Supporting Information

Synthesis, Characterization, and Evaluation of Pluronic-based β-Cyclodextrin Polyrotaxanes for Mobilization of Accumulated Cholesterol from Niemann-Pick Type C Fibroblasts

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Contents

S.1: β-CD:Pluronic PRTx ^{1}H NMR spectra

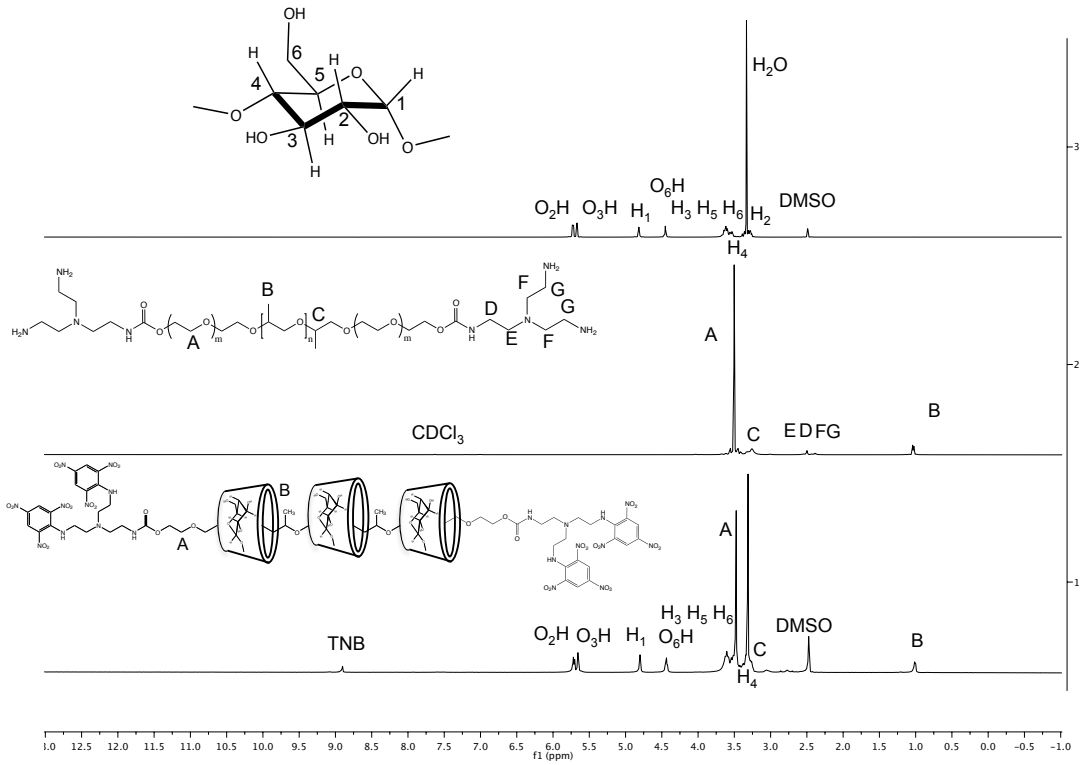


Fig. S.1.1: ^{1}H NMR spectra of (1) β-CD, (2) F68-TREN, (3) β-CD:F68 PRTx

β-CD:F68 PRTx ^{1}H NMR: (400Mhz, DMSO-d_6, 22°C) δ 8.9-8.89(br. s., 8H, TNB), 5.74-5.63 (m, 196H, O_2H, O_3H of CD), 4.83-4.77 (s, 98H, H_1 of CD), 4.46-4.39 (t, 98H, O_6H of CD), 3.65-3.55 (m, 196H, H_3, H_6, H_5, H_4 of CD), 3.51-3.44 (m, 132H, PPO and PPG-CH_2-), 1.05-0.98 (d, 87H, PPG-CH_3)

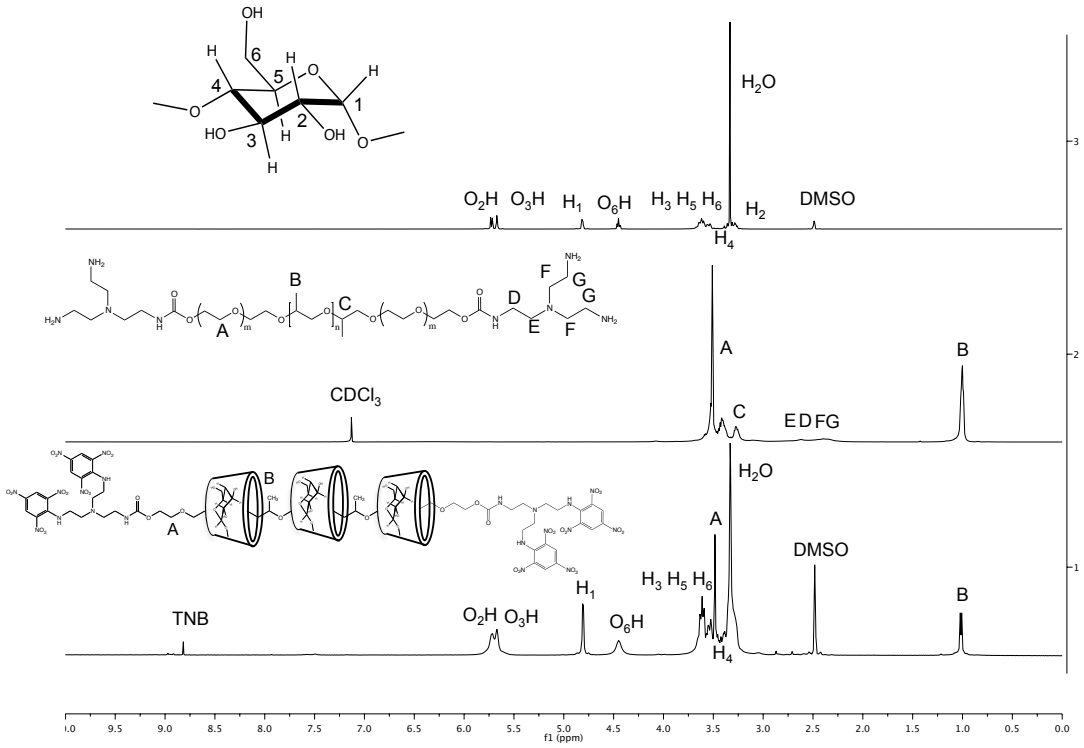


Fig. S.1.2: ^{1}H NMR spectra of (1) β-CD, (2) L64-TREN, (3) β-CD:L64 PRTx

β-CD:L64 PRTx ^{1}H NMR: (400Mhz, DMSO-d_6, 22°C) δ 8.891-8.814 (br. s., 8H, TNB), 5.76-5.6 (m, 168H, O_2H, O_3H of CD), 4.84-4.76 (s, 84H, H_1 of CD), 4.52-4.38 (t, 84H, O_6H of CD), 3.67-3.59 (m, 336H, H_3, H_6, H_5, H_4 of CD), 3.51-3.43 (m, 194H, PPO and PPG-CH_2-), 1.05-0.95 (d, 90H, PPG-CH_3)

