

adebiotech

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#Proteinov2016

Synthelis

Your Cell-Free Partner

Systemes d'expression "cell-free":

De nouvelles possibilités pour la production de protéines

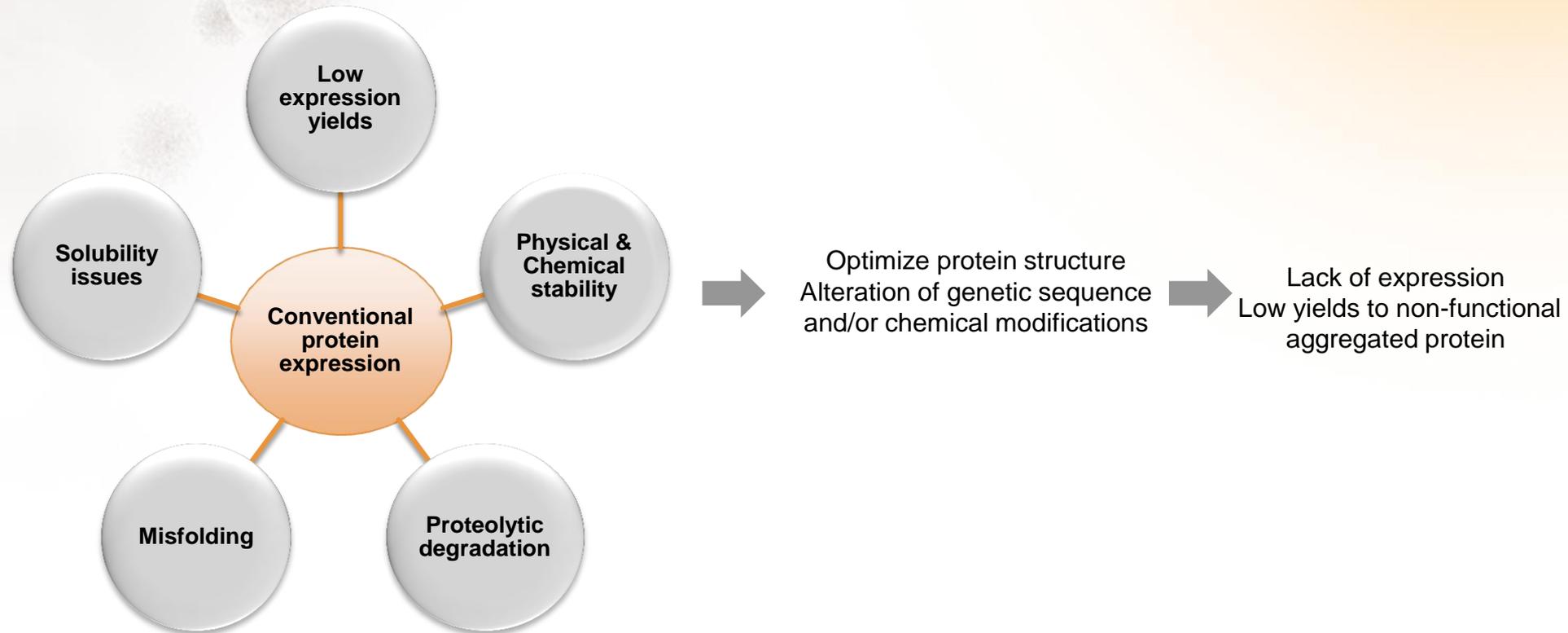
Dr. Sandra CORTES
CSO Synthelis

Cell free
expert

www.synthelis.com

Challenge

Solution to produce difficult-to-express proteins using conventional *in vivo* methods



Cell-free expression system (CFES)
Complementary approach to overcome these bottlenecks

