Supplementary information for:

Studying the interaction of carbohydrate-protein on the dendrimer-modified solid support by microarray-based resonance light scattering assay

Xiaomei Li[a,b], Jingqing Gao[a,b], Dianjun Liu[a] and Zhenxin Wang[*a]

[a] *State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, 130022, P. R. China*

[b] *Graduate School of the Chinese Academy of Sciences, Beijing, 100039, P. R. China*

[*] *Corresponding author. Fax:+86-431-85262243; E-mail: wangzx@ciac.jl.cn*

Supplement information: Experimental section and Fig. S1-S11

Experimental section

Materials and reagents

Hydrogen tetrachloroaurate trihydrate ($HAuCl_4 \cdot 3H_2O$), ethylenediamine (EDA) core poly(amidoamine) starburst generation 4 (G4) dendrimers with 64 surface primary amino groups (PAMAM, 10% (w/w) stock solution in methanol), glutaraldehyde solution (GA, 25% aqueous solution), amino modified monosaccharides (4-aminophenyl α-D-mannopyranoside (Man-α), 4-aminophenyl β-D-galactopyranoside (Gal-β) and 4-aminophenyl α-D-glucopyranoside (Glc-α)), maltose (Mal), mannose (Man), glucose (Glc), biotinylated concanavalin A from *Canavalia ensiformis* (Con A-biotin), avidin, and bovine serum albumin (BSA) were obtained from Sigma Corp. (USA). Streptavidin was purchased from Promega Co. (USA). Biotinylated *Ricinus communis* agglutinin (RCA 120-biotin) was obtained from Vector Laboratory Ltd. (Burlingame, CA). Peptides (CALNN and CALNNGK-(biotin)G) were purchased from Scilight Biotechnology Ltd. (Beijing, China). Lactose (Lac) and Sucrose (Suc) were obtained from DingGuo Ltd. (Beijing, China). Aldehyde modified glass microscope slides (named as 2D slides) were obtained from CapitalBio Ltd. (Beijing, China). Other chemicals were analytical grade. Milli-Q water (18.2 MΩ.cm) was used in all experiments.

Preparation of peptide-stabilized nanoparticles

Thirteen nanometer gold nanoparticles were synthesized by the traditional "Turkevich-Frens" method.[47,48] Peptide-stabilized gold nanoparticles (biotin@GNPs) were prepared by the previously reported peptide capping procedure.[49,50] Generally,

an aqueous solution of peptide mixture (CALNN and CALNNGK(biotin)G) was added into the solution of 5 nM 13 nm gold nanoparticles to give a final concentration of total peptide of 1.5 mM. The molar ratio of CALNN and CALNNGK(biotin)G in the mixture is 9:1. After 1 h incubation, excess peptides were removed by repeated centrifugation at 13000 rpm (~16100 g, 3 times) using an Eppendorf centrifuge (Eppendorf, Germany). The purified gold nanoparticles were re-suspended in Milli-Q water and stored at 4 °C.

Generation of the PAMAM dendrimer modified surface

The aldehyde modified glass slides were incubated in a solution containing PAMAM (0.1% (w/w), 30 mL) in methanol for 12 h at room temperature under gentle agitation. Then, the slides were washed with 30 mL of methanol for 3 min (3 times) to remove excess dendrimers, dried by centrifugation (480 g for 1 min), and reacted with 30 mL of $NaBH_4$ (10 mM) for another 30 min. Subsequently, the modified surface was activated by 30 mL of GA (5% in PBS (pH 7.4, 50 mM PB, 0.15 M NaCl)) for 4 h, following washed with 30 mL of PBS (3 times) and 30 mL of water (3 times), respectively. Finally, the slides were dried by centrifugation (480 g for 1 min) and stored at 4 °C. The PAMAM modified slides were named as 3D slides.

Fabrication of carbohydrate microarrays

Amino modified monosaccharides (Man-α, Glc-α and Gal-β) with desired concentration were dissolved in 15 μL of spotting buffer (pH 7.4, 50 mM PB, 0.15 M NaCl supplemented with 15% (v/v) glycerol) and spotted on the 3D slides or the commercial 2D slides by a SmartArrayer 48 system (Capitalbio Ltd., Beijing, China)

under contact printing mode, respectively. After an overnight incubation under 75% humidity at 37 °C, the slides were rinsed with 50 mL of washing buffer (pH 7.4, 50 mM PB, 0.15 M NaCl supplemented with 0.1% (v/v) Tween-20; 3 times) and then immersed in 30 mL of blocking buffer (pH 7.4, 50 mM PB, 0.15 M NaCl supplemented with 1% (w/w) BSA and 0.1 M ethanolamine) at 30 °C for 1 h to de-active remaining free aldehyde groups, respectively.

