Electronic Supplementary Material (ESI) for Polymer Chemistry.
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Diblock brush-arm star copolymers via a core-first/graft-from approach using γ-cyclodextrin and ROMP: a modular platform for drug delivery

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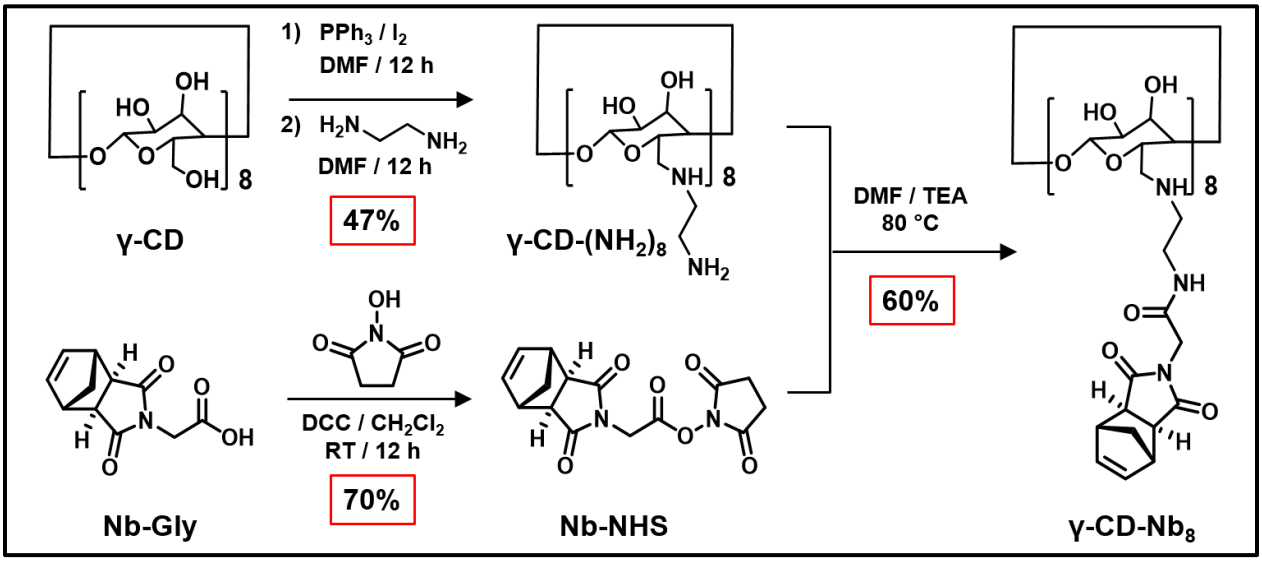
Section A. Materials / General Methods / Instrumentation

All reagents were purchased from commercial suppliers, used without further purification, and dried under vacuum prior to reaction unless stated otherwise. All reactions were performed under nitrogen (N_2) or argon (Ar) gas unless otherwise stated. Column chromatography was carried out on silica gel 60F (EMD Millipore, 0.040–0.063 mm). Polymerization of all polymers was performed under an inert atmosphere of UHP N_2 in glovebox using a modified Grubbs' 3rd generation catalyst that was prepared according to a previously reported protocol[1]. All nuclear magnetic resonance (NMR) spectra were recorded on Varian Inova-spectrometer at 25 °C, with working frequencies of 500 (^{1}H) and 125 (^{13}C) MHz. Chemical shifts are reported in ppm relative to the signals corresponding to the residual non-deuterated solvent: $CDCl_3$: δ_H = 7.26 and δ_C = 77.16 ppm; $(CD_3)_2SO$: δ_H = 2.50 ppm and δ_C = 39.52 ppm; D_2O: δ_H = 4.79 ppm. High-resolution mass spectrometry (HRMS) data was recorded on a Bruker maXis 4G UHR-TOF mass spectrometer. Infrared spectroscopy (IR) was performed on a Bruker Alpha Platinum ATR FT-IR spectrometer. Matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was recorded on Bruker Solaris 12T FT-MS, sample was prepared using 2´,4´,6´-trihydroxyacetophenone as matrix in DMF. Preparative gel permeation chromatography (GPC) analyses were performed on Japan Analytical Industry LaboACE instrument with one JAIGEL-2HR column and one JAIGEL-2.5HR column in tandem, running with dimethylformamide (DMF) at 8 mL/min. Analytical GPC analyses were performed on an Agilent 1260 Infinity setup with three PSS NOVEMA MAX Lux analytical 100 Å columns in tandem and in H_2O mobile phase (0.025 M Na_2SO_4) at 50 °C with 1.0 mL/min flow rate. The differential refractive index (dRI) of each compound was monitored using a Wyatt Optilab T-rEX detector and the light scattering (LS) of each compound was monitored using a Wyatt Dawn Heleos-II detector. Ultraviolet-visible-near infrared (UV-Vis-NIR) absorbance spectra were recorded on an Agilent Cary 5000 spectrophotometer with a PbSmart NIR detector. Fluorescence emission spectra were recorded

on a Nanolog UV-Vis-NIR spectrofluorimeter (excitation wavelength, 490 nm; excitation slit width, 4 nm; emission wavelength, 597, 542 nm; emission slit width, 5 nm). Dynamic light scattering (DLS) data were recorded on Malvern ZEN3600 equipment. Transmission electron microscopy (TEM) images were recorded on JEOL 2100F Transmission Electron Microscope, samples were prepared using UC-A on lacey, 400 mesh Cu TEM grid, and were stained by 2% uranyl acetate. Thermogravimetric analysis (TGA) was performed on a TA Instruments TGA5000 using a high temperature platinum pan and 3 mg of sample that was heated from 25 to 800 ºC at a rate of 10 ºC/min. Differential scanning calorimetry (DSC) was performed on a TA Instruments DSC2500 with 18–22 mg of sample massed into a Tzero aluminum pan, which was sealed with a Tzero hermetic lid. Samples were first equilibrated at 200 ºC for 10 minutes, followed by cooling to –50 ºC at 5 ºC/min, heating to 200 ºC at 5 ºC/min and cooling to –50 ºC at 5 ºC/min, data is reported from the second heating and cooling cycle. Powder X-ray diffraction (PXRD) was done using a Bruker d8 Advance X-ray diffractometer with zero background silicon sample holder (MTI), samples were prepared by grinding the as-synthesized polymers into a fine powder.

Section B. Synthetic Protocols

1) Synthesis of γ-CD-Nb$_8$



Scheme S1. Synthetic Route of **γ-CD-Nb$_8$**

a) γ-CD-I$_8$

Based on literature,[2] a modified method was employed here. A solution of I_2 (3.60 g, 14.17 mmol, 15.32 equiv.) in DMF (anhydrous, 4.6 mL) was slowly added to a solution of PPh_3 (3.62 g, 13.80 mmol, 14.92 equiv.) in DMF (anhydrous,18 mL) at ambient temperature. After stirring for 30 min, γ-CD (1.2 g, 0.925 mmol, 1 equiv.) was added. The reaction was heated up to 70 °C and stirred for 24 h. After cooling down, a suspension of CH_3ONa (0.87 g, 16.10 mmol, 17.41 equiv.) in CH_3OH (6 mL) was added dropwise and stirred at ambient temperature for 30 min to quench the reaction. Once completed, the organic solvent was removed. The crude material was re-dissolved in a minimal amount of DMF and precipitated by adding Me_2CO. The precipitation was performed three times to yield the desired compound as a white powder (1.2 g, 59% yield). ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ_H 5.97 (s, 16H), 5.03 (d, J = 3.7 Hz, 8H), 3.82 (d, J = 9.1 Hz, 8H), 3.61 (dd, J = 14.6, 8.1 Hz, 16H), 3.41 (dd, J = 18.1, 9.5 Hz, 16H), 3.27 (d, J = 9.2 Hz, 8H). ^{13}C NMR (125 MHz, $(CD_3)_2SO$): δ_C 101.94, 85.25, 72.38, 71.81, 71.08, 9.25.

b) ***γ-CD-(NH$_2$)$_8$***

γ-CD-I$_8$ (0.50 g, 0.2297 mmol, 1 equiv.) was dissolved in ethylenediamine (13.78 g, 15.3 mL, 1000 equiv.). The reaction was stirred at 80 °C for 12 h. After completion of the reaction, the organic solvent and excess ethylenediamine were removed. The crude material was re-dissolved in a minimal amount of H_2O and precipitated by adding Me_2CO. The solid was collected and lyophilized to yield the product as a pale-yellow powder (0.30 g, 80% yield). ^{1}H NMR (500 MHz, D_2O): δ_H 5.36 – 5.01 (br, 8H), 4.19 – 3.79 (br, 16H), 3.63 – 3.41 (br, 16H), 3.04 – 3.69 (br, 32H). MALDI-TOF (m/z): found, 1655.9 $[M+Na]^+$; 1633.9 $[M+H]^+$; 1595.8 $[M+Na]^+$-60 (M_1, $NH_2CH_2CH_2NH_2$); 1573.8 $[M+H]^+$-M_1; 1535.7 $[M+Na]^+$-2M_1; 1513.8 $[M+H]^+$-2M_1; 1475.7 $[M+Na]^+$-3M_1; 1453.7 $[M+H]^+$-3M_1; 1393.6 $[M+H]^+$-4M_1. This data matches the previously reported data.[3]

c) ***Nb-Gly***

A modified method was conducted here according to the literature.[4, 5] *cis*-5-Norbornene-exo-2,3-dicarboxylic anhydride (3.28 g, 0.02 mol, 1 equiv.) and glycine (1.50 g, 0.02 mmol, 1 equiv.) were added to a 14/20 neck, 50 mL round bottom flask and heated to 140 °C for 30 min (melt). After completion of the reaction, the crude mixture was cooled to room temperature to yield the desired compound as a white solid without further purification (4.78 g, quantitative). ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 6.30 (s, 2H); 4.27 (s, 2H); 3.31 (s, 2H); 2.76 (s, 2H); 1.60 (d, J = 10.0 Hz, 1H); 1.50 (d, J = 9.9 Hz, 1H).^{13}C NMR (125 MHz, $CDCl_3$): δ_C 177.38, 171.95, 138.11, 48.16, 45.55, 42.97, 39.27.

d) ***Nb-NHS***

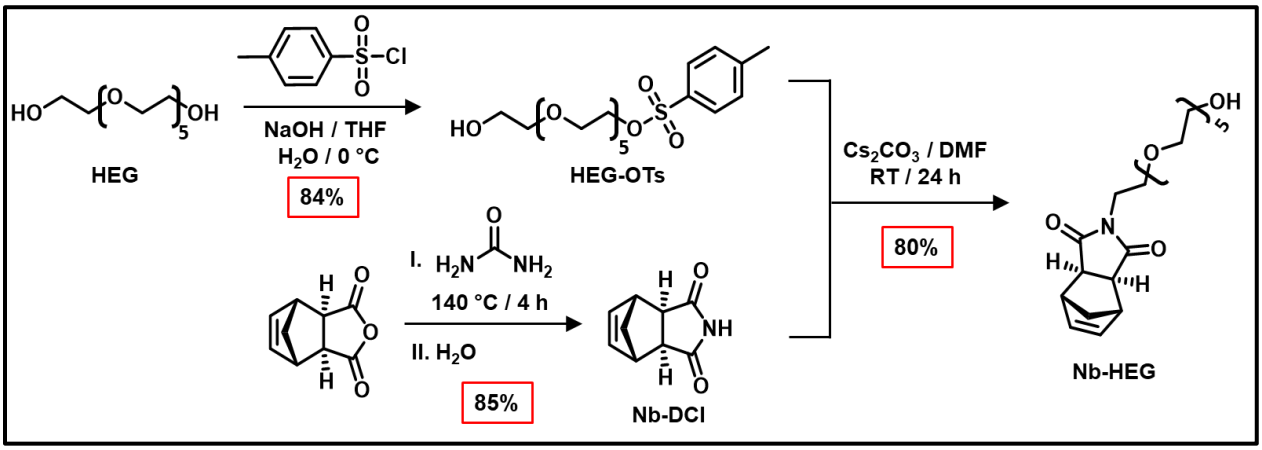
Based on a previous literature method,[5] *N,N′*-dicyclohexylcarbodiimide (DCC, 1.85 g, 8.96 mmol, 1 equiv.) and **Nb-Gly** (2.00 g, 8.96 mmol, 1 equiv.) were dissolved in CH_2Cl_2 (anhydrous, 15 mL) and stirred at room temperature for 15 min until a precipitate formed. *N*-hydroxysuccinimide (1.13 g, 9.86 mmol, 1.1 equiv.) was added to the precipitated solution and stirred at room temperature for 12 h. After filtration of DCU byproduct, the crude product was recrystallized with EtOAc and

CH_2Cl_2 to yield the product as a white solid (2.0 g, 70 % yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 6.30 (t, J = 1.8 Hz, 2H); 4.57 (s, 2H); 3.39 – 3.26 (m, 2H); 2.83 (s, 4H); 2.78 (d, J = 1.1 Hz, 2H); 1.61 (s, 2H); 1.56 (d, J = 10.0 Hz, 1H), 1.51 (d, J = 10.1 Hz, 1H).^{13}C NMR (125 MHz, $CDCl_3$): δ_C 176.46, 168.26, 163.26, 138.12, 48.16, 45.62, 43.00, 37.27, 25.67.

e) γ-CD-Nb$_8$

To the mixture of **γ-CD-(NH$_2$)$_8$** (93.9 mg, 0.057 mmol, 1 equiv.) and **Nb-NHS** (150 mg, 0.471 mmol, 8.2 equiv.), DMF (anhydrous, 3 mL) and triethylamine (300 µL) were added. The reaction was stirred at 80 °C for 12 h. After completion of the reaction, the organic solvent was removed, the crude product was re-dissolved in minimal amount of DMF and precipitated by adding Me_2CO. The precipitation was performed three times to yield the product as a brown solid (111 mg, 60% yield). ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ_H 8.19 – 7.79 (m, 8H), 6.30 (s, 16H), 5.22 – 4.75 (m, 8H), 4.67 – 4.14 (m, 8H), 4.13 – 3.46 (m, 32H), 3.43 – 1.96 (m, 112H), 1.82 – 1.57 (m, 8H), 1.40 – 1.25 (m, 8H). IR spectroscopy, see Figure S3.

