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Supplementary Information

Carbohydrate microarrays fabricated on poly(2-methylacrylic acid)-based substrates for analysis of carbohydrate-protein interactions

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1. Fluorescence wash-off analysis

5-FITC cadaverine aqueous solutions in concentration ranging from 1 nM to 100 μM were spotted on NHS-activated pMAA-modified slides. Each spot was about 1.5 mm in diameter. The spotting volume for each spot was 320 nL. The slides were then placed in a sealed humid dark chamber at room temperature for at least 12 h. The slides were dried in the air and imaged by array scanner, then they were washed by water, dried by N_2 stream and imaged again by array scanner.

The number of 5-FITC cadaverine that remain bound to the surface (N) is calculated by equation S1.

$$N = \frac{cVN_A F_{aft}}{F_{be_f}} \qquad (S1)$$

Where *c* denotes the spotting concentration; V denotes the spotting volume for each spot; N_A is Avogadro's Number; F_{bef} and F_{aft} denote the fluorescence intensity before and after washing, respectively. Each spot was about 1.5 mm in diameter.

2. Determination of anti-adsorption ability of pMAA-based substrates

The pMAA-modified glass slide was immersed in an aqueous solution of EDC (70 mM) and NHS (40 mM) for 30 min at room temperature. After being rinsed three times with water, the glass slide was then immersed in EOA aqueous solution (1 M, pH=8.60) for at least 3 h at room temperature to deactivate the surfaces. After washed by water three times and dried under a N_2 stream, the glass slide was divided into two incubation chambers sealed with polydimethylsiloxane or non-trace tapes. The chamber 1 was incubated with ConA-FITC (100 μg/mL) dissolved in Tris-HCl buffer (pH=7.60) for 30 min, and the chamber 2 was incubated with ConA-FITC (100 μg/mL) dissolved in KHP buffer (pH=4.00) for 30 min, respectively. After rinsed three times by water and dried under a N_2 stream, the fluorescence intensity of chamber 1 and chamber 2 area was recoded.

3. Calculation of $K_{D,surf}$ values

For the determination of glycan-protein binding affinity on pMAA-based substrates, the binding intensity was plotted against the concentration of protein by fitting curves to equation (S2) [1]

$$F = \frac{F_{max}[P]}{[P]+K_{D,surf}} \qquad (S2)$$

where F_{max} denotes the maximum fluorescence intensity; [P] is the concentration of protein in solution; $K_{D,surf}$ is the equilibrium dissociation constant for protein and immobilized glycan.

4. Polysaccharide modification

The amino groups on polysaccharides (e.g., heparin and fucoidan) were modified by reductive amination according to literature. [2] The polysaccharide was dissolved in acetic acid to 10 mg/mL, then the 2-(4-aminophenyl)ethylamine was added to make its final concentration to 100 mM. The mixed solution was incubated in sealed cubes for 1 h at 37 °C. The freshly prepared reducing reagent, borane dimethylamine complex (100 mM) was added to reaction solution then incubated for 1 h at room temperature. Sample was then heated at 50 °C for 1 h under the protection of N_2. Finally, samples after modification were diluted by H_2O and transferred to ultrafiltration tubes with 3 kDa molecular weight cut-off. After centrifuging for 30 min at 10000 rmp, the samples containing modified polysaccharides were obtained.

5. Typical AFM images of pMAA-modified glass substrates

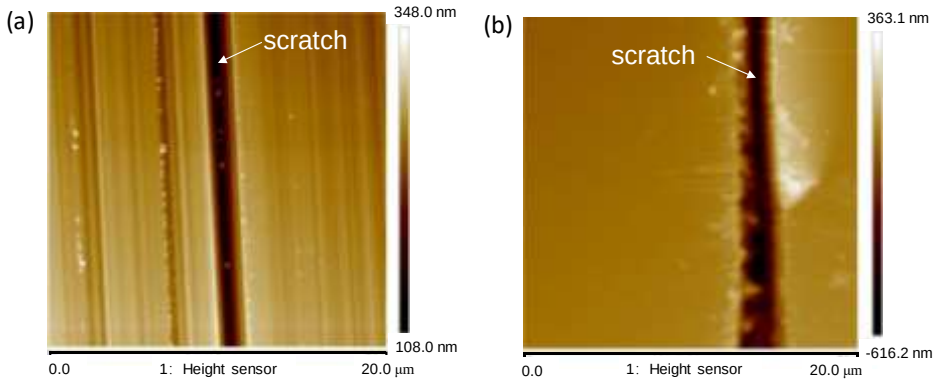

Figure S1 Typical AFM images of pMAA-modified glass substrates during thickness measurement. The polymerization time of pMAA was (a) 3 h and (b) 7 h, respectively.

6. Variation among the different batches of microarray preparation

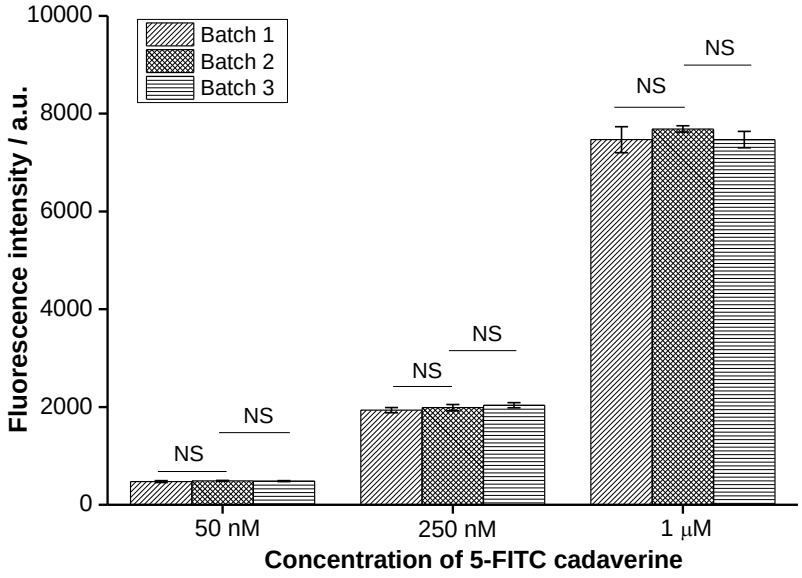

Figure S2 Fluorescence intensity of 5-FITC cadaverine microarrays in different batches. The spotting concentration of 5-FITC cadaverine was 50 nM, 250 nM, and 1 μM. The data represent mean ± SD, and determined by t-test (confidence level 95%). NS, not significant.

