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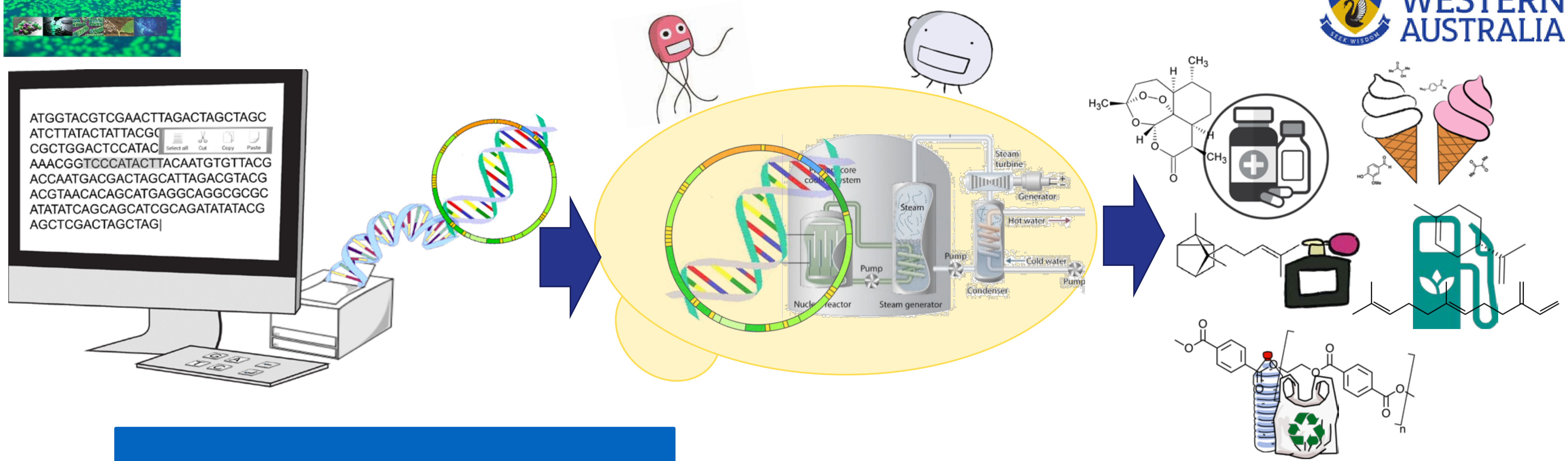


Engineering Microbial Cells as Chemical Factories II

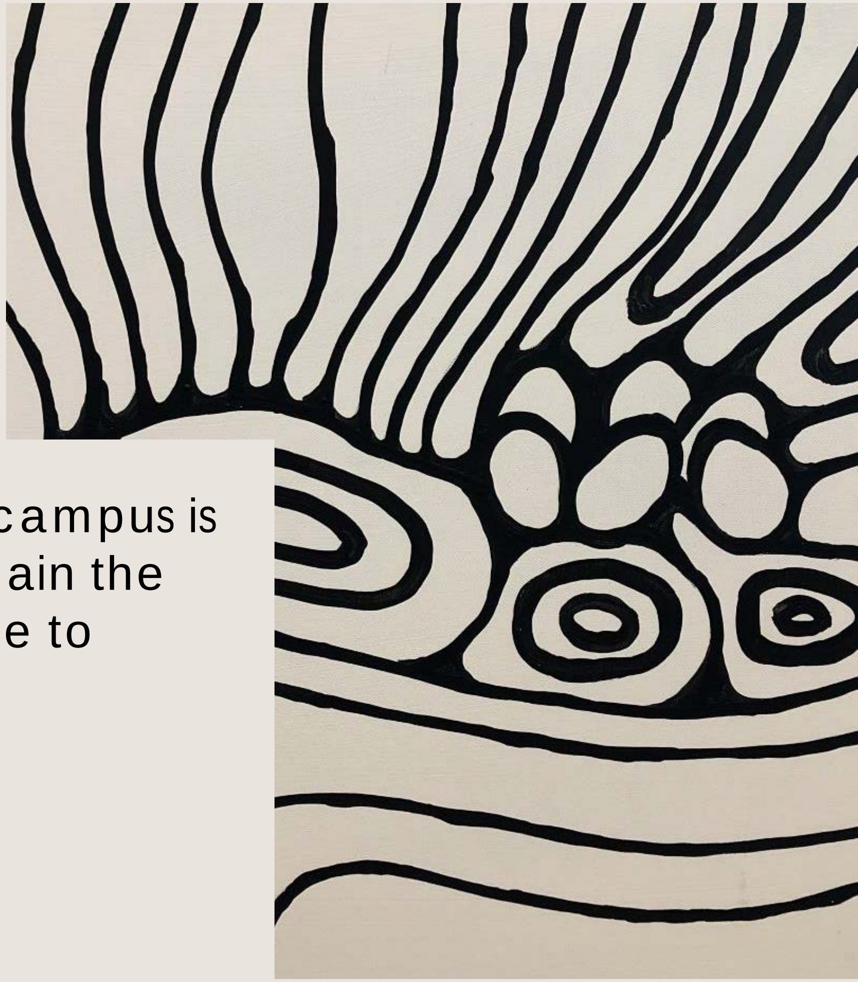
Applications: from fuels, fine chemicals to pharmaceuticals/natural products

S2 2020

Dr Heng Chooi / School of Molecular Sciences

Acknowledgement
of country

The University of Western Australia acknowledges that its campus is situated on Noongar land, and that Noongar people remain the spiritual and cultural custodians of their land, and continue to practise their values, languages, beliefs and knowledge.



Artist: Dr Richard Barry Walley OAM

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Microbial Chemical Factories: Applications

1. Biofuels
2. Biomonomers for polymers
3. Advanced Pharmaceutical Intermediates
4. Pharmaceutical Drugs
5. Genomics-driven Drug Discovery

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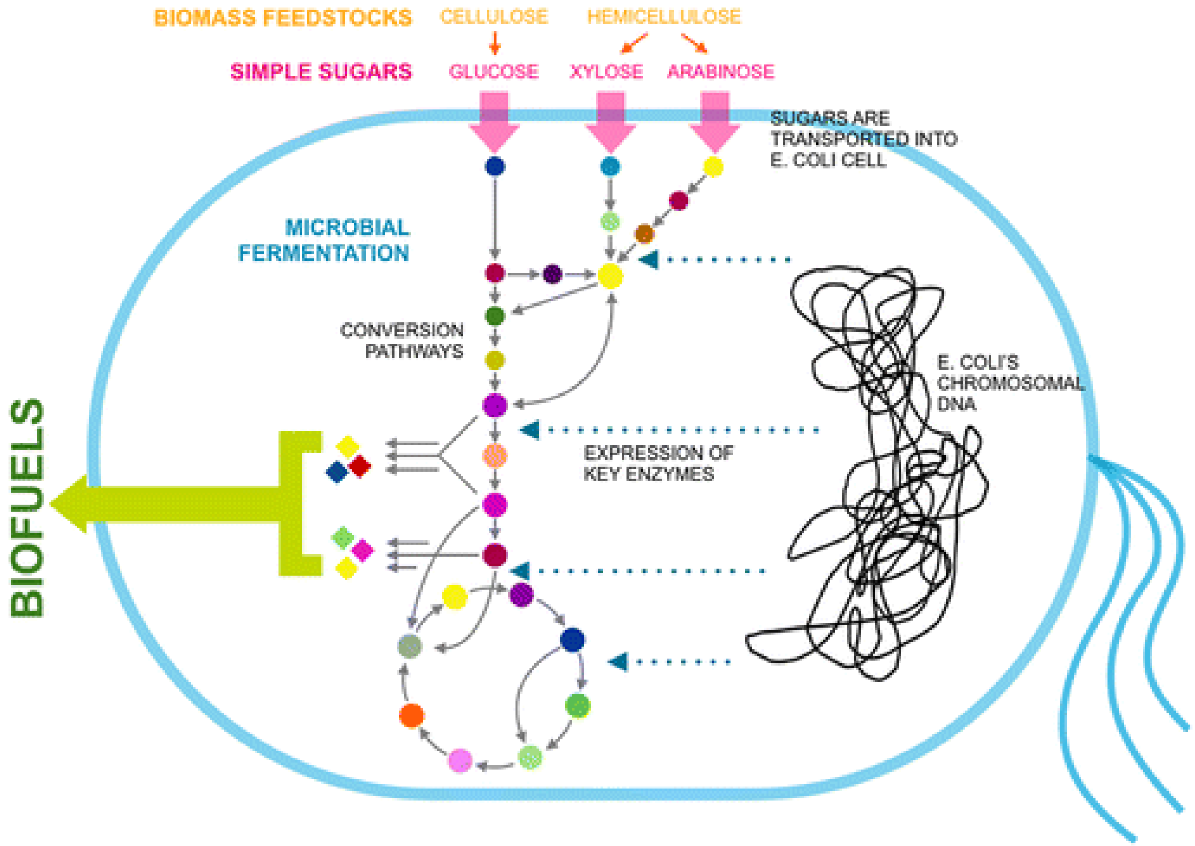
Biofuels



Kung et al. 2012 *ACS Syn Biol*

- **Fermentative Short-Chain Alcohol Fuels**
- **Non-fermentative Alcohol Fuels**
- **Fatty Acid-Derived Fuels**
- **Isoprenoid-Derived Fuels**

