Building Microbial Chemical Factories

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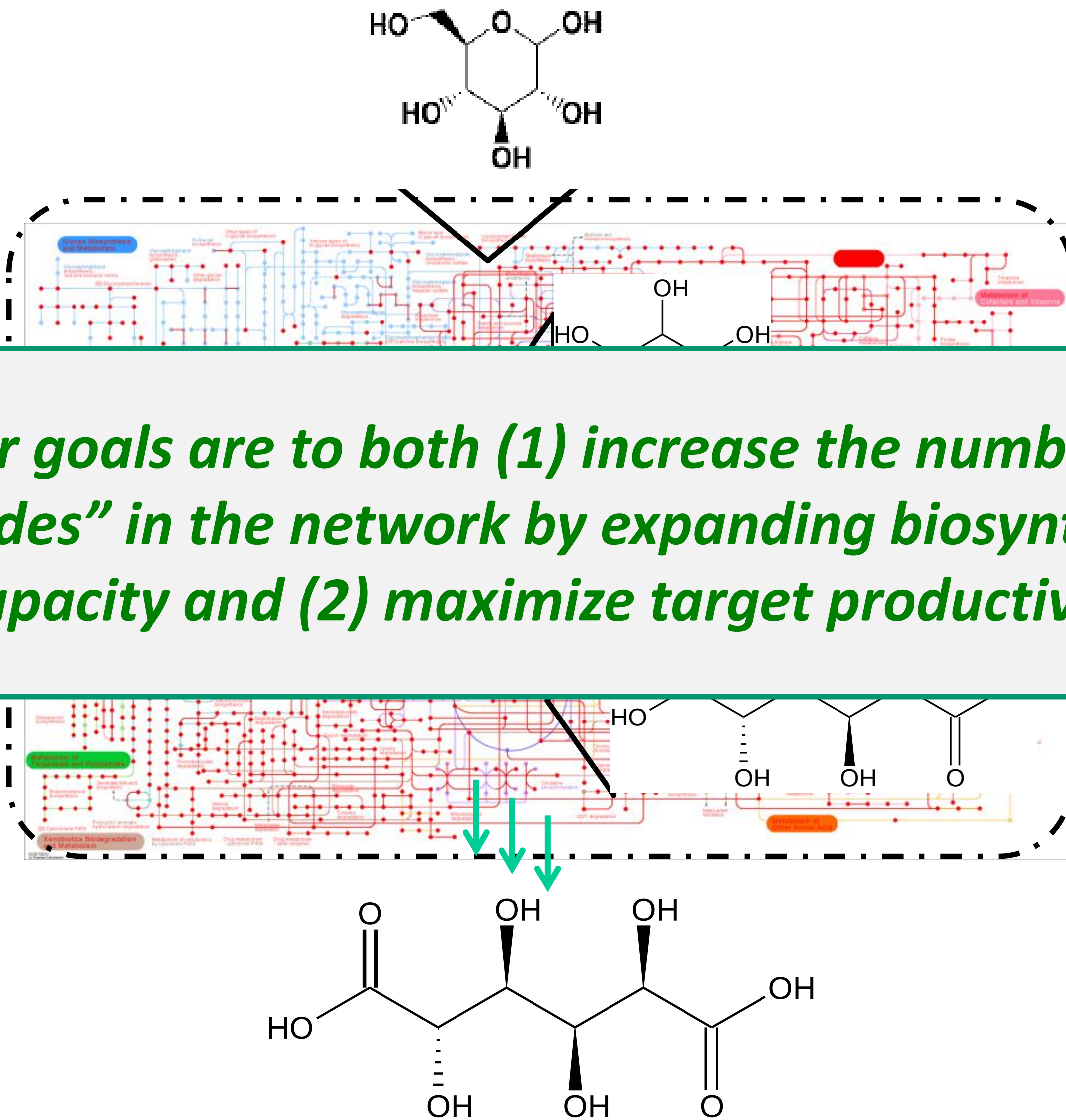
***The presenter declares competing financial interests.**

Microbial Chemical Synthesis

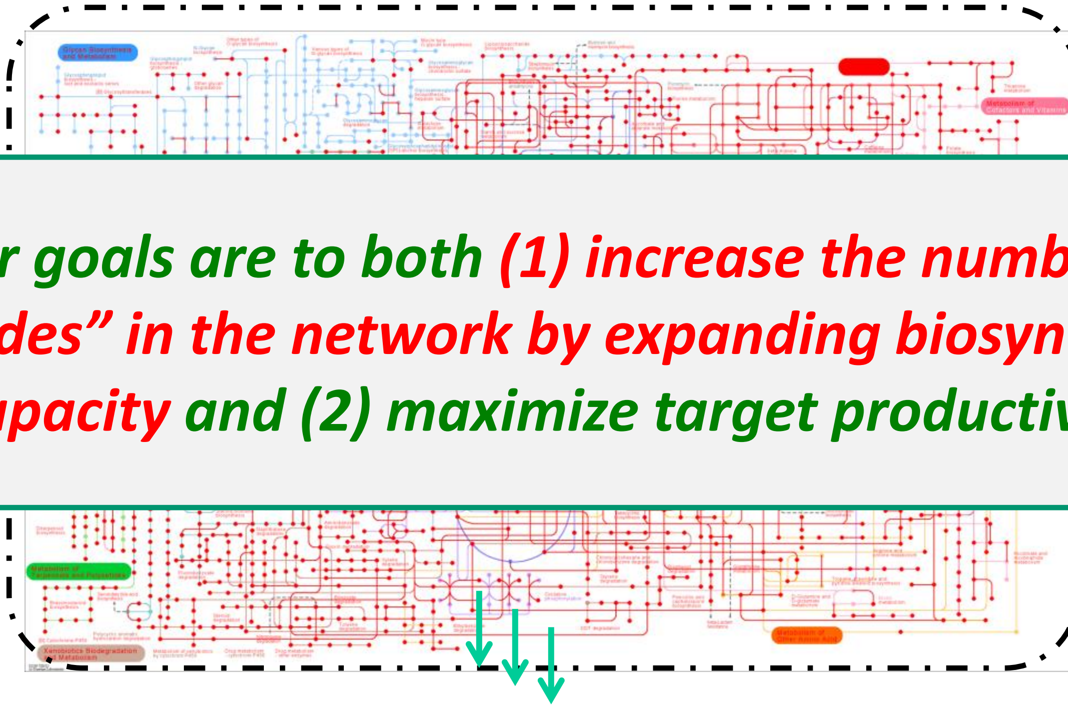


Byproduc

Our goals are to both (1) increase the number of “nodes” in the network by expanding biosynthetic capacity and (2) maximize target productivity.

owth

Microbial Chemical Synthesis



Byproduc **owth**

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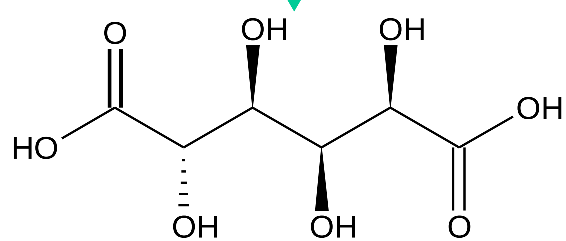
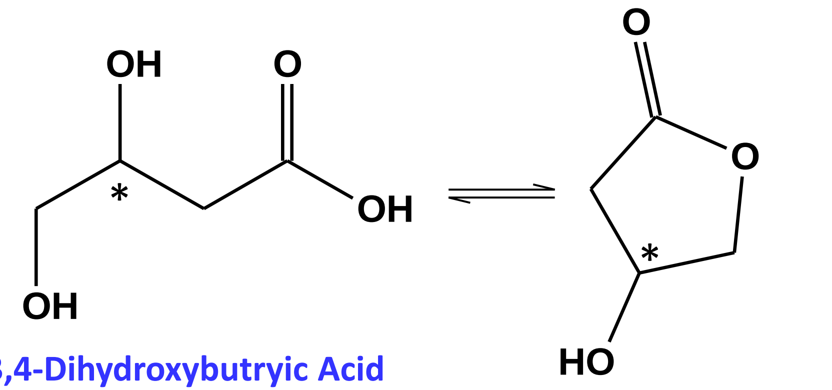


3-Hydroxybutyrolactone (3-HBL)

- Key Intermediate in Chiral Synthesis of Solvents (e.g. Furan Derivatives) and Pharmaceuticals (e.g. Statins)

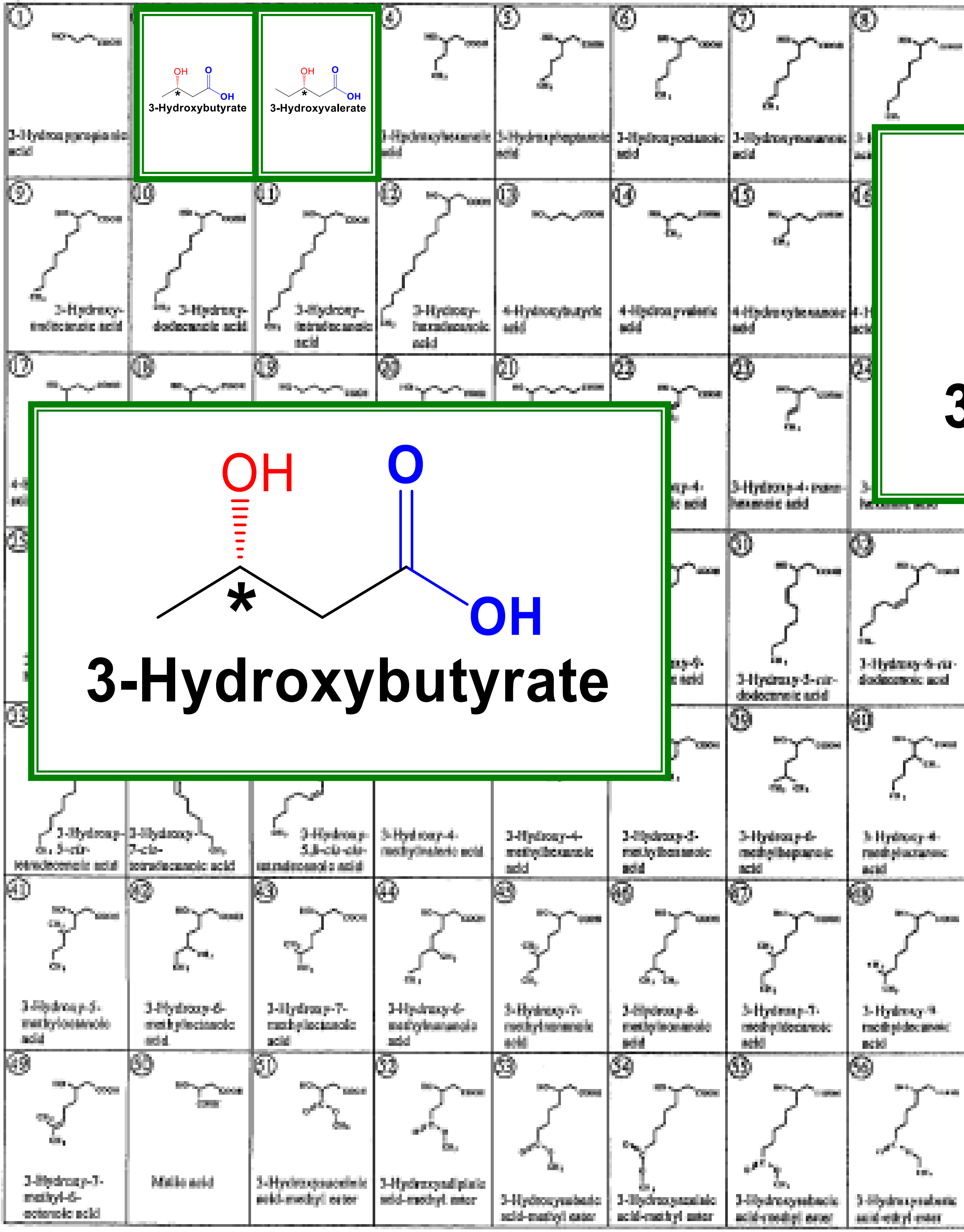
- Wholesale Cost ~ $450/kg ($20-50/gram for lab-scale quantities)

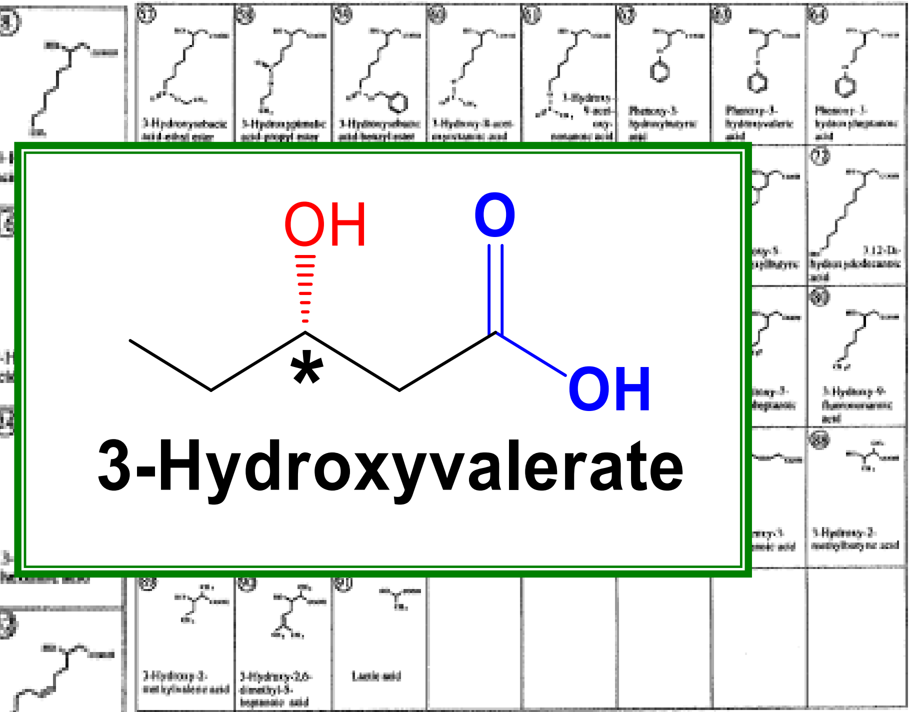
- **No Known Biological Routes towards DHBA or 3-HBL.**



