Electronic Supplementary Information

Insights into the structure and nanomechanics of the Quatsome membrane by force spectroscopy measurements and molecular simulations

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ESI 1. QS vesicle structural characterization

Cholesterol (Chol):CTAB (1:1) QS were prepared either in ultrapure water (QS_H_2O) or in PBS/NaCl pH 7.4 (94 mM NaCl, 4 mM PBS) buffer solution (QS_PBS). From dynamic light scattering (DLS), the average hydrodynamic diameter (*d*) and zeta potential (ζ-pot) of the obtained QS were determined (Table S1). The samples were also imaged using Cryo-TEM (Fig. S1).

Dynamic light scattering (DLS). Size distribution and zeta potential (ξ-pot) of QT vesicles suspensions were analysed by DLS using a Zetasizer Nanoseries instrument Nano-ZS (Malvern Instruments, UK). Measurements were carried out on a minimum of two samples from three independent runs. The measurements were performed at room temperature (RT) without dilution. Results are given in percentage of intensity of scattered light.

Cryogenic transmission electron microscopy (Cryo-TEM). QSs morphology was assessed by Cryo-TEM using a JEM-2011 microscope (JEOL Ltd., Tokyo, Japan) operating at 120 kV. The samples were frozen by plunge freezing in liquid ethane and stored in liquid nitrogen until loaded onto a cryogenic sample holder (Gatan 626 CTH). The Cryo-TEM images were acquired below -175 ºC. Images were recorded on a Gatan 724 CCD camera under low-dose conditions using Digital Micrograph 3.9.2. (Gatan Inc.).

Table S1. Hydrodynamic diameter (*d*), polydispersity index (PDI) and z-potential (ζ-pot) values of Chol:CTAB QS_H_2O and QS_PBS.

QS vesicular sample	*d* (nm)	PDI	ζ-pot (mV)
QS_H_2O	90 ± 2	0.23 ± 0.01	85 ± 4
QS_PBS	152 ± 3	0.39 ± 0.01	43 ± 2

The hydrodynamic diameter and PDI for QS prepared and measured in PBS/NaCl were higher than in water. The ions in the buffer solution can affect both the geometrical diameter (the actual size) of the vesicles, but also the hydrodynamic diameter, as they may not coincide. The ions from the solution can alter the interaction between Chol and CTA^+, but also the solvation shell, affecting the diffusion of the vesicles in suspension leading to an overestimated size by DLS. This is also seen in the strong reduction in the ζ-pot values in PBS/NaCl respect to water. From Cryo-TEM measurements (Fig. S1) we observed that in general QS_PBS are bigger than QS_H_2O, and less rounded in shape.

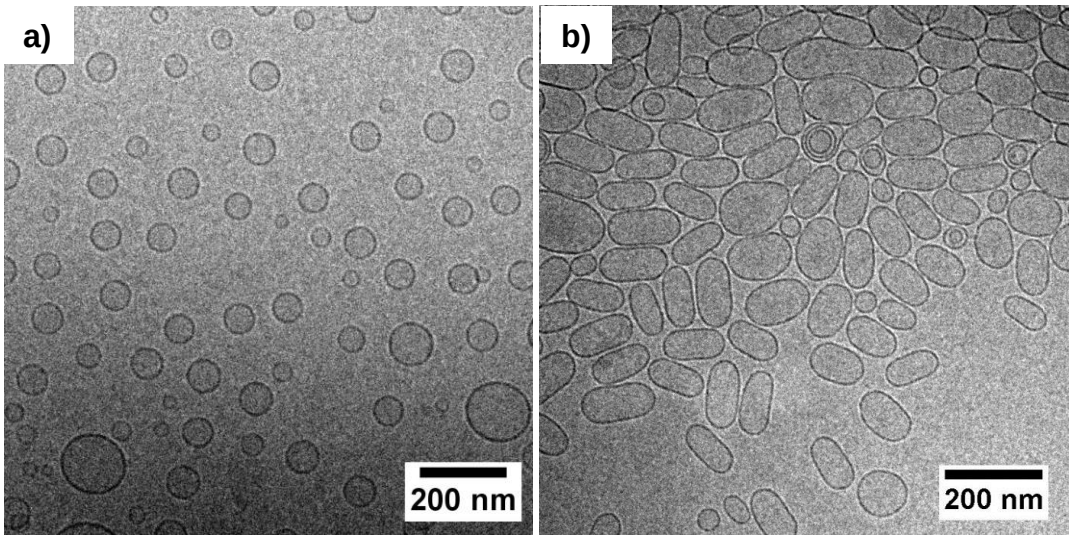

Fig. S1 Cryo-TEM images of Chol:CTAB QS_H_2O (a) and QS_PBS (b).