Further reading: Kung et al. From Fields to Fuels: Recent Advances in the Microbial Production of Biofuels. *ACS Syn Biol* https://doi.org/10.1021/sb300074k

Hijacking cell primary metabolism for biofuel production

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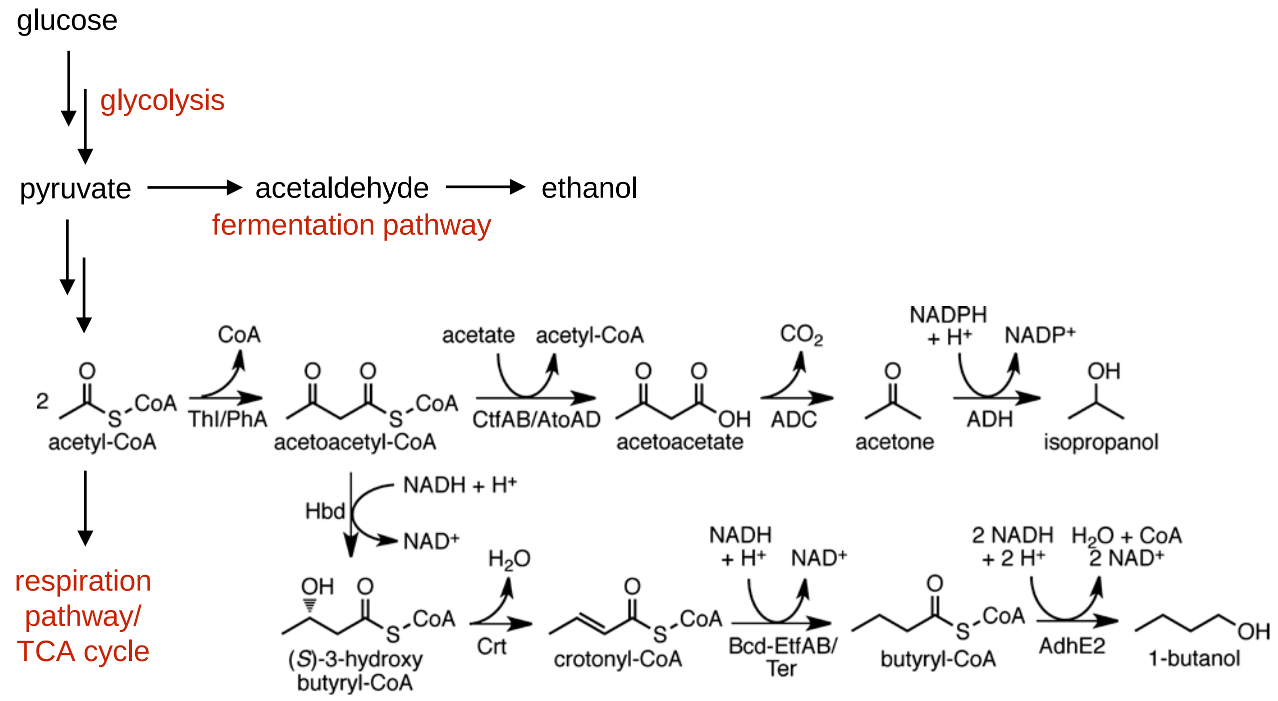
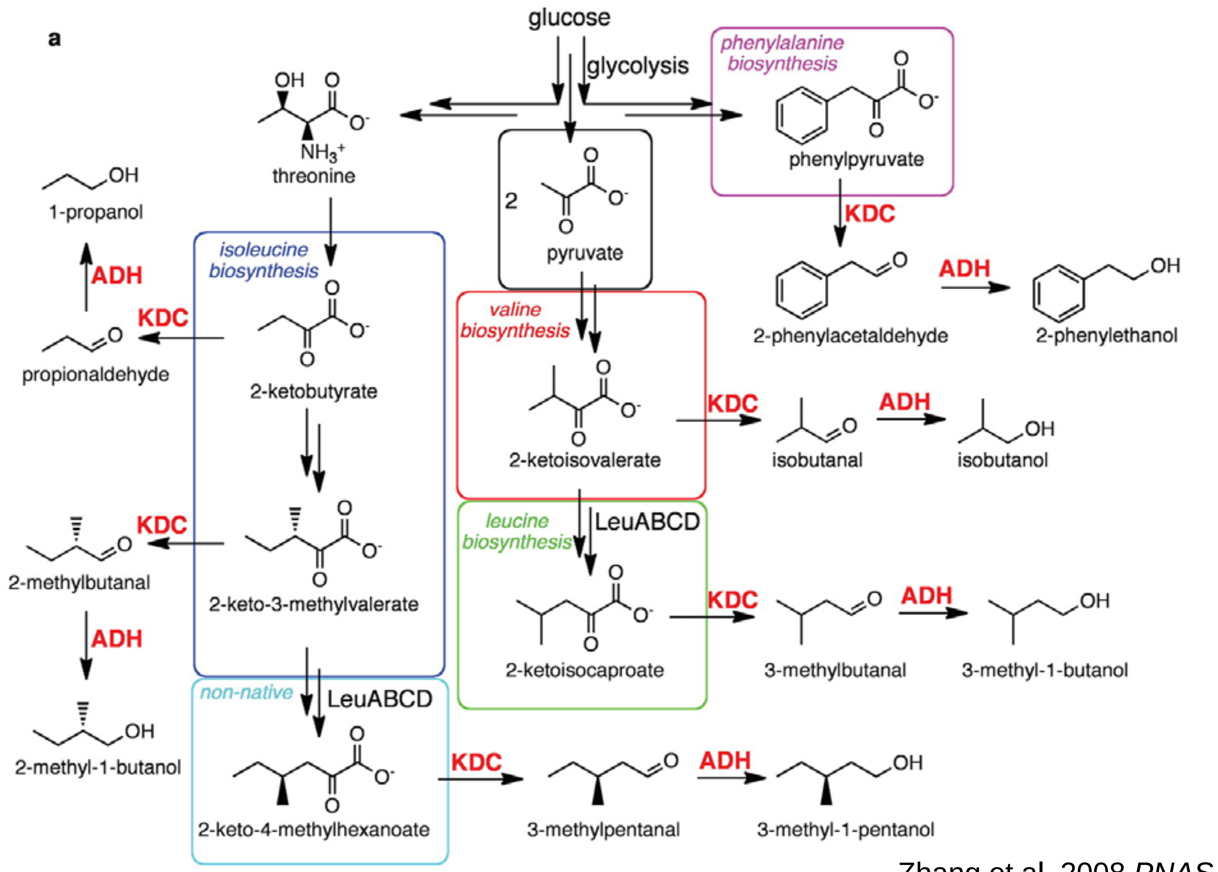