3,4-Dihydroxybutryic Acid (DHBA)

3-Hydroxybutyrolactone (3-HBL)

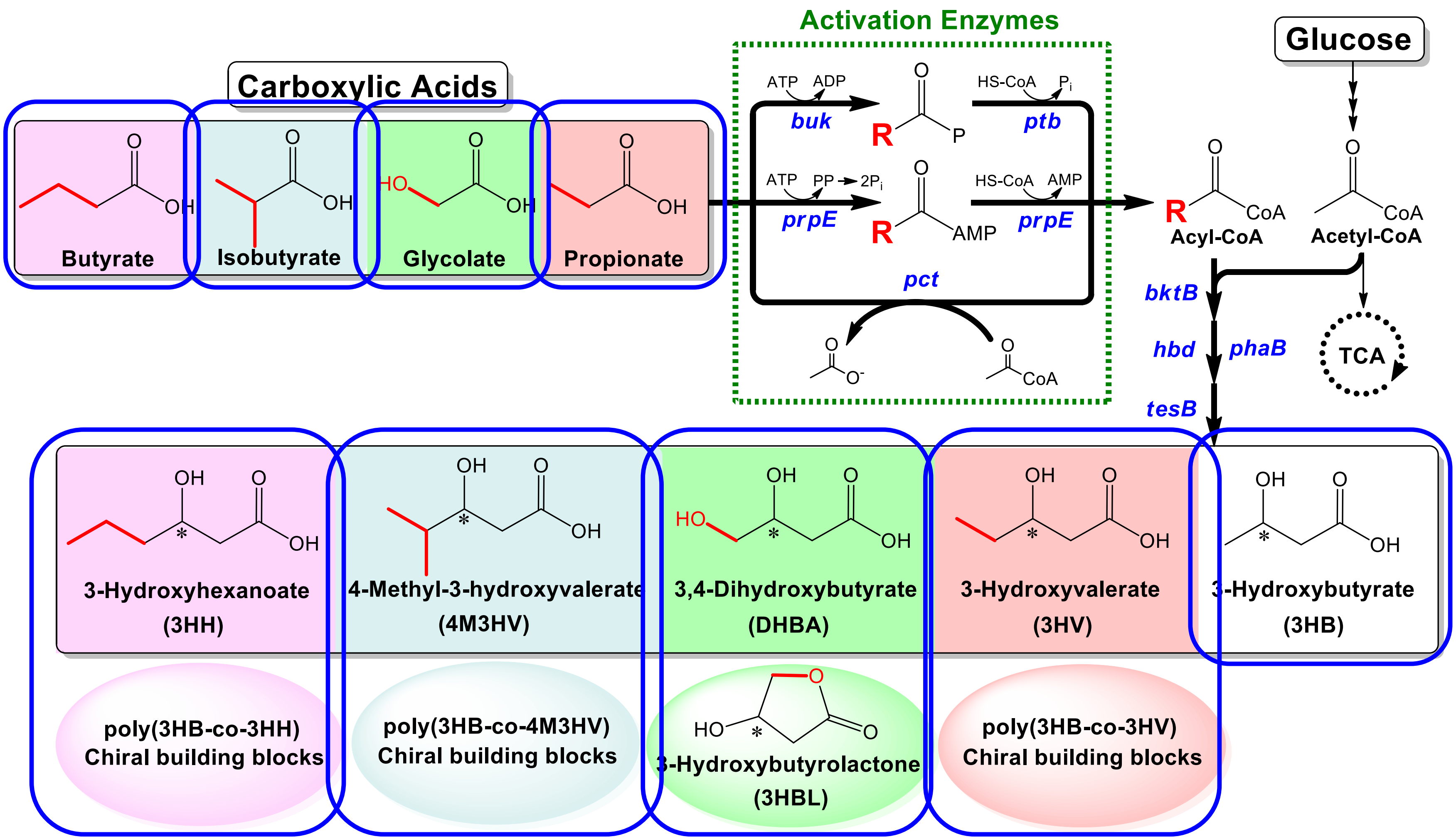




- Many hydroxyacids have been made as constituents of intracellular PHAs (biopolymers).
- Very few have been produced economically as free monomers.

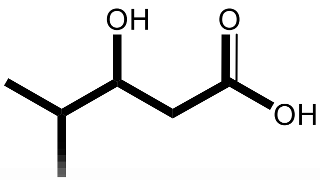
Steinbüchel *et al*, FEMS Microbiol. Lett., 1995, 128:219-228.

Synthesis of Chiral 3-Hydroxycarboxylic Acids

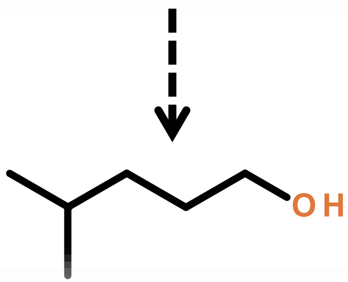


Martin et al, 2013. *Nat. Commun*. 4:1414.

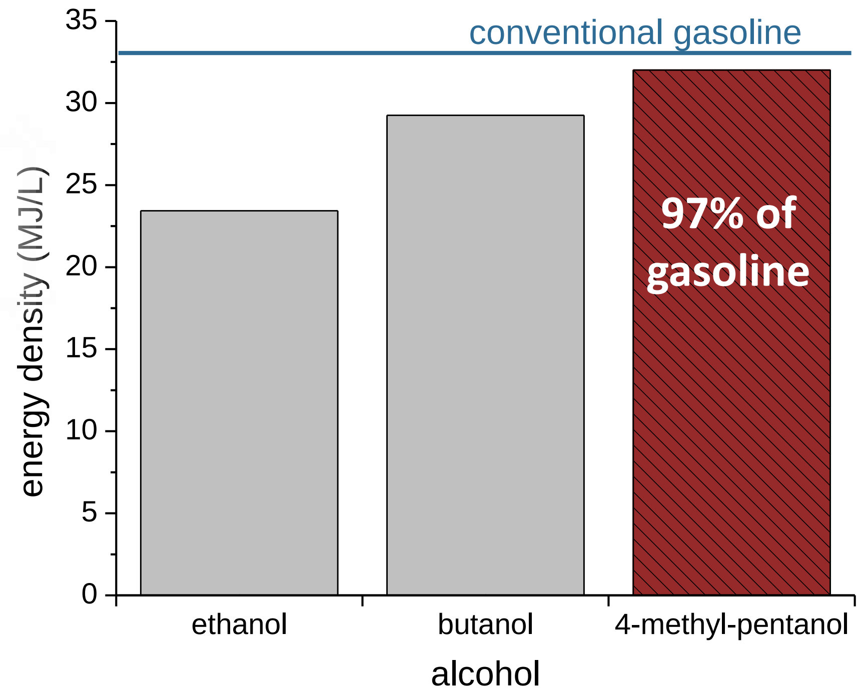
Accessing new molecules through Biology: 4-Methylpentanol



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



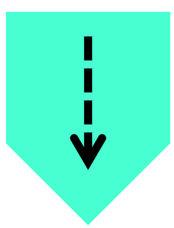
4-methyl-pentanol (4MP)



Mimicking n-butanol (CoA-dependent) biosynthesis

Theoretical maximum energy yield of 94.6%

glucose



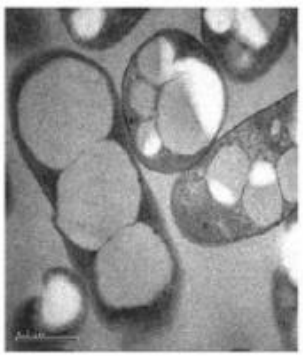
4MP

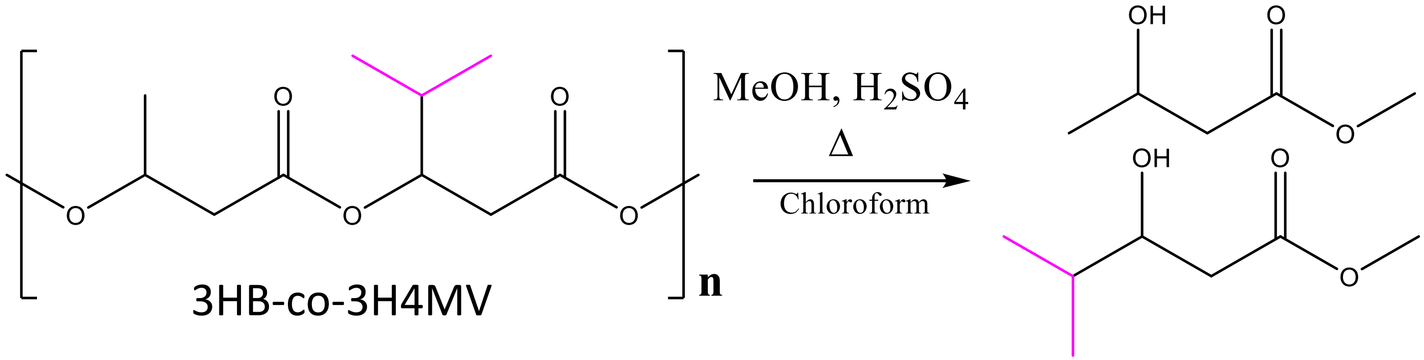
- Final pathway employed **10 genes** from **8 different organisms**
- Max **selectivity of ~80%** for 4MP alcohol

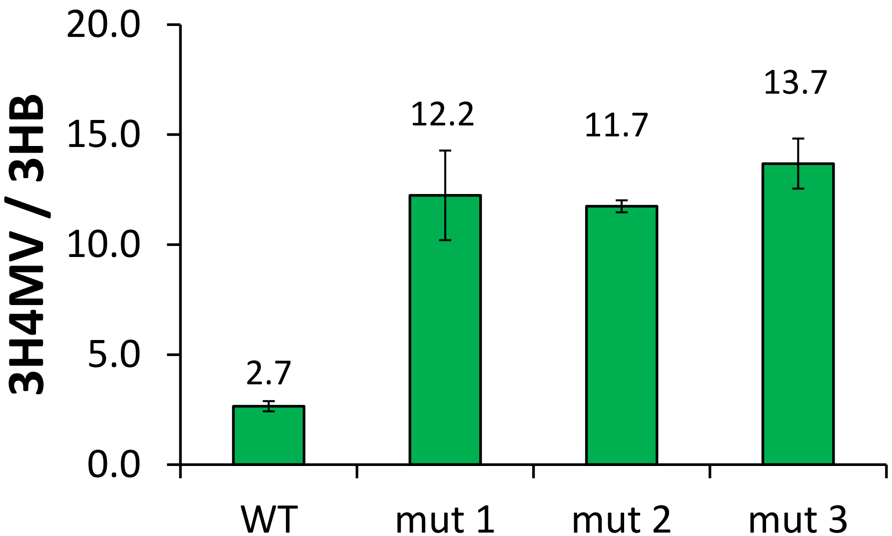
Sheppard, et al, 2014. *Nat. Commun*. 5:5031.

Protein Engineering for Increased Selectivity

- Rational protein design (in collaboration with Bruce Tidor, MIT-BioE)





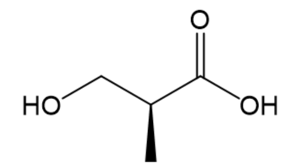


4-5x fold improvement in selectivity over wild-type enzyme

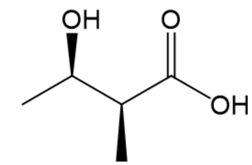
Bonk et al, 2018.
Biotechnol. Bioeng. 115:2167–2182

Designer Polymers for Biodegradation

Allow Biology to do what it does best…



(2S)-3HIB



(2S,3R)-3H2MB

Biologically synthesize biopolymer building blocks
Lab(s) - Prather

Chemically polymerize building blocks and predict properties via *in silico* techniques
Lab(s) – Brad Olsen

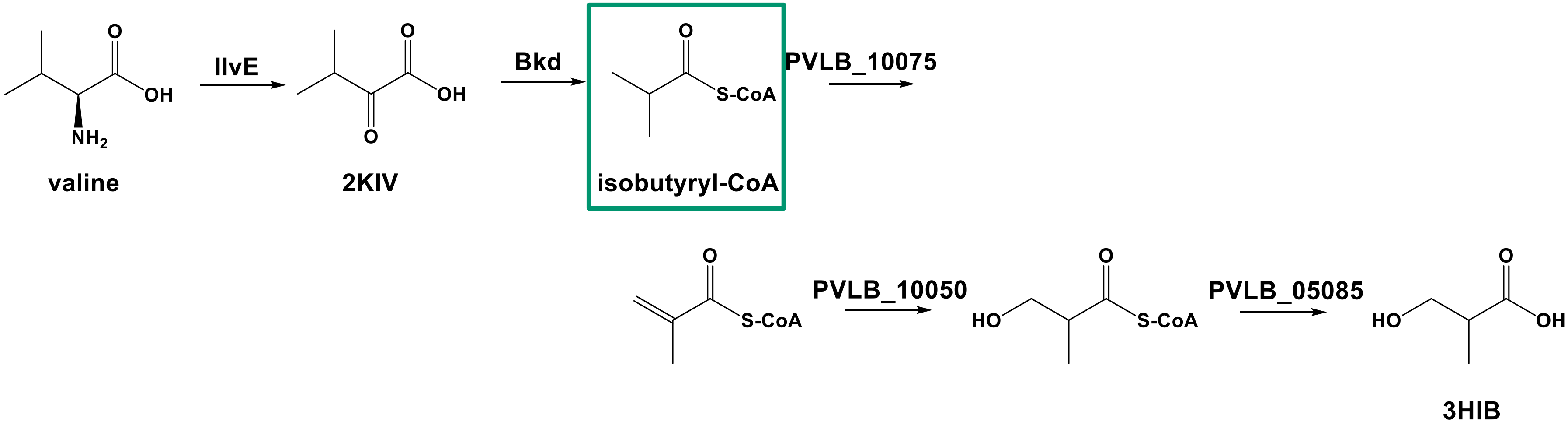
Determine photo and biodegradability properties of biopolymers
Lab(s) – Brad Olsen, Desiree Plata

