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Electronic Detection of Lectins Using Carbohydrate Functionalized Nanostructures: Graphene *versus* Carbon Nanotubes

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Abstract

Here we investigated the interactions between lectins and carbohydrates using field-effect transistor (FET) devices comprised of chemically converted graphene (CCG) and single-walled carbon nanotubes (SWNTs). Pyrene- and porphyrin-based glycoconjugates were functionalized noncovalently on the surface of CCG-FET and SWNT-FET devices, which were then treated with 2 μ M of nonspecific and specific lectins. In particular, three different lectins (PA-IL, PA-IIL and ConA) and three carbohydrate epitopes (galactose, fucose and mannose) were tested. The responses of 36 different devices were compared and rationalized using computer-aided models of carbon nanostructure/glycoconjugate interactions. Glycoconjugates surface coverage in addition to one-dimensional structures of SWNTs resulted in optimal lectin detection. Additionally, lectin titration data of SWNT- and CCG-based biosensors were used to calculate lectin dissociation constants (K_d) and compare them to the values obtained from the isothermal titration microcalorimetry (ITC) technique.

Keywords

carbon nanotubes; graphene; carbohydrate; lectin; sensors

Because of their unique physical and chemical properties, single-walled carbon nanotubes (SWNTs) and more recently graphene have attracted considerable interest for the development of biosensors.^{1–5} These carbon nanostructures are both just one atom thick, and their electronic properties are extremely sensitive to adsorption of chemical species on their

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Supporting Information Available Experimental details of UV-vis-NIR spectroscopy and AFM imaging, FET experiment of CCG and lectin interaction, UV-vis-NIR spectroscopy, AFM and fluorescence microscopy imaging, FET experiments of CCG and SWNT with pyrene-glycoconjugates functionalization, titration experiment of CCG and SWNTs with pyrene-glycoconjugates functionalization, FET experiments of CCG with porphyrin-glycoconjugates functionalization, Langmuir isotherm of CCG FET and SWNT FET data, experimental details for treatment of ITC data, and characterizations of glycoconjugates **1a–c** by 1D and 2D NMR and high-resolution mass spectrometry were included in the Supporting Information. This material is available free of charge *via* the Internet at <http://pubs.acs.org>.

surface. They have been successfully configured into field-effect transistor (FET) devices, which can be used for ultrasensitive detection of many chemical and biological molecules including proteins.^{2,3}

Lectins are a class of nonimmune proteins that bind mono- and oligosaccharides reversibly and with high specificity.⁶ Lectins have weak interaction with carbohydrates, in the forms of glycoprotein, glycolipids and glycans in biological systems with dissociation constants (K_d) in the millimolar to micromolar range.^{7–9} These interactions are involved in various biological processes, including cell-cell communication, pathogen binding, tumor cell metastasis, and immune responses. It is important to understand and mimic carbohydrate and bacterial lectins interactions as the foundation of pathogen detection and prevention of bacterial infection.^{10–12}

Although carbon nanostructures have been functionalized using both covalent and noncovalent methods to induce specific lectin affinity,^{13–17} noncovalent functionalization route preserves physicochemical properties (*i.e.* electrical conductivity) required for FET device operation. Utilizing this methodology, we have recently reported the noncovalent functionalization of SWNTs with glycosylated porphyrins for detection of lectins with a SWNT-based FET device.¹⁸

In this work, we further investigated carbohydrate-lectin interactions with both SWNTs and chemically converted graphene (CCG), towards developing carbon nanostructure-based biosensors. In addition to glycosylated porphyrins, we also used pyrene-based glycoconjugates with both nanostructures. It should be mentioned here that among polynuclear aromatic compounds,¹⁹ pyrene derivatives are recognized as highly effective labelling agents that are capable of forming π - π stacking interactions with graphitic surfaces.^{20–22} To date, pyrene-SWNT conjugate systems have been implemented with monosaccharides^{15,17} or glycodendrimers²³ for lectin biosensing, drug delivery or interfacing with living cells. Here we investigated SWNT-FET and CCG-FET devices noncovalently functionalized with pyrene- and porphyrin-based glycoconjugates carrying β -D-galactose, α -D-mannose and α -L-fucose for nanoelectronic detection of lectins PA-IL, PA-IIL and concanavalin A (ConA).

