



**HAL**  
open science

## Cell-free systems Gene expression outside of the cell

Olivier Borkowski

► **To cite this version:**

Olivier Borkowski. Cell-free systems Gene expression outside of the cell. Engineering school. AgroParisTech - INRAE - Campus Agro Paris-Saclay, France. 2023. hal-04336158

**HAL Id: hal-04336158**

**<https://hal.science/hal-04336158>**

Submitted on 11 Dec 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

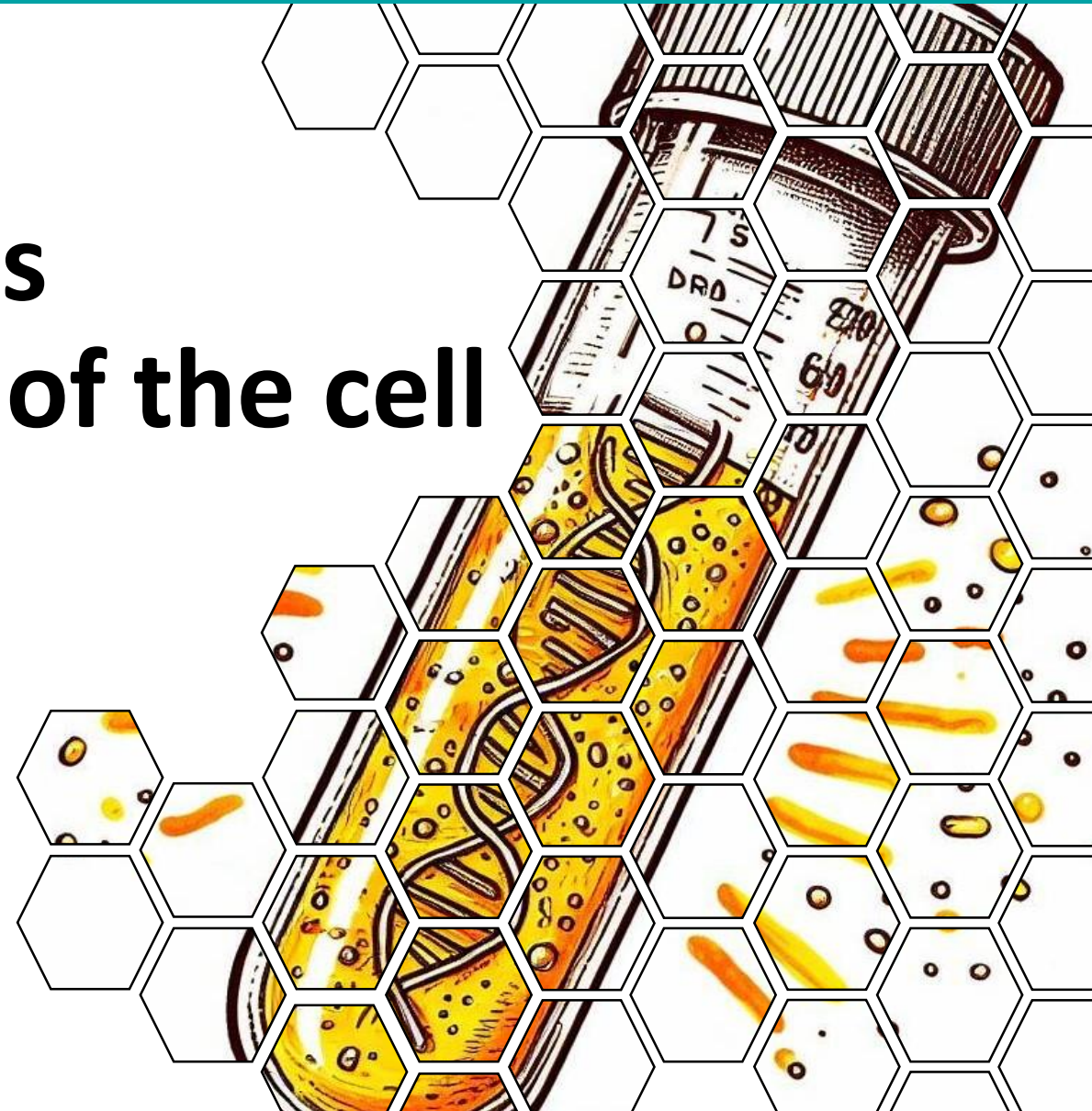
L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Cell-free systems

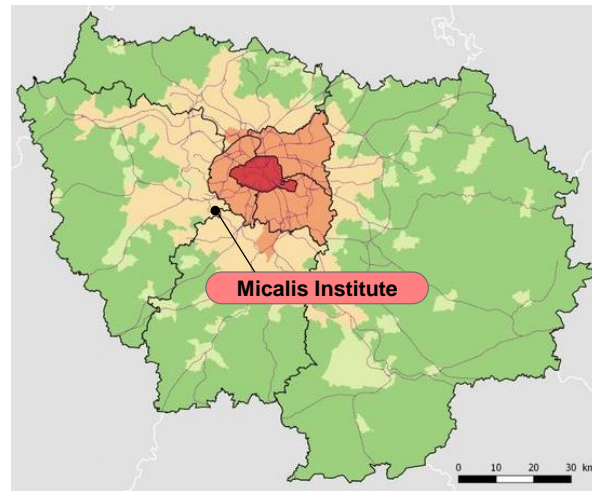
## Gene expression outside of the cell

**Olivier Borkowski, PhD**

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



## Micalis Institute



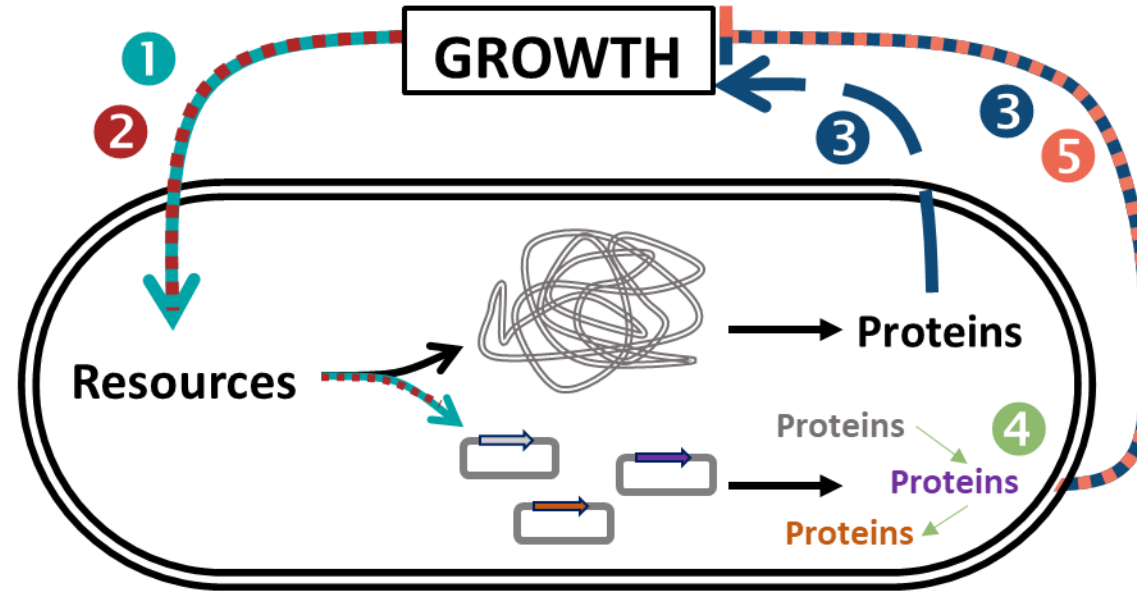
### 21 Research Teams

- 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:

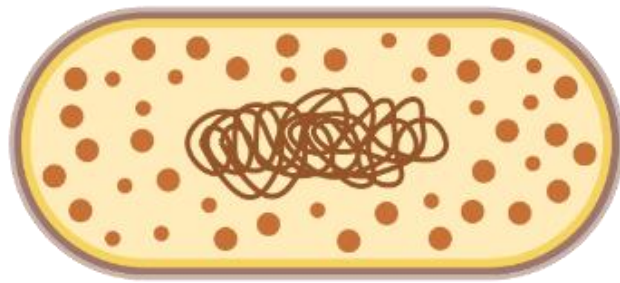
- Link between bacterial growth and nutrient concentrations in culture media (Monod, 1942)
- Link between the macromolecular composition and the cell doubling time (Schaechter et al., 1958)
- Linear relationships between RNA/DNA, RNA/protein, protein/DNA and growth rate (Bremer & Dennis, 1974)
- Increasing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

**-> New technology allows novel experiments**

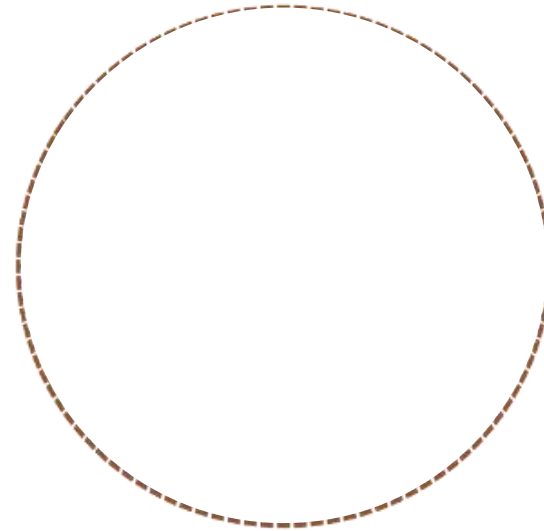
## Bacterial Physiology

Experimental setup

Genomic regulations



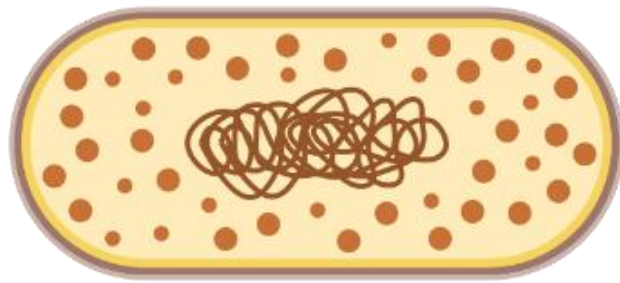
*E. coli* living cells



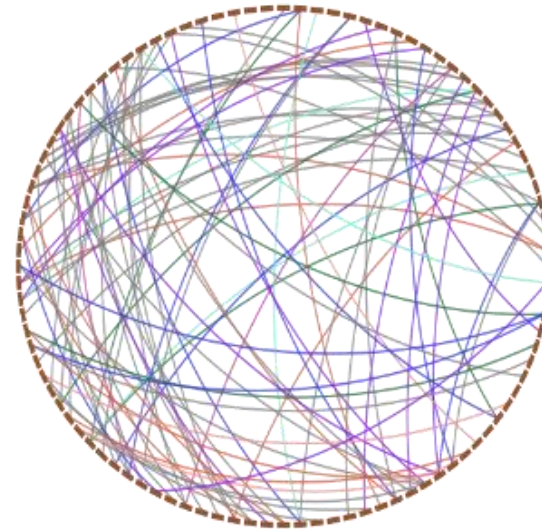
## Bacterial Physiology

Experimental setup

Genomic regulations



*E. coli* living cells



**Intertwined Effects**

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



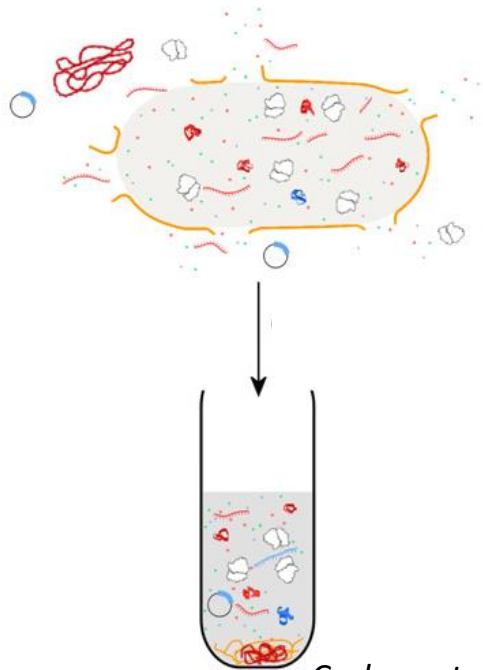
# Cell-free reaction:

## 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:

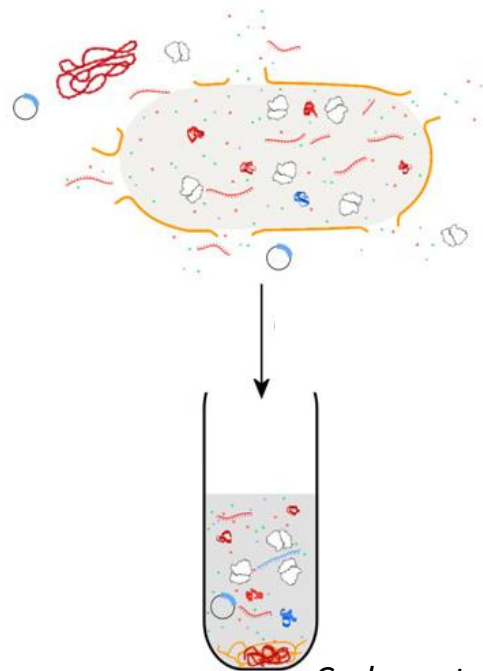
## Protein expression in an open system

---

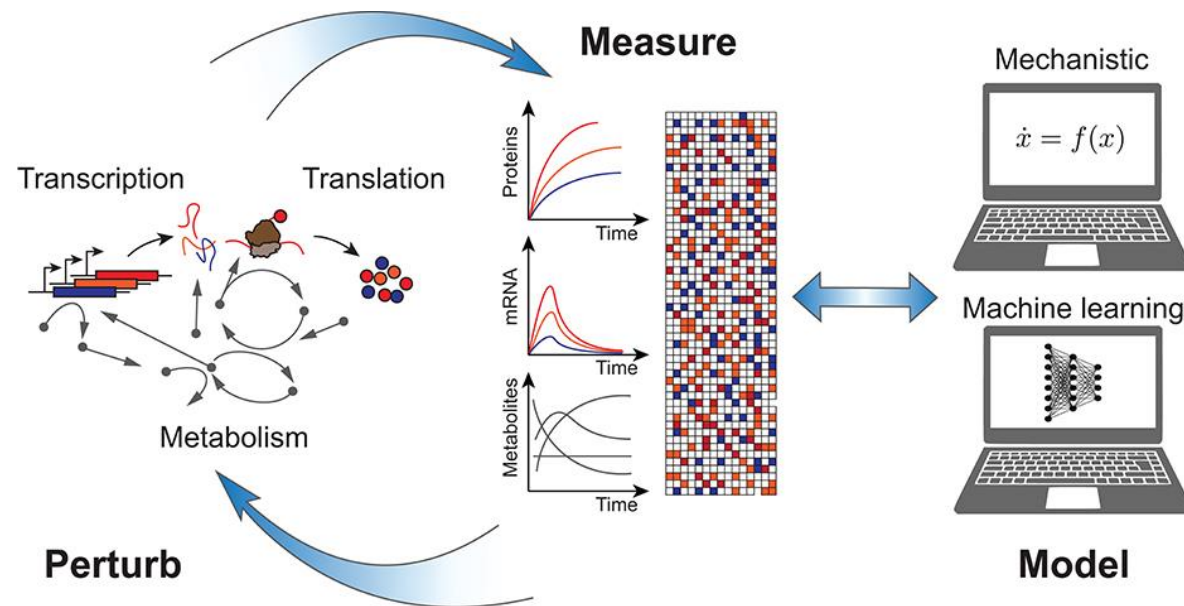
---

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\*

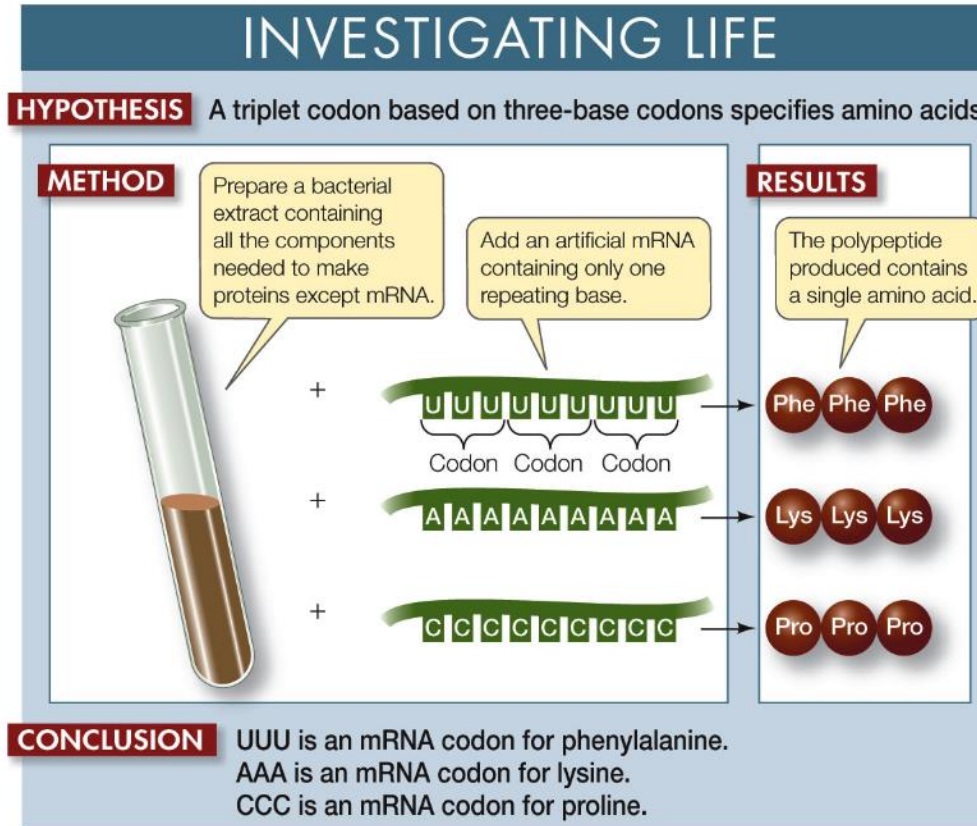
NATIONAL INSTITUTES OF HEALTH, BETHESDA, MARYLAND

*Communicated by Joseph E. Smadel, August 3, 1961*

A stable cell-free system has been obtained from *E. coli* which incorporates C<sup>14</sup>-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.<sup>1</sup> 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:

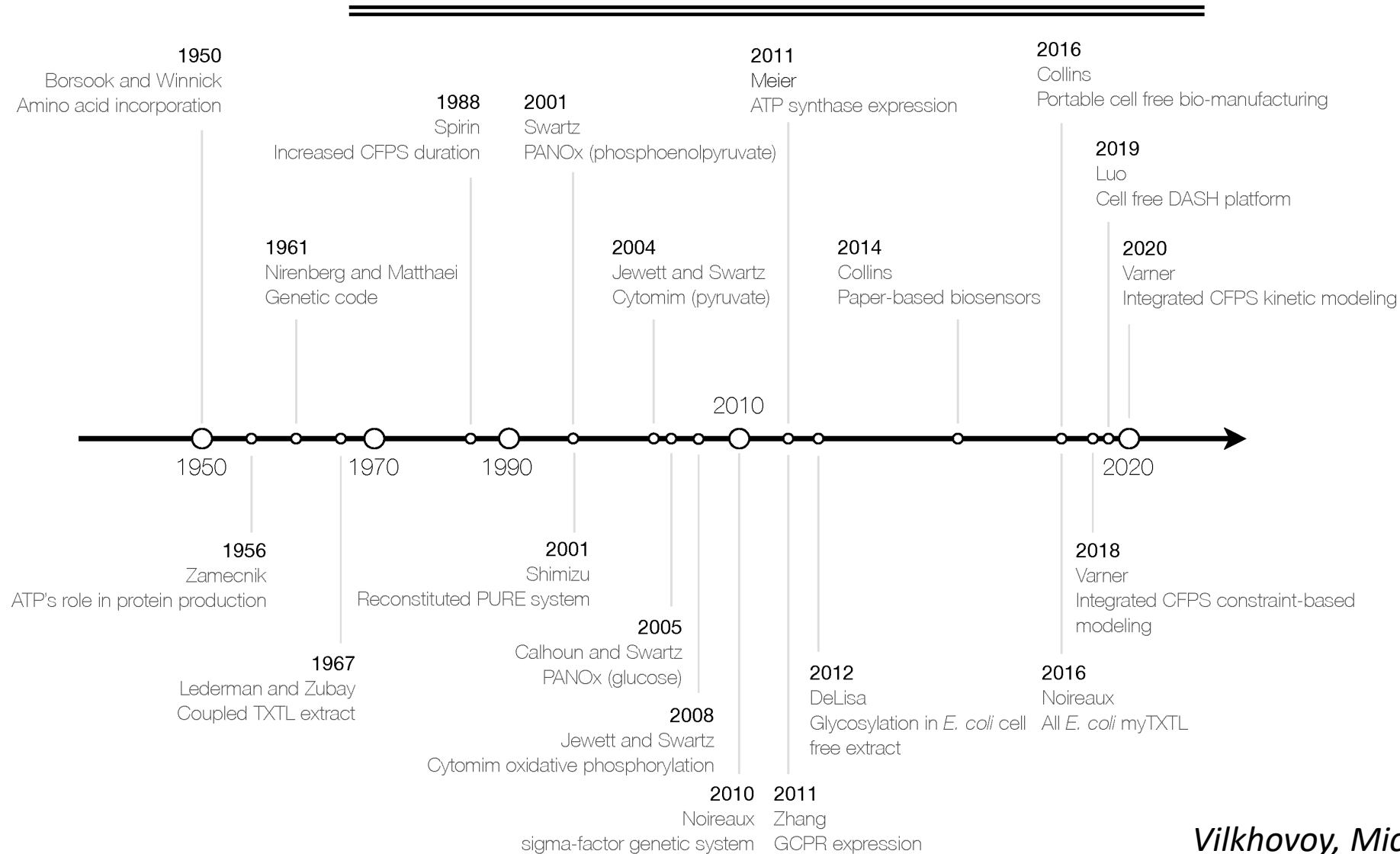
## Nobel prize for the interpretation of the genetic code and its function in protein synthesis



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

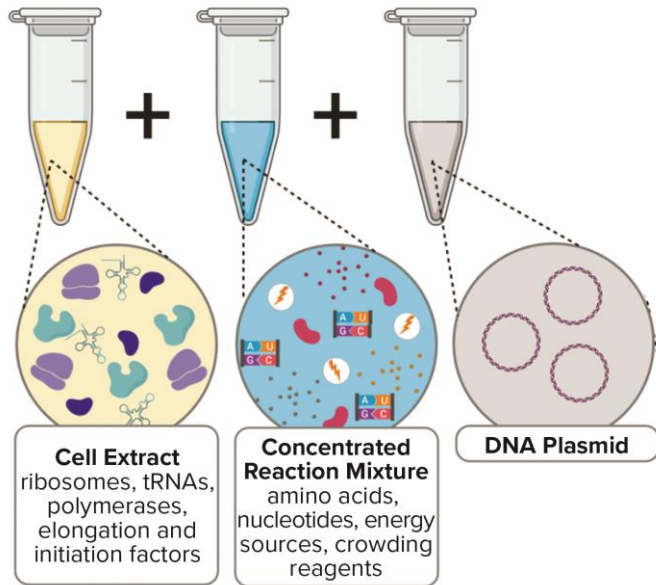


# Cell-free reaction:

## What is the composition of a cell-free reaction?

---

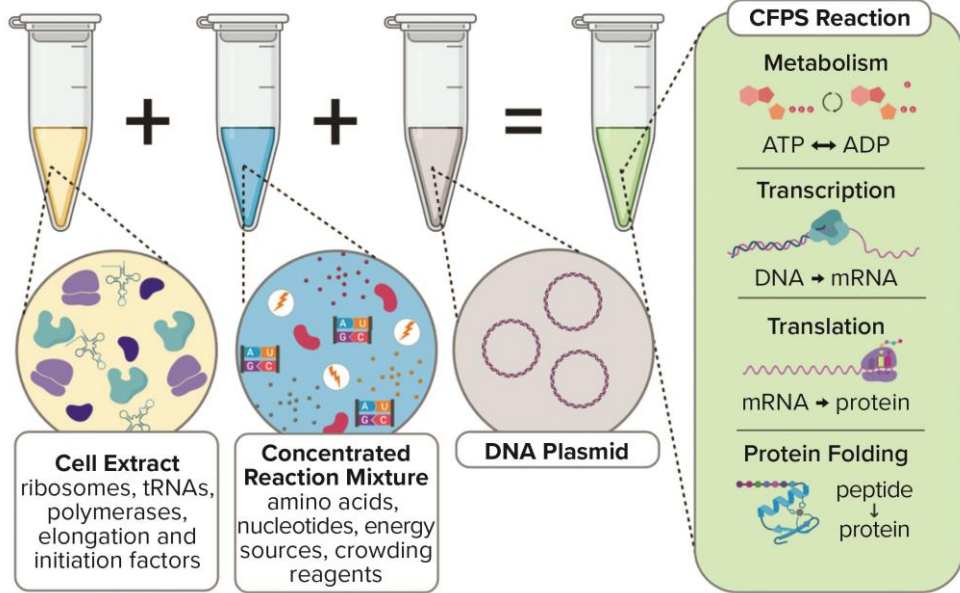
---



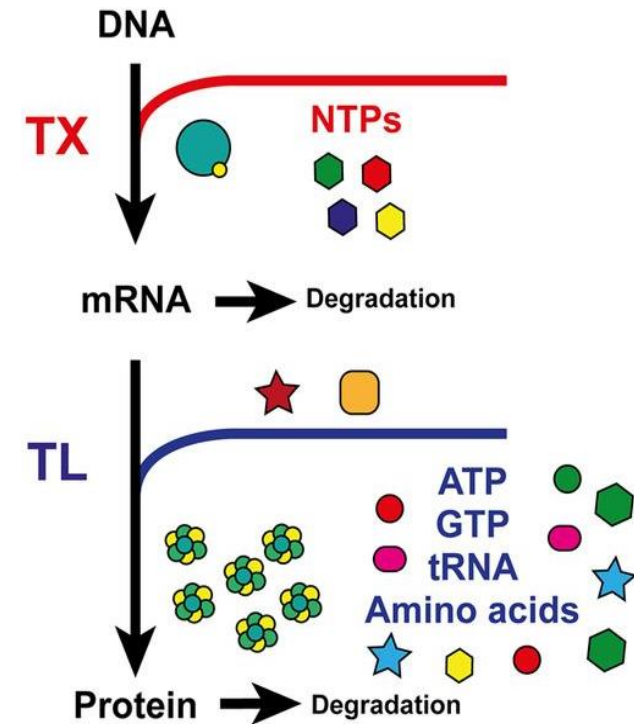
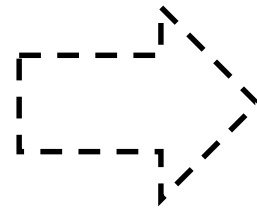
*Melinek B, et al. BioProcess International (2020)*

# Cell-free reaction:

## What is the composition of a cell-free reaction?



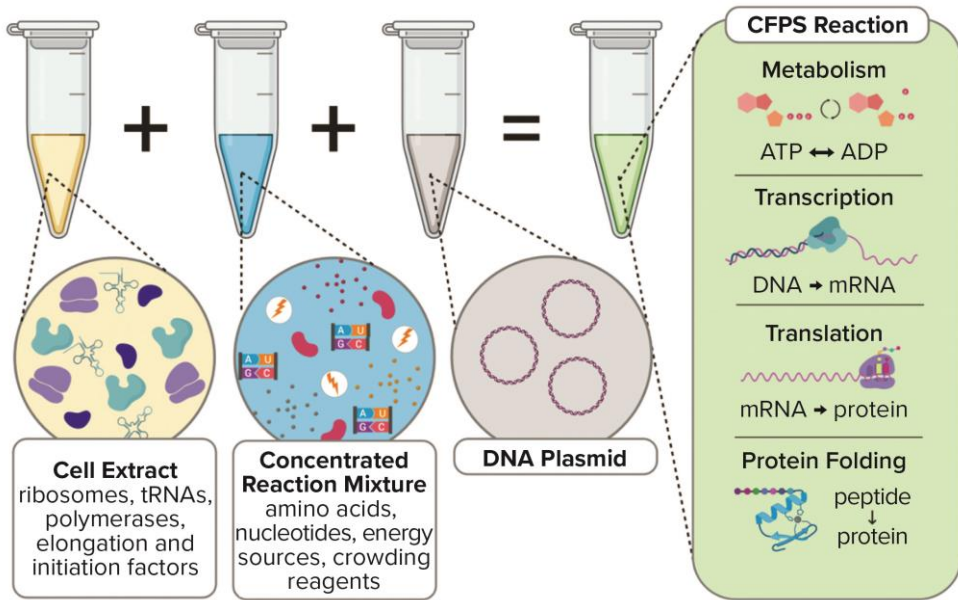
Melinek B, et al. *BioProcess International* (2020)



