Biomimetic Artificial Ion Channels Based On β-Cyclodextrin - ESI

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Materials

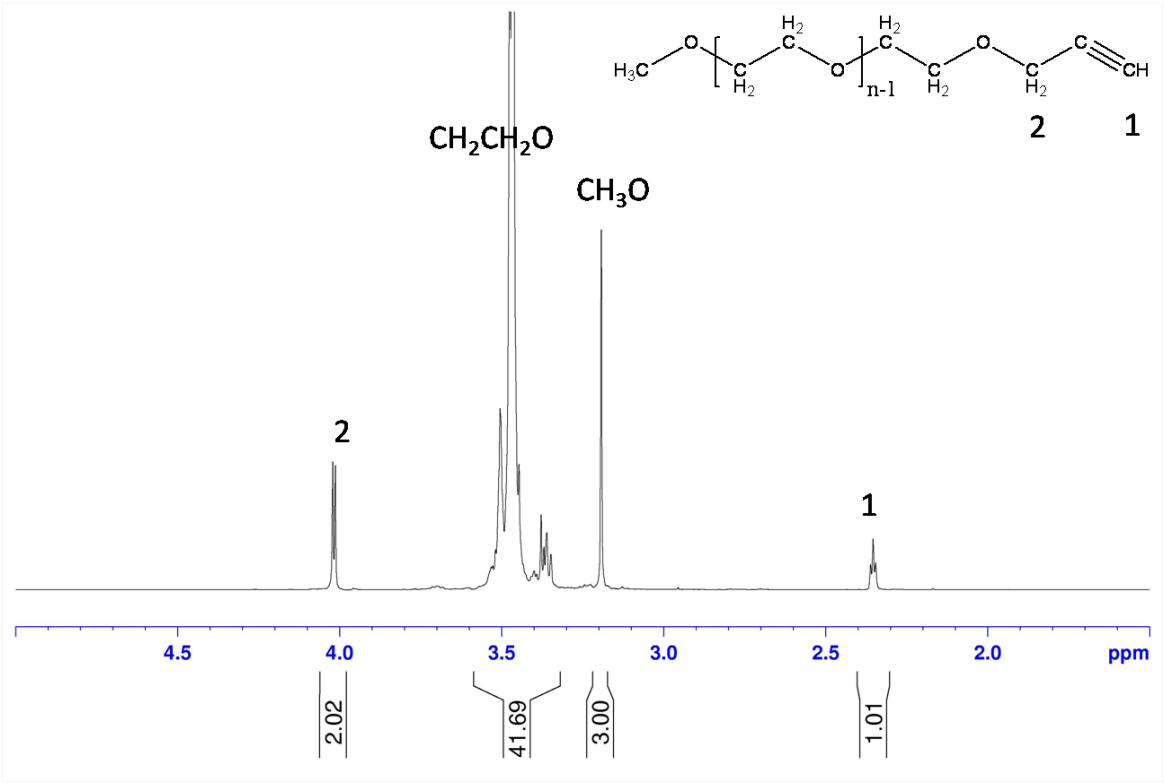
β-Cyclodextrin was purchased from Wacker and dried under vacuum at 80 °C during 24 h. Chemicals were purchased from commercial suppliers and used as received in their highest purity. All solvents were used as supplied without further purification, with the exception of CH_2Cl_2 dried on CaH_2. Distilled water was used in all experiments.

Syntheses of the star-shaped ethylene oxide oligomers/polymers via "click-chemistry" (CD-triazole-PEG)

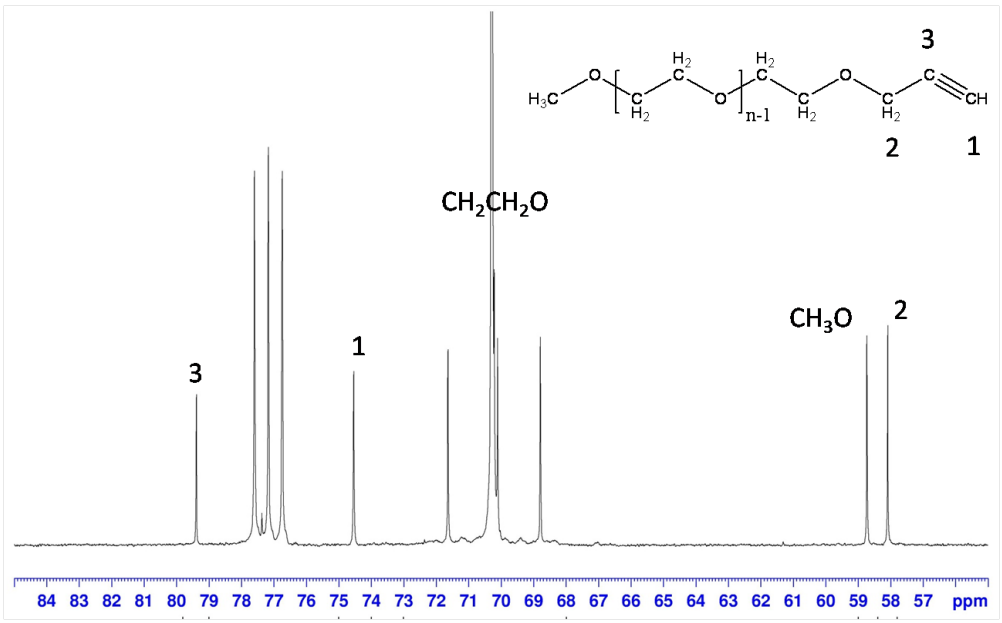
Synthesis of α-methoxy-ω-propargyl PEG[1]

In a round-bottom flask, 800 mg (20 mmol) of sodium hydroxide NaOH was added to a solution of methoxy poly(ethylene glycol) MPEG-OH (10 mmol) in toluene (10 mL). 2.4 mL of a solution of propargyl bromide (20 mmol, 80 wt. % in toluene) was dropped in the medium and the reaction was stirred during 24 h at 60°C under N_2 atmosphere. The solution was evaporated under a reduced pressure and 50 mL of water was added. The pH is brought at 7 by addition of hydrochloric acid HCl 3M. The polymer was extracted three times with 200 mL of CH_2Cl_2. The organic layer was dried over magnesium sulfate, then evaporated and finally dried under vacuum.

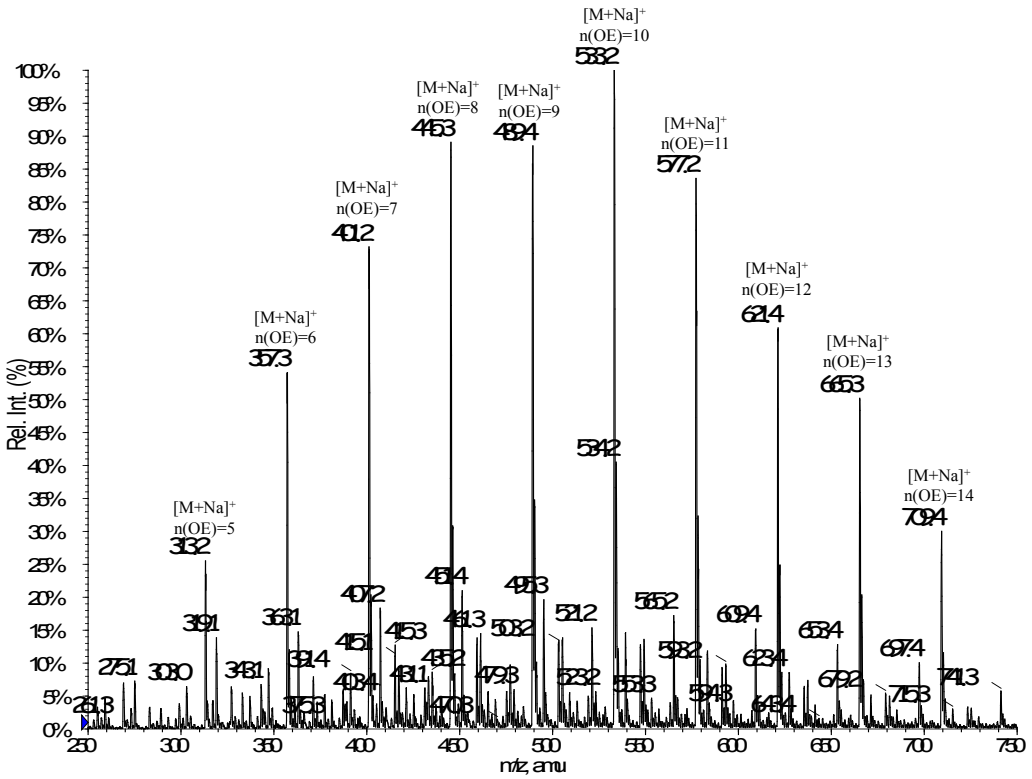
Yield: 90% - ^{1}H NMR (300MHz, $CDCl_3$) : < = 2.35 (t, 1H, ≡C*H*), 3.19 (s, 3H, C*H*$_3$O), 3.47 (m, C*H*$_2$C*H*$_2$O), 4.01 (d, 2H, -C*H*$_2$-C≡) - ^{13}C NMR (75MHz, $CDCl_3$): < = 57.97 (-*C*H$_2$-C≡), 58.60 (*C*H$_3$O), 70.10 (*C*H$_2$*C*H$_2$O), 74.50 (≡*C*H), 79.29 (-C≡).



^{1}H NMR of α-methoxy-ω-propargyl-PEG$_{550}$ ($CDCl_3$, 25°C).



^{13}C NMR of α-methoxy-ω-propargyl-PEG$_{550}$ ($CDCl_3$, 25°C).