Introduction

1897 Eduard Buchner
Cell-free fermentation
1907 Nobel Prize



↓
1961 Nirenberg's
discovery of genetic code
1968 Nobel Prize

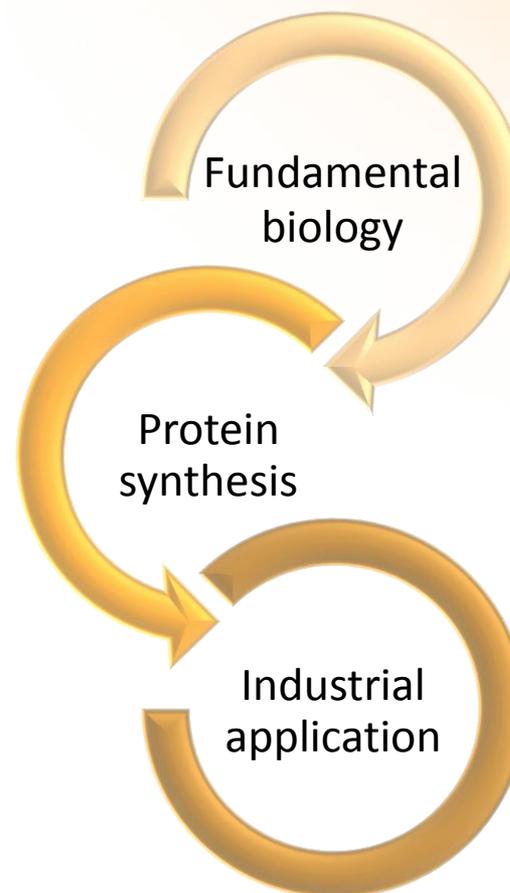


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1984
Crude extract optimization

