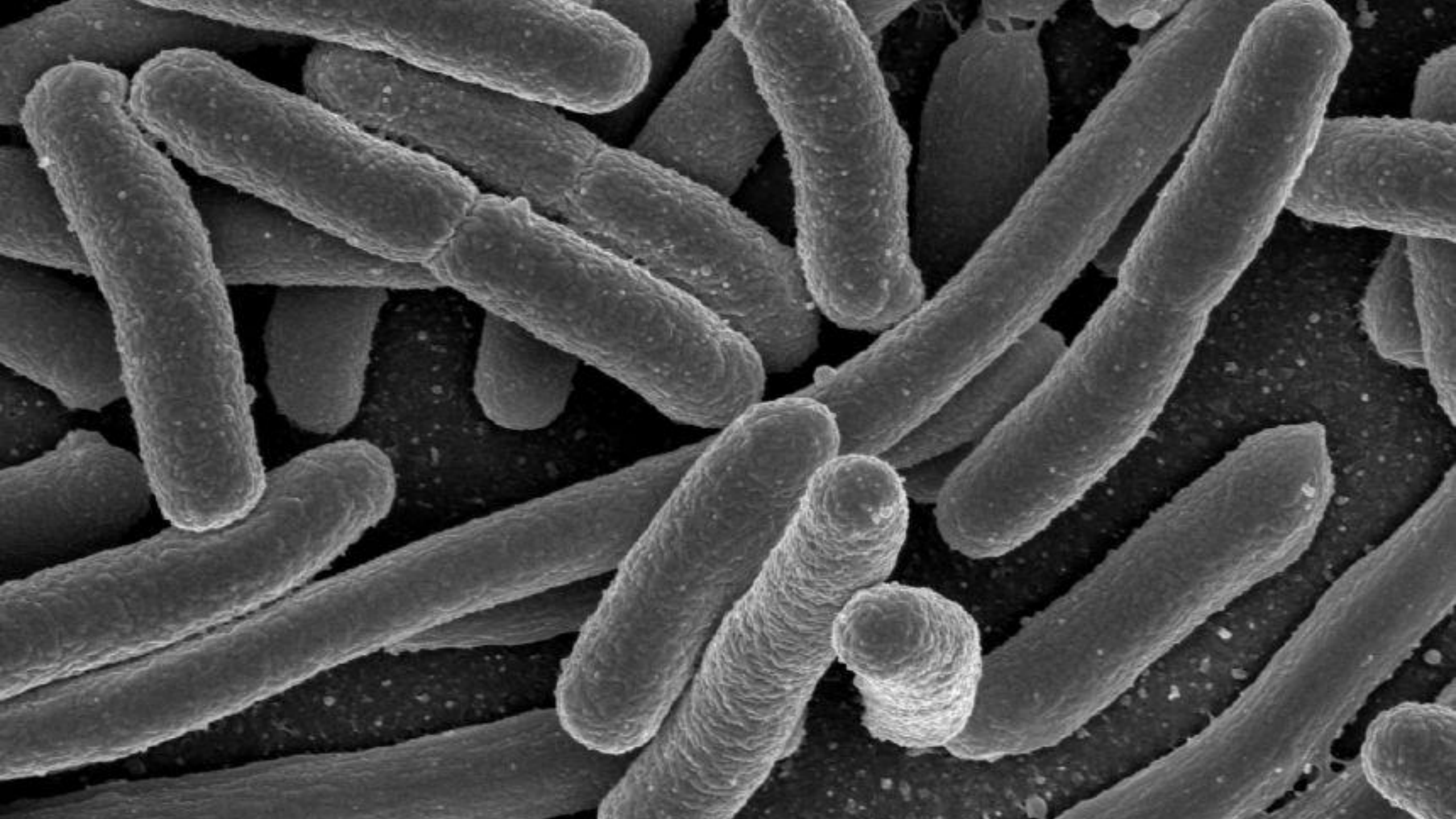
Lab on Cell-free Synthetic Biology - EE-490(j)

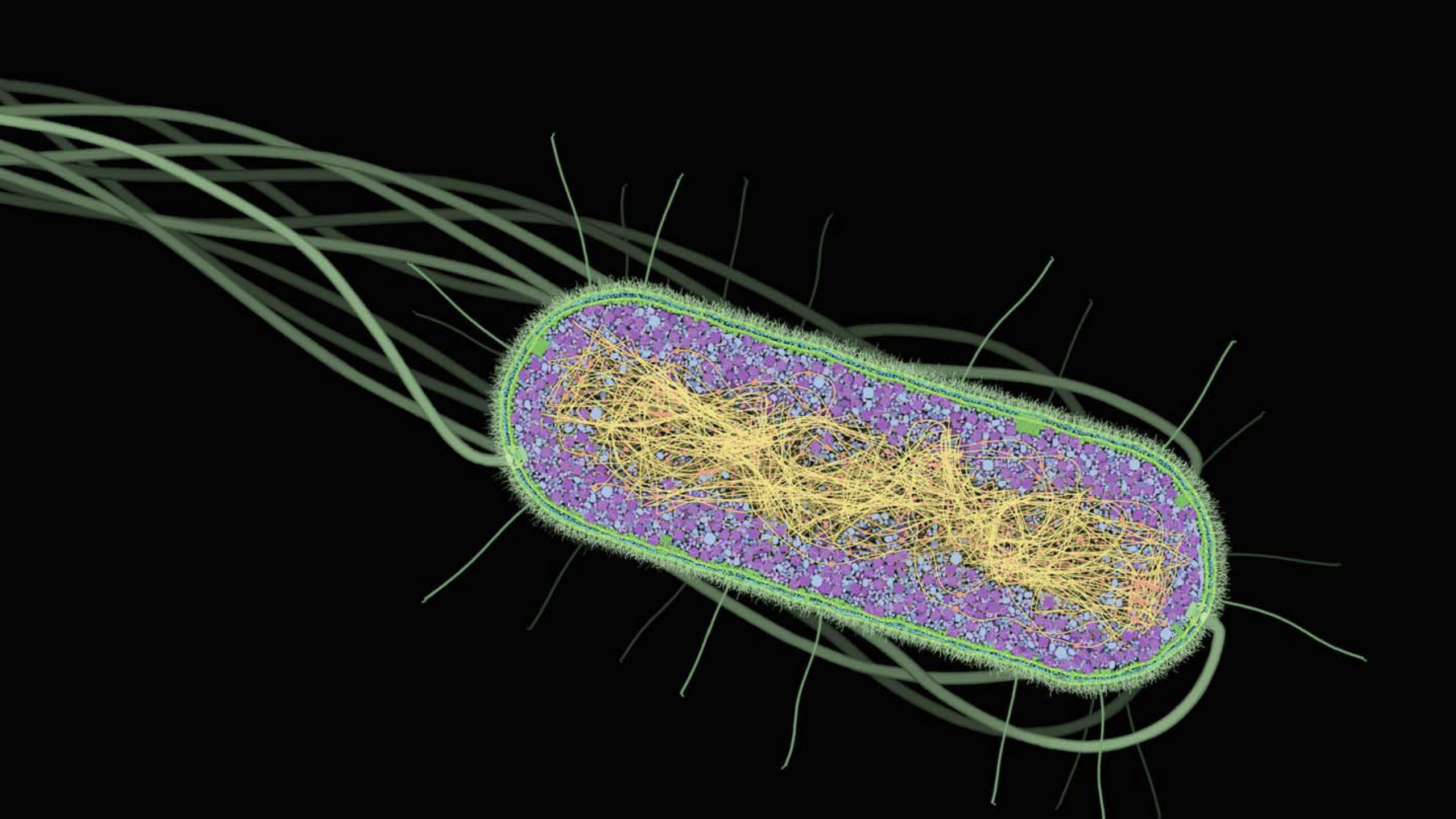
Prof. Sebastian Maerkl

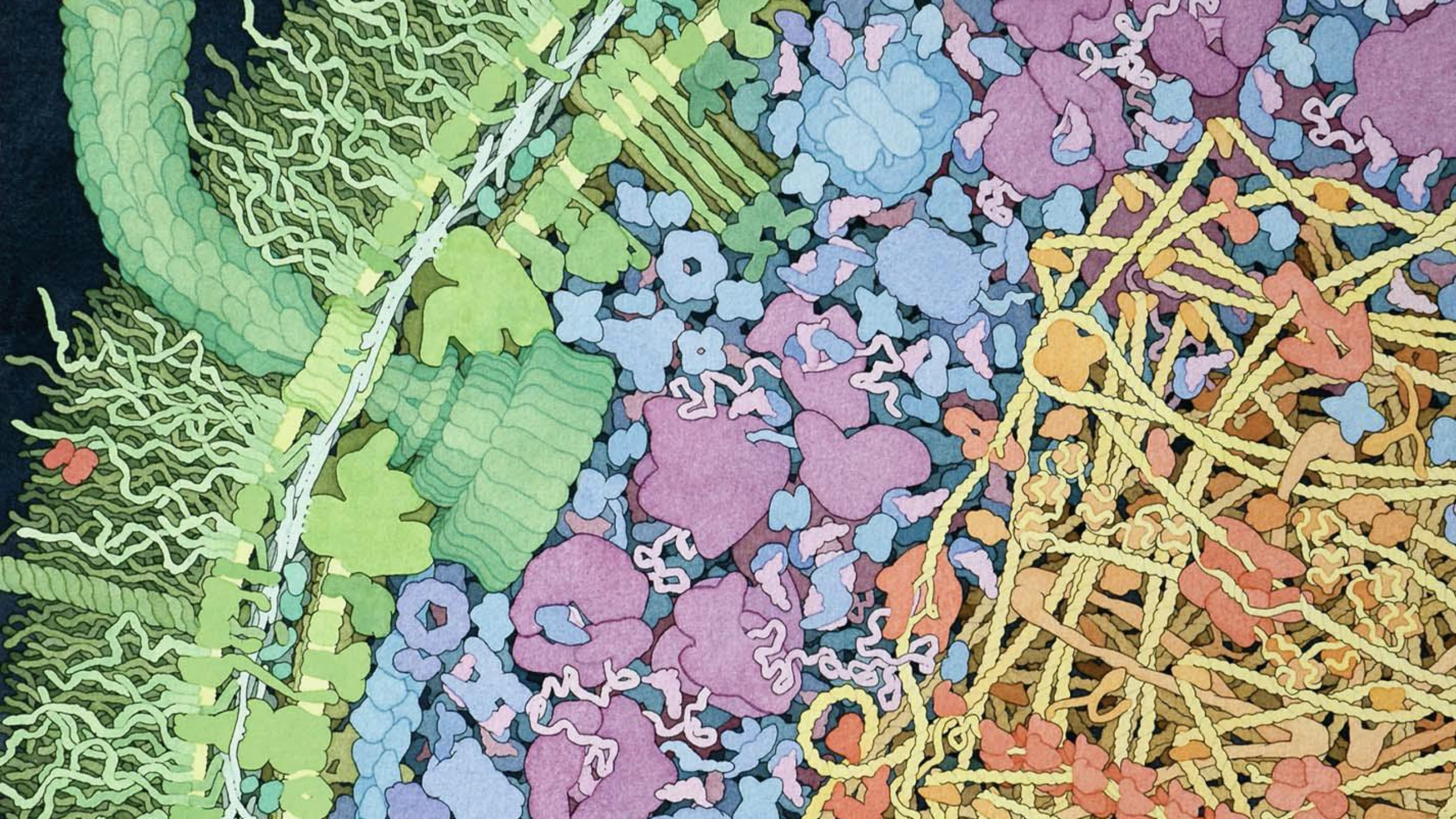
09.09.2025

Course content

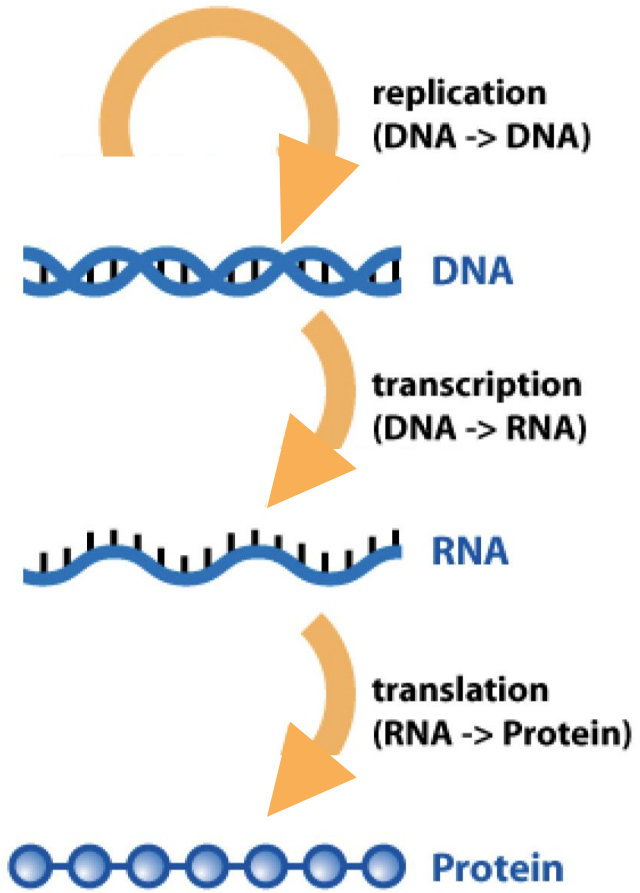
- Basic molecular biology experiments (Week 2-3)
  - Plasmid transformation
  - Protein expression
  - PCR
- Preparation of cell-free protein expression components
  - Onepot PURE purification (Week 4-6)
  - Ribosome production (Week 7-8)
  - PURE reaction (Week 9)
- Project (Week 10-12)





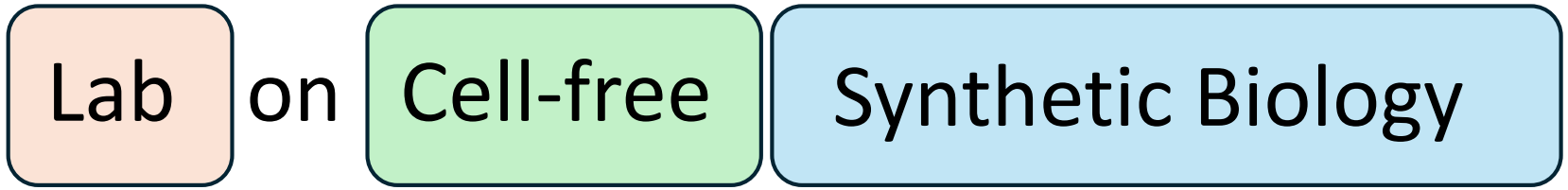


Central dogama



DNA Learning Center

CSH Cold Spring Harbor Laboratory

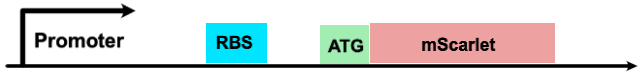

Lab
on
Cell-free
Synthetic Biology

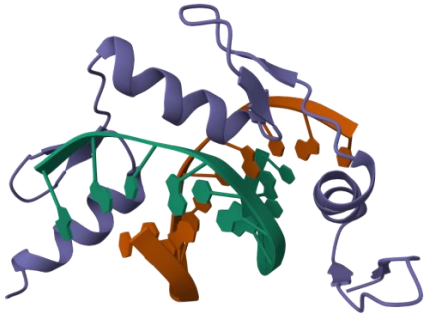
Synthetic Biology

Synthetic biology is an interdisciplinary field that applies engineering principles to design and construct new biological parts, devices, and systems, or redesign existing ones, for useful purposes.

