Rational Design of Microbial Chemical Factories

Enzymes as Interchangeable Parts

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Microbes as Chemical Factories

- Antibiotics/Antimicrobials
- Other therapeutics (lovastatin)

- Amino Acids
- Organic Acids

- Improvement of natural producers

Improvement of Natural Producers

http://www.kevinhenkes.com/images/fun/print/mouse_maze.jpg

Microbes as Chemical Factories

- Antibiotics/Antimicrobials
- Other therapeutics (lovastatin)

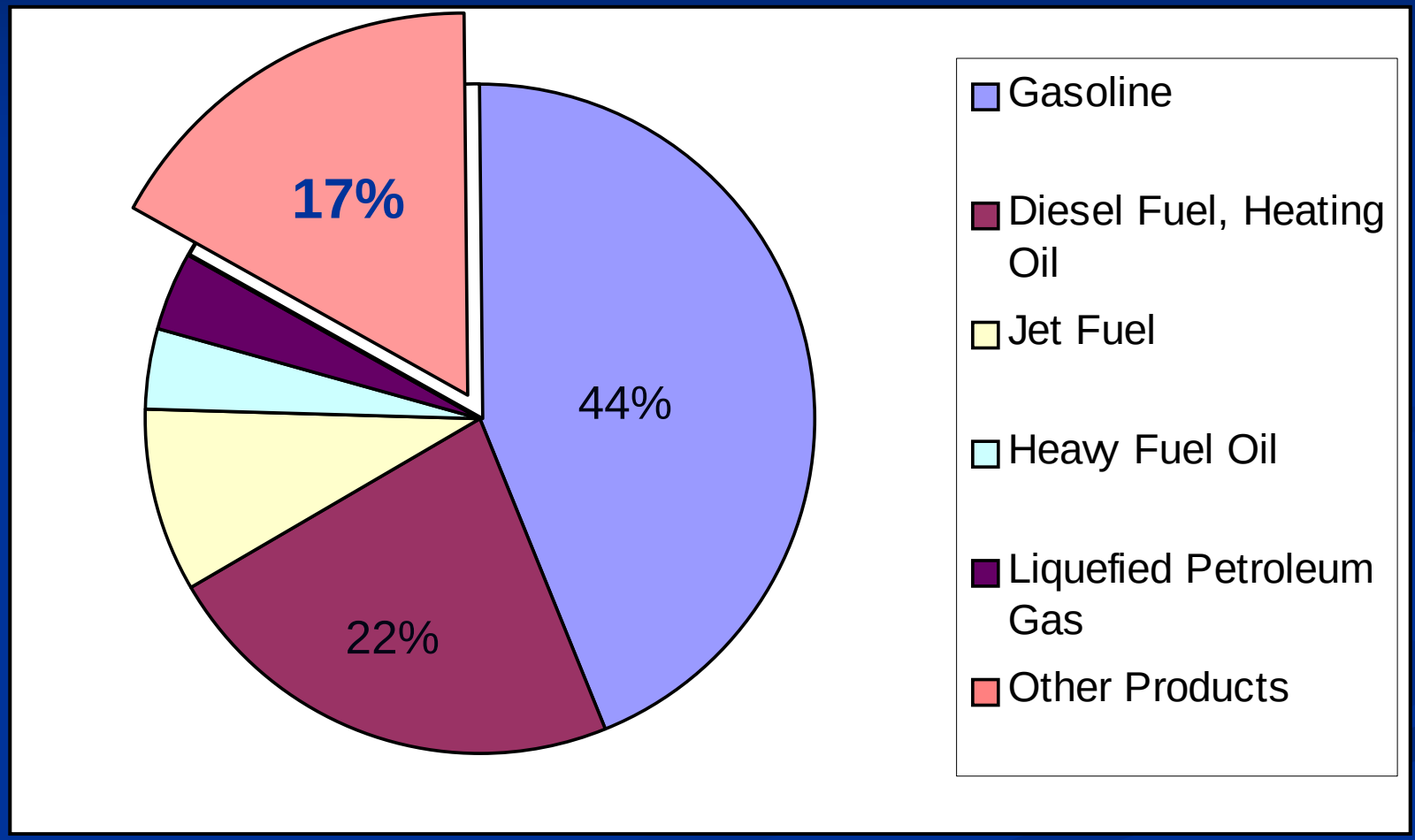
- Amino Acids
- Organic Acids

- Improvement of natural producers

- 1,3-Propanediol
- Artemisinic Acid

- Re-constitution of natural pathways in unnatural hosts

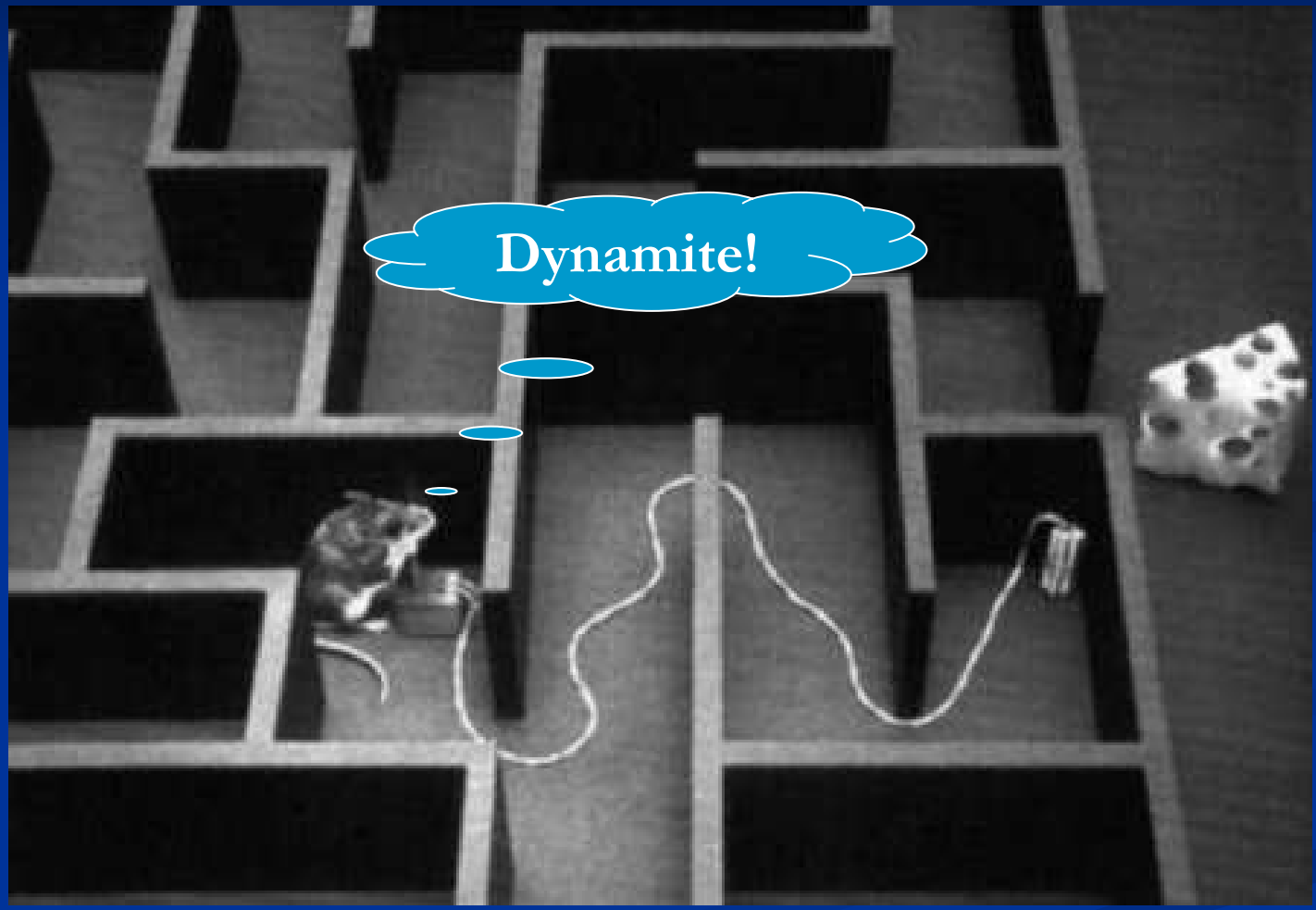
A continued (and increasing?) need for microbial chemical factories



Product distribution per barrel of crude oil, US average
Source: Energy Information Administration, Dept of Energy, April 2007

