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Supplementary information

Supramolecular Replication of Peptide and DNA Patterned Arrays

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Experimental Methods and Supplementary Figures.

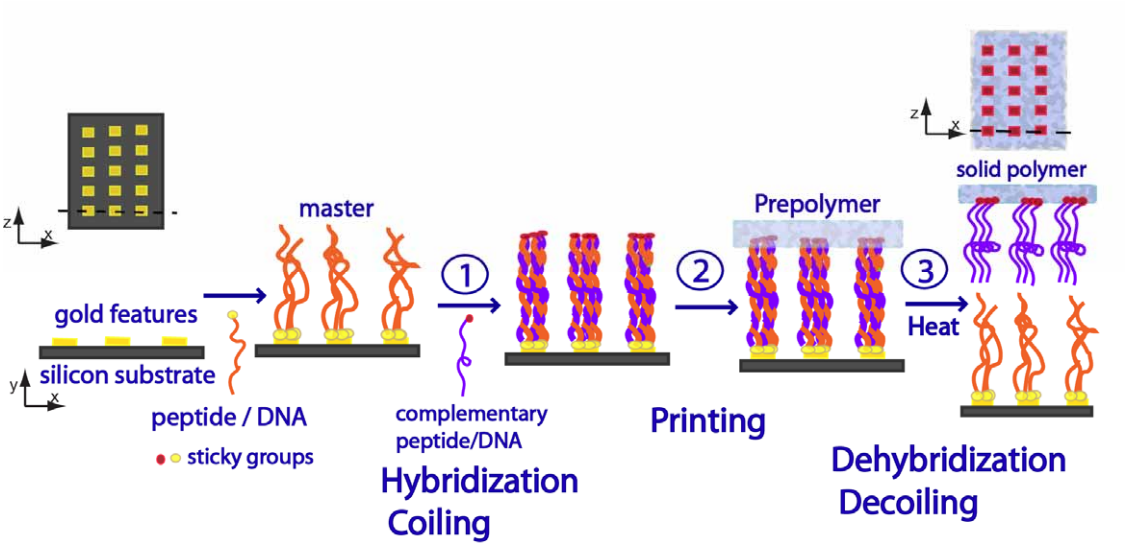

Fig. S1. Schematic illustration showing the steps involved in the Liquid Supramolecular Nanostamping (LiSuNS).

Experimental Methods

***Coiled-coil peptide synthesis*:** Peptides were synthesized by standard 9*H*-(f)luoren-9-yl(m)eth(o)xy(c)arbonyl (Fmoc) solid phase techniques. Amino acids and resin were purchased from Novabiochem (UK) and all other reagents for solid phase synthesis were obtained from AGTC Bioproducts (UK). Peptides were dried in a vacuum and purified by reverse-phase HPLC (Gilson) using a 20%- 65% water/acetonitrile gradient containing of 0.1% v/v TFA. The identities of each coiled-coil were confirmed by Mass Spectrometry (Micromass Ltd., Altrincham, UK).

Peptide sequences:

E: Ac- E VSALEKE VSALEKE VSALEKE VSALEK GGGC $CONH_2$, and **K**: Ac-CGGG K VSALKEK VSALKEK VSALKEK VSALKE $CONH_2$ were designed based on the work described by Litowski & Hodges. (Litowski, J.R. & Hodges, R.S. Designing Heterodimeric Two-stranded α-Helical Coiled-coils: Effects of Hydrophobicity and α-helical Propensity on Protein Folding, Stability, and Specificity, The Journal of Biological Chemistry 2002, 277 (40), 37272-37279 and Triplet, B.; Yu, L.; vBautista, L.V.; Wong, W.Y.; Irvin, R. T.; Hodges, R.S. Engineering a de novo-designed coiled-coil heterodimerization domain for the rapid detection, purification and characterization of recombinantly expressed peptides and proteins, Protein Engineering 1996, 9, 1029-1042).

Three glycines (G) were introduced into the sequence design to space the coiling domain from the cysteine residue (C) that acts as an anchor to the surfaces.

The secondary structure of the peptides was evaluated by Circular Dichroism Spectroscopy (CD) (Fig 1b).

CD Spectra were recorded on a Dichroism Spectropolarimeter Jasco 715. The temperature was maintained a 20 ºC by a water bath. CD spectra were the average of four scans obtained by collecting data at 0.1 nm intervals from 290 nm to 190 nm. The results

were expressed as mean residue molar ellipticity [θ] with units of deg cm^2/ dmol and calculated from the following equation:

$$[\theta] = ([\theta]_{obs} \times MRW) / (10lc) \qquad \text{Equation 1}$$

Where $[\theta]_{obs}$ is the ellipticity measured in millidegrees, MRW is the mean residue molecular weight (molecular weight of the peptide divided by the number of amino acid residues), c is the peptide concentration in mg/mL (1 mg/mL), and l is the optical path length of the cell in cm. Peptide solutions were prepared in PBS buffer (50 mM PO_4, 100 mM KCl, pH 7.0).

Both **E** and **K** exhibit a random coil spectrum with a broad minimum around 200 nm, but when mixed (**E/K)** form heterodimers that exhibit a typical α-helical spectra with minima at 208 and 222 nm characteristic of the coiled-coil structure.(J.R. Litowski and R.S. Hodges. *J. Biol. Chem.*, 2002, **277**, 37272-37279) The ratio of $[\theta]_{222}$/ $[\theta]_{208}$ was found to be 0.93.