Synthetic Biology

Parts





Systems

NAND

A	B	Output
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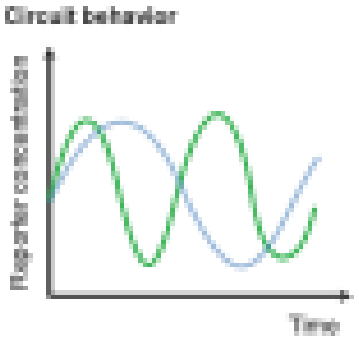
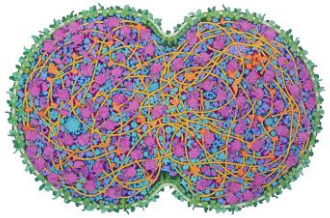
1	0	1
0	1	1
1	1	0

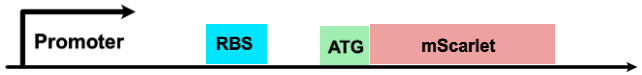


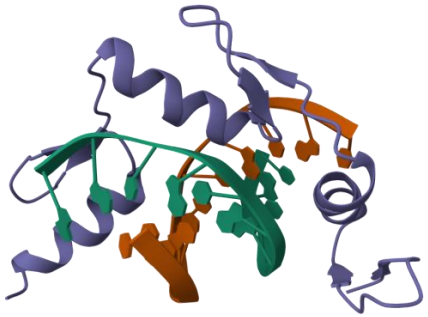
Organism



Synthetic Biology

Parts





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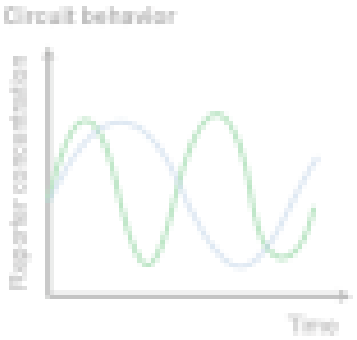

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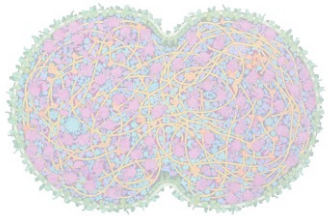

Design a DNA template to express protein

TAATACGACTCACTAT

AGGAGG

Design a DNA template to express protein

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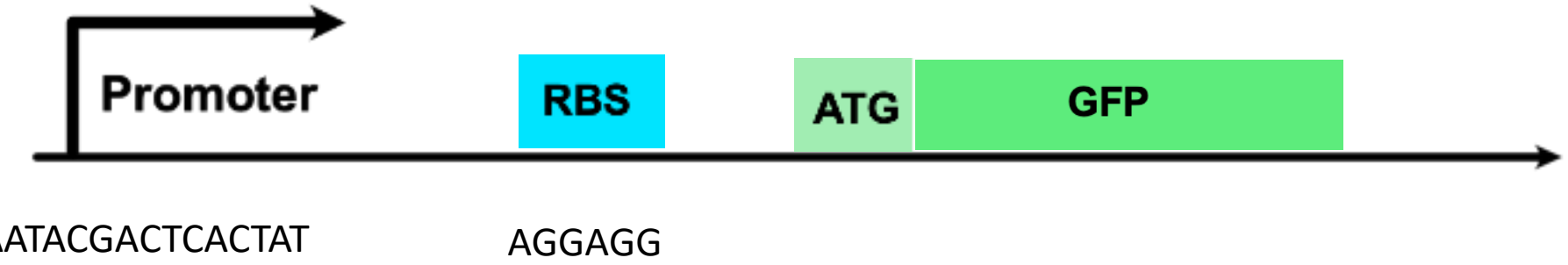
Design a DNA template to express protein



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Design a DNA template to express protein

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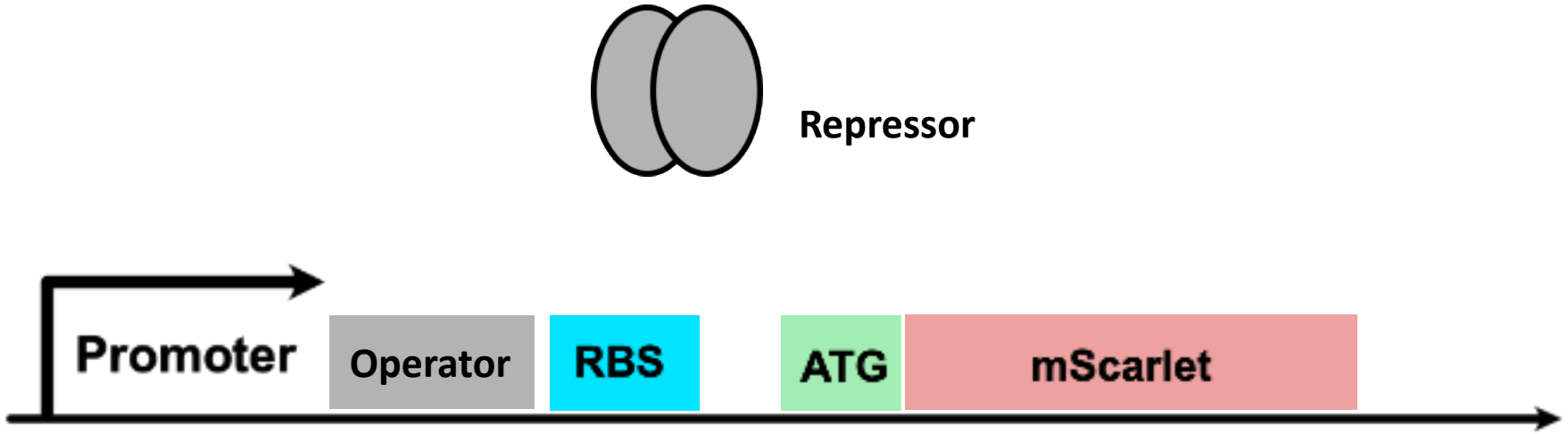
Regulate protein expression



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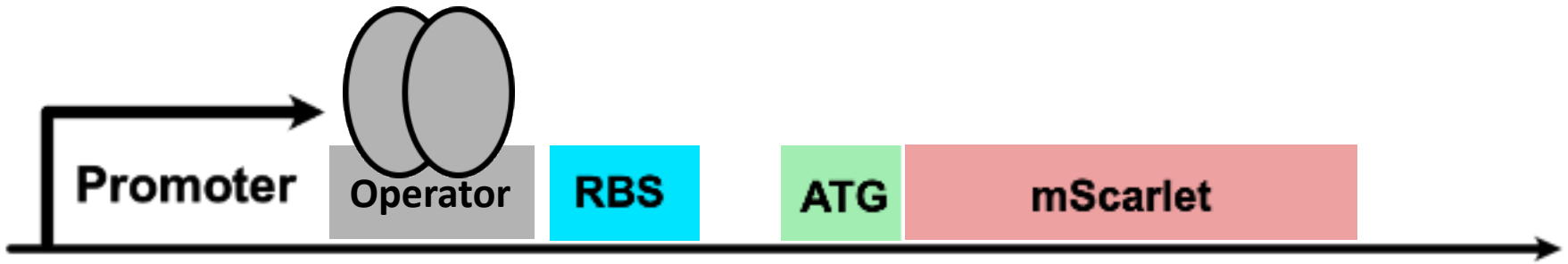
Regulate protein expression



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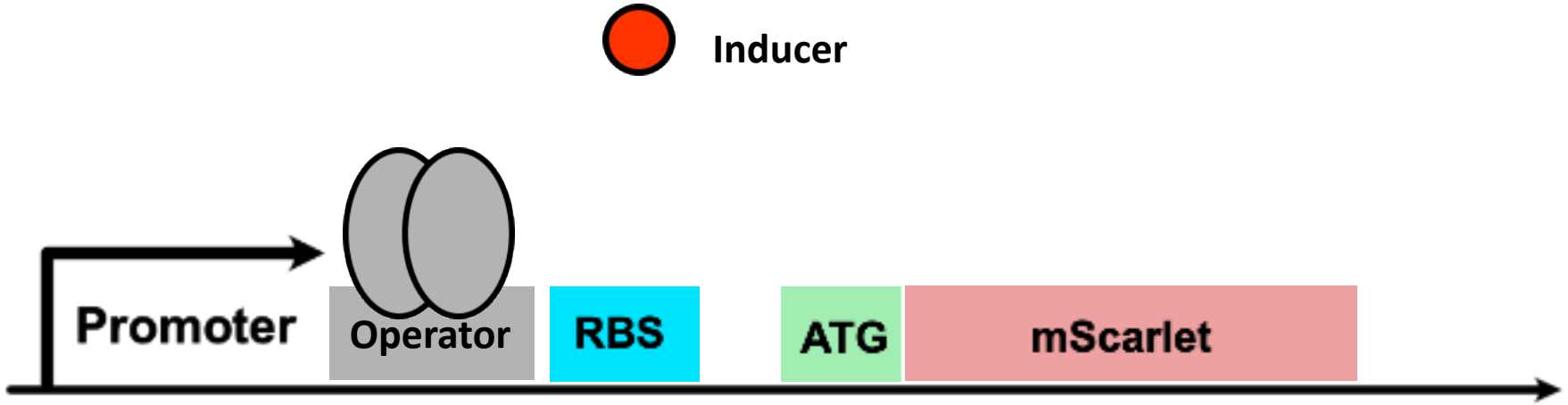
Regulate protein expression



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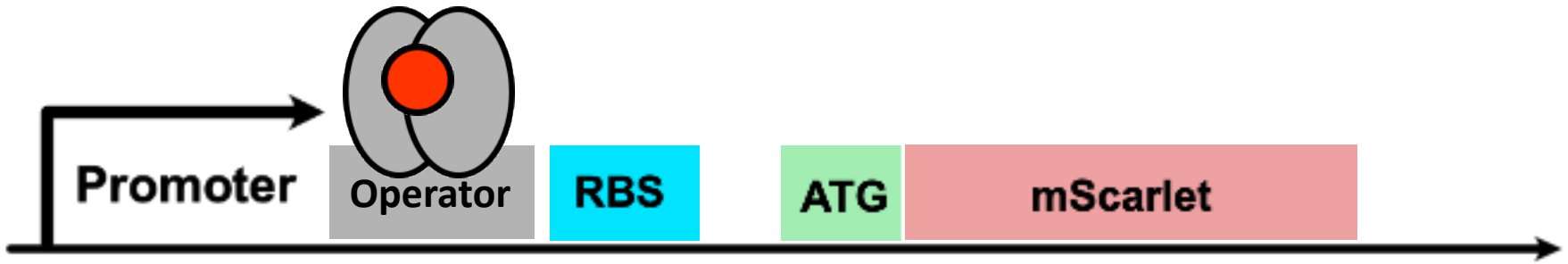

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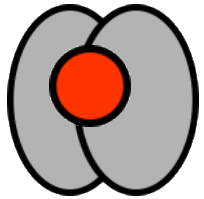
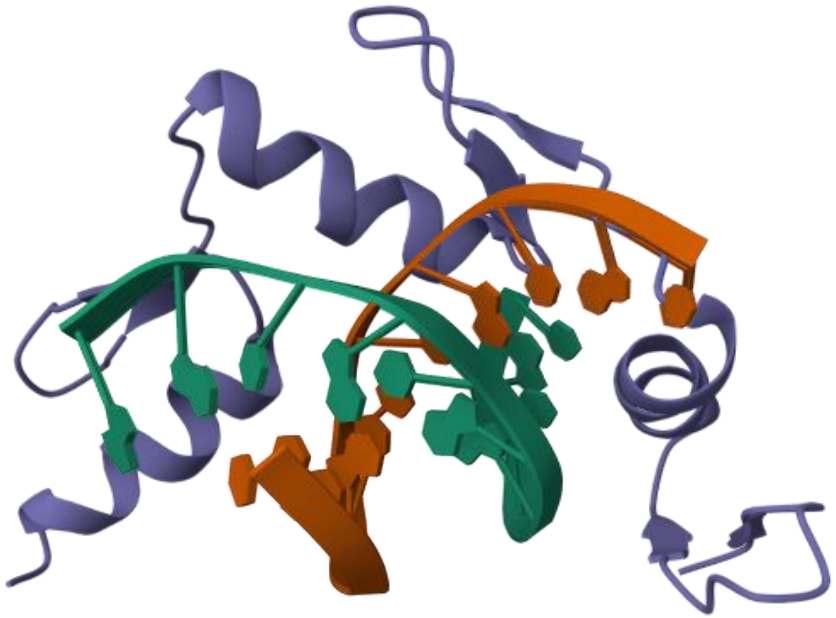


