Supporting Information

Fabrication of Glyconanoparticle Microarrays

*Qi Tong,[1] Xin Wang,[1] Hui Wang,[1,2] Takuya Kubo,[3] Mingdi Yan[1,2]**

[1]Department of chemistry, Portland State University, P.O. Box 751, Portland, Oregon, 97207

[2]Department of chemistry, University of Massachusetts Lowell, Lowell, MA 01854

[3]Graduate School of Environmental Studies, Tohoku University, Aoba 6-6-20, Aramaki, Aobaku, Sendai 9808579, Japan

Correspondence: Mingdi Yan, Department of Chemistry, University of Massachusetts Lowell, 1 University Ave., Lowell, MA 01854

E-mail: mingdi_ yan@uml.edu

Table of Contents

1. Experimental section

Materials. D-(+)-Mannose (Man, >98%), D-(+)-glucose (Glc, >98%), D-(-)-fructose (Fru, >98%) 3-aminopropyltrimethoxysilane (APTMS), 3-glycidyloxysilylpropyl trimethoxy silane (GOPTS), were obtained from TCI America. Anthrone (97%), fluorescein isothiocyanate isomer I (FITC, 90%), tetraethyl orthosilicate (TEOS), phosphate buffered saline (PBS) tablet, HEPES, Con A (lectin from *Canavalia ensiformis* (jack bean), Type IV) were purchased from Sigma-Aldrich. Polystyrene (PS) was purchased from Scientific Polymer Products, Inc. Absolute ethanol (200-proof) was purchased from Pharmco-AAPER. Poly(allylamine) (PAAm) hydrochloride, poly(2-ethyl-2-oxazoline) (PEOX) were obtained from Alfa Aesar. 2-*O*-α-D-Mannopyranosyl-D-mannopyranose (Man 2, 96.3%) and 3,6-di-*O*-(α-D-mannopyranosyl)-D-mannopyranose (Man 3, >99%) were obtained from V-labs Inc. (Covington, LA). Ammonium hydroxide (NH_4OH), hydrogen peroxide (H_2O_2) and methanol (HPLC grade) were obtained from Fisher Scientific. All chemicals were used as received without purification. Water used was from a Milli-Q ultrapure water purification system. Silicon wafers were obtained from Wafernet, Inc.

Microarray images were recorded on a GenePix 4100A microarray scanner with 532-nm laser (Molecular Devices Corporation, Union City, CA). Dynamic light scattering (DLS) experiments were carried out using Horiba LB-550 DLS Nano-Analyzer. UV-vis spectra were recorded on Perkin-Elmer Lambda 45 UV-vis spectrometer. AFM images were obtained on an atomic force microscope (Nanoscope III, Veeco).

Synthesis of silica nanoparticles (SNPs). SNPs were synthesized following a modified protocol from the classic Stöber method.[1] Briefly, TEOS (2.8 mL) was added to 200-proof absolute ethanol (34 mL) followed by NH_4OH (25%, 1.4 mL). The reaction was allowed to proceed at room temperature for 24 h with vigorous stirring to yield a colloidal solution.

Functionalization of SNPs with PFPA-silane. PFPA-silane (80 mg), synthesized following a previously reported procedure,[2] was added directly to the Stöber solution prepared above and the mixture was stirred at room temperature overnight. The mixture was then refluxed under stirring at 78 °C for 1 h. PFPA-functionalized SNPs (10 mL) were isolated by centrifugation at 12,000 rpm and then redispersion

in the fresh solvent (10 mL) by sonication. This centrifugation/redispersion procedure was repeated three times with ethanol and twice with acetone.

Conjugation of carbohydrates onto SNPs. The as-prepared solution of PFPA-functionalized SNPs in acetone (5 mL) was placed in a flat-bottom dish, and an aqueous solution of carbohydrate (10 mg/mL, 1 mL) was added. The mixture was covered with a 280-nm long-path optical filter (WG-280, Schott Glass) and was irradiated with a 450 W medium-pressure Hg lamp (Hanovia) for 10 min under vigorous stirring. The filter was used to avoid crosslinking and degradation of materials. Excess carbohydrate was removed by centrifugation and then redispersion by sonicating in fresh Milli-Q water, repeated three times.

