Aalto University
School of Chemical
Technology

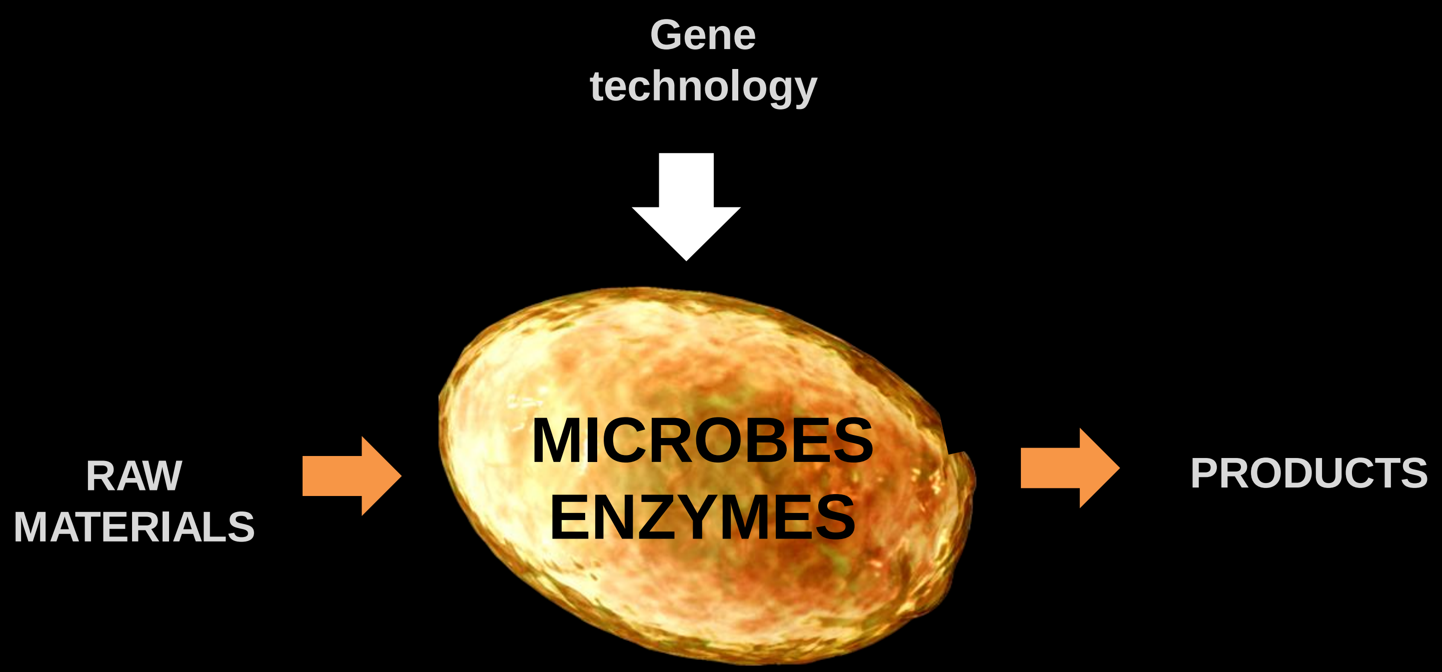
Synthetic biology (Course CHEM-E8125), spring 2022

Synbio biotech examples

Prof. Merja Penttilä

A key synbio application potential is in CIRCULAR BIOECONOMY – towards a bio-based society

Industrial biotechnology



Efficient production of only the wanted product in closed bioreactors

A single unit operation

Ambient temperatures and pressures, no toxic catalysts

Atmos-
pheric CO_2

H_2

Industrial
flue gases

Synthesis
gas

Methane,
methanol

Industrial
sidestream
sugars

Ligno-
cellulose

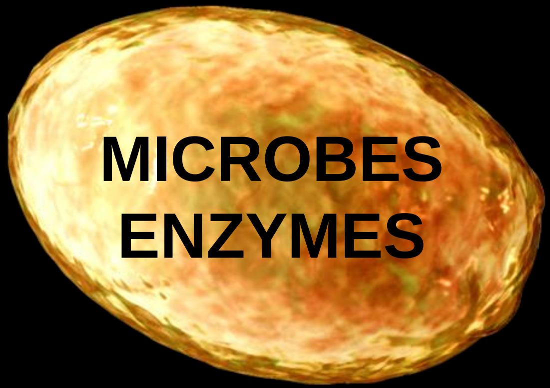
Food in-
dustry
sidestreams

Packaging
and textile
waste

Versatile use of non-fossil raw materials and unique possibilities to broaden the product range

Natural synthesis power
Evolution power
Reaction specificity

RAW
MATERIALS



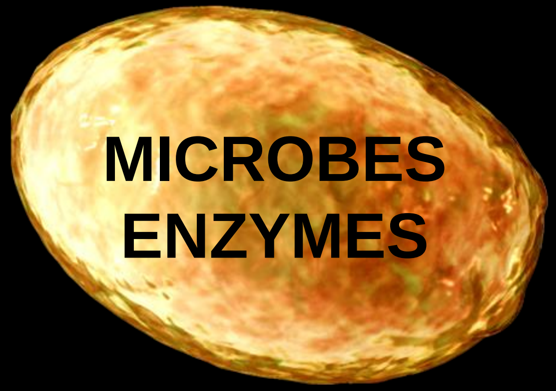
PRODUCTS

- Atmospheric CO_2 + H_2
- Industrial flue gases
- Synthesis gas
- Methane, methanol
- Industrial sidestream sugars
- Lignocellulose
- Food industry sidestreams
- Packaging and textile waste

Versatile use of non-fossil raw materials and unique possibilities to broaden the product range

Natural synthesis power
Evolution power
Reaction specificity

RAW MATERIALS →



→ PRODUCTS

Engineering biology using DNA as a code - Synthetic DNA

Faster process development

SYNTHETIC BIOLOGY

- Computer-aided design of production strains
- Rapid construction and testing of strains using automation and robotics
- New reactions, new products, more efficient processes

Atmos-pheric CO_2

H_2

Industrial flue gases

Synthesis gas

Methane, methanol

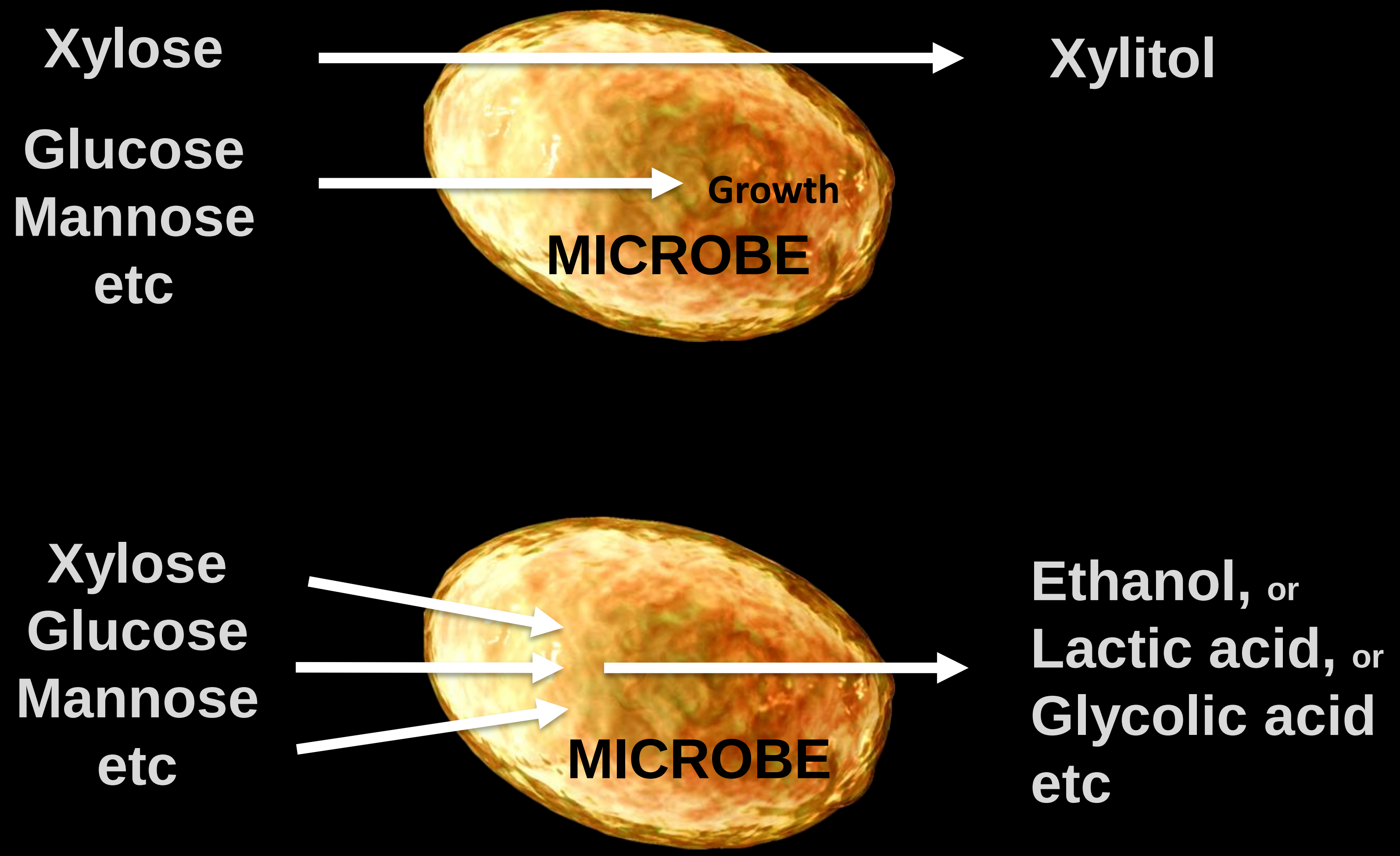
Industrial sidestream sugars

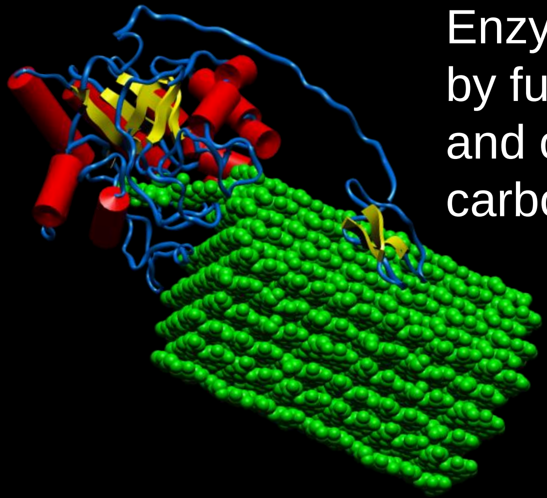
Ligno-cellulose

Food in-dustry sidestreams

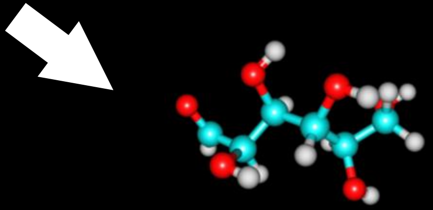
Packaging and textile waste

Heterogenous raw material → **Single (pure) product**



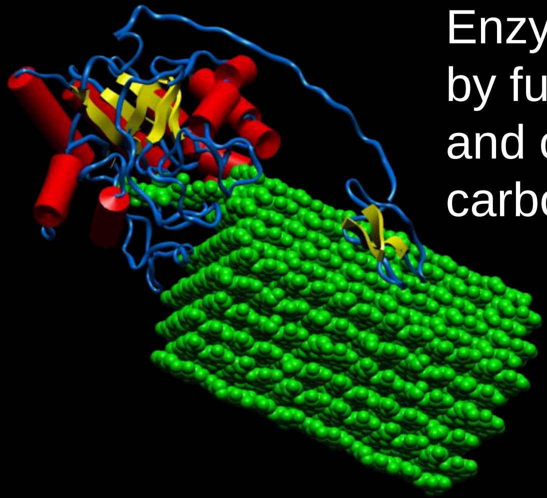


Enzymes produced
by fungi digest cellulose
and other plant biomass
carbohydrates to sugar



Sugar

Merja Penttilä



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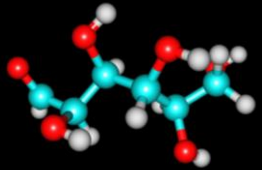

Sugar

Baker’s yeast
- A cell factory

Ethanol

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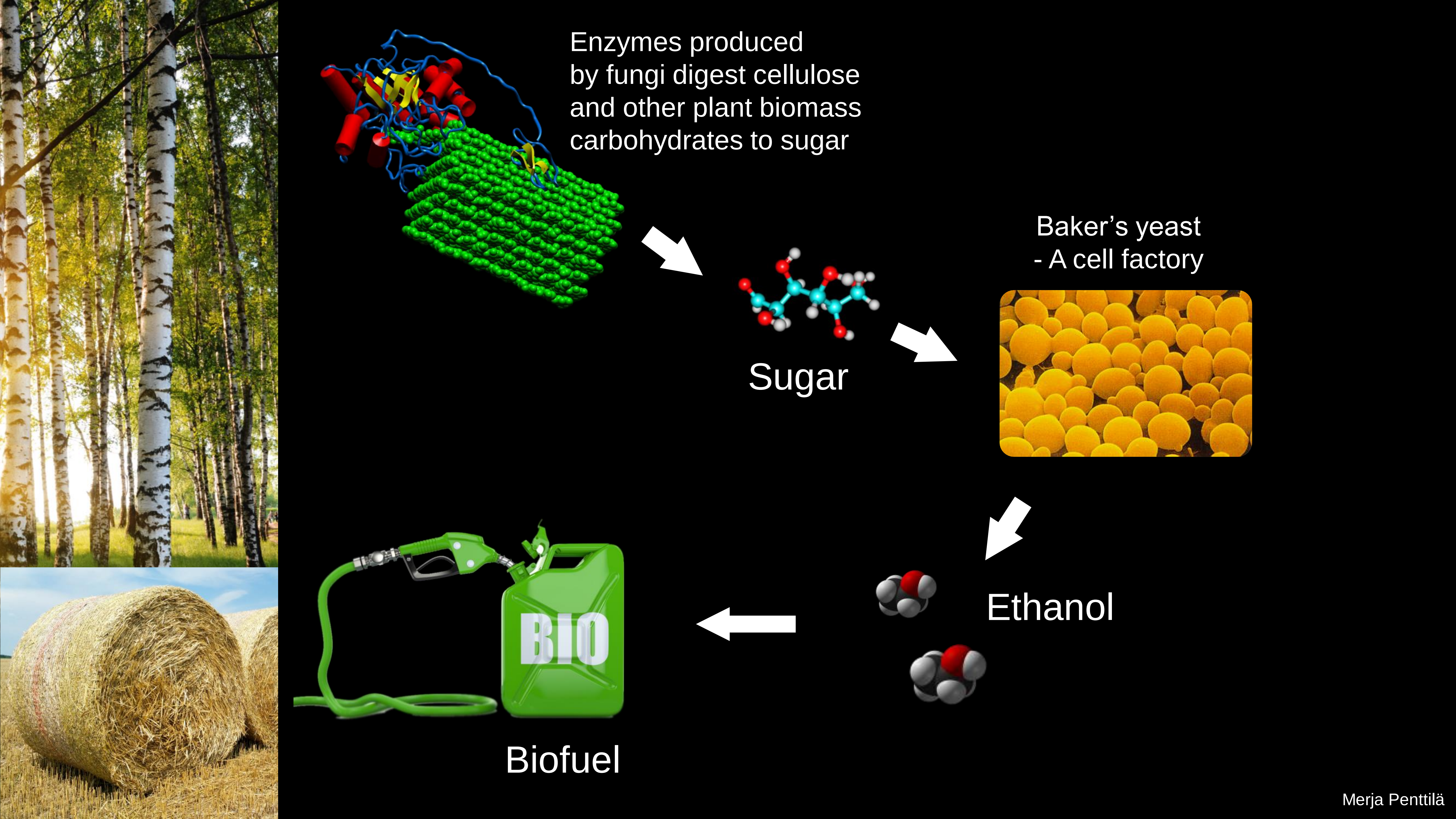
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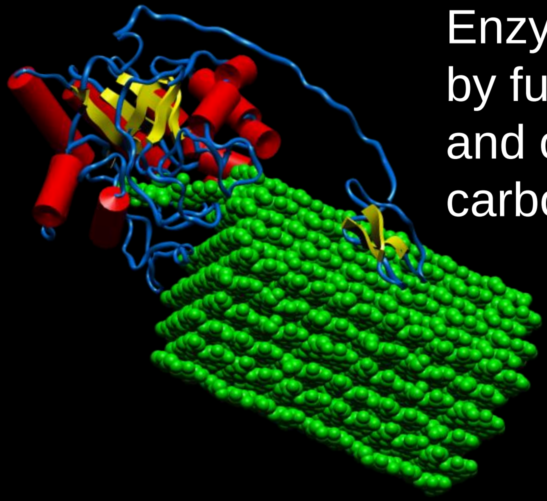
Sugar
Baker's yeast
- A cell factory
Ethanol
BIO
Biofuel
Merja Penttilä



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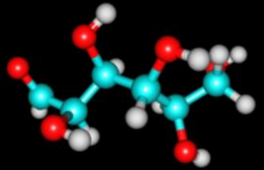

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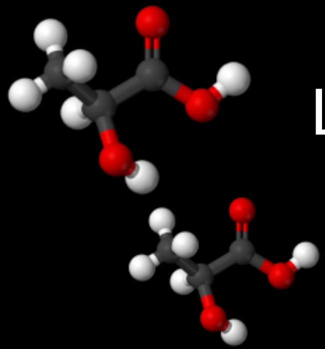

Lactic acid

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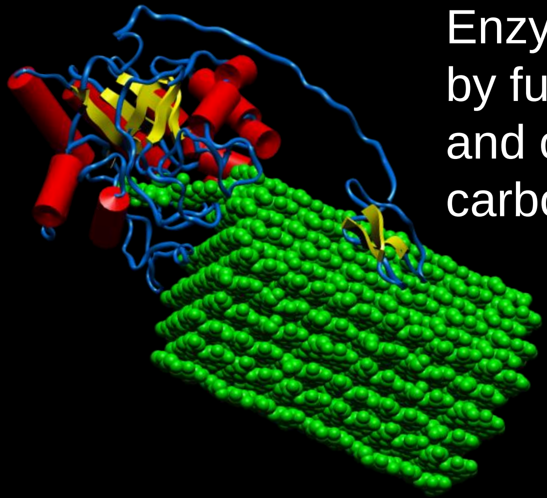

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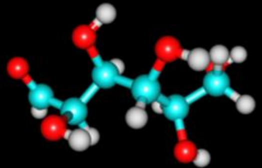

Sugar

Baker's yeast
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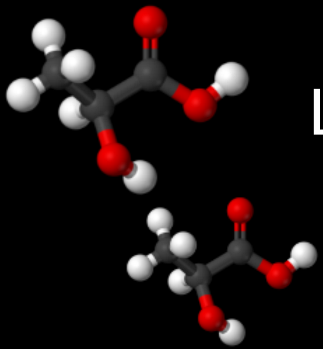

Lactic acid

Bioplastic PLA

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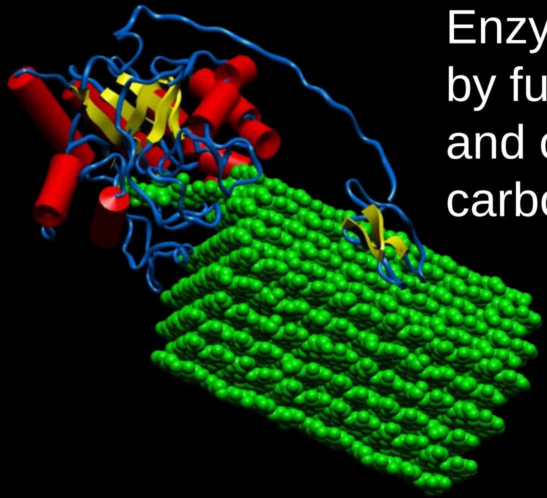

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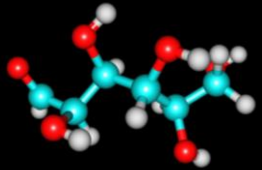


Sugar

Baker’s yeast
- A cell factory

Xylitol
Vanillin
Terpenes

Insulin
Artemisin
Opioids
etc

Food &
Feed Protein

Silk
PHB
Hyaluronan
Alginate
Isoprene
etc

Lactic acid
Succinic acid
Itaconic acid
Acrylic acid
Muconic acid
etc

Ethanol
Butanol
Biodiesel
Jet fuels
etc

Merja Penttilä

VTT has experience in ENZYMATIC HYDROLYSIS of many different biomasses

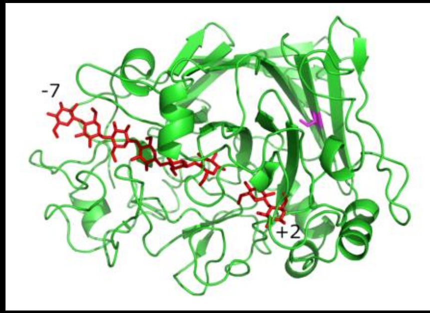



Steam exploded or hydrothermally treated, acidic pretreatment

- Softwood
- Hardwood
- Wheat straw
- Wheat bran
- Sugar cane bagasse
- Grass silage

From alkaline pretreatment

- Softwood
- Hardwood
- Wheat straw
- Wheat bran
- Sugar cane bagasse
- Waste wood/recycled wood
- Green biomasses, grass silage



Other

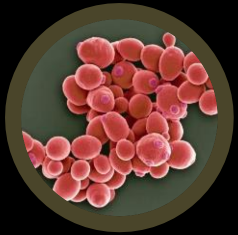
- Waste fiber
- Spent grain
- Municipal waste (sorted, mixed)
- Sludges from paper mills
- Solid recovered fuel (SRF)



Production host engineering at VTT

Microbes & products

VTT



Bacteria

Escherichia coli
Clostridium ljungdahlii
Synechocystis (cyanobacteria)
Rhodococcus opacus

Yeasts

Saccharomyces cerevisiae
Kluyveromyces lactis
Kluyveromyces marxianus
Yarrowia lipolytica
Scheffersomyces stipitis
Pichia kudriavzevii
Candida sonorensis
Pichia membranefaciens
Candida methanosorbosa
Cryptococcus curvatus

Filamentous fungi

Trichoderma reesei
Aspergillus niger
Aspergillus oryzae
Mucor circinelloides

Organism selection

Organism development

Process development

Scale-up and piloting

Chemicals

Ethanol
Butanol
Triacylglycerids & derivatives

Lactic acid
Glycolic acid
Xylonic acid
Arabinoic acid
Galactaric acid
Glucaric acid

Xylitol
Pigments
Isoprene
γ-terpinene
Ent-pimaradiene
Alcaloids
Styrene

Proteins

Industrial enzymes
Material proteins
Antibodies
Food proteins
Feed proteins

KORVAA headphones, made from microbially produced materials

Reach:
>350 mill.
readers

MICROBIAL BIOPLASTIC PLA
The 3D printed biodegradable plastic PLA is made from lactic acid that is produced by the yeast *Saccharomyces cerevisiae.*

ENZYMATICALLY PRODUCED CELLULOSE
The microbial and enzymatically produced cellulose is naturally lignin free.