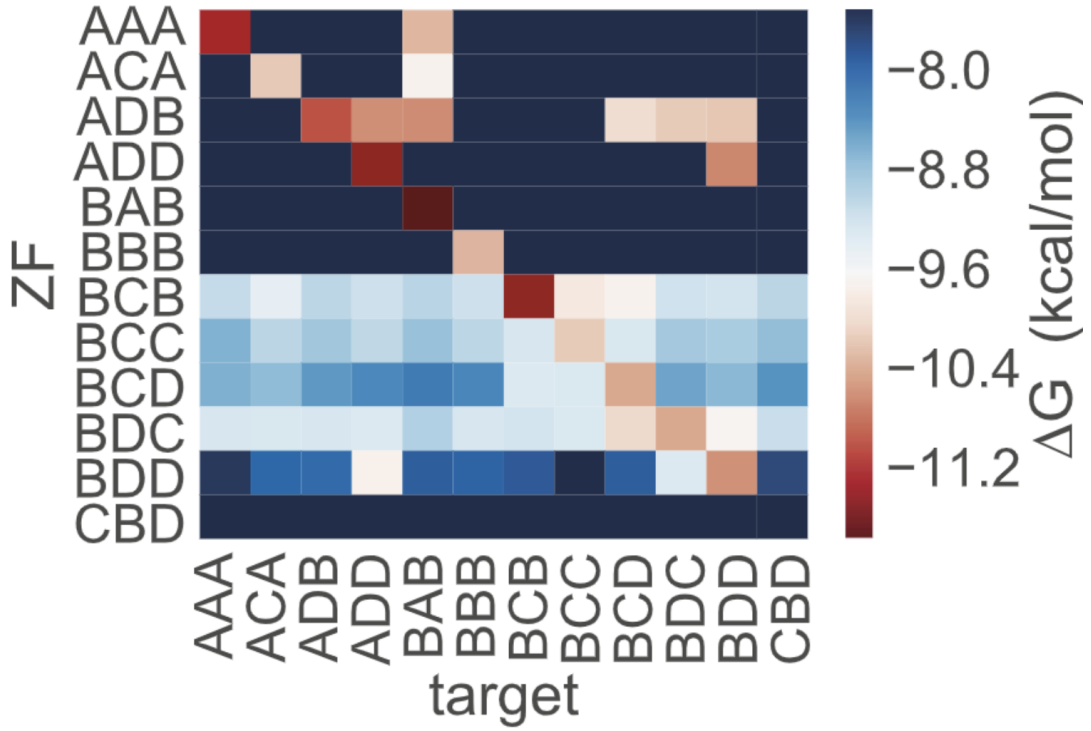
Repressor : LacI
Inducer : IPTG

TAATACGACTCACTAT

AGGAGG

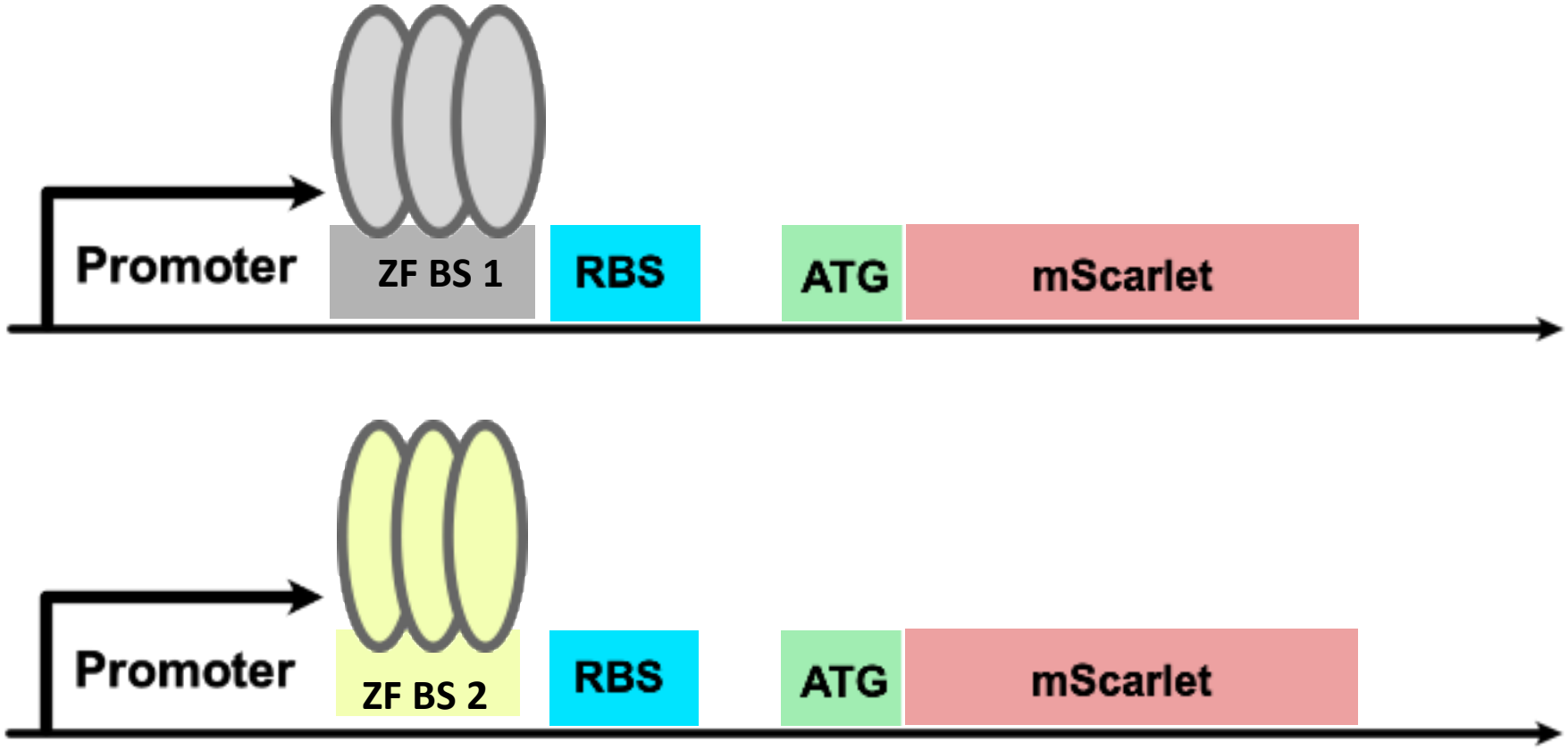
Zinc Finger protein





Swank, Z., Laohakunakorn, N., & Maerkl, S. J. (2019). Cell-free gene-regulatory network engineering with synthetic transcription factors. *Proceedings of the National Academy of Sciences*, *116*(13), 5892-5901.

Application of Zinc finger – Programmable repressor



Application of Zinc finger – Programmable repressor

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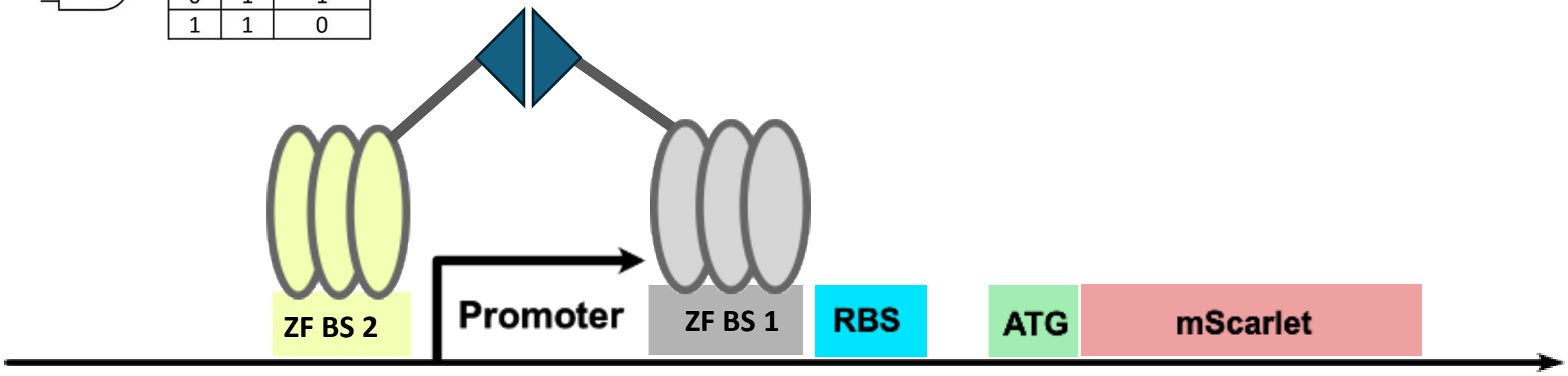

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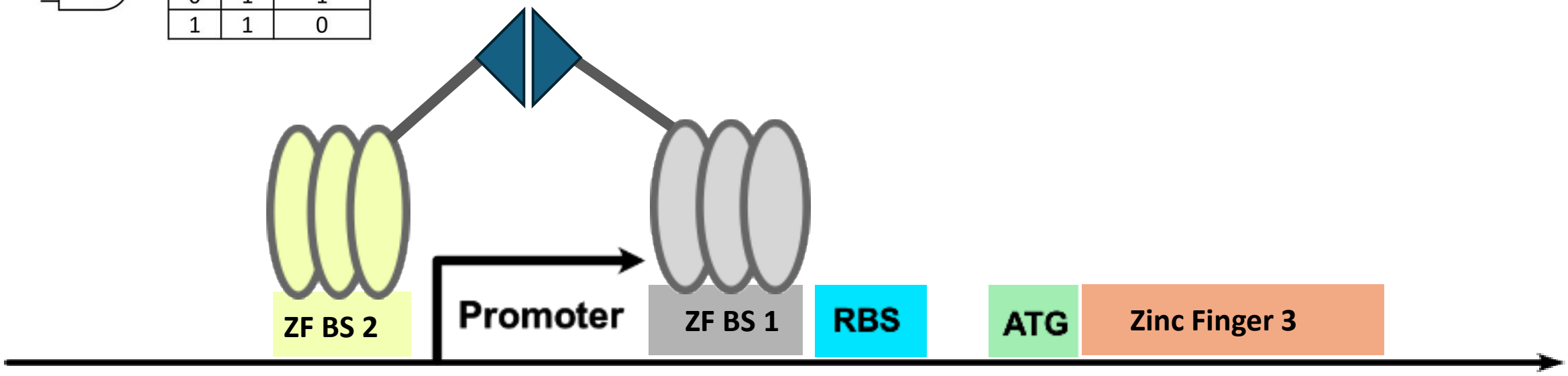
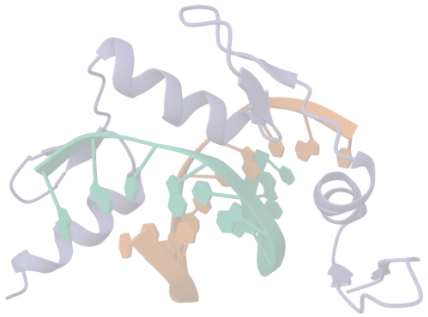


Synthetic Biology

Parts



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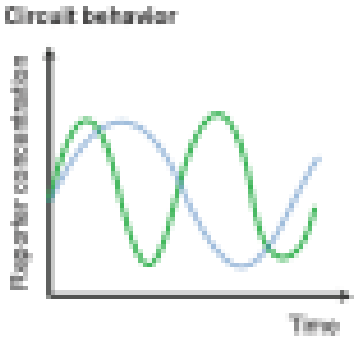