Goals

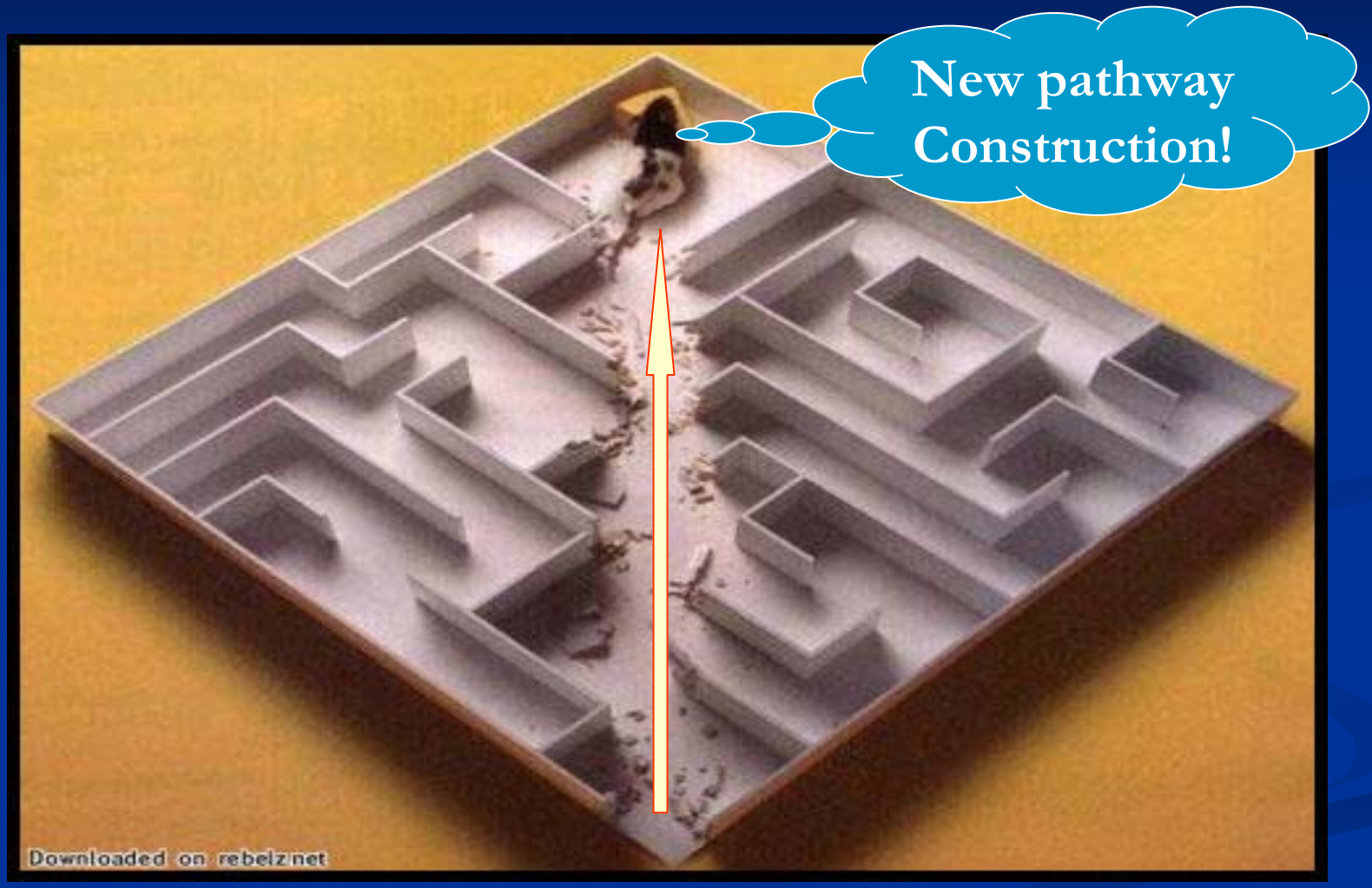
Novel Pathway Design



http://www.jokebandit.com/wp-content/uploads/2008/02/smart-mouse-in-maze-genius-rodent-using-dynamite.jpg&imgrefurl

Goals

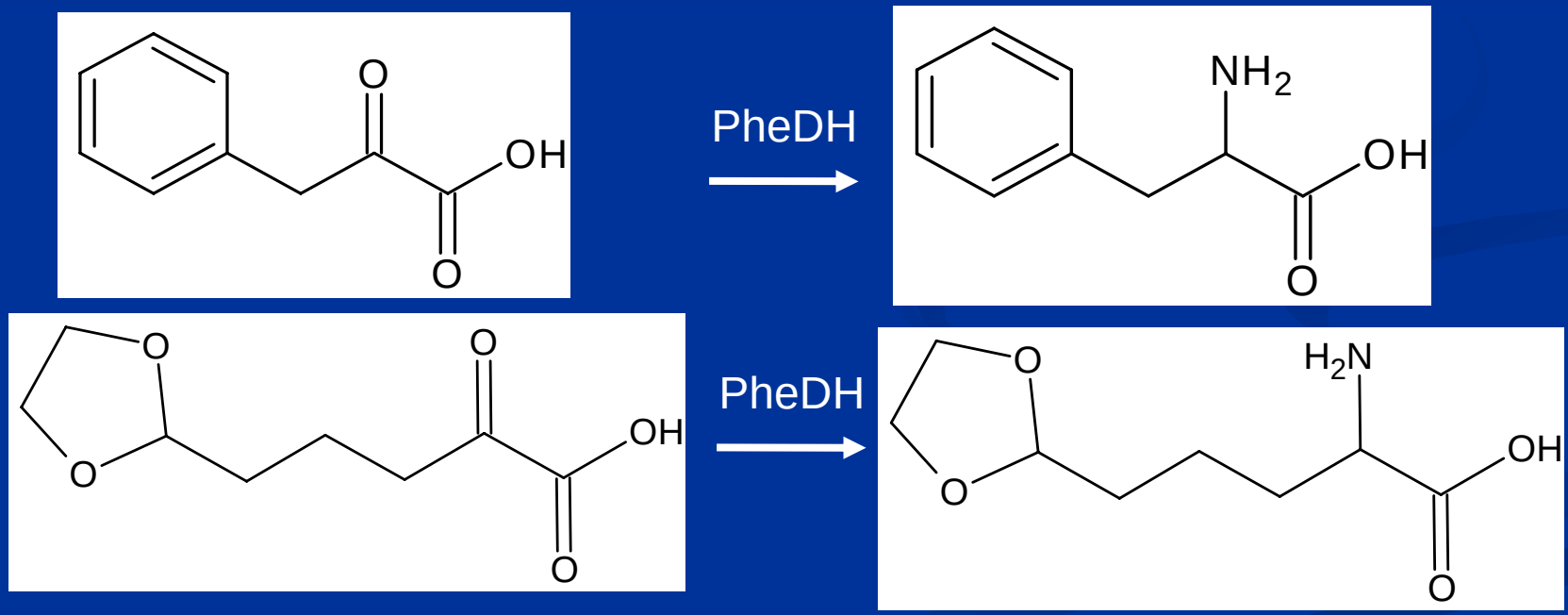
Novel Pathway Design



http://www.functionalhandstrength.com/images3/mouse_maze.jpg

Biocatalysis – Extending the Unnatural Product Spectrum

- Rely on functional specificity across broad substrate range
- Unnatural substrates → Unnatural products



PheDH = phenylalanine dehydrogenase

Hanson et al. 2000. *Enzyme Microb. Tech.* **26**:348-358

Retro-biosynthetic Pathway Design

- Biocatalysis (& Bioprospecting) – "Parts" selection; Metabolic Engineering – "Systems" assembly, analysis
- Design and assemble *in vivo* series of biocatalytic conversions
  - Tie starting reactants to cellular metabolism (intermediates, or carbon sources)
- Existing biosynthetic pathway algorithms
  - Hatzimanikatis and Broadbelt – EPFL-Lausanne [Switzerland], Northwestern U. [USA] (synthesis, degradation)
  - Wackett and Ellis – UM-BBD [Minnesota, USA] (degradation)
  - w/A. Jaramillo – École Polytechnique* [France]
- Elucidation of Design Principles
- Development of Design and Assembly Tools ("Devices")

**Bioinformatics.* 2008. 24:2554-2556

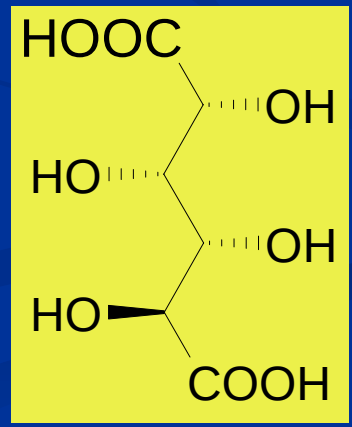
Target Compound

D-Glucaric Acid

- ✓ Found in fruits, vegetables, and mammals.
- ✓ Studied for cholesterol reduction and cancer chemotherapy.
- ✓ Starting material for new nylons and hyperbranched polyesters.

  : a top value-added chemical from biomass (PNNL & NREL)
- ✓ Currently produced by chemical oxidation of starch

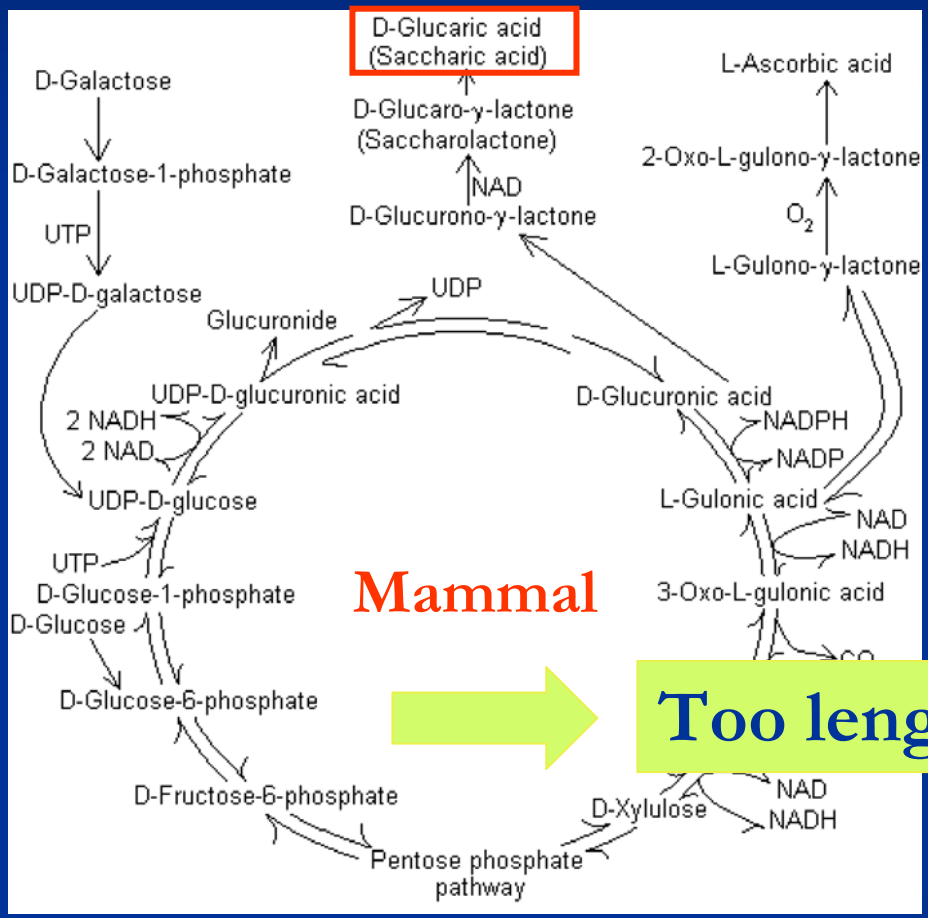
  → nonselective and expensive process
- ✓ No known microbial pathway



Rationale for This Research

Existing Pathway

Mimicking the existing pathway*?



