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Cell-free systems Gene expression outside of the cell

Olivier Borkowski

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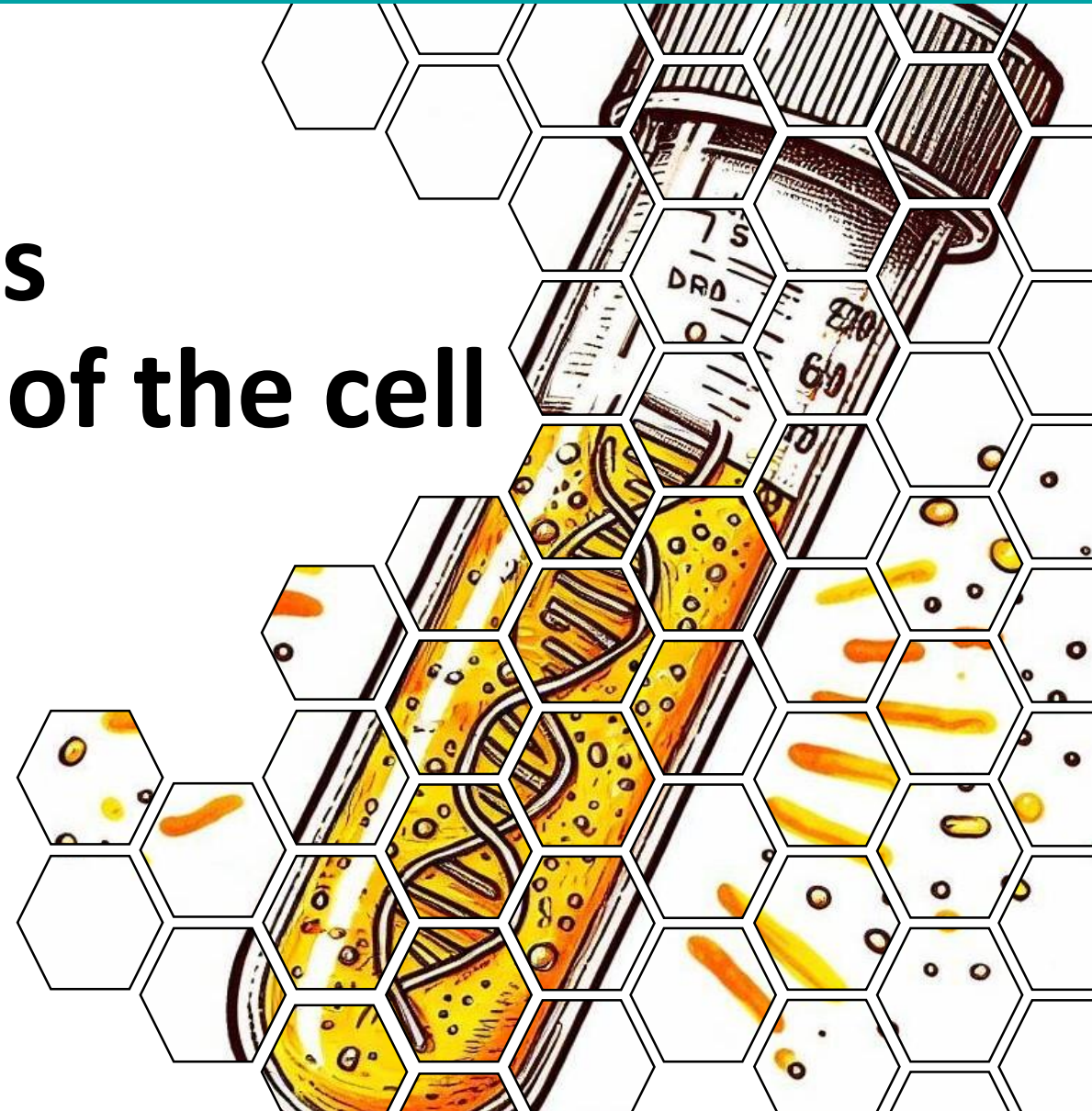
HAL Authorization

Cell-free systems

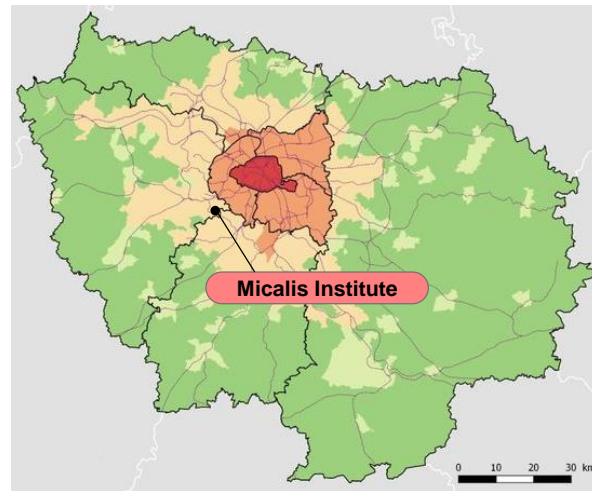
Gene expression outside of the cell

Olivier Borkowski, PhD

Permanent researcher in Synthetic Biology
Institut Micalis, INRAE - AgroParisTech - Univ. Paris-Saclay, France
SyBER team
Email: Olivier.Borkowski@inrae.fr



Micalis Institute



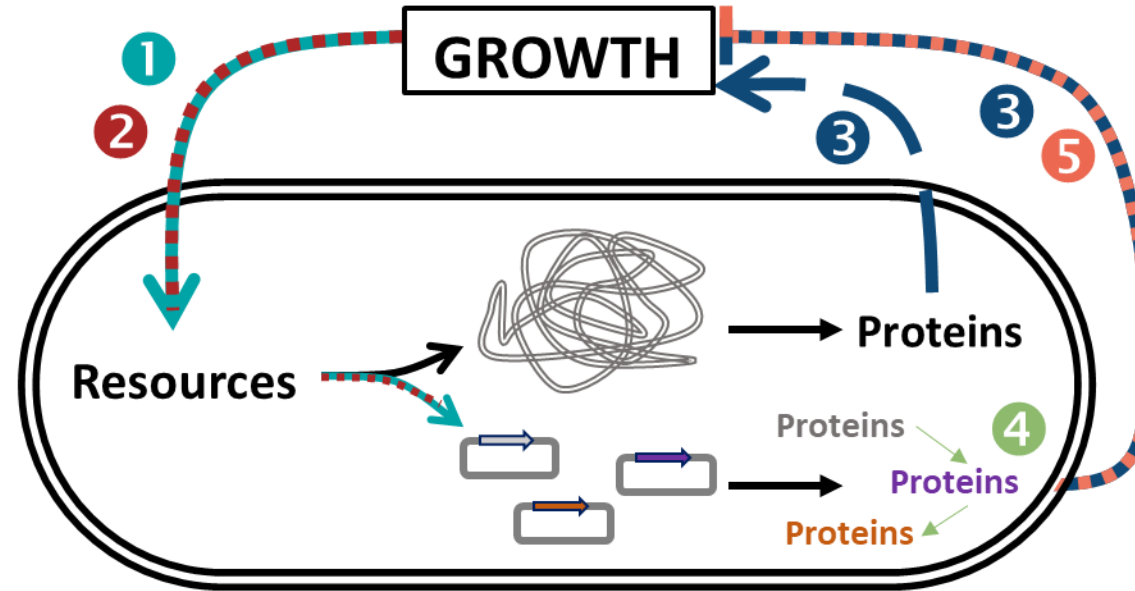
21 Research Teams

- 340 people
- 130 scientists and engineers
- 140 PhD students and post-docs
- 70 scientific and administrative personnel

Themes

- (1) Emergence, control, and adaptation of pathogenic microorganisms
- (2) Microbial food and intestinal ecosystems
- (3) Synthetic and systems biology

Understand and use the relationship between protein production and bacterial physiology



Bacterial Physiology

Bacterial physiology is a branch of biology that aims to understand overarching principles of cellular reproduction:

What are the fundamental principles underlying the regulation and coordination of biosynthesis in a given growth environment?

-> Bacterial growth law

Bacterial Physiology

Bacterial physiology is a branch of biology that aims to understand overarching principles of cellular reproduction:

What are the fundamental principles underlying the regulation and coordination of biosynthesis in a given growth environment?

-> Bacterial growth law

Some major principles studied in bacterial physiology:

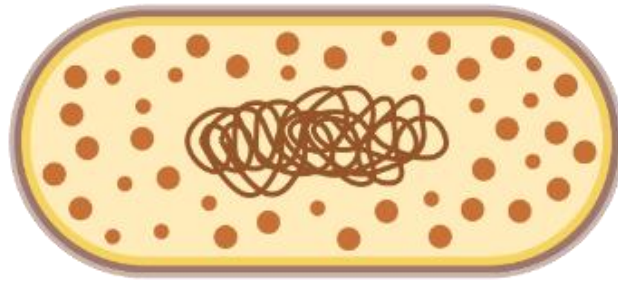
- Link between bacterial growth and nutrient concentrations in culture media (Monod, 1942)
- Link between the macromolecular composition and the cell doubling time (Schaechter et al., 1958)
- Linear relationships between RNA/DNA, RNA/protein, protein/DNA and growth rate (Bremer & Dennis, 1974)
- Increasing allocation of cellular resources to ribosome synthesis with increasing growth rate (Hwa & Scott, 2010)

-> New technology allows novel experiments

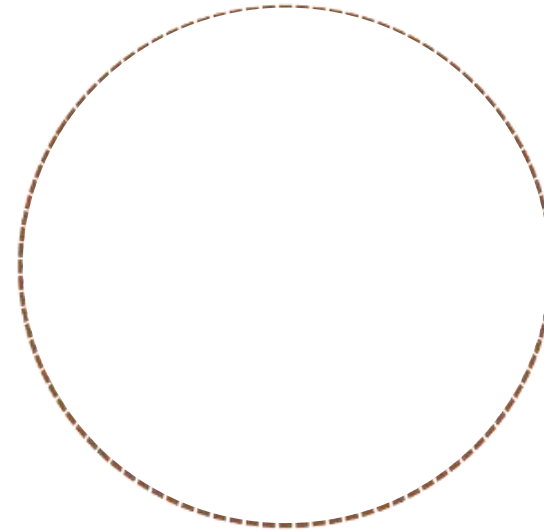
Bacterial Physiology

Experimental setup

Genomic regulations



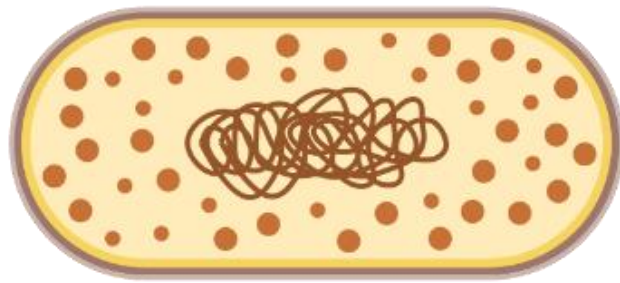
E. coli living cells



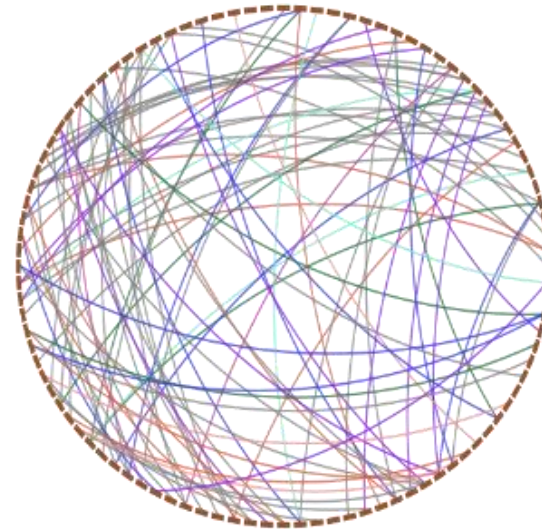
Bacterial Physiology

Experimental setup

Genomic regulations



E. coli living cells



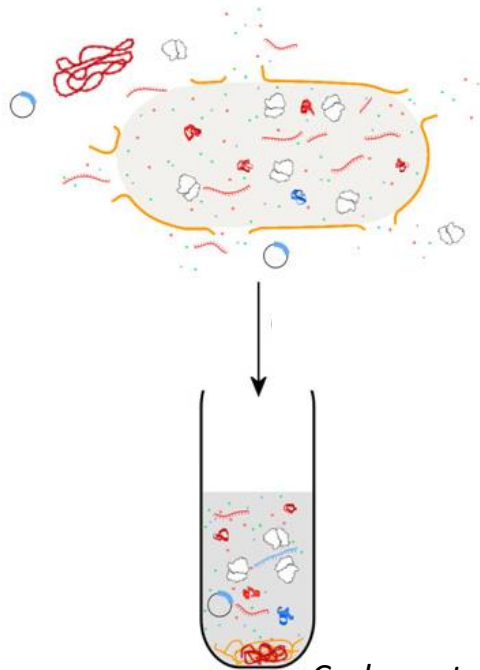
Intertwined Effects

-> New technology to decouple the many effects observed in living cells

Cell-free reaction:

Protein expression in an open system

A direct manipulation of the environment of protein production because it is an open system:



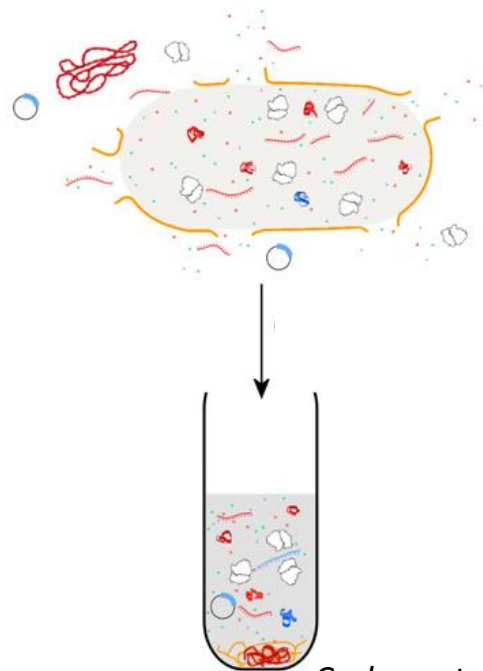
Carlson et al. Biotechnology advances (2012)

Cell-free reaction:

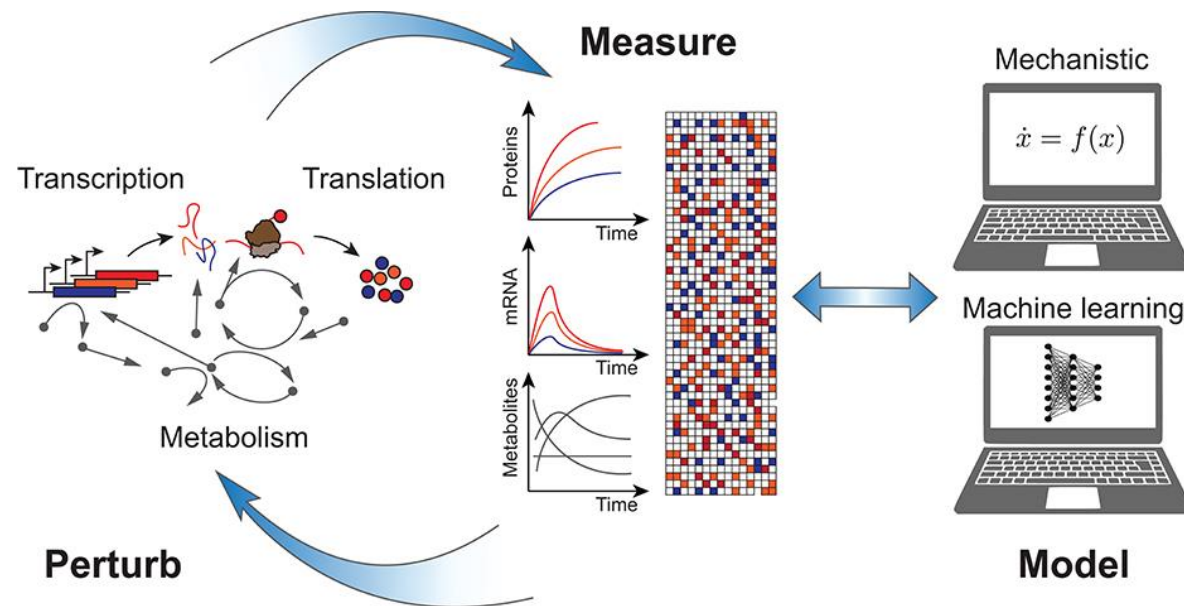
Protein expression in an open system

A direct manipulation of the environment of protein production because it is an open system:

- An alternative technology to study central process observed in living cells (replication, transcription, translation, degradation, metabolism)
- A versatile technology for bioengineering (Use in Synthetic Biology)



Carlson et al. *Biotechnology advances* (2012)



Nadanai, L. *Frontiers in Bioengineering and Biotechnology* (2020)

1. Description of Cell-Free Systems

History: First use of cell-free

What is the composition of a cell-free reaction?

Advantages of producing protein outside of the cell

Measurements in 384-well plate

Current existing protocol to produce bacterial cell extract

Alternative cell-free approach, the pure system

2. Current Applications of Cell-Free Systems in Academia and Industry

Cell-free reaction:

First major use of cell-free

Cell-free was first used in the 1960's by Nirenberg and Matthaei to understand the genetic code

*THE DEPENDENCE OF CELL-FREE PROTEIN SYNTHESIS IN E. COLI
UPON NATURALLY OCCURRING OR SYNTHETIC
POLYRIBONUCLEOTIDES*

BY MARSHALL W. NIRENBERG AND J. HEINRICH MATTHAEI*

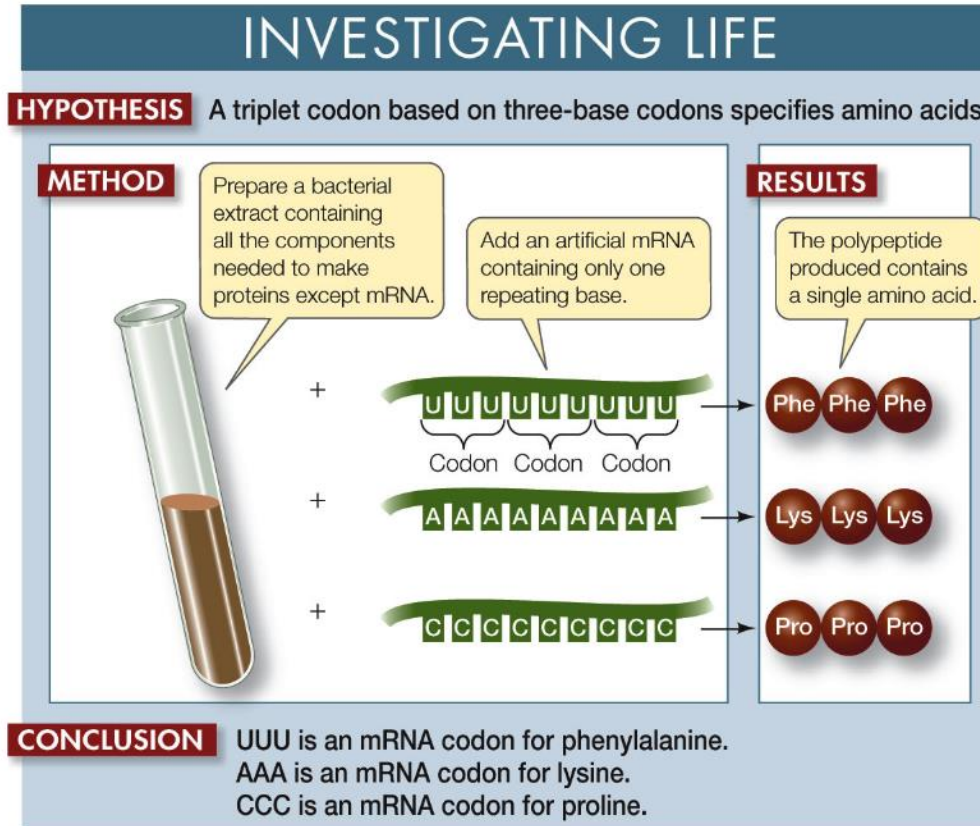
NATIONAL INSTITUTES OF HEALTH, BETHESDA, MARYLAND

Communicated by Joseph E. Smadel, August 3, 1961

A stable cell-free system has been obtained from *E. coli* which incorporates C¹⁴-valine into protein at a rapid rate. It was shown that this apparent protein synthesis was energy-dependent, was stimulated by a mixture of L-amino acids, and was markedly inhibited by RNAase, puromycin, and chloramphenicol.¹ The present communication describes a novel characteristic of the system, that is, a requirement for template RNA, needed for amino acid incorporation even in the

Cell-free reaction:

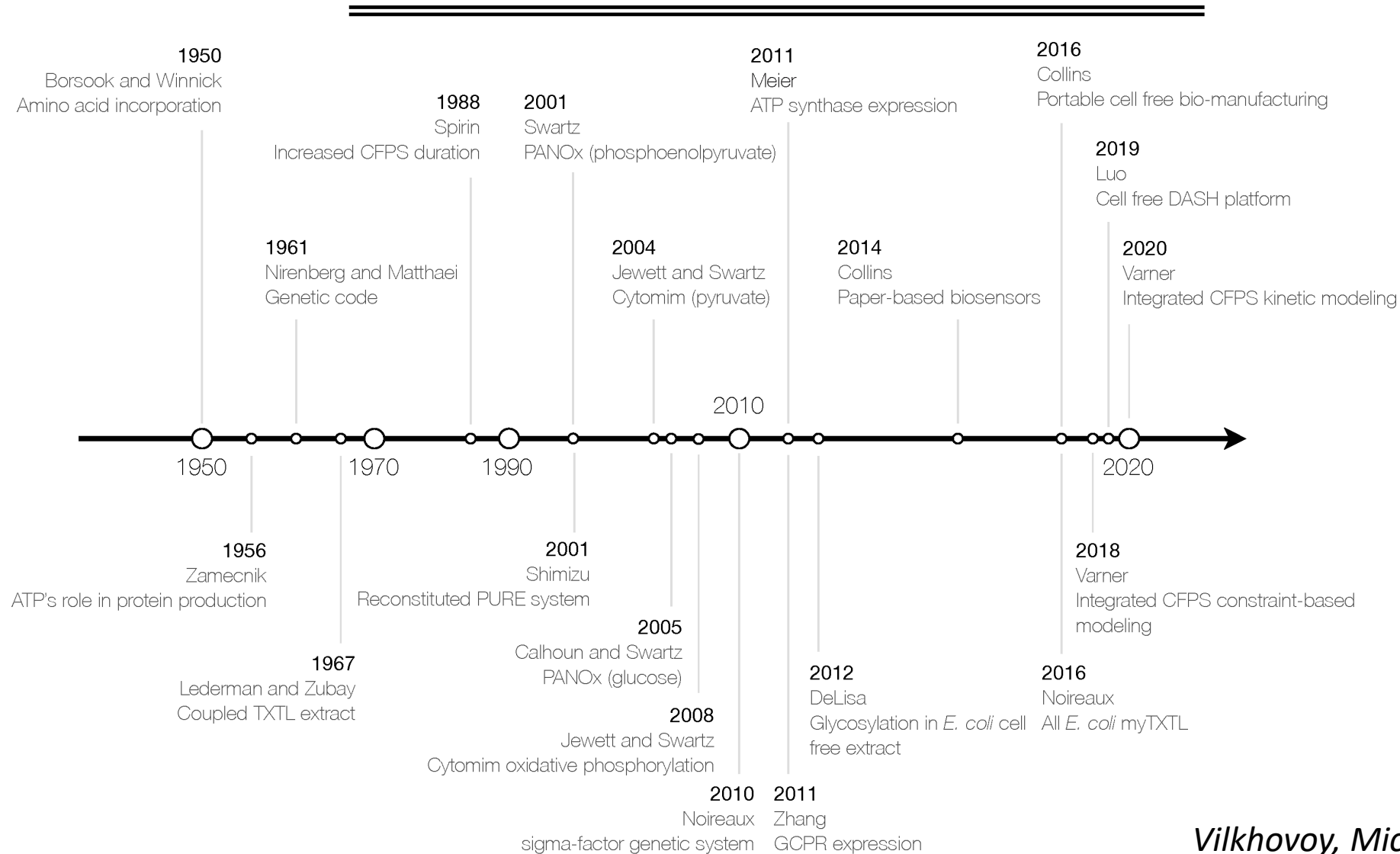
Nobel prize for the interpretation of the genetic code and its function in protein synthesis



They made a synthetic RNA only with the base uracil (poly U – codon UUU) and tested it in 20 tubes, each one with a different radioactive amino acid.

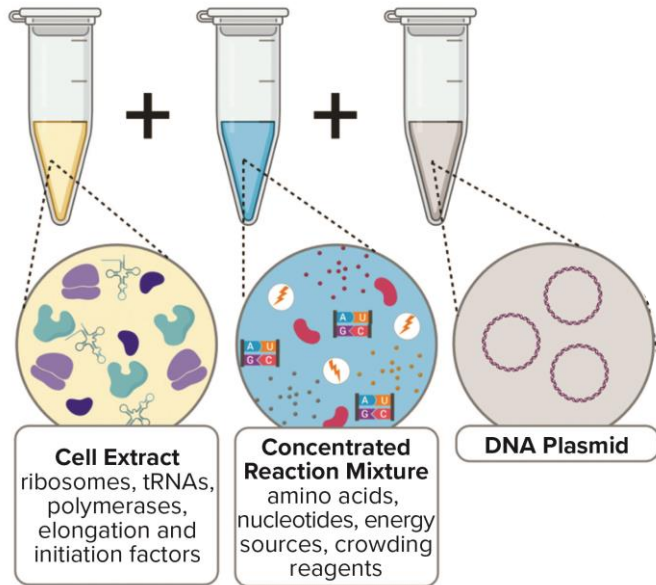
Cell-free reaction:

History



Cell-free reaction:

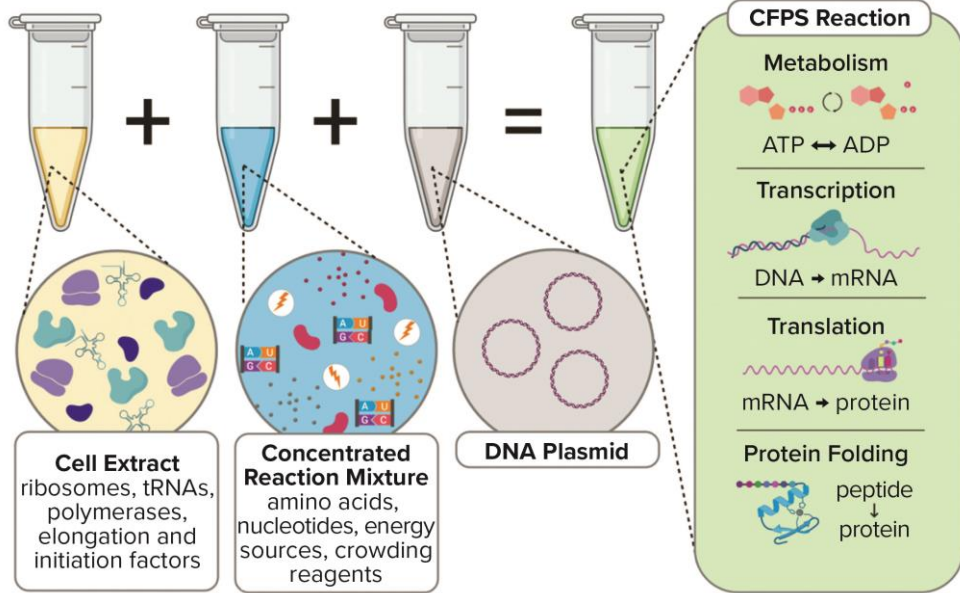
What is the composition of a cell-free reaction?



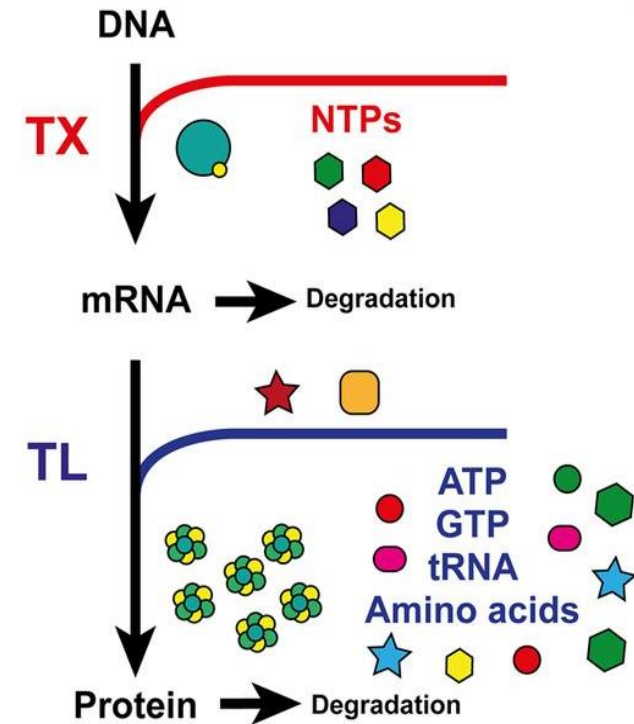
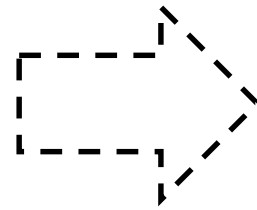
Melinek B, et al. BioProcess International (2020)

Cell-free reaction:

What is the composition of a cell-free reaction?

