Electrostatic Assemblies of Single-Walled Carbon Nanotubes and Sequence-Tunable Peptoid Polymers Detect a Lectin Protein and Its Target Sugars

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ABSTRACT: A primary limitation to real-time imaging of metabolites and proteins has been the selective detection of biomolecules that have no naturally occurring or stable molecular recognition counterparts. We present developments in the design of synthetic near-infrared fluorescent nanosensors based on the fluorescence modulation of single-walled carbon nanotubes (SWNTs) with select sequences of surface-adsorbed N-substituted glycine peptoid polymers. We assess the stability of the peptoid-SWNT nanosensor candidates under variable ionic strengths, protease exposure, and cell culture media conditions and find that the stability of peptoid-SWNTs depends on the composition and length of the peptoid polymer. From our library, we identify a peptoid-SWNT assembly that can detect lectin protein wheat germ agglutinin (WGA) with a sensitivity comparable to the concentration of serum proteins. To demonstrate the retention of nanosensor-bound protein activity, we show that WGA on the nanosensor produces an additional fluorescent signal modulation upon exposure to the lectin’s target sugars, suggesting the lectin protein remains active and selectively binds its target sugars through ternary molecular recognition interactions relayed to the nanosensor. Our results inform design considerations for developing synthetic molecular recognition elements by assembling peptoid polymers on SWNTs and also demonstrate these assemblies can serve as optical nanosensors for lectin proteins and their target sugars. Together, these data suggest certain peptoid sequences can be assembled with SWNTs to serve as versatile optical probes to detect proteins and their molecular substrates.

KEYWORDS: Single-walled carbon nanotubes, peptoids, nanosensors, protein detection

Single-walled carbon nanotubes (SWNTs) have emerged as promising signal transduction elements for molecular imaging owing to their relatively tissue-transparent optical properties, photostability, low toxicity when functionalized, and nanometer size.1−3 SWNTs exist in many distinct chiralities described by the chiral vector of the carbon lattice which dictates the SWNT fluorescence excitation and emission wavelengths, making SWNTs suitable for ratiometric detection.4,5 SWNTs notably fluoresce through exciton recombination in the near-infrared (NIR), a region of the electromagnetic spectrum following visible wavelength photon scattering by biological tissues, but prior to absorption of photons by water.6−7 This NIR window exhibits minimal photon attenuation, suitable for deep tissue bioimaging applications.6 Frustrated SWNTs exhibit a hydrophobic π-conjugated surface lattice and require polymer or surfactant encapsulation both for colloidal stability and for radiative exciton recombination-based NIR fluorescence. This NIR fluorescence is sensitive to the SWNT local dielectric environment and charge transfer.2,9 Several mechanisms have been proposed for the fluorescence modulation of SWNTs including Fermi level shifting through redox-active analyte adsorption, exciton disruption in response to analyte binding, solvatochromic shifting due to perturbation of the polymer wrapping, and analyte-activated polymer switching.10−11

The encapsulation of SWNTs with biomolecules and biopolymers can therefore serve a dual purpose of conferring biocompatibility, but can also enable molecular recognition for biological analytes of interest. In particular, SWNTs can be functionalized to be selective and sensitive optical nanosensors via surface adsorption by polymers with a method known as corona phase molecular recognition (CoPhMoRe).11 In this technique, polymers are electrostatically pinned to the surface of SWNT and adopt a specific conformation that can bind and optically recognize a small molecule via selective modulation of...
the SWNT exciton recombination rate (intensity change) or band gap (wavelength shift). To date, polymer-SWNT nanosensors have been created with DNA oligomers, peptides, and fluorescein-, rhodamine-, or carbodiimide-derived polymers as the nanosensor corona phase. These polymer-SWNT nanosensors have been successful in the detection of DNA hybridization, neurotransmitters, vitamins, cellular metabolites, chemotherapeutics, and proteins. However, detection of proteins remains a challenging task, owing to the complexity of these larger analytes. Several methods exist in which recognition is conferred through a His-tagged protein tethered to polymer-SWNT through biocconjuction. These methods rely on the naturally occurring molecular recognition of capture proteins and antibodies involved in protein detection. Conversely, SWNT CoPhMoRe is an attractive synthetic platform that does not rely on biologically derived molecular recognition elements for optical detection of protein analytes, with Rap1, HIV-1, fibrinogen, and insulin protein SWNT-based CoPhMoRe nanosensors developed recently.

