Supporting Information

Self assembly of bivalent glycolipids on single walled carbon nanotubes and their specific molecular recognition properties

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Content:

1. Materials:

Single walled carbon nanotubes (Pristine, P-2) were purchased from Carbon solutions, Inc., CA, USA. Chemicals were purchased from Aldrich and were used without further purifications. Solvents DMF, DCM, acetone and ethanol were purchased from Merck, dried and distilled according to literature procedures. DMF for AFM analysis was analytical grade also obtained from Merch. Analytical TLC was performed on commercial plates coated with silica gel GF254 (0.25 mm). Silica gel (100-200 mesh) was used for column chromatography. High-resolution mass spectra were obtained from a Q-TOF instrument by electro spray ionisation (ESI) technique. ^{1}H and ^{13}C NMR spectral analyses were performed on a spectrometer operating at 300 and 75 MHz, respectively, and the residual solvent signal was used as the internal standard. The following abbreviations were used to explain the multiplicities: s, singlet; d, doublet; t, triplet; dd, doublet of doublets; m, multiplet; band, several overlapping signals and br., broad. Millipore water (Milli Q-plus system, R >18 mΩ, pH 5.5) was used to prepare the buffer solution. Lectin Con A (salt-free lyophilised powder) and FITC-Con A were purchased from Sigma.

2. Glycolipid synthesis:

To a suspension of the appropriate 2-*O*-hexadecyl glycerol derivative[1], $Hg(CN)_2$, $HgBr_2$ and molecular sieves (4Å) in CH_2Cl_2, a solution of 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide in CH_2Cl_2 was added under stirring at room temperature and under argon atmosphere.[2] The mixture was stirred for 36 h, then it was filtered through celite, washed with CH_2Cl_2 and the organic layer was washed with aq. $Na_2S_2O_3$ (10%), aq. $NaHCO_3$ (5%) and H_2O. The CH_2Cl_2 layer was dried (Na_2SO_4), filtered, concentrated *in vacuo* and the residue was purified (SiO_2, petroleum ether/EtOAc) to afford the *O*-benzoyl-protected glycolipids. A suspension of protected glycolipid in MeOH was admixed with NaOMe / MeOH (0.5 M, 0.5 mL) and left stirring for 12 h, then neutralised with Amberlite IR-120 resin (H^+ form), filtered and the filtrate concentrated *in vacuo*. The resulting gummy syrup was triturated with diethyl ether and further lyophilised to afford the glycolipid as white foamy powder.

GL-1: Yield: 58 %. $[\alpha]_D^{25} = +35^o$ (*c* = 1.0, MeOH). ^{1}H NMR (300 MHz, DMSO–d_6): δ 4.75 (m, 4 H), 4.58 (br s, 4 H), 4.48 (m, 2 H), 3.62–3.42 (band, 11 H), 1.42 (m, 2 H), 1.22 (br s, 26 H), 0.83 (t, 3 H, *J* = 6.9 Hz). ^{13}C NMR (75 MHz, DMSO–d_6): δ 100.3, 79.2, 73.9, 71.0, 70.3, 69.5, 66.9, 66.4, 61.2, 31.4, 29.7, 29.1, 29.0, 28.7, 25.6, 22.1, 14.1. HR-MS *m/z*: calculated for $C_{31}H_{60}O_{13}Na$: 663.3932, found: 663.3915 (M + Na). Elemental analysis: calculated for $C_{31}H_{60}O_{13} + H_2O$: C 56.50, H 9.42; found: C 56.14, H 9.62.

GL-2: Yield: 61 %. $[\alpha]_D^{25} = +31^o$ (*c* = 1.0, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.74 (m, 4 H), 4.61 (br s, 4 H), 4.42 (m, 2 H), 3.67–3.34 (band, 19 H), 1.43 (m, 2 H), 1.26 (br s, 26 H), 0.89 (t, 3 H, *J* = 6.9 Hz). ^{13}C NMR (75 MHz, DMSO–d_6): δ 99.92, 73.86, 70.94, 70.52, 70.29, 69.78, 68.23, 67.12, 66.92, 66.61, 61.25, 31.29 - 22.10, 13.97. HR-MS *m/z*: calculated for $C_{35}H_{68}O_{15}Na$: 751.4456, found: 751.4489 (M+Na). Elemental analysis: calculated for $C_{35}H_{68}O_{15} + 2H_2O$: C 54.97, H 9.42, found: C 54.19, H 9.20.

