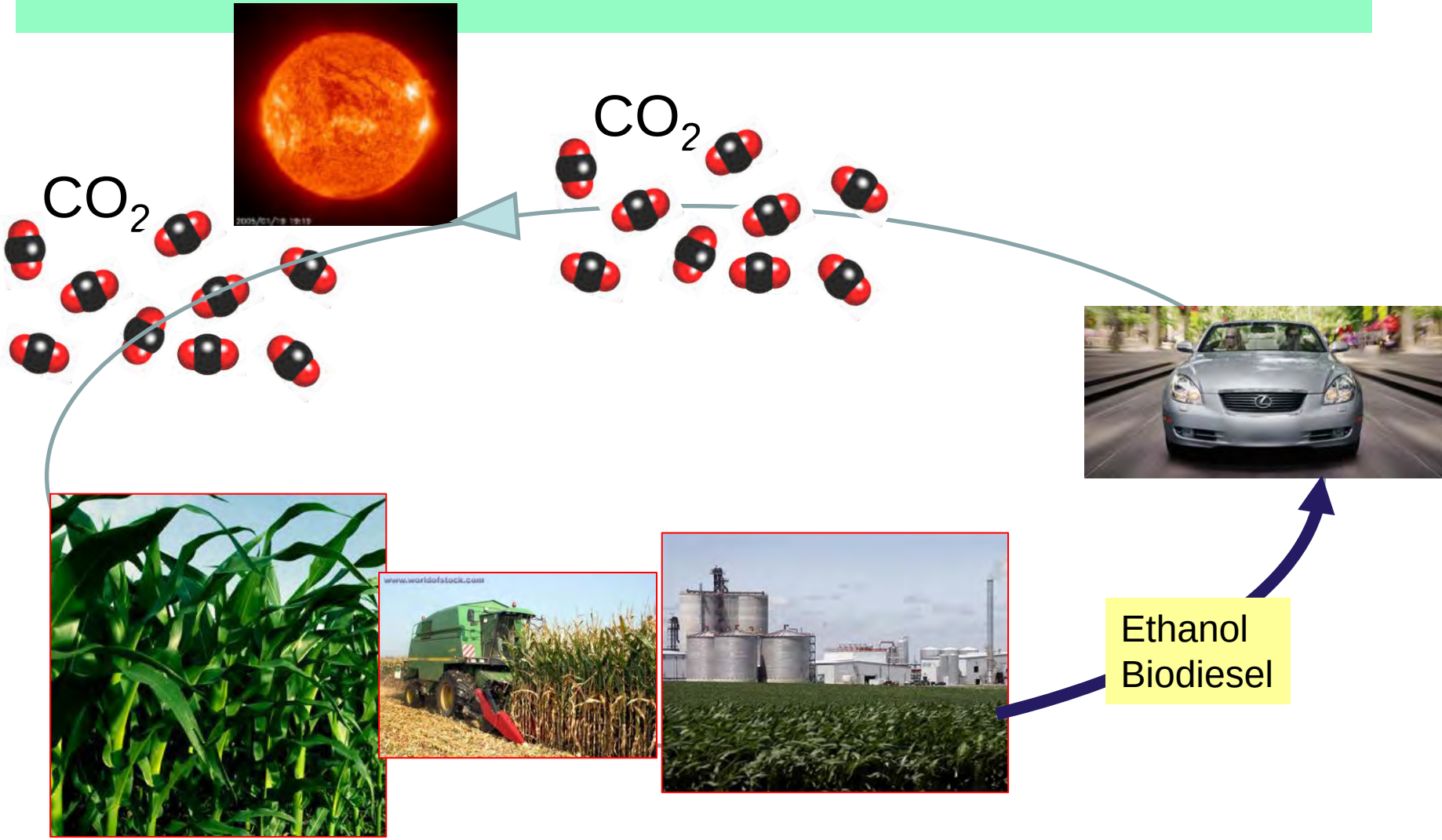
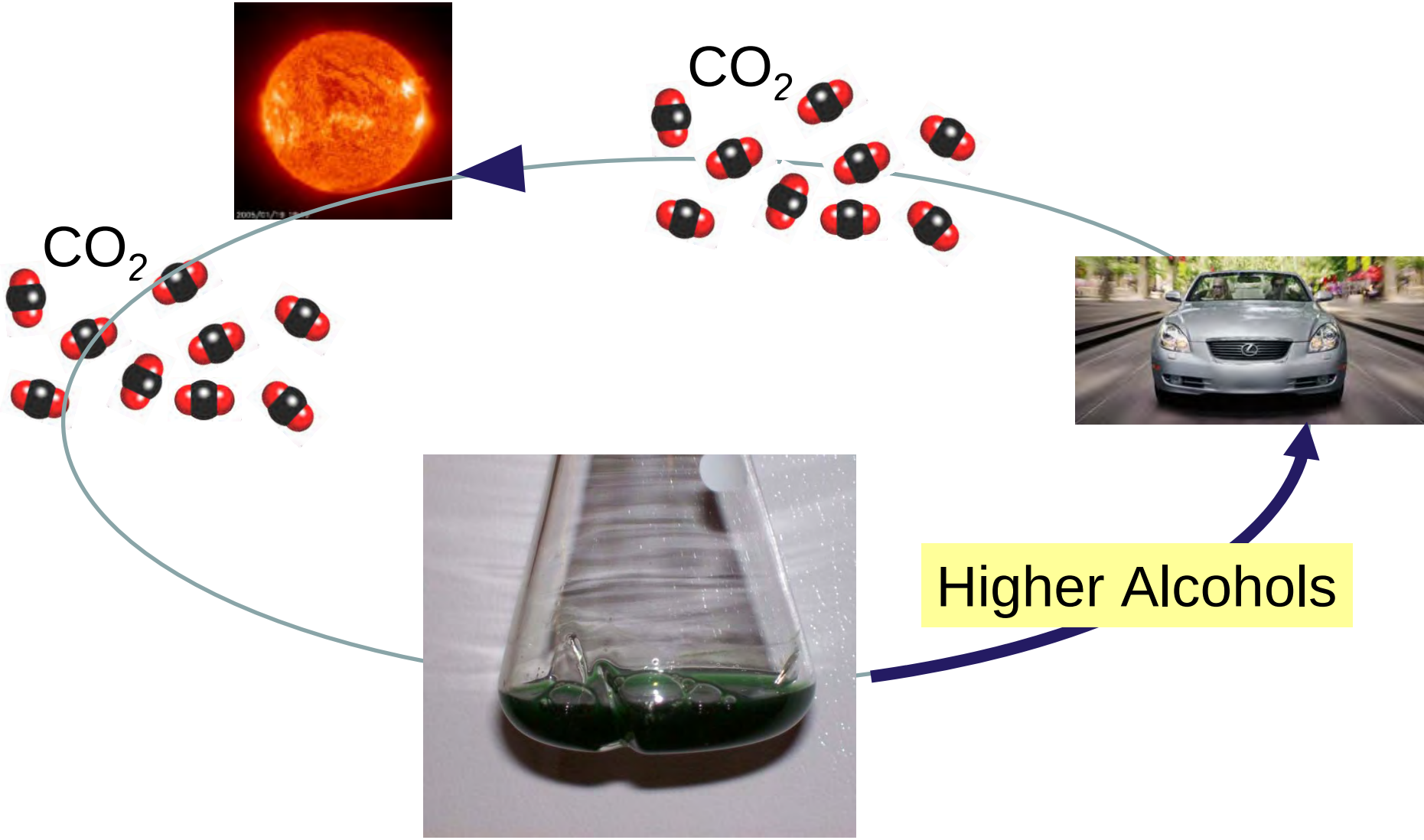
Photo-Synthetic Biology for Fuels

James C. Liao
Chemical and Biomolecular Engineering
University of California, Los Angeles

The Current Biofuel Cycle



The Direct Solar Fuel Cycle

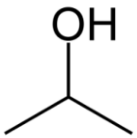
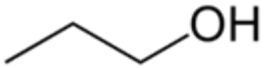
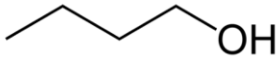
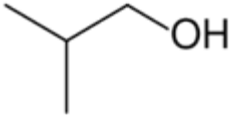
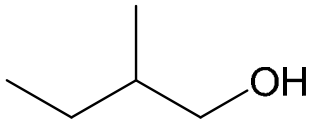
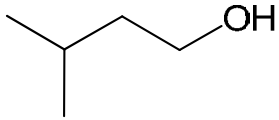


Biofuels

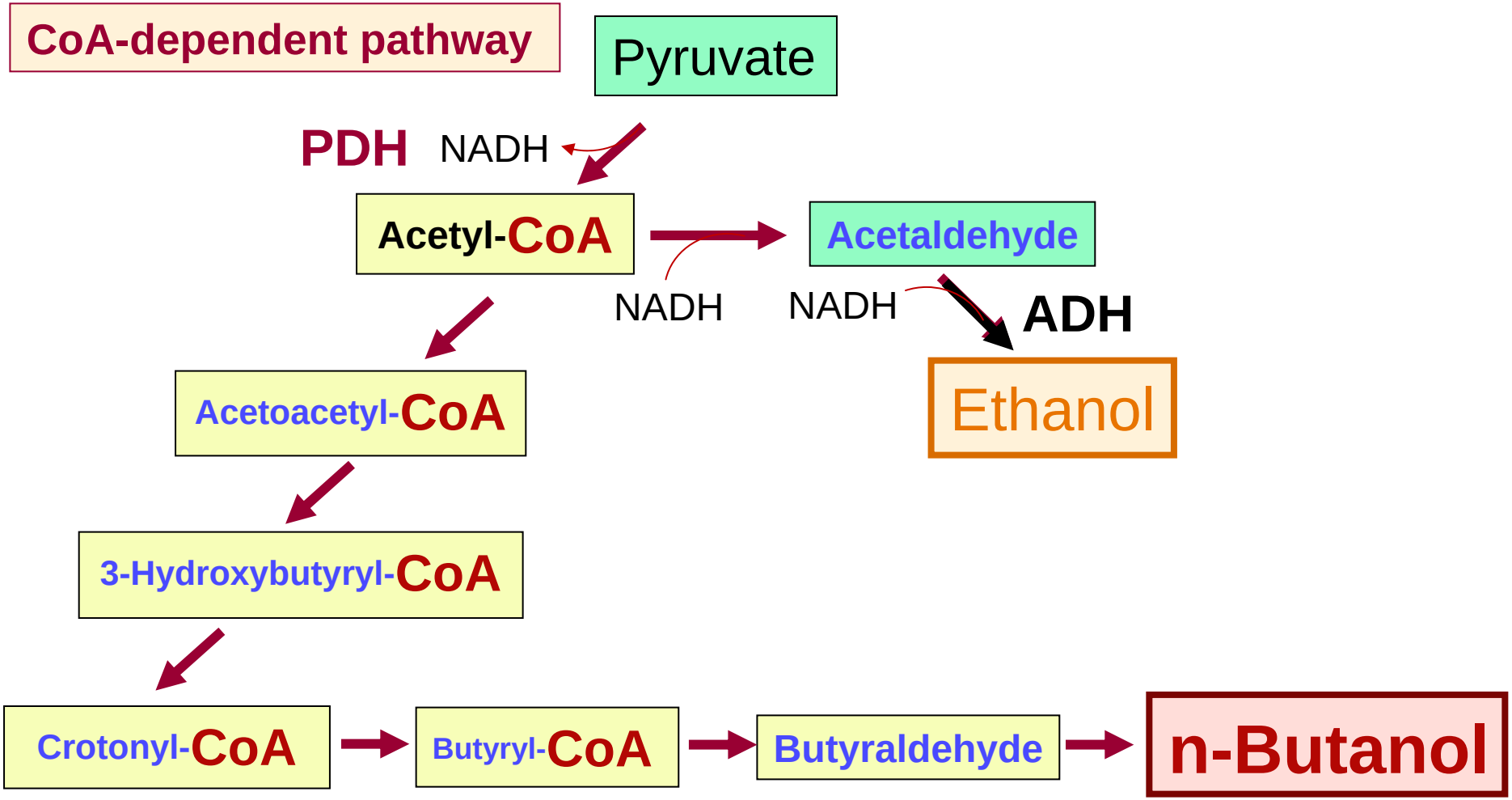
	Ethanol	Long chain alcohols/alkanes
Energy content	Low	High
Hygroscopicity	High	Low
Vehicle retrofitting?	Yes	No
Vapor pressure	High	Low
Production yield	High	Zero/Low

