Supporting Information

Acid-Sensitive Polypseudorotaxanes Based on Ortho Ester-Modified Cyclodextrin and Pluronic F-127

Ran Ji, Jing Cheng, Cheng-Cheng Song, Fu-Sheng Du,* De-Hai Liang and Zi-Chen Li*

Beijing National Laboratory for Molecular Sciences, Key Laboratory of Polymer Chemistry and Physics of Ministry of Education, College of Chemistry & Molecular Engineering, Peking University, Beijing 100871, China.

Materials. Pluronic F127 (Alfa, Germany) and triethylamine (TEA) were used as received. β-CD was recrystallized from deionized water and dried in vacuum for 5 h at 40 ^{o}C prior to use. EMD-CD was prepared following the reported procedure;[1] the substitution degree (DS) of the ortho ester at 6-OH was determined by comparing the peak intensities of **C1** proton of the CD ring at 4.86 ppm to that of the proton **A** (-CH_3 of F127) at 1.05 ppm **(Figure 1)**. Tetrahydrofuran (THF) and toluene were distilled over sodium prior to use. Dichloromethane and ethanol were distilled over CaH_2 and magnesium, respectively, prior to use. $CDCl_3$ and DMSO-d_6 were treated with anhydrous K_2CO_3 and CaH_2, respectively, for all of the ^{1}H NMR measurements.

NMR Measurements. ^{1}H NMR spectra (400 MHz) of EMD-CD, F127 and the polypseudorotaxanes (**PPR1**-**PPR4**) in DMSO-d_6 were recorded on a Brucker ARX 400 MHz spectrometer. ^{1}H NMR spectra of the PPR aggregates hydrolyzed in deuterated buffers with various pHs for different times were recorded on the Varian Mercury Plus 300 MHz NMR spectrometer.

Preparation of PPRs. Take the host-guest (H/G) system with a feed ratio of $n_{EMD\text{-}CD}$:n_{F127} = 27:1 (**PPR4** in Table 1) as an example. EMD-CD (554 mg, 275 μmol)[2] and F127 (129 mg, 10.2 μmol) were dissolved in 10 mL of ethanol in a flask and stirred at 40 ^{o}C for 12 h. The solvent was then removed by rotary evaporation at reduced pressure to get a semitransparent film at the bottom of the flask. Then, deionized water (50 mL with ~1% TEA, 4 ^{o}C,) was added into the flask which was ultrasonicated for ~5 min to get a turbid dispersion. This

dispersion was incubated at 4 ^{o}C for 24 h, affording a white precipitate. After centrifugation, the upper supernatant was collected and lyophilized to afford a white powder which is mainly the Pluronic F127 as analyzed by ^{1}H NMR in DMSO-d_6 (**Figure S1**). The precipitate was washed with 10 mL of deionized water (~4 ^{o}C) twice and lyophilized, affording a white powder (**PPR4**) in a 76% yield.

The other PPRs (**PPR1**-**PPR3**) were prepared following the same procedure but at different feed ratios. The H/G ratios in the PPRs were determined by comparing the peak intensities of the methyl proton (A) of F127 at 1.05 ppm to that of the **C1** proton of EMD-CD at 4.86 ppm in the ^{1}H NMR spectra (**Figure 1** and **S2**). For the PPRs formed in other organic solvents, the same experimental procedure was applied.

Critical Aggregation Concentration (CAC). CACs of the PPRs (**PPR1**-**PPR3**) were measured by the fluorescence method using pyrene as a probe at 37 ^{o}C. PPR powder (10 mg) was re-dispersed in 10 mM phosphate buffer (PB, pH 8.4,) under sonication and incubated for 12 h at 37 ^{o}C. A series of the PPR dispersions with gradient concentrations were obtained by stepwise dilution of the parent dispersion (**1.0 mg/mL**) using the same PB. 10 μL of pyrene solution in THF (1.0×10^{-4} mol/L) was added into each PPR dispersion (2.0 mL) followed by equilibration at 25 ^{o}C overnight prior to the measurements. The final concentration of pyrene in the dispersion was 5.0×10^{-7} mol/L. A Hitachi F4500 fluorescence spectrometer was used to get the excitation spectra from 300 to 360 nm at 37 ^{o}C by monitoring the fluorescence intensity at 390 nm. I_{338}/I_{333} ratio was calculated by comparing the emission intensities at excitation wavelengths of 338 nm and 333 nm, respectively (**Figuse S6**).