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

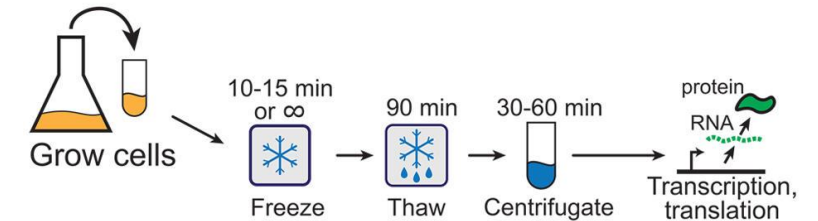


### French press

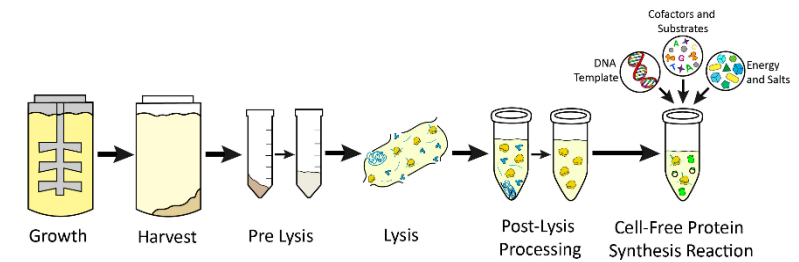


## Chemical disruption

### Autolysis



### Lysozyme

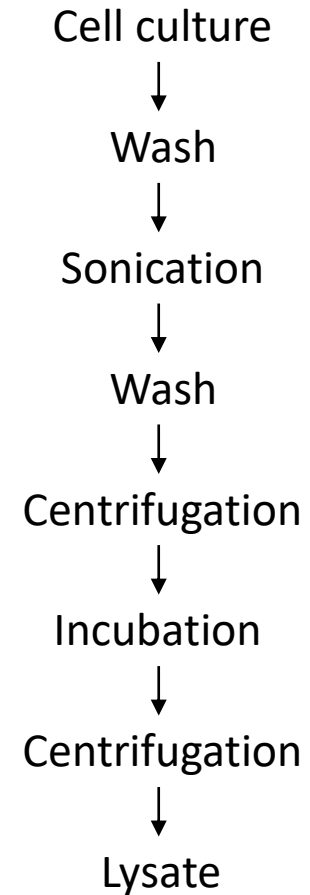
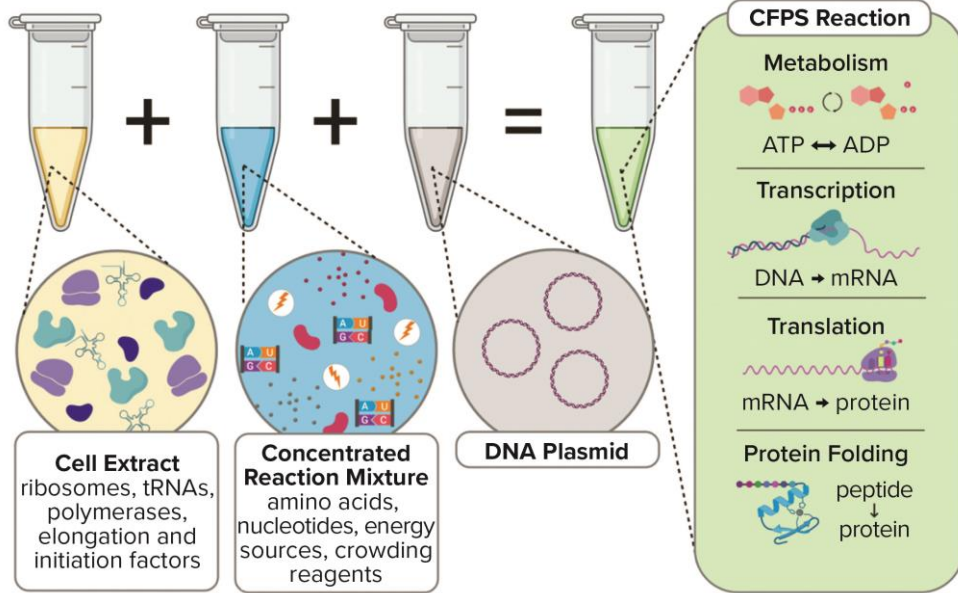


# Cell-free reaction:

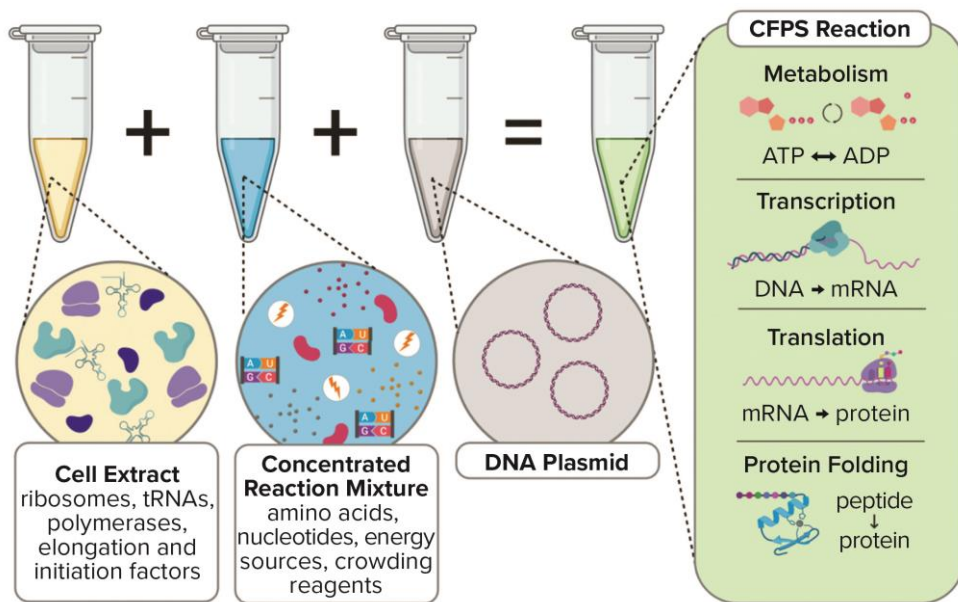
## Protocol for home-made cell-extract preparation

---

---



# 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)

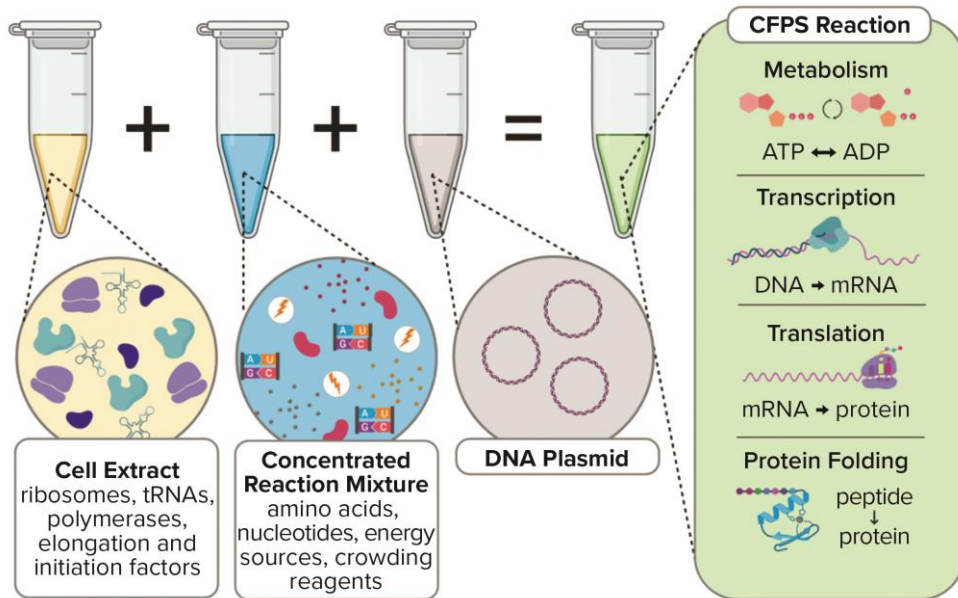
Translation  
Translation  
Ribosome maintenance  
Ribosome stability  
A precursor for formylmethionine (fMet)  
Ribosome activity /RNA stability  
Regenerate ATP via glycolysis  
Regenerate ATP via glycolysis  
Regenerate ATP  
regulating glycolysis  
Transcription

# Cell-free reaction:

## Measurements in 384-well plate

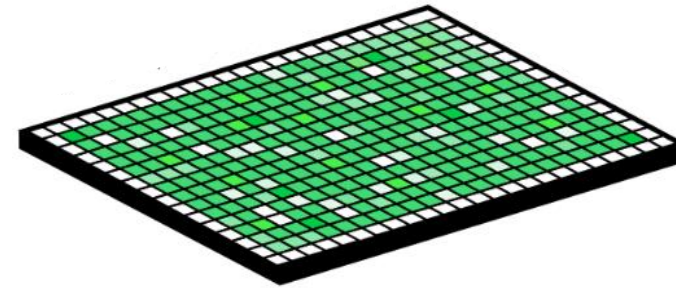
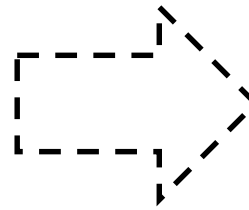
---

---



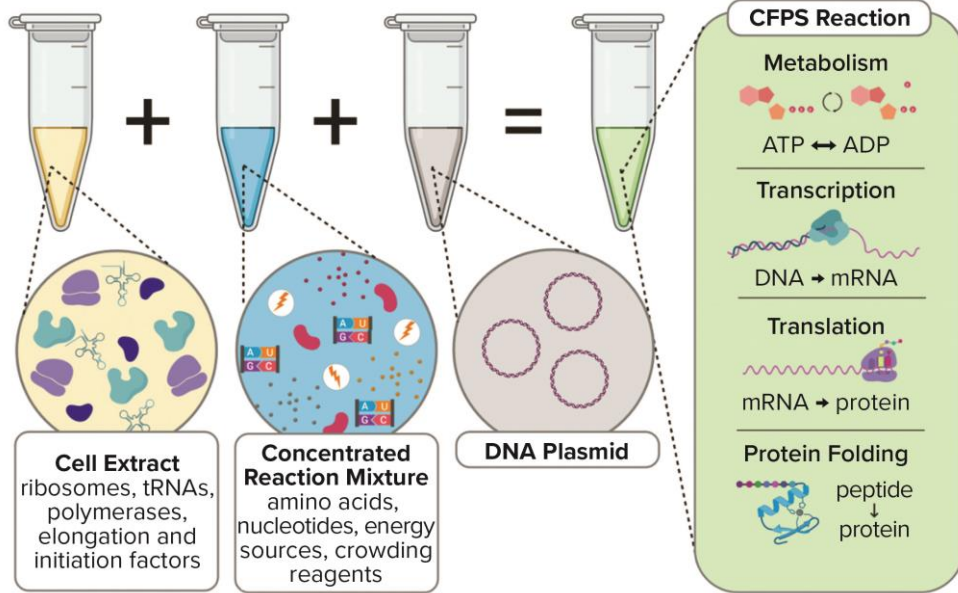
Melinek B, et al. *BioProcess International* (2020)

1 to 20  $\mu$ l reactions  
(in 384-well)



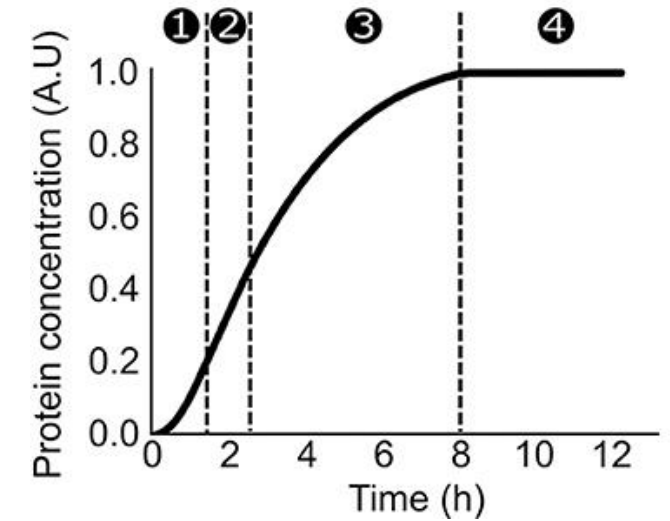
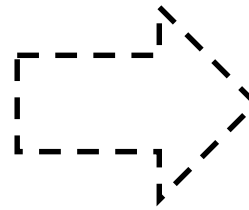
# Cell-free reaction:

## Measurements in 384-well plate

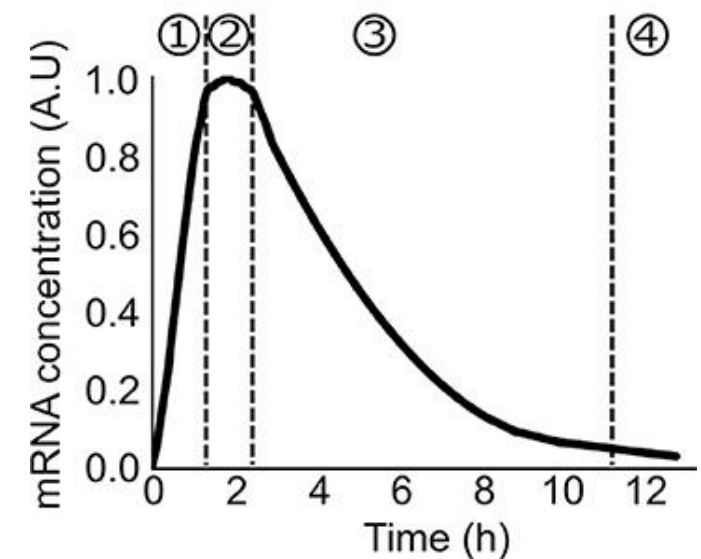


Melinek B, et al. *BioProcess International* (2020)

### Translation

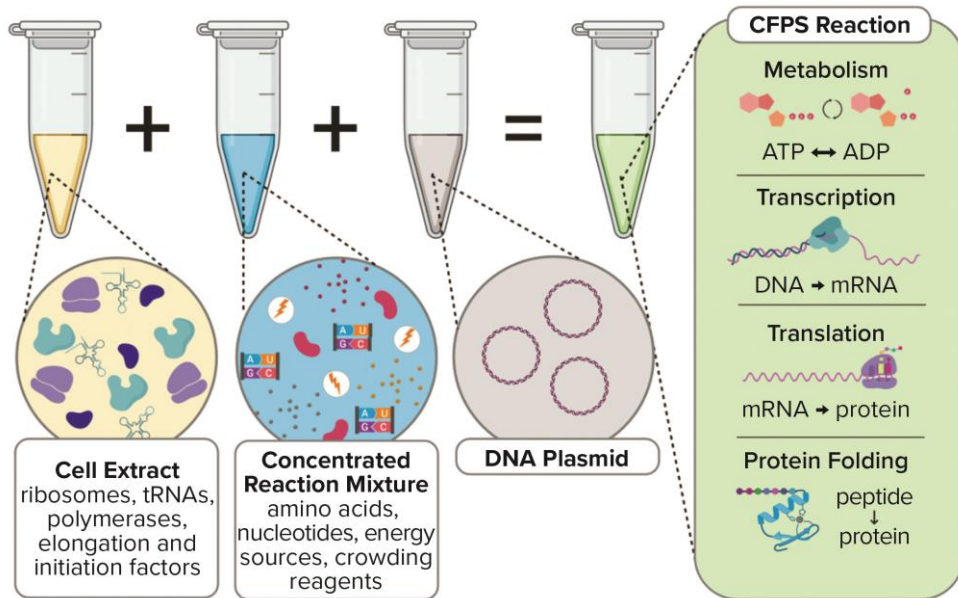


### Transcription



# Cell-free reaction:

## Current existing protocol to produce bacterial cell extract



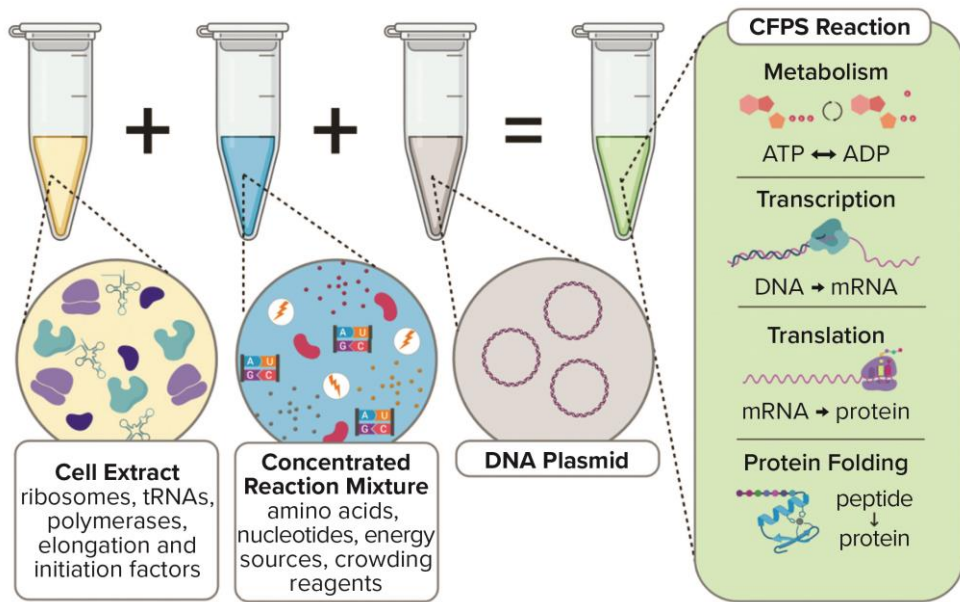
Melinek B, et al. *BioProcess International* (2020)

**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



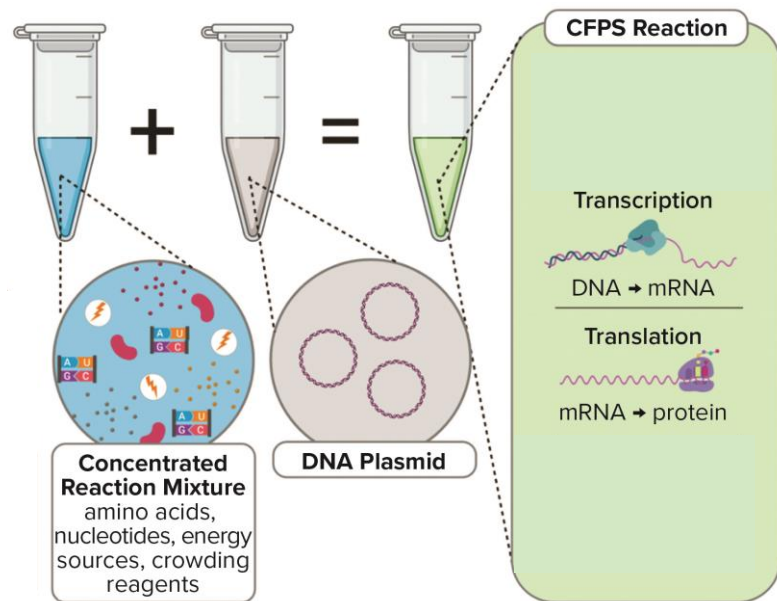
Melinek B, et al. *BioProcess International* (2020)

	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

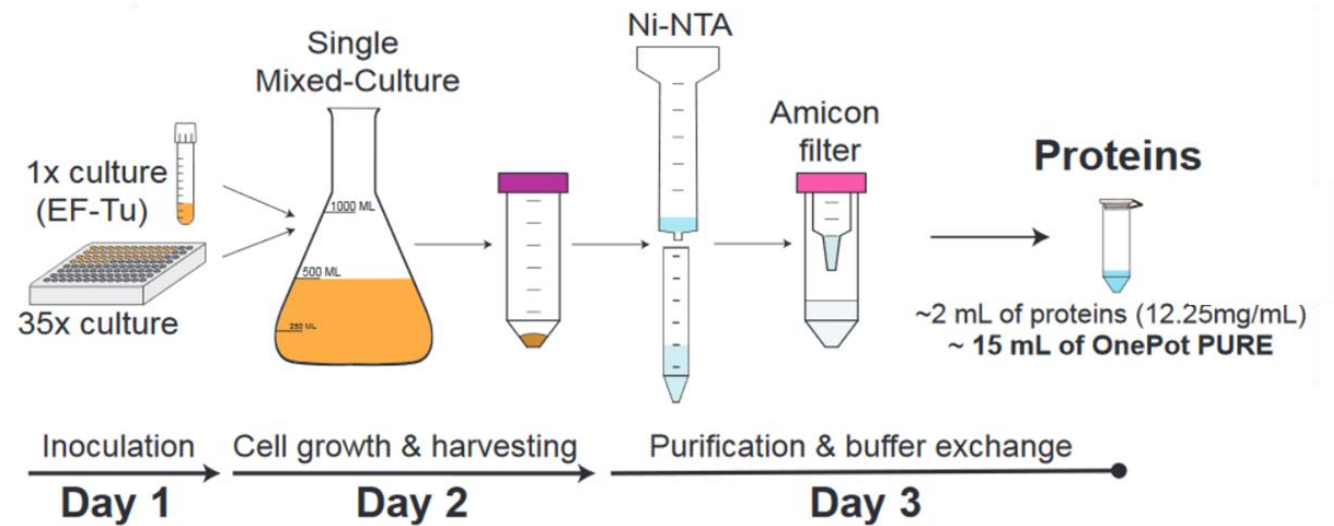
# Cell-free reaction:

## 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)<sub>6</sub>-tagged 70S ribosomes purified using affinity chromatography



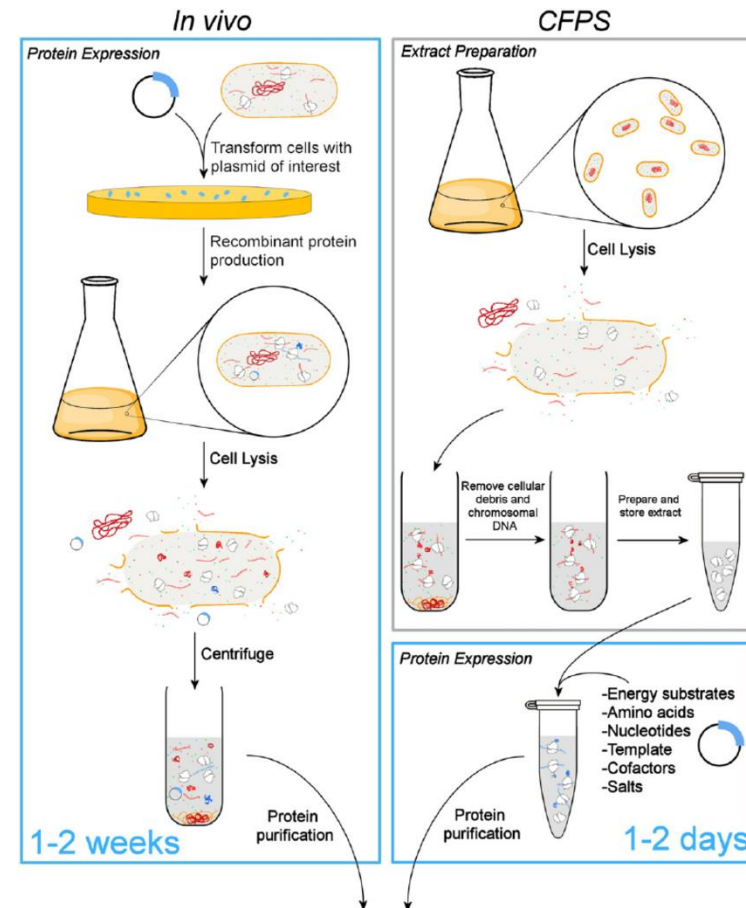
Lavickova, Barbora, et al. *ACS synthetic biology* (2019)



# 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

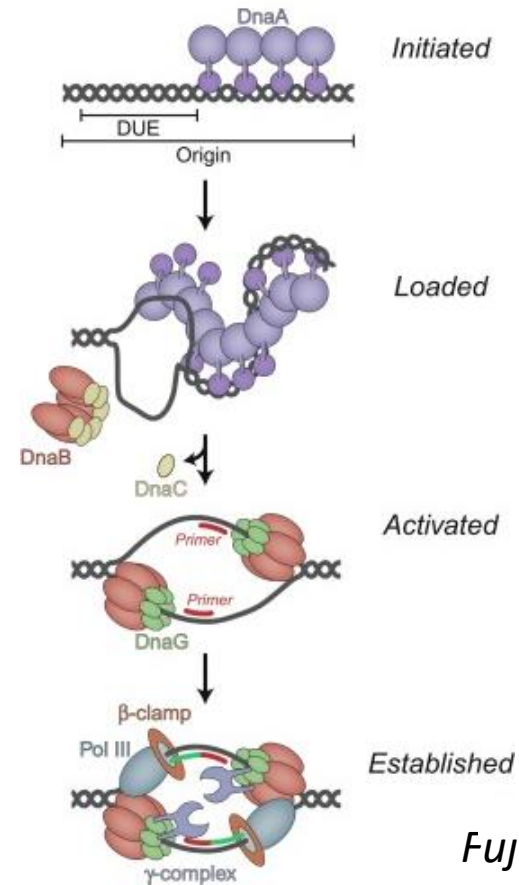
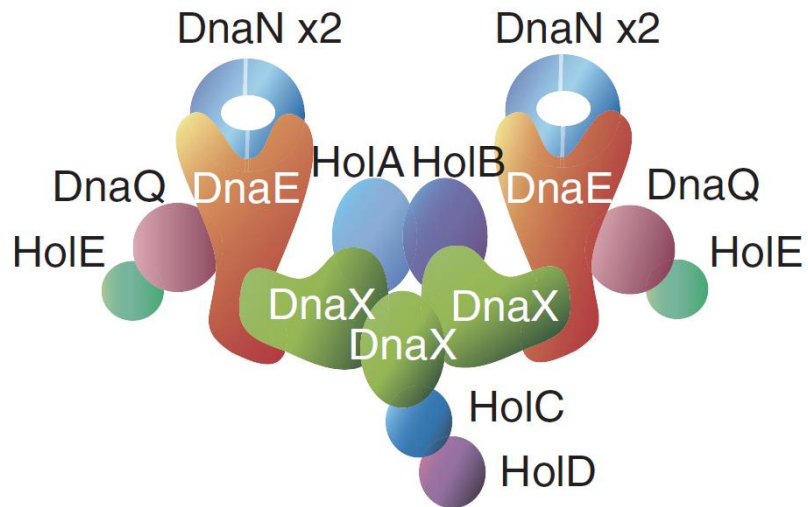
---

---

### - 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

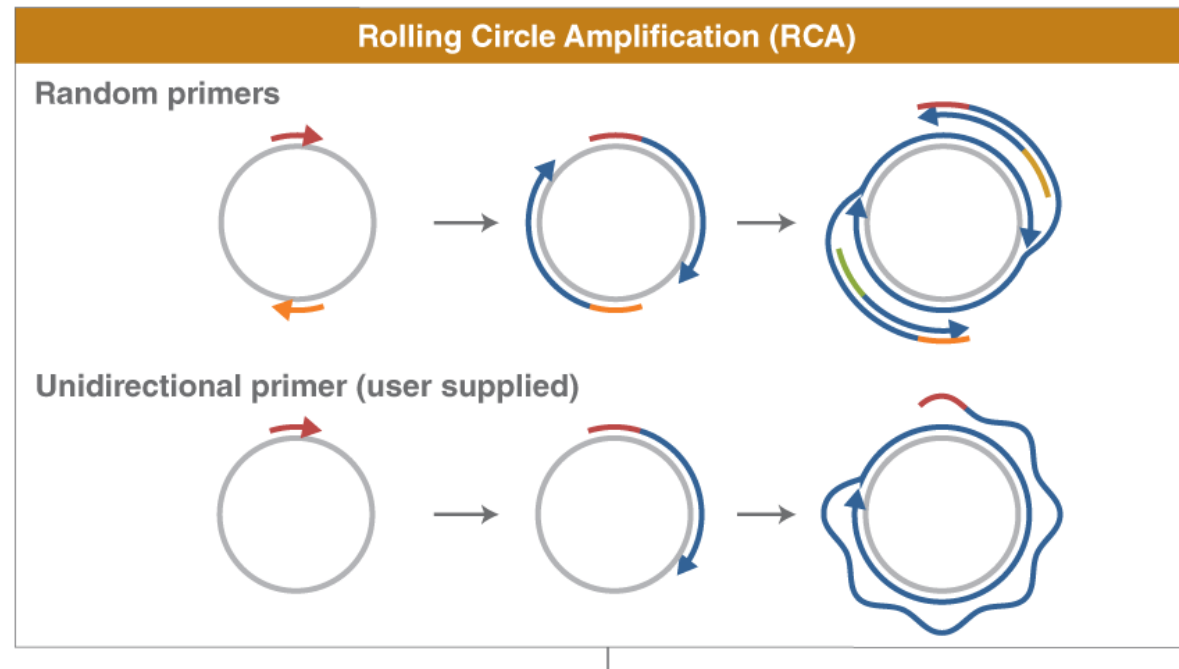
---

---

### - 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

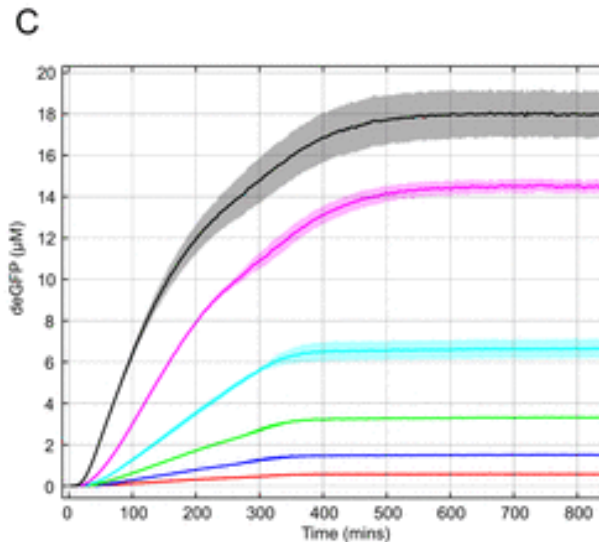
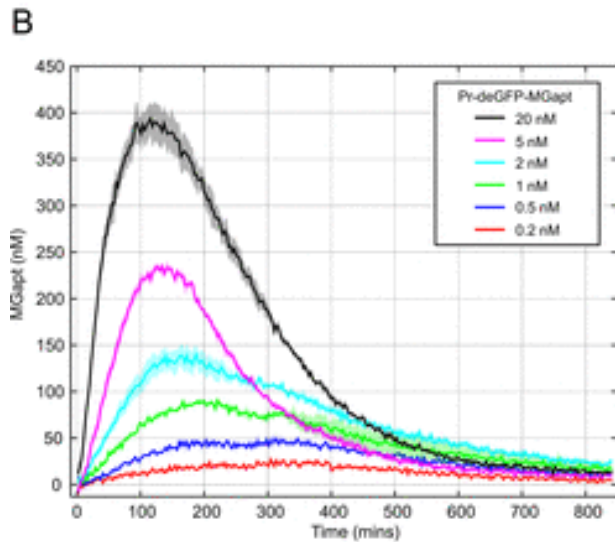
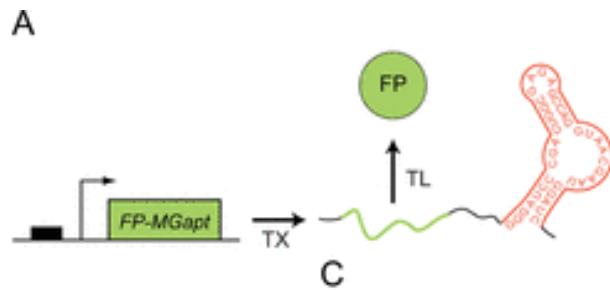
*Libicher, K, nat comms (2020)*

