

## **SUPPORTING INFORMATION**

# **Beam Pen Lithography as a New Tool for Spatially Controlled Photochemistry, and its Utilization in the Synthesis of Multivalent Glycan Arrays**

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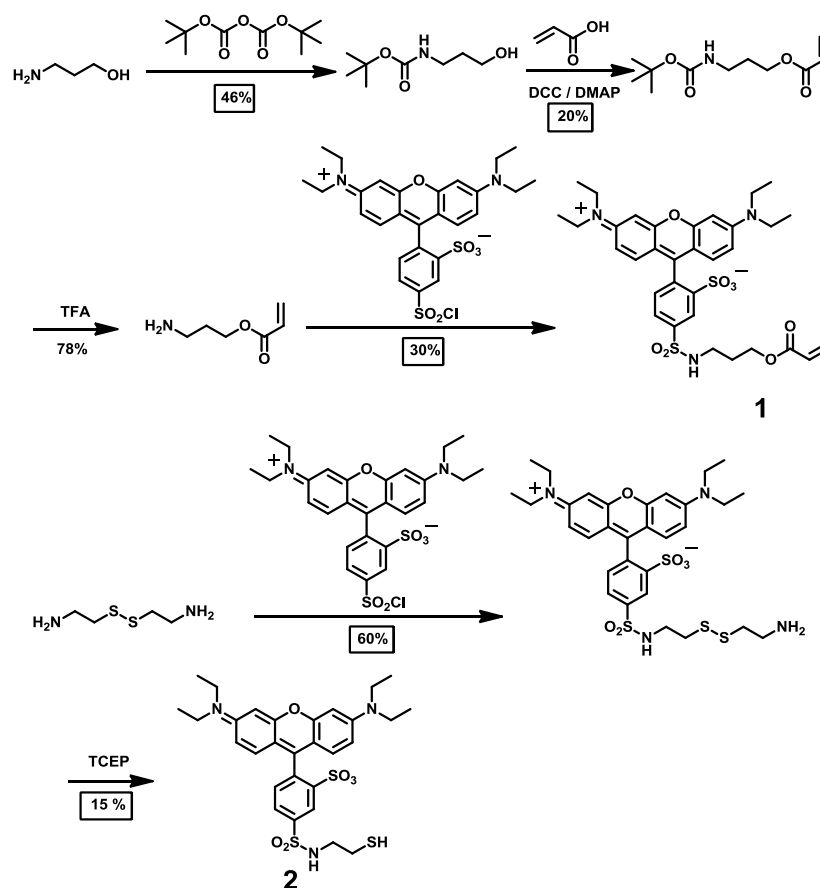
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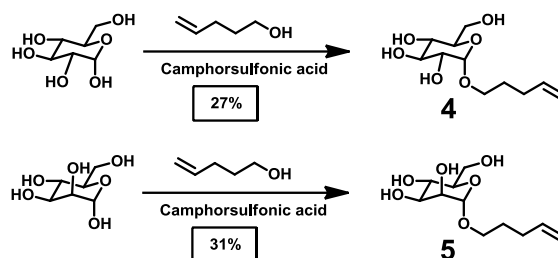
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## 1. Organic Synthesis

**General methods.** Cy3-labelled ConA was purchased from Protein Mods (USA). All solvents and reagents were purchased from Aldrich or VWR and dried prior to use. Solutions were prepared from nanopure water purified from Milli-Q plus system (Millipore Co.), with a resistivity  $> 18 \text{ M}\Omega \text{ cm}^{-1}$ . Compounds **1**<sup>1</sup>, **2**<sup>1</sup>, **4**<sup>2</sup> and **5**<sup>3</sup> were prepared according to published literature procedures. Compound **3** was purchased from Aldrich. Rhodamine-labeled PNA was purchased from VWR and Cy3-ConA was purchased from Protein Mods. Thin-layer chromatography was carried out using aluminum sheets pre-coated with silica gel 60 (EMD 40 - 60 mm, 230 - 400 mesh with 254 nm dye). All reactions were carried out under an inert  $\text{N}_2$  atmosphere using standard Schlenk techniques or an inert-atmosphere glovebox unless otherwise noted. Deuterated solvents were purchased from Cambridge Isotope Laboratories Inc. and used as received. NMR spectra were obtained on a Bruker AVANCE 400 MHz spectrometer. All chemical shifts are reported in ppm units with reference to the internal solvent peaks for  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts, and all spectral data were consistent with their reported literature values. High-resolution mass spectrometry analyses were carried out on an Agilent 6200 LC/MSD TOF system.



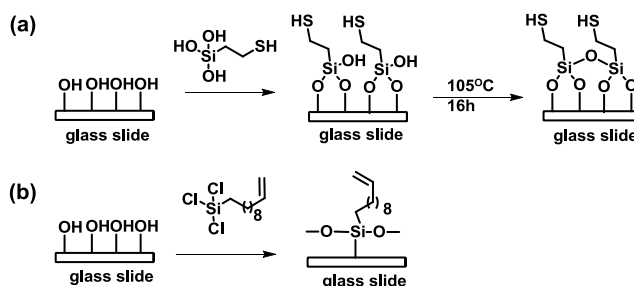
**Scheme S1.** Preparation of **1** and **2**.



**Scheme S2.** Preparation of **4** and **5**.

## 2. Monolayer Preparation

**General methods.** Microscope glass slides were purchased from VWR. All other chemicals and materials were purchased from Aldrich, and all chemicals were used as received. Metals were evaporated using Bal-Tec MED 020 Coating System.



