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Carbohydrate Materials Discovery: Towards a Post-Cellulosic Future

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AFOSR Progress Report

Carbohydrate Materials Discovery: Towards a Post-Cellulosic Future

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Carbohydrate Materials Discovery: Towards a Post-Cellulosic Future

ABSTRACT. Carbohydrates could provide attractive solutions to critical energy, defense, and material challenges because they are abundant, naturally sourced, and renewable. Despite these attributes, carbohydrates remain relatively unexplored as feedstock for advanced materials or in the context of nanotechnology because of the difficulty inherent to determining their superstructure, studying their recognition, and in preparing even simple oligosaccharides. To realize the full potential of carbohydrates as feedstock for advanced materials and nanotechnologies, the means by which carbohydrate binding and assembly are studied must be reevaluated entirely. To this end, we have developed a new class of carbohydrate receptors – termed the Bear Paw Dimers – that recognize non-glucosidic monosaccharides by accessing biomimetic binding modes, and thereby achieve high affinities at biologically-relevant solvents and temperatures. Moreover, because of the modularity of this scaffold, its molecular structure can be manipulated easily to test new hypotheses regarding fundamental aspects of glycan-binding. In addition, a new platform – the glycopolymer-modified plasmon field effect transistor – has been used to study binding in an environment intended to recreate the glycan density and presentation common to biological interfaces, and this platform has the most sensitive detection limit for carbohydrate binding proteins yet reported as a result of the combination of cooperative, multivalent binding and amplified electrical output. Future efforts will be devoted to (1) expanding the scope of novel carbohydrate binders, (2) multiplexing the glycopolymer-modified plasmon field effect transistor for combinatorial studies, and (3) perturbing the assembly of hierchical carbohydrate materials.

A. Introduction and Objectives

The aim of this research program is to explore carbohydrates as a potential solution to critical energy, defense, and material challenges by exploring the structure, binding, assembly, and equilibrium dynamics of abundant saccharide feedstocks. The proposed approach to achieving this is based on three ideas: (1) Specific noncovalent binding is a key tool for exploring and attenuating the assembly of hierarchical, carbohydrate-based materials, (2) carbohydrate are very complex, and that their structure and complexity can only be understood through combinatorial datasets and model-systems that reduce glycans to their simplest elements, and (3) because of their structural diversity, carbohydrates themselves could potentially alter the assembly of hierarchical carbohydrate-materials. *Thus, the overarching aim of this project will be achieved by controlling the structure, binding, and assembly of accessible carbohydrate feedstock.*

The specific project goals are:

- Develop molecules to investigate carbohydrate binding and assembly
- Create glycopolymer platforms for studying carbohydrate recognition on surfaces
- Alter material properties of reconstituted carbohydrates using carbohydrate additives

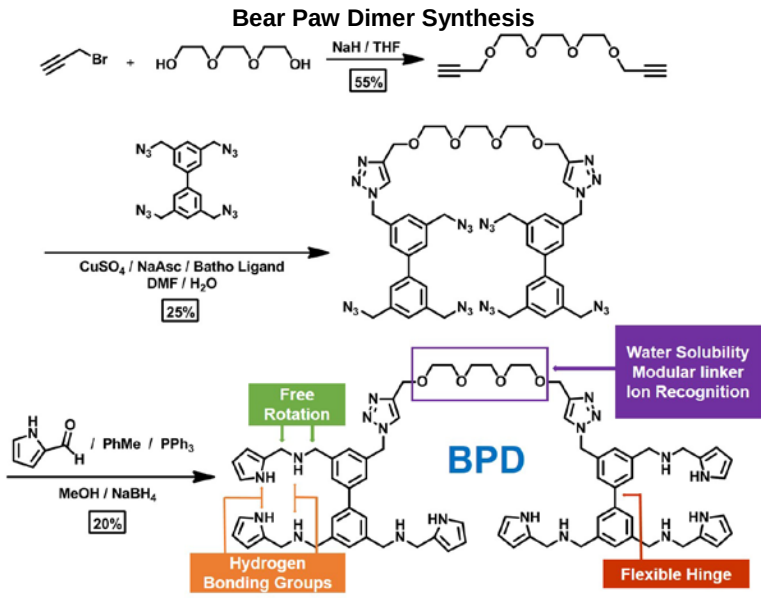
B. Progress Towards Project Goals

B. 1. Carbohydrate Receptors for Understanding Binding And Assembly

The surface of every eukaryotic cell is coated with a layer of glycolipids, glycoproteins, and glycopolymers – termed the glycocalyx – and binding events involving these oligosaccharides mediate a wide variety of biological events, including cell-cell communication, immunological response, cell-pathogen interactions, and material assembly. Cell-surface glycosylation patterns are unique and accessible identifiers of cell-type. For example, α-mannose (α-Man) is overexpressed on the surface of human liver, lung, and prostate, cells whereas α-galactose (α-Gal) is abundant on human colon, pancreas, and medulla cells. So synthetic molecules that recognize with some preference specific mono- and oligosaccharides in the glycocalyx could be used for understanding how cell-surface glycosylation is used to transmit extracellular information within complex biological networks. Alternatively, these same molecules could be used to atenuate assembly in hierarchical, carbohydrate-based materials. Although α-Man and α-Gal are abundant on cell-surface glycans, their epimer, α-glucose (α-Glc) is almost entirely absent from cell surface because it occurs in such high concentration in the blood and cytoplasm, and, as such, understanding cell-surface communication must explore recognition to non-glucosides. Despite the importance of the glycocalyx to biology, designing synthetic carbohydrate receptors that bind non-glucosides is considered an insurmountable challenge. In most cases, synthetic carbohydrate receptors achieve binding that primarily occurs through H-bonding in a very competitive solvent, select between complex molecules that differ sometimes by only the orientation of a single stereocenter, and access cooperative and multivalent binding pathways that amplify avidity and selectivity.

Despite these difficulties, a significant number of synthetic carbohydrates receptors – including those that bind in water – have been developed. Generally, these fall into two classes: those that bind through the formation of boronate esters and others that are rigid scaffolds that bind entirely

through noncovalent contacts. The former bind monosaccharides possessing syn-diols with high binding affinity in water (10^3-10^4 M^{-1}), and particularly noteworthy example are the chiral diboronic acid receptors by Shinkai that bind D-Fructose and D-Glucose with K_a of ~10^4 M^{-1}. The drawback of these is the bound substrate rapidly rearranges into its isomer to form a more thermodynamically stable complex in a dynamic recognition process, so that nonspecific recognition of other monosaccharides may become an issue in biological systems, but using principle component analysis, could be used for sugar sensor. The latter organize polar and nonpolar domains around a rigid scaffold, and particularly important examples include porphyrin conjugates, podand receptors, encapsulating receptors, and the temple- classes of receptors developed by Davis that bind glucosides in aqueous solvents with association constants of 130 M^{-1}. The applications for both these classes of receptors are manifold, monitoring of blood glucose, the early detection of disease biomarkers such as sialyl Lewis X antigen and TF antigen, and the site-specific imaging of cancer cells, still dominated by glucose and sialic acid binders, but for applications including cell-surface targeting, carbohydrate based nanotechnology, or characterizing the structure of complex oligosaccharides, there remains a need to continue developing synthetic carbohydrate receptors with a binding preference for non-glucosides.



