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# 3000788 Intro to Comp Molec Biol

## Lecture 26: Synthetic biology

Fall 2025



**Sira Sriswasdi, PhD**

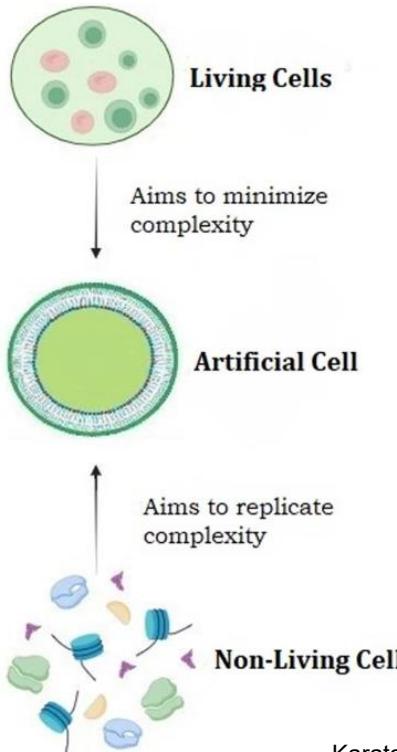
- Research Affairs
- Center of Excellence in Computational Molecular Biology (CMB)
- Center for Artificial Intelligence in Medicine (CU-AIM)

# Today's agenda

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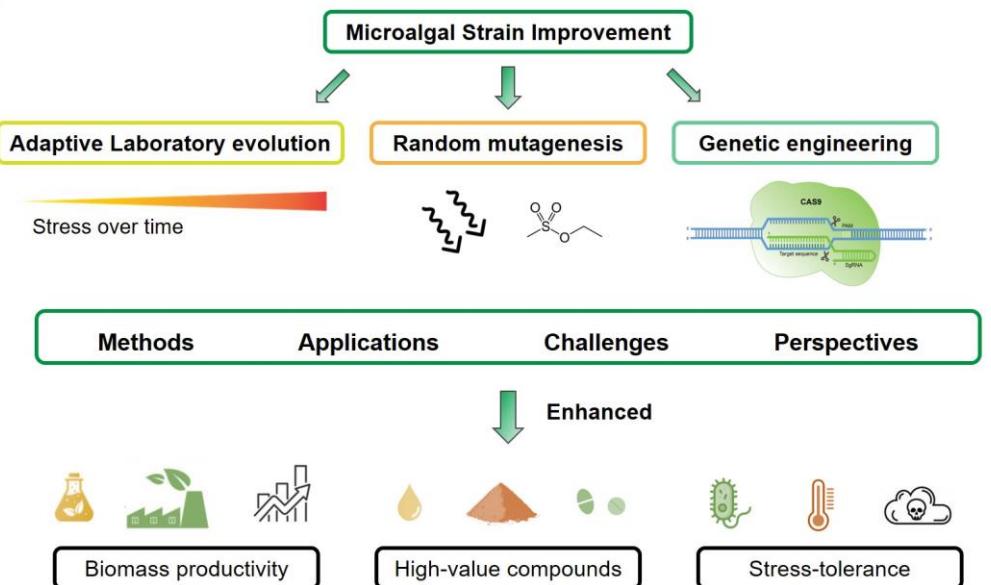
- Goals of synthetic biology
- Modular design of life
  - Genetic circuit
  - Metabolic engineering
- Computation in synthetic biology

# Goals of synthetic biology



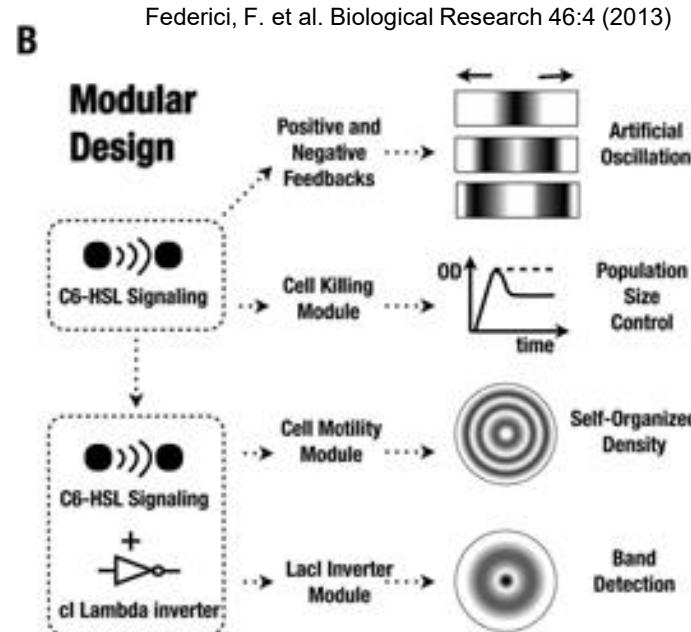
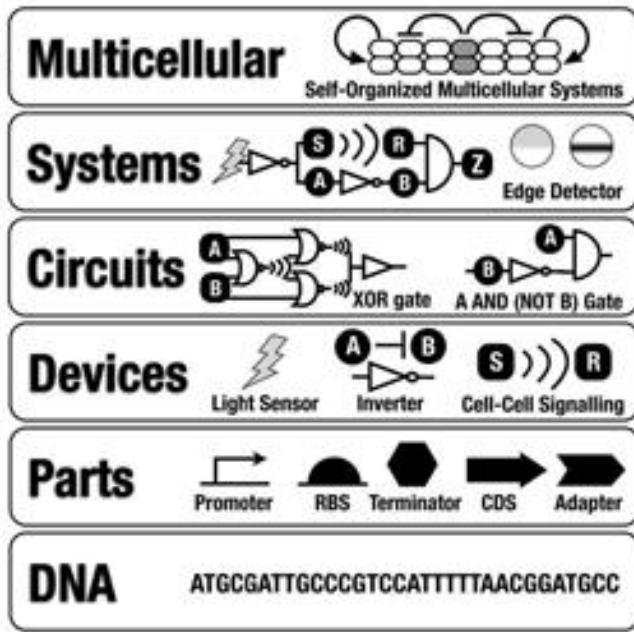
- Design and building of artificial biological systems (processes, organisms, etc.)
- To understand the mechanisms of life
- To develop biological products and biological factories to address medical, agricultural, and environmental needs

# Comparison to genetic engineering



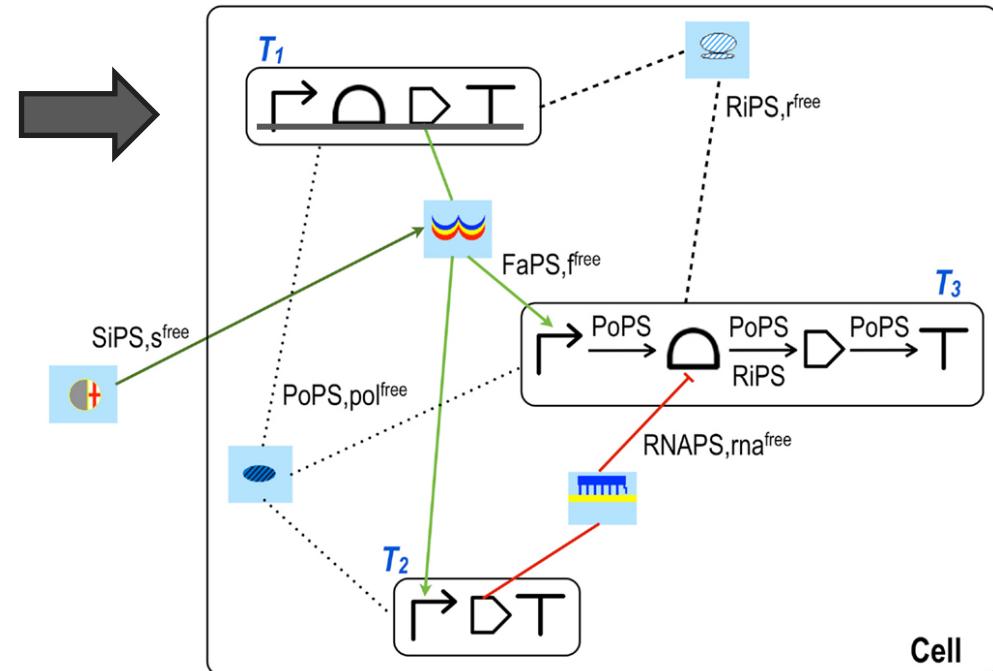
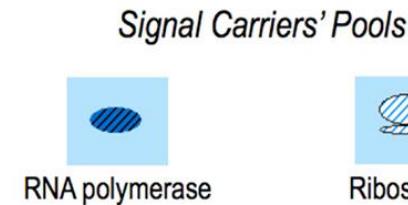
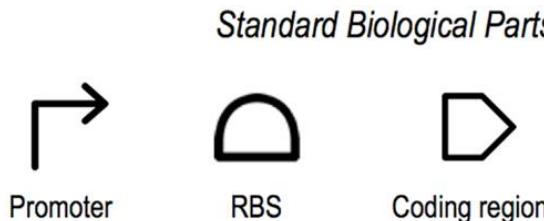
- Genetic engineering is about modifying an intact natural system to derive the desired phenotypes
  - Synthetic biology is about designing or controlling the whole system