Hanai et al. 2007 *Appl Env Microbiol*
Kung et al. 2012 *ACS Syn Biol*

Redirecting acetyl-CoA to production of C3-C4 chain alcohols

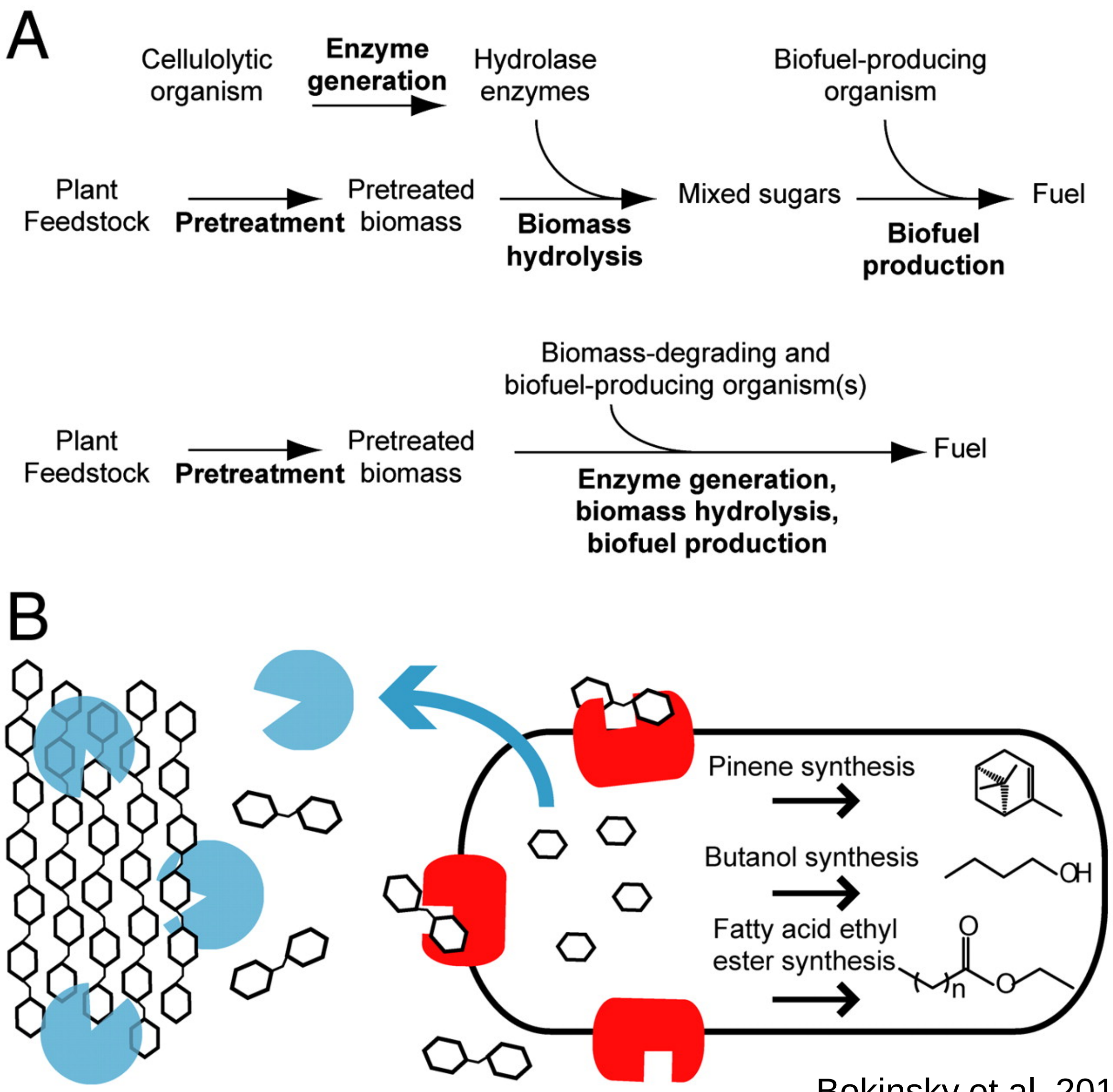


Zhang et al. 2008 *PNAS*
Kung et al. 2012 *ACS Syn Biol*

Hijacking amino acid biosynthesis for production of branched chain alcohols

James Liao group at UCLA (now in Academia Sinica, Taiwan)

Two-in-One Biofuel Producer



Bokinsky et al. 2011 *PNAS*

A strain of *Escherichia coli* has been genetically engineered to break down switchgrass into sugars, and then convert those sugars into three types of biofuel.

This consolidated process could lower the cost of producing fuels from biomass.

Jay Keasling group at UC Berkeley

Figure 2. Biosynthetic pathways for fermentative short-chain alcohol production.

Table 1. Summary of Titers and Yields for Select Fuels

fuel	pathway used	host organism	titer (g/L)	yield (%)	ref
isopropanol	alcohol (fermentative)	*E. coli*	143	67	6
1-butanol	alcohol (fermentative)	*E. coli*	30	70	11
isobutanol	alcohol (non-fermentative)	*E. coli*	50	68	14
branched alcohols[a]	alcohol (from protein hydrolysates)	*E. coli*	4.0	56	25
FAEE	fatty acid	*E. coli*	0.674	9.4	36
FAEE	fatty acid	*E. coli*	1.5	28	40
methyl ketones	fatty acid	*E. coli*	0.38	58	42
alkanes/alkenes	fatty acid	*E. coli*	0.3	3.5	43
farnesol	isoprenoid	*E. coli/S. cerevisiae*	0.135/0.145	ND	57/55
bisabolene	isoprenoid	*E. coli/S. cerevisiae*	0.9	4	67

[a]Includes isobutanol, 2-methyl-1-butanol, and 3-methyl-1-butanol.

Kung et al. 2012 *ACS Syn Biol4*

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Value per Volume of Bulk vs. Fine chemicals

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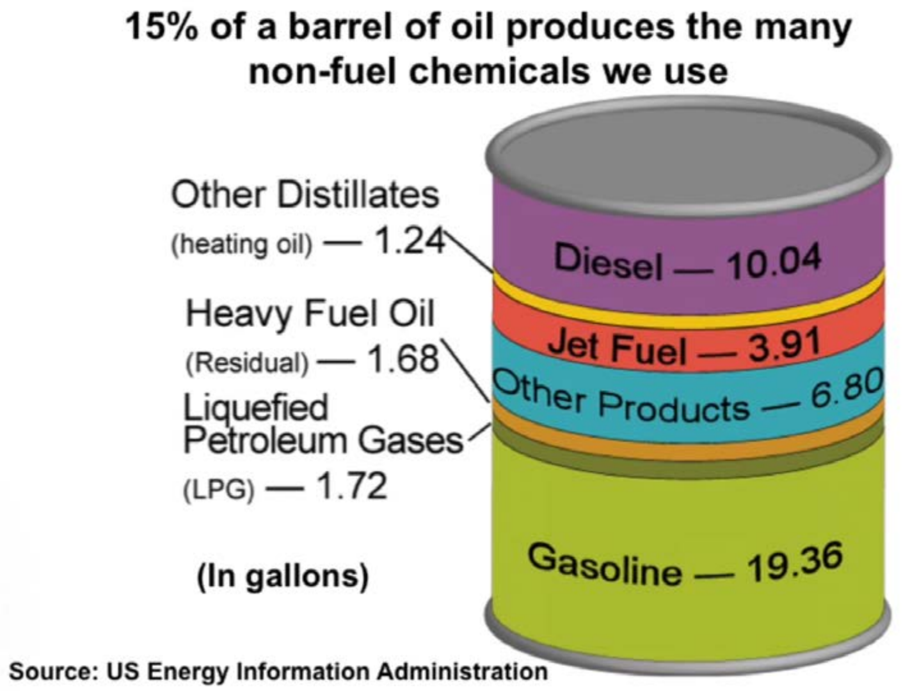
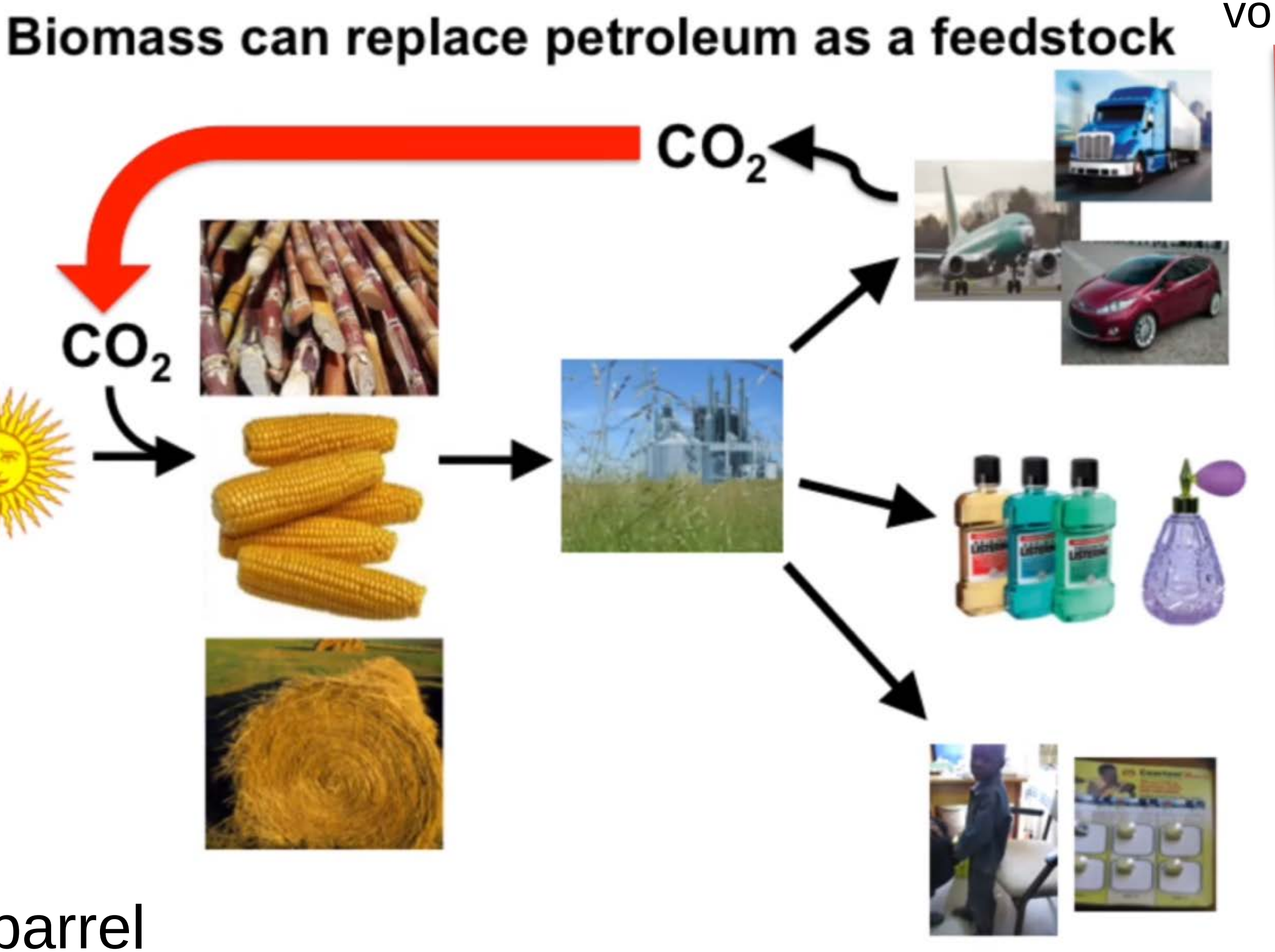
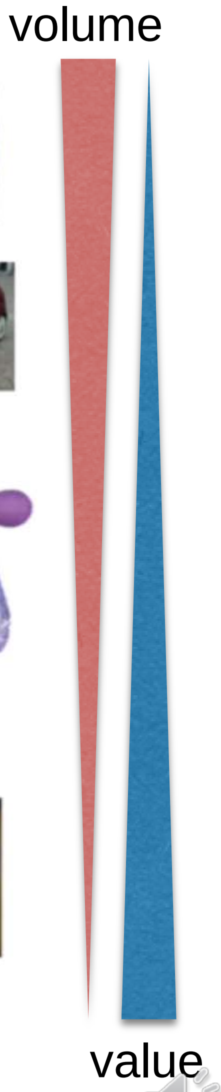




