Integrated and Dynamic Multispectroscopic *In Situ* Imaging of Plant Metabolism at the Level of Subcellular Compartments

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Project Goals: This multi-disciplinary team is developing new integrated multi-spectral imaging technologies that will assess and quantitatively model metabolic processes that are non-symmetrically distributed at the cellular and subcellular levels of plant organs. The imaging technology is being developed in the context of computational capabilities that will integrate multi-spectral images with genome scale modeling and thus contribute to the better understanding how biomass-based biofuel producing metabolic pathways are interconnected and controlled within topological constraints in spatially defined membrane-bounded regions within plant cells.

This project is developing and applying integrated molecular imaging technologies that can be used to monitor membrane lipid remodeling. Understanding the remodeling of membrane lipid topology in plant cells has major consequence in optimizing plant biomass productivity. The integrated molecular imaging technologies is being developed in the biological context of autophagy that remodels membrane lipid topologies that control spatially defined subcellular regions within plant cells and optimizes plant biomass productivity in response to environmental signals. Genetic stocks that will enable the dissection of membrane lipid dynamics have been identified and analyzed to identify specific target lipid molecules for molecular imaging. Analytic technologies for imaging these specific target lipid molecules via fluorescence, Raman and mass spectroscopy have been established. These imaging technologies are being developed in the context of computational capabilities that will integrate the multi-spectral images with genome scale models. We have established an infrastructure that ensures transparent collaboration among the students of different collaborating groups. Defined tasks to fulfill the goals of the project, and these are expanded as follows.

Task 1: Genetic and biochemical analysis of defined autophagy and lipid metabolism genes. The initial analyses focused on identifying specific lipid molecules that are affected by the autophagy induced dynamics of cellular membranes. The rationale being that these specific lipid molecules will be targeted for imaging via the technologies that will be developed in Tasks 2-4.

Task 2: Develop and apply in situ optical imaging platforms. The team has demonstrated the first-of-its-kind, red-releasing photocage. Photocages are compounds that release a cargo or generate a change in a signal when exposed to light. For use in plant systems, it is desirable to design photocages that release their cargo when exposed to a range of visible light wavelengths

that are not interfered by the endogenous optically active biomolecules (e.g., chlorophylls, carotenoids). This technology should enable multiple cargos to be released independently with different wavelengths of light or to increase the penetration depth of the light that generates the signal. Longer wavelengths of light are associated with deeper penetration depths in tissues, so a red-release photocage has this benefit. The team synthesized and demonstrated the use of the red photocage in a biological system.

Task 3: Chemical synthesis and tuning of self-destructing fluorophores for the in situ visualization of dynamic events. A new class of fluorescent chemical imaging probes capable of in situ imaging have been synthesized. In particular, we have designed and synthesized a new class of photocages derived from BODIPY dyes capable of dynamic fluorescence imaging using visible light. These probes release compounds with visible light irradiation with wavelengths spanning the visible and entering the near-IR.

Task 4: Spatial mapping of metabolites via mass-spectrometry. We have focused on optimizing the performance of atmospheric pressure mass spectrometry imaging (MSI) and are applying this imaging technology to spatially map specific lipid-metabolites. These optimizations have reduced the laser spot size to approximately 50 μm, as compared to the 125 μm spot size that was available at the start of the project. In addition, we have focused on finding a technique that can be used to integrate information from different imaging platforms, i.e. mass spectrometry, Raman and fluorescence microscopy, and optical microscopy, which is essential for multimodal image comparison. This is especially important because MSI has far lower image resolution than optical imaging techniques.

Task 5: Develop computational imaging visualization platform. We are establishing a database with a visualization platform that the broader research community in different fields of chemistry, chemical engineering, biochemistry, and biology can readily access, comprehend and explore the data obtained from the combined and diverse analytical chemistries in the context of the biological materials under study.

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