Multivalency enables dynamic supramolecular host-guest hydrogel formation

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Synthesis of 1-tosyl-1H-imidazole.

To *para*-toluenesulfonyl chloride (10.0 g, 52.5 mmol) in dry CH_2Cl_2 (100 mL) at 0°C and under argon atmosphere was slowly dropwise added imidazole (8.0 g, 118 mmol, in 50 mL dry CH_2Cl_2). The reaction was allowed to slowly warm to room temperature overnight. The next day, solvent was removed *in vacuo*, and the resulting solids were dissolved in warm hexanes for recrystallization. The title compound oiled out of solution, then slowly crystallized after keeping at 4°C overnight. The crystals were filtered off and washed with cold hexanes, yielding 1-tosyl-*1H*-imidzole (9.2 g, 77% yield, TLC R_f = 0.25 50:50 hexanes:EtOAc). ^{1}H NMR in

$CDCl_3$: δ (ppm) 7.99 (1H, *t*, 1.1 Hz), 7.81 (2H, *d*, 8.6 Hz), 7.35 (2H, *d*, 8.4 Hz), 7.28 (1H, *t*, 1.5 Hz), 7.07 (1H, *t*, 1.2 Hz), 2.43 (3H, *s*).

Synthesis of 6-O-Monotosyl-β-cyclodextrin.

β-Cyclodextrin (2.0 g, 1.76 mmol) and 1-tosyl-*1H*-imidazole (470 mg, 2.11 mmol) were dissolved in water (20 mL) and stirred vigorously for 6 hours. Then, a 20 wt% solution of NaOH (2.86 mL, 572 mg, 14.3 mmol) was added dropwise to the stirred solution and allowed to react for 15 minutes. Solids from the reaction mixture were removed via filtration, then NH_4Cl (2.0 g, two portions) was added to induce precipitation of the products. The precipitate was removed via filtration and washed with H_2O (2x), acetone (2x), $CHCl_3$ (2x), then acetone (2x) and dried in air. The remaining solids were recrystallized from hot H_2O (filtering the hot solution to remove insoluble), and put at 4°C overnight to facilitate precipitation. Small powdery crystals were removed via filtration and then washed with H_2O (2x) and acetone (2x) then dried in the oven overnight yielding 6-O-Monotosyl-β-cyclodextrin (569 mg, 25% yield). ^{1}H NMR in *d*$_6$-DMSO: δ (ppm) 7.75 (2H, *d*, 8.2 Hz), 7.43 (2H, *d*, 8.2 Hz), 5.85–5.61 (14H, *m*), 4.87–4.81 (5H, *m*), 4.76 (2H, *t*, 3.1 Hz), 4.50 (3H, *m*), 4.43 (2H, *q*, 5.6 Hz), 4.37–4.30 (2H, *m*), 4.19 (1H, *dd*, 6.7, 11.3 Hz), 3.80–3.20 (40H, *m*), 2.42 (3H, *s*). ESI-MS $C_{49}H_{76}O_{37}S$ calcd. $[M+H]^+ = 1289.39$ obs. 1289.43.

Synthesis of 6-(6-aminohexyl)amino-6-deoxy-β-cyclodextrin (CD-HA).

CD-tosyl (2.30 g, 1.78 x 10^{-3} mol) and 1,6-hexadiamine (8.4 g, 7.23 x 10^{-2} mol) were weighed into a round bottom flask equipped with a stirrer. Anhydrous DMF (100 mL) was added to the solids and flask was capped with a rubber septum. Reaction solution was deoxygenated with nitrogen for 30 min before being left to react at 80 °C for 18 h. Reaction solution was cooled to r.t. and added dropwise to a cold acetone bath (500 mL). Precipitated white solid was collected

via centrifugation and supernatant was decanted. This precipitation step was repeated in cold acetone, followed by twice in diethyl ether. White precipitated was then dried in a vacuum oven overnight at r.t. before analyzed via NMR.

