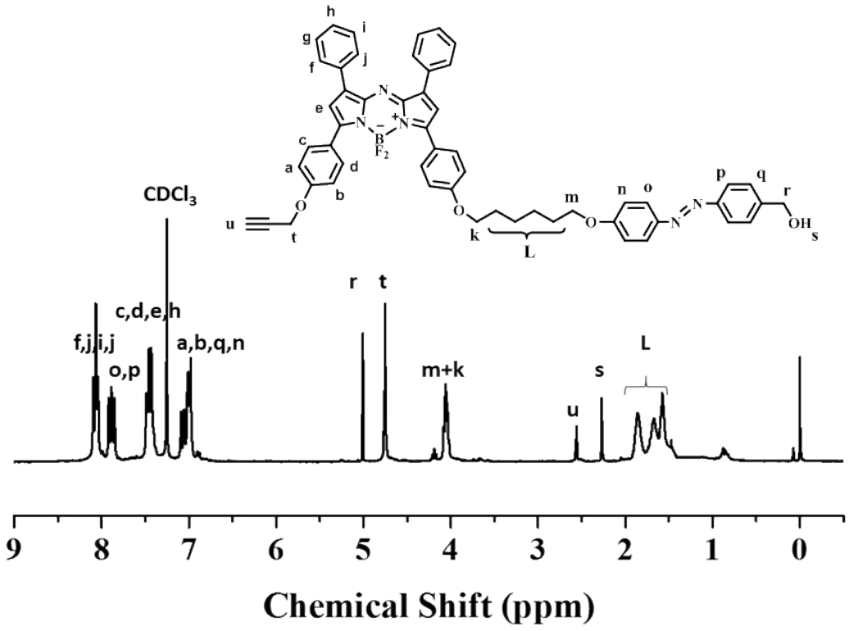


Figure S1. ^{1}H NMR spectrum of Alkynyl-AzaBODIPY-AZO-Hydroxyl in $CDCl_3$.

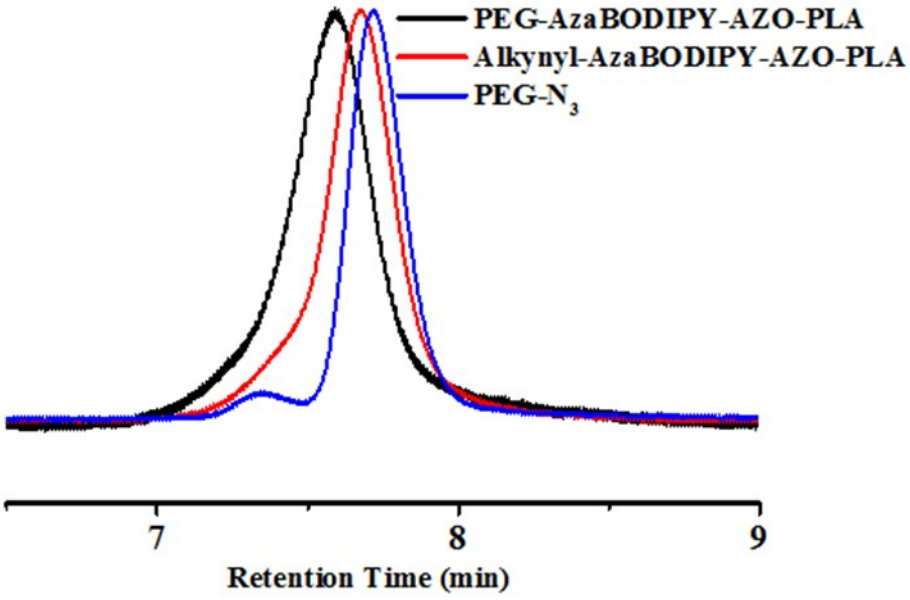


Figure S2. GPC traces of PEG_{398}-AzaBODIPY-AZO-PLA_{144}, PEG_{20000}-N_3 and Alkynyl-AzaBODIPY-AZO-PLA.

