Supplementary Material (ESI) for Soft Matter

Materials and methods

Lipid dissolution in mineral oil Egg-PC lipids (P3556, Sigma) are dissolved at 100mg/ml in a chloroform: methanol (9:1) solution and used at least a week after powder dissolution. This solution is then diluted 5 times in the same solvent in 25 ml cleaned bottles in a 10% humidity environment within a plastic glove box. The solvent is evaporated under vacuum for 20 minutes to obtain a white film at the bottom of the bottle. The bottles are then filled with mineral oil (3516 Sigma) and stored for 1h at room temperature before being sonicated for 1h keeping in a bath of temperature lower than 40°C, to obtain a stable lipid in oil solution of 0.5 mM that can be used the following day and up to 3 days after preparation.

Aqueous solutions In a typical experiment, sucrose-glucose density contrast is used at 100mM. All solution are filtered using 0.22 micron filter prior to use. It is crucial to match the osmotic pressure of the inner and outer fluid. Higher concentrations (up to 1M) were used for actin filaments, hemoglobin or red blood cells encapsulation.

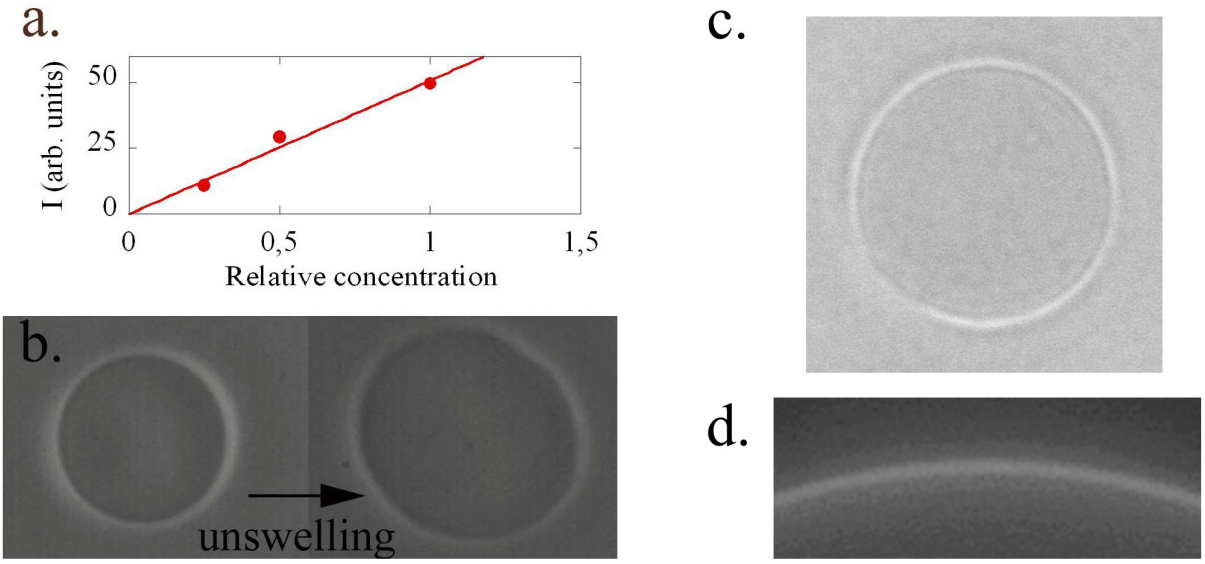
Chamber design and injection capillaries The chamber is made of the tops of two petri dishes glued together to form a closed cylinder 35 mm in diameter, with an an aperture of ~1 cm in diameter cut in the top so that the capillarycan be introduced. The chamber is fixed on a rotating motor. Capillaries are hydrophobized by silane coating. Capillaries diameters range from 2 to 25 microns depending on the vesicle size to be obtained.

Vesicles production The motor can be rotated on a range from 10 to 50 turn a second. In a typical experiment, the motor rotates at a rate of 30 turns/sec. The chamber is filled first with 1 ml of the DAS (100mM glucose solution), 3.5 of the LOS and 1 ml of decane. The EAS is injected from the capillary in decane at pressure ranging from 300 to 800 mBar with a pressure system (fluigent), for 5 minutes. Importantly, the motor should then be gradually stopped and the outer solution containing the vesicles is gently withdrawn with a micropipette.

Alpha hemolysin (H9395, Sigma) is dissolved at 1mg/ml in 10mM hepes containing 150mM KCl. During experiment, 1µl of this solution is diluted in 100 µl of a glucose solution and matched in osmolarity with the EAS. This solution is then added in an observation chamber, on top of a 200 µl solution of vesicles encapsulating fluorescin. Fluorescence pictures are taken before and after addition of alpha hemolysin.

Formula for the Flying time, τ_F, i.e. the time spent by a droplet in the LOS layer:

If one computes the Reynolds number, that is the ratio of the inertial forces over the viscous forces, one finds at the most $Re \approx 10^{-2}$, i.e. for the larger centrifugal force and the larger droplet size. In the limit of low Reynolds number, the droplet velocity is given by the balance between the viscous force and the external force, here the centrifugal force. For a radius r, one can infer $v(r)=r(2\Delta\rho R^2\omega^2)/9\eta$. Thus, the flight time τ_F for droplets produced at r_0, to travel through a layer thickness e, is given by the integral between r_0 and r_0+e of $1/v(r)$: $\tau_F=9\eta/(2\Delta\rho\, R^2\omega^2)\ln(1+e/r_0)$.



Supplemental Figure 1 **A** Increase in fluorescence intensity as a function of the concentration in Dextran-RITC, relative to a reference concentration equal to 0.5 mM, **B** . Images of a vesicle before (left) and after deswelling in a hypertonic solution. A movie of fluctuating vesicle is provided in supplemental information. The vesicle on the right has deflated into a disk because of gravity (the inner fluid being heavier than the outer one). **C**. cDICE vesicle imaged with a x100 (NA 1.3) objective and a condenser (NA 1.1) in bright field. **D.** Part of PKH (Sigma) labeled membrane imaged with a fluorescent microscope (x100, NA 1.3).