# Modular design in synthetic biology

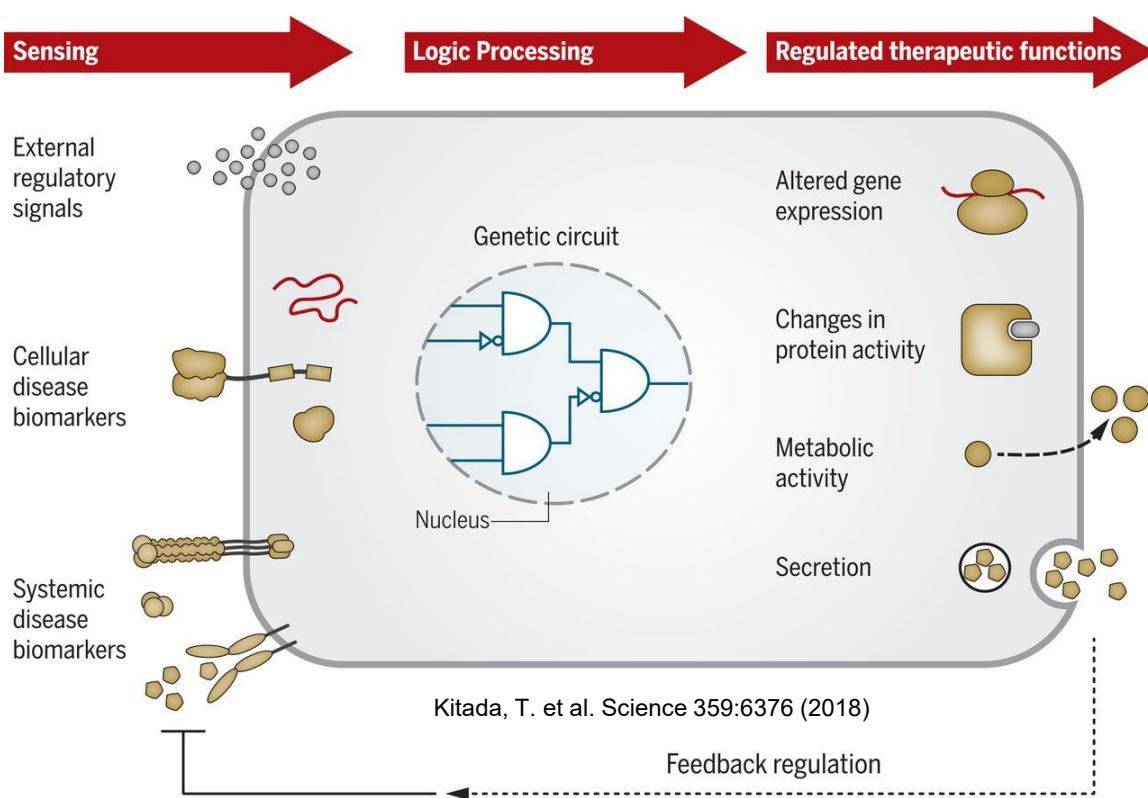


- Treat biological system like an electronics

# Biological parts

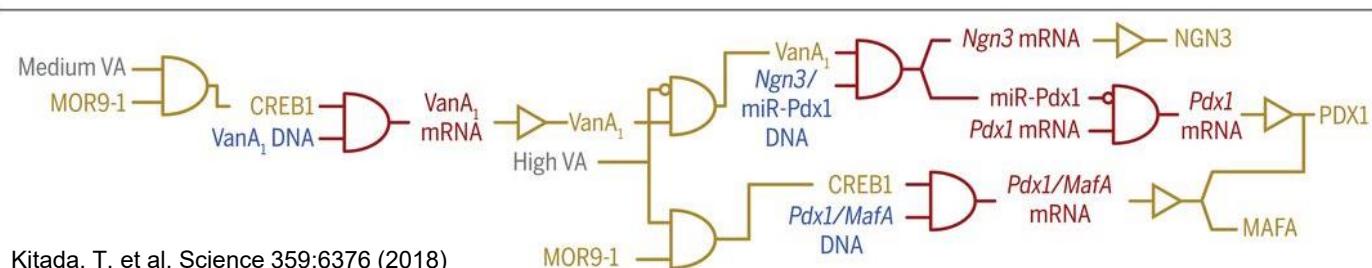
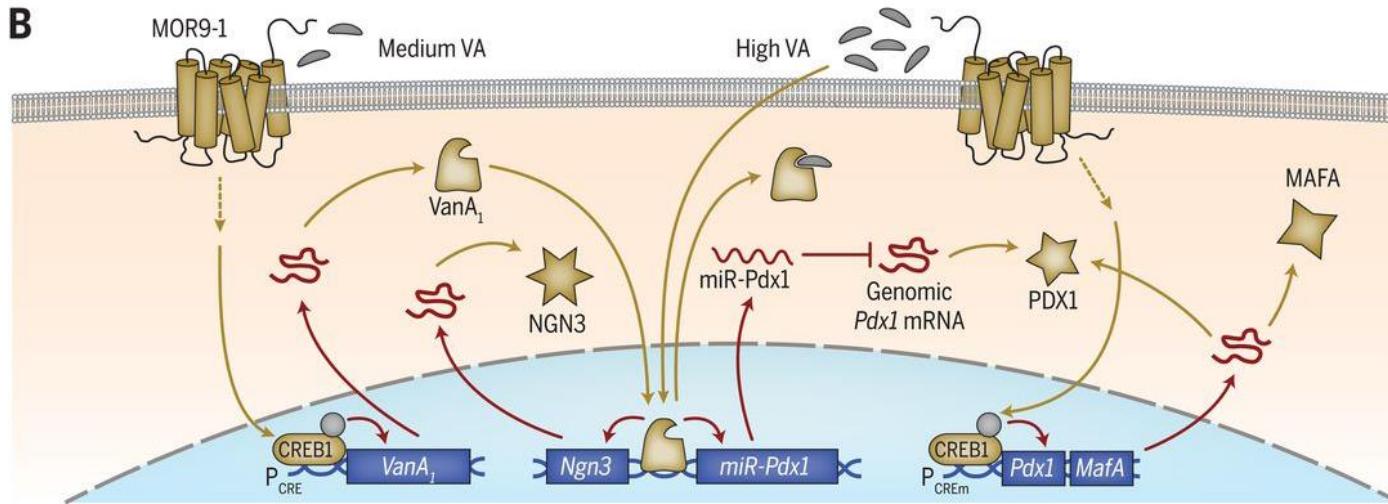


# DNA programming

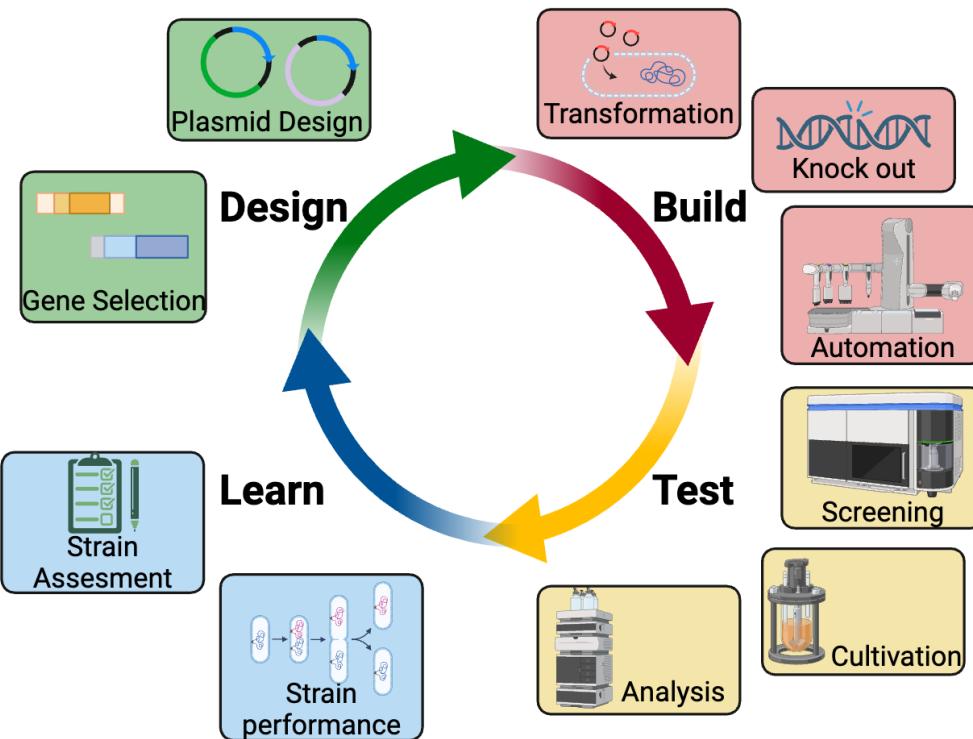


- Cell = computer
- Genetic circuit processes inputs and produce outputs
- Customize genetic circuits and put inside the cell for testing

# A complex genetic circuit



# Design-build-test-learn cycle



- Synthetic biology integrates computational design with experimental validation
- Cycle of hypothesis testing and hypothesis generation
- **Good computational design saves cost!**



# Biological parts

# Registry of standard biological parts

Name	Description	Length	Created by	Documentation	Type	Status
BBa_K808000	araC-Pbad - Arabinose inducible regulatory promoter/repressor unit	1209	Valentina Herbring, Sebastian Palluk, Andreas Schmidt	1033281	Regulatory	In stock
BBa_K2607001	HB-EGF/Tar Receptor (HT) Device	1836	Andrea Laurentius	191593	Composite	It's complicated
BBa_K2607000	DiphTox (DT)	254	Andrea Laurentius	183996	Protein_Domain	It's complicated
BBa_J04450	RFP Coding Device	1069	Tamar Odle	115288	Reporter	In stock
BBa_K3187028	Sortase A7M (Ca <sup>2+</sup> -independent variant)	450	iGEM TU_Darmstadt 2019	96929	Coding	Not in stock
BBa_J23100	constitutive promoter family member	35	John Anderson	83466	Regulatory	In stock
BBa_R0062	Promoter (luxR & HSL regulated -- lux pR)	55	Vinay S Mahajan, Voichita D. Marinescu, Brian Chow, Alexander D Wissner-Gross and Peter Carr	77850	Regulatory	In stock
BBa_K857000	acetaldehyde dehydrogenase	951	yale yuen	73967	Coding	It's complicated
BBa_K801060	(+)-Limonene synthase 1 with Strep-tag and yeast consensus sequence.	1708	Lara Kuntz	68635	Coding	In stock
BBa_K3128009	RFP protein under PLac promoter (with two restriction sites around the reporter)	1055	Lucas PINERO	67891	Reporter	Not in stock