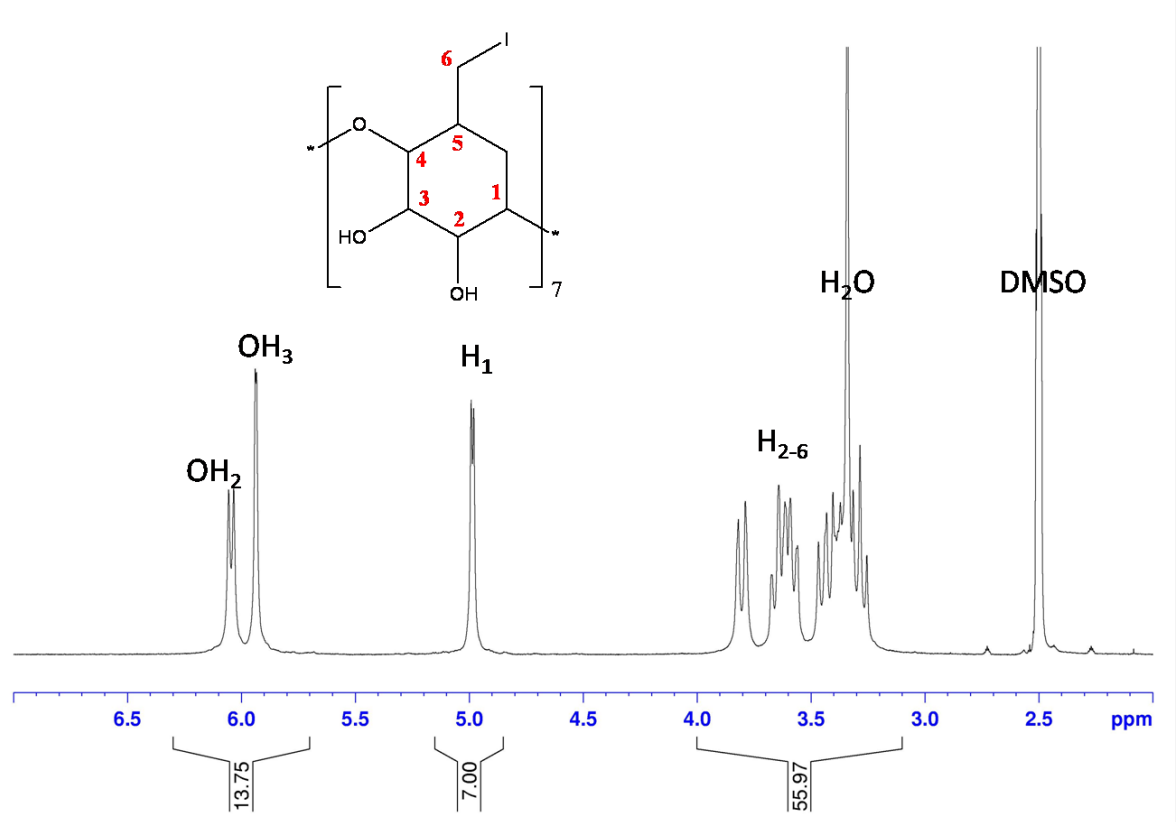


ESI-MS of α-methoxy-ω-propargyl-PEG_{550} at 0.2 mg/mL in MeOH 5% NaI.

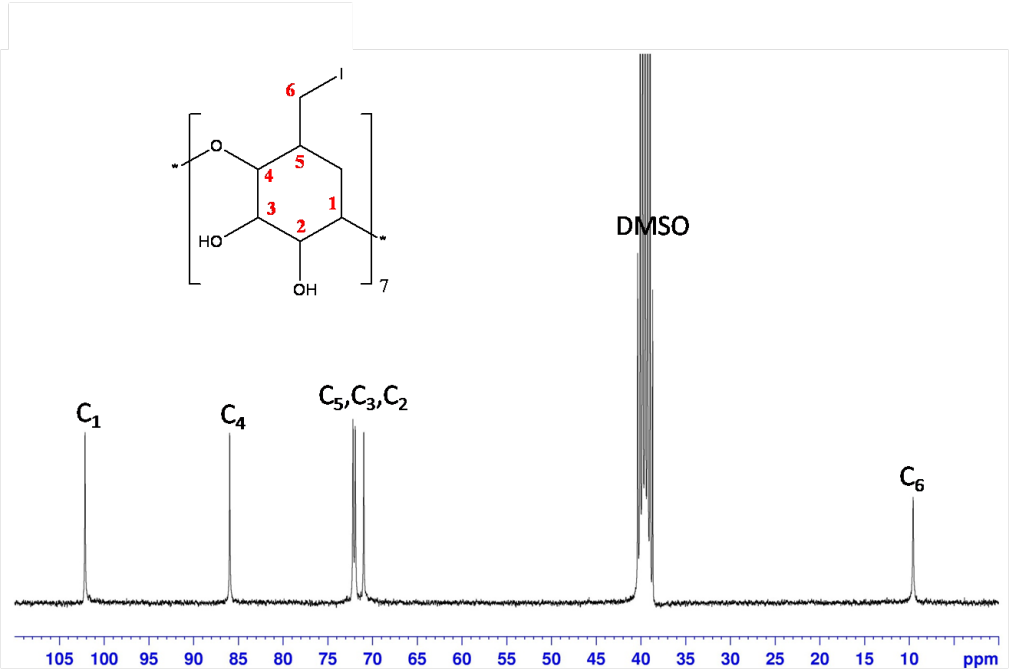
Synthesis of per-(6-deoxy-6-iodo)-β-CD[2]

PPh_3 (23.1 g, 88 mmol) was dissolved in dry DMF (90 mL) in a 250 mL flask equipped with a condenser under N_2. During the heating at 50°C, I_2 was added carefully (22.3 g, 88 mmol). β-cyclodextrin (5 g, 4.4 mmol) was then added to the dark brown solution and the solution was stirred at 70°C for 18h under N_2. After the reaction, the solution was concentrated under reduced pressure by the removal of DMF. The reaction mixture was then cooled to room temperature. A solution of NaOMe in methanol (5.6 g in 80 mL) was prepared under nitrogen in an ice bath and carefully introduced in the reaction vessel, by cooling. The mixture was stirred few minutes, then precipitated in 600 mL of methanol. The insoluble yellow product was filtered and washed with methanol, until the solvent becomes colorless. The product was dried at 80°C under vacuum overnight.

Yield: 90% - ^{1}H NMR (DMSO-d_6): < = 3.10 – 3.90 (m, 42H, *H*2, *H*3, *H*4, *H*5, *H*6), 4.98 (d, 7H, *H*1), 5.93 (d, 7H, O*H*3), 6.03 (d, 7H, O*H*2). ^{13}C NMR (DMSO-d_6): < = 9.57 (*C*6), 70.98 (*C*2), 71.93 (*C*3), 72.18 (*C*5), 85.96 (*C*4), 102.14 (*C*1).



^{1}H NMR of per-(6-deoxy-6-iodo)-β-CD(DMSO-d_6, 25°C).

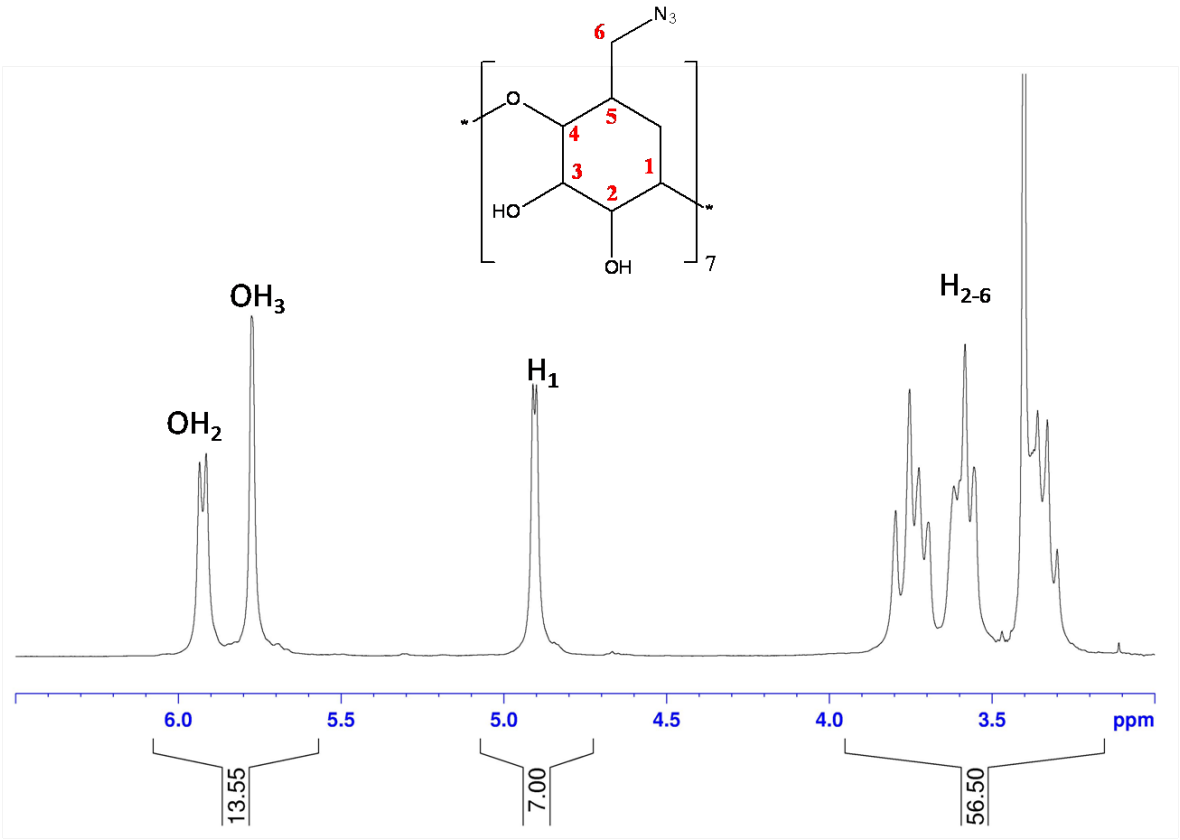


^{13}C NMR of per-(6-deoxy-6-iodo)-β-CD(DMSO-d_6, 25°C).