Higher alcohols

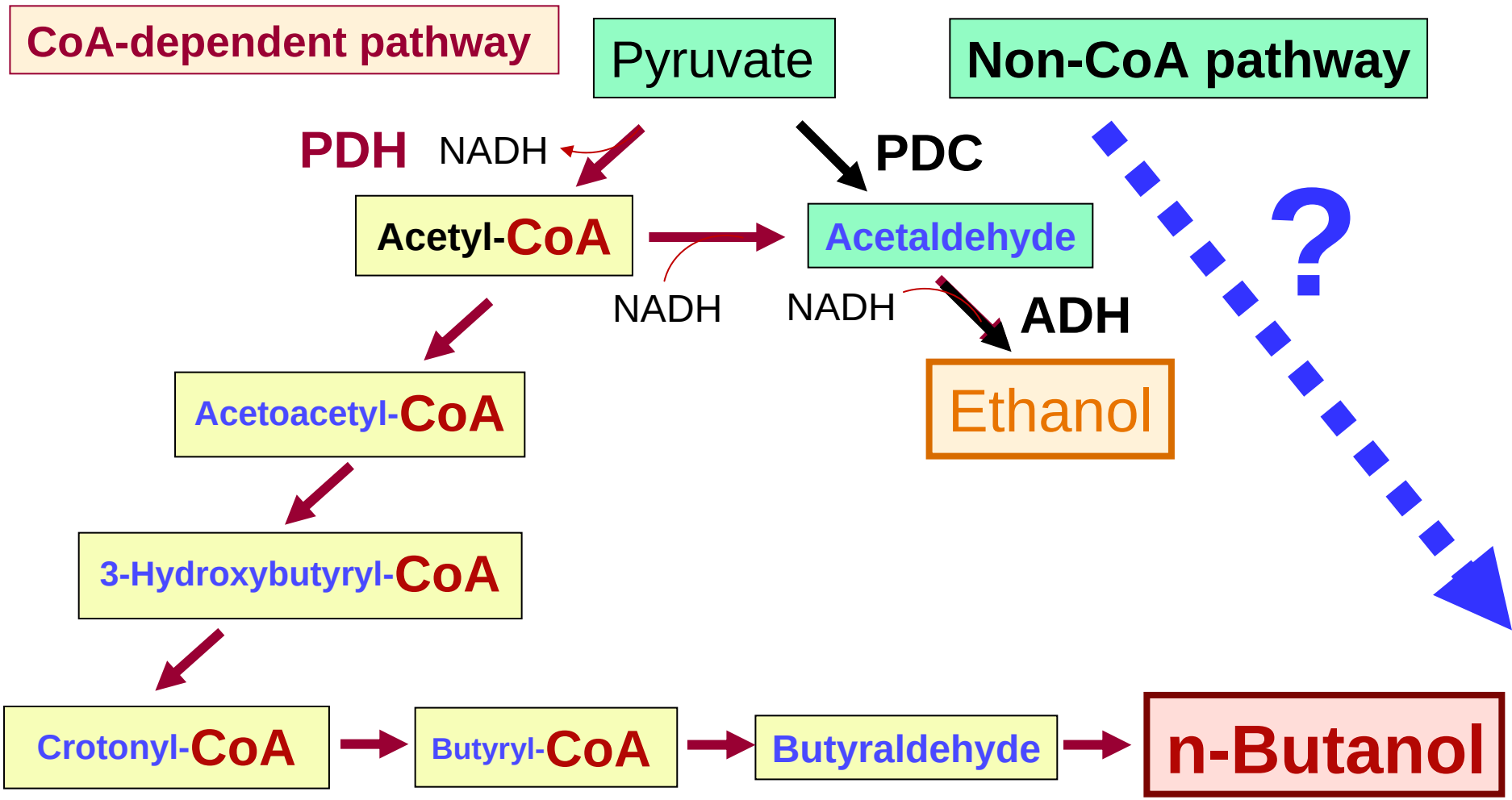
Native producers?

yes	Iso-propanol	
no	n-propanol	
yes	n-butanol	
no	Iso-butanol	
no	2-methyl-1-butanol	
no	3-methyl-1-butanol	

Pathways for alcohol synthesis

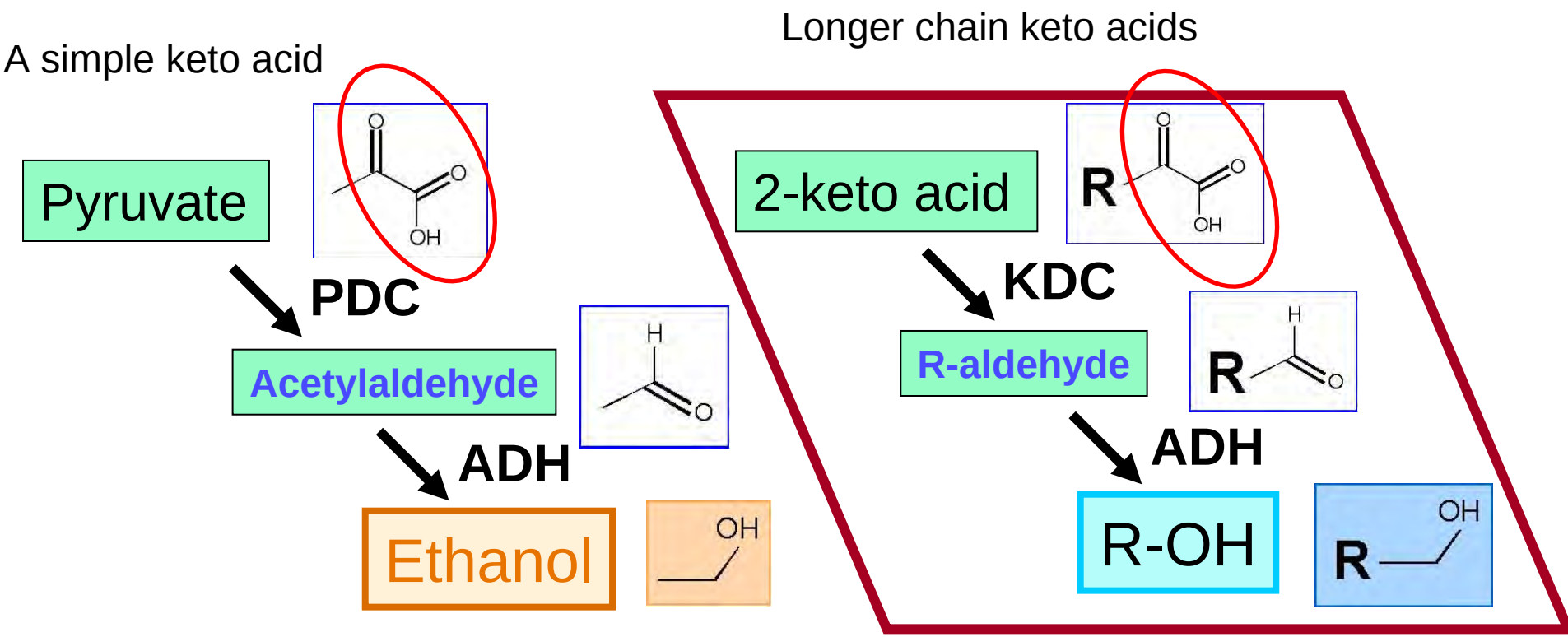


Alternative Pathways to Make Alcohols



Atsumi *et al.* ***Nature*** **2008**

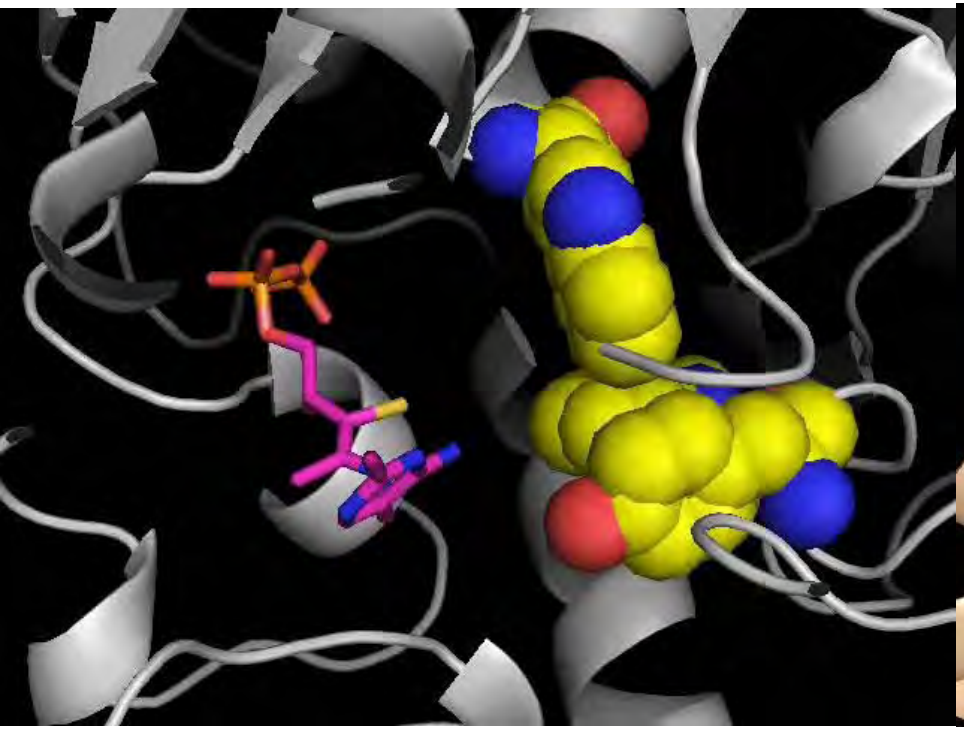
Generalization of keto acid decarboxylase chemistry