2) Synthesis of Nb-HEG



Scheme S2. Synthetic Route of **Nb-HEG**

a) HEG-OTs

This compound was reported previously.[6-8] Briefly, hexaethylene glycol (HEG, 15.00 g, 53.13 mmol, 1.75 equiv.) was dissolved in THF (15 mL) and cooled to 0 °C. A solution of NaOH (1.82 g, 45.53 mmol, 0.57 equiv.) in H_2O (15 mL) was added slowly to the cooled reaction mixture. A

solution of *p*-toluenesulfonyl chloride (5.78 g, 30.32 mmol, 1.0 equiv.) in THF (120 mL) was added dropwise. The resulting solution was stirred for 3 h at 0 °C. After completion of the reaction, the crude mixture was poured onto ice, CH_2Cl_2 was added, the organic layer was washed with H_2O and brine, and the organic layer was dried over Na_2SO_4. The compound was further purified by flash column chromatography (silica gel, 100:1 CH_2Cl_2:MeOH) to yield the desired compound as colorless oil (11.2 g, 84% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 7.77 (d, J = 8.3 Hz, 2H); 7.34 – 7.30 (m, 2H); 4.15 – 4.12 (m, 2H); 3.71 – 3.57 (m, 20H); 3.56 (s, 4H); 2.88 (s, 1H); 2.42 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 144.86, 133.10, 129.90, 128.05, 72.58, 70.80, 70.68, 70.64, 70.62, 70.60, 70.59, 70.40, 69.35, 68.75, 61.80, 21.72.

b) ***Nb-DCI***

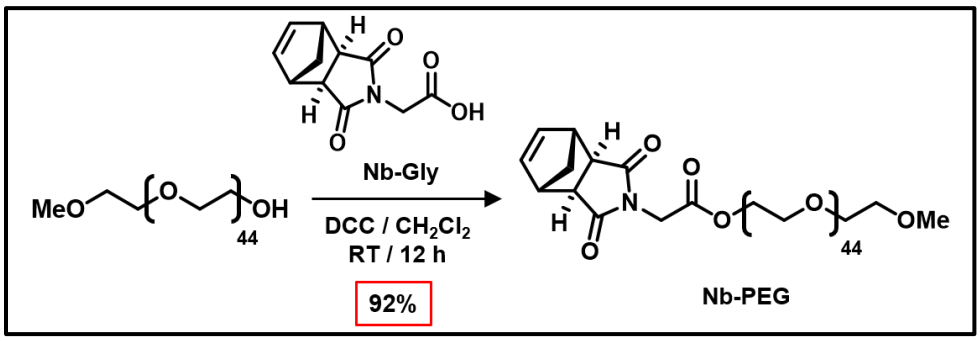
According to literature,[9, 10] a modified method was used to synthesize this compound. *cis*-5-Norbornene-exo-2,3-dicarboxylic anhydride (2.00 g, 12.20 mmol, 1 equiv.) and urea (1.47 g, 24.40 mmol, 2 equiv.) were added to a 14/20 neck, 25 mL round bottom flask and heated to 140 °C for 4 h (melt). After completion of the reaction, H_2O (10 mL) was added and the solution was heated until a homogeneous solution formed. The resulting solution was cooled to room temperature and crystals were collected via filtration and washed several times with H_2O to yield the desired product as a white, crystalline solid (**Nb-DCI**, 1.70 g, 85% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 6.28 (t, J = 1.7 Hz, 2H); 3.29 (m, 2H); 2.74 (m, 2H); 1.57 (d, J = 9.9 Hz, 1H); 1.46 (d, J = 10.0 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 178.26, 137.90, 49.32, 45.31, 43.06.

c) ***Nb-HEG***

Nb-DCI (1.08 g, 6.60 mmol, 1.1 equiv.) and Cs_2CO_3 (9.77 g, 30.00 mmol, 5 equiv.) were dissolved in DMF (anhydrous, 15 mL) and the solution was stirred at room temperature for 1 h. A solution of **HEG-OTs** (2.60 g, 6.00 mmol, 1 equiv.) in DMF (anhydrous, 20 mL) was added and stirred at room temperature for an additional 24 h. After completion of the reaction, the resulting unwanted precipitate was removed via filtration, followed by removal of the organic solvent. The residue was

re-dissolved in CH_2Cl_2, washed with H_2O, and dried over Na_2SO_4. The compound was further purified by flash column chromatography (silica gel, 100:1 CH_2Cl_2: MeOH) to yield the desired product as a colorless oil (2.05 g, 80% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 7.77 (d, J = 8.3 Hz, 2H); 7.34 – 7.30 (m, 2H); 4.15 – 4.12 (m, 2H); 3.71 – 3.57 (m, 20H); 3.56 (s, 4H); 2.88 (s, 1H); 2.42 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 144.86, 133.10, 129.90, 128.05, 72.58, 70.80, 70.68, 70.64, 70.62, 70.60, 70.59, 70.40, 69.35, 68.75, 61.80, 21.72. ESI-MS (m/z), calculated for $C_{21}H_{33}NO_8$, 427.2206; found, 450.2122 $[M+Na]^+$.

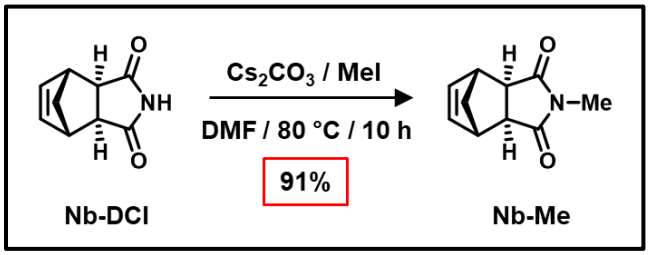
3) Synthesis of Nb-PEG



Scheme S3. Synthetic Route of **Nb-PEG**

To the mixture of **Nb-Gly** (4.38 g, 0.0198 mmol, 1.5 equiv.), poly(ethylene glycol) methyl ether (M_n=2000, 26.4 g, 0.013 mmol, 1 equiv.), *N,N′*-dicyclohexylcarbodiimide (DCC, 3.30 g, 0.016 mmol, 1.2 equiv.), and 4-dimethylaminopyridine (DMAP, 0.38 g, 0.003 mmol, 0.24 equiv.), CH_2Cl_2 (anhydrous, 50 mL) was added. After stirring at room temperature for 24 h, the DCU byproduct was filtered, the crude product was purified by preparative GPC with DMF as eluent to yield the product as a white solid (26.5 g, 92% yield). ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ_H 6.32 (t, J = 1.5 Hz, 2H), 4.21 – 4.18 (m, 4H), 3.66 – 3.63 (m, 2H), 3.61 – 3.58 (m, 3H), 3.57 – 3.48 (m, 160H), 3.43 (m, 3H), 3.38 – 3.35 (m, 3H), 3.24 (s, 4H), 3.13 (s, 2H), 1.60 (d, J = 9.4 Hz, 2H), 1.36 (d, J = 9.7 Hz, 2H). ESI-MS (m/z): calculated for $C_{14}H_{16}NO_5(C_2H_4O)_{44}$, 2217.2669; found, 2217.2710 $[M+H]^+$.

4) Synthesis of Nb-Me



Scheme S4. Synthetic Route **of Nb-Me**

This compound was synthesized using a modified procedure from literature.[11] **Nb-DCI** (0.2 g, 1.23 mmol, 1 equiv.) and Cs_2CO_3 (0.802 g, 2.46 mmol, 2 equiv.) were dissolved in DMF and stirred for 30 min. Methyl iodide (0.524 g, 0.23 mL, 3.69 mmol, 3 equiv.) was added and the resulting mixture was heated to 80 °C for 10 h. After completion of the reaction, the resulting unwanted precipitate was filtered, and the organic solvent was removed. The residue was re-dissolved in DCM, washed with H_2O, and dried over Na_2SO_4 to yield the desired product as a white solid (198 mg, 91% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 6.27 – 6.25 (m, 2H); 3.26 – 3.24 (m, 2H); 2.95 (s, 3H); 2.68 (s, 2H); 1.52 – 1.48 (m, 1H); 1.21 – 1.16 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 178.26, 137.86, 48.07, 45.25, 43.03, 24.73.

5) Synthesis of CD-(HEG$_5$)$_8$

A solution of modified Grubbs 3rd generation catalyst (G3G, 0.1 M) was freshly prepared in DMF. G3G (0.0245 mL, 1.78 mg, 2.45 µmol, 8 equiv.) was added to a solution of **γ-CD-Nb$_8$** (1.56 mM, 0.172 mL, 1.00 mg, 0.31 µmol, 1 equiv.) in DMF to give G3G: **γ-CD-Nb$_8$** ratio of 8:1. The resulting solution was stirred for 2 h at room temperature. Next, **Nb-HEG** (10.49 mg, 245.4 µmol, 80 equiv.) was added and stirred at room temperature for 5 h. After the completion of the polymerization, the reaction was quenched by the addition of excess ethyl vinyl ether (EVE) and stirred for 30 min, then the excess EVE and DMF were removed by vacuum to yield the star polymer as a brown solid.

6) Synthesis of CD-(HEG$_7$)$_8$

A solution of modified Grubbs 3rd generation catalyst (G3G, 0.1 M) was freshly prepared in DMF. G3G (0.0245 mL, 1.78 mg, 2.45 μM, 8 equiv.) was added to a solution of **γ-CD-Nb$_8$** (1.56 mM, 0.172 mL, 1.00 mg, 0.31 μmol, 1 equiv.) in DMF to give G3G: **γ-CD-Nb$_8$** ratio of 8:1. The resulting solution was stirred for 2 h at room temperature. Next, **Nb-HEG** (20.99 mg, 49.09 μmol, 160 equiv.) was added and stirred at room temperature for 5 h. After the completion of the polymerization, the reaction was quenched by the addition of excess ethyl vinyl ether (EVE) and stirred for 30 min, then the excess EVE and DMF were removed by vacuum to yield the star polymer as a brown solid.

7) Synthesis of CD-(HEG$_{13}$)$_8$

A solution of modified Grubbs 3rd generation catalyst (G3G, 0.1 M) was freshly prepared in DMF. G3G (0.0216 mL, 1.96 mg, 2.70 μmol, 8 equiv.) was added to a solution of **γ-CD-Nb$_8$** (1.56 mM, 0.189 mL, 1.10 mg,0.34 μmol, 1 equiv.) in DMF to give G3G: **γ-CD-Nb$_8$** ratio of 8:1. The resulting solution was stirred for 2 h at room temperature. Next, **Nb-HEG** (34.63 mg, 81.00 μmol, 240 equiv.) was added and stirred at room temperature for 5 h. The reaction was quenched by the addition of excess ethyl vinyl ether (EVE) and stirred for 30 min, then the excess EVE and DMF were removed by vacuum to yield the star polymer as a brown solid.

8) Synthesis of CD-(HEG$_{17}$)$_8$

A solution of modified Grubbs 3rd generation catalyst (G3G, 0.1 M) was freshly prepared in DMF. G3G (0.0245 mL, 1.78 mg, 2.45 μmol, 8 equiv.) was added to a solution of **γ-CD-Nb$_8$** (1.56 mM, 0.172 mL, 1.00 mg, 0.28 μmol, 1 equiv.) in DMF to give G3G: **γ-CD-Nb$_8$** ratio of 8:1. The resulting solution was stirred for 2 h at room temperature. Next, **Nb-HEG** (52.45 mg, 122.7 μmol, 400 equiv.) was added and stirred at room temperature for 5 h. The reaction was quenched by the addition of excess ethyl vinyl ether (EVE) and stirred for 30 min, then the excess EVE and DMF were removed by vacuum to yield the star polymer as a brown solid.