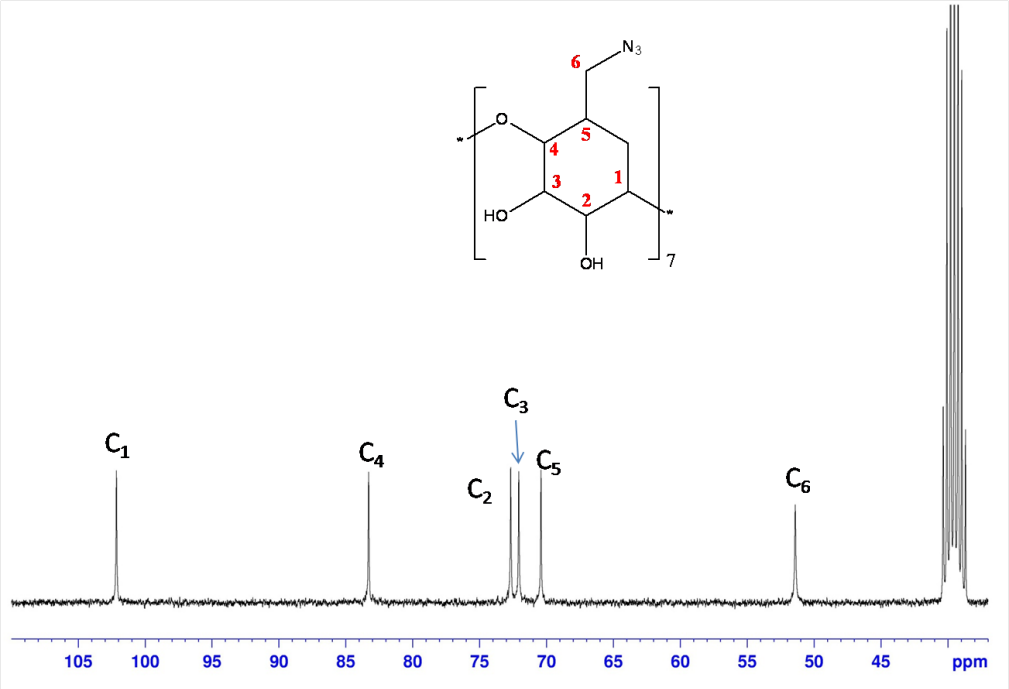
Synthesis of per-(6-deoxy-6-azido)-β-CD[2]

Per-(6-deoxy-6-iodo)-β-cyclodextrin (4 g, 2.1 mmol) was dissolved in DMF (70 mL), in a 250 mL flask equipped with a condenser under N_2. NaN_3 (1.4 g, 21 mmol) was added to the medium. The solution was stirred at 60 °C for 20 h. The solution was then concentrated under reduced pressure. The mixture was precipitated in 500 mL of water. The white insoluble product was filtered and washed with large excess of water. The product was dried at 80°C under vacuum overnight.

Yield: 90% - ^{1}H NMR (DMSO-d_6): < = 3.20 – 3.80 (m, 42H, *H*2, *H*3, *H*4, *H*5, *H*6), 4.90 (d, 7H, *H*1), 5.78 (d, 7H, O*H*3), 5.92 (d, 7H, O*H*2). ^{13}C NMR (DMSO-d6): < = 51.43 (CH_2-N_3), 70.44 (*C*5), 72.10 (*C*3), 72.71 (*C*2), 83.30 (*C*4), 102.16 (*C*1). ESIMS (MeOH): Calcd for $(M+Na^+)^+$ $C_{42}H_{63}O_{28}N_{21}Na$: 1332.4; Found: 1332.8 uma.



^{1}H NMR of per-(6-deoxy-6-azido)-β-CD(DMSO-d_6, 25°C).

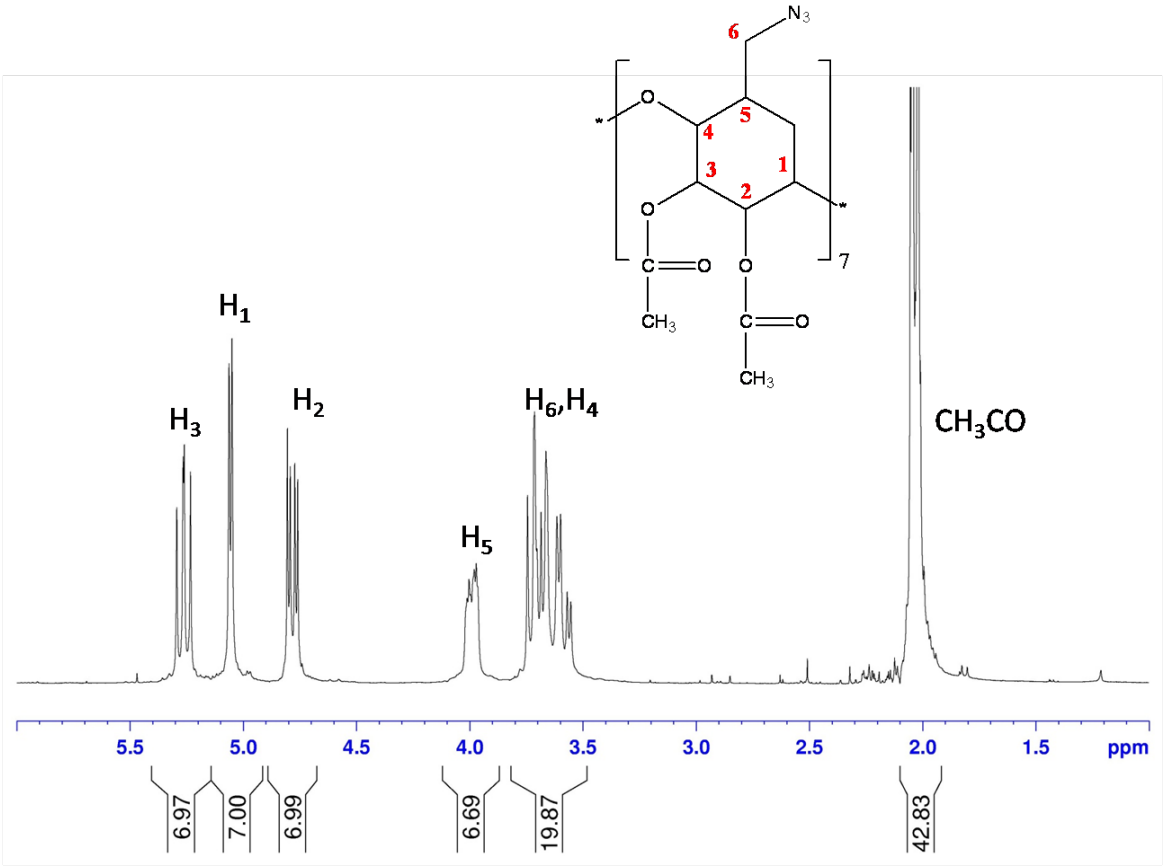


^{13}C NMR of per-(6-deoxy-6-azido)-β-CD(DMSO-d_6, 25°C).

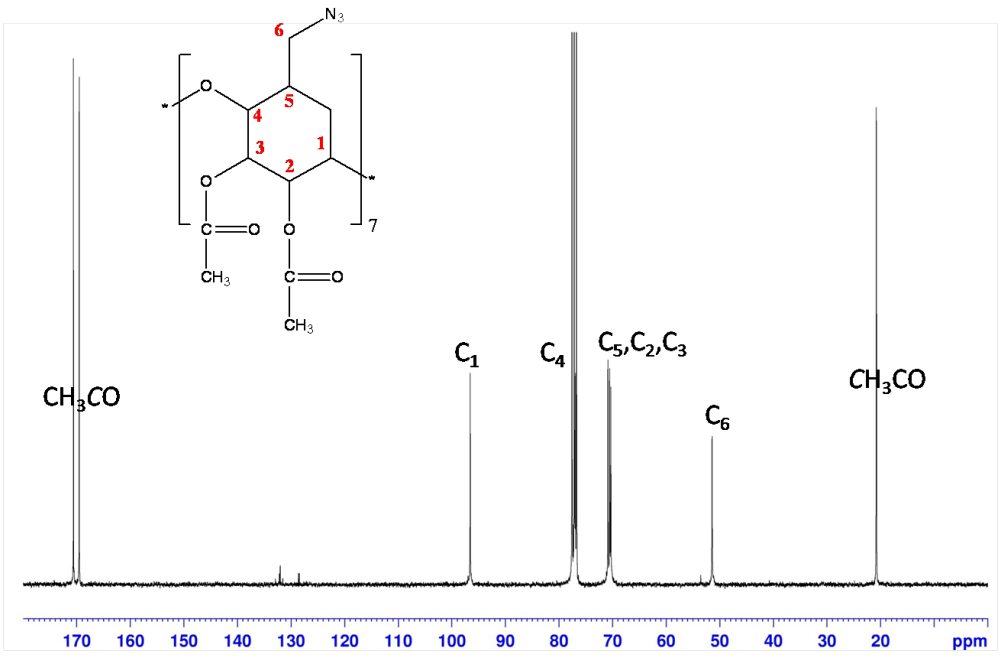
Synthesis of per-(2,3-di-O-acetyl)-(6-deoxy-6-azido)-β-cyclodextrin[3]

Per-(6-deoxy-6-azido)-β-cyclodextrin (2 g, 1.52 mmol) was dissolved in dry pyridine (35 mL) in a 250 mL flask. Acetic anhydride (25 mL) and a catalytic amount of 4-dimethylaminopyridine were added. The solution was stirred at 70 °C for 24 h under N_2. The residue was dissolved in 150 mL of CH_2Cl_2 and washed with HCl 1M (3x100 mL), followed by saturated $NaHCO_3$ (3x100 mL) and water (3x100 mL). The organic layer was dried over magnesium sulfate, filtered, and evaporated under a reduced pressure to remove all of dichloromethane. The product was dried at 80°C under vacuum overnight.

