Supporting Information

Near-Infrared Light Sensitive Polypeptide Block Copolymer Micelles for Drug Delivery

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1. Synthesis and Characterization

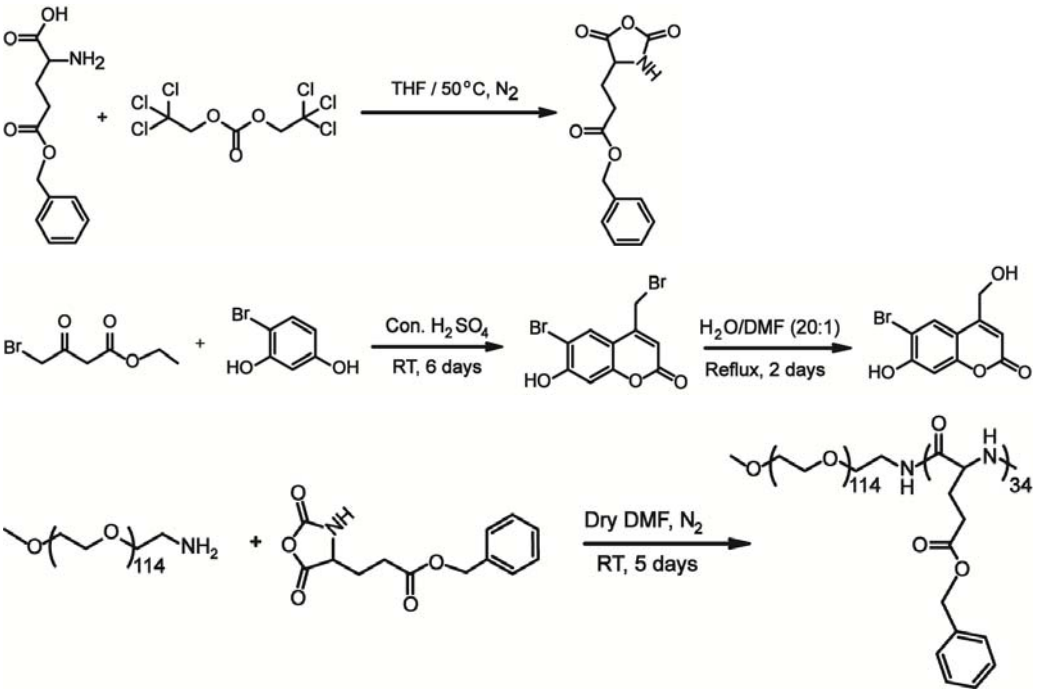
1.1 Materials

L-glutamic acid γ-benzyl ester (Aldrich, ≥ 99.0%), triphosgene (Aldrich, 98%), anhydrous N,N-dimethylformamide (DMF, Aldrich, ≥ 99.8%,) dichloromethane (DCM Aldrich, 99.99) N,N'-dicyclohexylcarbodiimide (EDC, Fluka, 99%), 4-dimethylaminopyridine (DMAP, Aldrich, ≥ 99%), 4-bromoresorcinol (Aldrich), ethyl 4-chloroacetoacetate (Aldrich, ≥ 99.9%), α-Methoxy-ω-amino-PEO macro initiator (Aldrich, Mn = 5000 $g \cdot mol^{-1}$) were used as received. Tetrahydrofuran (THF), and dimethylsulfoxide (DMSO) were obtained from commercial sources and purified according to standard procedure. Milli-Q water with resistivity of 18 mΩ was used for the release experiments. Fluoresence dye nile red (NR), antibacterial drug Rifampin (RIF) were purchased from Aldrich and used as received. PBS buffer purchased from EMD. Paclitaxel (PTX) was a kind gift from Prof. Jean-Christophe Leroux (ETH).

