1. Introduction to Metabolic Engineering

Metabolic engineering is the practice of optimizing genetic and regulatory processes within cells to increase the cell's production of a desired small molecule or protein product of interest.

Metabolic engineers manipulate the biochemical networks cells use to convert raw materials into molecules necessary for the cell's survival. Metabolic engineering specifically seeks to:

1. Mathematically model biochemical networks, calculate the yield (product divided by substrate) of valuable products, and identify parts of the network that constrain the production of these products of interest.
2. Use genetic engineering techniques to modify the biochemical network to relieve constraints limiting production. Metabolic engineers can then model the modified network to calculate the new product yield and identify new constraints (back to 1).

Resources for metabolic engineering:

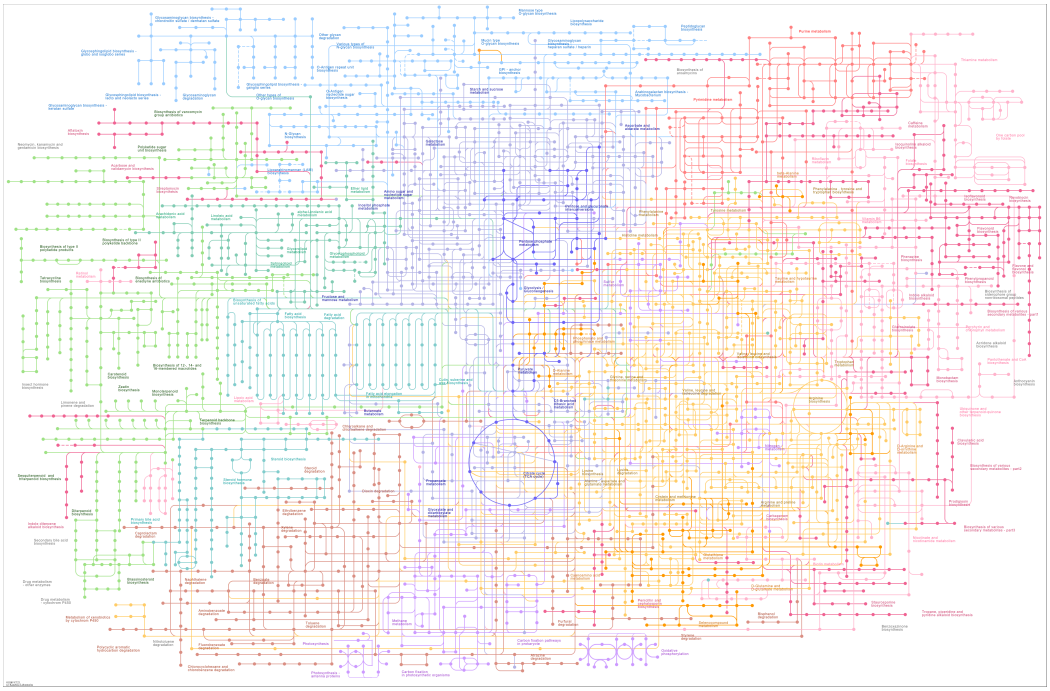
- Bailey JE. Toward a science of metabolic engineering. Science. 1991 Jun 21;252(5013):1668-75. doi: 10.1126/science.2047876. PMID: 2047876.
- Stephanopoulos G, Vallino JJ. Network rigidity and metabolic engineering in metabolite overproduction. Science. 1991 Jun 21;252(5013):1675-81. doi: 10.1126/science.1904627. PMID: 1904627.

Resources for biochemical network information

- Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 2000 Jan 1;28(1):27-30. doi: 10.1093/nar/28.1.27. PMID: 10592173; PMCID: PMC102409.
- Karp, Peter D et al. "The BioCyc collection of microbial genomes and metabolic pathways." Briefings in bioinformatics vol. 20,4 (2019): 1085-1093. doi:10.1093/bib/bbx085
- Gama-Castro, Socorro et al. "RegulonDB version 9.0: high-level

integration of gene regulation, coexpression, motif clustering and beyond." Nucleic acids research vol. 44,D1 (2016): D133-43. doi:10.1093/nar/gkv1156

Fig 1. The overall metabolic map from the KEGG database. Each dot (*node*) is a metabolite, each line (*edge*) is a metabolic reaction.