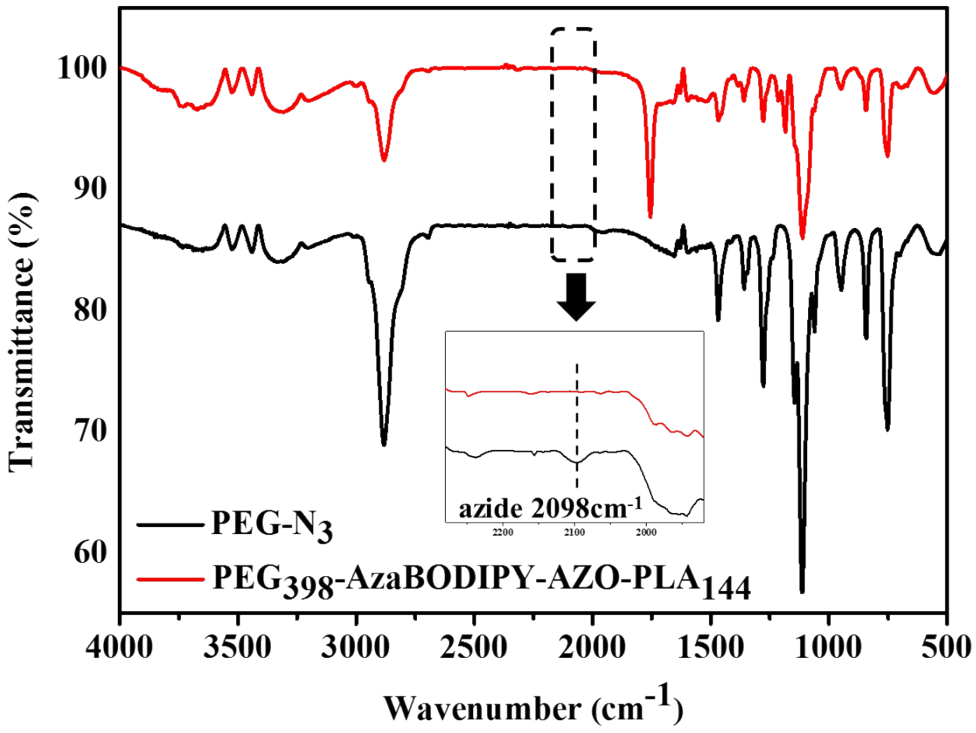


Figure S3. FT-IR spectra of PEG-N_3 and PEG_{398}-AzaBODIPY-AZO-PLA_{144}.

