Supporting Information

Supramolecular Hydrogels Formed by Pyrene-Terminated Poly(ethylene glycol) Star Polymers through Inclusion Complexation of Pyrene Dimer with γ-Cyclodextrin

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1. Experimental Section

Materials

PEG star polymers, PEG-8A(10k) and PEG-8A(20k) (hexaglycerol poly(ethylene glycol) ether, molecular weight 10 and 20 kDa, respectively) were obtained from NOF company (Japan). 1-Pyrenemethylamine chloride (95%) was purchased from Aldrich. 1,1’-carbonyldiimidazole (CDI) was obtained from Aldrich. Triethylamine (99%) was purchased from Lancaster. Diethyl ether, methanol, and tetrahydrofuran (THF) were purchased from Merck. α-CD, β-CD, and γ-CD were supplied by TCI (Tokyo, Japan). DMSO-\textsubscript{d}_6 (99.9%) used as solvent in the NMR measurements was obtained from Aldrich. THF was distilled under CaH\textsubscript{2} to remove traces of water. Anhydrous DMF from Aldrich was used as received.

Synthesis of Pyrene-Terminated PEG Star Polymers

PEG-8A-Py(20k). PEG-8A(20k) (1.0 g, 0.050 mmol) was dehydrated at 100 °C in vacuo overnight. In a 150 ml 3-neck flask fitted with a funnel, CDI (1.3 g, 8.0 mmol) was dissolved in 40 ml of anhydrous THF. The dehydrated PEG-8A(20k) was dissolved in 50 ml of anhydrous THF and added dropwise to the CDI solution with stirring in N\textsubscript{2} environment. The mixture was stirred at room temperature for 12 hours, followed by precipitation from 900 ml of diethyl ether. The precipitate was centrifuged and used for next reaction without drying.

1-Pyrenemethylamine chloride (2.0 g, 7.1 mmol) was dissolved in 20 ml of methanol, and heated at 50 °C for 2 h after adding triethylamine (2.1 g, 21 mmol). The resulting mixture was precipitated in cold water. The precipitate was centrifuged and lyophilized to give 1-pyrenemethylamine.

In a 100-ml flask fitted with a pressure equalizing funnel, 1-pyrenemethylamine (0.28 g, 1.2 mmol) was dissolved in 40 ml of DMF. The activated PEG-8A(20k) (1.08 g, 0.05mmol) was dissolved in 30 ml of DMF and added slowly to the solution of 1-pyrenemethylamine with stirring in N\textsubscript{2} atmosphere. The mixture was stirred at room temperature for 12 hours, and heated at 70 °C for
another 6 hours. The light yellow mixture was precipitated in 800 ml of diethyl ether. The precipitate was dissolved in methanol, and further purified by column chromatography with Sephadex LH-20. The pyrene-terminated PEG star polymer PEG-8A-Py(20k) was obtained as light yellow waxy powder (0.82 g, yield 75%). ¹H NMR (400 MHz, DMSO-d₆, 22 °C): δ=7.93-8.48 (m, ca. 58H, CH of pyrene, d of PEG-8A-Py(20k)), 4.88-4.98 (d, ca. 13H, pyrene-CH₂-NH, c of PEG-8A-Py(20k)), 4.05-4.15 (s, ca. 15H, pyrene-CH₂-NH-COO-CH₂, b of PEG-8A-Py(20k)), 3.49 (s, ca. 1823H, OCH₂CH₂, a of PEG-8A-Py(20k)). Anal. Calcd for C₁₀₅₂H₁₉₀₄O₄₆₄N₈: C, 57.20; H, 8.67; N, 0.51. Found: C, 57.05; H, 8.98; N, 0.52.