Scheme 1. Preparation of the Bear Paw Dimer (BPD) through multistep organic synthesis. The molecular design enables saccharide recognition in water as a result of the dimeric structure and the water soluble oligoethylene glycol linker.

Generally, the synthetic receptors that bind through noncovalent interactions are designed by following the principle of preorganization developed by Cram, who showed that binding affinity is increased in rigid receptors because the entropic penalty upon binding associated with reorganization is minimized. This design strategy is consistent with Fisher's "lock-and-key" model of protein binding, which assumes that both enzyme and substrate have rigid conformations that lead to an ideal fit with relatively high K_as. Glycan binding proteins – like lectins or the periplasmic binding proteins – are classical examples of the more nuanced "induced fit" model, where enzyme flexibility and substrate influence dictate the structure of the enzyme-substrate complex. Typically, glycan binding proteins are characterized by promiscuity – they will bind many monosaccharides with weak 1:1 binding – and overcome the avidity and selectivity challenges by achieving binding enhancement of up to 10^6 M^{-1} by accessing cooperative and multivalent binding pathways. This phenomena, termed the "cluster-glycoside effect", demonstrates how small differences in binding

affinity are amplified within the dense-forest of saccharides on the cell-surface. Thus, developing synthetic carbohydrate receptors that bind with some specificity towards non-glucosidic monosaccharides may require approaches towards receptor design that reconsider the role of preorganization and the meanings of selectivity and specificity.

To this end, we have reported previously a highly flexible synthetic carbohydrate receptor – referred to as the "Bear Paw" – that possesses four aminopyrroles organized around biaryl core that binds β-Man selectively in chloroform through H-bonding and C−H•••π interactions in concert with multivalent and cooperative equilibria. Like natural glycan binding proteins, this receptor is promiscuous and forms 1:1 complexes with all monosaccharides assayed, and selectivity as high as 16.8:1 α-Man:α-Gal and 1.5:1 α-Man:β-Glc is achieved as a result of 2:1 and 1:2 receptor:substrate complexes. This receptor demonstrates the potential of flexible scaffolds for addressing the unmet challenge of creating synthetic carbohydrate receptors that possess non-glucosidic selectivities, but the major drawback of this design however is that binding has been demonstrated only in chloroform, it has poor water solubility, and low binding affinities that arise from weak H-bonding may not translate to a more competitive solvent environment. Herein, we report a novel synthetic carbohydrate receptor that builds upon this earlier design to create a flexible, water-soluble molecule that is a dimer of the Bear Paw receptor whose two parts are linked by an oligoethylene glycol chain. The result is a molecule – termed the Bear Paw Dimer (BPD) – that binds carbohydrates, including non-glucosides, with high K_a and in aqueous environments, thereby serving as a powerful platform to study and understand the recognition and material properties of carbohydrates.

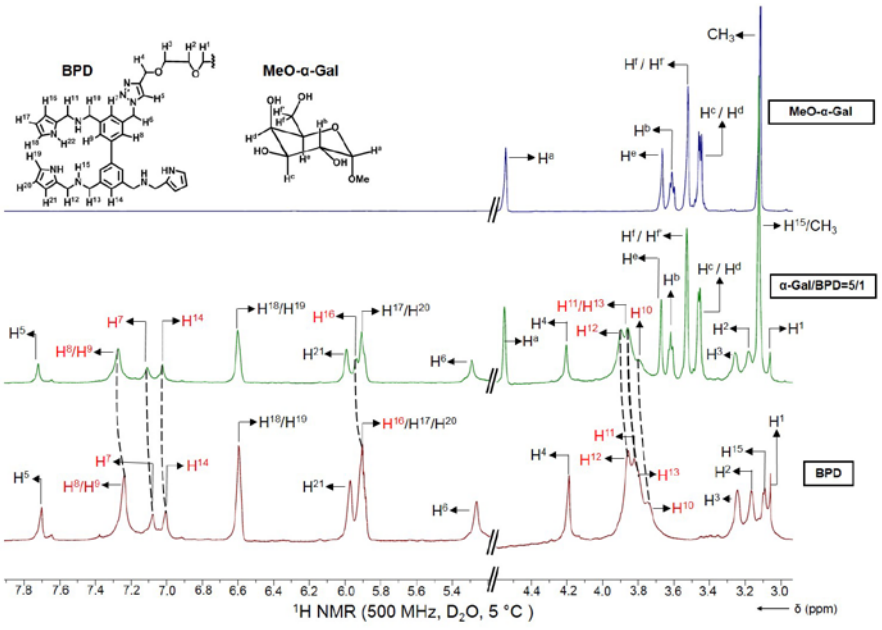


Figure 1. Titration of a solution of α-MeO-Gal into an aqueous solution containing BPD, which causes the peaks corresponding to the receptor to shift (indicated by dashed line), reflecting changes in chemical environment upon the formation of a host-guest complex.

BPD Design & Synthesis. The rational design of synthetic molecules that bind non-glucosides in water through entirely noncovalent approaches has proven particularly challenging because (1) binding is dominated by H-bonding in a competitive solvent and relatively weak C−H•••π contacts, (2) the challenging task of

distinguishing between complex molecules that may differ only by the orientation of a single stereocenter, and (3) the difficulty of designing a host that encompasses the saccharide guests to overcome the weak binding. To address this challenge, the original Bear Paw (BP) host was conformationally flexible, so that it could sample thermodynamic space and form complexes with a large number of monosaccharide guests in a noncompetitive organic solvent. Extensive NMR analysis and computational modeling found that this molecule bound saccharides through a variety of binding pathways, including both 2:1 and 1:2 BP:Man complexes, with the 2:1 complex showing a preference for α-anomers and the 1:2 complex preferring β-anomers. Importantly, binding of the 2:1 BP:α-Man involved only three of the four aminopyrrolitic arms on each host were involved in binding, which suggested a dimeric design for a water-soluble, high binding affinity carbohydrate receptor, the BPD. The BPD replaces the unused aminopyrrolitic position on each monomer with an oligoethylene glycol linker that also increases substantially the water solubility of the host. It was anticipated that these structural changes would increase both selectivity and binding affinity compared to BP because linking the two binding units of the receptor together would decrease the entropic penalty of bringing together three molecules into a host-guest complex, and also selectivity would increase because the previously observed 2:1 receptor:saccharide structures preferred α-anomers.

The BPD was prepared in two steps from the previously reported tetrazide precursor (**Scheme 1**). First, the two subunits are linked with the alkyne-terminated oligoethylene glycol chain via a Cu^{I}-catalyzed azide-alkyne Huisgen reaction in the presence of copper sulfate, sodium ascorbate and bathocuproinedisulfonic acid disodium salt (Batho) to provide a precursor in 25 % yield. Finally, the BPD was obtained using a Staudinger amination, where all six aminorpyrrolitic arms – which are added to form H-bonds with the guest saccharides – are installed in 20 % yield. Characterization, including ^{1}H NMR, ^{13}C NMR, and high resolution mass spectrometry, are all consistent with the proposed molecular structures. Importantly, this brief synthesis is easily scaled to provide hundreds of milligrams of material, and is modular such that the length of the oligoethylene glycol spacer, biaryl linker, or H-bonding groups can be easily varied.

