SYNTHETIC BIOCHEMISTRY

Making Biofuels and Commodity Chemicals the Cell-Free Way

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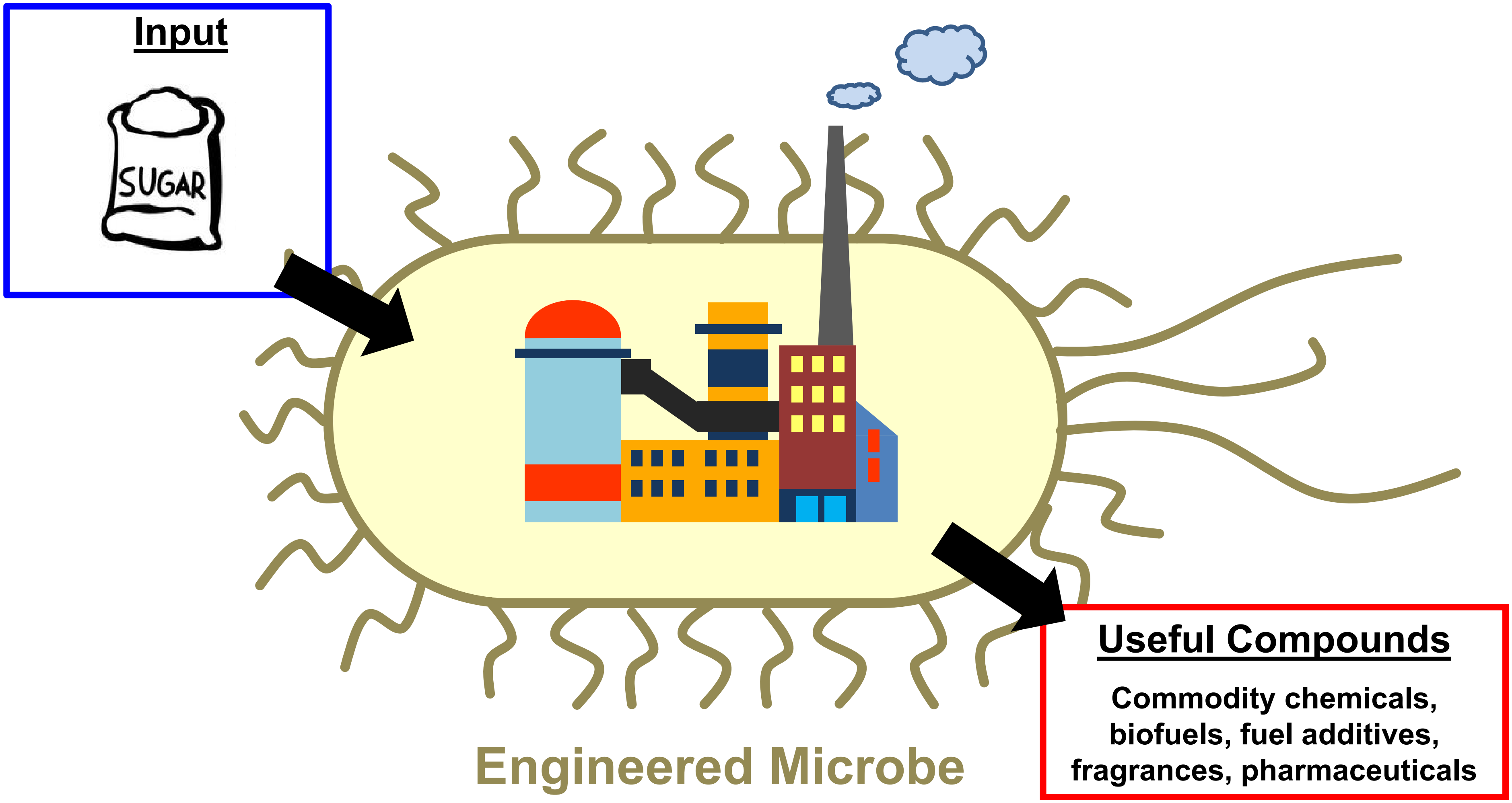
Synthetic Biology/Metabolic Engineering



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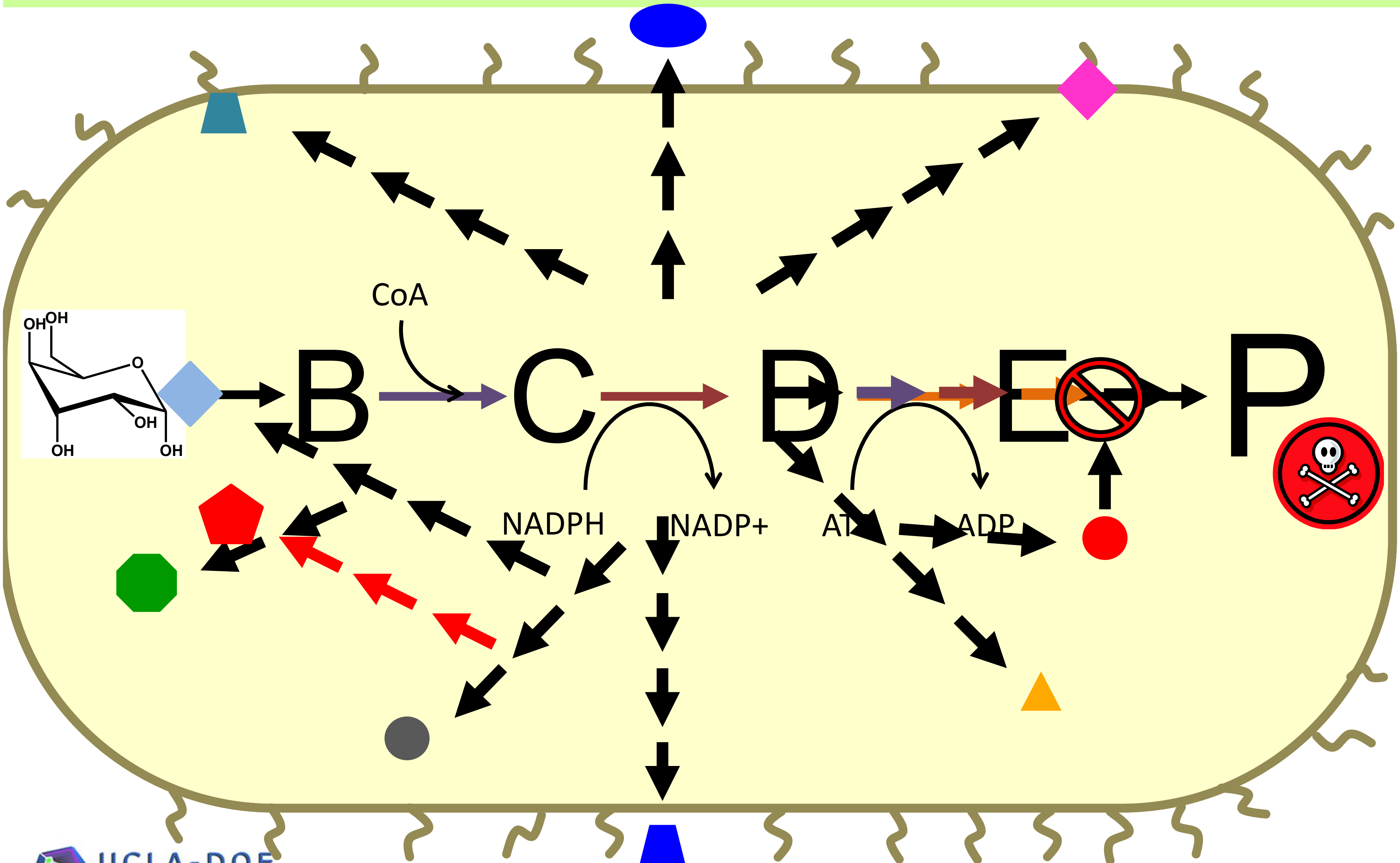
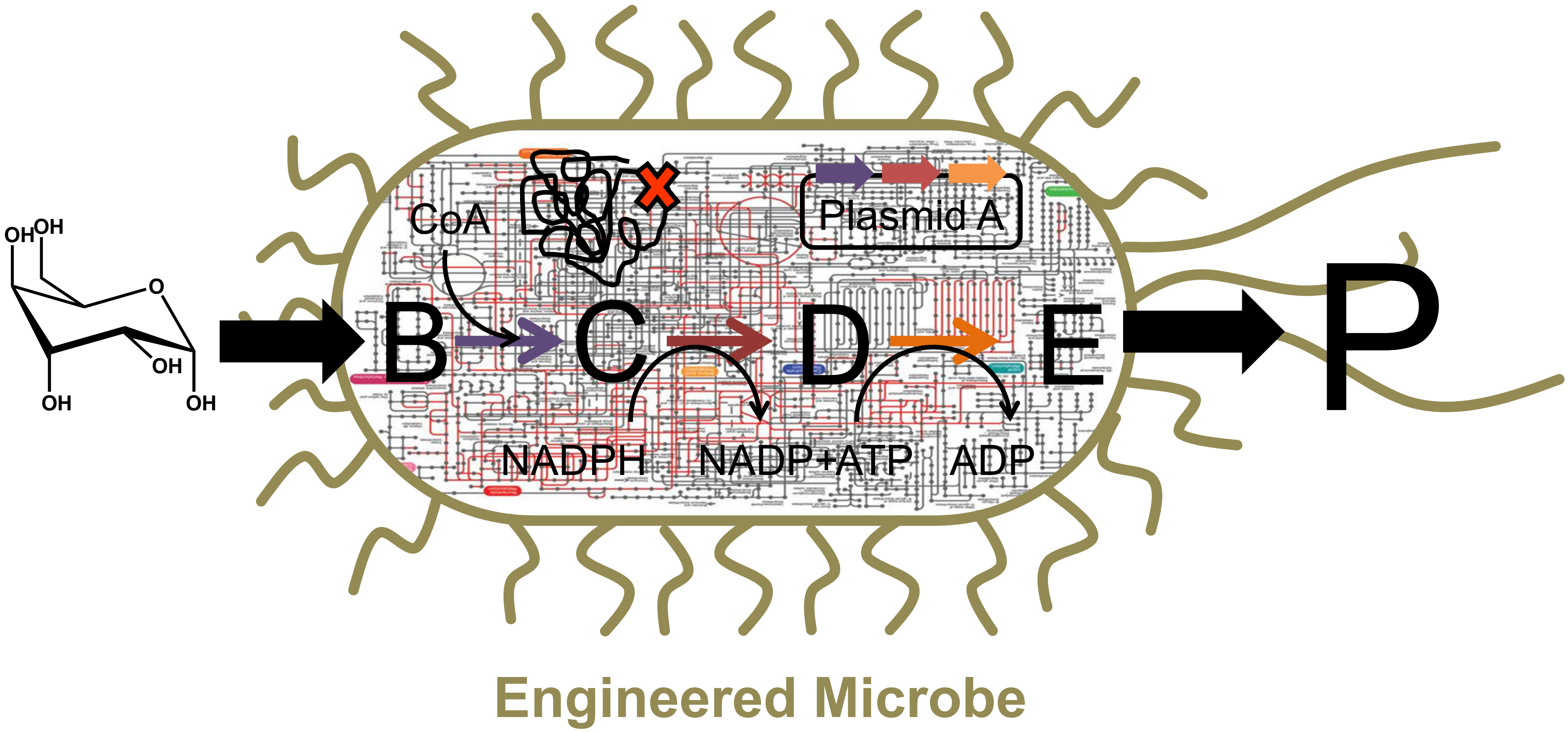
The Problem(s) with Cells