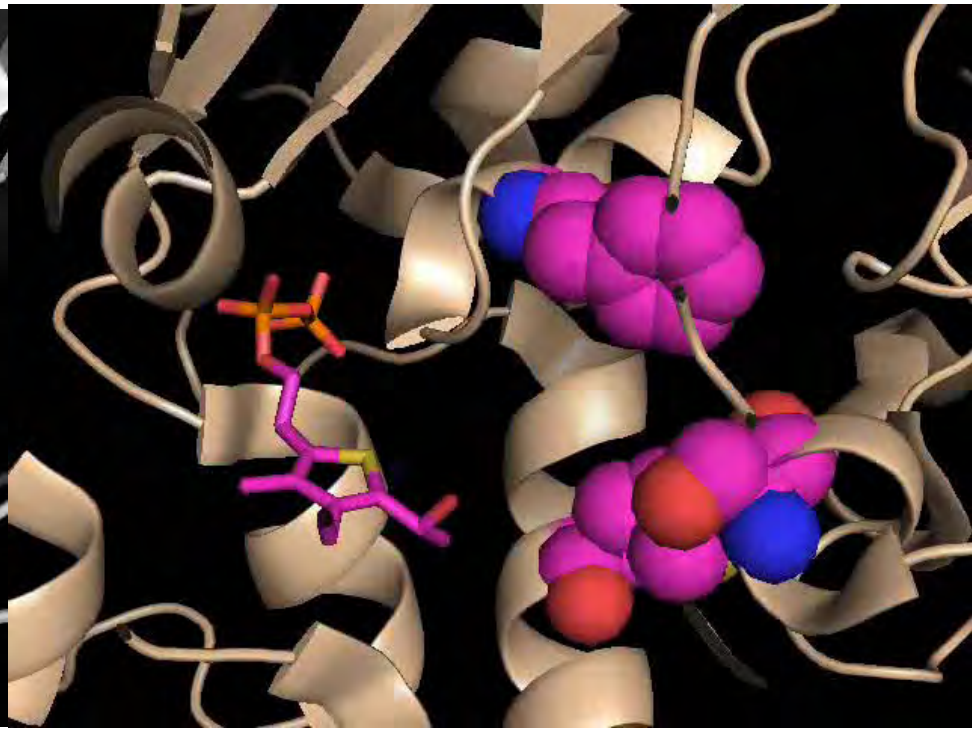
Ehrlich, F. Ber. Dtsch. Chem. Ges.1907

Opening the active site

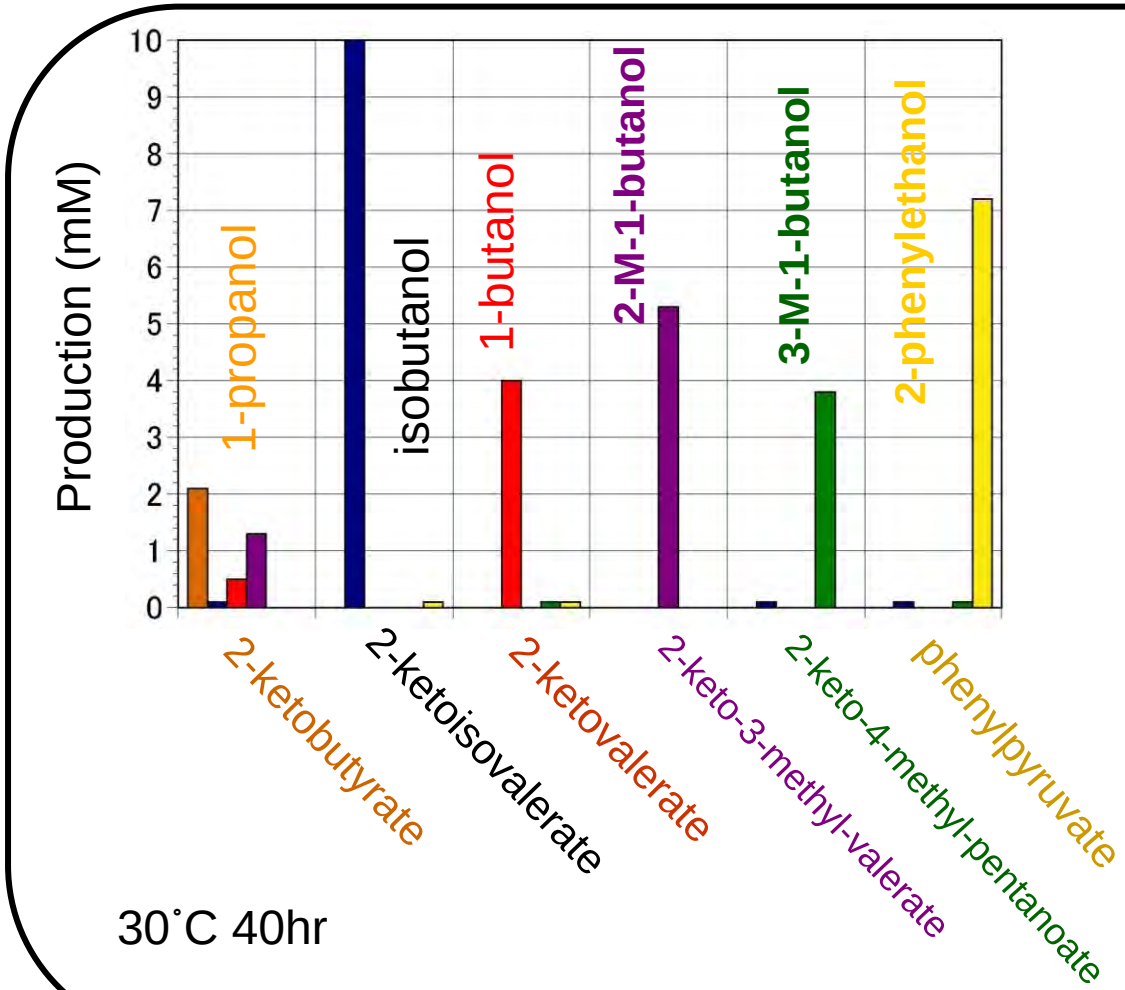
Zymomonas mobilis PDC



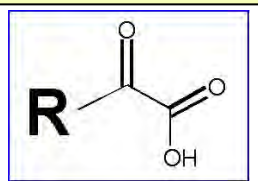
Lactococcus lactis KdcA



Production of Higher Alcohols in *E.coli* (*from* 2-keto acids)