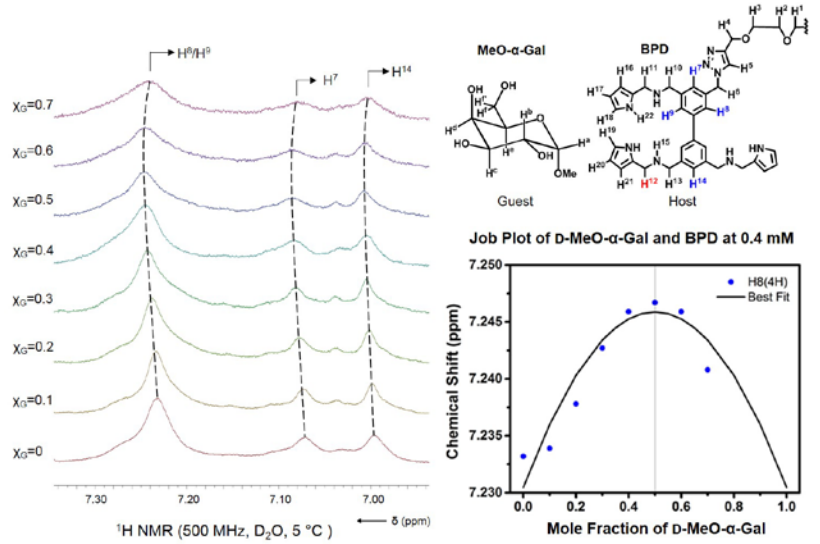


Figure 2. A Job plot indicates that the BPD binds MeO-α-Gal in a 1:1 stoichiometry, demonstrated by a peak occurring at Mole Fraction = 0.5.

BPD Binding Studies. Titrations involving BPD and various monosaccharides were carried out using variable temperature ^{1}H NMR in D_2O to determine the K_as and selectivity of this receptor in aqueous environments (**Figure 1**). Initially, binding was studied between D-monosaccharides that are methylated at the anomeric oxygen because (1) methylation halts the equilibration between α- and β- anomers that would complicate analysis, and (2) non-equilibriating glycans are more reflective of the carbohydrates prevalent on the cell-surface than those possessing anomeric hydroxyl groups. In line with convention, we refer to the monosaccharides possessing anomeric methyl groups as "non-reducing sugars", and those possessing anomeric hydroxyl groups as "reducing sugars". Upon increasing the concentration of monosaccharide to an aqueous solution of BPD, the equilibrium is driven towards a host-guest complex, whose exchange between bound and unbound states is fast on the NMR timescale. As such, the ^{1}H NMR peaks that correspond to the protons of the receptor shift to reflect their changing chemical environment. To ensure that binding occurred, titrations were originally carried out at 5 ^{o}C because reducing temperatures decreases the contributions of entropy, which generally disfavors binding. For every reducing sugar that was titrated, ^{1}H NMR peak shifts characteristic of host-guest complex formation occurred. The magnitudes of the peak shifts – even upon complete association – were not large (<0.4 ppm), but this is not unanticipated in our system that binds primarily through H-bonding: the receptor protons that are monitored by ^{1}H NMR are saturated by H-bonds when solvated and also when involved in the host-guest complexes, and as consequence do not experience a dramatic change in the chemical environment.

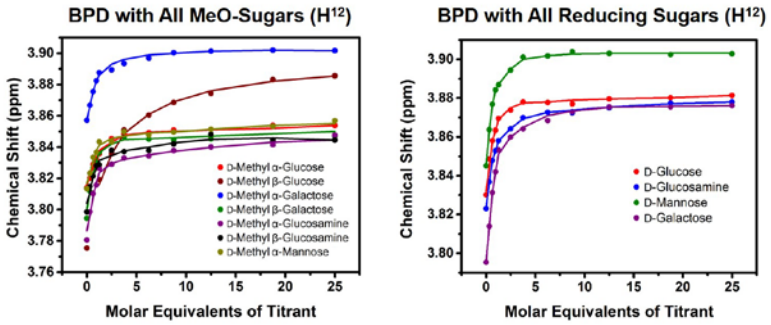
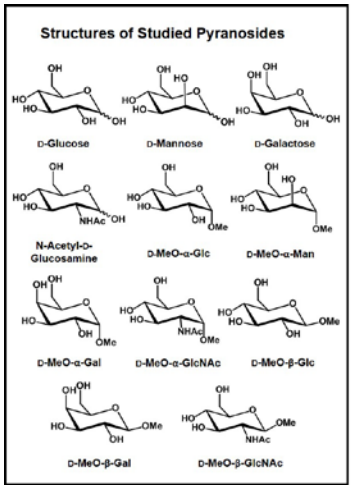


Figure 3. Association constants are determined by fitting ^{1}H NMR peak shift fittings to a 1:1 association model. For each titration all peak shifts are fitted simultaneously, but only the peak shifts for H^{12} of BPD are shown for all reducing and non-reducing sugars measured.

The changes in the ^{1}H NMR peaks upon addition of non-reducing sugars can be used to determine K_as, but only once the binding dynamics are determined. The original Bear Paw receptor bound monosaccharides by accessing simultaneously a number of cooperative and multivalent binding pathways, and as a consequence did not bind guests in a 1:1 stoichiometry. So both the dynamics and the stoichiometry of the BPD:sugar host-guest complexes were determined. Initially, the propensity of the BPD to dimerize was determined by dilution experiments, and it was found that at concentrations below 1 mM, no dimerization occurred. Therefore BPD dimerization does not contribute significantly to association thermodynamics under the conditions that were explored during binding studies. To determine the stoichiometry of binding between non-reducing sugars and BPD, a Job plot was prepared (**Figure 2**), which involves altering the mole fraction (χ) between host and guest, while keeping overall concentration of host and guest constant. The peak of the inverse parabola at χ = 0.5 indicates that the

Association Constants (*K*) Between BPD and Pyranosides



Titrants (5 °C)	log(*K*) (M^{-1})	Δ*G* (kcal/mol)
*Ca^{2+}	3.66 ± 0.05	−4.67 ± 0.07
*D-MeO-α-Gal	3.83 ± 0.04	−4.88 ± 0.05
*D-MeO-β-Gal	3.15 ± 0.02	−4.02 ± 0.02
*D-MeO-α-Glc	3.68 ± 0.04	−4.69 ± 0.05
*D-MeO-β-Glc	3.14 ± 0.01	−4.57 ± 0.02
*D-MeO-α-GlcNAc	3.89 ± 0.04	−4.95 ± 0.05
*D-MeO-β-GlcNAc	3.08 ± 0.03	−3.92 ± 0.05
*D-MeO-α-Man	3.00 ± 0.02	−3.83 ± 0.02
*D-Gal	4.00 ± 0.03	−5.10 ± 0.05
*D-Glc	4.36 ± 0.06	−5.55 ± 0.07
*D-GlcNAc	3.80 ± 0.03	−4.83 ± 0.02
*D-Man	4.13 ± 0.03	−5.26 ± 0.02

*Error values are derived from the NMR error bounds.