- Catalog of annotated sequences and molecules
- Enable standardized assembly of synthetic biological system

# Synthetic biology open language

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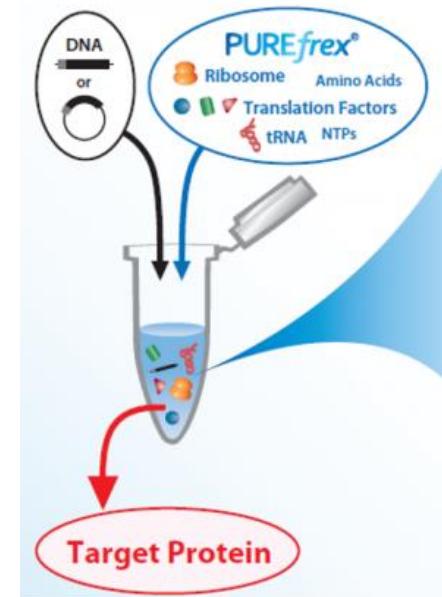
↗ promoter	→ primer binding site
▷ cds	restriction site
▷ ribosome entry site	] blunt restriction site
T terminator	└ 5'sticky restriction site
□ operator	┌ 3'sticky restriction site
■ insulator	= 5'overhang
X ribonuclease site	= 3'overhang
Y rna stability element	= assembly scar
Z protease site	☒ signature
Q protein stability element	█████ engineered region
O origin of replication	

- **Part:** Piece of functional DNA
  - Promoter, CDS, UTR
- **Device:** Collection of parts with defined function
  - Gene
- **System:** Combination of device that perform high-level tasks
  - Pathway

# Chassis for synthetic biology

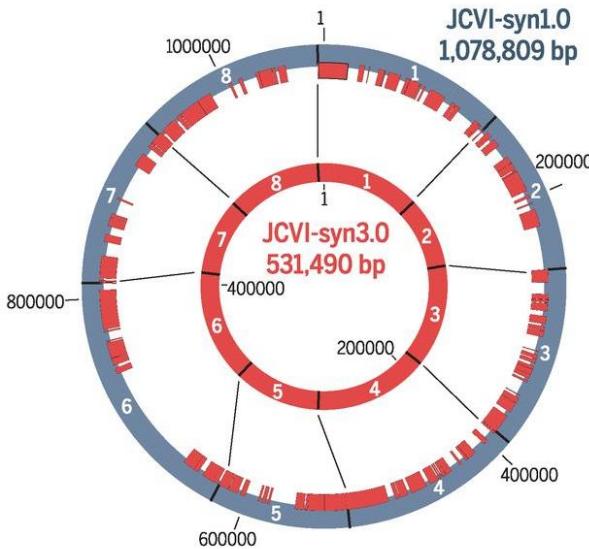
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- Harmless organisms with well-characterized genomes, easy to grow, and predictable phenotypes
- Bacteria: *E. coli*, *B. subtilis*
- Yeast: *S. cerevisiae*
- Plant, algae, and mammalian systems are available
- Cell free systems: crude extract, purified components

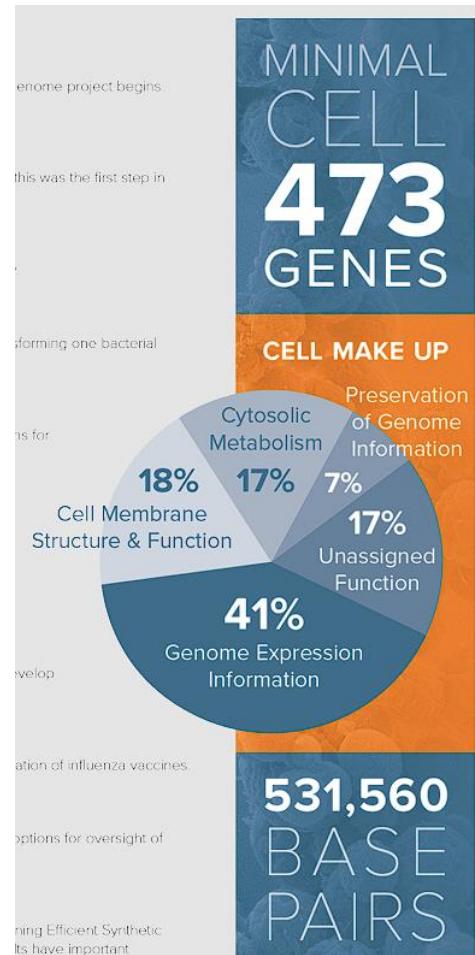


# Minimal genome project

- 2010-2016
- Based on bacterium *Mycoplasma mycoides*
- Reduced from 1 Mb to 531 kb (473 genes)
- Still retain 149 genes with unknown functions



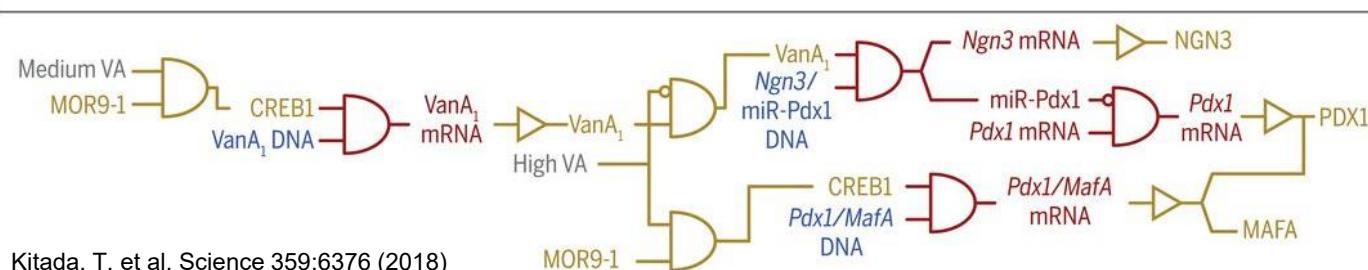
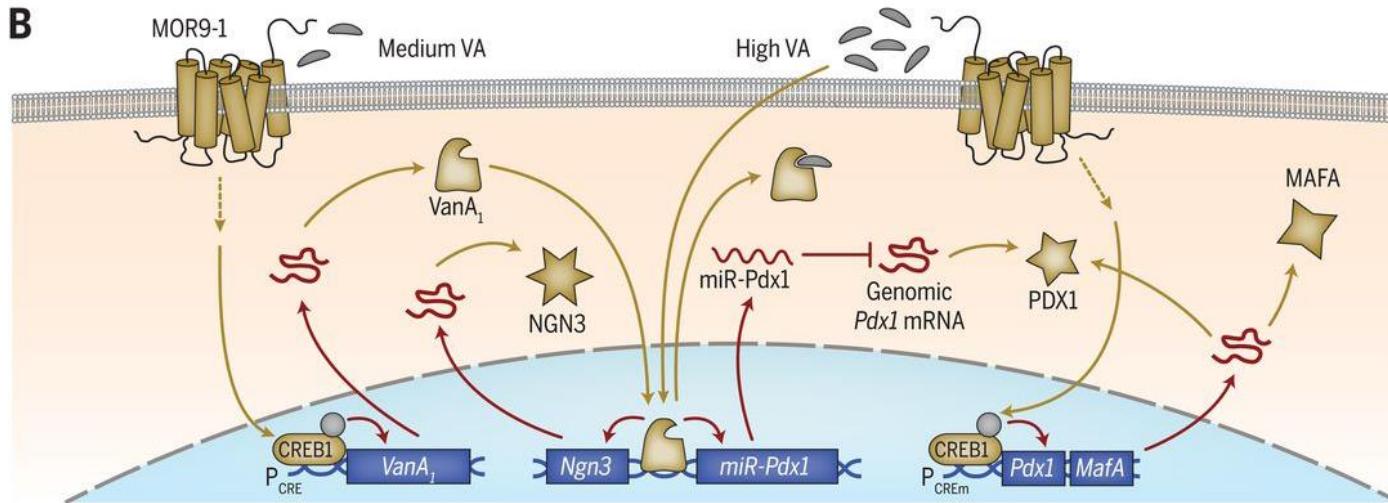
Hutchinson III, C.A. et al. Science 351:aad6253 (2016)