COMPOSITE OF FUNGAL MYCELIUM AND BACTERIAL CELLULOSE
This material consists of mycelium, the cells of the fungus *Trichoderma reesei*, which is grown in a bioreactor and mixed with microbially produced cellulose. The dried composite is hard and light.

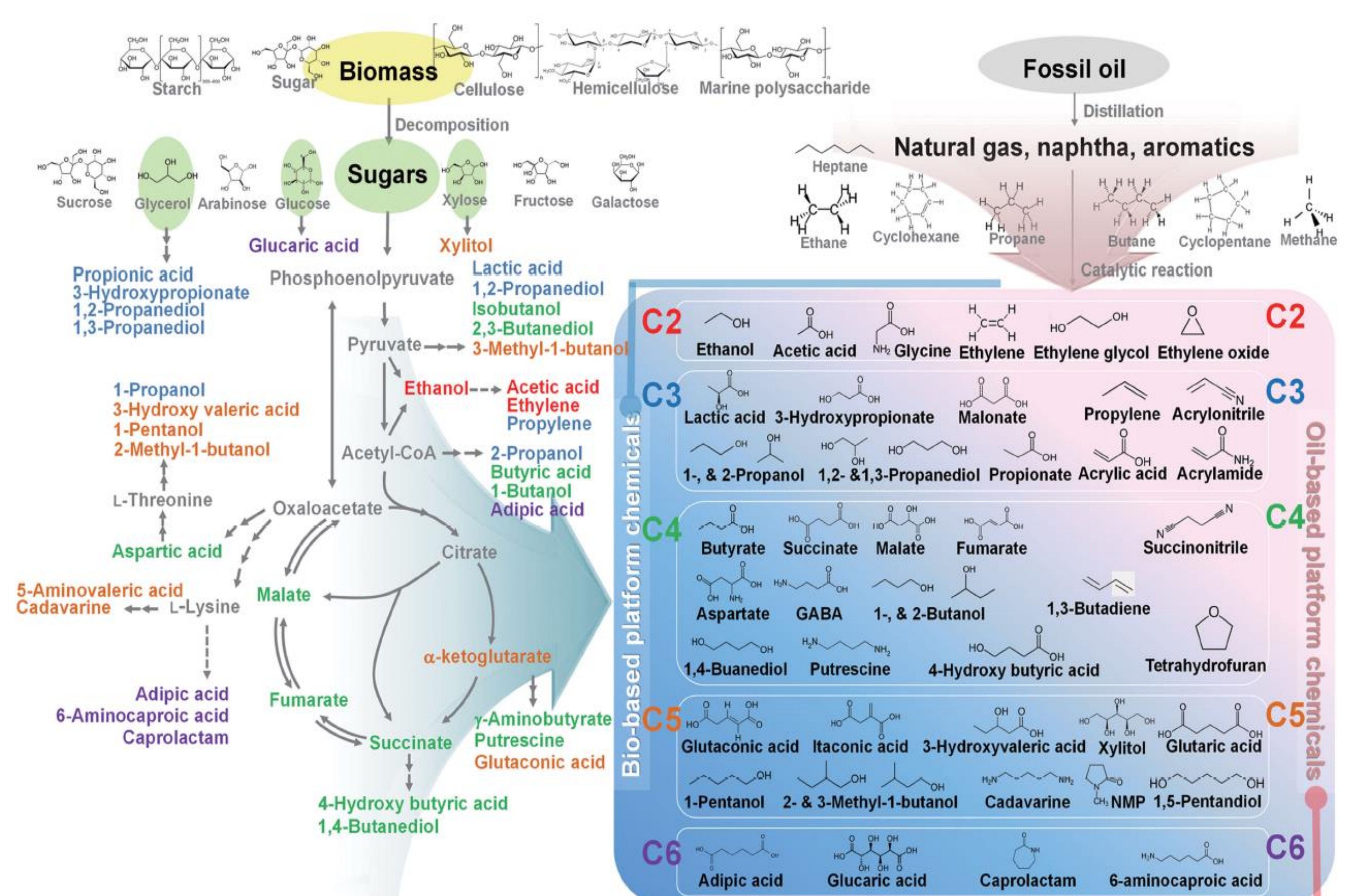
FUNGAL MYCELIUM
The growth of the fungus *Phanerochaete chrysosporium* creates a leather-like material.

BIOSYNTHETIC SPIDER SILK
Sustainable microbially produced silk protein.

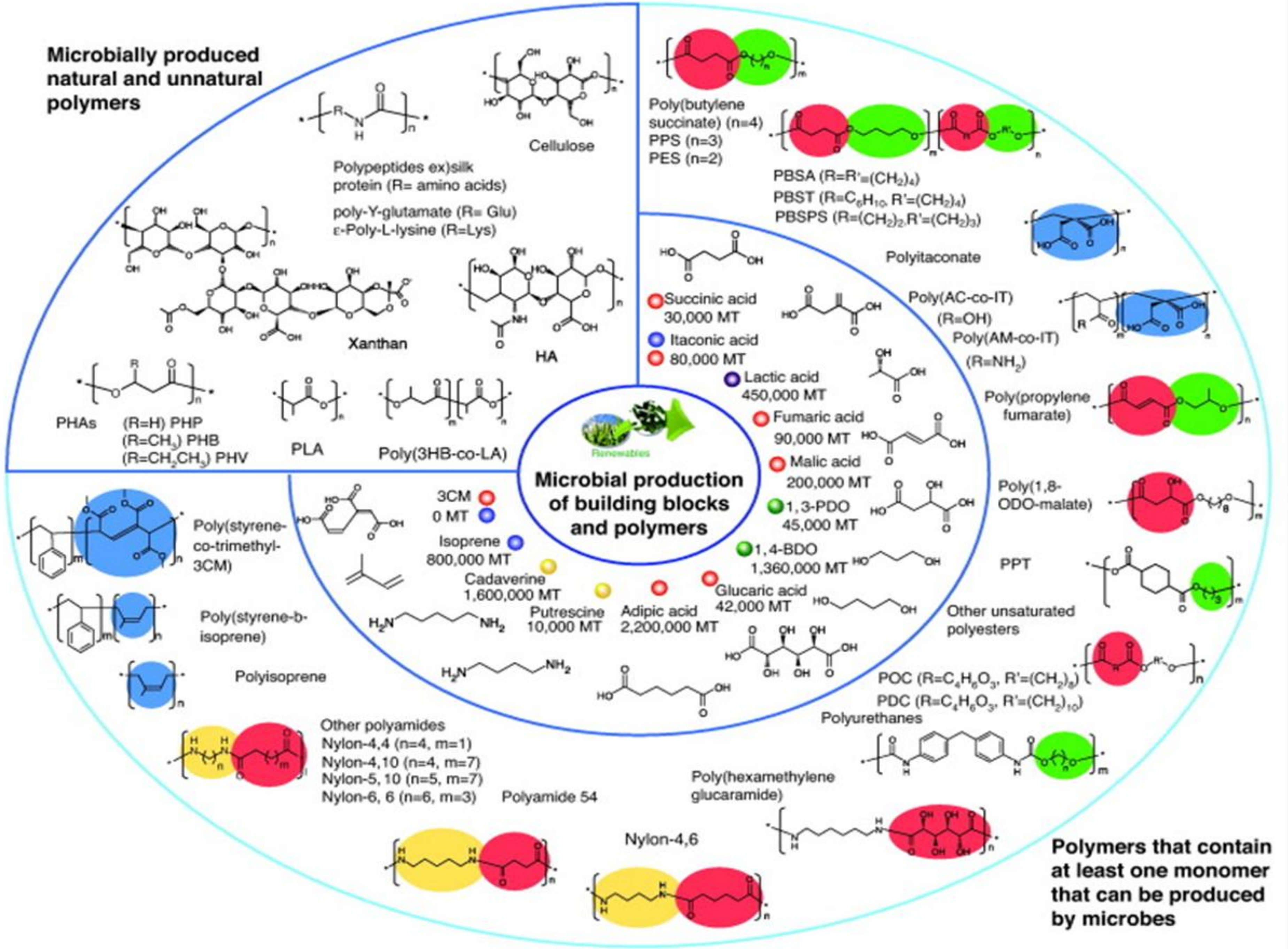
PROTEIN FOAM AND PLANT CELLULOSE
A foaming protein, hydrophobin, is produced by the fungus *Trichoderma reesei.* It is nature’s strongest “bubble-maker” which aids fungal cells to grow into air from a moist soil.

VTT and Aalto University researches, design company Aivan, Nina Pulkkis

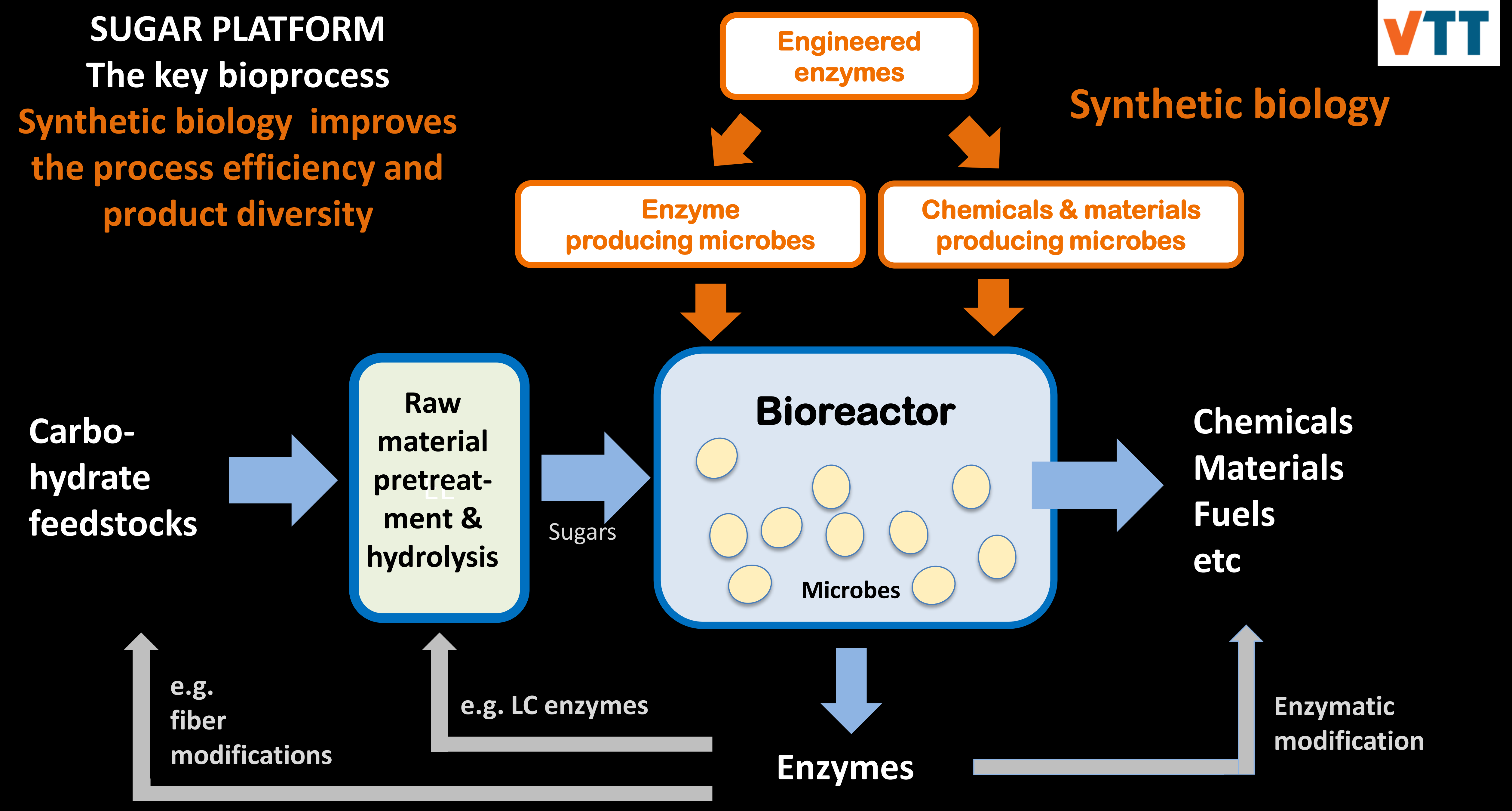
Cell chemistry can be harnessed for production of platform chemicals that can replace oil-based compounds – and for many new complex molecules difficult to synthetize chemically



Biotechnology and Bioengineering, 109: 10, 2437-2459

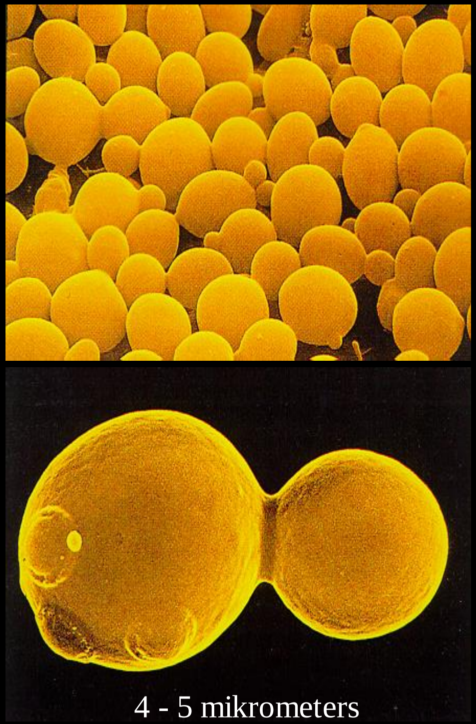


Lee et al. 2011. Curr. Opinion in Biotechn. 22: 758-767


SUGAR PLATFORM
The key bioprocess
Synthetic biology improves the process efficiency and product diversity
Engineered enzymes
Synthetic biology
Enzyme producing microbes
Chemicals & materials producing microbes
Carbo-hydrate feedstocks
Raw material pretreat-ment & hydrolysis
Sugars
Bioreactor
Microbes
Chemicals
Materials
Fuels
etc
e.g. fiber modifications
e.g. LC enzymes
Enzymes
Enzymatic modification

Bioreactors for ethanol and lactic acid can be more than 1000 m^3 in size

Saccharomyces cerevisiae baker’s yeast is a robust process organism



Biotechnology is suited also for very large scale

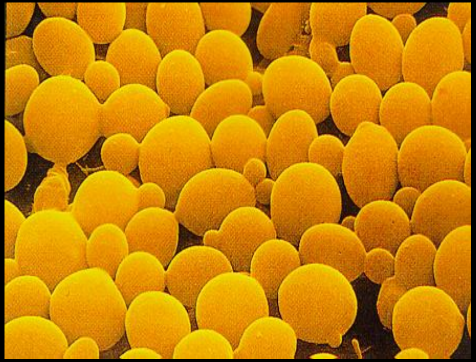
Industrial production is established for various products

10^8 small cell factories fit in one liter

4 - 5 mikrometers

Bioreactors for ethanol and lactic acid can be more than 1000 m^3 in size

Saccharomyces cerevisiae* baker's yeast is a robust process organism



4 - 5 mikrometers

Synthetic chassis vs. use of synbio tools in "natural" hosts?

- Process robustness is important – traditionally difficult to engineer
  - Low pH, T, raw material or product tolerance, pressure, oxygen variation, growth rate
- Natural organisms, even non-conventional ones may provide beneficial features and natural biodiversity (e.g. lipid production, acid tolerance, difficult to engineer pathways)
- Host is critical for achieving high production yields, rates and titres

A difficult question: Synthetic chassis or a favoured host, or a new natural one?

Does the Yeast 2.0 make a difference?

Needs in industrial production

- Replacement of fossil resources with renewable ones (plant biomass, photosynthesis) in production of chemicals, materials and fuels
  - Engineering of substrate utilisation pathways & photosynthetic organisms

- Equivalent products to petrochemicals by microbial fermentation
  - Metabolic engineering, heterologous pathway expression

- Novel, better products through biotechnology (materials, drugs etc)
  - Combinatorial pathways, novel enzyme catalysts, strong novel biodegradable materials

- Efficiency of production (titer, rate, yield)
  - Cut-off side reactions, increase flux, engineer cellular energetics & redox; predictive cellular modelling,.. thermodynamics, chemical biology etc

- Improve process robustness
  - Mutagenesis, product efflux, stress biomarkers, ...

Synthetic biology targets

- Host strains that have predictable behaviour and are easy to manipulate (“minimalistic” chassis)
- New product pathways (balanced redox and energy, minimal carbon loss = carbon economy)
- Controllable and efficient expression (expression modules and circuits with synthetic designed elements)
- Novel chemistry (protein engineering, combinatorial biochemistry)
- Control of process robustness (intracellular sensors and control loops)

Design-Build-Test-Learn (DBTL) cycle of synthetic biology

Automation of strain engineering

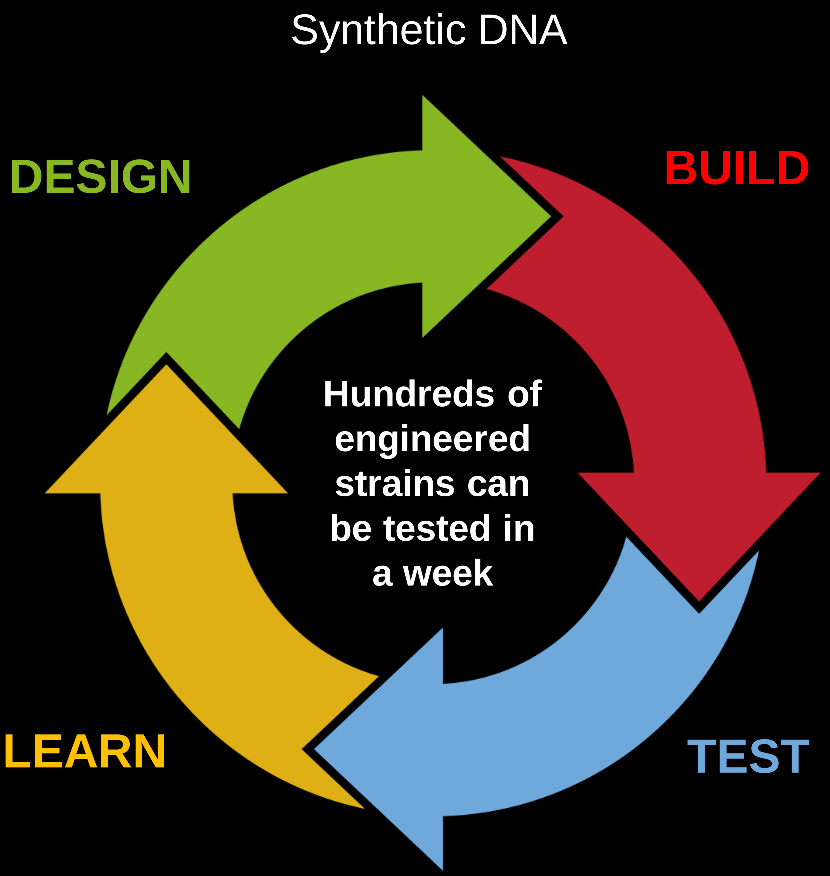
(ultimately towards a robot scientist)

The Design-Build-Test-Learn cycle of Synthetic Biology

Engineering biology using DNA as a code

Design
Cells and their parts are designed using computational tools

Analysis and decisions
Machine learning algorithms can help the researcher to analyse and understand measured data.



Building of production strains
Synthetic DNA is delivered to the cells using genome editing tools such as CRISPR.

Cultivation and measurement
Robots are cultivating the strains and carry out measurements. The results are automatically stored in databases.