Determination of carbohydrate density on GNPs. A previously developed colorimetric method was followed to determine the density of carbohydrates immobilized on SNPs.[3] Calibration curves were obtained for each carbohydrate where carbohydrate solutions of various concentrations were incubated with anthrone/sulfuric acid and the absorbance at 620 nm were measured.[4] A freshly-prepared anthrone solution in concentrated H_2SO_4 (0.5 wt%, 1 mL) was added to a carbohydrate solution in water (0.5 mL) in an ice bath under shaking. The solution was then heated to 100 °C and stirred for 10 min. After cooled to room temperature, the UV-vis spectra of the resulting solutions were recorded.

GNPs were subjected to the same assay where solutions of the GNPs in Milli-Q water (1 mg/mL, 0.5 mL) were treated with anthrone/H_2SO_4. Background adsorption due to SNPs was accounted for by treating SNPs solution of the same concentration with anthrone/ H_2SO_4, and the absorbance at 620 nm was subtracted from that of the GNPs. The amount of surface-bound carbohydrate was then computed from the corresponding calibration curves.

Synthesis of FSNPs. FITC (39 mg, 0.10 mmol) was mixed with APTMS (20 μL, 0.10 mmol) in absolute ethanol (100 mL), and the solution was stirred at 42 °C for 24 h to yield the FITC-silane precursor. The solution (5 mL) was mixed with TEOS (2.8 mL), and the mixture was added to absolute ethanol (34 mL) followed by NH_4OH (25%, 2.8 mL). The reaction was allowed to proceed at room temperature overnight with vigorous stirring to yield a bright yellow colloidal solution.

Functionalization of SNPs with epoxy groups. GOPTS (40 μg, 170 nmol) was added directly to the Stöber solution (20 mL) prepared above, and the mixture was stirred at room temperature overnight. The mixture was then refluxed while stirring at 78 °C for 1 h. The epoxy-functionalized FSNPs were isolated by centrifugation and then redispersion in the fresh solvent by sonication. This centrifugation/redispersion procedure was repeated three times with ethanol and twice with the PBS buffer (pH 7.4). The PBS buffer was prepared by dissolving one phosphate buffered saline tablet (Sigma) in Milli-Q H_2O (200 mL).

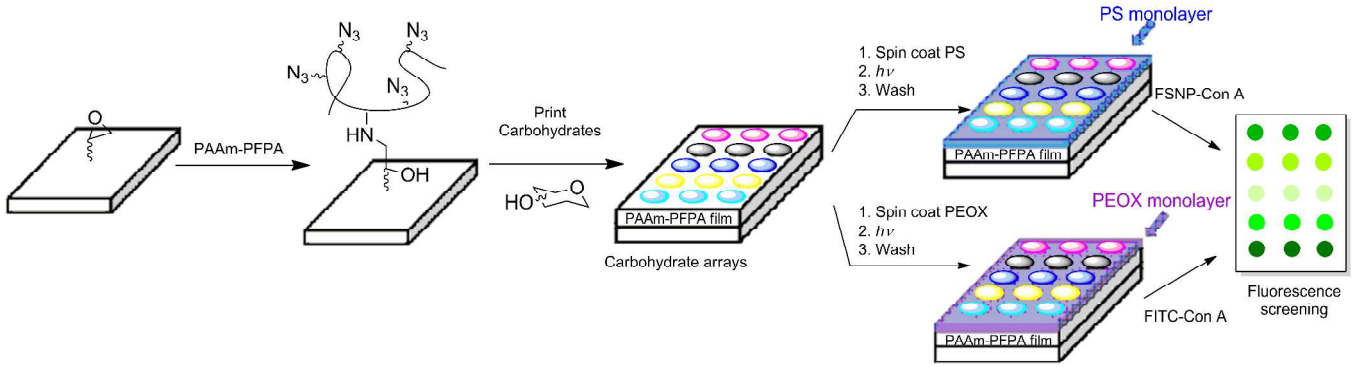
Con A Conjugation. Con A (20 μg) was added into the epoxy-FSNP solution prepared above, and the mixture was stirred for 3 h. The product, FSNP-Con A, was washed 3 times with PBS buffer containing $CaCl_2$ (0.1 mM) and $MnCl_2$ (0.1 mM).