Protein recognition assay

After blocking step, each array was incubated with biotin-modified lectins, Con A and RCA 120, which were diluted to the desired concentrations with 20 μL of probe buffer A (pH 7.4, 50 mM PB, 0.15 M NaCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$, 0.5% (w/w) BSA and 0.1% (v/v) Tween-20). Following 1 h incubation at 30 °C, the slides were subjected to a series of rinsing steps: (1) 50 mL of washing buffer for 3 min (3 times); (2) 50 mL of PBS buffer for 3 min (3 times) and (3) 50 mL of Milli-Q water for 3 min (3 times), respectively; and dried by centrifugation (480 g for 1 min). Then, the microarrays were incubated with 20 μL avidin or streptavidin solution (50 μg/mL in probe buffer B (pH 7.4, 50 mM PB, 0.15 M NaCl supplemented with 0.5% (w/w) BSA and 0.1% (v/v) Tween-20)), washed and dried as the previously described. Subsequently, the microarray were treated with 20 μL peptide-stabilized gold nanoparticles (3 nM in probe buffer B) for 1 h at 30 °C, then washed and dried as the previously described.

Saccharide competitive assay

The various concentrations of Con A were firstly mixed with different saccharides

(Man, Glc, Mal, Suc or Lac) in probe buffer A, respectively. Then, 20 μL of the mixtures were immediately applied to the carbohydrate microarrays. After incubation at 30 °C for 1 h, the microarrays were treated as the previously described.

Detection and data analysis

After labeled by gold nanoparticles, the slides were detected by a ArrayIt SpotWare Colorimetric Microarray Scanner (Telechem. International Inc., USA). According to the manufacturer's preset parameters, all images were collected with broad spectrum white light source. The background originating from the slide was recorded and subtracted from each image prior to evaluation. The mean value and standard deviation of the signals were determined for the 20 spot replicates per sample, respectively. The detection limit was determined to be the concentration where signal/standard deviation = 3 was reached. To determine the linear ranges of the curves, the range of concentrations that best fitted the linear equation $y = mx + b$ were specified.

Calculation of $K_{D,surf}$ values

$K_{D,surf}$ values are calculated from the equation (1)[18]:

$$R = \frac{R_{max}[P]}{[P] + K_{D,surf}} \qquad (1)$$

R_{max} is the maximum RLS intensity; $K_{D,surf}$ is the equilibrium dissociation constant for surface carbohydrate and the corresponding binding lectin. The equation (1) can be converted to equation (2):

$$\frac{[P]}{R} = \frac{[P]}{R_{max}} + \frac{K_{D,surf}}{R_{max}} \qquad (2)$$

Make a straight line of $[P]/R$ to $[P]$, the slope and intercept of the line are $k=1/R_{max}$, $b=K_{D,surf}/R_{max}$, respectively. Therefore, we can obtain the $K_{D,surf}$ value by b/k.

Calculation of K_i values

K_i values are calculated from the equation (3)[18]:

$$R = \frac{R_{max}[P]}{[P] + K_{D,surf}\left[1 + \frac{[I]}{K_i}\right]} \qquad (3)$$

$[I]$ is the concentration of the inhibitor (competitor); K_i is the inhibition constant. The equation (3) can be converted to equation (4) and (5):

$$\frac{[P]}{R} = \frac{[P]}{R_{max}} + \frac{K_D^{'}}{R_{max}} \qquad (4)$$

in which

$$K_D^{'} = K_{D,surf}\left[1 + \frac{[I]}{K_i}\right] \qquad (5)$$

Make a straight line of $[P]/R$ to $[P]$, we can obtain the $K^{'}_D$ values. Then, the K_i values can be calculated by substituting the $[I]$, $K_{D,surf}$ values to the equation (5).

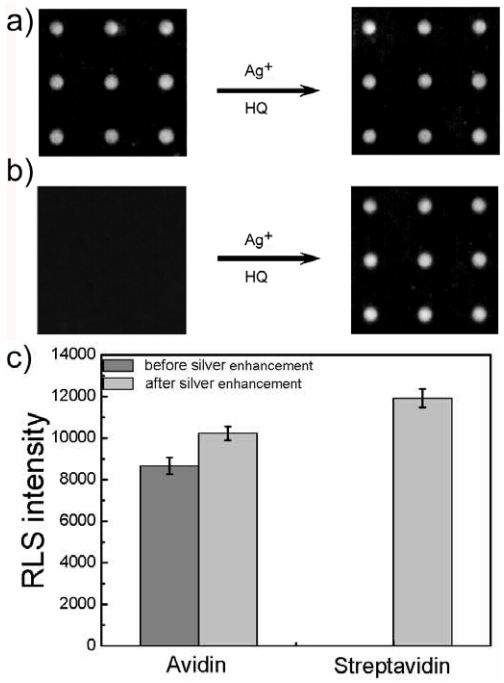

Fig. S1 RLS images of the 3D microarrays before and after silver enhancement step (a and b) and corresponding data analysis (c). The biotin@GNP (3 nM in probe buffer B) was attached on the Man-α with Con A pair by 50 μg/mL avidin (a) and 50 μg/mL streptavidin (b), respectively. The concentration of Man-α is 250 μM in the spotting solution, and the concentration of Con A is 2 μg/mL in the probe buffer A solution, respectively.