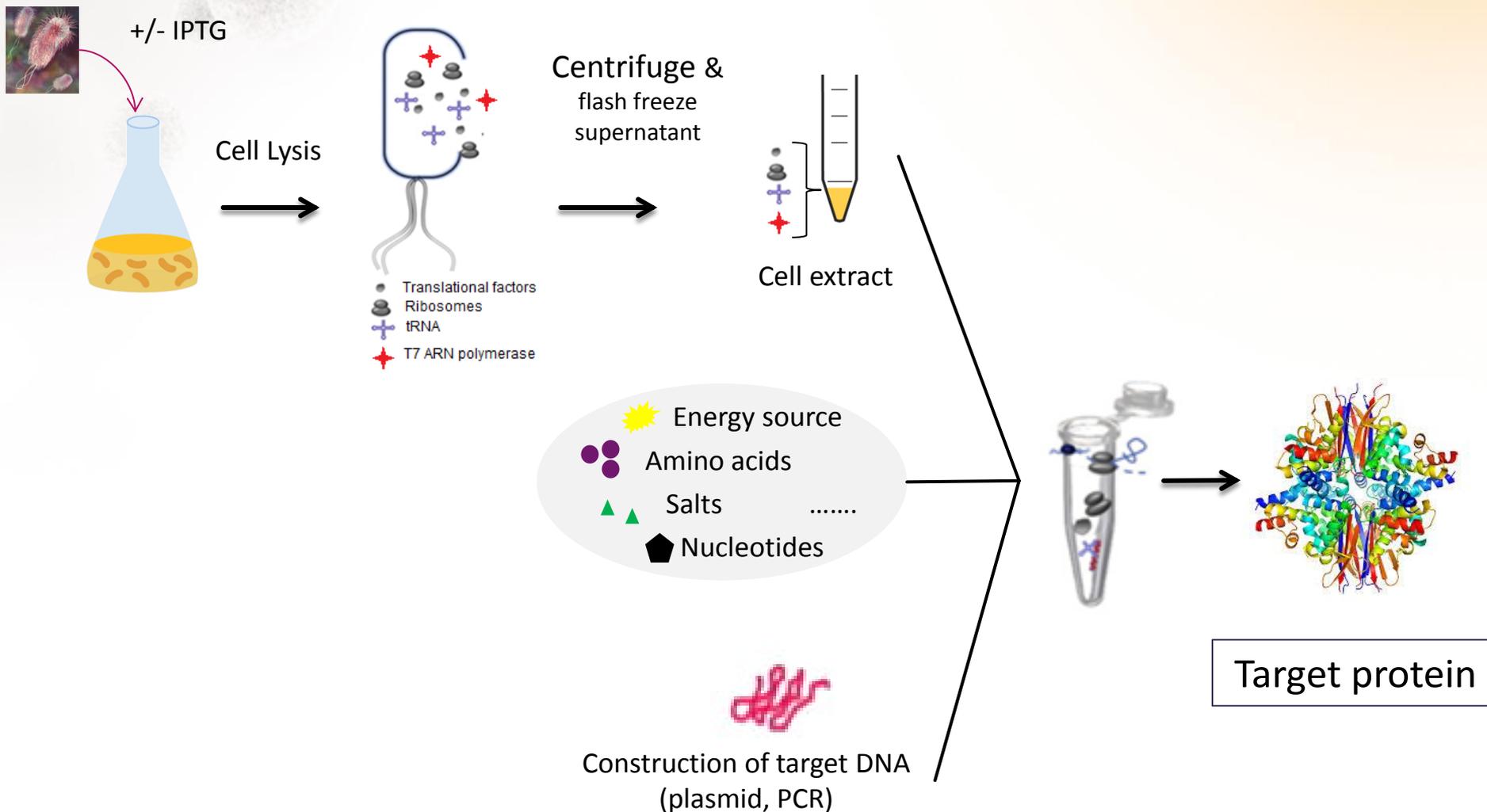
↓
1990
mRNA structure

↓
2003
Vector optimization