**Scheme S3.** Preparation of (a) thiol-terminated glass surfaces, and (b) alkene-terminated glass surfaces.

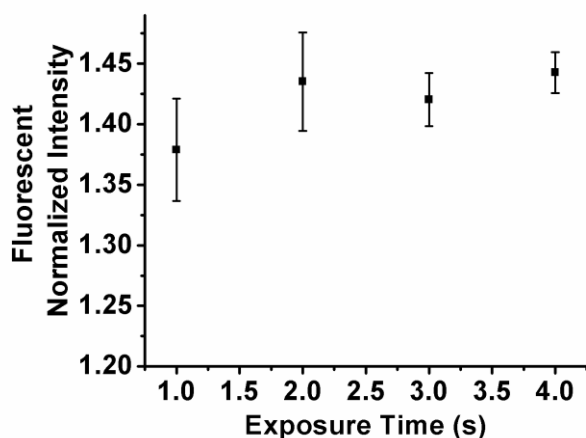
**Preparation of thiol-terminated glass surfaces.**<sup>4</sup> Glass slides were cleaned by sonication in 1 M HNO<sub>3</sub>, H<sub>2</sub>O, and EtOH for 15 min each. After drying under a N<sub>2</sub> stream, the glass slides were incubated in a 20 mL vial containing 0.8 mL (3-Mercaptopropyl)trimethoxysilane (3-MPTS) in 18 mL toluene at 37 °C with gentle agitation for 4 h. Then the glass samples were removed and rinsed thoroughly with toluene, EtOH / toluene (1:1), and absolute EtOH. Finally the samples were cured in oven at 105 °C for 18 h and were stored in MeOH at 4 °C until used.

**Preparation of alkene-terminated glass surfaces.**<sup>1</sup> Glass slides were cleaned by sonication in pentane, Acetone, and H<sub>2</sub>O for 15 min. Subsequently glass slides were immersed in a piranha solution (3:1 H<sub>2</sub>SO<sub>4</sub>: 30% H<sub>2</sub>O<sub>2</sub> (aq)) for 30 min. After washing thoroughly with H<sub>2</sub>O and dried with an N<sub>2</sub> stream, the surfaces were incubated in a stirred toluene solution containing 0.10% 10-undecenyl trichlorosilane for 2 h. Finally the alkene-terminated glass surfaces were washed with EtOH and H<sub>2</sub>O.

## 3. Fluorescence Microscopy

**General Methods.** Fluorescence intensity profiles of the patterns were obtained using a Nikon Eclipse Ti fluorescence microscope ( $\lambda_{\text{ex}}$  = 532-587 nm,  $\lambda_{\text{obs}}$  = 608-683 nm), and extracted by NIS-elements software (Nikon Instruments, Inc.). Exposure times ranged from 1 – 4 s, depending on the brightness of the arrays. To compare data taken with different exposure times, the normalized fluorescence intensity was obtained by dividing the maximum fluorescence intensity by the background fluorescence intensity, and, as a result a normalized fluorescence value of 1 indicates that no signal is detectable above the background

fluorescence. It is possible that the ratio of signal-to-background can vary with exposure time, so to test the effect of exposure time on the normalized fluorescence of features on the microarray, fluorescence images of a surface patterned with **4** and bound to Cy3-modified ConA were taken at different exposure times. It was found the normalized fluorescence in this time range was independent of the camera capture time (Figure S1).



**Figure S1.** The influence of camera capture time on the normalized fluorescence intensity of a surface patterned with **4** and subsequently exposed to Cy3-labelled ConA.

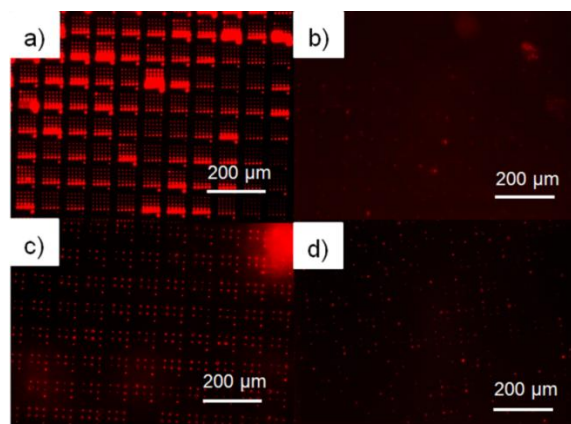
## 4. Polymer Pen Lithography

**General Methods.** To prepare the pen arrays for inking, they were exposed to O<sub>2</sub> plasma (Harrick PDC-001, 30 s, medium power, ~200 mTorr) to render the surfaces of the pen-arrays hydrophilic. Then 4 drops of the ink solution, comprised of the thiol, acrylate, methacrylate, or alkene ink (0.80 mg), PEG (2,000 g mol<sup>-1</sup>, 5 mg mL<sup>-1</sup>) and DMPA (0.30 mg, 1.17 mmol) in 1 mL 80:20 THF:H<sub>2</sub>O that was sonicated to ensure solution homogeneity, were spin coated (2,000 rpm, 2 min) onto the PPL pen array. A Park XE-150 scanning probe microscope equipped with a PPL head (Park Systems Corp.), XEP custom lithography software, and an environmental chamber capable of controlling humidity were used for PPL writing at a humidity of 78%-82% at room temperature. The tip array was leveled by optical methods with respect to the substrate surface using an xy tilting stage. A dot array was printed by bringing the tip array into contact with the thiol- or alkene-terminated glass surface, whilst varying the dwell times from 50 – 100,000 ms at 80 – 85% humidity. The surface was placed under the UV light (3 mW cm<sup>-2</sup>) for 5 h, washed thoroughly with 50 ml EtOH and H<sub>2</sub>O, sonicated in EtOH and dried with N<sub>2</sub>.

