LOSCHMIDT LABORATORIES

Metabolic engineering

Engineering and optimizing genetic, epigenetic and biochemical processes to increase the cells' production

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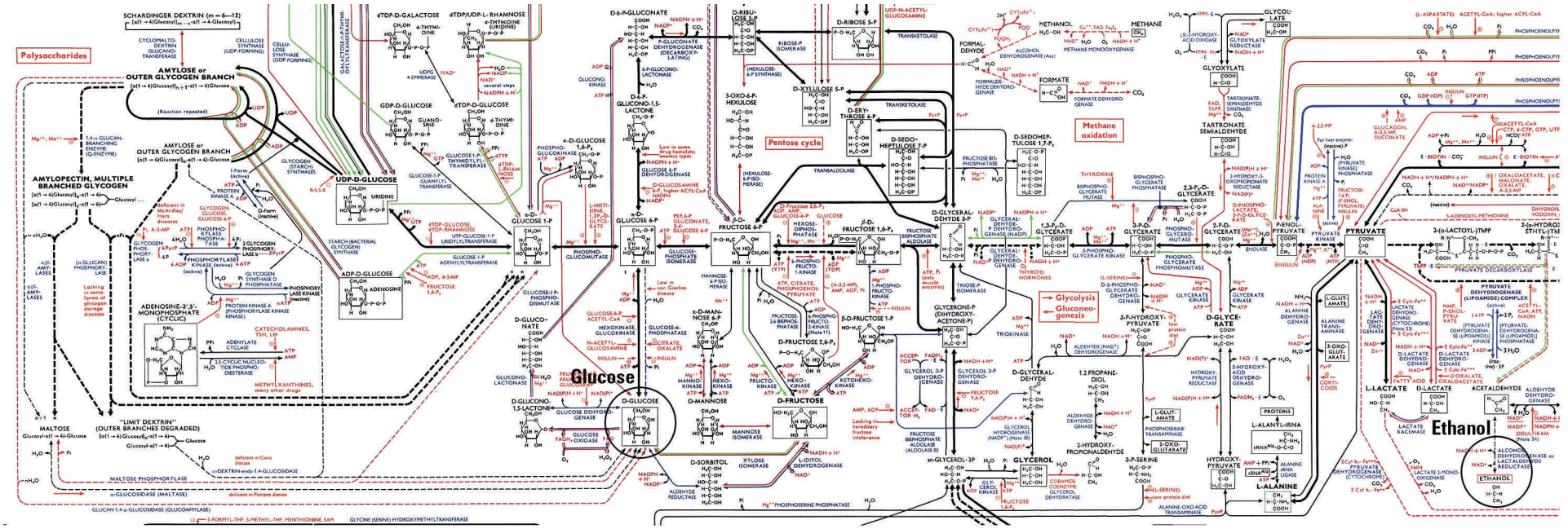
What will we talk about

- Metabolic engineering
  - definition, goals and applications
- Metabolomics and systems biology
  - concepts, methodology, limitations,
- Synthetic epigenetics, epigenetic therapy
  - definition, goals and applications
- Synthetic biology in drug discovery
  - Emerging technologies, unmet challenges

What is metabolic engineering?

- Metabolic engineering is basically meant for the production of chemicals, fuels, pharmaceuticals, and medicine by altering the metabolic pathways.
- Metabolic engineering is motivated by commercial applications by which we can improve the developing strains for production of useful metabolites. This method requires overexpression or downregulation of certain proteins in a metabolic pathway, such that the cell produces a new product.



https://doi.org/10.1016/j.tibs.2018.05.004

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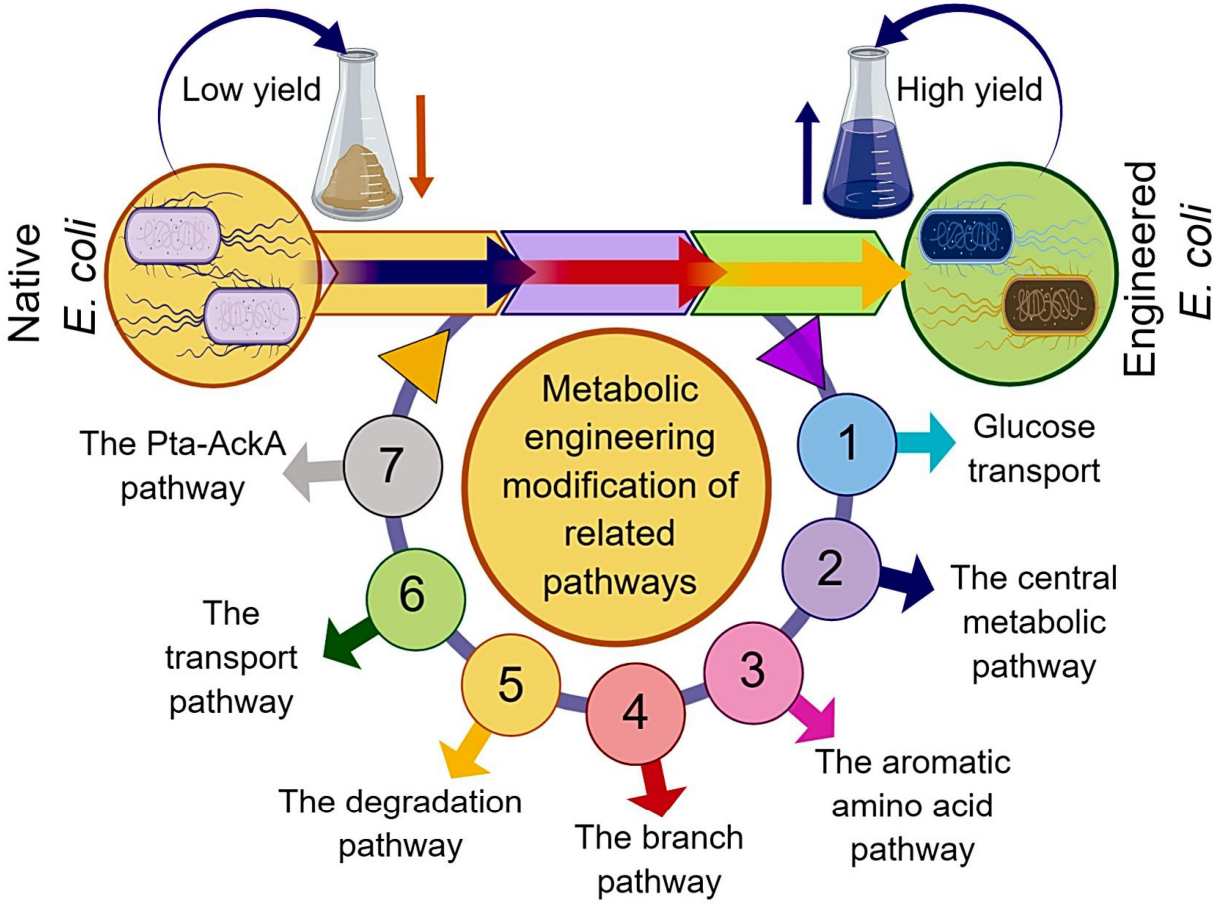
- First step for successful engineering requires the complete understanding of host cell for genetic modifications. The effect of genetic manipulation on growth should also be examined. The genetic manipulation may negatively effect on metabolic burden.
- To achieve the product several methods, such as elimination of competitive pathway and toxic byproduct and expression of a heterologous enzyme to improve the synthesis of products, are used for the modification of the host cell.
- In classical metabolic engineering approach, genetic manipulation is based on the prior knowledge of enzyme network pathway and its kinetics; on the other hand, in inverse metabolic engineering the environmental or genetic conditions are considered for the desired phenotype for genetic manipulation.
- A number of approaches are used for secondary metabolites improvement using bacteria in metabolic engineering, such as (1) heterologous expression of gene clusters, (2) regulatory networks pathway engineering, (3) gene insertion or deletion, and (4) stimulation using certain substrates.
- Therefore, metabolic engineering is currently used as cell factories for production of amino acids, biofuels, pharmaceuticals, bioplastics, platform chemicals, silk proteins, etc.

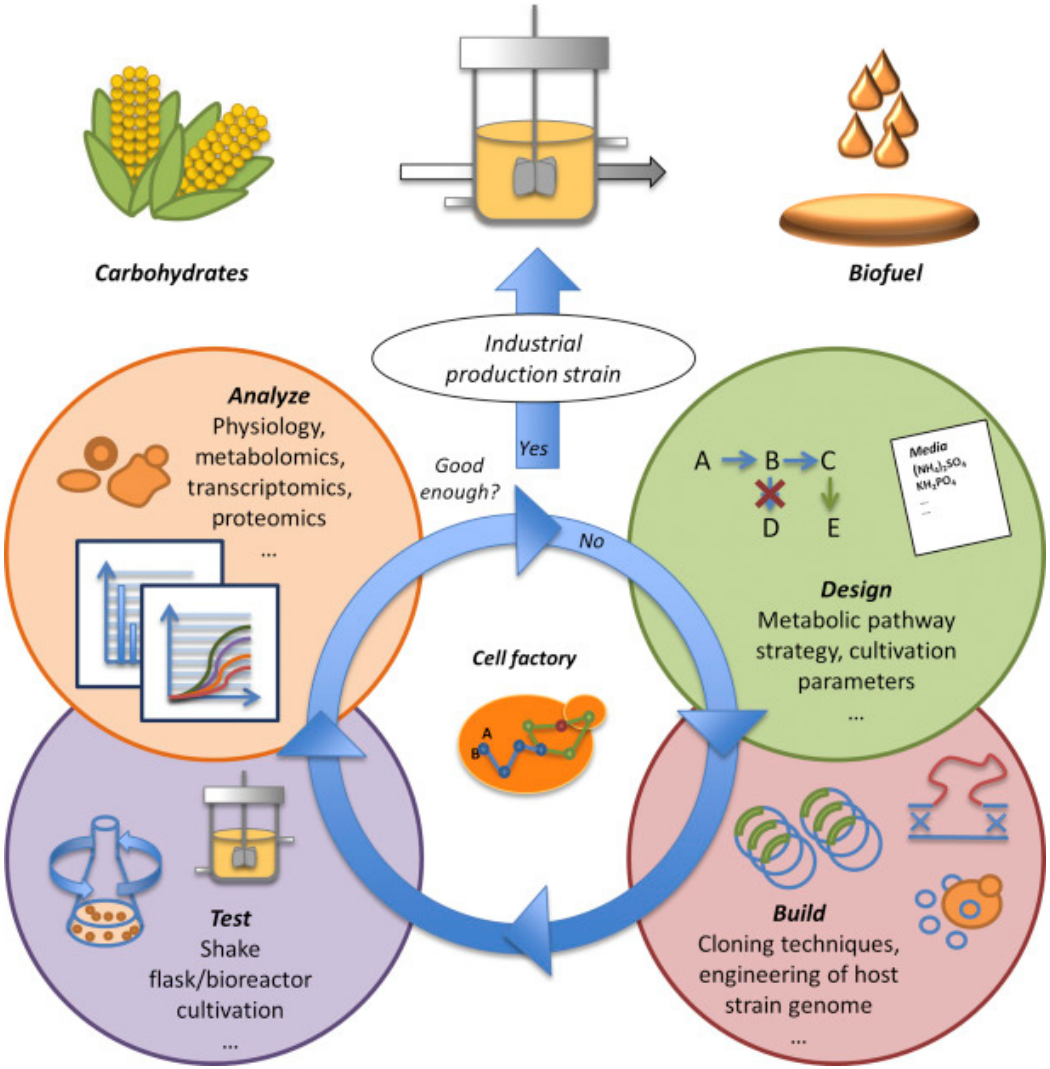
https://doi.org/10.1016/j.tibs.2018.05.004

Goals of metabolic engineering

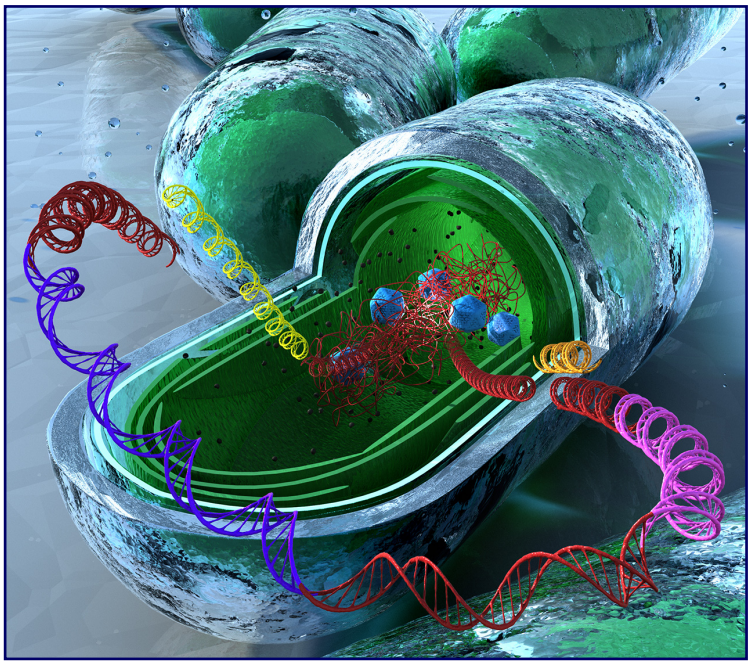


https://www.mdpi.com/2227-9717/7/4/213

Metabolic engineering workflow

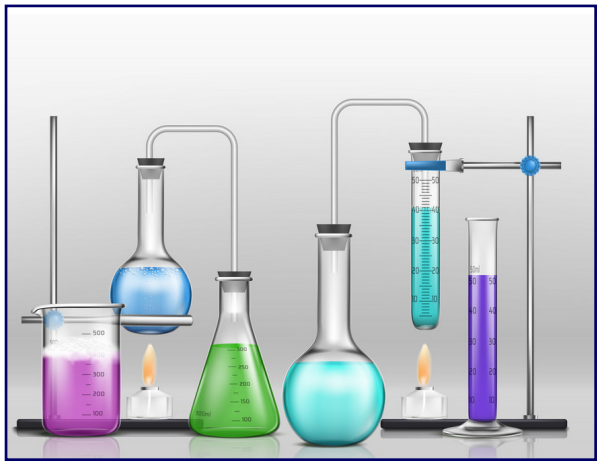


Why do we need to build a synthetic metabolism of the future?



The power of synthetic biology

Synthetic chemistry 20th century



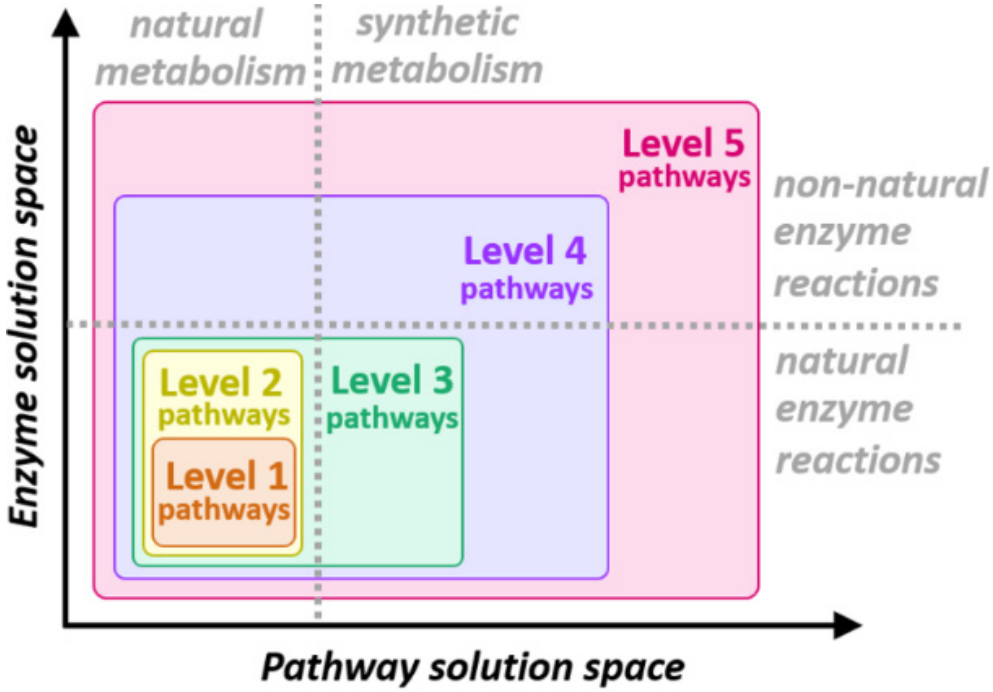
Synthetic biology 21st century

Biological systems are:
- self-optimizing
- self-repairing
- self-propagating

Biological systems operate:
- in a sustainable fashion
- environmentally friendly

Five different levels of metabolic engineering

https://www.sciencedirect.com/science/article/pii/S1367593116302071



Level 1	Optimization of natural pathway in natural host
Level 2	Transfer and exchange of natural (sub-)pathways in new host
Level 3	Synthetic pathway created from known reactions
Level 4	Synthetic pathway created from novel reactions engineered from known enzymes
Level 5	Synthetic pathway created from novel reactions from *de novo* engineered enzymes

- The enzyme solution space describes the number of possible enzymes reactions available for a given strategy while the pathway solution space corresponds to the number of possible pathways that can be constructed.
- While level 1, 2 and 3 metabolic engineering efforts do not differ in enzyme solution space, because they all rely on known enzymes, level 4 and 5 metabolic engineering efforts provide new enzymes created through enzyme engineering or *de novo* protein design.

Central questions of metabolic engineering

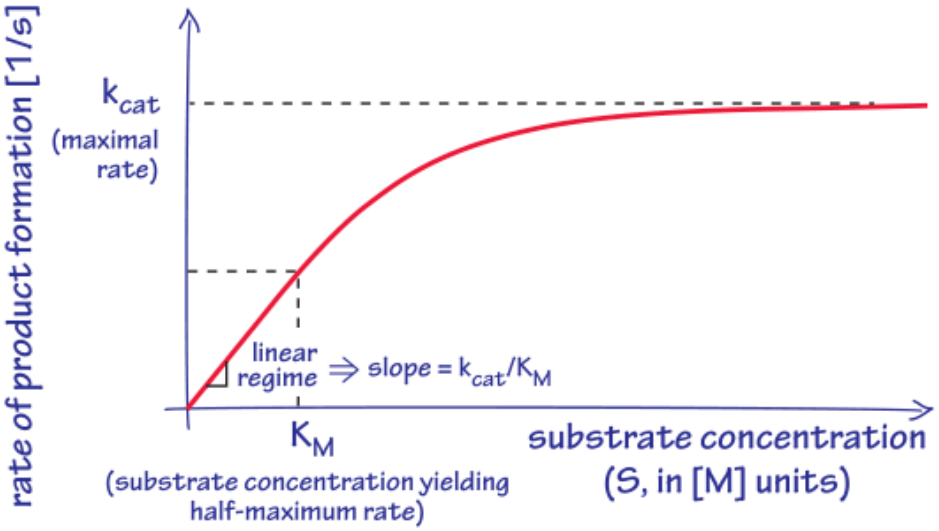
How to design synthetic metabolic networks?

How to realize synthetic metabolic networks?

How to optimize synthetic metabolic networks?

How to transplant synthetic metabolic networks?

Measuring the efficiency of enzymes

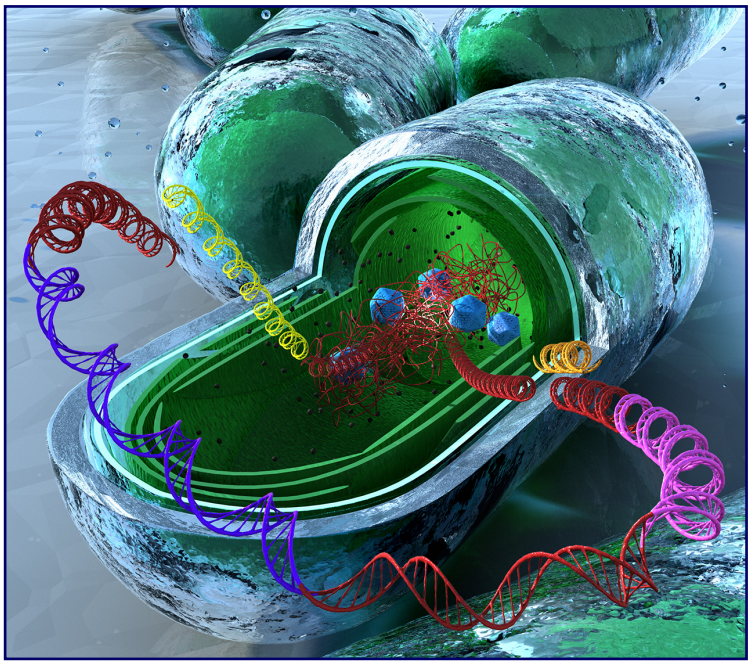


***kcat* = Turnover number (s^{-1})**
Chemical conversions of substrate molecules per second per active site

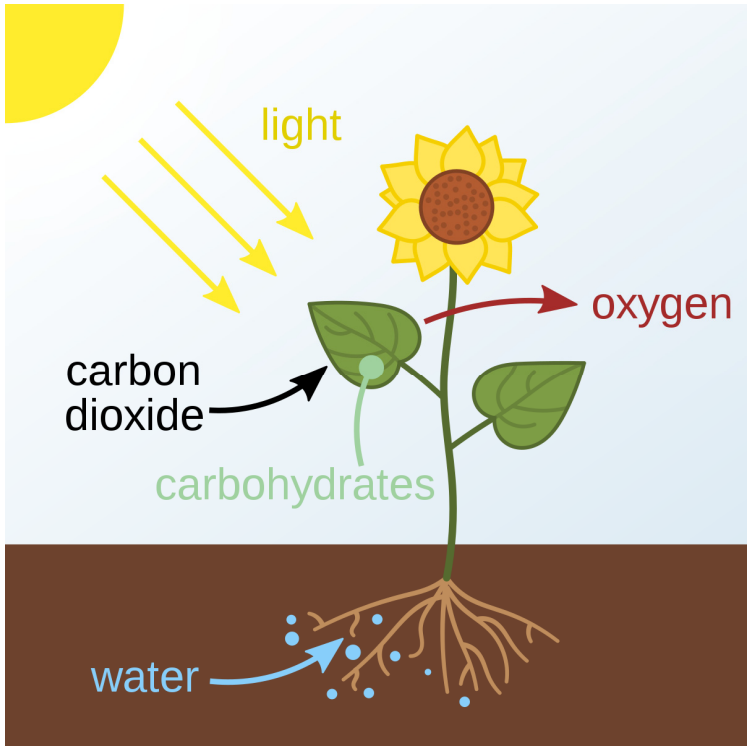
***Km* = Michaelis constant (M^{-1})**
Substrate concentration at half maximum activity

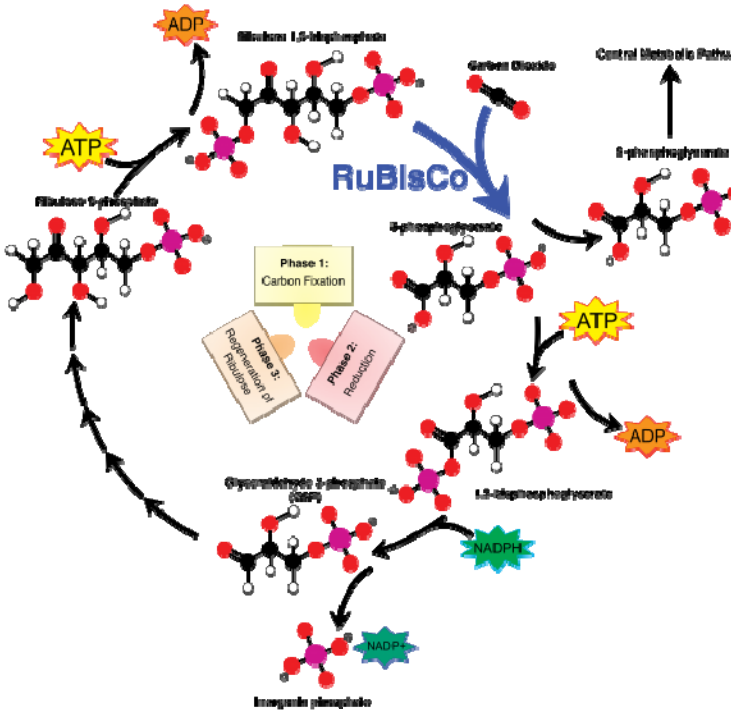
***kcat* / *Km* (M^{-1} s^{-1})**
Catalytic efficiency

Examples of metabolic engineering



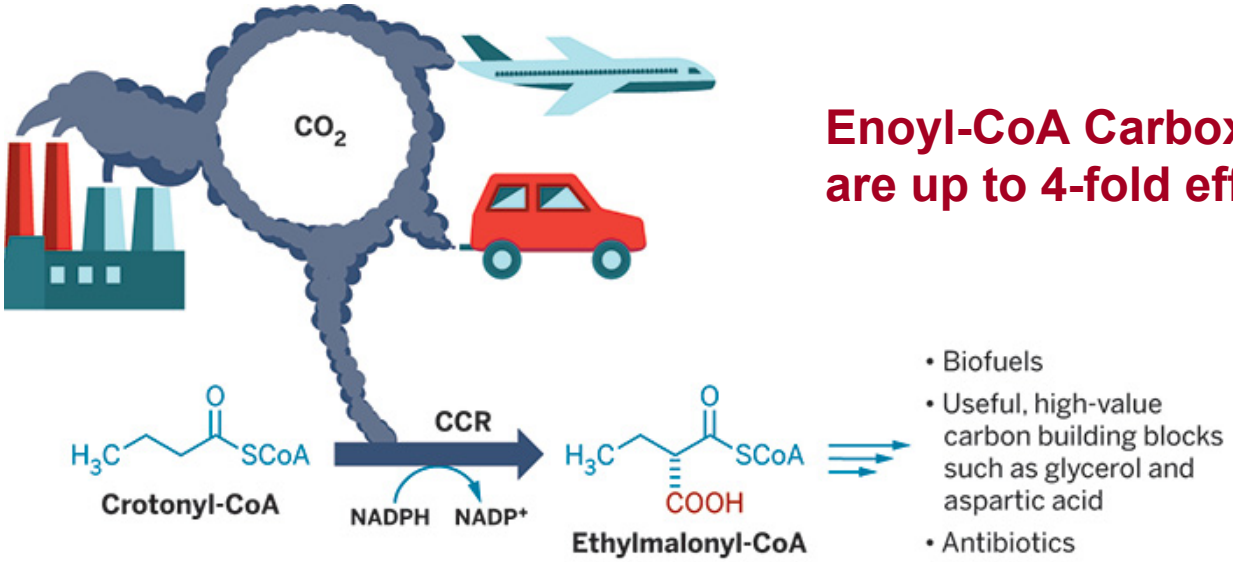
Example 1: a fixation of carbon dioxide





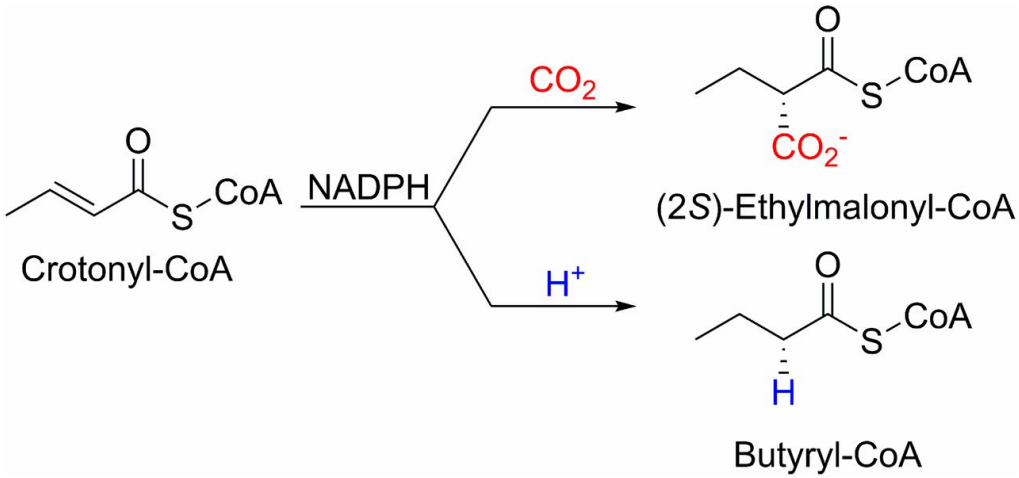
A fixation of carbon dioxide in vitro: an example of ME

Carbon dioxide (CO_2) is an important carbon feedstock for a future green economy. This requires the development of efficient strategies for its conversion into multicarbon compounds. We describe a **synthetic cycle for the continuous fixation of CO_2 in vitro**. The crotonyl-coenzyme A (CoA)/ethylmalonyl-CoA/hydroxybutyryl-CoA (**CETCH**) cycle is a reaction network of **17 enzymes** that converts CO_2 into organic molecules at a rate of 5 nanomoles of CO_2 per minute per milligram of protein. The CETCH cycle was drafted by **metabolic retrosynthesis**, established with enzymes originating from **nine different organisms** of all three domains of life, and optimized in several rounds by **enzyme engineering** and **metabolic proofreading**. The CETCH cycle adds a seventh, synthetic alternative to the six naturally evolved CO_2 fixation pathways, thereby opening the way for in vitro and in vivo applications.

