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Supporting Information

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"Biomimetic engineering of carbon nanotubes using cell surface mucin mimics"

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General Procedure for Self-Assembly of Mucin Mimics on Carbon Nanotubes.

High-purity SWNTs (HiPCO, >95%) were purchased from Carbon Nanotechnologies Inc., and high-purity of MWNTs (CVD, >95%) were purchased from Nanolab. In a typical preparation experiment, 1 mg of as-produced carbon nanotubes was suspended in 5 mL of aqueous C_{18}-MM solution (0.1%~0.5%). The mixture was sonicated using a water-bath sonicator for 1 h. First, insoluble material was removed by low-speed centrifugation at 3,000 x g for 30 min, and the product suspension was decanted from the insoluble material. Then the excess free mucin mimic polymer was removed by dialysis of the suspension in a polycarbonate membrane against deionized water for 24 h. The resulting C_{18}-MM-NTs (C_{18}-α-MM-SWNTs, C_{18}-β-MM-SWNTs, C_{18}-α-MM-MWNTs, and C_{18}-β-MM-MWNTs) formed stable suspensions in aqueous solution.

Control experiment I: As-produced carbon nanotubes were treated under the same conditions but without C_{18}-MMs. The resulting suspensions were not stable and the carbon nanotubes precipitated after several h.

Control experiment II: Mucin mimics without C_{18} lipid tails were sonicated with carbon nanotubes in water for 1 h. The resulting suspensions were not stable and the carbon nanotubes precipitated after several h.

Scanning Electron Microscopy (SEM) Imaging.

SEM images of C_{18}-α-MM-SWNTs were obtained on a JEOL 6400 field emission SEM operated at 5 keV. Samples were deposited onto a silicon wafer and then allowed to dry in air.

Atomic Force Microscopy (AFM) Imaging.

The sample solution was deposited in a 1 μL droplet onto a silicon wafer and then allowed to dry in air. Tapping mode was used to acquire the images under ambient conditions (Molecular Imaging, Pico SPM).

Transmission Electron Microscopy (TEM) Imaging.

TEM images of C_{18}-α-MM-SWNTs were obtained on a JEOL 2011 microscope operating at electron energy of 100 keV. Samples were prepared by depositing the suspension onto grids, allowing the grids to absorb for 2 min, and then staining the material with 0.5% methylamine vanadate in H_2O (Nanovan, Nanoprobes Inc., Yaphank, NY).

^{1}H NMR Spectra of C_{18}-α-MM-SWNTs.

The ^{1}H NMR spectra of C_{18}-α-MM-SWNTs (Figure S2) was obtained on a Bruker DRX-500 MHz spectrometer. The C_{18}-α-MM-SWNTs were lyophilized to remove H_2O, and re-suspended in D_2O as solvent. Chemical shifts were referenced to the protonated solvent peak (DOH). The spectrum in D_2O showed identifiable signals for the polymer backbone and pendant GalNAc residues.

HPA-FITC Binding Assays

Helix pomatia agglutinin conjugated with fluorescein isothiocyanate (HPA-FITC) was obtained from EY-Laboratories. *N*-acetylgalactosamine (GalNAc) was obtained from Sigma.

A 1 mL solution of HPA-FITC (100 μg/mL) in buffer (0.10 M Tris, and 0.15 M NaCl, pH 8.0) was added to the suspensions of mucin mimic-coated nanotubes (C_{18}-α-MM-SWNTs or C_{18}-β-MM-SWNTs) in H_2O (1 mL). An additional 0.5 mL buffer was added and the reactions were incubated for 1h at rt in the dark. GalNAc inhibition of HPA binding to C_{18}-α-MM-SWNTs was tested by pre-incubating 1 mL of HPA-FITC solution in buffer (100 μg/mL) with 0.5 ml of GalNAc in buffer (200 mg/mL) for 1h at rt in the dark. This pre-incubated solution was added to the suspension of C_{18}-α-MM-SWNTs in H_2O (1 mL), and the resulting solution was incubated for 1h at rt in the dark, as described above. After incubation, the solutions were all subjected to dialysis against the buffer for 48 h. The dialyzed solutions were analyzed at 510-550 nm using a fluorescence microplate reader (excitation wavelength 492 nm). Spectra were corrected for background fluorescence by subtracting the fluorescence spectrum of C_{18}-α-MM-SWNTs or C_{18}-β-MM-SWNTs alone.

