



# 3000788 Intro to Comp Molec Biol

## Lecture 26: Synthetic biology

Fall 2025



**Sira Sriswasdi, PhD**

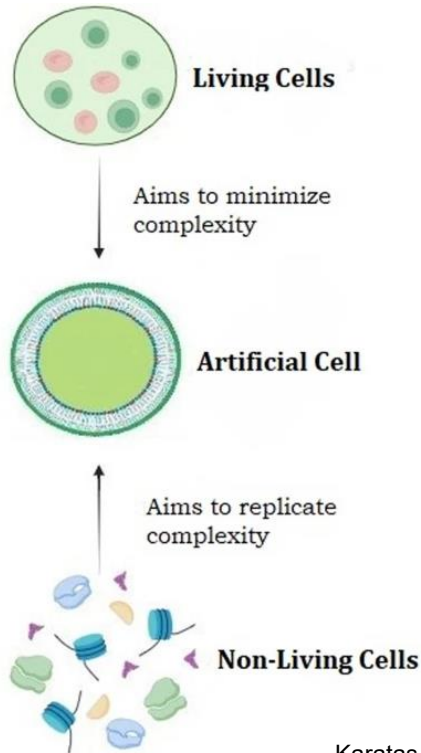
- Research Affairs
- Center of Excellence in Computational Molecular Biology (CMB)
- Center for Artificial Intelligence in Medicine (CU-AIM)

# Today's agenda



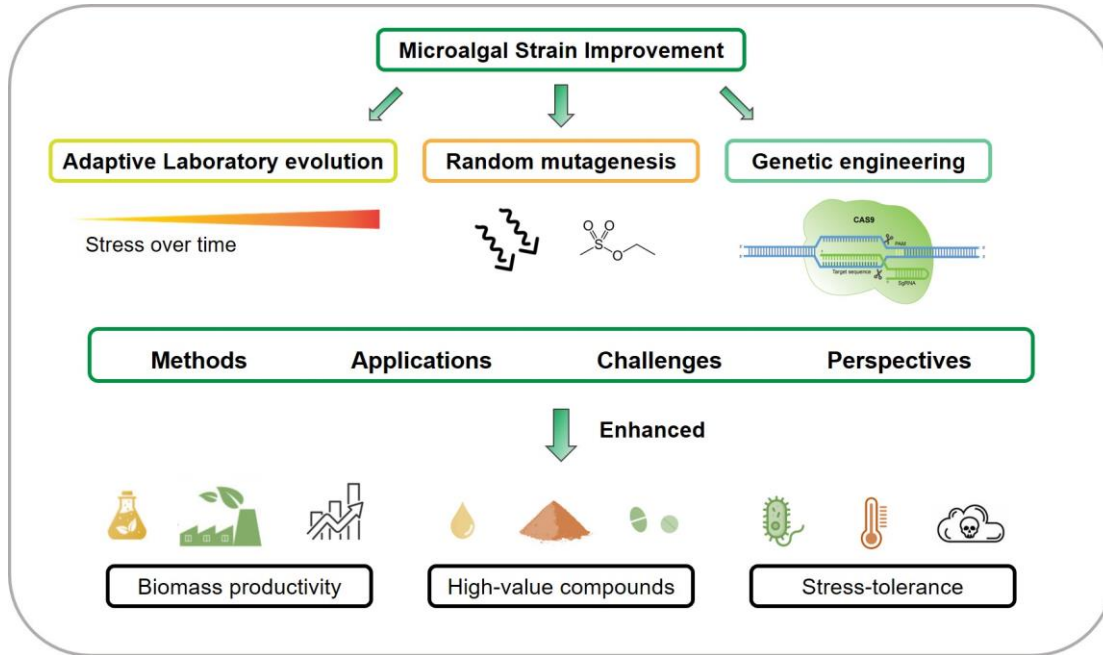
- Goals of synthetic biology
- Modular design of life
  - Genetic circuit
  - Metabolic engineering
- Computation in synthetic biology

# Goals of synthetic biology



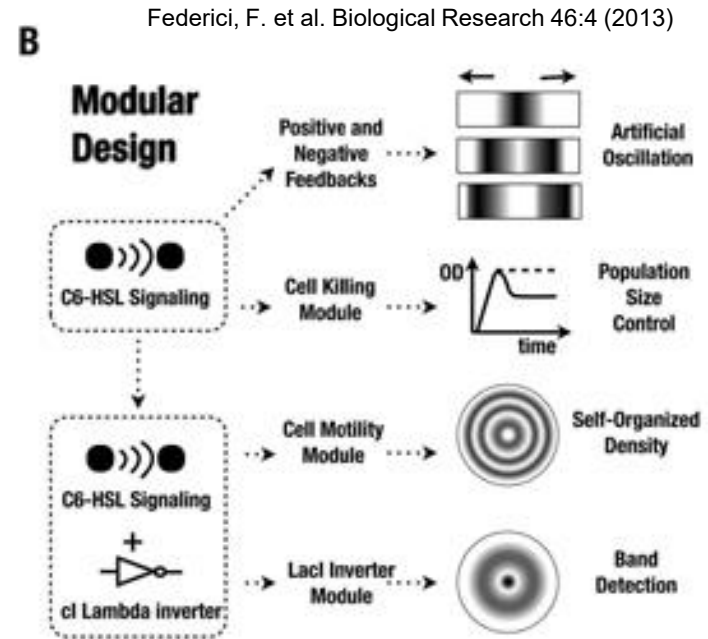
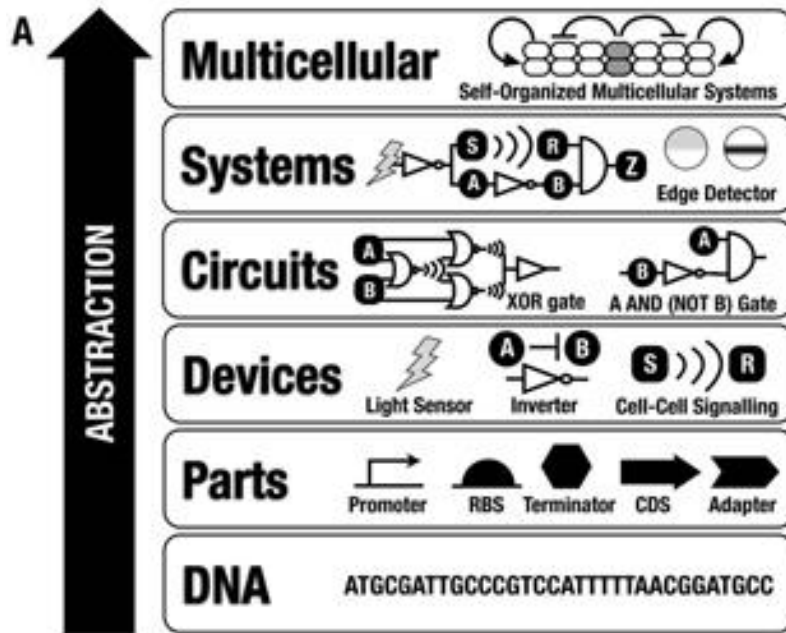
- Design and building of artificial biological systems (processes, organisms, etc.)
- To understand the mechanisms of life
- To develop biological products and biological factories to address medical, agricultural, and environmental needs