Of the polymers leveraged for SWNT nanosensor design, biopolymers such as polynucleotides and peptides are often preferred owing to sequence tunability with which they can be synthesized or produced by bio-organisms. However, polynucleotides and peptides are susceptible to enzymatic degradation by nucleases or proteases, respectively, and as such, their conditional stability limits long-term use in complex biological systems. Conversely, synthetic polymers may require lengthy synthesis and are difficult to control in terms of sequence, length dispersity, and structural tunability. Furthermore, both biopolymers and synthetic polymers are limited by a lack of monomer sets for creating chemical diversity. Therefore, future advancements in the area of synthetic protein nanosensors will require generation of biomimetic polymers amenable to facile synthesis, with a large monomer space, which are also resistant to enzymatic degradation. To this end, herein we synthesize a library of peptoids, N-substituted glycine polymers, to serve as molecular recognition elements for proteins when adsorbed on SWNT surfaces. Peptoids are sequence-defined synthetic polymers that draw inspiration from peptides, the building blocks of proteins, with synthesis that is amenable to robotic automation and a large monomer space of primary amines. Peptoids are created through stepwise solid-phase submonomer synthesis with high sequence specificity and can include a wide variety of nonproteinogenic chemical functionalities such as alkynes, glycosylation, and fluorophores. Peptoids are resistant to proteases and can remain stable in the body for day-long time periods. Peptoids have been shown to self-assemble into supramolecular nanosheets that are capable of specific multivalent interactions with enzymes and proteins such as kinases, lectins, and Shiga toxins. Because peptoids are designable and tunable proteomimetic materials, they are well suited to address the current need of chemical diversity of SWNT polymer coronas.

We present the development of peptoid polymer-SWNT (peptoid-SWNT) assemblies and their characterization for implementation as protein nanosensors. We first investigate the primary interactions of the peptoid polymer with SWNT and present our findings on the stability of these peptoid-SWNT assemblies upon exposure to long-term laser illumination, variable solution ionic strength conditions, complex biological media, and proteases. We next show that certain peptoid-SWNT assemblies can serve as nanosensors through secondary interactions to enable moderately selective and sensitive WGA lectin protein detection. We further show that peptoid-SWNT nanosensors have the capacity for ternary analyte interaction and detection, where the WGA protein tethered to the nanosensor can in turn detect WGA’s target sugars. Our work informs key design parameters for the noncovalent adsorption of peptoids with SWNT and their development as molecular recognition elements for protein detection and ternary analyte interactions.

**Peptoid Polymer Design for Surface Adsorption to SWNT.** Peptoids are created with an automated submonomer approach that provides ease of synthesis and control over polymer length and sequence (Scheme 1). The chemical sequence of the peptoid is dictated by the selective order in which variable side chain groups are added to the growing peptoid chain via primary amines.

**Scheme 1. Peptoid Synthesis with the Solid-Phase Two-Step Submonomer Method.**

![Scheme 1](image)

To gauge the possibility of creating peptoid-SWNT assemblies for use as protein nanosensors, we first elucidated the peptoid design parameters necessary for rendering colloidal stable peptoid-SWNT assemblies. We synthesized a small library of 11 amphiphilic peptoid sequences, outlined in Table 1 (see Figure S1 for full chemical structures), of variable length to form peptoid-SWNT assemblies, while other peptoids do not suspend SWNT at pH 7.

<table>
<thead>
<tr>
<th>Peptoid Name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block36</td>
<td>(Naa-Npe)-(Nce-Npe)</td>
</tr>
<tr>
<td>DB1</td>
<td>(Naa-Nia)-(Nce-Nia)</td>
</tr>
<tr>
<td>DB2</td>
<td>(Naa-Nhis)-(Nce-Nhis)</td>
</tr>
<tr>
<td>Pep1</td>
<td>(Nta-Nta)</td>
</tr>
<tr>
<td>Pep2*</td>
<td>(Nta-Nta)</td>
</tr>
<tr>
<td>Pep3</td>
<td>(Nta-Nta)</td>
</tr>
<tr>
<td>PA28</td>
<td>(Naa-Nta)</td>
</tr>
<tr>
<td>PC18</td>
<td>(Naa-Nta)</td>
</tr>
<tr>
<td>PC28*</td>
<td>(Naa-Nta)</td>
</tr>
<tr>
<td>PC30*</td>
<td>(Naa-Nta)</td>
</tr>
<tr>
<td>ProLoop*</td>
<td>PC18-(Nbu-Nbu-Npp-Npp-Nbu-Npp)-PC18</td>
</tr>
</tbody>
</table>