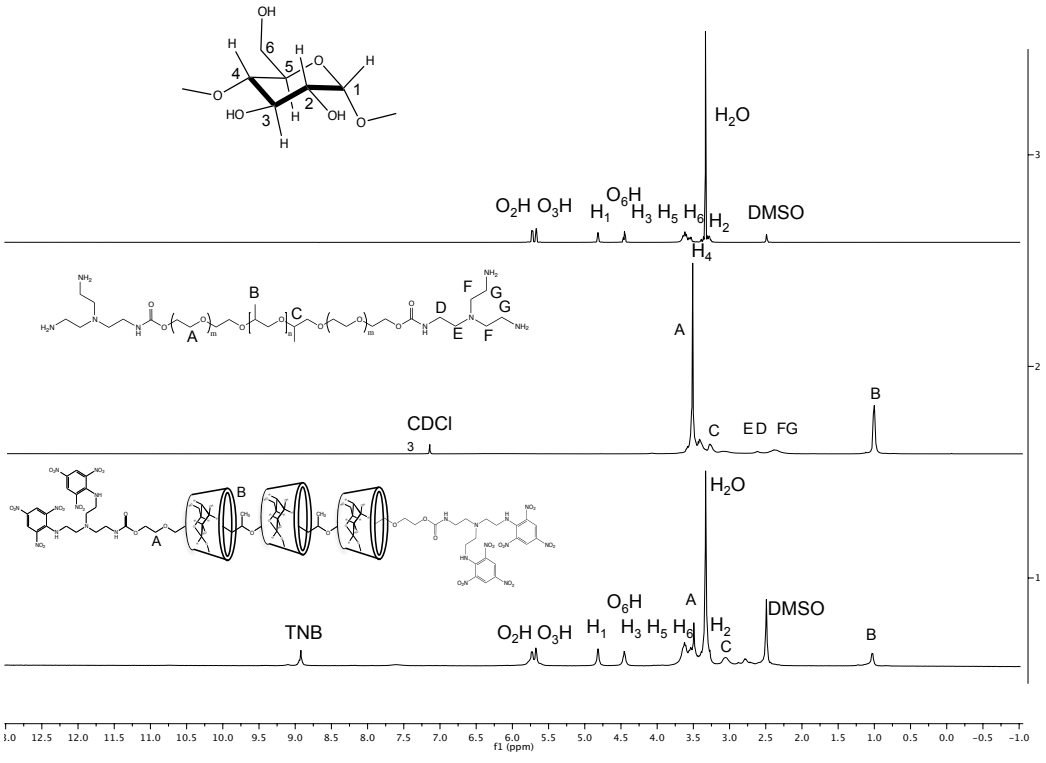


Fig. S.1.3: ^{1}H NMR spectra of (1) β-CD, (2) L35-TREN , (3) β-CD:L35 PRTx

β-CD:L35 PRTx NMR: (400Mhz, DMSO-d_6, 22°C) δ 8.93-8.91(br. s., 8H, TNB), 5.75-5.65 (m, 98H, O_2H, O_3H of CD), 4.84-4.78 (s, 49H, H_1 of CD), 4.45 (t, 49H, O_6H of CD), 3.67-3.59 (m, 532H, H_3, H_6, H_5, H_4 of CD), 3.51-3.48 (m, 995H, PPO and PPG-CH_2-), 1.06-0.98 (d, 48H, PPG-CH_3)

S.2: β-CD:Pluronic PRTx 2D NOESY Spectra

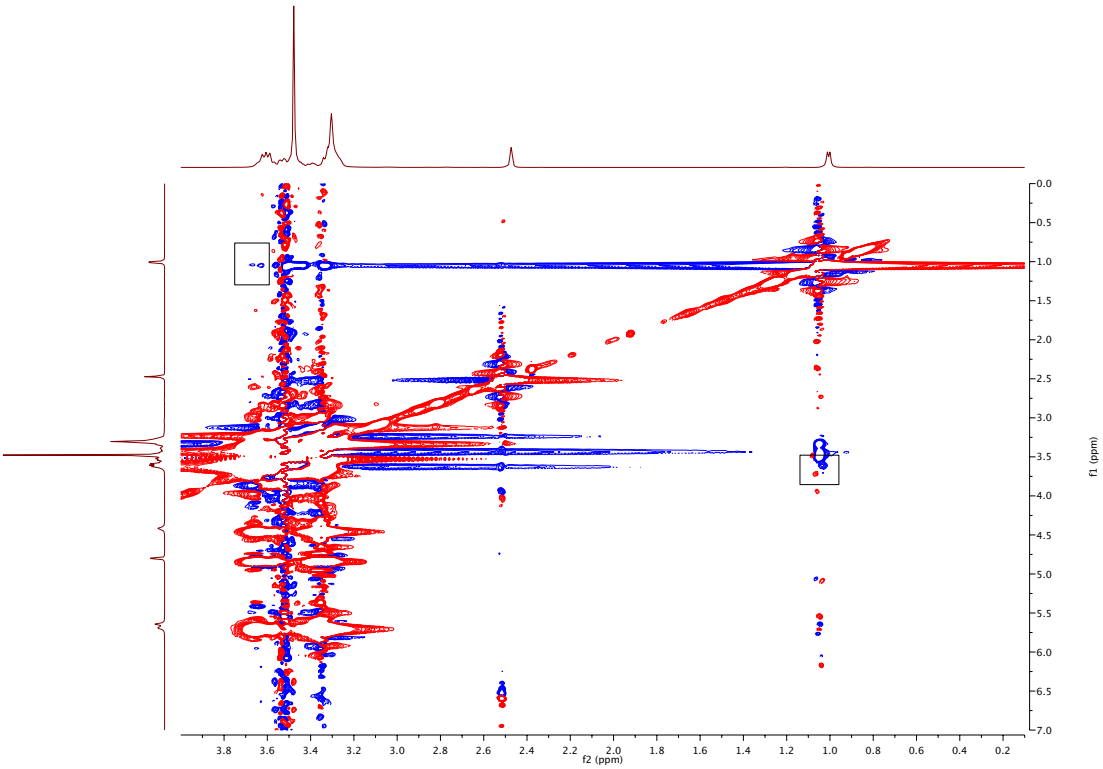


Fig. S.2.1: Full NOESY Spectra β-CD:F127 Spectra taken on a 500 MHz Bruker DRX500 spectrometer with a 5mm TBI probe.

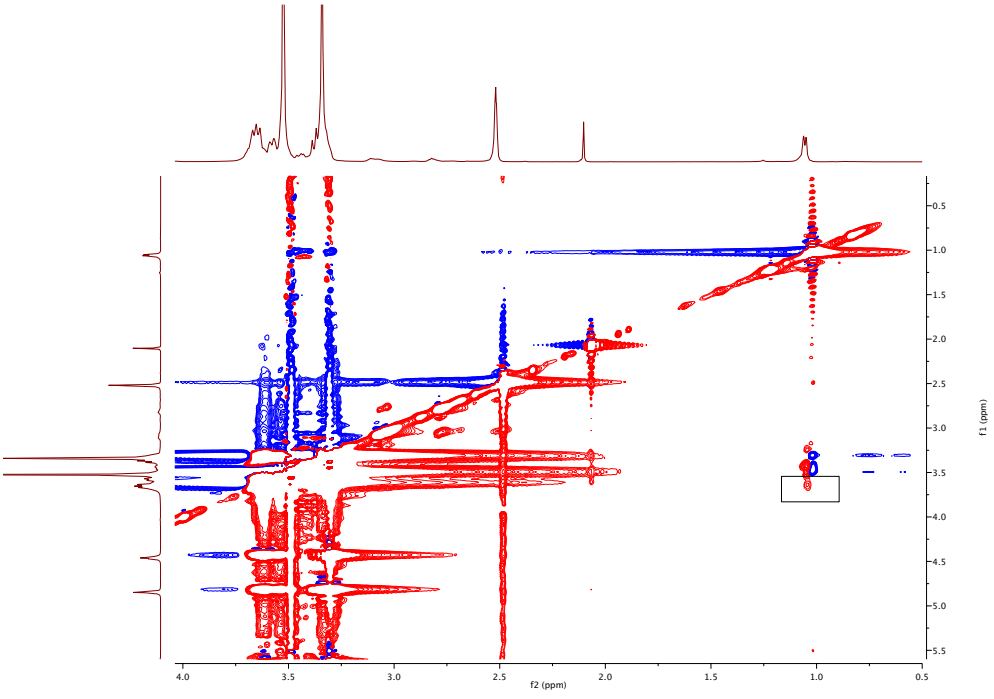


Fig. S.2.2: NOESY Spectra β-CD:F68 PRTx. Spectra taken on a 500 MHz Bruker DRX500 spectrometer with a 5mm TBI probe