Lectin PA-IL and PA-IIL are produced in *Pseudomonas aeruginosa* together with other secondary metabolism virulence factors under quorum-sensing control and are involved in the infection process as demonstrated by the murine model.^{24–26} *P. aeruginosa* bacteria are involved in cystic fibrosis lung infections, community-infections like otitis and pneumonia, and hospital-acquired infections.²⁷ PA-IL (12 kDa) is composed of four subunits of 121 amino acids and binds galactose plus its derivatives. PA-IIL (11 kDa) is composed of four subunits of 114 amino acids and binds fucose and other related monosaccharides. ConA (purified from jack-bean, *Canavalia ensiformis*, 25 kDa) lectin is a tetrameric lectin, which binds mannose and glucose. These three lectins require divalent cations for sugar-binding activity. Calcium ions mediate the binding of PA-IL and PA-IIL to their specific sugar moiety,^{28,29} while calcium and manganese maintain the active conformation of a loop in ConA.³⁰

RESULTS AND DISCUSSION

Our synthesis of pyrene-based glycoconjugates (**1a**, **1b**, **1c**) and porphyrin-based glycoconjugates (**2a**, **2b**, **2c**)¹⁸ was achieved through 1,3-dipolar cycloaddition between alkynylated pyrene or porphyrin derivatives and azido-functionalized carbohydrates. The conjugation through Cu(I)-catalyzed alkyne-azide cycloaddition (CuAAC)^{31–33} was preferred to the direct carboxylic acid-amine condensation methodology, which usually

requires a significant excess of either reagent and the use of stoichiometric amounts of coupling reagents. In contrast, CuAAC only requires a catalytic amount of copper iodide and consumes a small excess of carbohydrate derivative (1.5 equivalents). The reaction of 1-pyrenebutyric acid **3** with propargyl amine afforded the corresponding amide **4**³⁴ in nearly quantitative yield. Subsequently CuAAC was performed between alkyne **4** and glycosylated azides **5a–c** under microwave activation^{35–39} to obtain the acetylated pyrene-functionalized carbohydrate derivatives **6a–c**. The final deprotection step of acetyl groups was achieved by solvolysis to afford the desired hydroxylated pyrene glycoconjugates **1a**, **1b**, **1c** in high yields (Scheme 1). The three glycoconjugates were fully characterized by 1D and 2D NMR and high-resolution mass spectrometry (see Supporting Information). This synthetic methodology has provided three glycosylated probes (β -D-galactose, α -D-mannose, α -L-fucose) ready to be immobilized on SWNTs or CCG for the detection of lectins with three different specificities (Table 1). The linker length was chosen on the basis of solubility, flexibility and commercial availability of a desymmetrized triethyleneglycol.

Field-effect transistor (FET) devices comprising of a CCG or SWNT network were fabricated by patterning interdigitated microelectrodes (source-drain spacing of 10 μ m) on top of 200 nm oxide layer on Si substrates using photolithography and e-beam evaporation of 30 nm Ti and 100 nm of Au (Figure 1a). Figure 1b depicts the schematic illustration of liquid-gated FET configuration with either SWNTs or CCG as the conducting channel. CCG was prepared as described elsewhere^{40–42} and was employed as conducting channel in these FETs. CCG and SWNTs were deposited onto the device using an a.c. dielectrophoresis (DEP) method⁴³ (see Experimental Section). Carbon nanostructures were deposited between fingers of interdigitated microelectrodes, and the DEP method facilitated the alignment of SWNTs.¹⁸

To selectively detect lectins, SWNT-FET and CCG-FET devices were noncovalently functionalized with pyrene-based glycoconjugates. The noncovalent functionalization of carbon nanostructures with pyrene-based glycoconjugates was performed by incubating the Si chip with FET devices in 20 μ M solution of the glycoconjugates (in deionized water) for two hours followed by rinsing with deionized water three times. Upon interaction with pyrene-based glycoconjugates (**1a–c**), a decrease in the FET device conductance with a slight negative shift in gate voltage was observed (Figure 1c and 1d). The decrease in device conductance was attributed to electron donation from pyrene molecules to SWNTs or CCG conducting channels. The interactions were also studied by ultraviolet-visible-near infrared (UV-Vis-NIR) absorption spectroscopy, which indicated the attachment of glycoconjugate to the carbon nanostructures (Figure S1).