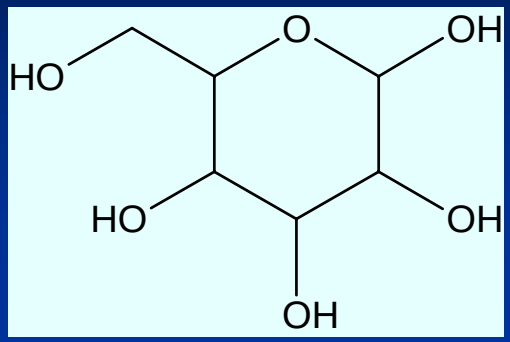
Mammal

Too lengthy!

* D-glucuronic acid pathway in mammals. Adapted from *Encycl. of Chem. Tech.*

Synthetic Pathway

PW1 (Assembly of Natural Enzymes)



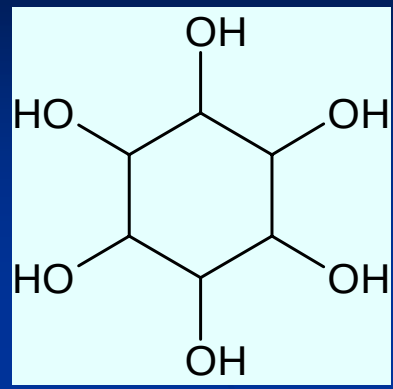
glucose

EC 5.5.1.4
S. cerevisiae myo-inositol 1-phosphate synthase*

CLONED

glucose-6-phosphate

myo-inositol-1-phosphate

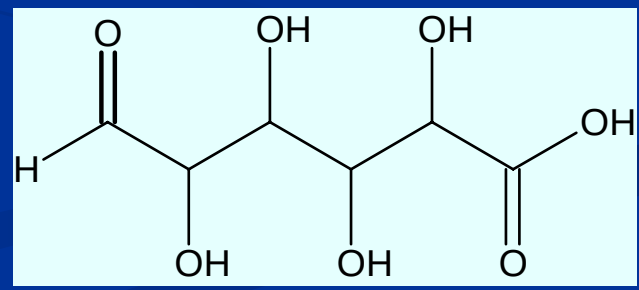


myo-inositol

SYNTHESIZED

EC 1.13.99.1
myo-inositol oxygenase

Mouse



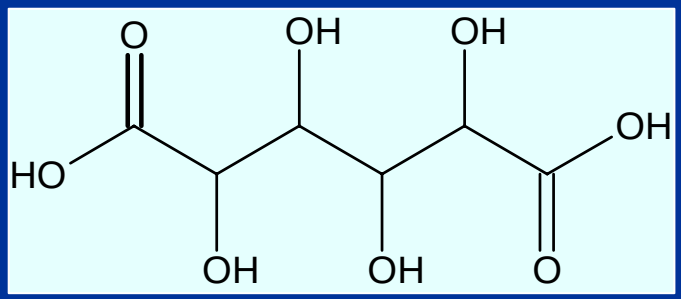
glucuronic acid

EC 1.1.1.203

P. syringae uronate dehydrogenase

NADH + H^+

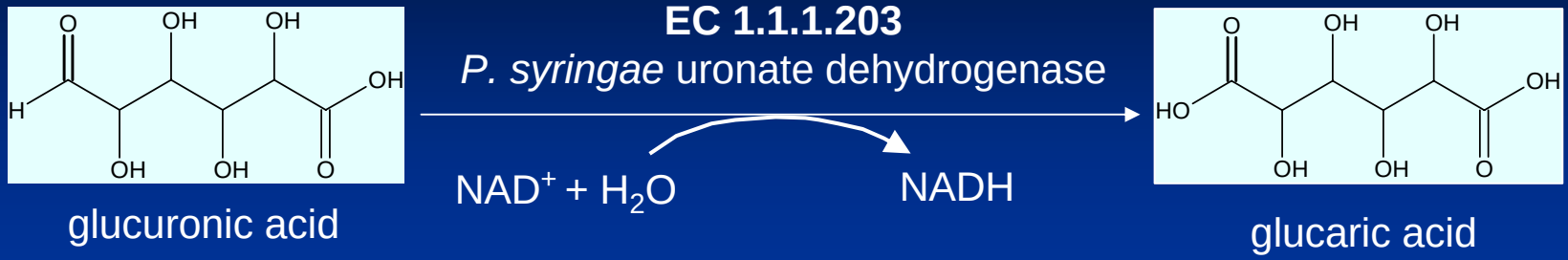
$NAD^+ + H_2O$



glucaric acid

* 3 steps, 1 cloned activity in *E. coli* (Frost Lab, *JACS*, **121**:3799)

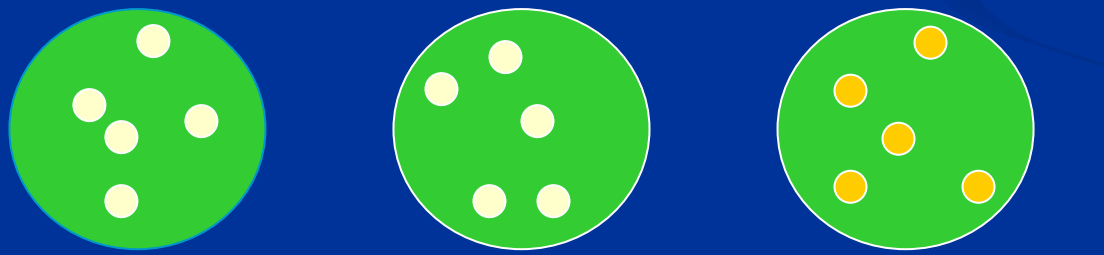
Results – 3rd Step



Uronate Dehydrogenase (UDH) from gDNA library

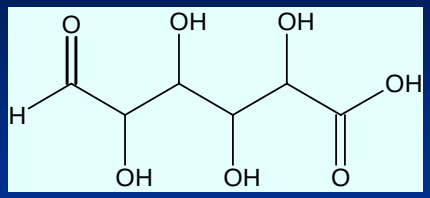
- MG1655 mutant (*uxaC* KO)

  uxaC KO mutant ➔ w/ functional UDH



LB or glucose | glucaric acid | glucuronic acid

Results – 3rd Step



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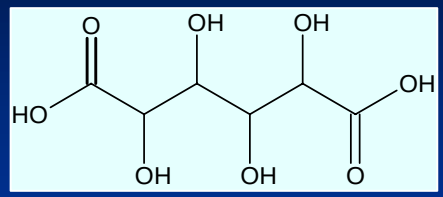
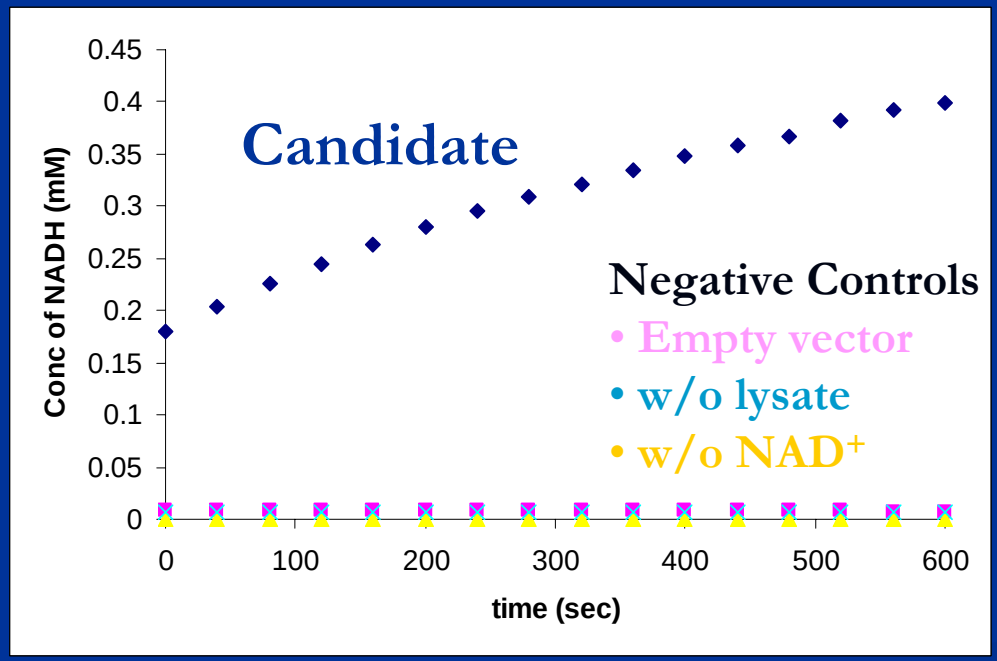
P. syringae uronate dehydrogenase

$NAD^+ + H_2O$ → $NADH$



glucaric acid



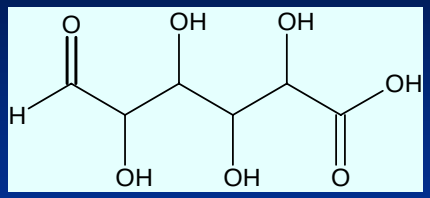
At low concentration,

Rate ∝ [glucuronic acid]

