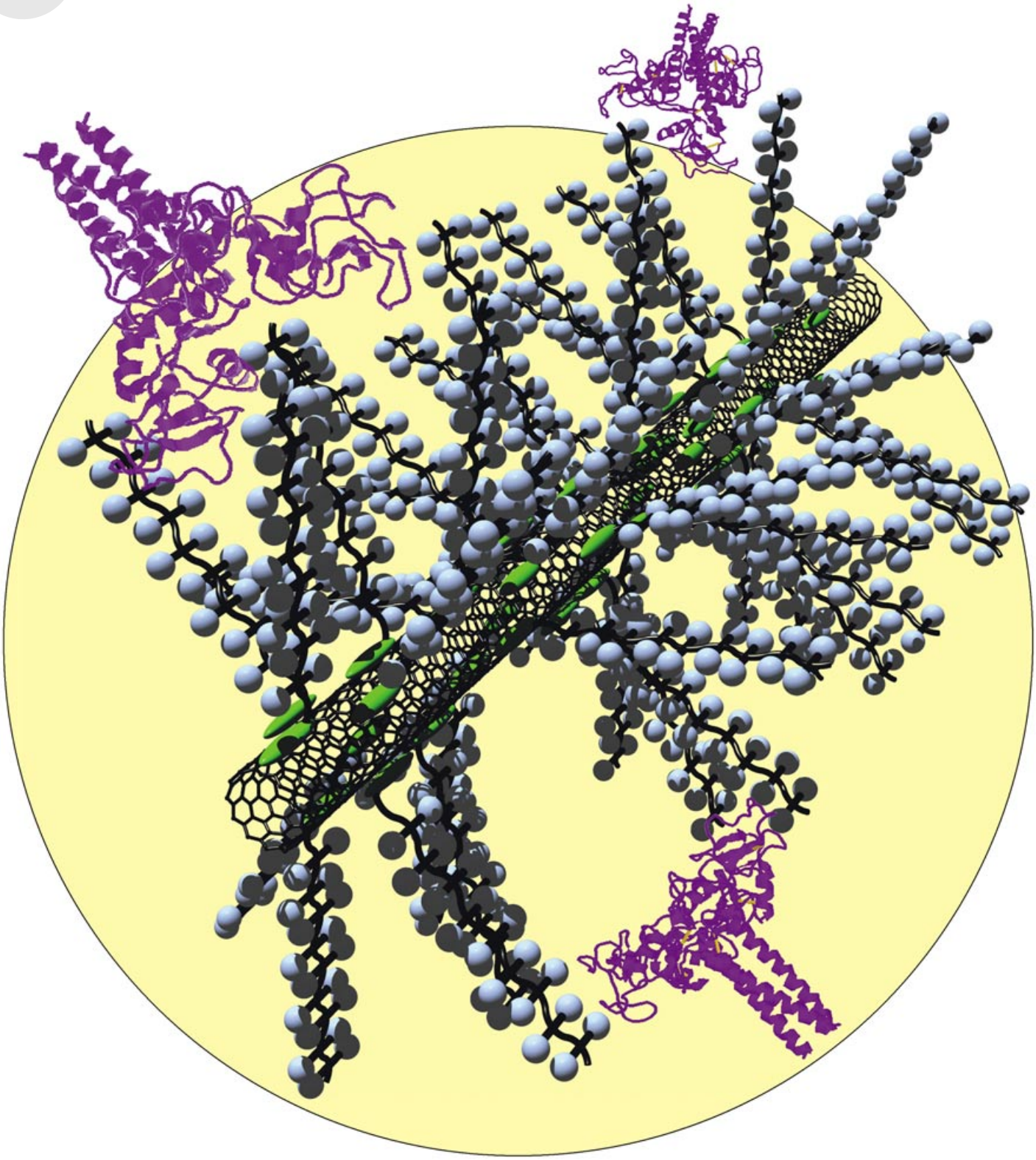
Communications



Carbohydrate-functionalized polymers designed to mimic the structures of mucin glycoproteins assembled on carbon nanotubes are water soluble and can be functionalized with ligands for specific receptor binding. For more information see the Communication by A. Zettl, C. R. Bertozzi et al. on the following pages.

