Electronic Supplementary Information

Covalently bound monolayer patterns obtained by plasma etching on glass surfaces

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Experimental Section

General

Chemicals were purchased from Sigma Aldrich/Merck unless mentioned otherwise. Knittel (Germany) borosilicate glass cover slips (18x18 mm, No. 1) were used as glass substrates for functionalization experiments. Hyper pure polished silicon wafers (76.2 mm diameter, purchased from University Wafer, USA) were used as silicon masters. A photomask template containing patterns, with different feature and spacing sizes, was designed and ordered from JD photo data (UK). Deionized (DI) water was used in all the experiments. Cyanine 5-diadamantane (Cy5-Ad_2) was synthesized by Dr. Mark Rood from the Interventional Molecular Imaging lab at the Leiden University Medical Centre (LUMC, Leiden, the Netherlands).[1]

Instrumentation

For the preparation of the silicon master, a standard UV-lithography set-up was used. The negative photoresist on the silicon wafer was irradiated with a UV lamp at λ = 350 nm at 50% intensity. An Inseto Plasma Etch, Inc. PE-25 benchtop air plasma cleaner was used for plasma cycles of 1 min at its maximum RF plasma power of 100 W with an air flow of ~5-10 cc/min, which allowed for a vacuum pressure of 200-250 mTorr within the chamber during plasma treatment. An RC6 Chemistry Hybrid Pump from Vauubrand (vacuum of 2 x 10^{-3} mbar) was used for applying high vacuum condition in desiccators. The functionalized glass surface were imaged in air, using a Leica DMi8 epifluorescence microscope with 40X (oil immersion), 10X, 5X or 2.5X magnification objective lens, beam intensity of 100% and an exposure time of 278 ms. The Cy5 fluorescence was excited at a wavelength (λ_{ex}) of 590-650 nm and emission (λ_{em}) was collected at 662-738 nm (using the Y5 filter cube), rhodamine B isothiocyanate (RITC) at λ_{ex} of 541-551 nm and λ_{em} of 662-738 nm (RHOD filter cube). The results were analyzed with FIJI software (ImageJ).[2] The images were rotated in such a way that the patterns were positioned vertically. Then, a square was drawn over the patterns and the averaged gray values were collected within that square. The profile plots were also corrected for fluorescent beam curvature by acquiring profile plots of a fully fluorescent Cy5 slide with the same objective (see Figure S3), normalizing this curvature plot, and then multiplying the fluorescent intensity of line profile plots by the inverse of the curvature plot. The corrected gray values (a.u.) were then normalized for the maximum value and plotted against the distance (μm).

Glass surface functionalization

The general glass surface functionalization with β-cyclodextrin was performed as described by Onclin *et al*.[3] Glass microscope cover slips were cleaned and oxidized with piranha solution (H_2SO_4 (95-98%)/ H_2O_2 (35%), 3.33:1 v/v; *Warning! Piranha solutions must be handled with caution as they may unexpectedly detonate*) for 45 minutes, rinsed with large amounts DI water, and dried under N_2. The glass slides were placed in a high vacuum, pre-heated desiccator together with a glass vial containing

1 mL of 3-aminopropyltriethoxysilane (APTES, 99%) and placed in an oven at 70° C overnight for chemical vapor deposition (CVD) of APTES. Following amine monolayer formation, the glass slides were removed from the desiccator and rinsed with toluene (HPLC grade, VWR, the Netherlands) and dichloromethane (DCM, VWR). The glass slides were then cured for at least 1 hour in the oven at 70°C. Next, the glass slides were immersed in 0.1 M 1,4-phenylene diisothiocyanate (PDITC, TCI Chemicals Belgium) in anhydrous toluene (max 0.002% H_2O, VWR) for 2 hours under argon atmosphere to yield isothiocyanate bearing layers. Following the immersion, the surfaces were rinsed with toluene and DCM and incubated in 0.72 mM heptakis amino β-cyclodextrin (β-CD, Cyclodextrin Shop, the Netherlands) in aqueous solution at pH 8.0 (reached by adding small amount of 1 M NaOH). This incubation was carried out for at least 2 hours. Surfaces were then rinsed with DI water and dried with nitrogen.

