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NSF Engineering Research
Center for Biorenewable Chemicals

Chemicals from Biorenewables: Creating a New Catalytic Platform

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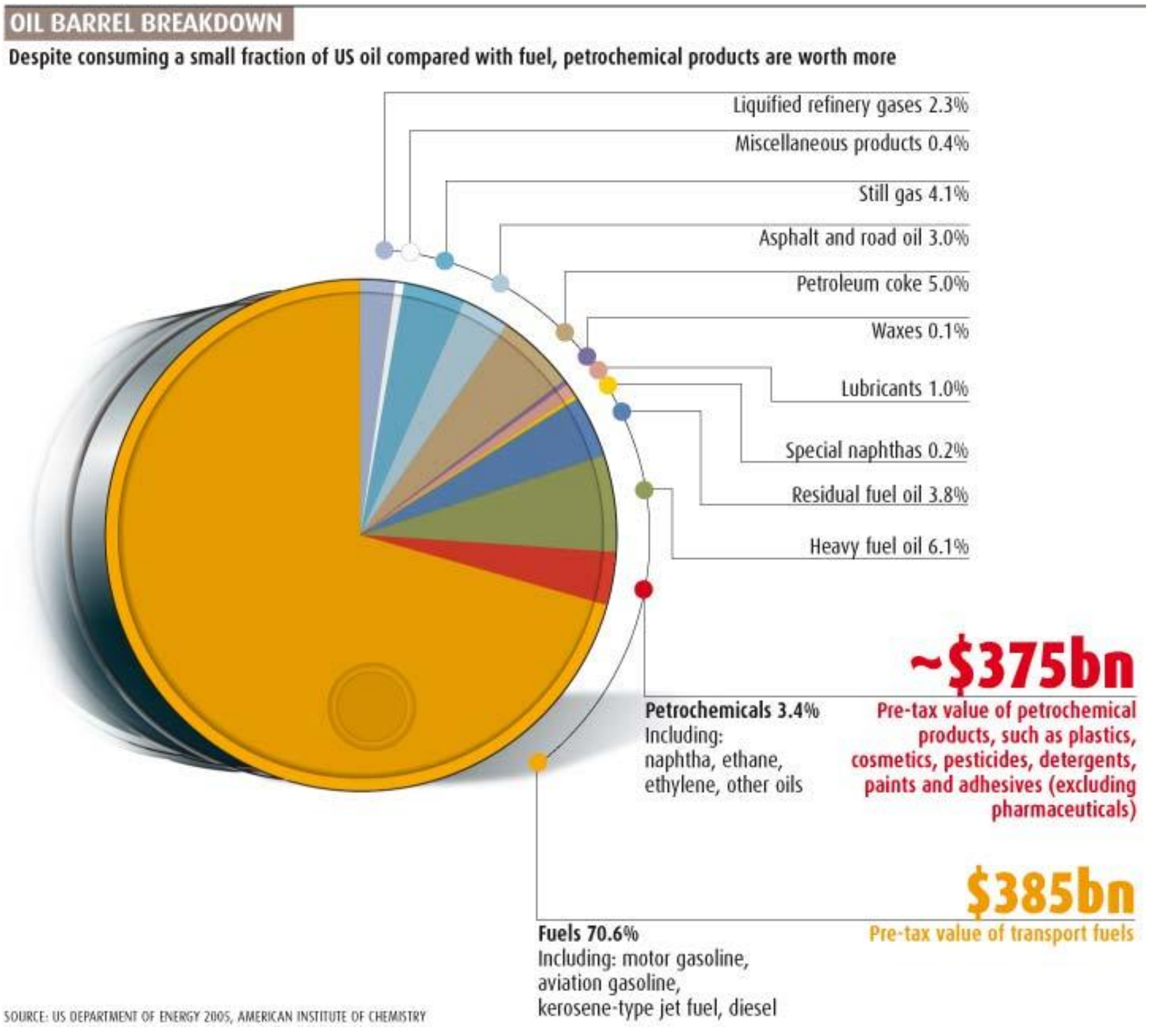
Center for Biorenewable Chemicals

Value of Petrochemicals



OIL BARREL BREAKDOWN

Despite consuming a small fraction of US oil compared with fuel, petrochemical products are worth more

SOURCE: US DEPARTMENT OF ENERGY 2005, AMERICAN INSTITUTE OF CHEMISTRY

Value per %

$110bn

$5bn

Discontinuities

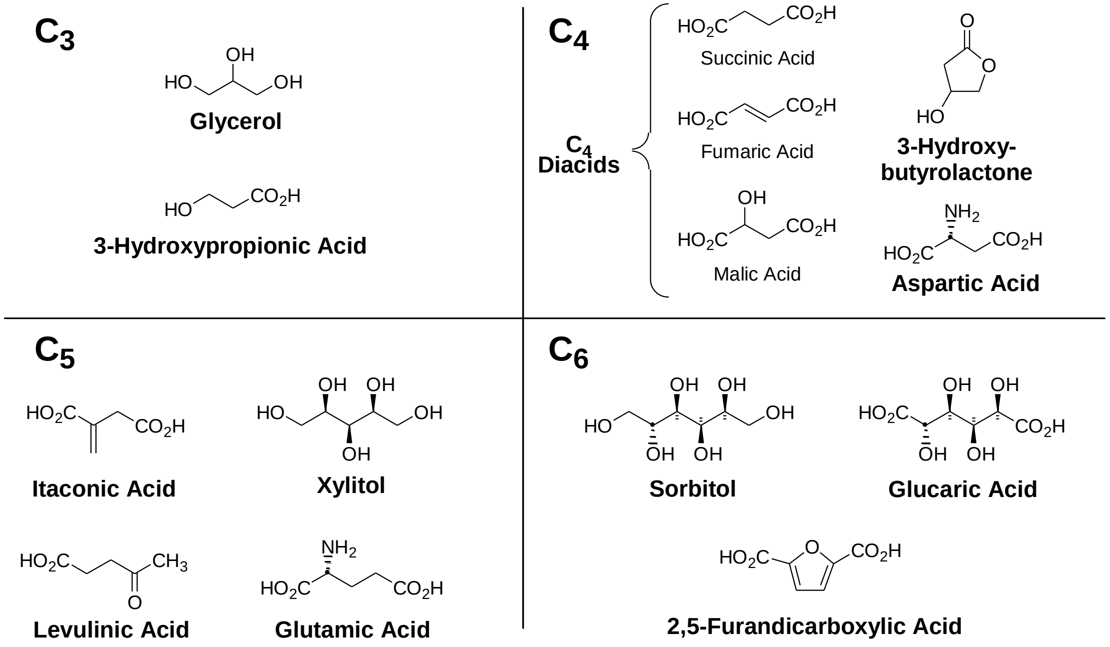
- **Disconnected biocatalysis/chemical catalysis technology**

→ *Center-sized effort needed*

- **Single product development (fractured market)**

→ *Generalized approach needed*

Platform Chemicals



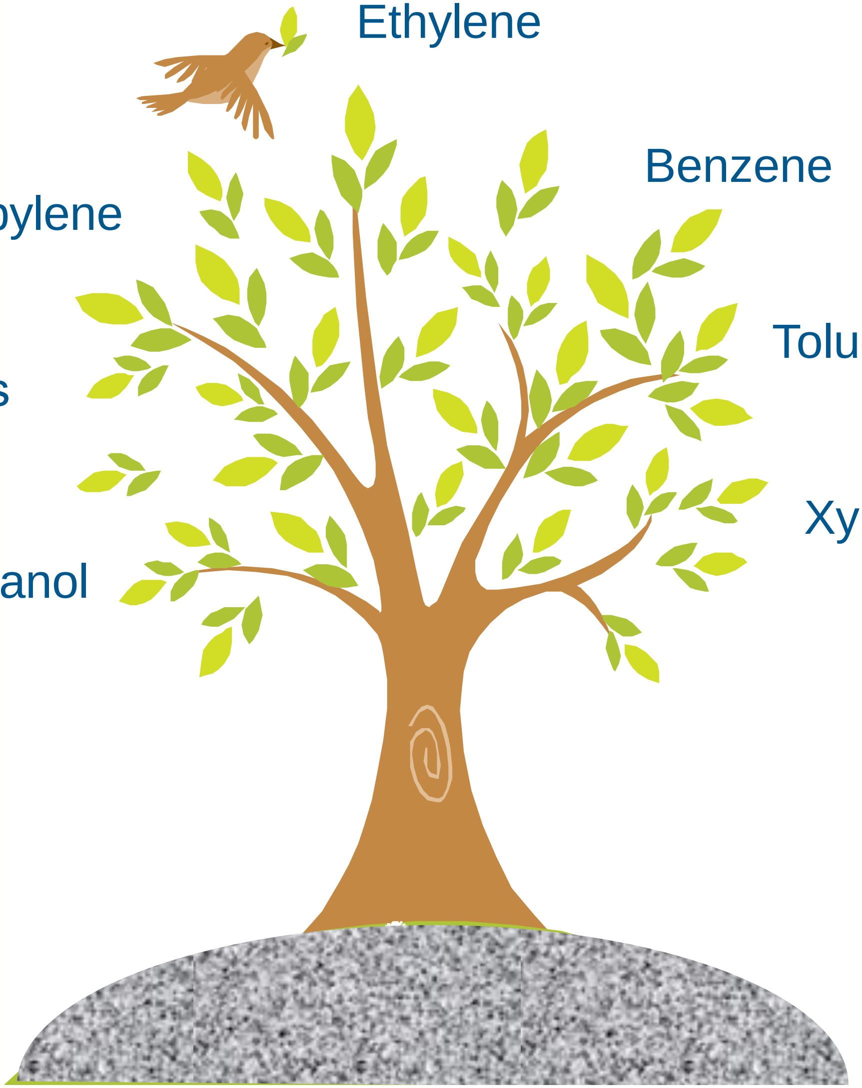
Werpy et al., *Top Value Added Chemicals from Biomass*, U.S. DOE, 2004

Ethanol
Glycerol
Lactic Acid
Hydroxypropionic Acid
Sorbitol

Furans
Biohydrocarbons
Succinic Acid
Levulinic Acid
Xylitol

Bozell and Peterson, *Green Chem.*, **12,** 539-554, 2010

Petrochemicals



Ethylene

Propylene

Benzene

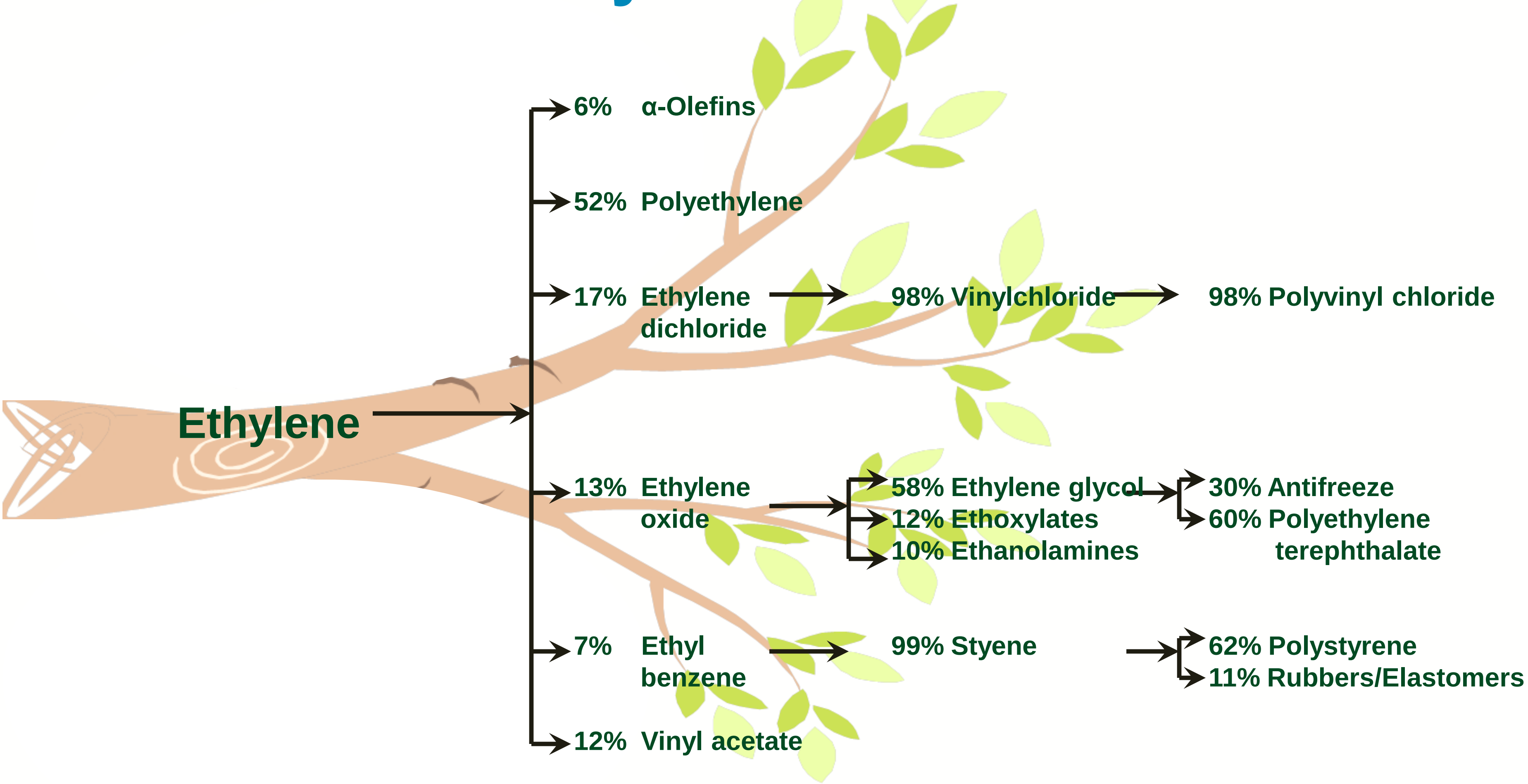
Toluene

C_4's

Xylene

Methanol

Ethylene and Derivatives



Ethylene

- 6% α-Olefins
- 52% Polyethylene
- 17% Ethylene dichloride → 98% Vinylchloride → 98% Polyvinyl chloride
- 13% Ethylene oxide
  - 58% Ethylene glycol
    - 30% Antifreeze
    - 60% Polyethylene terephthalate
  - 12% Ethoxylates
  - 10% Ethanolamines
- 7% Ethyl benzene → 99% Styene
  - 62% Polystyrene
  - 11% Rubbers/Elastomers
- 12% Vinyl acetate