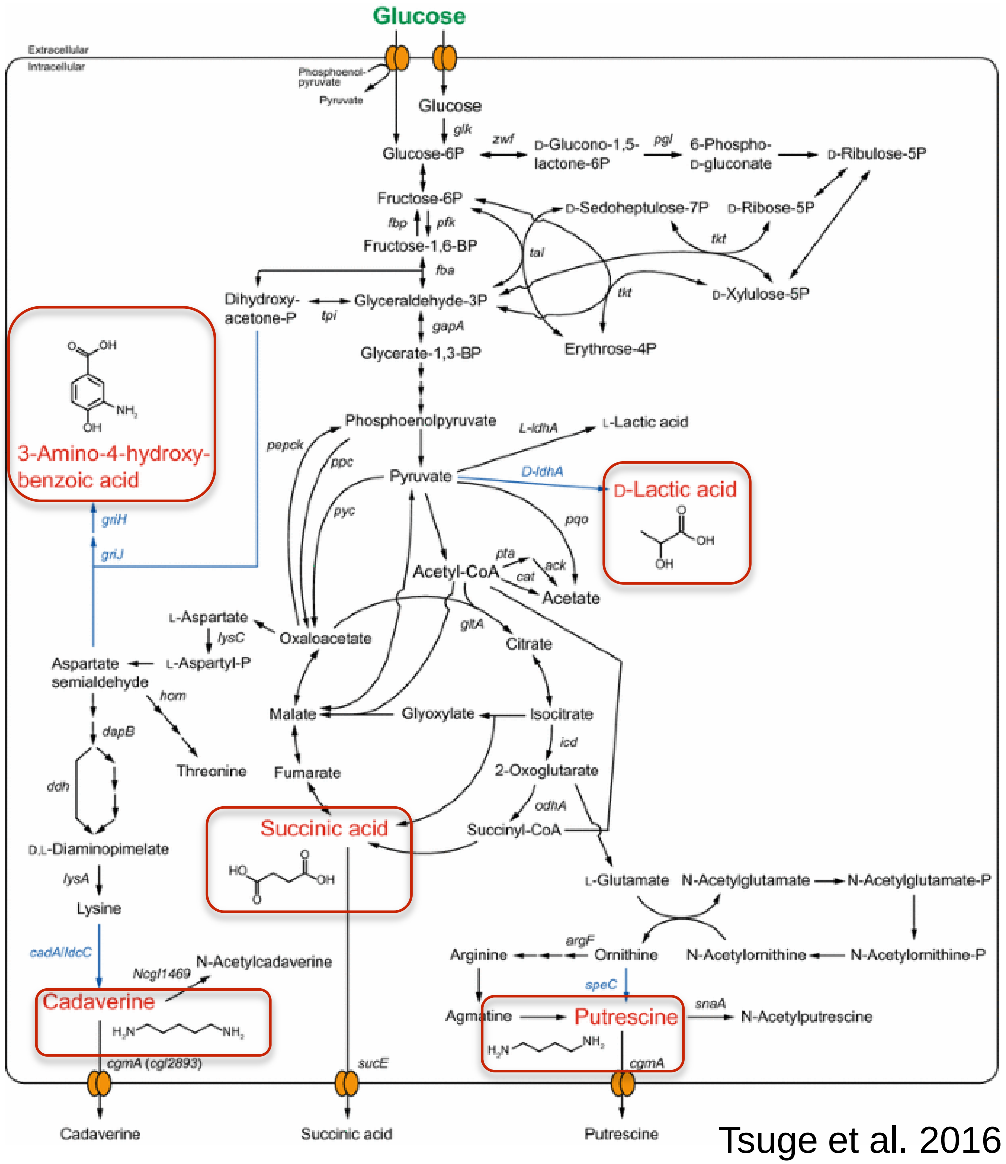


USD $150 per barrel → $30 per barrel

iBiology lecture by Jay Keasling

Supplemental reading: https://www.technologyreview.com/2018/05/10/2851/the-scientist-still-fighting-for-the-clean-fuel-the-world-forgot/
https://www.nature.com/scitable/blog/eyes-on-environment/the_biofuel_controversy/

Biomonomers for polymers

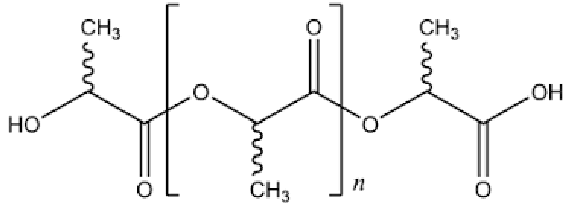


Tsuge et al. 2016 *Microb Cell Fact*

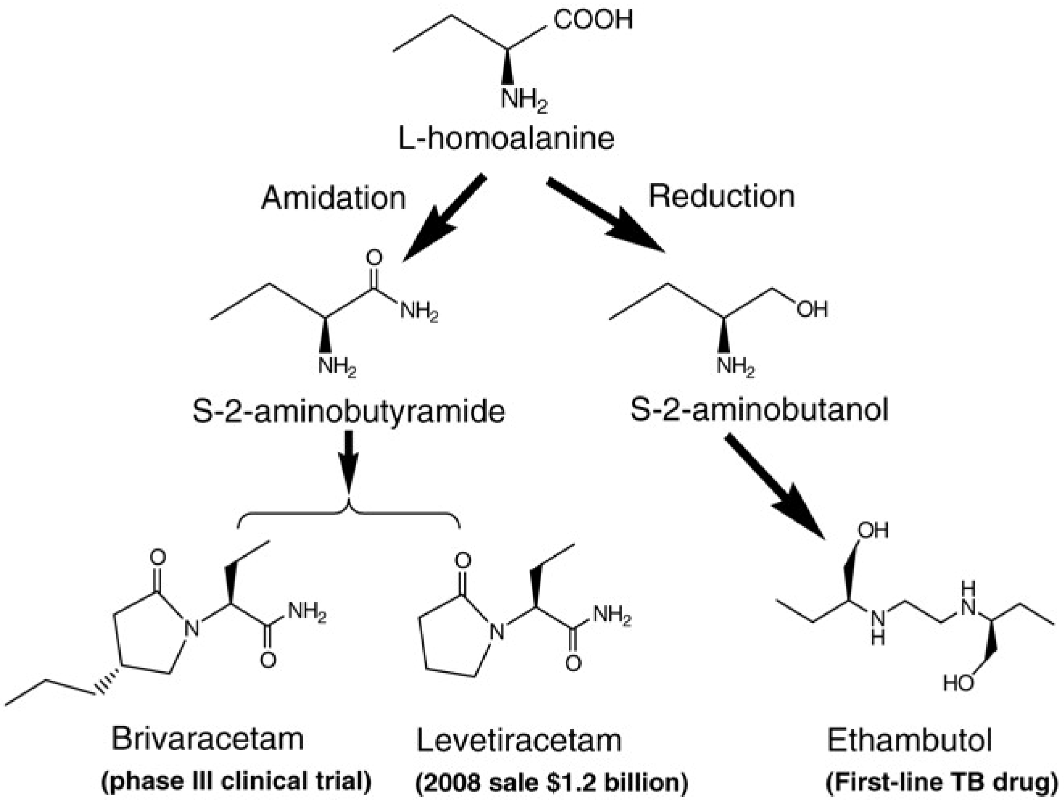
- D-Lactic acid → Poly-L-lactic acid (PLLA): only successful 100 % bio-based polymer that is industrialised

- 3-Amino-4-hydroxybenzoic acid (3, 4-AHBA) → poly-benzoxazole: can be made into commercial textile with extremely high thermal and mechanical properties