Synthesis of PAAm-PFPA. An aqueous solution of PAAm hydrochloride (10 mg) in water (2 mL) was mixed with a solution of PFPA-NHS (5 mg/mL) in pyridine (2 mL), and the mixture was stirred for 24 h at room temperature.

Preparation of PAAm-PFPA surface. Silicon wafers were cut into 1×1 inch pieces, cleaned in the piranha solution (3:1 v/v conc. H_2SO_4/H_2O_2) at 80-90 °C for 1 h, washed in boiling water for three times at 30 min each, and then dried under nitrogen. Caution: the piranha solution reacts violently with organic solvents. The cleaned silicon wafers were treated with a solution of GOPTS in toluene (12.6 mM) for 4 hours, rinsed with toluene and dried with nitrogen. The slides were then treated with the PAAm-PFPA solution prepared above at 50 ℃ for 5 h, sonicated in dilute HCl (0.1 M) for 5 min followed by pyridine/H_2O (1:1 v/v) for 5 min.

Fabrication and fluorescence imaging of GNP and carbohydrate microarrays (Scheme 1, Scheme 1S). Solutions of GNPs or carbohydrates (10 mg/mL) were prepared in Milli-Q H_2O, and were printed onto the PAAm-PFPA-functionalized wafers using a robotic printer (BioOdyssey Calligrapher miniarrayer; Bio-Rad Laboratories, Inc.) from a 384-well plate using a solid pin (310s). Each spot was printed 5 times (each print delivered ~1 nL solution), and the distance between each spot was programed at 900 μm. Printing conditions were as follows: humidity 60%; volumn of solution in the source plate: 70

μL; temperature:20 ℃; spot size: 400 μm. The wafers were dried under vacuum for 1 h. A solution of PS in chloroform (5 mg/mL) was spin-coated on the wafers and the samples were irradiated with the medium-pressure Hg lamp for 9 min in the presence of the optical filter. The samples were sonicated in chloroform and H_2O for 5 min each, and dried with nitrogen. The microarrays were then incubated in a solution of FSNP-Con A in PBS (pH 7.4, containing 0.1 mM of $CaCl_2$, and 0.1 mM $MnCl_2$) (0.5 mg/mL) at room temperature overnight, rinsed with fresh PBS buffer 3 times, and dried under nitrogen. The samples were scanned using a Genepix 4100A microarray scanner at excitation of 532 nm. Image analysis was carried out with Axon Genepix Pro 5.1 analysis software (Molecular Devices Corporation, Union City, CA)



Scheme 1S. Fabrication of carbohydrate microarrays and interactions with FSNP-Con A or FITC-Con A.

For microarrays probed with FITC-ConA, GNP and carbohydrate solutions were printed on the PAAm-PFPA wafers, dried for 1 h and spin-coated with a solution of PEOX 200,000 in chloroform (10 mg/mL). The wafers were then irradiated for 9 min, sonicated in chloroform followed by water for 5 min each, and dried with nitrogen. The wafers were incubated in a solution of FITC-Con A in HEPES (pH 7.5, 10 mM containing NaCl (100 mM), $CaCl_2$ (0.1 mM), and $MnCl_2$ (0.1 mM)) (0.5 mg/mL) overnight, rinsed with HEPES buffer 3 times, and dried with nitrogen.