Plasma microcontact patterning (PμCP)

Stamps were fabricated by using a replica molding technique from Whitesides.[4] A silicon master was first fabricated by spin-coating at 1500 rpm with SU-8 2025 photoresist (to yield 50 μm thick photoresist layer) from Microchem and then treated using UV photolithography with a mask containing 150 μm patterns and 50 μm pattern spacing. The silicon master and a separate glass vial containing 100 μL mL of trichloro(1H, 1H, 2H, 2H-perfluorooctyl)silane (PFOTS, 97%) were introduced into a desiccator under vacuum for overnight CVD. After incubation with PFOTS, the wafer was cleaned with isopropanol and dried with nitrogen. Stamps were prepared by casting a 10:1 (w/w) mixture of poly(dimethylsiloxane) (PDMS) and curing agent (Sylgard 184, Dow Corning) onto the silicon master with 150 μm patterns and 50 μm pattern spacing. After overnight curing at 70 °C, the PDMS stamps were cut out the master to ca 0.75 cm^2 and sonicated in ethanol to remove low molecular weight PDMS. The PDMS stamps were then brought into conformal contact with the freshly prepared functionalized glass surfaces. The glass substrates were then placed in the plasma cleaner at high energy for 4 cycles of 1 minute each. Surfaces were rinsed again with DI water and dried with nitrogen.

Fluorescence microscopy

APTES patterned glass surface were incubated with 1 mM RITC dissolved in methanol for 5 minutes. The surface was then rinsed thoroughly with methanol to remove excess RITC and then dried with nitrogen. RITC stained APTES patterns were then visualized with the microscope. For β-CD functionalized surfaces, 0.28 μM of Cy5-Ad_2 in phosphate buffered saline (PBS: 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, pH 7.4) was incubated on the glass surface for 15 minutes. The surface was then rinsed thoroughly with DI water and dried under a stream of nitrogen before imaging with the microscope.