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Biomimetic Synthesis

Biomimetic Engineering of Carbon Nanotubes by Using Cell Surface Mucin Mimics**

Xing Chen, Goo Soo Lee, A. Zettl, and Carolyn R. Bertozzi**

One of the most exciting applications of nanoscale science and technology is in the exploration of biological systems.[1] Carbon nanotubes (CNTs) have unique structural, mechanical, and electrical properties, and consequently numerous potential applications in biology, including sensing,[2] imaging,[3] and scaffolding for cell growth.[4] Few of these applications have yet been realized, however, because of the incompatibility of the CNT surface, which is hydrophobic and prone to nonspecific bioadsorption, with biological components such as cells and proteins. In addition, the aqueous environment required for biological materials are not suitable for unfunctionalized CNTs.[5] In nature, cells are faced with a similar challenge of resisting nonspecific biomolecule interactions while engaging in specific molecular recognition. These functions can be simultaneously fulfilled by mucin glycoproteins, defined by their dense clusters of O-linked glycans. Here we describe a biomimetic surface modification of CNTs using glycosylated polymers designed to mimic natural cell-surface mucins. CNTs modified with mucin mimics were soluble in water, resisted nonspecific protein binding, and bound specifically to biomolecules through receptor–ligand interactions. This strategy for biomimetic surface engineering provides a means to bridge nanomaterials and biological systems.

Mucins coat the surfaces of numerous cell types[6] and present epitopes for receptor-mediated cell–cell recognition.[7] Their dense glycosylation confers rigidity to the polypeptide backbone and thereby extends the mucin polymer well above the cell surface.[8] In addition, the glycans provide for strong hydration and passivation against biofouling. We have recently developed glycosylated polymers that share many properties with natural mucins (Figure 1a).[9] In native mucins, the clustered α-*N*-acetylgalactosamine (α-GalNAc) residues proximal to the peptide are the major contributors to the rigidification of peptide backbones. Although the core α-GalNAc residue is usually elaborated with additional sugars, removal of those sugars does not affect the mucin architecture.[10] The importance of the α-GalNAc residue for mucin structure has also been suggested by NMR analysis of synthetic glycopeptides.[11] Accordingly, we designed a synthetically tractable mucin mimic in which α-GalNAc residues were linked through an oxime bond to a poly(methyl vinyl ketone) [poly(MVK)] backbone (Figure 1a). The synthesis involved chemoselective ligation of poly(MVK) with an aminooxy-functionalized GalNAc analogue.[12] Light-scattering analysis indicated that the α-GalNAc-conjugated polymers adopt a rigid extended structure in water, similar to native mucin, whereas the unconjugated polymers adopt a more conventional globular structure.[9]

We introduced a C_{18} lipid at one end of a mucin mimic polymer with a molecular weight of about 75000 g mol^{-1} to enable surface modification of CNTs (Figure 1b; see Supporting Information for synthetic details). The hydrated diameter of the lipid-terminated mucin mimic was determined by light-scattering analysis to be about 54 nm. Lipids are known to self-assemble on the surface of CNTs through hydrophobic interactions in the presence of water[13] and lipid-functionalized glycopolymers have been shown to form ordered arrays on graphite surfaces.[14] We envisioned that lipid-functionalized mucin mimics could assemble on CNTs in a similar manner as the organization of native mucins in the cell membrane, with the glycosylated polymers projecting into the aqueous medium (Figure 1c).

We first subjected single-walled carbon nanotubes (SWNTs) to ultrasonication in the presence of an aqueous solution of the C_{18}-functionalized mucin mimic bearing α-GalNAc residues (C_{18}-α-MM, Figure 1b). The SWNTs were fully solubilized during the procedure (Figure 2a), which suggests the formation of a hydrophilic surface coating. A similar procedure was applied to multiwalled carbon nanotubes (MWNTs) with the same outcome (Figure 2c). The suspensions of C_{18}-α-MM-functionalized CNTs were stable for more than three months, while unfunctionalized CNTs precipitated very quickly (within hours) in aqueous solutions (Figure 2b and d). The C_{18} lipid on the mucin mimic polymer was essential to solubilize the CNTs: they rapidly precipitated from solutions of polymers identical to the above mucin mimics but lacking the lipid tail (not shown). These observations are consistent with a model in which C_{18}-α-MMs coat the CNTs as shown in Figure 1c.