*Peptoids denoted with an asterisk (*) successfully adsorb on SWNT to form peptoid-SWNT assemblies, while other peptoids do not suspend SWNT at pH 7.*
N-(2-aminoethyl)glycine (Nae); and negatively charged: N-(2-carboxyethyl)glycine (Nce) (Scheme 2). We included several di-block peptoids similar to previous antibody-mimetic nanosheet forming peptoids such as Block36 (Nae-Npe)₉-(Nce-Npe)₉, DB1 (Nae-Nia)₉-(Nce-Nia)₉, and DB2 (Nae-Nph)₉-(Nce-Nph)₉. We synthesized several polar but uncharged peptoid polymers such as Pep1 (Nte-Npe)₁₄, Pep3 (Nme-Npe)₁₄, as well as a hybrid charged and uncharged polar polymer Pep2 (Nte-Npe-Nce-Npe)₁₂. We included a positively charged polymer, PA28 (Nae-Npe)₁₆, and negatively charged polymers of alternating Npe and Nce (PC) monomers with final polymer lengths of 18, 28, and 36 (PC18, PC28, and PC36, respectively), and an anchor-loop peptoid consisting of a (Nce-Npe)₉ “anchor” peptoid sequence that can adsorb to the carbon nanotube flanking a synthetic 6-monomer loop consisting of 3 N-butylglycine (Nbu) and 3 N-(N′-pyrolidinonylpropyl)glycine (Npp) monomers ((Nce-Npe)₉-Nbu-Nbu-Npp-Npp-Nbu-Npe-(Nce-Npe)₉, and further abbreviated as ProLoop). The loop segment within the ProLoop peptoid interacts semi-selectively with wheat germ agglutinin (WGA), a 36 kDa lectin, a sugar-binding protein, as assessed with a FRET binding assay (see Figure S2 for peptoid loop selectivity and sensitivity FRET screen results). WGA was chosen as a target protein analyte since it is a commercially procurable lectin protein that does not require special handling, making it ideal for proof-of-principle experiments. Lectins have an important biological function binding to glycans that act as molecular beacons for viral and bacterial infection of cells in several diseases and as biomarkers on cancer cells. Furthermore, WGA was previously shown to bind to glycosylated loops on peptoid nanosheet self-assembly and therefore serves as a good system for experimental validation of peptoid-SWNT-based recognition of protein targets.

The peptoid library was evaluated for each peptoid’s ability to adsorb to SWNTs. Peptoid-SWNT adsorption was attempted with solution-phase probe-tip sonication of peptoids and SWNT (see Materials and Methods in the Supporting Information), and adsorption efficacy was confirmed with UV–vis-NIR absorbance spectroscopy and NIR fluorescence spectroscopy (Figure 1, Figure S3). UV–vis-NIR absorbance was used to measure the sample optical density, from which the yield of colloidally stable peptoid-SWNT assemblies can be calculated (see Materials and Methods in the Supporting Information). NIR fluorescence spectroscopy further confirms peptoid adsorption as only stable peptoid-SWNT assemblies exhibit NIR fluorescence. Probe-tip sonication was performed in 50 mM borate buffer (pH 9.2), and peptoid-SWNT assemblies were diluted in phosphate buffered saline (PBS, pH 7.4) prior to spectroscopy and fluorescence measurements, to represent physiological conditions in preparation for downstream biological applications in protein detection. Four of the 11 peptoids stably adsorb onto SWNTs and remained stable at physiological pH, with successful peptoid-SWNT assemblies labeled with an asterisk (*) in Table 1. Adsorption efficiency was compared by measuring the UV–vis-IR absorbance spectrum of the assembly, where a higher absorbance is indicative of a higher peptoid-SWNT yield (Figure S3). We found that peptoid polymer length affects the effectiveness of peptoid-SWNT adsorption. To assess the effect of peptoid length on peptoid-SWNT stability, we compared peptoids comprised of the same carboxyethyl-phenethyl repeat with lengths of 18, 28, and 36 monomers. We found the

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**Figure 1.** Platform for peptoid-SWNT assembly. (a) No NIR fluorescence is observed prior to adsorption of peptoids to SWNT surfaces. (b) Ultrasonication promotes the formation of peptoid-SWNT assemblies with a distinct NIR fluorescence spectrum that is (c) modulated by the addition of a target protein (blue) that binds to the protein-recognition loop (red). Schematics not to scale.

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**Scheme 2. Monomers Used To Create the Peptoid Polymers in This Study**

**Monomer Sidechains**

- **Nonpolar**: Npe, Nph, Nia, Nbu, Npp, Nme
- **Polar**: Nte, Nce, Nia, Nph, Nme
- **(+)-charge**: Nae
- **(-)-charge**: Nph
- **(-)-charge**: Nce

“Peptoid polymers can be created using a combination of monomers with different polarities and charge.
peptoid-SWNT adsorption efficiency is roughly proportional to peptoid length: PC36 (yield = 165.1 mg/L) ≈ PC28 (194.9 mg/L) > PC18 (92.2 mg/L). We posit that longer peptoid polymers adsorb more strongly to SWNTs owing to the increased number of contacts made between a longer peptoid polymer and the surface of the SWNT. We also found that peptoid hydrophilicity is an important contributor to peptoid-SWNT adsorption efficiency. Pep1, Pep2, Pep3, and PC28 peptoids are of the same 28 monomer length, with alternating monomers of aromatic phenyl and hydrophilic monomers of either triethyloxy, monoethoxy, or carboxyethyl. We show that the efficiency of peptoid adsorption to SWNT follows the trend of PC28 > Pep2 > Pep1 ≈ Pep3, with Pep1 and Pep3 unable to suspend SWNT, and follows the trend of peptoid hydrophilicity as observed by HPLC (Figure S4). Lastly, we note that peptoid charge also facilitates peptoid-SWNT colloidal stabilization: Both the peptoid backbone and the SWNT are nonpolar and hydrophobic and require hydrophilicity by charge or polarity to create stable peptoid-SWNT assemblies in aqueous buffer. Of the charged 28-mer peptoids, the highly negatively charged PC28 best adsorbed to SWNT with a yield of 194.9 mg/L, whereas the less charged Pep2 adsorbed least with a yield of 19.8 mg/L and the neutrally charged Pep1 and Pep3 failed to adsorb onto SWNT. PA28, with positively charged monomers, showed low adsorption to SWNT putatively due to the difficulty of protonation of the polymer’s primary amines.38 We tested this hypothesis by attempting to suspend SWNT with PA28 at low pH conditions and found we could only maintain stable peptoid-SWNT assemblies at very acidic conditions (100 mM acetate buffer pH < 5), a regime not suitable for biological applications. Therefore, we find that certain charged monomers can confer colloidal stabilization of SWNT.

