Poly(ethylene glycol)-Poly(vinyl alcohol)-Adamantanate: Synthesis and Stimuli-Responsive Micelle Properties

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Supporting information

Experimental Methods

Materials and Methods. All solvents were reagent grade, purchased from commercial sources, and used without further purification, except DMSO, which was dried over CaH_2 under N_2, filtered and distilled under reduced pressure. 1-Adamantanecarbonyl chloride (Ad-COCl), 4-hydroxybenzaldehyde, Na_2CO_3, 1,1-carbonyldiimidazole (CDI), β-CD, trifluoroacetic acid (TFA) and p-toluenesulfonyl acid (TSA) were obtained from Aldrich-Sigma, Inc and used as received. PVA (Mowiol® 4-98, Mw ~27,000) was purchased from Acros Organics, Inc. ^{1}H NMR spectra were recorded on a 400 MHz Bruker ARX400 spectrometer at 20 °C. Chemical shifts were referenced to the residual protonated solvent peak. Polymer micelles were prepared by sonicating a 2 mg/mL solution of PEG-PVA-Ad in water or phosphate buffered saline (PBS, 20 mM Na_2HPO_4/NaH_2PO_4, 12 0 mM NaCl, pH 7.4) using a Heat Systems-Ultrasonic W350 unit, fitted with a 1/8” microtip, in pulsed mode (50% duty cycle) at 50 Watts for 15 min at 25 °C.

Synthesis of 4-adamantanecarbonylate benzaldehyde (1): To a solution of 4-hydroxybenzaldehyde (2.44g, 20 mmol) in CH_2Cl_2 (10 mL) was added 3 mL Et_3N. The solution was cooled on an ice bath prior to dropwise addition of an Ad-COCl (5.94g, 30 mmol) solution in THF (10 mL). After 6 h, the solvent was removed using a rotary evaporator. The residue was dissolved in 50 mL Et_2O and washed three times with 1 M Na_2CO_3 and saturated NaCl solution. The ethereal solution was dried over Na_2SO_4 before evaporation under reduced pressure to give a pale yellow solid. Yield = 5.11g (90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.99 (s, 1H), 7.91 (d, *J* = 8.4 Hz, 2H), 7.23 (d, *J*=8.0 Hz, 2H), 2.09-2.05 (m, 9H), 1.81-1.74 (m, 6H).

Synthesis of PVA-Ad (2): PVA (27kD) (460 mg, 10 mmol) was dissolved in 10 mL dry DMSO before addition of Compound **1** (568 mg, 2.0 mmol) and 25 mg TSA. The solution was stirred for 2 d at 50 °C before pouring into acetone (300 mL) to produce a fine white precipitate that was gathered by filtration. The precipitate was washed with acetone and dried under vacuum to yield a stable white solid. Yield =

0.84 g (85%). ^{1}H NMR (400 MHz, d_6-DMSO): δ = 7.42 (w, 2H), 7.03 (w, 2H), 5.51 (s, 1H), 4.66-4.02 (m, 3.9H), 3.96-3.74 (m, 6.5H), 2.02-1.95 (m, 9H), 1.70 (m, 6H), 1.59-1.21 (m, 13H).

Synthesis of PEG-PVA-Ad: CDI (1.62 g, 10 mmol) in DMSO (10 mL) was added dropwise to a solution of **2** (0.72 g) in 10 mL DMSO and the mixture stirred for 1 d at 20 °C. The product was isolated by three cycles of addition of dry ether to a DMSO solution, followed by redissolution of the solid and re-precipitation to give Compound **3**. This product was dissolved in 10 mL DMSO and NH_2-PEG_{750}-OMe (7.5g, 10 mmol) was added before stirring the solution for 12 h at 20 °C. The product was dialyzed (Spectra/Por Membrane, MWCO 6000-8000) against DMSO and 18 MΩ deionized water, three times each, to remove any low MW impurities. After solvent removal by lyophilization, the polymer was redissolved in DMSO, precipitated with ether, and the PEG-PVA-Ad product isolated as a pale yellow solid. Yield = 1.2g. ^{1}H NMR (400 MHz, H_2O): δ = 7.61-7.45 (br, ph), 7.12-7.01 (br, ph), 5.2-4.6 (br, m), 4.01-3.05 (br, m), 2.2-1.5 (br).