…and fuse with the best of Chemistry

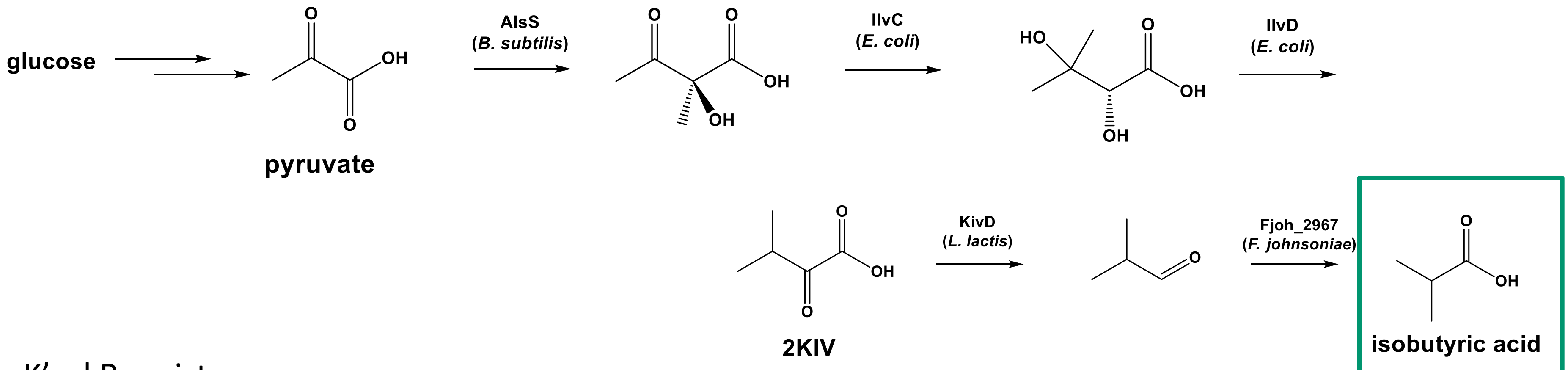
Color & Comfort

Inspiration from branched-chain amino acid catabolism

P. taiwanensis VLB120 Valine Catabolism



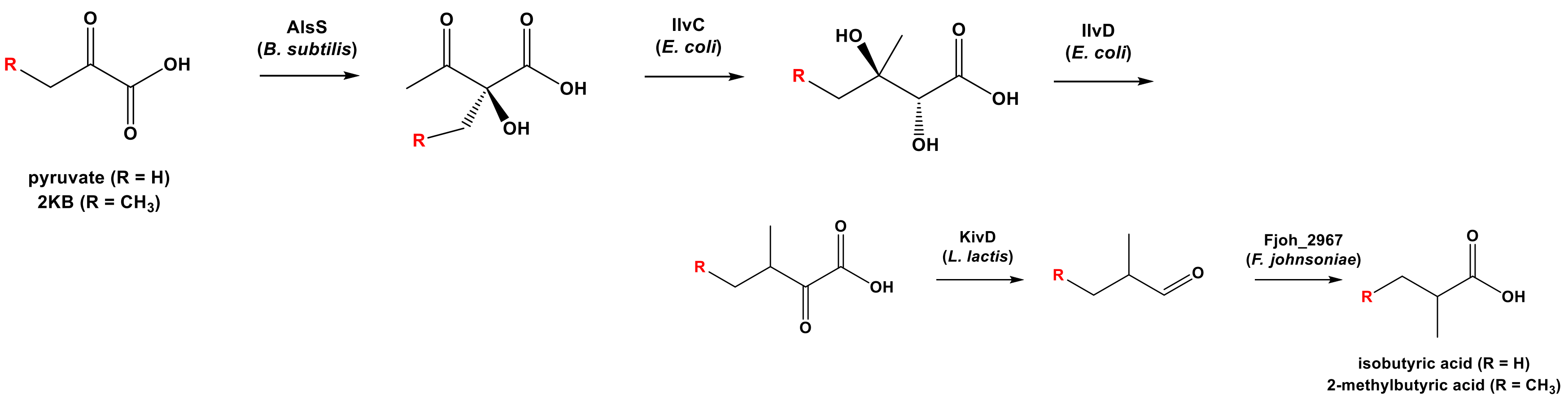
Isobutyric Acid Biosynthesis (Sheppard, M. *et al., Nat. Comm.*,***5*** (2014).) :



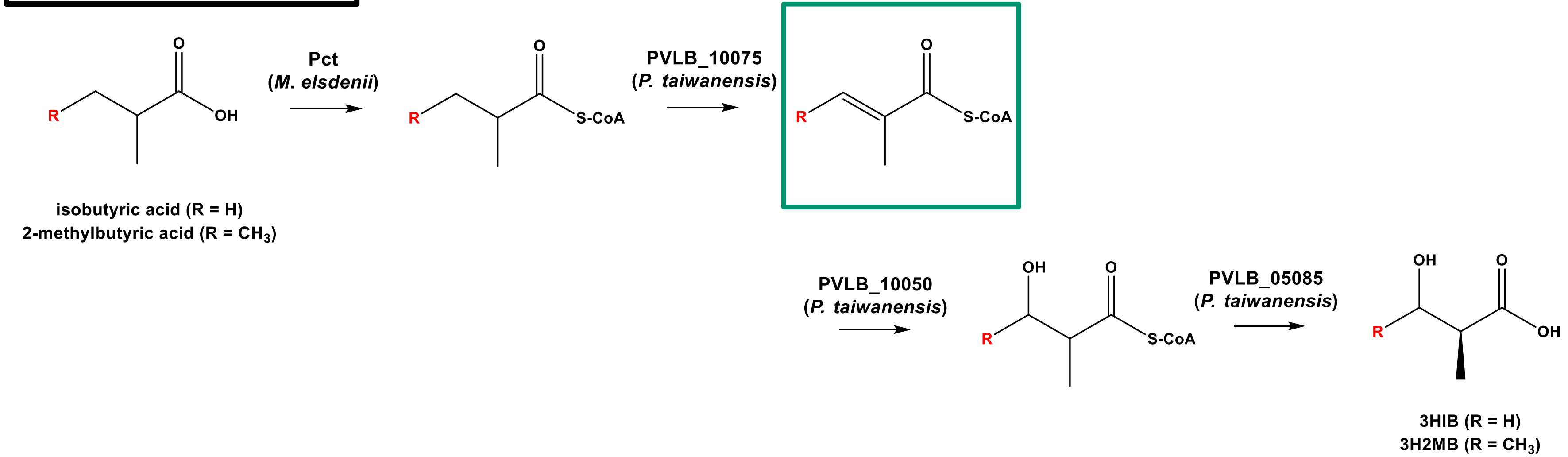
K'yal Bannister

A Platform Pathway to α-Substituted 3HAs

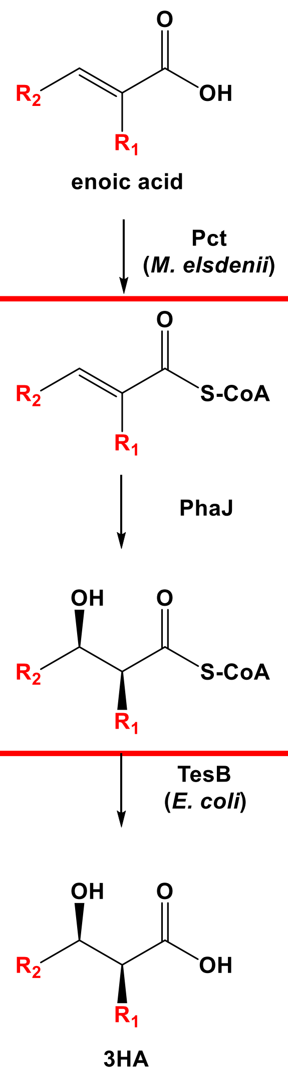
Module 1

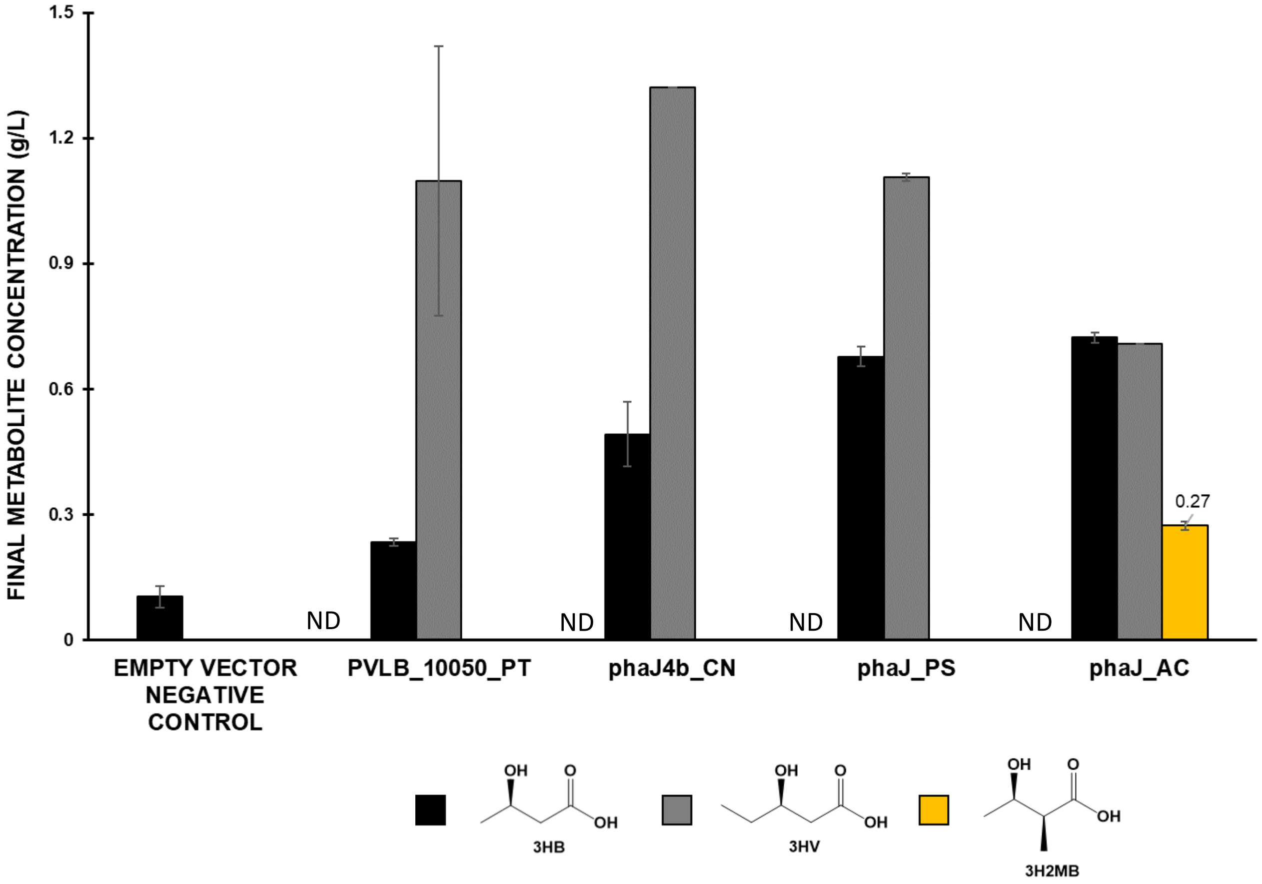


Module 2



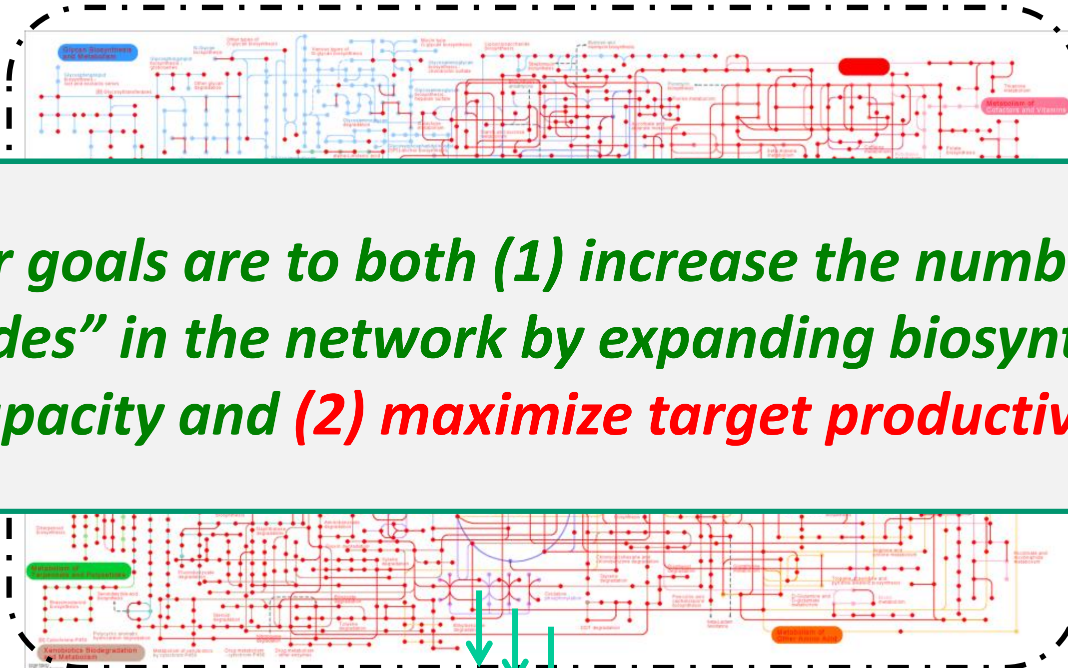
3H2MB Synthesis from Tiglic Acid





PhaJ from *Aeromonas caviae* enables product synthesis

Microbial Chemical Synthesis



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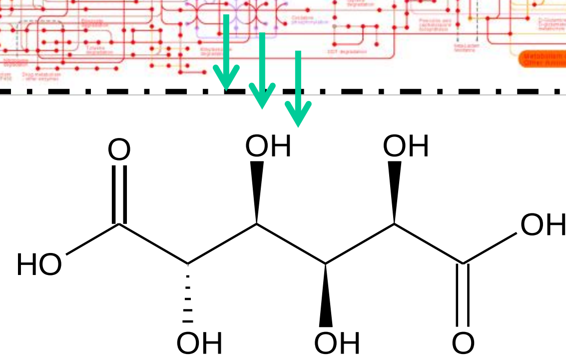