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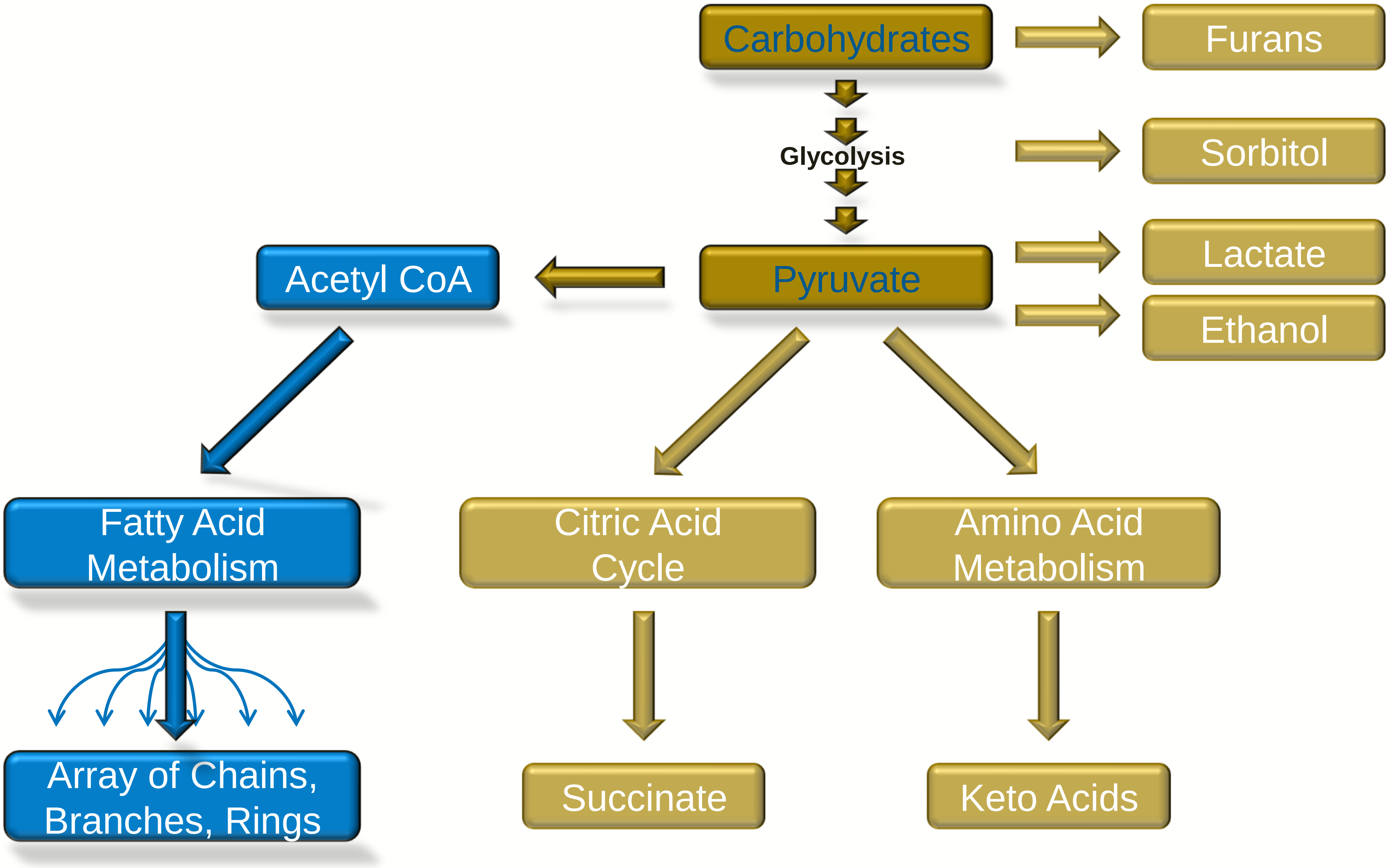
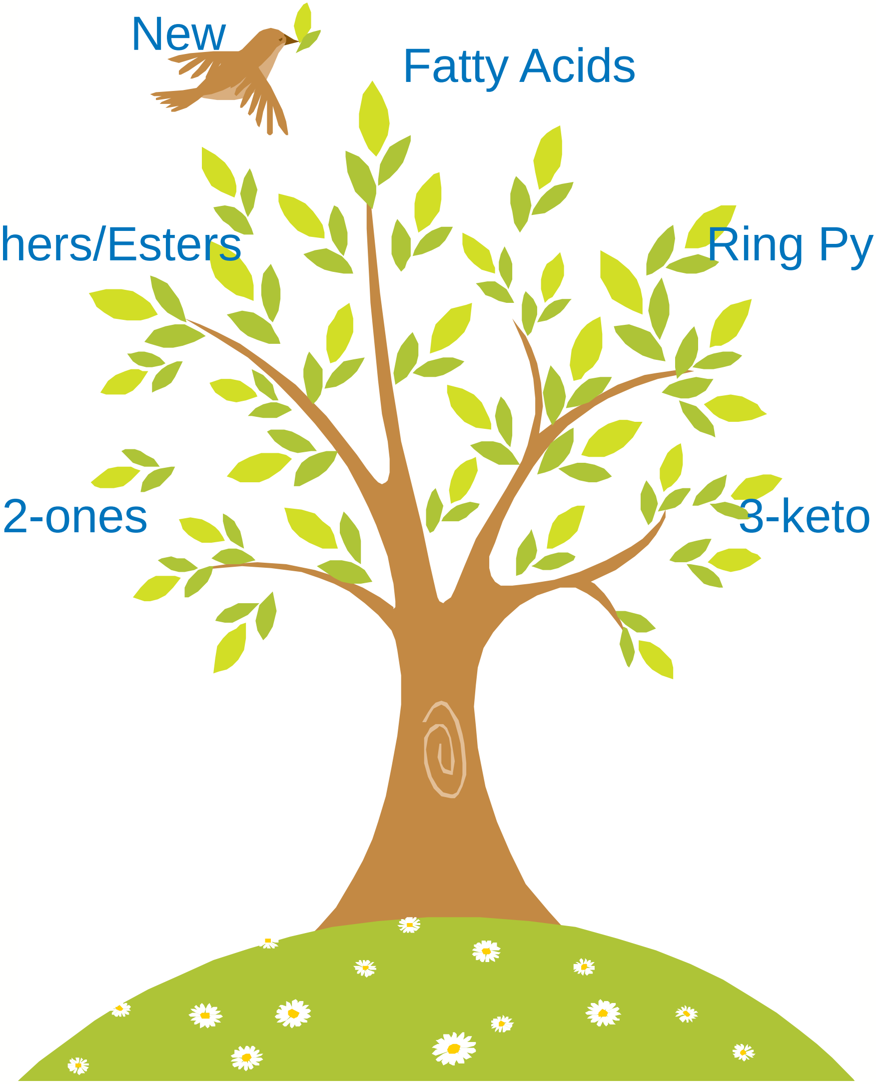
Pathways to Biorenewables



Biobased Chemicals



New

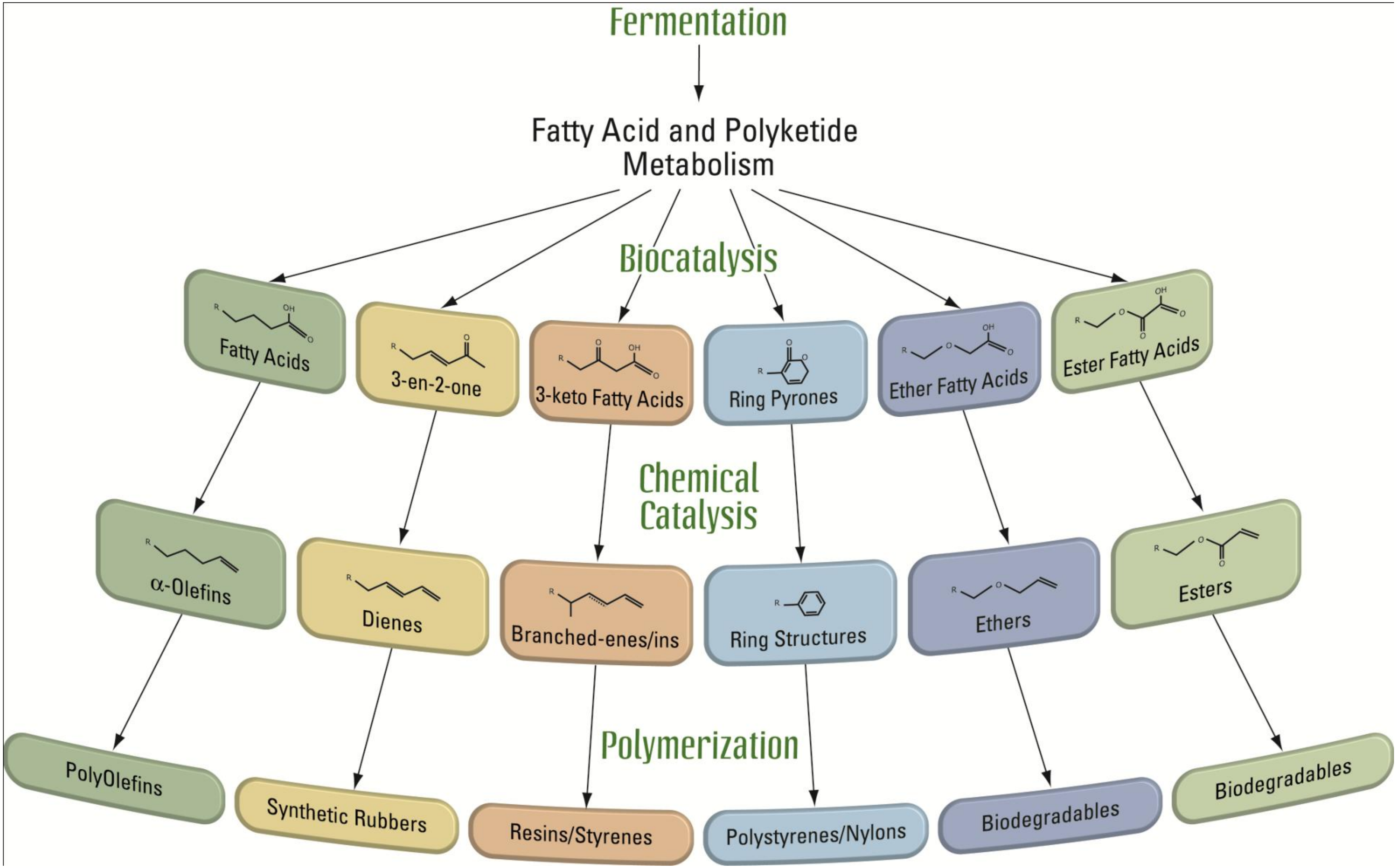
Fatty Acids

Ethers/Esters

Ring Pyrones

3-en-2-ones

3-keto Fatty Acids



Fermentation

Fatty Acid and Polyketide Metabolism

Biocatalysis

Fatty Acids

3-en-2-one

3-keto Fatty Acids

Ring Pyrones

Ether Fatty Acids

Ester Fatty Acids

Chemical Catalysis

α-Olefins

Dienes

Branched-enes/ins

Ring Structures

Ethers

Esters

Polymerization

PolyOlefins

Synthetic Rubbers

Resins/Styrenes

Polystyrenes/Nylons

Biodegradables

Biodegradables

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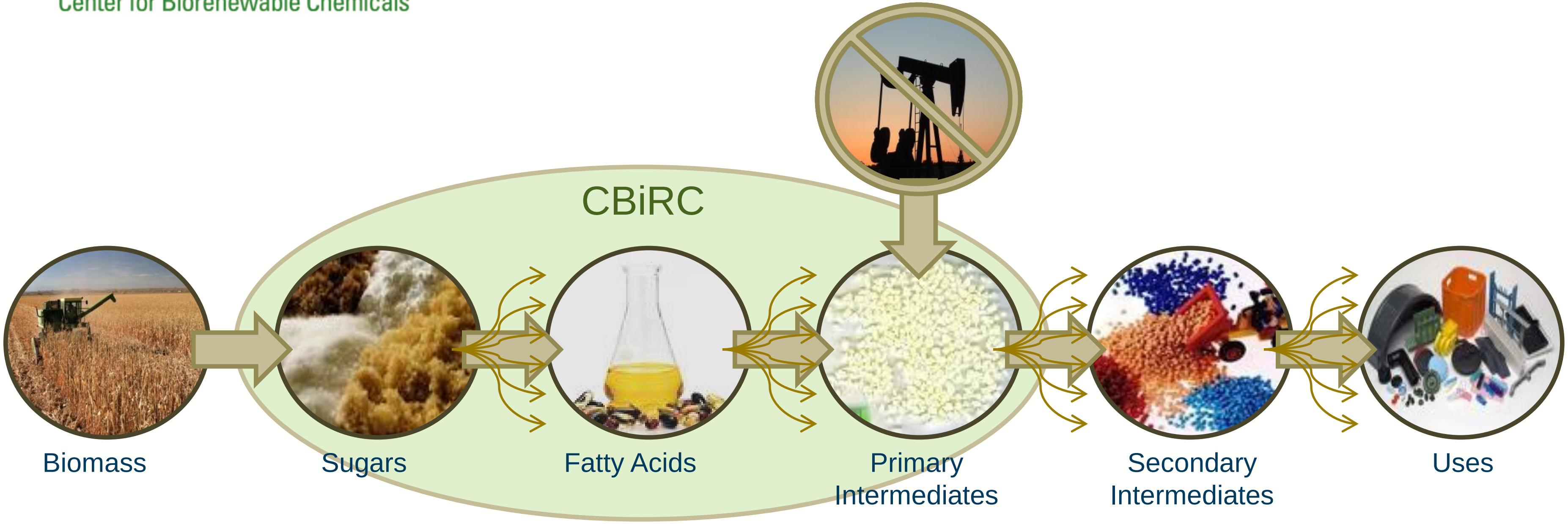
Value Chain



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Biomass → Sugars → Fatty Acids → Primary Intermediates → Secondary Intermediates → Uses

An array of bio-based opportunities:

- **Polymers, Paints, Coatings, Resins, Industrial Chemicals**
- **Packaging, Bottles, Containers, Inks, Dyes**
- **Adhesives, Sealants, Construction Chemicals**
- **Surfactants, Cleaning Agents, Specialty Chemicals**
- **Food additives, Flavorings, Fragrances, Cosmetics**

