Metabolic modelling and genetic circuit design in synthetic biology

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VTT Technical Research Centre of Finland Ltd

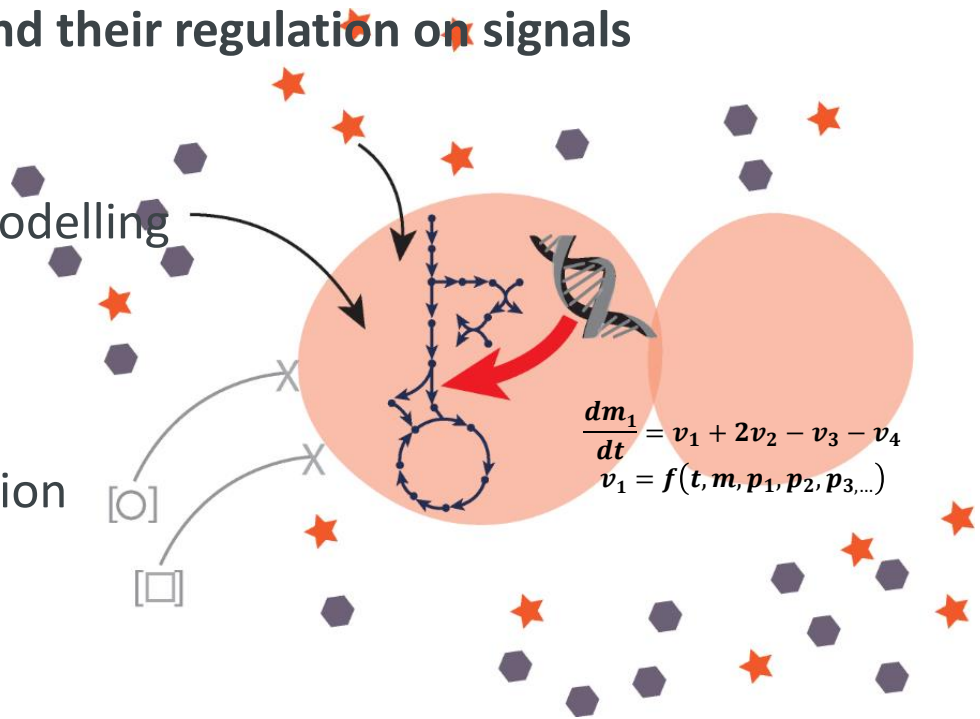
Scope

Background

- Synthetic biology adopts **engineering approach** for developing biological systems
- Biological systems include **functions and their regulation on signals** of the system state and prerequisites
- Engineering approach involves rational design using computational modelling

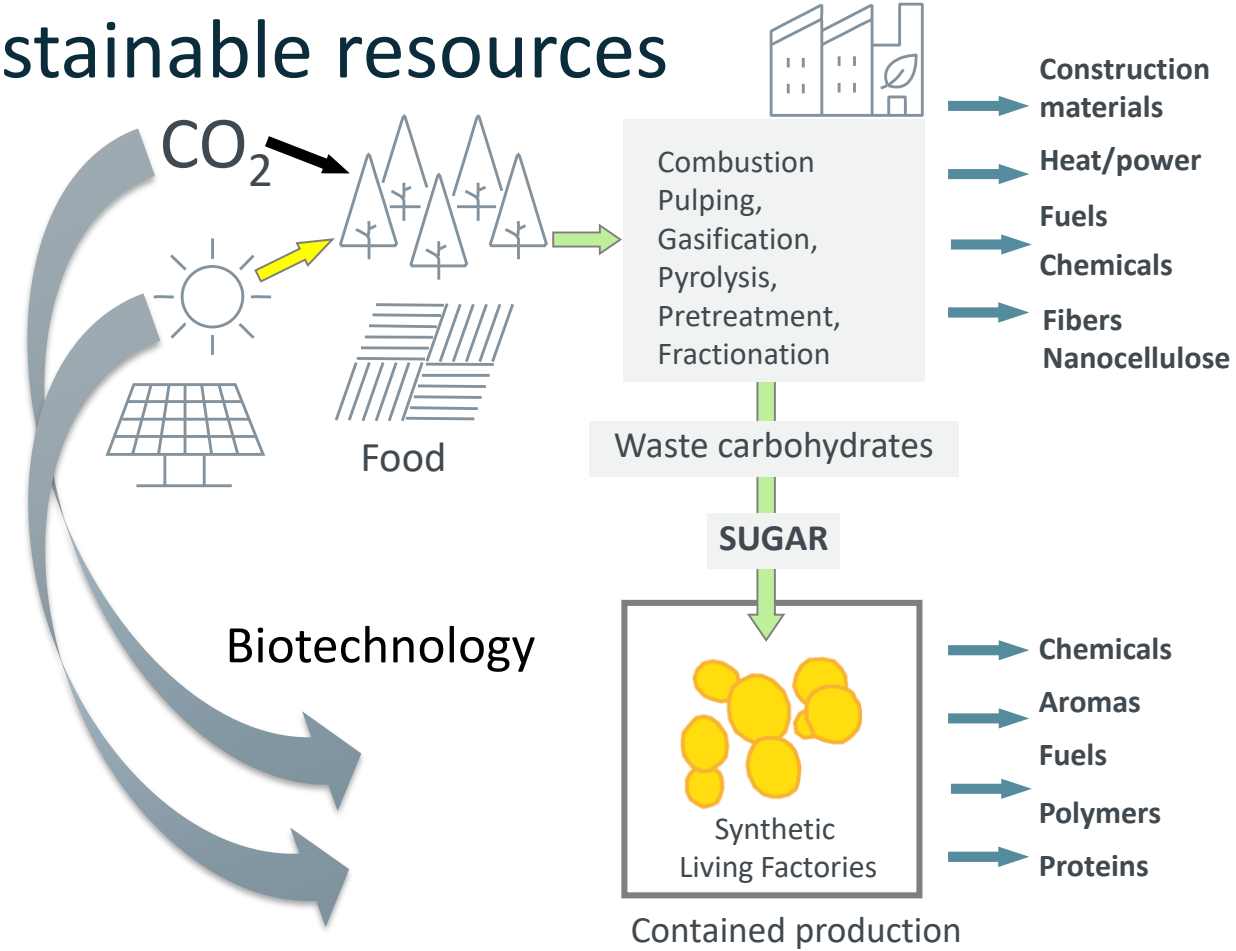
Lecture will introduce

- Metabolic modelling for designing synthetic pathways and host optimization
- Design of synthetic control circuits



Metabolic modelling for synthetic pathway design

Metabolism of living factories allow for valorising sustainable resources



Enormous possibilities of microbial bioconversions! Everything possible with synthetic biology?

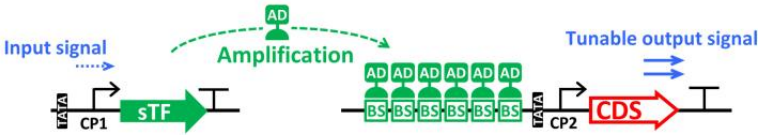
Synthetic biology tools enable creating new to nature living factories

Genome editing tools

www.genomicseducation.hee.nhs.uk/news/item/411-genome-editing-talking-to-patients/

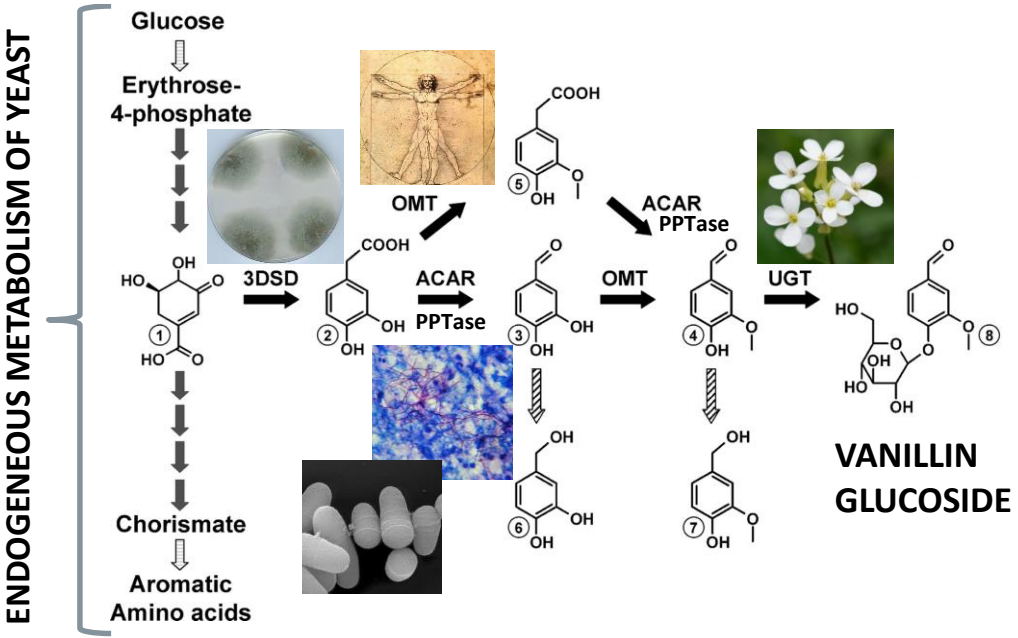
Universal synthetic expression system

Proprietary VTT Ltd



Rantasalo A. et al. Nucleic Acids Res. 2018

Synthetic vanillin production pathway



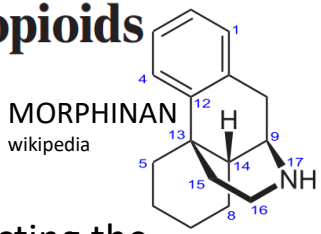
Hansen EH. et al. Appl. Environ. Microbiol. 2009

Synthetic pathway and strain optimization for opioid synthesis in yeast

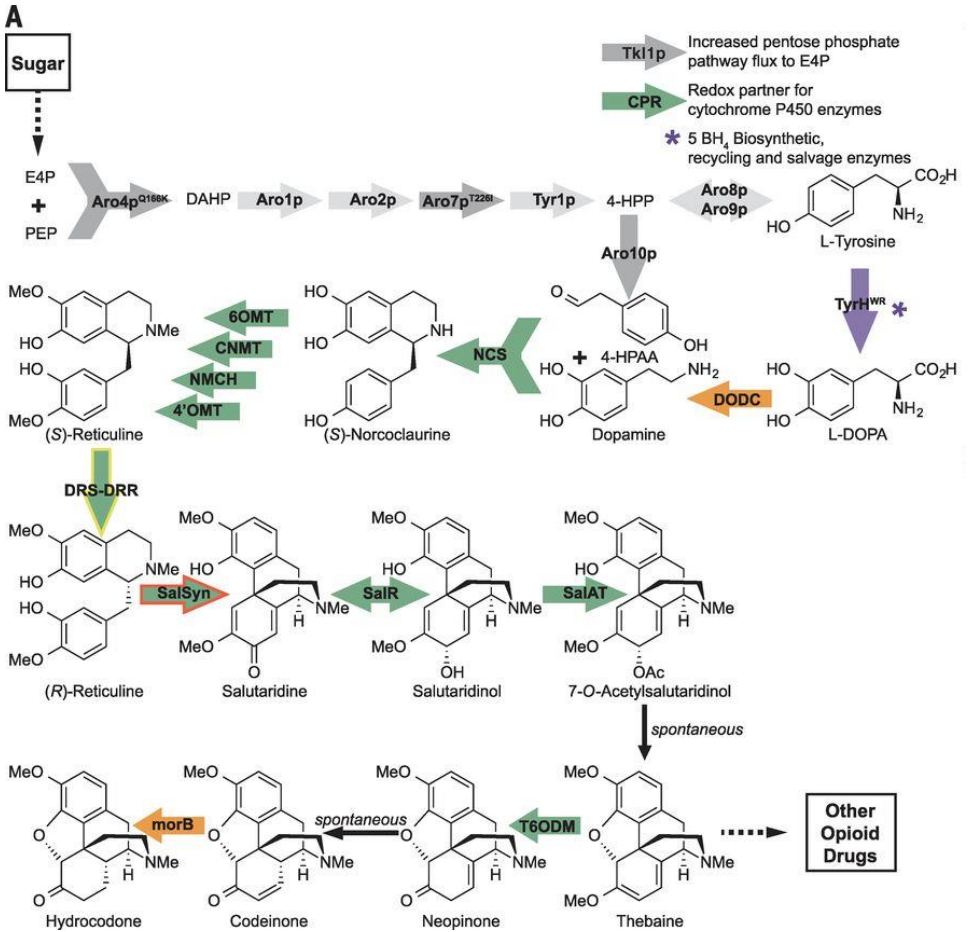
SYNTHETIC BIOLOGY

Complete biosynthesis of opioids in yeast

Stephanie Galanie,[1] Kate Thodey,[2] Isis J. Trenchard,[2] Maria Filsinger Interrante,[2] Christina D. Smolke[2]*



- Enzyme engineering for correcting the processing and increasing the activity of the key pathway cytochrome P450
- Expression of 21 heterologous enzymes from plants, mammals, bacteria, and yeast (color codes)
- Overexpression of two native yeast enzymes
- Deletion of one native yeast gene



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

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

LETTER

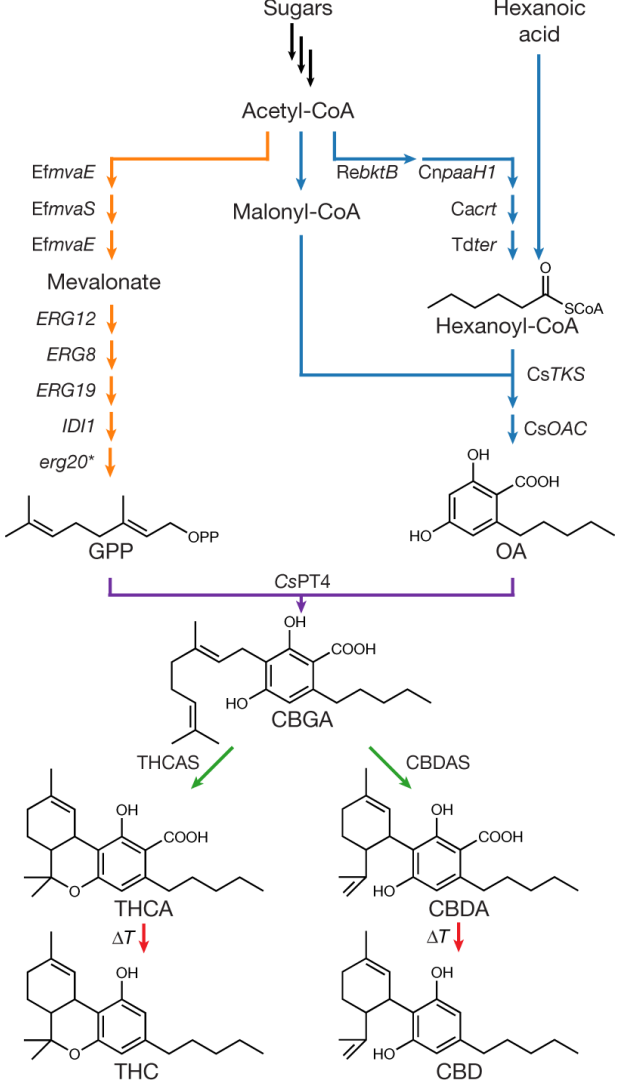
https://doi.org/10.1038/s41586-019-0978-9

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]*

7 MARCH 2019 | VOL 567 | NATURE | 123

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



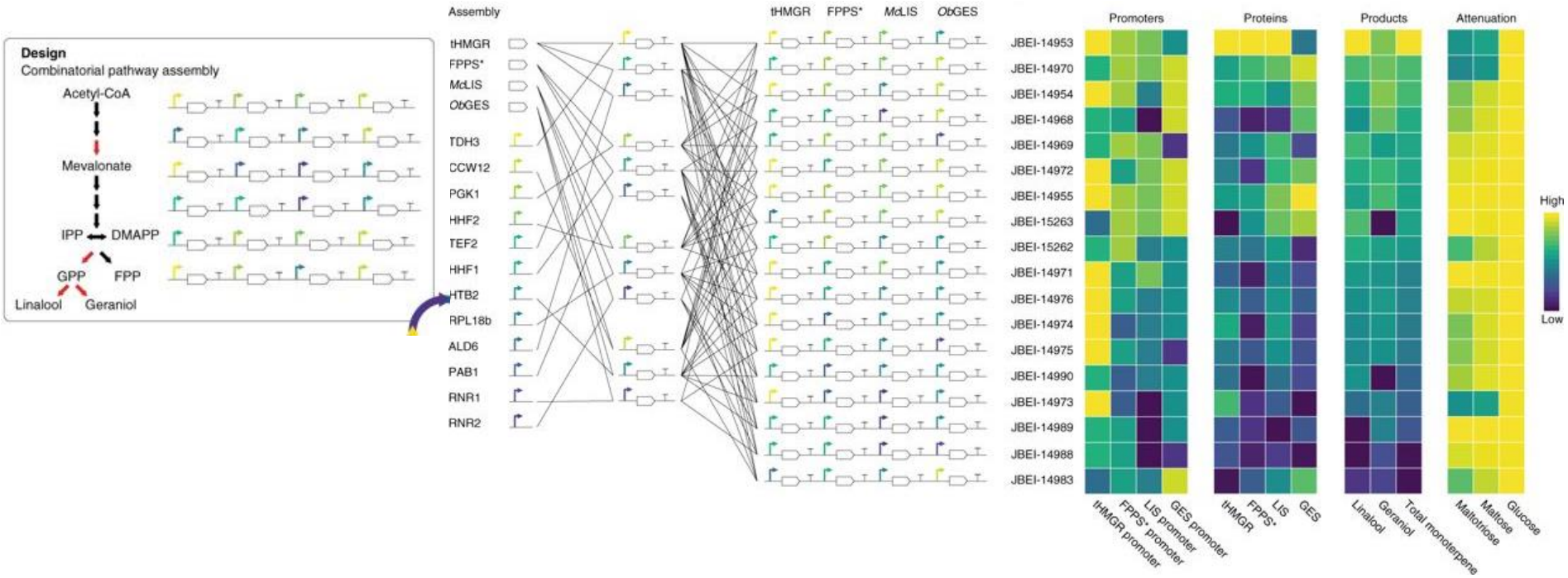
Hops aroma synthesis in yeast through combinatorial optimization of synthetic pathway

nature COMMUNICATIONS

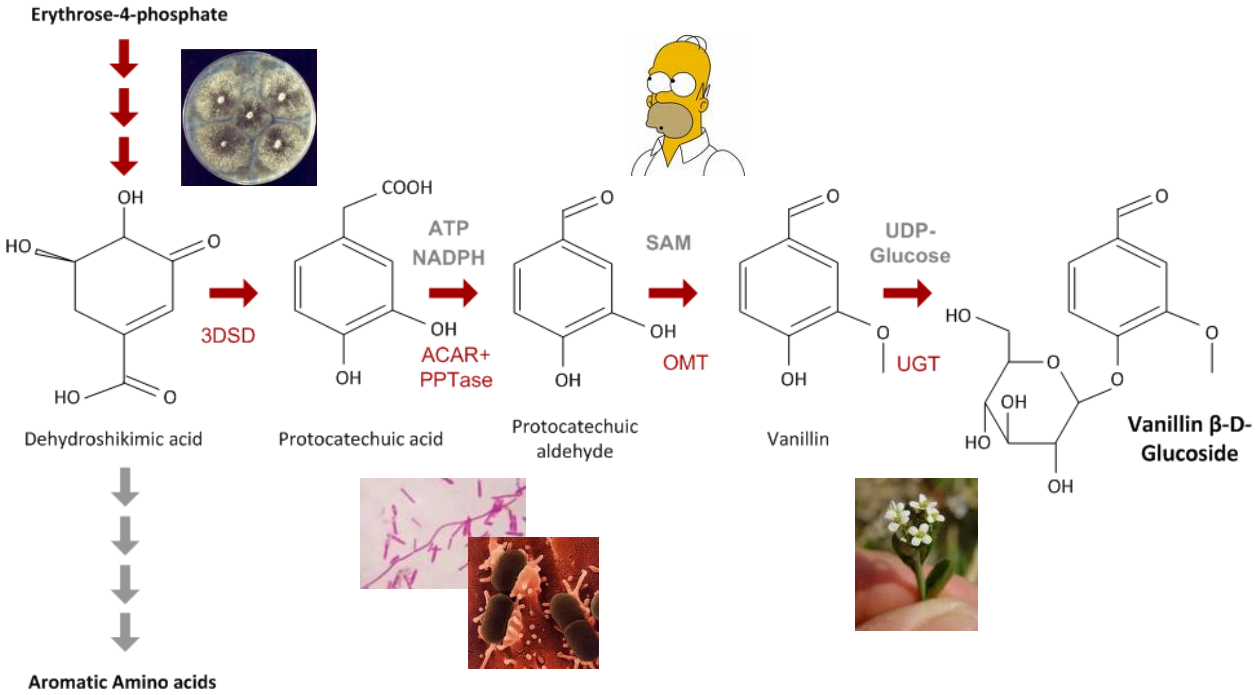
Industrial brewing yeast engineered for the production of primary flavor determinants in hopped beer