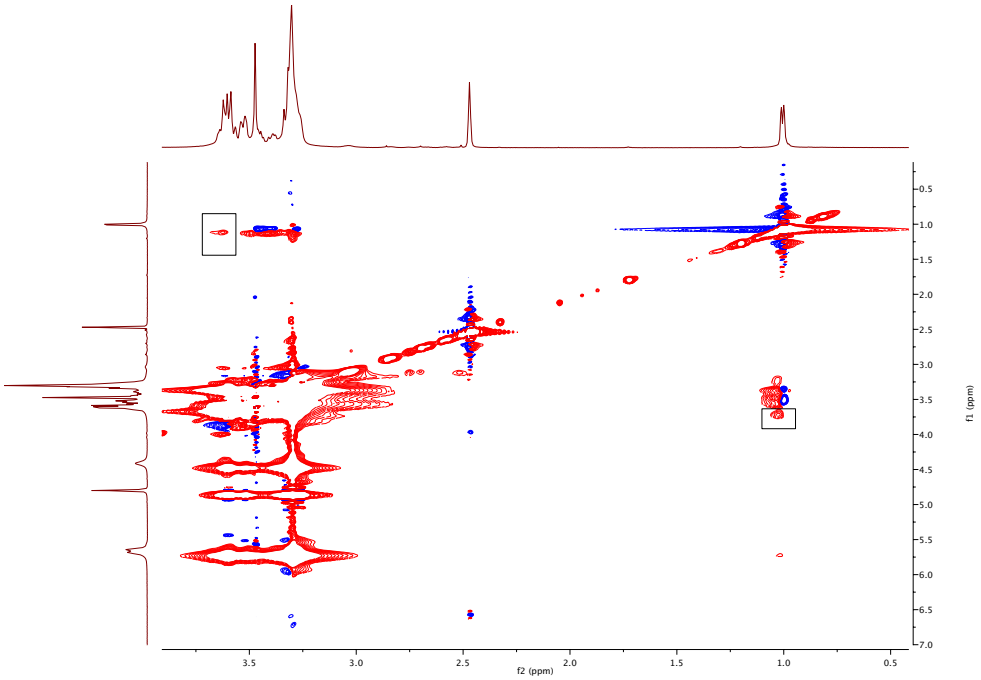


Fig. S.2.3: NOESY Spectra β-CD:L64 PRTx. Spectra taken on a 500 MHz Bruker DRX500 spectrometer with a 5mm TBI probe.

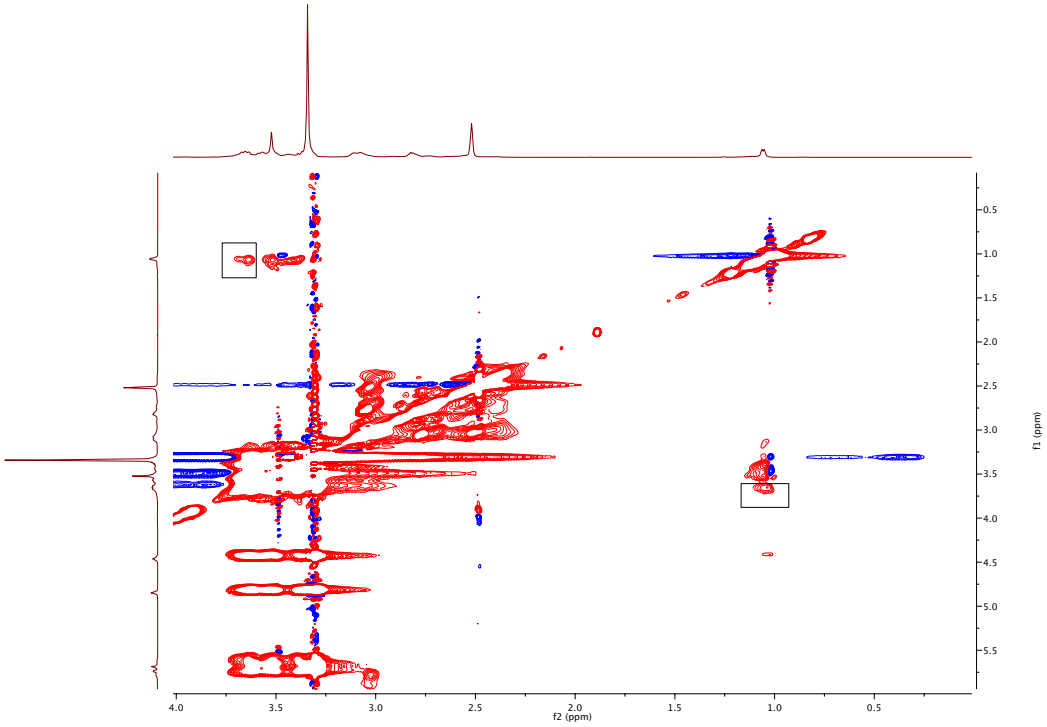


Fig. S.2.4: NOESY Spectra β-CD:L35 PRTx. Spectra taken on a 500 MHz Bruker DRX500 spectrometer with a 5mm TBI probe.