9) Synthesis of CD-(HEG$_{23}$)$_8$

A solution of modified Grubbs 3rd generation catalyst (G3G, 0.1 M) was freshly prepared in DMF. G3G (0.0221 mL, 1.60 mg, 2.21 μmol, 8 equiv.) was added to a solution of **γ-CD-Nb$_8$** (1.56 mM, 0.155 mL, 0.90 mg, 0.28 μmol, 1 equiv.) in DMF to give G3G: **γ-CD-Nb$_8$** ratio of 8:1. The resulting solution was stirred for 2 h at room temperature. Next, **Nb-HEG** (66.10 mg, 154.6 μmol, 560 equiv.) was added and stirred at room temperature for 5 h. The reaction was quenched by the addition of excess ethyl vinyl ether (EVE) and stirred for 30 min, then the excess EVE and DMF were removed by vacuum to yield the star polymer as a brown solid.

10) Synthesis of CD-(HEG$_7$-PEG$_9$)$_8$

A solution of modified Grubbs 3rd generation catalyst (G3G, 0.1 M) was freshly prepared in DMF. G3G (0.0245 mL, 1.78 mg, 2.45 μmol, 8 equiv.) was added to a solution of **γ-CD-Nb$_8$** (1.56 mM, 0.172 mL, 1.00 mg, 0.31 μmol, 1 equiv.) in DMF to give G3G: **γ-CD-Nb$_8$** ratio of 8:1. The resulting solution was stirred for 2 h at room temperature. Next, **Nb-HEG** (10.49 mg, 24.54 μmol, 80 equiv.) was added and stirred at room temperature for 5 h, then **Nb-PEG** (54.42 mg, 24.54 μmol, 80 equiv.) was added and stirred for 3 h. The reaction was quenched by the addition of excess ethyl vinyl ether (EVE) and stirred for 30 min. Then, the excess EVE and DMF were removed by vacuum to yield the star polymer as a brown solid. For binding affinity, drug release and cytotoxicity studies, the polymer was first dialyzed against DMF (RC dialysis tubing, 1 kDa molecular weight cut-off, 38 mm flat-width) to remove the G3G catalyst residue, and then dialyzed against H_2O to remove DMF, the polymer inside the dialysis tubing was collected and lyophilized for 24 h.

11) Synthesis of CD-(HEG$_5$-PEG$_{14}$)$_8$

A solution of modified Grubbs 3rd generation catalyst (G3G, 0.1 M) was freshly prepared in DMF. G3G (0.0245 mL, 1.78 mg, 2.45 μmol, 8 equiv.) was added to a solution of **γ-CD-Nb$_8$** (1.56 mM, 0.172 mL, 1.00 mg, 0.31 μmol, 1 equiv.) in DMF to give G3G: **γ-CD-Nb$_8$** ratio of 8:1. The resulting

solution was stirred for 2 h at room temperature. Next, **Nb-HEG** (20.99 mg, 49.09 μmol, 160 equiv.) was added and stirred at room temperature for 5 h, then **Nb-PEG** (108.83 mg, 49.09 μmol, 160 equiv.) was added and stirred for 3 h. The reaction was quenched by the addition of excess ethyl vinyl ether (EVE) and stirred for 30 min, then the excess EVE and DMF were removed by vacuum to yield the star polymer as a brown solid.

12) Synthesis of CD-(HEG$_9$-PEG$_{13}$)$_8$

A solution of modified Grubbs 3rd generation catalyst (G3G, 0.1 M) was freshly prepared in DMF. G3G (0.0245 mL, 1.78 mg, 2.45 μmol, 8 equiv.) was added to a solution of **γ-CD-Nb$_8$** (1.56 mM, 0.172 mL, 1.00 mg, 0.31 μmol, 1 equiv.) in DMF to give G3G: **γ-CD-Nb$_8$** ratio of 8:1. The resulting solution was stirred for 2 h at room temperature. Next, **Nb-HEG** (31.48 mg, 73.63 μmol, 240 equiv.) was added and stirred at room temperature for 5 h, then **Nb-PEG** (163.25 mg, 73.63 μmol, 240 equiv.) was added and stirred for 3 h. The reaction was quenched by the addition of excess ethyl vinyl ether (EVE) and stirred for 30 min, then the excess EVE and DMF were removed by vacuum to yield the star polymer as a brown solid.

13) Synthesis of CD-(HEG$_9$-Me$_{10}$-PEG$_{10}$)$_8$

A solution of modified Grubbs 3rd generation catalyst (G3G, 0.1 M) was freshly prepared in DMF. G3G (0.0245 mL, 1.78 mg, 2.45 μmol, 8 equiv.) was added to a solution of **γ-CD-Nb$_8$** (1.56 mM, 0.172 mL, 1.00 mg,0.31 μmol, 1 equiv.) in DMF to give G3G: **γ-CD-Nb$_8$** ratio of 8:1. The resulting solution was stirred for 2 h at room temperature. Next, **Nb-HEG** (10.49 mg, 24.54 μmol, 80 equiv.) was added and stirred at room temperature for 5 h, then **Nb-Me** (4.35 mg, 24.54 μmol, 80 equiv.) was added and stirred at room temperature for 1 h, and **Nb-PEG** (54.42 mg, 24.54 μmol, 80 equiv.) was added and stirred for 3 h. The reaction was quenched by the addition of excess ethyl vinyl ether (EVE) and stirred for 30 min, then the excess EVE and DMF were removed by vacuum to yield the star polymer as a brown solid.

14) Synthesis of CD-(HEG$_{11}$-Me$_9$-PEG$_{15}$)$_8$

A solution of modified Grubbs 3rd generation catalyst (G3G, 0.1 M) was freshly prepared in DMF. G3G (0.0245 mL, 1.78 mg, 2.45 µmol, 8 equiv.) was added to a solution of **γ-CD-Nb$_8$** (1.56 mM, 0.172 mL, 1.00 mg, 0.31 µmol, 1 equiv.) in DMF to give G3G: **γ-CD-Nb$_8$** ratio of 8:1. The resulting solution was stirred for 2 h at room temperature. Next, **Nb-HEG** (20.99 mg, 49.09 µmol, 160 equiv.) was added and stirred at room temperature for 5 h, then **Nb-Me** (4.35 mg, 24.54 µmol, 80 equiv.) was added and stirred at room temperature for 1 h, and **Nb-PEG** (108.83 mg, 49.09 µmol, 160 equiv.) was added and stirred for 3 h. The reaction was quenched by the addition of excess ethyl vinyl ether (EVE) and stirred for 30 min, then the excess EVE and DMF were removed by vacuum to yield the star polymer as a brown solid.

Section C. Spectroscopic Characterization

1) Nuclear Magnetic Resonance (^{1}H NMR)

a) Core

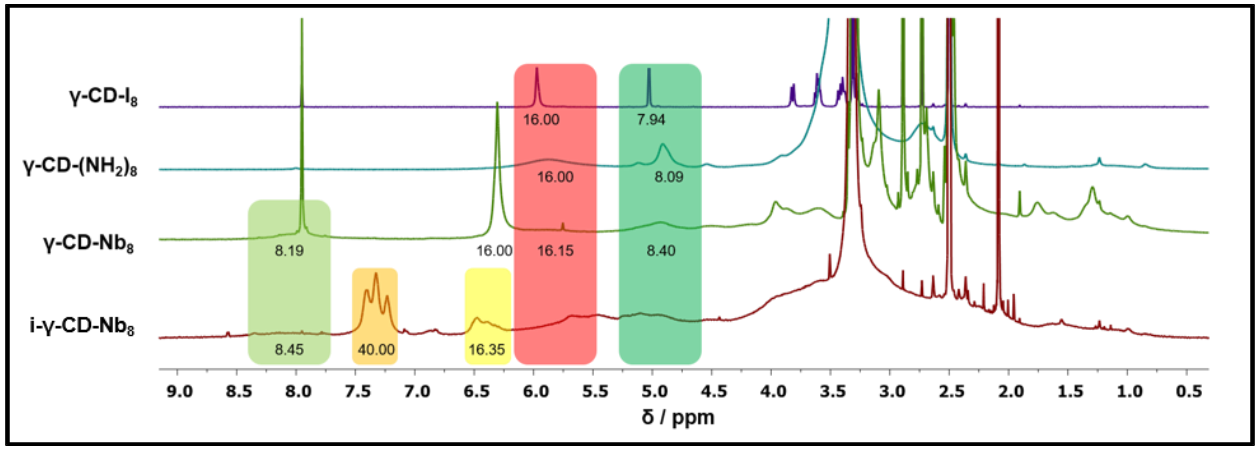


Figure S1. ^{1}H NMR (500MHz, 25 ºC, $(CD_3)_2SO$) of **γ-CD-I$_8$**, **γ-CD-(NH$_2$)$_8$**, **γ-CD-Nb$_8$**, and the initiated core **i-γ-CD-Nb$_8$**. Colors: light green = amide protons; orange = styrene protons; yellow = styrene olefin protons; red and darker green = secondary cyclodextrin OH protons.

b) Monomers/Core Conversion to Star Polymers

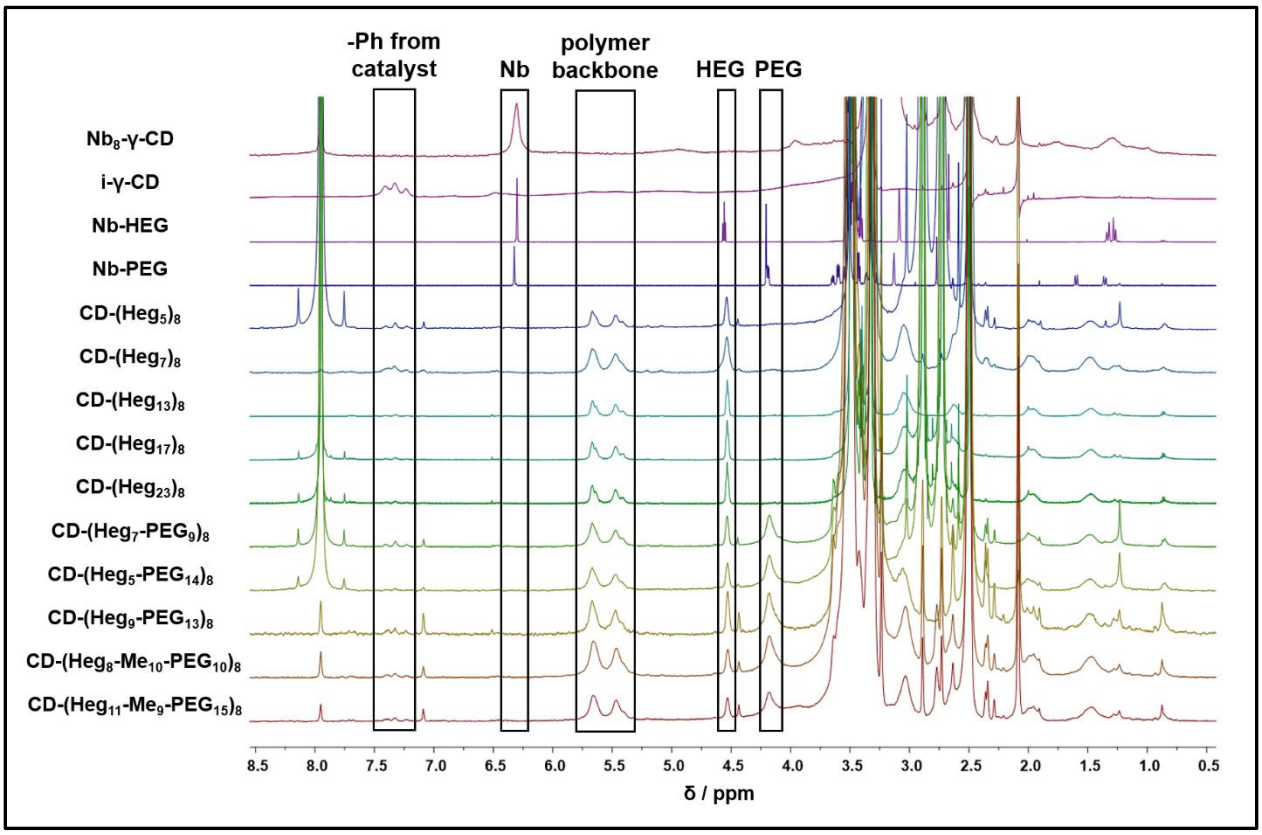


Figure S2. ^{1}H NMR (500 MHz, 25 ºC, $(CD_3)_2SO$) of monomers, core, and polymers.