1.2 Characterizations

^{1}H-NMR spectra data were recorded at 400 MHz (Bruker Spectrometer), at room temperature. ^{1}H chemical shifts are reported relative to residual $CHCl_3$ (δ 7.24 ppm), DMSO (δ 2.50), or $(CH_3)_2CO$ (δ 5.32). DLS experiments were performed on a Brookhaven goniometer (BI-200) equipped with a highly sensitive avalanche photodiode detector (Brookhaven, BI-APD), a digital correlator (Brookhaven, TurboCorr) that calculates the photon intensity autocorrelation function $g^2(t)$, a helium-neon laser (wavelength λ = 632.8 nm), and a thermostat sample holder. The autocorrelation data was fitted using the CONTIN algorithm to determine the diameters of suspended micelles/assemblies, and the change in scattered light intensity was measured at 90°. UV-vis absorption and steady-state fluorescence emission spectra were recorded using a UV-vis (Varian Cary 50 Bio) and a fluorescence spectrophotometer (Varian Cary Eclipse), respectively. The excitation wavelength was 540 nm for NR (excitation and emission slit widths set at 5 nm, and the scan rate at 10 nm s^{-1}). Gel permeation chromatography (GPC) measurements were conducted on a Waters system equipped with a refractive index detector (RI 410), a photodiode array detector (PDA 996) and one column (Styragel 5HE, 7.8 mm x 300 mm). THF was used as the eluent (elution rate, 1 mL min^{-1}), and polystyrene standards were used for calibration. Micellar aggregates and dissociation after NIR irradiation were examined using a Hitachi H-7500 transmission electron microscope (TEM) operating at 80 KV. Samples for TEM observations were prepared by casting one drop of the micellar solution on carbon-coated copper grid and dried at room temperature. A 0.50 wt% PEO_{114}-*b*-$P(LGA_{0.62}$-*co*-$COU_{0.38})_{34}$ micelle suspension was diluted fivefold with DI water prior to imaging. A 10 μL aliquot was then placed on a carbon grid that had been glow discharged and allowed to sit for 1 min before blotting with filter paper. Next, 10 μL of a 2 % uranyl acetate solution was placed on the grid and left to stand for 10 min. The staining solution was removed by gentle soaking in water for 3 s followed by blotting off with filter paper. The samples were air dried prior to imaging at ambient temperature. In the case of one-photon absorption of ultraviolet (UV) light,

photons were generated by a spot-curing system combined with a UV filter centered at 365 nm; intensity 70 mW cm^{-2}.



Scheme 1. Synthesis of *γ*-Benzyl *L*-glutamate *N*-carboxyanhydride, 6-Bromo-7-hydroxy-4-hydroxymethylcoumarin and poly(ethylene oxide)-*block*-poly(benzyl-L-glutamic acid) (PEO-*b*-PBLG)

1.3. Synthesis of monomers and polymers

The scheme for the syntheses monomers and diblock copolymers is shown in Scheme 1.

1.3.1. Synthesis of (BLG-NCA) monomer. The monomer of BLG-NCA, (*γ*-Benzyl *L*-glutamate *N*-carboxyanhydride) was synthesized as follows: 1 g (4.2 mmol) of *γ*-benzyl *L*-glutamate (vacuum dried) in 20 mL of dry ethyl acetate (freshly distilled ethyl acetate over CaH_2) was placed in a 100 mL two-necked round-bottomed flask fitted with a magnetic stirrer, condenser and nitrogen inlet. Then 0.54 g (1.8 mmol) of triphosgene in 8 mL of dry ethyl acetate was added drop wise into the *γ*-benzyl *L*-glutamate solution. The reaction mixture temperature was brought to 50 °C. After 3 h, the reactants were completely soluble and the reaction was cooled down to room temperature. The monomer was obtained by recrystallization from ethyl acetate/hexane. Yield: 10.2 g (92%).
^{1}H ($CDCl_3$): δ ppm= 7.10-7.42 (Ar), 6.62 (NH), 5.04 (Ar-CH2), 4.29 (α-CH), 2.50 (*γ*-CH_2), 2.16 (*β*-CH), 2.03 (*β*-CH)

1.3.2. Synthesis of 6-Bromo-7-hydroxy-4-hydroxymethylcoumarin. Ten grams (53 mmol) of 4-bromoresorcinol, ethyl 4-chloroacetoacetate (10 mL, 74 mmol) in concentrated sulfuric acid (100 mL), and was stirred for 6 days at room temperature. The reaction mixture was poured into ice and stirred for 2 h to give a fine precipitate. The precipitate was filtrered, washed with cold water, and suspended in 20 mL of DMF and 300 mL of water. The suspension was refluxed for 2 days. The resulting solution was cooled and evaporated to give 268.7 mg (90 % yield) of 13 as solid.

