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Preparation of Membrane-Mimicking Lamellar Structures by Molecular Confinement of Hybrid Nanocomposites

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Supporting Information

Experimental Section

Materials

Metallacarborane cesium [3-cobalt(III) bis(1,2-dicarbollide)]$^-$, Cs[COSAN], called also COSAN, was purchased from Katchem (Prague, Czech Republic).

Preparation of Na[COSAN].4H_2O: The cationic exchange resin (Amberlite IR120, H form) was purchased from Acros Organics. Cs[COSAN] was converted to Na[COSAN]: First, we charged the resin by passing 250 mL of 3 M HCl followed by rinsing with water. The column was loaded by sodium cation by passing 3M NaCl for preparation of Na[COSAN] solution through resin, and then rinsed by around 500 mL of water (the eluent was tested if it contains traces of NaCl by $AgNO_3$ solution). Solid Cs[COSAN] was dissolved in a mixture of acetonitrile/water (50:50) and this solution (around 100 mL) was passed repeatedly through (drop by drop) a cation exchanging resin loaded with desired cation. The solvent was finally evaporated, and the crude product was leaving to dry in a desiccator overnight. Water content in Na[COSAN] was determined by TGA to be 16%.

THF (Sigma–Aldrich) was distilled from $AlLiH_4$, Methanol was dried with Mg and Br_2, distilled and kept over molecular sieves 3A, NaN_3 (99.5 % - Sigma Aldrich), HBr in Glacial Acetic Acid (33 % wt. Acros), NaH (60 % wt. dispersion in oil Sigma Aldrich), Na metal (99.9% Acros), Propargyl Bromide solution (80 % wt. in toluene, Sigma Aldrich), N,N,N',N'',N''-Pentamethylendiethylenetriamine (PMDETA) (99%, Sigma Aldrich), CuBr (99 %, Sigma Aldrich), methoxy poly(ethyleneoxide) mPEG (M_n = 2000 g/mol, 5000 g/mol), poly(ethyleneoxide) PEO (M_n = 2000 g/mol, 4000 g/mol) (99% Sigma Aldrich) and other solvents (p.a., Lach-ner) were used as received.

Synthesis

1-bromo-2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside synthesis: In 100 mL round bottom flask with magnetic stirbar a 5 g (13 mmol) of 1,2,3,4,6-penta-O-acetyl glucopyranoside was disolved in dry chloroform. 8.6 mL (45 mmol) of 33 % HBr in acetic acid was added slowly at 0°C. After addition the reaction mixture was sealed with drying and kept overnight. End of reaction was checked by TLC chromatography in 3/2 Hex/EtOAc (reactant R_f = 0.23, product R_f = 0.43). 50 mL of DCM was added to reaction mixture, extracted twice with cold distilled water and twice with 10 % K_2CO_3 solution. Organic phase was dried over anhydrous $MgSO_4$ and concentrated in vacuo. Yellowish powder was recrystalized twice from mixture 1/9 Et_2O/Heptane, 4.76 g of white powder was obtained, yield 87 %. ^{1}H NMR (300 MHz, $CDCl_3$)

δ: 6.61 (d, J = 4.1 Hz), 5.56 (t, J = 9.8 Hz), 5.15 (t, J = 10.1 Hz), 4.84 (dd, J_1 = 10.1 Hz, J_2 = 4.1 Hz), 4.37-4.27 (m), 4.17-4.09 (m), 2.11 (s) 2.10 (s), 2.06 (s), 2.04 (s).

1-azido-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside synthesis: 2 g of 1-bromo-2,3,4,6-tetra-O-acetyl-α-D- glucopyranose and 0.4 g of NaN_3 were dissolved in 20 mL of DMF. Reaction was kept for 24 h at room temperature. 50 mL of EtOAc was added and inorganic NaBr salt precipitated from solution. Reaction mixture was filtrated and extracted with saturated KCl solution four times. Organic phase was dried over molecular sieves 3A and concentrated in vacuo. 1.29 g of white powder was obtained, yield = 79 %. **^{1}H NMR (300 MHz, $CDCl_3$) δ:** 5.21 (t, J = 9.4 Hz), 5.09 (t, J = 9.9 Hz), 4.94 (t, J = 9.2 Hz), 4.63 (d, J_1 = 8.8 Hz), 4.21 (ddd, J_1 = 16.5, J_2 = 7.7 Hz, J_3 = 4.7 Hz), 3.79 (m), 2.09 (s) 2.06 (s), 2.02 (s), 1.99 (s).

1-azido-1-deoxy-D-glucopyranose synthesis: Acetyl deprotection was performed according to Zemplen. Briefly, 940 mg (2.52 mmol) of 1-azido-2,3,4,6-tetra-O-acetyl-D-glucopyranoside was dissolved in 5 mL of anhydrous methanol and 50 μL of freshly prepared methanolic solution of MeONa was added to the solution. The reaction mixture was left standing at room temperature for 15 min. After deprotection, the methanolic solution was neutralized by adding Dowex 50WX2, filtrated and dried over molecular sieves 3A. Methanol was evaporated on a rotavapor. The product was placed in vacuum desiccator for 48 h (91 % Yield). **^{1}H NMR (300 MHz, D_2O) δ**: 4.62 (d, J = 8.8 Hz, 1H), 3.79 (dd, J_1 = 12.3 Hz, J_2 = 2 Hz, 1H), 3.71 (dd, J_1 = 12.5 Hz, J_2 = 5.6 Hz, 1H) 3.45 – 3.29 (m, 2H), 3.24 (t, J = 8.9 Hz, 1H).

General procedure for Synthesis of alkyne terminated PEO/mPEO: 2 g (1 mmol) of methoxy poly(ethylene glycol) (M_w = 2000 g/mol) (5 mmol, 5-fold excess in case of mPEO, 10-fold excess in case of PEO) and 0.2 g of NaH were weighted in oven dried schlenk flask equiped with magnetic stirbar and PTFE septa. Schlenk flask was evacuated for 20 minutes and flushed with argon for 5 minutes. 30 mL of dry THF was added under positive argon pressure, the reaction mixture was cooled in an ice water bath and propargyl bromide solution was added dropwise under positive argon pressure. After addition, the reaction mixture was warmed to room temperature and left overnight. The reaction was stopped by addition of 2M HCl until neutral pH. THF was evaporated under reduced pressure and the organic residue was re-dissolved in 40 mL of DCM. The organic phase was extracted with a saturated NaCl solution for three times and dried over anhydrous $MgSO_4$. The DCM solution was concentrated in vacuo and added dropwise to slowly stirred excess of cold Et_2O. A white precipitate was isolated on Buchner funnel and dried in vacuo. **$mPEO_{45}$-A:** Yield = 75 %. **^{1}H**

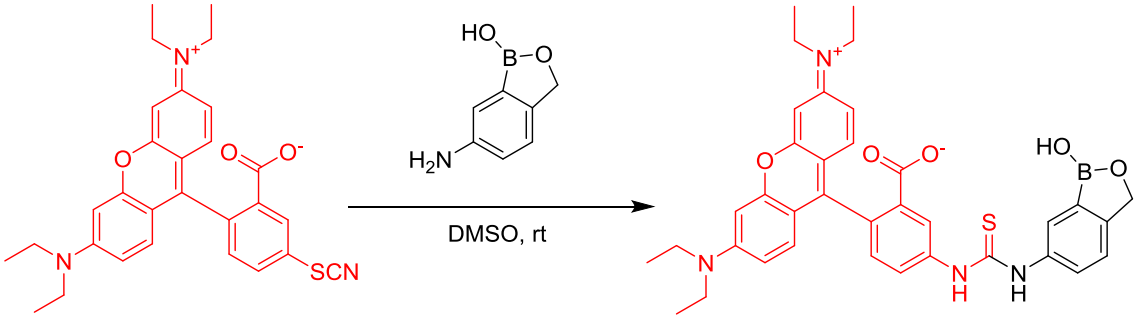
NMR (300 MHz, $CDCl_3$) δ(ppm): 4.21 (d, J = 2.4 Hz), 3.64 (s), 3.38 (s), 2.44 (t, J_1 = 2.4 Hz, J_1 = 2.4 Hz), GPC (THF): M_n = 2676, M_w = 2791, $Đ$ = 1.04. **$mPEO_{114}$-A:** Yield = 74 %. **^{1}H NMR (300 MHz, $CDCl_3$) δ(ppm):** 4.21 (d, J = 2.4 Hz), 3.65 (s), 3.38 (s), 2.44 (t, J_1 = 2.4 Hz, J_1 = 2.4 Hz), GPC (THF): M_n = 5861 g/mol, M_w = 6190 g/mol, $Đ$ = 1.06. **A-PEO_{45}-A:** Yield = 69 %. **^{1}H NMR (300 MHz, $CDCl_3$) δ(ppm):** 4.21 (d, J = 2.4 Hz), 3.65 (s), 2.44 (t, J_1 = 2.4 Hz, J_1 = 2.4 Hz), GPC (THF): M_n = 2848, M_w = 2953, $Đ$ = 1.04. **A-PEO_{90}-A:** Yield = 71 %. **^{1}H NMR (300 MHz, $CDCl_3$) δ(ppm):** 4.21 (d, J = 2.4 Hz), 3.65 (s), 2.44 (t, J_1 = 2.4 Hz, J_1 = 2.4 Hz), GPC (THF): M_n = 5163 M_w = 5448, $Đ$ = 1.06.

General procedure for CuAAC click reaction of propargylated PEO/mPEO with 1-azido-1-deoxy-D-glucopyranose: The ratio of alkyne/azide in reaction mixture for all synthesized polymers was kept 1/1.1. The reactants were transferred to a Schlenk flask equipped with magnetic stir bar. The flask was sealed with a three-way stopcock and flushed with argon for 5 min. The reactants were dissolved in 10 mL of acetone/water 10/1 mixture and 0.100 μL of PMDETA was added using a stainless-steel syringe under positive argon pressure. The reaction mixture was degassed in three freeze-thaw cycles with liquid nitrogen. Finally, a catalytic amount (0.5 – 2 mg) of CuBr was quickly added under positive argon pressure. The reaction mixture flask was placed in an oil bath preheated to 55 °C for 24-48 h. Subsequently, the polymer solution was dried over molecular sieves 3A, and acetone was evaporated at reduced pressure. The organic residue was dissolved in 20 mL of DCM and passed through a short plug of basic Al_2O_3 three times to remove residual copper. Metal free solution was added dropwise to a slowly stirred excess of cold Et_2O. The white precipitate was collected by centrifugation at 2500 rpm for 20 min. **$mPEO_{45}$-Glc: 50% yield. ^{1}H NMR (300 MHz, D_2O) δ(ppm):** 8.28 (s, 1H), 5.76 (d, J = 9.2 Hz, 1H), 4.73 (s, 2H), 4.00 (t, J = 9.1 Hz, 1H), 3.69 (s, 180H), 3.90-3.78 (m, 1H) 3.49-3.44 (m, 1H), 3.37 (s, 3H), GPC (THF): M_n = 2787 g/mol, M_w = 2891 g/mol, $Đ$ = 1.04. **$mPEO_{114}$-Glc: 62% yield. ^{1}H NMR (300 MHz, D_2O) δ(ppm):** 8.28 (s, 1H), 5.76 (d, J = 9.2 Hz, 1H), 4.73 (s, 2H), 4.00 (t, J = 9.1 Hz, 1H), 3.69 (s, 180H), 3.90-3.78 (m, 1H) 3.49-3.44 (m, 1H), 3.37 (s, 3H), GPC (THF): M_n = 5585 g/mol, M_w = 6022 g/mol, $Đ$ = 1.08. **Glc-PEO_{45}-Glc: 53% yield. ^{1}H NMR (300 MHz, D_2O) δ(ppm):** 8.26 (s, 2H), 5.74 (d, J = 9.2 Hz, 2H), 4.71 (s, 4H), 3.98 (t, J = 9.1 Hz, 2H), 3.88-3.77 (m, 2H) 3.66 (s, 186H) – overlapping signals of PEO protons and few pyranose ring hydrogens, 3.44 (m, 2H) GPC (THF): M_n = 2774 g/mol, M_w = 2925 g/mol, $Đ$ = 1.05. **Glc-PEO_{90}-Glc: 69% yield. ^{1}H NMR (300 MHz, D_2O) δ(ppm):** 8.27 (s, 2H), 5.74 (d, J = 9.2 Hz, 2H), 4.71

(s, 4H) – overlapped with D_2O, 3.98 (t, J = 9.1 Hz, 2H), 3.88-3.77 (m, 2H) 3.66 (s, 186H) - overlapping signals of PEO protons and few pyranose ring hydrogens, 3.44 (m, 2H) GPC (THF): M_n = 4979 g/mol, M_w = 5371 g/mol, $Đ$ = 1.08.

GB179 fluorescence probe: GB179 was prepared by Bohumír Gruner (IIC, Řež near Prague, Czechia). Synthesis and probe characterization are given in our previous paper [1].