2) Infrared Spectroscopy (IR)

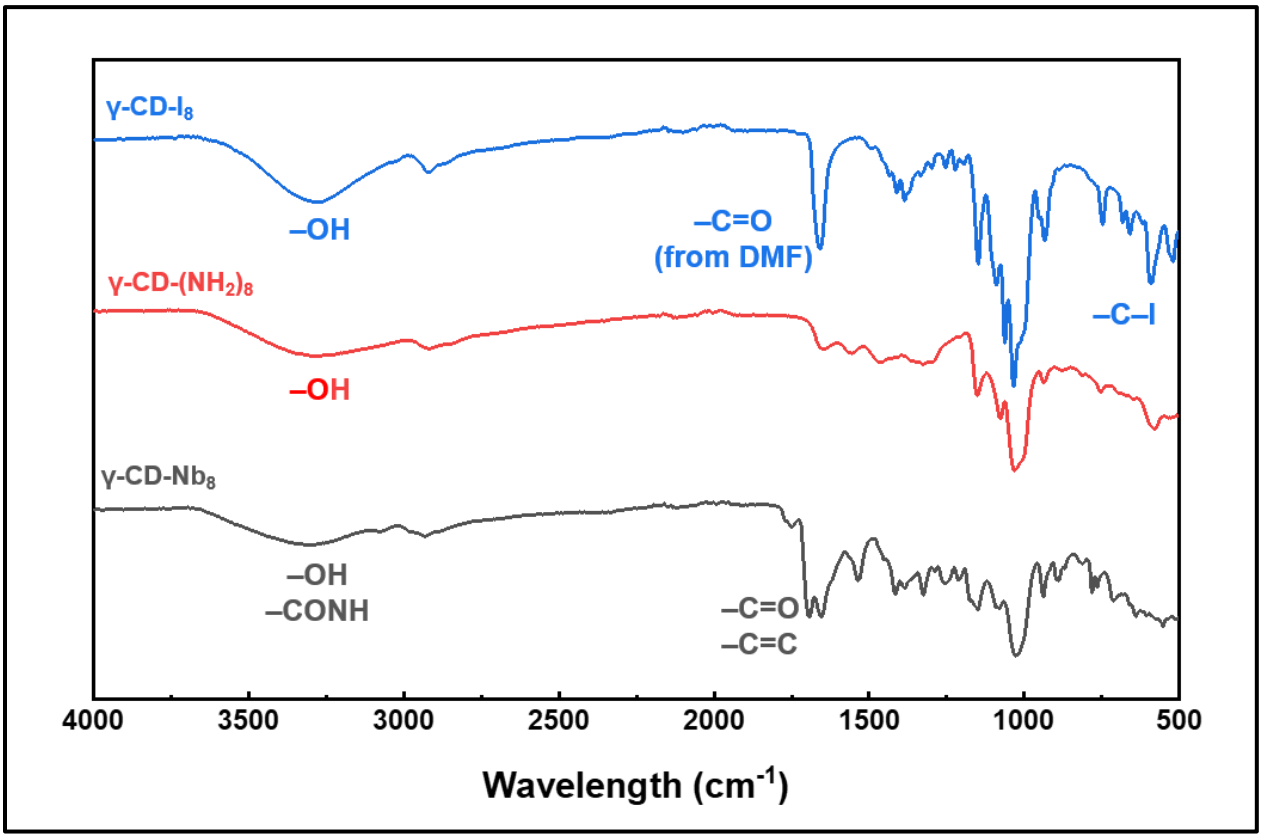


Figure S3. Solid-state IR of **γ-CD-I_8**, **γ-CD-$(NH_2)_8$**, **and γ-CD-Nb_8**.

3) Gel Permeation Chromatography (GPC)

a) GPC Traces of Star Polymers with Homo-block and Diblock Arms

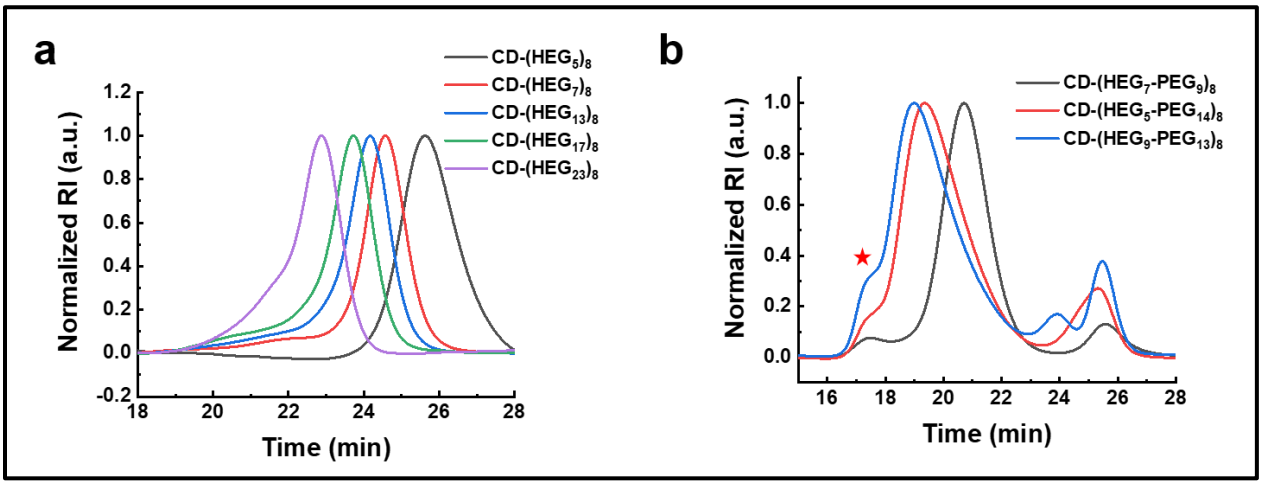


Figure S4. GPC traces of star polymers with **a)** homo-block arms; **b)** diblock arms. Data was collected using three PSS NOVEMA MAX Lux analytical 100 Å columns in tandem and H_2O mobile phase (0.025 M Na_2SO_4) running at 50 ºC. The red star indicates the formation of aggregates at the column threshold.

b) GPC Traces of Star Polymers with Triblock Arms

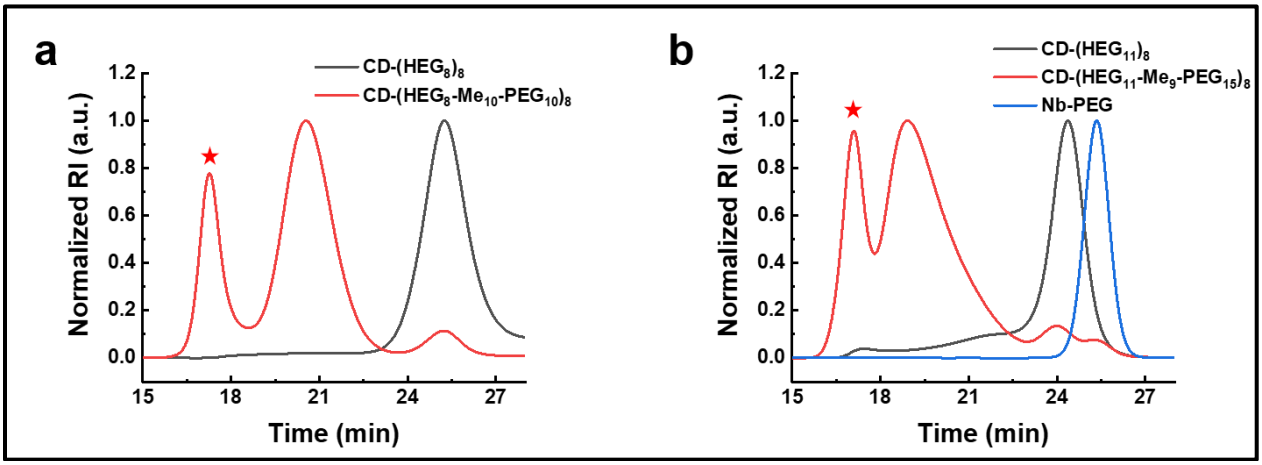


Figure S5. GPC traces of triblock star polymers **a**) **CD-(HEG$_8$-Me$_{10}$-PEG$_{10}$)$_8$**; **b**) **CD-(HEG$_{11}$-Me$_9$-PEG$_{15}$)$_8$**. Data was collected using three PSS NOVEMA MAX Lux analytical 100 Å columns in tandem and H_2O mobile phase (0.025 M Na_2SO_4) running at 23 ºC. The red star indicates the formation of aggregates at the column threshold.

c) Determination of dn/dc Values of Star Polymers

To calculate the dn/dc values of star polymers, different known concentrations (0.2 mg/mL, 0.4 mg/mL, 0.6 mg/mL, 0.8 mg/mL, 1.0 mg/mL) of **CD-(HEG$_7$)$_8$** and **CD-(HEG$_7$-PEG$_9$)$_8$** in the exact same solvent (0.025 M Na_2SO_4 in H_2O) were prepared. The samples were directly injected into the Optilab (U)T-rEX detector without any filtration using a syringe pump (flow rate 0.33 mL/min). The dRI was collected for each injection, and the slope of the dRI-concentration plot describes the dn/dc value of the star polymer.

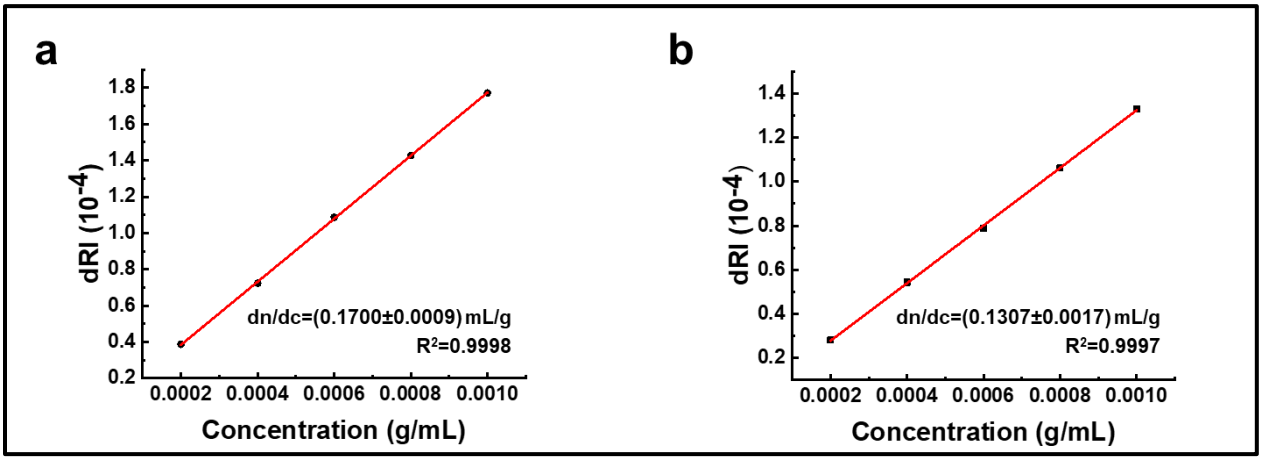


Figure S6. dn/dc determination of star polymers **a**) homo-block arms and **b**) diblock arms.

Based on the acquired data, 0.1700 was used as the dn/dc to calculate the M_w and M_n for star polymers with homo-block arms, and 0.1307 was used for all diblock-arm star polymers. For polymers, dn/dc may depend on the molar mass of the polymer, but when it is above certain molar mass (5-10 kDa), the dn/dc can be considered constant (From Wyatt Technical Note 4000, Batch dn/dc Measurements). Since the star polymer we synthesized are at least 20 kDa, we assume the dn/dc value is very similar if the polymers contain same repeating units, and the only difference is the length of each arm.

d) GPC Trace of γ-CD-Nb$_8$ Initiated with 2 Equiv. Catalyst

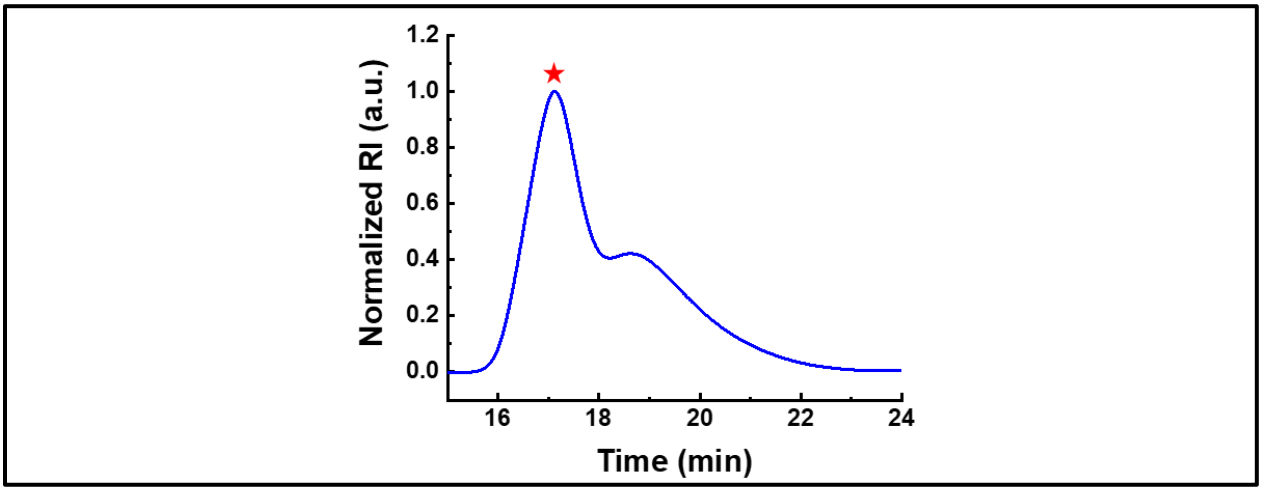


Figure S7. GPC trace of **γ-CD-Nb$_8$** initiated with 2 equiv. catalyst and polymerized to generate **CD-(HEG$_{30}$)$_8$**. Data was collected using three PSS NOVEMA MAX Lux analytical 100 Å columns in tandem and in H_2O mobile phase (0.025 M Na_2SO_4) running at 23 ºC. The red star indicates the formation of aggregates at the column threshold.