Solution: Synthetic Biochemistry



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Synthetic Biochemistry

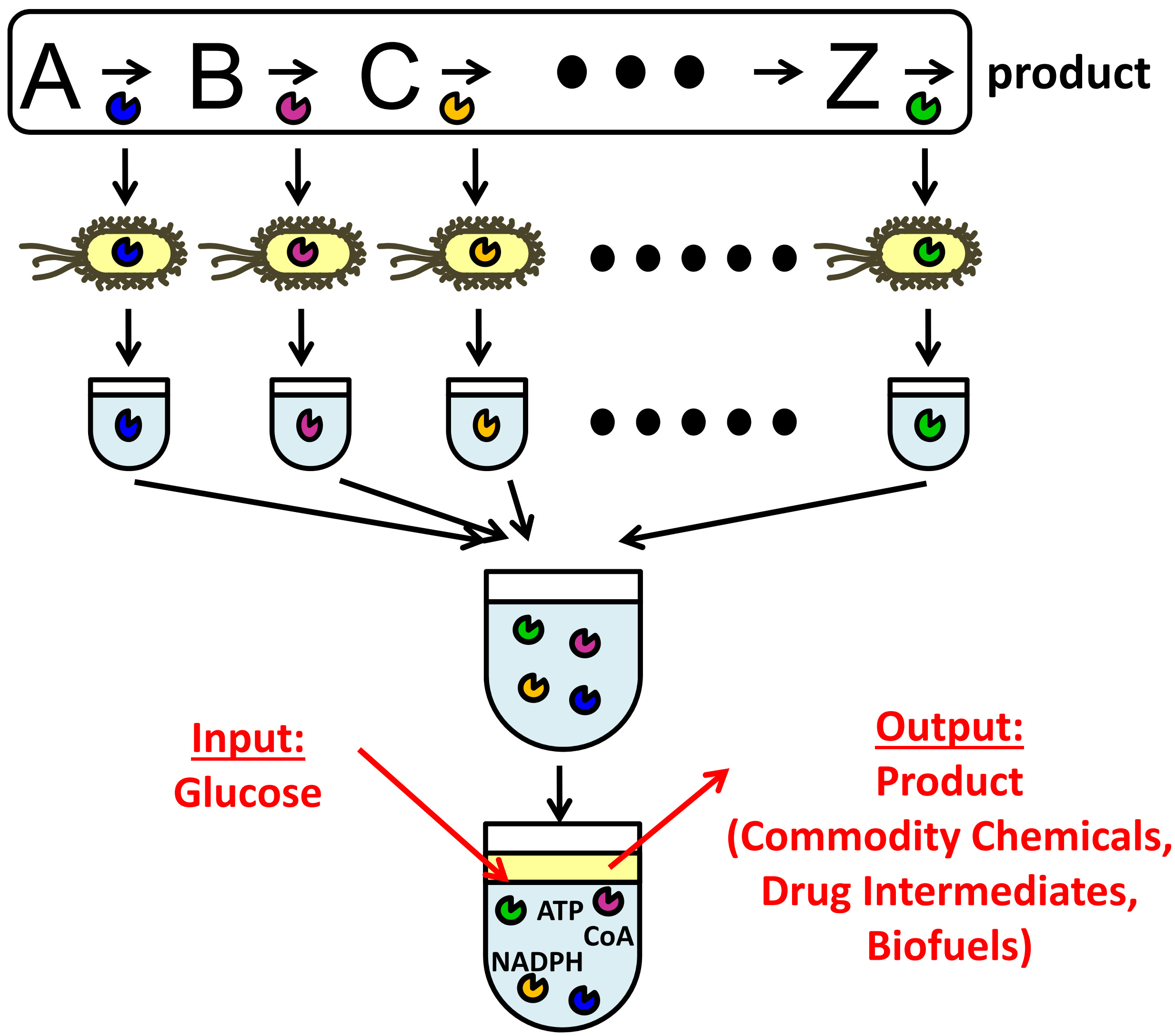
1. Design Biochemical Transformation

2. Clone/Express Enzymes

3. Purify/Isolate Enzymes

4. Mix Enzymes

5. Run Bioreactor



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Main Advantages

- High yields
- Easy optimization
- Can redesign central metabolism
- Toxicity not a concern
- Easier product purification
- Potential for much higher productivity

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Main Challenges

- Enzyme cost
- Enzyme stability
- Cofactor Recycling and Maintenance

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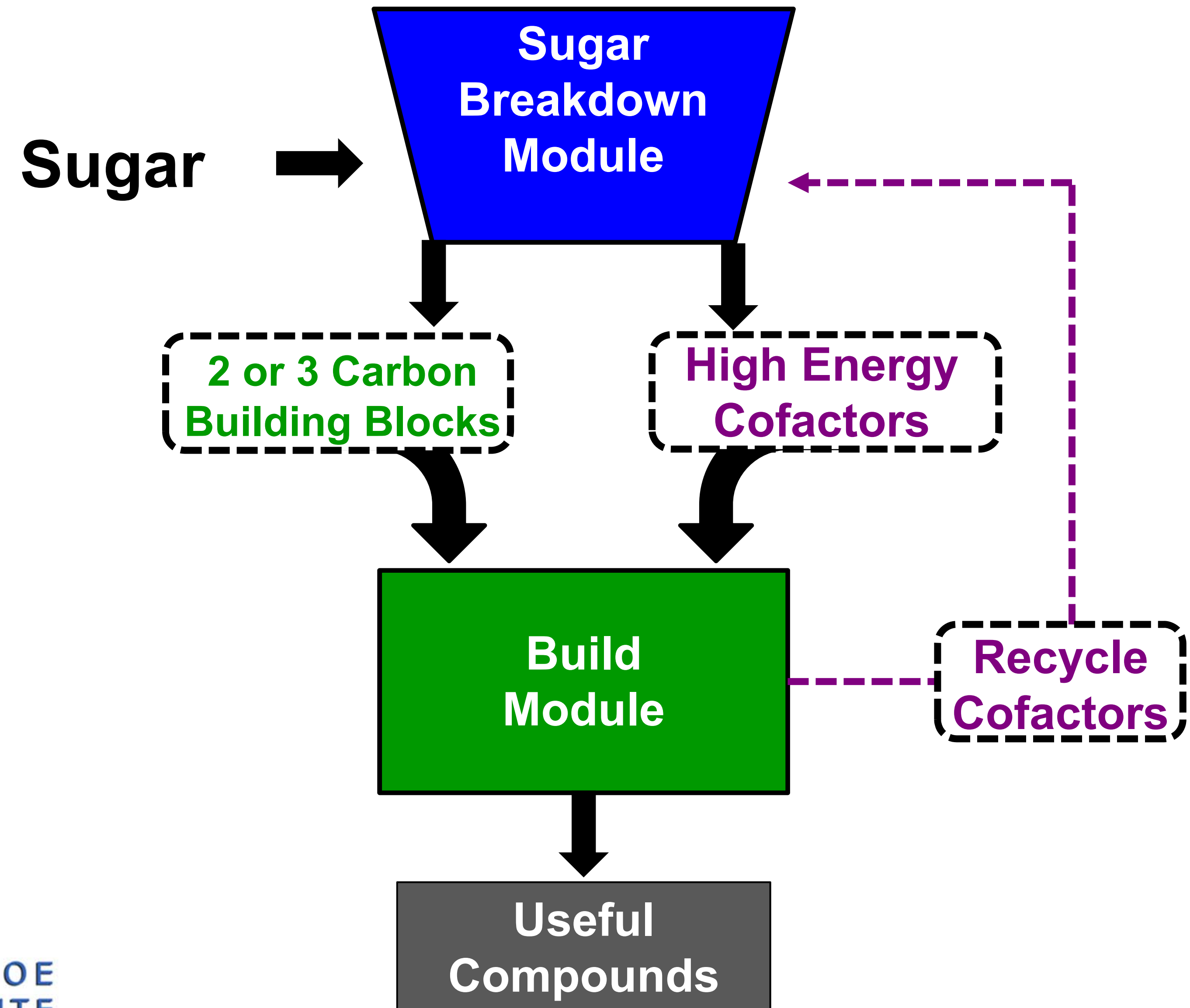
Basic Synthetic Biochemistry System