Silver Enhancement. After being treated with gold nanoparticles, silver enhancer (1:1 mixed (1 ml total volume) solutions A ($AgNO_3$) and B (hydroquinone) (Sigma-Aldrich)) was applied to each microarray for 6 min and washed with water (3 times). After silver deposition, the slides were detected with ArrayIt SpotWare Colorimetric Microarray Scanner (TeleChem. International Inc. USA). According to manufacturer's preset parameters, all images were collected with broad spectrum white light source.

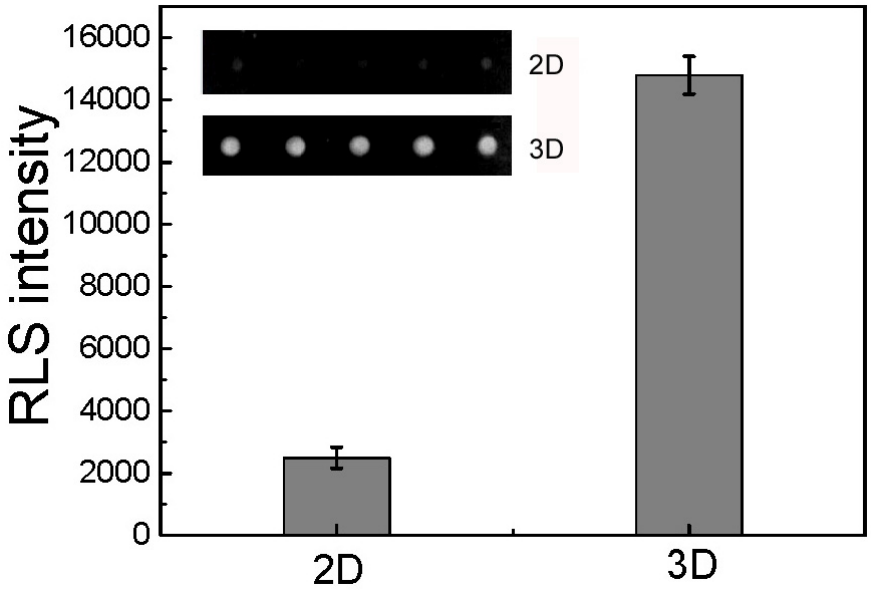

Fig. S2 RLS images of the 2D and 3D microarrays and corresponding data analysis. The biotin@GNP (3 nM in probe buffer B) was attached on the Man-α with Con A pair by 50 μg/mL avidin. The concentration of Man-α is 1 mM in the spotting solution, and the concentration of Con A is 2 μg/mL in the probe buffer A solution, respectively. The strong RLS signal in 3D microarray format may due to the formation of gold nanoparticle clusters on the slide surface since RLS intensity of single 13 nm gold nanoparticle is relatively low.