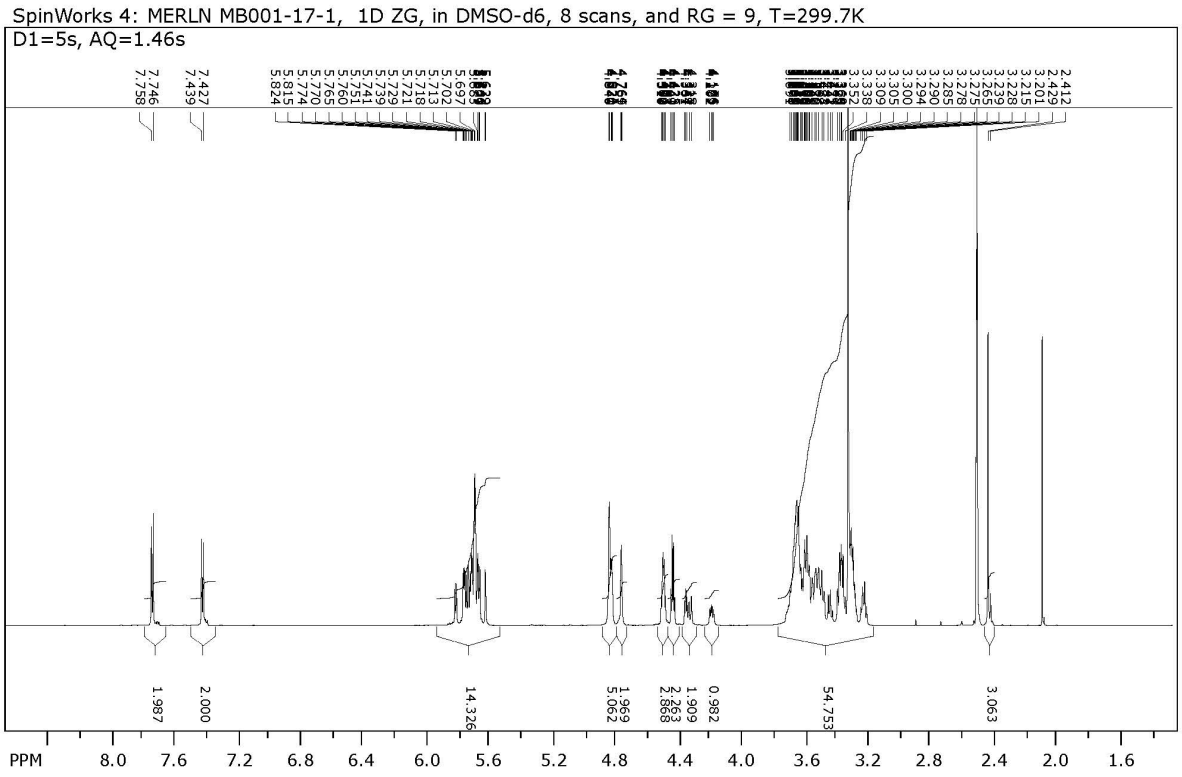


Figure S1. ^{1}H-NMR spectrum (d_6-DMSO) of 6-*O*-monotosyl-*β*-cyclodextrin

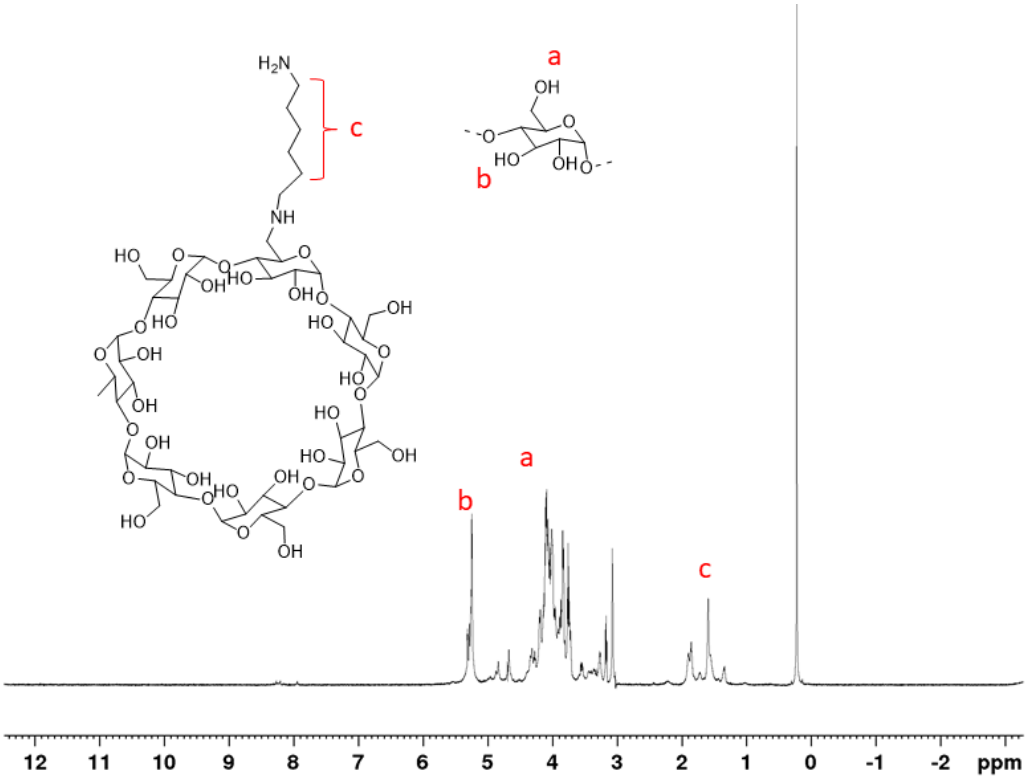


Figure S2. ^{1}H-NMR spectrum (D_2O) of CD-HA.