^{1}H-NMR (CD_3OD) δ ppm = 7.83 (Ar), 6.86 (Ar), 6.40 (Ar), 4.78 (CH_2).

1.3.3. Synthesis of Poly(ethylene oxide)-*block*-poly(benzyl-L-glutamic acid) (PEO_{114}-*b*-$PBLG_{34}$). In a flame dried 25 mL round-bottomed flask, the amino-terminated polyethylene oxide (PEO-NH_2) was dissolved in dry DMF under nitrogen atmosphere. In another flask, the BLG-NCA monomer was dissolved in dry DMF under nitrogen. Then the monomer solution was added drop wise to the polymer solution by syringe. The resulting mixture was stirred at room temperature for 5 days. Afterwards, the solvent was removed under vacuum and the residue was dissolved in chloroform. The resulting diblock copolymer was obtained as a white solid after reprecipitation with cold diethyl ether.
^{1}H-NMR ($CDCl_3$): δ ppm= 8.10 (NH), 7.10-7.40 (Ar), 4.88-5.18 (Ar-CH2), 3.80-4.04 (α-CH), 3.48-3.79 (CH_2CH_2O), 3.33 (CH_3), 2.00-2.70 (*β*-CH_2 and *γ*-CH_2) ppm.

1.3.4. Synthesis of Poly(ethylene oxide)-*block*-poly(L-glutamic acid) (PEO_{114}-*b*-$PLGA_{34}$). The benzyl groups of PEO-PBLG were removed by hydrogenation with a palladium catalyst (Pd/C, 10%) and H_2 in THF/methanol in a flame dried 100 mL round-bottomed flask, at room temperature for 24 h. The palladium catalyst was filtered off and the solvent removed in vacuum. The residue was dissolved in deionized water and dialysed in deionized water for a week. The polymer was precipitated by adding diethyl ether, solvents were removed under vacuum and the resulting polymer was dried at low pressure and room temperature for 24 h. Yield: 0.278 g (92%).
^{1}H (DMSO-*d*6) δ ppm: 3.90-4.24 (α-CH), 3.34- 3.64 (CH_2CH_2O), 3.33 (-OMe) 1.60-2.40 (40H, β-CH2 and γ-CH2) ppm.

1.3.5. Synthesis of poly(ethylene oxide)-*block*-[poly(L-glutamic acid)-*co*-(6-bromo-7-hydroxycoumarin-4-ylmethoxycarbonyl)-L-glutamate)] (PEO_{114}-*b*-$P(LGA_{0.62}$-*co*-$COU_{0.38})_{34}$). In a 25 mL round-bottomed flask, 100 mg of the deprotected block copolymer (PLGA-*b*-PEO) was dissolved in 2 mL of dry DMF. In another 25 mL round-bottomed flask, the 6-Bromo-7-hydroxy-4-hydroxymethylcoumarin (2 eq), the DMAP (0.6 eq) and the EDC (1 eq) were dissolved in 3 mL of dry DMF and transferred to the polymer solution by syringe. Then the mixture was stirred at room temperature for 24 h. The polymer solution was filtered off to remove the precipitate. The polymer was precipitated by adding diethyl ether and dried under vacuum.
^{1}H (DMSO-*d*6) δ ppm: 8.18 (Ar), 7.25 (Ar), 6.79 (Ar), 5.03~4.93 (Ar-OH), 4.51-4.25 (-CH_2), 4.20-3.90 (α-CH), 3.34-3.64 (CH_2CH_2O), 3.33 (-OMe) 2.53-1.60 (β-CH_2 and γ-CH_2) ppm.