After glycoconjugate functionalization, the FET devices were treated with non-specific lectin (control) and then with specific binding lectins (both 2 μ M of lectins in PBS with 5 μ M Ca^{2+}). Calcium ions were added to mediate the binding of lectins to their specific sugar moiety.^{28–30} As presented in Figure 1c, SWNT-FET device functionalized with pyrene-glycoconjugate **1a** was treated with 2 μ M non-specific (ConA) and specific (PA-IL) lectins (in PBS with 5 μ M Ca^{2+}). Upon treatment with nonspecific lectin, the transfer characteristics of the device remained unaffected. A decrease in device conductance was observed after treatment with specific lectin, which indicated selective interaction between the lectin and glycoconjugate. The same experiment with α -D-mannose and α -L-fucose pyrene-based glycoconjugate **1b** and **1c** also demonstrated a similar trend in response and selectivity (Figure S2).

To compare the CCG- and SWNT-FET devices for lectin detection, similar experiments were performed on pyrene glycoconjugates **1a–c** functionalized CCG-FET devices as well. Figure 1d shows the response of the **1a** functionalized CCG-FET devices to various lectins.

Compared to p-type SWNT-FET devices, CCG-FET had an ambipolar transfer characteristic and demonstrated different lectin responses at negative and positive gate voltages (p-type and n-type regions). To be consistent with the SWNT data, we compared the response in the p-type region only. Upon incubation with non-specific lectin (ConA), there was a small decrease in conductance at negative gate voltages, while the decrease in the positive voltage region was larger. When treated with the galactose specific lectin (PA-IL), a decrease was observed primarily at the negative gate voltages. Incidentally, the maximum selectivity for the CCG-FET devices was observed at the p-type region. The same experiments with α -D-mannose and α -L-fucose pyrene-based glycoconjugates **1b** and **1c** showed similar response as the CCG/**1a** configuration (Figure S3). As an additional control, when bare CCG-FET devices were exposed to ConA, the device conductance decreased due to the non-specific adsorption (Figure S4).

Figure 1e summarizes the responses of all tested SWNT and CCG biodetectors to specific and nonspecific lectins (**1b**, **1c**, **2a**, **2b** and **2c** data presented in Supporting Info). In all cases, we plotted the responses at negative gate voltages ($V_g = -0.5$ V), which allowed direct comparison between the two types of devices. For pyrene-based receptor molecules, SWNT-FET devices demonstrated a larger response and better selectivity for lectin detection than CCG. This may be attributed to the greater sensitivity to lectin adsorption on the transport properties of 1D SWNT than 2D CCG.^{44,45} We also compared the performance of SWNT-porphyrin and CCG-porphyrin (**2a**, **2b**, **2c**) biodetectors and again SWNT-FET devices showed higher selectivity and response. Although glycoconjugates can have different interactions with either SWNTs or CCG, 1D systems should be generally more sensitive to the lectin binding (Scheme 2). Because lectin molecules have a comparable size to the diameter of SWNT, even one molecule can effectively alter the electrical conductivity of SWNTs compared to significantly larger CCG flakes.

Since the better response of SWNTs may also be due to carbohydrate accessibility, computational modeling was performed to further understand the interaction between glycoconjugates, carbon nanomaterials, and lectins. Figure 2 illustrates molecular models of the interactions between the different carbon nanomaterials associated with galactosylated glycoconjugates and PA-IL lectin. Semiconducting SWNTs were built with chiral indices $m=14$ and $n=4$ as described previously,⁴⁶ which resulted in nanotubes with 1.3 nm diameter. As displayed in Figure 2a and 2b, the diameter of the SWNT is much smaller than the size of the lectin. The stacking of pyrene or porphyrin moieties of the glycoconjugates on the SWNT yielded galactose residues that are easily accessible for the lectins. Alternatively, stacking the same molecules on CCG resulted in galactose residues that were in close proximity to the carbon sheet and therefore less accessible for lectin binding. This is particularly true for porphyrin conjugates as displayed in Figure 2d. Therefore, it can be concluded that the SWNT system provides more flexibility for the sugar moieties to interact with lectin leading to higher sensitivity than the CCG system.

