

Electronic Supporting Information

Biodegradable and pH-sensitive polymersome with tuning permeable membrane for drug delivery carrier

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1. Materials

Methoxypoly(ethylene glycol) (PEG) ($M_n=2,000$) was purchased from Aldrich and dried for 2 h at 80 °C under vacuum. Carboxylic acid-modified PEG (PEG-COOH) was prepared as reported elsewhere.¹ Succinic anhydride, N,N'-dicyclohexyl carbodiimide (DCC), 4-(dimethyl amino) pyridine (DMAP), anhydrous dioxane, anhydrous dichloromethane (DCM), anhydrous tetrahydrofuran (THF), 3-amino 1-propanol (AP), 1,4-butanediol diacrylate (BD), stannous octoate ($\text{Sn}(\text{Oct})_2$), and D,L-lactide (LA) were used as received (from Aldrich). Diethyl ether was supplied by Samchun chemicals (Korea).

2. Synthesis

2.1. Synthesis of pH-sensitive poly(β -amino ester) (PAE)

pH-sensitive poly(β -amino ester) was synthesized by Michael addition polymerization.² Briefly, BD (18 g, 90 mmol) and AP (6.9 g, 90 mmol) were placed in a one-neck round-bottom flask. The solution was reacted at 100 °C for 5 h. After completion, the viscous liquid was dissolved in DCM and precipitated in diethyl ether. The product was dried at 30 °C for 2 d.

2.2. Synthesis of PEG-grafted poly(β -amino ester) (PAE-g-PEG):

To synthesize the PEG-grafted poly(β -amino ester), PEG-COOH was coupled to PAE containing a hydroxyl group using DCC and DMAP. PEG-COOH (7.7 g, 3.7 mmol) and PAE (10 g, 36.6 mmol) were placed in a two-neck round-bottom flask and dried at 80 °C for 2 h. After dissolving the mixture in DCM (60 mL), DCC (1.5 g, 7.4 mmol) and DMAP (0.9 g, 7.4 mmol) were added to the solution. The reaction mixture was left to stand at room temperature for 24 h under N₂ gas. After filtering to remove the insoluble dicyclohexyl urea (DCU), PAE-g-PEG was obtained by precipitation in diethyl ether.

2.3. Synthesis of PEG and PLA-grafted poly(β -amino ester) (PAE-g-PEGLA)

The ring-opening polymerization of PLA was performed in the presence of $\text{Sn}(\text{Oct})_2$ as a catalyst at 130 °C for 8 h with constant stirring under a nitrogen atmosphere. The product was dissolved in DCM and precipitated in diethyl ether. The synthetic route is shown in Scheme S1.

3. Characterization

The copolymer compositions were determined by ^1H NMR (Varian Unity Inova-500NB, 500 MHz) in CDCl_3 containing 0.03 v/v tetramethylsilane as a reference. Fig. S1 shows the ^1H NMR spectrum of the copolymer. The M_n and polydispersity index (PDI) were confirmed by gel permeation chromatography (GPC) (Shodex-KF 801, 802.5, and KF 803) using THF as the eluent at a flow rate of 1.0 mL/min under RI detection (Shodex RI-101). Calibration was carried out using poly(methyl methacrylate) standards (Shodex) over the molecular weight range of 2,000–49,600.

4. Polymersome preparation

In a typical procedure, 100 mg of the copolymer was first dissolved in 1 mL THF, and DI water (9 mL) was added to the polymer solution. After vigorous stirring for 10 min, the resulting giant polymersome suspension was dialyzed against a pH 7.4

phosphate buffered saline (PBS) solution for 12 h to remove the organic solvent. For the preparation of nano-sized polymersome, 10 mg of the copolymer was dissolved in 1 mL THF and DI water (10 mL) was added. After stirring for 10 min, the solution was dialyzed against a pH 7.4 PBS for 12 h.

