Biosensors

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Reversible Control of Carbon Nanotube Aggregation for a Glucose Affinity Sensor**

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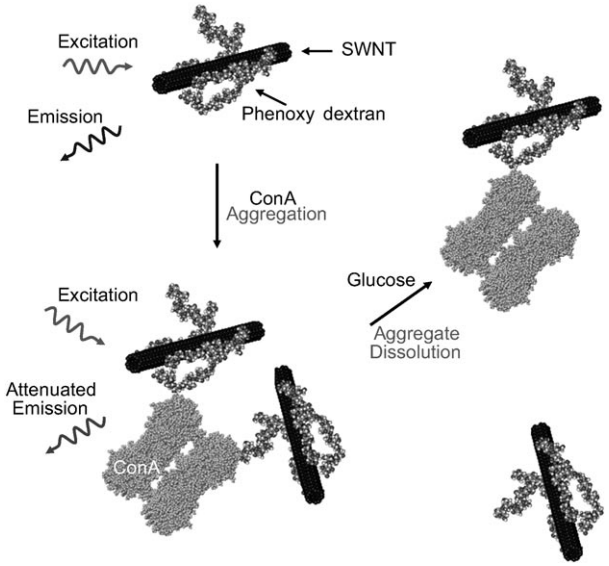
Single-walled carbon nanotubes (SWNT) have garnered much interest as sensing elements[1–6] owing to their quasi-one-dimensional electronic structure, which renders them sensitive to surface adsorption events.[7,8] The majority of studies have been based on SWNT field effect transistors;[4–6,9–11] however, recently we demonstrated that SWNT near-infrared (NIR) photoluminescence (PL) can be used in the selective detection of small molecules[1,2] and nucleic acids.[3] Although most conventional fluorophores fluoresce in the visible range where tissue and biological media emit a strong autofluorescent background[12] and are strongly absorbing[13,14] and scattering, SWNT-PL occurs in the NIR[15] where these complications are minimized. Additionally, SWNT remain photostable, unlike other NIR fluorophores.[16] A central challenge in the development of SWNT fluorescence sensors is designing a sensing scheme that retains the nanotube optical properties. Covalent functionalization of the nanotube disrupts the electronic structure and SWNT-PL;[17,18] thus, only noncovalent methods can be used. Herein we describe the design and function of the first SWNT based affinity sensor for glucose. Previously, nanotubes have been utilized in electronic biosensors[11] and glucose detection.[9,10]

A nanotube sensor was fabricated following the reaction path outlined in Scheme 1. Equilibrium binding between proteins and a target analyte has been used in a number of biosensors.[19–26] Generally, the sensors operate by taking advantage of the competitive binding between a target analyte and analogue for a protein binding site. The resulting signal change is from fluorescence resonance energy transfer between the analogue and a fluorophore-labeled protein. For our system, the SWNT fluorophores are initially suspended in aqueous solution with a phenoxy-derivatized dextran, a polysaccharide that functions as a glucose analogue. The addition of 8–15 μM Concanavalin A (ConA), a plant lectin with four saccharide binding sites at pH 7.4, induces aggregation of the dextran–nanotube complexes and a decrease in SWNT-PL. The introduction of 3–11 mM glucose causes aggregate dissolution and PL recovery owing to competitive binding between the glucose and dextran for ConA binding sites.



Scheme 1. Representation of SWNT affinity sensor operation.

Modification of the dextran with hydrophobic moieties is necessary to observe stable nanotube suspension. Dialysis of 1 wt% dextran (64–76 kDa, unmodified) and a sodium cholate suspended SWNT mixture against 10 mM phosphate-buffered saline (PBS) removes the cholate[1,3] but results in SWNT aggregation and PL quenching.[15] Hydrophobic dextran derivatives (**3**) were synthesized and used to functionalize the nanotubes (**4**, Scheme 2). The phenoxy content (wt%) in compound **3** affects the nanotube dispersability and optical properties. Figure 1a shows the PL spectra of **4** synthesized with a phenoxy content of **3** ranging from 3.5 to 8.3 wt%. The normalized PL intensity increases with

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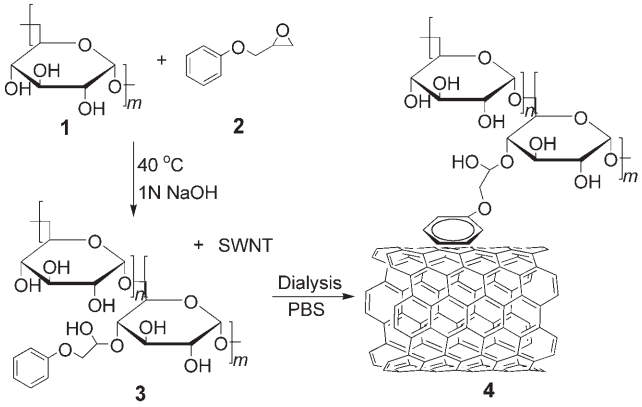

Scheme 2. Reaction of dextran (**1**) with 1,2-epoxy-3-phenoxypropane (**2**) to make hydrophobic dextran derivatives (**3**) that are used to noncovalently functionalize SWNT making a dextran–SWNT complex (**4**).