Figure 4. Association constants K_a between BPD and various pyranosides that were determined at 5 °C in D_2O by fitting changes in ^1H NMR peak positions upon titration.

host:guest binding stoichiometry is 1:1 (experiments at high-mole fraction of BPD could not be carried out because of receptor solubility). With this this information, a 1:1 binding model can be used to determine K_a from the changes in the NMR peak shifts ($\Delta\delta$) in response to increasing concentration of monosaccharide guest. To quantify the K_a for any particular BPD-monosaccharide pair, the fits between the binding model and the ^1H NMR data were found by minimizing the model to all peak shifts simultaneously to reduce the fit error, thereby providing K_as with high precision. **Figure 3** shows the fits between $\Delta\delta$ of H^{12} of BPD upon addition of non-reducing and reducing sugars, and the binding model used to determine K_a, showing that (1) like native lectins, the BPD is promiscuous and binds all monosaccharides examined, and (2) the ^1H NMR peak shifts could be fit to determine quantitatively the K_as. The K_as between BPD and the various reducing and non-reducing sugars in D_2O at 5 °C are provided in **Figure 4**. It is important to note that the experimentally determined K_as are comparable in magnitude to the strongest synthetic lectins reported in the literature, however these previously reported systems generally bind only glucosides, whereas our aim was to develop a receptor that also associates to non-glucosides. The higher binding affinity for reducing sugars compared to non-reducing sugars arises because of the additional H-bond that can occur with the free hydroxyl group at the anomeric position, and this hypothesis is supported by computational modeling.

To quantify the full binding thermodynamics – specifically the entropy (ΔS^o) and enthalpy (ΔH^o) of binding – the titrations are currently being repeated at various temperatures, and the resulting K_as will be subjected to a van't Hoff analysis. Initial results (**Figure 5**) of the van't Hoff analysis between MeO-α-Gal and BPD explore host-guest complex formation from 5 °C to 35 °C. These data provide ΔS^o = −7.1 e.u. and ΔH^o = −3.3 cal•mol^{-1}. Remarkably, the K_a for the *binding between BPD and MeO-*

Thermodynamic Analysis

(a) **Binding Constants**

D-MeO-Gal / BPD	
Temp (°C)	Log(K)
5.26	4.0314
10.07	3.9156
14.89	3.7268
35.48	3.0299

(b) **Van't Hoff Analysis**

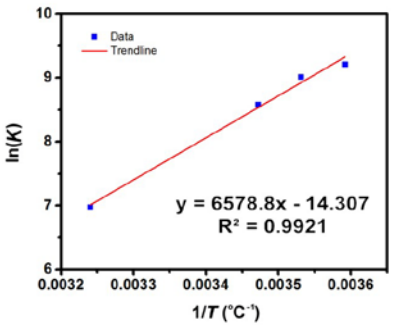


Figure 5. (a) Binding constants between BPD and MeO-α-Gal at various temperatures determined by ^1H NMR titrations in D_2O. (b) van't Hoff plot for the binding between BPD and MeO-α-Gal.

α-Gal exceeds 1000 M^{-1} at 35 °C, suggesting these probes could be used to study carbohydrate recognition in living systems. We anticipate that the binding studies, including full van't Hoff analysis between BPD and all reducing and non-reducing sugars, will conclude by then end of May 2016.

Conclusions and Significance. In the first year of this project, we have developed a new receptor that has the following important attributes: (1) it is water soluble, so it can be used to study carbohydrate recognition in biologically relevant environments, (2) it is a promiscuous binder as a result of its biomimetic design, that seeks to access cooperative and multivalent pathways, and can therefore be used to study non-glucosidic glycans, like those most prevalent on cell surfaces, and (3) has a K_a > 1000 M^{-1} at 35 °C in water, which is, to the best of our knowledge, the highest affinity synthetic carbohydrate receptor with a recognition strength comparable to natural proteins. In addition, synthetic preparation of the BPD is simple and modular so the chemical structure can be modified easily to add functionality or test new theories regarding carbohydrate binding and assembly.

Future Directions. There are two common types of lectins, S-lectins and C-lectins, where in the latter, H-bonds are mediated by Ca^{2+} ions as bridges. We intend to use the BPD scaffold to understand and utilize Ca^{2+}-mediated carbohydrate binding in natural materials and systems. To this end, we are pursuing two goals, currently, (1) measure BPD-sugar binding in the presence of Ca^{2+} ions, and (2) expand the length of the oligoethylene glycol linker to optimize the system for Ca^{2}-mediated binding. Regarding the former, we have begun to study the effects of Ca^{2+} ions on the binding between BPD and various monosaccharides (**Figure 6**). We have found that the presence of Ca^{2+} ions affects both binding affinity, and in the presence of reducing sugars, the ratio between α/β anomers. However, in the original BPD design, the oligoethylene glycol chain is likely too short to optimally bind both Ca^{2+} and monosaccharides, and so we are preparing BPD derivatives with longer

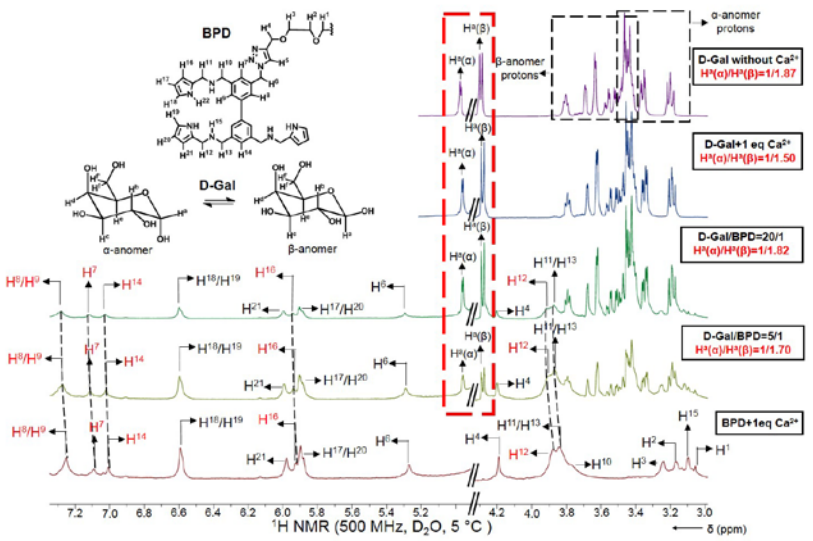


Figure 6. Titration of a solution of D-Gal into an aqueous solution containing BPD and Ca^{2+}, which causes the peaks corresponding to the receptor to shift (indicated by dashed line), reflecting changes in chemical environment upon the formation of a host-guest complex. The anomeric protons are indicated by the dotted red line, showing the change in α/β equilibrium upon complex formation.

oligoethylene glycol chains using the same synthetic scheme that was developed for BPD (**Scheme 1**).

Collaborations. We have recently begun a collaboration on this project with Dr. Chia-Suei Hung (AFRL/RXAS) and Dr. Wendy Goodson (AFRL/RXAS) at the Air Force Research Laboratory, Wright-Patterson AFB. The aim of this collaboration is to determine whether BPD can be used as a fluorescent probe to detect biofilm formation. To this end, we have measured the fluorescence properties of BPD, and found that BPD exhibits strong fluorescence (λ_{ex}= 350 nm, λ_{em}= 420 nm) as a result of the 6 aminopyrrole groups on the receptor. In addition, 25 mg of BPD has been sent to WPAFB for testing.

B. 2. Carbohydrate-Surface Interactions: Glycocalyx Mimics

In the original proposal for this work, the focus on surface chemistry had several aims: (1) create new chemistry that mimics the density and orientation of carbohydrates that occurs in the glycocalyx, and study binding within this environment, (2) develop a screening platform for finding carbohydrate-binding carbohydrates, and (3) develop a new tool for creating molecular arrays with the chemical and structural diversity of the cell surface. The effort devoted to developing a new printing platform in this project has been dropped because it is now part of the MURI grant "4D Nanolithography" (DoD 15RT0675) which was awarded in 2015.