GL-3: Yield: 37 %, R_f = 0.5 (MeOH / $CHCl_3$ = 1: 4). $[\alpha]_D^{25} = +24^o$ (*c* = 1.0, MeOH). ^{1}H NMR ($CDCl_3$ + DMSO-d_6, 300 MHz): δ 4.82 (br s, 1 H), 3.8–3.3 (band, 21 H), 1.52 (m, 2 H), 1.26 (br, 26 H), 0.87 (t, 3 H, J = 7. 2 Hz). ^{13}C NMR ($CDCl_3$ + DMSO-d_6, 75 MHz): δ 100.03, 77.58, 72.79, 72.44, 71.41, 70.65, 70.58, 70.52, 70.27, 67.64, 66.32, 61.82, 61.11, 31.73, 29.90, 29.49, 29.46, 29.44, 29.33, 29.3, 29.1, 25.89, 22.5, 14.1. HR-MS *m/z*: calculated for $C_{29}H_{58}O_{10}Na$: 589.3928, found: 589.3946 (M+Na). Elemental analysis: calculated for $C_{29}H_{58}O_{10}$: C 61.46, H 10.31, found: C 61.24, H 10.28.

3.Carbon nanotubes oxidation (cutting):

SWNTs were purchased from Carbon Solutions Inc. and were used as purchased. For every 50 mg of full length SWNT, 150 mmol of 3 M nitric acid (HNO_3) was added and then refluxed for 24 h. This was followed by sonication in a mixture of 25 mL of concentrated HNO_3 and 75 mL concentrated sulfuric acid (H_2SO_4) over 8h. The water in the sonicator was cooled using ice to maintain a temperature of approximately 20°C. The SWNT/acid mixture was then diluted with approximately 400 mL of deionised water. The cut nanotubes were filtrated through a 0.22 µm PTFE filter and washed with MilliQ water several times. The cut nanotubes were dried in vacum oven and stored in desicator.

4. SWNTs solubilisation studies: A suspension of as-prepared/cut- SWNTs (0.5 mg/mL) in glycolipid solution (10 mM, above the CMC) was sonicated for 1 h at room temperature. The sonicated sample was centrifuged at 10000 g for 30 min. The upper 70-80 % of supernantent was then carefully decanted and then supernatent was used for various characterization studies and Con A binding studies.

5. Supplementary figures:

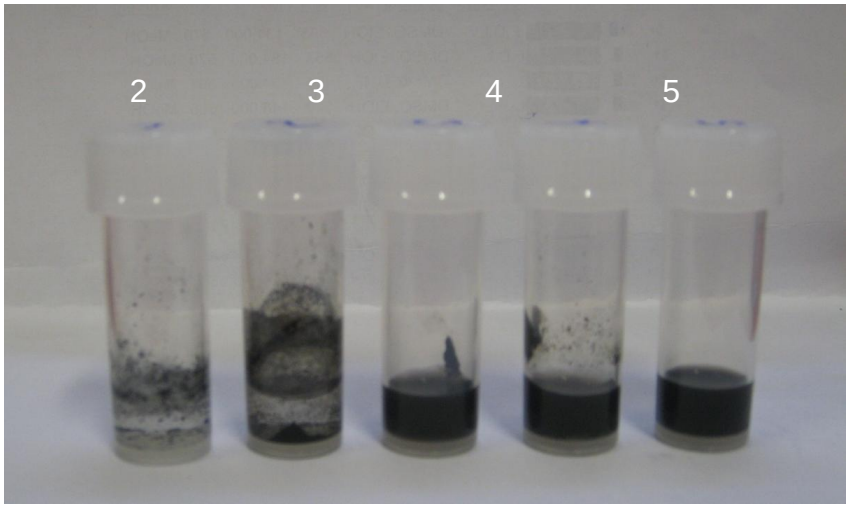

Figure S1: Photograph showing as-prepared, uncut SWNT in water (1-2), uncut SWNT in an aqueous solution containing GL-2 (3), GL-1 (4) and GL-3 (5).