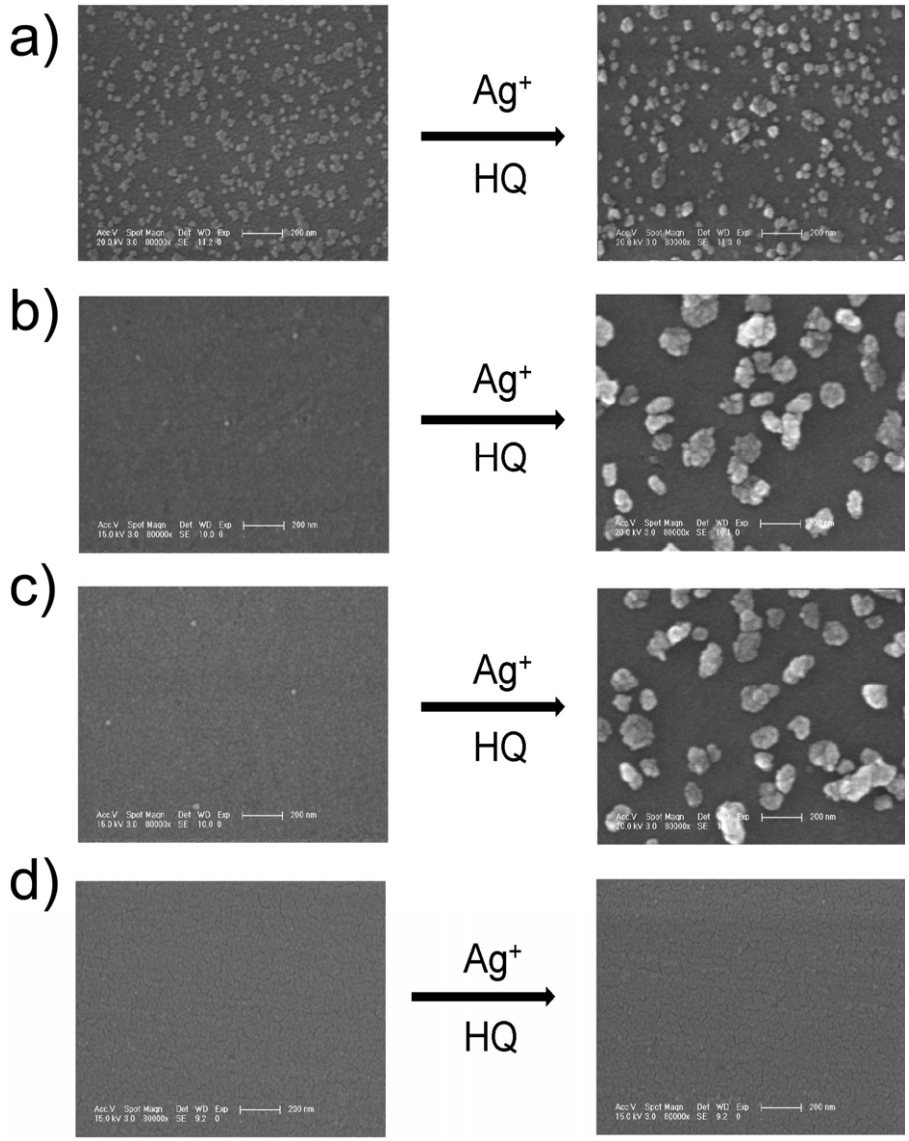

Fig. S3 The Scanning electronic microscopy (SEM) micrographs of the 3D microarrays before and after silver enhancement step. The biotin@GNP (3 nM in probe buffer B) was attached on the Man-α with Con A pair by 50 μg/mL avidin on the 3D slide (a) and the 2D slide (b), or by 50 μg/mL streptavidin on the 3D slide (c), respectively. (d) The biotin@GNP (3 nM in probe buffer B) was attached on the Gal with Con A pair by 50 μg/mL avidin on the 3D slide. The concentration of Man-α or Gal is 250 μM in the spotting solution, and the concentration of Con A is 2 μg/mL in the probe buffer A solution, respectively. The scale bar is 200 nm.

Before silver enhancement step, gold nanoparticle clusters are clearly observed on the 3D microarray surface when avidin is used as a linker (as shown in Fig. S3a†). In contrast, microarray surfaces generated in the control experiments are very smooth (as shown in left images of Fig. S3b and S3c).

After silver enhancement step, large nanoparticles (about 100 nm in diameter) have been formed because of silver deposition on the gold seeds (Fig. S3b and S3c).

No any particles were observed on the (d), indicating that the assay has good selectivity.

