Supporting Information

Fabrication of Carbohydrate Microarrays through Boronate Formation

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Materials. Free reducing oligosaccharides were purchased from Carbosynth and Dextra. All chemicals were purchased from Sigma-Aldrich and Acros and used without further purification. Lectins were purchased from Sigma and Vector Laboratories. 5-amino-2-hydroxylphenyl boronic acid (AHMPBA) was purchased from Combi-Blocks Inc. Anti-dextran monoclonal antibody (clone DX1) was purchased from StemCell Technologies. Fibroblast growth factor 2 (FGF-2) and biotin conjugated rabbit anti-FGF-2 antibody were purchased from US Biological Inc. Antibody to Lex (clone P12) was purchased from GeneTex. Antibody to SLex (clone KM93) and Cy3-conjugated goat anti-mouse IgG and goat anti-mouse IgM (μ chain) were purchased from Millipore. Vantage nuclease-free aldehyde slides were purchased from CEL Associates, Inc.

Preparation of boronic acid-coated glass slides. The hydrophilic BSA based amine glass slides were produced by immersing the aldehyde slides into 5% BSA (w/v) solution at 4 ℃ for 5 h and then 25% glutaric dialdehyde in H_2O (Acros) at 4 ℃ for 5 h. Above BSA-dialdehyde coating procedures were repeated for three times to create triple BSA hydrophilic layer. After washing with deionized water, the slides were dried by centrifugation. The slides were kept in a Pap Jar containing a solution of glutaric anhydride (0.25 M) and pyridine (0.25 M) in DMSO with gentle shaking for 5 h. The slides were washed with deionized water, and then dried by centrifugation. Subsequently, the slides were immersed in a solution of 3-aminophenylboronic acid (APBA, 100 mM), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC, 100 mM), hydroxybenzotriazole (HOBt, 100 mM) and *N*,*N*-diisopropylethylamine (DIPEA, 100 mM) in DMSO. After gentle shaking for 7 h, the slides were washed with DMSO and deionized water. The slides were dried by centrifugation and stored at 4 ℃ until use.

Printing of arrays. Microarray printing was performed by robotic contact printing with an AD1500 Arrayer (BioDot) fitted with Stealth Pins SMP3 (ArrayIt Corporation). Carbohydrates (10 mM for oligosaccharides, 50 μg/ml for polysaccharides) were dissolved in 300 mM sodium phosphate buffer (pH 8.5) and distributed in 0.2 ml PCR tube (20 μL per sample). The glycan arrays were fabricated by printing of approx. 6 nL of carbohydrate samples onto boronic acid-coated glass slides with a 0.4 mm pitch between spots. The needles dwell time in the wells was 5 seconds and the pins underwent 3 wash cycles in between visits. Thereafter the slide was placed into a humidity chamber at 4 ℃ for 16 h or under microwave irradiation for 2 min. The slide was washed with TBST (20 mM, pH 7.4, 0.1% Tween 20) and deionized water, and immersed in 3% BSA (w/v) solution at room temperature for 1 h. The slides were washed with TBST and deionized water, and then dried by

centrifugation.

Lectin and antibody staining. The blocked slides were divided into 16 blocks each with a 9 × 9 mm^2 format by an incubation blocker. Incubation followed a two-step procedure, in which at first solutions of biotin-labeled lectins ConA (10 μg/ml), RCA120 (10 μg/ml), AAL (10 μg/ml) and MAA (20 μg/ml) in TBST (30 μL per well) and antibodies dextran (1:100), Lewis X (1:50) and sialyl Lewis X (1:50) were placed in the relevant wells. For ConA binding, $MnCl_2$ and $CaCl_2$ were added at final concentrations of 1 mM. Then Cy3-conjugated streptavidin and Cy3-conjugated secondary antibodies (1:200) in TBST (5 μg/mL, 30 μL per well) was placed in all wells for detection. The slides were incubated for 1 h at 4 ℃ in a humidity chamber for each incubation step. In between incubations the slides were washed with TBST, rinsed with deionized water and dried by centrifugation. The slides were finally scanned by using an ArrayWoRx biochip scanner (Applied Precision) to visualize fluorescence. Fluorescence intensity was analyzed using ArrayVision 8.0 software from Imaging Research, Inc.

