Generating Selective Saccharide Binding Affinity of Phenyl Boronic Acids using Single Walled Carbon Nanotube Corona Phases**
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Abstract: Saccharides recognition is challenging due to their low affinity for substrates, yet this recognition is critical for human immunity and glycobiology. Here, we demonstrate that a polymer or surfactant corona phase surrounding a single walled carbon nanotube can substantially modify the selectivity of pre-adsorbed phenyl-boronic acids (PBA) for mono-, di- and poly-saccharides. A library of 17 PBAs including carboxy, nitro, and amino PBA with ortho-, meta- or para-substitutions are used to generate 144 distinct corona phases. Six in particular demonstrate significantly increased selectivity to specific saccharides including ribose (0.42, mol/total mol), arabinose (0.36), and glucose (0.25), but unusually diminished binding to fructose (0.02). Recognition proceeds by saccharide adsorption into the corona, followed by PBA reaction in a consecutive 2nd order reaction. The results extend to larger saccharides such as glycosaminoglycans, suggesting promise for protein glycosylation.

Unlike the three other classes of molecules that comprise human life, namely nucleic acids, amino acids, and lipids, carbohydrates remain exceedingly difficult to characterize.[1] The display of multiple hydroxyl groups is often difficult to recognize from clusters of solvating water molecules.[2] Even lectins, a family of natural proteins known to bind carbohydrates, demonstrate relatively low avidity in aqueous solution.[3] Synthetic receptors for specific carbohydrate recognition[4] constitute a challenging yet highly impactful area of research. In this work, we demonstrate that the corona phase around a single walled carbon nanotube (SWNT) can substantially modify the otherwise weak selectivity of phenyl boronic acids for mono-, di- and polysaccharides.

The covalent pair-wise interaction between boronic acids and 1,2- or 1,3-diols in aqueous systems is known to be rapid and reversible, and well-studied for binding for saccharide recognition[5]. The 1,2 or 1,3-diols present on saccharides provide a potential scaffold for these interactions, as demonstrated for various boronic acid assays for saccharides.[6] The effective dissociation constant, KD, of such interactions tends to vary by less than a factor of 2-4 across typical mono-saccharides, with a slight preference for fructose (KD = 0.23mM for phenylboronic acid[7], 3.7mM for anthrylboronic acid[8], and 0.16mM for indolyboronic acid[9]). Our previous study[10] has shown that specific boronic acids adsorbed on the surface of SWNTs can be used to modulate the photoluminescence of SWNT in response to glucose. However the impact of the corona phase on BA binding has not been previously explored. In this work, we advance the unexpected result that BA selectivity towards various saccharides can be directed substantially using the corona phase, either via suppression or enhancement of the surface mediated reaction with adsorbed BA. This control offers a new route to engineer carbohydrate recognition.

We investigated 8 different surfactant/polymer corona environments including sodium dodecyl sulfate (SDS), sodium dodecylbenzenesulfonate (SDBS), sodium
cholate (SC), polyoxyethylene (100) stearyl ether (Brij-S100), polyoxyethylene-polyoxypropylene block copolymer (Pluronic-F127), polyethylene glycol 8 membered branched polymer (PPEG8), polysodium 4-styrenesulfonate (PSS), and polyanetholesulfonic acid sodium salt (PASA). For all corona phases investigated, the concentration of the polymer or surfactants was selected to be above the critical micelle concentration (for small molecule surfactants) or critical aggregation concentration (for amphiphilic polymers). At 1 wt %, which is used often in the literature[11], this criterion is satisfied, and hence selected for this. We further modified these phases by non-covalent adsorption of 17 different phenyl boronic acids generating a library of 144 distinct corona phases (including PBA-free controls). We screened this set against an analyte library of 10 saccharides and 5 glycosaminoglycans. We evaluated phase for its ability to colloidal disperse SWNT in aqueous suspension, rejecting those producing instability (Figure 1). We also evaluated the relative solvatochromic shift, and the surface coverage. Saccharide responses are shown to occur only within corona phases that have allowed phenyl boronic acids adsorb and pre-quench the SWNT emission (Figure 1). This trend is observed for all corona phases explored in this work for the mono- and disaccharides in the library. Hence, the molecular recognition is shown to occur within the phase itself, and is a measure of its selectivity towards specific saccharides.

The surface coverage of a polymer or surfactant on the SWNT can be calculated using the shift in optical transition Eii of individual SWNT relative to a reference state by measuring the optical absorption or emission spectrum. Applying a previously developed mathematical model[12], an accessible surface area with respect to water, a molecule considerably smaller than the saccharides considered in this work is calculated as described in the supporting information. SDS, SC, and SDS molecules with lower molecular weight yielded the bluest shift of the SWNT absorption spectra compared with polymers, in agreement with prior results.[13] The calculated surface coverage of SC on SWNT is 75%, as reported previously[14], while polymer corona phases considered in this work display a lower surface coverage below 70%. Intuitively, longer chain structures, linear or branched, result in lower inaccessible surface area due to steric effects, consistent with these data.