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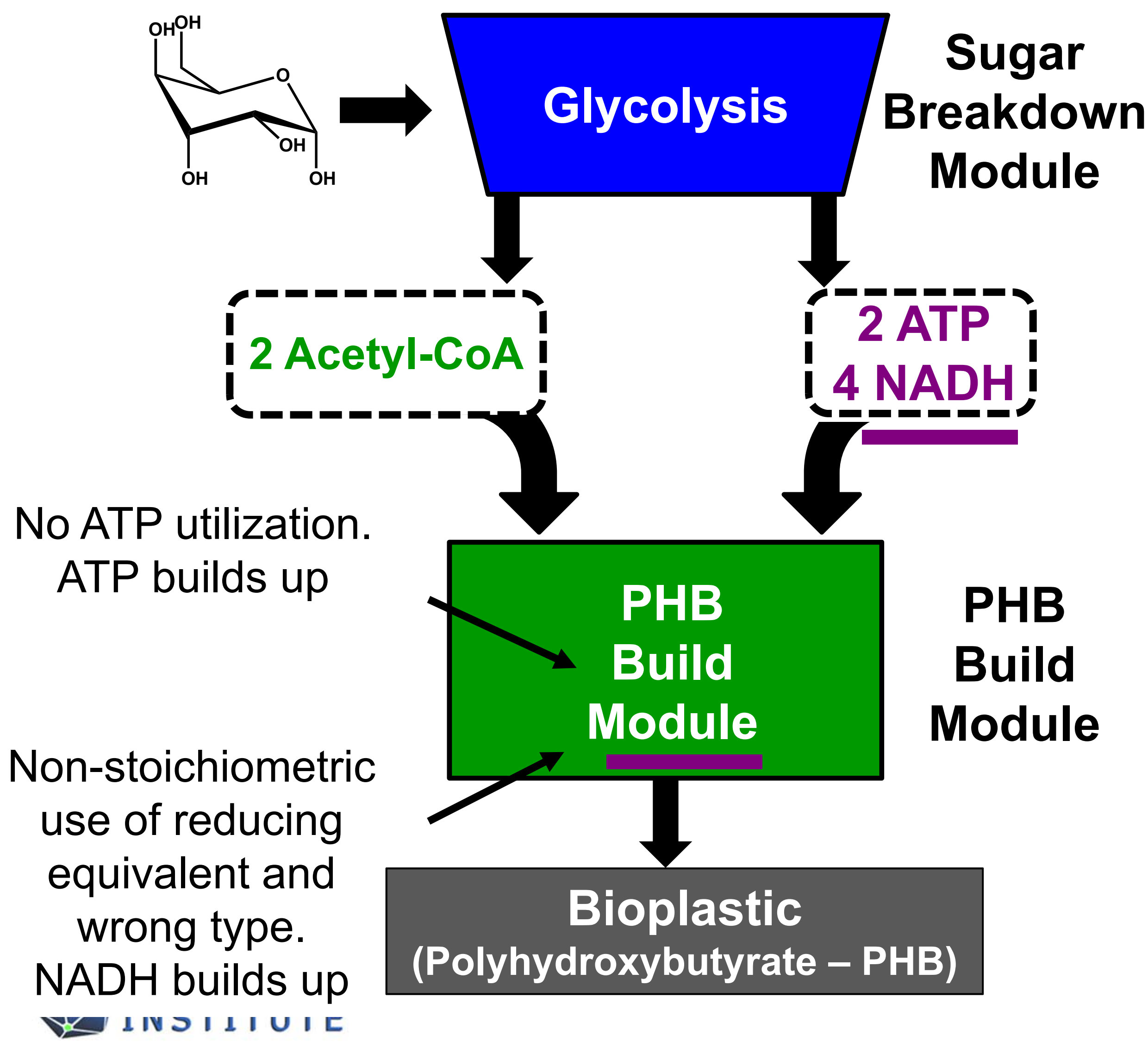
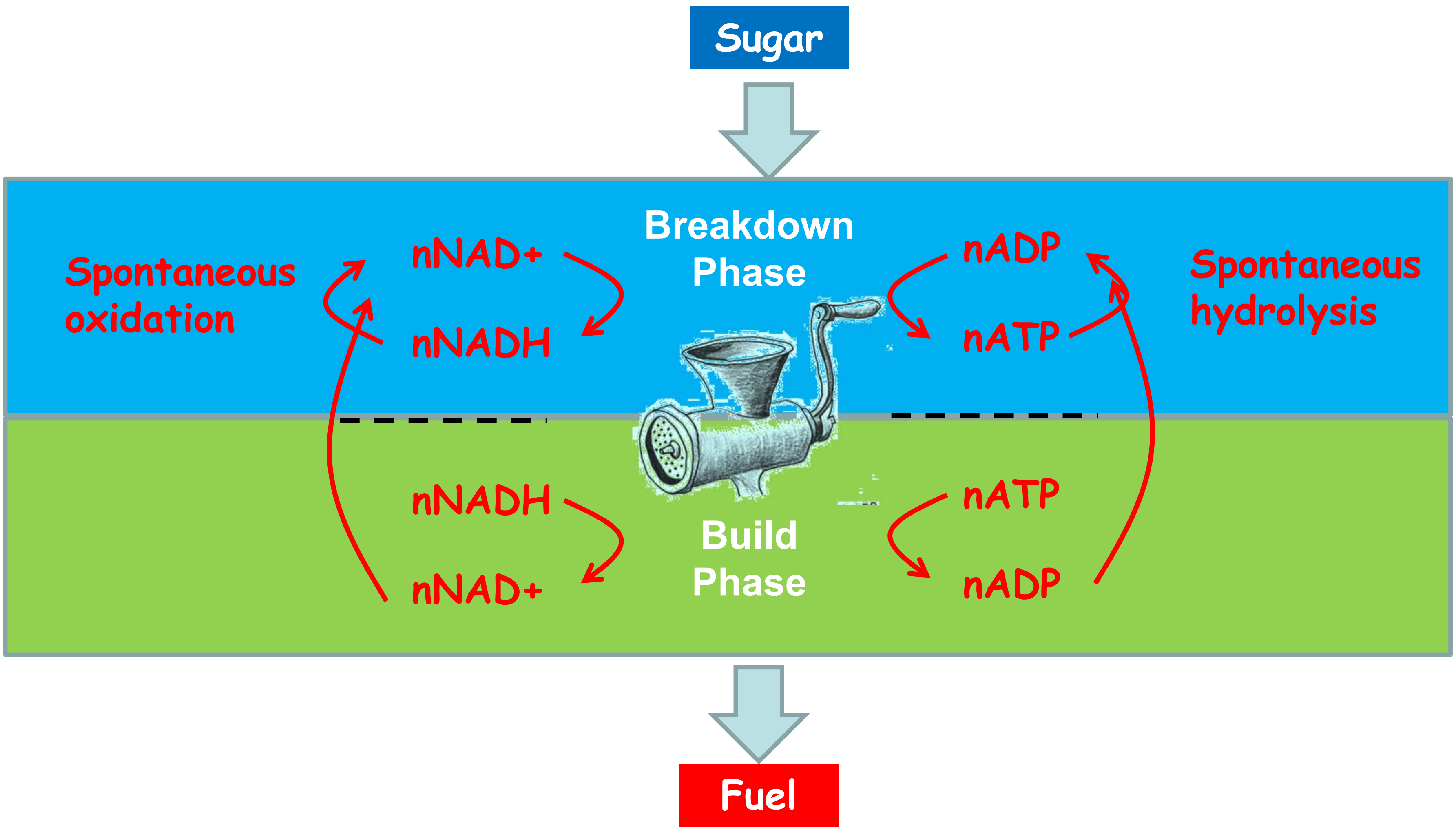
Cofactor Balancing/Recycling Problem



Perfect cofactor balance is not sustainable



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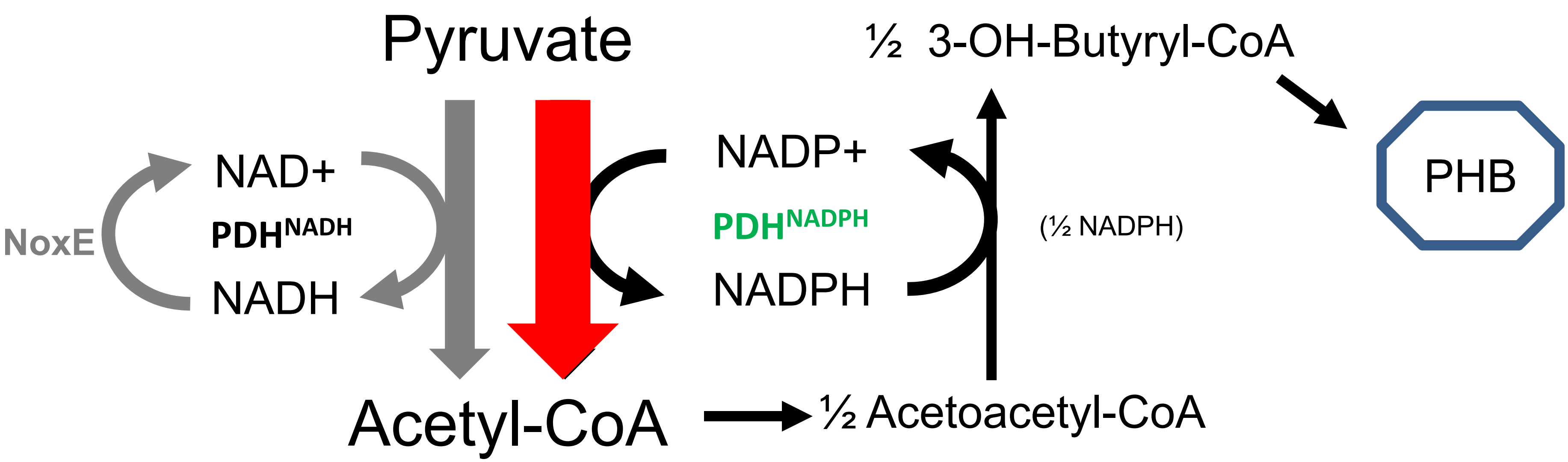
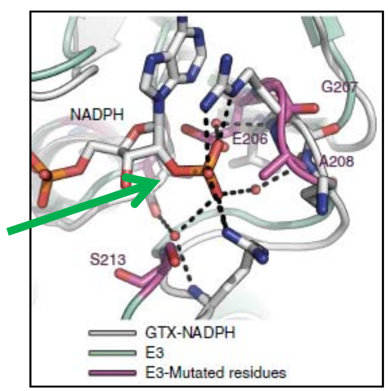
“Purge Valve” System for Regulating NADPH Levels