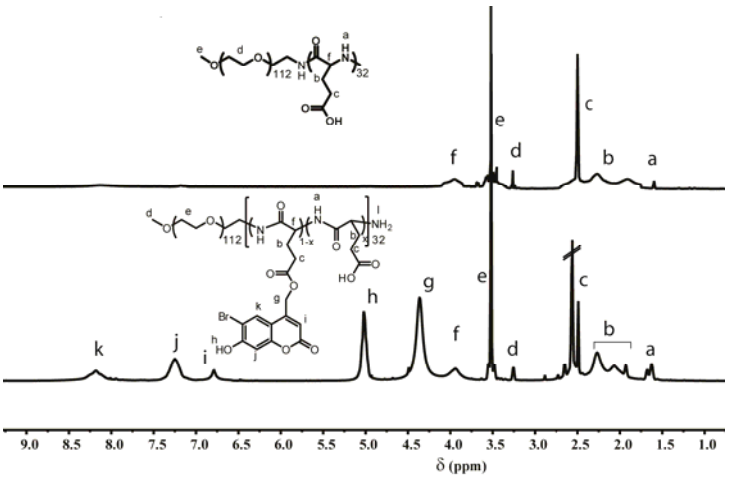


Fig. S1 ^{1}H NMR spectra of (PEO_{114}-*b*-$PLGA_{34}$) and PEO_{114}-*b*-$P(LGA_{0.62}$-*co*-$COU_{0.38})_{34}$ diblock copolymers.

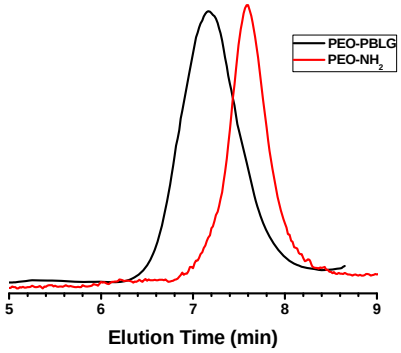


Fig. S2 GPC traces of PEO-NH_2 macroinitiator and (PEO_{114}-*b*-$PBLG_{34}$) copolymer

1.4. Critical Micelle Concentration (CMC) determinations

One hundred milligrams of copolymer was dissolved in 6 mL DMSO and added dropwise (1 droplet/3 s) into 5 mL Milli-Q water under ultrasonication. DMSO and a part of the water were removed under dialysis for overnight, and pure Milli-Q water was added to reach a typical micelle concentration of 10 mg mL^{-1}. From this stock solution, 0.5 mL of different micelle solutions were prepared with concentrations ranging from 0.0001 to 10 mg mL^{-1} followed by addition of 20 µL of a Nile Red (NR) stock solution in acetone (1 mg mL^{-1}), gentle hand shaking, and acetone evaporation. The critical micellar concentration (CMC) was determined by fluorescence measurements of NR-loaded micelles at the inflection point on the spectrometry plots representing the maximum emission wavelength as a function of the copolymer concentration (Fig. S3). Excitation of the hydrophobic NR with 550 nm light in an aqueous medium resulted in a relatively low fluorescence with a λ_{max} of 660 nm. However, if the dye resides in a hydrophobic environment, such as the interior of a micelle, its fluorescence emission intensity increased dramatically and experienced a blue shift.

