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Are giants unilamellar phospholipid vesicles really unilamellar? A two photon microscopy study.

32nd Annual Meeting of the Biophysical Society, Kansas City, Missouri, 1998. *Biophys J.* 1998; 74(2 Pt 2): A374, Th-Pos163. Abstract

Unilamellar liposomes are used for several purposes such as transport studies and drug delivery. We want to utilize such liposomes composed of a single bilayer for the study of domain coexistence and for the estimation of the domain size. A variety of methods for their preparation are described in the literature. We used several of those methods and we used fluorescence microscopy and electron microscopy to assess the number of lamellae present in the vesicles following each preparation. Using some of the methods reported in the literature, we were able to obtain only a small percent of unilamellar vesicles in a population mainly composed of multilamellar vesicles. By using two-photon microscopy to image the vesicles labeled with 2-dimethylamnino-6-lauroylnaphthalene (LAURDAN), we observed that the vesicle lamellar structure also depends upon the phase state of the lipids. In some cases, we were able to assess the number of lamellae present in each vesicle in a preparation based on the quantization of the intensity. By a comparison with red blood cell membrane intensity, labeled with LAURDAN together with the vesicles, we could estimate which vesicle, or which portion of them, may be composed of a single bilayer. We must point out that using the sectioning capability of the two-photon excitation microscopy to image LAURDAN labeled vesicles, we obtain an impressively clear representation of the vesicle complex internal structure. Supported by the National Institutes of Health (RR03155).