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Supporting Information

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Host-Guest Interaction Mediated Polymeric Core-Shell Assemblies: Versatile Nanocarriers for Drug Delivery

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Experimental Section

Materials

L-Aspartic acid β-benzyl ester was purchased from Sigma (St. Louis, USA). Triphosgene was obtained from Fisher (USA). β-Benzyl-L-aspartate N-carboxyanhydride (BLA-NCA) was synthesized according to literature.[1] α-Methoxy-ω-amino-polyethylene glycol (MPEG-NH_2) with average molecular weight (M_W) of 5000 was purchased from Laysan Bio, Inc. (Alabama, USA), and used without further purification. Ethylenediamine (EDA) was purchased from Sigma (St. Louis, USA) and distilled over CaH_2 under decreased pressure. Pyrene (≥99%) and β-cyclodextrin (β-CD, ≥98%) were purchased from Sigma-Aldrich Co. (USA) and used as received. The method established by Baussanne et al. was employed to synthesize 6-monotosyl β-CD.[2] Coumarin 102 was obtained from Acros Organics (New Jersey, USA). 1,6-Diphenyl-1,3,5-hexatriene (DPH), poly(D,L-lactide) (PDLLA) with inherent viscosity 0.55-0.75 dL/g and branched polyethylenimine (PEI) with M_W of 25, 000 were purchased from Sigma (St. Louis, USA). Adamantane-1-carboxylic acid (ADCA, 97%) was purchased from Maybridge Trevillett (Tintagel, England).

Synthesis of PEG-*b*-polyaspartamide containing EDA unit (PEG-PEDA)

The PEG-block-poly(β-benzyl L-aspartate) (PEG-*b*-PBLA) copolymer was synthesized as reported by Harada et al.[3] Briefly, BLA-NCA was polymerized in DMF at 40 ºC by the initiation from the terminal primary amino group of MPEG-NH_2 to obtain PEG-*b*-PBLA. The degree of polymerization (DP) of PBLA was calculated to be 15 based on ^{1}H NMR spectroscopy. PEG-*b*-PEDA was prepared through the quantitative aminolysis reaction of PEG-*b*-PBLA in dry DMF at 40 ºC in the presence of 50-fold the molar concentration of EDA.[4] After 48 h, the reaction mixture was dialyzed against deionized water (MWCO: 3500), and the final aqueous solution was lyophilized to obtain white powder. Based on ^{1}H NMR determination, the DP of PEDA block is 12.

Synthesis of PEG-*b*-PCD

The β-CD containing copolymer (**2**) was synthesized by a nucleophilic reaction. Briefly, lyophilized PEG-*b*-PEDA (600 mg) and 5 fold excess amount of 6-monotosyl β-CD were reacted in 30 ml anhydrous DMSO. After 5 days, the reaction mixture was dialyzed against 0.1 N NaOH for 2 days to remove unreacted 6-monotosyl β-CD, and then dialyzed against distilled water for 2 days. After filtered through a 0.22 μm syringe filter, the resultant aqueous solution was lyophilized to obtained brown powder.

Synthesis of poly(β-benzyl L-aspartate) (PBLA)

PBLA was synthesized according to a reference.[5] Briefly, 1.5 g BLA-NCA was dissolved in 30 ml anhydrous dioxane at -30ºC, into which appropriate amount of n-hexylamine was added to achieve a

molar ratio of monomer to initiator of 20:1. Polymerization was performed at room temperature (22ºC) for 5 days. After precipitated from diethyl ether, the polymer was dissolved in dichloromethane and precipitated from diethyl ether again. The resultant powder was dried under vacuum. The number-average molecular weight determined by Matrix Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) mass spectrometer is about 2000.

Preparation of host-guest assemblies based on PEG-*b*-PCD

PEG-*b*-PCD based polymeric assemblies containing small molecules including pyrene, coumarin 102 and indomethacin (IND) were prepared by dialysis method. Briefly, mixtures of a small molecule substance and the copolymer with a certain weight ratio were co-dissolved in dimethylsulfoxide (DMSO) at 50ºC with a final polymer concentration of 10 mg/ml. This solution was placed into a dialysis tubing (MWCO 6-8 kDa) for dialysis against deionized water for 24 h at 25ºC. The outer aqueous solution was renewed every 30 min for the first 2 h, and then every 2 h for the remaining period of time. After filtering through a 0.45 μm syringe filter, the dialysis solution was adopted for further experiments. The weight content of pyrene or coumarin 102 was quantified by UV-Vis measurement.

For assemblies based on PEG-*b*-PCD and a hydrophobic polymer, the same procedure was employed. In brief, PEG-*b*-PCD, PBLA, or PDLLA was separately dissolved in DMSO at 50ºC. Mixture solution with appropriate weight ratio of PEG-*b*-PCD/PBLA or PEG-*b*-PCD/PDLLA was dialyzed against deionized water for one day. Further characterizations were performed after the dialysis solution was filtered through a 0.45 μm syringe filter.

For the preparation of polyion complex (PIC) like assemblies, PEG-*b*-PCD and ADCA (2 fold in excess of that of β-CD group) were co-dissolved in 0.05 M NaOH aqueous solution, which was dialyzed (MWCO: 6-8 kDa) against deionized water for 1 day and then lyophilized. The dried product (8.0 mg) was dissolved in deionized water (4.0 ml), into which 5.0 mg PEI in 1.0 ml water was added dropwise under sonication. Solution thus obtained was subject to dialysis against deionized water for 1 day. Further measurements were performed after the dialysate was filtered through a 0.22 μm syringe filter.

In vitro release study

A certain amount of lyophilized IND-containing assemblies was dissolved into deionized water, and placed into dialysis tubing, which was immerged into 30 ml PBS (0.1M, pH 7.4) or PBS containing 0.05 M ADCA. At predetermined time intervals, 4.0 ml release medium was withdrawn, and fresh PBS was added. IND concentration in the release buffer was determined using UV at 280 nm.

