# High-resolution secondary ion mass spectrometry (SIMS) imaging of lipid distributions in the plasma membrane

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Cell membrane organization is critical to healthy biological processes and those associated with disease. Though the cellular levels of cholesterol and sphingolipids affect protein clustering, signaling complex formation, and ultimately cell function, the mechanisms for this lipid-mediated cellular function are unknown. According to the prevailing hypothesis, favorable sphingolipid-cholesterol interactions induce the formation of lipid rafts, which are plasma membrane domains that are enriched with cholesterol, sphingolipids, and distinct membrane proteins. This cholesterol- and sphingolipid-dependent compartmentalization of the plasma membrane was postulated to regulate protein activity by modulating each protein's proximity to potential binding partners. However, until recently, the cholesterol and sphingolipid distribution in the plasma membrane was a mystery. To address this issue, my lab has developed a method that uses high-resolution secondary ion mass spectrometry (SIMS), which is performed with a Cameca NanoSIMS 50, to map the $^{15}$N-enrichment and $^{18}$O-enrichment from metabolically labeled $^{15}$N-sphingolipids and $^{18}$O-cholesterol on the surfaces of mammalian cells. Using this approach, we discovered that sphingolipids are concentrated in micrometer-scale domains, but cholesterol is evenly distributed within the plasma membrane. By imaging the effects of drugs on the sphingolipid distribution, we determined the sphingolipids were confined within distinct plasma membrane domains by the cytoskeleton and its associated proteins. I will show how we used this approach to evaluate the long-standing hypotheses that influenza hemagglutinin co-localizes with lipid rafts, and that the influenza virus assembles and buds from lipid rafts. These experiments provide a new understanding of plasma membrane organization, a fundamental issue in cell biology.