2. DLS characterization of GNPs

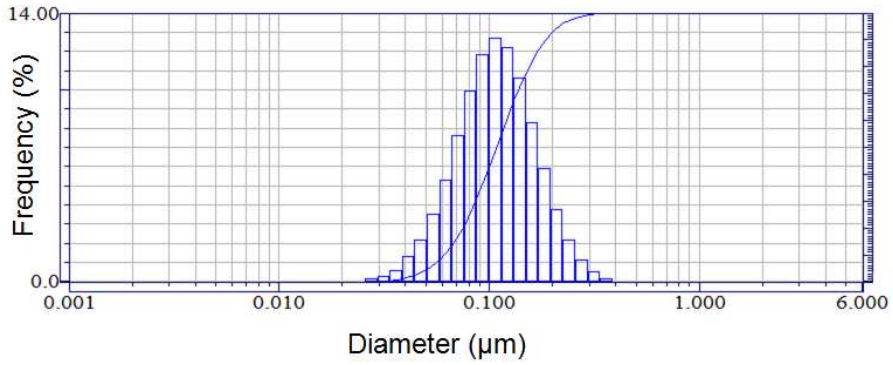


Figure S1. Particle size distribution of Glc-GNPs measured by DLS. All other GNPs showed similar histograms as the one above.

3. Determination of immobilized carbohydrate density and coupling yield

Average diameter of SNPs: d=118 nm

Surface area of each SNP: $S = \pi d^2 = 4.37 \times 10^4 \text{ nm}^2$

Volume of each SNP: $V = \frac{\pi d^3}{6} = 8.60 \times 10^5 nm^3 = 8.60 \times 10^{-16} cm^3$

Density of SNP: $\rho = 2.3 g/cm^3$

Concentration of SNP: 1 mg/mL

In 0.5 mL of the SNP solution, the number of SNPs:

$$\frac{0.5 \times 10^{-3} g}{2.3 g / cm^3 \times 8.60 \times 10^{-16} cm^3} = 2.5 \times 10^{11}$$

Using the projection area of each carbohydrate molecule obtained by ChemDraw 3D,[3] e.g. 0.24 nm^2 for Man, the maximal number of Man on each Man-SNP:

$$N_{\max} = \frac{4.37 \times 10^4 nm^2}{0.24 nm^2} = 18.2 \times 10^4$$

The coupling yield is:

$$N_{\exp} / N_{\max} \times 100\%$$

where (N_{exp}) is the number of carbohydrate ligands on GNP obtained experimentally, determined using the anthrone/H_2SO_4 assay described in the Experimental Section above.

Table S1. Ligand density and coupling yield of GNPs

Ligand	N_{exp} ($\times 10^4$)	N_{max} ($\times 10^4$)	Coupling yield (%)
Fru	5.96	18.2	33
Glc	6.38	18.2	35
Man	6.79	18.2	34
Man 2	3.05	6.62	46
Man 3	2.86	5.68	50

4. Fluorescence images of Man-GNP microarrays

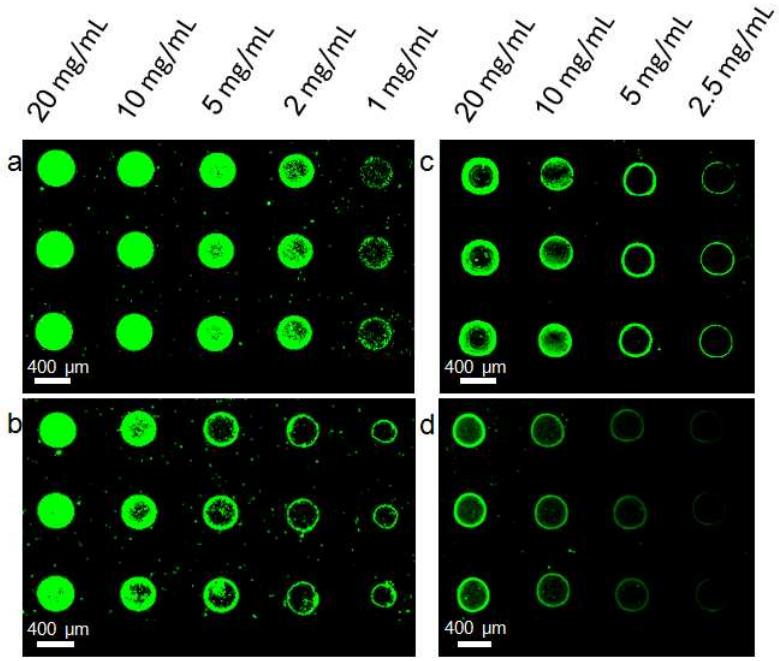


Figure S2. Fluorescence images of Man-GNP microarrays probed with (a) FSNP-Con A or (b) or FITC-Con A. In c and d, no polymer coating was used and the Man-GNP microarrays were incubated with BSA before treating with FSNP-Con A (c) or FITC-Con A (d). Solutions of Man-GNP were printed at various concentrations indicated above the images.