S.3: β-CD:Pluronic PRTx GPC Traces

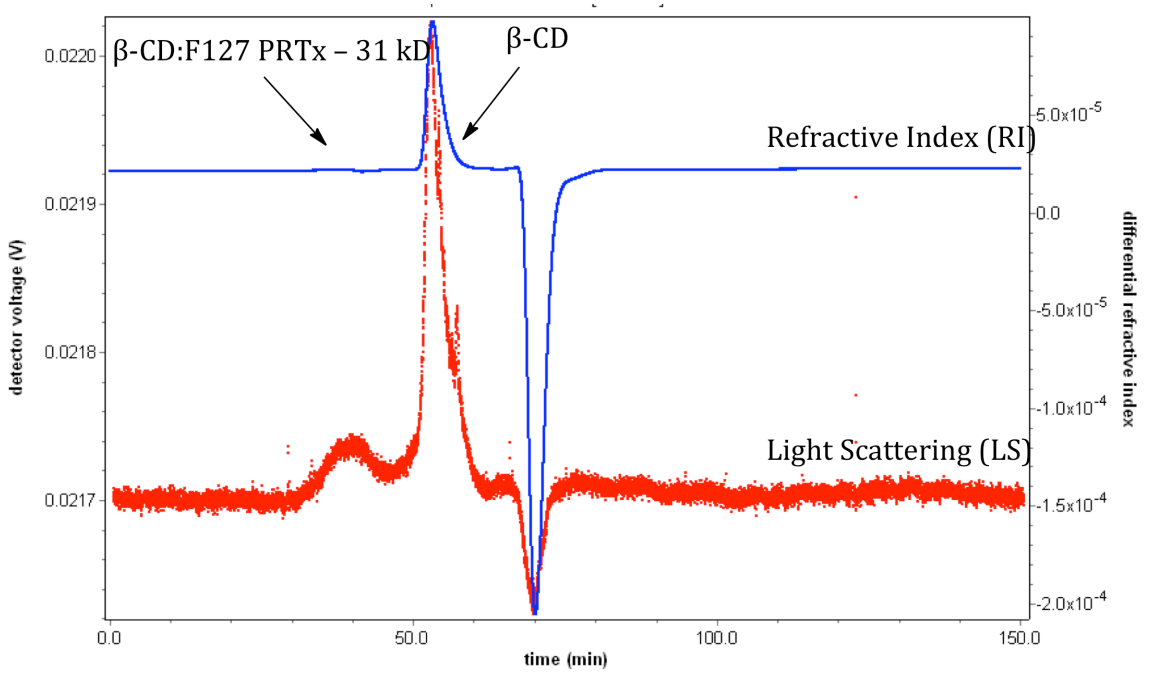


Fig. S.3.1: β-CD:F127 PRTx GPC Traces

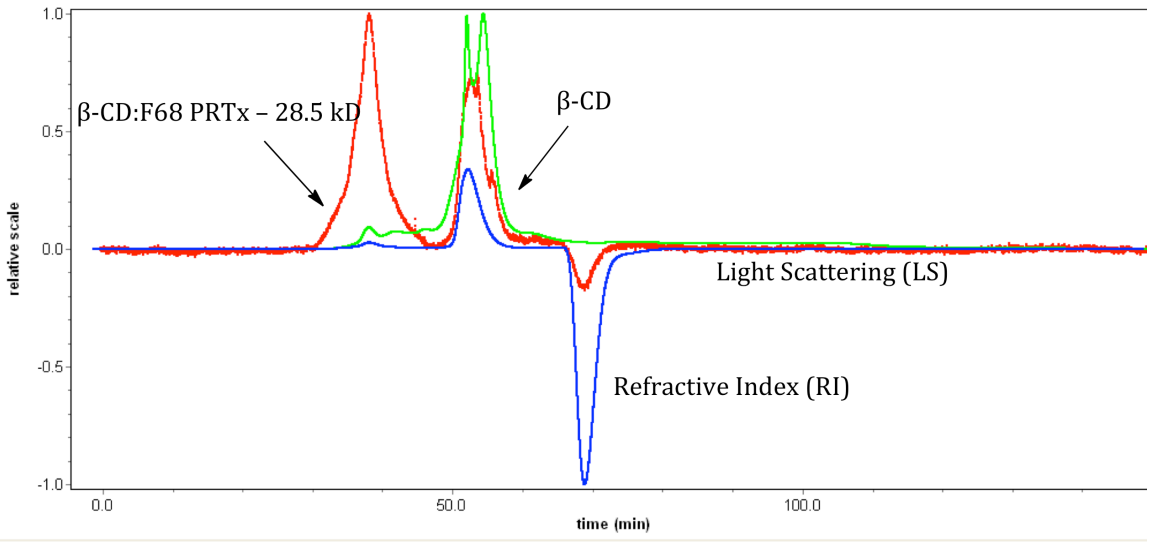


Fig. S.3.2: β-CD:F68 PRTx GPC Traces

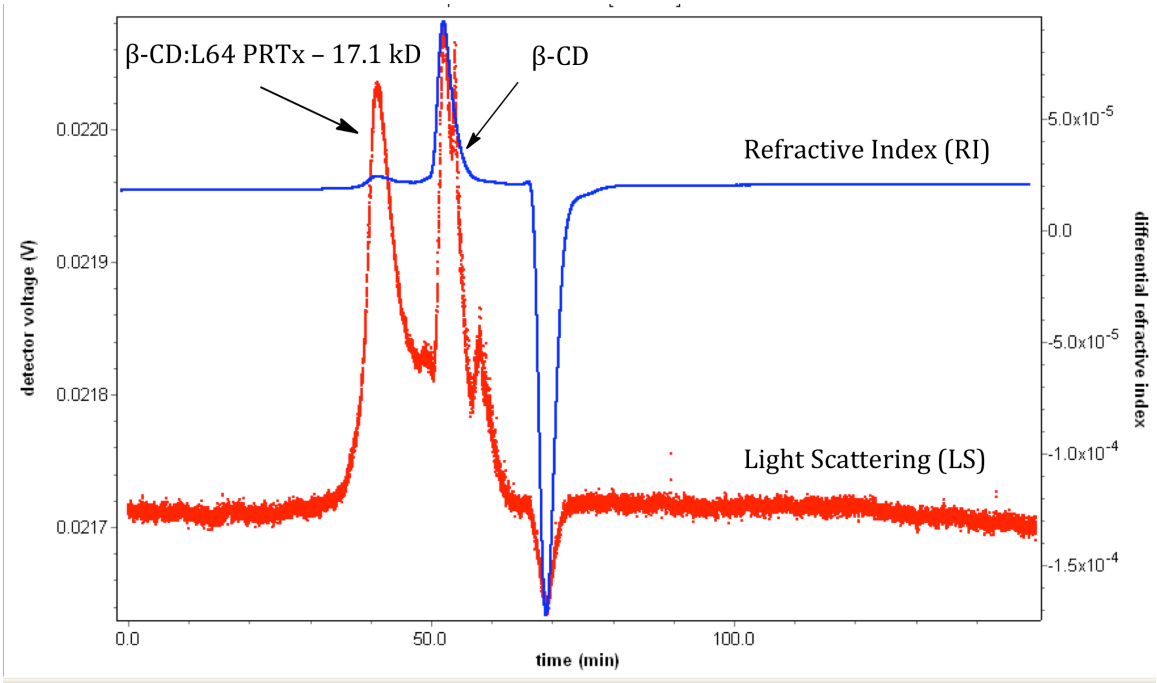


Fig. S.3.3: β-CD:L64 PRTx GPC Traces

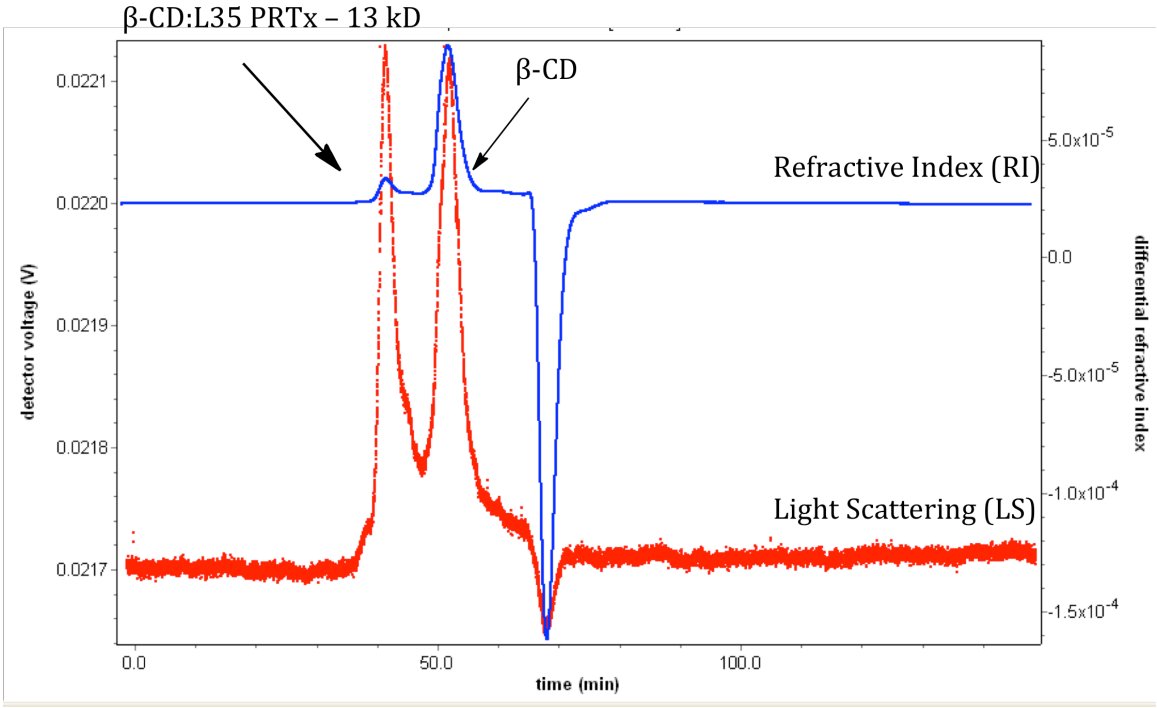


Fig. S.3.4: β-CD:L35 PRTx GPC Traces

S.4: β-CD:Pluronic PRTx AFM Images

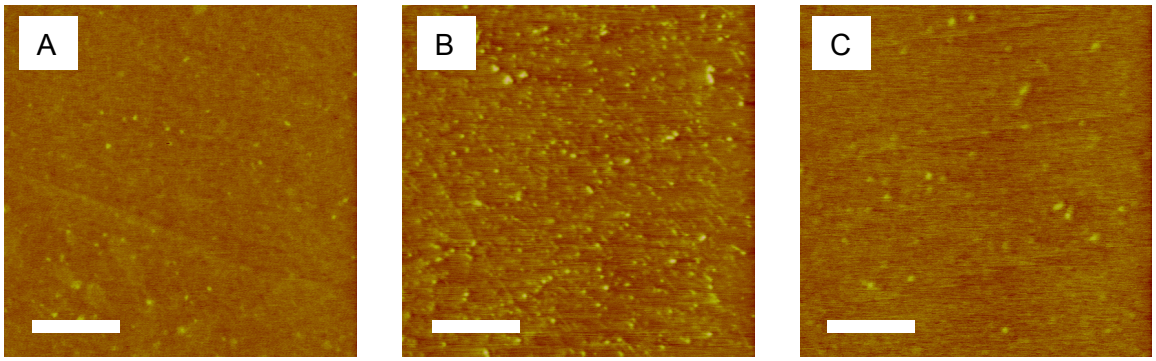