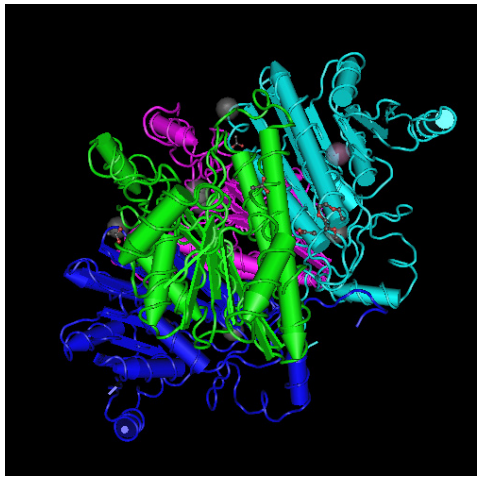


Enoyl-CoA Carboxylases/reductases are up to 4-fold efficient than RuBisco

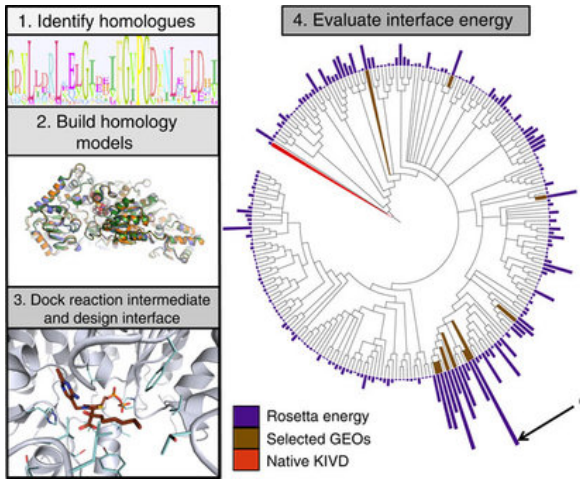
Expanding the biosynthetic space of ECR reactions



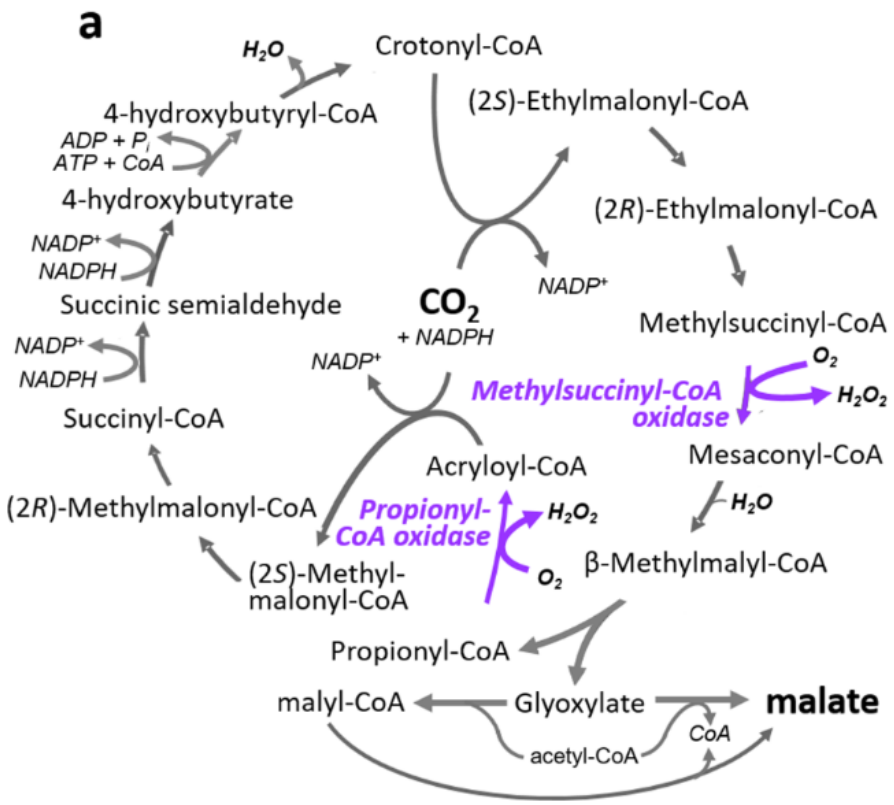
Enzyme engineering



Genome mining



Designing and realizing a new synthetic metabolic pathway



Design principles:

- Cyclic topology
- Carbon-splitting reactions
- Biochemically possible reactions

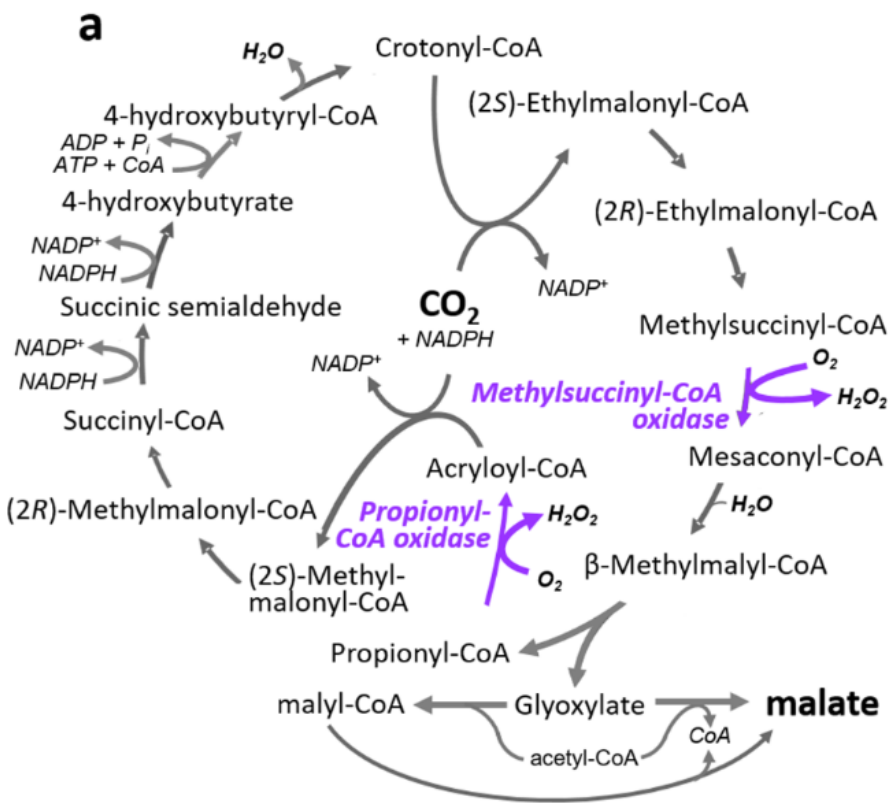
Evaluation criteria:

- Kinetically favoured
  Fast and efficient enzyme reactions
- Thermodynamically favoured
  Energy per CO_2 molecule fixed
- Thermodynamically feasible
  All equilibrium constants ≥1

The CETCH cycle for CO_2 fixation. Important enzymes that were engineered to establish the cycle are highlighted in purple.

https://science.sciencemag.org/content/354/6314/900

Designing and realizing a new synthetic metabolic pathway



Finding the parts:

- Searching enzyme databases
- Testing enzyme homologs
- (Re)-engineering enzymes

NCBI

PubMed

1987 2019 BRENDA

The Comprehensive Enzyme Information System

STRENDA

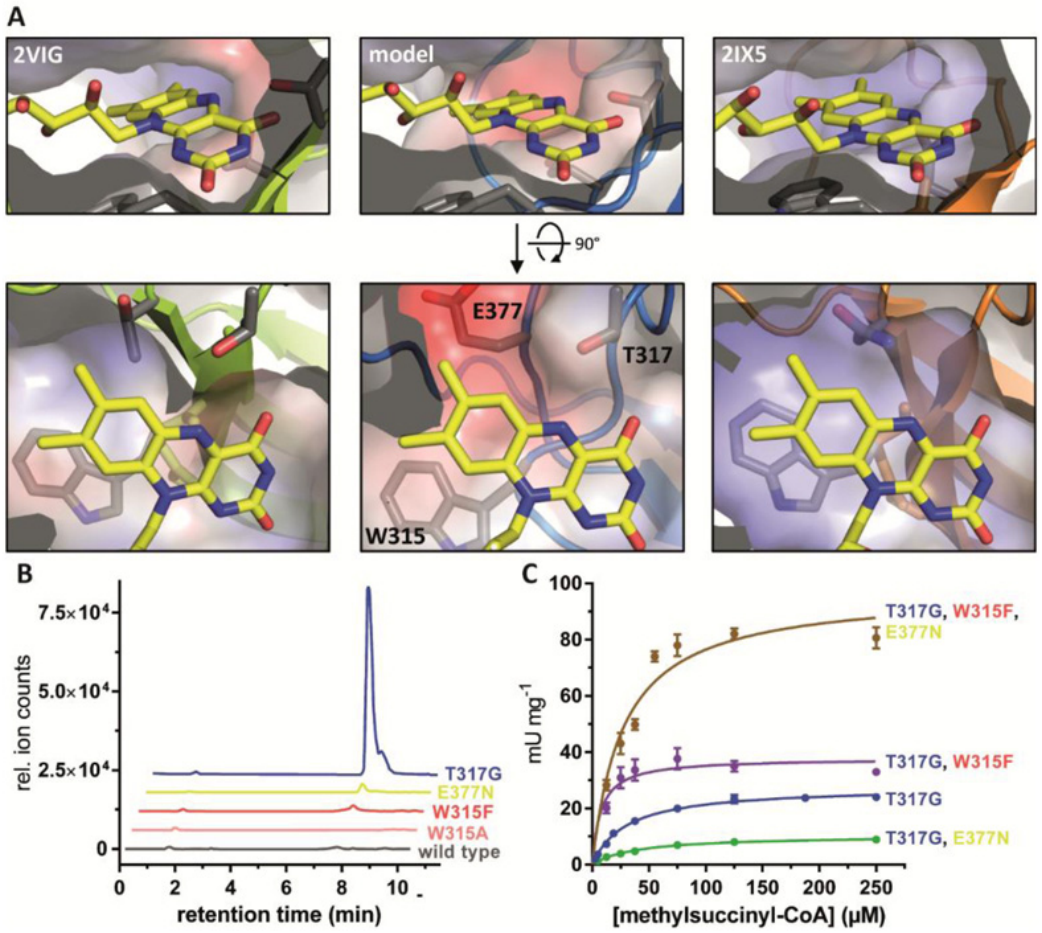
The CETCH cycle for CO_2 fixation. Important enzymes that were engineered to establish the cycle are highlighted in purple.

https://science.sciencemag.org/content/354/6314/900

Key role of protein design in metabolic engineering

Structure guided engineering of Mcd into a Mco

(**Methysuccinyl-CoA Dehydrogenase into Methylsuccinyl-CoA oxidase**)



https://science.sciencemag.org/content/354/6314/900

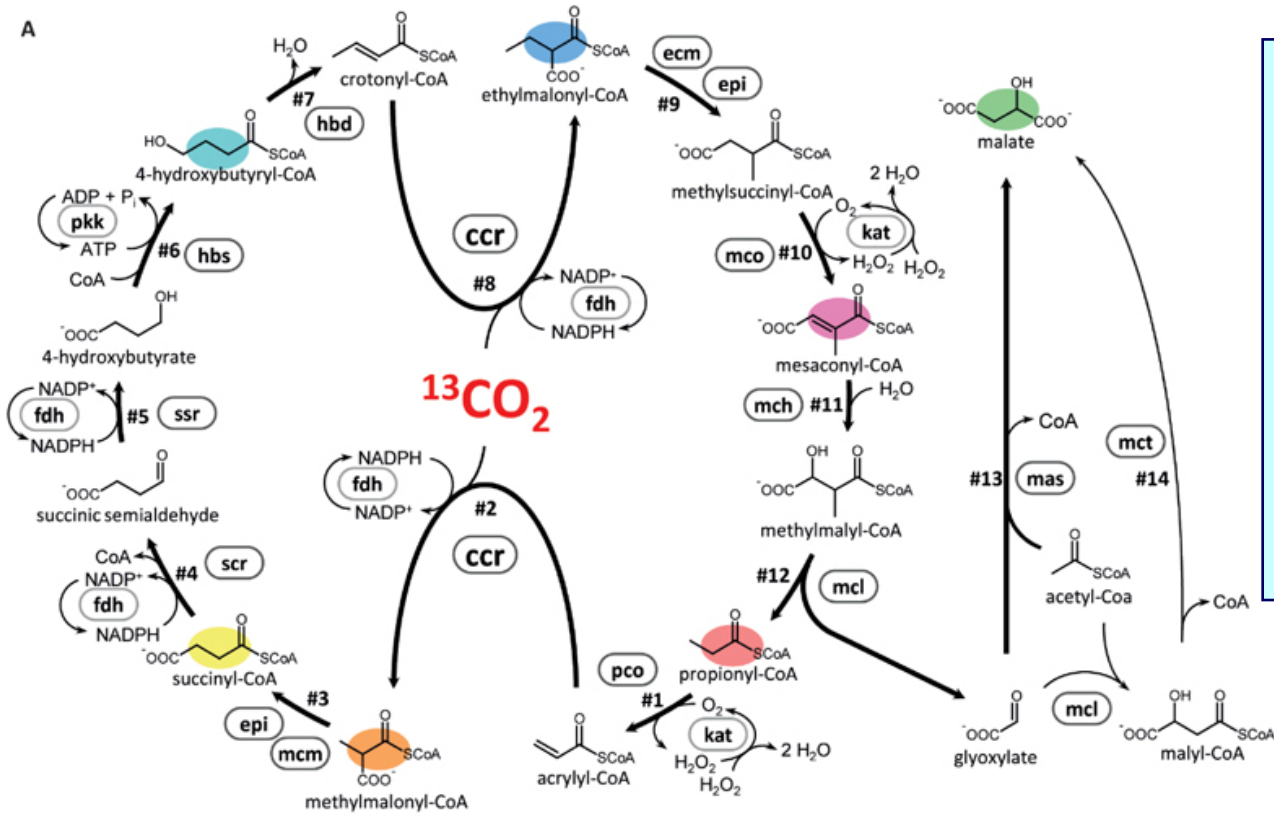
A) Active site comparison of the human short-chain acyl-CoA dehydrogenase (PDB: 2VIG, green backbone) with a model of Mcd from *Rhodobacter sphaeroides* (blue backbone, modeled by the SWISS-MODEL server with 2VIG as template) and the short-chain acyl-CoA oxidase 4 from *Arabidopsis thaliana* (PDB: 2IX5, orange backbone). The three residues that were targeted to introduce oxidase activity into Mcd are highlighted.

B) HPLC-MS based analysis of wild-type and different single active-site mutants for oxidation of methylsuccinyl-CoA into mesaconyl-CoA with molecular oxygen as electron acceptor. The screen identified three substitutions (W315F, T317G and E377N) that were combined into double and triple mutants.

C) Michaelis-Menten graphs of single, double and triple mutants characterized in more detail. The triple mutant showed best kinetic parameters.

The CETCH cycle

The crotonyl-coenzyme A (CoA)/ethylmalonyl-CoA/hydroxybutyryl-CoA (**CETCH**) cycle

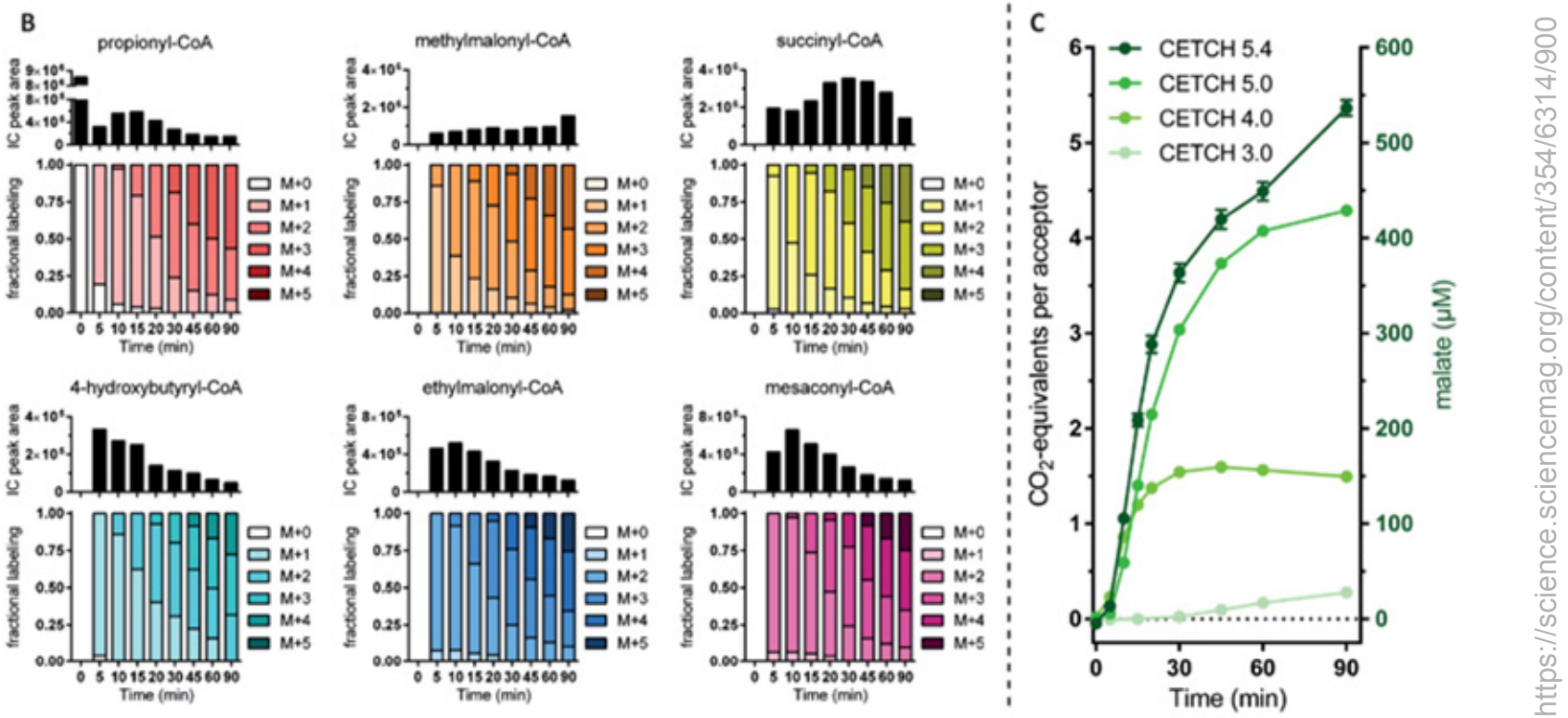


- **17 different enzymes**
- **9 different organisms**
  - *Methylobacterium*
  - *Rhodobacter*
  - *Clostridium*
  - *E. coli*
  - *Nitrosopumilus*
  - Human liver
  - etc.
- **3 engineered enzymes**

(**A**) Topology of the CETCH cycle (version 5.4), including proofreading and cofactor regenerating enzymes.

https://science.sciencemag.org/content/354/6314/900

Dynamics of key intermediates of CETCH over 90 minutes

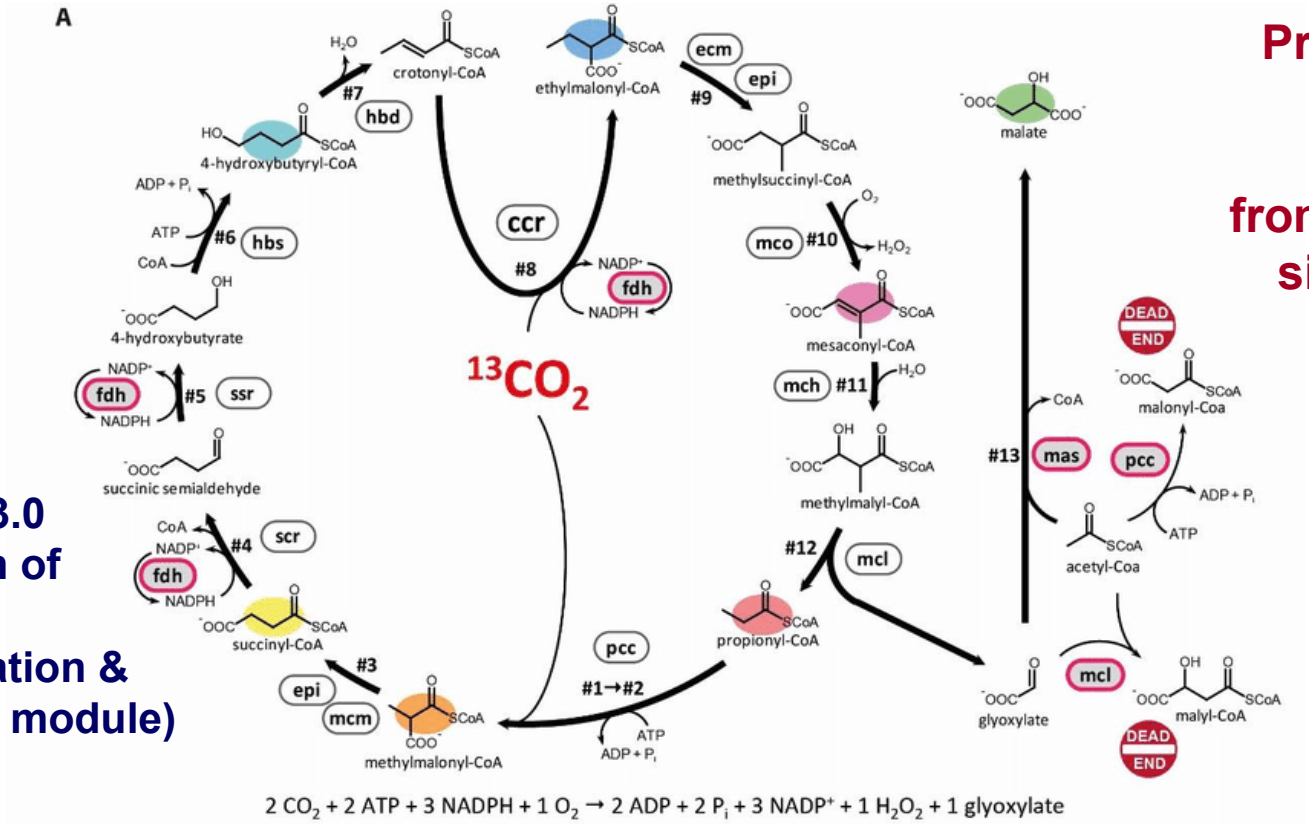


B) Shown are the levels of six different intermediates, as well as their fractional labeling patterns from the incorporation of $^{13}CO_2$ for each turn of the cycle.

C) Left x-axis: CO2-fixation efficiency of the CETCH cycle over the course of its optimization. CO_2-fixation efficiency is defined as the CO_2-equivalents fixed per acceptor molecule in the cycle (i.e., starting amount of propionyl-CoA). Right x-axis: Absolute malate concentration formed over the course of 90 minutes in CETCH 5.4. The final assay (0.52 ml) contained 2.3 mg ml^{-1} protein of cycle core enzymes plus 0.8 mg ml^{-1} auxillary enzymes and produced 540 µM malate over 90 min, which corresponds to 1080 µM fixed CO_2.

How to optimize metabolic pathways?