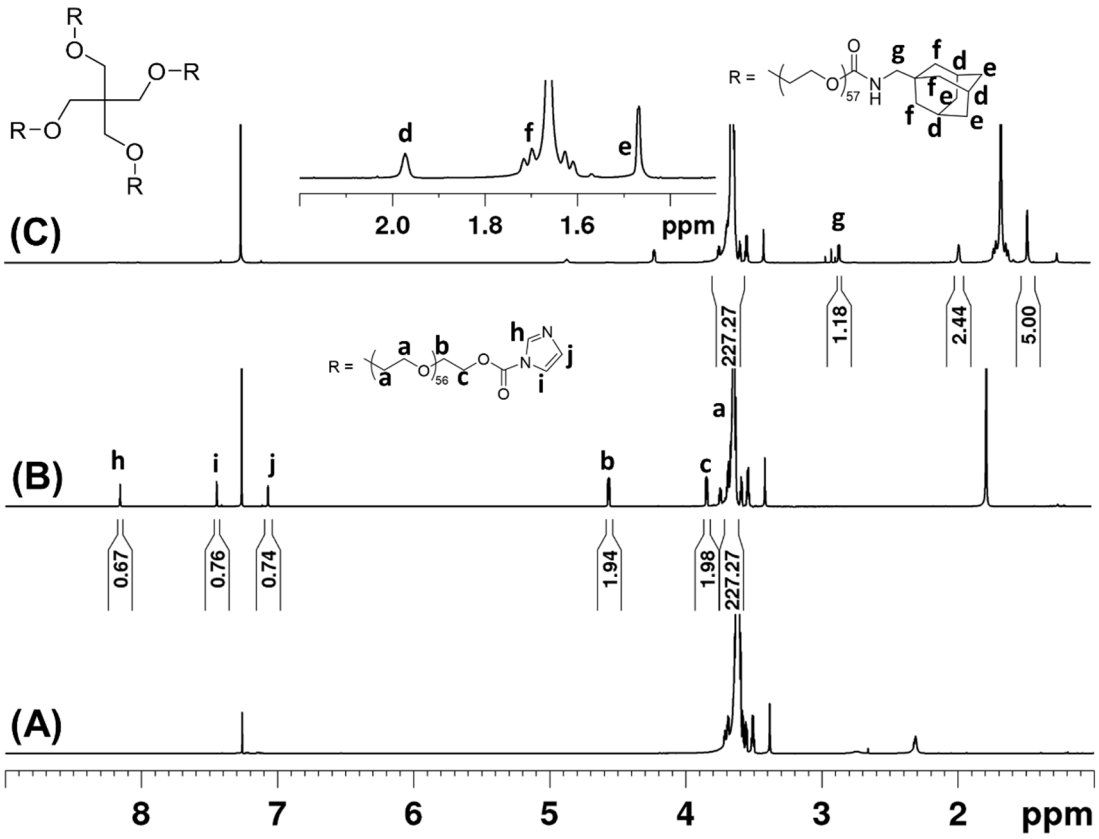


Figure S3. ^{1}H-NMR spectrum ($CDCl_3$) of (A) unreacted PEG4, (B) PEG4-CDI, and (C) PEG4-ADA. The appearance of CDI specific peaks (h, i, and j) and shifts in the α and β methylene protons (b,c) were observed after reaction of PEG4 with CDI. Following reaction of PEG4-CDI with adamantane methylamine, appearance of adamantane specific peaks (d, e, f, and g) and disappearance of CDI peaks were observed.

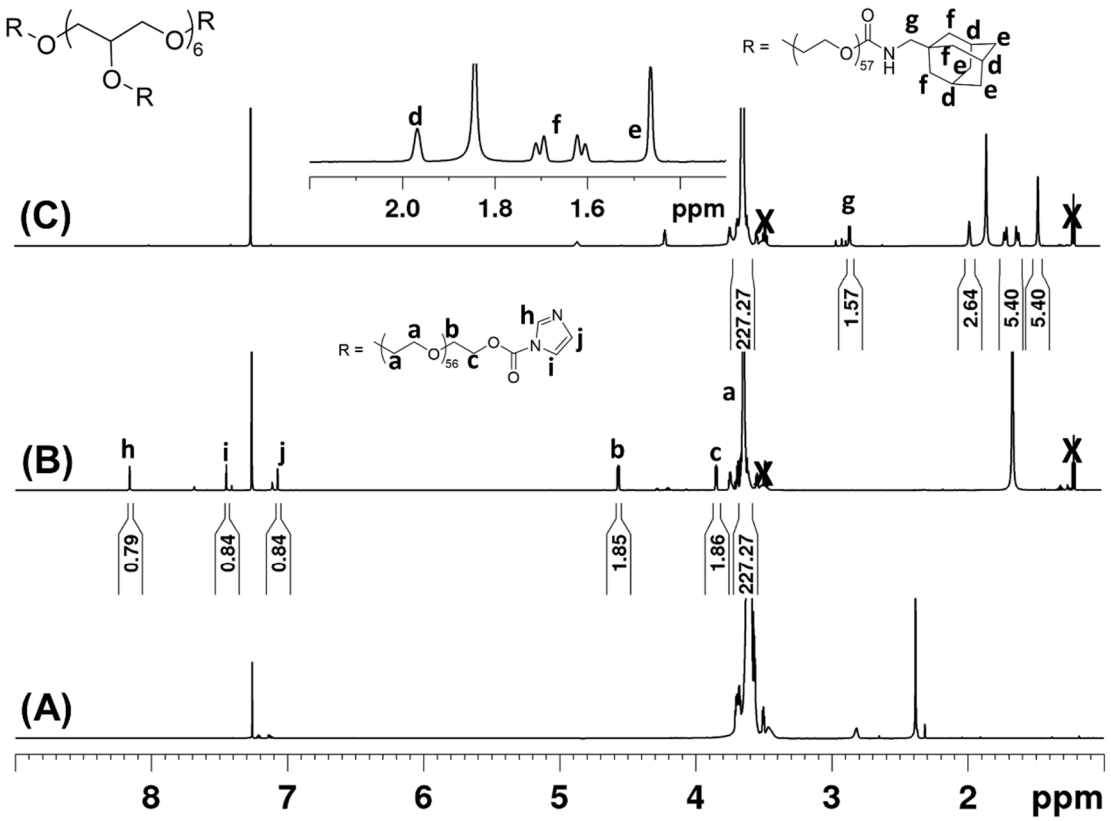


Figure S4. ^{1}H-NMR spectrum ($CDCl_3$) of (A) unreacted PEG8, (B) PEG8-CDI, and (C) PEG8-ADA. The appearance of CDI specific peaks (h, i, and j) and shifts in the α and β methylene protons (b,c) were observed after reaction of PEG8 with CDI. Following reaction of PEG8-CDI with adamantane methylamine, appearance of adamantane specific peaks (d, e, f, and g) and disappearance of CDI peaks were observed. X denotes presence of residual diethyl ether.