Computation

Data is the fuel -
the higher the quality of data, the more we learn and the better we can predict

Automation

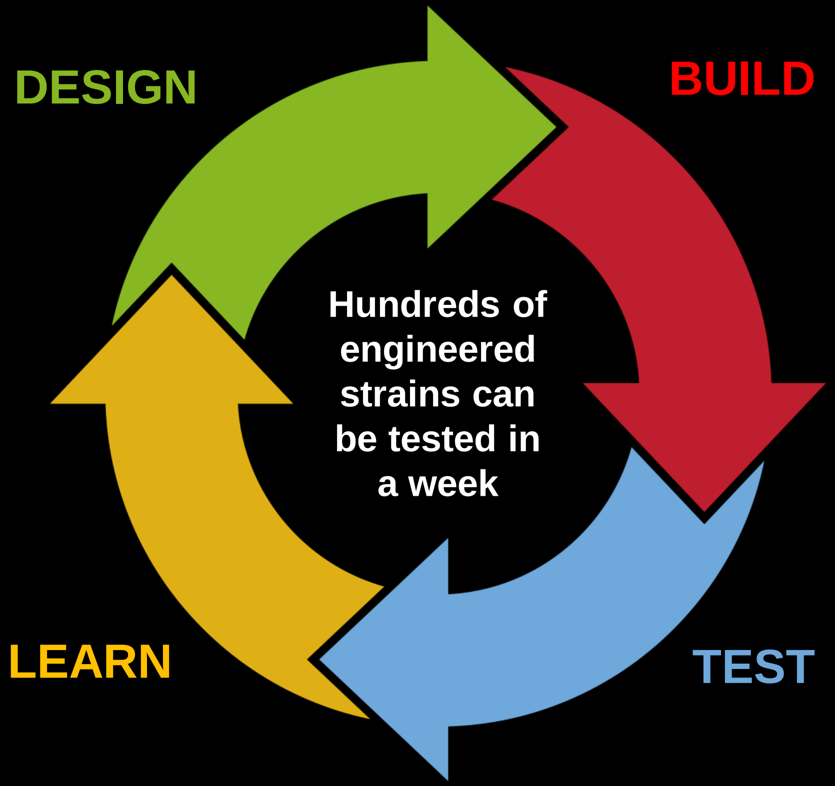
The Design-Build-Test-Learn cycle of Synthetic Biology

Engineering biology using DNA as a code

Design
- Mining for best genes from databases
- Design of cell biochemistry for high product yields
- Novel reactions

Analysis and decisions
- Mastering cell complexity using AI
- Prediction of new engineering targets

Synthetic DNA



Building of production strains
- CRISPR
- Designed control of growth and production
- Automated cell engineering

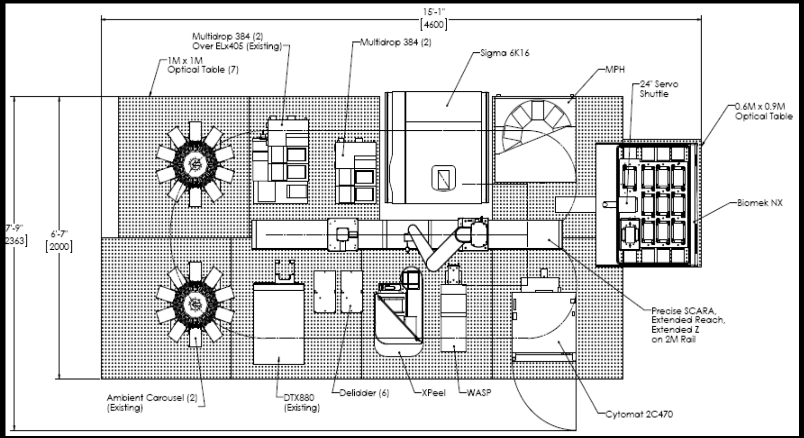
Cultivation and measurement
- High throughput screening robotics
- Fully automated, parallel small-scale bioreactor cultures
- On-line analytics

Computation

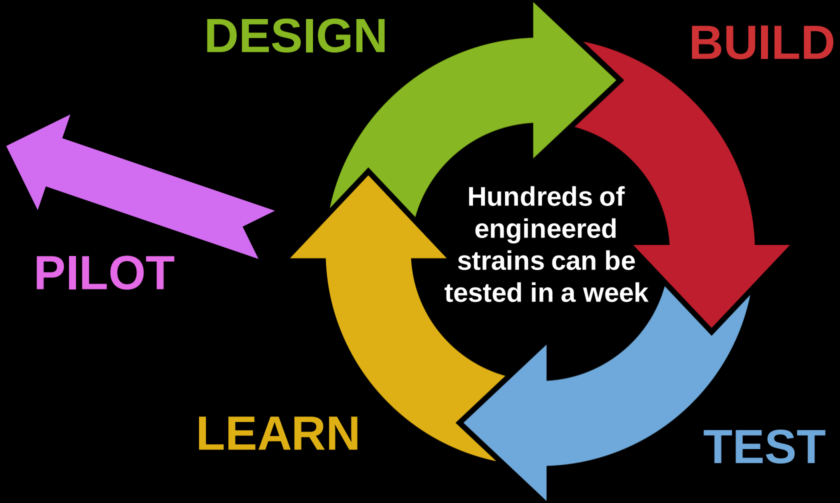
Data is the fuel -
the higher the quality of data, the more we learn and the better we can predict

Automation

Aalto-VTT national Bioeconomy infrastructure: From synthetic biology to piloting



A versatile computing platform for design, prediction and analysis



A robotic platform for efficient DNA assembly, transformation and strain screening

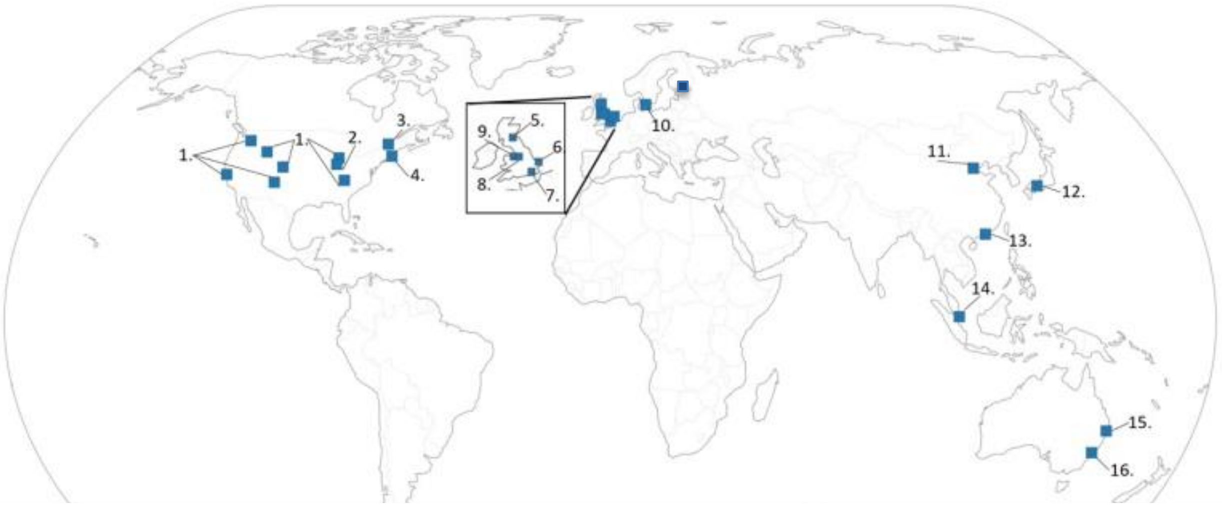
Controlled parallel bioreactor systems with automated sampling and analytics

International Consortia

EBRC

Engineering Biology Research Consortium (USA)

Global Biofoundries Alliance



IBISBA

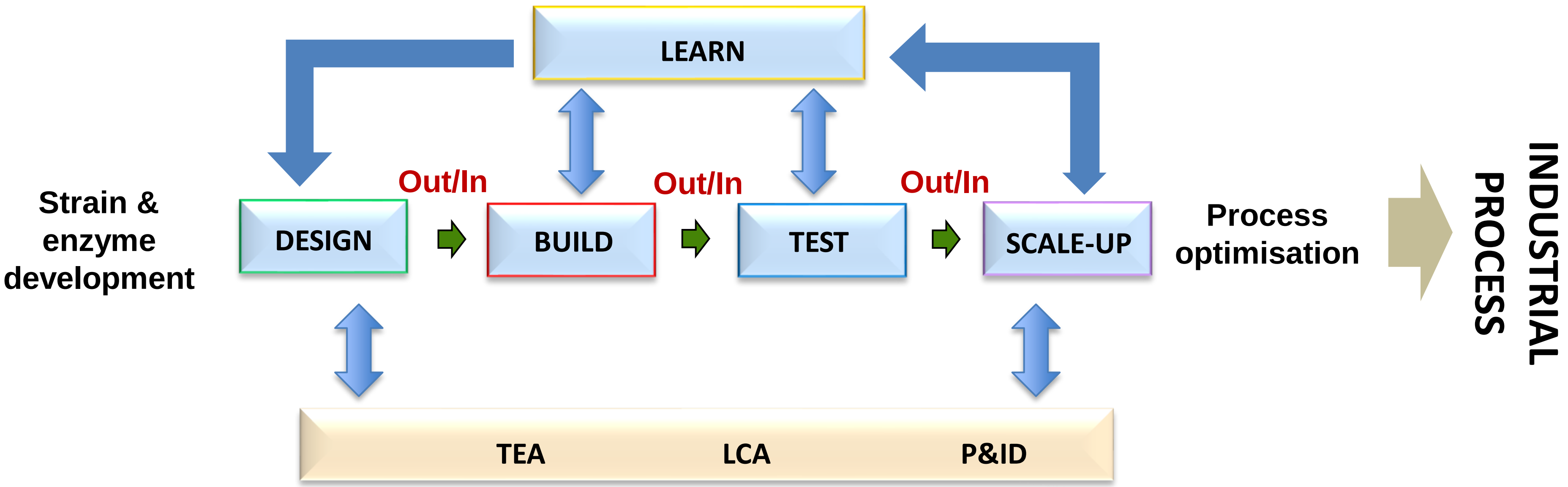
ESFRI Strategy Report on Research Infrastructures ROADMAP 2018

EU-IBISBA
Industrial Biotechnology Innovation and Synthetic biology Accelerator

EU project IBISBA

Aim to accelerate biotechnology development through excellence in capabilities and infrastructure

– from biocatalyst design to bioprocess



TEA, technoeconomic analysis
LCA, life cycle analysis
P&ID, piping and instrumentation diagram

From distributed capabilities
to harmonised seamless services

- **Dissection of tasks** that are needed to carry out projects – computational and wet lab

- For creation of a **hierarchical structures of modular tasks** that can be combined to make **seamless workflows** (for automation) and for tracing back experiments
- For **harmonizing the Protocols** so that highest quality of results are obtained similarly in different labs. The Input to the next phase is verified with go/no go criteria (the devil is in the details!)
- **Experimental and computational verification** of key steps and parameters (that are good examples for most biotech cases)

- Making biology engineerable

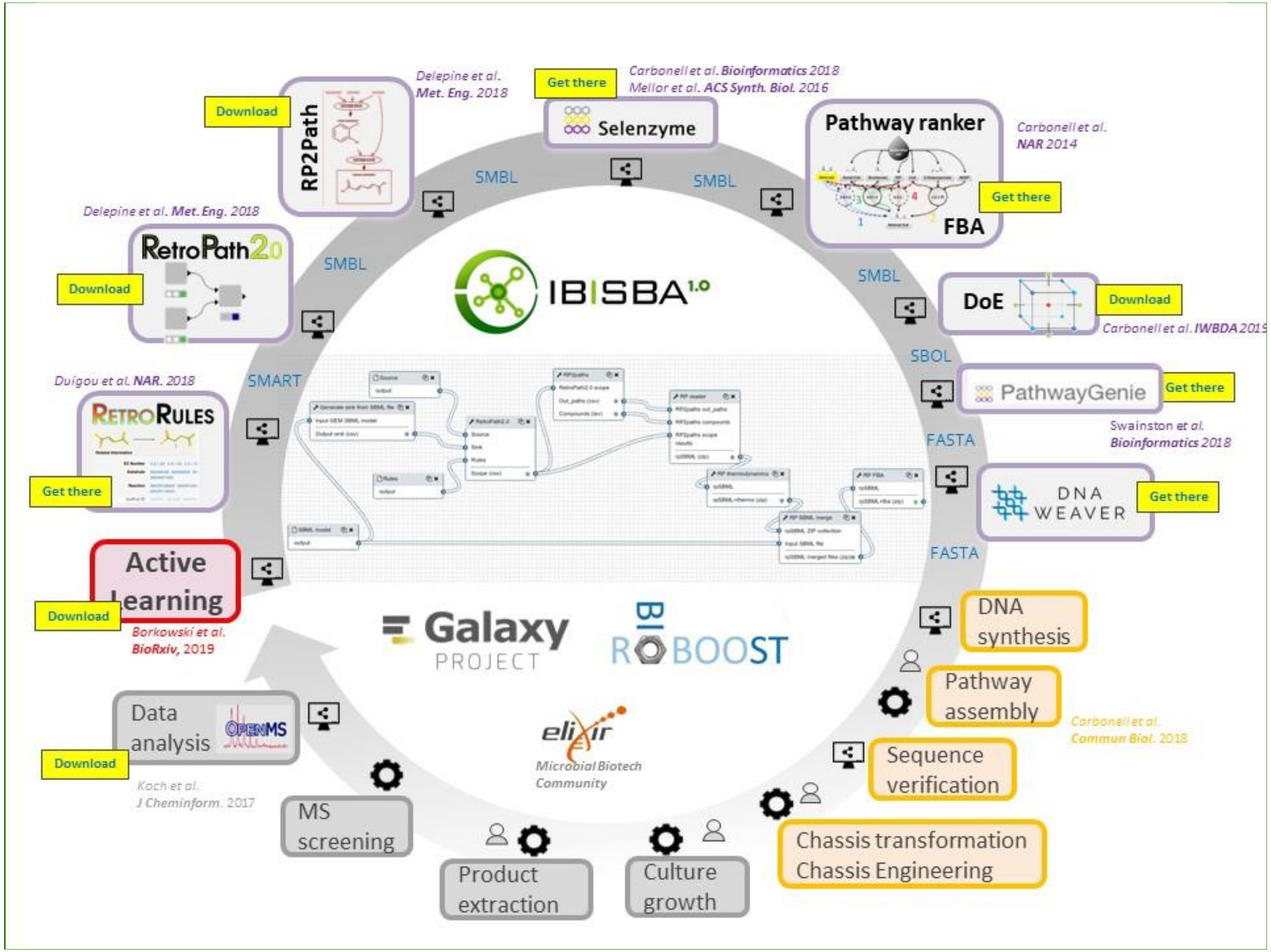
IBISBA
Workflow steps with protocols

TasCu

- Design
  - Execution of Design
    - Design production strain
      - Information search
      - Computational metabolic design
        - Computational product pathway design
          - Enumerate pathway options (e.g. Retropath)
          - Score pathways without chassis
        - Chassis embedment
          - Receive input from product pathway design
          - Map metabolites between pathway and chassis
          - Add production pathway to chassis SBML in silico
          - Fill metabolic gaps
          - Screen potential substrates in silico
          - Screen growth conditions
          - Growth-product coupling (e.g. OptKnock, RobustKnock, Minimal Cut Sets)
          - Calculate expected yields
          - Estimate productivities
        - Evaluate and choose pathways
      - Genetic design for chassis
    - Select and/or design enzyme
    - Design DNA constructs for expression host
    - Design growth medium and cultivation conditions
  - Criteria for successful outcome of #Design
- Build
- Test
- Learn
- Upscale

Protocols linked to tasks

IBISBA Workflow platform

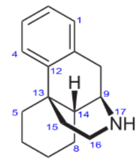


Synbio examples for biotechnology

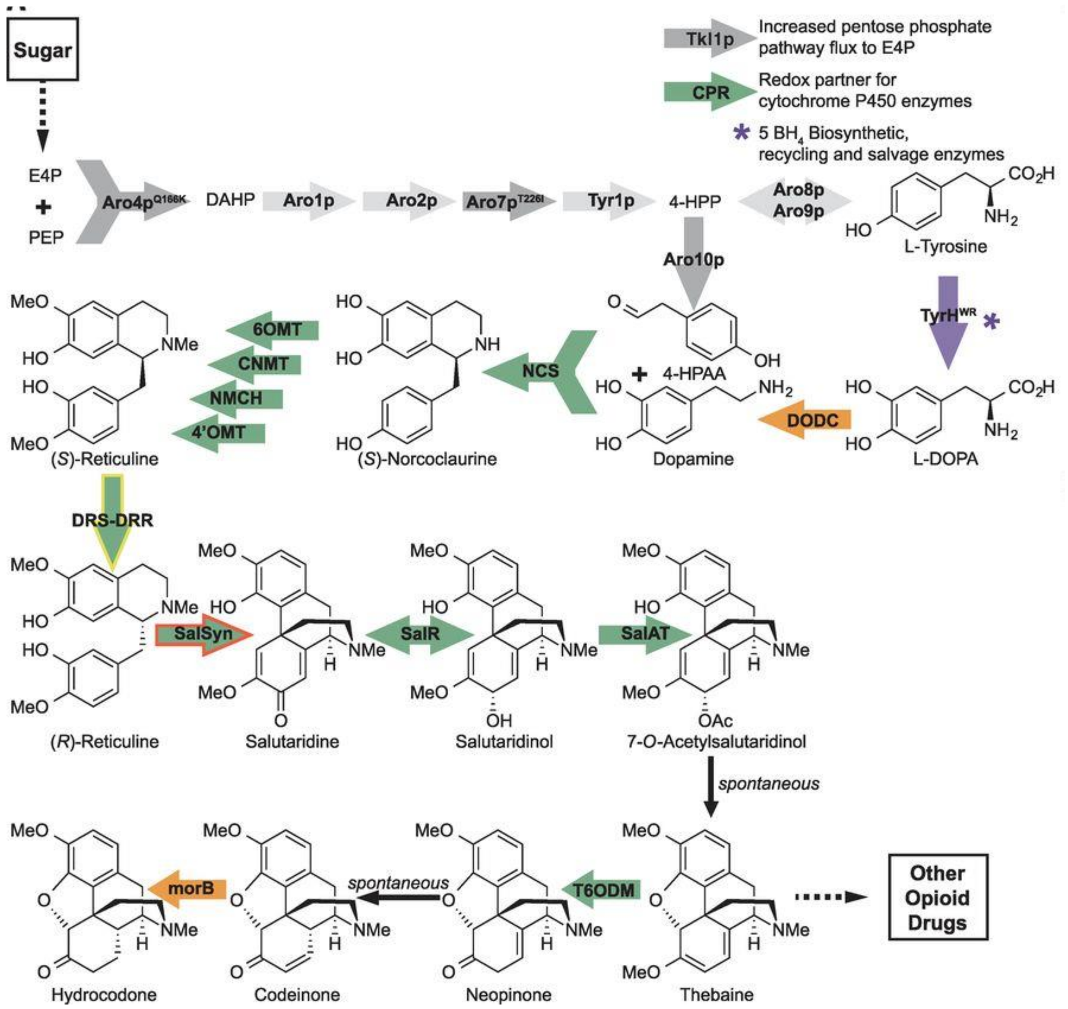
Synthetic pathway and strain optimization for opioid synthesis in yeast

Stephanie Galanie et al. Science 2015;349:1095-1100

- Overexpression of two native yeast enzymes
- Deletion of one native yeast gene
- Expression of 21 heterologous enzymes from plants, mammals, bacteria, and yeast (color codes)
- P450 enzyme (SalSyn) engineering to obtain a fusion protein for correct glycosylation and activity



MORPHINAN



Biosynthetic scheme for production of thebaine and hydrocodone from sugar. Thebaine is a starting material for many opioid drugs through biosynthetic and semisynthetic routes. Block arrows indicate enzyme-catalyzed steps. **Light gray arrows**, unmodified yeast enzymes; **dark gray arrows**, overexpressed and modified yeast enzymes; **purple arrows**, mammalian (*Rattus norvegicus*) enzymes; **orange arrows**, bacterial (*Pseudomonas putida*) enzymes; **green arrows**, plant (*Papaver somniferum*, *P. bracteatum*, *Coptis japonica*, *Eschscholzia californica*) enzymes. Yellow outline highlights DRS-DRR; **red outline** highlights engineered SalSyn. E4P, erythrose 4-phosphate; PEP, phosphoenolpyruvate; DAHP, 3-deoxy-d-*arabino*-2-heptulosonic acid 7-phosphate; 4-HPP, 4-hydroxyphenylpyruvate; 4-HPAA, 4-hydroxyphenylacetaldehyde; BH_4, 5,6,7,8-tetrahydrobiopterin; Tkl1p, transketolase; CPR, cytochrome P450 reductase; $Aro4p^{Q166K}$, DAHP synthase; Aro1p, pentafunctional *arom* enzyme; Aro2p, bifunctional chorismate synthase and flavin reductase; $Aro7p^{T226I}$, chorismate mutase; Tyr1p, prephenate dehydrogenase; Aro8p, aromatic aminotransferase I; Aro9p, aromatic aminotransferase II; Aro10p, phenylpyruvate decarboxylase; $TyrH^{WR}$, feedback inhibition–resistant tyrosine hydroxylase (mutations R37E, R38E, W166Y); DODC, l-DOPA decarboxylase; NCS, (*S*)-norcoclaurine synthase; 6OMT, norcoclaurine 6-*O*-methyltransferase; CNMT, coclaurine *N*-methyltransferase; NMCH, *N*-methylcoclaurine hydroxylase; 4′OMT, 3′-hydroxy-*N*-methylcoclaurine 4′-*O*-methyltransferase; DRS-DRR, 1,2-dehydroreticuline synthase-1,2-dehydroreticuline reductase; SalSyn, salutaridine synthase; SalR, salutaridine reductase; SalAT, salutaridinol 7-*O*-acetyltransferase; T6ODM, thebaine 6-*O*-demethylase; morB, morphinone reductase.

Cannabinoid synthesis in yeast

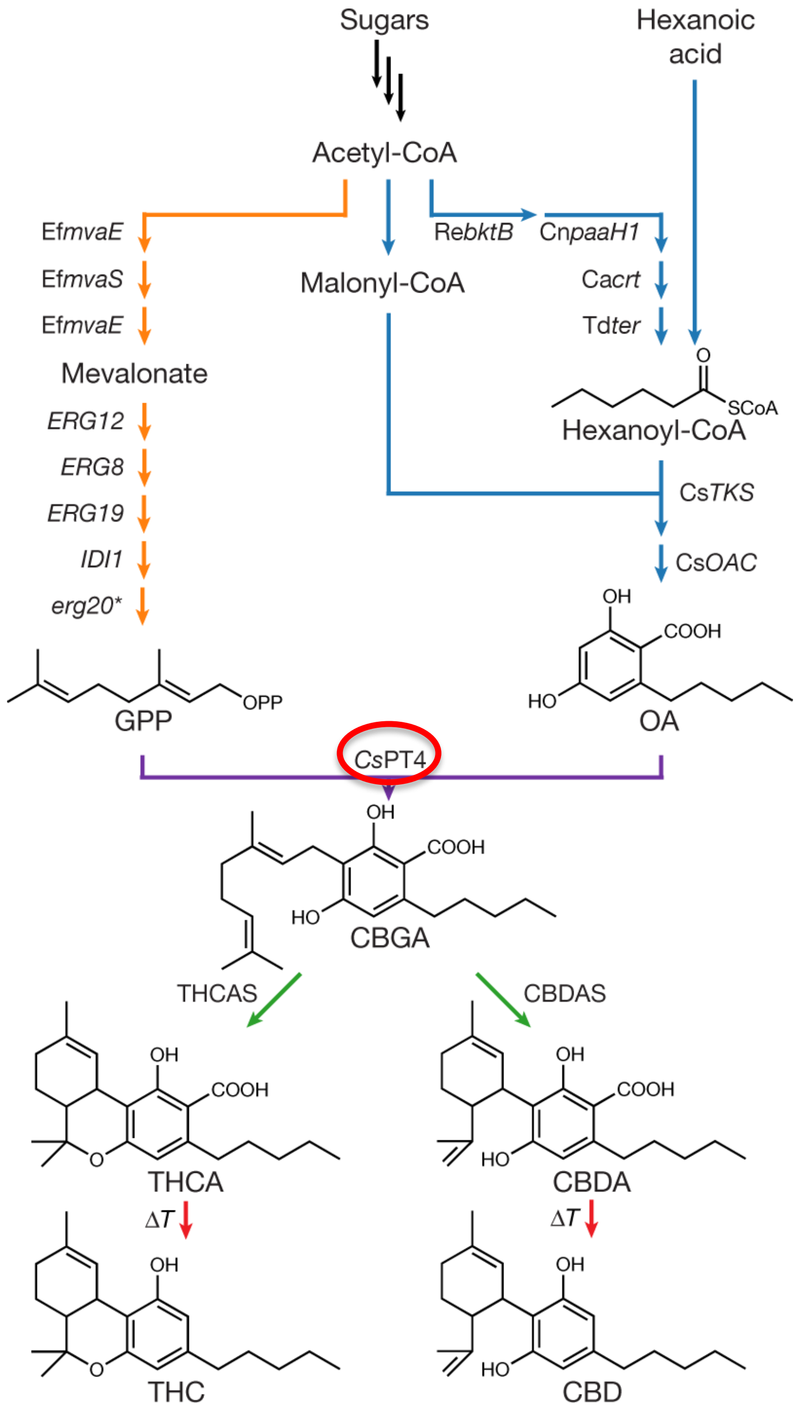
Nature 2019, vol 567:123

Complete biosynthesis of cannabinoids and their unnatural analogues in yeast

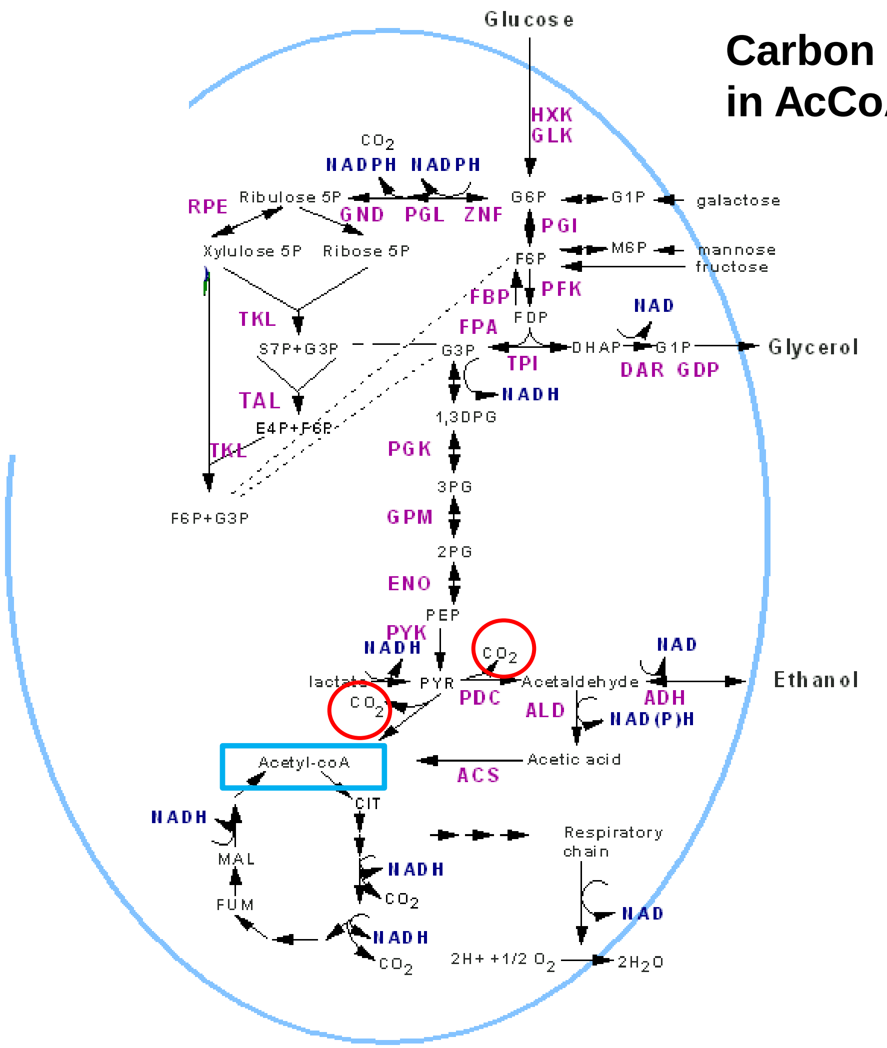
Xiaozhou Luo[1,15], Michael A. Reiter[1,2,15], Leo d'Espaux[3,12], Jeff Wong[3,12], Charles M. Denby[1,13], Anna Lechner[4,5,14], Yunfeng Zhang[1,6], Adrian T. Grzybowski[1], Simon Harth[3], Weiyin Lin[3], Hyunsu Lee[3,7], Changhua Yu[3,5], John Shin[3,4], Kai Deng[8,9], Veronica T. Benites[3], George Wang[3], Edward E. K. Baidoo[3], Yan Chen[3], Ishaan Dev[3,4], Christopher J. Petzold[3] & Jay D. Keasling[1,3,4,5,10,11*]