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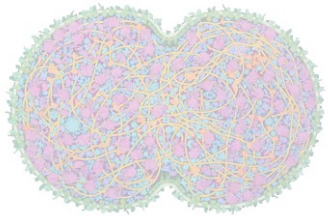
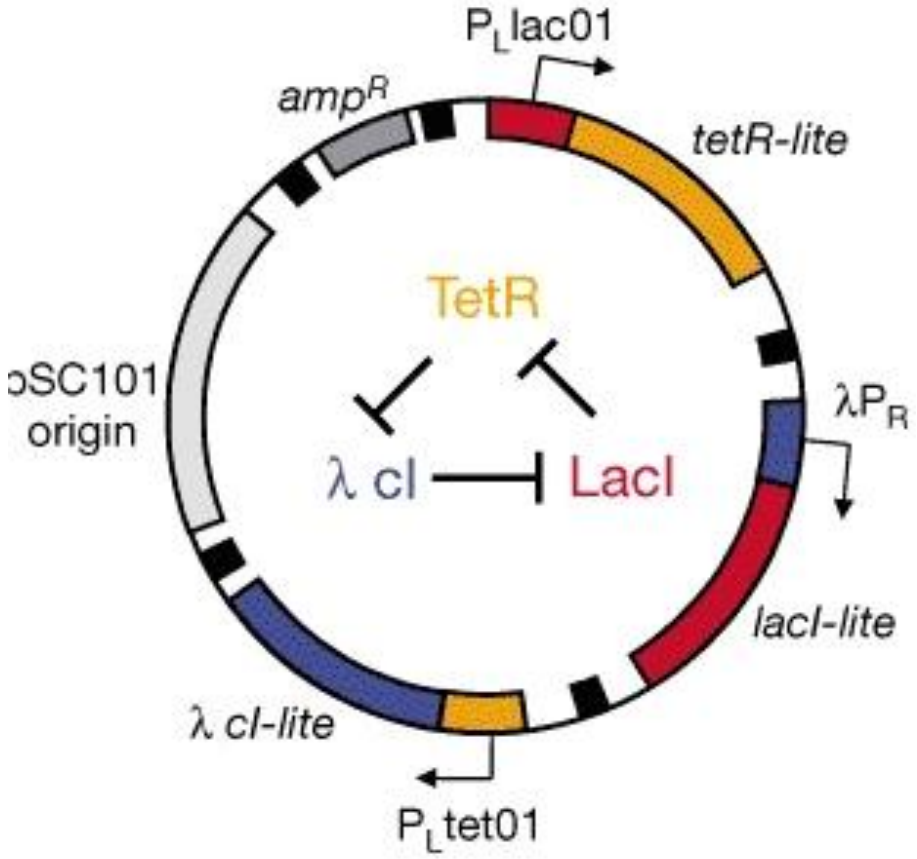
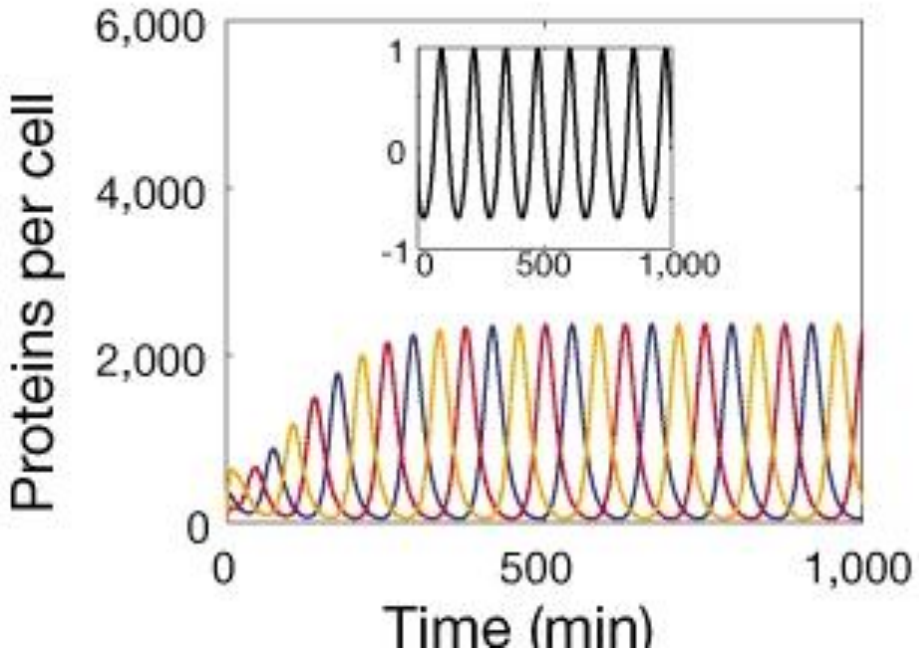
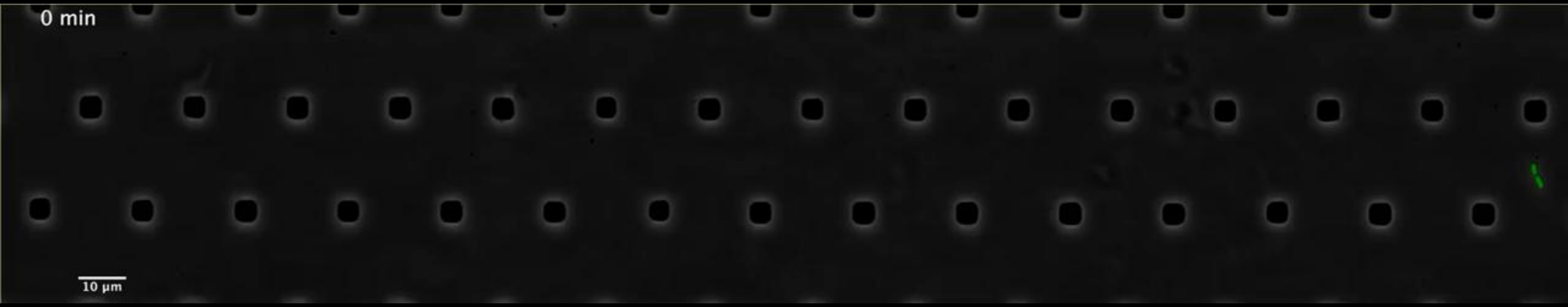


Repressilator

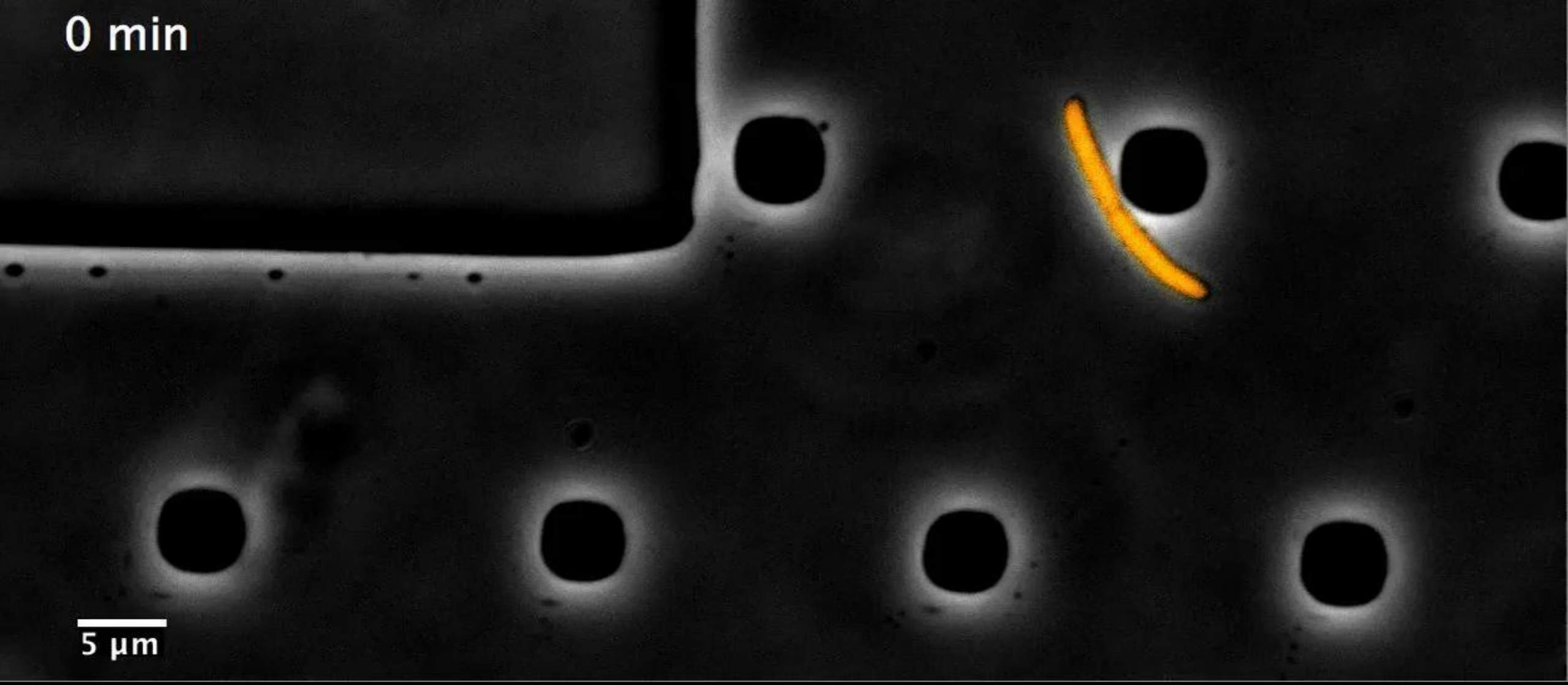




Elowitz, M. B., & Leibler, S. (2000). A synthetic oscillatory network of transcriptional regulators. *Nature*, *403*(6767), 335-338.



Niederholtmeyer, H., Sun, Z. Z., Hori, Y., Yeung, E., Verpoorte, A., Murray, R. M., & Maerkl, S. J. (2015). Rapid cell-free forward engineering of novel genetic ring oscillators. *elife*, *4*, e09771.



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Synthetic Biology

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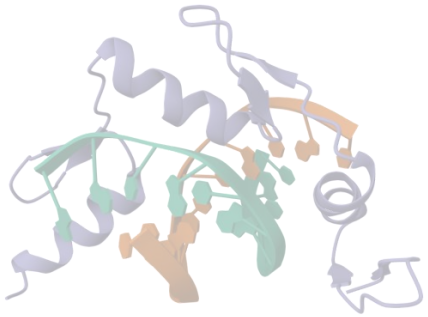

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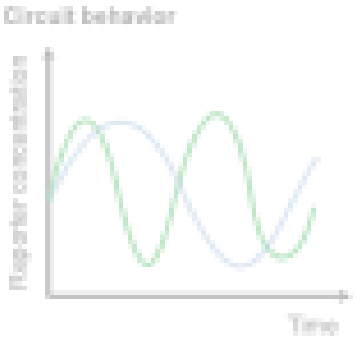

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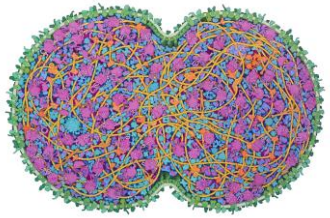
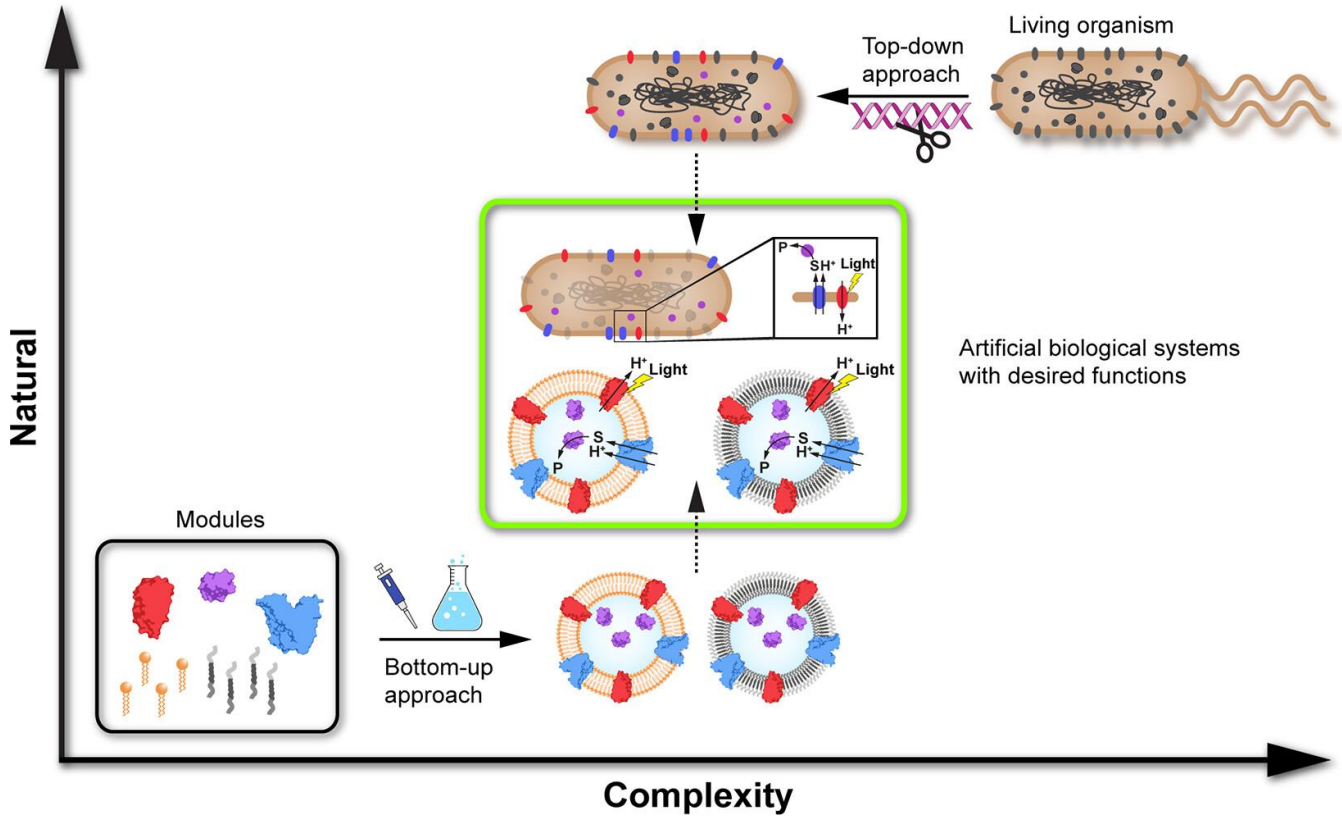
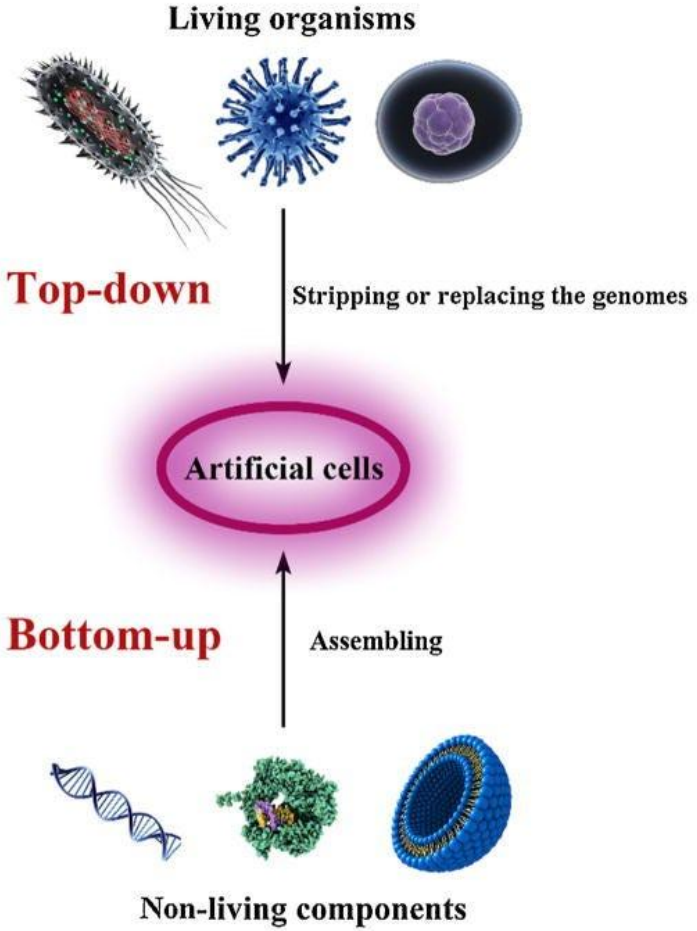