RhBB fluorescence probe: In dry round-bottom flask with magnetic stirrer a 40 mg of Rhodamine B isothiocyanate (7.46×10^{-2} mmol) was dissolved in 3 mL of dry DMSO, the flask was sealed with silicone septa and flushed with argon for 5 min. 12 mg of (5-amino-2-hydroxymethylphenyl)boronic acid (8.05×10^{-2} mmol) was dissolved in dry DMSO (2 mL), this solution was transferred to rhodamine B solution by cannula under argon atmosphere. The reaction (Scheme S1) was left for 24 h. at ambient temperature. The reaction mixture was subjected to column chromatography with SiO_2 as stationary phase and a gradient mobile phase starting from pure EtOAc up to solvent mixture MeOH/EtOAc 4/1. Fraction with lowest R_f = 0.15 contained product amongst other rhodamine derivatives. Low boiling solvents were evaporated and residual DMSO was eliminated by repeated lyophilization with water (DMSO/water = 1/2). Red solid was vacuum dried for 48 h. The dried product was purified by preparative TLC with gradient eluent, starting from pure EtOAc and finishing with mixture EtOAc/MeOH 1/9. Each solvent run was kept through whole height of TLC plate and to last solvent mixture NH_4OH was added for easier elution of thiourea complex. The desired fraction (R_f = 0.1) was cut out of the plate and extracted with MeOH (+50 µL of NH_4OH) for 2 hours. The liquid phase was filtered of and evaporated at reduced pressure. The product was 5 mg of dark red solid, yield = 10 %. ^{1}H NMR (600 MHz, MeOD) δ (ppm): 8.10-6.74 (bm, 12H), 5.49 (s, 2H), 3.7-3.5 (m, 8H), 1.36-1.23 (m, 12H) ^{11}B NMR (600 MHz, MeOD) δ (ppm): 6.86 (s), 2.24 (s).



Scheme S1: Schematic representation of synthetic pathways used in this work.

Sample preparation

The solutions of PEO, PEO_m-glc and glc-PEO_n-glc (ca. 2 g/L) with Na[COSAN] (molar ratio of COSAN-to-PEO segment up to 1.7) in 0.1 M NaCl for cryoTEM, light scattering and ^1H NMR were prepared by (i) slow (consecutive), or (ii) quick (at once) addition of concentrated Na[COSAN] solution (ca. 300 mM); Samples for SAXS/WAXS analysis were prepared in similar way but with polymer concentration 10 g/L.

The samples for preliminary fluorescence measurements were prepared as follows:

(i) GB179 (ca. 4 μL of 5 mM methanol solution) and RhBB (10 μL of 1 mM methanol solution) fluorescence probes were added to PEO_m-glc and glc-PEO_n-glc solutions (ca. 1 g/L) in 0.1 M NaCl for GB179 and in 0.1 M NaCl + 5 mM $NaHCO_3$ (pH around 8.3) for RhBB. After that, 300 mM Na[COSAN] was added to polymer solutions to have PEO-segment : COSAN : RhBB ratio = 1000 : 1000 : 1, or PEO-segment : COSAN : GB179 ratio = 500 : 500 : 1. (ii) The second set of samples were prepared in the same way but GB179 and RhBB probes were added after mixing of the polymers with COSAN. The probe concentration is very low in all samples. Due to the kinetics aspects of a polymeric nanocomposite formation, the staining of both probes may depend on the preparation protocol, e.g., whether the probe was added first to the pure polymer or after formation of the nanostructures with Na[COSAN]. This effect is important mainly for RhBB probe that should be attached only to the accessible ends of telechelic PEO chains.

50 μL of the probe containing samples was deposited on glass plates (treated by piranha solution) and freely leave to evaporate. The solid film was measured by fluorescence microscopy.

Preliminary FCS experiments:

In our Fluorescence Correlation Spectroscopy (FCS) study, the interaction between RhBB and telechelic PEO-glc was confirmed by the decrease in the relative diffusion coefficients: D(RhBB) > D(glc-PEO-glc/RhBB) > D(glc-PEO-glc/COSAN/RhBB).

The samples for studies in cell cultures were prepared as follows:

Stock solutions: (i) 10 mg/mL Na[COSAN].$4H_2O$ in 0.9 weight-% NaCl; (ii) 2 mg/mL glc-PEO_{45}-glc in 0.9 weight-% NaCl; (iii) 1 mg/mL glc-PEO_{45}-glc + 10.1 mg Na[COSAN].$4H_2O$ (PEO-segment : COSAN ratio = 1000 : 1000) in 0.9 weight-% NaCl; (iv) 1 mg/mL glc-PEO_{45}-glc or 1 mg/mL PEO_{114}-glc + 10.1 mg Na[COSAN].$4H_2O$ + 0.025 mg/mL GB179 (PEO-segment : COSAN : GB179 ratio = 1000 : 1000 : 1) in 0.9 weight-% NaCl; (v) 2 mg/mL glc-PEO_{45}-glc + 8 mg Na[COSAN].$4H_2O$ + 0.125 mg/mL GB179 (PEO-segment : COSAN : GB179 ratio = 1000 : 200 : 2) in 0.9 weight-% NaCl; (vi) 1 mg/mL glc-PEO_{45}-glc

or 1 mg/mL PEO_{114}-glc + 10.1 mg Na[COSAN].$4H_2O$ + 0.1 mg/mL RhBB (PEO-segment : COSAN : GB179 ratio = 1000 : 1000 : 6) in 0.9 weight-% NaCl; (vii) 2 mg/mL glc-PEO_{45}-glc + 8 mg Na[COSAN].$4H_2O$ + 0.125 mg/mL GB179 (PEO-segment : COSAN : GB179 ratio = 1000 : 200 : 4) in 0.9 weight-% NaCl.

Methods

Size exclusion chromatography (SEC) analyses were performed on an Agilent Technologies 1100 Series apparatus fitted with a UV/Vis Diode Array Detector (DAD) and a Differential Refractometer. Chromatograms from the refractometer are reported. A series of three PL-gel (polystyrene-divinylbenzene) columns (Mixed B, Mixed C and Mixed E, Polymer Laboratories, UK) and tetrahydrofuran (THF) (flow rate 0.7 mL min^{-1}) were used. A set of polystyrene (PS) standards (Polymer Laboratories, UK) was used for column calibration. The following apparent (relative to PS) molecular weight characteristics are reported: number-average molecular weight, M_n, weight-average molecular weight, M_w, and the corresponding averages of degrees of polymerisation (DP) X_n and X_w, respectively, dispersity index $Đ = M_w/M_n$, molecular weight corresponding to the apex of the SEC peak, M_p, and the molecular weight corresponding to the i-th slice of the SEC chromatogram, M_i.

Dynamic Light Scattering (DLS) and Static Light Scattering (SLS). The light scattering setup (ALV, Langen, Germany) consisted of a 633 nm He-Ne laser, an ALV CGS/8F goniometer, an ALV High QE APD detector, and an ALV 5000/EPP multibit, multitau autocorrelator. DLS data analysis was performed by fitting the measured normalized intensity autocorrelation function $g_2(t) = 1 + \beta|g_1(t)|^2$, where $g_1(t)$ is the electric field correlation function, t is the lag-time and β is a factor accounting for deviation from the ideal correlation. An inverse Laplace transform of $g_1(t)$ with the aid of a constrained regularization algorithm (CONTIN) provides the distribution of relaxation times, $\tau A(\tau)$. Effective angle- and concentration-dependent hydrodynamic radii, $R_H(q,c)$, were obtained from the mean values of relaxation times, $\tau_m(q,c)$, of individual diffusive modes using the Stokes-Einstein equation. To obtain true hydrodynamic radii, the data have to be extrapolated to a zero scattering angle. Since the refractive index increment, dn/dc, is unknown for almost all of the samples, we evaluated in such cases only the light scattering intensity extrapolated to zero scattering angle as the measure proportional to molar mass of polymeric nanoparticles.

Cryo-Transmition Electron Microscopy (cryo-TEM). Glow discharge (Emitech KX100, 2 min/25 mA) treated Quantifoil R2/2 holey carbon copper grid with the hole size of 2 μm was transferred into an environmental chamber of FEI Vitrobot having room temperature and 100

% humidity. 3 μL of sample solution was applied on the grid which was blotted for 1.5 seconds and then shot to 1/1 mixture of liquid ethane and propane of temperature -180 °C. The grid with vitrified sample film was cryotransferred into a FEI Tecnai 12 transmission electron microscope with Gatan 910 cryotransfer holder at temperature ca. -185 °C. Bright-field TEM was performed using an acceleration voltage of 120 kV and images were recorded on Gatan Ultrascan 1000 CCD camera.

^{1}H NMR spectroscopy. ^{1}H NMR spectra were measured on a Varian ^{UNITY}*INOVA* 400 in deuterium oxide (99.5 %; Chemotrade, Leipzig, Germany). Spectra were referenced to the solvent signal (4.80 ppm).

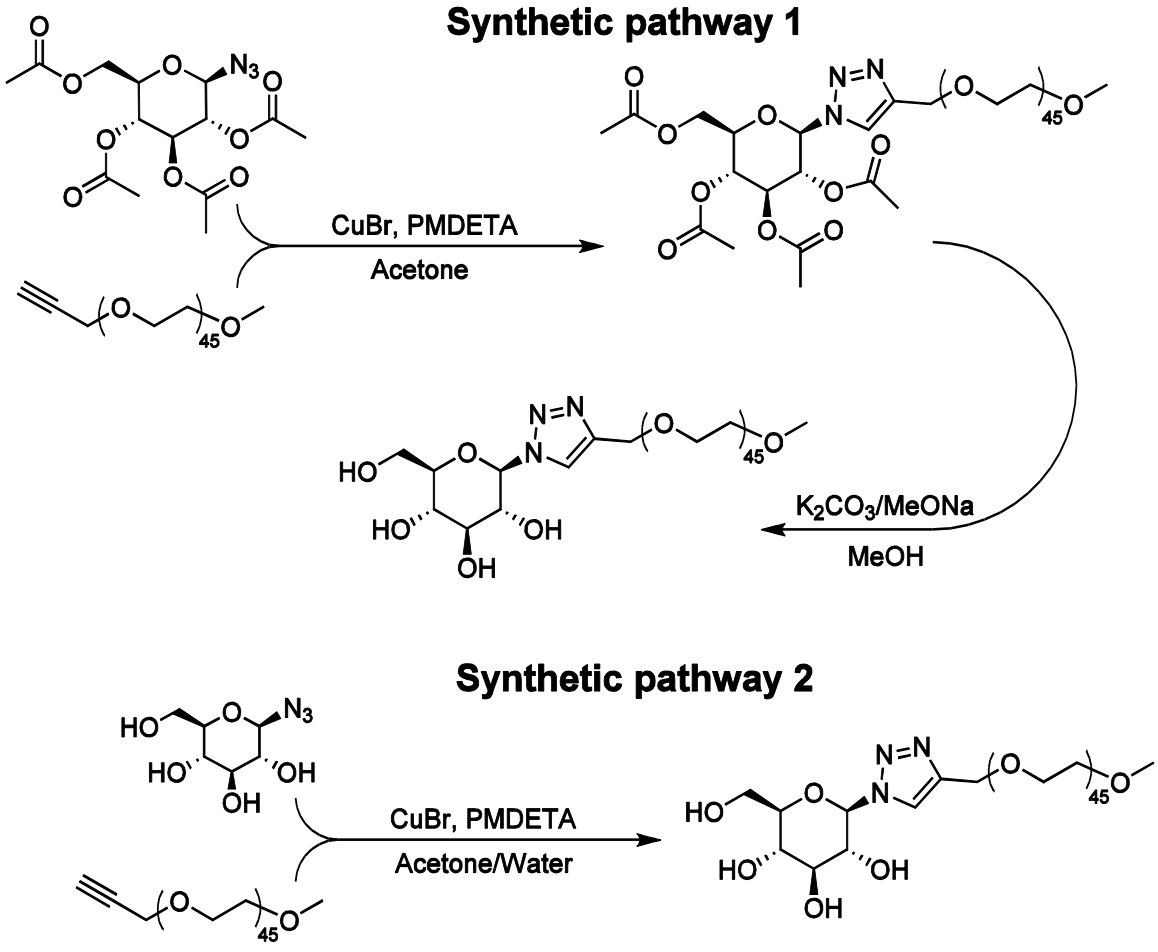
In vitro cell viability/cytotoxicity assay. 5×10^4 HeLa cells (human cervical carcinoma cell line) were seeded in 100 μL of media into 96-well flat-bottom plates (TPP, Czech Republic) 24 h before adding the particles or COSAN. Their concentrations varied in the range 0.5 – 500 $\mu g \cdot mL^{-1}$ of final concentration of COSAN. The cells were cultivated for 72 h in 5 % CO_2 at 37°C. Then 10 μL of alamarBlue® cell viability reagent (Life Technologies, Czech Republic) was added to each well and incubated for 4 h at 37°C. The active component of the alamarBlue reagent resazurin was reduced to the highly fluorescent compound resorufin only in viable cells. Its fluorescence was detected in the plate reader Synergy Neo (Bio-Tek, Czech Republic) using excitation at 570 nm, emission at 600 nm. As a control, the cells cultivated in medium without COSAN of particles were employed. Three wells were used for each concentration. The assay was repeated three times independently.