The CNTs coated with mucin mimics (C_{18}-α-MM-SWNTs, for example) were directly characterized by atomic force microscopy (AFM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). As produced,

[*] Prof. A. Zettl
Department of Physics
University of California and Materials Sciences Division
Lawrence Berkeley National Laboratory
Berkeley, CA 94720 (USA)
Fax: (+1) 510-643-8497
E-mail: azettl@socrates.berkeley.edu
Prof. C. R. Bertozzi
Departments of Chemistry and Molecular and Cell Biology, and Howard Hughes Medical Institute
University of California, and
Materials Sciences Division, Lawrence Berkeley National Laboratory
Berkeley, CA 94720 (USA)
Fax: (+1) 510-643-2628
E-mail: crb@berkeley.edu
X. Chen, Dr. G. S. Lee
Department of Chemistry, University of California and Materials Sciences Division, Lawrence Berkeley National Laboratory
Berkeley, CA 94720 (USA)

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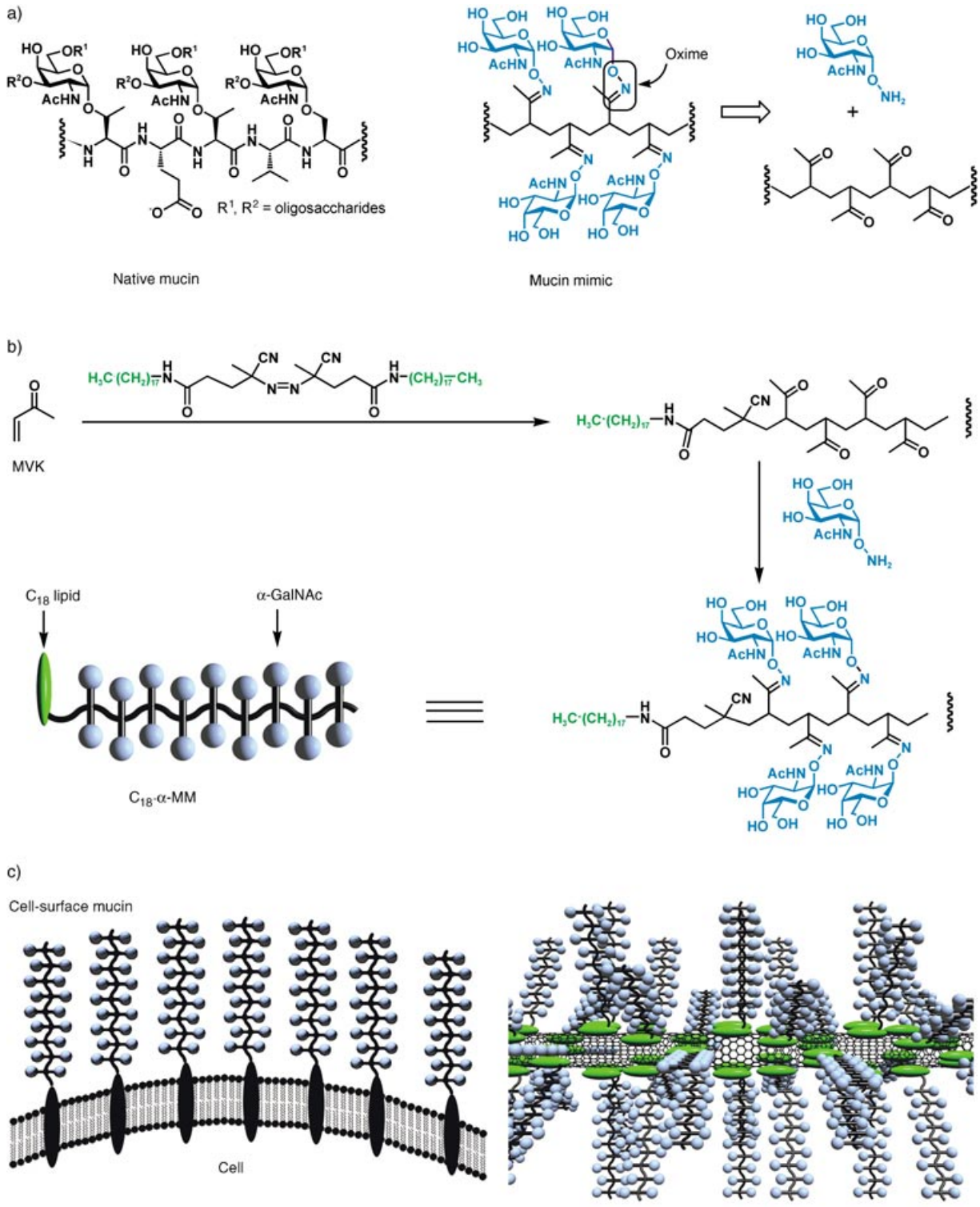

