In vivo thermodynamic analysis of metabolic networks and engineered pathways

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Project Goals: This project will integrate advanced mass spectrometry, computational modeling, and metabolic engineering to develop and experimental-computational approach for the *in vivo* genome-scale determination of Gibbs free energies (ΔG) in metabolic networks suitable for high-throughput thermodynamic profiling of engineered organisms and emerging model systems.

Thanks to continuing breakthroughs in genome editing and metabolic engineering, the range of industrially useful organisms that can transform renewable biomass resources into biofuels or bioproducts will continue to expand. Thus, there is an increasing need for genome-scale, high-throughput tools to characterize metabolic capabilities of engineered organisms, identify new routes for bioproduct synthesis, and uncover the foundational principles that drive these complex biological systems.

Thermodynamic analysis of metabolic networks has emerged as a powerful new tool for pathway design and metabolic engineering whose full potential still remains to be realized. Thermodynamics constrains the kinetics of biochemical reactions and determines enzyme efficiency in metabolic pathways. Specifically, a pathway with a strong thermodynamic driving force will achieve larger flux with less total enzyme than a pathway closer to thermodynamic equilibrium. It will also be less susceptible to product feedback inhibition, thereby reaching higher final titers. Thermodynamic analysis can therefore provide unique insights in synthetic pathway design by pinpointing the enzymes whose expression will have the largest effect on flux, by identifying kinetic and thermodynamic bottlenecks in engineered pathways, or by predicting the most efficient metabolic route for product biosynthesis while ruling-out unfavorable ones. Although the usefulness of thermodynamic analysis in pathway engineering is now widely recognized, we currently lack an experimental framework for high-throughput thermodynamic profiling of metabolic networks. This project will develop an experimental-computational approach for the *in vivo* genome-scale determination of Gibbs free energies (ΔG) in metabolic networks suitable for high-throughput thermodynamic profiling of engineered organisms and emerging model systems. This project will result in the construction of experimentally-derived models that quantitatively define trade-offs between energy efficiency of biosynthetic pathways and their overall catalytic rates.

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