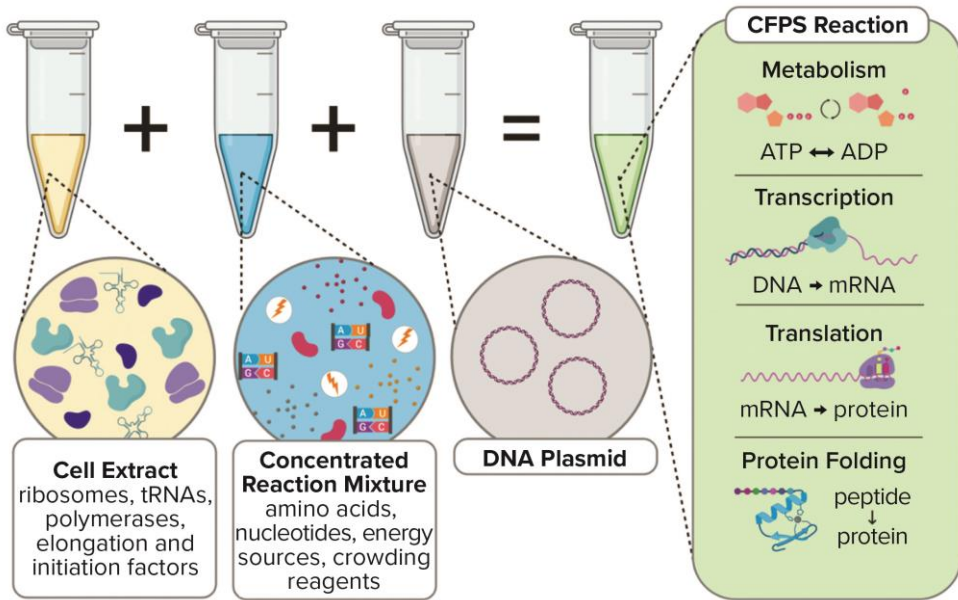


Melinek B, et al. *BioProcess International* (2020)



Seok Hoon Hong et al. *Frontiers in Chemistry* 2014

Cell-free reaction: Cell-extract preparation



Melinek B, et al. *BioProcess International* (2020)

Physical disruption

Sonication

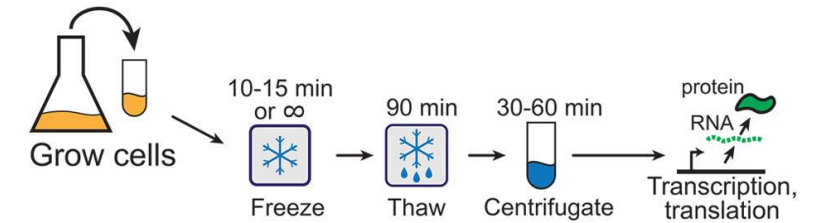


French press

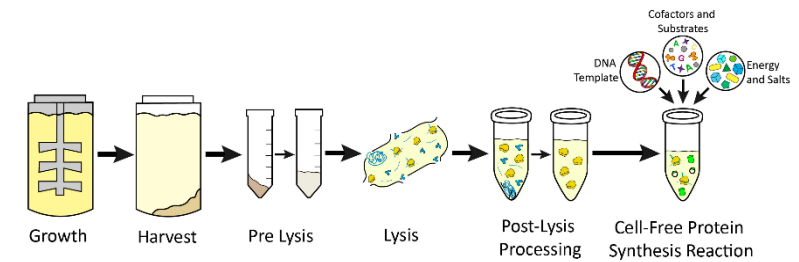


Chemical disruption

Autolysis

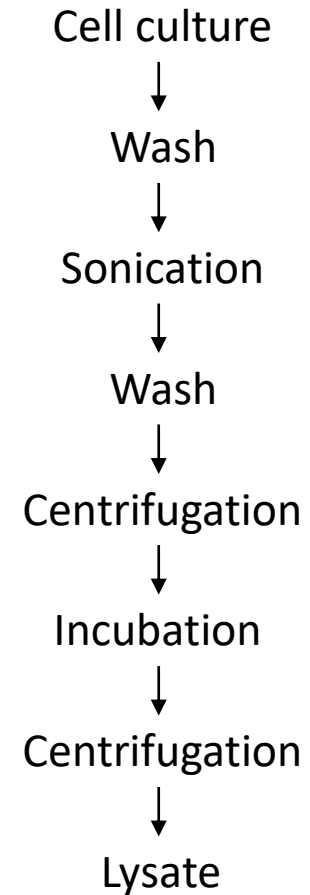
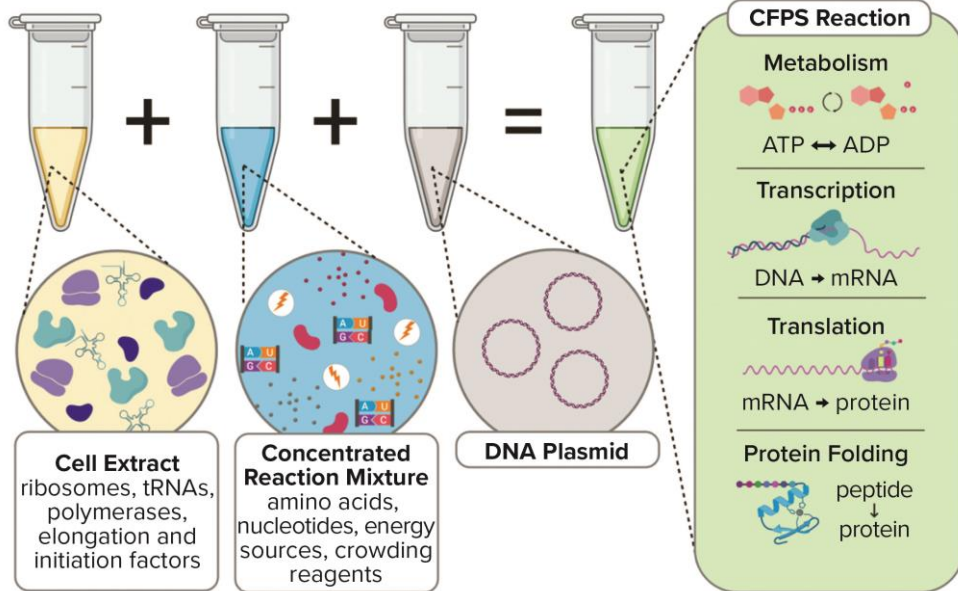


Lysozyme

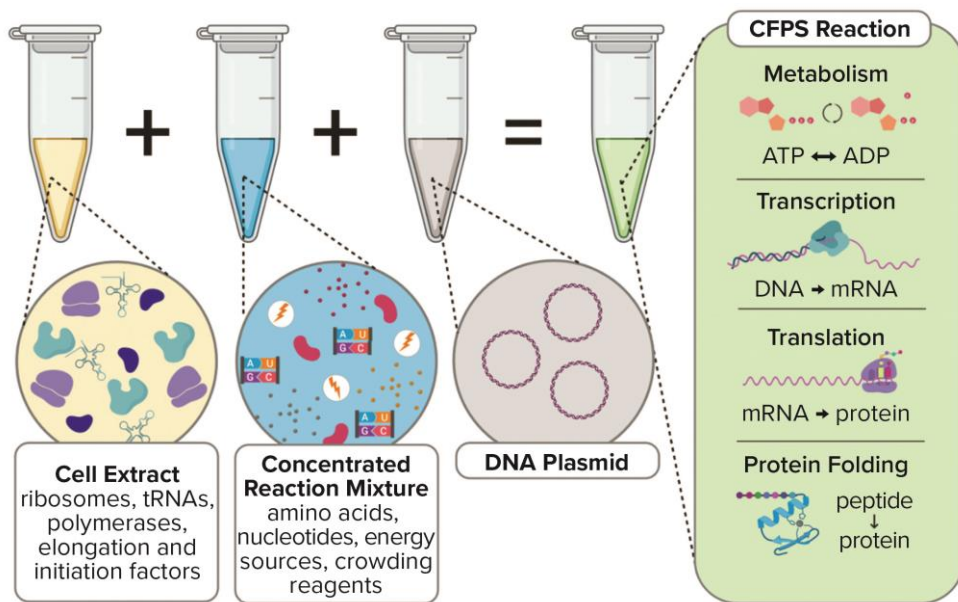


Cell-free reaction:

Protocol for home-made cell-extract preparation



Cell-free reaction: Buffer composition



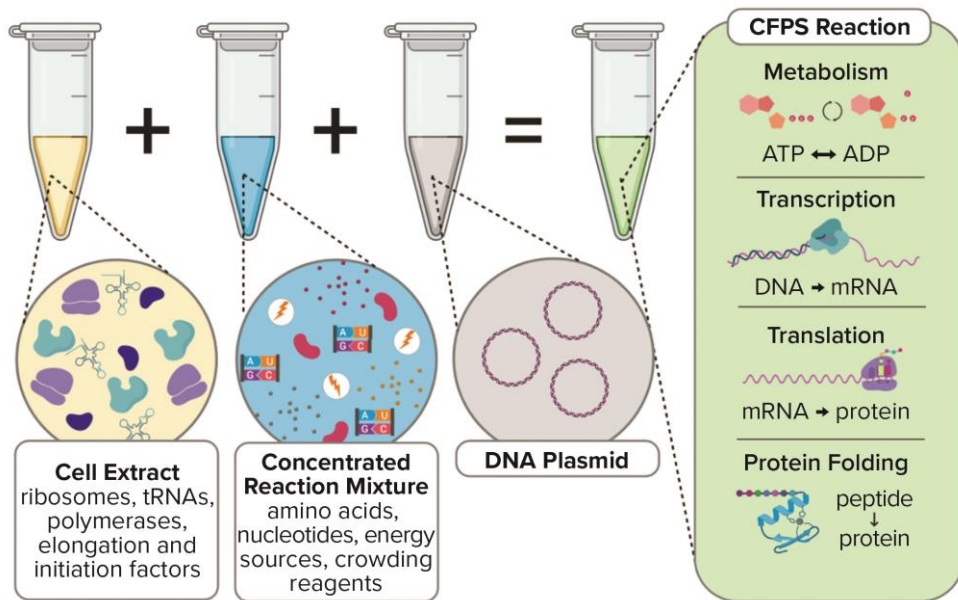
Melinek B, et al. *BioProcess International* (2020)

Amino Acid
tRNA
Mg-glutamate
K-glutamate
Folnic Acid
Spermidine
Coenzyme A
NAD
3-PGA
AMPC
Nucleoside triphosphate (NTP)

Translation
Translation
Ribosome maintenance
Ribosome stability
A precursor for formylmethionine (fMet)
Ribosome activity /RNA stability
Regenerate ATP via glycolysis
Regenerate ATP via glycolysis
Regenerate ATP
regulating glycolysis
Transcription

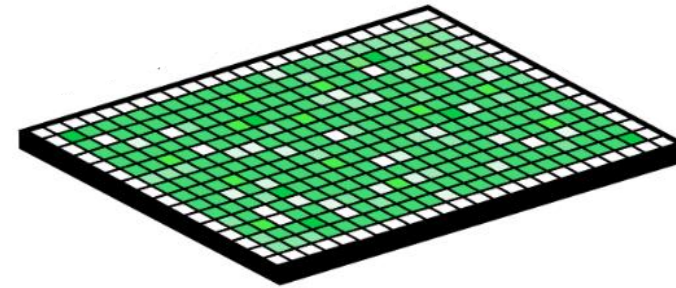
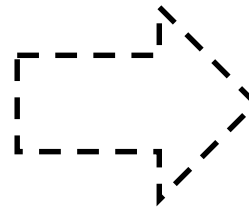
Cell-free reaction:

Measurements in 384-well plate



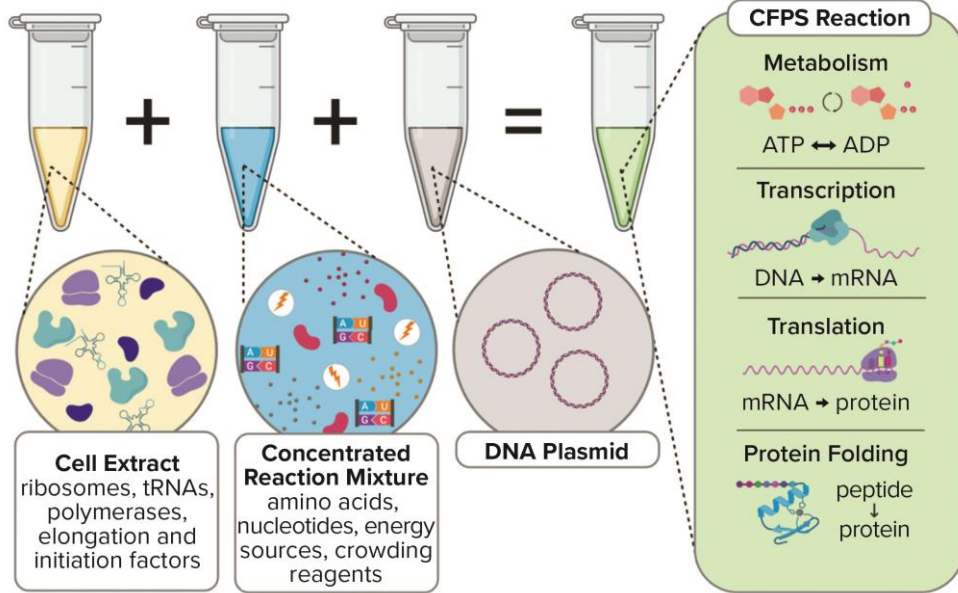
Melinek B, et al. *BioProcess International* (2020)

1 to 20 μ l reactions
(in 384-well)



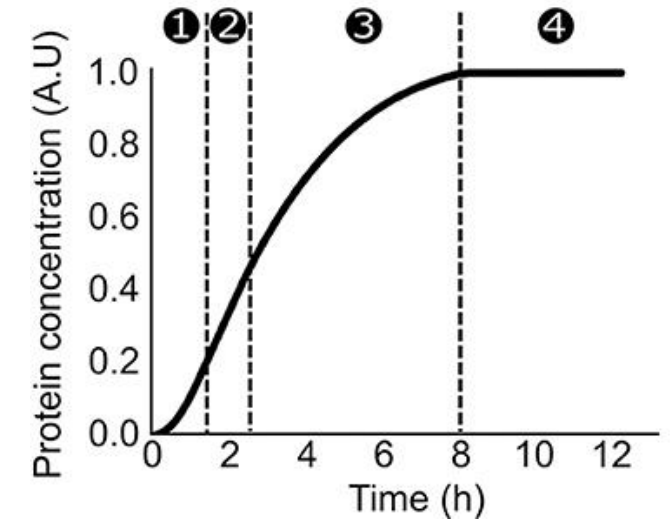
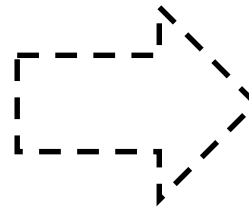
Cell-free reaction:

Measurements in 384-well plate

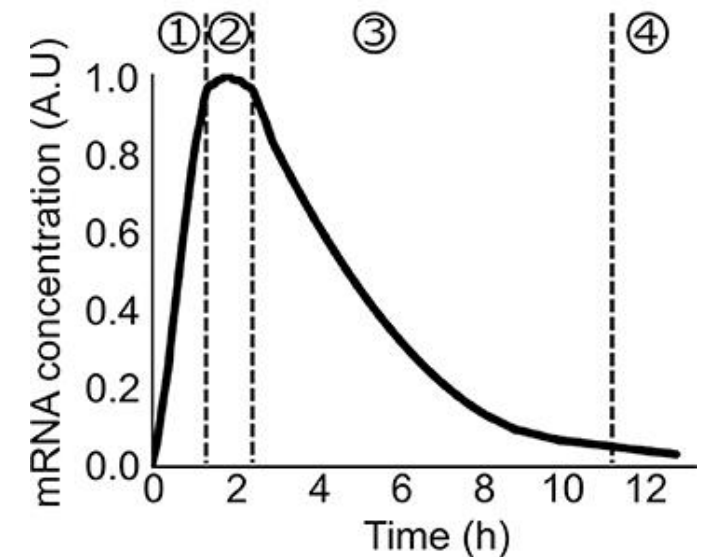


Melinek B, et al. *BioProcess International* (2020)

Translation

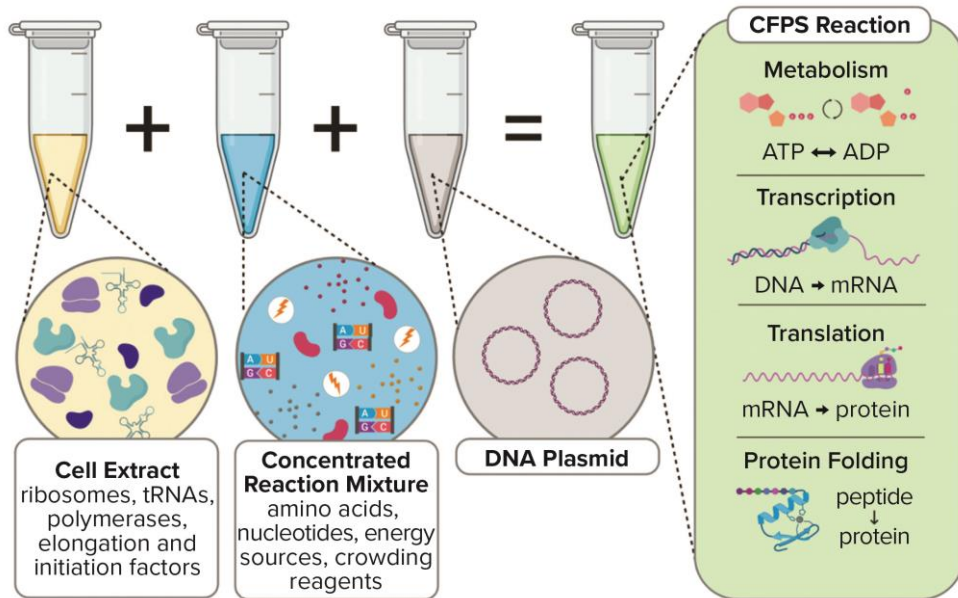


Transcription



Cell-free reaction:

Current existing protocol to produce bacterial cell extract



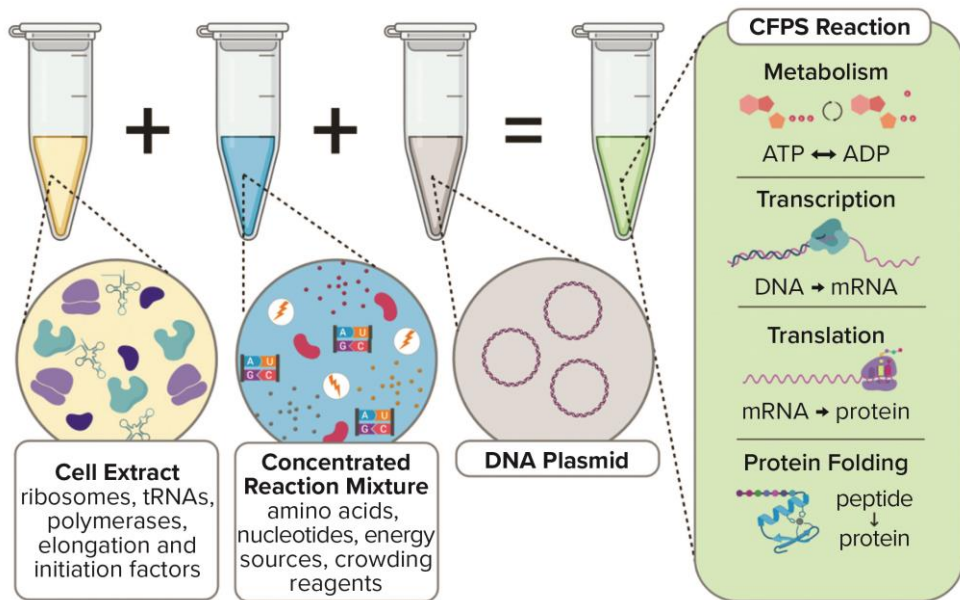
Melinek B, et al. *BioProcess International* (2020)

Table 1. Current existing protocols to produce cell-free lysates from bacteria cells

Organism	Bacteria Phylum
<i>E. coli</i>	Proteobacteria
<i>B. subtilis</i>	Firmicutes
<i>L. lactis</i>	Firmicutes
<i>B. megaterium</i>	Firmicutes
<i>C. autoethanogenum</i>	Firmicutes
<i>V. natriegens</i>	Proteobacteria
<i>P. putida</i>	Proteobacteria
<i>E. fergusonii</i>	Proteobacteria
<i>S. enterica</i>	Proteobacteria
<i>K. oxytoca</i>	Proteobacteria
<i>C. glutamicum</i>	Actinobacteria
<i>S. venezuelae</i>	Actinobacteria

Cell-free reaction:

Types of cell-free extract



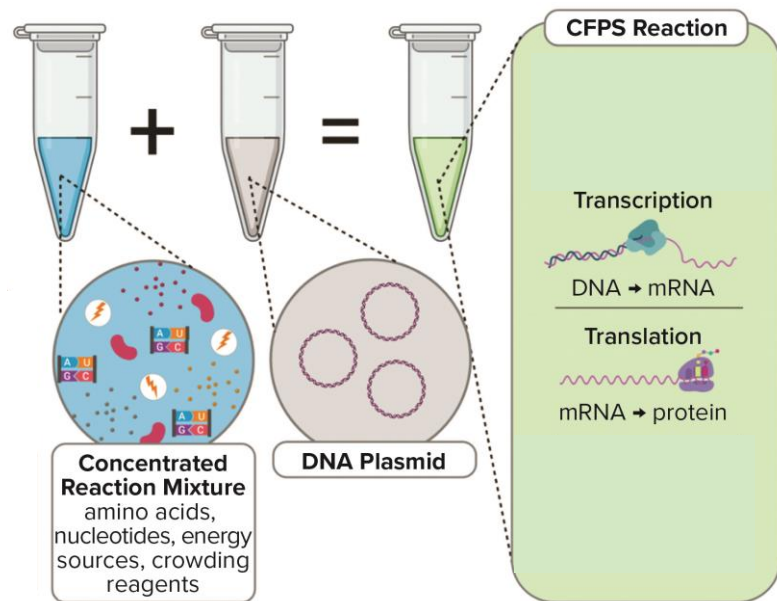
Melinek B, et al. *BioProcess International* (2020)

	Soluble proteins	Membrane proteins	Glycoproteins
Archae / Protozoa	✓		
<i>E.coli</i>	✓		
Wheat Germ	✓		
Yeast	✓		✓ Addition of microsomes
Rabbit Reticulocyte	✓		✓ Addition of exogenous microsomes necessary
Tobacco	✓	✓	✓
Insect	✓	✓	✓
CHO / Human	✓	✓	✓

Anne Zemella et al. *ChemBioChem* 2015

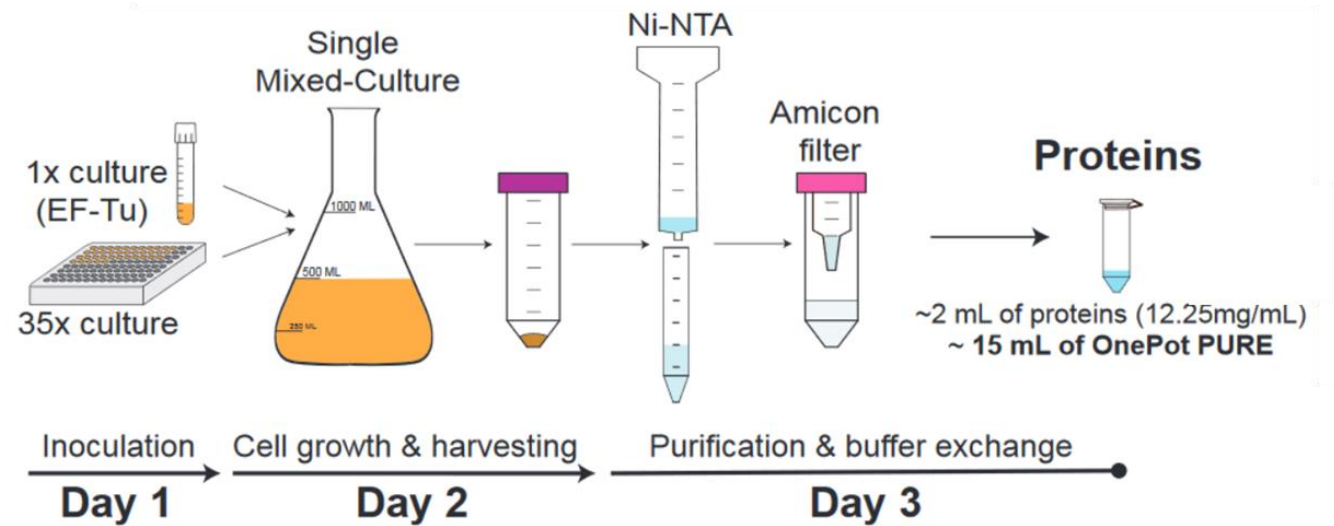
Cell-free reaction:

Alternative cell-free approach, the pure system



Melinek B, et al. *BioProcess International* (2020)

36 PURE protein components produced using the OnePot method + tetra-(His)₆-tagged 70S ribosomes purified using affinity chromatography

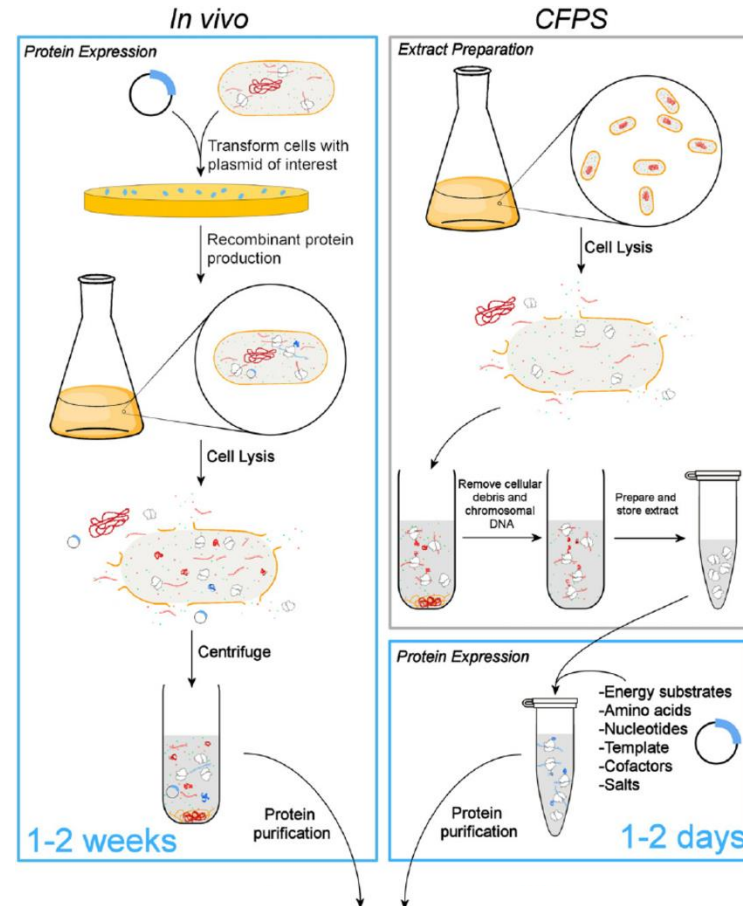


Lavickova, Barbora, et al. *ACS synthetic biology* (2019)

Cell-free reaction:

Advantages of producing protein outside of the cell

- Fast reaction to produce protein
- No need for transformation
- No GMO
- No interaction with a living host
(No mutation, No burden)
- Decoupling effect
(e.g. Metabolic burden vs production burden)
- Low volume of reaction
- Higher level of control: Amount of DNA, Composition of the reaction.
- Work with Linear DNA



Current Applications of Cell-Free Systems in Academia and Industry

Fundamental science: cell-free to understand living cells

Applied Science: Bridging Theory to Practice

Industrial Applications: Transforming Knowledge into Innovation

Cell-free reaction:

Current uses of cell-free system

Fundamental science: cell-free to understand living cells

- Central functions
 - DNA replication
 - Transcription and translation process
 - Metabolism and energy production
- Resource competition *in vitro*
 - Production burden
 - Decouple production and metabolic burden
- Construction hybrid systems
 - Multi-species systems
 - Nonstandard (unnatural) amino acids
 - Eukaryote properties (Glycolysation)
- Constructing artificial cells

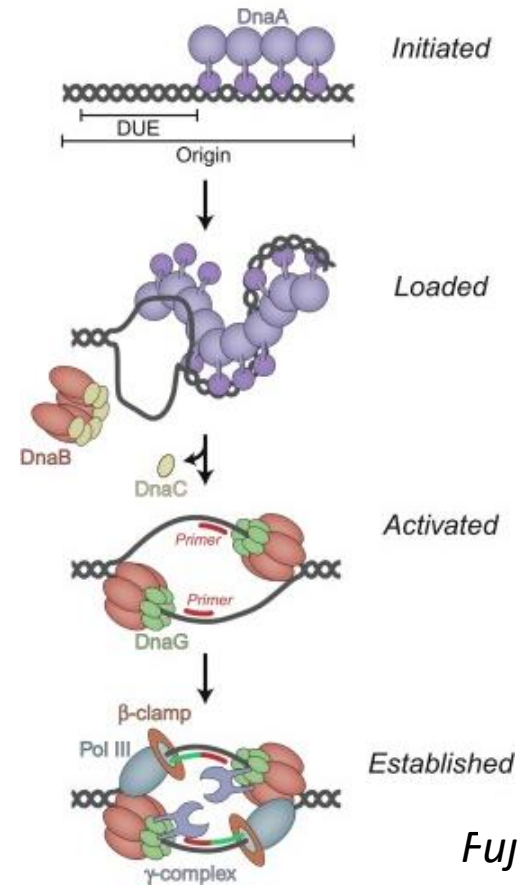
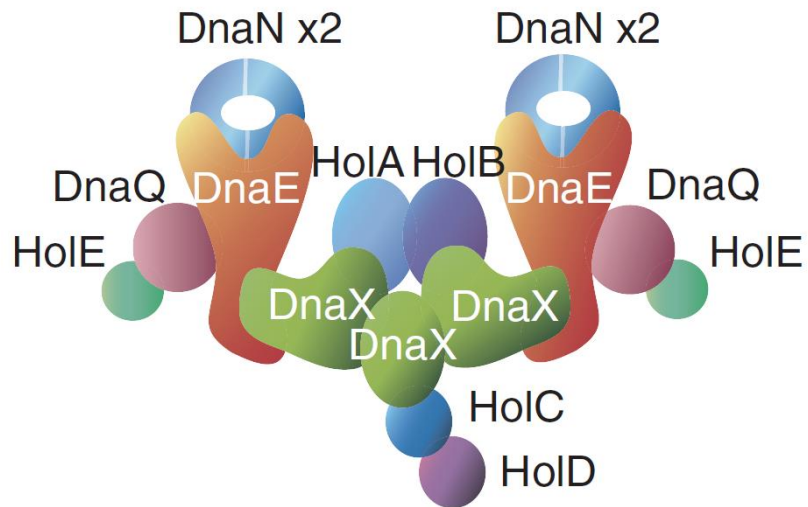
Cell-free reaction:

Fundamental science: cell-free to understand living cells

- Central functions

DNA replication

DNA replication system reconstructed by expressing 13 central genes encoding initiator (*dnaA*), DNA helicase (*dnaB*), helicase loader (*dnaC*), RNA primase (*dnaG*) and DNA polymerase III.



Omitting *dnaC* (one of the 13 genes) is the most important effect.

