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Quantitative Analysis of Membrane Composition by Secondary Ion Mass Spectroscopy

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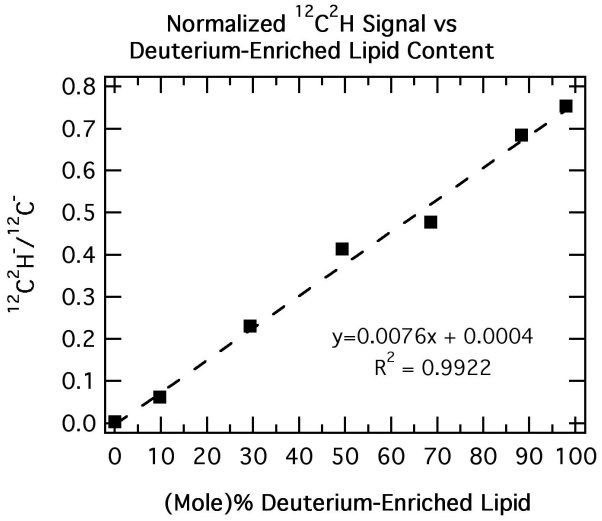
Investigations of the lateral organization within membranes hinge upon the ability to differentiate one component of interest from another. Typically, fluorophores are conjugated to specific components and the organization is probed with fluorescence microscopy. However, bulky labels may change the physical properties of the components they are attached to, and only the labeled component can be visualized. We have developed an approach to explore the lateral composition of supported lipid bilayers that employs an isotopic labeling strategy and high-resolution secondary ion mass spectroscopy (SIMS), which is performed with a NanoSIMS 50 (Cameca). Lateral resolution as high as 50 nm is possible with very high sensitivity. Here, we present a method to quantify isotopically labeled components within membranes. Homogeneous supported lipid bilayers that systematically varied in their deuterium-enriched lipid (1-palmitoyl-D_{31}-2-oleoyl-*sn*-glycero-3-phosphocholine) content were freeze-dried and examined with the NanoSIMS 50. The normalized $^{12}C^{2}H^{-}$ secondary ion signal intensity ($^{12}C^{2}H^{-}/^{12}C^{-}$) had an excellent linear correlation with the amount of deuterium-enriched lipid within the sample (see figure). This relationship may be exploited to obtain quantitative information on microdomains within a membrane by creating similar calibration curves for multiple, unique, isotopically labeled components within the sample.



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