Polymer Micelle Preparation. To prepare polymer micelle solutions, PEG-PVA-Ad was dispersed in 18 MΩ deionized water or PBS by 1/8" microtip probe sonication in pulsed mode (50% duty cycle) at 50 Watts for 15 min at 20 °C. For pyrene solubilization fluorescence measurements, the polymer micellar solutions were prepared using a saturated pyrene solution in distilled water during the sonication process. These pyrene-containing polymer micelle samples were further diluted with a saturated pyrene solution in distilled water to produce the target PEG-PVA-Ad concentration. The PEG-PVA-Ad concentrations were varied from 600 to 2.4 mg/L for subsequent pyrene solubilization measurement via fluorescence spectroscopy.

Fluorescence Measurements. Pyrene fluorescence spectra were obtained in 18 MΩ deionized water using a Yobin Yvon Fluoromax 4 spectrofluorimeter to probe the solubilization properties of the PEG-PVA-Ad dispersions. For pyrene excitation spectra measurements, the emission and excitation slit widths were set at 3 and 1.5 nm, respectively. For pyrene excitation spectra, the excitation wavelength was set at 390 nm.

Size Distribution Analysis. The polymer micelle size distributions were evaluated by dynamic light scattering (DLS) at 20 °C using a Zetasizer Nano S (Malvern Instruments Ltd.) particle size analyzer with a 90° scattering angle.

Atomic Force Microscopy (AFM). The nanoparticles were imaged using a MultiMode AFM (Veeco, USA) in tapping mode on dry samples that were mounted on mica and imaged at 0.5 or 1 Hz scan rates.

The AFM tips (PPP-NCH, Nanoscience Instruments, Inc., USA) used had a typical radius of 7 nm or less. Samples were prepared by transferring 2 mL of polymer solution onto the mica surface, followed by drying overnight at 20 °C.

Kinetic Analysis of PEG-PVA-Ad Hydrolysis by ^{1}H NMR. PVA-Ad (50mg) was dispersed by sonication in 2 mL D_2O for 10 min, 22.6 mg β-CD was added to this solution. The ^{1}H NMR spectrum of this solution was recorded before addition of 0.5 mL of 200 mM acetate buffer, pH 4.0. ^{1}H NMR spectra of this mixture were recorded periodically during incubation of the sample for 2 d at 25 °C. Acetate buffer in D_2O was prepared by mixing glacial acetic acid (47 mL) and sodium acetate (15 mg) in 5 mL D_2O to give a pH = 4.0 solution.