# Comparison to genetic engineering



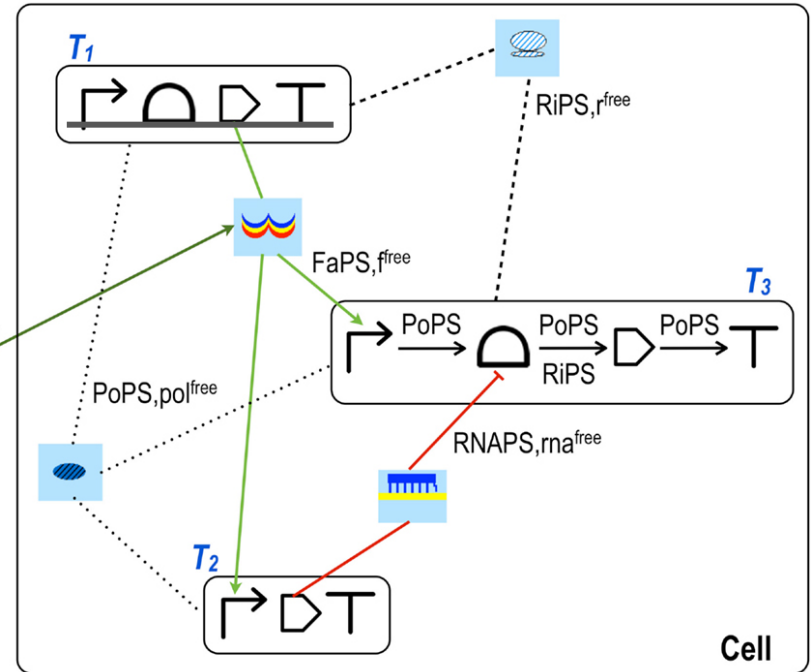
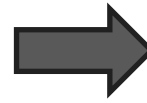
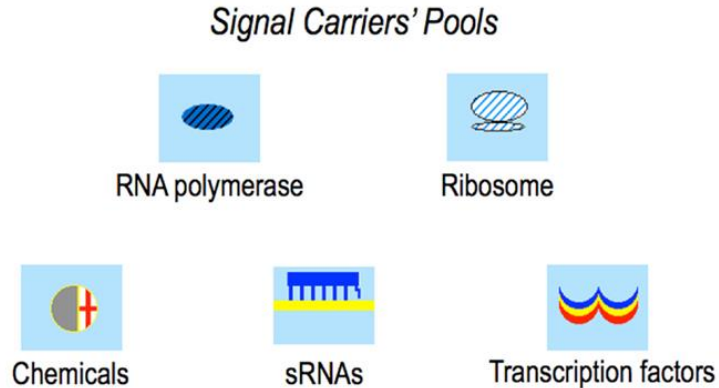
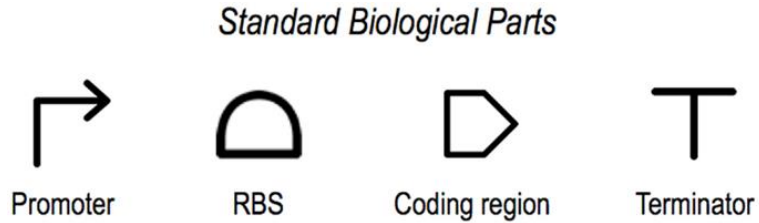
- Genetic engineering is about modifying an intact natural system to derive the desired phenotypes
- Synthetic biology is about designing or controlling the whole system

# Modular design in synthetic biology

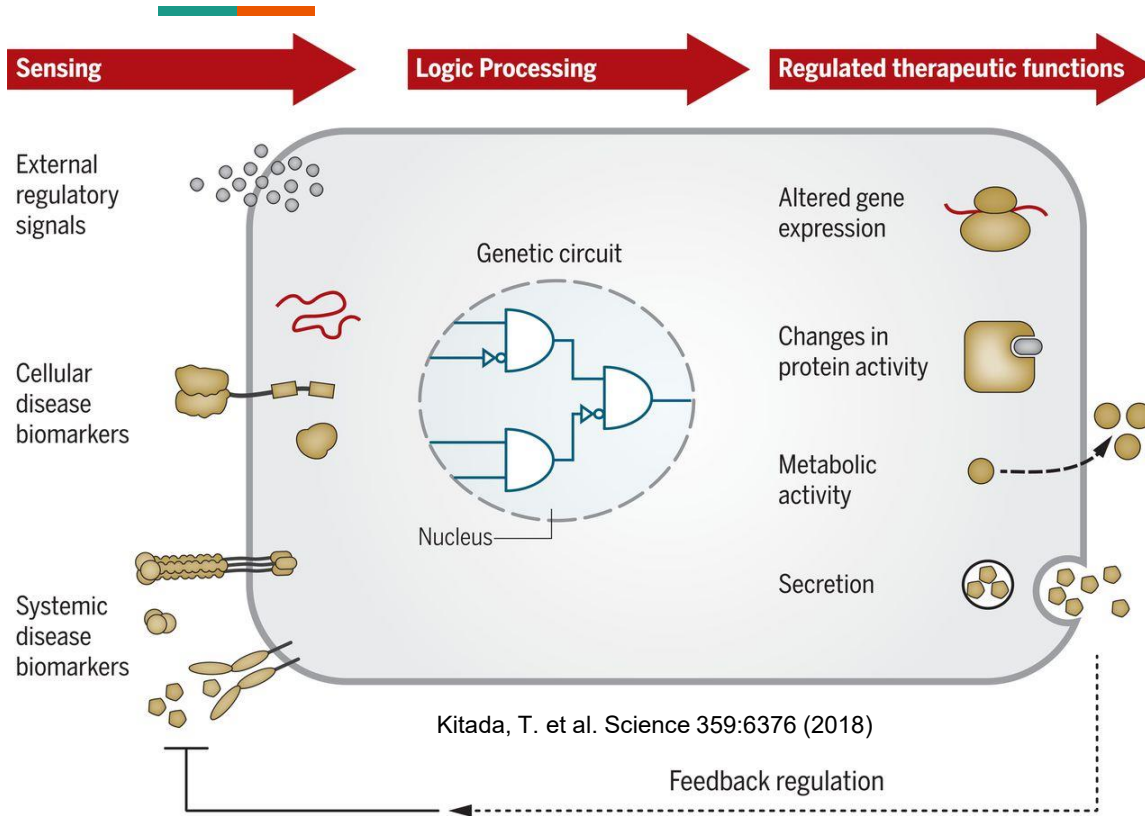


- Treat biological system like an electronics

# Biological parts

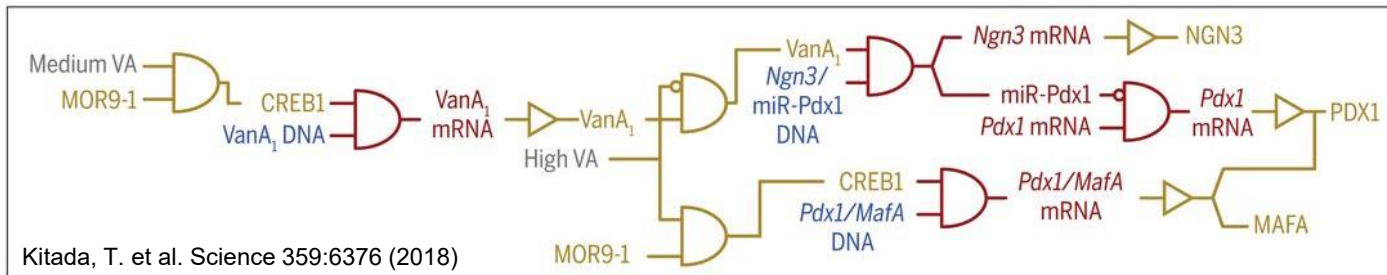
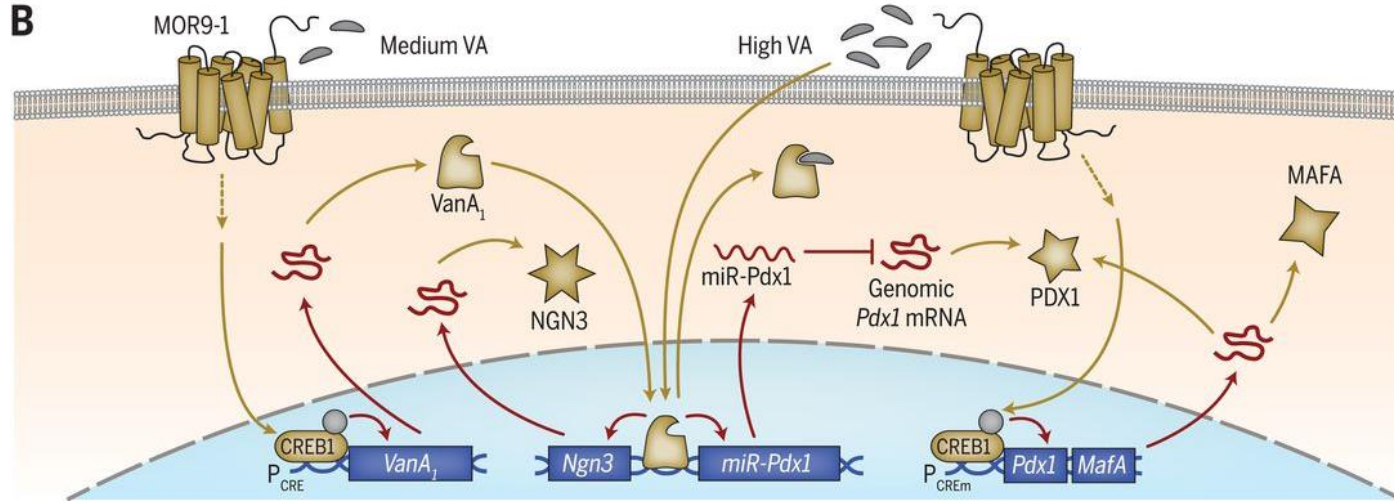