PDH^{NADPH}

Altered active site to accept PO_4

Nature Comm., 2014

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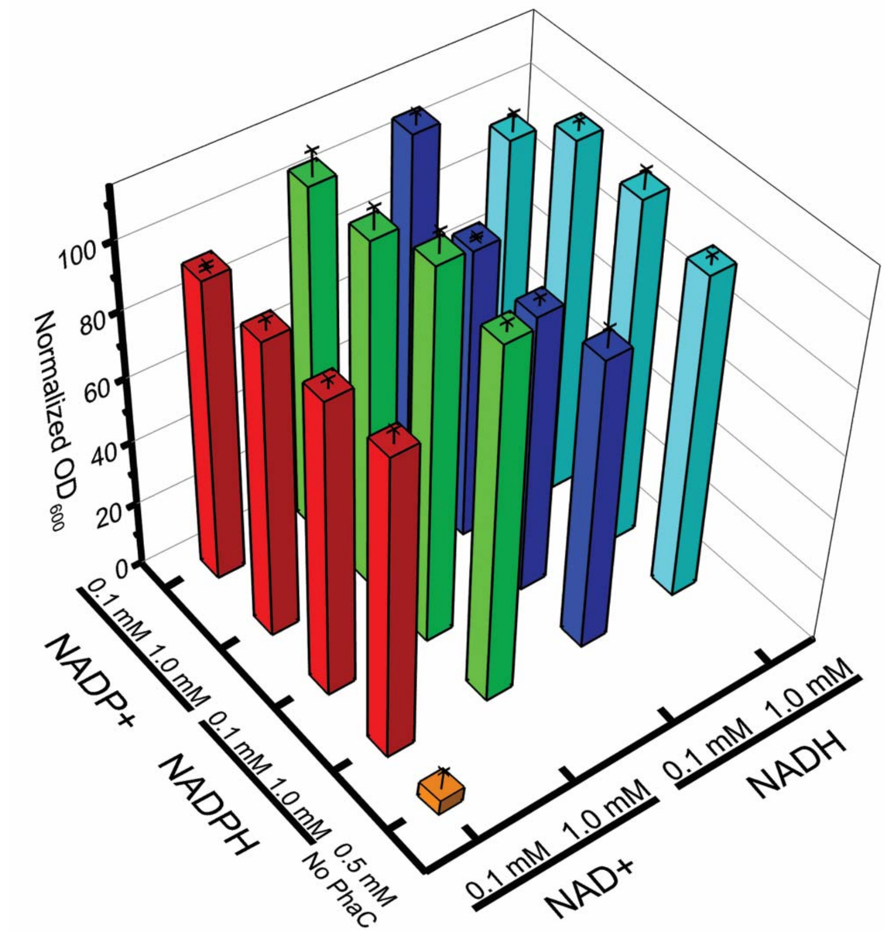
Operation of the Purge Valve

Pyruvate → **PHB**

6 Enzyme Pathway

High yields that are Robust to NAD(P)+/NAD(P)H Levels



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Nature Comm., 2014

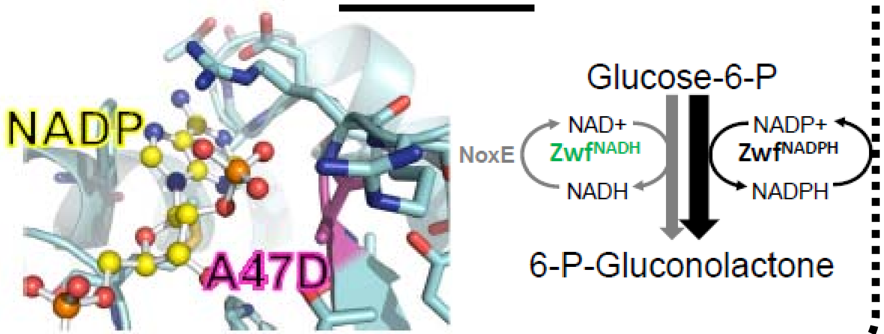
Synthetic Biochemistry Platform: Alternative Purge Valves

PBG Pathway requires additional purge valve nodes

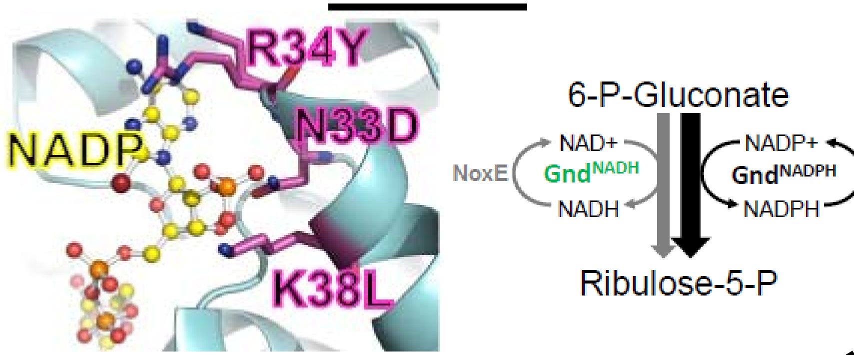
Purge Valve Concept

NAD+ Dependent Reductase
NADP+ Dependent Reductase
NADH oxidase (NoxE)

GsZwf



GtGnd



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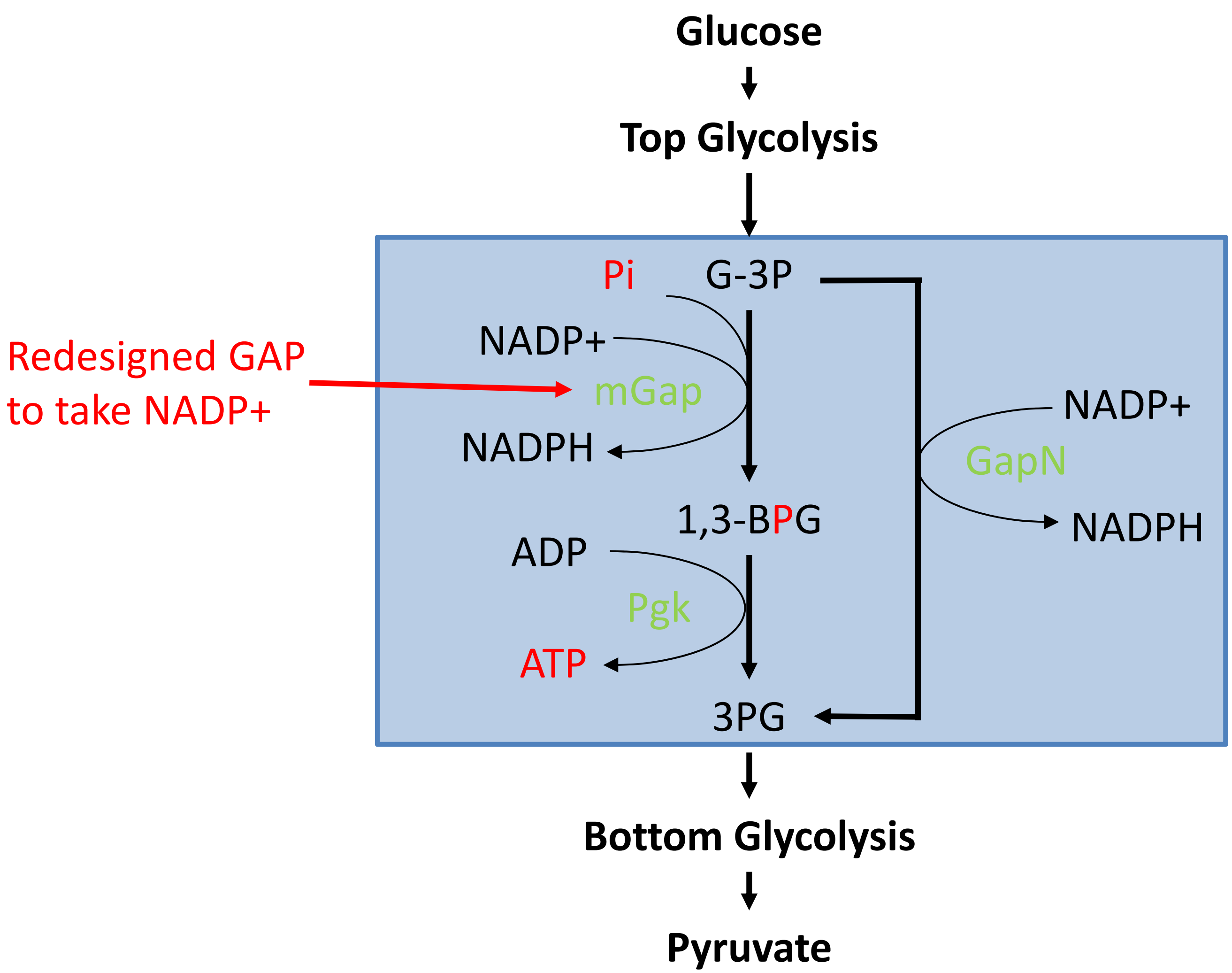
Molecular Rheostat for Maintaining ATP levels