7. Anti-nonspecific adsorption property of pMAA-based substrates

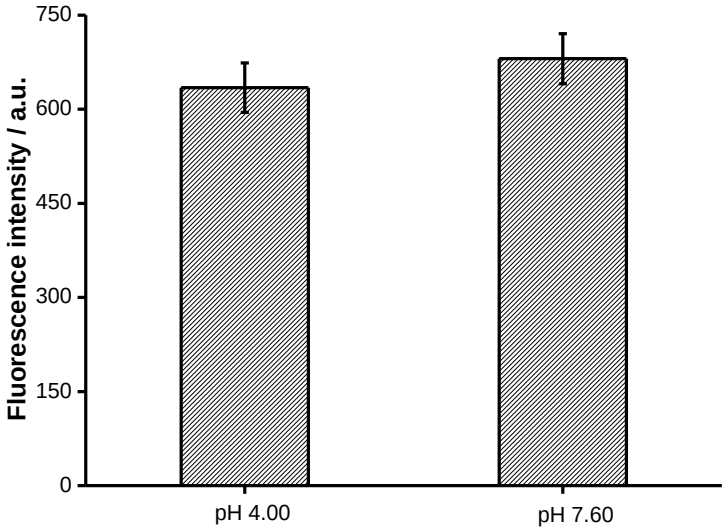

Figure S3 Background signals caused by nonspecific adsorption of ConA-FITC on pMAA-based substrates. ConA-FITC (pI=5.00) was diluted in KHP buffer (pH=4.00) and Tris-HCl buffer (pH=7.60), respectively. The pMAA-based substrates were obtained after the blocking of NHS-activated pMAA-modified substrates with EOA.

8. Fitting curves of mannose-ConA recognition

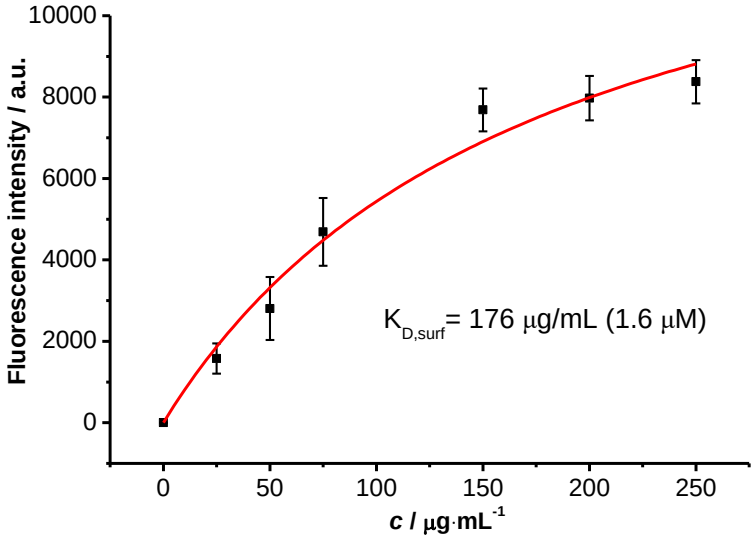

Figure S4 Binding signals of α-Man with a series of ConA-FITC solutions. The fitting curves were obtained by fitting to Langmuir isotherms (equation S2). α-Man (100 mM) in spotting solution was spotted on pMAA-based substrates.

9. The successful immobilization of amino-modified polysaccharides

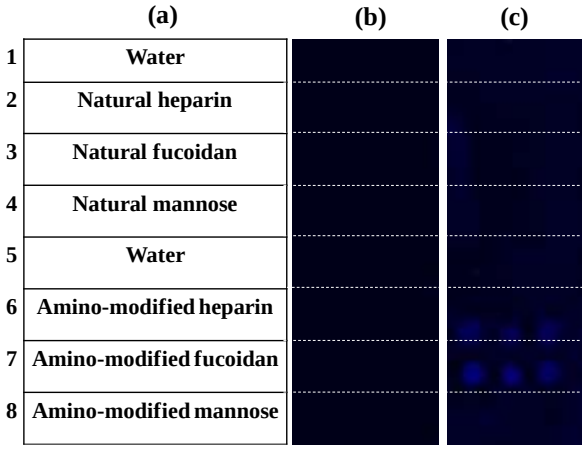

Figure S5 (a) the layout of samples on carbohydrate microarray. Fluorescence image of microarray before (b) and after (c) incubating with S-His tag and labeled by Ab-His tag-FITC.

Reference

[1] P.H. Liang, S.K. Wang, C.H. Wong, Quantitative analysis of carbohydrate-protein interactions using glycan microarrays: Determination of surface and solution dissociation constants, Journal of the American Chemical Society, 129 (2007) 11177-11184.

[2] J.H. Seo, C.S. Kim, B.H. Hwang, H.J. Cha, A functional carbohydrate chip platform for analysis of carbohydrate-protein interaction, Nanotechnology, 21 (2010) 8.