Multiplexed Silicon Nanophotonic Biosensing via Immobilized Protein Glycoconjugates

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Abstract and Introduction:

Cell surface interactions are responsible for a myriad of biological processes, including cell signaling, cell growth, morphology and host-pathogen interactions. Many of these cellular functions are mediated by cell surface carbohydrates and glycoconjugates—including glycolipids, glycoproteins and extracellular polysaccharides. In particular, carbohydrates frequently act as receptors in the biochemical mechanisms involved in intercellular communication. Because glycans play an integral role in cellular biology, our interests lie in developing methods to better understand the biological functionality of glycans. One technology that can potentially offer such insight is silicon nanophotonic biosensors.

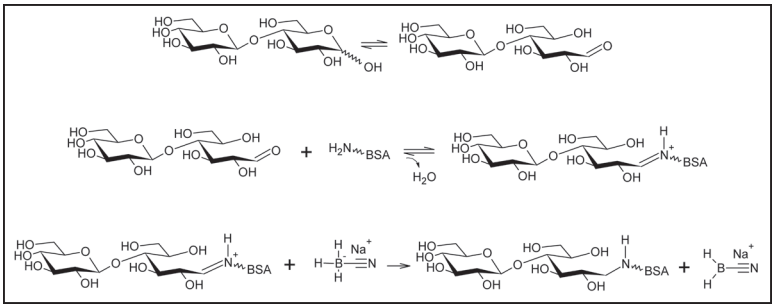

Figure 1: Reductive amination reaction scheme (lactose, BSA).

This project focuses on the integration of glycan receptors with silicon nanophotonic biosensors through the development of an optimized (i.e., low cost and time) protein-carbohydrate conjugation and immobilization scheme. Protein-carbohydrate conjugates were used to present glycans of biological interest on silicon photonic platforms for the purpose of developing functional biosensors for glycomics applications. Both disaccharide-protein and tetrasaccharide-protein conjugates were employed to validate the bioconjugation strategy, and bioactivity was confirmed by the binding of relevant plant lectins (carbohydrate-binding proteins) ricin (RCA) and concanavalin A (ConA). Finally, we demonstrated facile immobilization of oligosaccharide-protein conjugates for the specific and multiplexed detection of lectin analytes in solution using an integrated silicon nanophotonics biosensing platform.

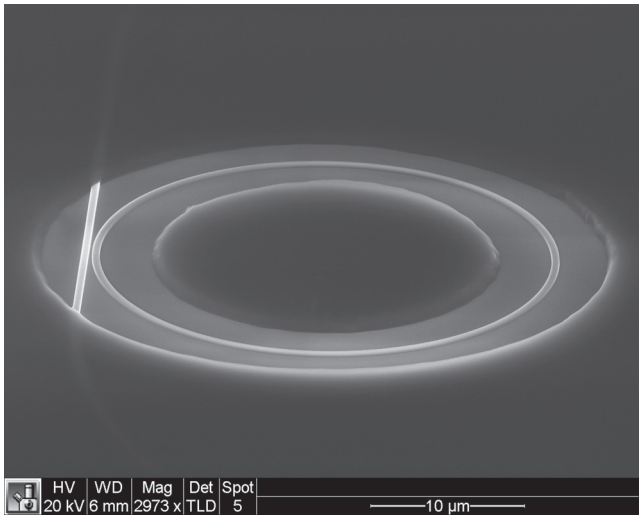

Figure 2: Microring SEM image (Courtesy of M. Hochberg). As light travels within the linear waveguide, photons of frequency matching a resonant mode of the micro-ring are coupled into the ring. Moreover, the rings are constructed such that the coupled light experiences a phase shift of π. Any light coupled into the ring thus destructively interferes with incoming light in the linear waveguide, resulting in a sharp negative peak in the optical power spectrum density of the linear waveguide.

Methods:

General Procedure for Reductive Amination. Reductive aminations were carried out as described by Gildersleeve, et al. [1]. Lactose and maltotetrose were conjugated to bovine serum albumin (BSA) to form BSA-galactose and BSA-maltotriose (BSA-M3) respectively.

BSA (150 mg/ml), sodium borate (200 mM solution, pH 8.5), sodium sulfate (3M solution), saccharide (20 mM solution), water, and sodium cyanoborohydride (3M solution) were mixed then incubated at 56°C for 96 hours. Samples were then dialyzed against water for two days. The reaction scheme is shown in Figure 1.