Synthetic pathway required for efficient precursor (hexanoyl-CoA) production

Introduced also a gene for a previously undiscovered enzyme with geranylpyrophosphate:olivetolate geranyltransferase activity (CsPT4)
(known natural producer gene gave no activity)

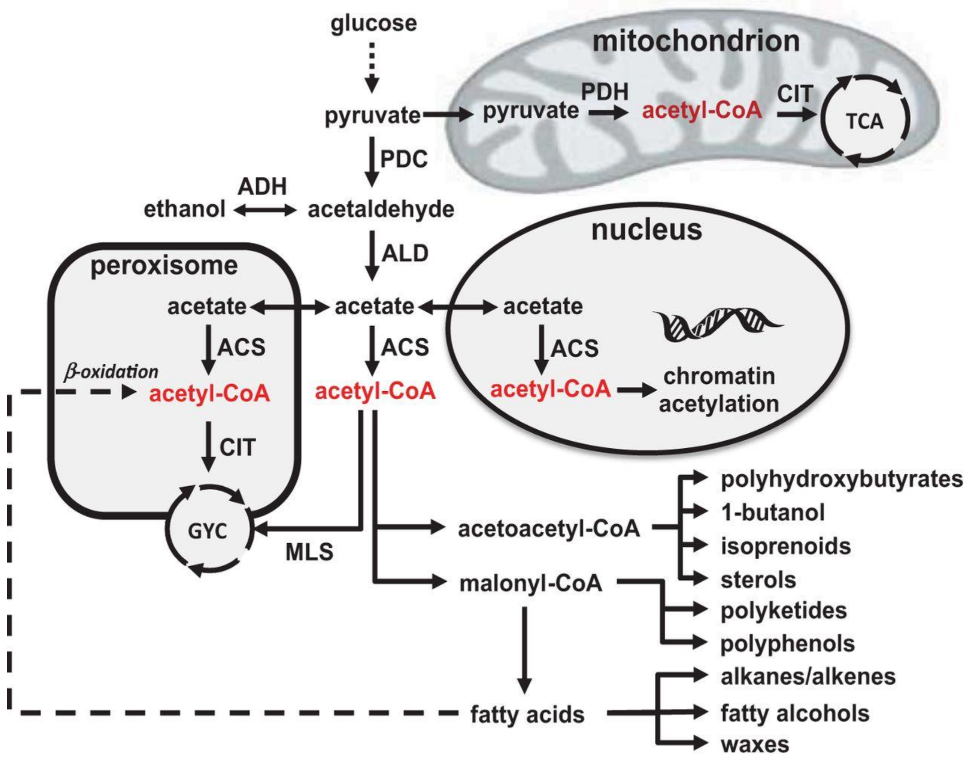


Synthetic non-oxidative glycolysis
– prevention of carbon loss in AcCoA formation (1)



Carbon is lost in AcCoA formation

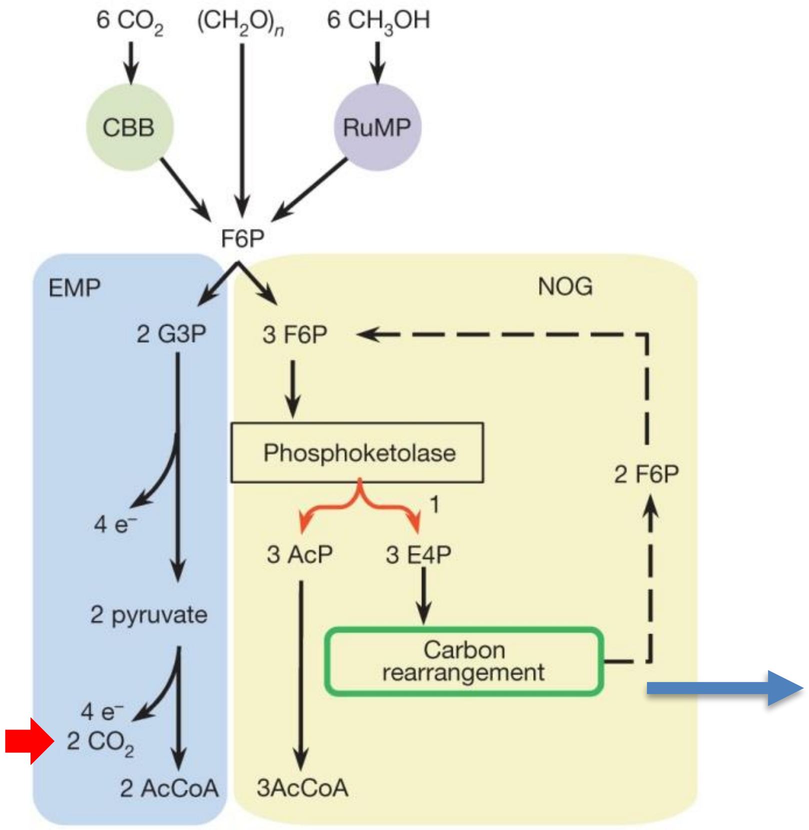
AcCoA is a key intermediate in product pathways

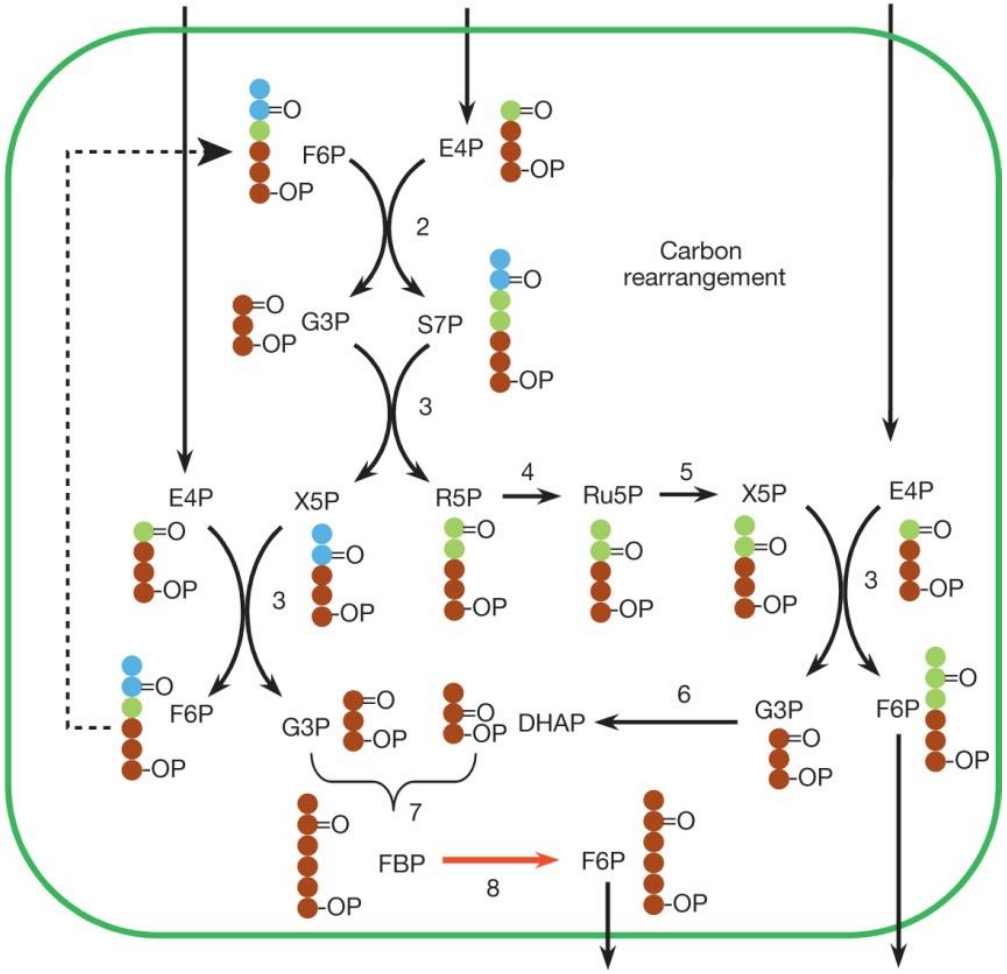


Bogorad et al. (2013). Synthetic non-oxidative glycolysis enables complete carbon conservation. Nature 502, 693-697.

Synthetic non-oxidative glycolysis
– prevention of carbon loss in AcCoA formation (2)

C1 or sugar as carbon sources





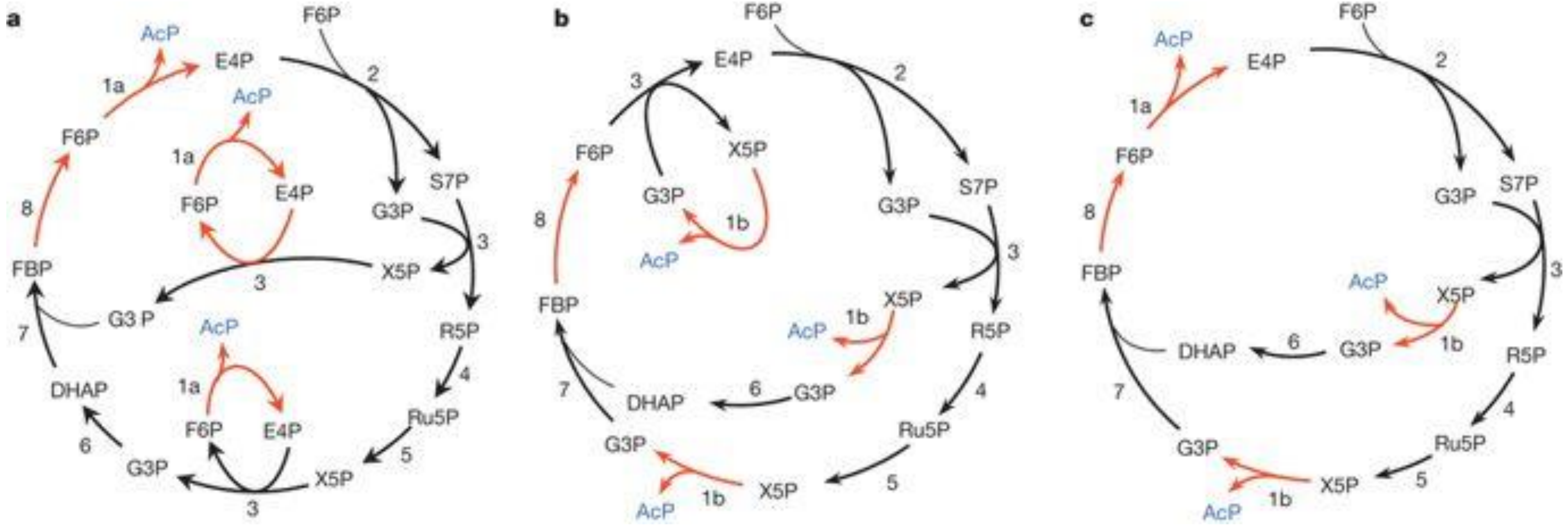
Bogorad et al. (2013). Synthetic non-oxidative glycolysis enables complete carbon conservation. Nature 502, 693-697.

Enzyme numbers: 1, phosphoketolase; 2, Tal; 3, Tkt; 4, Rpi; 5, Rpe; 6, Tpi; 7, Fba; 8, Fbp. DHAP, dihyroxyacetone phosphate; Ru5P, ribulose 5-phosphate.

Synthetic non-oxidative glycolysis – prevention of carbon loss in AcCoA formation (3)

PHOSPHOKETOLASE:

D-fructose 6-phosphate + phosphate -> acetyl phosphate + D-erythrose 4-phosphate + H_2O
D-xylulose 5-phosphate + phosphate -> acetyl phosphate + D-glyceraldehyde 3-phosphate + H_2O
D-sedoheptulose 7-phosphate + phosphate -> acetyl phosphate + D-ribose 5-phosphate + H_2O



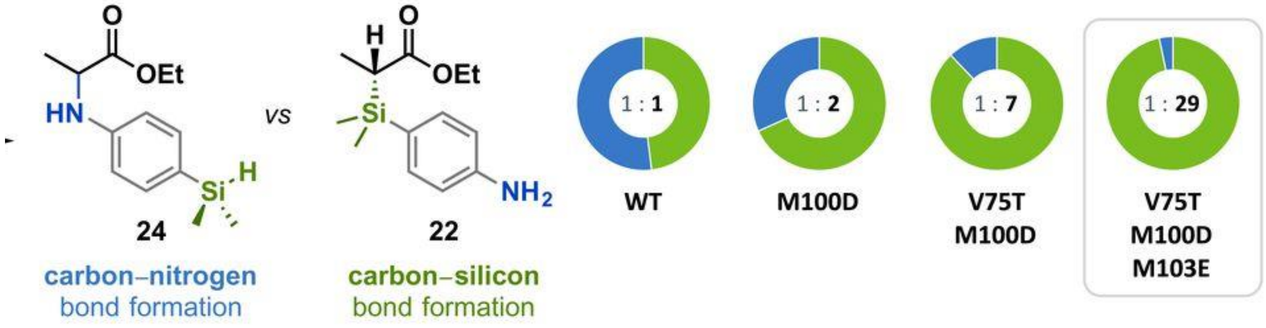
Phosphate acetyl transferase (PTA): CoA + acetyl phosphate -> acetyl-CoA + phosphate

Bogorad et al. (2013). Synthetic non-oxidative glycolysis enables complete carbon conservation. Nature 502, 693-697.

Engineering for C-Si bonds

Silicon based life ?

- at least biochemicals

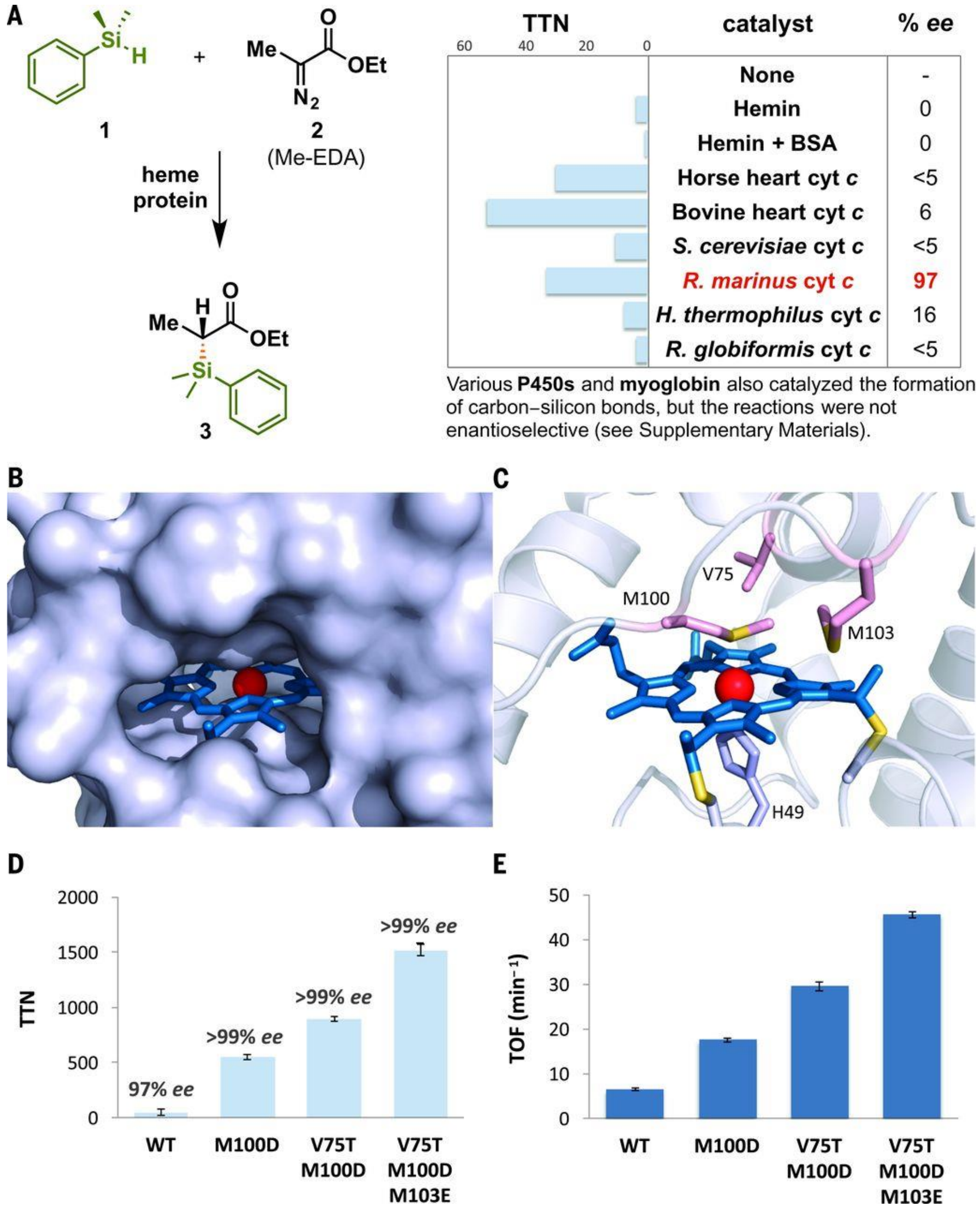


- Silicon is the second most abundant element on Earth, after oxygen
- It is not found in biochemistry but life based on silicon (instead of carbon) has been suggested as alternative (e.g. in space)
- Frances Arnold and her group were able to create C–Si bonds in living *E.coli* by engineering an enzyme of *Rhodothermus marinus* from Icelandic hot springs using (only 3 rounds!) directed evolution
- Si has both metal and non-metal properties
  - > enzyme: cytochrome C (heme Fe^{2+}), an electron transfer protein that does not perform a catalytic function in nature
- The engineered reaction is 15-fold more efficient than with chemical catalysts with certain Si compounds

President Sauli Niinistö is giving the Millenium Technology Prize 2016 to Frances Arnold (California Institute ofTechnology, USA). Figure M. Penttilä

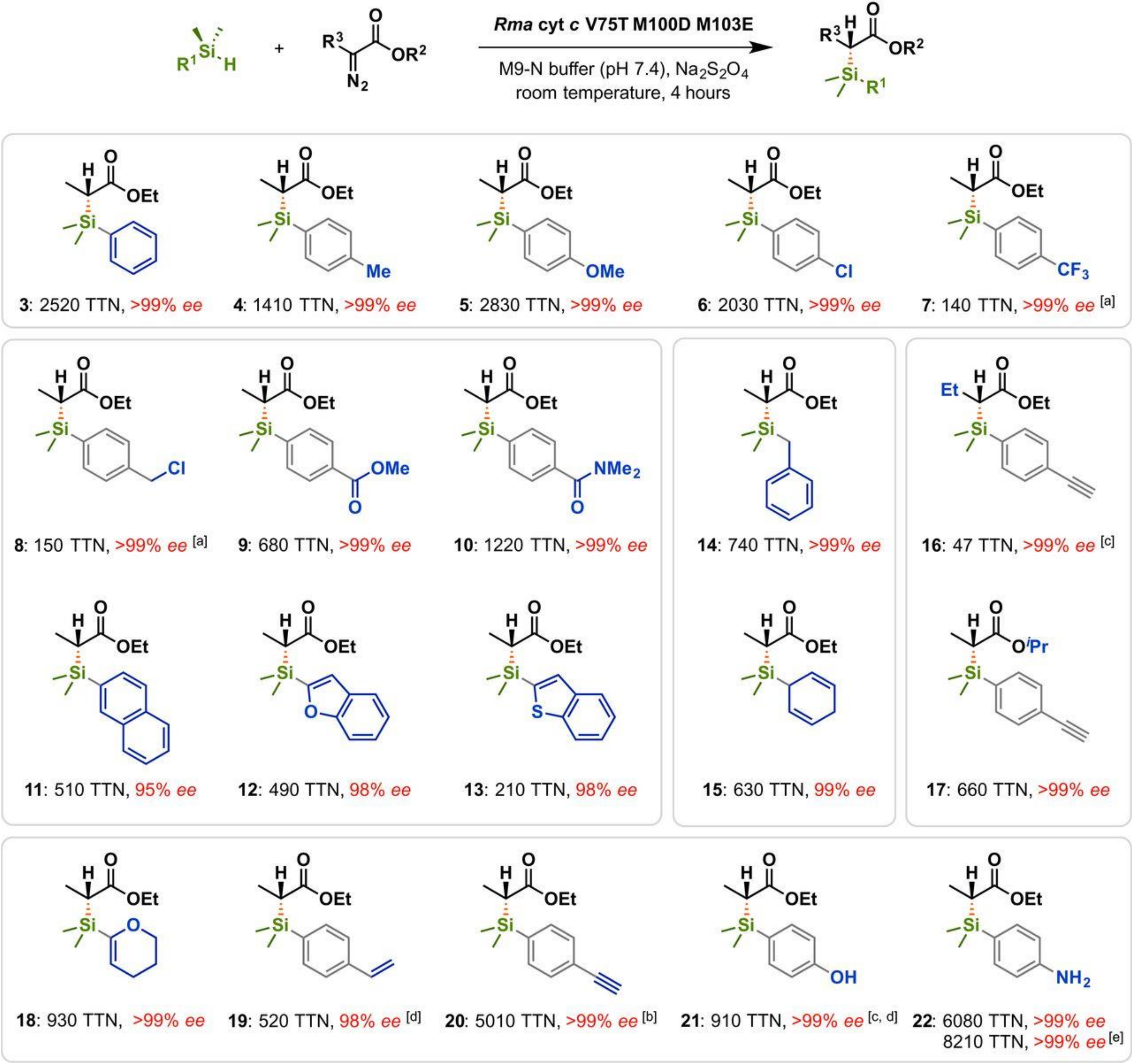
S. B. Jennifer Kan, Russell D. Lewis, Kai Chen, Frances H. Arnold. Directed evolution of cytochrome c for carbon–silicon bond formation: Bringing silicon to life. Science 25 November 2016. Vol 354 (6315). !048-1051.