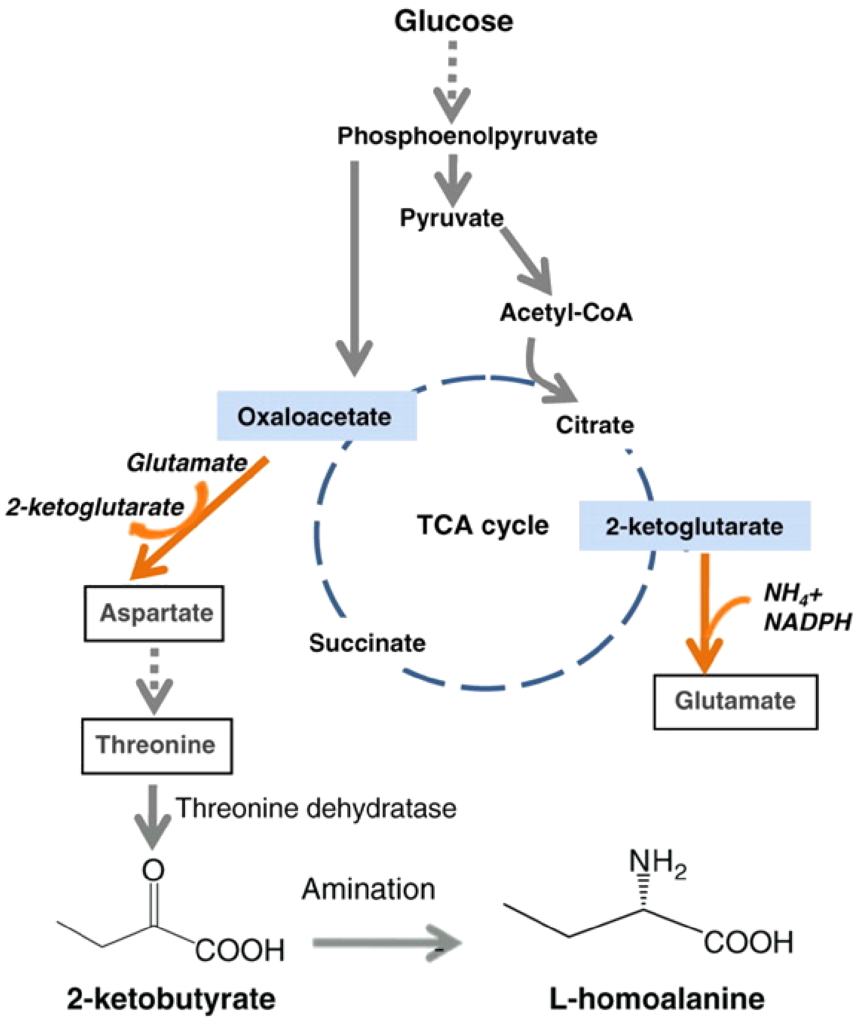
- Succinic acid (butanedioic acid) is utilized as a building block for several commercially important polymers, such as polybutylene succinate adipate. Moreover, in combination with diamines, putrescine, and cadaverine, succinic acid can also be used to produce 100 % bio-based nylon materials.



Advanced Pharmaceutical Intermediates



L-homoalanine as a valuable API



Redirecting threonine to L-homoalanine

Zhang et al. PNAS 2010

Combine directed evolution and pathway engineering

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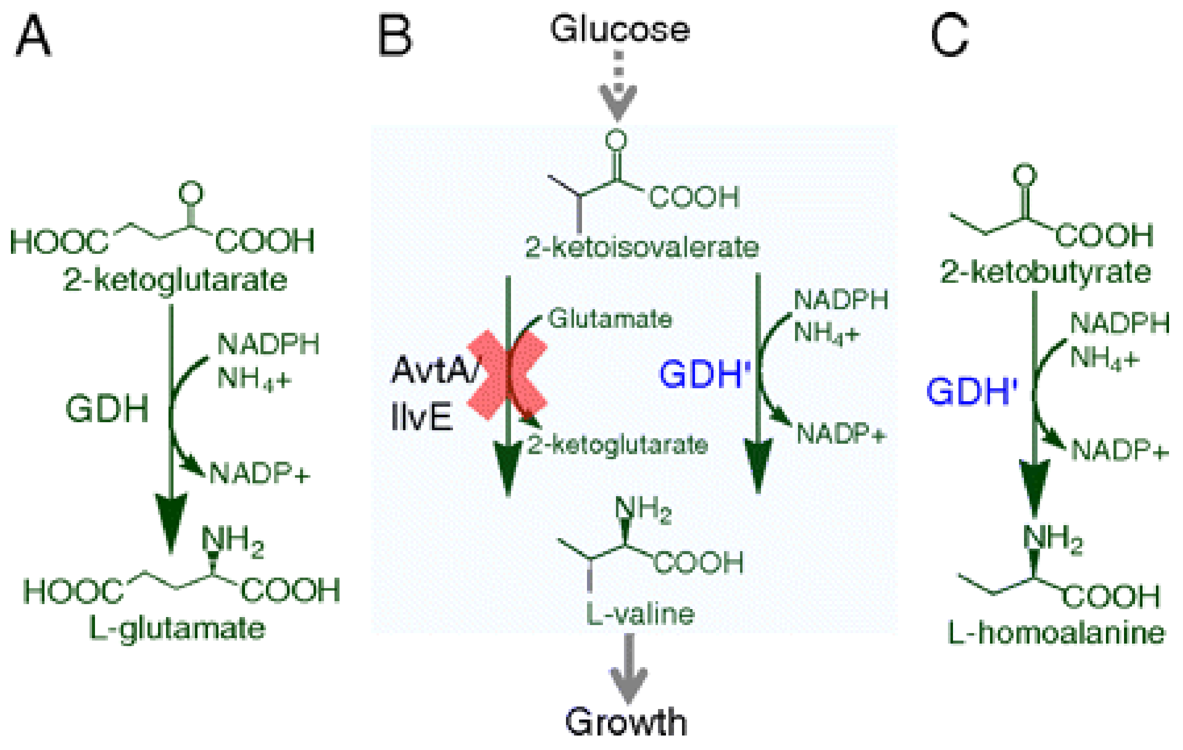
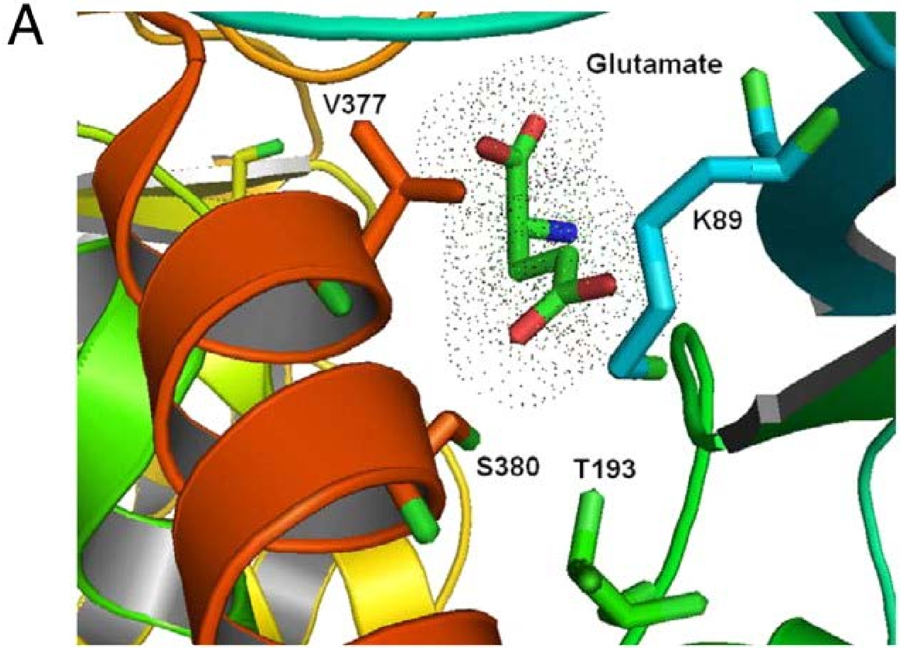
Directed evolution: Generate a survival-based auxotrophy screen for site-saturation mutagenesis of glutamate deaminase



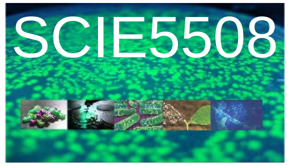


B

	K92	T195	V377 S380
E. coli	YKGGM	VFTGKG	GVATSGL
C. symbiosum	YKGGL	VLTGKA	GVLVSGF
	K89	T193	V377 S380

site-saturation mutagenesis with randomized NNK codon

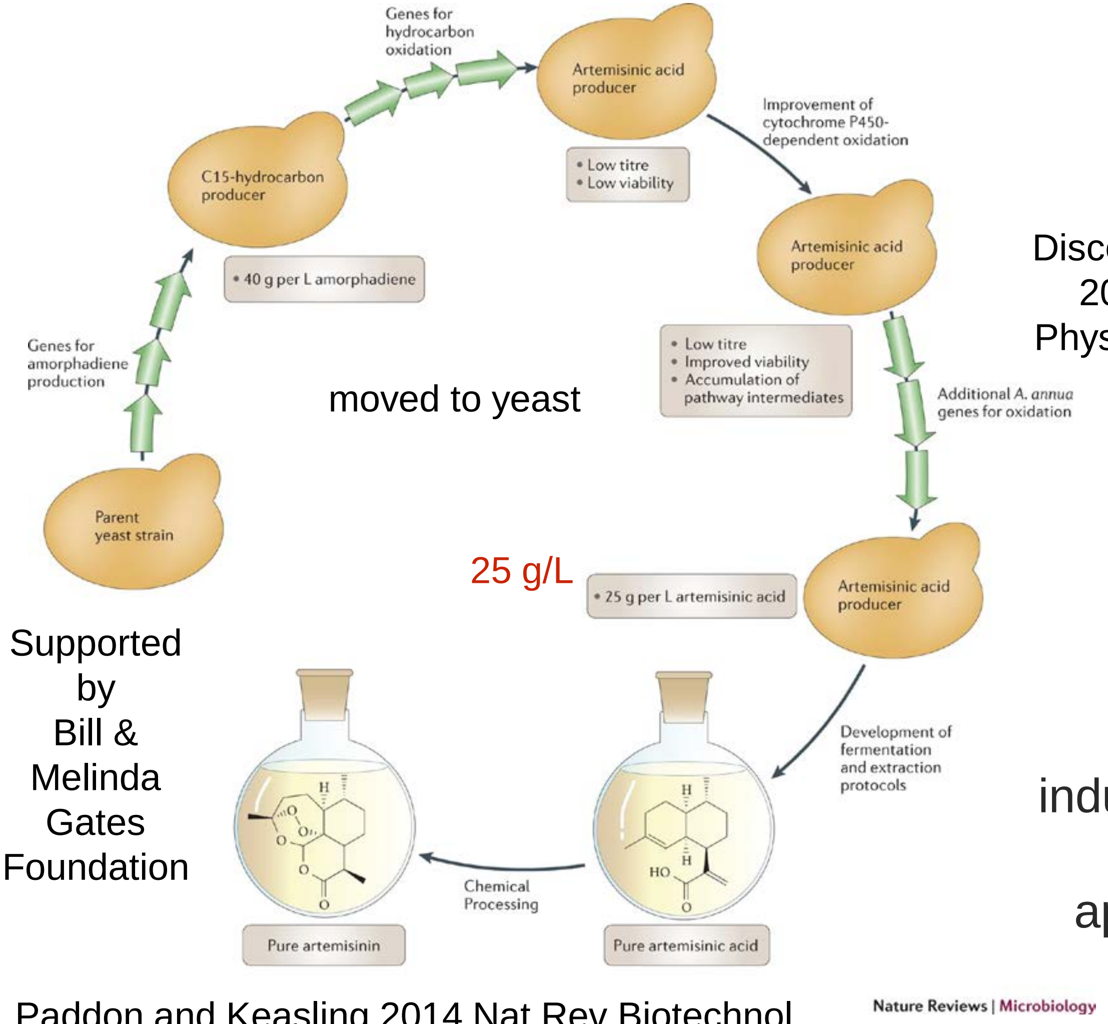
Pharmaceutical Drugs: Antimalarial Artemisinin