# DNA programming

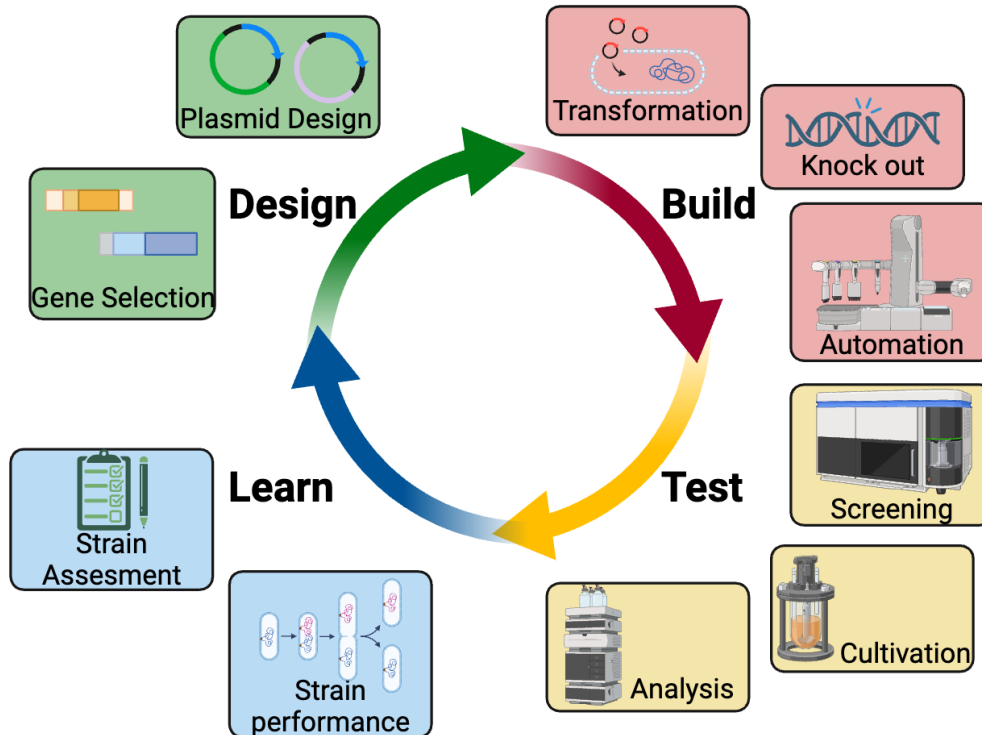


- Cell = computer
- Genetic circuit processes inputs and produce outputs
- Customize genetic circuits and put inside the cell for testing

# 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	Documentaion	Type	Status
<a href="#">BBa_K808000</a>	araC-Pbad - Arabinose inducible regulatory promoter/repressor unit	1209	Valentina Herbring, Sebastian Palluk, Andreas Schmidt	1033281	Regulatory	In stock
<a href="#">BBa_K2607001</a>	HB-EGF/Tar Receptor (HT) Device	1836	Andrea Laurentius	191593	Composite	It's complicated
<a href="#">BBa_K2607000</a>	DiphTox (DT)	254	Andrea Laurentius	183996	Protein_Domain	It's complicated
<a href="#">BBa_J04450</a>	RFP Coding Device	1069	Tamar Odle	115288	Reporter	In stock
<a href="#">BBa_K3187028</a>	Sortase A7M (Ca <sup>2+</sup> -independent variant)	450	iGEM TU_Darmstadt 2019	96929	Coding	Not in stock
<a href="#">BBa_J23100</a>	constitutive promoter family member	35	John Anderson	83466	Regulatory	In stock
<a href="#">BBa_R0062</a>	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
<a href="#">BBa_K857000</a>	acetaldehyde dehydrogenase	951	yale yuen	73967	Coding	It's complicated
<a href="#">BBa_K801060</a>	(+)-Limonene synthase 1 with Strep-tag and yeast consensus sequence.	1708	Lara Kuntz	68635	Coding	In stock
<a href="#">BBa_K3128009</a>	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

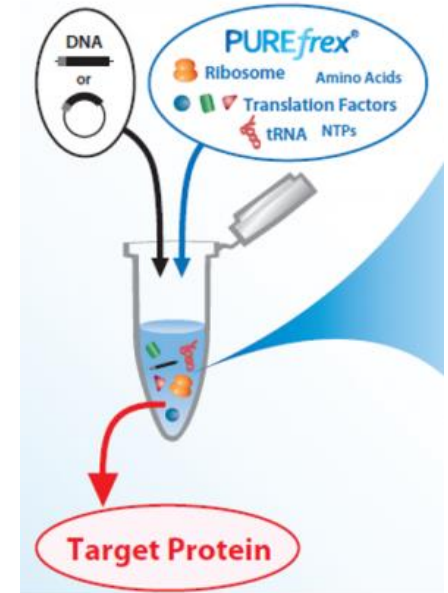
# Synthetic biology open language

 promoter	 primer binding site
 cds	 restriction site
 ribosome entry site	 blunt restriction site
 terminator	 5'sticky restriction site
 operator	 3'sticky restriction site
 insulator	 5'overhang
 ribonuclease site	 3'overhang
 rna stability element	 assembly scar
 protease site	 signature
 protein stability element	 engineered region
 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

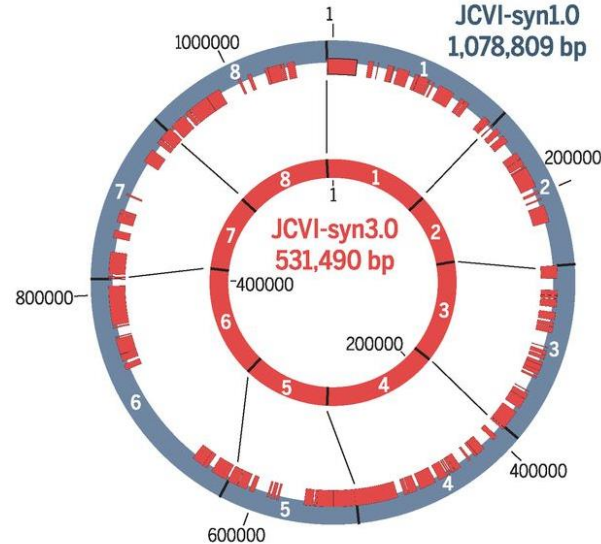
# Chassis for synthetic biology

- 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

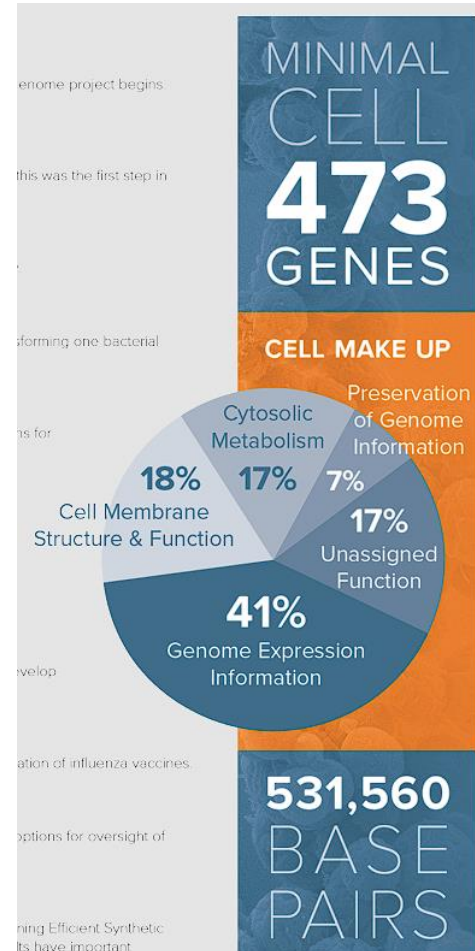


# Minimal genome project

- 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)