Fig S.4.1: AFM Images of (A) β-CD:F68 PRTx at 10^{-5} mg/mL, (B) β-CD:L64 PRTx at 10^{-5} mg/mL, and (C) β-CD:L35 PRTx at 10^{-9} mg/mL. Scale bar represents 500 nm.

S.5: β-CD:Pluronic PRTx MALDI Mass Spectrometry

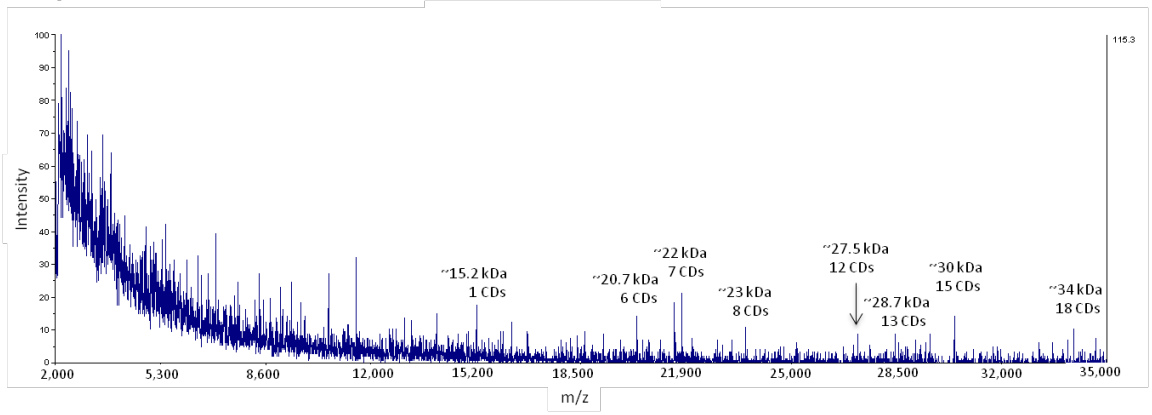


Fig. S.5.1: β-CD:F127 PRTx MALDI Mass Spectrometry. Laser power was 6500.

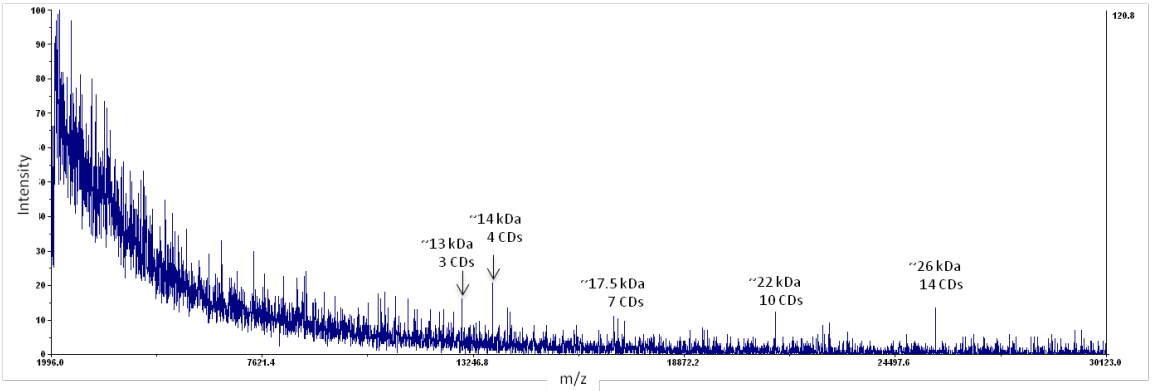


Fig. S.5.2: β-CD:F68 PRTx MALDI Mass Spectrometry. Laser power was 6500.

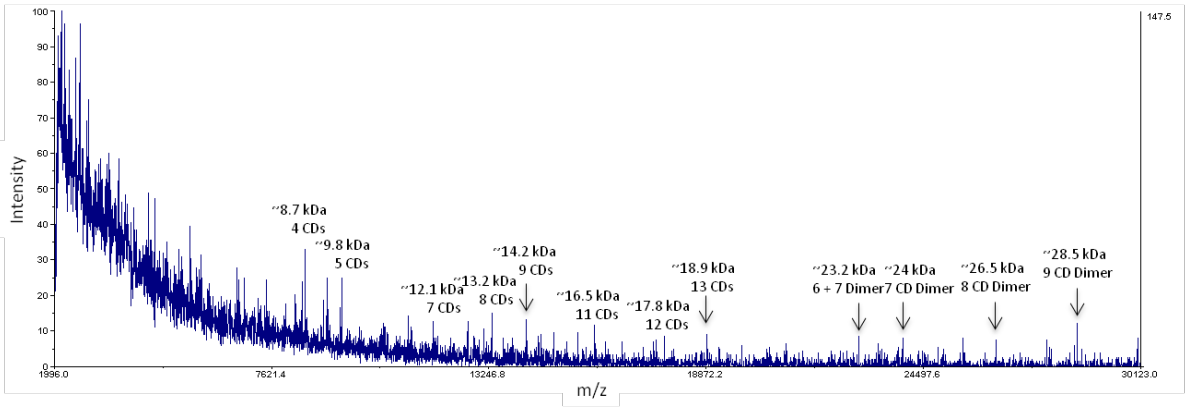


Fig. S.5.3: β-CD:L64 PRTx MALDI Mass Spectrometry. Laser power was 6500.