Yield: 72% - ^{1}H NMR ($CDCl_3$): < = 2.04 (d, 42H, $COCH_3$), 3.50 – 3.80 (m, 21H, *H*6, *H*4), 3.90 – 4.10 (m, 7H, *H*5), 4.80 (dd, 7H, *H*2), 5.07 (d, 7H, *H*1), 5.28 (t, 7H, *H*3). ^{13}C NMR ($CDCl_3$): < = 20.83 ($COCH_3$), 51.32 (CH_2-N_3), 70.42 (*C*3), 70.64 (*C*2), 70.96 (*C*5), 77.07 (*C*4), 96.67 (*C*1), 169.57 ($COCH_3$), 170.61 ($COCH_3$).



^{1}H NMR of per-(2,3-di-O-acetyl)-(6-deoxy-6-azido)-β-cyclodextrin ($CDCl_3$, 25°C).

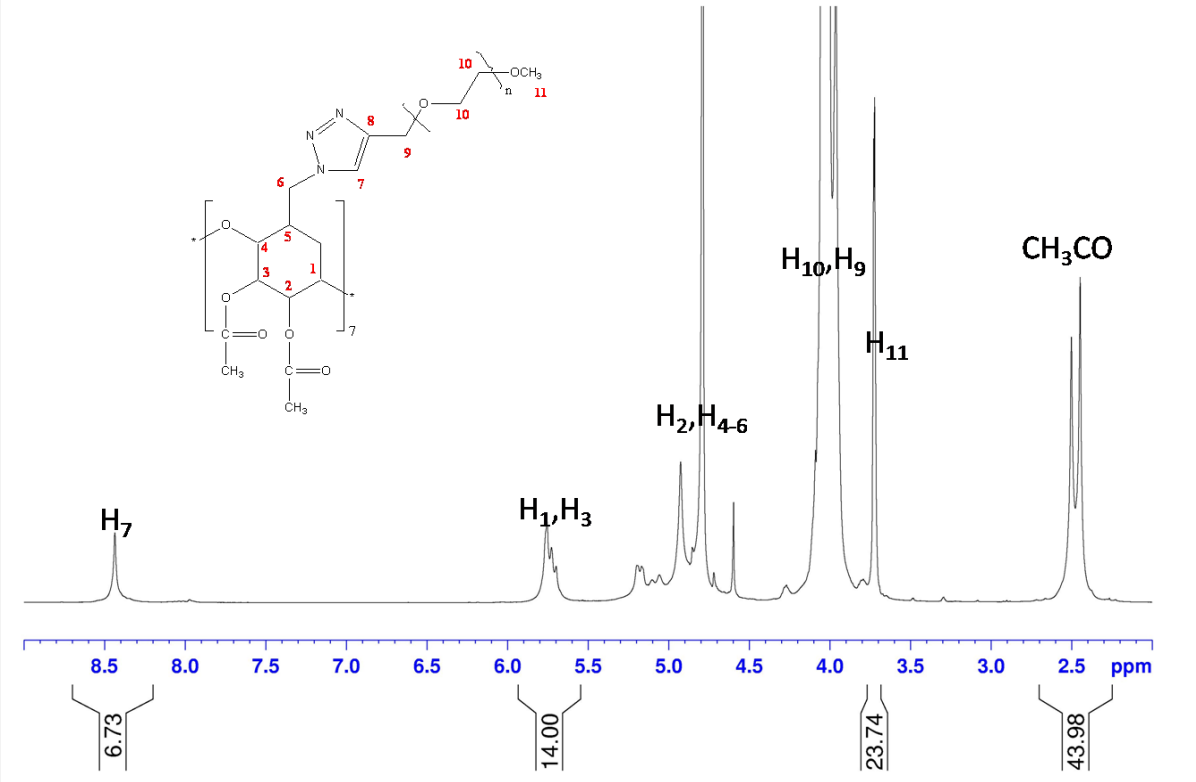


^{13}C NMR of per-(2,3-di-O-acetyl)-(6-deoxy-6-azido)-β-cyclodextrin ($CDCl_3$, 25°C).

Synthesis of per-(2,3-di-O-acetyl)-6-methoxyPEG-6-(1,2,3-triazole)-β-cyclodextrins[4]

α-methoxy-ω-propargyl PEG(2 mmol) (M_{PEG}=550, 1100 or 2000 g.mol^{-1}) was dissolved in dry DMF (10 mL). To this solution, per-(2,3-di-O-acetyl)-(6-deoxy-6-azido)-β-cyclodextrin (500 mg, 0.26 mmol) and hydrated copper sulphate (500 mg, 2 mmol) was added subsequently. A freshly prepared solution of sodium ascorbate (850 mg, 4 mmol, 3M) dissolved in water was dropped and the reaction was stirred 48h at room temperature. After evaporation of solvents, the residue was dissolved in 300 mL of CH_2Cl_2 and washed with 3x200 mL of ammonium hydroxide 5N. The organic layer was dried over magnesium sulfate, filtered, and evaporated under a reduced pressure. The product was dried at 50°C under vacuum overnight.

Yield: 80% - ^{1}H NMR (D_2O, 60°C): < = 2.48 (d, 42H, COC*H*$_3$), 3.72 (s, 21H, *H*11), 4.04 (m, 294H, *H*10, *H*9), 4.60-5.34 (m, 35H, *H*2, *H*4, *H*5, *H*6), 5.75 (d, 14H, *H*1, *H*3), 8.44 (s, 7H, *H*7).^{13}C NMR (D_2O, 60°C): < = 21.19 (CO*C*H$_3$), 51.13 (*C*6), 59.07 (*C*11), 64.19 (*C*9), 66-76 (*C*2, *C*3, *C*5, *C*10), 77.65 (*C*4), 97.40 (*C*1), 127.56 (*C*7), 145.17 (*C*8), 172.52 (*C*OCH$_3$).



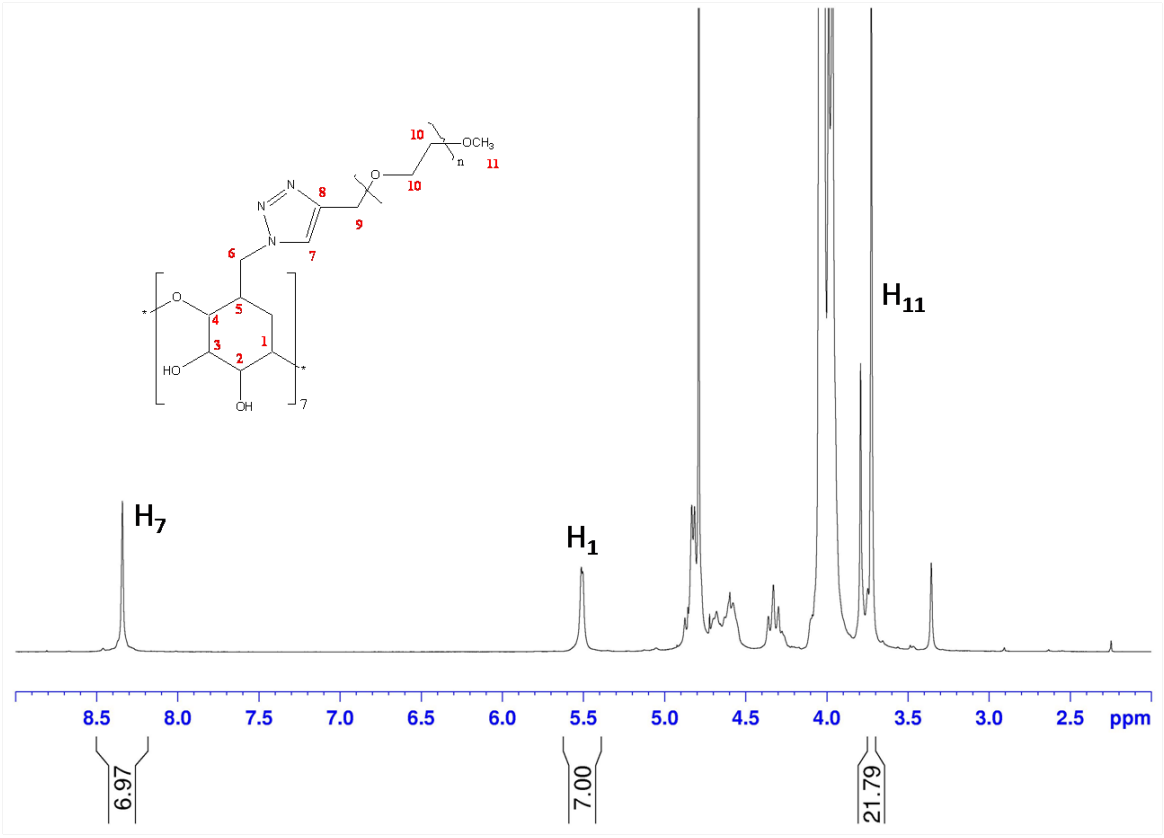
^{1}H NMR of per-(2,3-di-O-acetyl)-6-methoxyPEG$_{550}$-β-cyclodextrin (D_2O, 60°C).