To obtain evidence of the attachment of glycoconjugates onto the carbon nanostructure surfaces and the interaction between glycoconjugates and lectins, atomic force microscopy (AFM) imaging was performed at different stages of functionalization. The height of bare SWNTs was observed to be around 3–4 nm indicating the presence of SWNT bundles (Figure 3a). After functionalization with α -D-mannose glycoconjugates **1b**, the total height increased to 5–7 nm (Figure 3b). After incubation with the non-specific lectin PA-IL, the increase in height was 1–3 nm (Figure 3c). On the other hand, after exposing the glycoconjugate functionalized SWNTs to the specific binding lectin (ConA for α -D-mannose), an increase in height of more than 10 nm was observed (Figure 3d). Bare CCG was observed to be 0.67 ± 0.15 nm in thickness (Figure 3e), which indicated the presence of single sheet structure. After functionalization with α -D-mannose glycoconjugates **1b**, the

total height increased to 2.44 ± 0.35 nm (Figure 3f). When incubating with the non-specific lectin PA-IL, the increase in height was around 1 nm (Figure 3g). After exposing the glycoconjugate functionalized CCG to specific binding lectin (ConA for α -D-mannose), an increase in height to 8.25 ± 1.73 nm was observed (Figure 3h). The height measurements obtained by AFM were in good agreement with the literature values.⁴⁷ Typically, ConA was observed as a tetramer in solution at $\text{pH} \geq 7$, and the molecular dimensions of tetramer are $60 \times 70 \times 70$ Å (Protein DataBank, 1CN1) as determined from X-ray diffraction studies. It should be mentioned here that, as can be visualized from Figures 3d and 3h, SWNTs are covered continuously while the binding sites are scattered randomly on CCG surface. This indicates that SWNT system provides more adsorption sites for lectins, which is consistent with the computer modelling studies. Additionally, the basal plane of CCG could contain oxygen functional groups (*i.e.* epoxides and tertiary alcohols),⁴² which could hinder the π - π stacking of polynuclear aromatic compounds (glycoconjugates) and result in the lower lectin coverage. It should be mentioned here that the attachment of glycoconjugates to the surfaces of the nanostructure can effectively prevent the nonspecific binding of the lectin. For example, in Figures 3c and 3d, although PA-IL can adsorb nonspecifically onto the poly-L lysine decorated mica surface and form agglomerates, no evidence of nonspecific binding onto the **1b**-decorated surfaces of the carbon nanostructure was observed. The high coverage of lectins on SWNTs in SWNT-FET devices was further illustrated by fluorescence microscopy. Figure 4 depicts the fluorescence image of α -D-mannose functionalized SWNT-FET device incubated with fluorescently labeled lectin ConA-Alexa488, which shows specific binding to α -D-mannose. SWNTs (aligned by DEP between fingers of interdigitated electrodes) clearly exhibit high fluorescence indicating high coverage of ConA. Due to the fluorescence quenching effect of graphene,⁴⁸ no representative image for graphene flakes was obtained using fluorescence microscopy. The preferential lectin binding in addition to its pronounced effect on the electrical conductivity (Scheme 2) can explain the observed larger response and better selectivity of SWNT-FET devices.

Additionally, the sensitivity of SWNT-FET and CCG-FET devices was investigated by plotting the G vs V_g for β -D-galactose functionalized device (control measurements with $10 \mu\text{M}$ ConA) for varying concentration (2 nM to $10 \mu\text{M}$) of specific lectin PA-IL (Figure S6). The response of both types of devices to $10 \mu\text{M}$ specific lectin PA-IL is almost two times higher than the response to $10 \mu\text{M}$ nonspecific lectin ConA, which demonstrates good selectivity. Figures 5a, 5c and 5e depict the biosensor calibration plot derived from multi-concentration experiments (data from three devices) for SWNT/**1a**, CCG/**1a** and CCG/**1c**, respectively. The detection ability of the devices was also tested in the mixed sample of specific and non-specific lectins. The device showed similar signal compared to the pure sample of specific lectin (Figure S7).