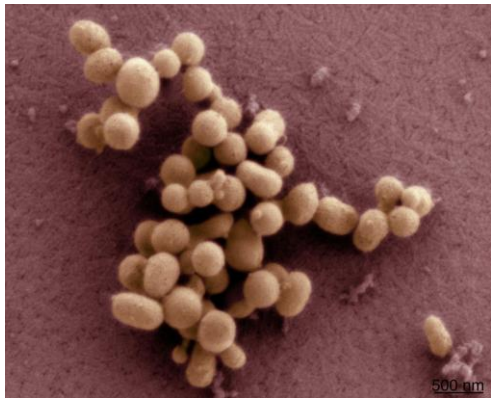
Bottom-up vs. top-down approach



Hirschi, Chem. Rev., 2022

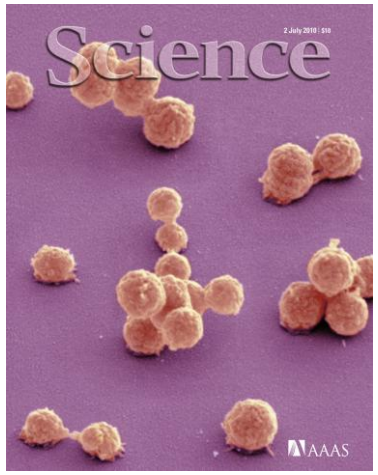


Minimal synthetic bacterial cell



Mycoplasma mycoides

~1000 genes



2010

JCVI-syn1.0
(synthetic genome)

2016

JCVI-syn3.0

473 genes
smallest genome of any self-replicating organism

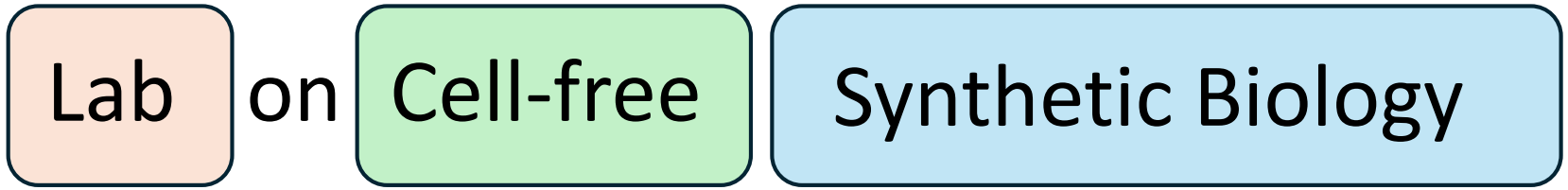
Hutchison III, Clyde A., et al. "Design and synthesis of a minimal bacterial genome." *Science* 351.6280 (2016): aad6253.

Can we build a cell from bottom-up approach?

PURE

Protein synthesis Using Recombinant Elements

Proteins the can make proteins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Cell-free Protein Synthesis (CFPS)

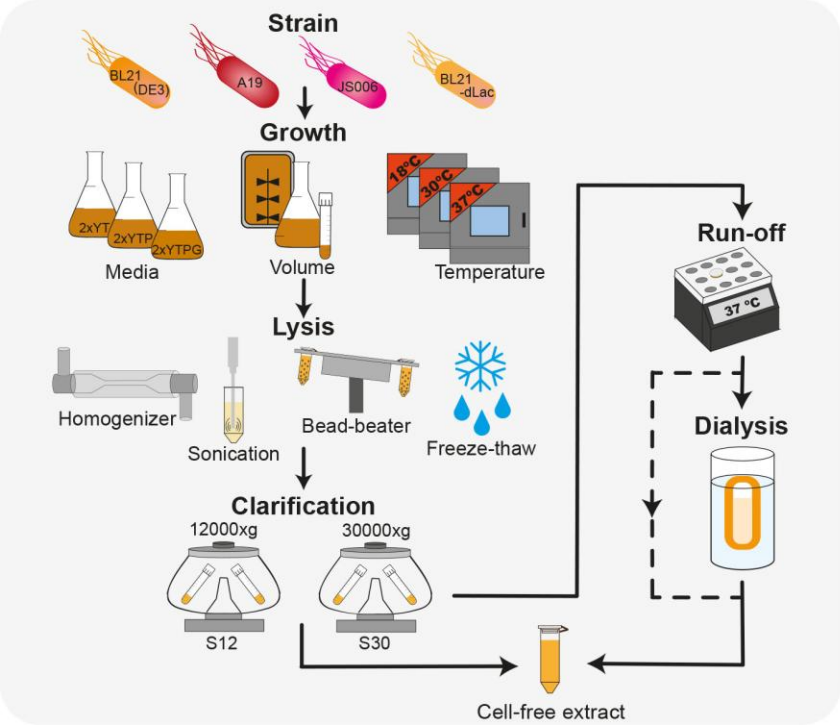
- Also referred to as:
  - Cell-free gene expression (CFE)
  - *In vitro* transcription and translation (IVTT or TX-TL)

- Synthesis protein without living cells
- Take the cellular transcription/translation out of the cell

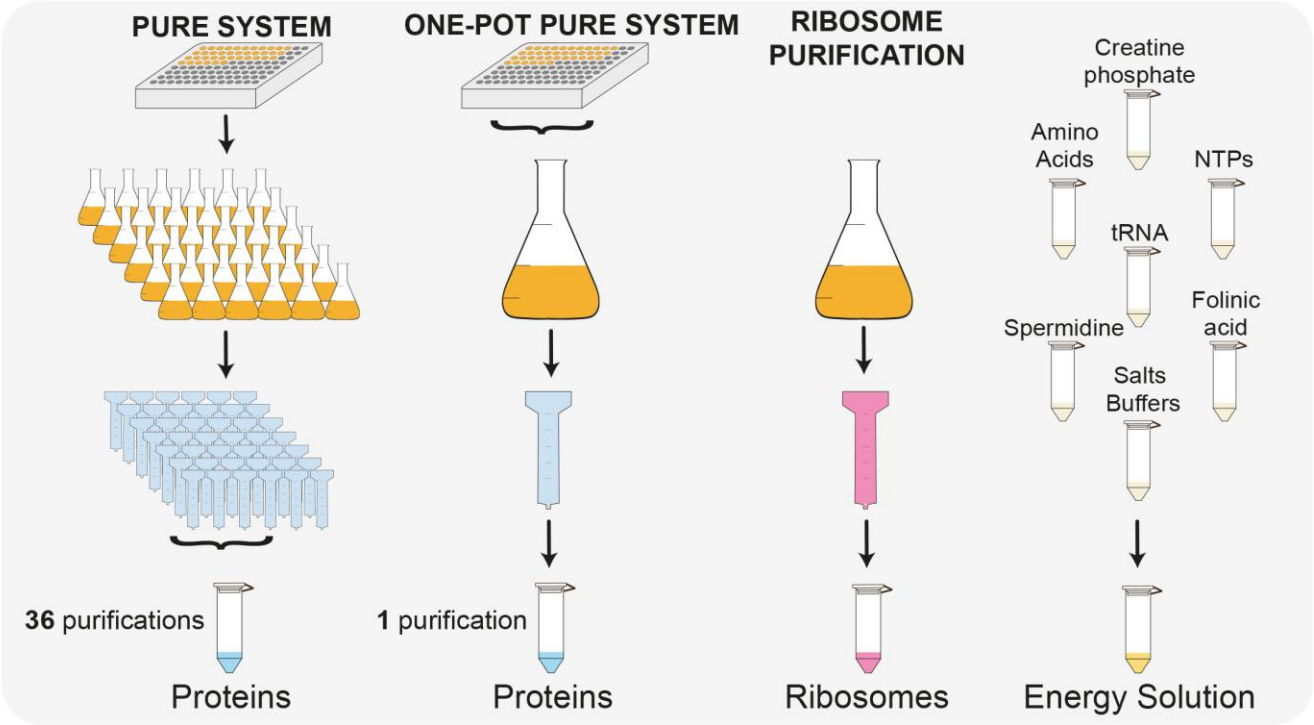
- PURE
  - A reconstituted cell-free transcription-translation platform

Lysate vs PURE

Lysate



PURE



adapted from: Laohakunakorn et al., Frontiers in Bioengineering and Biotechnology, 2020

Lysate vs. PURE

Lysate

- High yield
- Cost-effective
- Quick setup
- Many different systems available
- Post-translational modifications (eukaryotic lysates)
- Scalable

→ Black box

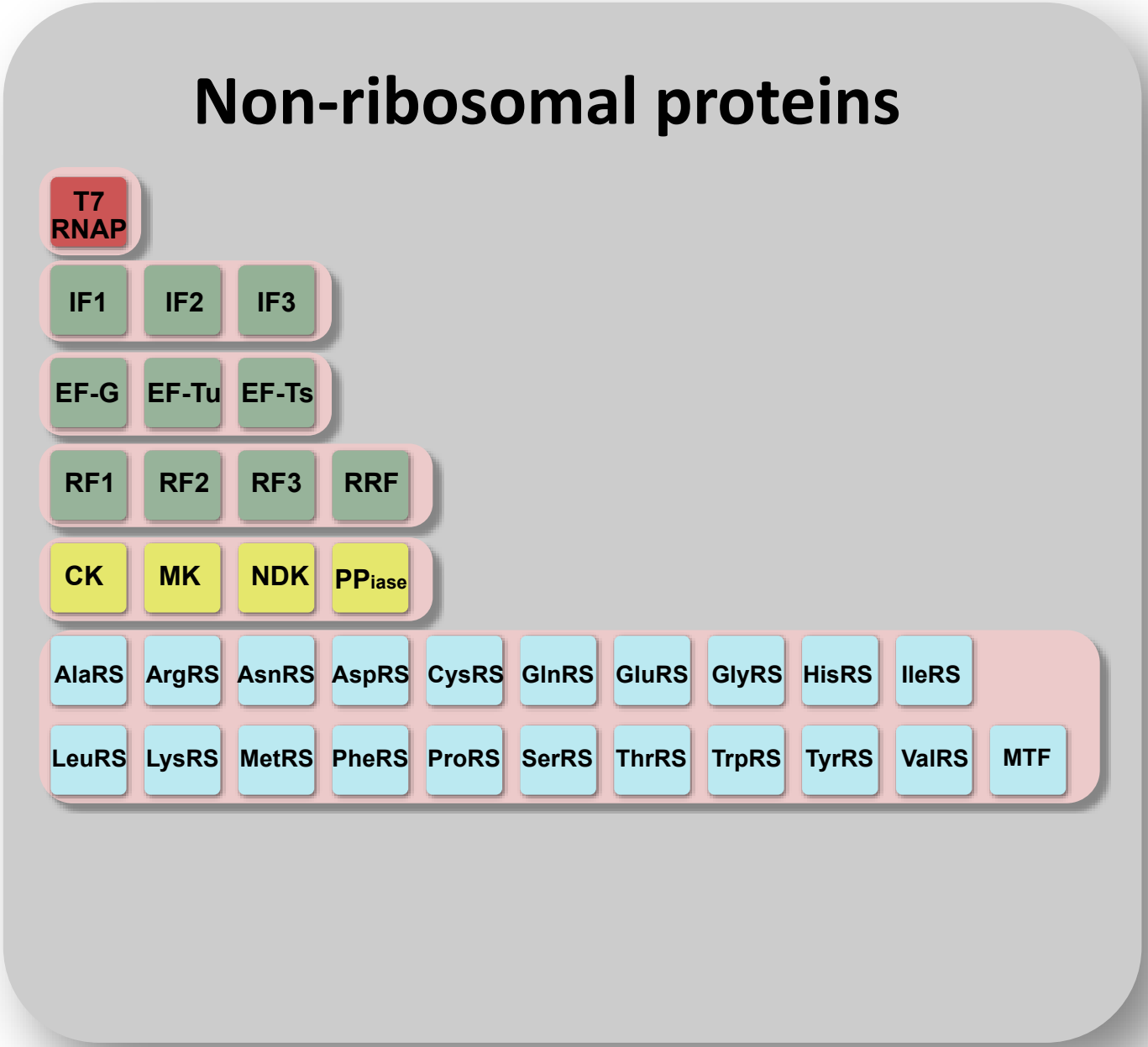
PURE

- Only *E. coli*-based
- Low background activity (endogenous nucleases or proteases)
- Minimal cross-reactions (less interference from host proteins or metabolic pathways)
- Ideal for synthetic biology
- Customizability
- Well-defined and comparably well understood