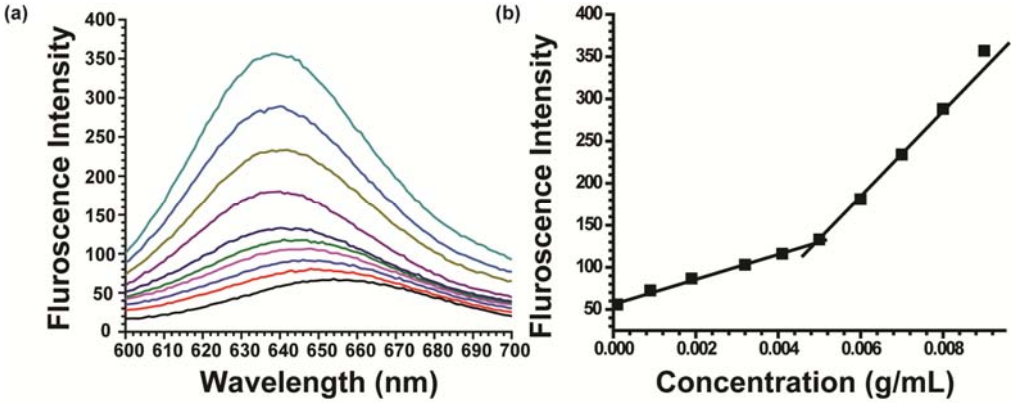


Fig. S3 (a) Fluorescence spectra of Nile red with various concentration of PEO_{114}-*b*-P($LGA_{0.62}$-*co*-$COU_{0.38}$)$_{34}$ in water; (b) Fluorescence intensity of Nile red as a function of the concentration of the diblock polymer.

1.5. Drug Loading Measurements

Drug loading is characterized by the drug loading content (LC) and the drug loading efficiency (LE) defined as follows:

$$\text{LC (\%)} = \frac{\text{wt of drug in micelles}}{\text{total weight of micelles}} \times 100$$

$$\text{LE (\%)} = \frac{\text{wt of drug in micelles}}{\text{total weight of drug}} \times 100$$

The amount of drug in the micelles is measured with UV–vis spectroscopy by dissolving drug-loaded micelles in methanol. UV absorbance is monitored at a wavelength of 470 nm for Rifampicin and 238 nm for Paclitaxel. Drug concentration is determined by calibration with a series of standards of known concentrations of drugs in the same solvent. The total weight of the micelles is determined by removing water from the aqueous solution and weighing the sample.

2. Photosensitivity of BCP micelle under UV light (one-photon)

To demonstrate one-photon absorption, miccellar solution of PEO_{114}-*b*-P($LGA_{0.62}$-*co*-$COU_{0.38}$)$_{34}$ was irradiated with UV light and compared to samples that were not irradiated. A 0.5 mL micelle solution of PEO_{114}-*b*-P($LGA_{0.62}$-*co*-$COU_{0.38}$)$_{34}$ (10 mg mL^{-1}) was placed in cuvette and irradiated with UV light (365 nm, 70 mW cm^{-2}) for certain periods of time. Irradiation of UV light yielded photocleavage of coumarin moieties accompanied by a dramatic decrease in the flurosence at 468 nm (Fig. S4a). Fig. S4b shows the plot of the normalized fluorescence emission intensity of coumarin at 468 nm versus UV light irradiation time. After a prolonged irradiation time (37 min), the emission band disappeared almost completely indicating that the photoreaction was complete. The apparent kinetics of the UV light-induced (one-photon, 365 nm, 70 mW cm^{-2}) photoreaction in the micellar solution was best fitted by first-order kinetics, as judged from the mono-exponential fit of the data. The time constant was 11.2 minutes.