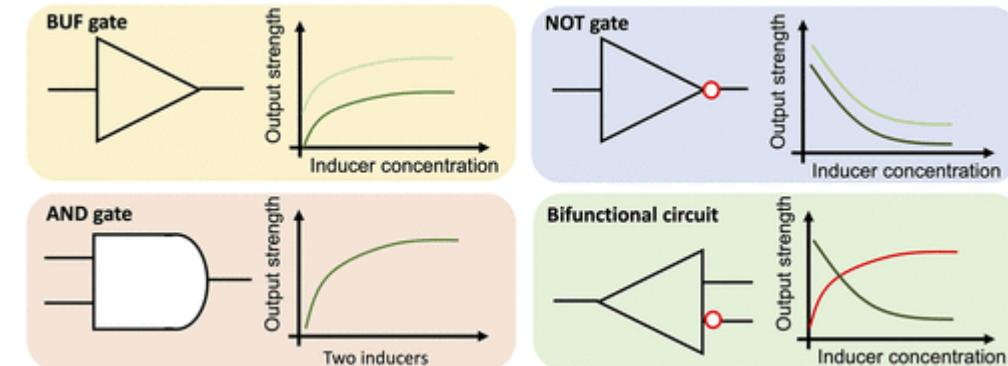


# Genetic circuits

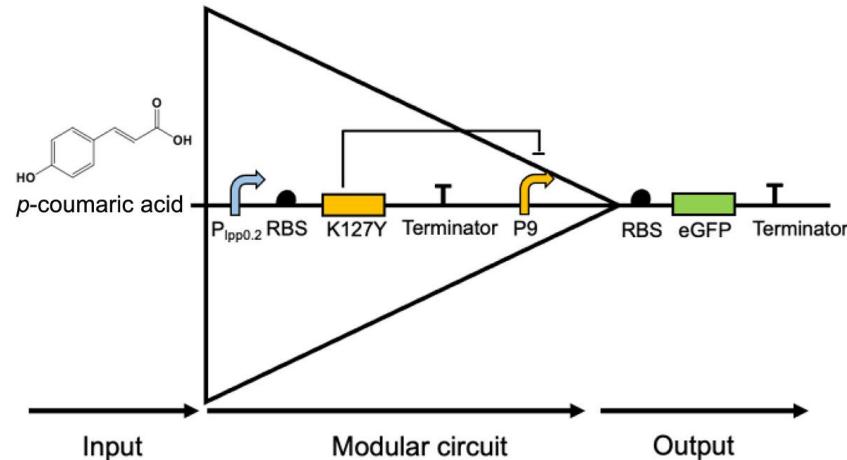
# Genetic circuit



# Genetic logic gates

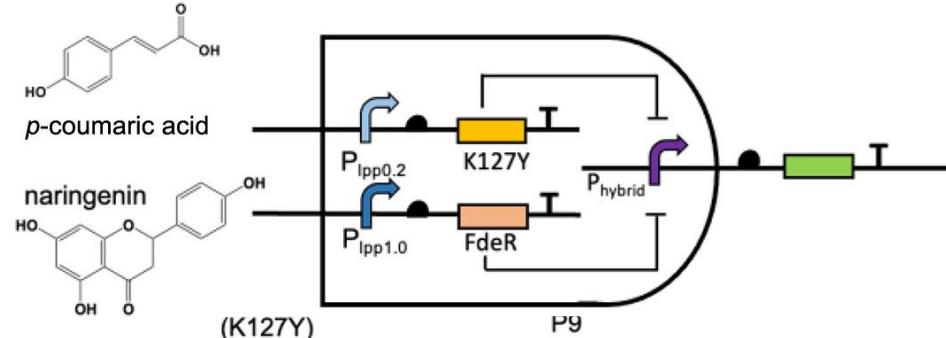


Jiang, T. et al. ACS Synthetic Biology 12:3730-3742 (2023)

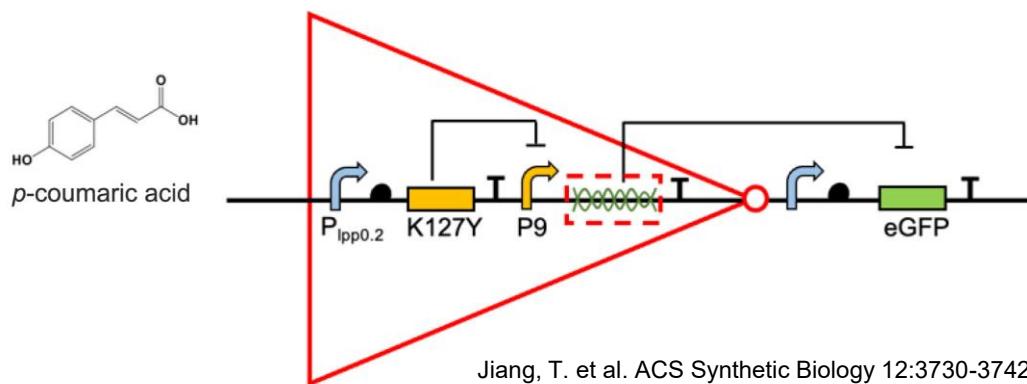


- Combining activation and repression to create customize genetic circuits that respond to specific input compounds
- Tune the system to achieve the desired input-response curve
  - By changing biological parts

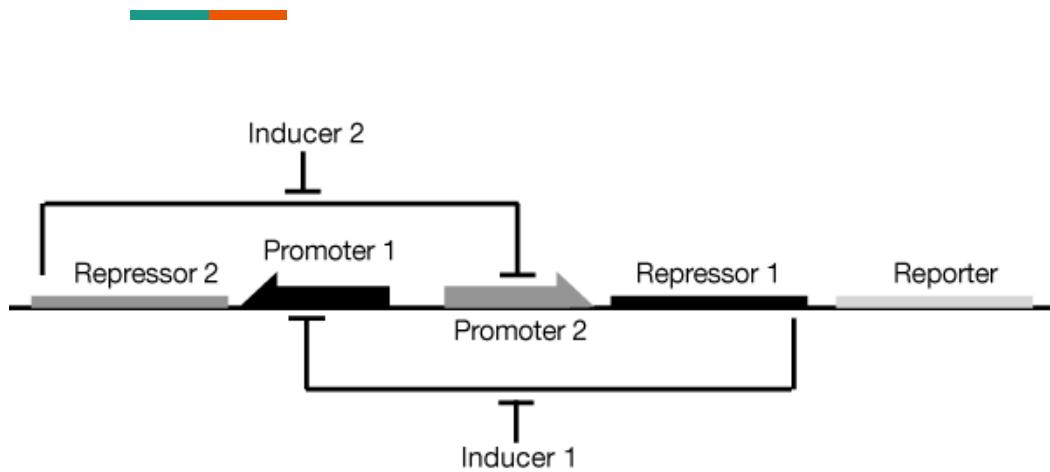
# More examples of genetic logic fates



- **AND gate:** two compounds stop the repression of a common downstream gene
- **NOT gate:** a compound unlocks the repression of the repressor of the downstream gene

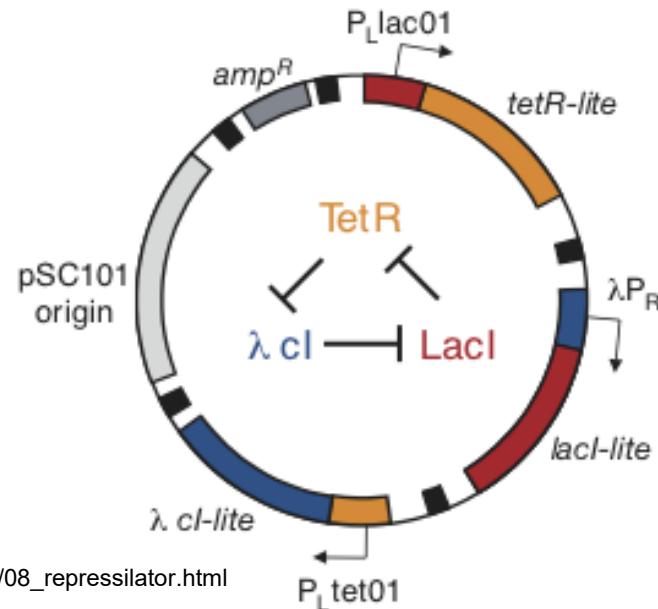


# Toggle switch and oscillator



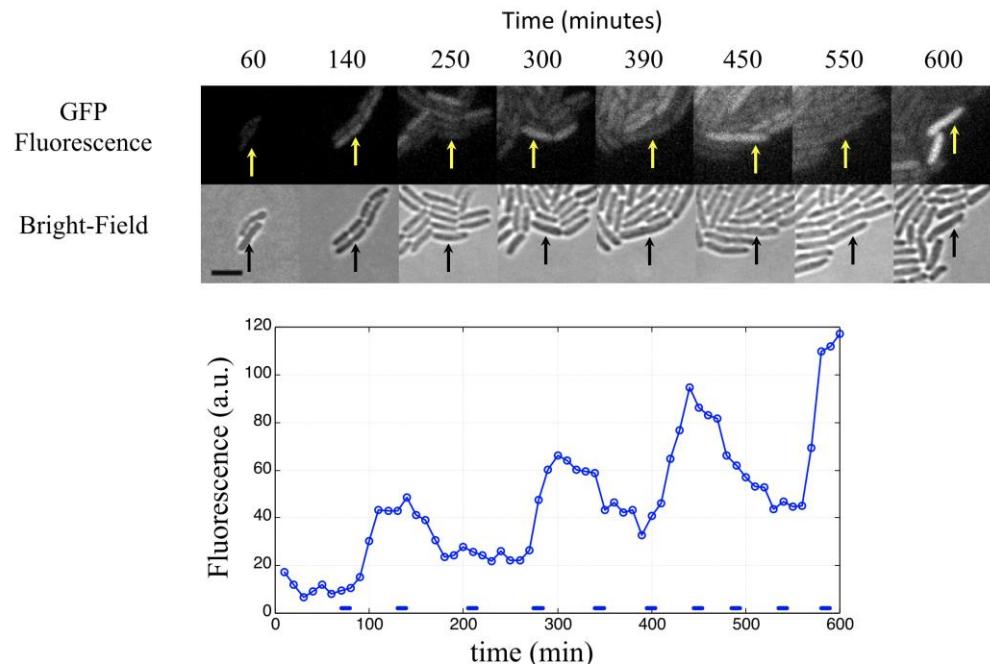
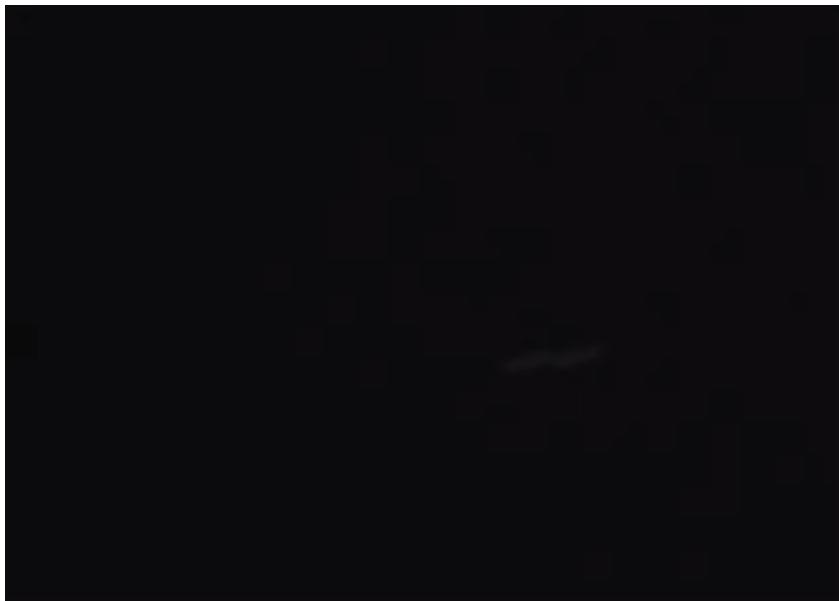
Gardner, T.S. et al. Nature 403:339-342 (2000)