CETCH 3.0 (addition of NADPH regeneration & read-out module)

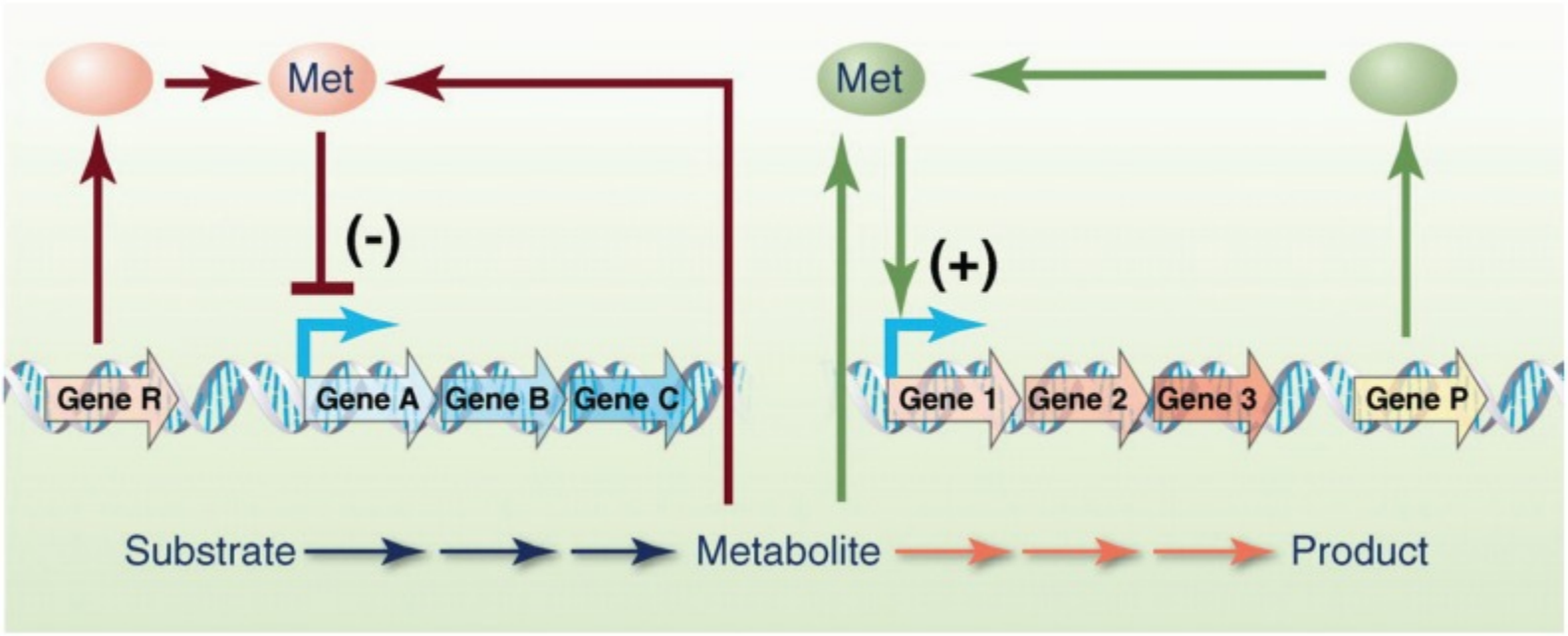


Production of dead-end metabolites from unwanted side reaction

(A) Topology of the CETCH cycle version 3.0. The newly added enzymes Fdh (NADPH regeneration) and Mas (read-out module) converting the primary CO_2-fixation product glyoxylate into malate are boxed in red.

https://doi.org/10.1016/j.tibs.2018.05.004

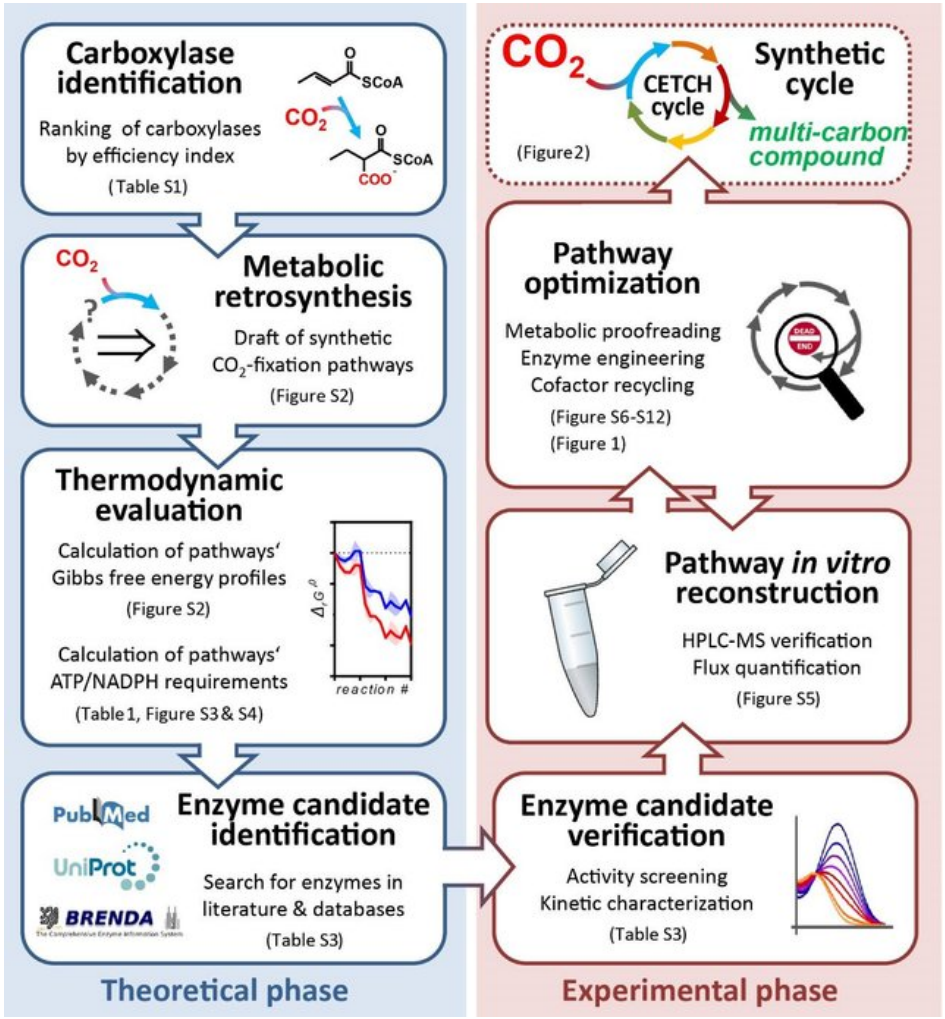
Use of synthetic regulators to modulate metabolic pathways that have a toxic intermediate



Regulatory proteins or RNAs bind the toxic metabolite and down-regulate the biosynthetic pathway and up-regulate the consumption pathway.

https://www.ncbi.nlm.nih.gov/books/NBK84444/

Strategy for the design and realization of the CETCH cycle, a synthetic pathway for CO_2-fixation

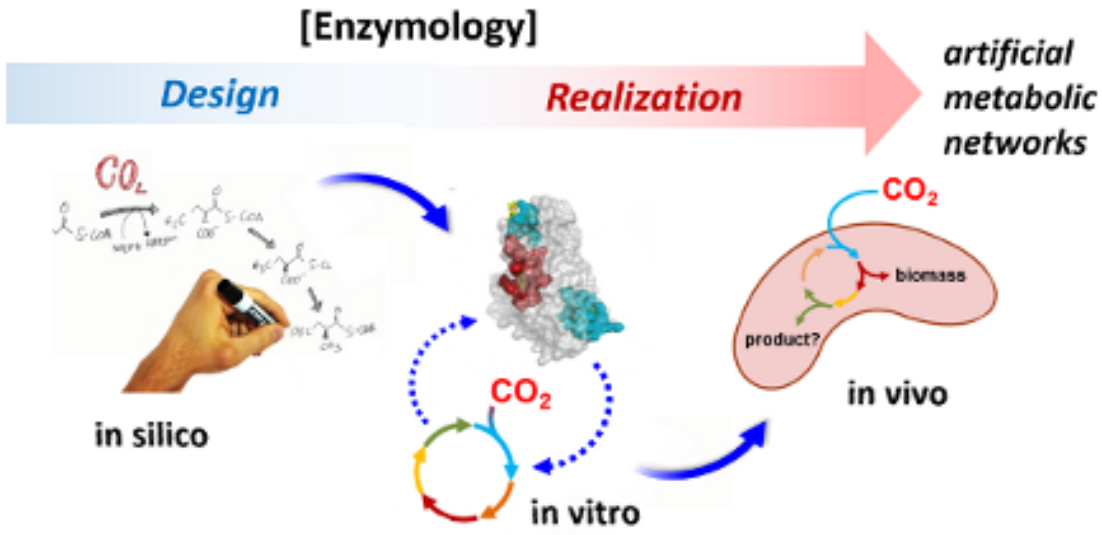


CETCH cycle:
4 ATP molecules per CO2 (pyruvate)

Calvin cycle:
7 ATP molecules per CO2 (pyruvate)

https://www.beilstein-journals.org/bjoc/articles/15/49

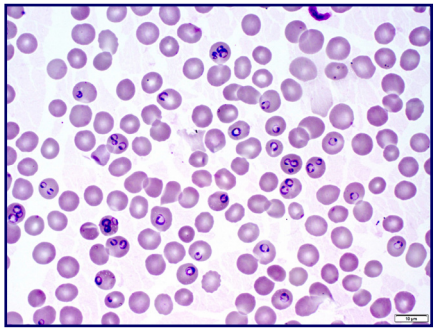
Next steps: Transplanting the CETCH cycle

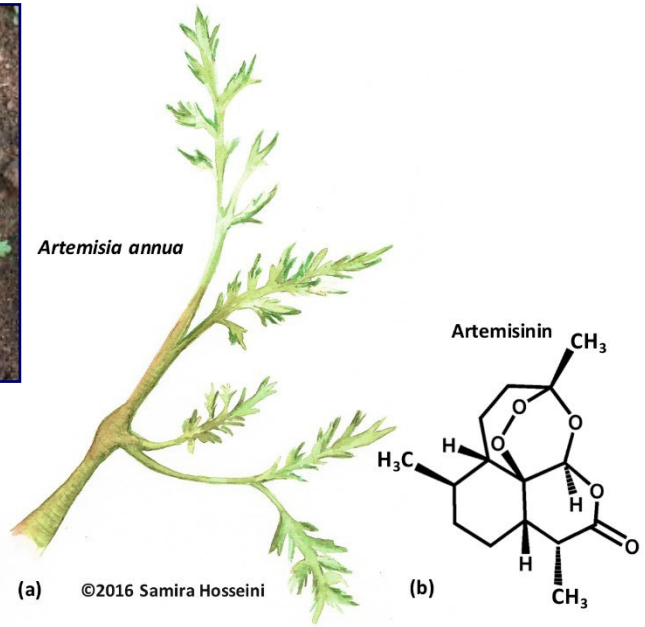


https://www.beilstein-journals.org/bjoc/articles/15/49

Artemisinin: a metabolic engineering success story

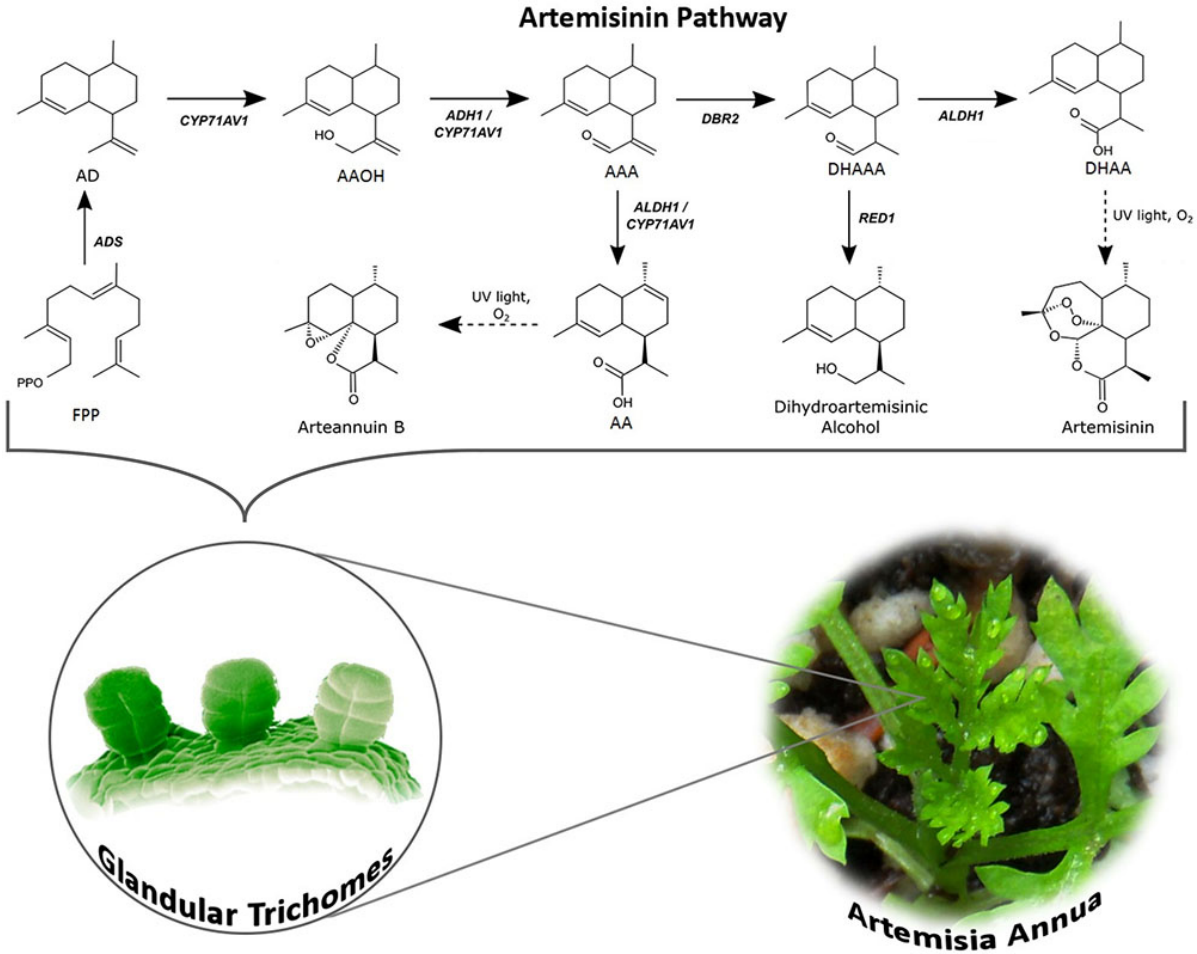
Malaria is caused by parasites of the *Plasmodium* species, primarily *Plasmodium falciparum* and *Plasmodium vivax*. Almost one million deaths from malaria and over 200 million new infections are recorded annually, primarily among young children in the developing world. In recent years, malaria parasites have developed resistance to all inexpensive drugs available in those areas, rendering these drugs ineffective. In response, the World Health Organization recommended the use of alternative treatments called artemisinin-based combination therapies (ACTs). This report describes progress toward the development of a semisynthetic production process whereby an artemisinin precursor is produced by engineered yeast (*Saccharomyces cerevisiae)* in large-scale fermentation processes, and the precursor is then chemically converted to artemisinin.





https://www.pnas.org/content/109/3/E111/1

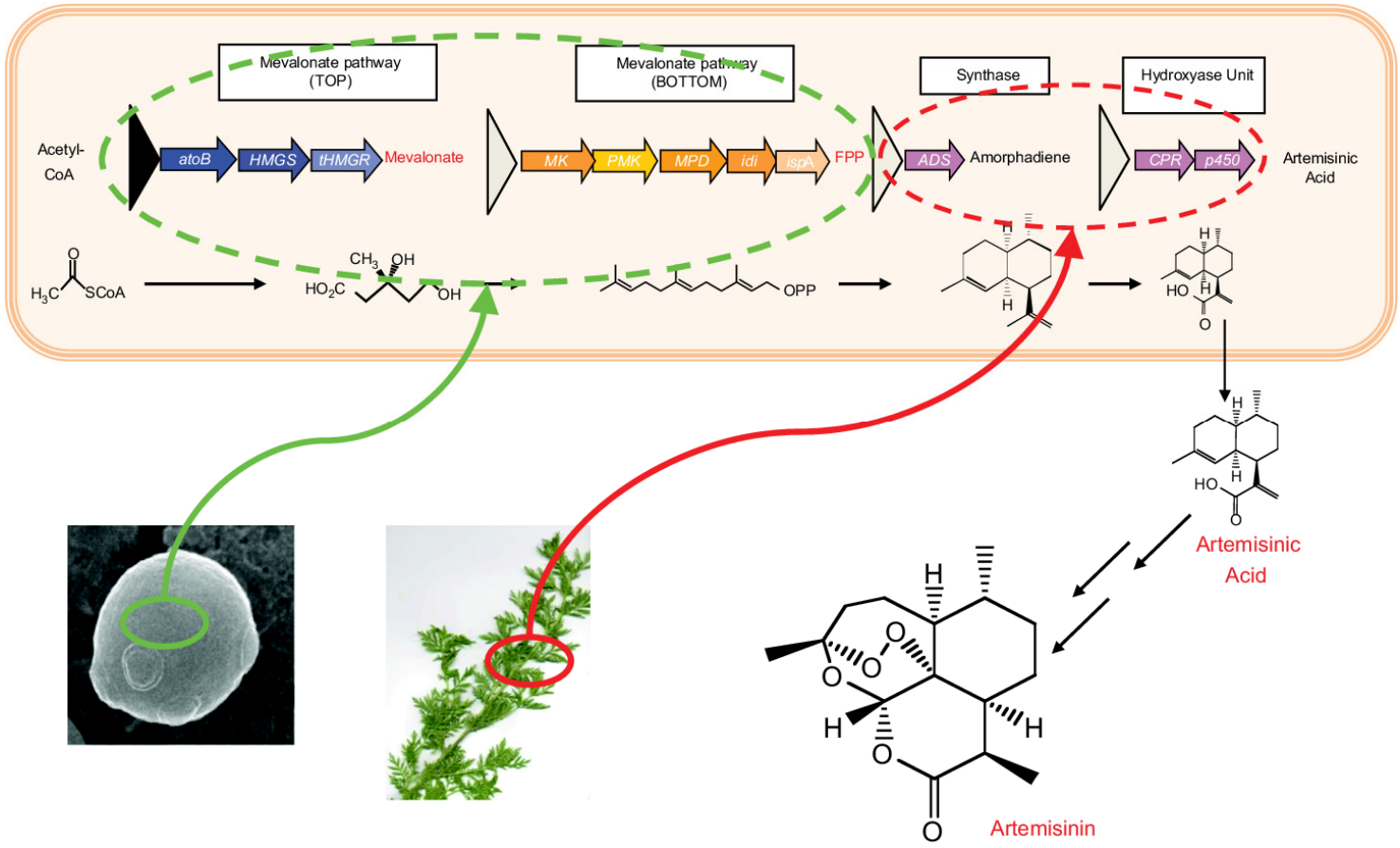
Artemisinin biosynthesis in *Artemisia annua*



Artemisinin biosynthesis pathway occurs in the glandular trichomes of *Artemisia annua*. The pathway intermediates are defined as FPP, farnesyl diphosphate; AD, amorpha-4,11-diene; AAOH, artemisinic alcohol; AAA, artemisinic aldehyde; AA, artemisinic acid; DHAAA, dihydroartemisinic aldehyde; DHAA, dihydroartemisinic acid.

https://www.frontiersin.org/articles/10.3389/fpls.2017.01966/full

Reconstruction of artemisinin pathway in yeasts



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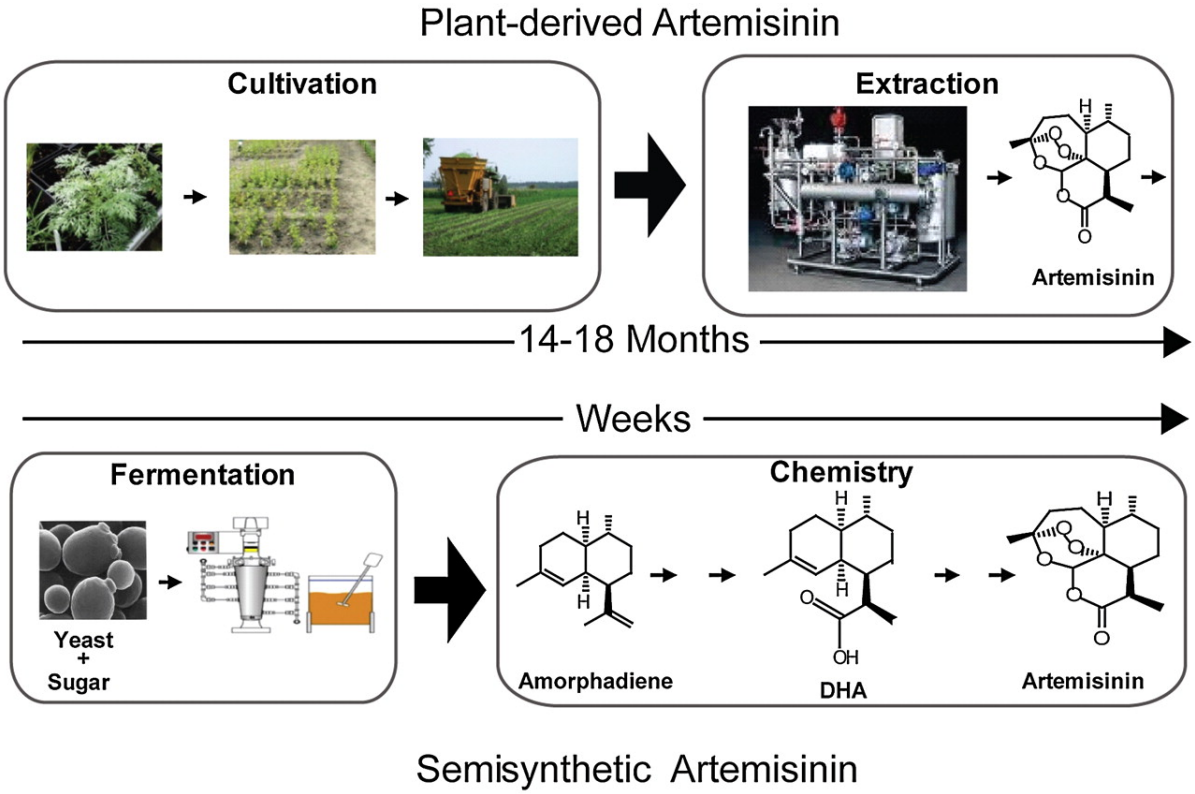
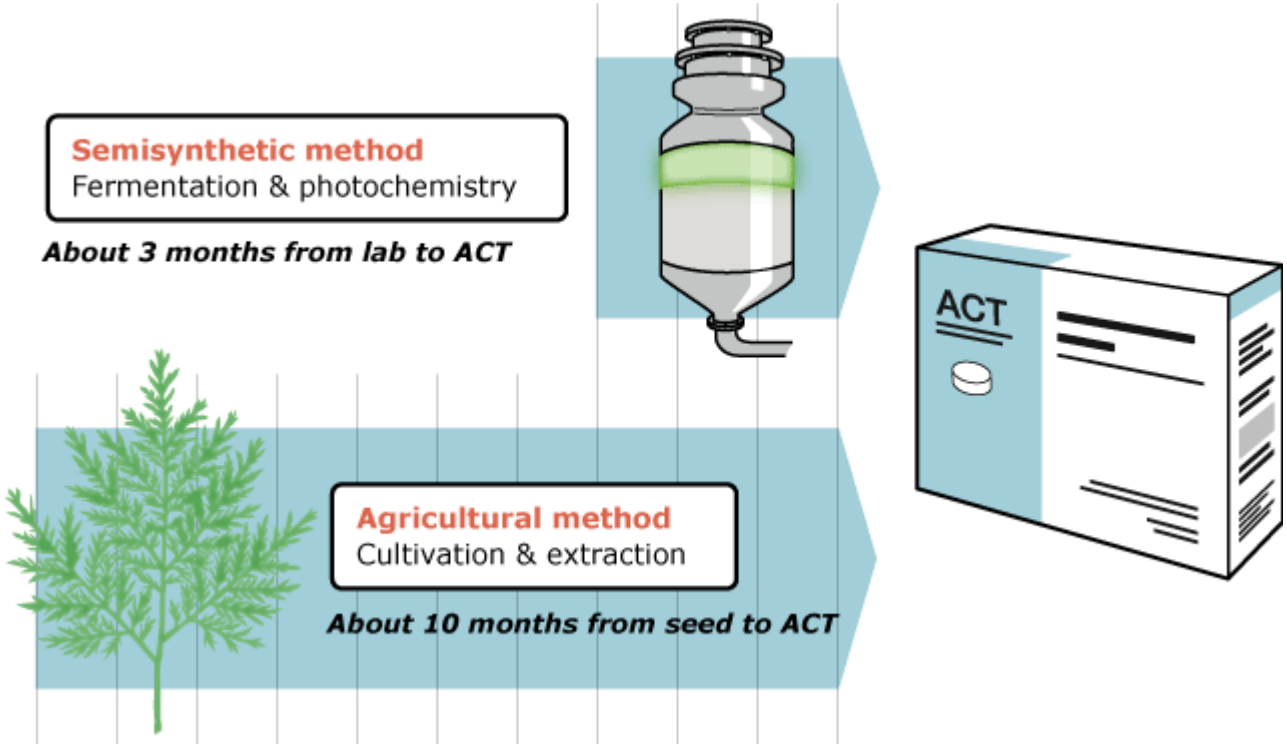
Artemisinin: a metabolic engineering success story



Production of plant-derived artemisinin compared to semisynthetic artemisinin. Production of plant-derived artemisinin takes from 14 to 18 months from planting to production. Plant-derived artemisinin requires cultivation of *A. annua*, followed by extraction of artemisinin from the leaves and conversion to artemisinin derivatives for incorporation into antimalarial ACT medication. Semisynthetic artemisinin, by contrast, uses engineered yeast to produce amorphadiene in fermentations. The amorphadiene extracted from the fermentor is chemically converted to dihydroartemisinic acid and then to artemisinin derivatives for incorporation into ACT drugs. The entire process could be accomplished in weeks.

https://www.pnas.org/content/109/3/E111/1

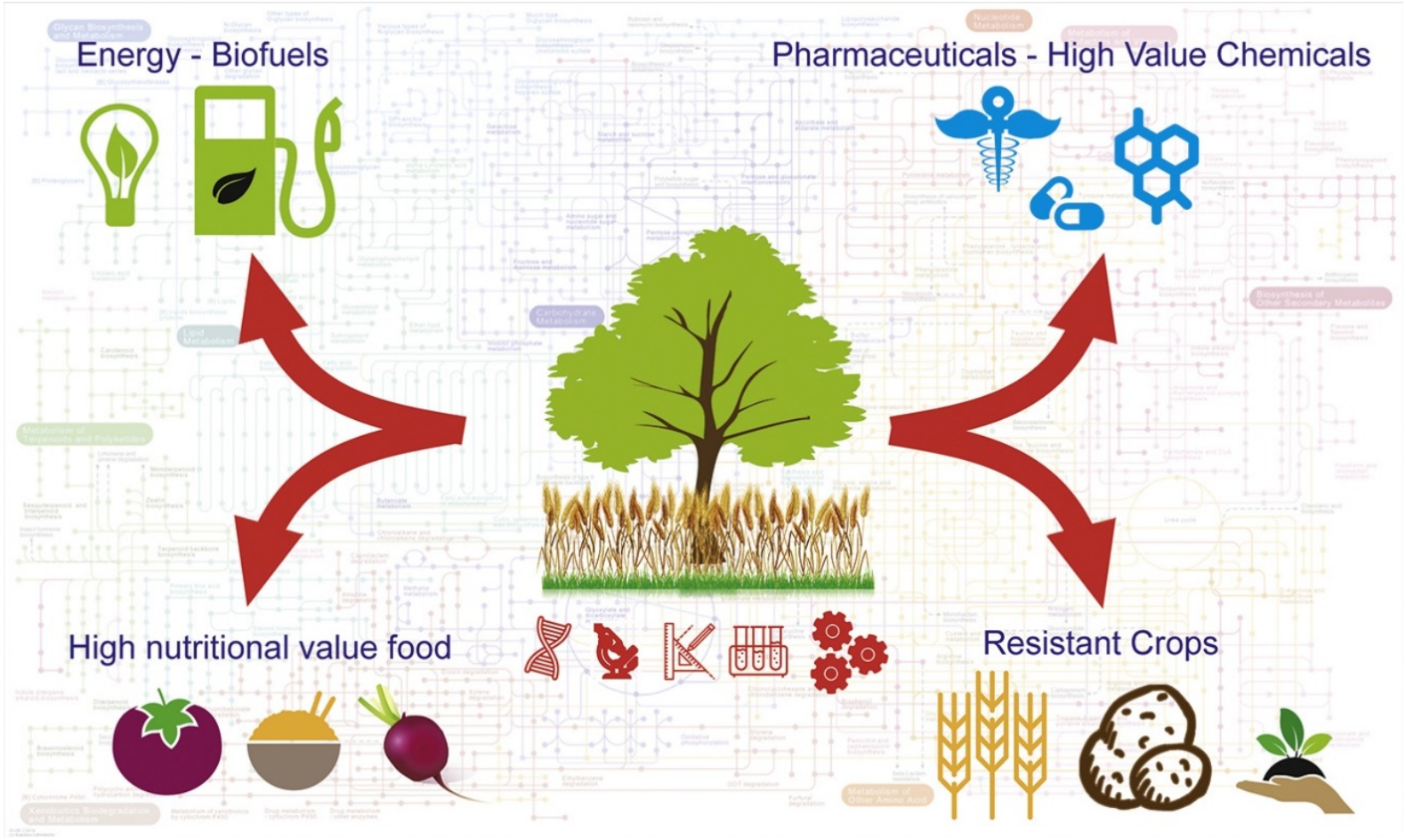
Making artemisinin to fight malaria



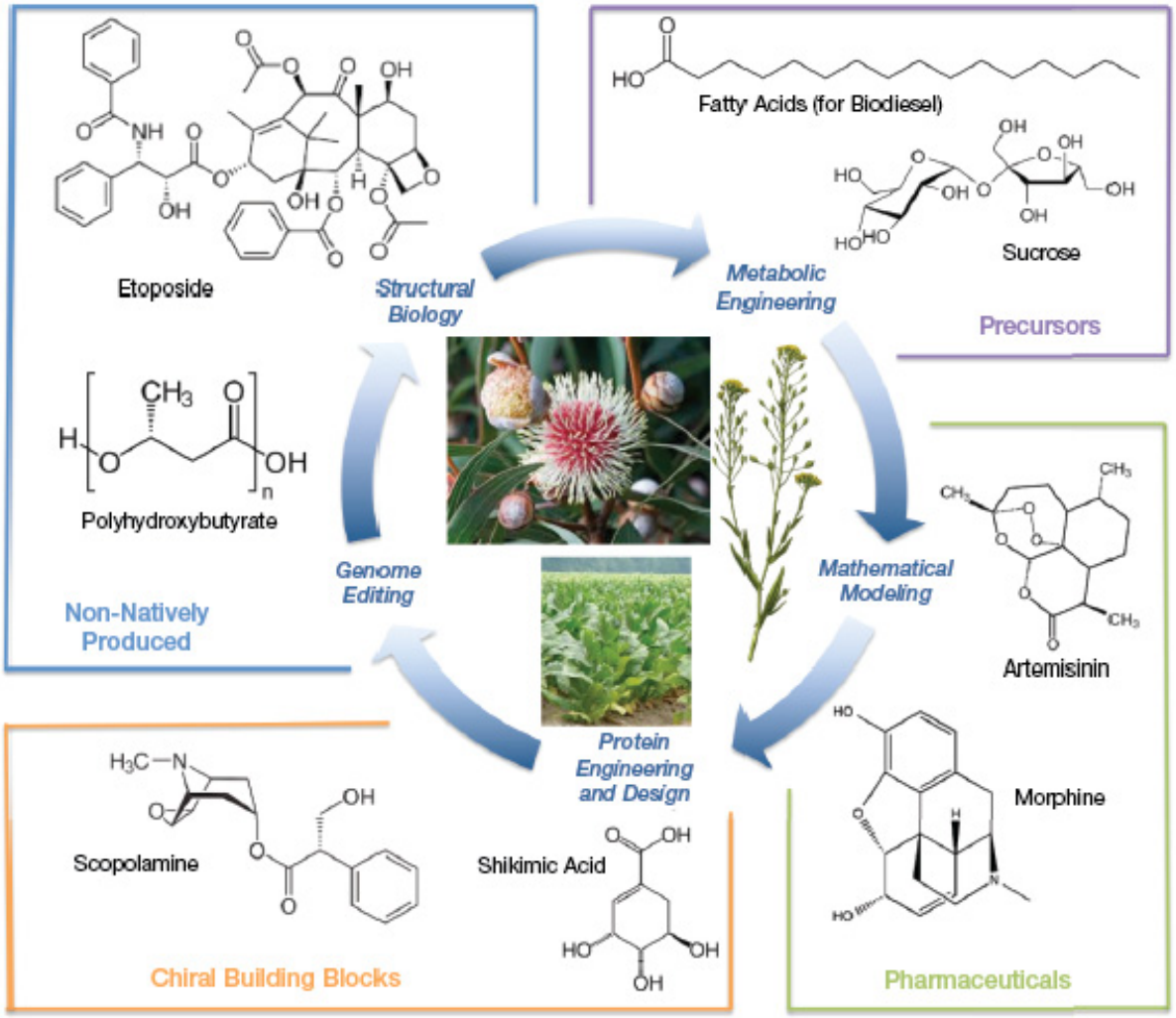
As for artemisinin itself, almost 100 tonnes of it are needed each year to treat malarial infections, however, there are still great challenges in finding cost-effective ways of producing artemisinin. The main way of producing artemisinin used to be by extraction from *Artemisia annua* plant itself, however, the costs of growing the plant and the efficiency of the process (5 kg or artemisinin per 1,000 kg of dried leaves) has never been sufficient to meet the global demands. Therefore, the producers have now focussed on looking for new synthetic ways of making the drug. With a financial backup from Melinda and Bill Gates foundation a company called Amyris and its collaborators have created a yeast strain capable of making artemisinic acid (a precursor for artemisinin) in fermentation reactors at relatively high yields. Even so, the cost of producing artemisinic acid in yeast is still quite high making it difficult to obtain for the poorer countries in Asia and Africa, which are usually most afflicted by malaria.