Supplementary Figures

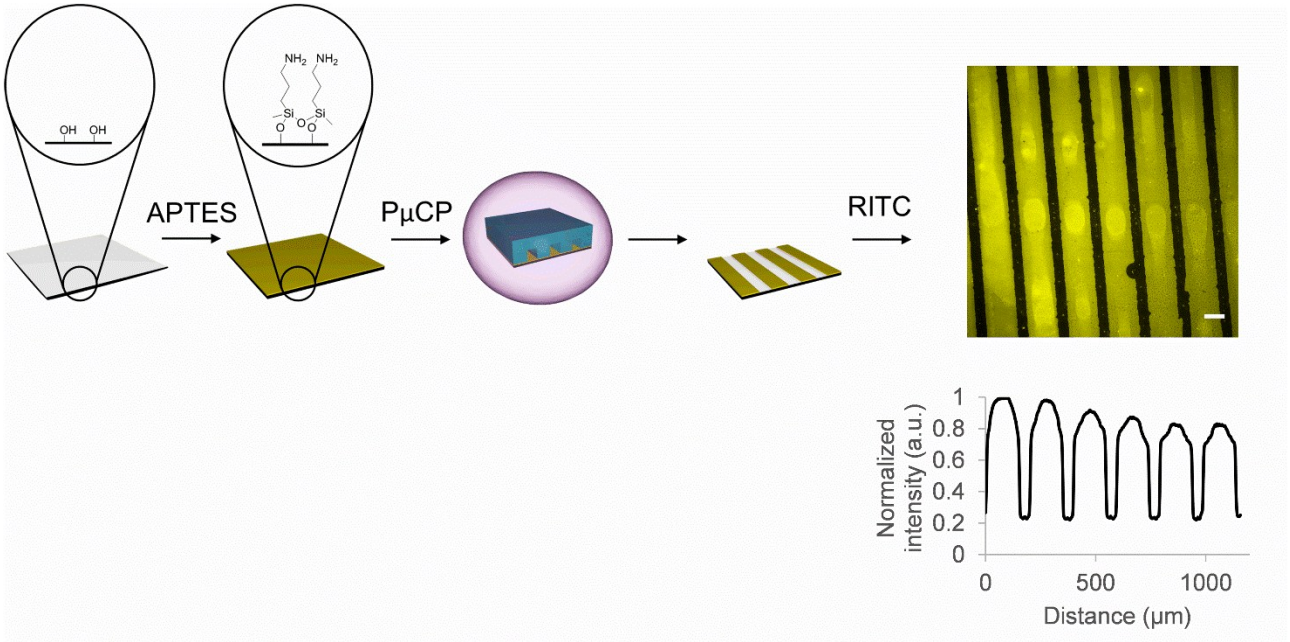

Figure S1: Validation of APTES patterns after PμCP (Exp. 1) using RITC. The scale bar in the microscope image is 100 μm. The profile plot below is already corrected for beam curvature.

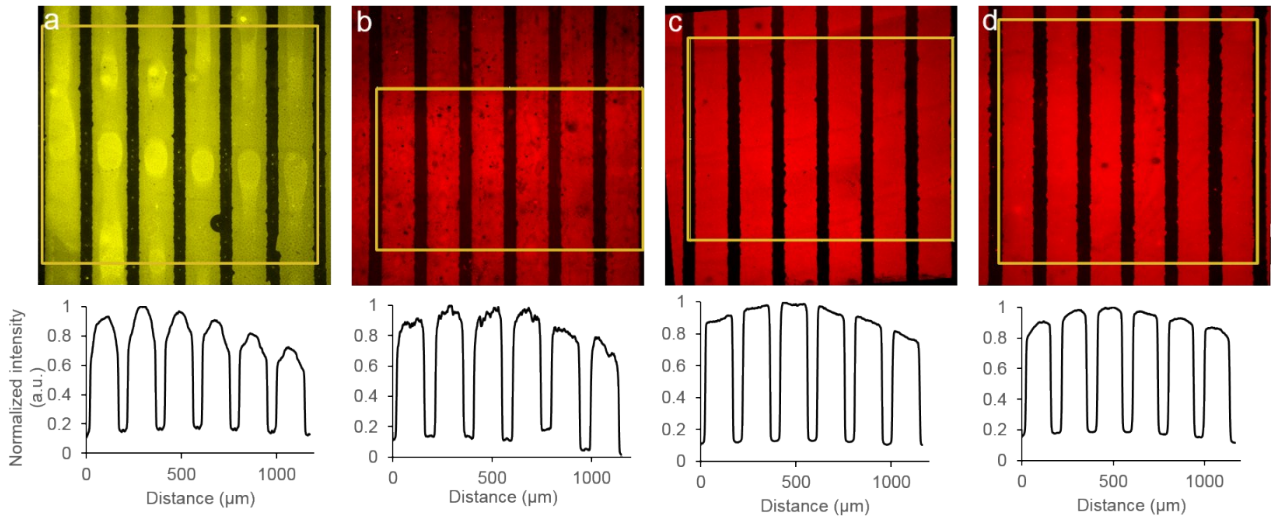

Figure S2: Images before beam curvature correction. a) APTES PμCP and RITC, b) Exp. 1: APTES PμCP -PDITC-β-CD and Cy5-Ad_2, c) Exp. 2: APTES-PDITC-β-CD PμCP and Cy5-Ad_2 and d) Exp. 3: APTES-PDITC-β-CD-Cy5-Ad_2 PμCP. The yellow outline on each image marks the area used for creating the profile plots in Fiji.