Initial tried in *E. coli*…

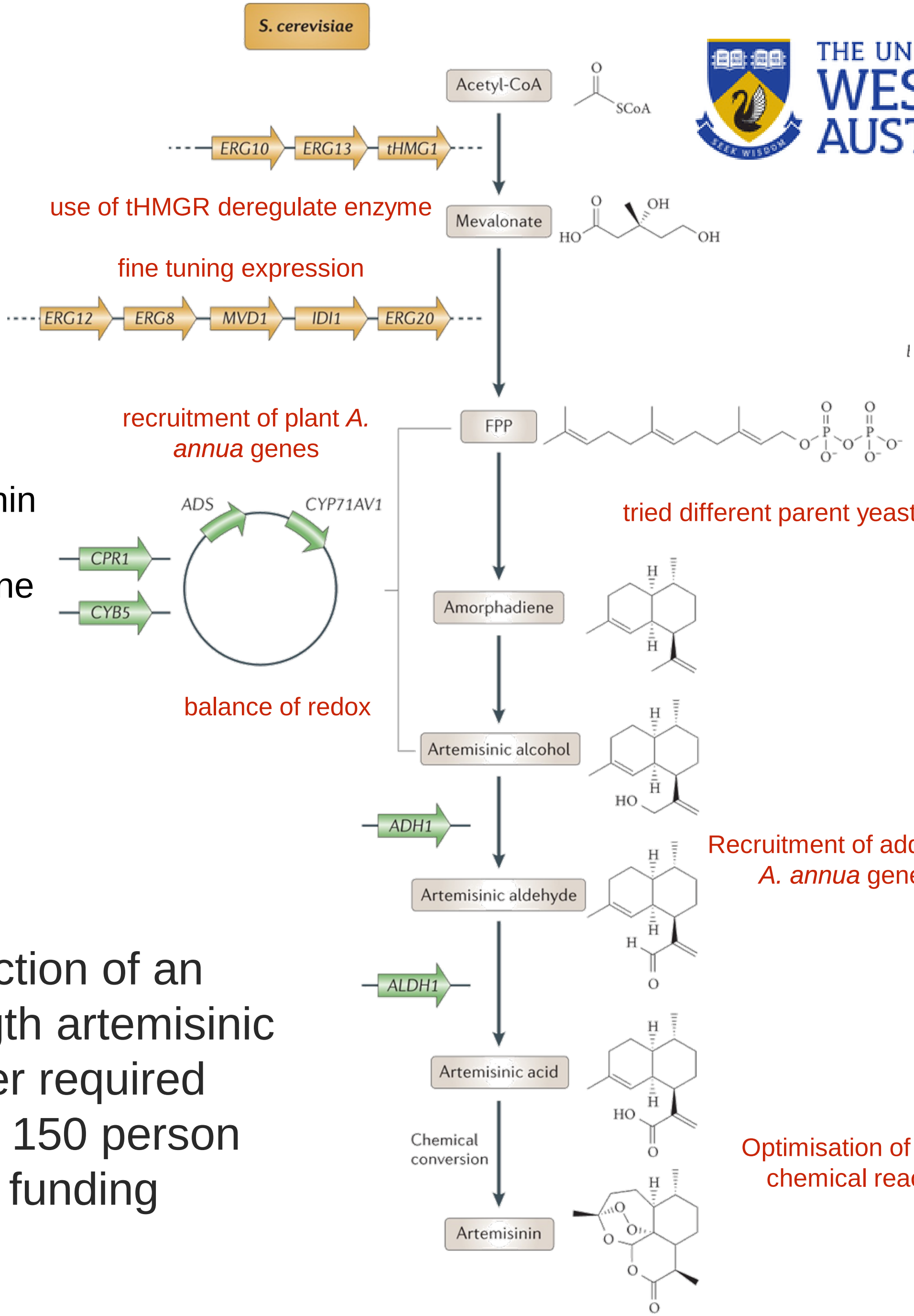
moved to yeast

25 g/L



Supported by Bill & Melinda Gates Foundation

Youyou Tu
Discovered Artemisinin
2015 Nobel Prize Physiology & Medicine



use of tHMGR deregulate enzyme

fine tuning expression

recruitment of plant *A. annua* genes

tried different parent yeast strains

balance of redox

Recruitment of additional *A. annua* genes

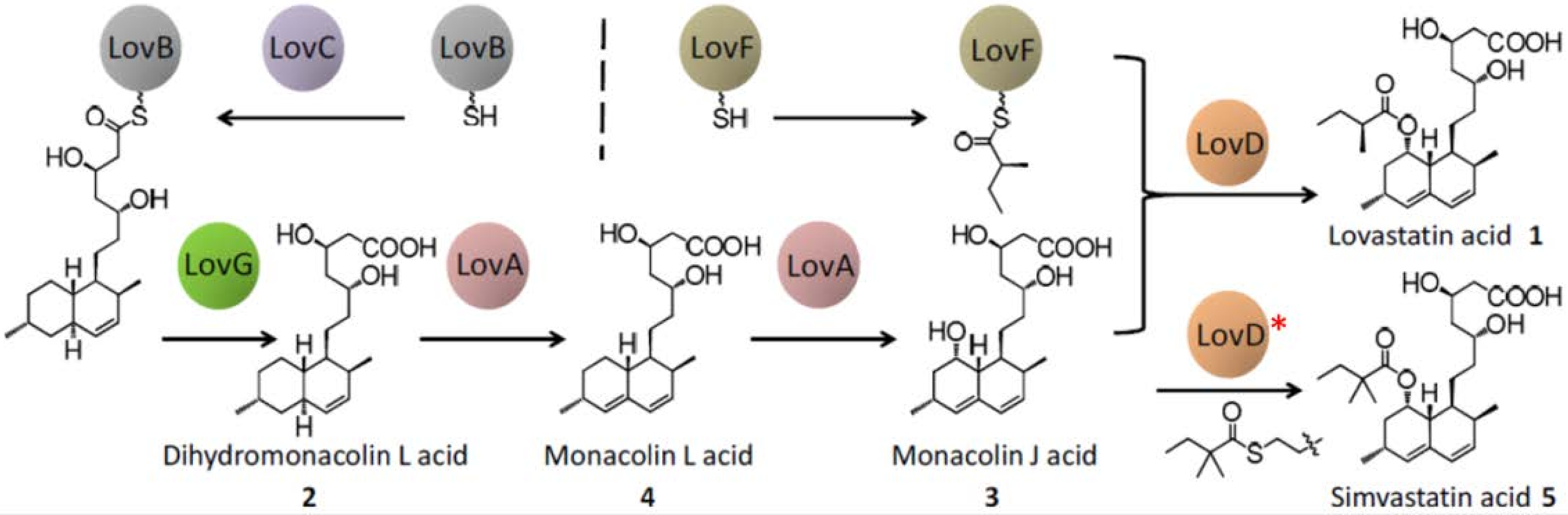
Optimisation of photo-chemical reaction

The construction of an industrial-strength artemisinic acid producer required approximately 150 person years and funding

Paddon and Keasling 2014 Nat Rev Biotechnol
https://www.nature.com/articles/nrmicro3240#Sec5

Pharmaceutical Drugs: Semisynthetic Anticholesterol Simvastatin

Lower production cost; environmental friendly – e.g. simvastatin



Engineered production of Simvastatin in yeast with synthetic biology
(provisional patent application no. US 61/812,942)

Genes cloned from the lovastatin producing fungus *Aspergillus terreus.* Combining pathway reconstruction and protein engineering (LovD acyltransferase) in yeast.

