## Lab on a Chip

# Supplemental Information for

### Microfluidic chip with pillar array for controlled production and observation of

## lipid membrane nanotubes

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### **Supplementary Methods**

### Fabrication of SU-8 micropillars

SU-8 micro-pillars were fabricated by photolithography on 25 mm diameter round glass cover slides. The width of the micro-pillars was 15  $\mu$ m, separated from each other by 10, 20, 30, and 40  $\mu$ m, as it is depicted in **Fig. S1A**. Glass cover slides were dehydrated first at 220 °C, followed by SU-8 2005 deposition at a maximum velocity of 2000 rpm s<sup>-1</sup>. After that, the samples were baked at 65 °C for 8 min and 95 °C for 14 min. Samples were then exposed to 5 mJ·s<sup>-1</sup> UV light (365 nm) for 20 s in 4 pulses and baked for 3 min at 65 °C and 6 min at 95 °C. The non-polymerized photoresist was then removed by immersing the samples in the SU-8 developer. A final hard-baking step was performed for 30 min at 150 °C.

## Fabrication of ionogel micropillars

The solution of the ionogel (IO) was comprised of a monomer (NIPAAm), a photoinitiator (DMPA), a crosslinker (mBAAm), and the ionic liquid (IL) DCA<sup>1-3</sup>. The molar ratio for NIPAAm:DMPA:mBAAm:DCA prepolymer solution was 40:4:1:25, and it was obtained by mixing 452 mg NIPAAm, 30.9 mg mBAAm and 30 mg DMPA in 1.5 mL of DCA IL. Some of the prepolymer solution components may produce eye irritation and skin burns, as specified in their MSDS; thus, specific protective equipment should be used during prepolymer solution manipulation, polymerization, and washing steps. For long time storage at 4 °C, the solution was covered with aluminum foil to protect it from light.

Previous reports showed that the covalent attachment of ionogels to a substrate improved with the chemical functionalization of the substrate. For the production of the IO microstructures, the coverglass was functionalized by 30 min oxidation with air plasma followed by vapor deposition of 3-(trimethoxysilyl)propyl methacrylate for 2 h. Then 50  $\mu$ L of the prepolymer was spread over the functionalized glass using a film applicator to form a 10  $\mu$ m thin layer. The ionogel film was exposed to 460 mJ of 365 nm wavelength UV light through the photomask. The photomask and the sample were in direct contact during polymerization. The power of the lamp was set at 20 %, since lower or higher values resulted in poor resolution and excess of polymerization, respectively. We used four cycles of 5 s with 30 s gaps between the cycles in both cases. Using light pulses, instead of continuous light exposure, increased the resolution of the micro-pillars pattern. To remove any residual IL, the formed micropillars were thoroughly washed three times with isopropanol and water. The pillars were then heated to 45 °C to induce IO shrinkage and the consequent expelling of any possible unbound IL from the poly-NIPAAM network.

On the other hand, it is well known that the solvent percentage influences the resolution of the photopatterning process<sup>4</sup>, and the evaporation of a part of the solvent is required during the fabrication process. ILs do not evaporate, so the percentage of IL in the polymer precursor solution of the ionogel can be decisive for the resolution of the micropillar arrays. The final ILs ratio used for the experiments was an adequate compromise to form a solution that could homogeneously spread over the substrate while providing a proper pattern resolution.

The DCA IO array of IO micropillars with a 15  $\mu$ m width was correctly produced with this protocol. The anchoring of the microstructures to the substrate was strong. The patterned IO micro-pillars remained attached to the surface even after being washed with isopropanol, water, and soap. However, the resolution of DCA IO patterns was worse than the resolution of SU-8 patterns. DCA IO patterns with micro-pillars at 10  $\mu$ m pitch was hardly achievable. In contrast, well-defined micro-pillars were obtained all over the photo-polymerized surface (2 mm x 2 mm) when the interpillar distance was 20, 30, or 40  $\mu$ m.