5. Confocal laser scanning microscopy

Confocal laser scanning microscopy (CLSM) of the 1% (w/v) polymersome suspension was carried out on a Carl Zeiss LSM510 microscope. To confirm the vesicular structure, the polymersome suspension (1 mL) was mixed with an amphiphilic fluorescent dye (FM1-43, 10 μ L of a 1 mg/mL solution in DI water), and left to stand for 1 h to allow the dye to absorb into the hydrophobic membrane prior to imaging. The image was obtained at λ_{ex} 488 nm and λ_{em} 580–610 nm. To examine the permeability of the dye through the polymersome membrane, 1 mL fluoresceinamine solution (pH 7.4 PBS, 0.1 mg/mL) was added to the polymersome suspension (pH 7.4 PBS, 1 mL). The solution pH was adjusted to pH 5.5 and kept at room temperature for 1 h before observing CLSM image at λ_{ex} 488 nm and λ_{em} 510–550 nm.

6. Transmission electron microscopy

The morphology of nano-sized polymersome was observed by transmission electron microscopy (TEM), using a PhilipsCM200 transmission electron microscope, operated at an acceleration voltage of 80 kV, after negatively staining the nano-sized polymersomes with 2% (wt./vol.) uranyl acetate. Samples for Cryo-TEM measurement were prepared as follows: Nano-sized polymersome solution was loaded onto holey carbon film-supported grids. Vitrobot (FEI) was used to make a thin aqueous film blotting with filter paper and the immediate plunging into liquid ethane. The frozen grids were stored in liquid nitrogen and transferred to a cryotransfer holder (Gatan) under liquid nitrogen at approximately $-180\text{ }^{\circ}\text{C}$. Images were recorded on a CCD camera (2k, Gatan) using a Tecnai F20 field emission gun electron microscope operated at 200 kV (FEI) with low dose mode.

7. Calcein leakage

Calcein loaded polymersome was prepared using calcein containing DI water during solvent exchange method. THF was removed from dialysis against pH 7.4 PBS for 12 h and unloaded calcein was removed by passing through a Sephadex G-50 column using pH 7.4 PBS solution. Calcein loaded polymersome solution (5 mL) was diluted with pH 7.4 PBS (45 mL) and solution pH was adjusted to each pH. Final samples were diluted

(5 times) with pre-adjusted PBS and Triton X containing PBS, respectively. The fluorescence change was monitored at 495 and 515 nm as excitation and monitoring wavelength. The percent leakage of polymersome was defined as

$$\% \text{ Leakage} = \frac{R_t - R_0}{1 - R_0}$$

where R_0 and R_t mean the initial and intermediate fluorescence intensity ratio, respectively. R_0 and R_t were calculated from dividing the fluorescence intensity of diluted solution in PBS to the fluorescence intensity of diluted solution in Triton X containing PBS.

8. Fluorescence study

Ionization property of pH-sensitive polymersome was investigated via 2-(*p*-toluidino) naphthalene-6-sulfonic acid (TNS) fluorescence changes. TNS (0.1 mg) was added to 10 mL diluted polymersome suspension (0.1 mg/mL) and the solution was titrated to each pH. The TNS fluorescence was determined using Spectramax M5 (Molecular devices) at excitation and emission wavelengths of 321 and 445 nm, respectively.

9. Dynamic light scattering

Nano-sized polymersome sample was prepared as follows: 50 mg of the copolymer was dissolved in 5 mL THF and DI water (10 mL) was added. After stirring for 10 min, pH 7.4 PBS (40 mL) was added to the solution. THF was removed from evaporation at room temperature for 30 min.

The hydrodynamic radius (size) of nano-sized polymersome at pre-adjusted pH was measured by DLS (dynamic light scattering, Malvern Instrument Ltd. Series 4700) with a helium laser at 633 nm and a digital correlator. When the difference between the measured and calculated baselines was less than 0.1%, the correlation function was deemed acceptable. The scattering angle was fixed at 90°. The concentration of the solution was kept at 0.1 mg/mL.

10. Degradation

Degradations of nano-sized polymersomes were investigated in PBS solution at pH 7.4 and pH 5.5. Previously prepared polymersome solution (1 mg/mL) was placed in shaking bath at 37 °C. Freeze-dried sample at predetermined time interval was analyzed by GPC.