Engineering plant metabolic pathways

- Plant metabolic pathways can be reconstituted in heterologous hosts.
- Metabolism in crop plants can be engineered to improve the production of biofuels.
- Crops can be engineered to express metabolic pathways that improve human health

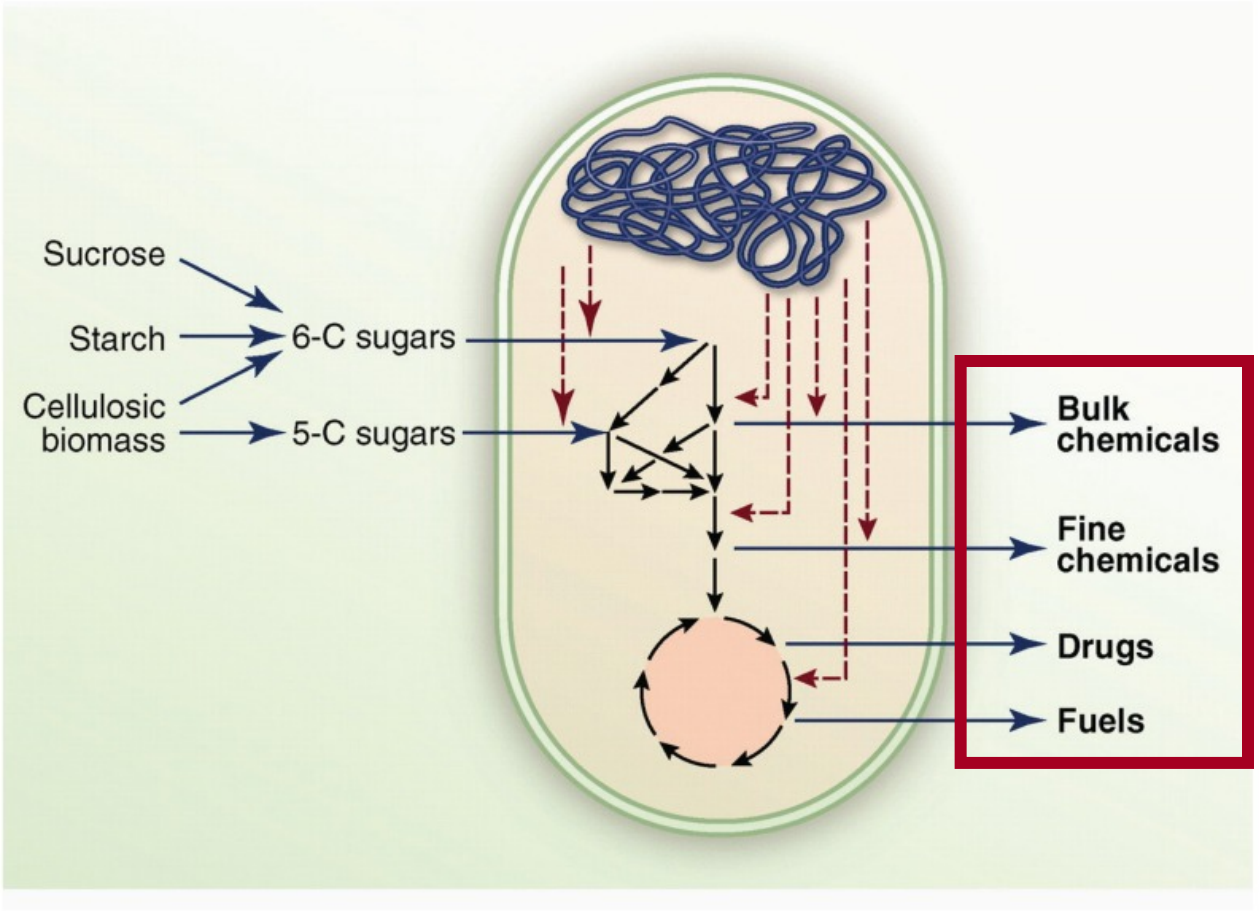


Plant metabolic engineering for chemicals, fuels, and precursors



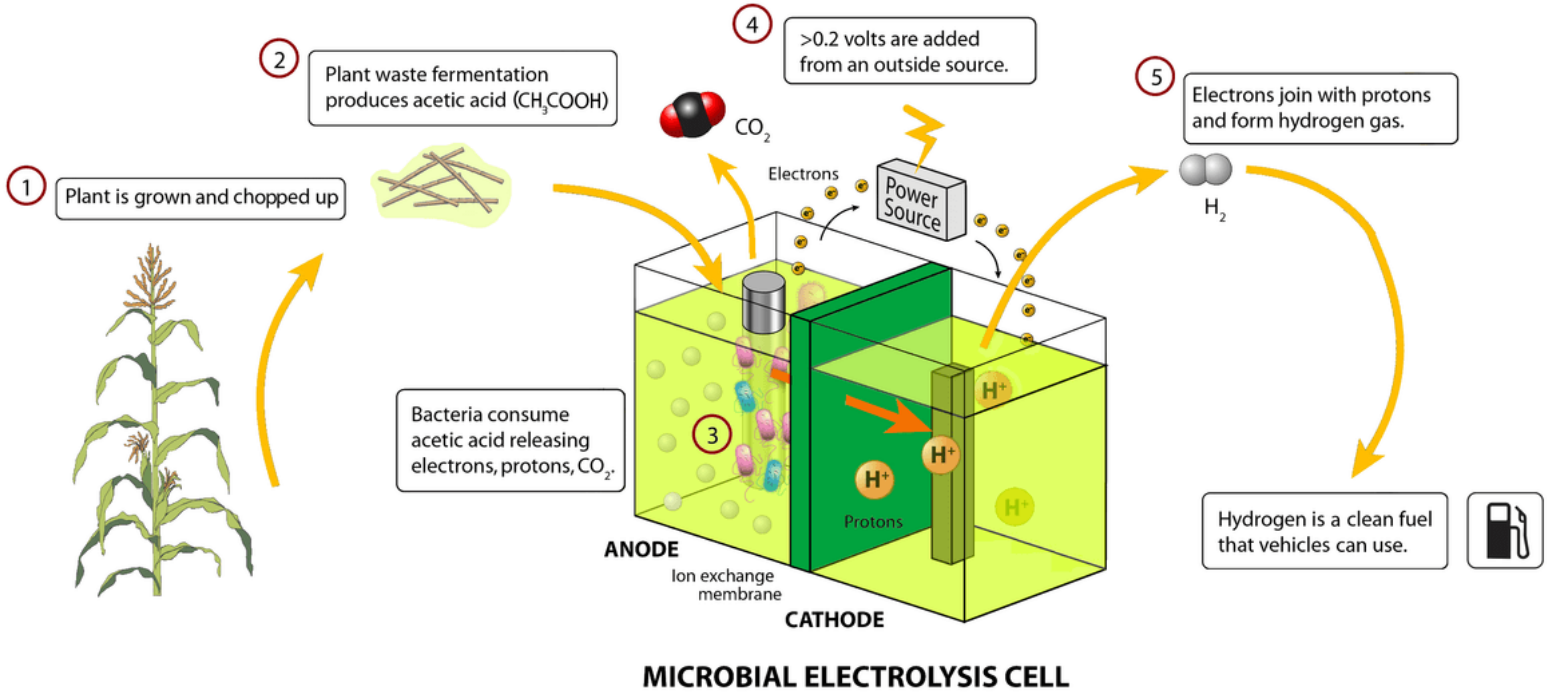
Many different chemicals and fuels can be produced from plants; the photos in the center are several representative species. The outer boxes show various chemicals that can be produced from plants, including shikimic acid and morphine. The inner ring shows different enabling technologies that facilitate production routes for these and other fuels and chemicals.

Conversion of sugars to chemicals by means of microbial catalysts



https://www.ncbi.nlm.nih.gov/books/NBK84444/

Biological fuel cells (BFCs) and the bio-production of hydrogen



Biological power systems have many advantages over traditional chemical systems due to low-temperature operation and non-hazardous materials. These systems have the potential to eliminate the transport and storage of large quantities of hydrogen because the hydrogen is created in-situ. Also, the BFC eliminates the occurrence of catalyst poisoning over time due to the use of biological catalysts. These naturally-occurring systems hold the future of energy production for portable and stationary systems.

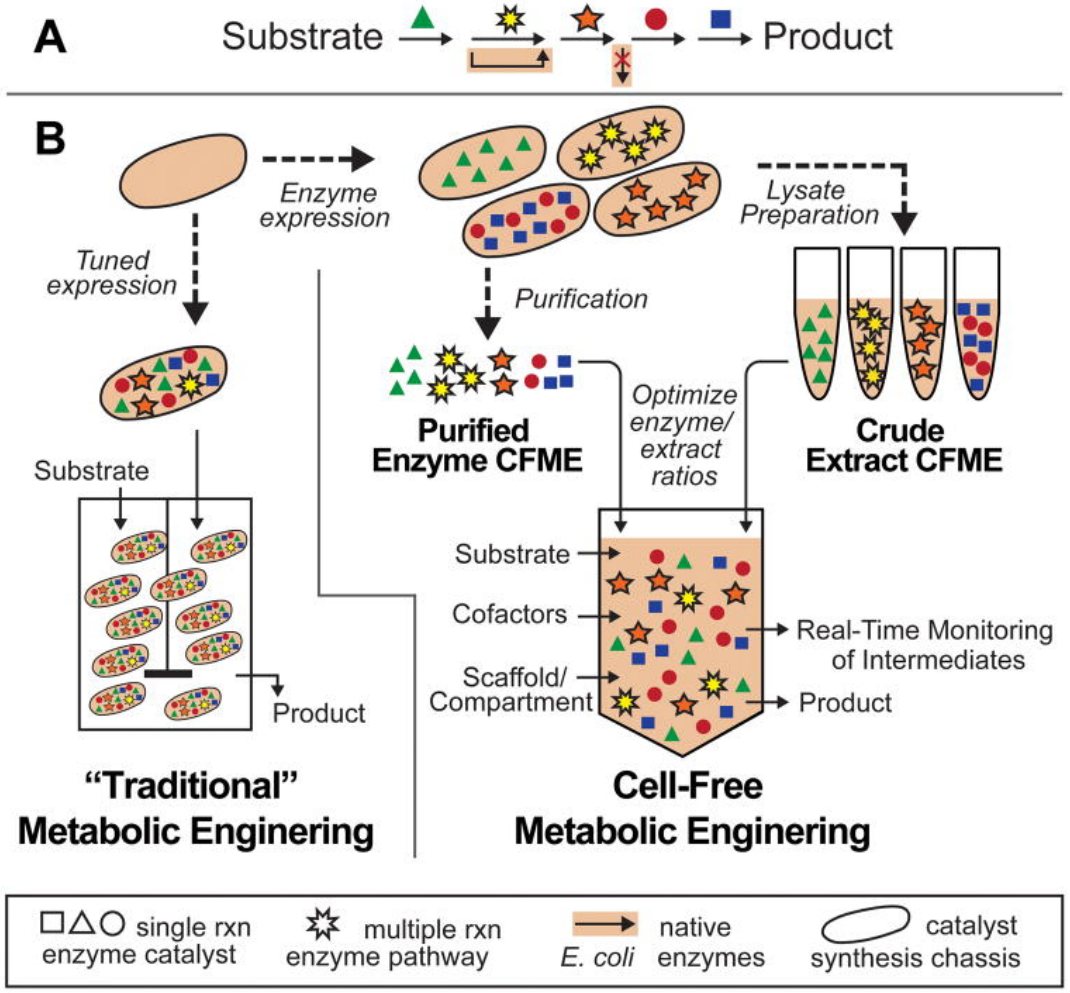
https://www.fuelcellstore.com

How algae could change our world

https://www.forbes.com/sites/jenniferhicks/2018/06/15/see-how-algae-could-change-our-world/#f6653cc3e466

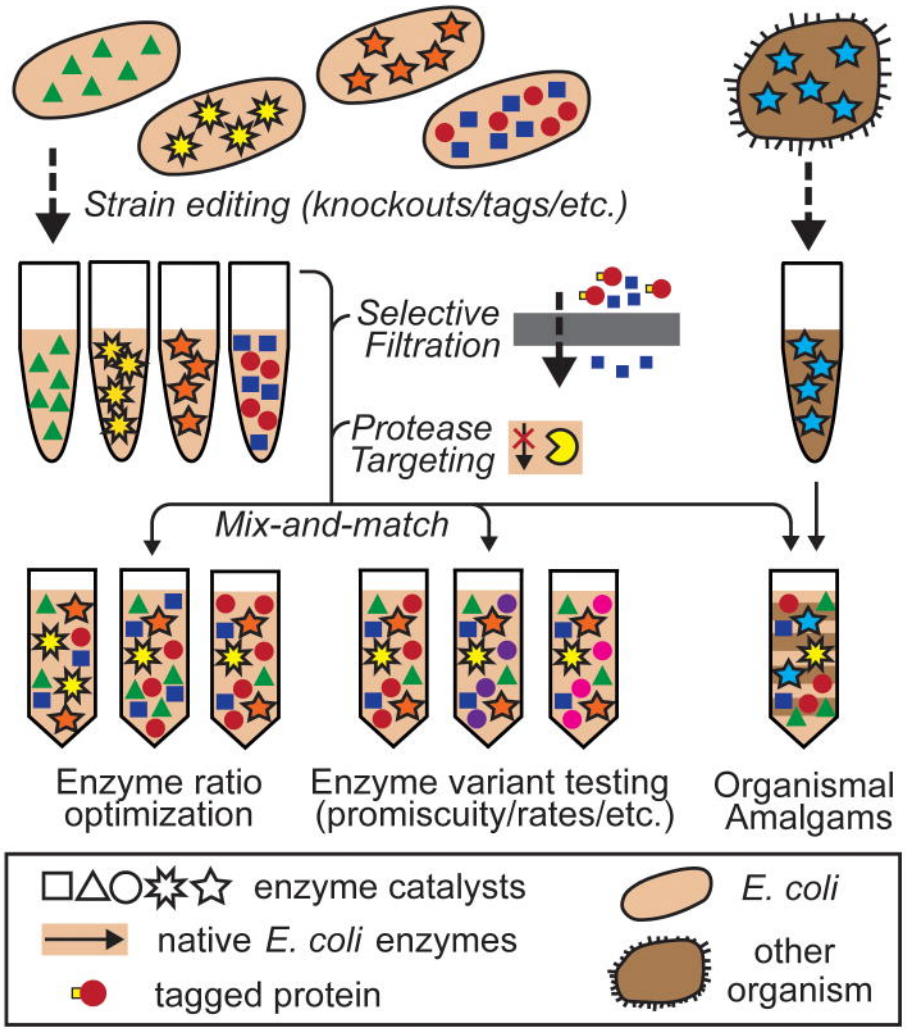
Cell-free metabolic engineering: biomanufacting beyond the cell

Paradigms for metabolic engineering (**A**) sample pathway (**B**) traditional and cell-free approaches.



Industrial biotechnology and microbial metabolic engineering are poised to help meet the growing demand for sustainable, low-cost commodity chemicals and natural products, yet the fraction of biochemicals amenable to commercial production remains limited. Common problems afflicting the current state-of-the-art include **low volumetric productivities, build-up of toxic intermediates** or products, and byproduct losses via competing pathways. To overcome these limitations, **cell-free metabolic engineering (CFME)** is expanding the scope of the traditional bioengineering model by using *in vitro* ensembles of catalytic proteins prepared from purified enzymes or crude lysates of cells for the production of target products.

Cell-free metabolic engineering: biomanufacturing beyond the cell



New opportunities to use crude cell lysates for pathway optimization and debugging.

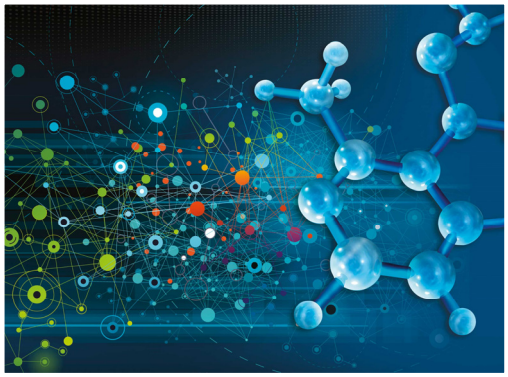
http://europepmc.org/articles/pmc4314355

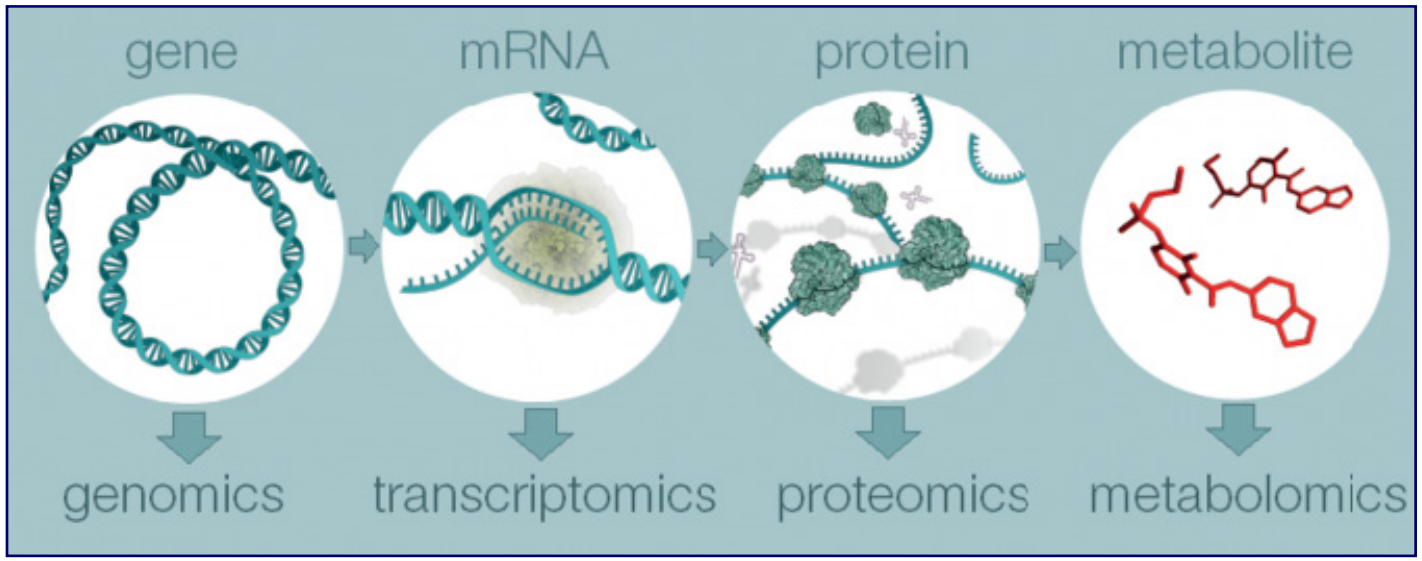
Metabolomics

Concepts
Methods
Applications

Flux analysis and metabolomics for metabolic engineering

Metabolomics is the large-scale study of small molecules, commonly known as metabolites, within cells, biofluids, tissues or organisms. Collectively, these small molecules and their interactions within a biological system are known as the metabolome.



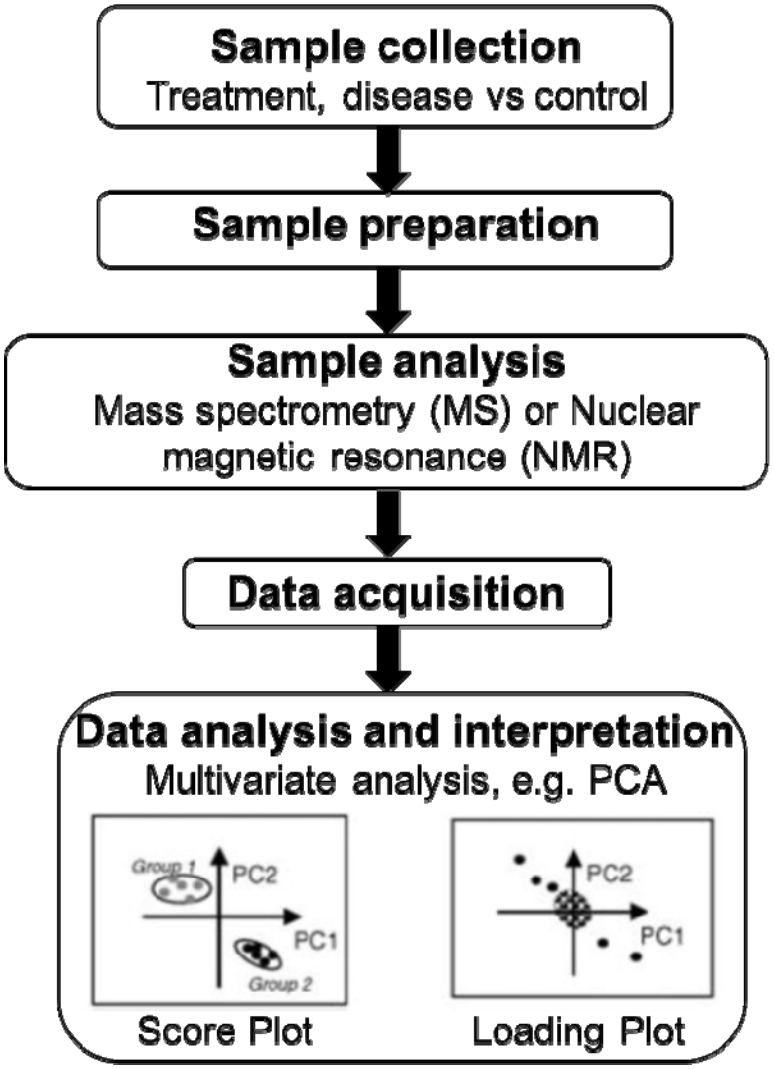


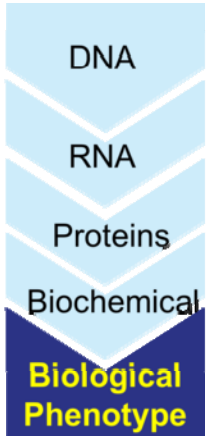
Metabolome

- **Metabolome** refers to the complete set of small-molecule **(<1.5 kDa**) metabolites (such as metabolic intermediates, hormones and other signalling molecules, and secondary metabolites) to be found within a biological sample, such as a single organism.

- Although the metabolome can be defined readily enough, it is not currently possible to analyse the entire range of metabolites by a single analytical method.