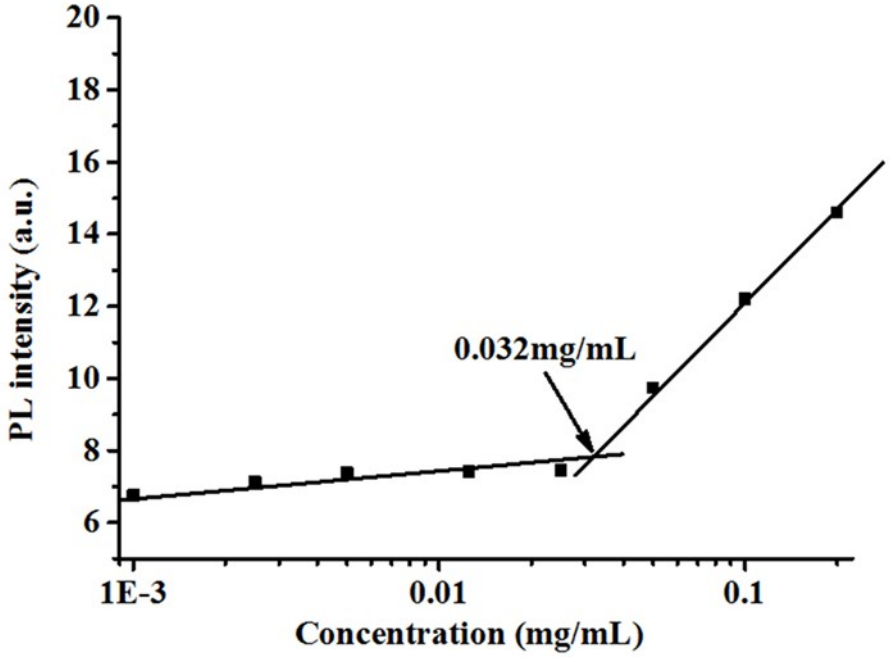


Figure S4. Fluorescent intensity of Nile Red at 630 nm (λ_{ex} = 550 nm) versus PEG_{398}-AzaBODIPY-AZO-PLA_{144} concentration (mg mL^{-1}).

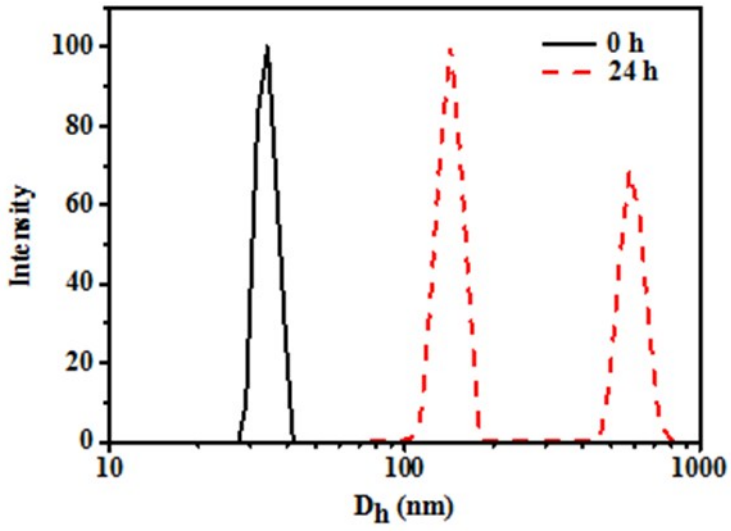


Figure S5. DLS results for the hydrodynamic diameter (D_h, DLS) distributions in PBS solutions of untreated PEG_{398}-AzaBODIPY-AZO-PLA_{144} micelles (D_h = 35 nm, PDI = 0.130) and the micelles after cultivation for 24 h (D_h = 227 nm, PDI = 0.288) in DT Diaphorase human and NADPH (PBS, pH = 7.4) at 37 °C.

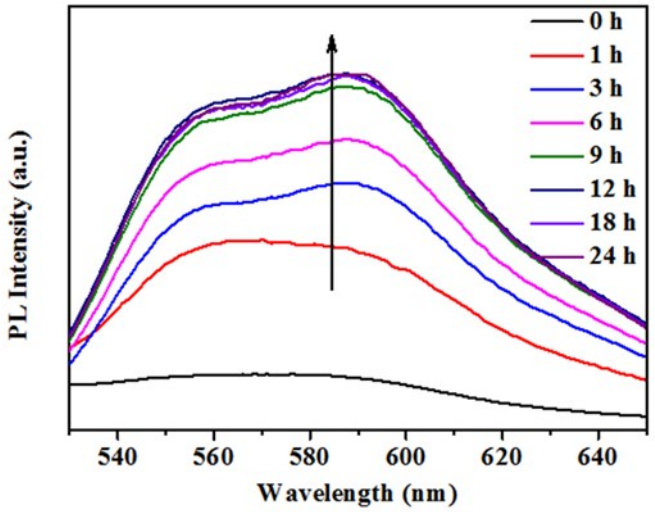


Figure S6. Fluorescence change of DOX encapsulated in PEG398-AzaBODIPY-AZO-PLA144 micelles in PBS (pH 7.4) at 37 oC after azoreductase treatment for different time (micelle concentration: 0.3 mg/mL, λex = 480 nm).