Figure 1. a) The structural features of native mucin (left) as well as the designed and synthesized mucin mimic (right). Natural mucins are characterized by dense clusters of O-linked glycans bound to Ser/Thr residues of the polypeptide. The α-GalNAc residues attached to the Ser/Thr residues are elaborated with additional sugars but only the initial GalNAc residues are required for the mucin structure. b) Synthesis of C_{18}-α-MM. The C_{18} lipid was conjugated to 4,4′-azobis(4-cyanopentanoic acid) (ACPA) and the amide-linked product was used to initiate radical polymerization of MVK to produce C_{18}-poly(MVK). C_{18}-α-MM was obtained by chemoselective ligation of C_{18}-poly(MVK) with aminooxy-GalNAc. c) A model for the self-assembly of C_{18}-MMs on the surface of carbon nanotubes (right), which is similar to the proposed arrangement of cell-surface mucins (left).

SWNTs usually exist as bundles that are heavily entangled with one another to form three-dimensional (3D) networks. After functionalization with C_{18}-α-MM, the entangled SWNT bundles dissociate to form much finer bundles and even

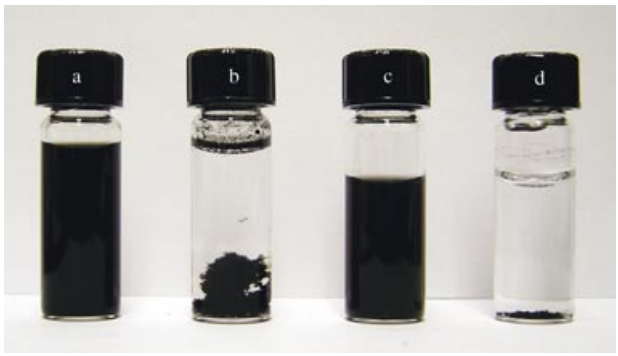

Figure 2. Photographs of vials containing a) a stable suspension of C_{18}-α-MM-SWNTs in H_2O after more than three months, b) as-produced SWNTs in H_2O, which precipitate in several hours, c) stable suspension of C_{18}-α-MM-MWNTs in H_2O after more than three months, d) as-produced MWNTs in H_2O, which precipitate over several hours.

individual nanotubes, as observed by all three imaging techniques. AFM permitted analysis of the modified tubes at ambient pressure and in an hydrated form, which is important for maintaining the structure of the mucin coating. As shown in Figure 3a, tapping mode AFM images on a silicon substrate revealed C_{18}-α-MM-SWNTs with fairly uniform diameters of 65–70 nm. The idealized model shown in Figure 1c would predict a diameter on the order of 100 nm, roughly twice that of the polymer mimiking the mucin (the SWNT itself is only ca. 2 nm in diameter). However, distortions to the soft mucin coating imposed by the AFM tip or substrate, or a nonperpendicular angle of projection of the mucin polymer from the SWNT surface could account for the discrepancy.

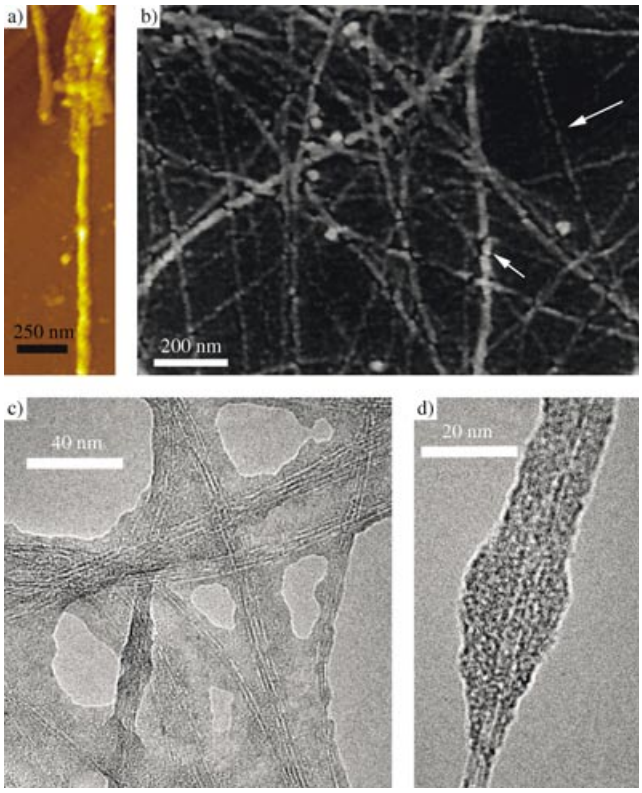

Figure 3. a) Tapping mode AFM image of C_{18}-α-MM-SWNTs on a silicon substrate. b) SEM image of C_{18}-α-MM-SWNTs. The arrows indicate damaged regions of the mucin mimic coating caused by the electron beam. c), d) TEM images of C_{18}-α-MM-SWNTs stained with 0.5% methylamine vanadate.