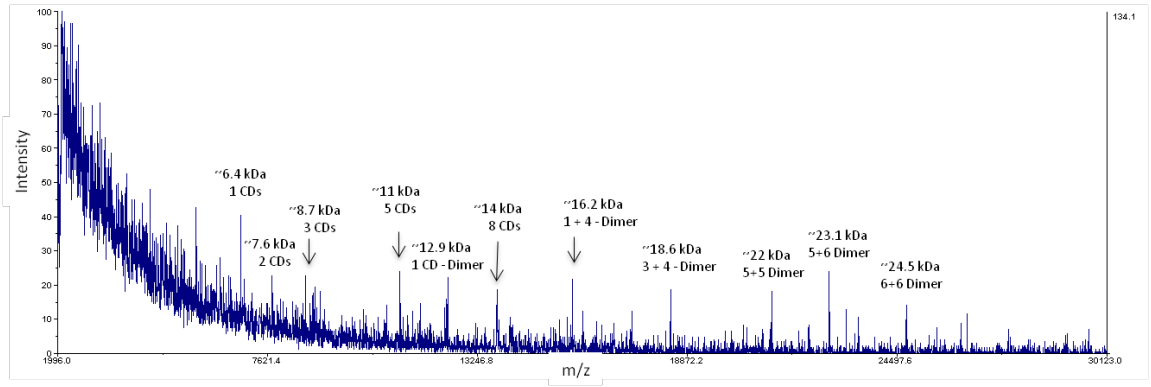


Fig. S.5.4: β-CD:L35 PRTx MALDI Mass Spectrometry. Laser power was 6500.

S.8: Filipin Staining Images

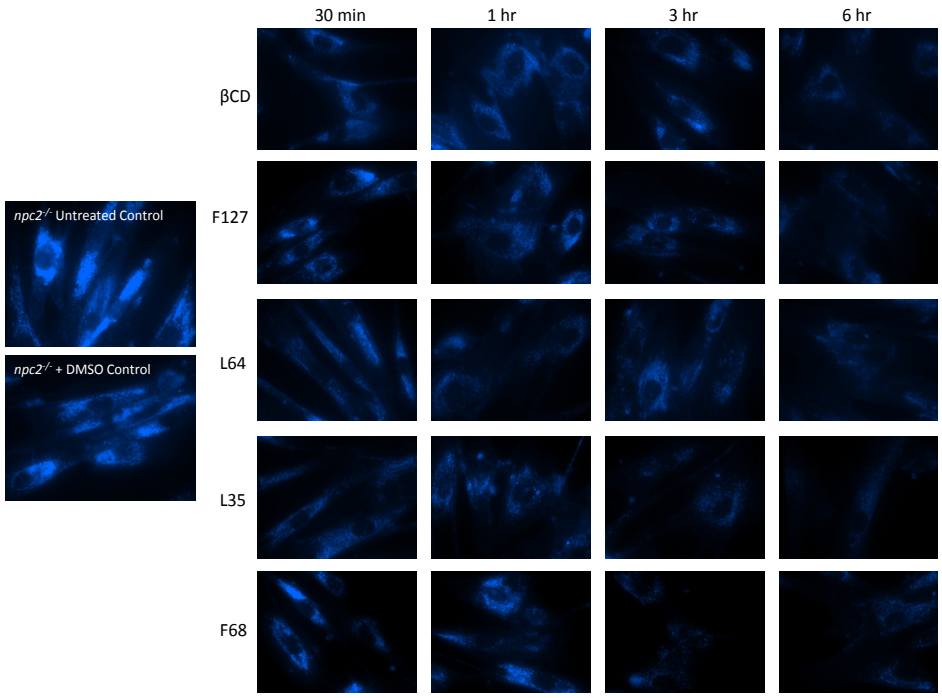


Fig. S.6: : Filipin staining experiments showing cholesterol efflux from *npc2*$^{-/-}$ fibroblasts. Images at left show decrease in filipin staining from 0.5 to 6 hr.

S.7: Synthesis of Unthreaded F68-TNB

TREN-terminated F68 polymer was synthesized as described. 0.26 mL (4 eq) of TNBS (10% solution in H_2O) was added to an aqueous solution of 0.2 g F68-TREN terminated polymer. Reaction mixture was stirred for 18 h, precipitated in ether and collected by centrifugation. Product was then dissolved and re-precipitated into ether (2 x 150 mL). Product was collected by centrifugation and dried on Hi-vac. Product was collected as a yellow/orange solid (0.198g, 90.4% yield).

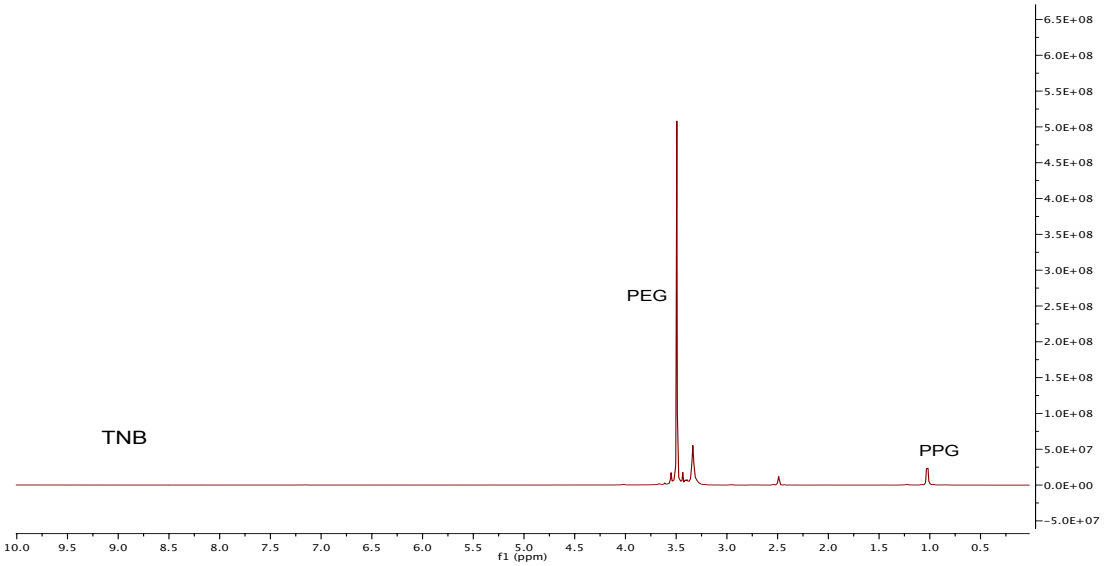


Fig. S.7: Unthreaded F68-TNB

S.8: Synthesis of Short PEG-TNB

0.1 g 2,2'-(Ethylenedioxy)bis(ethylamine) was dissolved in THF. TNBS (4.2 mL, 2.5 eq) was activated with 1 mL 5% $NaHCO_3$ and added to the THF solution. Reaction was allowed to stir for 24 h with a yellow/orange precipitate forming within 1 h. Precipitate was collected by vacuum filtration and washed with THF (3x 25 mL). Product was isolated as an orange powder (158 mg, 40.8% yield).
ESI-MS: Found 569.39 m/z

