Supplementary Material (ESI) for Lab on a Chip

Electronic supplementary information (ESI) for:

Multiplexed inkjet functionalization of silicon photonic biosensors

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Materials

Bovine serum albumin (BSA) was purchased from EMD Chemicals (Gibbstown, NJ). Sulfo-SMCC, TCEP Resin and Zeba Desalting Columns were purchased from Pierce (Rockford, IL). Filter microcentrifuge tuges were purchased from Corning, Inc (Corning, NY). Sugar-thiols and OEG-thiols were synthetically derived as previously described. [1-3] AlexaFluor488-conjugated streptavidin was purchased from Invitrogen Corp. (Carlsbad, CA). Concanavalin-A (ConA) was purchased from MP Biomedical (Solon, OH). RNase B was purchased from New England Biolabs (Ipswich, MA). Ricin (RCA) was purchased from EY Laboratories (San Mateo, CA). Griffithsin was generously provided by Dr. Barry R. O'Keefe of the Molecular Targets Laboratory of the National Cancer Institute. All other chemical reagents were purchased from Sigma-Aldrich (St. Louis, MO) and used as received without further purification. Millipore-filtered water was used for all aqueous solutions and buffers. All

binding experiments were performed in phosphate-buffered saline (PBS; 150mM NaCl, 10mM phosphate, pH 7.4) or HEPES2+ buffer (20mM HEPES, 150mM NaCl, 1mM $CaCl_2$, 1mM $MnCl_2$, pH 7.3) as noted.

Synthesis of BSA-glycoconjugates

BSA-glycoconjugates were fabricated to provide a facile method to immobilize and display sugars on individual silicon ring resonators. Thiolated sugars (mono-mannose, galactose, lactose residues) and thiolated oligoethelyne glycol (OEG) were attached to the free amines of the BSA by way of the heterobifunctional cross-linker sulfo-SMCC. BSA was dissolved in PBS at an initial concentration of 10 mg/ml and activated for thiol conjugation by incubating for one hour at room temperature with sulfo-SMCC mixed at a final concentration of 1.15mM. Free sulfo-SMCC was removed using desalting columns, following the manufacturer's instructions for a buffer exchange into PBS. Sugar- and OEG-thiols, at a starting concentration of 4 mM in water, were reduced for one hour at room temperature in an equal volume of TCEP resin slurry. Prior to addition of the sugar- and OEG-thiols, the TCEP resin suspension buffer was removed by centrifugation at 1500xG for one minute and removed by pipette. In order to keep the resin suspended in solution, the microcentrifuge tubes containing the mixture were shaken at 1500 rpm on a shaker. Immediately following reduction, the sugar- and OEG-thiols were separated from the TCEP resin using filtration microcentrifuge tubes and centrifuging at 1500xG for one minute. Equal volumes of each of the 4mM sugar- and OEG-thiols were mixed with the 10 mg/ml maleimide-activated BSA and incubated for two hours at room temperature. After conjugation, free sugar- and OEG-thiols were removed using desalting columns, following the manufacturer's instructions for a buffer exchange into PBS.

A bicinchoninic acid assay (Pierce; Rockford, IL) was performed on the BSA conjugates (BSA-mannose, BSA-lactose, BSA-galactose) to determine the protein concentration, at which point they were aliquoted and stored at -20^{o}C at a 4 mg/ml concentration of BSA. Prior to printing, BSA conjugates were diluted in PBS to a working concentration of 0.5 mg/ml.

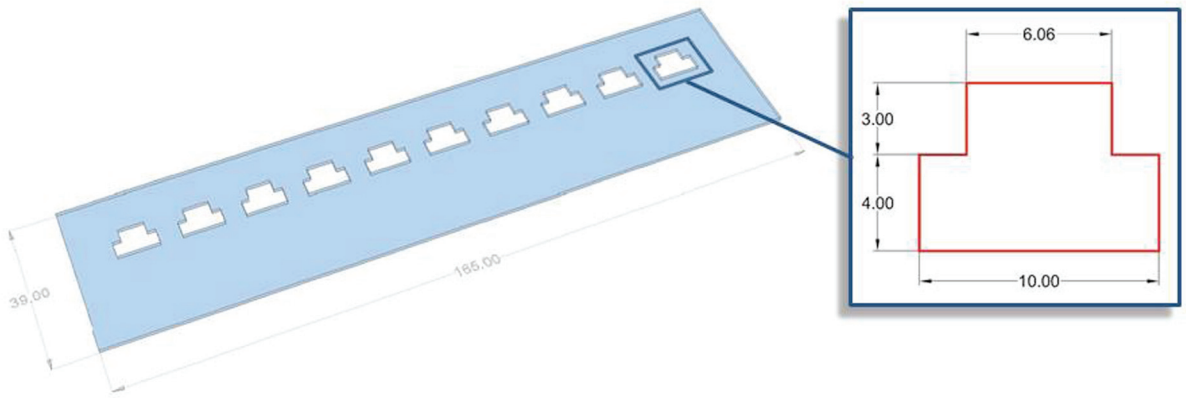

Fig. S1. The multi-chip holder secures 10 microring resonator chips in place to be functionalized using the Scienion S3 Flexarrayer non-contact printer. The dimensions of each slot are shown on the right.

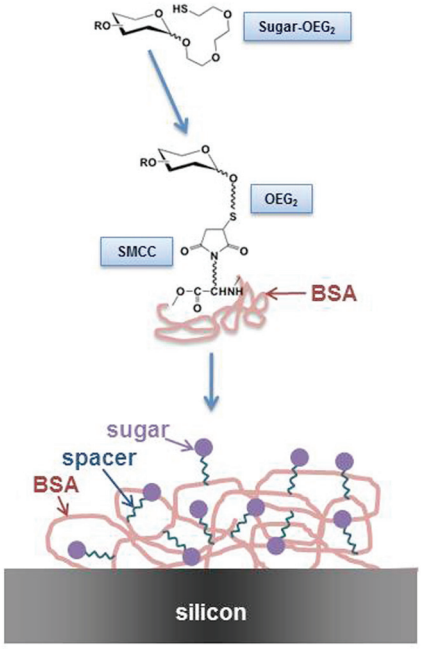

Fig. S2. Sugars are initially synthesized with a spacer (OEG_2) and a functional group (thiol) so that they can be covalently immobilized to support structures – in this case, the sugars are immobilized to bovine serum albumin (BSA) using the heterobifunctional cross-linker sulfo-SMCC. The neoglycoconjugates are then passively adsorbed onto the silicon surfaces for biosensing.

References

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2. D. Ratner and P. Seeberger, *Curr Pharm Des*, 2007, **13**, 173-183.
3. M. Dhayal and D. Ratner, *Langmuir*, 2009, **25**, 2181-2187.