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

Olivier Borkowski

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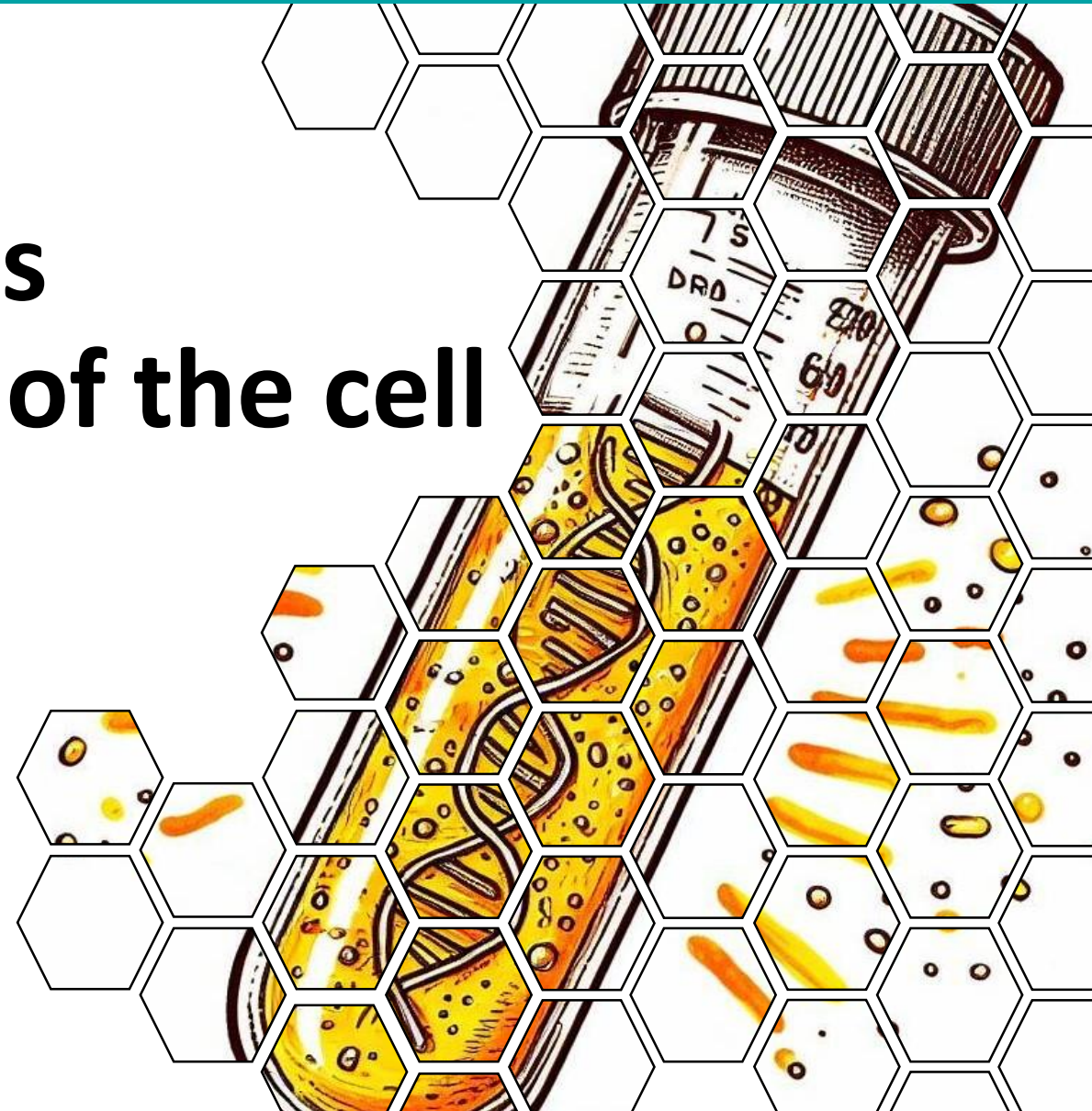
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# Cell-free systems

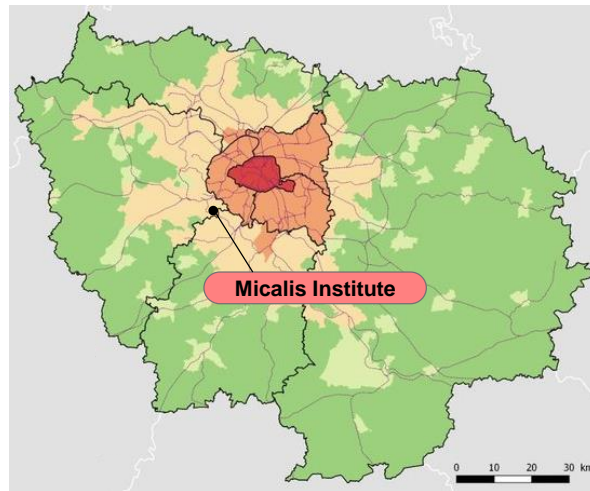
## Gene expression outside of the cell

**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



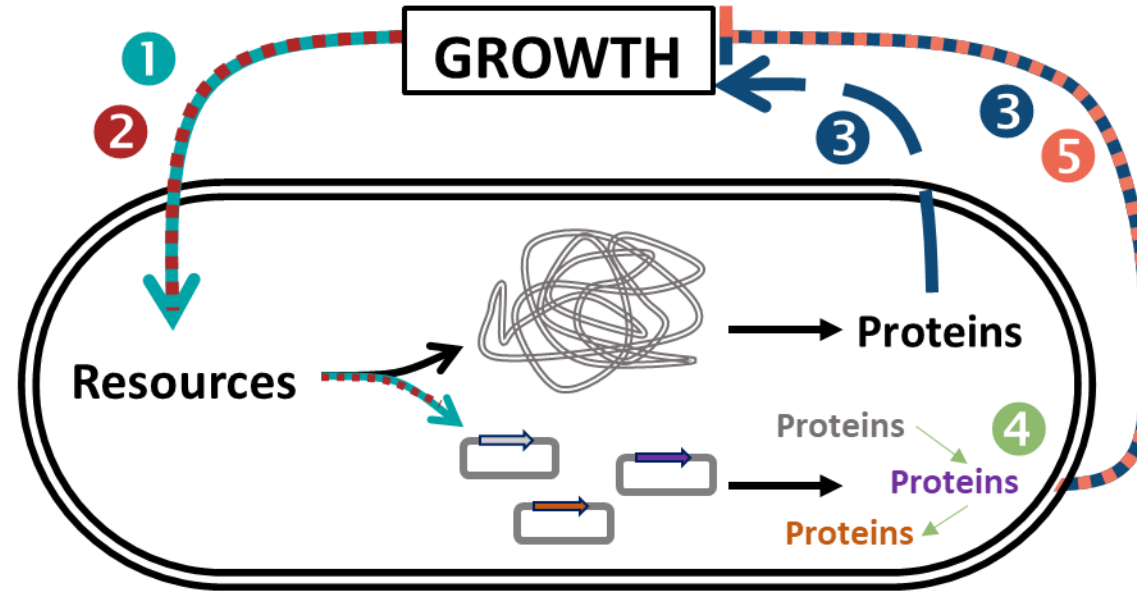
### 21 Research Teams

- 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:

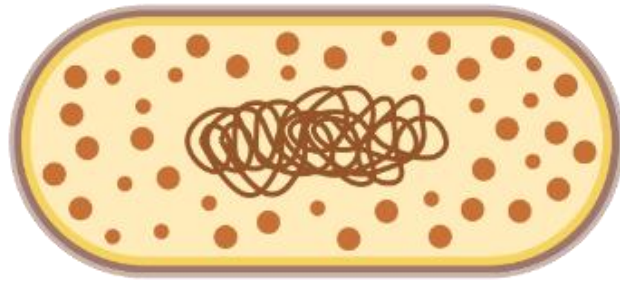
- Link between bacterial growth and nutrient concentrations in culture media (Monod, 1942)
- Link between the macromolecular composition and the cell doubling time (Schaechter et al., 1958)
- Linear relationships between RNA/DNA, RNA/protein, protein/DNA and growth rate (Bremer & Dennis, 1974)
- Increasing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

**-> New technology allows novel experiments**

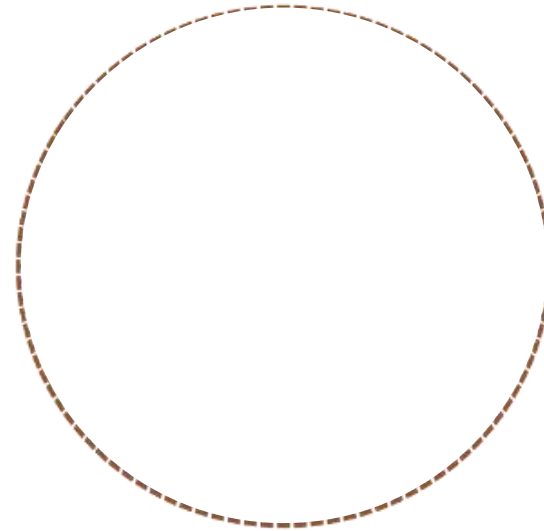
## Bacterial Physiology

Experimental setup

Genomic regulations



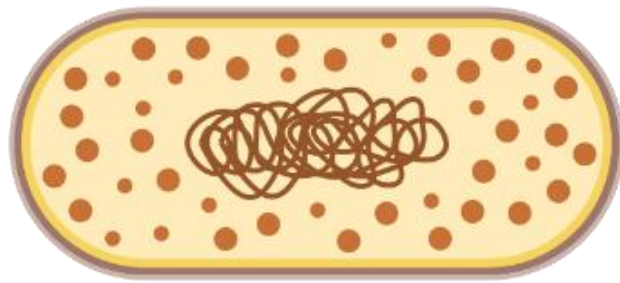
*E. coli* living cells



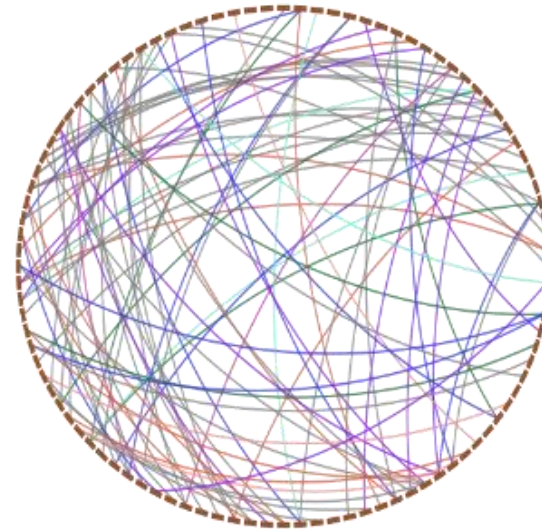
## Bacterial Physiology

Experimental setup

Genomic regulations



*E. coli* living cells



**Intertwined Effects**

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



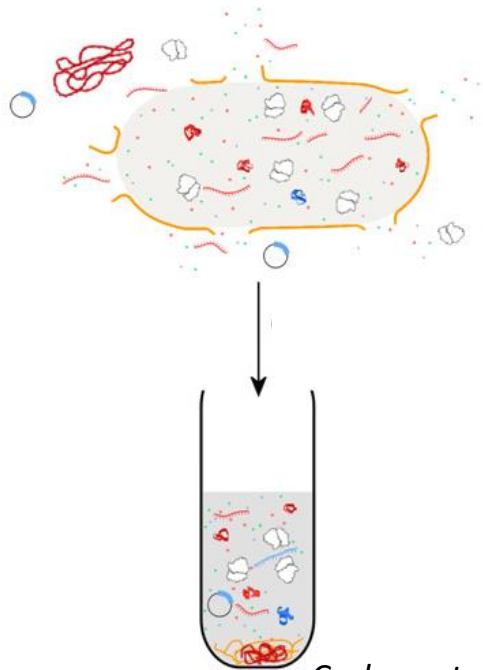
# Cell-free reaction:

## Protein expression in an open system

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



*Carlson et al. Biotechnology advances (2012)*

# Cell-free reaction:

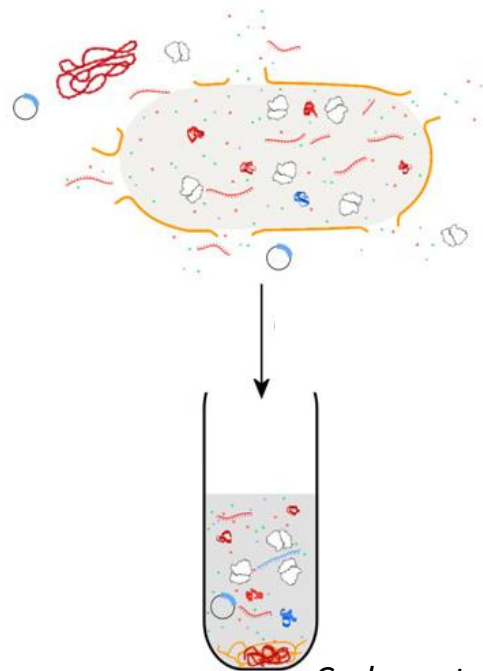
## Protein expression in an open system

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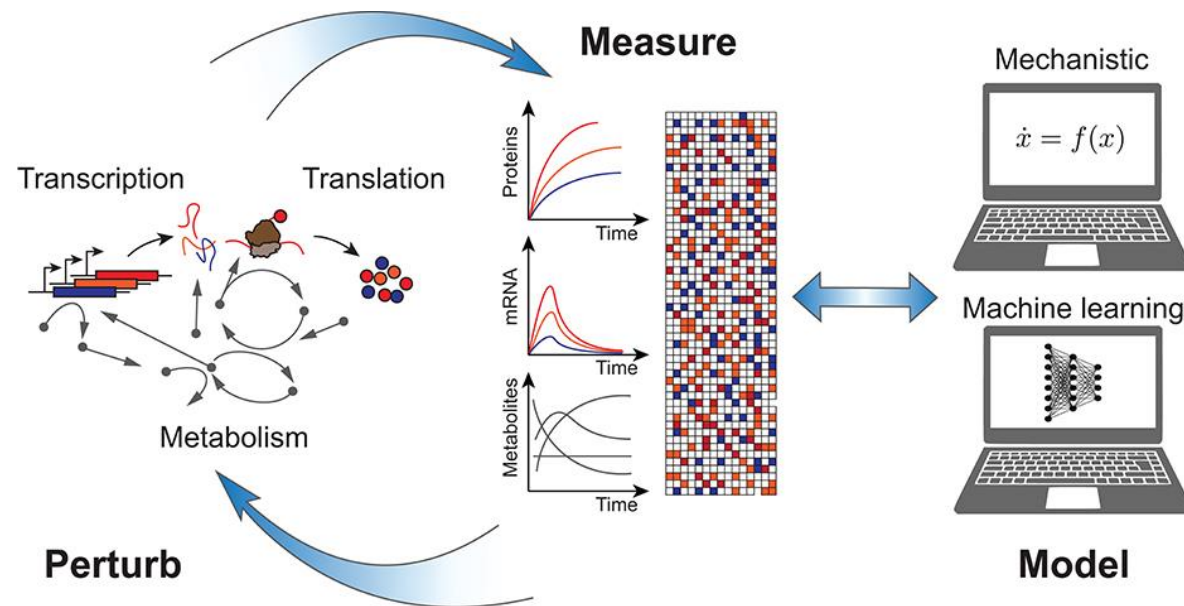
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A direct manipulation of the environment of protein production because it is an open system:

- 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

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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\*

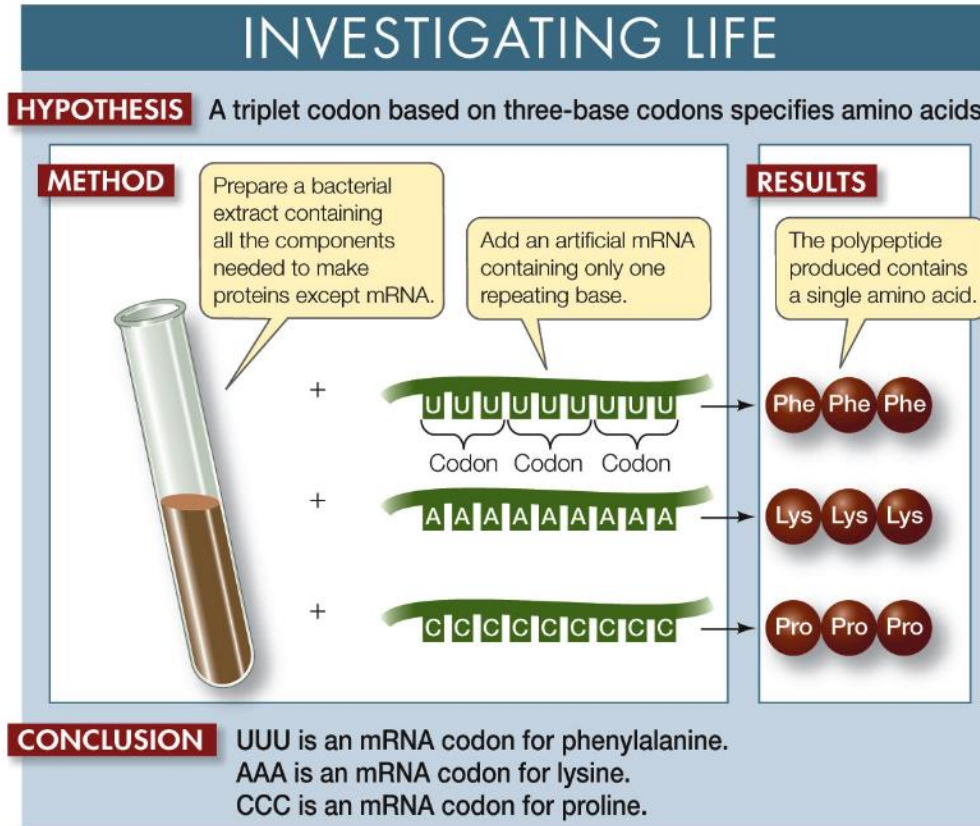
NATIONAL INSTITUTES OF HEALTH, BETHESDA, MARYLAND

*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:

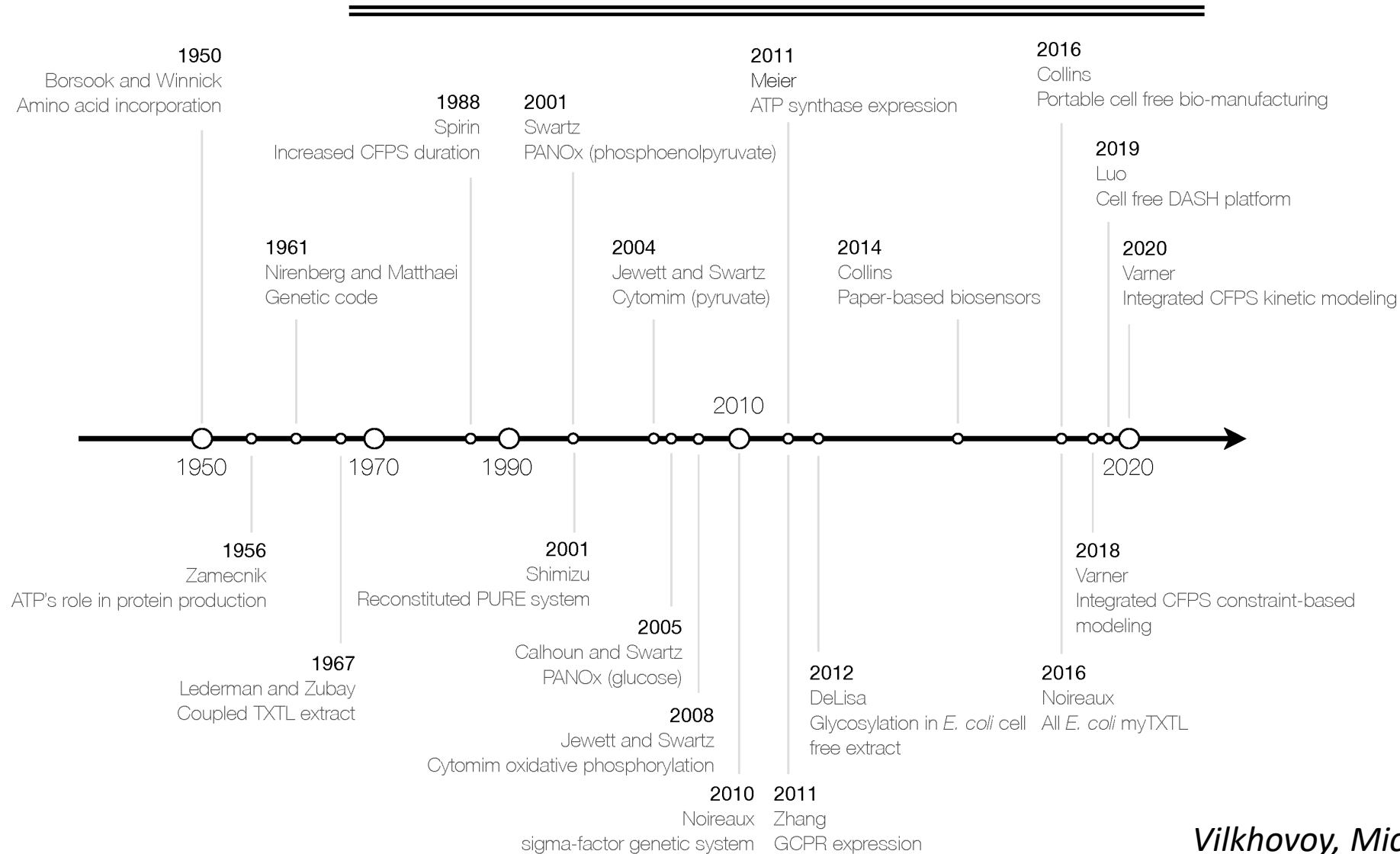
## Nobel prize for the interpretation of the genetic code and its function in protein synthesis



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

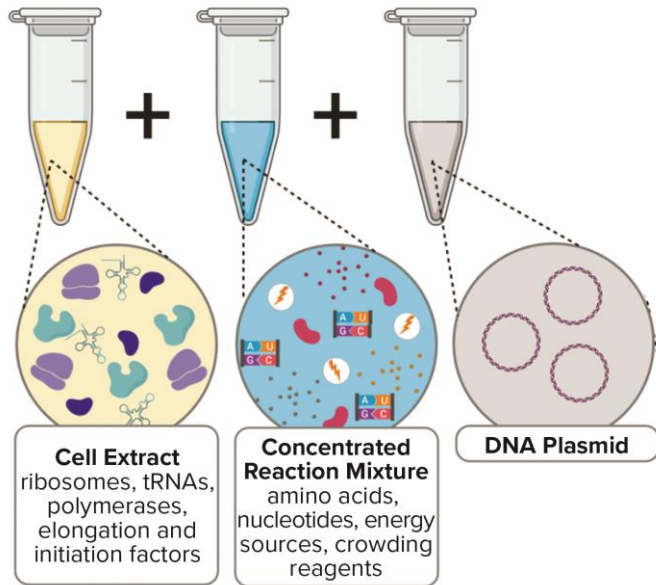


# Cell-free reaction:

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

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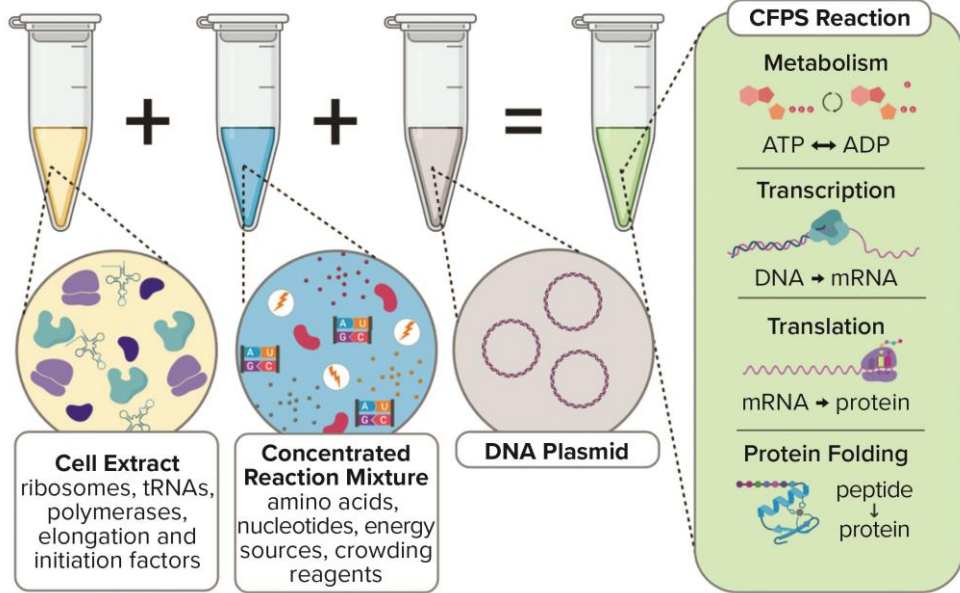
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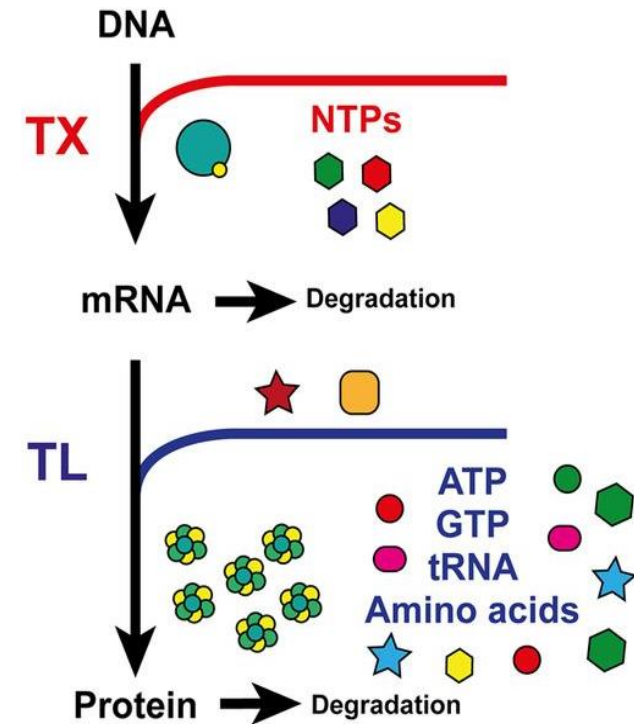
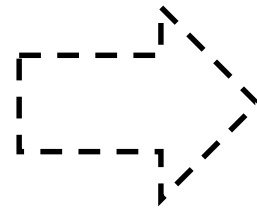
*Melinek B, et al. BioProcess International (2020)*

# Cell-free reaction:

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



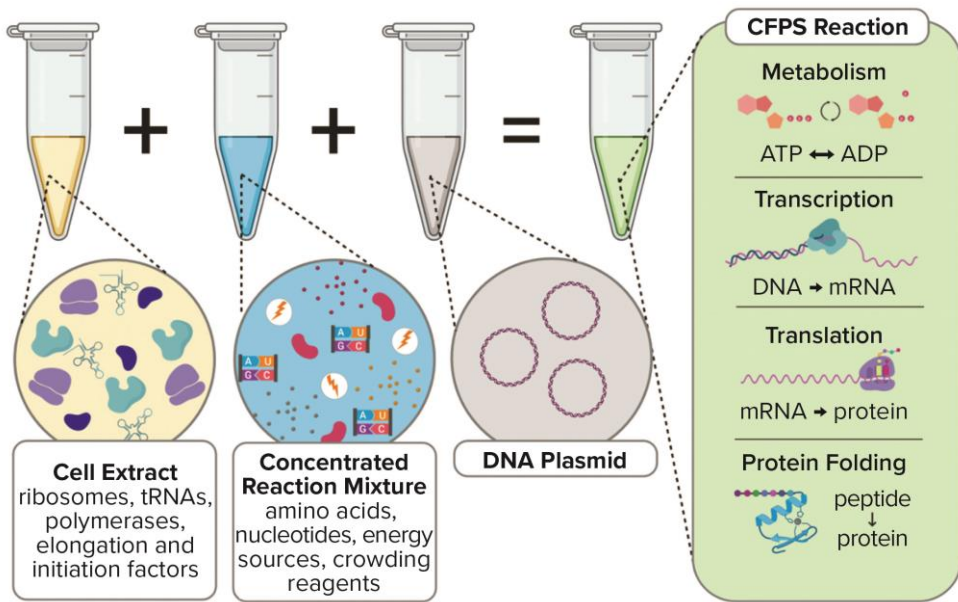
Melinek B, et al. *BioProcess International* (2020)



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



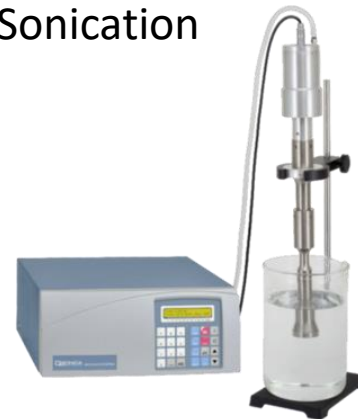
# Cell-free reaction: Cell-extract preparation