→ Less of a black box

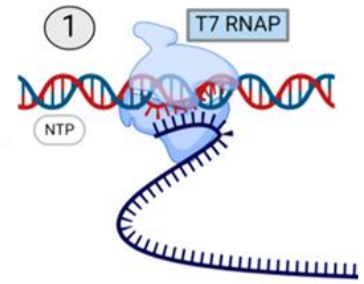
PURE

- 36 non-ribosomal proteins
- Ribosomes
- tRNAs
- Small molecules



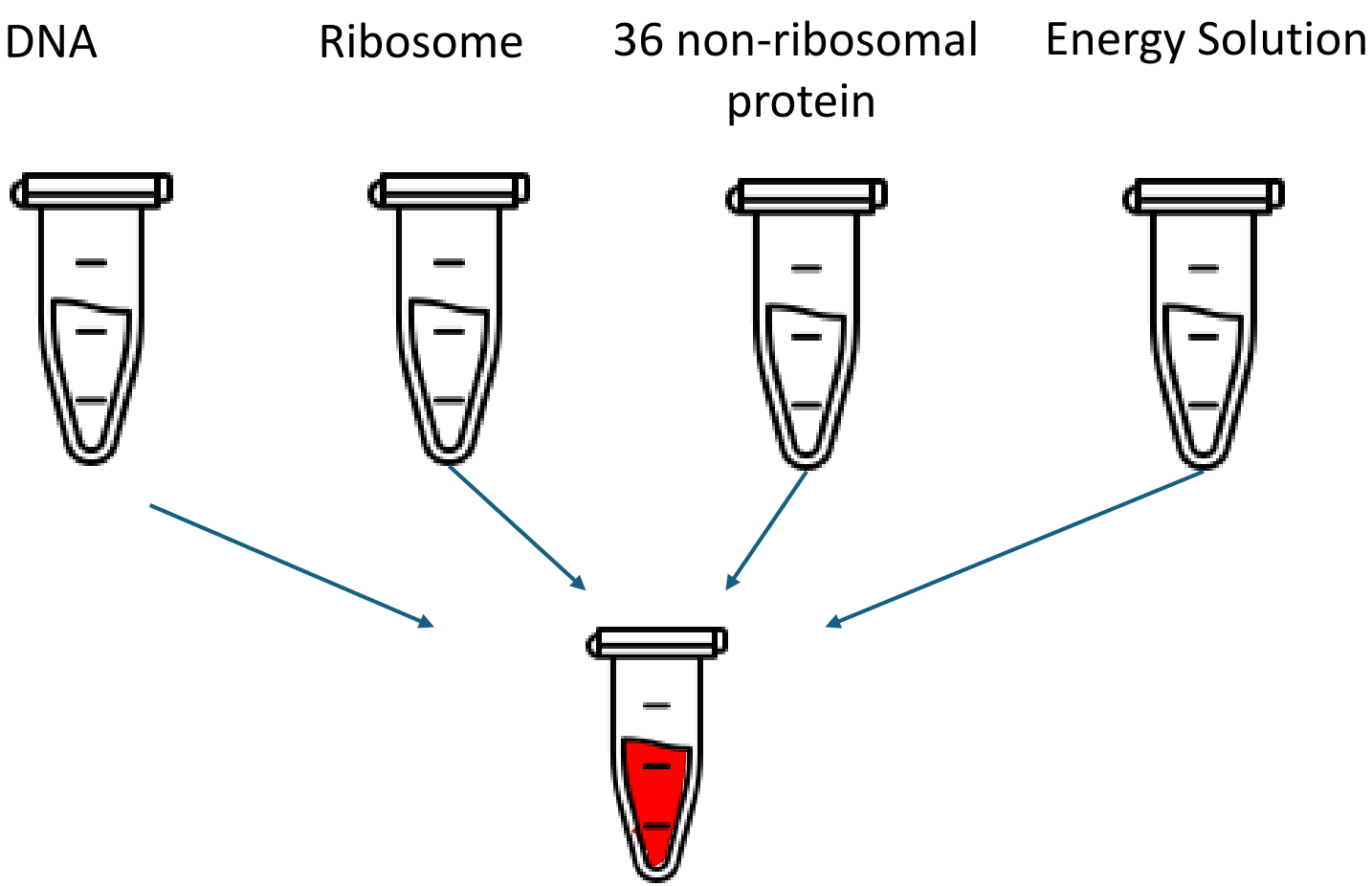
Shimizu, Yoshihiro, et al. "Cell-free translation reconstituted with purified components." *Nature biotechnology* 19.8 (2001): 751-755

PURE



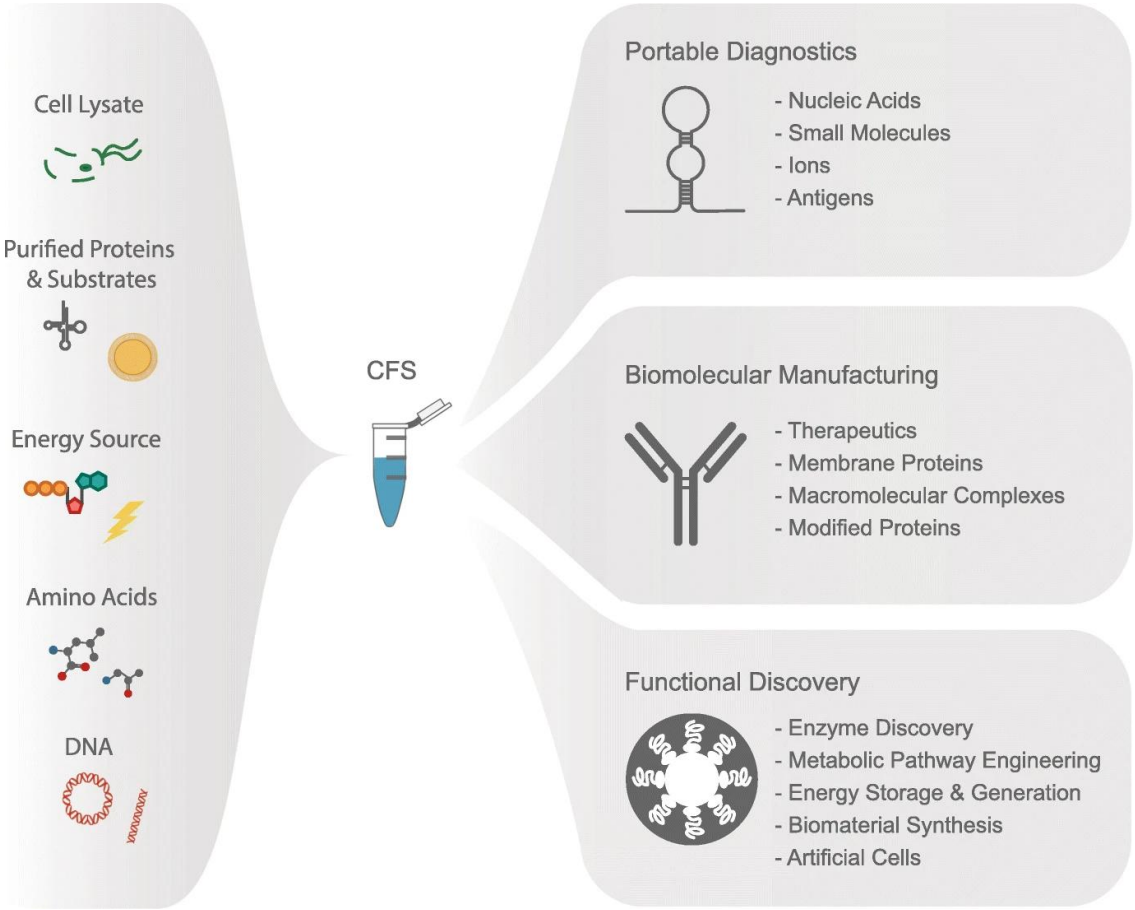
Ganesh & Maerkl, ACS Synth. Biol., 2024

Set up a PURE reaction



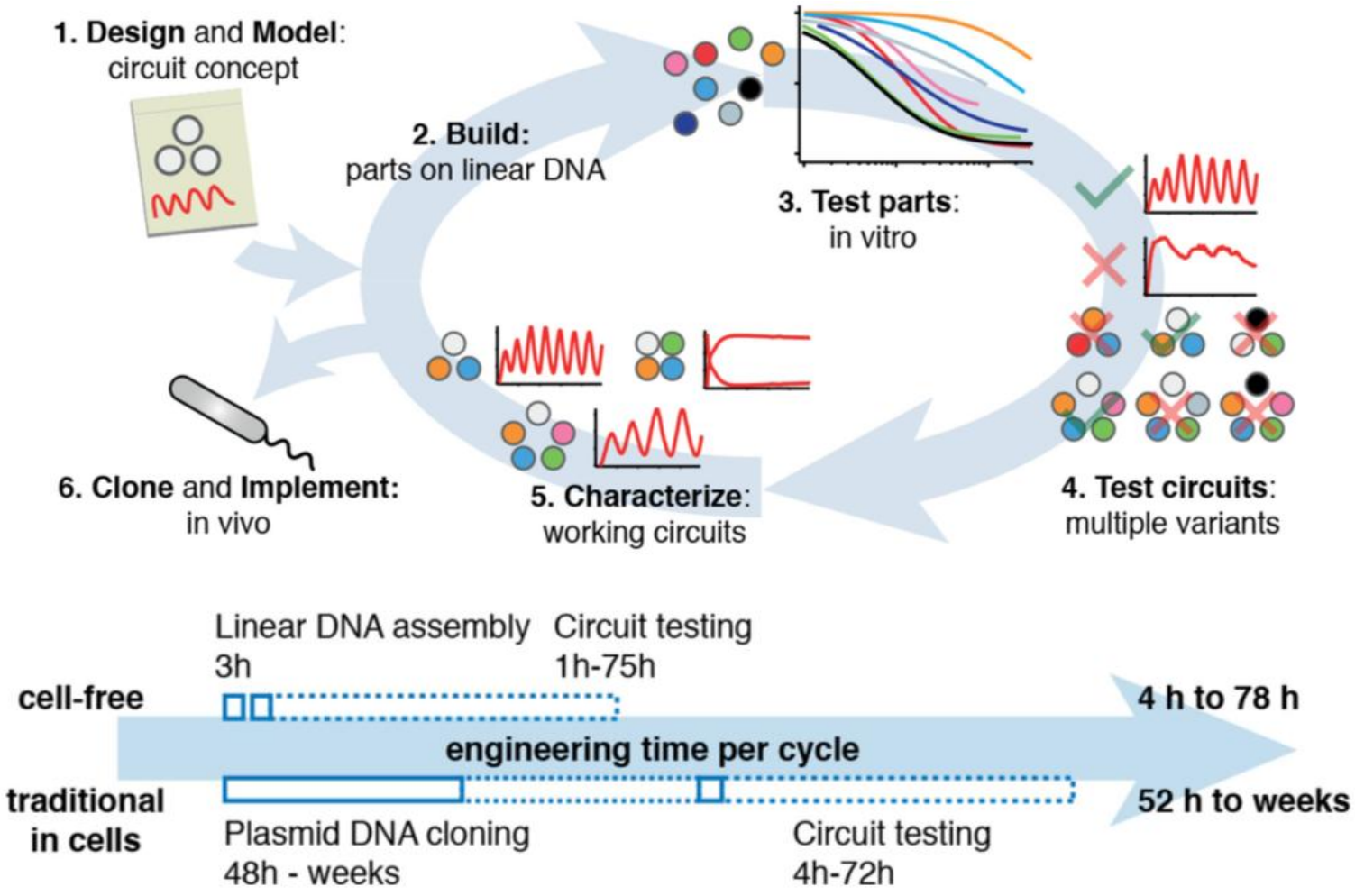
Applications

- Basic science: understanding biological systems better
- Rapid prototyping
- Diagnostics
- Therapeutics
- Building a synthetic cell



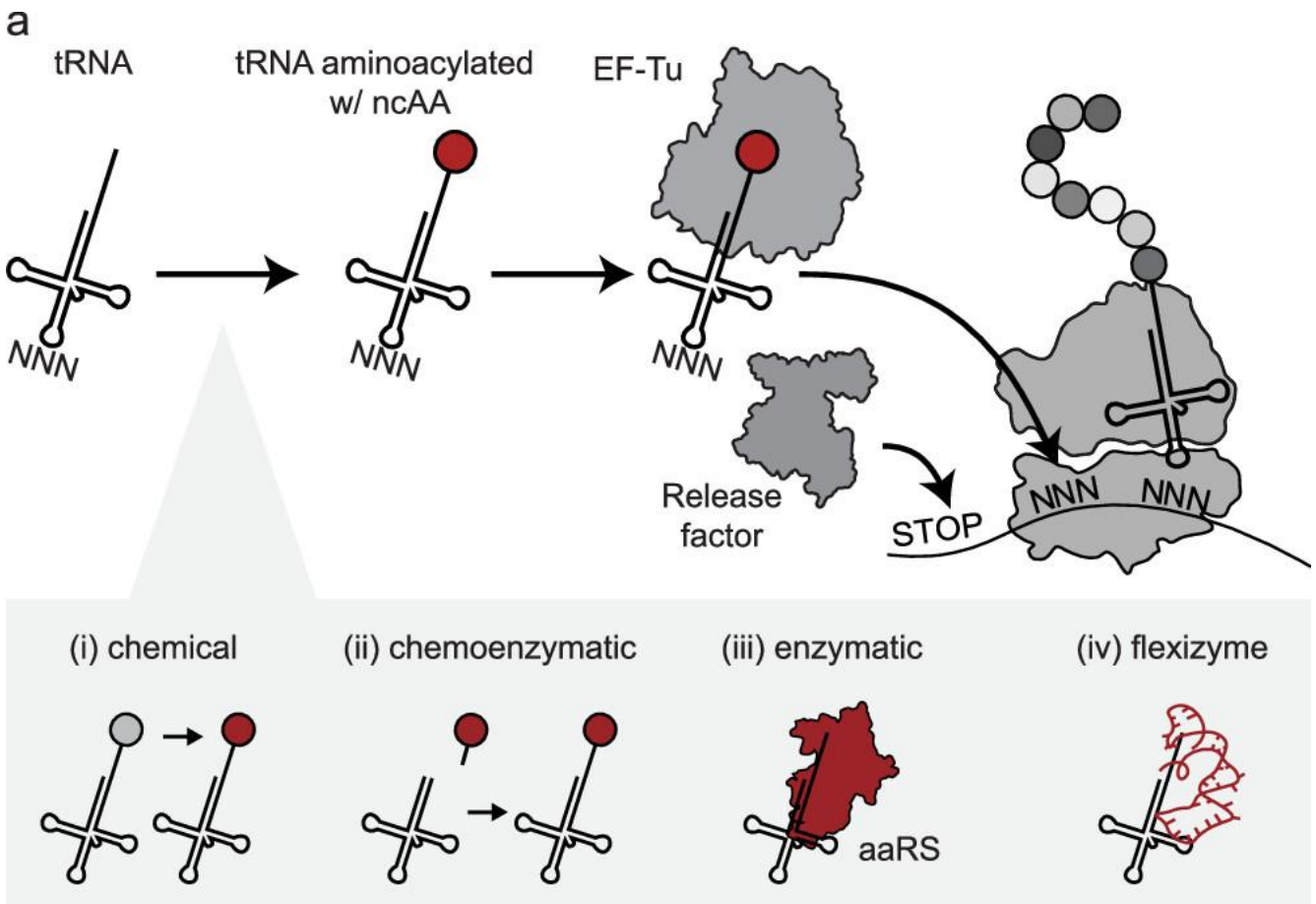
Tinafar 2019

Rapid prototyping



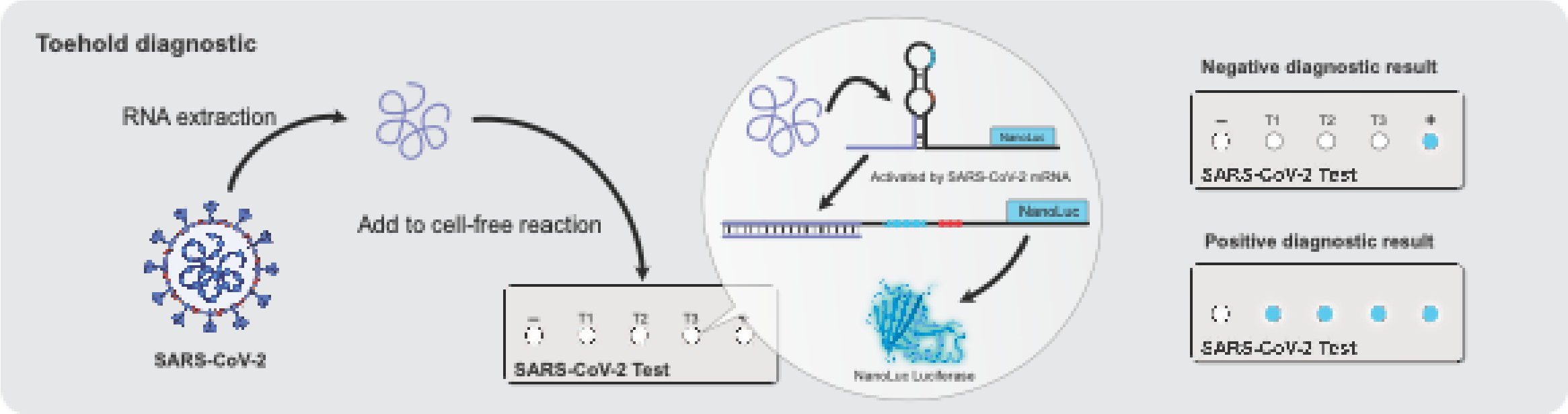
Niederholtmeyer et al. eLife 2015.