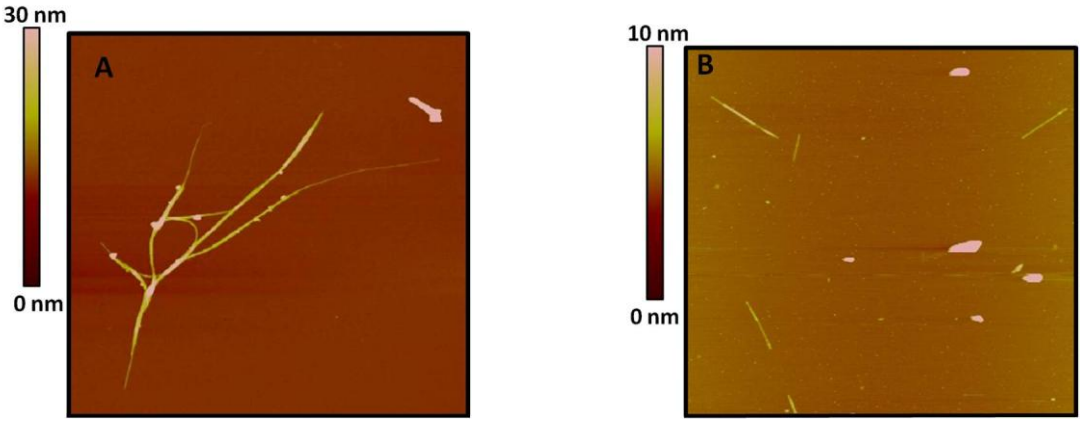

Figure S2: Tapping mode AFM height images of uncut-SWCTs (left) and cut-SWNTs (right) deposited on mica. A) uncut-SWNTs, image scale 4 µm x 4 µm, B) cut-SWNTs, image scale 3.5 µm x 3.5 µm.

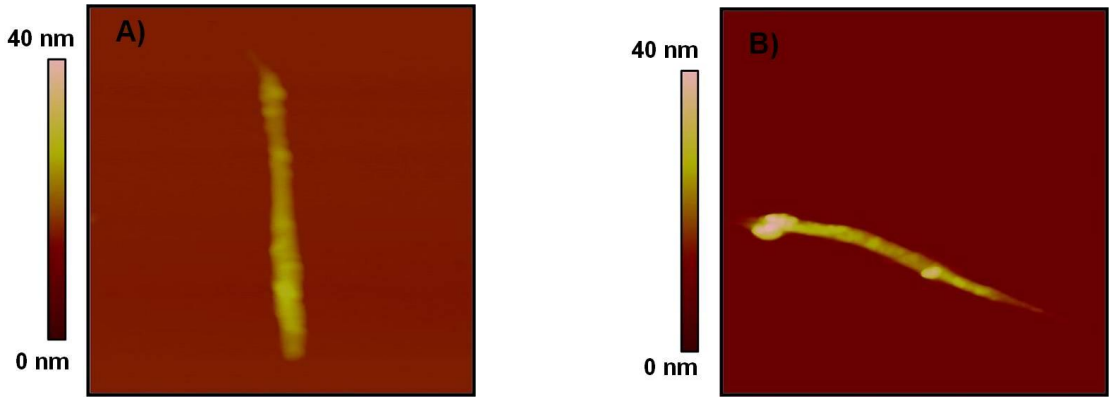

Figure S3: Tapping mode AFM height images of GL-coated cut-SWNT deposited on mica. A) GL-2 coated SWNT, image scale 450 nm x 450 nm; B) GL-3 coated SWNT, image scales 1200 nm x 1200 nm.

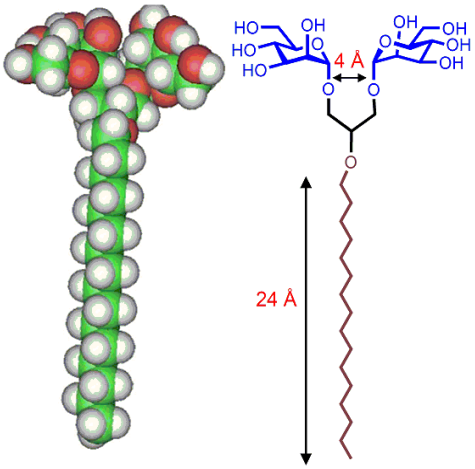

Figure S4: Sybyl 7.0 energy minimised structures of glycolipid **GL-1 (**total height of GL-1 is approximately 3.1 nm).