Monitoring Interfacial Lectin Binding with Nanomolar Sensitivity

The binding events between glycan binding proteins, termed lectins, and cell-surface glycans – including glycopolymers, glycoproteins, and glycolipids – are the subject of increasing research focus because of their critical role in intercellular communication, immune response, and disease progression. Glycan recognition within the glycocalyx – the 1 – 100 μm layer of glycans that coat the surface of every eukaryotic cell – is inherently interfacial and as a consequence, multivalency and cooperativity play an outsized role in determining binding strength and selectivity within this dense forest of carbohydrates. To mimic and understand how glycan-lectin recognition occurs in biology, these events must be studied on surfaces, where aspects of the 3D structure of the glycocalyx that influence the association between receptor and ligand can be reproduced. While many instruments for studying interfacial binding exist, the complexity of glycan recognition imposes stringent demands upon their detection sensitivity and ligand presentation. Firstly, these platform for monitoring binding must possess a wide dynamic range, and be able to detect binding at low lectin concentrations. While many glycan-lectin interactions are strong (K_a ~ 10^5 – 10^6 M^{-1}), they are usually far weaker than normal protein-substrate interactions, with K_a typically on the order of 10^3 – 10^1 M^{-1}. Secondly, lectins are normally promiscuous proteins, and bind most monosaccharides weakly in solution. Lectin-substrate specificity is achieved by accessing multivalent and cooperative interactions that occur in the dense matrix of the cell surface, which can increase K_a by up to 10^6 M^{-1}. Thus, to understand lectin-glycan binding in biological systems, the glycans on a sensor surface must be presented with the appropriate valency, density, and orientation to capture dynamics that may occur within the glycocalyx. Therefore, a sensing platform that can detect binding between glycans and lectins that are present in nanomolar

concentrations or quantify differences in binding affinities would be an important tool in advancing the rapidly developing fields of glycobiology, carbohydrate nanotechnology, and in the search for new carbohydrate binding systems.

Current advances in the fabrication of nanostructures have contributed significantly to the development of materials with unique plasmonic properties, whose applications include photovoltaic devices, biomedical sensors, and point of care diagnostics. One such plasmonic device that could contribute to studying glycan binding is the localized surface plasmon resonance (LSPR) sensor, which is a label-free tool that monitors molecular binding in real-time, in small-volume samples, and with picomolar sensitivity when K_a is high ($>10^7\ M^{-1}$) . The biological systems that have been analyzed by LSPR include protein-substrate, nucleotide-nucleotide, antigen-antibody, and glycan-lectin binding. All of the aforementioned examples employ the conventional colorimetric LSPR architecture, where detecting binding involves monitoring changes of reflectance or transmittance of the plasmonic nanostructures. Despite the sensitivity of LSPR, a drawback of these devices is that they require optical detection, which precludes facile integration into microfabricated chips. Moreover, the measured transmittance or reflectance in LSPR sensors is also affected by the medium surrounding the nanostructures, which can produce false readings when the nanostructures and surrounding medium have overlapping feature in their absorption spectrum.

The plasmon FET monitors the plasmonic absorption of Au nanoparticles (NPs) on the device surface by converting the induced plasmonic hot electrons to electrical current, which is accompanied by an amplification of the hot plasmonic electrons that overcome the Schottky junction formed by Au and InGaZnO (IGZO) (**Figure 7a(i)**). Applying a gate voltage bias accelerates the entry of electrons into the n-channel of the plasmon FET (**Figure 7a (ii)**) by producing a vertical potential difference, which drives the electrons that must otherwise diffuse thermally through the IGZO film to reach to the n-channel. Thermal diffusion alone would result in decreased hot electron current as a result of recombination processes and the short mean-free-path of hot electrons in IGZO. Drain current enhancement occurs when excess plasmonic carriers enter the FET current channel (**Figure 1a (iii)**), and as a consequence of this current enhancement, the sensor is sensitively dependent to the changes in the plasmonic absorption. The distinction between the plasmonic FET platform and conventional LSPR is that the former uses light for excitation, but electronic detection, whereas the latter uses both optical excitation and detection. The plasmon FET only detects the plasmonic signal from the nanostructure absorption, which eliminates the parasitic signal from media that occurs in conventional LSPR sensors. As a result of these attributes, the plasmon FET offers 5-fold higher signal-to-noise ratio than conventional LSPR sensors, which makes them ideal for low noise applications – such as glycan-lectin recognition, where binding is inherently weak and complex. Thus, when combined with appropriate surface chemistry, the plasmon FET could lead to breakthroughs in understanding cell-surface binding in an environment intended to mimic directly the interfacial structure of the glycocalyx.

We have developed a platform that can detect binding between glycopolymers appended to Au NPs on the plasmon FET surface and lectins in solution (**Figure 7a**), even when the latter are present in only picomolar concentrations. The glycopolymer coating on the Au NPs is

designed to mimic the glycan density and presentation in the glycocalyx so that the observed binding captures cooperative and multivalent dynamics that are more representative of cell surface recognition than would occur in glycan monolayers. The unique combination of electronic detection and polymer surface chemistry provides a sensing platform with high substrate specificity and that is two orders of magnitude more sensitive than conventional fluorescence detection on a surface composed of identical glycopolymers. This increase in detection sensitivity is particularly important in the context of glycobiology, where binding interactions are weak and lectins are present at exceedingly low concentrations, and as such, this platform – that combines new device architectures with synthetic glycopolymers – has the potential to make important contributions to understanding cell-surface glycan recognition.

Glycopolymer Plasmon FET

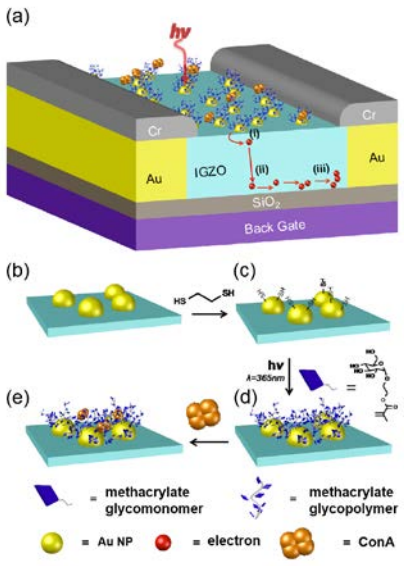


Figure 7. a) A plasmonic field effect transistor (FET) for sensing lectins is coated with a layer of Au NP-glycopolymer conjugates. Increases in plasmon absorption with binding of lectins to the glycopolymers increase the electrical current (dimensions are not proportional): (i) Hot electron transfer from Au NPs to IGZO layer by overcoming the Schottky junction. (ii) The applied gate voltage assists the migration of hot electrons into the current channel of the plasmon FET. (iii) The number of charge carriers increases in the IGZO layer, thereby enhancing the detected signal. Sensor fabrication: b) Au NPs are self-assembled onto the plasmon FET; c) 1,2-ethanedithiol is adsorbed onto the Au NPs; d) glucosyloxyethyl methacrylate is incubated onto the Au NP under UV light (λ = 365 nm) in the presence of a photoinitiator for 2 h to form the glycopolymer via a thiol-acrylate photopolymerization; e) binding of the lectin ConA to the glycopolymers on the Au NP surfaces.