The finding could help chemists to develop new pharmaceuticals and industrial catalysts — and perhaps explain why evolution has almost completely shunned silicon.



Heme protein–catalyzed carbon–silicon bond formation.(**A**) Carbon–silicon bond formation catalyzed by heme and purified heme proteins. (**B**) Surface representation of the heme-binding pocket of wild-type *Rma* cyt c (PDB ID: 3CP5). (**C**) "Active site" structure of wild-type *Rma* cyt c showing a covalently bound heme cofactor ligated by axial ligands H49 and M100. Amino acid residues M100, V75, and M103 residing close to the heme iron were subjected to site-saturation mutagenesis. (**D**) Directed evolution of *Rma* cyt c for carbon–silicon bond formation [reaction shown in (A)]. Experiments were performed using lysates of *E. coli* expressing *Rma* cyt c variant (OD_{600} = 15; heat-treated at 75°C for 10 min), 10 mM silane, 10 mM diazo ester, 10 mM $Na_2S_2O_4$, 5 vol % MeCN, M9-N buffer (pH 7.4) at room temperature under anaerobic conditions for 1.5 hours. Reactions were done in triplicate. (**E**) Carbon–silicon bond forming rates over four generations of *Rma* cyt c. Single-letter abbreviations for the amino acid residues are as follows: D, Asp; E, Glu; M, Met; T, Thr; and V, Val. TTN, total turn over number.

S. B. Jennifer Kan, Russell D. Lewis, Kai Chen, Frances H. Arnold. Directed evolution of cytochrome c for carbon–silicon bond formation: Bringing silicon to life. Science 25 November 2016. Vol 354 (6315):1048-1051.



Scope of *Rma* cyt c V75T M100D M103E-catalyzed carbon–silicon bond formation. Standard reaction conditions: lysate of *E. coli* expressing *Rma* cyt c V75T M100D M103E (OD_{600} = 1.5; heat-treated at 75°C for 10 min), 20 mM silane, 10 mM diazo ester, 10 mM $Na_2S_2O_4$, 5 vol % MeCN, M9-N buffer (pH 7.4) at room temperature under anaerobic conditions. Reactions performed in triplicate. [a] OD_{600} = 5 lysate. [b] OD_{600} = 0.5 lysate. [c] OD_{600} = 15 lysate. [d] 10 mM silane. [e] OD_{600} = 0.15 lysate.

Can be used already for *in vitro* enzymatic catalysis. Will take some time to make larger scale production with cells possible?

S. B. Jennifer Kan, Russell D. Lewis, Kai Chen, Frances H. Arnold. Directed evolution of cytochrome c for carbon–silicon bond formation: Bringing silicon to life. Science 25 November 2016. Vol 354 (6315):1048-1051.

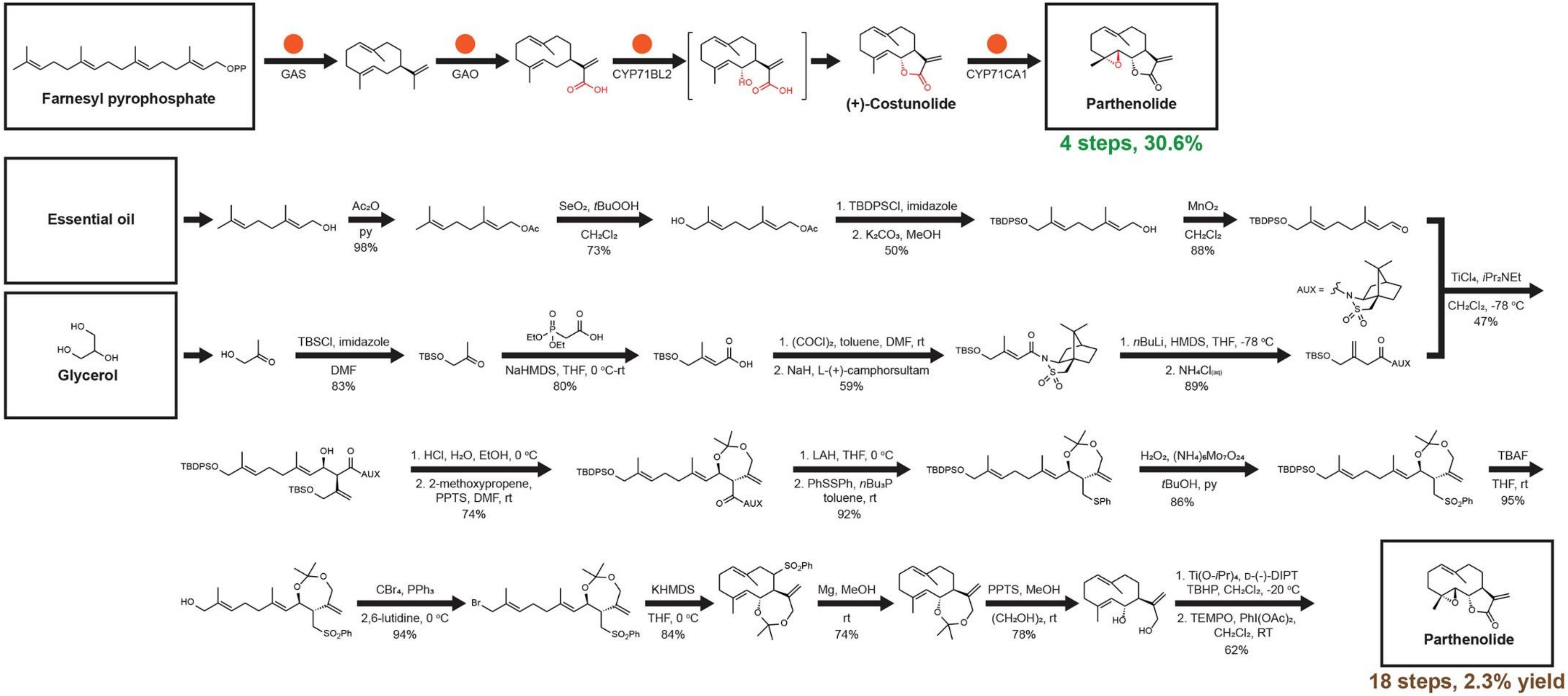
Read this article

Retrosynthetic design of metabolic pathways to chemicals not found in nature

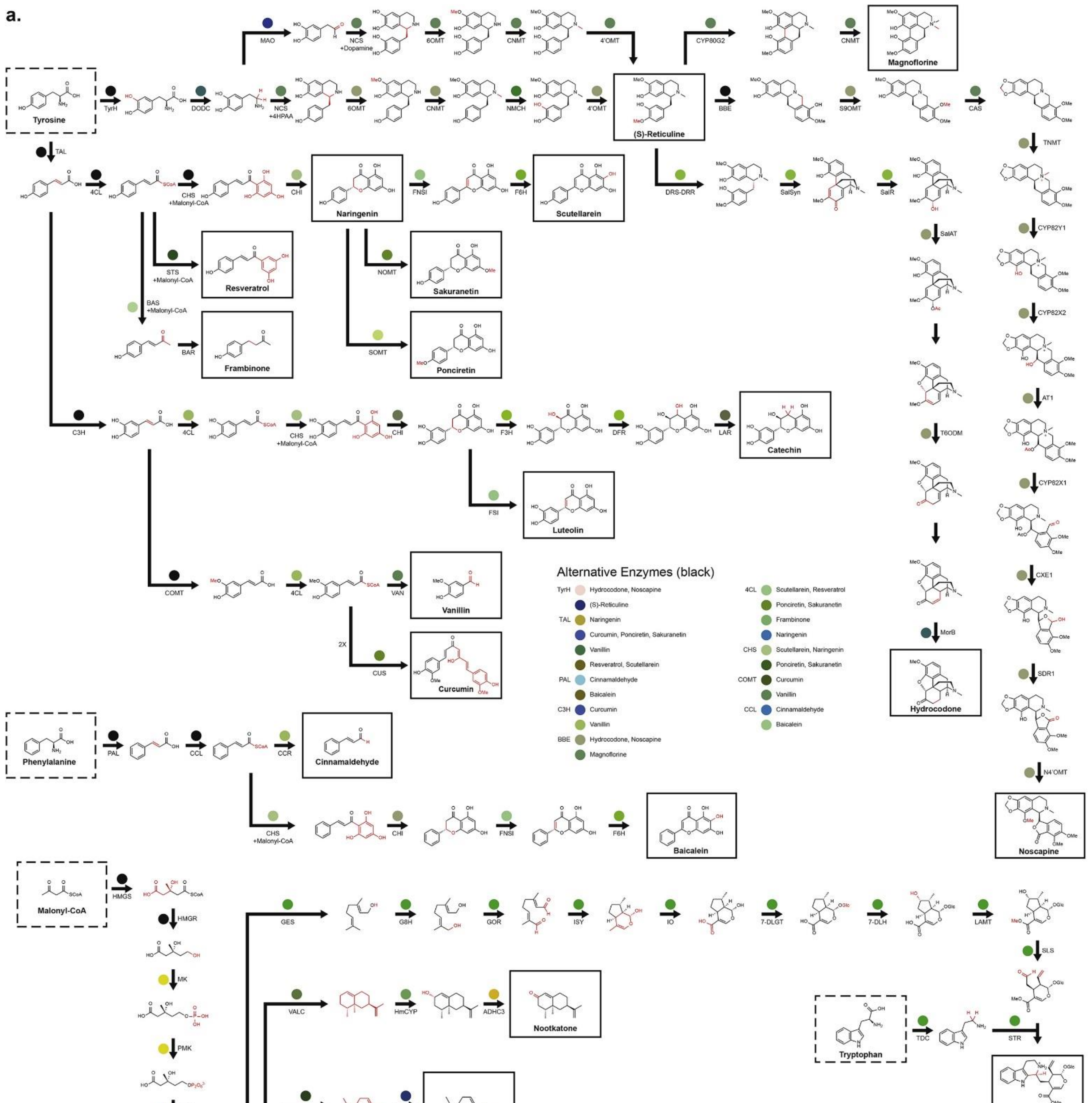
Geng-MinLin, Robert Warden-Rothman & Christopher A.Voigt
Current Opinion in Systems Biology 14, 82-107 (2019)

https://doi.org/10.1016/j.coisb.2019.04.004

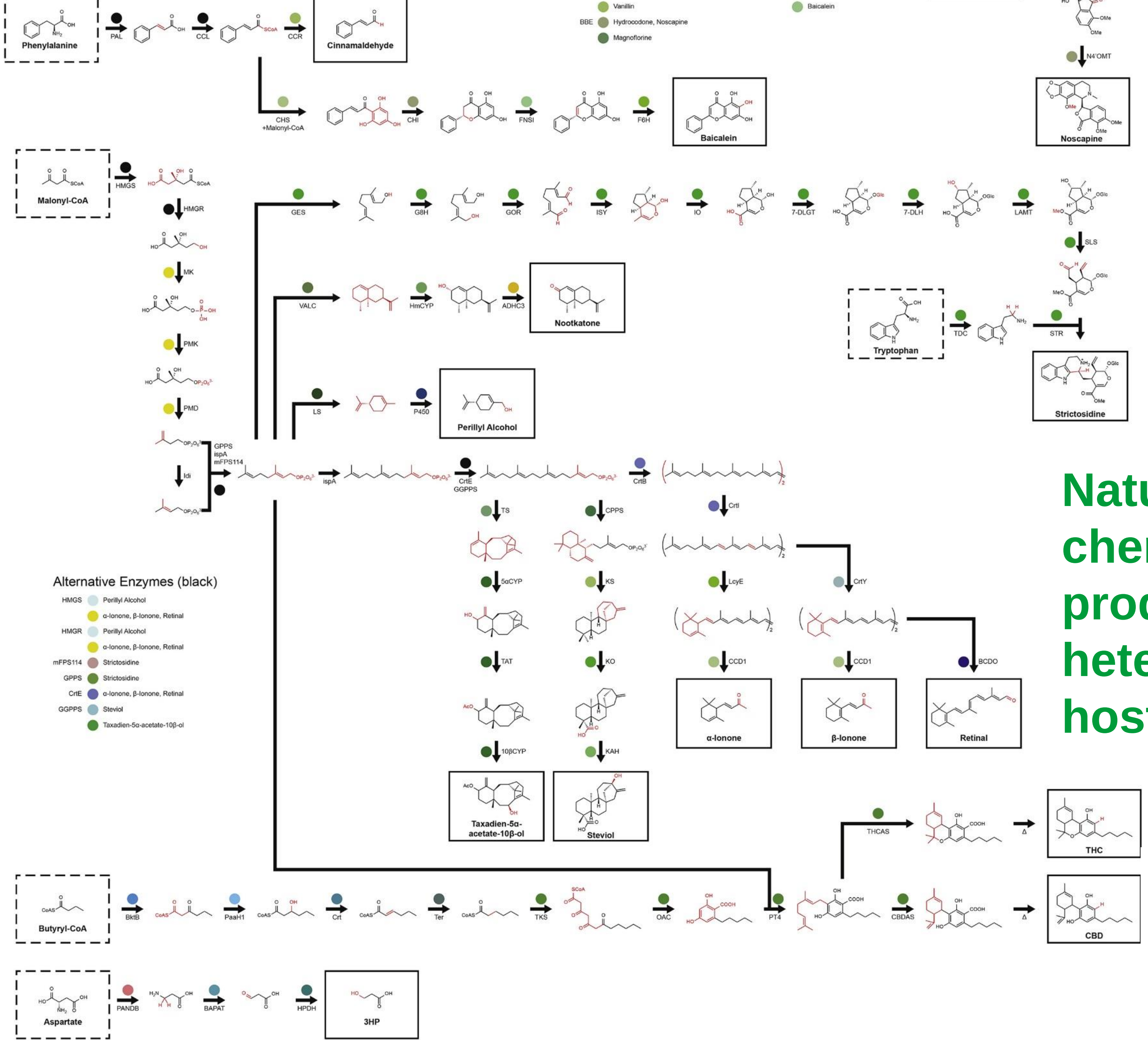
Biochemistry vs. Chemistry



Comparison of metabolic (from FPP) and chemical routes to parthenolide. The pathway has been identified and transferred from its native organism (*Tanacetum parthenium*) to yeast and the theoretical yield of the biosynthetic route is shown (0.306 g/g **glucose**).

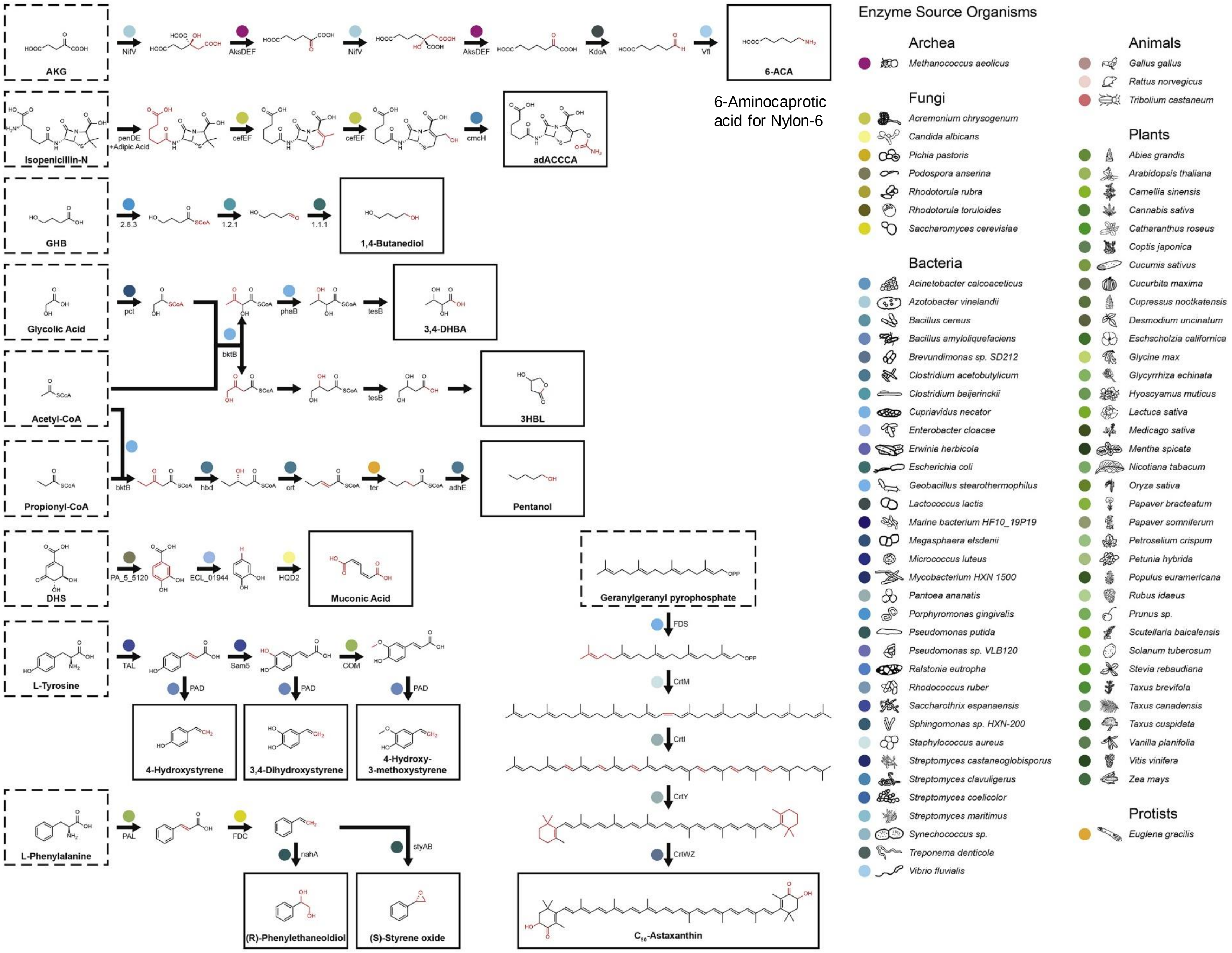


Natural chemicals produced in a heterologous host



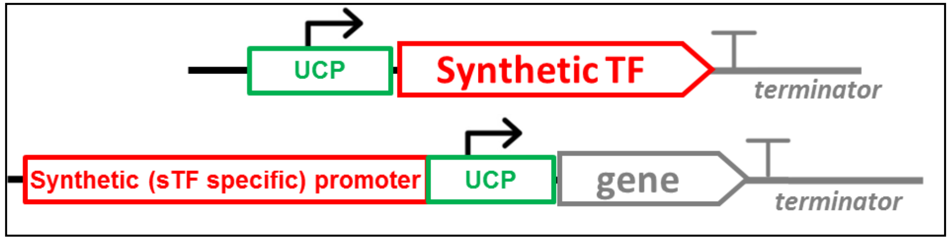
Natural chemicals produced in a heterologous host

Retrosynthesis for xenobiotic compounds, not found in nature



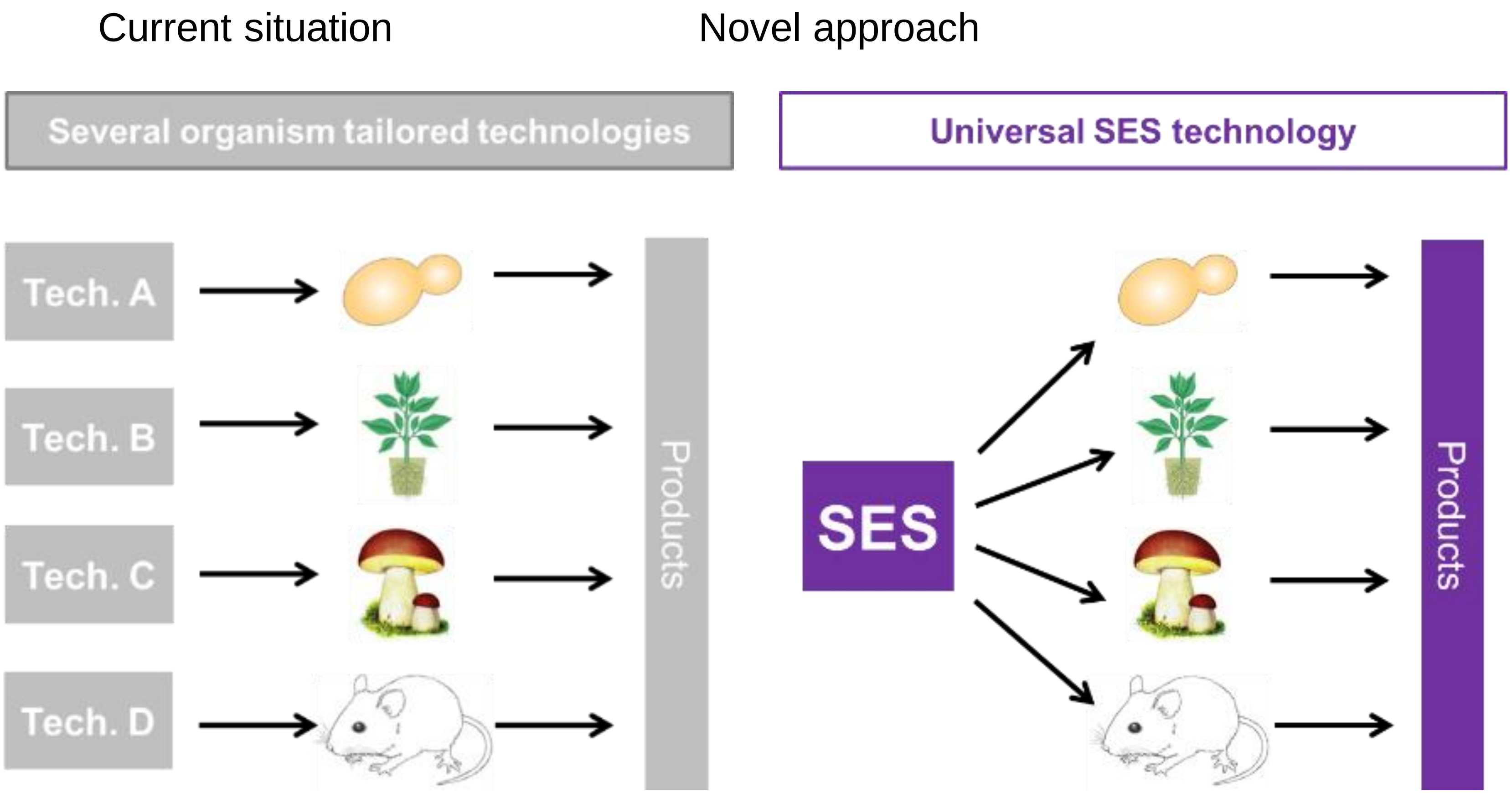
Synthetic promoters and control circuits for biotechnology
- VTT example