Yoon et al, 2009. *J. Bacteriol.* 191(5):1565-1573

Results – 3rd Step

PW1



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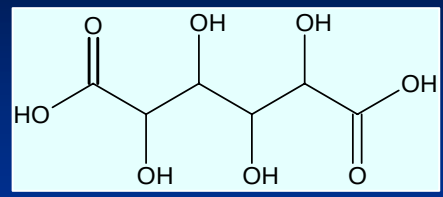
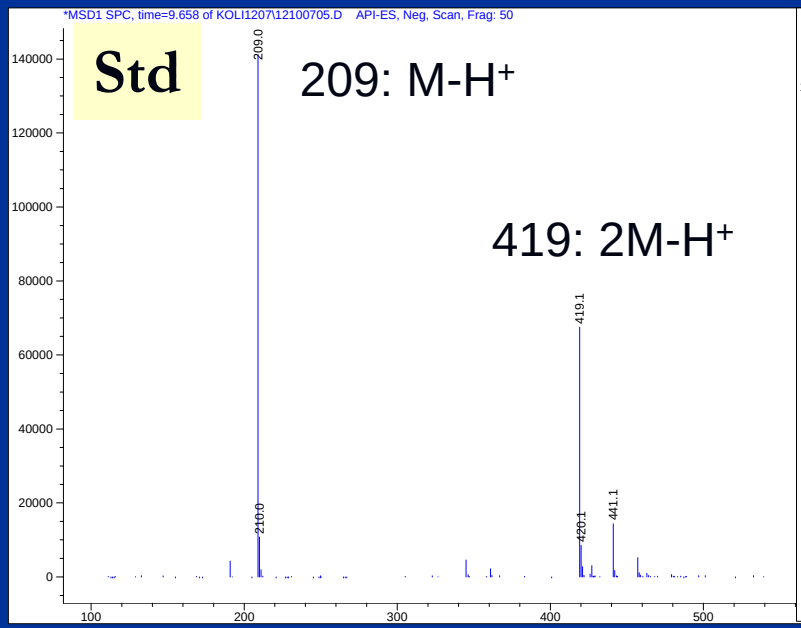


glucaric acid



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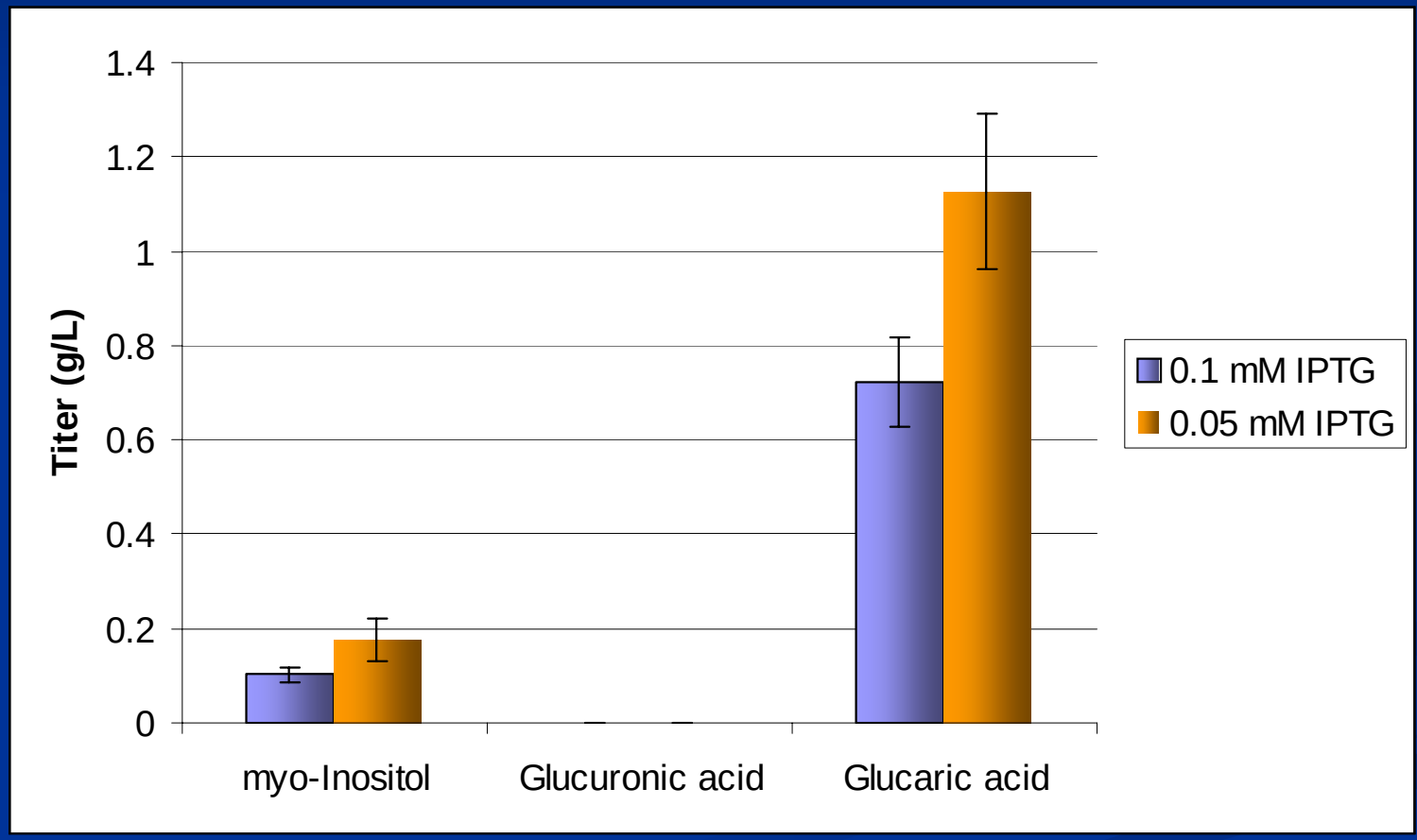
Results – All Three Steps

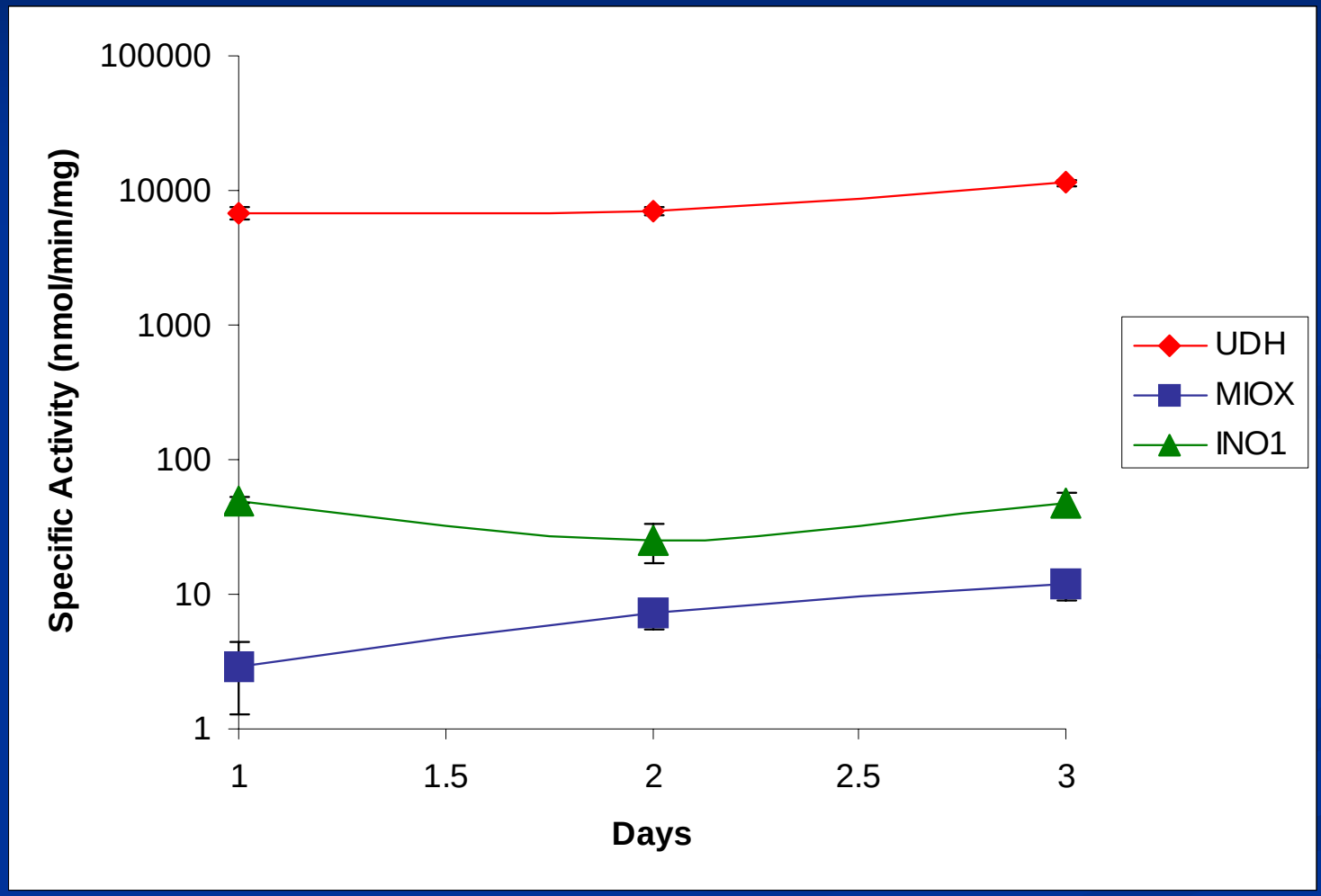
Co-expression of All Three Genes in *E. coli*

