Probing Lipid Accumulation in Organelles of Interest Using Secondary Ion Mass Spectrometry and Complementary Imaging Techniques

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The cellular plasma membrane is a selectively permeable bilayer of lipids that separates the cell from its surroundings. In eukaryotic cells, cholesterol-containing lipid membranes also separates the cell into organelles, which are membrane-bound compartments for specialized cellular functions. The relative distributions of cholesterol and different lipid species between organelles is tightly controlled by intracellular transport and enzymes that synthesize and degrade lipids. Defects in lipid degradation and transport can be life-threatening. For example, Niemann-Pick disease type C is a fatal, neurodegenerative disease in which mutations in either of the cholesterol transport proteins, NPC1 or NPC2, impairs cholesterol export from endosomes [1, 2]. This causes cholesterol and sphingolipids to accumulate in late endosomes, which alters protein trafficking [3, 4]. Yet, the mechanisms for lipid-mediated cellular (dys)function are poorly understood. This is due, in part, to the difficulty of semi-quantitatively imaging cholesterol and lipid species of interest in specific organelles.

Secondary ion mass spectrometry (SIMS) performed in a depth profiling mode has enabled imaging the distributions of cholesterol or distinct lipid species in three dimensions (3D) within individual cells [5-7]. Though SIMS imaging has revealed the accumulation of cholesterol and distinct lipid species within subcellular compartments, which organelle these compartments correspond to could not be identified. Organelle-specific labels that produce distinctive ions are needed to enable detecting the organelles or interest with SIMS. Labels that also allow detection with complementary imaging approaches, such as fluorescence microscopy, would maximize the amount of information that can be acquired from the cells.

Here we describe our progress in the development of organelle-specific labels that can be detected with SIMS and fluorescence microscopy. We have previously demonstrated that the commercially available ER-Tracker Blue-White stain enables the complementary imaging of the endoplasmic reticulum, which is the organelle where lipids and proteins are biosynthesized. Because the ER-Tracker Blue-White stain is a fluorine-containing fluorophore that accumulates in the endoplasmic reticulum, this stain allows the endoplasmic reticulum to be imaged with fluorescence microscopy, and located with SIMS according to the distinctive $^{19}F^-$ secondary ions it produces [8]. We are also developing a strategy for the detection of any organelle of interest with both fluorescence microscopy and SIMS. Our approach harnesses molecular biology techniques that enable fusing small enzymes that catalyze the covalent attachment of a small substrate to themselves. As a proof of concept, we transfected CHO-K1 cells so they stably express a small enzyme fused to a protein that resides in late endosomes. This small enzyme selectively and covalently reacts with a small molecule ligand that we functionalized for detection with both fluorescence microscopy and SIMS. By performing fluorescence microscopy, atomic force microscopy, and SIMS on the same cells, the location of the late endosomes within the cell can be visualized in 3D. Subsequent studies that use this cell line and the functionalized ligands will enable identifying the abundances of lipid species of interest within the late endosomes using SIMS. This approach can also be extended to other organelles of interest [9].

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