- **Lead Institution**
  - Iowa State University
- **Partner Institutions**
  - Rice University
  - University of California - Irvine
  - University of New Mexico
  - University of Virginia
  - University of Wisconsin
- **Affiliated Institutions**
  - The Salk Institute
  - University of Michigan
- **International Institutions**
  - Abo Akademi University (Finland)
  - Fritz Haber Institute (Germany)
  - Technical University of Denmark
  - Technische Universiteit (Netherlands)

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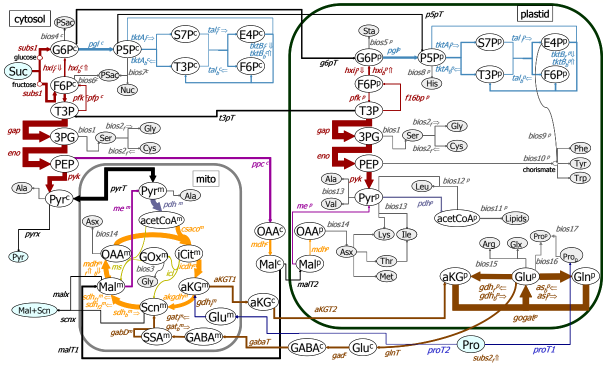
Research Areas



New Biocatalysts for Pathway Engineering

David Oliver *(ISU)*
Basil Nikolau *(ISU)*
Joe Noel *(Salk)*
Eran Pichersky *(UMich.)*
Tom Bobik *(ISU)*
Peter Reilly *(ISU)*
Eve Wurtele *(ISU)*

Microbial Metabolic Engineering

Jackie Shanks *(ISU)*
Nancy Da Silva *(UCIrv.)*
Ka-Yiu San *(Rice)*
Julie Dickerson *(ISU)*
Ramon Gonzalez *(Rice)*
Laura Jarboe *(ISU)*
Suzanne Sandmeyer *(UCIrv)*
Costas Maranas *(PSU)*

Chemical Catalyst Design

Robert Davis *(UVa)*
Brent Shanks *(ISU)*
Jim Dumesic *(UWisc)*
Abhaya Datye *(UNM)*
Matt Neurock *(UVa)*
George Kraus *(ISU)*
Klaus Schmidt-Rohr *(ISU)*
Keith Woo *(ISU)*

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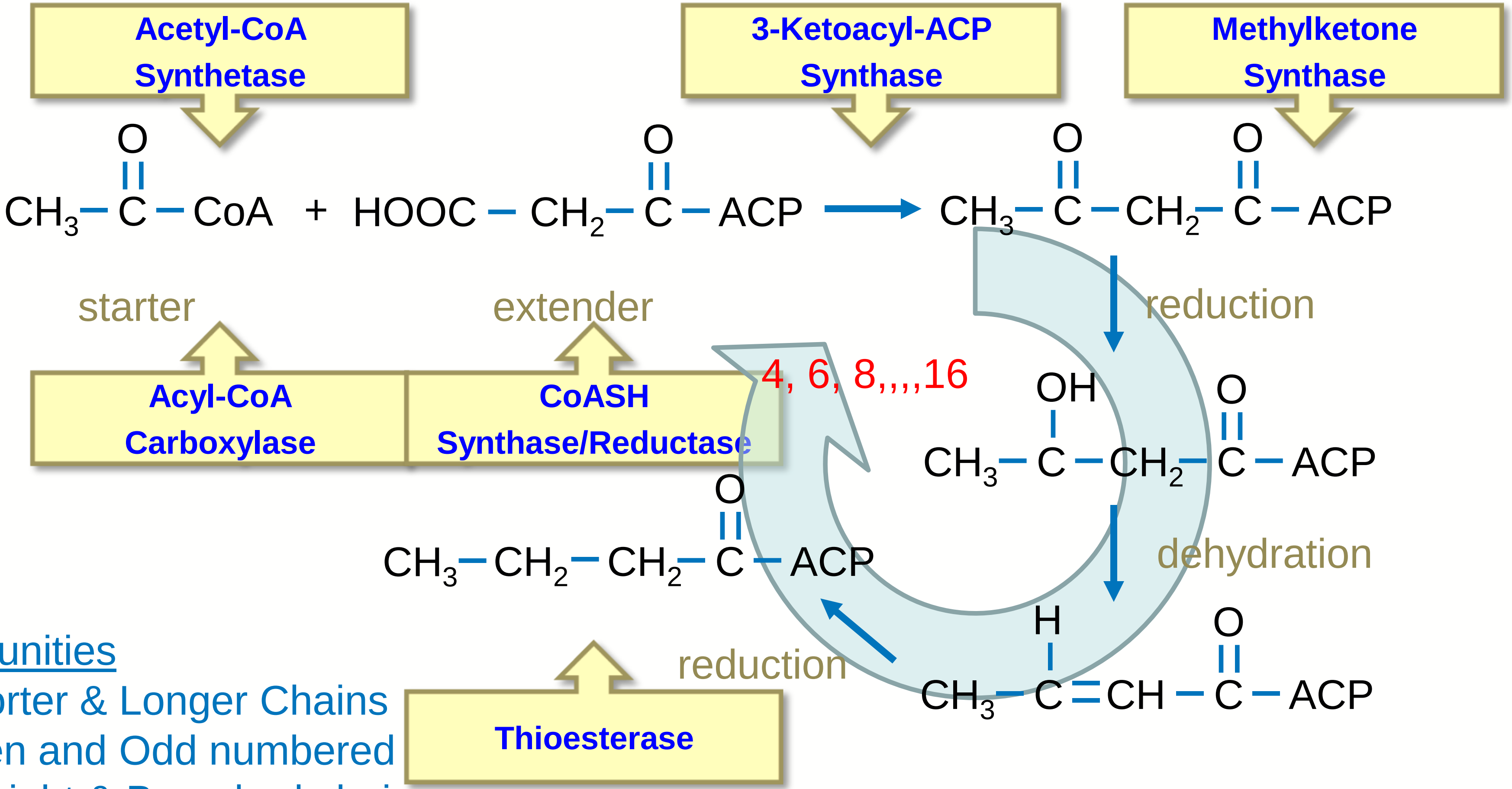
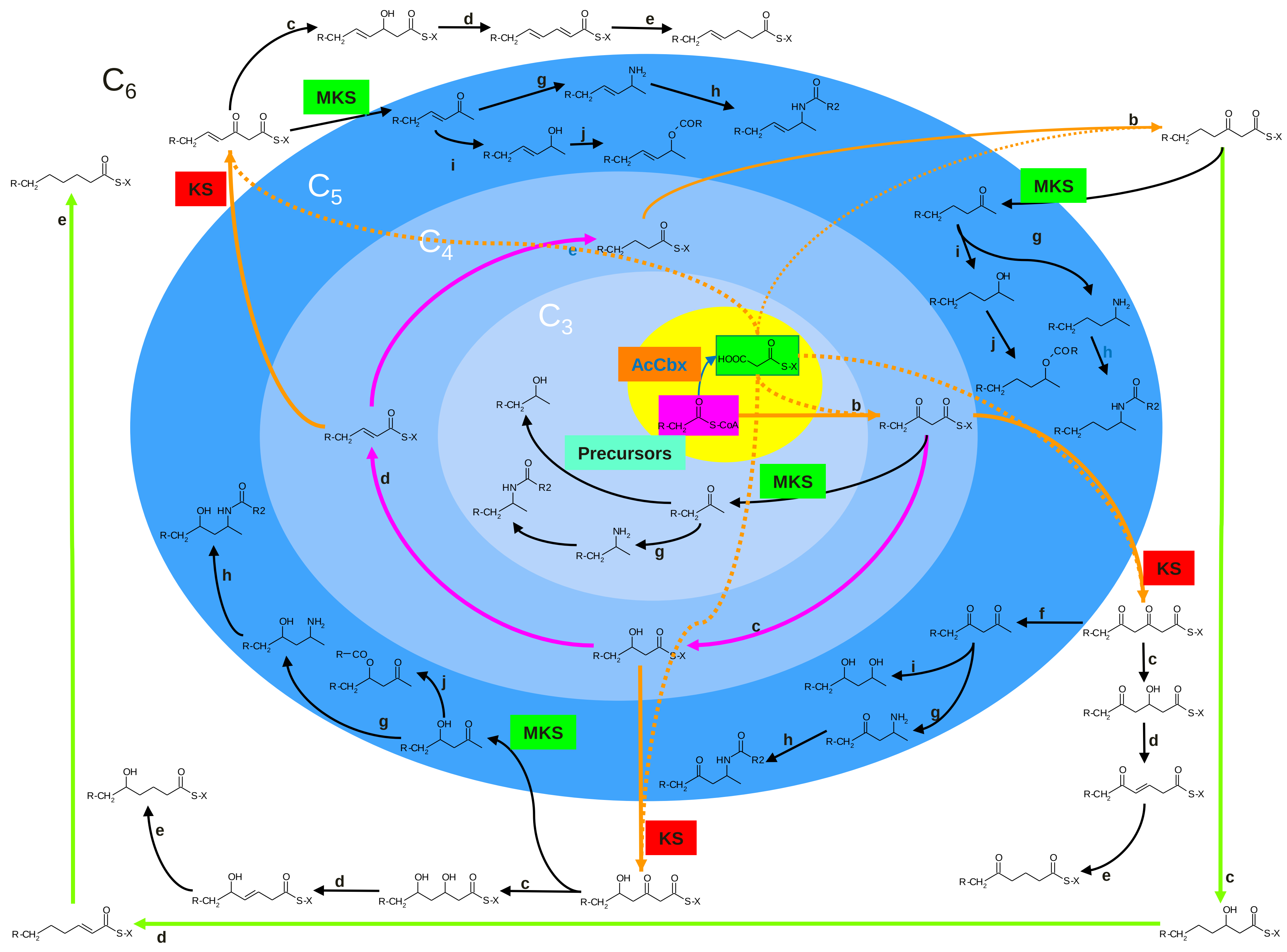
Fatty Acid Biosynthesis



<u>Opportunities</u>

1. Shorter & Longer Chains
2. Even and Odd numbered
3. Straight & Branched chains
4. Diversified Chemistry



C_6
C_5
C_4
C_3
MKS
MKS
MKS
MKS
MKS
KS
KS
KS
AcCbx
Precursors
HOOC
S-X
R-CH$_2$
S-CoA
b
c
d
e
f
g
h
i
j
OH
NH$_2$
HN
R2
COR
R—CO
O

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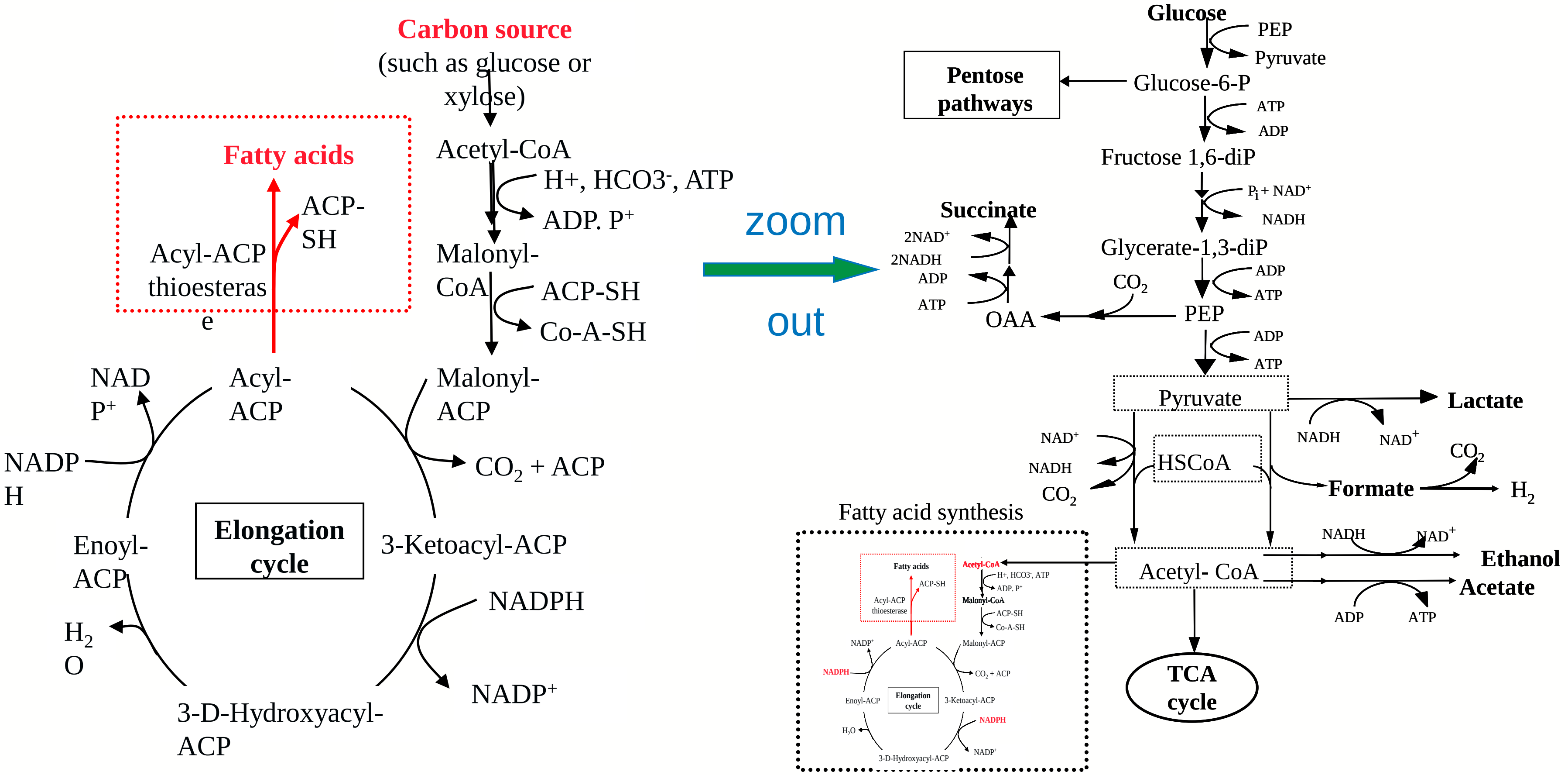
Microbial Engineering