Several peptoids did not adsorb to SWNT. Peptoids lacking aromatic hydrophilic residues, such as DB1, did not suspend SWNT presumably due to a lack of π−π interactions. Conversely, peptoids with aromatic groups such as Block36 have previously been shown to self-assemble into supra-molecular nanosheets driven by zwitterionic stabilizing interactions of the charged groups, hydrophobic interactions, and π−π stabilization between the aromatic rings of the peptoid.34 Thus, when Block36 is adsorbed to SWNTs, we propose that peptoid zwitterionic and hydrophobically driven self-interactions dominate over peptoid-SWNT interactions, resulting in unstable SWNT assemblies in favor of spontaneous formation of peptoid nanosheets as confirmed by AFM (Figure S5). DB2 also did not adsorb to SWNT, presumably due to the larger steric hindrance of phenyl monomers compared to the 2-phenylethyl monomers.

From our initial screen, we identified repeats of (Nce-Npe) as highly stable peptoid sequences for SWNT stabilization at physiological pH and utilized this Nce-Npe repeat as an “anchor” for peptoid adsorption to SWNT. Next, from FRET binding assays between WGA and libraries of peptoid sequences, we identified a 6-monomer peptoid segment that demonstrated high binding affinity for lecin protein WGA (Figure S2). We inserted this WGA-binding segment between Nce-Npe anchor repeats to form the ProLoop peptide (Table 1). We also identified ProLoop as a stable peptoid for SWNT adsorption with a moderate yield of 103.1 mg/L. Thus, the ProLoop-SWNT assembly and the PC36-SWNT assembly with equal numbers of (Nce-Npe) monomers as ProLoop were selected for downstream stability characterization and for use as fluorescent WGA protein nanosensors.

Peptoid-SWNT Nanosensor Fluorescence Stability. To validate peptoid-SWNT assemblies as fluorescent protein nanosensors, we next demonstrated the stability of peptoid-SWNT assemblies in a range of conditions suitable for bioimaging applications. We first examined the effect of salt and buffer conditions, as such parameters have been shown to affect the stability of SWNT assemblies.39–41 We also examined the stability of the SWNT assemblies to continuous laser exposure and protease activity.

To test salt stability, peptoid-SWNT assemblies were incubated overnight in sodium chloride (NaCl) solutions ranging in concentration from 1 mM to 500 mM, and peptoid-SWNT fluorescence was subsequently measured (Figure 2a). PC28 and PC36 peptoid-SWNT assemblies remained colloidal stable and exhibited NIR fluorescence under a 1 mM to 500 mM NaCl range of ionic strengths. NaCl concentrations higher than 500 mM destabilized the peptoid-SWNT assemblies and induced SWNT aggregation, leading to a loss of SWNT fluorescence. Pep2-SWNT assemblies were unstable in NaCl solutions with concentrations higher than 10 mM and formed visible aggregates at higher ionic strength (Figure S6). Recent work demonstrates that DNA-SWNT fluorescence is proportional to ionic strength,40 thus we compared fluorescence of peptoid-SWNT assemblies under the above-mentioned salt concentrations. In concurrence with prior results, peptoid-SWNT assemblies show high NIR

**Figure 2.** Peptoid-SWNT stability. (a) PC36-SWNT fluorescence increases with increasing ionic strength, suggesting (b) peptoid surface coverage of SWNT increases with ionic strength. (c) Fluorescence of peptoid-SWNT assemblies under continuous laser irradiation shows ProLoop-SWNT is the most stable assembly in this study. (d) ProLoop-SWNT exposure to trypsin or proteinase K (Pro K) for 24 h reveals no loss of fluorescence. Error bars denote standard deviation (n = 6).
fluorescence at higher ionic strengths, presumably due to a tighter association between the peptoid and the SWNT. Conversely, at lower ionic strengths, peptoids are putatively more loosely associated with the surface of the SWNT, resulting in a lower SWNT fluorescence (Figure 2b). Additionally, we tested the effect of salt composition on the fluorescence of peptoid-SWNT assemblies. Recently, divalent salts have been shown to induce a wavelength shift in the fluorescence spectra of DNA-SWNT assemblies. In these studies, it is hypothesized that solvatochromic shifts are due to induced conformational changes in the DNA backbone along the SWNT and correlated with the stiffness of the polymer backbone. We observed significant wavelength shifts in the fluorescence spectra of PC28 and PC36 peptoid-SWNT assemblies upon addition of CaCl₂ salt and minor shifts in the fluorescence spectra of ProLoop and Pep2 peptoid-SWNT assemblies (Figure S7), suggesting that multiple factors, of which salts may be but one, affect peptoid flexibility and thus binding stability on SWNT.