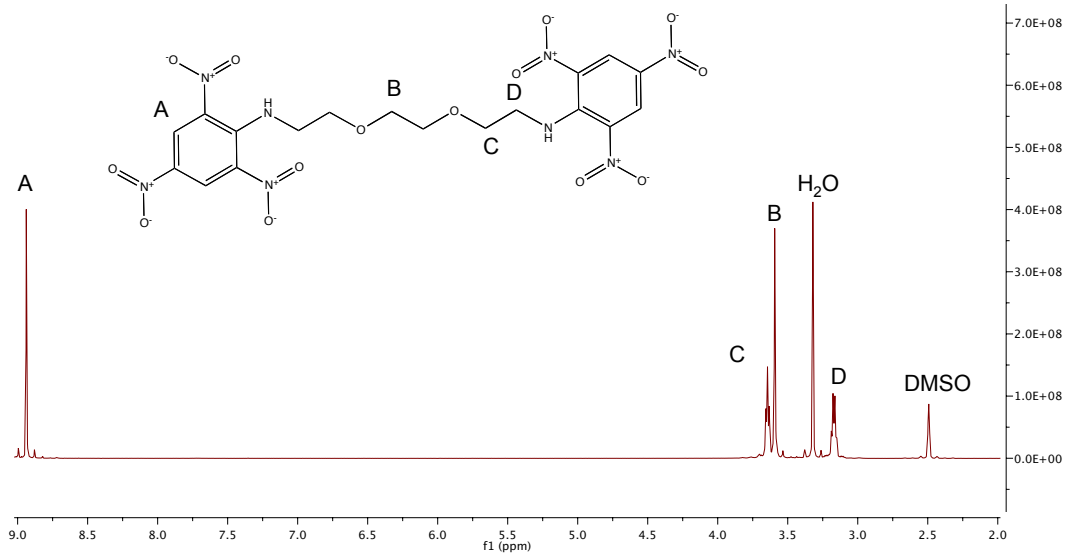


Fig. S.8: ^{1}H NMR Short PEG – TNB

S.9: Stability in DMSO and Media

Two solutions of ~ 12 mg β-CD:F127 PRTx were prepared in 0.2 mL DMSO. The PRTx solutions were then diluted to 10 mL with either PBS buffer or Minimum Essential Medium at pH 7.4 before incubation for 1 hr at 37 °C. After incubation, the samples were dialyzed against for 3 d with water changes approximately every 12 h. Samples were then frozen, lyophilized, and collected as yellow-orange powders. Analyzed as described in the manuscript, integration of the H_1 protons for each sample showed 20.9, 21.1 and 20.5 protons for DMSO/media incubated, DMSO/buffer incubated and control PRTx respectively. This corresponds to ~3 CDs for each sample and no apparent degradation of the samples in DMSO or media.

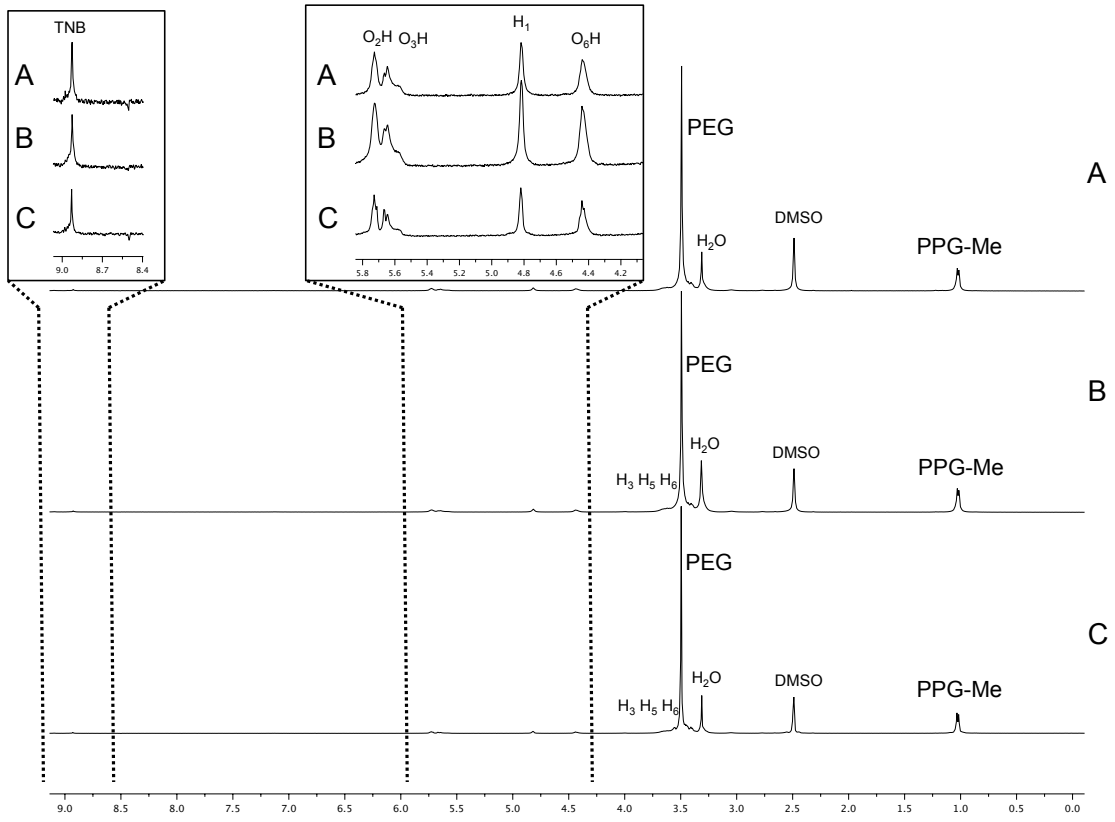
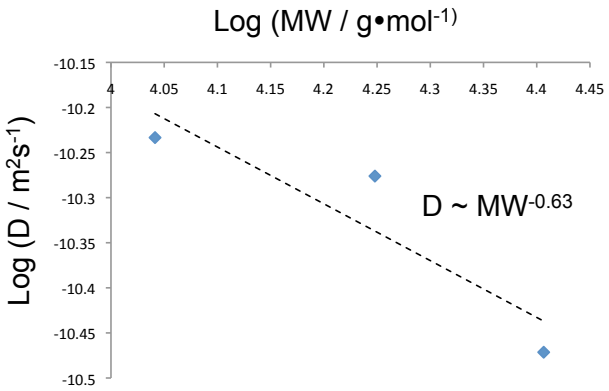
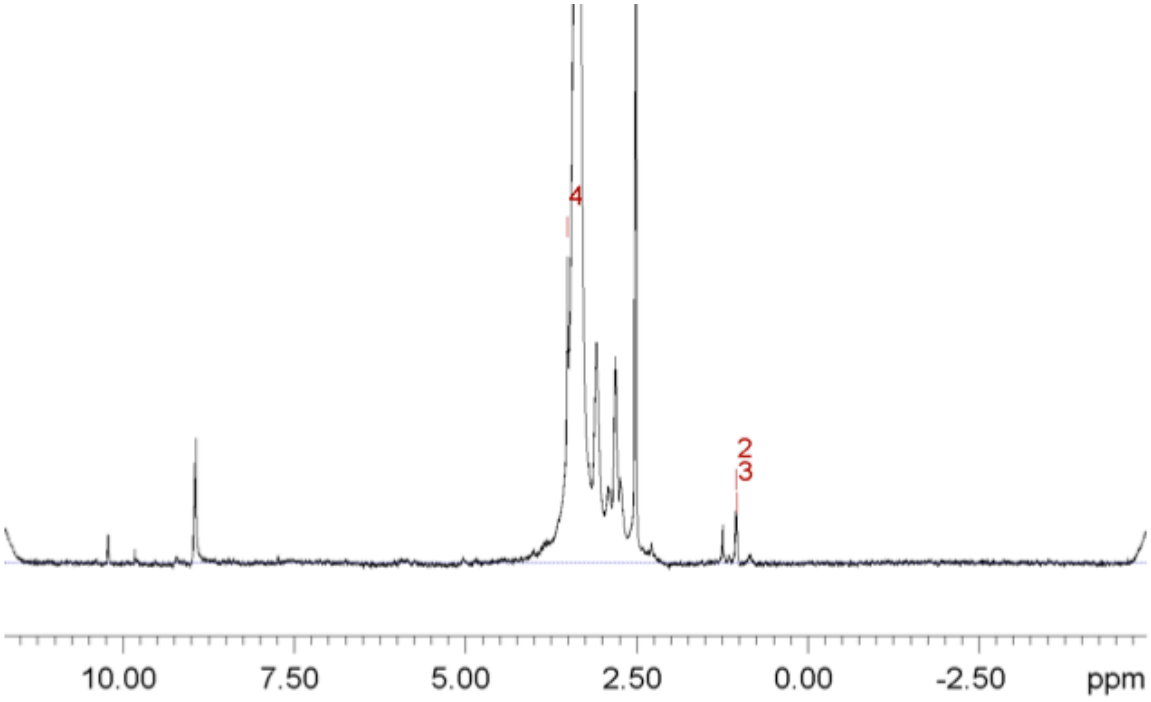