Laser Light Scattering (LLS). Before LLS measurements, the PPR aggregates were obtained by re-dispersing of the PPR powder in PB (pH 8.4, 10 mM) under sonication and equilibration at 37 ^{o}C for 12 h. The bluish dispersion (1.0 mg/mL) was filtered into a dust-free vial through a Millipore PVDF membrane (0.45 μm). Both static light scattering (SLS) and dynamic light scattering (DLS) experiments over a scattering angular range of 20-150^{o} were measured by a commercialized spectrometer (BI-200SM Goniometer, Holtsville, NY). A 17 mW Helium-Neon laser (Newport Corp., CA, USA) operated at 633 nm was used as light

source and a BI-TurboCo Digital Correlator (Brookhaven Instruments Corp.) was used to collect and process the data. The root mean-square radius of gyration (R_g) was obtained by

$$\frac{HC}{R_{vv}(\theta)} = \frac{1}{M_w}\left[1 + \frac{1}{3}R_g^2 q^2\right] + 2A_2C \qquad H = \frac{4\pi^2 n^2 (\frac{dn}{dc})^2}{N_A \lambda^4} \qquad q = \frac{4\pi n}{\lambda}\sin(\frac{\theta}{2})$$

where the Rayleigh ratio $R_{vv}(\theta)$ was measured as the angular dependence of the excess absolute time-averaged scattered intensity in SLS and N_A, n, d_n/d_C, λ and θ was the Avogadro's number, the solvent refractive index, the specific refractive index increment, the wavelength of light in a vacuum, and the scatting angle, respectively. The hydrodynamic radius (R_h) was calculated by using the Stokes-Einstein equation $D = k_B T / 6\pi\eta R_h$ where D was obtained by extrapolating $\overline{\Gamma} / q^2$ to zero angle. Laplace inversion program, CONTIN, was used to obtain Γ at different angles, by normalizing the line width distribution $G(\Gamma)$ obtained from DLS measurements.

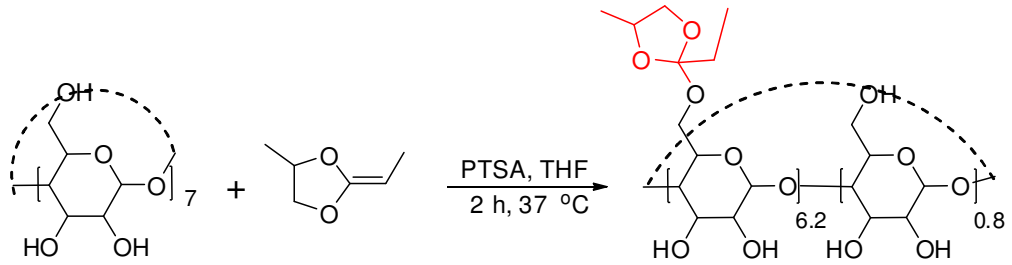
Transmission Electron Microscopy (TEM) and Freeze-Fracture TEM (FF-TEM). The **PPR1-PPR4** aggregate dispersions (**1.0 mg/mL**) were obtained by re-dispersing the PPR powders in deionized water (with ~1% TEA) under sonication and equilibration at 37 ^{o}C for 12 h. The specimens were prepared by dropping 10 μL of the dispersion on one piece of copper mesh. After 60 s, most of the liquid was removed by blotting with a filter paper, and the remnant water on the copper mesh was evaporated at room temperature for 15 min. $UO_2(Ac)_2$ aqueous solution (5 μL, 2 wt%, adjusted to pH 7.4 prior to use) was dropped on the dispersion covered copper mesh to enhance the contrast via negative staining. After 90 s, most of the staining liquor was removed by blotting with a filter paper and the specimen was dried at room temperature for 12 h. The TEM equipment (JEOL JEM-2100) with an acceleration voltage of 200 kV was used to get the TEM images.

For FF-TEM of the **PPR3** aggregate, one drop of the dispersion (1.0 mg/mL in water with ~1% TEA) was added on a piece of copper mesh and covered quickly with another copper mesh, then followed by quickly immersing in liquid propane. A freeze-fracture apparatus (Balzers BAF400, Germany) was used to fracture and replicate the sample at -140

^{o}C. After Pt/C was deposited on the sample fracture surface, the organic components were dissolved by acetone. The Pt/C replica was moved onto a fresh copper mesh and observed by the aforementioned TEM equipment with an acceleration voltage of 200 kV.

Acid-Triggered Dissociation Monitored by LLS. The scattered intensity *vs* time plots of the **PPR3** dispersions (1.0 mg/mL) were measured at different pHs on a particle size analyzer (ZETA PALS, Brookhaven Instruments Corp.) equipped with a temperature controller and a 35 mW He-Ne solid-state laser (λ = 660 nm, detection angle: 90^{o})**.** pH of the **PPR3** dispersion was adjusted to 7.4 by adding 2.0 M PB (pH = 7.4). The dispersion was thoroughly mixed by shaking for 20 s; the scattered intensity was measured at 37 ^{o}C and used as for 0 h time point. The normalized intensity (%) was defined as I_t/I_0, where I_t and I_0 denotes the scattering intensity at a specific hydrolysis time and at the 0 h time point, respectively. Acetate buffer (5.0 M, pH = 5.0) was used to adjust pH of the dispersion to 5.0. All dispersions and the buffers were filtered through a Millipore PVDF membrane (0.45 μm) before the measurements.