We next subjected each peptoid-SWNT assembly to an hour-long imaging experiment to probe peptoid-SWNT stability to continuous laser exposure. Excitation of peptoid-SWNT samples by a 77 mW 721 nm laser for SWNT spectroscopy stabilizes to a temperature of 37°C in the sample well within 1 min of laser irradiation (Figure S8). Peptoid-SWNT assemblies did not show wavelength shifts during the hour-long spectral measurement with largely chirality-independent changes in fluorescence (Figure S9). Our results showed that the ProLoop-SWNT construct exhibits the most stable fluorescence with a negligible 2.1 ± 6.6% (mean ± standard deviation (SD)) increase in fluorescence after 1 h of laser illumination (Figure 2c). In contrast, PC28 and PC36 both showed moderate fluorescence perturbations of -12.2 ± 8.9% (mean ± SD) and -36.8 ± 17.8% (mean ± SD) decrease in fluorescence, respectively, while Pep2-SWNT showed the largest fluorescence perturbation with a -69.7 ± 27.1% (mean ± SD) decrease in fluorescence. We note that peptoid-SWNT stability trends observed herein follow trends in peptoid-SWNT adsorption efficiency, although the ProLoop-SWNT exhibited moderate adsorption efficiency and the highest stability. The superior fluorescence stability and the incorporation of a WGA molecular recognition loop sequence led us to choose the ProLoop-SWNT assembly as an exploratory candidate for use as an optical nanosensor to detect WGA protein. Additionally, we tested the stability of the ProLoop-SWNT nanosensor against two proteases: trypsin and proteinase K. These proteases were chosen for their ability to digest a wide range of peptide sequences, specifically positively charged, aromatic and aliphatic amino acids, which mimics the function of the major proteases in the human body. We found that a 24 h incubation of peptoid-SWNT in proteases yielded a minimal loss of fluorescence or absorbance and hence no significant peptoid proteolysis (Figure 2d, Figures S10 and S11).

**ProLoop-SWNT Nanosensors Exhibit Fluorescence Modulation in Response to Wheat Germ Agglutinin Protein.** The ProLoop-SWNT assembly was assessed for use as a fluorescent nanosensor for WGA protein, a sugar-binding lectin, via binding of WGA to the ProLoop peptoid sequence (Figure S2). ProLoop-SWNT nanosensors were diluted to a concentration of 5 mg/L, and the baseline fluorescence (I₀) was measured. WGA was added to the nanosensor, and subsequent fluorescence (I) was measured at consecutive time points. We found that the ProLoop-SWNT nanosensor exhibited a decrease in fluorescence upon the introduction of WGA. The fluorescent response was time dependent and
reached equilibrium within 60 min following addition of 10 μM WGA (Figure 3a). We next characterized the ProLoop-SWNT nanosensor sensitivity to WGA through a concentration screen of 0.1 μM to 14.9 μM WGA. We measured the ProLoop-SWNT nanosensor change in fluorescence 60 min following the protein addition. Several models for SWNT nanosensor responses have been developed that take into account exciton diffusion or molecular interactions. Given that our data (AFM, FRET, absorption) are largely based on molecular binding affinity between WGA and the ProLoop-SWNT construct, we modeled our data using an equilibrium binding curve with Langmuirian adsorption. Assuming a single SWNT construct, we modeled our data using an equilibrium molecular binding affinity,

\[ \frac{I - I_0}{I_0} = \frac{a(K[WGA])^n}{(K[WGA])^n + 1} + b \]  

(1)

In eq 1, I denotes the ProLoop-SWNT nanosensor intensity after 60 min, I₀ denotes the initial nanosensor intensity at 0 min, [WGA] is the concentration of WGA in the system, K is the equilibrium binding constant, and a and b denote scaling factors (full model derived in Figure S12). Since WGA is composed of two symmetric monomeric units, we assumed the Hill coefficient (n), which denotes the cooperativity of the analyte bound, to be 2 for the fit to converge (goodness of fit of the model is discussed in Figure S12). Using nonlinear least-squares fitting, the model parameters were found to be a = −14.47, K = 0.192 μM⁻¹, b = 0.009, and R² = 0.83. With this equilibrium model of WGA binding to ProLoop-SWNT, we calculated the limit of detection for WGA binding to ProLoop-SWNT as 3.4 μM (Figure S12), a concentration comparable to the average serum concentration of common blood proteins such as albumin (600 μM), IgG (100 μM), and fibrinogen (7.5 μM).[40] The ProLoop-SWNT nanosensor response was confirmed to be in response to WGA protein, rather than protein contaminants, and also confirmed by atomic force microscopy showing WGA proteins attached to the surface of the nanosensor (Figures S13 and S14).

