1F32ES027324, P20-GM103546, P30GM103338 and J Murdock Charitable Trust.

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Advanced STED Microscopy of the Membrane Organization in Activating T-Cells

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Nanoscale organization of the membranes of living cells plays crucial roles in numerous vital processes, including during the activation of T-cells and their formation of the immunological synapse. However, the exact nature and function of reorganization of lipids during this key initiating event remain unclear. To gain further insight into this process, we employed two techniques that probe complementary properties of the membranes at the molecular level: 1) super-resolution STED-FCS to reveal detailed picture of the diffusion of the lipids, with additional information on spatial heterogeneity provided by the scanning mode; and 2) spectral (super-resolution STED) imaging with environment-sensitive membrane probes, i.e. fluorescence microspectroscopy, to map differences in local molecular order within the lipid bilayer. Using these methods, we monitored diffusion properties of lipids and molecular order of the plasma membrane of (Jurkat) T-cells over space and time upon their activation, revealing marked differences in lipid diffusion and molecular order. For the most informative and robust description of the latter, we systematically analyzed the benefits and pitfalls of the established representations, i.e. phasors, generalized polarization, and lineshape description.

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STED-FCS Reveals Diffusional Heterogeneity of Lipids and GPI-Anchored Proteins in the Plasma Membrane and Actin Cytoskeleton Free Plasma Membrane Vesicles

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The plasma membrane is a highly complex structure with nanoscale heterogeneities. The diffusion of proteins and lipids in the cellular membrane is an important measure for its membrane heterogeneity and can be used to characterise transit interactions of molecules. In this study, we employ super-resolution STED-microscopy in combination with fluorescence correlation spectroscopy (STED-FCS) to investigate the diffusional properties of lipid probes and fluorescently labelled GPI-anchored proteins (GPI-APs) in the plasma membrane of living cells and compare our findings to the diffusional behaviour in actin cytoskeleton free cell-derived plasma membrane vesicles (GPMVs). In the cellular plasma membrane, we find a variety of different diffusion characteristics (hindered diffusion such as hop and confined diffusion) but this hindered diffusion is mostly abolished in GPMVs for phospho- and sphingolipids. However, domain-like diffusion of the ganglioside GM1 persists in GPMVs. STED-FCS measurements of GPI-AP revealed also actin-dependent domain-like diffusion in the live cell plasma membrane (free diffusion in GPMVs), yet fluorescence cross-correlation spectroscopy (FCCS) indicated no co-diffusing GPI-APs in cells. This study underlines the strong influence of the cortical actin cytoskeleton on the plasma membrane organisation of most (but not all) of the membrane molecules.

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Using Laurdan and Spectral Phasor Analysis to Study Erythrocytes Membrane Solubilization

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Erythrocytes are widely used as a model system for membrane studies due to their relatively simple structure (they lack nuclei and organelles having only the plasma membrane), their convenient experimental manipulation and availability. However, the high hemoglobin content inside the red blood cells may be a problem for the use of fluorescent membrane probes. Hemoglobin content inside erythrocytes is around 20 mM and the molecules near the inner membrane surface would be the responsible for the quenching of fluorescent dyes such as 1,6-diphenyl-l,3,5-hexatriene (DPH) and 12-(9-anthroyl) stearic acid (AS). Laurdan (6-lauroyl,1-2-dimethylamino naphthalene), a fluorescent probe commonly used to study membrane structure is being used in studies in whole erythrocytes successfully. However, in order to avoid data misinterpretation due to the quenching effect of hemoglobin, authors either work at constant hematocrit (constant hemoglobin concentration or they work with hemoglobin depleted erythrocytes (ghosts). Taking this into account, the aim of this work was to explore the use of Laurdan in the presence of hemoglobin and during hemolysis. We studied the changes promoted in the membrane of rabbit red blood cells by the sucrose monoester of myristic acid, β-D-fructofuranosyl-6-O-myristoyl-α-D-glucopyranoside (MMS). We followed the interaction before and after hemolysis using FLIM (phasor plots) and Spectral imaging (spectral phasor). Our data indicate that at sublytical concentration of surfactant (20 μM MMS), there is a decrease of about 35% in erythrocytes size, without changes in Laurdan lifetime or emission spectra. As hemolysis progress, Laurdan lifetime data are not informative due to the presence of hemoglobin but Laurdan spectral phasor analyses clearly show an increase in membrane fluidity promoted by MMS.

Acknowledgements Fondecyt #1140454 (S.S.), Fondecyt #1080412 (G.G.), Beca de Doctorado Conicyt (C.S.). PICT 2657/2013 (VH). # FIS2015-70339-C2-2-R (M.P.L.). LFD (8P41GM103540).

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Computational Insights into Fuels and Chemicals Extraction from Microbial Biorefineries

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Over the past two decades, substantial investments have been made in engineering microorganisms to produce specific fuels and chemicals as part of the global bioeconomy. Many target molecules accumulate intracellularly, and a challenge is how to effectively extract the product from the cells without needing to destroy them due to the barrier imposed by the cell membrane. For some hydrophobic compounds, an organic overlay is an effective strategy for nondestructive product extraction, although the relationship between functional groups on the product and the rate of extraction are not well understood. Through both biased and unbiased molecular dynamics simulations for a range of fatty acyl compounds and terpenoids, we directly compute permeability coefficients for different steps of the extraction process. Via comparative analysis between the calculated permeability coefficients and observed interactions between the compounds and the membrane, we determine how the rate limiting steps vary depending on product chemistry. For instance, fatty aldehydes are found to transfer very rapidly across the membrane bilayer relative to alcohols, although their comparable rate of extraction into the organic phase makes them equally effective at extraction from the cell. In assessing the terpenoids, it is found that in general a modestly hydrophilic product improves desorption rates into an organic phase sufficiently to make up for their lower bilayer crossing rate. With this new insight, we can more effectively engineer microorganisms towards the production of these modestly hydrophilic fuel precursors or chemicals.

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Interactions of Poly(Ionic Liquid) Nanoparticles with Giant Unilamellar Vesicles

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When approaching the complex field of drug delivery, one crucial aspect to consider is the interaction that occurs between particles and cellular membranes, as this is the first point of contact between cells and external material. The same can also be said of anti-microbial/bacterial agents. To progress within these bio-medical fields, new interactions between particles and membranes must be explored. One such novel system is the poly(ionic liquid) (PIL) nanoparticles, first reported by in JACS 133:17556, 2011. Polymer-based nanoparticles have an increasing presence in research due to their attractive properties such as flexible functionality; when this is coupled with the additional properties afforded by ionic liquids, a new realm of