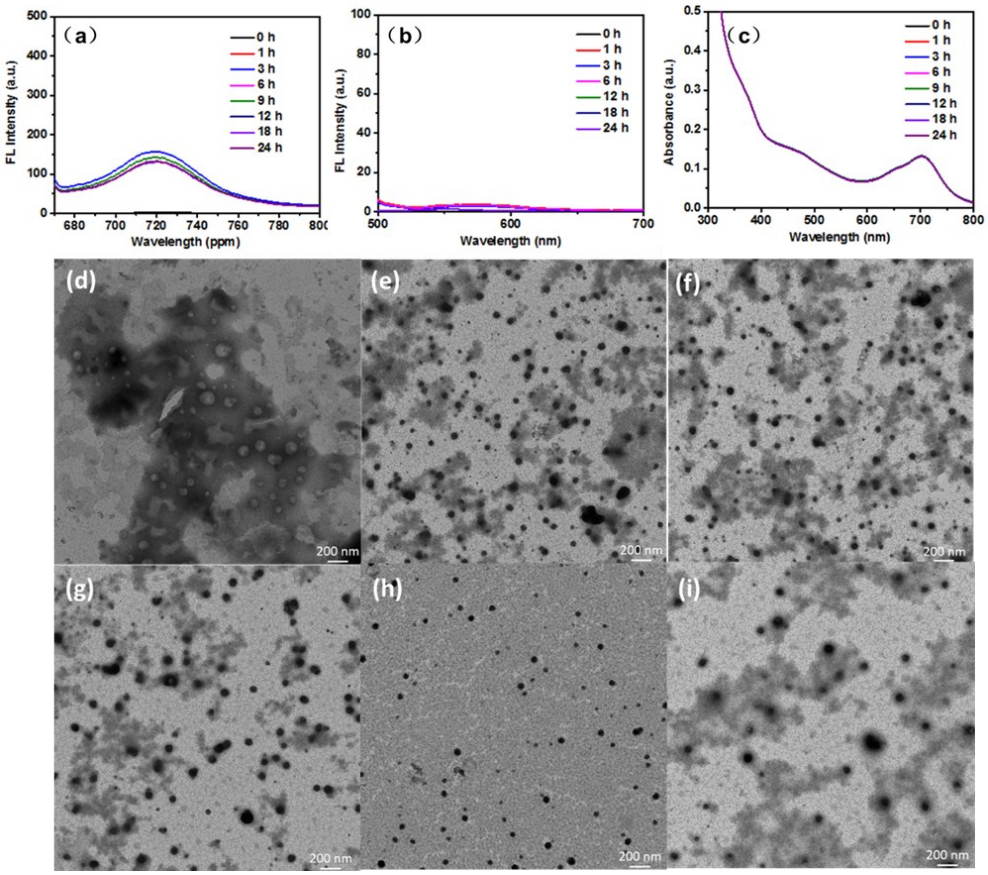


Figure S7. Fluorescence emission spectra for the DOX-loaded PEG_{398}-AzaBODIPY-AZO-PLA_{144} micelles in PBS solution without azoreductase treatment for different time periods excited by (a) 650 nm and (b) 480 nm (0.3 mg mL^{-1} micelles in PBS at 37 °C); (c) UV-vis spectra of the DOX-loaded PEG_{398}-AzaBODIPY-AZO-PLA_{144} micelles in PBS solution without azoreductase treatment for different time periods (0.3 mg mL^{-1} micelles in PBS at 37 °C); TEM images of DOX-loaded PEG_{398}-AzaBODIPY-AZO-PLA_{144} micelles without azoreductase treatment for (d) 0 h (e) 3 h (f) 6 h (g) 9 h (h) 12 h (i) 24 h (0.3 mg mL^{-1} micelles in PBS at 37 °C).

