Supporting Information:

Collective Conformations of DNA Polymers Assembled on Surface Density Gradients

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DNA handling: PCR, purification, quantification, etc.

Double stranded DNA (dsDNA) fragments were produced using polymerase chain reaction (PCR) using Phusion™ High-Fidelity DNA Polymerase (Finnzymes) and Taq SuperTherm DNA polymerase (JMR Holdings). To make the comparison between the different DNA brushes valid, all the different lengths were created using a single primer 5' end labeled with an Alexa Fluor® 488 dye while changing the biotin modified primer (Integrated DNA Technologies). DNA was purified using Promega Wizard® SV Gel and PCR Clean-Up System according to protocol, and eluted into

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DNase-free water. The purified DNA were cut from 0.7% agarose gel and cleaned using Promega Wizard® SV Gel and PCR Clean-Up System. The concentration of the DNA was measured (NanoDrop 1000) followed by conjugation with streptavidin (SA) in a molecular ratio of 1:1 in 1X PBS[2]. Excess SA was reduced using Microcon Centrifugal Filter Devices YM-100 (milipore), ensuring that more than 90% of the free SA was removed. The final DNA concentration of all of the samples was 100 nM.

The labeling of SA was done according to the protocol of Alexa Fluor® Protein Labeling Kit (Invitrogen). Lyophilized SA (Thermo Scientific) was dissolved in PBS 1x buffer (pH 7.4) to concentration of 1 mg/mL. 100 μL of 1 M sodium bicarbonate (pH 8.3) were added to 1 mL of SA solution. The protein solution was transferred to a vial of reactive dye. The reaction mixture was stirred for 3 hr at room temperature. The protein was purified on a size exclusion column (Bio-Rad BioGel P-30 fine size exclusion purification resin) to give labeled SA with dye to protein molar ratio of 1:1.

Biochip methodology

In short, the biochip is based on a self-assembled monolayer comprising a tri-functional molecule with a poly-ethylene-glycol (PEG) backbone, a silicon-dioxide binding group at the surface-proximal end, and a protected amine at the surface-distal end[1]. Glass slides and prisms were initially cleaned in ethanol heated to $70^{\circ}C$ for 10 minutes followed by cleaning with RCA solution (H_2O_2:NH_3:H_2O; 1:1:4) heated to $70^{\circ}C$ for 10 minutes. The glass slides were thoroughly dried under nitrogen flow. Since the prisms were re-used, buffered HF was applied to ensure that silicon-oxide surface is uniform. The clean glass slides and prisms were incubated with solution of 0.2 mg/mL of the

modified PEG in toluene for 30 minutes before being thoroughly washed with toluene and dried under nitrogen flow.

The de-protection of the PEG monolayer with the desired pattern was performed by placing the biochip on a translational stage coupled to an inverted microscope (Zeiss Axiovert 200M). UV light (350-365nm, EXFO Xcite-120Q) was passed through the rectangular field-stop of the microscope serving as a mask and focused on the substrate with a 60x objective (water immersion 1.2 NA, Olympus). The stage was automatically moved to the desired location and a shutter was opened to allow UV light to reach the substrate. Irradiating specific UV doses onto the biochip was done by setting the dwell time. The dwell time (in seconds) was calculated by dividing the desired UV dose (in mJ/cm^2) by the measured flux of the UV source (in mW/cm^2). In order to create discrete patterns, the microscope stage was moved between pre-determined locations and the dwell time was increased at each spot. To create continuous density graded patterns, the microscope stage was programed to continuously move along a single axis while increasing the dwell time at each step.

Attachment of biotin to the de-protected PEG monolayer was done by incubating a fresh solution of 0.5 mg/mL biotin-sulfo-NHS (Pierce) in borate buffer (pH 8.6) for 20 min. Assembly of the SA-DNA conjugates or labeled-SA surface layer was done by incubating the biotinylated surface with a droplet of 1-2μL of solution containing the desired specie in a PBS 1x buffer at 15$^{\circ}C$ for 3 hours. All of the gradients patterns were created in the same fashion on the same prism and imaged on the same day. Each type of specie was added to a different spot on the prism, incubated to allow brush buildup and then extensively washed in buffer solution.

Imaging

EpiFL and TIRF images were taken by a 16 BIT cooled EMCCD imaging camera (Andor iXon EM+), mounted on an Olympus upright microscope (Olympus BX51WI), equipped with optical filters (Chroma) suitable for Alexa Fluor® 488. The prisms (Zell Quarzglass) were mounted onto a temperature controlled holder and kept at $20^{\circ}C$, in an open bath configuration. The bath was stable for hours, enabling imaging of all the different species under the same conditions. A prism-based TIRF setup was constructed where a Ar-Kr laser (Innova 70, Coherent Inc.) was inserted into a single-mode optical fiber (Oz optics), mounted onto a goniometer and directed at 90 degrees to the prism surface. To ensure the same illumination for all the molecules, regardless of their position on the prism, the laser optical fiber was mechanically coupled to the microscope. A water immersion 60x objective (Olympus) was dipped into the bath in order to take the images. Two types of images were taken, an epi-fluorescent image, taken with an illumination system (EXFO X-Cite 120Q) of the microscope, followed by imaging of the labeled species using the laser light source (488 nm).

(1) A. Buxboim, M. Bar-Dagan, V. Frydman, D. Zbaida, M. Morpurgo, and R. Bar-Ziv. A single-step photolithographic interface for cell-free gene expression and active biochips. *Small*, 3(3):500–510, 2007.

(2) A. Buxboim, S.S. Daube, and R. Bar-Ziv. Ultradense synthetic gene brushes on a chip. *Nano letters*, 9(2):909–913, 2009.