[http://be150.caltech.edu/2019/handouts/08\\_repressilator.html](http://be150.caltech.edu/2019/handouts/08_repressilator.html)

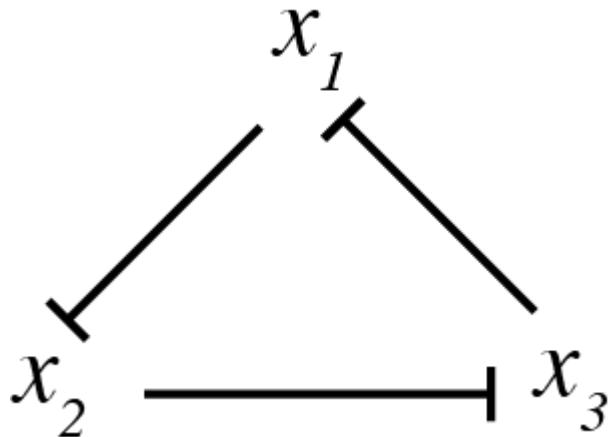


- **Repressilator:** At least 3 genes repressing each other in cycle
  - High LacI → Low TetR → High λ cl → Low LacI →

# Oscillating gene expression



# Recap: Using ODE to understand circuit dynamics



$$\frac{dx_1}{dt} = \frac{\beta}{1 + (x_3/k)^n} - \gamma x_1,$$

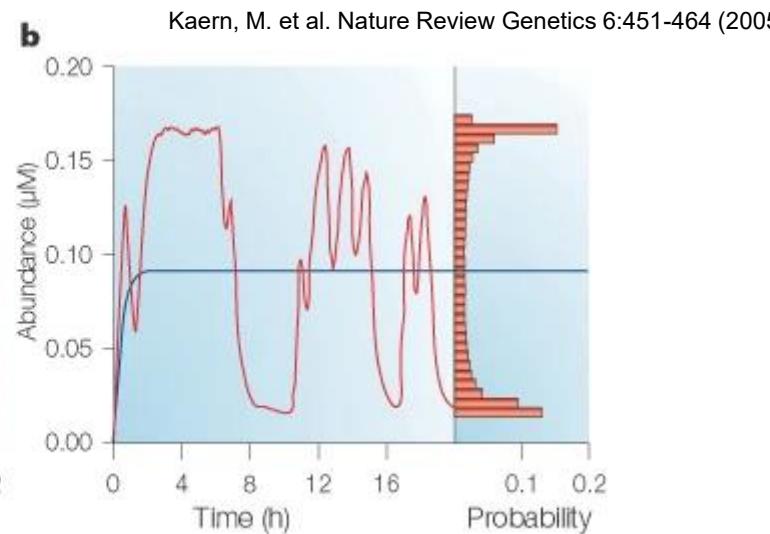
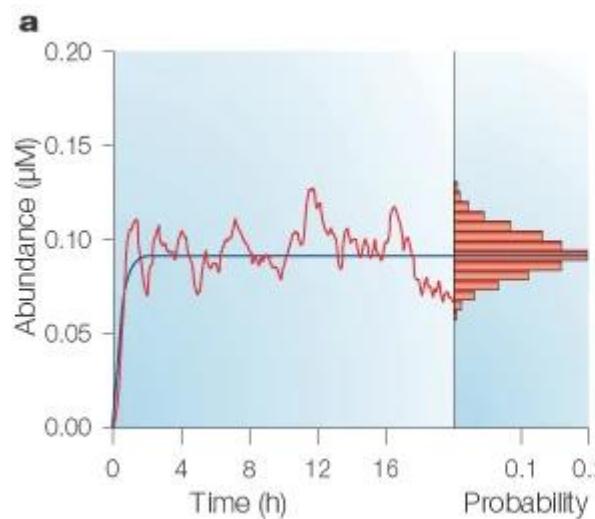
$$\frac{dx_2}{dt} = \frac{\beta}{1 + (x_1/k)^n} - \gamma x_2,$$

$$\frac{dx_3}{dt} = \frac{\beta}{1 + (x_2/k)^n} - \gamma x_3.$$

[http://be150.caltech.edu/2019/handouts/08\\_repressilator.html](http://be150.caltech.edu/2019/handouts/08_repressilator.html)

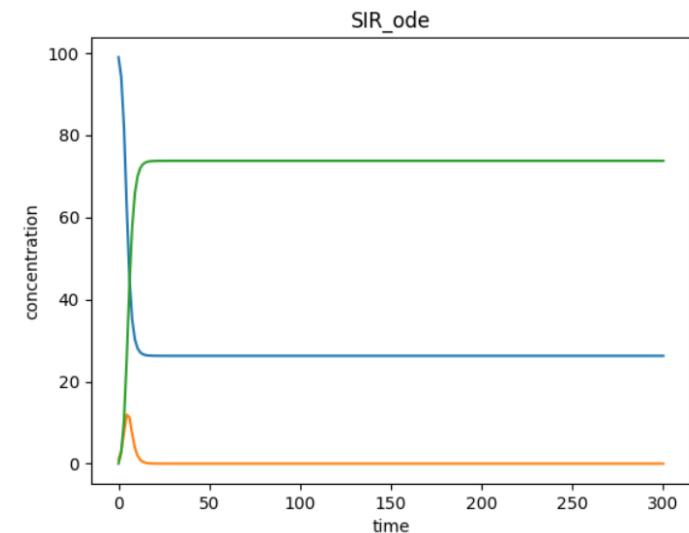
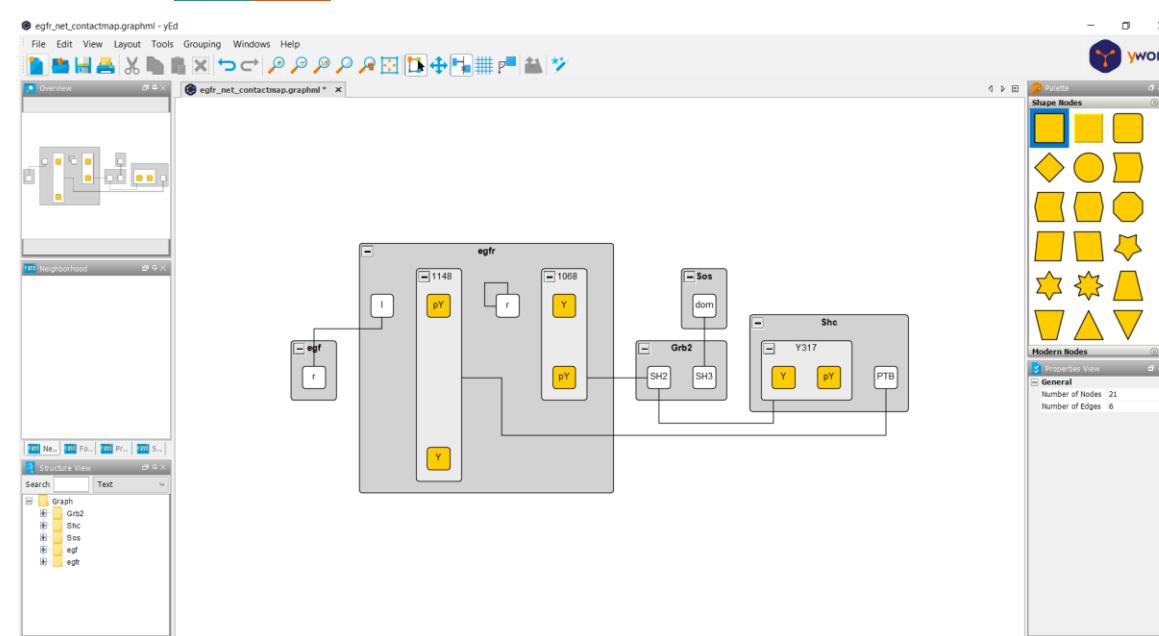
- Can optimize the repression strengths to control the period length and maximum expression level

# Stochasticity in real biological system



- Low molecular density and promoter occupancy can induce stochasticity in gene expression dynamics
  - Example: Promoter stays in ON and OFF state for extended period