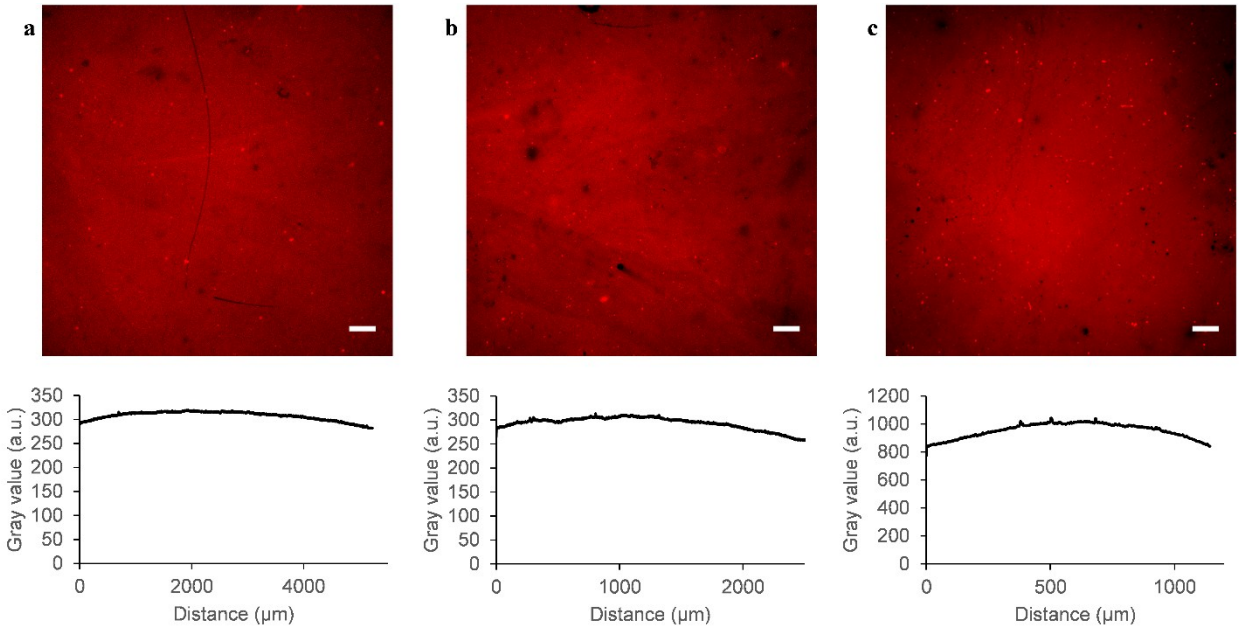

Figure S3: Profile plots of beam curvature of Cy5-Ad_2 fluorescent glass slides using a) 2.5x objective, b) 5x objective, and c) 10x objective. These plots were used to correct for the beam curvature in line profile plots by first normalizing the intensity values, then inverting the values and multiplying them with profile plot intensity values from Figure S4 for the constituent objective. Scale bars in images are a) 400 µm, b) 200 µm and c) 100 µm