Synthesis of per-6-methoxyPEG-6-(1,2,3-triazole)-β-cyclodextrins[5]

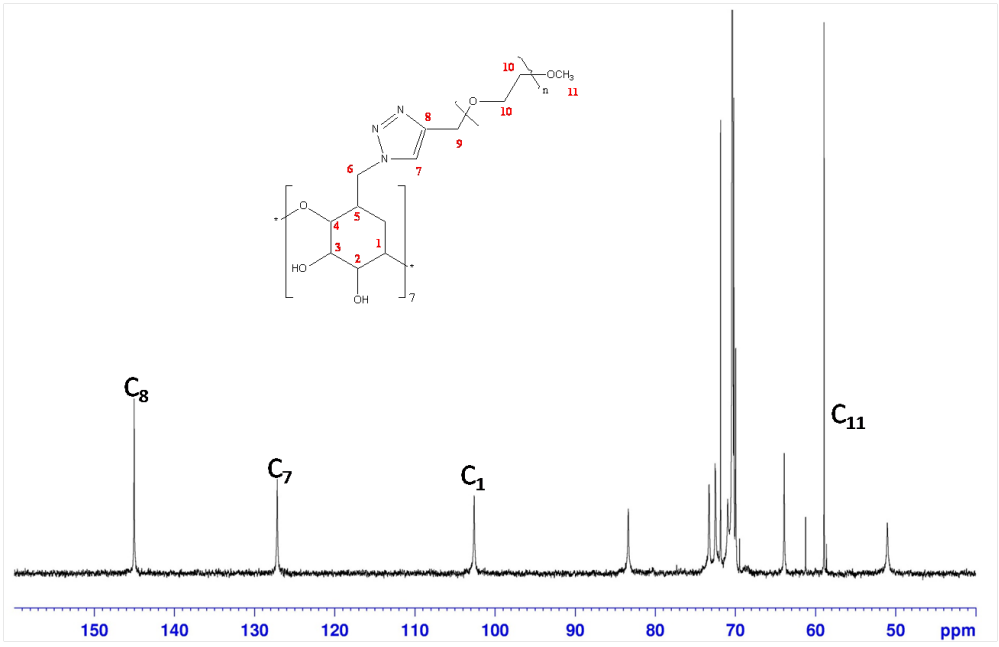
250 mg of per-(2,3-di-O-acetyl)-6-methoxypoly(ethylene glycol)-6-(1,2,3-triazole)-β-cyclodextrins were dissolved in 5 mL of a solution of sodium methoxide in methanol 1M and stirred overnight at room temperature. The product was dialyzed against methanol, concentrated, and dried at 50°C under vacuum overnight.

Yield: 85% - ^{1}H NMR (D_2O): < = 3.73 (s, 2H, *H*11), 3.79 (m, 7H, *H*4), 4.04 (m, 294H, *H*2, *H*10), 4.33 (t, 7H, *H*3), 4.50-5.00 (m, 35H, *H*5, *H*6, *H*9), 5.50 (d, 7H, *H*1), 8.34 (s, 7H,

*H*7).[13]C NMR (D_2O): < = 51.05 (*C*6), 58.93 (*C*11), 63.93 (*C*9), 69-74 (*C*2, *C*3, *C*5, *C*10), 83.38 (*C*4), 102.59 (*C*1), 127.16 (*C*7), 145.02 (*C*8).



[1]H NMR of per-6-methoxyPEG$_{550}$-β-cyclodextrin (D_2O, 60°C).



[13]C NMR of per-6-methoxyPEG$_{550}$-β-cyclodextrin (D_2O, 60°C).

Synthesis of per-(2,3-di-O-heptyl)-6-methoxyPEG-6-(1,2,3-triazole)-β-cyclodextrins(CD-triazole-PEG)[6]

The CD-triazole-PEG was achieved in a two-step procedure. Sodium hydride NaH (28 mmol) was added at 0°C to a solution of per-(6-methoxypoly(ethylene glycol)-6-(1,2,3-triazole))-β-CD in THF/DMF 50/50 (0.5 mmol in 90 mL of THF/DMF 50/50). The mixture was stirred 20 h at room temperature. Heptylbromide (28 mmol) was added dropwise and the mixture was stirred for 6 days at room temperature under N_2. In a second step, NaH (28 mmol) was added to the reaction mixture followed by an addition of heptylbromide (28 mmol) 20 h later. After 6 more days, methanol was added to neutralize the excess of sodium hydride and the solvents were evaporated under reduced pressure. The product was dialyzed against methanol, concentrated, and dried at 50°C under vacuum overnight.

Yields: 60% - ^{1}H NMR (DMSO-d_6, 60°C – Figure S1): < = 0.80 (m, 42H, *H*G), 1.20 (m, 112H, *H*F,*H*E, *H*D, *H*C), 1.45 (m, 56H, *H*B), 3.23 (s, 21H, *H*11), 3.50 (m, 315H, *H*A, *H*2, *H*3, *H*4, *H*10), 4.50 (m, 35H, *H*5, *H*6, *H*9), 5.40 (d, 7H, *H*1), 7.95 (s, 7H, *H*7) - ^{13}C NMR (DMSO-d_6, 60°C – Figure S2): < = 13.49 (*C*G), 21.87 (*C*F), 25.45 (*C*E), 28.56 (*C*D), 29.78 (*C*C), 31.23 (*C*B), 49.90 (*C*6), 57.87 (*C*11), 63.47 (*C*9), 69.75 (*C*10), 71.23 (*C*A), 72.31 (*C*5), 79.53 (*C*2, *C*3, *C*4), 96.89 (*C*1), 125.29 (*C*7), 143.73 (*C*8).

Structural Characterization

NMR spectra were recorded on a Bruker Advance AM300 spectrometer (^{1}H 300 MHz and ^{13}C 75 MHz) using DMSO-d_6, $CDCl_3$ or D_2O as solvents at 25°C or 60°C (Figures S1-S2).

For the Diffusion-Ordered SpectroscopY (DOSY) experiments (Figures S3-S4), the maximum field gradient strength was calibrated using a homemade Plexiglas phantom (8 mm ± 0.01 length and a width equal to the inner diameter of the NMR tube) inserted in a H_2O filled NMR tube and using the pulse program calibgp. The linear plot of the obtained gradient strengths against the gradient strength setting (GPZ1) used gave a maximum field gradient strength equal to 56.8 G.cm^{-1}. The temperature calibration of the spectrometer was performed with a sample of 100% CH_3OH in the temperature range between 298K and 313K. The accuracy of the calibrations was checked by measuring the self-diffusion coefficient of a mixture H_2O/D_2O (10% / 90% in moles) at 25 °C. The DOSY experiments were carried out using the stegp1s pulse sequence with a linear gradient of 16 steps between 2% and 95%. Before each diffusion experiment, the proton relaxation times were determined in order to correctly set the D1 parameter of the DOSY sequence and the length of the gradient δ and the diffusion time Δ were optimized for each analyzed product. All the DOSY experiments were carried out at 25°C and the concentration used for each sample was equal to 16 mg.mL^{-1} in DMSO-d_6. The mathematical treatment of the data was performed as previously described.[7]

Mass spectra (ESIMS) were recorded with an API2000 spectrometer. Solutions of CD derivatives were prepared in MeOH.

Molar masses were determined by size exclusion chromatography (SEC) with THF as eluent, the concentration of the polymer being 3 mg.mL^{-1} (Figure S5). The column used is a Styragel column (type HR 4E) from Waters. SEC was calibrated using poly(ethylene oxide) standards. The software used for the data analysis was OmniSEC (Viscotek).

Determination of the Critical Aggregation Concentration of CD-triazole-PEG$_{550}$ – Figure S6

The size of the CD-triazole-PEG$_{550}$star polymer, at various concentrations in buffer solutions (KCl 1M, Hepes 5 mM, pH=7.4 and KCl 1M, sodium citrate 5 mM, pH=3) was determined using a dynamic light scattering detector (Zetasizer nano ZS, ZEN 3500, Malvern) operating at a wavelength of 532 nm. Measurements were made at 25°C at a scattering angle of 173°. Each measurement was repeated triplicate.

BLM (Black Lipid Membrane) conductance measurements[8]

A film of a 1% solution of diphytanoylphosphatidylcholine in decane is spread across a 150 µm wide hole, drilled in a Delrin wall separating two chambers of a measurement device. Each chamber contains 1 mL of a 1M KCl, 5mM Hepes (pH 7.4) solution or 1 mL of a 1M KCl, 5mM sodium citrate (pH 3). After thinning of the decane film in order to get a planar bilayer, electrical current measurements are conducted to assess the formation of channels through the membrane when adding different quantities of star polymers from a stock solution into the two chambers.

Data acquisition: The ionic current through the membrane is measured with a BLM 120 amplifier (Biologic, Axon Instruments). Data are filtered at 300 Hz and acquired at 1,500 Hz with the Measurement Computing Digitizer (Axon Instruments), in order to minimize the noise. Before each experiment, the capacity of the membrane is measured in order to provide an estimation of the membrane thickness.

Data analysis: An all-points histogram of the electrical traces are generated using Igor Pro. The occurrence of the histogram thus represents the number of time the current measurement appears in the considered trace. This histogram has therefore a main peak centered at a current representing the current of the pores formed in the membrane. For different traces, we measure different pores currents which are multiple of a single pore current.