# Cell-free reaction:

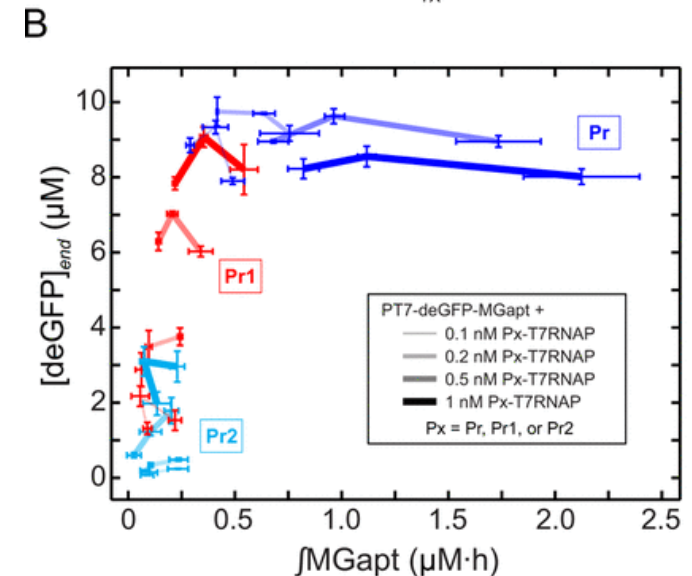
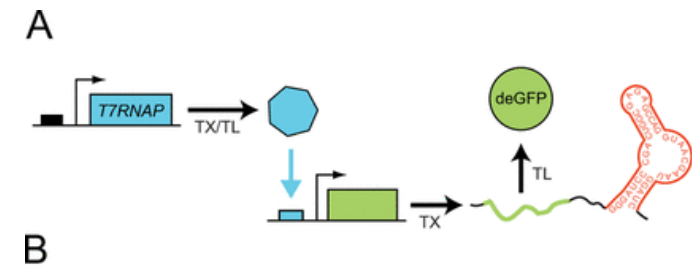
## Fundamental science: cell-free to understand living cells

### - Central functions

#### Transcription process: T7 polymerase



Gene circuit performance characterization



# Cell-free reaction:

## Fundamental science: cell-free to understand living cells

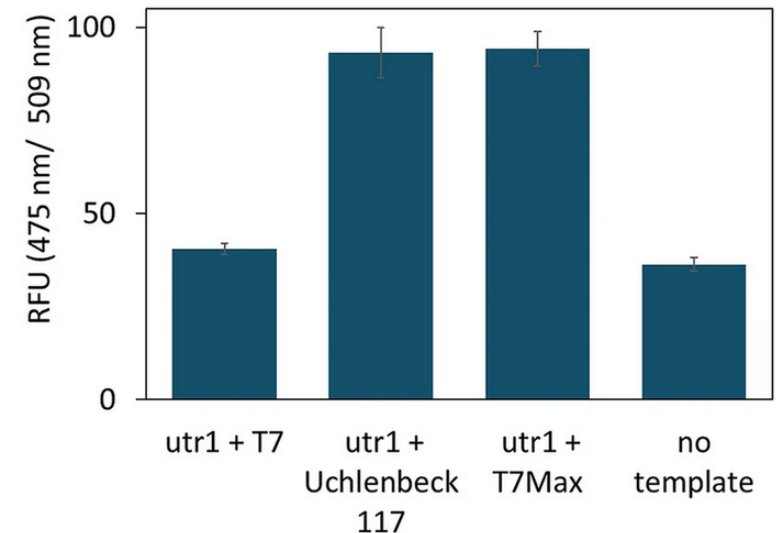
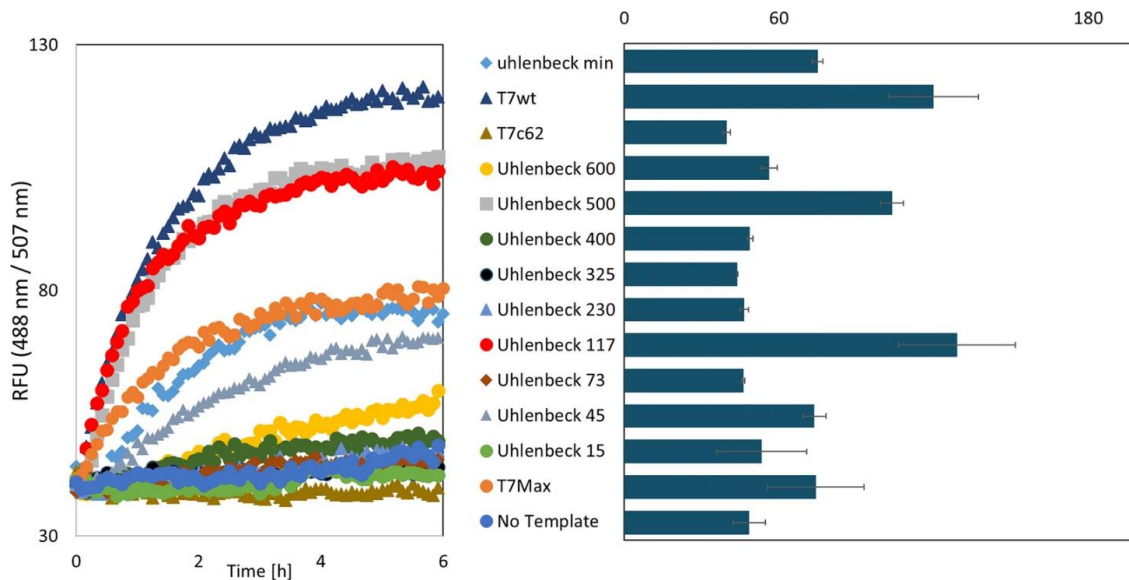
### - Central functions

**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



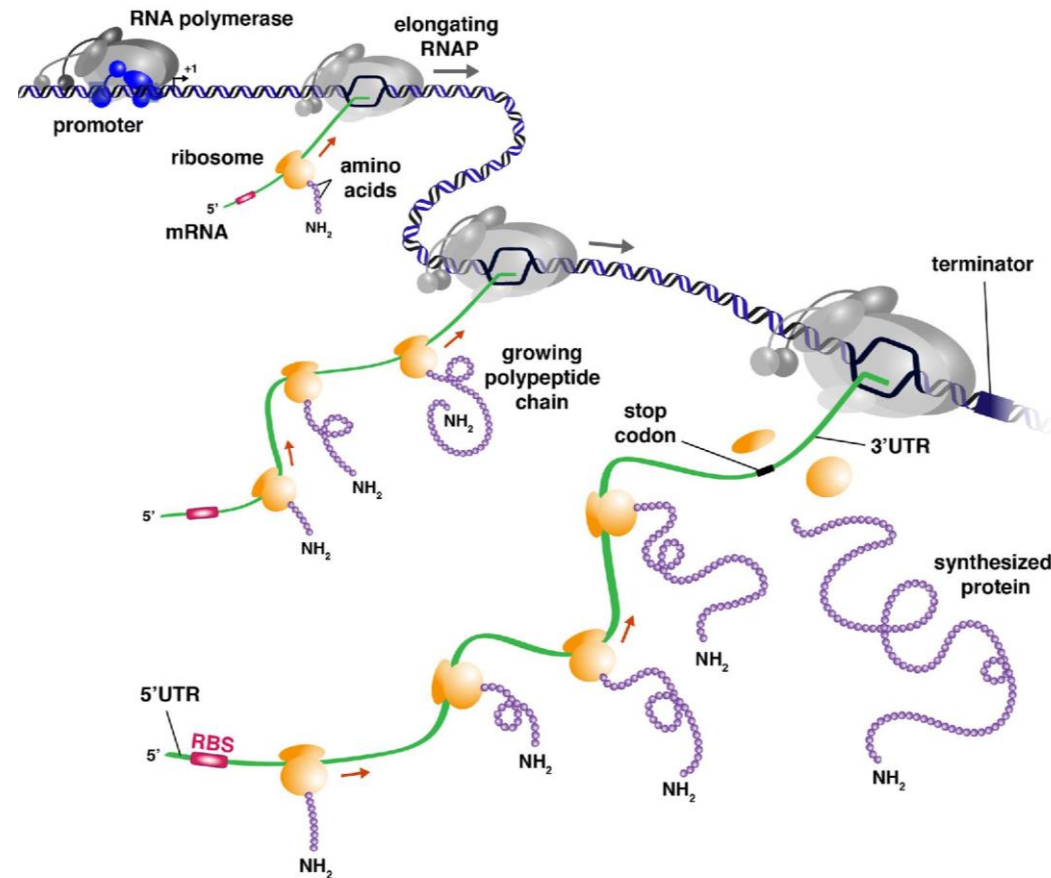


# Cell-free reaction:

## Fundamental science: cell-free to understand living cells

### - Central functions

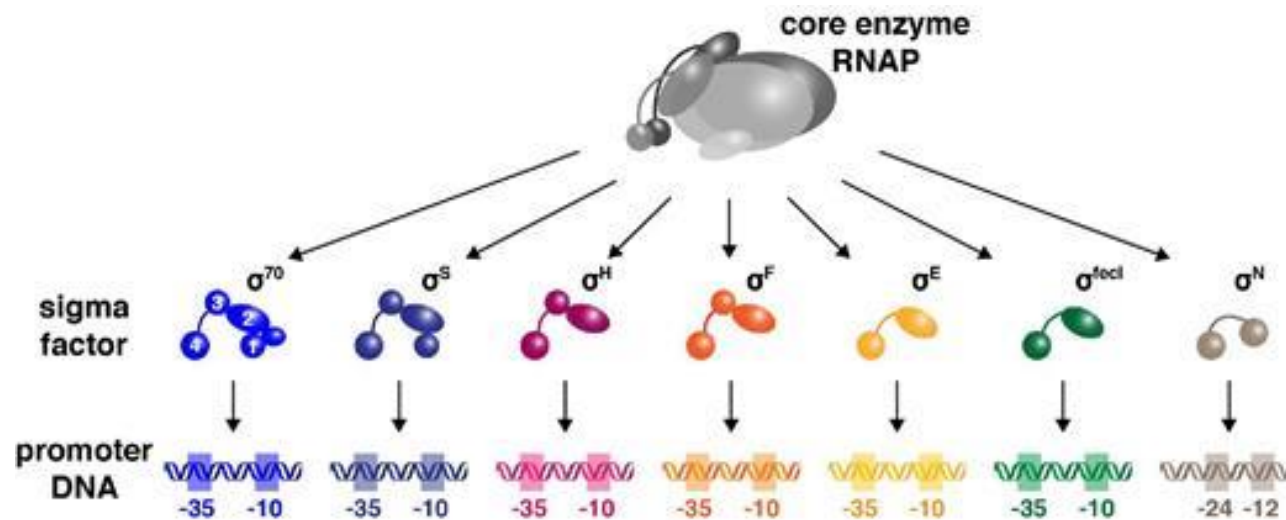
#### Transcription



# Cell-free reaction:

## Fundamental science: cell-free to understand living cells

- Central functions
- Transcription



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

# Cell-free reaction:

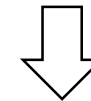
## Fundamental science: cell-free to understand living cells

### - 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



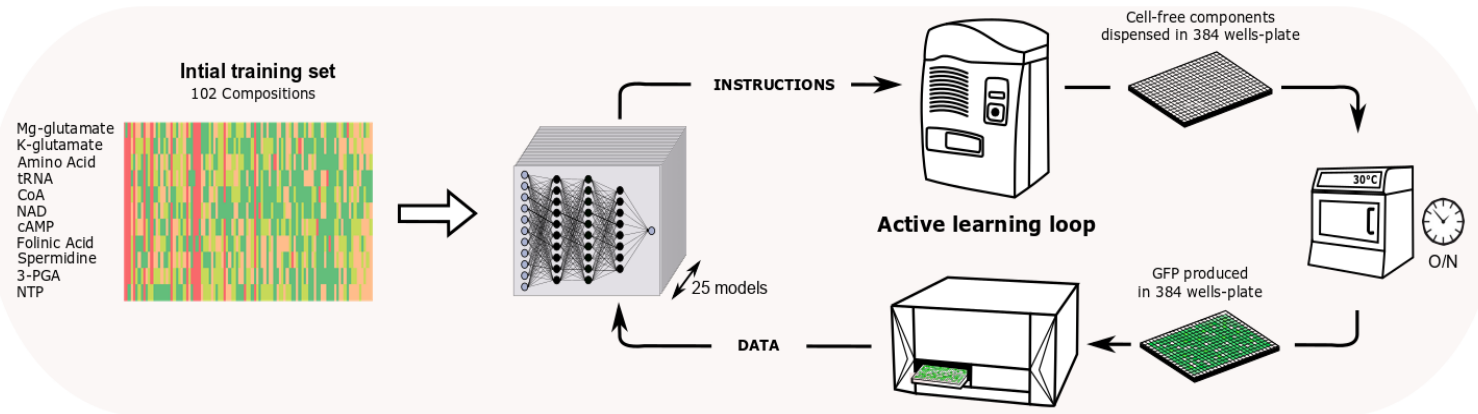
- Can we improve protein production for a specific lysate without increasing the price of cell-free reaction?
- Can we provide efficient predictions of protein production?
- Can we highlight the critical parameters involved in protein production?

# Cell-free reaction:

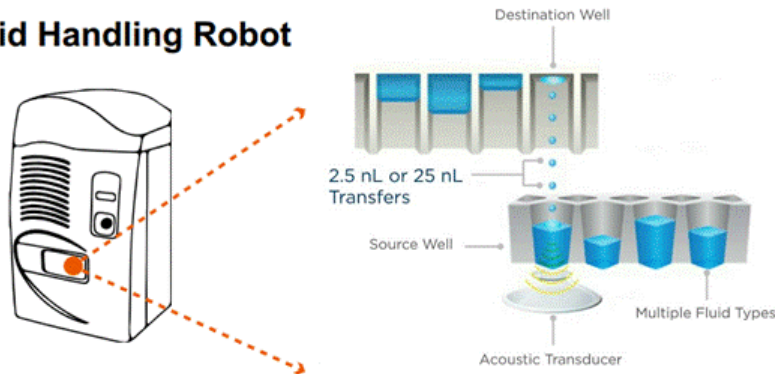
## Fundamental science: cell-free to understand living cells

### - Central functions

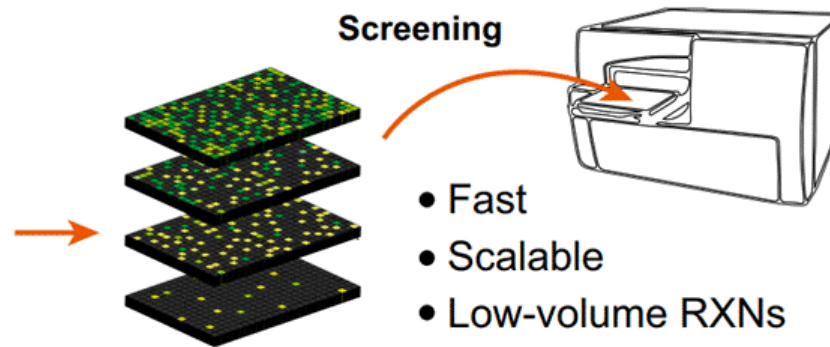
#### Translation: Optimizing translation process



### Liquid Handling Robot



### Screening

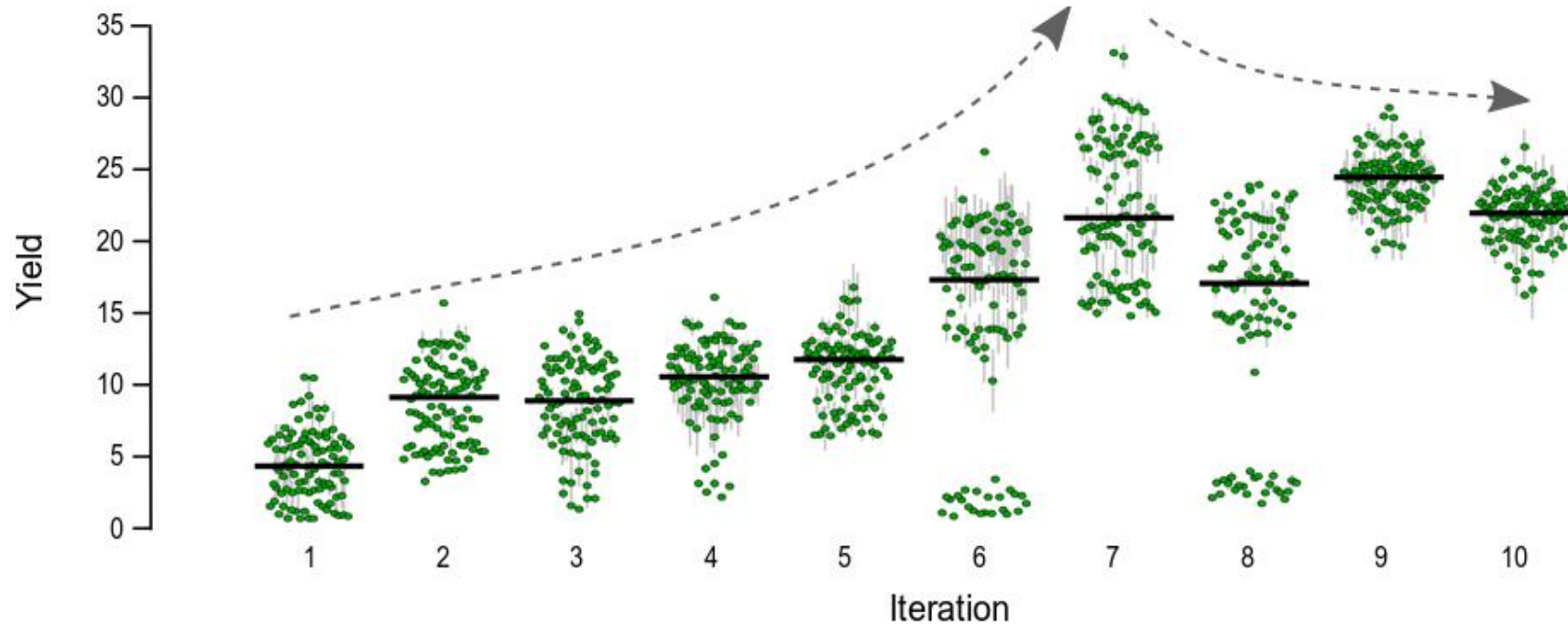


# Cell-free reaction:

Fundamental science: cell-free to understand living cells

- Central functions

Translation: Optimizing translation process

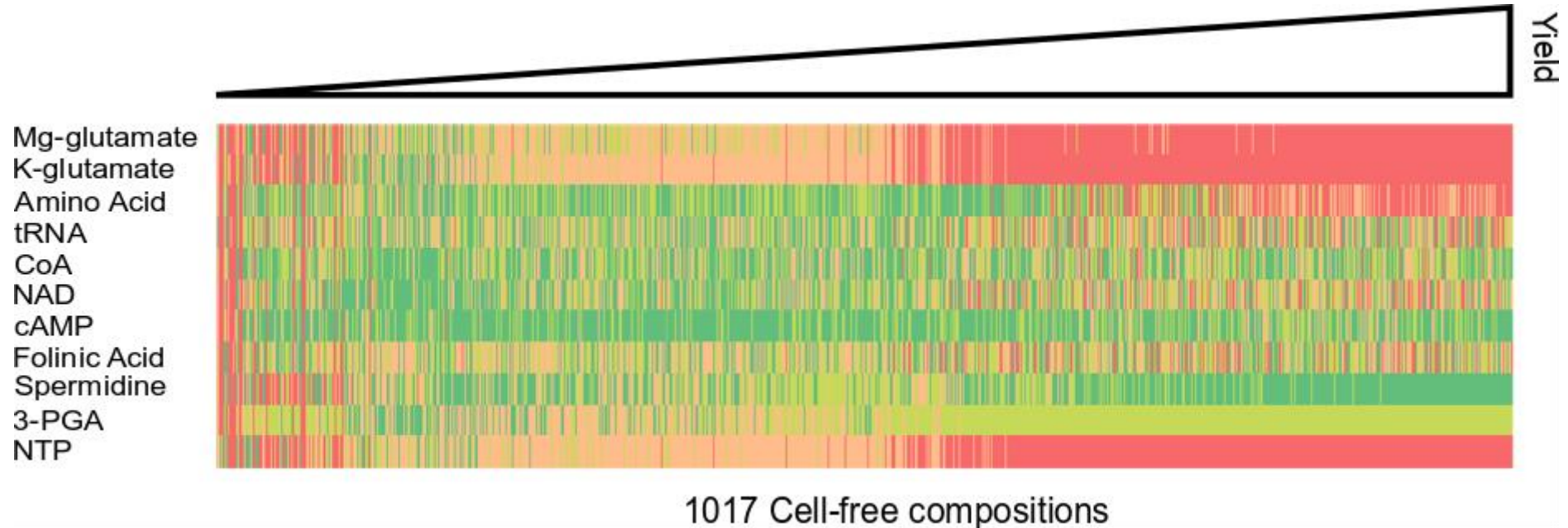


# Cell-free reaction:

## 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:

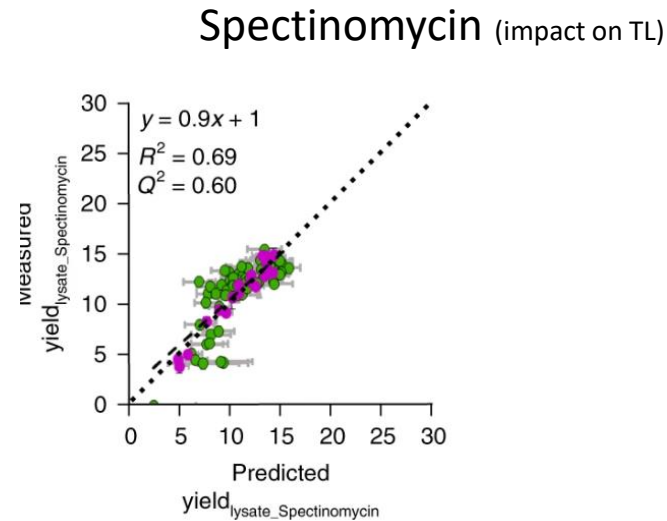
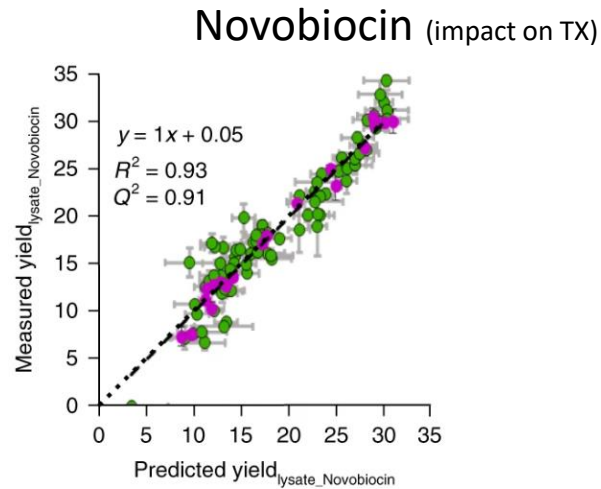
## Fundamental science: cell-free to understand living cells

---

---

### - Central functions

Translation: Optimizing translation process



### Transcription vs translation:

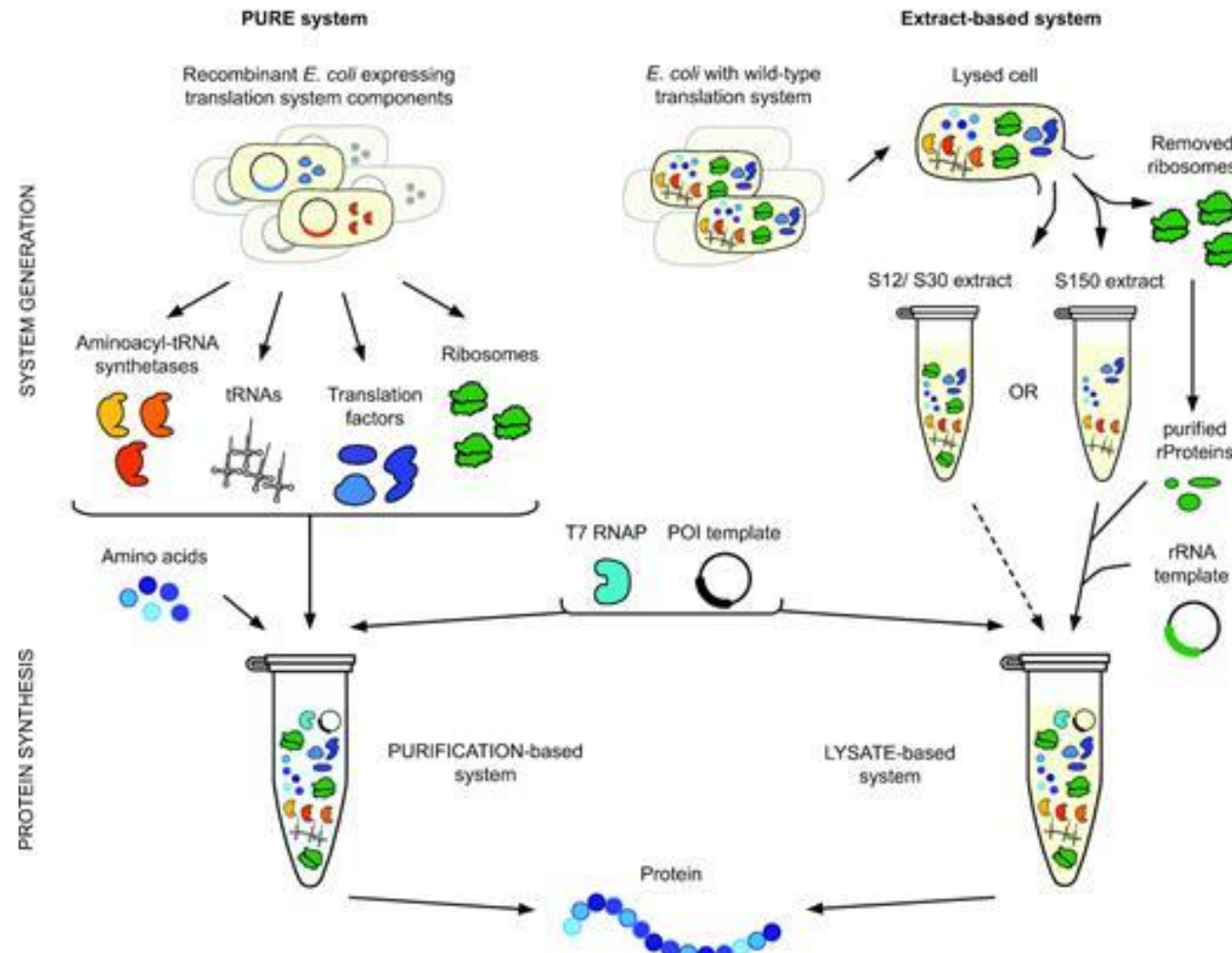
Our data suggests that we mainly improved translation via composition optimization

