Supporting Information

Block Copolymer of Zwitterionic Polyphosphoester and Polylactic acid for Drug Delivery

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Synthesis of glutamic acid-cysteamine (Glu-Cya) (Scheme S1)

[(Boc-Glu-OtBu)-Cya]$_2$. Boc-L-glutamic acid 1-tert-butyl ester (Boc-Glu-OtBu, 10.0 g, 33.3 mmol) was dissolved in 20 mL anhydrous dichloromethane, then added to the solution of hydroxysuccinimide (NHS) (4.2 g, 36.5 mmol) and *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC•HCl) (7.0 g, 36.5 mmol) in 80 mL CH_2Cl_2. The mixture was maintained at room temperature overnight, and the impurities were removed by washing with deionized water, saturated sodium bicarbonate solution and saturated sodium chloride solution. Subsequently, (Boc-Glu-OtBu)NHS (5.8 g, 14.1 mmmol) was dissolved in 50 mL anhydrous CH_2Cl_2, then to this solution was added cystamine dihydrochloride (1.3, 5.9 mmol) and anhydrous triethylamine (1.5 g, 13.4 mmol) in CH_2Cl_2. The reaction was performed at room temperature for 36 h, and the resultant solution was washed with deionized water, dried over anhydrous magnesium sulfate. The organic solvent was removed and the product was dried under vacuum to a constant weight at room temperature. The crude product was further purified by silica gel column chromatography with methanol/chloroform (v/v, 2/98) as the eluent. The purified product was lyophilized with a yield of 70%. Electrospray ionization mass spectrometry (ESI-MS) was performed on Proteome X-LTQ (ThermoFisher Scientific, USA). The theoretical molecular mass of [(Boc-Glu-OtBu)-Cya]$_2$ is [M] = 694 and the ESI-MS result shows [M+H$^+$] = 694.96. Nuclear magnetic resonance (NMR) spectra were recorded on

AVANCE III 400 MHz with direct cryoprobe. ^{1}H NMR ($CDCl_3$, ppm): δ 6.88 (t, H), 5.34 (d, H), 4.15 (m, H), 3.58 (m, 2H), 2.84 (t, 2H), 2.31 (t, 2H), 2.14 (m, H), 1.89 (m, H), 1.46 (s, 3H), 1.44 (s, 3H).

Glu_2-Cya_2. [(Boc-Glu-OtBu)-Cya]$_2$ (1.0 g) was dissolved in 5 mL of anhydrous CH_2Cl_2, then 5 mL of trifluoroacetic acid was added, and the solution was further stirred at 25 °C for 5 h. The mixture was concentrated and dried under vacuum at room temperature, then dissolve in deionized water and the incomplete deprotected products were remove by extraction with CH_2Cl_2. The purified product was lyophilized with a yield of 85%. The theoretical molecular mass of Glu_2-Cya_2 is [M] = 410 and the ESI-MS result shows [M+H$^+$] = 411.12. ^{1}H NMR (D_2O, ppm): 4.02 (t, H), 3.48 (t, 2H), 2.82 (t, 2H), 2.45 (m, 2H), 2.18 (m, 2H).

Glu-Cya. To 0.4 g (0.1 mmol) of Glu_2-Cya_2 dissolved in 12.6 mL of deionized water , dimethyl formamide and hydrochloric acid (v/v/v, 1/3/0.2), tributyl phosphine (1.0 g, 5 mmol) was added. The reaction was carried out at room temperature overnight. The resultant solution was dried under vacuum, dissolve in deionized water and the unreacted tributyl phosphine were remove by extraction with CH_2Cl_2. The purified product was lyophilized with a yield of 80%. The theoretical molecular mass of Glu_2-Cya_2 is [M] = 206 and the ESI-MS result shows [M+H$^+$] = 207.10.

Characterization of drug-loaded nanoparticles

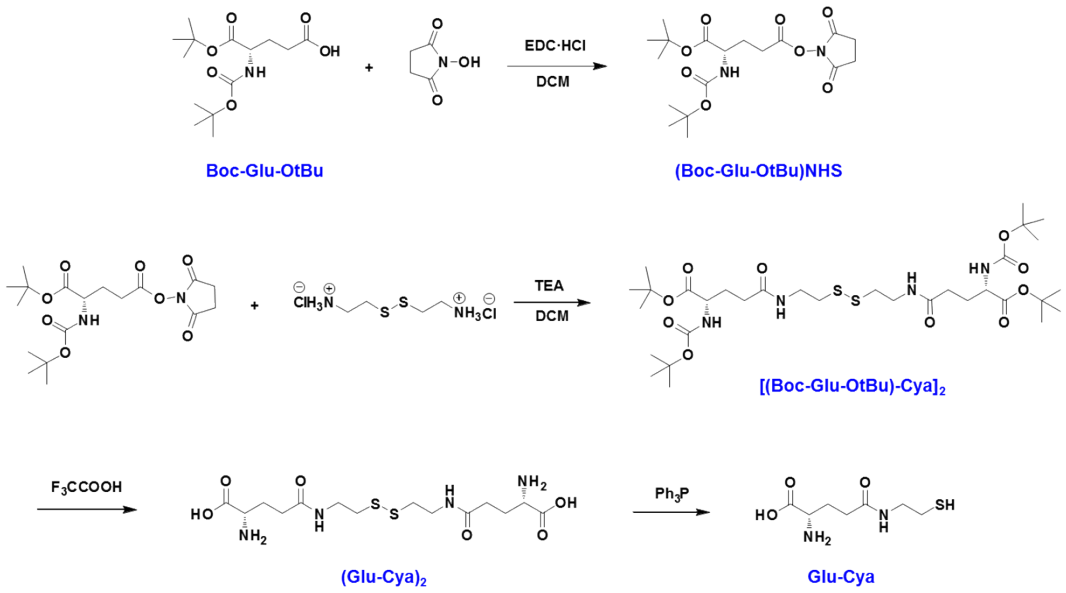
The size, size distribution and zeta potential of particles in aqueous solution were measured by dynamic light scattering (DLS) carried out on a Malvern Zetasizer Nano ZS90 with a He-Ne laser (633 nm) at 90° collecting optics. The data were analyzed by Malvern Dispersion Technology Software 4.20. All samples were prepared in aqueous solution at a concentration of 1.0 mg/mL and filtered through Millipore 0.45 μm filter prior to measurements. Transmission electron microscopy (TEM) measurements were performed on a JEOL 2010 transmission electron microscope with an accelerating voltage of 200 kV. The samples were prepared by pipetting a drop of the solution onto a 230 mesh copper grid coated with carbon and allowing the sample to dry in air before measurements.