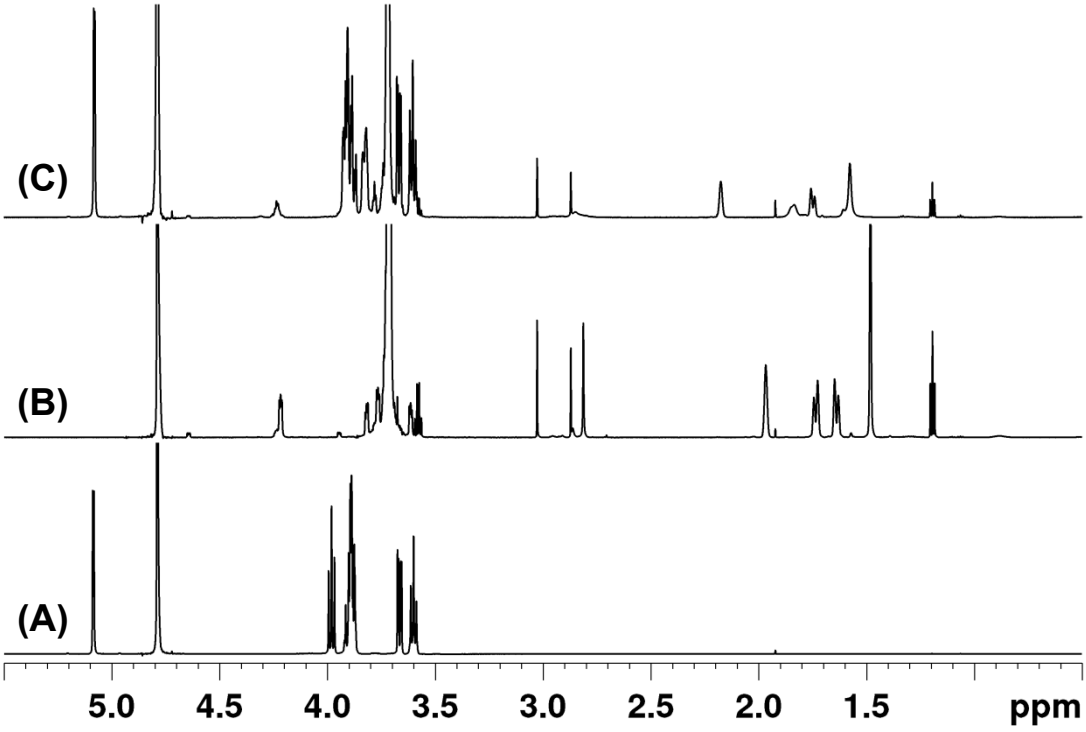


Figure S5. ^{1}H-NMR spectrum (D_2O) of (A) β-CD, (B) PEG2-ADA, and (C) 1.25 mM of PEG2-ADA: β-CD.

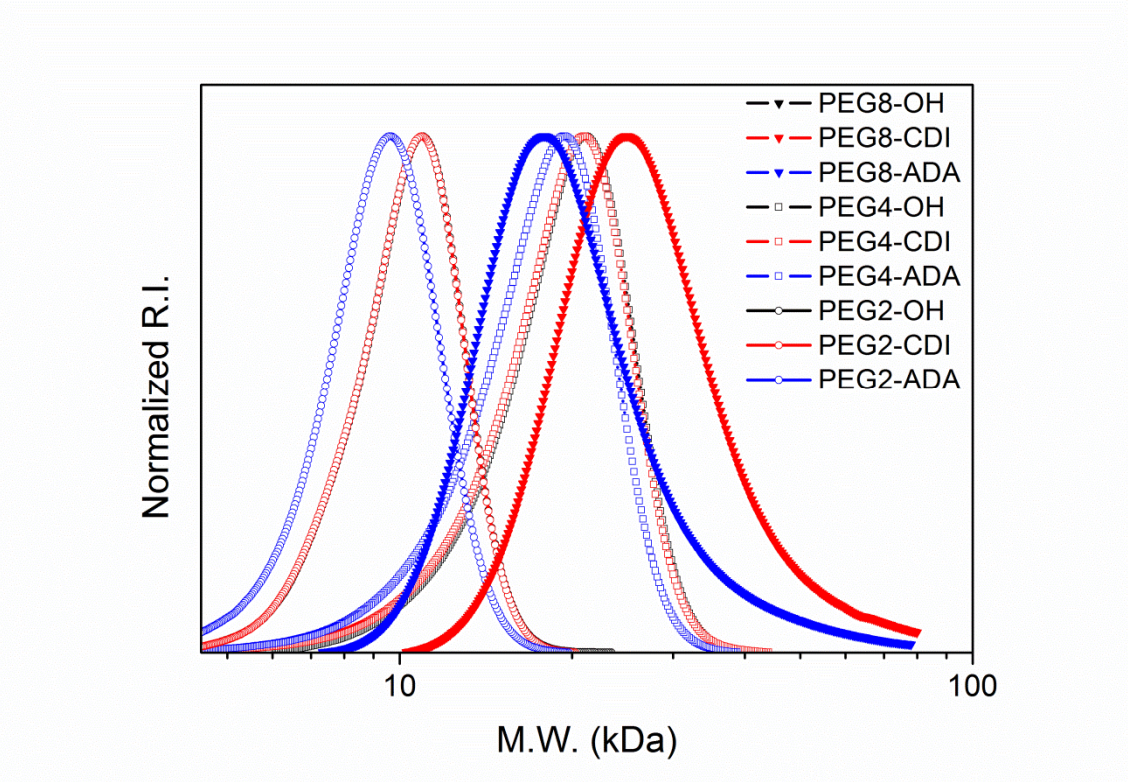


Figure S6. GPC traces of PEG2, PEG4, and PEG8 before and after reaction with CDI and subsequently adamantane methylamine.