Internalization of particles. The HeLa cells were cultivated for 24 h in 5 % CO_2 at 37°C on 35 mm glass bottom dish with 4 chambers, 20 mm microwell, #1 cover glass (0.13-0.16 mm) (Bio-Port Europe s.r.o., Czech Republic). The particles or COSAN were added at final concentration 200$\mu g \cdot mL^{-1}$. After 2 and 24 h the cells were visualized using the Olympus laser scanning confocal microscope FV10-ASV (Olympus, Czech Republic).

Further comments on polymer synthesis

Synthesis of alkyne terminated PEO: Alkyne terminated PEO were obtained by simple Williamson ether synthesis as published by Bell et al. [2]. These reactions were easily done and the synthesized polymers were almost quantitatively modified with the alkyne group as confirmed by the ^{1}H NMR end group integration. The procedure did not affect the polymer dispersity, which was confirmed by GPC.

Synthesis of glucose terminated PEO by CuAAC: We tried two pathways for this synthesis; the first pathway was coupling of the alkyne-PEOs with acetyl-protected glc and subsequent deprotection of acetyl groups by conventional methods (K_2CO_3 or NaOMe). Second pathway was deprotection of the glycopyranoside and subsequent coupling with the alkyne-PEOs. Both pathways are shown in Scheme S2.



Scheme S2: Schematic representation of synthetic pathways used in this work.

Polymers prepared by the first pathway had high yields (80%) but the GPC measurments indicated a decrease of Molecular weight and large increase of dispersity index for both deprotection agents as shown in Figure S1A-B. Polymers prepared by second pathway did not exhibit higher dispersity index and had almost the same distribution as their precursors

(Figure S1C). All four polymers were therefore prepared by second pathway to ensure their uniformity. Overall yields (50 %) were lower because the deprotected glucose tends to adsorb on basic alumina compared to its acetyl protected counterpart. GPC traces for all four polymers are shown in Figure S1D.

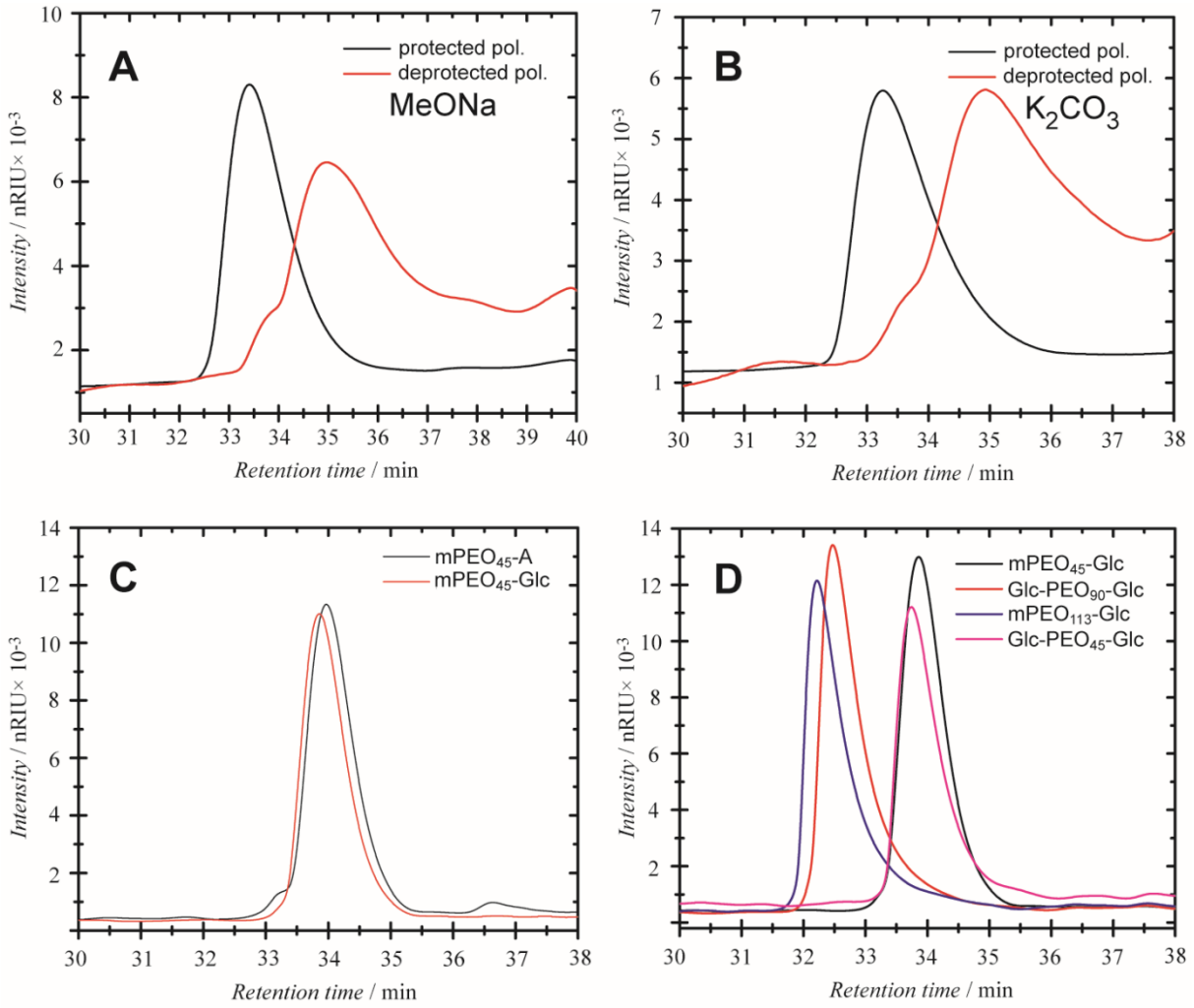


Figure S1: (A) Pathway 1, GPC traces of polymer before deprotection with MeONa and after. (B) Pathway 1, GPC traces of polymer before deprotection with K_2CO_3 and after. (C) Pathway 2, GPC traces of $mPEO_{45}$-A and click product $mPEO_{45}$-Glc. (D) GPC traces of glucose terminated PEOs prepared by the second pathway.