The Hill plot represents the open probability P_0 of single ion channel versus the polymer concentration. The obtained profile gives information about the stoichiometry of the assembly and gives then information of the number of molecules involved in the pore formation, by the equation (1).

$$\log P_0 = n \log C - n \log K_D \qquad (1)$$

with n the Hill coefficient and K_D the dissociation constant.

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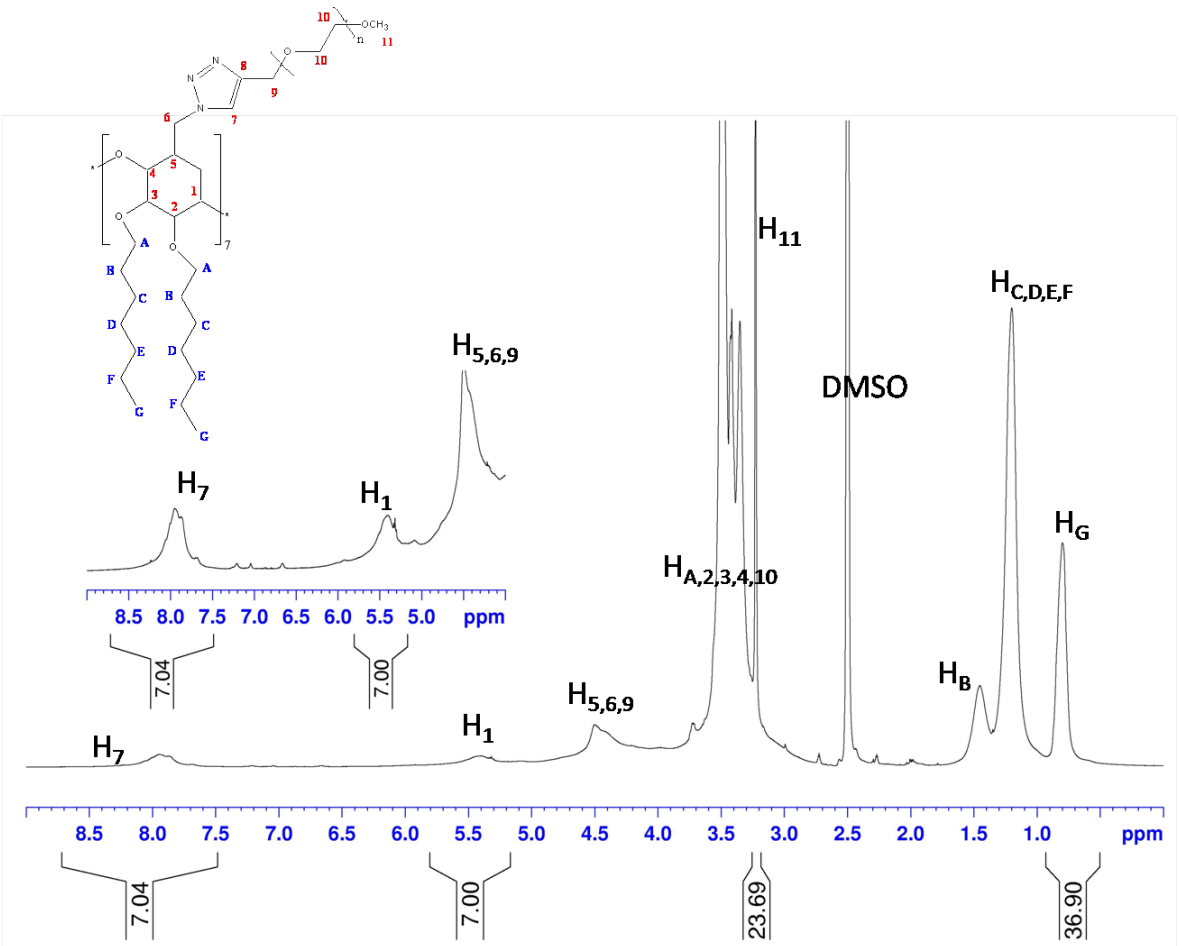

Figure S1. ^{1}H NMR of CD-triazole-PEG$_{550}$ - per-(2,3-di-O-heptyl)-6-methoxyPEG-6-(1,2,3-triazole)-β-CD (M_{PEG}=550 g.mol^{-1}) (DMSO-d$_6$, 60°C).

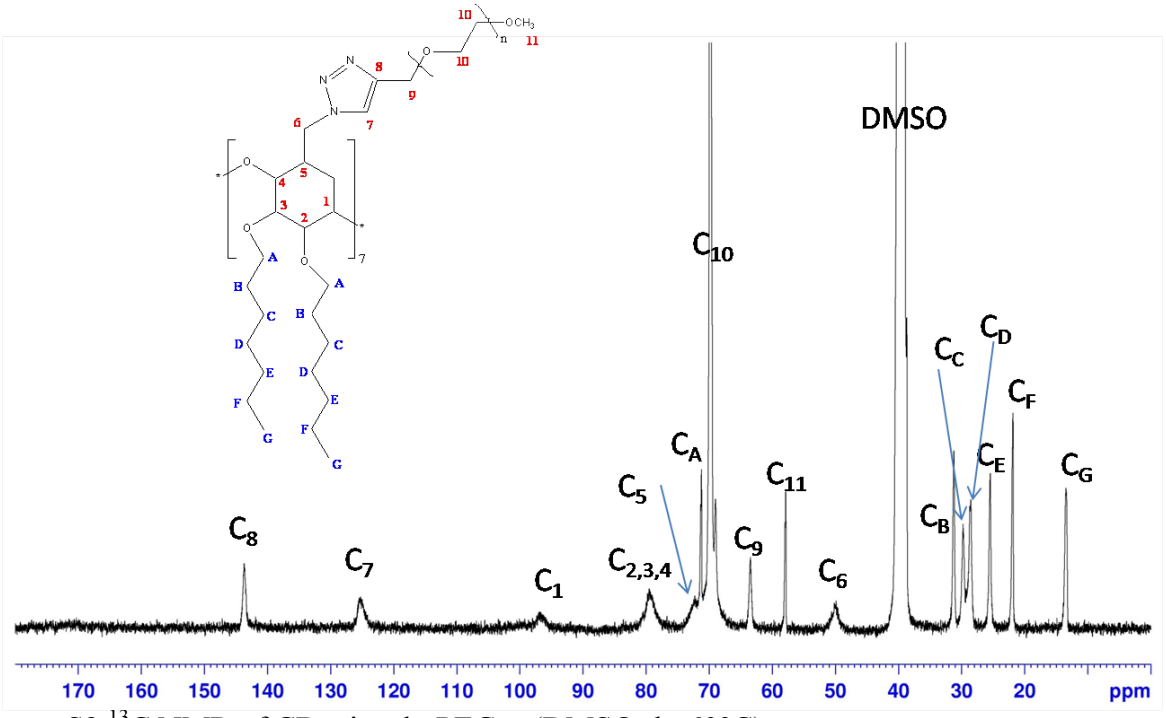

Figure S2.^{13}C NMR of CD-triazole-PEG$_{550}$(DMSO-d$_{6,}$ 60°C).

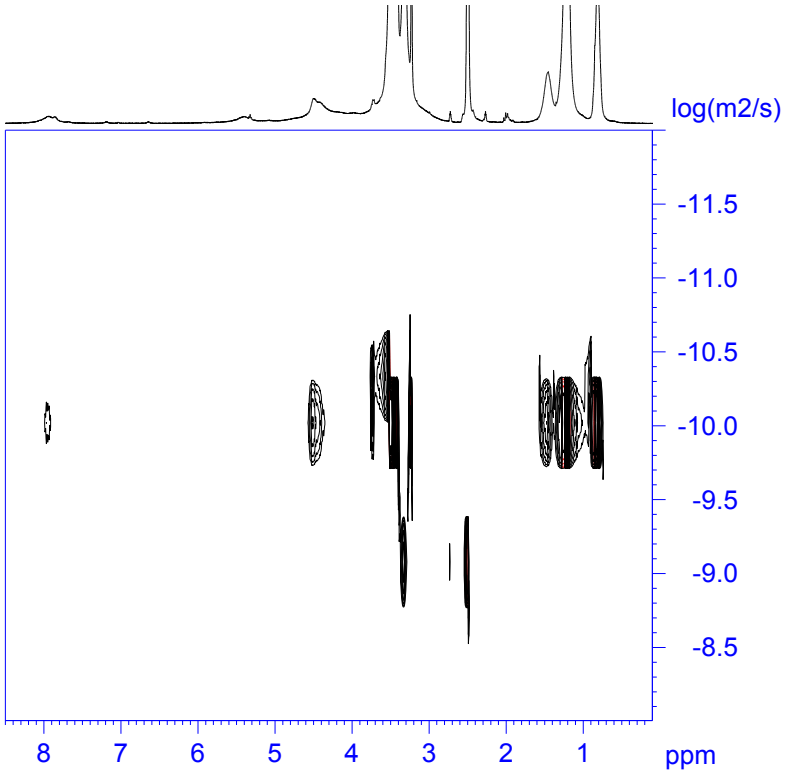

Figure S3.DOSY NMR of CD-triazole-PEG$_{550}$(DMSO-d$_{6,}$ 25°C).