SES
Orthogonal Synthetic Expression System for fungi



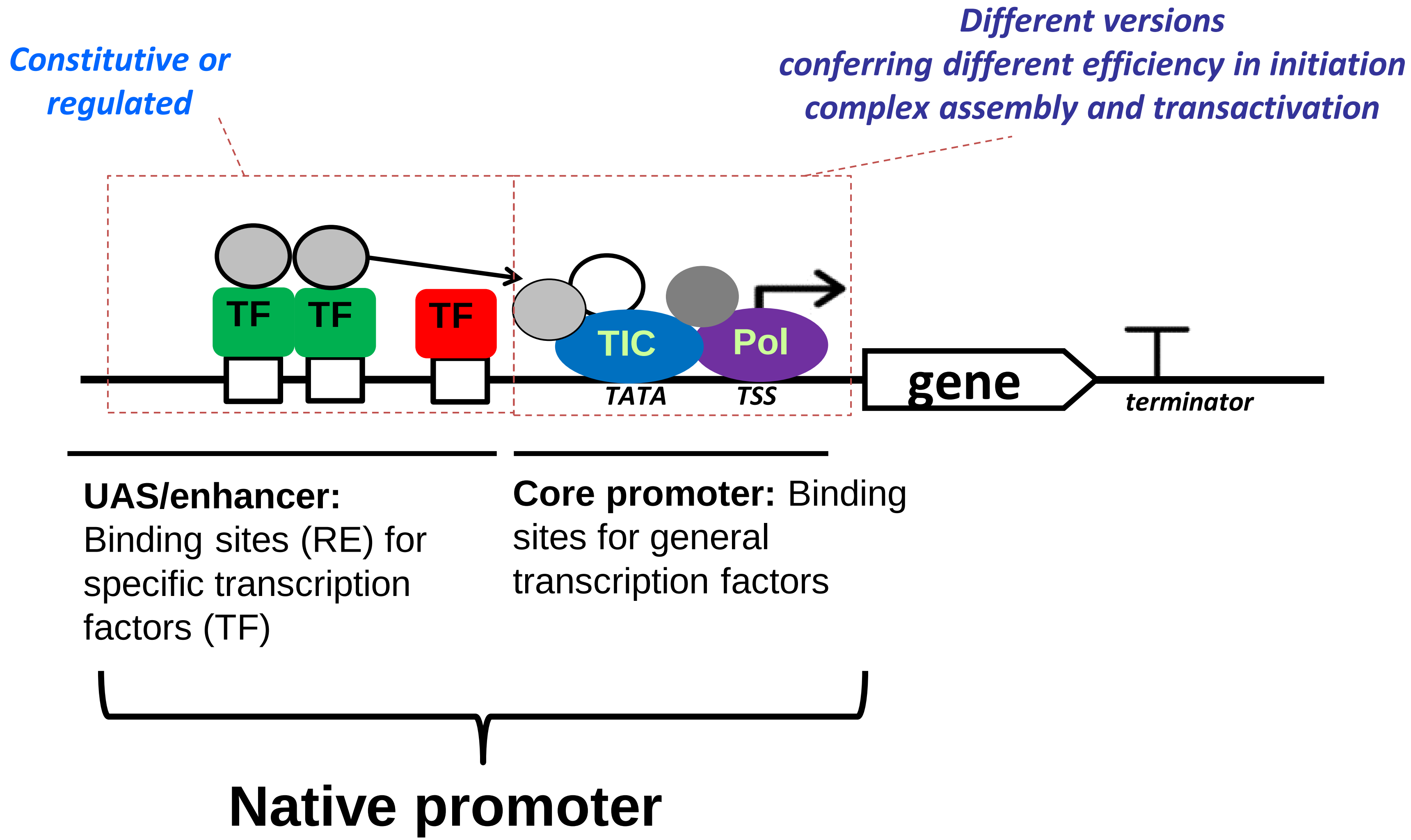
- Tunable controllable promoters, driving different expression levels
- Constitutive, inducible or repressable
- Orthogonal, not responding to host's background regulation
- Enables memory
- Functional over several fungal species

Anssi Rantasalo, Joosu Kuivanen, Jussi Jäntti, Dominik Mojzita /VTT

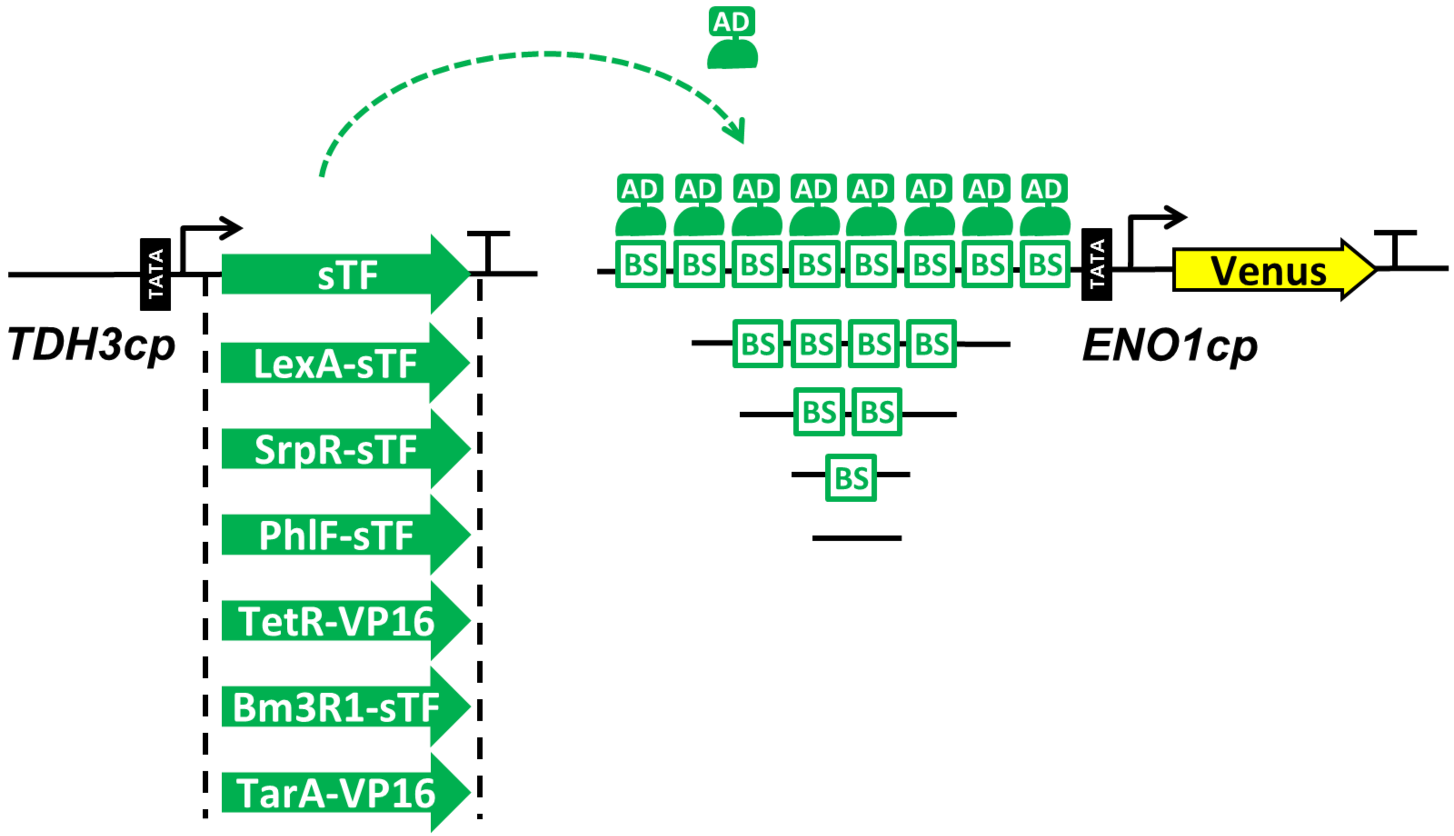


Current situation
Novel approach
Several organism tailored technologies
Universal SES technology
Tech. A
Tech. B
Tech. C
Tech. D
Products
SES
Products

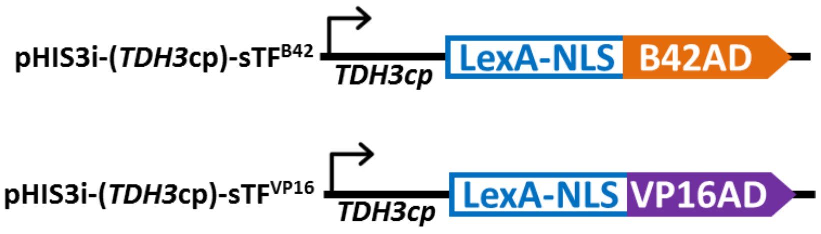
Eukaryotic gene expression

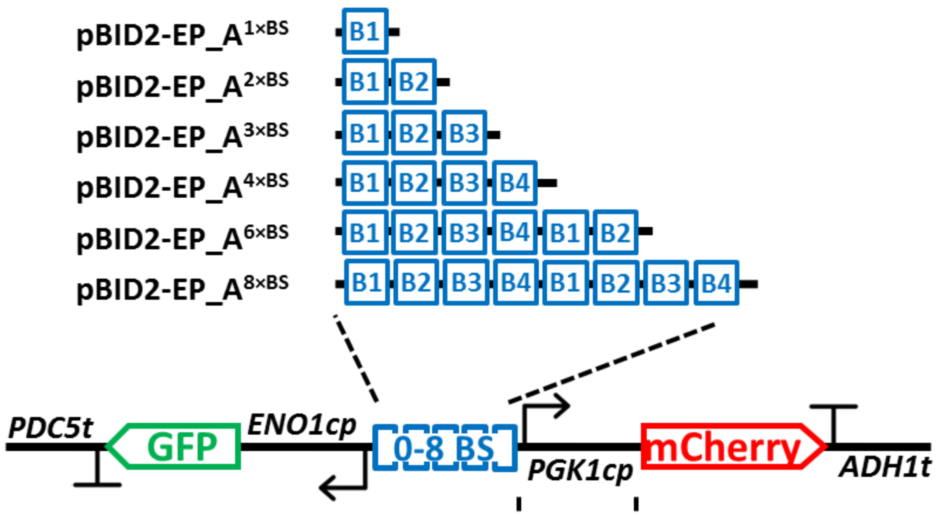


Synthetic gene expression system



Development of a tunable expression system for *S. cerevisiae*

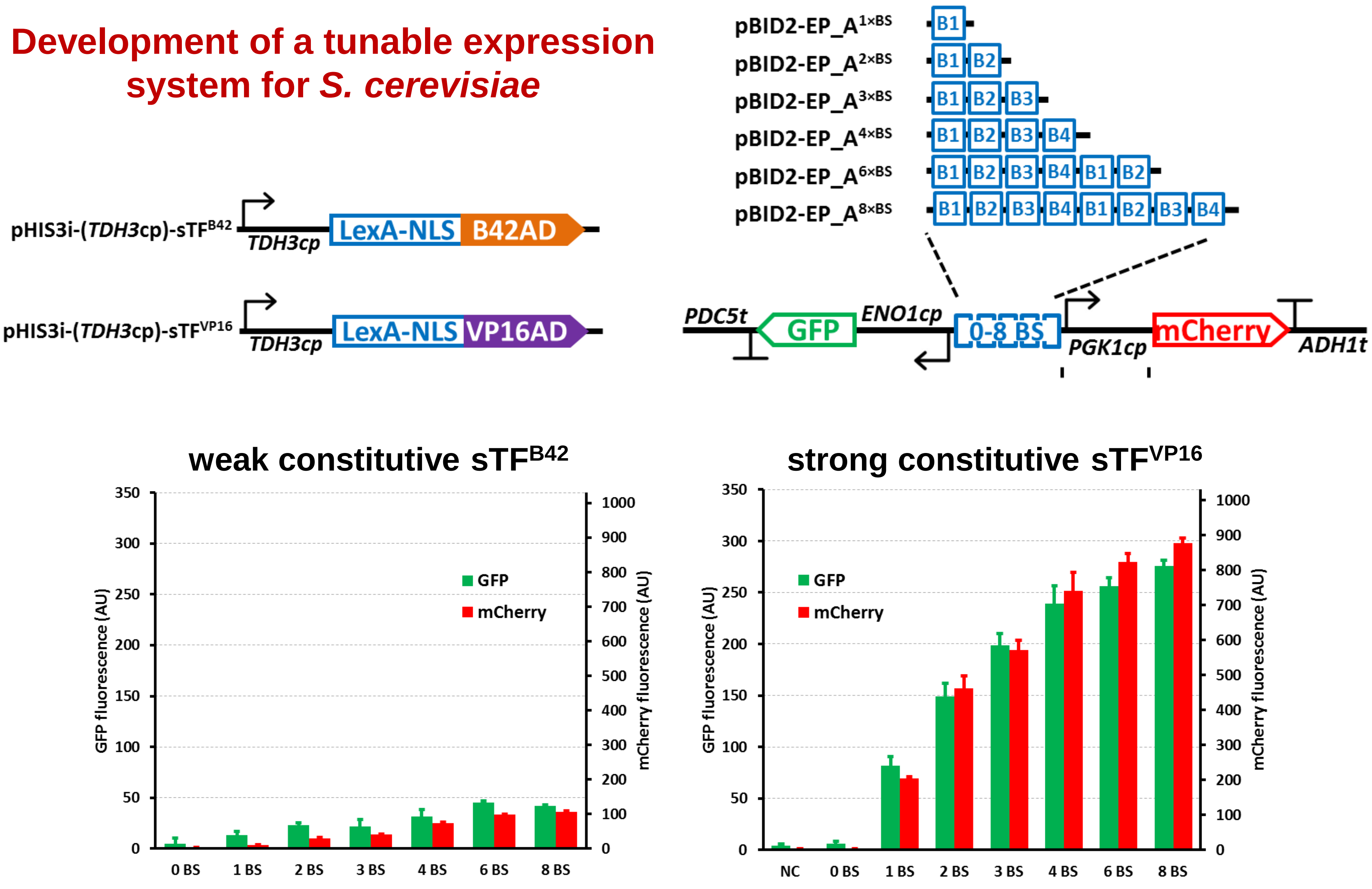




Bidirectonal synthetic promoter

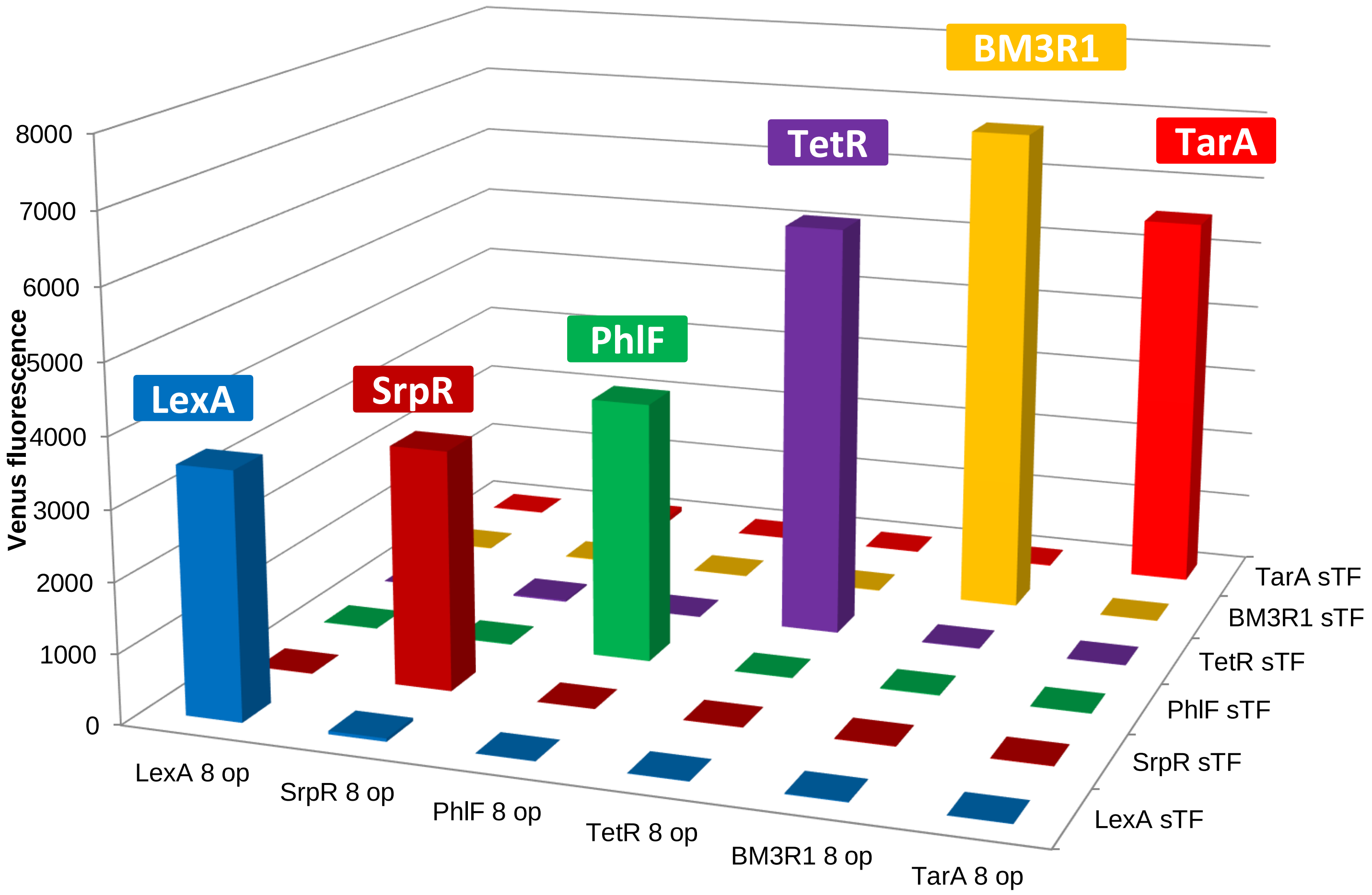
Rantasalo et al. (2016) PLoS One

Development of a tunable expression system for *S. cerevisiae*



Rantasalo et al. (2016) PLoS One

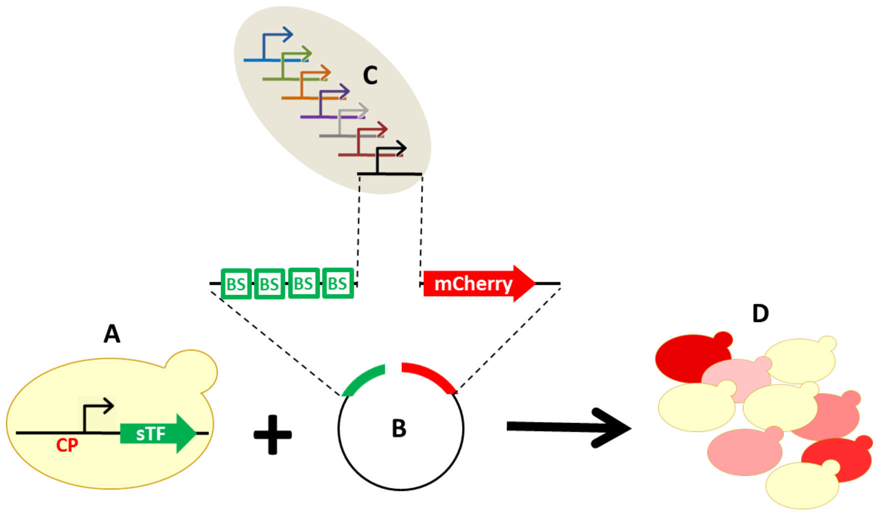
Orthogonality matrix – test of the sTFs' specificity



- Mathematical models of the different expression circuits

Universial core promoters for different fungi

- Core promoters of highly expressed genes from various organisms (as gBlocks).
- gBlocks assembled *in vivo to a* CEN-type plasmid in a yeast strain constitutively expressing LexA-based sTF.
- Strains analyzed for red fluorescence.
- A few new strong (universal) core promoters selected.