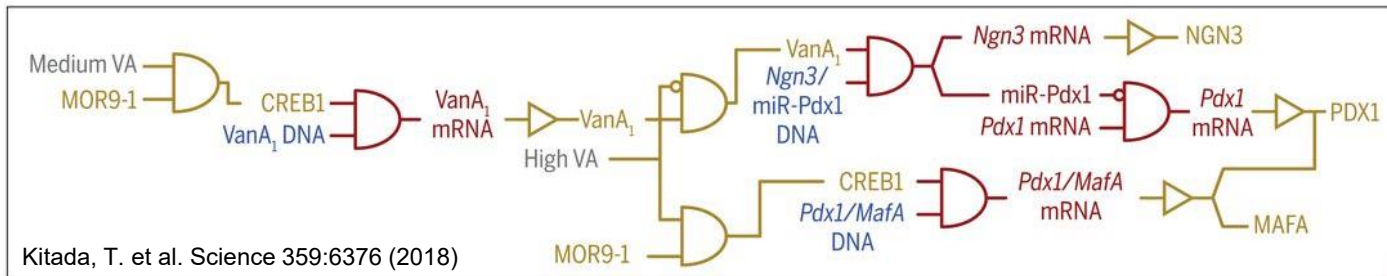
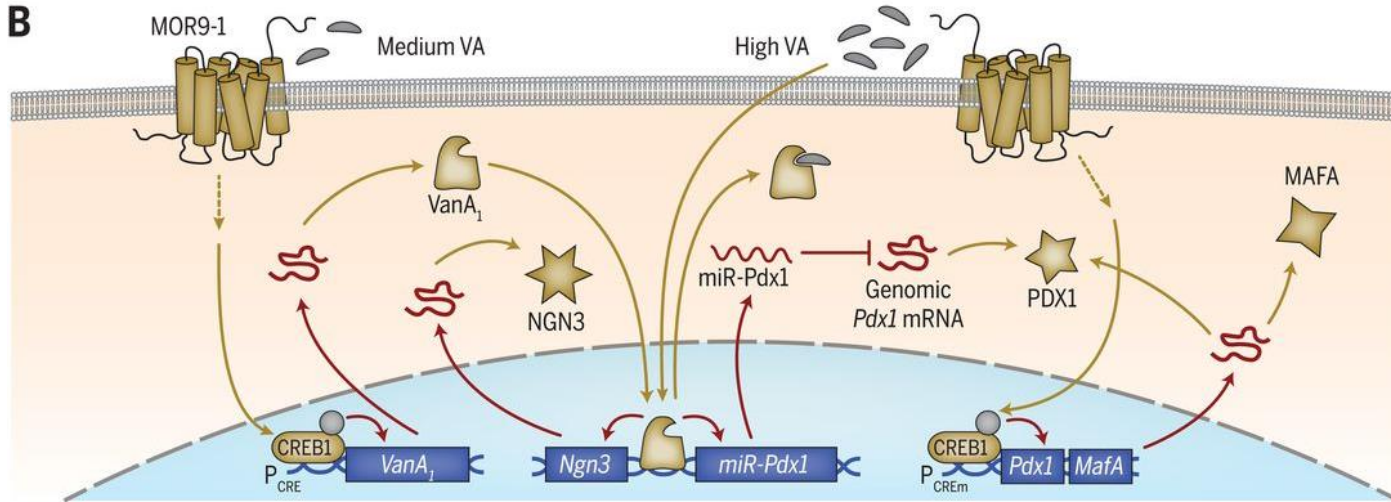




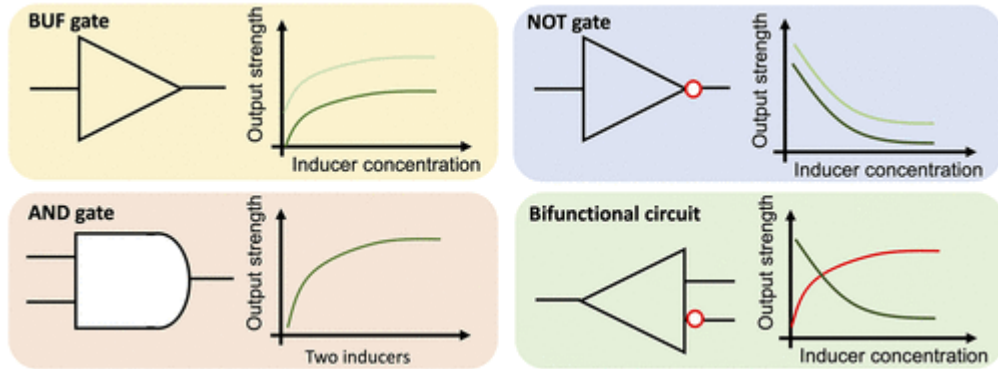
# Genetic circuits

# Genetic circuit

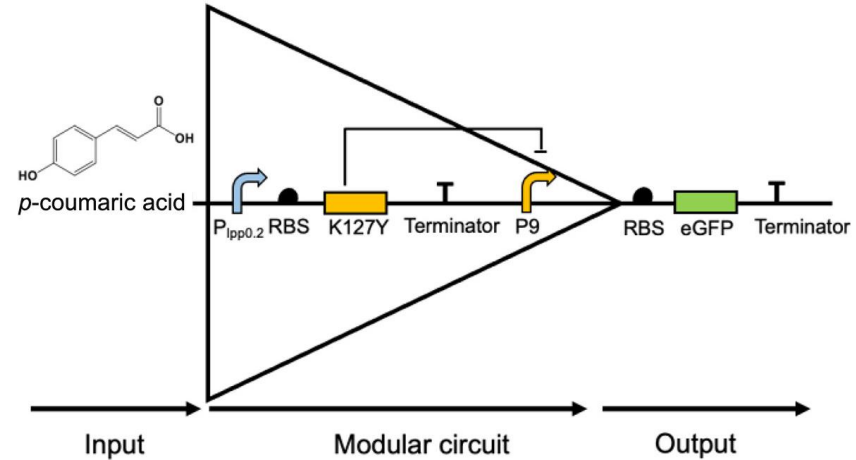
**B**



# Genetic logic gates

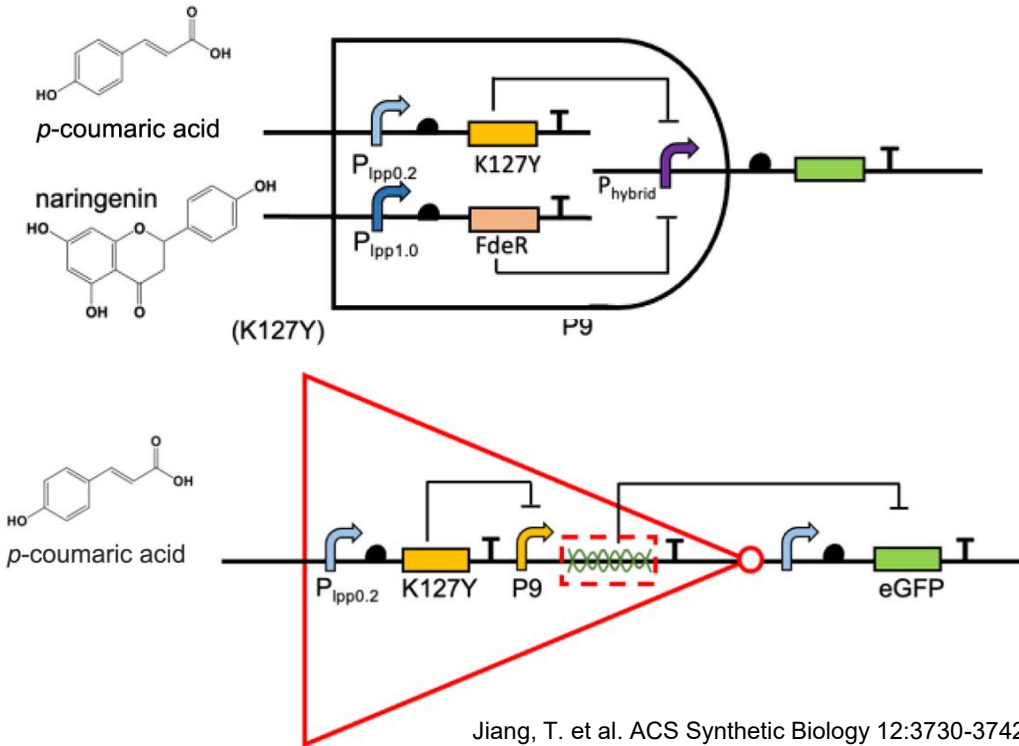


Jiang, T. et al. ACS Synthetic Biology 12:3730-3742 (2023)