### Control Experiments

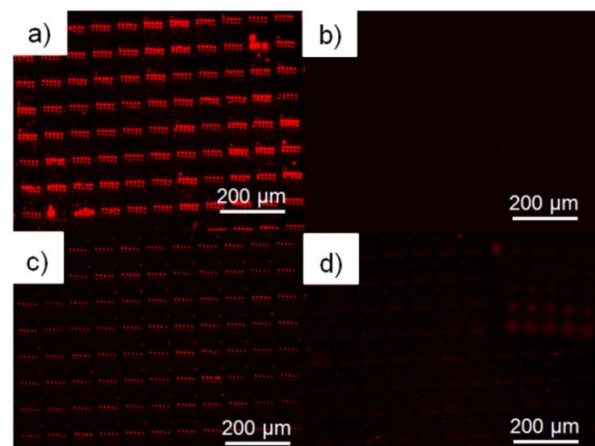
**Rhodamine-methacrylate (1) Printed onto Bare Glass Surfaces and onto Thiol-terminated Glass Surfaces Without UV Exposure.** In the first control experiment, an ink mixture containing **1** (0.80 mg, 1.2 mmol), PEG (2,000 g mol<sup>-1</sup>, 5 mg mL<sup>-1</sup>) and DMPA (0.30 mg, 1.17 mmol) was deposited onto a bare glass surface by PPL, and ink deposition was confirmed by fluorescence microscopy (Figure S2a). Following UV exposure, no fluorescent pattern was observed (Figure S2b). Alternatively, **1** was patterned onto the thiol terminated glass surface, and deposition was confirmed by fluorescence microscopy (Figure

S2c), and then washed with 50 mL EtOH and H<sub>2</sub>O without exposure to UV light. Some dim patterns could still be seen on the alkene terminated glass surface even without UV light (Figure S2d).



**Figure S2.** (a) Fluorescence microscopy image (Nikon Eclipse Ti,  $\lambda_{\text{ex}}$ = 532-587 nm,  $\lambda_{\text{em}}$ = 608-687 nm) of an array of **1** patterned by PPL on a bare glass slide with dwell times of 50, 500, 5,000, 50,000 and 100,000 ms before washing and (b) after washing. Fluorescence microscopy image ( $\lambda_{\text{ex}}$ = 532-587 nm,  $\lambda_{\text{em}}$ = 608-687 nm) of an array of **1** patterned by PPL on an thiol-terminated glass slide (no UV exposure) before washing (c) and (d) after washing.

**Rhodamine-thiol (2) Printed onto Bare Glass Surfaces and onto Alkene-terminated Glass Surfaces Without UV Exposure.** In the second set of control experiments, an ink mixture containing **2** (0.80 mg, 1.2 mmol), PEG (2,000 g mol<sup>-1</sup>, 5 mg mL<sup>-1</sup>) and DMPA (0.30 mg, 1.17 mmol) was deposited onto a bare glass surface by PPL, and ink deposition was confirmed by fluorescence microscopy (Figure S3a). Following UV exposure, no fluorescent pattern was observed (Figure S3b). Alternatively, **2** was patterned onto the alkene terminated glass surface, and deposition was confirmed by fluorescence microscopy (Figure S3c), and then washed with 50 mL EtOH and H<sub>2</sub>O without exposure to UV light. Some weak patterns could still be seen on the alkene terminated glass surface even without UV light (Figure S3d).



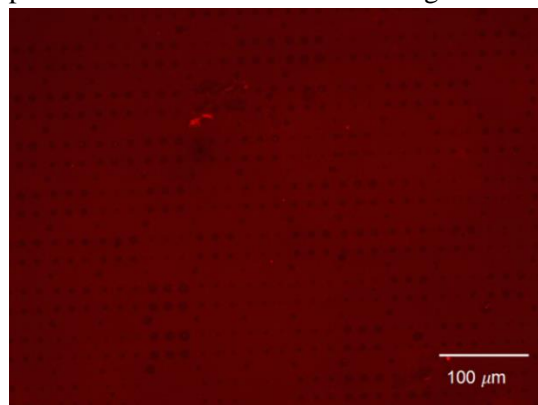
**Figure S3.** (a) Fluorescence microscopy image (Nikon Eclipse Ti,  $\lambda_{\text{ex}}$ = 532-587 nm,  $\lambda_{\text{em}}$ = 608-687 nm) of an array of **2** patterned by PPL on a bare glass slide with dwell times of 50, 500, 5,000, 50,000 and 100,000 ms before washing and (b) after washing. Fluorescence microscopy image ( $\lambda_{\text{ex}}$ = 532-587 nm,  $\lambda_{\text{em}}$ = 608-687 nm) of an array of **2** patterned by PPL on an alkene-terminated glass slide (no UV

exposure) with dwell times of 50, 500, 5,000, 50,000 and 100,000 ms before washing (c) and (d) after washing.

## 5. Beam Pen Lithography

**Rhodamine-methacrylate (1) Printing onto Thiol-terminated Glass Surfaces.** BPL pen arrays were fabricated following the reported procedure.<sup>5</sup> To prepare the pen arrays for inking, they were exposed to O<sub>2</sub> plasma (Harrick PDC-001, 30 s, medium power, ~200 mTorr) to render the surface of the BPL pen-arrays hydrophilic. Then 4 drops of the ink solution, comprised of **1** (1.6mg, 2.4 mmol), PEG (2,000 g mol<sup>-1</sup>, 5 mg mL<sup>-1</sup>) and DMPA (0.60 mg, 2.34 mmol) in 0.8 mL 80:20 THF:H<sub>2</sub>O that had been sonicated to ensure solution homogeneity were spin coated (2,000 rpm, 2 min) onto the BPL pen array. A Park XE-150 Scanning probe microscope equipped with a PPL head (Park Systems Corp.), XEP lithography software, and an environmental chamber capable of controlling humidity were used for BPL writing at a humidity of 66%-72% at room temperature. The tip array was leveled by optical methods with respect to the substrate surface using an xy tilting stage. The ink mixture was patterned into dot arrays with 1s dwell time. After printing, AFM-controlled BPL tip array was brought back to each spot and exposed for different times (2, 5, 10, and 20 min) under the 365-nm UV lamp (90 mW), and the substrate was subsequently washed and sonicated with 50 ml EtOH and H<sub>2</sub>O.