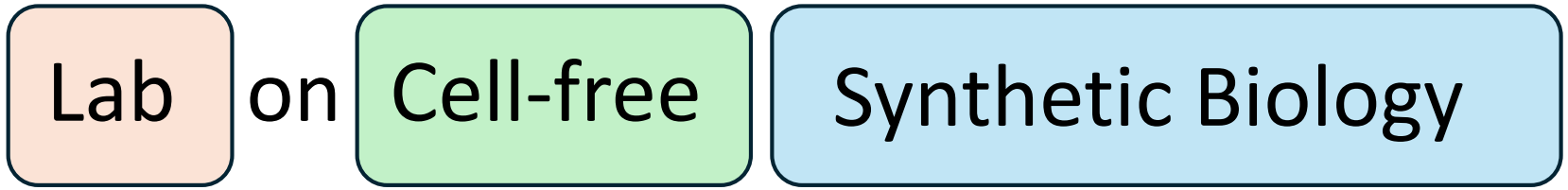
Incorporation of Noncanonical Amino Acids into Proteins



Hunt, Chem. Rev., 2024

Diagnostic


Toehold diagnostic
RNA extraction
SARS-CoV-2
Add to cell-free reaction
SARS-CoV-2 Test
Activated by SARS-CoV-2 mRNA
NanoLuc
NanoLuc Luciferase
Negative diagnostic result
SARS-CoV-2 Test
Positive diagnostic result
SARS-CoV-2 Test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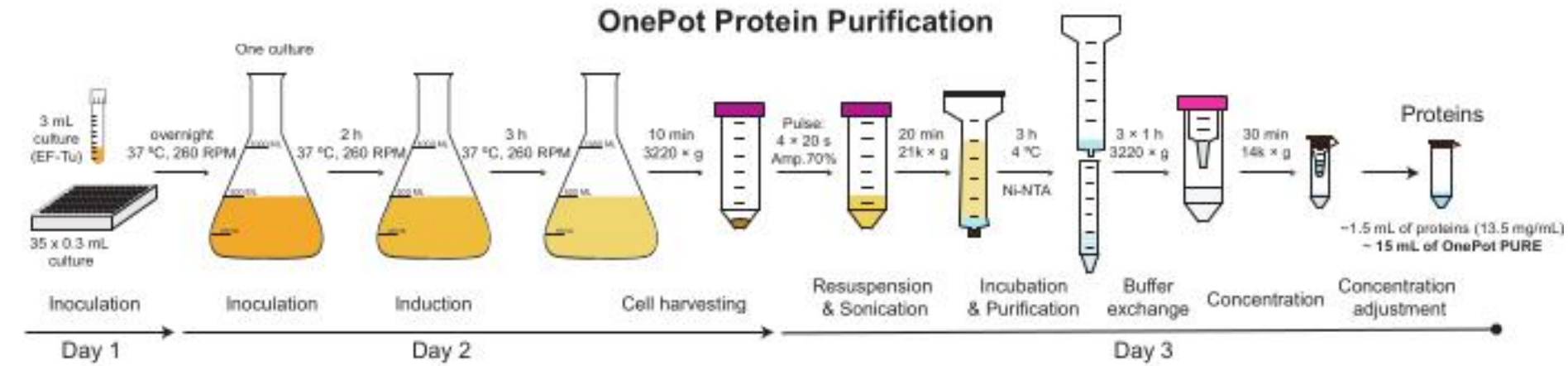
Synthetic Biology

OnePot PURE protduciton




Inoculation


Induction


Resuspended cells

Sonication

Lyse cells

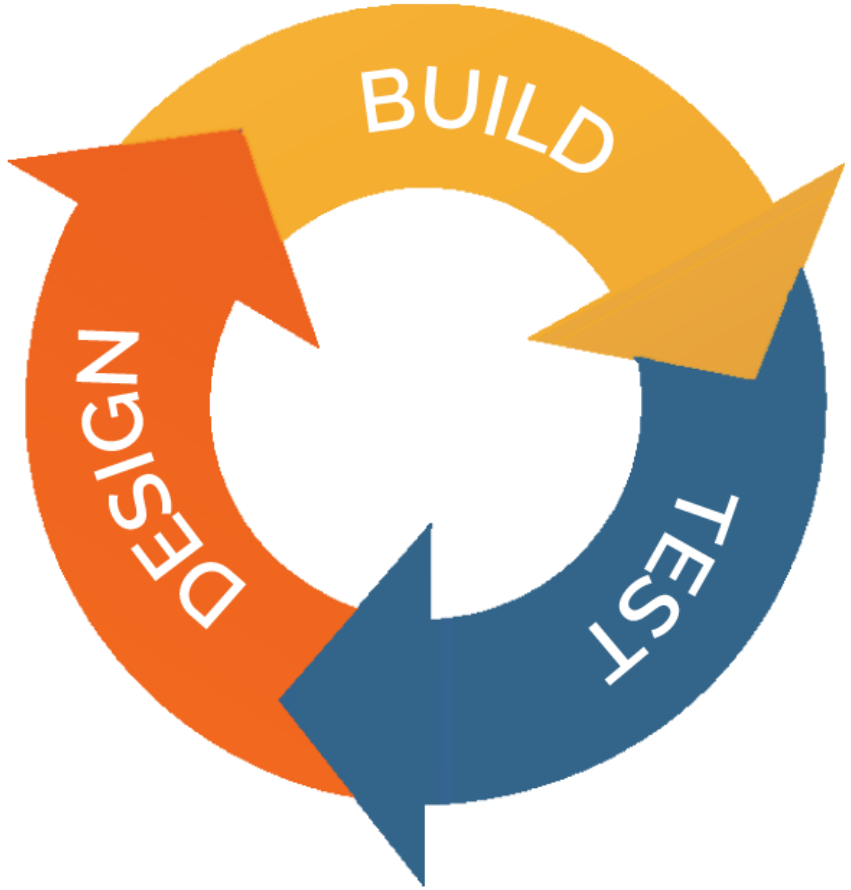
Lysate


Purification


Buffer exchange

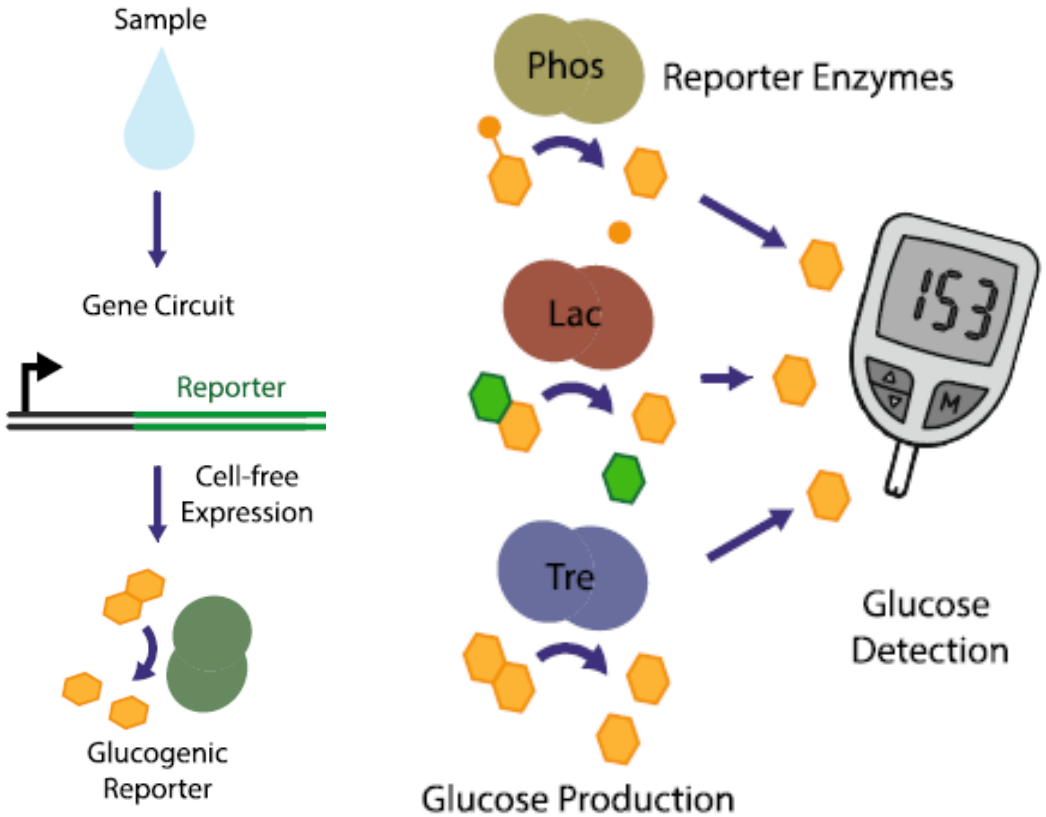
Project

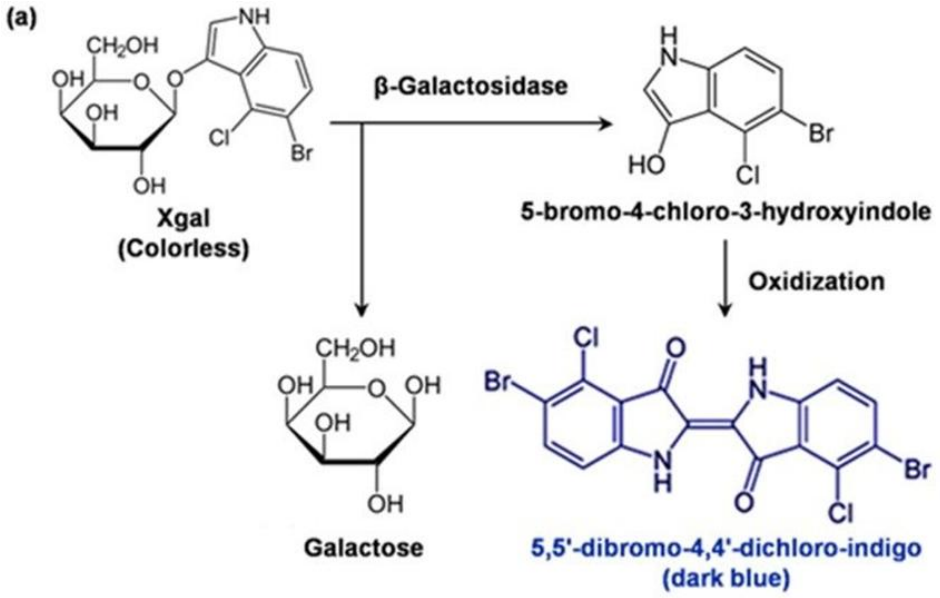
- Structure of report
  - Introduction
  - Method
  - Result
  - Discussion

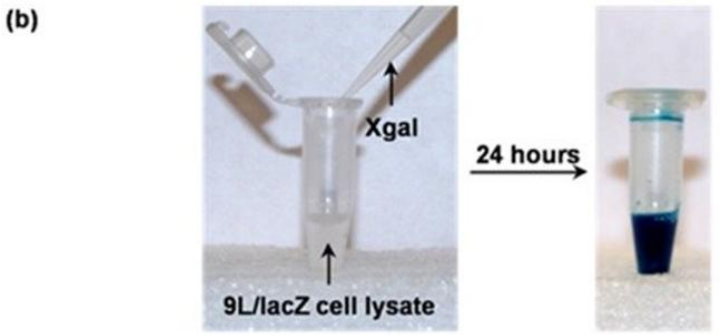


Design your gene fragment(s)