pH-Responsive Hydrolysis of Ortho Ester Monitored by ^{1}H NMR. The white PPR powder as prepared by the aforementioned procedure was dispersed in deuterated PB (1.0 mM, pD 8.4) to afford stable dispersion (20 mg/mL) under ultrasonication for 10 min. 0.8 mL of the dispersion was charged into a NMR tube, and the ^{1}H NMR spectrum was measured and used as for 0 time point. After adding 10 μL of 0.5 M deuterated buffer (pH 7.4), the dispersion was quickly mixed and the ^{1}H NMR spectra were recorded at desired time points (**Figure S9**). For the hydrolysis experiments at pD 6.5 or 5.6, the same procedure was applied.



Scheme S1. Synthesis of EMD-CD. PTSA: *p*-toluenesulfonic acid.

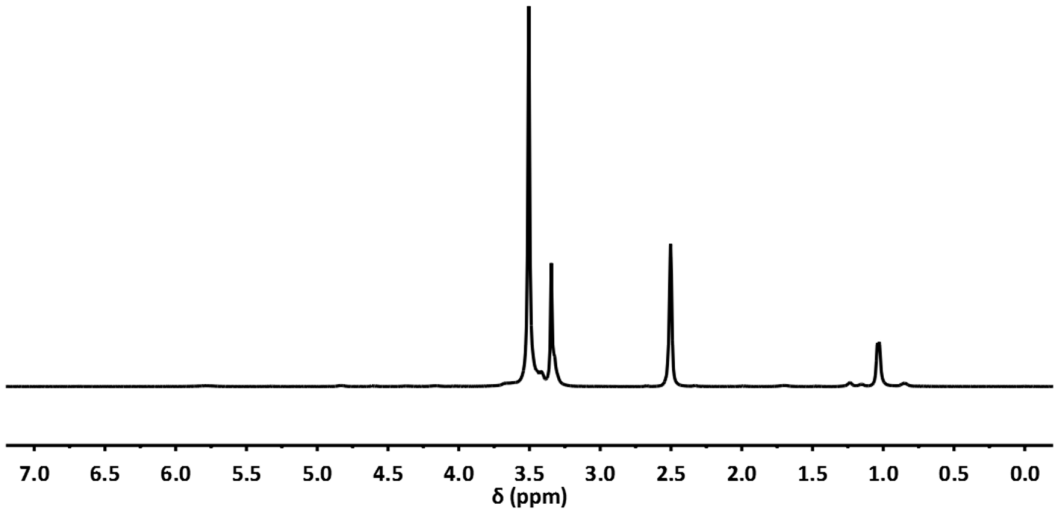
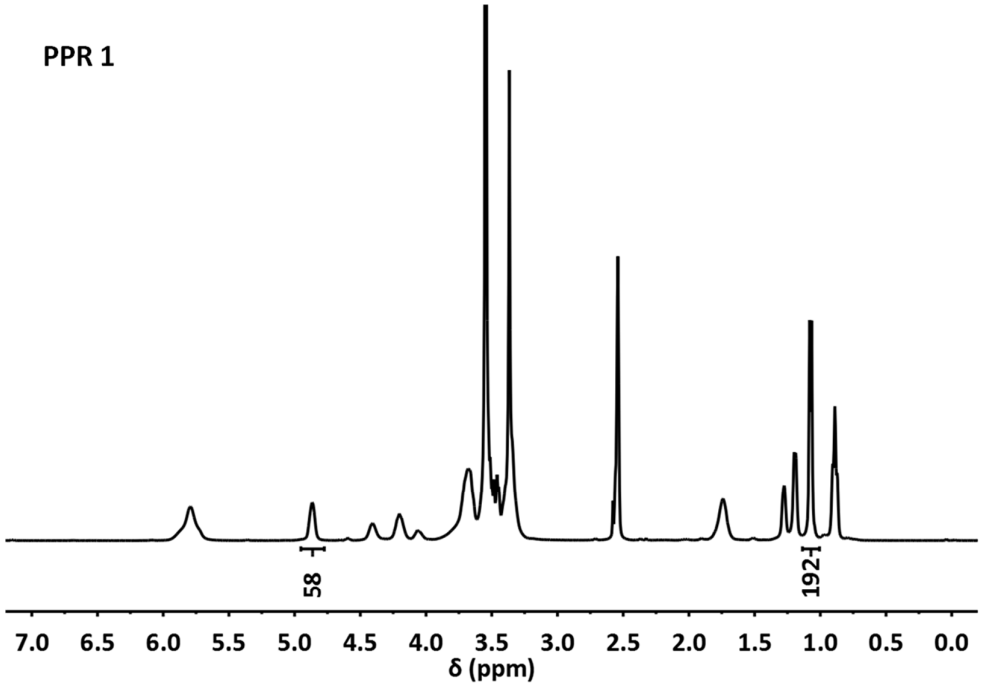
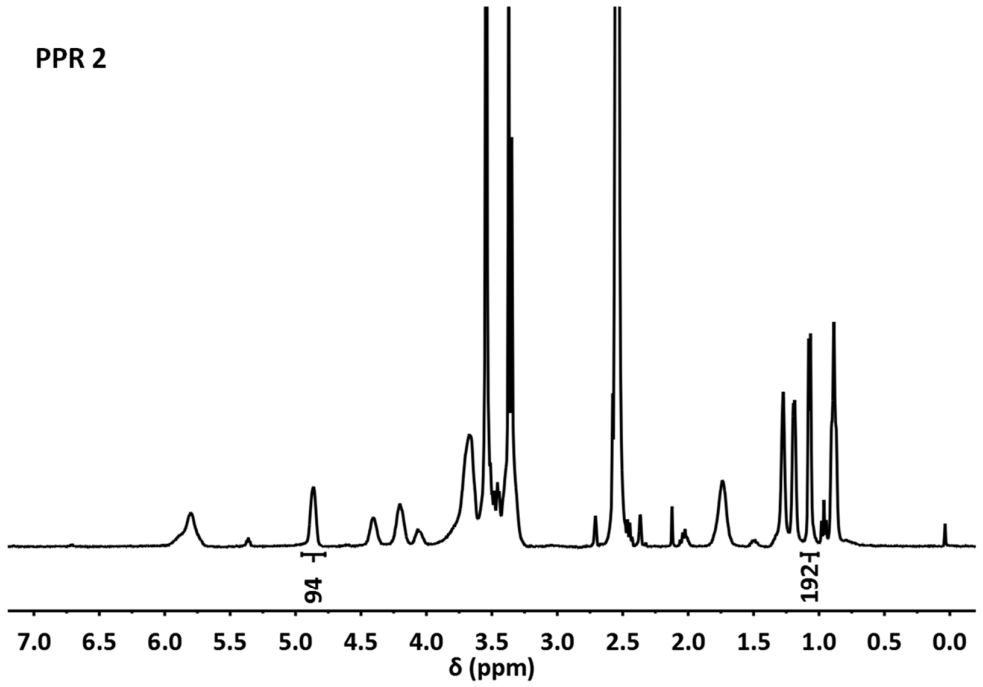