4) Dynamic Light Scattering (DLS)

a) Star Polymers with Diblock Arms

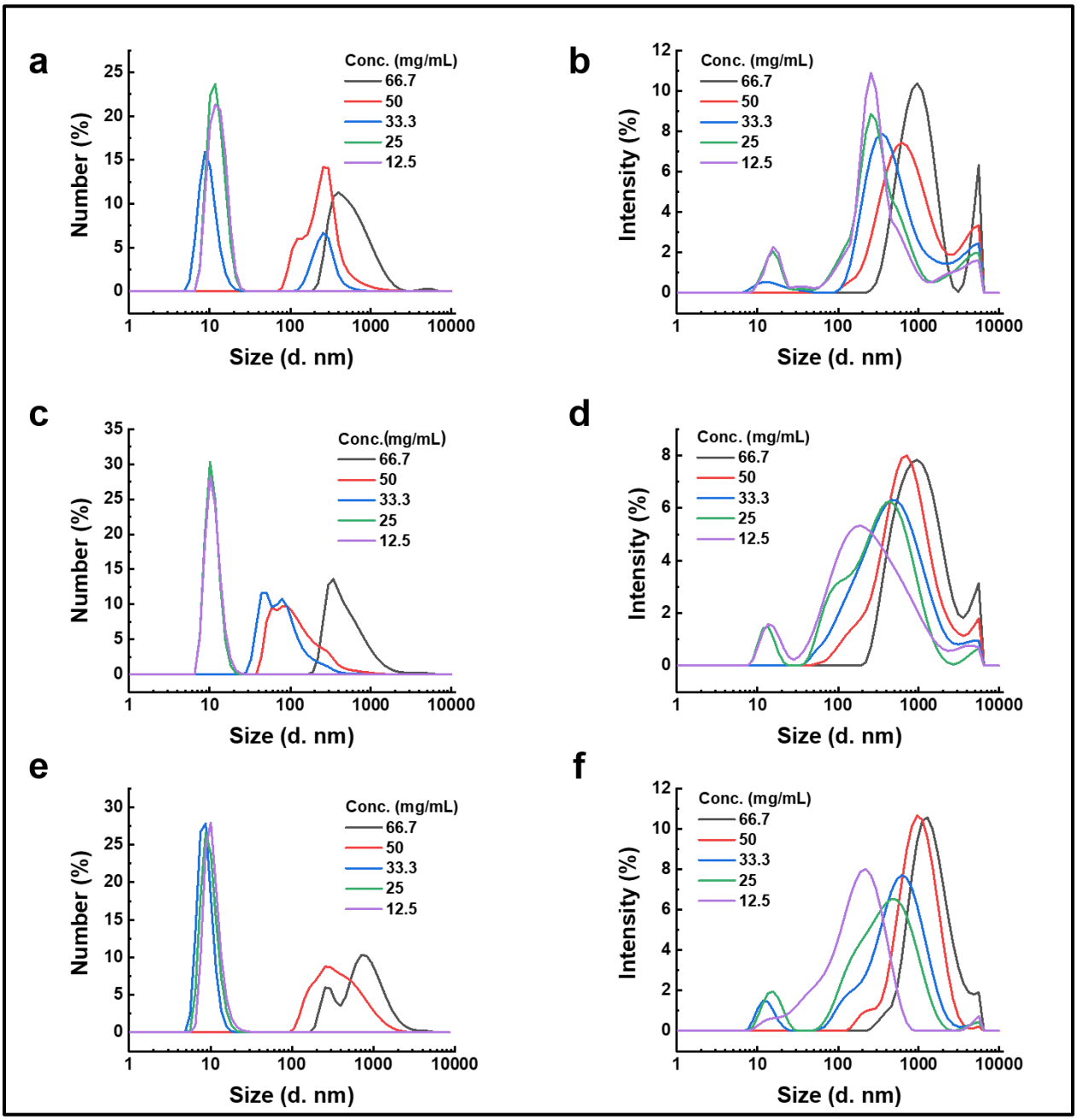


Figure S8. DLS data of star polymers with diblock arms in H_2O at 25 ºC at different concentrations. **a**, **b**) number and intensity distribution of **CD-(HEG$_7$-PEG$_9$)$_8$**; **c**, **d**) number and intensity distribution of **CD-(HEG$_5$-PEG$_{14}$)$_8$**; **e**, **f**) number and intensity distribution of **CD-(HEG$_9$-PEG$_{13}$)$_8$**.

b) Star Polymers with Triblock Arms

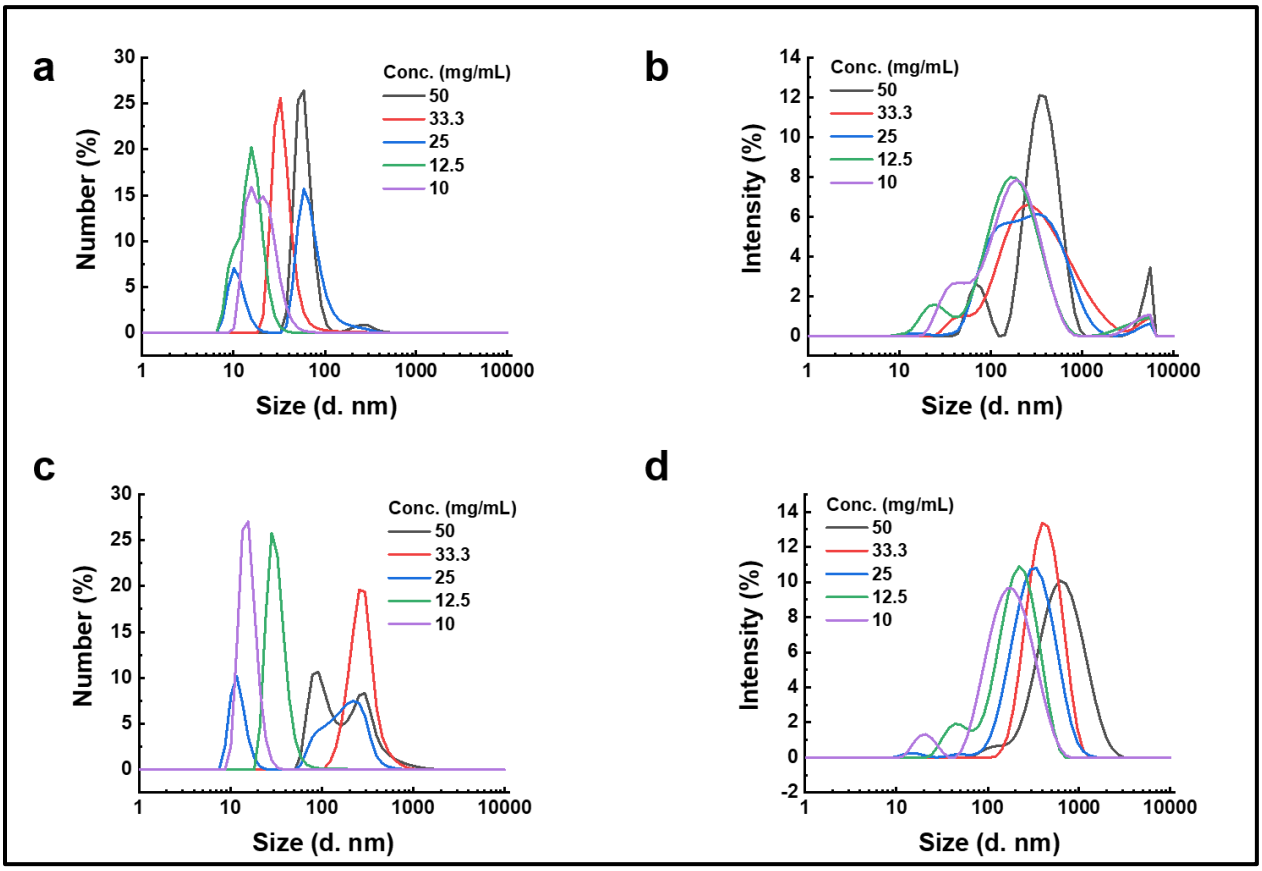


Figure S9. DLS data of star polymers with triblock arms in H_2O at 25 ºC at different concentrations. **a**, **b**) number and intensity distribution of **CD-(HEG$_8$-Me$_{10}$-PEG$_{10}$)$_8$**; **c**, **d**) number and intensity distribution of **CD-(HEG$_{11}$-Me$_9$-PEG$_{15}$)$_8$**.

Section D Spectrometric Characterization

1) Matrix Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF)