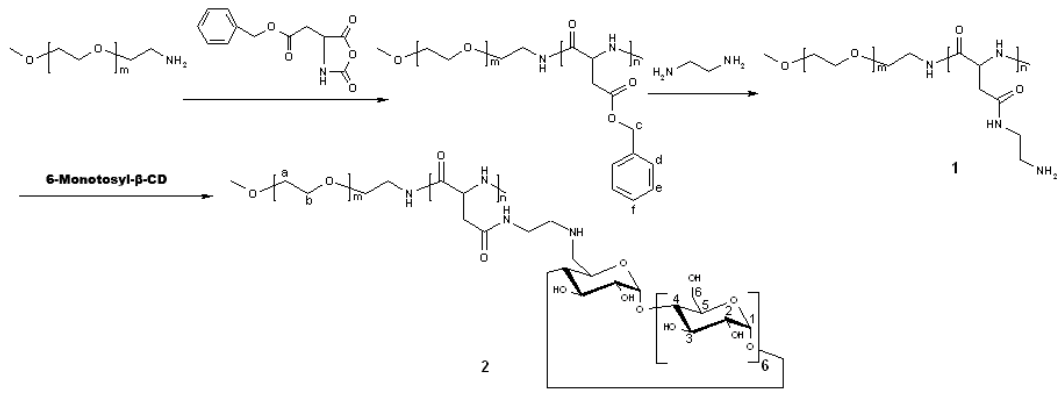
Measurements

^{1}H and ^{13}C NMR spectra were recorded on a Varian INOVA-400 spectrometer operating at 400 MHz. The MALDI-TOF mass spectrum of PBLA was acquired with a Waters Micromass TofSpec-2E run in linear mode. Dithranol (purchased from Aldrich Chemical) was used as a matrix, and tetrahydrofuran as a solvent for both matrix and polymer. The dried-droplet method was employed in sample preparation.[6] Using pyrene as fluorophore, steady-state fluorescence spectra were measured on JASCO FP-6200 fluorescence spectrophotometer with a slit width of 5 nm for both excitation and emission. All spectra were run on air-equilibrated solutions. For fluorescence emission spectra, excitation wavelength was set at 339 nm, and for excitation spectra, the emission wavelength was 390 nm. The scanning rate was set at 125 nm/min. All tests were carried out at 25ºC. Sample solutions were prepared as described previously.[7] In brief, the aqueous copolymer solutions containing pyrene (6.0×10^{-7} M) were incubated at 50ºC for 12 h and subsequently allowed to cool overnight to room temperature. UV measurements were performed on a UV-Visible Spectrophotometer (JASCO V630). The wavelength was set at 339 and 389 nm for pyrene and coumarin 102 respectively.

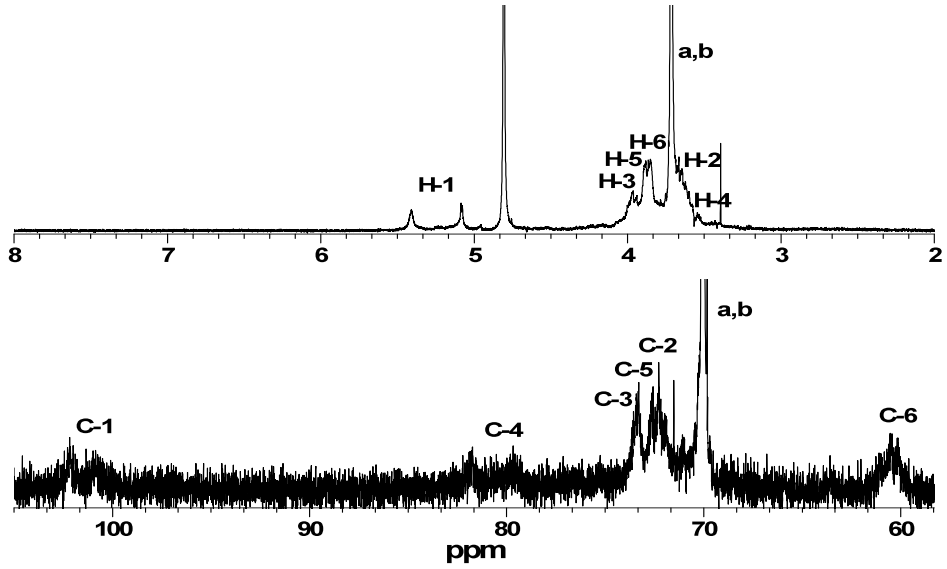
Fluorescence anisotropy (r) was determined with a Fluoromax-2 fluorimeter equipped with an auto-polarizer accessory. The monochromator slits were set at 5.0 nm. 1,6-Diphenyl-1,3,5-hexatriene (DPH) was used as fluorescence probe. The excitation wavelength was 360 nm, while the emission wavelength was 430 nm. The fluorescence anisotropy was calculated according to the relationship $r=(I_{VV}-GI_{VH})/(I_{VV}+2GI_{VH})$, where $G=I_{VH}/I_{HH}$ is an instrumental correction factor and I_{VV}, I_{VH}, I_{HV}, and I_{HH} refer to the resultant emission intensities polarized in the vertical or horizontal detection planes (second subindex) upon excitation with either vertically or horizontally polarized light (first subindex).[8] To prepare sample solutions, a known amount of DPH in methanol was added to 10.0 ml volumetric flasks and the methanol was evaporated. To each flask was then added a stock sample solution, which was heated at 50ºC for 12 h and cooled overnight to room temperature. The DPH concentration was kept at 1.0×10^{-6} M.

DLS measurements for the assemblies in aqueous solution were performed with a Malvern Zetasizer Nano ZS instrument at 25ºC. Atomic force microscopy (AFM) observation was carried out on a NanoScope IIIa-Phase Atomic Force Microscope connected to a NanoScope IIIa Controller using an EV scanner. Samples were prepared by drop-casting the dilute solution onto freshly cleaved mica. All the images were acquired under a tapping mode. Transmission electron microscopy (TEM) observation was carried out on a JEOL-3011 high resolution electron microscope operating at an acceleration voltage of 300 kV. Samples were prepared at 25ºC by dipping the grid into the aqueous solution of assemblies, and extra solution was blotted with filter paper. After the water was evaporated at room temperature for several days, samples were observed directly without any staining. Formvar coated copper grids, stabilized with evaporated carbon film, were used. SEM images were taken on a Field Emission Scanning Electron Microscope (XL30 FEG, Phillips) after a gold layer was coated using a sputter coater (Desk-II,

Denton vacuum Inc., Moorstown, NJ) for 60 s. Samples were prepared by coating aqueous solution of assemblies onto freshly cleaved mica, and water was evaporated at room temperature under normal pressure.



(a)



(b)

Figure S1. (a) Synthesis of PEG-*b*-PCD; (b) ^{1}H NMR spectrum in D_2O (top) and ^{13}C NMR spectrum in DMSO-d_6 (bottom) of PEG-*b*-PCD.