**Control Experiments.** In the control experiment, ink mixture containing **1** (1.6 mg, 2.4 mmol), PEG (2,000 g mol<sup>-1</sup>, 5 mg mL<sup>-1</sup>) and DMPA (0.60 mg, 2.34 mmol) was deposited onto a thiol-terminated glass surface by BPL without UV exposure and following identical procedure described above. No fluorescent patterns were observed on the bare glass surface (Figure S4).

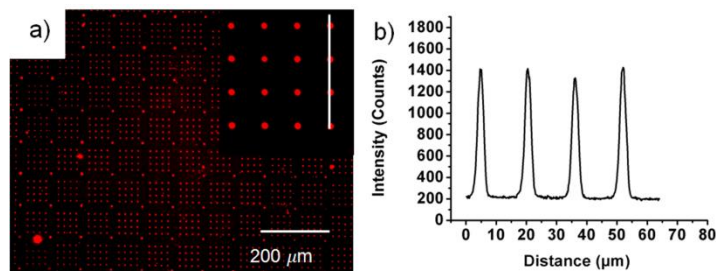


**Figure S4.** Fluorescence microscopy image (Nikon Eclipse Ti,  $\lambda_{\text{ex}}$  = 532-587 nm,  $\lambda_{\text{em}}$  = 608-687 nm) of an array of **1** patterned by BPL onto a thiol-terminated glass slide with 1s dwell time (no UV exposure) after washing.

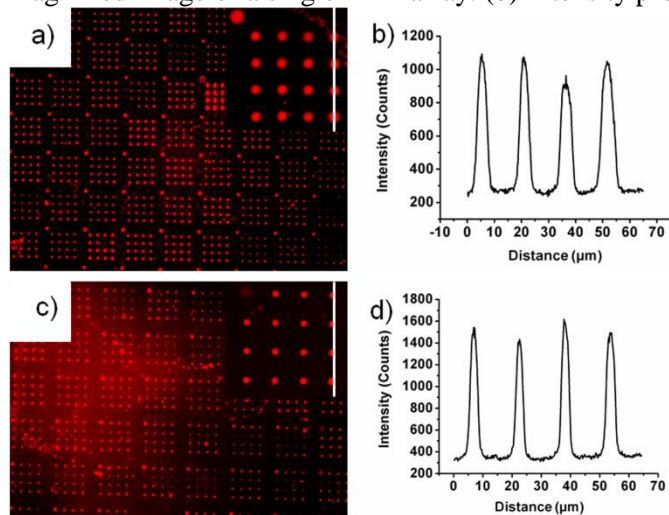
## 6. Lectin Binding

**Preparation of Carbohydrate Arrays by the PPL-induced Thiol-ene and Acrylate photopolymerization Reactions.** To prepare the pen arrays for inking, they were exposed to O<sub>2</sub> plasma (Harrick PDC-001, 30 s, medium power, ~200 mTorr) to render the surfaces of the pen-arrays hydrophilic. Subsequently 4 drops of the ink solution, comprised of **3** (2.18 mg, 10 mM), PEG (2,000 g mol<sup>-1</sup>, 5 mg mL<sup>-1</sup>) and DMPA in 0.8 mL 80:20 THF:H<sub>2</sub>O that was sonicated to ensure solution homogeneity, were spin coated (2,000 rpm, 2 min) onto the pen PPL array. A Park XE-150 Scanning probe microscope

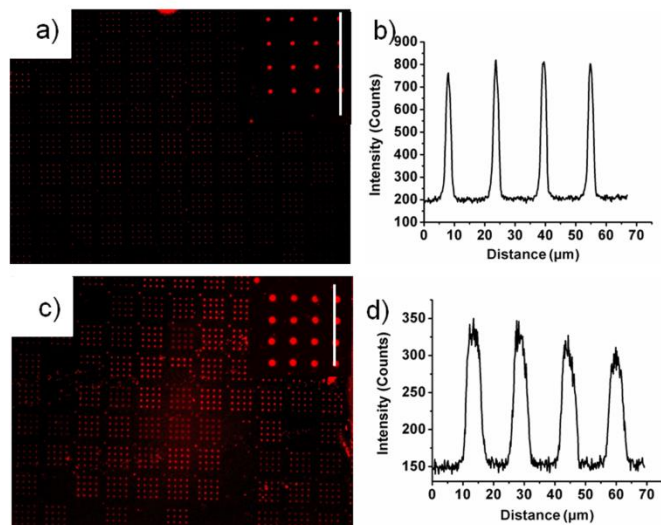
equipped with a PPL head (Park Systems Corp.), XEP lithography software, and an environmental chamber capable of controlling humidity were used for PPL writing at a humidity of 78%-83% at room temperature. The tip array was leveled by optical methods with respect to the substrate surface using a xy tilting stage. After placed under the UV light ( $3 \text{ mW cm}^{-2}$ ) for 5 hours, the slide was washed with 30 ml THF and 30 ml  $\text{H}_2\text{O}$  and dried with  $\text{N}_2$ . Then the slide was immersed in bovine serum albumin (1%) solution for 2 hours and washed 3 times with aqueous phosphate buffer (10 mM, pH 7.4, 0.005% Tween 20). After drying with  $\text{N}_2$ , the slide was immersed in Cy3-ConA solution (phosphate buffered saline, 10 mM, pH 7.4, 0.1mM  $\text{Ca}^{2+}$ , 0.1mM  $\text{Mn}^{2+}$ ) of varying concentrations ( $21.7 \times 10^{-6}$ ,  $10.8 \times 10^{-6}$ ,  $5.4 \times 10^{-6}$ ,  $3.0 \times 10^{-6}$ ,  $1.7 \times 10^{-6}$ ,  $4.3 \times 10^{-7}$ ,  $2.1 \times 10^{-7}$  M, Figures S11-S17) for 5 h at  $4^\circ\text{C}$ , washed 3 times with aqueous phosphate buffer (10 mM, pH 7.4, 0.005% Tween 20), and dried with  $\text{N}_2$ . Cy3-ConA concentration was based on a monomer  $M_w$  of 26,000.<sup>6,7</sup> The carbohydrate arrays of **4** and **5** were prepared following the same procedure.