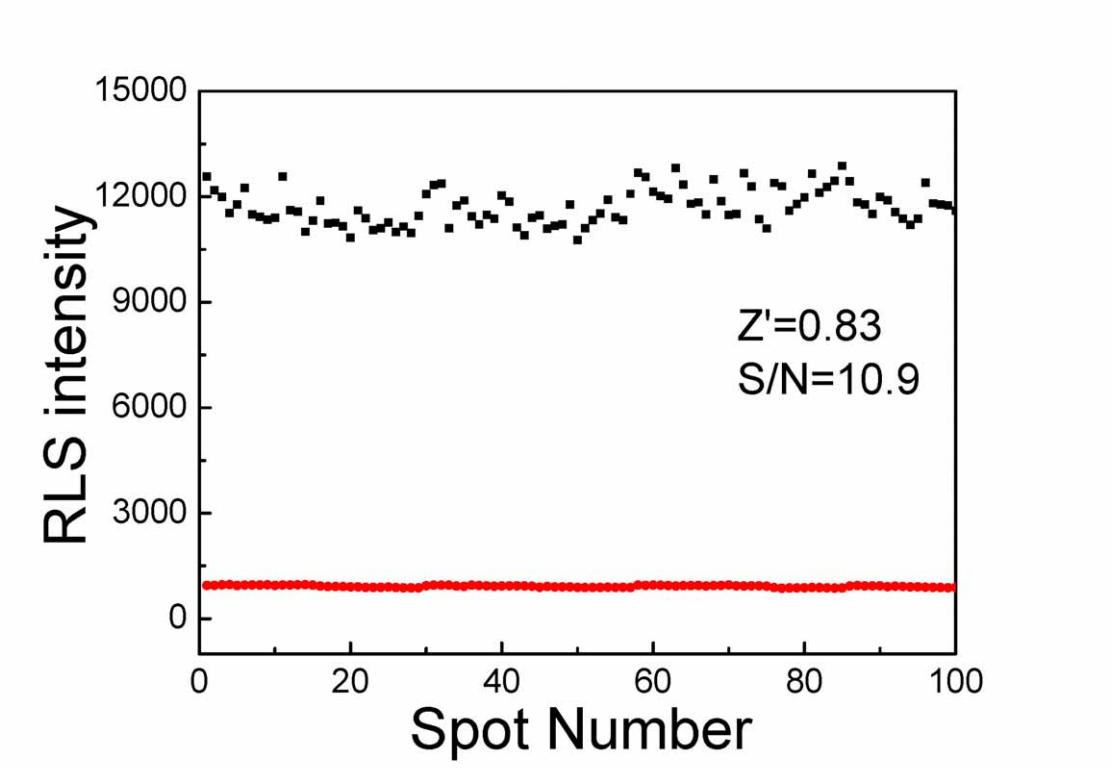

Fig. S4 Evaluation of the assay performance. The observed RLS intensities (black squares) of Gal-β with RCA 120 are shown in comparison with background measurements (red dots). The mean values and standard deviations extracted from the data were used to calculate the S/N ratio and Z' factor. The spots without immobilized Gal-β were used as control measurements. The data is derived from 2 slides (four 5×5 subarrays) which were run on 2 days. The concentration of Gal-β is 10 mM in spotting solution, the concentration of RCA 120 is 2 μg/mL in probe solution, and the concentration of biotin@GNPs is 3 nM in labeling solution.

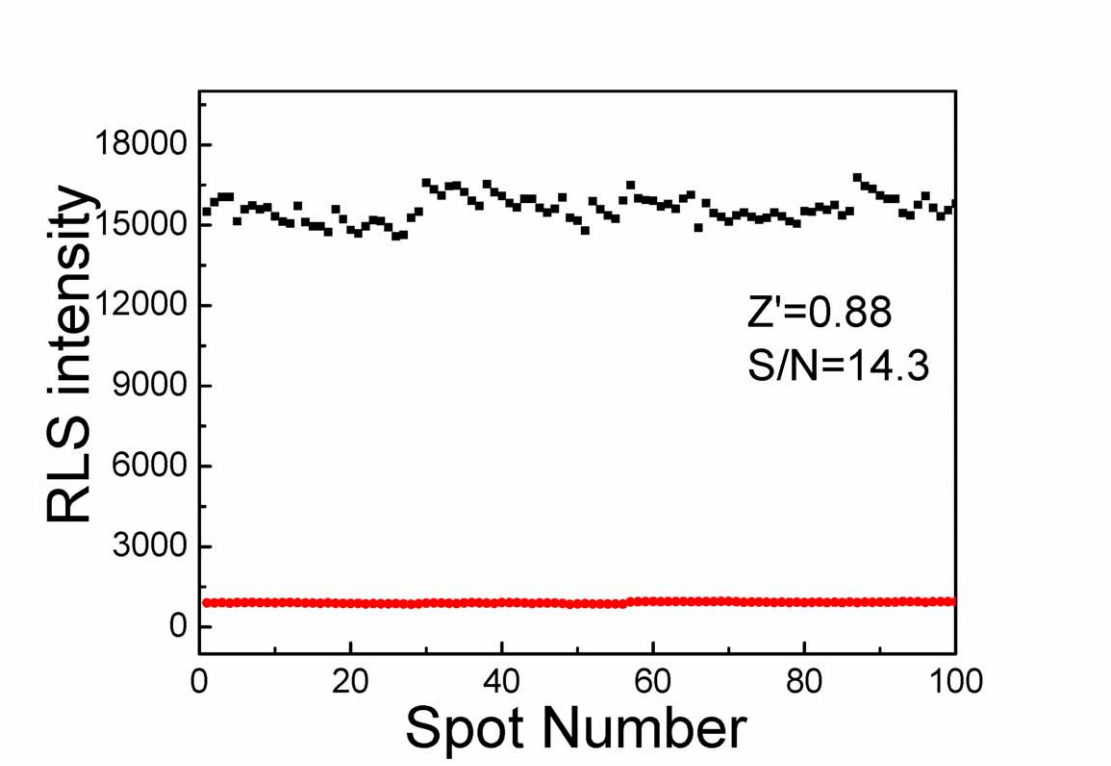

Fig. S5 Evaluation of the assay performance. The observed RLS intensities (black squares) of Man-α with Con A are shown in comparison with background measurements (red dots). The mean values and standard deviations extracted from the data were used to calculate the S/N ratio and Z' factor. The data is derived from 2 slides (four 5×5 subarrays) which were run on 2 days. The spots without immobilized Man-α were used as control measurements. The concentration of Man-α is 10 mM in spotting solution, the concentration of Con A is 2 μg/mL in probe solution, and the concentration of biotin@GNPs is 3 nM in labeling solution.