11. Critical aggregate concentration

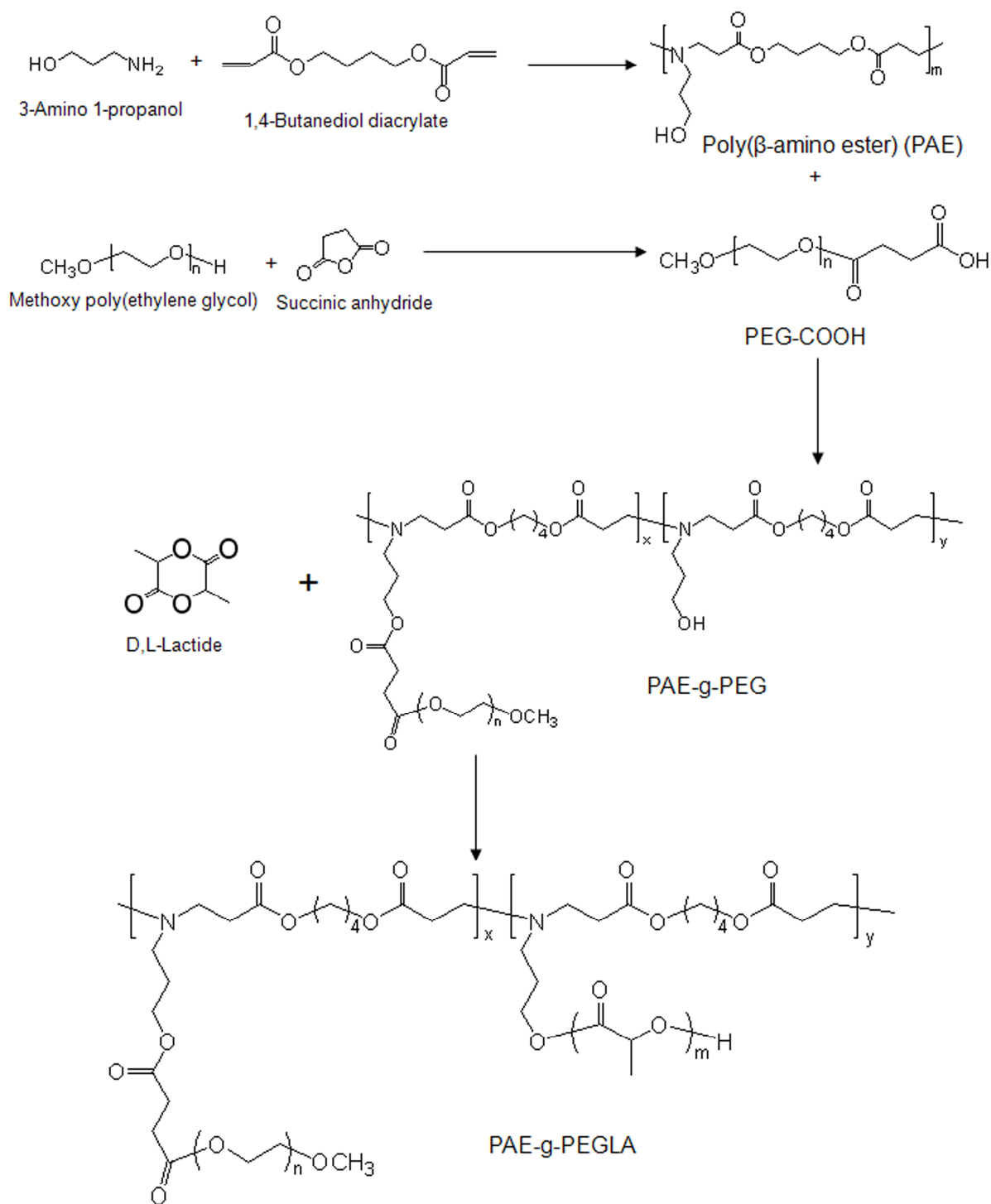
The critical aggregate concentration (CAC) was determined using a fluorescence spectrometer (Aminco Bowoman Series 2; Aminco Bowoman, Urbana, IL) with pyrene as the hydrophobic dye. A stock solution of pyrene in THF (200 μ L) was added to the PBS (200mL) solution and the THF was removed by heating at 60 $^{\circ}$ C for 2 h. The final pyrene concentration was 1×10^{-6} M. Nano-sized polymersome solution (1 mg/mL) using pyrene containing PBS (1 mg/mL) was prepared according to previously described method. Final polymersome solution was diluted with pyrene containing PBS solution and the excitation spectrum of pyrene was recorded at 392 nm.

