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Melanie A Cheng, Qiaoqiao Ruan, Nicholas P Barry, Sophia Y Breusegem, Nabil Halaihel, Makoto Inoue, Moshe Levi, Enrico Gratton, and William W Mantulin.

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Abstract

Lipid rafts are discrete regions in the plasma membrane which are composed of lipids that exist in the liquid ordered state, and are believed to function as platforms for protein and lipid transport. These structures are reported to be present in numerous membrane systems, including brushborder membranes in kidney cells. This research aims to study the membrane dynamics in brushborder membranes and membrane fractions of rat renal proximal tubular cells. Fluorescence (Laurdan) provided direct visualization of GUVs formed through electroformation of raft and non-raft fractions of intact renal brushborder membranes. Flotation through a density gradient (Optiprep) separated the raft and non-raft fractions without the use of a detergent. Two-photon scanning microscopy of the GUVs formed from the raft fraction showed uniform fluorescence intensity images with some non-fluorescent domains of a few microns in size. Previous GUV studies of raft fractions obtained through detergent extraction yielded vesicles devoid of domains. Membrane fluidity measured as Laurdan Generalized Polarization (GP) function was monitored across a physiological temperature range (25°-42°C) in the GUVs. The GP in the raft fraction indicated a less fluid phase than in the non-raft fraction. This result is consistent with the compositional differences in these membrane fractions, particularly in terms of sphingomyelin and cholesterol content. Scanning fluctuation correlation spectroscopy performed on the intact brushborder membrane revealed the diffusion rate of NaPi-II cotransporter, a brushborder membrane specific protein, to be consistent with the mobility of a membrane associated protein. Supported by the NIH, PHS 5 P41-RRO3155, and by UIUC.