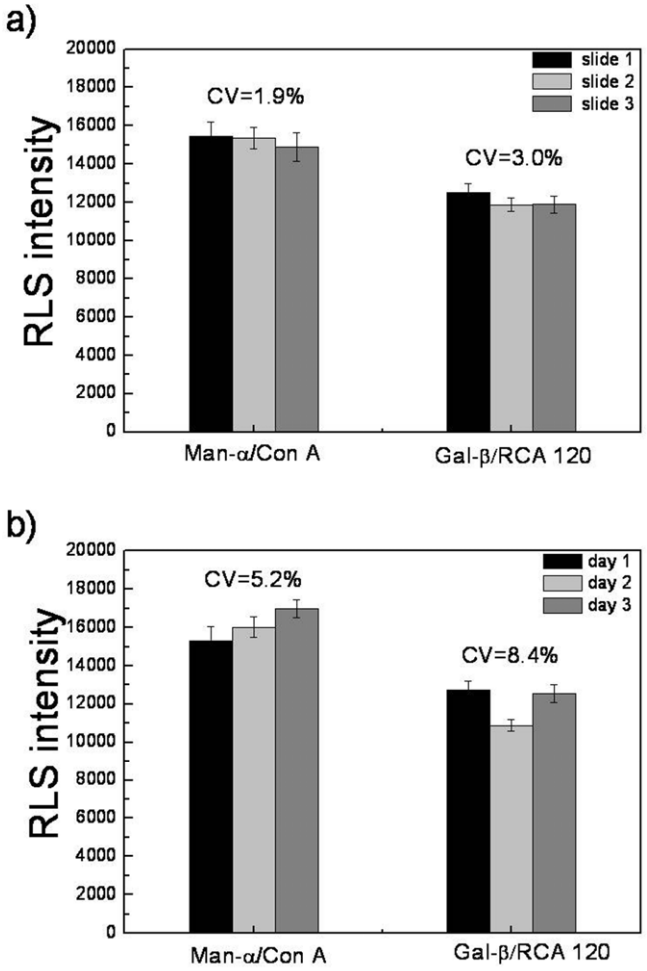

Fig. S6 Investigation of assay reproducibility. Both Man-α/ConA and Gal-β/RCA 120 interaction were tested in three different batches (a) and in three different days (b). The coefficient of variation (CV) values of the RLS intensity in different batches (a) for Man-α/ConA and Gal-β/RCA 120 are 1.9% and 3.0%, while the CV values for them in different days (b) are 5.2% and 8.4%, respectively.

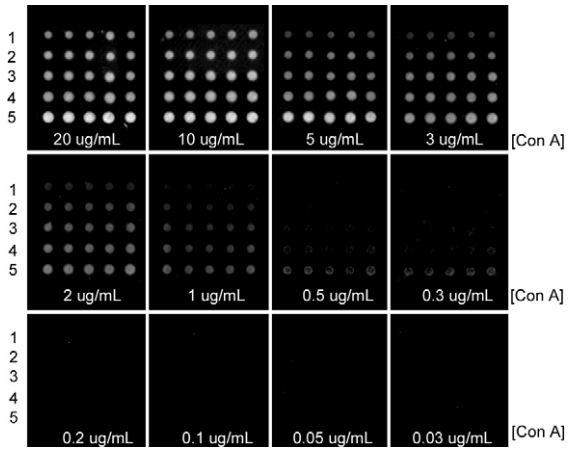

Fig. S7 RLS images of the binding of immobilised Man-α with various concentrations (from 30 ng/mL to 20 μg/mL, as shown in the images) of Con A in the probe solution. The concentrations of Man-α in the spotting solution are 0.1 (line 1), 0.3 (line2), 1 (line 3), 3 (line 4), and 10 mM (line 5), respectively.