Figure S1. ^{1}H NMR spectrum of residual compounds in the supernatant (for the preparation of **PPR4**) after lyophilization. Solvent: DMSO-d_6. Pluronic F127 is the main component with little EMD-CD.


PPR 1
58
192
7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
δ (ppm)


PPR 2
94
192
7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
δ (ppm)

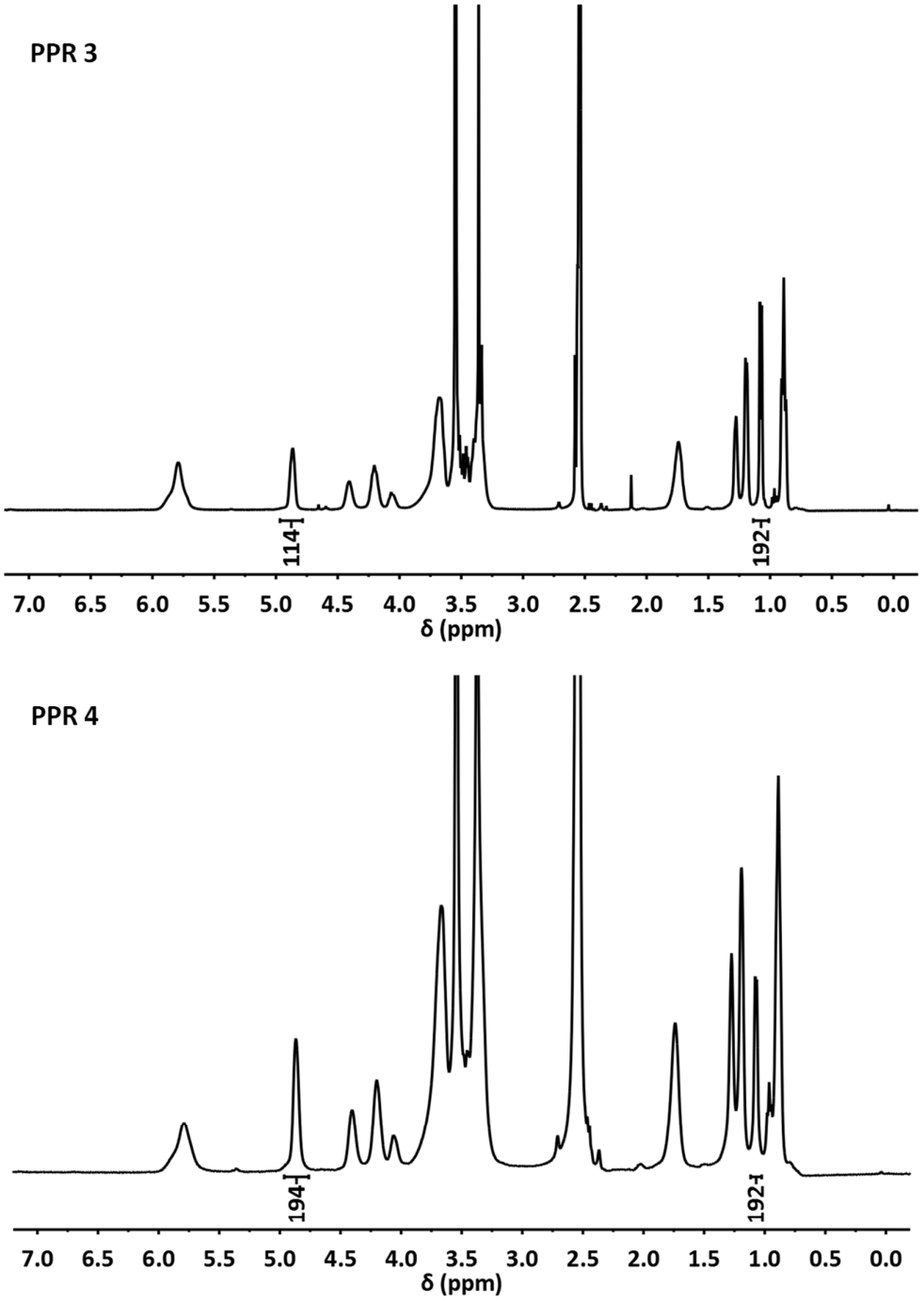