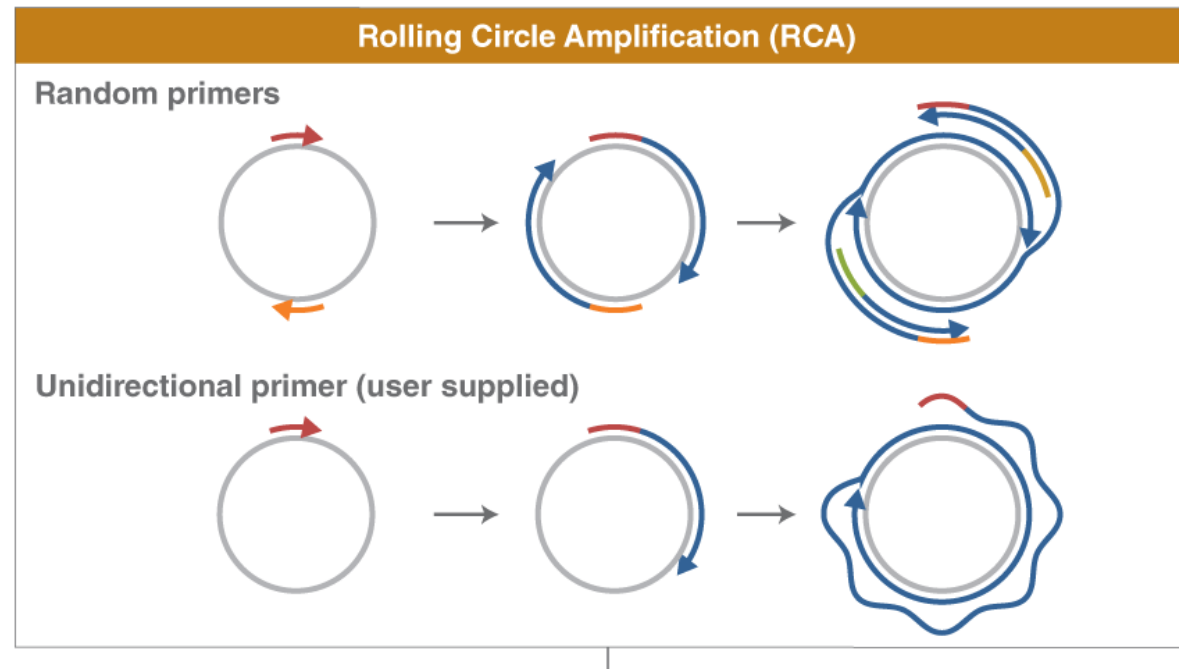
Cell-free reaction:

Fundamental science: cell-free to understand living cells

- Central functions

DNA replication

DNA replication system reconstructed by expressing 1 gene of the bacteriophage phi 29 DNA replication .



Use for replication of large DNA genomes

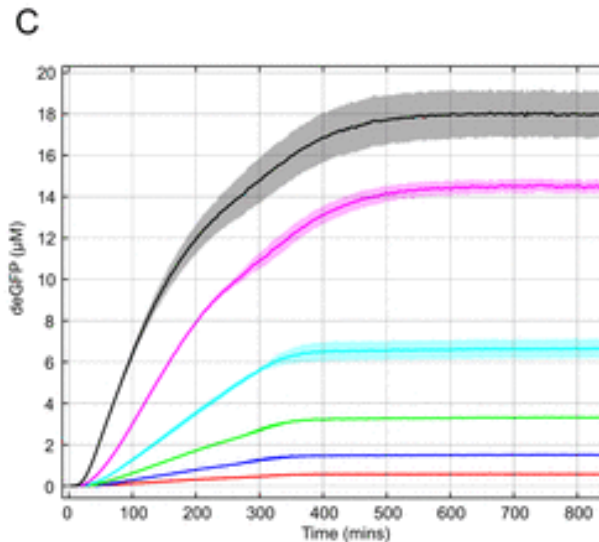
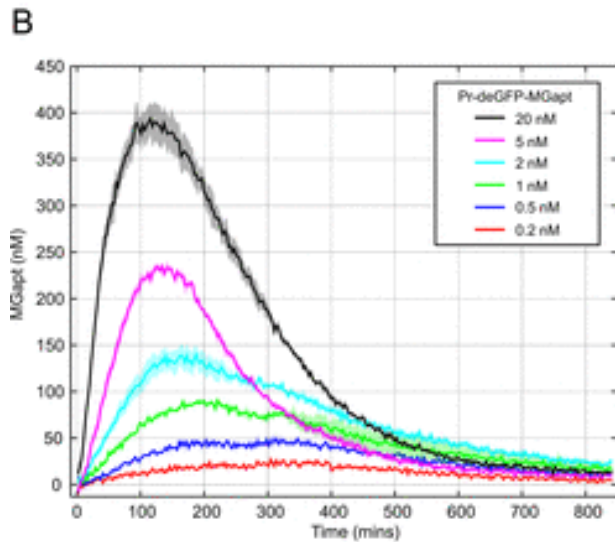
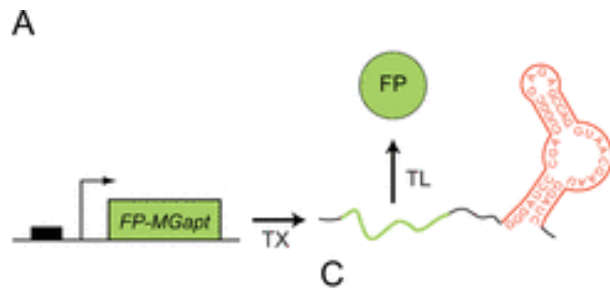
Libicher, K, nat comms (2020)

Cell-free reaction:

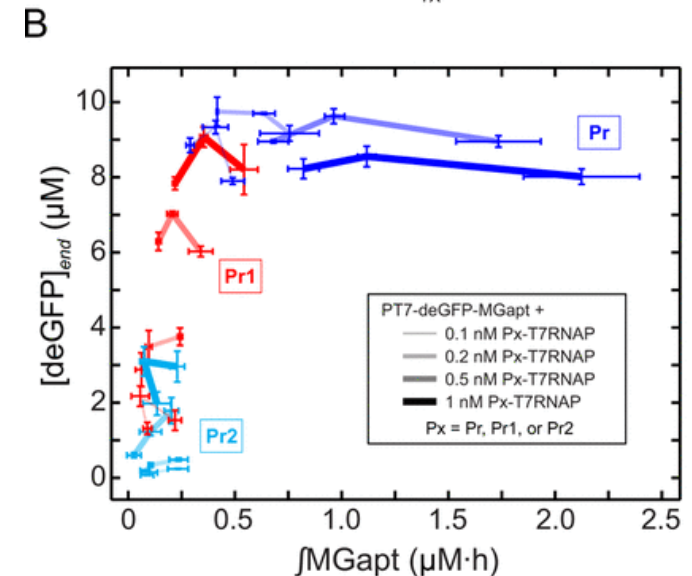
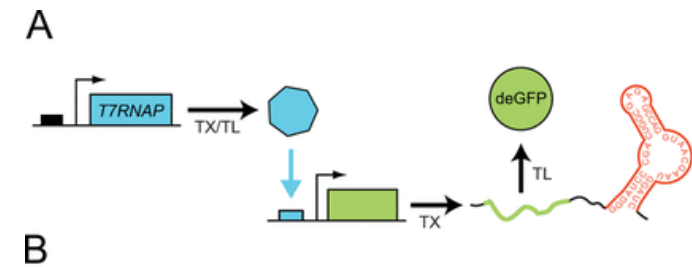
Fundamental science: cell-free to understand living cells

- Central functions

Transcription process: T7 polymerase



Gene circuit performance characterization



Cell-free reaction:

Fundamental science: cell-free to understand living cells

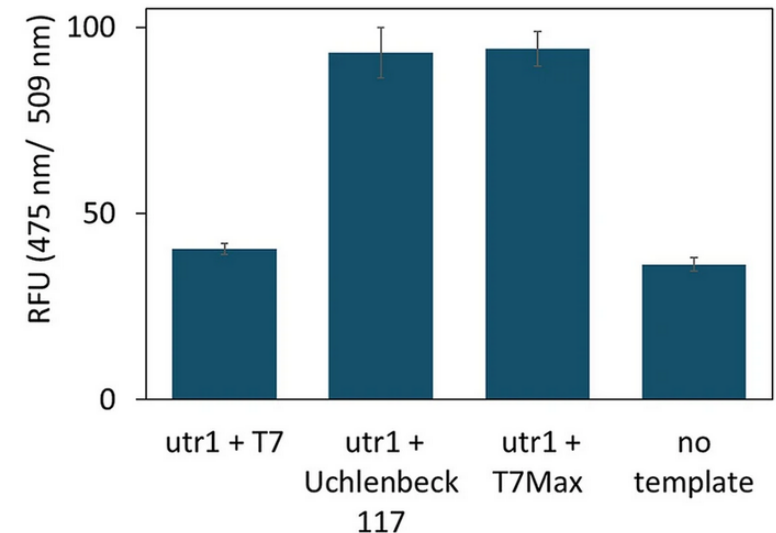
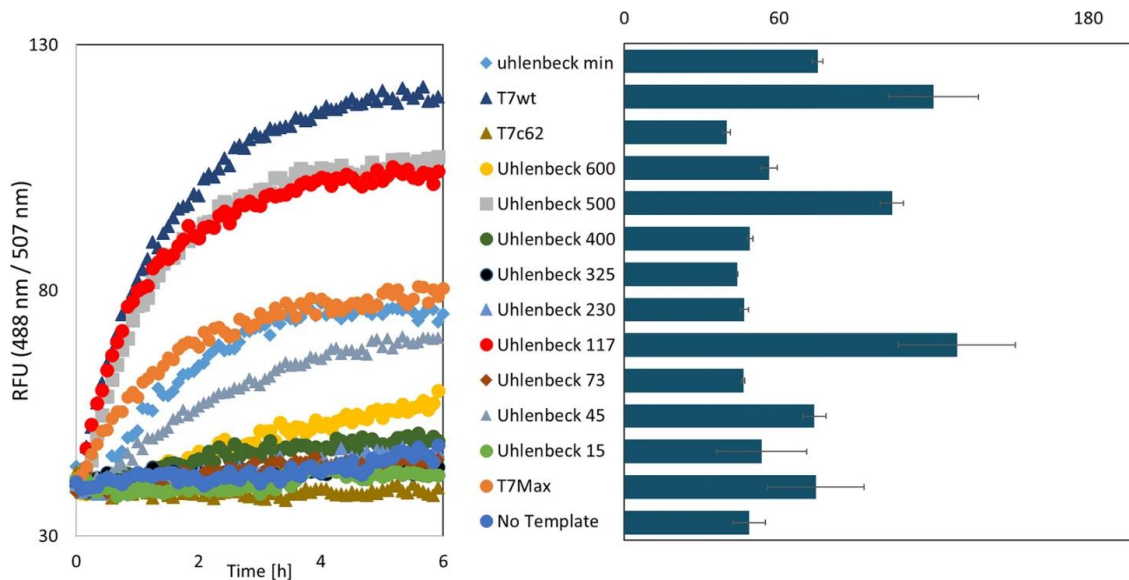
- Central functions

Transcription process: modified promoter to recruits more T7 polymerase

Control environment: Fixed purified T7 RNA polymerase concentration, Fixed plasmid concentration, Fixed lysate and buffer concentration (ribosome, NTPs, Amino Acides etc..)

One changing elements = promoter sequence

Specific effects measured = Protein production, RNA production and DNA template degradation

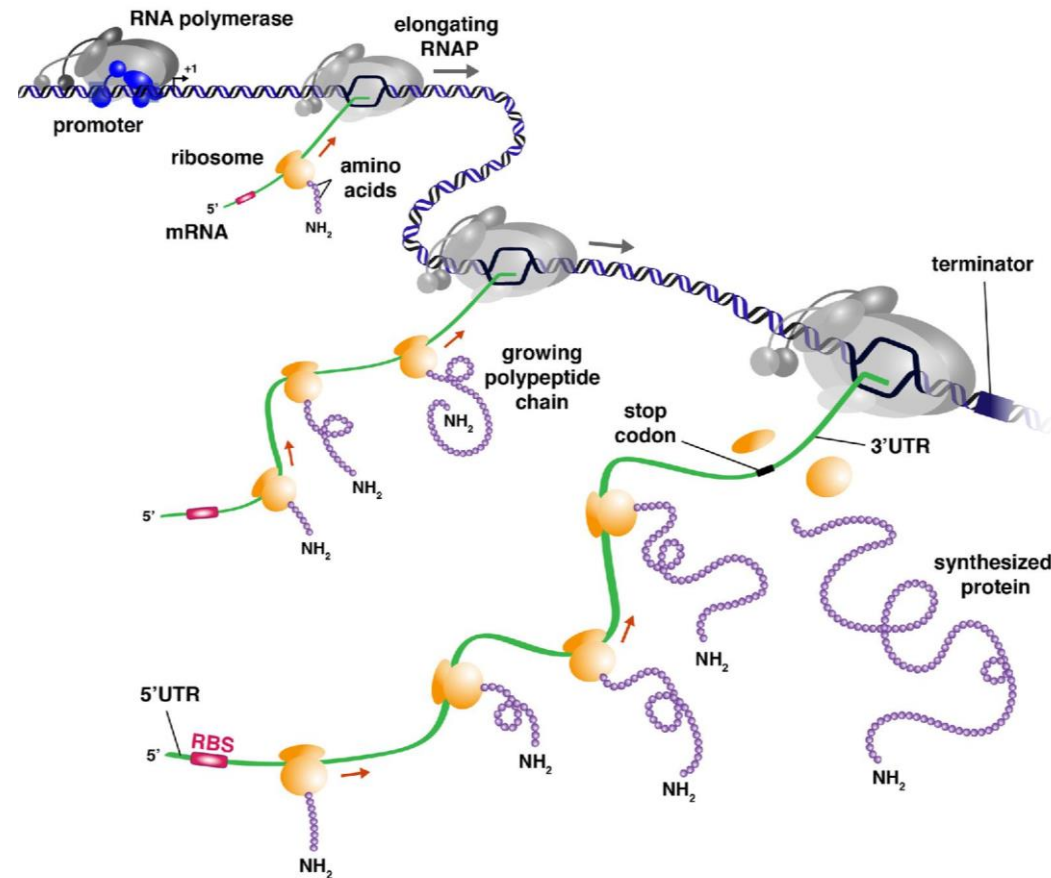


Cell-free reaction:

Fundamental science: cell-free to understand living cells

- Central functions

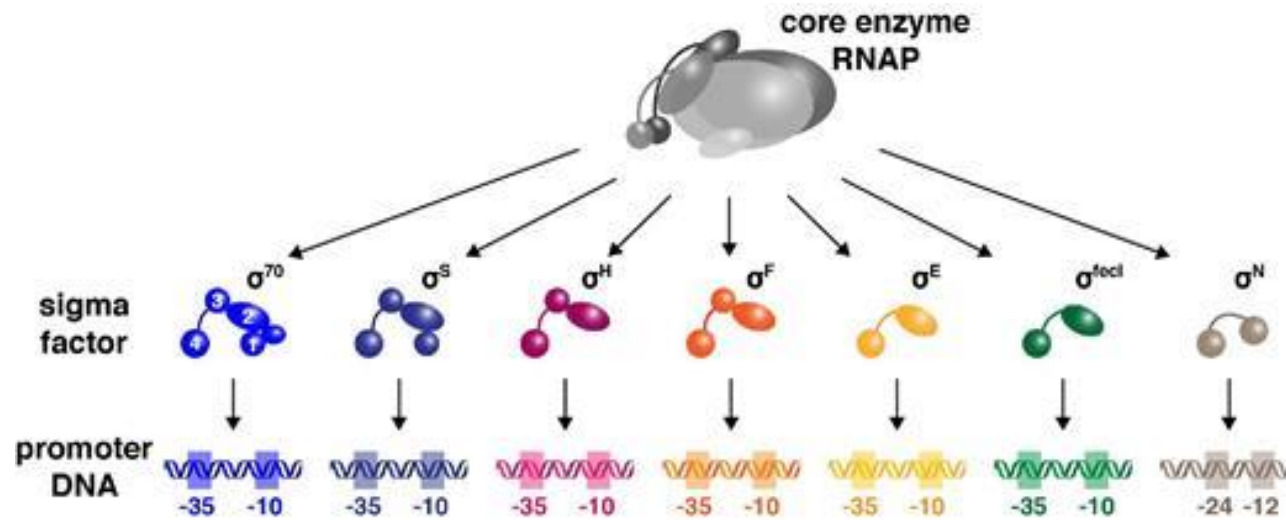
Transcription



Cell-free reaction:

Fundamental science: cell-free to understand living cells

- Central functions
- Transcription



σ-factor	Function	Group
σ^D/σ^{70}	Housekeeping genes	1
σ^S/σ^{38}	Stationary phase	2
σ^H/σ^{32}	Heat shock response	3
σ^F/σ^{28}	Motility genes	3
σ^E/σ^{24}	Extracytoplasmic function	4
σ^{Ecd}/σ^{19}	Extracytoplasmic function	4
σ^N/σ^{54}	Nitrogen metabolism	

Cell-free reaction:

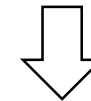
Fundamental science: cell-free to understand living cells

- Central functions

Translation: Optimizing translation process

Component	Concentration			
Mg-glutamate (mM)	0.4	1.2	2	4
K-glutamate (mM)	8	24	40	80
Amino Acid (mM)	0.15	0.45	0.75	1.5
tRNA (mg/ml)	0.02	0.06	0.1	0.2
CoA (mM)	0.026	0.078	0.13	0.26
NAD (mM)	0.033	0.099	0.165	0.33
cAMP (mM)	0.075	0.225	0.375	0.75
Folinic Acid (mM)	0.0068	0.0204	0.034	0.068
Spermidine (mM)	0.1	0.3	0.5	1
3-PGA (mM)	3	9	15	30
NTP (mM)	0.15	0.45	0.75	1.5

Combinatorial space = $4^{11} = 4\,194\,304$ compositions



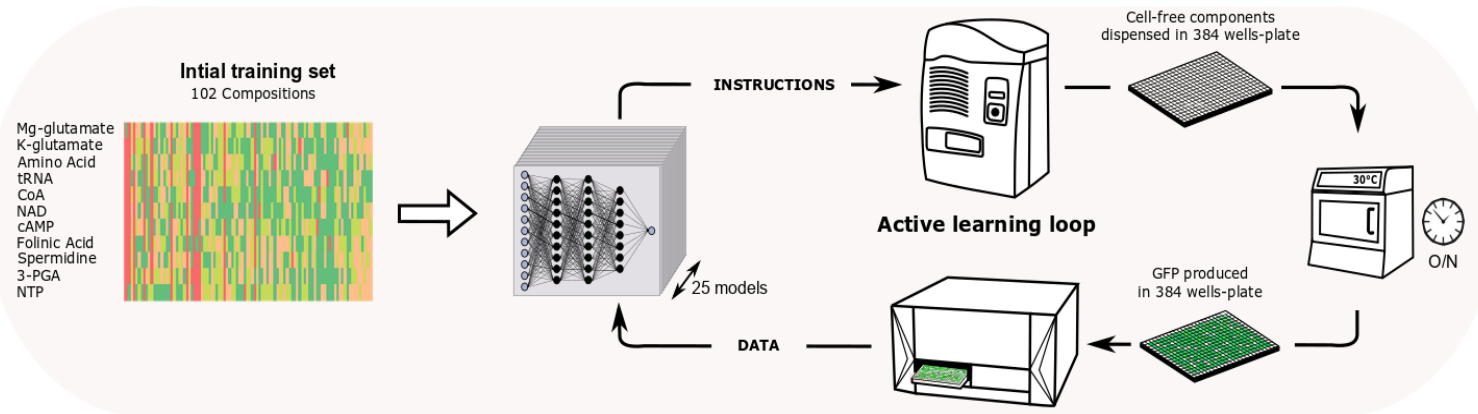
- Can we improve protein production for a specific lysate without increasing the price of cell-free reaction?
- Can we provide efficient predictions of protein production?
- Can we highlight the critical parameters involved in protein production?

Cell-free reaction:

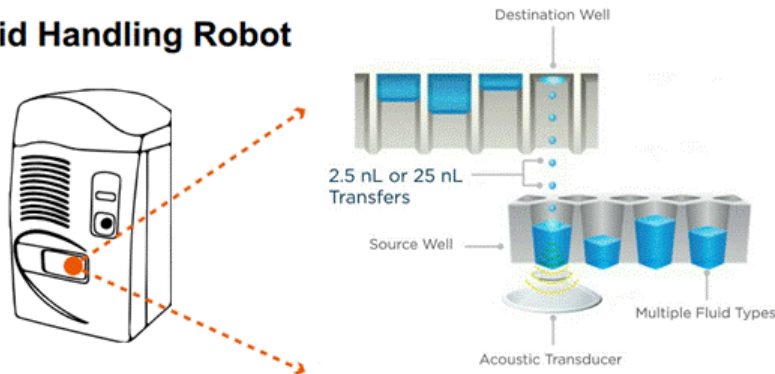
Fundamental science: cell-free to understand living cells

- Central functions

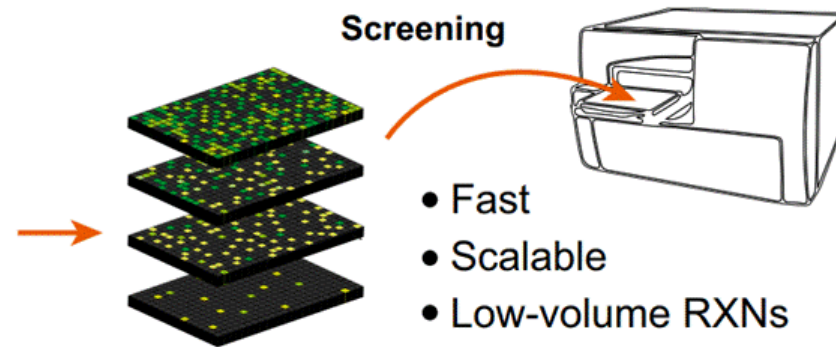
Translation: Optimizing translation process



Liquid Handling Robot



Screening

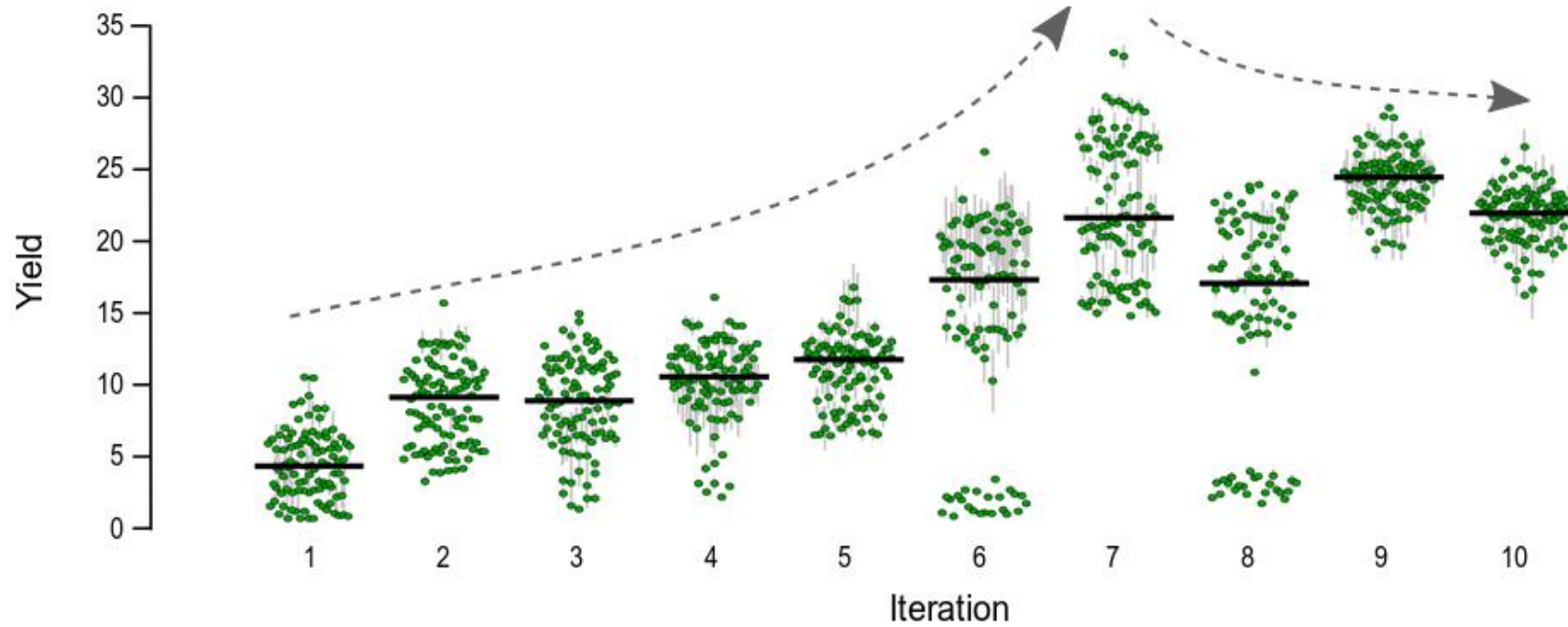


Cell-free reaction:

Fundamental science: cell-free to understand living cells

- Central functions

Translation: Optimizing translation process

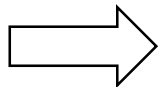
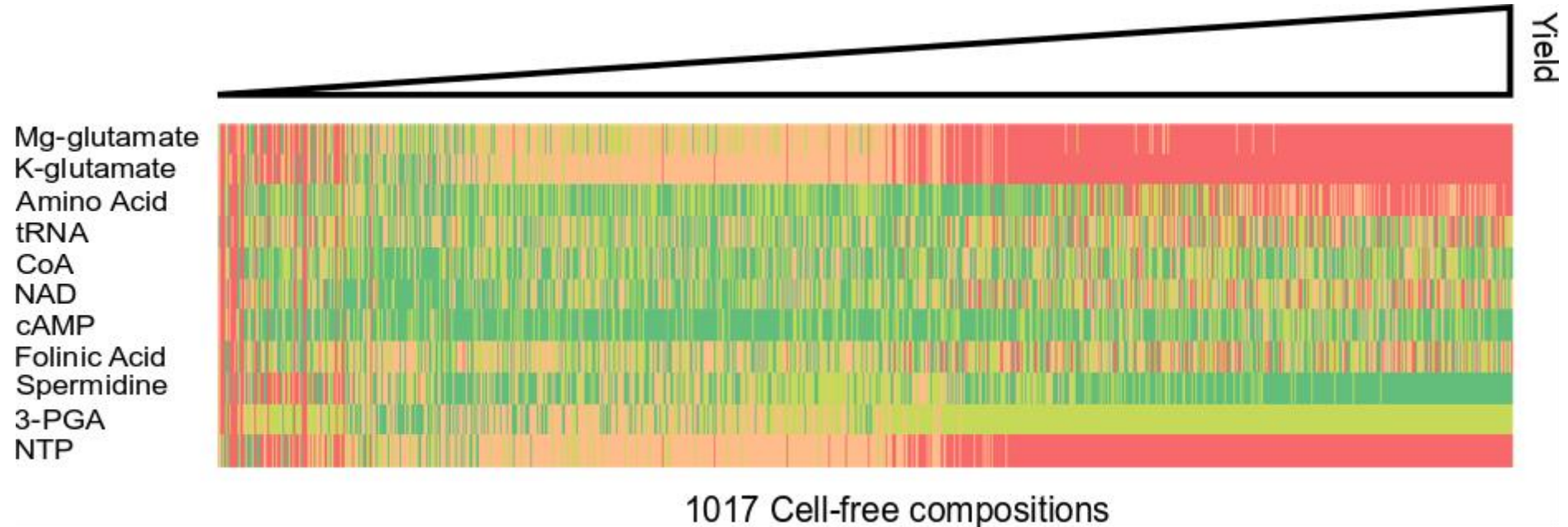


Cell-free reaction:

Fundamental science: cell-free to understand living cells

- Central functions

Translation: Optimizing translation process



Components that improve yield at high concentration: Mg-glutamate, K-glutamate, Amino Acids and NTPs

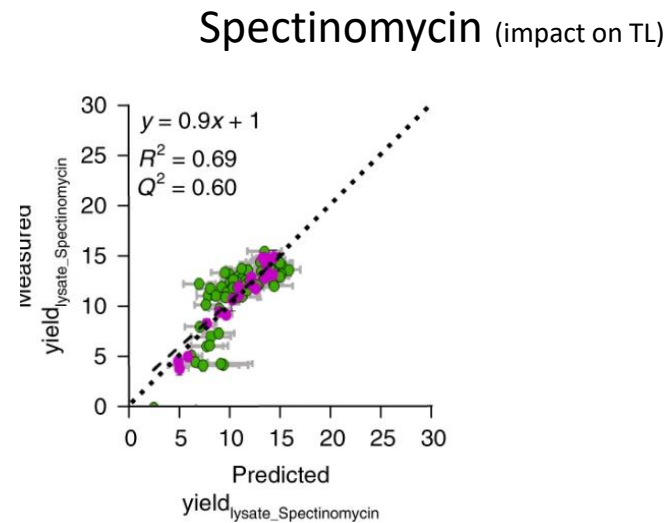
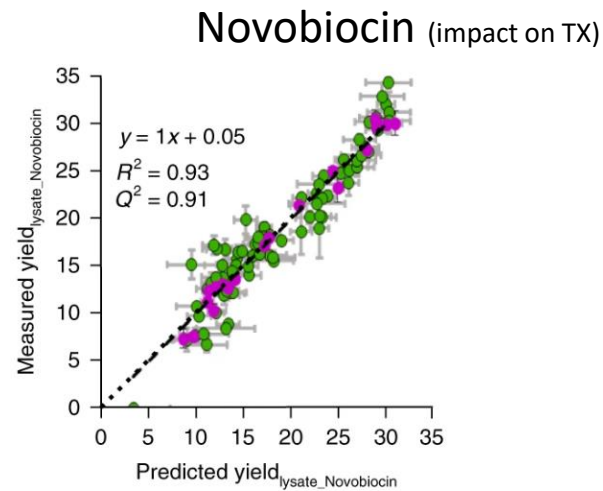
Components that improve yield at low concentration: Spermidine, 3-PGA

Cell-free reaction:

Fundamental science: cell-free to understand living cells

- Central functions

Translation: Optimizing translation process



Transcription vs translation:

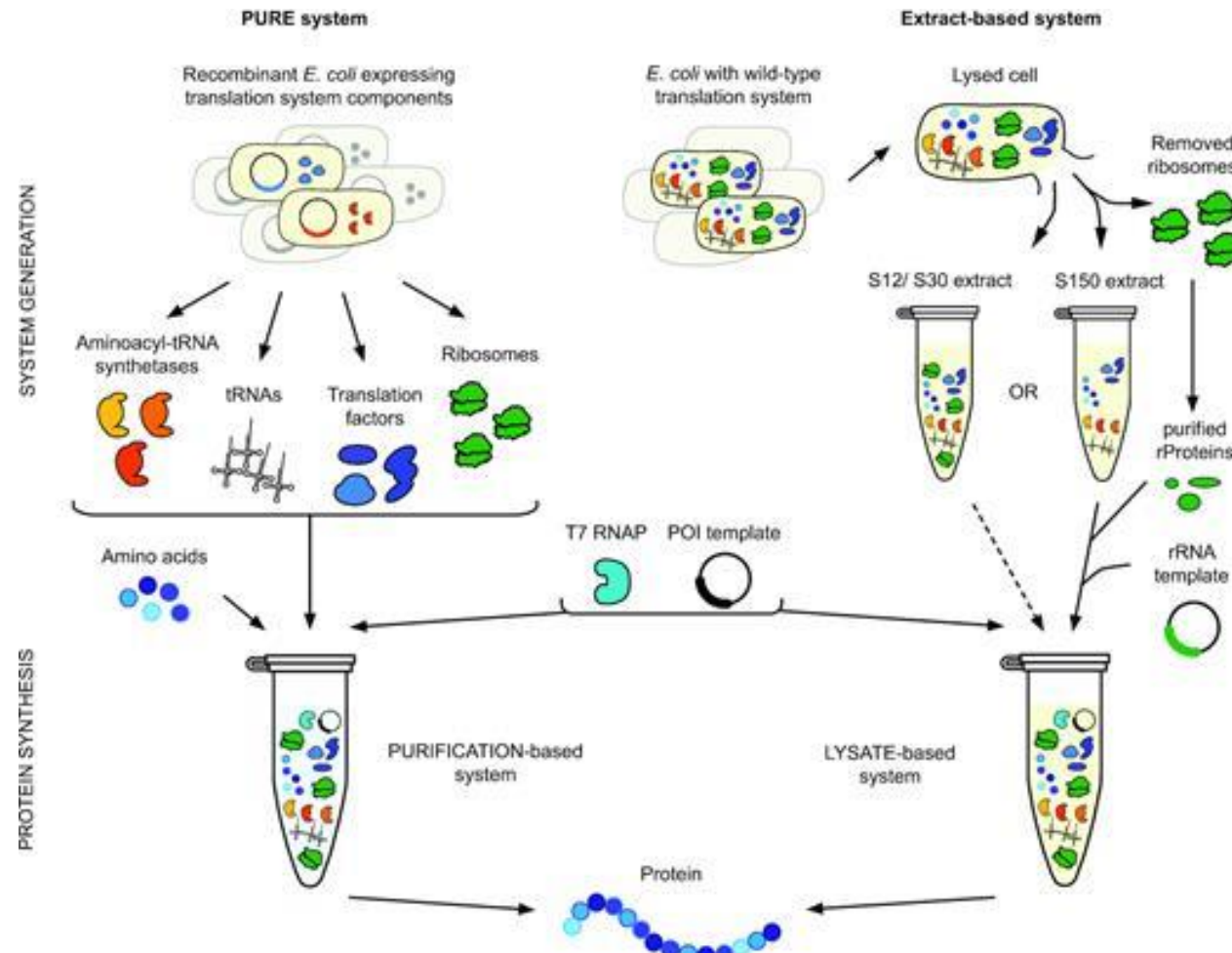
Our data suggests that we mainly improved translation via composition optimization

Cell-free reaction:

Fundamental science: cell-free to understand living cells

- Central functions

Translation: engineering translation machinery

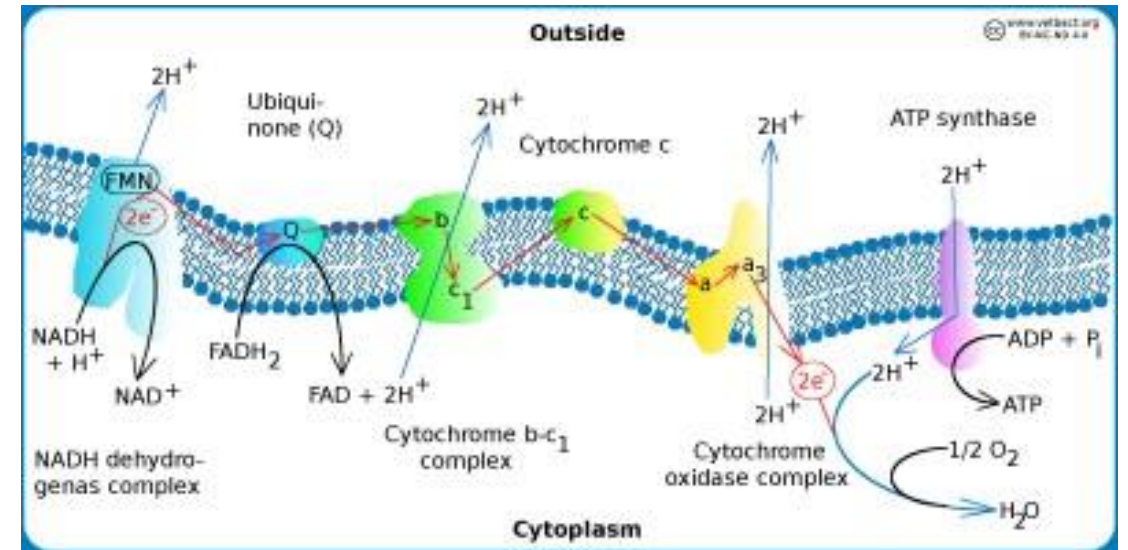
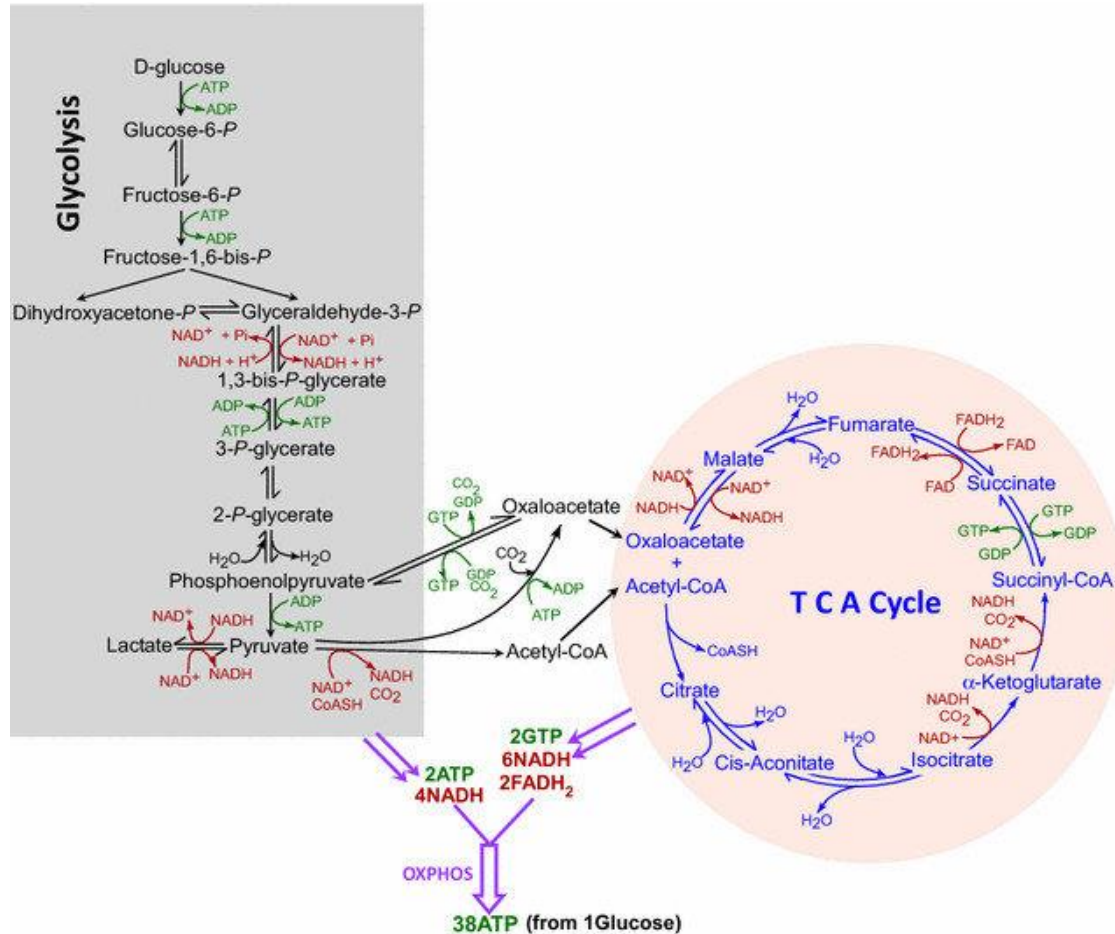


Cell-free reaction:

Fundamental science: cell-free to understand living cells

- Central functions

Metabolism and energy production



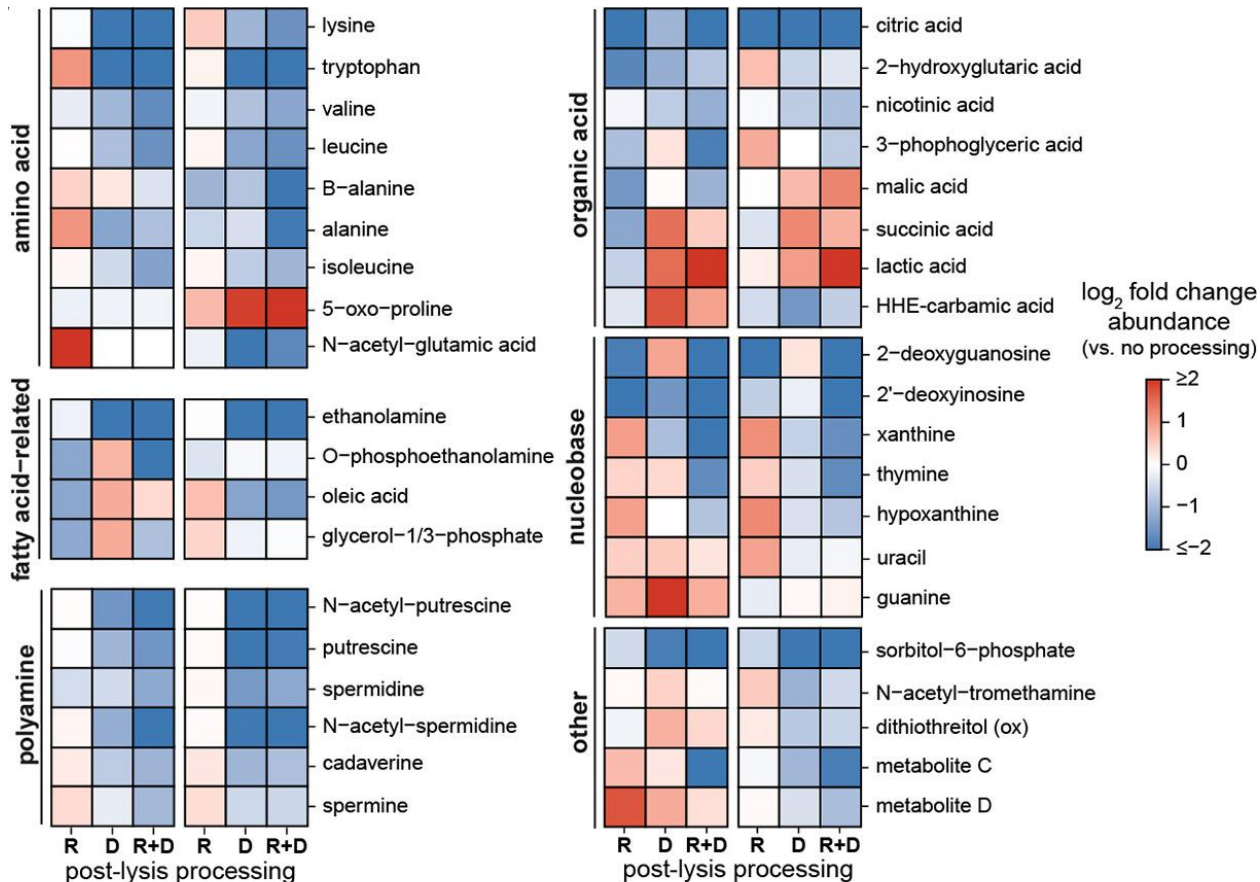
Cell-free reaction:

Fundamental science: cell-free to understand living cells

- Central functions

Metabolism and energy production

Study of composition in metabolites in different cell-free process

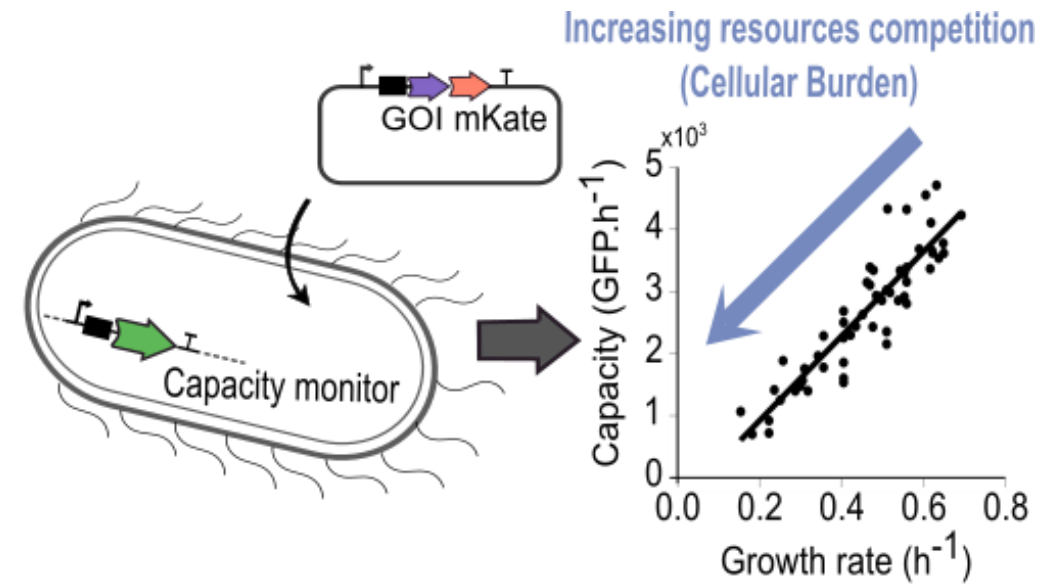
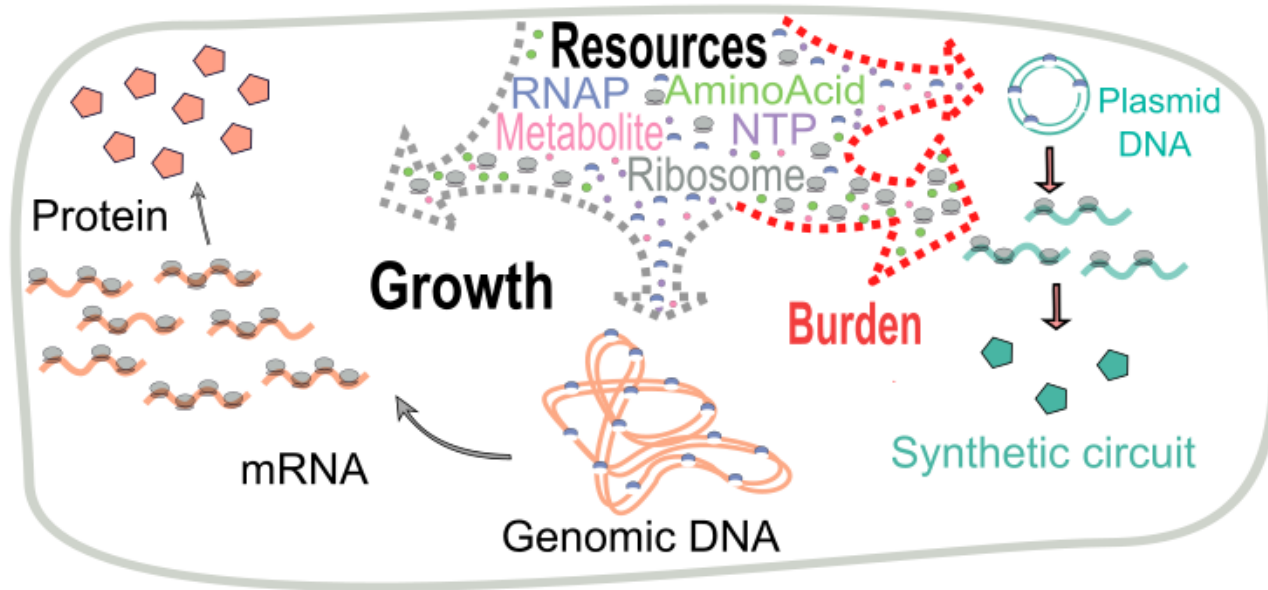


-> improve cell-free efficiency, supplemented with proteins to observe the impact on each part of the metabolism

Cell-free reaction:

Fundamental science: cell-free to understand living cells

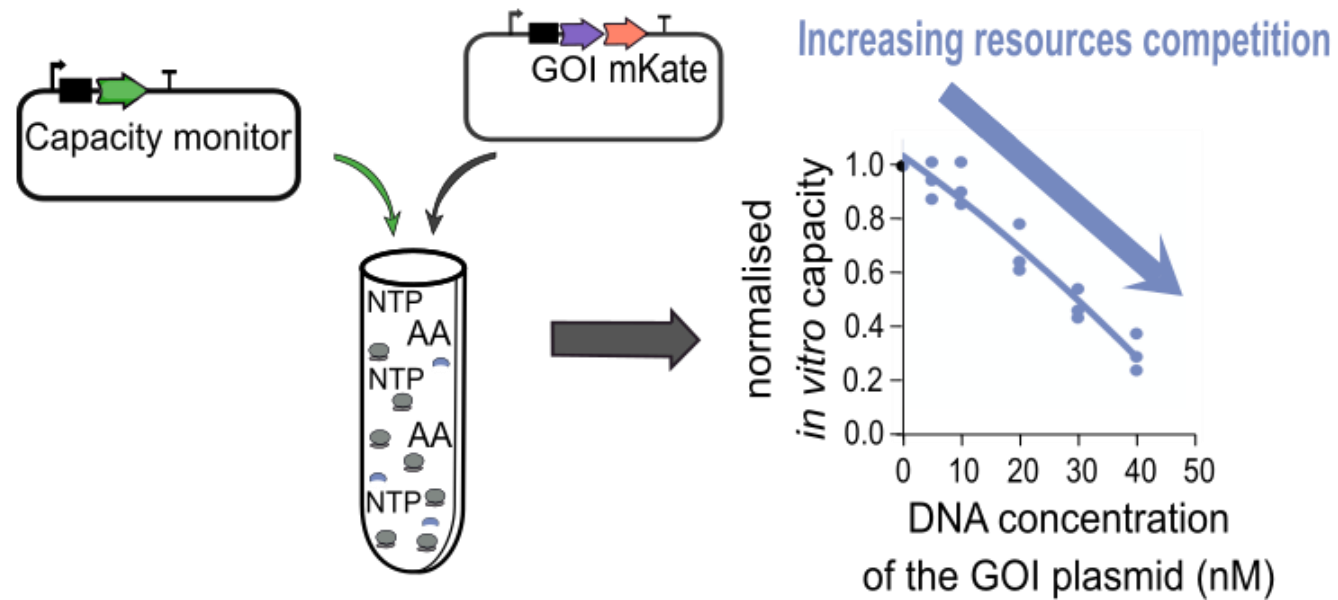
- Resource competition *in vitro*
- Production burden



Cell-free reaction:

Fundamental science: cell-free to understand living cells

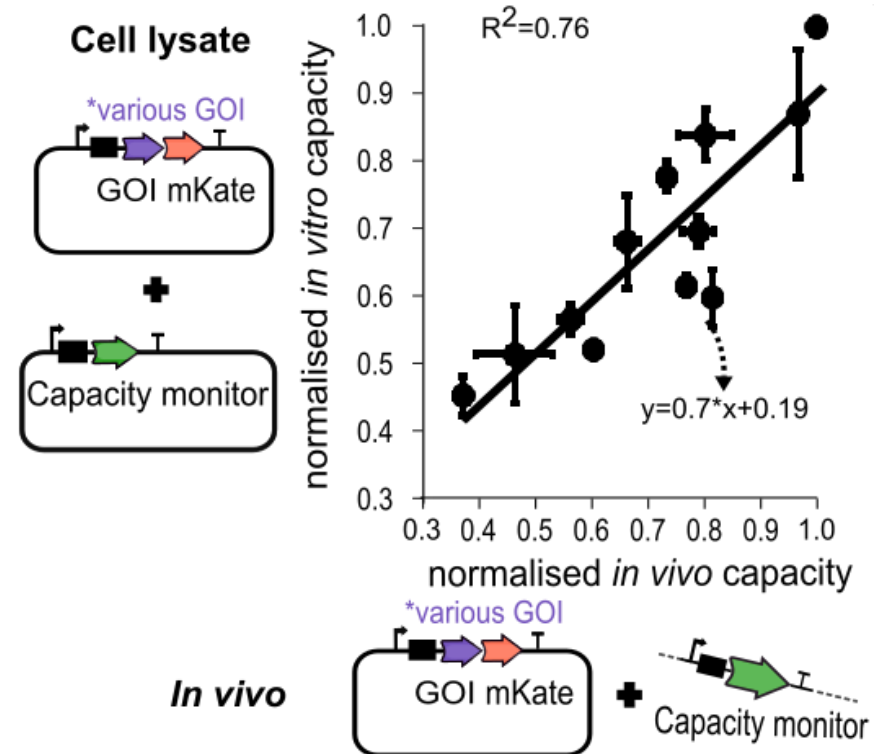
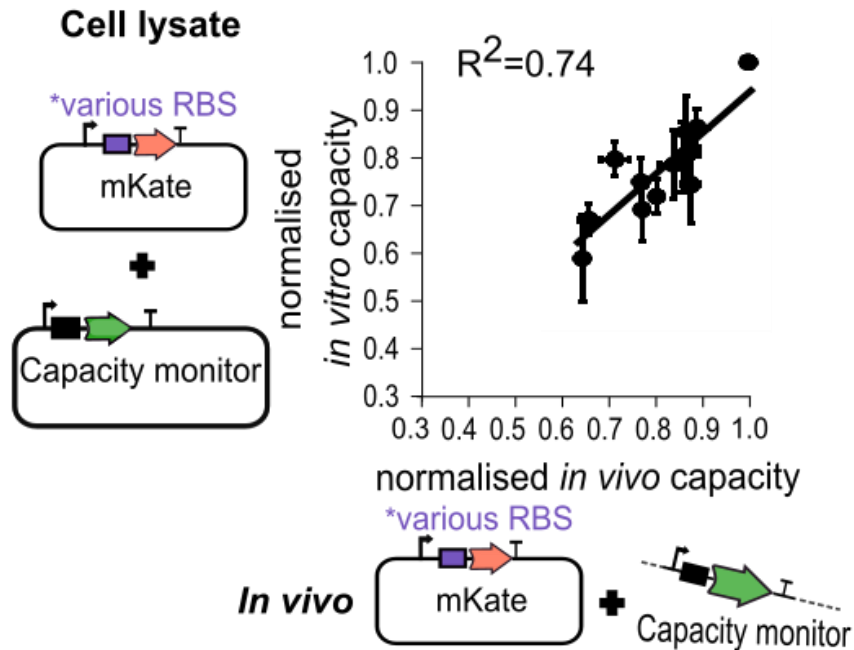
- Resource competition *in vitro*
Production burden



Cell-free reaction:

Fundamental science: cell-free to understand living cells

- Resource competition *in vitro*
Production burden

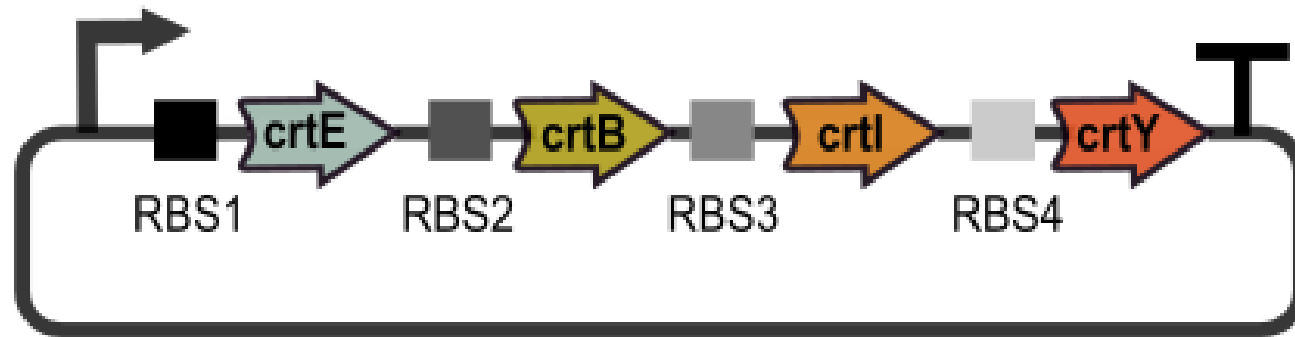


Cell-free reaction:

Fundamental science: cell-free to understand living cells

- Resource competition *in vitro*

Decouple production and metabolic burden



Farnesyl Pyrophosphate

Phytoene

Beta-carotene



Geranylgeranyl Pyrophosphate

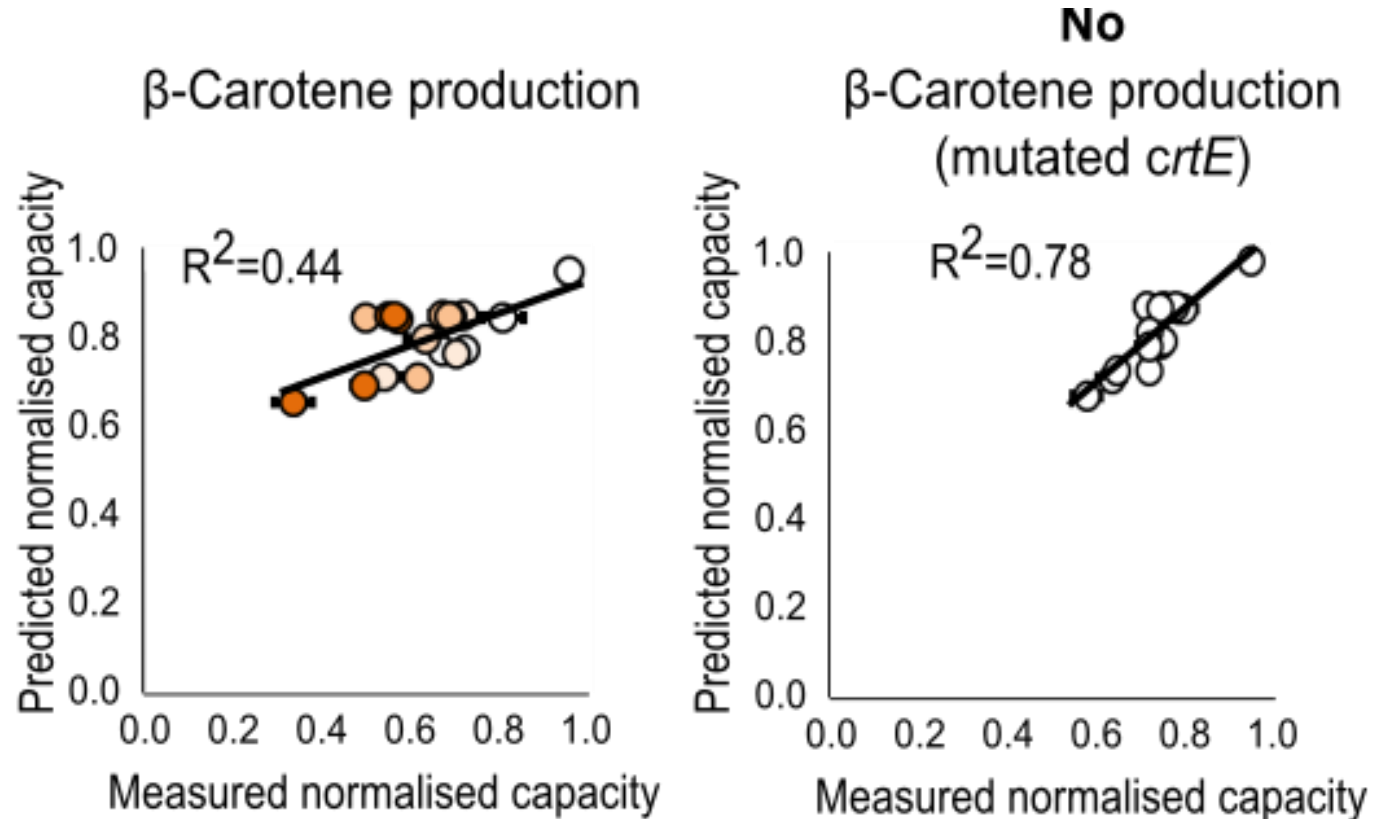
Lycopene

Cell-free reaction:

Fundamental science: cell-free to understand living cells

- Resource competition *in vitro*

Decouple production and metabolic burden

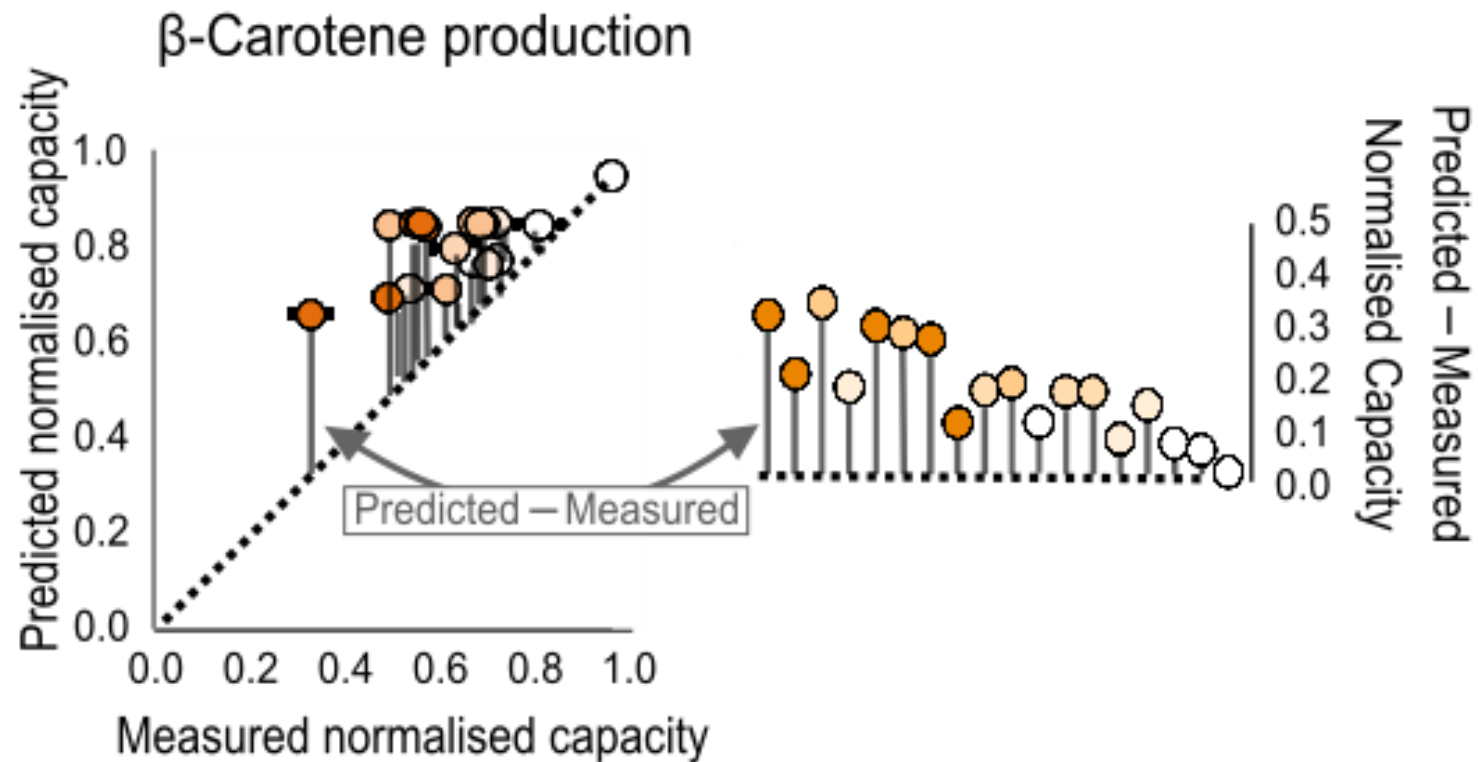


Cell-free reaction:

Fundamental science: cell-free to understand living cells

- Resource competition *in vitro*

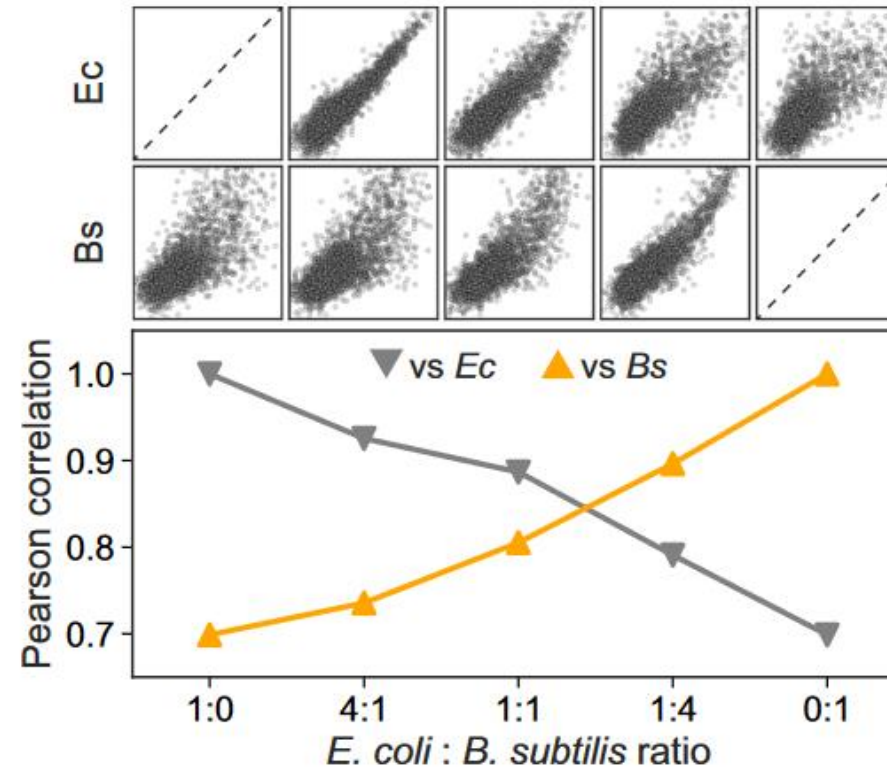
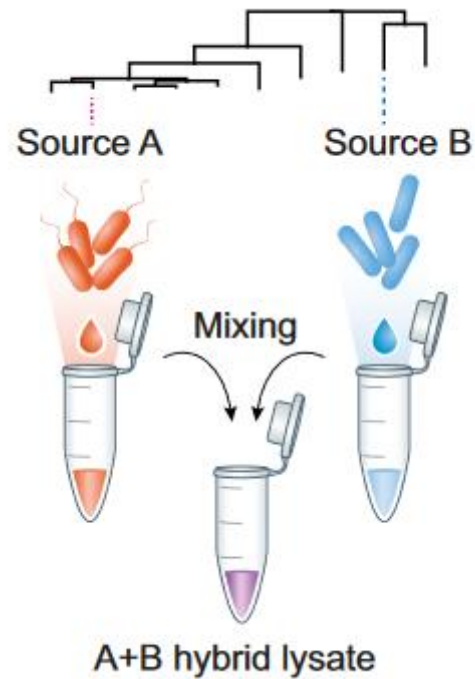
Decouple production and metabolic burden



Cell-free reaction:

Fundamental science: cell-free to understand living cells

- Construction hybrid systems
- Multi-species systems

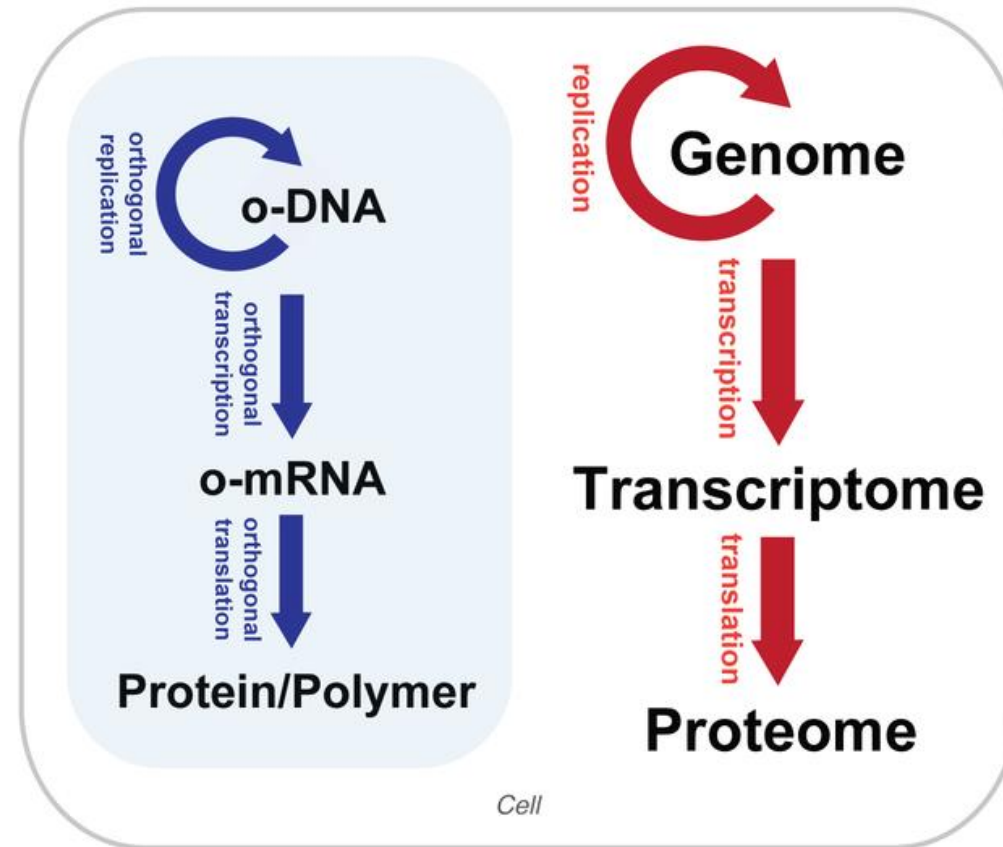


Cell-free reaction:

Fundamental science: cell-free to understand living cells

- Construction hybrid systems

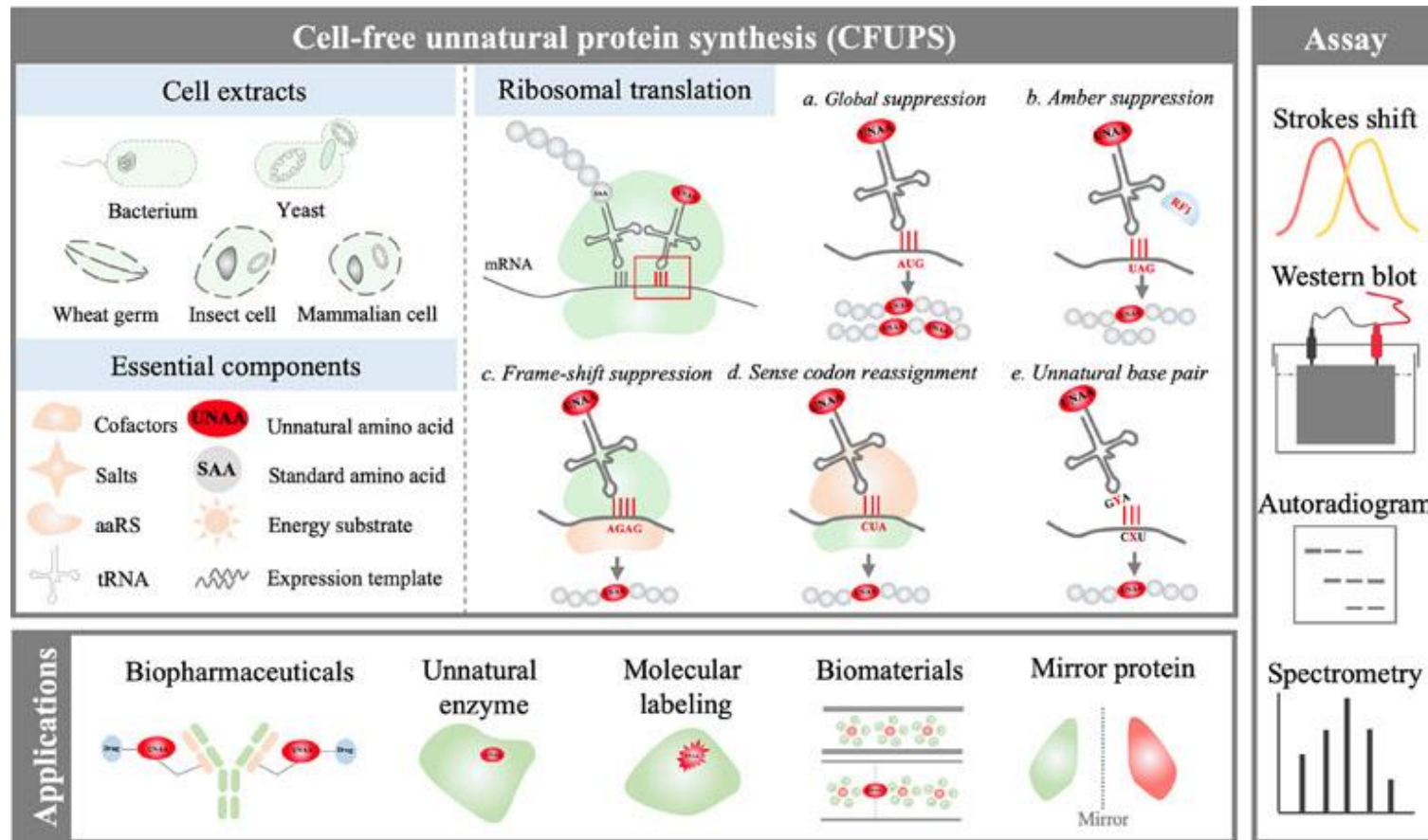
Nonstandard (unnatural) amino acids, more orthogonality for a better predictability



Cell-free reaction:

Fundamental science: cell-free to understand living cells

- Construction hybrid systems
- Nonstandard (unnatural) amino acids

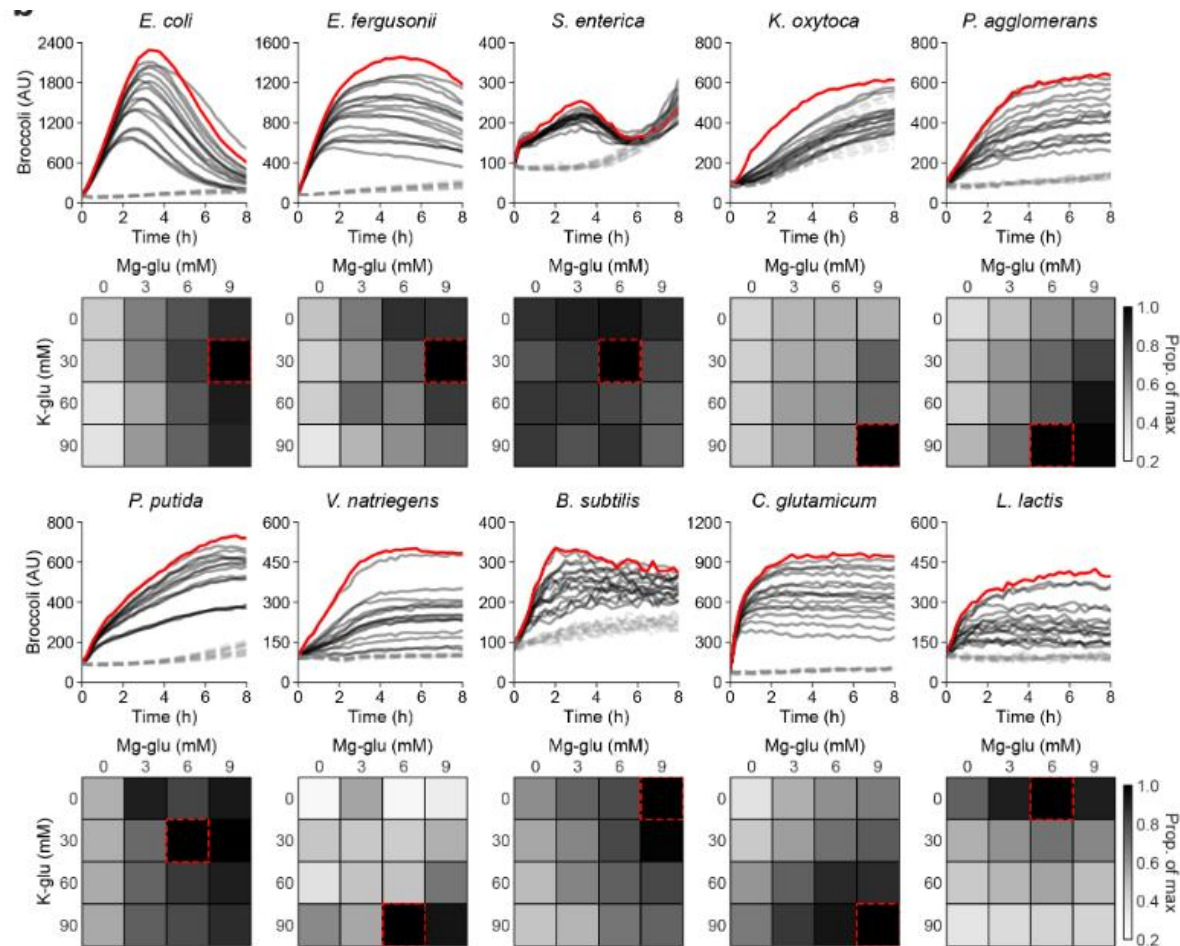


Cell-free reaction:

Fundamental science: cell-free to understand living cells

- Construction hybrid systems

Multi-species systems

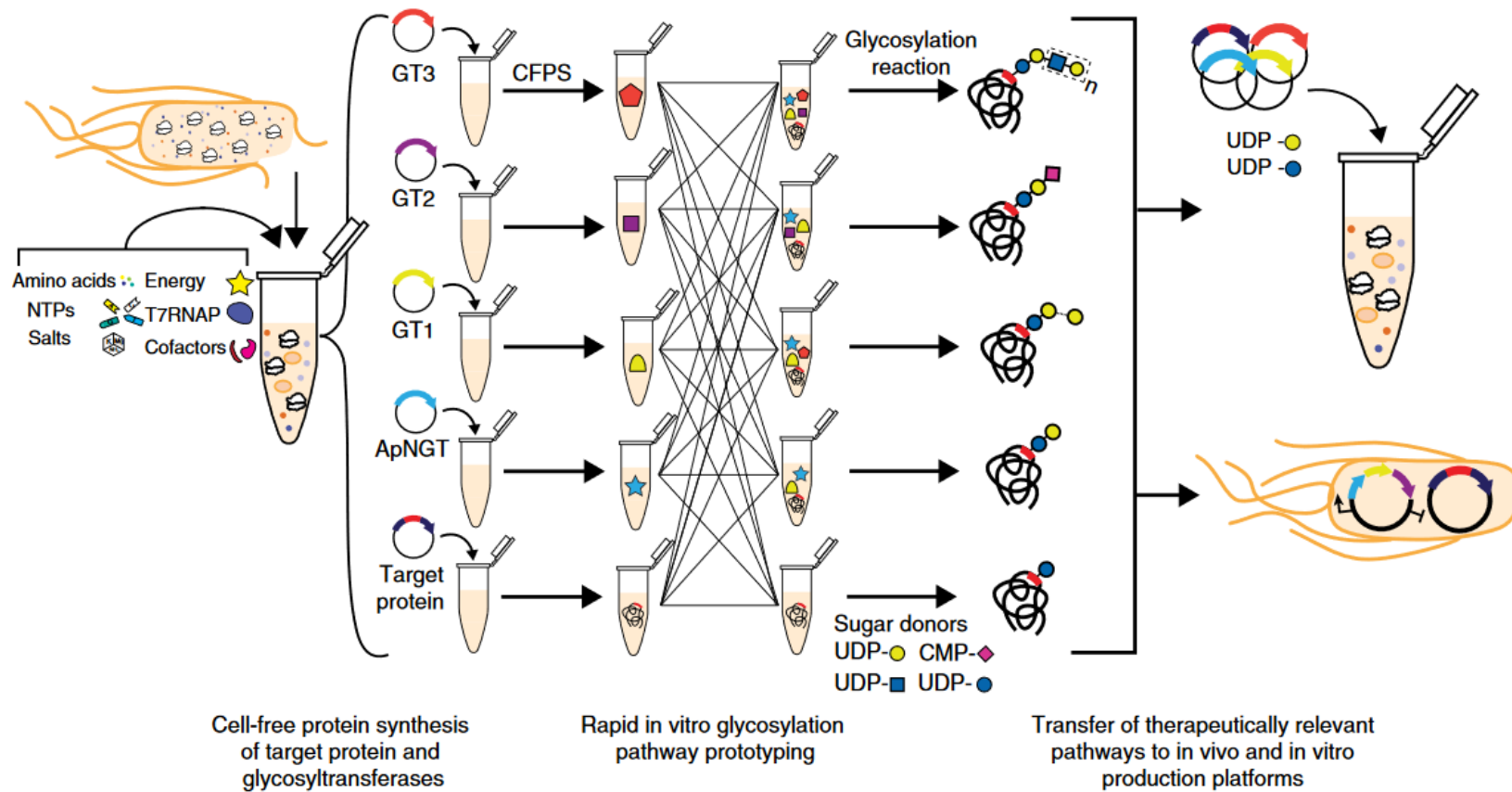


Cell-free reaction:

Fundamental science: cell-free to understand living cells

- Construction hybrid systems

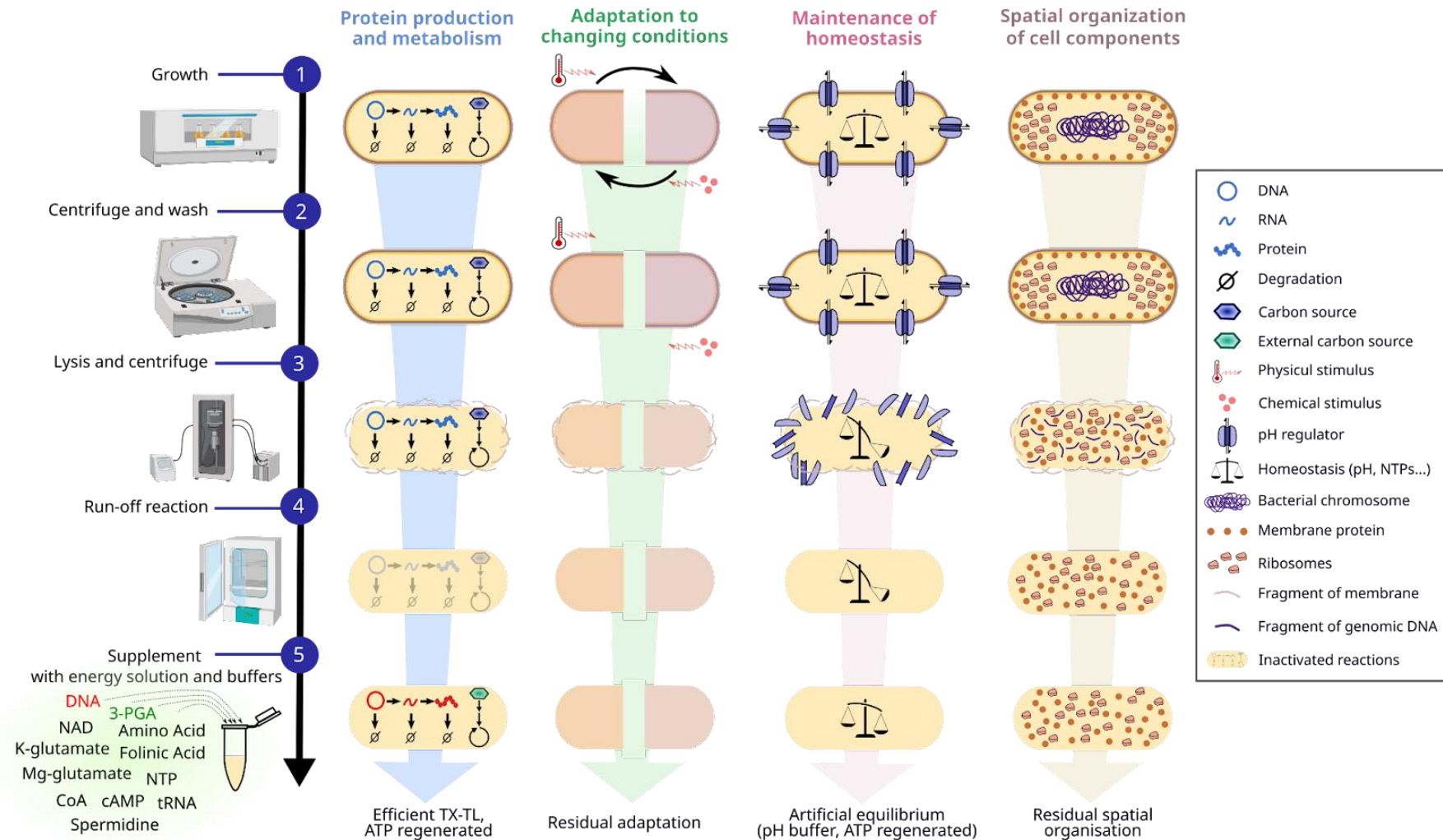
Eukaryote properties (Glycolysation)



Cell-free reaction:

Fundamental science: cell-free to understand living cells

- Constructing artificial cells

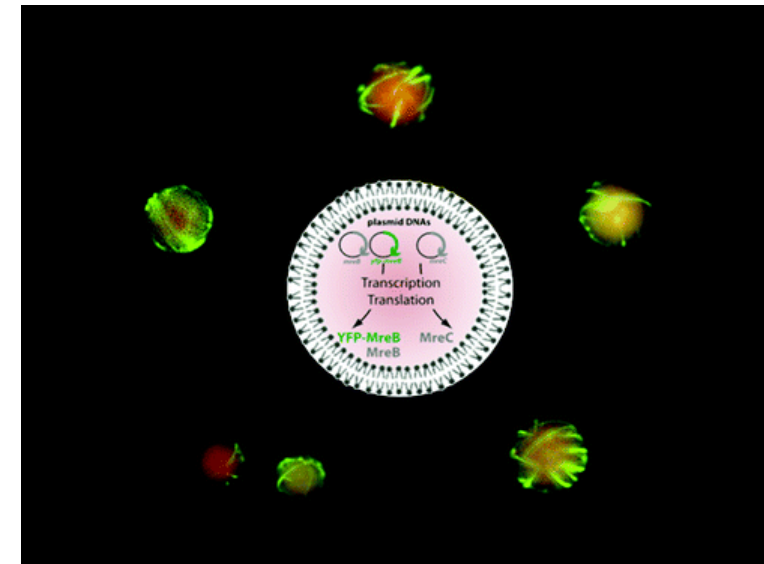
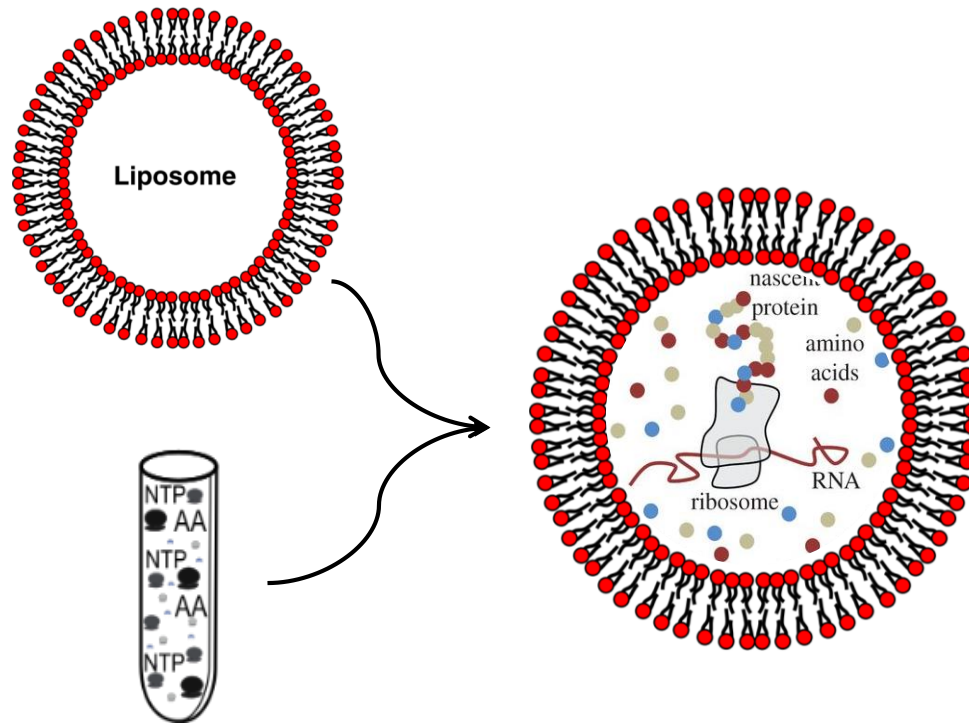


Cell-free reaction:

Fundamental science: cell-free to understand living cells

- Constructing artificial cells

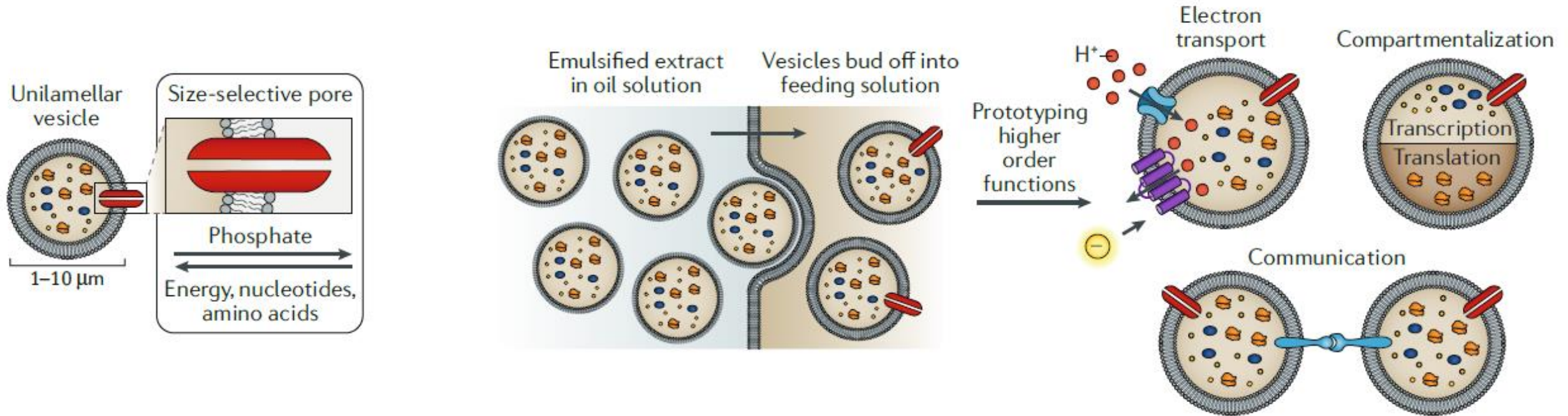
Liposomes



Cell-free reaction:

Fundamental science: cell-free to understand living cells

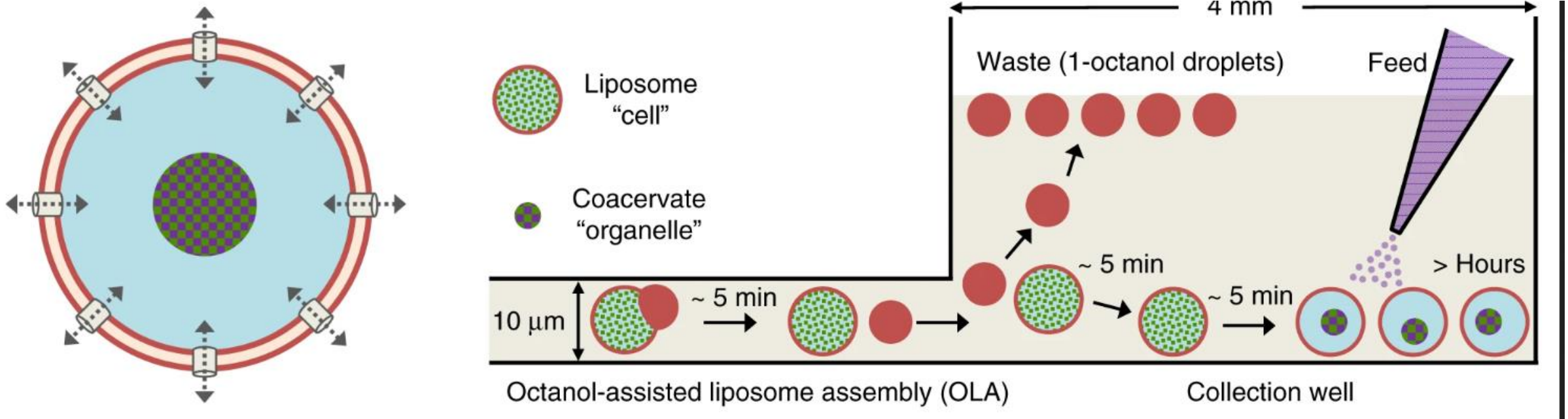
- Constructing artificial cells: Communication / exchange with the environment