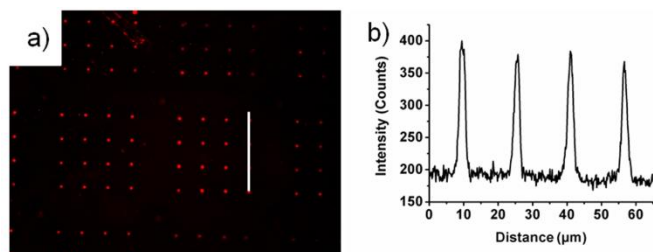
**Figure S5.** (a) Fluorescence microscopy image (Nikon Eclipse Ti,  $\lambda_{\text{ex}} = 532\text{-}587 \text{ nm}$ ,  $\lambda_{\text{em}} = 608\text{-}683 \text{ nm}$ ) of a surface patterned with **3** and exposed to a solution of  $2.17 \times 10^{-5} \text{ M}$  Cy3-modified ConA. The inset is a magnified image of a single  $4 \times 4$  array. (b) Intensity profile of the white line in (a).



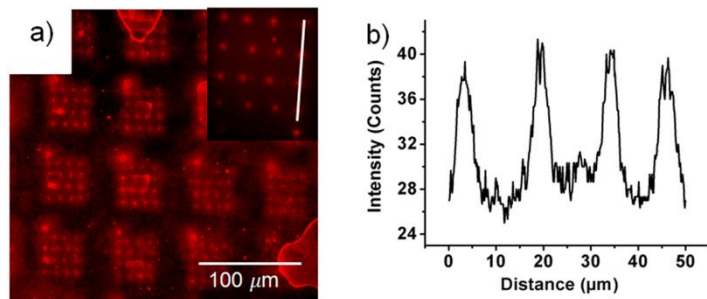
**Figure S6.** (a) Fluorescence microscopy image (Nikon Eclipse Ti,  $\lambda_{\text{ex}} = 532\text{-}587 \text{ nm}$ ,  $\lambda_{\text{em}} = 608\text{-}683 \text{ nm}$ ) of a surface patterned with **3** and exposed to a solution of  $1.08 \times 10^{-5} \text{ M}$  Cy3-modified ConA. The inset is a magnified image of a single  $4 \times 4$  array. (b) Intensity profile of the white line in (a). (c) Fluorescence microscopy image (Nikon Eclipse Ti,  $\lambda_{\text{ex}} = 532\text{-}587 \text{ nm}$ ,  $\lambda_{\text{em}} = 608\text{-}683 \text{ nm}$ ) of a surface patterned with **3** and exposed to a solution of  $5.4 \times 10^{-6} \text{ M}$  Cy3-modified ConA. The inset is a magnified image of a single  $4 \times 4$  array. (d) Intensity profile of the white line in (c).



**Figure S7.** (a) Fluorescence microscopy image (Nikon Eclipse Ti,  $\lambda_{\text{ex}} = 532\text{-}587\text{ nm}$ ,  $\lambda_{\text{em}} = 608\text{-}683\text{ nm}$ ) of a surface patterned with **3** and exposed to a solution of  $3.0 \times 10^{-6}\text{ M}$  Cy3-modified ConA. The inset is a magnified image of a single  $4 \times 4$  array. (b) Intensity profile of the white line in (a). (c) Fluorescence microscopy image (Nikon Eclipse Ti,  $\lambda_{\text{ex}} = 532\text{-}587\text{ nm}$ ,  $\lambda_{\text{em}} = 608\text{-}683\text{ nm}$ ) of a surface patterned with **3** and exposed to a solution of  $1.7 \times 10^{-6}\text{ M}$  Cy3-modified ConA. The inset is a magnified image of a single  $4 \times 4$  array. (d) Intensity profile of the white line in (c).

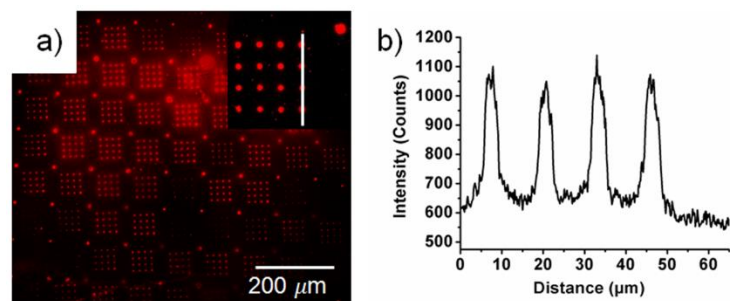


**Figure S8.** (a) Fluorescence microscopy image (Nikon Eclipse Ti,  $\lambda_{\text{ex}} = 532\text{-}587\text{ nm}$ ,  $\lambda_{\text{em}} = 608\text{-}683\text{ nm}$ ) of a surface patterned with **3** and exposed to a solution of  $4.3 \times 10^{-7}\text{ M}$  Cy3-modified ConA. (b) Intensity profile of the white line in (a).