Carbon source
(such as glucose or xylose)
Acetyl-CoA
H+, HCO3-, ATP
ADP. P+
Malonyl-CoA
ACP-SH
Co-A-SH
Malonyl-ACP
CO$_2$ + ACP
3-Ketoacyl-ACP
NADPH
NADP+
3-D-Hydroxyacyl-ACP
H_2O
Enoyl-ACP
Elongation cycle
NADPH
NADP+
Acyl-ACP
Fatty acids
ACP-SH
Acyl-ACP thioesterase

zoom out

Glucose
PEP
Pyruvate
Glucose-6-P
Pentose pathways
ATP
ADP
Fructose 1,6-diP
P_i + NAD$^+$
NADH
Glycerate-1,3-diP
ADP
ATP
CO$_2$
PEP
OAA
ATP
ADP
2NADH
2NAD$^+$
Succinate
ADP
ATP
Pyruvate
NADH
NAD$^+$
Lactate
NAD$^+$
NADH
CO$_2$
HSCoA
Formate
CO$_2$
H$_2$
Fatty acid synthesis
Acetyl- CoA
NADH
NAD$^+$
Ethanol
Acetate
ADP
ATP
TCA cycle

Fatty acids
Acetyl-CoA
H+, HCO3-, ATP
ADP. P+
ACP-SH
Acyl-ACP thioesterase
Malonyl-CoA
ACP-SH
Co-A-SH
NADP+
Acyl-ACP
Malonyl-ACP
NADPH
CO$_2$ + ACP
Enoyl-ACP
Elongation cycle
3-Ketoacyl-ACP
NADPH
H$_2$O
NADP+
3-D-Hydroxyacyl-ACP

Strategy: prune the network by eliminating competitive pathways
co-factoring engineering – NAD(P)H, CoA/acetyl-CoA

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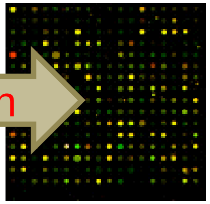
Metabolic Engineering

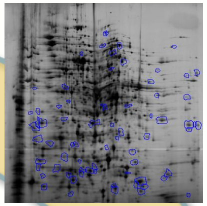
Strain Selection and Evolution

Omics and Flux Analysis

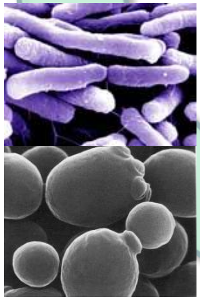

Metabolic Evolution

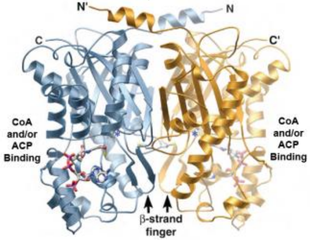
Characterization


Transcriptomics

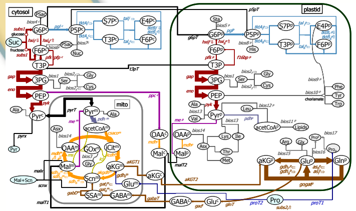

Proteomics

Information

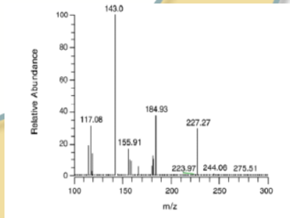

Strain Selection and
Transformation



Gene Targets
(from Thrust 1)



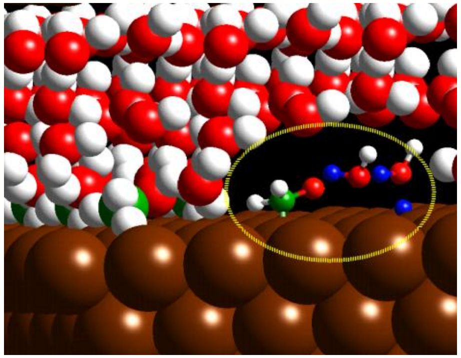
Flux Analysis



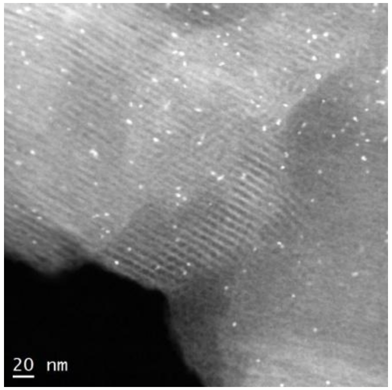
Metabolomics

Chemical Catalyst Design

- **Developing the catalytic “tool chest”**
  - Existing biorenewable molecules
  - Model compounds from biological catalysis



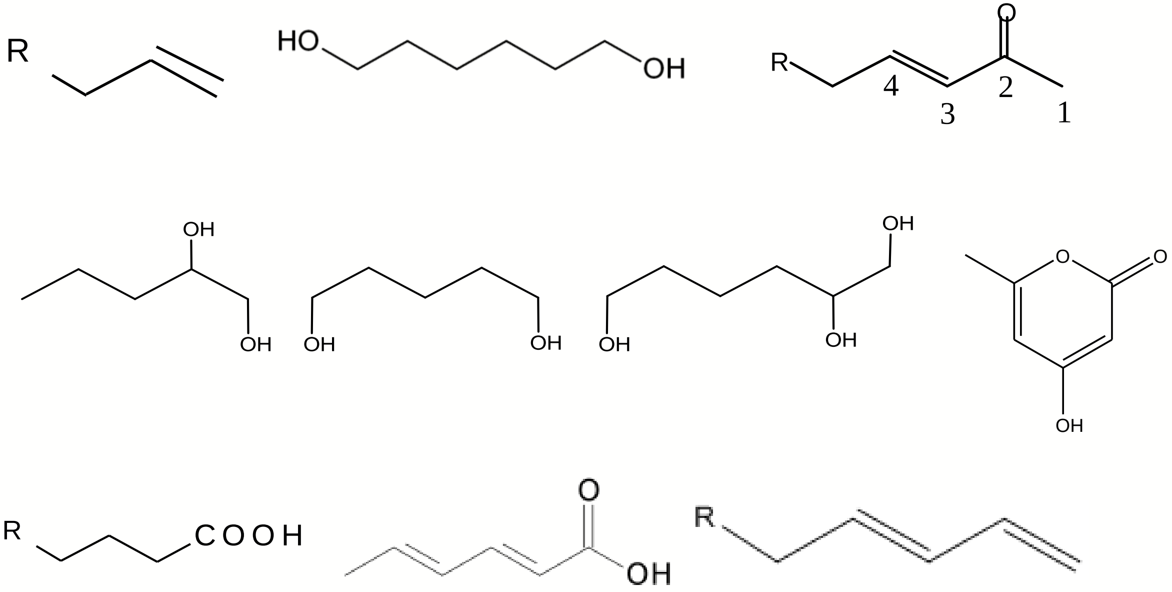
- **Developing integrated conversion systems**
  - Reaction systems with new biorenewable substrates



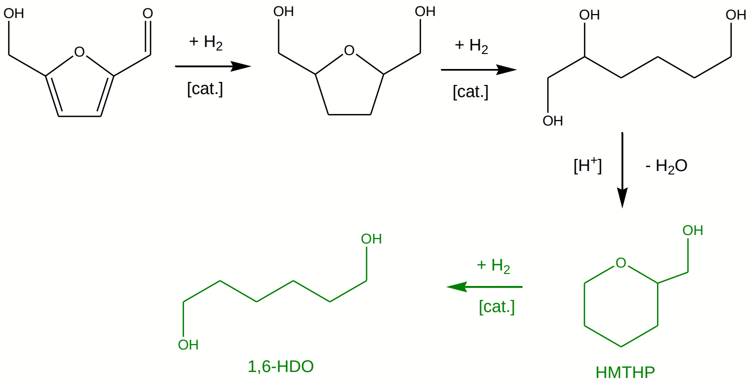
Catalytic Toolbox

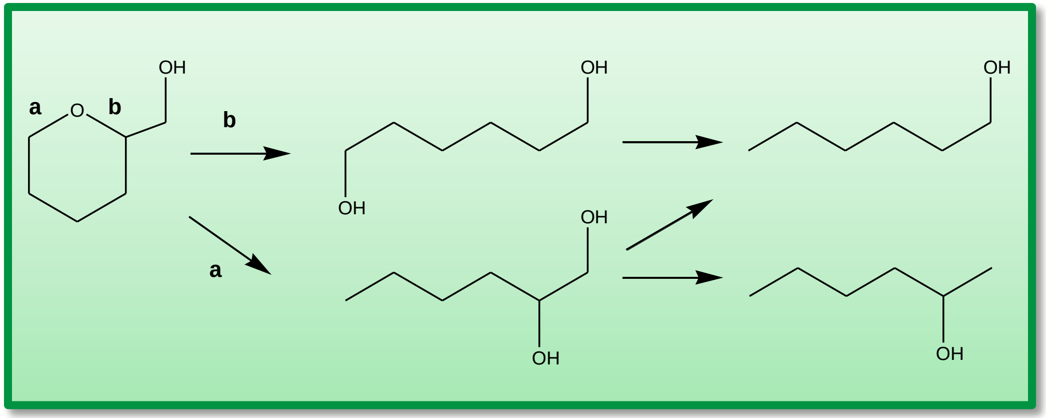
Selective Chemical Catalysis

1. Decarboxylation
2. Hydrogenation
3. Ring opening
4. Conjugation
5. Dehydration
6. *Stable catalysts*
7. *Bifunctional catalysts*



Selective Pyran Ring-Opening

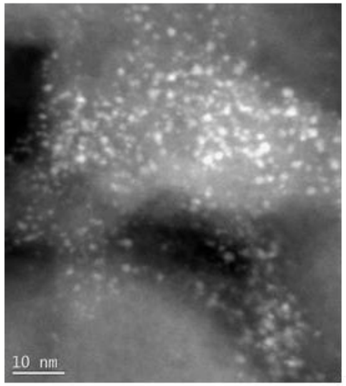


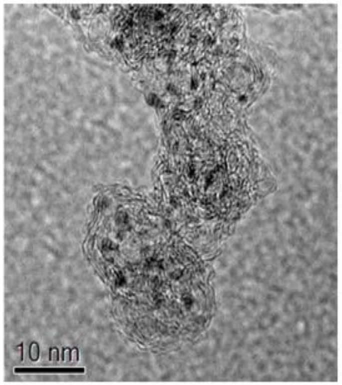


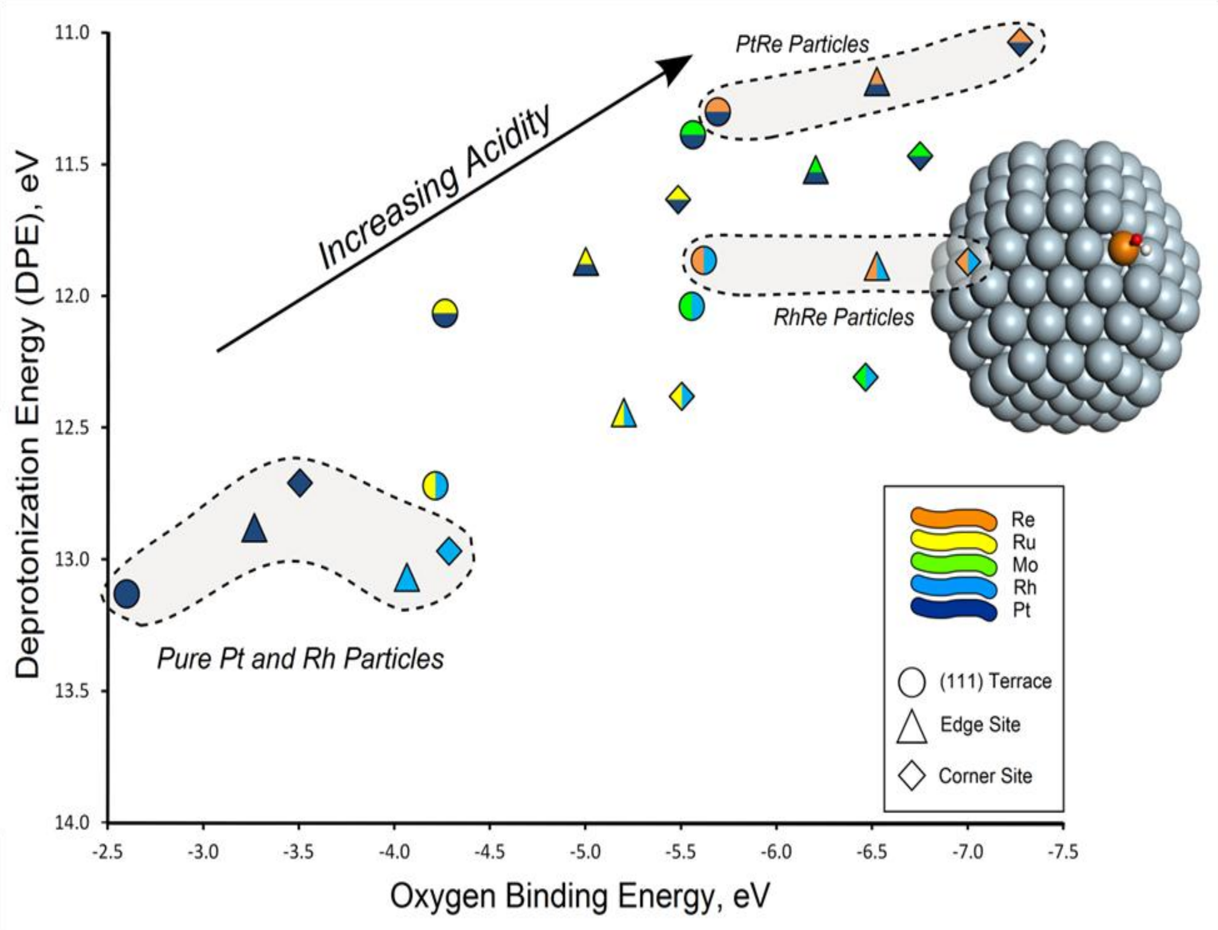
Catalyst	Time (h)	Catalyst Mass (g)	Conversion (%)	Selectivity (%)		Rate ($\mu molg^{-1}min^{-1}$)
				1,6-HDO	1,2-HDO	
4wt% Rh/C‡	4.5	0.2	7	46	12	8
3.6wt% MO_x/C‡	12	0.2	2	0	0	0.6
1.8wt% $M'O_x$/C‡	12	0.2	1	0	0	0.3
4wt% Rh-MO_x/C*	4.5	0.08	55	86	0	153
4wt% Rh-$M'O_x$/C*	12	0.2	55	84	0	22