Metabolomics: methodology





Genomics

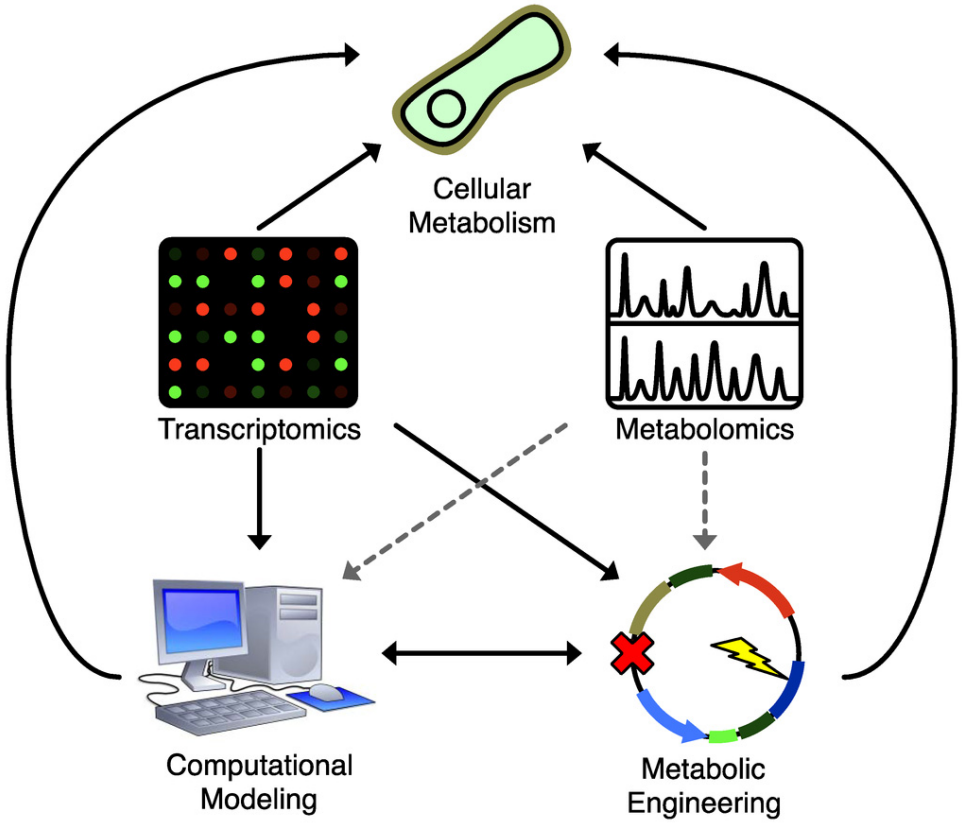
Transcriptomics

Proteomics

Metabolomics

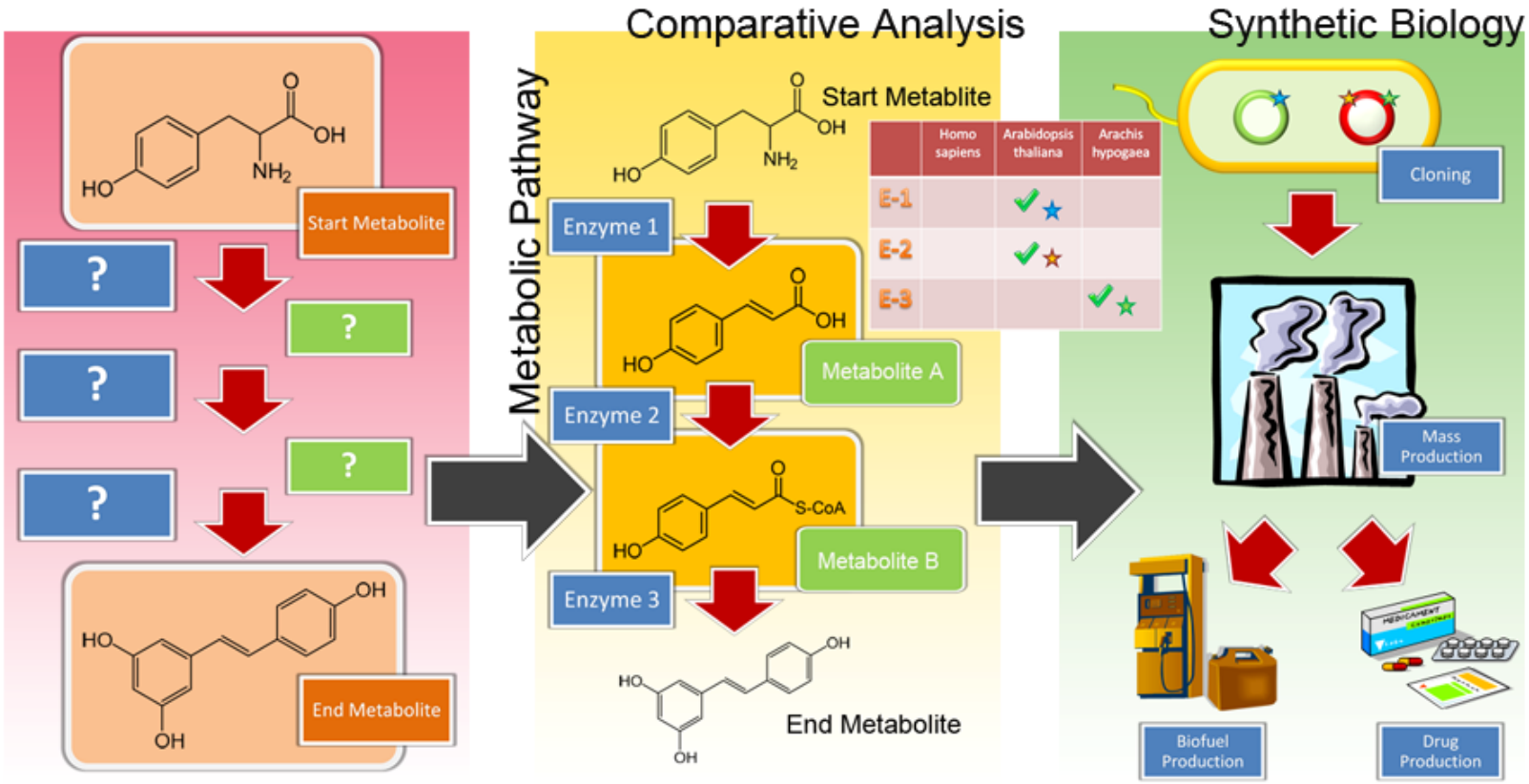
- Gas chromatography-mass spectrometry (GC-MS)
- NMR spectroscopy

Understanding cellular metabolism



Applications of various techniques to understanding and manipulating cellular metabolism. Solid lines represent widely used strategies, dashed lines represent underused strategies. Both metabolomics and transcriptional profiling provide a direct readout that helps enable a deeper understanding of cellular metabolism, but only transcriptional profiling has seen widespread application to enhance standard computational modeling and metabolic engineering strategies. Integrating metabolomics data into metabolic engineering and computational modeling strategies would help bridge gaps in biochemical knowledge and improve our ability to control cellular metabolism.

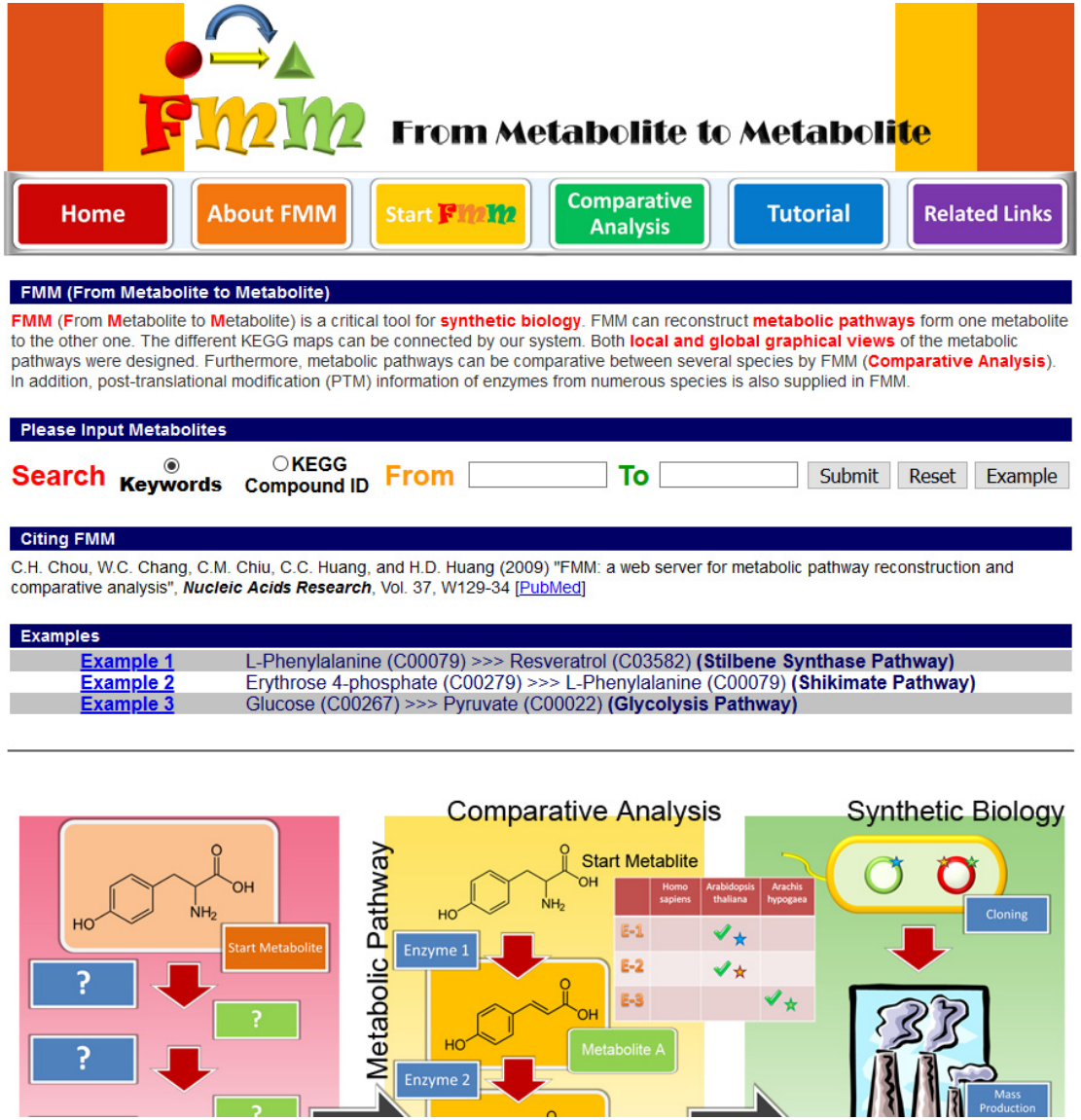
From Metabolite to Metabolite (FMM) is a critical tool for metabolic engineering



http://fmm.mbc.nctu.edu.tw/

FMM (**F**rom **M**etabolite to **M**etabolite) is a critical tool for **synthetic biology**. FMM can reconstruct **metabolic pathways** from one metabolite to the other one. The different KEGG maps can be connected by our system. Both **local and global graphical views** of the metabolic pathways were designed. Furthermore, metabolic pathways can be comparative between several species by FMM (**Comparative Analysis**). In addition, post-translational modification (PTM) information of enzymes from numerous species is also supplied in FMM.

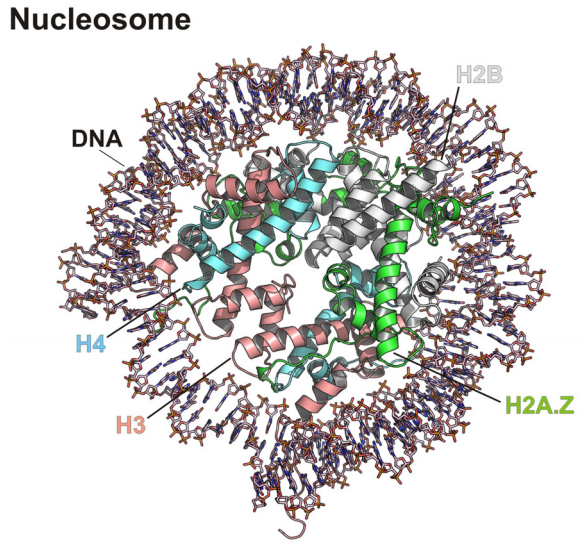
FMM: a software tool for metabolic pathway design



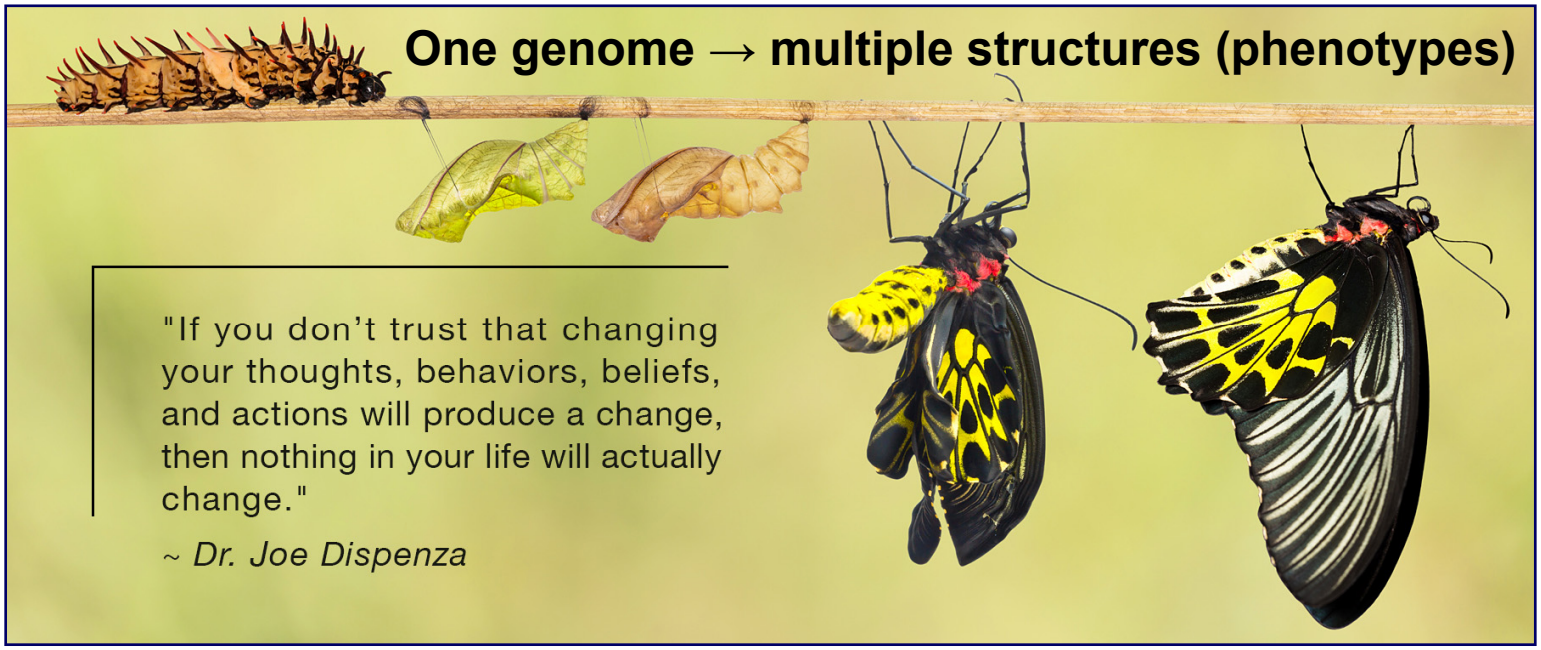
http://fmm.mbc.nctu.edu.tw/

Synthetic epigenetics

Concepts
Methods
Applications

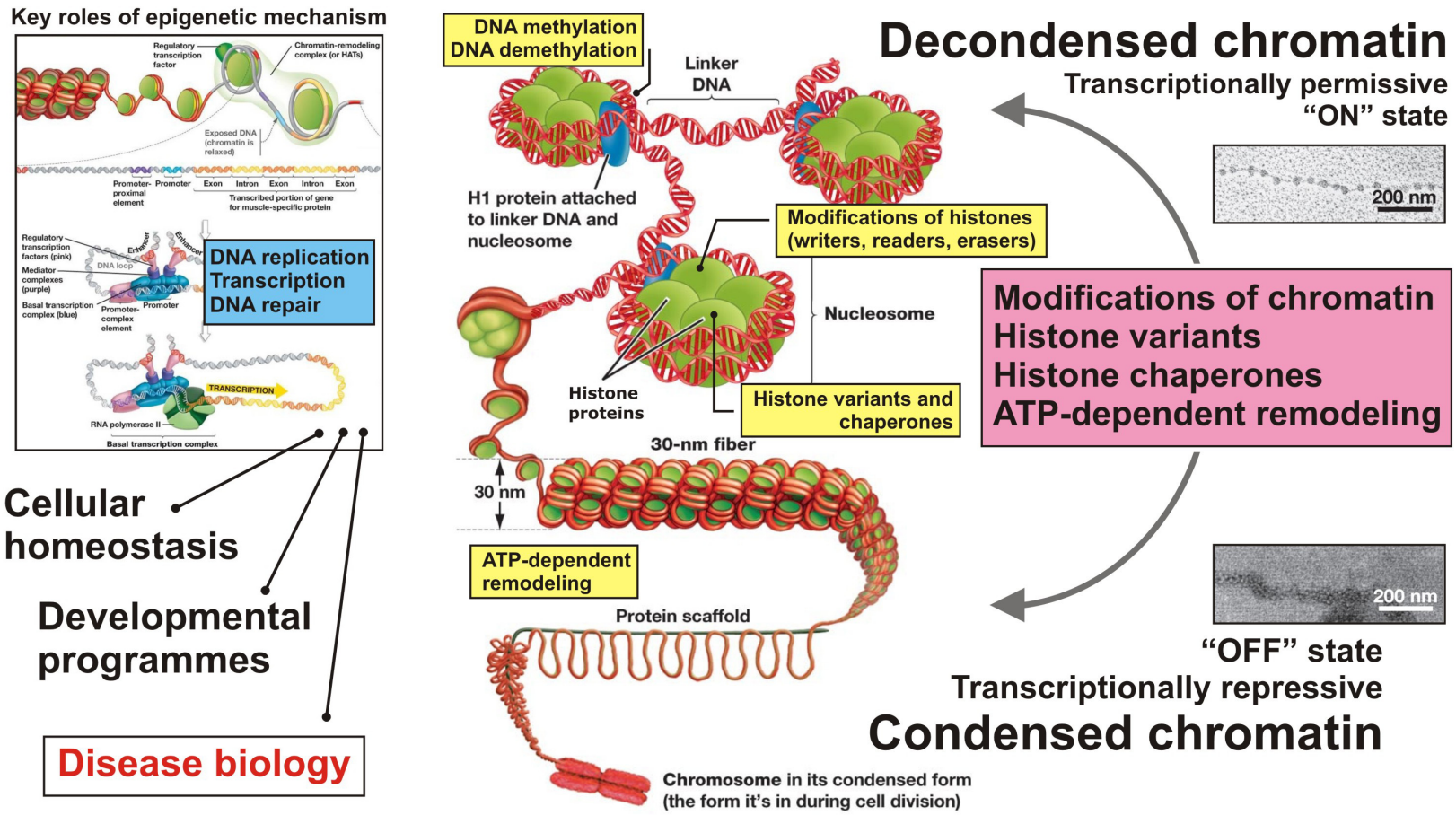


What is epigenetics?

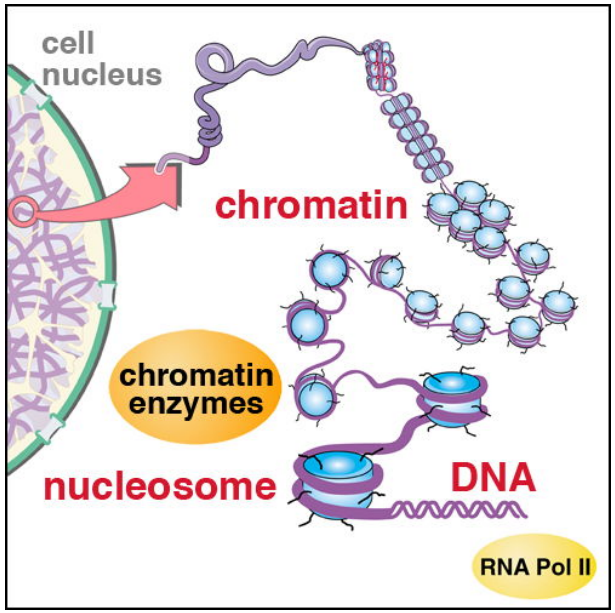


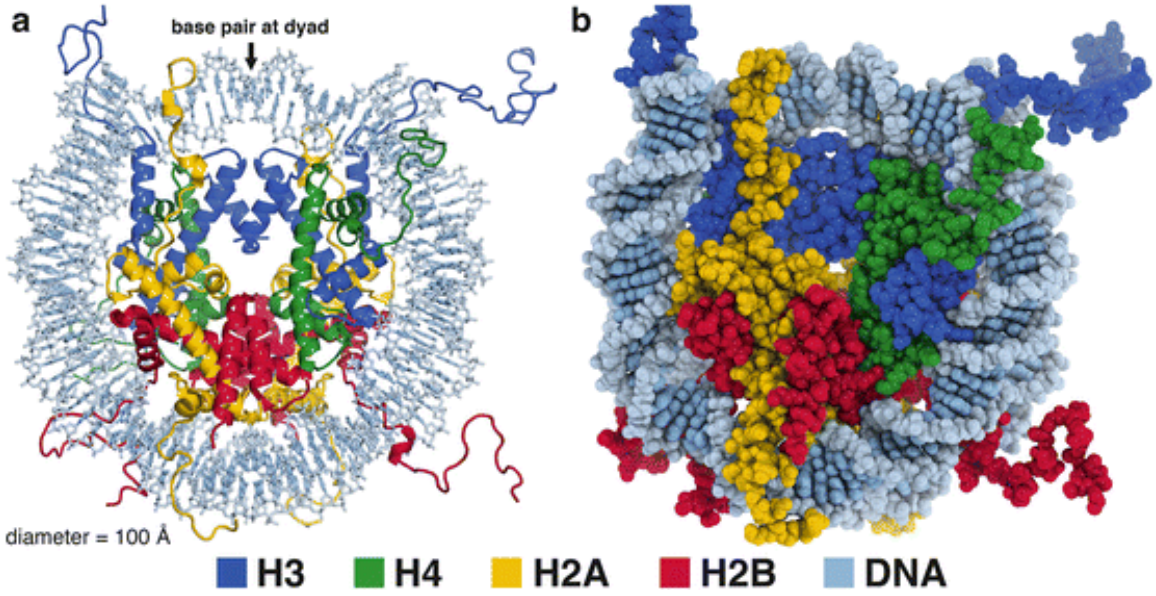
- Changes in gene expression (turning a gene on) and gene silencing (turning a gene off), which do not change the underlying DNA sequence, are collectively referred to as **EPIGENETICS**.
- Metamorphosis is a perfect example of a power of epigenetic control of gene expression.
- Organisms have silenced genes and activated genes.
- Genes can be switched on or off rapidly with needs of adaptation.
- These clever mechanisms point to creation and intelligent design.

Epigenetic regulatory mechanisms

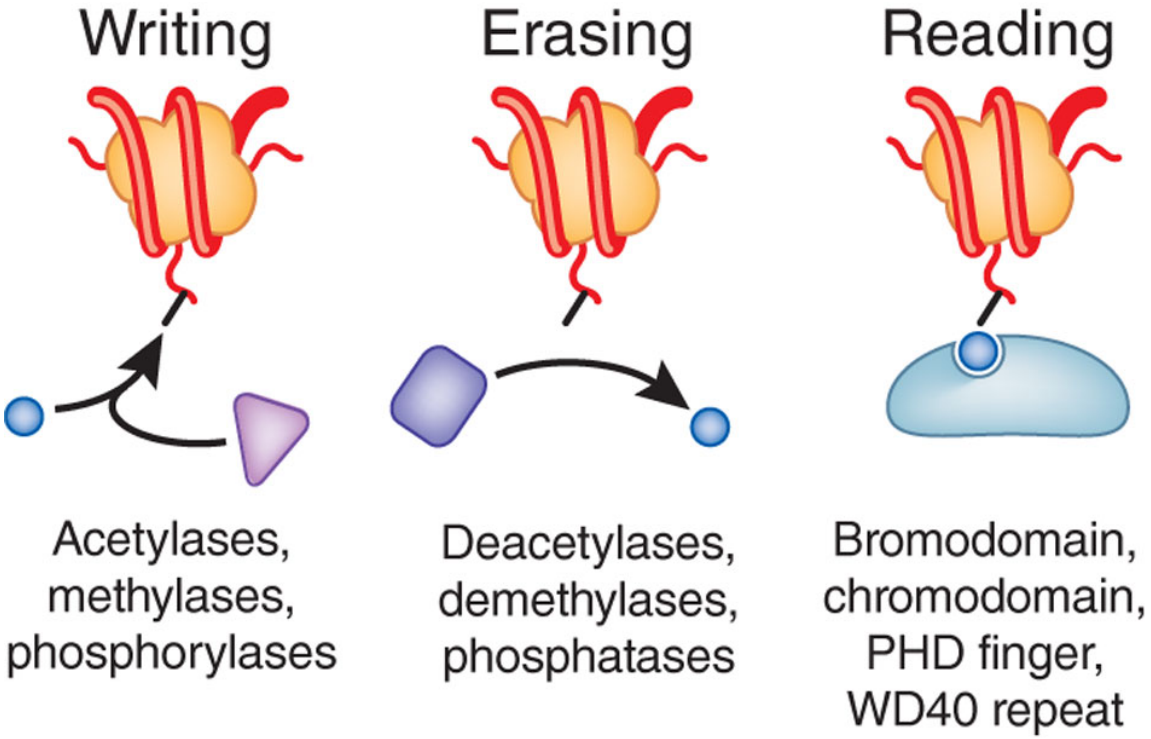


Chromatin structure: a basic building unit (nucleosome)

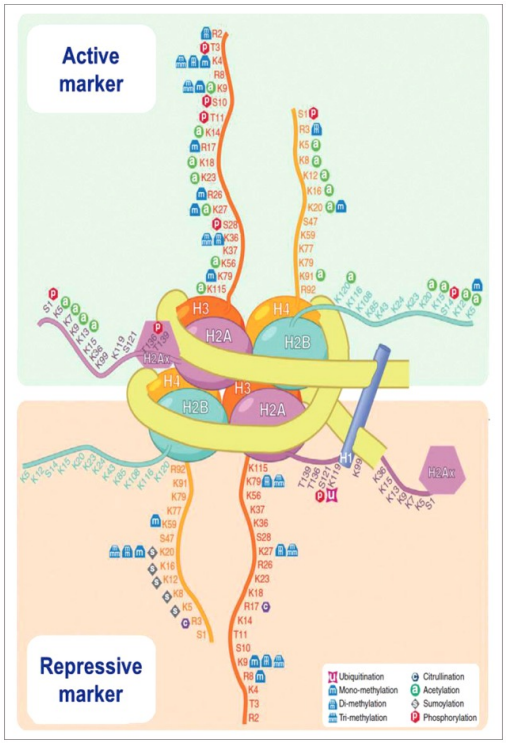


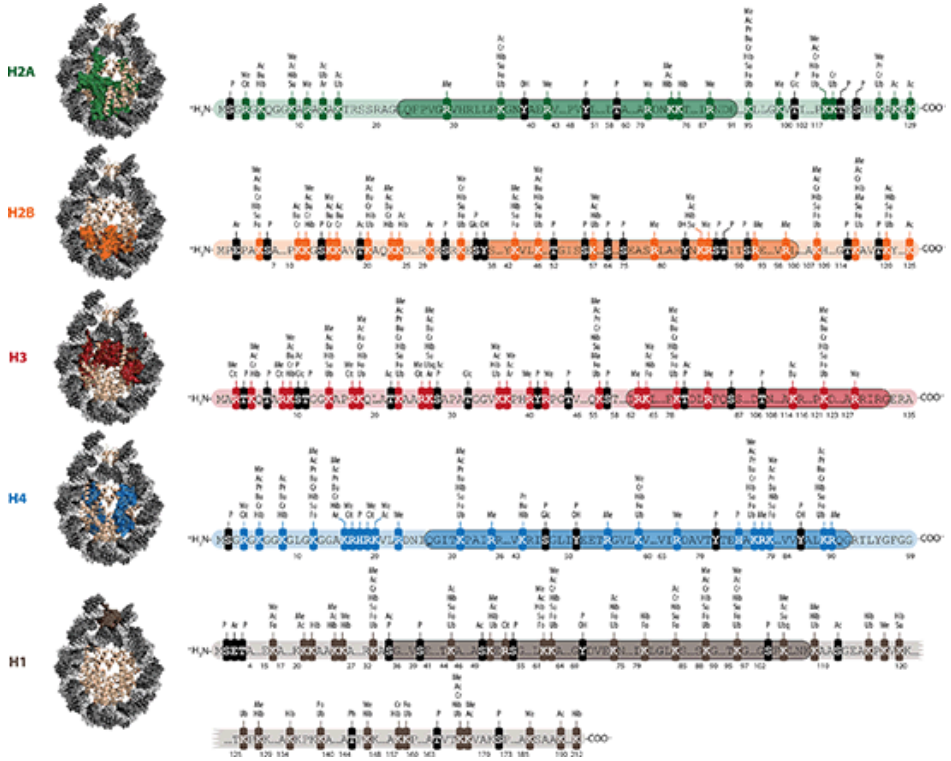


Key epigenetic regulators



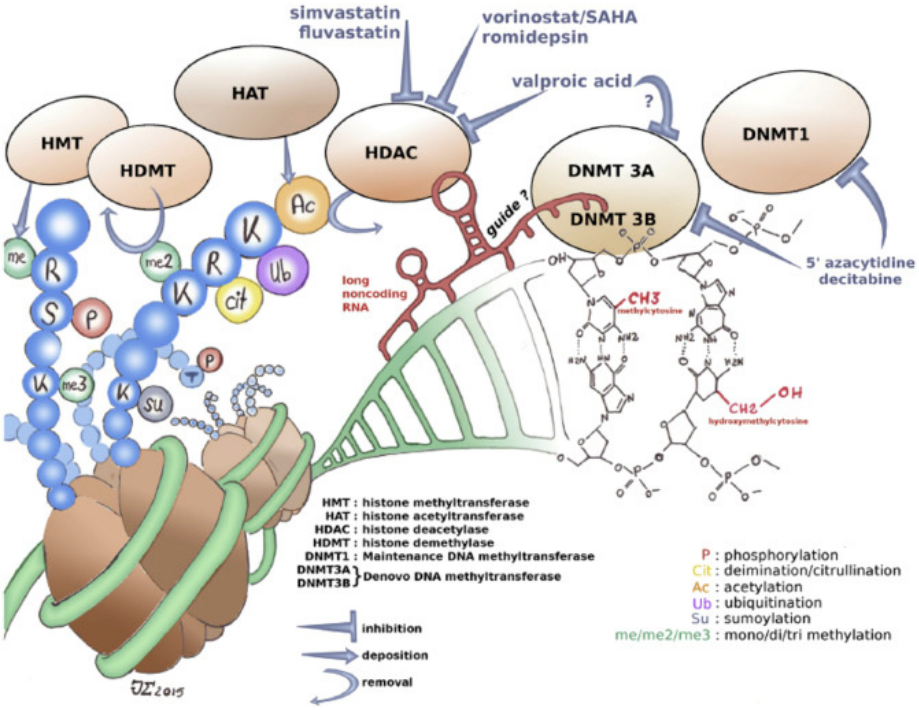
The histone code



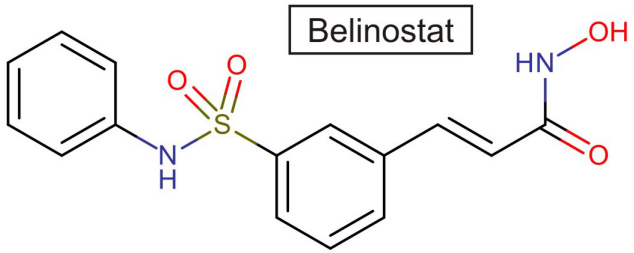


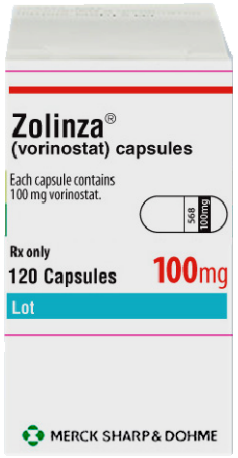
- The **histone code** is a hypothesis that the transcription of genetic information encoded in DNA is in part regulated by chemical modifications to histone proteins, primarily on their unstructured ends.
- Together with similar modifications such as DNA methylation it is part of **the epigenetic code**.