Other Pharmaceuticals and Neutraceuticals

Examples:

- Opioids/hydrocodone (analgesics)
- Cannabinoids (analgesics)
- Noscapine (antitussive)
- Vindoline, precursor towards vinblastine (anticancer)
- Taxadiene, precursor towards paclitaxel (anticancer)
- Resveratrol (antioxidant/antiaging)

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Synthetic Biology-Aided Natural Product Discovery

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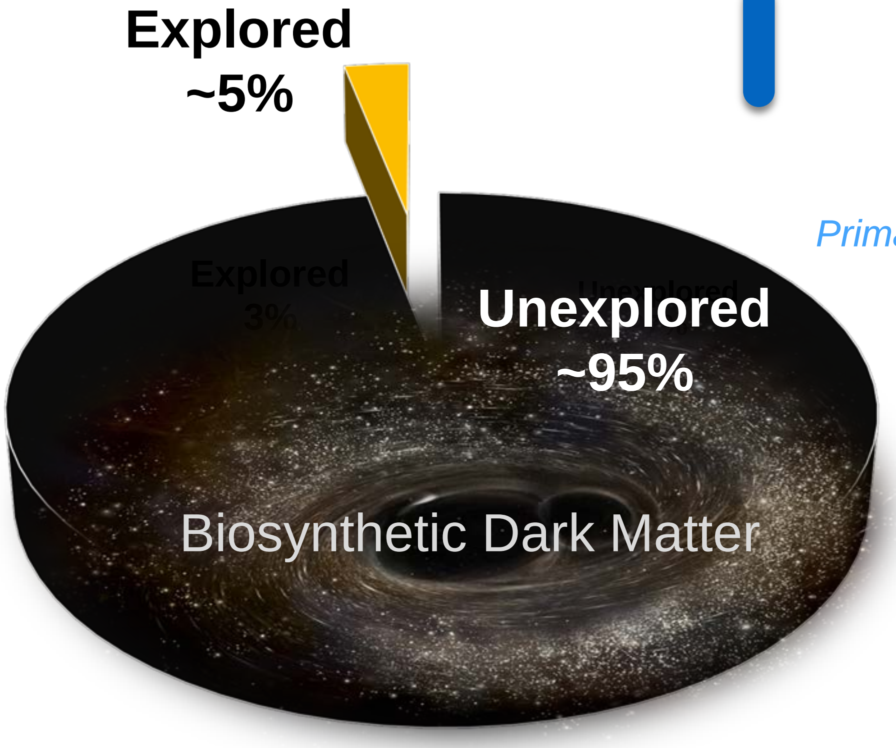

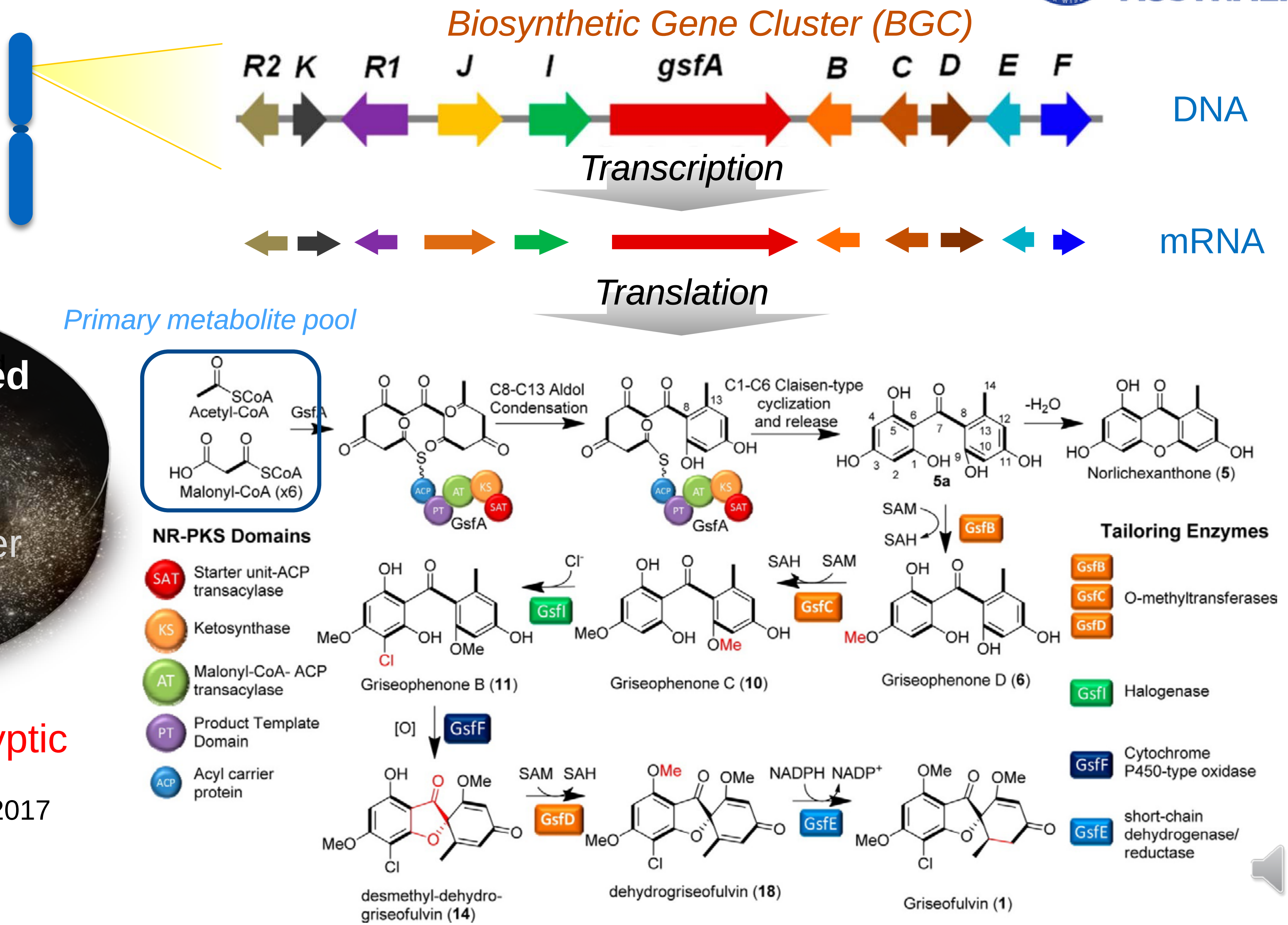

Most microbial BGCs are cryptic

Tang and Walsh, Nat Prod Biosynthesis 2017
Chooi et al. Chem. Biol. 2010
Cacho et al. ACS Chem Biol. 2013

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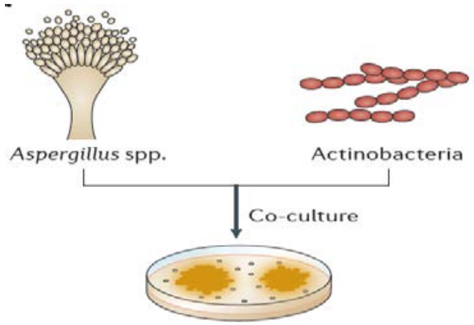
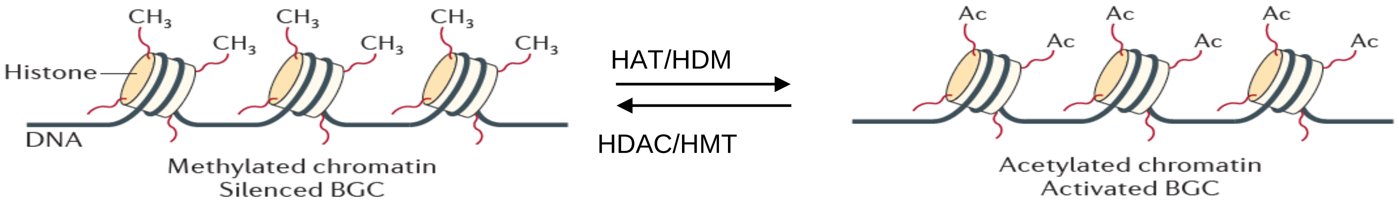
Approaches to access cryptic BGCs

A1) non-genetic/culture-based



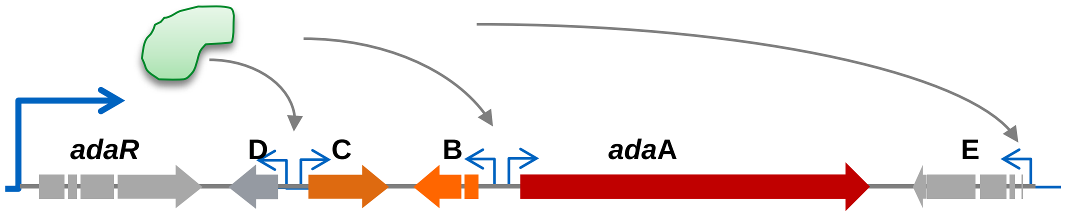
A2) Pleiotropic Genetic/epigenetic approaches

