How to make and trap a pseudo-vesicle with a micropipette

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SUPPLEMENTARY INFORMATION

Fluorescence imaging, image analysis

For the experiments with αHL nanopores, the pseudo-vesicles contain carboxyfluorescein and are imaged through epifluorescence with a Leica macroscope. Images (Fig. 1a) are acquired every 4 minutes and analyzed as follows. First the image is binarized and we determine the centroid (x_c, y_c) of the white region using the *regionprops* function of Matlab (Fig. 1b). The edges of the white region are determined and we fit a circle to the bottom part ($y < y_c$) of the pseudo-vesicle (see Fig. 1c). The internal (*resp.* external) intensity I_{in} (*resp.* I_{out}) is taken as the average $\langle I(r < 0.5R)\rangle$ (*resp.* $\langle I(r > R)\rangle$). The contrast is then computed as $\Gamma = \frac{I_{in}-I_{out}}{I_{in}+I_{out}}$. We plot on Fig. 1d the normalized contrast $\Gamma/\Gamma(t=0)$ as a function of time, for all performed experiments, with or without αHL.

For the experiment with fluorescent oil (red channel) and fluorescent lipids (green channel), the pseudo-vesicles are imaged through confocal microscopy (Spinning Disc Xlight V2, Gataca systems) with a 4x objective. To determine the radial intensity profile, we first manually click on the green channel image to specify three points on the pseudo-vesicle membrane. The position of these three points is then automatically refined by looking at an intensity maximum in its vicinity (blue points in Fig. 1e). We determine the circle of radius R passing through these 3 points (red dotted line in Fig. 1e). The radial intensity is determined by averaging in a 3 pixel width ring, restricted to the bottom half of the pseudo-vesicle Fig. 1f. The intensity is normalized as $I_{norm} = \frac{I(r)-<I(r<0.5R)>}{I_{cap}+<I(r<0.5R)>}$, where $< I(r < 0.5R) >$ is a measure of the averaged intensity inside the pseudo-vesicle, and I_{cap} is the intensity in the oily cap region. Corresponding radial profiles for both red and green channels are plotted on Fig. 1g. Taking the average over 8 replicates gives the Fig. 2c of the main text.

Surface tension effects

We have measured the surface tension between the internal phase and the oil+lipid phase using pending drop experiments. We used a Nemesys syringe pump to slowly grow a droplet of the internal aqueous phase through a thin cylindrical tip, inside the oil+lipid phase (*See* Fig. 2a). The droplet is imaged in transmission using a LED array and a monocular loop. Recorded images are analyzed (*See* Fig. 2b) using the *Drop Analysis* ImageJ Plugin [1]. Experiments have been performed in the absence or in the presence of αHL nanopores, up to a concentration of 100 μg/mL. We plot on Fig. 2c the variations of the measured surface tension γ_{ow} as a function of the nanopore concentration [αHL]. The value of γ_{ow} decreases tenfold, from ≈3 mN/m down to ≈0.3 mN/m as the nanopore concentration is increased, from 0 to 10 $\mu g/mL$. Above this concentration, γ_{ow} remains constant. This decrease of surface tension is due to the non-specific adsorption αHL monomers at the oil/water interface (Note that this is different from the heptamerization of αHL monomers in lipid bilayers leading to the formation of a transmembrane pore). In our system, a consequence of this surface tension decrease is that the oil cap tends to wet and spread on the aqueous inner droplet (*See* Inset of Fig. 2b) . Experimentally, this wetting correlates with a strong decrease of the pseudo-vesicle stability. The success rate of stable pseudo-vesicle formation for [αHL]>20 μg/mL is below 1%, which hinders the use of this protocol to insert nanopores in the vesicle membrane. As an alternative, we used SUVs decorated with αHL nanopores, dispersed in the internal aqueous phase. In this case, αHL monomers are already heptamerized in the SUV membrane, which prevents them from decreasing too strongly the oil/water surface tension.