High-performance liquid chromatography (HPLC) analyses

HPLC analyses were performed using a Waters HPLC system consisting of Waters 1525 binary pump, Waters 2475 fluorescence detector, 1500 column heater and a Symmetry C18 column. HPLC grade acetonitrile/water (pH 2.7, adjusted by $HClO_4$) at volume ratio 1/1 was used as the mobile phase at 30 °C with a flow rate of 1.0 mL min^{-1}. Fluorescence detector was set at 465 nm for excitation and 570 nm for emission and linked to Breeze software for data analysis. Linear calibration curves for concentrations in the range of 0.1 - 100 μg/mL were constructed using the peak areas by linear regression analysis.

In vitro drug release

The drug-loaded nanoparticles was performed by the dialysis against the release medium (pH 7.4 Phosphate Buffer Solution). 2 mL of DOX-loaded nanoparticles was sealed in the dialysis tubing. The tubing was immersed in 15.0 mL of the release medium at 37 °C, followed by an shaking at a speed of 100 rpm. All of the release medium was taken out at predetermined time. After the remove of used medium, the same volume of fresh release medium was immediately supplemented into the release tank. The DOX content in the release medium were determined by HPLC as above. The release rate (RR, %) was calculated with the formula: $RR=(Wi/Wtotal)\times100\%$, where Wi is the measured amount of DOX in release medium at the time-point, and Wtotal is the total amount of DOX in the equal volume of nanoparticles before the dialysis.



Scheme S1. Synthetic route for cysteamine-glutamic acid. TEA: triethylamine, EDC: *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride, DCM: dichloromethane, Ph_3P: tributyl phosphine.

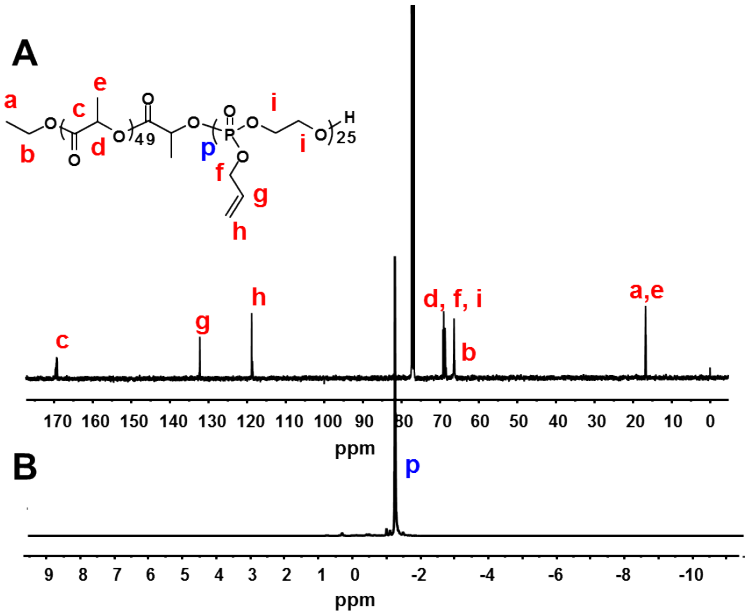


Fig. S1. ^{13}C NMR and ^{31}P NMR spectra of PLA-*b*-PAEP.

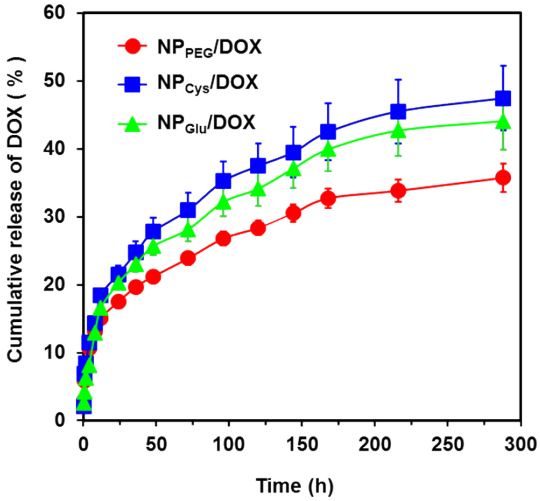


Fig S2. Cumulative release or DOX from NP_{PEG}/DOX, NP_{Cys}/DOX and NP_{Glu}/DOX. Data are shown as means ± s.d. (n=3).