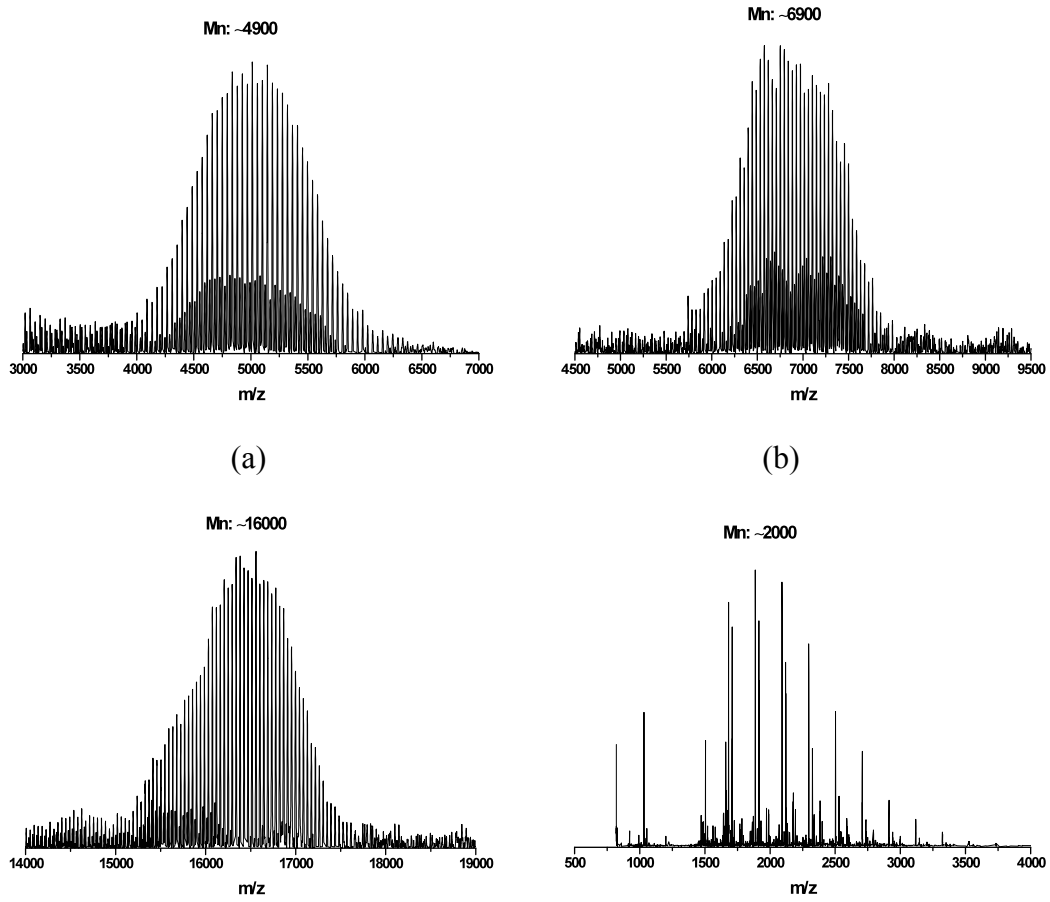


Figure S2. MAIDI-TOF spectra of (a) MPEG-NH_2, (b) PEG-*b*-PEDA, (c) PEG-*b*-PCD, and (d) PBLA.

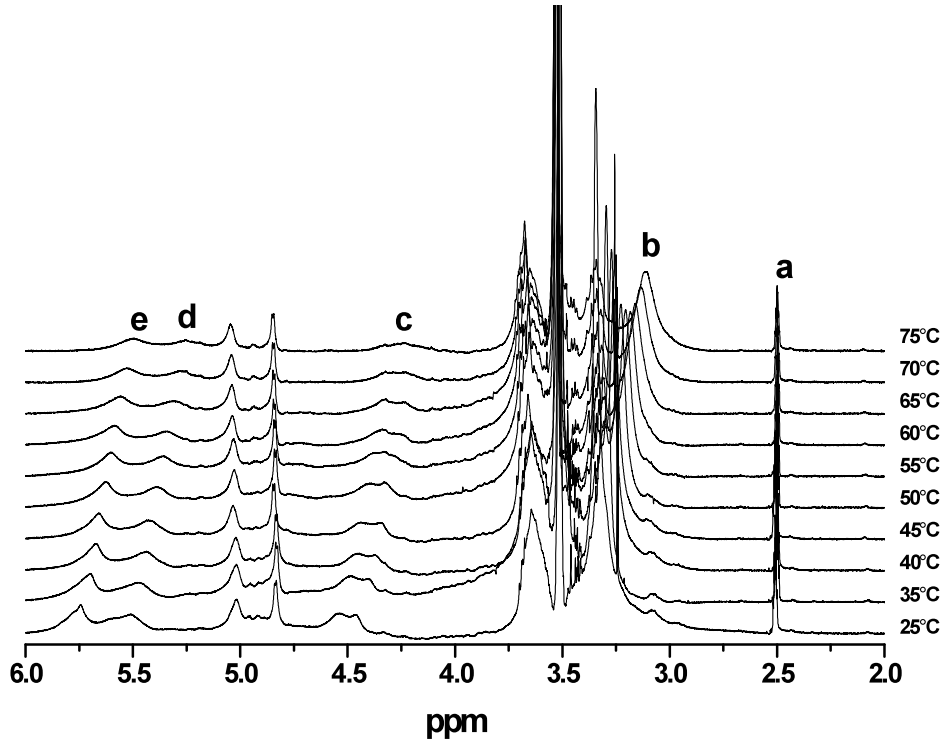
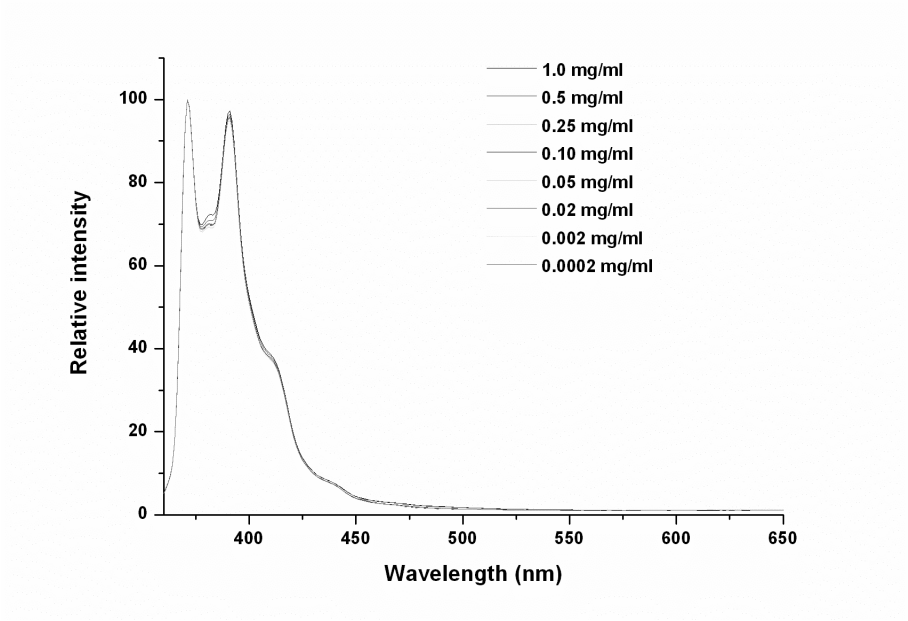
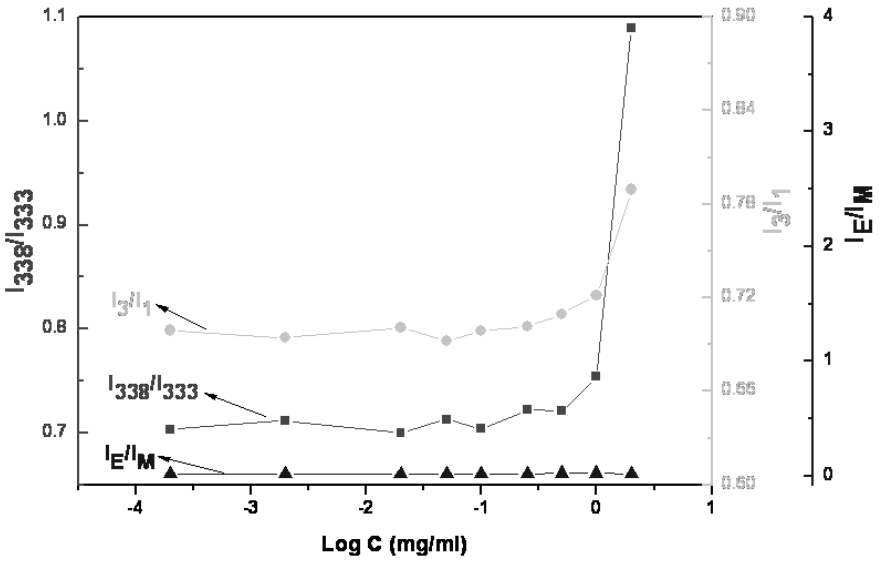