Cell-free reaction:

Fundamental science: cell-free to understand living cells

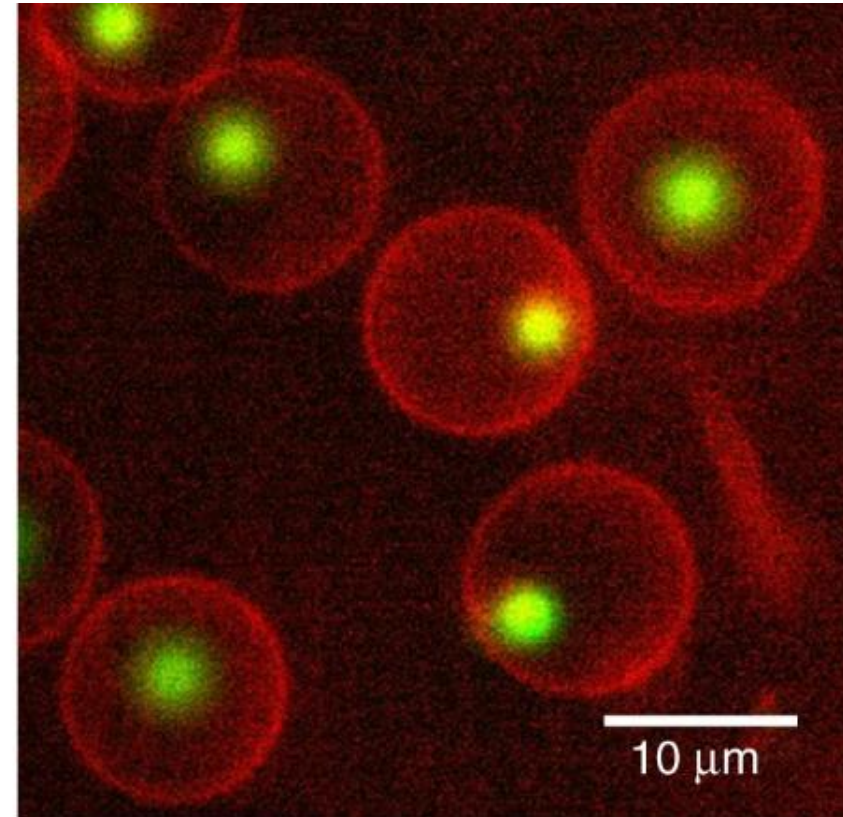
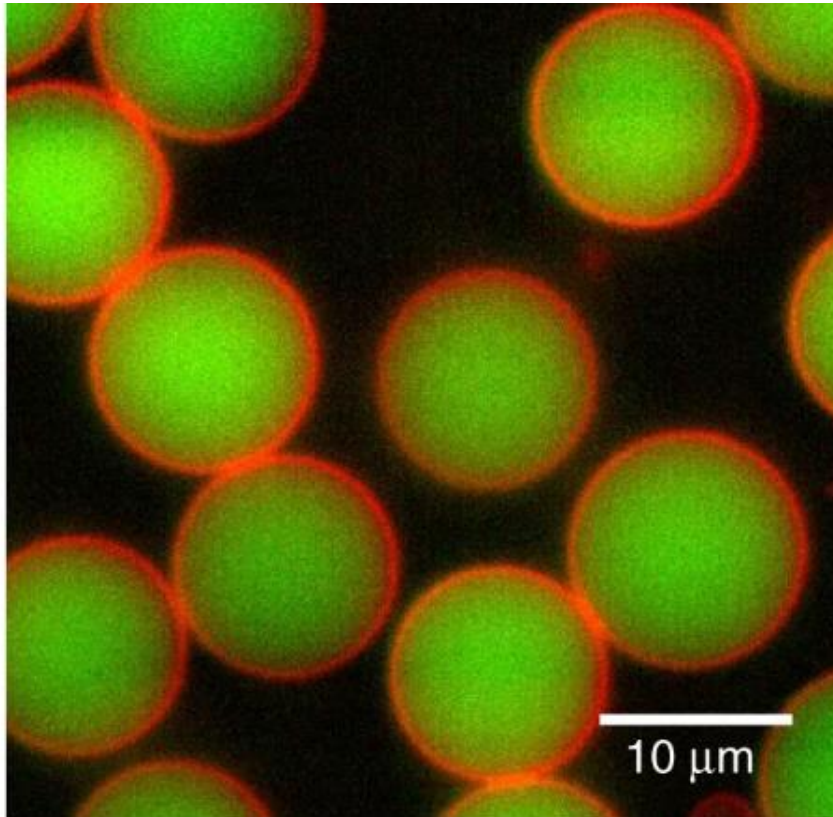
- Constructing artificial cells : compartmentation



Cell-free reaction:

Fundamental science: cell-free to understand living cells

- Constructing artificial cells : compartmentation



Cell-free reaction:

Current uses of cell-free system

Applied Science: Bridging Theory to Practice

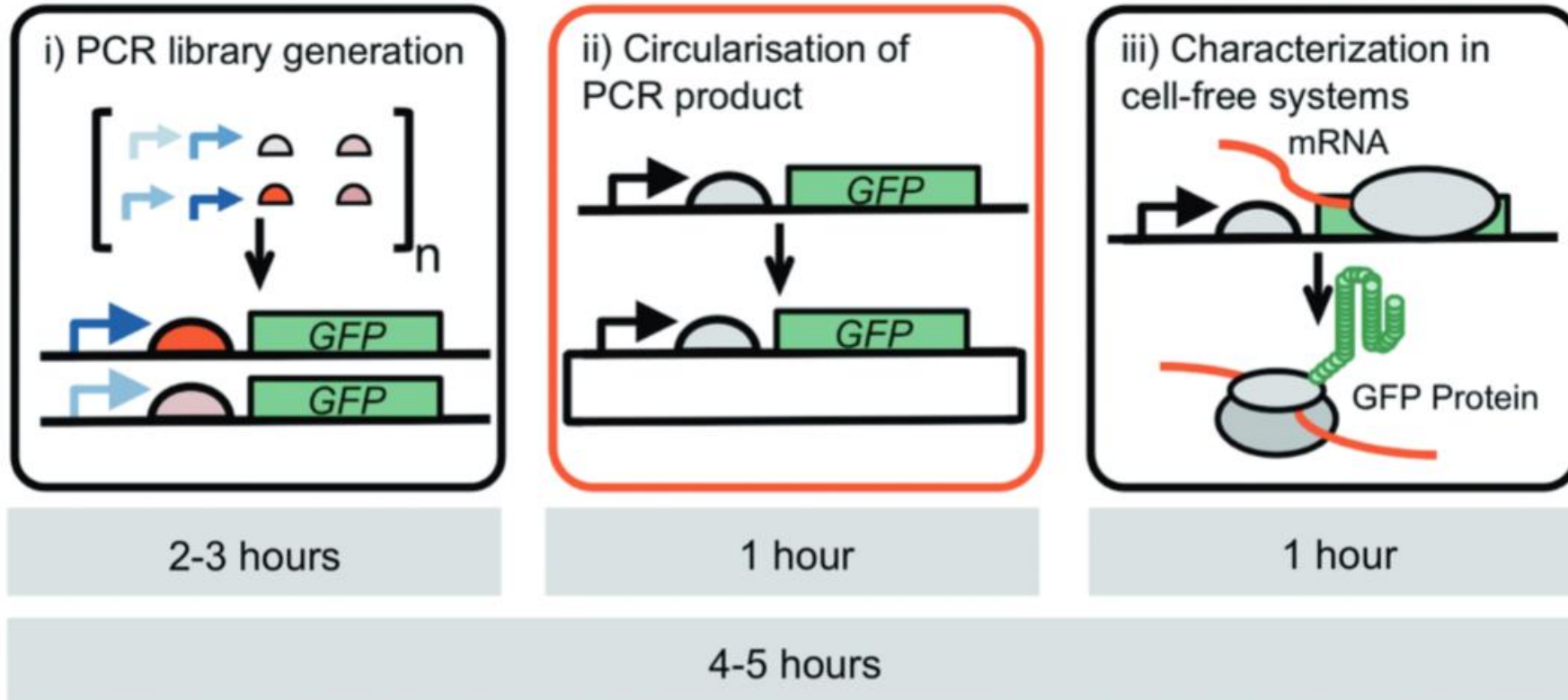
- Prototyping genetic circuits and pathways
 - Genetic parts
 - Metabolic engineering
- Developing biosensors
 - Optimize cell-free biosensor
 - Biocomputing
 - Materials and cell-free
 - Medical diagnostics
 - Pollution diagnostics
- Bio-machine assembly
 - Phages selection
 - Antibodies

Cell-free reaction:

Applied Science: Bridging Theory to Practice

- Prototyping genetic circuits and pathways

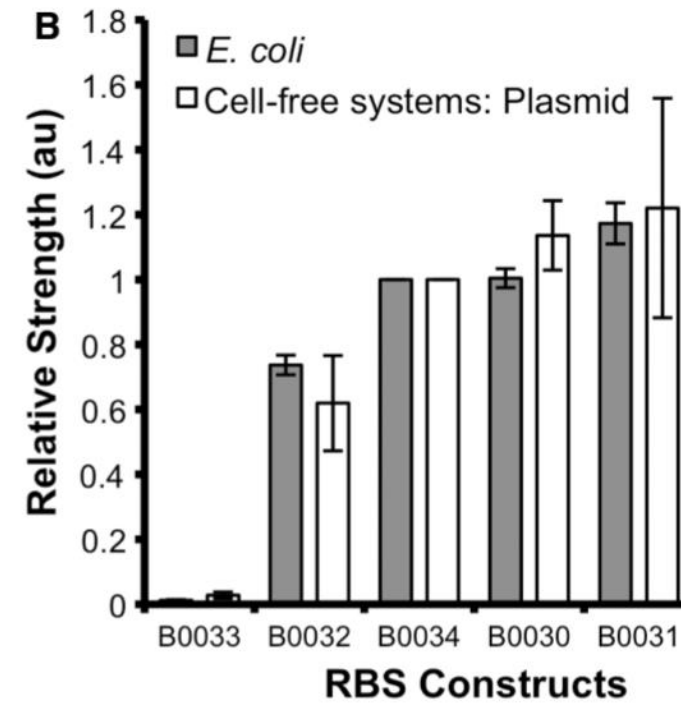
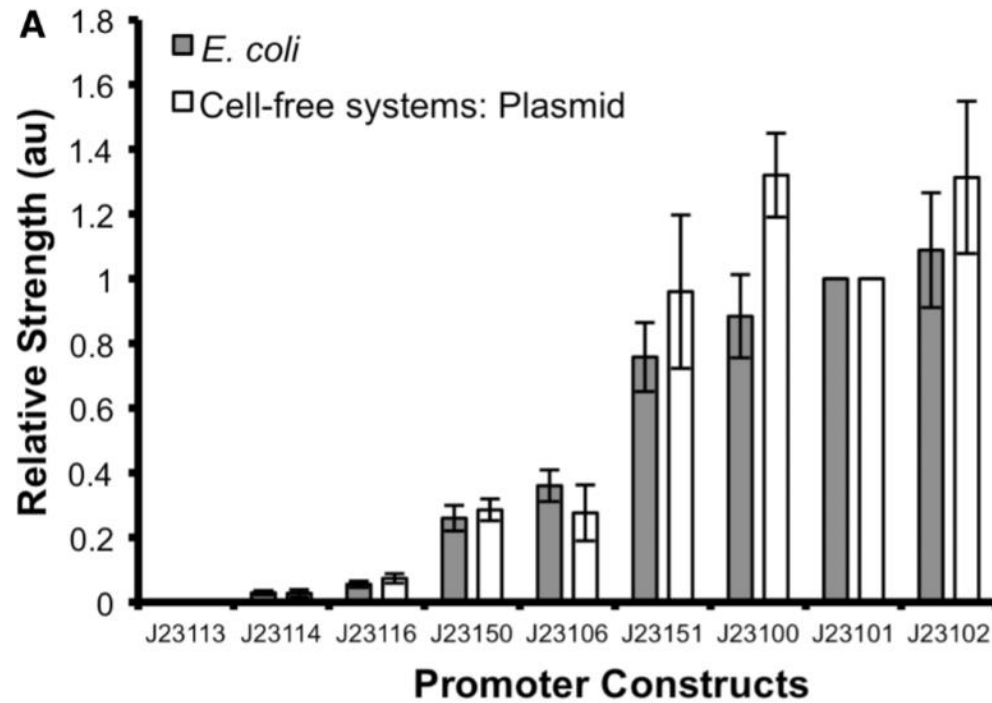
Genetic parts



Cell-free reaction:

Applied Science: Bridging Theory to Practice

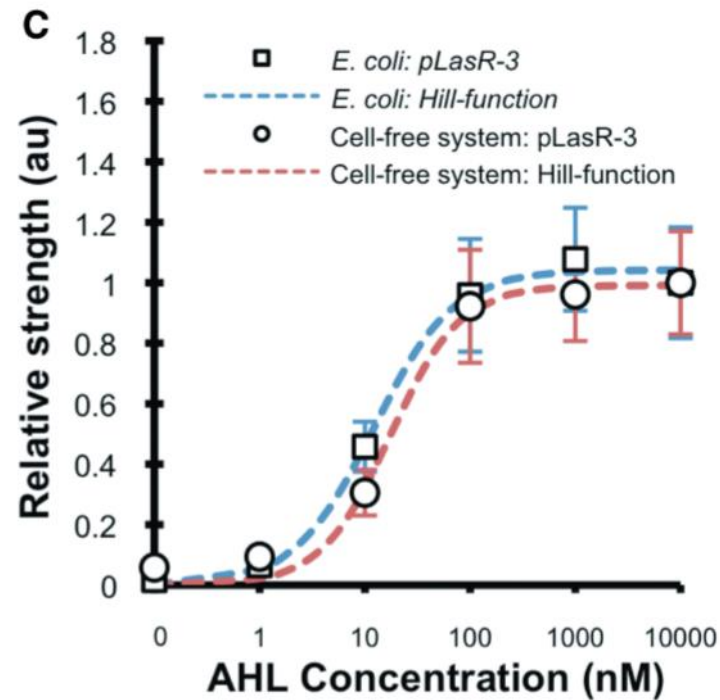
- Prototyping genetic circuits and pathways
- Genetic parts



Cell-free reaction:

Applied Science: Bridging Theory to Practice

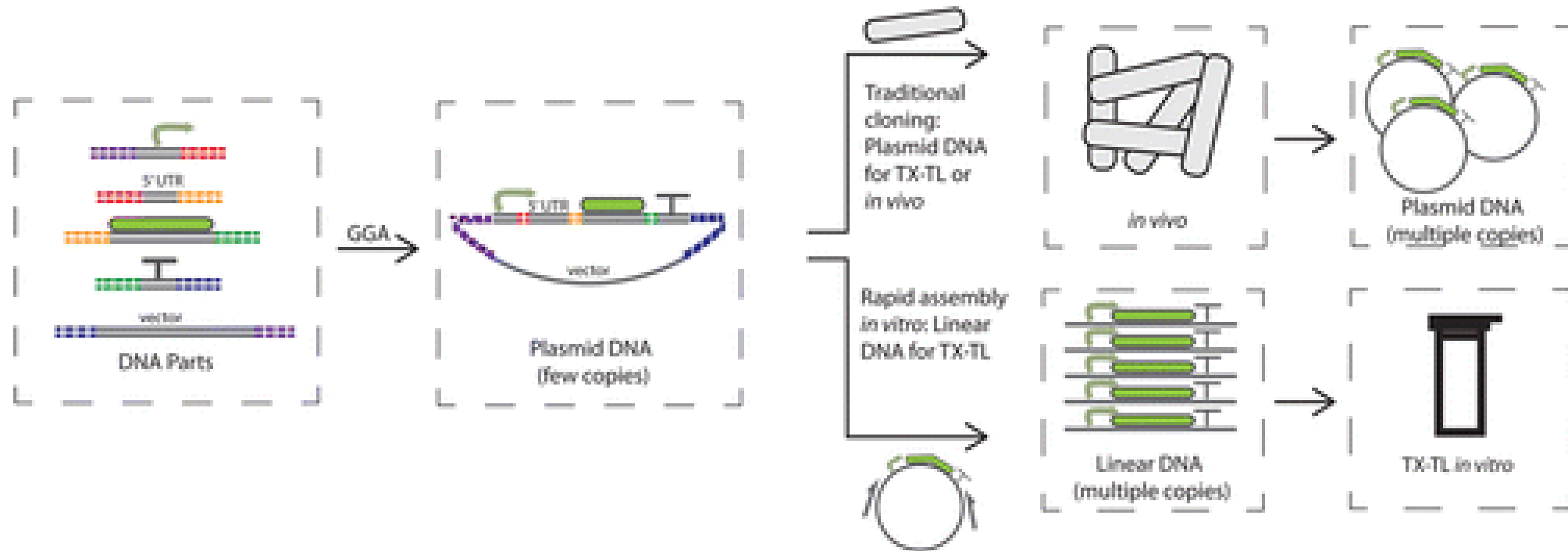
- Prototyping genetic circuits and pathways
Genetic parts: regulated systems



Cell-free reaction:

Applied Science: Bridging Theory to Practice

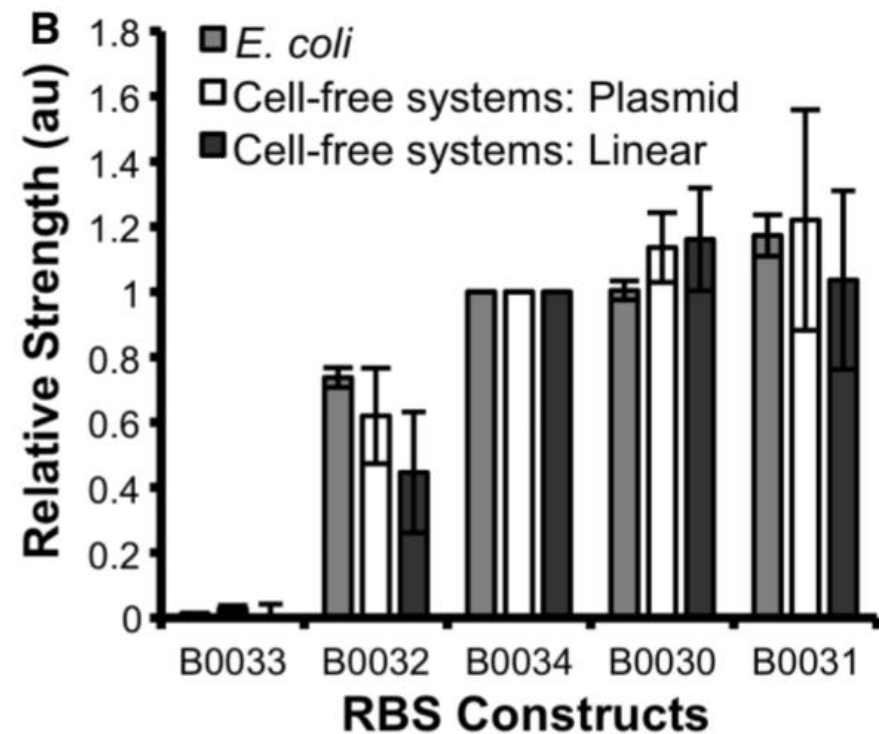
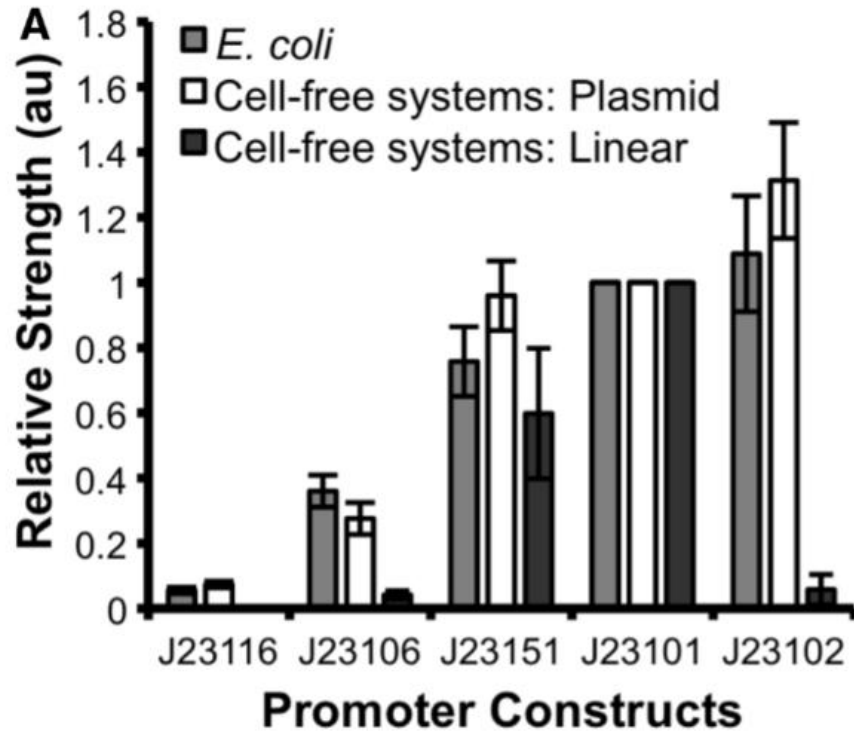
- Prototyping genetic circuits and pathways
Genetic parts: Linear fragment vs plasmid



Cell-free reaction:

Applied Science: Bridging Theory to Practice

- Prototyping genetic circuits and pathways
- Genetic parts: Linear fragment vs plasmid

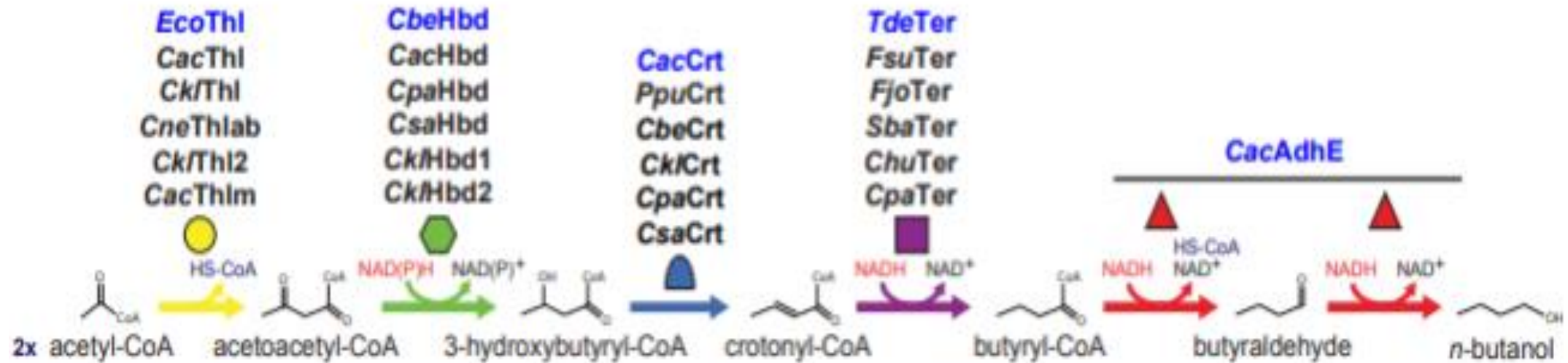


Cell-free reaction:

Applied Science: Bridging Theory to Practice

Prototyping genetic circuits and pathways

Metabolic engineering: combinatorial exploration



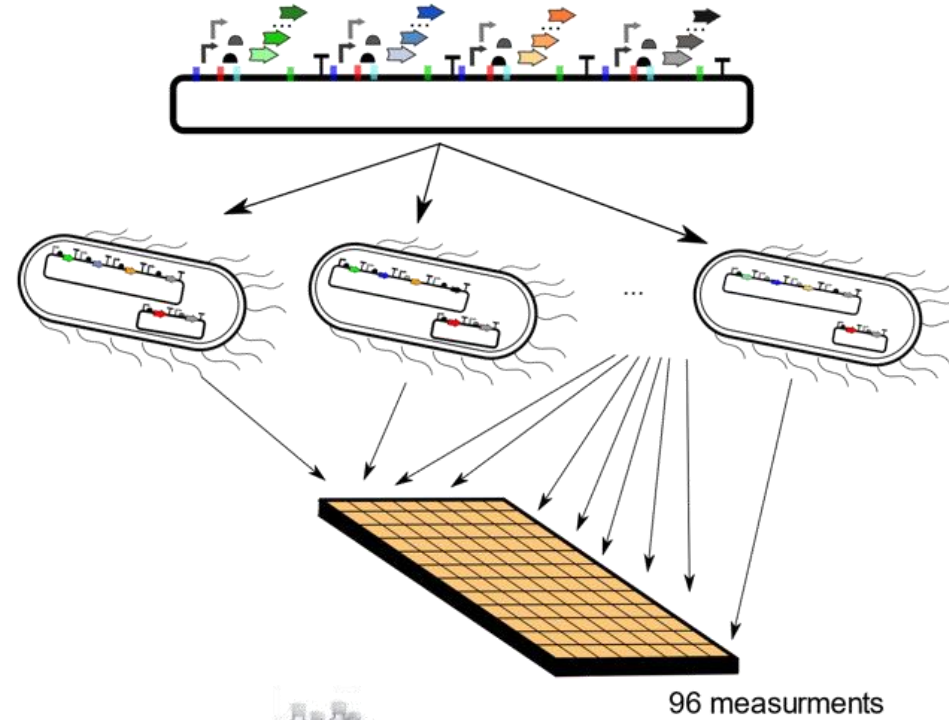
Example of : 6⁴ combinations

Cell-free reaction:

Applied Science: Bridging Theory to Practice

Metabolic engineering: combinatorial exploration

6^4 plasmids

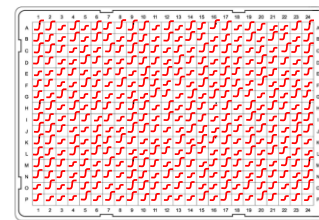
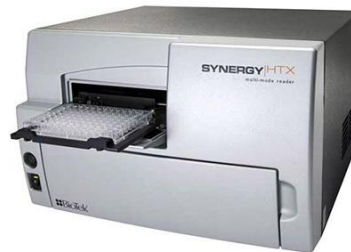
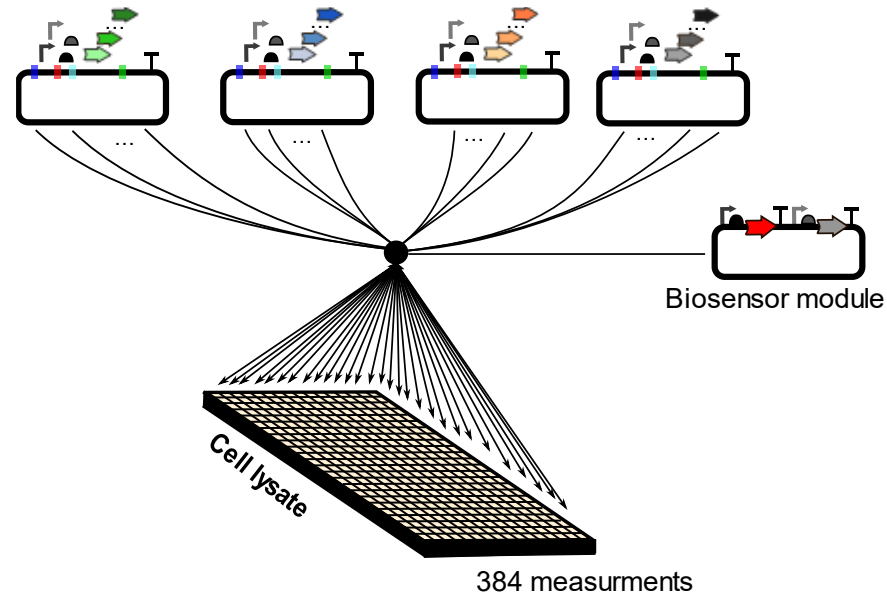


Cell-free reaction:

Applied Science: Bridging Theory to Practice

Metabolic engineering: combinatorial exploration

$6 \times 4 = 24$ plasmids

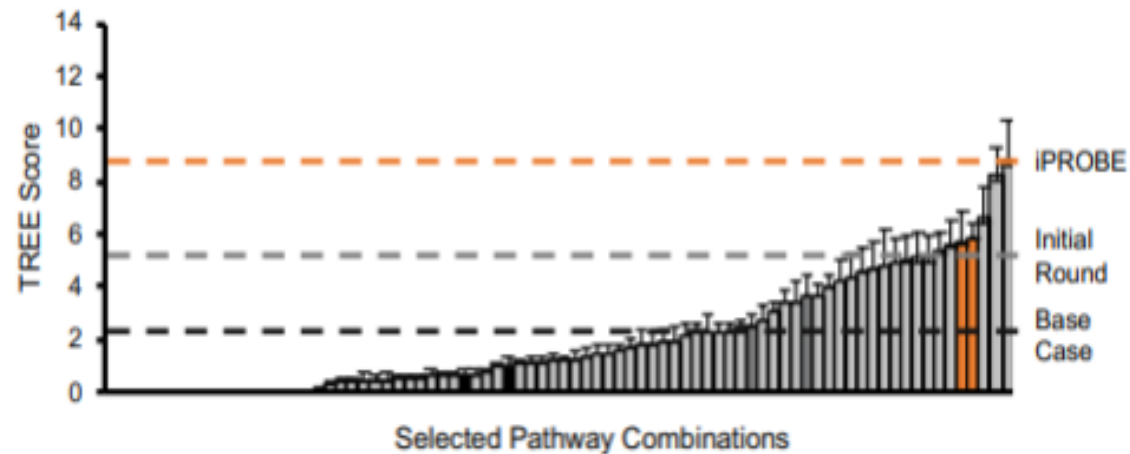
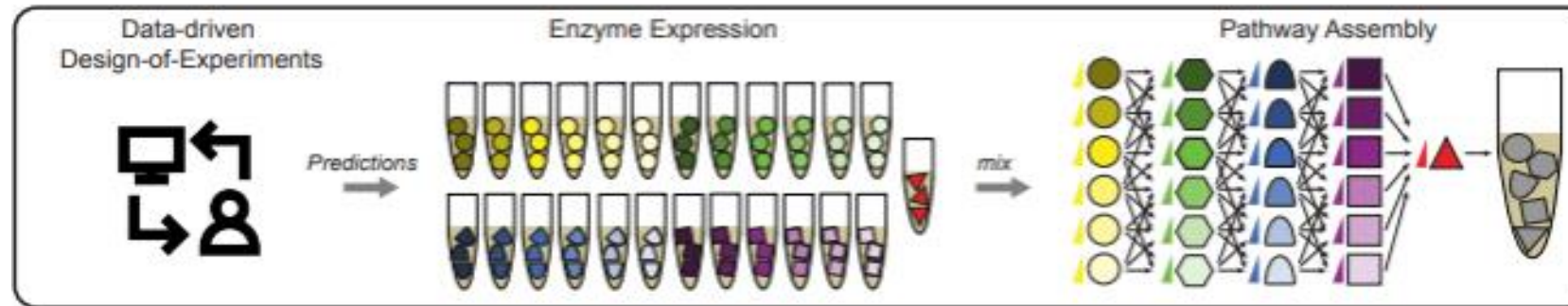