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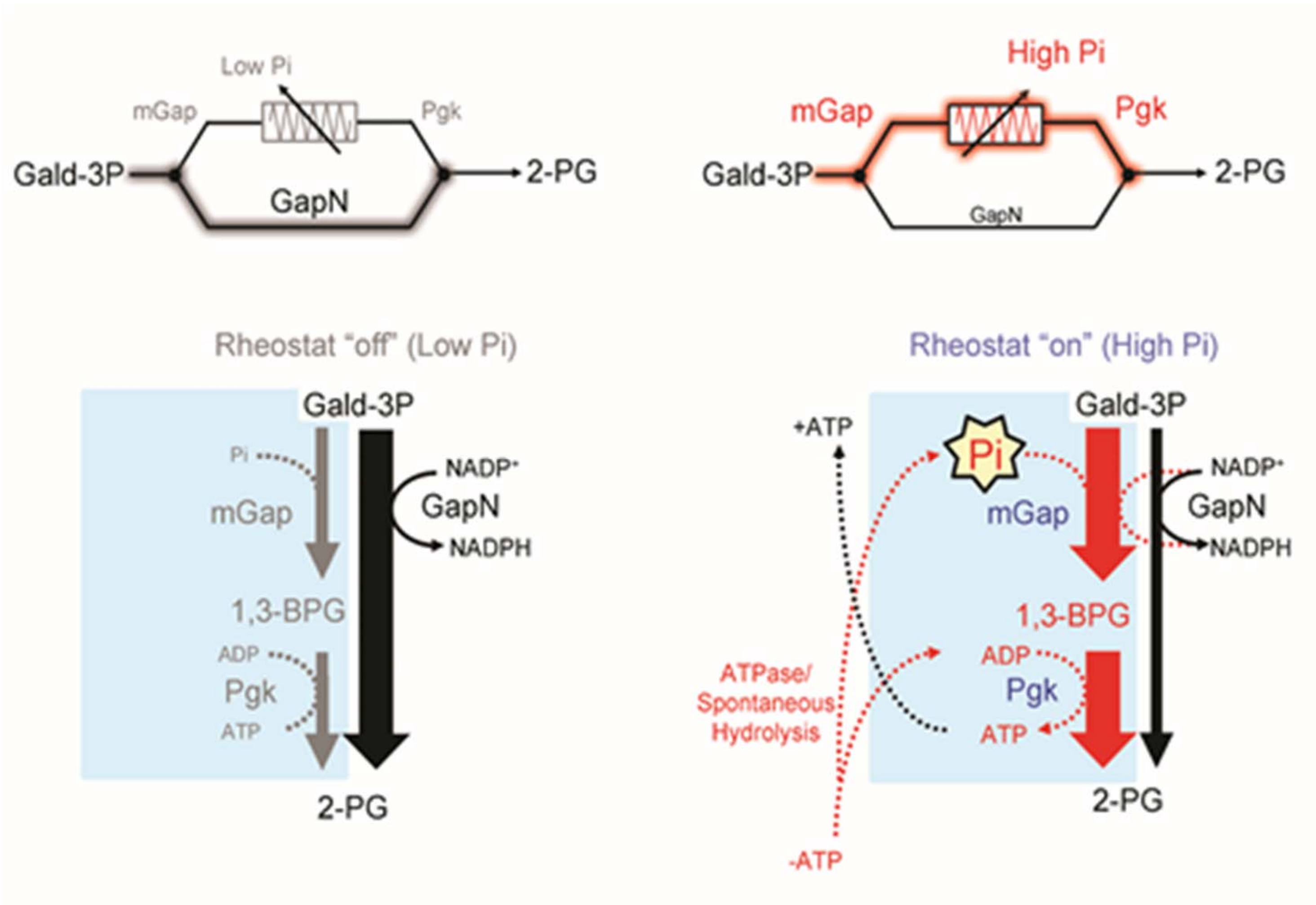
Nature Chem. Biol., 2017

Molecular Rheostat for Maintaining ATP levels



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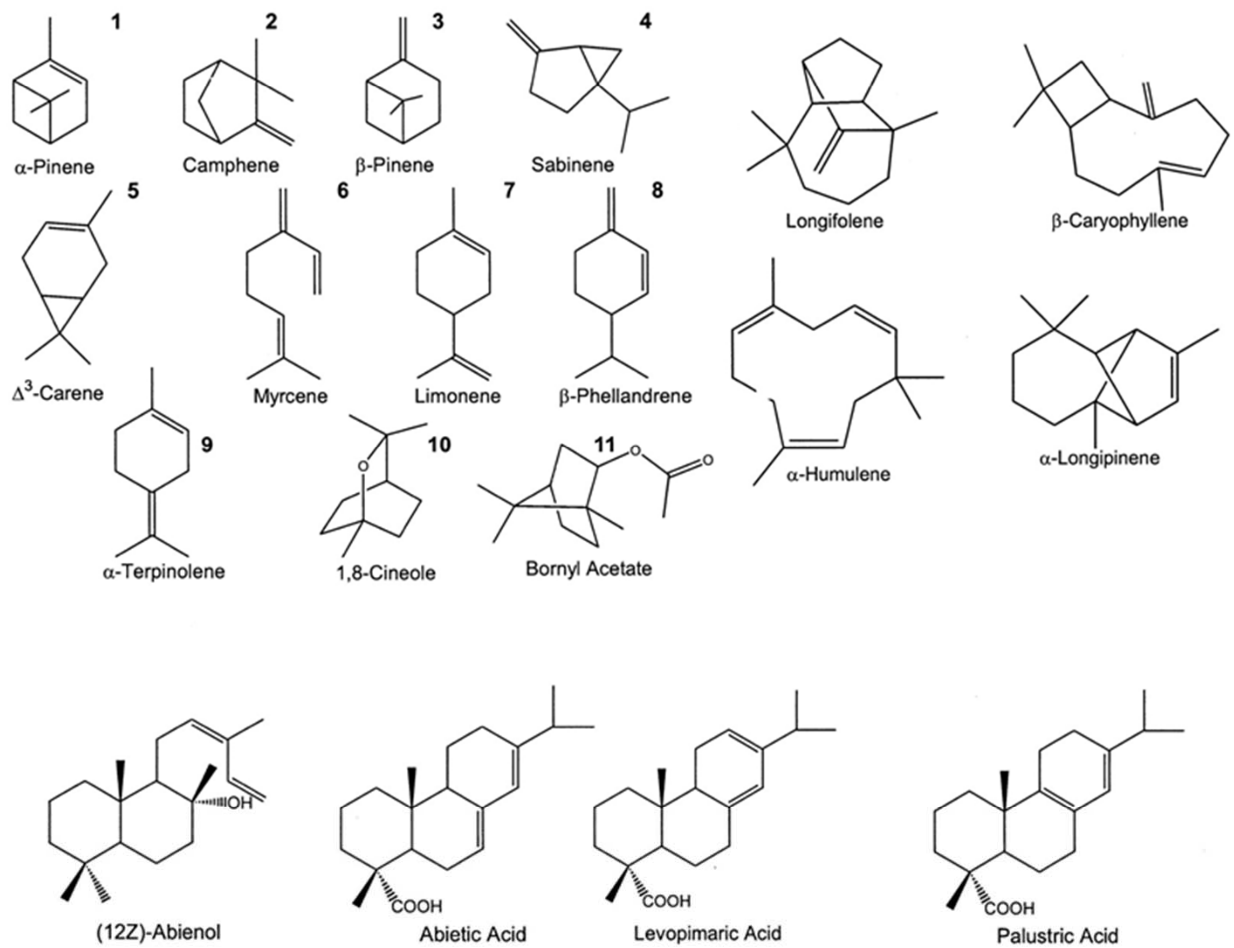
Nature Chem. Biol., 2017

Terpenes:

A vast array of compounds derived from a common building block



Many uses:

Fragrances, insect repellents, lubricants, biofuels, pheromones, drug precursors, etc.

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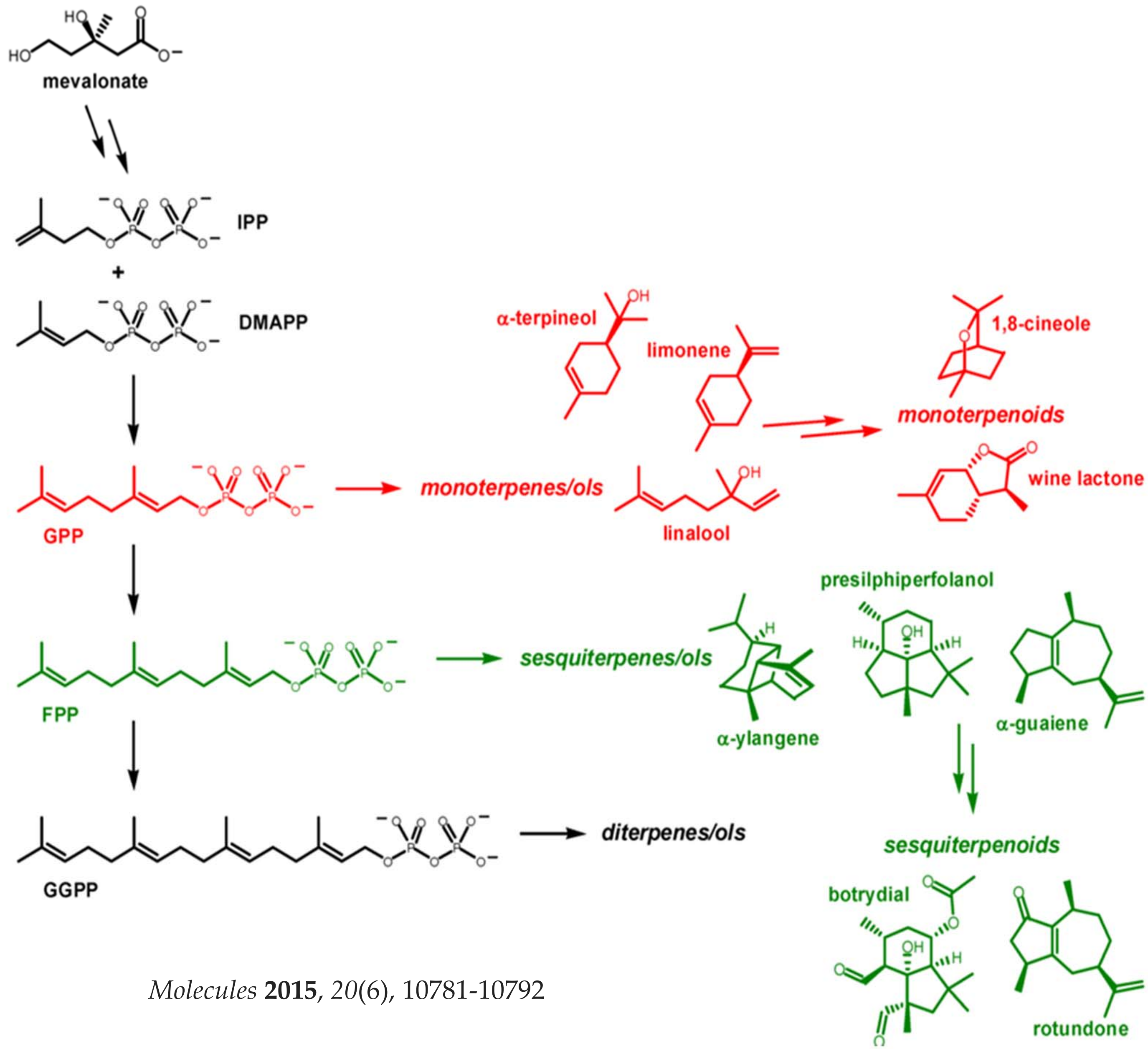
One pathway to huge variety of useful compounds

5-carbon building blocks

10-carbon building blocks

15-carbon building blocks

20-carbon building blocks



Molecules **2015**, *20*(6), 10781-10792

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Examples of terpene production in microorganisms

