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Electronic Supplementary Information (ESI)

Macroscopic supramolecular assembly to fabricate multiplexed DNA patterns for potential application in DNA chips

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Experiment Details:

Materials and Methods

The following chemicals were used as supplied: (3-aminopropyl) triethoxysilane (APTES), glutaraldehyde (GA) (aq, 25 wt%), poly(diallyldimethylammonium chloride) (PDDA) (aq, 20 wt%, MW 200000) and poly(acrylic acid) (PAA (aq, 25 wt%, MW 240000) from Sigma-Aldrich; polyethyleneimine (PEI) (branched, MW 1800) from Alfa Aesar; photoresist of SU8 2005 and its developer (propylene glycol monomethyl ether acetate, PGMEA) from MicroChem; Fe_3O_4 magnetic nanoparticles (MNPs) with an average diameter of 20 nm from Beijing DK Nanotechnology Co. Ltd. (Beijing, China). All solutions were prepared with PBS (pH=7.4) containing Na_2HPO_4/NaH_2PO_4 (100 mM) and NaCl (150 mM). In all DNA hybridization processes, extra Mg^{2+} (5 mM) in the form of $MgCl_2$ was added. All DNA sequences listed as follows are purchased from Sangon Biotech. Co. Ltd. (Shanghai, China).

Instruments and Characterization

A mask alignment system for photolithography (G-25, Chengdu Xinnanguang Mechanical Equipment Co., Ltd., China), scanning electron microscope (Hitachi, SU1510), UV-visible spectrophotometer (Hitachi U-3900H) and confocal microscope (Leica TCS SP5) were used for the preparation and characterization of the disk building blocks and the surface modification of DNA and microarrays. The fluorescence intensity data were analyzed using ImageJ software.

S1. Sequences of all used DNA

MSA-DNA:

5'-NH_2-$(CH_2)_6$ TTTTTTTTTTTTTTTTTTTT**ATTCAGGATTCTCAACTCGTA**-3'

M

Prob-DNAs

Prob1: 5'-NH_2-$(CH_2)_6$ **TACGAGTTGAGAATCCTGAAT**AGTCAGTGTGGA-3'

M' **P1**

Prob2: 5'-NH_2-$(CH_2)_6$ **TACGAGTTGAGAATCCTGAAT**TACCACATCATC-3'

M' **P2**

Prob3: 5'-NH_2-$(CH_2)_6$ **TACGAGTTGAGAATCCTGAAT**TCTGCACACCTC-3'

M' **P3**

TAR-DNAs

TAR1: 5'-GCTAGAGATTT**TCCACACTGACT**-3'

F1' **P1'**

TAR2: 5'-TCAGTTATATG**GATGATGTGGTA**-3'

F2' **P2'**

TAR3: 5'-CGGAGTGTCAA**GAGGTGTGCAGA**-3'

F3' **P3'**

Fluo-DNAs

Fluo1: 5’-AAATCTCTAGC-Cy3-3’

F1+chromophore Cy3

Fluo2: 5’-CATATAACTGA-FAM-3’

F2+chromophore FAM

Fluo3: 5’-TTGACACTCCG-ROX-3’

F3+chromophore ROX

ERR-DNAs

ERR1: 5’-GCTAGAGATTGTCCACACTGACT-3’

Error 1

ERR2: 5’-TCAGTTATATGTATGATGTGGTA-3’

Error 2

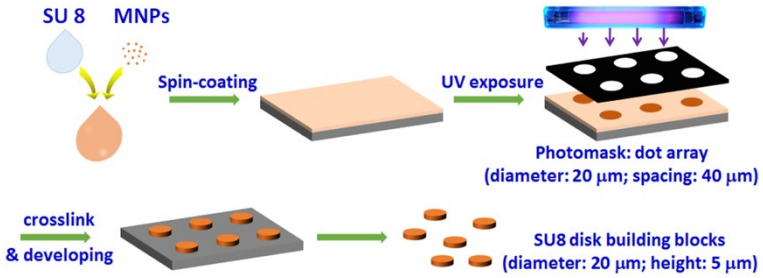
ERR3: 5’-CGGAGTGTCAATAGGTGTGCAGA-3’

Error 3

S2. Fabrication of magnetic-responsive disk building blocks

The magnetic-responsive disk building blocks were fabricated through a photolithography method using a photoresist of SU-8 2005, as schematically illustrated in **Scheme S1**. First, powders of Fe_3O_4 MNPs were mixed in the dense liquid of the

SU-8 2005 photoresist with a weight/volume ratio of 8 mg/mL. Silicon wafers were washed with a mixed solution of H_2O_2 (30 wt%) and H_2SO_4 (98%) (v/v=3:7) and deionized water, followed by drying at 120 °C for 30 min. Second, the photoresist mixed with the Fe_3O_4 MNPs was spin-coated onto the silicon wafer 6000 rpm for 40 s, and the coating was prebaked by drying at 65 °C for 15 min and subsequently at 90 °C for 30 min. Third, exposure was carried out under a photo mask of a dot array (diameter: 20 μm; spacing: 20 μm) for 35 s using 365-nm UV irradiation with an intensity of 3.3 mW/cm^2 on a photo-alignment system, followed by post-baking at 65 °C for 15 min and subsequently at 95 °C for 40 min. Fourth, the exposed wafer was immersed in PGMEA developer for 60 s, washed with isopropyl alcohol and dried under a nitrogen flow. Finally, separate disk-like building blocks (**Figure S1**: diameter: 20 μm; height: 5 μm) were harvested in water by gentle scraping with a rubber knife.