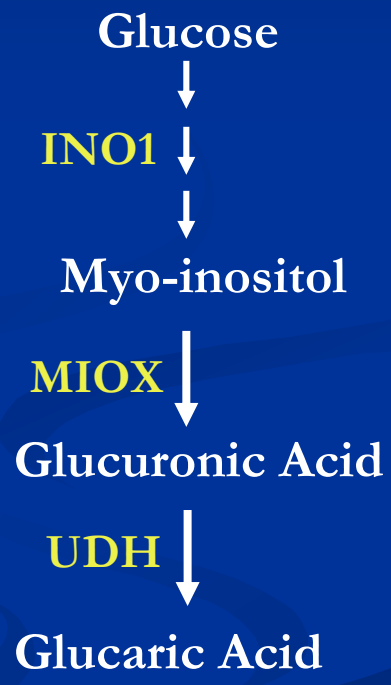


Moon et al, 2009. *Appl. Environ. Microbiol.* 75(3):589-595

Results – All Three Steps

Rate Limiting Step – MIOX → No Glucuronic acid





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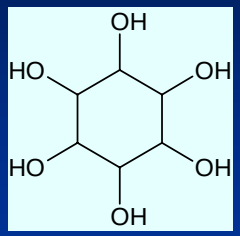
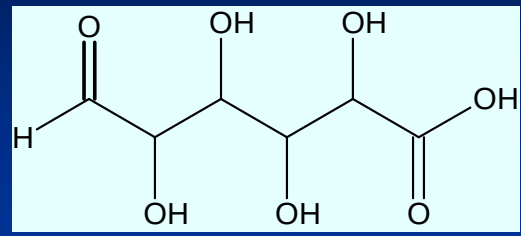
Results – 2nd Step



myo-inositol

EC 1.13.99.1
Mouse *myo*-inositol oxygenase (MIOX)

O_2 H_2O



glucuronic acid

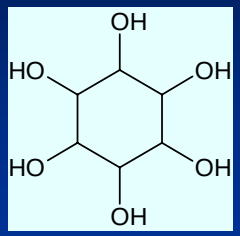
Culture Conditions	Activity at 6 hr (nmol/min/mg)	Activity at 24 hr (nmol/min/mg)	Glucuronic Acid (g/L)
+ MI	430	76	0.44
- MI	28	15	N/A

➔ Intrinsic instability of MIOX*

➔ High MI production by INO1 is needed.

* ***BBRC*, 324, p1386**

Results – 2nd Step



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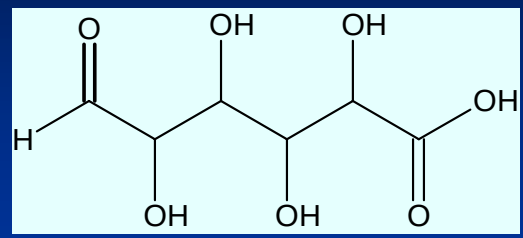

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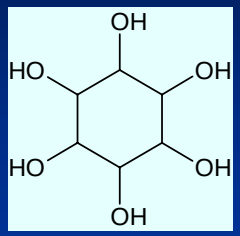

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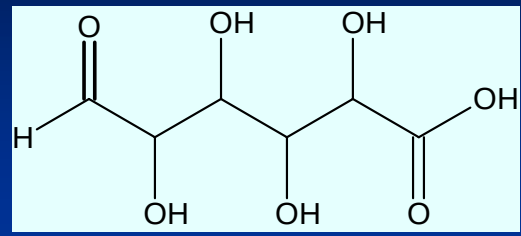

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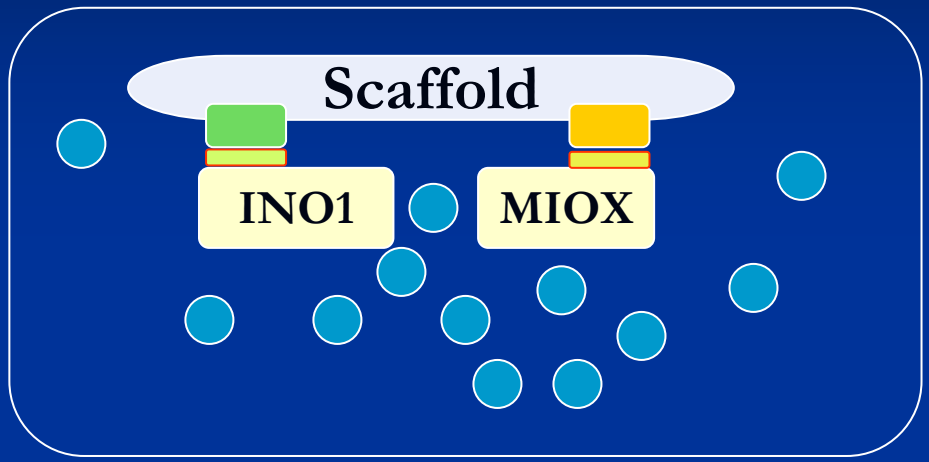
Not a very well-characterized part

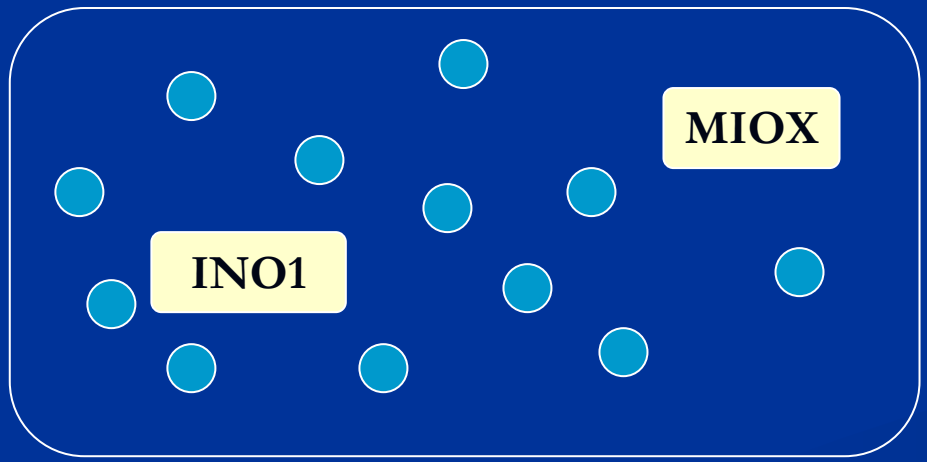
* ***BBRC*, 324, p1386**

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

Engineering "Parts"

Co-localization (Collaboration w/ Dr. John Dueber, SynBERC*)

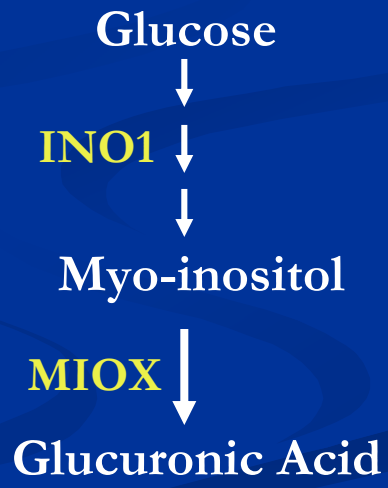




→ Higher $[MI]_{local}$

→ Better activation of MIOX

→ Faster conversion



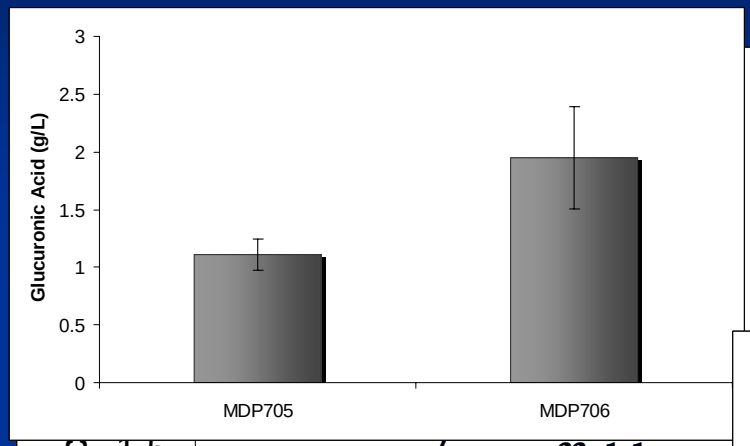
MI = *myo*-Inositol

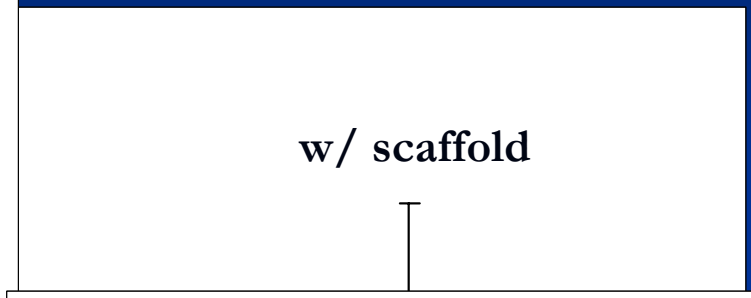
* QB3, University of California, Berkeley, USA

Engineering "Parts"