Figure S3. ^{1}H NMR spectra of PEG-*b*-PCD in DMSO-d_6 measured at various temperatures. Note the temperature dependent chemical shift of peaks corresponding to H_2O and hydroxyl groups in β-CD, which is a well documented phenomenon. Chemical shift was referenced according to DMSO peak at 2.5 ppm. For sample preparation, PEG-*b*-PCD was first dissolved in DMSO-d_6 by heating to 60ºC. After cooled to room temperature (about 23ºC), spectra at various temperatures were acquired. Assignments of selected peaks as labeled in the figure: **a**, residual solvent (DMSO) peak; **b**, trace of H_2O in DMSO-d_6; **c**, proton signal from OH group connected with **C-6** of cyclodextrin as shown in Figure 1Sb; **d**, proton signal from OH group connected with **C-3**; **e**, proton signal from -OH group connected with **C-2**.



(a)



(b)

Figure S4. Normalized emission spectra of pyrene in aqueous solutions containing various concentrations of β-CD. (a), emission spectra with an excitation wavelength at 339 nm; (b), plots of I_{338}/I_{333}, I_3/I_1 and I_E/I_M as a function of β-CD concentration. I_{338}/I_{333} -- the intensity ratio of the (0, 0) band in pyrene excitation spectrum; I_3/I_1 -- the intensity ratio between the third and first vibrational bands in pyrene emission spectrum; I_E/I_M -- the intensity ratio of the excimer (475 nm) to monomer (371 nm) in emission spectrum. [Pyrene]=6.0×10^{-7} M.

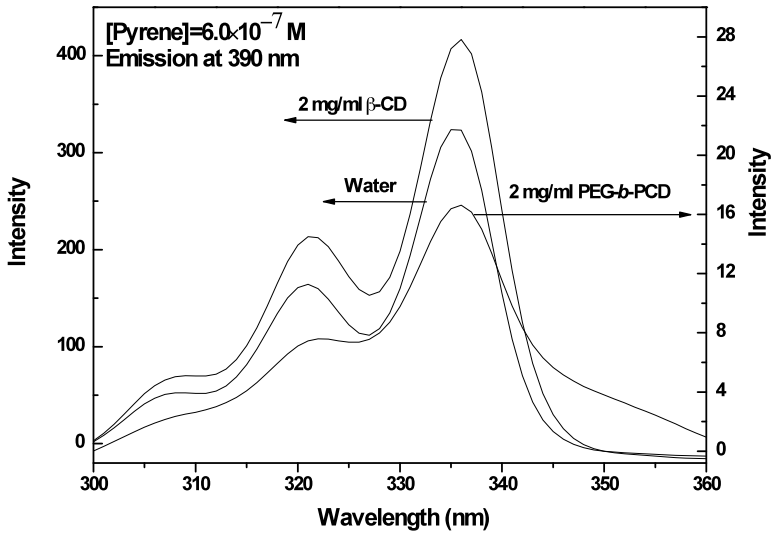


Figure S5. Excitation spectra of pyrene in aqueous solutions containing various substances. Introduction of β-CD leads to an enhancement of fluorescence intensity of pyrene in aqueous solution, while the shape of excitation spectrum is scarcely altered by the β-CD. On the other hand, the addition of PEG-*b*-CD substantially reduces the fluorescence intensity of pyrene and alters the shape of the excitation spectrum; the spectrum becomes broad and the excitation edge shifts towards long wavelength. The increase in excitation intensity when CD is added is probably due to the dissolution of pyrene adsorbed to the walls of container considering the inclusion effect of CD to pyrene.