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

## Physical disruption

### Sonication

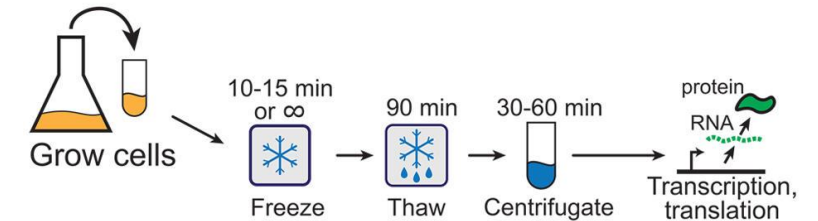


### French press

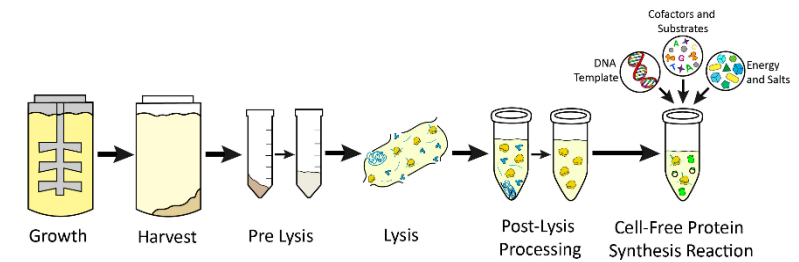


## Chemical disruption

### Autolysis



### Lysozyme

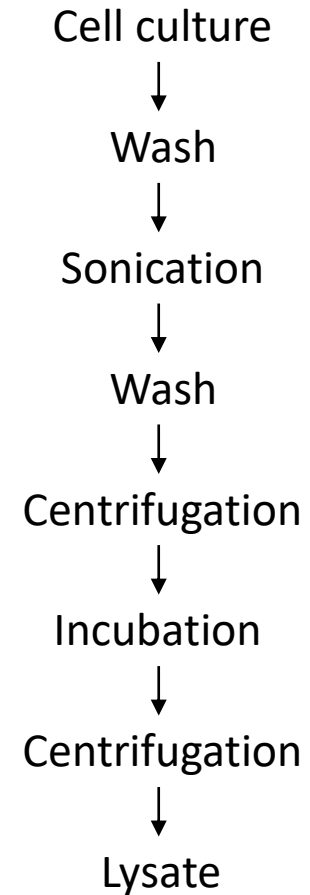
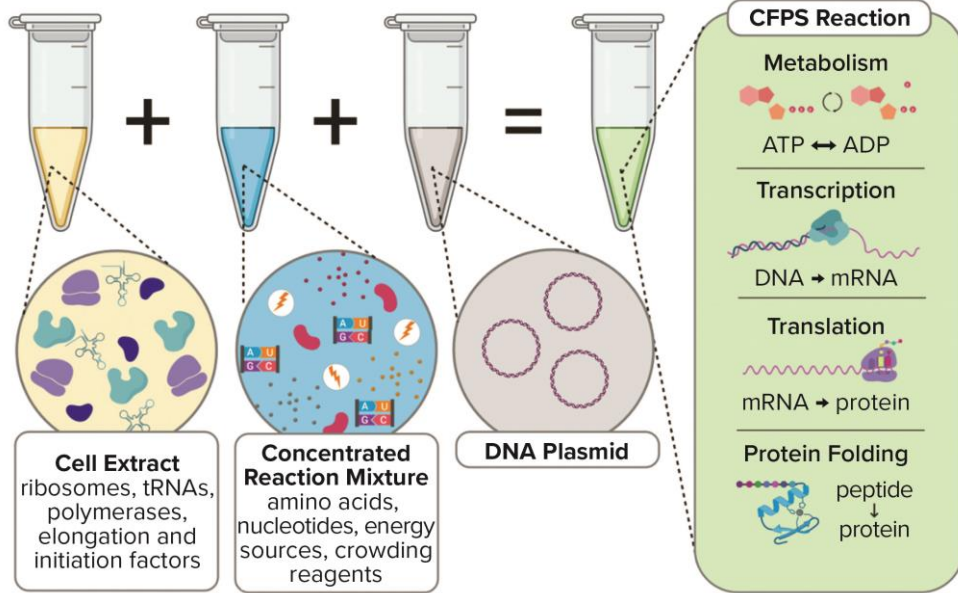


# Cell-free reaction:

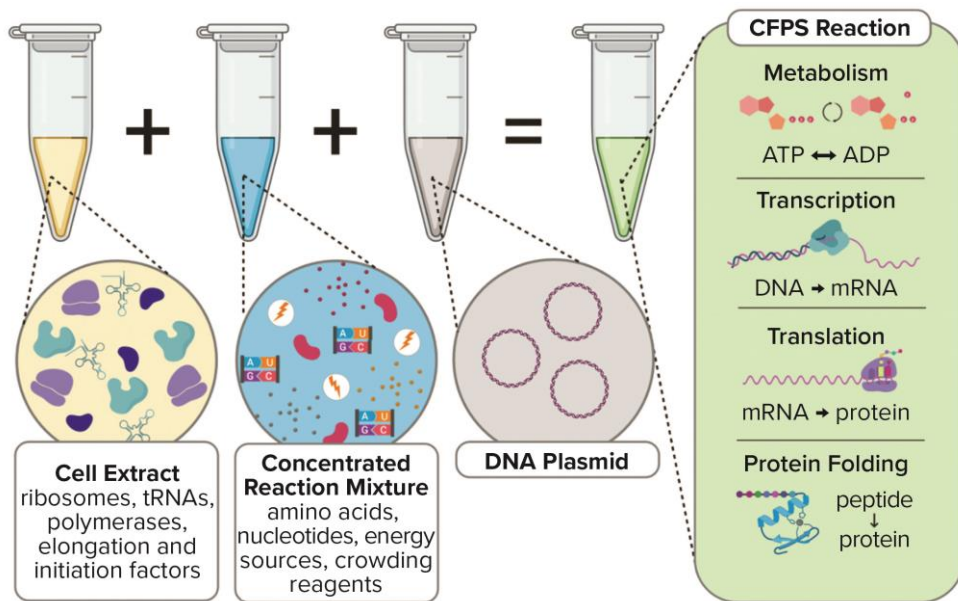
## Protocol for home-made cell-extract preparation

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# 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)

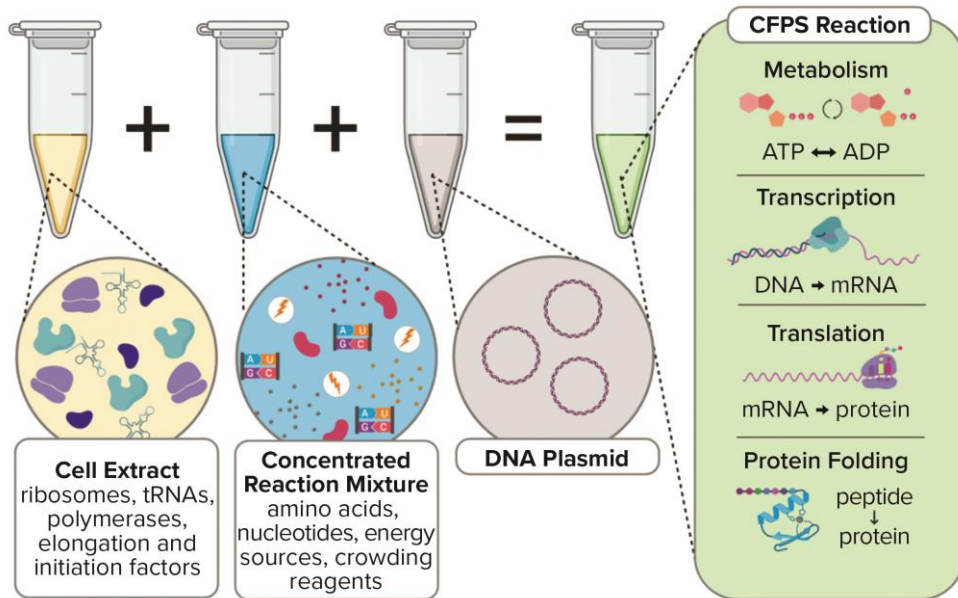
Translation  
Translation  
Ribosome maintenance  
Ribosome stability  
A precursor for formylmethionine (fMet)  
Ribosome activity /RNA stability  
Regenerate ATP via glycolysis  
Regenerate ATP via glycolysis  
Regenerate ATP  
regulating glycolysis  
Transcription

# Cell-free reaction:

## Measurements in 384-well plate

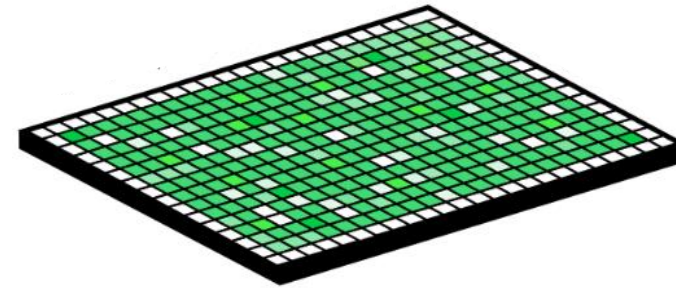
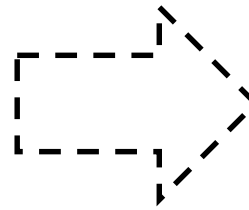
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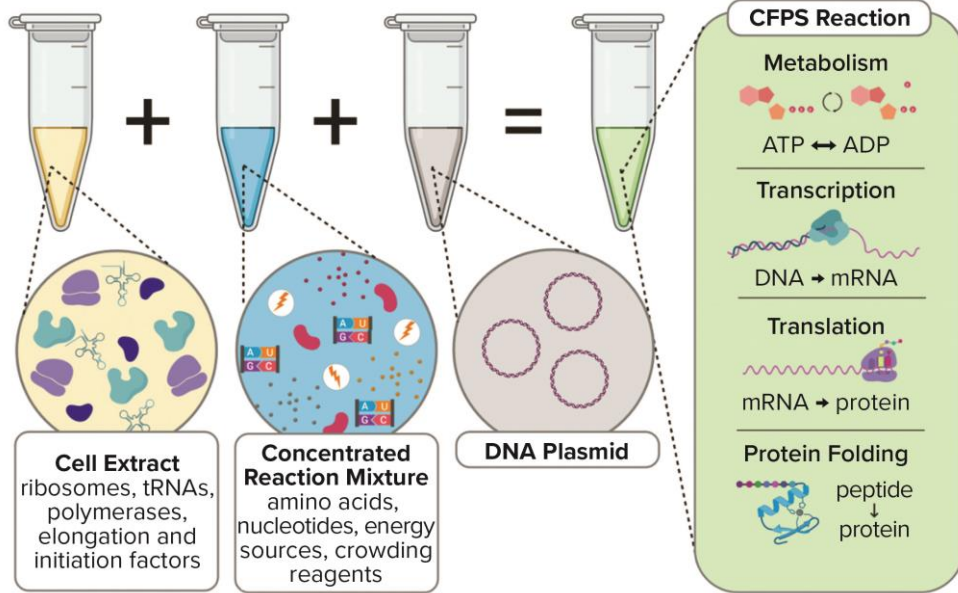
Melinek B, et al. *BioProcess International* (2020)

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



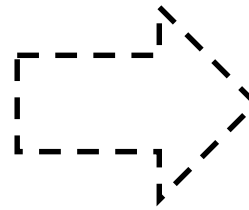
# Cell-free reaction:

## Measurements in 384-well plate

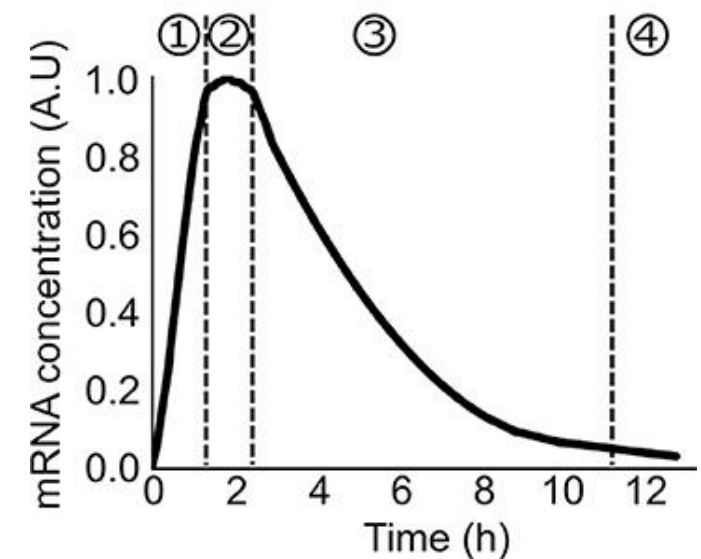
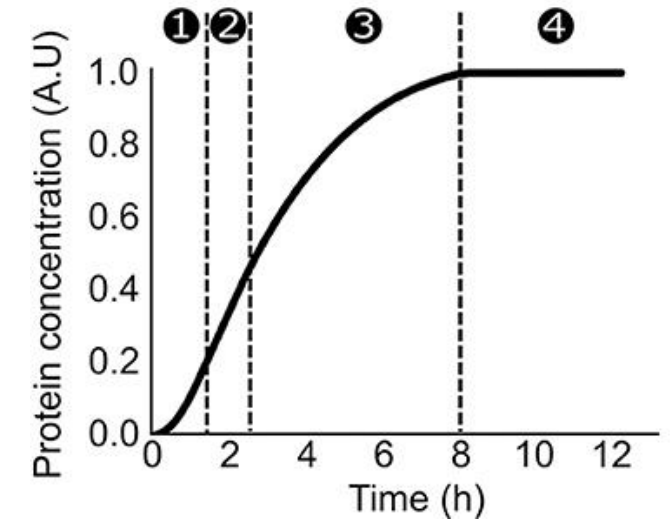


Melinek B, et al. *BioProcess International* (2020)

### Translation

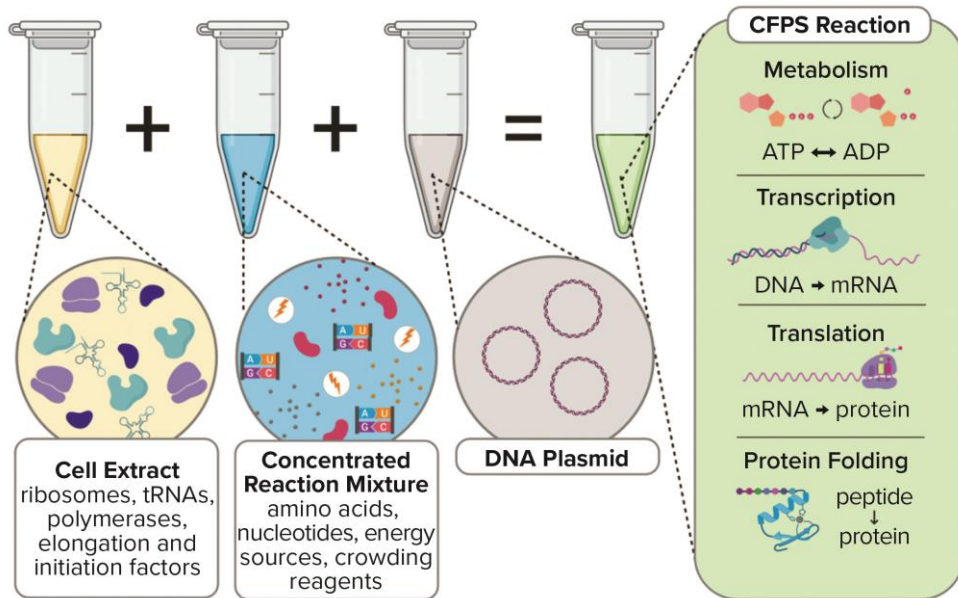


### Transcription



# Cell-free reaction:

## Current existing protocol to produce bacterial cell extract



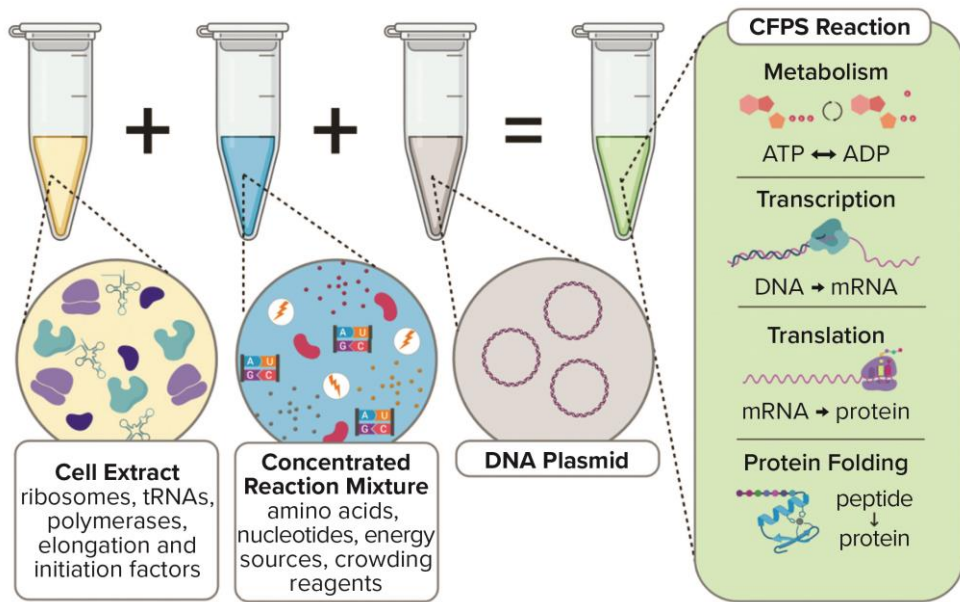
Melinek B, et al. *BioProcess International* (2020)

**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



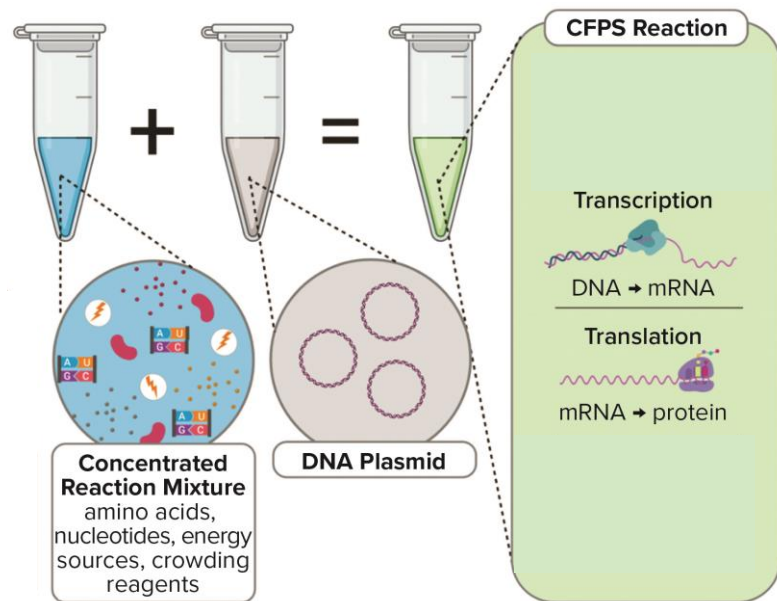
Melinek B, et al. *BioProcess International* (2020)

|                     | 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

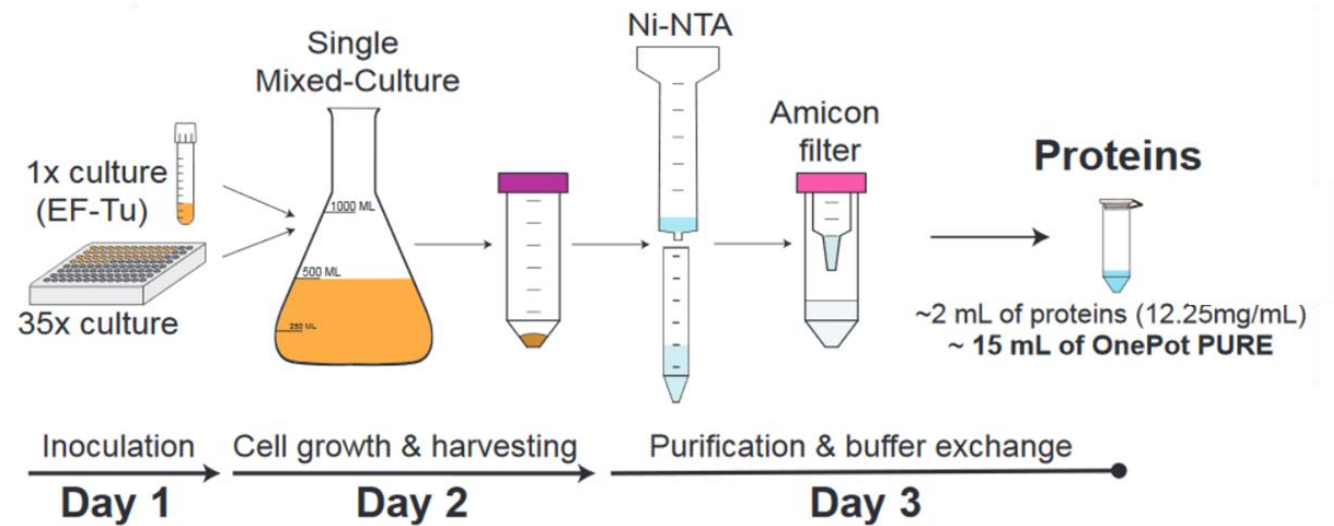
# Cell-free reaction:

## 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



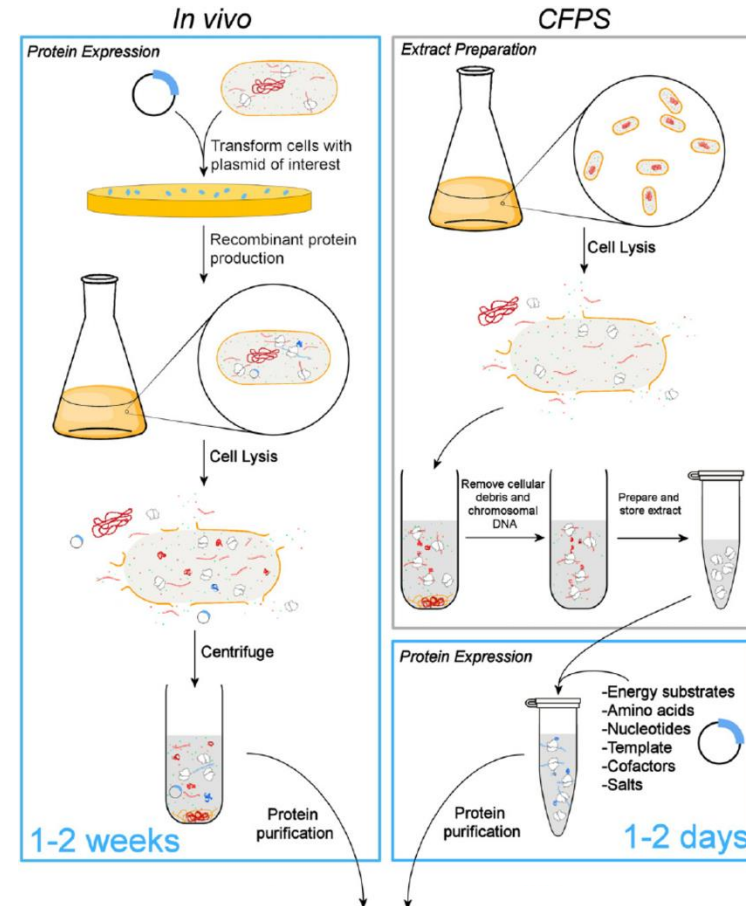
Lavickova, Barbora, et al. *ACS synthetic biology* (2019)



# 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

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### 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

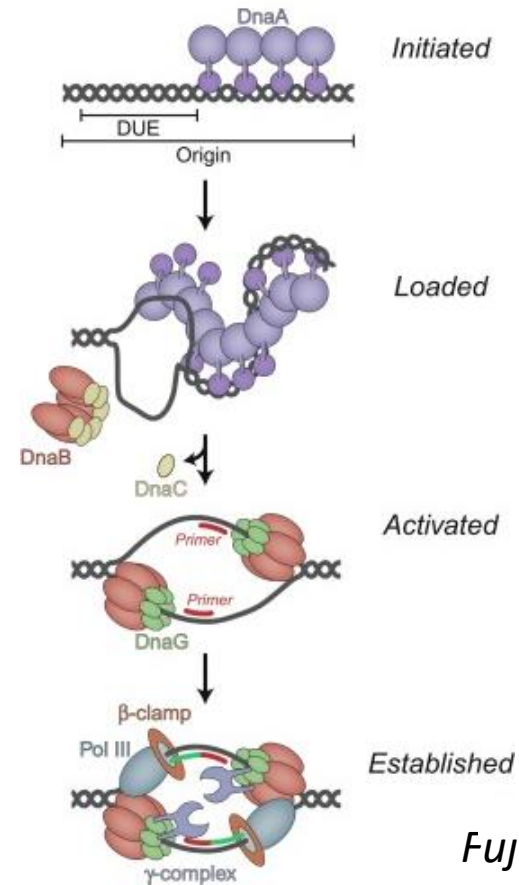
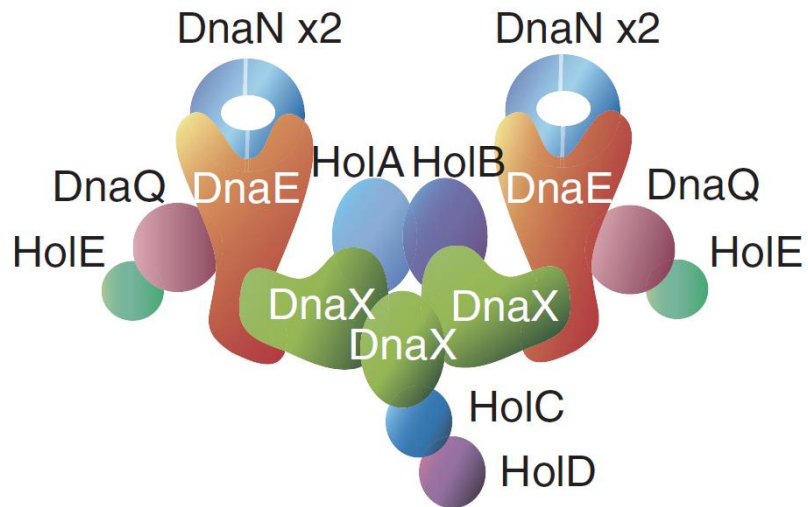
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### - Central functions

#### 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

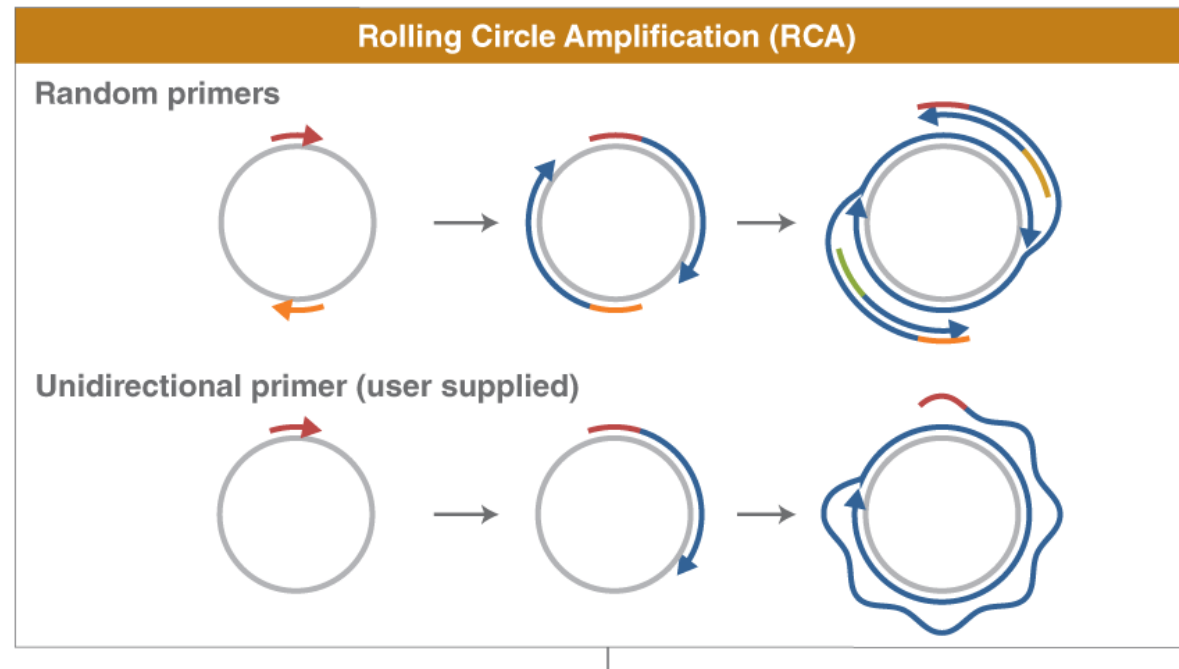
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### - Central functions