The construction of the lectin-binding plasmon FET involved microfabrication of the device followed by surface-functionalization with synthetic glycopolymers (**Figure 7b-e**). The plasmon FET was fabricated by following previously reported protocols, and the surface was functionalized by the photochemical synthesis of glycan brush polymers with pendant α-D-glucose residues from the Au NPs on the device surface, resulting in a dense carbohydrate forest that mimics the glycocalyx. Briefly, a heavily doped n-type Si substrate (the back gate) with 50 nm of thermally grown SiO_2 film was prepared, and a n-type IGZO film, which serves as the active semiconductor channel (30 μm × 100 μm), was deposited and patterend on top of the SiO_2. The Au NPs are incorporated using a thermal reflow based self-assembly method on top of the IGZO surface, such that they are physically separated from all three electrodes of the plasmon FET. To make the plasmon FET specific towards glucose-binding lectins, the Au NPs were functionalized with methacrylate polymer brushes with pendant D-glucose residues at each repeat unit following a previously reported surface-polymerization approach. Briefly, a drop of 1,2-ethanedithiol solution (1.5 mM in EtOH) was placed onto the IGZO surface to produce thiol-capped Au NPs (**Figure 7c**). After washing with EtOH (10 mL), the substrate was placed in a solution of glycosyloxyethyl methacrylate (1 mM in EtOH) along with the photoinitiator 2,2-dimethoxy-2-phenylacetophenone (1.56 mM), and the surfaces were

exposed to 365 nm light for 2 h to grow the glycopolymers from the Au NPs by a thiol-methacrylate radical photopolymerization mechanism.

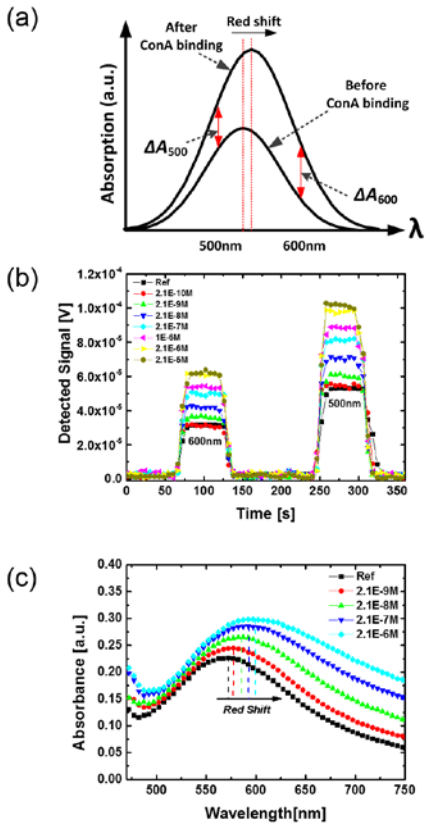


Figure 8. a) Absorption spectrum of the Au NPs in solution, before (bottom curve) and after (top curve) binding of the target analytes. After binding of the analyte to the NP surface, the absorption spectrum broadens, increases in intensity, and redshifts. ΔA_{600} and ΔA_{500} are the changes in absorption in response to binding at 600 and 500 nm, respectively. b) Detected voltage signal through the plasmon FET under two different color excitations (500 and 600 nm) with different concentrations of ConA (black line shows the reference plasmon FET before binding of ConA). c) Absorption spectra using the conventional spectrometer LSPR-based sensing (control experiment) with different concentrations of ConA (the dashed lines show the redshift upon increasing ConA concentration).

To demonstrate the ability to detect lectins in solution, the plasmon FETs with the α-D-glucose side-chain functionalized glycopolymers were exposed to solutions containing different concentrations of the glucose-binding lectin concanavalin A (ConA). The binding between ConA and glycans is a widely studied model system in glycobiology that is often used to validate new lectin-sensing platforms because ConA is widely available, its specificity is well understood, and its K_a towards different monosaccharide substrates has been quantified. To study the binding of ConA onto the plasmon FET, the monochromated input modulated light was delivered onto the sensor surface by an optical fiber with a 45° incident angle. The sensors were biased with 20 V of drain-source voltage and 25 V of gate voltage, which produced approximately 4 μA of drain-source current that is passed through a resistor in series with the plasmon FET. The resulting voltage across the resistor is read by the lock-in amplifier, and is used as the measure of binding between polymer and lectin because voltage increases with the association of the protein to the glyocpolymers on the sensor surface. Binding assays were run by immersing the sensor into the ConA solution in phosphate buffer saline (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 0.5 mM $CaCl_2{\bullet}2H_2O$, 0.5 mM $MnCl_2$) at 4 °C for 4 h (see supporting information for details). The sensors were removed from solution and dried for analysis. To study the plasmon FET response to the ConA solution, measurements were taken at two different excitation wavelengths (500 & 600 nm). To measure the voltage signal generated by the sensor, the plasmon FETs were kept in the dark for 1 min, and then illuminated by 600 nm light for 1 min. Following another 2 min in the dark, the plasmon FET was exposed to 500 nm light for 1 min.. The plasmonic absorption peak of the Au NPs is 560 nm before ConA binding, and the plasmonic absorption peak red shifts to 610 nm when ConA binds to the polymers on the Au NP surface because the presence of the protein increases the effective refractive index of the medium surrounding the Au NPs. The

change in absorption spectrum of Au NPs at two wavelengths (ΔA_{600}, ΔA_{500}) due to ConA binding represents the spectral shift of the plasmonic absorption and is an indicator of binding as illustrated in **Figure 8a**, where ΔA_{600nm} and ΔA_{500nm} is the change in absorption spectrum at 600 and 500 nm, respectively.

Following confirmation that the glycopolymer-modified plasmon FET could detect the presence of ConA, the sensor was exposed to solutions with varying ConA concentration to determine the dynamic range and detection limit of this platform. The measured optical response of the sensor towards solutions with different concentrations of the ConA is shown in **Figure 8b**. Higher concentration of ConA drives complexation, which increases the voltage signal, broadens and red-shifts the absorption spectrum, and ultimately increases signal current. The observed detection limit of the plasmon FET towards ConA – below which no protein could be detected – is 1.0×10^{-10} M, and the sensor saturates at 2.1×10^{-5} M, indicating that the plasmon FET has a five-decade dynamic range. To confirm that the detected signal change occurs in response to the interaction of the lectins to the glycans immobilized onto the Au NPs, a plasmon FET without Au NPs was fabricated. Upon exposure of the glycan-free sensor to the ConA solution, no change in signal was observed, confirming that the previously observed signal changes were the result of lectin-glycan binding modulating the plasmonic signal.

Binding Data for Plasmon FET

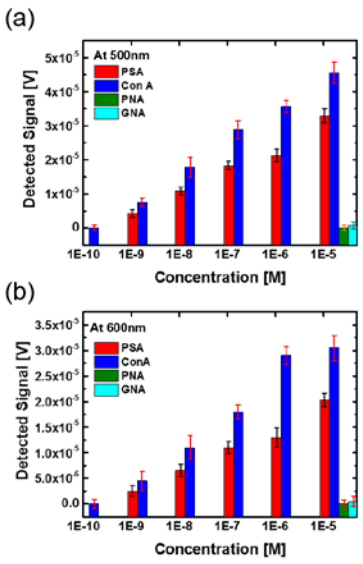


Figure 9. Plasmon FET average voltage change signal with different target proteins (ConA, PSA, PNA and GNA) at a) 500 nm and b) 600 nm excitation at varying protein concentrations. The error bars are a standard deviation from the mean and measured in a single sensor 5 different times.