ESI 2. AFM-FS on lipid membranes

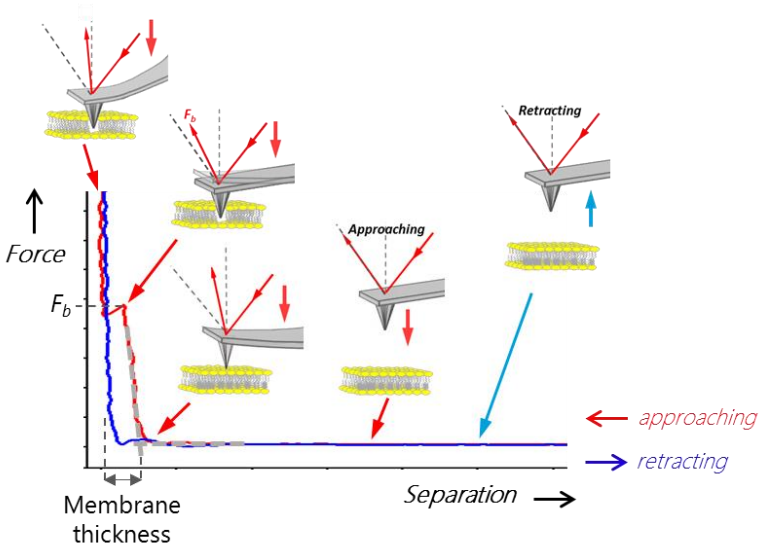

Fig. S2 Force-separation curve in an AFM-FS experiment, during approach (red) and retract (blue). The bilayer penetration by the AFM tip is shown as a sharp discontinuity during approach, at the breakthrough force F_b. The distance between the first tip-membrane contact and the final contact with the underlying substrate (in the approach curve) is used to calculate the membrane thickness. The onset of the tip-membrane contact was defined at the intersection between the baseline and the extrapolation of the slope of the elastic deformation region of the curve. The schematics of the different approach and retract steps are shown.

ESI 3. Mechanical properties of DOPC:Chol (80:20) membrane

***Liposome and SLB preparation*.** DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine) was purchased from Avanti Lipids. DOPC:Chol (80:20 molar ratio) liposomes (LP) were prepared using the DELOS-SUSP method.[1, 2] Supported lipid bilayers were obtained by vesicle fusion onto freshly cleaved mica surfaces (mica discs, Ted Pella, Redding, CA). 100 µL of LP suspension (2.1 mg ml^{-1}) were deposited on to the mica for 30 min at RT. Afterwards, the samples were rinsed several times with PBS/NaCl buffer to remove unfused vesicles, keeping always the samples hydrated.

***Liposome membrane characterization*.** SLBs composed by DOPC:Chol (4:1) in PBS buffer pH 7.4 were morphologically and nanomechanically characterized. As observed in Fig. S3A, a homogeneous topography was observed for the lipid mixture displaying a membrane thickness of 5.0 ± 0.4 nm, comparable to SQMs in PBS buffer. The AFM-FS measurements show force-separation curves displaying the typical sharp breakthroughs for a fluid-like system (Fig. S3B) and leading to a unimodal F_b distribution centered at the mean value of 8.5 ± 2.3 nN (Fig. S3C). This results are in agreement with previously reported ones, where DOPC:Chol SLBs were explored varying the concentration of Chol into the lipid membrane.[3]

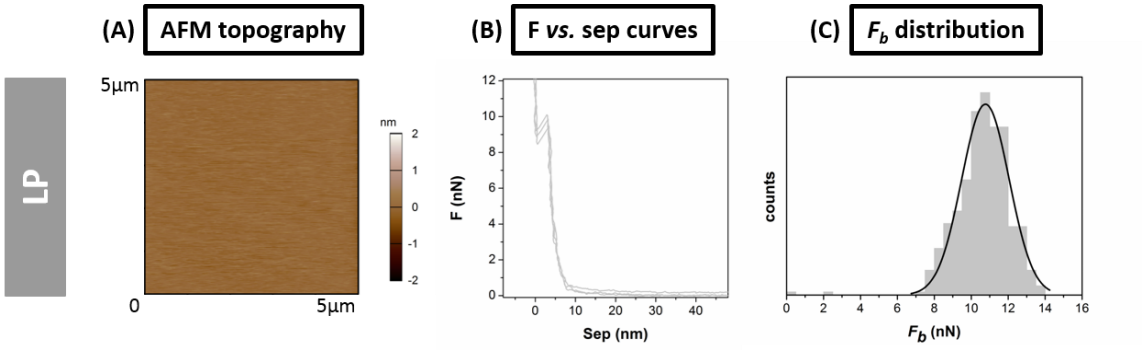

Fig. S3 AFM AC-mode topographical image (A) and AFM-FS results (force-separation curves (B) and the corresponding F_b distribution (C)) for DOPC:Chol (80:20) SLB on mica in PBS/NaCl pH 7.4 at RT.