Figure S2. ^{1}H NMR spectra of the PPRs with different EMD-CD/F127 molar ratios (**PPR1-PPR4** in DMSO-d_6). n_H:n_G ratio = $I_{4.86}$/7, assuming that each F127 chain has 64 PO units. The PPRs were completely dissociated in DMSO.

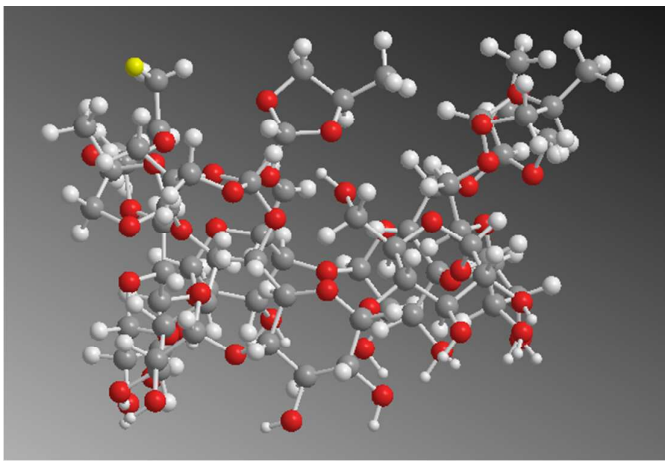


Figure S3. 3D Molecular structure of EMD-CD simulated via MM2 method.

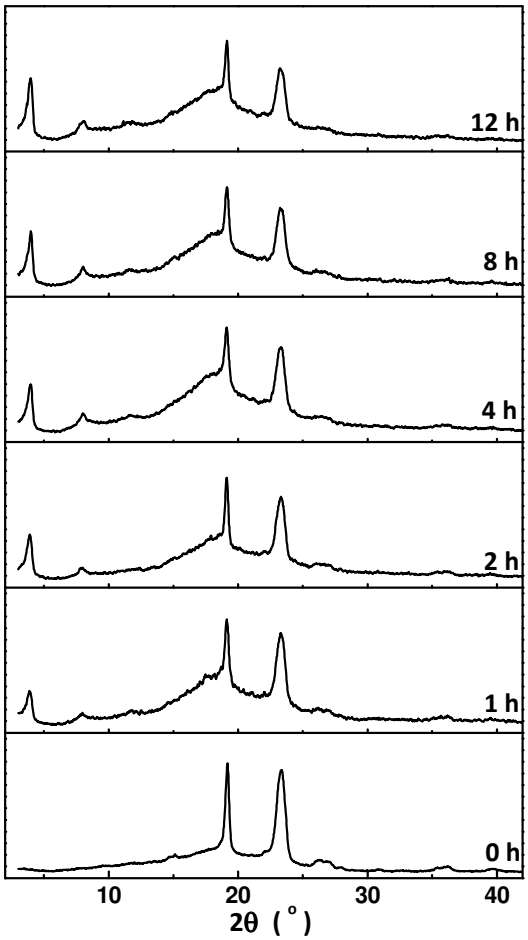


Figure S4. WAXD patterns of the PPRs prepared in ethanol at different threading times. The patterns are normalized to the peaks of PEO.