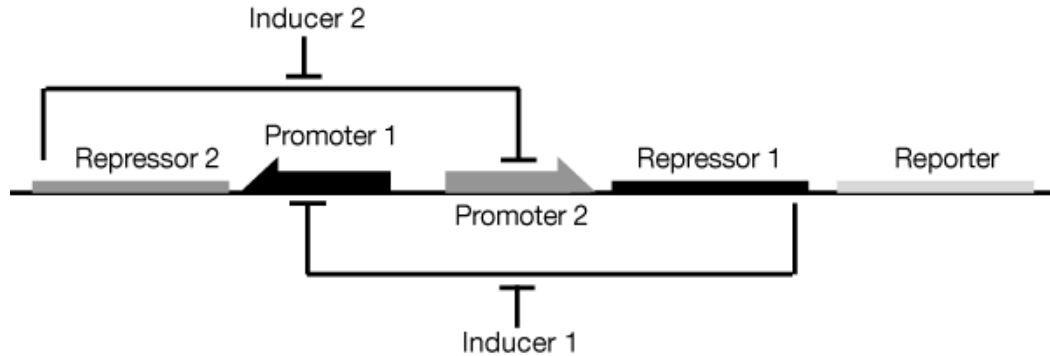
- Combining activation and repression to create customized genetic circuits that respond to specific input compounds
- Tune the system to achieve the desired input-response curve
  - By changing biological parts

# More examples of genetic logic gates



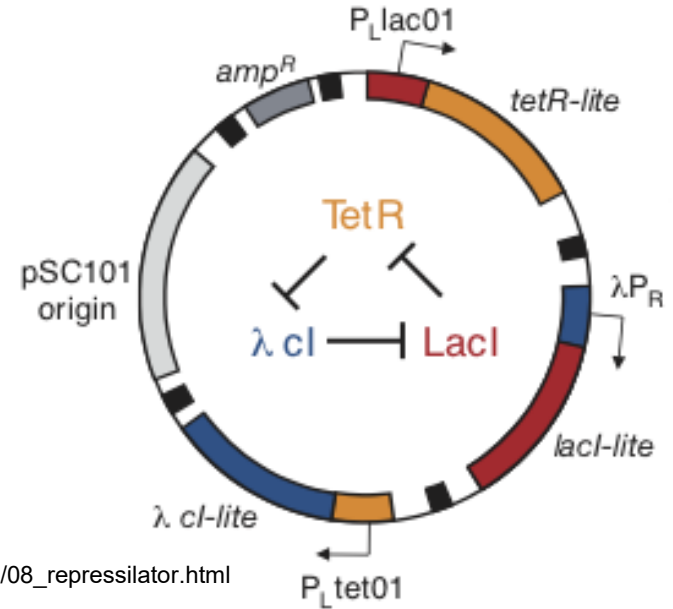
- **AND gate:** two compounds stop the repression of a common downstream gene
- **NOT gate:** a compound unlocks the repression of the repressor of the downstream gene

# 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)

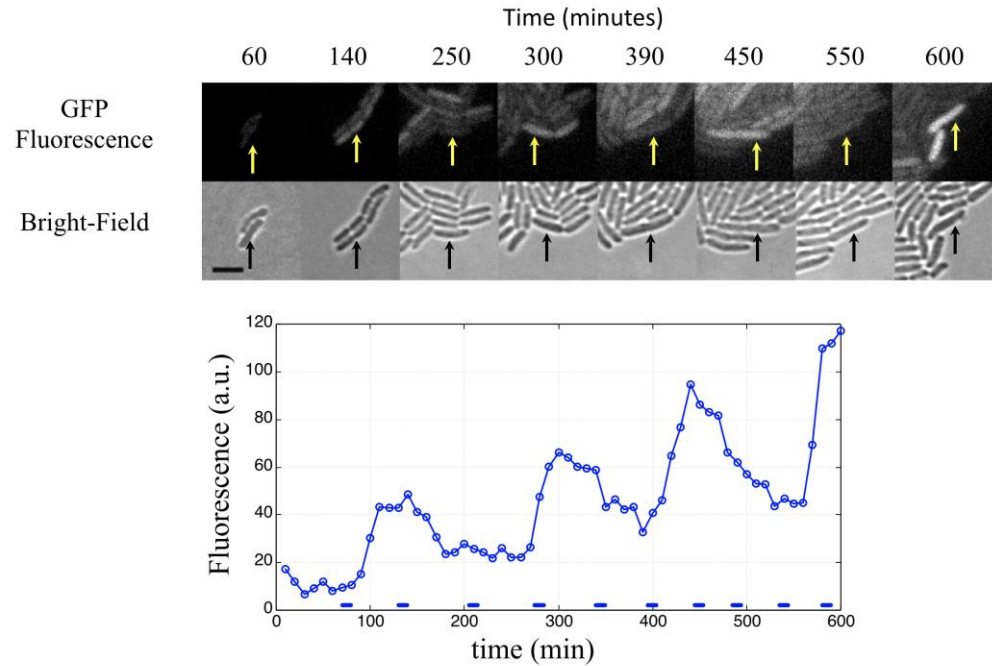


- **Repressilator:** At least 3 genes repressing each other in cycle
  - High LacI  $\rightarrow$  Low TetR  $\rightarrow$  High  $\lambda$  cl  $\rightarrow$  Low LacI  $\rightarrow$

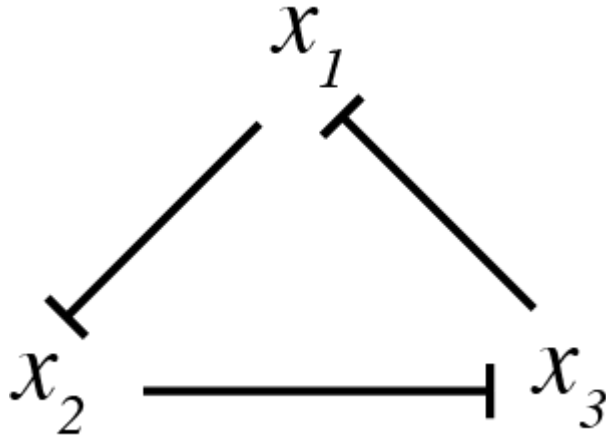
# Oscillating gene expression



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



## Recap: Using ODE to understand circuit dynamics



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

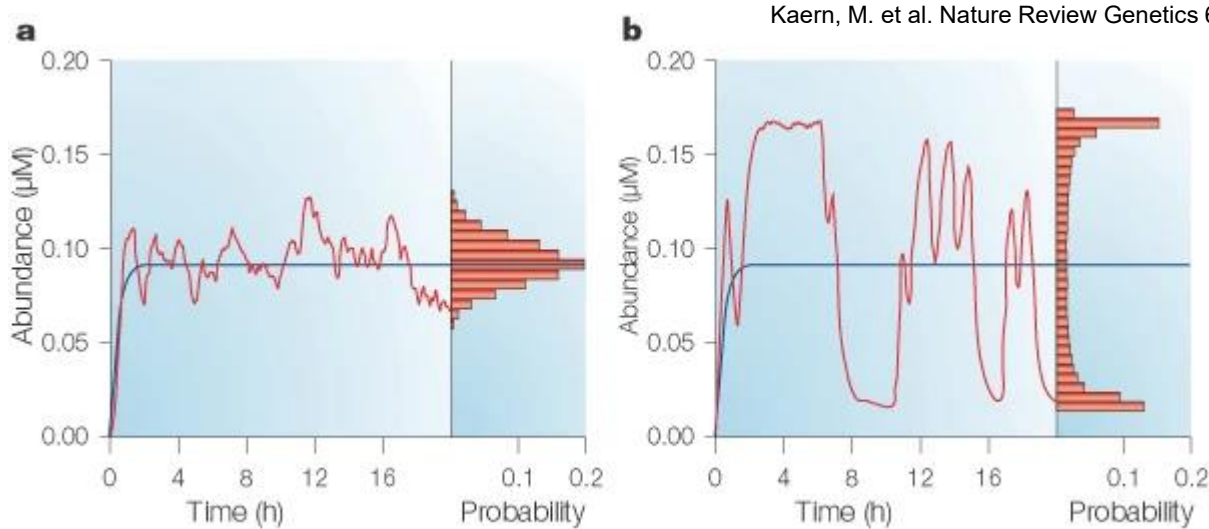
$$\frac{dx_1}{dt} = \frac{\beta}{1 + (x_3/k)^n} - \gamma x_1,$$

