Transforming Cellular Factories with Synthetic Biology

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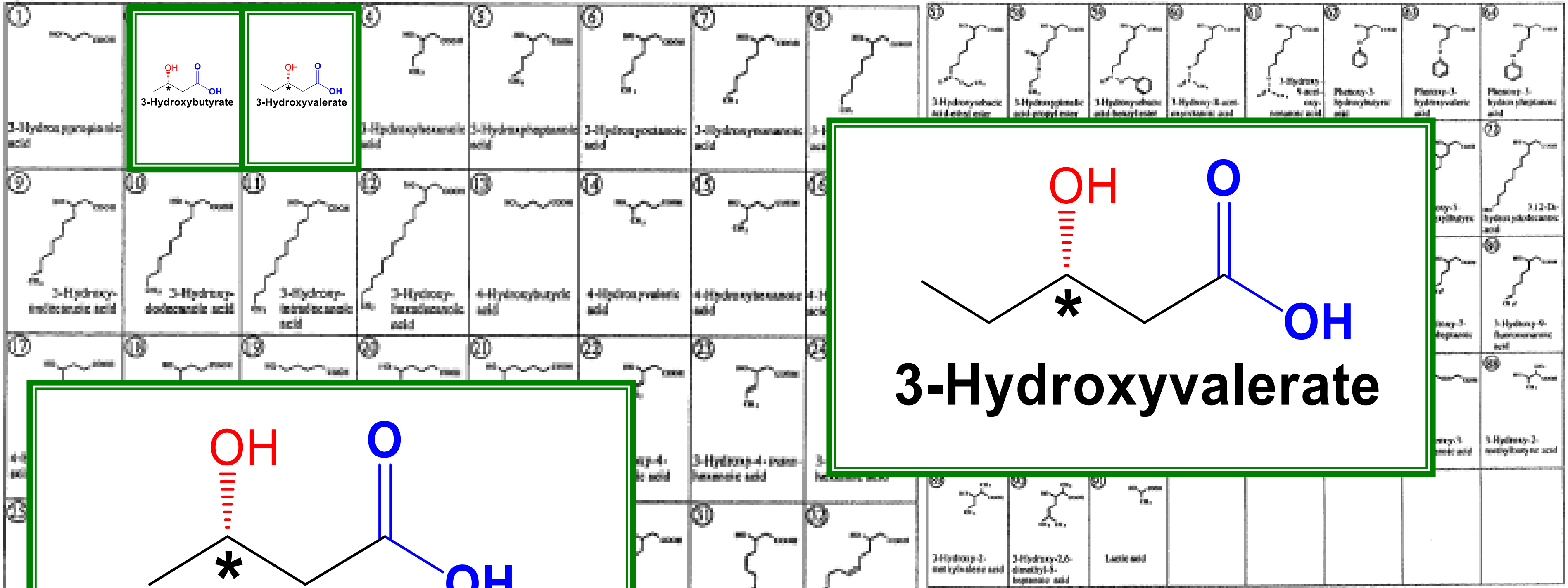
Let's start with a different kind of factory…

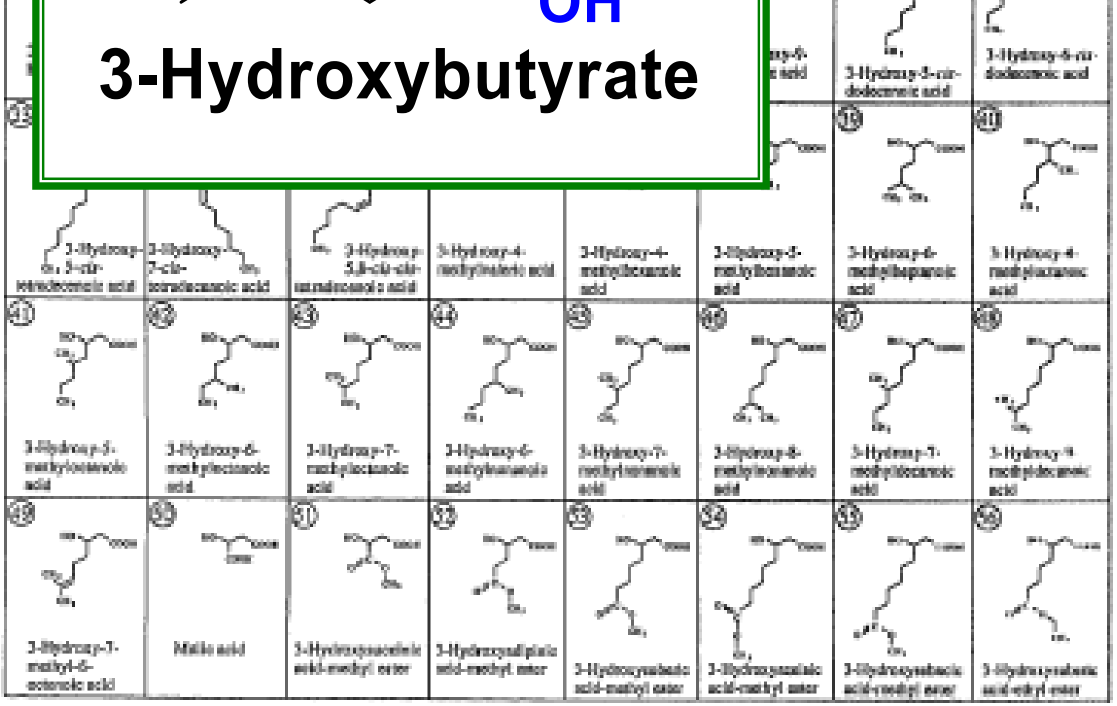
Intro to Chemical Engineering

Which *feedstocks* are viable compared to resources (including wastes!) that are available?

What level of precision, flexibility, robustness can be achieved in the transformative steps?

What *can* we produce compared to what we need to produce?

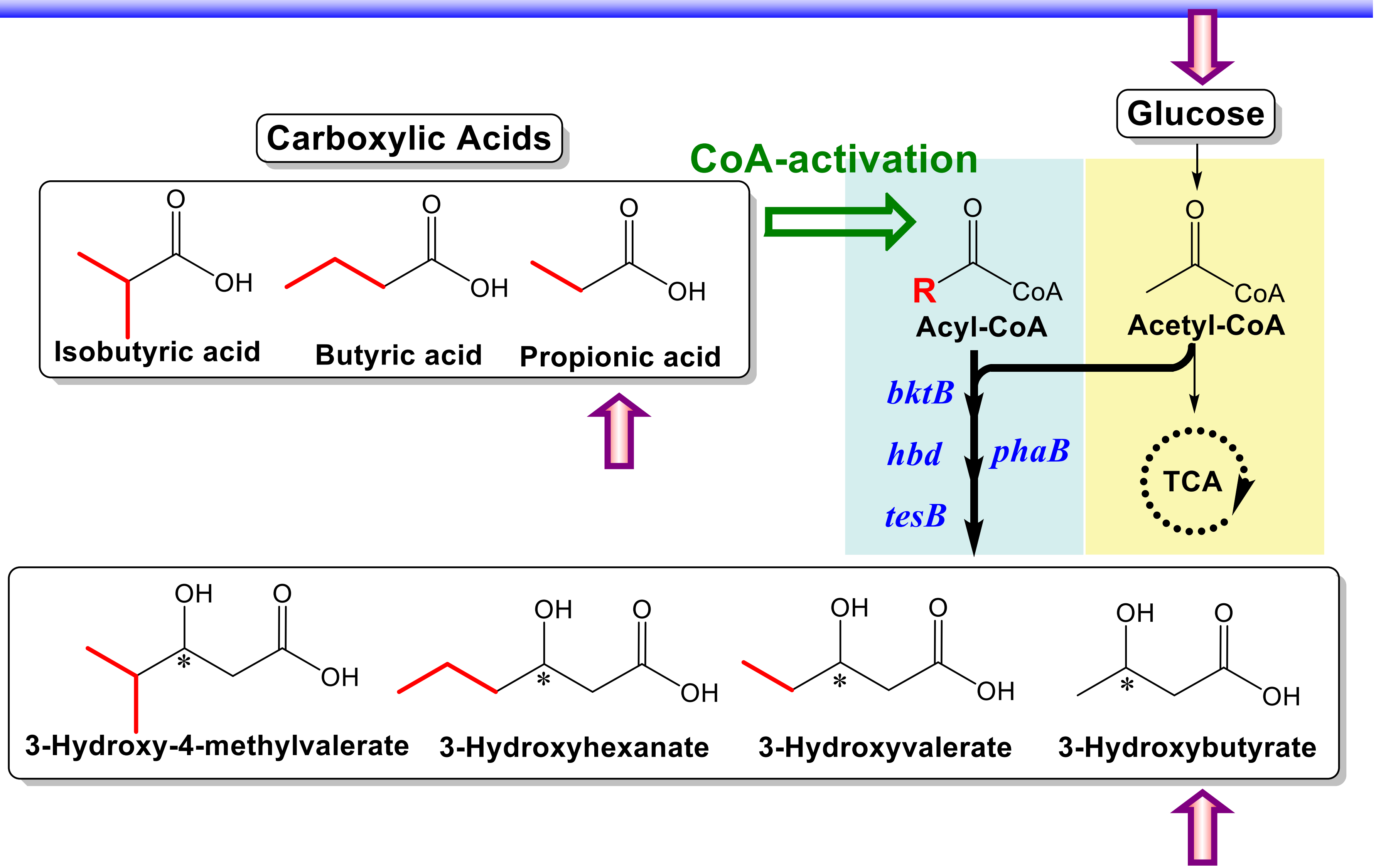




- Many hydroxyacids have been made as constituents of intracellular PHAs (biopolymers).
- Very few have been produced economically as free monomers.

Steinbüchel *et al*, FEMS Microbiol. Lett., 1995, 128:219-228.

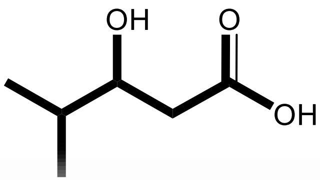
Extending the Product Spectrum



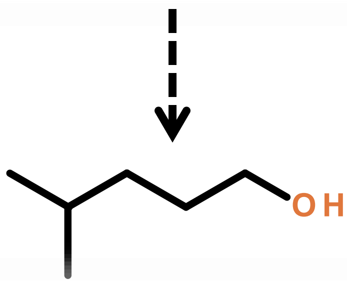
~300 mg/L **4Me-(*R*)-3HV** ~[illegible] mg/L **(*R*)-3HH** ~2 g/L **(*R*)-3HV** ~3 g/L **(*R*)-3HB**

~2 g/L **(*S*)-3HV** ~2 g/L **(*S*)-3HB**

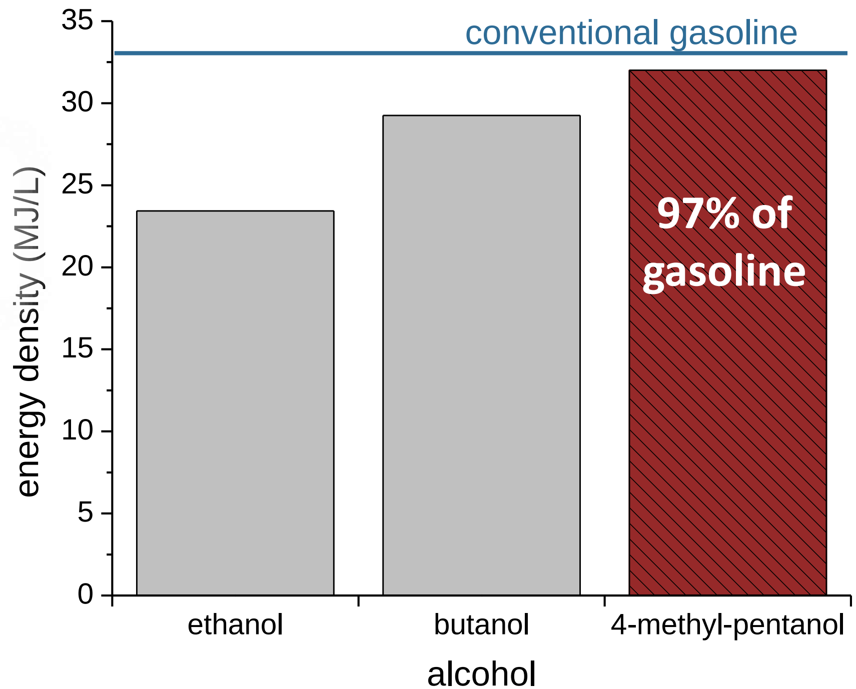
Accessing new molecules through Biology: 4-Methylpentanol



3-hydroxy-4-methyl-valerate (3H4MV)



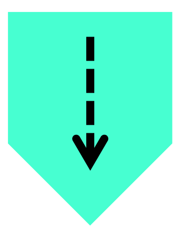
4-methyl-pentanol (4MP)



Mimicking n-butanol (CoA-dependent) biosynthesis

Theoretical maximum energy yield of 94.6%

glucose

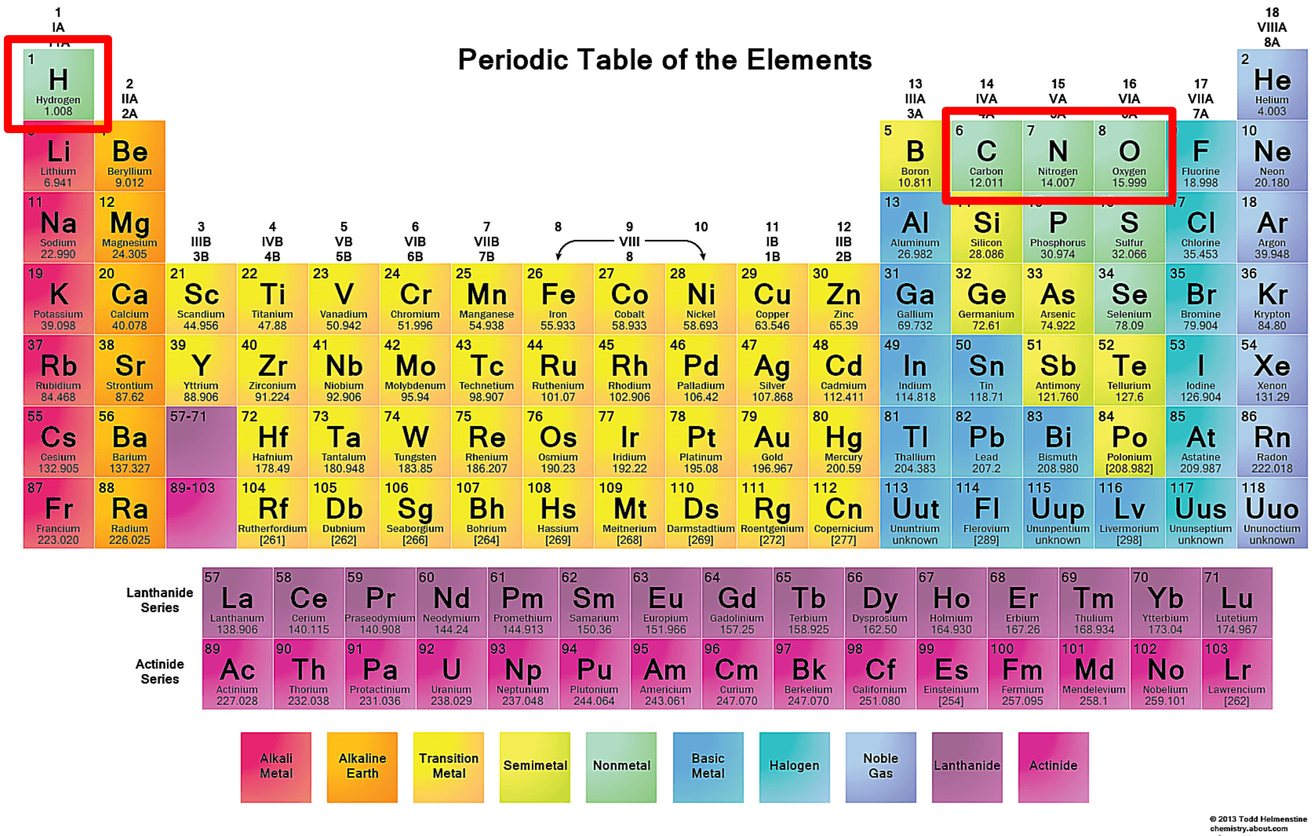


4MP

- Final pathway employed **10 genes** from **8 different organisms**
- Max **selectivity of ~80%** for 4MP alcohol

Sheppard, et al, 2014. *Nat. Commun*. 5:5031.

