Introduction:
Through recent advancements in optics and semiconductor fabrication, silicon photonics has lent itself to the development of a versatile class of real-time, label-free biosensors. One of the most promising biosensing platforms to emerge from this field is the silicon photonic ring resonator array. The array used in these studies consisted of 32 microrings, each of which had been fabricated onto a silicon-on-insulator chip using complementary metal oxide semiconductor (CMOS) processing [1]. Light from a tunable laser was coupled into each ring cavity via an adjacent waveguide, and a photodetector was used to measure the resonant spectra of each microring [2]. Biomolecular samples were then introduced to the array using syringe pumps and on-chip microfluidics [1]. When biomolecules bind at or near a ring cavity functionalized with its complimentary ligand, they induce a shift in the ring’s refractive index, and thus, a shift in its resonant wavelength [2]. Using data acquisition software, these shifts can be measured in real-time—allowing us to both detect and quantify biomolecular binding.

A fundamental challenge to this technology is our limited understanding of how surface chemistries influence biomolecular interactions at the biosensor interface. As a simple validation model for the platform, we investigated the binding of the protein, Concanavalin A (ConA), to mannose-functionalized ring resonators. To examine how chemical modification affects biomolecular binding, we implemented two different methods to immobilize the mannose ligand onto the silicon surface. We then used atomic force microscopy (AFM) to characterize silicon substrates modified for biomolecular detection and to investigate the binding of biomolecules to the biosensor surface. Real-time binding experiments offered further insight into how immobilization methods influenced the performance of the biosensing platform.

Experimental Procedure:
Surface Functionalization. The ring resonator chips were first cleaned in a piranha solution (1:1 sulfuric acid: hydrogen peroxide), then dried under a stream of nitrogen. Mannose was then immobilized either covalently using silanization chemistry, or non-covalently via physical adsorption of a bovine albumin (BSA)-mannose conjugate. Covalent immobilization of mannose began with the activation of the silicon oxide surface by immersing the silicon chips in a 1% (v/v) epoxy-silane in toluene solution for six hours. The chips were then incubated in 0.2 mM D-mannose-(OEG)2-SH for 12 hours. To prevent non-specific binding to the biosensing surface, functionalized substrates were blocked with 1 µM BSA for one hour. Functionalized chips were then allowed to equilibrate in HEPES buffer for at least eight hours. On separate silicon chips, mannose was instead non-covalently immobilized onto the surface by immersing cleaned substrates in a 0.5 mg/mL solution of BSA-mannose for 90 minutes at 37°C. The surface was then thoroughly rinsed in deionized (DI) water and dried under a nitrogen stream.

Protein Adhesion. For the purpose of AFM characterization, both the covalently and non-covalently functionalized...
substrates were incubated in a 1 µM solution of ConA for 30 minutes at room temperature. Chips were then rinsed under de-ionized (DI) water and blown with nitrogen. Alternatively, some covalently and non-covalently functionalized ring resonator chips were used to monitor protein binding in real-time. Different concentrations of ConA were flowed directly over ring resonator chips, and binding was quantified by measuring shifts in the resonant wavelengths of each microring.

Characterization. AFM images were acquired in tapping mode using a Dimension 3100 Atomic Force Microscope. A FESP tip was used to obtain topographic maps of the silicon surface after each stage of surface modification (i.e., cleaning, mannose immobilization, ConA binding). Nanscope software was used to measure surface roughness by averaging three 250 × 250 nm areas from each image.

Results and Conclusions:
Preliminary AFM characterization indicates that the methods used to immobilize mannose influenced the adhesion of protein to the biosensor surface. AFM scans showed a homogeneous distribution of ConA on surfaces modified with covalently-bound mannose (see Figure 2d). Conversely, surfaces modified with physically adsorbed BSA-Mannose showed aggregation and uneven distribution of ConA (see Figure 2b), with some images (not shown) displaying little or no protein binding. After ConA binding, covalently-functionalized surfaces were found to have a surface roughness of 0.69 ± 0.04 nm, whereas non-covalently functionalized surfaces were found to have an average roughness of 0.97 ± 0.11 nm. As uniform biomolecular adhesion is critical for a reliable and reproducible biosensing device, the lower surface roughness suggests that covalent immobilization is optimal for the detection and adhesion of ConA.

ConA binding was also detected in real-time using ring resonators that were functionalized by means of either silane chemistry or physical adsorption (see Figures 3 and 4). Results were characteristic of specific binding and demonstrate the ring resonator’s ability to detect carbohydrate-mediated protein adhesion. Binding profiles showed larger response curves for ring resonators functionalized with covalently immobilized mannose, suggesting that the covalent immobilization of mannose enhanced protein-surface interactions.

These results demonstrate the ring resonator platform’s potential as a versatile bioanalytical tool. By further optimizing the surface chemistries used for detecting ConA and other more clinically-relevant biomolecules, this novel biosensing technology will be poised to renovate conventional paradigms for disease diagnostics.

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