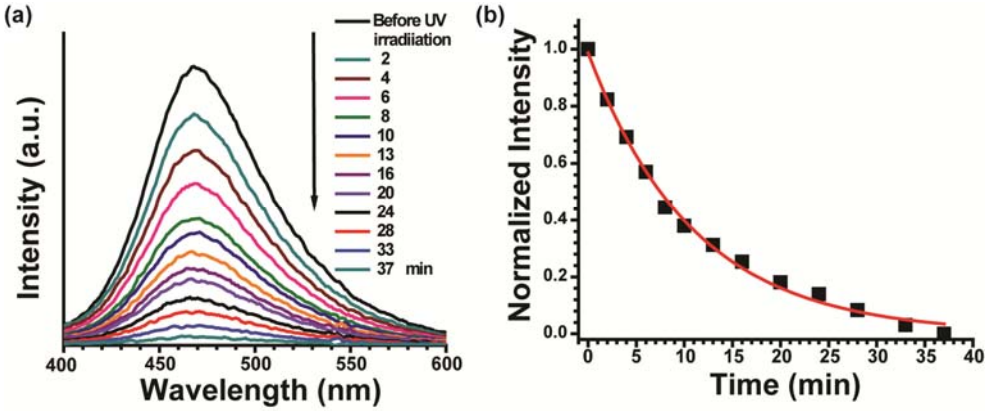


Fig. S4 (a) Fluorescence emission spectra (λ_{exc}=380 nm) of a micellar solution of PEO_{114}-*b*-P($LGA_{0.62}$-*co*-$COU_{0.38}$)$_{34}$ (10 mg/mL) exposed to 360 nm UV light, 70 mW cm^{-2}. (b) Normalized fluorescence emission intensity of coumarin at 468 nm as a function of cumulative time of NIR irradiation.

3. NIR Light Triggered Release of Nile Red

A NR loaded BCP micellar solution (~ 0.3 mL) was placed in a dialysis cap integrated with a quartz cuvette filled with ~3.7 mL of water (Fig. 1b). The release of Nile Red (NR) molecules from NIR-disrupted micelles through diffusion across the membrane was monitored by recording the change of absorbance at 640 nm in the solution outside the dialysis cap. In order to increase the solubility of NR in the receiving phase, a PEO-PPO-PEO triblock copolymer (Pluronic F-127, 1 mg mL^{-1}) was dissolved in the solution inside the receiving phase of cuvette (PPO is polypropylene oxide). Fig. S5a, fluorosence spectra of NR, obtained from the solution underneath the dialysis cup, for the micellar solution after NIR irradiation are compared to the spectrum for the same micellar solution without NIR irradiation after being placed in the dialysis cap for 63 h. Fig. S5b where the normalized NR released into the solution with and with out irradiation of NIR is plotted as a function of time.

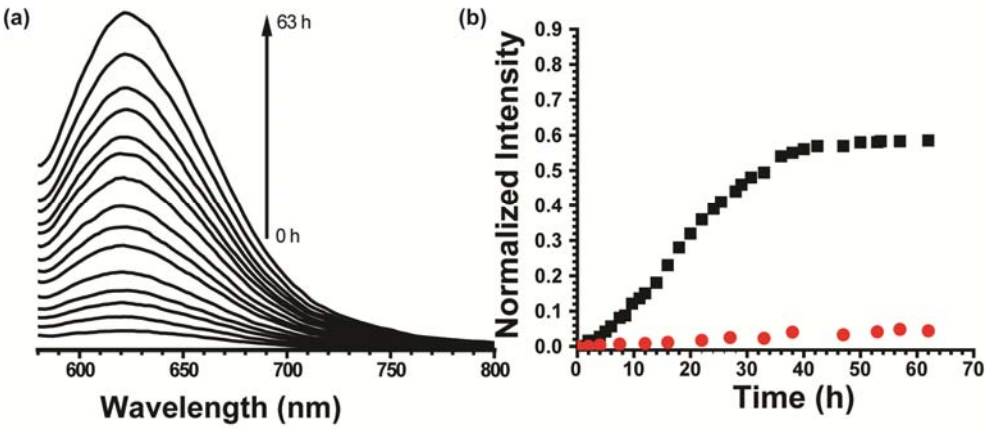


Fig. S5 (a) Change in the fluorescence emission spectra (λ_{exc}=640 nm) over time of nile red (NR) released from micelles of PEO_{114}-*b*-P($LGA_{0.62}$-*co*-$COU_{0.38}$)$_{34}$ after NIR irradiation (220 min). (b) Cumulative release profiles of NR from dye-loaded PEO_{114}-*b*-P($LGA_{0.62}$-*co*-$COU_{0.38}$)$_{34}$ micelles before and after NIR irradiation, the release spectrum for the micellar solution without NIR irradiation after 63 hrs also shown for comparison.