Charles M. Denby[1,2], Rachel A. Li[2,3,4], Van T. Vu[5], Zak Costello[2,4,6], Weiyin Lin[1,2], Leanne Jade G. Chan[2,4], Joseph Williams[7], Bryan Donaldson[8], Charles W. Bamforth[7], Christopher J. Petzold[2,4], Henrik V. Scheller[2,3,9], Hector Garcia Martin[2,4,6] & Jay D. Keasling[1,2,4,5,10,11]

NATURE COMMUNICATIONS | (2018)9:965



A synthetic pathway introduced in yeast for vanillin production



Hansen *et al.*, AEM, 2009
Brochado *et al.*, 2010

Slide from Dr. Kiran Patil

EMBL

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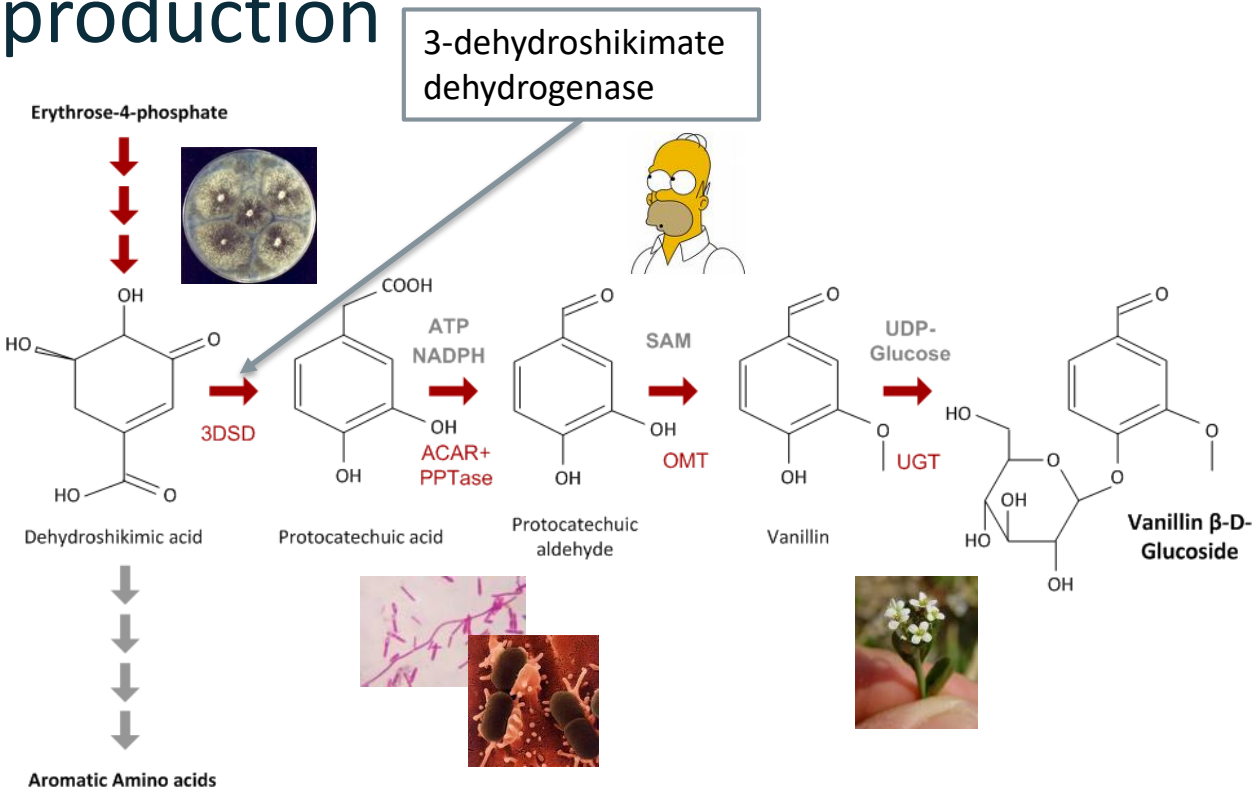

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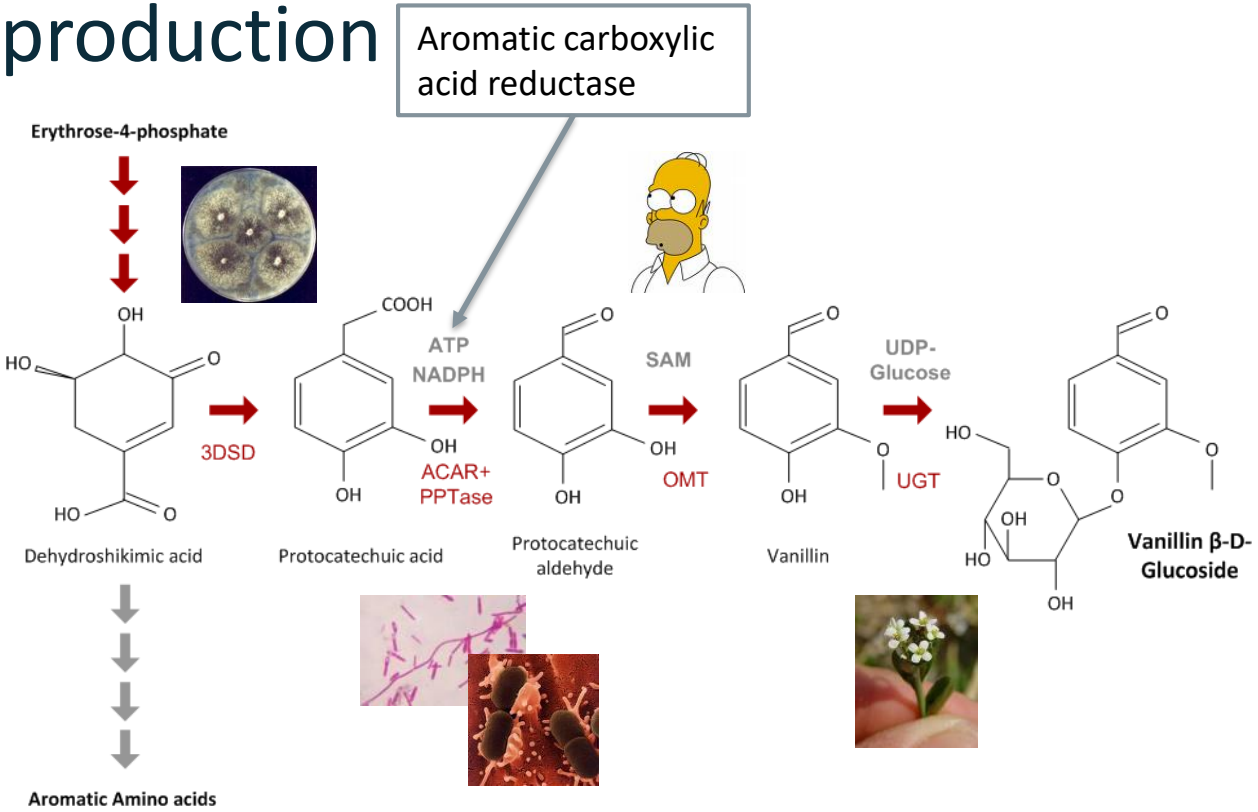

Hansen *et al.*, AEM, 2009
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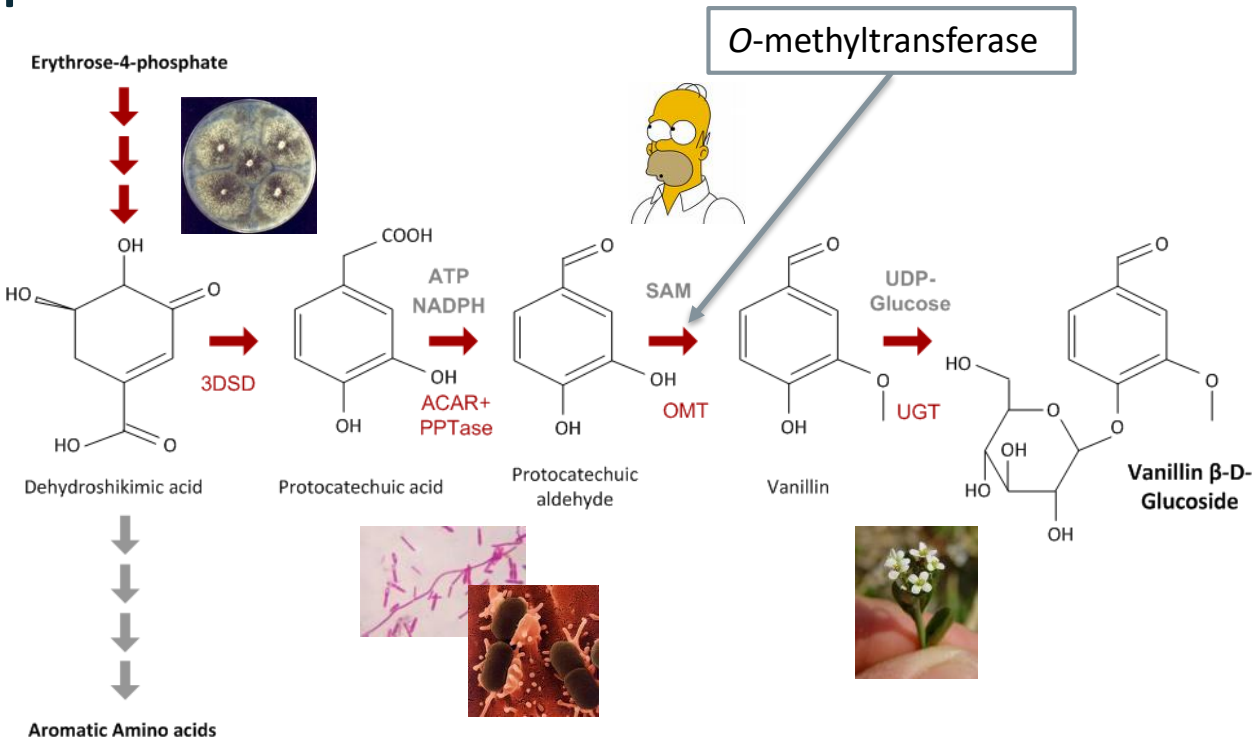

Hansen *et al.*, AEM, 2009
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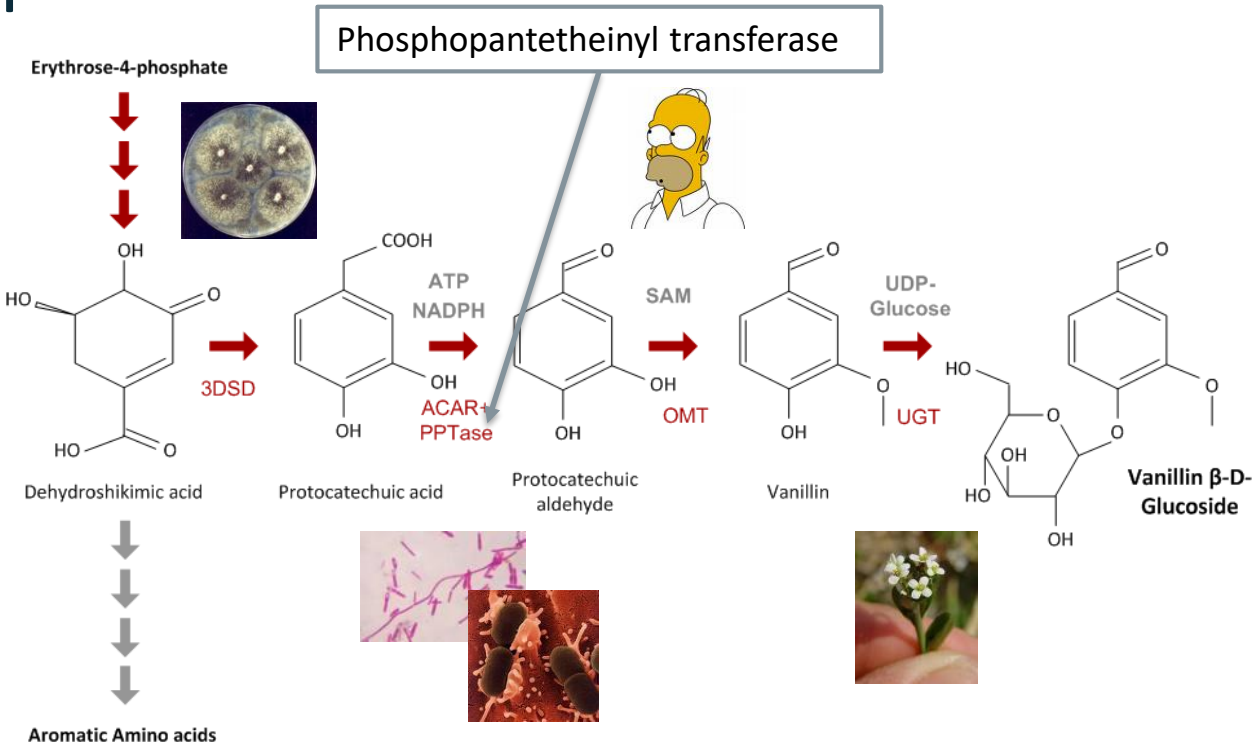

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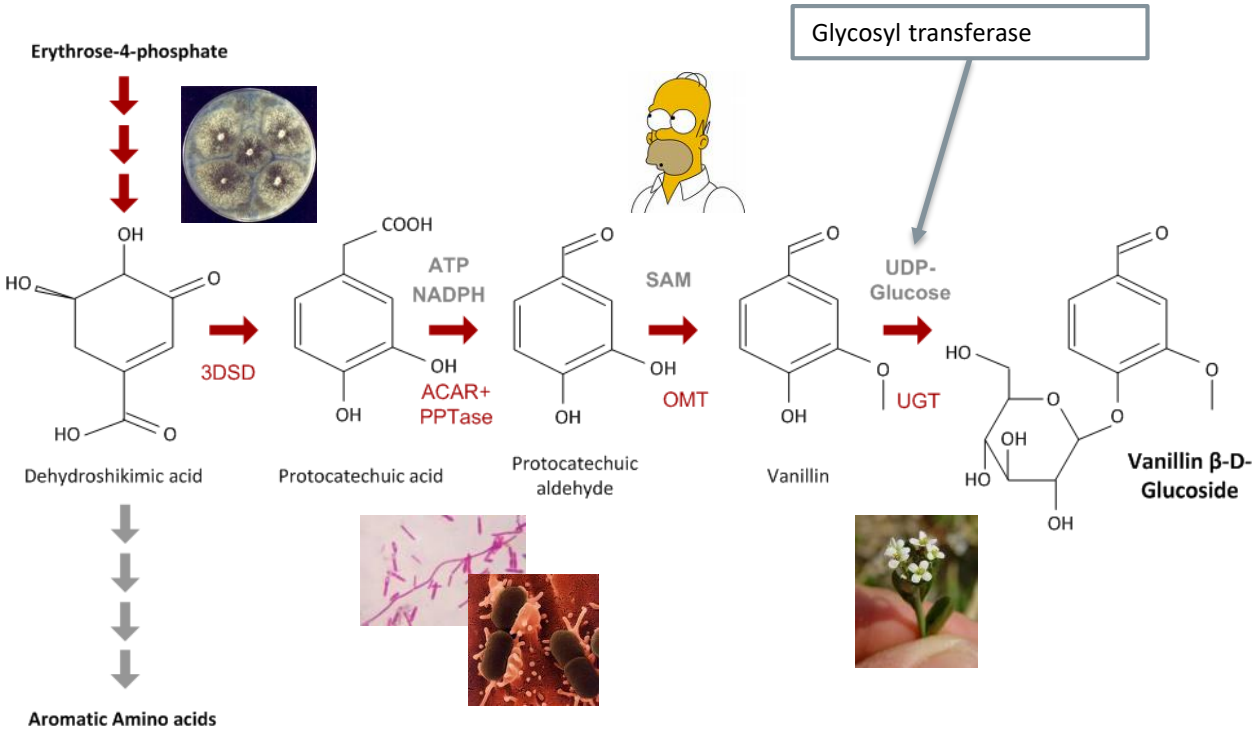