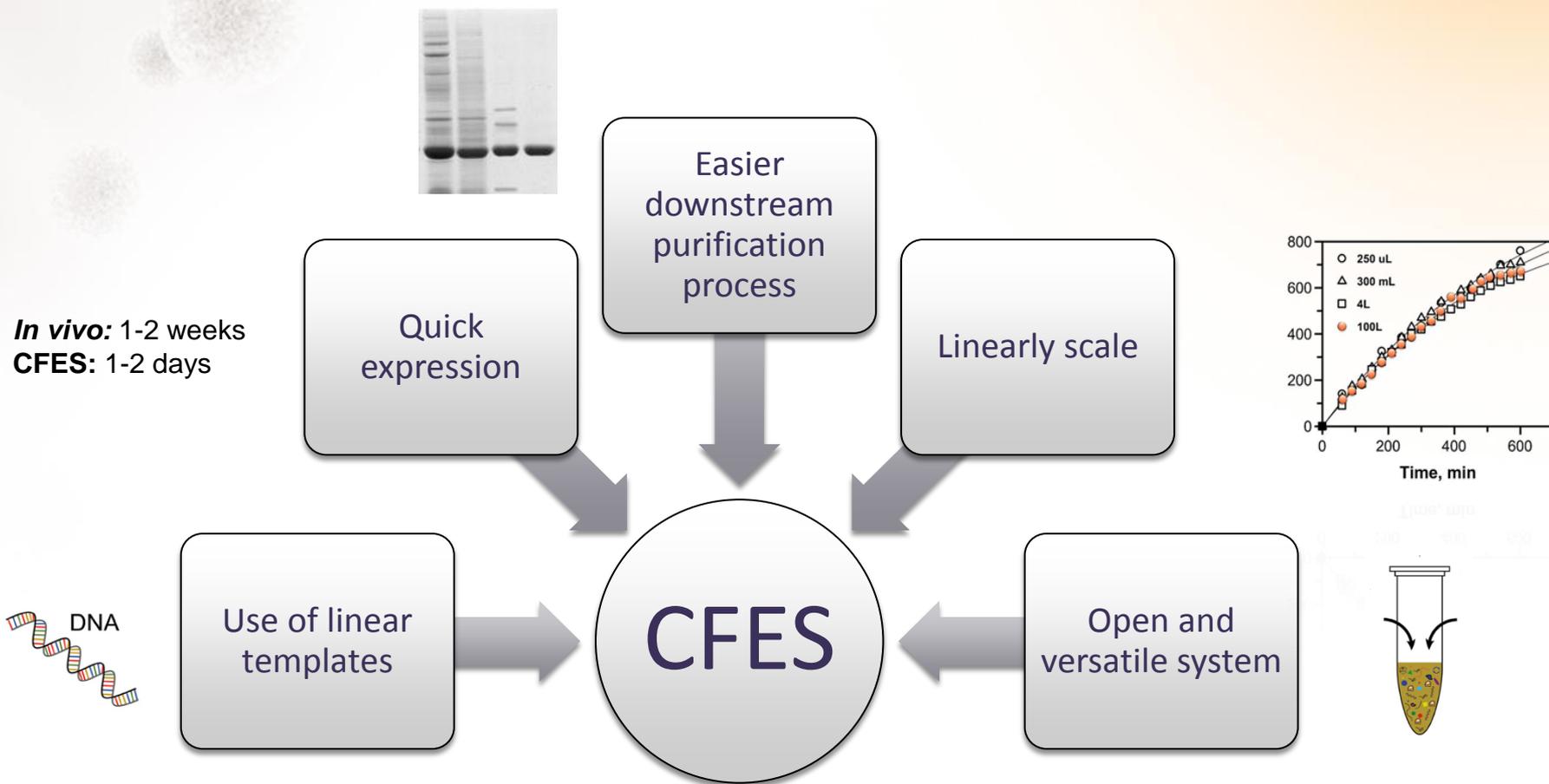
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2011
100L scale up - SUTRO