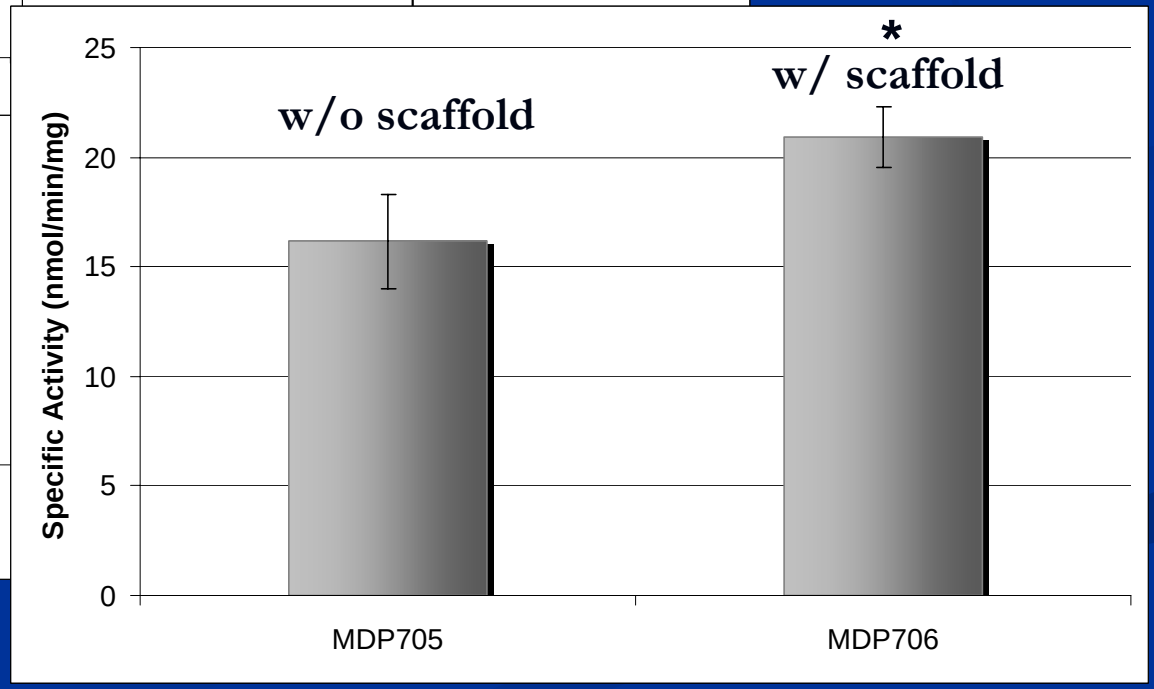
PW1

Co-localization → Better Activation of MIOX?





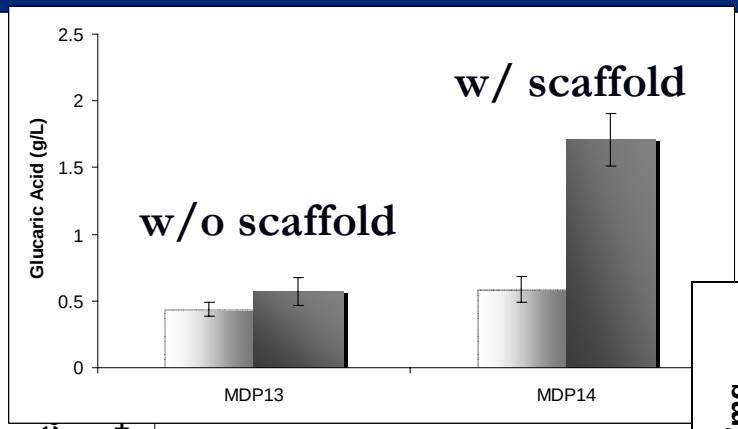
P-value = 0.032



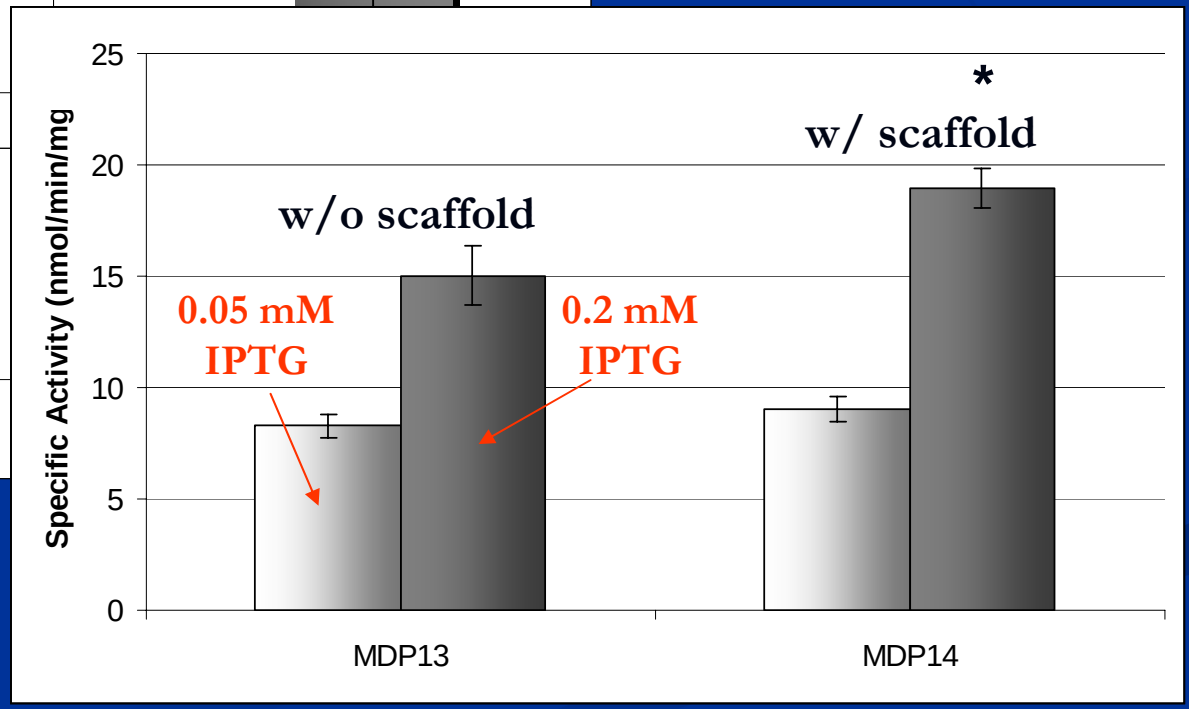
0.5 mM IPTG

Engineering "Parts"

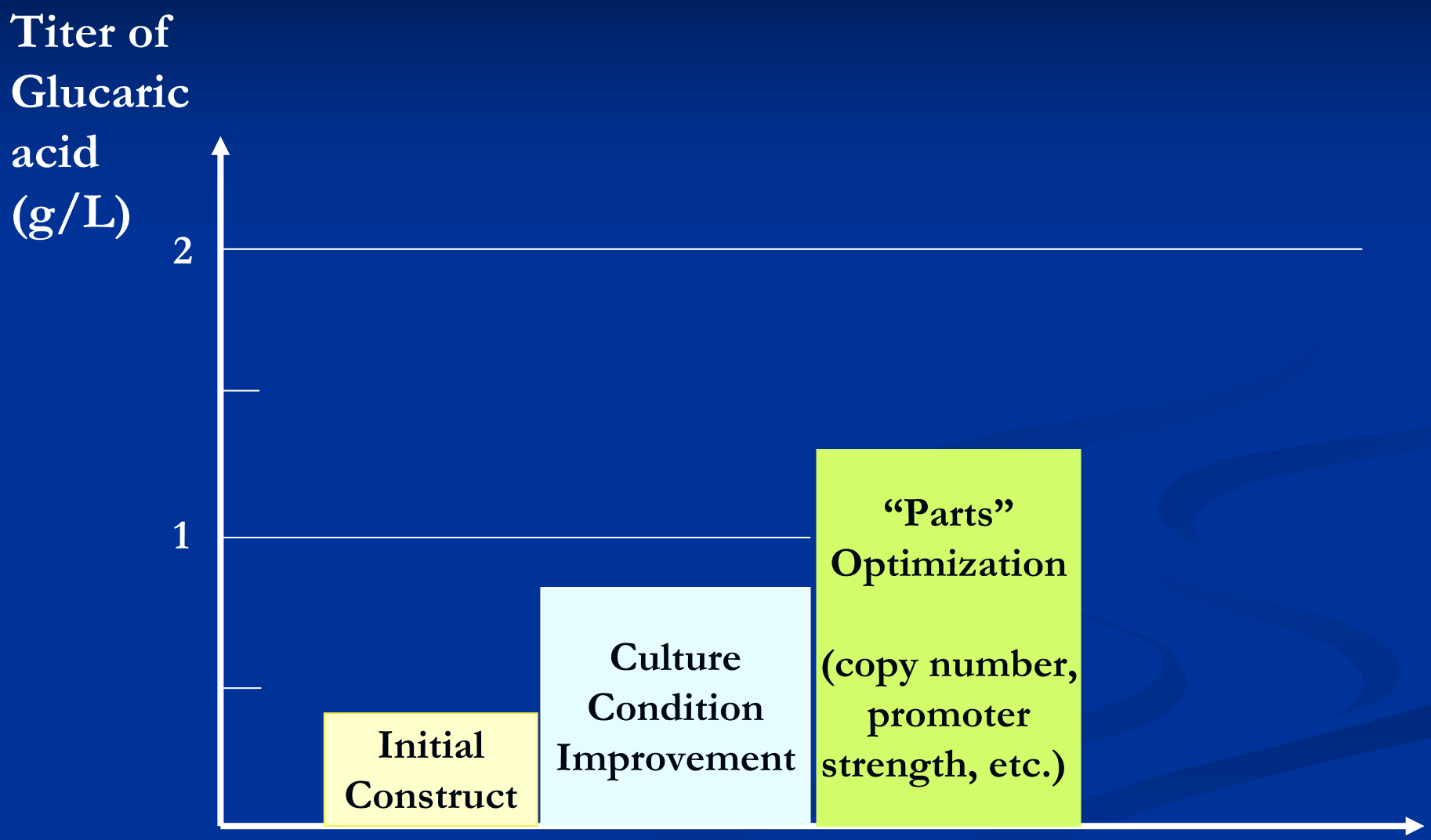
Co-localization → Better Activation of MIOX