Hydrogenolysis of HMTHP over monometallic‡ and promoted* catalysts; atomic ratios: 5wt% HMTHP in H_2O, 120 °C, 8 MPa H_2

Highlights: *Discovery (T3)*



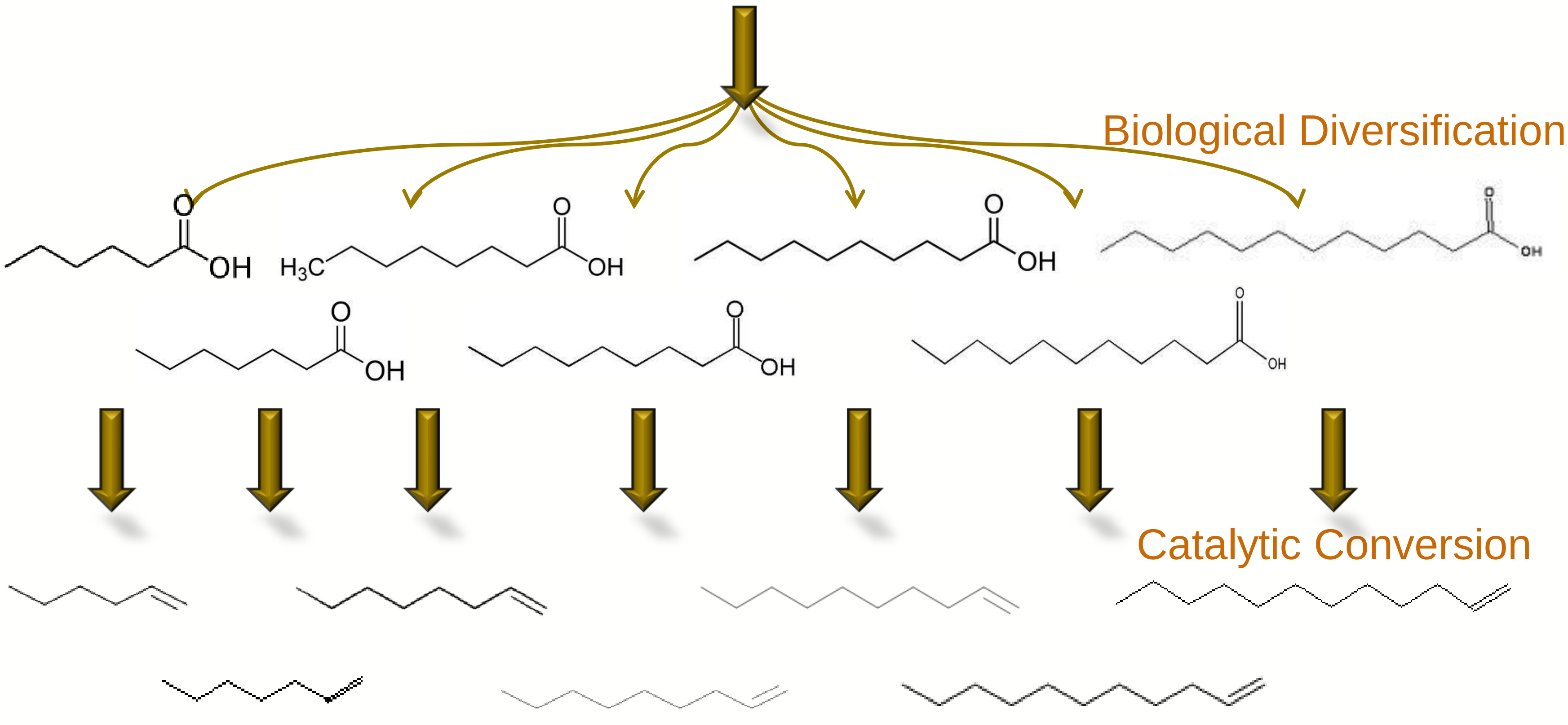




Matrix with Testbeds

		Carboxylic Acids	Pyrones	Bi-Functionals	Discovery
THRUST 1	1.1		●	●	
	1.2				●
	1.3	●		●	●
	1.4			●	●
	1.5			●	●
	1.6	●			
THRUST 2	2.1	●	●		●
	2.2	●	●		●
	2.3	●	●		●
	2.4	●	●		●
	2.5	●	●		●
THRUST 3	3.1				●
	3.2		●		●
	3.3	●			
	3.4			●	●
	3.5				●
	3.6			●	●
	3.7				●
	3.8				●
	3.9		●		
LCA		●	●	●	

Carboxylic Acid Testbed



Sugars

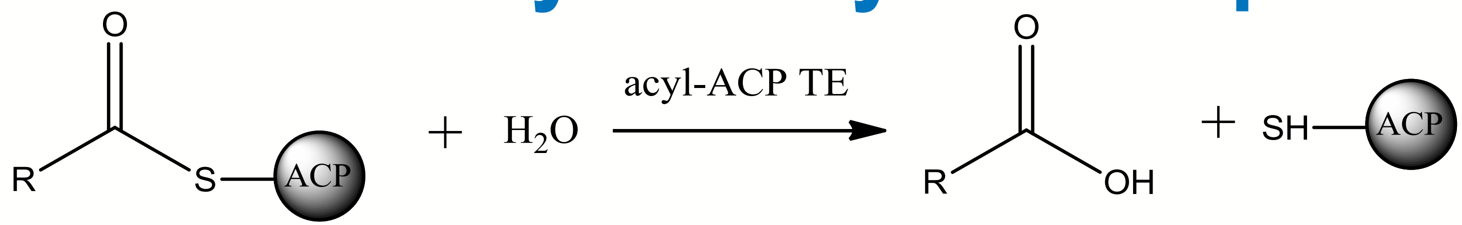
Biological Diversification

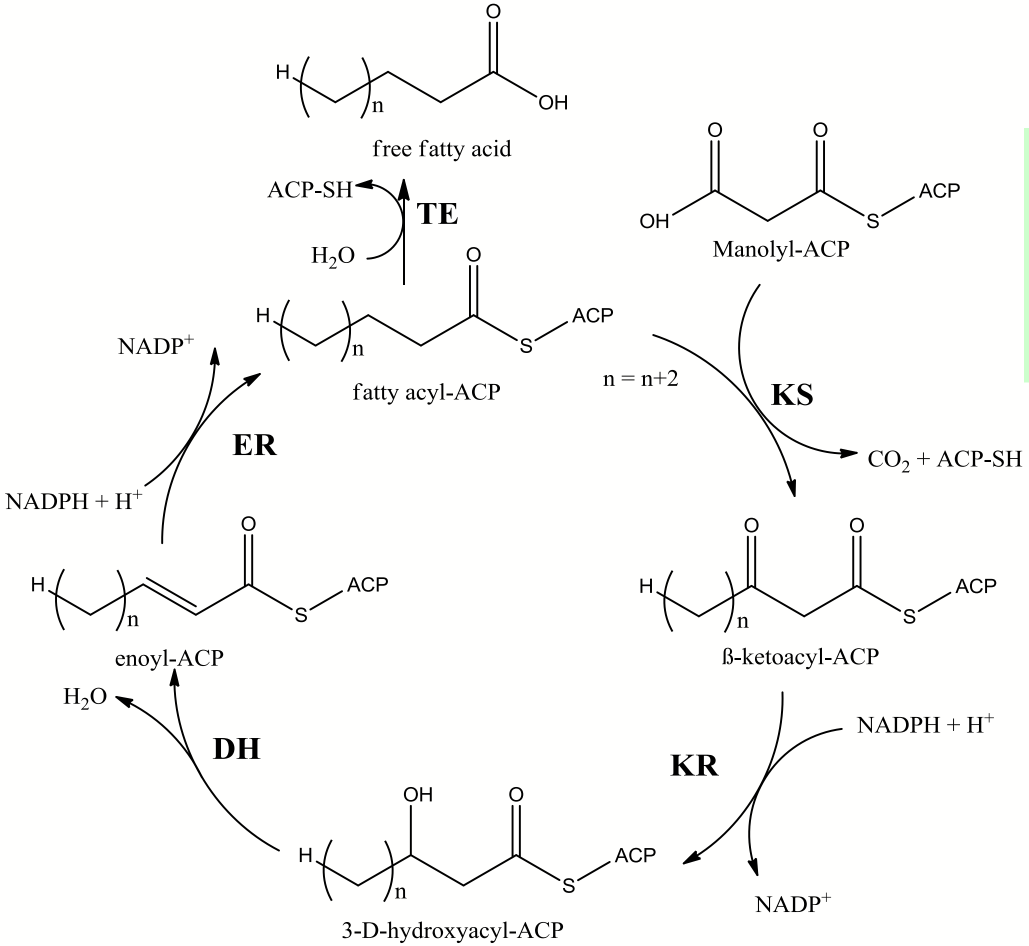
Catalytic Conversion

Hexene Heptene Octene Nonene Decene Undecene Dodecene etc.

α-Olefins

Fatty acid synthesis pathway



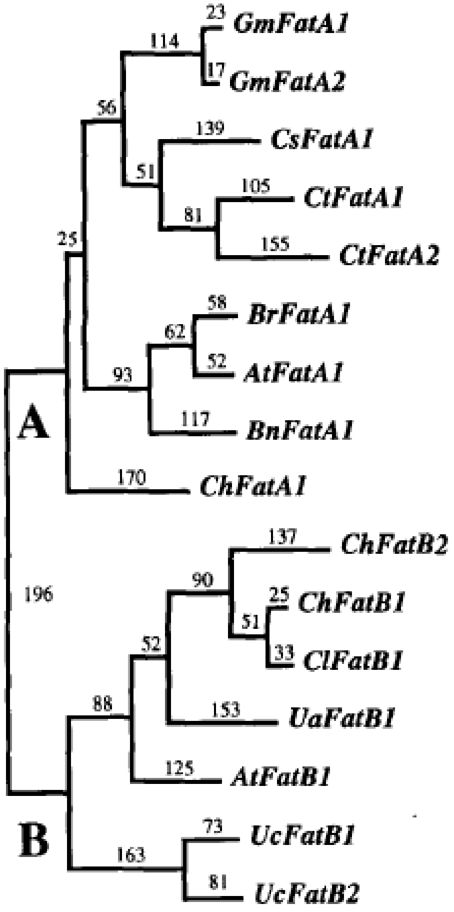


Acyl-ACP thioesterase

Terminates FAS elongation

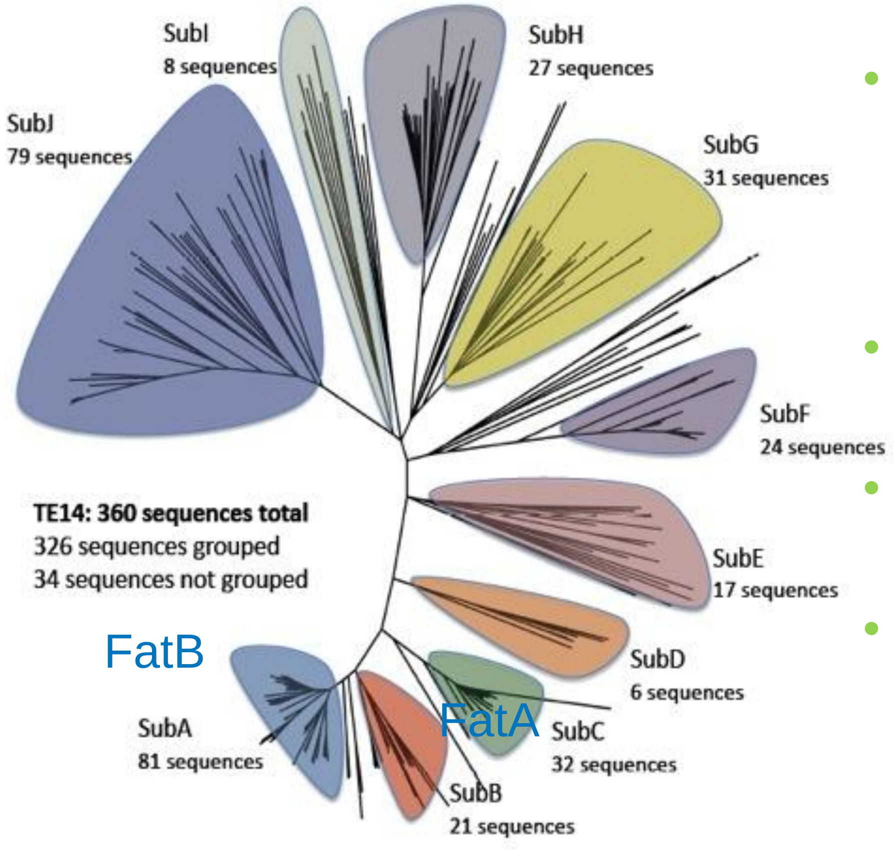
TEs known prior to CBiRC

Name	**organism**	**substrate specificity**
CpFatB1	*Cuphea palustris*	**8:0**/10:0
CpFatB2	*Cuphea palustris*	**14:0**/16:0
ChFatB1	*Cuphea hookeriana*	14:0/**16:0**/18:0/18:1
ChFatB2	*Cuphea hookeriana*	8:0/**10:0**
UcBTE	*Umbellularia californica*	**12:0**/14:0
UaFatB1	*Ulmus americana*	8:0/**10:0**/12:0/14:0/**16:0**/18:0
MfFaB1	*Myristica fragrans*	14:0/**16:0**/18:0
AtFatA	*Arabidopsis thaliana*	**18:1**
GmFatA	*Garcinia mangostana*	**18:1**/18:0
MtFatA	*Macadamia tetraphylla*	**18:1**/16:1