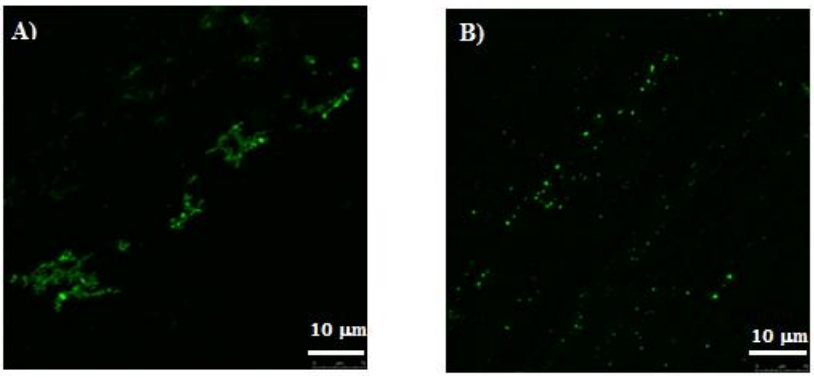

Figure S5: Confocal microscopy images of GL-1 coated SWNT after incubation with FITC-labelled Con A (left), and FITC-labelled Con A only (right).

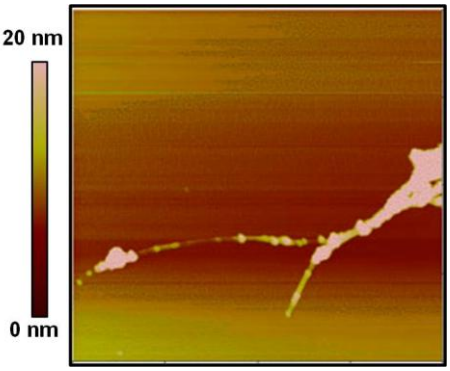

Figure S6: Tapping mode AFM height image of GL-1 coated uncut-SWNT after incubation with ConA on mica surface. Image scale 4 µm x 4 µm.

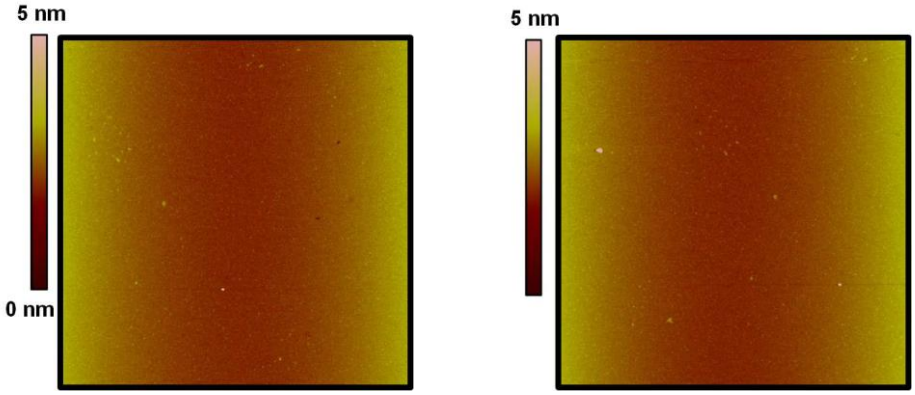

Figure S7. AFM height images of the mica surface after deposition of a DMF droplet (drop-casting method) without (left) and with (right) sonication of the solution, Image scale 5 µm x 5 µm. [In order to avoid the artefacts that are usually observed upon organic solvent evaporation on mica surface, the high-purity solvent DMF (99%, Aldrich) was dried over CaH_2 and further filtered through 0.22 µm syringe filters. 20 µL of DMF was placed on freshly cleaved mica surface and sample was dried in oven at 60 ^{o}C for 12 h, then AFM images were acquired in tapping mode. Pure DMF deposited on mica showed a flat surface devoid of any nanostructures, even when the solvent was sonicated for 15-20 min before deposition].

References:

1. B. N. Murthy, N. H. Voelcker and N. Jayaraman, *Glycobiology*, 2006, **16**, 822-832.
2. R. K. Ness, H.G. Fletcher Jr, C.S. Hudson, *J. Am. Chem. Soc.*, 1950, **72**, 2200-2205.