The Need for New Products

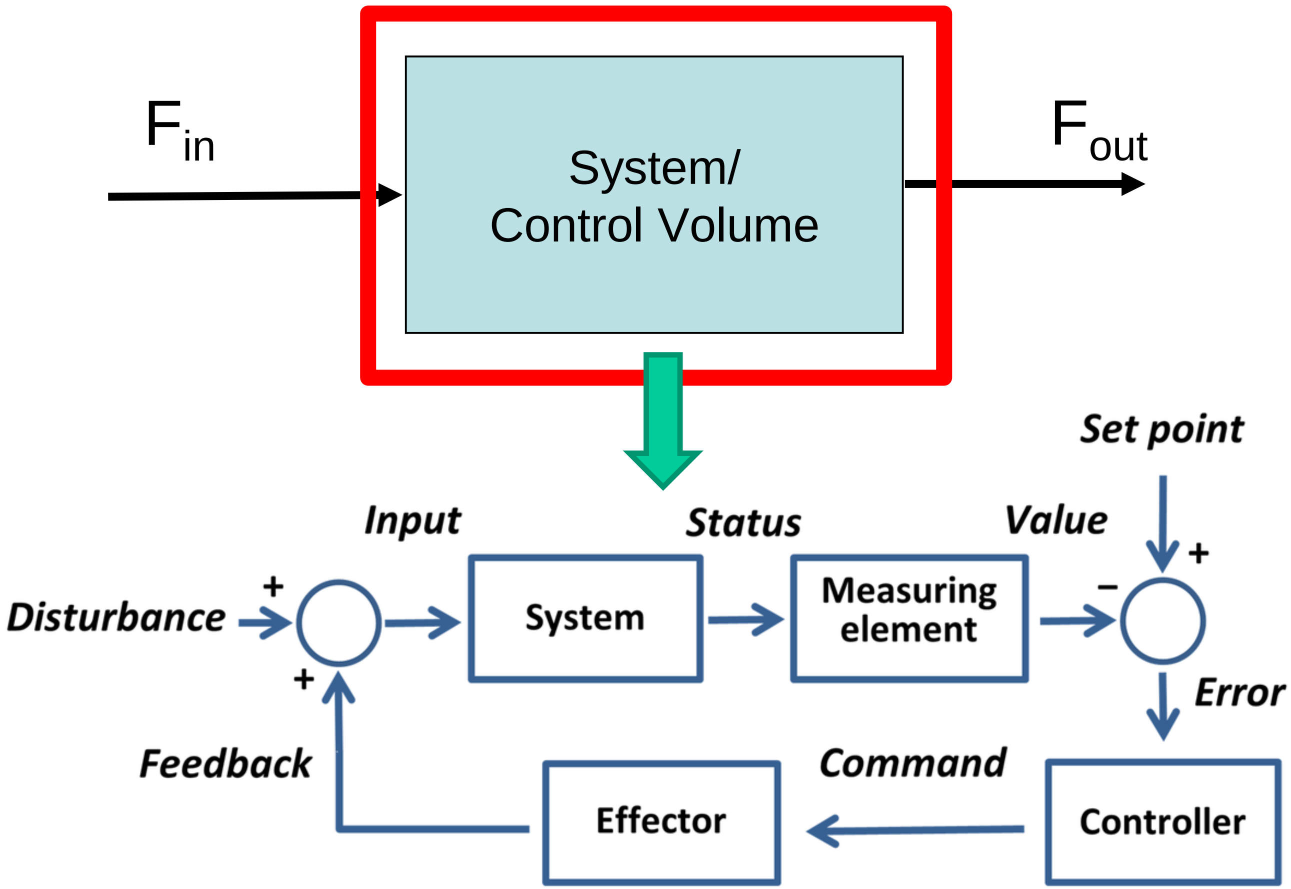


- Increase diversity wrt atomic composition, molecular weight (e.g., materials), structural complexity….
- *Are there unique opportunities to build hybrid production systems?*

Back to the generic System…

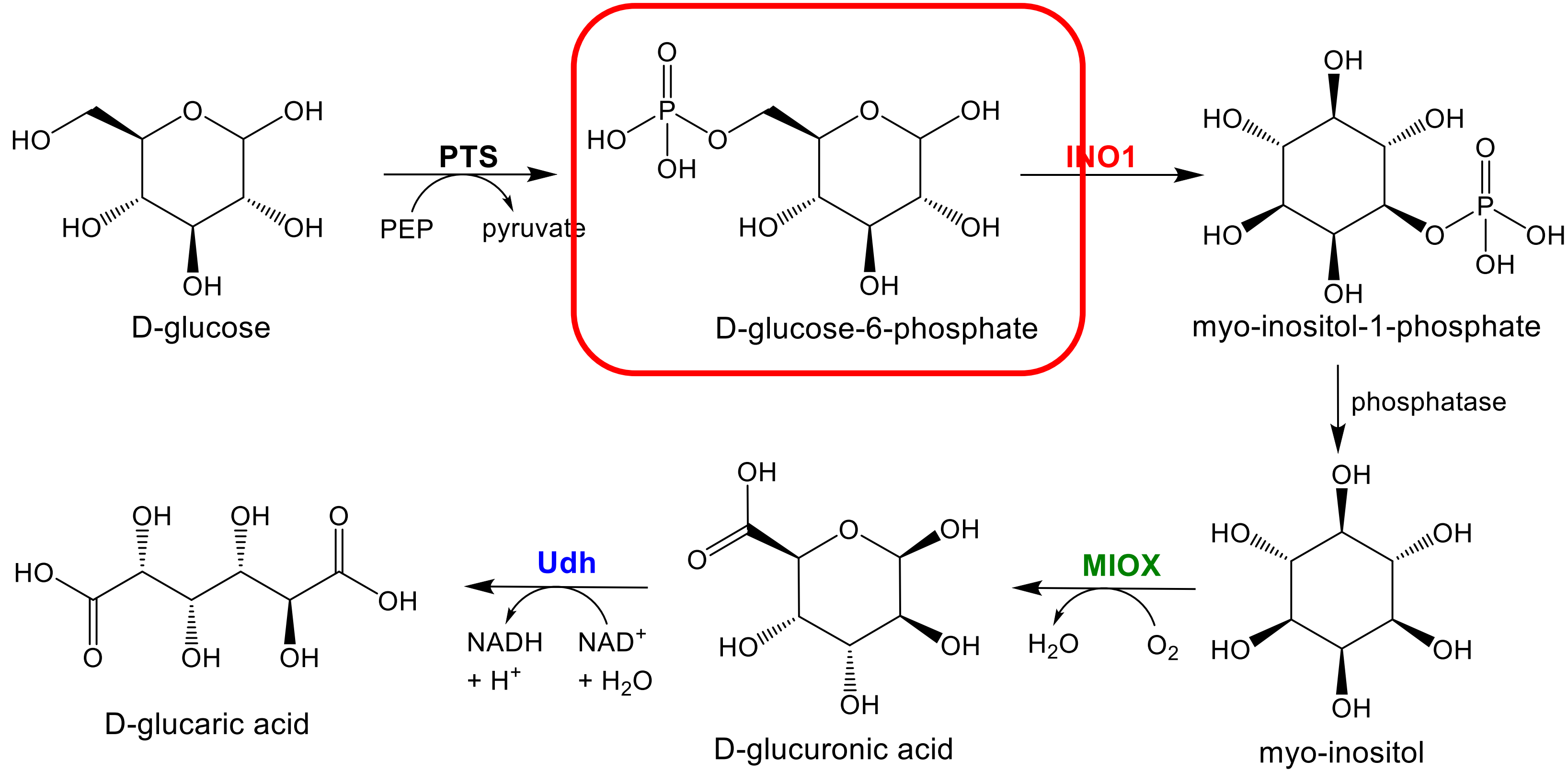
Considering Feedstocks

Integrating Process Control into Cellular Factories



- How can we most effectively design and integrate "process control" circuits into cellular factories?

Glucaric Acid Production in *E. coli*



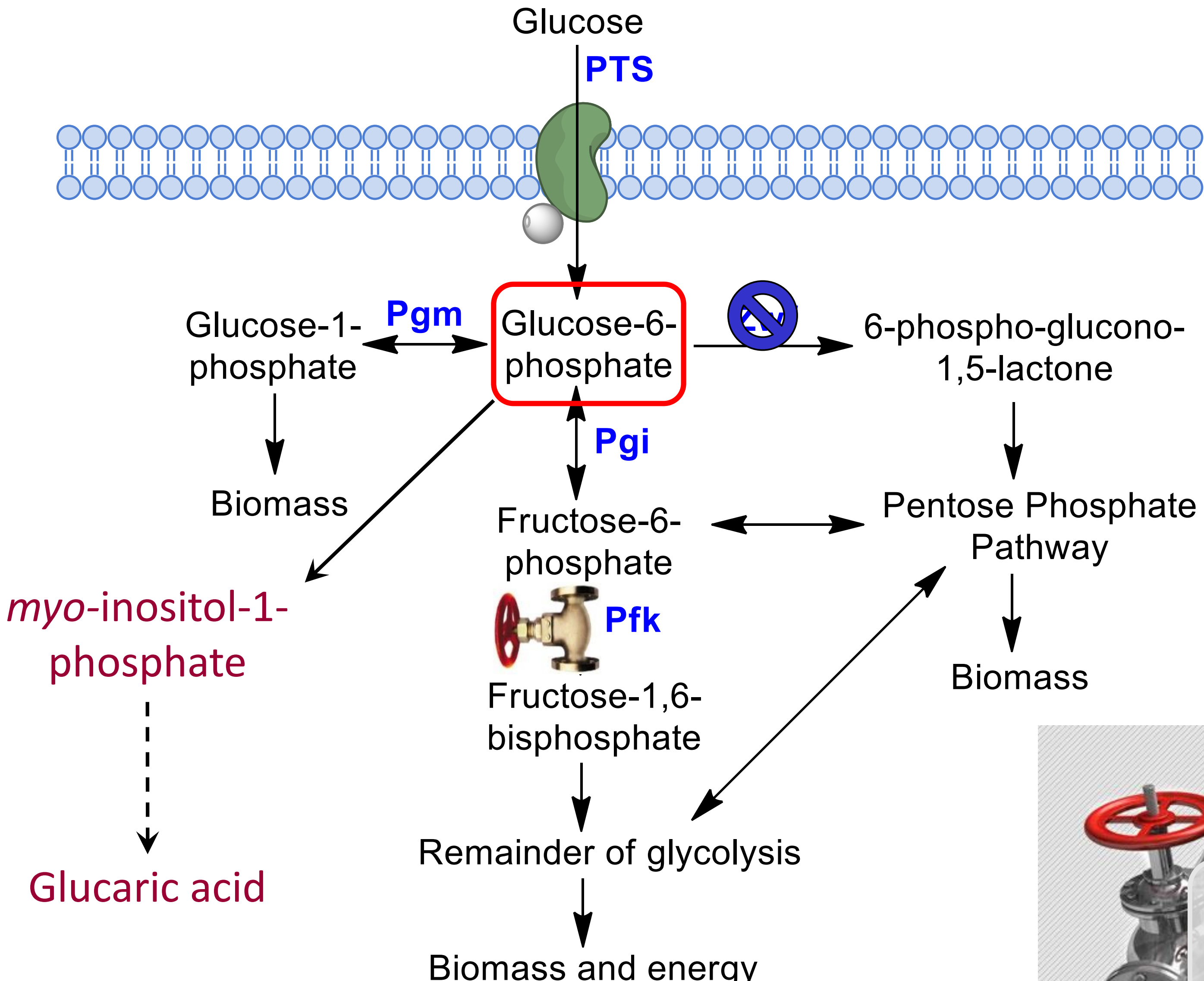
- US DOE "top value-added" chemical
- Used in pharmaceuticals, materials, deicers, detergents…..

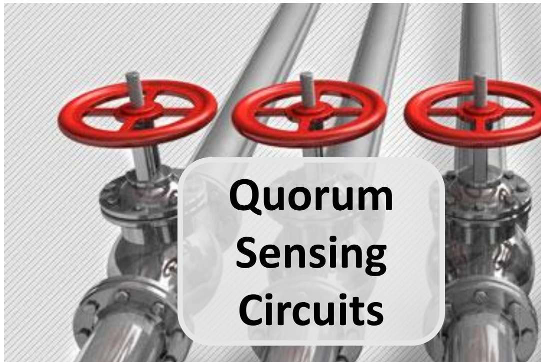
INO1 *S. cerevisiae* (yeast)
MIOX *M. musculus* (mouse)
Udh *P. syringae* (bacterium)

Moon, T. S., *et. al. Appl Environ Microbiol.* (2009)

Yoon, S.-H., *et al*. *J. Bacteriol*. (2009)

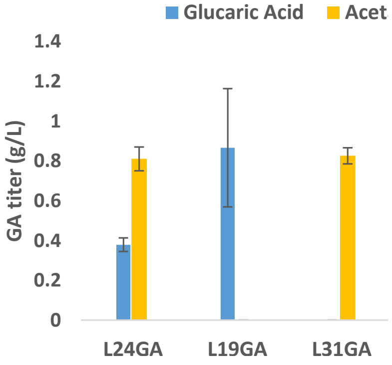
Manipulating Glucose Metabolism

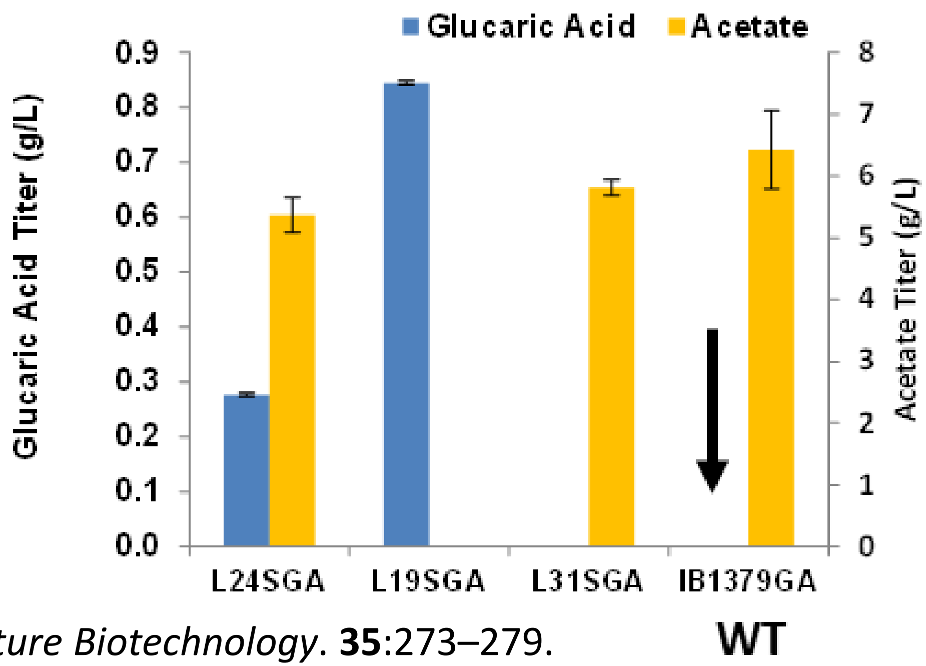




Metabolite Valves Across Scales

m2p-labs BioLector **250-ml shake flasks** **Infors 3-L benchtop reactor**

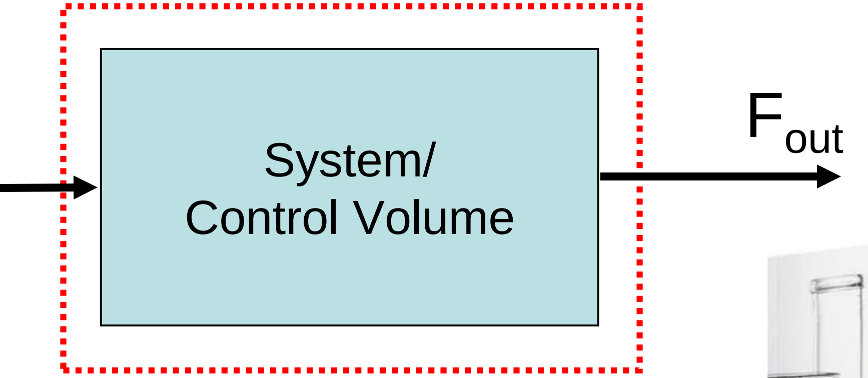


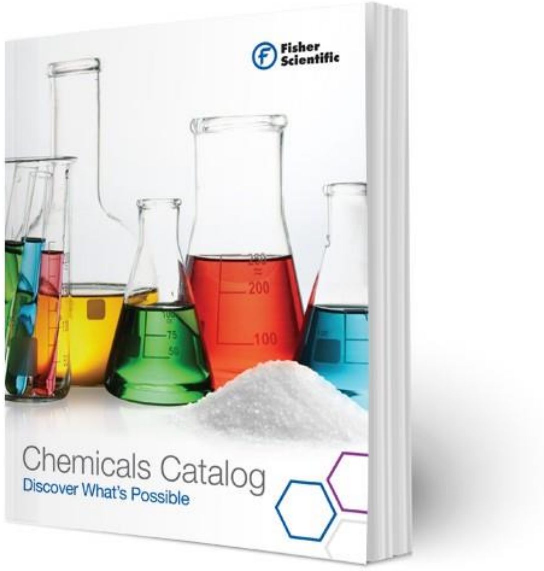


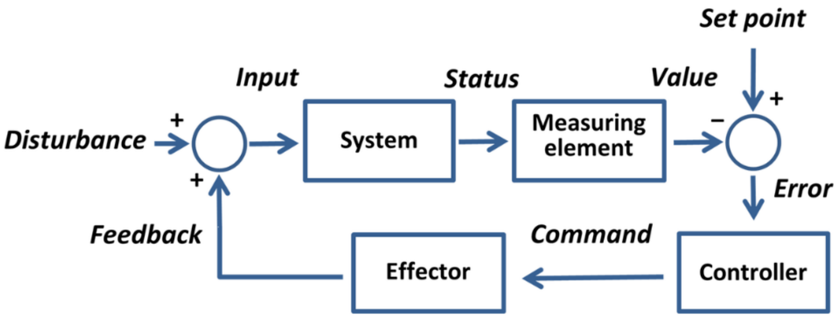
Glucaric acid ir … sistent across scales

Gupta *et al*. 2017. *Nature Biotechnology*. **35**:273–279.