Most phenyl boronic acids appear to quench SWNT PL, provided they are able to access the corona phase and adsorb to the SWNT surface. Taking an empirical approach, we created a “heat map” to demonstrate the diversity of fluorescence intensity modulation caused by different phenyl boronic acids and subsequent saccharide analytes. Here, red corresponds to a “turn-on” response or increasing intensity while blue is a quenching response or decreasing intensity (Figure 1). For each corona phase, the last column in each map is the initial quenching from the specific BA. For SDS, we observe that, 3CPBA, 4CPBA, 4SCPBA, 5B3CPBA, 5F3CPBA, 5N3CPBA, 4NPBA and 3APBA result in about 50% fluorescence quenching of SWNT wrapped by SDS molecules. This accessibility appears to reflect this loose or fluxional packing. The SDS corona phase, when combined with these specific BA species, appears to favor boronate complexes with pentose saccharides. Other responses of note are sucrose (30%, for 4CPBA and 4NPBA) and N-acetyl-D-glucosamine (20%, 3CPBA, 4CPBA, 4SCPBA, and 5B3CPBA). It is worth noting that
All "turn-on" responses occur on the condition of pre-quenching of SWNT by a corresponding BA.

Both SDS and SDBS are linear-chain surfactants. However, most boronic acids fail to penetrate the SDBS corona and interact with SWNT. These results agree with previous findings, where the aromatic ring of SDBS molecule is believed to provide enhanced dispersive ability due to π-π interactions with the SWNT surface.[15] According to the results from MD simulation[15b], the tail segments and most aromatic rings are positioned close to the SWNT surface while the sulfonate headgroups are exposed to water. These studies point to the sizable π-π interactions between the aromatic ring of SDBS and SWNT as enhancing its binding. We find this consistent with a much less permeable corona phase. Consistent with our recovery mechanism, the lack of BA pre-quenching means few if any saccharides can be detected. Furthermore, saccharide recognition indicates that any sugar response occurs where the corona phase has to allow boronic acid to get through it, to sit on the tube and initially pre-quench the SWNT. By contrast, SC is a bile salt with a relatively stiff steroidal backbone that imparts characteristic hydrophobic and hydrophilic faces. Previous studies[14b, 16] have shown that this bifacial nature of SC causes a tightly packed monolayer on SWNT. Our results confirm that SC is a “tight” wrapping agent with almost no responses from boronic acids or sugars, which reflects its nearly featureless heat map.

The assembly of short chained small molecule surfactants on SWNT including SDS, SDBS, and SC has been proposed to result in encapsulation via a cylindrical micelle[17], adsorption of hemimicelles[15a, 18], or random adsorption[19], depending on the surfactant concentration[20] and nanotube diameter. Alternatively, Brij-S100, Pluronic-F127, and PPEG8 are amphiphilic block copolymers with molecular weights close to or more than 5KDa. For these polymers, the dispersion mechanism for SWNT is distinct from small molecule surfactants. It has been proposed that two modes of interaction between dispersing polymers and SWNT include the “wrapping mode”[21] and polymer-decorated “kinetic mode”[22]. Here, our experimental results indicate that the wrapping mechanism appears more consistent for these corona phases. In the case of Pluronic-F127, the heat map is featureless except for 5B3CPBA and 3APBA, which cause slight PL quenching of the SWNT. In contrast, Brij-S100, 5B3CPBA, 5N3CPBA, 2APBA, and 3APBA result in more than 30% fluorescence quenching of SWNT, with then arabinose and N-acetyl-D-glucosamine addition recovering more than 20% fluorescence intensity. This selectivity is remarkable given the compositional similarity among the saccharides tested. In further contrast, most of the examined boronic acids can quench the fluorescence of SWNT wrapped by PPEG8, and arabinose and ribose appear to be the most detectable sugars. These three polymer examples, in conjunction with the three surfactants, illustrate that the corona phase environment can have a profound effect on the selectivity of saccharide interaction with the adsorbed BA, and may constitute a new direction in the engineering of molecular recognition. The corona phase can influence selectivity in a variety of ways, including as a molecularly selective, steric barrier to specific species, and by modifying the micro-environment to favor or disfavor electrostatic or van der Waals interactions.
The cases of PSS and PASA are distinct from the polymers mentioned above due to their permanently charged backbone with aromatic pendant groups, which allow both π-π interactions to bond the polymer with the SWNT backbone while maintaining water solubility. Such polymers may not be efficient in dispersing SWNT from the raw form because of a decreased ability to promote an "unzippering" process of the SWNT bundle during ultrasonic agitation[23]. Such polymers are also self-avoiding due to their intrinsic and permanent charge. Hence, their wrapping is predicted to be quite sparse, with large areas of the SWNT surface exposed to solvent (water). In agreement, we find that PSS and PASA present the most colorful heat maps suggesting easy accessibility of PBA and subsequent saccharides. While most boronic acids give a quenching response in PSS-SWNT solution, 2CPBA, 4CLBA, 1,4-BDBA, and 4CYBA result in a unique "turn-on" response. Similarly, adding 2CPBA or 2NPBA to PASA-SWNT can also enhance the fluorescence intensity.