Calculations for estimation of degree of functionalization of CD on alginate with ^{1}H NMR using DMF as an internal standard:

Mass of NMR sample	3	mg
Volume of NMR sample	500	uL
[DMF] in sample	6.5×10^{-3}	M
Integration of spacer (8H)	1.41	
Integration for 1 H	0.17625	
[CD-HA]	1.1×10^{-3}	M
No. of mol of CD-HA	5.7×10^{-7}	mol
MW of CD-HA	1233	
Mass of CD-HA	0.702	mg
Mass of alginate	2.300	mg
MW of alginate	197.9000	
No. of mol of alginate	1.2×10^{-5}	mol
% functionalization	4,90	%
Concentration of CD-HA on alginate	0.19	mmol/g

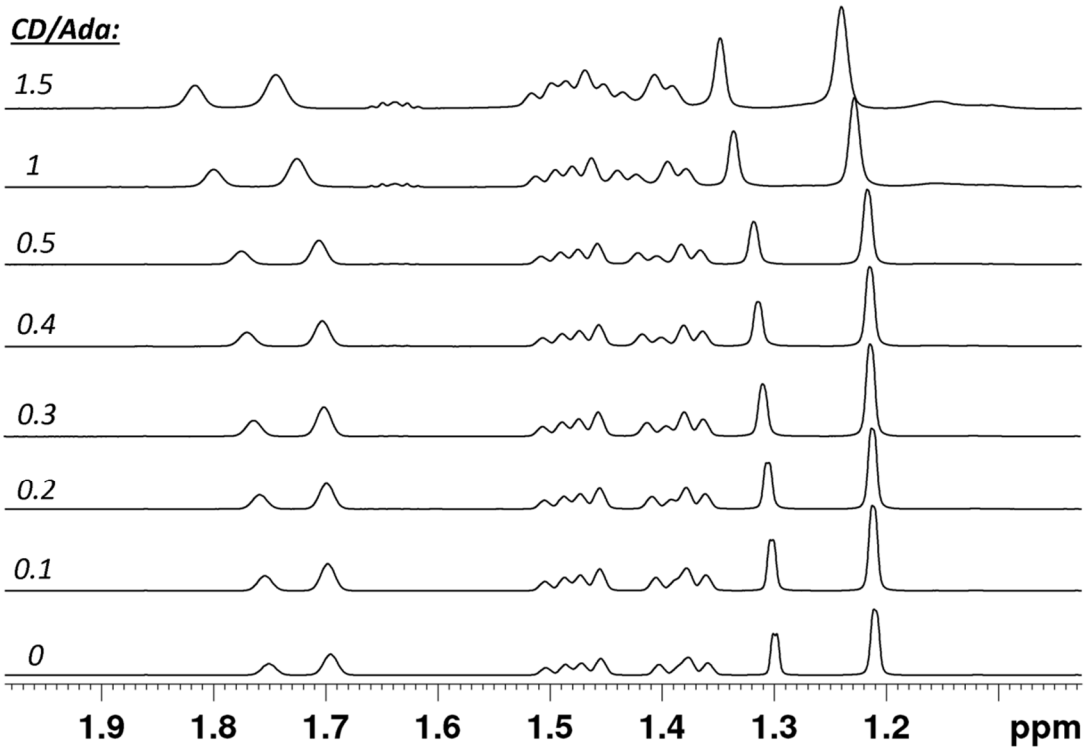


Figure S7. ^{1}H NMR spectra of PEG4-ADA (guest) titrated with Alg-CD (host). Chemical shifts of ADA peaks (1.2 and 1.7 ppm) were monitored for determination of binding constant. [ADA] in all samples is 2 mM.

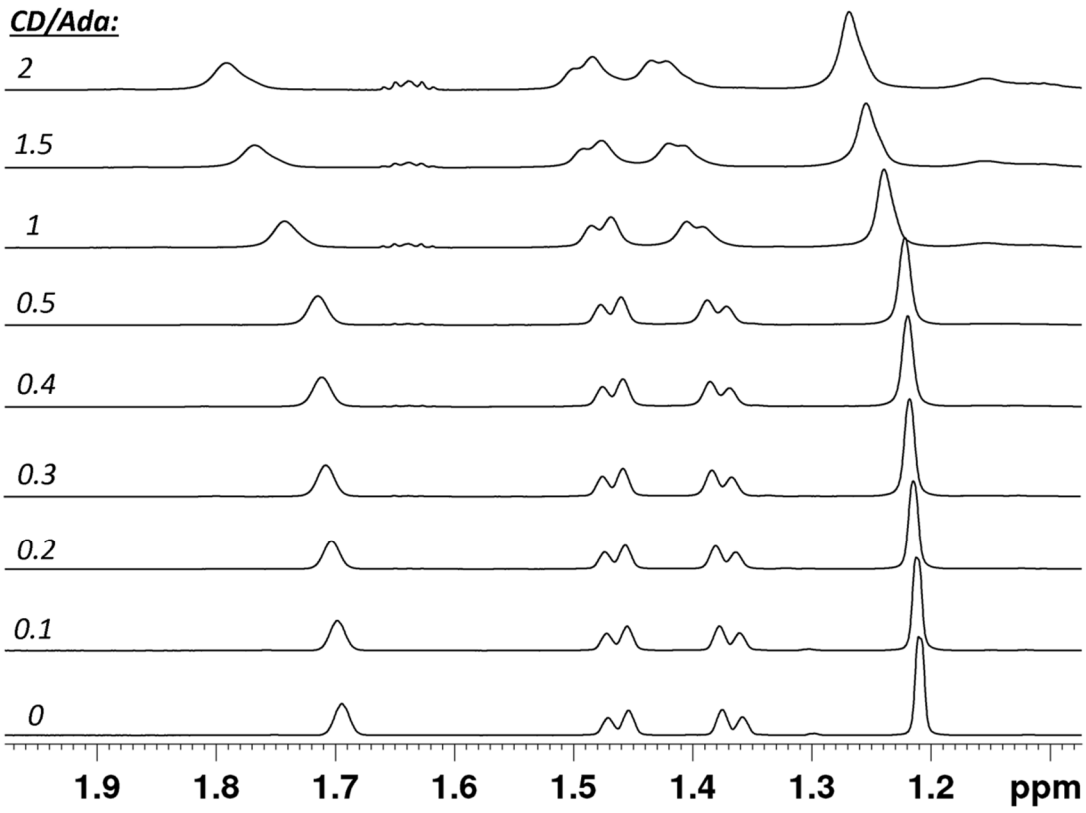


Figure S8. ^{1}H NMR spectra of PEG2-ADA (guest) titrated with Alg-CD (host). Chemical shifts of ADA peaks (1.2 and 1.7 ppm) were monitored for determination of binding constant. [ADA] in all samples is 2 mM.

