

227. Studying lipid accumulation mechanism in oleaginous yeast using hyperspectral SRS microscopy

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Project Goals: Our research will combine advanced imaging, single-cell systems biology, and metabolic engineering approaches to understand the lipid accumulation mechanism of oleaginous yeast, and to decouple nitrogen regulation and sugar utilization on lipid production for lignocellulosic advanced biofuel production. The proposed research will focus on the analysis of transcriptomic data of single cells that will be correlated with the quantitative measurement of lipid production in vivo. Through genetic association study we will identify genes and transcriptional factors that correlate with lipid accumulation, and further verify these potential candidate genes or genome regions that contribute to microbial lipid accumulation by metabolic engineering.

The Xie group at Harvard University develops advanced imaging and sequencing techniques to quantitatively characterize the genome, transcriptome, and metabolome of single cells. Using these novel tools, we hope to understand lipid accumulation mechanism of oleaginous yeasts and identify yeast mutants that are capable of high lipid production using biomass hydrolysates. Although the mechanism of lipid production has been explored with genome and multi-omic analysis and documented recently in certain yeast species such as *R. toruloides*, the exact regulatory network governing lipid accumulation to maximize the microbial lipid production has not yet been completely unraveled. In addition, it has been reported that the content, morphology (i.e., number and size), and chemical composition of lipid droplets produced by oleaginous yeast vary in different strains and under different growth conditions. To understand the lipid production mechanism and identify genetic features responsive to lipid accumulation in the presence of pentose and nitrogen, we propose to develop an automated chemical imaging and single cell transcriptomics method to correlate the lipid accumulation with the transcriptional profiles at the single-cell level.

Our first goal is to develop a high throughput single cell imaging technique that can visualize and quantify lipid production of yeasts under a wide variety of culture conditions and with different sugar sources. Our group has pioneered stimulated Raman scattering (SRS) microscopy, a technique that visualizes molecules based on their intrinsic vibrational contrasts without any need for labels. It offers exciting new opportunities in studying lipid production in vivo with high spatial and temporal resolution based on C-H vibrational signature from lipids. The number and size of lipid droplets within yeast cells can be easily quantified. However, it has limited chemical resolvability due to the strong spectral overlap among many biomolecular species in the C-H region. We tackled this challenge with two technical innovations: 1) hyperspectral SRS imaging uses spectral information to distinguish closely related lipid species; 2) deuterium tracing SRS exploits deuterium labeled metabolic substrates to quantify their conversion to cellular components. We have demonstrated the capability of these novel imaging approaches in both single cell organisms such as yeasts as well as multicellular organisms such as *C. elegans*. Triglycerides and cholesteryl ester, two major neutral lipids, can be distinguished based on their spectral difference in the C-H region. Uptake of different fatty acids into individual lipid droplets in living cells can be quantified with deuterium tracing. We further demonstrated that lipogenesis can be monitored at subcellular resolution by using deuterated glucose as the metabolic substrate. These unprecedented capabilities open up new ways to understand lipid accumulation in oleaginous yeast. We will build a flow cytometry platform that uses SRS signal from lipids to screen for single cells with high lipid production. In combination with single cell sequencing, we expect to unravel the genetic elements that regulate yeast

lipid accumulation and engineer mutants with robust and high yield lipid production.

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