Titre
~112 mg l^{-1}
55 mg l^{-1}
1·3 g l^{-1}
61·9 mg l^{-1}
1·7 mg l^{-1}
0·97 g l^{-1}
32·4 mg l^{-1}
~27 μg g^{-1} DCW
80 mg l^{-1}
20 mg l^{-1}
3·05 g l^{-1}
145·7 mg l^{-1}
135·5 mg l^{-1}
380 mg l^{-1}
1·1 g l^{-1}
~305 μg l^{-1}
~912 mg l^{-1}
~994 mg l^{-1}
~10 mg l^{-1}
0·6 mg l^{-1}

Table ...production time of isoprenoid-based biofuels production from engineered microbes

Isopren...	Titre	Rate of production	Time	References
Isopent...	~112 mg l^{-1}	N.A.	43 h	Withers *et al.* (2007)
	55 mg l^{-1}	N.A.	N.A.	Chou and Keasling (2012)

Amorphadiene
(Artemisinin precursor)

Titer: ~40 g/L

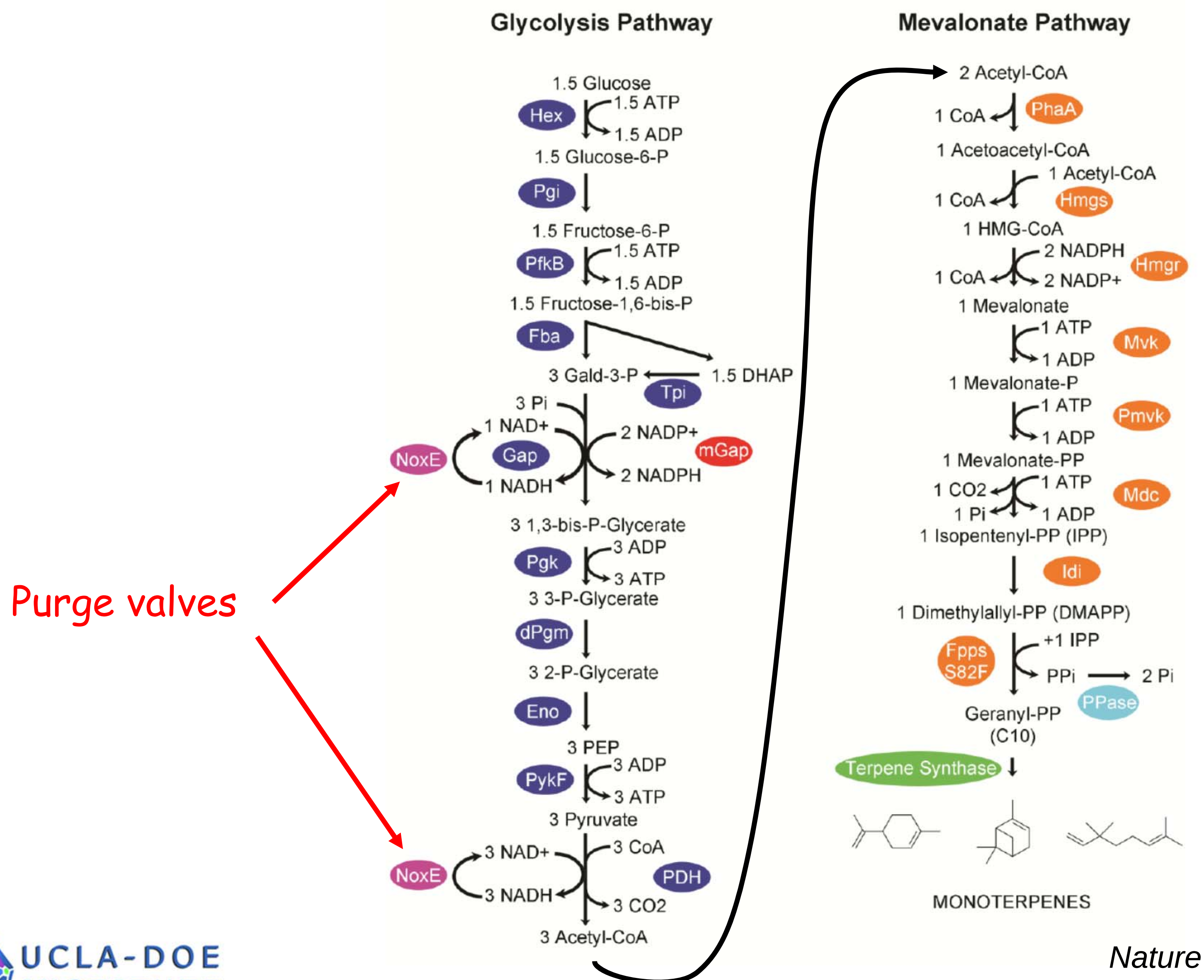
Yield: 17 %

>150 person years of effort

(PNAS, 2012 Jan 17;109(3))

Journal of Applied Microbiology **119**, 605–619 © 2015

Synthetic Biochemistry System for the Production of Terpenes



Nature Comm., 2017

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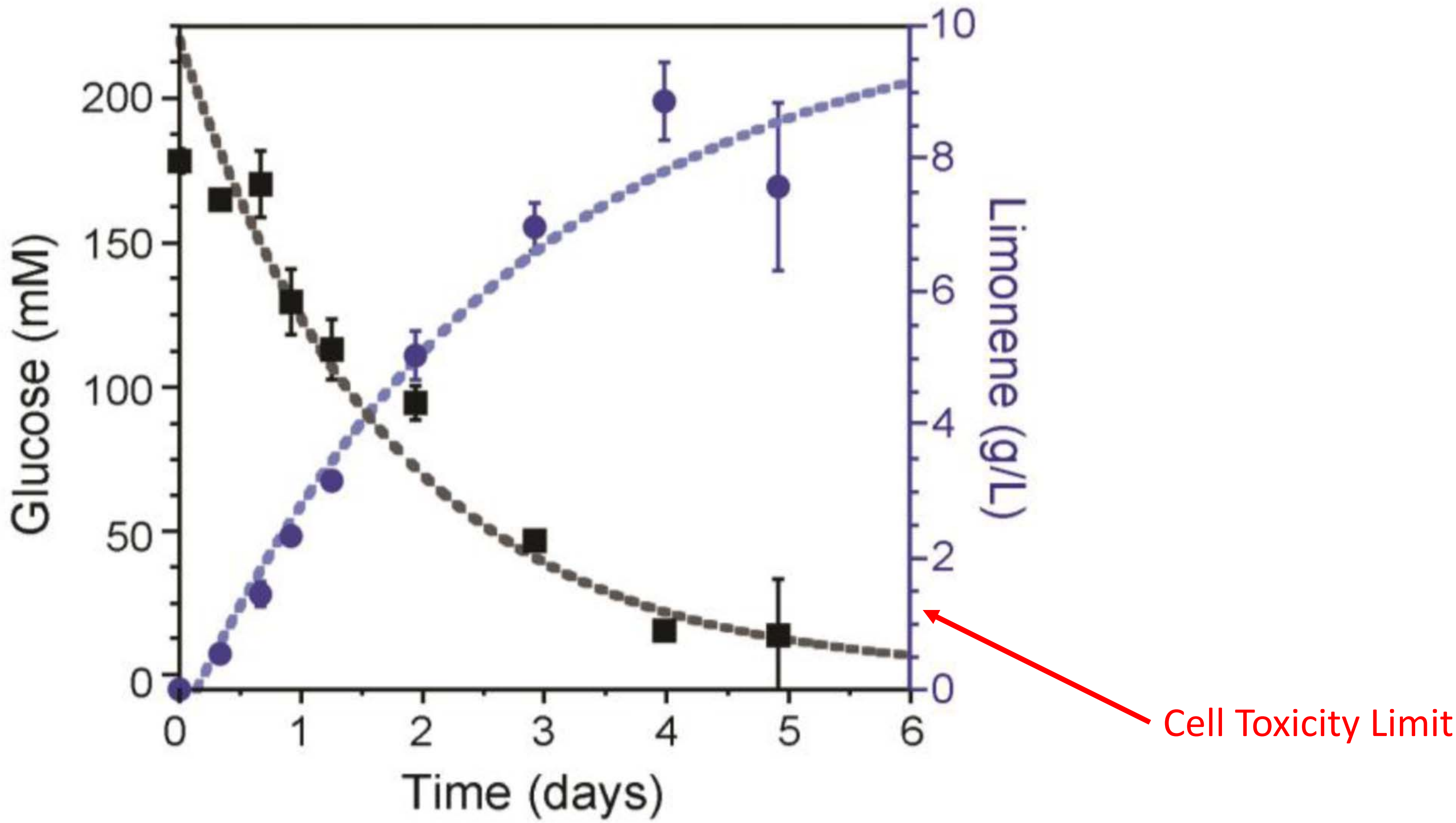
Sustainable, high yield production of Limonene