5. AFM characterization of GNP microarrays

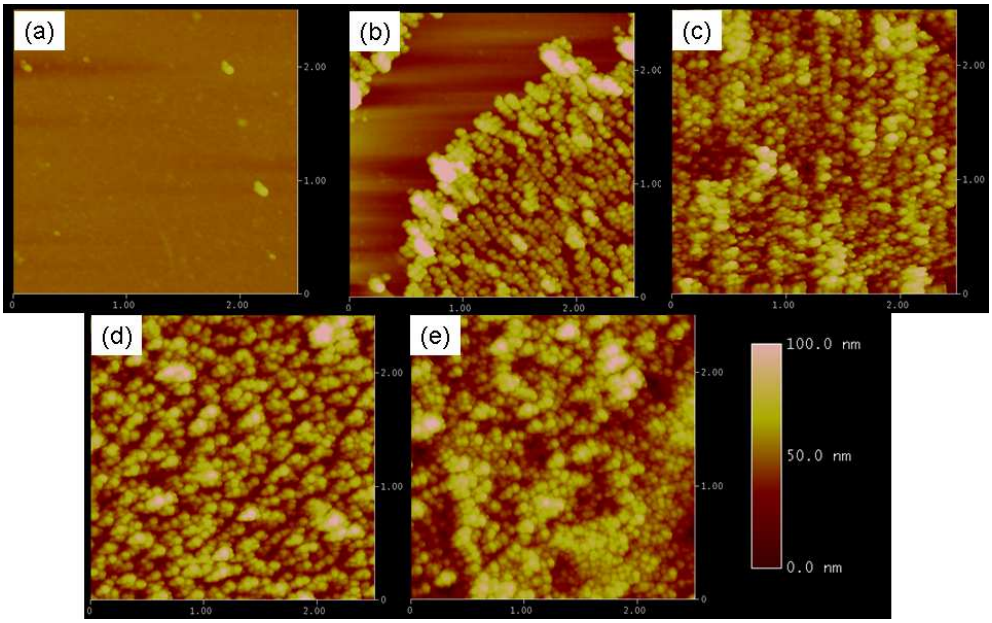


Figure S3. AFM images of (a) PAAm-PFPA, and immobilized Man-GNPs printed from solutions of (b) 2.5 mg/mL, (c) 5 mg/mL, (d) 10 mg/mL, and (e) 20 mg/mL, respectively. The topographic scales (bottom right) are the same for all images.

6. Binding affinity of free ligands with Con A

Table S2. The association constant (K_a) of carbohydrates with Con A[5,6]

Carbohydrate ligand	K_a (M^{-1}) ($\times 10^3$)
Glc	0.560
Man	2.13
Man 2	41.7
Man 3	337

References

(1) Stöber, W.; Fink, A. *J. Colloid Interface Sci.* **1968**, *26*, 62-69.

(2) Yan, M.; Ren, J. *Chem. Mater.* **2004**, *16*, 1627-1632.

(3) Wang, X.; Ramström, O.; Yan, M. *J. Mater. Chem.* **2009**, *19*, 8944-8949.

(4) Wang, X.; Ramström, O.; Yan, M. *Anal. Chem.* **2010**, *82*, 9082-9089.

(5) Mandal, D. K.; Kishore, N.; Brewer, C. F. *Biochemistry* **1994**, *33*, 1149-1156.

(6) Schwarz, F. P.; Puri, K. D.; Bhat, R. G.; Surolia, A. *J. Biol. Chem.* **1993**, *268*, 7668-7677.