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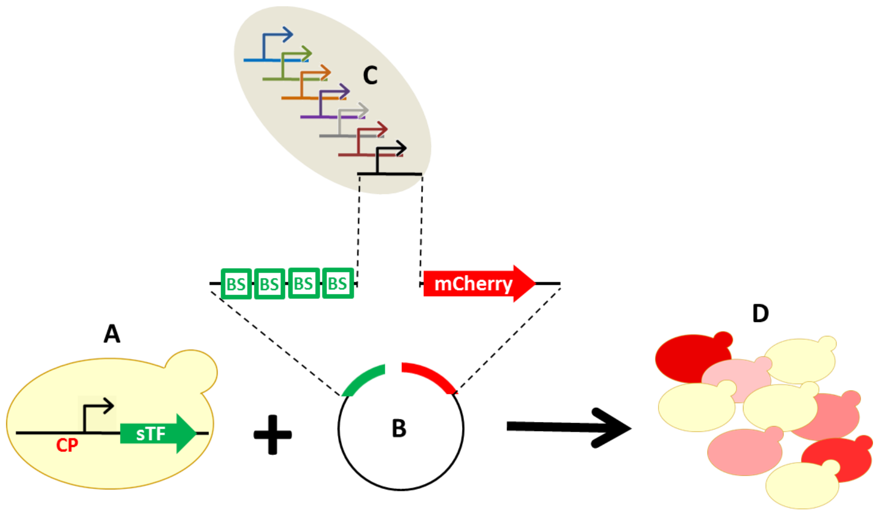
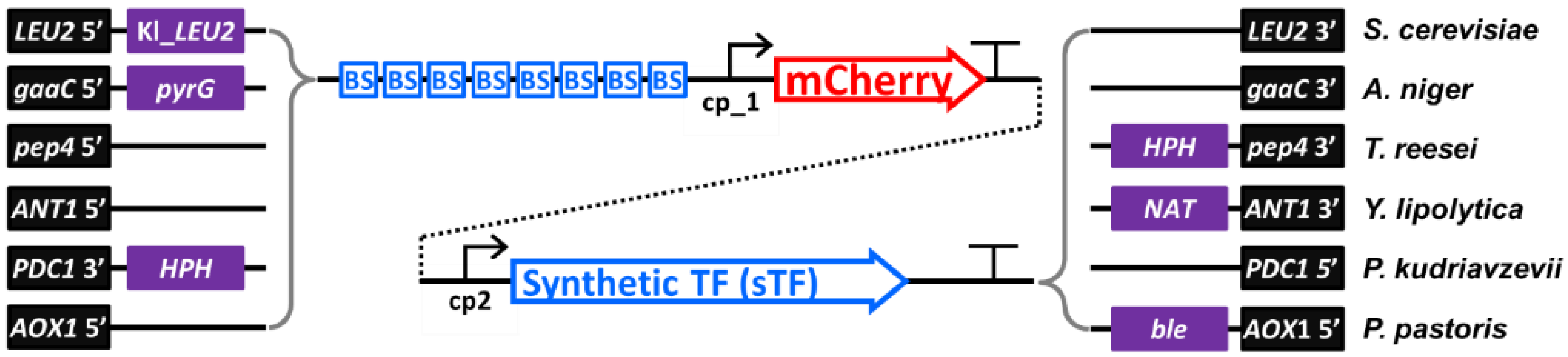
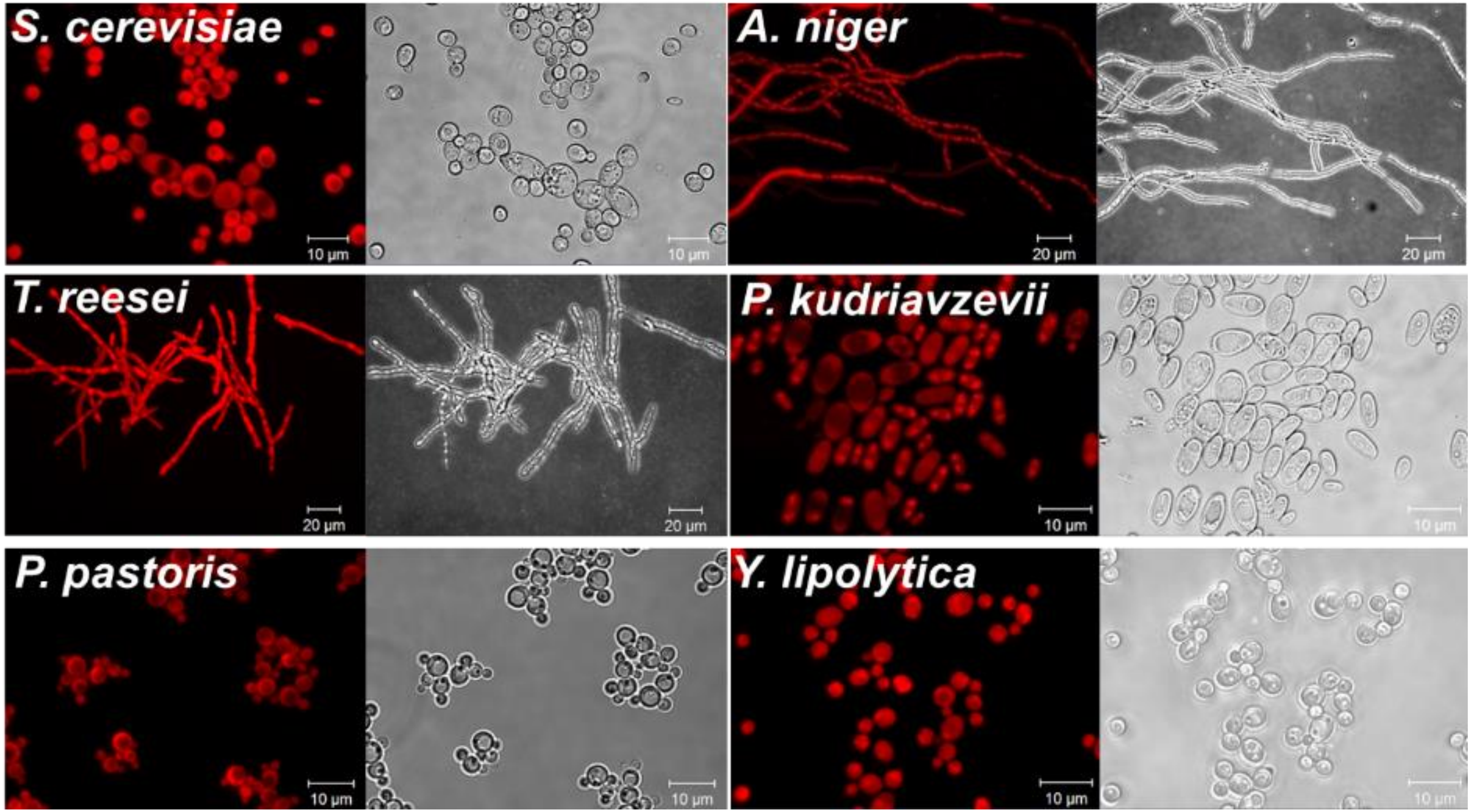




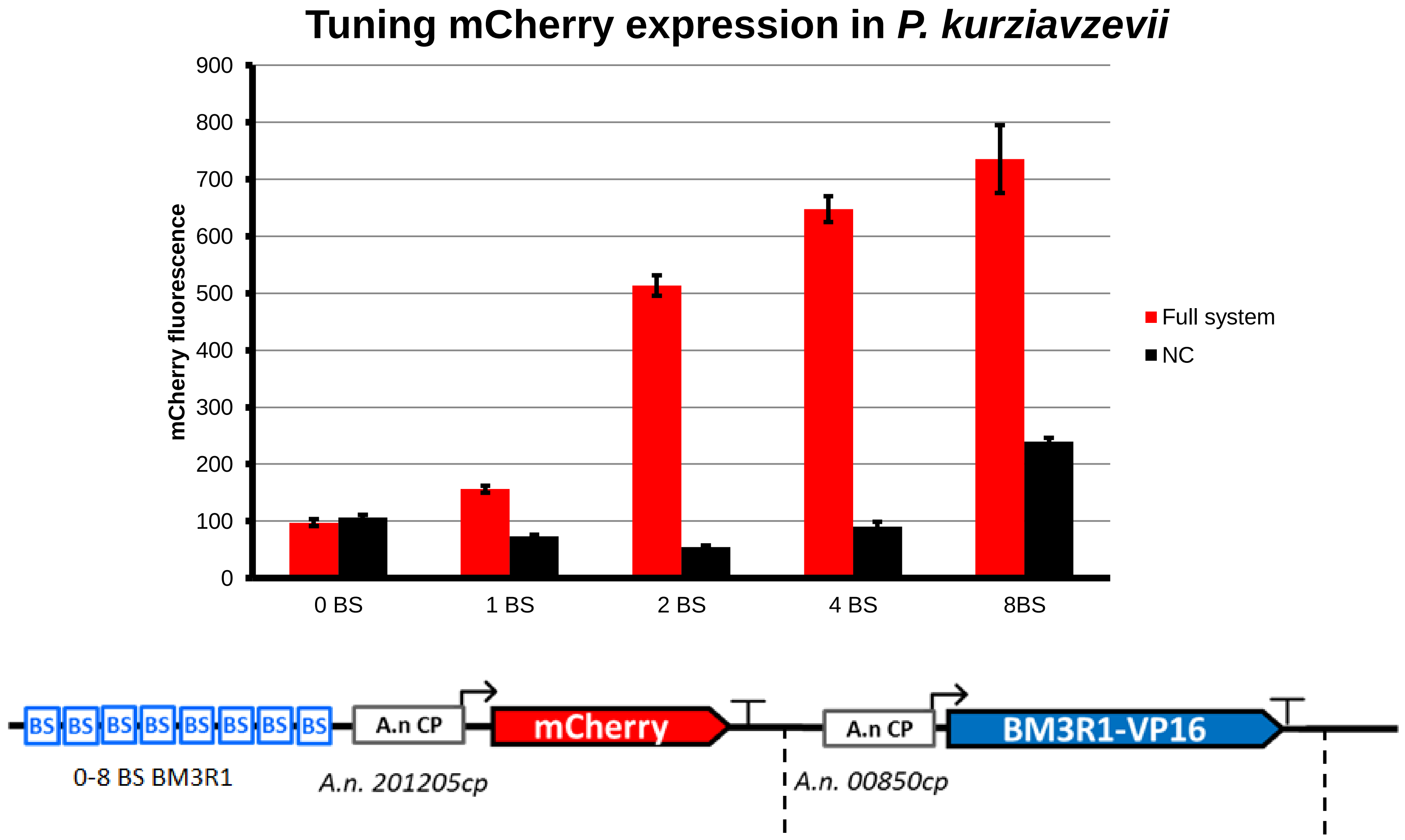
- The best performing core promoters (CP) from the screen used for the construction of transfrerable expression cassettes
- Two different CPs used for the sTF and mCherry expression

SES is functional in several fungal species



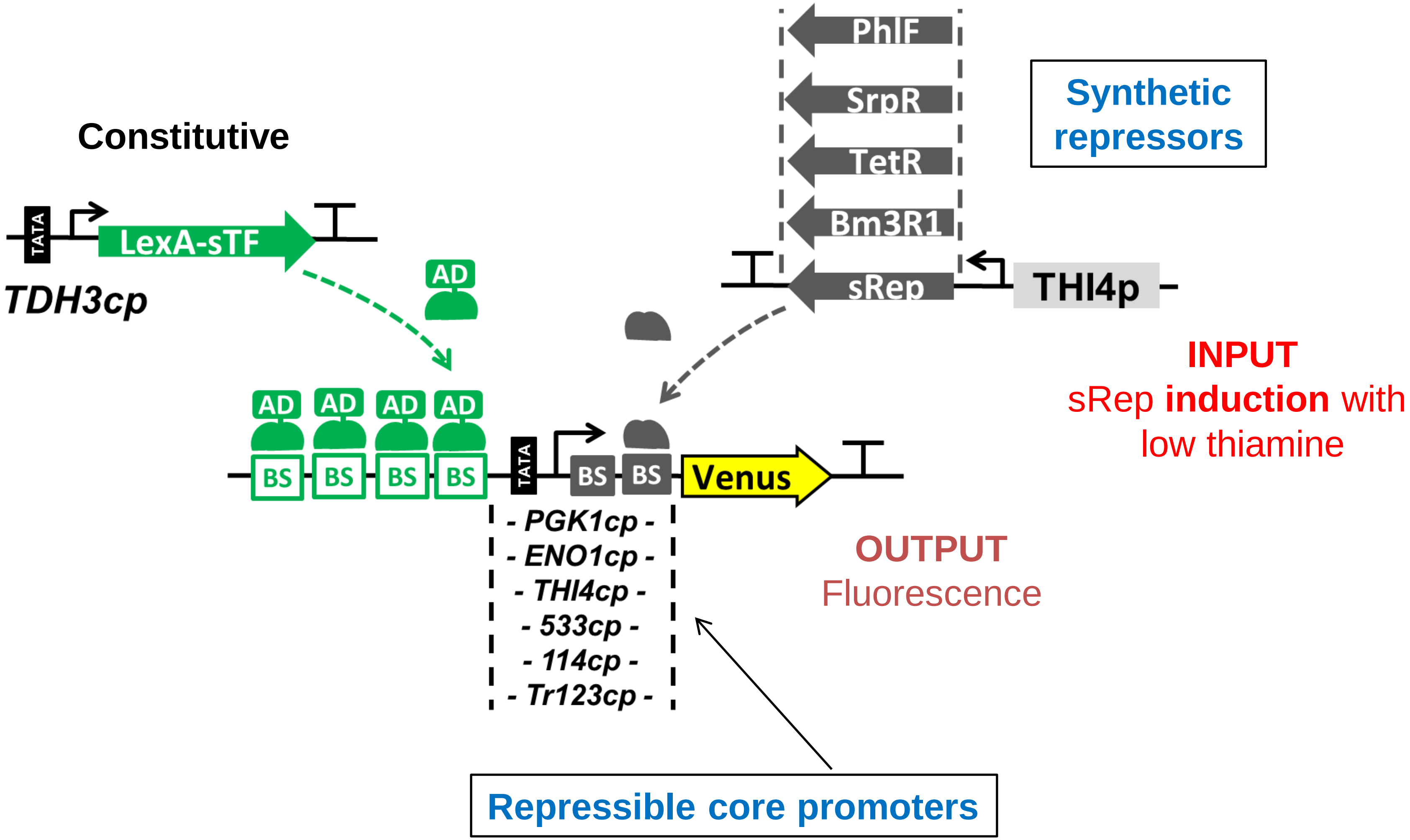
Fluorescence microscopy (mCherry)
Stable and homogenous expression in all cells/species

Tuning expression with SES promoters in *Pichia kurziavzevii*



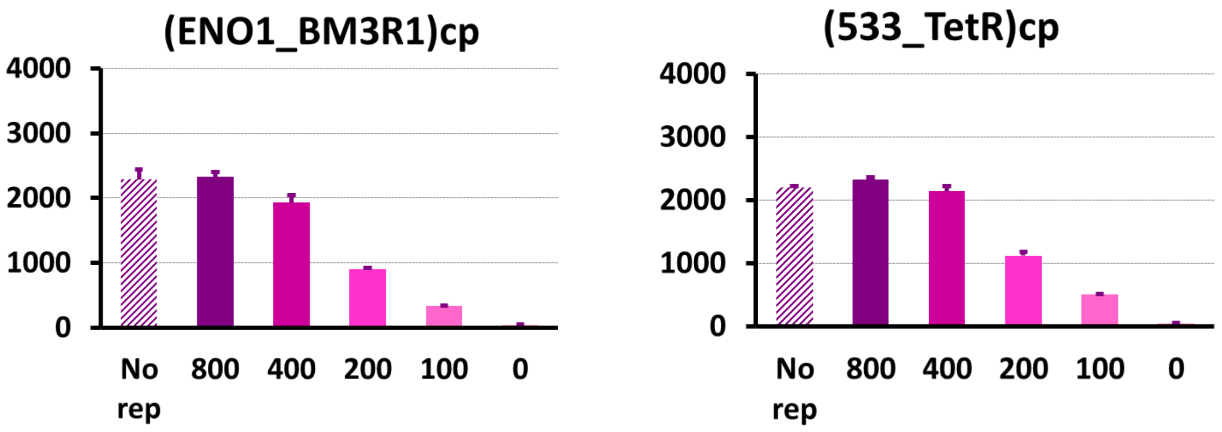
NC = without TF

Downregulation of the synthetic promoter with a synthetic repressor (sRep)

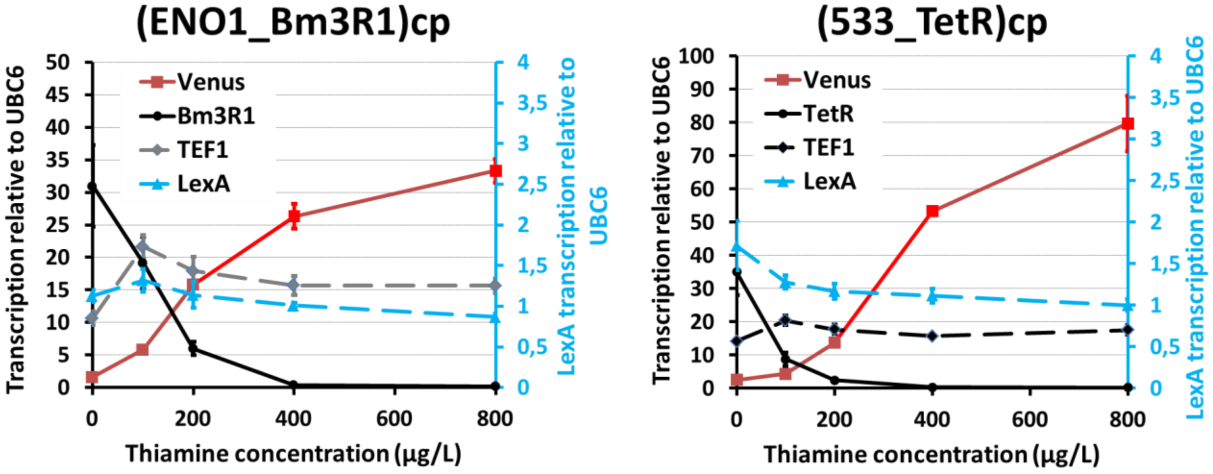


Downregulation of gene expression with synthetic repressor (sRep) in *S.crevisiae*

Fluorescence



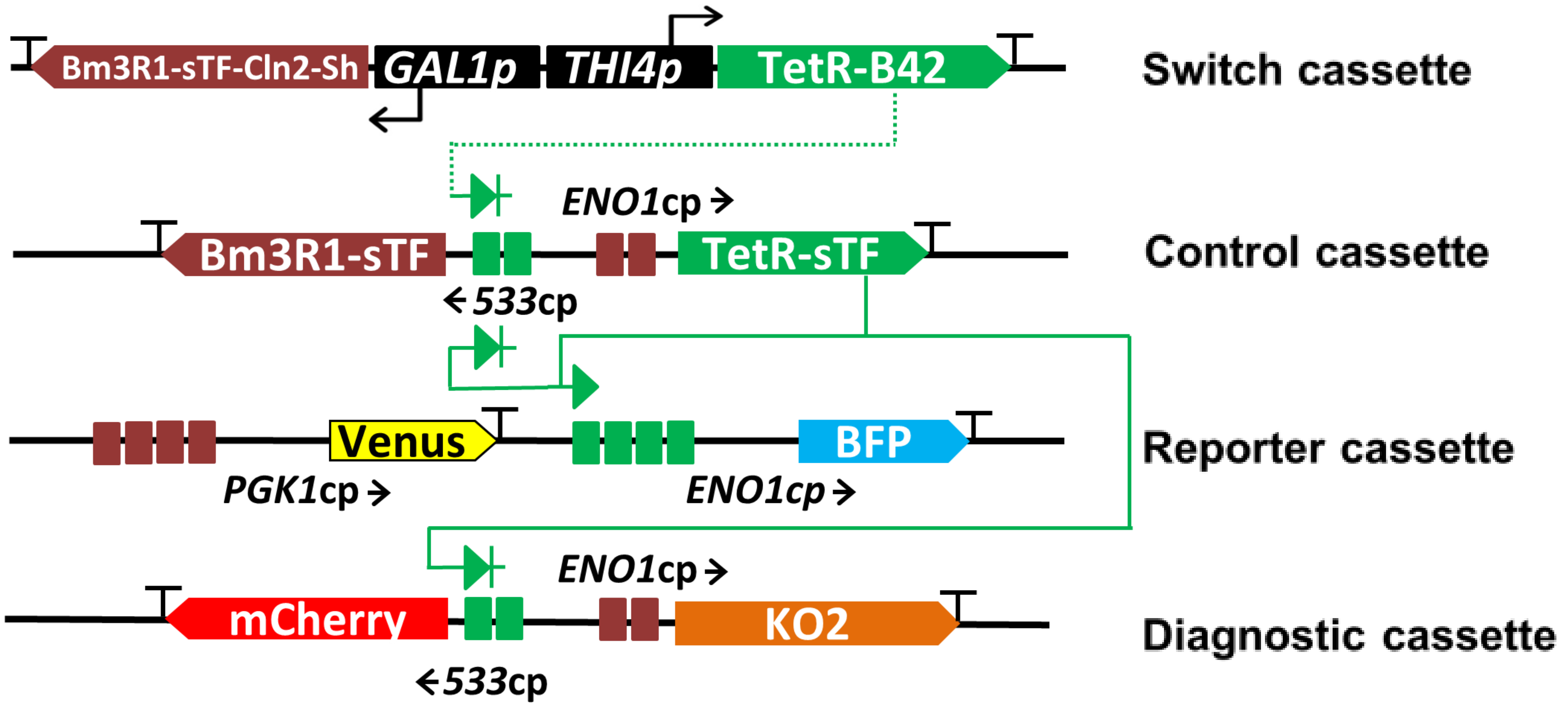
Transcription



Repression of Venus expression with sRep

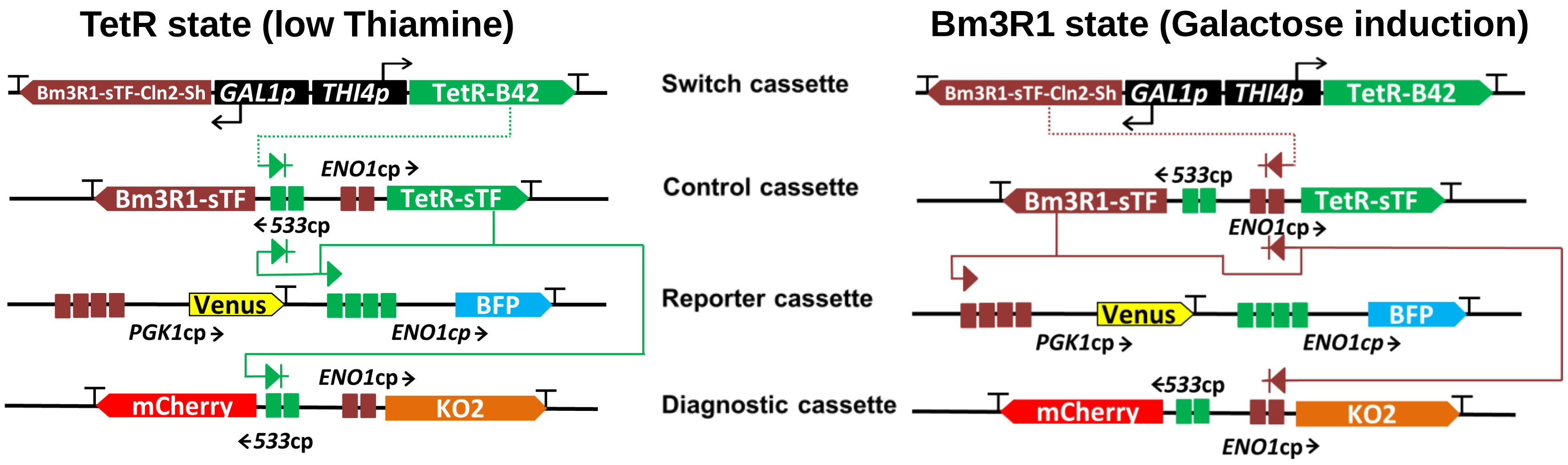
Bi-stable switch – Design

based on well-characterized orthogonal DNA parts



Bi-stable switch – Test

putting the system through series of tests to assess its robustness



Bi-stable switch – Test

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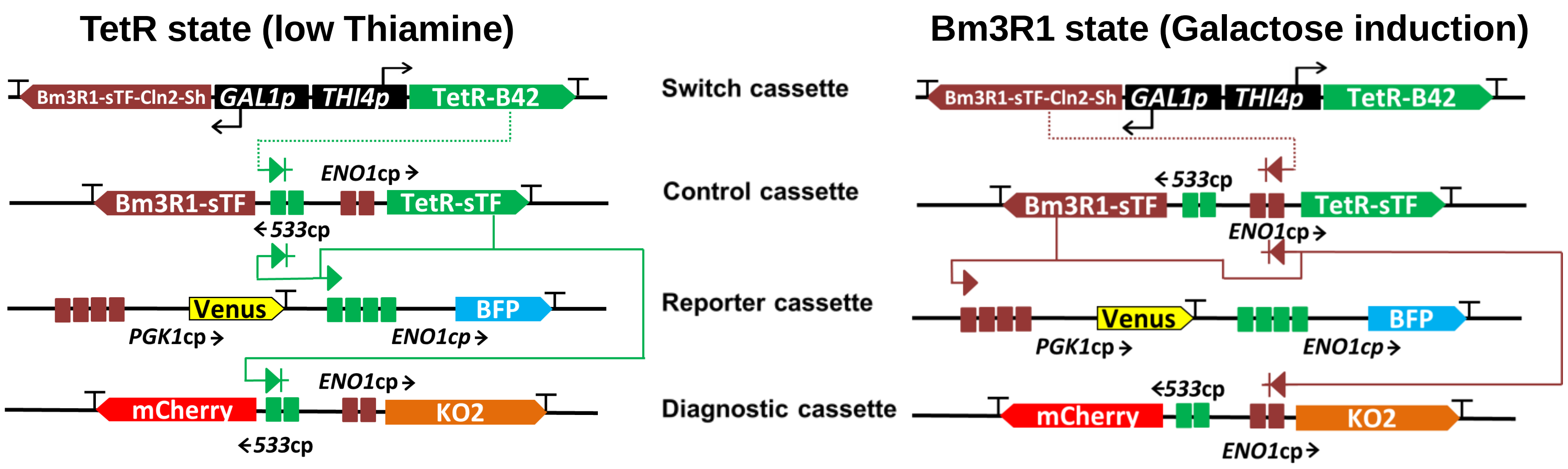
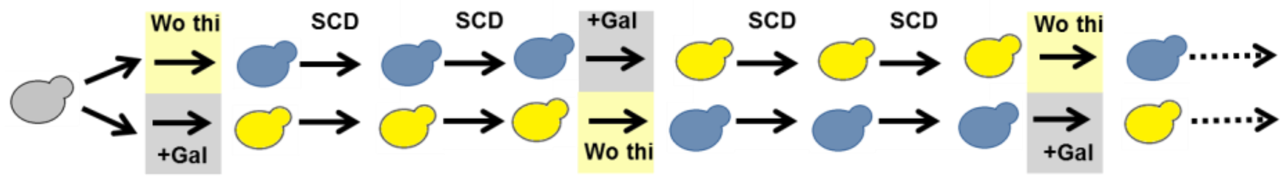
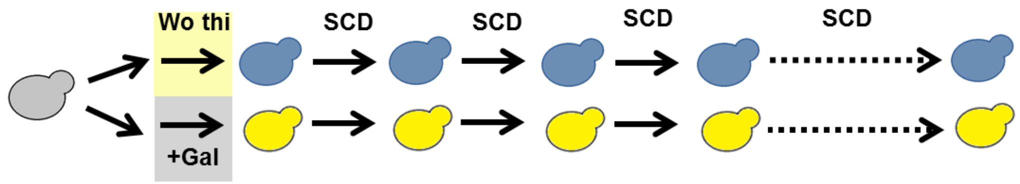