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

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

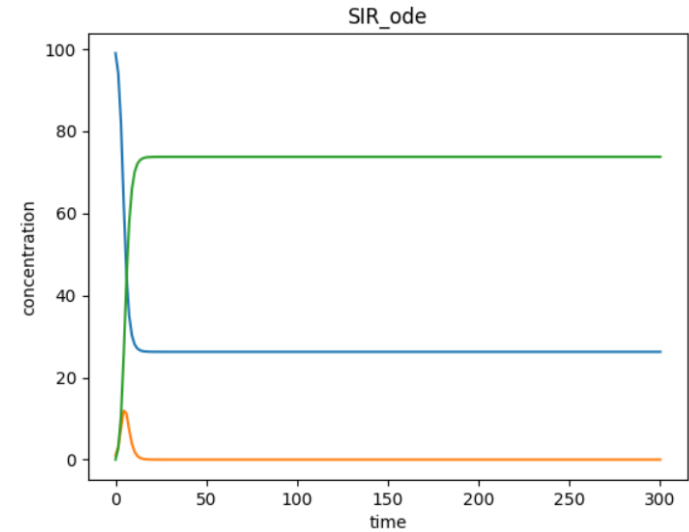
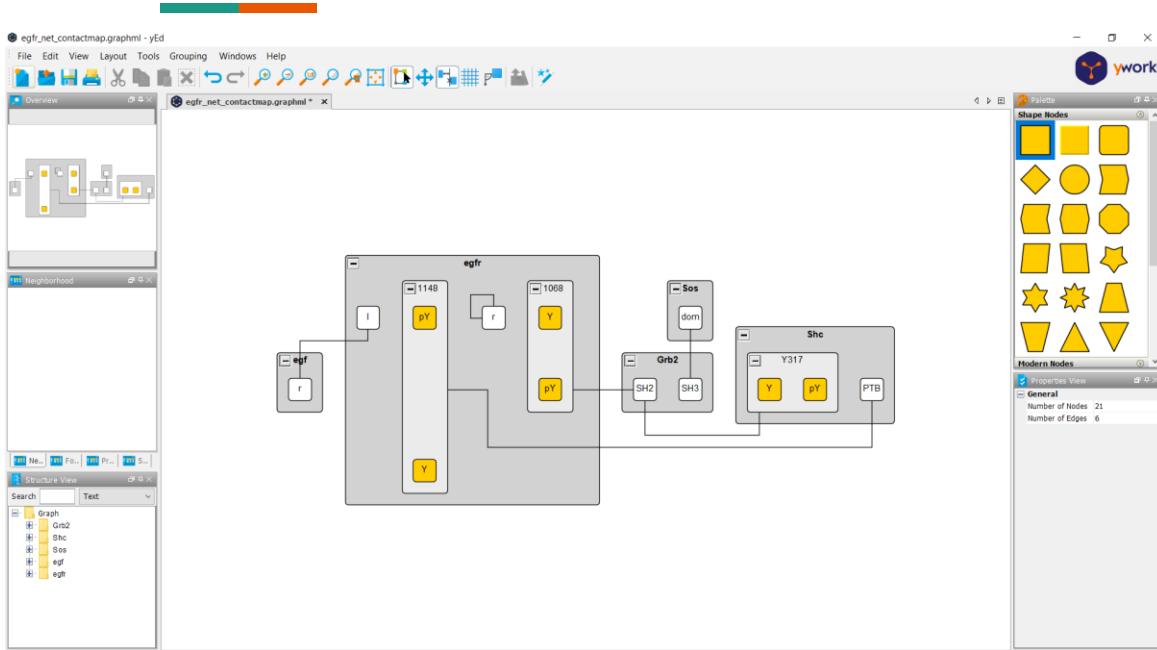
- Can optimize the repression strengths to control the period length and maximum expression level

# 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



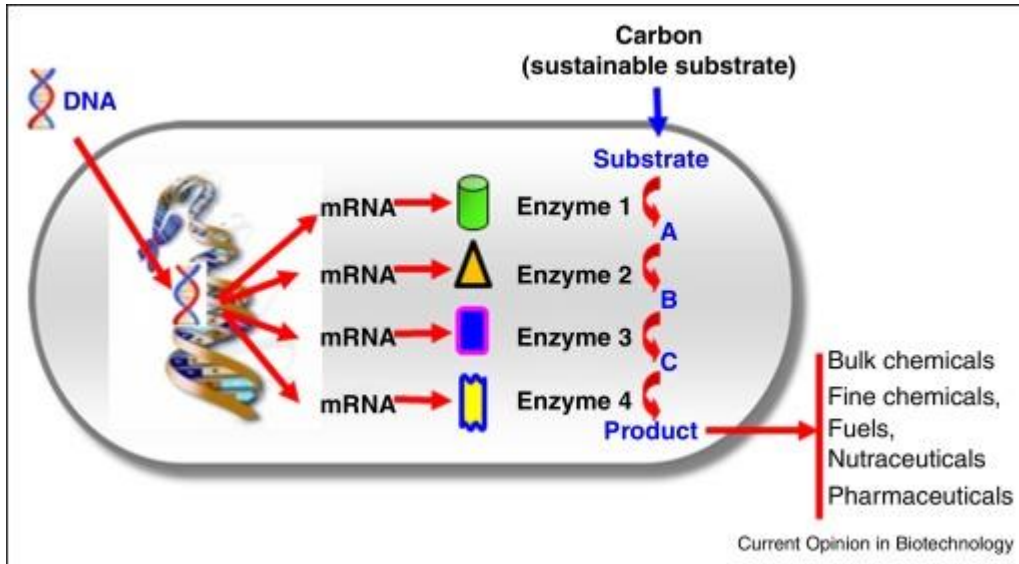
<https://bionetgen.org/>

- Tools for designing and simulating biochemical reaction circuits



# Metabolic engineering

# Metabolite as system's endpoints

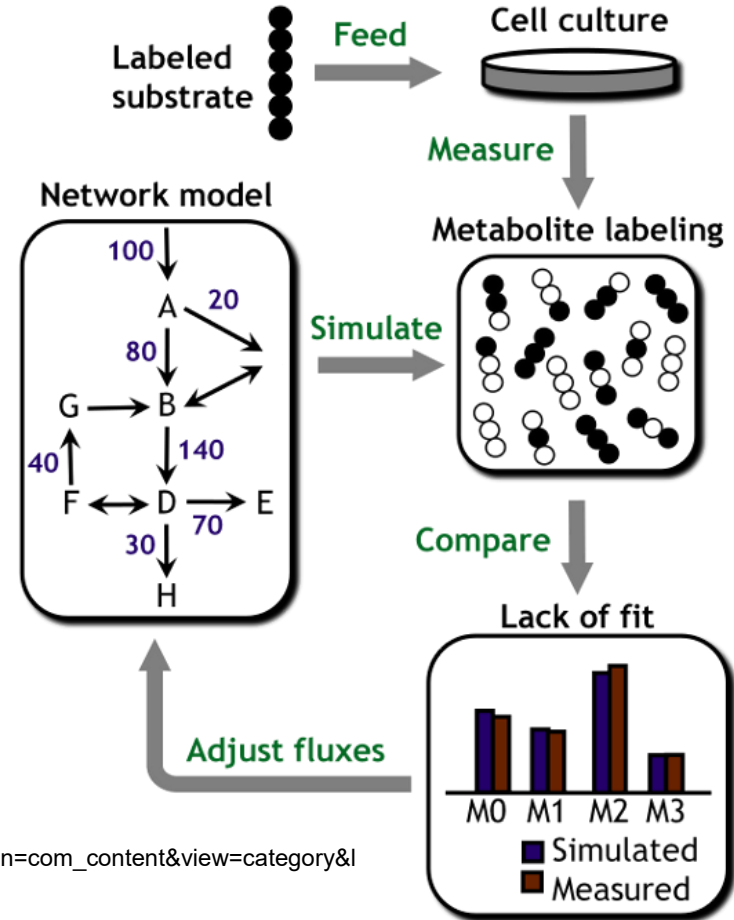


Zhu, Q. and Jackson, E.N. Current Opinion in Biotechnology 36:65-72 (2015)