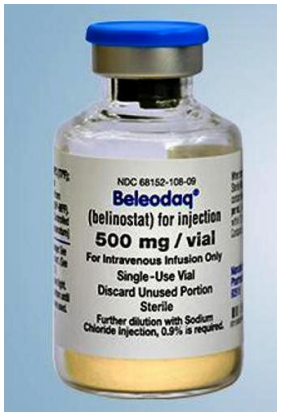
Epigenetic therapy (targeting epigenome)



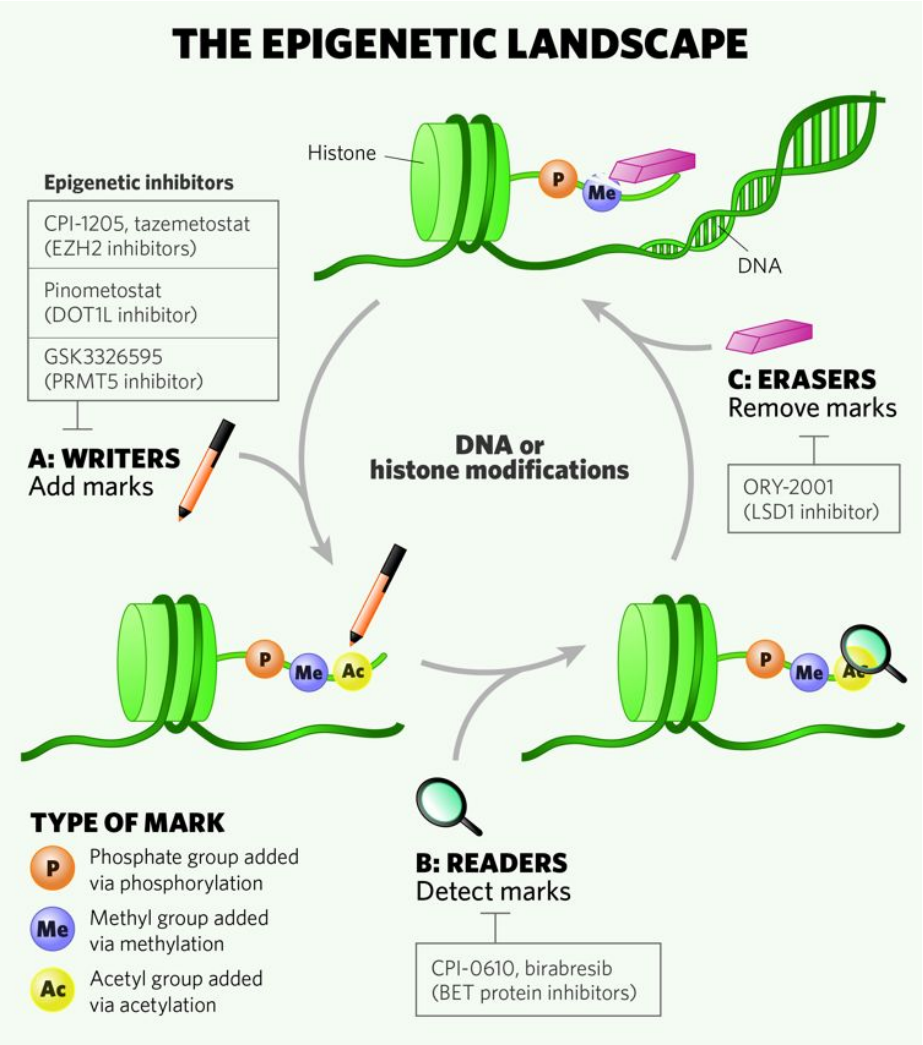
Epigenetic therapy is the use of drugs or other epigenome-influencing techniques to treat medical conditions. Many diseases, including cancer, heart disease, diabetes, and mental illnesses are influenced by epigenetic mechanisms, and epigenetic therapy offers a potential way to influence those pathways directly.







Epigenetic therapy (targeting epigenome)



There is a growing awareness that epigenetic dysregulation plays a significant role in many types of cancer, and that is fuelling an increasing number of studies into drugs that target epigenetic regulators.

Such regulatory proteins fall into three main categories: writers, readers and erasers. Writers 'mark' histones and DNAs by adding chemical groups, indicating either transcription, replication or repair. Modifications include acetylation, phosphorylation and methylation. Readers recognize and act upon the modifications, whereas erasers remove them (see 'The epigenetic landscape').

Several drugs that inhibit epigenetic writers and erasers have been approved by the US Food and Drug Administration (FDA) for the treatment of cancer. These include DNA methyltransferase and histone deacetylase (HDAC) inhibitors. Further candidates, including inhibitors of acetyl-lysine readers (bromodomain-containing proteins), are undergoing clinical evaluation for efficacy in different cancer settings.

Cell

ARTICLE | VOLUME 176, ISSUE 1, P227-238.E20, JANUARY 10, 2019

Engineering Epigenetic Regulation Using Synthetic Read-Write Modules

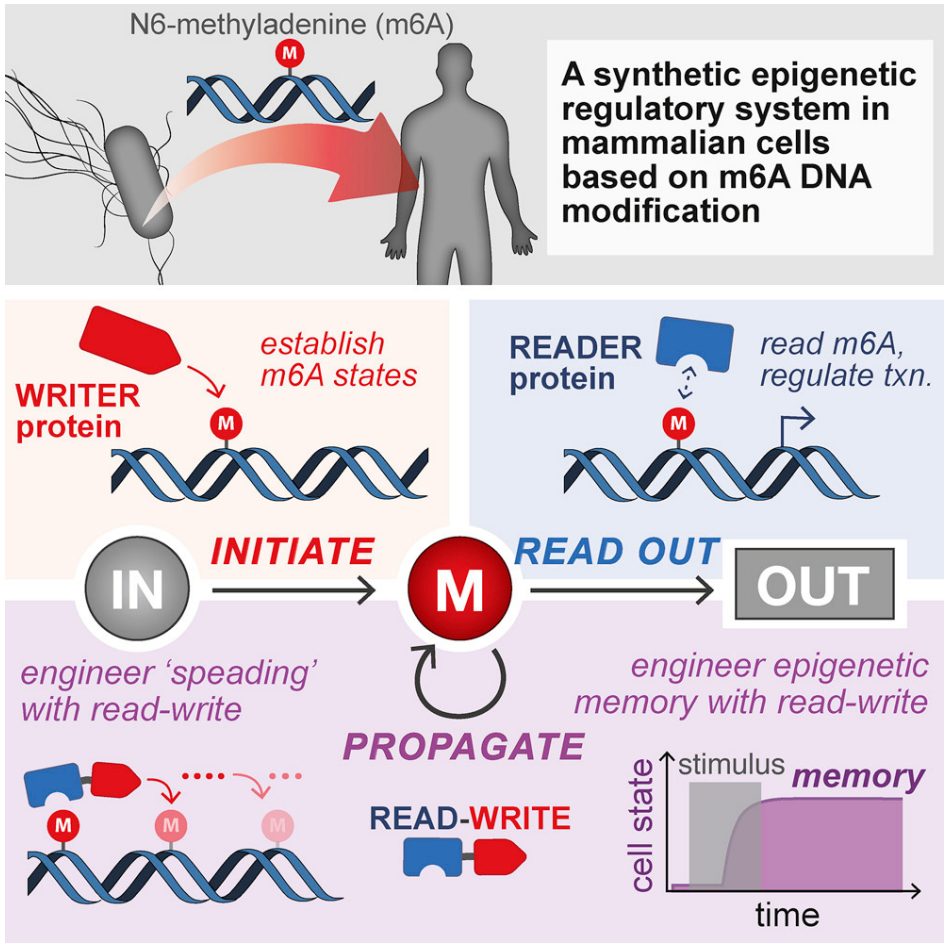
Minhee Park • Nikit Patel • Albert J. Keung • Ahmad S. Khalil [5] • Show footnotes

Published: December 06, 2018 • DOI: https://doi.org/10.1016/j.cell.2018.11.002 •

Highlights

- A synthetic epigenetic regulatory system in human cells using m6A DNA modification
- Engineered writers and readers of m6A enable construction of regulatory circuits
- Read-write circuits drive spatial propagation and hallmarks of chromatin spreading
- Read-write circuits enable epigenetic memory of transcriptional states

Engineering epigenetic regulation using synthetic read-write modules

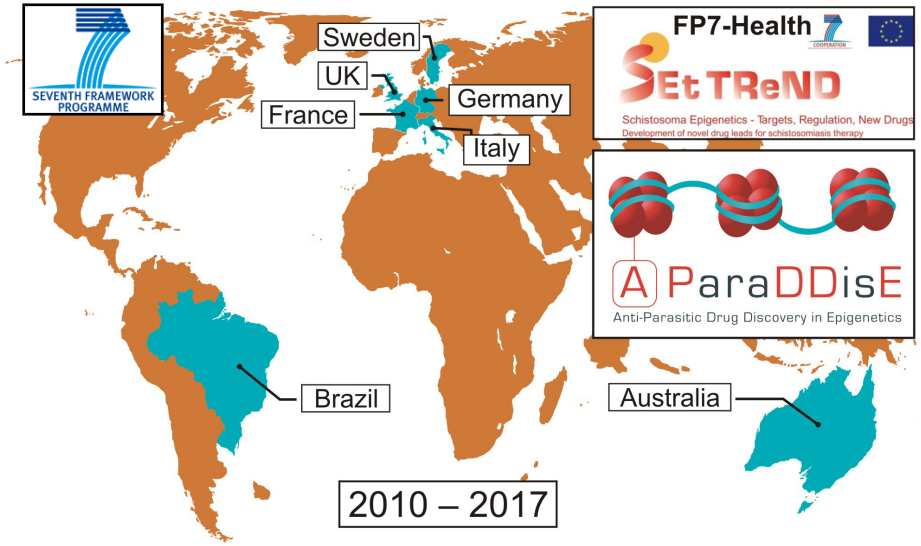


https://www.cell.com/cell/pdf/S0092-8674(18)31461-2.pdf

- Regulatory networks involving molecular writers and readers of chromatin marks are thought to control the epigenetic programs.
- Guided by this common principle, an **orthogonal epigenetic regulatory system** in mammalian cells using **N6-methyladenine (m6A)**, a DNA modification not commonly found in metazoan epigenomes, was established.
- The system utilizes synthetic factors that write and read m6A and consequently recruit transcriptional regulators to control reporter loci.
- Inspired by models of chromatin spreading and epigenetic inheritance, we used our system and mathematical models to construct regulatory circuits that induce m6A-dependent transcriptional states, promote their spatial propagation, and maintain epigenetic memory of the states. These minimal circuits were able to program epigenetic functions *de novo*, conceptually validating "read-write" architectures.

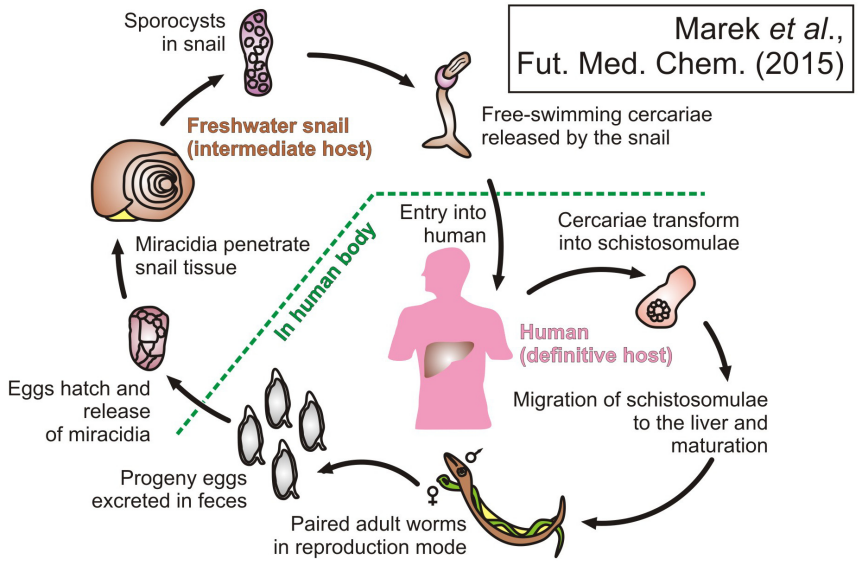
Anti-parasitic drug discovery in epigenetics

Exploring epigenetic mechanisms and regulations in schistosome parasites



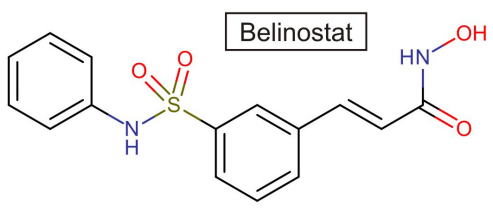
- 207 millions people infected worldwide
- More than 280,000 deaths annually
- Mass treatment with praziquantel (PZQ)
- PZQ-resistant parasites reported

Why to target parasites' epigenetics?

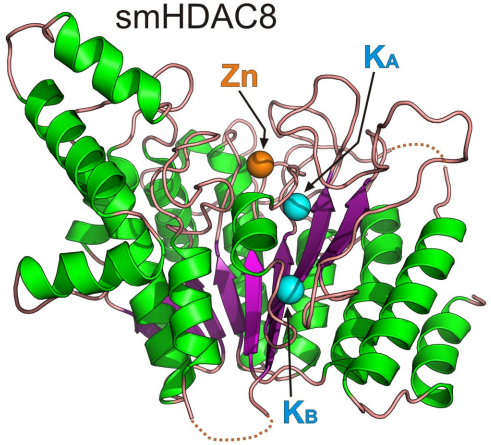


A 'piggyback' strategy in drug discovery

- HDACs – the most explored epigenetic targets
- Four HDAC inhibitors approved by FDA

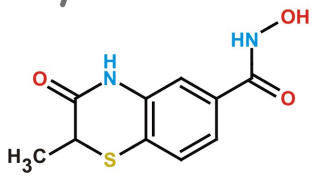


Fine-tuning small-molecule epigenetic inhibitors



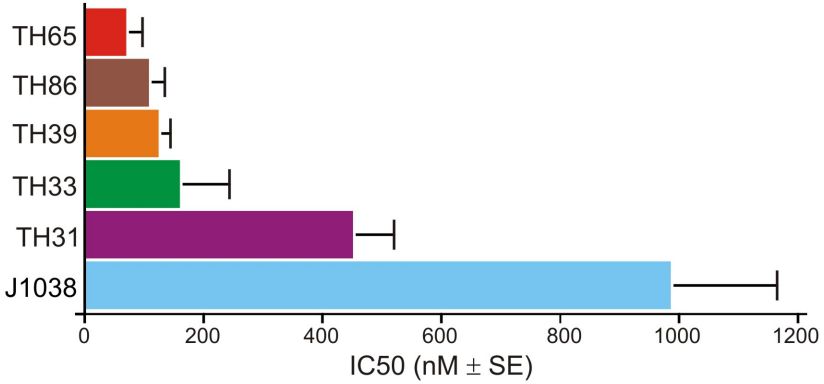
Marek *et al.*, 2013
Stolfa*, Marek* *et al.*, 2014
Kannan *et al.*, 2014
Marek *et al.*, 2015
Heimburg *et al.*, 2016

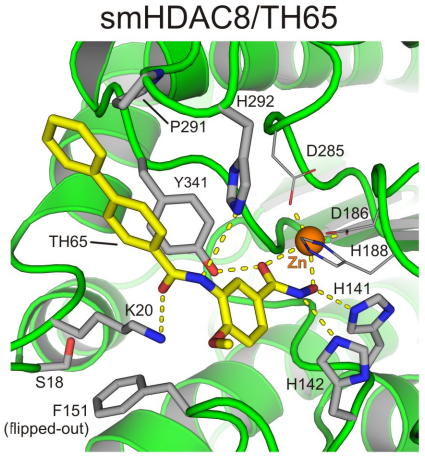
Lead optimization

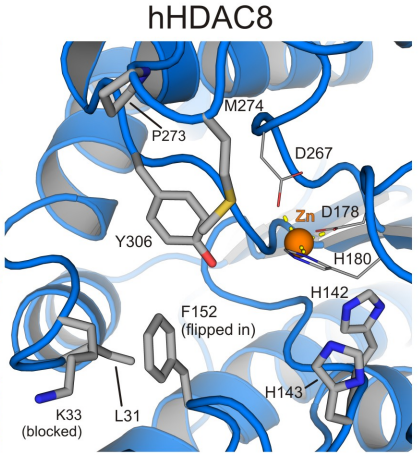


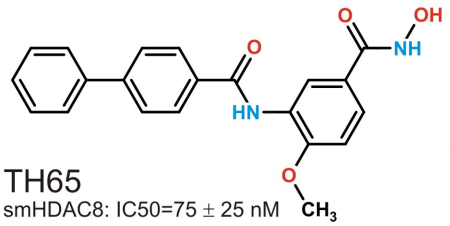
J1038
smHDAC8: IC50 = 1.48 ± 0.5 μM

Virtual screening

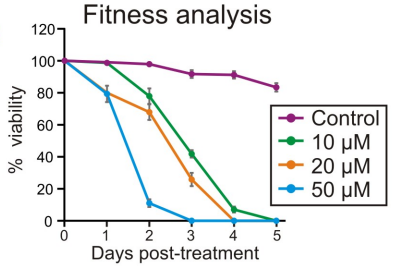


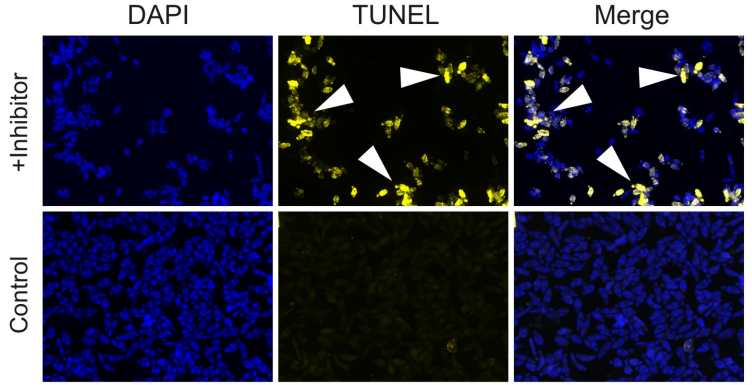






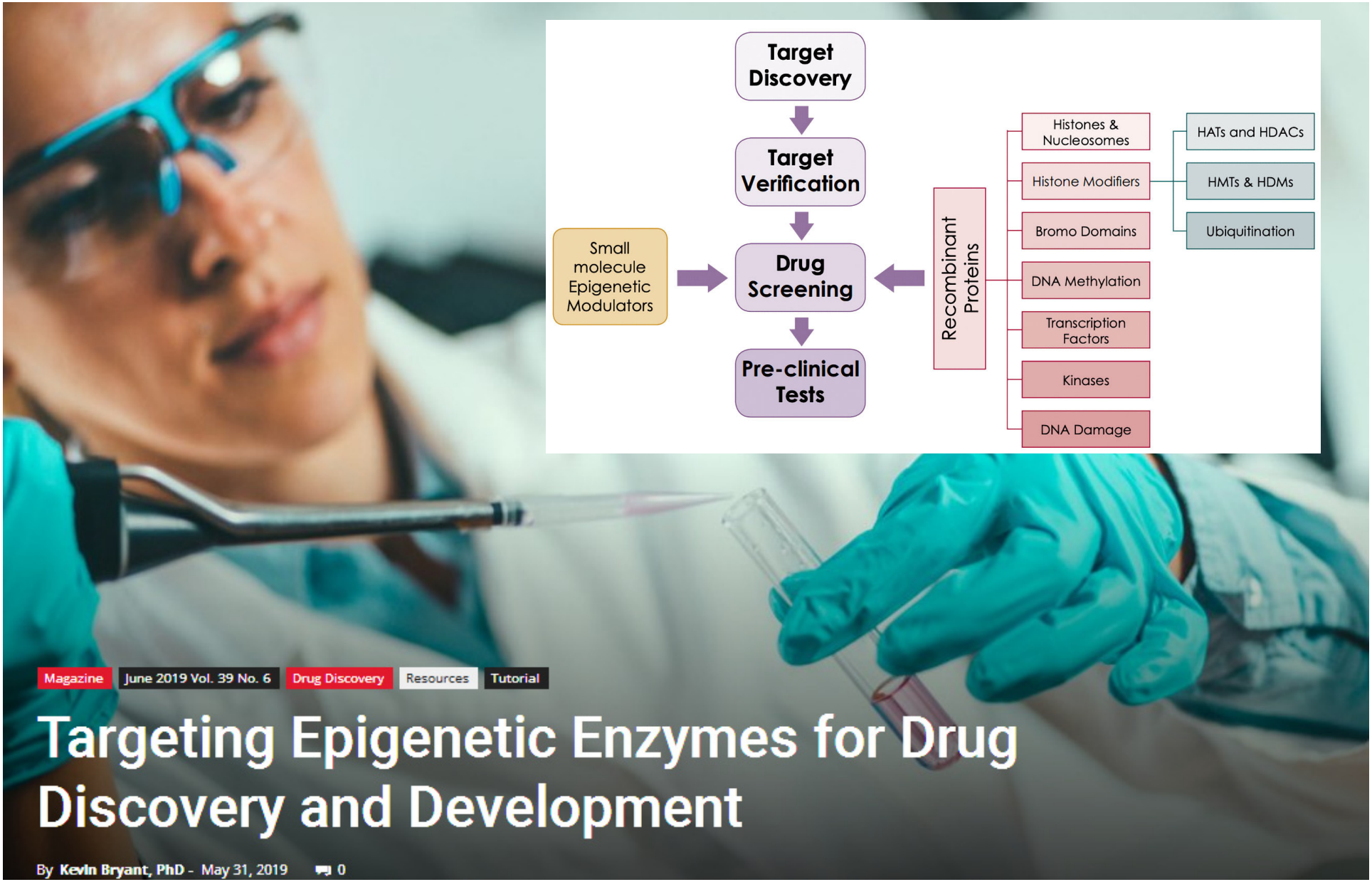
TH65
smHDAC8: IC50=75 ± 25 nM



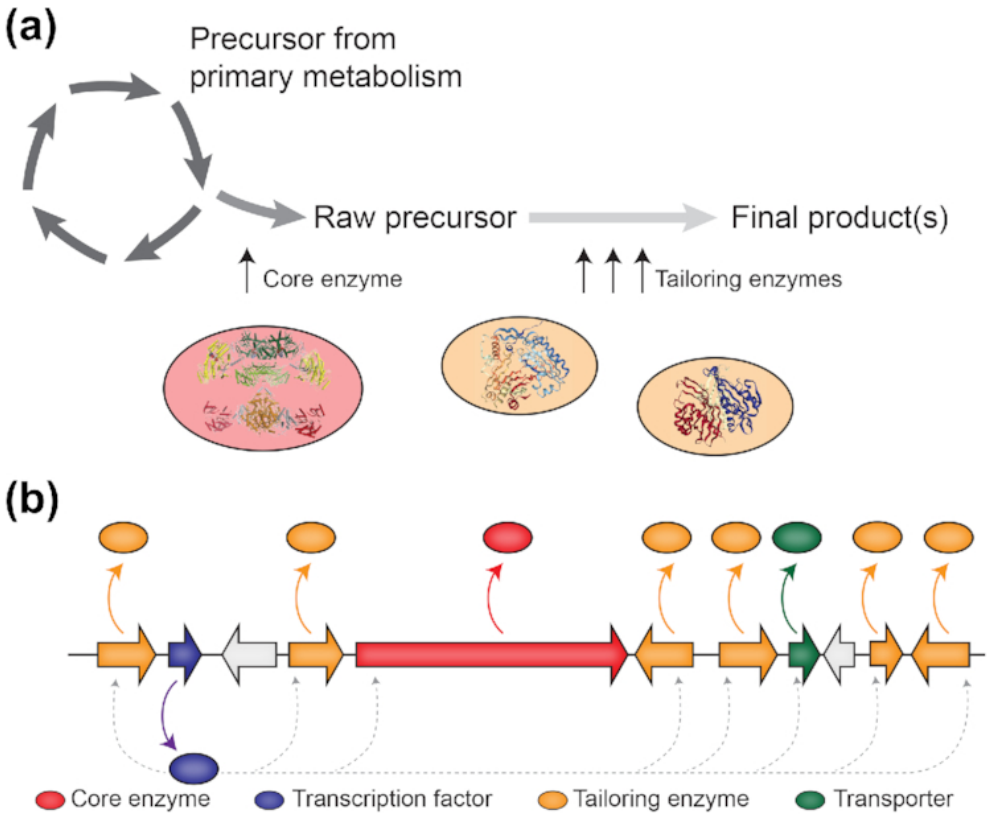


HDACi-triggered apoptosis

Targeting epigenetic enzymes for drug discovery



Secondary metabolites are synthesised by biosynthetic pathways that are encoded by gene clusters

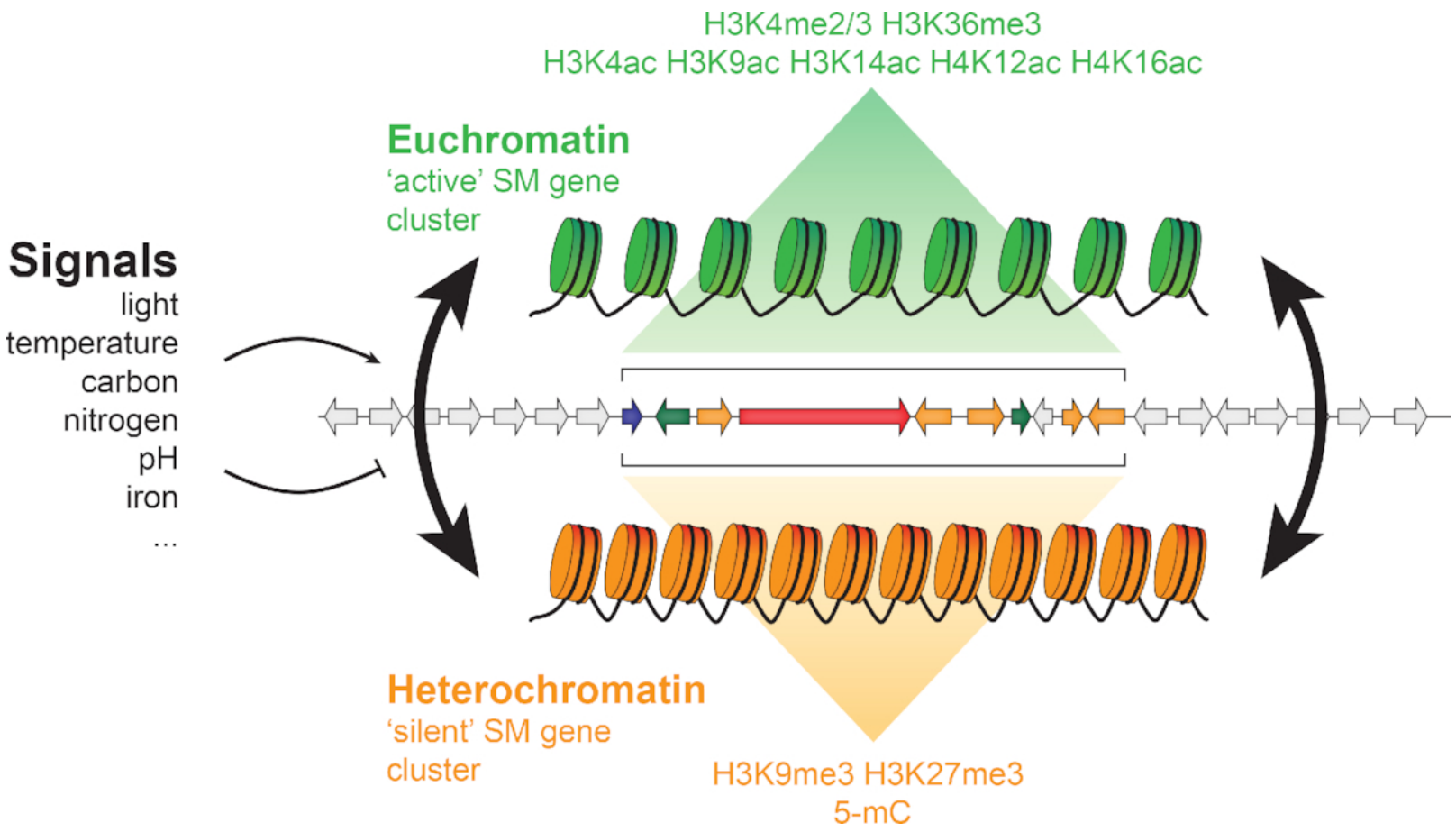


A) Secondary metabolites (SMs) are synthesised from few precursors by biochemical pathways centred around characteristic core enzymes (red). Core enzymes often contain multiple domains or modules that operate conjointly to support the synthesis of the precursor. The precursor is usually further modified by tailoring or decorating enzymes (orange) to produce the final active compound, which is subsequently exported by transporters (green).