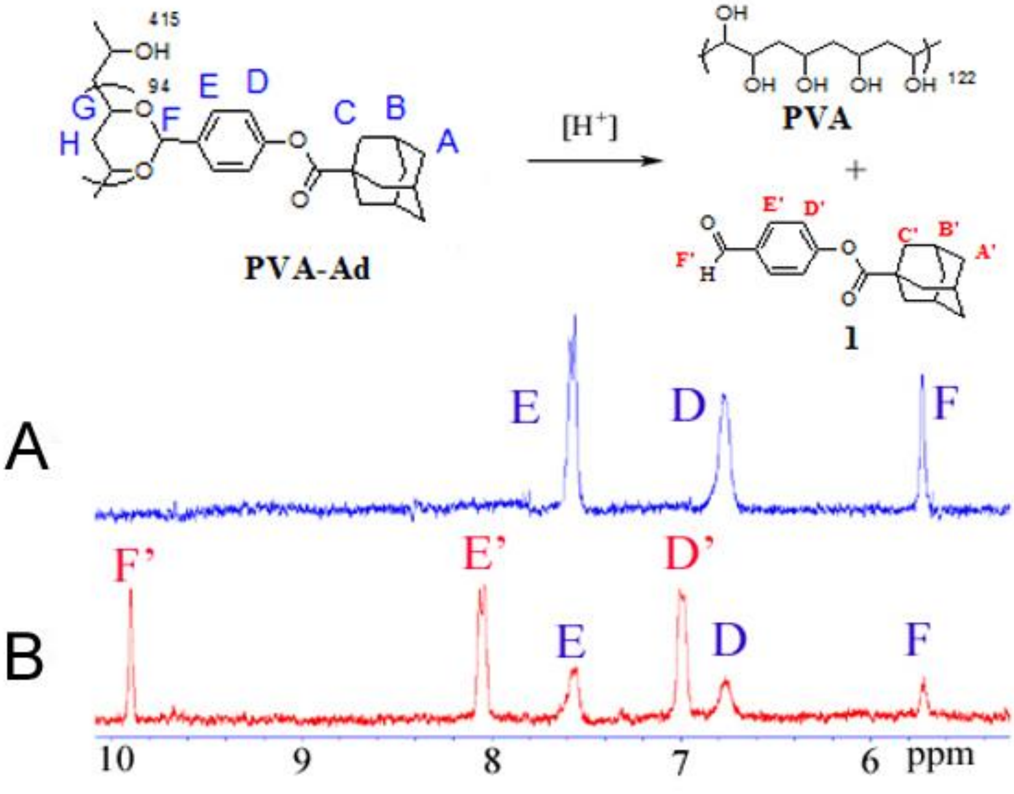


Figure S1. 400 MHz ^{1}H NMR spectra of Compound **2** with βCD in pH 4.0 buffer at time = 0 (A) and after 2 days (B) at 20 °C. Compound **2** was dispersed at 100 mg/L in 5 mM CH_3CO_2Na/CH_3CO_2H buffer, pH 4.0 in D_2O as described above.

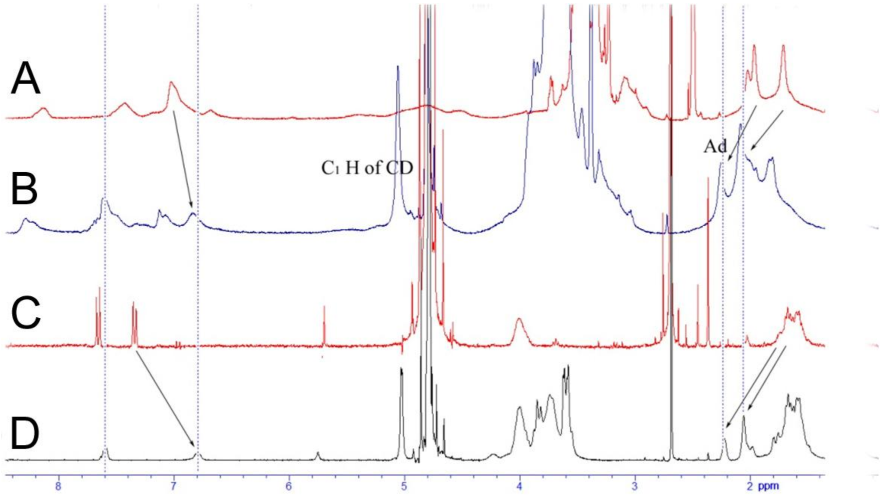
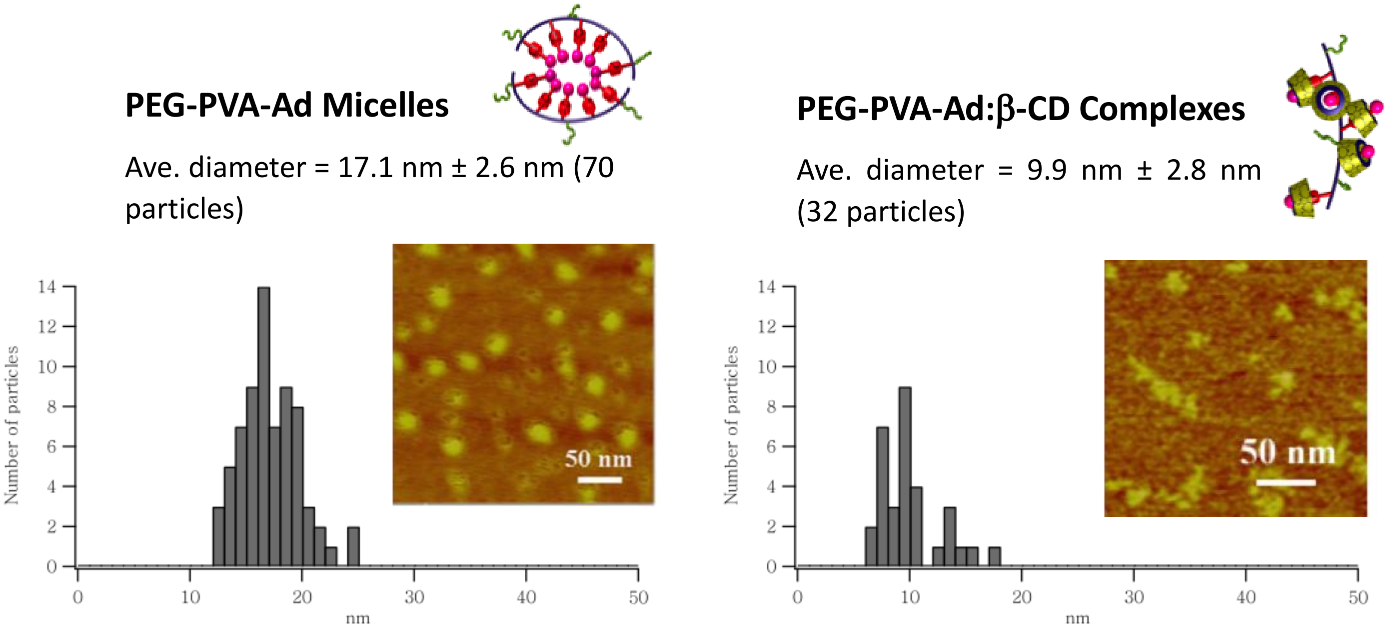