NMR Characterization

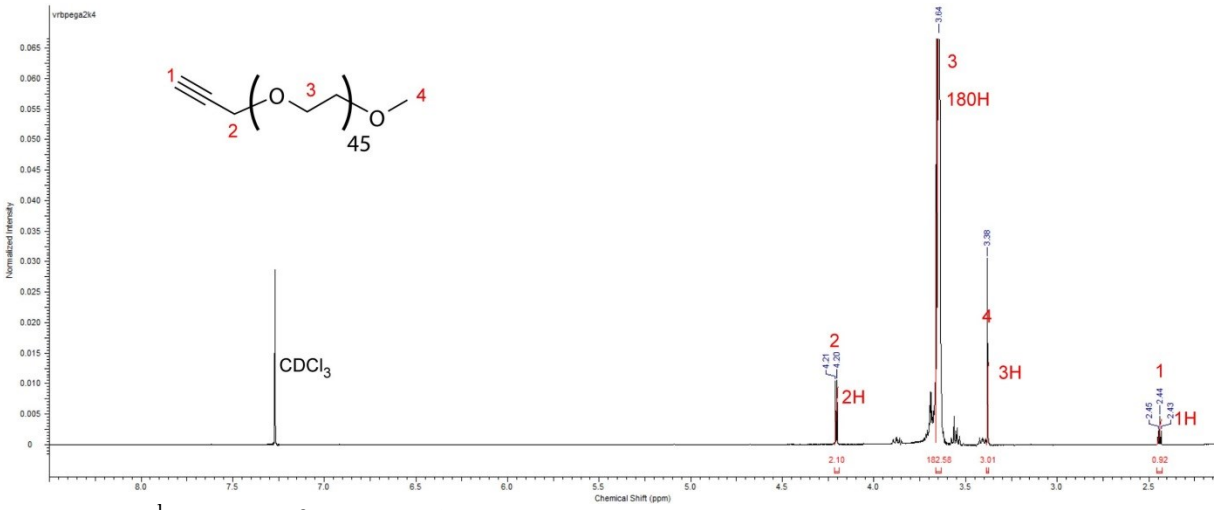


Figure S2: ^{1}H NMR of mPEO$_{45}$-A

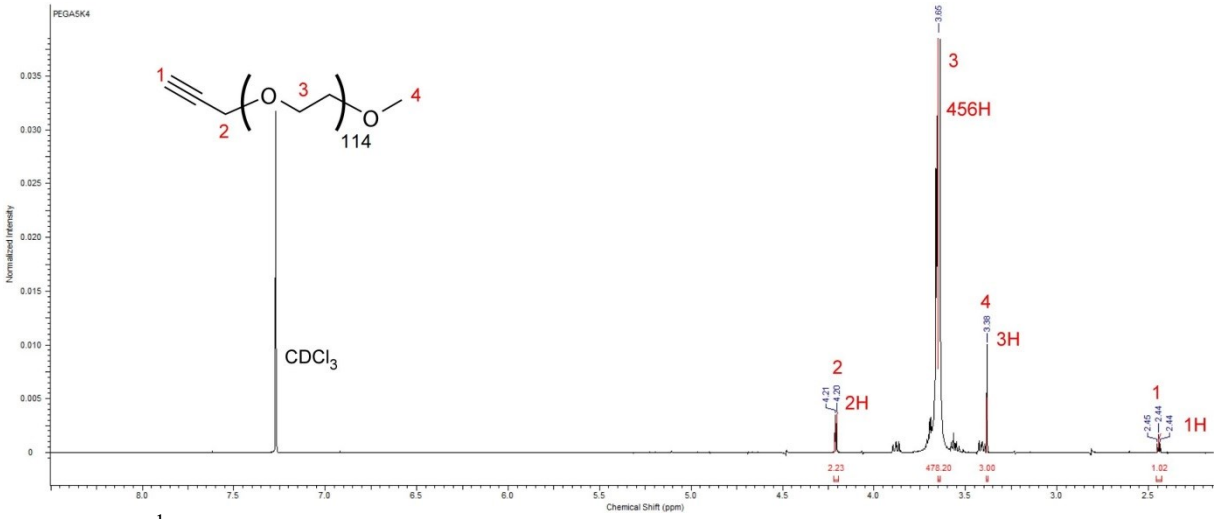


Figure S3: ^{1}H NMR of mPEO$_{114}$-A

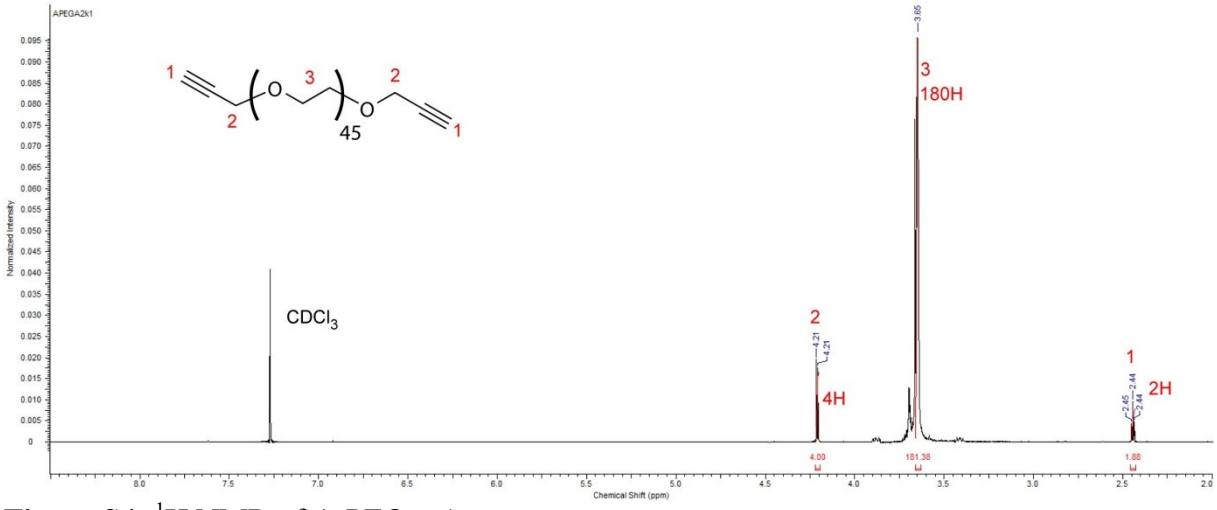


Figure S4: ^{1}H NMR of A-PEO$_{45}$-A

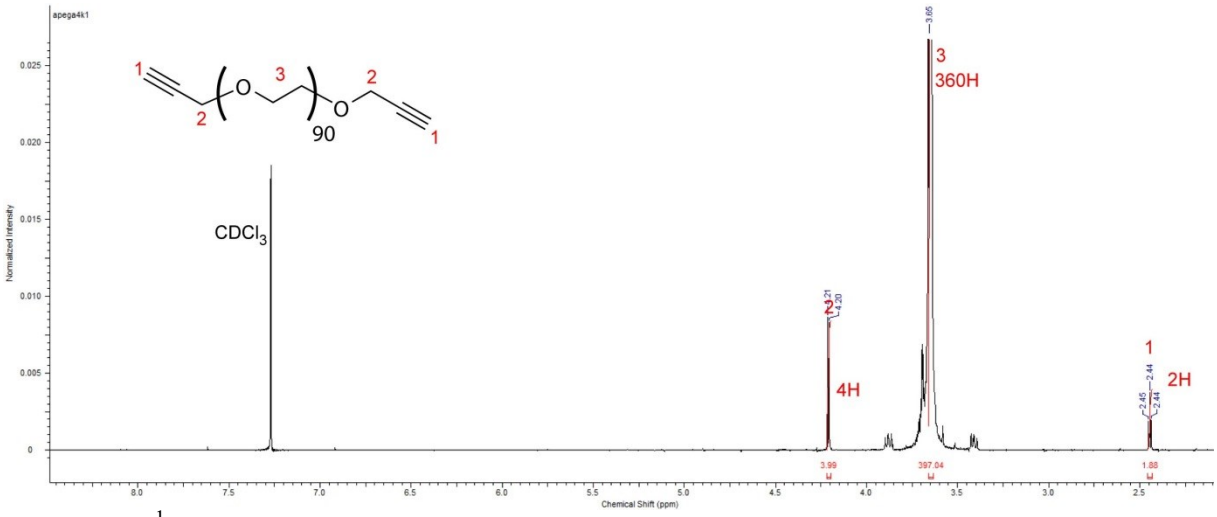


Figure S5: ^{1}H NMR of A-PEO$_{90}$-A

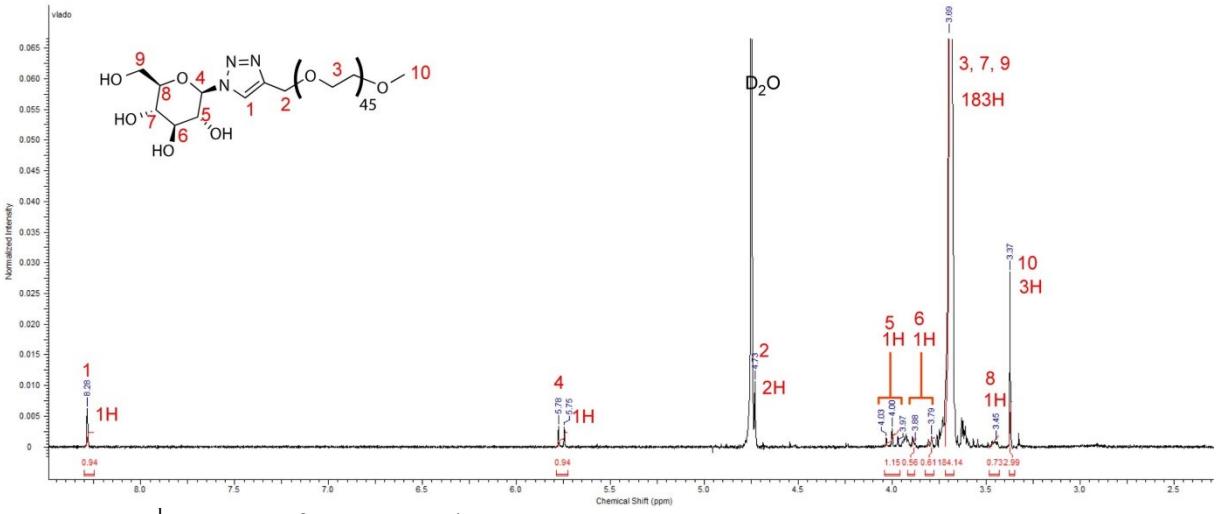


Figure S6: ^{1}H NMR of mPEO$_{45}$-Glc

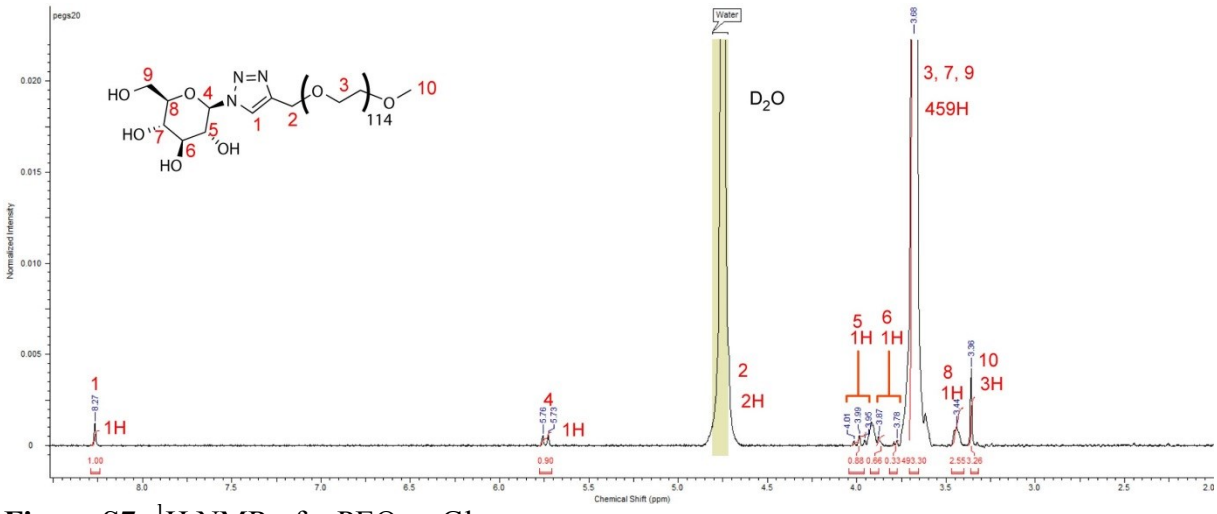


Figure S7: ^{1}H NMR of mPEO$_{114}$-Glc

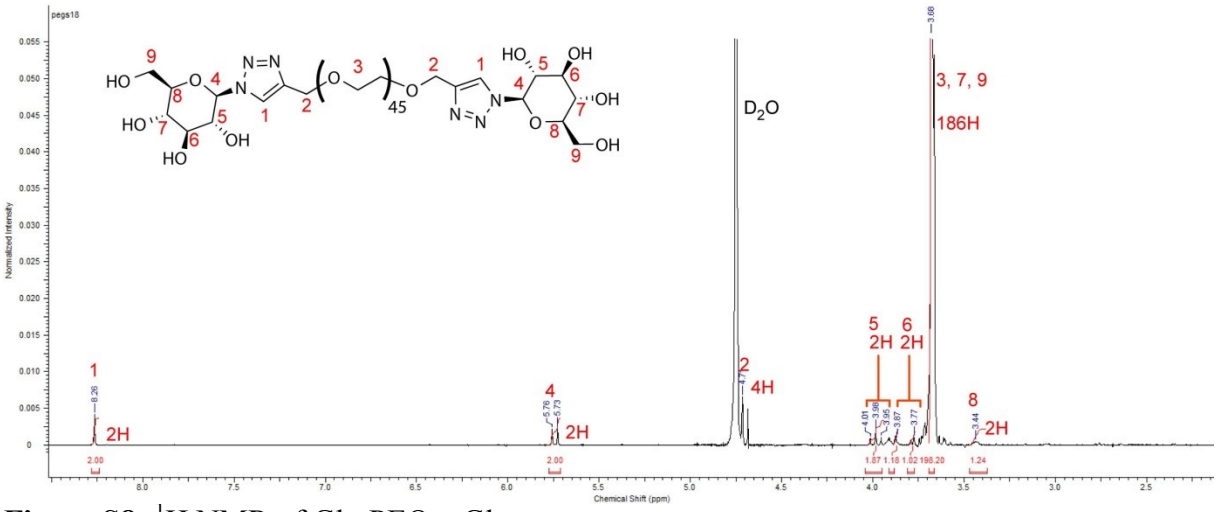


Figure S8: ^{1}H NMR of Glc-PEO$_{45}$-Glc

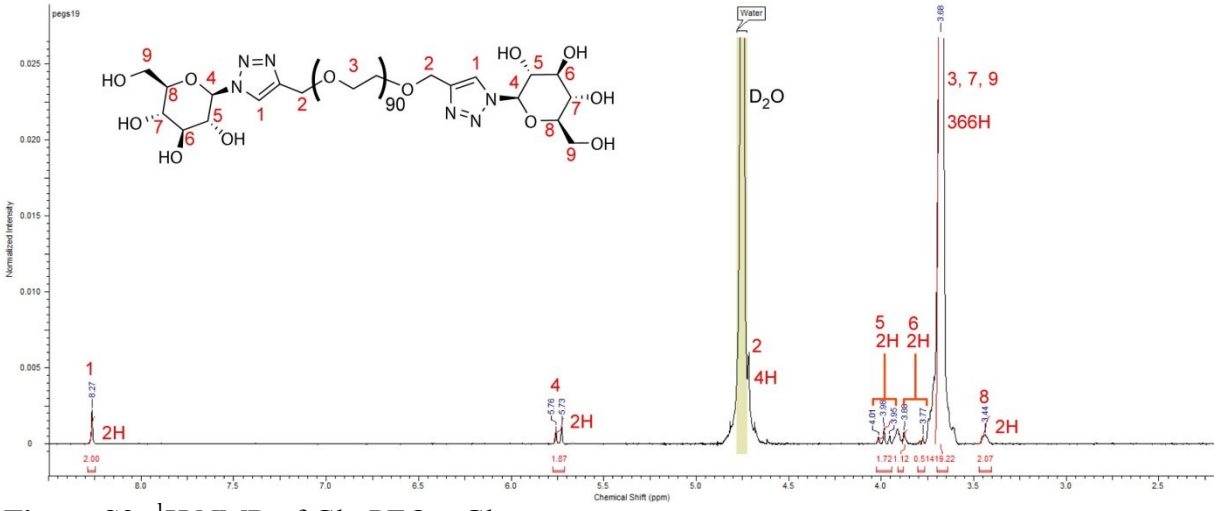


Figure S9: ^{1}H NMR of Glc-PEO$_{90}$-Glc

GPC Characterization

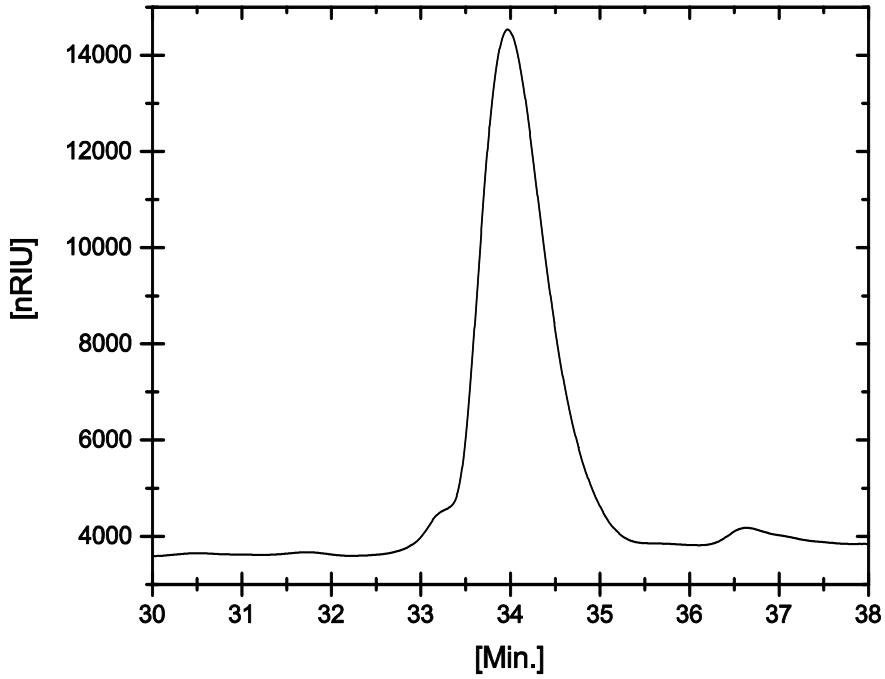


Figure S10: GPC chromatogram of $mPEO_{45}$-A

