

Abstract Submitted
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Time-Correlated Single-Photon Counting Fluorescence Imaging of Lipid Domains In Raft-Mimicking Giant Unilamellar Vesicles¹ JAMES CLARKE², Trinity University, KWAN CHENG, Trinity University, ORRIN SHINDELL, University of Texas at Austin, EXING WANG, UT- Health Science Center at San Antonio — We have designed and constructed a high-throughput electrofusion chamber and an incubator to fabricate Giant Unilamellar Vesicles (GUVs) consisting of high-melting lipids, low-melting lipids, cholesterol and both ordered and disordered phase sensitive fluorescent probes (DiIC12, dehydroergosterol and BODIPY-Cholesterol). GUVs were formed in a 3 stage pulse sequence electrofusion process with voltages ranging from 50mVpp to 2.2Vpp and frequencies from 5Hz to 10Hz. Steady state and time-correlated single-photon counting (TCSPC) fluorescence lifetime (FLIM) based confocal and/or multi-photon microscopic techniques were used to characterize phase separated lipid domains in GUVs. Confocal imaging measures the probe concentration and the chemical environment of the system. TCSPC techniques determine the chemical environment through the perturbation of fluorescent lifetimes of the probes in the system. The above techniques will be applied to investigate the protein-lipid interactions involving domain formation. Specifically, the mechanisms governing lipid domain formations in the above systems that mimic the lipid rafts in cells will be explored.

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