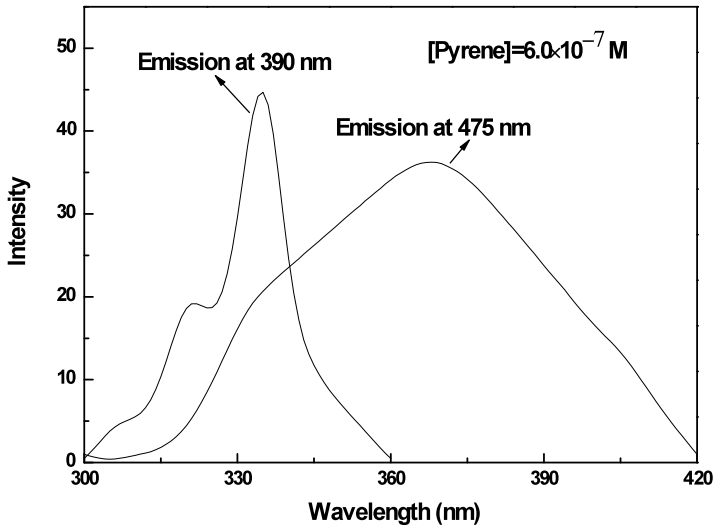
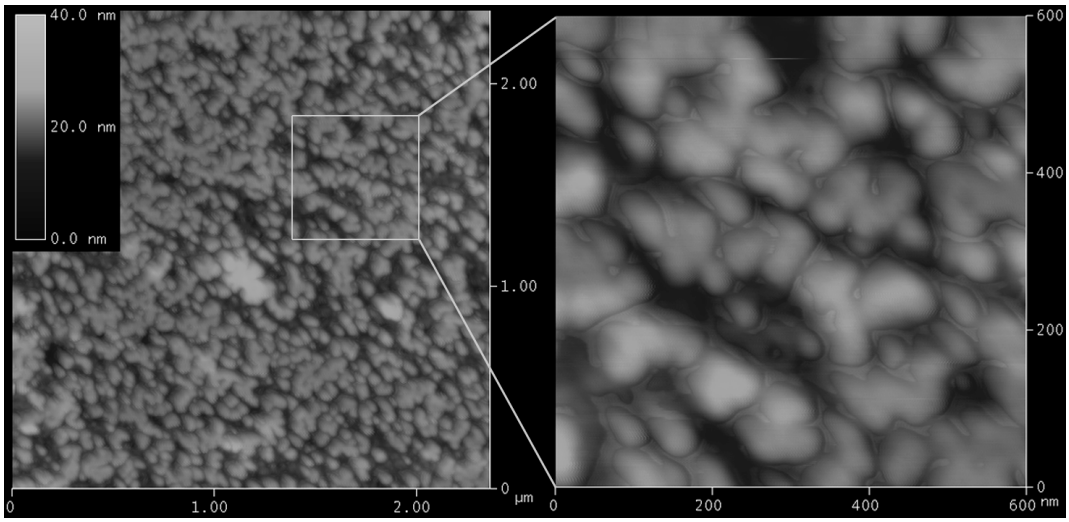
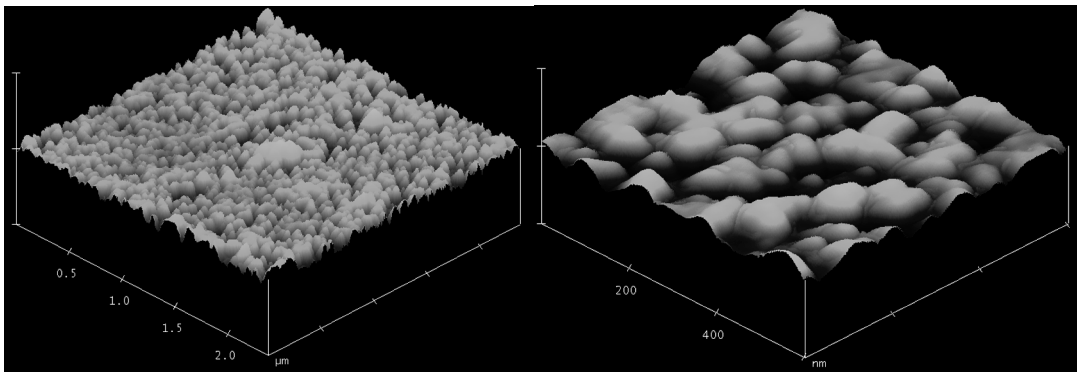


Figure S6. Fluorescence excitation spectra of pyrene in aqueous solution in the presence of PEG-*b*-PCD (1.0 mg/ml), which were monitored at 390 and 475 nm separately.



(a)



(b)

Figure S7. Tapping-mode atomic force microscopy (AFM) images of assemblies based on PEG-*b*-PCD and pyrene. (a) Height and (b) 3D images. Total concentration was 2 mg/ml

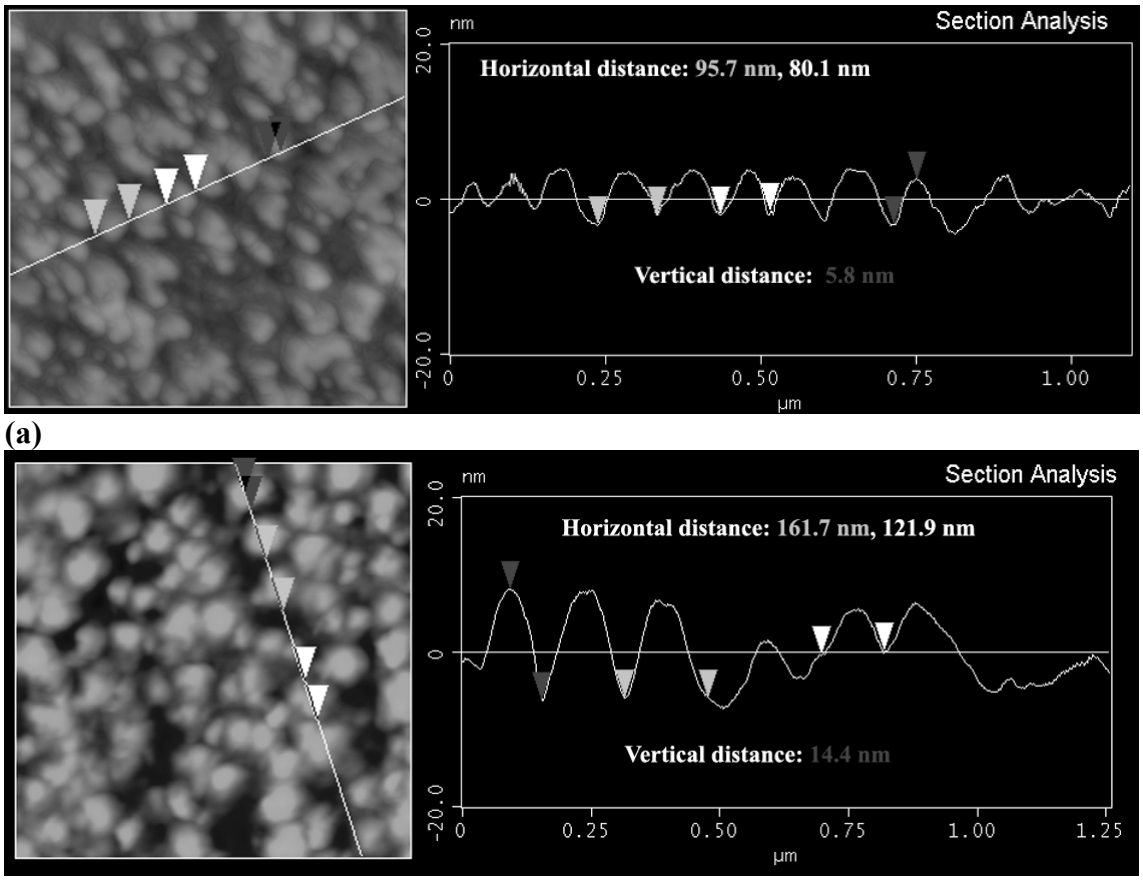


Figure S8. Cross-sectional analysis of PEG-*b*-PCD assemblies containing (a) pyrene and (b) coumarin 102. For assemblies containing coumarin 102, the ratio of diameter to height is about 7~15.