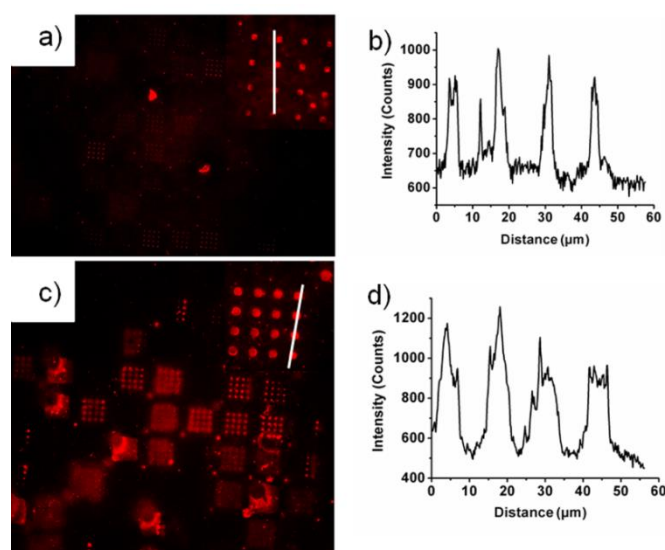


**Figure S9.** (a) Fluorescence microscopy image (Nikon Eclipse Ti,  $\lambda_{\text{ex}} = 532\text{-}587\text{ nm}$ ,  $\lambda_{\text{em}} = 608\text{-}683\text{ nm}$ ) of a surface patterned with **4** and exposed to a solution of  $2.17 \times 10^{-5}\text{ M}$  Cy3-modified ConA. The inset is a magnified image of a single  $4 \times 4$  array. (b) Intensity profile of the white line in (a).





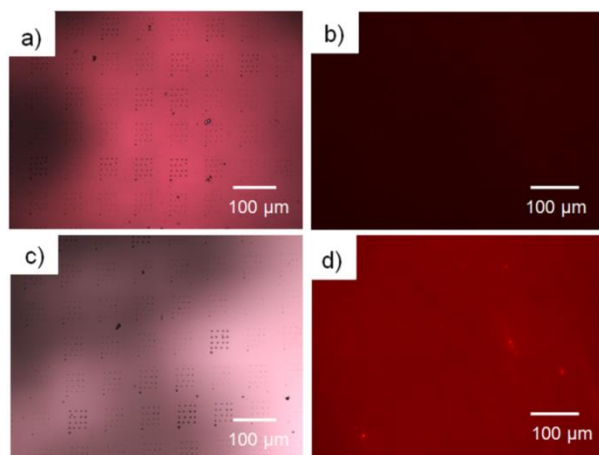
**Figure S10.** (a) Fluorescence microscopy image (Nikon Eclipse Ti,  $\lambda_{\text{ex}} = 532\text{-}587\text{ nm}$ ,  $\lambda_{\text{em}} = 608\text{-}683\text{ nm}$ ) of a surface patterned with **5** and exposed to a solution of  $2.17 \times 10^{-5}\text{ M}$  Cy3-modified ConA. The inset is a magnified image of a single  $4 \times 4$  array. (b) Intensity profile of the white line in (a).



**Figure S11.** (a) Fluorescence microscopy image (Nikon Eclipse Ti,  $\lambda_{\text{ex}} = 532\text{-}587\text{ nm}$ ,  $\lambda_{\text{em}} = 608\text{-}683\text{ nm}$ ) of a surface patterned with **5** and exposed to a solution of  $1.08 \times 10^{-5}\text{ M}$  Cy3-modified ConA. The inset is a magnified image of a single  $4 \times 4$  array. (b) Intensity profile of the white line in (a). (c) Fluorescence microscopy image (Nikon Eclipse Ti,  $\lambda_{\text{ex}} = 532\text{-}587\text{ nm}$ ,  $\lambda_{\text{em}} = 608\text{-}683\text{ nm}$ ) of a surface patterned with **5** and exposed to a solution of  $5.4 \times 10^{-6}\text{ M}$  Cy3-modified ConA. The inset is a magnified image of a single  $4 \times 4$  array. (d) Intensity profile of the white line in (c).

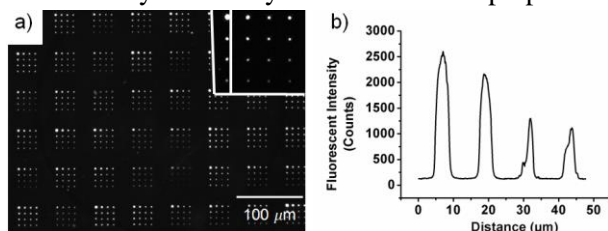
### Control Experiments.

BPL-printed microarrays of **3** and **4** were printed following the aforementioned procedure and immersed in a phosphate-buffered saline solution (10 mM, pH 7.4, 0.1mM  $\text{Ca}^{2+}$ , 0.1mM  $\text{Mn}^{2+}$ ) of Rhodamine-labeled PNA ( $2.17 \times 10^{-5}\text{ M}$ ), which is a galactose-specific lectin that does not bind mannose or glucose, for 5 h. Following washing with aqueous phosphate buffer (10 mM, pH 7.4, 0.005% Tween 20), three times, no visible fluorescence was observed. This experiment supports the conclusion that fluorescence in the Cy3-labelled ConA exposed arrays of **3** and **4** arises from ConA- glucose binding (Figure S12).

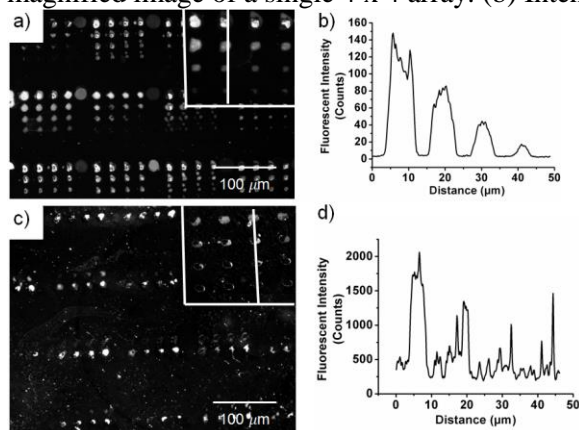


**Figure S12.** (a) Optical image of the printing of **3** in a PEG matrix on a thiol-terminated glass slide by PPL-induced thiol-acrylate reaction. (b) Fluorescence image of **3** on a thiol-terminated glass slide after Rhodamine-PNA interaction. (c) Optical image of the printing of **4** in a PEG matrix on a thiol-terminated glass slide by PPL-induced thiol-alkene reaction. (d) Fluorescence image of **4** on a thiol-terminated glass slide after immersion in a Rhodamine-labeled PNA solution.