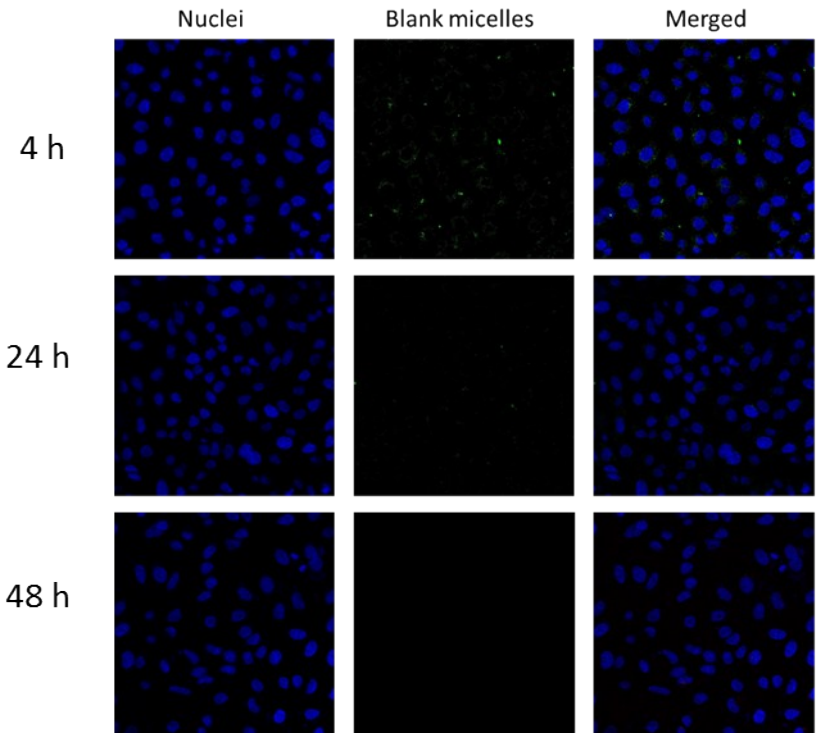


Figure S8. CLSM images of A549 cells after incubation with blank micelles (100 μg/mL) for 4h, 24 h, and 48 h. The cell nuclei was stained by DAPI.

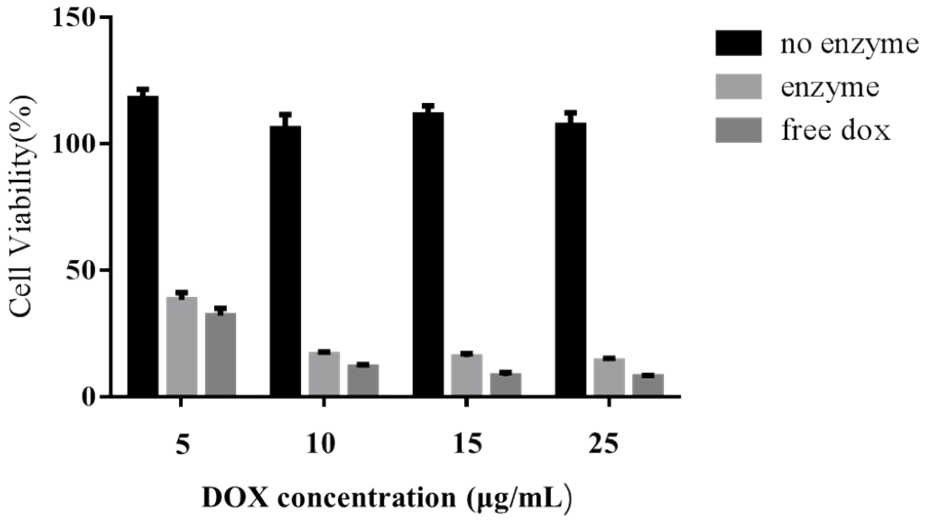


Figure S9. Cell cytotoxicity of DOX-loaded micelles without azoreductase (black), DOX-loaded micelles with azoreductase (gray) and DOX•HCl (gray-black) against A549 cells at various concentrations of DOX.

References

[S1] J. Murtagh, D. O. Frimannsson and D. F. O'Shea, *Org. Lett.*, 2009, **11**, 5386-5389.