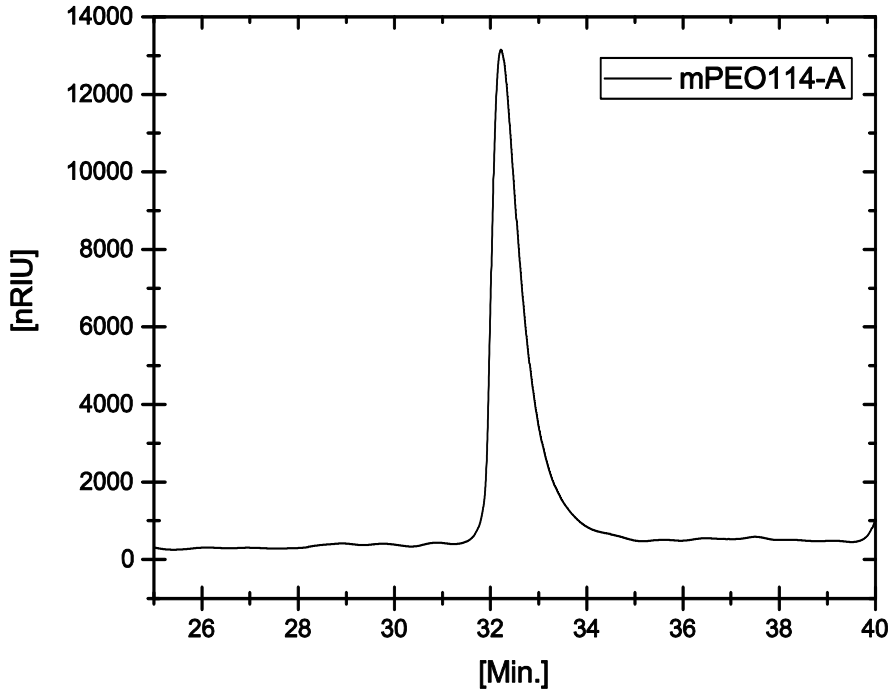


Figure S11: GPC chromatogram of $mPEO_{114}$-A

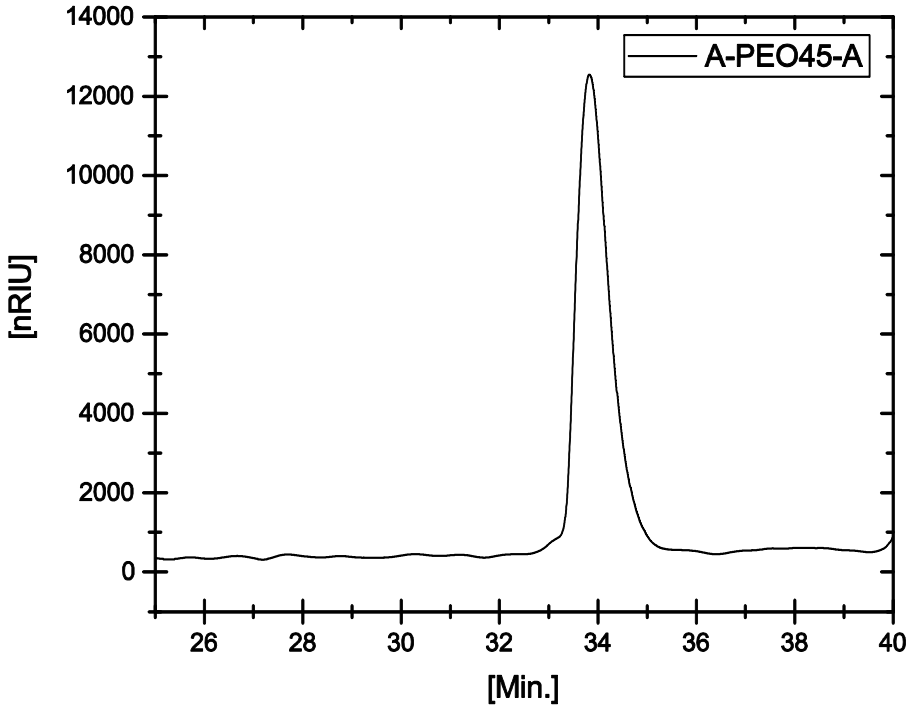


Figure S12: GPC chromatogram of A-PEO_{45}-A

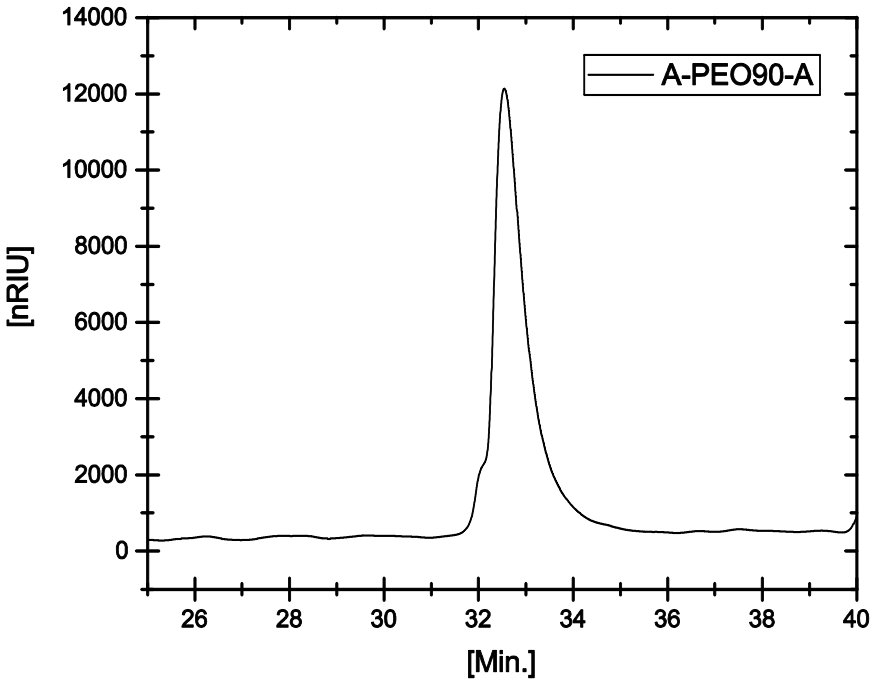


Figure S13: GPC chromatogram of A-PEO_{90}-A

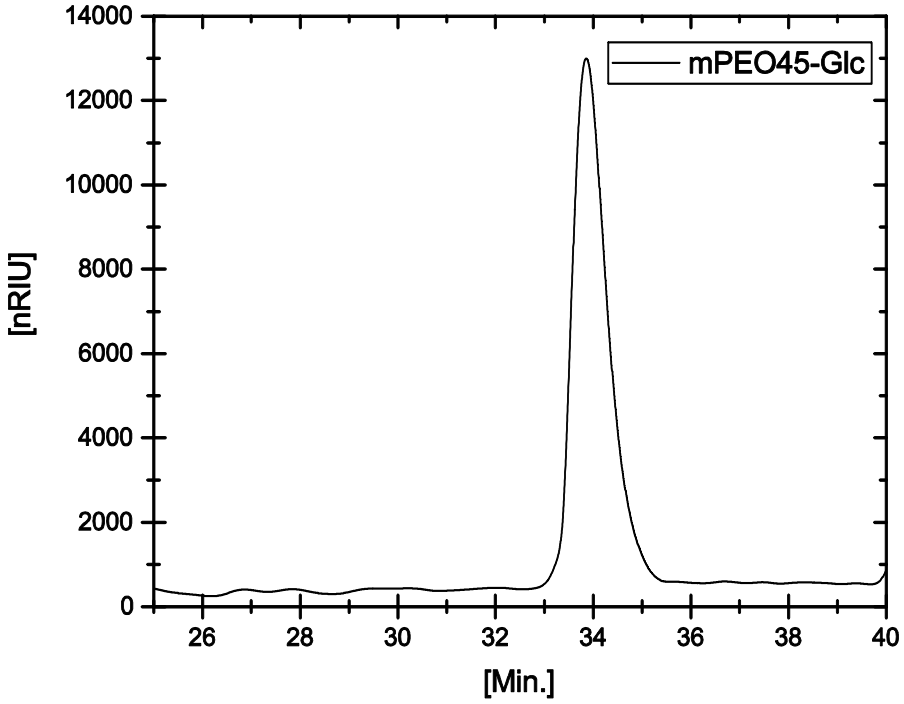


Figure S14: GPC chromatogram of $mPEO_{45}$-Glc

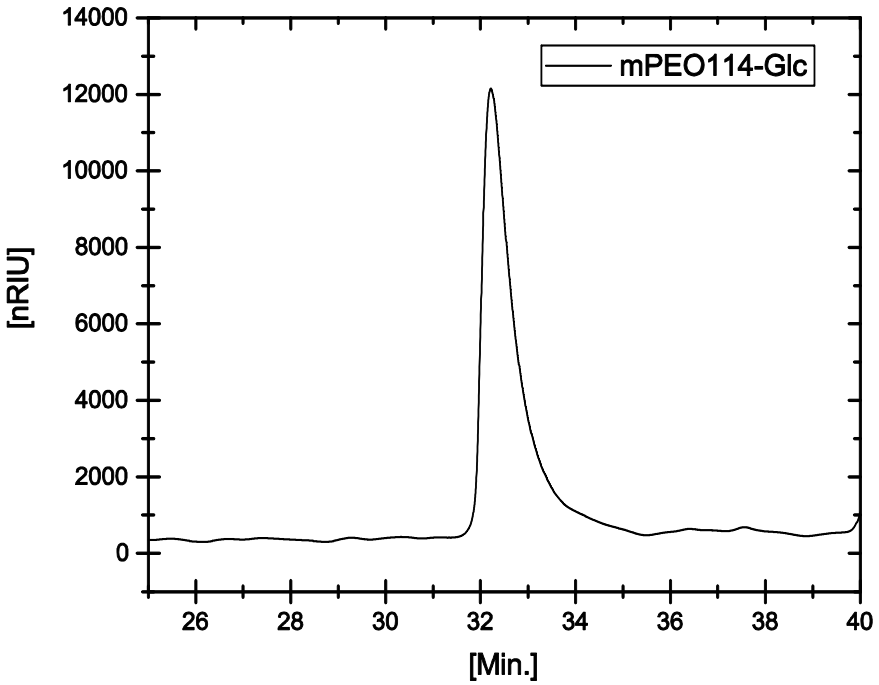


Figure S15: GPC chromatogram of $mPEO_{114}$-Glc

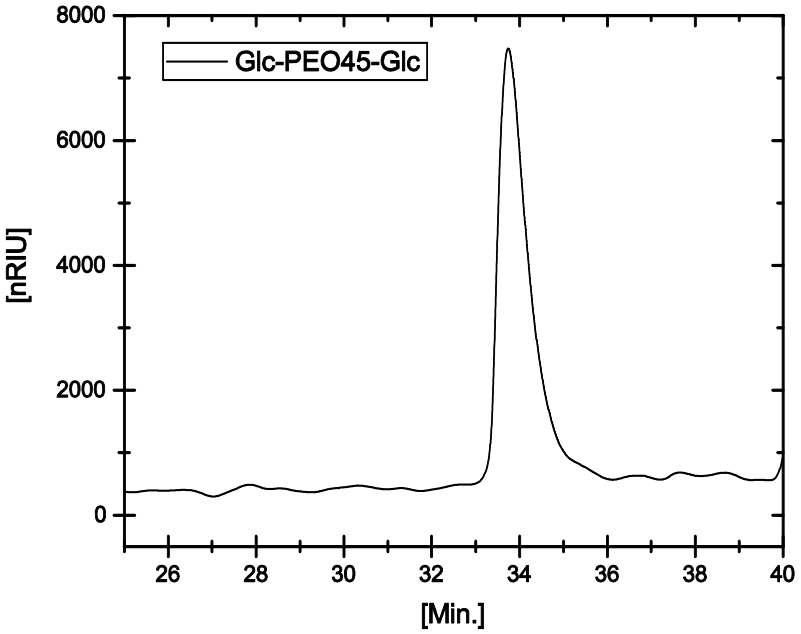


Figure S16: GPC chromatogram of Glc-PEO_{45}-Glc

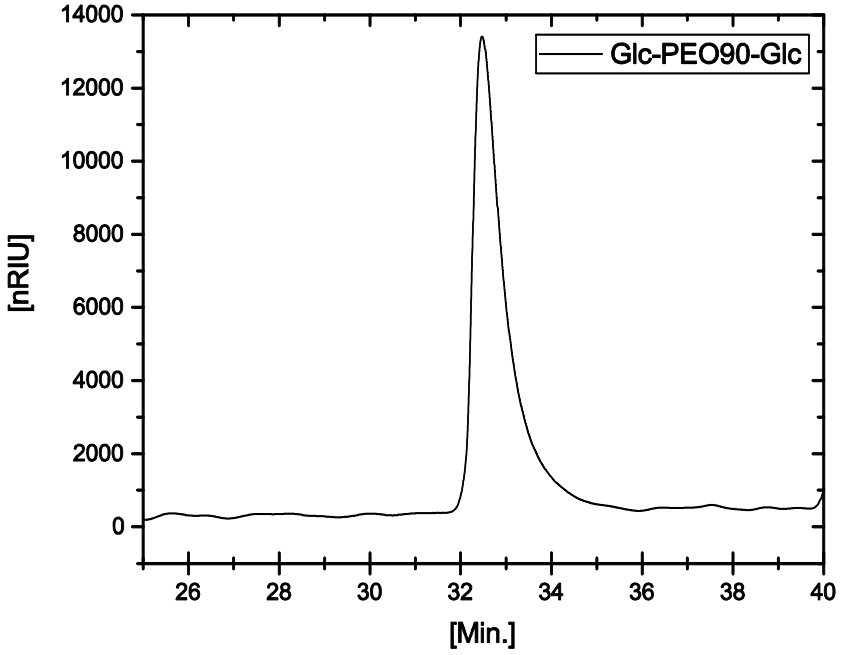


Figure S17: GPC chromatogram of Glc-PEO_{45}-Glc

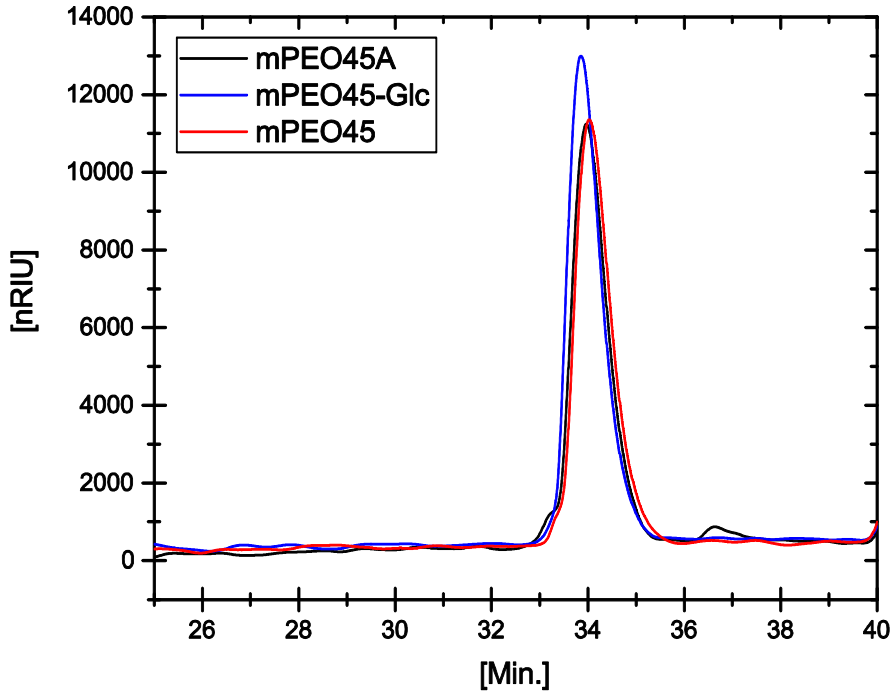


Figure S18: GPC traces of polymers after each reaction step in pathway 2 towards synthesis of mPEO45-Glc.