Schematic overview of CFES



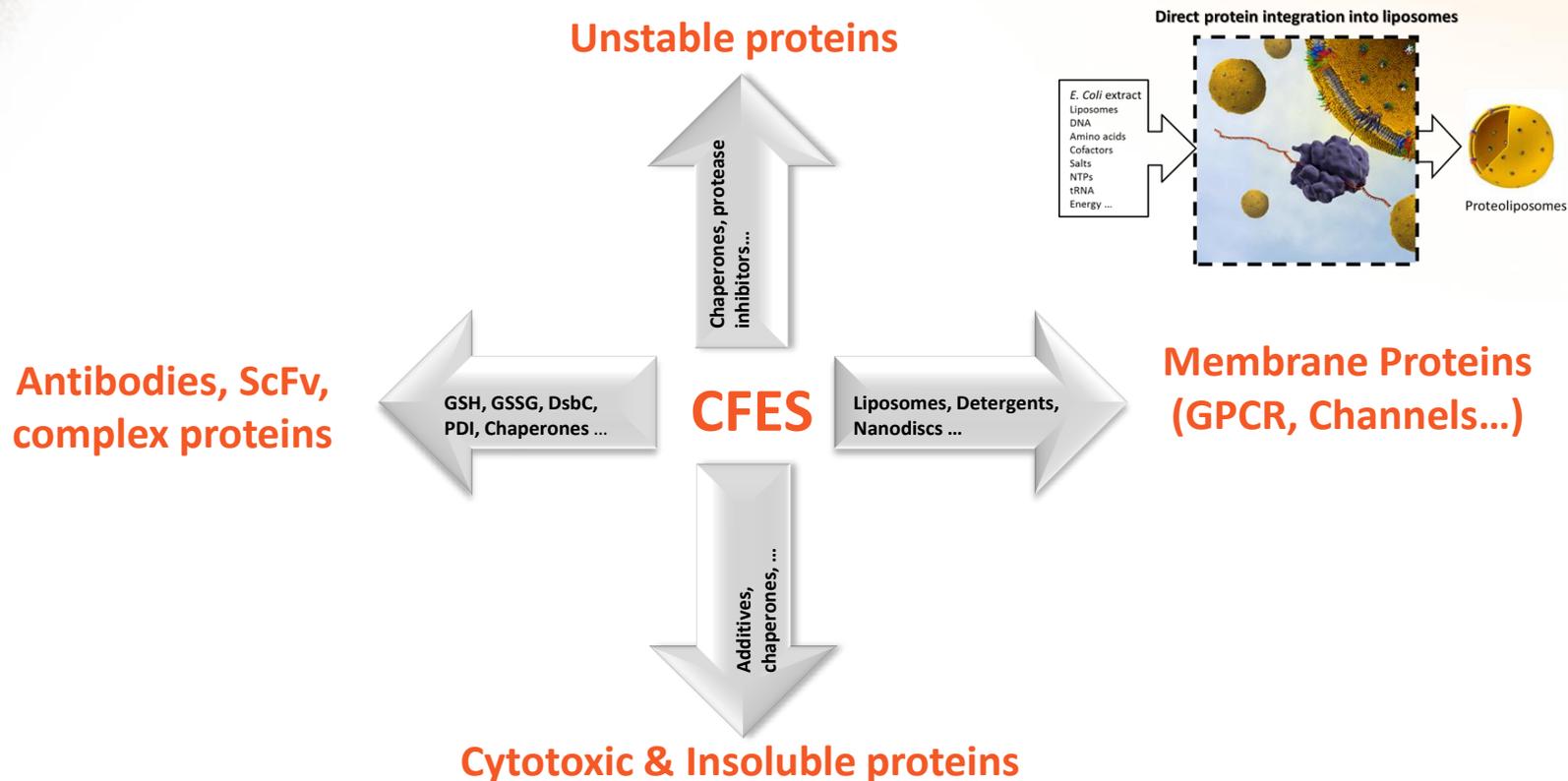
CFES : Open and customizable system



Solution for difficult-to-express proteins

Poorly expressed, insoluble, cytotoxic or subject to proteolysis *in vivo*....

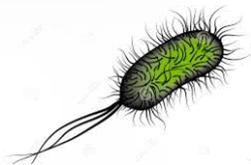
Cell-free protein expression strategies can overcome these problems and give access to a larger number of expressed proteins



Comparison of CFES platforms

Prokaryotic vs eukaryotic cell-free expression. While prokaryotic expression enables higher yields and is more cost efficient, eukaryotic expression offers more advanced features.

Prokaryotic



Easy, cost-efficient, high yield

No post-translational modifications

Wheat Germ



High yield

Complexity, poor post-translational modifications

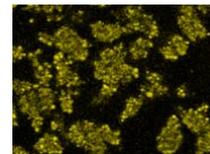
Insect cells / Rabbit reticulocytes



Post-translational modifications

Low yield, expensive

Mammalian cells

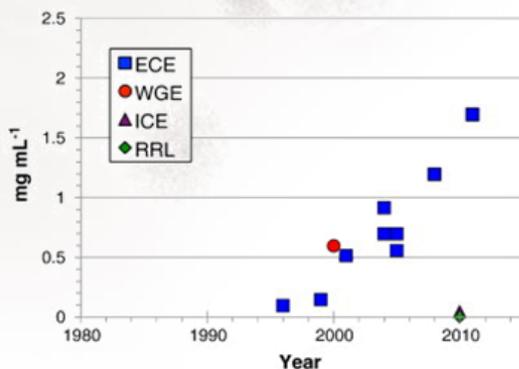


Post-translational modifications

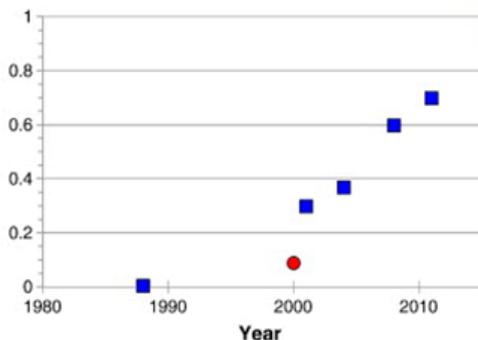
Low yield, expensive, complexity

Technological advances in CFES

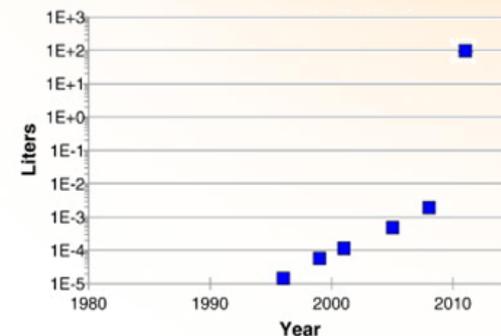
Biggest improvements for E.coli and WG



Protein Yield (Batch)