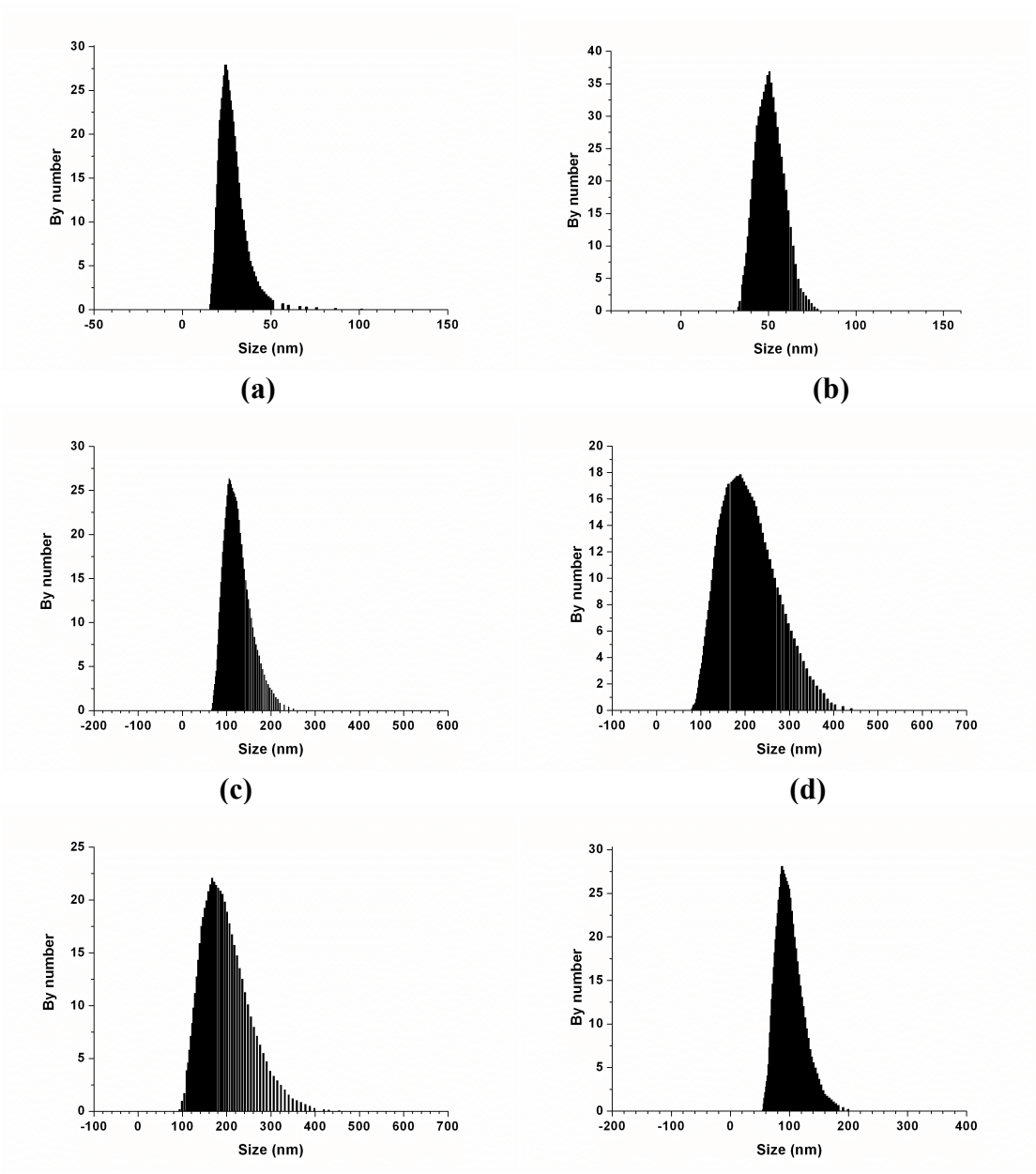
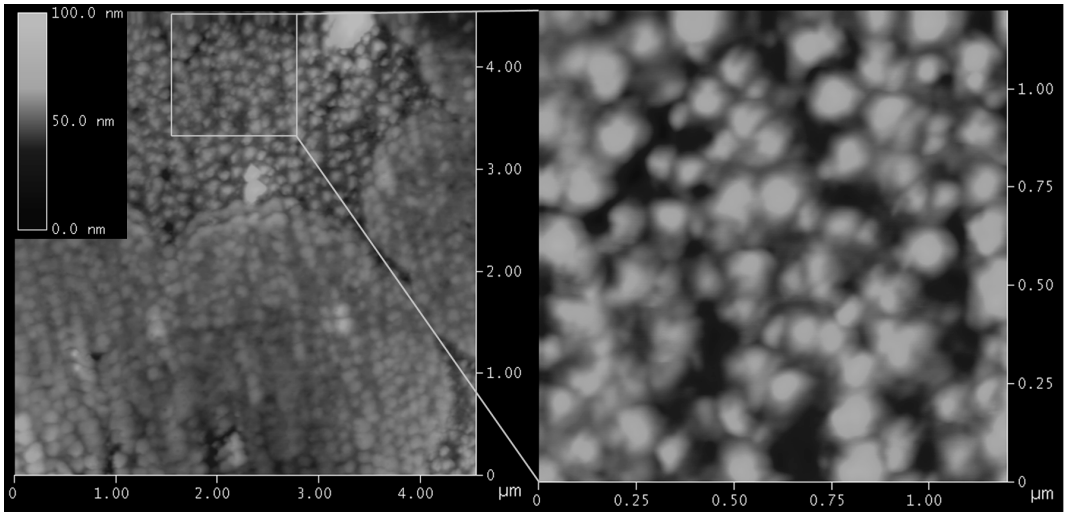
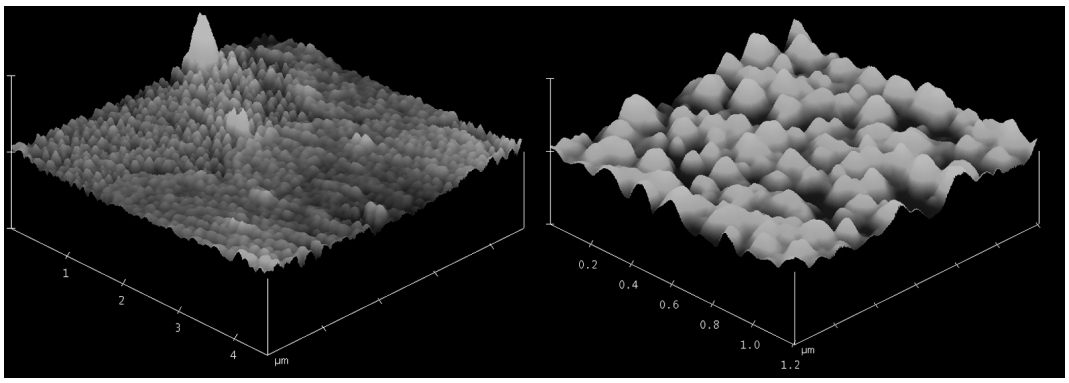