Transforming Cellular Factories

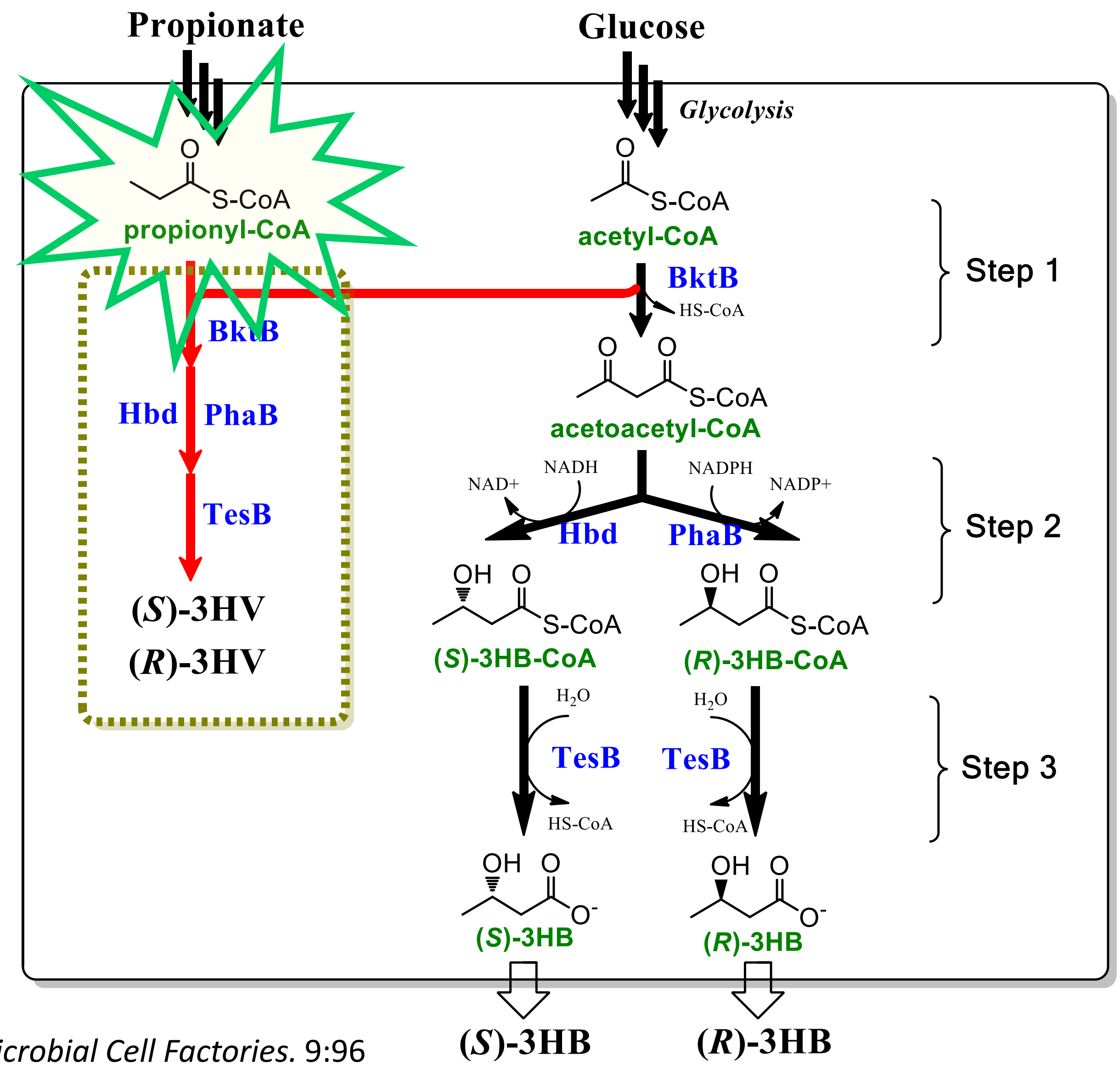






A Pathway for 3HB/3HV Biosynthesis

E. coli

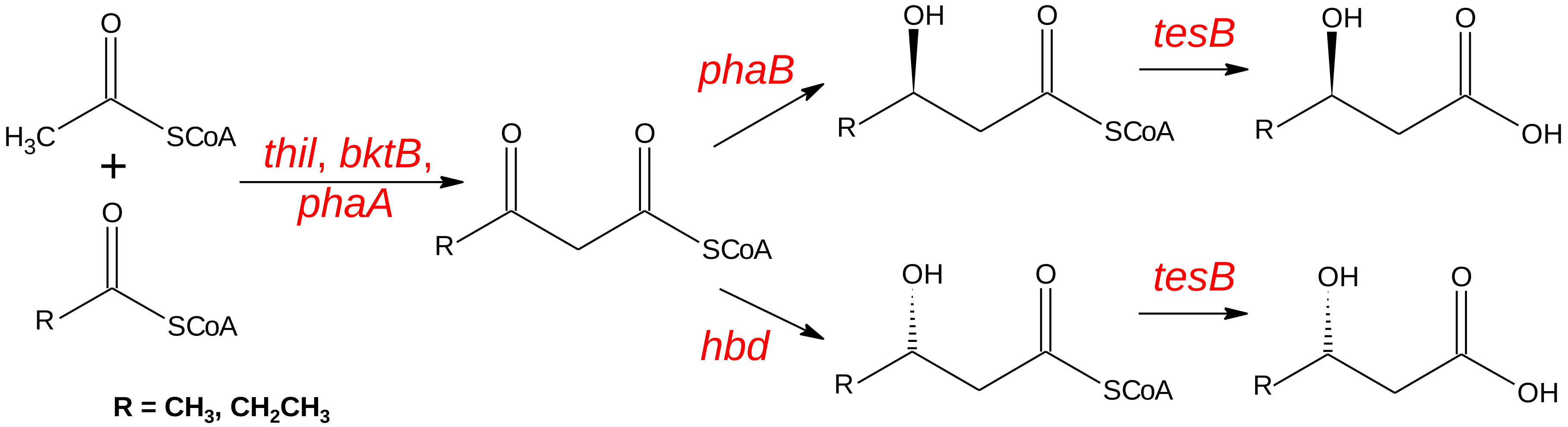


Tseng et al, 2010, *Microbial Cell Factories.* 9:96

Tseng et al, 2009. *Appl. Environ. Microbiol.* 75(10):3137-3145. **>2 g/L *R*- or *S*- 3HB**

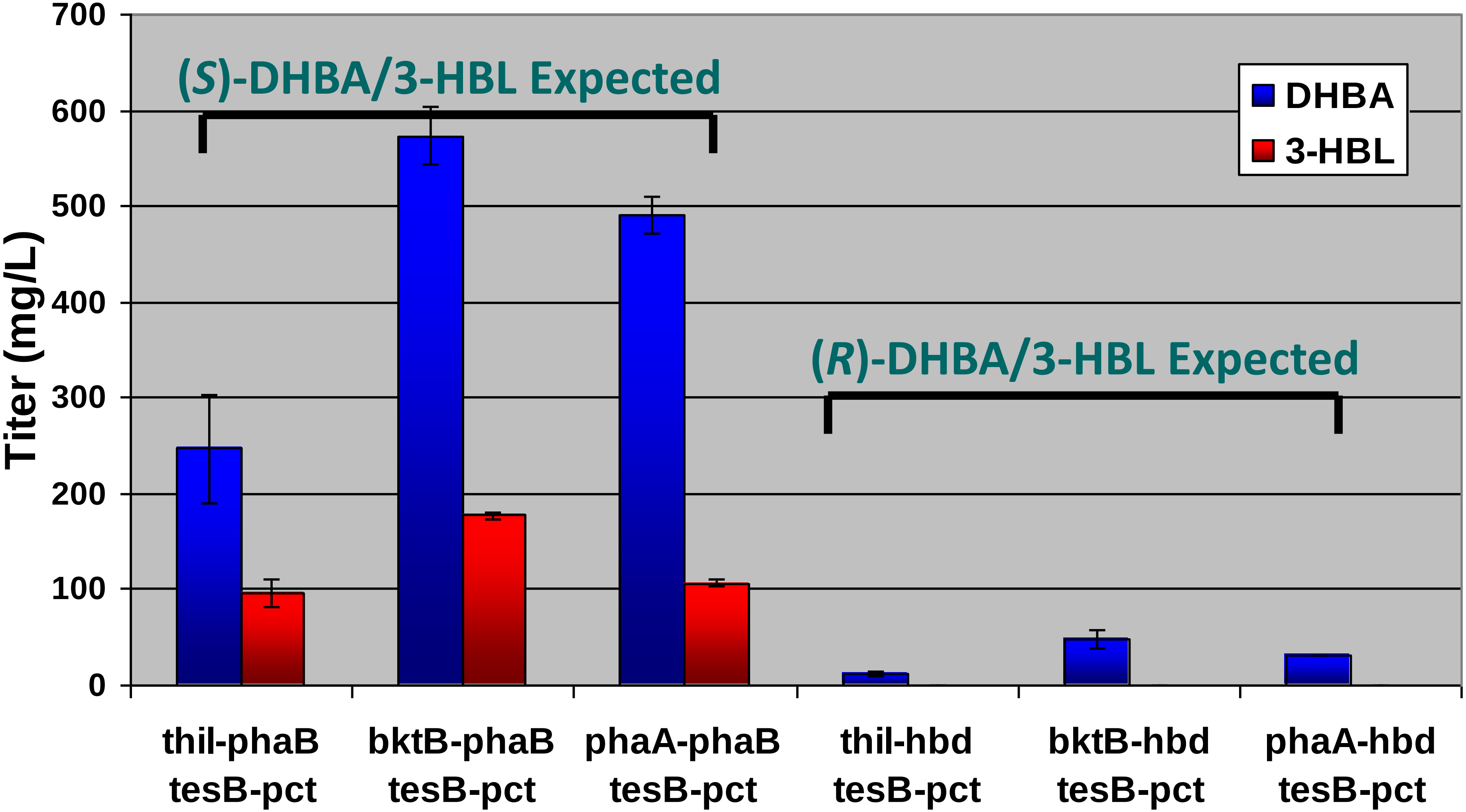
From 3HB/3HV to DHBA?

3-hydroxybutyrate & 3-hydroxyvalerate biosynthetic pathway



Production of 3,4-Dihydroxybutyrate (DHBA)

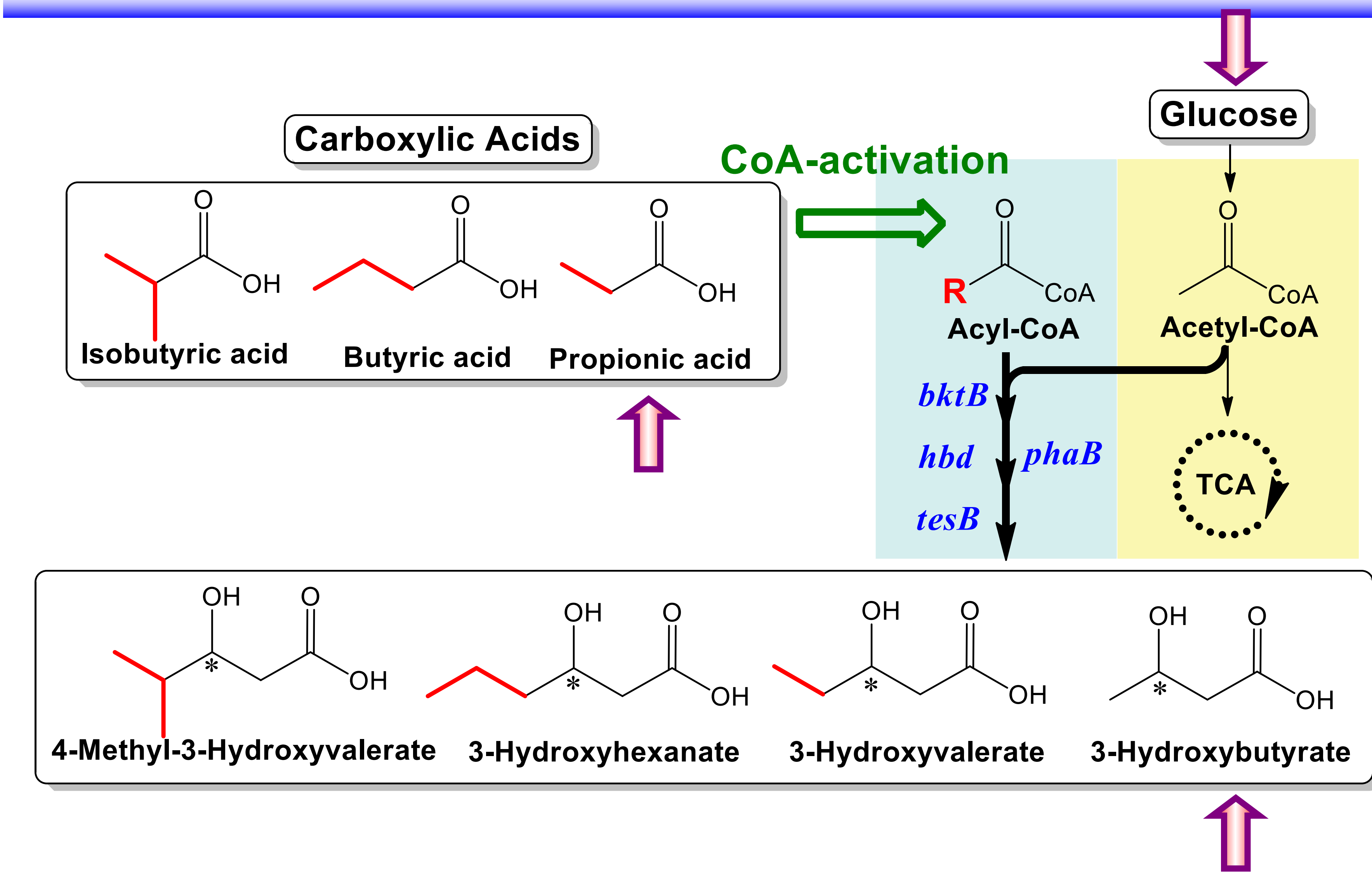
Glucose + Glycolate



All cultures were *E. coli* MG1655(DE3) *end*A$^-$ recA$^-$ grown in LB for 72 hours and were supplied with glycolate.