Hansen *et al.*, AEM, 2009
Brochado *et al.*, 2010

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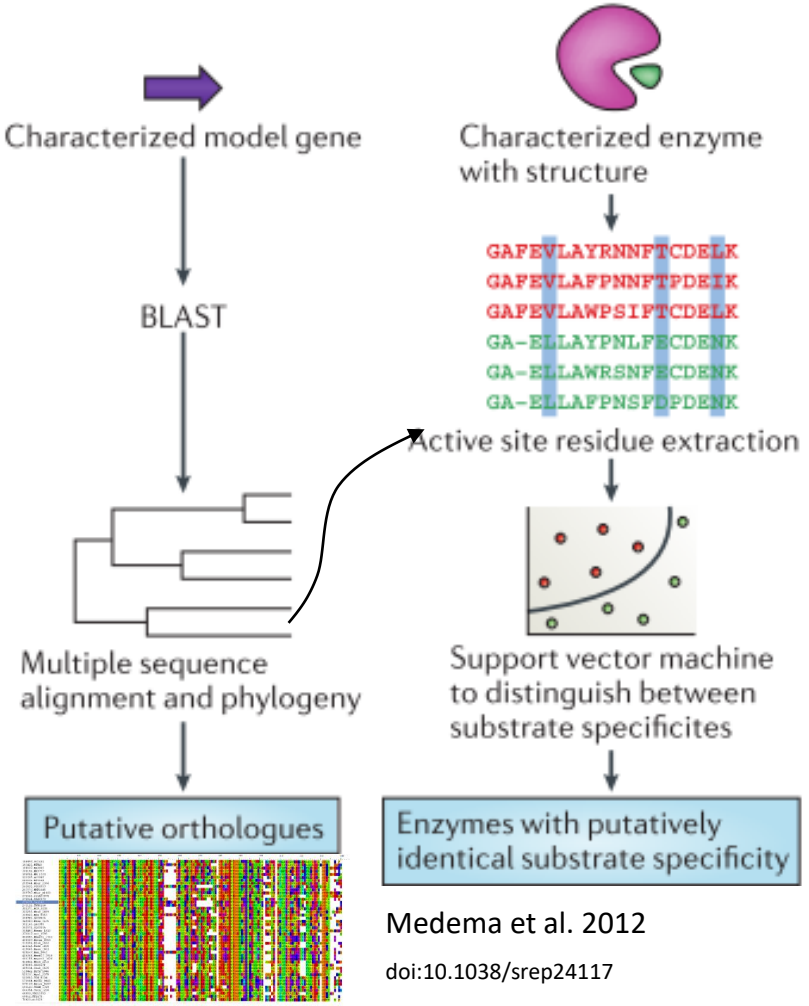
EMBL

Selecting the best enzyme candidates

- Gene/protein databases include references to enzyme mechanisms (e.g. EC numbers)
- Further candidates (orthologs) by genome mining of sequence databases
- Screening candidate performances



Medema et al. 2012

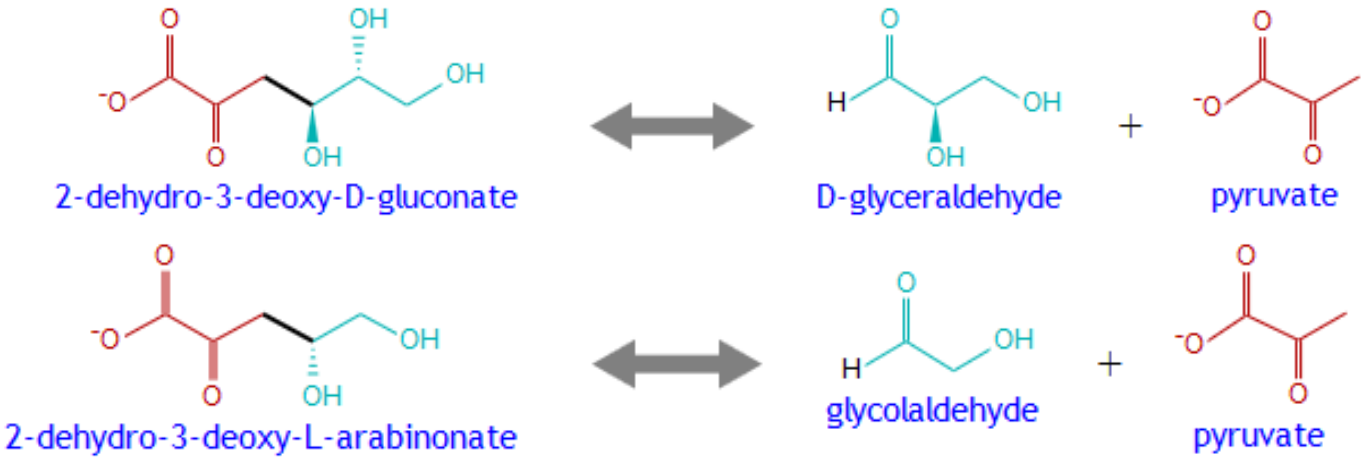
doi:10.1038/srep24117

doi:10.1038/nrmicro2717

Enzyme promiscuity as a source for new activities

- Enzymes are often capable of catalyzing alternative reactions
  - catalytic promiscuity = catalyze more than one reaction
  - substrate promiscuity = substrate ambiguity

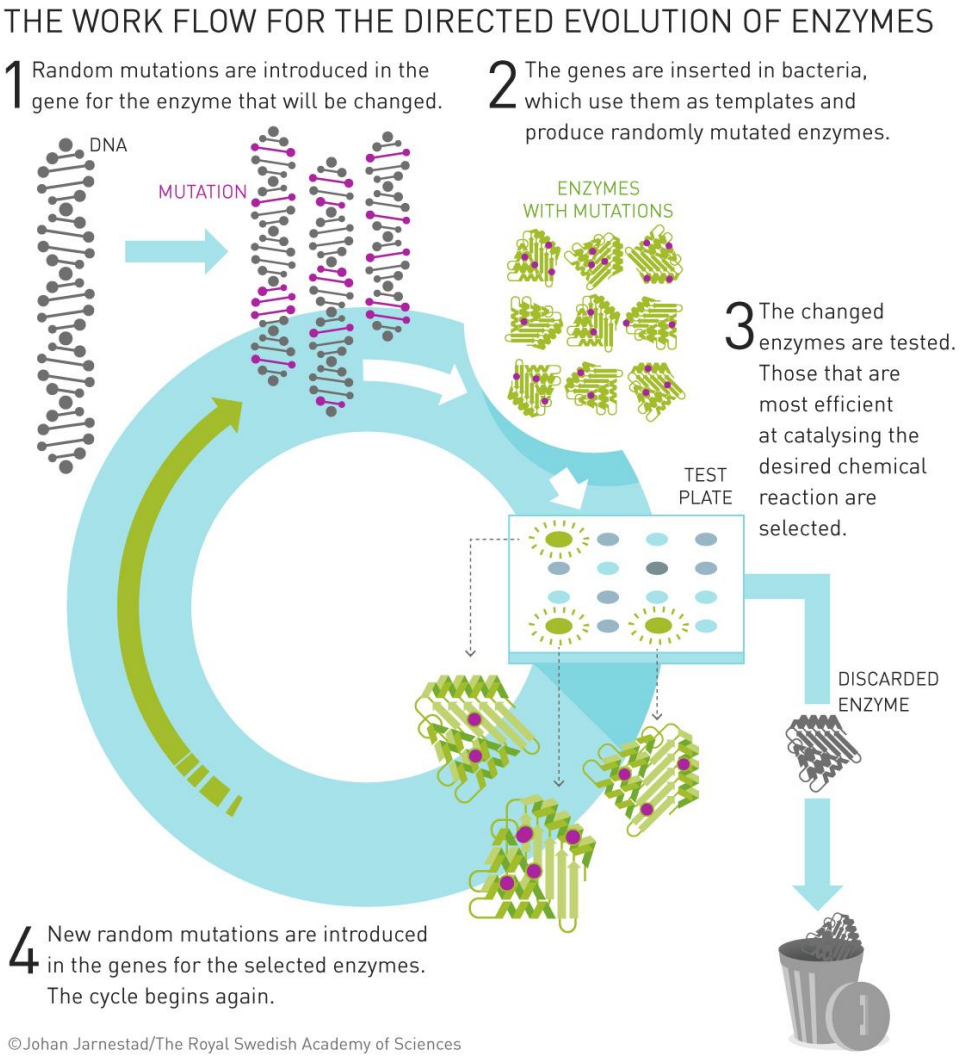
Reactions catalyzed by KDG aldolase from *Sulfolobus solfataricus*



http://www.jbc.org/content/279/42/43886.full.html
http://www.jbc.org/content/285/44/33701.long

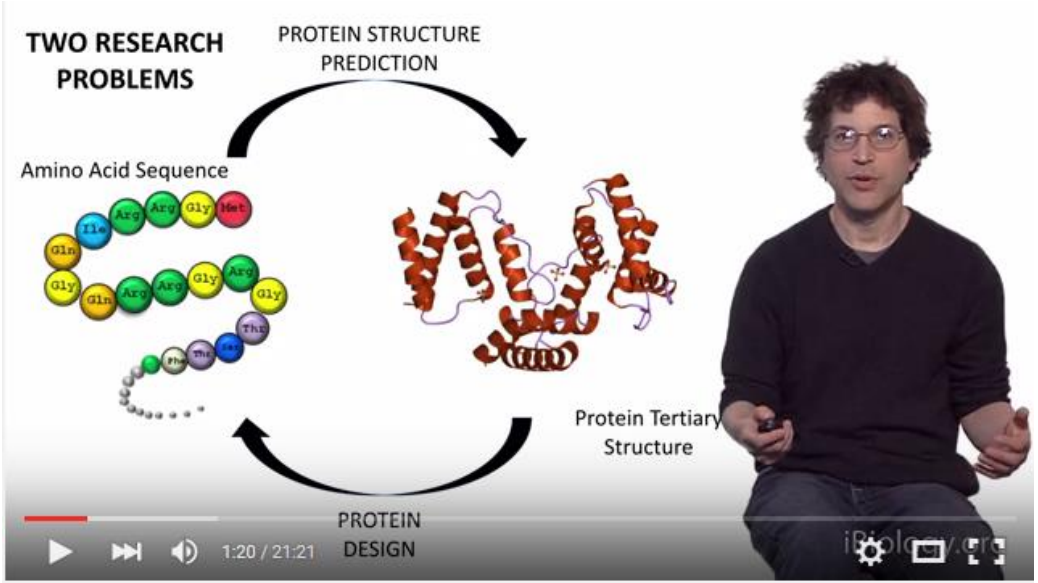
Frances H. Arnold received the Noble prize for protein directed evolution in 2018

https://www.quantamagazine.org/frances-arnold-george-smith-and-gregory-winter-win-chemistry-nobel-for-directing-evolution-20181003/



Design of new-to nature proteins

Which structure is needed for the desired function?



https://www.youtube.com/watch?v=0LetJMbu7uY

David Baker, PhD, Director of the Institute for Protein Design
"His research group is a world leader in computational protein design and protein structure prediction."
Rosetta computational prediction and design method

More recent talk: "The coming age of de novo protein design"
https://www.youtube.com/watch?v=z2YHy_bsiGU

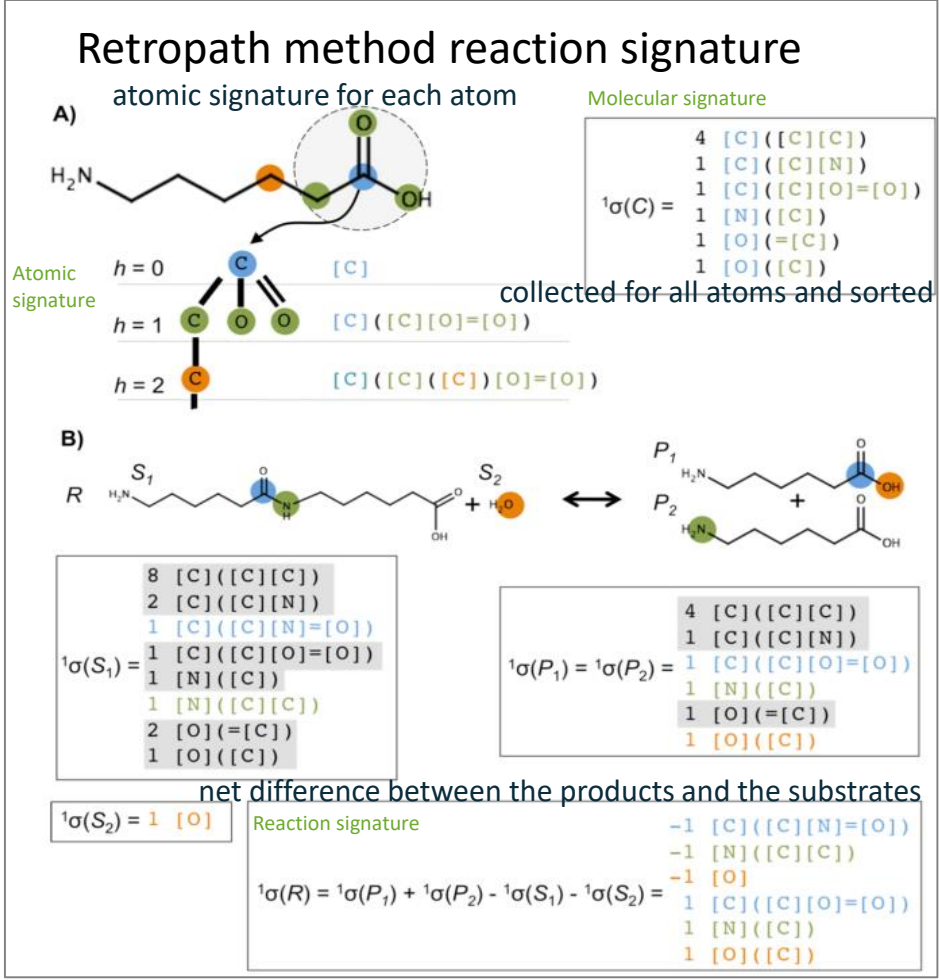
Reaction rules extend the reaction space to novel reactions

- How reaction rules are defined, differs by algorithm
- Estimate similarities to known reactions (i.e. similarities of reactants)
- Assume that if the core of the reaction (where the bonds break) remains the same then an enzyme could be found/built for the novel reaction
- Define different dimensions of the core

Table 1 Reactions in the EMRS

height *h*	reactions	% increase from canonical
2	9083	17.72%
3	7882	2.15%
4	7800	1.09%
5	7752	0.47%
6	7725	0.12%
canonical	7716	0%

Number of novel generated putative reactions in the EMRS for different heights *h*.

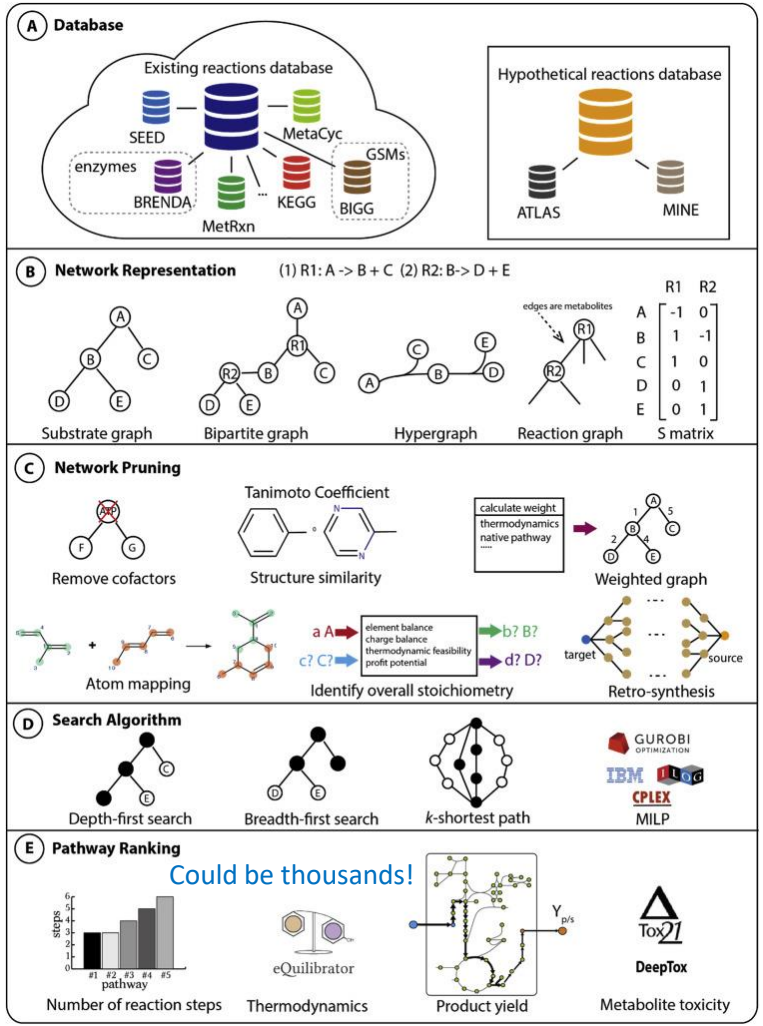


Carbonell, P., Planson, A.-G., Fichera, D., & Faulon, J.-L. (2011). A retrosynthetic biology approach to metabolic pathway design for therapeutic production. BMC Systems Biology, 5(1), 122.

General workflow for designing synthetic metabolic pathways

- (A) Construct a (extended) reaction database
- (B) represent it as a network, and (C) prune it
- (D) Choose the search algorithm for path enumeration (depends inherently on network representation)
- (E) Rank and select the candidates

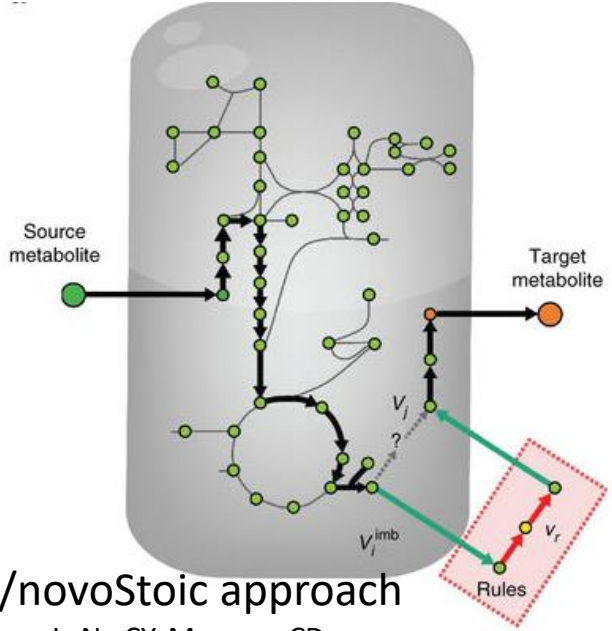
- Host selection?
- Strain design with the pathway(s)?
- Find enzymes
  - Find best natural sequence
  - Design new-to-nature proteins



Kumar et al. (2017) https://doi.org/10.1016/j.synbio.2017.11.002

rePrime reaction rules recruited for missing reaction steps

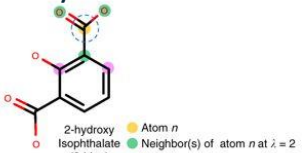
Moiety balances complement component balances as constraints in MILP formulation



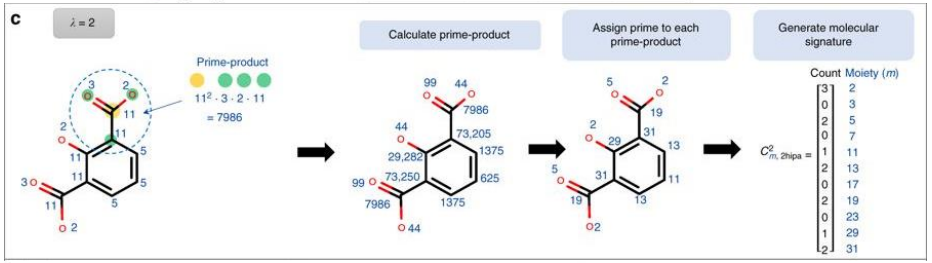
rePrime/novoStoic approach

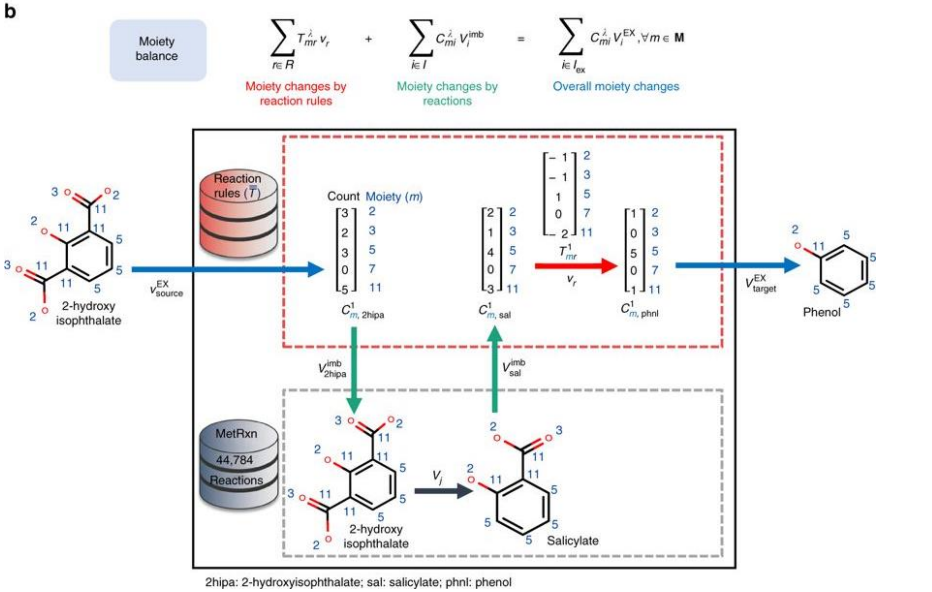
Kumar A, Wang L, Ng CY, Maranas CD.
Nat Commun. (2018) 9(1):184.
doi: 10.1038/s41467-017-02362-x.