To compare directly the sensitivity of the glycopolymer-modified plasmon FET to more conventional sensing platforms, we studied ConA binding to the same glycopolymers on a spectrometer-based LSPR sensor with optical detection. The LSPR surface was prepared by depositing Au NPs onto a glass substrate in the same reforming procedure used in the plasmon FET, and the optical absorption spectrum was measured using a conventional optical spectrometer. Following the same protocols used to synthesize the glycopolymers and assess binding to the plasmon FET, absorption spectra were measured following exposure to solutions containing varying concentrations of ConA (**Figure 8c**). By comparing the signal change at each wavelength with increasing ConA concentration for the plasmon FET and the LSPR sensors, we observed that the plasmon FET generates a larger signal response than the LSPR at the same protein concentration. The calculated slope of the least squares regression of the relationship between ConA concentration and signal response at 500 nm indicates that the plasmon FET induces a 5 times larger signal response than the spectrometer-based LSPR sensor. At 600 nm, where the control LSPR sensor shows the highest level of sensitivity, the plasmon FET presents a two-fold larger signal response. Therefore, the plasmon FET, as a consequence of its amplified electrical readout, offers an

enhanced sensitivity towards lectin concentration changes than conventional, spectrometer-based LSPR sensors.

The glycopolymer-modified plasmon FET was also exposed to solutions containing different lectins to demonstrate that the variations in binding affinities between these proteins for glucose known from solution studies would manifest as differences in signal response at equivalent concentrations. In addition to ConA, the plasmon FET was exposed to solutions containing peanut agglutinin (PNA), galanthus nivalis agglutinin (GNA), and pisum sativum agglutinin (PSA). While ConA is known to possess the strongest binding towards glucose of the lectins analyzed, PSA also binds glucose, albeit with lower affinity, whereas neither PNA nor GNA bind glucosides. **Figure 9a,b** show the voltage signals generated by each sensor against different concentrations of each protein at 500 nm and 600 nm, respectively. A lower signal current was observed for PSA compared to ConA for all concentrations. No increase in signal current was observed when the sensor was exposed to PNA and GNA solutions at the high concentration of 2.1×10^{-5} M, and these observations are consistent with expectations based on previously known binding trends.

To determine quantitatively the K_as between the lectins and the glycopolymers on the plasmon FET surface, the current response for each lectin solution was fit to the Langmuir isotherm (1),

$$\Delta R/\Delta R_{max} = (K_a[P])/(1+K_a[P]) \qquad (1)$$

where ΔR is the device response (the change in detected voltage), ΔR_{max} is the maximum response (maximum change in detected voltage), K_a is the binding constant, and [*P*] is the lectin concentration. The Langmuir isotherm is the widely accepted method for determining the strength of association between glycans and lectins in microarray- and SPR-based sensors, and applying this model provides numbers that can be compared across platforms. To obtain the K_a values, the data points were plotted against lectin concentration; the points were fitted according to Eq. 1; K_as are reported as averages from all concentrations; and errors are presented as a standard deviation from the mean value. The K_a for the binding between ConA on the glycopolymer on the plasmon FET surface was determined to be $1.66 \pm 0.14\times10^{7}$ M^{-1} at 500 nm excitation and $1.28 \pm 0.1 \times 10^{7}$ M^{-1} at 600 nm excitation. The K_a for the binding of PSA onto the plasmon FET surface was $6.53 \pm 0.58 \times 10^{6}$ M^{-1} at 600 nm excitation, and $5.81 \pm 0.43 \times 10^{6}$ M^{-1} at 500 nm. The larger value of the K_a for ConA than for PSA is consistent with the trend observed for solution binding. For PNA and GNA, no shifting in the absorption spectrum or increased current was observed, which was expected because neither of these two lectins are known to bind glucose in solution. This control experiment confirms that the observed voltage changes in the sensor are the direct result of lectin-carbohydrate binding rather than nonspecific adsorption. Importantly, the K_a value for the binding between ConA and the glycopolymers on the plasmon FET surface exceeds by nearly four orders-of-magnitude the K_a value of 2.3×10^{3} M^{-1} for the association between ConA and α-D-methoxyglucopyranoside in aqueous solution, and to the best of our knowledge is stronger than any previously reported binding of ConA to glucosides.

Why is this glycopolymer-modified plasmon FET so sensitive towards lectins, and how does this platform provide insight into how multivalency of the glycopolymers contribute to the observed binding dynamics? The binding of ConA to surface immobilized ligands has been studied extensively, and the increased avidity with increasing glycan density and valency is well-established. Using octomeric-dendritic mannoside ligands, for example, a two-fold enhancement in binding was observed compared to monovalent surfaces, resulting in K_as of 2 x 10^6 M^{-1} and a detection limit of 10 nM (0.26 μg mL^{-1}) . The binding of ConA to α-glucose on surface is highly dependent on carbohydrate spacing, and using microarrays the K_a was found to be as high as 8.8 x 10^6 M^{-1} when the glucosides were immobilized in a multivalent fashion on the protein BSA and at high BSA surface density. The K_a of 1.66 ± 0.14×10^7 M^{-1} that we observed between ConA and the glycopolymers on the plasmon FET are higher than these previously reported values, and this unprecedented avidity can be understood by considering the mechanism of ConA binding to multivalent ligands. The four binding sites of ConA are separated by 6.5 nm, and binds multivalent ligands in solution with negative cooperativity as a result of diminishing entropy with each subsequent binding event. As a consequence, the increase in avidity between multivalent ligands and ConA is sensitively dependent on ligand structure. It has been observed previously that ConA attaches to multivalent brush polymers through a bind-and-jump mechanism, where the protein attaches and detaches as it works its way along the polymer backbone towards the surface. Thus, with increasing surface density, avidity increases because of cross-linking between chains. In addition, when studying ConA binding to multivalent polymers, K_a increased with increasing polymer length because sufficiently long polymers could simultaneously interact with the different subunits of the protein. We have shown previously that the glycopolymers prepared under these thiol-acrylate radical photopolymerization conditions are ~1 μm in length, and are therefore sufficiently long to bridge multiple binding sites simultaneously, although the possibility of cross-linking cannot be excluded. These interaction with multiple binding sites simultaneously likely prevent the bind-and-slide behavior observed with shorter polymers or multivalent ligands that cannot bind multiple subunits of ConA simultaneously – like dendrimers or BSA conjugates – thereby leading to the significant avidity enhancement. Thus the picomolar detection sensitivity towards ConA of this glycopolymer-plasmon FET compared to previously used SPR and microarray methods is the result of a combination of the amplified detection signal and enhanced surface avidity.

Conclusions and Significance. The significance of the glycopolymer-modified plasmon FET is that this platform measures quantitatively interfacial interactions with high sensitivity and in an environment that is designed to mimic the glycocalyx.Thus the plasmon FET surface can be modified with glycopolymers to study carbohydrate recognition at sub-nanomolar concentrations. The recently developed plasmon field effect transistor was combined with multivalent glycopolymer brushes, resulting in the most sensitive binding reported between ConA and glucosides. The increased sensitivity relative to conventional optical LSPR or fluorescence sensors occurs because of electronic amplification enabled by the plasmon-to-electric conversion, which eliminates the need for the optical read-out, in combination with the high avidity to the long polymers.