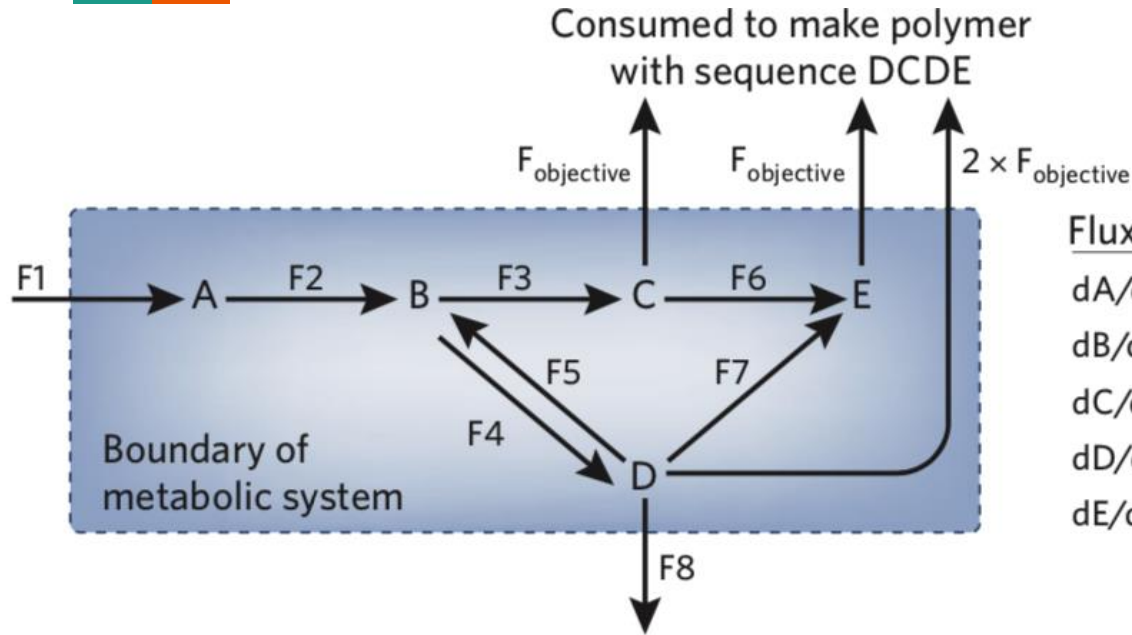
- In the context of cells as biological factories, metabolites are key endpoint products
- Processing of metabolites involve many proteins and intermediate products
- Can we identify the equilibrium of the cell?

# 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



- 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

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



# Recon3D: database of human metabolism network

**VIRTUAL METABOLIC HUMAN**

Home Browse Map navigator Download Help Quick search Search

Human

Search all enter search term Search

13543 reactions found

Abbreviation	Description	Formula
<a href="#">10FTHF5GLU[]</a>	5-Glutamyl-10Fthf Transport, Lysosomal	10thf5glu[c] -> 10thf5glu[]
<a href="#">10FTHF5GLU[m]</a>	5-Glutamyl-10Fthf Transport, Mitochondrial	10thf5glu[m] -> 10thf5glu[c]
<a href="#">10FTHF6GLU[]</a>	6-Glutamyl-10Fthf Transport, Lysosomal	10thf6glu[c] -> 10thf6glu[]
<a href="#">10FTHF6GLU[m]</a>	6-Glutamyl-10Fthf Transport, Mitochondrial	10thf6glu[m] -> 10thf6glu[c]
<a href="#">10FTHF7GLU[]</a>	7-Glutamyl-10Fthf Transport, Lysosomal	10thf7glu[c] -> 10thf7glu[]
<a href="#">10FTHF7GLU[m]</a>	7-Glutamyl-10Fthf Transport, Mitochondrial	10thf7glu[m] -> 10thf7glu[c]
<a href="#">10FTHF[]</a>	10-Formyltetrahydrofolate Lysosomal Transport via Diffusion	10thf[c] <=> 10thf[]

<< < Page 1 of 271 > >> Displaying 1 - 50 of 13543 Change page size OK Download

4138 metabolites found

Abbreviation	Name	Charged Formula
<a href="#">10thf</a>	10-Formyltetrahydrofolate	C20H21N7O7
<a href="#">10thf5glu</a>	10-Formyltetrahydrofolate-[Glu](5)	C40H45N11O19
<a href="#">10thf6glu</a>	10-Formyltetrahydrofolate-[Glu](6)	C45H51N12O22
<a href="#">10thf7glu</a>	10-Formyltetrahydrofolate-[Glu](7)	C50H57N13O25
<a href="#">11_cis_ret[]</a>	Fatty Acid 11-Cis-Retinol	C20H29OFULLR2CO
<a href="#">11docrt[]</a>	Cortexolone	C21H30O4
<a href="#">11docrt[m]</a>	Deoxycorticosterone	C21H30O3

Curated and predicted  
metabolic reactions and  
stoichiometry

# Integration of omics data in metabolic flux analysis



- 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



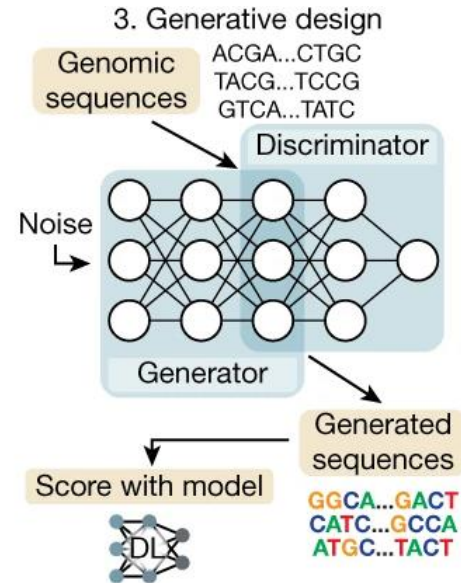
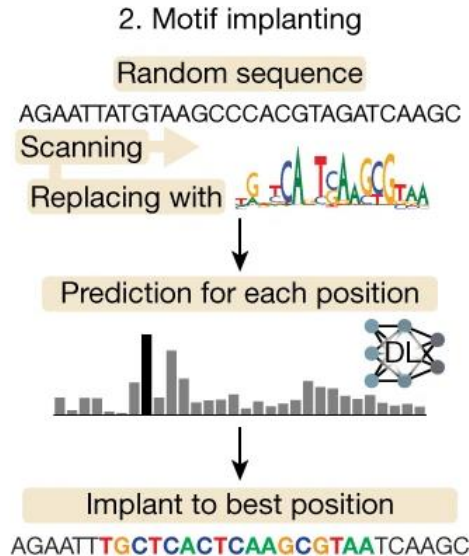
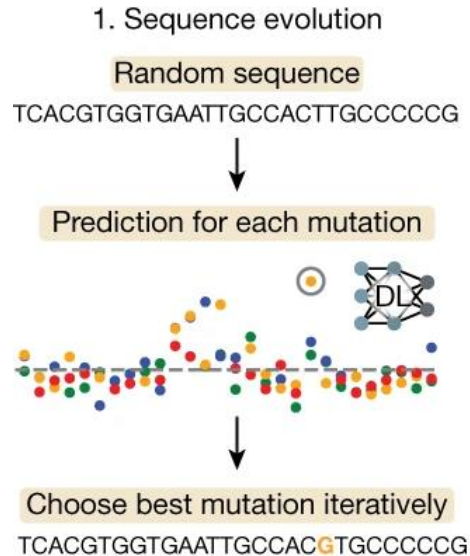
# Computation in synthetic biology