References

1. S. Zalipsky, C. Gillon and A. Zilkha, *Eur. Polym. J.*, 1983, **12**, 1177.
2. J. J. Green, G. T. Zugates, N. C. Tedford, Y-H. Huang, L. G. Griffith, D. A. Lauffenburger, J. A. Sawicki and R. Langer, D. G. Anderson, *Adv. Mater.*, 2007, **19**, 2836.

Table S1. The molecular weight and structure of polymers calculated from ^1H NMR.

Polymers	M_n	No. of PEG	No. of PLA
PAE	3,822	-	-
PAE-g-PEG	5,922	1	-
PAE-g-PEGLA _{0.4}	11,538	1	13
PAE-g-PEGLA _{0.7}	15,282	1	13
PAE-g-PEGLA _{1.3}	22,822	1	13
PAE-g-PEGLA _{2.5}	38,682	1	13



Scheme S1. Schematic diagram of the synthesis and chemical structure of the copolymers.

Fig. S1 ^1H NMR spectrum of PEG and PLA-grafted poly(β -amino ester) (PAE-g-PEGLA_{0.7}).

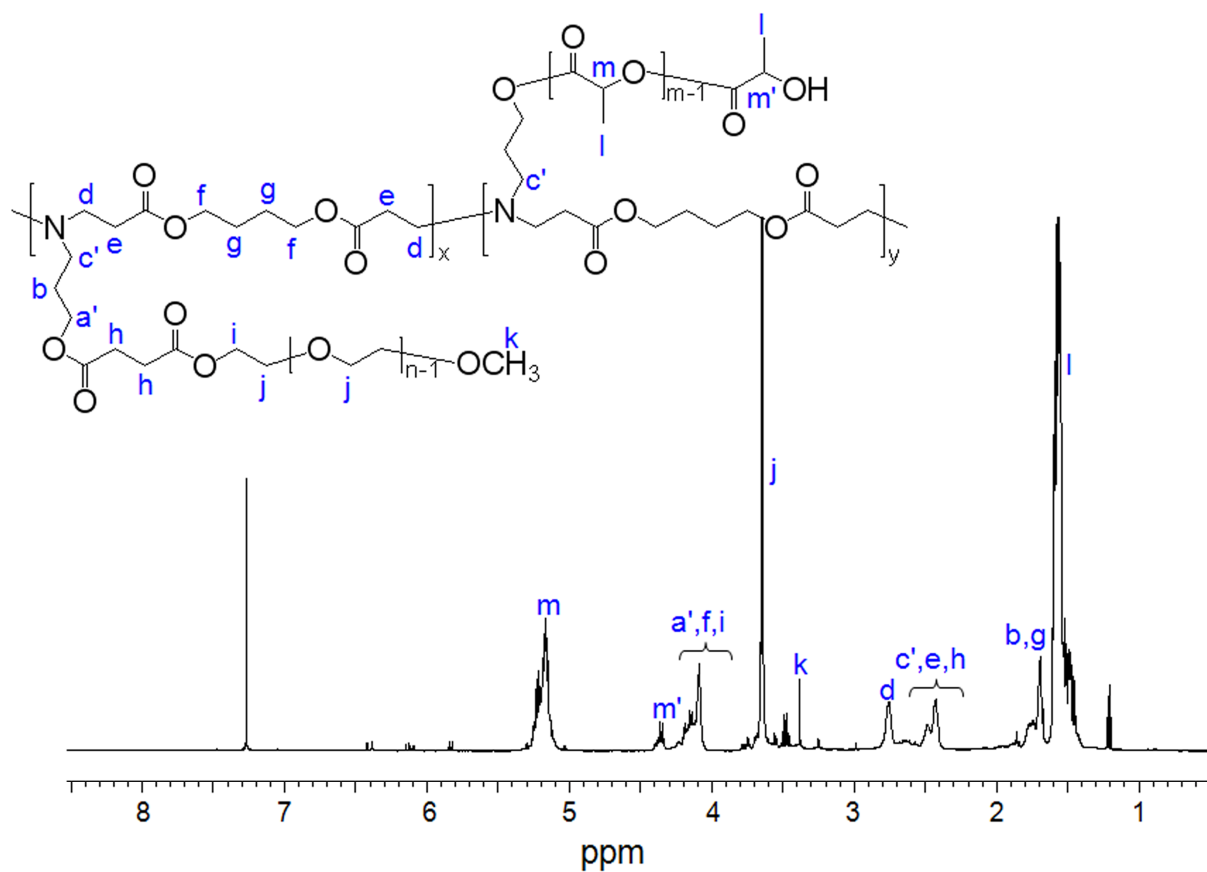


Fig. S2 Size distribution of PAE-g-PEGLA_{0.7} nano-sized polymersome in PBS solution at a) pH 7.5 and b) pH 5.5. The concentration of polymersome was 0.1 mg/mL.

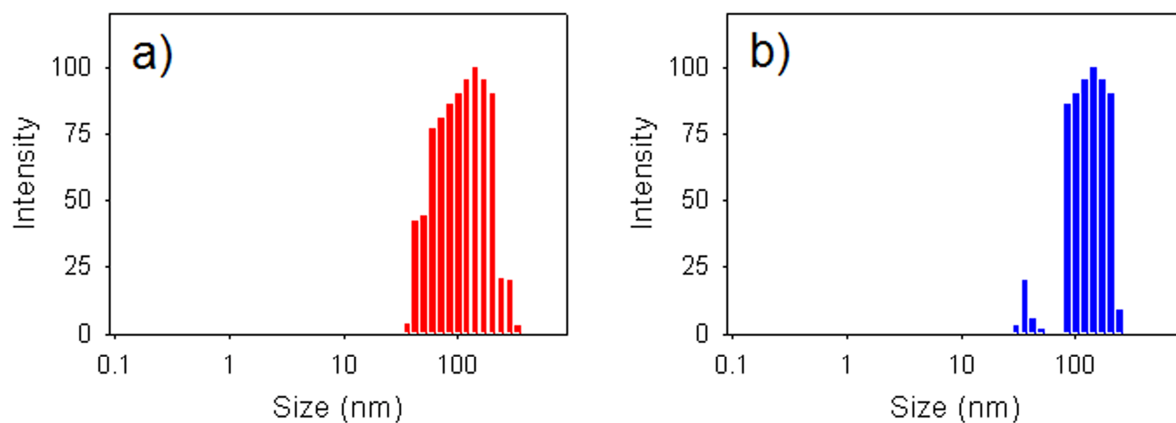


Fig. S3 TEM image of nano-sized polymersome stained with uranyl acetate (scale bar =30 nm) prepared using PAE-g-PEGLA_{0.7}.