Future Directions. Binding between lectins and glycans in the glycocalyx is profoundly different than free in solution, and understanding these differences may provide insight into carbohydrate-directed assembly and information transfer. But there are few platforms enable researchers to probe these interactions, and how parameters unique to interfaces, such as density, orientation, and the totality of the chemical environment, affect binding. Thus, we intend to expand this aspect of the project in three important directions: (1) Increase the number of glycopolymers immobilized on the plasmon FET by varying the composition of the monosaccharide monomers, (2) study how spacing, orientation, and the presence of fillers affect binding, and (3) use the plasmon FET to determine whether the K_a of BPD increases at interfaces by accessing cluster-glycoside effects. Because of the flexibility and large number of binding groups that arise from the unique structure of the BPD, we anticipate that this molecule will exhibit a substantial increase in binding affinity within the dense forest of carbohydrates on the surface.

Collaborations. We are currently working with Prof. Sung-Jin Kim in the Department of Electrical Engineering at the University of Miami to build glycopolymer-modified plasmon FETs. The aims of this collaboration are to study binding of BPD in an environment that mimics the cell surface and the second is to multiplex the platform so we can study binding onto substrates with significantly greater complexity. This collaboration has already resulted in a submitted manuscript.

B. 3. Altering Assembly of Carbohydrate Feedstock

The final goal of this project – altering the hierarchical assembly of reconstituted carbohydrate feedstock – is dependent upon having new carbohydrate-binding molecules, and having platforms to study binding to carbohydrates, and as such this goal is dependent upon other parts of the project is less advanced. In Year 1, we have developed a new class of molecules for studying carbohdrates – the BPD – and we have also developed a method to understand carbohydrate recognition in a biomimetic environment – the glycopolymer modified plasmon FET. In Year 2 of the project we intend to pursue two goals regarding the assembly and manipulation of carbohydrates: (1) study the reconstitution of carbohydrate feedstock in the presence of molecules that bind to glycans, and (2) search for new carbohydrate feedstock.

Collaborations. We have received samples of tube worm mucus from Prof. Dmitri Deheyn at the Scripps Oceanographic Institute as a source for non-traditional carbohydrate-containing feedstock.

C. Personnel Supported

Personnel Supported	**Percentage of Salary Provided by Grant**
Adam Braunschweig, PI	0.5% (0.6 Summer Month)
Yeting Zheng, Graduate Student	100% (6 Months)
M. Fernando Bravo, Graduate Student	100% (6 Months)

D. Publications

[2] Shokri Kojori, H.; Ji, H.; Paik, Y.; Braunschweig, A. B.*; Park, S. J.* "Monitoring Interfacial Lectin Binding With Nanomolar Sensitivity Using a Plasmon Field Effect Transistor" *In Revision.*

[1] Xu, H.; Zheng, Y.; Munro, C. J.; Ji, Y.; Braunschweig, A. B.* "Carbohydrate Nanotechnology: Hierarchical Assembly and Molecular Logic Using Nature's Other Information Carrying Biopolymers" *Current Opinion in Biotechnology,* **2015**, *34*, 41 – 47.

E. Presentations

[12] Hunter College, Chemistry Department Colloquium, 21 April, 2016
"Carbohydrate Nanotechnology"

[11] University of Florida, Chemistry Department Colloquium, 12 April, 2016
"Carbohydrate Nanotechnology"

[10] Florida International University, Department Colloquium, 23 March, 2016
"On the quantitative determination of binding constants: Implications on supramolecular chemistry, surface science, and solar energy harvesting"

[9] University of Massachussetts, Amherst Chemistry Department Colloquium, 24 February, 2016
"Chasing Emergence: A Supramolecular Approach Towards Optically and Biologically Active Nanosystems"

[8] Tufts University Department of Chemistry Colloquium, 23 February 2016
"Chasing Emergence: A Supramolecular Approach Towards Optically and Biologically Active Nanosystems"

[7] Emory University, Department of Chemistry Colloquium, 15 February, 2016
"Chasing Emergence: A Supramolecular Approach Towards Optically and Biologically Active Nanosystems"

[6] City University of New York, Advanced Science Research Center, 11 February 2016
"Chasing Emergence: A Supramolecular Approach Towards Optically and Biologically Active Nanosystems"

[5] AFOSR Natural Materials and Systems & Extremophiles Annual Program Review, 7-11 December, 2015, Ft. Walton Beach, FL
"Carbohydrate Materials Discovery: Towards a Post-Cellulosic Future"

[4] IX International Congress on Chemical Sciences, Technology and Innovation (Quimicuba' 2015)
Nano and Supramolecular Chemistry Symposium
"3D Nanolithography with Force and Light-Accelerated Reactions"

[3] 250th American Chemical Society National Meeting, Boston, MA 16 – 20 August 2015
Organic Division Young Academic Investigators Symposium
"Increasing the Scope of Organic Reactions for Tailoring the Biotic/Abiotic Interface"

[2] 15th European Symposium on Organic Reactivity, Kiel, Germany, 31 August – 5 September 2015

Journal of Physical Organic Chemistry Award for Early Excellence in Physical Organic Chemistry Award Lecture
"Supramolecular Polymers and the Subtleties of Molecular Recognition"

[1] Gordon Research Conference on Physical Organic Chemistry, Holderness, NH, 21-26 June, 2015
Journal of Physical Organic Chemistry Award for Early Excellence in Physical Organic Chemistry Award Lecture
"Addressing the surface chemistry bottleneck with force- and light-accelerated reactions"

F. Student Theses Defended

[1] Yeting Zheng, UM M.A. 2016

G. Consultative/Advisory Functions

Proposal Reviewer

Deutsche Forschungsgemeinschaft, Bio Division Review Panel National Science Foundation, Army Research Office

Manuscript Reviewer

Accounts of Chemical Research, ACS Applied Materials and Interfaces, ACS Macro Letters, ACS Nano, Angewandte Chemie International Edition, Applied Physics A: Materials Science & Processing, Chemical Communications, Chemical Science, Chemistry – A European Journal, Journal of the American Chemical Society, Journal of Applied Polymer Science, Langmuir, Macromolecules, Nanoscale, RSC Advances, Small, Supramolecular Chemistry

H. New Invention Disclosures and Patents

Patents Issued

Braunschweig, A. B.; Rieth, S.; Miner, M. R. "Carbohydrate Selective Receptors" *US 9,296,688 B2*

I. Honors/Awards

Braunschweig; PI

[1] Royal Society of Chemistry *Chemical Society Reviews* Emerging Investigator 2016
[2] ***Journal of Physical Organic Chemistry Award for Early Career Excellence in Physical Organic Chemistry 2015***
[3] ACS Organic Chemistry Young Innovator 2015
[4] UM Provost's Research Award 2015
[5] *Polymer Chemistry* Emerging Investigator 2015

Peurifoy, Samuel R.; Undergraduate Researcher

[1] RISE Fellowship, Germany, 2015

[2] Winner Clinton Global Initiative University Resolution Project Social Venture Challenge 2015
[3] ***National Defense Science and Engineering Graduate Fellowship*** 2016-2019
[4] American Chemical Society Undergraduate Award in Organic Chemistry 2016

Zhou, Yujia; Undergraduate Researcher
[1] ***Goldwater Fellowship*** 2016-2018
[2] Beyond the Book Summer Research Fellowship 2016