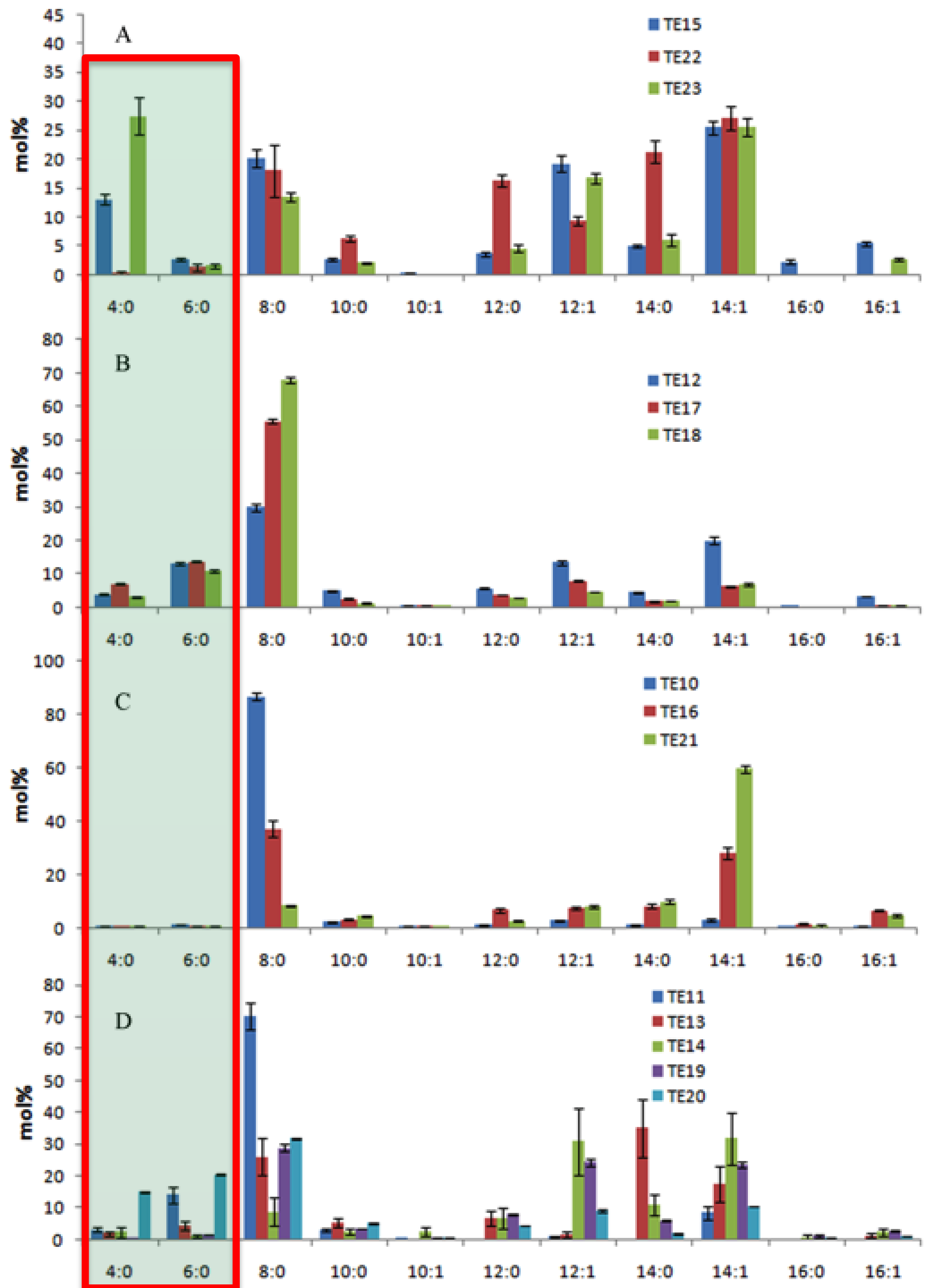
- **~30 plant acyl-ACP TEs had been functionally characterized**
- **Classified into two classes based on substrate specificity**
    - fatA and fatB

Computationally identified diverse TEs (n=360) by phylogenetic analysis



~ 360 sequences
90% sequences are not characterized

- **24 representative sequences distributed across the TEs subfamilies were selected for characterization**
- **They were codon-optimized for the expression in *E. coli***
- **The transit peptides of plant TEs were removed by PCR**
- **All TEs were expressed with pUC57 (lacZ promoter) in *E. coli fadD* mutant strain**



Substrate specificities of bacterial acyl-ACP TEs

Activity against very short chains

e.g., TE15, TE20, TE23

- ➢ 4-carbon acyl-ACP
- ➢ 6-carbon acyl-ACP

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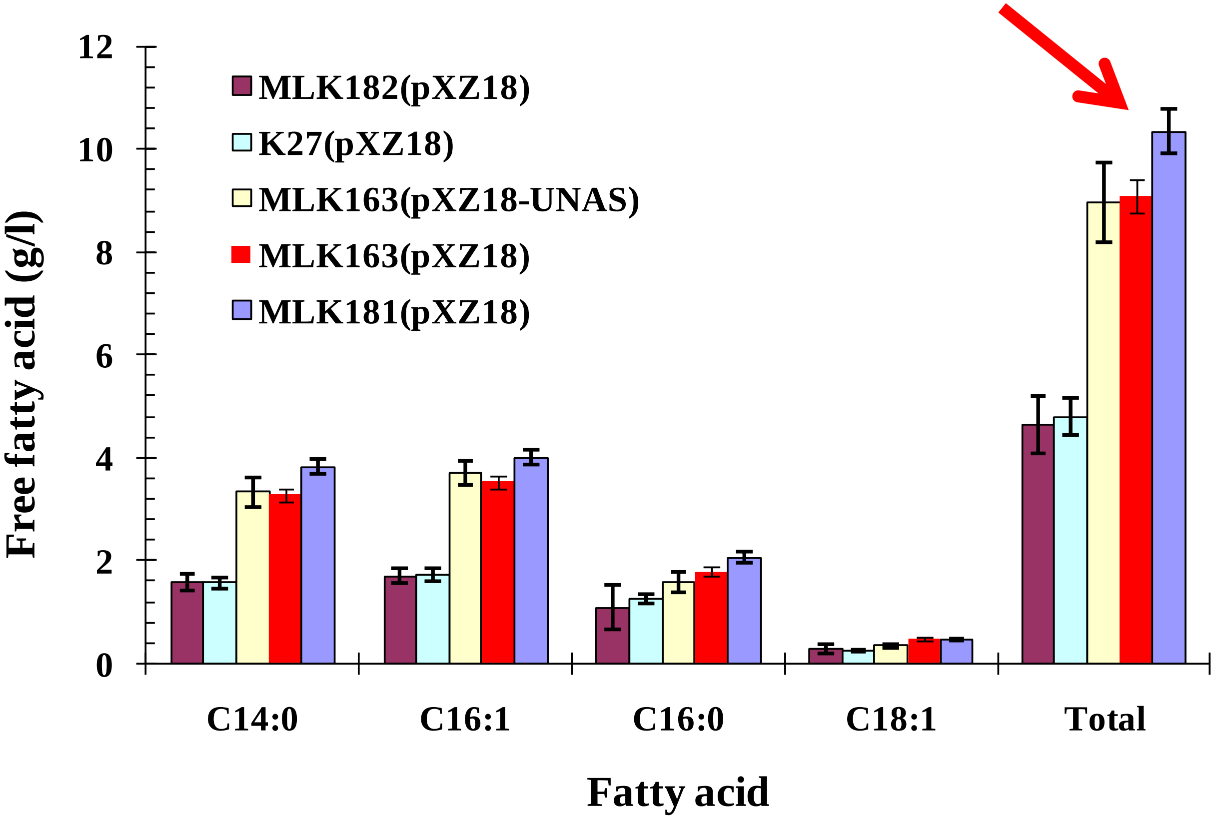
Further Strain Improvement

Strong Medium Chain Thioesterase (pXZ18)

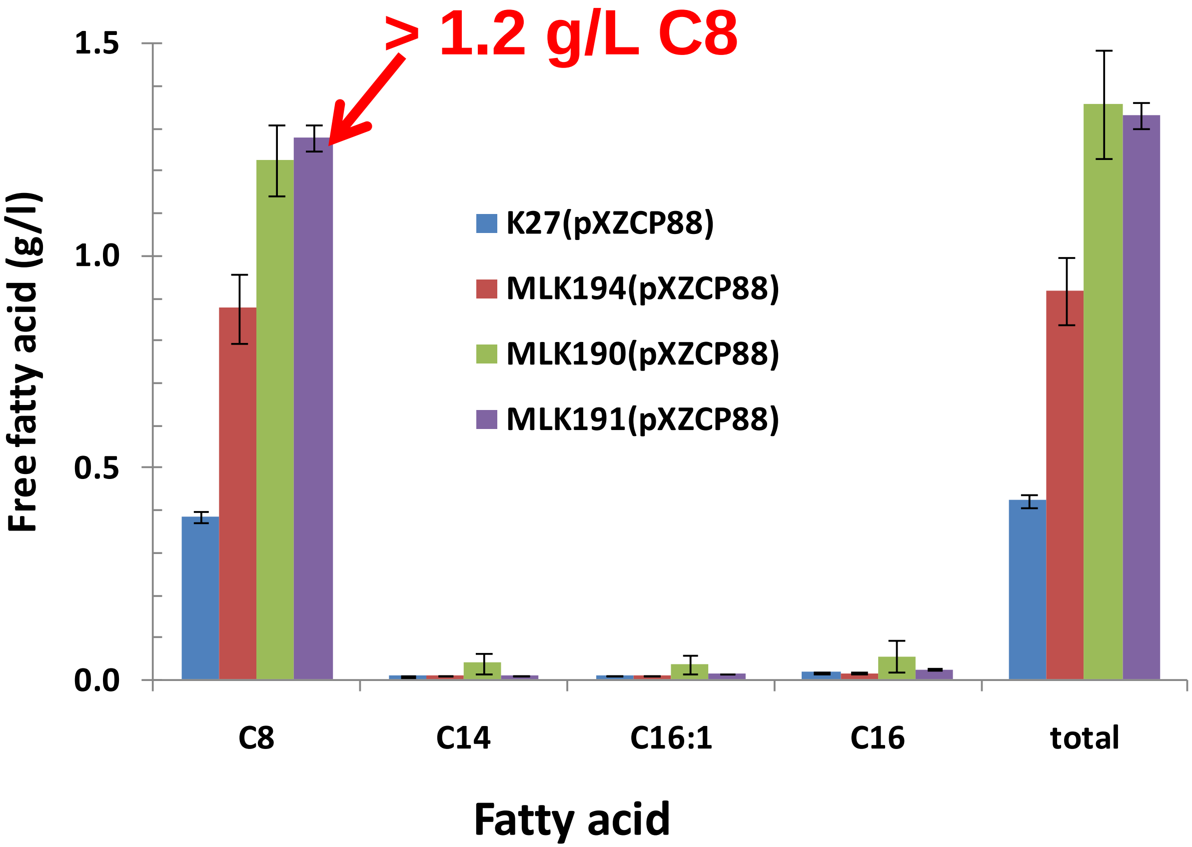
- Five new strains were developed
- Highest Titer:
  - >10 g/L
- Highest Yield:
  - > 0.2 g/g (from 50 g/L of glucose)
  - > 50% max theoretical yield



Host Strain Improvement
CP88 - Modified Acyl-ACP Thioesterase

- Three new host strains were developed
- Highest Titer:
  - >1.2 g/L
- Highest Yield
  <u>24 hrs:</u>
  - ~ 0.2 g/g
  - ~ 50% max theoretical yield

  <u>48 hrs: </u>~ 0.1 g/g


> 1.2 g/L C8

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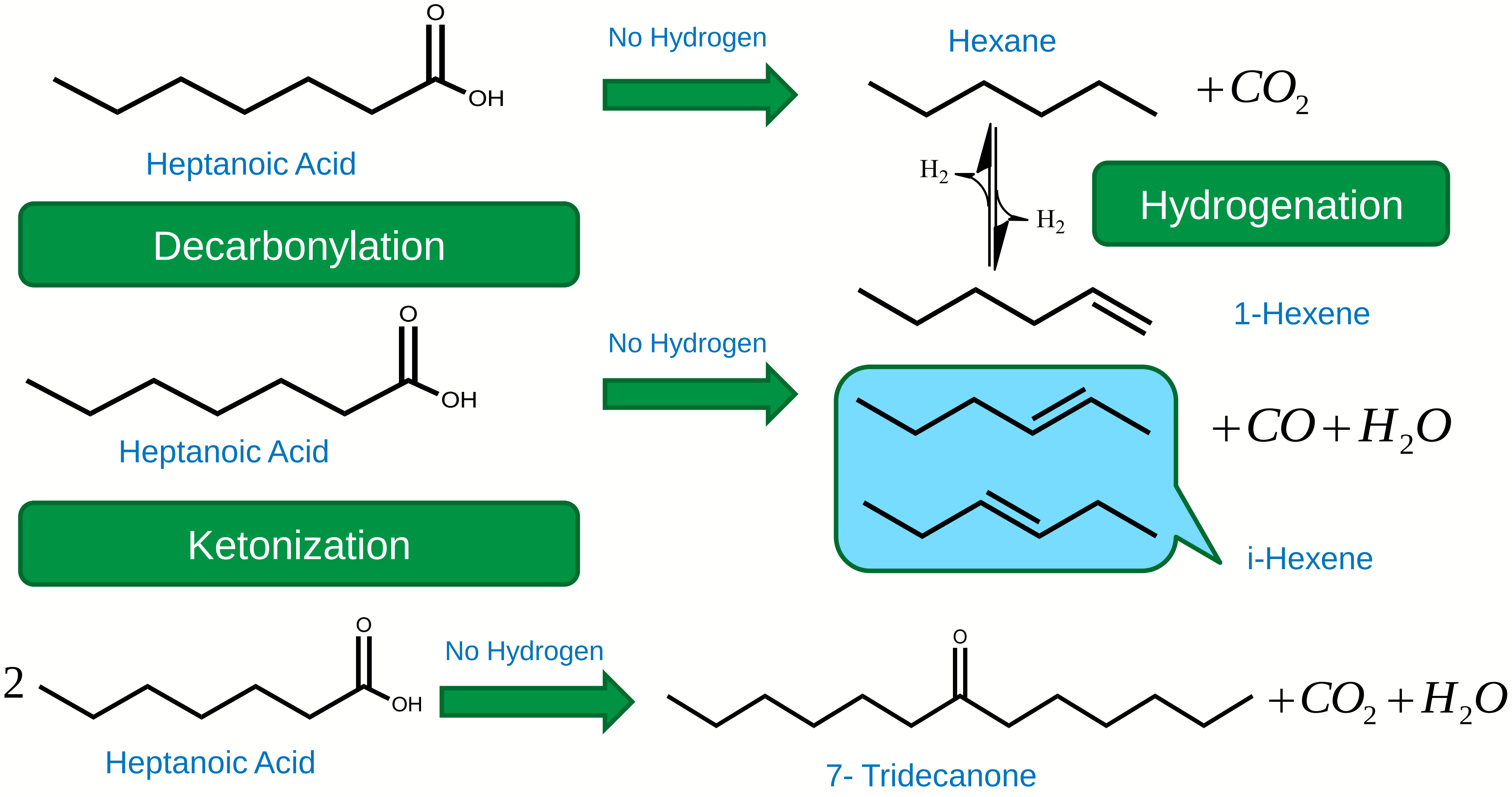
Deoxygenation Reactions