Figure S9. Particle size distribution of PEG-*b*-PCD based assemblies containing (a) pyrene, (b) coumarin 102; (c) PBLA, PBLA:PEG-*b*-PCD=1:20; (d) PBLA, PBLA: PEG-*b*-PCD=8:20; (e) PDLLA, and (f) ADCA and PEI.



(a)



(b)

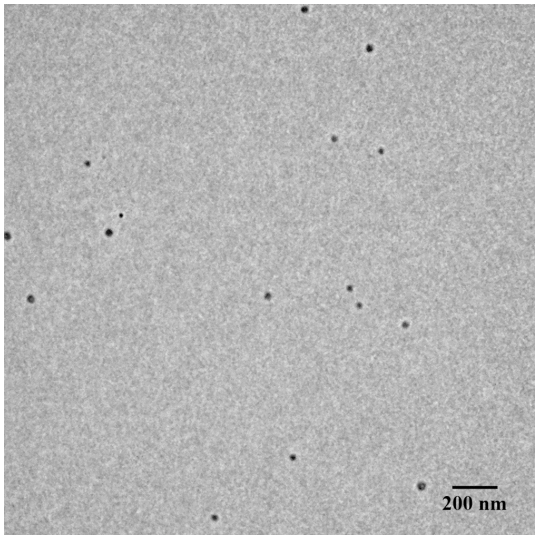


Figure S10. Tapping-mode AFM images of assemblies based on PEG-*b*-PCD and coumarin 102 (1.2 wt.%): (a) Height and (b) 3D images. Sample concentration was 2 mg/ml. Statistical analysis based on AFM images show a mean number-average diameter of 108.4 nm. (c) TEM image of assemblies based on PEG-*b*-PCD containing coumarin 102. TEM images reveal a mean size of ~38 nm.

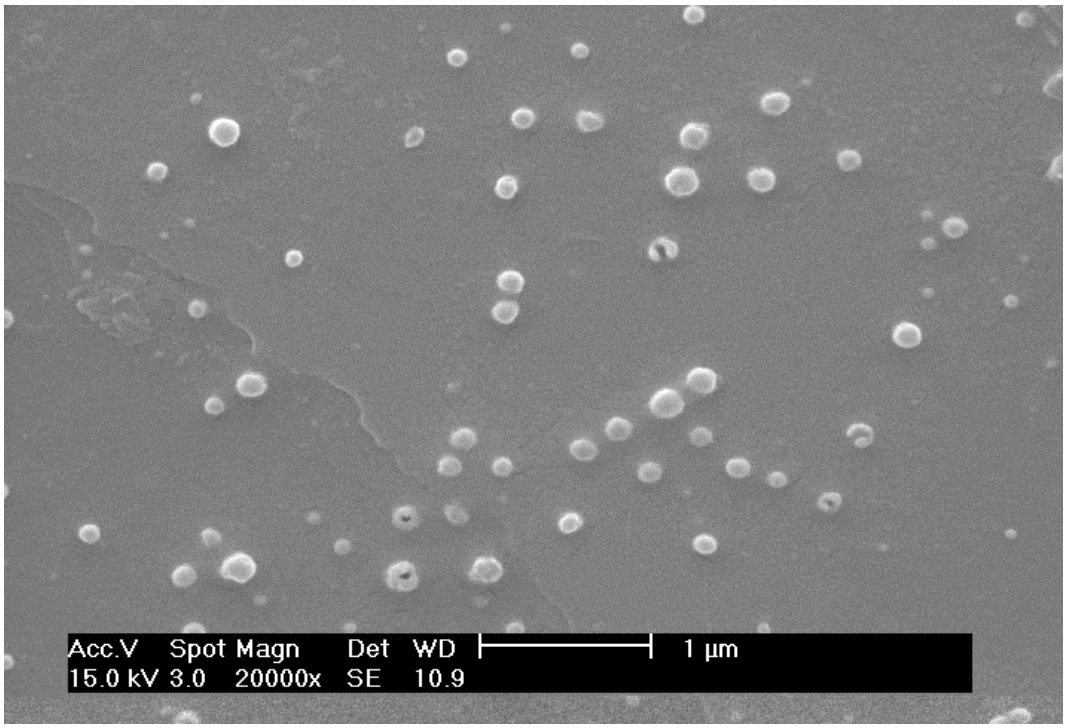


Figure S11. Field emission scanning electron microscopy (FE-SEM) image of assemblies based on PEG-*b*-PCD and PBLA with a weight ratio of 20:1 (PEG-*b*-PCD: PBLA).

