Spreading of porous vesicles submitted to osmotic shocks: The role of aquaporins

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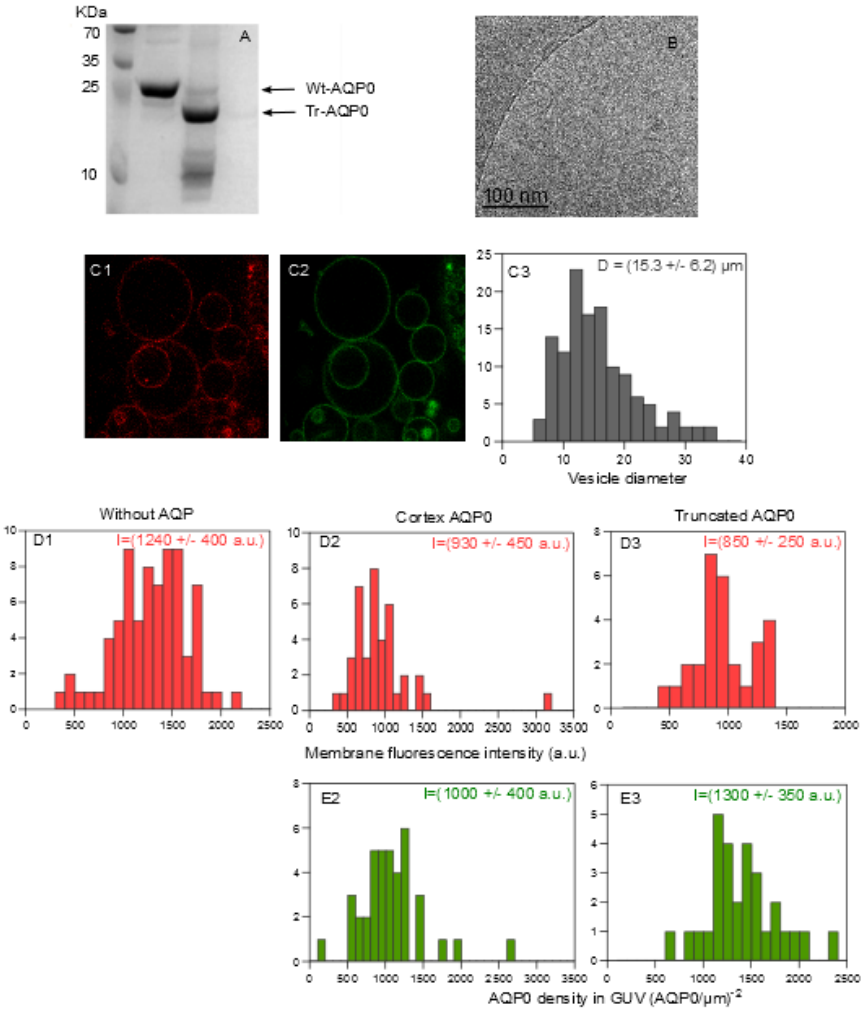
SUPPORTING INFORMATIONS

S1: Vesicle characterization:

To check the GUV unilamelarity, we grow GUV with lipid composition of 99.75% EPC and 0.25 % TR-DHPE which are known to produce unilamellar vesicle (1). The fluorescence intensity of these GUVs was measured at their equatorial plane using confocal microscopy. In the absence of AQP0, the average intensity is: 1240 ± 400 a.u. (Fig. S1.D1) If the presence of AQP0 would lead to the formation of multilayers of lipid bilayer in the GUV, the intensity would be equal to N*1240 (where N would be the number of bilayers). We found that for Wt-AQP0 the intensity is equal to 930 ± 450 and 850 ± 250 for Tr-AQP0 (FIG.S1.D2 and D3) implying that GUVs are unilamellar and that the presence of AQP0 in the membrane does not produce multilamellar vesicles.

To quantify the AQP0 density in the GUV, we also used confocal microscopy of AQP0 labeled with Alexa-488 (Fig. S1.C2). The protein density, *D*, could be calculated using: $D = \frac{I.A_{gain}}{N_f.F}$ where I is the measured fluorescence intensity of the AQP0-Alexa488, A_{gain} is the calibration coefficient, F a correction factor and N_f the labeling efficiency of AQP0 (see

(2) for details). The AQP0 density was found to be equal to 1000 ± 400 and 1300 ± 250 AQP0/μm^2 for WT and Tr-AQP0 respectively.



Legend Figure S1: Characterization of the GUV.

(A) SDS-PAGE gel of Wt and Tr-AQP0 purified from the eye lens of sheep. Tr-AQP0 has been obtained by chymotrypsin digestion of the Wt-AQP0.