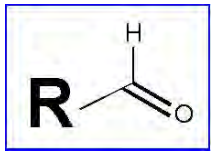


Long Chain 2-keto acids

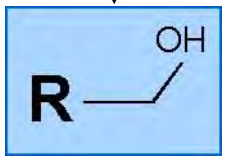


↓ **KDC**

R-aldehyde

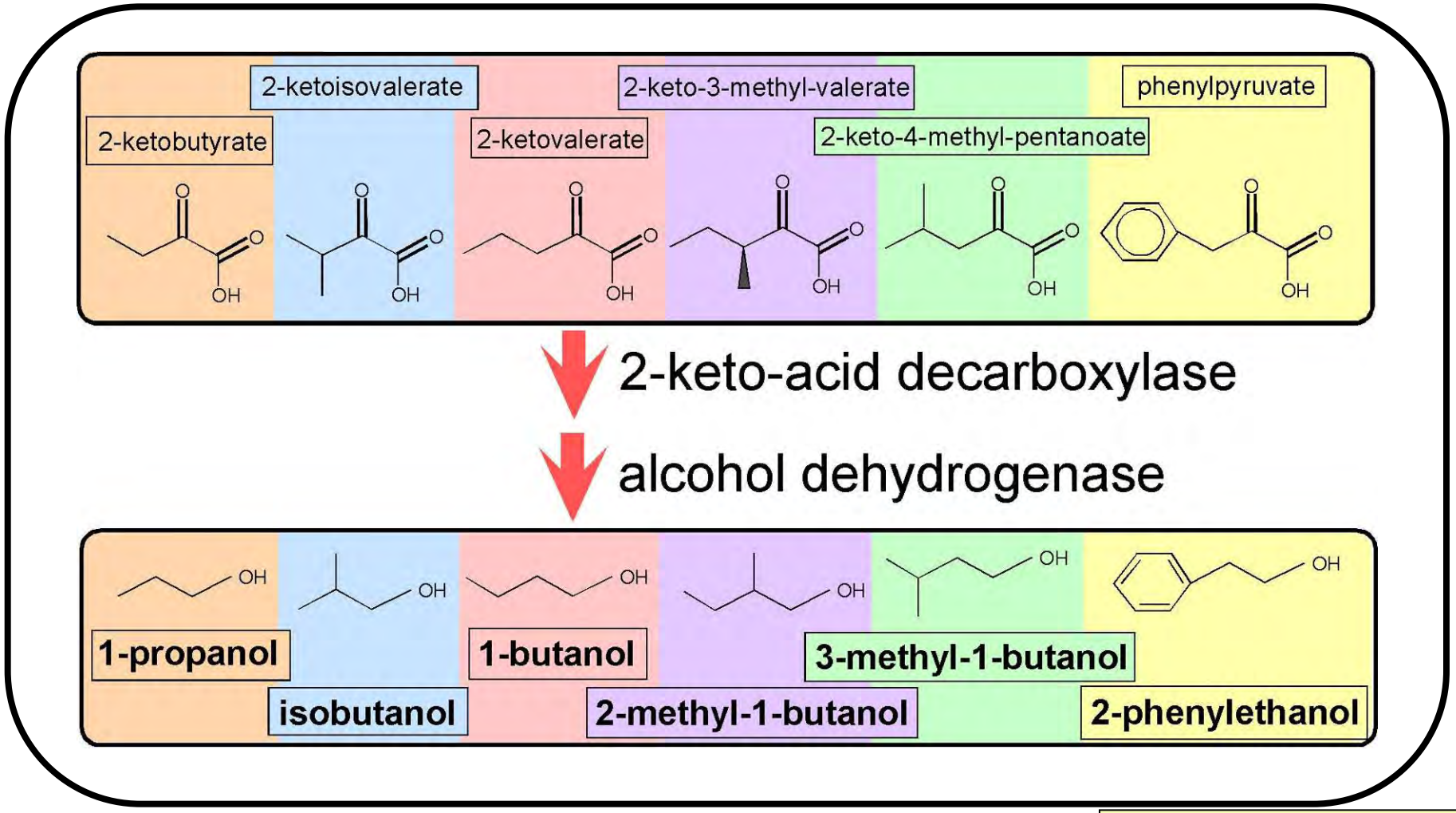


↓ **ADH**



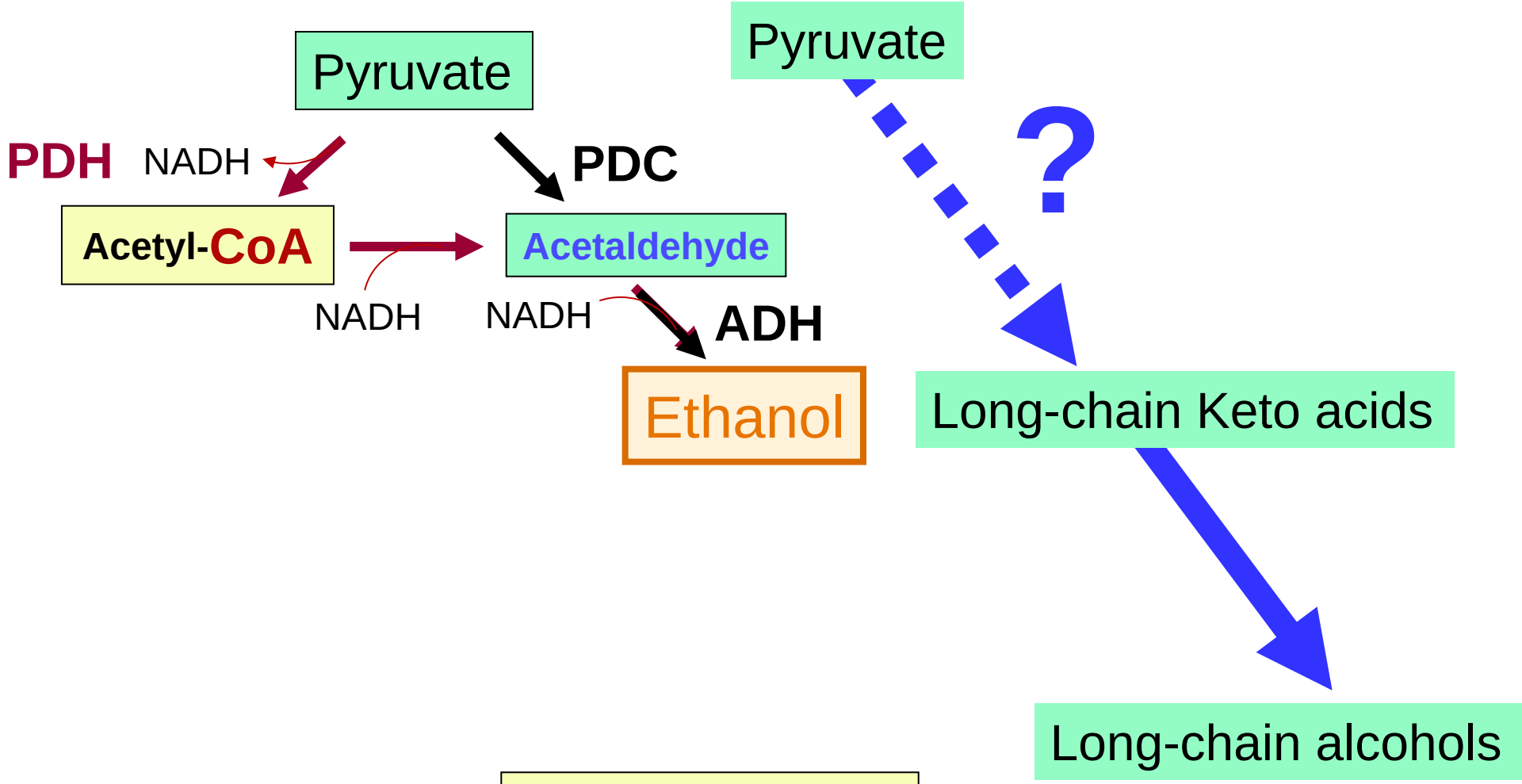
Atsumi *et al.* ***Nature*** **2008**

Further generalization of alcohol-producing chemistry



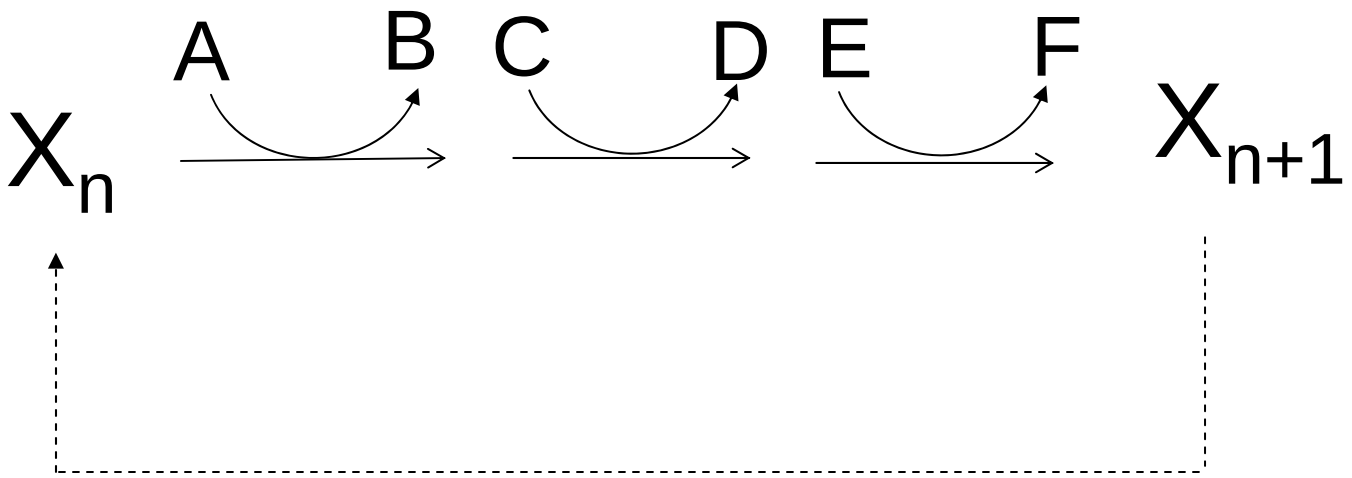
Atsumi *et al.* ***Nature*** **2008**

Alternative Pathways to make Alcohols

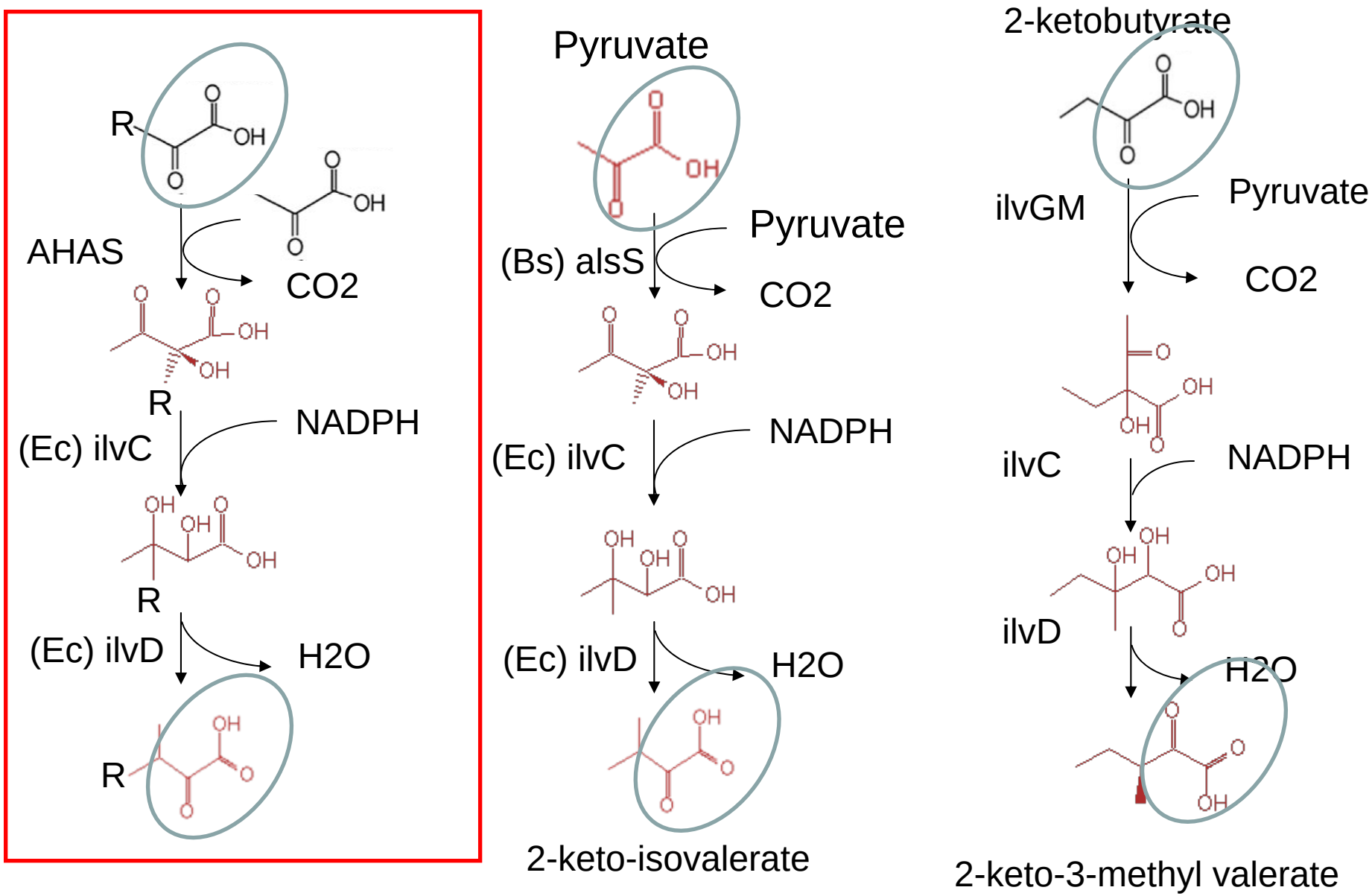


Atsumi *et al. Nature* 2008

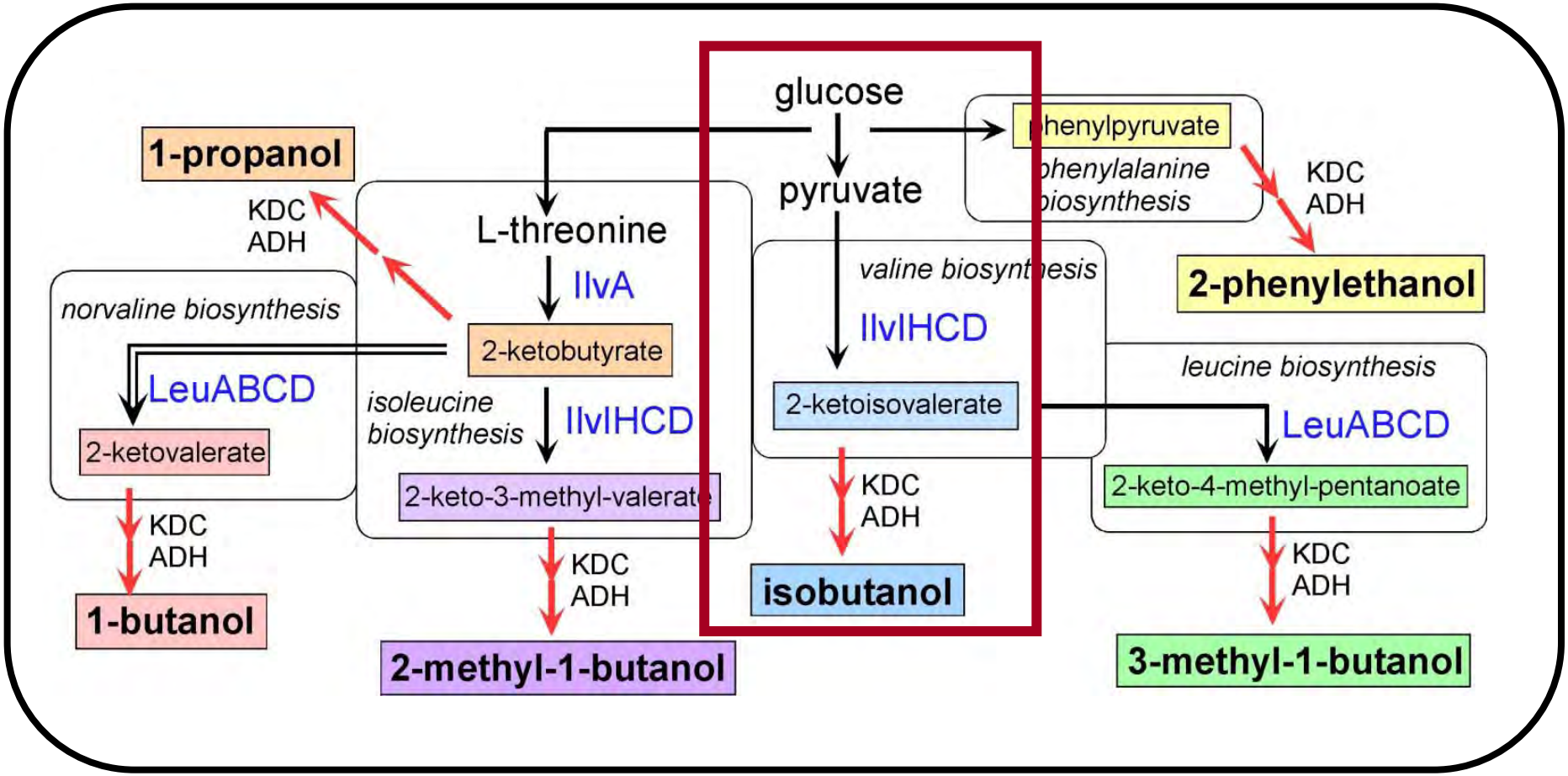
Non-polymeric Chain Elongation



Aceto-Hydroxy Acid Synthase (AHAS) Chain Elongation

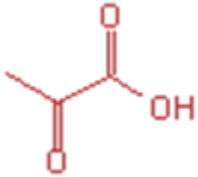


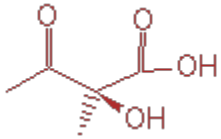
Novel Pathways for C4, C5 Alcohol Synthesis

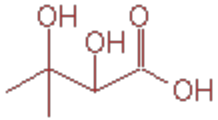


Atsumi *et al.* ***Nature*** **2008**

Isobutanol Synthesis







Pyruvate → CO2 + NADH → Ethanol

46x2/180 =0.51

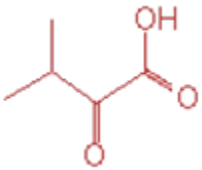
ilvIH: Pyruvate → acetolactate + CO2 (also ilvMG, ilvNB)