# Genome design



- 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

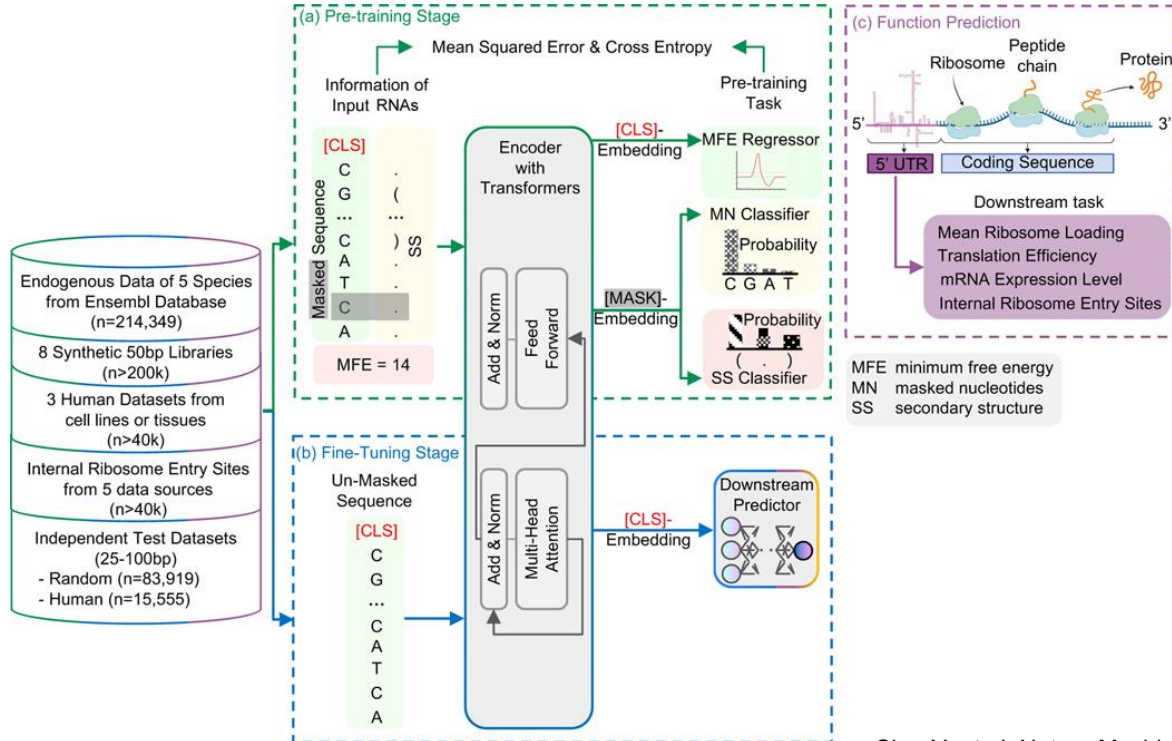
# Computer-aided design of DNA



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

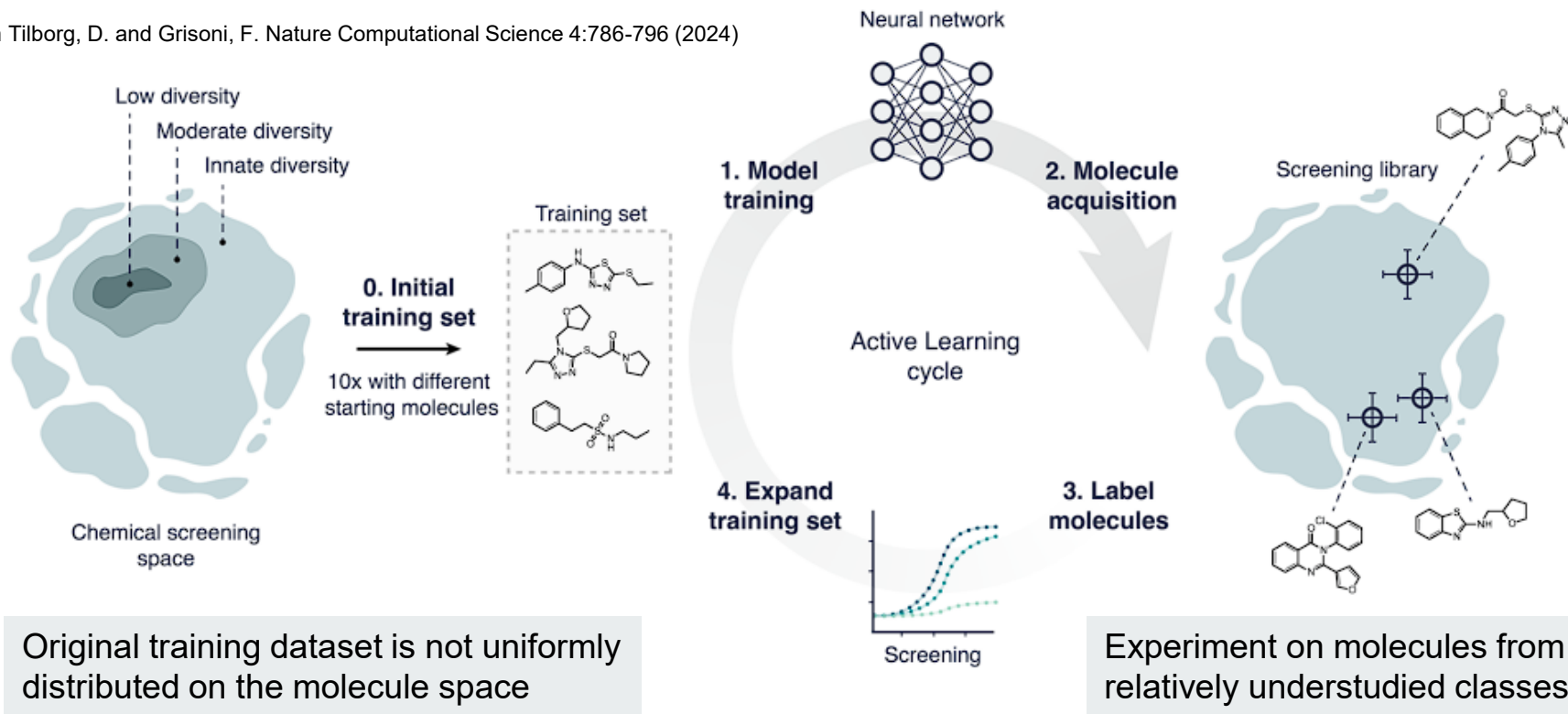
# Data-driven design-build-test-learn cycle



- 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

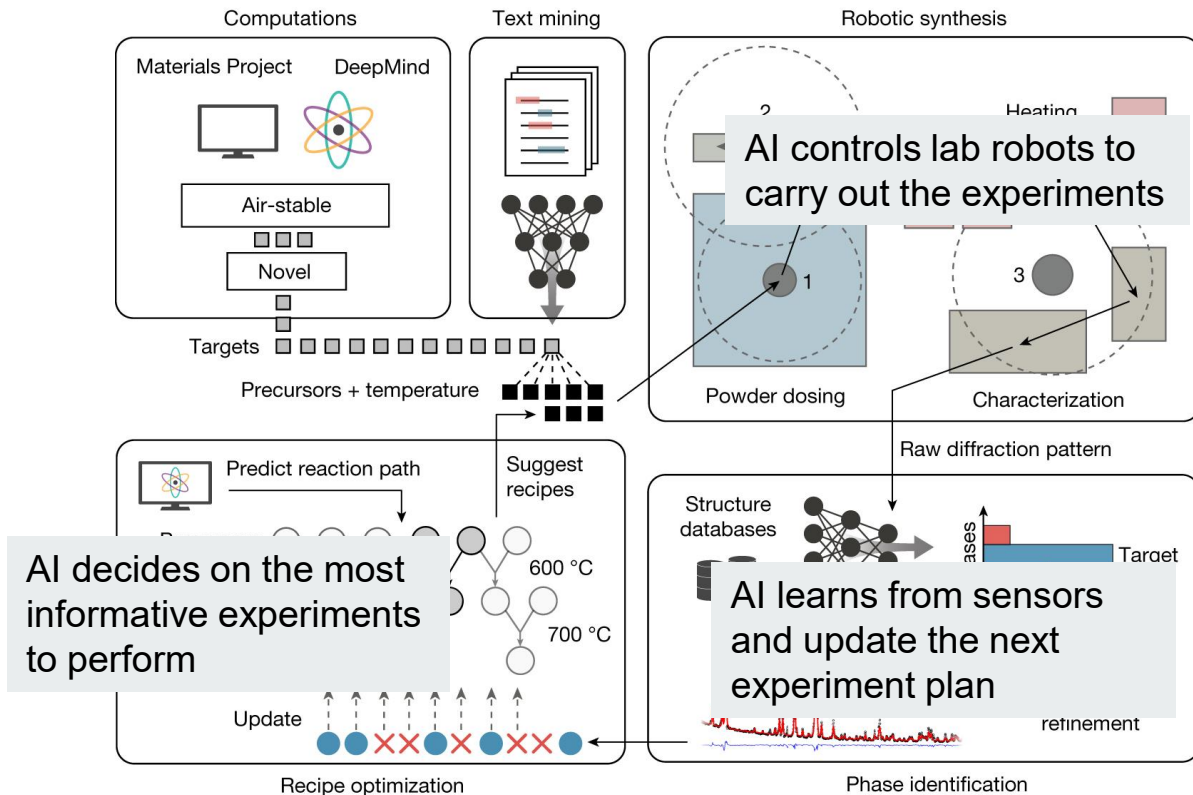
# Active learning approach for molecular design

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



# Automated experiments

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



# Summary



- 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?



- See you next time