Scheme S1. The fabrication process of SU-8 photoresist building blocks.

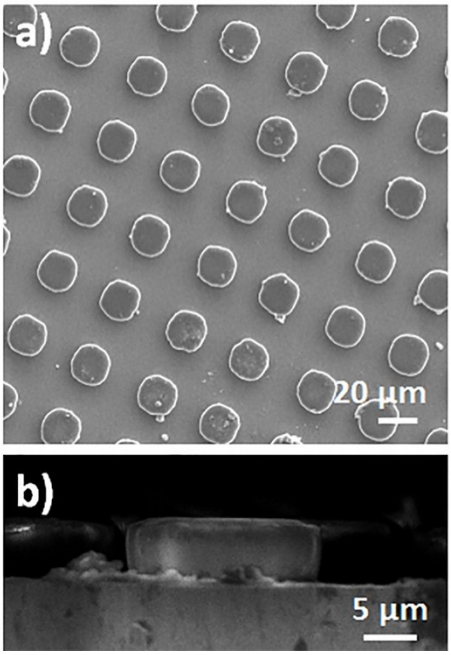
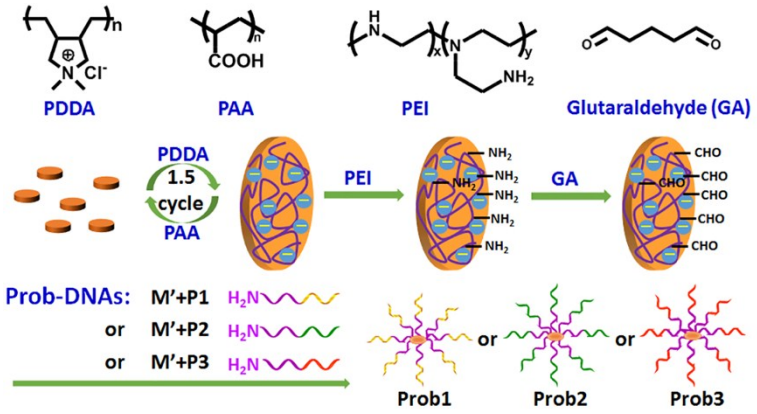


Figure S1. SEM images of the as-prepared SU-8 photoresist building blocks

S3. Surface modification of the building blocks and the substrate

The as-prepared disk-like building blocks were modified with DNA strands through the procedure shown in **Scheme S2**. To overcome the inert nature of the hydrophobic surfaces of the disks, we induced the formation of polyelectrolyte layers for the modification of amino groups. (1) The disks were immersed in PDDA polycation solution (aq, 1 mg/mL) for 30 min to obtain a layer of positive charges and then washed with alternating centrifugation at 2000 rpm to separate the disks from the solutions or water and redispersed in water. (2) The disks were alternately immersed in PAA polyanion solution (aq, 1 mg/mL) for 20 min and PDDA for 20 min for 1.5 cycles, with each immersion followed by rinsing assisted by centrifugation, leading to two bilayers of PDDA/PAA on the disks. (3) To induce the formation of amino groups, the disks were immersed in PEI solution (aq, 1 mg/mL) for 30 min. (4) After washing, a GA

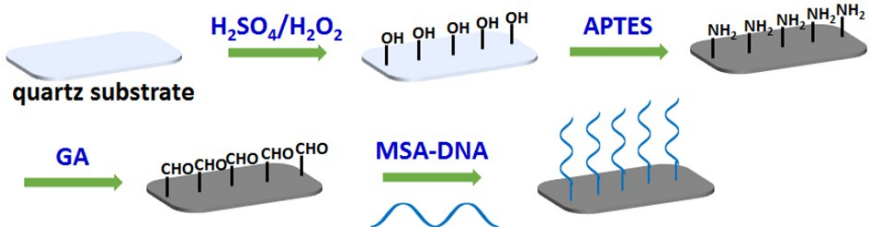
solution (5% v/v, water) was added, and the disks were immersed for 30 min to obtain aldehyde groups as most of the amino groups modified on the disks reacted with the excess GA molecules. (5) After rinsing with deionized water, the disks were redispersed in 500 μL of PBS solution, and Prob-DNAs were added to a final concentration of 4 μM and kept for 24 h to covalently link the terminal amino groups on the Prob-DNAs with the aldehyde groups on the disk surfaces.



Scheme S2. Surface modification process of SU-8 photoresist building blocks.