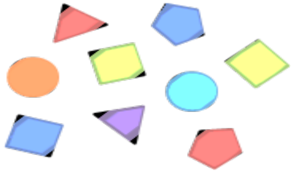


Rutledge & Challis 2010, Nat Rev Microbiol

A) Global
B) Pathway-specific

B1) Pathway-specific TF overexpression

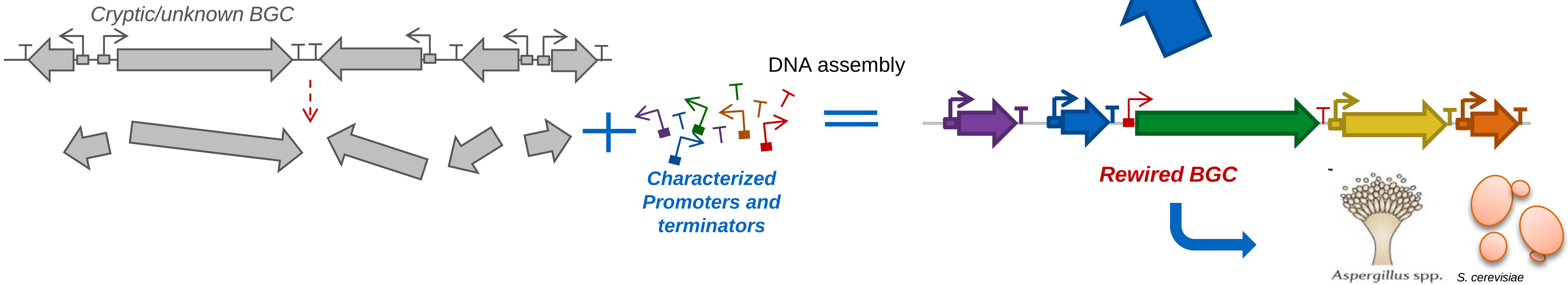




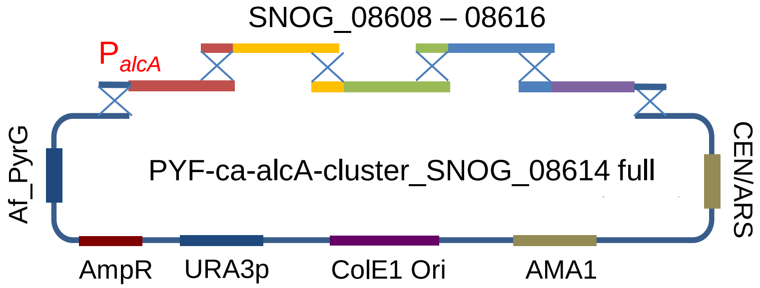
Novel/cryptic NPs

Molecules are linked to biosynthetic gene cluster

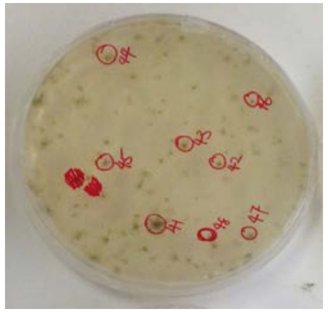
B2) Pathway reconstruction



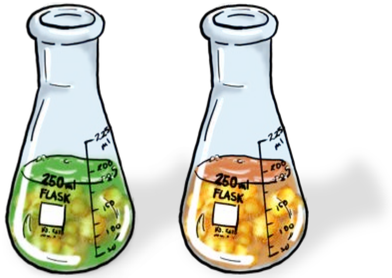
Reconstructing the biosynthesis of a light activated plant toxin from a wheat pathogen *Paarastagonospora nodorum* using yeast-fungal artificial chromosome (YFAC) system



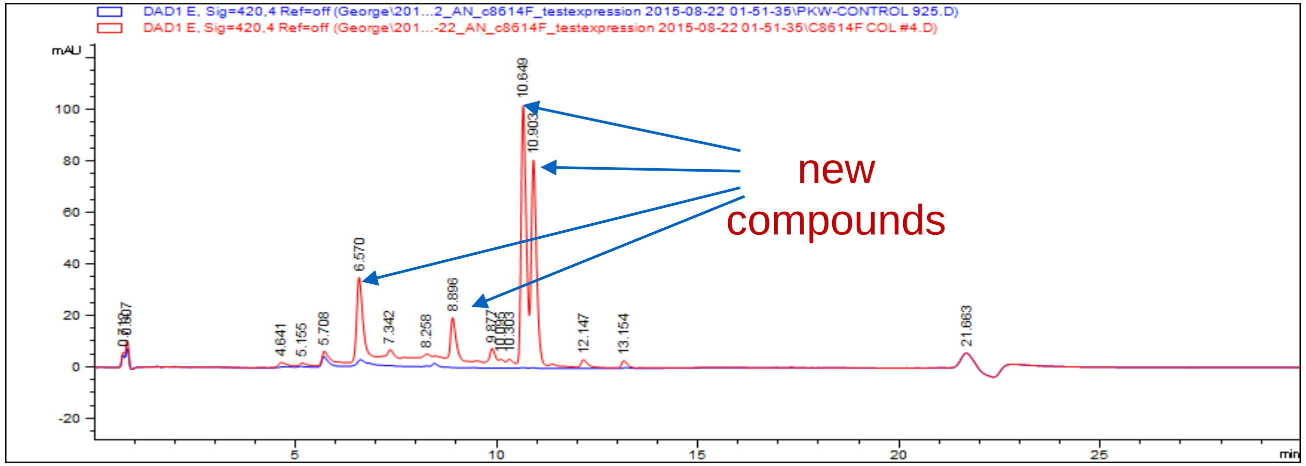
Reconstruction of a nine-gene pathway on a fungal artificial chromosome

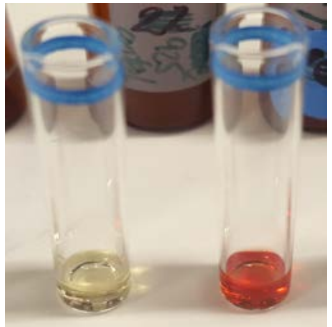


A. nidulans



Expression in *A. nidulans*





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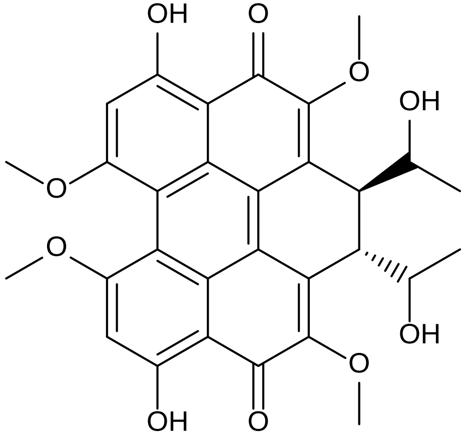
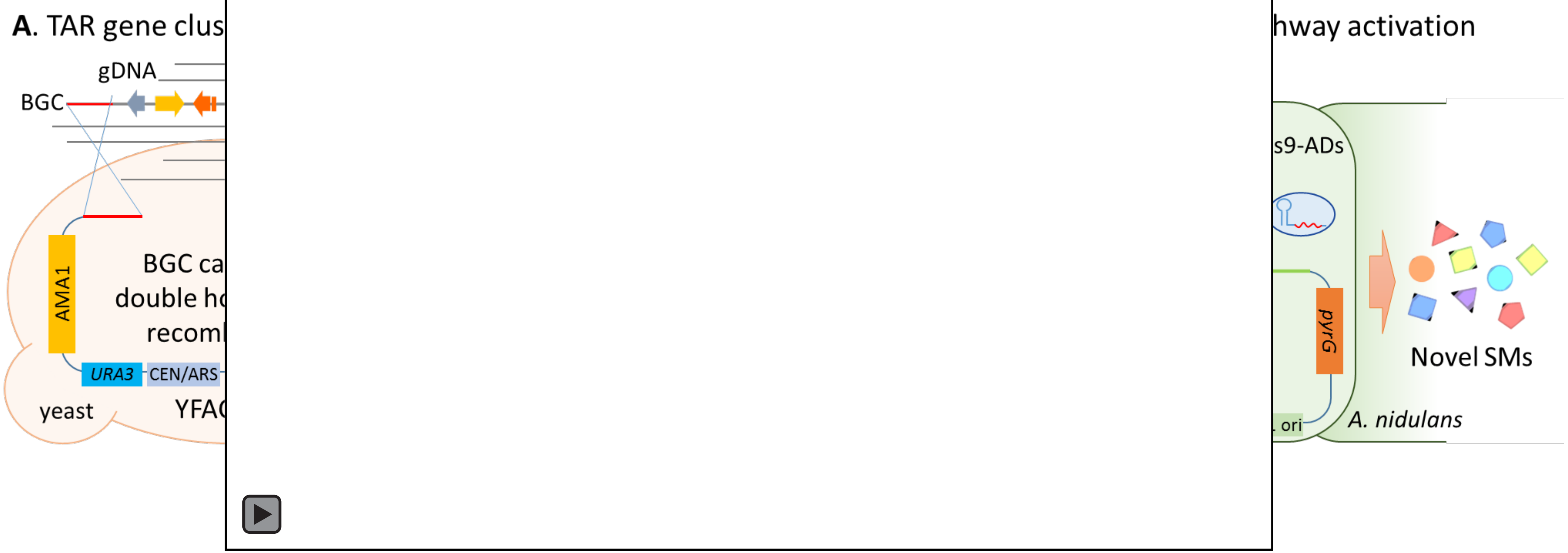


elsinochrome C

Chooi et al. Env. Microb. 2015
Hu et al. Chem Sci. 2013

CRISPR activation of cryptic BGCs



- New technologies required to access the “biosynthetic dark matter”
- Opportunities for drug discovery. Unveil small molecules involved in pathogen-host interactions (crops and humans).

Research in Chooi lab
Chooilab.org

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Other applications of Microbial Synthetic Biology

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The broad areas:

- Biodegradation: e.g. xenobiotic/microplastic degradation
- Biosensing: e.g. sensing environmental pollutants or illicit drugs
- Therapeutics: e.g. engineered bacteriophage as antibacterial agent
- Biocontrol: e.g. engineered endophytic microbe protecting plants against pathogens