# BioNetGen / Tellurium / COPASI



<https://bionetgen.org/>

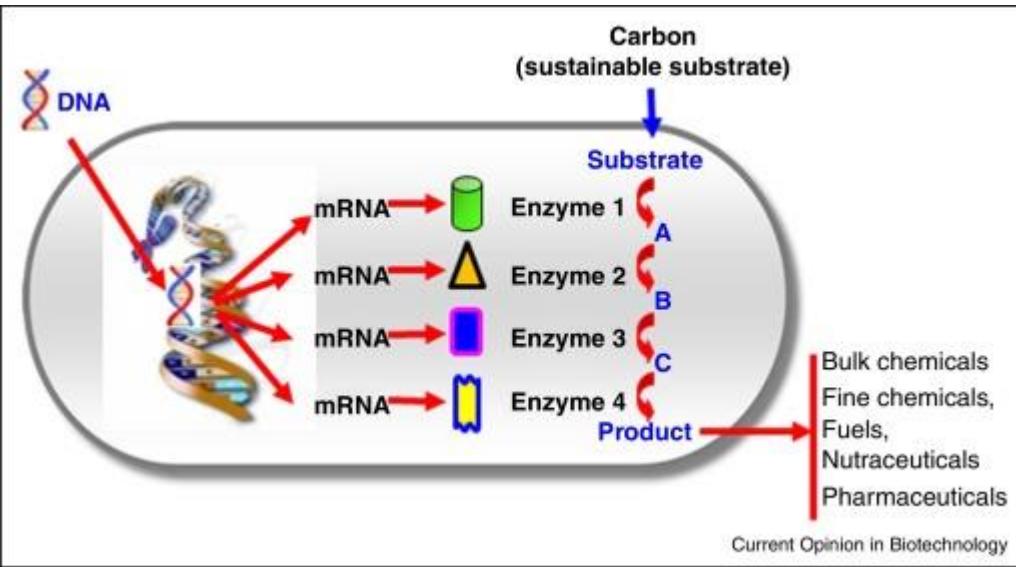
- Tools for designing and simulating biochemical reaction circuits



# Metabolic engineering

# Metabolite as system's endpoints

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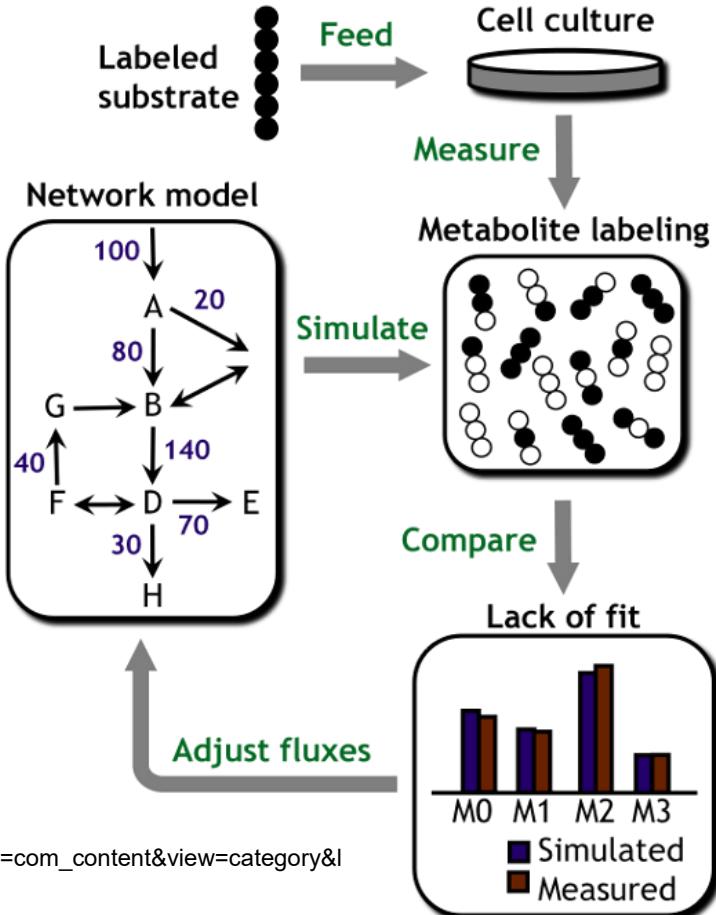


Zhu, Q. and Jackson, E.N. Current Opinion in Biotechnology 36:65-72 (2015)

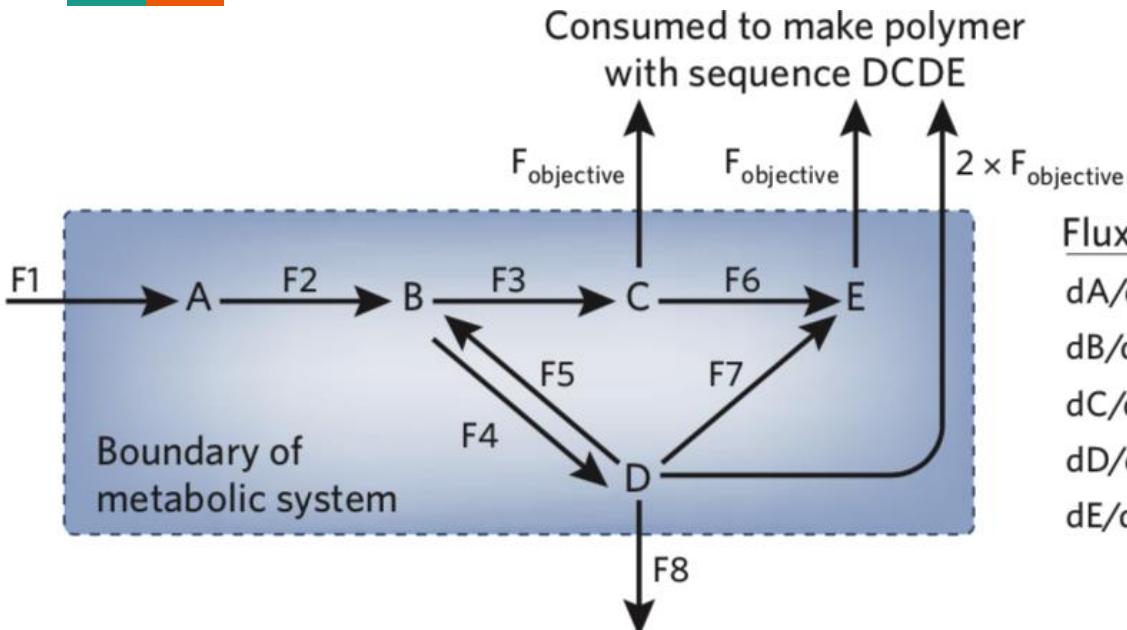
- In the context of cells as biological factories, metabolites are key endpoint products
- Processing of metabolites involve many proteins and intermediate products
- Can we identify the equilibrium of the cell?

# Metabolic flux modeling

- Modeling of chemical reactions that process various metabolites inside the cell
  - Follow isotopes from the initial substrate that are incorporated into downstream metabolites
  - Integrate with stoichiometry and network model
- More differential equations!



# Flux balance analysis



Rabinowitz, J.D. and Vastag, L. Nature Chemical Biology 8:497-501 (2014)

## Flux balance equations

$$dA/dt = F_1 - F_2 = 0$$

$$dB/dt = F_2 - F_3 - F_4 + F_5 = 0$$

$$dC/dt = F_3 - F_6 - F_{\text{objective}} = 0$$

$$dD/dt = F_4 - F_5 - F_7 - F_8 - 2F_{\text{objective}} = 0$$

$$dE/dt = F_6 + F_7 - F_{\text{objective}} = 0$$

- Flux balance is achieved when the level of all metabolites are unchanged
- Calculated by setting all derivatives to zero