Cell-free reaction:

Applied Science: Bridging Theory to Practice

Prototyping genetic circuits and pathways

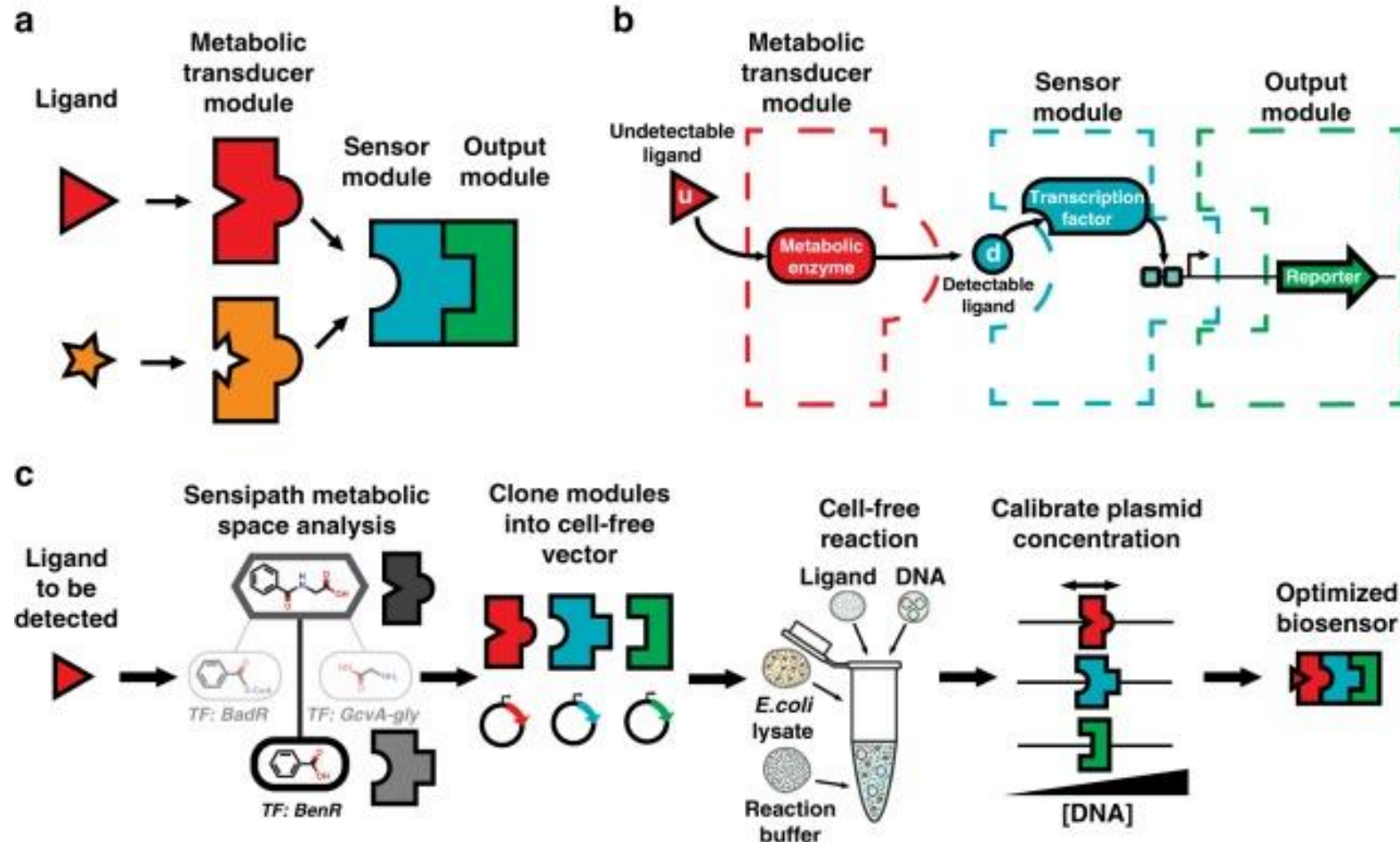
Metabolic engineering: combinatorial exploration



Cell-free reaction:

Applied Science: Bridging Theory to Practice

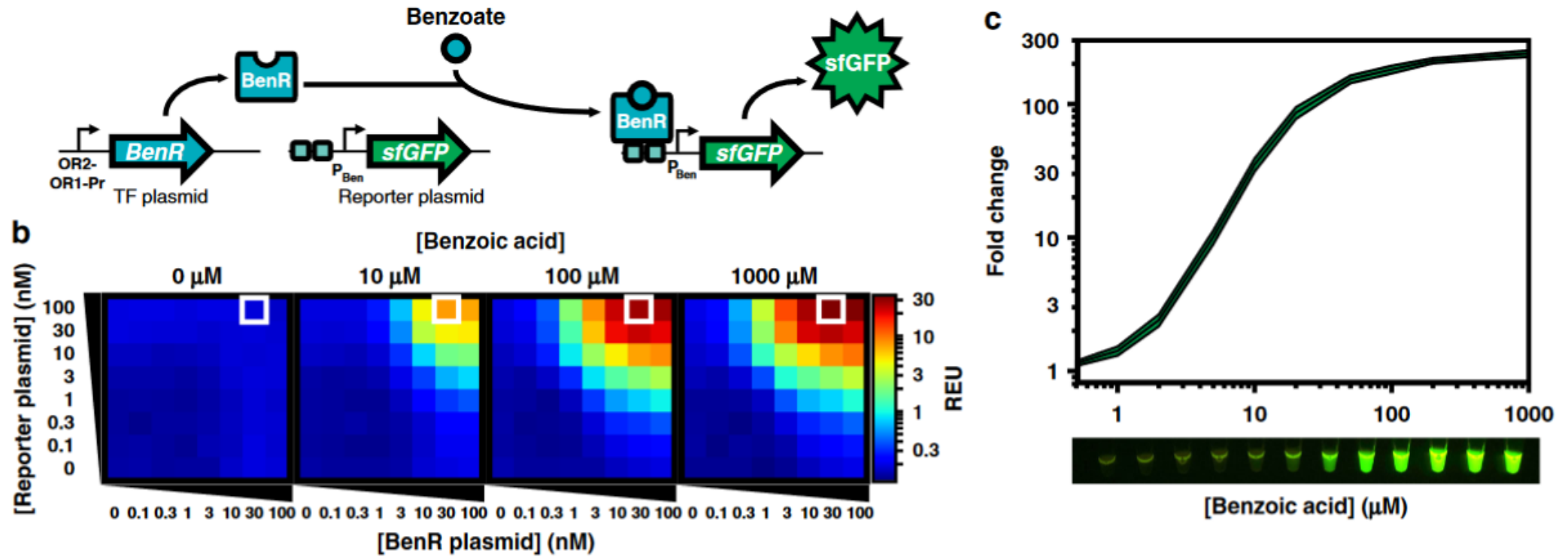
- Developing biosensors



Cell-free reaction:

Applied Science: Bridging Theory to Practice

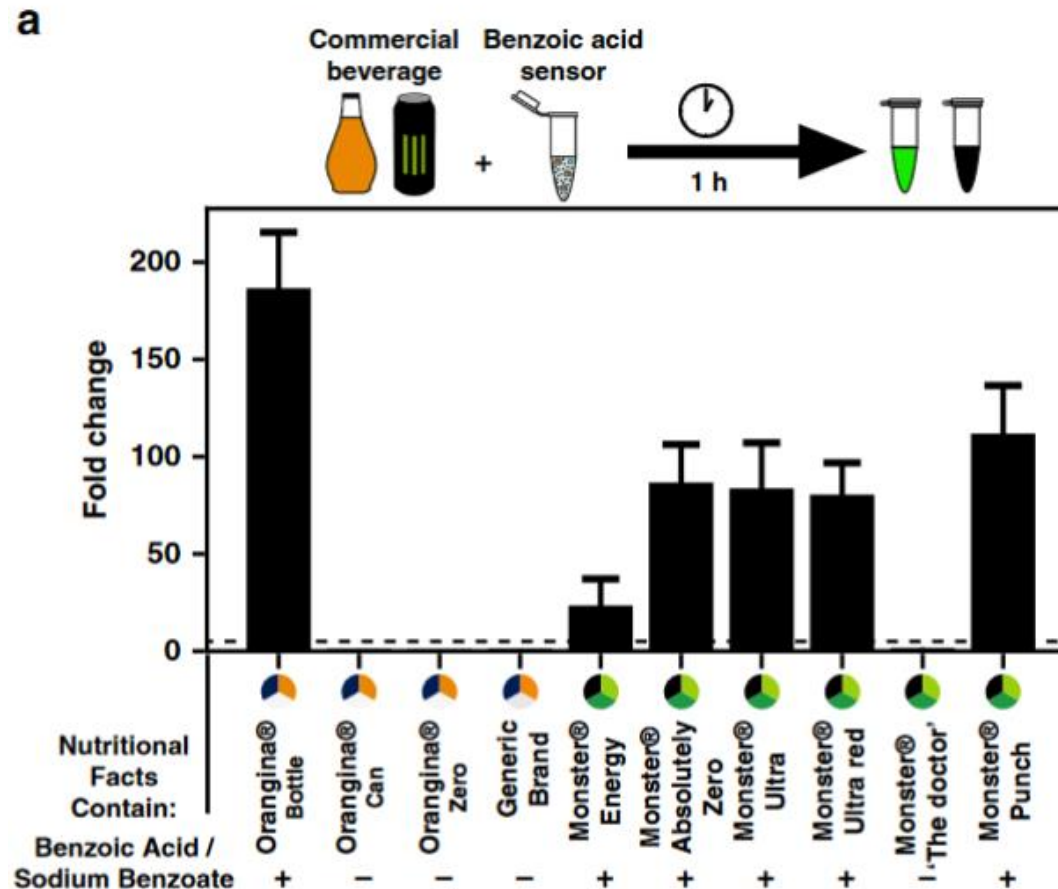
- Developing biosensors



Cell-free reaction:

Applied Science: Bridging Theory to Practice

- Developing biosensors



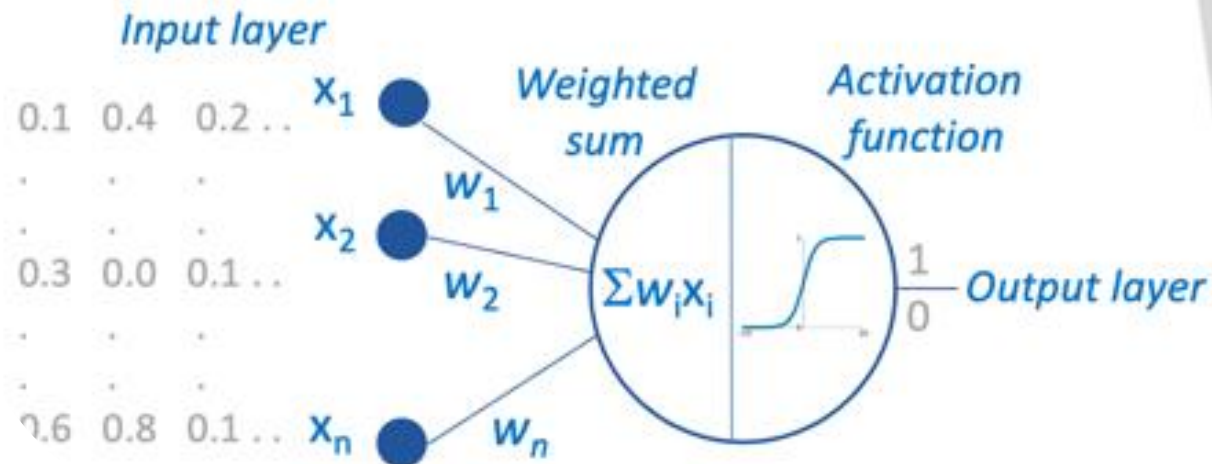
Cell-free reaction:

Applied Science: Bridging Theory to Practice

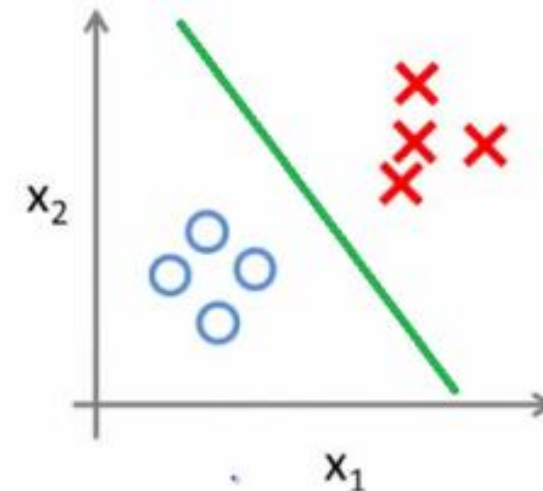
Developing biosensors
Biocomputing



Perceptron weights (w_i) are learned *in silico* to increase classifier accuracy



Binary classification:



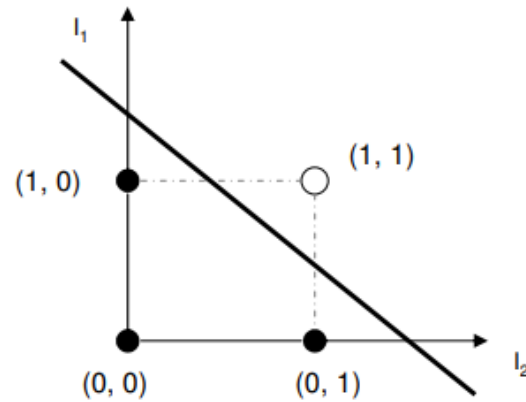
Cell-free reaction:

Applied Science: Bridging Theory to Practice

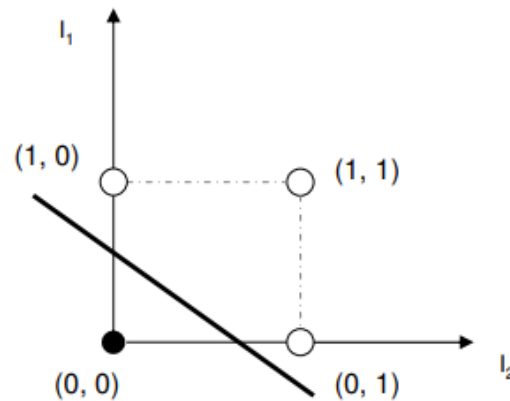
Developing biosensors

Biocomputing: logic gates

AND		
I_1	I_2	out
0	0	0
0	1	0
1	0	0
1	1	1



OR		
I_1	I_2	out
0	0	0
0	1	1
1	0	1
1	1	1

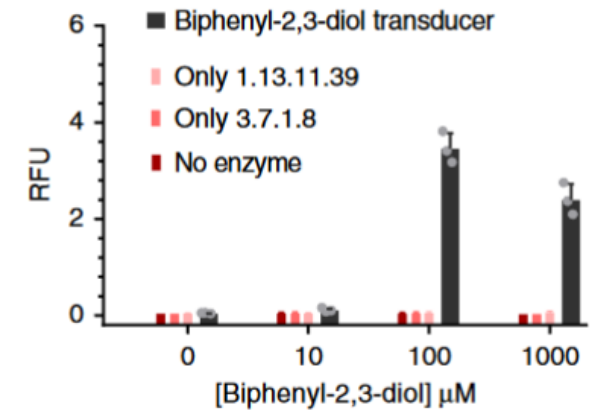
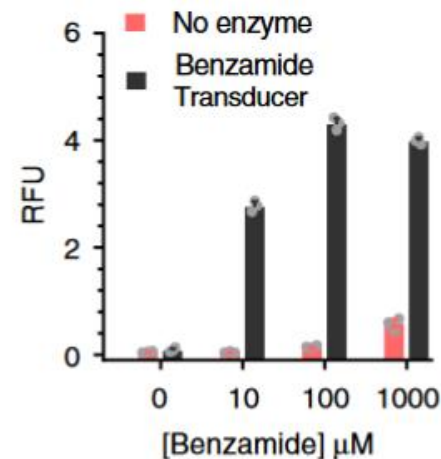
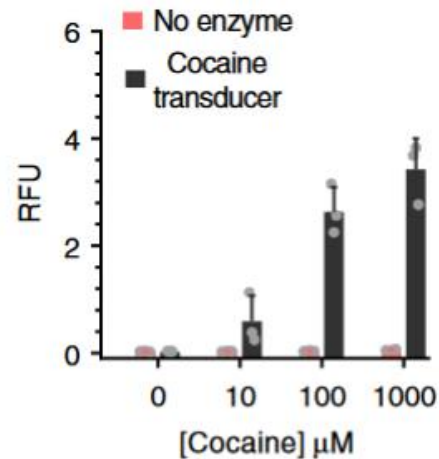
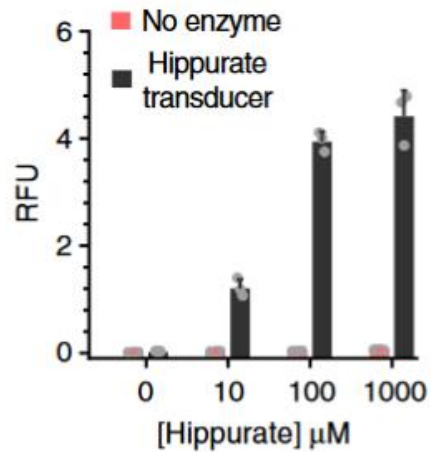
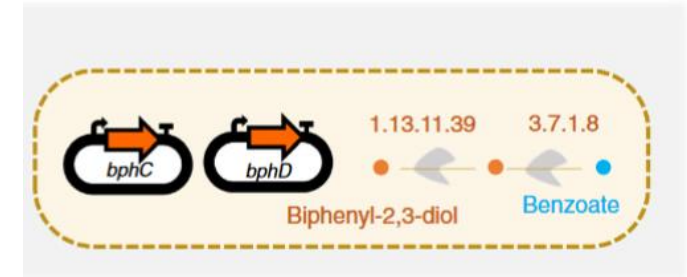
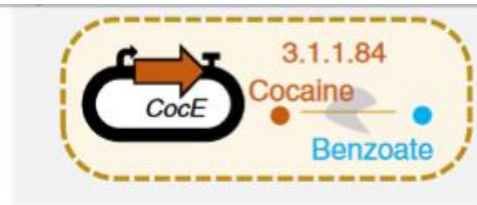
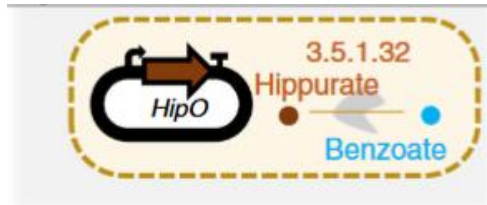


Cell-free reaction:

Applied Science: Bridging Theory to Practice

Developing biosensors

Biocomputing: 4 biosensors

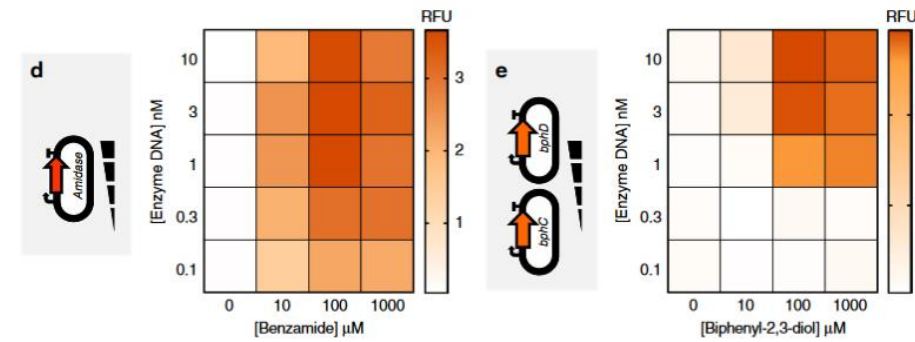
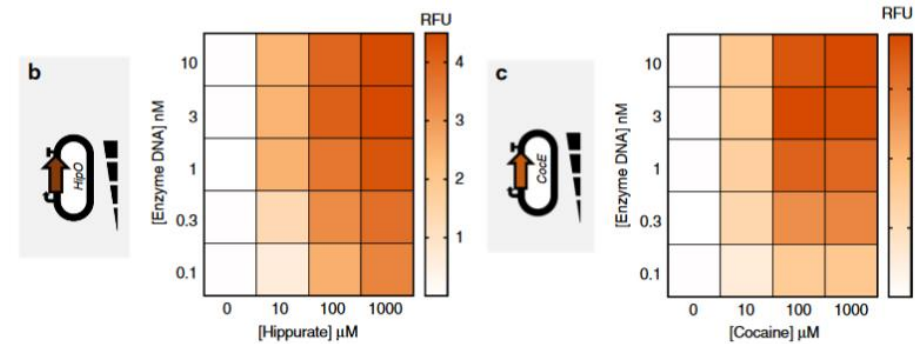
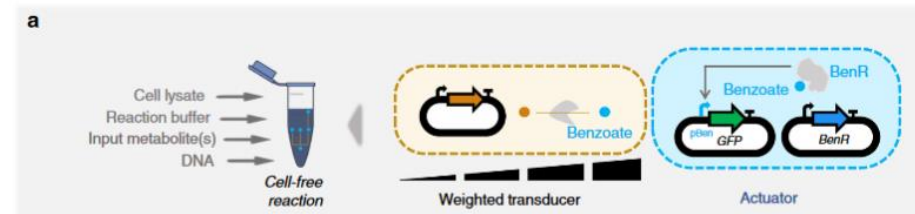


Cell-free reaction:

Applied Science: Bridging Theory to Practice

Developing biosensors

Biocomputing: tuning the weights

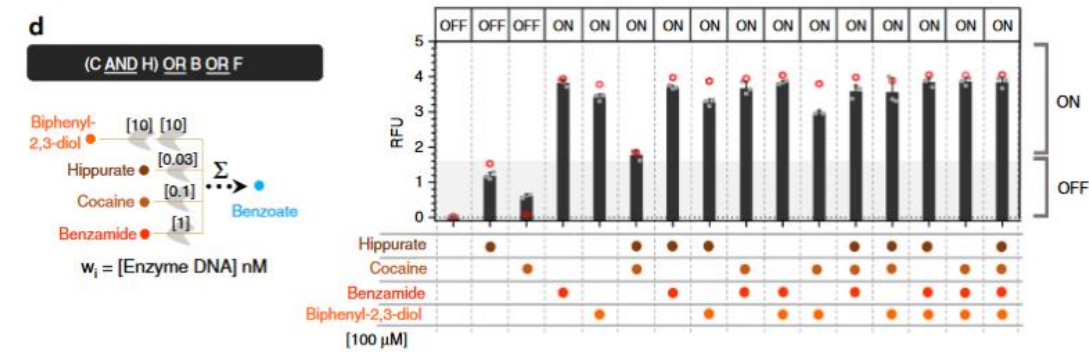
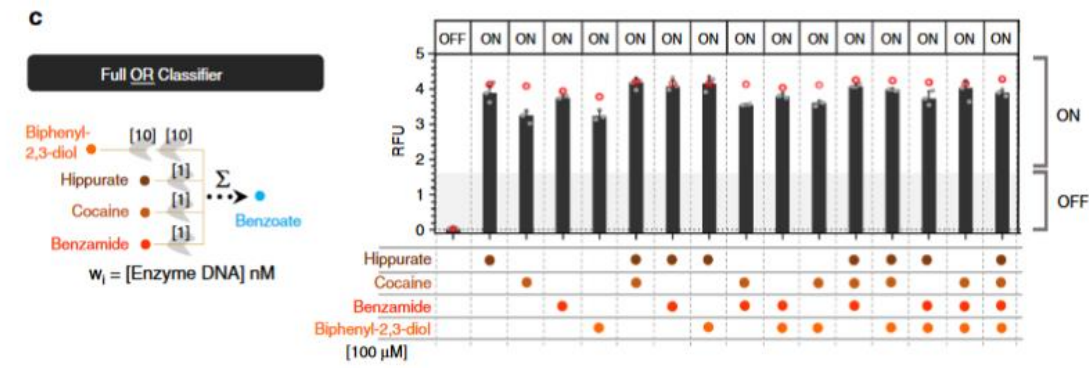
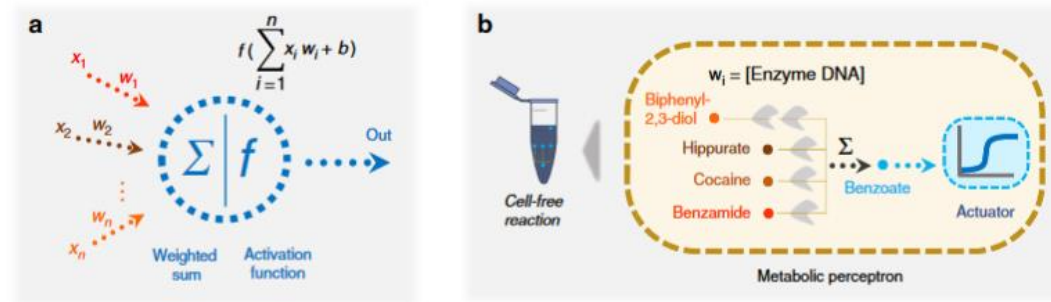


Cell-free reaction:

Applied Science: Bridging Theory to Practice

Developing biosensors

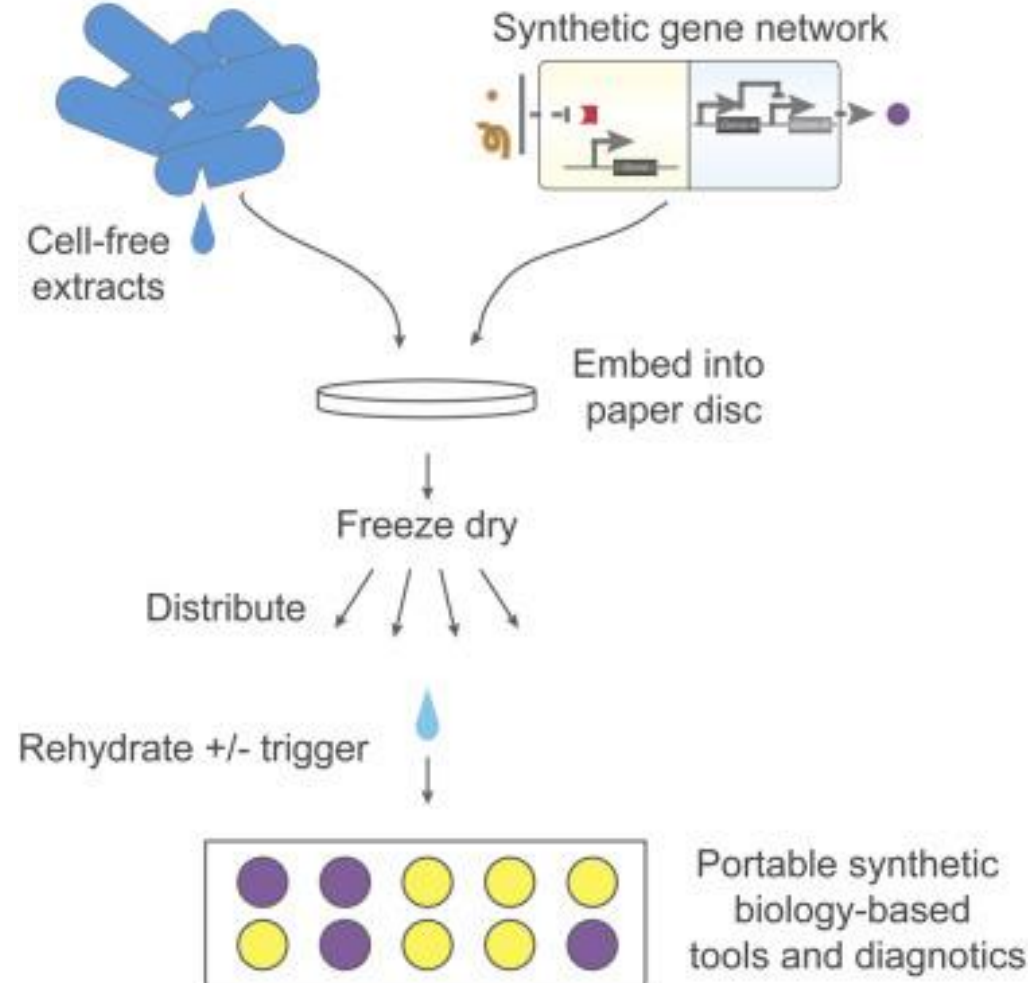
Biocomputing: perceptron



Cell-free reaction:

Applied Science: Bridging Theory to Practice

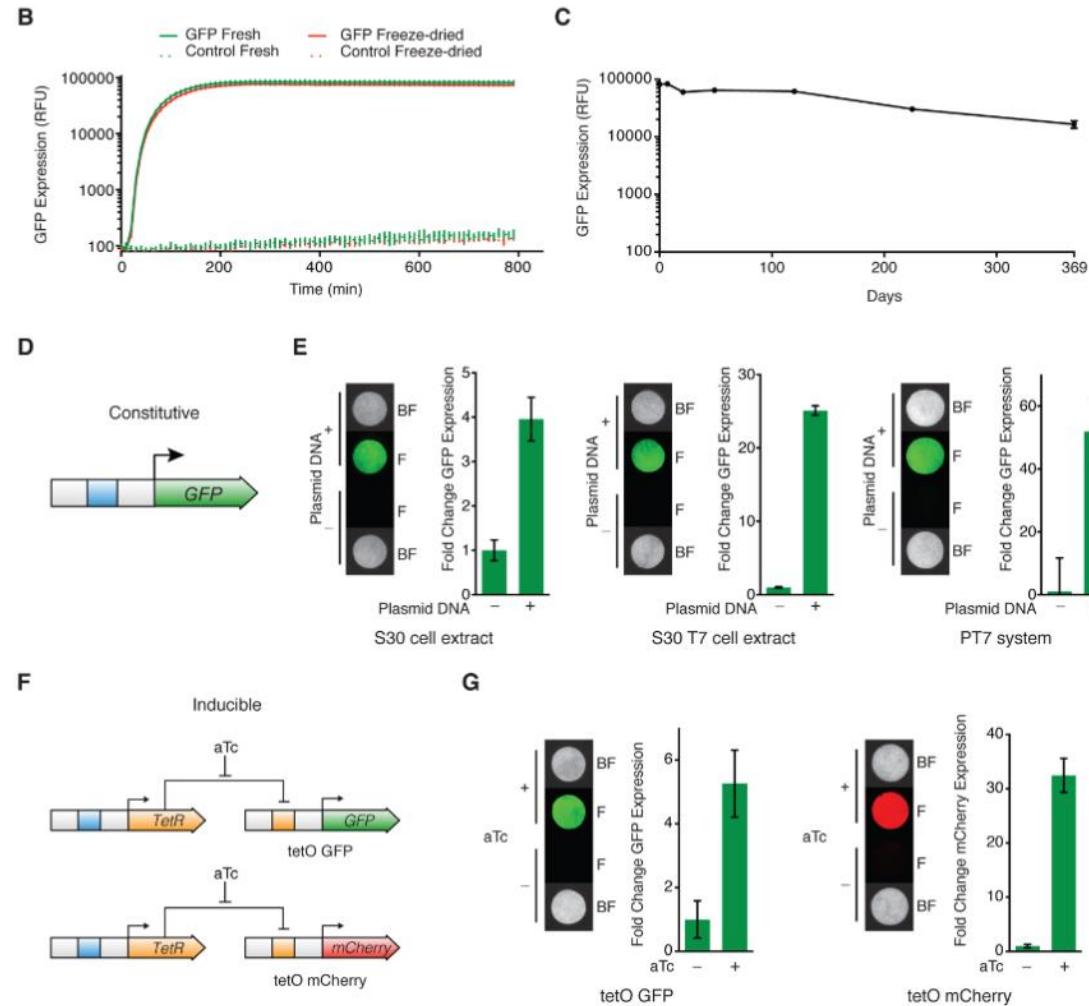
- Developing biosensors
Medical diagnostics



Cell-free reaction:

Applied Science: Bridging Theory to Practice

- Developing biosensors
- Medical diagnostics



Cell-free reaction:

Applied Science: Bridging Theory to Practice

- Developing biosensors
Materials and cell-free

Johnson & Johnson's QuickFire Challenge
Johnson & Johnson was Live • Follow

BioFabrics
Synthetic Biology + Textiles

Biosensor for Zika Virus on Thread

Thread Alone - Zika + Zika

Synthetic Biology Biosensor

INPUT → Environment-responsive promoter → Gene → Gene → Gene → OUTPUT

- Engineered gene circuits
- Transcriptional changes

Response to Target

Clean Dangerous Splash Detected

Integration with Internet of Things
#ChampionsOfScience

Wearable Diagnostics?