To further understand the kinetics of the lectin-glycoconjugate interactions and to determine the dissociation constant (K_d) of the interaction, $C_{\text{lectin}}/(\Delta\theta/\theta_0)$ as a function of C_{lectin} were plotted, where C_{lectin} is the lectin concentration and $\Delta\theta/\theta_0$ is the relative change in conductance after lectin attachment (Figure 5).^{49,50} A linear relationship was obtained, which indicated a Langmuir isotherm adsorption, and the **1a**/PA-IL K_d was calculated for SWNT-FET and CCG-FET as 135 nM and 106 nM, respectively. **1c**/PA-IL K_d was calculated for CCG-FET as 20 nM. These K_d values were compared with the values obtained by isothermal titration microcalorimetry (ITC) to explore the correlation between surface and solution binding (Table 2). The titration of the galactose and fucose pyrene-based conjugate (**1a** and **1c**) by their specific lectins (PA-IL and PA-IIL) followed by ITC (Figure 6a and 6b) indicated the occurrence of two binding events probably due to additional interaction of the pyrene aromatic group with a hydrophobic region on the lectins as previously evidenced for PA-IL.^{51,52} Data treatment was performed using a the two sites

model (see Supporting Information S7), and only values for the carbohydrate binding sites were discussed below.

For PA-IL interacting with the galactose pyrene-based conjugate **1a**, the binding event in the galactose binding site had a dissociation constant of 0.1 mM, which was in agreement with data obtained on PA-IL/galactosides,⁵³ but is much weaker than the K_d of 106 nM and 135 nM obtained by FET measurement, when glycoconjugate **1a** is attached to CCG or SWNT, respectively (Figures 5b and 5d). This result was likely due to the chelating effect that has been evidenced for PA-IL binding to multivalent glycoconjugates with a two to three orders of magnitude increase in affinity when comparing multivalent ligands to monovalent ones.^{37, 53–55} Binding of PA-IL to glycoconjugates on surfaces or membranes has also been demonstrated to be more efficient than to monovalent compounds in solution.⁵⁶ The data obtained from the ITC experiment on porphyrin **2a**/PA-IL were in the range of 100 nM for both the solution and FET experiments, in agreement with the chelating effect since compound **2a** is tetravalent.

For PA-III interacting with fucose pyrene-based conjugate **1c**, a dissociation constant (K_d) of 50 nM was obtained for the fucose binding site, which was stronger than the one measured for methyl α -L-fucoside (430 nM).⁵⁷ The K_d value of **1c**/PA-III measured by FET experiment in Figure 5f was calculated as 20 nM and was consistent with the above mentioned K_d value. No chelating effect has been observed for **1c**/PA-III binding.

ITC demonstrated that the carbohydrate moiety of the pyrene glycoconjugate is efficiently recognized by the corresponding lectin. The additional interaction of pyrene and the hydrophobic regions of the lectin occurs only in solution; therefore, this was not an issue in the FET experiment, where the pyrene moiety was stacked on the graphitic surface of either SWNT or CCG and not accessible for binding. Moreover, the K_d values obtained by ITC and FET are well correlated, which provided evidence of the effectiveness of carbon nanomaterial-based FET systems to detect carbohydrate-lectin interactions.

CONCLUSION

In conclusion, we have demonstrated the detection of interactions between glycoconjugates and bacterial lectins using SWNT-FET and CCG-FET devices functionalized with two receptor molecules, namely porphyrin and pyrene-based glycoconjugates. The interactions between lectins and glycoconjugates were transduced as conductance changes measured by SWNT-FET and CCG-FET devices. SWNT-FET showed larger response and better selectivity than CCG-FET, and we propose that this difference was the result of 1D and 2D structural difference between the two materials as well as more favorable lectin binding to sugar moieties attached to SWNT surface. Because this nano-electronic detection platform has the potential to be employed for the rapid identification of bacterium in samples consisting of water systems, soils, or human specimens, this technology upon further development may aid in preventing disease outbreaks and preserving public health.