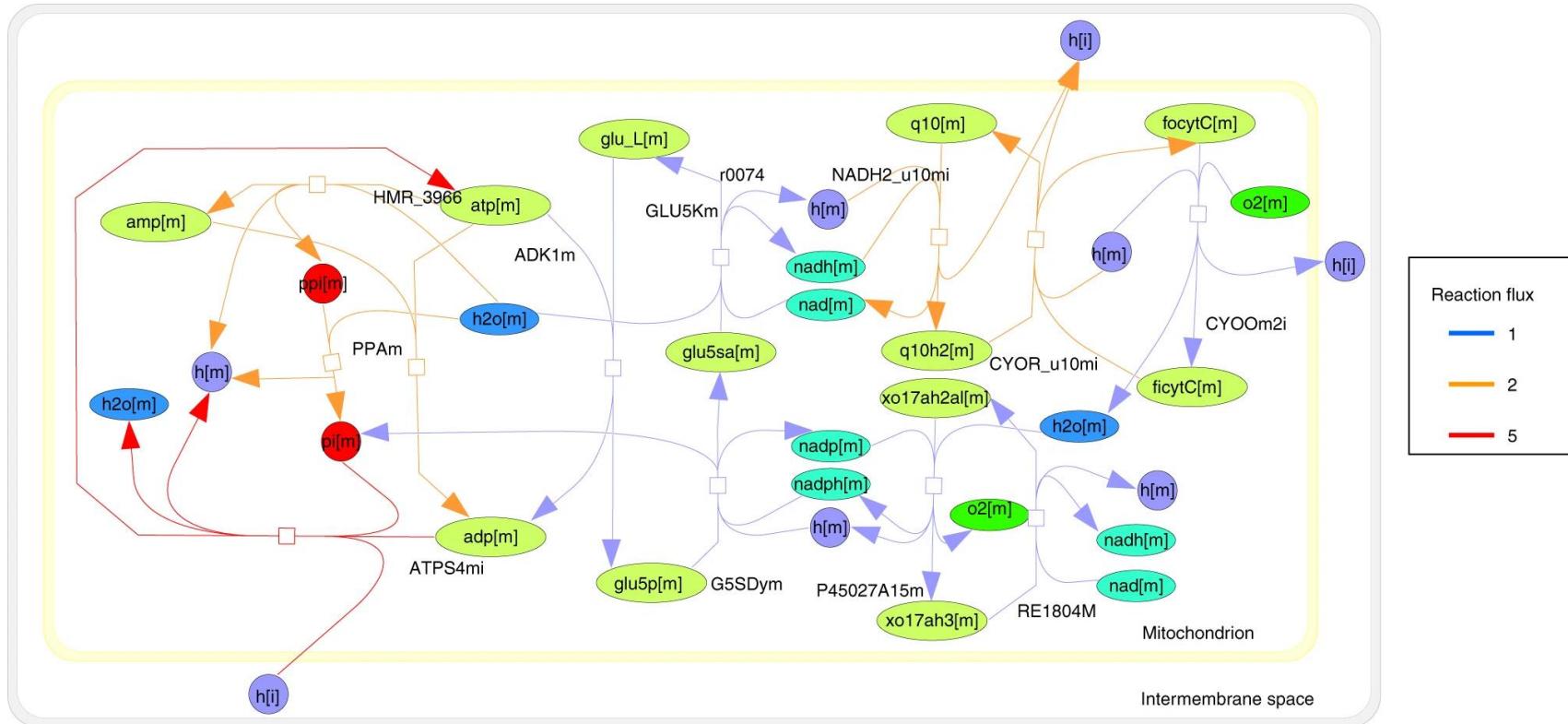
# Enzyme and reaction optimization

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- Alter metabolic flux to maximize objectives such as biomass production
- Identify reactions to modify
  - Calculate target stoichiometry and reaction rate through flux model
- Identify alternative enzymes that achieve the targets

# COBRA for metabolic flux analysis

Heirendt, L. et al. Nature Protocols 14:639-702 (2019)



# Recon3D: database of human metabolism network

The screenshot shows the Virtual Metabolic Human (VMH) web interface. At the top, there is a navigation bar with links for Home, Browse, Map navigator, Download, Help, Quick search, and a search bar. Below the navigation bar, there is a diagram of the human metabolism network with various pathways and metabolites. A search bar at the bottom left allows users to search all reactions or enter a specific search term.

**13543 reactions found**

Abbreviation	Description	Formula
<a href="#">10FTHF5GLU<sub>l</sub></a>	5-Glutamyl-10Fthf Transport, Lysosomal	$10\text{fthf5glu}[\text{c}] \rightarrow 10\text{fthf5glu}[\text{l}]$
<a href="#">10FTHF5GLU<sub>m</sub></a>	5-Glutamyl-10Fthf Transport, Mitochondrial	$10\text{fthf5glu}[\text{m}] \rightarrow 10\text{fthf5glu}[\text{c}]$
<a href="#">10FTHF6GLU<sub>l</sub></a>	6-Glutamyl-10Fthf Transport, Lysosomal	$10\text{fthf6glu}[\text{c}] \rightarrow 10\text{fthf6glu}[\text{l}]$
<a href="#">10FTHF6GLU<sub>m</sub></a>	6-Glutamyl-10Fthf Transport, Mitochondrial	$10\text{fthf6glu}[\text{m}] \rightarrow 10\text{fthf6glu}[\text{c}]$
<a href="#">10FTHF7GLU<sub>l</sub></a>	7-Glutamyl-10Fthf Transport, Lysosomal	$10\text{fthf7glu}[\text{c}] \rightarrow 10\text{fthf7glu}[\text{l}]$
<a href="#">10FTHF7GLU<sub>m</sub></a>	7-Glutamyl-10Fthf Transport, Mitochondrial	$10\text{fthf7glu}[\text{m}] \rightarrow 10\text{fthf7glu}[\text{c}]$
<a href="#">10FTHf<sub>l</sub></a>	10-Formyltetrahydrofolate Lysosomal Transport via Diffusion	$10\text{fthf}[\text{c}] \leftrightarrow 10\text{fthf}[\text{l}]$

Displaying 1 - 50 of 13543 | Change page size | OK | Download

**4138 metabolites found**

Abbreviation	Name	Charged Formula
<a href="#">10fthf</a>	10-Formyltetrahydrofolate	C20H21N7O7
<a href="#">10fthf5glu</a>	10-Formyltetrahydrofolate-[Glu](5)	C40H45N11O19
<a href="#">10fthf6glu</a>	10-Formyltetrahydrofolate-[Glu](6)	C45H51N12O22
<a href="#">10fthf7glu</a>	10-Formyltetrahydrofolate-[Glu](7)	C50H57N13O25
<a href="#">11_cis_retfa</a>	Fatty Acid 11-Cis-Retinol	C20H29OFULLR2CO
<a href="#">11docrtsl</a>	Cortexolone	C21H30O4
<a href="#">11docrtstrn</a>	Deoxycorticosterone	C21H30O3

Curated and predicted metabolic reactions and stoichiometry

# Integration of omics data in metabolic flux analysis

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- Changes in gene and protein expression affect metabolic flux
  - Know from omics profiles
  - Calculate variability in flux (e.g., lower and upper bounds)
- Context-specific metabolic flux analysis
  - Cell type and condition
- OptKnock/FastKnock: identify genes to knockout to achieve flux state



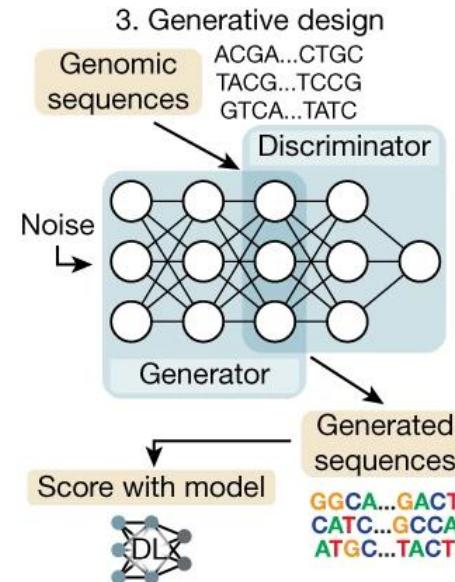
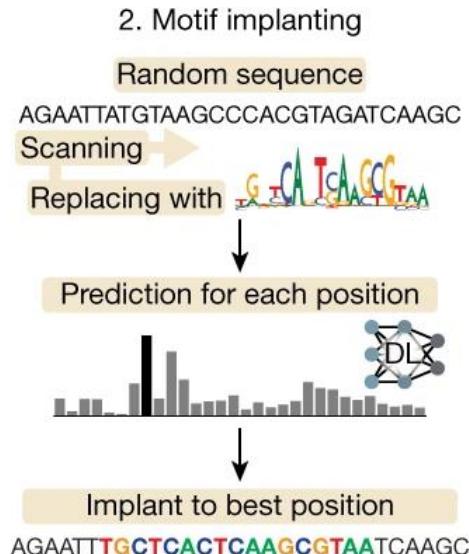
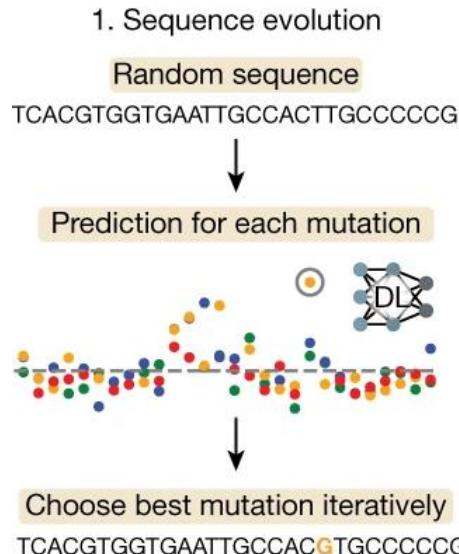
# Computation in synthetic biology

# Genome design

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- Creating genes and regulatory elements with the desired properties
  - Protein function
  - mRNA secondary structure and stability
  - Protein and non-coding RNA binding
- Recombination of existing DNA sequences
  - Connecting existing regulatory elements and promoters to CDS
  - Inserting known binding motifs
- Computational search
  - Random mutation + property prediction

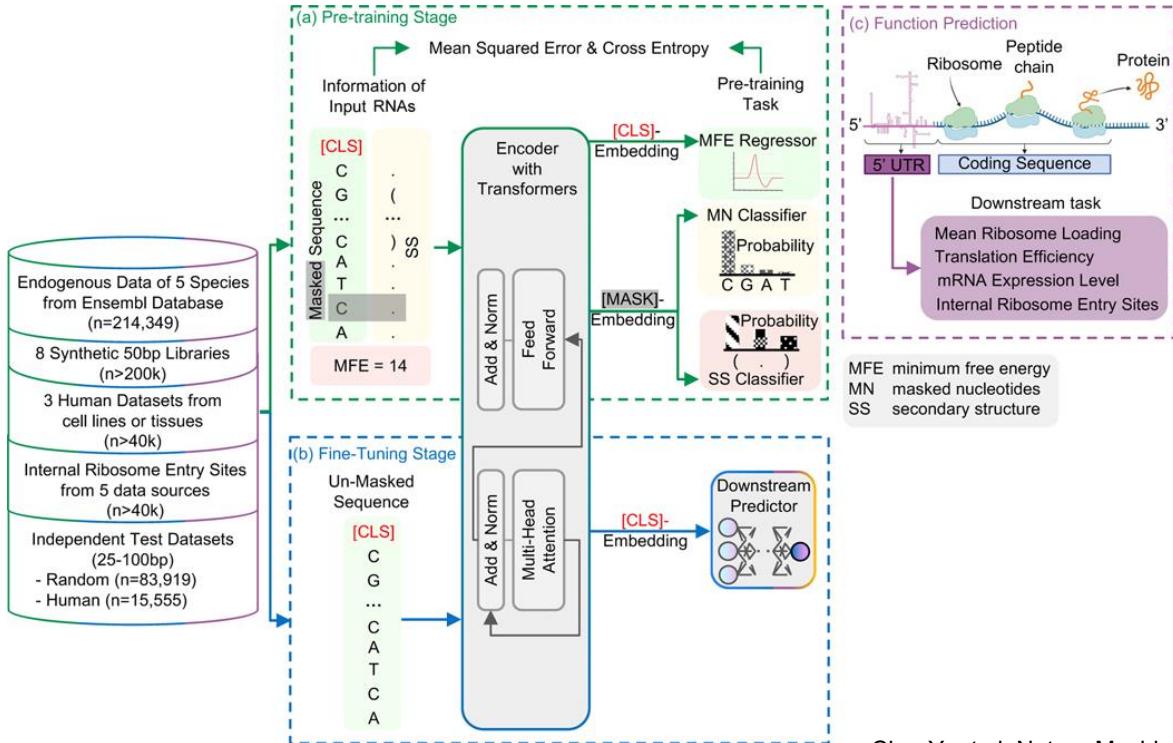
# Computer-aided design of DNA



Taskiran, I.I. et al. Nature 626:212-220 (2024)