Martin et al, 2013. *Nat. Commun.* 4:1414

Extending the Hydroxyacid Pathway



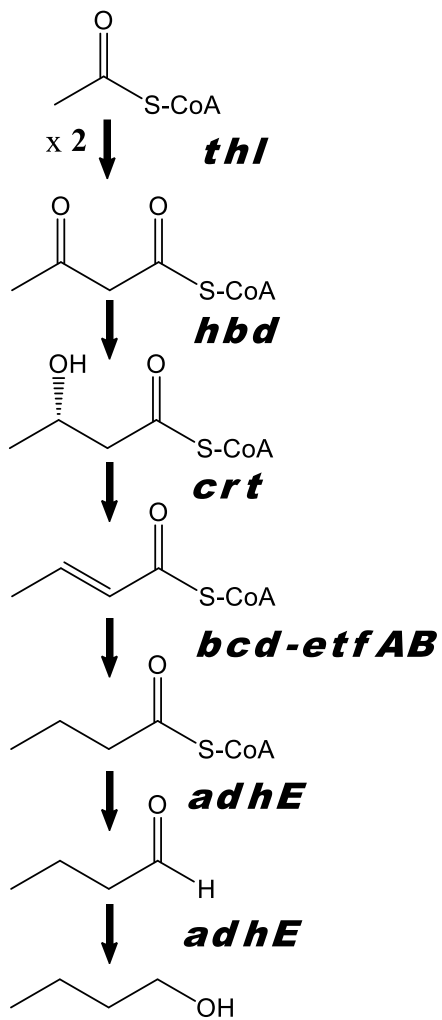
~200 mg/L **4Me-(*R*)-3HV** ~40 mg/L **(*R*)-3HH** ~2 g/L **(*R*)-3HV** ~3 g/L **(*R*)-3HB**

~2 g/L **(*S*)-3HV** ~2 g/L **(*S*)-3HB**

Martin et al, 2013. *Nat. Commun.* 4:1414

Synthesis of Products from Fuel Pathways

***Clostridial* ABE Pathway**

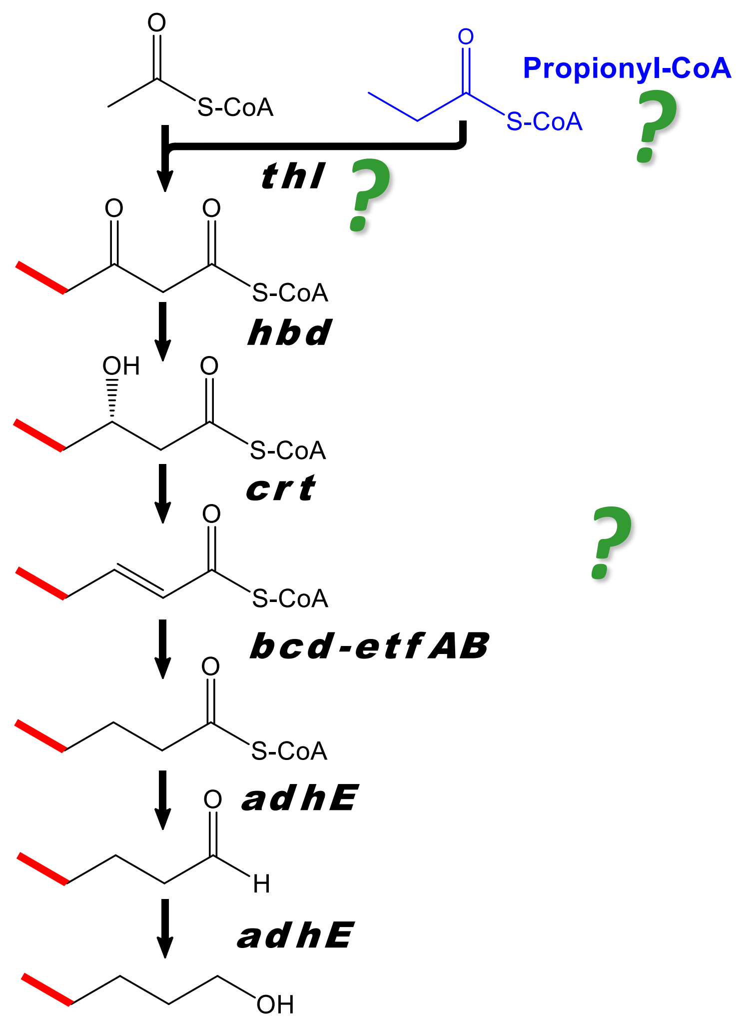


Synthesis of Novel Biofuels

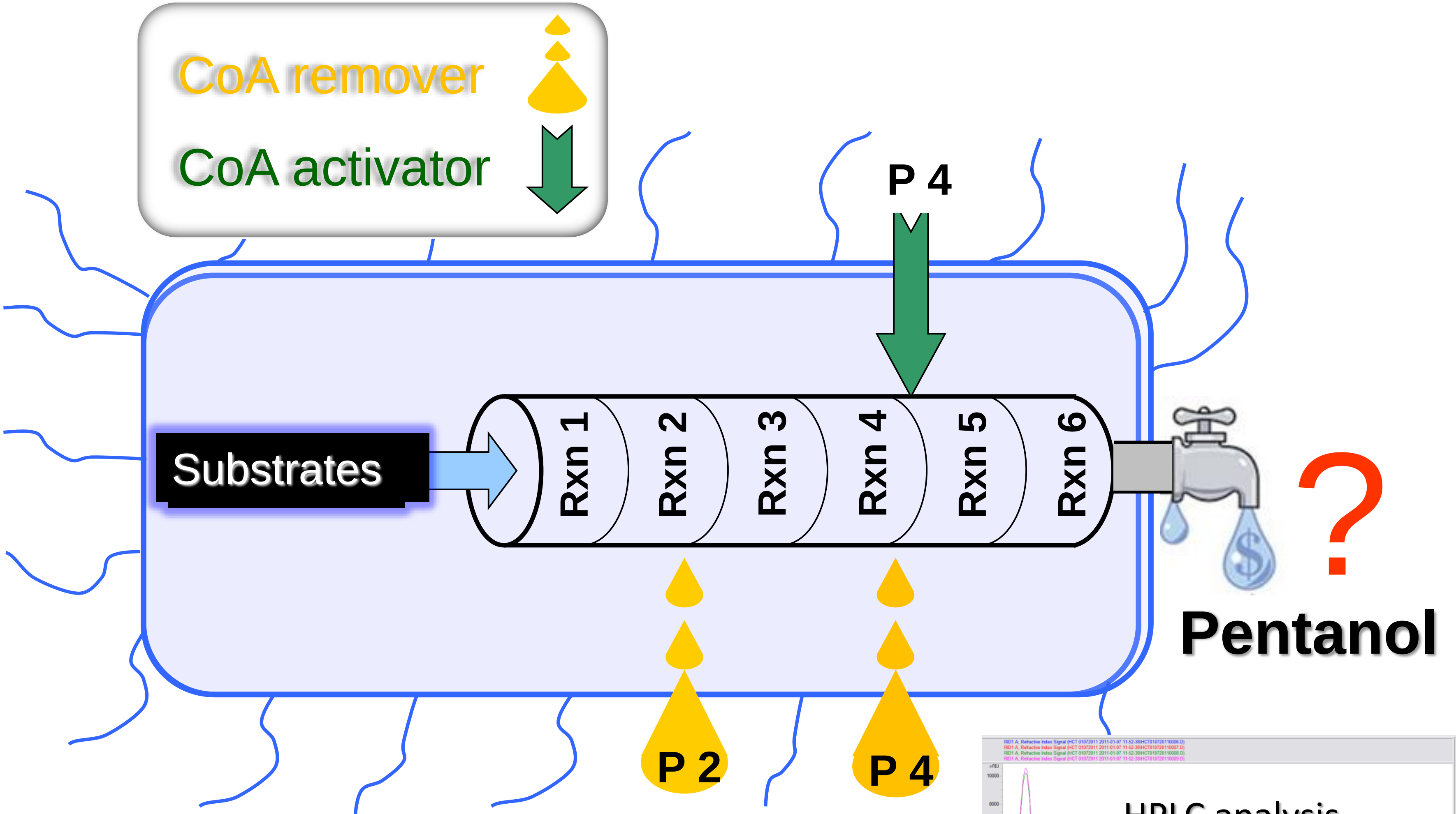
Key Questions:

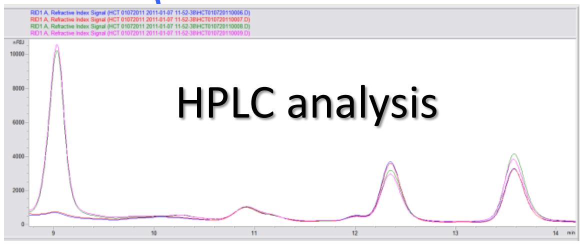
1. Supply of building block (propionyl-CoA)
2. Condensation reaction of C2 + C3
3. Acceptance of 5-carbon substrates for the remaining pathway enzymes

Proposed Pentanol Pathway



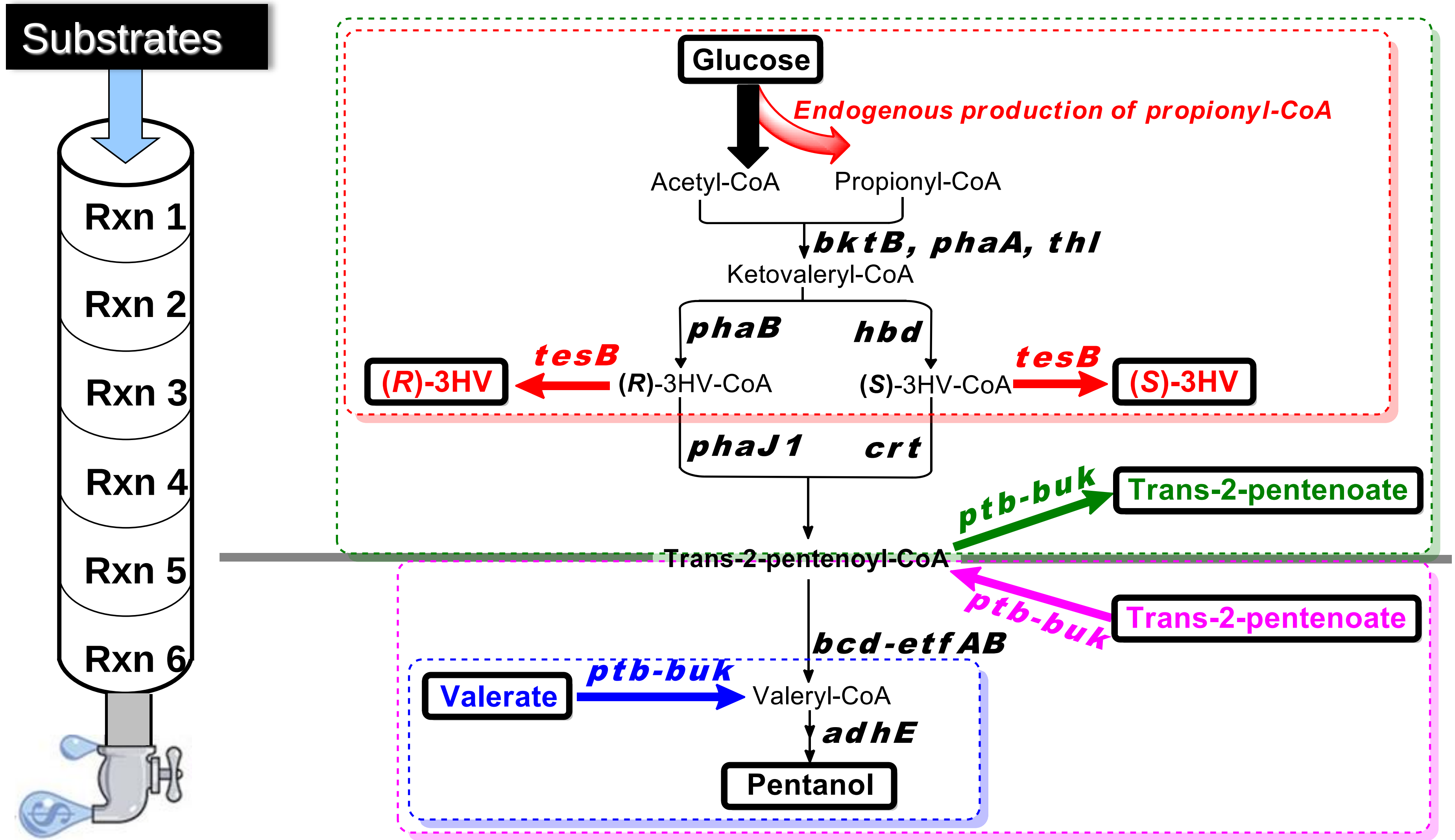
Bypass method for pathway modularization





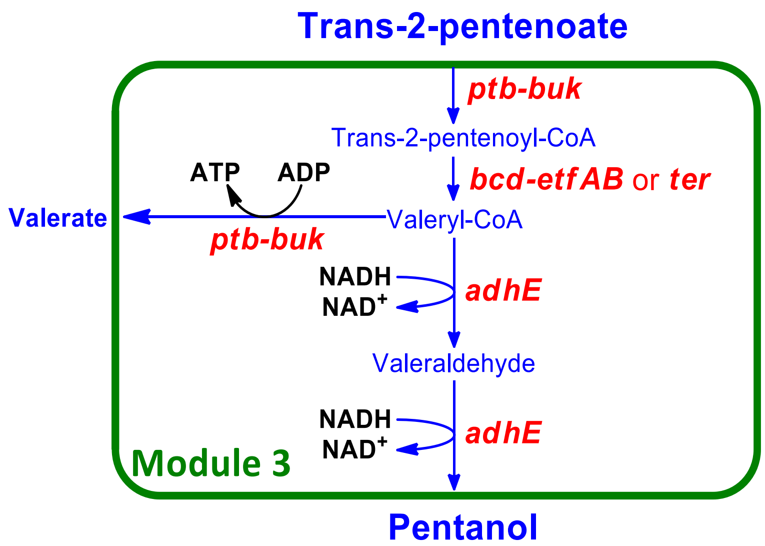
Tseng and Prather, 2012. *PNAS,* 109:17925-30.

Metabolic Pathway Design

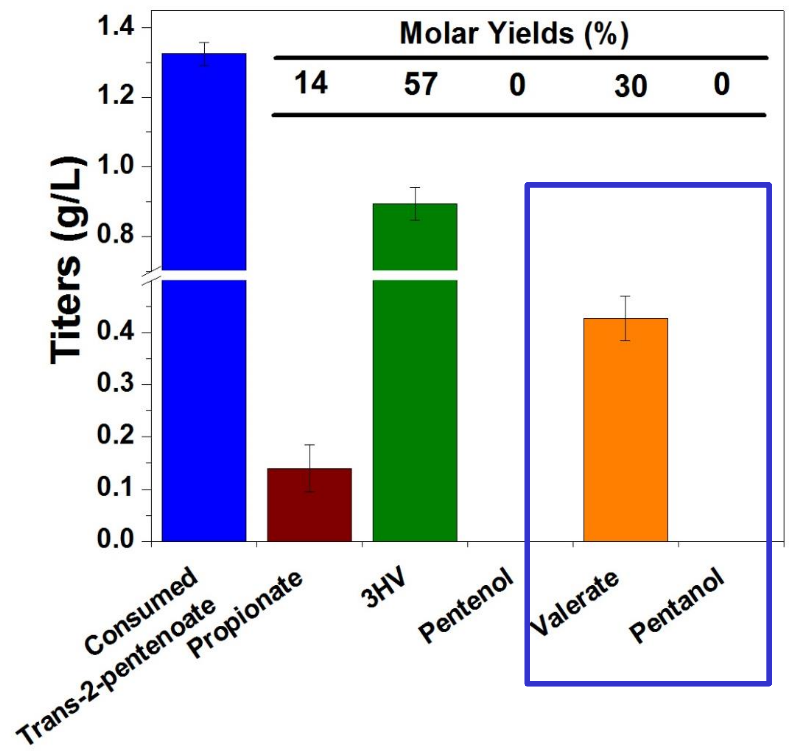


Tseng and Prather, 2012. *PNAS*, 109:17925-30.

Pentanol Synthesis from Trans-2-pentenoate



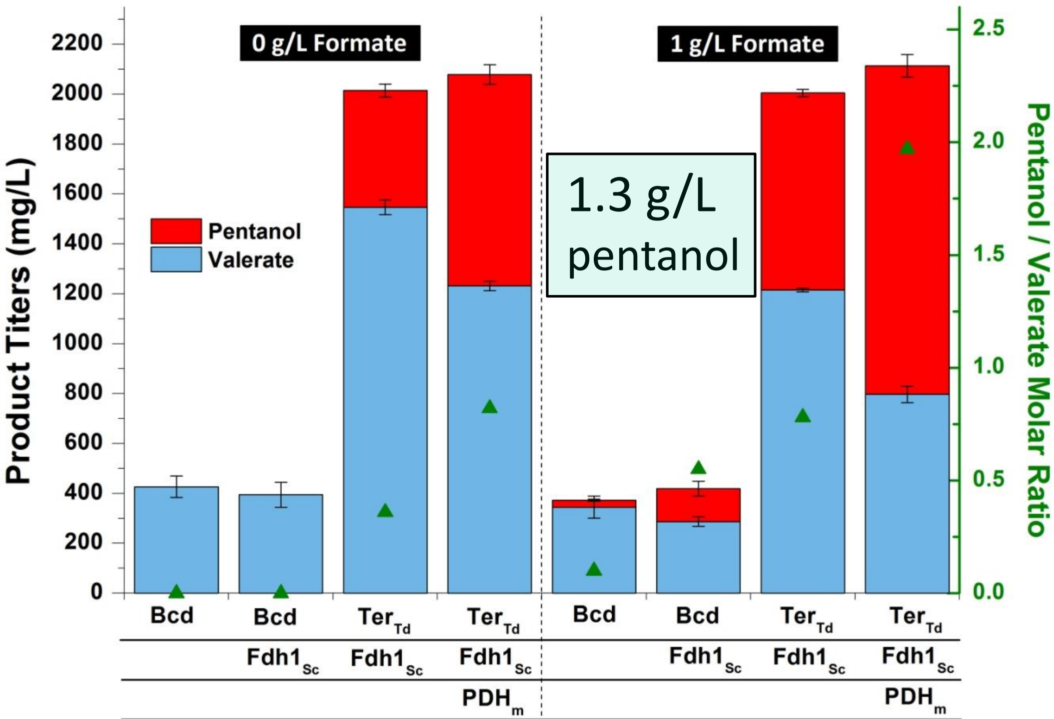
- Synthesis of 1 pentanol requires 2 NADH
- Synthesis of 1 valerate generates 1 ATP



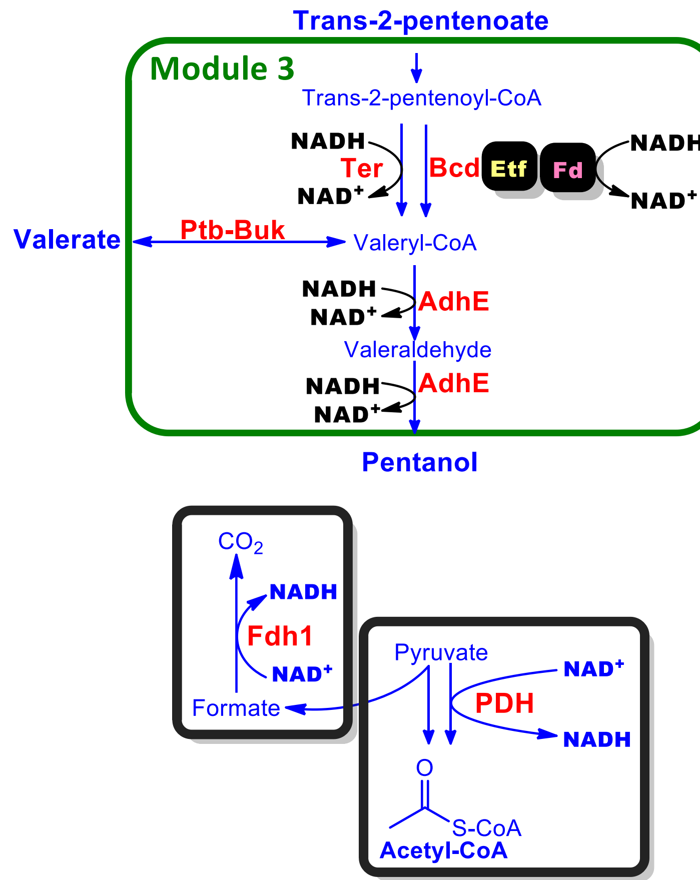
Hypothesis: Cofactor limitation prevents synthesis of desired end product, causing a "short-circuit"

Tseng and Prather, 2012. *PNAS*, 109:17925-30.