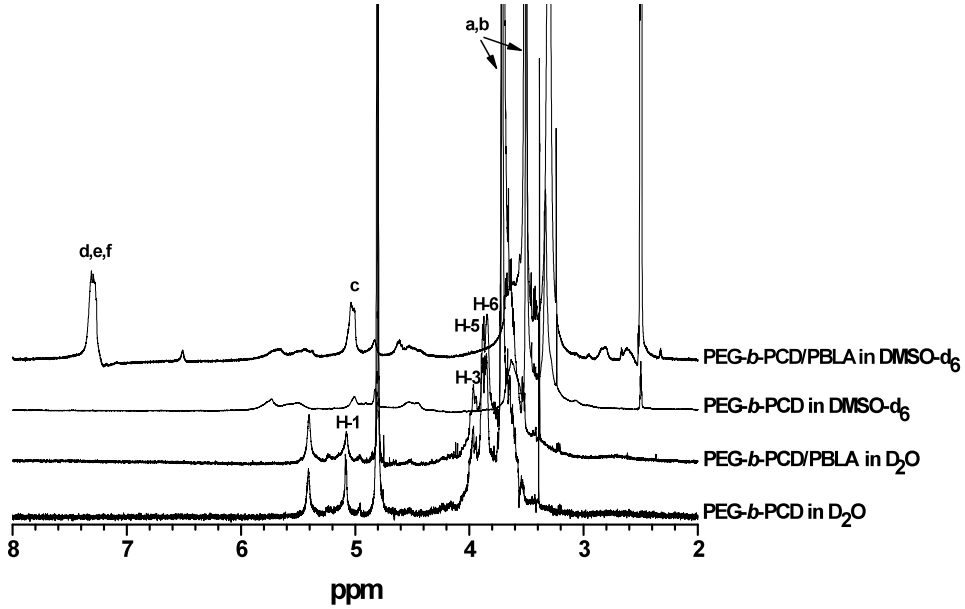


Figure S12. ^{1}H NMR spectra of PEG-*b*-PCD and PEG-*b*-PCD based assemblies containing PBLA in D_2O or DMSO-d_6. For the NMR characterization, PEG-*b*-PCD assemblies with PBLA were prepared by dialysis and the resultant aqueous solution was lyophilized. The dried sample was dissolved into D_2O, and ^{1}H NMR spectrum was acquired. The same sample was subjected to NMR measurement after it was lyophilized and dissolved in DMSO-d_6.

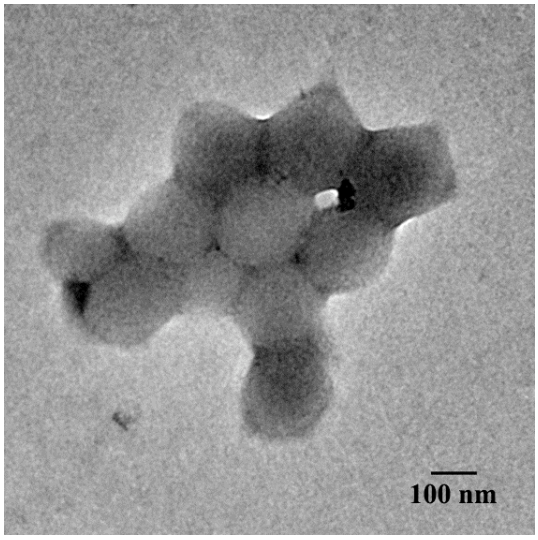


Figure S13. TEM image of assemblies based on PEG-*b*-PCD and PDLLA. The mean size calculated form TEM images is 153.6 nm, which well agrees with 184.3 nm determined by DLS (Figure S9e).

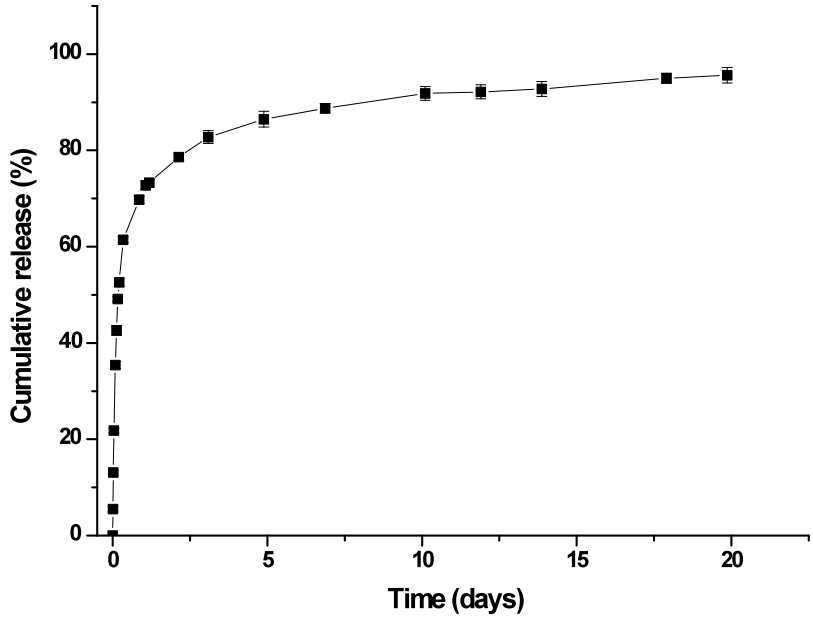


Figure S14. In vitro release of coumarin 102 from assemblies based on PEG-*b*-PCD and PBLA with a weight ratio of 20:2. Coumarin 102 loading: 9.8 wt.%.

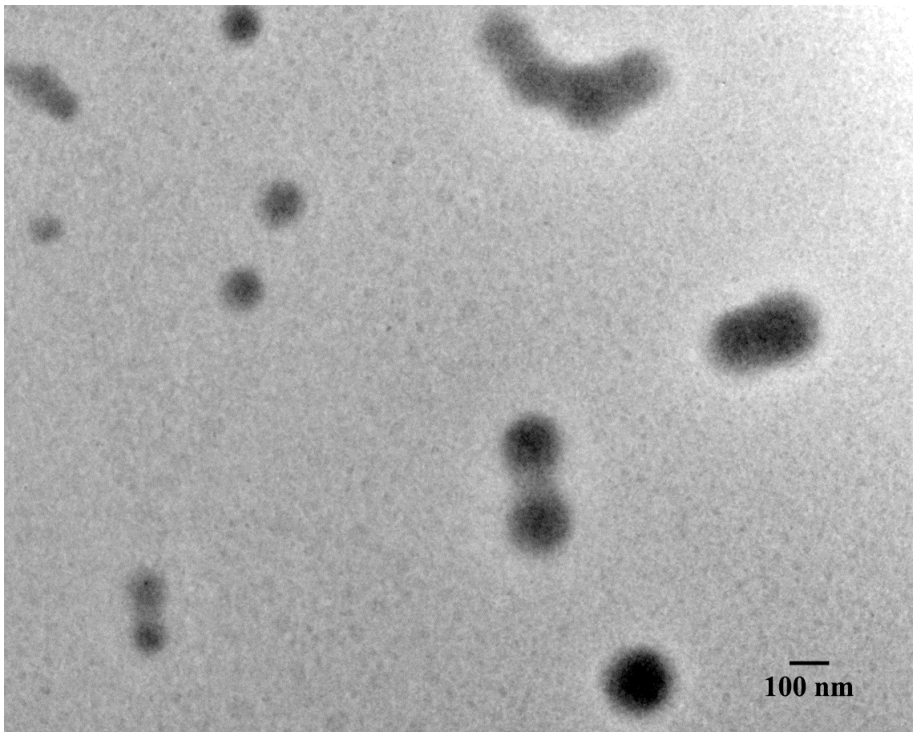


Figure S15. TEM image of assemblies based on PEG-*b*-PCD and PBLA of 15:6 in the presence of cetyltrimethylammonium bromide (CTAB).

References

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