Decarboxylation

Heptanoic Acid → No Hydrogen → Hexane $+ CO_2$

Hydrogenation

Decarbonylation

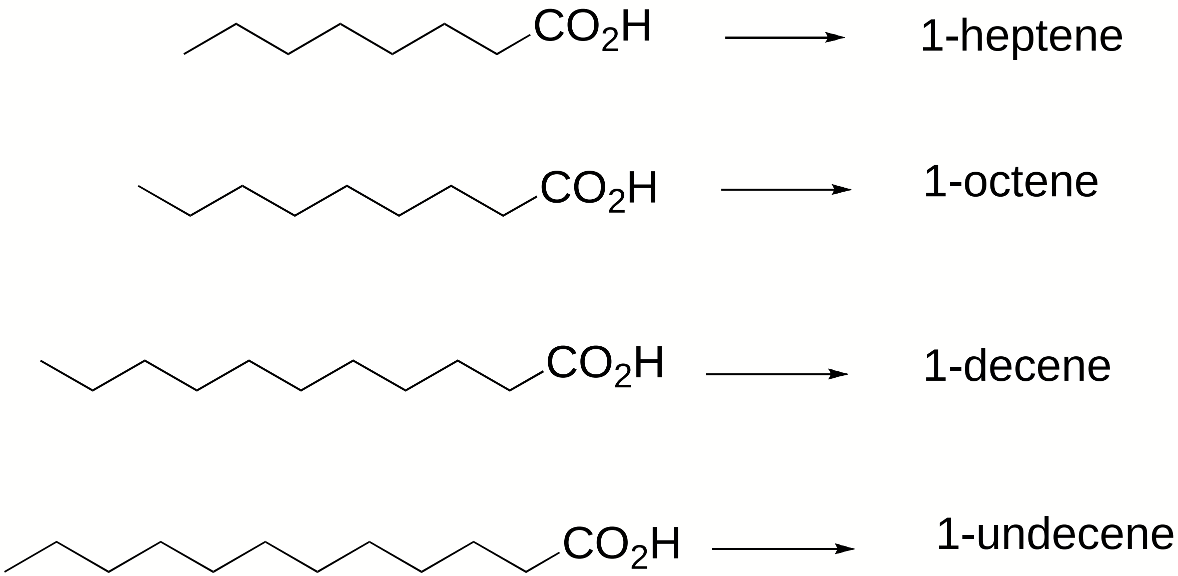
Heptanoic Acid → No Hydrogen → 1-Hexene, i-Hexene $+ CO + H_2O$

Ketonization

2 Heptanoic Acid → No Hydrogen → 7- Tridecanone $+ CO_2 + H_2O$

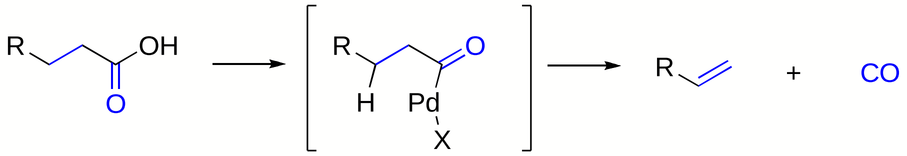
Decarboxylation of fatty acids

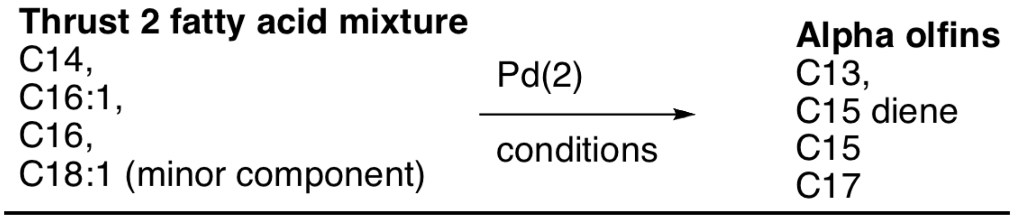
Fatty Acids to Alpha-Olefins



yields 45-65%

Alpha Olefins from Fatty Acids from Thrust 2





Thrust 2 fatty acid mixture
C14,
C16:1,
C16,
C18:1 (minor component)

Pd(2)
conditions

Alpha olfins
C13,
C15 diene
C15
C17

Composition	Grams	Conversion (brsm)
C14 = 34% C16 = 25 % C16:1 = 36 % C18 = 5 %	3.7 g	57%

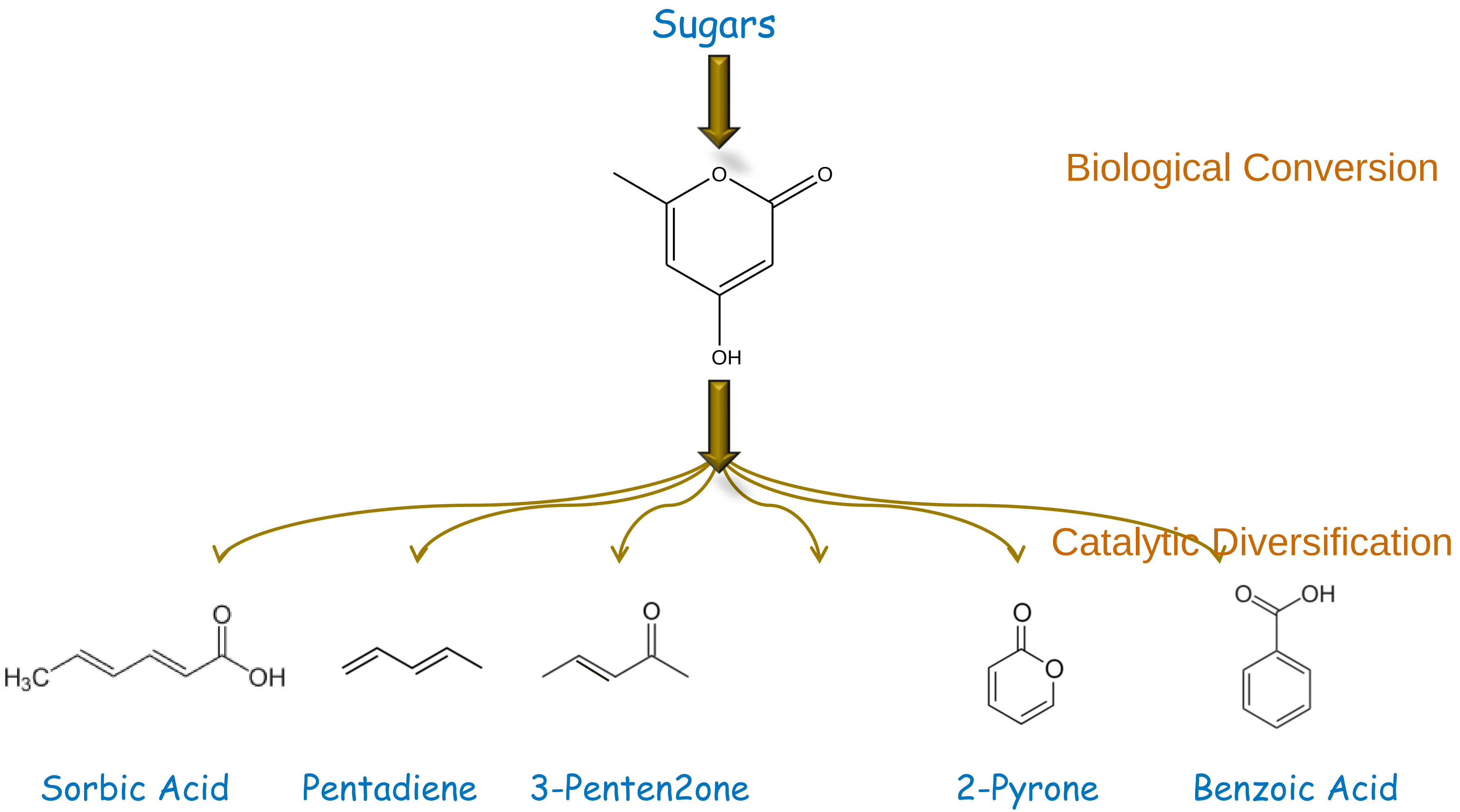
57% conversion – remainder is starting material
Products characterized by gc-ms

Reaction conducted at reduced pressure

Recent Status

Value	**Carboxylic Acid Testbed @ 18 mo.**	**Carboxylic Acid Testbed @ 30 mo.**
Titer	2.1 g/L	12 g/L
Productivity	0.044 g/L/h	0.27 g/L/h
$Y_{ferment}$	0.14 g/g *(35% max theoretical)*	0.20 g/g *(50% max theoretical)*
Y_{cat}	*65% (model material)*	~70% (model material) 57% (T2 material)

Pyrone Testbed



Sugars

Biological Conversion

Catalytic Diversification

Sorbic Acid

Pentadiene

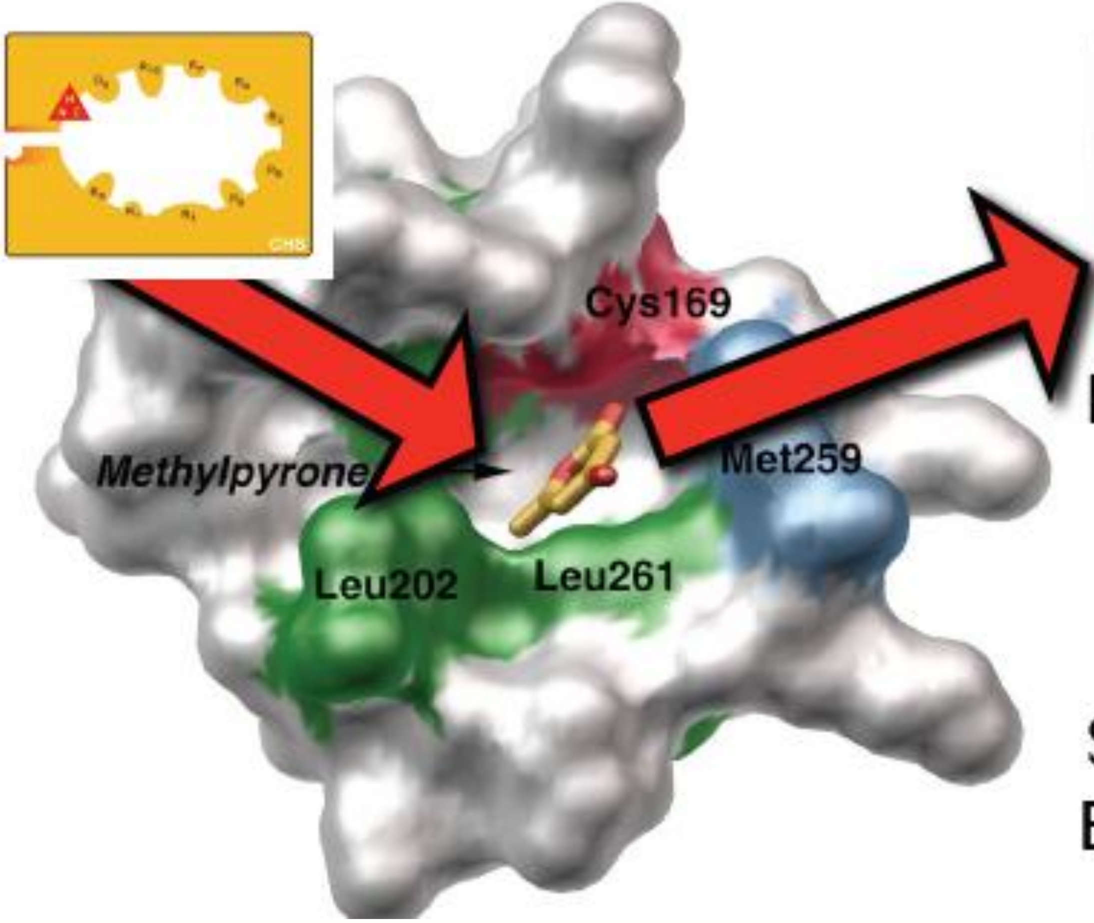
3-Penten2one

2-Pyrone

Benzoic Acid

2-Pyrone Synthase Active Site

Binding
Pocket
Still Too
Large for
High
Turnover



Successful
Rational Re-
Design of
Active Site
and
Surrounding
Environment

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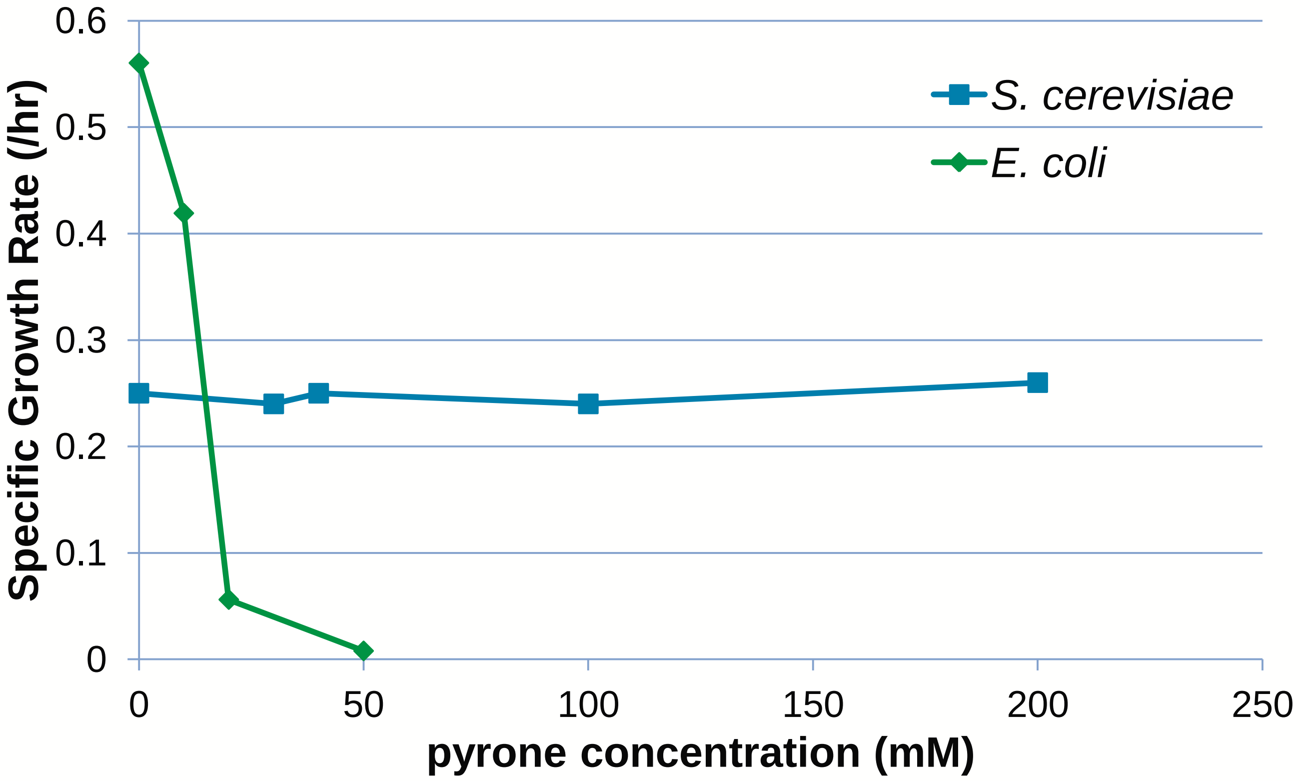
Pyrone Toxicity