Fig S.9: 1H NMR spectra for A) PRTx incubated with DMSO/Minimum Essential Media, B) PRTx incubated with DMSO/PBS Buffer and C) control PRTx sample.

S.10 Diffusion Ordered Spectroscopy

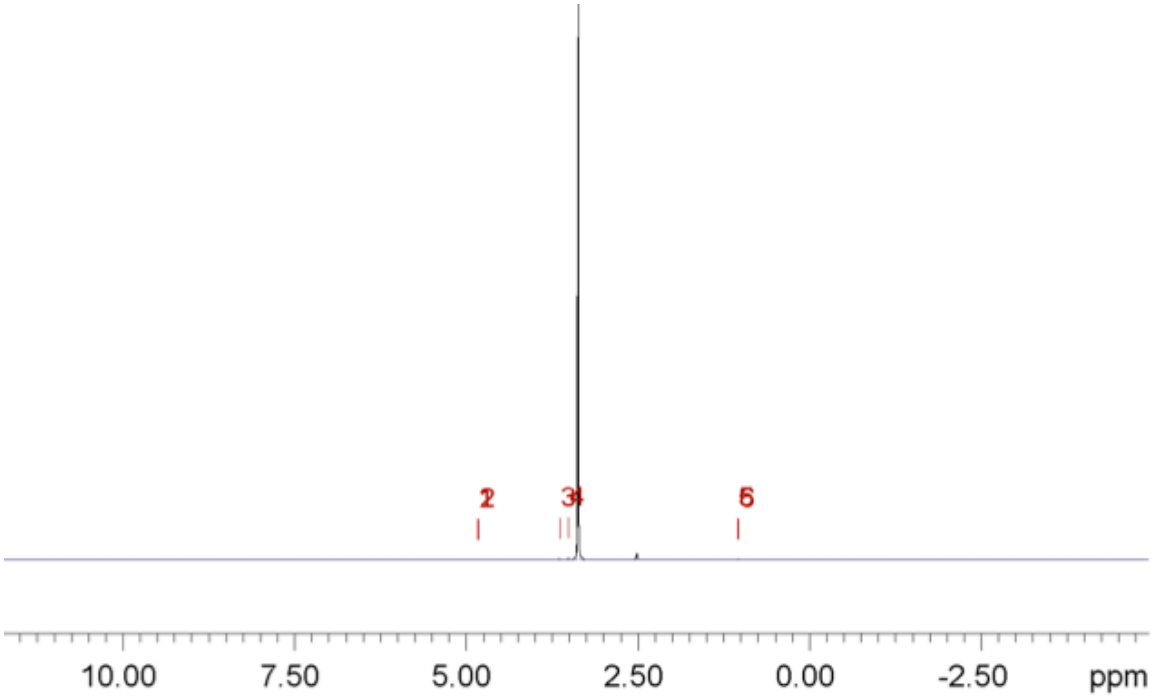


S.10.1: Self-diffusion coefficients vs. molecular weight for β-CD:L35 PRTx, β-CD:L64 PRTx, and β-CD:F68 PRTx. Dashed line is a least squared fit to the data.



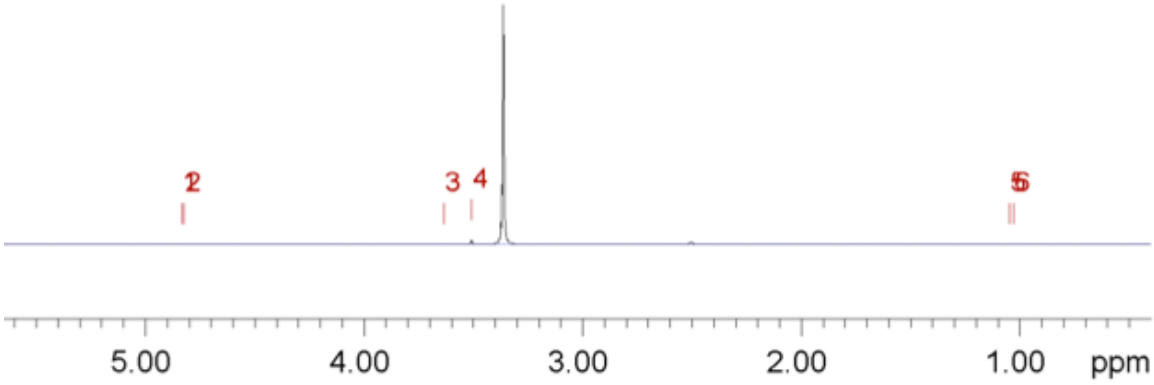
Peak name	F2 [ppm]	A	error	D [m2/s]	error	A	error	D [m2/s]	error
2	1.049	1.32e+05	2724	5.84e-11	1.959e-12	0.00	0.000	0.00	0.000
3	1.029	1.31e+05	2714	5.80e-11	1.968e-12	0.00	0.000	0.00	0.000
4	3.512	1.64e+05	4694	5.77e-11	2.075e-12	2.17e+05	1.469e+04	7.84e-10	7.504e-11

S.10.2: Spectra and self-diffusion coefficients for β-CD:L35 PRTx. Measured peaks are marked in red in the spectra above. Chemical shift, diffusion constants and error are listed in the table below.



Peak name	F2 [ppm]	A	error	D [m2/s]	error
1	4.836	3.55e+05	1178	9.86e-11	7.476e-13
2	4.826	3.35e+05	1161	1.00e-10	7.894e-13
3	3.637	4.99e+05	2698	1.02e-10	1.256e-12
4	3.507	9.34e+05	8701	5.68e-11	1.246e-12
5	1.048	3.47e+05	1047	5.30e-11	3.807e-13
6	1.029	3.75e+05	1350	5.38e-11	4.601e-13

S.10.3: Spectra and self-diffusion coefficients for β-CD:L64 PRTx. Measured peaks are marked in red in the spectra above. Chemical shift, diffusion constants and error are listed in the table below.



Peak name	F2 [ppm]	A	error	D [m2/s]	error
1	4.836	3.46e+05	2016	9.32e-11	1.241e-12
2	4.825	3.49e+05	1833	9.82e-11	1.176e-12
3	3.635	4.68e+05	3339	9.54e-11	1.554e-12
4	3.508	4.60e+06	4.098e+04	3.60e-11	8.395e-13
5	1.049	2.99e+05	1285	3.38e-11	3.880e-13
6	1.030	2.93e+05	1732	3.49e-11	5.464e-13

S.10.4: Spectra and self-diffusion coefficients for β-CD:F68 PRTx. Measured peaks are marked in red in the spectra above. Chemical shift, diffusion constants and error are listed in the table below.