B) In fungi, genes encoding core (red) and tailoring enzymes (orange) as well as transporters (green) involved in SM efflux or self-protection are often physically inked in the genome, defining a so-called gene cluster organization

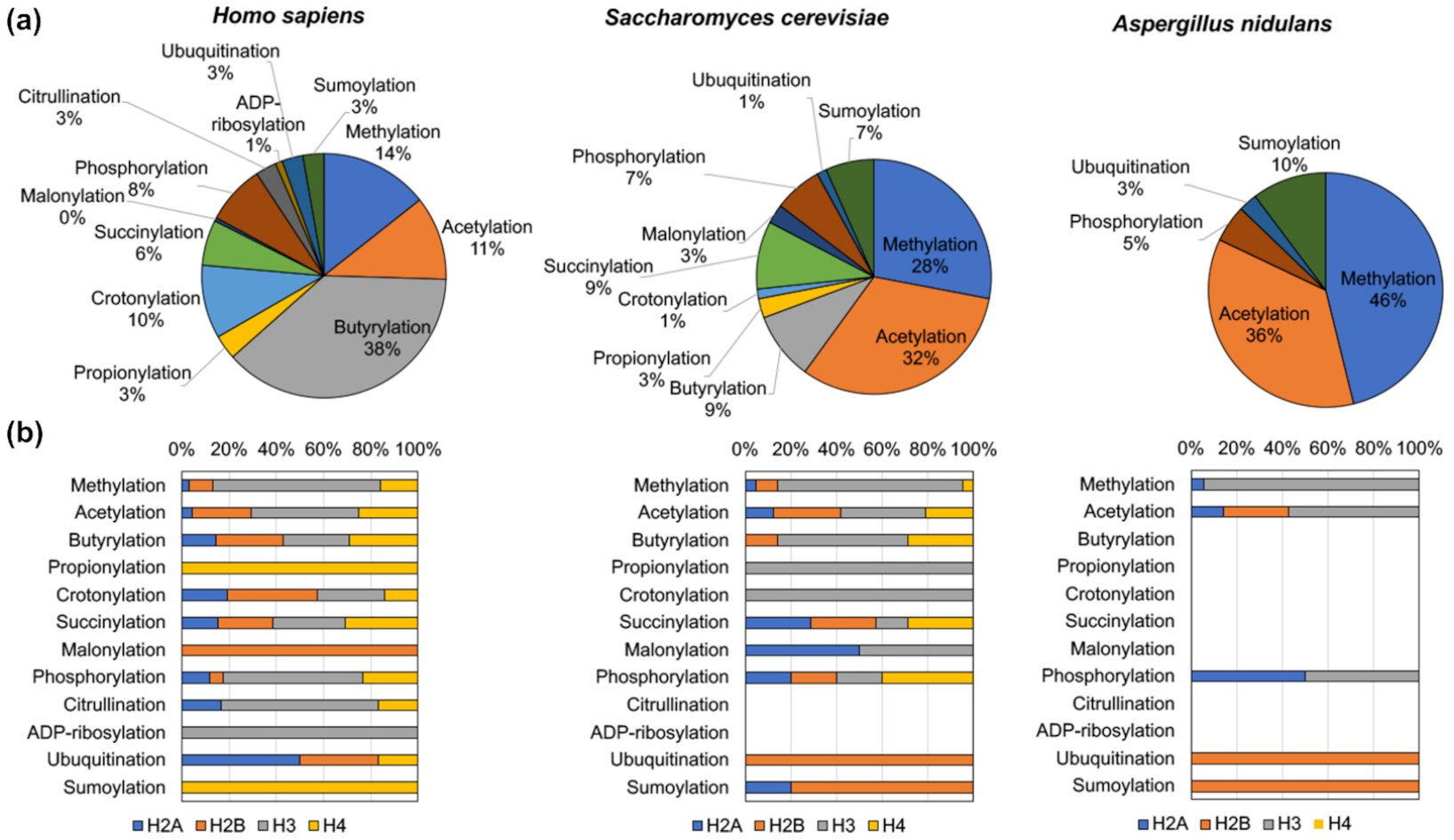
https://academic.oup.com/femsre/advance-article/doi/10.1093/femsre/fuz018/5521207

Secondary metabolites are synthesised by biosynthetic pathways that are encoded by gene clusters



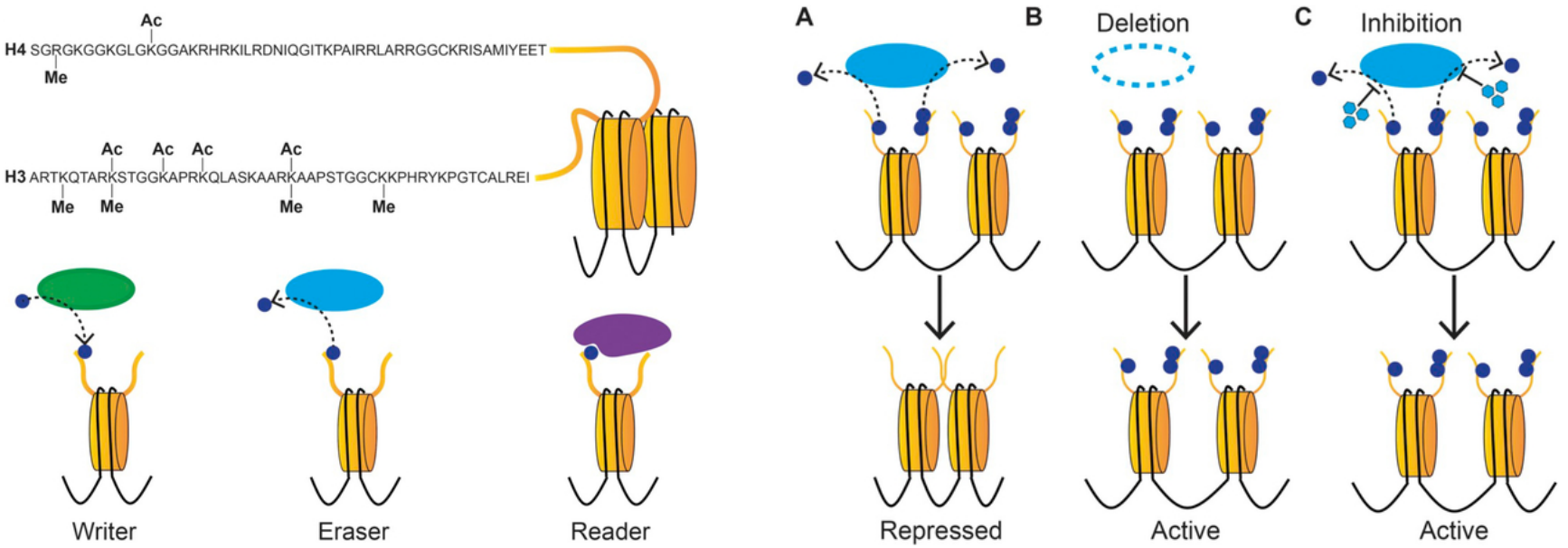
https://academic.oup.com/femsre/advance-article/doi/10.1093/femsre/fuz018/5521207

Histone post-translational modifications in human, yeast, and the secondary metabolite producing *Aspergillus*



https://academic.oup.com/femsre/advance-article/doi/10.1093/femsre/fuz018/5521207

How epigenetic machinery influences secondary metabolism in fungi



Strategies for interfering with chromatin regulation. A) Action of an epigenetic eraser under wild-type conditions. This enzyme removes the activating modifications represented by the blue dots, which leads to more condensed, repressed chromatin where BGC are often found. B) Deletion of the eraser prevents the removal of the activating modifications, and the chromatin remains open and active, allowing for expression of genes which are typically repressed. C) Adding chemical inhibitors (represented by the light blue hexagons) which prevent the eraser from removing the activating PTM. This leads to a similar outcome as deletion of the enzyme, and allows for expression of genes which are typically repressed.

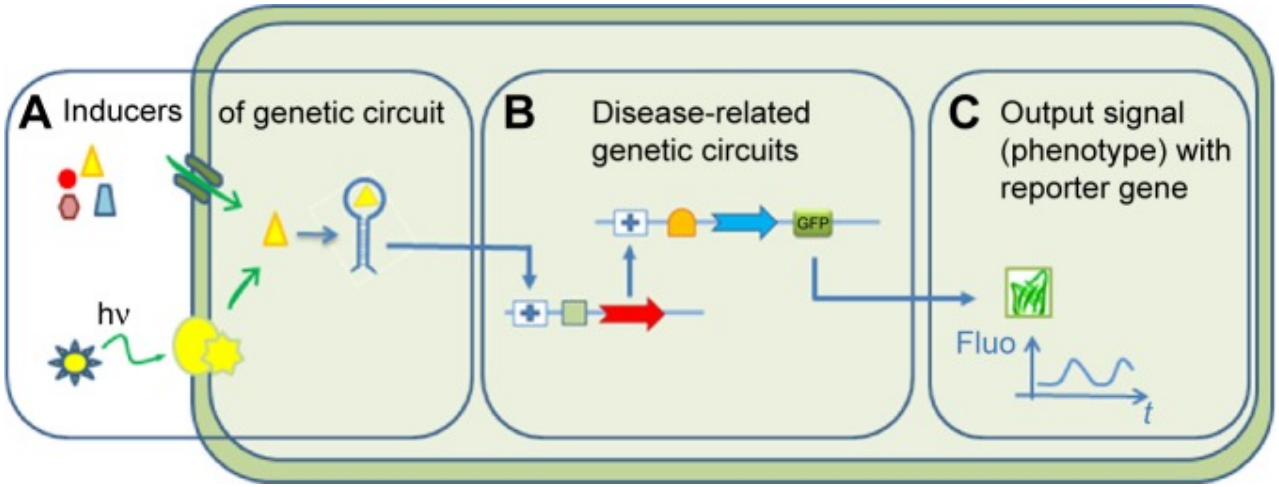
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6685777/

Synthetic biology in drug discovery

Concepts
Methods
Applications

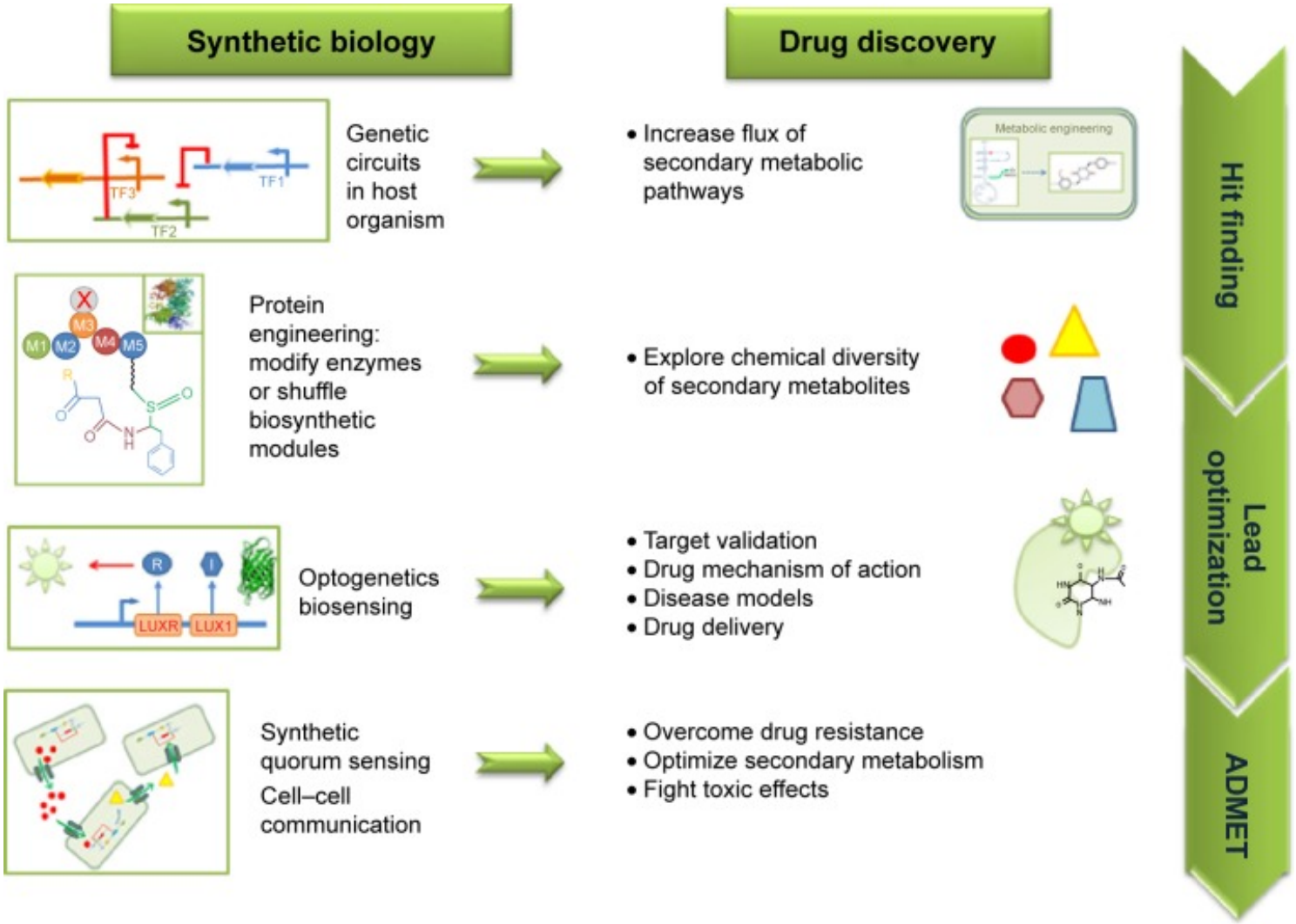
Concepts behind synthetic biology tools in drug discovery

- With the recent advanced genome editing, molecular biology, and protein engineering tools, synthetic biology has focused its aim at creating biological devices that can produce controlled phenotypes from a given input, such as a molecular or light switch.
- The design of genetic circuits in synthetic biology is used in pharmaceutical research not only for bioproduction of drugs by microorganisms but also to support the different steps of drug development.

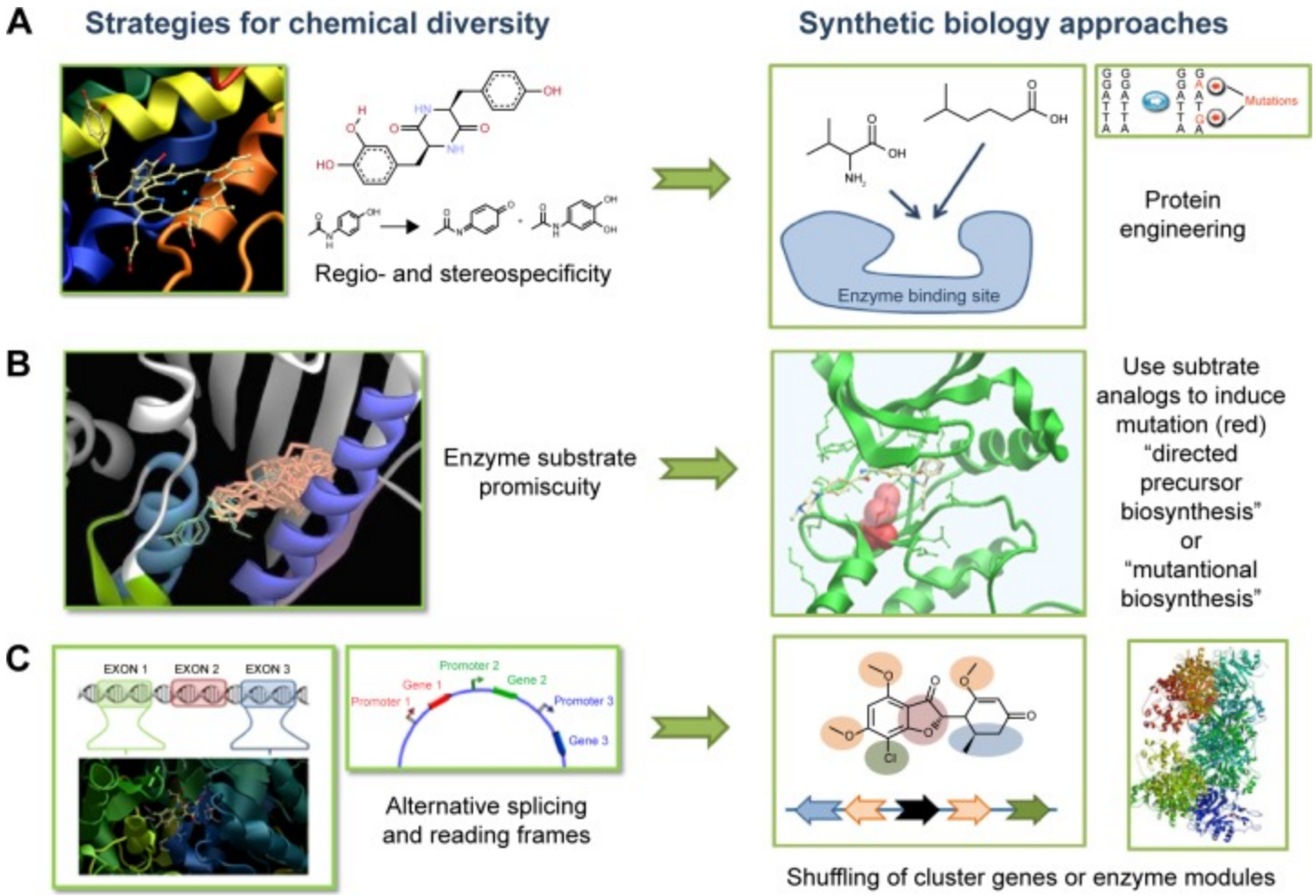


(A) Inducers of gene expression using light or small molecules (nutrient, drugs, cell messengers, etc)
(B) Gene circuit to control expression of specific genes
(C) Reporter genes to control output signals related to a disease phenotype

Synthetic biology tools in various steps of drug discovery

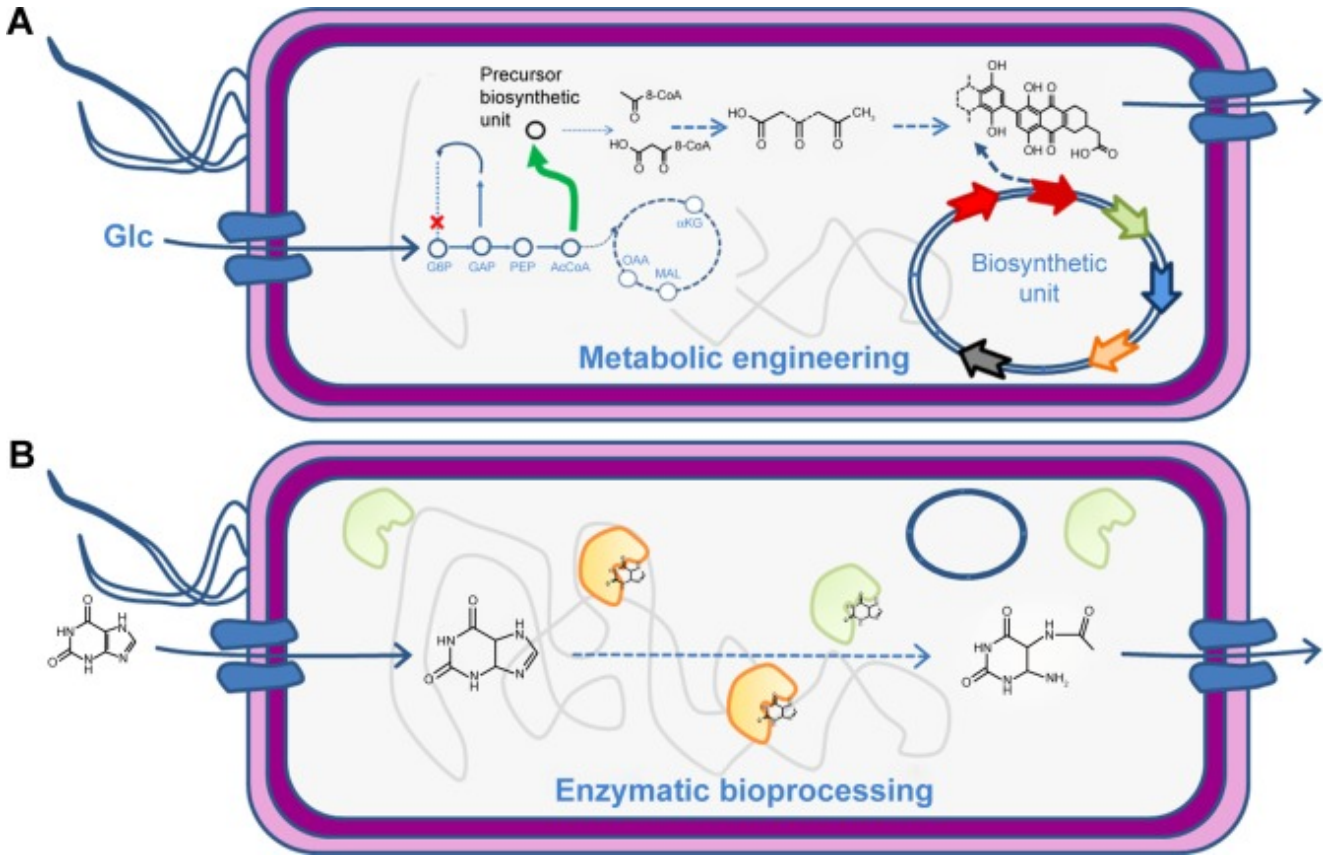


Protein engineering for exploring chemical diversity



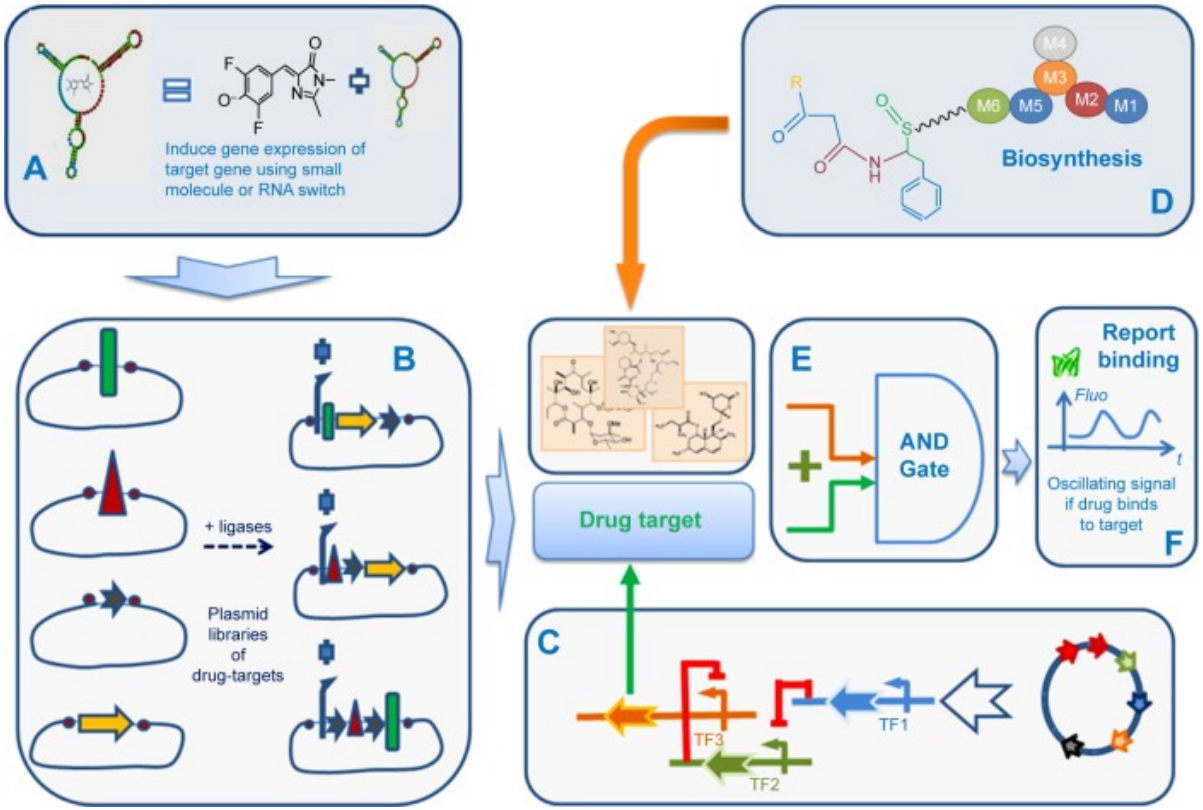
(A) Modify enzyme specificity by single amino acid mutations in binding site
(B) Use substrate analogs to induce mutation on selected enzyme
(C) Use alternating spliced isoforms to modify arrangement of enzymatic modules

Metabolic engineering for drug production



Production of secondary metabolites through heterologous genetic circuits (**A**) or by modifying chemical precursor from the milieu with enzyme present in the host organism (**B**).