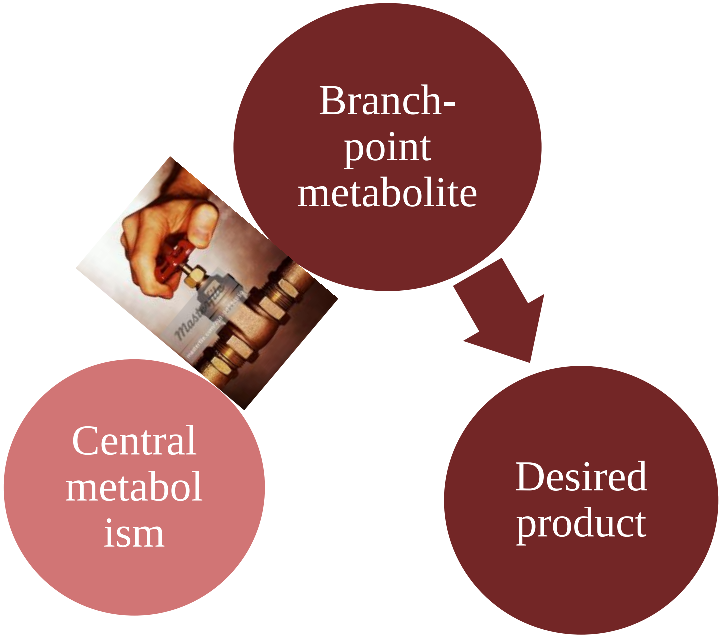
Building Metabolite Valves

✓ Achieved tunable, dynamic control of endogenous gene expression → inducible protein degradation*

✓ Utilized a pathway-independent (small molecule inducible) mechanism

- Still required user intervention

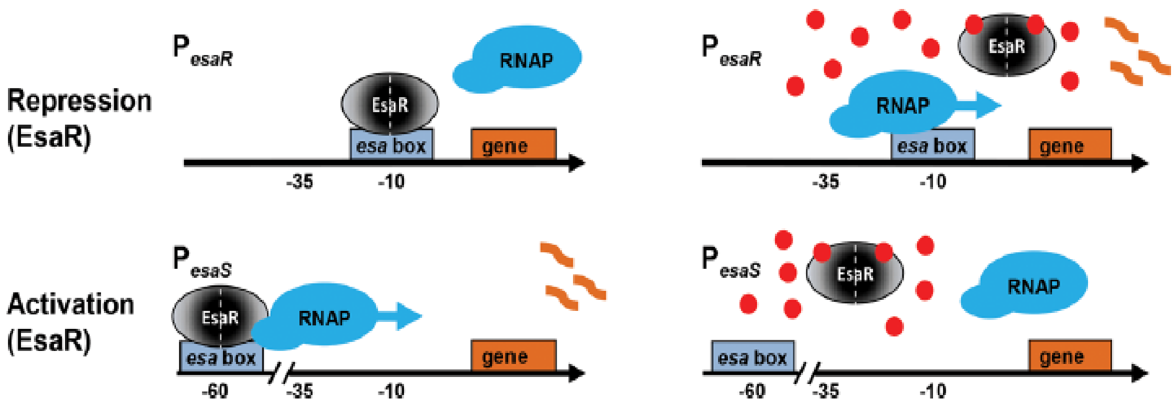
→ ***Autonomous Control***



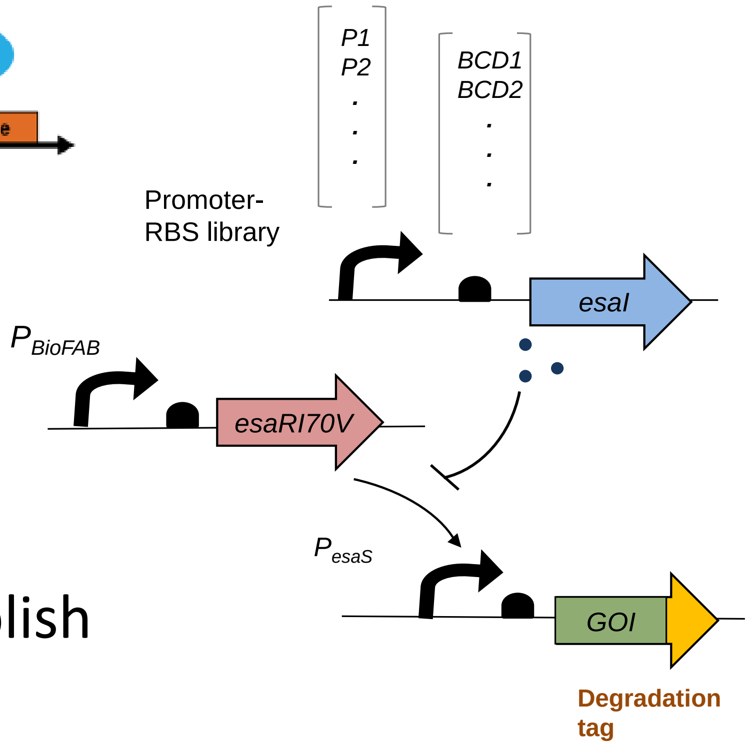
*Brockman and Prather, 2015. *Metabolic Engineering,* 28:104-113.

Turning to Quorum Sensing

Pantoea stewartii Esa System*



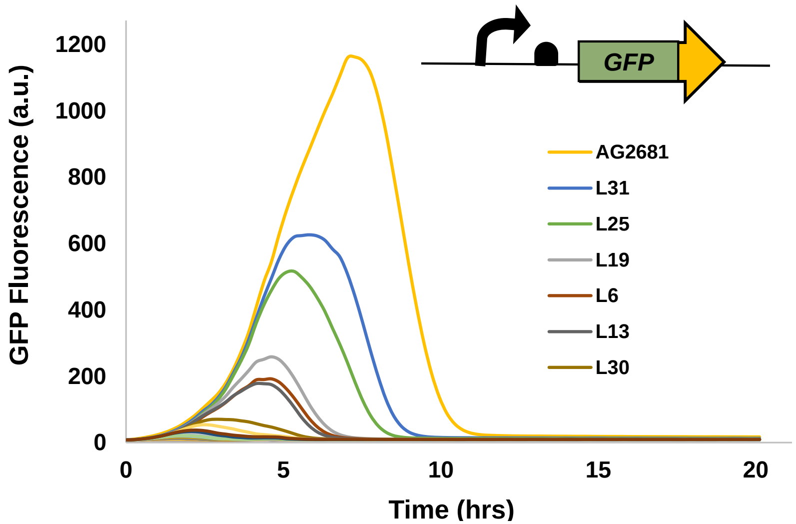
- **EsaI** – AHL synthase
- **EsaR** – Regulator Protein
- **P_{esaR}/P_{esas}** – Cognate Promoters

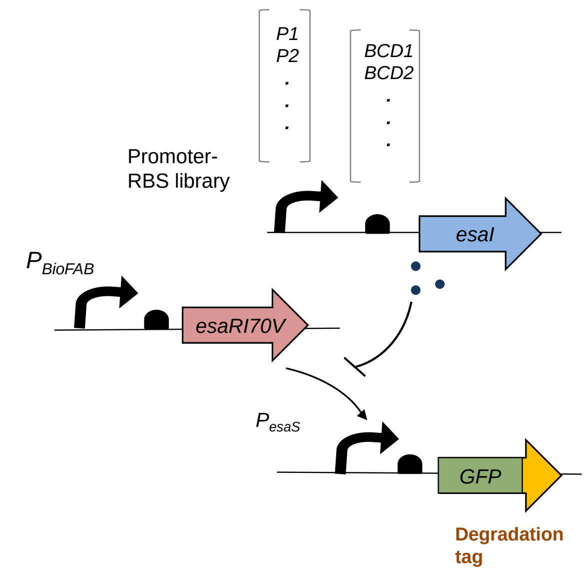


- Utilize the P_{esaS} promoter system
- Build an *esa*I expression library to establish tunability

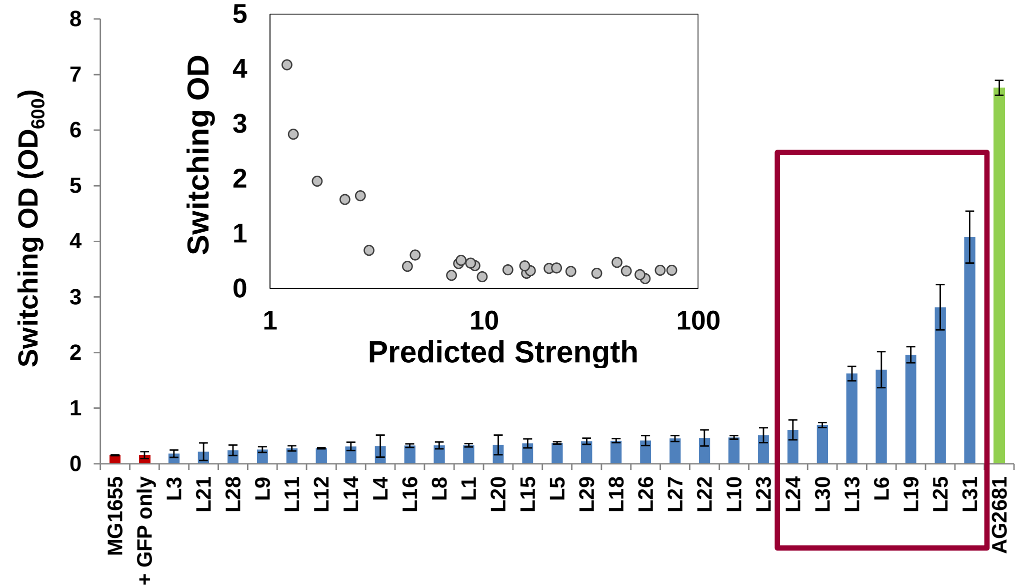
*Shong *et al*. 2013. *ACS Chemical Biology*. **8**:789–795 (Cynthia Collins, RPI).

EsaI Library Characterization



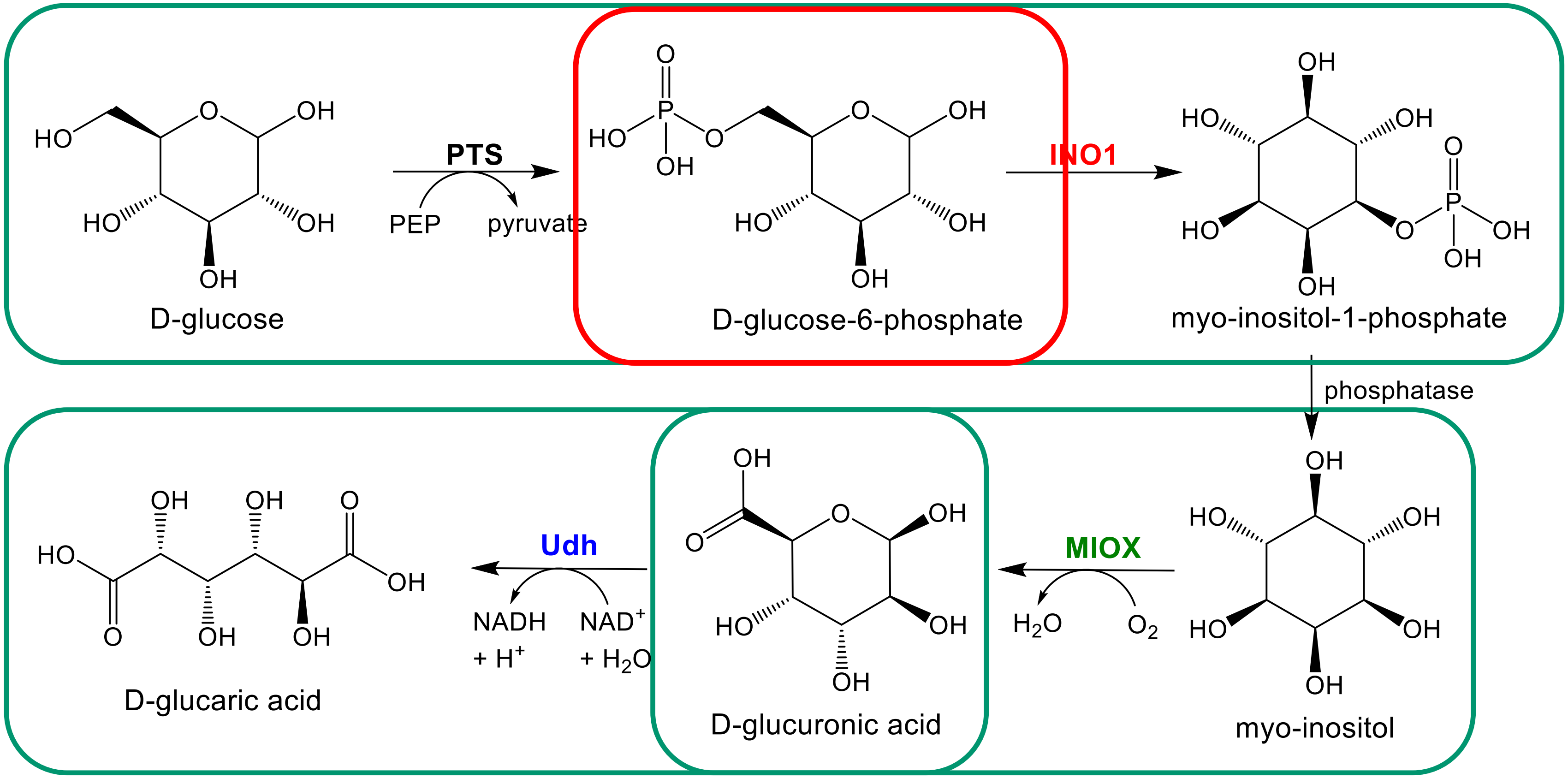


- Excellent agreement of part performance with prediction
- *Only lowest expression levels provide graded response*