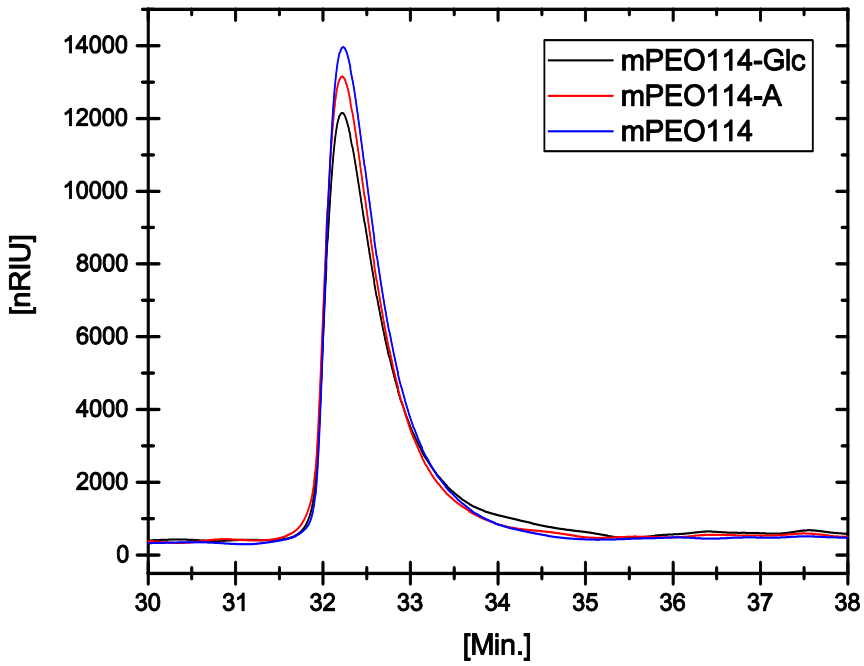


Figure S19: GPC traces of polymers after each reaction step in pathway 2 towards synthesis of $mPEO_{114}$-Glc.

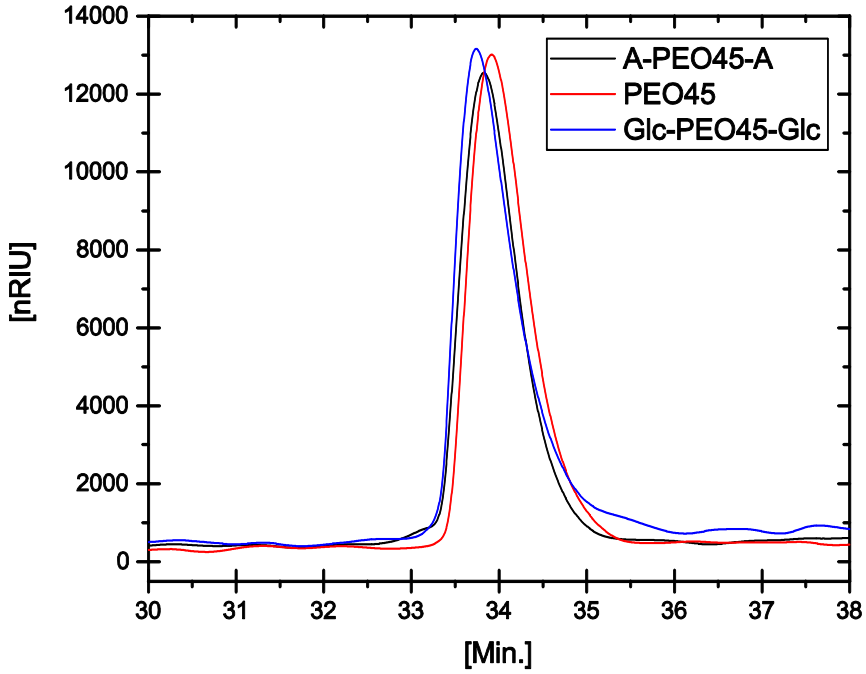


Figure S20: GPC traces of polymers after each reaction step in pathway 2 towards synthesis of Glc-PEO_{45}-Glc.

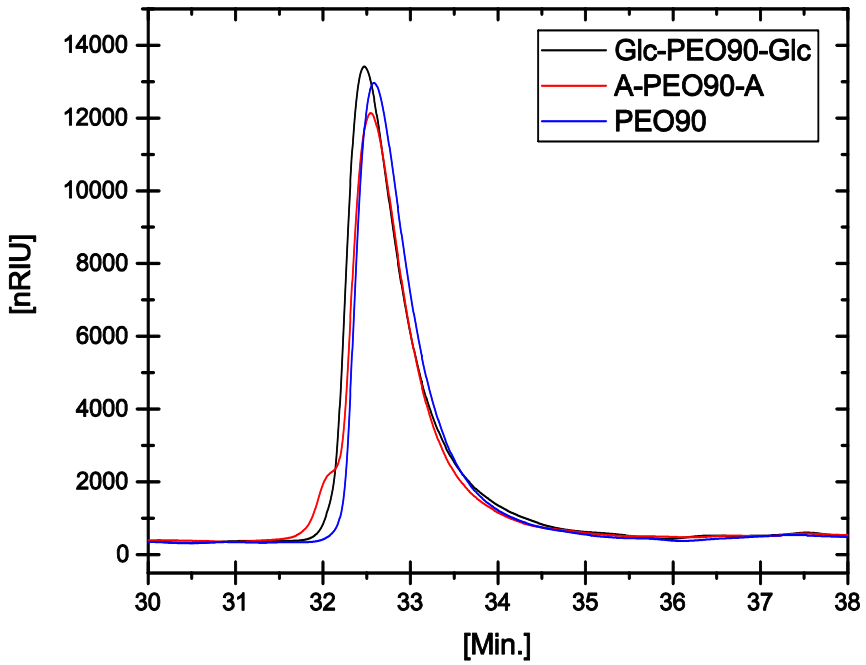


Figure S21: GPC traces of polymers after each reaction step in pathway 2 towards synthesis of Glc-PEO_{90}-Glc.

PEO/COSAN complexation (1-3):
(1) Detection and effect of Mw

Metallacarborane to PEO segment ratio has been evaluated for Na[COSAN]/PEO in bulk as ca. 1:14 by ss-NMR in the equilibrium state for polymer of high molecular weight (M_w ~ 20,000 g/mol).[3] Because the studied samples have lower degree of polymerization than in our earlier studies and are dispersed in water as nanoparticles, we investigated influence of M_w on COSAN to PEO segments stoichiometry. Fraction of PEO segments involved in the composite formation was calculated from diminishing of ^{1}H NMR signal of PEO as described in our previous works.[4] For PEO samples with M_w ranging from 350 g/mol to 20,000 g/mol, it is shown in Figure S22. The curves for 20,000 overlaps with that for 2,000 suggesting only limited impact of molar mass and also of chain-ends (-OH and/or -OCH_3) of PEO on COSAN complexation. The polymer is saturated when there is ca. 5-10 PEO segments per 1 COSAN cluster. The differences are obvious for PEO oligomers (350 g/mol and 750 g/mol), where PEO and PEO/Na[COSAN] are liquid-like. Such liquid phase can accumulate (solubilize) higher amount of COSAN (2-3 PEO segments per 1 cluster) than the solid nanocomposite. The experiments also confirmed our assumptions that all the PEO samples rapidly precipitated (underwent phase transition) after the addition of COSAN.

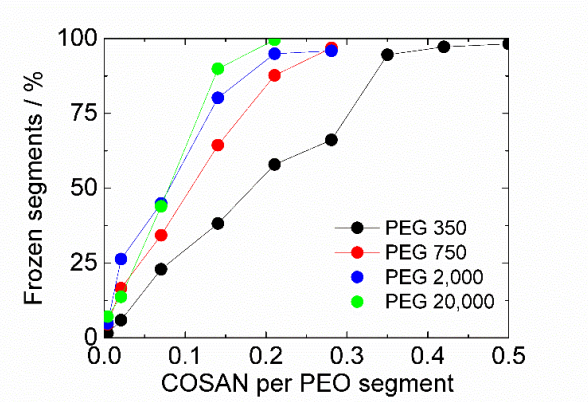


Figure S22: Fraction of frozen segments of PEO (molecular weights, M_w, assigned within the graph) derived from diminishing of PEO signal in ^{1}H NMR spectra after addition of Na[COSAN] in 0.1 M NaCl.

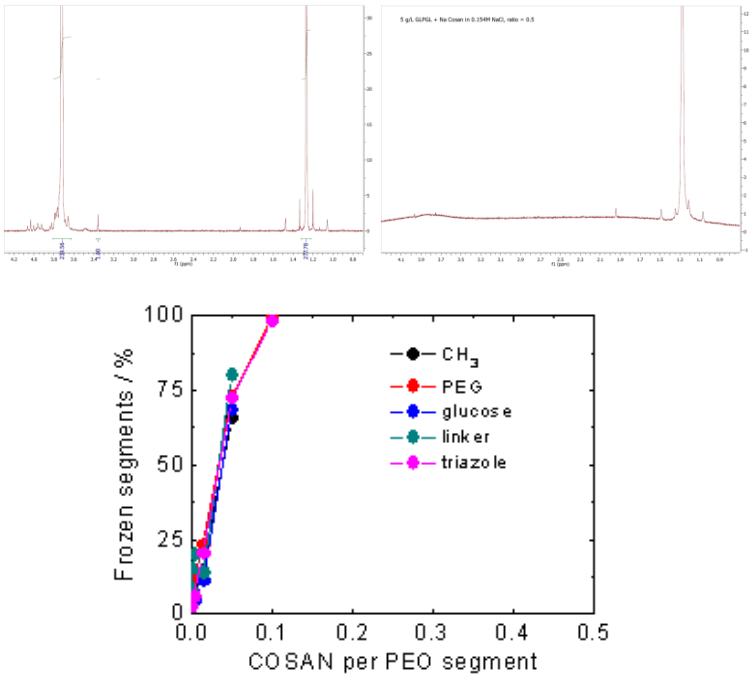


Figure S23: (**A**) 1H NMR spectrum of Glc-PEO_{45}-Glc in 0.154 M NaCl aqueous solution, and (**B**) 1H NMR spectrum of Glc-PEO_{45}-Glc and COSAN (ξ = 0.5) in 0.154 M NaCl aqueous solution. (**C**) Fraction of frozen segments and end groups (glucose, methoxy, glucose, and triazole linker) of PEO_{45}-Glc after addition of COSAN in 0.154M NaCl.
All results suggested that all signals of the polymer diminished upon addition of COSAN resulting in completely flat NMR spectrum.