0.3 Adjusted energy yield

ilvC: acetolactate + NADPH → 2,3 dihydoxy-isovalerate

ilvD: 2,3 dihydoxy-isovalerate → 2-keto-isovalerate + H2O

ilvE: 2-keto-isovalerate + glutamate → valine + 2-KG

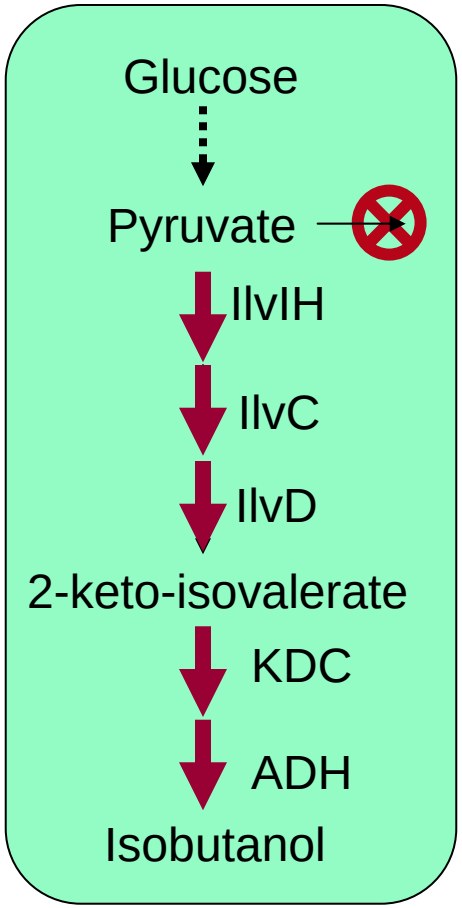


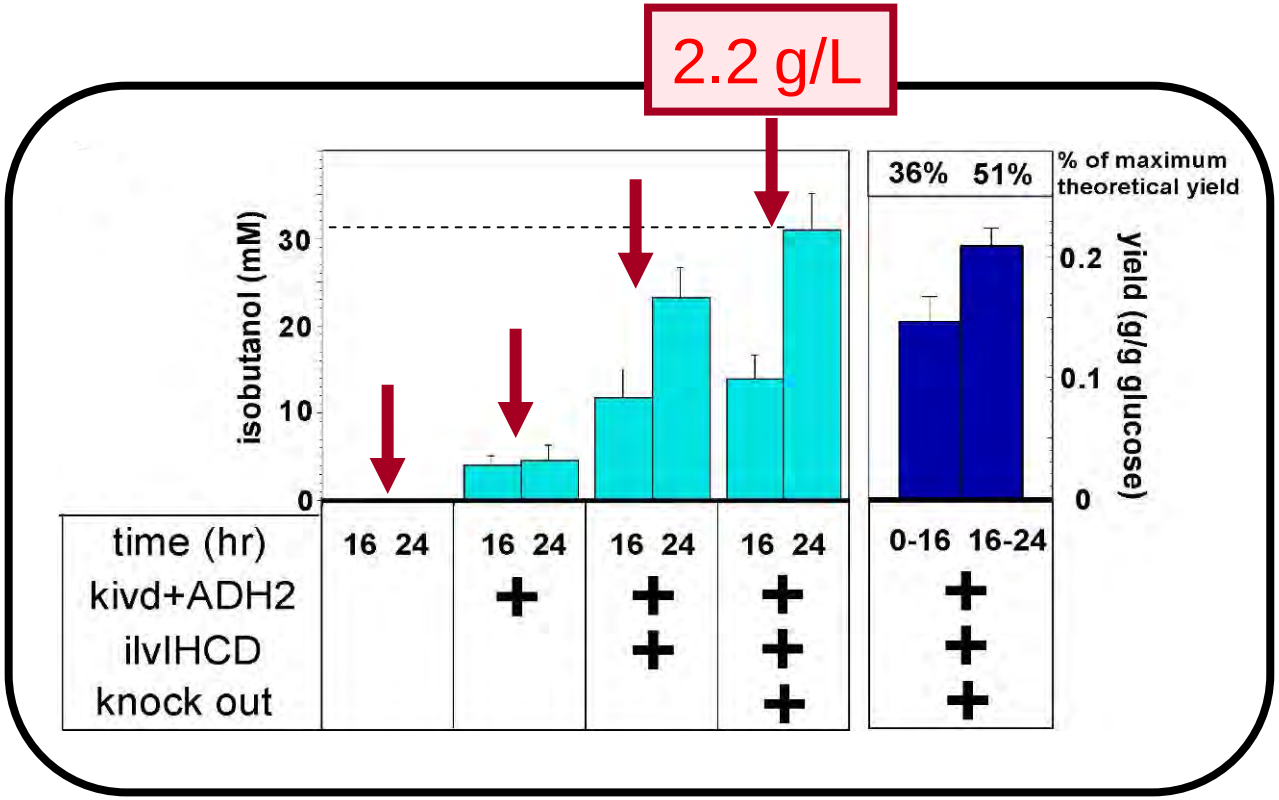
2-keto-isovalerate → CO2 + NADH → Iso-butanol