We also employed ARS (Alizarin Red S) to study the selectivity of saccharide binding to the examined boronic acids.[24] The equilibrium between ARS and ARS-PBA complex can be perturbed by adding saccharide, which results in a fluorescence intensity change. The corresponding visible fluorescence "heat map" (Figure 1a) using ARS presents the monotonous selectivity towards fructose, in agreement with the previous observation,[10a] The signal to noise appears to be too low to resolve the binding of other saccharides tested but the KD values are typically of the same order but greater than fructose.

The scatter plot (Figure 2) of absolute sugar response vs. absolute boronic acid response reveals that there are no saccharide responses in the absence of an initial boronic acid adsorption as shown in the triangular region. Four possible interacting steps can exist for the mechanism explored in this work, all of which can be coupled reversibly through equilibria as depicted in Figure 3a. Briefly, boronic acid (BA) may adsorb on the surface of SWNT to form BA-SWNT complex with a binding constant, K1. After saccharide addition, both homogeneous BA and BA-SWNT can bind with it to form saccharide-BA and saccharide-BA-SWNT complexes with different binding constants, K2 and K3. At the same time, saccharide-BA could potentially be adsorbed onto the surface of SWNT to form the same saccharide-BA-SWNT complexes with a binding constant K4. Several pieces of evidence indicate that the dominate pathway is step (1) followed by (2). The observation that no saccharide response was observed for any of the systems explored for which there was not an initial BA was quenching in forming the complex supports this pathway. Kinetic measurements also indicate that the (1) to (2) pathway dominates the recognition mechanism. Transient spectrophotoluminescent experiments (Figure 3b) conducted after adding boronic acids show a fluorescence intensity decrease with the time. Addition of the saccharide results in a near immediate jump in restored intensity arriving to stability within 30 minutes. When a pre-incubated saccharide-BA complex was added into SWNT solution, however, the fluorescence intensity change was substantially smaller (Figure 3c). These results indicate that K1 is larger than K4, and that only boronic acids adsorbed on the surface of SWNT can modulate the fluorescence of SWNT. This pathway is also supported by the observation (Figure 1) that no saccharide response was noted when there was no initial BA quenching in
forming the complex. 4-carboxyphenylboronic acid (4CPBA) recognition of arabinose (Figure 3d) was further analyzed to obtain forward and reverse kinetic rates of binding by assuming a single-site surface adsorption of 4CPBA on SWNT as described in the supporting information. This yielded the forward and the reverse rate constants of 0.0164 M$^{-1}$·s$^{-1}$ and 0.001s$^{-1}$ respectively, which leads to the KD of 60.9μM.

To demonstrate the relationship between the fluorescence intensity change and sugar concentration, a calibration curve is measured and demonstrated based on the response of CS_A on 3CPBA+PASA-SWNT complex. The calibration curve displays a non-linear change of the detection signal with the analyte concentration. The Langmuir adsorption equation provides a good fit to the experimental data (Figure 4).

In summary, we have shown that a nanoparticle corona phase can offer a new aspect of boronic acid chemistry for tuning their selectivity for specific saccharides. We demonstrate that a polymer or surfactant corona phase surrounding a single walled carbon nanotube can substantially modify the selectivity of various pre-adsorbed phenyl-boronic acids (PBA) for mono-, di- and poly-saccharides. The use of a library of 17 PBAs to generate 144 distinct corona phases can serve as a template for future approaches to screen specifically designed polymer corona phases to enhance selectivity and avidity for saccharides and protein glycoforms. Of these, we select 6 that demonstrate significantly increased selectivity to specific saccharides including ribose (0.42, mol/total mol), arabinose (0.36), glucose (0.25), and sucrose (0.23) and also unusually diminished binding to fructose (0.02). The kinetic measurements demonstrate that the mechanism of recognition proceeds by saccharide adsorption into the nanotube corona, followed by surface reaction with the phenyl boronic acid in a series consecutive second order reversible reaction. This type of selective binding shows promise for detecting higher molecular weight saccharides suggesting a compelling approach to detecting glycosylated proteins.