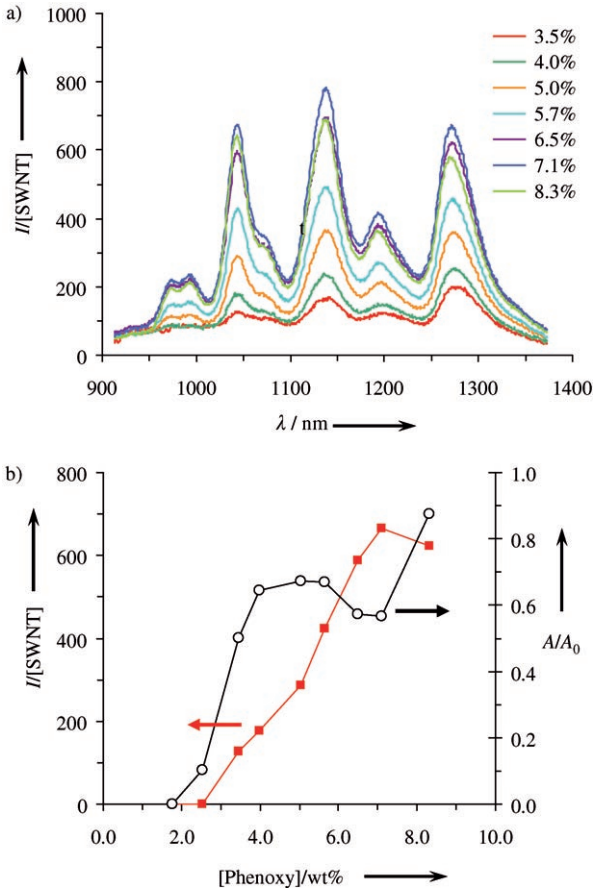

Figure 1. Effect of the amount of phenoxy (wt%) in (**3**) on SWNT suspension and colloidal stability. a) PL spectra normalized to nanotube concentration for complex **4** for varying phenoxy content (wt%) in **3**. b) The relationship between nanotube PL and nanotube concentration (I/[SWNT]; squares) and the ratio of suspended SWNT concentration to initial SWNT concentration (measured by absorption, A/A_0) before dextran adsorption (circles) versus phenoxy content (wt%) in **3**.

phenoxy content of **3** (Figure 1b), with the fraction of nanotubes in solution remaining constant above a phenoxy content of 3.5 wt%. Examination of SWNT photoabsorption (see Supporting Information) shows that nanotube absorption transitions are broadened and red-shifted as the phenoxy content decreases. This is due to either an increase in the number of small bundles or to the localization of water near the surface of the nanotube.[27] For all sensing experiments, **4** synthesized with a phenoxy content of 8.5 wt% was used.

Nanotube PL decreases with increasing ConA concentration concomitant with aggregate formation. The transient PL intensity of an aliquot of **4** during additions of 1.8 μM ConA followed by 3.7 mM additions of glucose is shown in Figure 2a. The first three ConA additions caused no PL

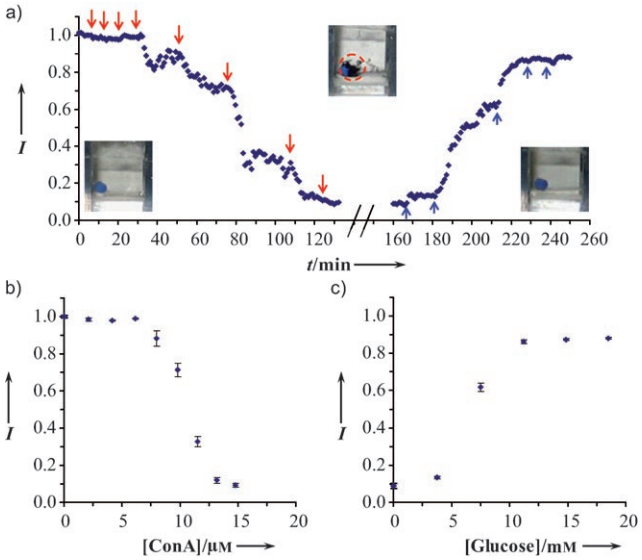

Figure 2. Response of **4** to ConA and glucose. a) Nanotube PL intensity versus time for 1.8 μM additions of ConA (red arrows) and 3.7 mM additions of glucose (blue arrows). Insets show pictures of the aggregation state of the solution initially (left), after 14.7 mM ConA (middle), and upon subsequent addition of 18 mM glucose (right). The steady-state nanotube PL intensity is plotted versus ConA concentration (b) and glucose concentration (c).