PEG-8A-Py(10k). PEG-8A-Py(10k) was prepared from PEG-8A(10k) (1.0 g, 0.10 mmol), CDI (2.6 g, 16.0 mmol), and 1-pyrenemethylamine (0.56 g, 2.4 mmol) in similar procedures. Yield, 0.86 g, 73%. ¹H NMR (400 MHz, DMSO-d₆, 22 °C): δ=7.93-8.48 (m, ca. 61H, CH of pyrene, d of PEG-8A-Py(10k)), 4.88-4.98 (d, ca. 14H, pyrene-CH₂-NH, c of PEG-8A-Py(10k)), 4.05-4.15 (s, ca. 15H, pyrene-CH₂-NH-COO-CH₂, b of PEG-8A-Py(10k)), 3.49 (s, ca. 909H, OCH₂CH₂, a of PEG-8A-Py(10k)). Anal. Calcd for C₅₉₈H₉₅₄O₂₃₂N₈: C, 59.48; H, 8.33; N, 0.93. Found: C, 58.37; H, 8.56; N, 1.05.

Measurements and Characterizations

¹H NMR spectra were recorded on a Bruker AV-400 NMR spectrometer (400 MHz) at room temperature. Chemical shift of polymers were referenced to δ=2.5 ppm for DMSO.

Gel permeation chromatography (GPC) tests were conducted with a Shimadzu SCL-10A and LC-8A system with two Phenolgel columns (50 and 1000 Å) in series and a refractive index detector. THF was used as eluent at a rate of 0.30 mL/min at 40 °C. Monodispersed poly(ethylene glycol) standards were used to obtain a calibration curve.

Fluorescence measurements were carried out on a Shimadzu RF-5301PC spectrofluorometer. The excitation wavelength was set to 340 nm. All measurements were performed at 25 °C.
Absorption spectra were recorded on a Shimadzu UV-2501PC UV-vis spectrophotometer. The absorption values were recorded at 340 nm at 298 K.

Rheological tests were performed on a Brookfield DV-III Rheometer with a sample adaptor SSA15/7R and a temperature controlling unit at 25 ºC. Measurements were carried out at a shear rate of 3 rpm. Concentration of polymers were set to 15 wt % of the whole mixture, while α, β, γ-CD concentrations were adjusted accordingly.

2. Synthesis of Pyrene-Terminated PEG Star Polymers

The pyrene-terminated PEG star polymers were prepared from 8-armed PEG star polymers. The hydroxy ends of the PEG star polymers were converted into pyrene ends according to the procedures shown in Scheme S1. The purity and molecular structures of the resultant pyrene-terminated PEG star polymers were confirmed by 1H NMR spectroscopy and elemental analysis. As an example, Figure S1 shows the 1H NMR spectrum of PEG-8A-Py(20). All protons of the PEG core segments, ethylene oxide unit next to pyrene, the methylene group attached to pyrene, and the aromatic protons of pyrene were observed. The ratio of the integral intensity of the peaks showed that ca. 81% of hydroxy end groups were converted to pyrene ends. GPC results also showed that the resultant pyrene-terminated PEG star polymers have larger molecular size than their respective starting star polymers.
Scheme S1. Synthesis of pyrene-terminated PEG star polymers

Figure S1. $^1$H NMR of PEG-8A-Py(20k) in DMSO-$d_6$. 
3. Viscosity Changes of PEG-8A-Py(10k) upon Addition of α-, β-, and γ-CDs

The hydrogels and the gelation processes were further studied by viscosity measurements. Figure S2 shows the viscosity of PEG-8A-Py(10k) aqueous solution and its viscosity changes upon addition of α-CD, β-CD, and γ-CD, respectively. All four samples, the pure PEG-8A-Py(10k) and its mixtures with α-CD, β-CD, and γ-CD, contained the same concentration of PEG-8A-Py(10k) (15 wt %). The molar ratio of pyrene terminal to CD in the mixtures was also kept at 2:1, or the CD concentration was kept at 76.0 mM. The results showed that the addition of α-CD did not change the viscosity, while β-CD slightly lowered the viscosity of PEG-8A-Py(10k). These results are in accordance with the observation that α-CD does not interact with any part of PEG-8A-Py(10k), while β-CD can include a single pyrene moiety into its cavity, and then weaken the hydrophobic interaction between pyrene moieties. However, the addition of γ-CD resulted in a huge change in viscosity of the PEG-8A-Py(10k) solution because of the formation of the supramolecular hydrogel.

![Figure S2](image-url)