Similarly as before reaction rules extracted from known reactions



Distance (λ)	1	2	3
Moiety	C		
# unique moieties	50	298	1100
# reaction rules	826	1929	6043





How to choose pathways for experimental implementation?

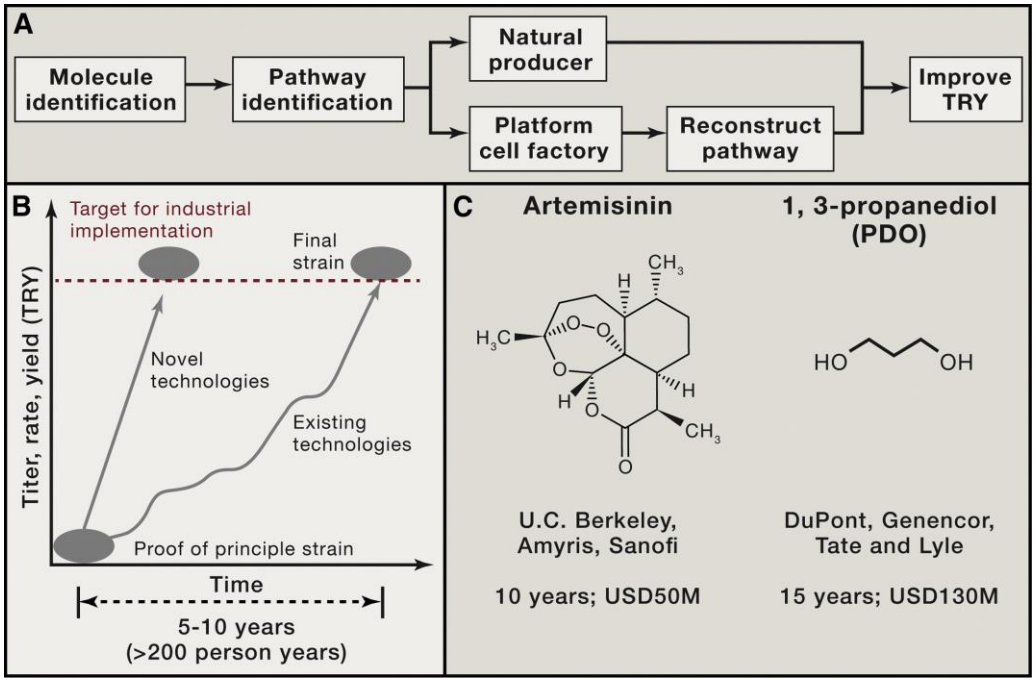
Criteria for ranking

- Yield
- Thermodynamics
- Pathway length
- Number of new-to-nature reactions
- Possible host
- Toxicity

Metabolic modelling for optimizing host metabolism

”From the first drop to the first truckload”

Microbial production routes have been demonstrated for many compounds, industrial processes for less many



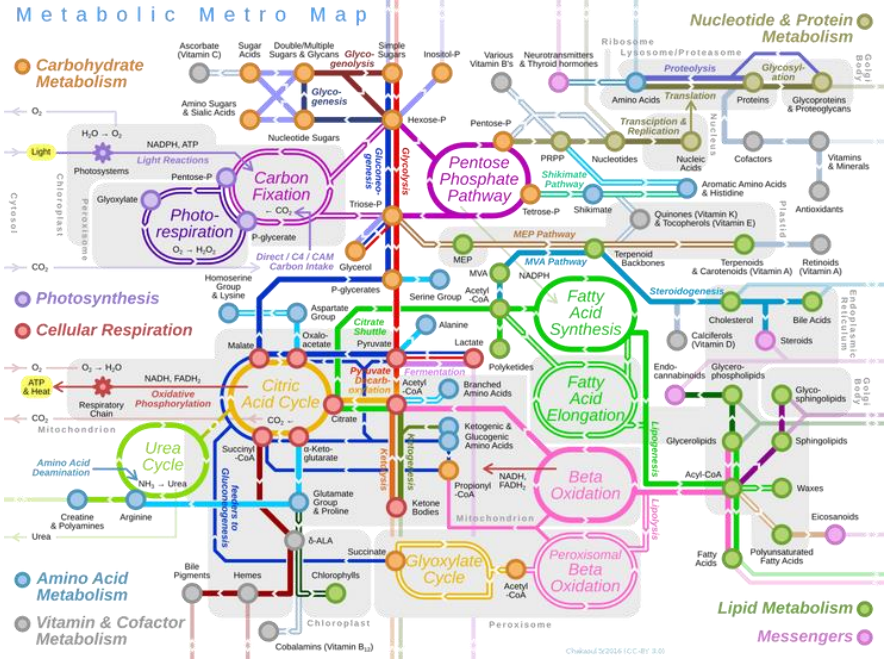
Nielsen J and Keasling JD. *Cell* 2016 164, 1185-1197DOI: (10.1016/j.cell.2016.02.004)

Review on possibilities: Lee SY et al. (2019) https://www.nature.com/articles/s41929-018-0212-4


YIELD
TITER
PRODUCTIVITY

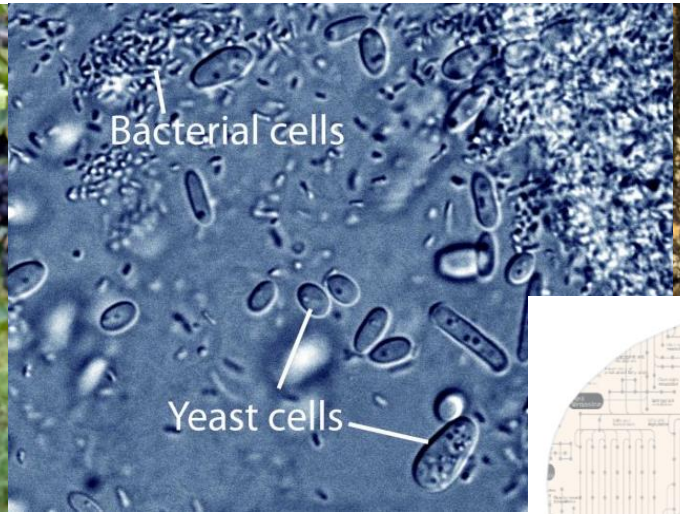
Microbial metabolism has immense biochemical conversion capabilities but it also serves essential functions for cells

- Catabolic metabolism generates energy
- Anabolic metabolism provides macromolecular building blocks
- Metabolism maintains cellular homeostasis (e.g. redox, pH, T, waste)



Wikipedia

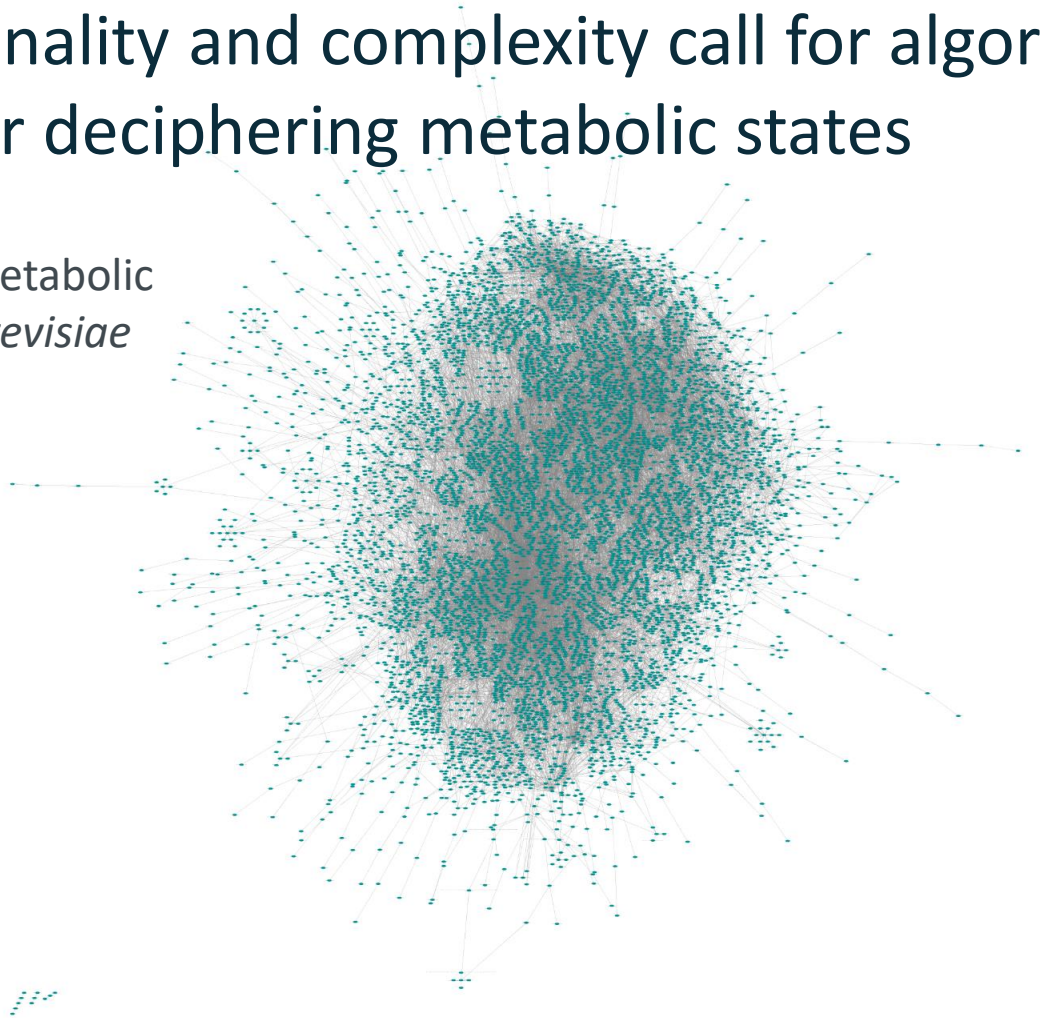
Distribution of resources in microbial metabolism is optimized for survival and growth



Mass conservation, enzymes, enzyme levels, affinities for substrates, kinetics, thermodynamics set how the resources are distributed

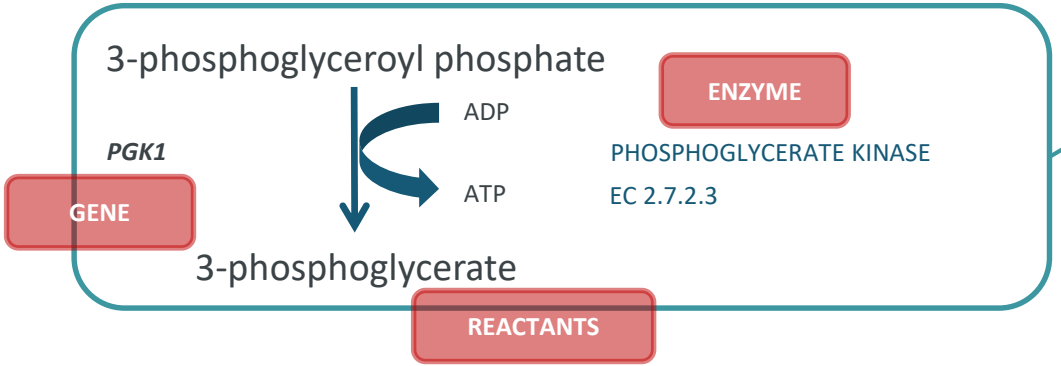
High dimensionality and complexity call for algorithmic approaches for deciphering metabolic states

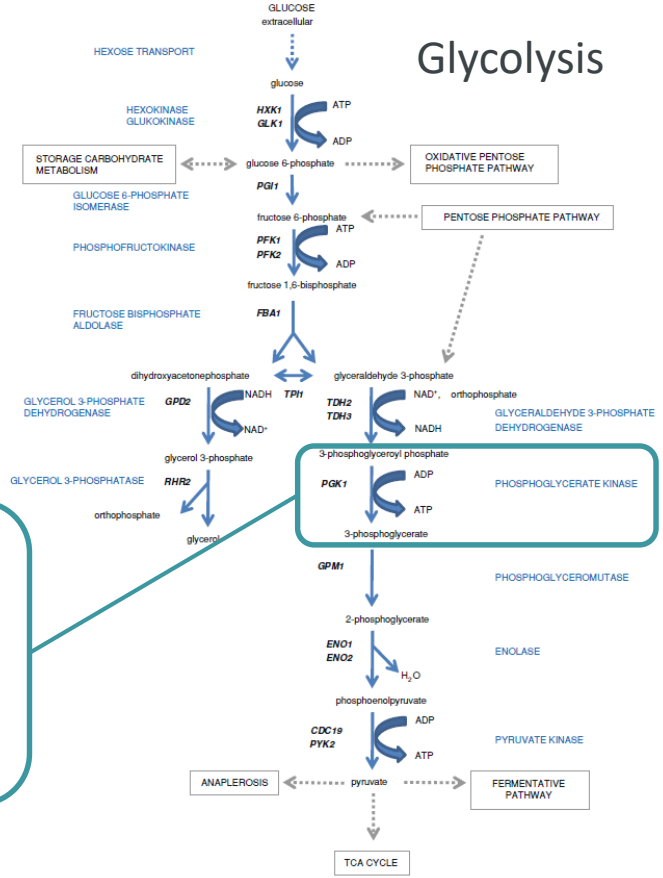
Genome-scale metabolic network of *S. cerevisiae*



Features of metabolic reactions

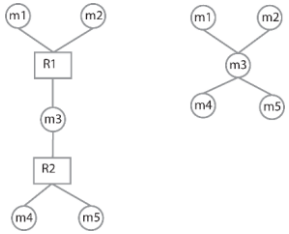
- Reactants: substrate(s) and product(s)
- Stoichiometry
- Enzyme catalyzing the reaction (EC number)
- Gene(s) encoding the enzyme (AND/OR rules)
- Rate law (~enzymatic mechanism)





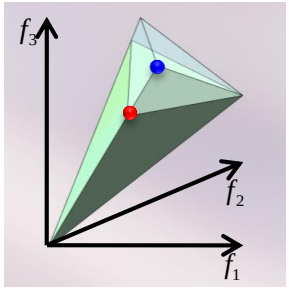
Models of metabolism

Graphs



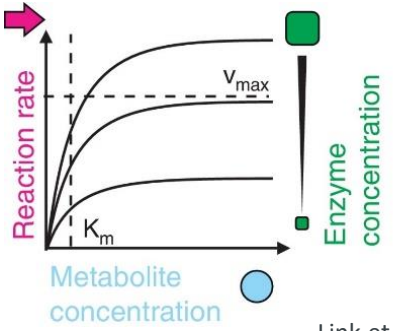
- Path finding, analyses of network structure

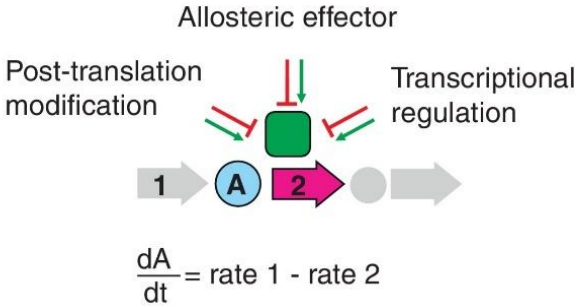
Constraint-based models



- Simulations of metabolic steady states constrained by mass conservation and thermodynamic laws

Kinetic models





Link et al. 2014

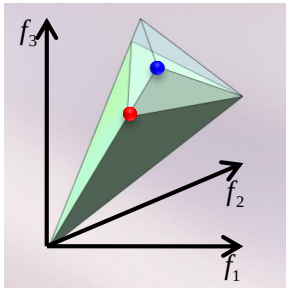
- Detailed predictions of metabolic dynamics requiring rate law and parameter information

Models of metabolism

Graphs

- Path finding, analyses of network structure

Constraint-based models



- Simulations of metabolic steady states constrained by mass conservation and thermodynamic laws

Kinetic models

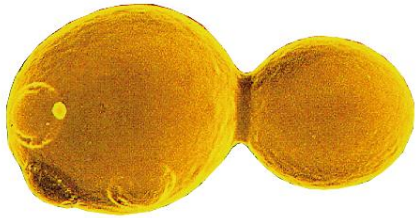
Link et al. 2014

- Detailed predictions of metabolic dynamics requiring rate law and parameter information

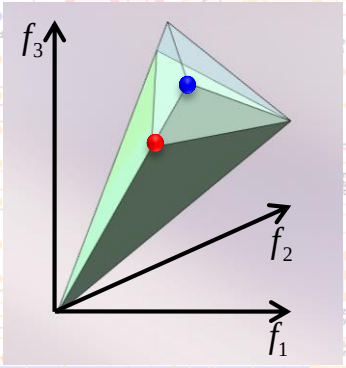
Genome-scale metabolic models can capture the translation of genotype to phenotype

Stoichiometric models

Also called constraint-based models



Few thousand reactions encoded in the genome (as ~1000 metabolic genes)



Mass balance constraints + Biological optimality principles → Metabolic phenotype (LP/QP problems)