We next assessed the selectivity of the ProLoop-SWNT nanosensor by measuring the fluorescence response of ProLoop-SWNT upon exposure to a small library of other protein targets of various sizes and isoelectric points (Table S1), including two other lectins: protein A, peanut agglutinin (PNA), concanavalin A, bovine serum albumin (BSA), NeutrAvidin, and lysozyme (Figure 3c). PNA is a lectin that binds selectively to galactose, and concanavalin A (ConA) is a lectin that binds selectively to mannose. Both BSA and NeutrAvidin are proteins known to bind the surface of SWNT, whereas protein A is a negatively charged protein at physiological pH, and lysozyme is a positively charged protein at physiological pH.[44,47] This modest protein library afforded a screen for ProLoop-SWNT selectivity toward WGA. We found that ProLoop-SWNT nanosensors show the highest magnitude in fluorescence decrease of −34.5 ± 13.9% (mean ± SD) upon addition of 10 μM WGA, with minor fluorescence modulation upon addition of 5 mg/mL of protein A, 10 mg/mL of BSA, 5 mg/mL of NeutrAvidin, or 5 mg/mL of lysozyme (2.0 ± 8.2%, −10.3 ± 12.5, −13.2 ± 5.2, and −17.3 ± 6.4% (mean ± SD), respectively). Conversely, ProLoop-SWNT showed an increase in fluorescence upon the addition of 10 mg/mL of PNA (22.3 ± 3.9, mean ± SD) and 10 mg/mL of ConA (33.9 ± 1.1, mean ± SD). Interestingly, fluorescence changes upon addition of PNA and ConA were both near immediate and also largest in magnitude for the least stable peptoid-SWNT assembly, Pep2-SWNT. These results suggest ProLoop-SWNT interactions with these two lectins are through a different and possibly nonspecific mechanism than the one described for WGA. PC28-SWNT and Pep2-SWNT assemblies also showed a decrease in fluorescence response upon addition of 10 μM WGA (~27.9 ± 18.1% and ~36.3 ± 10.0% (mean ± SD), respectively). However, addition of proteins, buffer, or nothing to PC28-SWNT and Pep2-SWNT also induced monotonic decreases in fluorescence, indicating that fluorescence modulation of PC28 and Pep2 constructs derives primarily from their lower colloidal stability. Furthermore, the magnitude of response of PC28-SWNT correlates with the protein isoelectric point, suggesting the negative monomers of the PC28 anchor interact more strongly with proteins that have higher isoelectric points (Neutravidin, WGA, and lysozyme). Therefore, ProLoop-SWNT with its 6-monomer loop exhibited the highest selectivity toward WGA protein, concurrent with our FRET peptoid-WGA binding data (Figure S2).

Probing the Interaction Between Peptoid-SWNTs and WGA. To further understand the interactions of WGA with peptoid-SWNT assemblies, we studied the fluorescence change of the assemblies upon surface perturbation by surfactant. Previous studies have shown that surfactants including sodium dodecyl sulfate, sodium cholate (SC), and sodium dodecylbenzenesulfonate can bind to the exposed SWNT surface of polymer-SWNTs and exclude water from the SWNT surface.[17] This water exclusion causes a change in the dielectric environment of the nanosensors and induces a solvatochromatic shift that can be implemented to study the accessibility of the SWNT surface in a polymer-SWNT construct.

Addition of 0.5% (w/v) SC to ProLoop-SWNT nanosensors induced a solvatochromatic blue shift in fluorescence for most nanotube chiralities, with the peaks at shorter fluorescence emission wavelengths showing the largest peak wavelength perturbation (Figure 3d) with peak wavelength shifts ranging from 0.97 to 11.76 nm. This wavelength shift was also exhibited by the other peptoid-SWNT assemblies and can be characterized based on SWNT chirality (Figure S15). Notably, Pep2-SWNT was only minimally perturbed by SC addition, showing on average a <1 nm shift after the addition of SC (Figure S15). This is likely due to the triethyl ether side chains in Pep2 that resemble polyethylene glycol (PEG) and may limit accessibility of SC to the SWNT surface. PEG is often used as an antifouling and biocompatibility coating on biological device and nanoparticle surfaces, and antifouling peptides have previously been synthesized to recreate this property of PEG or other antifouling polymers.[48] Perhaps similarly, the triethyl ether monomers of the peptoid polymer may create an antifouling polymer brush along the surface of the Pep2-SWNT assembly. Thus, in future implementations of peptoid-SWNT nanosensors, antifouling properties may be engineered into the construct with the addition of PEG-like monomers to the peptoid.