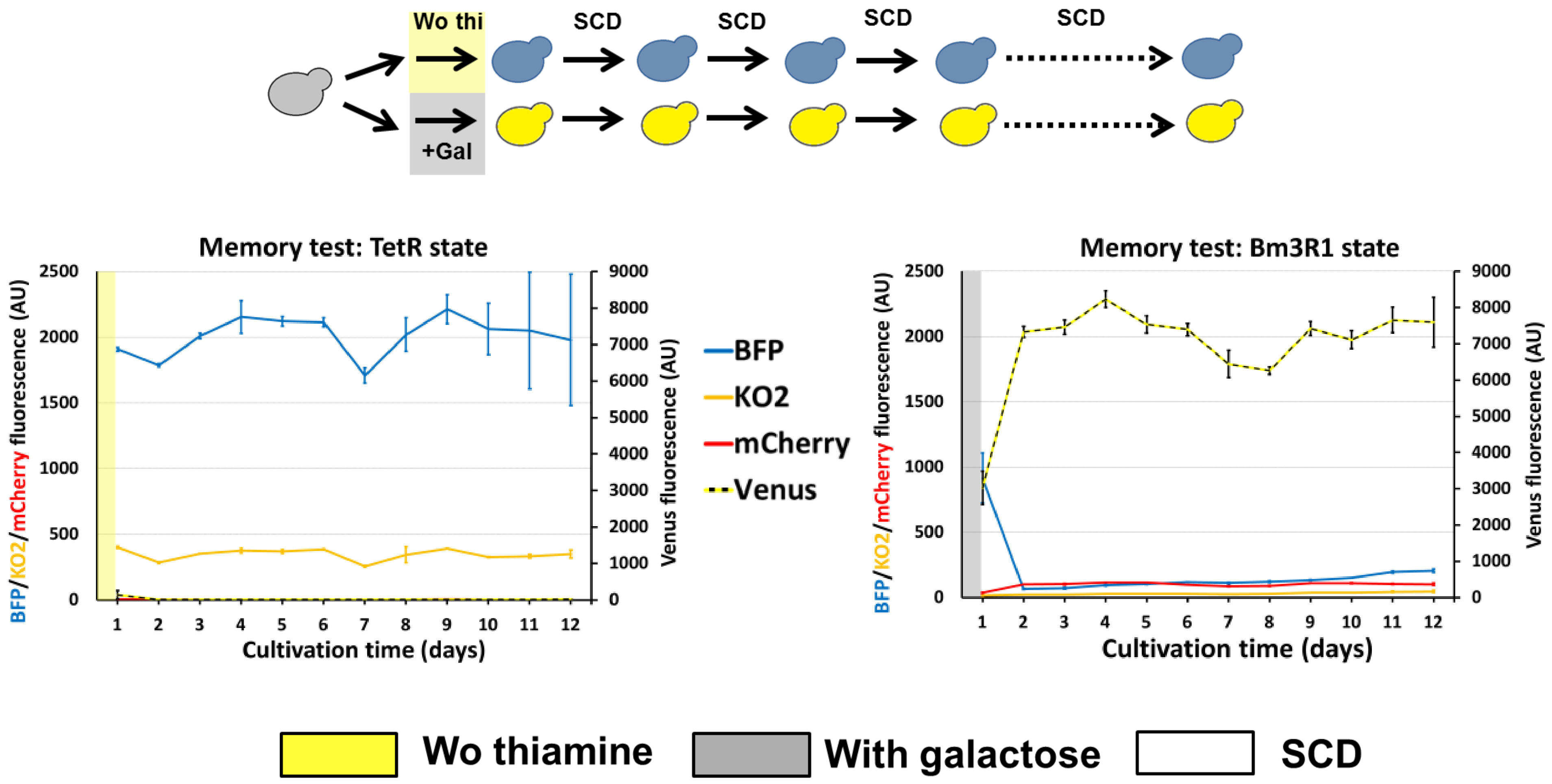
Repeated switches



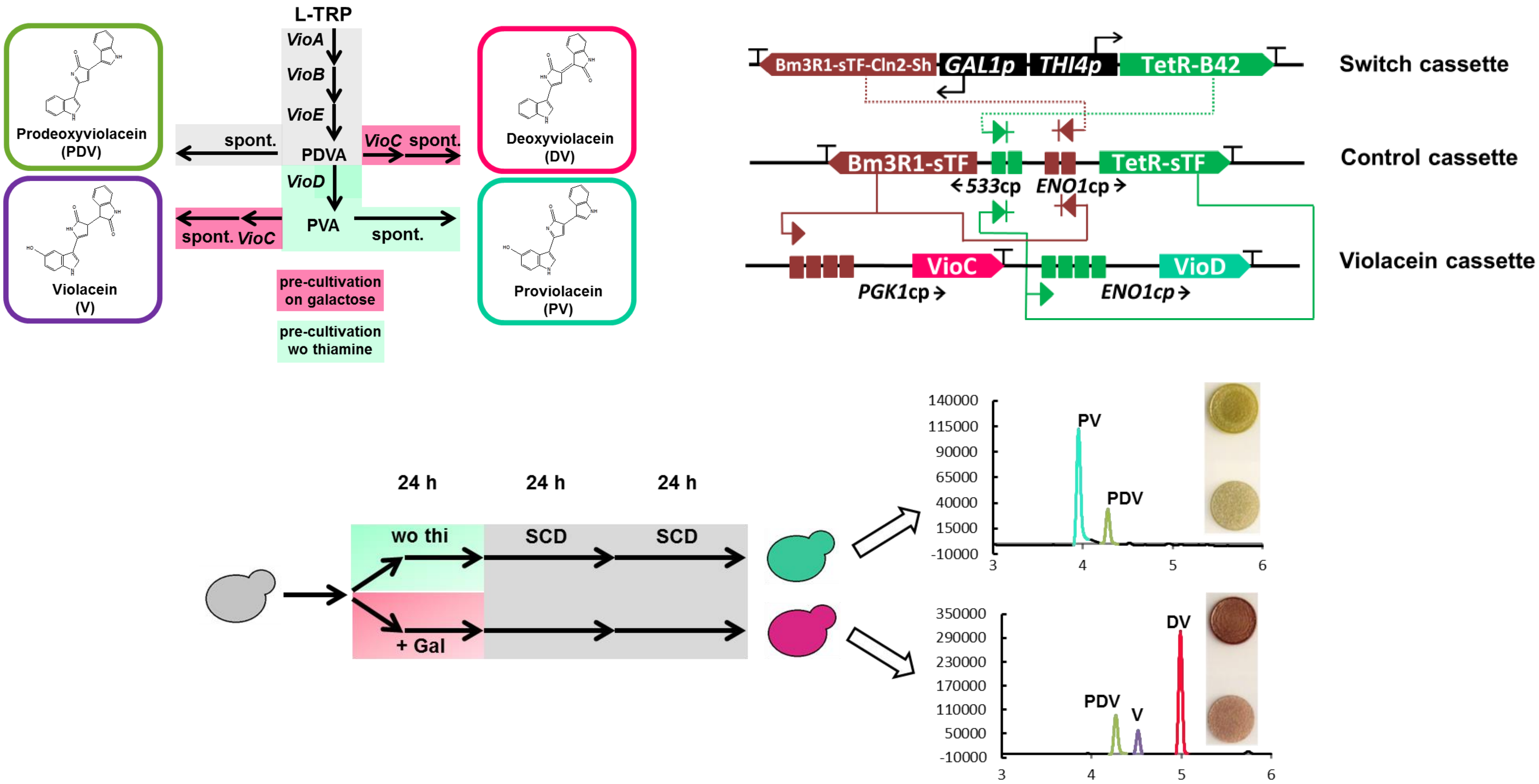
Memory



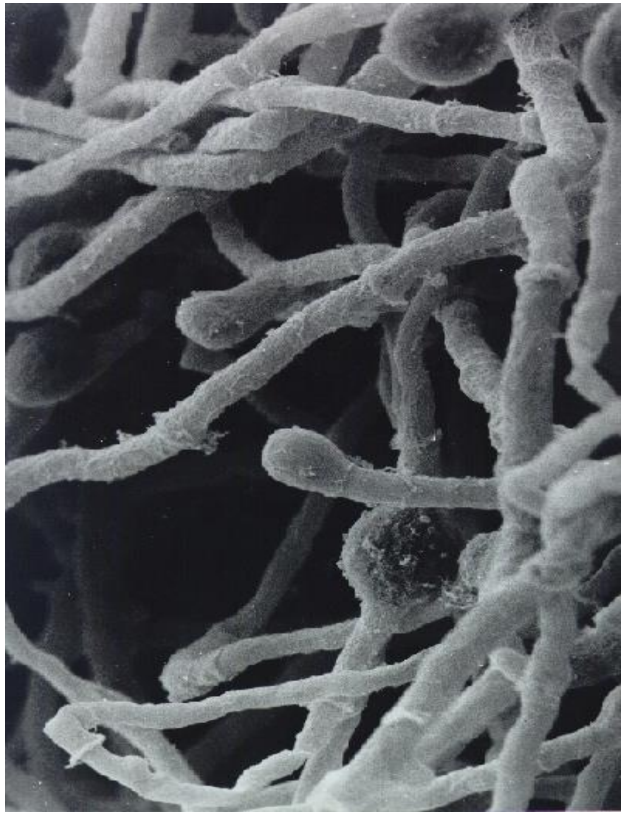
Bi-stable switch – Memory Test



Bi-stable circuit for metabolic pathway switching - Violacein pathway in *S.cerevisiae*

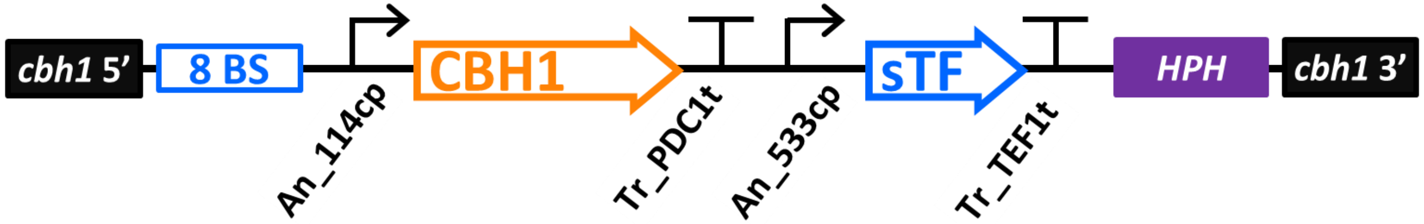


Using SES in protein production in *Trichoderma reesei*

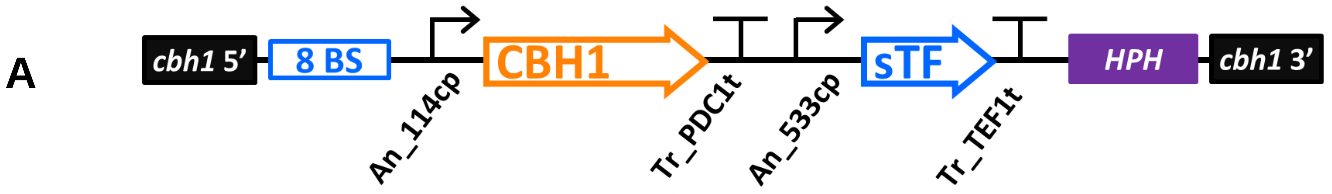


Production on glucose enables a more pure product

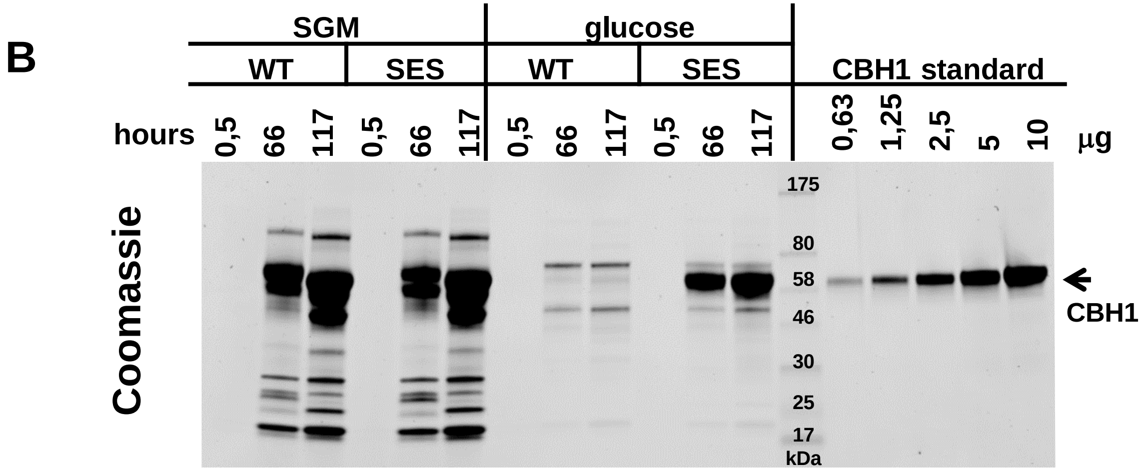
Dominik Mojzita, Mari Valkonen, Marika Vitikainen,
Chris Landowski et al, VTT



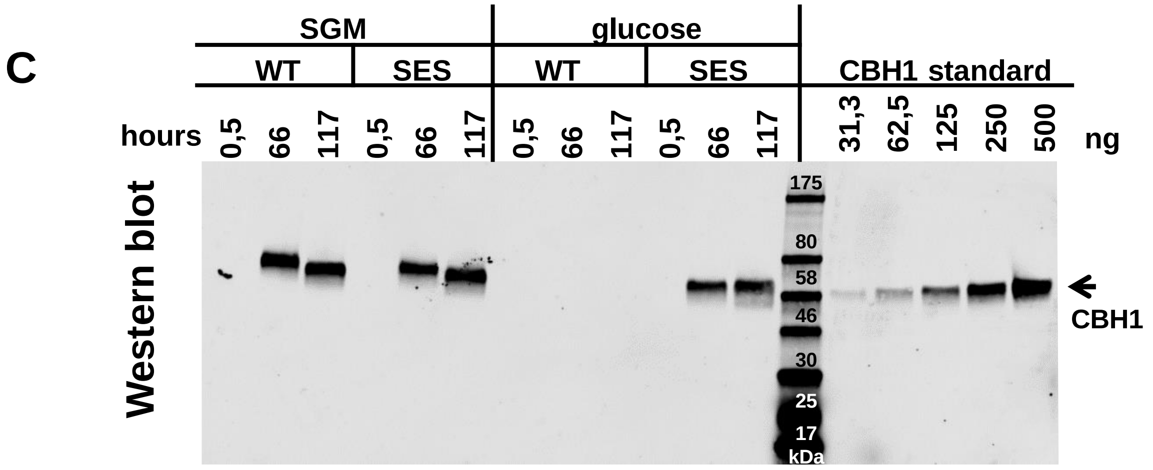
CBHI production in *Trichoderma reesei* with SES



Constitutive expression



SGM	WT	0,5 h	0,6 g/L
		66 h	9,4 g/L
		117 h	17,8 g/L
	SES	0,5 h	0,6 g/L
		66 h	8,4 g/L
		117 h	17,6 g/L
Glucose	WT	0,5 h	<0,5 g/L
		66 h	0,9 g/L
		117 h	1,2 g/L
	SES	0,5 h	<0,5 g/L
		66 h	3,7 g/L
		117 h	5,9 g/L



SGM	WT	0,5 h	<0,1 g/L
		66 h	4,9 g/L
		117 h	4,8 g/L
	SES	0,5 h	<0,1 g/L
		66 h	4,3 g/L
		117 h	5,4 g/L
Glucose	WT	0,5 h	<0,1 g/L
		66 h	<0,1 g/L
		117 h	<0,1 g/L
	SES	0,5 h	<0,1 g/L
		66 h	3,2 g/L
		117 h	4,1 g/L

Synthetic Biology for a Sustainable Bioeconomy – A Roadmap for Finland

Suomeksi

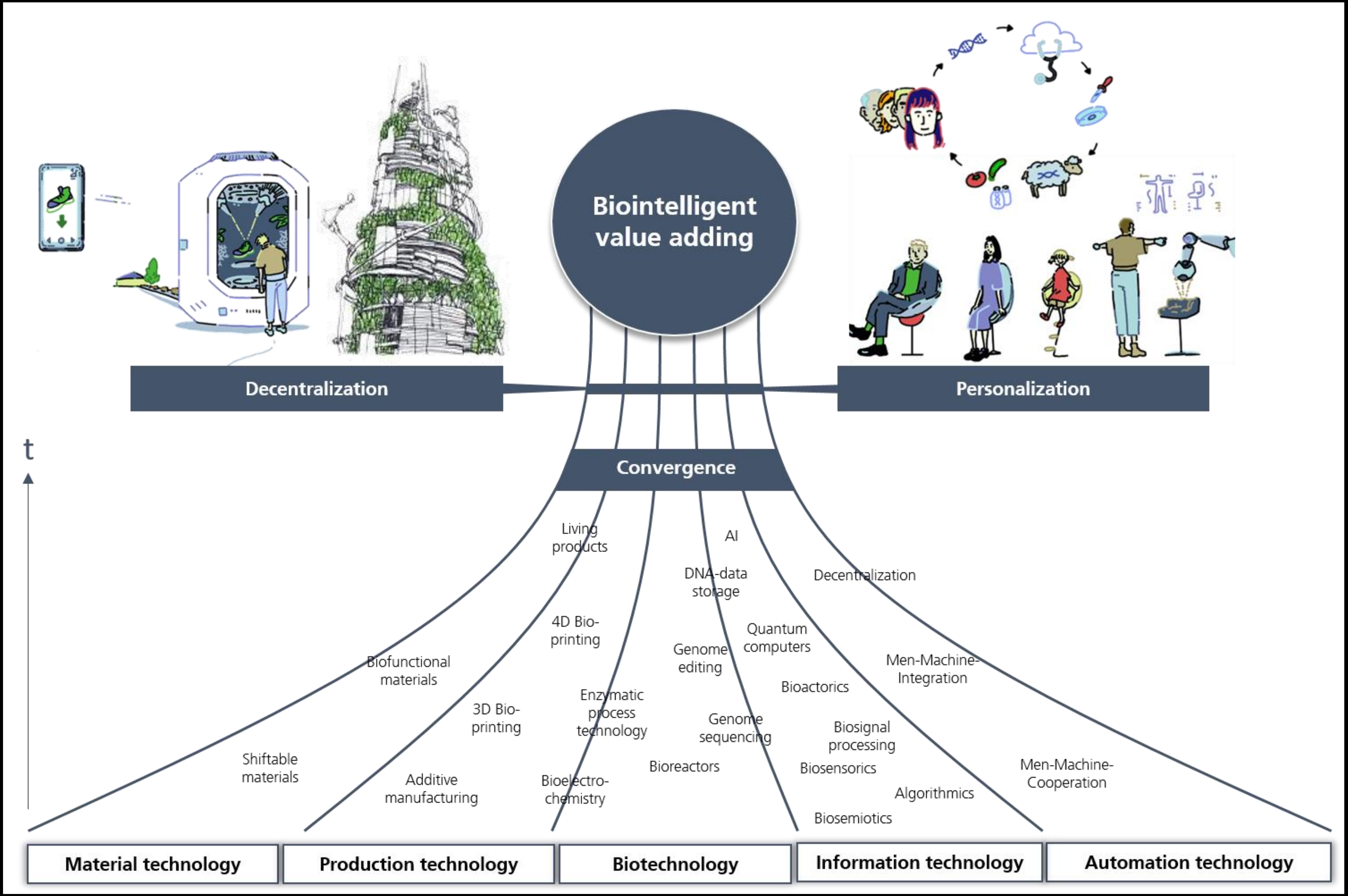
https://www.vttresearch.com/sites/default/files/julkaisut/muut/2017/syntheticbiologyroadmap.pdf

In English

https://www.vttresearch.com/sites/default/files/julkaisut/muut/2017/syntheticbiologyroadmap_eng.pdf

English version at MyCourses

Technology convergence in the context of a biological transformation



Towards Biointelligent Manufacturing

EU Manufacturing Platform

Merja Penttilä