Sv = 0

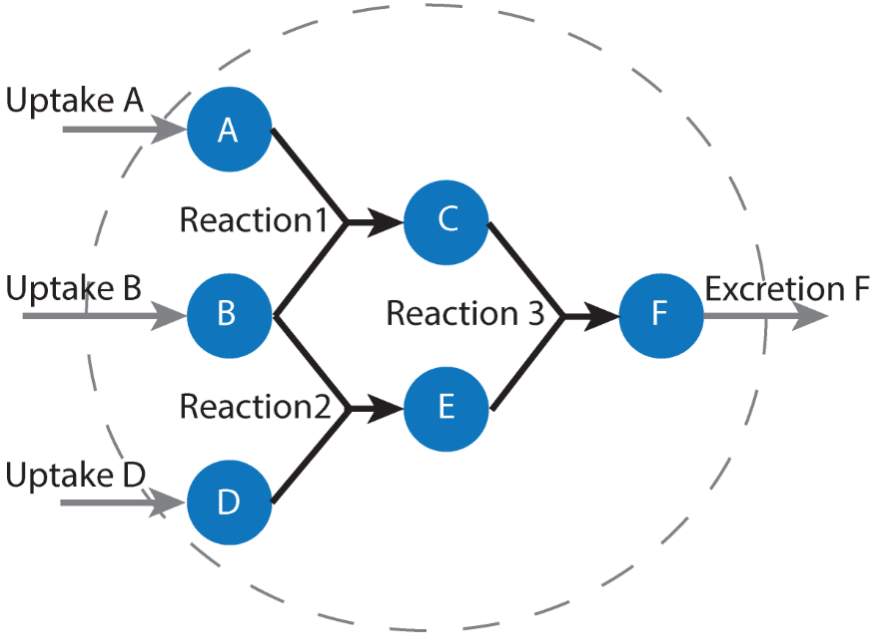
e.g. growth

Flux balance analysis (FBA): Varma and Palsson, 1993; Varma and Palsson, 1994

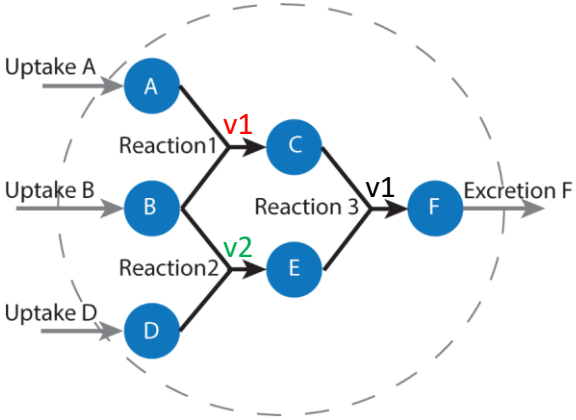
Slide modified from Dr. Kiran Patil

EMBL

Flux balance analysis (FBA) with a toy constraint-based metabolic model



Metabolite mass balances form linear constraints on metabolic fluxes under steady state



Reaction equations

Reaction 1: A + 2 B -> C
Reaction 2: B + D -> E
Reaction 3: C + E -> F
⋮ ⋮

Metabolite mass balances

A: Uptake A -v1
B: Uptake B -2 v1 -v2
C: v1 -v3
D: Uptake D -v2
E: v2 -v3
F: v3 -Excretion F

n Reactions

m Metabolites	R1	R2	R3 ...
A	-1	0	0 ...
B	-2	-1	0 ...
C	1	0	-1 ...
D	0	-1	0 ...
E	0	1	-1 ...
F	0	0	1 ...
⋮			

m x n Stoichiometric matrix

x

Fluxes

v1
v2
v3
...

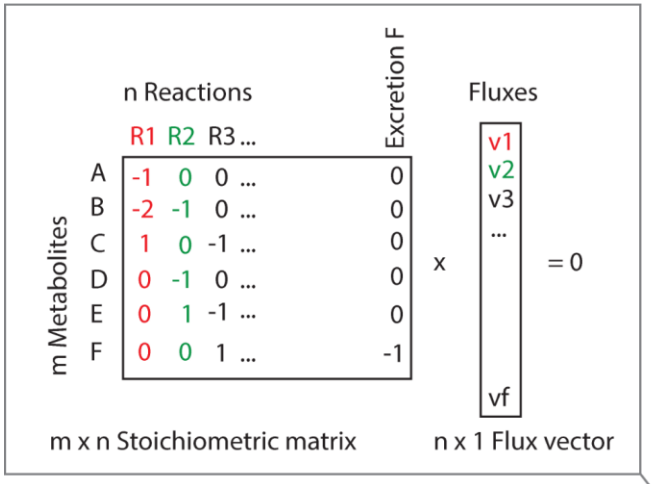
n x 1 Flux vector

= dM/dt
(time derivatives of metabolite concentrations)

Under steady state
= 0

Steady state assumption renders system linear and free from kinetic parameters

Objective function defined for identifying points of optimality in the space of feasible states



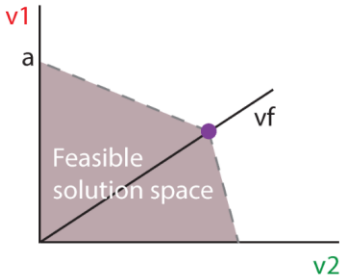
Objective function for optimizing excretion of F

$c'v =$ [0 0 0 ... 1] x [v1, v2, v3, ..., vf]

Sets vf to be optimized

Check tutorial: Orth, J. D., Thiele, I., & Palsson, B. Ø. (2010). What is flux balance analysis? Nat Biotechnol, 28(3), 245–248. doi:10.1038/nbt.1614.What

Optimizing the excretion of F using linear programming



$\max c \cdot v,$

s.t.

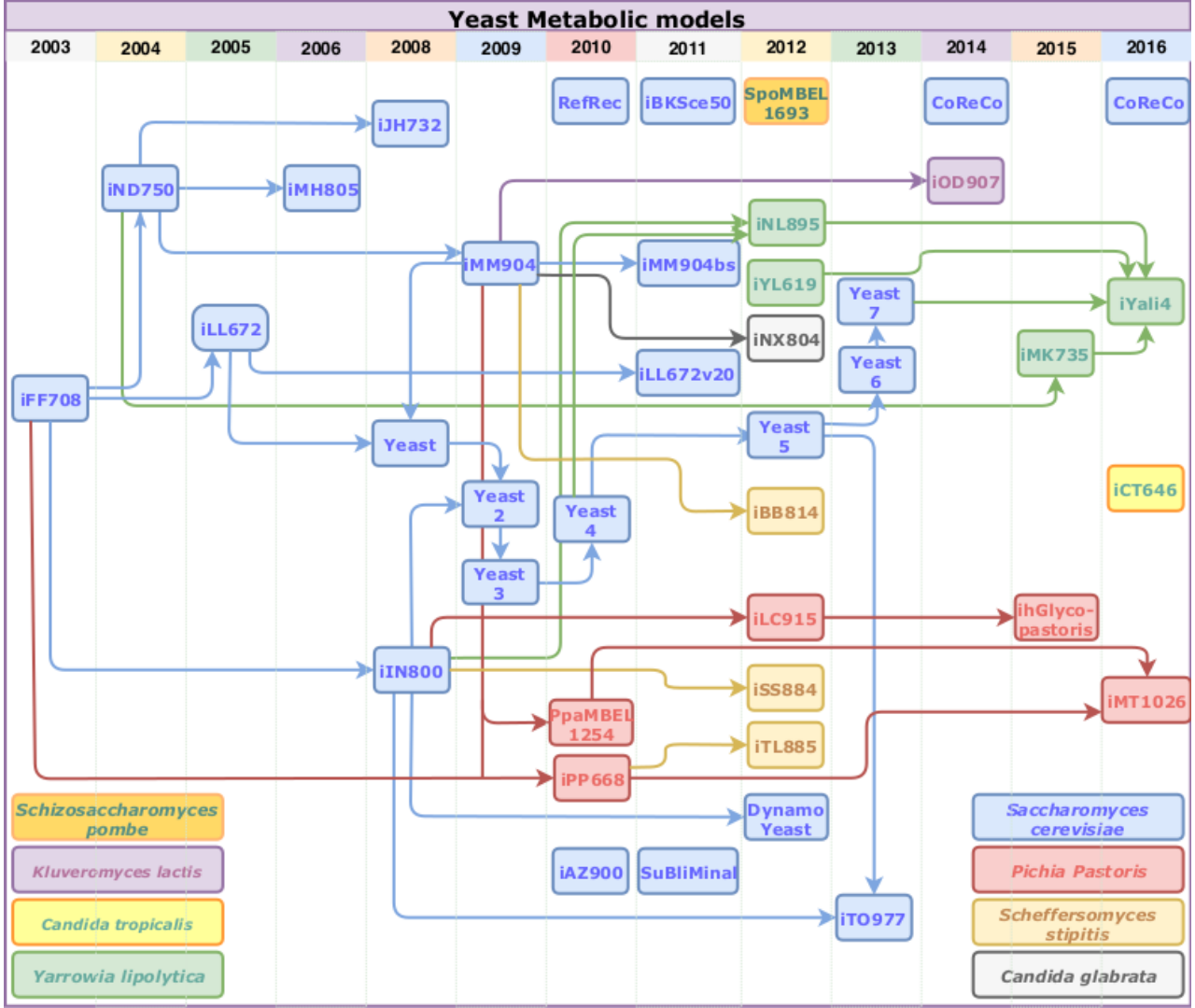
$S v = 0$ Mass conservation constraints

$0 \le v1 \le a$

$0 \le v2 \le \infty$ Thermodynamic and capacity constraints

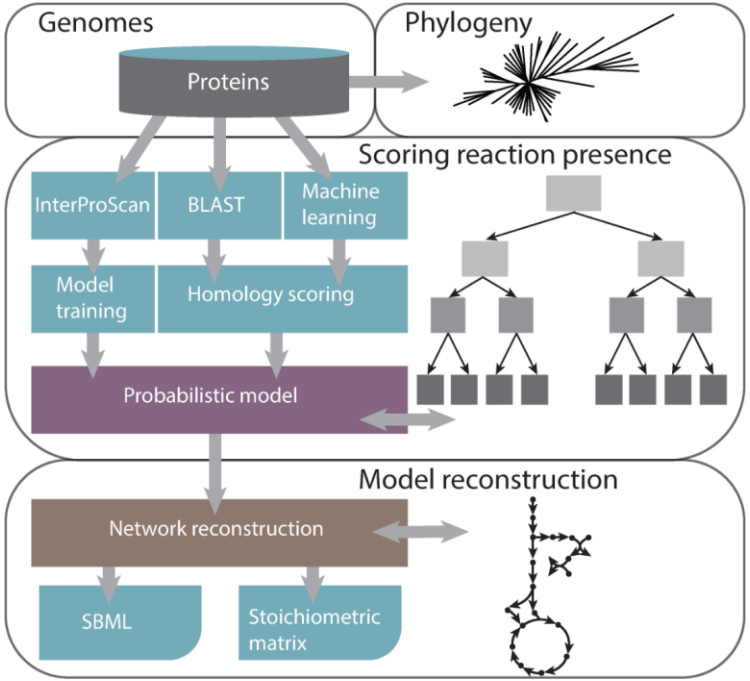
Defining alternative objective functions allows exploring the capabilities of the network

Manually curated models available for model organisms

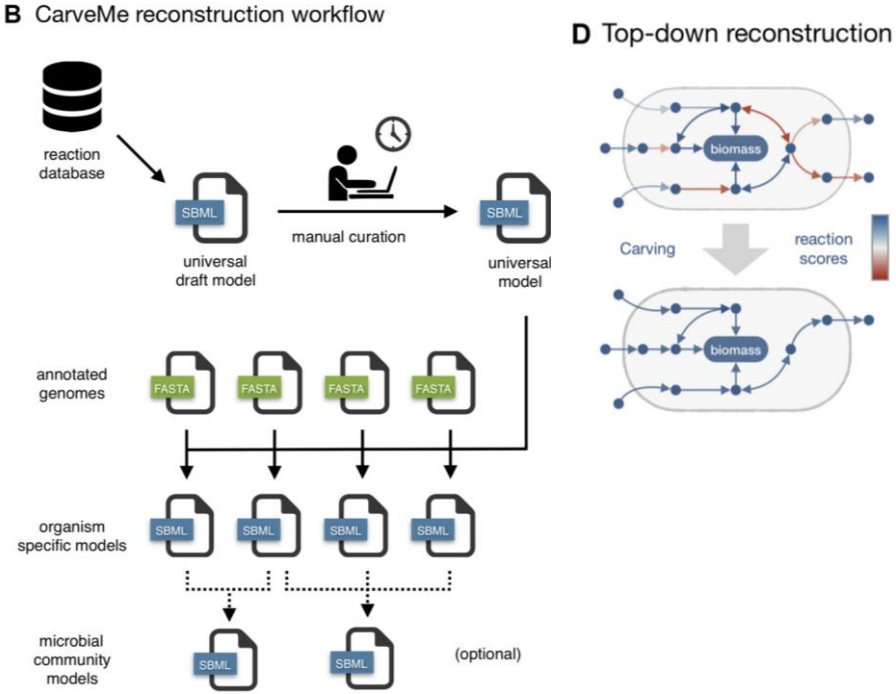


Genome-scale metabolic model reconstruction automatically from genome

Comparative reconstruction with CoReCo (Pitkänen et al. 2014; Castillo et al. 2016)

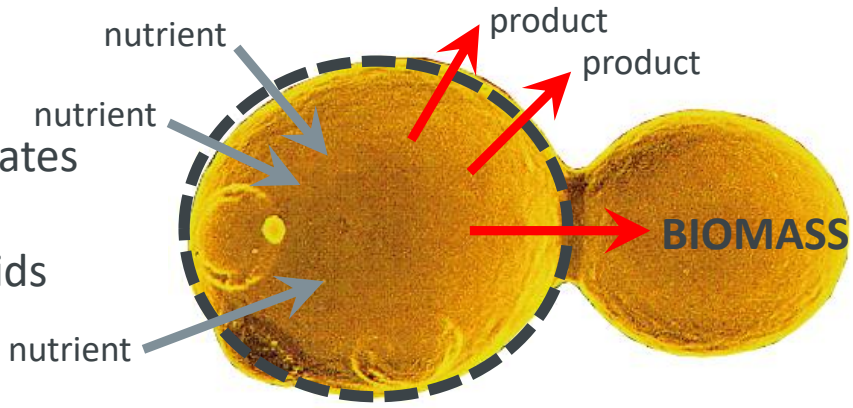


Top-down reconstruction with CarveMe (Machado et al. 2018)



What are cells made of?
Defining biomass equation

Protein
Carbohydrates
Lipids
Nucleic acids



Proportions and exact compositions are species, strain, and condition dependent

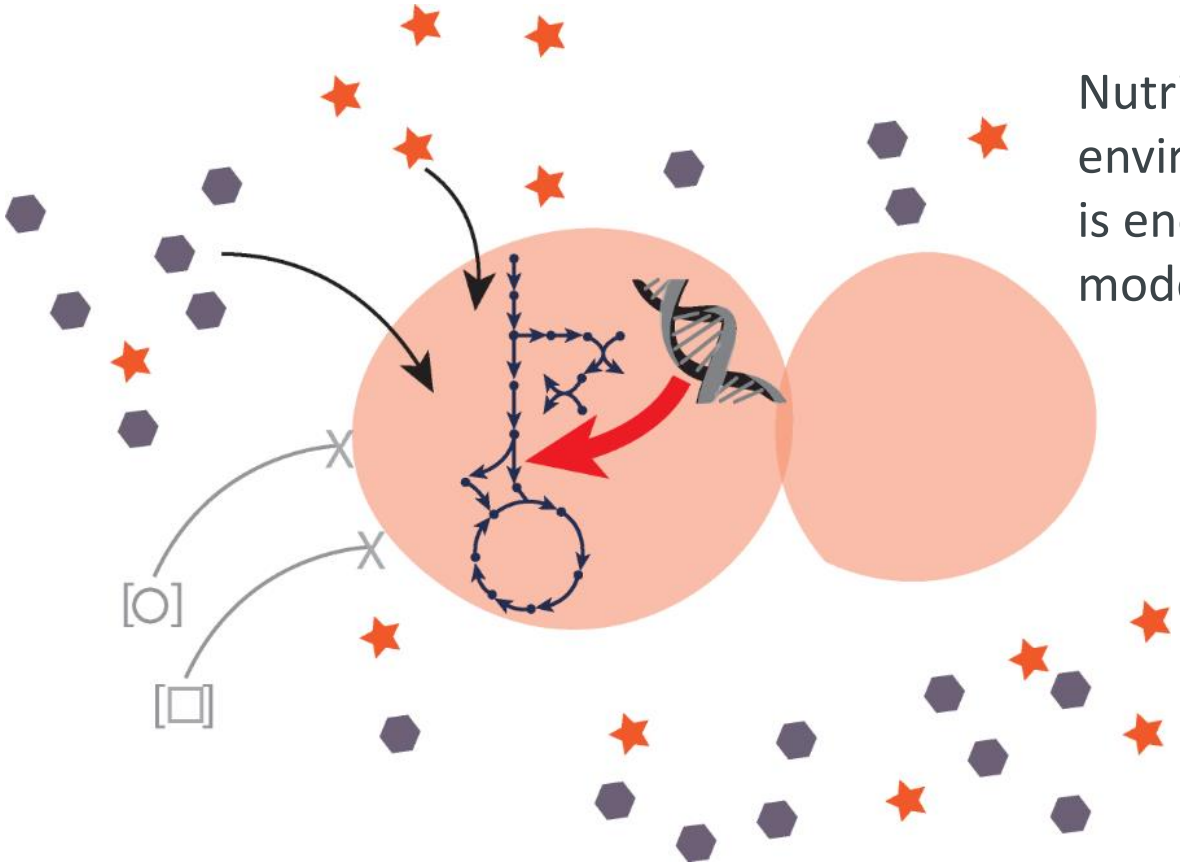
Biomass equation commonly describes the energy and redox balancing requirements of synthesizing macromolecules

Dilution of other intracellular metabolites due to cell division is neglectable and omitted in simulations

Universally Essential Cofactors in Prokaryotes
Xavier JC et al. (2017) Metab Eng.

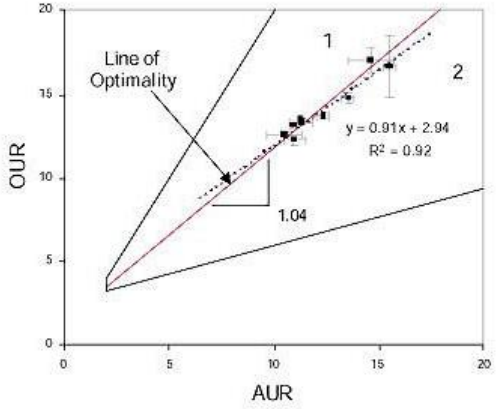
Organic cofactor(s)	BOFs of manually-curated GEMs (1)	A. Biosynthesis genes are essential (2)	B. Participates in essential reactions (3)	C. Reviewed Evidence: ModelSEED (4)	C. Reviewed Evidence: Literature (5)	Essentiality	Functional role
NAD(H)						Universal	Transport and transfer of hydride groups.
NADP(H)						Universal	Transport and transfer of hydride groups.
S-adenosyl-methionine						Universal	Universal methyl donor; generator of deoxyadenosyl radicals.
FAD						Universal	Electron transfer, radical and photoreceptor-induced reactions.
Pyridoxal 5p						Universal	Electrophilic catalyst
Coenzyme A						Universal	Transport and transfer of acyl groups
C1 carriers (derivatives of H(4)-MPT or H(4)folate)						Universal	Transport and donation of C1 units
Thiamin diphosphate						Universal	Making and breaking bonds between C and S, O, H and N atoms, and most notably C-C bonds
FMN						Universal	Electron transfer, radical and photoreceptor-induced reactions.

