118. Isotopically nonstationary ^{13}C flux analysis of isobutyraldehyde production in *Synechococcus elongatus*

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Project Goals: This project aims to apply ^{13}C flux analysis to identify strategies that will increase photosynthetic biofuel production in engineered cyanobacteria.

Recent studies have demonstrated the feasibility of converting energy from sunlight and carbon from CO_2 directly into biofuels using photosynthetic microorganisms. Despite the advances made in cyanobacterial biofuels production, the productivities achieved by cyanobacterial fermentations are currently too low for industrial feasibility and few tools are available that specifically address the challenges of redirecting and enhancing metabolic flux in photosynthetic microbes.

Our group is developing novel approaches that use isotope tracers and metabolic flux analysis (MFA) to quantitatively assess *in vivo* metabolic phenotypes of photoautotrophic hosts. Although ^{13}C is the preferred isotope tracer for mapping central carbon metabolism in heterotrophic hosts, photoautotrophs assimilate carbon solely from CO_2 and therefore produce a uniform steady-state ^{13}C-labeling pattern that is insensitive to fluxes. However, transient measurements of isotope incorporation following a step change from unlabeled to labeled CO_2 can be used to map photoautotrophic fluxes by applying newly developed techniques of isotopically nonstationary MFA (INST-MFA). We have recently developed a novel software package called INCA to facilitate model generation and computational solution of INST-MFA models, which is now publicly available to the scientific community [1]. We have also established experimental protocols for performing $^{13}CO_2$ labeling experiments and mass isotopomer analysis that are required for INST-MFA of autotrophic hosts [2, 3].

To establish proof-of-concept, we first applied ^{13}C INST-MFA to map fluxes in the model cyanobacterium *Synechocystis* sp. PCC 6803 growing under photoautotrophic conditions [4]. Comparison of the INST-MFA flux map to theoretical values predicted by a linear programming model revealed inefficiencies in photosynthesis due to oxidative pentose phosphate pathway and malic enzyme activity. Our ongoing work involves extending the ^{13}C INST-MFA approach to examine engineered strains of *Synechococcus elongatus* PCC 7942, with the goal of identifying novel genetic targets that control production of isobutyraldehyde (IBA, a direct precursor of isobutanol). Quantification of photosynthetic carbon fluxes in IBA-producing cyanobacteria is expected to pinpoint pathway bottlenecks that can be subsequently removed in further rounds of metabolic engineering, thus leading to maximal productivity by redirecting flux into biofuel- producing pathways.

References

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