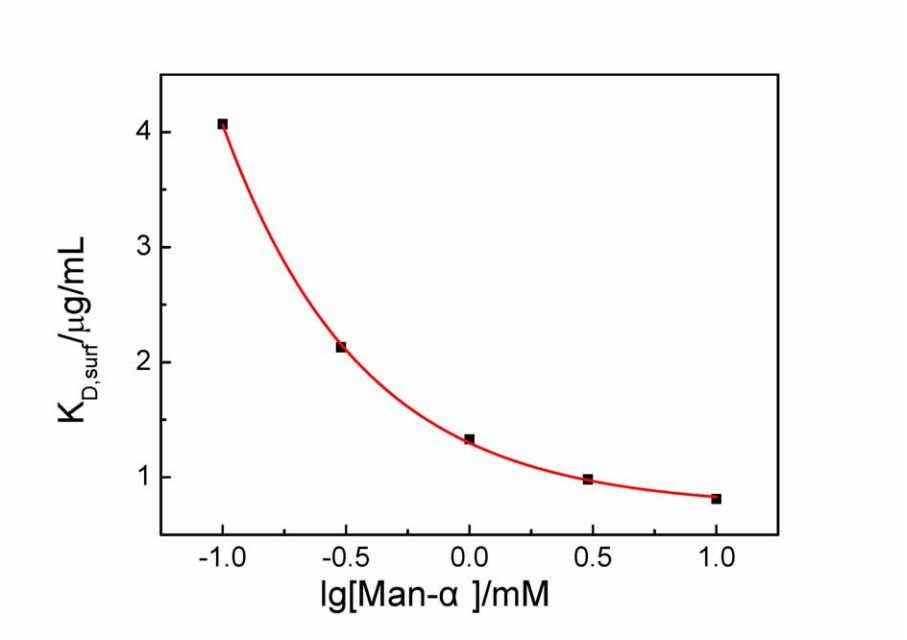

Fig. S8 The $K_{D,surf}$ value as a function of the concentration of Man-α in the spotting solution.

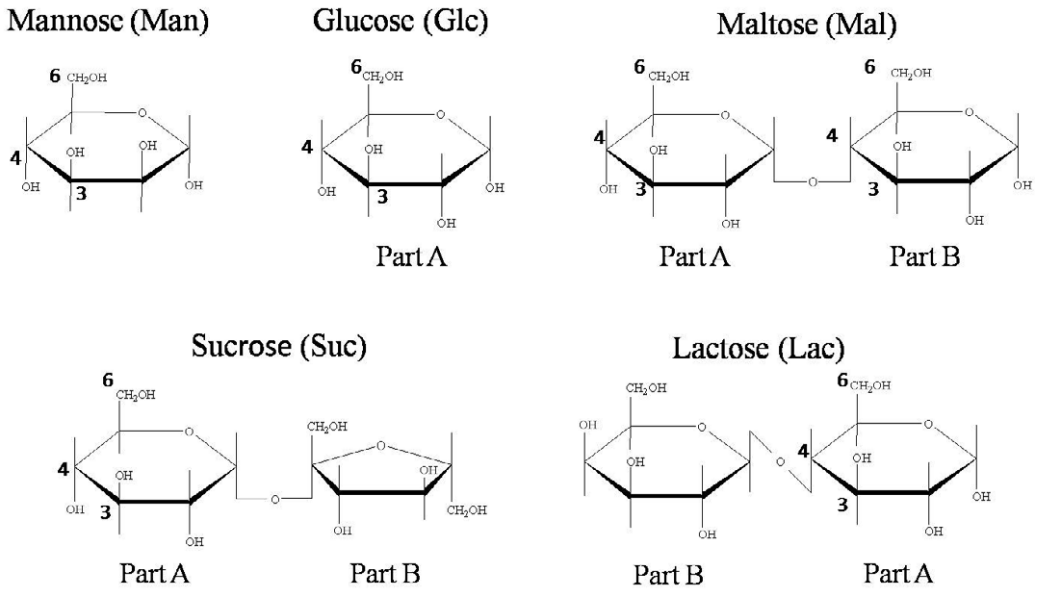

Fig. S9 Molecular structures of the carbohydrates.