EXPERIMENTAL SECTION

Materials and methods

All reagents were procured commercially (highest purity available for reagent grade compounds) and used without further purification. Solvents were distilled over CaH₂ (CH₂Cl₂), Mg/I₂ (MeOH) or purchased as the highest purity available (DMF). Reactions were performed under an argon atmosphere. Reactions under microwave activation were performed on a Biotage Initiator system. Thin-layer chromatography (TLC) was carried out on aluminum sheets coated with silica gel 60 F₂₅₄ (Merck). TLC plates were inspected by

UV light ($\lambda = 254$ nm) and developed by treatment with a mixture of 10% H_2SO_4 in EtOH/ H_2O (95:5 v/v) followed by heating. Silica gel column chromatography was performed with silica gel Si 60 (40–63 μm). NMR spectra were recorded at 293 K, unless otherwise stated, using a 300 MHz or a 400 MHz Bruker spectrometer. Chemical shifts are referenced relative to deuterated solvent residual peaks. The following abbreviations are used to explain the observed multiplicities: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet and bs, broad singlet. Complete signal assignments were based on 1D and 2D NMR experiments (COSY, HSQC and HMBC). High-resolution (HR-ESI-QToF) mass spectra were recorded by using a Bruker MicrOTOF-Q II XL spectrometer in the positive ionization mode. Optical rotations were measured using a Perkin Elmer polarimeter, and values are given in $10^{-1} \text{ deg}\cdot\text{cm}^2\cdot\text{g}^{-1}$. The carbohydrate azido-derivatives **5a**,^{37,58} **5b**,⁵⁹ and **5c**^{36,60} were previously described in the literature and prepared accordingly.

Single-walled carbon nanotubes (SWNTs) were procured from Carbon Solutions Inc. Reduced graphene oxide (RGO), a.k.a. chemically converted graphene (CCG), was prepared as described elsewhere.^{40–42} Briefly, graphite oxide was synthesized utilizing a modified Hummers' method on graphite flakes (Sigma Aldrich) that underwent a preoxidation step.⁴¹ Graphite oxide (~0.125 wt%) was exfoliated to form graphene oxide *via* 30 minutes of ultrasonication followed by 30 minutes of centrifugation at 3400 revolutions per minute (r.p.m.) to remove unexfoliated graphite oxide (GO). Graphene oxide was then reduced to CCG with hydrazine hydrate (Sigma Aldrich) following the reported procedure.^{40,42} Note that hydrazine is extremely flammable and toxic, and proper laboratory technique should be employed when handling this chemical.

PA-II and PA-III lectins were produced in recombinant form, purified on affinity column, extensively dialyzed and lyophilized as described previously.^{53,61} ConA (25 kDa) lectin was purchased from Sigma and was used without further purification.

Synthesis of the pyrene-glycoconjugates **1a**, **1b**, **1c**

General procedure for 1,3-dipolar cycloaddition (Method A)—The alkyne-functionalized pyrene derivative **4**, copper iodide, DIPEA and carbohydrate azido-derivatives **5a–c** in DMF were introduced in a Biotage Initiator 2–5 mL vial. The vial was flushed with argon and the solution was sonicated for 30 seconds. The vial was sealed with a septum cap and heated at 110°C for 30 min under microwave irradiation (solvent absorption level : High). After uncapping the vial, the crude mixture was evaporated and then purified by flash silica gel column chromatography to afford the desired acetylated pyrene glycoconjugates **6a–c**.

General procedure for deacetylation (Method B)—The acetylated pyrene glycoconjugates **6a–c** were suspended in distilled MeOH, ultra-pure water and ultra-pure triethylamine (10:1:1, v/v/v). The mixture was stirred under argon at room temperature for 1 to 3 days. Solvents were evaporated off then co-evaporated with toluene. The residue was dissolved in ultra-pure water (5 mL) and lyophilized to afford pure hydroxylated pyrene glycoconjugates **1a**, **1b** and **1c**.