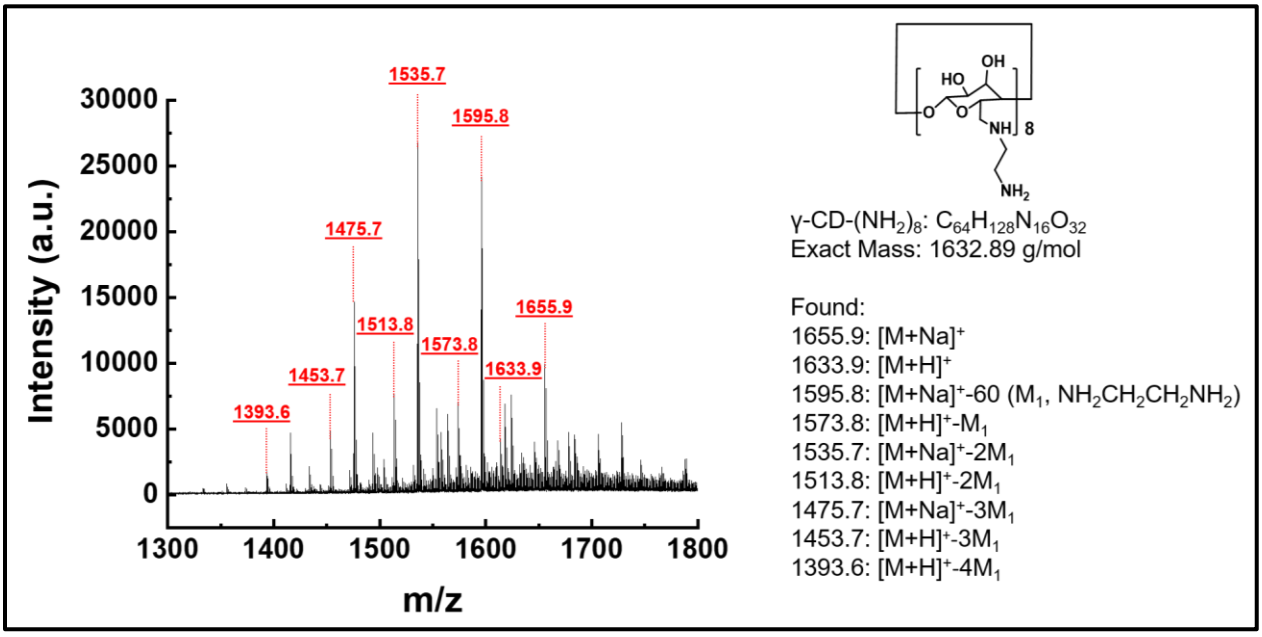


Figure S10. MALDI-TOF MS of **γ-CD-$(NH_2)_8$.**

Section E. Thermal Characterization

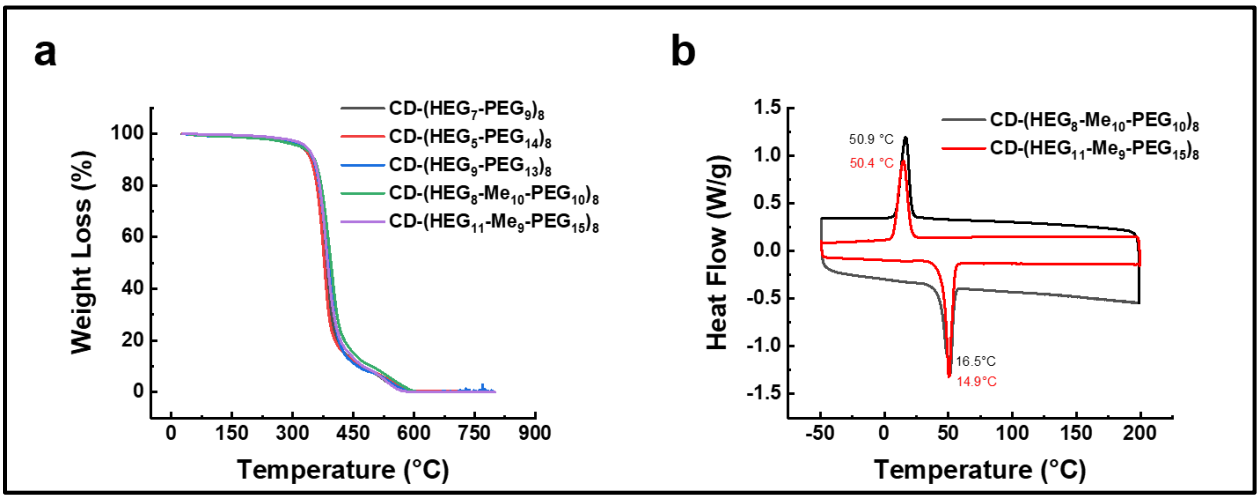
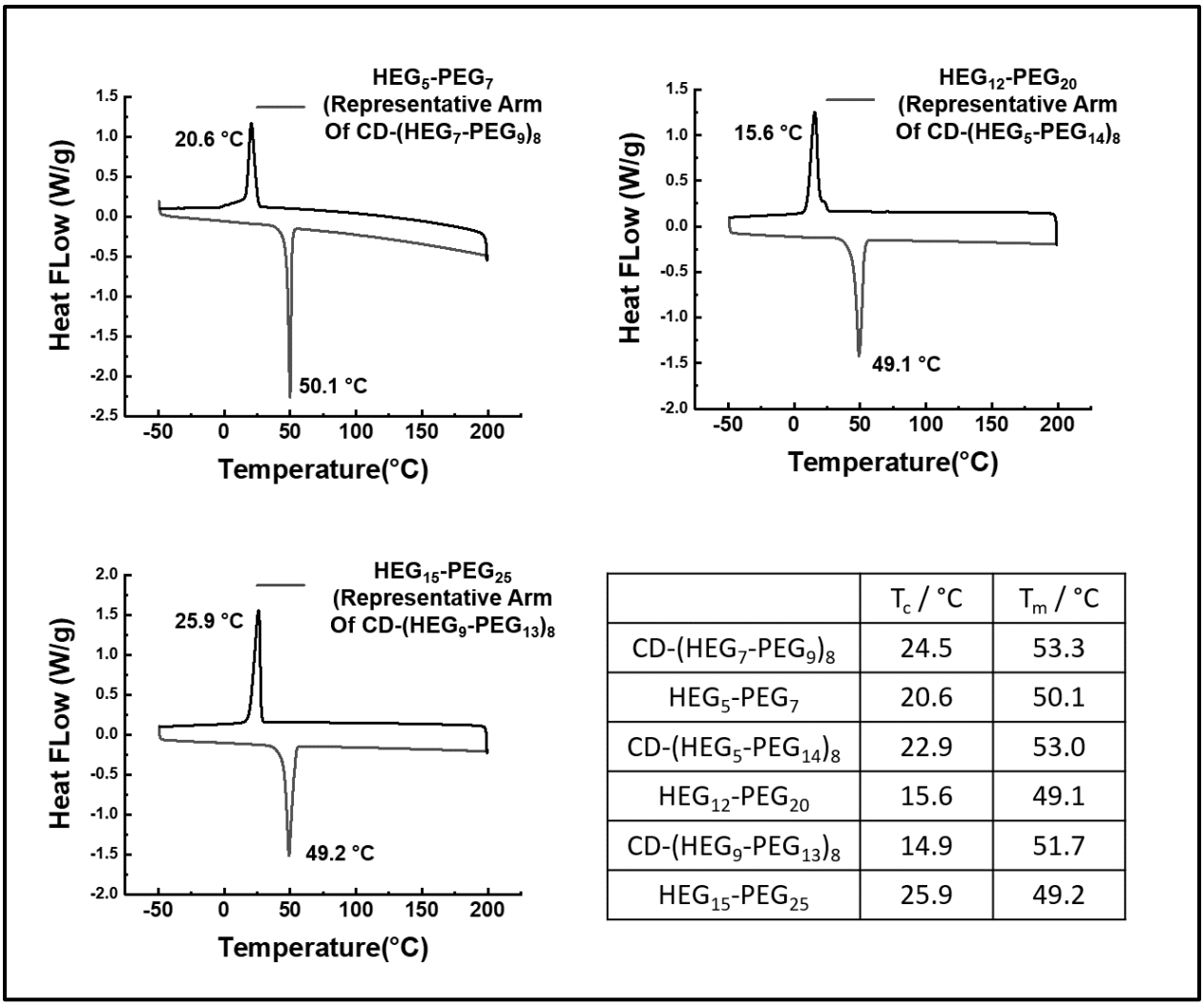


Figure S11. a) TGA data of star polymers with diblock and triblock arms; **b**) DSC data of star polymers with triblock arms.



	T_c / °C	T_m / °C
CD-$(HEG_7\text{-}PEG_9)_8$	24.5	53.3
$HEG_5\text{-}PEG_7$	20.6	50.1
CD-$(HEG_5\text{-}PEG_{14})_8$	22.9	53.0
$HEG_{12}\text{-}PEG_{20}$	15.6	49.1
CD-$(HEG_9\text{-}PEG_{13})_8$	14.9	51.7
$HEG_{15}\text{-}PEG_{25}$	25.9	49.2

Figure S12. DSC data of the arms of each diblock-arm star polymer, **a**) **CD-$(HEG_7\text{-}PEG_9)_8$**; **b**) **CD-$(HEG_5\text{-}PEG_{14})_8$**; **c**) **CD-$(HEG_9\text{-}PEG_{13})_8$**.

Section F. Characterization of Binding Affinities

1) ^{1}H NMR Titration of Native Cyclodextrins and DOX·HCl

To an NMR tube, 1 mL of a 1 mM solution of cyclodextrin in D_2O was added. 1 μL of a 100 mM solution of doxorubicin hydrochloride (DOX·HCl) in $(CD_3)_2SO$ (0.1 equiv.) was added for each addition. After 1.0 equiv. (10 additions), 2 μL (0.2 equiv.) of the DOX·HCl was added. The chemical shift of H_3 and H_5 was collected for each addition, and the binding affinity was calculated through **http://supramolecular.org/,** the process of data analysis was described in previous literature.[12] All titrations were done three times under same condition, and the average of the binding affinity acquired from each titration experiment is reported, the output data is the screenshot of one of the titrations.

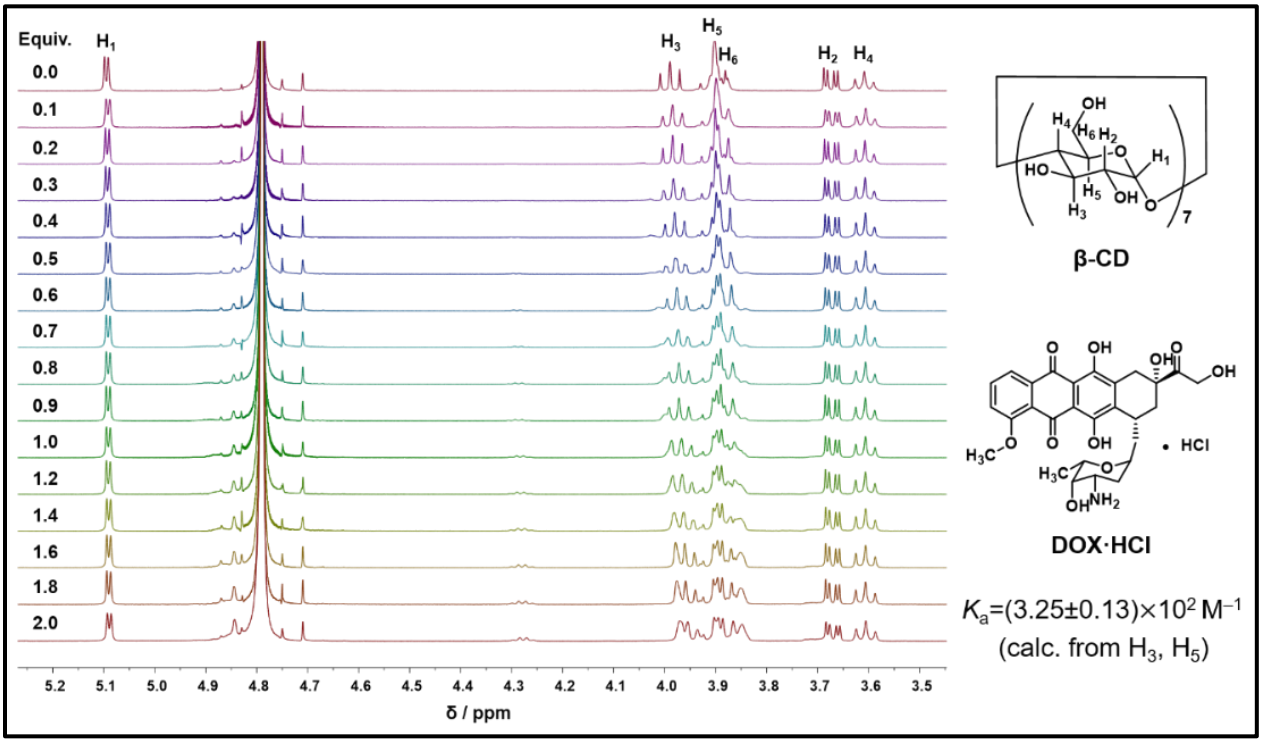


Figure S13. ^{1}H NMR titration (500 MHz, 25 ºC) between native β-CD and DOX·HCl.

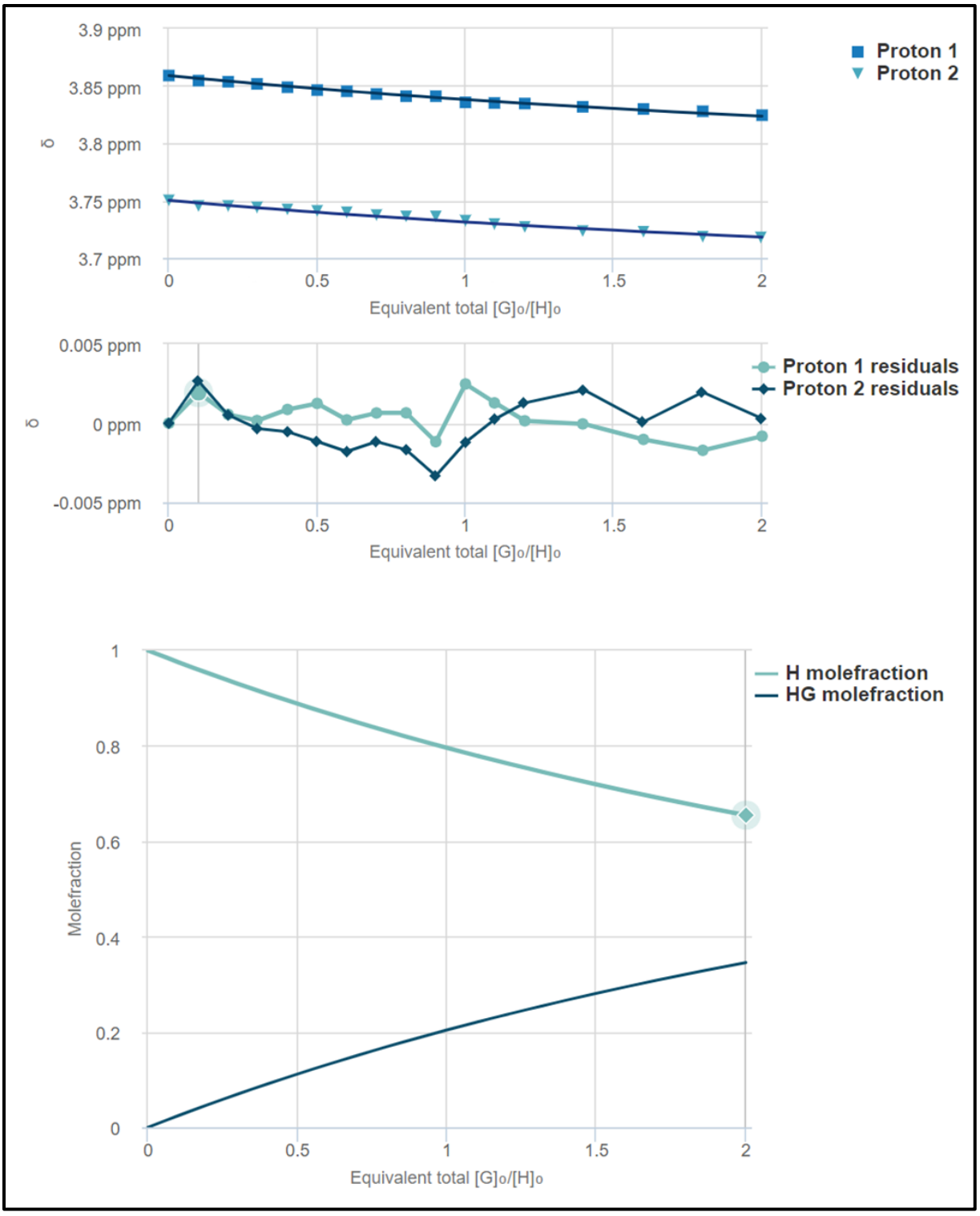


Figure S14. Output data from supramolecular.org of the ^{1}H NMR titration (500 MHz, 25 ºC) between native β-CD and DOX·HCl. (Proton 1 and Proton 2 are referring to H_3 and H_5 shown in Figure S13.) The top figure tells the change of chemical shifts of each proton over the addition of drug DOX·HCl. The mid figure gives us the residuals of each proton, indicating the good fit of experimental data. The bottom figure states the mole fractions of host molecule (H, β-CD) and the resulting complex (HG, β-CD⊃ DOX·HCl).