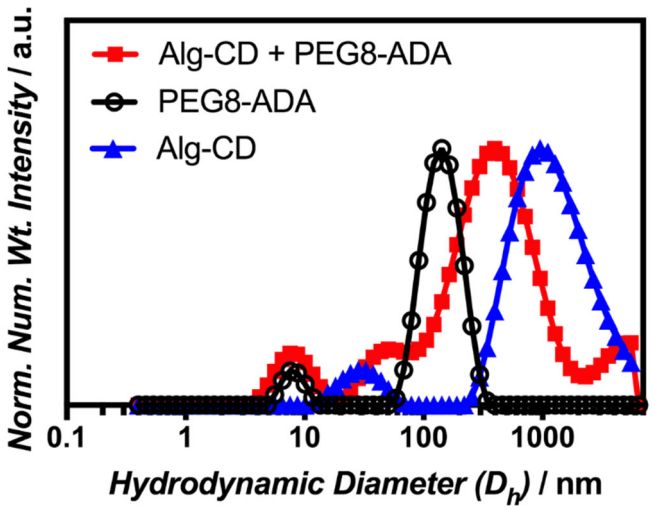


Figure S9. DLS measurements of Alg-CD, PEG8-ADA, and the supramolecular complex (1:1) in deionized water at 25 °C. Samples were measured in concentrations of 60 µM host and guest.

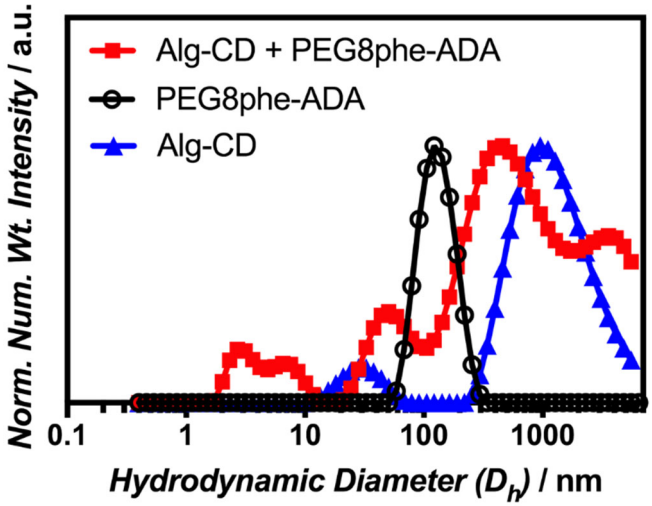


Figure S10. DLS measurements of Alg-CD, PEG8phe-ADA, and the supramolecular complex (1:1) in deionized water at 25 °C. Samples were measured in concentrations of 60 µM host and guest.

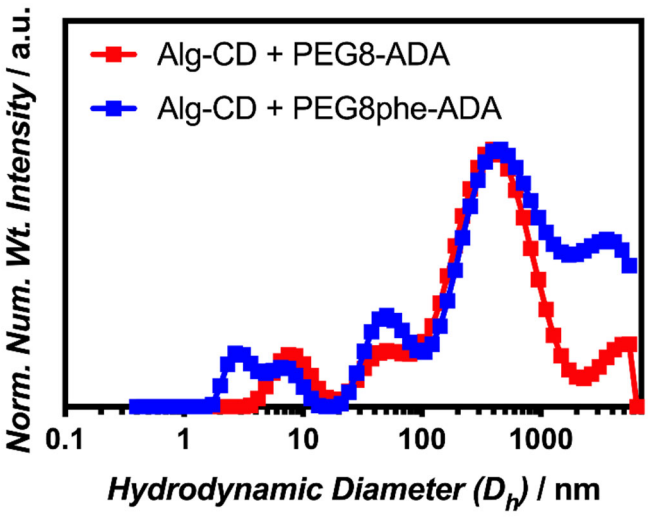


Figure S11. DLS results of Alg-CD/PEG8-ADA and Alg-CD/PEG8phe-ADA mixtures.

DLS was initially employed to investigate the formation and aggregate size of the Alg-CD/PEG8-ADA and Alg-CD/PEG8phe-ADA host-guest complexes at lower concentrations (60 μM). Intensity plots revealed the size of Alg-CD was approximately 1000 nm, while the two 8-arm adamantane functionalized PEGs, PEG8-ADA and PEG8phe-ADA, were approximately 110 nm. When the Alg-CD was mixed with PEG8-ADA, a product with a multimodal distribution was observed. In comparison, the use of the PEG8phe-ADA provided a product with an even broader multimodal distribution (Figure S11).