Pentanol Synthesis from Trans-2-pentenoate



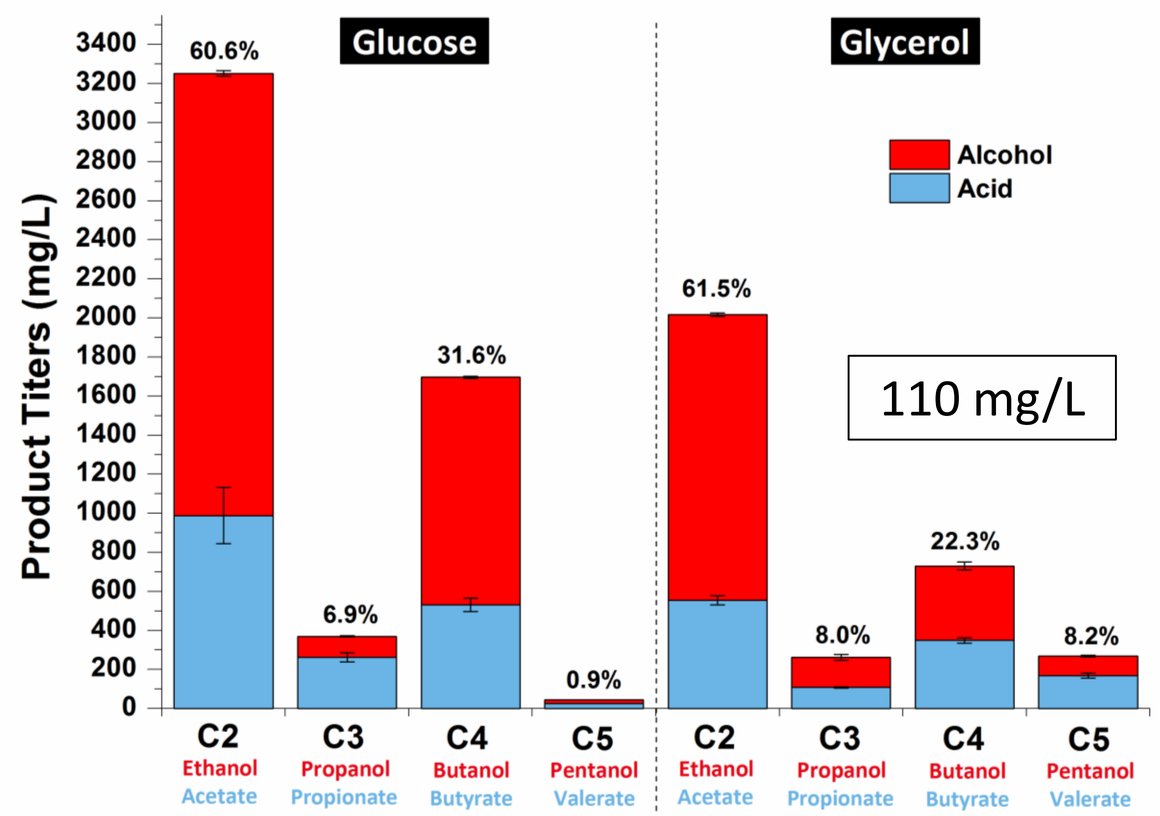
In addition to the indicated enzymes, Ptb-Buk and AdhE were also over-expressed

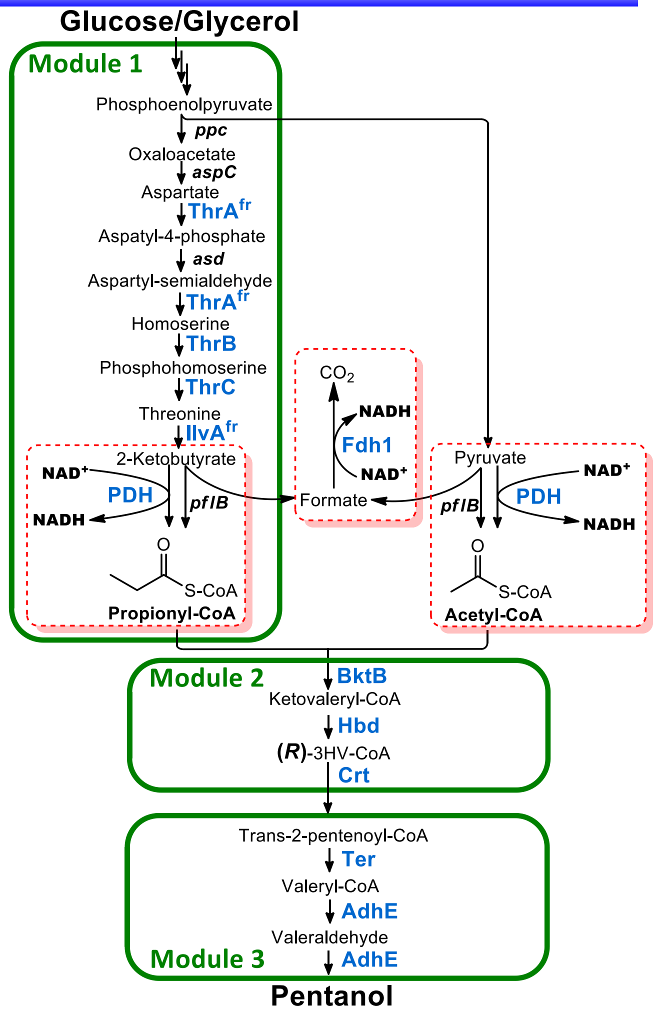


NADH limitation can be addressed by the use of Ter along with over-expression of Fdh1 and PDH$_m$

Tseng and Prather, 2012. *PNAS,* 109:17925-30.

Pentanol Synthesis via Module 1 + 2 + 3





<u>Ongoing Challenges</u>:

1. Increase alcohol to acid ratios
2. Reduce byproduct formation (C2, C3, and C4)

Changing the paradigm* – from "CH_2" to "CH_2O"

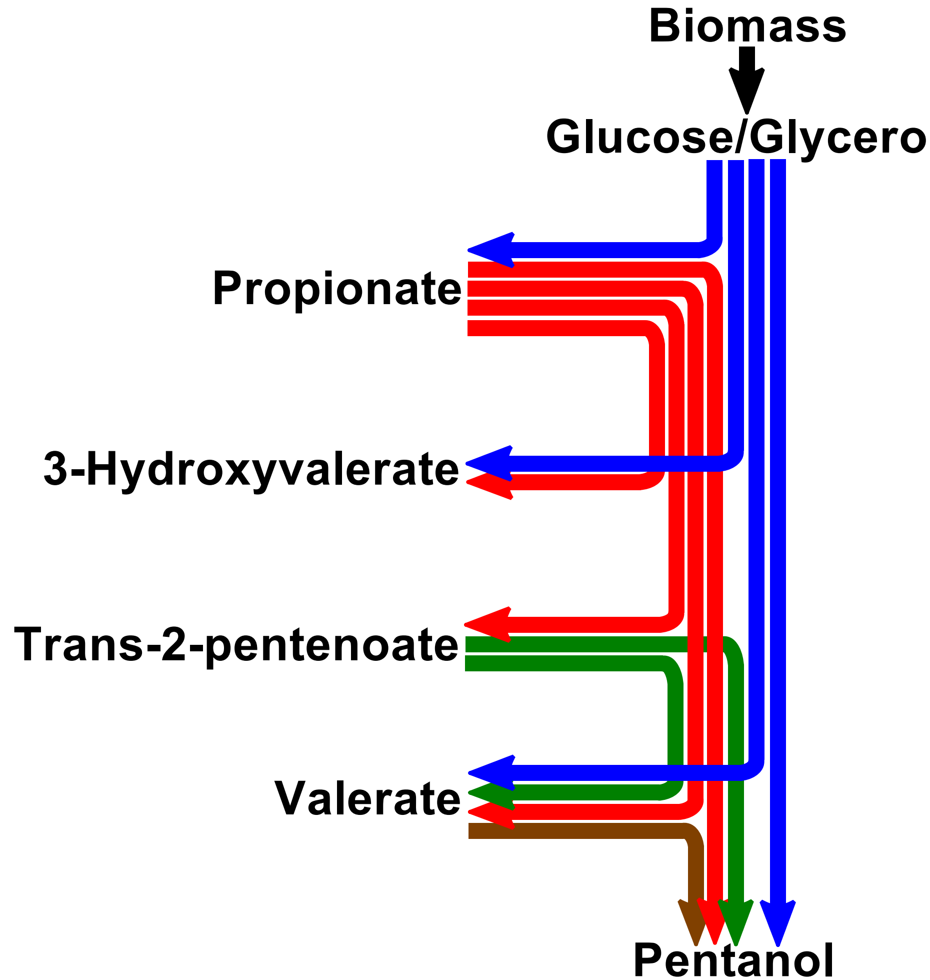
Biomass

Bio- Refinery

Bio-fuels

Ethanol, Butanol
branched chain alcohols

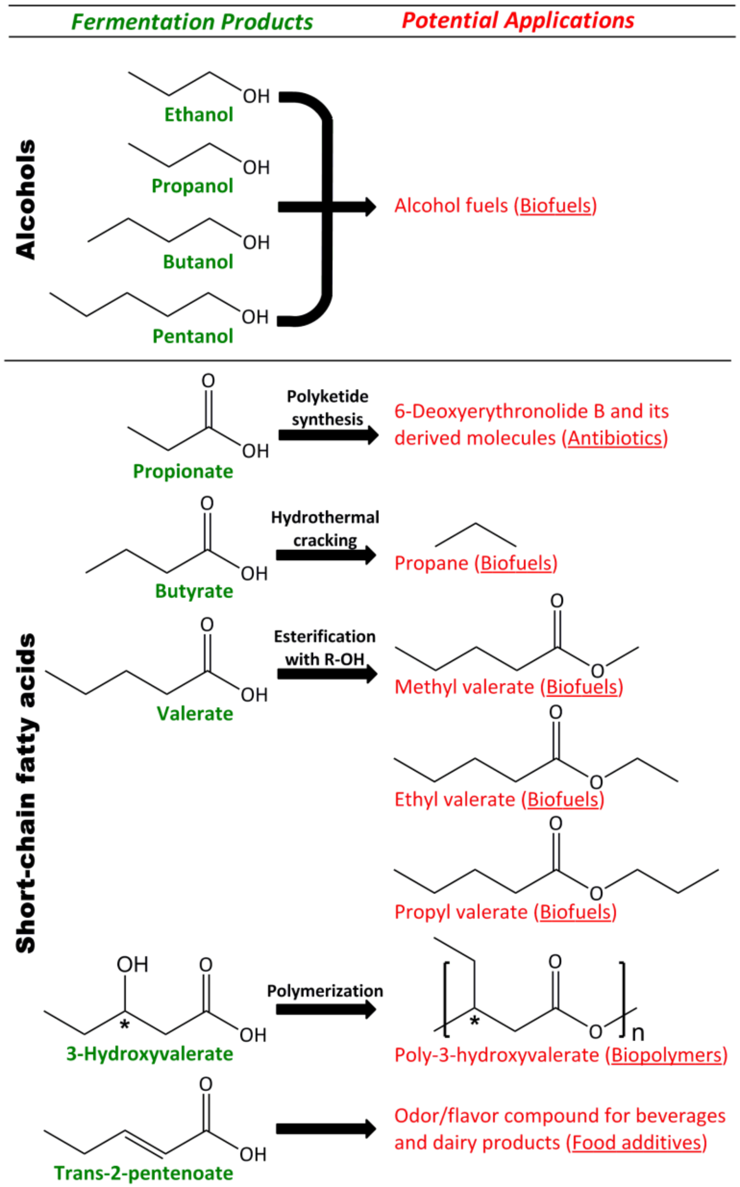
Value added Bio-chemicals

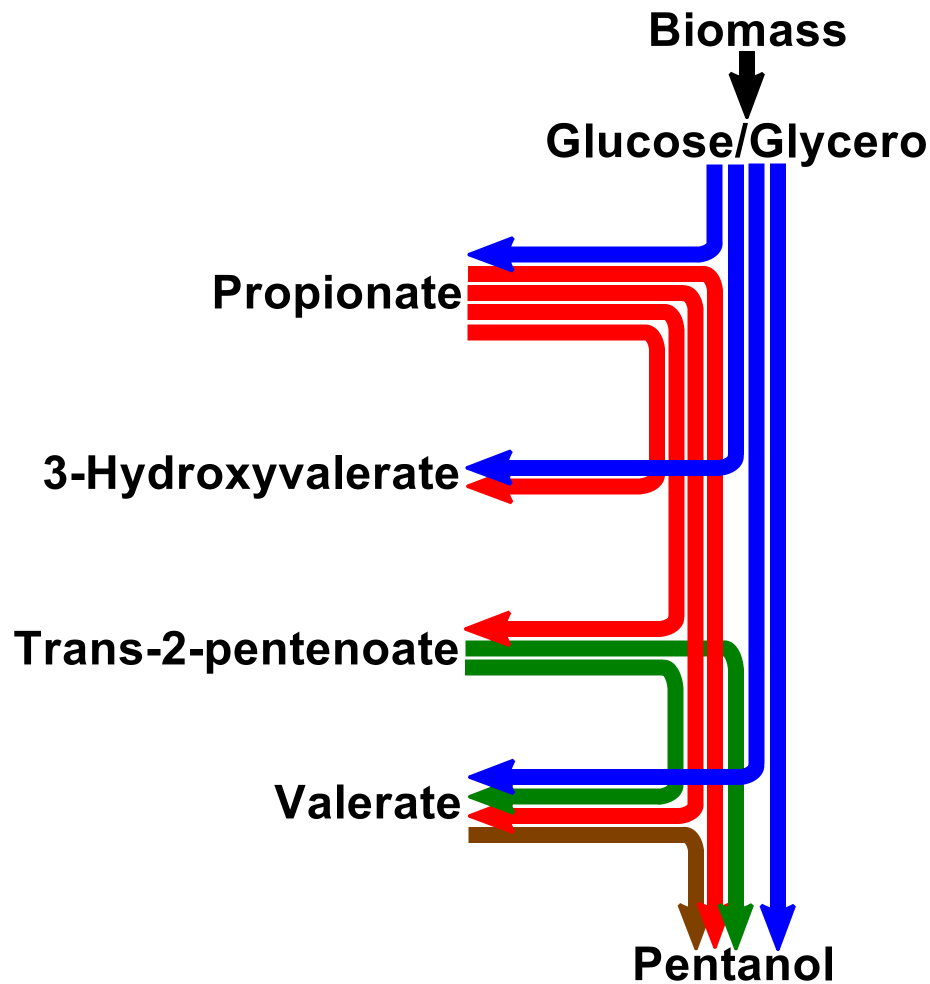


**Prof. B. D. Olsen*

Tseng and Prather, 2012. *PNAS,* 109:17925-30.