# Cell-free reaction:

## Fundamental science: cell-free to understand living cells

### - Central functions

#### Translation: engineering translation machinery



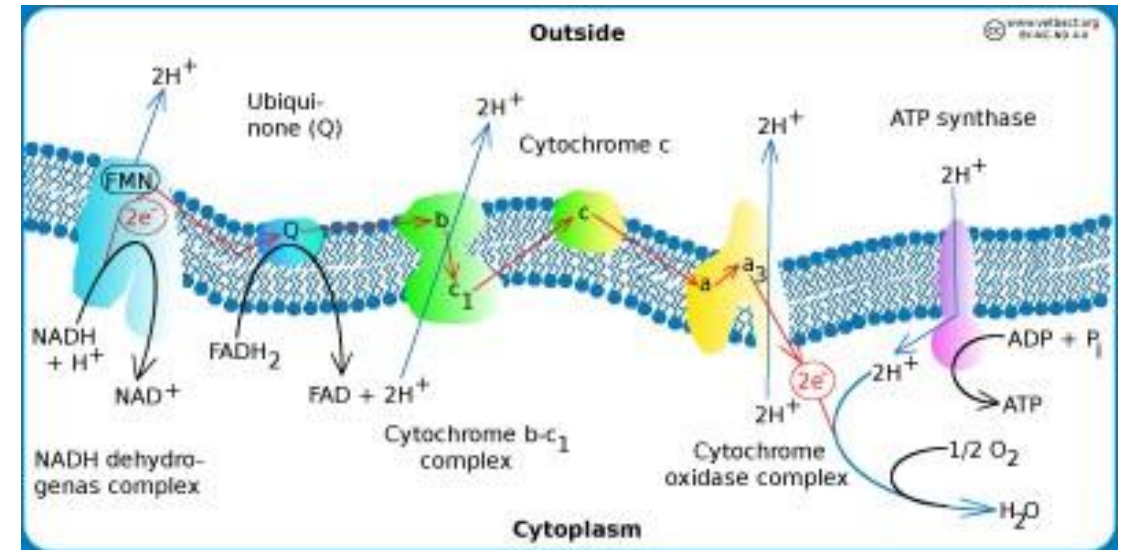
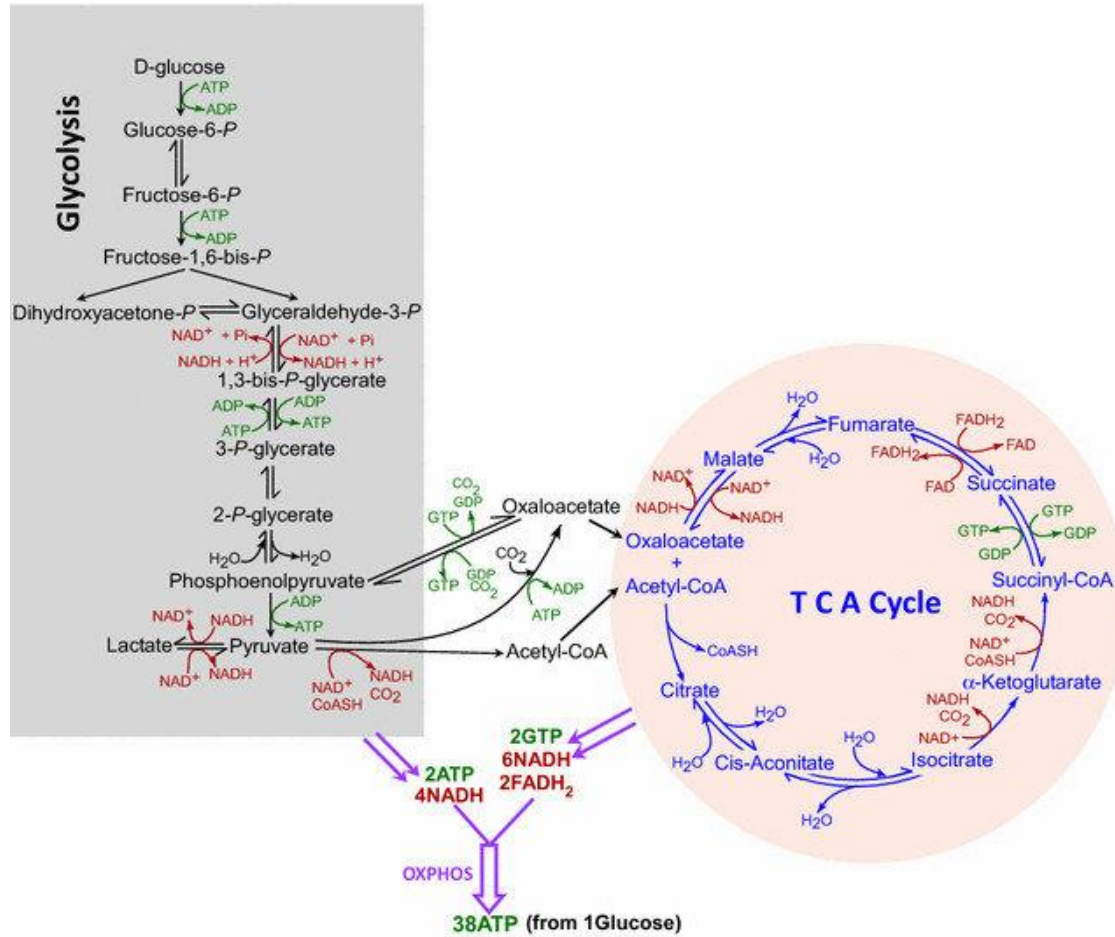


# Cell-free reaction:

## Fundamental science: cell-free to understand living cells

### - Central functions

### Metabolism and energy production



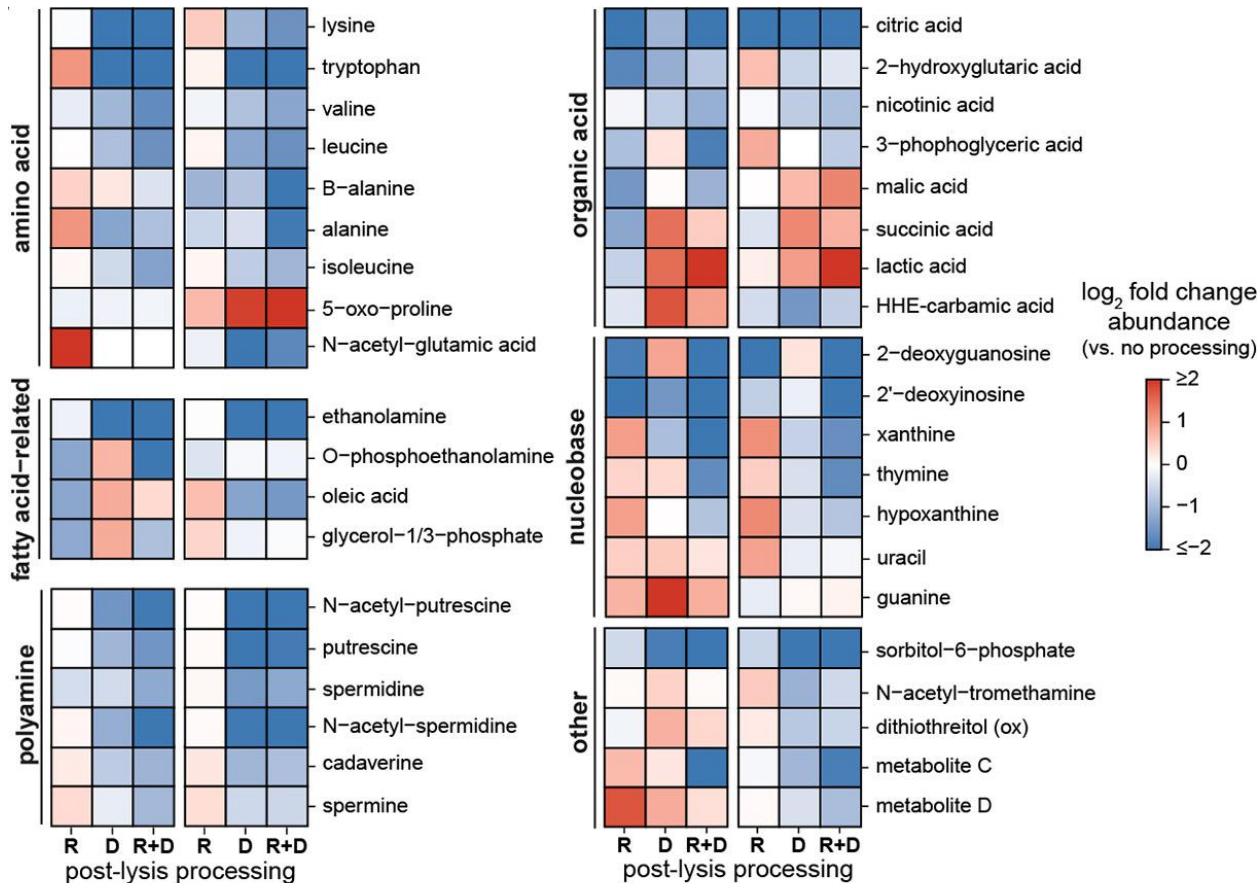
# Cell-free reaction:

## Fundamental science: cell-free to understand living cells

### - Central functions

### Metabolism and energy production

Study of composition in metabolites in different cell-free process

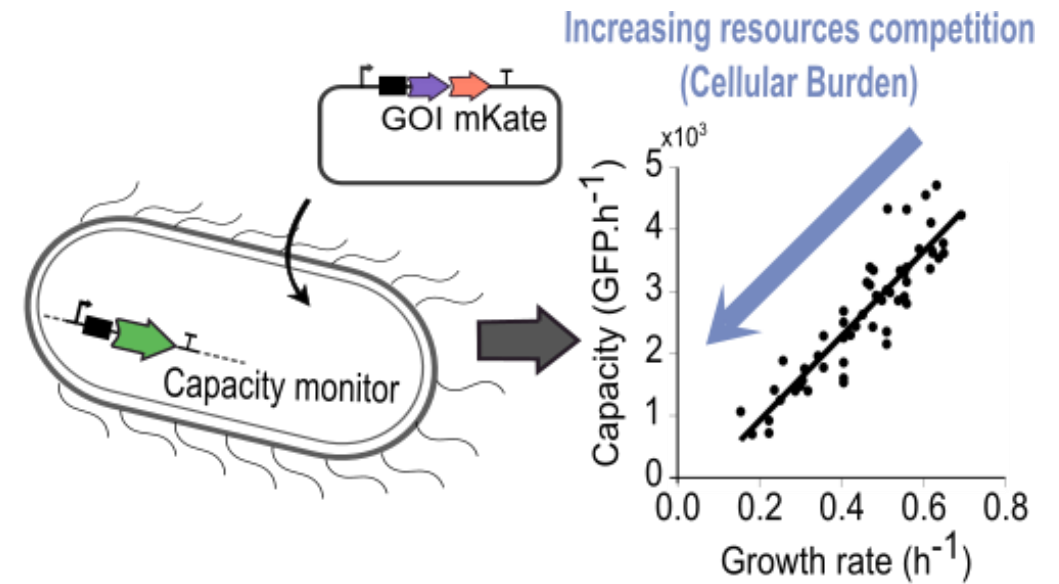
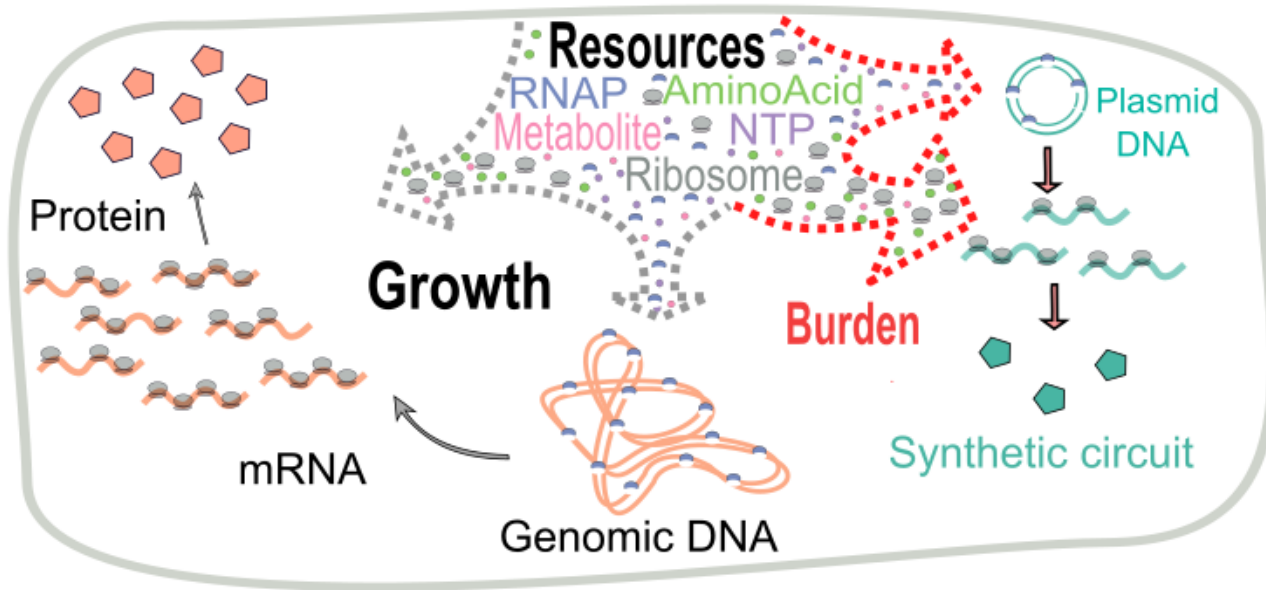


-> improve cell-free efficiency, supplemented with proteins to observe the impact on each part of the metabolism

# Cell-free reaction:

## Fundamental science: cell-free to understand living cells

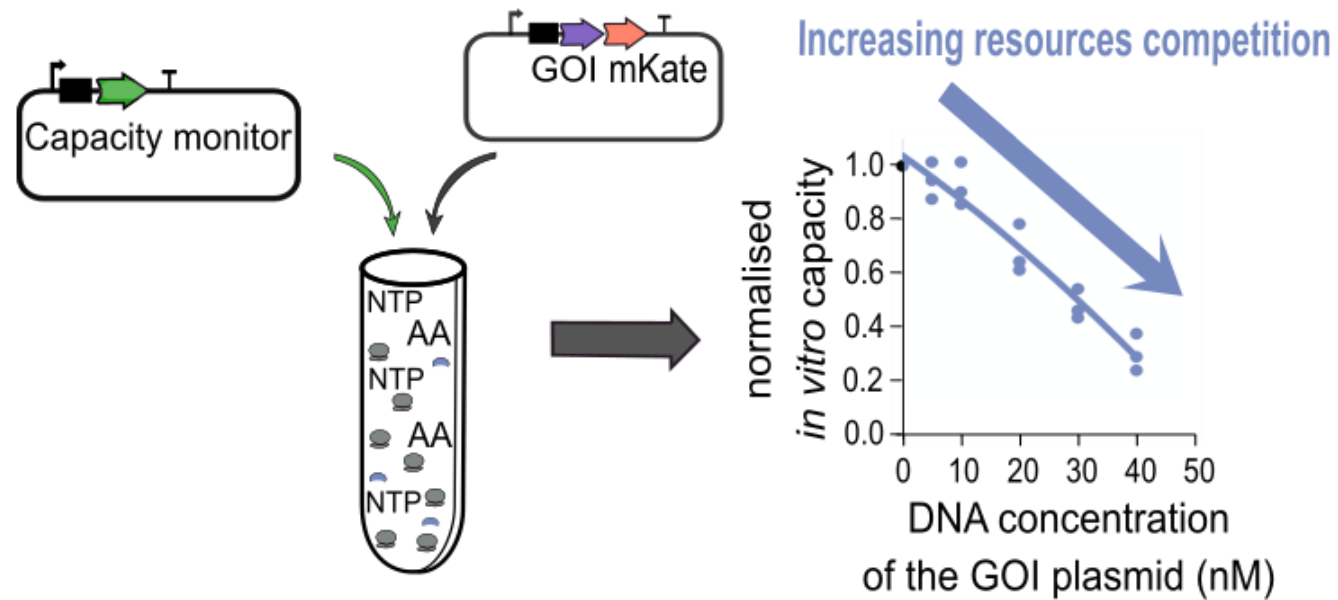
- Resource competition *in vitro*  
Production burden



# Cell-free reaction:

## Fundamental science: cell-free to understand living cells

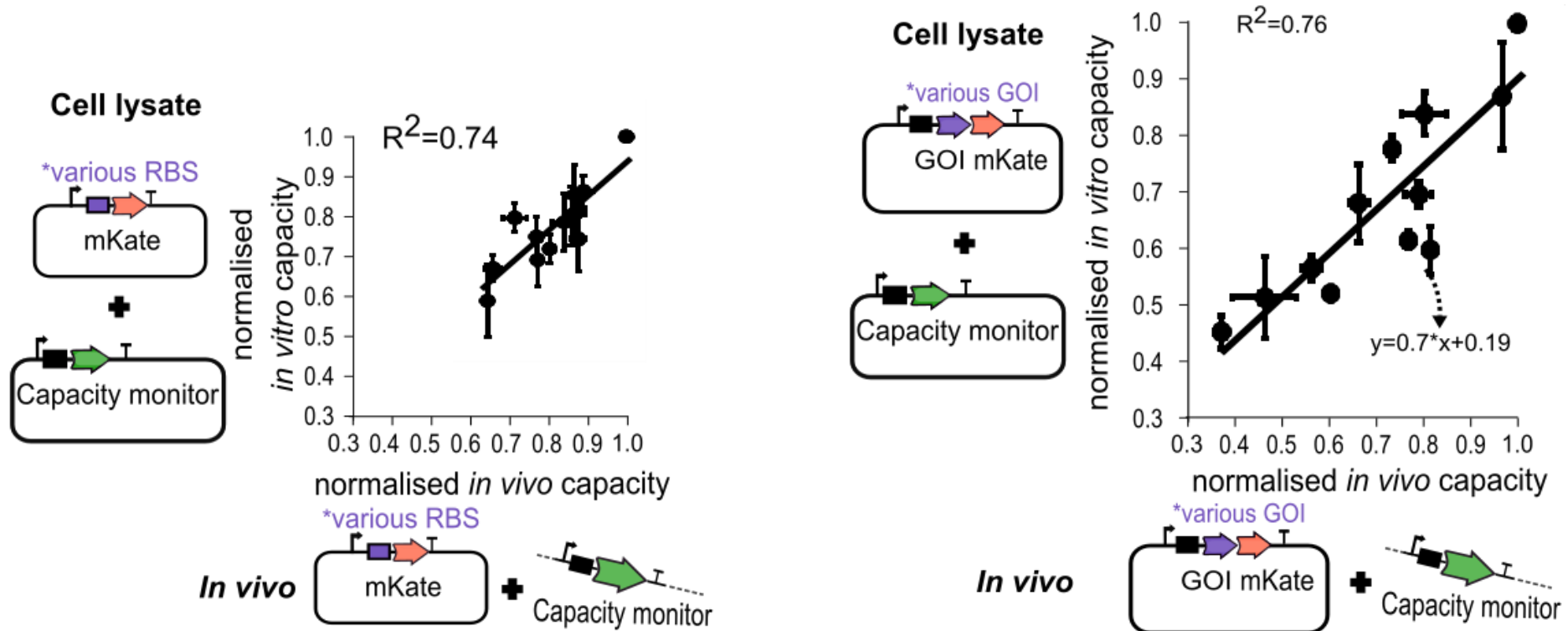
- Resource competition *in vitro*  
Production burden



# Cell-free reaction:

## Fundamental science: cell-free to understand living cells

- Resource competition *in vitro*  
Production burden

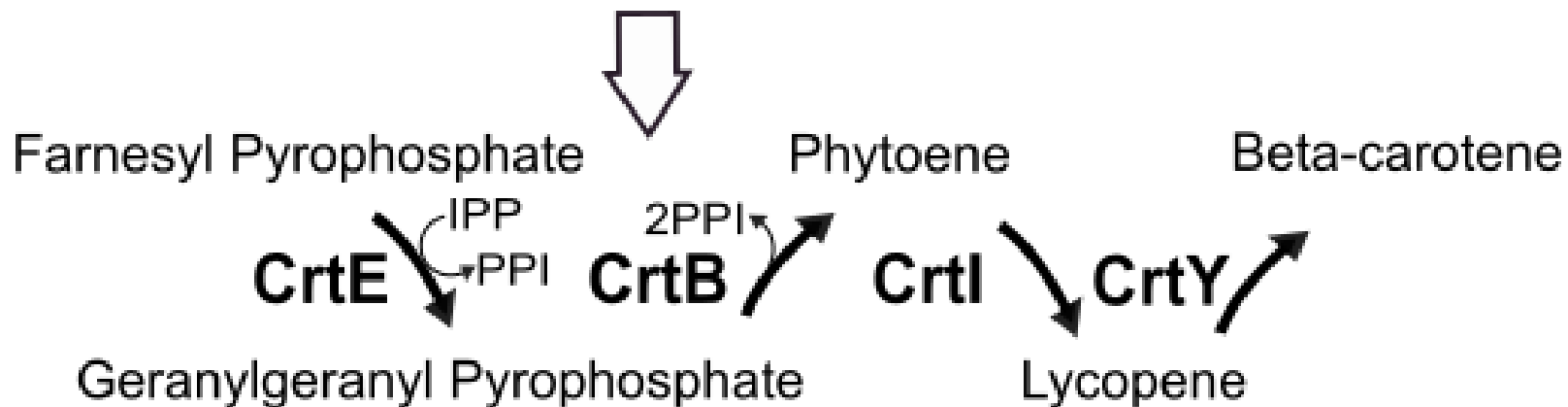
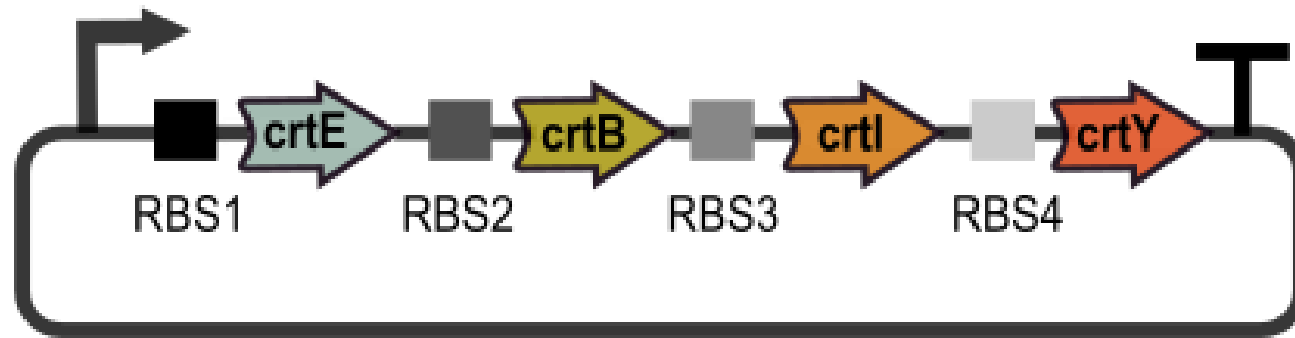


# Cell-free reaction:

## Fundamental science: cell-free to understand living cells

- Resource competition *in vitro*

Decouple production and metabolic burden



# Cell-free reaction:

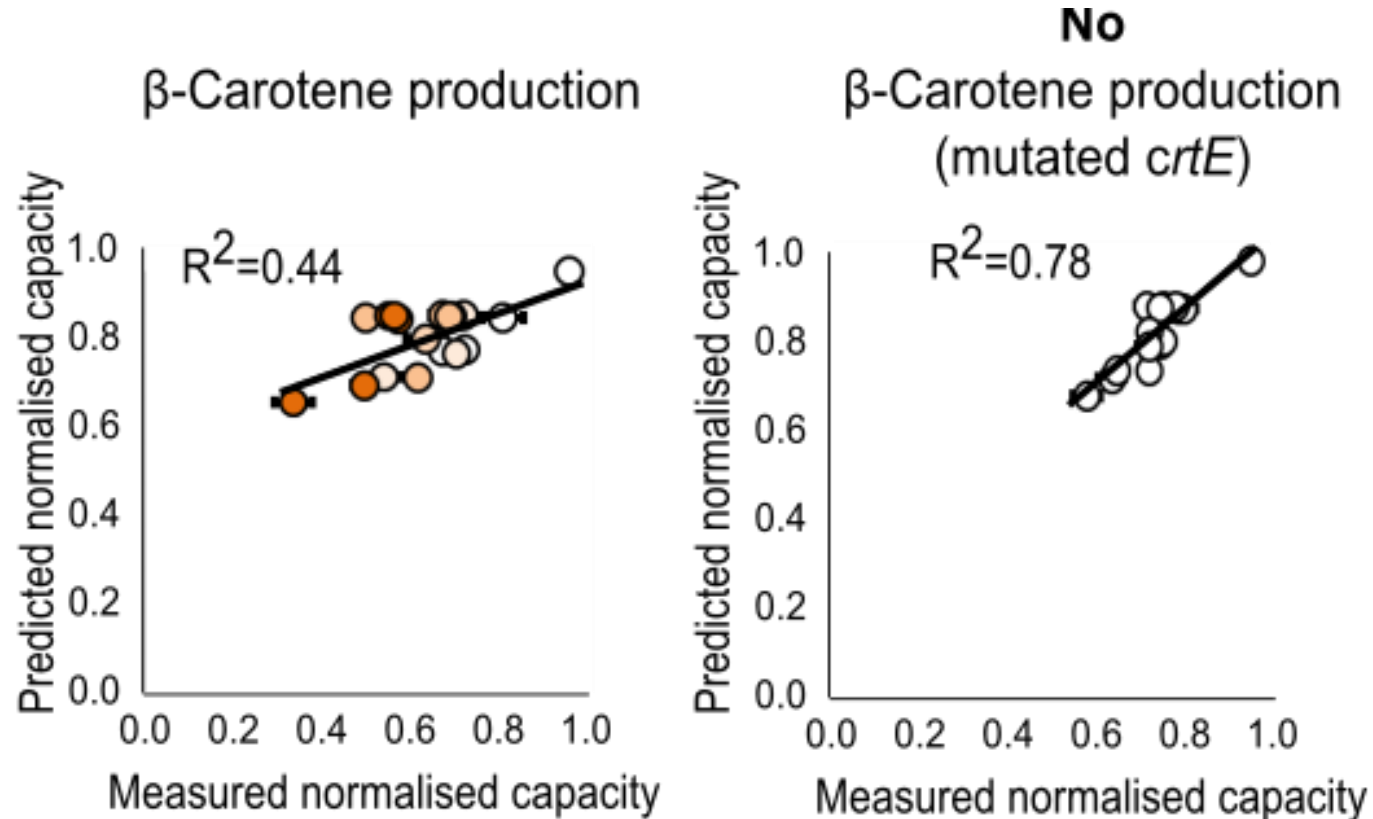
## Fundamental science: cell-free to understand living cells