FET measurements

Metal interdigitated devices (Au/Ti, 100 nm/30 nm) with interelectrode spacing of 10 μm were patterned on a Si/SiO₂ substrate using conventional photolithography. Each chip (2 mm $\times$ 2 mm) containing six identical devices was then set into a 40-pin ceramic dual in-line package (CERDIP), wire-bonded using Au wire, and isolated by epoxying the device's inner cavity. SWNTs were deposited onto each interdigitated microelectrodes pattern by a.c. dielectrophoresis (DEP) method from a suspension in *N,N*-dimethylformamide (DMF)

(Agilent 33250A 80 MHz Function/Arbitrary Waveform Generator, with an applied a.c. frequency (10 MHz), bias voltage (8 V_{pp}), bias duration (60 s)).¹⁸ CCG devices were prepared using the same DEP technique but with different parameters (a.c. frequency (300 kHz), bias voltage (10 V_{pp}), bias duration (120 s)).⁴³

The electrical performance of each device was investigated using an electrolyte gated FET device configuration. The conductance of the FET device was tuned using phosphate buffer solution (PBS) electrolyte as a highly effective gate. Two Keithley 2400 sourcemeters were employed for FET measurements. A small fluid chamber (1 mL) was placed over the FET device to control the liquid environment using PBS at pH 7. A liquid gate potential (−0.75 V to +0.75 V) with respect to the grounded drain electrode was applied using an Ag/AgCl (3 M KCl) reference electrode submerged in the gate electrolyte. The drain current of the device was measured at a constant source-drain voltage (50 mV). Transfer characteristics (conductance (G) *versus* gate voltage (V_g)) were measured.

Functionalization of the FET devices with each glycoconjugate was performed by incubating the chips in 20 μM of the glycoconjugates solution (in deionized water) for 2 hr followed by rinsing three times with double-distilled water. After testing the transfer characteristics, the chips were incubated for 40 min in different concentrations of lectin solutions that were prepared in PBS with 5 μM CaCl₂ and subsequently washed three times with PBS solution. For each glycoconjugate functionalized device, non-specific lectins were tested first and subsequently washed away. The specific lectin was then added, and the final transfer characteristics were tested again in the configuration mentioned above.

Modeling method

SWNT and graphene sheet were generated using Nanotube Modeler Software (JCrystalSoft, <http://jcrystal.com>). A semiconducting SWNT with length of 10 nm and diameter of 1.3 nm was built using the chiral indices $m=14$ and $n=4$ as described previously.⁴⁶ A graphene sheet with dimension 5×10 nm² was built using the chiral indices $m=20$ and $n=0$. Zinc tetraphenyl porphyrin (ZnTPP) was built starting from the structure of Zn-protoporphyrin IX complexed with cytochrome C (PDB code 1I54). Pyrene and porphyrin galactosylated conjugates were built using the molecular editor in Sybyl software (Tripos, Inc. St Louis), and structures were minimized with the Tripos force-field.⁶² Galactosylated porphyrin **2a** was manually docked on the SWNT and sheet followed by energy minimization, while keeping the carbon structures fixed. Galactose linkers were adapted in order to generate distances between pair of galactose residues of approximately 3 nm compatible with binding to PA-IL adjacent sites. Final docking was performed by fitting galactose residues in proper location or the binding sites as previously described.³⁷ A different procedure was used for the two galactosylated pyrene **1a** that were first docked in two adjacent protein binding sites, before stacking the pyrene rings on the SWNT or graphene, with final energy minimization of the linker moiety.