ESI 4. SQMs from QS_H_2O after changing to PBS/NaCl

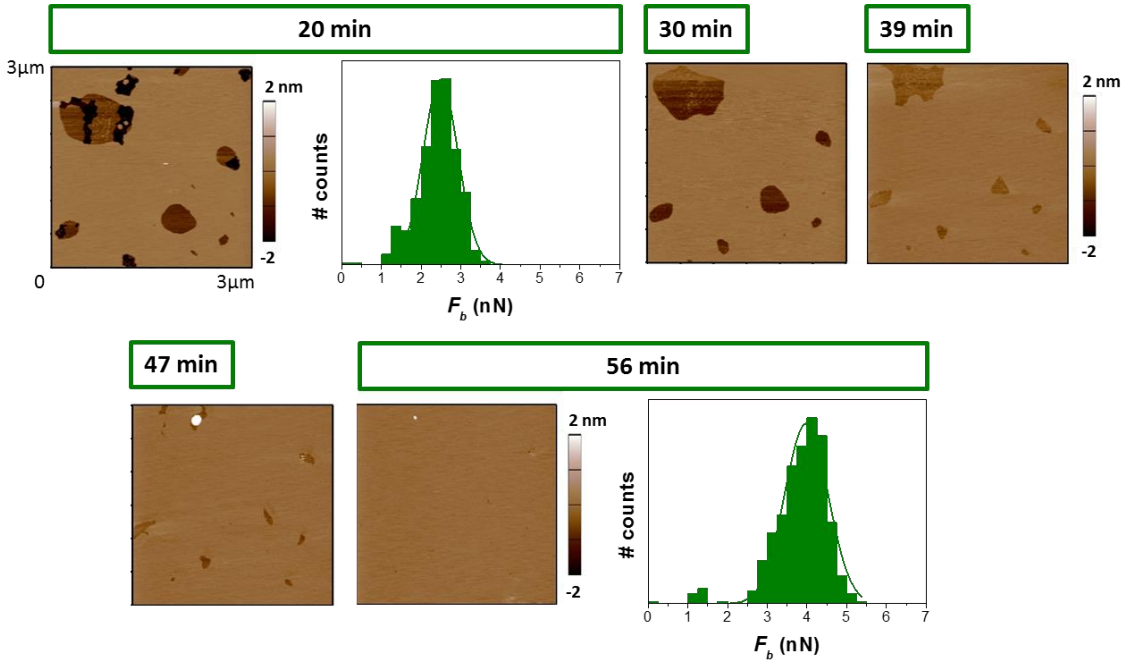

Fig. S4 Consecutive AFM AC mode topographical images and F_b distributions for SQM on mica from QS_H_2O vesicles, after replacing the medium with PBS/NaCl (94 mM NaCl, 4 mM PBS, pH 7.4) (QS_H_2O + PBS) at RT. The time described in the figure represents the minutes after the first PBS/NaCl rinsing.

ESI 5. SQMs from QS_PBS with T

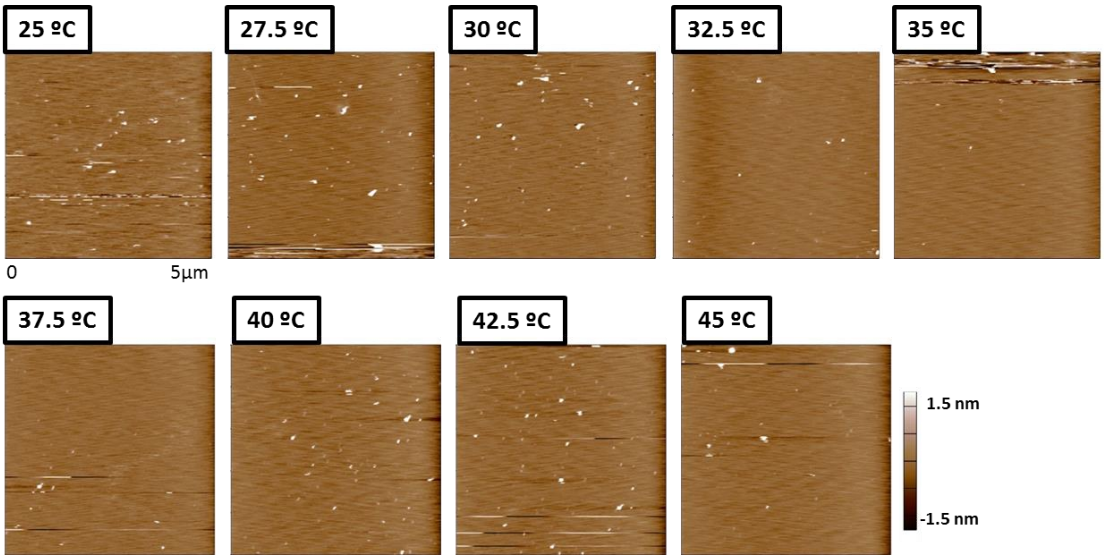

Fig. S5 AC AFM topographical images for QS_PBS membrane supported on mica in PBS/NaCl pH 7.4, while increasing the experimental temperature.