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

Glucaric Acid Production in *E. coli*

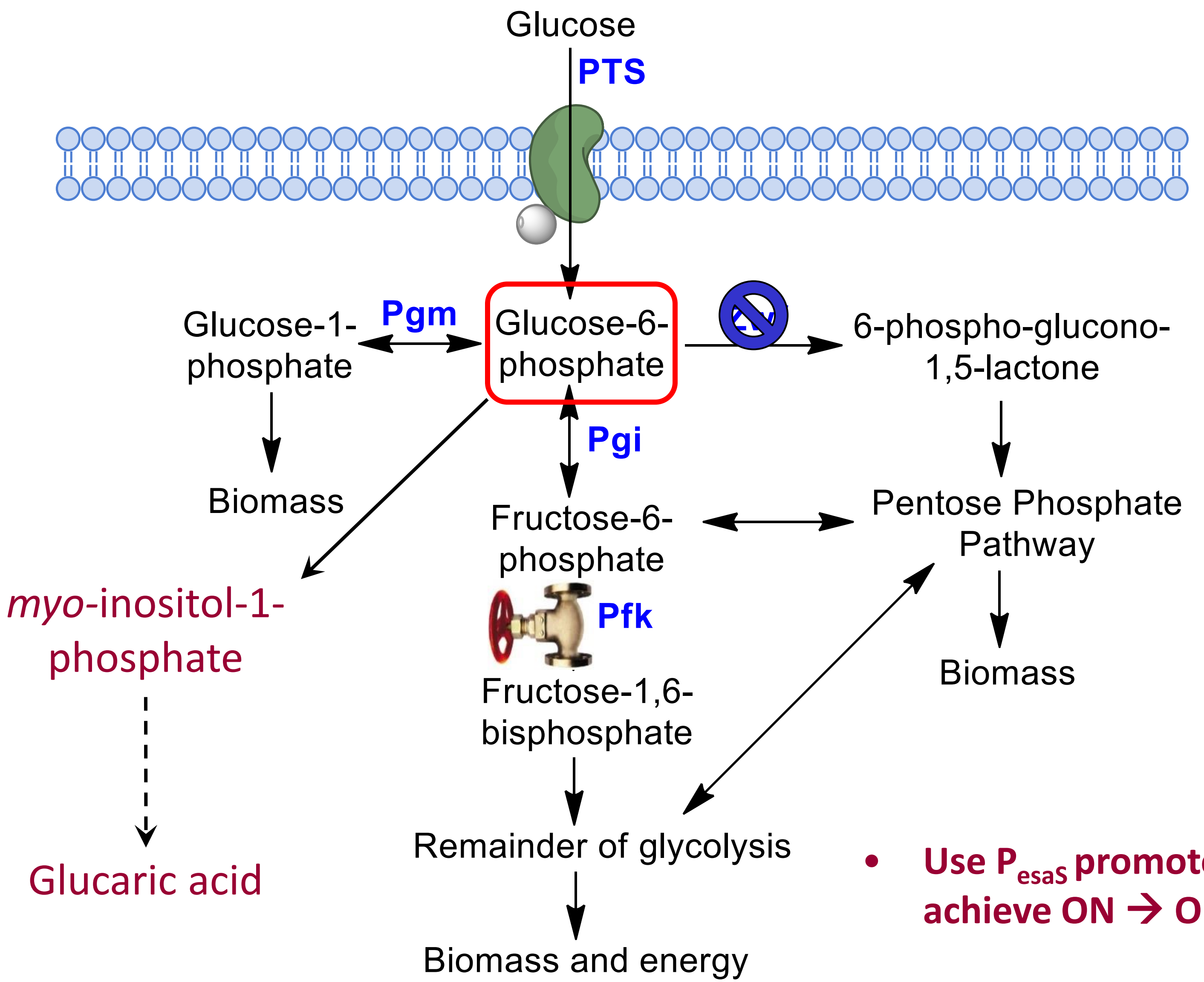


- DOE top "value-added" chemical
- Pharma, water treatment, materials

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



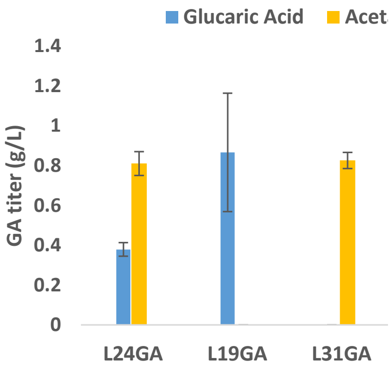
- **Use P_{esaS} promoter system to achieve ON → OFF switching**

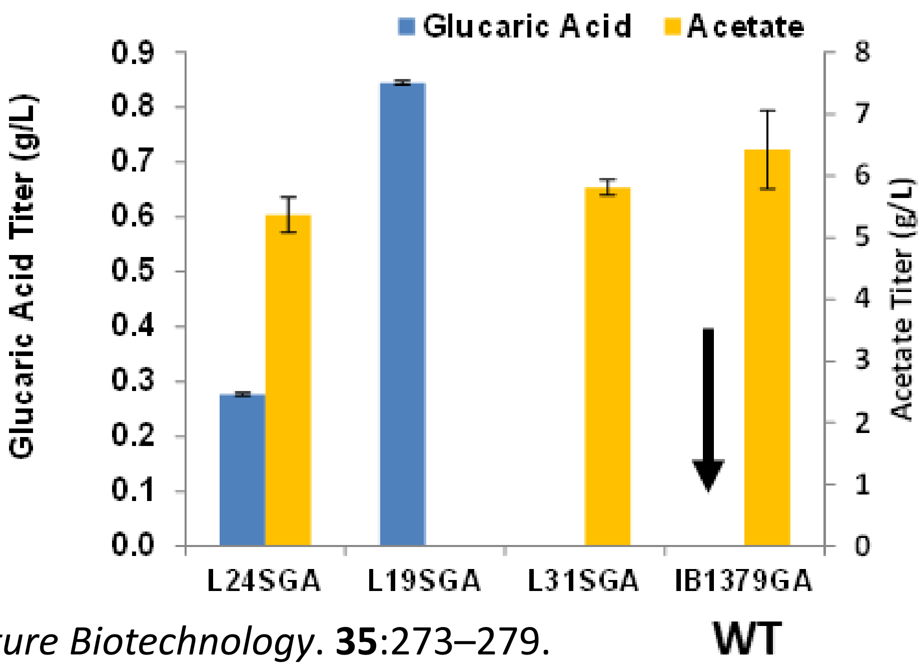
Metabolite Valves Across Scales

m2p-labs BioLector

250-ml shake flasks

Infors 3-L benchtop reactor

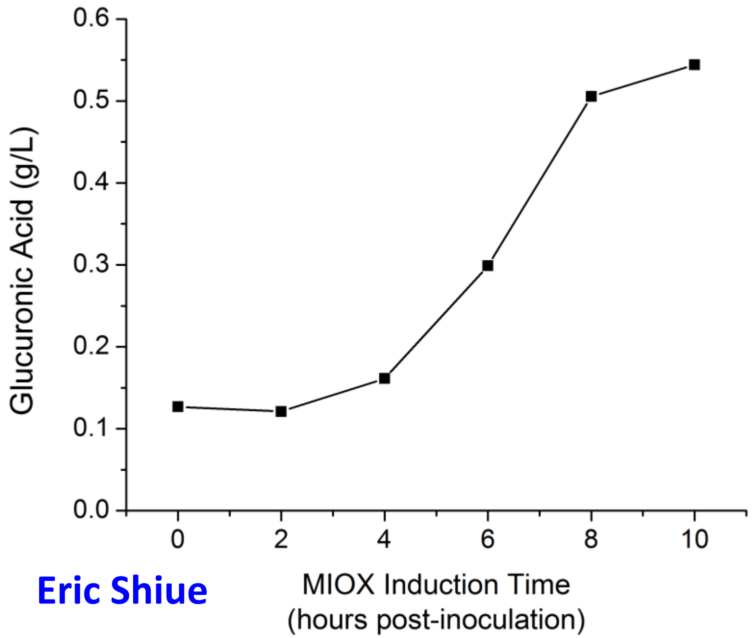




Glucaric acid ir … sistent across scales

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

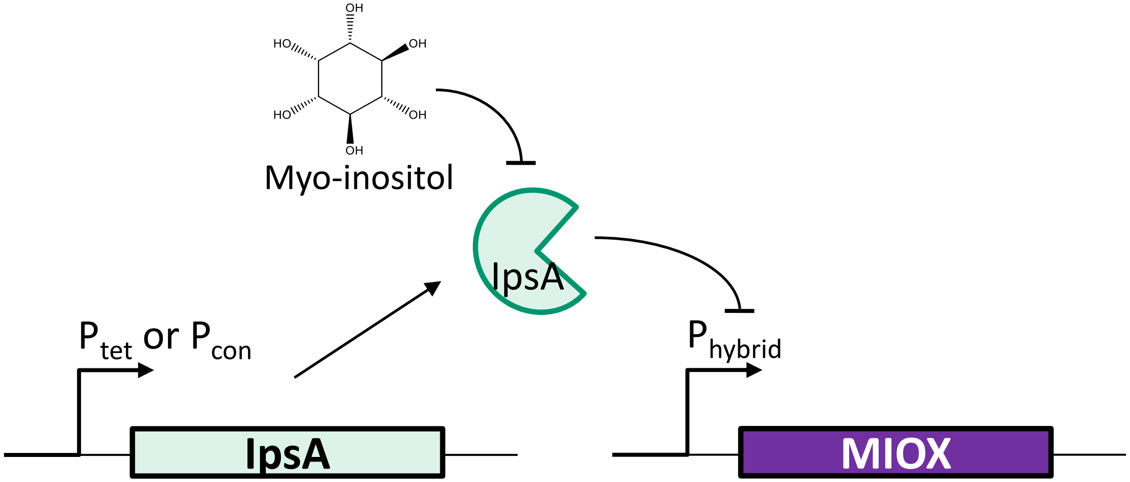
An Additional Control Point – MIOX



Eric Shiue

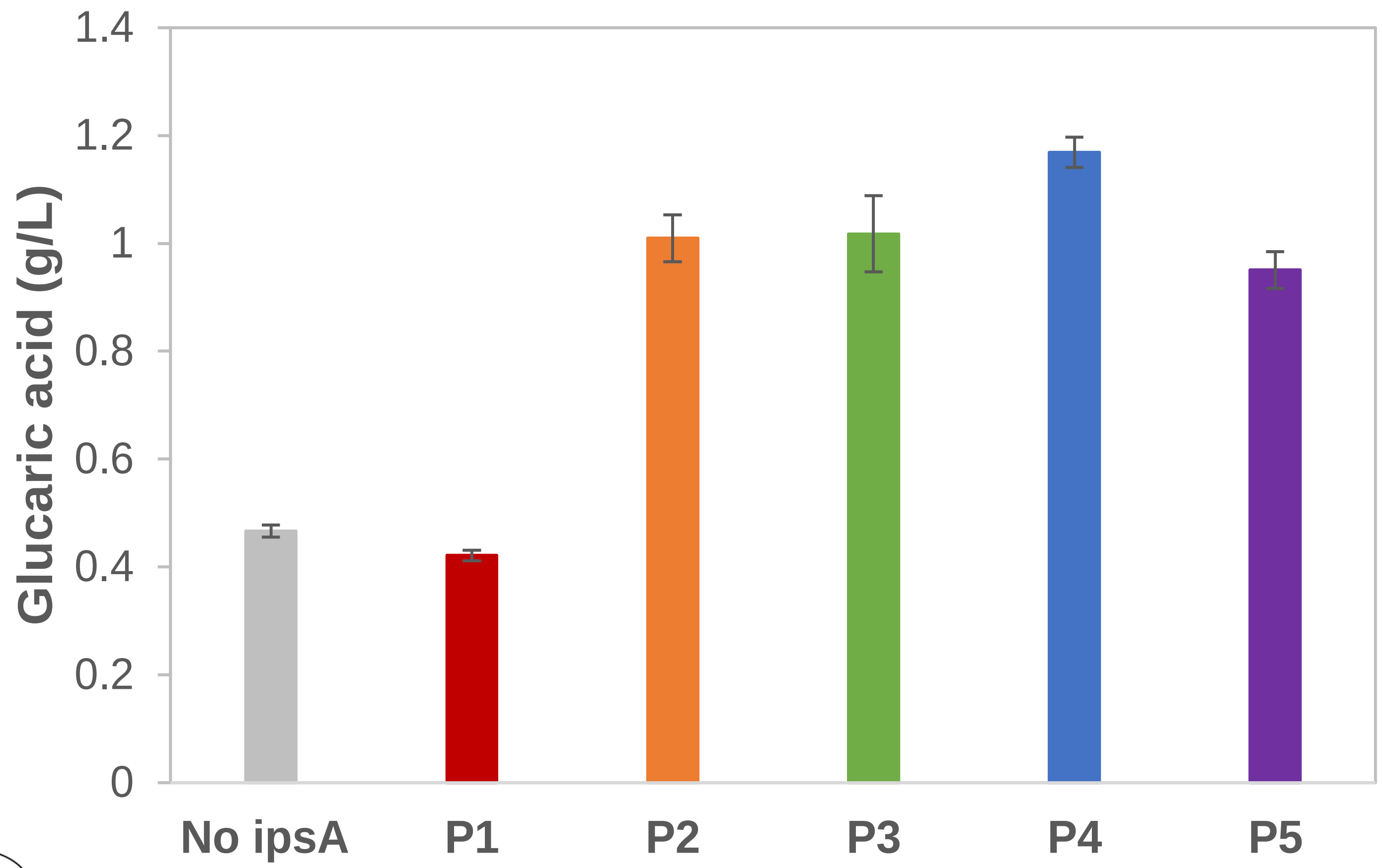
- MIOX activity is increased when cultured in the presence of *myo*-inositol (MI), unstable*
- Product titers are higher when MIOX induction is delayed