#### DNA replication

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



Use for replication of large DNA genomes

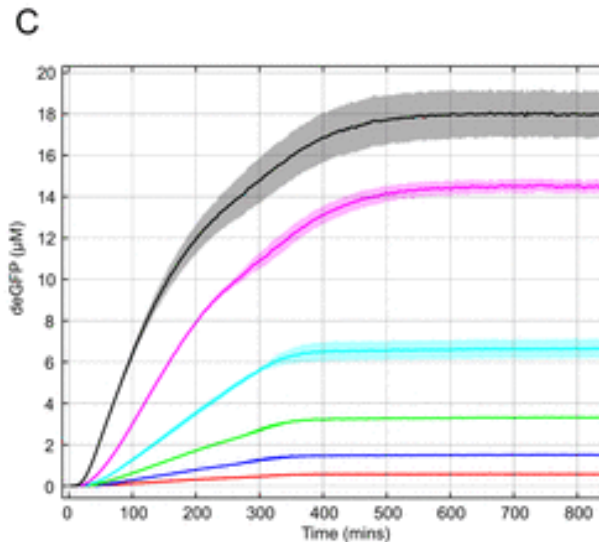
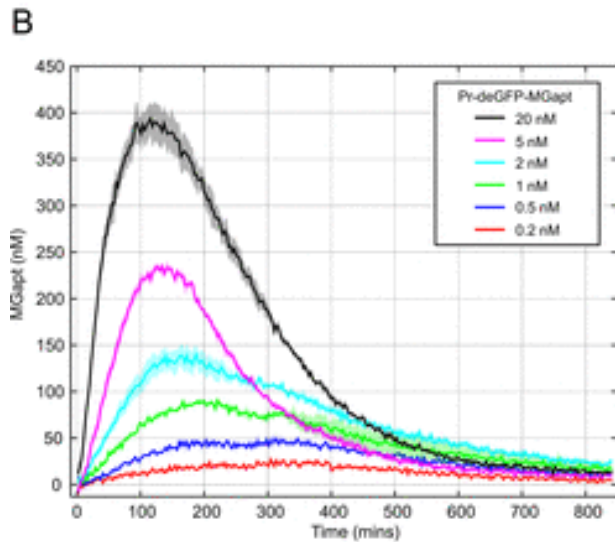
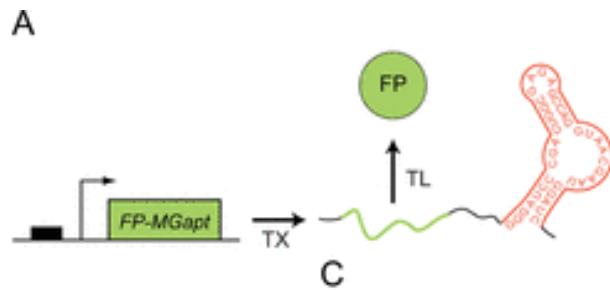
*Libicher, K, nat comms (2020)*

# Cell-free reaction:

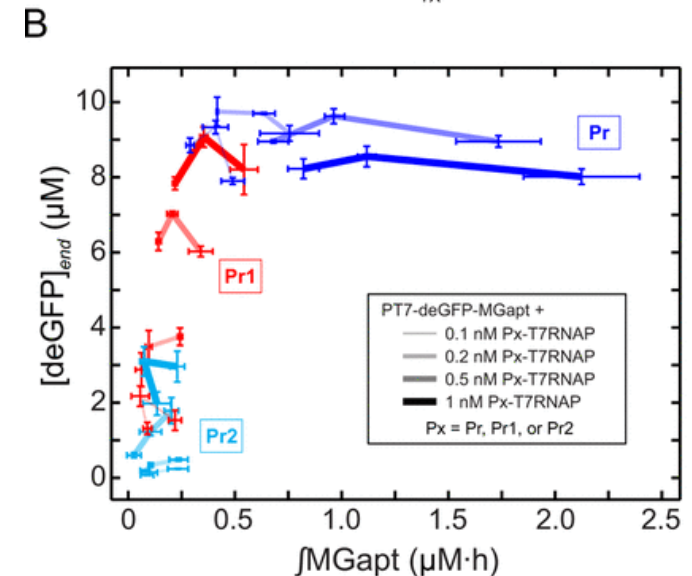
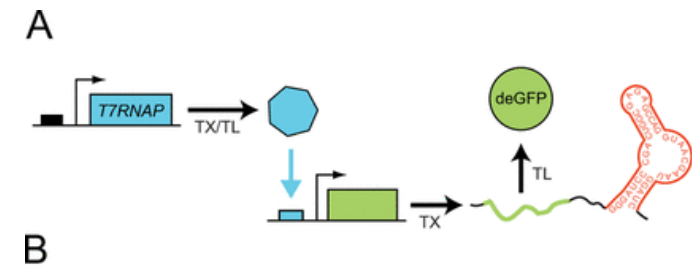
## Fundamental science: cell-free to understand living cells

### - Central functions

#### Transcription process: T7 polymerase



Gene circuit performance characterization



# Cell-free reaction:

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

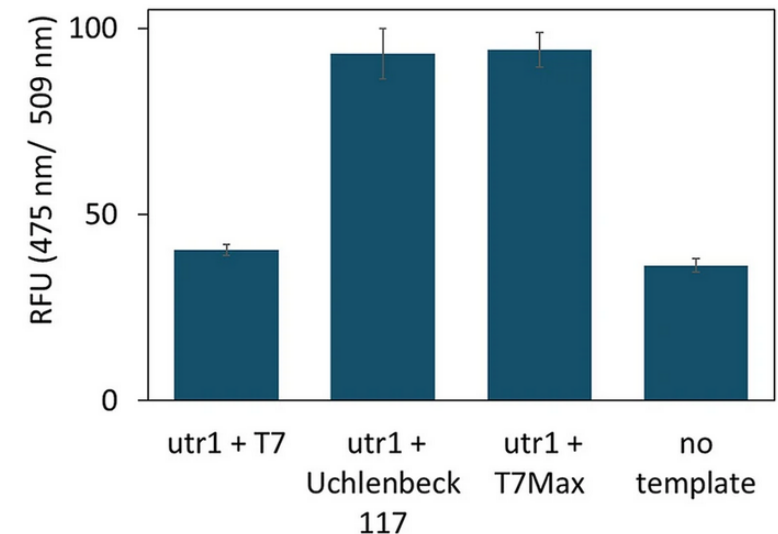
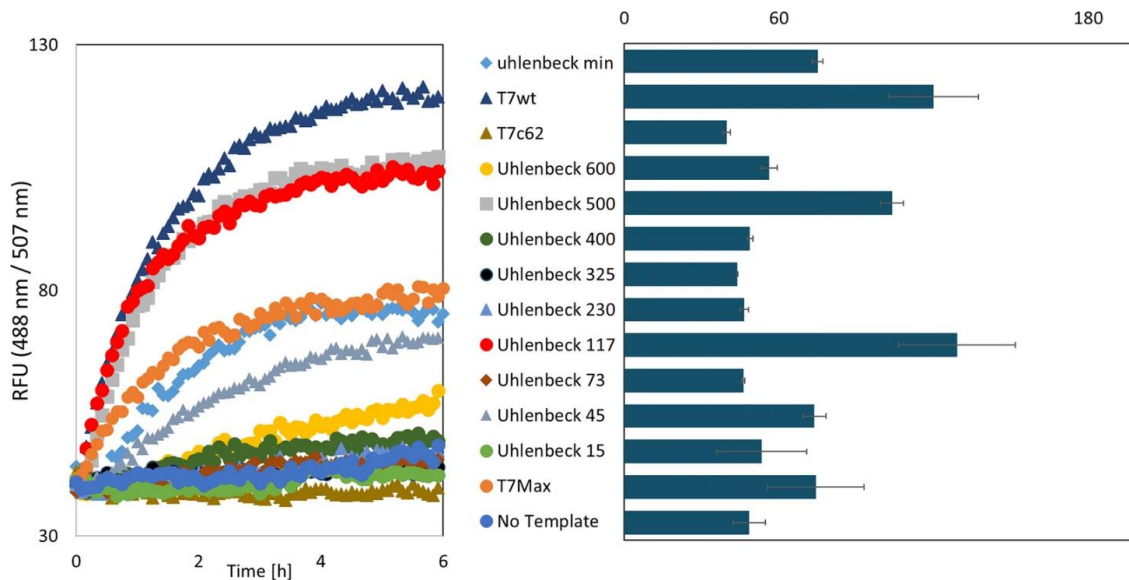
### - Central functions

**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



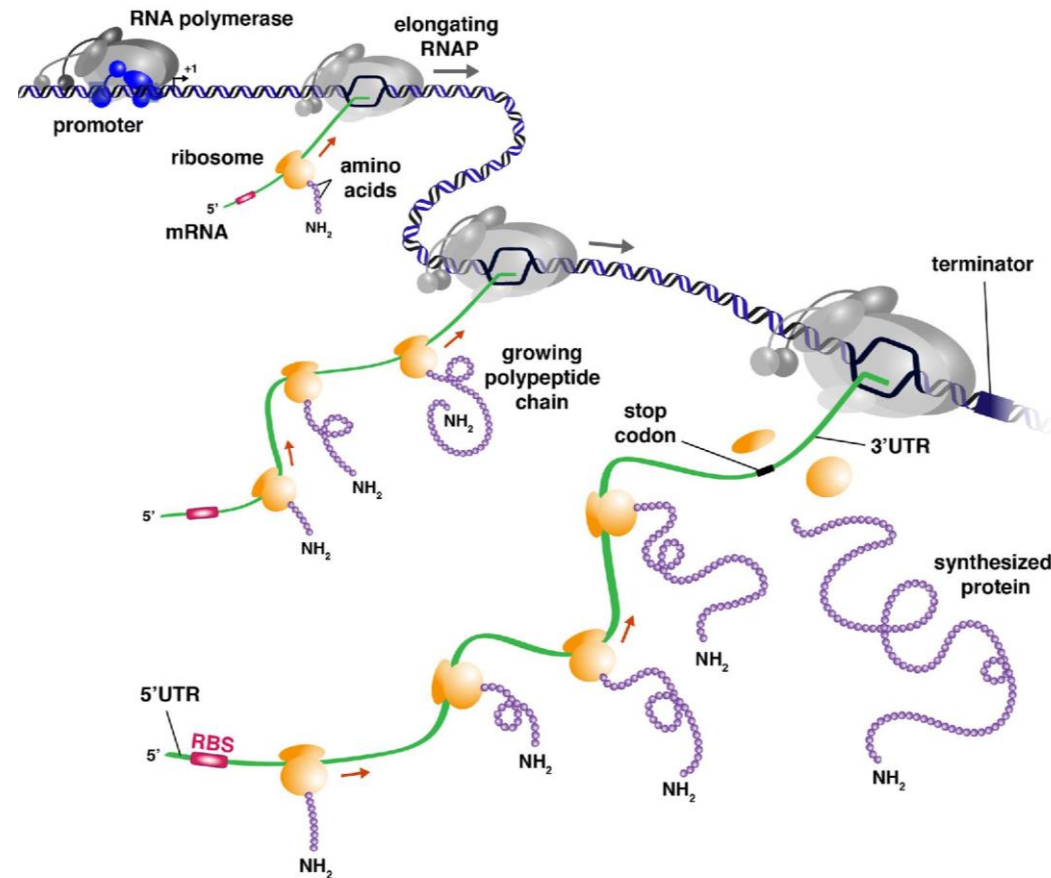


# Cell-free reaction:

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

### - Central functions

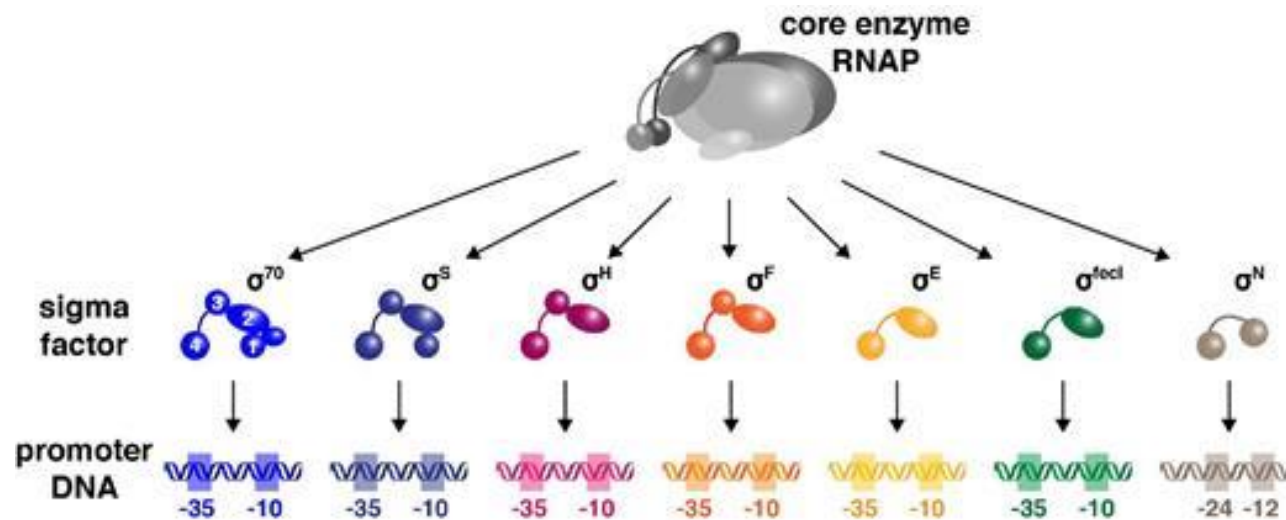
#### Transcription



# Cell-free reaction:

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

- Central functions
- Transcription



| σ-factor                   | Function                  | Group |
|----------------------------|---------------------------|-------|
| $\sigma^D/\sigma^{70}$     | 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     |
| $\sigma^{Ecd}/\sigma^{19}$ | Extracytoplasmic function | 4     |
| $\sigma^N/\sigma^{54}$     | Nitrogen metabolism       |       |

# Cell-free reaction:

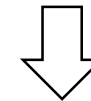
## Fundamental science: cell-free to understand living cells

### - 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



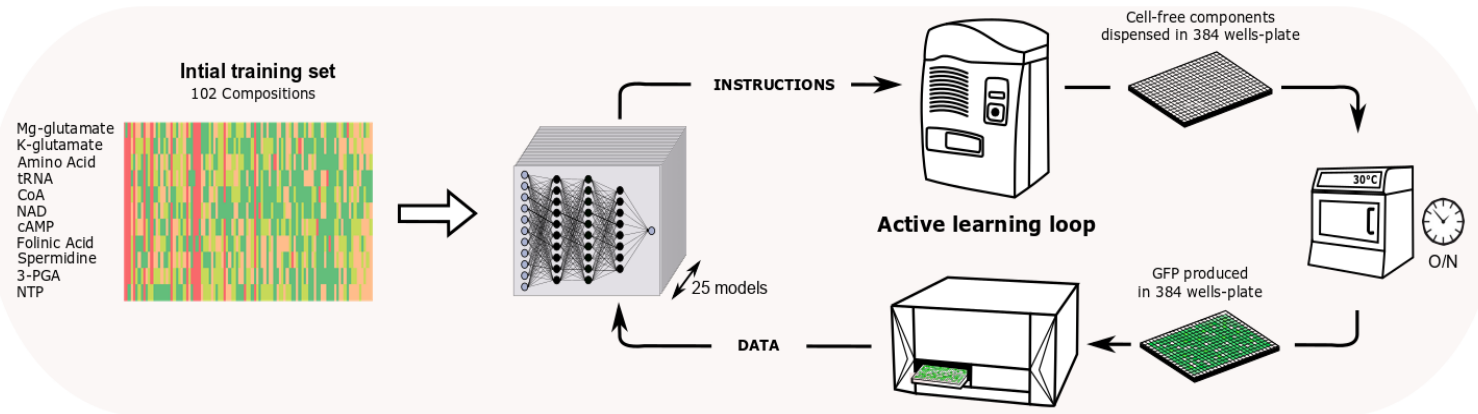
- Can we improve protein production for a specific lysate without increasing the price of cell-free reaction?
- Can we provide efficient predictions of protein production?
- Can we highlight the critical parameters involved in protein production?

# Cell-free reaction:

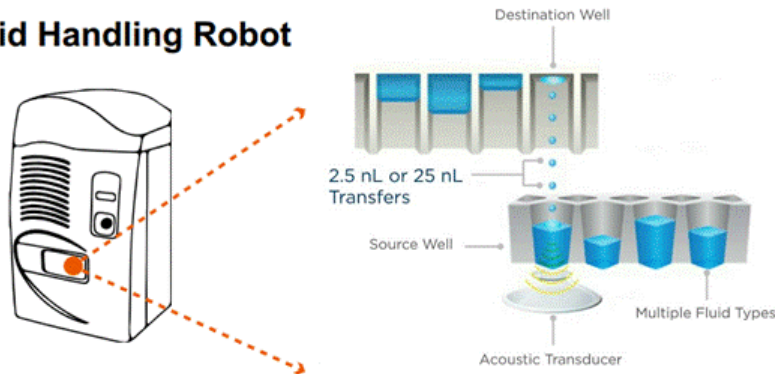
## Fundamental science: cell-free to understand living cells

### - Central functions

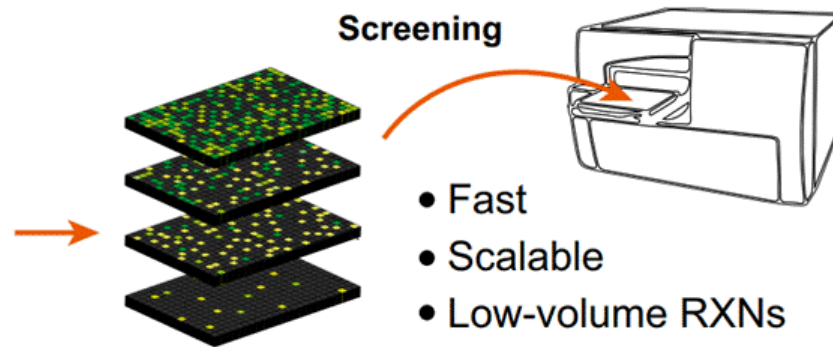
#### Translation: Optimizing translation process



### Liquid Handling Robot



### Screening

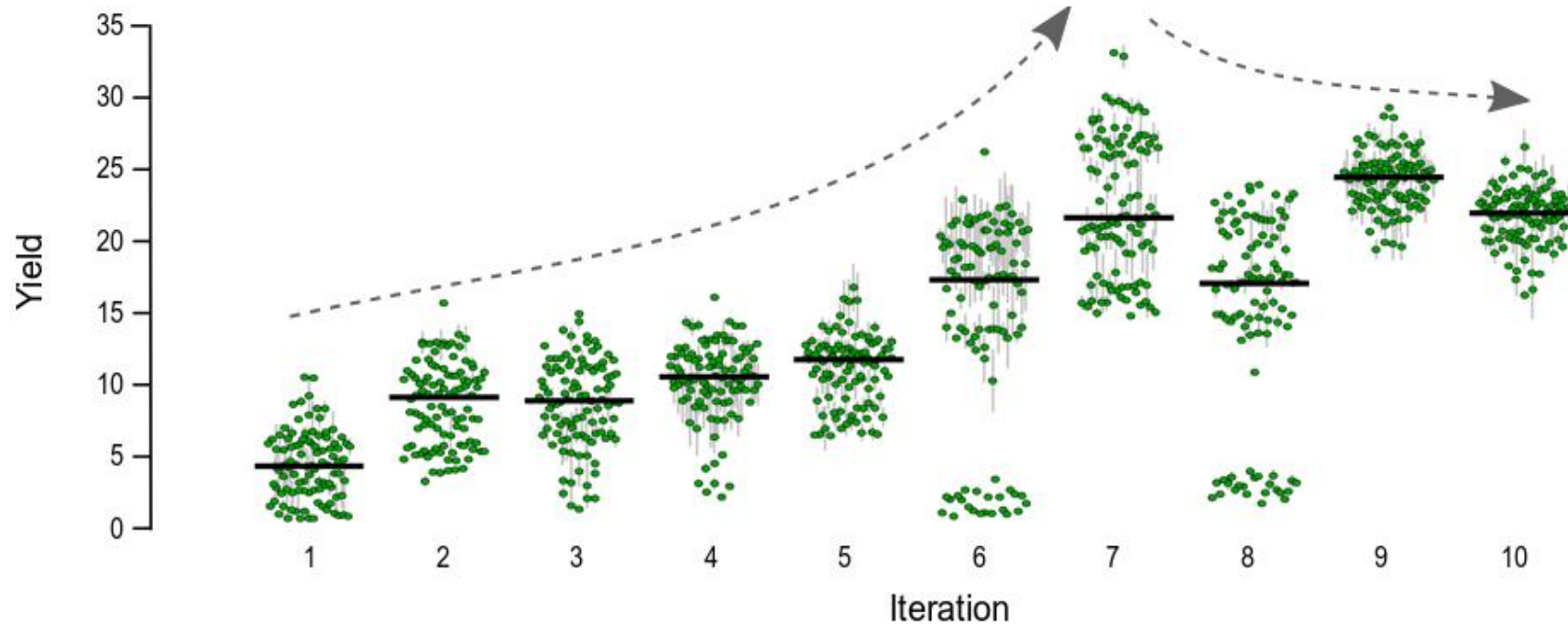


# Cell-free reaction:

Fundamental science: cell-free to understand living cells

- Central functions

Translation: Optimizing translation process

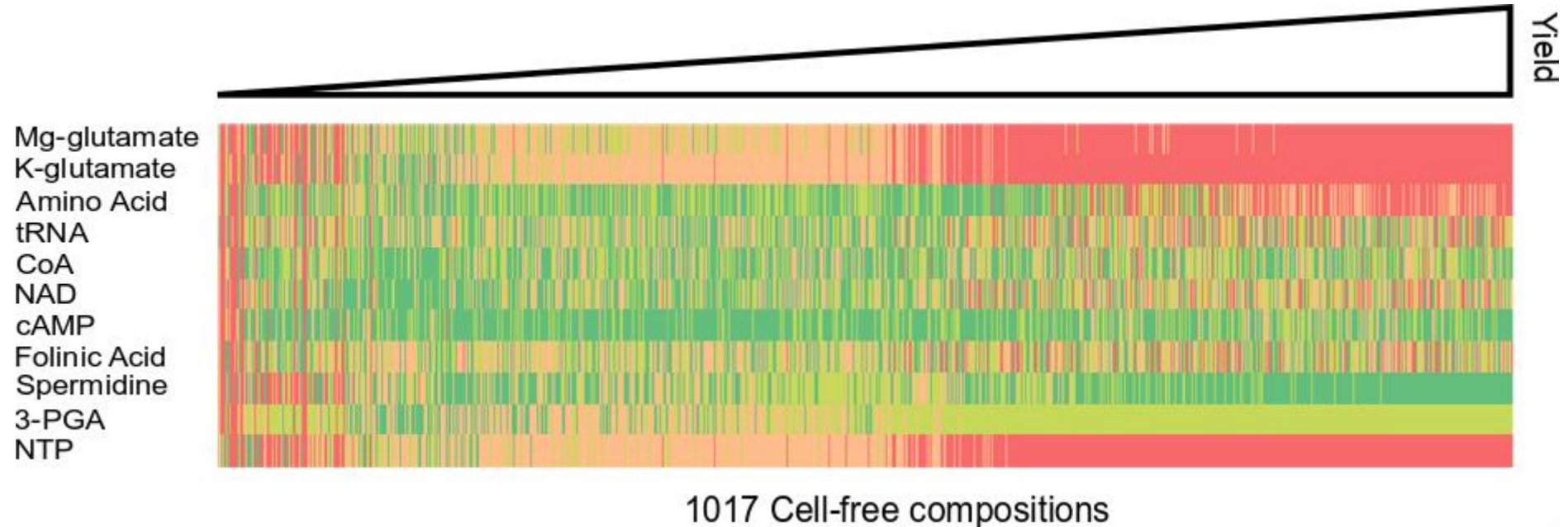


# Cell-free reaction:

## 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:

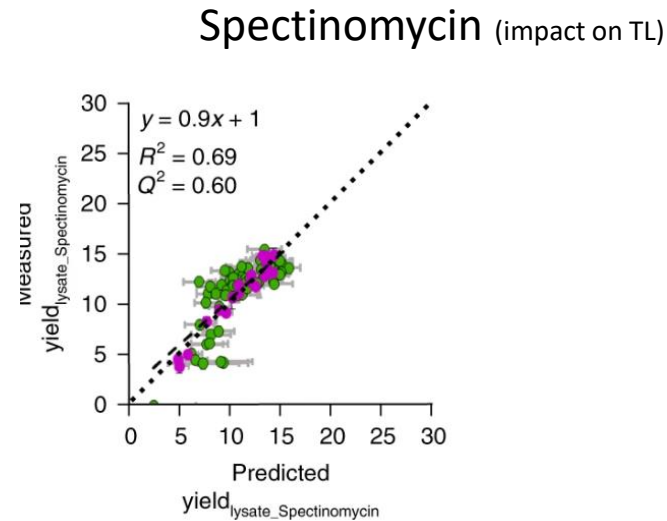
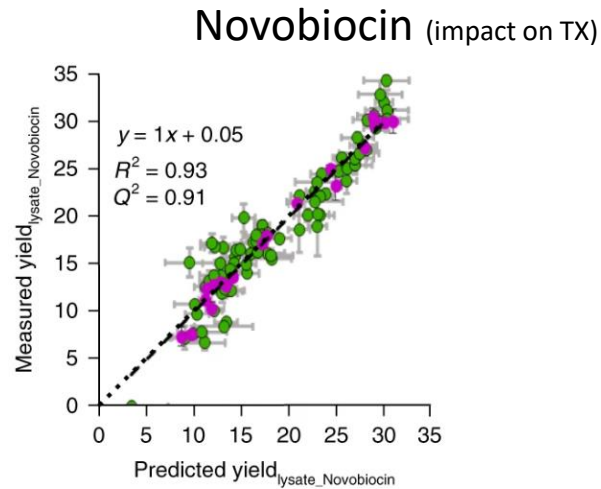
## Fundamental science: cell-free to understand living cells

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### - Central functions

Translation: Optimizing translation process



### Transcription vs translation:

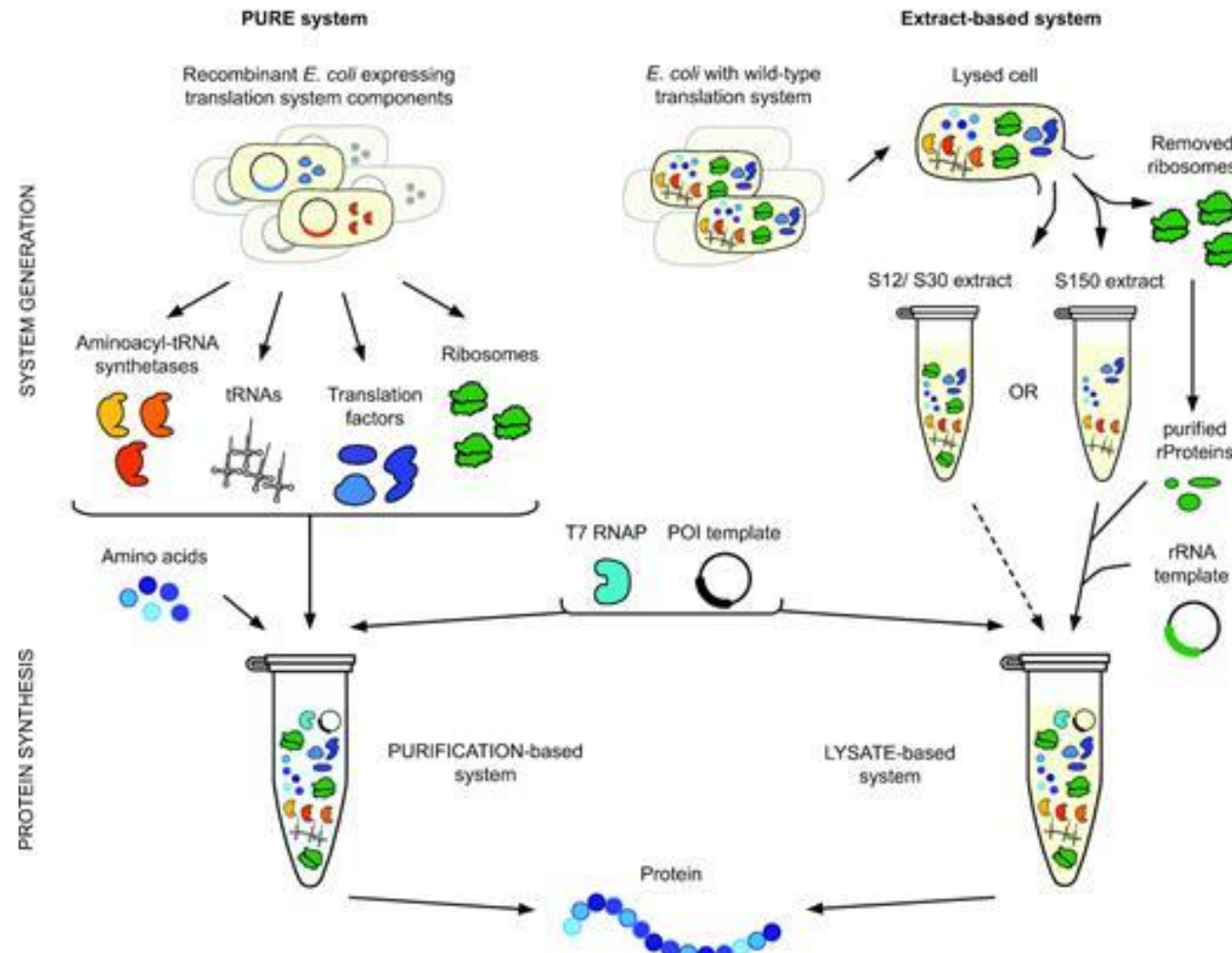
Our data suggests that we mainly improved translation via composition optimization