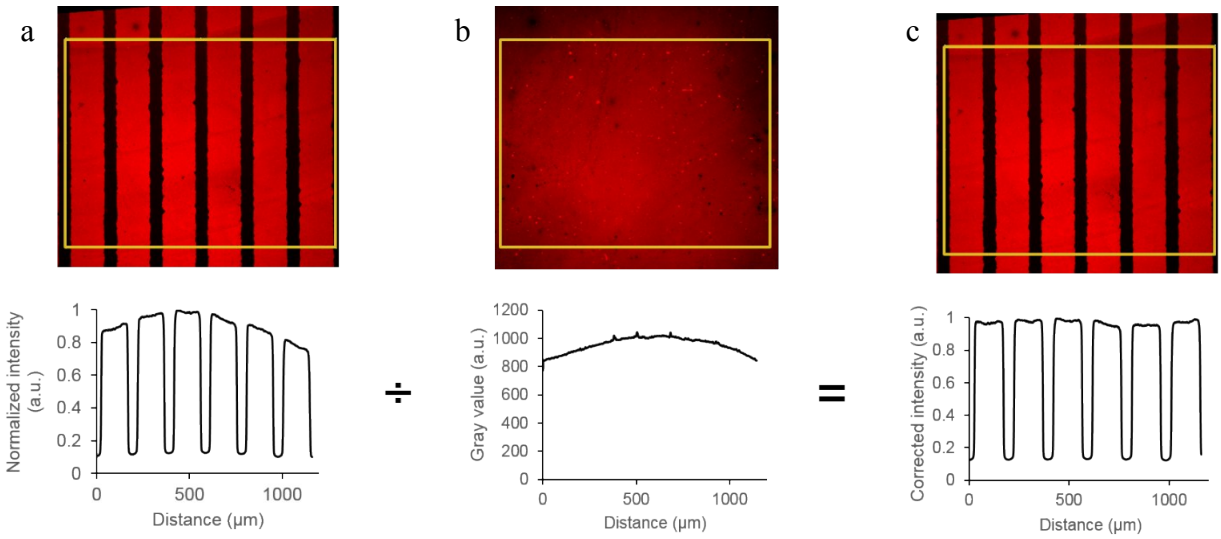

Figure S4: Scheme depicting how the beam correction was carried out over an image using Figure S3c and Figure S4c as an example. a) non-corrected, normalized intensity shown in profile plot. b) intensity plot of a fully covered slide. c) corrected profile plot after dividing profile plot of a) with normalized profile plot of c).

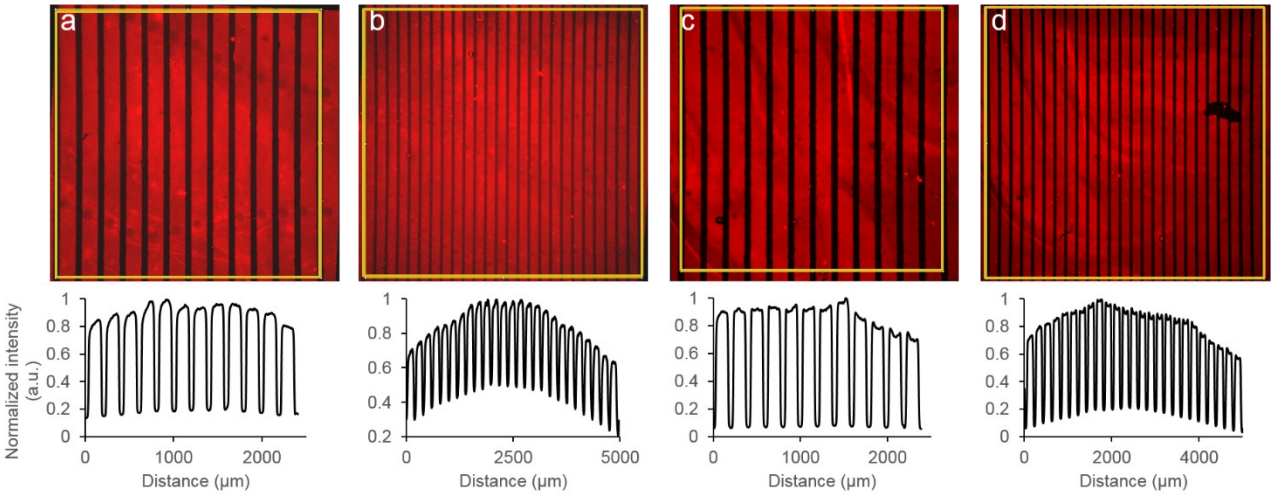

Figure S6: Larger overview of experiments from Figure 2 showing images of Experiment 1 with 2.5x (a) and 5x objective (b) and Experiment 2 with 2.5x (c) and 5x objective (d). Scale bars for 2.5x objective images are 400 µm and for 5x objective images are 200 µm. Constituent profile plots for images (a)-(d) are before beam curvature correction. Yellow outline on each images mark the area used for creating the profile plot