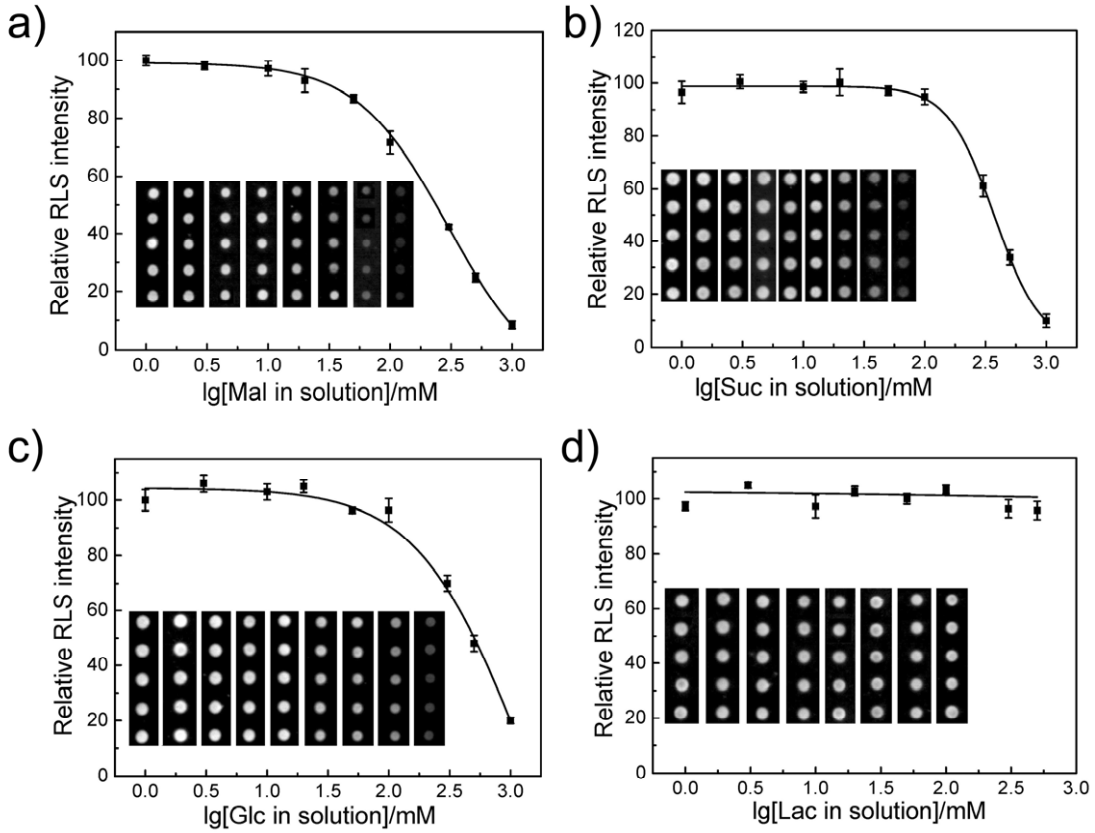

Fig. S10 The effect of various concentrations of carbohydrates (from 1 mM to 1M (500 mM for Lac)) in the probe solution on the light scattering images of the 3D microarrays and corresponding IC_{50} curves of the carbohydrates, respectively. The signals have been corrected for background noise and normalized to the relative RLS intensity obtained in the absence of correspondent carbohydrate.

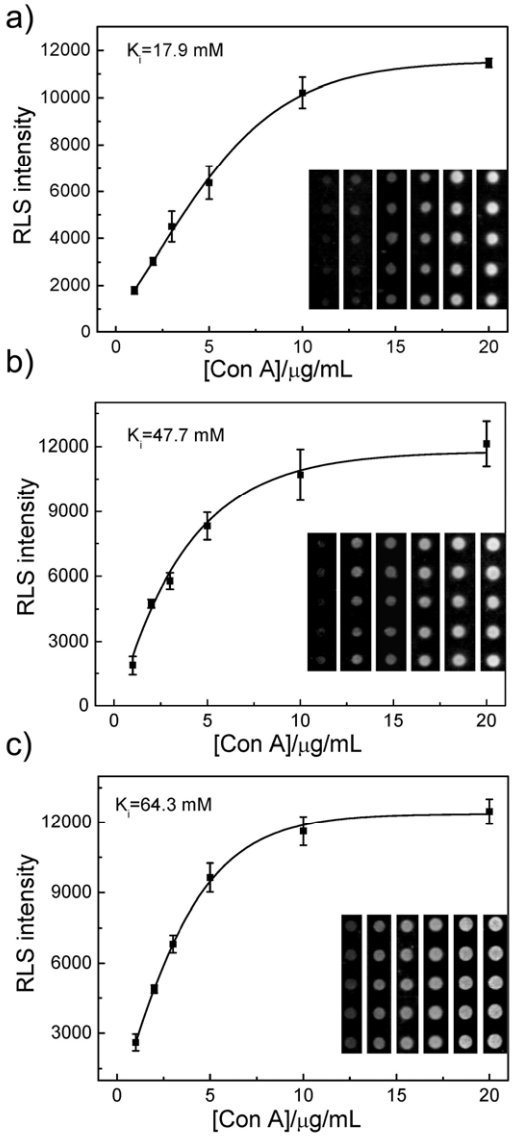

Fig. S11 The effect of various concentrations of Con A (from 1 μg/mL to 20 μg/mL) in the probe solution on the light scattering images of the 3D microarrays and corresponding K_i curves in the presence of Mal (a) Suc (b) and Glc (c), respectively. The concentration of Man-α is 10 mM in the spotting solution.