# Cell-free reaction:

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

### - Central functions

### Translation: engineering translation machinery



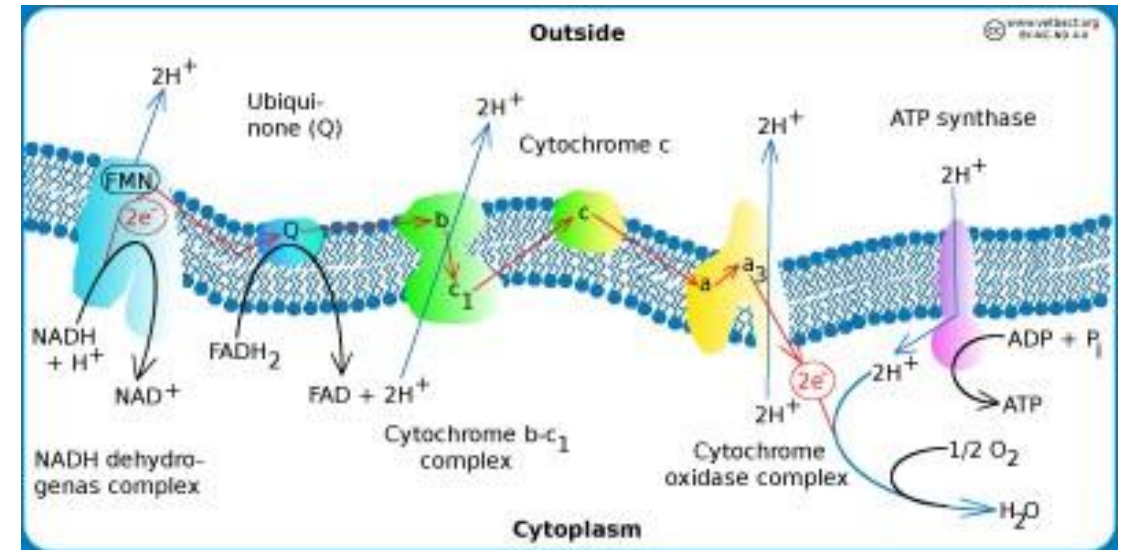
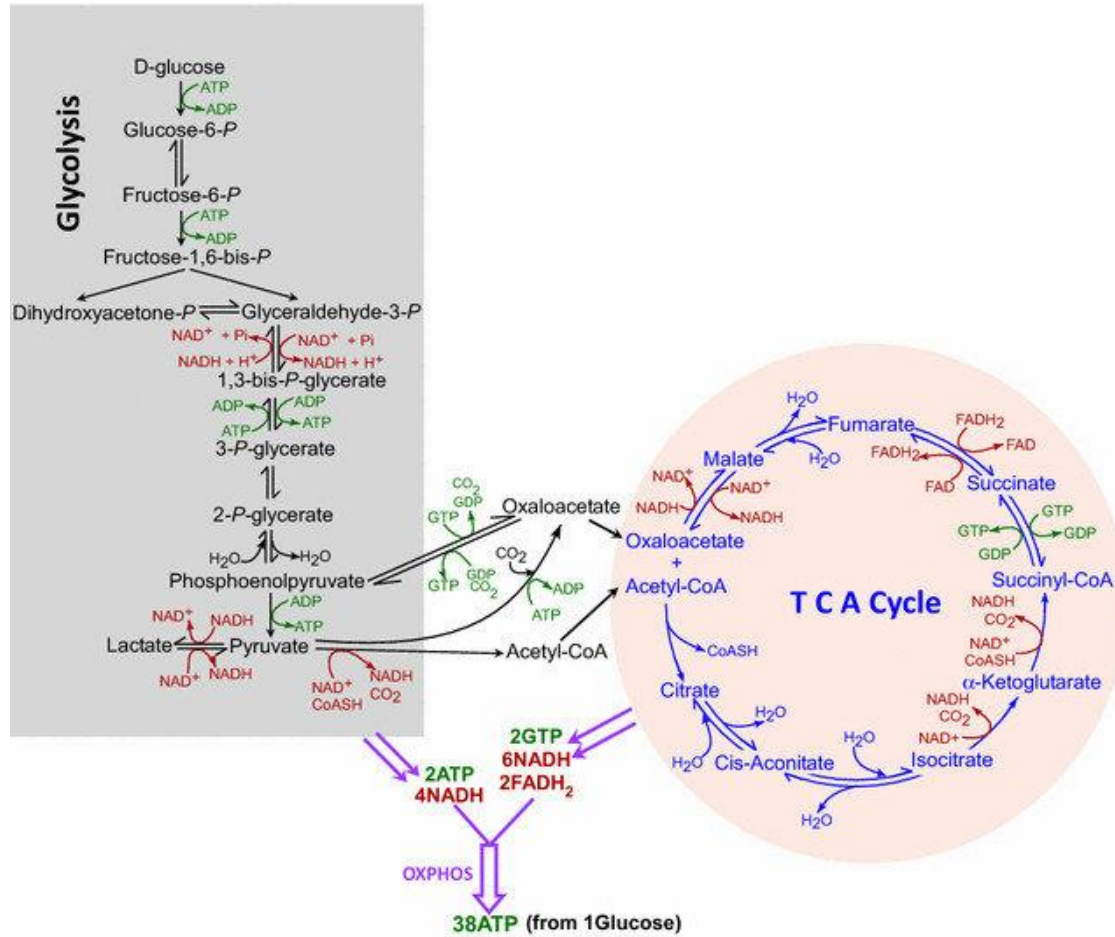


# Cell-free reaction:

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

### - Central functions

### Metabolism and energy production



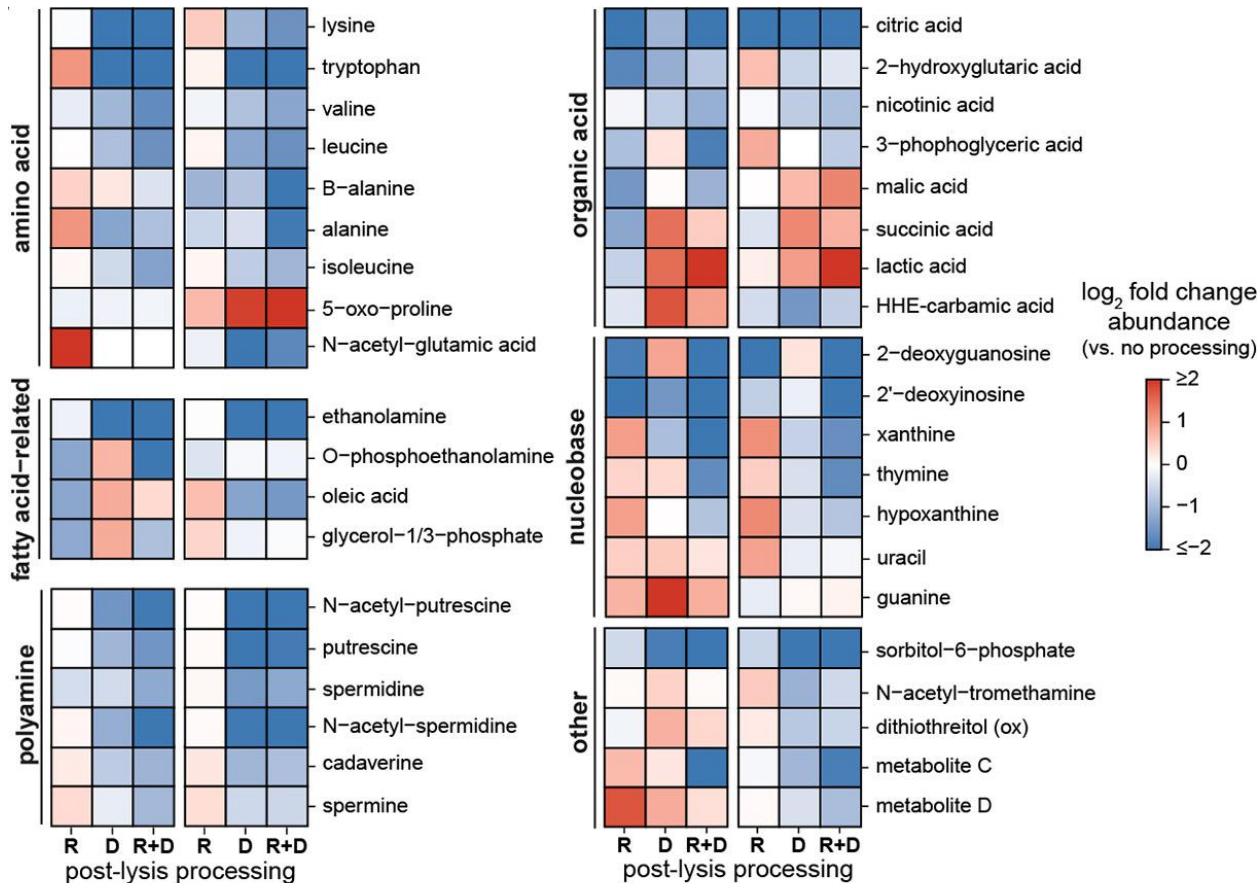
# Cell-free reaction:

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

### - Central functions

### Metabolism and energy production

Study of composition in metabolites in different cell-free process

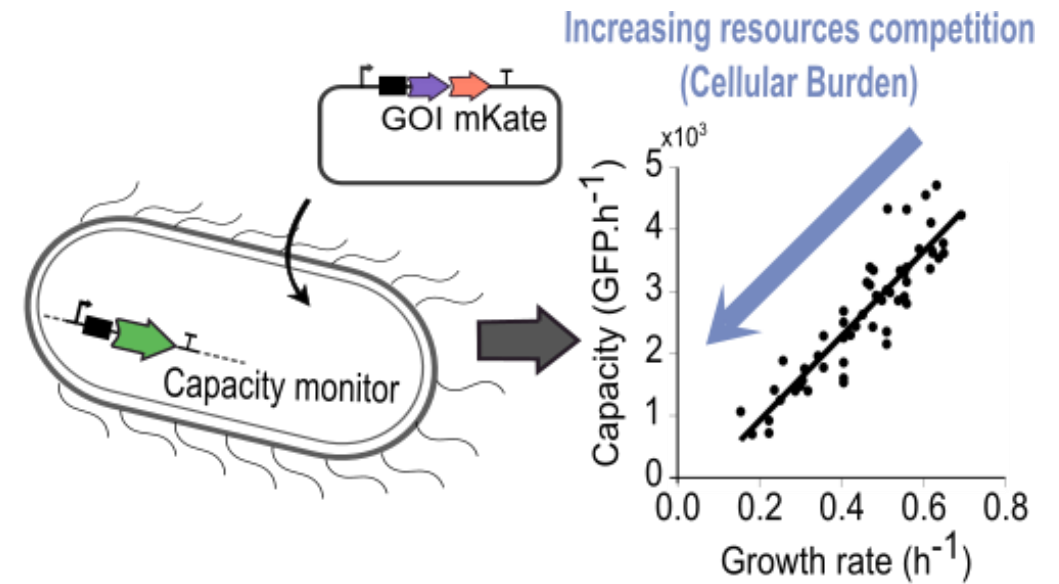
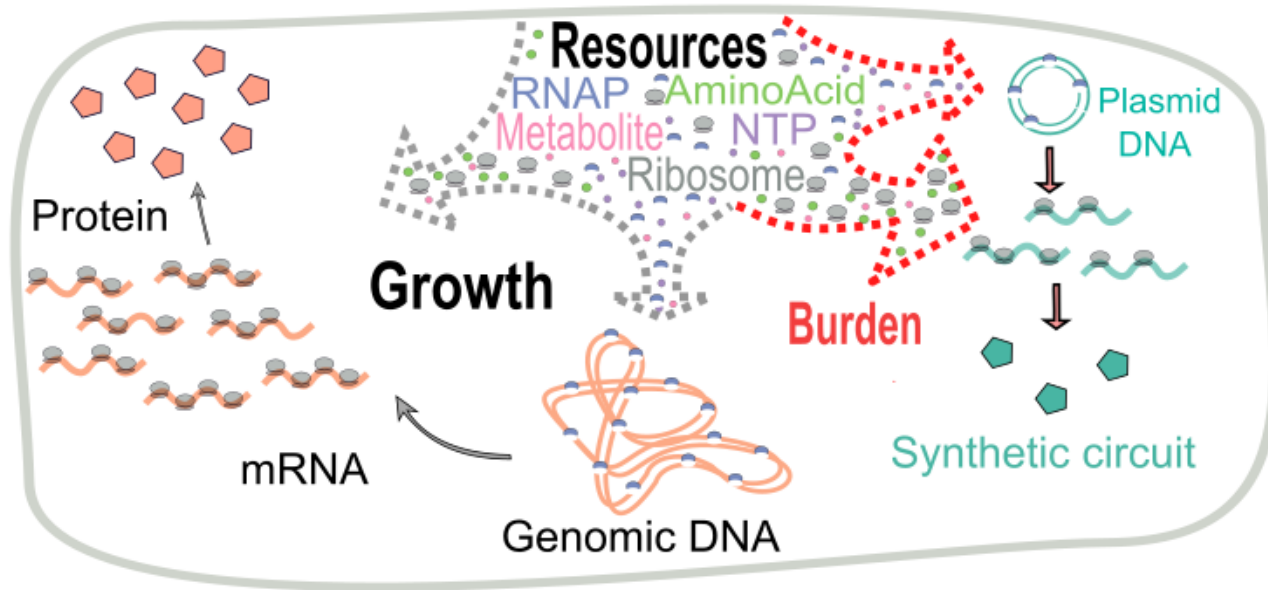


-> improve cell-free efficiency, supplemented with proteins to observe the impact on each part of the metabolism

# Cell-free reaction:

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

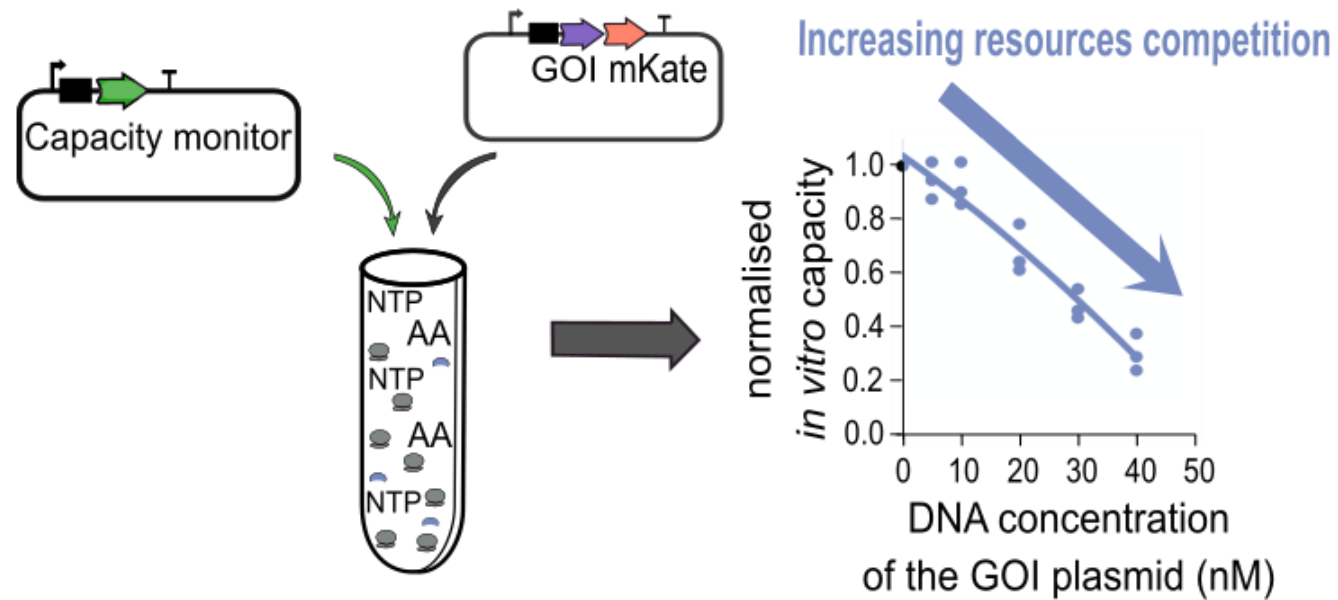
- Resource competition *in vitro*
- Production burden



# Cell-free reaction:

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

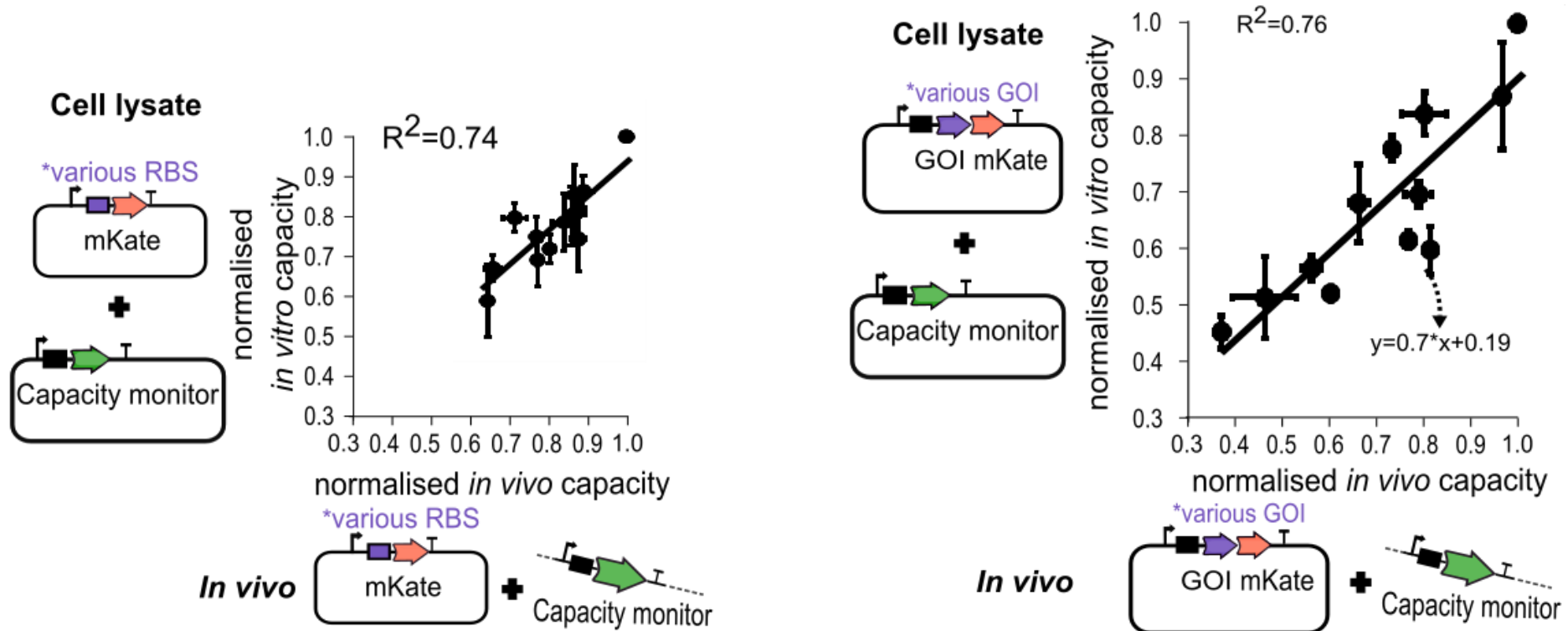
- Resource competition *in vitro*  
Production burden



# Cell-free reaction:

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

- Resource competition *in vitro*  
Production burden

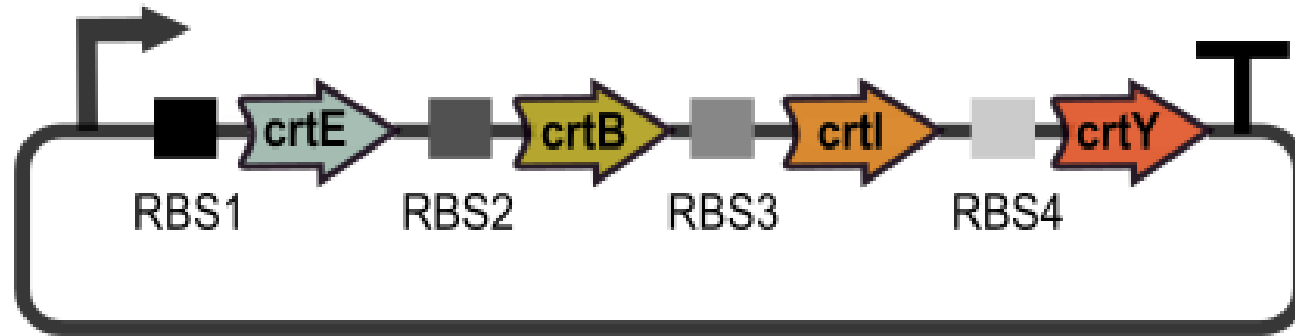


# Cell-free reaction:

## 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:

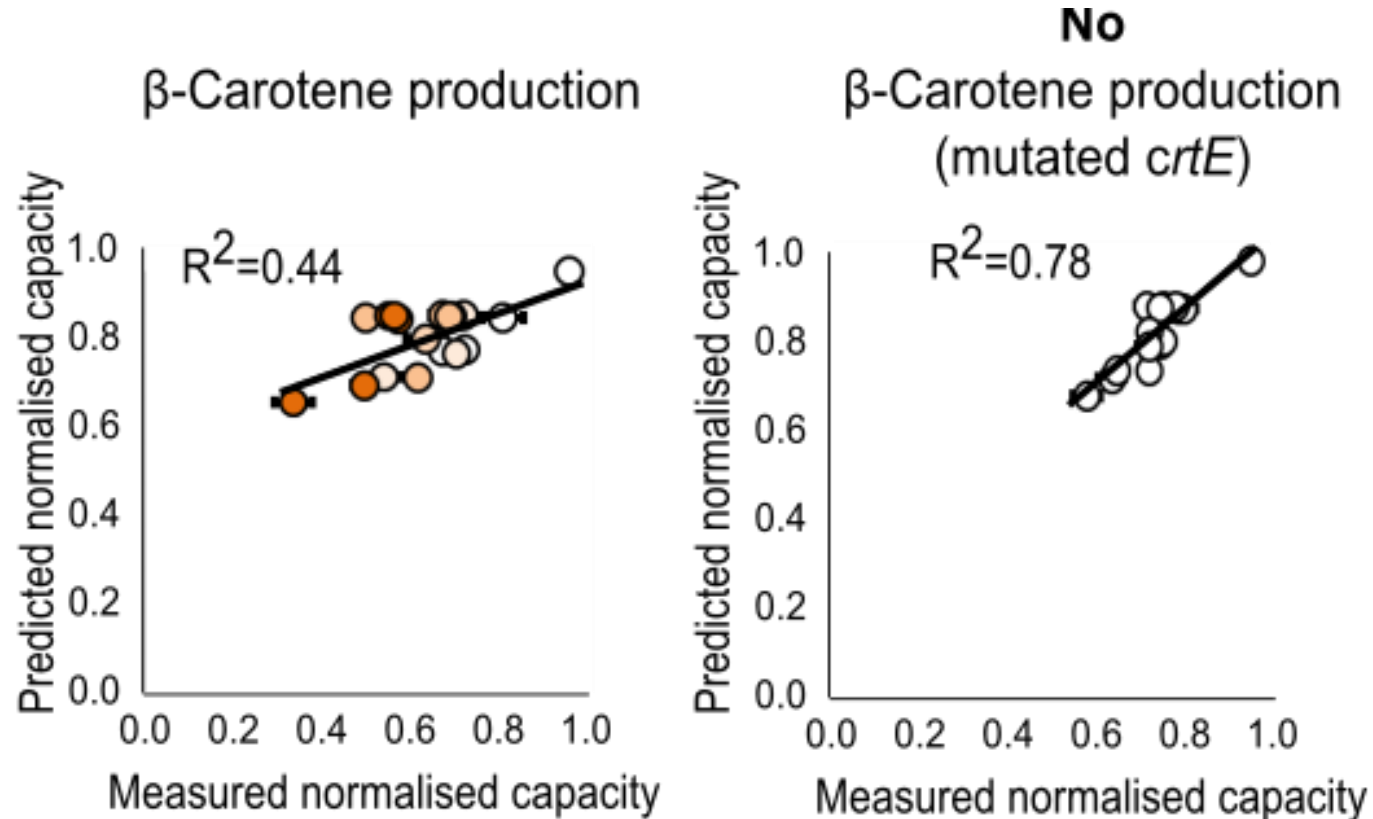
## Fundamental science: cell-free to understand living cells

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- Resource competition *in vitro*

