Title
A two photon Microscopy study of raft membranes from rat kidney proximal tubular cells reconstituted into giant unilamellar vesicles (GUVs)

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Rafts are discrete regions in the plasma membrane that are believed to function as platforms for protein and lipid transport. This research aims to study the structure of rat renal proximal tubular cell membranes using techniques used in characterizing artificial membranes. The study involves the direct visualization of GUVs formed from raft fractions of integral rat kidney brush border membranes obtained through detergent extraction with Triton X-100 at 4°C. Imaging of GUVs obtained from detergent resistant membranes (DR) labeled with the fluorescent probe Laurdan using two-photon scanning microscopy resulted in fluorescence intensity images showing an even distribution of Laurdan at physiological temperatures. No domain formation was observed in the DR membranes, whereas membrane domains were clearly present in intact brush border membranes. Membrane fluidity was characterized in terms of Laurdan Generalized Polarization (GP) function and was observed to be in a fairly rigid phase across the temperature range in which the DR GUVs were observed (42°C-20°C), which is consistent with the characterization of rafts as regions in the membrane in the liquid ordered phase.