SEM analysis of C_{18}-α-MM-SWNTs also provided evidence of the mucin coating. Exposure of the sample to the 5-keV electron beam led to visible sites of damage along the SWNT surface, as indicated by the arrows in Figure 3b. This result was expected based on the sensitivity of organic species to decomposition induced by an electron beam. The damage induced by the electron beam increased with longer exposure times, and culminated in near-complete destruction of the coating after 15 minutes (see the Supporting Information). By contrast, unmodified CNTs are fairly stable under the electron beam, and showed no visible change in surface morphology after prolonged exposure.

TEM analysis of C_{18}-α-MM-SWNTs provided a direct visualization of the mucin coating (Figure 3c and d). The thickness of the coating varied from 10 to 25 nm under these conditions, in which the sample is dehydrated under high vacuum. The coating was associated with the CNT surface (Figure 3c), and was not observed in regions of the image lacking CNTs. A single tube (or small bundle) coated with C_{18}-α-MM is shown in Figure 3d. The coating was not entirely uniform along the length of this SWNT, perhaps the result of damage induced by the electron beam (100 keV) or collapse of the mucin polymers under high vacuum. In this regard, AFM imaging is a superior technique for visualizing mucin-coated SWNTs as they would exist in a functionally relevant aqueous environment.

On cells, mucins serve the dual role of molecular recognition and resistance to biofouling. We sought to determine if these same functions could be realized in the context of the nanotube surface. The C_{18}-α-MM coating introduces α-GalNAc residues onto the CNT surface, which could be recognized by an α-GalNAc-specific receptor such as the lectin *Helix pomatia* agglutinin (HPA).[15] To test this we incubated C_{18}-α-MM-SWNTs with a solution of HPA conjugated with fluorescein isothiocyanate (HPA-FITC; shown schematically in Figure 4a I). The C_{18}-α-MM-SWNTs were dialyzed to remove excess HPA-FITC and then analyzed for bound lectin by fluorescence spectroscopy. The C_{18}-α-MM-SWNTs showed significant fluorescence, which was attributed to bound fluorescein (trace I of Figure 4b). HPA-FITC labeling of the C_{18}-α-MM-SWNTs was inhibited when 0.2 M free GalNAc was present in solution (Figure 4a III and trace III of Figure 4b), thus confirming that fluorescent labeling was dependent on the receptor–ligand interaction.

In addition, we prepared a similar mucin mimic in which the GalNAc residues were conjugated to the polymer backbone through a β-anomeric linkage (C_{18}-β-MM; see Supporting Information for synthetic details). This mucin mimic was physically identical to its α-linked counterpart but should not be capable of binding HPA.[16] We coated SWNTs with C_{18}-β-MM and incubated them with HPA-FITC (shown schematically in Figure 4a II). No significant fluorescence labeling was observed (trace II in Figure 4b), thus indicating that the lectin