Decouple production and metabolic burden

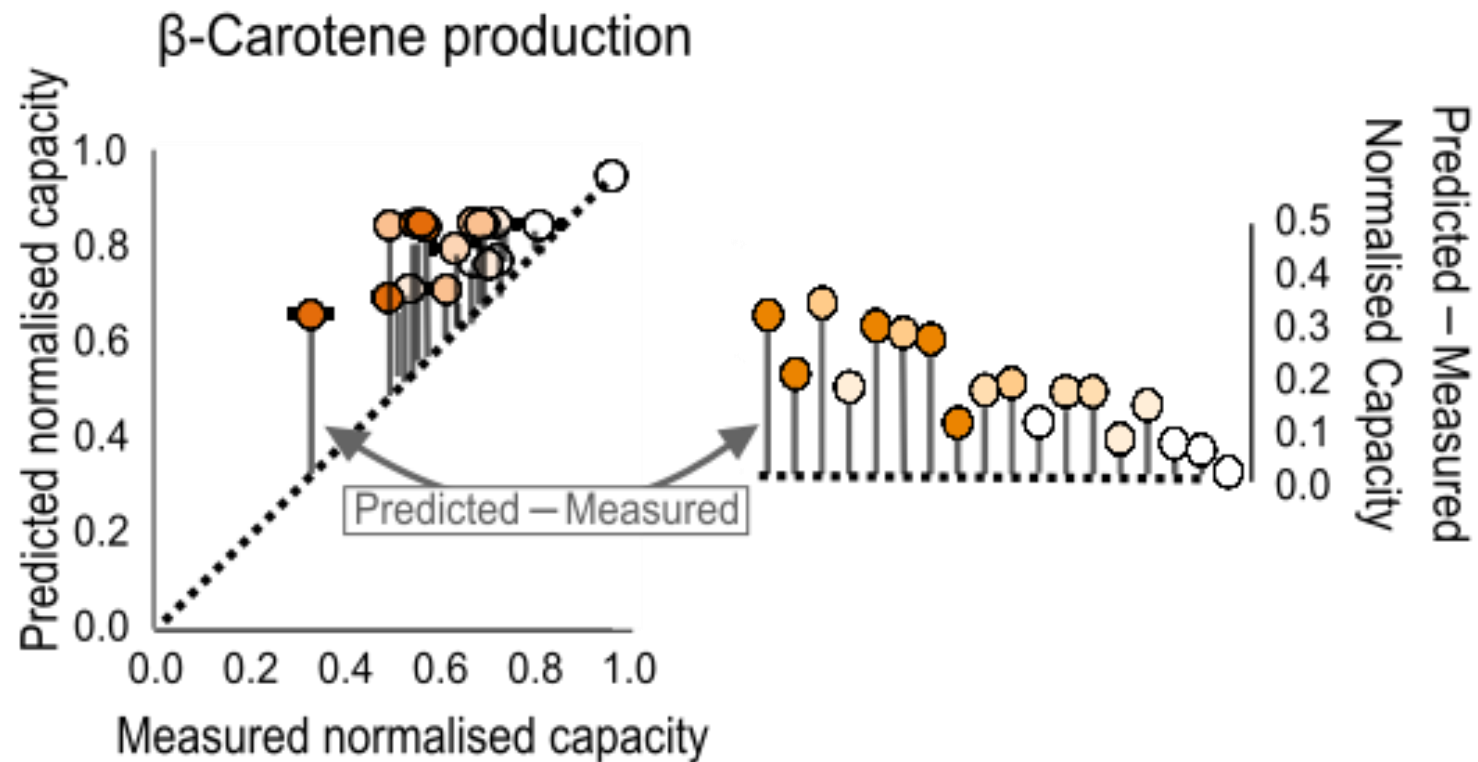


# Cell-free reaction:

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

- Resource competition *in vitro*

Decouple production and metabolic burden

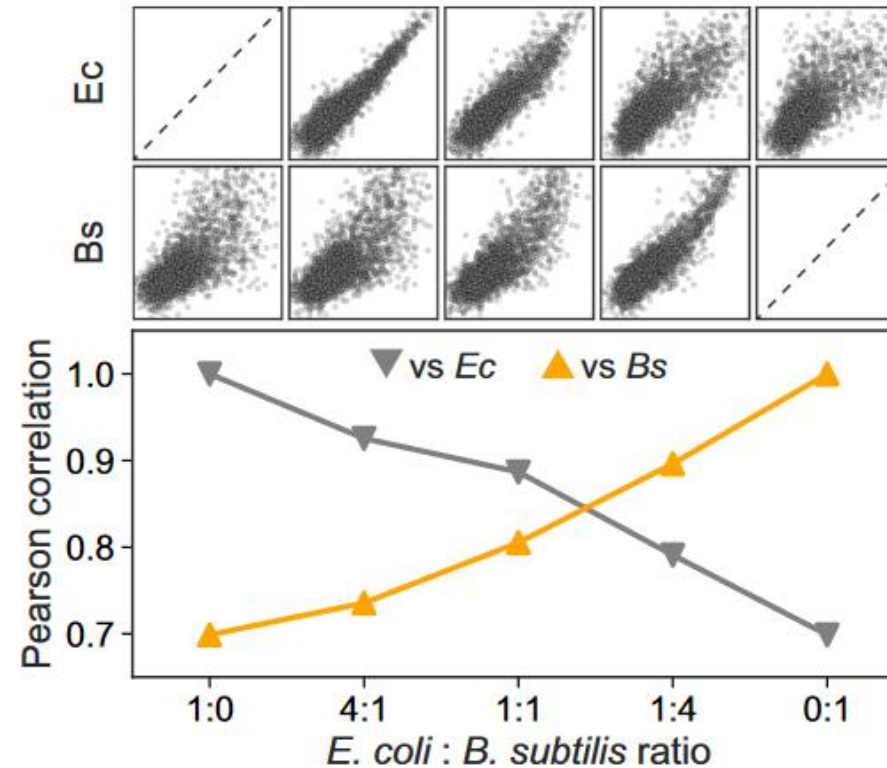
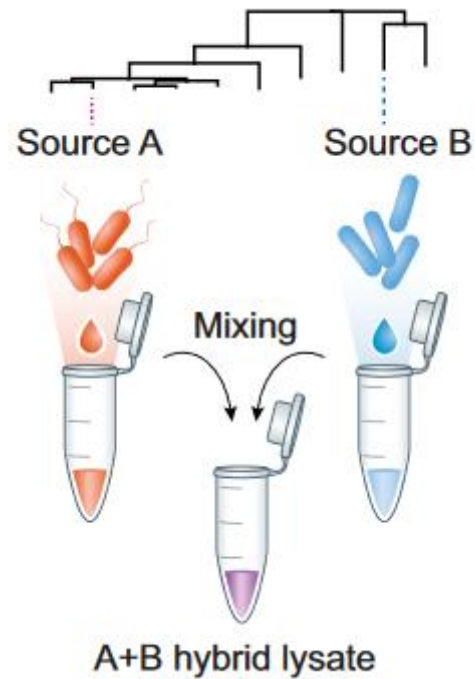




# Cell-free reaction:

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

- Construction hybrid systems
- Multi-species systems



# Cell-free reaction:

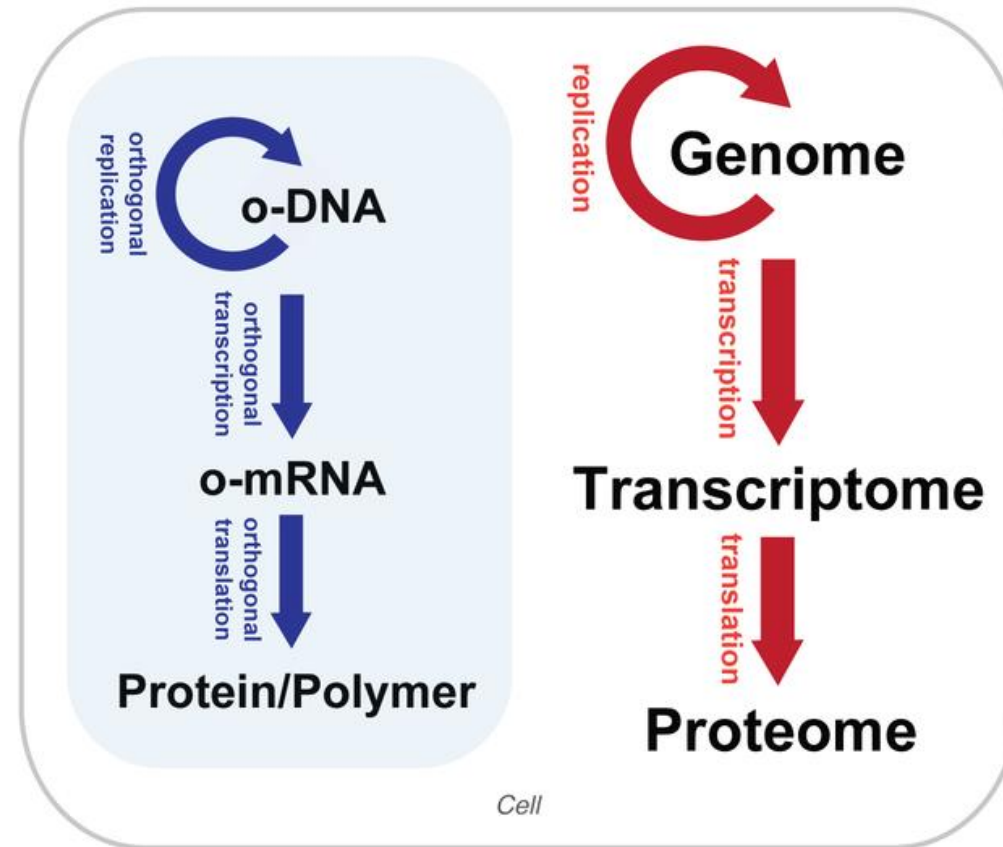
## Fundamental science: cell-free to understand living cells

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- Construction hybrid systems

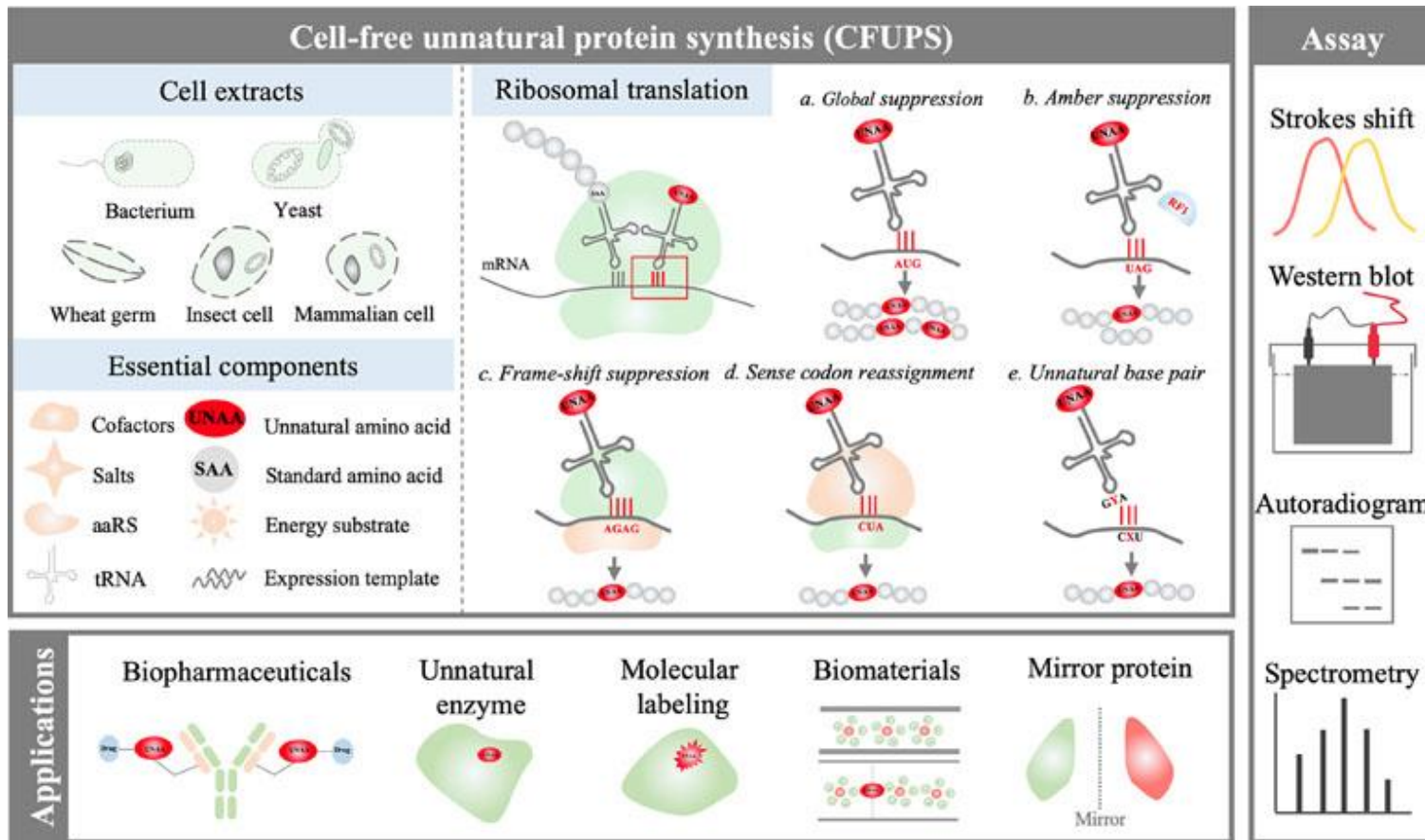
Nonstandard (unnatural) amino acids, more orthogonality for a better predictability



# Cell-free reaction:

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

- Construction hybrid systems
- Nonstandard (unnatural) amino acids

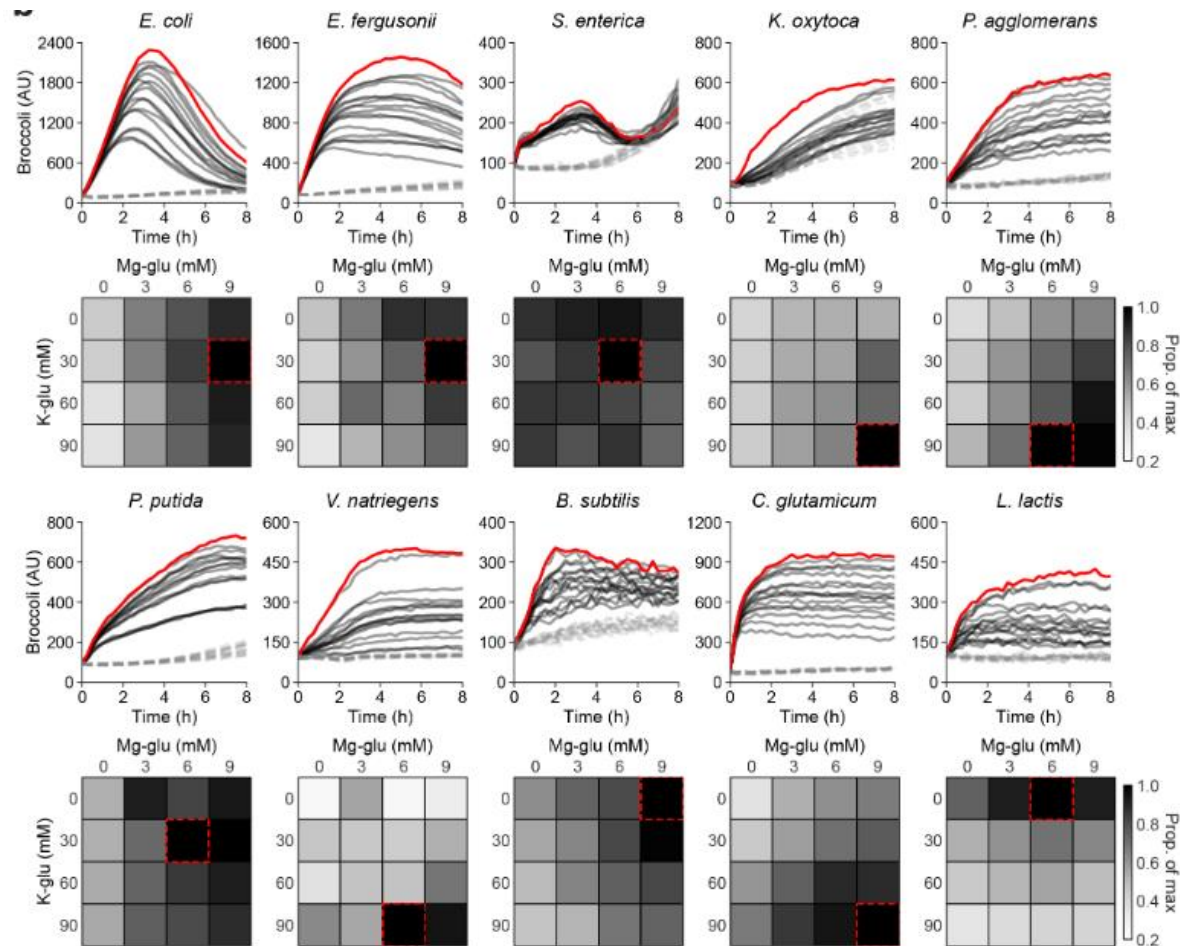


# Cell-free reaction:

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

- Construction hybrid systems

Multi-species systems

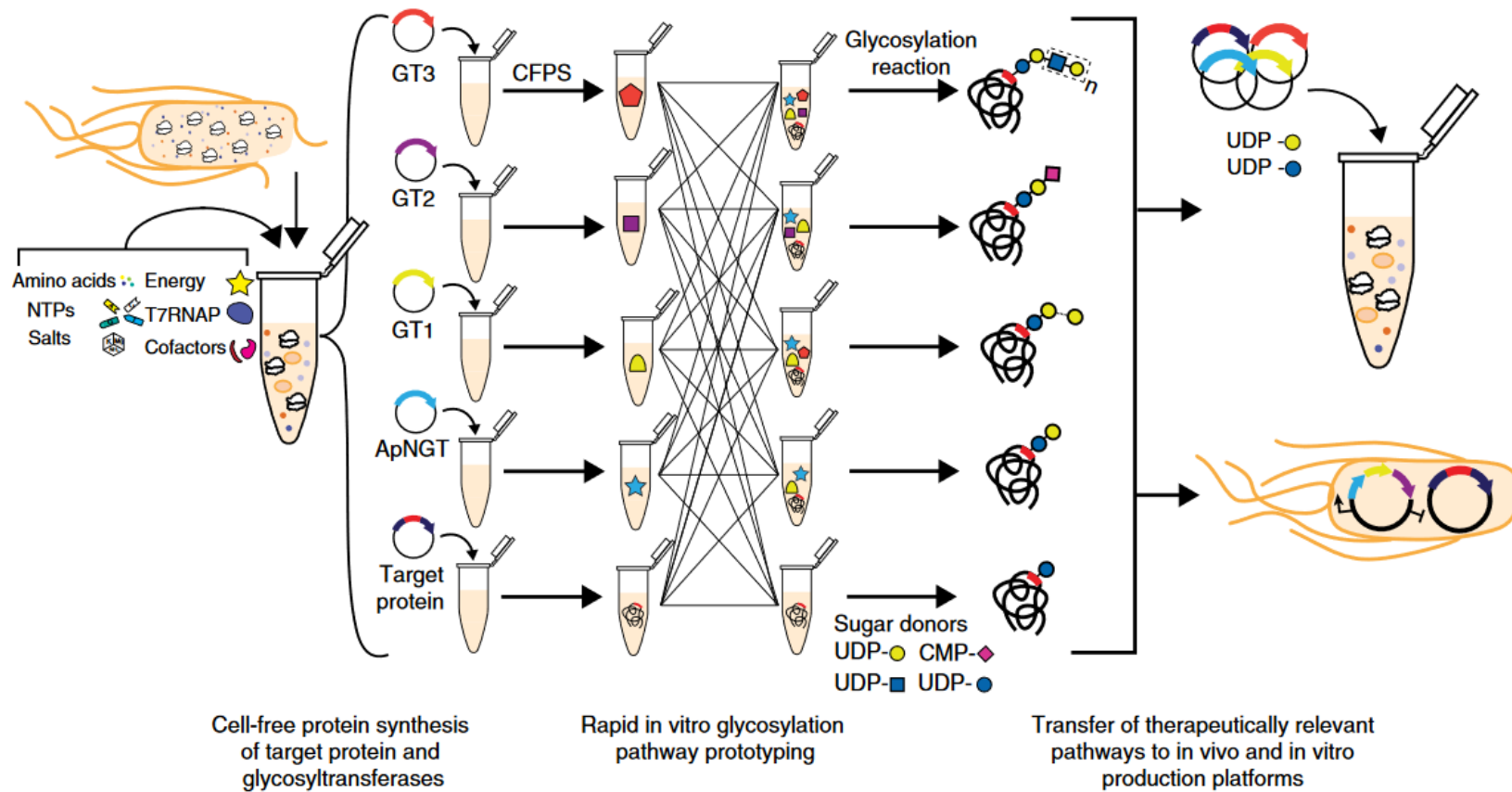


# Cell-free reaction:

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

- Construction hybrid systems

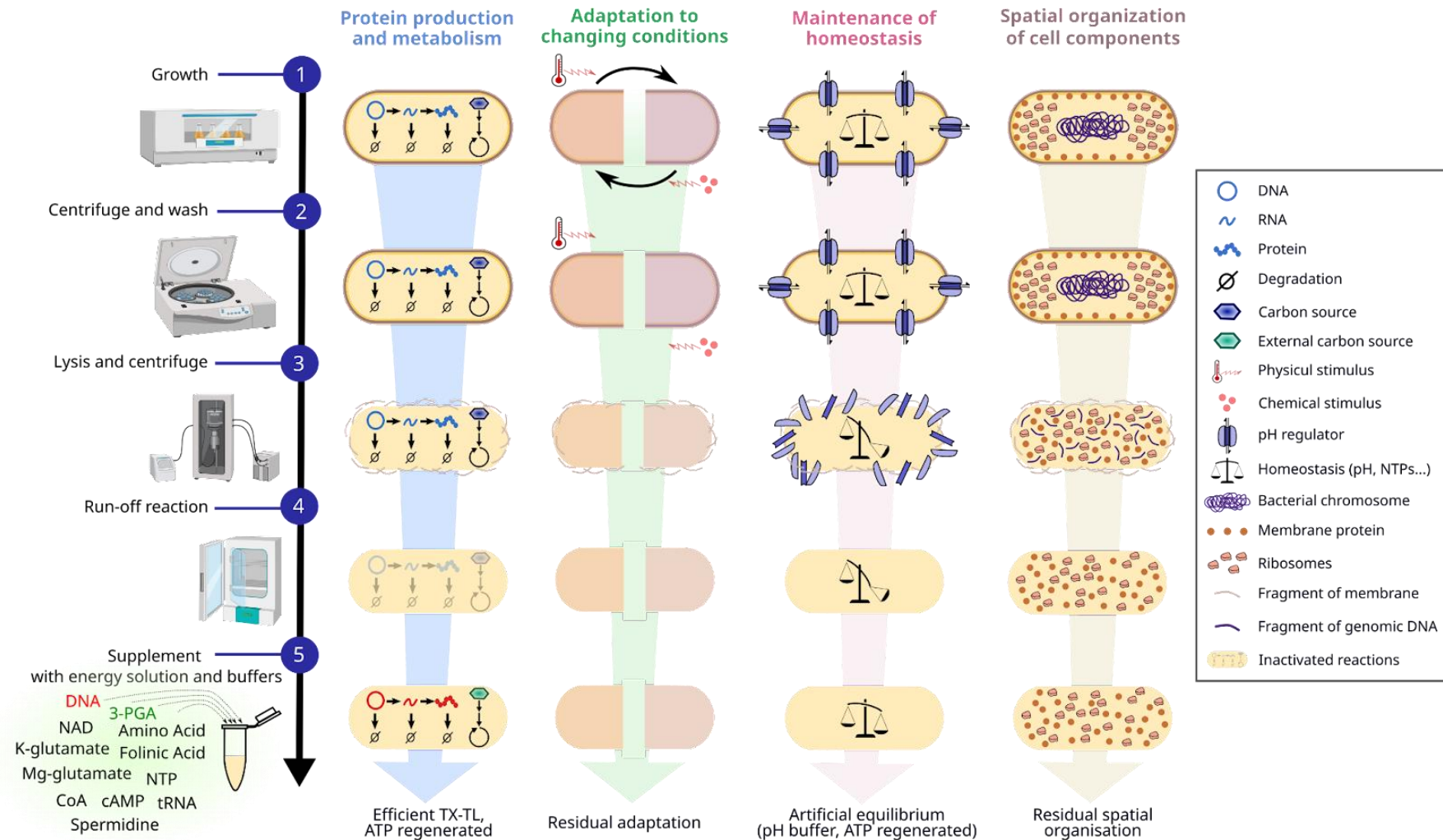
Eukaryote properties (Glycolysation)



# Cell-free reaction:

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

### - Constructing artificial cells



# Cell-free reaction:

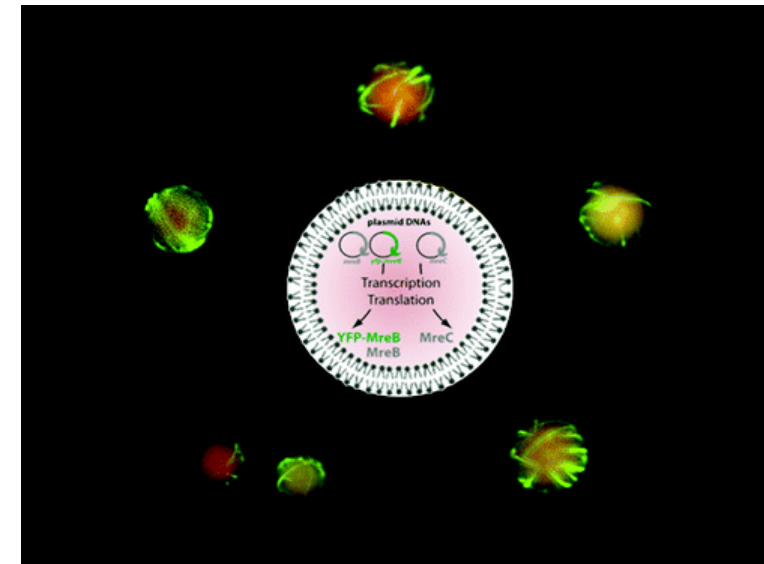
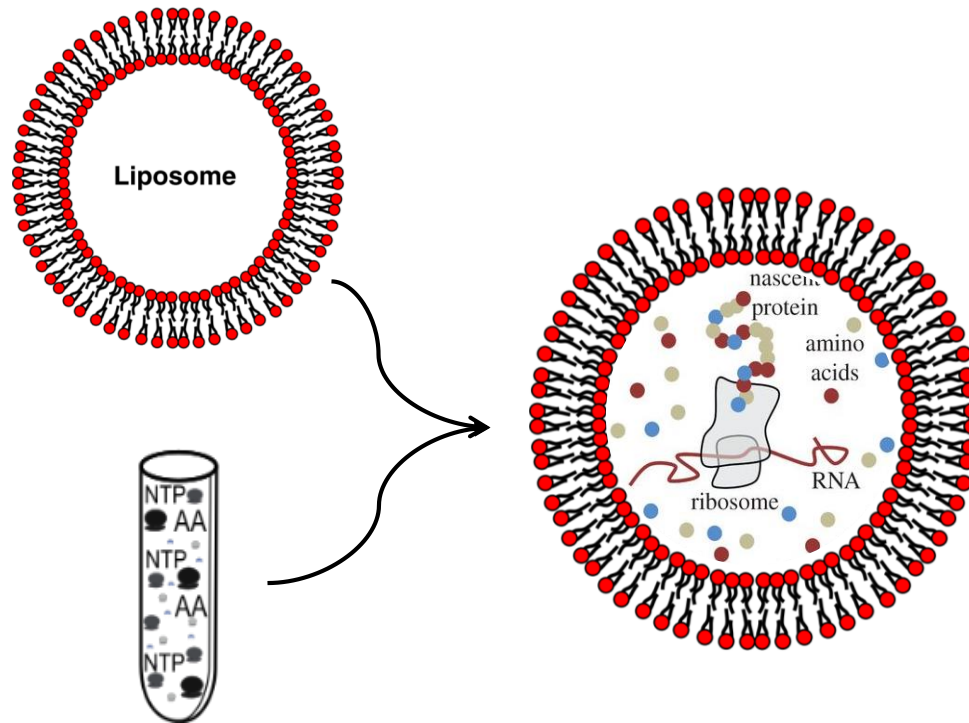
## Fundamental science: cell-free to understand living cells

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- Constructing artificial cells