ESI 6. MD simulations of a QS bilayer.

Addition of salt (NaCl)

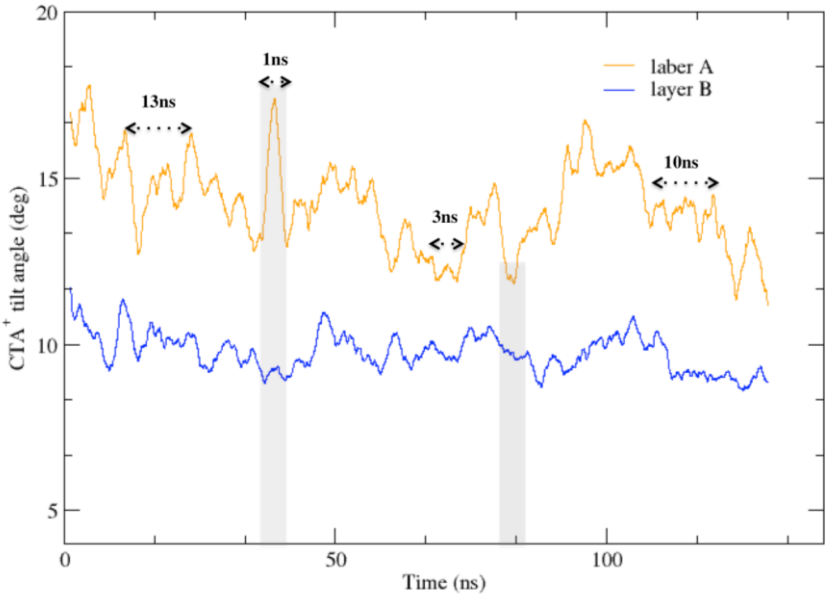

Fig. S6 Results obtained in MD simulations of a QS bilayer at 25 ºC with added salt (NaCl) (see Experimental section). The plot shows the tilt angle for CTA^+ molecules (averaged over a leaflet) *vs*. time during the MD simulations. The data for each leaflet of the bilayer is indicated in a different color as in Fig. 9 in the main text.

Tilt angles of CTA^+ with water and with added salt

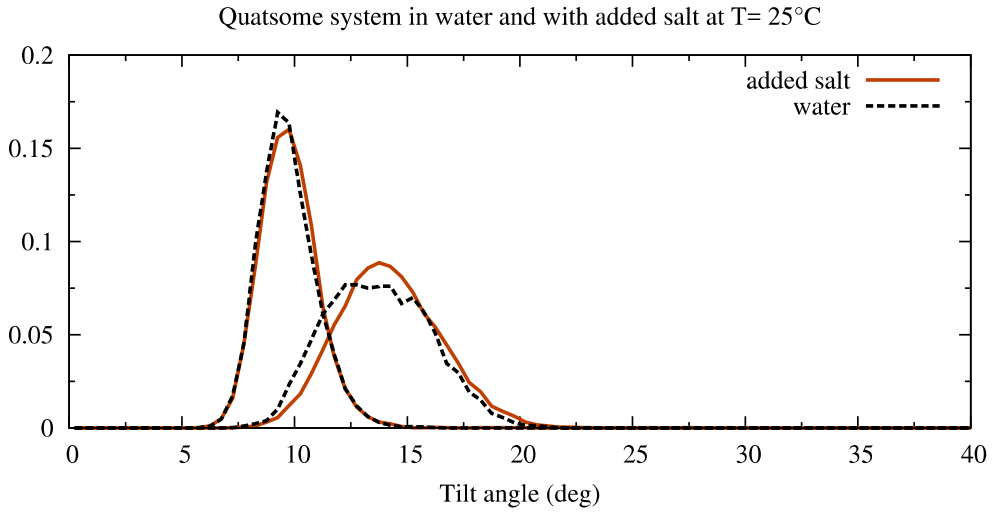

Fig. S7. Distribution of values of tilt angles of CTA^+ molecules for the QS system with water and with added salt (NaCl). Results show similar behavior in both cases pointing out two peaks corresponding to the different orientations described in the main article.

References

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3. L. Redondo-Morata, M. I. Giannotti and F. Sanz, *Langmuir*, 2012, **28**, 12851-12860.