- 25 g/L pyrone (TAL) does not inhibit *S. cerevisiae* growth
- < 2.5 g/L TAL significantly inhibits *E. coli* growth

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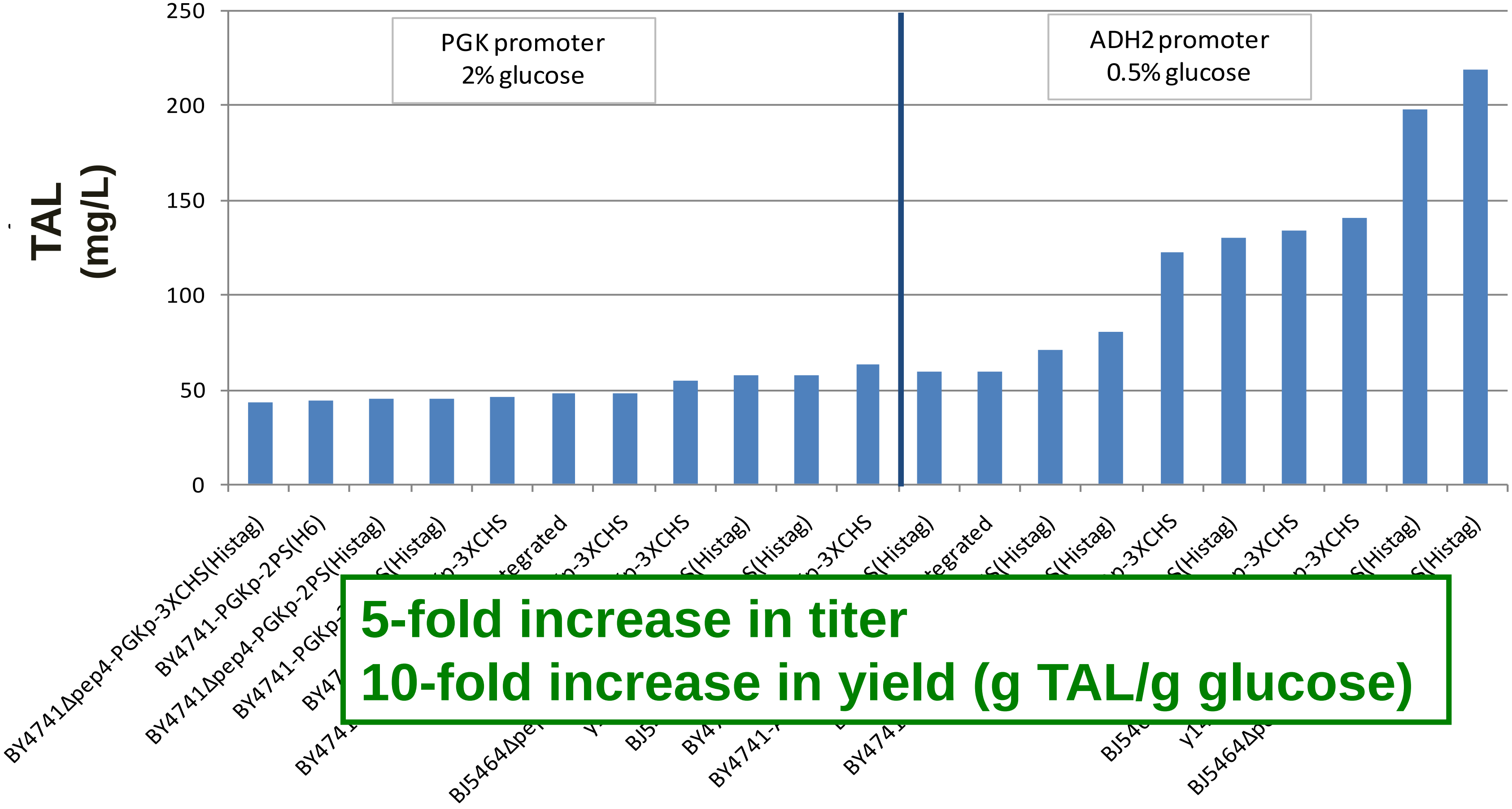
Overview



TAL Titer for Series of *S. cerevisiae* Strains

5-fold increase in titer
10-fold increase in yield (g TAL/g glucose)

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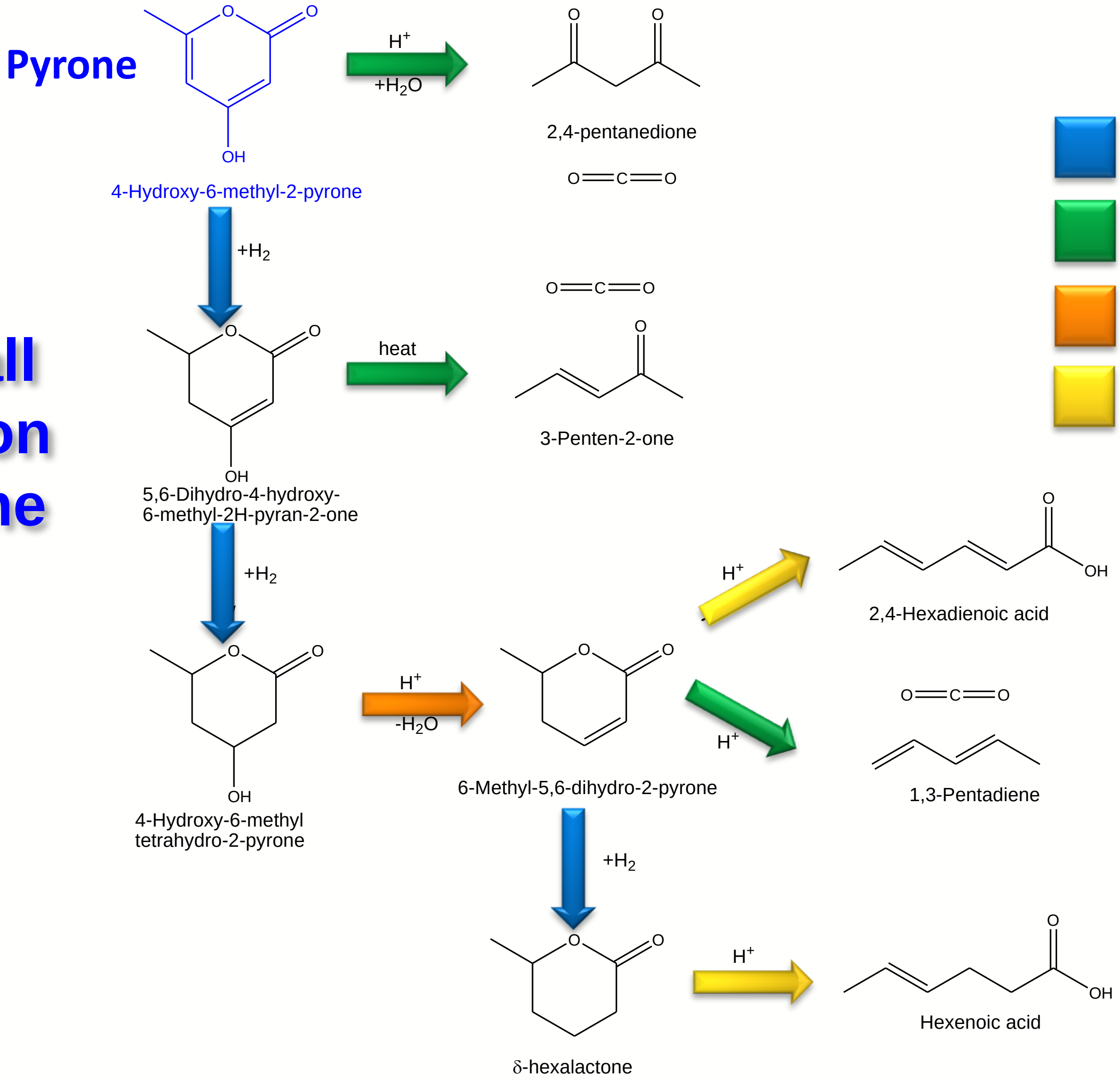
Overall reaction scheme



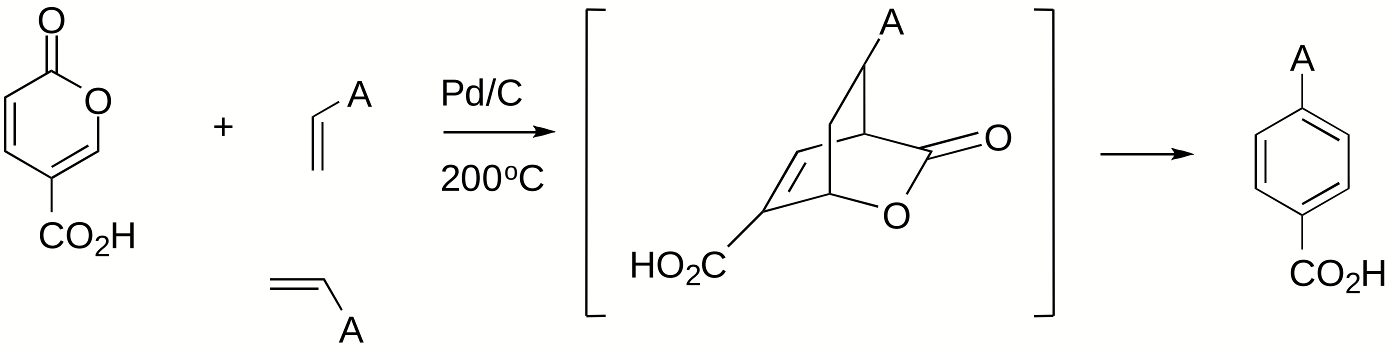
🟦 Hydrogenation

🟩 Decarboxylation

🟧 Dehydration

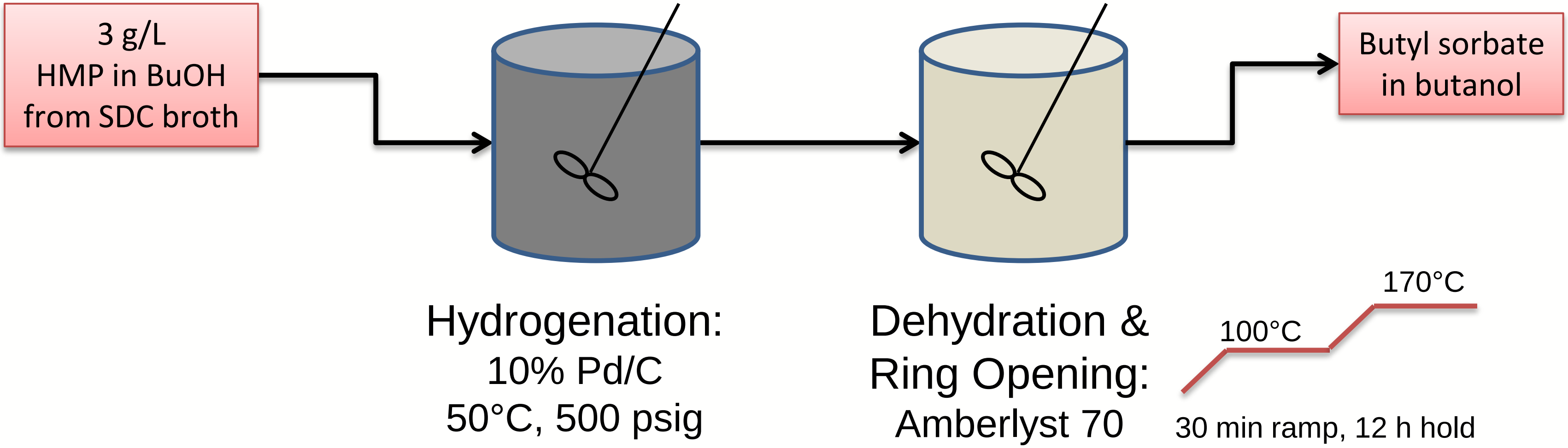
🟨 Ring-opening

Alkyl Benzoic Acids Directly from Alkenes



1-decene - 72%
1-heptene - 83%
allylbenzene - 79%
1-undecene - 69%

Catalytic Conversion of Pyrone from Simulated Fermentation Broth



3 g/L
HMP in BuOH
from SDC broth

Butyl sorbate
in butanol

Hydrogenation:
10% Pd/C
50°C, 500 psig

Dehydration &
Ring Opening:
Amberlyst 70

30 min ramp, 12 h hold

Feed	Hydrogenation Conv (%)	Selectivity to 4-HMTHP (%)	Selectivity to DHHMP (%)	Selectivity to BuSorb (%)
HMP/BuOH from water	>99	94	0	67
HMP/BuOH from SDC	>99	~60	0	~30

Recent Status

Value	**Pyrone Testbed @ 6 mo.**	**Pyrone Testbed @ 12 mo.**
Titer	0.072 g/L	0.22 g/L
Productivity	0.0015 g/L/h	0.005 g/L/h
$Y_{ferment}$	0.0036 g/g	0.044 g/g *(9% max theoretical)*
Y_{cat}	*Couldn't remove from fermentation media*	>60%

Acknowledgements

- National Science Foundation

NSF

- Industry Members

Allylix

ASHLAND
With good chemistry great things happen.

BASF
The Chemical Company

Chevron Phillips
Chemical Company LLC

Cibus

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