74/180 =0.41 g/g

0.37 Adjusted energy yield

Improvement of isobutanol production

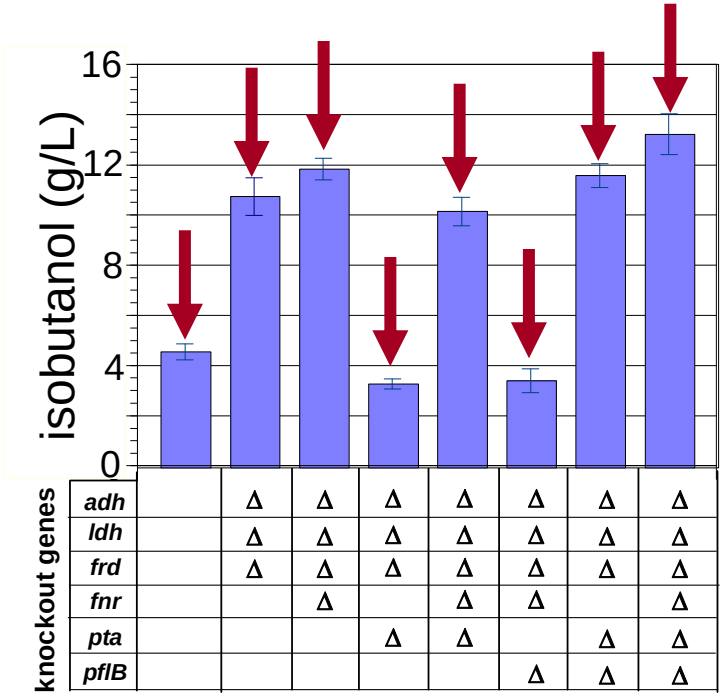




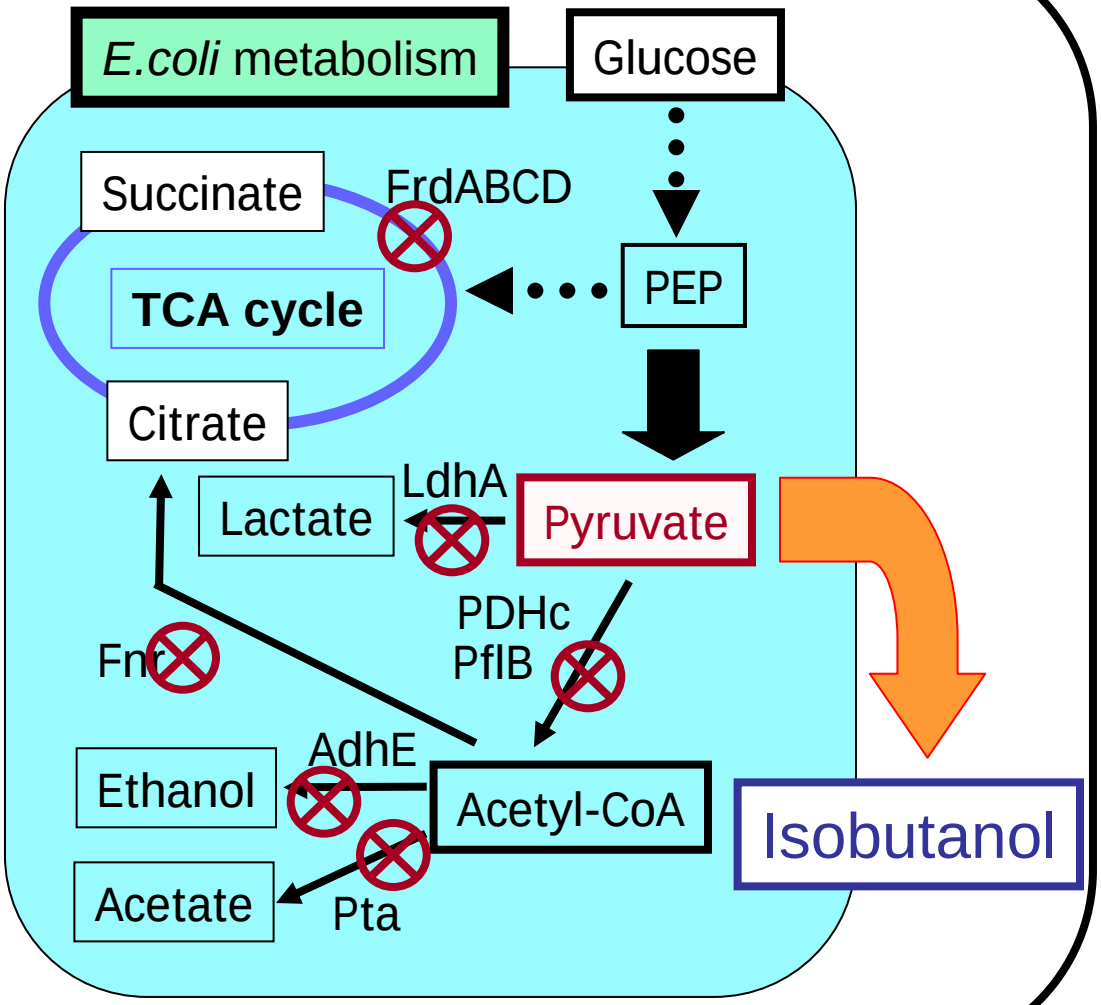
30˚C
M9 + 3.6% glc

Atsumi *et al.* ***Nature*** **2008**

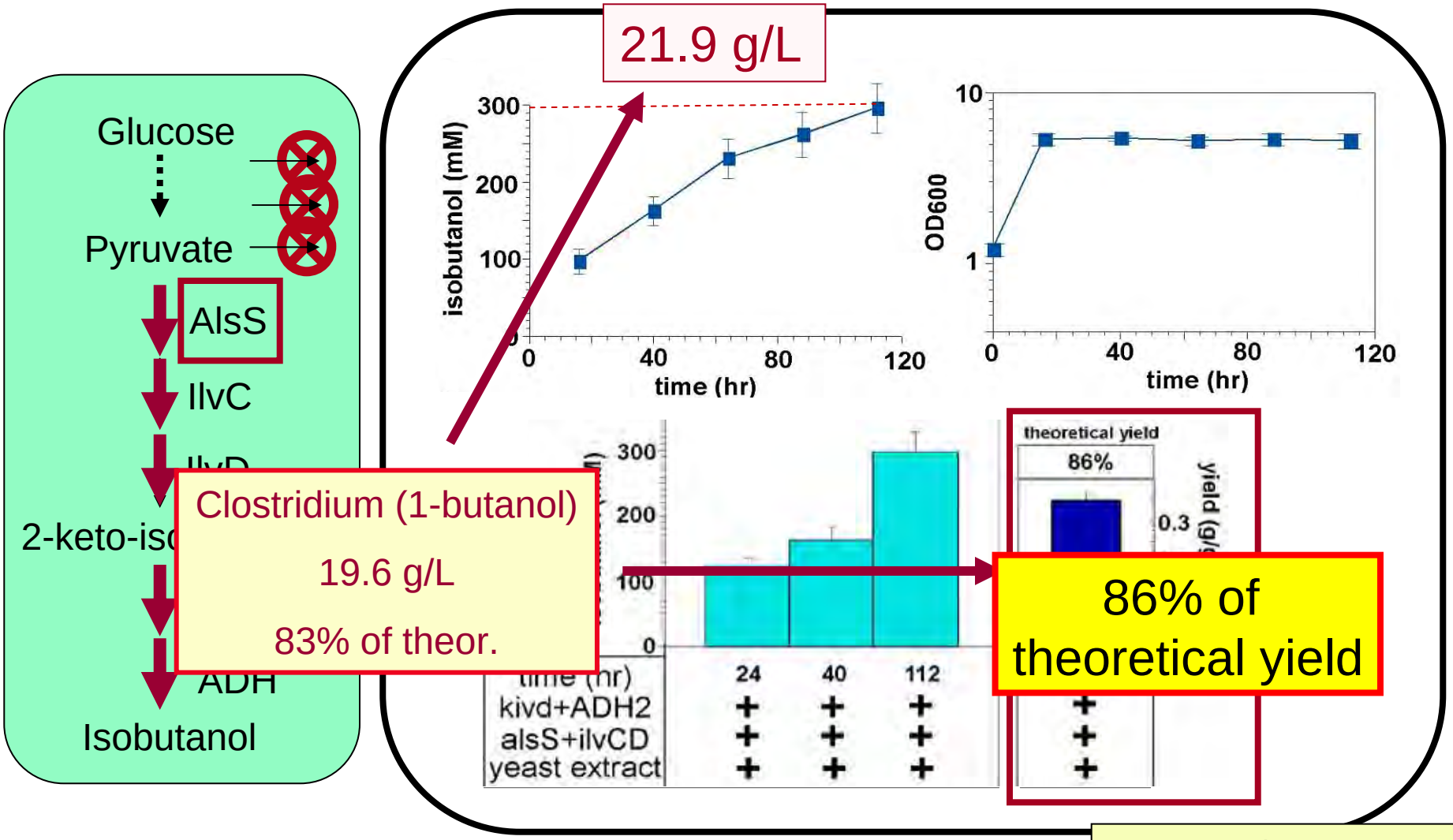
Effects of Gene Deletions



30˚C 64hr

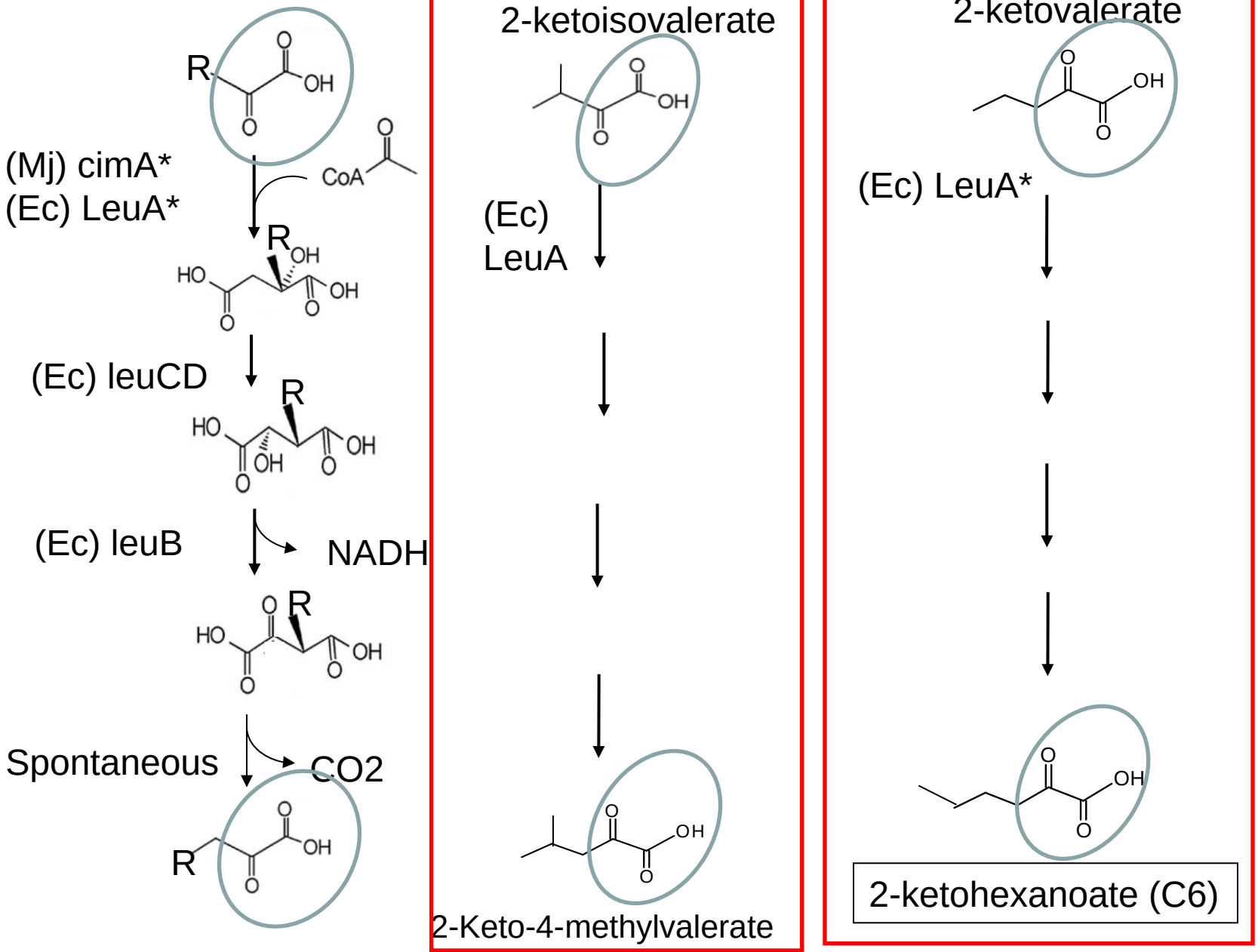


High yield production of isobutanol: 86% of Theoretical

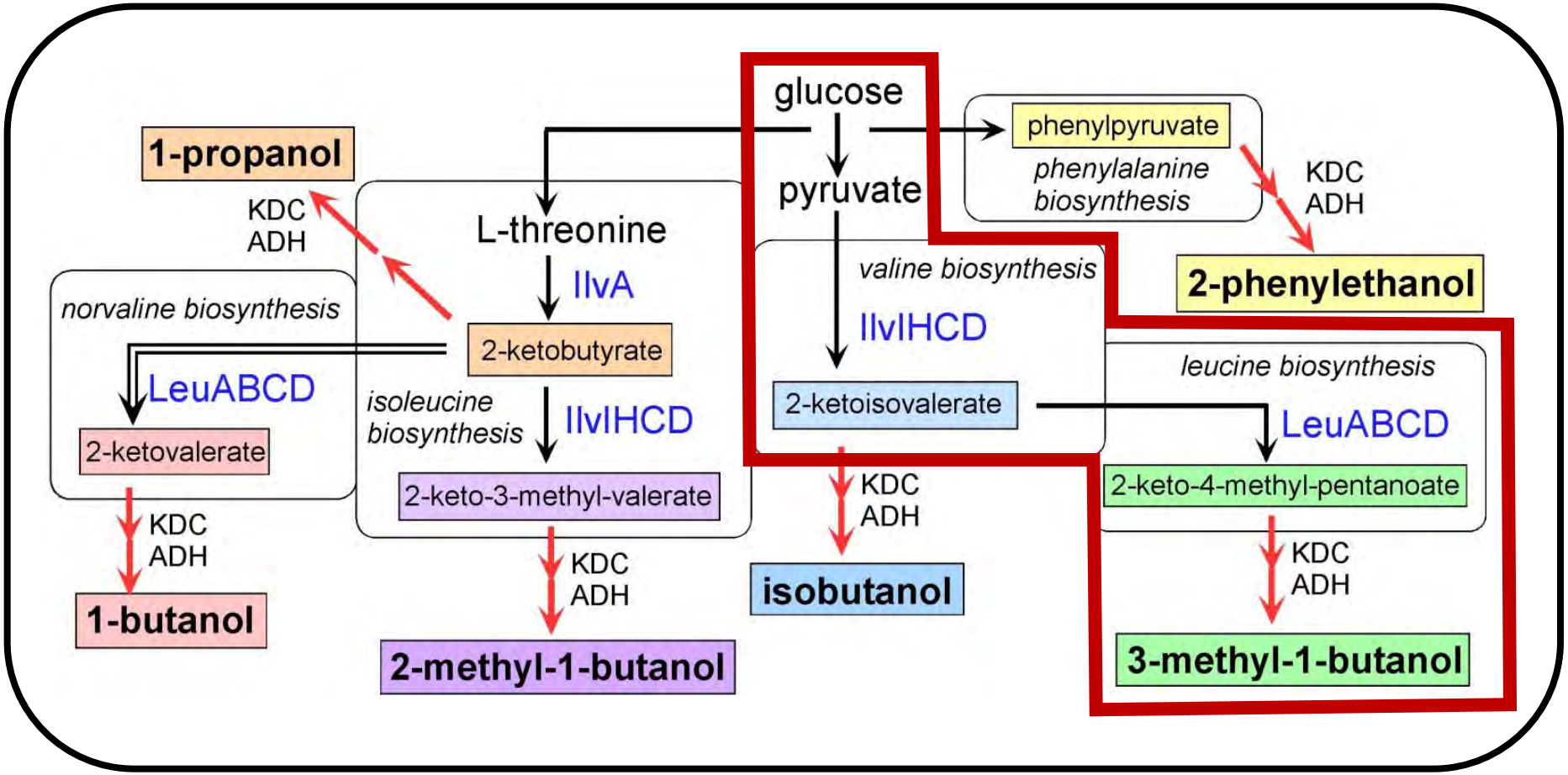


Atsumi *et al.* ***Nature*** **2008**

LeuABCD chain elongation



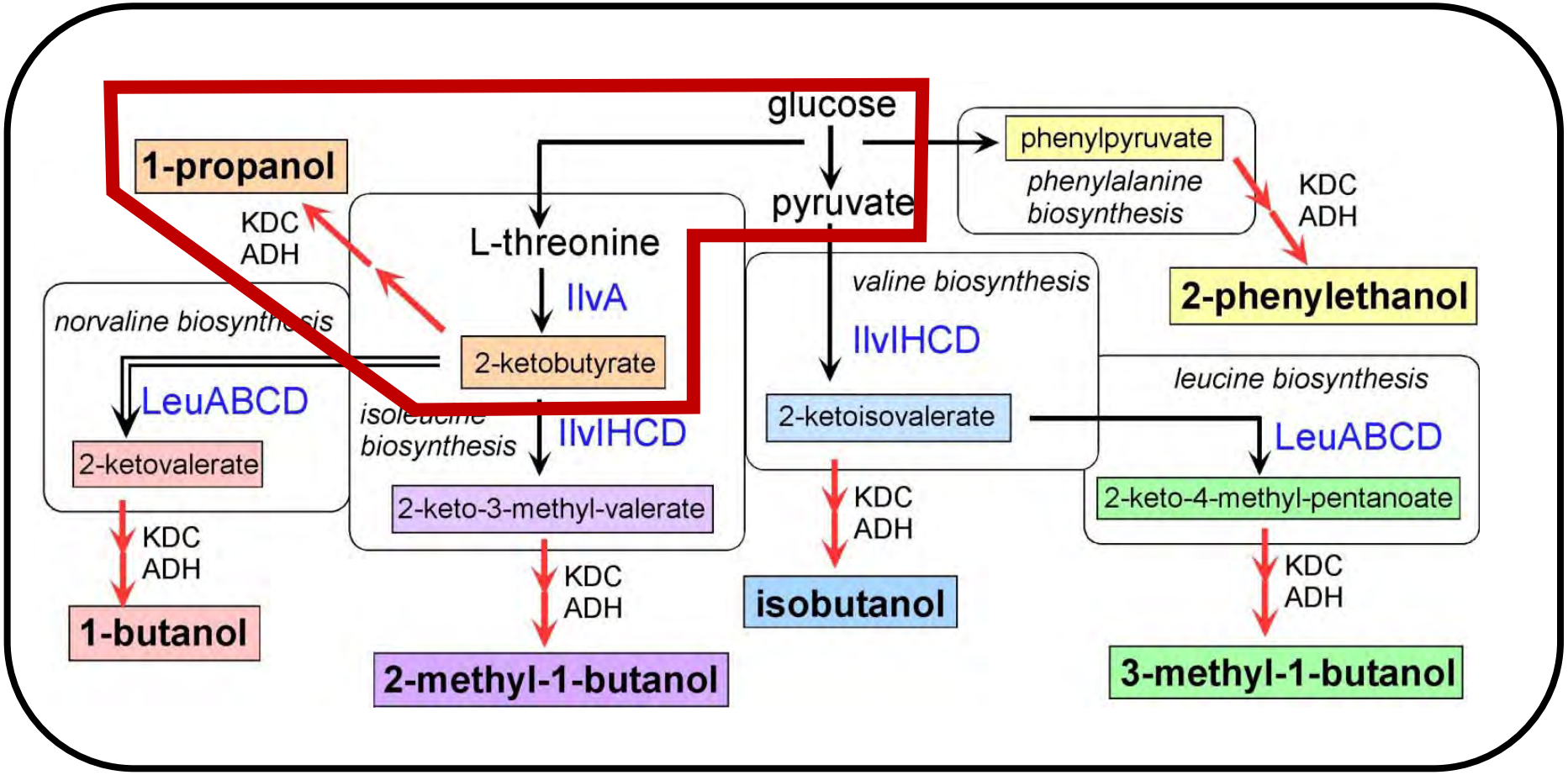
Novel Pathways for C5 alcohol synthesis



Connor and Liao, 2008 AEM

Atsumi *et al.* ***Nature*** **2008**

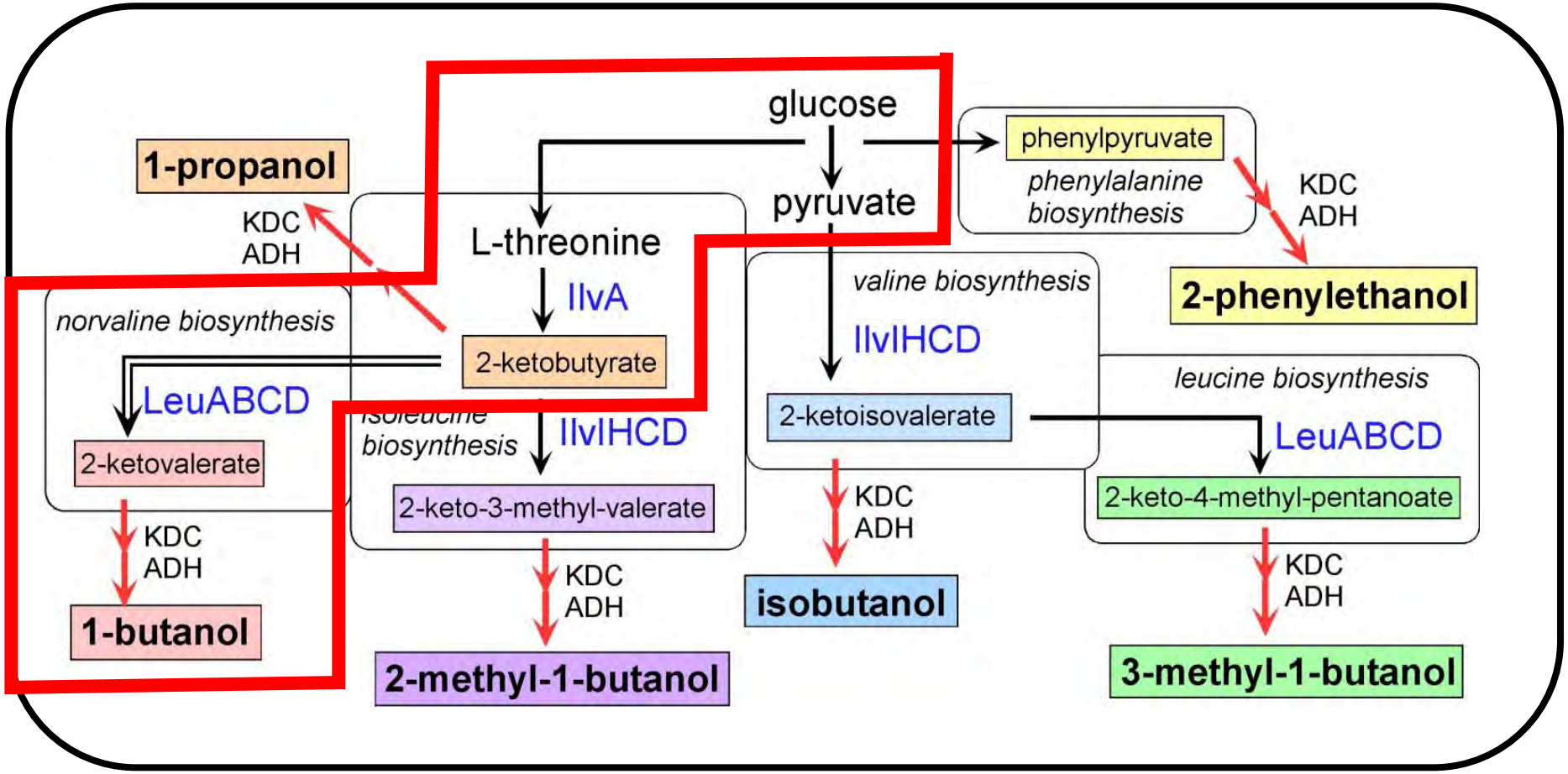