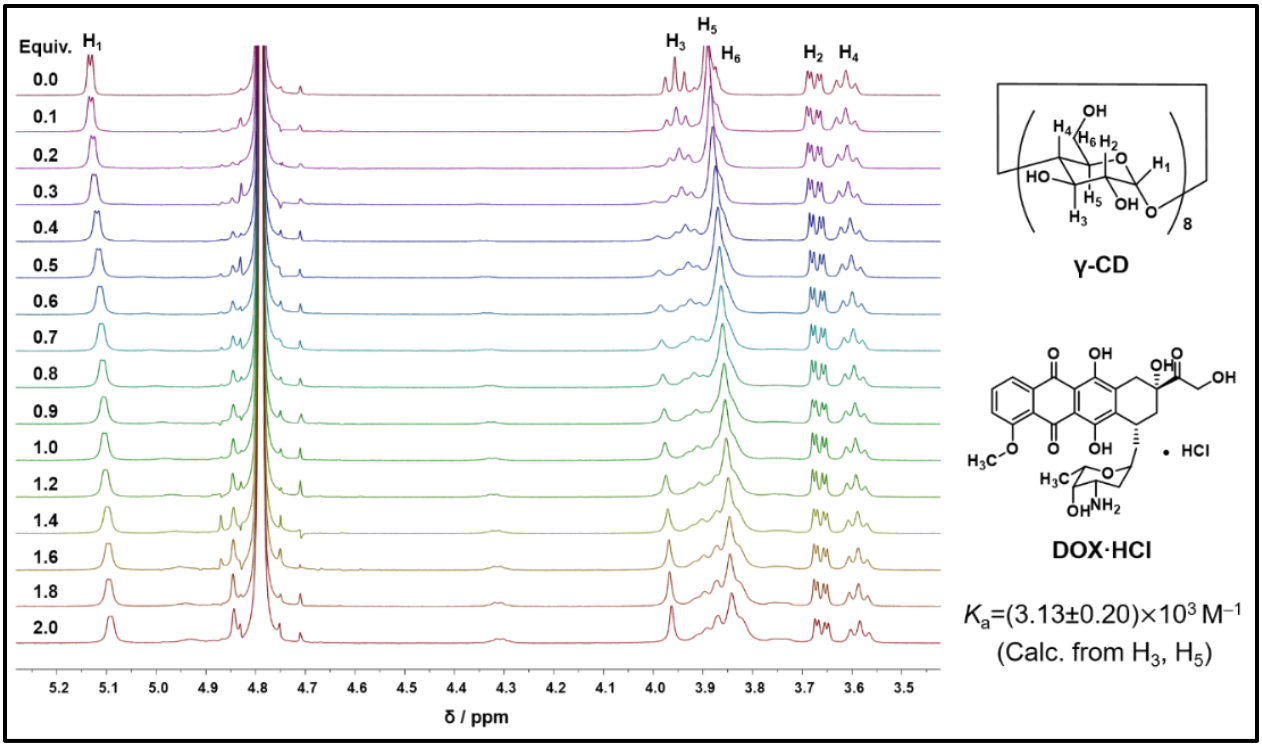


Figure S15. ^{1}H NMR titration (500 MHz, 25 ºC) between native γ-CD and DOX·HCl.

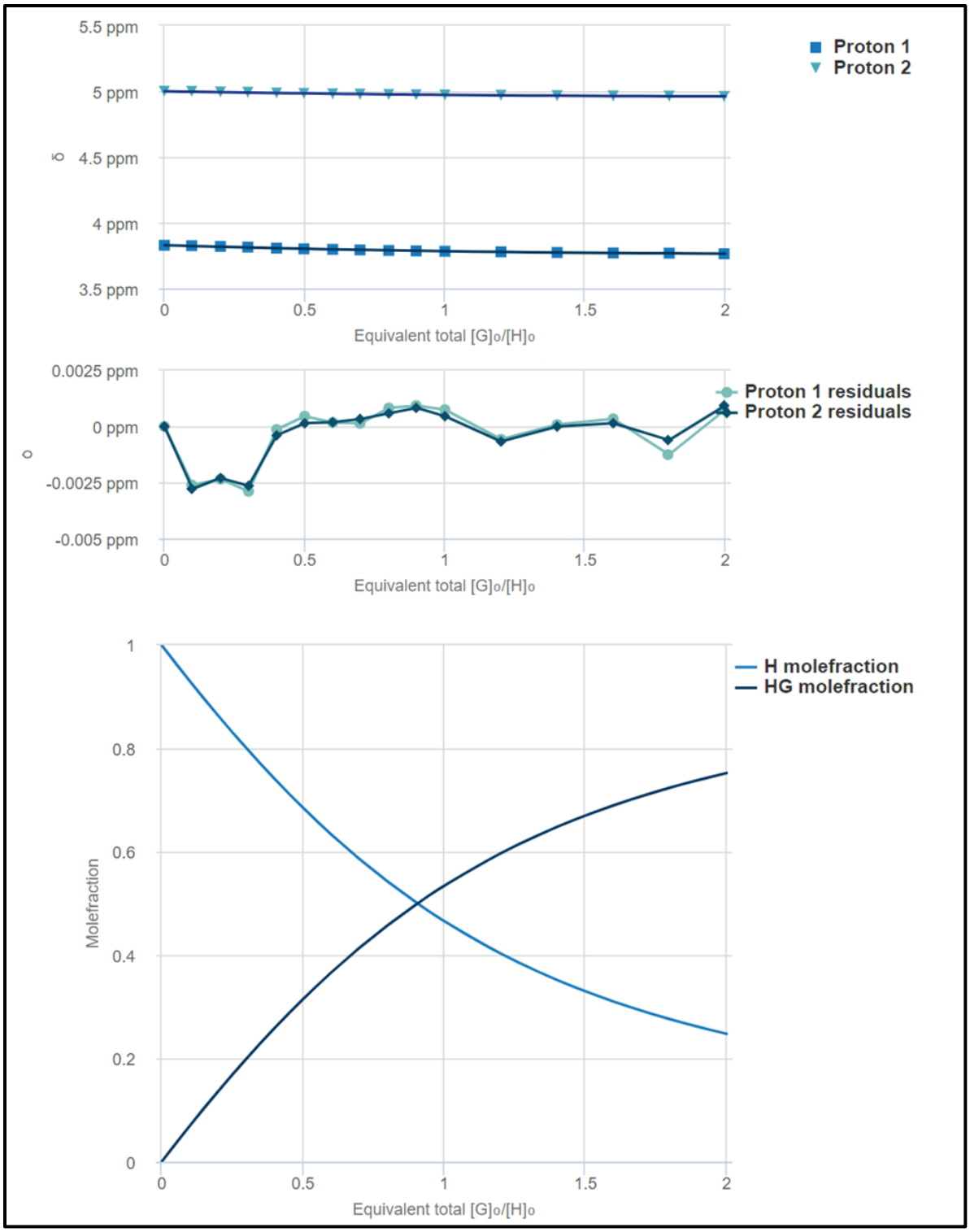


Figure S16. Output data from supramolecular.org of the ^{1}H NMR titration (500 MHz, 25 ºC) between native γ -CD and DOX·HCl. (Proton 1 and Proton 2 are referring to H_3 and H_5 shown in Figure S15.) The top figure tells the change of chemical shifts of each proton over the addition of drug DOX·HCl. The mid figure gives us the residuals of each proton, indicating the good fit of experimental data. The bottom figure states the mole fractions of host molecule (H, *γ*-CD) and the resulting complex (HG, *γ*-CD⊃DOX·HCl).

2) UV-Vis Titration of Native Cyclodextrin/Star polymer and DOX·HCl

To a UV-Vis cuvette, a solution of DOX·HCl in H_2O (20 μM, 600 μL) was added. A solution of **CD-(HEG$_7$-PEG$_9$)$_8$** in H_2O (1 mM, 1.2 μL for every 0.1 equiv. addition) was added to the cuvette, and the UV-Vis curve was obtained for each addition, and the absorbance at 592 nm, 497 nm and 293 nm was recorded. Binding affinity was calculated through **http://supramolecular.org/**. All titrations were done three times under same condition, and the average of the binding affinity acquired from each titration experiment is reported, the output data is the screenshot of one of the titrations.

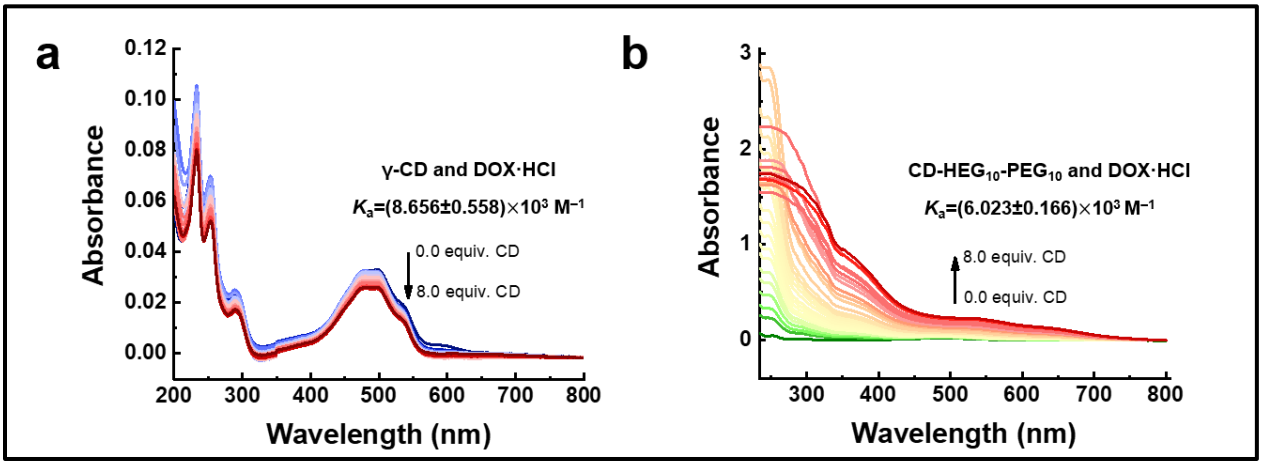


Figure S17. a) UV-Vis titration of γ-CD and DOX·HCl; **b)** UV-Vis titration of star polymer **CD-(HEG$_7$-PEG$_9$)$_8$** and DOX·HCl. All spectra were recorded at 23 ºC on an Agilent Cary 5000 spectrophotometer with a PbSmart NIR detector.