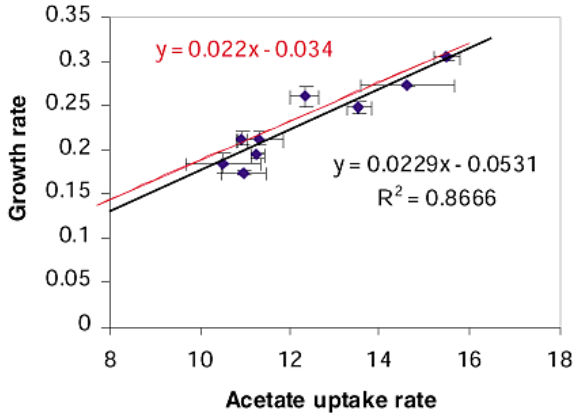
FBA simulations can be performed in a condition-dependent manner



Nutritional environment of cells is encoded in the model simulations

FBA simulations optimizing growth predict well experimental phenotypes





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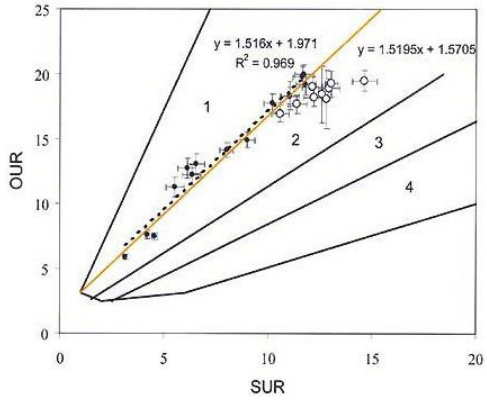
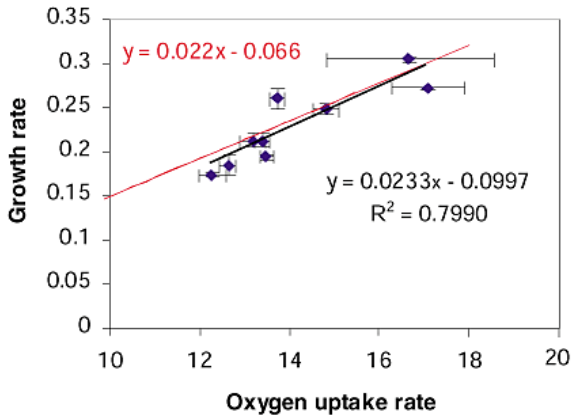
In silico predictions of *Escherichia coli* metabolic capabilities are consistent with experimental data

Jeremy S. Edwards[1,2], Rafael U. Ibarra[1], and Bernhard O. Palsson[1*]

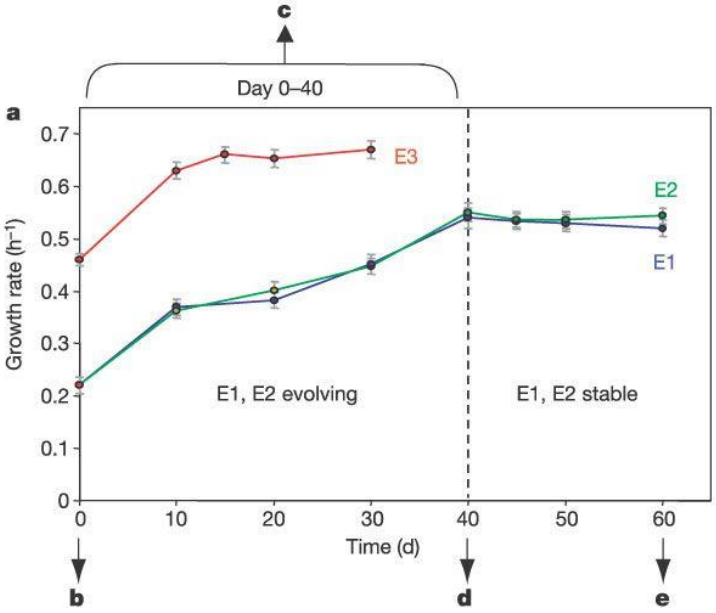
[1]Department of Bioengineering, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0412. [2]Current address: Department of Chemical Engineering, University of Delaware, Newark, DE 19716. *Corresponding author (palsson@ucsd.edu).

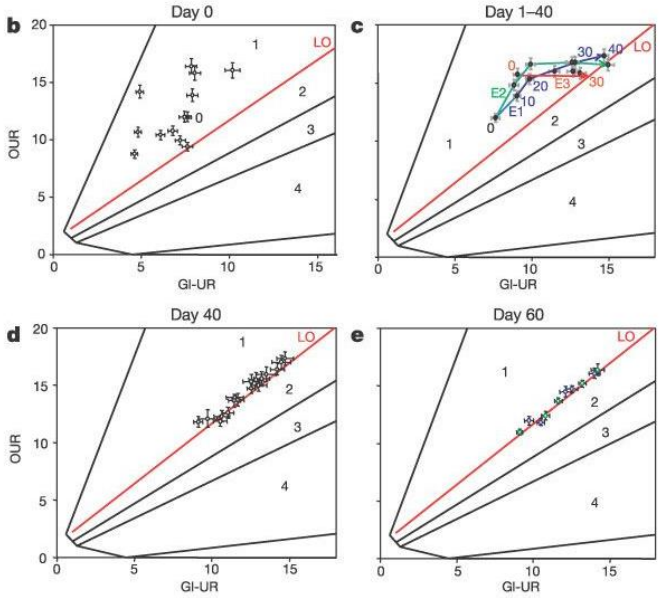




FBA simulates optimal metabolism achievable via adaptive evolution

Adaptive evolution of *E. coli* K-12 on glycerol moved the phenotype toward the FBA predicted optimality



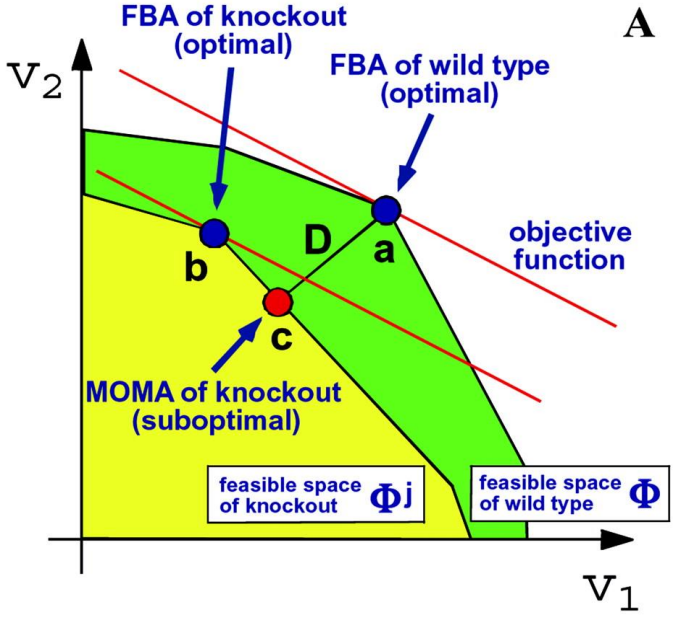


***Escherichia coli* K-12 undergoes adaptive evolution to achieve *in silico* predicted optimal growth**

Rafael U. Ibarra*†, Jeremy S. Edwards†‡ & Bernhard O. Palsson*

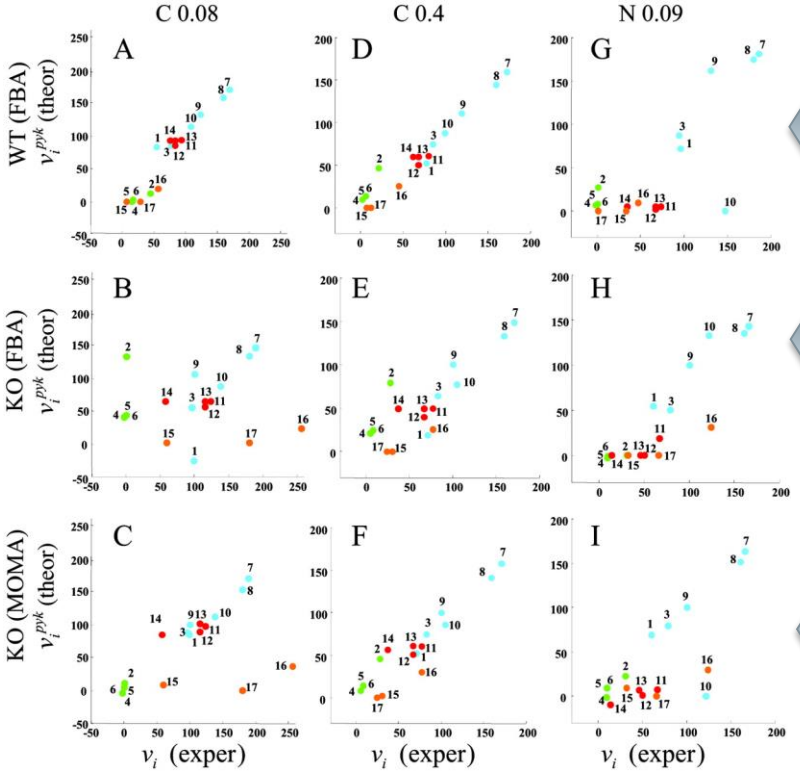
** Department of Bioengineering, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093-0412, USA*
‡ Department of Chemical Engineering, University of Delaware, Newark, Delaware 19716, USA
† These authors contributed equally to this work

Mutant phenotype simulations assuming not optimized metabolism



(A) The optimization principles underlying FBA and MOMA.

Three different experimental conditions



WT prediction with FBA is good

mutant prediction with FBA is less good

mutant prediction is improved with MoMA

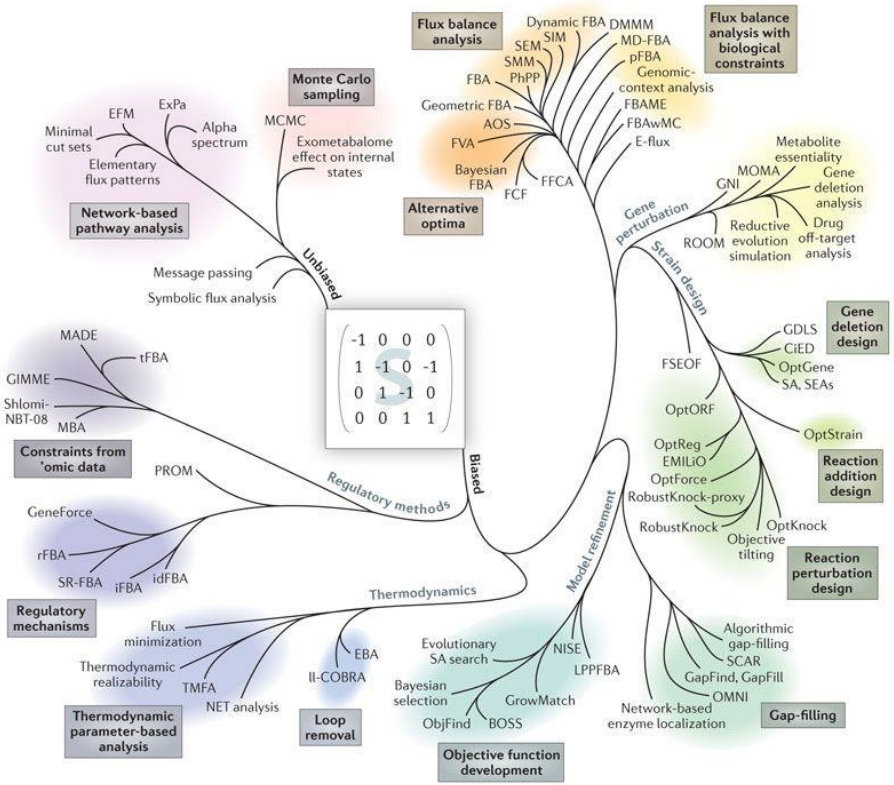
Comparison of FBA and MoMA predicted and ^{13}C-labelling determined flux values for E. coli delta *pyk* mutant

PNAS

Daniel Segrè et al. PNAS 2002;99:23:15112-15117

©2002 by National Academy of Sciences

A myriad of phenotype simulation methods using genome-scale metabolic models derive from FBA



Constraining the metabolic genotype–phenotype relationship using a phylogeny of *in silico* methods

Nathan E. Lewis[1], Harish Nagarajan[2] and Bernhard O. Palsson[1]

Platforms for genome-scale metabolic model manipulations and simulations

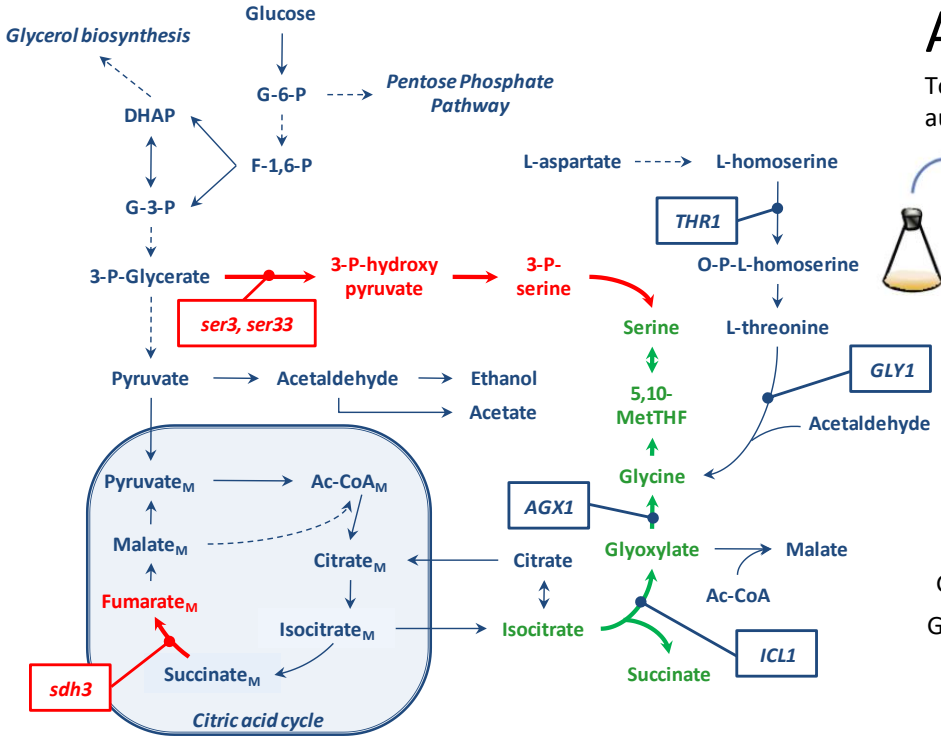
	Platform	Description	Link
For developers	COBRApy	Python package	https://opencobra.github.io/cobrapy/
	OpenCOBRA	Matlab functions	https://opencobra.github.io/cobratoolbox/stable/
	COBRA.jl	Julia package	https://opencobra.github.io/COBRA.jl/stable/
	Sybil	R-package	https://rdrr.io/cran/sybil/man/sybil-package.html
For end users	CAMEO	COBRApy compatible platform with *in silico* metabolic engineering tools	https://cameo.bio/
	BIOMET Toolbox	Web based platform with tools for reconstruction and analysis of models	http://biomet-toolbox.chalmers.se/
	MetaFlux	GUI or lisp API for model reconstruction and FBA	http://bioinformatics.ai.sri.com/ptools/metaflux.shtml
	OptFlux	Java based tool for *in silico* metabolic engineering	http://www.optflux.org/
	CellNetAnalyzer	GUI for model analysis using elementary flux modes approach, Matlab based	http://www2.mpi-magdeburg.mpg.de/projects/cna/cna.html

FBA derived simulations allow predicting genetic and environmental scenarios *in silico* before laboratory experiments

- Different genetic and environmental scenarios can be tested *in silico* before tedious laboratory experiments
  - Effect of different growth conditions
  - Effect of genetic modifications, e.g. deletion of gene(s)
- Simulations allow understanding of
  - Which reaction fluxes are essential for producing the given target metabolite?
  - Which options the cells have for generating energy (ATP) under the given environmental conditions?
  - Are there problems in cofactor balancing (NADH vs. NAD), should oxygenation be enhanced?

Growth-product coupling through metabolic network reduction

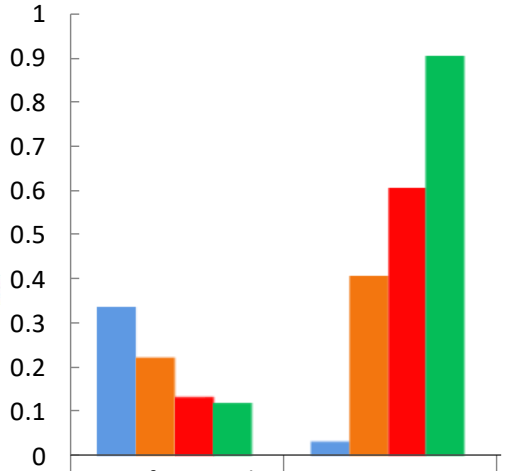
Proof of concept: succinate production in *S. cerevisiae*



Otero *et al.* PLoS One. (2013) **8**:e54144.

ALE

To recover from Gly auxotrophy



	Specific Growth Rate (1/h)	Maximum Titer (g/L)
Gly auxotrophic	REF	0.33	0.03
	8D	0.22	0.40
Gly prototrophic	8D Evolved	0.13	0.60
	8D Evolved + pICL1	0.12	0.90

Slide from Dr. Kiran Patil

Growth-product coupling elegantly aligns biological and engineering objectives through network reduction

Bi-level optimization

Design Objective

Find *k* deletions such that maximum product yield is achieved:

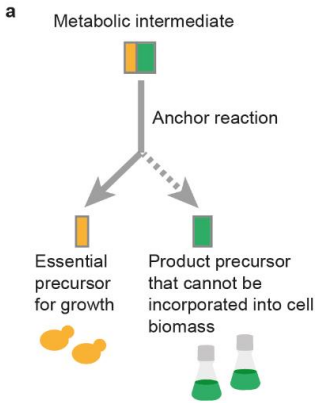
such that,

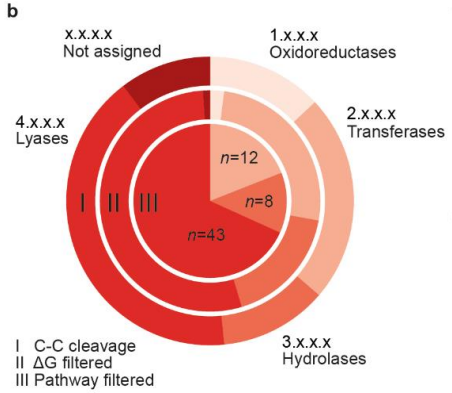
Biological Objective

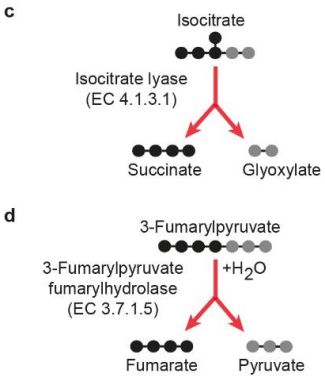
e.g. *flux is distributed for maximum growth*

Evolution driven objective

OptKnock: Burgard et al. (2003)
OptGene: Patil et al. (2005)







Jouhten P, Huerta-Cepas J, Bork P, Patil KR. *Metabolic anchor reactions for robust biorefining.* Metab Eng. (2017) **40**:1-4.