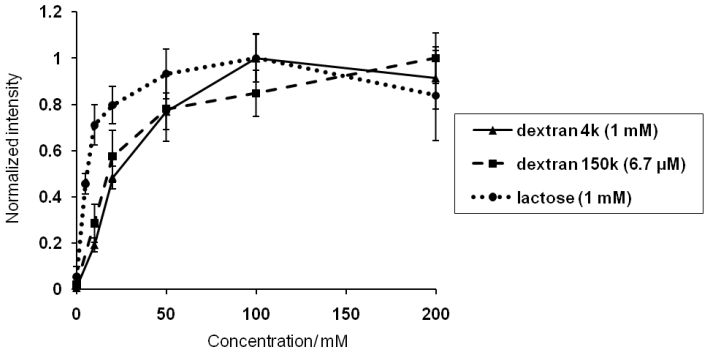

Figure S1. Immobilization of FITC labeled dextran-4k, dextran-150k, and lactose on the APBA-functionalized glass surfaces with various concentrations. The incubation conditions for dextran 4k and 150k are at 4 ^{o}C for overnight and that for lactose is microwave irradiation for 3 min. Data points represent the mean of experiments performed in 25 spots, and error bars show the standard deviations of the mean.

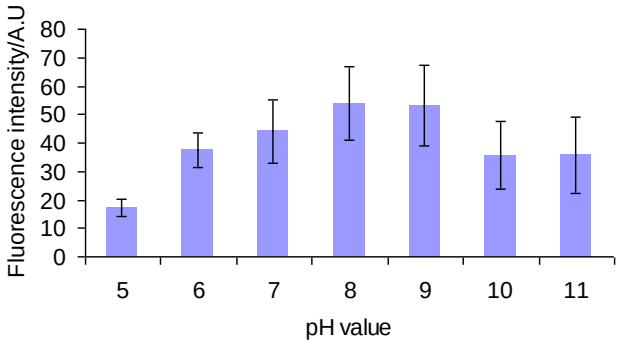

Figure S2. Immobilization of lactose–FITC in the phosphate buffer with different pH value on the APBA-functionalized glass surfaces. Data points represent the mean of experiments performed in 25 spots, and error bars show the standard deviations of the mean.

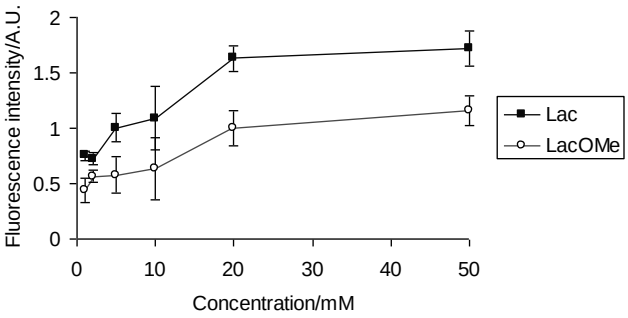

Figure S3. The various concentrations (1-50 mM) of methyl lactoside and lactose immobilized on the BA slides were probed with FITC-RCA_{120}.

