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**Pressure studies of giant unilamellar vesicles using two photon fluorescence microscopy.**  
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**Abstract**  
We used two-photon-fluorescence microscopy to investigate the effects of pressure on the lateral organization of lipids on the membrane of Giant Unilamellar Vesicles (GUVs). GUVs of the size of few microns were grown by electroformation onto platinum electrodes. After detaching the vesicles from the electrodes, they were sucked into transparent quartz capillaries with an inner diameter of 50 µm and an outer diameter of 360 µm. This novel high pressure cell for the microscope was recently described (J.D. Muller and E. Gratton, High pressure fluorescence correlation spectroscopy, *Biophysical Journal* (85) October 2003). Pressure can be applied to the vesicles inside the capillaries sealing one end and connecting the other to a high pressure pump. This system allows using high N.A. immersion objectives to observe the surface of the vesicles with high spatial resolution. It was shown that the behavior of single molecules and fluctuation experiments can be performed under high hydrostatic pressure. 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. For this reason, LAURDAN can be used to detect the phase state of the lipid membrane. The spectral shift of LAURDAN (of more than 40 nm between the liquid and the gel lipid phase) is quantified in the GUV images by using the GP function. Supported by the NIH, PHS 5 P41-RRO3155, and by UIUC.