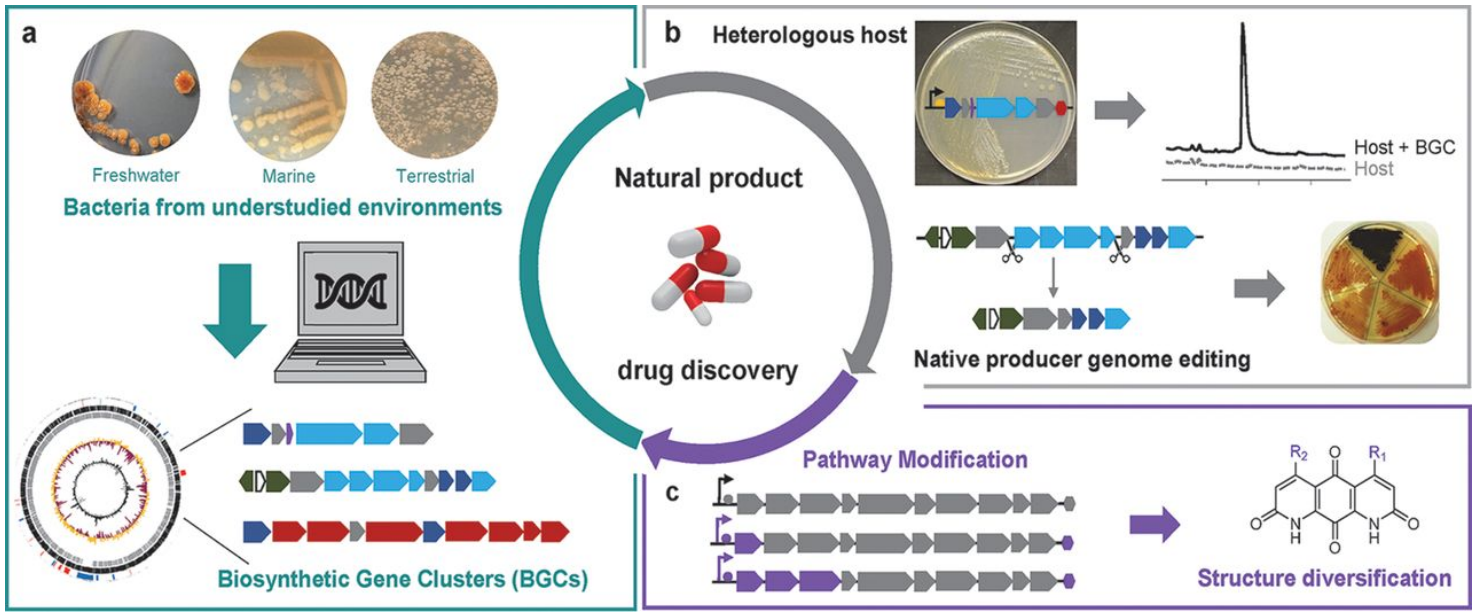
Conceptual SB pipeline for drug screening in synthetic cell



(**A**) A system to induce gene expression of protein target based on small molecule inducer and/or RNA-based switch for gene expression. (**B**) A combination of drug target from unique genes. (**C**) A genetic oscillator to focus readout on chosen drug target. (**D**) Generation of diverse drug candidate libraries from genetic shuffling of enzymatic modules. (**E**) A logical AND gate that gives output signal (**F**) if a drug candidate binds to biological target.

Natural products and synthetic biology

Genomic, genetic, and synthetic biology approaches towards natural product drug discovery



(a) Exploration of bacteria from understudied environments and taxa follows the hypothesis that different selective pressures may lead to the evolution of distinct chemistry. Genomes are sequenced and biosynthetic gene clusters (BGCs) are identified using bioinformatic tools

(b) BGCs of interest are selected. Heterologous expression in suitable hosts can be used to streamline natural product discovery. Genome editing of native producers offers an alternative approach to aid discovery and to study gene and BGC function

(c) Synthetic biology-driven pathway modification via BGC reprogramming contributes to structure diversification of natural products

With synthetic biology, drug discovery is going virtual

Questions

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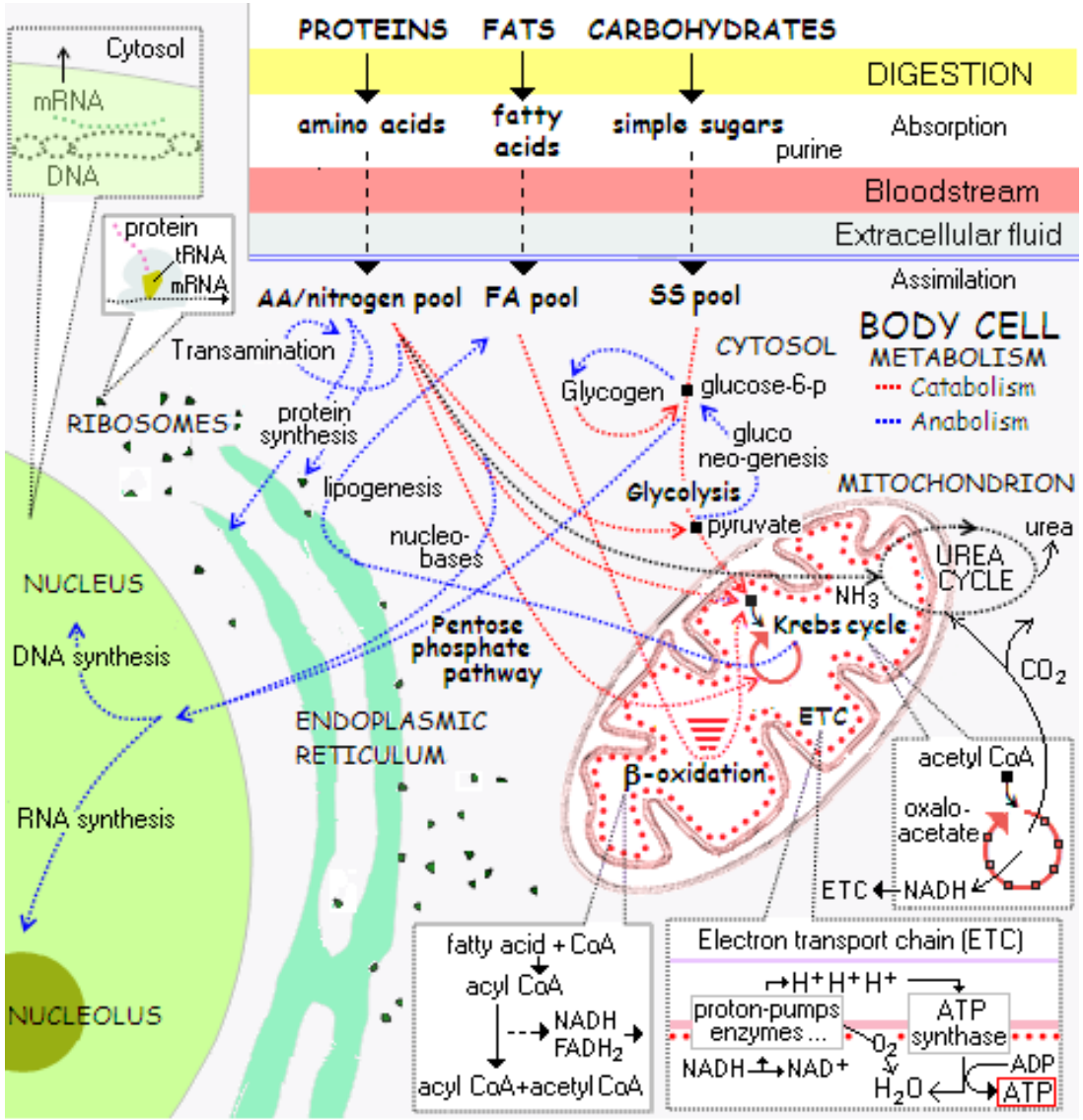
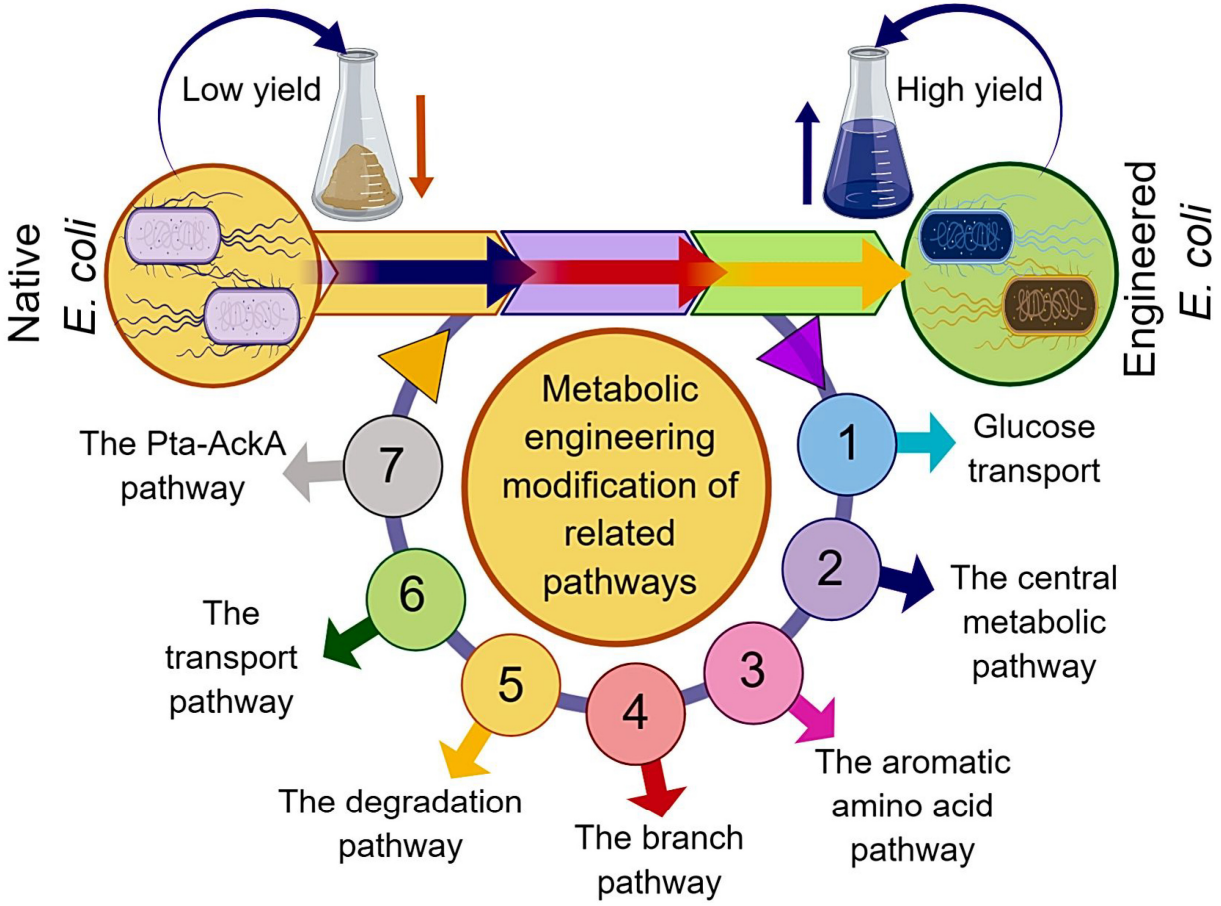
Supplementary materials

Molecules to metabolism



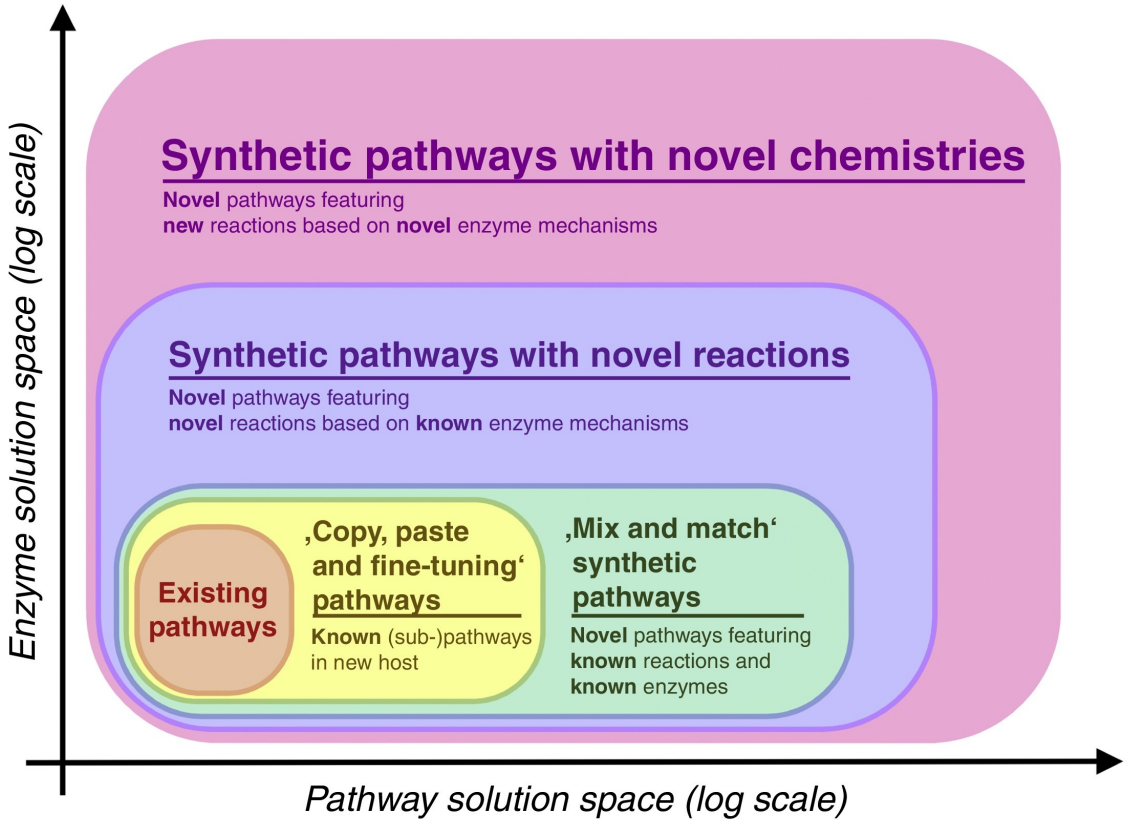
Metabolic engineering approaches for induced production of L-tryptophan using engineered *E. coli*



https://www.mdpi.com/2227-9717/7/4/213

Different levels of metabolic engineering

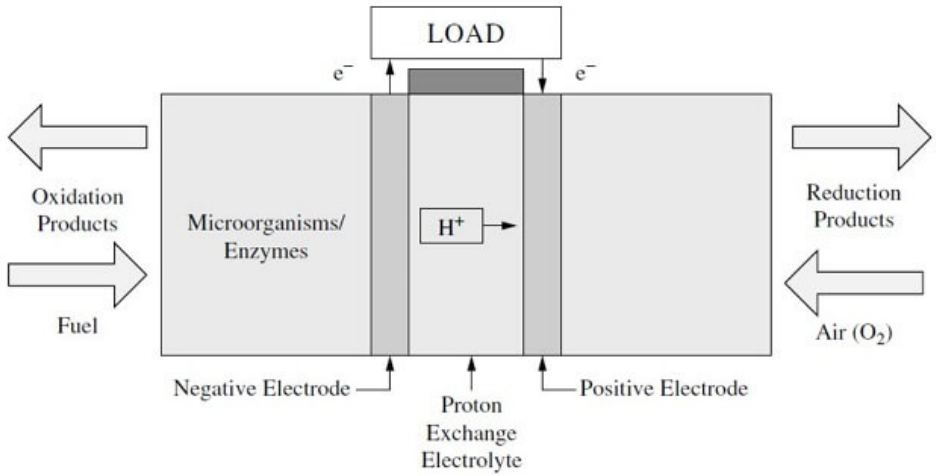
https://www.sciencedirect.com/science/article/pii/S1367593116302071



- The enzyme solution space describes the number of possible enzymes reactions available for a given strategy while the pathway solution space corresponds to the number of possible pathways that can be constructed.
- While level 1, 2 and 3 metabolic engineering efforts do not differ in enzyme solution space, because they all rely on known enzymes, level 4 and 5 metabolic engineering efforts provide new enzymes created through enzyme engineering or *de novo* protein design.

Biological fuel cells (BFCs) and the bio-production of hydrogen

A biological fuel cell (BFC) or microbial fuel cell (MFC) is a type of fuel cell that converts biochemical energy into electrical energy. Like other types of fuel cells, a biological fuel cell consists of an anode, a cathode, and a membrane that conducts ions. In the anode compartment, fuel is oxidized by microorganisms, and the result is protons and electrons. In the cathode compartment, ions are consumed, and the by-product is water. In BFCs, there is the redox reaction between the carbohydrate substrate (such as glucose and methanol) and the catalyst - which is a microorganism or enzyme. The biological fuel cell is illustrated in Figure 1. The main difference between a standard fuel cell and a BFC is the catalyst is a microorganism or enzyme. Therefore, noble metals are not needed for the catalyst in BFCs. The fuel cell operates in a liquid media in a near neutral environment and at a low temperature. The potential applications for biological fuel cells are (1) low power energy sources; (2) sensors based upon direct electrode interactions; and (3) electrochemical synthesis of chemicals.

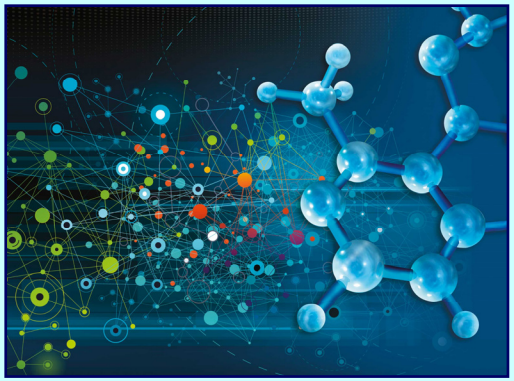


Metabolomics and systems biology

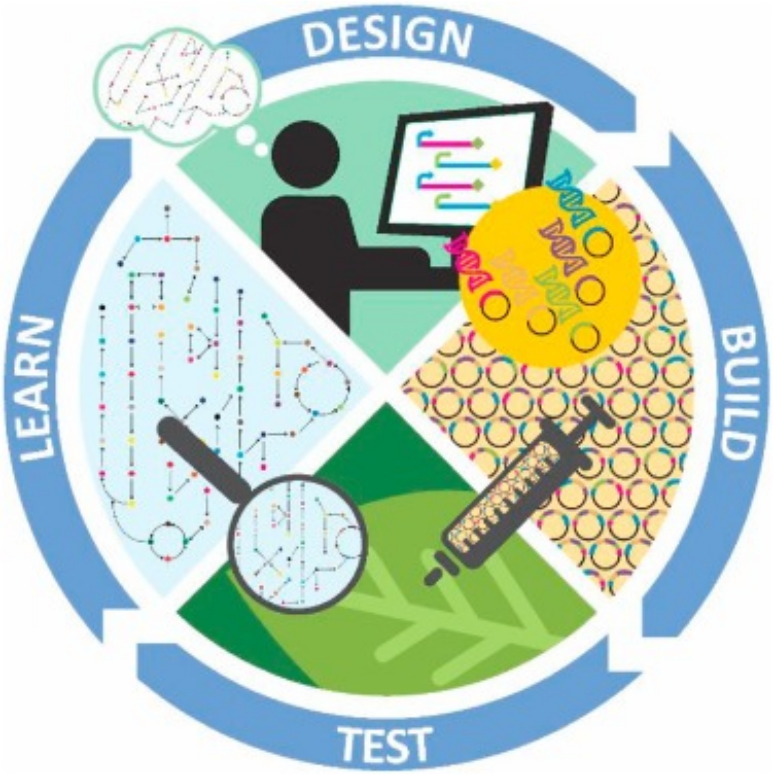
- **Metabolic engineering** is a field where the level of a specific metabolite is quite obviously a very important thing, because that metabolite is the valuable product that one is trying to produce. If knowing the level of one metabolite is useful, it stands to reason that knowing the levels of many metabolites --- immediate precursors and products of the target metabolite, as well as other related molecules --- should be even more useful.

- **Metabolomics** is a powerful approach because metabolites and their concentrations, unlike other "omics" measures, directly reflect the underlying biochemical activity and state of cells/tissues. Thus metabolomics best represents the molecular phenotype.

- Metabolomics provides a downstream, phenotypic snapshot of the cell that can be critical to **understanding metabolism and metabolic dynamics**.



Plant metabolic engineering and synthetic biology



Genetic improvement of crops started since the dawn of agriculture and has continuously evolved in parallel with emerging technological innovations. The use of genome engineering in crop improvement has already revolutionised modern agriculture in less than thirty years. Plant metabolic engineering is still at a development stage and faces several challenges, in particular with the time necessary to develop plant based solutions to bio-industrial demands. However the recent success of several metabolic engineering approaches applied to major crops are encouraging and the emerging field of plant synthetic biology offers new opportunities. Some pioneering studies have demonstrated that synthetic genetic circuits or orthogonal metabolic pathways can be introduced into plants to achieve a desired function. The combination of metabolic engineering and synthetic biology is expected to significantly accelerate crop improvement. A defining aspect of both fields is the design/build/test/learn cycle, or the use of iterative rounds of testing modifications to refine hypotheses and develop best solutions. Several technological and technical improvements are now available to make a better use of each design, build, test, and learn components of the cycle. All these advances should facilitate the rapid development of a wide variety of bio-products for a world in need of sustainable solutions.

How epigenetic machinery influences secondary metabolism in fungi

The discovery of chromatin as a central regulator of fungal secondary metabolism (SM) production significantly impacted on our understanding of the complex transcriptional regulation of biosynthetic gene clusters. The rapid progress in identifying the genetic determinants underlying this chromatin-based regulation provided new tools to activate silent gene clusters. Thus far, most efforts have focused on two well-known chromatin modifications, histone methylation and histone acetylation. However, most SM gene clusters remain untouched by any genetic or chemical modification of chromatin, and thus we still lack a comprehensive understanding on how chromatin modifications regulate SM gene clusters. Based on examples from other eukaryotes and the availability of novel technologies that allow to comprehensively study the composition and organisation of chromatin, we are now entering a new decade during which several outstanding questions can be answered. What is the contribution of other chromatin modifications to the regulation of SM gene clusters? How do chromatin modifications interact with each other to provide a tight and subtle regulation of SM gene clusters? How are chromatin modifications orchestrated in the different genomic sub-compartments, and what is the chromatin dynamic during interactions with other organisms? Answering these questions is important to advance our basic knowledge on chromatin-based regulation, but it will also provide the needed tools to successfully activate the wide diversity of fungal SM gene clusters. Exploitation of the fungal kingdom to the discovery and development of novel bioactive compounds would then enter a completely new era.

What is synthetic biology?

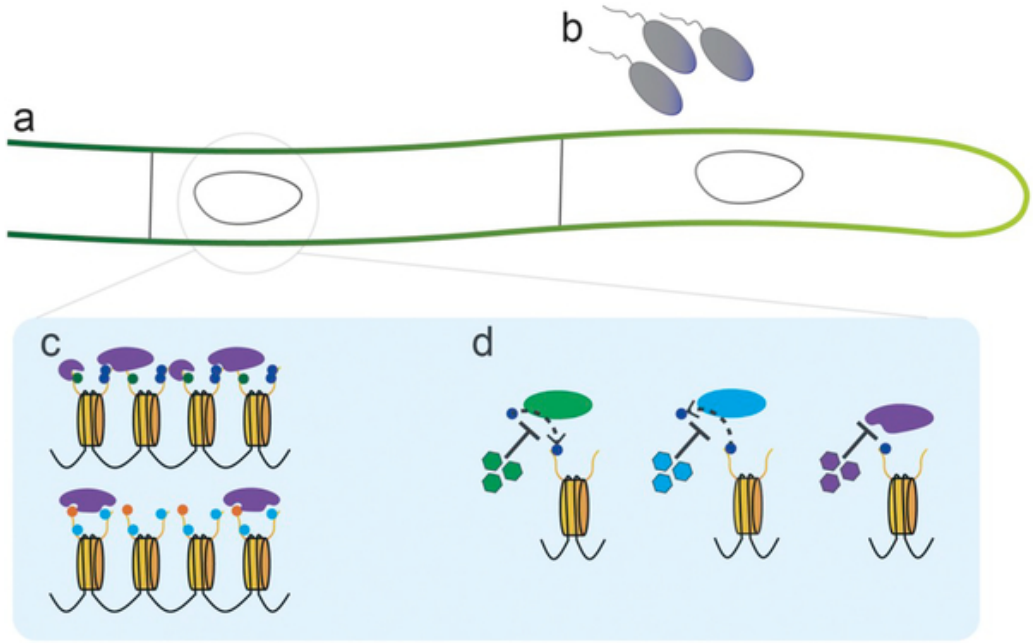
Synthetic biology is essentially the engineering counterpart to biology: whether it is building a novel genome or editing an existing one to add a new function or remove a problematic pathway, the idea is to use engineering principles to improve performance.

Synthetic biology got its first major push forward with two innovations, both published in 2000: a genetic toggle switch that lets researchers turn a gene on or off, and an oscillator that switches a gene on or off depending on the state of another specified gene or protein[3,4]. These were among the earliest biological building blocks and advanced the development of synthetic biology, just as the invention of the resistor served as an important part of the foundation for electrical engineering.

Today, the number of biological engineering tools like those switches has soared. The field's primary repository for these building blocks, the iGEM Registry, now has more than 20,000 different parts available for public use – many of them far more sophisticated than simple on/off switches. The rapid increase in biological parts has made it possible for researchers all over the world to get involved in synthetic biology and to contribute to the community's understanding of the engineering principles underpinning biological organisms.

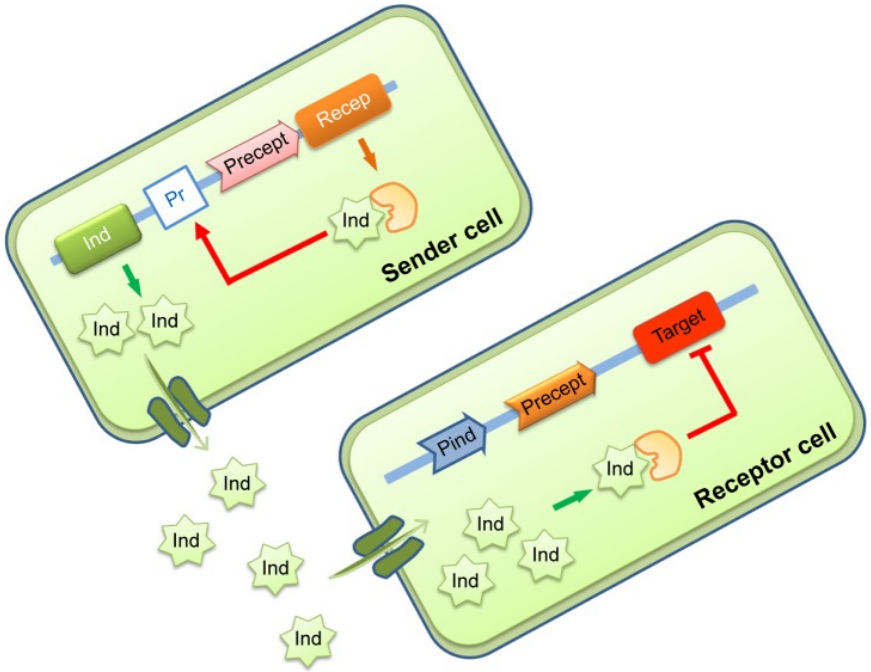
https://www.ddw-online.com/enabling-technologies/p315003-with-synthetic-biologydrug-discovery-is-going-virtual.html

How epigenetic machinery influences secondary metabolism in fungi



Areas of future research. Depiction of a fungal hyphae, which contains two nuclei represented by the ovals. Each letter represents an area that needs further research relating to chromatin regulation of secondary metabolism. “a” is labeling the fungus, because despite the extensive research that has occurred within *Aspergillus* and *Fusarium*, very few taxa of fungi have been studied for how their BGC are regulated by chromatin. “b” marks the bacterial interaction which has been demonstrated to regulate secondary metabolite production. “c” a zoomed in look in the nucleus, shows chromatin with various purple reading proteins. This represents an untapped resource of proteins which may be controlling secondary metabolism through recognition of PTM, and recruitment of writing or erasing enzymes. “d” represents the use of chemical inhibitors to prevent the actions of the histone modifying enzymes.

Design of synthetic quorum sensing in microbial consortium



The sender cell synthesizes a messaging molecule (inducer) that stimulates its receptor synthesized in the receiver cells. This complex triggers inhibition (or activation) of a target gene in a cell density-dependent manner.
Abbreviations: Ind, inducer; Pr, promotor; Precept, receptor's promoter.

- Designing of synthetic cell–cell communication network as a model to study persistence in bacteria and how to sensitize cells or to use it as a screening platform to target QS in bacterial communities
- QS is a cell–cell communication system that allows bacteria or microorganisms to synchronize expression of a particular gene in a cell density-dependent manner