Interestingly, addition of WGA to the ProLoop-SWNT assemblies prior to the addition of 0.5% SC eliminates the solvatochromatic shift, suggesting WGA stabilizes the ProLoop-SWNT assemblies against surfactant perturbation (Figure 3e).

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Prior work has confirmed that this stabilization effect represents a strong and selective binding interaction between the molecular analyte and the polymer-SWNT assembly. To confirm we may attribute the additional stability provided to ProLoop-SWNT assemblies by selective binding of WGA, we showed that WGA by itself does not associate strongly to SWNT and that attempted assembly of WGA with SWNT produced a highly unstable complex (Figure S16). These results suggest that the peptoid-SWNT assembly is necessary to stably bind WGA, and this binding of the target analyte further promotes surface stability of the nanosensor-protein complex.

**Peptoid-SWNT Stability in Biological Media.** We assessed whether peptoid-SWNT nanosensors retain their ability to detect WGA in complex biological environments. We showed that ProLoop-SWNT nanosensors retain their ability to respond to WGA in Dulbecco modified Eagle’s medium (DMEM), a common medium used for mammalian cell culture. Pre-incubation of the nanosensors with DMEM for 1 h retained ProLoop-SWNT nanosensor responsivity to 10 μM WGA (Figure 4a). DMEM-pre-incubated ProLoop-SWNT nanosensors show a fluorescence decrease of $-58.6 \pm 8.6\%$ (mean ± SD) upon the addition of WGA against a baseline decrease of $-29.3 \pm 0.1\%$ (mean ± SD). Baseline decreases are caused by dilution effects and some nanosensor instability in DMEM.

Lastly, we tested the responsivity of peptoid-SWNT nanosensors in serum conditions. Nanosensor response was attenuated in the presence of fetal bovine serum, with peptoid-SWNT nanosensors pre-incubated in serum for 24 h exhibiting unstable fluorescence upon laser illumination. The fluorescence of ProLoop-SWNT pre-incubated in serum decreased by $-12.6 \pm 6.4\%$ (mean ± SD) over the course of 1 h of spectrometry without the addition of WGA and decreased by $-20.6 \pm 7.3\%$ (mean ± SD) upon addition of WGA (Figure 4b). Our results demonstrate nanosensors can recognize WGA in serum conditions, albeit with reduced sensitivity. ProLoop-SWNT pre-incubated in NeutrAvidin, a protein that binds bare nanotube surfaces, and recombinant human serum albumin, the most abundant protein in serum, also showed an attenuated but statistically significant fluorescence decrease response to WGA (Figure S17). These results suggest that ProLoop-SWNT nanosensors can monitor protein dynamics in in vitro cell cultures, but that further optimization will be necessary to increase ProLoop-SWNT nanosensor activity in serum conditions.

**Single-Molecule Imaging of Peptoid-SWNT Nanosensors.** The fluorescence response of ProLoop-SWNT nanosensor to WGA can also be imaged with single ProLoop-SWNT nanosensors immobilized on a microscope slide to confirm the fluorescence decrease of nanosensors is not the result of aggregation. Negatively charged ProLoop-SWNT nanosensors were surface immobilized on a positively charged 3-aminopropyltriethoxysilane (APTES)-coated glass coverslip and imaged with NIR single nanosensor fluorescence microscopy (see Materials and Methods in the Supporting Information). Addition of WGA to a final protein concentration of 50 μM induces a fluorescence change in the saturated detection regime of the ProLoop-SWNT nanosensor. Although single nanosensors exhibit variable fluorescence...
intensity trajectories over time, the average of the regions of interest shows a significant decrease in fluorescence upon WGA addition $-14.5 \pm 2.0\%$ (mean $\pm$ SD) compared to a control sample using only water $-8.1 \pm 3.5\%$ (mean $\pm$ SD) (Figure 4c, Figure S18). Single nanosensor imaging suggests that the molecular recognition of individual ProLoop-SWNT nanosensors can be monitored optically for dynamic biological imaging.

**WGA on Peptoid-SWNT Enables Detection of WGA’s Target Sugars.** We further showed ternary nanosensor interactions of peptoid-SWNT nanosensors, post-interaction with WGA, and a secondary analyte through lectin-sugar interactions. WGA protein is a lectin, a sugar-binding protein, with specificity to two target sugars: N-acetylglutaminic acid (GlcNAc) and N-acetylenearaminic acid (NeuSAc). Previous work has shown the recognition of glucose, fucose, and GlcNAc when enzymes and lectin proteins are grafted to the surface of SWNTs. These studies showed that proteins can remain active when tethered to the surface of SWNT through a polymer intermediate, and we test to see if our synthetic peptoid system can emulate these findings.