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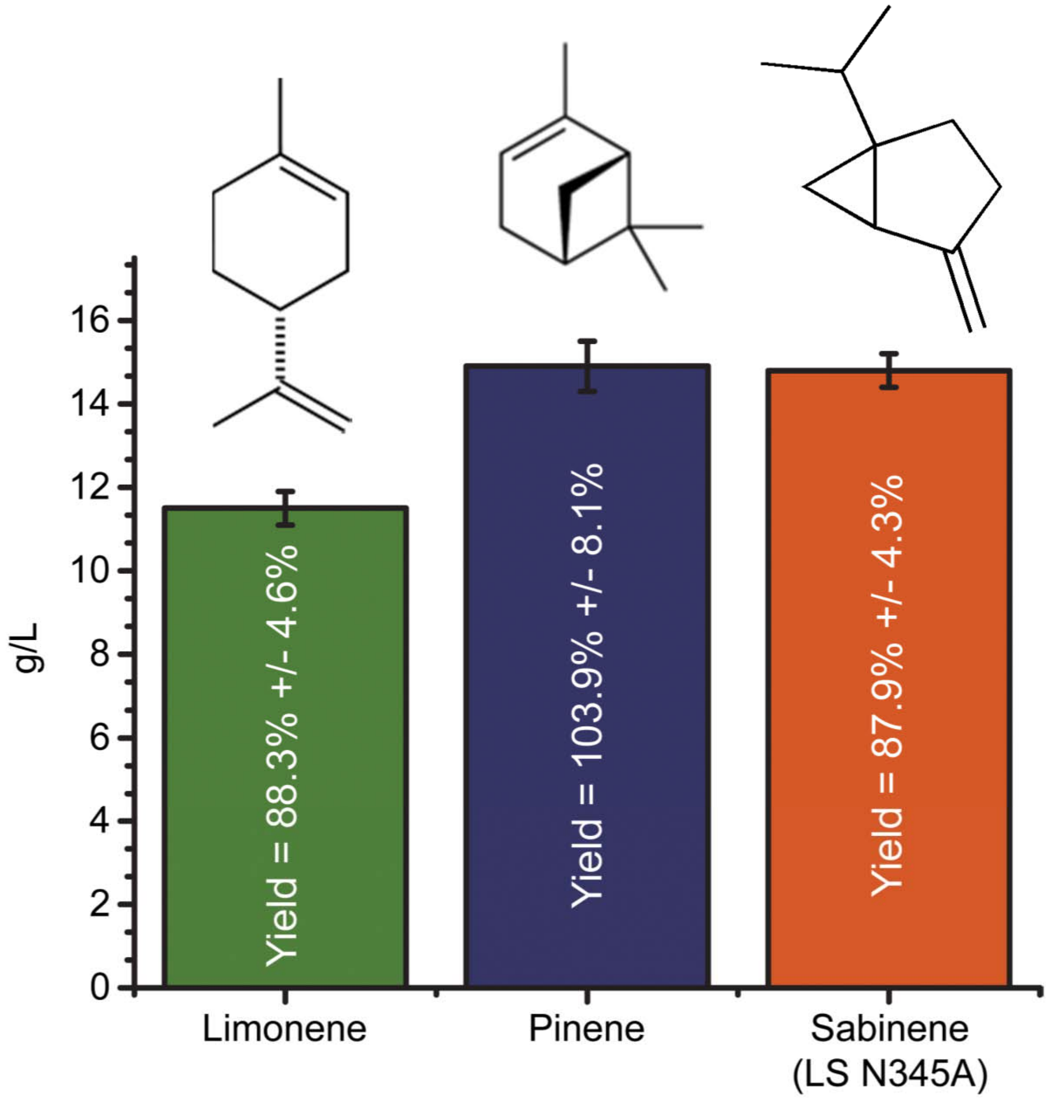
Production of Monoterpenes



Nature Comm., 2017

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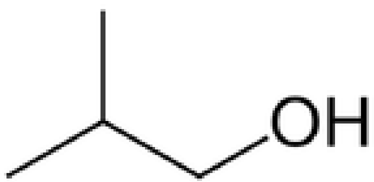
Isobutanol: Commodity chemical and biofuel



First produced in *E. coli* in Liao lab at UCLA
(Atsumi S et al. Nature. 2008;451(7174):86-9)

After strain engineering obtain:

- **Titer: 22 g/L**
- **Yield: 76%**
- **Productivity: 0.2 g/L/hr**

(Smith & Liao, Metab Eng. 2011, 13(6):674-81)

(Titers can go much higher with gas stripping)

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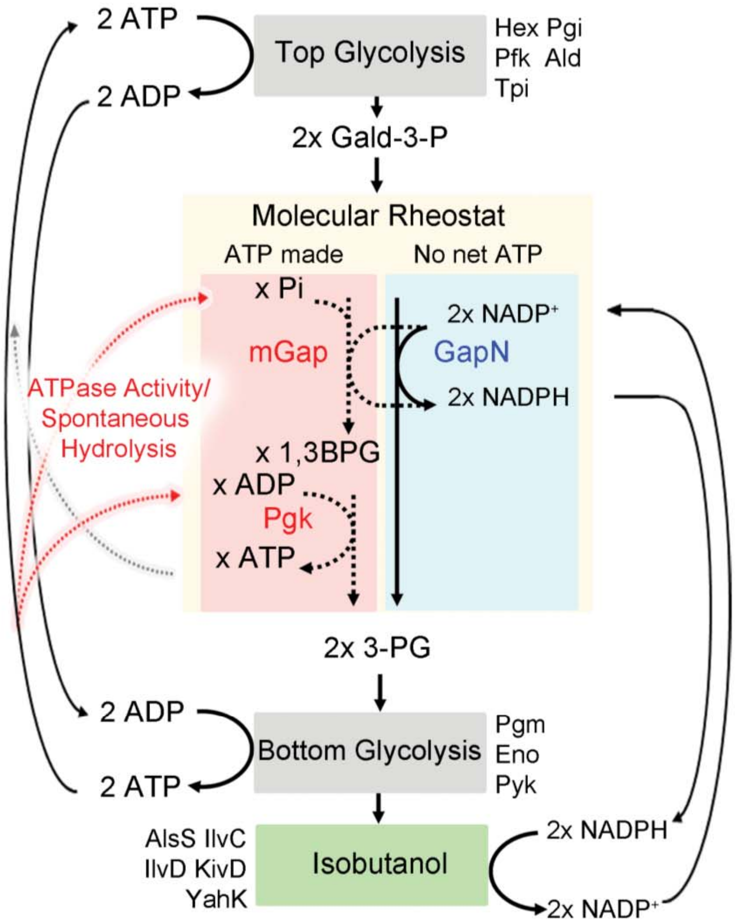
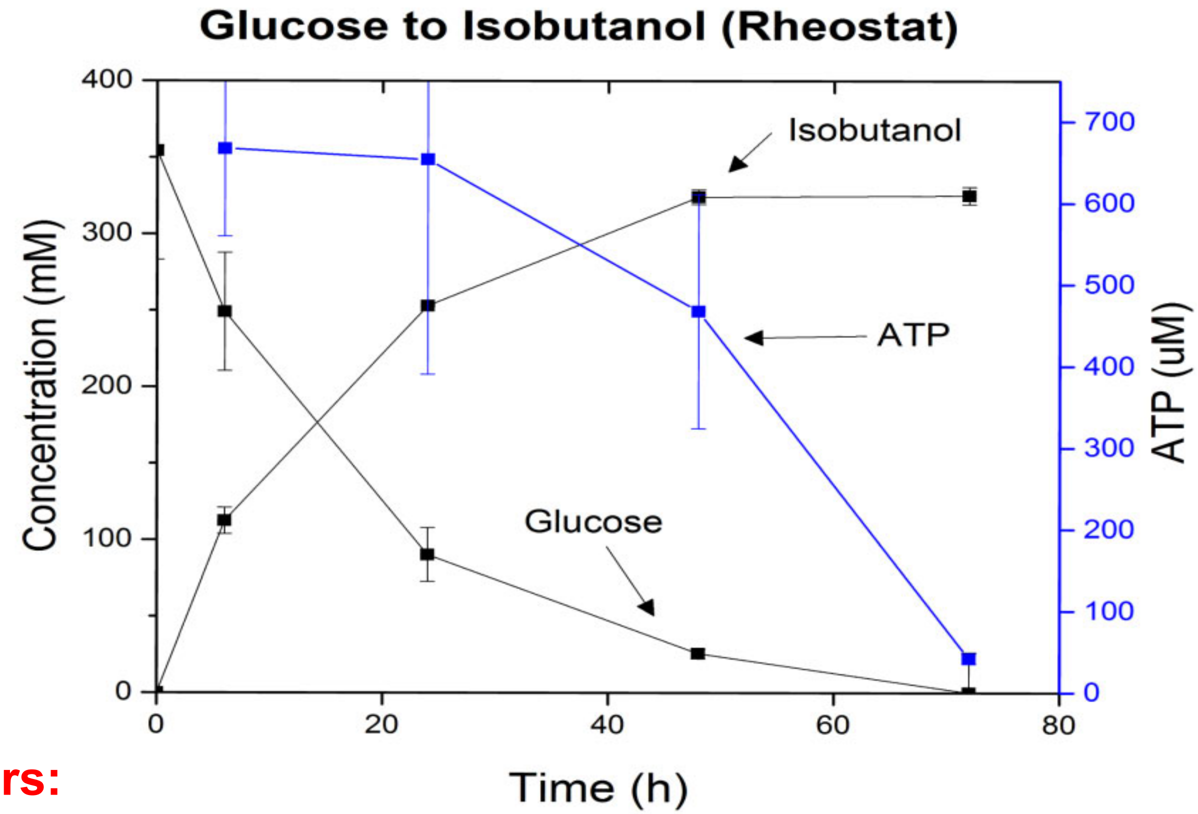
Isobutanol Production System

Regulated ATP Pathway



Nature Chem. Biol., 2017

Efficient production of isobutanol



Production parameters:

Titer: 24.1 ± 1.8 g/L
Yield: 91 %
Productivity: 1.3 g/L/hr

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Nature Chem. Biol., 2017

The Potential of Synthetic Biochemistry

Fermentation	Synthetic Biochemistry		
Ethanol Production	**Bioplastic**	**Monterpenes**	**Isobutanol**
Thousands of years of optimization	~1 person year	~1 person year	~1 person year
Productivity: ~2 g/L/hr	Prod.: 0.7 g/L/hr	Prod.: 0.2 g/L/hr	Prod.: 1.3 g/L/hr
Yield: 85 %	Yield: ~90%	Yield: ~96%	Yield: ~91%
Titer: ~100 g/L	Titer: 9 g/L	Titer: 12-16 g/L	Titer: 24 g/L
Time: 1-3 days	Time: 2-3 days (not continuous)	Time: > 4 days	Time: 2 days