Protein Synthesis Rate



Reaction Volume (Batch)

- Metabolic pathway (new energy substrates)
- Extract quality
- Simplifying extract preparation
- Stabilization of amino acids
- Effective template concentration
- Host
- Vector DNA



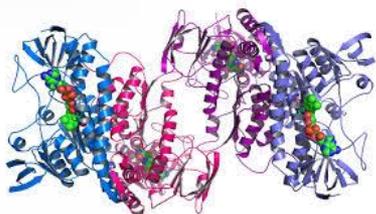
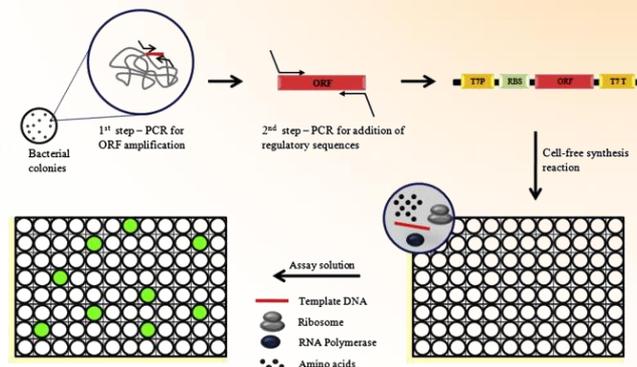
Increases in protein
synthesis rate
&
Increases in Batch
reaction duration



Increases protein yield
and reduced cost

Examples of technological applications

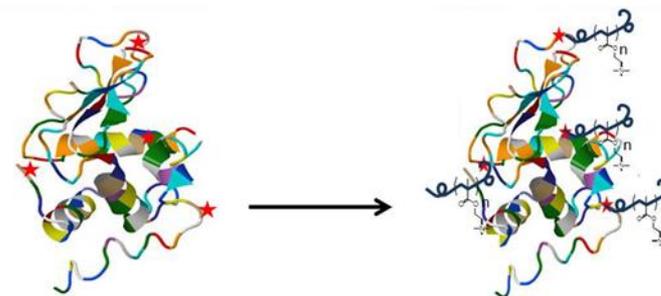
- **High-throughput screening** to identify targets before scale-up, to determine the best expression conditions



- Structural biology

Labeling of recombinant proteins with isotopic amino acids : Se-Met, ¹³C, ¹⁵N labeling

- **Protein engineering** to investigate protein stability / improve the catalytic properties of enzymes / understand its functionality