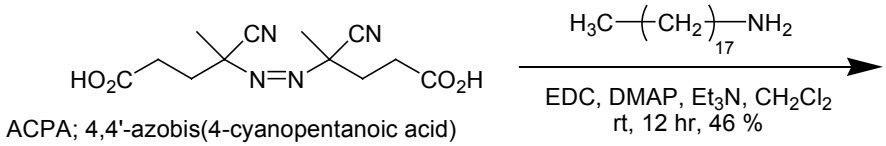
Synthesis of Mucin Mimics Used in This Report.

Synthesis of mucin mimics with α-GalNAc residues and no C_{18} lipid tail (α-MMs) was described previously.[Si]

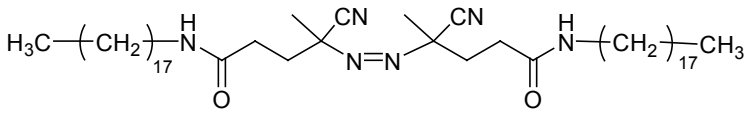
The synthesis of β-MMs was similar to α-MMs. The only difference was that β-aminooxy GalNAc was used instead of α-aminooxy GalNAc in the final step of the synthesis.

Synthesis of C_{18} functionalized mucin mimics with α-GalNAc residues (C_{18}-α-MMs).

Synthesis of C_{18}-tailed initiator (C_{18}-ACPA):



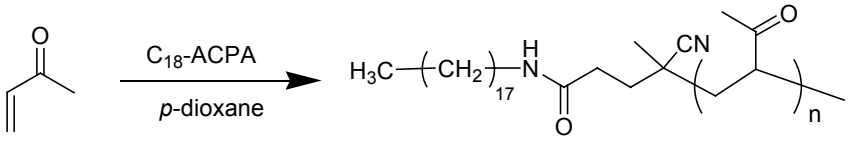
ACPA; 4,4'-azobis(4-cyanopentanoic acid)



C_{18}-ACPA

To a solution of ACPA (0.591 g, 2.11 mmol) in anhydrous CH_2Cl_2 (100 mL) in the dark at rt were added EDC (1.62 g, 8.44 mmol), DMAP (0.258 g, 2.11 mmol), Et_3N (1.20 mL, 8.61 mmol), and octadecylamine (2.28 g, 8.44 mmol). The reaction mixture was stirred overnight at rt. The reaction mixture was diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$ (aq.), water, and brine. The organic layer was dried over anhydrous $MgSO_4$. After evaporation of the solvent, the residue was separated by column chromatography (1:4 EtOAc/hexane) on silica gel to afford C_{18}-ACPA (0.76 g, 46 %) as a white solid.

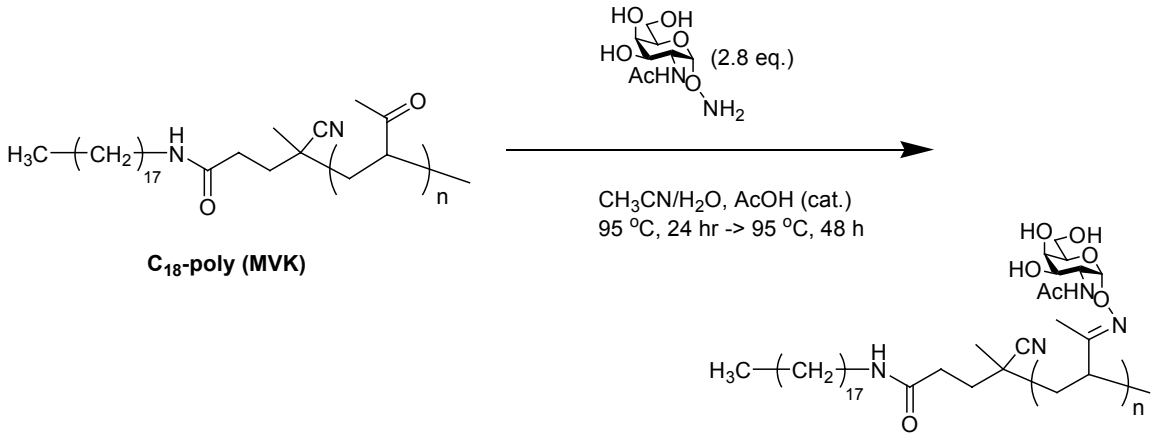
Synthesis of C_{18}-poly(MVK)



C_{18}-poly(MVK)