- Edit seed DNA sequence to achieve the target properties
- Conditional generative process

# UTR optimization



- Learn to predict masked nucleotide and secondary structure of known UTR
- Fine-tuned to predict ribosome loading, translation efficiency, and mRNA expression

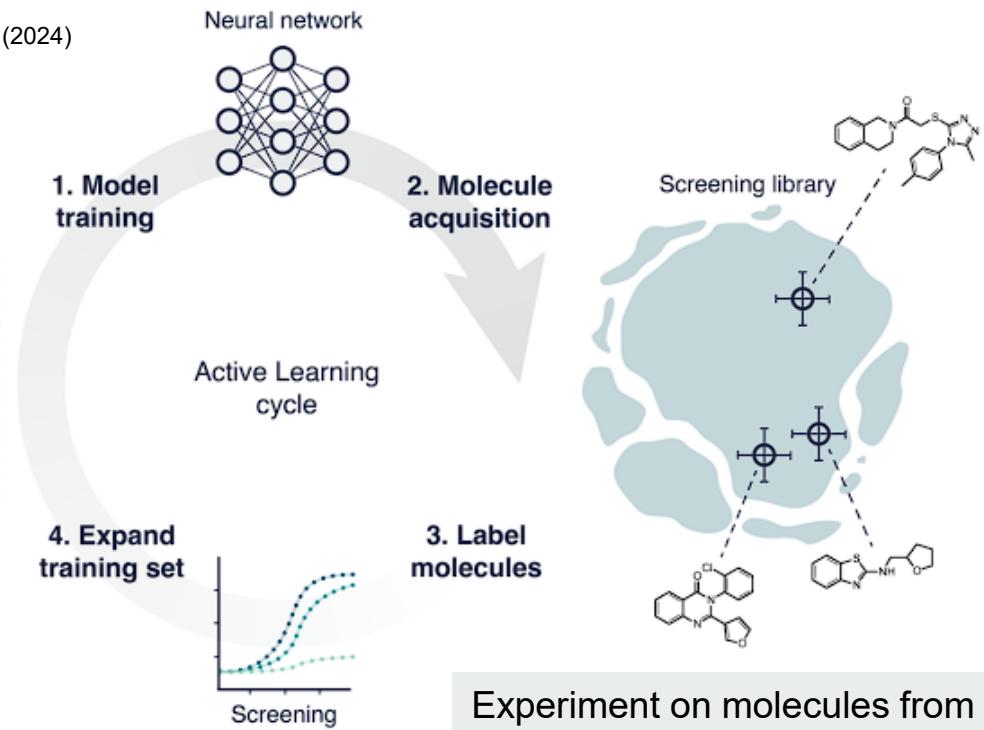
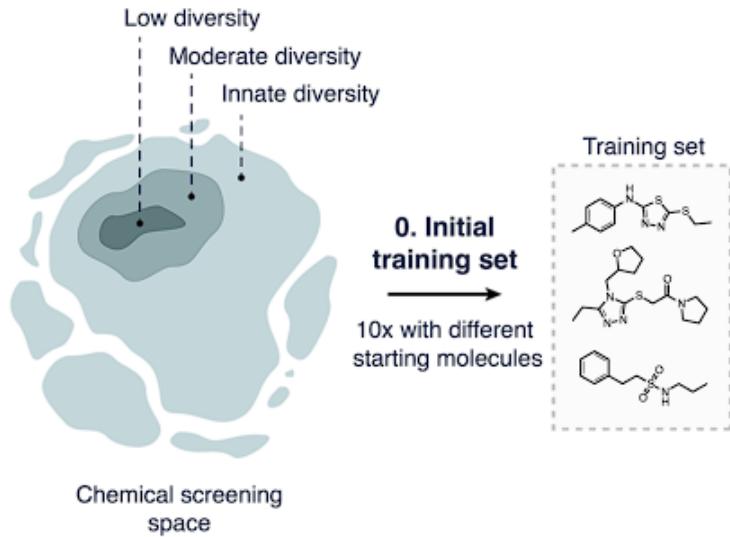
# Data-driven design-build-test-learn cycle

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- Design of molecules with specific properties using AI
- Predict impacts in biological system via mathematical modeling and simulation
- **Active learning:** Propose experiments that would provide the best information to improve knowledge and AI's performance
  - Based on current AI's errors
  - Based on the distribution of existing datasets

# Active learning approach for molecular design

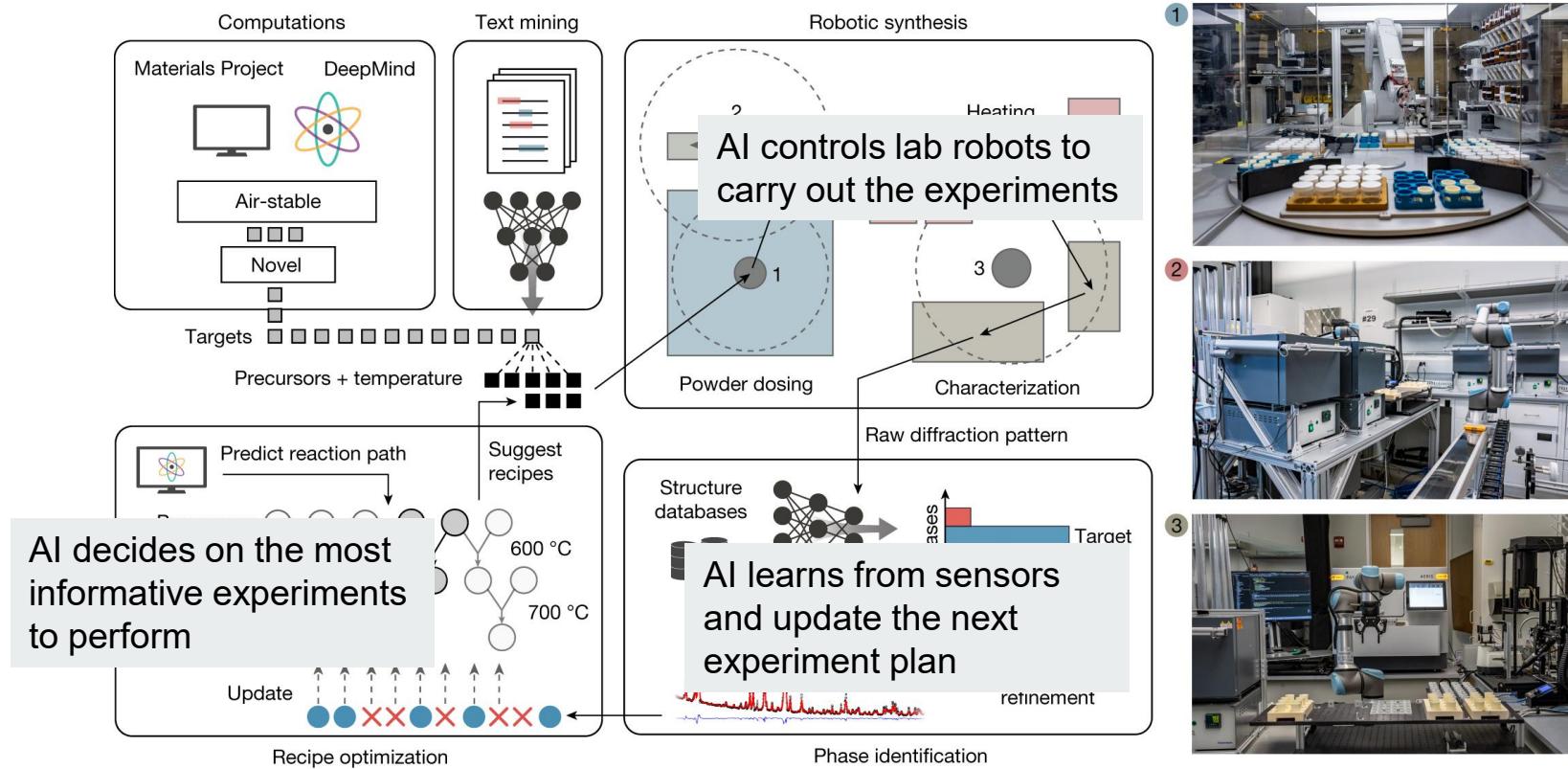
van Tilborg, D. and Grisoni, F. Nature Computational Science 4:786-796 (2024)



Original training dataset is not uniformly distributed on the molecule space

# Automated experiments

Szymanski, N.J. et al. Nature 624:86-91 (2023)



# Summary

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- Synthetic biology is about designing, manipulating, and understanding the working of a whole biological process and organism
- Genetic circuits and computation genome design
- Metabolic engineering and flux optimization
- Benefits from omics, mathematical modeling, and AI

# Any question?

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- See you next time