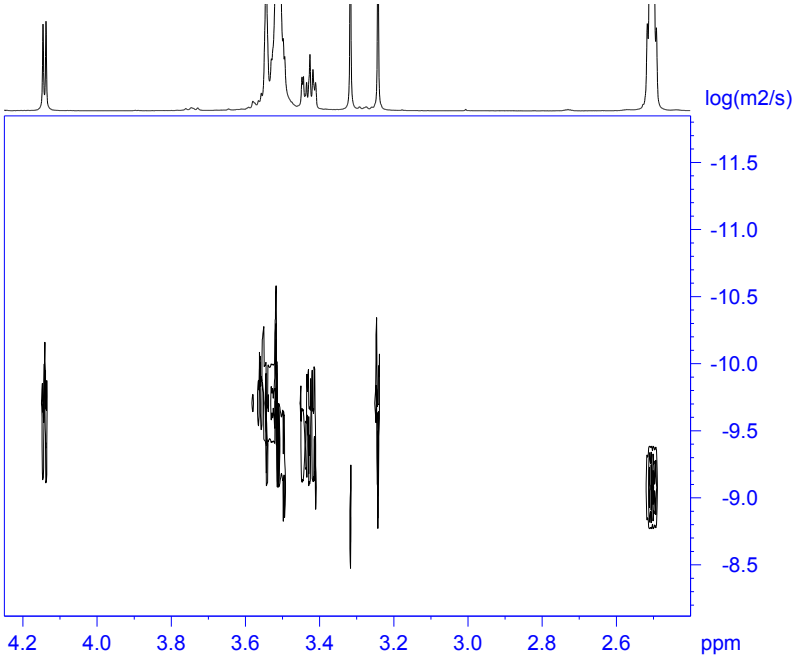

Figure S4.DOSY NMR of α-methoxy-ω-propargyl-PEG$_{550}$ (DMSO-d$_6$, 25°C).

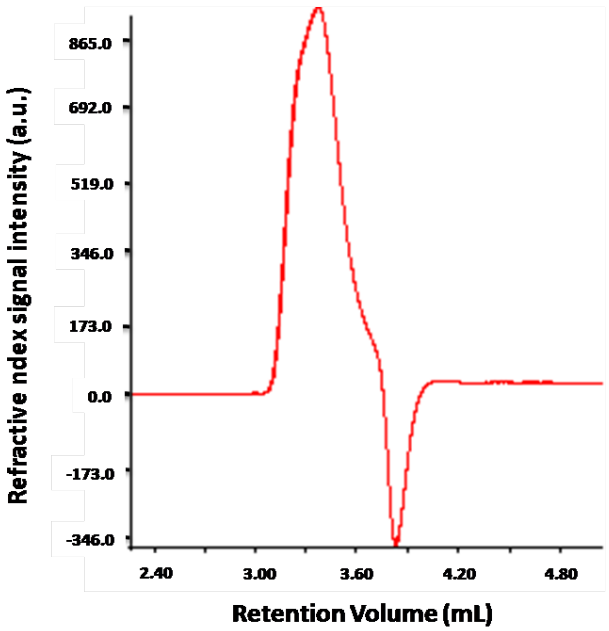

Figure S5.SEC chromatogram of per-6-methoxyPEG-6-(1,2,3-triazole)-β-CD (M_{PEG}=550 g.mol^{-1}) (THF, 25°C).

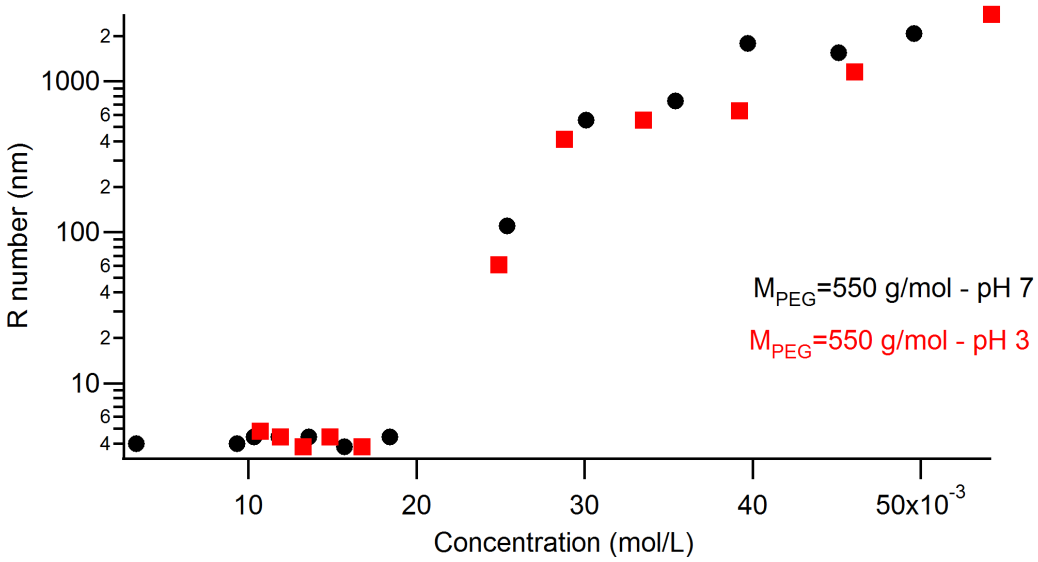

Figure S6. Sizemeasurements by DLS of CD-triazole-PEG_{550}in KCl 1M, Hepes 5 mM, pH=7 and in KCl 1M, sodium citrate 5 mM, pH=3.

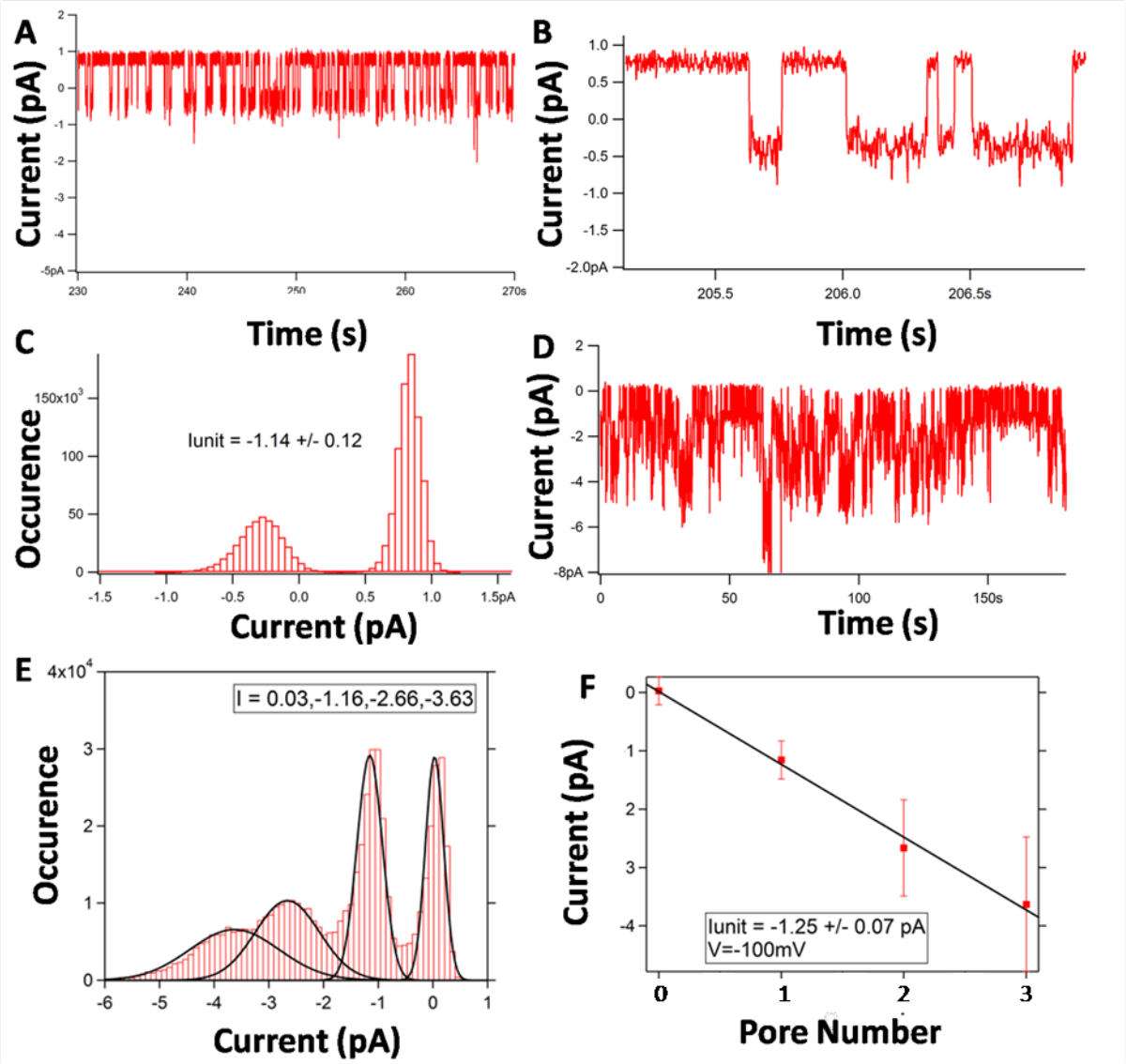

Figure S7. BLM conductance measurements and analysis of CD-PEG$_{530}$ -per-(2,3-di-O-heptyl)-6-PEG-β-CD ($\overline{Mn}$ =5000 g.mol^{-1} / M_{PEG}=530 g.mol^{-1}) - Concentration < 4.10^{-6} mol.L^{-1}– pH 7 – Applied voltage V= -100mV