---

---

- Resource competition *in vitro*

Decouple production and metabolic burden

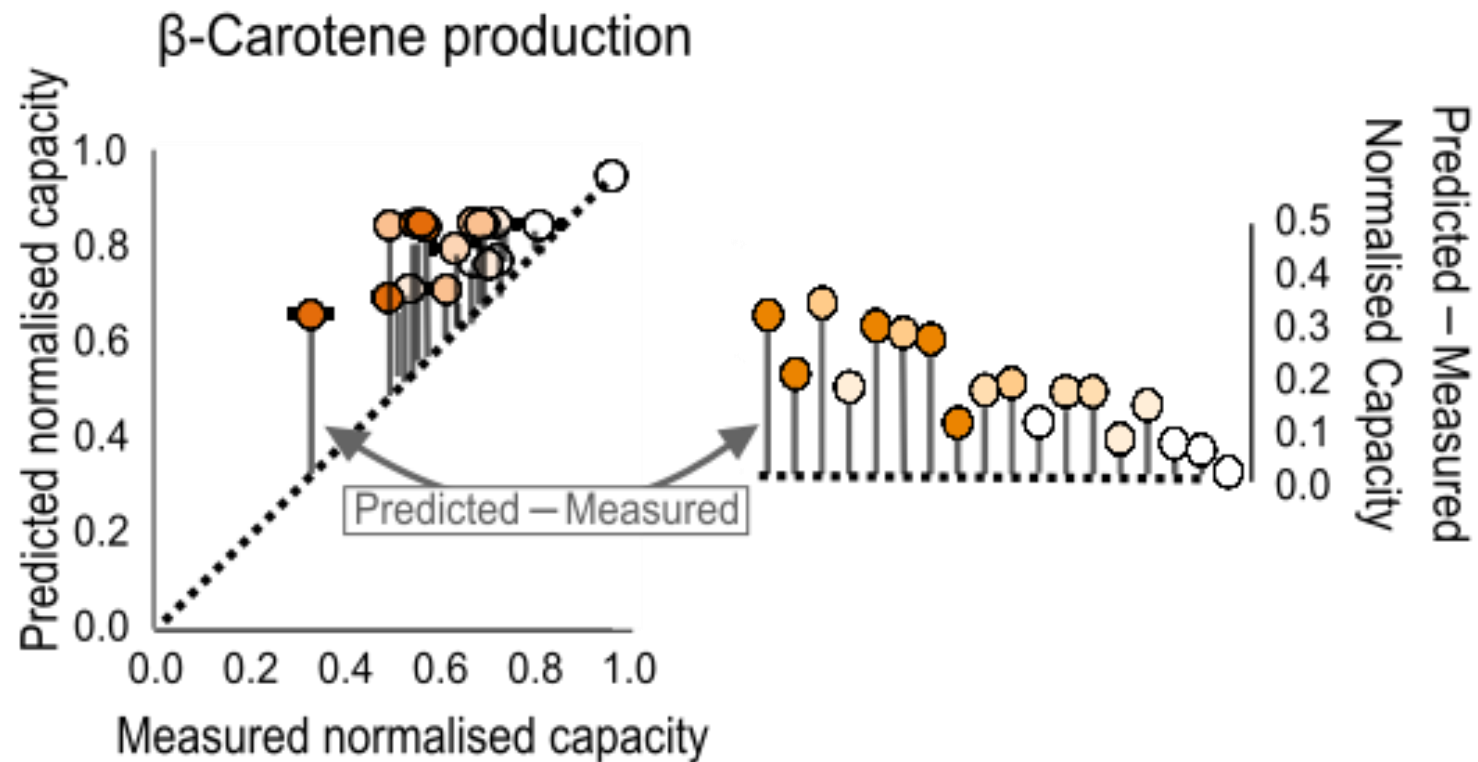


# Cell-free reaction:

## Fundamental science: cell-free to understand living cells

- Resource competition *in vitro*

Decouple production and metabolic burden

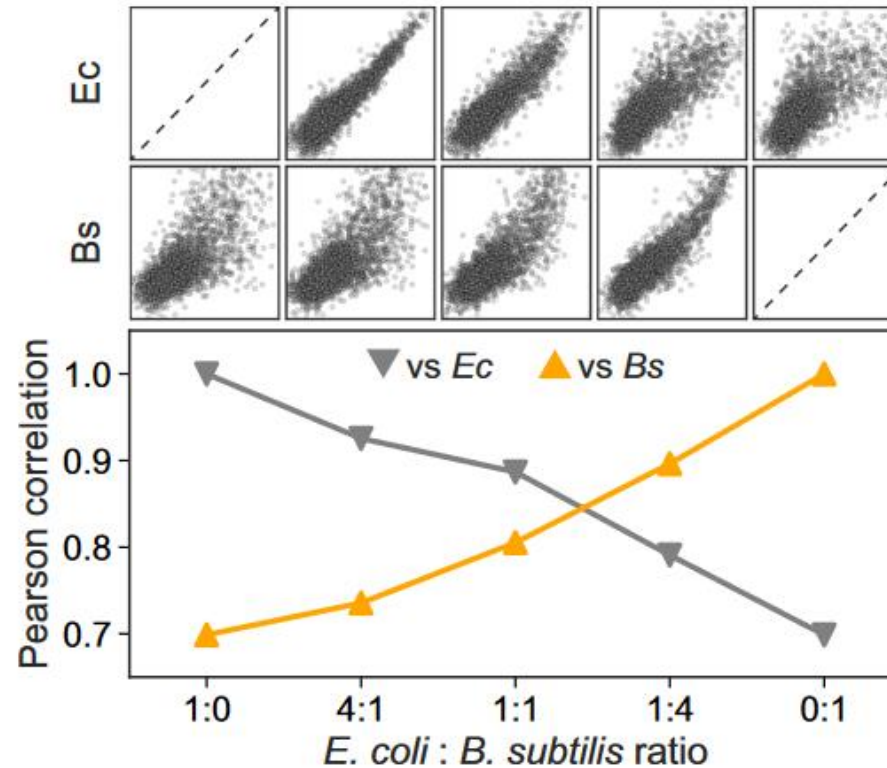
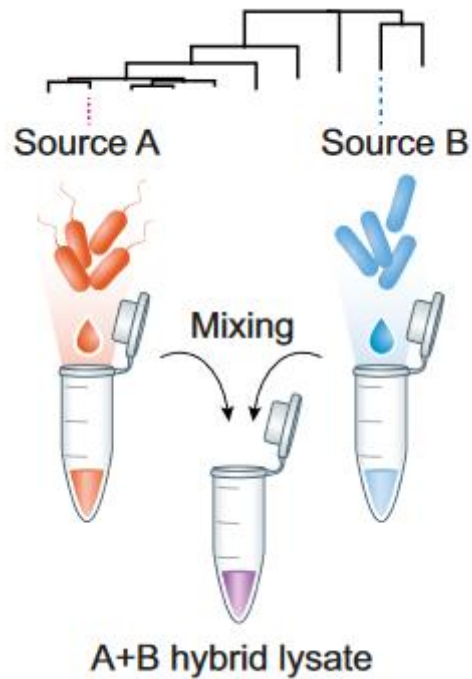




# Cell-free reaction:

## Fundamental science: cell-free to understand living cells

- Construction hybrid systems
- Multi-species systems



# Cell-free reaction:

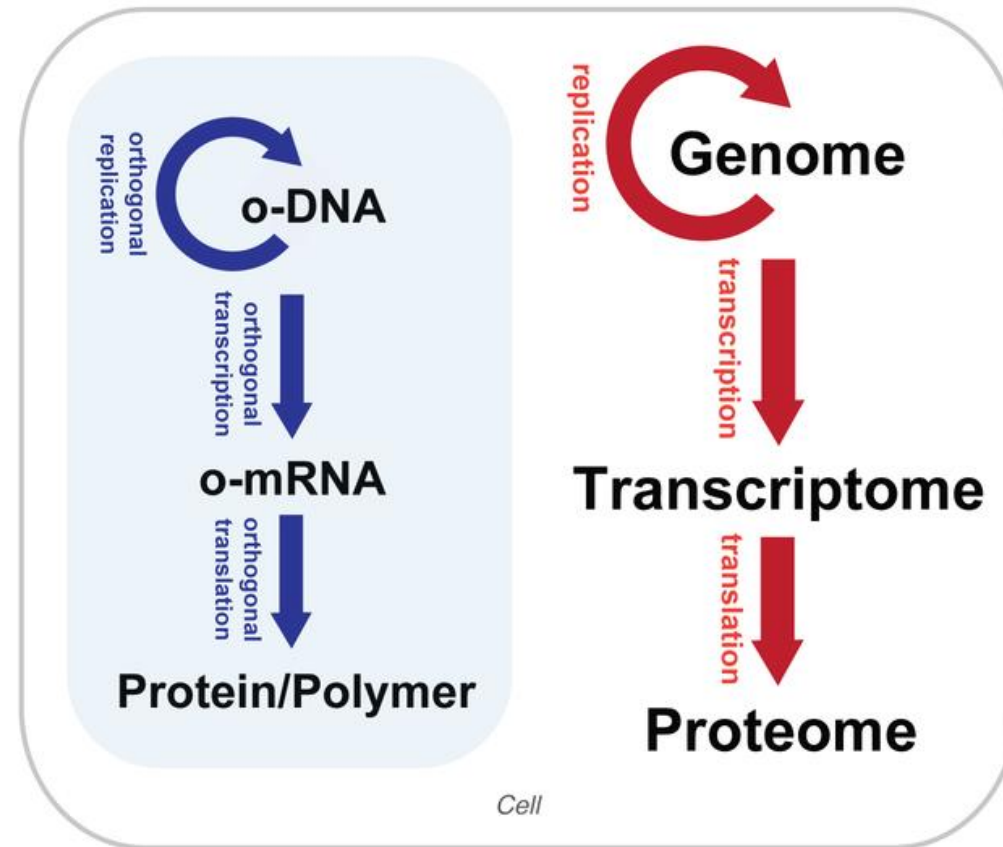
## Fundamental science: cell-free to understand living cells

---

---

- Construction hybrid systems

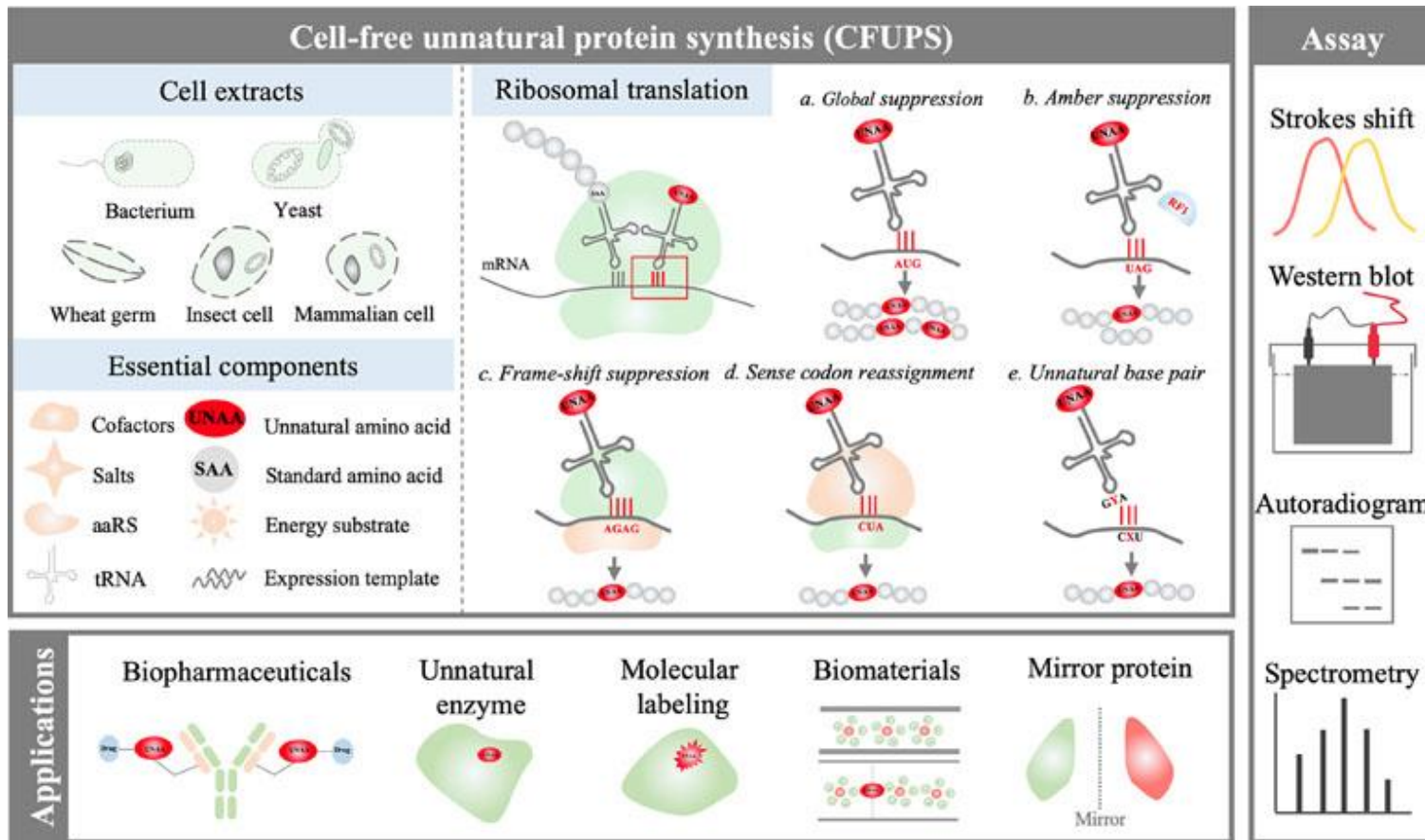
Nonstandard (unnatural) amino acids, more orthogonality for a better predictability



# Cell-free reaction:

## Fundamental science: cell-free to understand living cells

- Construction hybrid systems
- Nonstandard (unnatural) amino acids

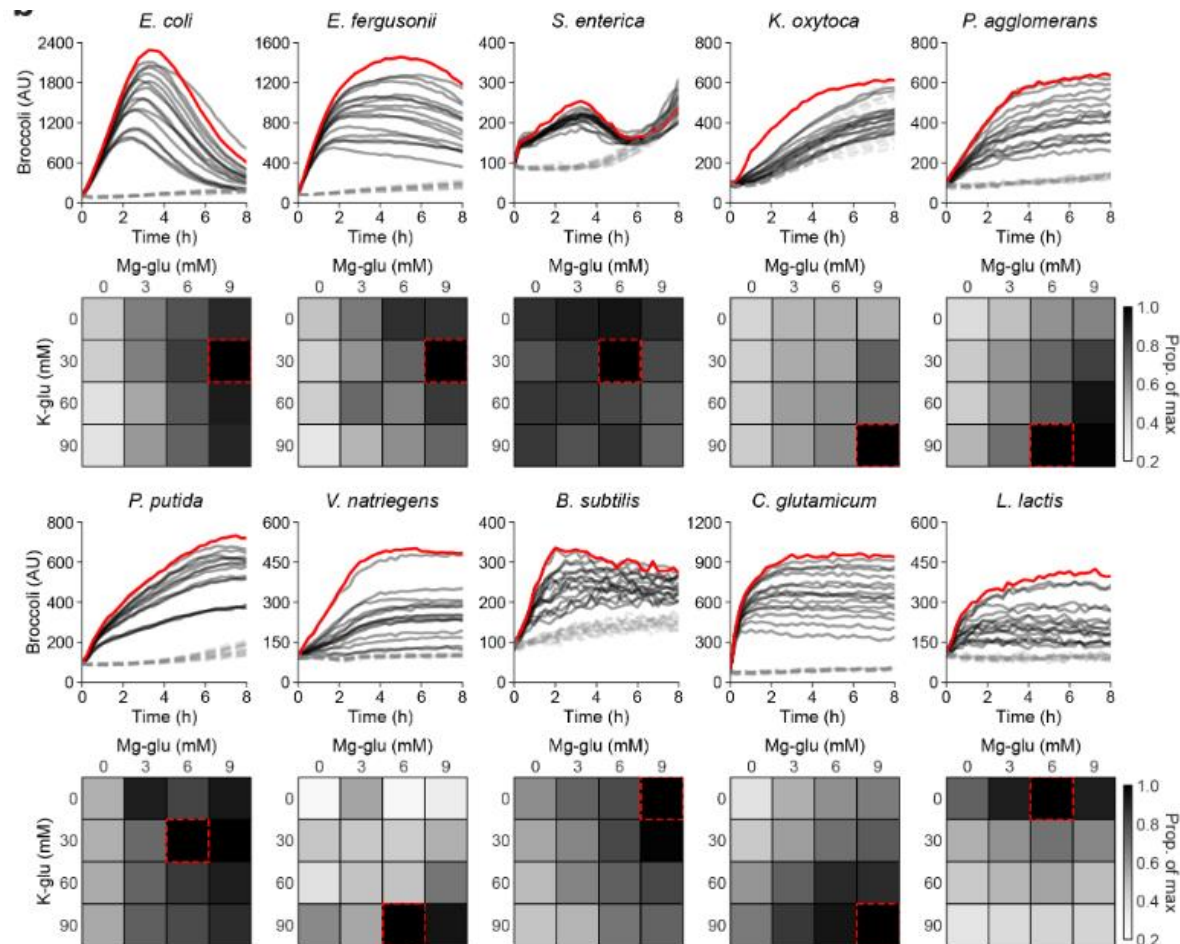


# Cell-free reaction:

## Fundamental science: cell-free to understand living cells

- Construction hybrid systems

Multi-species systems

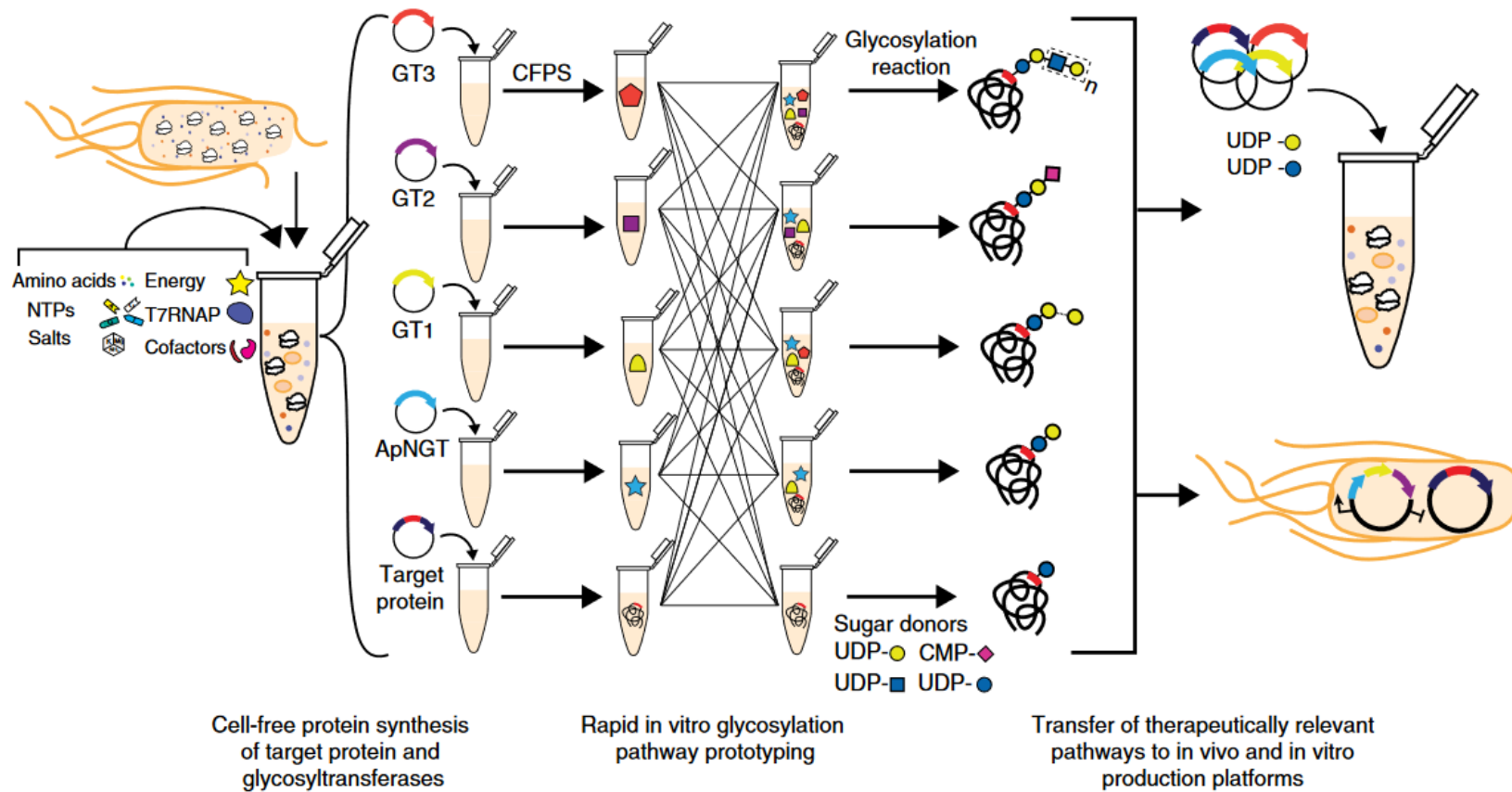


# Cell-free reaction:

## Fundamental science: cell-free to understand living cells

- Construction hybrid systems

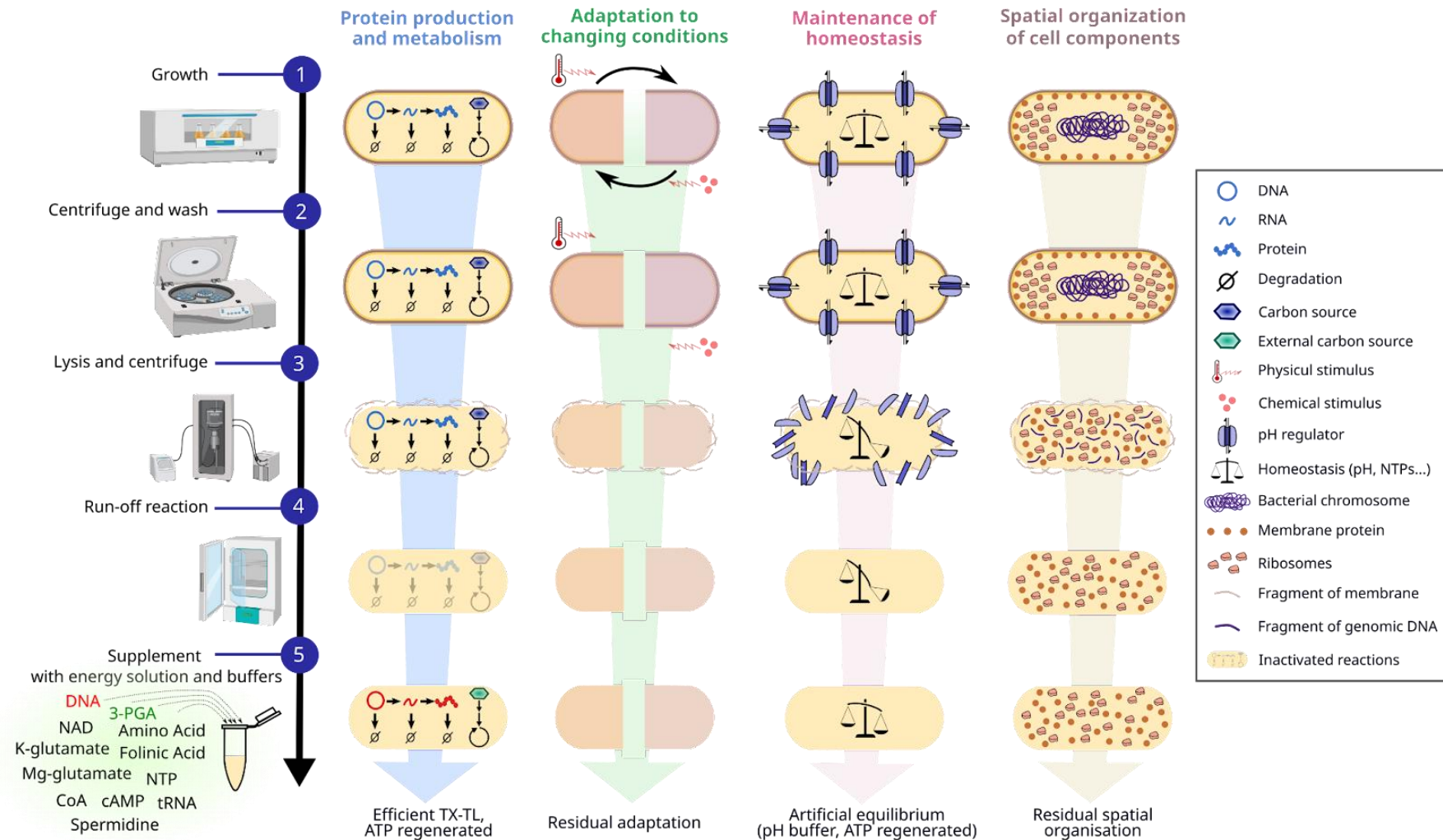
Eukaryote properties (Glycolysation)



# Cell-free reaction:

## Fundamental science: cell-free to understand living cells

### - Constructing artificial cells

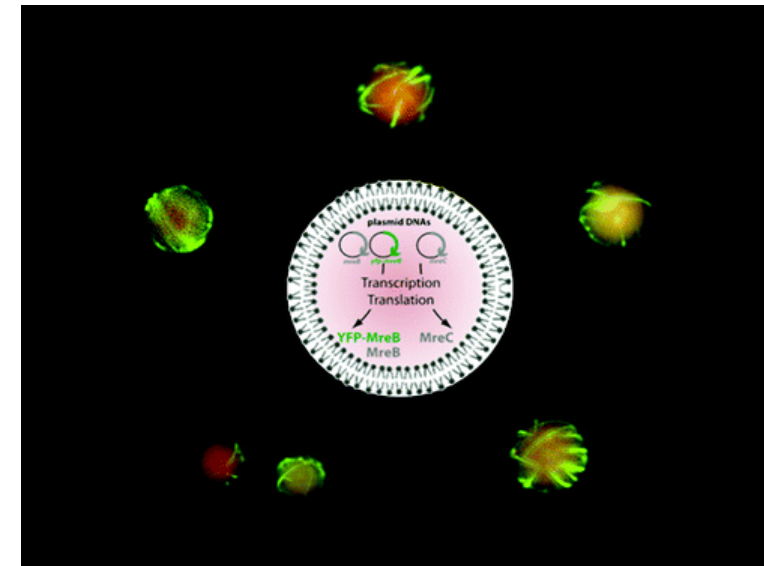
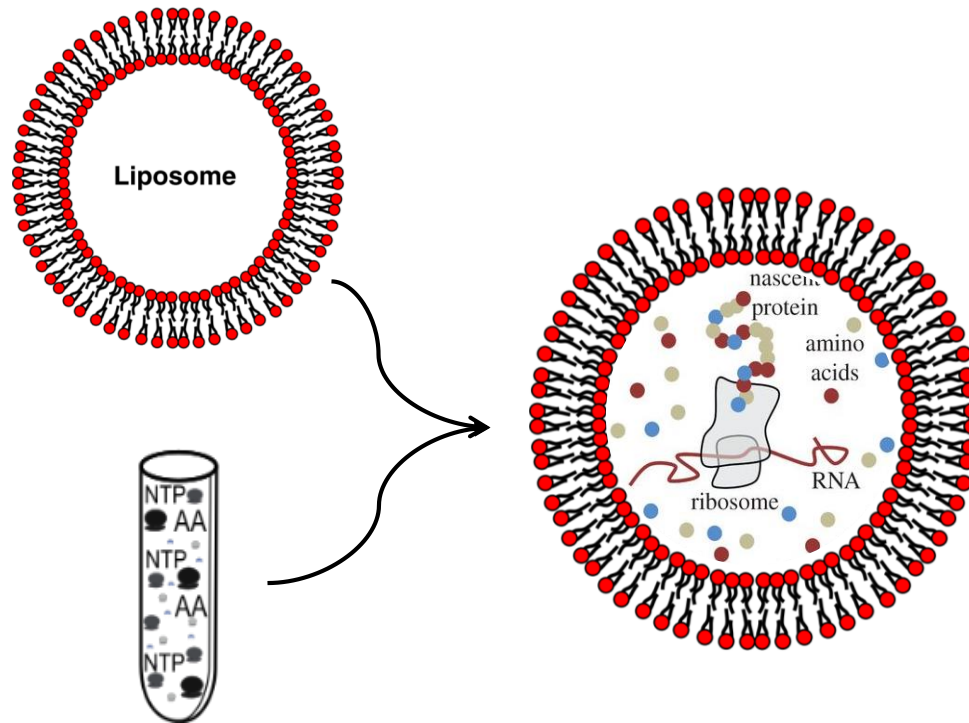