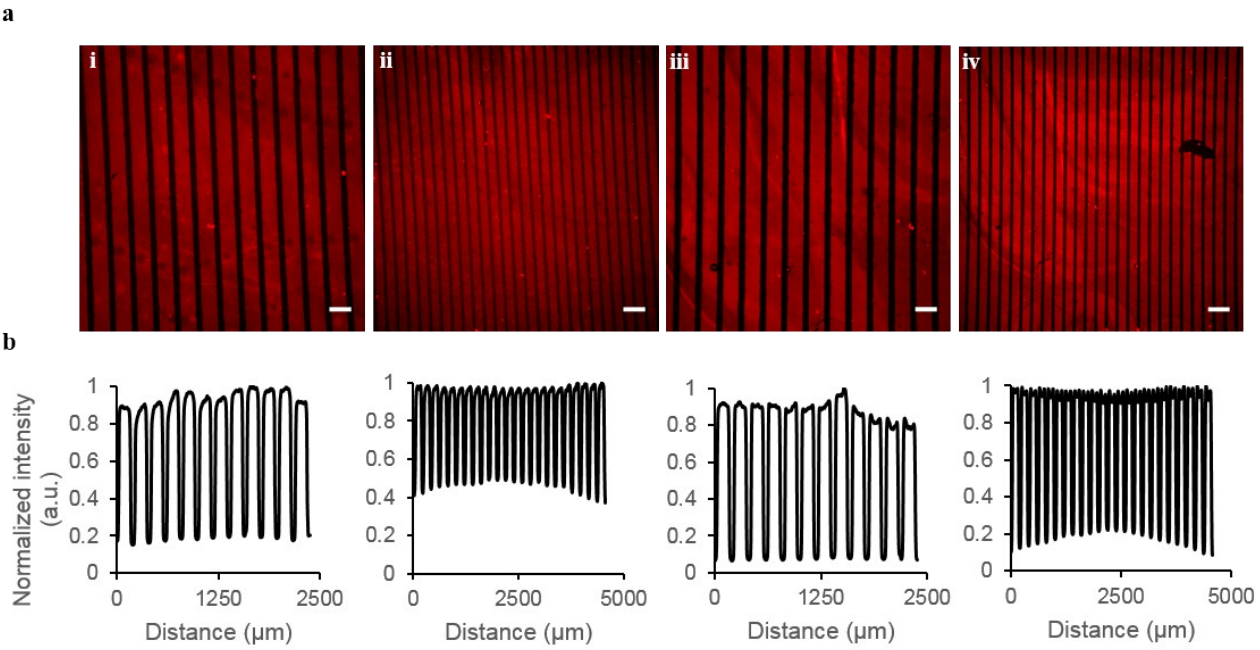

Figure S5: Correction of Figure S5 for beam curvature a) Larger overview of experiments from Figure 2 showing images of Experiment 1 with 5x (i) and 2.5x objective (ii) and Experiment 2 with 5x (iii) and 2.5x objective (iv). Scale bars for 5x objective images are 200 µm and for 2.5x objective images are 400 µm. b) Constituent profile plots for images (i)-(iv).