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[1] A. Daerr and A. Mogne, J. Open Res. Soft. **4** (2016).

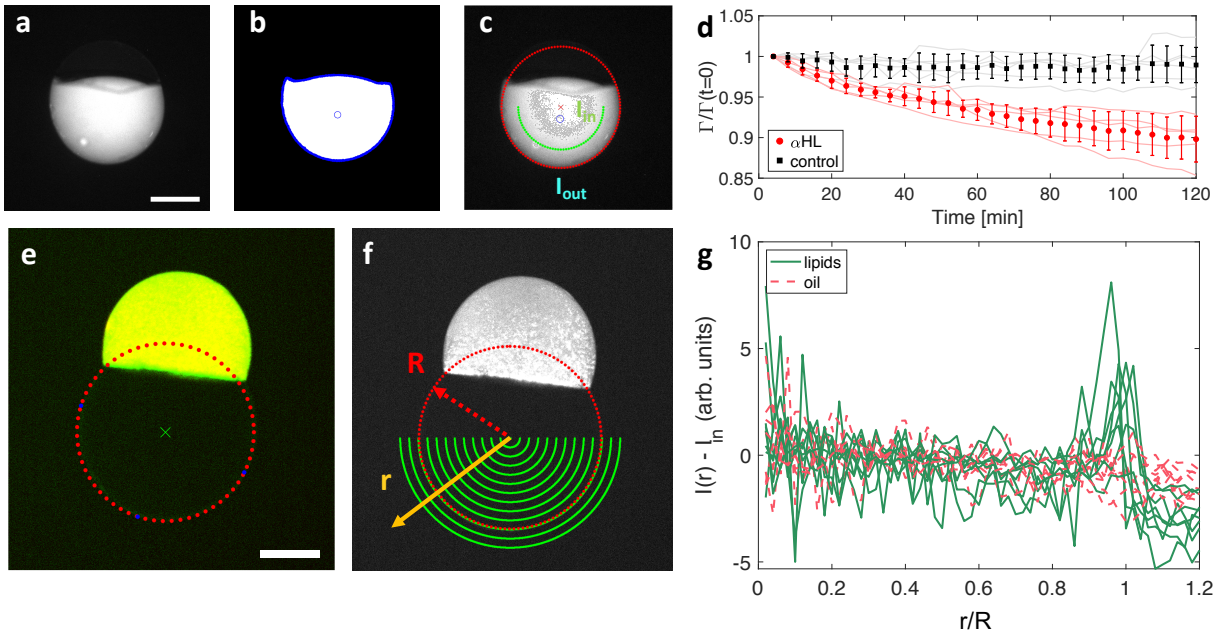

FIG. 1. a-c) Image analysis steps for contrast measurements. a) Raw epifluorescence imaging of a pseudo-vesicle. Scale bar = 200 μm. b) The image is binarized. The blue circle is the detected centroid of the white region. The edges of the binarized image are detected (blue dots). c) A circle is fitted to the lower part of the pseudo-vesicle's edge (red dots). The interior intensity is computed (within the green dashed semi-circle), the outside intensity is computed (within the blue half-rings). d) The normalized contrast is plotted over time. Solid lines represent individual experiments, symbols are averages (errorbars= standard deviation). e) Composite image of a pseudo vesicle, with a fluorescent oil phase (Nile Red, red) and fluorescent lipids (NBD-PC, green). Yellow indicates the addition of the two channels. We manually click on three points of the pseudo-vesicle's membrane (blue disks) and determine the corresponding circle (red dotted line). f) Sketch of the image analysis procedure. Fluorescence intensities in both green and red channels are measured in radial half rings. g) Fluorescence intensity, to which the inner central intensity I_{in} has been subtracted, as a function of the radial coordinate, for both green and red channels.