(2) Characterization of PEO/COSAN complex

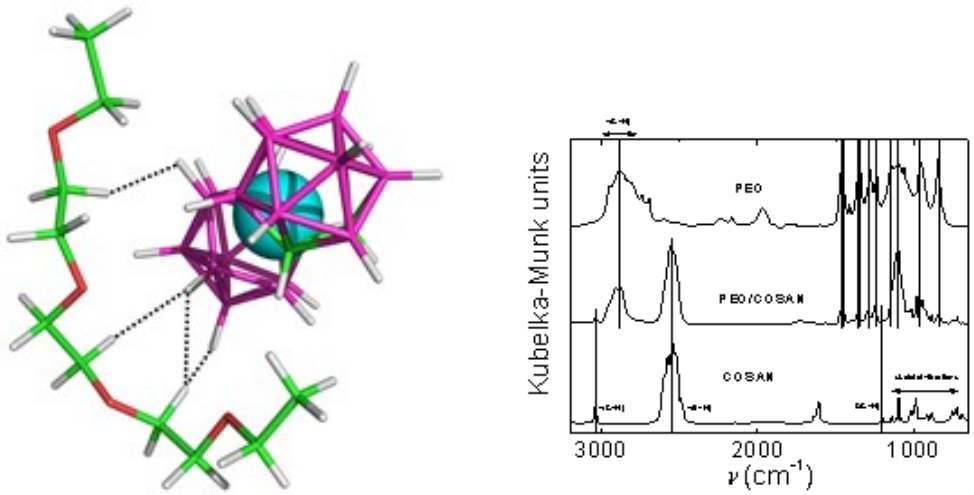
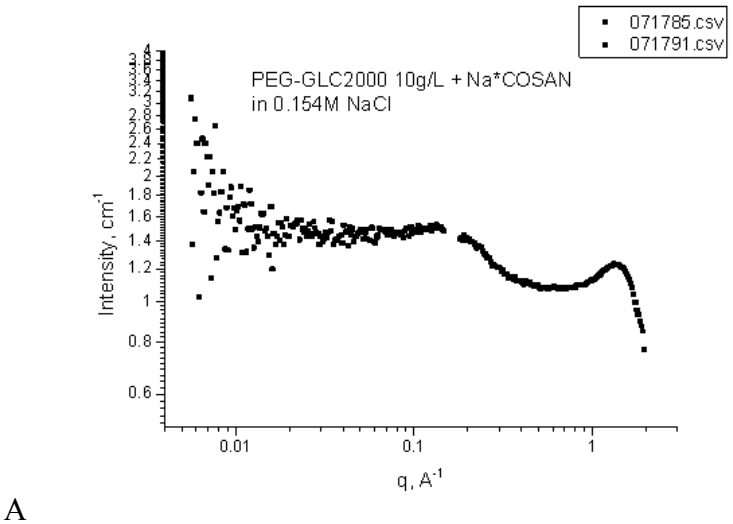
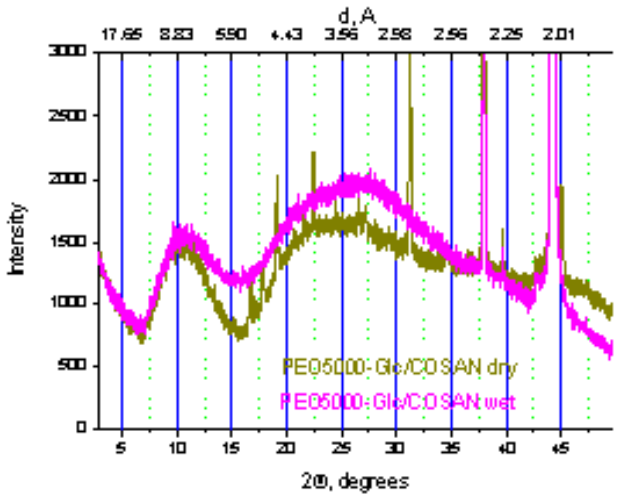


Figure S24: (**A**) Structure of optimized complex of ethylene oxide pentamer, EO_5, with COSAN anion from Quantum Chemistry calculations showing an example of dihydrogen bonds that occurred between hydridic H-atoms of metallacarborane clusters and positively charged H-atoms of polymer backbone B-H(δ-)…(δ+)H-C (details in Ref. [5]). (**B**) Comparison of FTIR spectra of poly(ethylene oxide), PEO, sodium salt of COSAN, and solid PEO/COSAN composite (details in Ref. [5]).

(3) Scattering/diffraction characterization of PEO-Glc/COSAN nanostructures in 0.154 M NaCl



A

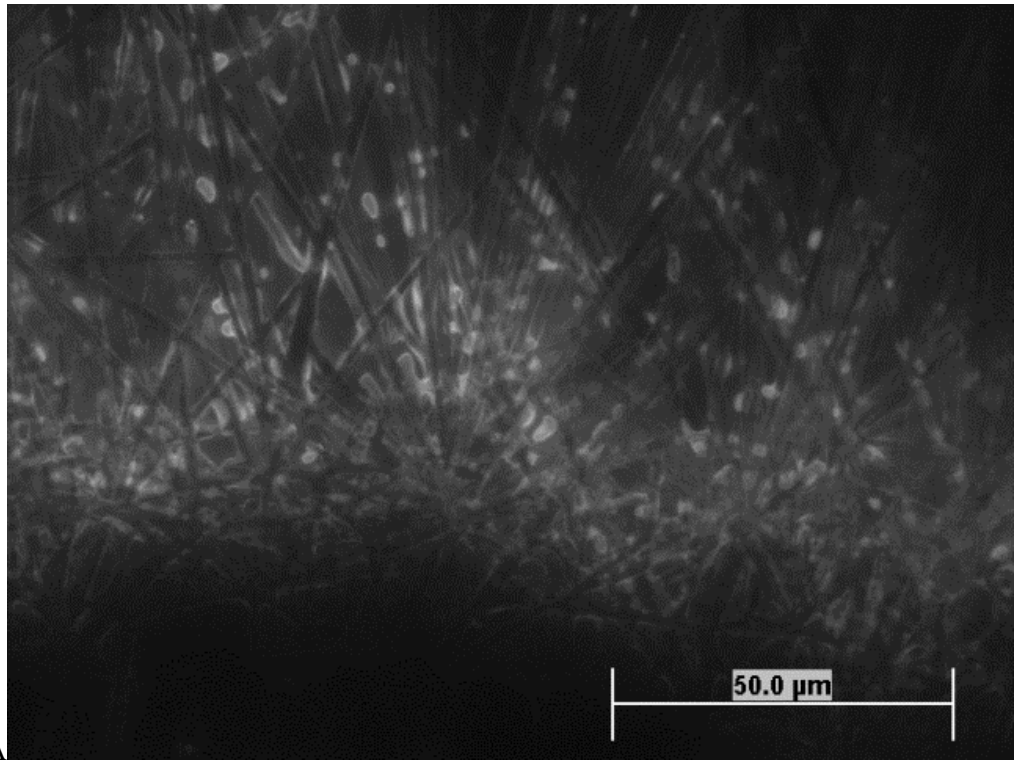


B

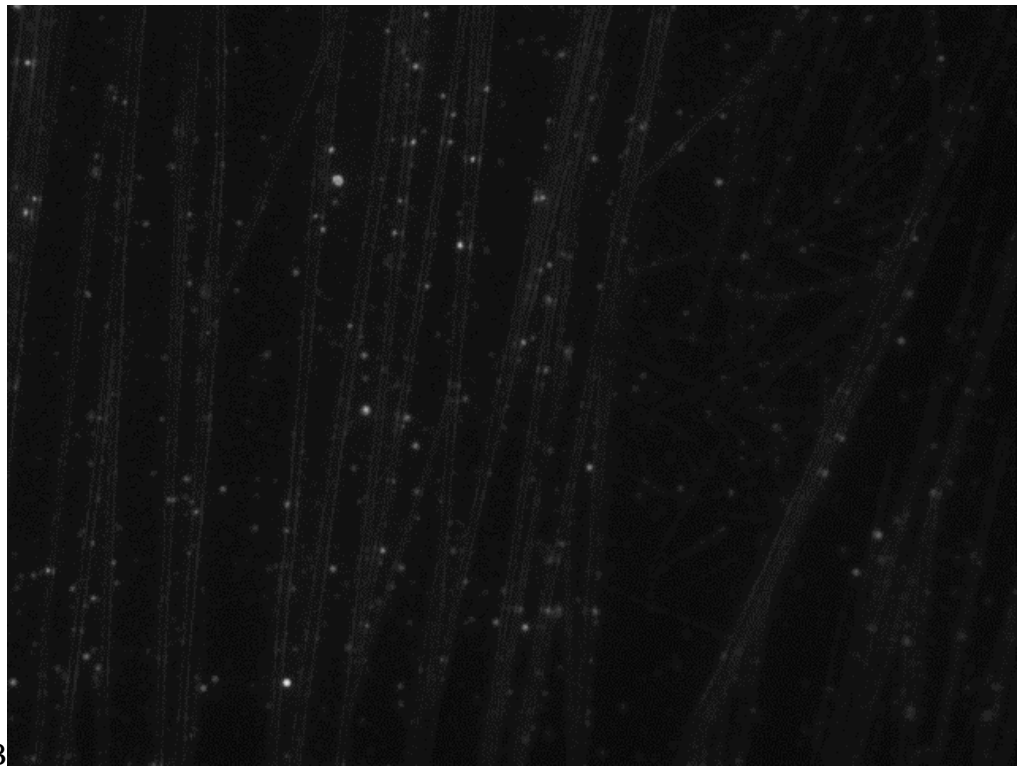
Figure S25: (**A**) Typical SAXS curve of PEO_{45}-Glc/COSAN nanostructure in 0.154 M NaCl aqueous solution with predominant features of COSAN aggregates. (**B**) Typical XRD spectrum of PEO_{90}-Glc/COSAN in 0.154 M NaCl solution (pink) and the same system, but after evaporation of water that leads to crystallization of COSAN and NaCl (yellow).

Fluorescence micrographs in solid state

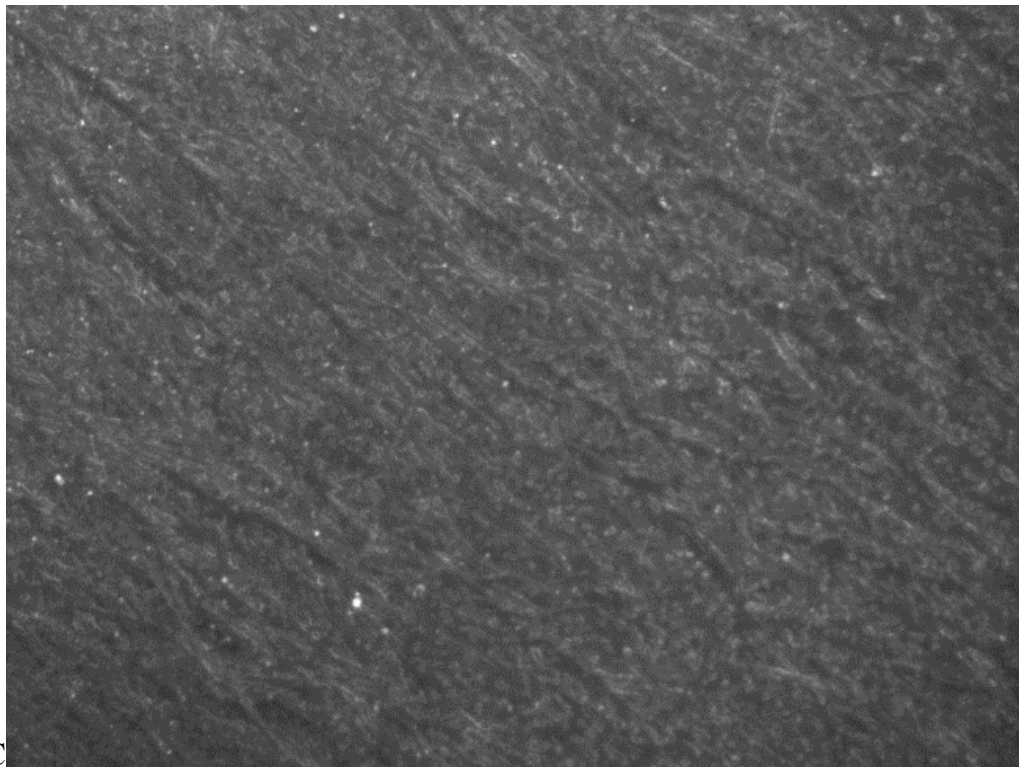
A



B



C



D

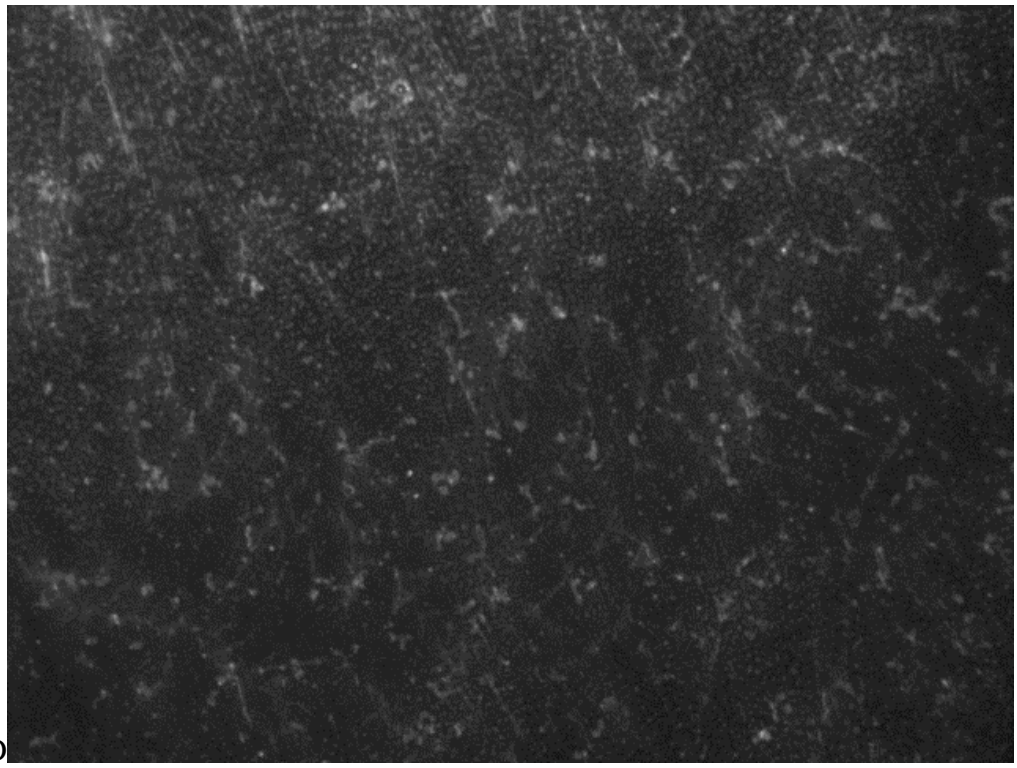


Figure S26. Fluorescence microscopy micrographs of PEO/glc/COSAN structures in 0.1 M NaCl dried on the glass plate for (A) glc-PEO_{90}-glc/COSAN/GB179, (B) PEO_{114}-glc/COSAN/RhBB, (C) glc-PEO_{45}-glc/COSAN/RhBB and (D) glc-PEO_{90}-glc/COSAN/RhBB.