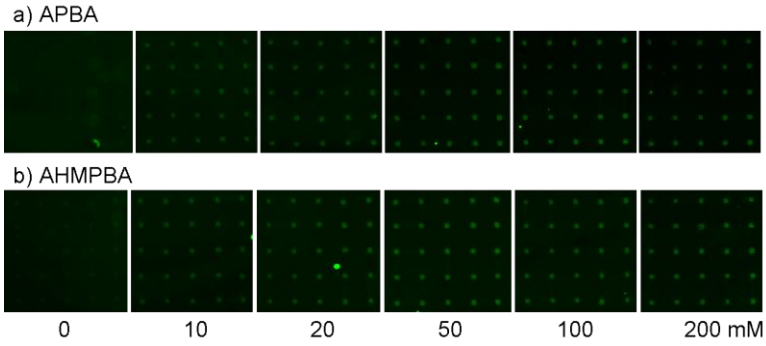

Figure S4. Immobilization of dextran 4k-FITC on the APBA and AHMPBA functionalized glass surfaces with various concentrations (0-200 mM).

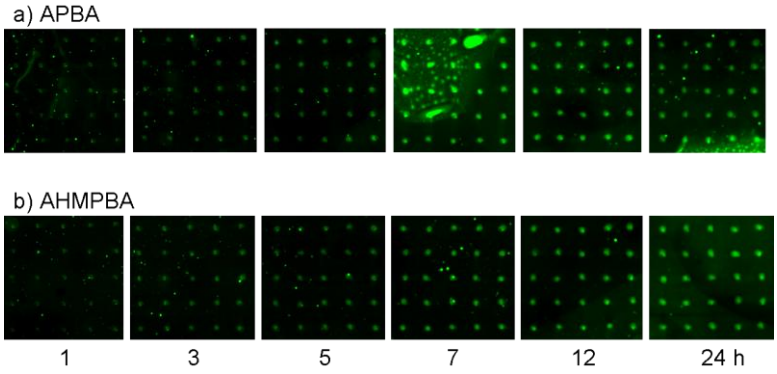

Figure S5. Immobilization of dextran 4k-FITC on the APBA and AHMPBA functionalized glass surfaces with various immobilization times (0-24 h).

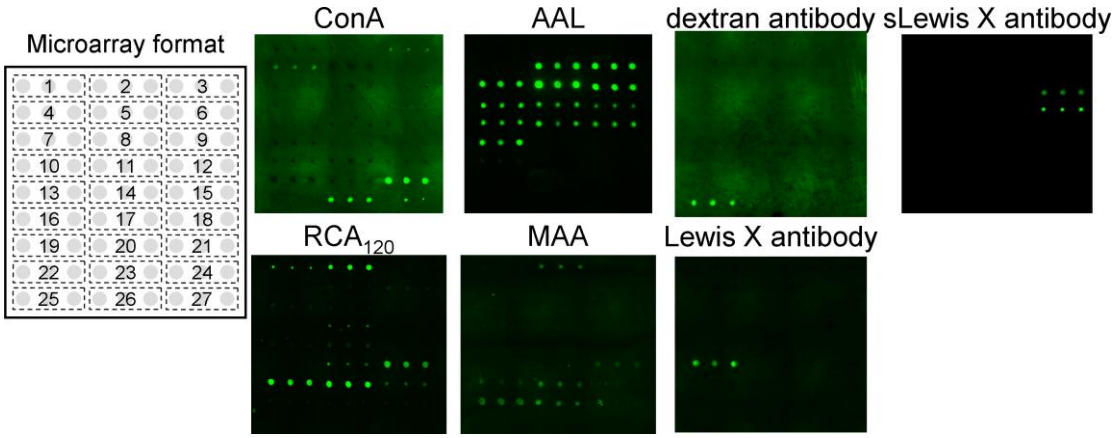

Figure S6. Array format: each array includes 16 replicated subarrays; in each subarray, 27 glycans were printed in 3 replicates each. Fluorescence images of carbohydrate microarrays containing 27 carbohydrates probed with biotinalyted lectins (a) ConA, (b) RCA_{120}, (c) AAL, and (d) MAA, followed by incubation with Cy3-streptavidin, and antibodies (e) dextran, (f) Lewis X and (g) sialyl Lewis X, followed by incubation with Cy3-conjugated secondary antibodies.