Mass Spectrometry. Matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) was used to characterize conjugation efficiency. More specifically, the molecular masses of conjugates were compared to that of bare BSA protein (66.5 kDa). Mass spectrometry was carried out on a Bruker Autoflex II MALDI-TOF instrument with 25% laser power in linear mode. We used α-cyano-4-hydroxycinnamic acid (CHCA) as the matrix for all the MALDI analysis. Samples were prepared by spotting 1 μl BSA/BSA-glycoconjugate and 1 μl CHCA onto MALDI target.

Silicon Microring Biosensing. Each microring structure is fabricated adjacent to a linear silicon waveguide. Figure 2 shows a scanning electron microscopy (SEM) image of the microring device. The resonant modes of a microring are directly proportional to the effective refractive index of the nearby media [3]. Any change in the refractive index (e.g. binding of a biomolecule) results in a change in the resonant frequency of the microring, and a corresponding shift in the negative peak of the linear waveguide optical power spectrum density.

To assess bioactivity, 1 μl BSA-glycoconjugate was first spotted onto the silicon surface via silicone masks, functionalizing the biosensor surface. The model lectins, RCA and ConA were used to test for bioactivity. RCA specifically binds to galactose residues (BSA-galactose) whereas ConA to mannose or glucose residues (BSA-M3). As buffer was passed over the chip via integrated fluidics, a laser diode was rastered across discrete grating couplers in order to interrogate microrings. Photo-detectors were used to determine the output band of least power and thus the resonant frequency of the microring.

Results and Discussion:

Mass spectrometry determined our conjugation ratio to be approximately seven sugars per BSA molecule (Table 1). Multiplexed analysis with both BSA-galactose and BSA-M3 functionalized on the sensor platform was performed successfully (Figure 3). Specific binding to RCA was observed only with BSA-galactose functionalized microrings, while only BSA-M3 microrings responded to ConA. We have demonstrated both a low-cost conjugation scheme and the immobilization of conjugates for the specific and multiplexed detection of lectin analytes in solution on integrated nanophotonic platforms. In addition to the utility of our method, the incorporation of masking and spotting furthers the cost effectiveness of this conjugation scheme (only 1 μl of reagent is required per spot, and effective conjugation is achieved within 96 hours).

Conjugate	m/z [kDa]	Sugar Molecular Weight [Da]	Ratio[a]
BSA	66.5	–	0
BSA-galactose	68.9	360.31	6.90
BSA-M3	71.6	666.58	7.65

Table 1: MALDI-MS Analysis of BSA, BSA-galactose, BSA-M3 and calculated ratio. [a = Calculated number of sugars bound per BSA.]

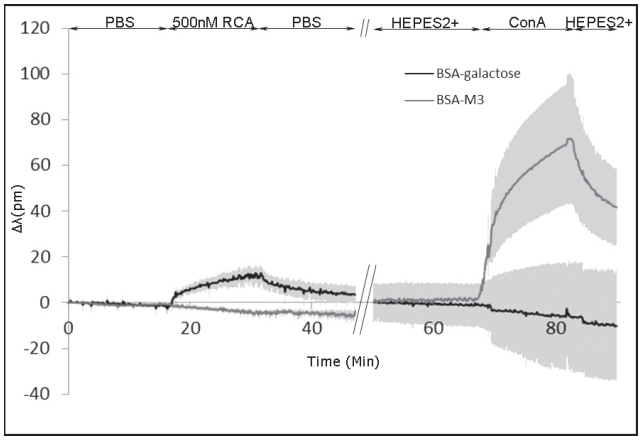

Figure 3: Microring association and dissociation curves for multiplexed testing of BSA-galactose and BSA-M3 functionalized rings with RCA and ConA. Double-hash marks at 50 minutes indicate renormalization from PBS buffer to HEPES2 + buffer.

Acknowledgements:

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References:

[1] Gildersleeve, J., O. Oyelaran, J. Simpson, and B. Allred. "Improved Procedure for Direct Coupling of Carbohydrates to Proteins via Reductive Amination." Bioconjugate Chem 19.7: 1485-490, 2008.

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[3] Washburn, A.L., L.C. Gunn, and R.C. Bailey. "Label-Free Quantitation of a Cancer Biomarker in Complex Media using Si Photonic Microring Resonators." Analytical Chem (2009): 091022153841077.