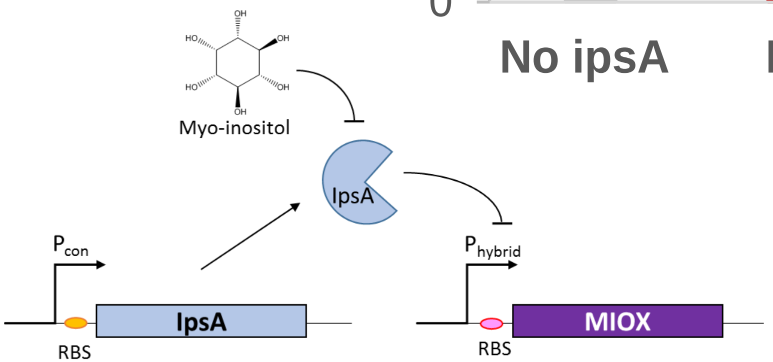
- MI-responsive regulator (IpsA) adapted from *C. glutamicum*
- Can be used to control MIOX induction



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

Metabolite-Driven Production of GA



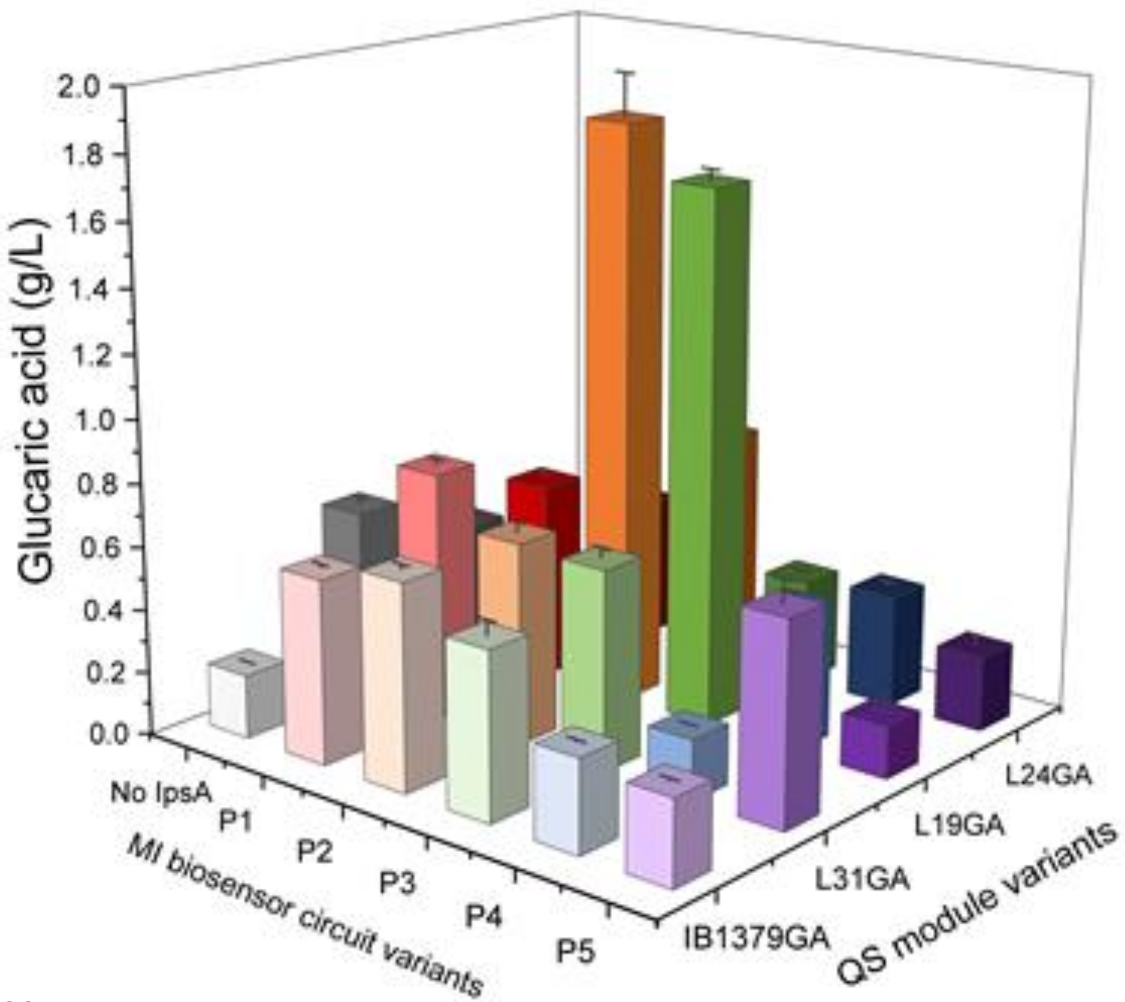


MI-driven MIOX expression results in increased glucaric acid production.

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

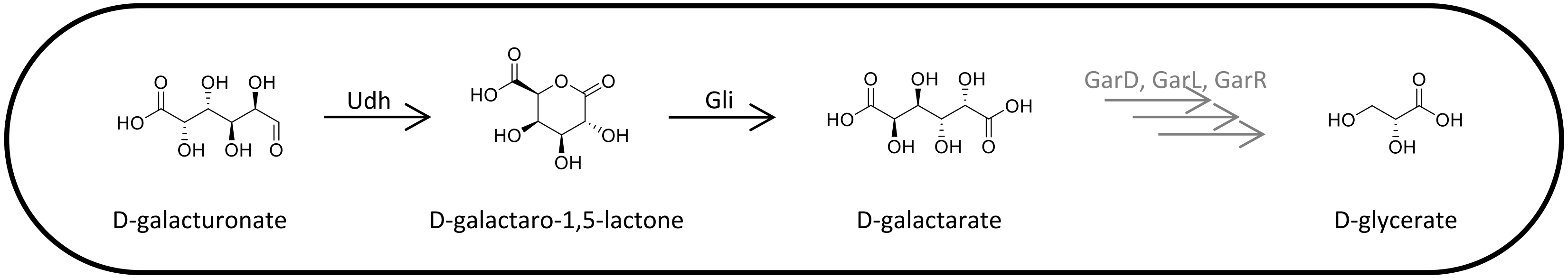
Layering Both Strategies

- Individual strategies increase 2- to 3-fold
- Layered approach increases titers 10-fold
- Highest reported titers to-date in K strains of *E. coli*

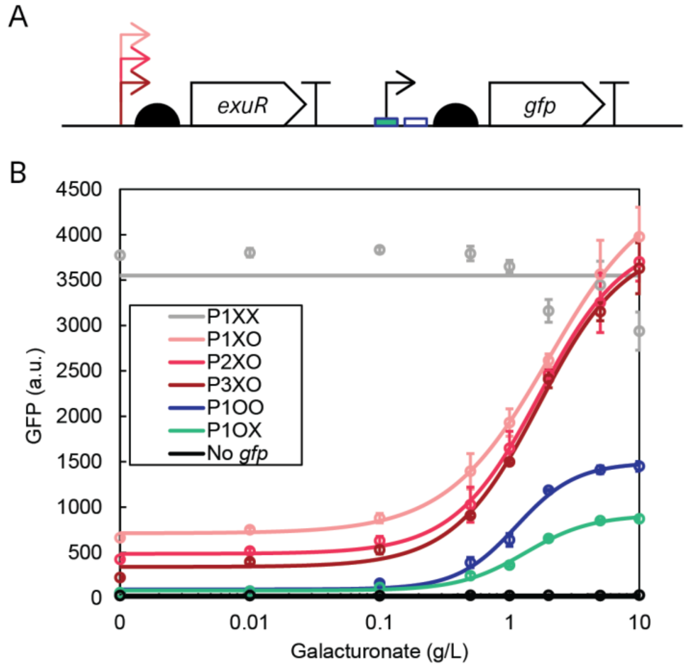


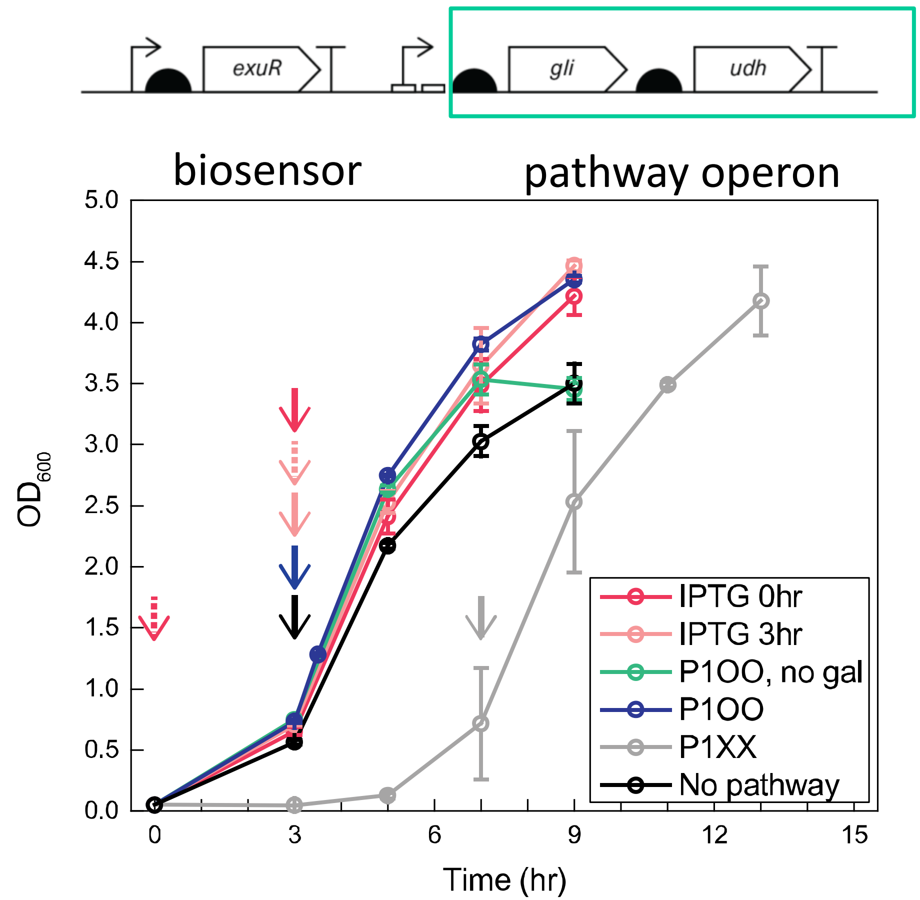
Doong *et al*. 2018. *PNAS.* **115**:2964-2969.

Substrate-Controlled Pathway Regulation



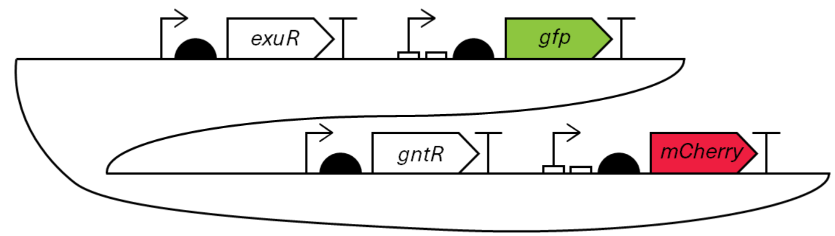
Fox and Prather. 2020. *J. Ind. Microbiol. Biotechnol.*, **47**:1075