Anhydrous *p*-dioxane (0.56 mL) was introduced into a 15-mL brown Schlenk tube containing MVK (0.56 mL, 6.73 mmol) and C18-ACPA (50 mg, 64 μmol) under Ar. The reaction mixture was stirred for 72 h at 95 °C after degassing by four freezing-and-thawing cycles. The reaction mixture was allowed to cool to rt, diluted with *p*-dioxane (5 mL), and then added dropwise to excess diethyl ether (1 L) with vigorous stirring. The precipitate was filtered and washed with diethyl ether. The oligomers of low molecular weight were removed by dialysis in *p*-dioxane. The polymer was reprecipitated in exess diethyl ether (1 L), and the precipitate was dried *in vacuo* at 60 °C overnight. The polymer was soluble in dichloromethane, chloroform, acetonitrile, acetone, THF, and *p*-dioxane and insoluble in diethyl ether and hexane. The polymer is atactic as determined by ^{1}H-NMR analysis.

Conjugation of α-aminooxy GalNc to C_{18}-poly (MVK)



C_{18}-poly (MVK)

C_{18}-α-MM

To a solution of C_{18}-poly(MVK) (6.0 mg, 86 μmol based on carbonyl number) in THF (3 mL) were added α-aminooxy GalNAc (56.5 mg, 239 μmol) and aqueous acetic acid (0.1%, 1 mL). After 24 h at 95 °C, the reaction mixture was allowed to cool to rt. All solvents were removed *in vacuo*, and then deionized water (2 mL) was introduced into the mixture to dissolve the partially ligated water-soluble polymer. After 48 h at 95 °C, the reaction mixture was allowed to cool to rt, dialyzed in water to remove excess α-aminooxy GalNAc, neutralized by anion exchange and lyophilized to afford a fluffy white solid.

Synthesis of C_{18} functionalized mucin mimics with β-GalNAc residues (C_{18}-β-MMs).

The synthesis of C_{18}-β-MMs was similar to C_{18}-α-MMs. The only difference was that β-aminooxy GalNAc was used instead of α-aminooxy GalNAc in the final synthetic step.

