Applying Secondary Ion Mass Spectrometry to Cellular and Model Membranes to Image Component Distribution and Quantify Composition

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Secondary ion mass spectrometry (SIMS) returns chemically specific data on membrane component distribution and composition. Of particular interest is a membrane protein's lipid environment, which may play a role in the protein's function. Herein, this thesis describes the synthesis of fluorine-functionalized colloidal gold immunolabels that facilitate the imaging of specific proteins in parallel with stable isotope labeled lipids in the cellular membrane using high resolution SIMS performed with a NanoSIMS (**Figure 1**). Further, the spatial statistical analysis tools are outlined and applied to NanoSIMS data to test the hypothesis of colocalization of the flu virus protein hemagglutinin and sphingolipids.

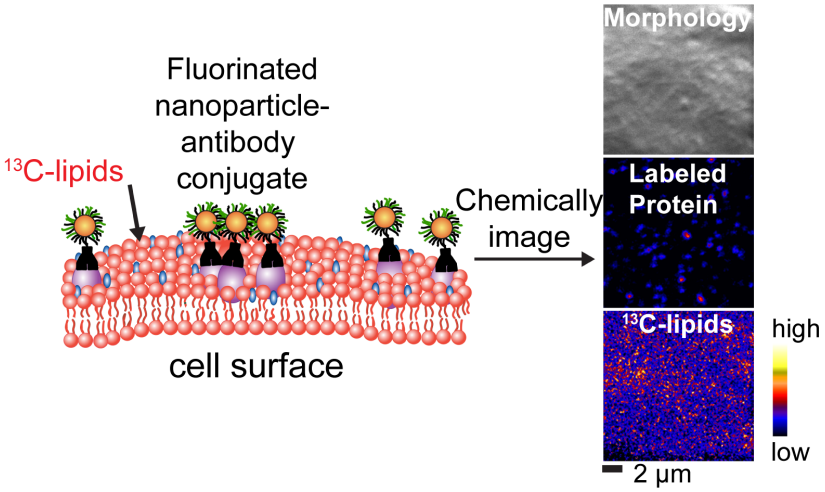

Figure 1 Schematic of protein and lipid labeling for NanoSIMS detection

While the NanoSIMS has very high lateral resolution, it requires isotope labels for component identification. Time-of-flight SIMS (TOF-SIMS) detects large portions of the mass spectrum, allowing species to be identified by their fragmentation pattern. Ionization yields in TOF-SIMS, however, limit the lateral resolution and the maximum m/z that can be used consistently for analysis. These challenges can be addressed using multivariate analysis (MVA). TOF-SIMS and the MVA method partial least squares discriminant analysis (PLS-DA) was used to identify the differentiation stage of hematopoietic stem cells. Since TOF-SIMS is a location specific technique, it can be used to investigate how varying chemical and material environments on one sample affect stem cell fate decisions. Another MVA method, partial least squares regression (PLSR), uses a calibrated model to return quantitative information from TOF-SIMS data. This was used to determine the concentration of cholesterol in homogeneous supported lipid membranes (**Figure 2**).

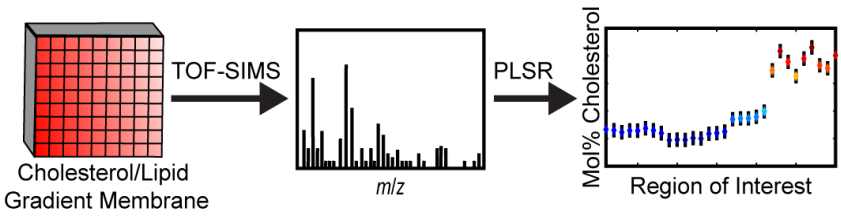

Figure 2 TOF-SIMS used to quantify the amount of cholesterol in a membrane with varying cholesterol content