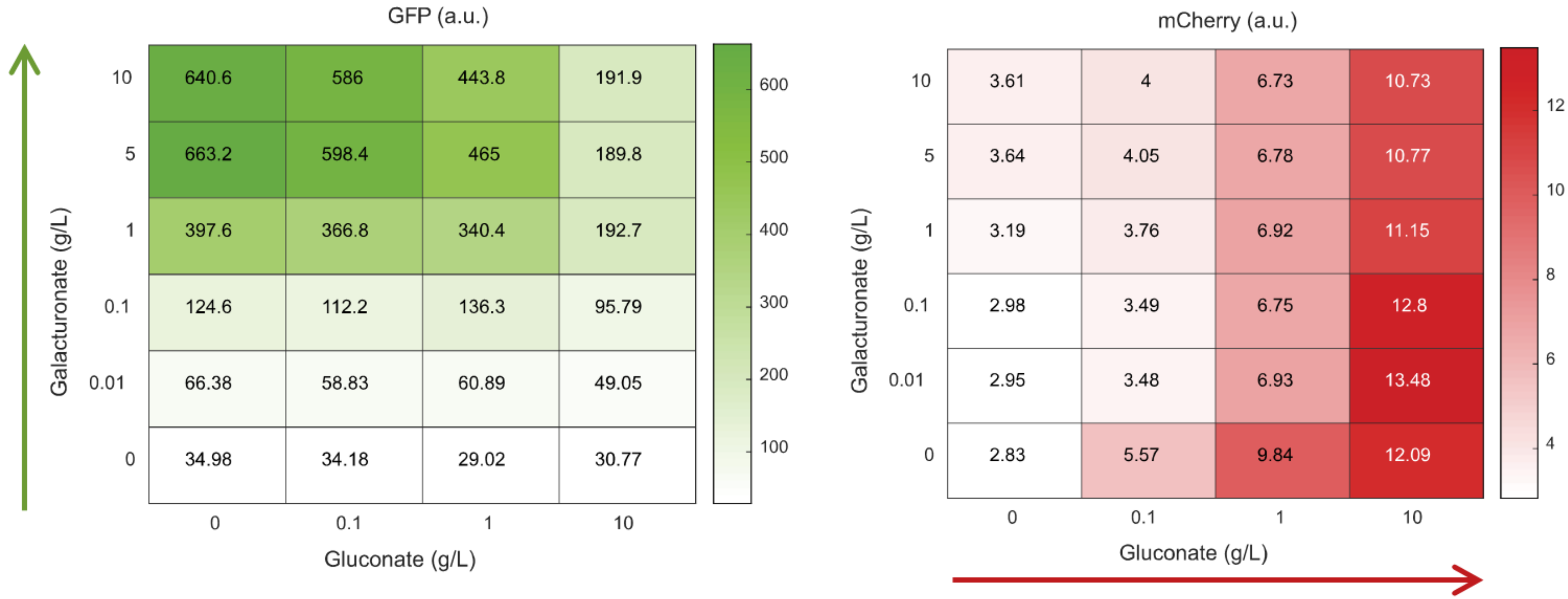




Ni *et al*. 2021. *Biotechnol. J.*, 2000433

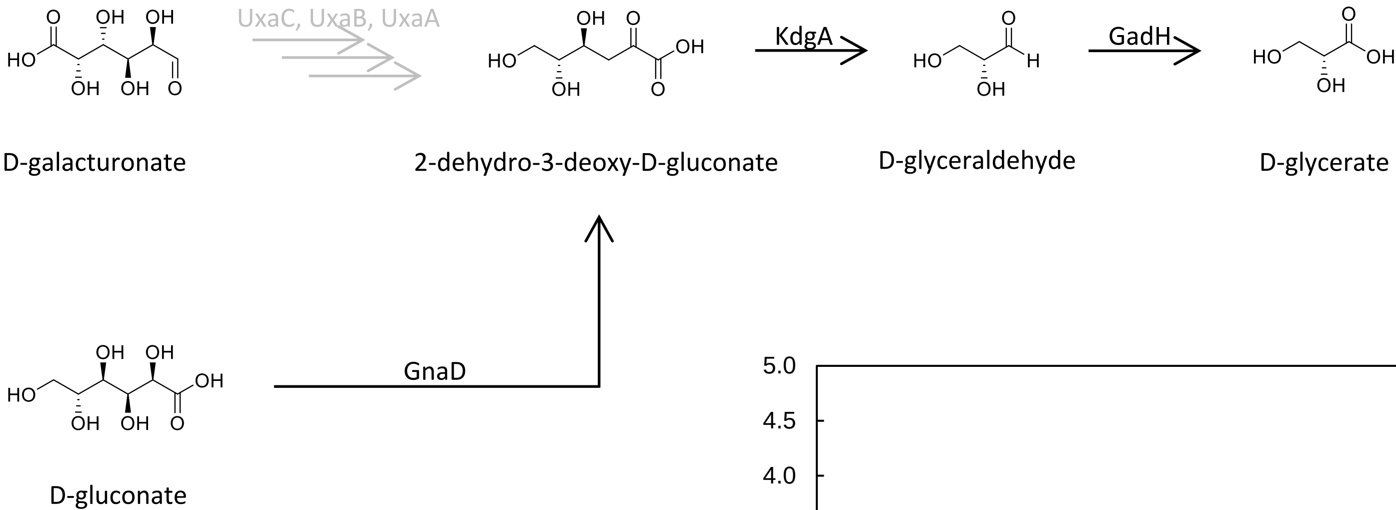
Orthogonal Sensor Construction for Mixed-Substrate Feeds*



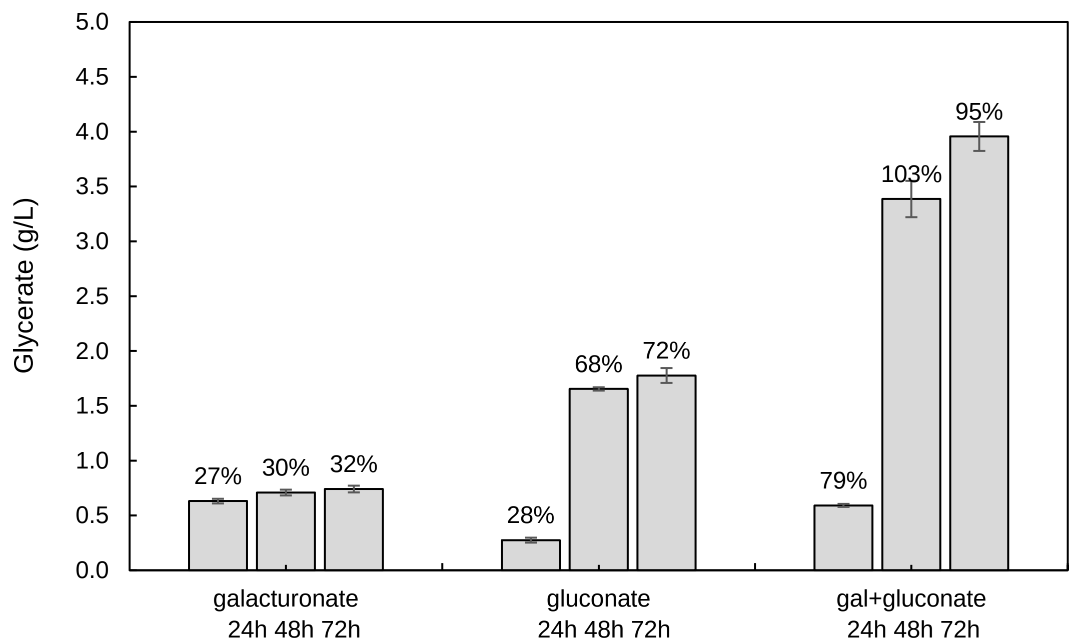


* Motivated by the need to valorization complex wastes such as food, MSW

Mixed Substrate-Single Product Fermentations



Mixed substrate use results in higher conversion, titer, and yield compared to single pathways



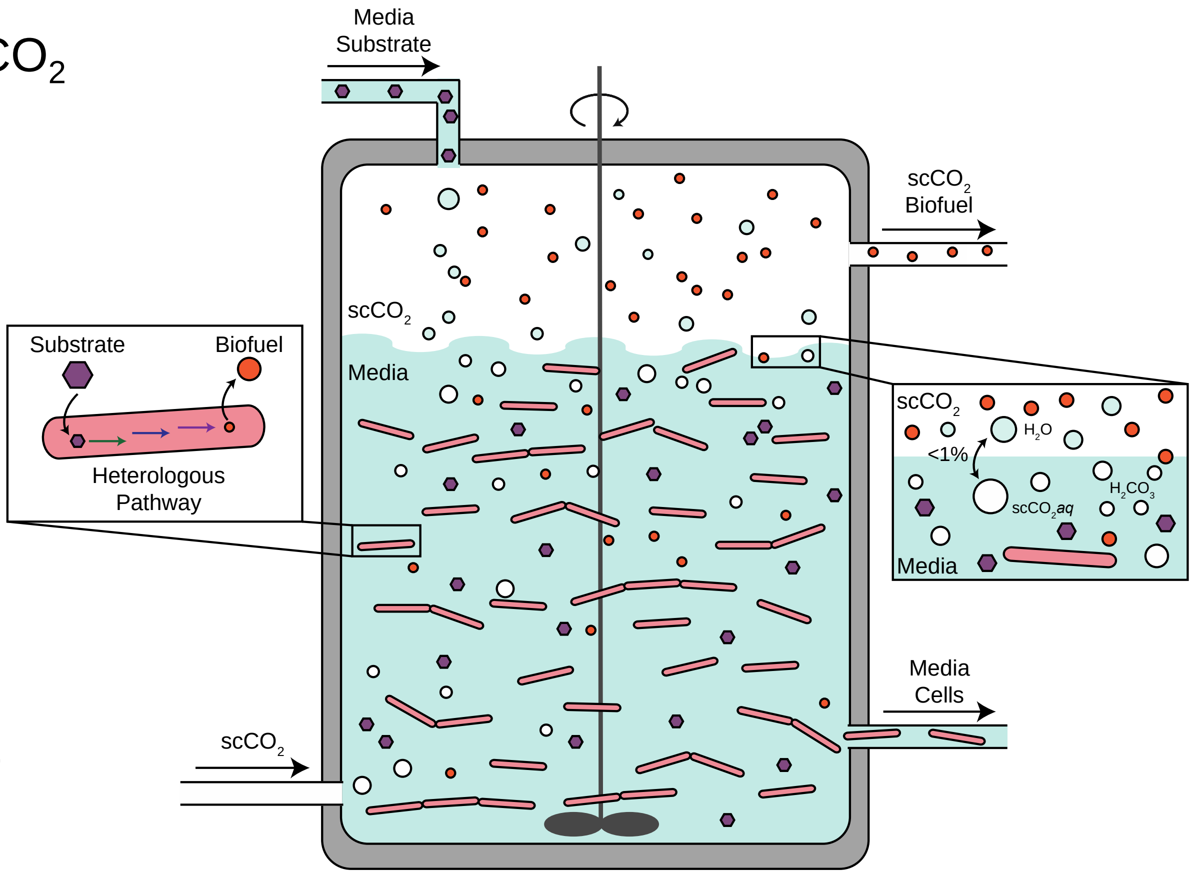
Cynthia Ni

Biofuels production using $scCO_2$-tolerant bacteria

Goal: isolate bacteria capable of robust growth under $scCO_2$, engineer them for biofuel production, and scale up the process

Advantages of growth in $scCO_2$
- Microbial sterility
- Product extraction

Why CO_2?
- Sustainable solvent
- Preferential extraction of desired products
- Concentration of final products
- Possible continuous stripping of product



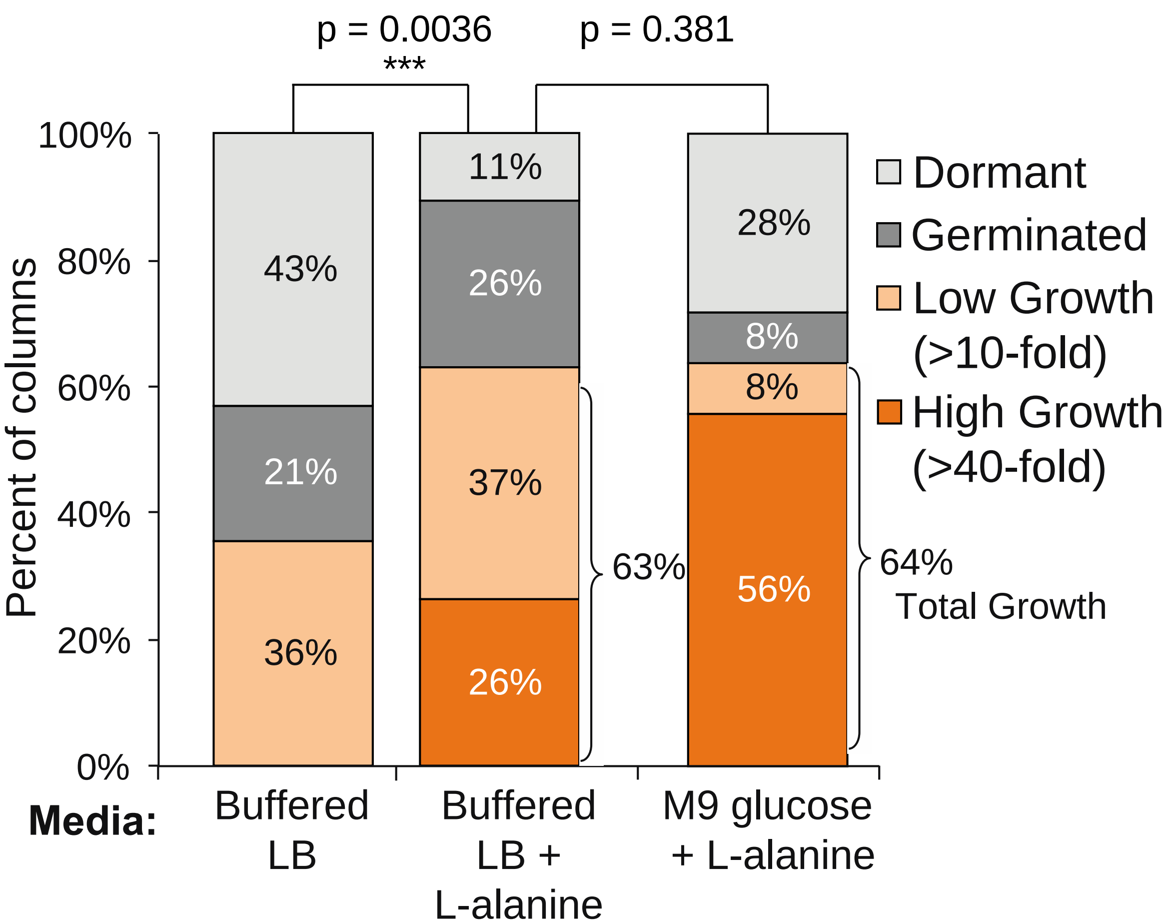
Bacillus megaterium strain SR7 isolated from natural CO_2 reservoir in Colorado