response and small aggregates began to become visible, whereas subsequent additions showed a decrease in SWNT-PL. The PL response times ranged from 12 to 20 minutes to reach steady state. The insets of Figure 2a show pictures before ConA addition (left), with no visible aggregation, and after the addition of 14.7 μM ConA (middle), when aggregation was evident. Subsequent additions of glucose result in dissolution of the aggregates (right) and recovery of SWNT-PL (Figure 2a), with the recovery response times ranging from 3 to 28 min to reach steady state. The steady-state PL response versus ConA concentration and glucose concentration is given in Figure 2b,c. We expect there is a portion of **3** free in solution and not adsorbed to the nanotubes. This source of unbound **3** competes for binding with ConA, and thus delays the onset of critical aggregation, when measurable decrease in SWNT-PL occurs. Removal of free phenoxy-derivatized dextran (**3**) causes the SWNT-PL to decrease at lower ConA concentrations. The steady-state PL recovery versus glucose concentration in Figure 2b shows the largest

increase, between 3.8 and 11 mM glucose. A commercially viable glucose sensor will be responsive to glucose concentrations between 2 and 30 mM.[28] The response range is ConA concentration dependent and can be tuned based on ConA concentration.[29] We note that additions of glycerol, a hydrogen-bond-breaking agent, cause no response in the Dextran–SWNT–ConA aggregate, indicating specificity of the system to glucose.

Dynamic light scattering suggests that **4** remains unchanged following aggregate dissolution. Scattering measurements were carried out on **4** before aggregation and after aggregate dissolution. Calculated diffusion coefficients for **4** are 6.96 $\mu m^2 s^{-1}$ and 5.74 $\mu m^2 s^{-1}$ before ConA addition and after aggregate dissolution, respectively. Comparison of diffusion coefficients for **4** before aggregation and after recovery with glucose shows the two values to be within 1 $\mu m^2 s^{-1}$, suggesting that **4** is not permanently altered during the aggregation/disaggregation process. The fact that the diffusion coefficient is slightly lower after recovery is not unexpected as ConA is still in the solution, of which some fraction is likely bound to **4**.

Removal of glucose, through dialysis, results in reformation of aggregates. Glucose was removed from the recovered sample using dialysis. Aggregates were visibly formed, although not as dense as those shown in Figure 2a. Additions of glucose resulted in aggregate dissolution; however, the sensor did not respond to subsequent cycles of glucose removal and addition. We speculate that ConA becomes denatured during the process resulting in a limited lifetime of the sensor, but that is beyond the scope of this Communication and protein stability will be addressed in a future study.

In conclusion, we have shown that it is possible to fabricate an affinity sensor based on SWNT-PL. We formed colloidally stable suspensions of dextran-coated SWNT by first covalently adding phenyl groups to dextran. Addition of ConA induces nanotube aggregation and decreases SWNT-PL, whereas subsequent additions of glucose break apart the aggregates, resulting in a restoration of SWNT-PL. These results demonstrate the first solution-phase nanotube affinity sensor. The methodology outlined herein is generic and can conceivably be extended to other analytes of interest.

Experimental Section

Materials: Single-walled carbon nanotubes were obtained from the Rice University research reactor (Run 107). ConA, BSA, 64–76 kD MW dextran, 1,2-epoxy-3-phenoxypropane, and glucose were obtained from Sigma Aldrich (St. Louis). Hydrophobic dextran derivatives were synthesized similar to previously published methods.[30] Phenoxy content was determined by absorption at 269 nm[31] in a Shimadzu UV-PC 3101 UV/Vis/NIR spectrophotometer. SWNTs were initially dispersed in an aqueous 2 wt % sodium cholate solution using previously published methods,[1,15] and hydrophobic dextran was adsorbed to the nanotube surface through dialysis.[1,3] All collected samples were centrifuged (16000 × g) for 30 min to remove unsuspended nanotubes.

Detection: Nanotube aggregation and PL monitoring were performed in a well-stirred cuvette. The solution was illuminated with the dispersed beam of a 632.8 nm HeNe laser. SWNT-PL was collected at 90° with a Princeton Array Spectrometer. Initially, 10 mg mL^{-1} BSA (50 μL) in PBS was added to prevent ConA adsorption to the walls of the cuvette.

Dynamic light scattering: A goniometer (Brookhaven Instruments BI-200 SM) with a 514 nm laser (Lexel Argon-Ion Model 95) was used with the detector at a 90° angle from the light source to the sample.

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