Slide modified from Dr. Kiran Patil

EMBL

FBA derived tools demonstrated in strain design

Methods for designing genetic engineering strategies (e.g. growth-product coupling) for wetlab metabolic engineering

- **gene deletion(s)**
  - OptKnock [Burgard, et al. 2003]
  - OptGene [Patil et al. 2005]
- **gene additions / deletions**
  - OptStrain [Pharkya, et al. 2004]
- **gene overexpressions / known down**
  - e.g. OptForce [Ranganathan, 2010]
- FSEOF for overexpression by scanning towards increasing production, [Choi et al. 2010]
- K-OptForce includes **kinetics**, [Chowdhury et al. 2014]
- tSOT, considers **gene expression data**, [Kim et al. 2016]

Yeast Genome-Scale Metabolic Models for Simulating Genotype-Phenotype Relations.
Castillo S, Patil KR, Jouhten P.
Prog Mol Subcell Biol. 2019;58:111-133. doi: 10.1007/978-3-030-13035-0_5.

Table 5.2 Examples of reported overproducer yeast strains whose development has been involved using genome-scale metabolic model simulation tools

Product	Species	Tools	Year	Ref.
Ethanol	*S. cerevisiae*	in house script (FBA)	2006	Bro et al. (2006)
Sesquiterpene	*S. cerevisiae*	MOMA, OptGene	2009	Asadollahi et al. (2009)
Vanillin	*S. cerevisiae*	MOMA, OptGene, OptKnock	2010	Brochado et al. (2010)
2,3-butanediol	*S. cerevisiae*	OptKnock	2012	Ng et al. (2012)
Fummaric acid	*S. cerevisiae*	FBA	2012	Xu et al. (2012)
Succinic acid	*S. cerevisiae*	OptGene	2013	Otero et al. (2013)
Tyrosine	*S. cerevisiae*	OptKnock	2013	Cautha et al. (2013)
Dihydroartemisinic acid	*S. cerevisiae*	MOMA, OptStrain, OptForce, OptKnock	2013	Misra et al. (2013)
Muconic acid	*S. cerevisiae*	FBA	2013	Curran et al. (2013)
Malate	*C. glabrata*	FBA	2013	Chen et al. (2013)
Triacetic acid lactone	*S. cerevisiae*	OptKnock	2014	Cardenas and Da Silva (2014)
Human recombinant protein	*P. pastoris*	FSEOF, MOMA	2014	Nocon et al. (2014)
Ethanol	*S. cerevisiae*	FBA, EMA	2014	Toro et al. (2014)
Acetoin	*C. glabrata*	FBA	2014	Li et al. (2014)
Amorphadiene	*S. cerevisiae*	MOMA, FBA	2014	Sun et al. (2014)
Succinate	*S. cerevisiae*	FBA	2014	Rosdi and Abdullah (2014)
3-hydroxypropionic acid	*S. cerevisiae*	FBA	2015	Borodina et al. (2015)
Patchoulol	*S. cerevisiae*	EMA	2015	Gruchattka and Kayser (2015)
Lipid	*Y. lipopytica*	FBA	2015	Kavscek et al. (2015)
Tyrosine	*S. cerevisiae*	OptKnock	2015	Gold et al. (2015)
β-Farnesene	*S. cerevisiae*	pFBA	2016	Meadows et al. (2016)
3-hydroxypropionic acid	*S. cerevisiae*	pFBA	2016	Kildegaard et al. (2016)
Muconic acid	*S. cerevisiae*	FBA	2016	Suastegui et al.

CORRECTED PROOF

Genetic circuit design

Genetic circuits can implement logic gates

Logic gates are circuits in which the relationship between the input and the output is based on a certain logic

They are basic building blocks of any digital systems

Name	NOT	AND	NAND	OR	NOR	XOR	XNOR
Alg. Expr.	$\overline{A}$	AB	$\overline{AB}$	$A+B$	$\overline{A+B}$	$A \oplus B$	$\overline{A \oplus B}$
Symbol							
Truth Table	A | X 0 | 1 1 | 0	B A | X 0 0 | 0 0 1 | 0 1 0 | 0 1 1 | 1	B A | X 0 0 | 1 0 1 | 1 1 0 | 1 1 1 | 0	B A | X 0 0 | 0 0 1 | 1 1 0 | 1 1 1 | 1	B A | X 0 0 | 1 0 1 | 0 1 0 | 0 1 1 | 0	B A | X 0 0 | 0 0 1 | 1 1 0 | 1 1 1 | 0	B A | X 0 0 | 1 0 1 | 0 1 0 | 0 1 1 | 1

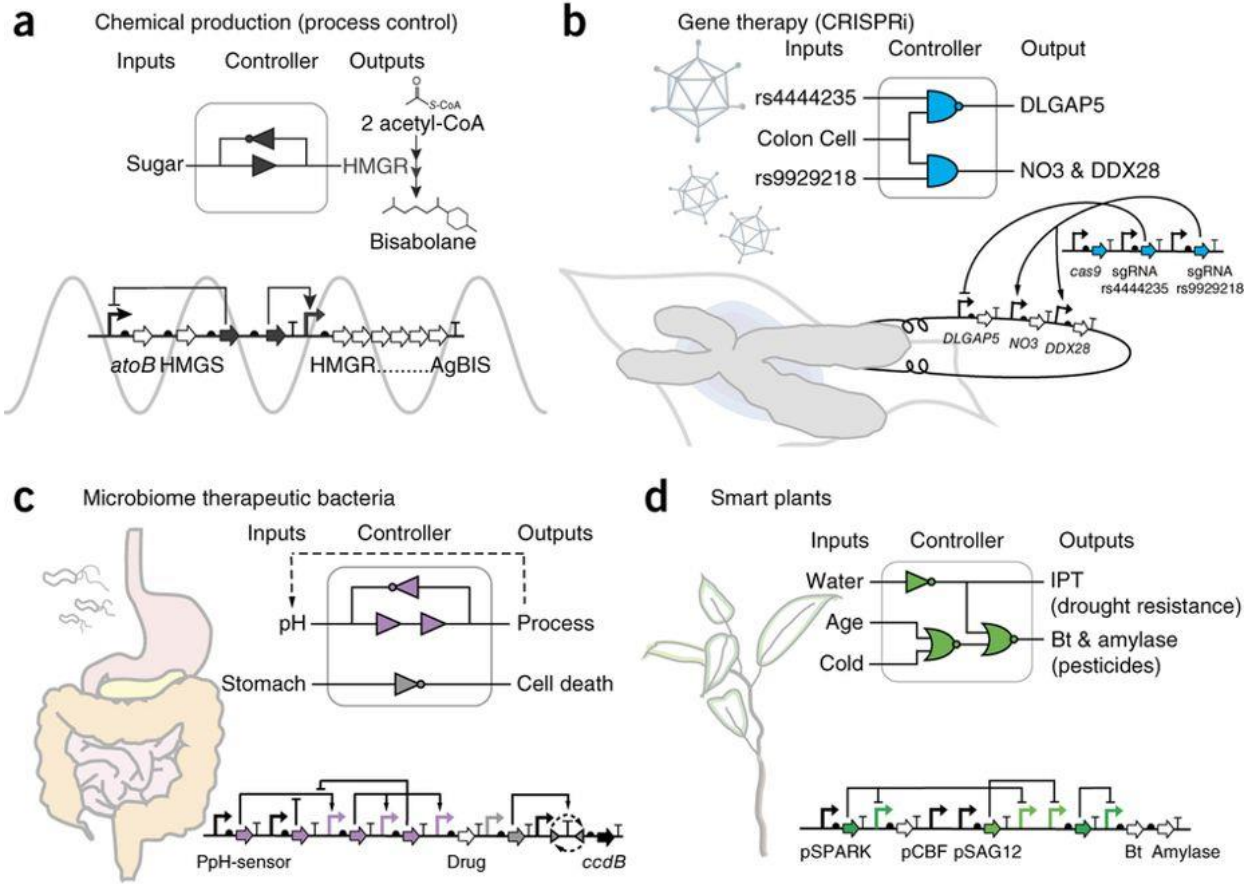
Slide modified from E. Czeizler

Principles of genetic circuit design

Jennifer A N Brophy & Christopher A Voigt

508 | VOL.11 NO.5 | MAY 2014 | NATURE METHODS

Potential uses of synthetic circuits

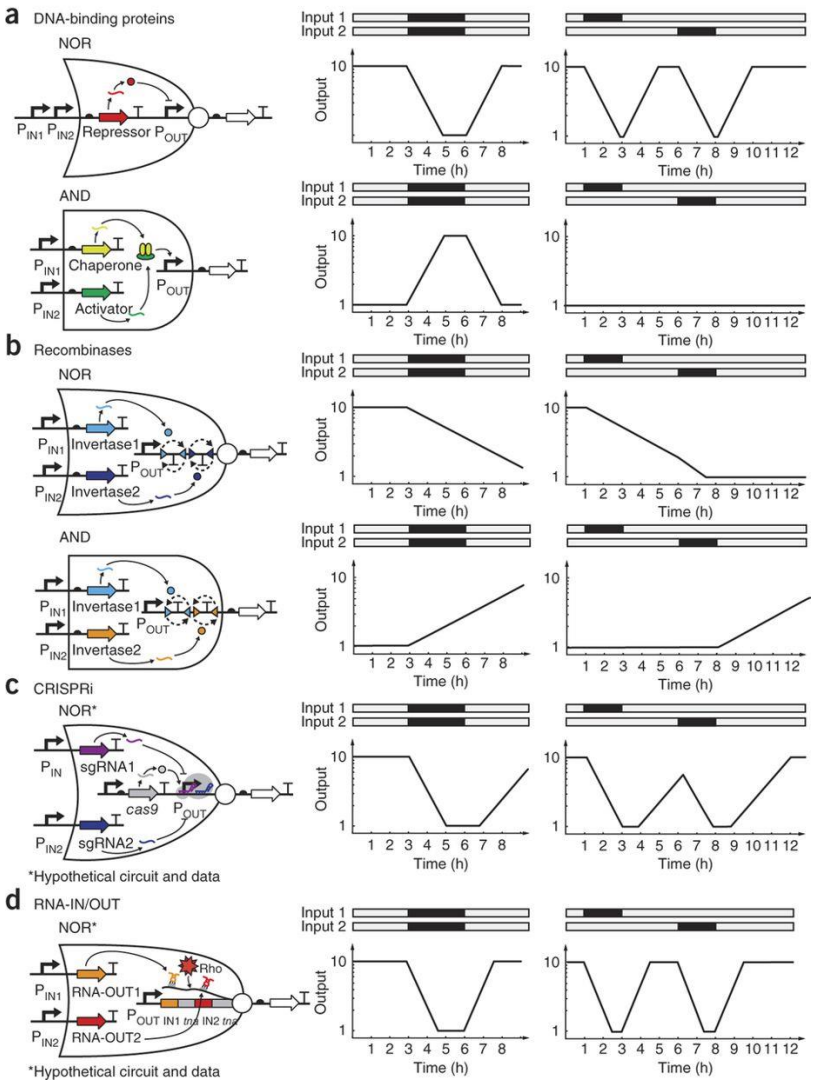


Principles of genetic circuit design
Jennifer A N Brophy & Christopher A Voigt
Nature Methods volume 11, pages 508–520 (2014)

Alternative regulators for transcriptional circuits

Examples of two input, single output circuits (left)

Response to simultaneous signals (middle) or sequential signals (right)



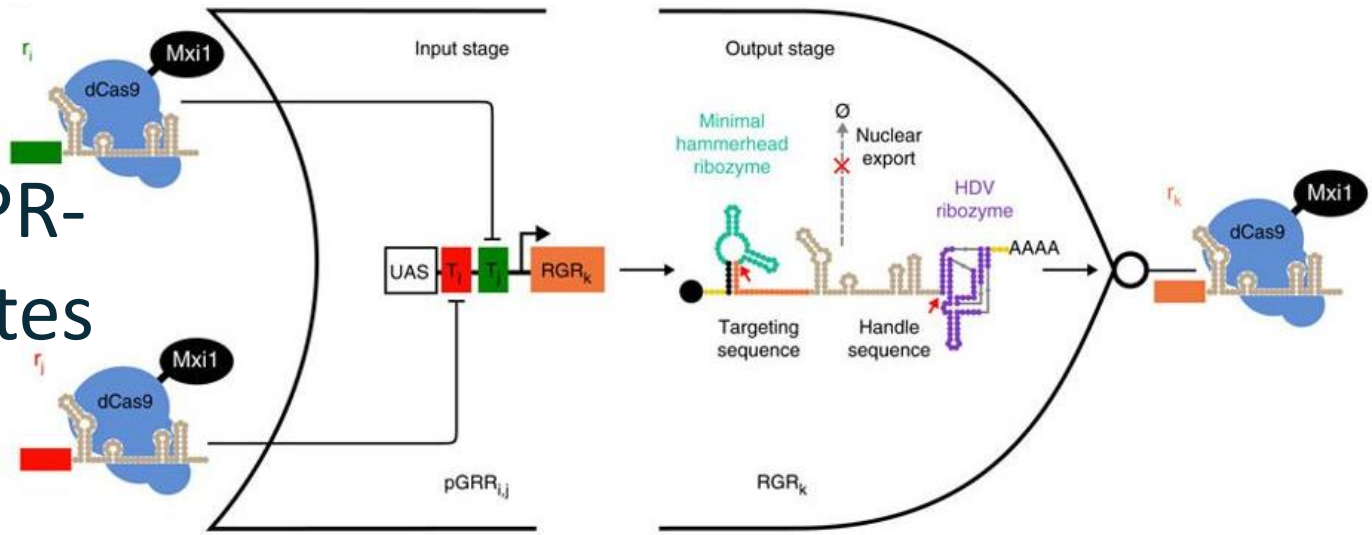
Relative promoter units (RPU)

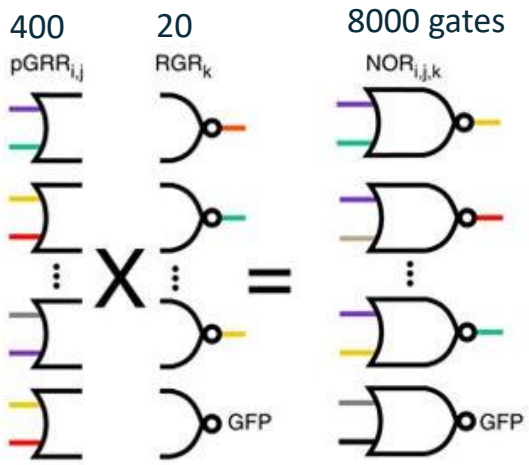
Principles of genetic circuit design
Jennifer A N Brophy & Christopher A Voigt
Nature Methods
volume 11, pages 508–520 (2014)

Combinatorial library of CRISPR-dCAS9 NOR-gates in yeast

Repression using Mxi1 fused to dCas9

Do they still leak?

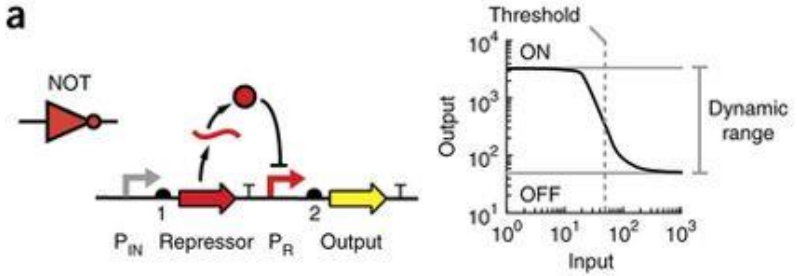




Digital logic circuits in yeast with CRISPR-dCas9 NOR gates. Miles W. Gander, Justin D. Vrana, William E. Voje, James M. Carothers & Eric Klavins Nature Communications volume 8, Article number: 15459 (2017)

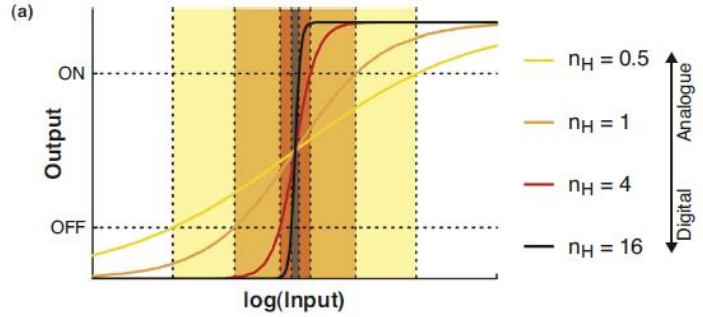
Circuit behavior tuning shifts the response function

Response function

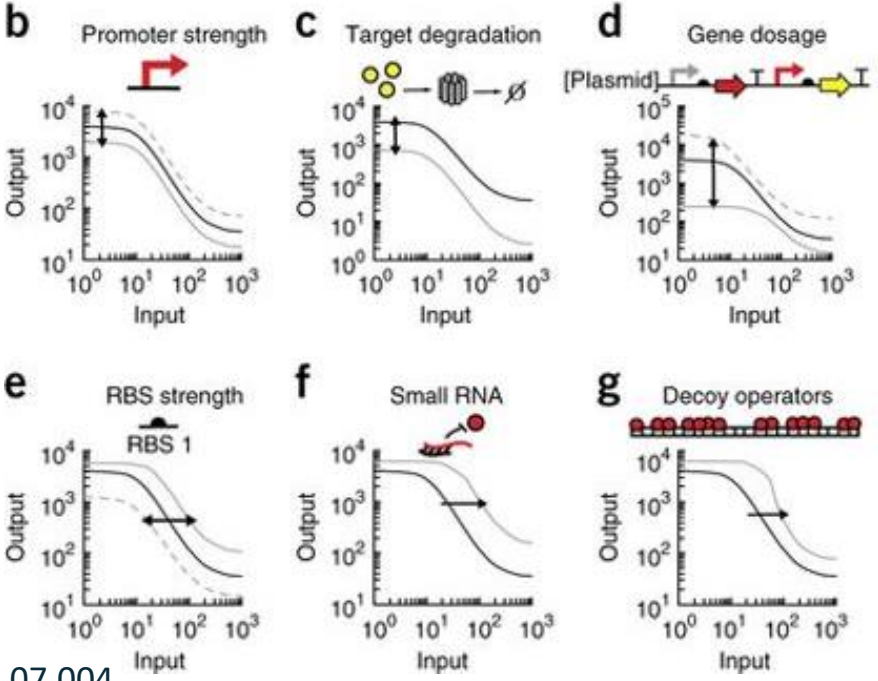


Relative promoter units (RPU)