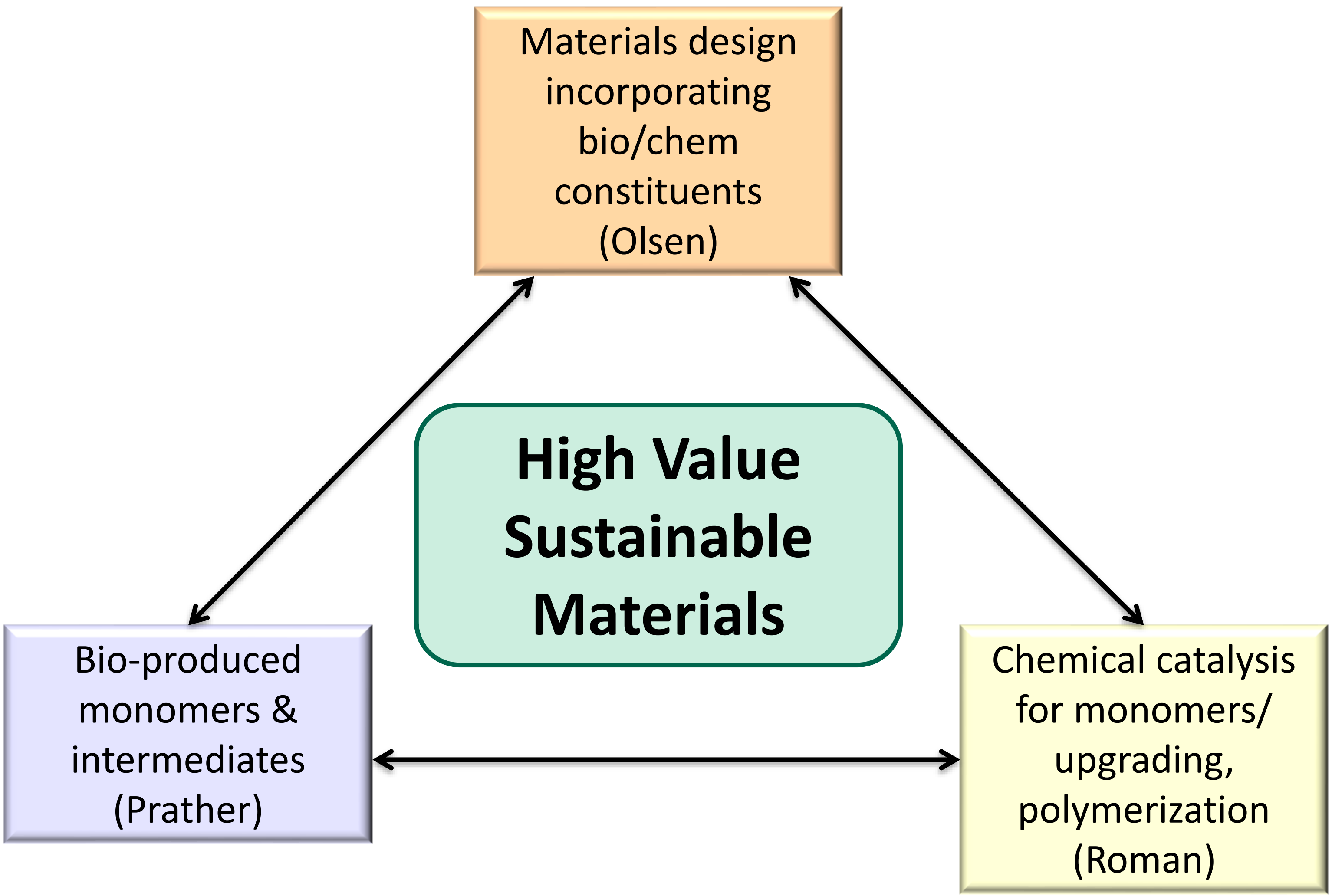
One Pathway, Many Products





Tseng and Prather, 2012. *PNAS*, 109:17925-30.

An Integrated Approach to Materials Design



Acknowledgments

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***The presenter declares competing financial interests.**

"Retro-biosynthetic" Pathway Design*

- Integration of Biocatalysis (enzyme selection) and Metabolic Engineering (systems assembly, analysis)

- (Others) On-going work on algorithms for biosynthetic pathway design

- Elucidation of Design Principles

- Development of Design and Assembly Tools

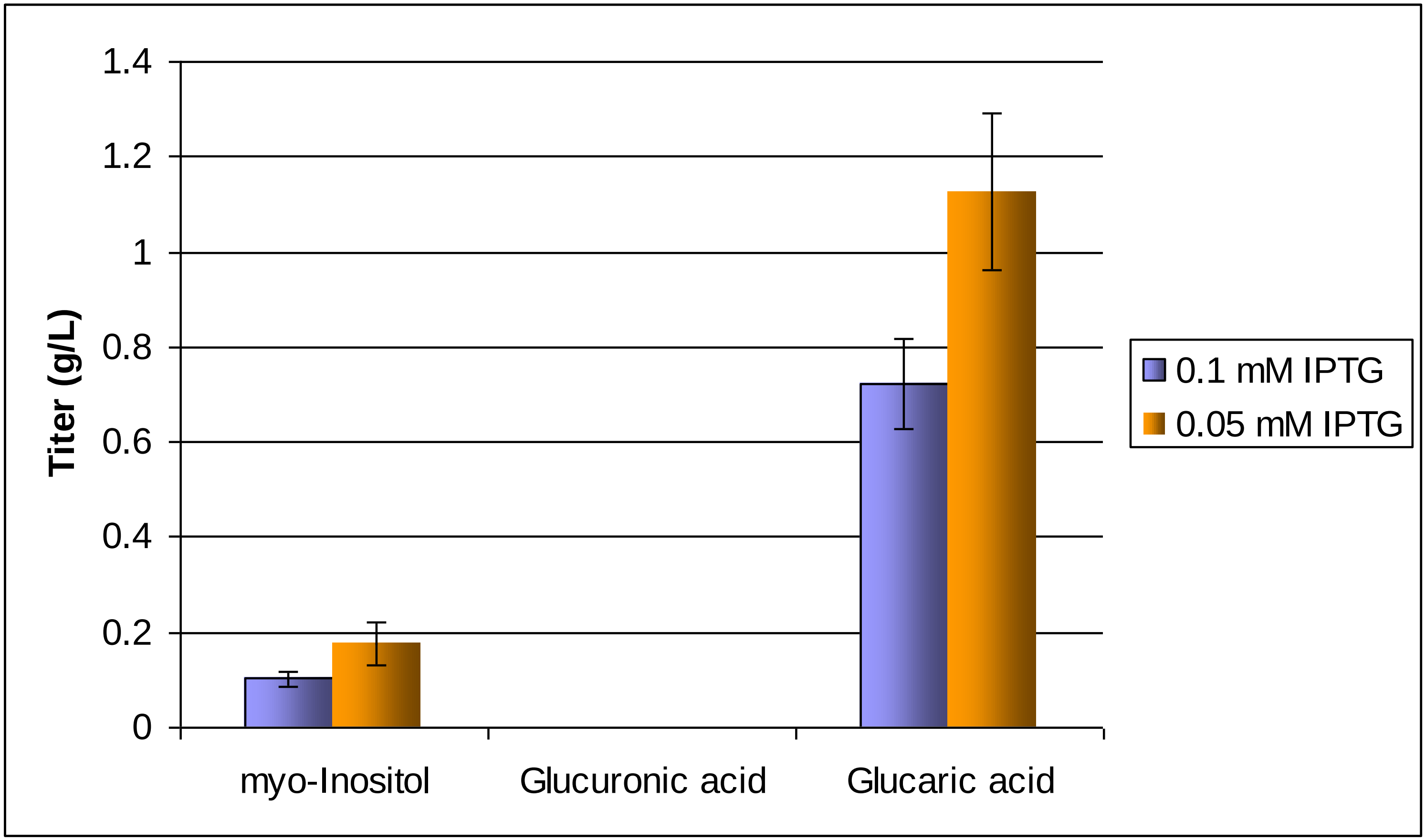
* *Curr. Opin. Biotechnol.* **2008. 19:468-474**

Candidates for Target Molecules

Building Blocks
1,4-diacids (succinic, fumaric and malic)
2,5-furan dicarboxylic acid
3-hydroxypropionic acid
aspartic acid
Glucaric Acid
glutamic acid
itaconic acid
levulinic acid
3-Hydroxybutyrolactone
glycerol
sorbitol
xylitol/arabinitol

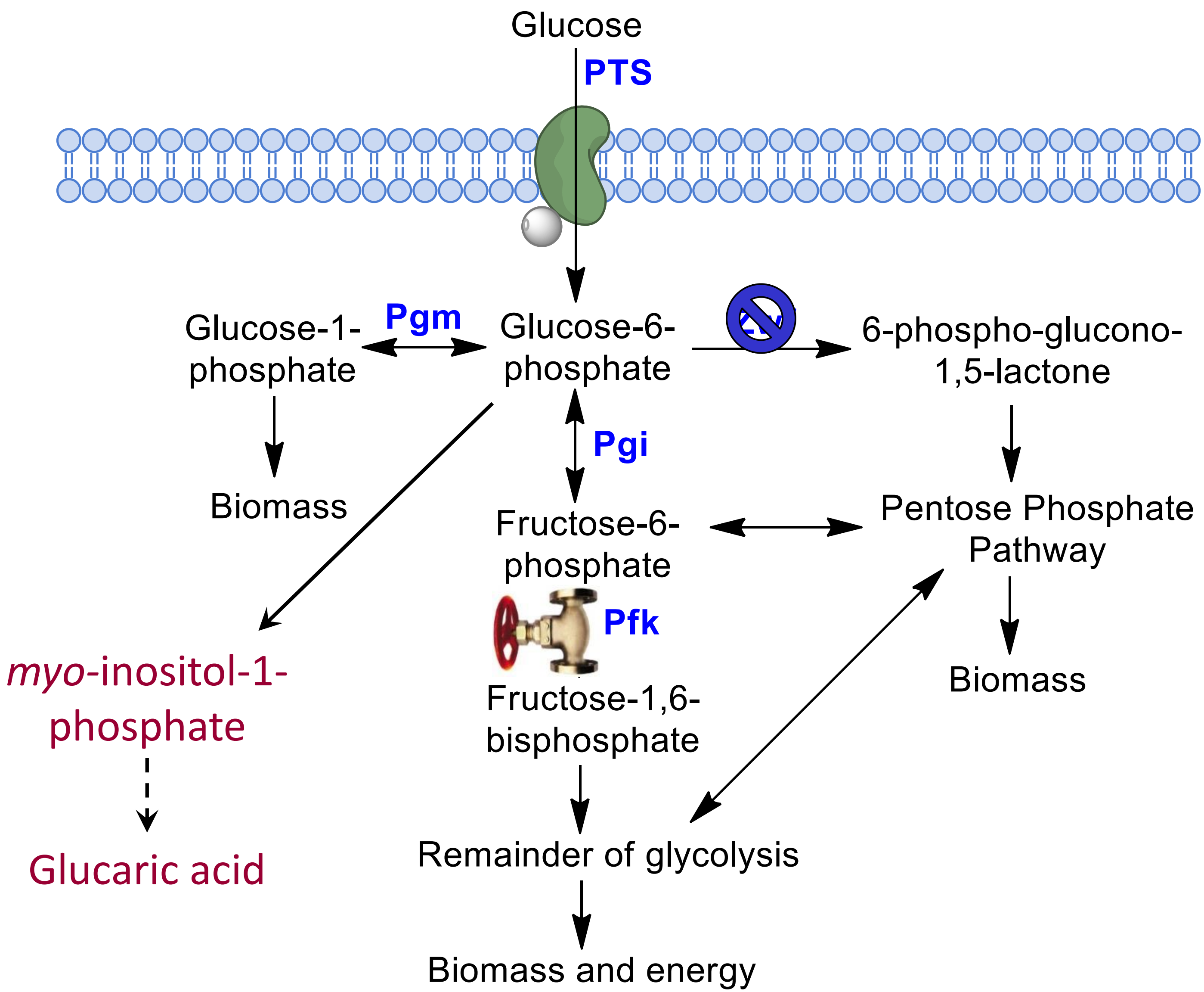
August 2004

Co-expression of 3 genes in *E. coli*



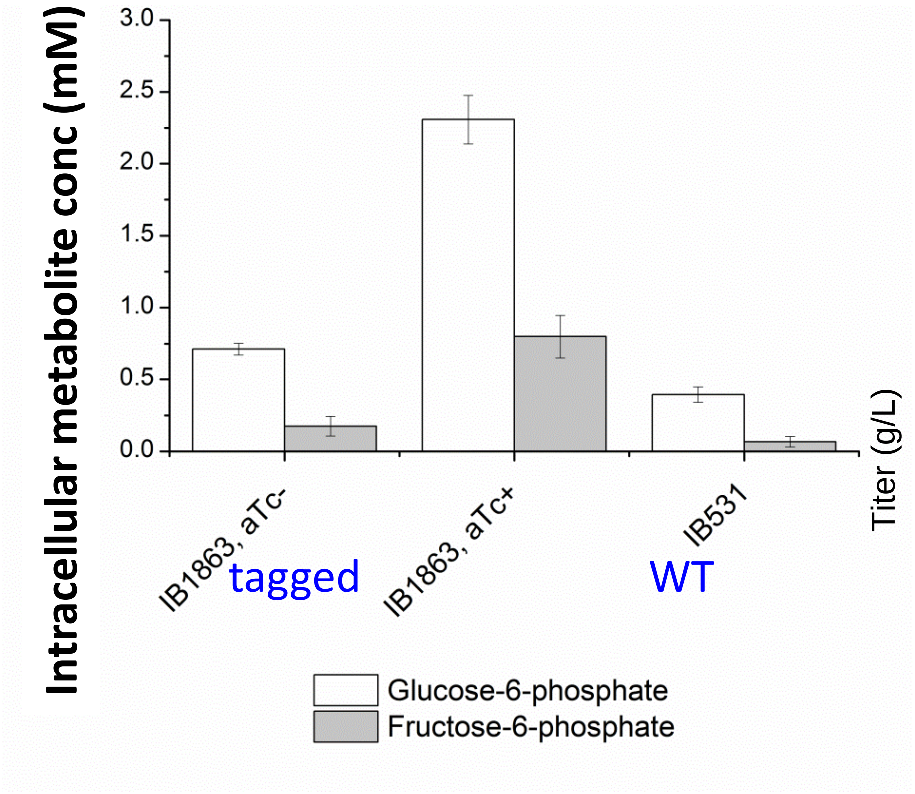
Moon et al, 2009. *Appl. Environ. Microbiol.* 75(3):589-595