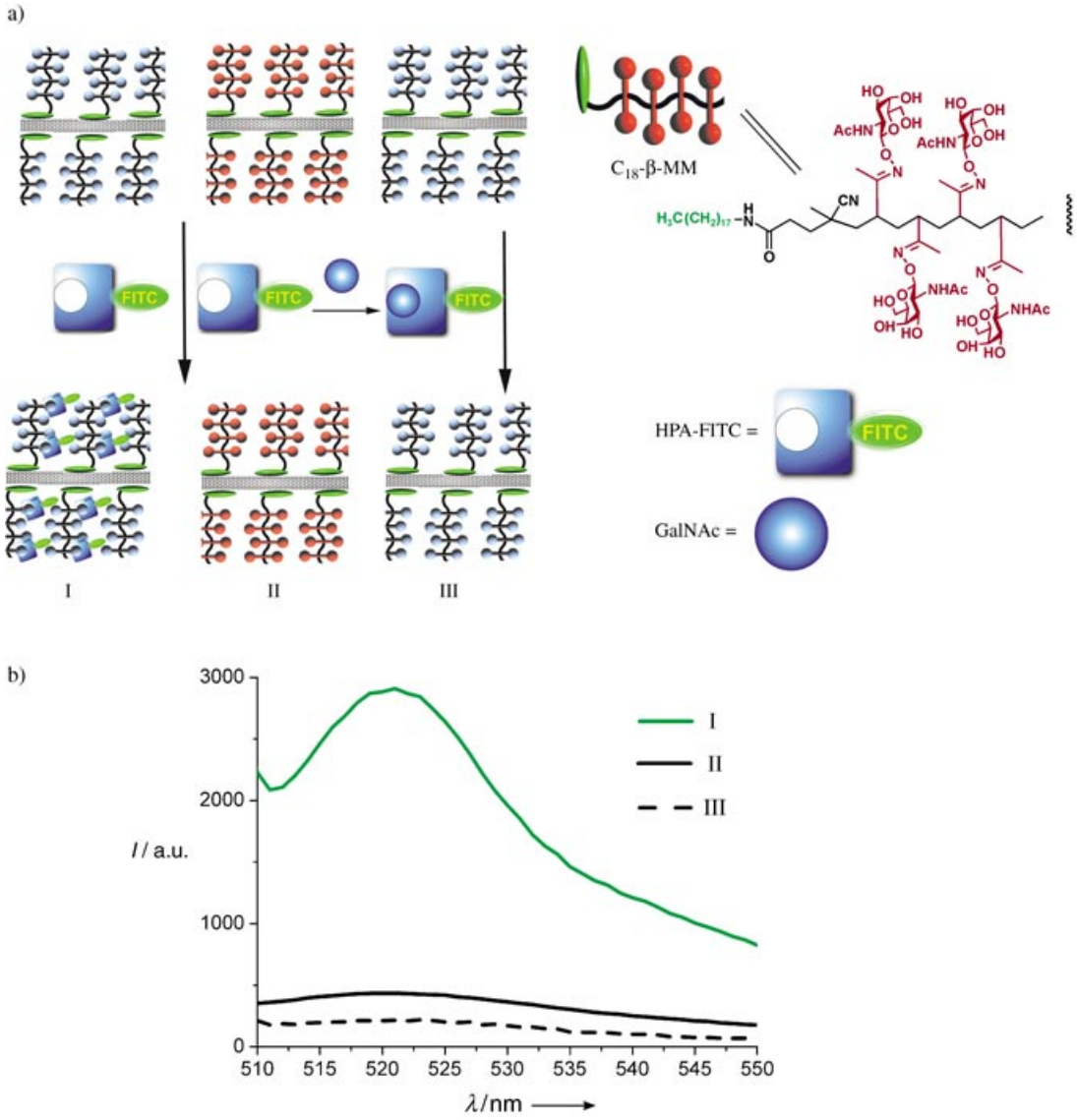

Figure 4. Specific binding of HPA to C_{18}-α-MM-coated nanotubes. a) Scheme for: I) specific binding of HPA to the surface of C_{18}-α-MM-SWNTs, II) lack of binding of HPA to C_{18}-β-MM-SWNTs, III) inhibition of HPA binding by soluble GalNAc. b) Fluorescence spectra (510–550 nm) showing the specific binding of HPA to the surface of C_{18}-α-MM-SWNTs. Trace I: spectrum showing bound HPA-FITC on the surface of C_{18}-α-MM-SWNTs; trace II: spectrum showing the lack of bound HPA to C_{18}-β-MM-SWNTs; trace III: spectrum showing fluorescence associated with HPA-FITC binding in the presence of soluble GalNAc. The excitation wavelength was 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. These data are representative of results observed in triplicate experiments.

does not interact with the tubes coated with the mucin mimic in the absence of its preferred ligand. These results demonstrate that CNTs coated with a mucin mimic can engage in specific molecular recognition with protein receptors and resist nonspecific protein binding.

In summary, we have developed a practical and general approach to engineering biomimetic surfaces on CNTs. The mucin mimics used in this study endowed the CNT surfaces with properties shared by cell surfaces, including the display of carbohydrates capable of molecular recognition. The synthetic process leading to the mucin mimic allows for the introduction of myriad functional epitopes, in addition to sugars, that could encode interactions with numerous receptor types. This work should facilitate the integration of CNTs into aqueous biological systems that include proteins and cells.

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