#### Fluorescence signal calibration for NT radii measurements

For assessing the NTs radii, the total fluorescence of the NT should be correlated with the total area of the membrane<sup>16</sup>. To construct a calibration curve that relates the fluorescence intensity of the membrane with its area, we used Supported Lipid Bilayers (SLBs) produced from the same lipid films as the ones used to produce the NTs (Fig. S3A). To prepare the SLBs, a coverglass was cleaned by sonication in isopropanol for 5 min followed by MilliQ water rinse and drying under an N<sub>2</sub> gas stream. The coverglass was then treated with air plasma for 1 min to get rid of impurities and promote the SLB formation. The coverglass was mounted into the microscopy chamber, which was placed on the inverted microscope stage and filled with the working buffer. The lipid-film covered microbeads were picked up by a fire-closed tip of a glass micropipette and deposited into the buffer of the microscopy chamber. The SLB formation was observed as a spill of the lipid films from the microspheres into the coverglass surface.

The plot profiles of the resulting SLBs were analyzed to find unilamellar regions of the SLB (see Fig. S3A, B). Importantly, the SLBs were imaged at the same fluorescence intensity and camera settings as the ones used in the NT experiments. Finally, the calibration curve was produced by plotting the total fluorescence intensities (measured with the Raw Intensity Density tool in Fiji software) versus the area of several regions of interest (ROIs) from the unilamellar SLB. The proportional coefficient, k, corresponds to the slope of the linear fit of such a plot (Fig. S3C).

#### NT radii calculation

The NT radii were calculated as reported previusly<sup>56</sup>. Briefly, NT micrographs with uniform and low background signal located near the focal plane were chosen. The background was first subtracted from the images using the rolling ball algorithm of the Fiji software<sup>7</sup>. If needed, the micrographs were rotated to align the NT along the horizontal or vertical axes. Upon selecting a narrow NT ROI of known length ( $L_{srr}$ , Fig. S3D), the ROI's integral fluorescence was calculated by using the Raw Intensity Density tool in Fiji software<sup>7</sup>. The total fluorescence intensity of the ROI ( $I_{sot}$ ) is related to the total membrane area (A) enclosed in the ROI as:

$$I_{ROI} = \alpha A,$$
 (eq. 1)

where  $\alpha$  is the proportional coefficient calculated from the unilamellar SLB calibration curve (Fig. S3C).

The area of a segment of a cylindrical NT is:

 $A = 2\pi L_{NT} r_{NT}, \qquad (\text{eq. 2})$ 

where  $L_{NT}$  is the length of the ROI, while  $r_{NT}$  is the NT radius. Then, the value of  $r_{NT}$  is:

$$r_{NT} = \frac{I_{ROI}}{2\pi L_{NT}\alpha} \qquad (eq.3).$$

#### **Supplementary Figures and Movies**



Fig. S1. Micropillars arrays and microfluidic device design. A. The photograph shows the coverglass with the three micropillars arrays. The schematics show the overall dimensions of the arrays. B. Zoom up of one of the arrays in (A) showing the different dispositions of the micropillars. The SEM images show the array zones with 10 and 20  $\mu$ m pillars separation. Scale bars are 10  $\mu$ m. C. Final microfluidic device with three separate channels, each for one of the arrays shown in (A).



Fig. S2: The integral fluorescence intensity of the NT remains constant near the focal plane. A. Several images of an NT near and at focus. B. Plot profile of the region selected in (A). The areas under each intensity peak (corresponding to each NT image in (A)) appear dashed. C. Areas in (B) normalized to their higher value. The last column shows the mean of all normalized area values (n = 6). The error bar is SD.



**Fig. S3:** NT radius assessment. **A**. Micrograph showing an SLB. **B**. Plot profile along the red line in (**A**) showing two levels of fluorescence that correspond to the unilamellar and the bilamellar regions of the SLB. **C**. Graph of the total fluorescence of four ROIs (like the one shown with a blue rectangle in (**A**)) from the unilamellar region of the SLB versus the corresponding ROI areas. The parameter  $\alpha$  corresponds to the slope of the linear fit through the graph points (shown in red). **D**. An example of an ROI used to calculate the radius of an NT.

**Movie S1:** Bead displacement in the 20  $\mu$ L min<sup>-1</sup> flow regime of the pump in the 40  $\mu$ m pillar separation zone of the SU-8 array.

**Movie S2:** Bead displacement in the 100  $\mu$ L min<sup>-1</sup> flow regime of the pump in the 40  $\mu$ m pillar separation zone of the SU-8 array.

Movie S3: The addition of 0.5  $\mu$ M Dyn1 to an NT in the presence of 1 mM GTP (at t = 0 s) leads to the NT membrane fission and its retraction towards the pillars.

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