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### Title

High hydrostatic pressure induced shape and lipid phase changes on giant unilamellar vesicles studied with two-photon excitation microscopy

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**High hydrostatic pressure induced shape and lipid phase changes on giant unilamellar vesicles studied with two-photon excitation microscopy.**

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**Abstract**

We used two-photon-fluorescence microscopy to investigate the effects of pressure on the morphology and lipid phase of Giant Unilamellar Vesicles (GUVs). GUVs of the size of few tens of microns are grown by electroformation. After detaching the vesicles from the electrodes, they are pulled into transparent quartz capillaries with an inner diameter of 50  $\mu\text{m}$  and an outer diameter of 375  $\mu\text{m}$ . Pressure can be applied to the vesicles inside the capillaries sealing one end and connecting the other to a high pressure pump. We used LAURDAN to label the GUVs. The emission spectrum of this fluorescent probe is sensitive to the degree of water penetration into the lipid membrane. The spectral shift of LAURDAN is quantified in the GUV images by using the GP function. After locating a vesicle in the capillary, we followed both the GP and shape changes due to high hydrostatic pressure. We observed GP and shape hysteresis when performing successive compression and decompression cycles. We performed pressure experiments on different types of lipids and we observed that structurally different lipids perform the phase transition at different pressures, while the general shape and GP behavior are similar. When the pressure is increased, the vesicle undergoes phase transition from the liquid crystalline phase to the gel phase and we observe a decrease in its volume of about 30%. During the decompression cycle, the vesicle loses its spherical shape as a result of the mismatch between its internal volume and surface and fluctuations in the membrane shape ... [truncated at 250 words]