Isothermal titration microcalorimetry (ITC)

Recombinant lyophilized lectins were dissolved in buffer (0.1 M Tris-HCl, 6 μM CaCl₂, pH 7.5) and degassed (see the Supporting Information for concentration details). Protein concentration was checked by measurement of optical density. Ligands were dissolved directly into the same buffer, degassed, and placed in the injection syringe. ITC was performed with a VP-ITC microcalorimeter (MicroCal Inc.). Lectins were placed into the 1.4478 mL sample cell at 25°C. Each titration was performed with 10 μL injections of ligands every 300 s. Data were fitted with MicroCal Origin 7 software according to standard procedures. Two or three independent titrations were performed for each ligand tested.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(a) Optical image of wirebonded Si chip with multiple photolithography patterned Au/Ti electrodes. Inset shows scanning electron microscopy (SEM) image of one set of the interdigitated electrodes (left, SWNT-FET; right, CCG-FET). (b) Schematic illustration of the liquid-gated FET configuration (left, SWNT-FET; right, CCG-FET). (c, d) Nanoelectronic detection of carbohydrate-lectin interactions: Conductance (G) vs gate voltage (V_g) of (c) bare SWNT-FET and (d) bare CCG-FET device after functionalization with β -D-galactose pyrene-based glycoconjugates (**1a**) and after attachment with 2 μ M non-specific (control) lectin ConA and 2 μ M specific lectin PA-IL. (e) Comparison of the normalized responses ($V_g = -0.5$ V) to 2 μ M control lectin and to 2 μ M

specific lectin for biosensors: SWNT+**1a, 1b, 1c** or **2a, 2b, 2c**, CCG+**1a, 1b, 1c** or **2a, 2b, 2c**. All measurements were performed in electrolyte-gated FET configuration in PBS (pH 7), Ag/AgCl reference electrode, with source-drain voltage of 50 mV. Lectin binding experiments were performed in the presence of 5 μM Ca^{2+} .

Figure 2. Computer-aided models of (a) SWNT/**1a**/PA-IL interaction, (b) SWNT/**2a**/PA-IL interaction, (c) CCG/**1a**/PA-IL interaction, and (d) CCG/**2a**/PA-IL interaction.

Figure 3.

Atomic force microscope (AFM) images of (a) bare SWNTs, (b) SWNT functionalized with **1b**, (c) after incubation with PA-IL (control) and (d) after ConA attachment; (e) bare CCG, (f) CCG functionalized with **1b**, (g) after incubation with PA-IL (control) and (h) after ConA attachment. Lectin attachment was performed in the presence of 5 μM Ca^{2+}

Figure 4. Fluorescence image of α -D-mannose glycoconjugates functionalized SWNT-FET device incubated with fluorescently-labelled lectin ConA-Alexa488.

Figure 5. Biosensor calibration plot: normalized change in the device conductance ($V_g = -0.5 \text{ V}$) versus PA-IL concentration of (a) SWNT-FET and (c, e) CCG-FET (data from three devices). Langmuir isotherm of (b) SWNT-FET data presented in panel (a), (d) for CCG-FET with their corresponding calculated lectin dissociation constants (K_d). (f) Langmuir isotherm of CCG/1c and PA-III interaction, with calculated lectin dissociation constant K_d .

Figure 6.

Titration of (a) PA-IL (0.05 mM) by compound **1a** (1.7 mM) and (b) titration of PA-IIL (0.05 mM) by compound **1c** (0.5 mM). Top: control (injection of glycoconjugate in buffer), middle: titration of lectin, bottom: integration of peaks with a fitting curve obtained from a two site model.

Scheme 1.

Synthesis of pyrene-based glycoconjugates **1a**, **1b**, **1c** and general structure of porphyrin-based glycoconjugates **2a**, **2b**, **2c**¹⁸

Scheme 2.
Schematic illustration of lectin adsorption effects on an individual SWNT *versus* a single CCG flake.

Table 1

Glycoconjugates used in this study with their respective specific and control (non-specific) lectins

Glycoconjugate	Lectin	
	Specific	Control
1a or 2a (galactose)	PA-IL	ConA
1b or 2b (mannose)	ConA	PA-IL
1c or 2c (fucose)	PA-III	PA-IL

Table 2Comparison of K_d values obtained by FET and ITC methods

Glycoconjugate	Lectin	FET		ITC
		SWNT	CCG	
1a/2a	PA-IL	135 nM /147.7 nM	106 nM	100 μ M ^a /133 nM
1c	PA-III		20 nM	50 nM ^a

^a K_d value for the carbohydrate binding site