Chemicals:

Phosphate buffered saline (PBS) and saline sodium citrate (SSC) were obtained from VWR. N-hydroxysuccinimide Fluorescein (NHS-Fluorescein) was received from Pierce Biotechnology Inc. 6-mercapto-1-hexanol (MH) was purchased from Sigma-Aldrich. Dithiothreitol (DTT) was purchased from Biochem. All DNA strands were purchased from Integrated DNA Technologies: Hexyl thiol (abbreviated as HS), acrylic phosphoramidite (abbreviated as Acry), rhodamine red (RhoR) were attached to the 5' end of the DNA sequences.

DNA sequences:

HS-A (or RhoR-A): 5'-TCC CAA AGA ACA GTG GTG GCT CAA GCT ACG GCC CCT CAT GAA AAT CCT GG-3'

Acry-A': 5'-TTT TTT TTT TTT CCA GGA TTT TCA TGA GGG GCC GTA GCT TGA GCC ACC ACT GTT CTT TGG GA-3'

Substrate preparation:

1) 10μm × 10μm gold square array: A silicon wafer coated with a commercially available photoresist (OCG 825) was exposed to UV light using an optical mask containing 10μm × 10μm square shaped holes, followed by the development of the exposed regions. Onto this substrate, a 5 nm chromium layer (adhesion layer) and subsequently 30 nm gold layer were evaporated. Lift-off was carried on in *N*-methyl-2-pyrrolidone.

2) Poly(dimethylsiloxane) (PDMS) prepolymer kit was purchased from Dow Corning (Sylgard 184). The silicon elastomer and the curing agent were mixed with a weight ratio of 10:1. This mixture was allowed to settle down to spontaneously remove the trapped air bubbles for 1 hour. Following this, it was poured into the Petri dish containing a hybridized master.

Preparation of peptide masters on gold features:

Peptides and DNA sequences were kept with excess dithiothreitol (DTT) for 30 min to disrupt any disulfide groups.

The substrates were treated with 0.15 mM aqueous solution of **K** for 12 hours. After cleaning with DI water and drying, the master was backfilled with 1mM mercaptohexanol (MH) for 30 min. Again, after washing with PBS and DI water, and drying, the master was treated with 0.15 mM aqueous solution of **E.** Before printing, the **K**-functionalised master was washed briefly with PBS and water, and blow dried. PDMS prepolymer was prepared as mentioned above and poured into the Petri dish containing a hybridized master. After curing at 60 ºC for 1.5 hours, the solidified polymer was separated from the master.

Preparation of DNA and peptide master on a gold slide:

Gold slides were purchased from Sigma-Aldrich (100 nm thickness with titanium adhesion layer, product code: 643246) and used as received. A microfluidic channel made of PDMS was placed onto this substrate. Thiol modified DNA molecules (purchased as disulfides) were reduced before use by dissolving 1 nmole of DNA in 100 µl of a 0.1M phosphate buffer (pH 7) containing solid phase dithiothreitol (DTT). The mixture was shaken at room temperature for 20 minutes and filtered through a syringe filter of pore size 0.2 µm (Life Sciences, poly(tetrafluoroethylene) membrane). After reduction, to reach the targeted ionic strength and concentration (5 µM), 1 M potassium phosphate buffer solution (pH 3.8) was added. This solution was injected into the outer channels and a 0.15 mM peptide **K** in PBS solution was injected into the single inner channel. After 24 hours, the PDMS mold was removed; the master was washed with DI water and placed into 1 mM MH solution for 30 minutes. After thorough washing with DI water, the master was first treated with 0.15 mM complementary peptide **E** in aqueous solution for 3 hours under a cover slip, and then washed with PBS and DI water. After blow drying, a 10 µM solution Acry-A' in 1 M NaCl/TE buffer (10 mM Tris buffer pH 7.2 and 1 mM ethylenediamine tetraacetic acid (EDTA)) was dropped to the surface and left for hybridization under a cover slip. The coiled/hybridized master was washed with 4×SSC, 2×SSC and DI water briefly, and dried before printing. PDMS prepolymer was prepared as mentioned above and poured into the Petri dish containing a hybridized master. After curing at 60 ºC for 1.5 hours, the solidified polymer was separated from the master.

Post-printing treatments on PDMS:

a) Peptide printing:

The printed PDMS substrate was peeled from the master, washed with DI water and treated with 0.15 mM of complementary fluorescent peptide in 10 mM PBS. After 3 hours, it was washed with PBS and DI water, and then imaged with a fluorescent microscope.

b) DNA/Peptide printing:

After separation from the master, the PDMS substrate was washed with water and treated with 1 µM RhoR-A (the complementary to the cDNA:Acry-A') in 1 M NaCl/TE buffer. After 3 hours, the substrate was washed with 4×SSC, 2×SSC, and DI water briefly, and treated with NHS-Fluorescein (1 mg/ml) in PBS for 3 hours. The substrate was washed with PBS and water and then imaged with a fluorescent microscope.

Characterization: Fluorescence microscopy images were obtained by a Zeiss Axioplan 2 with a mercury lamp. To image RhoR-A (fluorescently tagged DNA), a filter with excitation and collection at 470 and 515 nm, respectively was used. To image NHS-Fluorescein, the respective wavelengths were 456 and 590 nm.