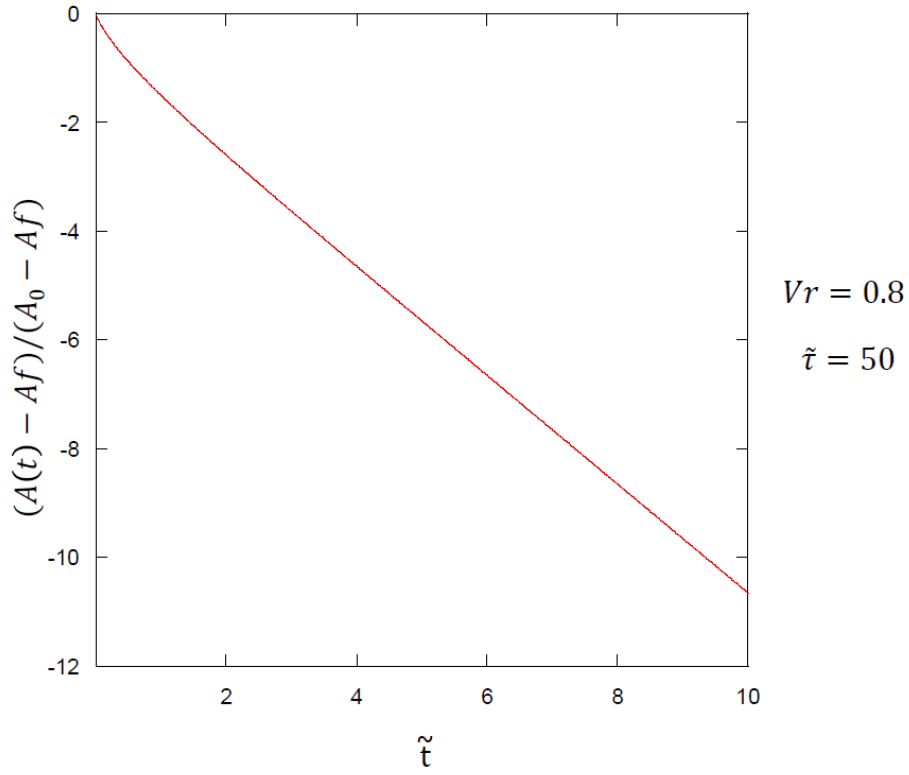
(B) Cryo-EM of SUV containing containing Wt-AQP0

(C) The lipids in the GUV are labeled with a Texas-Red-DHPE lipids (B1) whereas the AQP0 are labeled with an Alexa-488 maleimide (B2). The average diameter size of the GUV is equal to D = 15 ± 6 µm independent of the presence or absence of AQP0.

(D) The fluorescence intensity of the lipids has been measured for GUVs that do not contain AQP0 (C1) (known to be unilamellar) and for GUV with Wt-AQP0 (C2) and Tr-AQP0 (C3). The values are respectively equal to: 1240 ± 400, 930 ± 450 and 850 ± 250 a.u. The fluorescence intensity is normalized by the size of the GUV

(E) The fluorescence intensity of the AQP0 has been measured for GUV containing Wt-AQP0 and Tr-AQP0. The protein density of the Wt-AQP0 and Tr-AQP0 are almost identical and equal to 1000 ± 400 and 1300 ± 350 AQP0/μm^2.