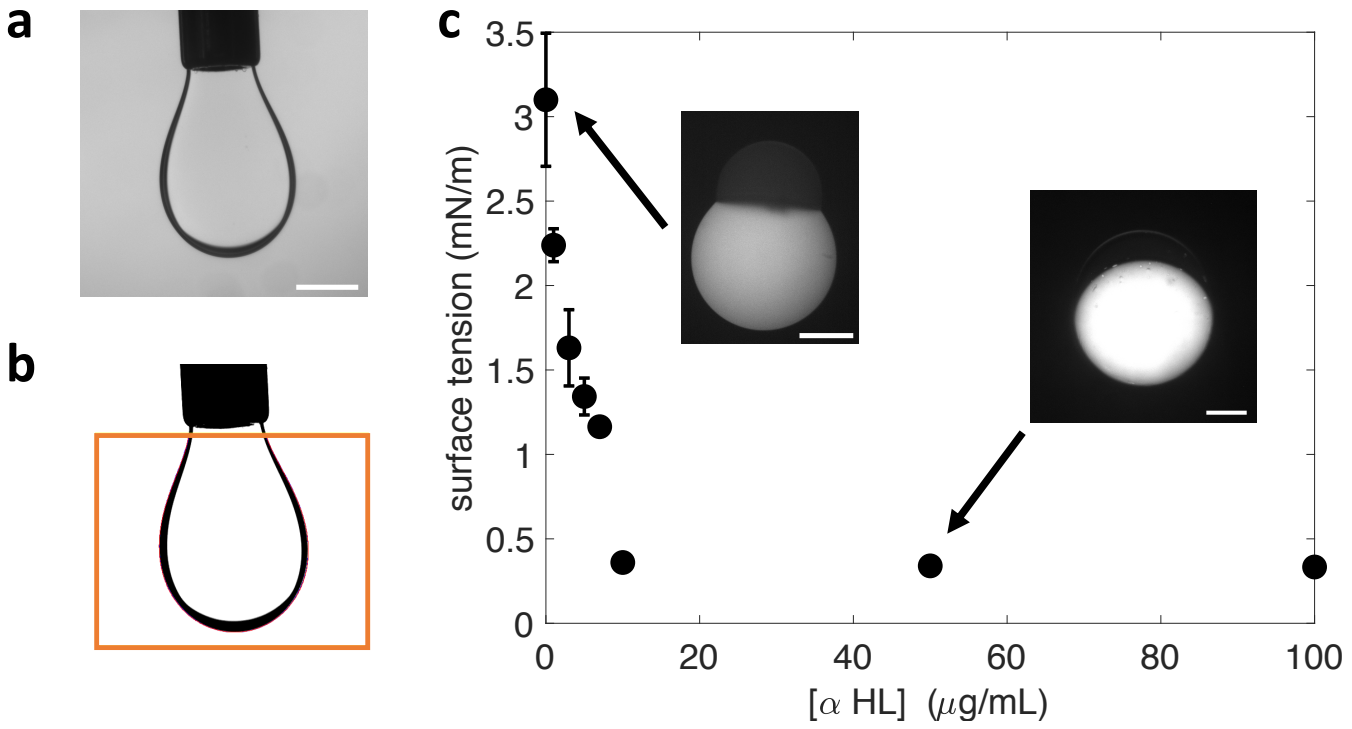

FIG. 2. a,b) Pending drop experiment with an aqueous droplet (internal phase, with [αHL] = 0.1 μg/mL) bathing in the oil+lipid mixture described above. In b) the image has been binarized and its shape (restricted to orange rectangle region of interest) is fitted to determine the surface tension, using the ImageJ plugin of [1]. Scale bar = 500 μm. c) Surface tension (deduced from the pending drops experiments) as a function of the nanopore concentration [αHL]. Inset: typical images of pseudo-vesicles trapped in an agar gel, at [αHL]= 0 and [αHL]=50 μg/mL, as indicated by the arrows.