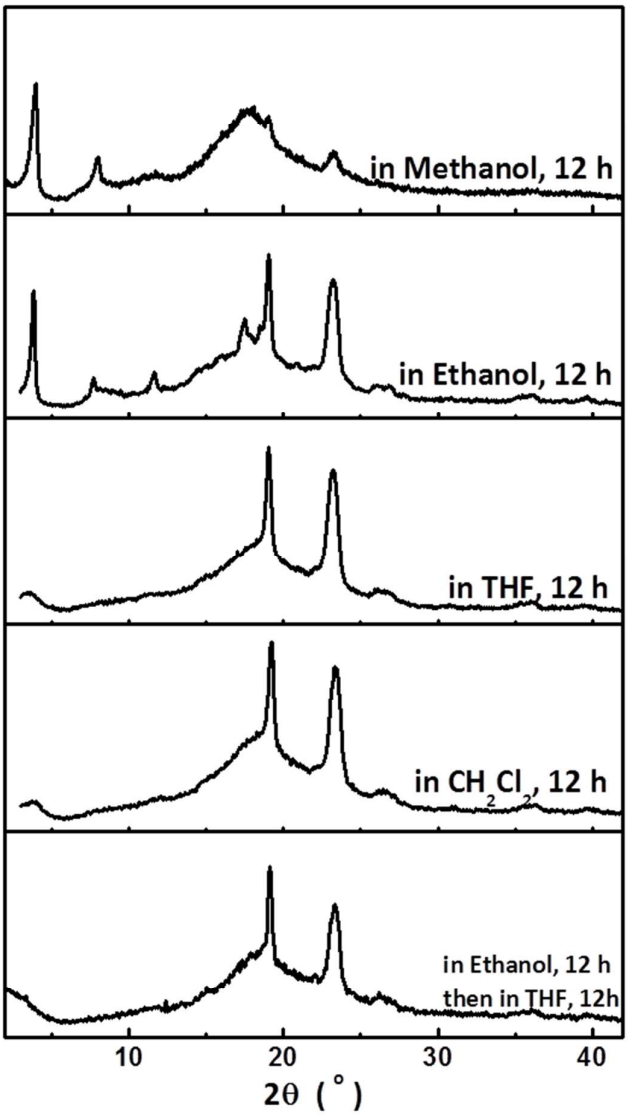


Figure S5. WAXD spectra of the PPRs prepared in different solvents. Inclusion time: 12 h.

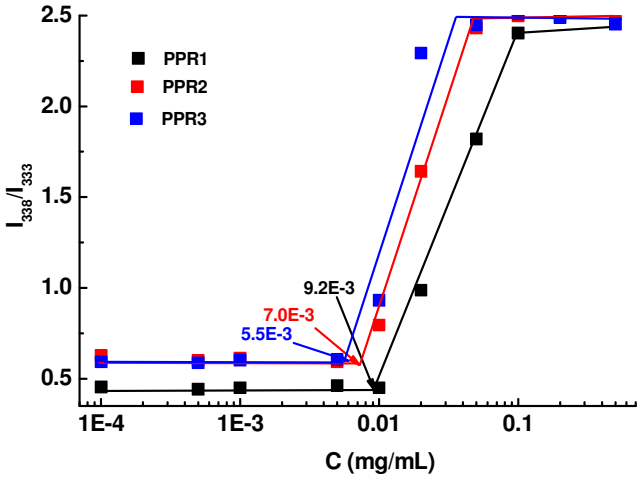


Figure S6. Concentration dependence of I_{338}/I_{333} ratio of pyrene in **PPR1**-**PPR3** aqueous dispersions (PB, 10 mM, pH 8.4). 37 ^{o}C; concentration of pyrene: 5.0×10^{-7} mol/L.