Novel Pathway for C3 alcohol synthesis



Shen and Liao 2008, Metabolic Engineering

Atsumi *et al.* ***Nature*** **2008**

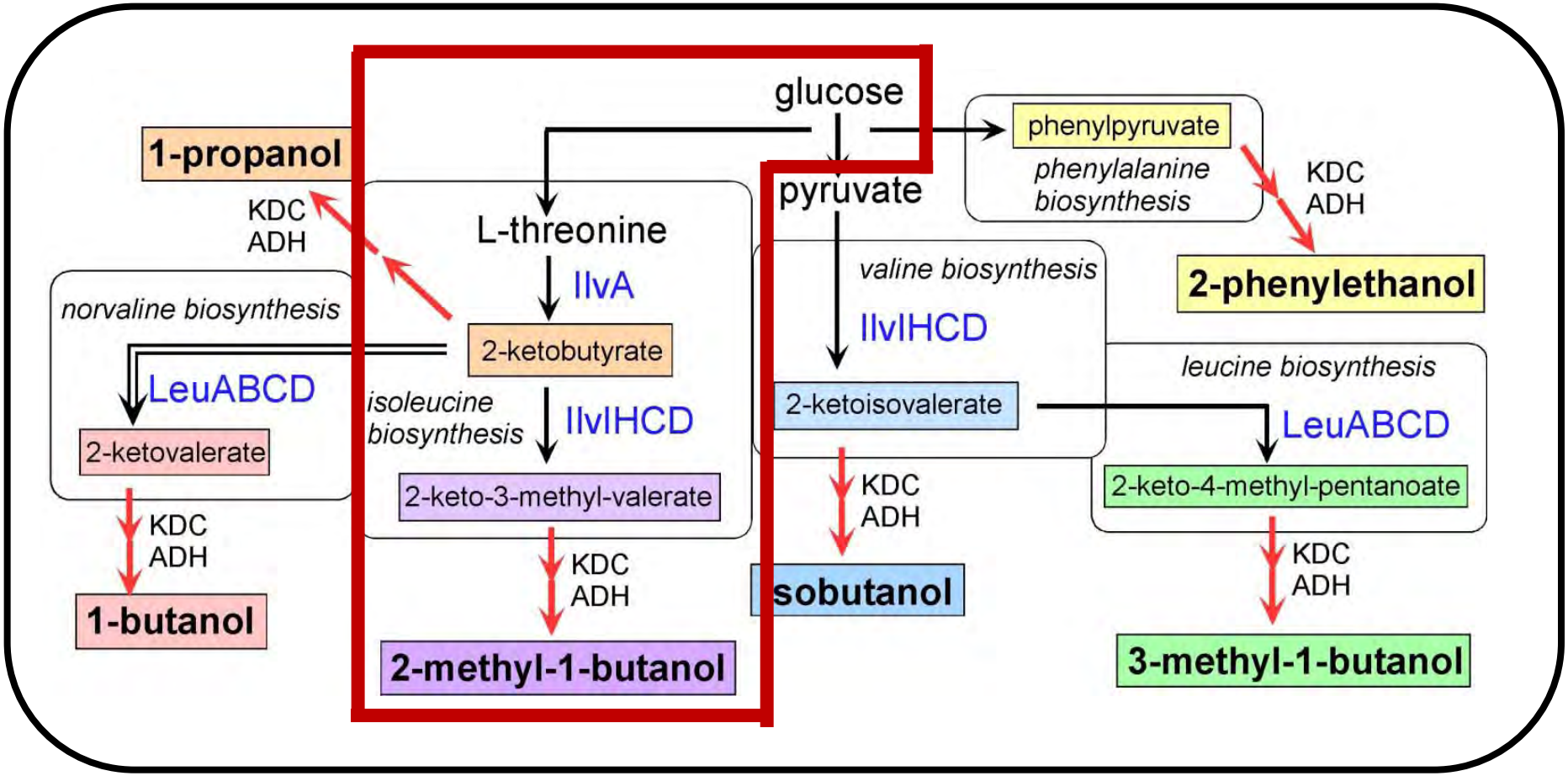
Novel Pathway for C4 alcohol synthesis



Shen and Liao 2008, Metabolic Engineering

Atsumi *et al.* ***Nature*** **2008**

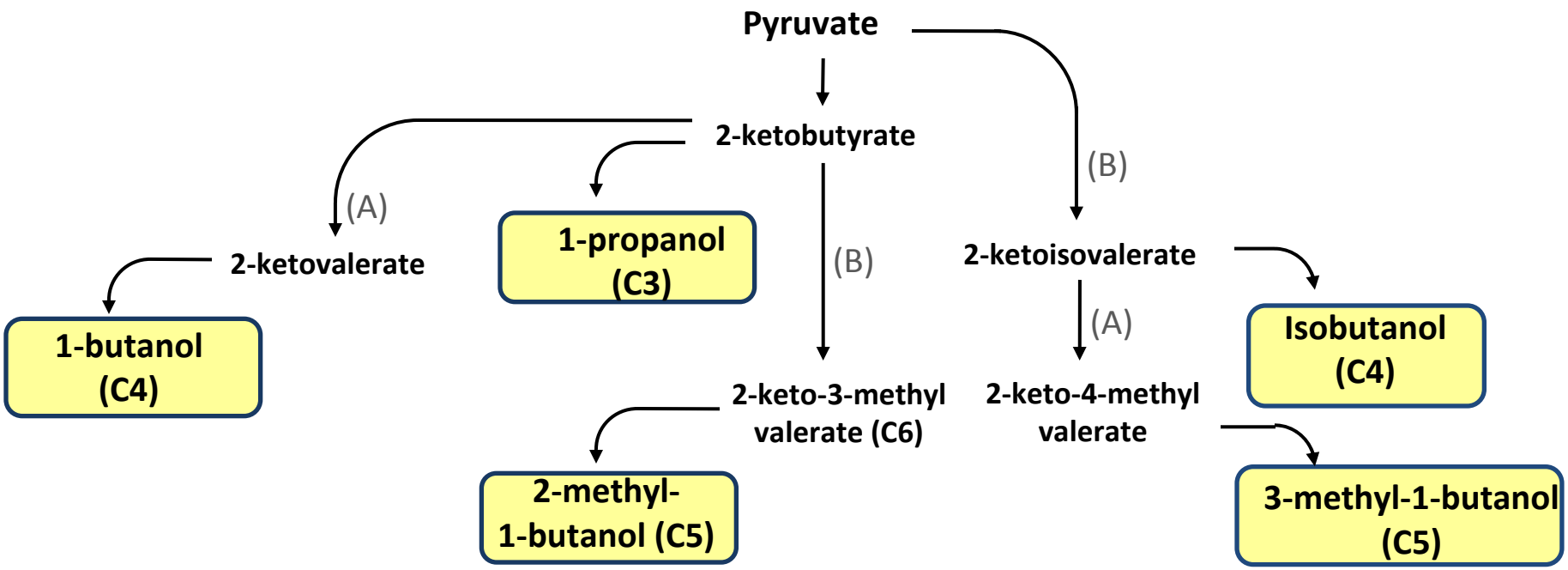
Novel Pathways for C5 alcohol synthesis



Cann and Liao, 2008 AMB

Atsumi *et al.* ***Nature*** **2008**

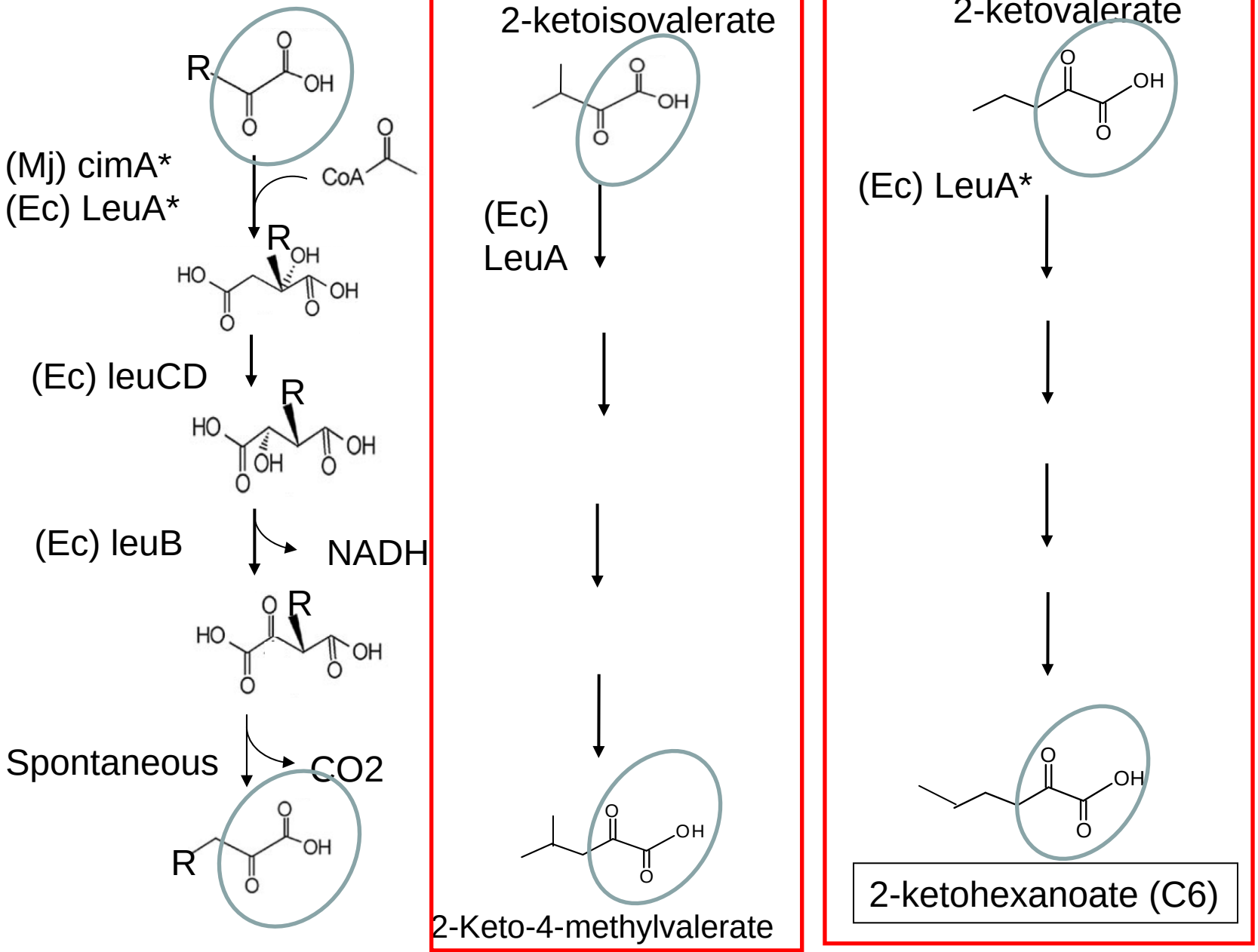
Synthetic Network for Higher Alcohols



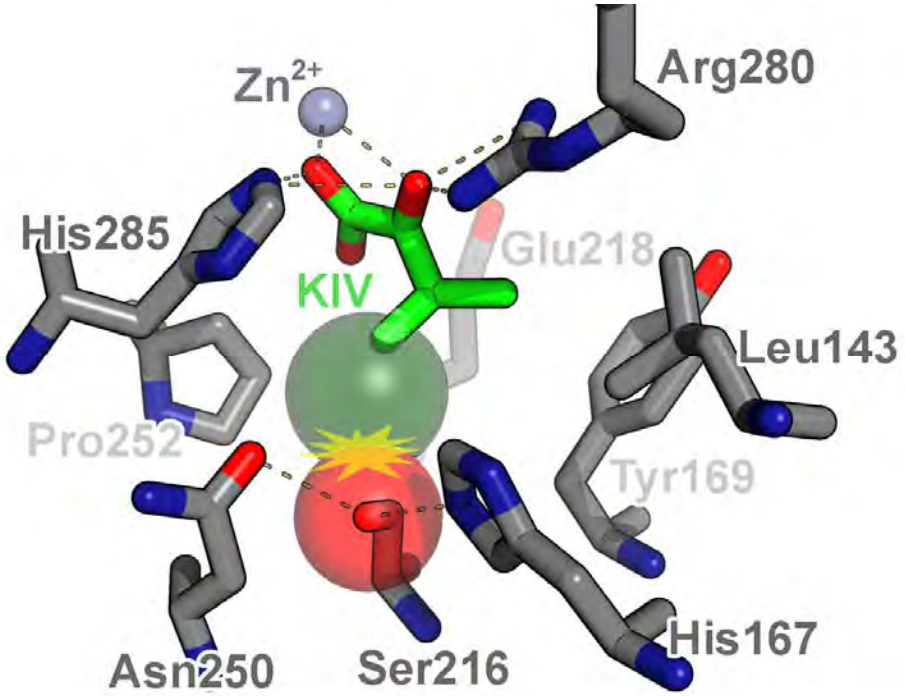
(A) Leucine biosynthesis (*leuABCD*)

(B) Isoleucine biosynthesis (*ilvGM*(ST)-*ilvCD*)

LeuABCD chain elongation