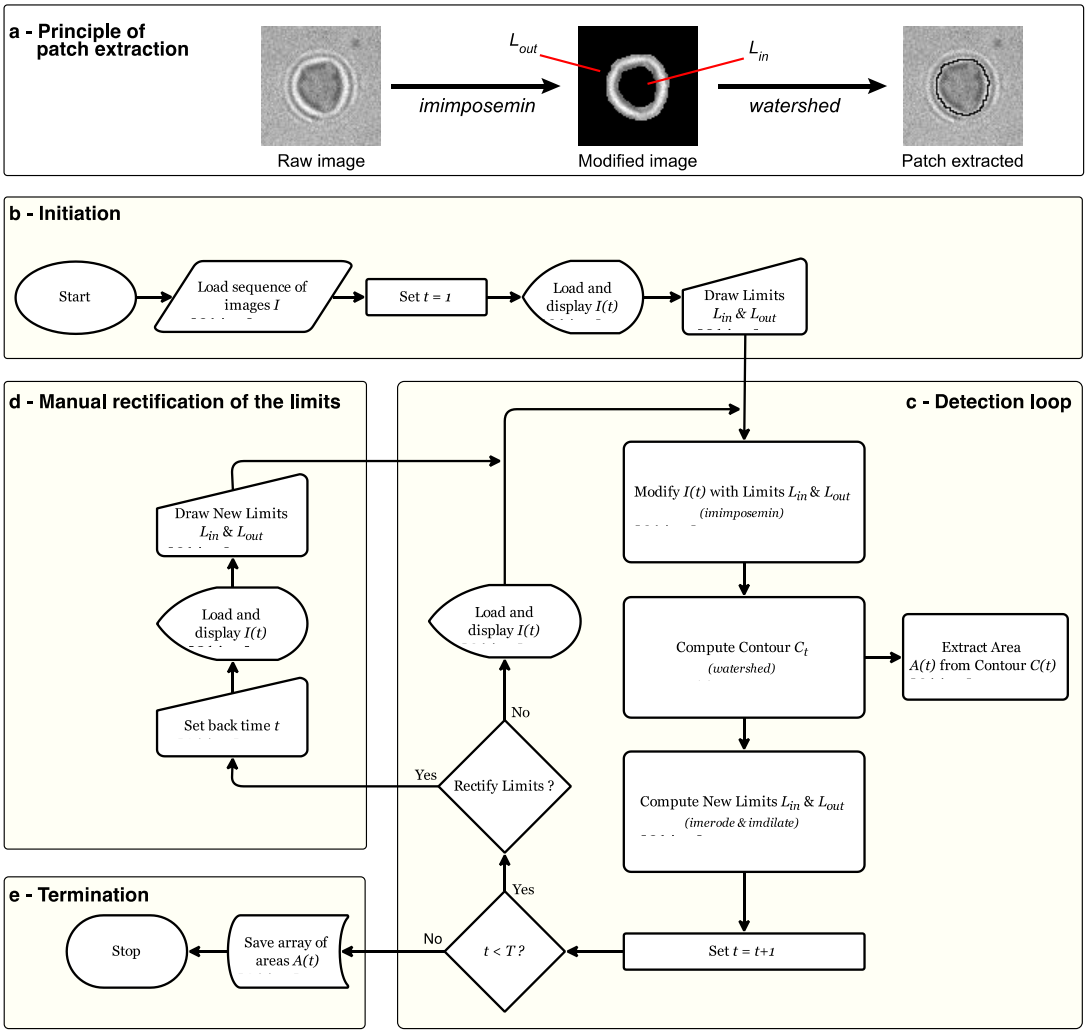
S2: Numerical solution of equation [12]



Legend Fig. S2: Numerical simulation of $\frac{A(t)-A_f}{A_0-A_f}$ as a function of $\tilde{t}$ defined with Eq. [14].

The validity of equation [13] was numerically checked. The variation of $\frac{A(t)-A_f}{A_0-A_f}$ is plotted on a log-log graph as a function of the non-dimensional time $\tilde{t}$ for an osmotic shock Vr=0.8 and a characteristic time $\tilde{\tau} = 50$. We clearly see that there is a linear relation between the relative variation of the area and the non-dimensional time except for very small time. This linear relation validates the equation [13].

S3: Analysis RICM-patch-gui.m.



The patch extraction algorithm is based on the existence of a bright ring around the adhering patch in RICM images (see also Figure 1). This bright ring allows efficient watershed detection [1]. Automatic contour detection may be hindered by fringe patterns typical of RICM imaging or defects of glass slide, but is improved here by iterative guiding under operator supervision. Inner and outer limits (L_{in} and L_{out}) of the adhering patch are generated from the contour *C(t-1)* extracted from the previous frame *I(t-1)*. These limits impose a "region of interest" where the watershed line has to be localized (a). The watershed line defines the contour *C(t)* of the patch of the current frame (from which the area *A(t)* is

calculated) and consequently inner and outer limits of the contour are updated (c). This iterative process is manually initiated by drawing inner and outer limits of the contour in the first frame (b). It can be stopped as soon as the watershed function fails to detect the contour. Then the operator manually rectifies inner and outer limits and the iterative process is resumed (d). The process ends when the loop reaches the end of the image sequence (e). Matlab morphomathematical functions used for patch extraction are indicated in italics.

1. Girard P, *et al.* (2004) A New Method for the Reconstitution of Membrane Proteins into Giant Unilamellar Vesicles. *Biophysical journal* 87(1):419-429.
2. Aimon S, *et al.* (2011) Functional Reconstitution of a Voltage-Gated Potassium Channel in Giant Unilamellar Vesicles. *PLoS ONE* 6(10):e25529.