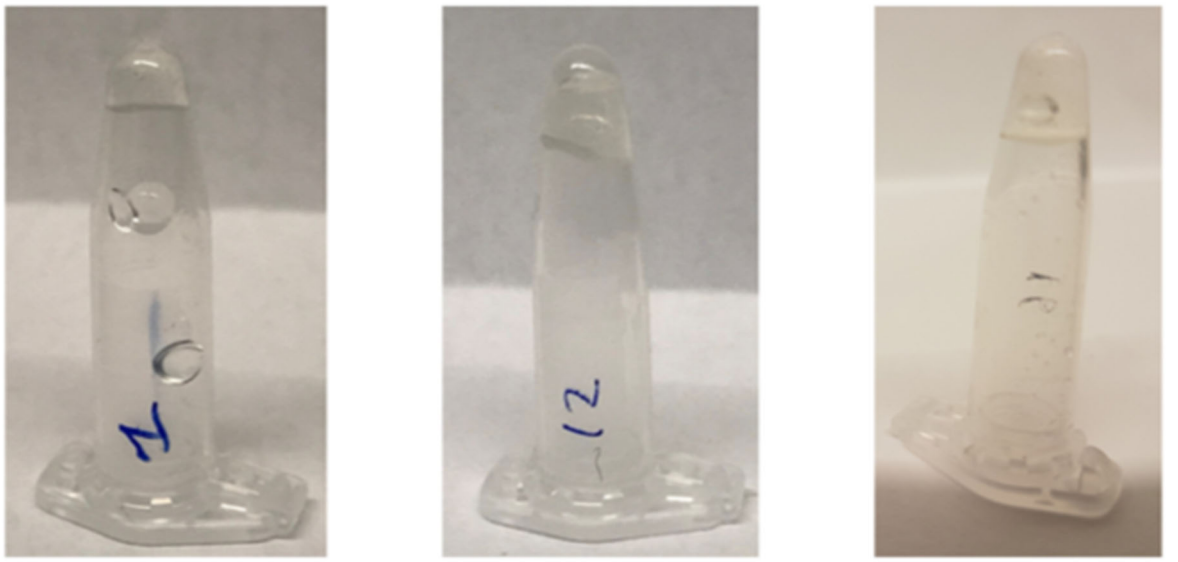
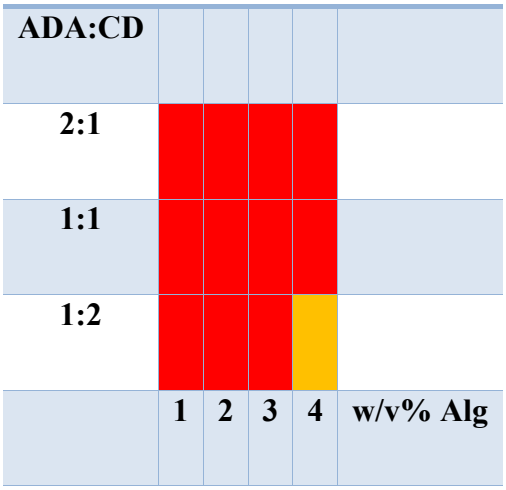


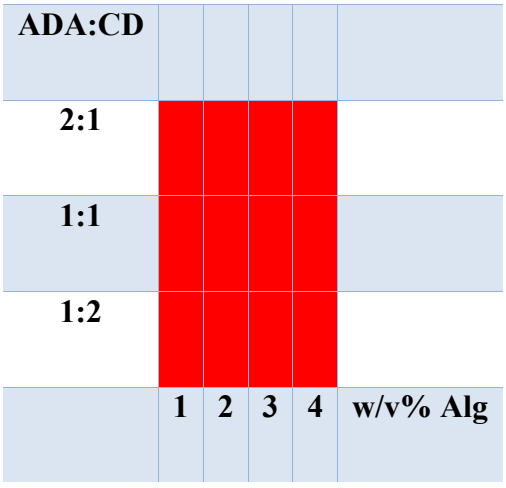
Figure S12. Visual representation of qualitative hydrogel classification. No hydrogel (example of 2wt% PEG2-ADA/Alg-CD with a 1:1 molar ratio of ADA to CD) means the sample was a viscous liquid. Weak means that the sample has gel-like properties but changes shape after mechanical shock. A strong hydrogel was able to retain its shape after mechanical shock.

Table S1. An overview of the gel precursor variables and the outcome of the gelation experiments for PEG2-ADA (4.6 kDa), PEG4-ADA (10 kDa), and PEG8-ADA (20 kDa) with varying weight percentages (w/v%) of alginate in the sample solution and molar ratio between ADA and CD. Gelation is the strength of the hydrogel formed by the above mentioned variables and represented by the color (red = no hydrogel, yellow = weak hydrogel, and green = good hydrogel)