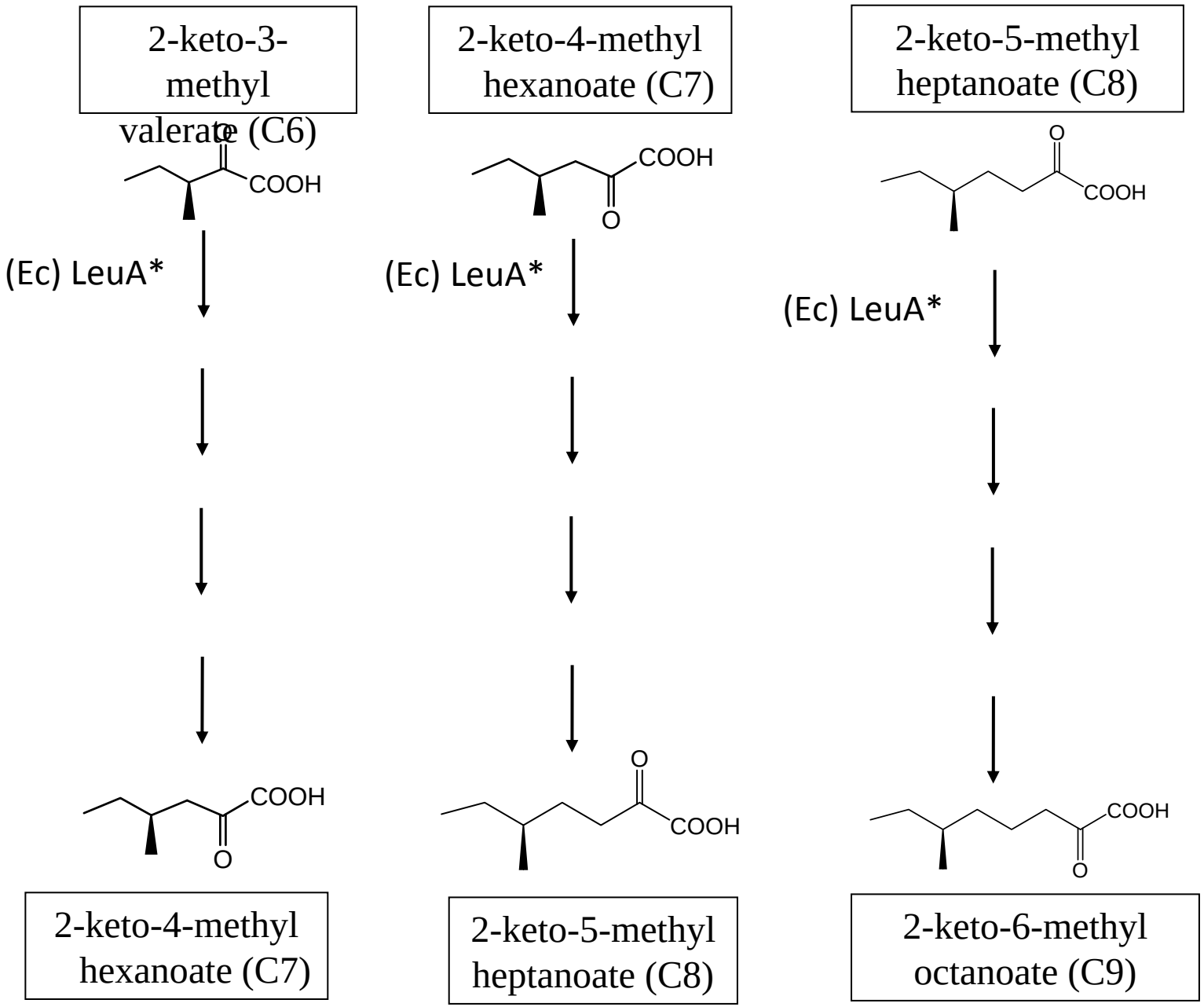


Engineering LeuA to produce non-natural alcohols

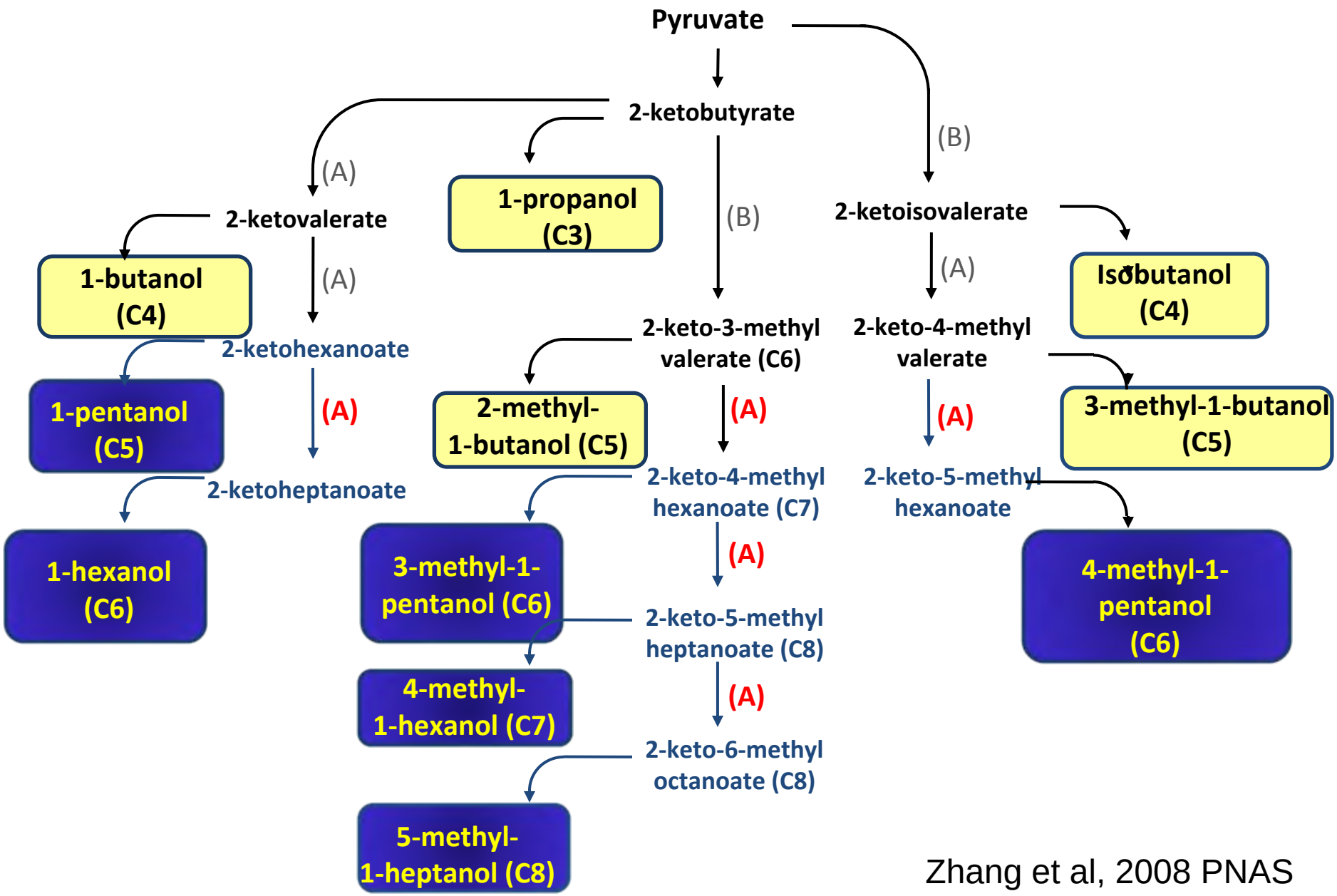


	His97	Ser139	Asn167
E. coli	RIHTFIATS	DVEFSCE	INIPDTV
S. typhimurium	RIHTFIATS	DVEFSCE	INIPDTV
M. tuberculosis	IVHFYNSTS	RFEYSPE	FNLPATV
	His167	Ser216	Asn250

Synthetic pathways for non-natural metabolites

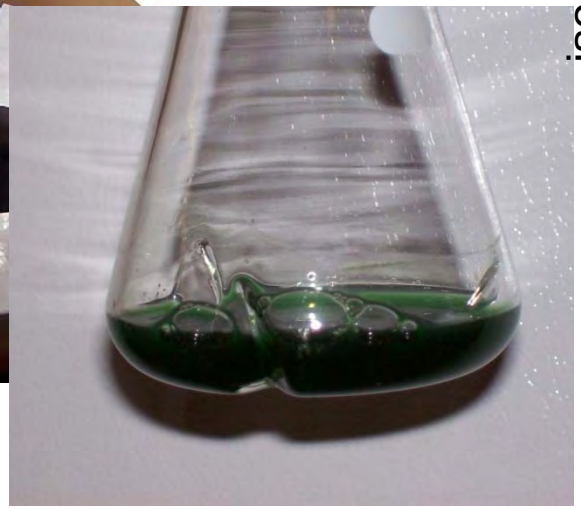
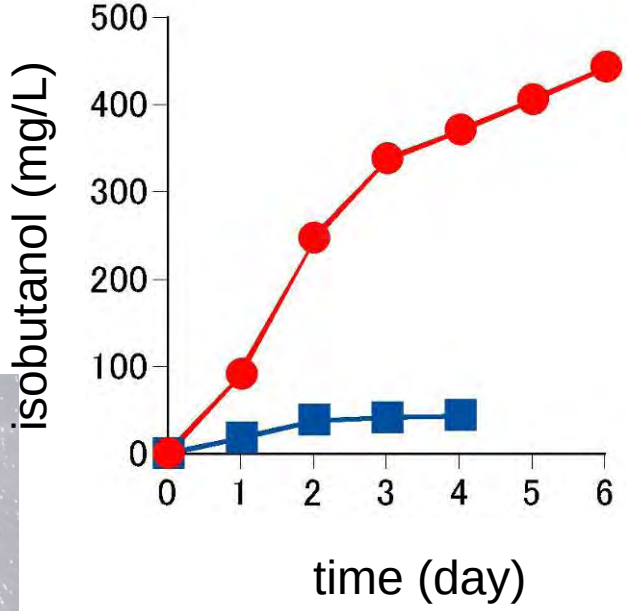


Synthetic Network for Higher Alcohols



Zhang et al, 2008 PNAS

What's new in this strategy?

- Harnesses cell's most abundant pathway for production of fuels and chemical stocks.
- Allows direct synthesis of fuels from cellulose and CO_2

CO_2 to isobutanol using Photosynthetic Bacteria