Figure S2. 400 MHz ^{1}H NMR spectra of β-CD-induced polymer micelle disruption. PEG-PVA-Ad in the (A) absence, and (B) presence of β-CD, Compound **2** in the (C) absence, and (D) presence of β-CD is also shown. All spectra were recorded in D_2O at 20 °C. Chemical shifts were referenced to HDO at 4.79 ppm.



PEG-PVA-Ad Micelles

Ave. diameter = 17.1 nm ± 2.6 nm (70 particles)

PEG-PVA-Ad:β-CD Complexes

Ave. diameter = 9.9 nm ± 2.8 nm (32 particles)

Figure S3. Statistical analysis of particle diameters determined by AFM (PEG-PVA-Ad contour length, ~110 nm). (Left) PEG-PVA-Ad in the absence of β-CD. (Right) PEG-PVA-Ad in the presence of β-CD. The samples were prepared by slowly evaporating the solvent (H_2O) overnight at 20 °C from a drop of solution placed on a mica surface. [PEG-PVA-Ad] = 6×10^{-5} mg/L; [Ad] = [CD].

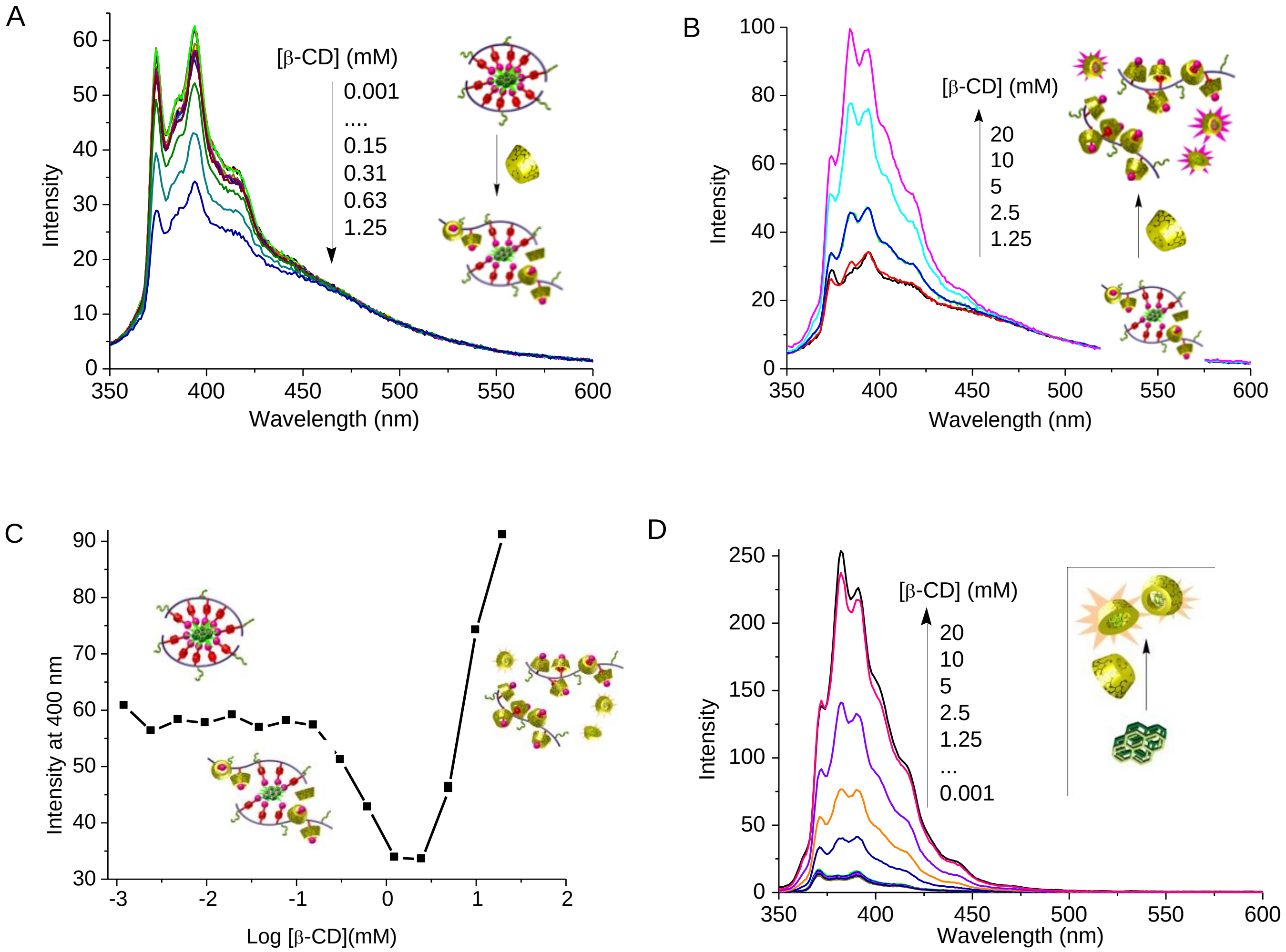


Figure S4. Pyrene fluorescence emission spectra in the presence of PEG-PVA-Ad upon addition of β-CD at concentrations ranging from 0.001 to 1.25 mM (A) and 1.25 to 20 mM (B). (C) Pyrene emission intensity at 400 nm as a function of log [β-CD]. (D) Pyrene fluorescence emission spectra upon addition of β-CD in the absence of PEG-PVA-Ad polymer. The excitation wavelength was fixed at 339 nm. The concentration of PEG-PVA-Ad in Panels A-C was 75 mg/L (i.e, above the CAC). The insets suggest a structure that is proposed to form under the conditions shown.