# Cell-free reaction:

## Fundamental science: cell-free to understand living cells

- Constructing artificial cells

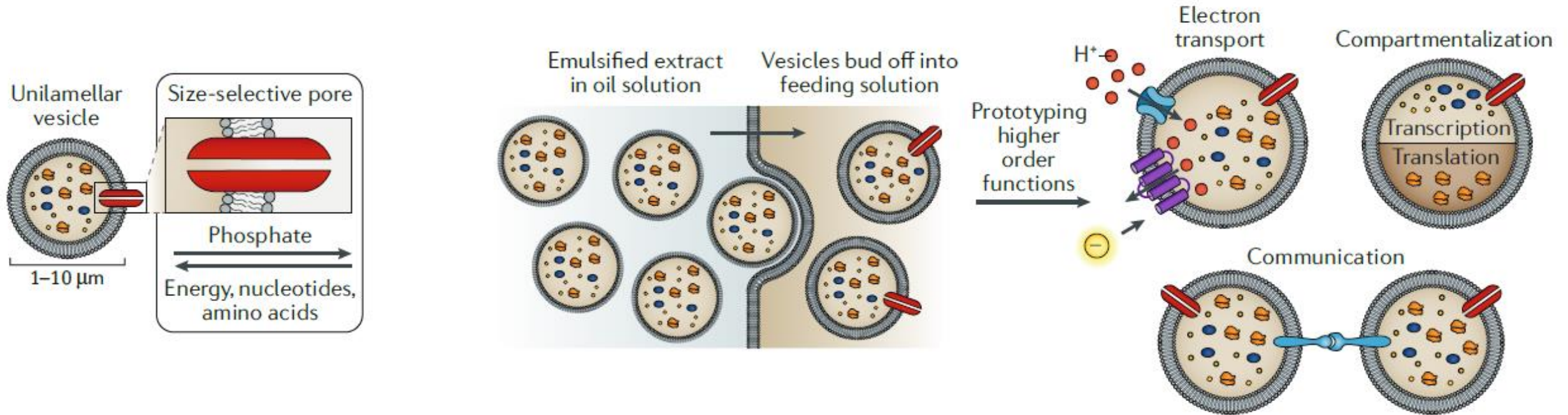
### Liposomes



# Cell-free reaction:

## Fundamental science: cell-free to understand living cells

- Constructing artificial cells: Communication / exchange with the environment

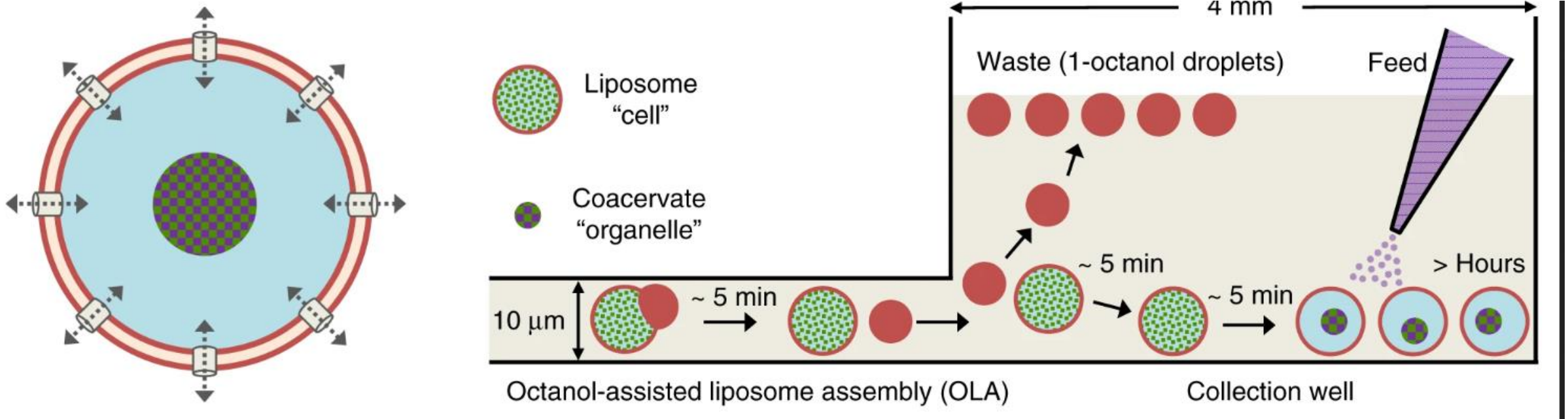




# Cell-free reaction:

## Fundamental science: cell-free to understand living cells

- Constructing artificial cells : compartmentation



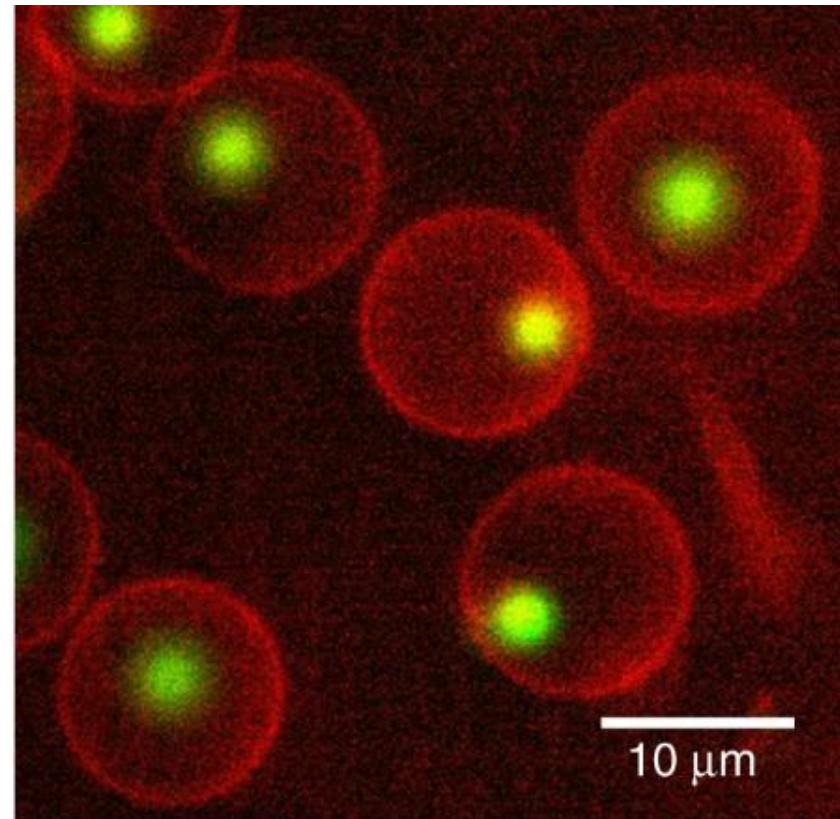
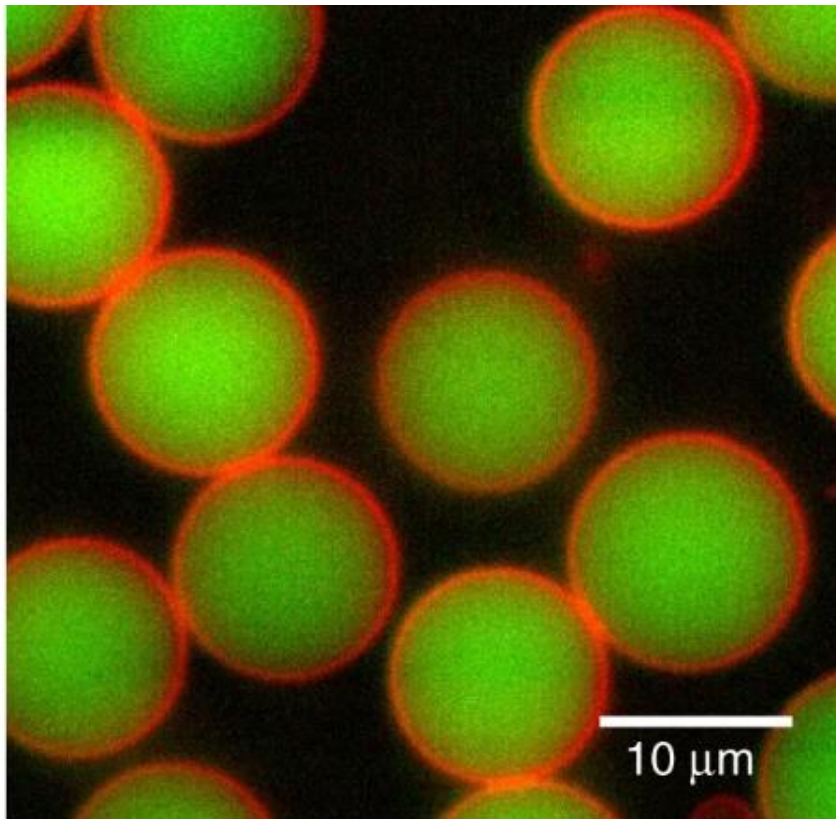
# Cell-free reaction:

## 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**

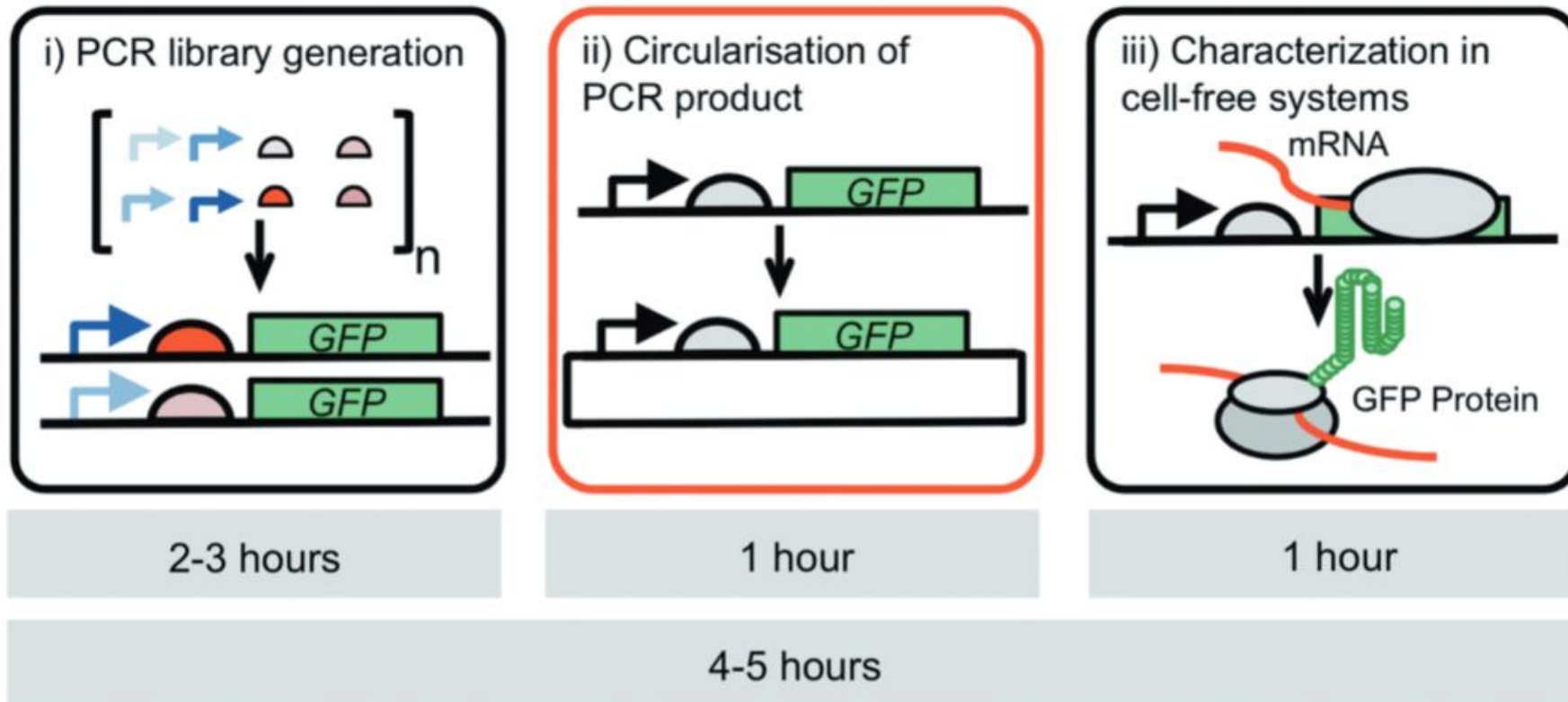
- Prototyping genetic circuits and pathways
  - Genetic parts
  - Metabolic engineering
- Developing biosensors
  - Optimize cell-free biosensor
  - Biocomputing
  - Materials and cell-free
  - Medical diagnostics
  - Pollution diagnostics
- Bio-machine assembly
  - Phages selection
  - Antibodies

# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

- Prototyping genetic circuits and pathways

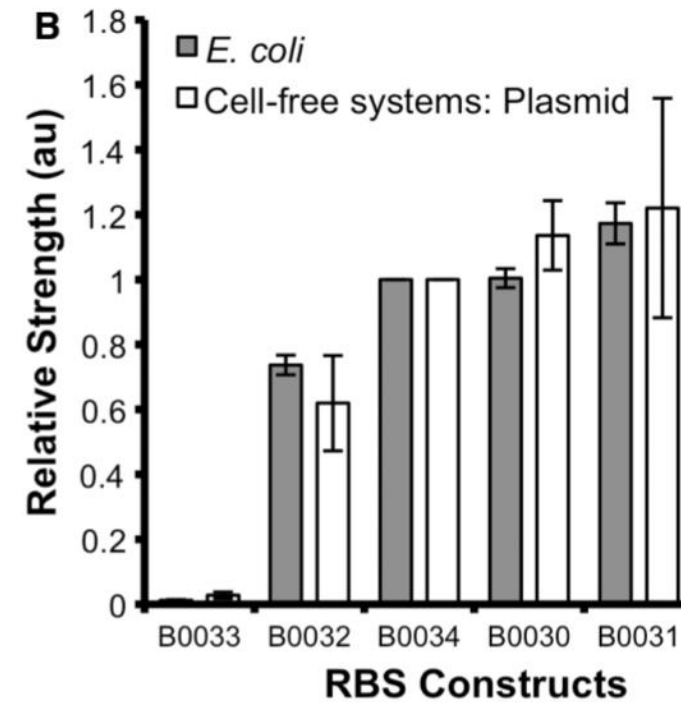
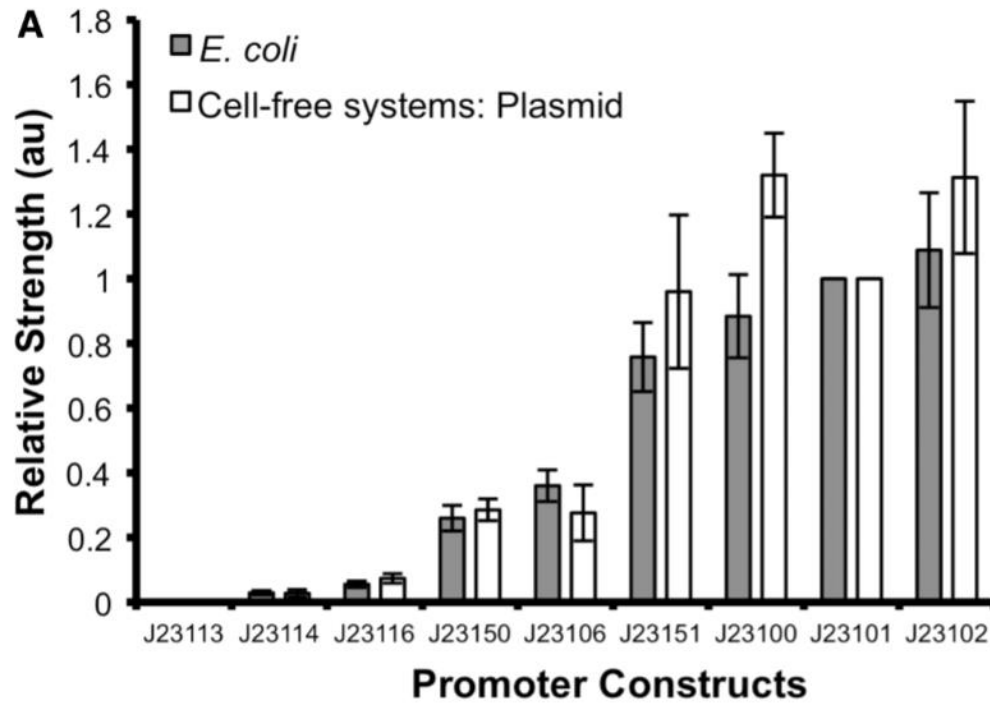
Genetic parts



# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

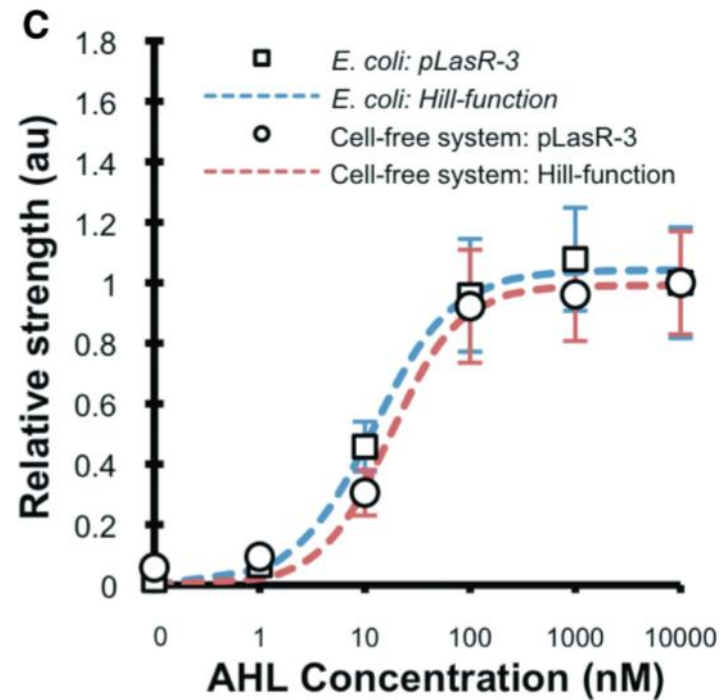
- Prototyping genetic circuits and pathways
- Genetic parts



# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

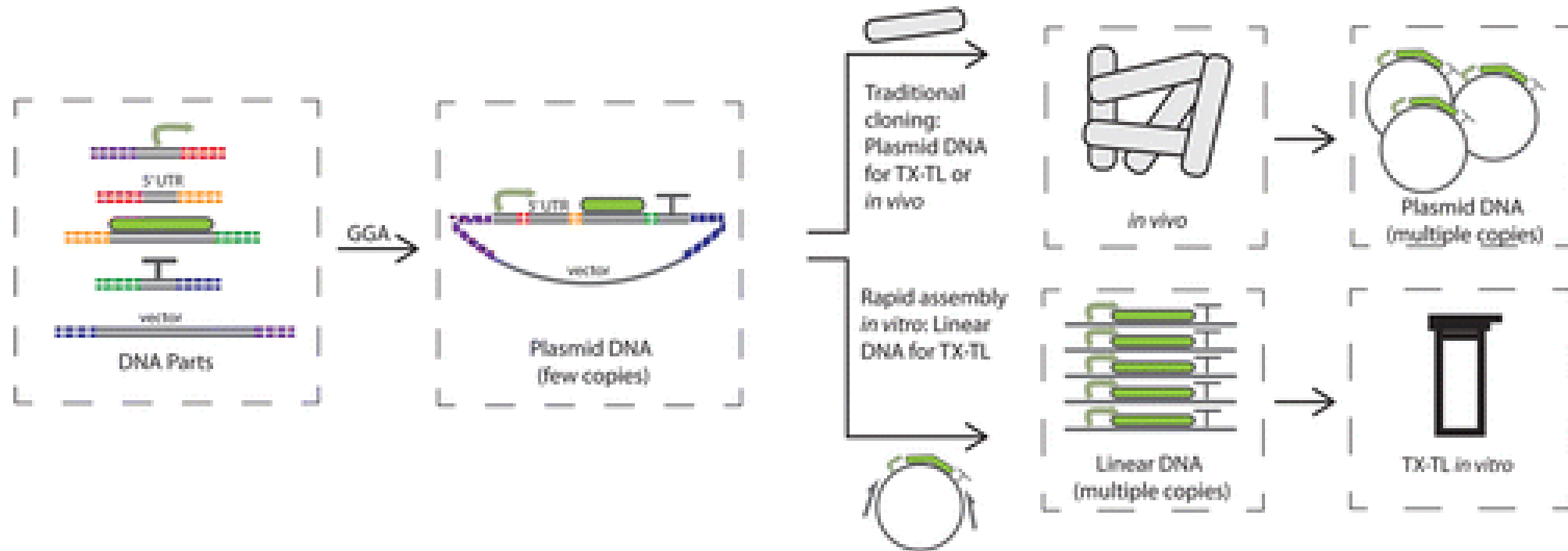
- Prototyping genetic circuits and pathways  
Genetic parts: regulated systems



# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

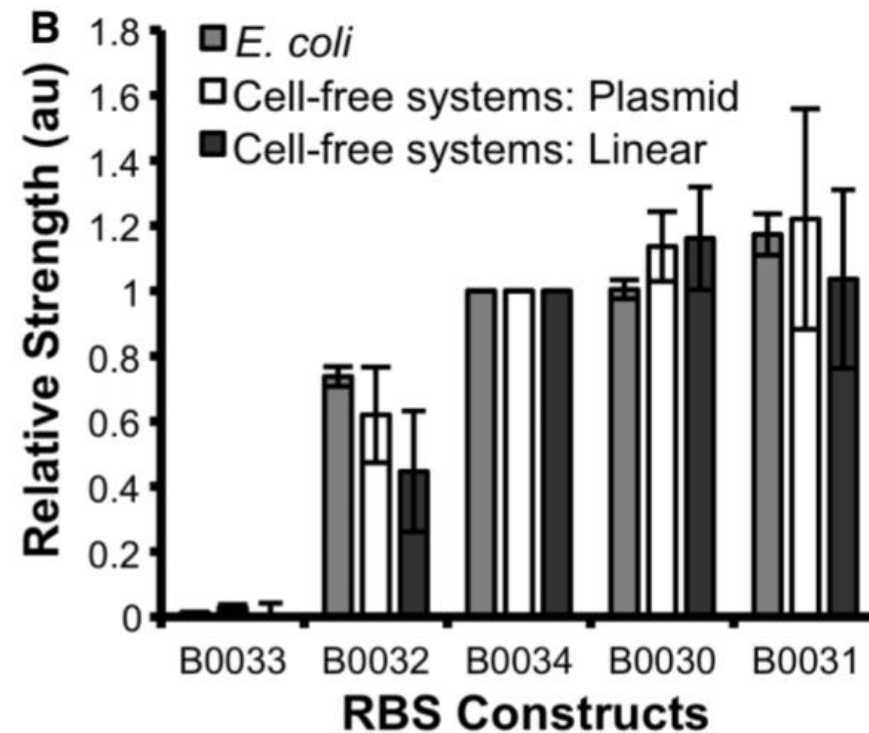
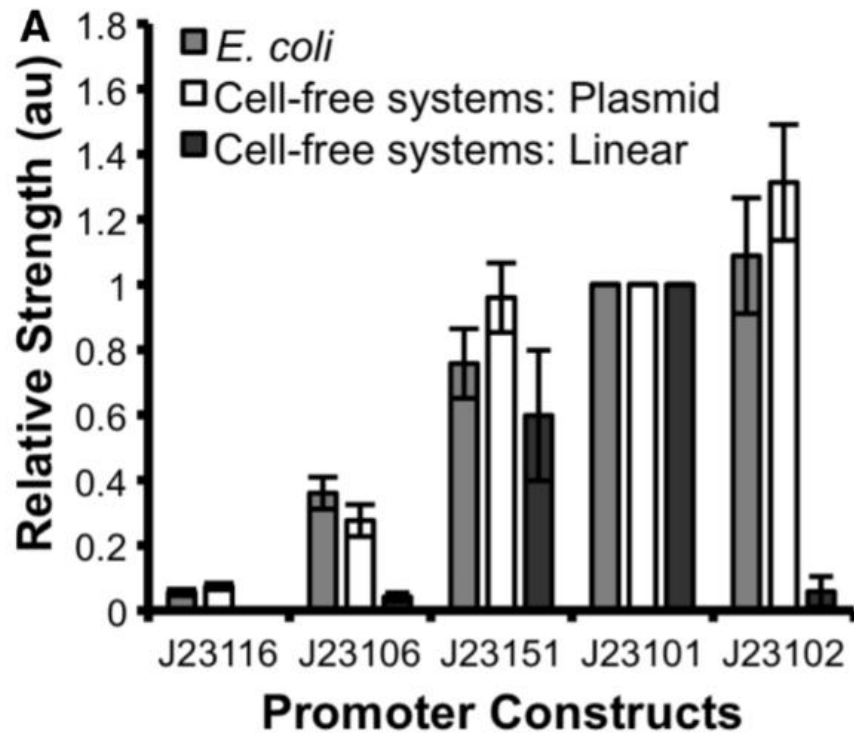
- Prototyping genetic circuits and pathways  
Genetic parts: Linear fragment vs plasmid



# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

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



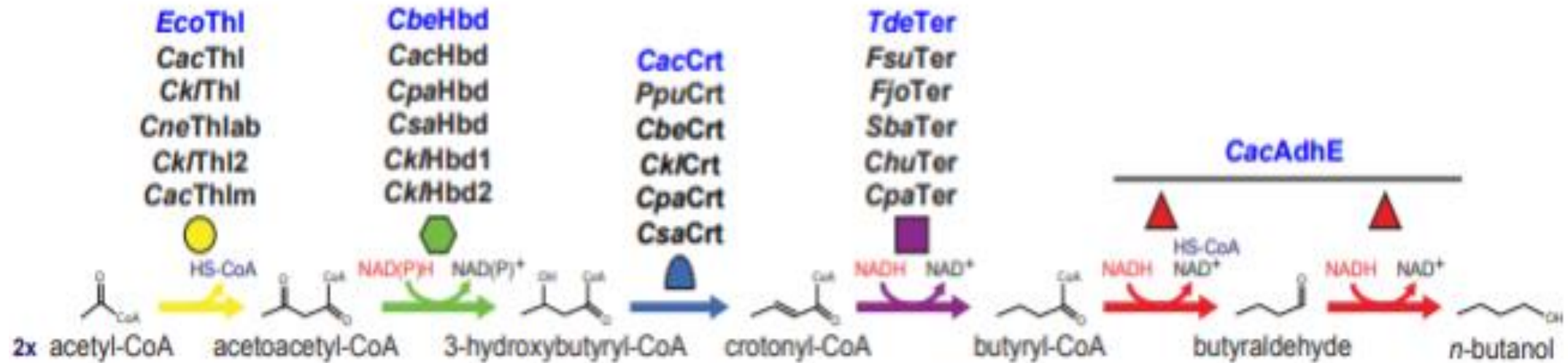


# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

Prototyping genetic circuits and pathways

Metabolic engineering: combinatorial exploration



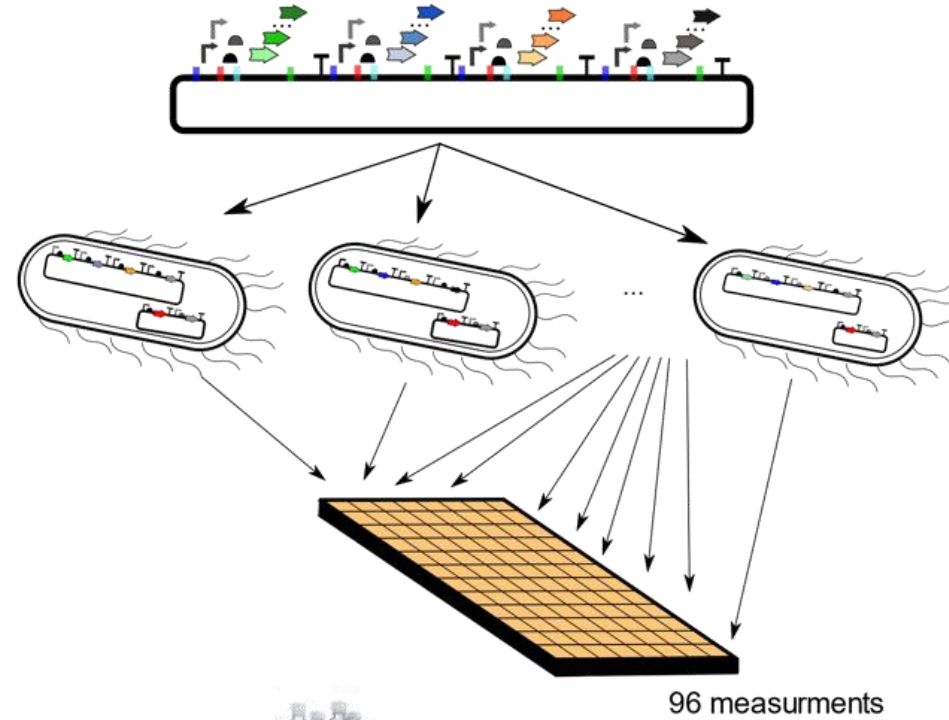
Example of : 6<sup>4</sup> combinations

# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

Metabolic engineering: combinatorial exploration

**$6^4$  plasmids**

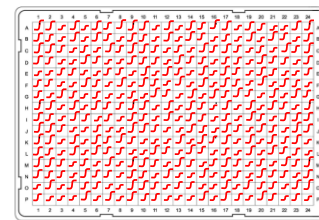
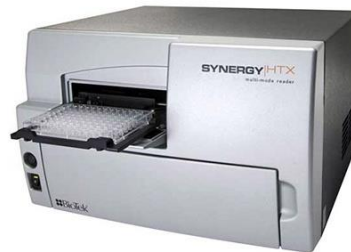
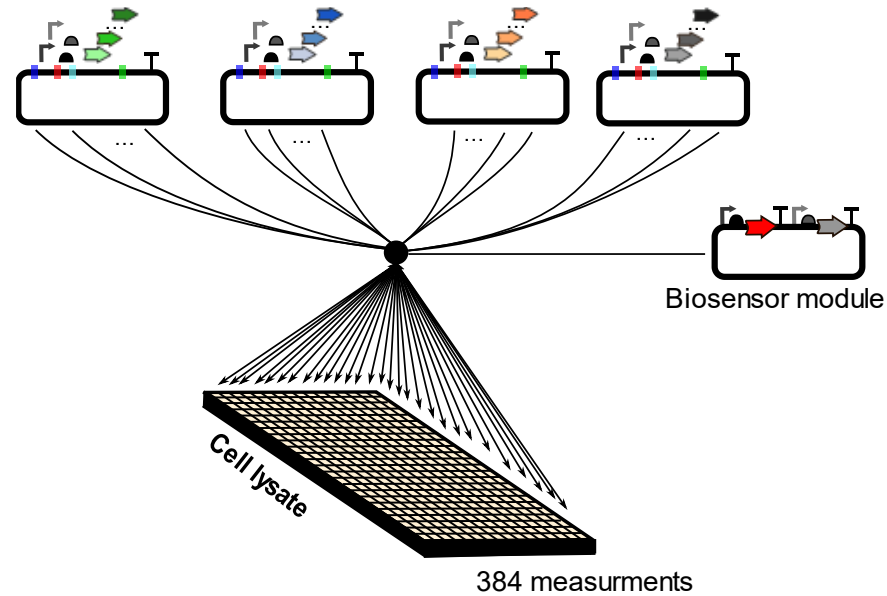


# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

Metabolic engineering: combinatorial exploration

$6 \times 4 = 24$  plasmids

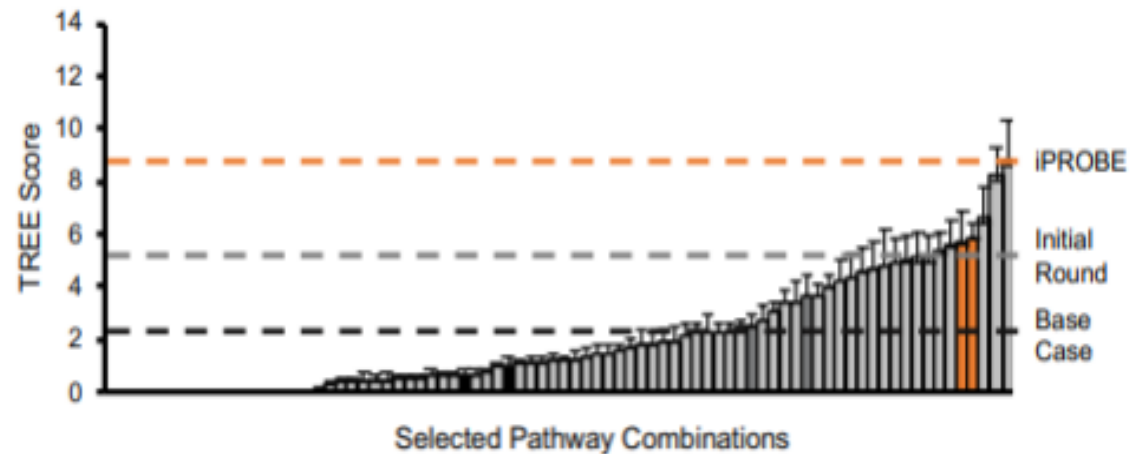
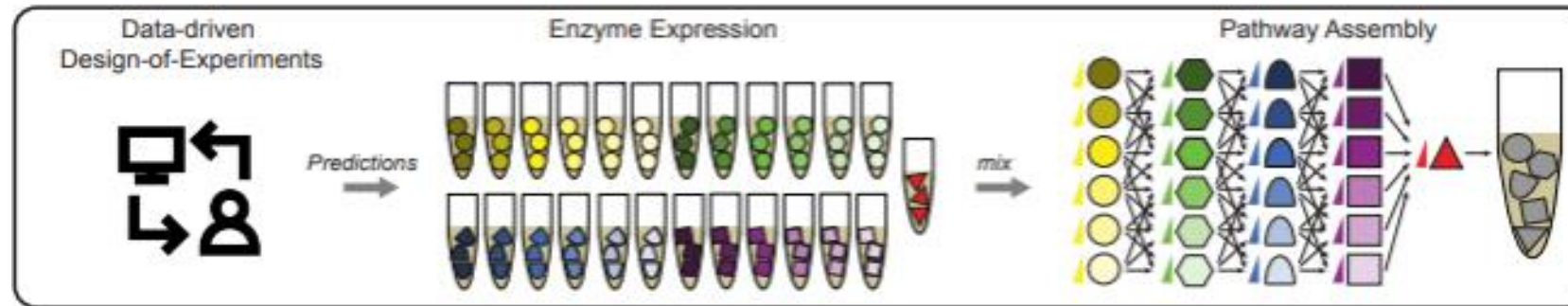


# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

Prototyping genetic circuits and pathways

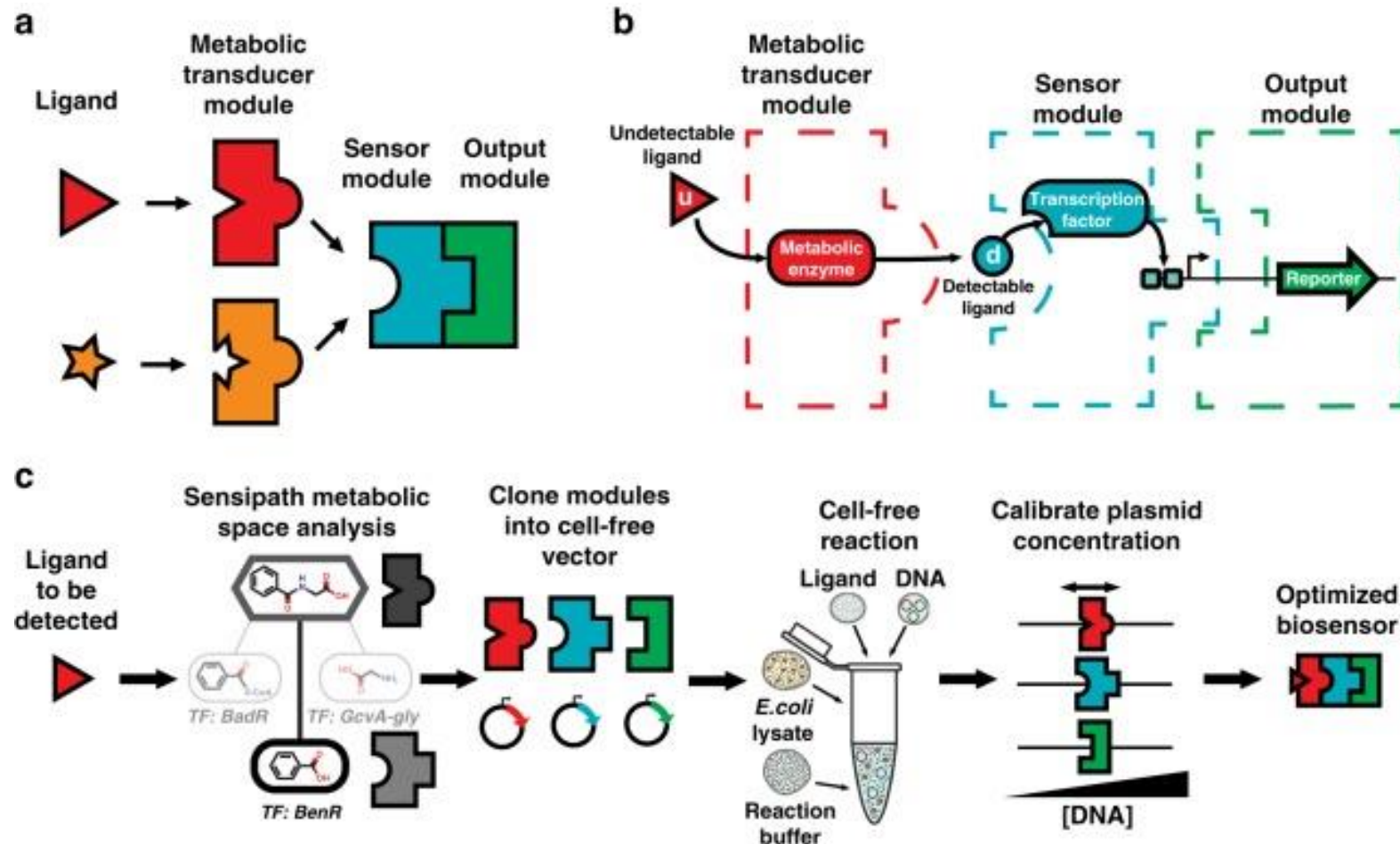
Metabolic engineering: combinatorial exploration



# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

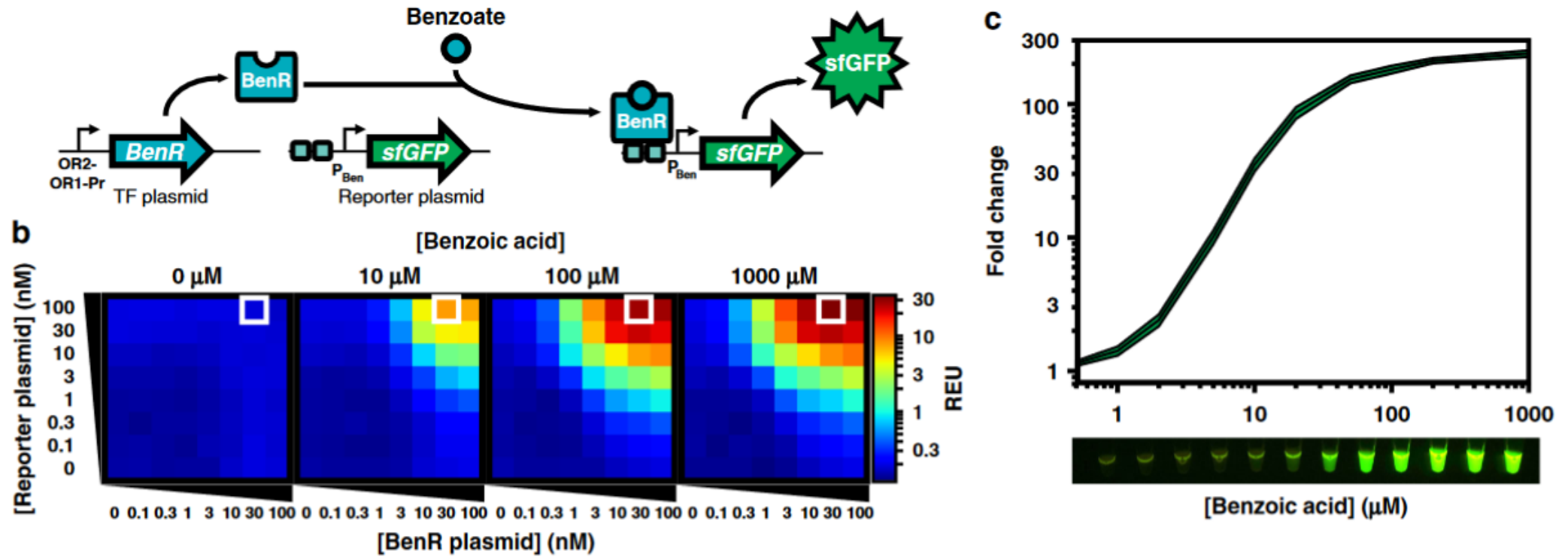
- Developing biosensors



# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

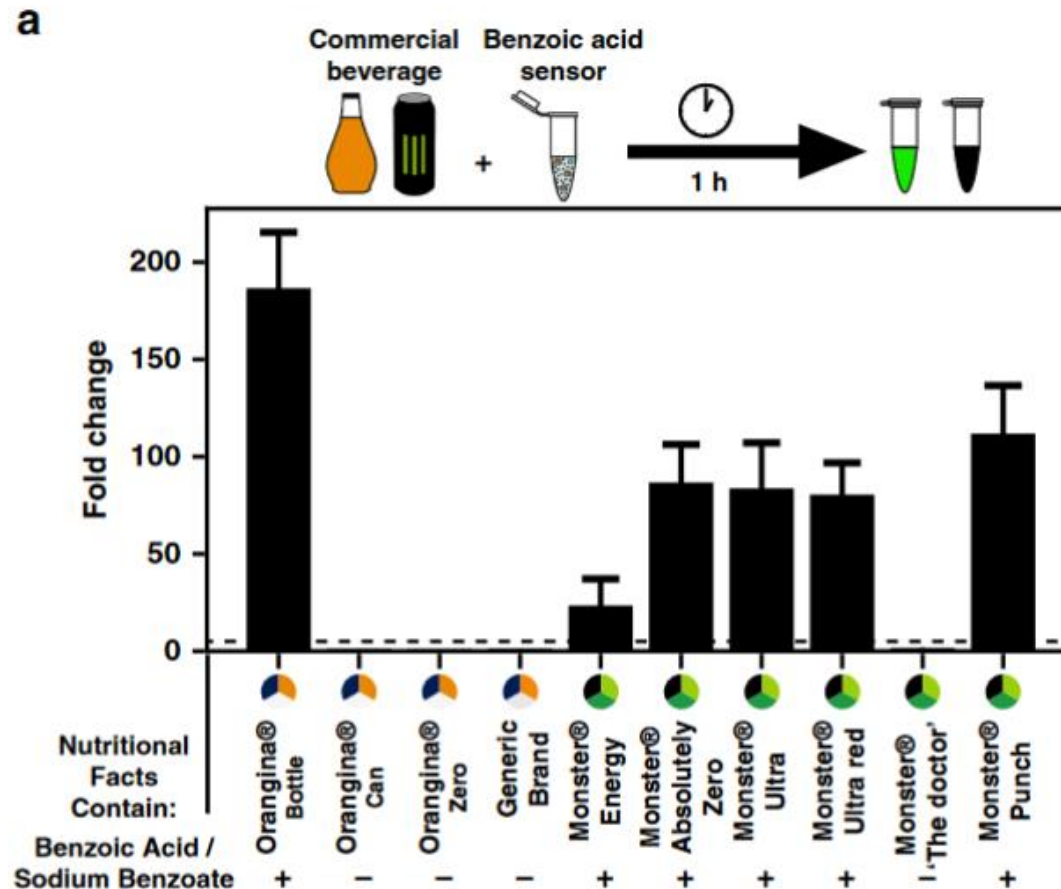
- Developing biosensors



# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

- Developing biosensors



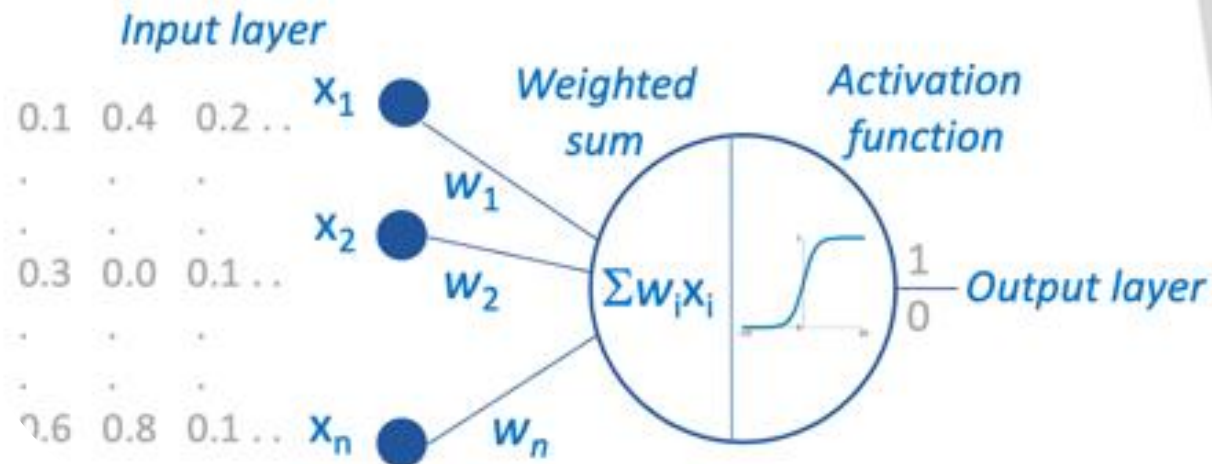
# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

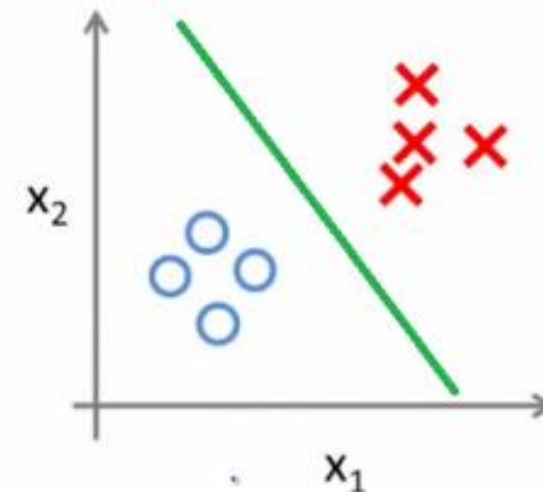
Developing biosensors  
Biocomputing



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



Binary classification:





# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

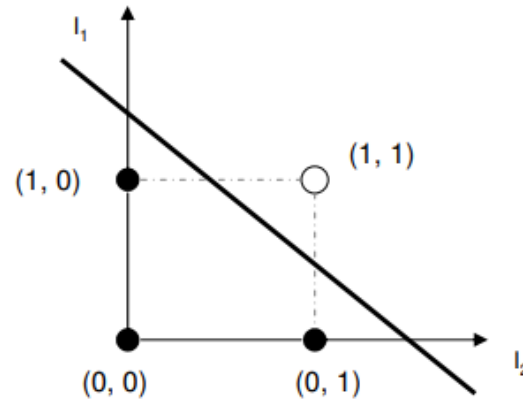
---

---

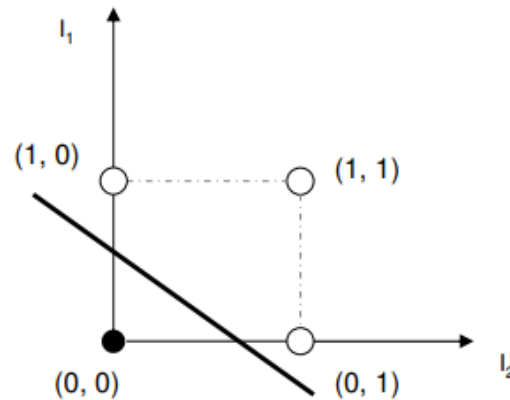
Developing biosensors

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

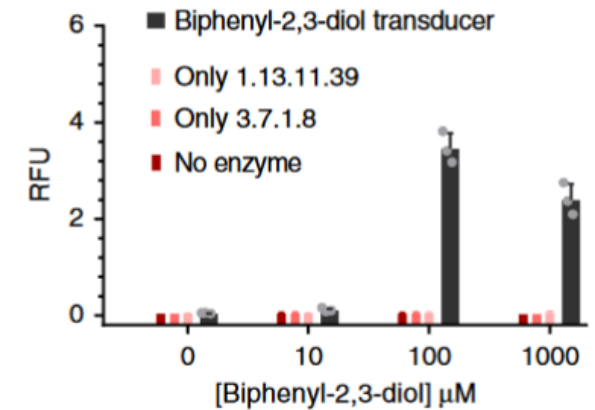
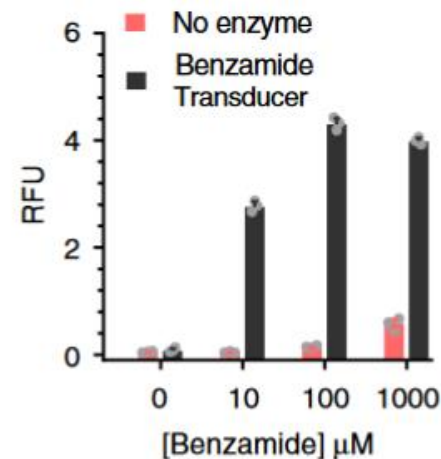
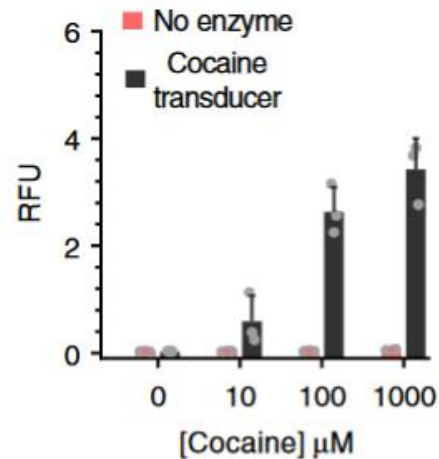
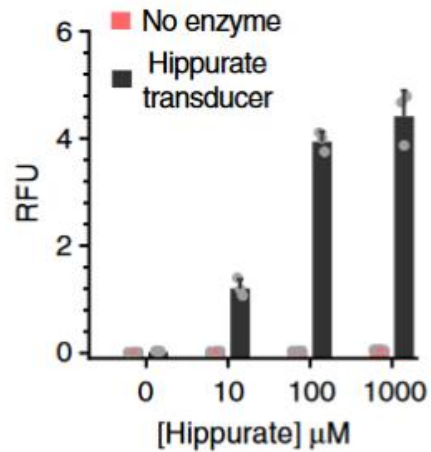
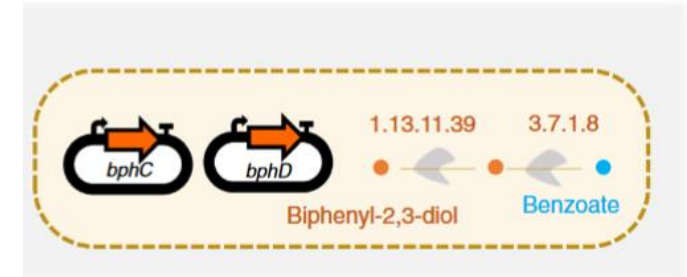
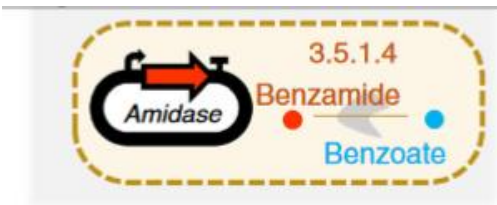
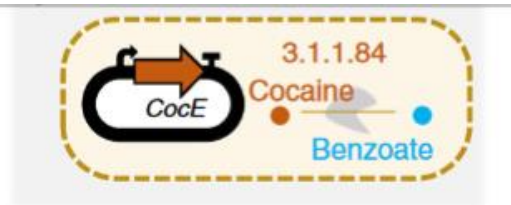
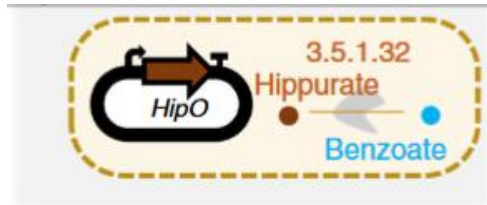


# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

Developing biosensors

Biocomputing: 4 biosensors

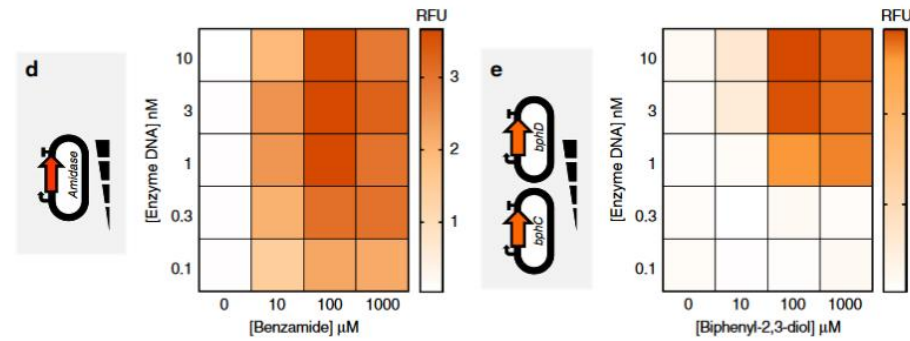
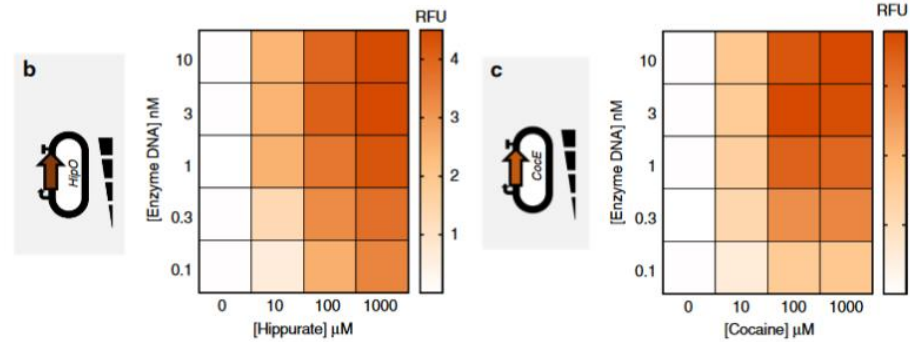
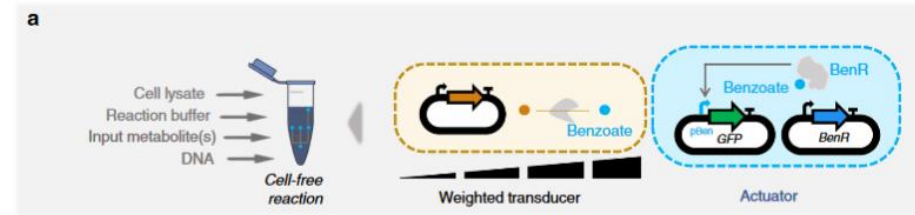


# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

Developing biosensors

Biocomputing: tuning the weights

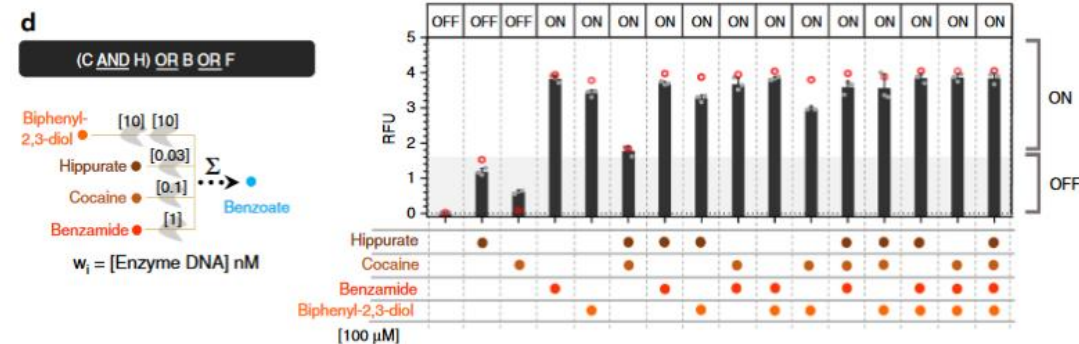
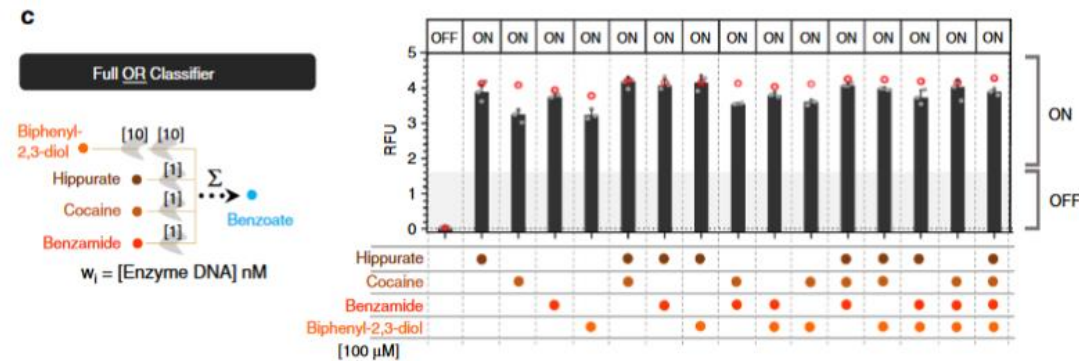
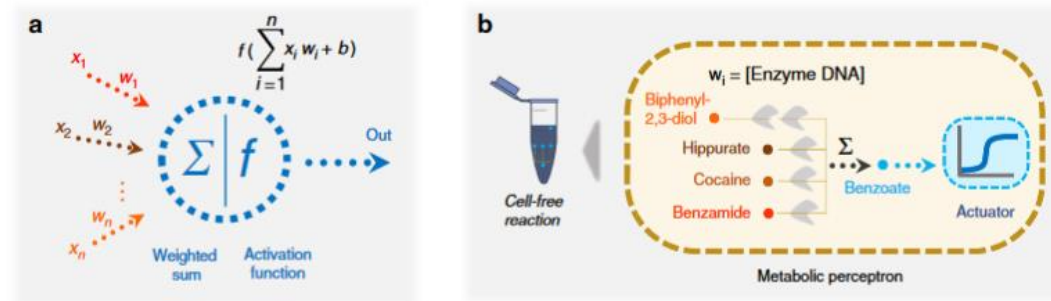


# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

Developing biosensors

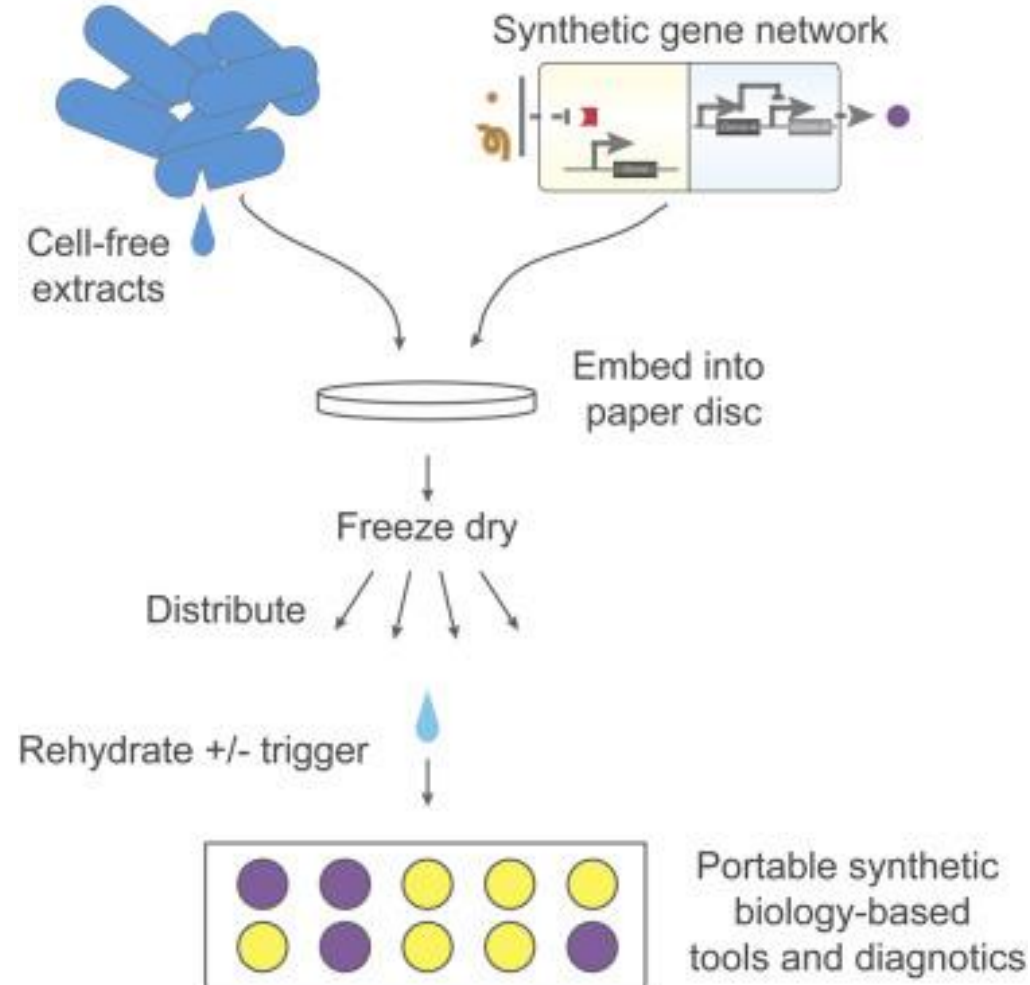
Biocomputing: perceptron



# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

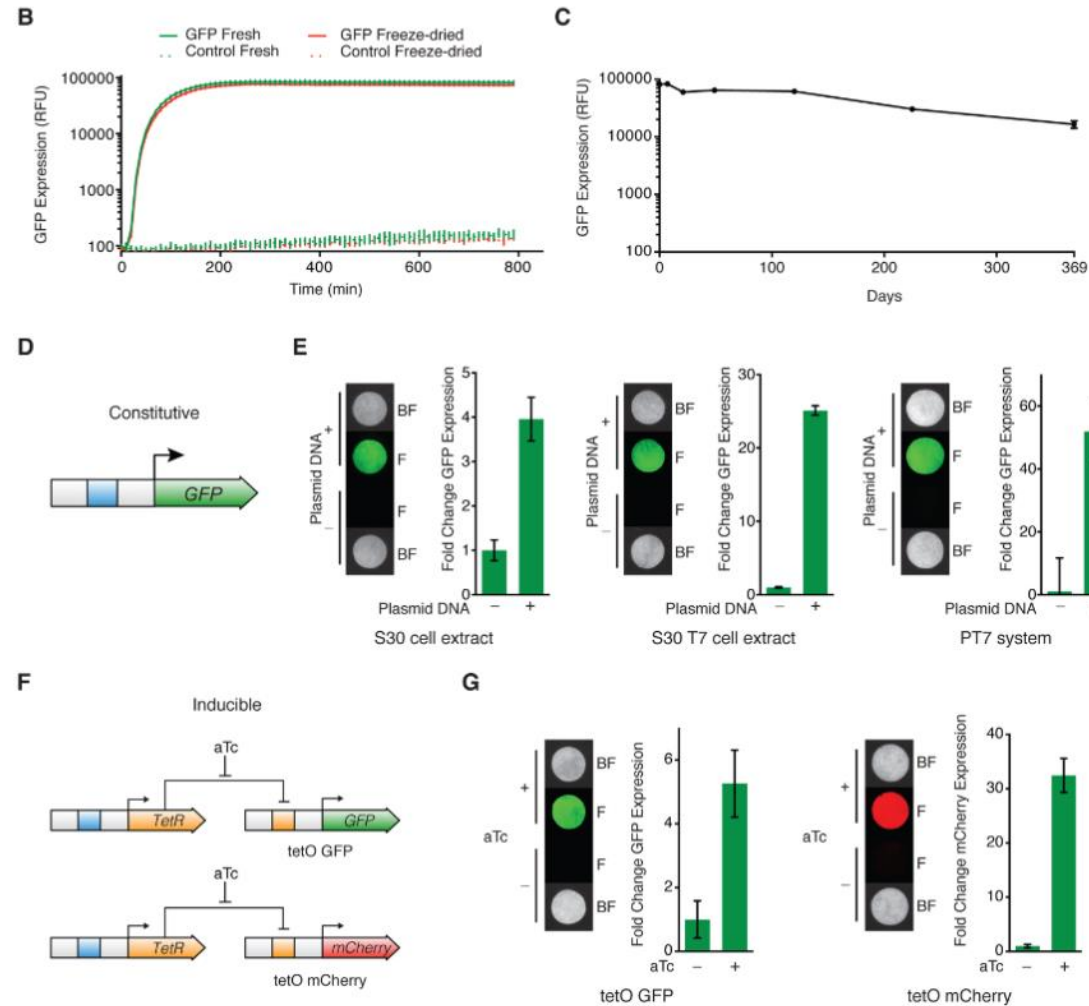
- Developing biosensors  
Medical diagnostics



# 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

**Biosensor Design**  
A U G C

**Cell-Free Reaction**

**Freeze Drying**

**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?**

**Paper-based Diagnostics**

**Engineered Biosensors**

**Animal Sentinels**

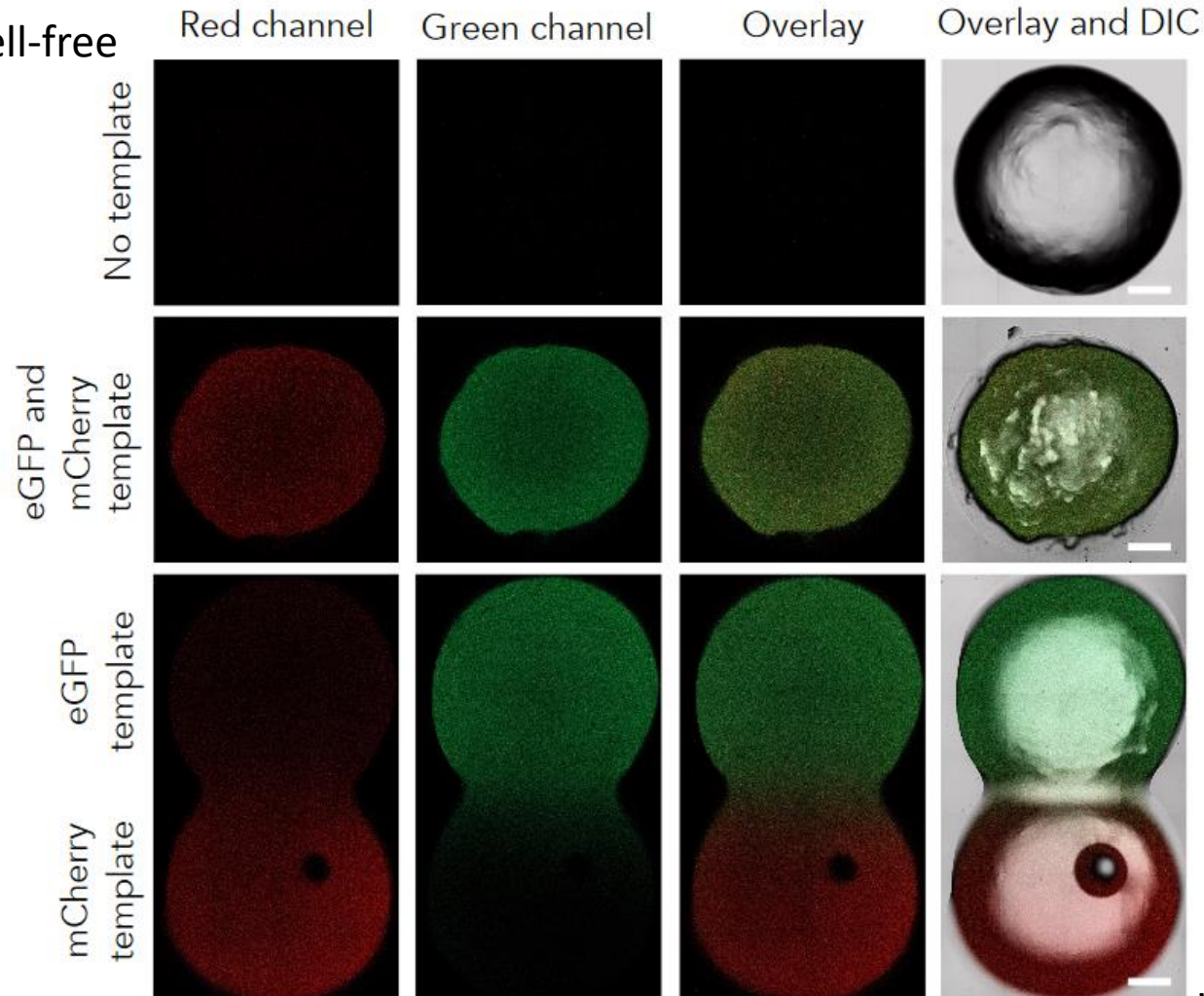
WYSS 3807 | HD | BROAD INSTITUTE | UNIVERSITY OF MICHIGAN

# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

- Developing biosensors

Materials and cell-free

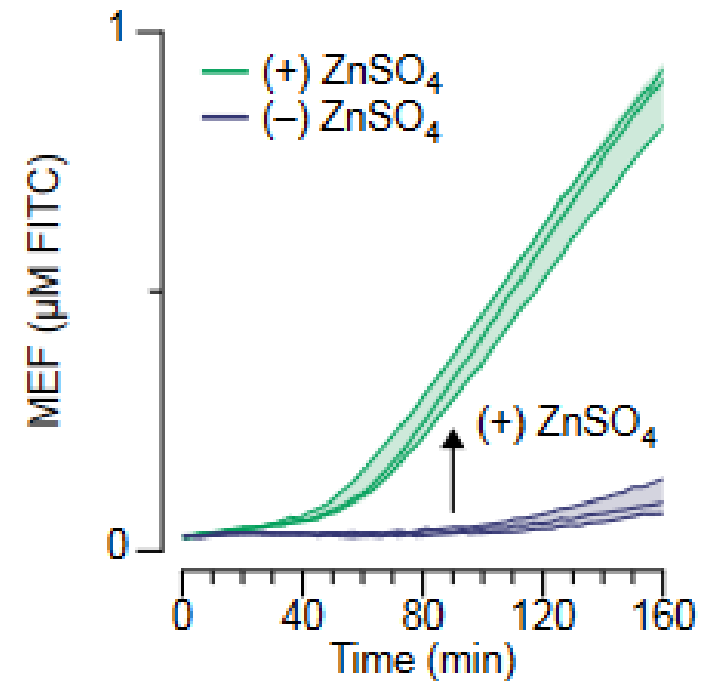
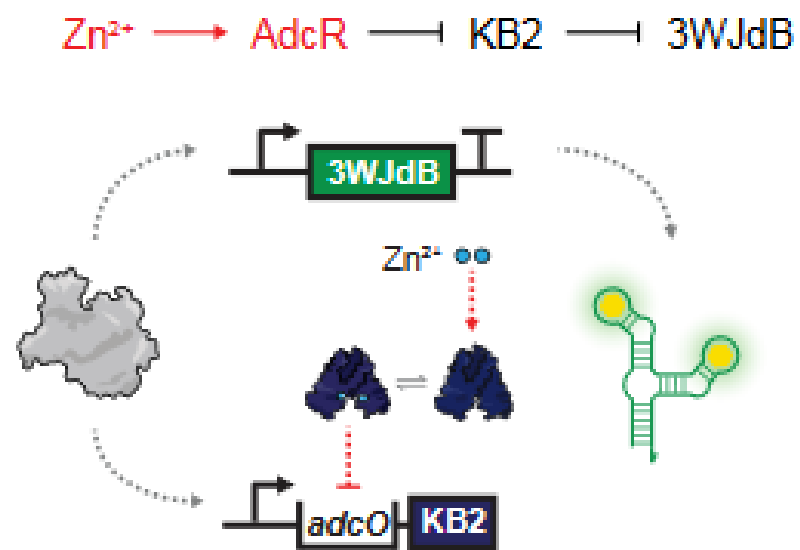




# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

- Developing biosensors
- Pollution detection

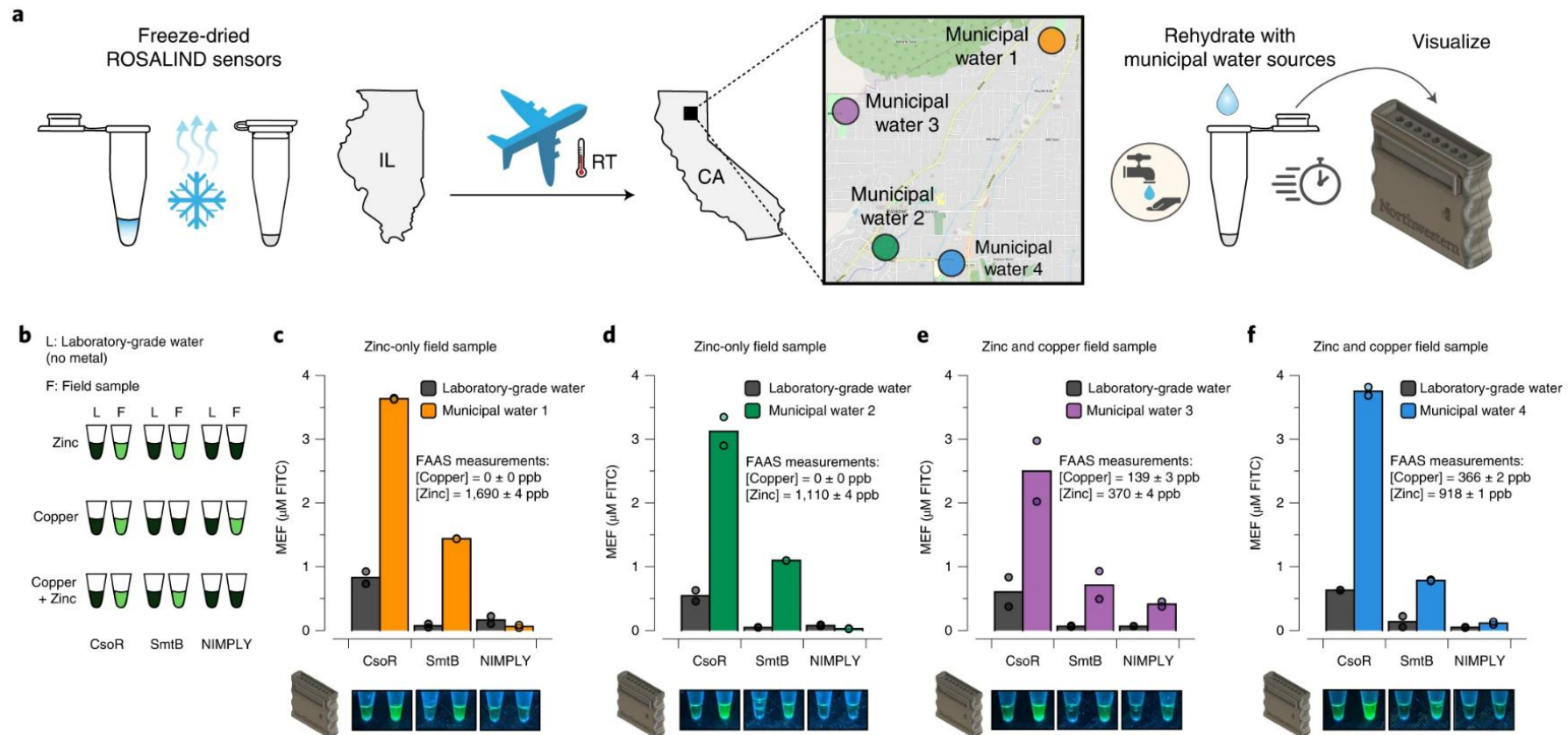


# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

- Developing biosensors

Pollution detection

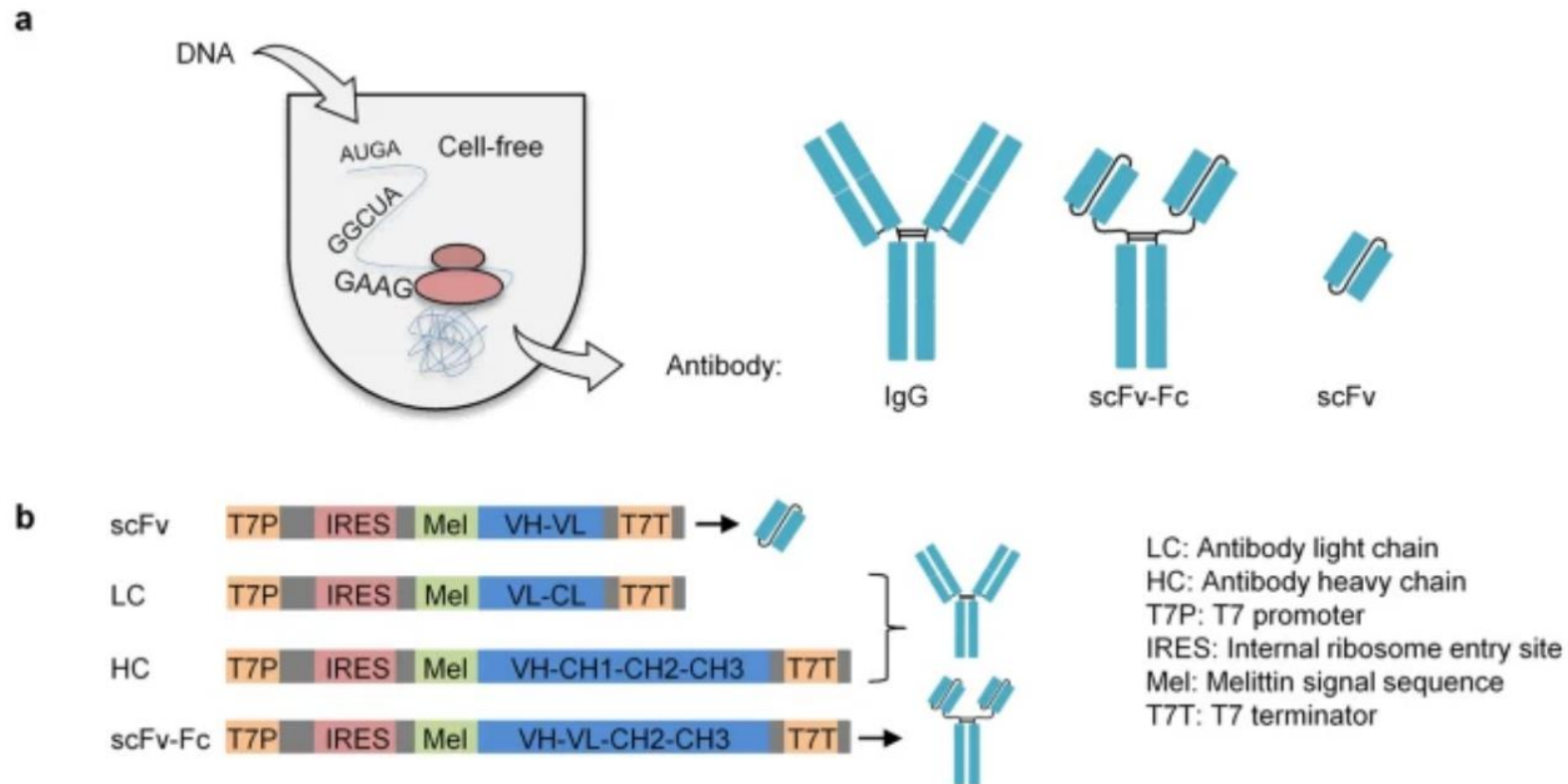


# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

- Bio-machine assembly

Antibodies (eukaryotic cell-free)



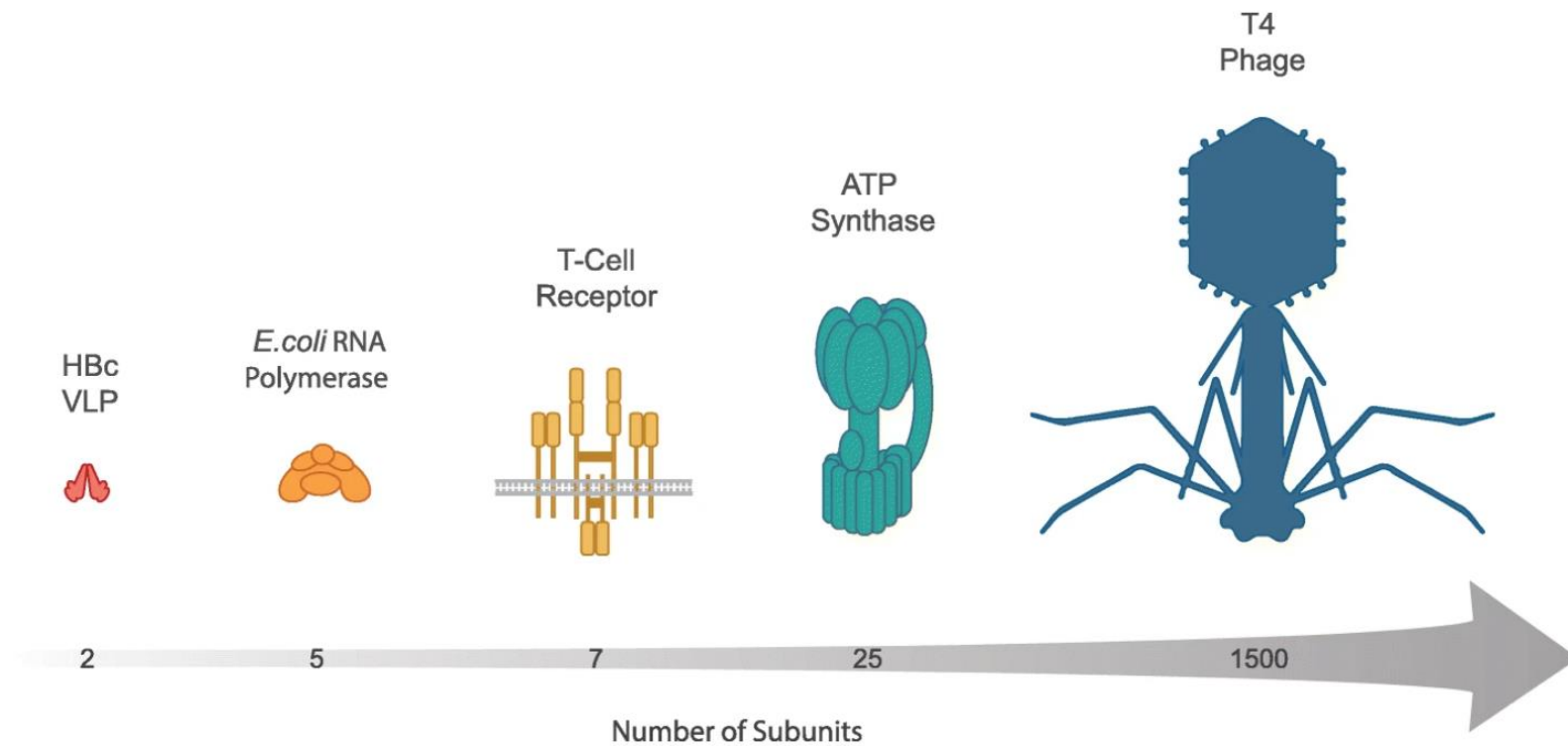
# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

---

---

- Bio-machine assembly
- Phages selection



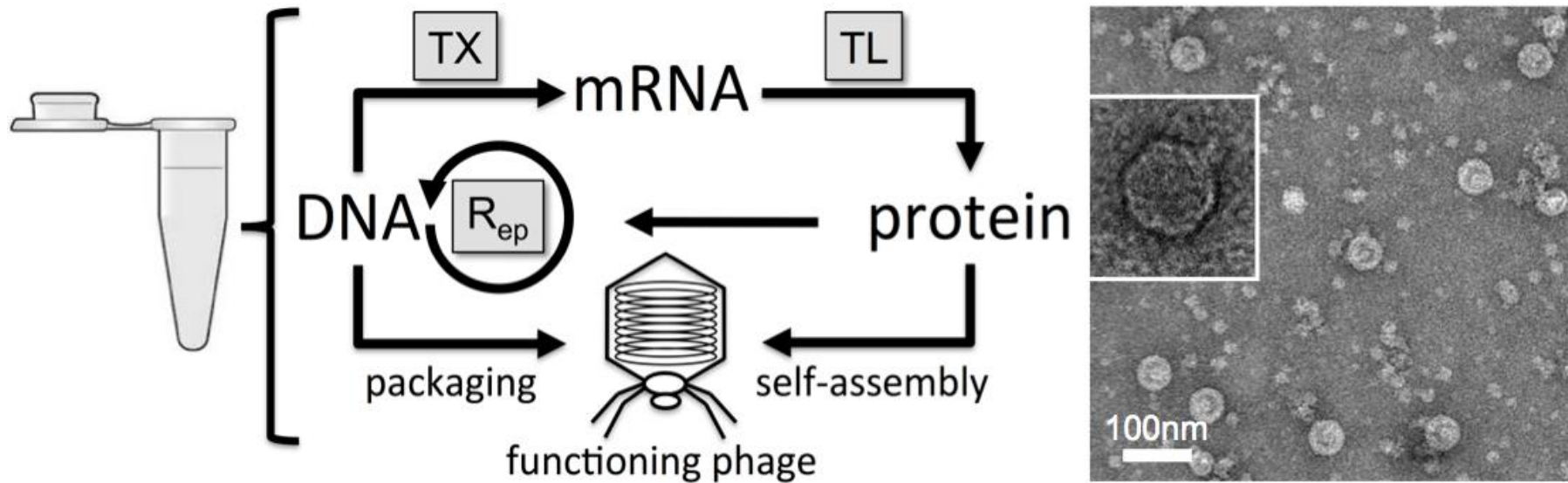
# Cell-free reaction:

## 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

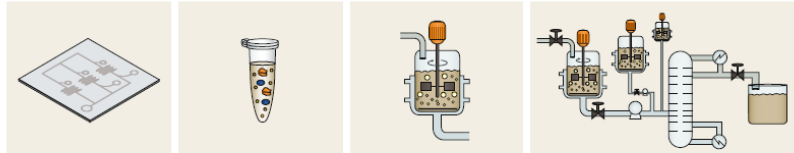
- Purified proteins
  - Tierra bioscience (California, USA)
  - CFS (Japan)
  - Cell-free (Ontario, Canada)

### Cell-free kit providers

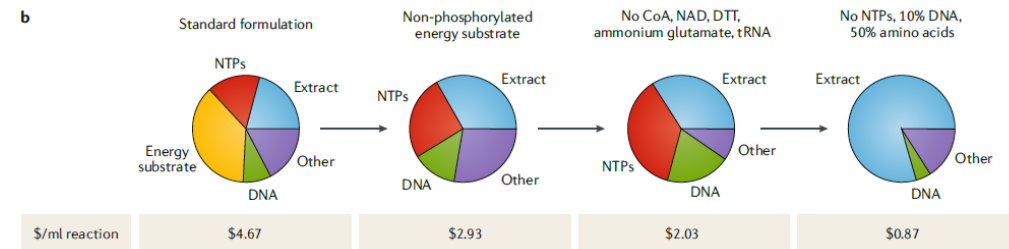
- New England Biolabs (Massachussets, USA)
- Thermo Fisher (Massachussets, USA)
- Biotechrabbit (Germany)
- arbor bioscience (Michigan, USA)

### 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



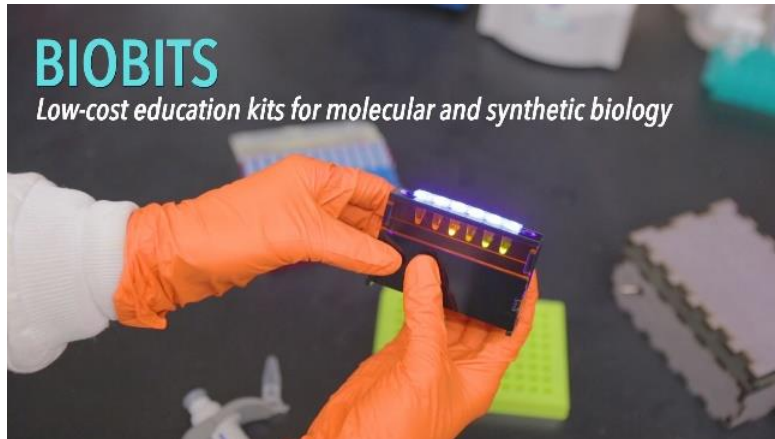
# Cell-free reaction:

## Additional use / challenges with cell-free

---

---

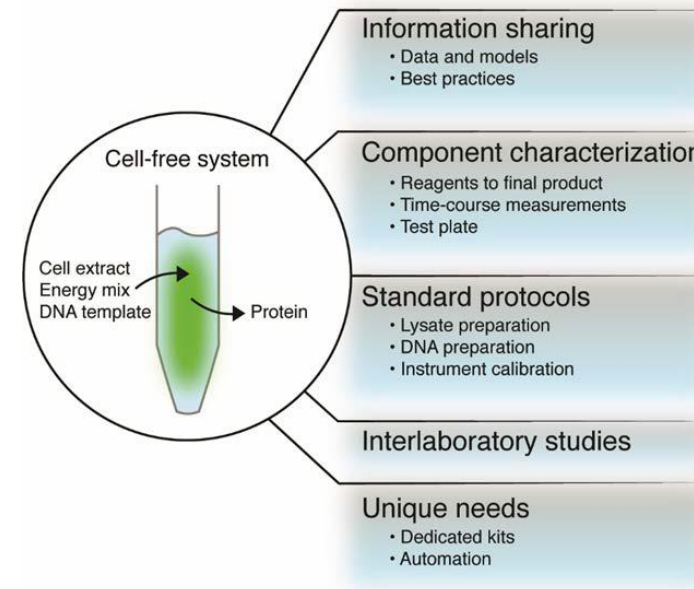
### Cell-free for education



myTXTL<sup>®</sup> Cell-Free Protein Expression kits for Igem

### National Institute of Standards and Technology

#### Towards reproducible cell-free systems





# Acknowledgements



Matthieu Jules

Léa Wagner

Olivier Delumeau

Vincent Sauveplane

**TOM ELLIS LAB**

Tom Ellis

**Guy-Bart Stan Lab**

Imperial College  
London

Brooke Rothschild-Mancinelli

Carlos Bricio

Francesca Ceroni

Michela Murgiano



**Bio-RetroSynth**

Jean-Loup Faulon



**Institut Pasteur**

Fabrice Agou

Mathilde Koch

Amir Pandi

Agnès Zettor