Cooperativity with higher Hill coefficients



ODE model simulated by tuning the relevant parameters



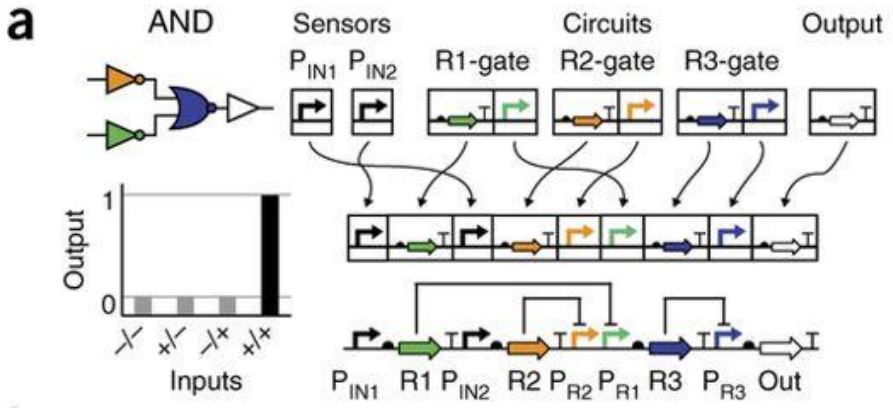
UP-DOWN shift

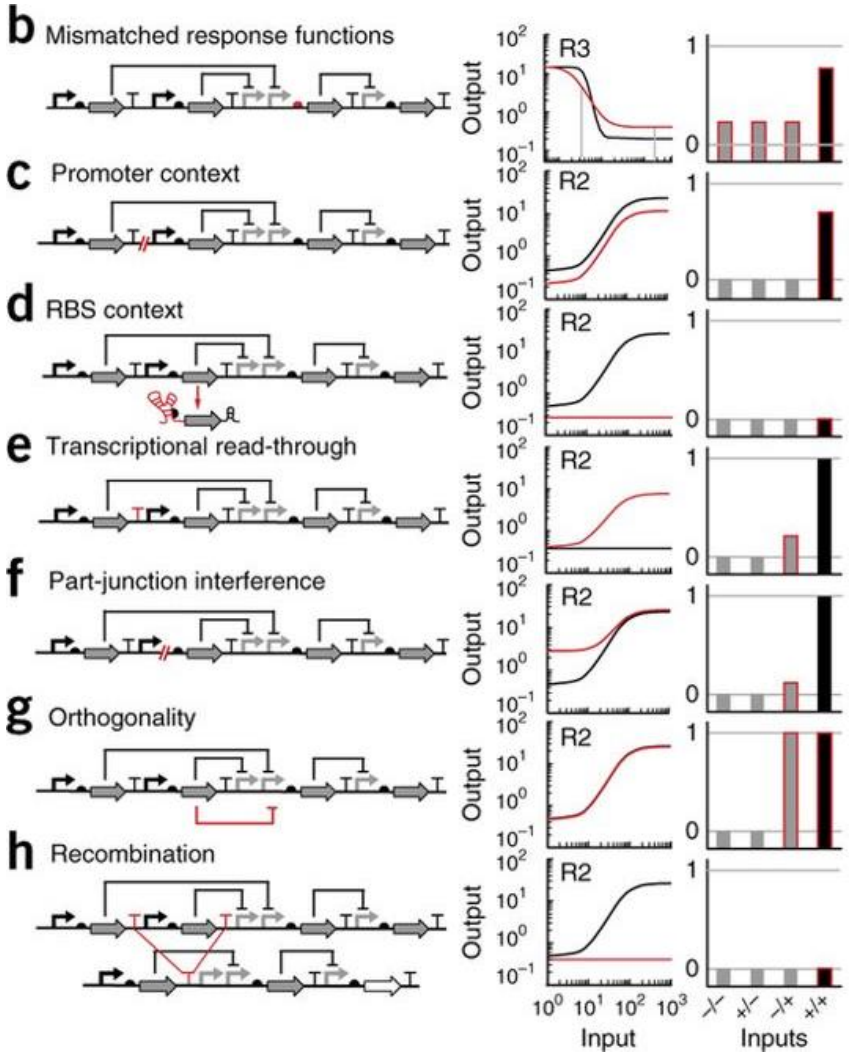
LEFT-RIGHT shift

Jennifer A N Brophy & Christopher A Voigt Nature Methods volume 11, pages 508–520 (2014)

Bradley et al. 2016: https://doi.org/10.1016/j.mib.2016.07.004

Possible failure modes when combined into larger circuits

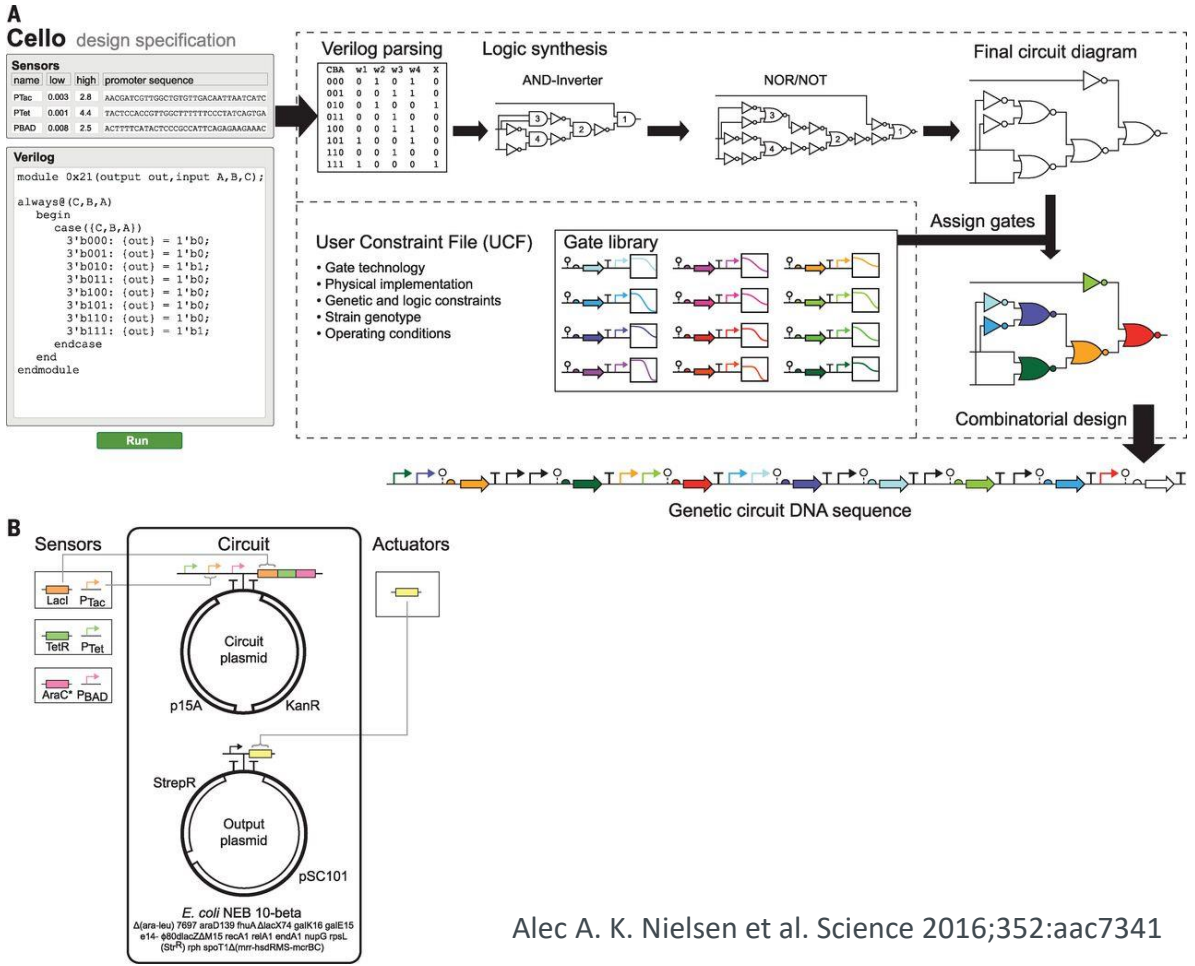




Jennifer A N Brophy & Christopher A Voigt
Nature Methods volume 11, pages 508–520 (2014)

Cello automates genetic circuit design (in *E. coli*)

A homework will give use a short tutorial as a presentation

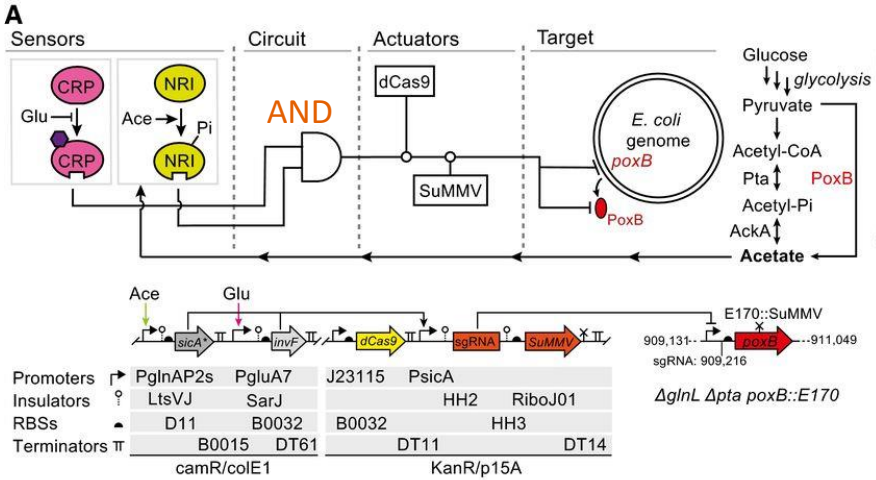


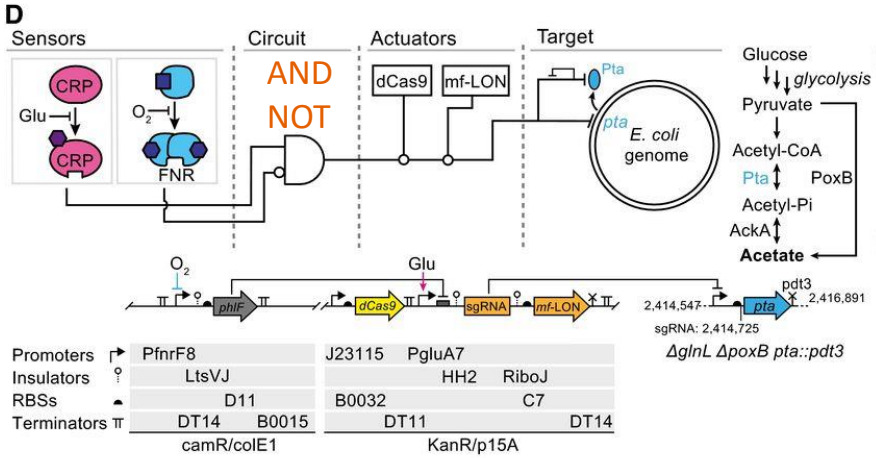
Alec A. K. Nielsen et al. Science 2016;352:aac7341

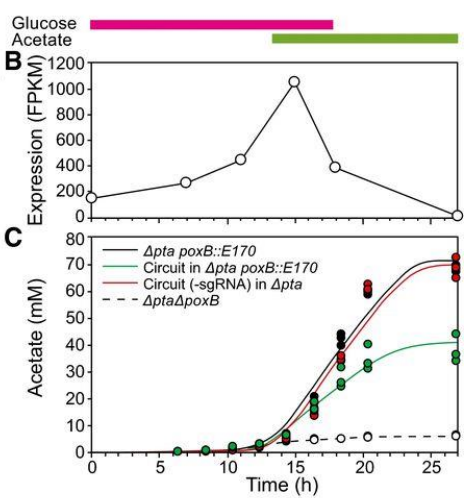
Dynamic control during a bioprocess by coupling sensors to circuits

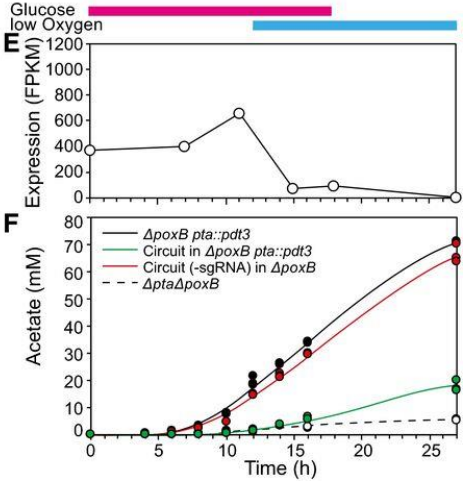
Controlling acetate excretion by *E. coli* on transcriptionl and translational level

Limiting issues:
Toxicity and instability !





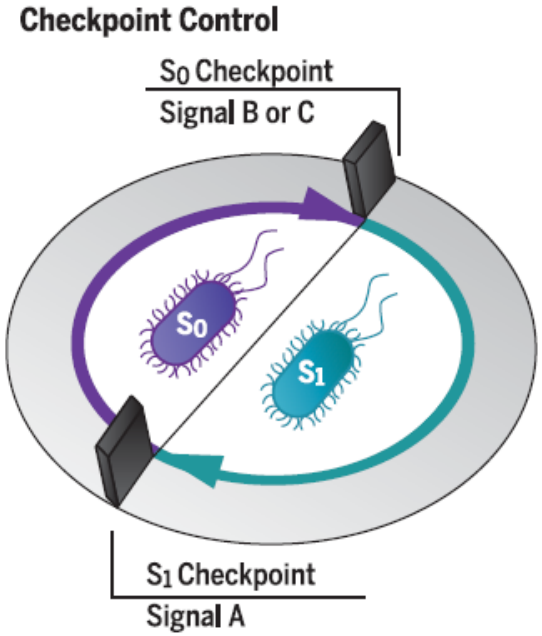


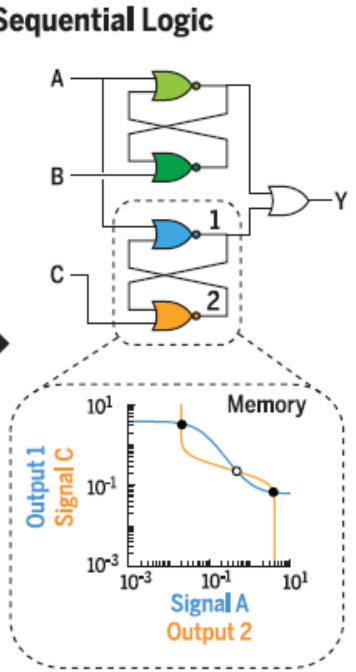


PoxB encodes pyruvate oxidase

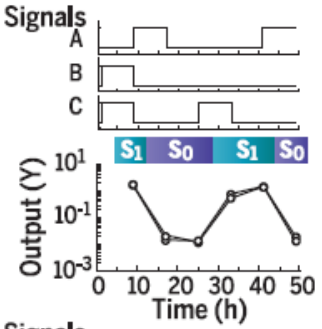
Felix Moser et al. Mol Syst Biol 2018;14:e8605

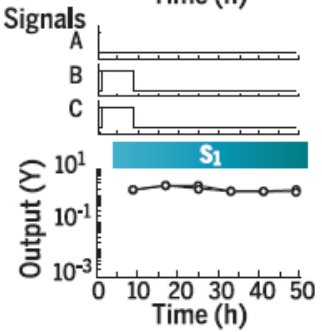
Programmable sequential logic for progress through discrete states





Switch state





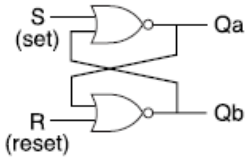
Hold state

RESEARCH ARTICLE SUMMARY

SYNTHETIC BIOLOGY

Cellular checkpoint control using programmable sequential logic

Lauren B. Andrews, Alec A. K. Nielsen, Christopher A. Voigt*



Set-Reset (SR) latch

Andrews *et al.*, *Science* **361**, 1217 (2018)

Homeworks

2. Circuit design using Cello web application

3. Synthetic pathway design and host optimization

Homework 2: Design genetic circuits using Cello web application

- Familiarize yourselves with the Cello software for genetic circuit design at http://cellocad.org/ and the original publication of the tool: http://science.sciencemag.org/content/352/6281/aac7341.long. The supplementary offers valuable information too: http://www.cellocad.org/suppinfo.html.
- Register as user and use Cello for designing two genetic circuits with three inputs and a single output.
- The circuit function is described as Verilog code for which the supplementary (link above) gives more information on. Use the default User Constraint File (UCF) provided by the web application.
- Present a tutorial of the tool using the two design cases made.
- In the presentation describe, using the Cello output, how the particular parts make up the desired circuit. Describe also how would you proceed if you where to implement the designs in cells.
- **Send the presentation to paula.jouhten@vtt.fi by 9 am on Monday 14th of April. Use the same email to contact in case of problems.**

References for Homework 2:

Cello software and Principles of genetic circuit design

- Nielsen AA, Der BS, Shin J, Vaidyanathan P, Paralanov V, Strychalski EA, Ross D, Densmore D, Voigt CA. Genetic circuit design automation. Science. 2016 Apr 1;352(6281):aac7341. doi: 10.1126/science.aac7341.
- Brophy JA, Voigt CA. Principles of genetic circuit design. Nat Methods. 2014 May;11(5):508-20. doi: 10.1038/nmeth.2926.

Homework 3: Design a producer strain hosting a synthetic production pathway

- Install Anaconda (Python 3.7 version, https://www.anaconda.com/distribution/) or miniconda, cplex solver (cplex (free student version or free trial): https://www.ibm.com/products/ilog-cplex-optimization-studio or gurobi (free academic): http://www.gurobi.com/), and use your python installation (Anaconda prompt) to install framed package using pip according to instructions: https://framed.readthedocs.io/en/latest/install.html (Python 3 should be fine though the docs talk about Python 2)
- Use the provided Jupyter Notebook exercises for developing Vanillin glucoside producing yeast *in silico.* If needed, tutorial for Jupyter notebooks can be found in: https://jupyter-notebook-beginner-guide.readthedocs.io/en/latest/
- Search for alternative synthetic pathways for Vanillin glucoside production using Retropath web application (http://xtms.issb.genopole.fr/) but for *E. coli*. Would any of the pathways be a good alternative pathway for yeast? How many pathways Retropath proposes? How do they differ? Which one is the most promising and why?
- Which reactants of the heterologous pathway are native metabolic intermediates of yeast?
- How could the host metabolism be modified for optimizing Vanillin glucoside production?
- Present your workflow, the heterologous pathway and it's interactions with the native metabolism, and your thoughts/ideas on the host optimization for improving Vanillin glucoside production for discussion.
- **Send the presentation to paula.jouhten@vtt.fi by 9 am on Monday 14th of April. Use the same email to contact in case of problems.**

References for Homework 3:

Retropath method and XTMS web server

- Carbonell, P., Parutto, P., Herisson, J., Pandit, S. B., & Faulon, J.-L. (2014). XTMS: Pathway design in an eXTended metabolic space. Nucleic Acids Research, 42(W1), 389–394. doi:10.1093/nar/gku362
- Carbonell, P., Parutto, P., Baudier, C., Junot, C., & Faulon, J.-L. (2014). Retropath: Automated pipeline for embedded metabolic circuits. ACS Synthetic Biology, 3(8), 565–577. doi:10.1021/sb4001273
- Delépine B, Duigou T, Carbonell P, Faulon JL. RetroPath2.0: A retrosynthesis workflow for metabolic engineers. Metabolic Engineering, 45: 158-170, 2018. doi: https://doi.org/10.1016/j.ymben.2017.12.002