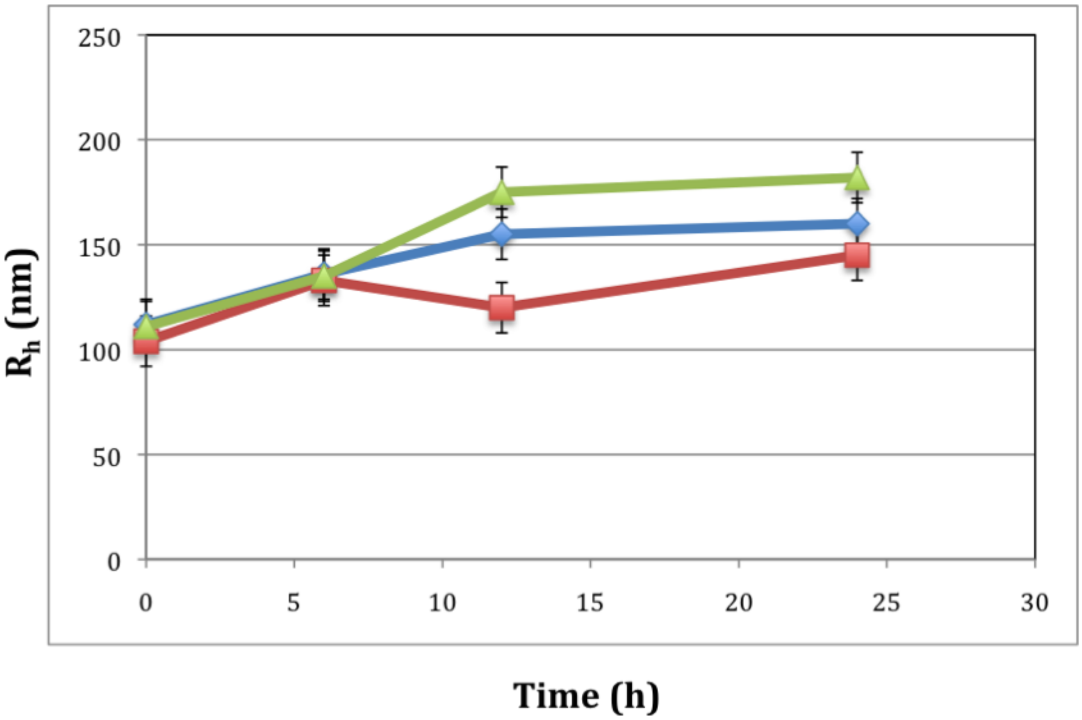


Figure S5. Particle size stability as a function of time in PBS at 20 °C (blue), 37 °C (red) and 150 mM NaCl (green).

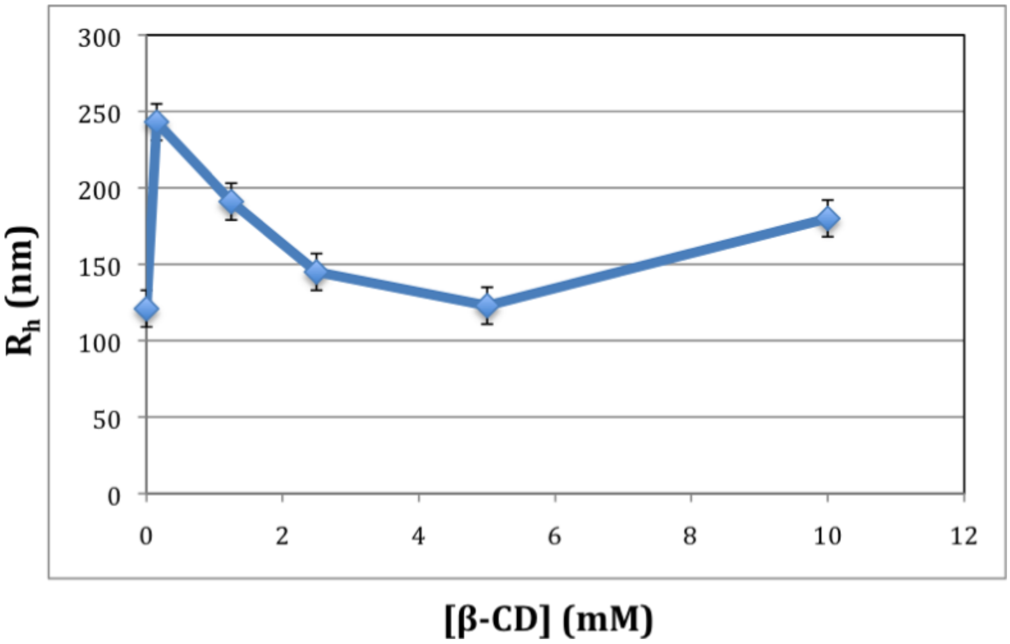


Figure S6. Hydrodynamic diameter as a function of [β-CD] in H_2O at 20 °C. Two populations of particles are observed between [β-CD] = 0.15 - 1.25 mM. The particle diameters are shown for the predominant species (>90%) in solution; the minor population ranged between 28 – 47 nm in diameter. This mixed population may be due to the presence of rod-like and spherical particles. A single population of species was observed above [β-CD] > 2.5 mM.