Dynamic Control of Metabolic Flux

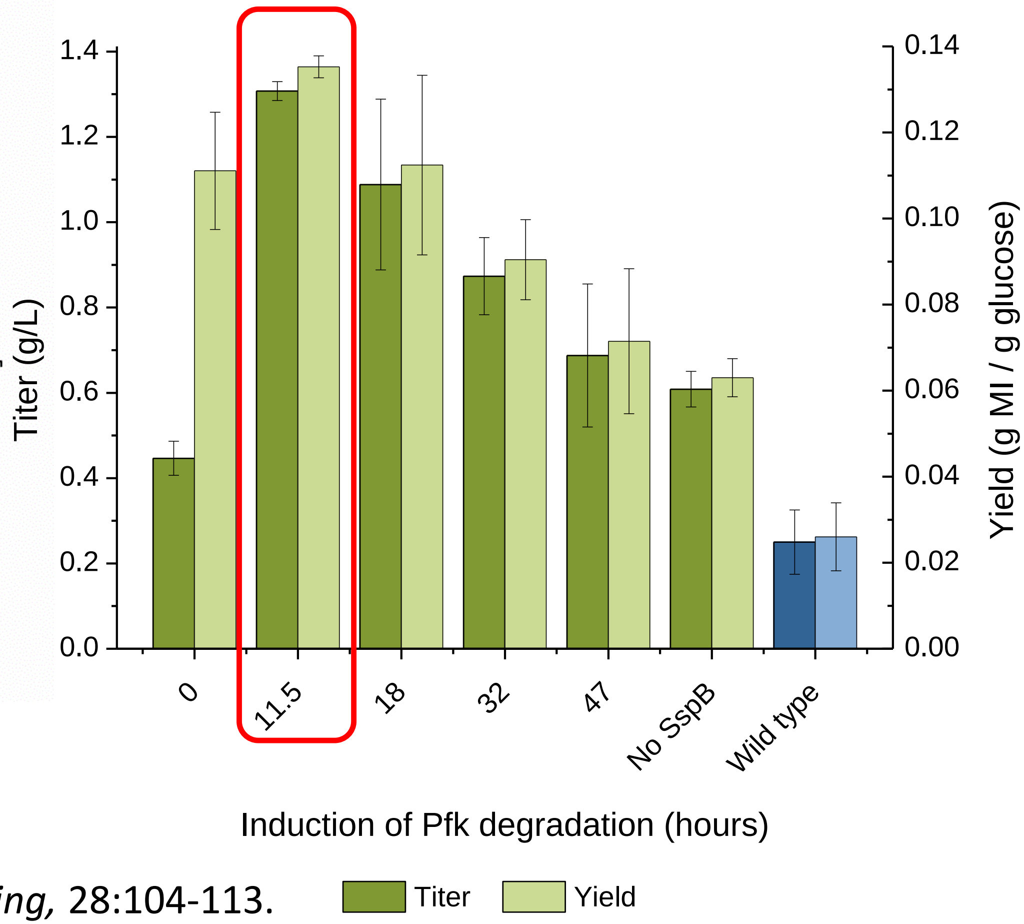


Dynamic Control of Metabolic Flux

Controlled degradation of PfkA
leads to increases in G6P pool size…

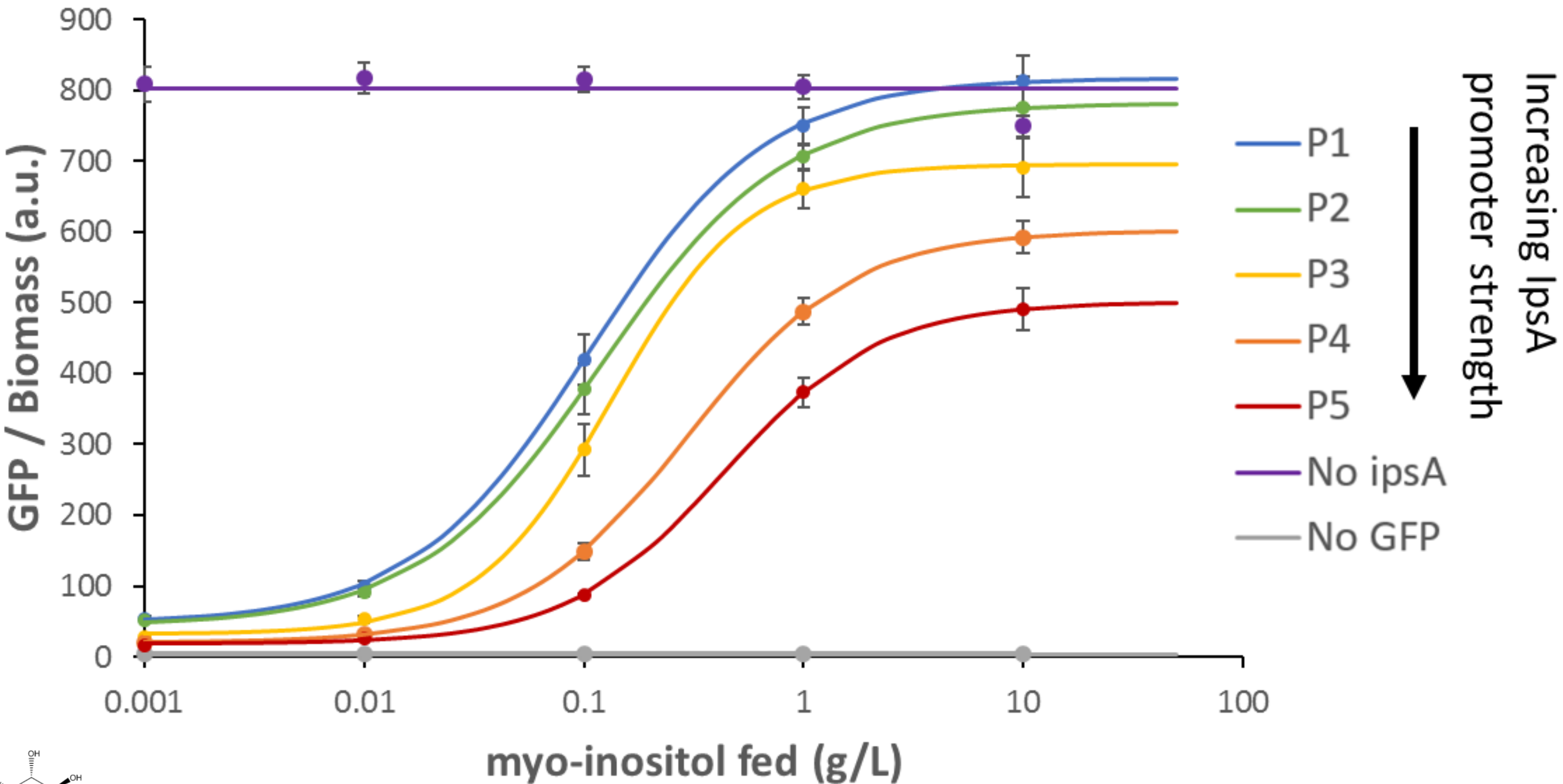


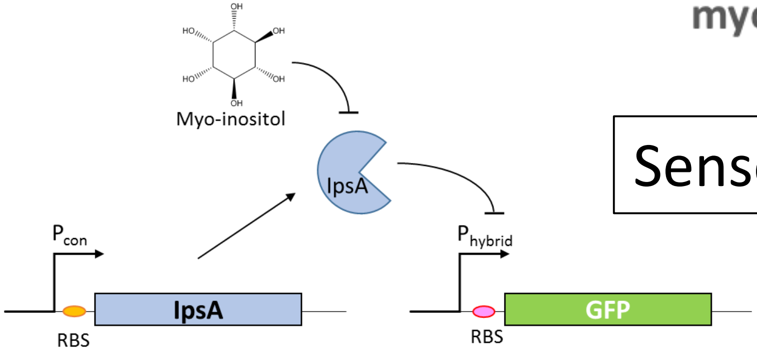
… and product titer/yield



Brockman and Prather, 2015. *Metabolic Engineering,* 28:104-113.

Circuit validation with exogenous MI

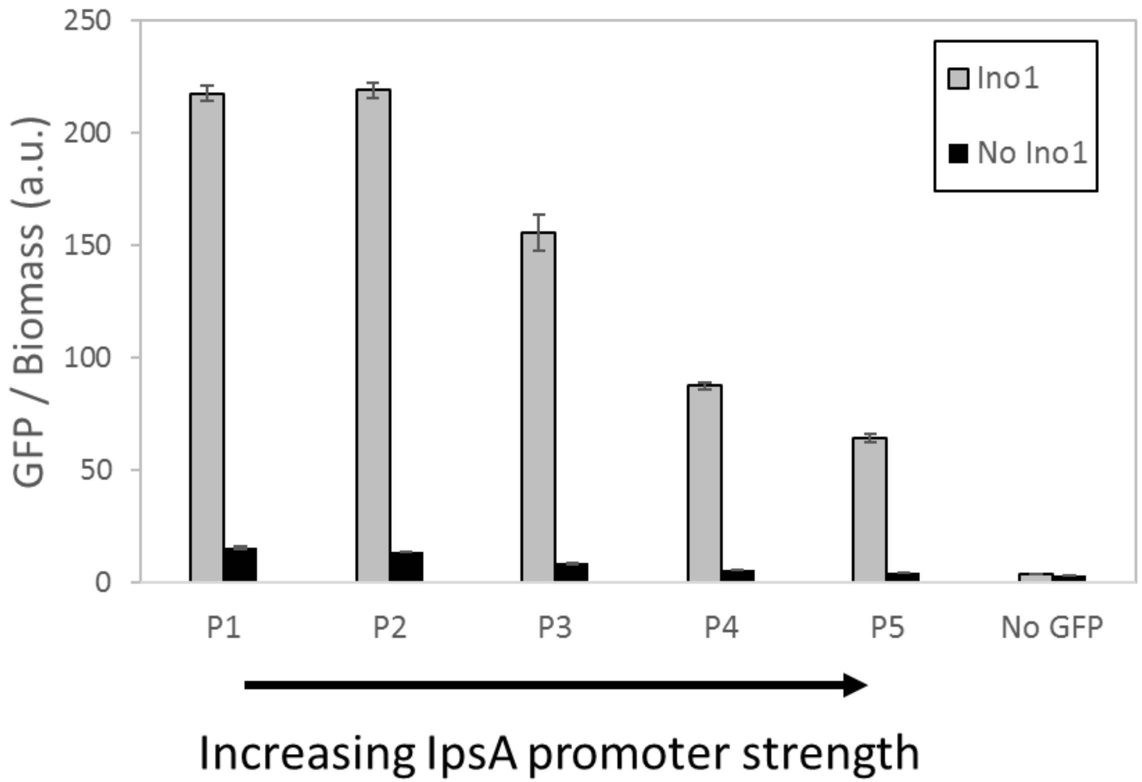


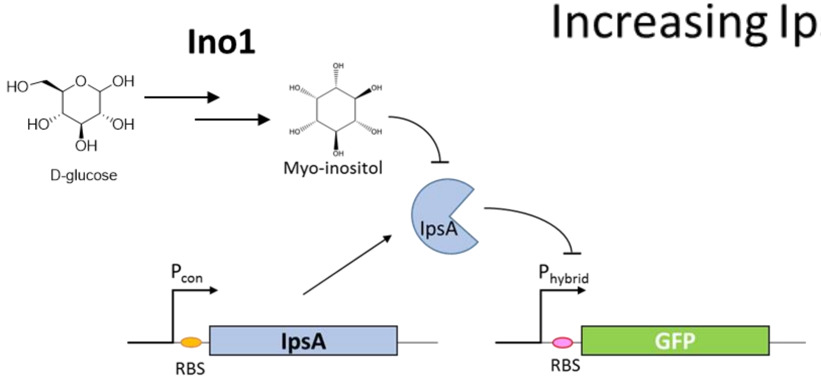


Sensor responds in dose-dependent manner

Doong *et al*. 2018. *PNAS.* **115**:2964-2969.

Circuit validation with endogenous MI

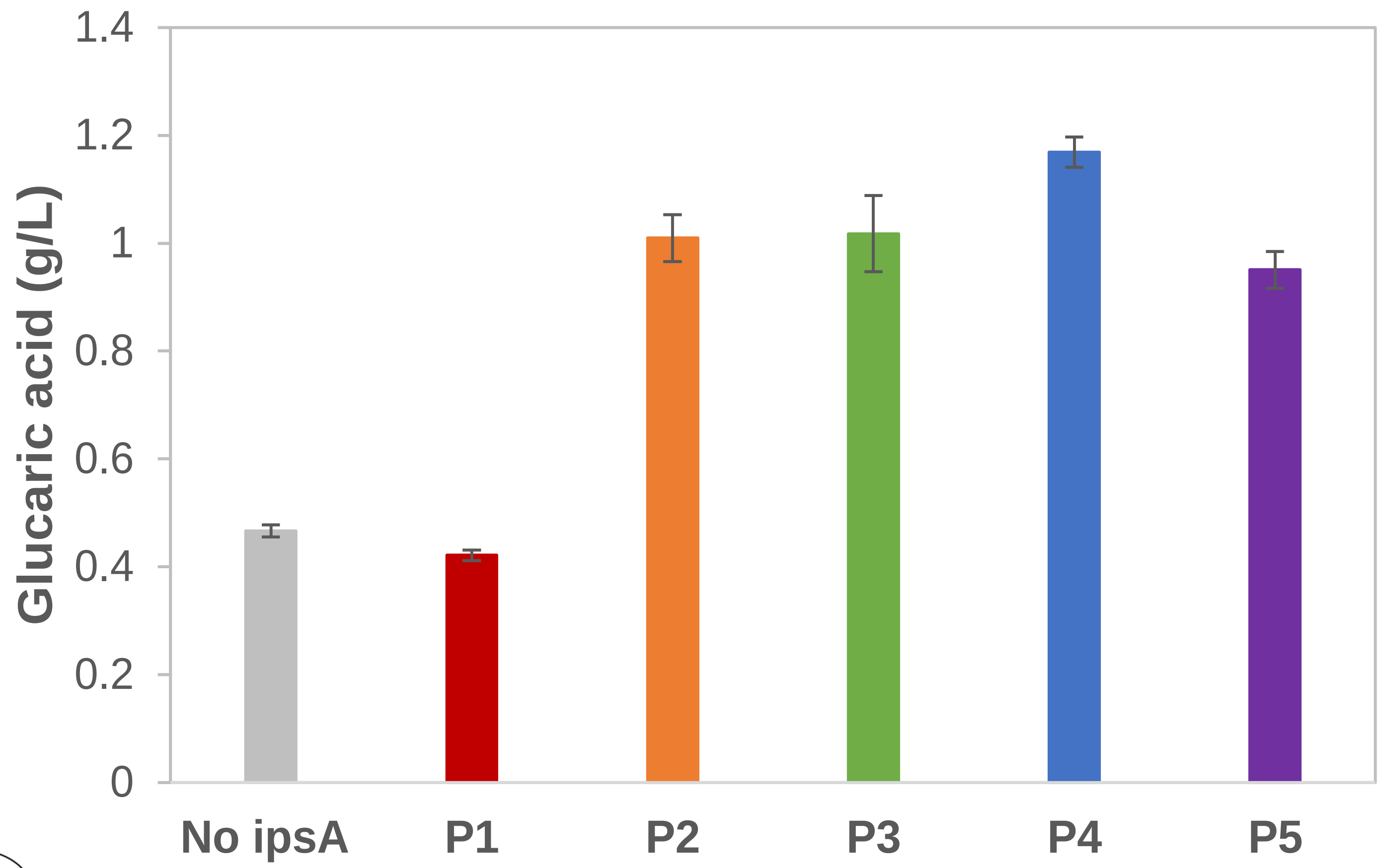


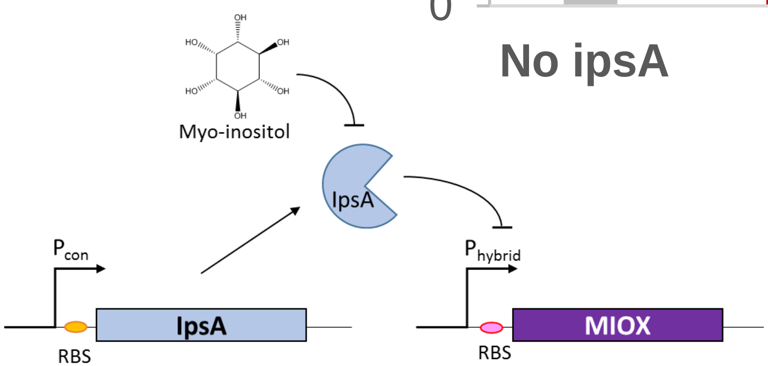


Sensor responds to MI produced from glucose by Ino1

Doong *et al*. 2018. *PNAS.* **115**:2964-2969.

Metabolite-Driven Production of GA



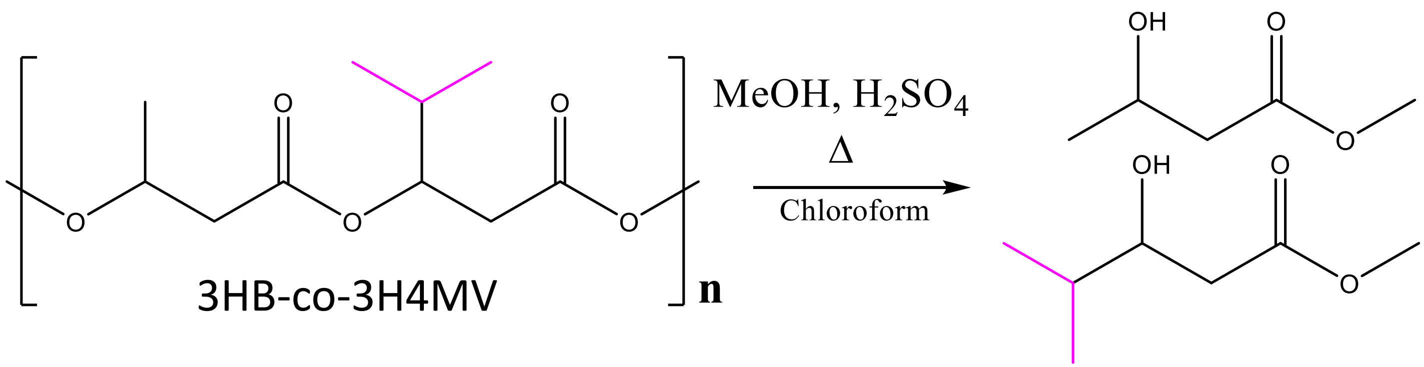


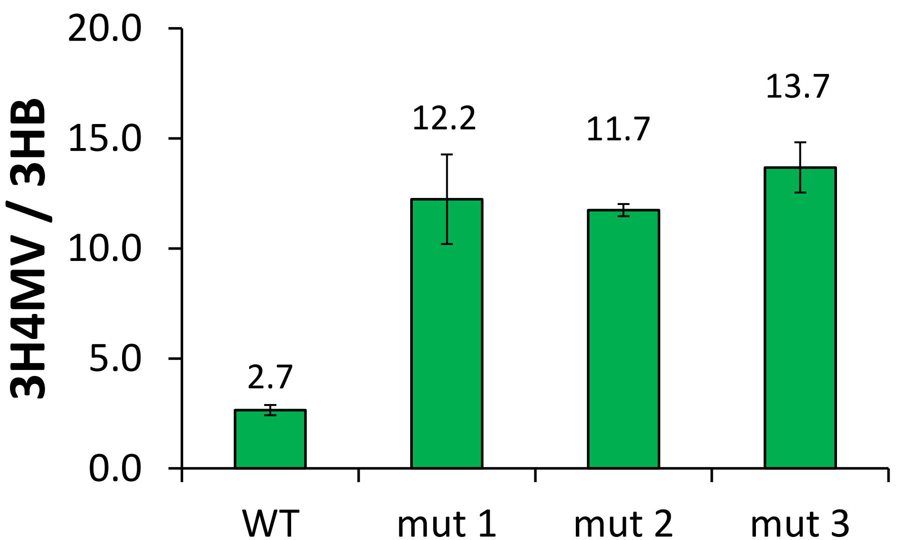
MI-driven MIOX expression results in increased glucaric acid production.

Doong *et al*. 2018. *PNAS.* **115**:2964-2969.