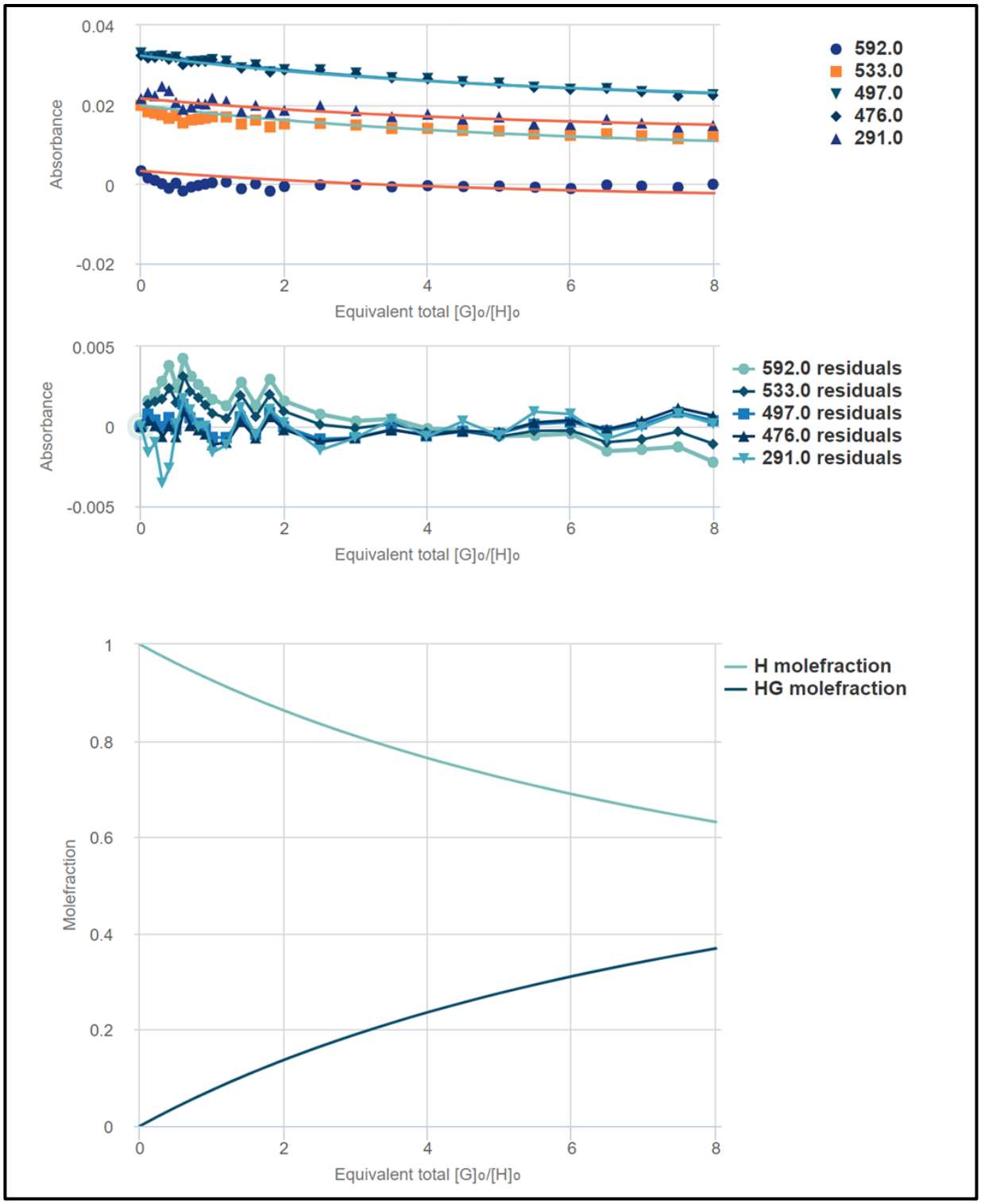


Figure S18. Output data from supramolecular.org of the UV-Vis titration between native γ-CD and DOX·HCl. The top figure tells the change of peak intensity at certain wavelength over the addition of drug DOX·HCl. The mid figure gives us the residuals of each proton, indicating the good fit of experimental data. The bottom figure states the mole fractions of host molecule (H, γ-CD) and the resulting complex (HG, *γ*-CD⊃DOX·HCl).

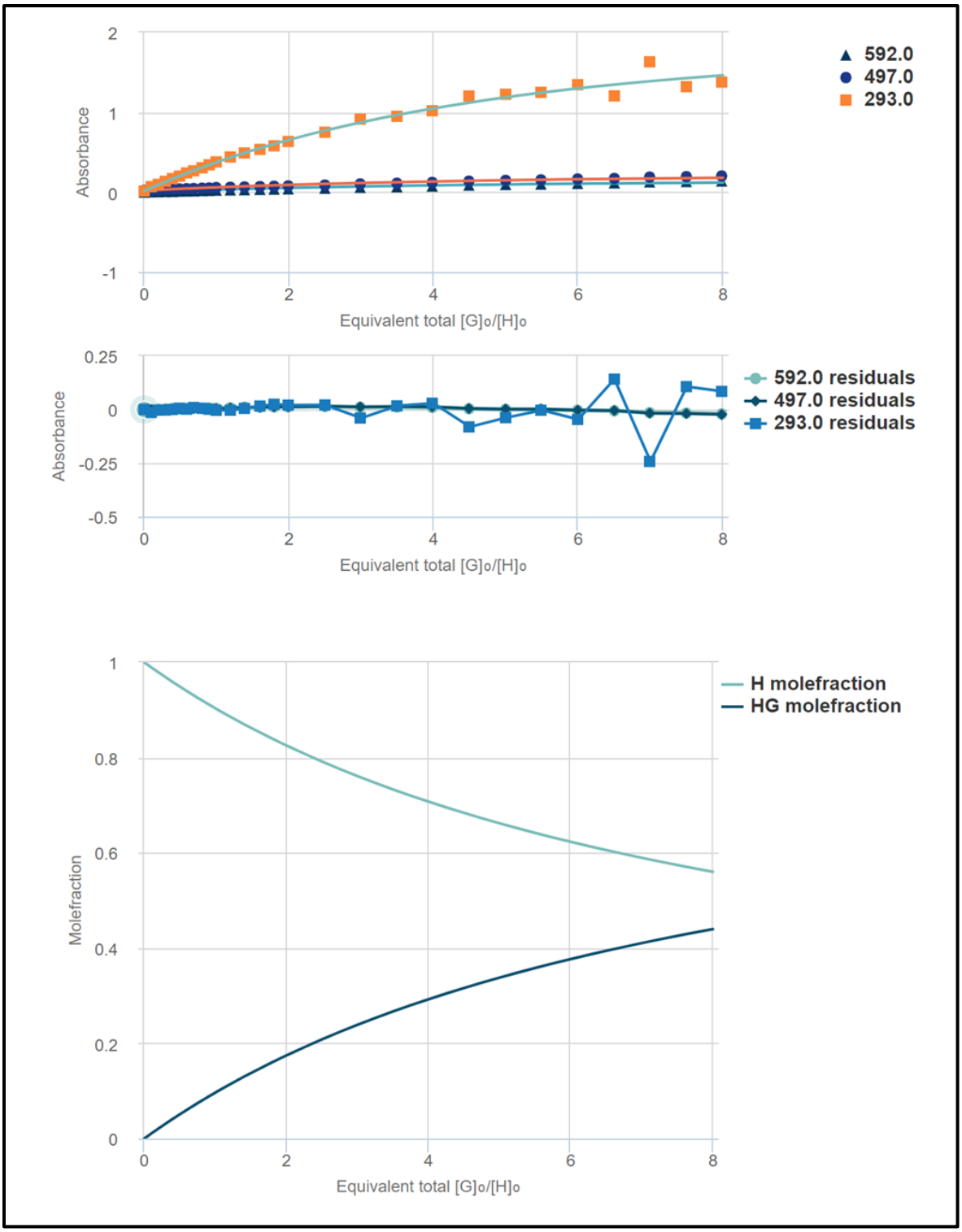


Figure S19. Output data from supramolecular.org of the UV-Vis titration between star polymer **CD-(HEG$_7$-PEG$_9$)$_8$** and DOX·HCl. The top figure tells the change of peak intensity at certain wavelength over the addition of drug DOX·HCl. The mid figure gives us the residuals of each proton, indicating the reasonably good fit of experimental data. The bottom figure states the mole fractions of host molecule *(H,* γ- **CD-(HEG$_7$-PEG$_9$)$_8$***)* and the resulting complex *(HG,* **γ-CD-(HEG$_7$-PEG$_9$)$_8$** ⊃DOX·HCl*).*

Section G. Determination of Drug Release Kinetics

The fluorescence intensity of different concentrations of DOX·HCl in DMSO/PBS (pH=7.4) 10/90 (v/v) was measured, and a linear intensity–concentration relationship was found between 1.2 nM–5 μM.

CD-(HEG$_7$-PEG$_9$)$_8$ (224.1 mg, 1.043 μmol, 1 equiv.) was dissolved in 10.43 mL DMSO/PBS (pH=7.4) 10/90 (v/v), DOX·HCl (0.60 mg, 1.043 μmol, 1 equiv.) was added and stirred for 30 min. The solution was transferred to dialysis tubing (RC dialysis tubing, 1 kDa molecular weight cut-off, 38 mm flat-width), and was placed in a beaker with 500 mL DMSO/PBS (pH=7.4) 10/90 (v/v). An aliquot (1 mL) was taken for fluorescence analysis at each time point. After each aliquot was removed, fresh DMSO/PBS (pH=7.4) 10/90 (v/v) solution (1 mL) was added to compensate for the volume loss. The percent of drug release of each aliquot was calculated based on the acquired fluorescence intensity and the standard curve.

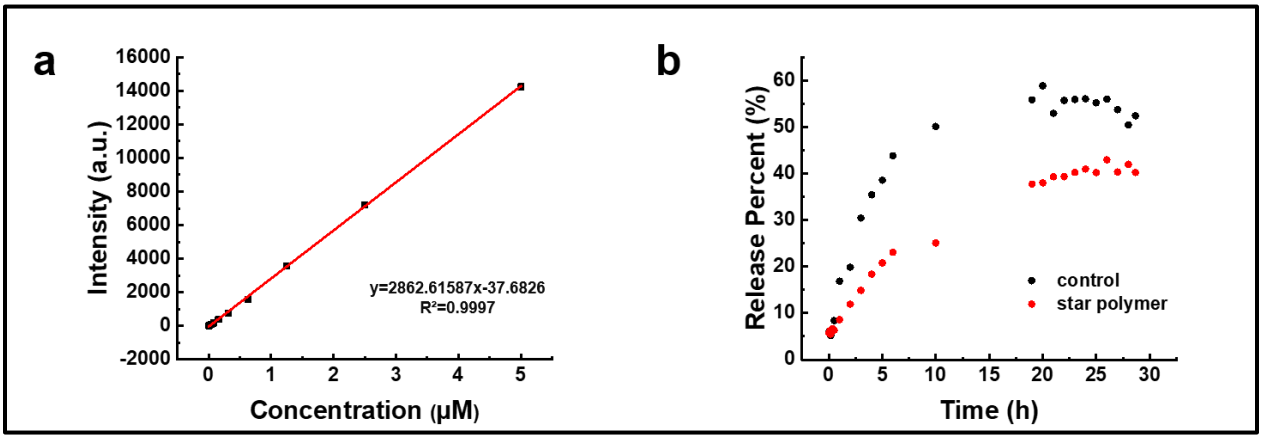


Figure S20. a) Fluorescence standard curve of DOX·HCl in DMSO/PBS (pH=7.4) 10/90 (v/v). **b)** Release kinetics of DOX·HCl directly from dialysis bag with and without (control) the presence of star polymer **CD-(HEG$_7$-PEG$_9$)$_8$**.

Section H. Cell Culture and Cytotoxicity Studies

1) Cell Culture

Human umbilical vein endothelial cells (HUVEC) and human breast cancer (MCF-7) cells were obtained from ATCC (American Type Culture Collection, USA) and were cultured as recommended. HUVEC cells were grown in vascular basal cell medium (ATCC) supplemented with 1% penicillin/streptomycin (100 units/mL and 0.1 mg/mL, respectively) and the endothelial cell growth kit-BBE (ATCC) which contains 0.2 % bovine brain extract, 5 ng/mL rh EGF, 10 mM L-glutamine, 0.75 units/mL heparin sulfate, 1 µg/mL hydrocortisone, 50 µg/mL ascorbic acid, and 2% fetal bovine serum. MCF-7 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat inactivated fetal bovine serum and 1% penicillin/streptomycin (100 units/mL and 0.1 mg/mL, respectively). All cells were maintained at 37 °C and 5% CO_2 in a humid incubator.

2) Cytotoxicity of Star Polymer Unloaded and Loaded with DOX·HCl

Cells (HUVEC and MCF-7, passaged 5 and 2 times, resp.) were seeded in a 96-well plate (5,000 cells/well, 100 µL) and incubated overnight to ensure proper attachment before treatment with various concentrations of empty **CD-(HEG$_7$-PEG$_9$)$_8$**, DOX·HCl loaded **CD-(HEG$_7$-PEG$_9$)$_8$**, and free DOX·HCl. Each polymer/drug combination and concentration were prepared and measured in 4 replicate wells. The final column contained 4 wells with cells (control) and 4 wells contained media only (blank). After 48 h of treatment, 100 µL of Celltiter Glo® (Promega) was added to each well and the luminescence was measured. Viability was calculated as follows:

$$\frac{Lum_{sample}\text{-}Lum_{blank}}{Lum_{control}\text{-}Lum_{blank}} \times 100 = \%\ Viability$$

Section I. References

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