Title: **Liquid phase segregation in lipid bilayers: an experimental and computational approach**

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The study of the eukaryotic cell membrane lipid bilayer and its functionality is of paramount importance to determine processes involved in signal transduction, transmembrane protein activity, etc… Up until the 1980s, the fluid mosaic was commonly used to describe cell membranes, but from there on the lipid raft model was proposed. According to the theory, the lipid composition of bilayers is not homogenous, but rather microdomains rich in saturated lipids and cholesterol which are more rigid and less permeable, and "float" in a liquid-disordered phase, rich in unsaturated lipids. The aim of this report is to study the structure of lipid bilayers with liquid phase segregation with both experimental and computational approaches. The bilayers mimic the complex, lipid-raft based structure of in vivo membranes via the formation of liquid phases in ternary vesicles.

Electroformation of lipid vesicles was carried out with three different compositions (DOPC, DPPC/CHOL, DOPC/DPPC/CHOL), whose amount and diameter were quantified using optical microscopy. DOPC vesicles are the smallest but most abundant, whereas DPPC/CHOL vesicles are the least abundant but the biggest. Ternary vesicles lie between the two extreme cases. Using fluorescence optical microscopy, liquid-ordered and disordered phases are observed, as well as occasionally pear-shaped vesicles due to differences in surface tension

Coarse-grained molecular dynamics simulations in explicitly hydrated nanometric boxes with periodic boundaries were carried out, at three different temperatures. DUPC was used as an unsaturated lipid, as well as two different saturated lipids with different aliphatic chain length, DLPC and DSPC and cholesterol. Liquid phase segregation was observed, as well as anticorrelation phenomena between bilayer leaflets in DSPC-rich bilayers. Furthermore, the lateral diffusion coefficient for each chemical species was calculated from the mean squared displacement of the last part of each simulation and compared and contrasted with experimental and other computational results to confirm they are realistic. DUPC is more mobile than their saturated counterparts, which fits the lipid raft model previously discussed.

Keywords: coarse grained, electroformation, fluorescence optical microscopy, lateral diffusion coefficients, lipid bilayer, liquid-disordered phase, liquid-ordered phase, molecular dynamics, vesicles