Synthelisis company

Expert in cell-free protein expression

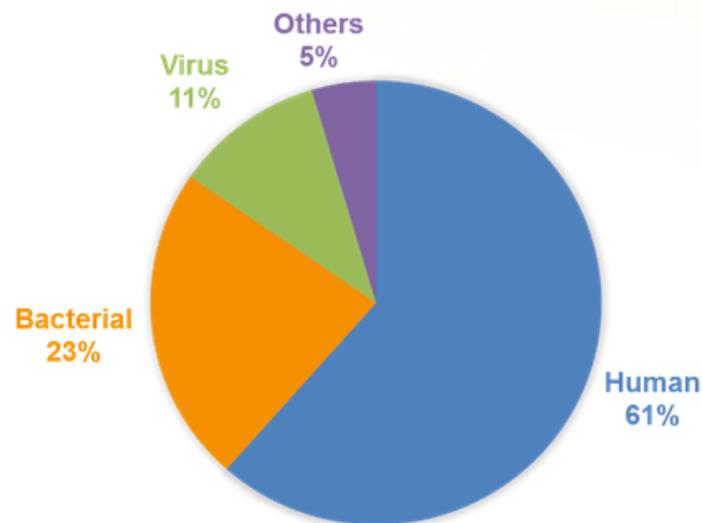
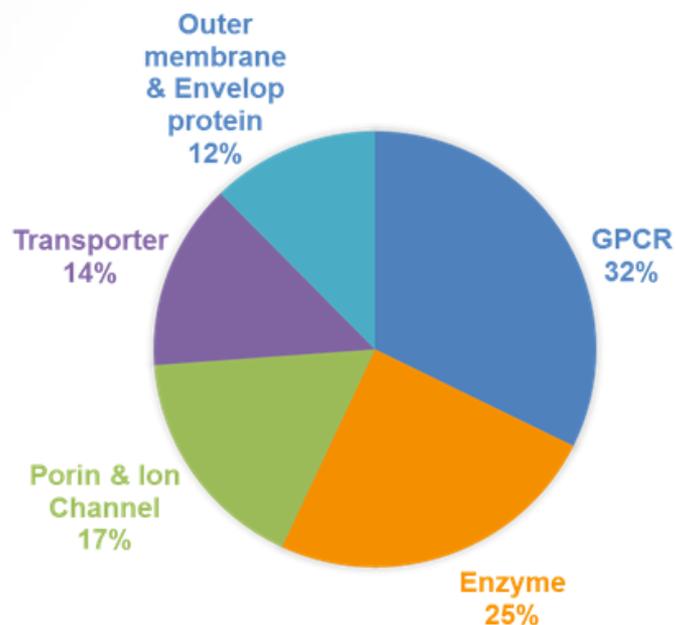


- Incorporated in January 2011
- Spin-off company from University Grenoble Alps (UGA - France)
- Proprietary technology and know-how in cell-free protein expression
- Expertise in development, production & characterization of difficult-to-express proteins: membrane, cytotoxic and soluble proteins
- Flexible formats: proteoliposomes, detergents, nanodiscs, etc.
- Service and off-the-shelf product offers

Track record : > 80 Successful projects

Proteins from different classes and origins produced by Cell free extract derived from *E.coli*

Some of these proteins are available in our catalog



Case study 1 : Tissue Factor (TF)

Problematic : Expression Yield & proper folding

Strategy : Express TF directly into liposomes by cell-free expression system. Define the best composition liposome to maintain activity

Structure & Protein function :

- In the full-size protein : 2 intrachain disulfide bridges
- A segment that crosses through the cell membrane and a small portion inside the cell.
- The portion outside is the part that interacts with the blood clotting machinery.

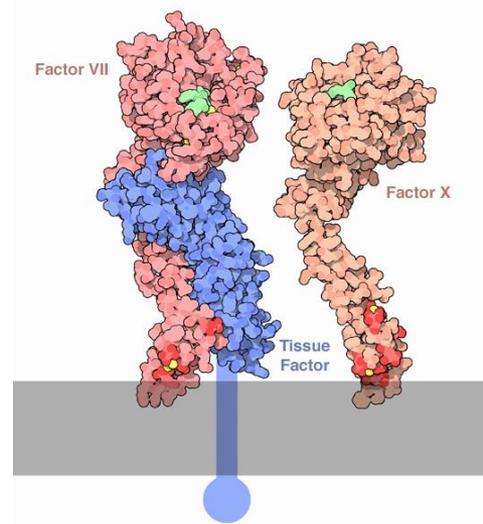
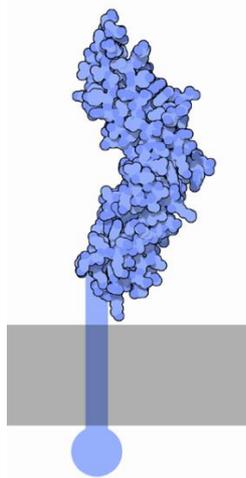
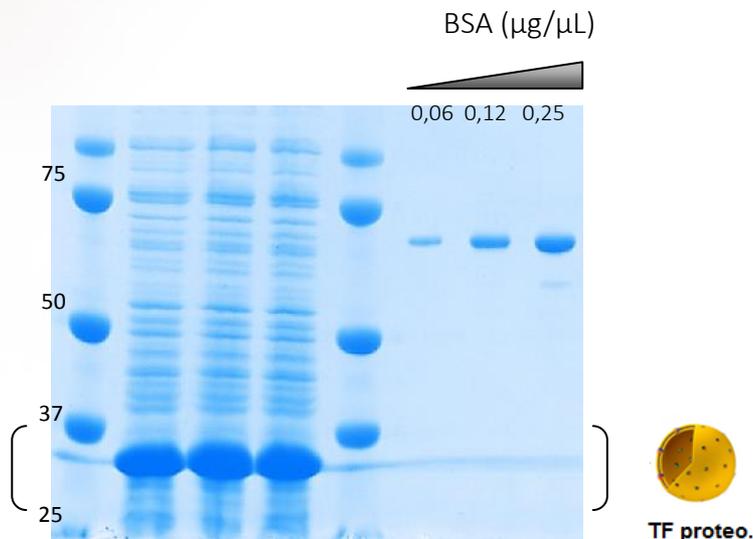