Additional micrographs of polymer/COSAN with HeLa cells

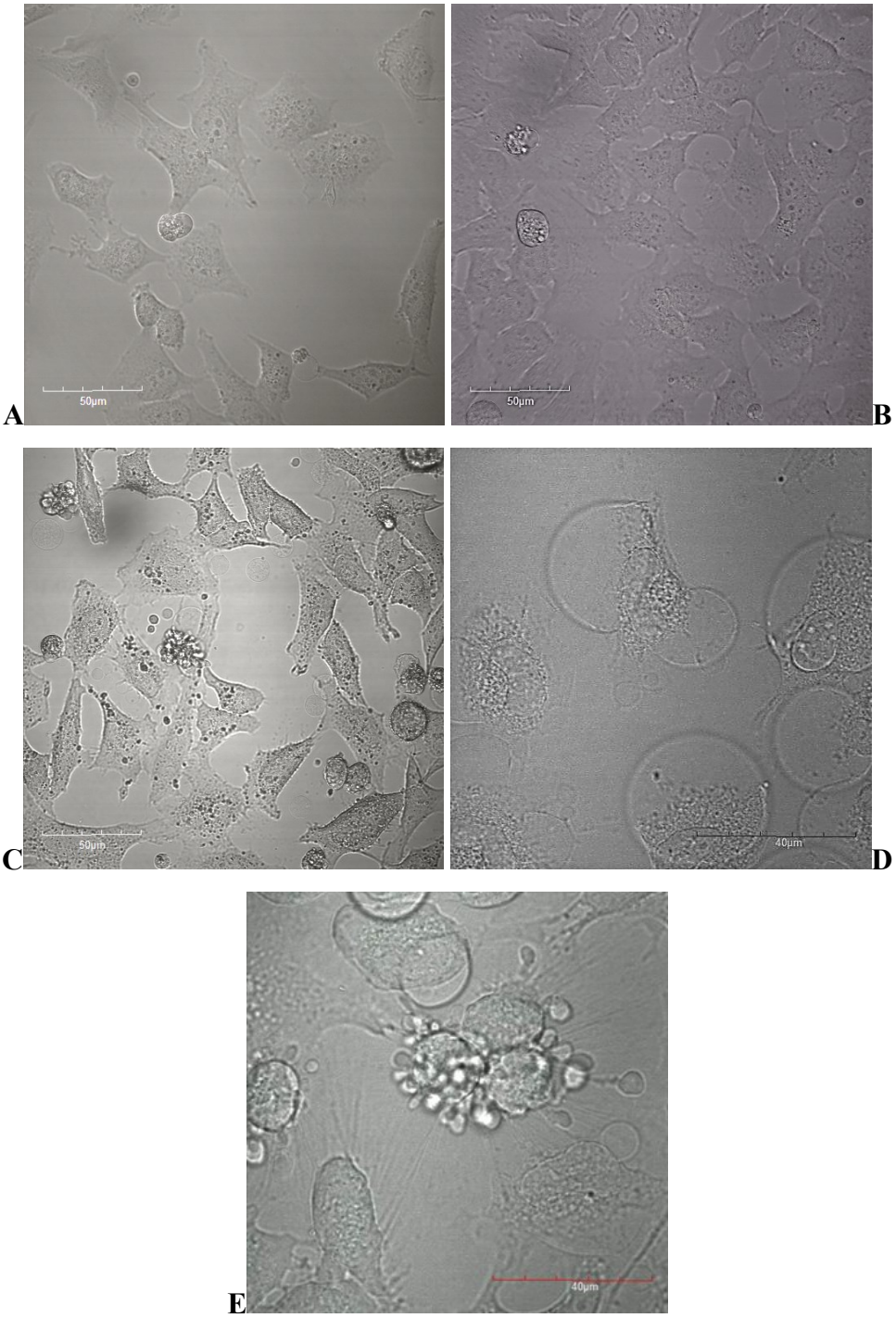


Figure S27: Bright field confocal microscopy micrographs of HeLa cells: (A) control, (B) incubated with pure polymer glc-PEO_{45}-glc (200 µg/mL) for 24h, (C) incubated with pure COSAN (10 µg/mL) for 24h, (D) detail of COSAN-induced vesicular structures decomposing cell and core walls, (E) Typical example of changes in cell morphology and the presence of apoptotic cells in samples incubated with nanoparticles 24h and longer, which prove the interaction of nanoparticles with the cells and their effect in higher concentrations. The apoptotic cell bodies are visible in the central part and in the left side of the picture.
It is evident that pure polymer does not lead to any changes of cells, while COSAN-containing cultures contain higher number of dead cell. The vesicle-or bubble-like structures

are observed in all COSAN-containing samples. The bubbles interact with cells leading to their decomposition. They probably composed by interaction of COSAN with incubation medium (buffer, proteins, etc.)

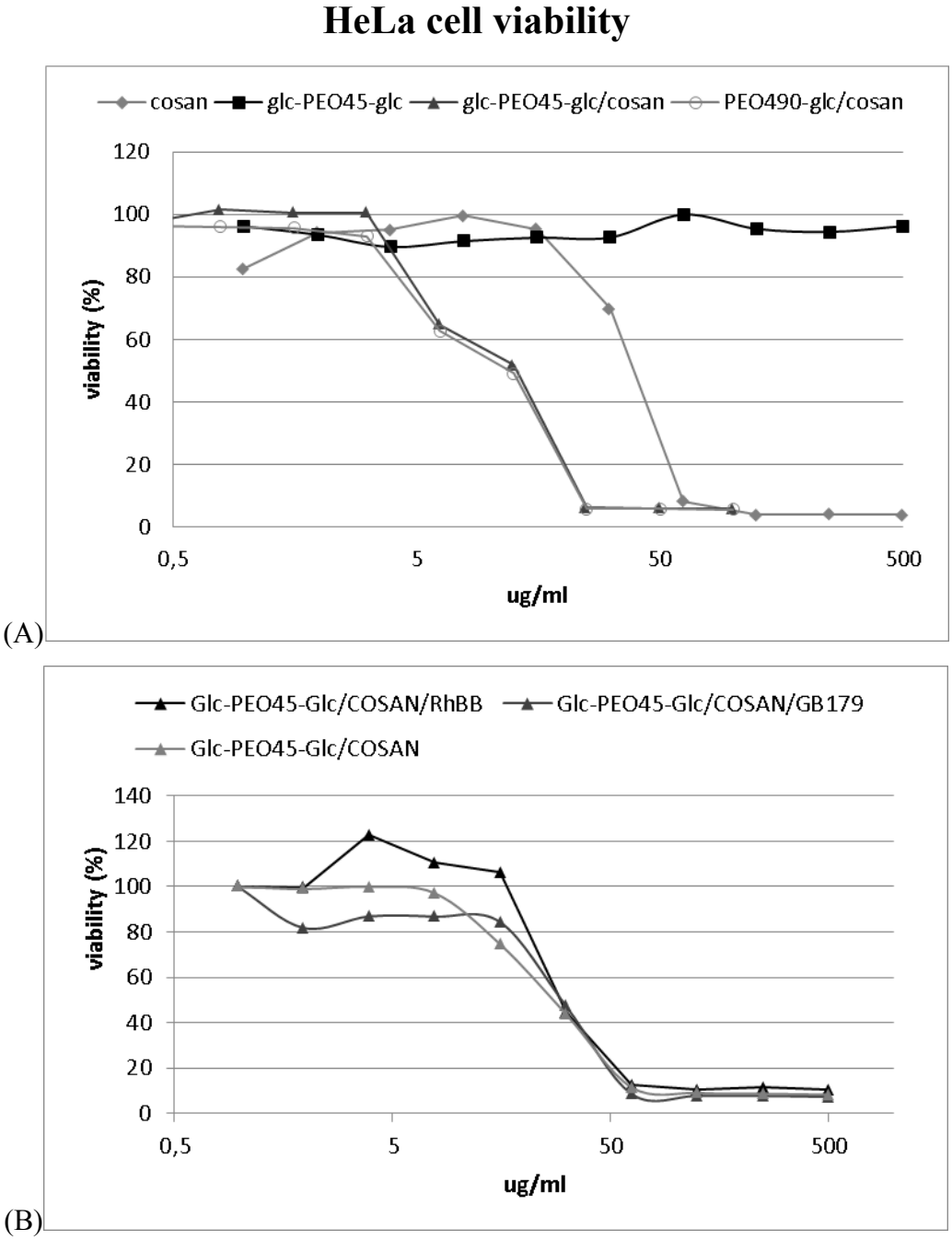


Figure S28: Cytotoxicity study with COSAN and PEO-glc polymer samples in HeLa cells culture: (A) Data for pure COSAN, pure polymer glc-PEO_{45}-glc, and glc-PEO_{45}-gl/COSAN and PEO_{114}-glc/COSAN nanostructures. (B) Comparison of nanostructures without fluorescence probes glc-PEO_{45}-glc/COSAN, and with fluorescence probes glc-PEO_{45}-glc/COSAN/GB179 and glc-PEO_{45}-glc/COSAN/RhBB. It is evident that all COSAN containing samples are slightly toxic to HeLa cells.

Comments on nanoparticles internalization in HeLa cells

The cell viability study documented that there is almost no influence of fluorescent probes on the cytotoxicity effect of polymer nanoparticles (Fig.S28B in SI), and that the morphology of studied cells did not depend on the presence of probes (it does depend on COSAN and nanoparticle presence).

Both probes has strong affinity to polymer and seemingly also to cells. Thus, their concentration in extracellular medium is low – no fluorescence signal seen there. However, GB179 is almost insoluble in water (solubility ca. 1e-5M [1]) and the solubility dramatically drops in saline (GB179 is sodium salt). It means that concentration of GB179 must be very low in extracellular medium in any case. In contrast, RhBB is more soluble. Therefore, higher concentration of RhBB in extracellular medium should be clearly detectable.

The nanoparticles formed the small compact objects colored by particular dye outside the cells after few hours' incubation without any color changes of extracellular media for both GB179 and RhBB. In the case of the massive probe release from the particles to the cell media, it should have been observable by fluorescence microscopy, especially for RhBB. However, we did not detect this change.

In our previous experiments, we studied the solubilization of metallacarborane probes such as GB179 to proteins or polymers placed inside the dialysis bags. The diffusion process and penetration through the dialysis membrane were extremely slow reaching the equilibrium in several weeks or months. Thus, we assume the active transport of the probes attached to nanoparticles into the cells. The exact mechanism is unknown to us, but it has not been the main research topic of our paper.

The probe release from nanoparticles upon the fusion with the cell membrane is also relatively slow process. That is why we can monitor the fluorescence of cells after one day incubation. Moreover, we observed the changes of the cell morphology in samples containing nanostructures even without the probes. The presence of apoptotic cells in samples incubated with nanoparticles for 24h and longer indicates the interaction of nanoparticles with the cells at higher concentrations. In Figure S27E (newly added to SI), there is an example of the cell morphology with visible apoptotic bodies in the cells in central part of the picture and in the left side of the picture.

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[3] J. Brus, A. Zhigunov, J. Czernek, L. Kobera, M. Uchman, P. Matejicek, *Macromolecules* **2014,** *47*, 6343-6354.

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[5] Matejicek P., Zednik J., Uselova K., Plestil J., Fanfrlik J., Nykanen A., Ruokolainen J., Hobza P., Prochazka K.: Stimuli-Responsive Nanoparticles Based on Interaction of Metallacarborane with Poly(ethylene oxide). *Macromolecules* **2009**, *42*, 4829-4837.