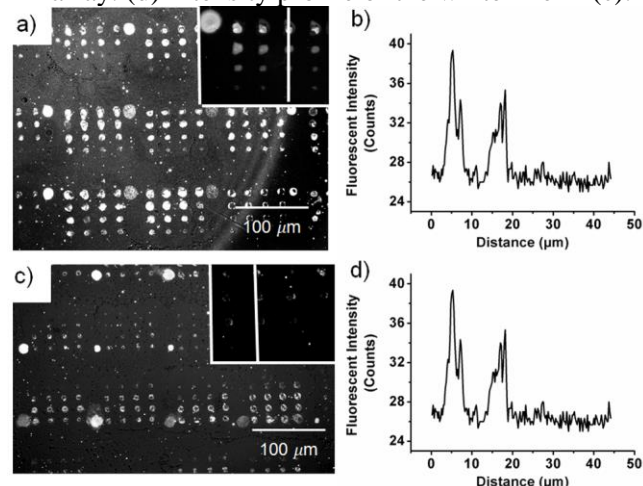
**Preparation of Carbohydrate arrays by BPL-induced Thiol-ene and Acrylate Photopolymerization Reactions.** To prepare the pen arrays for inking, they were exposed to O<sub>2</sub> plasma (Harrick PDC-001, 30 s, medium power, ~200 mTorr) to render the surface of the BPL pen-arrays hydrophilic. Then 4 drops of the ink solution, comprised of **3** (2.18 mg, 10 mM), PEG (2,000 g mol<sup>-1</sup>, 5 mg mL<sup>-1</sup>) and DMPA (0.80 mg, 3.12 mmol) in 1 mL 80:20 THF:H<sub>2</sub>O that had been sonicated to ensure solution homogeneity were spin coated (2,000 rpm, 2 min) onto the pen BPL array. A Park XE-150 Scanning probe microscope equipped with a PPL head (Park Systems Corp.), XEP lithography software, and an environmental chamber capable of controlling humidity were used for BPL writing at a humidity of 66%-72% at room temperature. The tip array was leveled by optical methods with respect to the substrate surface using an xy tilting stage. The ink mixture was patterned into dot arrays with 1s dwell time. After printing, the AFM-controlled BPL tip array was brought back to each spot and exposed for different times (2, 5, 10, and 20 min) under the 365-nm UV lamp (90 mW), and the substrate was subsequently washed with 50 ml EtOH and H<sub>2</sub>O and dried with N<sub>2</sub>. Then the slide was immersed in bovine serum albumin (1%) solution for 2 hours and washed 3 times with aqueous phosphate buffer (10 mM, pH 7.4, 0.005% Tween 20). After drying with N<sub>2</sub>, the slide was immersed in Cy3-ConA solution (phosphate buffered saline, 10 mM, pH 7.4, 0.1mM Ca<sup>2+</sup>, 0.1mM Mn<sup>2+</sup>) of varying concentrations (21.7x10<sup>-6</sup>, 5.4x10<sup>-6</sup>, 3.0x10<sup>-6</sup>, 1.7x10<sup>-6</sup>, 4.3x10<sup>-7</sup> M, Figures S20-S22) for 5 h at 4 °C, washed 3 times with aqueous phosphate buffer (10 mM, pH 7.4, 0.005% Tween 20), and dried with N<sub>2</sub>. The carbohydrate arrays of **4** and **5** were prepared following the same procedure.



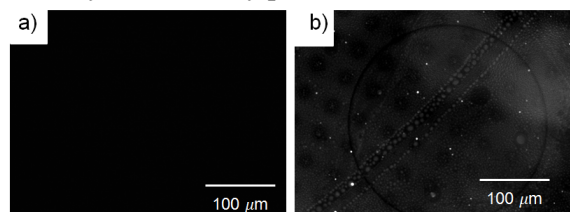
**Figure S13.** (a) Fluorescence microscopy image (Nikon Eclipse Ti,  $\lambda_{\text{ex}} = 532\text{--}587\text{ nm}$ ,  $\lambda_{\text{em}} = 608\text{--}683\text{ nm}$ ) of a surface patterned with **3** and exposed to a solution of  $21.7 \times 10^{-6}\text{ M}$  Cy3-modified ConA. The inset is a magnified image of a single  $4 \times 4$  array. (b) Intensity profile of the white line in (a).