Freedman, A.J.E et al. (2018) *Frontiers Microbiol.* 9:2152

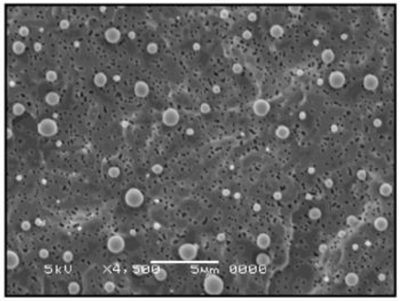
Improving growth under $scCO_2$

Inoculate reactors with spores of SR7

Modifications to media and supplements facilitate germination, growth



SR7 Spores

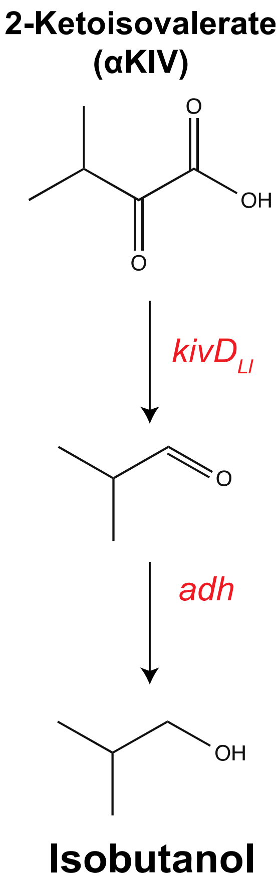


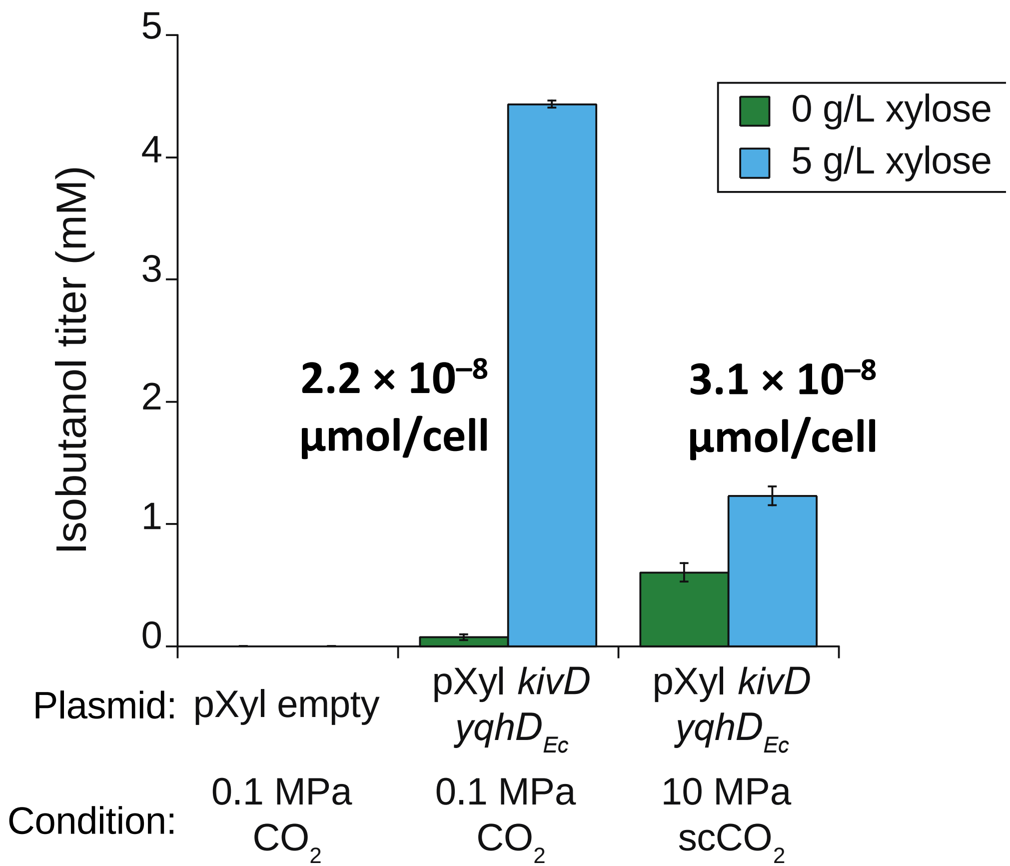
Adam Freedman
Thompson lab

Freedman, A.J.E et al. (2018) *Frontiers Microbiol.* 9:2152

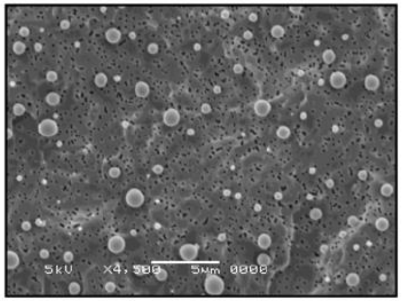
Isobutanol production in SR7 under $scCO_2$

SR7 pXyl KivD, YqhD; M9 medium + 5 mM αKIV
($scCO_2$ with 100 mM alanine)





SR7 Spores

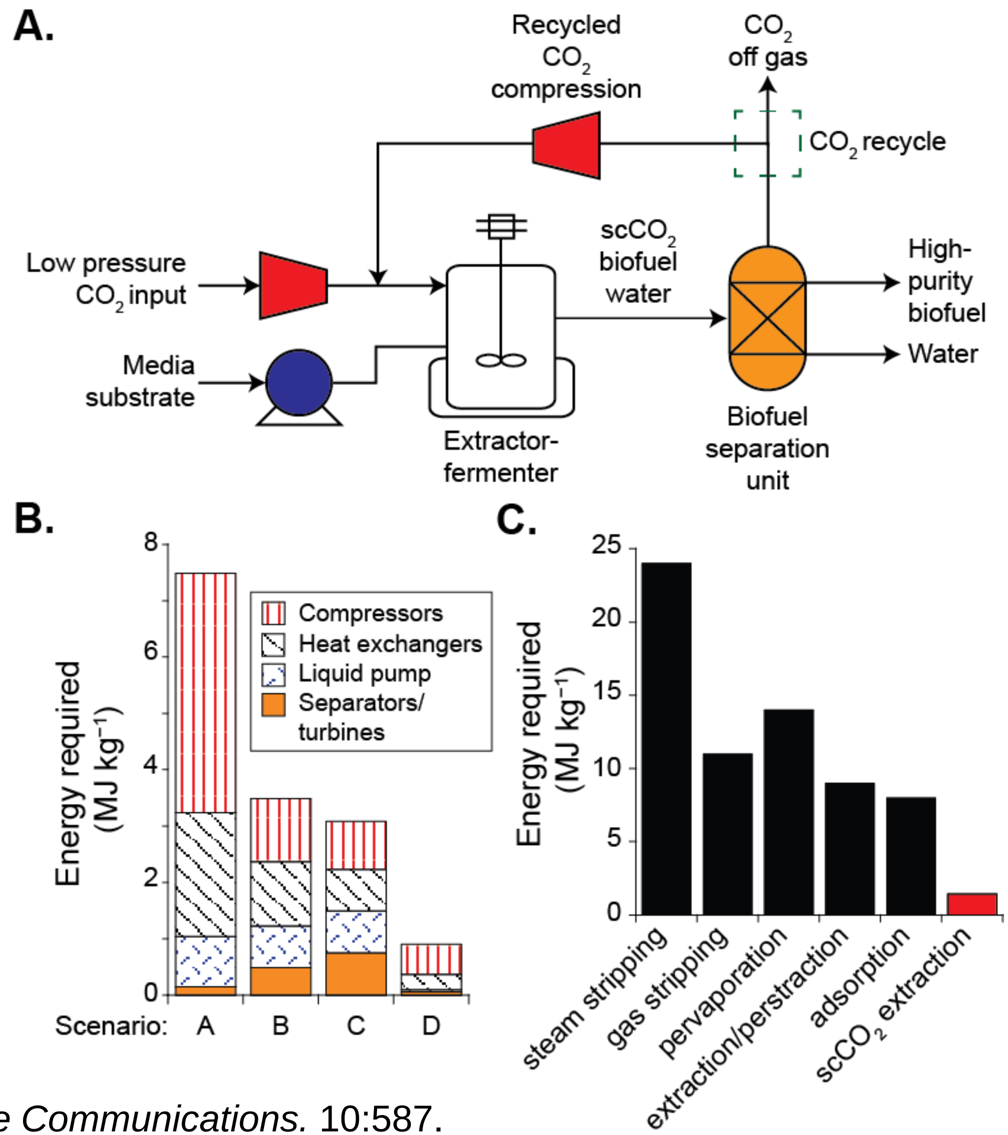


Production of isobutanol under $scCO_2$ in high metabolic activity cultures

Boock et al, (2019) *Nature Communications.* 10:587.

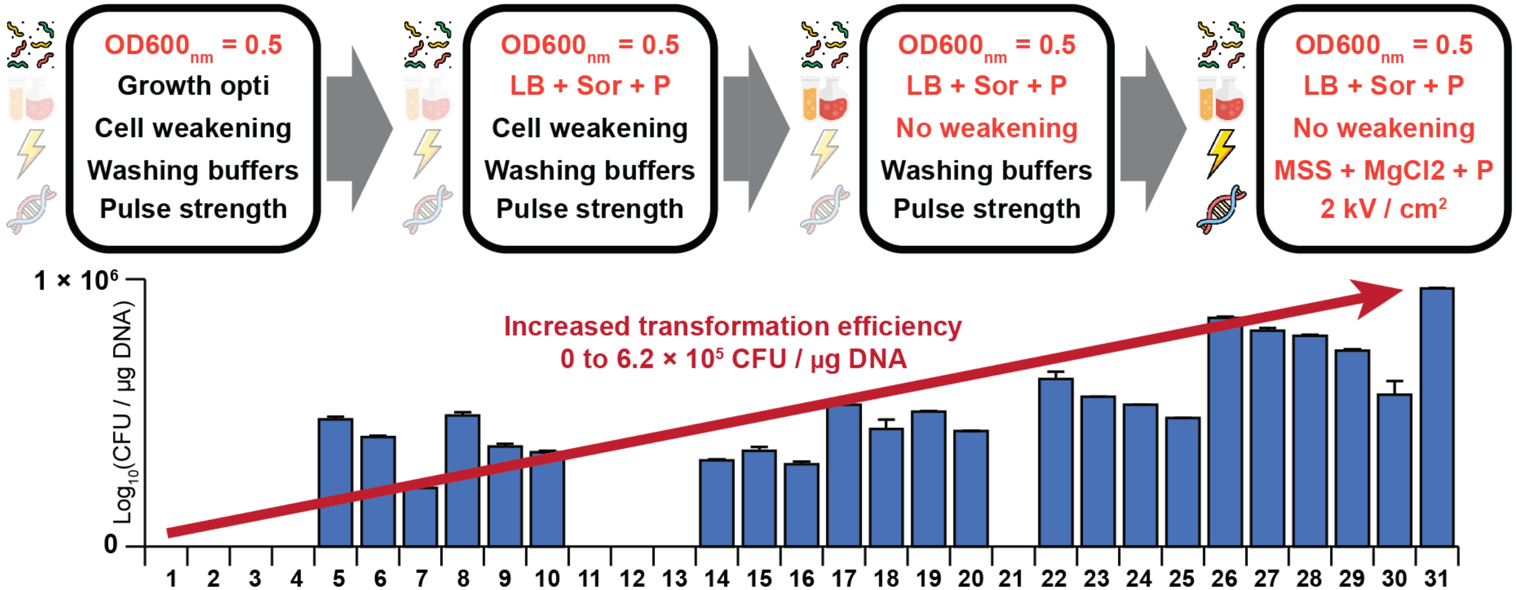
Bioprocess module of *in situ* extraction using $scCO_2$

ASPEN model of the energetics of $scCO_2$ bioprocessing



Boock et al, (2019) *Nature Communications.* 10:587.

Towards Biosystems Design



1 Water
2 Glycerol (10%)
3 PEG1000 (25%)
4 PEG8000 (25%)
5 Mannitol (0.25 M)
6 Sorbitol (0.25 M)
7 Sucrose (0.25 M)
8 Mannitol (0.50 M)
9 Sorbitol (0.50 M)
10 Sucrose (0.50 M)
11 Mannitol (0.75 M)
12 Sorbitol (0.75 M)
13 Sucrose (0.75 M)
14 Mannitol + Sorbitol (0.25 M)
15 Mannitol + Sucrose (0.25 M)
16 Sorbitol + Sucrose (0.25 M)
17 Mannitol + Sorbitol + Sucrose (0.25 M)
18 Mannitol + Sorbitol (0.50 M)
19 Mannitol + Sucrose (0.50 M)
20 Sorbitol + Sucrose (0.50 M)
21 Mannitol + Sorbitol + Sucrose (0.50 M)
22 Mannitol + Sorbitol + Sucrose (0.25 M) + $MgCl_2$(0.50 mM)
23 Mannitol + Sorbitol + Sucrose (0.25 M) + $MgCl_2$(1.00 mM)
24 Mannitol + Sorbitol + Sucrose (0.25 M) + $MgCl_2$(1.50 mM)
25 Mannitol + Sorbitol + Sucrose (0.25 M) + $MgCl_2$(2.00 mM)
26 Mannitol + Sorbitol + Sucrose (0.25 M) + NaPhosphate (0.50 mM)
27 Mannitol + Sorbitol + Sucrose (0.25 M) + NaPhosphate (1.00 mM)
28 Mannitol + Sorbitol + Sucrose (0.25 M) + NaPhosphate (1.50 mM)
29 Mannitol + Sorbitol + Sucrose (0.25 M) + NaPhosphate (2.00 mM)
30 Mannitol + Sorbitol + Sucrose (0.25 M) + NaPhosphate (2.50 mM)
31 Mannitol + Sorbitol + Sucrose (0.25 M) + MgCl2 (0.50 mM) + NaPhosphate (0.50 mM)

Transformation efficiency increased from a few colonies per µg DNA to levels acceptable for genome-scale engineering

Yoseb Song

Summary

- Biological synthesis is an effective tool for the production of diverse chemical products.
- Pathway optimization benefits from new tools to regulate metabolic flux and biosynthetic pathways.
- Non-model microbes open the door to new modes of bioprocess design that may be preferential for toxic (and useful) products.

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