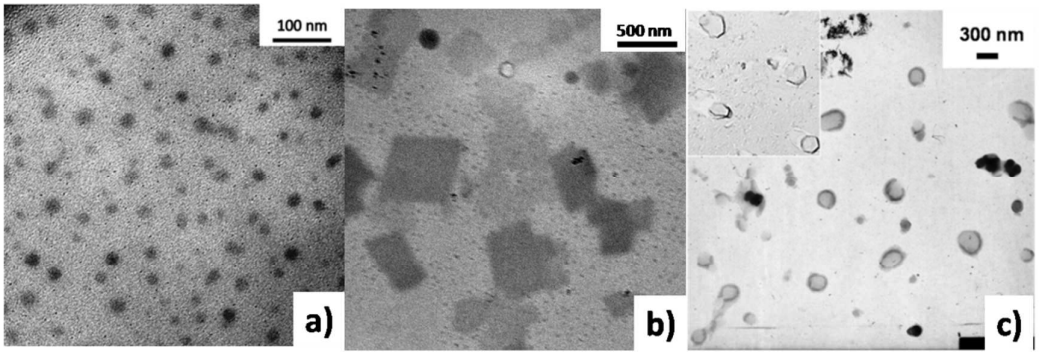


Figure S7. TEM images of (a) F127 micelle, (b) **PPR1** sheet-like aggregate, and (c) **PPR3** vesicular nanoparticle (inset: FF-TEM of **PPR3** vesicle) without staining. Concentration of the F127 micelle or the PPRs was 1.0 mg/mL in PB (10 mM, pH 8.4), 37 ^{o}C.

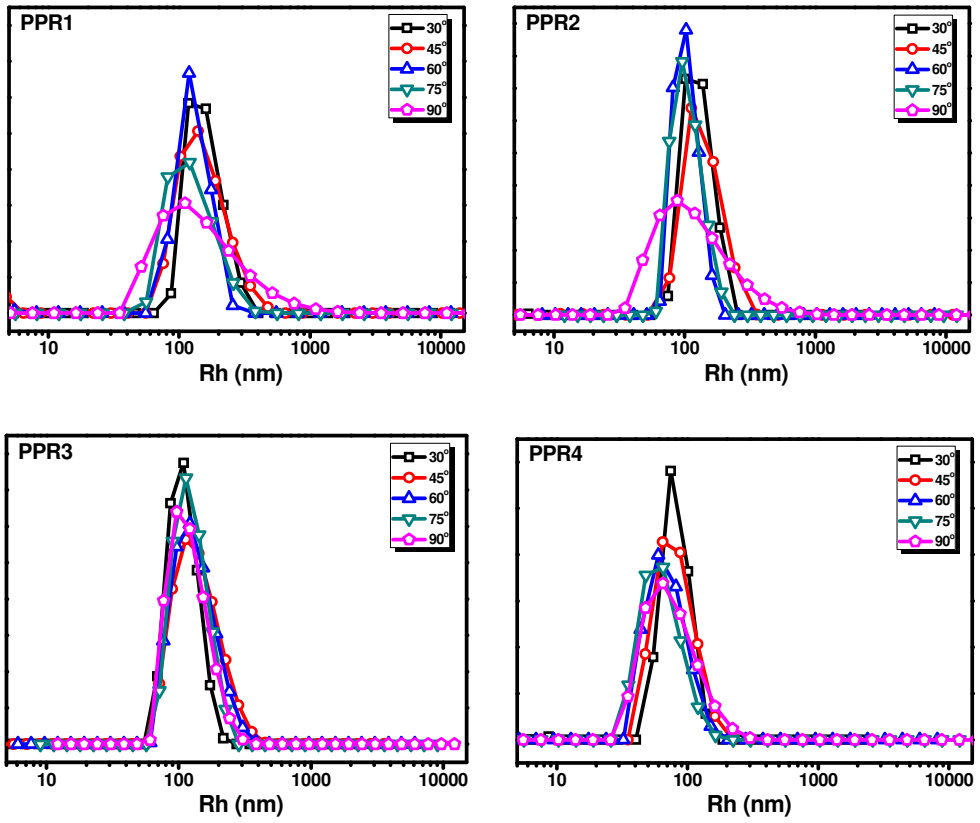


Figure S8. CONTIN analysis of the **PPR1**-**PPR4** dispersions (PB, 10 mM, pH 8.4) at 37 ^{o}C. Concentration of PPR: 1.0 mg/mL.