The surfaces of the substrates, as the platform of the microarrays, were modified using the process in **Scheme S3**. Quartz substrates ($10 \times 13 \times 1.0$ mm^3) were pretreated with a mixed solution of H_2O_2 (30 wt%) and H_2SO_4 (98%) (v/v=3:7) and deionized water. After drying under a nitrogen flow, the quartz substrates were kept in a toluene solution of an aminosilane, APTES (v/v=1:200), for 12 h to be modified with amino groups. The substrates were further washed with a solvent sequence of toluene, acetone and ethanol and immersed in a GA solution (aq, 0.5 wt%) for 30 min to induce the formation of aldehyde groups. Finally, the pretreated quartz substrates were exposed to 150 μL of the MSA-DNAs (4 μM) in PBS and kept for 24 h to covalently attach the

MSA-DNAs through their terminal amino groups to the aldehyde groups on the quartz substrates.



Scheme S3. Surface modification process of the substrate for MSA.

S4. Construction of the DNA microarray through MSA

One of the above quartz substrates modified with the MSA-DNAs intended to serve as the platform of the microarray was placed in a petri dish containing PBS and $MgCl_2$ (5 mM). A cleaned bare quartz substrate was placed next to the modified quartz as a control. Disks with a certain Prob-DNA (Prob1 or Prob2 or Prob3) were dropped onto the bare quartz for temporary "storage". We used a magnet (0.5 T) to lift one of the disks from the control quartz and transferred it to a designated location on the modified quartz as a result of the magnetic responsiveness of the disk building blocks. After sufficient hybridization between the M sequence on the MSA-DNA and the M' sequence on the Prob-DNA for 12 h, the disk was totally immobilized on the substrate and could not be moved by the applied magnetic force. Then, another disk was selected, placed and assembled in this manner of combining magnetic localization and DNA hybridization between complementary sequences. Through the above stepwise magnetic-assisted MSA, we obtained arbitrary patterns or arrays of the disks with flexible designs and adjustments independent of the template used. In this experiment,

all the presented DNA microarrays shared one pattern: the disks were aligned row by row with adjacent spacings of approximately 40~60 μm. The first row of disks was modified with Prob1-DNA, the second row was modified with Prob2-DNA, and the third was modified with Prob3-DNA.

S5. Detection of one, two and three DNA targets

We use TAR1-DNA as an example of detecting a single DNA target. We first prepared a fully mixed PBS solution containing $MgCl_2$ (5 mM); TAR1-DNA (4 μM); and all the Fluo-DNAs, each of which had a concentration of 4 μM. Then, the mixed solution was dropped onto an as-prepared microarray and kept for 12 h. After rinsing and drying, we used fluorescence microscopy to observe the microarray, with excitation at 495 nm, 550 nm and 575 nm corresponding to the excitation wavelengths of the chromophores Cy3, FAM and ROX, respectively. The conditions were identical for the singular detection of TAR2-DNA or TAR3-DNA; only the category of the DNA target was changed.

To detect two or three DNA targets, the above mixed solution only differed from containing a single DNA target to containing a combination of two or three TAR-DNAs. For example, in **Figure 1**d, the mixed PBS solution contained $MgCl_2$ (5 mM); TAR1-DNA (4 μM); TAR2-DNA (4 μM); and all the Fluo-DNAs, each of which had a concentration of 4 μM. The mixture was kept for 12 h, followed by identical washing and observation processes.

S6. Semi-quantification of the detection results.

We used a row of disks modified with Prob2-DNA for the semi-quantification. We prepared five 100-μL-PBS solutions containing $MgCl_2$ (5 mM); Fluo2-DNA (4 μM); and TAR2-DNA at varying concentrations of 0 nM, 10 nM, 100 nM, 1 μM and 4 μM. The five solutions were added to five similar as-prepared microarrays. The mixtures were kept for 12 h for sufficient hybridization. After rinsing and drying, we obtained the fluorescence images in **Figure 2a1-a4** (the blank group containing 0 nM TAR2-DNA showed no fluorescence), which were converted to grayscale images using ImageJ software (version 1.49v, National Institutes of Health, USA) to analyze the fluorescence intensity. For each concentration, we calculated the average fluorescence intensity of the three disks of each microarray from the grayscale images. A plot of the results versus the concentration of added TAR2-DNA are presented in **Figure 2b**. A linear correlation between the intensity products versus the logarithm of the concentration of TAR2-DNA was obtained, which served as a standard curve to determine the concentrations of the DNA targets in the test solutions.

In analytical chemistry, the limit of detection (LOD) is typically defined as three times the standard deviation of the blank. Taking the above five groups of quantified results as the blank, we calculated a standard deviation of 0.767, indicating the level of noise. Therefore, the LOD of the microarray was 2.3 nM.

S7. Detection of error: DNA sequences with one mismatched base pair.

Two groups of microarrays were used for detection in parallel: one was immersed in 100 μL of PBS solution containing the TAR-DNAs, all the Fluo-DNAs and $MgCl_2$ (5

mM), while the other was exposed to an almost identical solution, except the DNA to be detected was the ERR-DNAs. All DNA had a concentration of 4 μM. After hybridization for 12 h and sufficient rinsing, both microarrays were observed with fluorescence microscopy, and the obtained images were analyzed using ImageJ to obtain intensity information through the green or red channel.