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## Recommended alternatives (images)
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2. If your notebook is part of a git repository, place the imag
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2. Example biological products

There are (roughly) two categories of Biotechnology, industrial biotechnology, and medical biotechnology. Metabolic engineering plays a vital role in both sectors. Industrial biotechnology is typically consumer-focused, e.g., components of consumer products such as detergents, food products, and small-molecule chemical feedstocks. On the other hand, medical biotechnology develops molecules for human (and animal) health applications, e.g., antibodies, therapeutic proteins, vaccines, etc.

Industrial Biotechnology

Industrial enzymes: increasing at 6.3% annually to $8.5 billion in 2020

Medical Biotechnology

Protein therapeutics: increasing at 6.1% annually to $186 billion in 2020

Market size (2020): $195 billon

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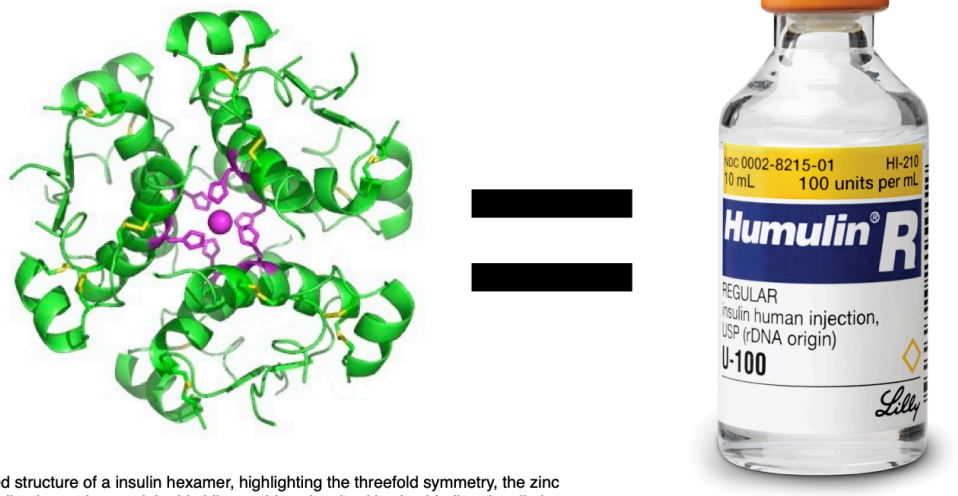
2.1 Monoclonal Antibodies (mAbs) and therapeutic proteins

Monoclonal antibodies (mAbs) are essential molecules for human health, e.g., cancer treatments such as Herceptin or everyday laboratory uses such as affinity reagents used Western blotting. In addition to mAbs, there are many therapeutic proteins, e.g., clotting factors or recombinant human insulin Humulin R products in the `biologics` space.

- Walsh G. Biopharmaceutical benchmarks 2018. Nat Biotechnol. 2018 Dec 6;36(12):1136-1145. doi: 10.1038/nbt.4305. PMID: 30520869

2.2 Origin story: The first biologic Humulin-R

Herbert Boyer et al. developed a portable expression system (circular DNA construct called a plasmid) to express the human insulin protein in *Escherichia coli* (Humulin R). He formed a company called Genentech and licensed the technology to Eli Lilly & Company in 1982. The rest is biotech history.



Estimated structure of a insulin hexamer, highlighting the threefold symmetry, the zinc ions holding it together, and the histidine residues involved in zinc binding. Insulin is stored in the body as a hexamer, while the active form is the monomer.