Project example 1:
Design a reporter for point-of-care diagnostics

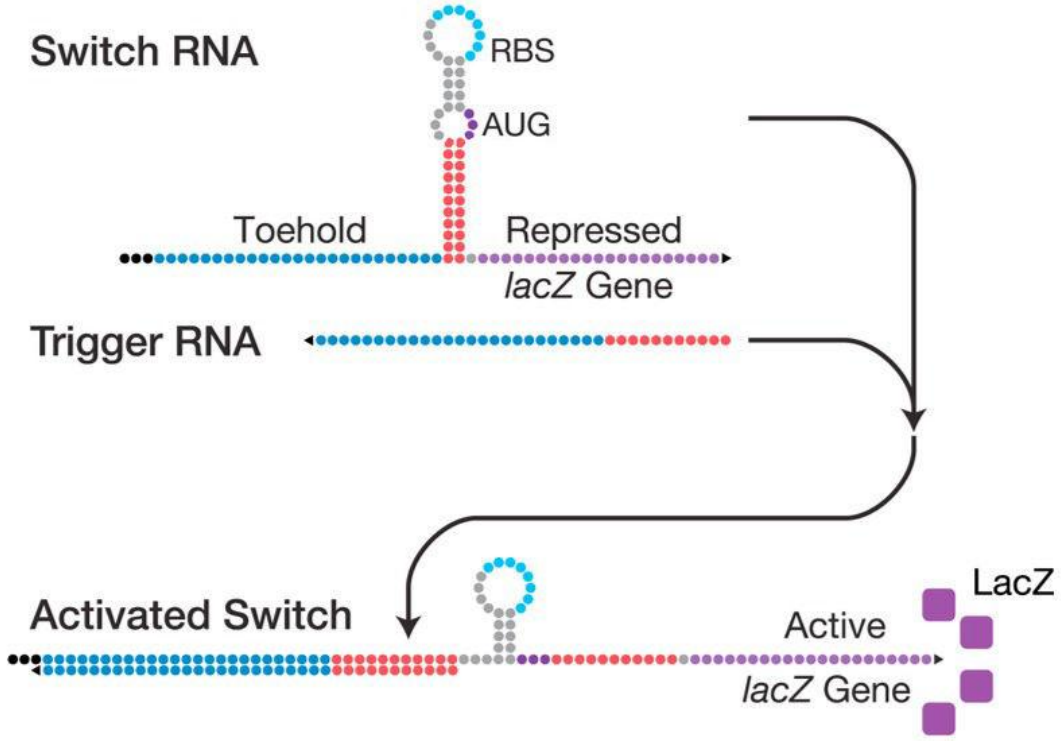




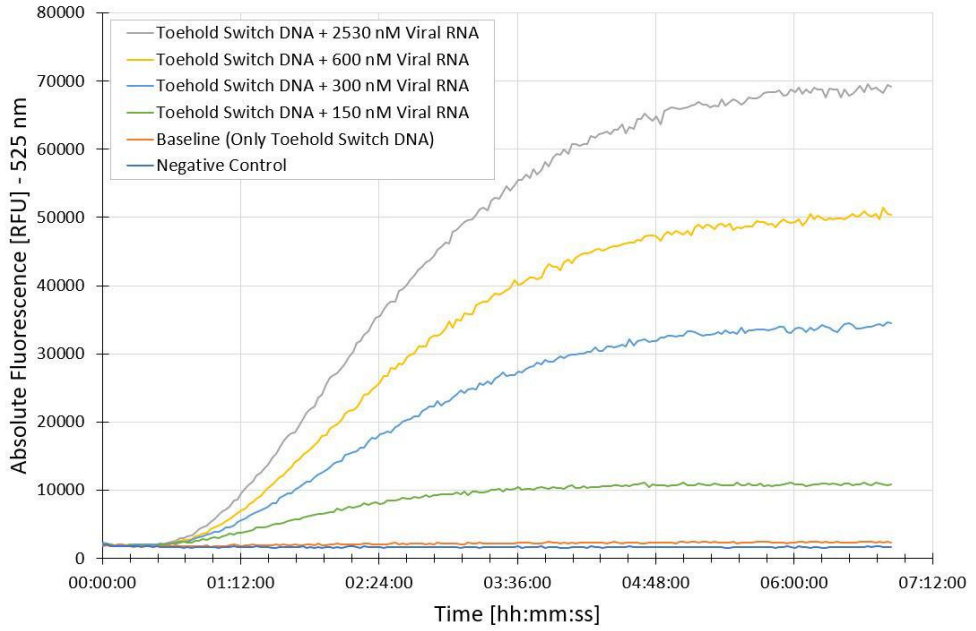


Amalfitano, Nat. Commun., 2021

Project example 2:
Detection of Zika virus via toehold switches

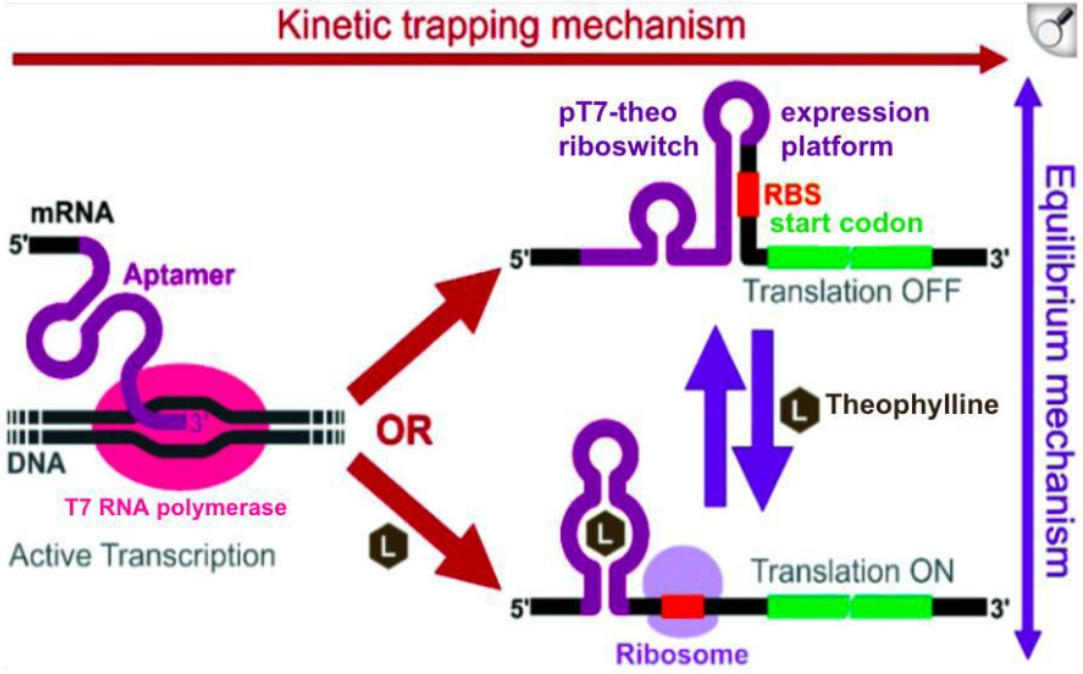


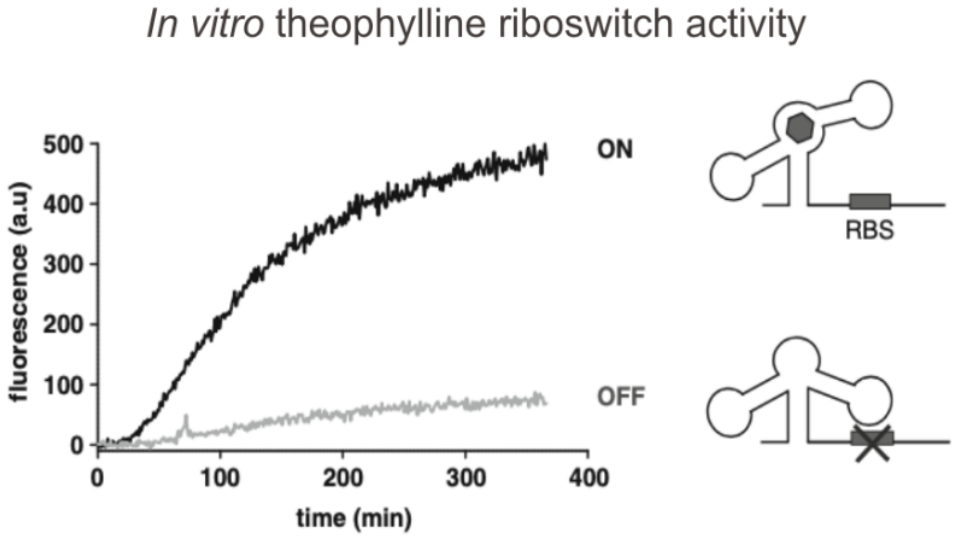
Pardee, Cell, 2016



Adapted from BINKOVÁ Helena, FILIMONOV Nicolas, BAUMANN Alexander, EE-490(j), 2024.

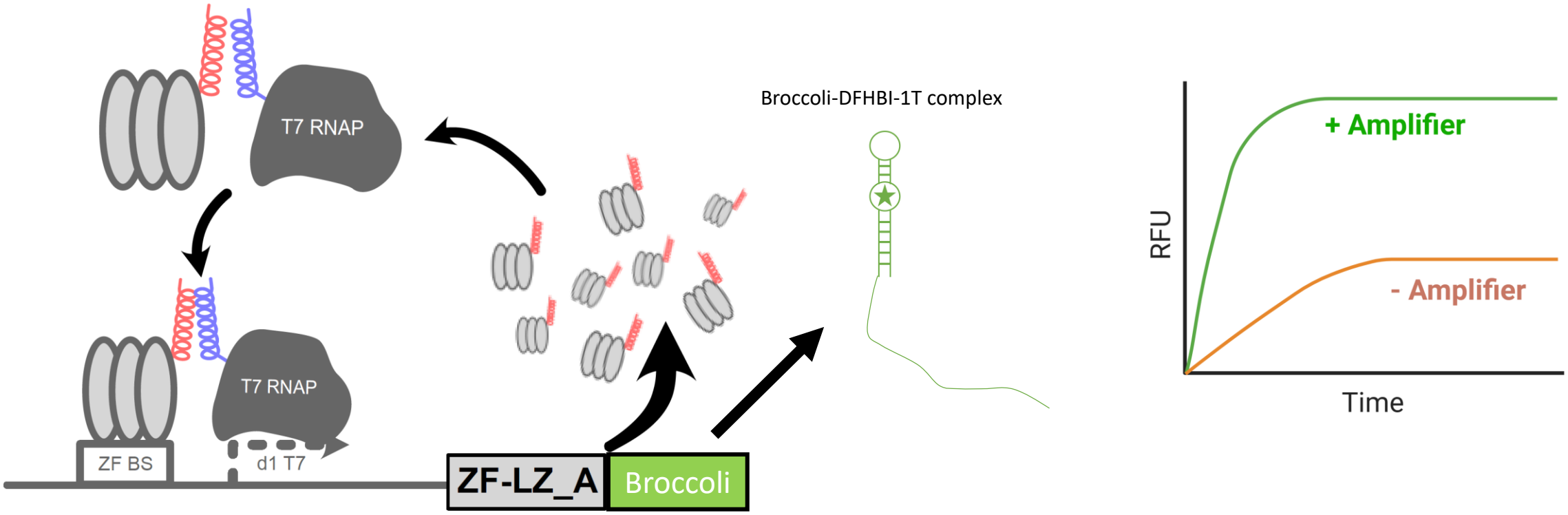
Project example 3:
Detection of theophylline via riboswitch





Tabuchi, RSC Chem. Biol., 2021

Project example 4:
Signal amplification via positive feedback loop



Lee, bioRxiv, 2025

What we will provide for project?

- 1-2 gene fragments you design (<1000bp)
- Reagents in LBNC (discuss with TA)
  - Small molecule sensor protein
  - Fluorescent protein DNA fragments
- Instruments in LBNC and DLL lab
  - Plate reader
  - Gel imager

What do you have to do?

- 2 presenations (before and after the project)
- 1 project report (per group)

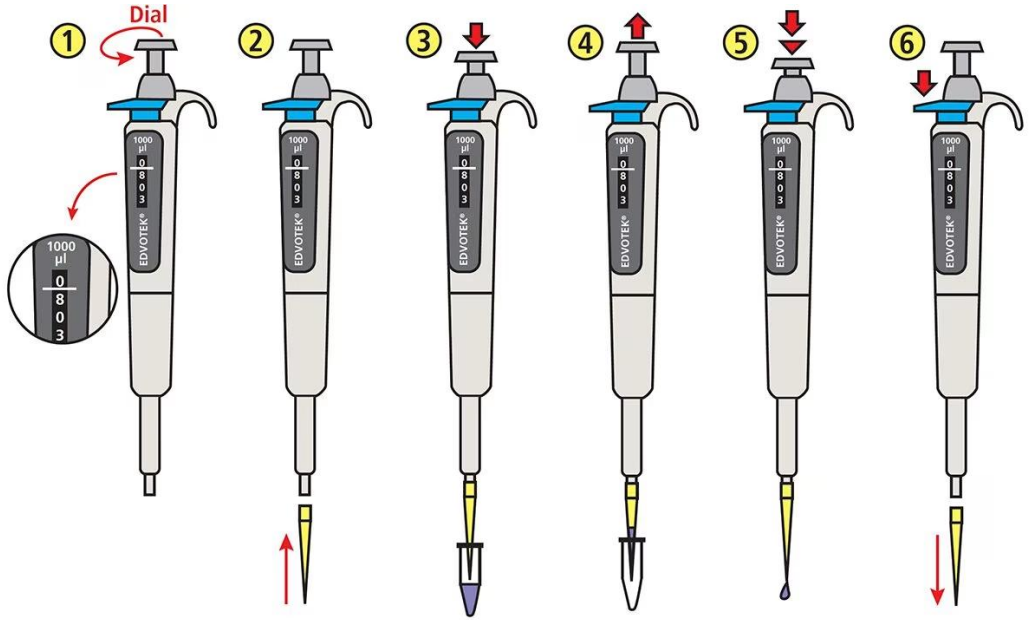
Schedule

	Lecture	Project
Week 1	Introduction to PURE	
Week 2	Genetics	Group allocation
Week 3	Biochemistry	
Week 4	Onepot PURE protein production	Discussion of project
Week 5	Onepot PURE protein purification	Discussion of project
Week 6	Onepot PURE protein adjustment	Project presentation
Week 7	Ribosome production	Project presentation
Week 8	Ribosome purification	Order gene blocks
Week 9	Ribosome adjustment	Project
Week 10	PURE functional test	Project
Week 11		Project
Week 12		Project
Week 13		Project presentation
Week 14	Final Exam	Final Exam

Grading method	
Attendance	25%
Lab Notebook	30%
Project	25%
Final exam	20%

How to use a micropipette?

1. **Choose the right pipette and set the volume**
   - Turn the dial to the amount you want.
2. **Load the tip**
   - Put on a disposable tip by firmly pressing the pipette into the tip box.
3. **Press the plunger**
   - Press down to the first stop.
4. **Draw up liquid**
   - Put the tip just under the surface of the liquid.
   - Slowly release the plunger to suck the liquid in.
5. **Dispense the liquid**
   - Press the plunger all the way to the second stop to push all the liquid out.
6. **Eject the tip**
   - Press the eject button to throw away the tip into the waste box.



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