Experimental Section

SWNT Dispersions: Powder SWNTs were individualized by dispersing them with the suspension procedures as follows. 10 mg SWNTs was dispersed in 30 mL distilled water at 1% surfactant/polymer concentration or 2% sodium cholate (SC) concentration. Samples were sonicated in a cold water bath with a 5 mm probe tip for 40 min at a power of 25 W, followed by ultracentrifugation for 4 hours at 30 000 rpm (~164 100 G) in Beckmann-Coulter Optima™ L-100 XP ultracentrifuge. Contamination of the supernatant with nanotube bundles was minimized by collecting only the top 80% of the supernatant.

Spectroscopic Characterizations: UV-vis-nIR spectra of all SWNTs solutions were obtained using SHIMADZU UV-3101PC scanning spectrophotometer. Visible fluorescence of ARS excited at 495 nm was recorded using Thermo Scientific Varioskan Flash Plate Reader; the emission wavelength was monitored from 520 nm to 700 nm. Near-infrared fluorescence of SWNTs solutions were measured using a custom-made near-infrared inverted microscope (Zeiss D.1 Observer) with a 50×/0.7 Zeiss objective, as described by previous publications.[1] Sample excitation was from a 785 nm photodiode laser, 450mW at the source, and 150 mW at the sample.
Visible Fluorescence Detection of Saccharide Response: All boronic acids were dissolved in DMSO at 0.1M concentration. All tests were performed using 96 well, 300 uL flat-bottom FALCON tissue culture plate. 10 uL boronic acid solution was added to 190 uL prepared ARS water solution (0.5mM), making the final concentration of boronic acid 0.5 mM. For the visible fluorescence test of saccharide response, 10.5 uL saccharides solution (1 M) was added to the mixture solutions, making the final concentration of sugars approximately 50 mM.

nIR Fluorescence Detection of Saccharide Response based on Polymer-SWNT Complexes: All boronic acids were dissolved in DMSO at 0.1M concentration. All saccharides were dissolved in PBS buffer at 1 M concentration, while glycosaminoglycans were dissolved in PBS buffer at 0.5% concentration. All tests were performed using 96 well, 300 uL flat-bottom FALCON tissue culture plate. 10 uL boronic acid solution was added to 190 uL prepared SWNT solution. The mixture solution was incubated for 30 minutes before the nIR fluorescence measurement. After then, 10 uL analyte solutions were added to measure the saccharide response.

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Figure 1. (a) Depiction of sugar detecting complex. The corona phase has to allow boronic acid to get through it, to sit on the tube and initially pre-quench the SWNT. In the presence of diols, both trigonal and tetrahedral forms of boron can bind with 1,2 or 1,3-diols to form diol-phenylboronate complexes with five- or six-membered
rings, respectively, which result in the photoluminescence intensity change of SWNT, (b)-(i) “Heat map” of fluorescence intensity change caused by boronic acids and sugar responses. In each matrix, x direction lists 10 saccharide analytes plus a response from only boronic acids, while y direction lists 17 boronic acids plus a control response in the absence of any boronic acids. In the matrix of PSS and PASA, 5 glycosaminoglycans were added to extend the x direction. In the matrix of ARS(h), the visible fluorescence “heat map” using ARS presents the monotonous selectivity towards fructose.

Figure 2. The scatter plot of absolute sugar response vs. absolute boronic acid response reveals more information about the relationship between the wrapping agents and photoluminescence properties of SWNT. The gray triangular shape indicates that no sugar response occurs where no boronic acid response on SWNT.
Figure 3. (a) Proposed 4-component equilibrium among SWNT, BA, and sugar. Our kinetic study indicates that $K_1$ to $K_2$ pathway is favorable in practice. (b) Transient Raman spectra analysis of SWNT fluorescence. Adding boronic acid will cause the fluorescence decrease, and adding sugar will cause the fluorescence increase. Several repeated operations are demonstrated using 4CPBA, 3CPBA, and 5N3CPBA. (c) When the pre-incubated sugar-BA complex was added into SWNT solution, the fluorescence intensity didn’t change much. These results demonstrate that $K_1$ is larger than $K_4$, and indicate that only boronic acids adsorped on the surface of SWNT can modulate the fluorescence of SWNT. (d) The adsorption of boronic acid onto the surface of SWNT could be described using a second-order reversible kinetic reaction. The dots are experimental data, while the curves are the modeling data.
Figure 4. Calibration curve is measured and presented based on the response of CS_A on 3CPBA+PASA-SWNT complex, which demonstrates a non-linear change of the detection signal with the analyte concentration. The Langmuir adsorption equation provides a good fit to the experimental data with a constant $A = 0.15$. 

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\frac{(I-I_0)}{I_0} = \frac{CA}{1 + CA}
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$A = 0.15$