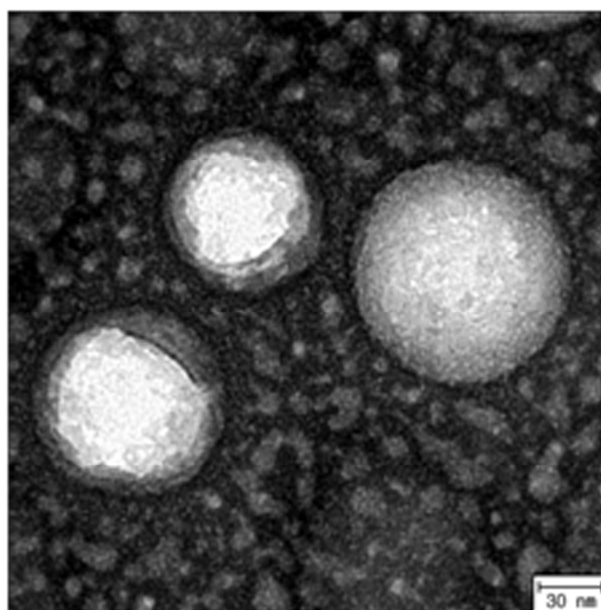


Fig. S4 Cryo-TEM image of nano-sized polymersome without staining (scale bar = 100 nm) prepared using PAE-g-PEGLA_{0.7}.

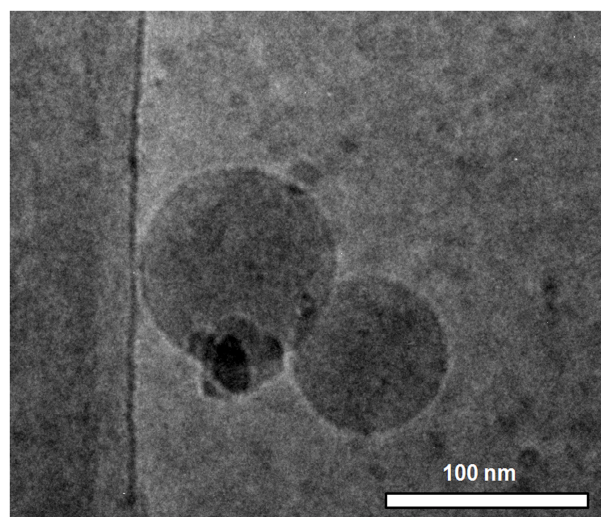


Fig. S5 Effect of pH on the TNS fluorescence of in a mixed TNS and nano-sized polymersome (PAE-g-PEGLA_{0.7}) solution. 0.01 mg/mL TNS was added to a 0.1 mg/mL polymersome solution and the pH was adjusted by titration.

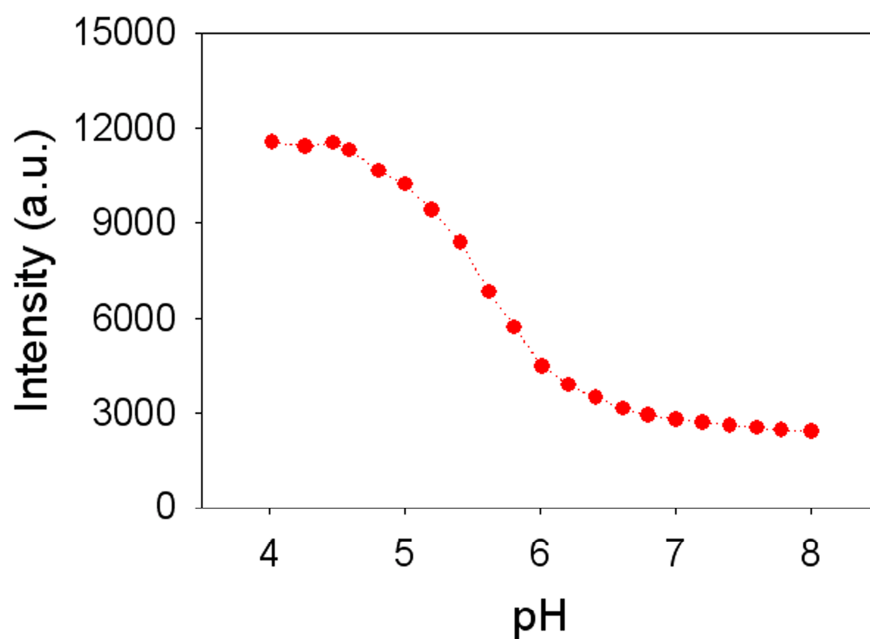


Fig. S6 Excitation spectra of pyrene in PBS (pH 7.4) in the presence of PAE-g-PEGLA_{0.7} nano-sized polymersome.