PEG2-ADA:Alg-CD



PEG4-ADA:Alg-CD



PEG8-ADA:Alg-CD

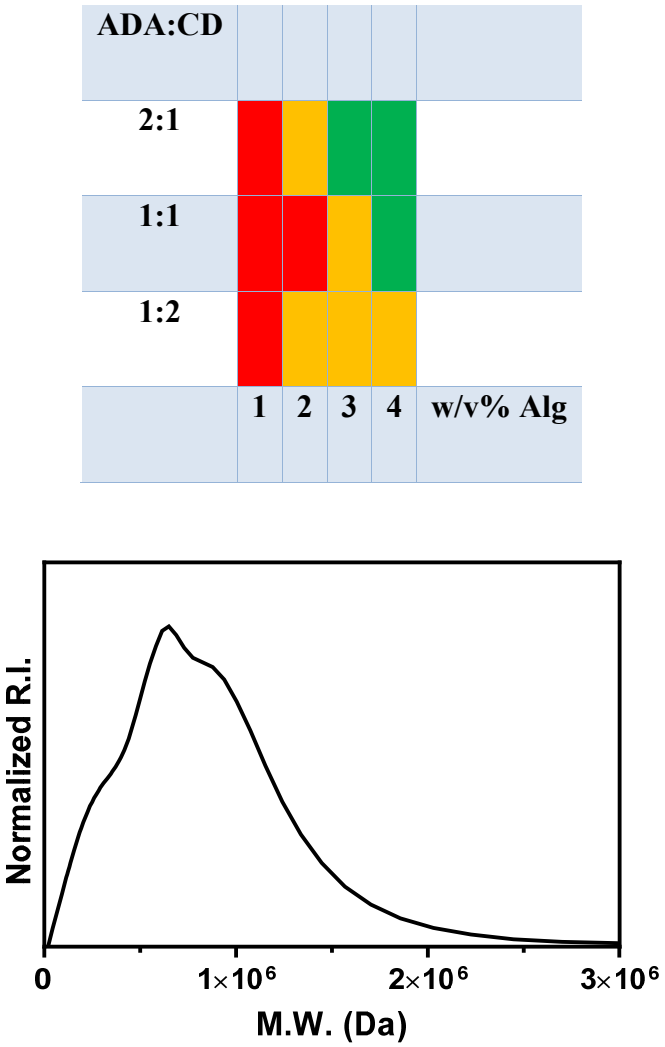


Figure S13. GPC trace of alginate in 100 mM sodium nitrate as mobile phase. Measurement was carried out on a Shimadzu Prominence LC equipped with refractive index and photodiode array detector. Sample was analyzed on a Tosoh G4000PWXL column (7.8 x 300 mm) with a flowrate of 0.4 mL/min. Poly(ethylene glycol) standards were used for calibration.

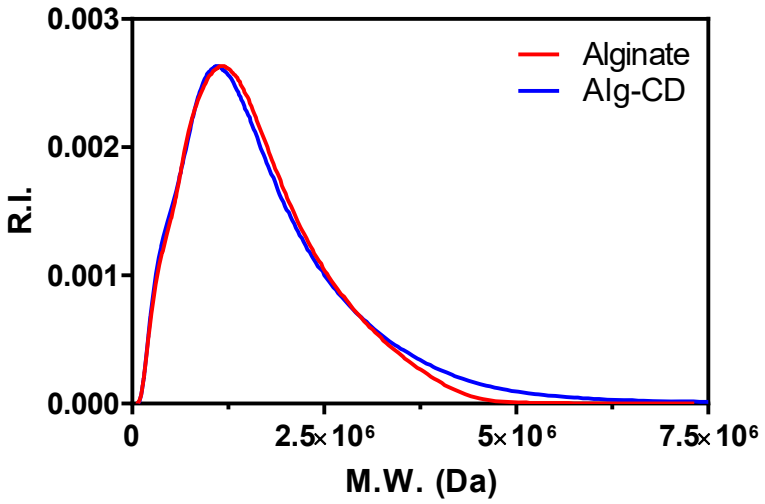


Figure S14. GPC traces of alginate and Alg-CD measured in H_2O (0.05% NaN_3) at 30 °C. Pullulan standards were used for calibration.

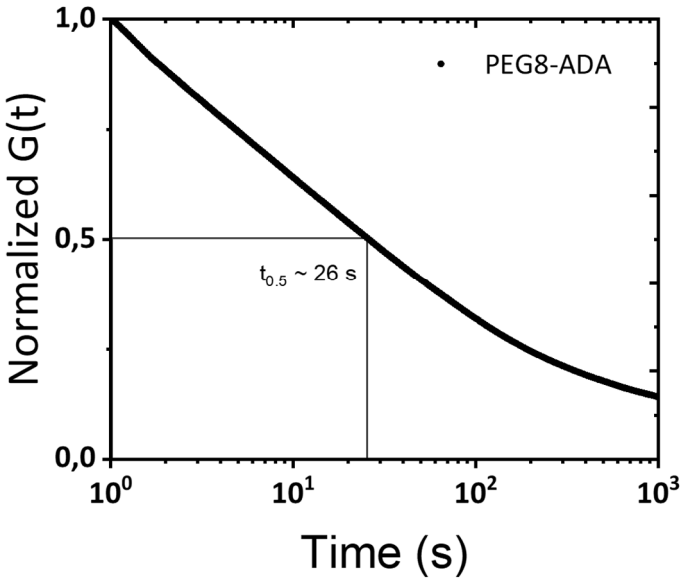


Figure S15. Stress relaxation of PEG8-ADA 4wt%. Stress relaxation measurements were performed at 20 °C and 2 % strain, after pre-equilibration.

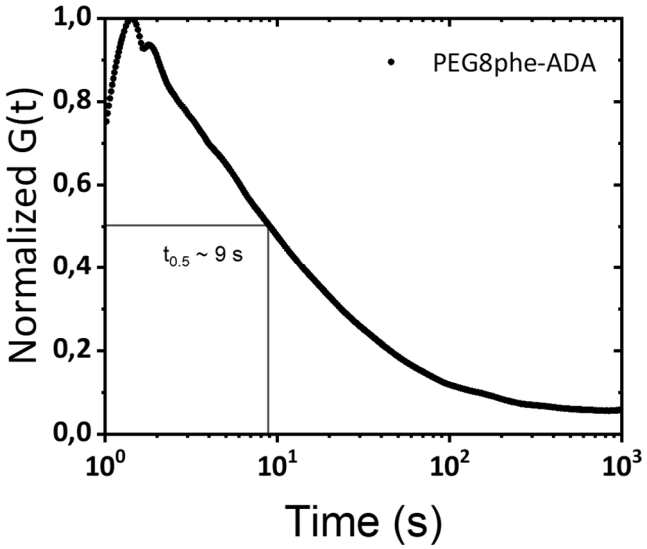


Figure S16. Stress relaxation of PEG8phe-ADA 4wt%. Stress relaxation measurements were performed at 20 °C and 2 % strain, after pre-equilibration