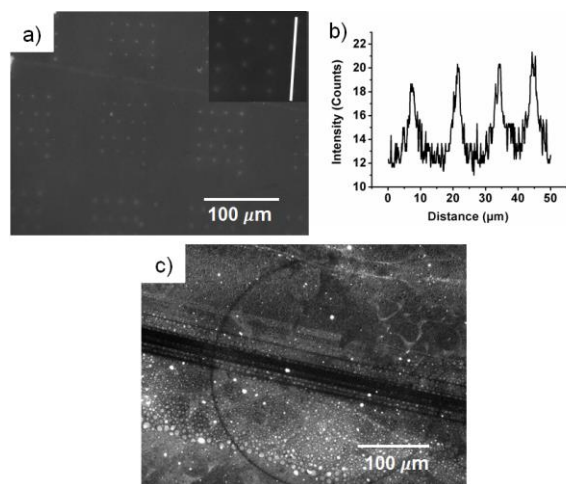
**Figure S14.** (a) Fluorescence microscopy image (Nikon Eclipse Ti,  $\lambda_{\text{ex}} = 532\text{--}587\text{ nm}$ ,  $\lambda_{\text{em}} = 608\text{--}683\text{ nm}$ ) of a surface patterned with **3** and exposed to a solution of  $5.4 \times 10^{-6}\text{ M}$  Cy3-modified ConA. The inset is a magnified image of a single  $4 \times 4$  array. (b) Intensity profile of the white line in (a). (c) Fluorescence microscopy image (Nikon Eclipse Ti,  $\lambda_{\text{ex}} = 532\text{--}587\text{ nm}$ ,  $\lambda_{\text{em}} = 608\text{--}683\text{ nm}$ ) of a surface patterned with **3** and exposed to a solution of  $3.0 \times 10^{-6}\text{ M}$  Cy3-modified ConA. The inset is a magnified image of a single  $4 \times 4$  array. (d) Intensity profile of the white line in (c).



**Figure S15.** (a) Fluorescence microscopy image (Nikon Eclipse Ti,  $\lambda_{\text{ex}} = 532\text{--}587\text{ nm}$ ,  $\lambda_{\text{em}} = 608\text{--}683\text{ nm}$ ) of a surface patterned with **3** and exposed to a solution of  $1.7 \times 10^{-6}\text{ M}$  Cy3-modified ConA. The inset is a magnified image of a single  $4 \times 4$  array. (b) Intensity profile of the white line in (a). (c) Fluorescence microscopy image (Nikon Eclipse Ti,  $\lambda_{\text{ex}} = 532\text{--}587\text{ nm}$ ,  $\lambda_{\text{em}} = 608\text{--}683\text{ nm}$ ) of a surface patterned with **3** and exposed to a solution of  $4.3 \times 10^{-7}\text{ M}$  Cy3-modified ConA. The inset is a magnified image of a single  $4 \times 4$  array. (d) Intensity profile of the white line in (c).



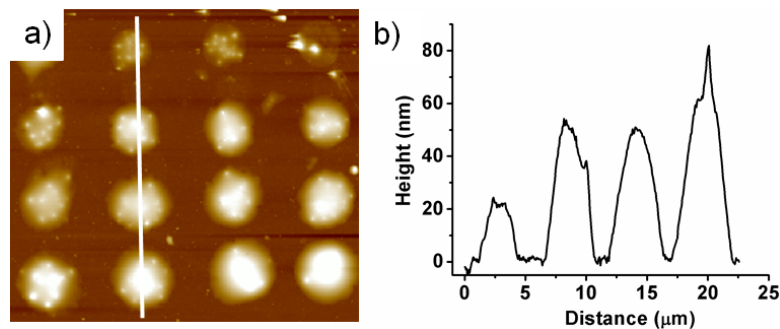
**Figure S16.** (a) Fluorescence microscopy image of **3** immobilized on a thiol-terminated glass slide after immersion in a  $21.7 \times 10^{-6}$  M Rhodamine-labeled PNA solution. (b) Fluorescence image of **4** immobilized on a thiol-terminated glass slide after immersion in a  $21.7 \times 10^{-6}$  M Rhodamine-labeled PNA solution.



**Figure S17.** (a) Fluorescence image of **5** immobilized on a thiol-terminated glass slide after immersion in a solution of  $21.7 \times 10^{-6}$  M Cy3-modified ConA. (b) Intensity profile of the white line in (a). (c) Fluorescence image of **5** immobilized on a thiol-terminated glass slide after immersion in a solution of  $21.7 \times 10^{-6}$  M Cy3-modified PNA.

## 8. Atomic Force Microscopy of Rhodamine Methacrylate Immobilized on the Surface

**1** was patterned onto the thiol-terminated glass surface by BPL, as described above, with a dwell time of 1s. After printing, AFM-controlled BPL tip array was brought back to each spot and exposed for different times (2, 5, 10, and 20 min) under the 365-nm UV lamp (90 mW), and the substrate was subsequently washed with 50 ml EtOH and H<sub>2</sub>O and dried with an N<sub>2</sub> stream. AFM characterization of the height profile of the features on the surface patterned with **1** after washing was performed on a Bruker Dimension Icon with an NCHR tip (NanoWorld, force constant /  $42 \text{ Nm}^{-1}$ ). AFM data analysis was performed using Nanoscope analysis software.



**Figure S18.** (a) AFM tapping mode image of a 4 x 4 pattern of **1** bound to the thiol-terminated glass slide. (b) AFM height profile of the white line in (a).

## 8. References

- (1) J. Mehlich and B. J. Ravoo *Org. Biomol. Chem.*, 2011, **9**, 4108.
- (2) J. R. J. Ruiz, G. Osswald, M. Petersen, and W. D. Fessner *J. Mol. Catal., B Enzym.*, 2001, **11**, 189.
- (3) I. Cumpstey, T. D. Butters, R. J. Tennant-Eyles, A. J. Fairbanks, R. R. France, and M. R. Wormald, *Carbohydr. Res.*, 2003, **338**, 1937.
- (4) C. M. Halliwell and A. E. G. Cass, *Anal. Chem.*, 2001, **73**, 2476.
- (5) F. W. Huo, G. F. Zheng, X. Liao, L. R. Giam, J. A. Chai, X. D. Chen, W. Y. Shim, and C. A. Mirkin, *Nat. Nanotechnol.*, 2010, **5**, 637.
- (6) A. R. Fraser, J. J. Hemperly, J. L. Wang, and G M Edelman, *Proc. Natl. Acad. Sci. USA.*, 1976, **73**, 790.
- (7) W. D. McCubbin and C. M. Kay, *Biochem. Biophys. Res. Commun.*, 1971, **44**, 101.