

4360 Challenges of Photoautotrophic Mfa: a Transient Isotopic Labeling Approach

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Metabolic flux analysis is well acknowledged as an important analysis tool for metabolic engineering. Several research groups have pioneered the development of experimental and computational methods to analyze steady state metabolic fluxes under heterotrophic and mixotrophic conditions. However, these steady state techniques cannot be applied to the purely autotrophic metabolic mode with a single carbon input to the system, e.g. photosynthetic growth on carbon dioxide. We have developed an overall scheme for MFA in photoautotrophic systems based upon steady-state intracellular metabolite concentration and transient isotopic data. We utilize isotopomer balancing methodology to simulate the dynamic labeling of intracellular intermediates of central carbon metabolism in *Synechocystis* sp. PCC 6803, a model photosynthetic prokaryote. We analyze the sensitivities of computed fluxes (observed transients) to the pool sizes and measurement errors. These insights are utilized for a comprehensive experimental design for autotrophic flux analysis. Preliminary experimental results of intracellular metabolite concentration measurements will be presented.