Innovations in Enzyme and Pathway Engineering for Cell-Free Production of Biofuels and High-Value Chemicals

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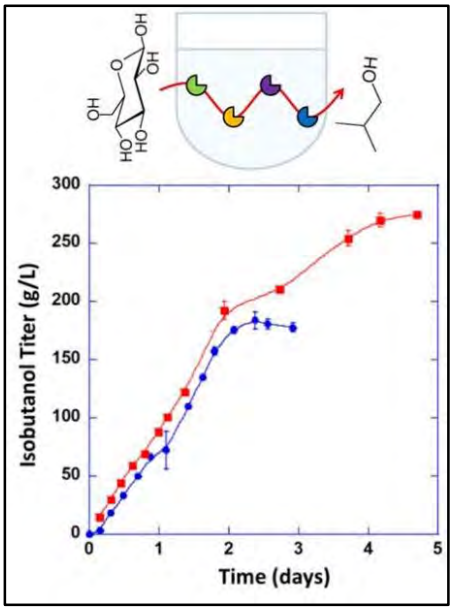
Project Goals: Research in the **UCLA-DOE Institute for Genomics and Proteomics** includes major efforts in engineering novel metabolic pathways involving complex combinations of enzymes and enzymatic materials. Cell-free systems are being developed, comprised in some cases of more than 20 enzymes, to produce metabolites ranging from biofuels to high value chemicals. New innovations in cofactor recycling have enabled high turnover processes. Future directions aim to drive carbon-negative pathways using electrical/redox inputs, and to develop novel protein engineering approaches for stabilizing key enzymes in the form of designed materials.

Abstract:

Metabolic engineering enables the production of diverse biochemical materials from microbial platforms. However, living cellular systems operate within a limited range of physical conditions, which can constrain the feasible space for pathway engineering. In addition, the exceptional complexity of native metabolic networks can confound optimization and complicate attempts to direct metabolic flux exclusively to desired products in the cell. In vitro, cell-free, systems of enzymes present opportunities for metabolic design that have only been partially explored. We have pursued cell-free or 'synthetic biochemistry' approaches to develop diverse production platforms in the laboratory. Exploiting the ability to systematically test enzymes and enzyme combinations from divergent origins, and to readily control and optimize concentrations, we have succeeded in producing systems that show high yield, titer and productivity. In one system, we have developed a 16 enzyme pathway that produces the biofuel isobutanol at >90% yield, productivity of 4 g/L/hr and a titer of 275 g/L, production parameters that exceed highly optimized cell-based fermentation parameters. In another we have created a pathway to make cannabinoids at titers that exceed published microbial production by almost 3 orders of magnitude. A further feature of the in vitro approach is high modularity. With multiple pathways now in production, the ability to



modify and combine different modules is accelerating the production of diverse products, including in the broad terpenoid space.

The simplifying advantages of synthetic biochemistry approaches are countered by separate and unique challenges, which our project team has been addressing through innovative methods. In recent work we have developed systems to deal with difficult problems of cofactor balance, through judicious combinations of cofactor-recycling enzymes and by re-engineering cofactor specificities. Another key challenge, in the absence of de-novo protein synthesis in vitro, is enzyme stability. Cost-effective systems require high enzyme activity and longevity, sometimes in solution conditions outside the scope of evolutionary pressures. We are meeting this important challenge through novel enzyme engineering strategies. In addition to traditional approaches aimed at stabilizing individual enzymes through systematic mutation, we are developing new schemes for stabilizing enzymes, alone and in functionally important combinations, through various forms of conformational confinement. Confinement of enzymes has been demonstrated to provide generally stabilizing effects on proteins, especially in harsh environments, and sequestration or co-assembly of multiple sequentially-acting enzymes offers special opportunities for improving the flux through engineered pathways. Our team is pioneering new protein material engineering and chemical biology tools to further advance those studies.

The long term vision of the project is to conceive and create ready-made enzyme combinations and enzymatic materials for biofuel and high value chemical production. We also expect our engineering explorations to reveal new insights into natural and unnatural enzymes systems, including their scope and limits.

Publications/preprints

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