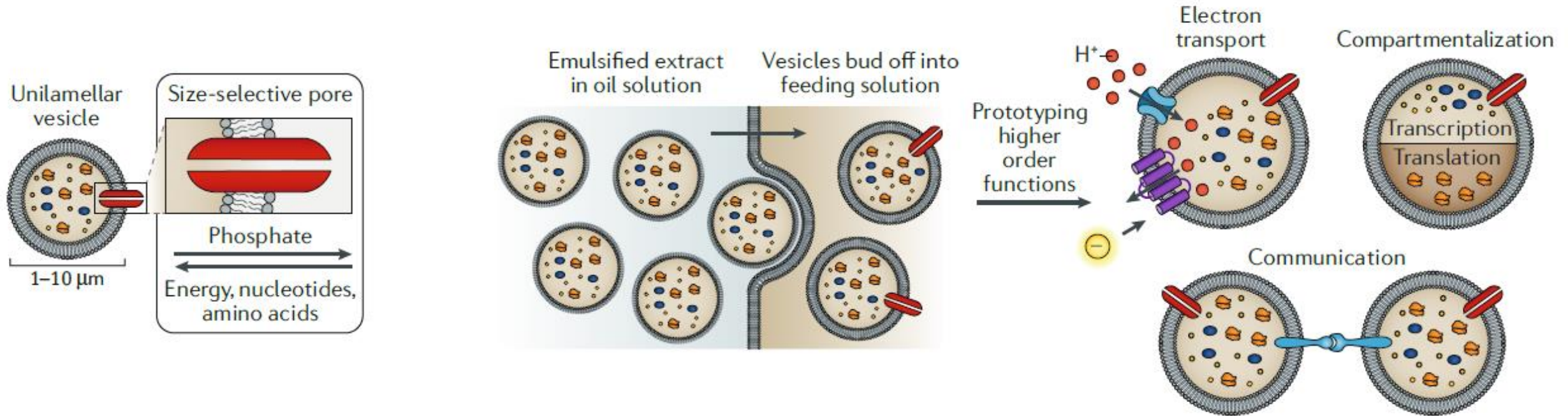
### Liposomes



# Cell-free reaction:

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

- Constructing artificial cells: Communication / exchange with the environment

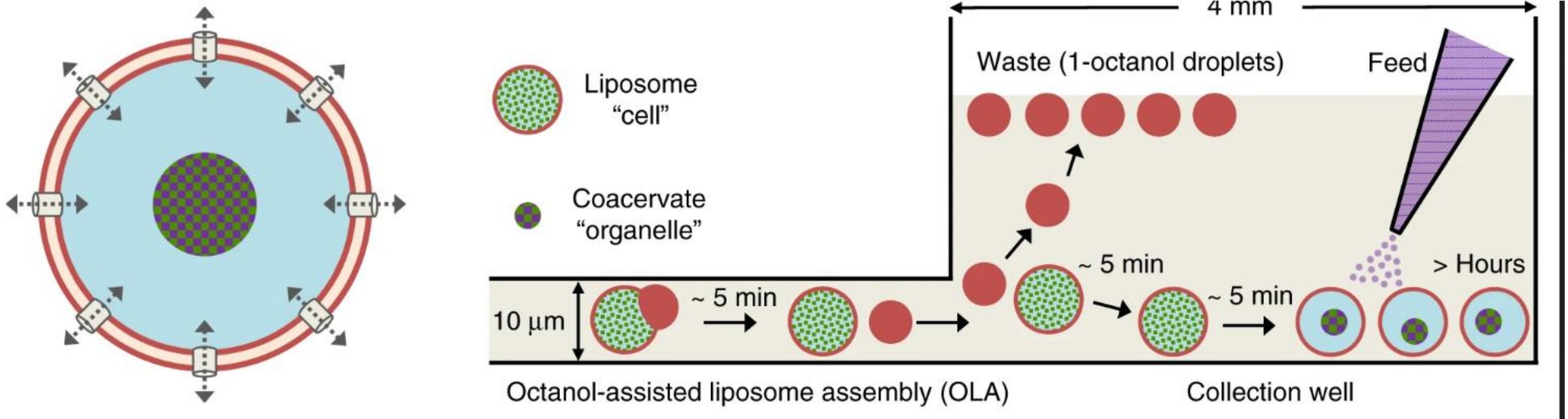




# Cell-free reaction:

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

- Constructing artificial cells : compartmentation



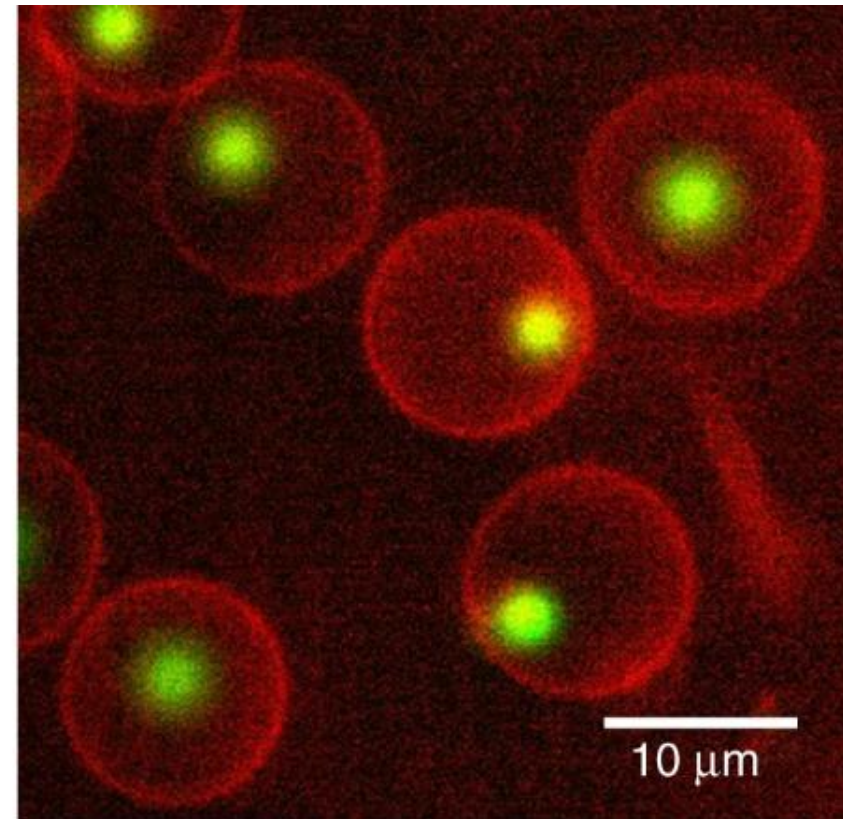
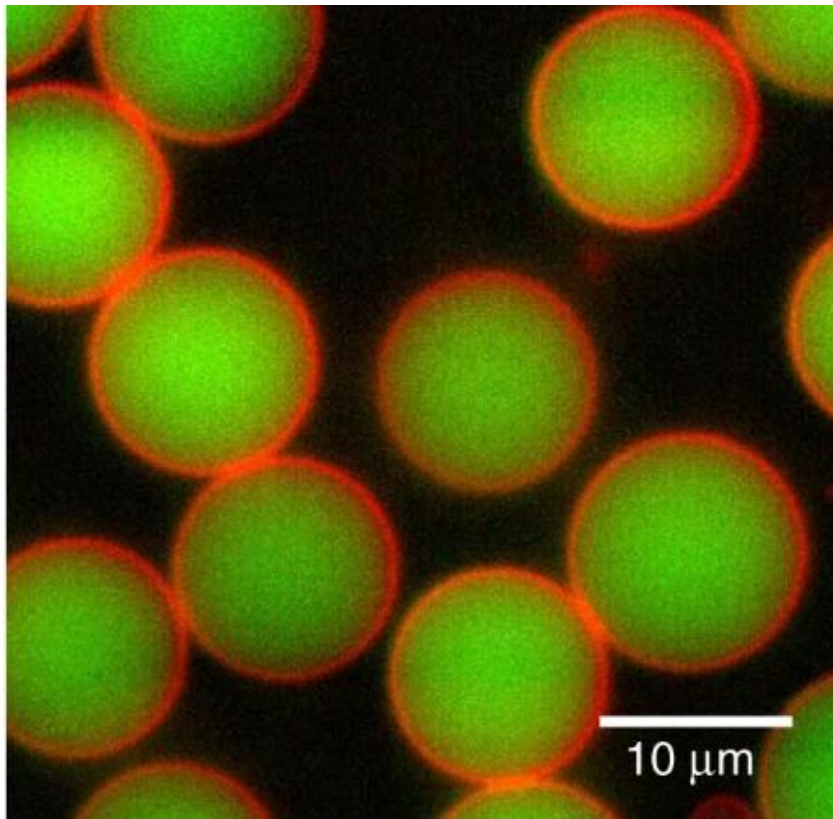
# Cell-free reaction:

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

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- Constructing artificial cells : compartmentation



# Cell-free reaction:

## Current uses of cell-free system

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### **Applied Science: Bridging Theory to Practice**

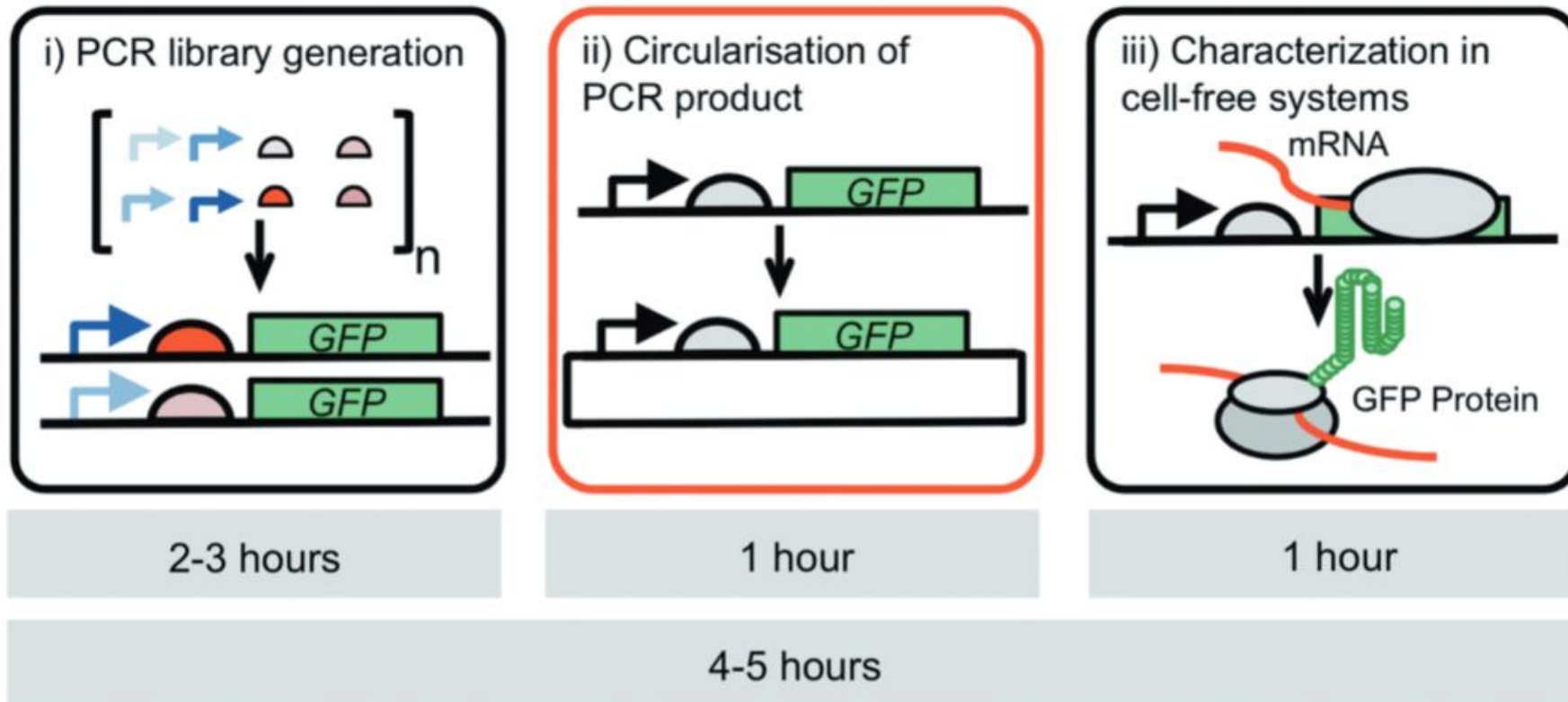
- Prototyping genetic circuits and pathways
  - Genetic parts
  - Metabolic engineering
- Developing biosensors
  - Optimize cell-free biosensor
  - Biocomputing
  - Materials and cell-free
  - Medical diagnostics
  - Pollution diagnostics
- Bio-machine assembly
  - Phages selection
  - Antibodies

# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

- Prototyping genetic circuits and pathways

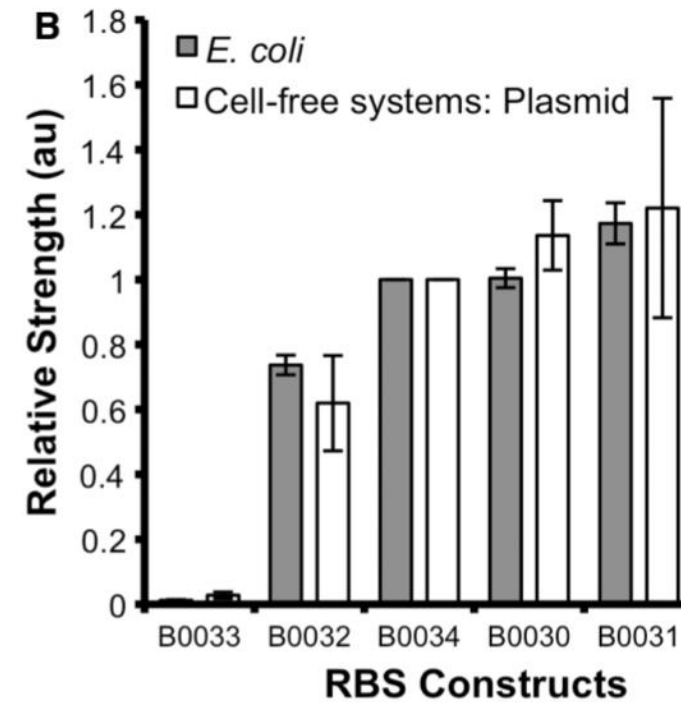
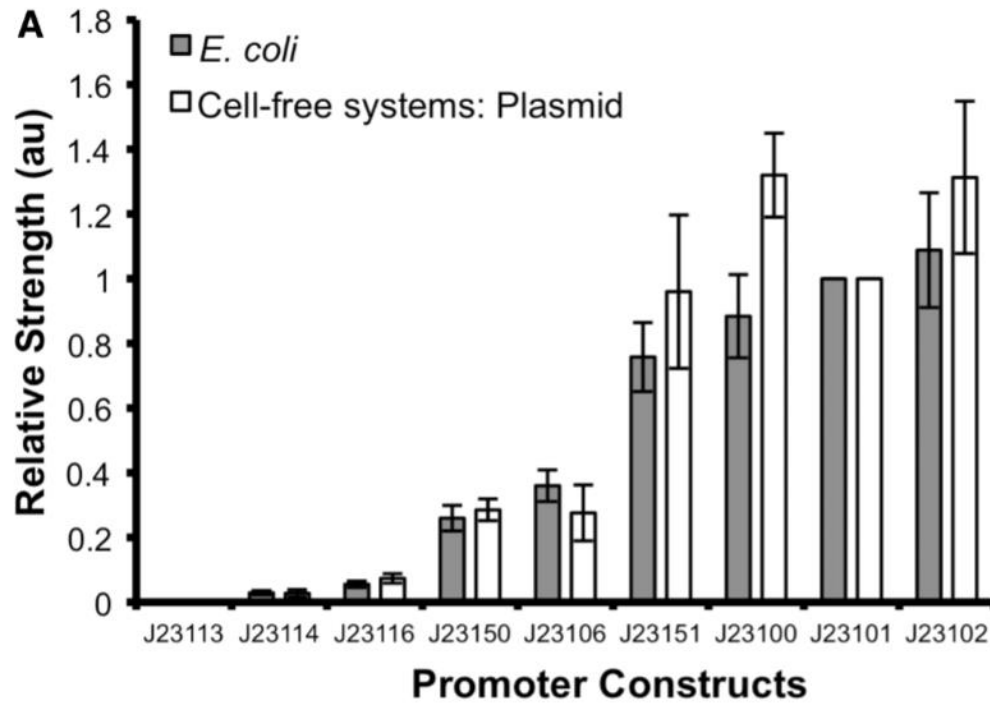
Genetic parts



# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

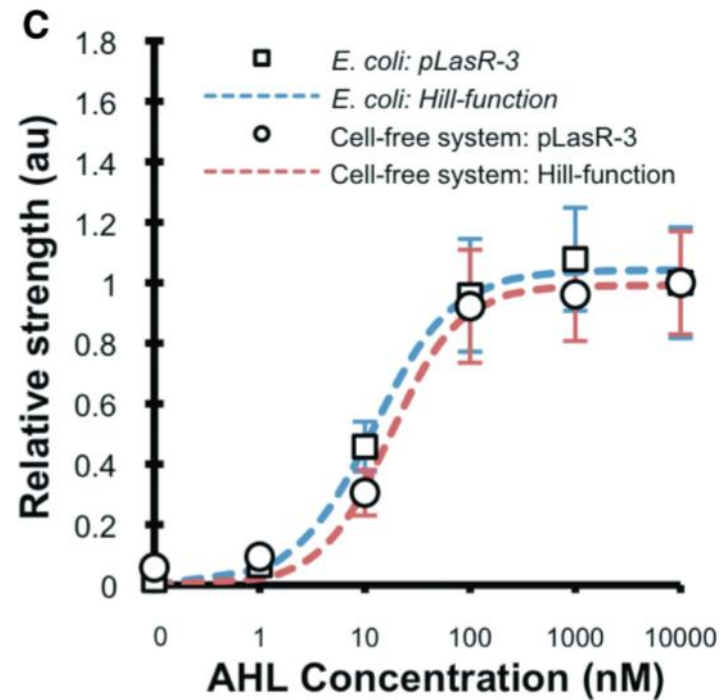
- Prototyping genetic circuits and pathways
- Genetic parts



# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

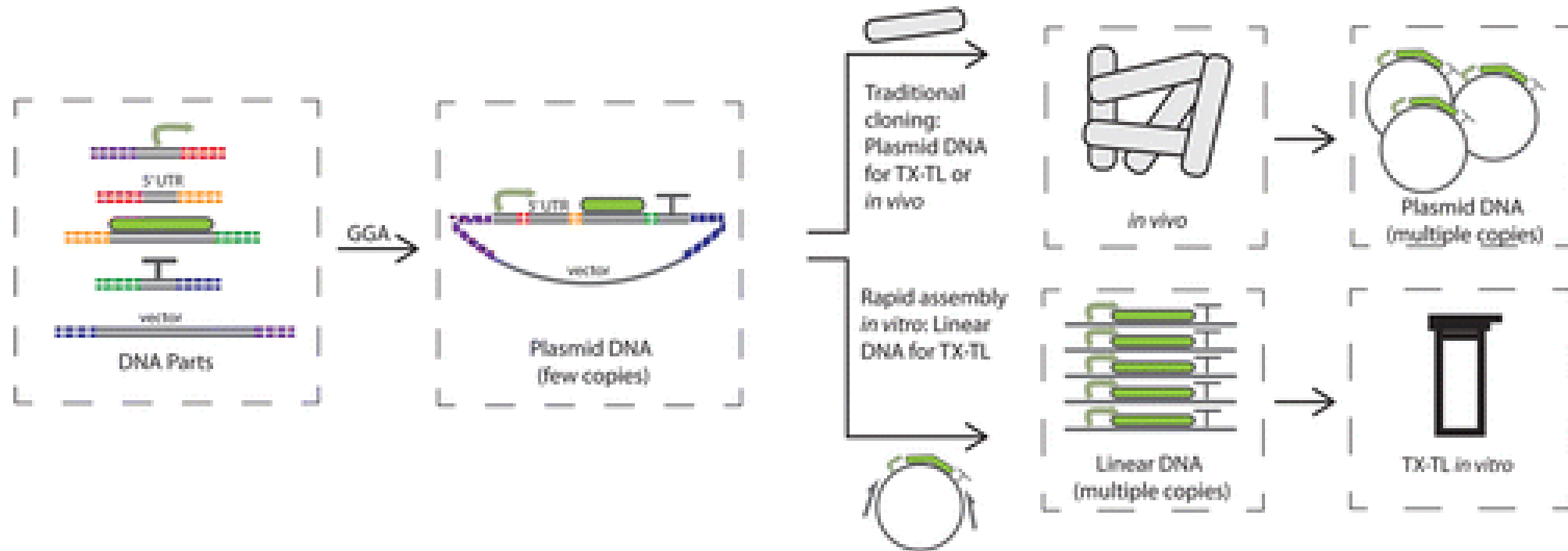
- Prototyping genetic circuits and pathways  
Genetic parts: regulated systems



# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

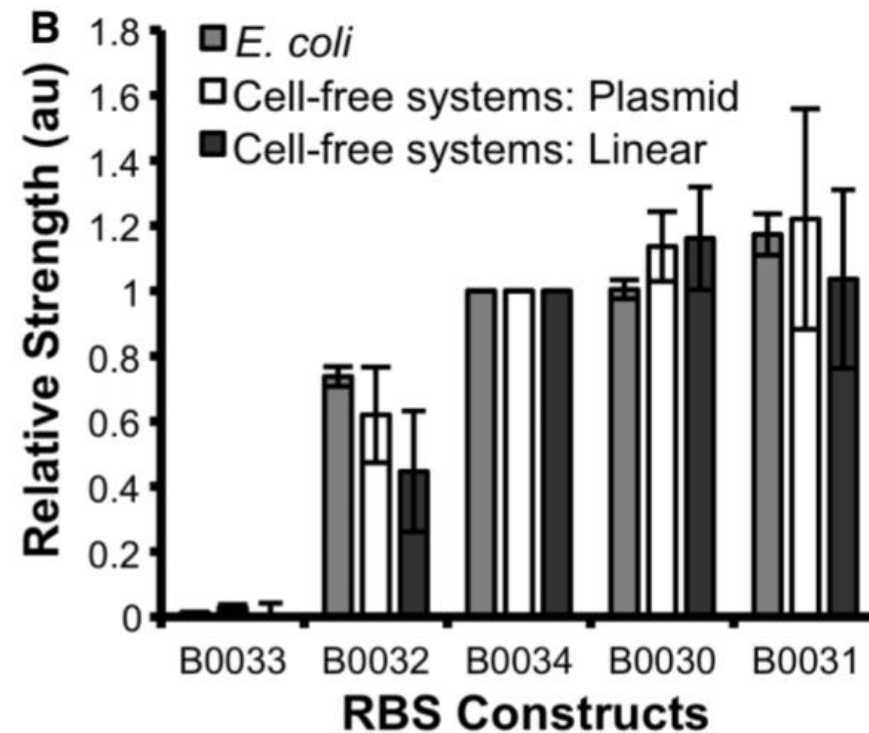
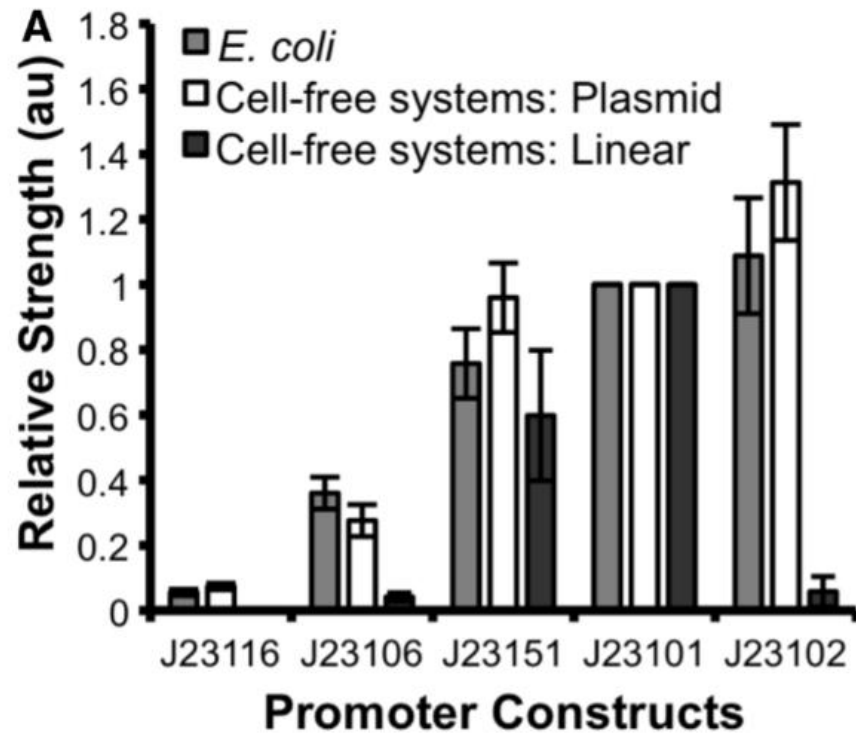
- Prototyping genetic circuits and pathways  
Genetic parts: Linear fragment vs plasmid



# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

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



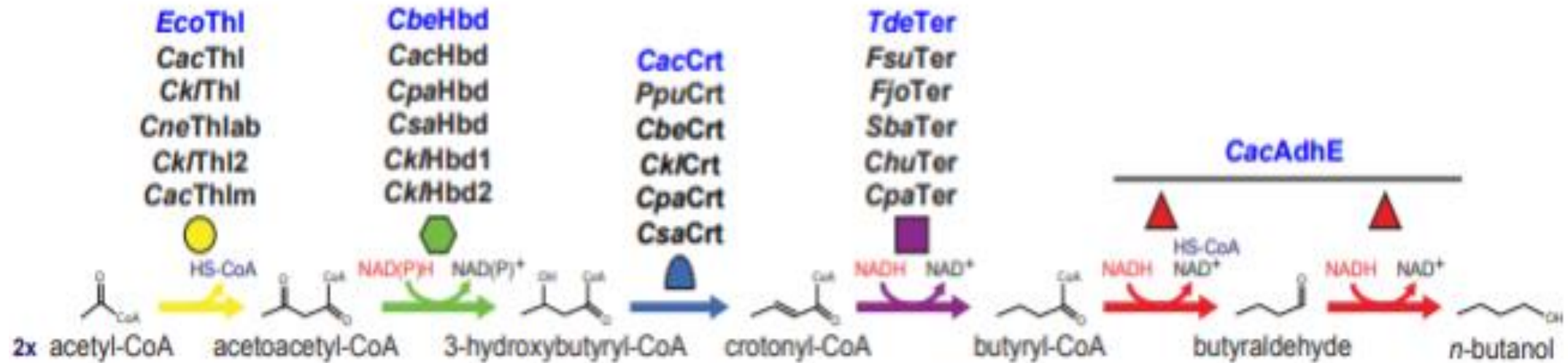


# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

Prototyping genetic circuits and pathways

Metabolic engineering: combinatorial exploration



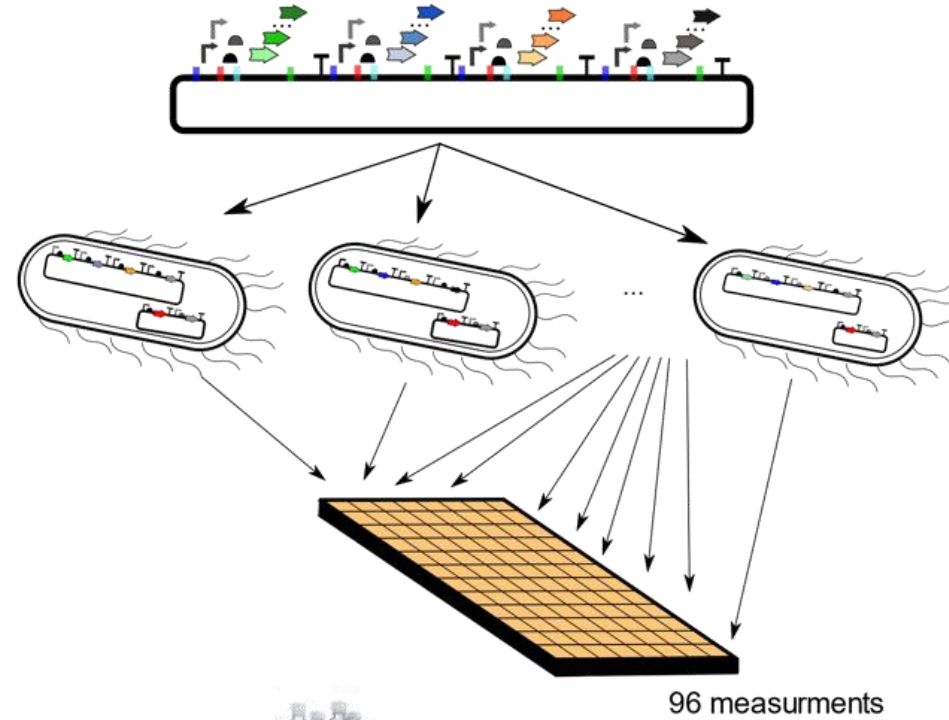
Example of : 6<sup>4</sup> combinations

# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

Metabolic engineering: combinatorial exploration

**6<sup>4</sup> plasmids**

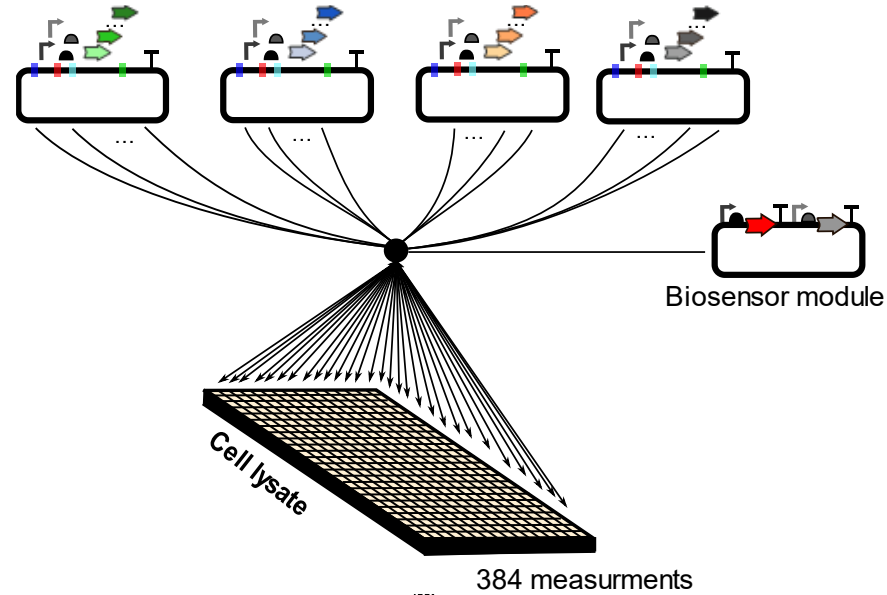


# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

Metabolic engineering: combinatorial exploration

$6 \times 4 = 24$  plasmids

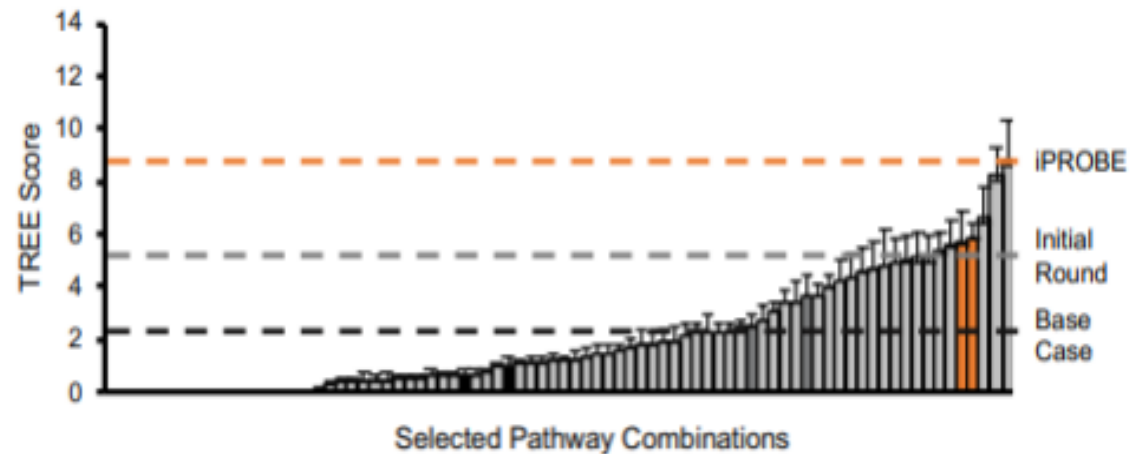
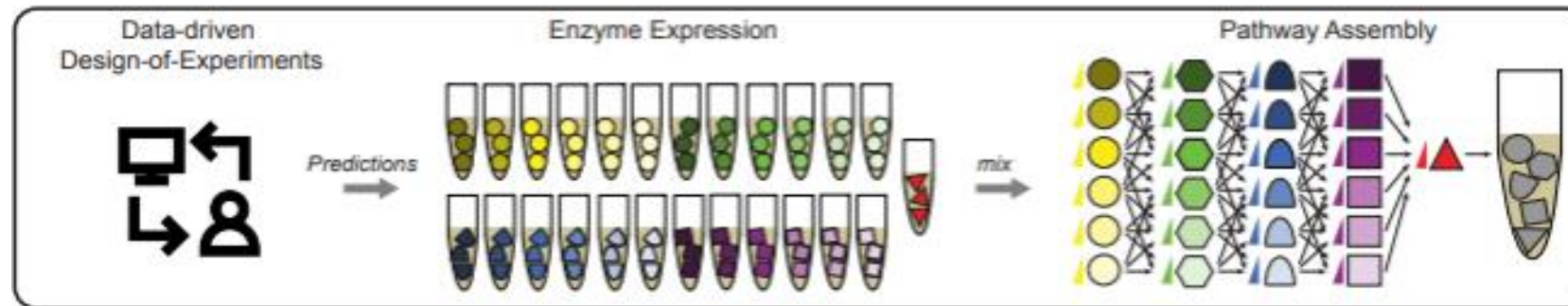


# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

Prototyping genetic circuits and pathways

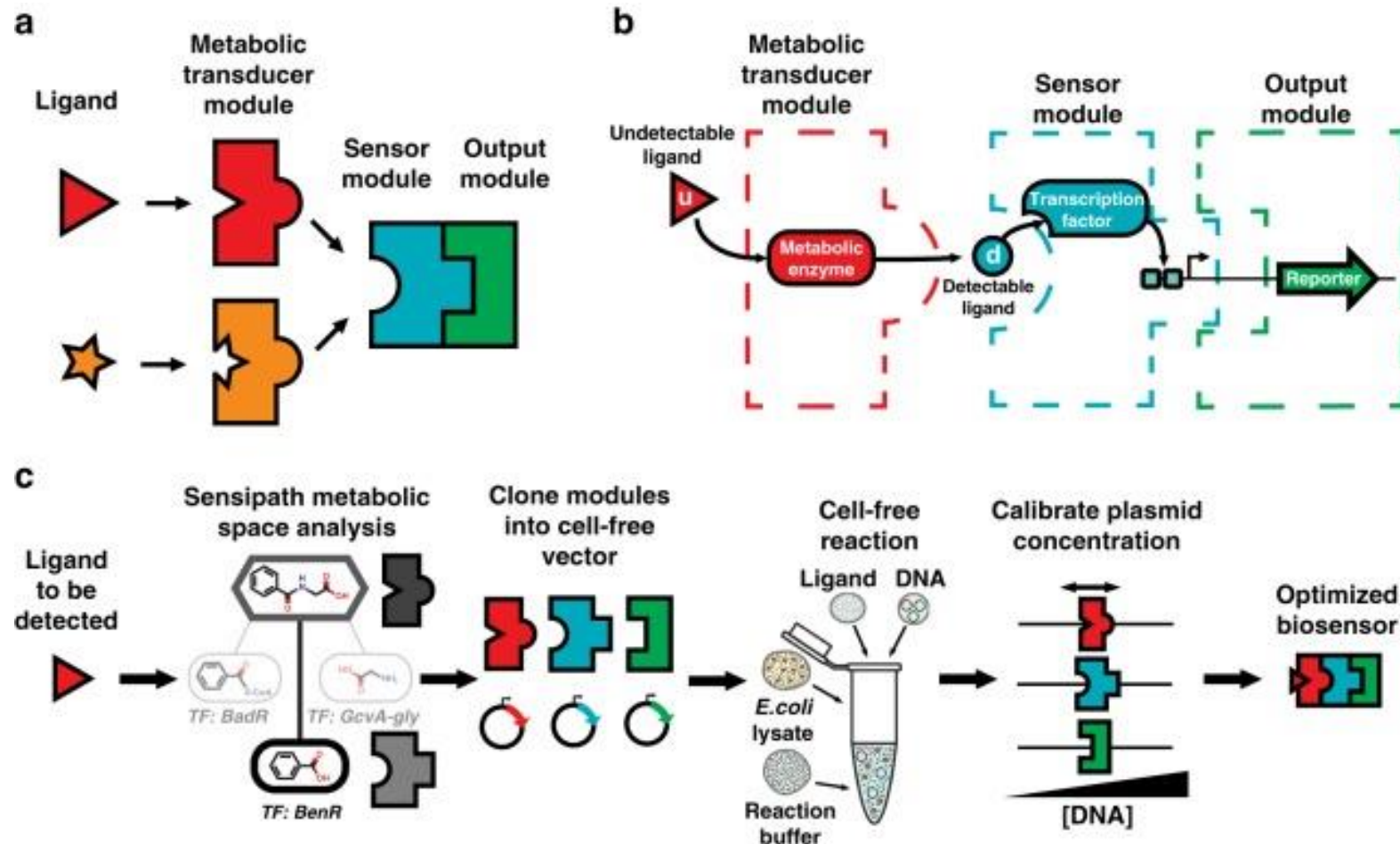
Metabolic engineering: combinatorial exploration



# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

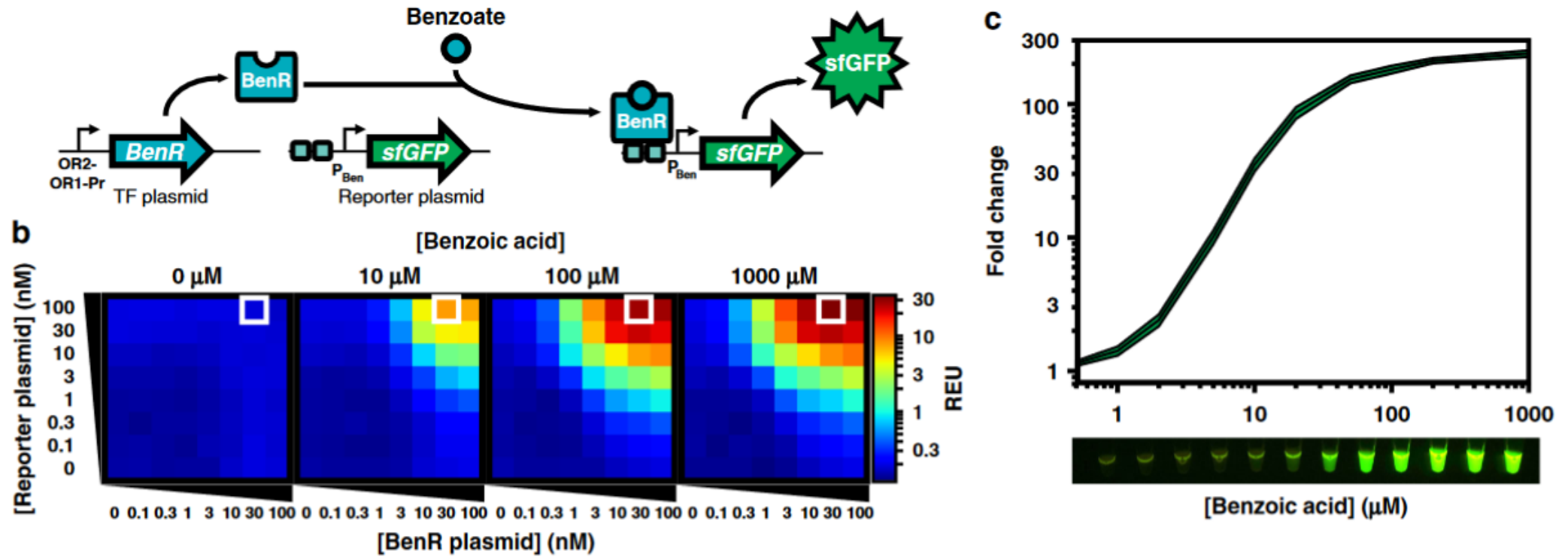
- Developing biosensors



# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

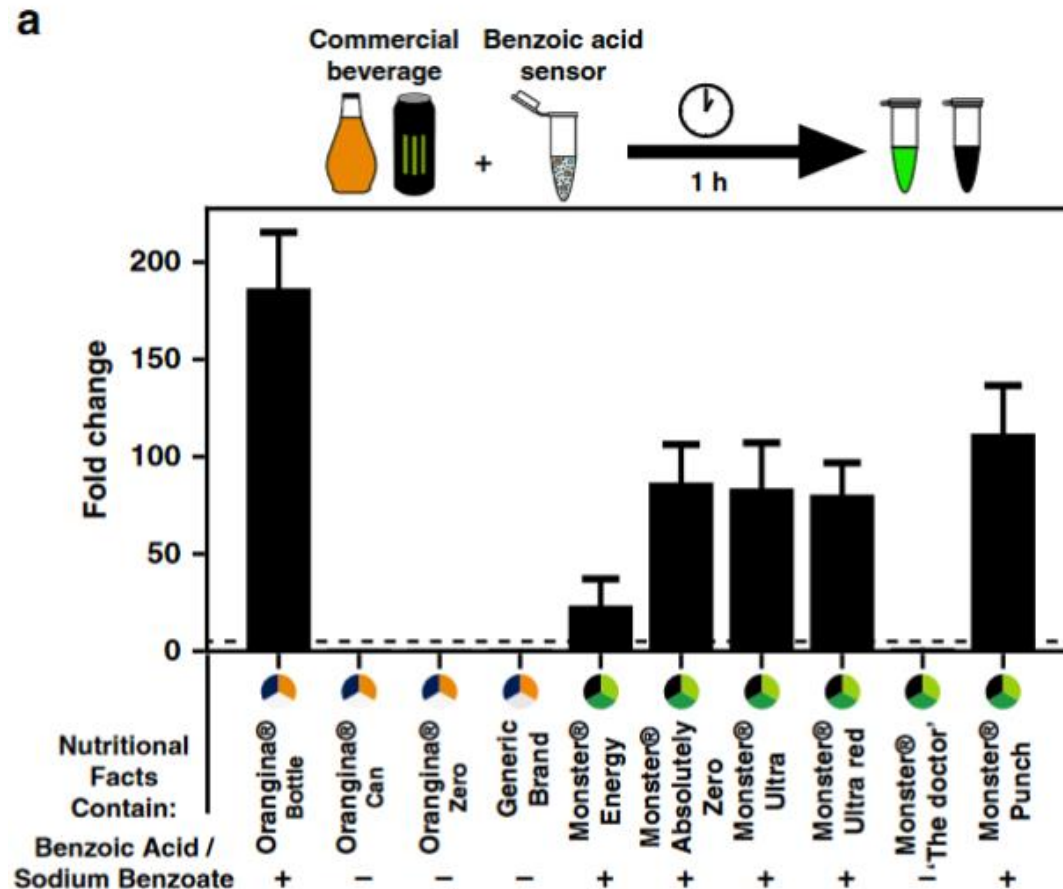
- Developing biosensors



# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

- Developing biosensors



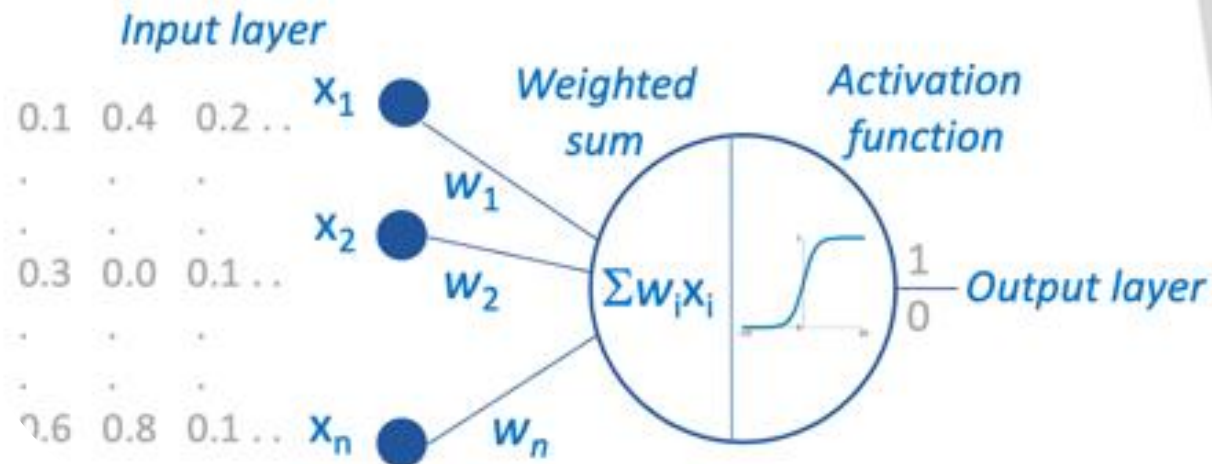
# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

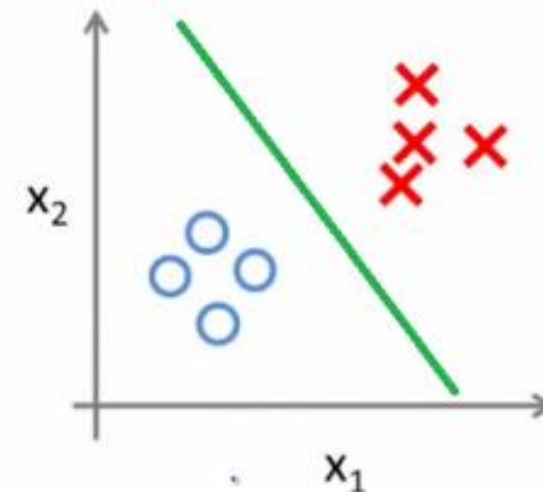
Developing biosensors  
Biocomputing



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



Binary classification:





# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

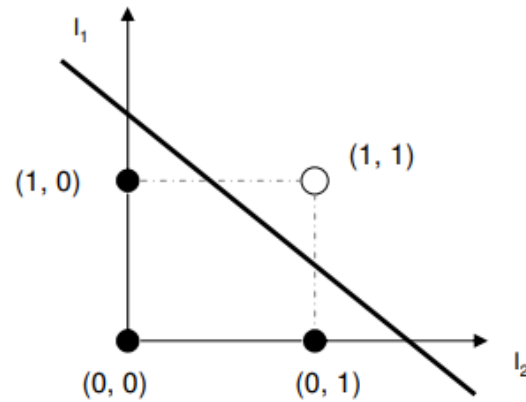
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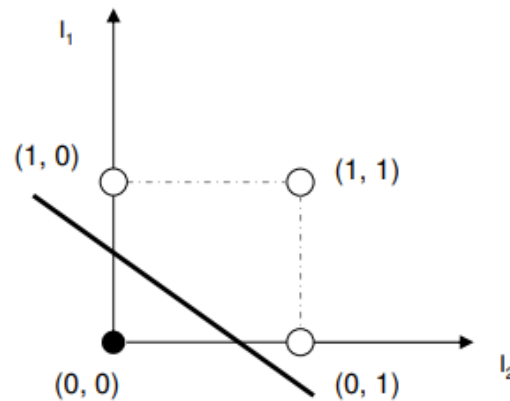
Developing biosensors

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   |

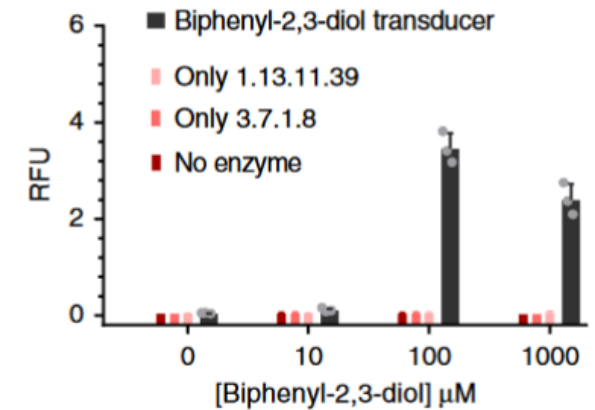
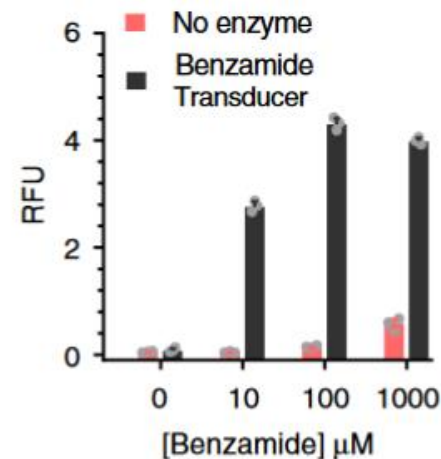
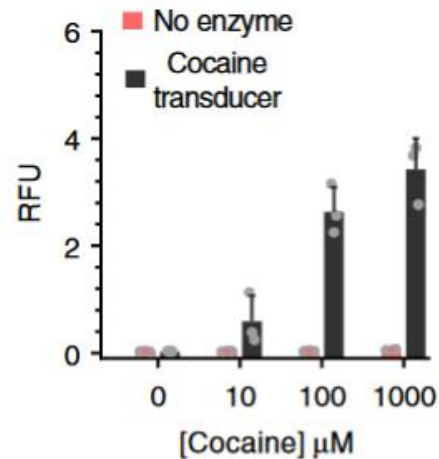
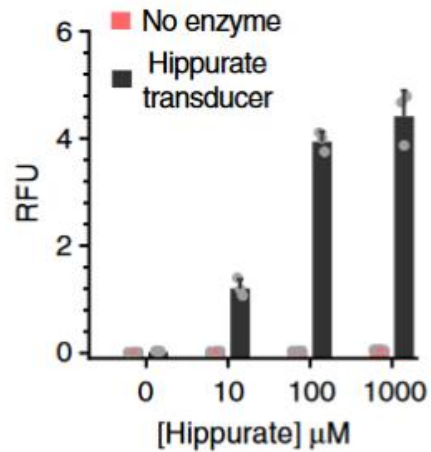
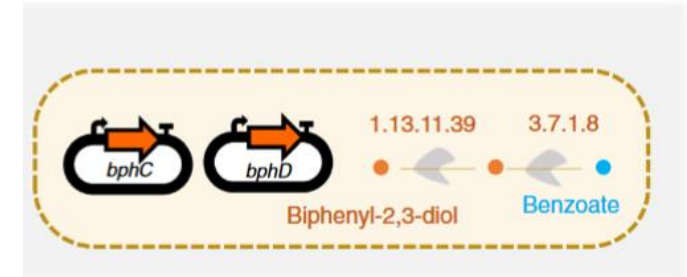
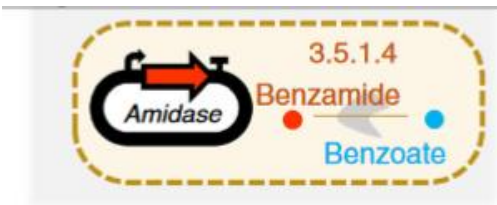
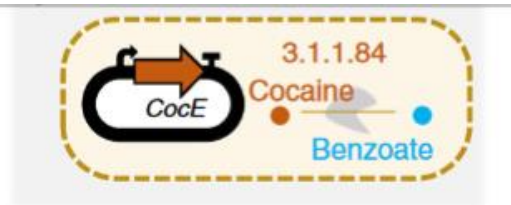
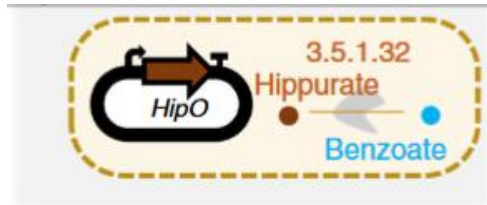


# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

Developing biosensors

Biocomputing: 4 biosensors

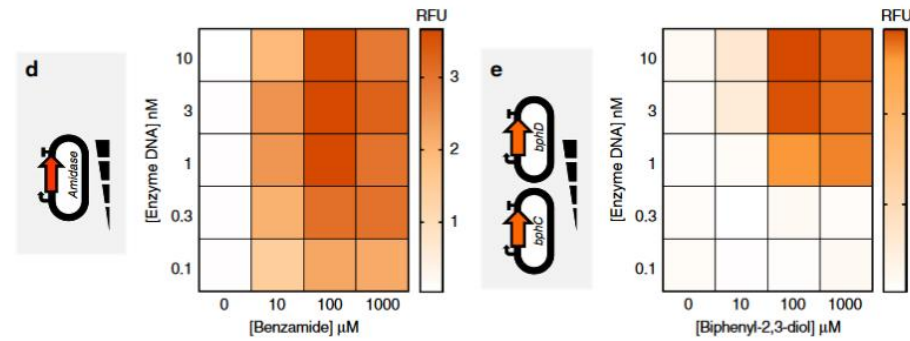
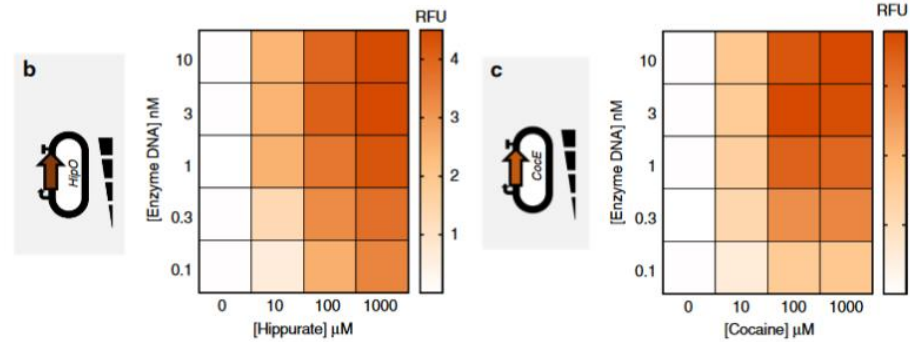
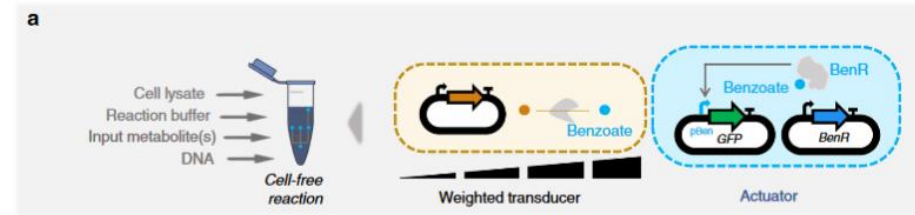


# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

Developing biosensors

Biocomputing: tuning the weights

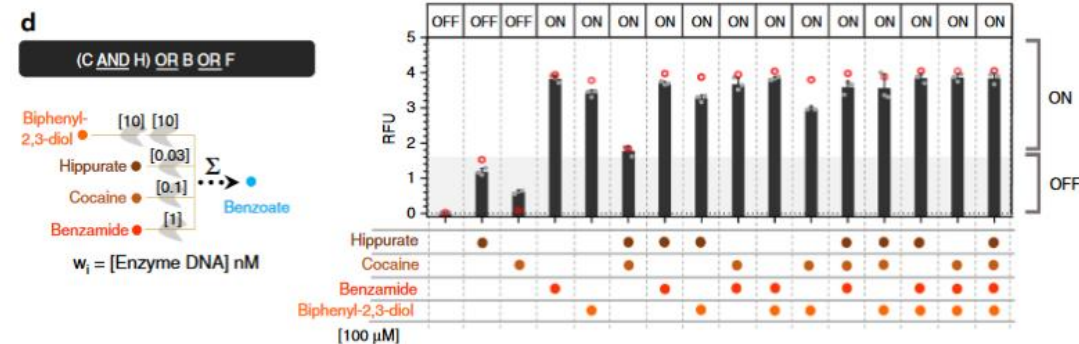
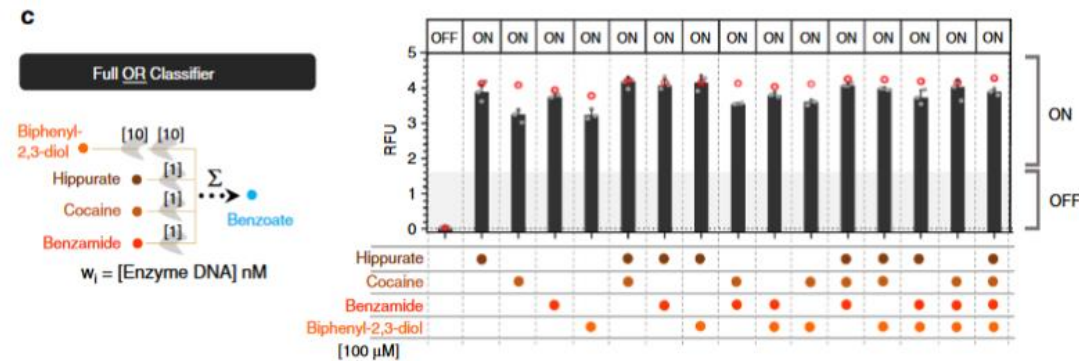
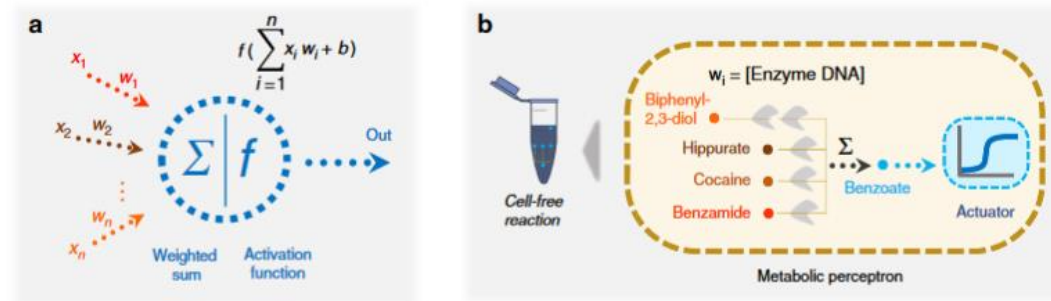


# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

Developing biosensors

Biocomputing: perceptron



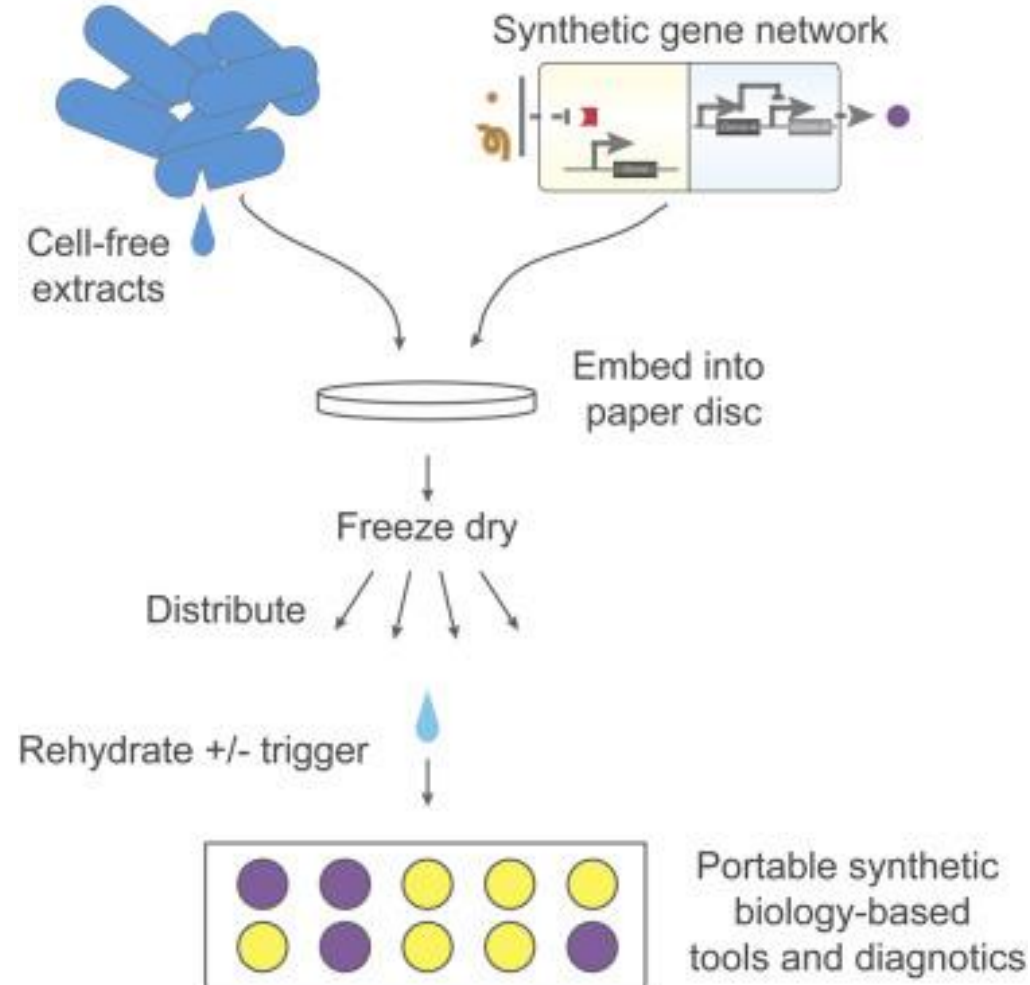
# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