Supporting Figures

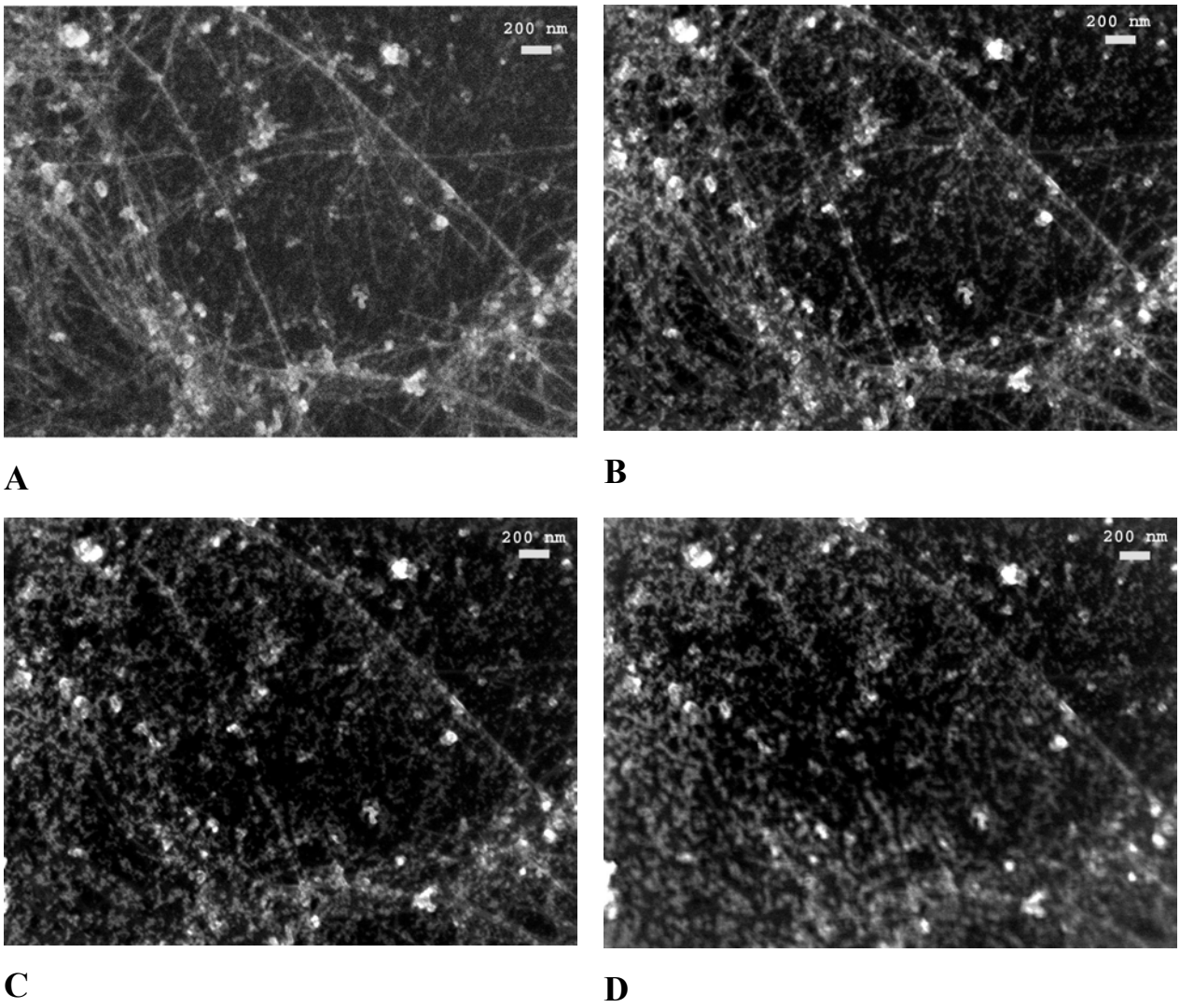


Figure S1. SEM images of C_{18}-α-MM-SWNTs. The surfaces of C_{18}-α-MM-SWNTs were damaged under the 5 keV electron beam. (A) t = 0 min; (B) t = 5 min; (C) t = 10 min; (D) t = 15 min. As shown in the pictures, the electron beam-induced damage increased with longer exposure times, culminating in near-complete destruction of the coating after 15 min.

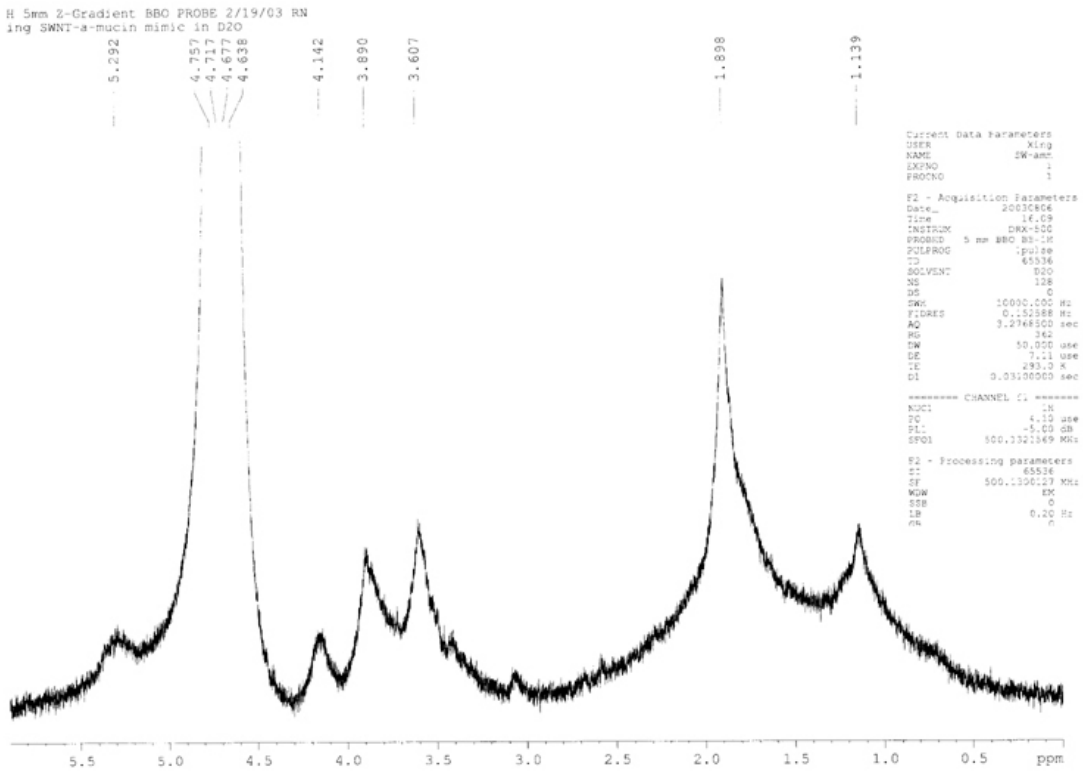


Figure S2. ^{1}H NMR spectrum of C_{18}-α-MM-SWNTs

Supporting Reference:

S1. Lee, G. S., Shin, Y., Choi, I., Hahn, H. & Bertozzi, C. R., submitted.