We tested the activity of WGA when bound to the surface of ProLoop-SWNT. We first incubated ProLoop-SWNT in 10 μM WGA for 1 hour before measuring its fluorescence spectrum. A panel of sugars commonly bound by lectins were tested including fructose, galactose, glucose, mannose, and fucose and complex sugars such as sucrose and mannitol in addition to the target sugars GlcNAc and NeuSAc. The addition of target sugars to WGA-saturated ProLoop-SWNT yielded a significant change in nanosensor fluorescence intensity (Figure 4d). This modulation of SWNT fluorescence intensity was not observed with other sugars that other lectins are known to bind including fructose, galactose, and glucose. Interestingly, we observe an off-target response of the WGA-saturated ProLoop-SWNT to fucose sugar, which might arise from orthogonal interactions between WGA and ProLoop-SWNT that are not specific to the sugar-lectin interaction, but note that the sugar response selectivity for WGA adsorbed to ProLoop-SWNT remains high with a 1 out of 9 rate for off-target sugar response.

We confirmed that WGA and ProLoop are both required and responsible for sugar-induced SWNT fluorescence modulation (Figure S19). We posit that sugar binding to the protein causes a protein conformational change or a perturbation to the corona phase of the ProLoop-SWNT (Figure 4e). Furthermore, we attributed this selective fluorescence response to the 6-monomer loop in the ProLoop peptoid, since WGA incubated with PC36-SWNT, which lacks this loop segment, does not respond to GlcNAc or NeuSAc (Figure S20). Additionally, ProLoop-SWNT pre-incubated with PNA and ConA did not yield a fluorescence response upon addition of their target sugars galactose and mannose, respectively. A common challenge in protein adsorption to nanoparticles for biosensing, enzymology, and protein delivery is a reduction in protein structure and activity. Notably, the specificity of WGA toward its target sugars suggests WGA remains active while tethered to the SWNT surface through ProLoop-SWNT. These results further suggest peptoid-SWNT assemblies can enable detection of both proteins and also their target molecular binders.

In summary, we present design principles and validate the modular platform for assembly of protein-like N-substituted synthetic glycine peptoid polymers with SWNTs. We demonstrated that certain peptoid sequences, namely ProLoop, exhibit the necessary stability to assemble on SWNT and exhibit robust photostability under conditions necessary for real-time imaging of proteins including variable buffer conditions, continuous laser illumination, and resistance to protease degradation. We further showed that ProLoop-SWNT can optically detect lectin protein WGA and can also selectively detect the lectin target sugars.

An outstanding question in nanosensor development is whether molecular recognition elements can be rationally designed for analytes of interest with synthetically tractable polymers. To this end, including peptoids in the repertoire of polymers for suspending SWNT realizes a new space of biomimetic polymers for the development of SWNT-based nanosensors. Peptoid polymers are resistant to protease degradation, leverage facile synthesis through automated solid-phase synthesis, and exhibit a large monomer space that is unavailable for conventional biopolymers. Our results suggest peptoid polymers are promising candidates for modular design of synthetic molecular recognition elements with nanomaterial substrates to serve as signal transduction elements. Our method of designing peptoid-SWNT nanosensors is fundamentally general for any protein target. The primary limitation is assessing peptoid-SWNT colloidal stability, since FRET screening of protein-binding peptoid loops can be accomplished upstream of peptoid-SWNT assembly. By elucidating design parameters for creating peptoids that can adsorb onto SWNT and assessing the contributing factors to peptoid-SWNT selectivity or sensitivity for WGA, our results set the groundwork for developing looped peptoid polymers for specific protein targets. In addition to the benefits of fluorescence detection of protein ligands, the ability to bind proteins to SWNTs with peptoid polymers could aid SWNT-based biomolecule delivery platforms. SWNTs have been shown to internalize across a wide range of biological membranes for delivery applications, thus the ability to graft proteins selectively to SWNTs without compromising endogenous protein function presents an enticing opportunity to deliver functional proteins to diverse biological systems. Previous work on peptoid polymers have shown success in selectively binding multivalent proteins as well enzymes with known ligands, thus expanding the library of potential protein analytes or delivery cargoes for use in our peptoid-SWNT platform.

Lastly, our work shows that peptoid-SWNT nanosensors can interact with proteins while preserving the protein’s inherent activity. We demonstrated that ProLoop-SWNT can bind WGA and can fluorescently respond to the two WGA target sugars GlcNAc and NeuSAc, demonstrating that the WGA protein remains active toward its target sugars even when bound to a peptoid-SWNT nanosensor. Despite prior reports of decreased or abolished protein activity upon protein adsorption to nanoparticles such as SWNT, it is possible that the maintenance of protein activity is due to the ProLoop-mediated binding of WGA, in lieu of WGA binding to the SWNT directly. Our results suggest peptoid polymers may serve a dual purpose of tethering proteins to SWNTs in a manner that enables protein detection and also preservation of the protein’s endogenous activity for future applications in surface science, enzymology, and protein delivery.
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