Metabolic pathways and metabolic engineering

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Metabolic engineering of *Clostridium thermocellum* for cellulosic ethanol production

By understanding and then modifying carbon and electron flux, we have increased ethanol yield in *C. thermocellum*. 
Major issues in applying metabolic engineering

- **Choice of platform organism**
  - Every host presents challenges

- **Start with easily modified, industrial organism**
  - Usually yeast or *E. coli*
  - Engineer for desired function → requires deep understanding of pathways to heterologously express
  - Control expression, mitigate toxicity of intermediates and products

- **Start with organism that has unique desired capabilities**
  - Develop genetic systems
  - Build understanding of metabolism, gene regulation, etc.
  - Engineer it to make only the compound of interest and be more robust
Current status of technology in metabolic engineering

• Developing new genetic systems is difficult but feasible

• Synthetic biology is allowing rapid progress on all fronts
  – DNA synthesis is relatively inexpensive, and cost is decreasing
  – Shifts the focus from tools to ideas, even in non-model organisms

• Metabolic models and other computational tools are becoming more advanced and could inform future strategies

• $^{13}$C labeling and other “fluxomics” could be broadly enabling, but are typically under-utilized
  – May be less useful for $\text{H}_2$ production because it can not directly follow electron flux, but still important
Barriers and challenges in metabolic engineering

• Knowledge
  – Often incomplete understanding of enzymatic pathways
  – Electron flux often less well understood than carbon flux

• Complexity
  – Regulatory pathways, including mechanisms of dynamic regulation
  – Allostery; either a hindrance or a tool
  – Multiple isozymes of key enzymes

• Models
  – Limited by the information you put into them

• Vision
  – Need clear idea of how to get where you want
Key needs in metabolic engineering

Near term

• Put existing pieces together as proof-of-principle
• Target applied metrics

Medium term

• Explore the basics of native pathways to increase foundational understanding
• Explore completely new approaches