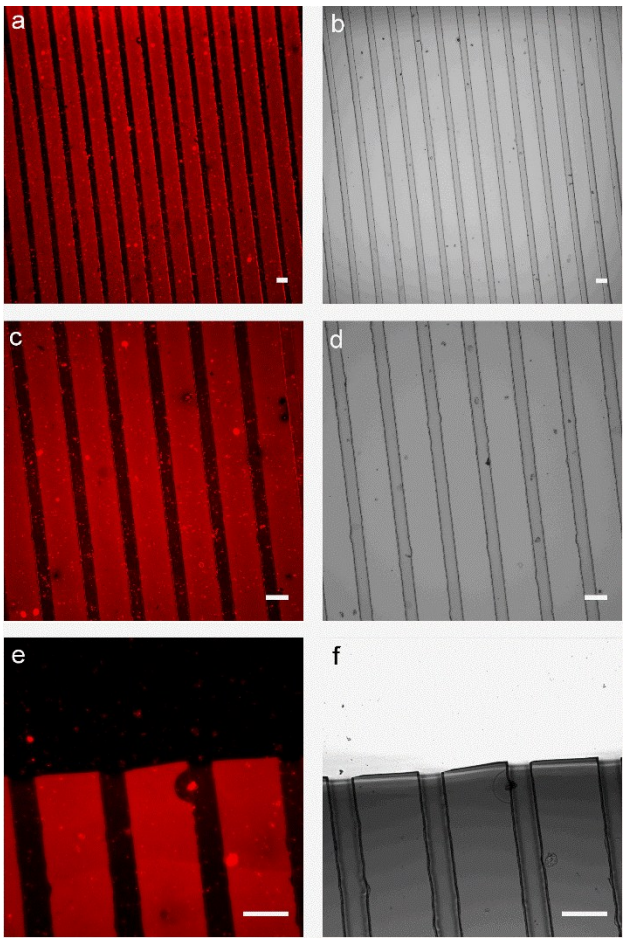

Figure S7: PμCP of β-CD surface after Cy5-Ad_2 immobilization with PDMS stamp still on top of patterned surface. 5x objective image with Cy5 (a) and TL-BF (b) filters. 10 objective images with Cy5 (c) andTL-BF filter (d) filters. 20x objective images with Cy5 (e) and TL-BF filters (f). All scale bars are 100 μm.

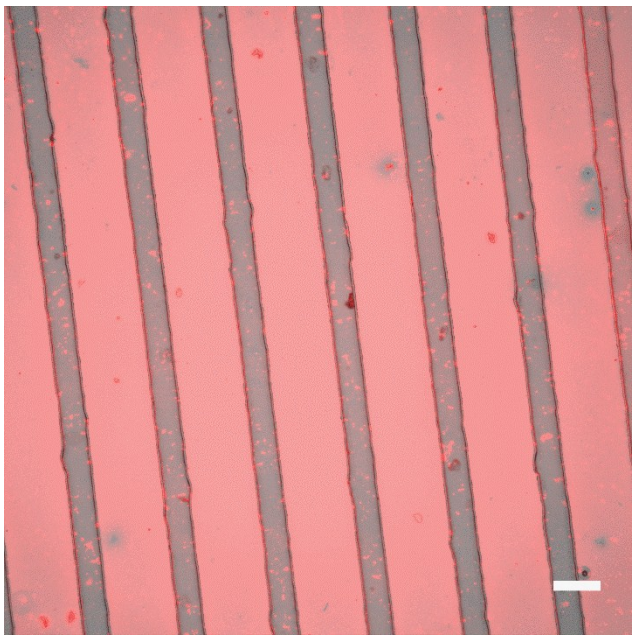

Figure S 8: Overlay of 10x objective images from Figure S7. Scale bar is 100 μm.

References

1. Rood, M. T. M.; Spa, S. J.; Welling, M. M.; ten Hove, J. B.; van Willigen, D. M.; Buckle, T.; Velders, A. H.; van Leeuwen, F. W. B., Obtaining control of cell surface functionalizations via Pre-targeting and Supramolecular host guest interactions. *Sci. Rep.* **2017,** *7*, 39908.
2. Schindelin, J.; Arganda-Carreras, I.; Frise, E.; Kaynig, V.; Longair, M.; Pietzsch, T.; Preibisch, S.; Rueden, C.; Saalfeld, S.; Schmid, B., Fiji: an open-source platform for biological-image analysis. *Nature methods* **2012,** *9* (7), 676-682.
3. Onclin, S.; Mulder, A.; Huskens, J.; Ravoo, B. J.; Reinhoudt, D. N., Molecular printboards: monolayers of beta-cyclodextrins on silicon oxide surfaces. *Langmuir* **2004,** *20* (13), 5460-5466.
4. Xia, Y.; Whitesides, G. M., Soft Lithography. *Angew. Chem. Int. Ed.* **1998,** *37* (5), 550-575.