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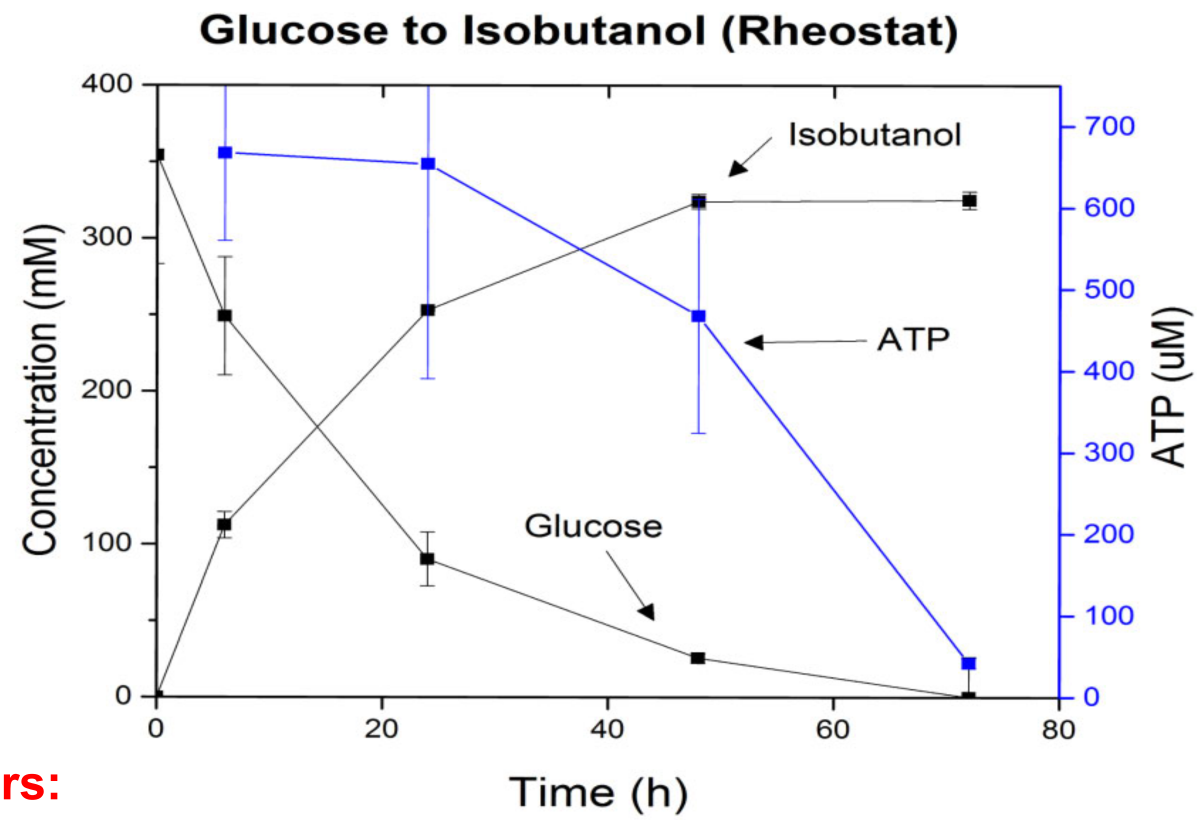
Efficient production of isobutanol



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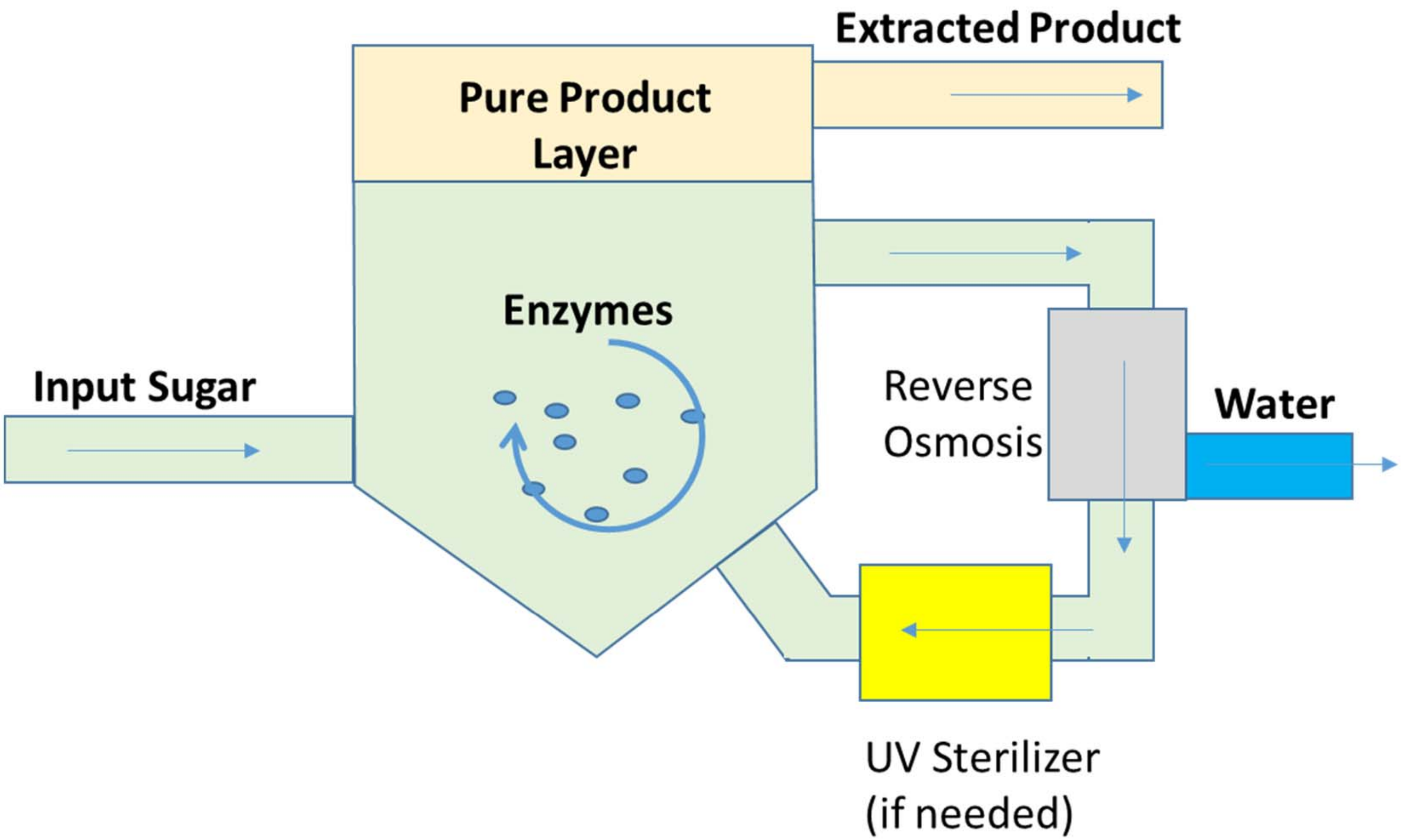
Cell Free Production System



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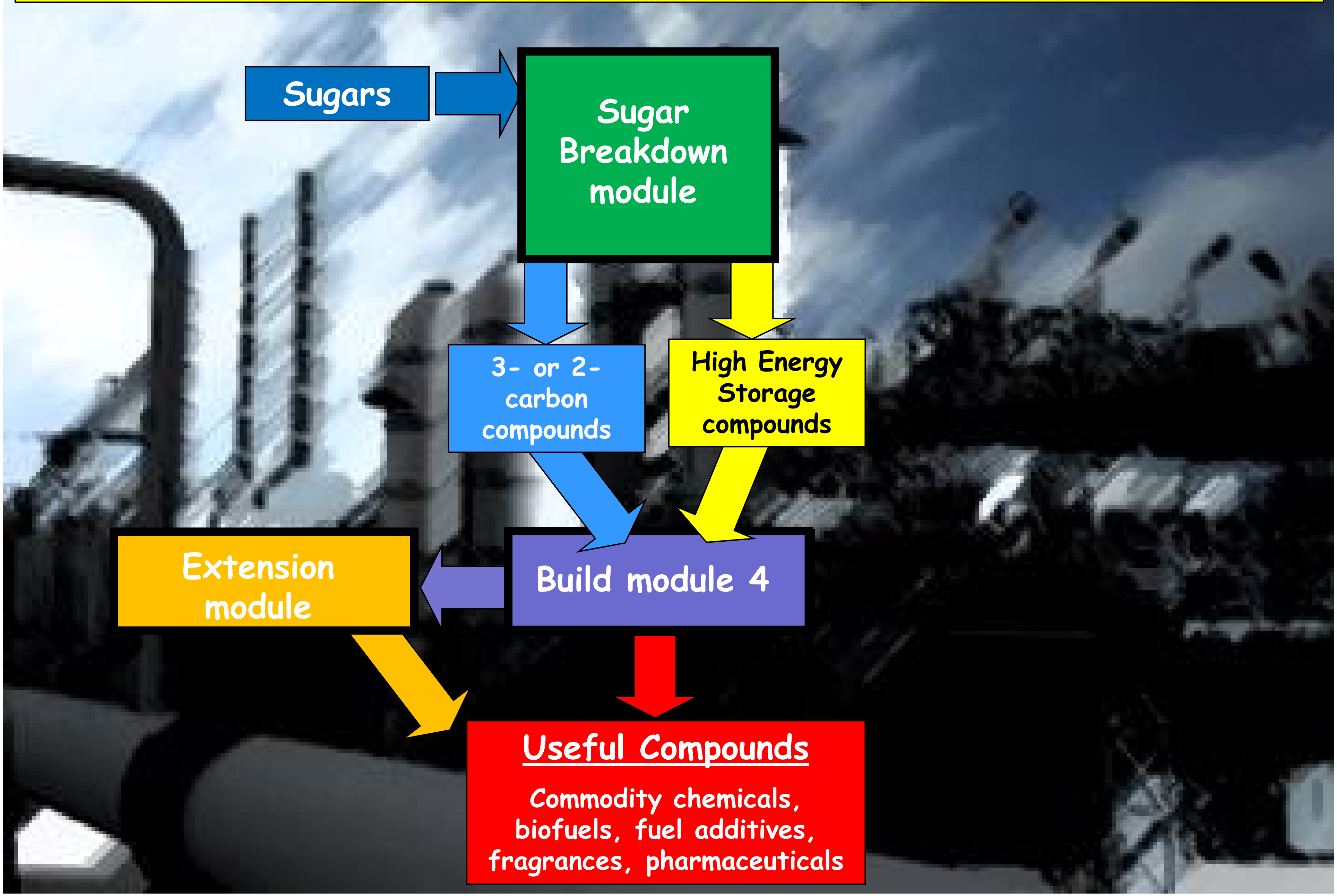
Modular Synthetic Biochemistry Units



Acknowledgements

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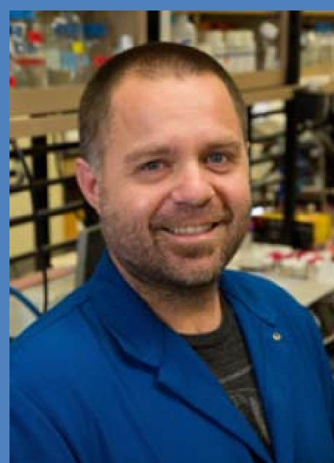

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