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ository and use a relative path: `PlutoUI.LocalResource("../ima
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Insulin references:

- Cohen SN, Chang AC, Boyer HW, Helling RB. Construction of biologically functional bacterial plasmids in vitro. Proc Natl Acad Sci U S A. 1973 Nov;70(11):3240-4. doi: 10.1073/pnas.70.11.3240. PMID: 4594039; PMCID: PMC427208.
- Riggs AD. Making, Cloning, and the Expression of Human Insulin Genes in Bacteria: The Path to Humulin. Endocr Rev. 2021;42(3):374-380. doi:10.1210/endrev/bnaa029
- Hirsch IB, Juneja R, Beals JM, Antalis CJ, Wright EE. The Evolution of Insulin and How it Informs Therapy and Treatment Choices. Endocr Rev. 2020;41(5):733-755. doi:10.1210/endrev/bnaa015

Insulin Litigation (since science isn't always *just* science):

- Fox JL. Insulin patent dispute revisits old biotechnology battleground. Nat Biotechnol. 1997 Apr;15(4):307. doi: 10.1038/nbt0497-307. PMID: 9094114.

3. What makes biology so cool? Choices. Many possible choices.

I became interested in biology, metabolism, models, etc because, unlike traditional chemical systems, biological systems are controlled. A cell can sense the world around them, take stock of their internal state and make choices, in essence, they can reprogram themselves to meet a changing world. There are fast choices that operate on a milli- or μ-second time scale (regulation of enzyme activity, called allosteric regulation) and slow choices (gene expression) which operate on a time scale of tens of minutes.

3.1 The origin story of omics. Yeast reprogramming

The promise of `omics` technologies was that we could measure everything, all at the same time, inside a population of cells. Promise and reality turned out to be a little different. The next wave of omics technology (occurring now) is that we can measure everything inside a single cell. But we have the same basic problem as before: what can we do with data that is noisy, and often has no direct physical interpretation e.g., has no units or is scaled, etc. From a traditional modeling perspective: not much. But still: it's cool.

- DeRisi JL, Iyer VR, Brown PO. Exploring the metabolic and genetic control of gene expression on a genomic scale. Science. 1997 Oct 24;278(5338):680-6. doi: 10.1126/science.278.5338.680. PMID: 9381177.

Yeast cells make a choice, by how and why? Fig. 3 Reproduced from DeRisi et al, Science 278, 1997.