Animal Sentinels || Engineered Biosensors | Paper-based Diagnostics

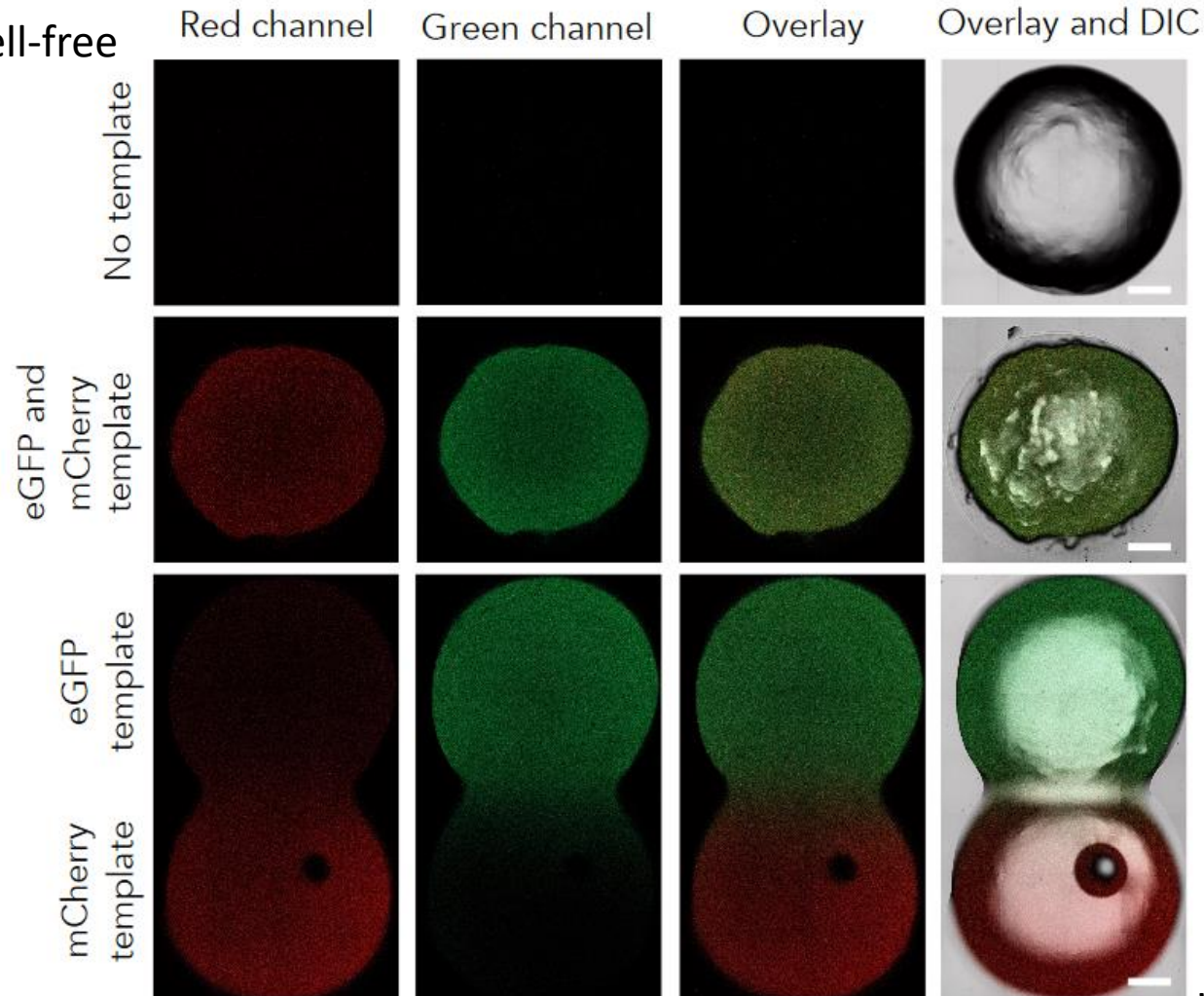
WYSS 3607 | BROAD INSTITUTE | UNIVERSITY OF MICHIGAN

Cell-free reaction:

Applied Science: Bridging Theory to Practice

- Developing biosensors

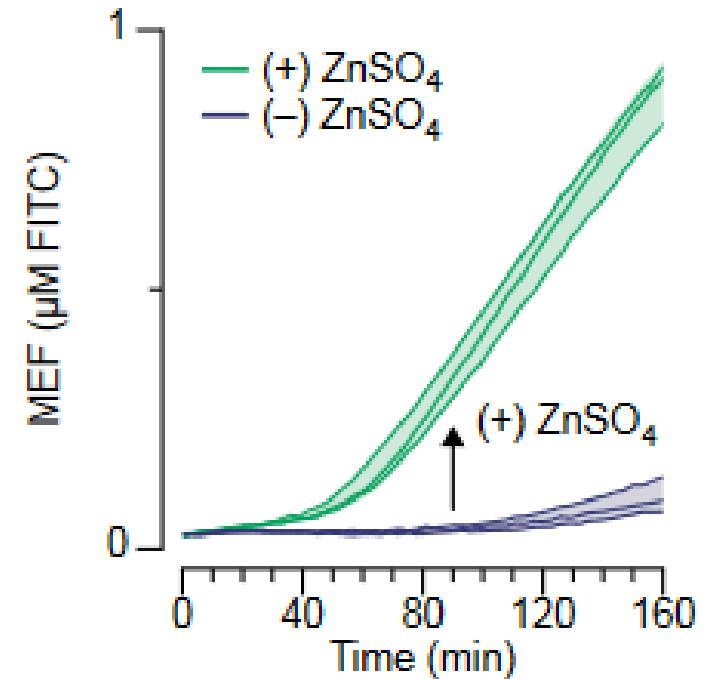
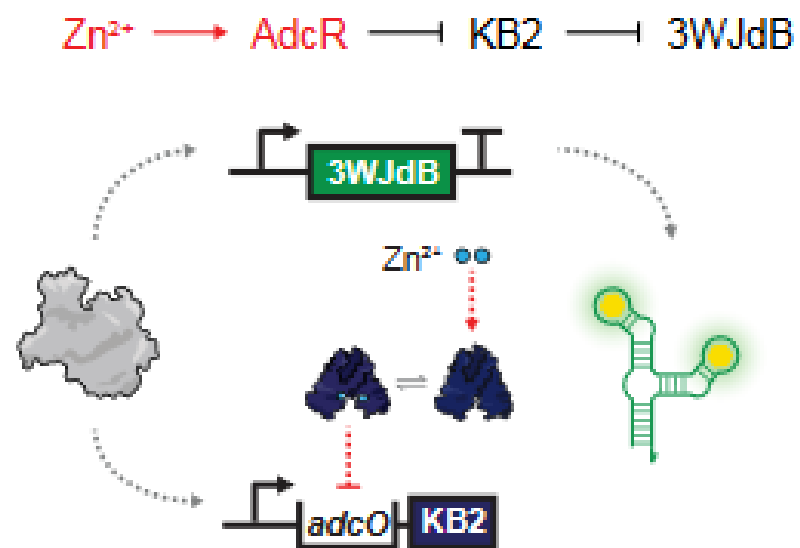
Materials and cell-free



Cell-free reaction:

Applied Science: Bridging Theory to Practice

- Developing biosensors
- Pollution detection

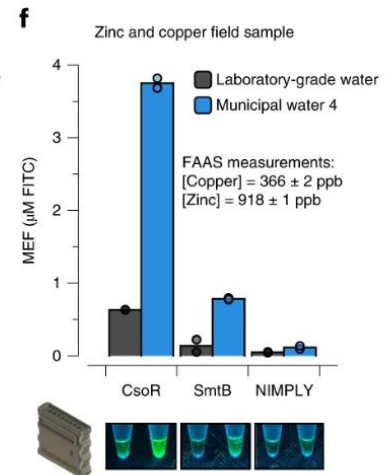
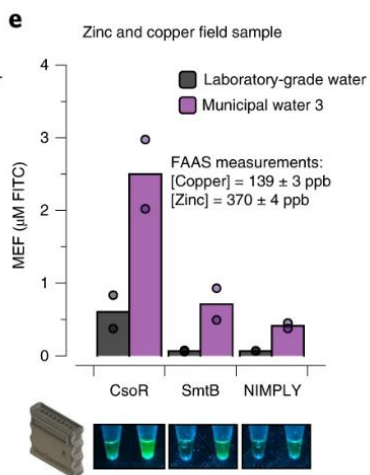
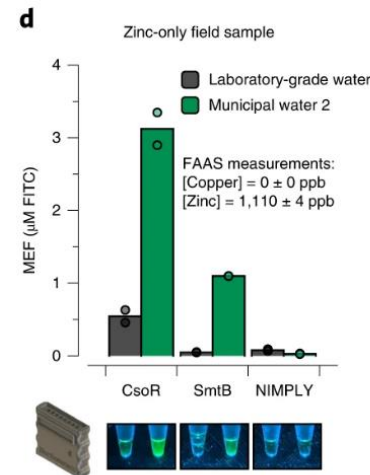
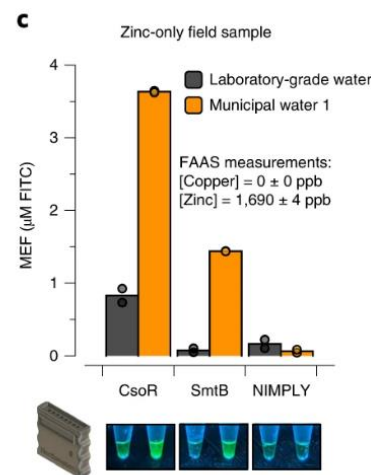
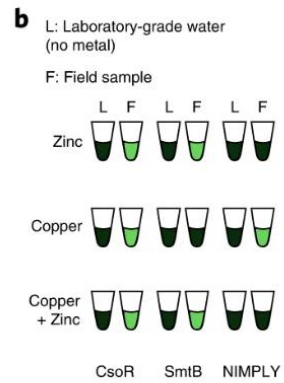
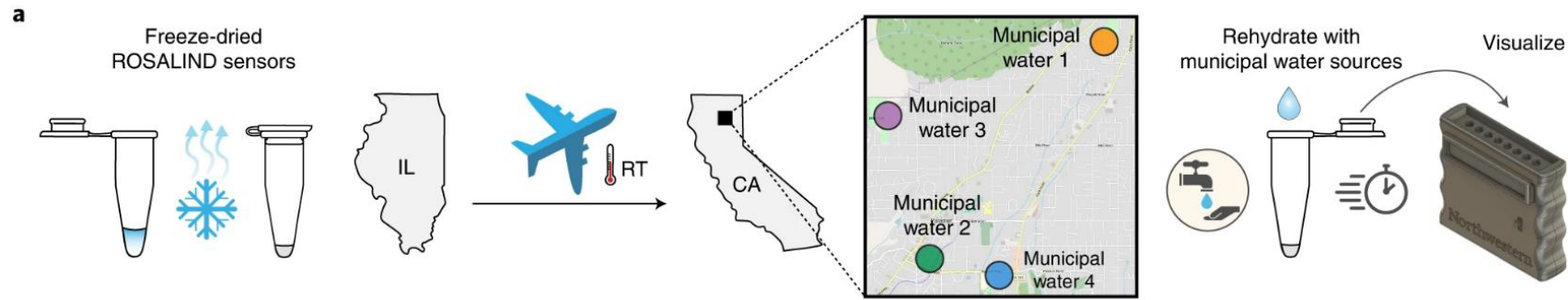


Cell-free reaction:

Applied Science: Bridging Theory to Practice

- Developing biosensors

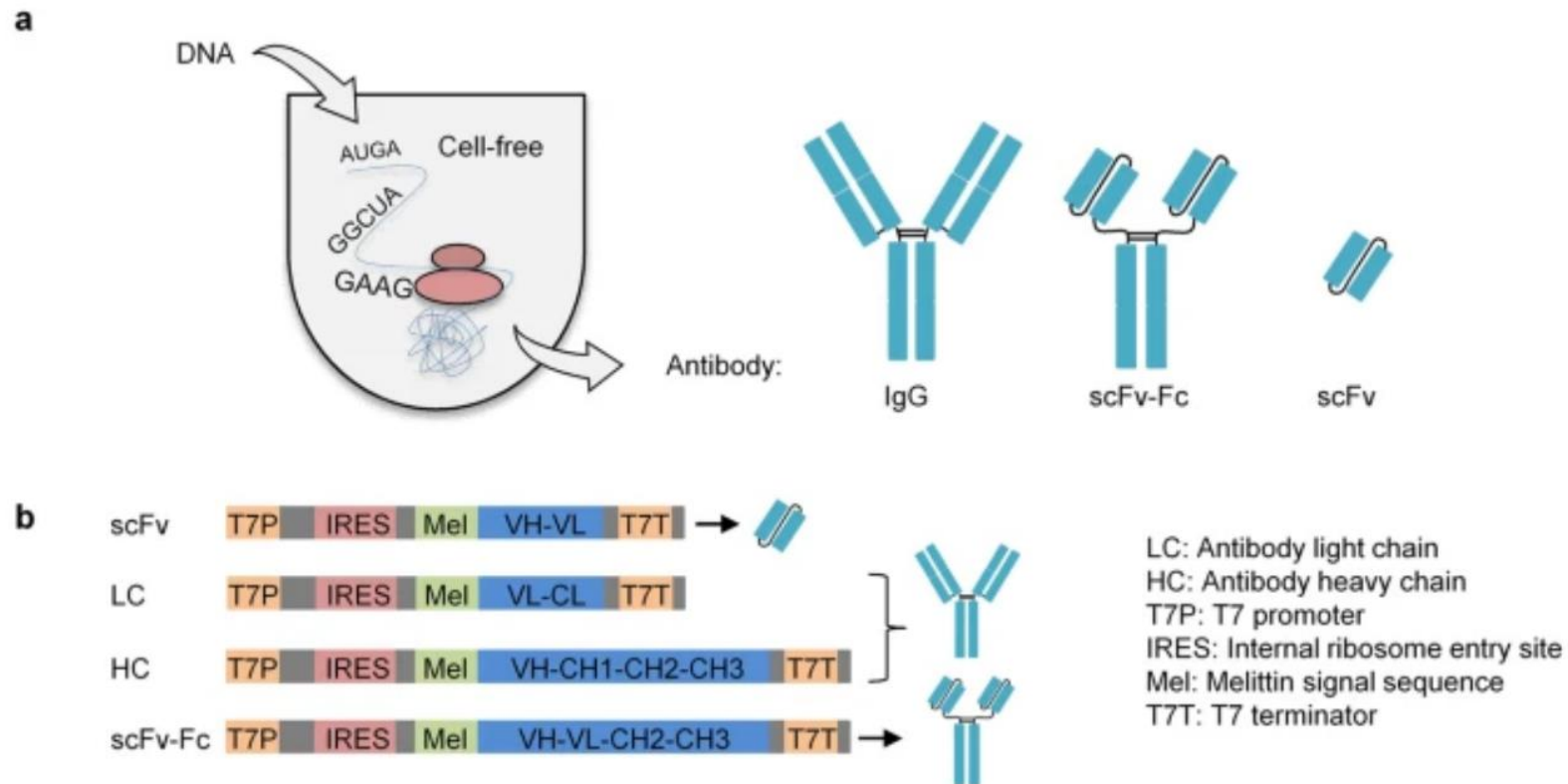
Pollution detection



Cell-free reaction:

Applied Science: Bridging Theory to Practice

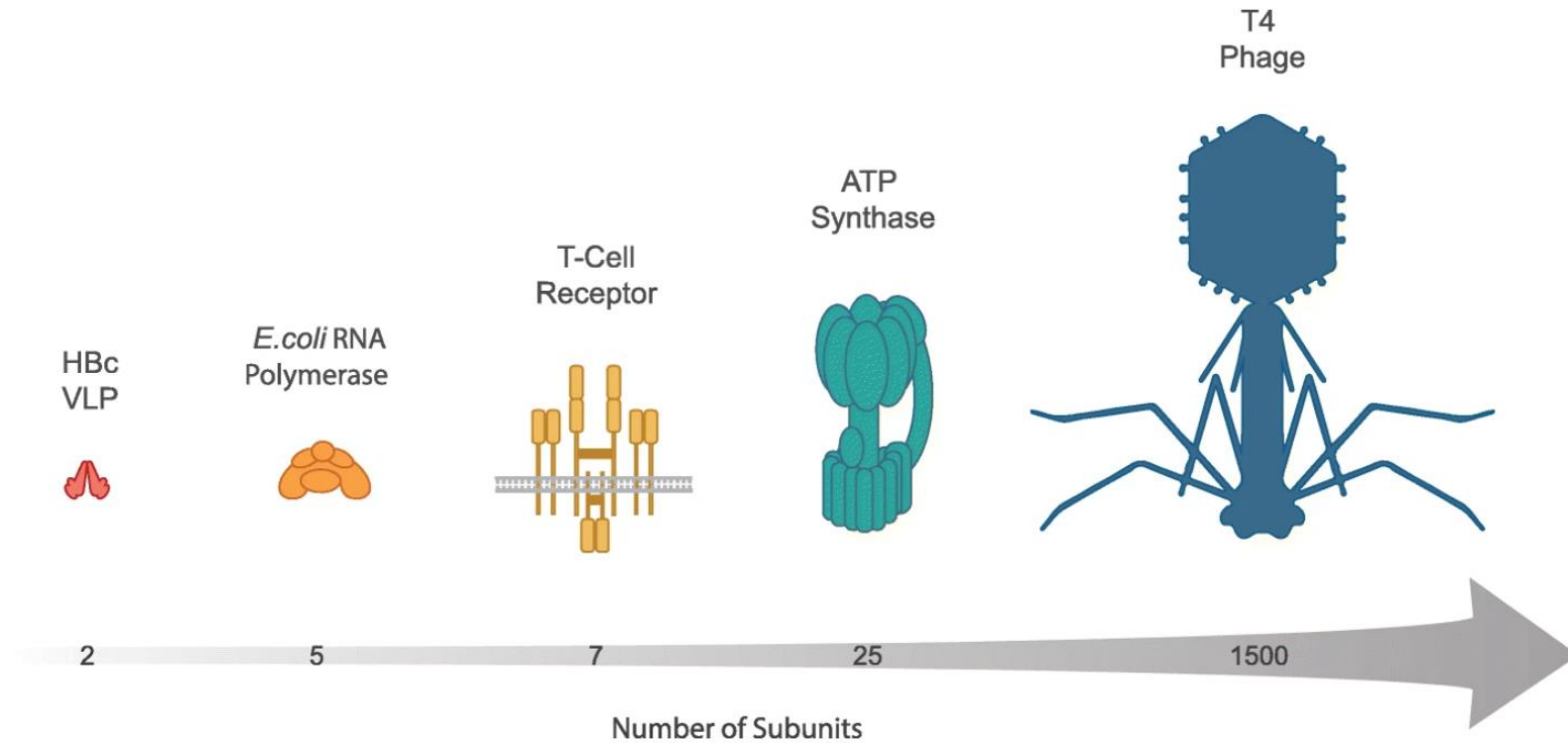
- Bio-machine assembly
- Antibodies (eukaryotic cell-free)



Cell-free reaction:

Applied Science: Bridging Theory to Practice

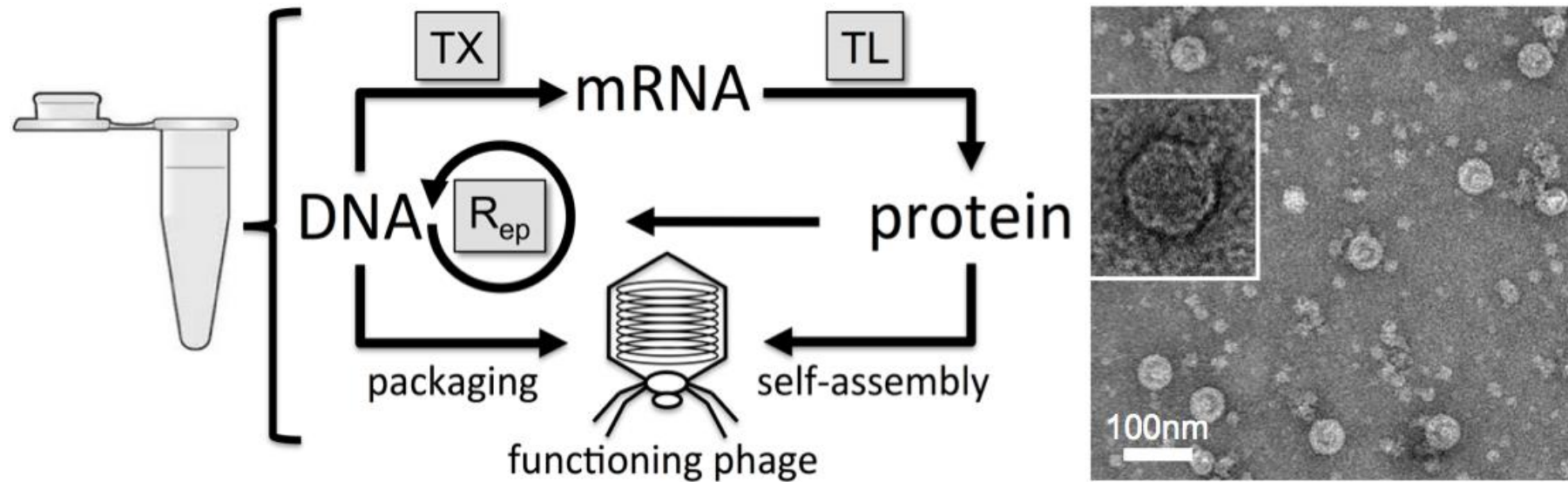
- Bio-machine assembly
- Phages selection



Cell-free reaction:

Applied Science: Bridging Theory to Practice

- Bio-machine assembly
Phages selection



Encoded by a 40 kbp DNA program composed of about 60 genes

Fundamental science: cell-free to understand living cells

Applied Science: Bridging Theory to Practice

Industrial Applications: Transforming Knowledge into Innovation

Cell-free reaction:

Industrial Applications: Transforming Knowledge into Innovation

Pharmaceuticals

- Monoclonal Antibodies & Antimicrobial Peptides
 - LenioBio (Germany)
 - Cube Biotech (Germany)
 - BioCat (Germany)
- Vaccines
 - Ntxpress (New Mexico, USA)
 - CPI (UK)
 - Sutro Biopharma (California, USA)
- Small Molecules
 - Creativebiomart (USA, Europe, Canada)
- Membrane Proteins
 - Creativebiomart (USA, Europe, Canada)
 - Desgin Pharma (Massachussets, USA)
 - Synthelis (France)

Pollution

- CO2 capture
 - Ensovi (Massachussets, USA)
- Biosensor
 - Stemloop (Illinois, USA)

On-Demand

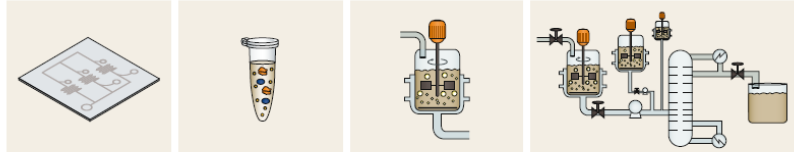
- Purified proteins
 - Tierra bioscience (California, USA)
 - CFS (Japan)
 - Cell-free (Ontario, Canada)

Cell-free kit providers

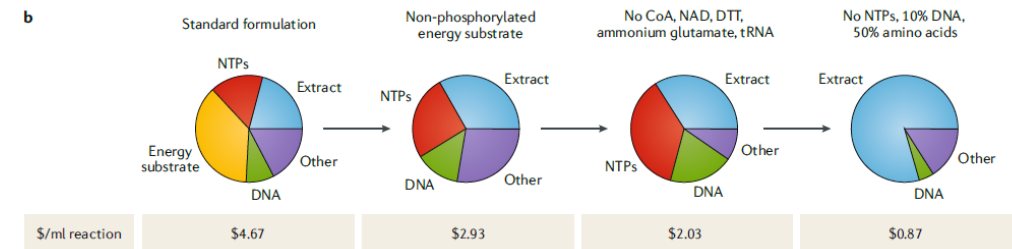
- New England Biolabs (Massachussets, USA)
- Thermo Fisher (Massachussets, USA)
- Biotechrabbit (Germany)
- arbor bioscience (Michigan, USA)

Scale-up

a



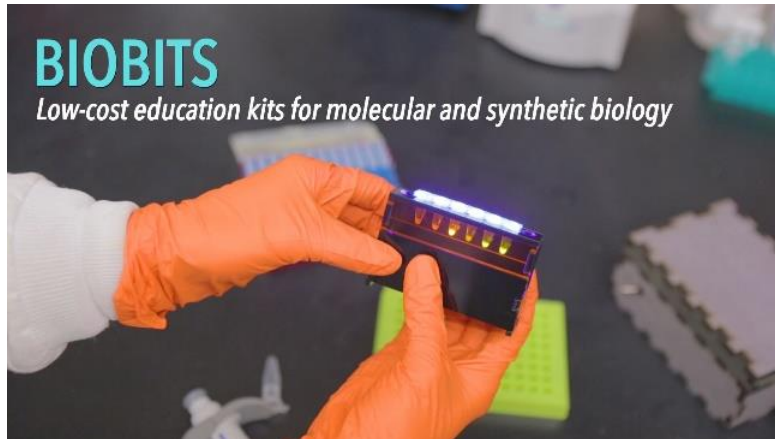
Approximate batch size	V = 1 µl	V = 1 ml	V = 100 l	V = 1,000 l (continuous operation)
Example product	Laboratory use T7 RNA polymerase	Point-of-use vaccine dose	Pharmaceutical antibody-drug conjugate	Commodity grade subtilisin
Commercial price (\$/mg)	\$13,250	\$200	\$5,500	\$0.022
Estimated cell-free yield (mg/ml reaction)	1.0	0.02	0.250	2.0
Estimated purification cost	0x	0x	10x	1x
Estimated breakeven cost (\$/ml reaction)	\$13,250	\$4	\$120	\$0.022



Cell-free reaction:

Additional use / challenges with cell-free

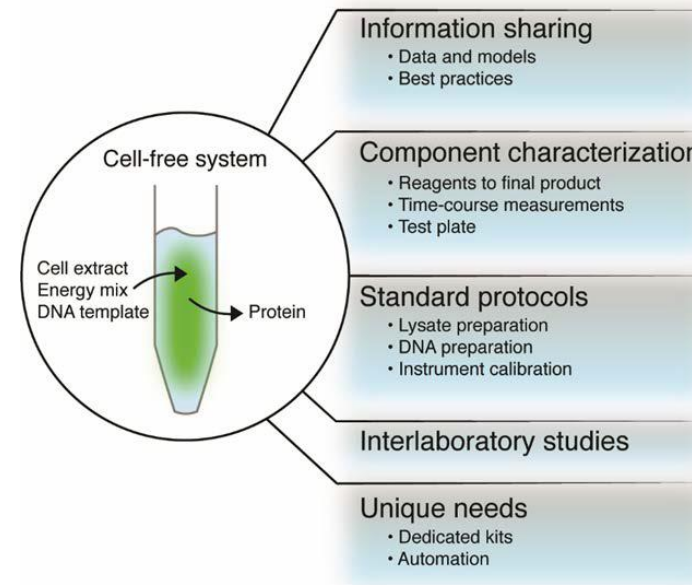
Cell-free for education



myTXTL[®] Cell-Free Protein Expression kits for Igem

National Institute of Standards and Technology

Towards reproducible cell-free systems



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