Protein Engineering for Increased Selectivity

- Rational protein design (w/B. Tidor, MIT-BE)
- Using insoluble PHA polymer as indicator of selectivity



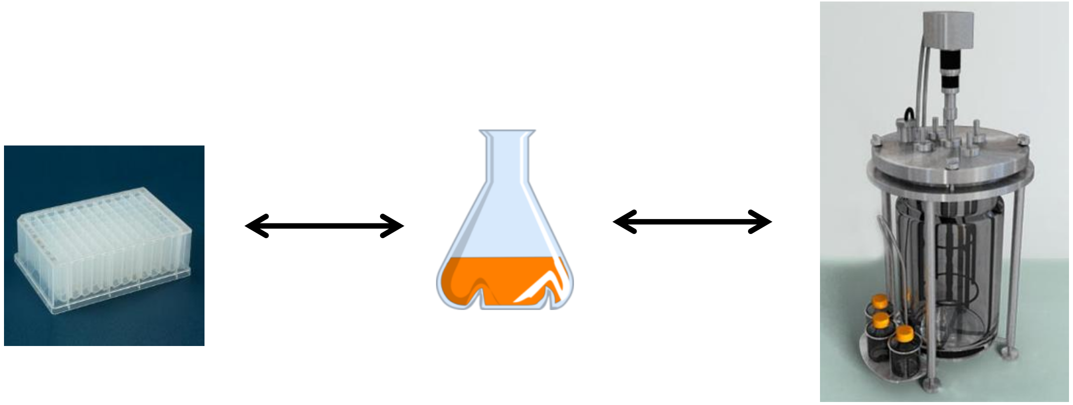


4-5x fold improvement in selectivity over wild-type enzyme

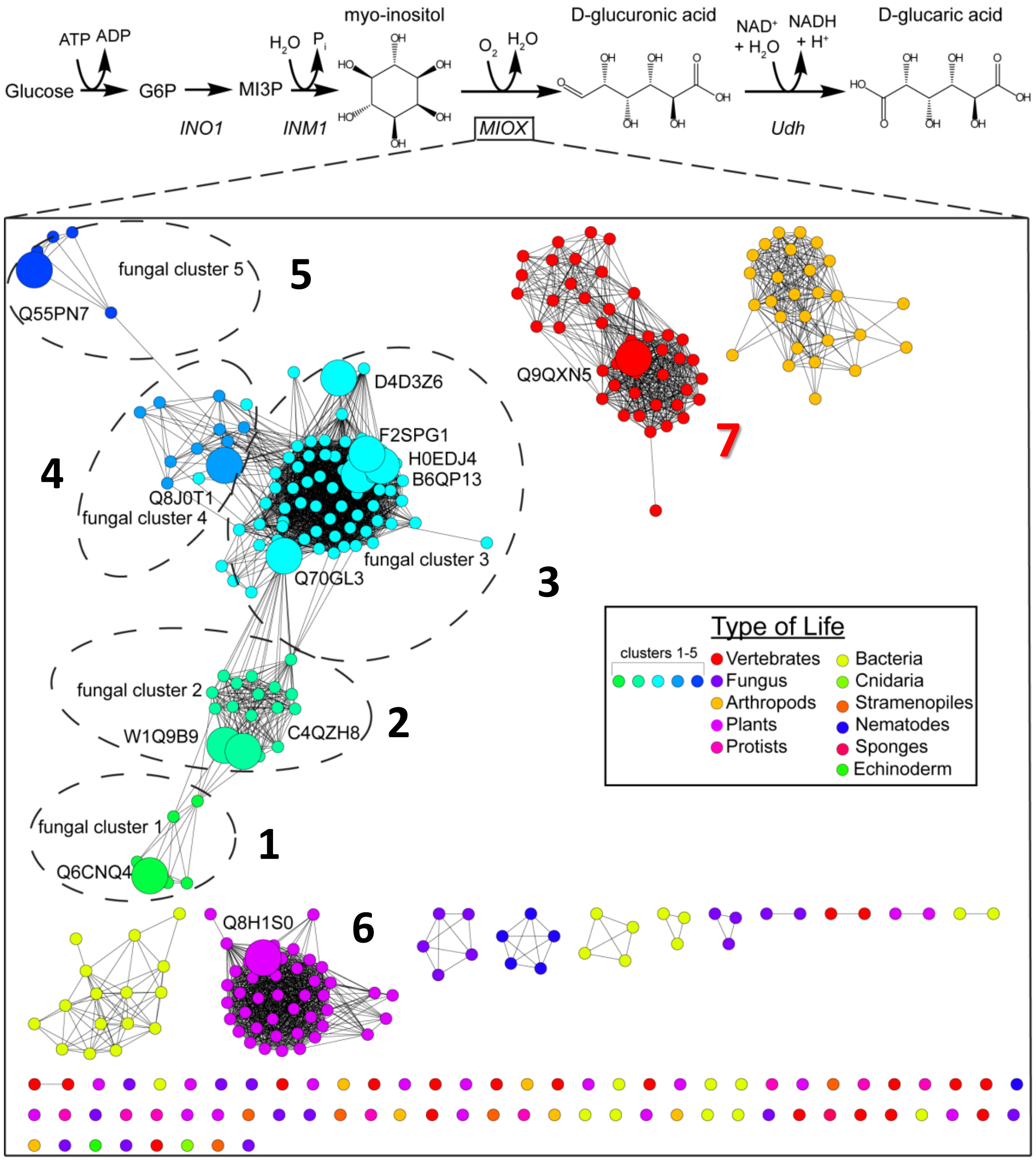
Bonk et al, 2018.
Biotechnol. Bioeng. 115:2167–2182

Evaluating Quorum-Sensing Devices Across Scales

- Developed ***Metabolite Valves*** based on Quorum-Sensing mechanisms
- Ultimate goal is application in large-scale production
- Small, medium and large scales important
  - *Development scale should be reliable*
- Especially relevant for QS due to reliance on extracellular molecular transport



Search Strategies for “Better” Enzymes



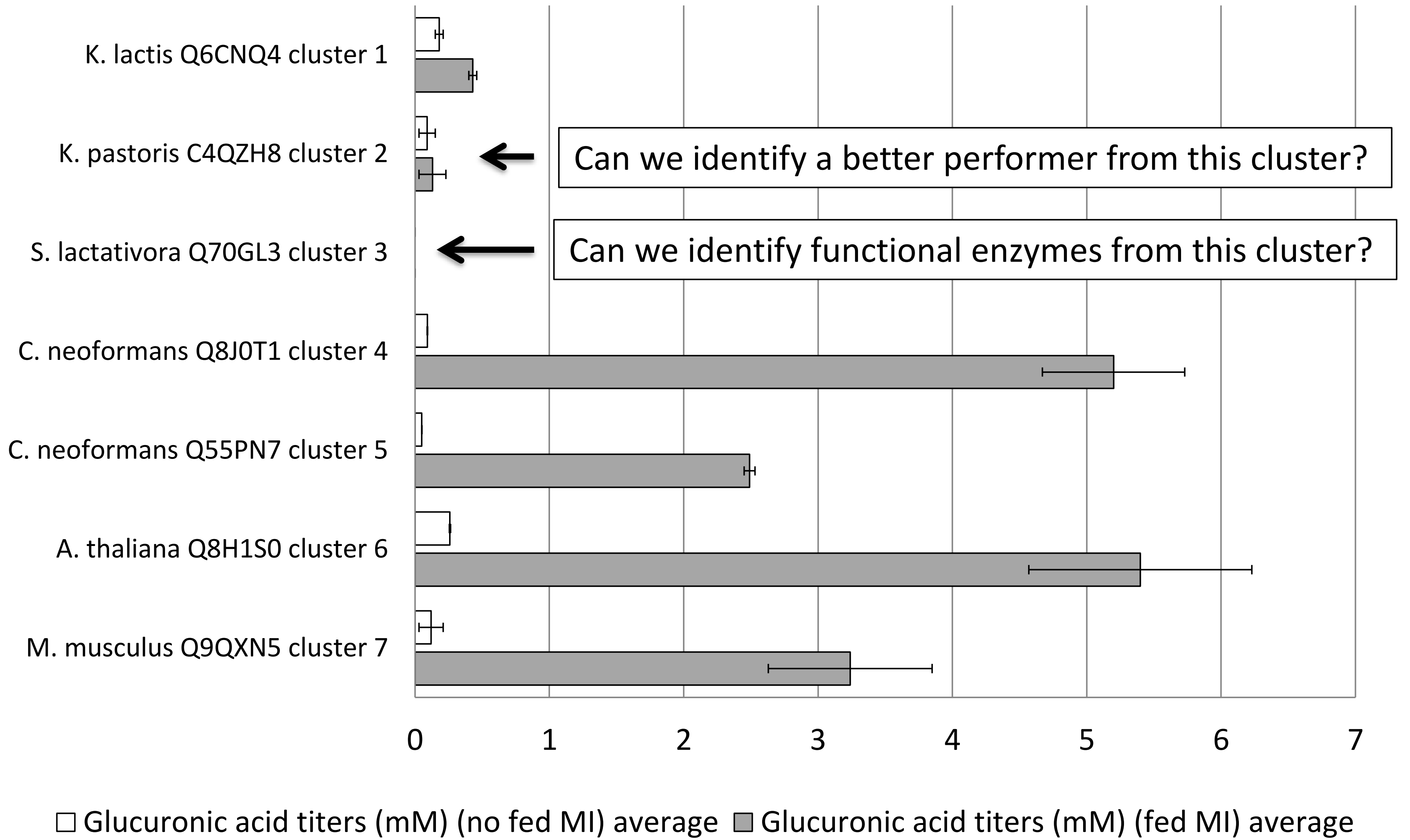
MIOX Enzyme (2^{nd} Step)

Network statistics

336 nodes
3207 edges
-log10(E)>120
Median %id = 67.6%
Median alignment length = 291

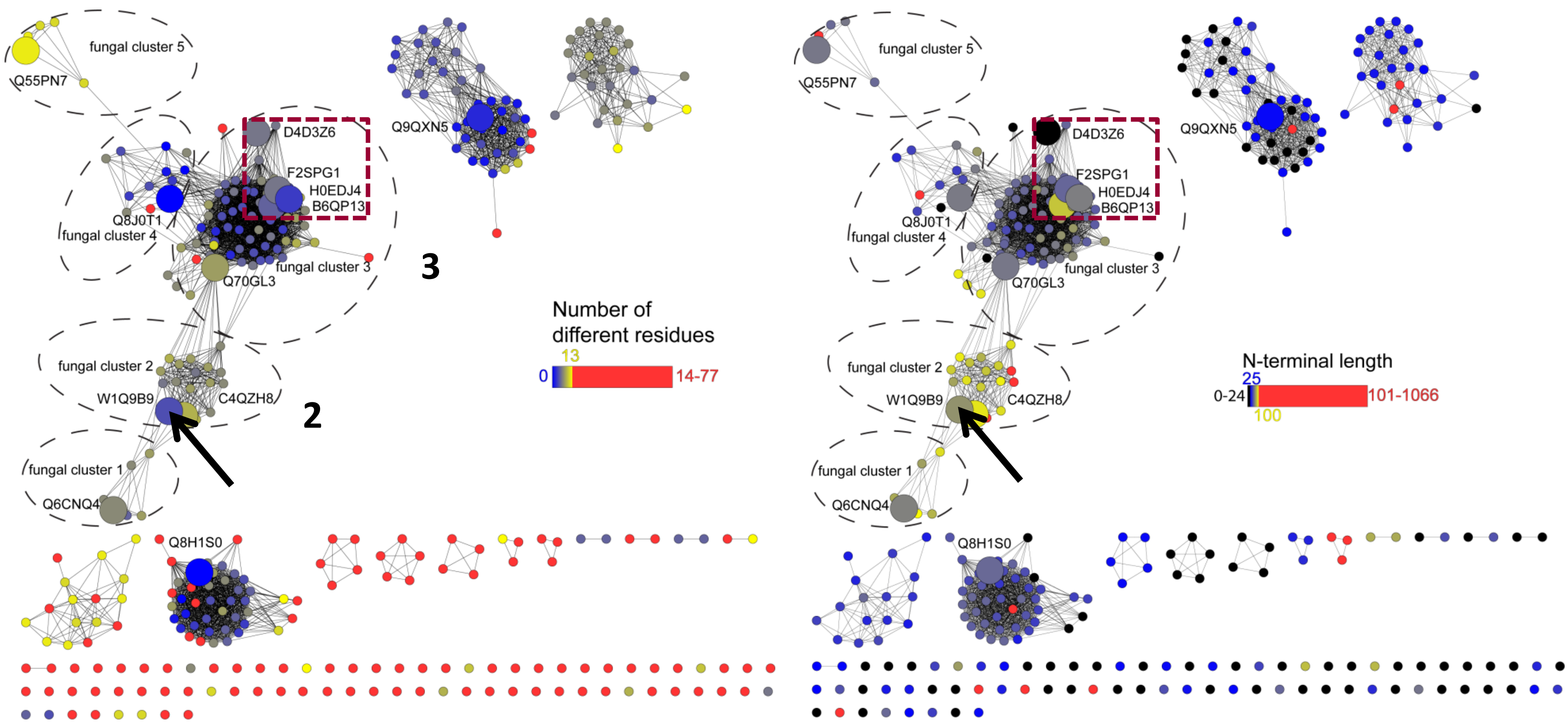
Michael Hicks

Variable glucuronic acid titers in a naïve test set

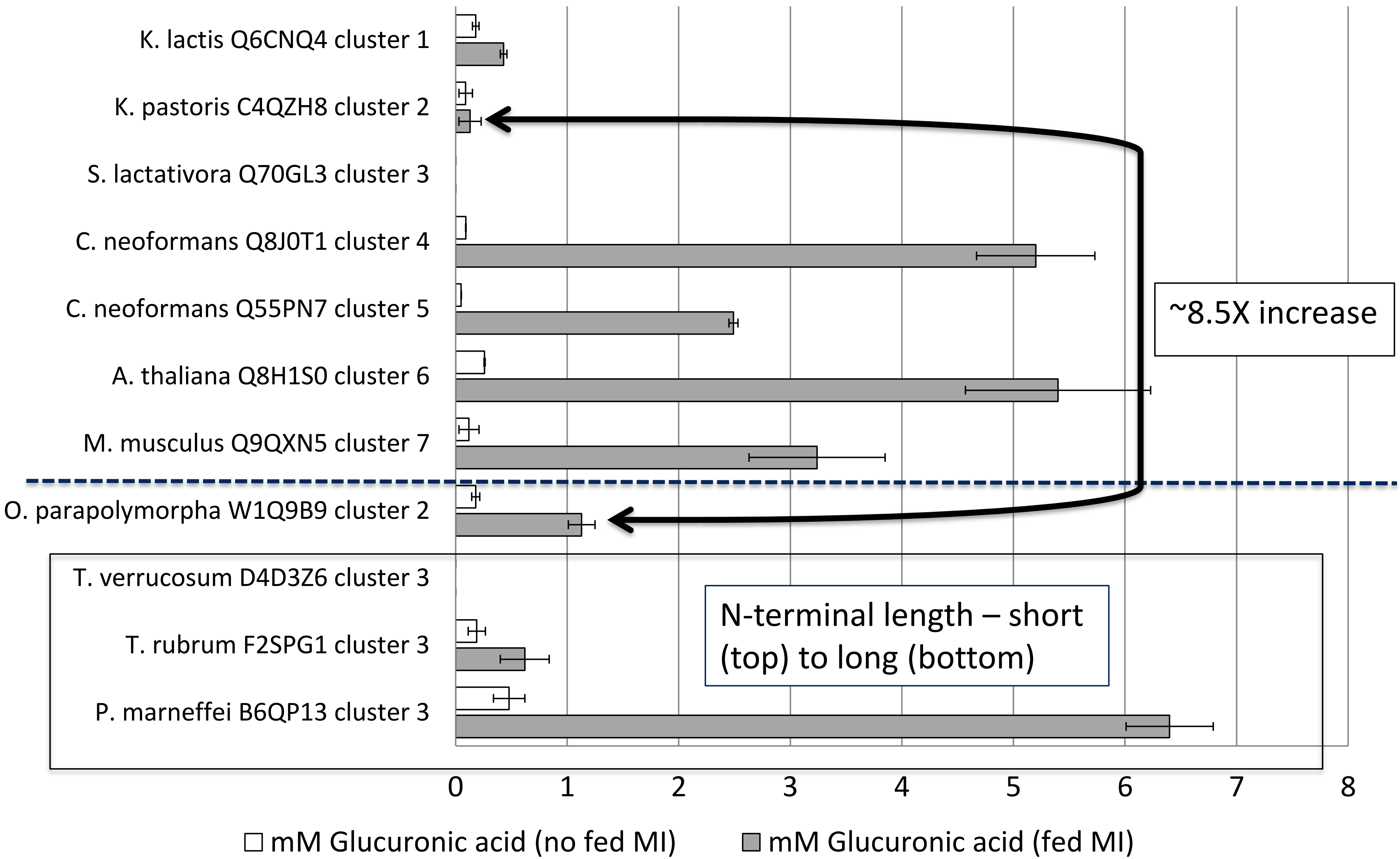


Choosing a new set of MIOX proteins

Active site residue conservation, N-terminal sequence length appear correlated with activity



Improved glucuronic acid titers in “informed” set



Summary

- Biological conversion is an effective tool for the production of chemical products.
- Pathway design enables access to unnatural structures, biosynthetic routes.
  - 3-Hydroxyacids
  - Saturated acids and alcohols
  - Glucaric acid
- New tools can be used to assemble, diagnose, and optimize pathways.

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