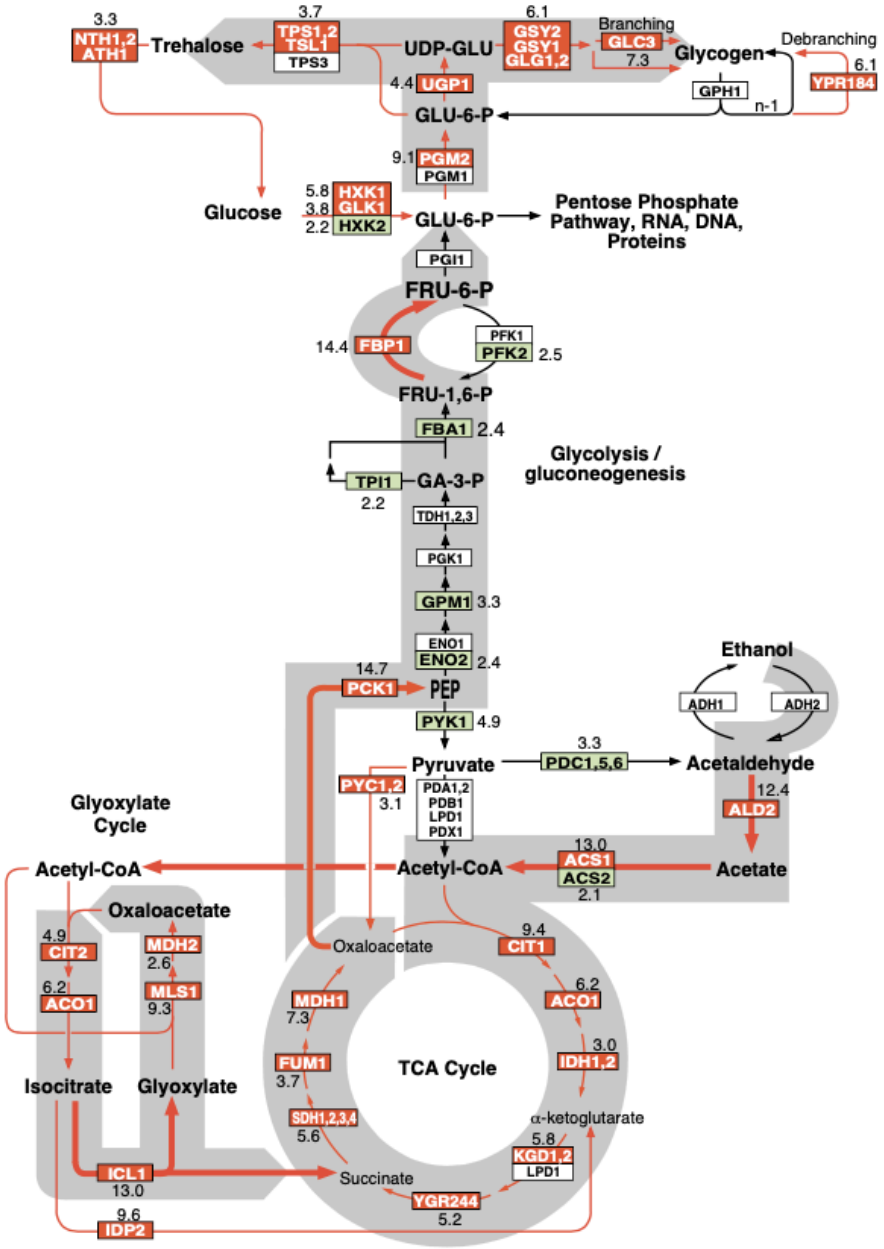

Fig. 3. Metabolic reprogramming inferred from global analysis of changes in gene expression. Only key metabolic intermediates are identified. The yeast genes encoding the enzymes that catalyze each step in this metabolic circuit are identified by name in the boxes. The genes encoding succinyl-CoA synthase and glycogen-debranching enzyme have not been explicitly identified, but the ORFs YGR244 and YPR184 show significant homology to known succinyl-CoA synthase and glycogen-debranching enzymes, respectively, and are therefore included in the corresponding steps in this figure. Red boxes with white lettering identify genes whose expression increases in the diauxic shift. Green boxes with dark green lettering identify genes whose expression diminishes in the diauxic shift. The magnitude of induction or repression is indicated for these genes. For multimeric enzyme complexes, such as succinate dehydrogenase, the indicated fold-induction represents an unweighted average of all the genes listed in the box. Black and white boxes indicate no significant differential expression (less than twofold). The direction of the arrows connecting reversible enzymatic steps indicate the direction of the flow of metabolic intermediates, inferred from the gene expression pattern, after the diauxic shift. Arrows representing steps catalyzed by genes whose expression was strongly induced are highlighted in red. The broad gray arrows represent major increases in the flow of metabolites after the diauxic shift, inferred from the indicated changes in gene expression.

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4. Conclusions

In this lecture we:

- Introduced Metabolic Engineering: using models and engineering principles to design the production of metabolic products
- Discussed two classes of biotechnology: industrial and medical biotechnology. Industrial biotechnology is primarily focused on consumer products, while medical biotechnology concentrates on human health products.
- Discussed modes of biological regulation (one of the reasons this problem is complicated). Regulation can occur on a fast scale (regulation of enzyme activity) and a slow scale (regulation of gene expression).

5. Next time

- We'll build our first mathematical model of coupled enzyme-catalyzed reactions and take stock of what we know and what we don't know.
- We'll introduce a life-changing way of thinking: Constraints based analysis

📚 Table of Contents


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    using PlutoUI

    # setup paths -
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    const _PATH_TO_FIGS = joinpath(_PATH_TO_NOTEBOOK,"figs")
    const _PATH_TO_SRC = joinpath(_PATH_TO_NOTEBOOK,"src")

    # return -
    nothing
end
```