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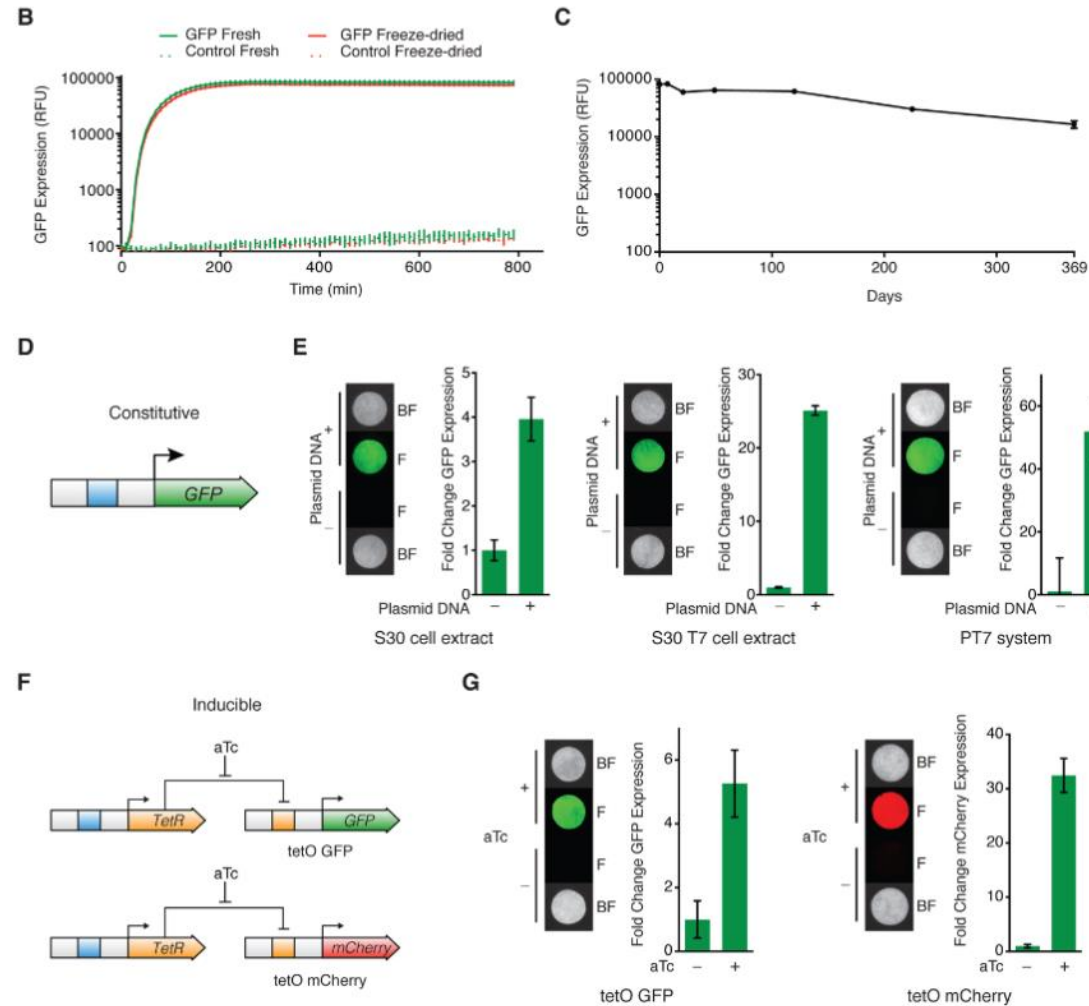
- Developing biosensors  
Medical diagnostics



# 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**

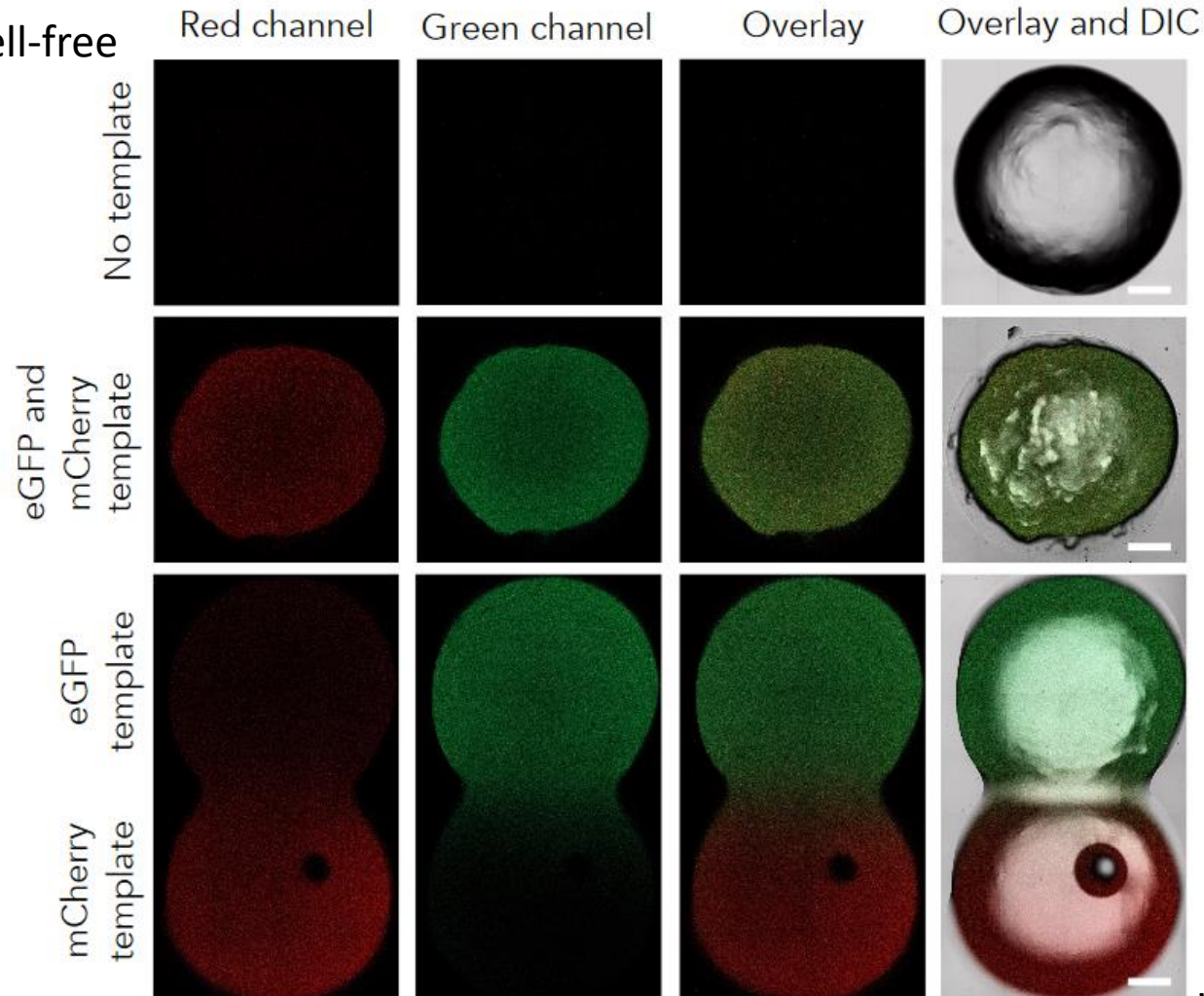
WYSS 3607 | HD | BROAD INSTITUTE | UNIVERSITY OF MICHIGAN

# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

- Developing biosensors

Materials and cell-free

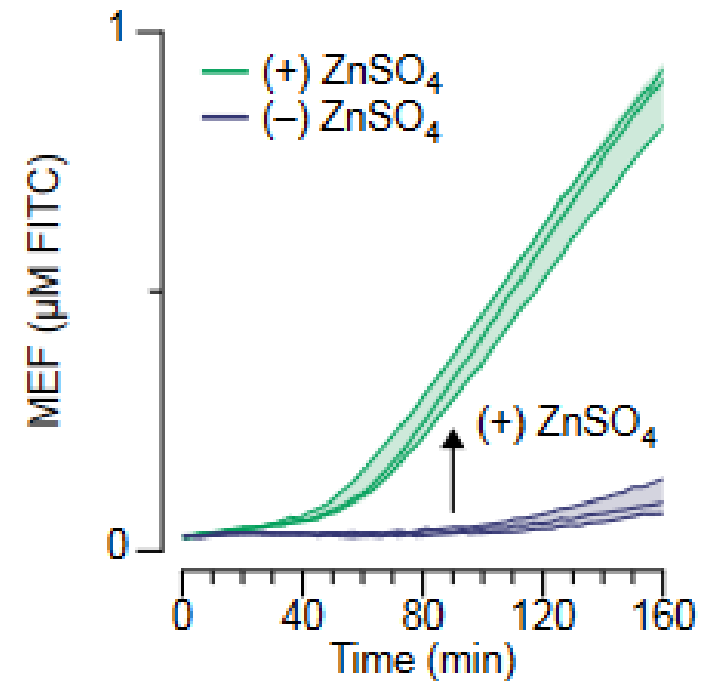
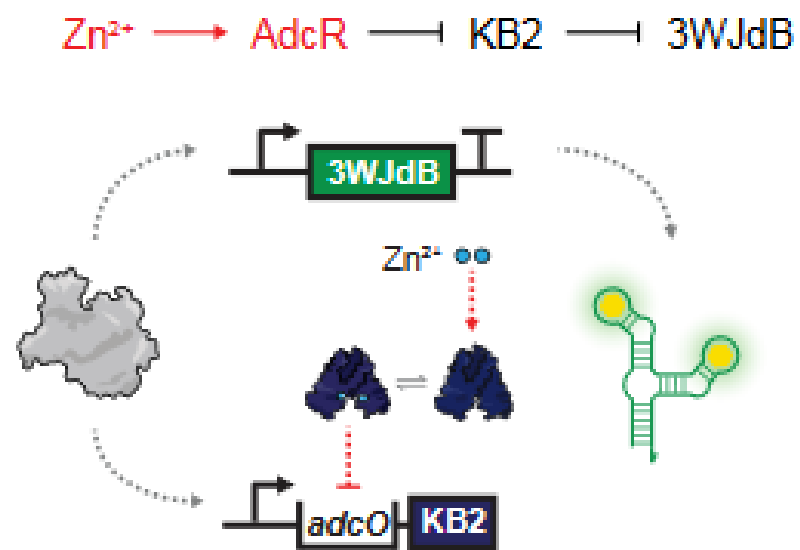




# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

- Developing biosensors
- Pollution detection

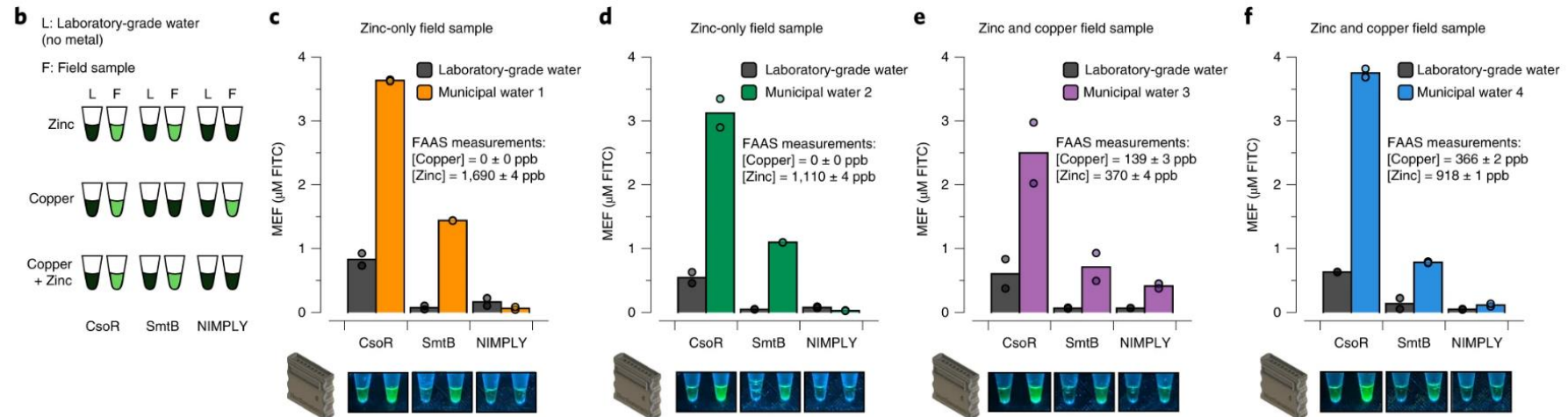
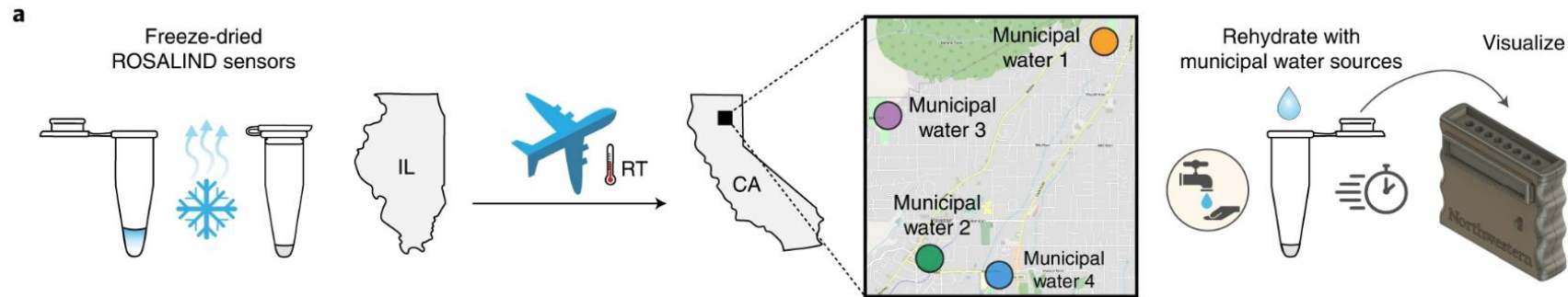


# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

- Developing biosensors

Pollution detection

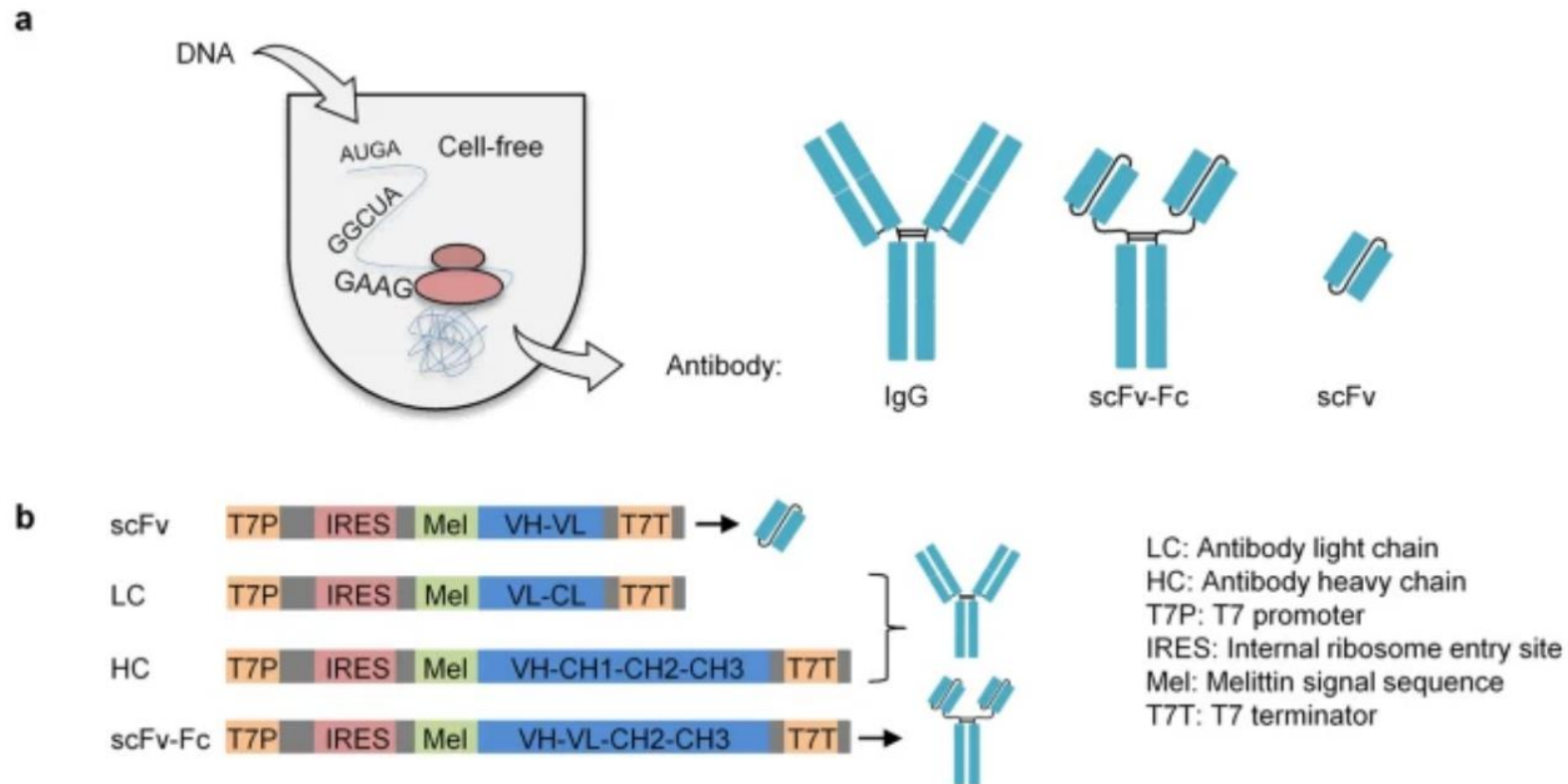


# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

- Bio-machine assembly

Antibodies (eukaryotic cell-free)



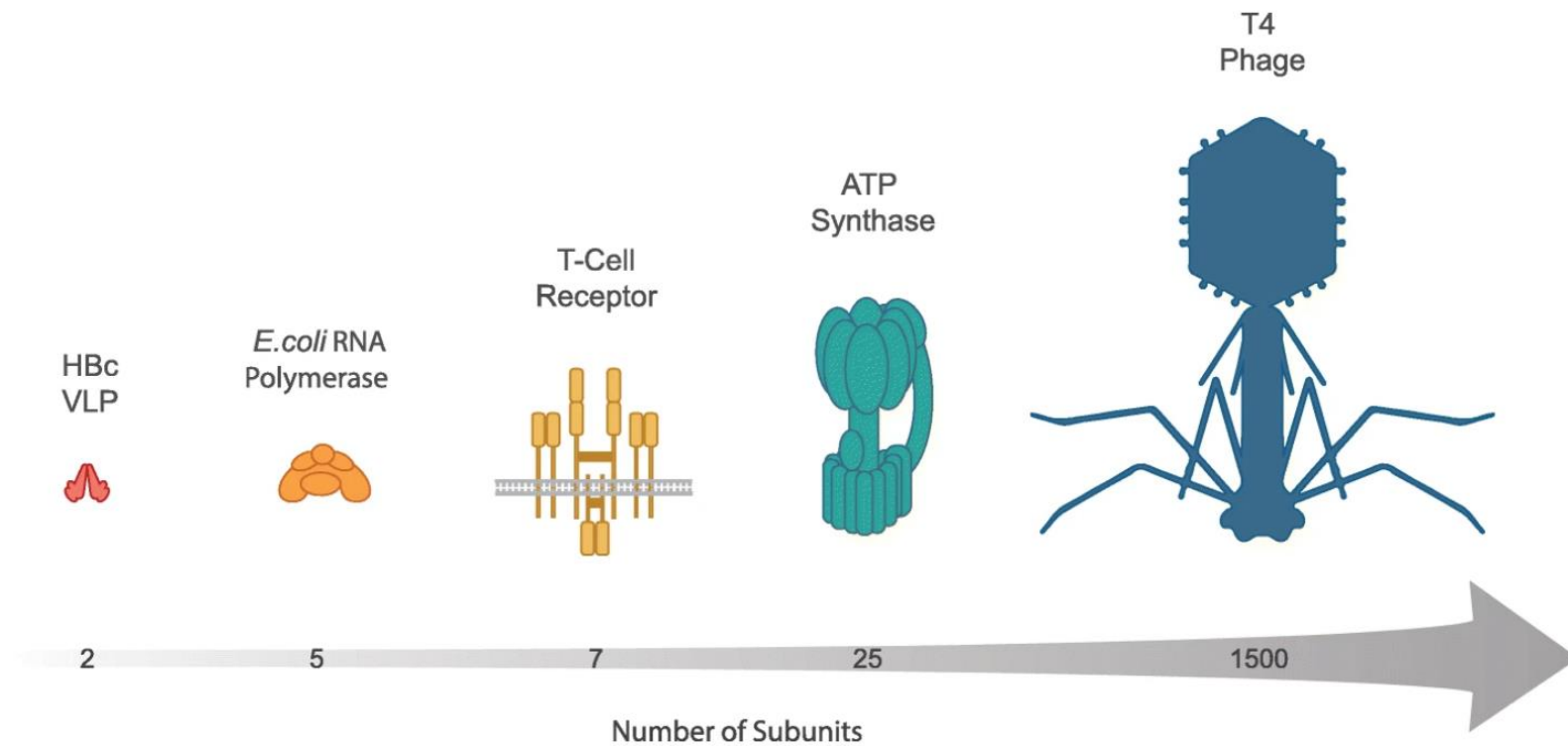
# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

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- Bio-machine assembly
- Phages selection



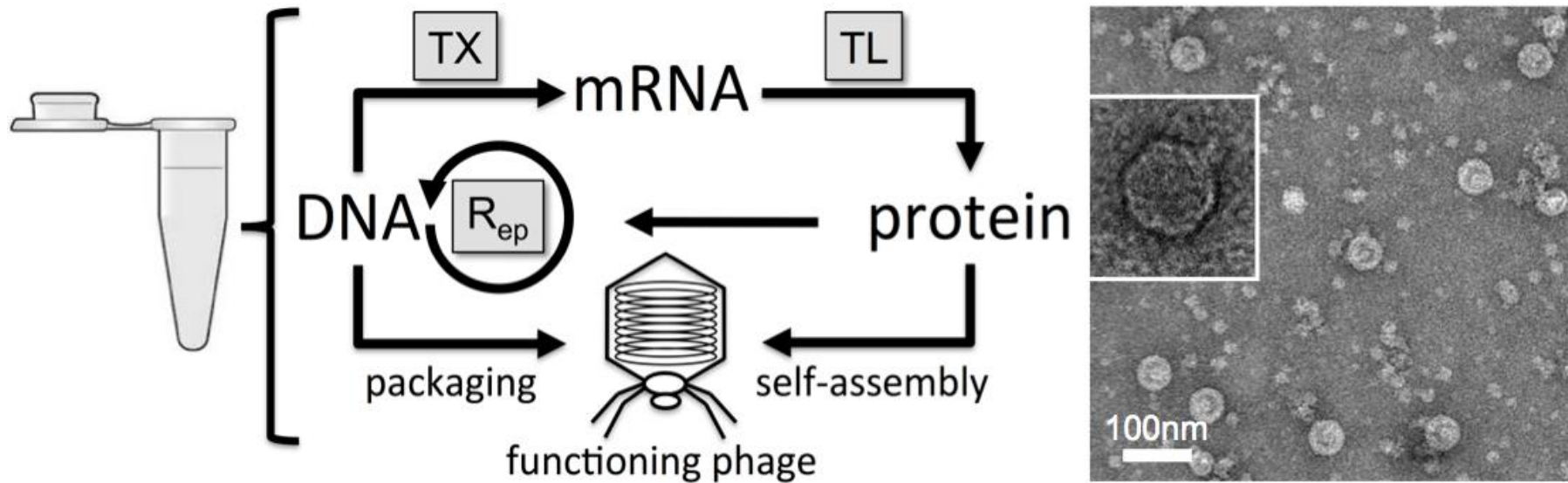
# Cell-free reaction:

## Applied Science: Bridging Theory to Practice

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- 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

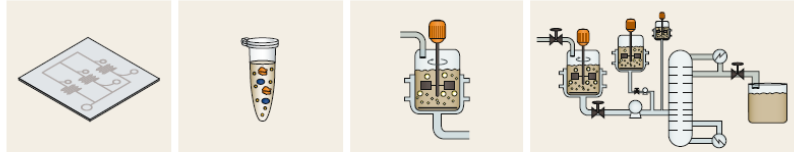
- Purified proteins
  - Tierra bioscience (California, USA)
  - CFS (Japan)
  - Cell-free (Ontario, Canada)

### Cell-free kit providers

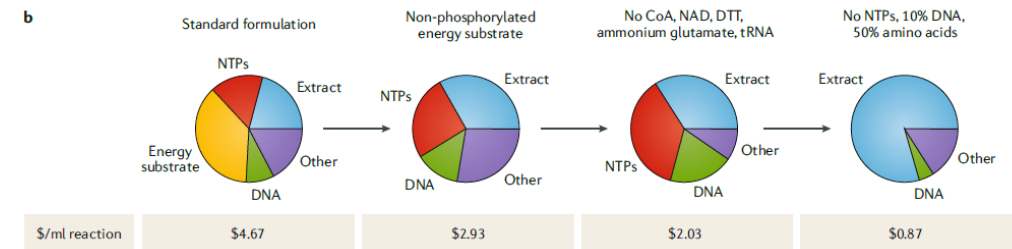
- New England Biolabs (Massachussets, USA)
- Thermo Fisher (Massachussets, USA)
- Biotechrabbit (Germany)
- arbor bioscience (Michigan, USA)

### 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                            |



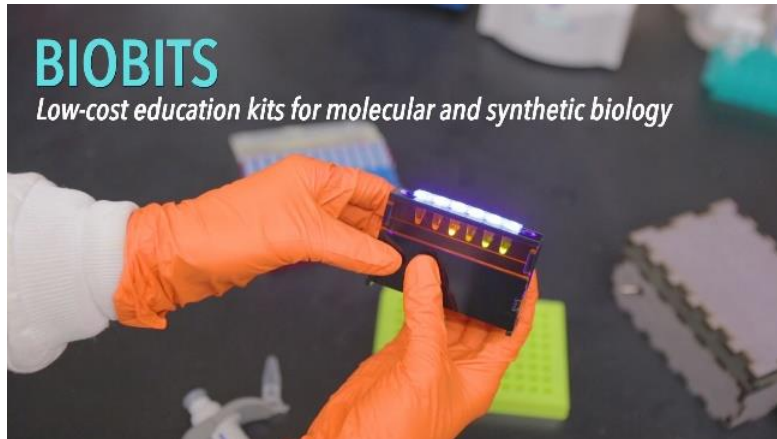
# Cell-free reaction:

## Additional use / challenges with cell-free

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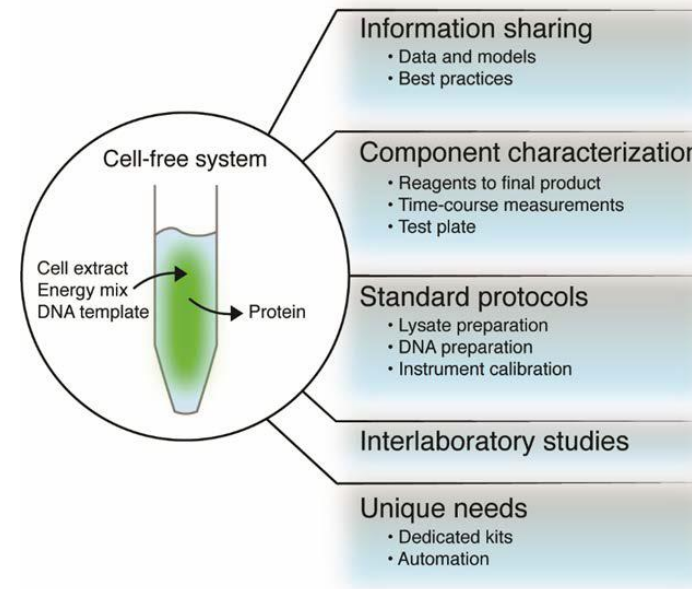
### Cell-free for education



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

### National Institute of Standards and Technology

#### Towards reproducible cell-free systems





# Acknowledgements



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