A) BLM electrical measurements – example 1

B) Zoom of the trace A

C) Number of events versus current corresponding to the trace A

D) BLM electrical measurements – example 2

E) Number of events versus current corresponding to the trace D

F) Current versus pore number

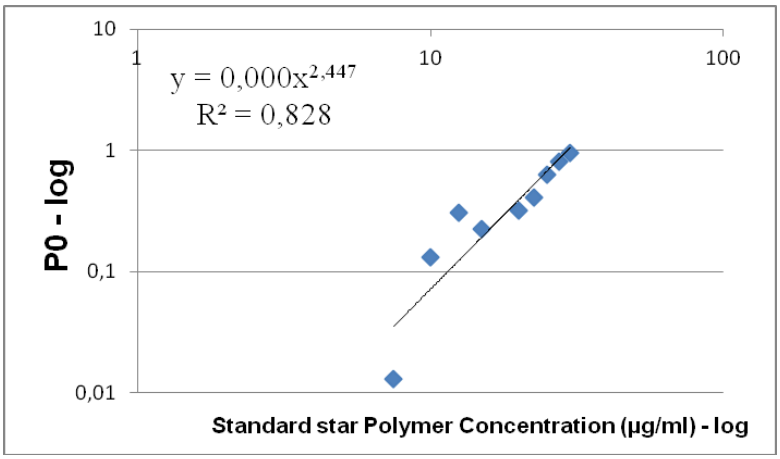

Figure S8. Open probability P_0 of single ion channel plotted as a function of CD-PEG_{530}concentration - experimental duration of BLM conductance measurements: 30 minutes - applied voltage V= -100mV

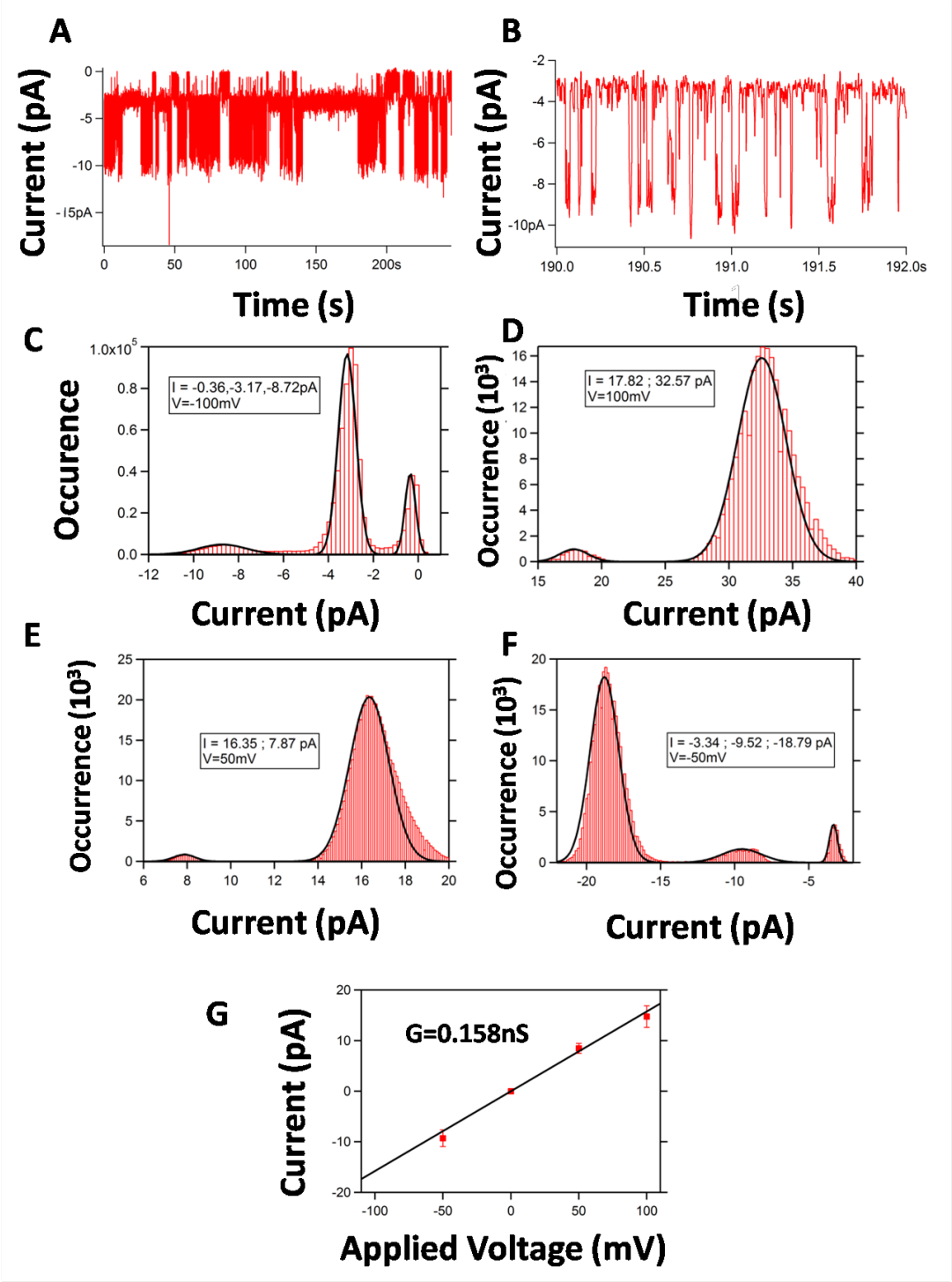

Figure S9. BLM conductance measurements and analysis for CD-PEG$_{530}$ - Concentration > 5.10^{-6} mol.L^{-1} – pH 7

A) BLM electrical measurements – Applied Voltage: -100mV
B) Zoom of the trace A
C) Number of events versus current corresponding to the trace A
D) BLM electrical measurements – Applied Voltage 100mV
E) BLM electrical measurements – Applied Voltage -50mV
F) BLM electrical measurements – Applied Voltage 50mV
G) I-V curve of the clusters

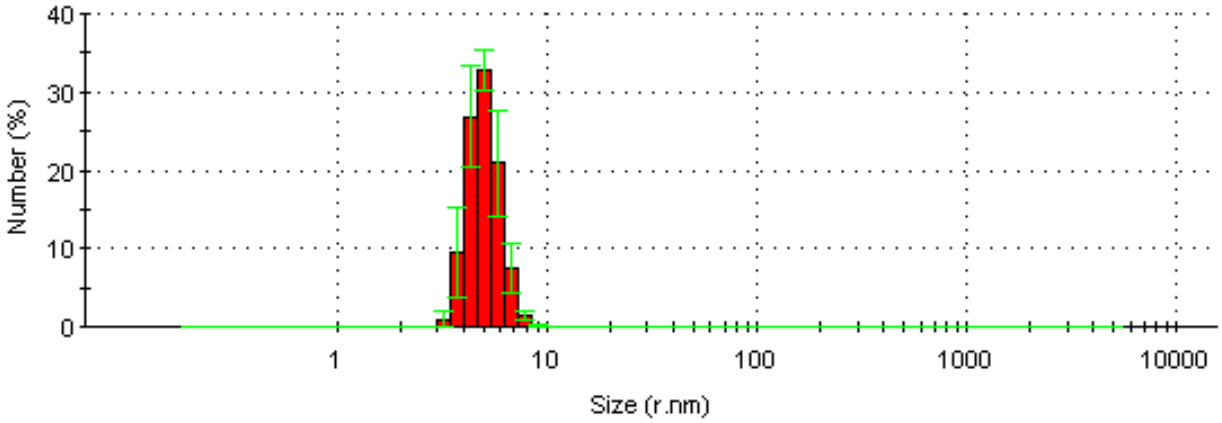

Figure S10. Sizemeasurements by DLS of CD-PEG$_{530}$in KCl 1M, Hepes 5 mM, pH=7 at $8.5.10^{-6}$ mol.L^{-1}.

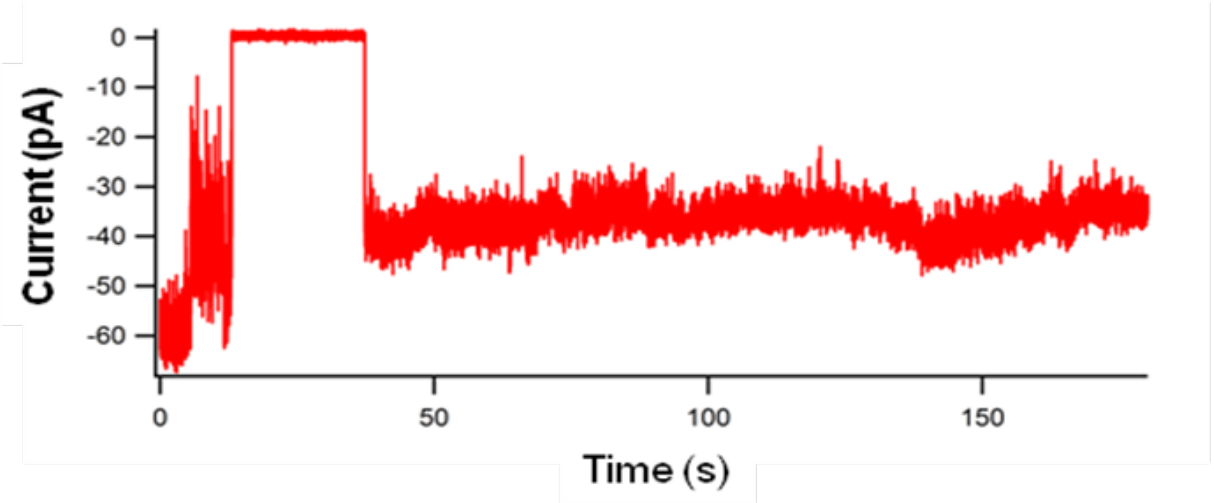

Figure S11. BLM conductance measurements for CD-PEG$_{2200}$at a concentration higher than $8.1.10^{-6}$ mol.L^{-1} – Current intensity versus time for an applied voltage: -100 mV