P-value = 0.013

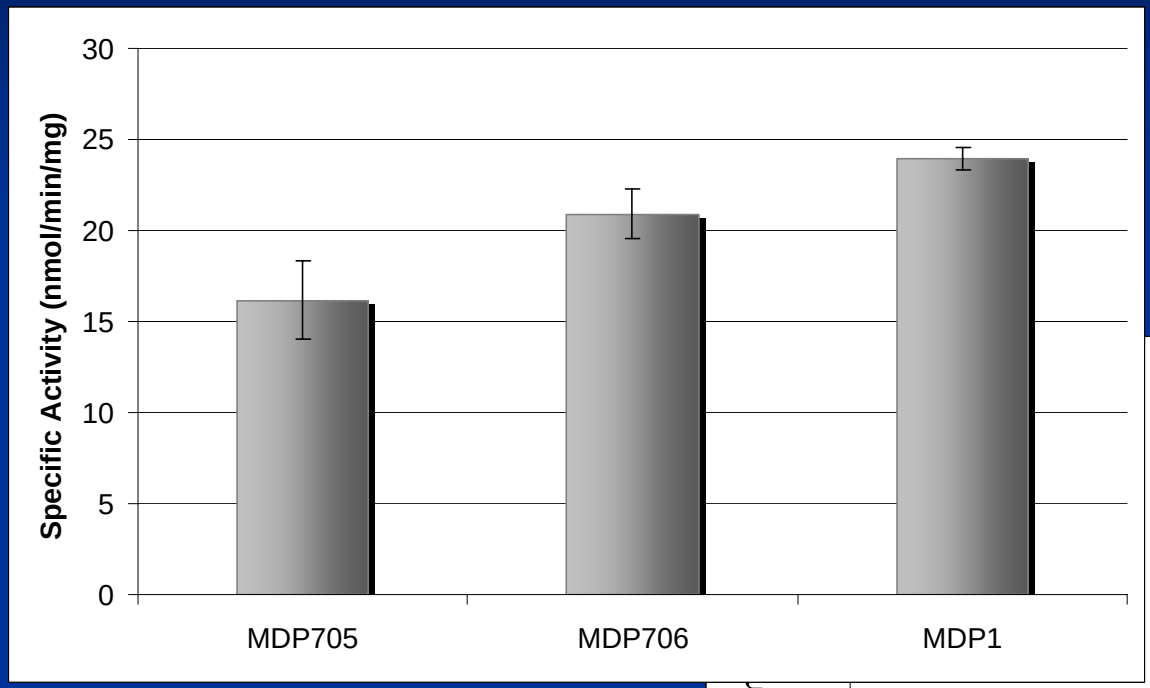


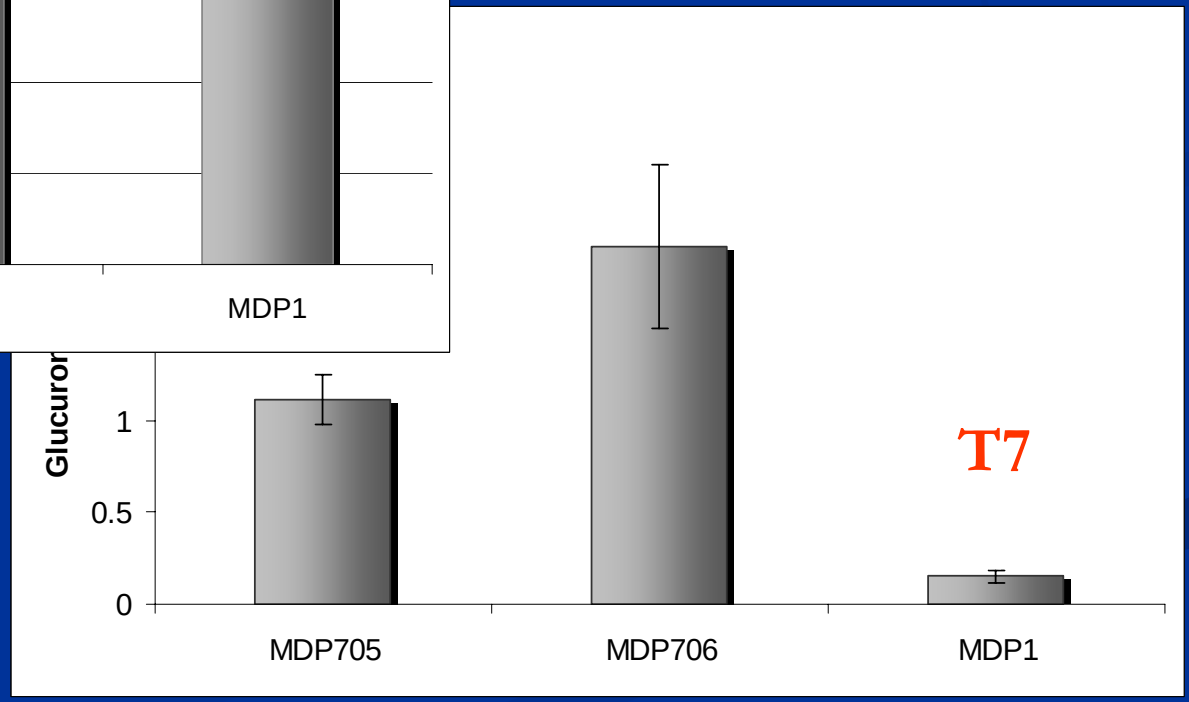
Optimization History



"Parts" Optimization

Effect of Promoter Choice





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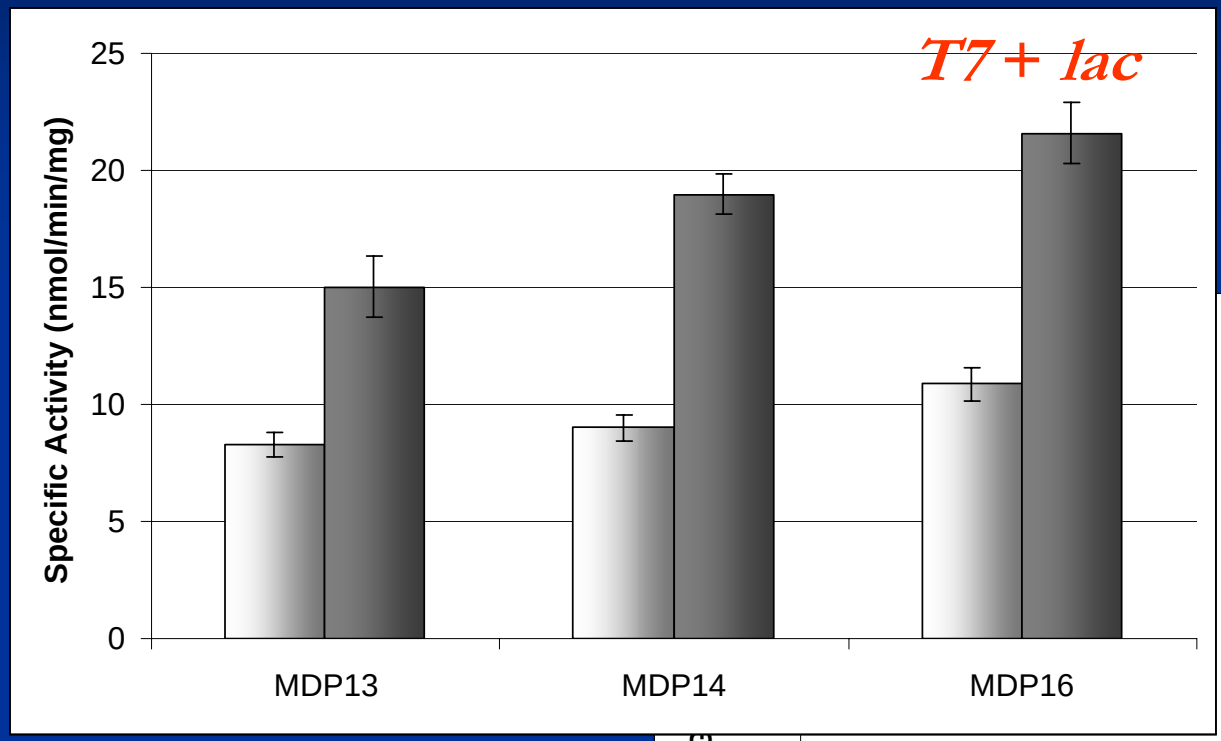
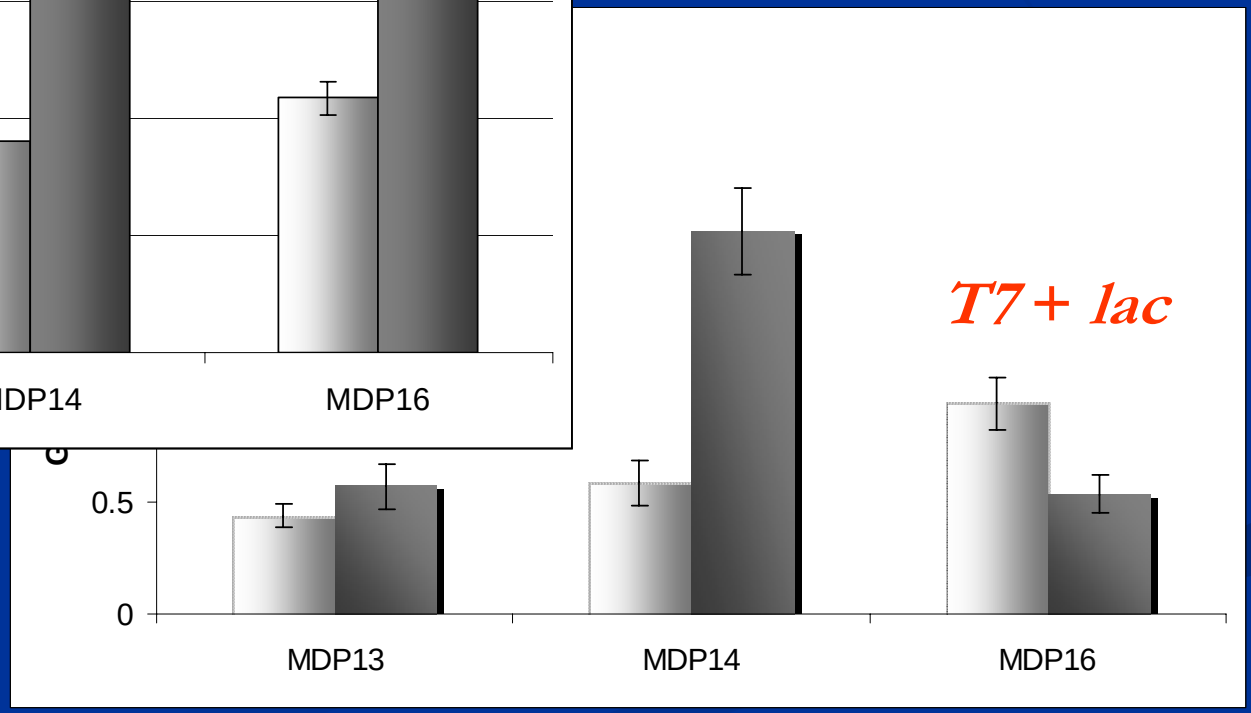
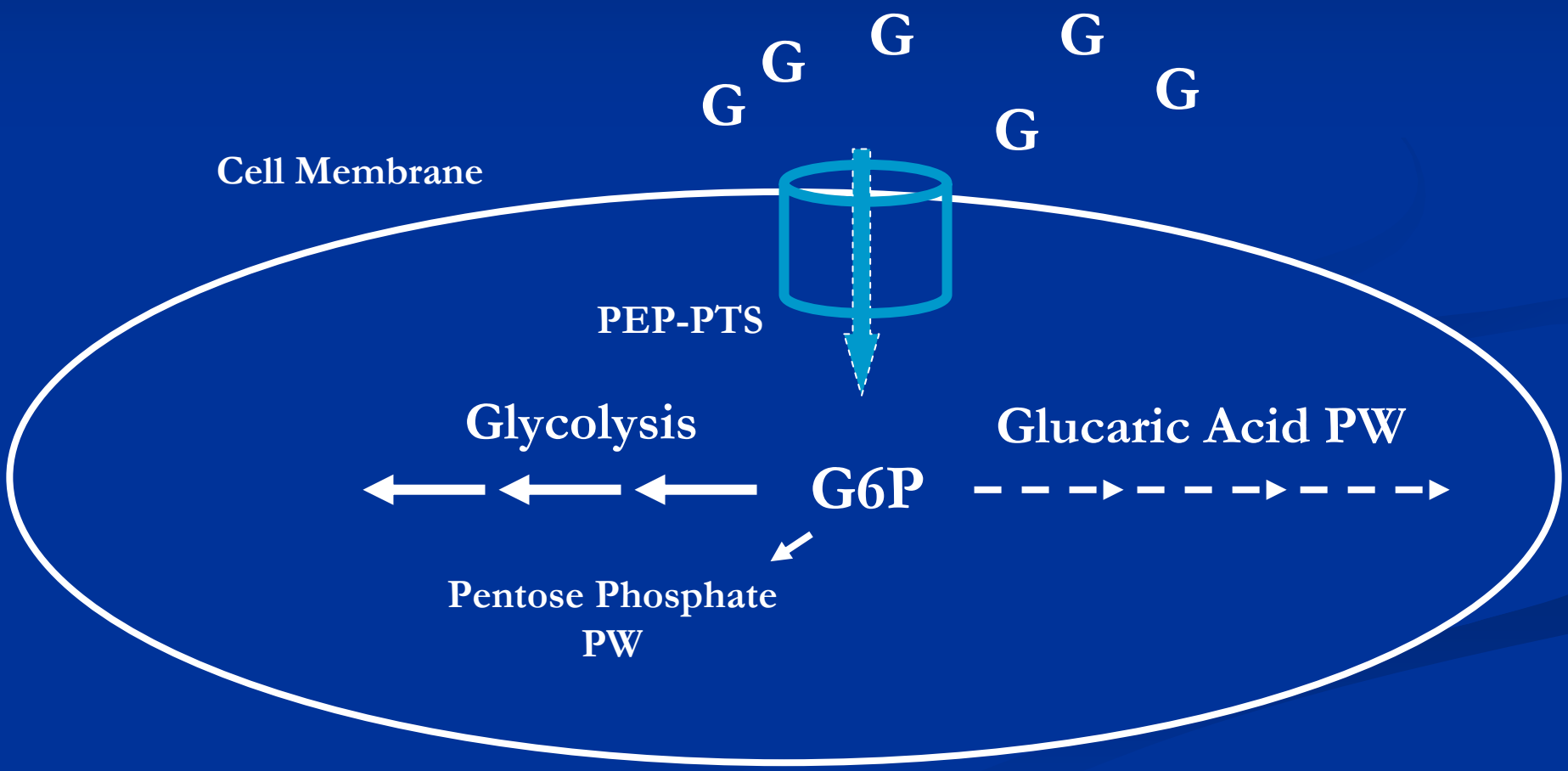




Challenges

Promoter Strength

➔ Too strong promoters / high induction cause burden.