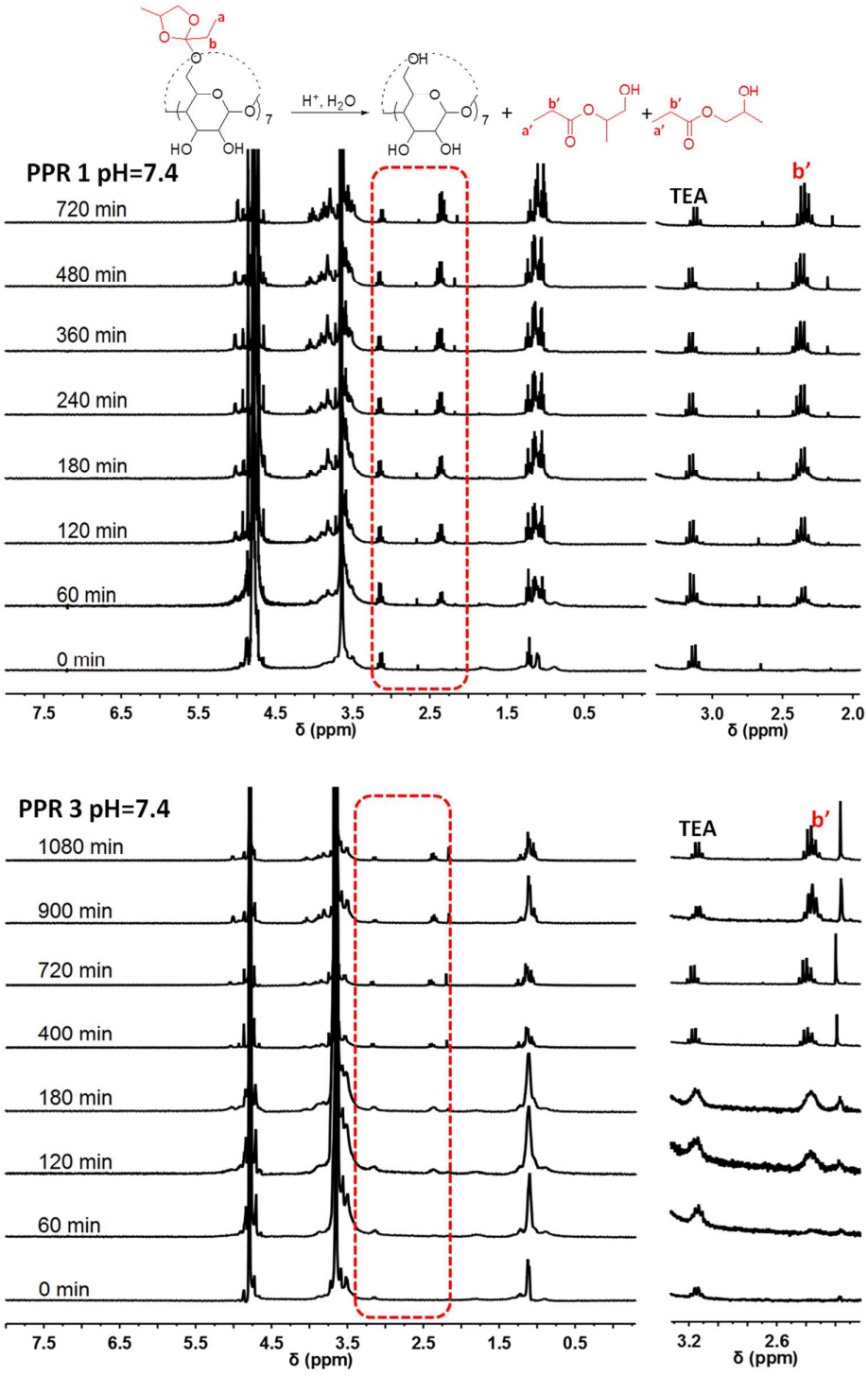


Figure S9. ^{1}H NMR spectra of **PPR1** and **PPR3** in deuterated PB (pH = 7.4) at different incubation times (PPR concentration: ~5 mg/mL; 37 ^{o}C). The remained amount of ortho ester was determined by comparing the peak intensities at 2.23 ppm at specific time to that of the fully hydrolyzed sample at 24 h. The peak at 3.15 ppm (-CH_3 of TEA) was used as a standard.

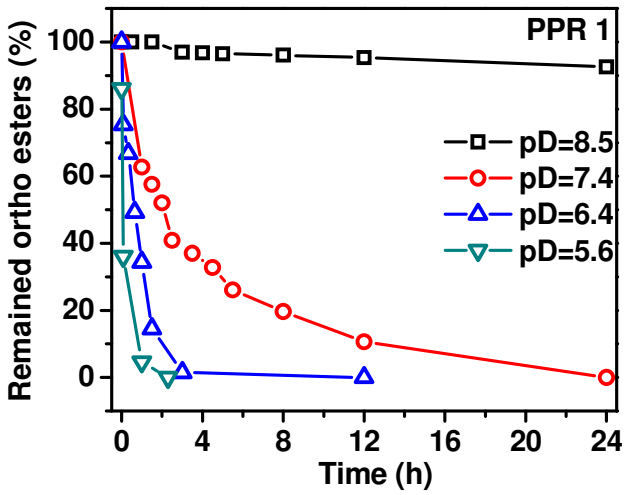


Figure S10. Effect of pH on hydrolytic kinetics of the ortho ester groups of **PPR 1**.

References and notes

1. R. Ji, J. Cheng, T. Yang, C. C. Song, L. Li, F.-S. Du and Z.-C. Li, *Biomacromolecules*, 2014, **15**, 3531-3539.

2. EMD sample contains ~7% organic solvents as determined by ^{1}H NMR spectroscopy.