Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.
This journal is © the Owner Societies 2017

Cation-induced shape remodeling of negatively charged phospholipid membranes

Z. T. Graber[a], Z. Shi[a], T. Baumgart[a]

Supplementary Information

Membrane deformation does not induce Ca^{2+} leakage

The presence of Ca^{2+} has a prominent impact on the shape of negatively charged membranes, morphing the virtually planar membrane of GUVs into a tubulated membrane with areas of high curvature. The tubulation of the membrane is reflected by other structural changes as well, from lipid condensation or clustering, to increased hydrophobicity. Given the significant structural impact of Ca^{2+} and the importance of an asymmetric distribution of Ca^{2+} across the membrane, it is important to ascertain whether Ca^{2+} permeability may be influenced by this structural change. To accomplish this, we utilized the Ca^{2+} sensitive fluorophore, Fluo-4, which increases in intensity in the presence of Ca^{2+}. Vesicles containing Fluo-4 were transferred into Ca^{2+} solution and the Fluo-4 intensity was monitored throughout the membrane deformation (see Fig. S1a and b). Fluo-4 intensity increases slowly throughout, independent of Ca^{2+}, due to bleaching of the fluorophore decreasing self-quenching. The intensity increases at the same rate before and after the membrane deformation occurs (marked by red arrow, Fig. S1b). Thus, membrane deformation does not induce Ca^{2+} leakage to any significant degree. In contrast, if a Fluo-4 containing vesicle is transferred to a Ca^{2+} solution in the presence of the Ca^{2+} ionophore A23187, the Fluo-4 intensity increases dramatically as Ca^{2+} is transported to the vesicle interior (see Fig. S1c and d). Again, the Fluo-4 intensity increase is independent of membrane deformation.

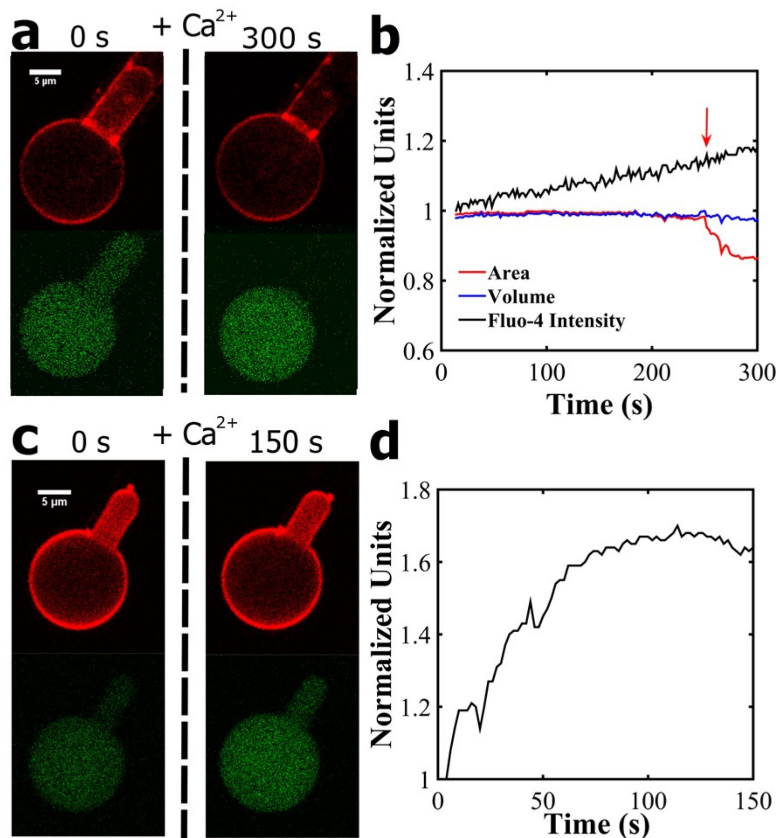

Figure S1. Membrane deformation does not influence Ca^{2+} leakage. (a) Confocal images showing GUVs (red) containing the Fluo-4 calcium sensitive dye (green) before and after transferring into 0.8 mM Ca^{2+} solution. After transferring into the Ca^{2+} solution the lipid area decreases (as shown by the loss of GUV area in the micropipette) while the Fluo-4 intensity increases. (b) Quantification of the apparent membrane area, volume, and Fluo-4 intensity within the GUV after transfer into the Ca^{2+} solution (values normalized to initial values). Fluo-4 intensity increases slowly over time, but is independent of the presence of Ca^{2+} (increase is likely due to reduced quenching as fluorophore slowly bleaches). Fluo-4 intensity does not change after membrane deformation occurs (denoted by the sudden decrease in apparent membrane area and marked with a red arrow), indicating that no Ca^{2+} leakage is induced by membrane deformation. (c) Confocal images of GUVs containing Fluo-4 before and after transfer into solution containing 0.8 mM Ca^{2+} solution and 1 nM A23187 calcium ionophore. Fluo-4 intensity increases sharply in the Ca2+ solution as the ionophore rapidly transports Ca^{2+} to the vesicle interior. (d) Quantified Fluo-4 intensity within the vesicle shows the dramatic increase in fluorescence due to Ca^{2+} transport to the interior of the vesicle. Fluo-4 intensity increases 60% within 100 s, while in the absence of the ionophore, Fluo-4 intensity only increases 20% over 300 s.