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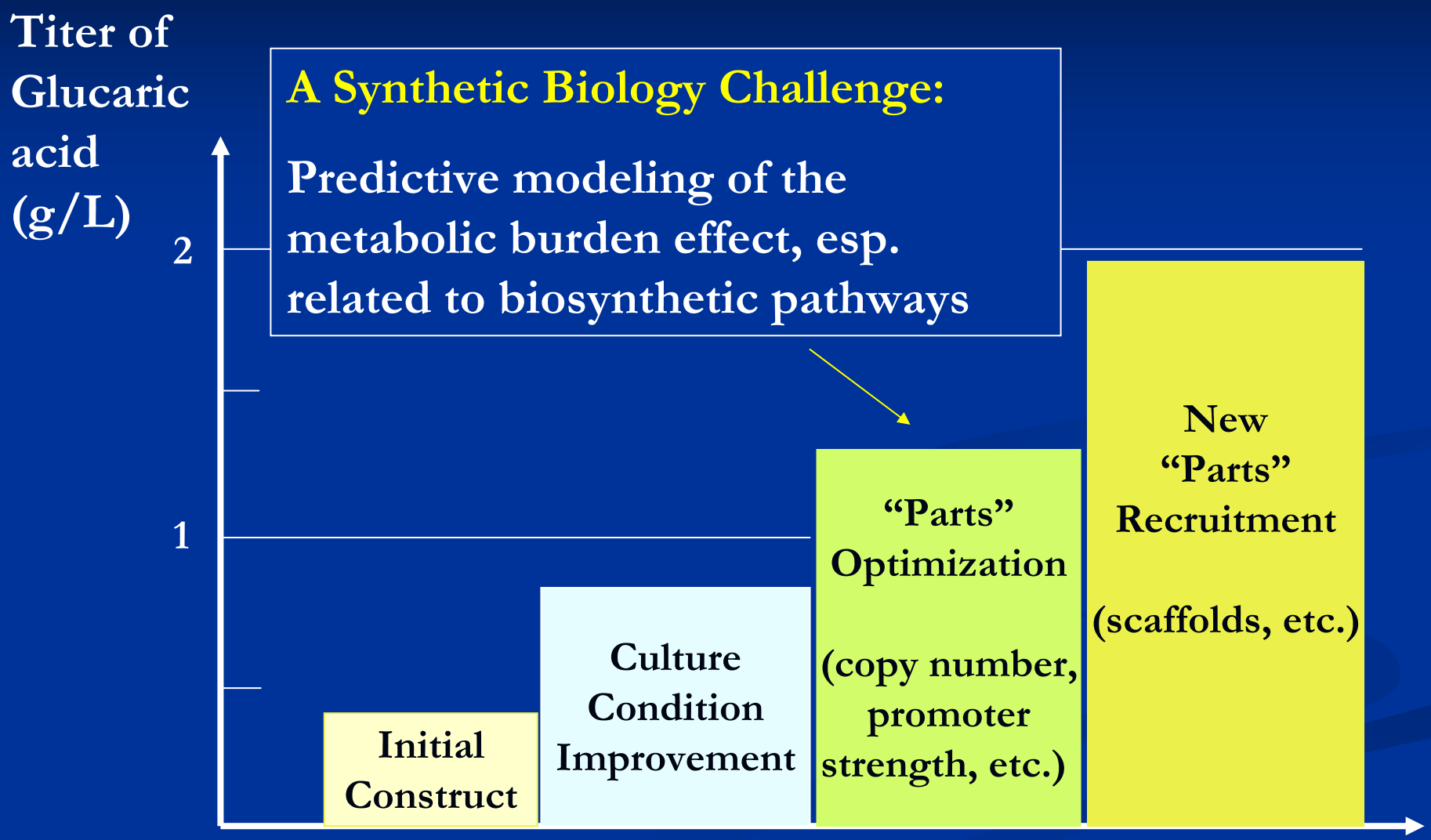

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SynBERC Synthetic Biology Engineering Research Center

The Prather Lab

Diana Bower

Dr. Effendi Leonard

Collin Martin

Tae Seok Moon

Neidi Negron Rodriguez

Dr. David Nielsen

Kevin Solomon

Hsien-Chung Tseng

Dr. Sang-Hwal Yoon (not picutred)

And many undergraduate researchers!

Collaborators

Alfonso Jaramillo

John Dueber