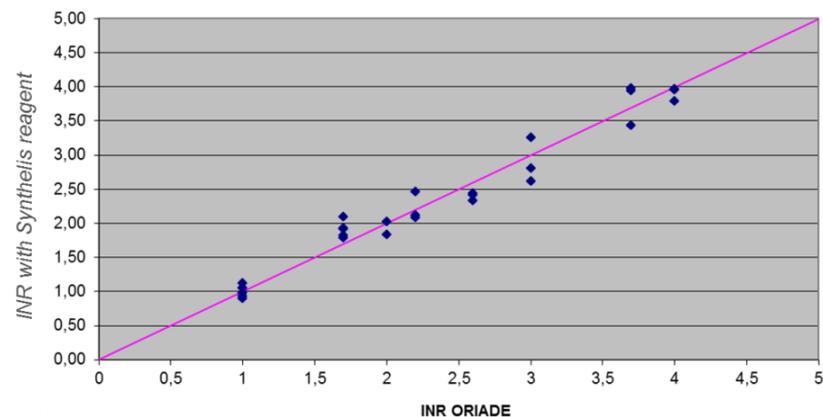
Image from <http://pdb101.rcsb.org/motm/75>

Case study 1 : Tissue Factor (TF)

Results: Expression Yield **700 µg/mL** & Blood clotting (coagulation) validated on healthy blood and pathologic blood



Expression Yield 700 $\mu\text{g}/\text{mL}$



Measurement of clotting speed

Expression yield *in vivo* expression system : 7 $\mu\text{g}/\text{mL}$

x100

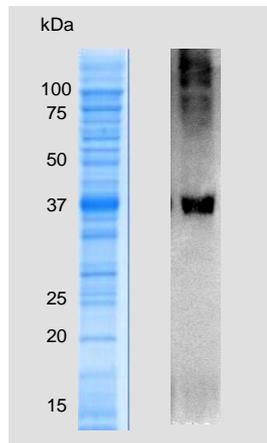
Case study 2 : C-X-C Chemokine receptor 4

Problematic : Expression Yield* & proper folding

Strategy : Express CXCR4 directly into liposomes by cell-free expression system.

Structure & Protein function :

- In the full-size protein :2 disulfide bridges, a 7 TM alpha-helical domains.
- Involved in hematopoiesis and in cardiac ventricular septum formation.
- Plays an essential role in vascularization and acts as a coreceptor (CD4 being the primary receptor) for human immunodeficiency HIV-1.



Produced in 3 formats

- ▶ Synthetic lipid proteoliposomes
- ▶ Detergent format
- ▶ Resolubilized from precipitates

Functional validation

- ▶ SPRi binding assays (HORIBA)
- ▶ Biolayer interferometry (Octet System – Pall Fortebio)
- ▶ Competition assay against a HEK cell line (CisBio)

Yield of Production by CFES : 200 µg/mL

x2000

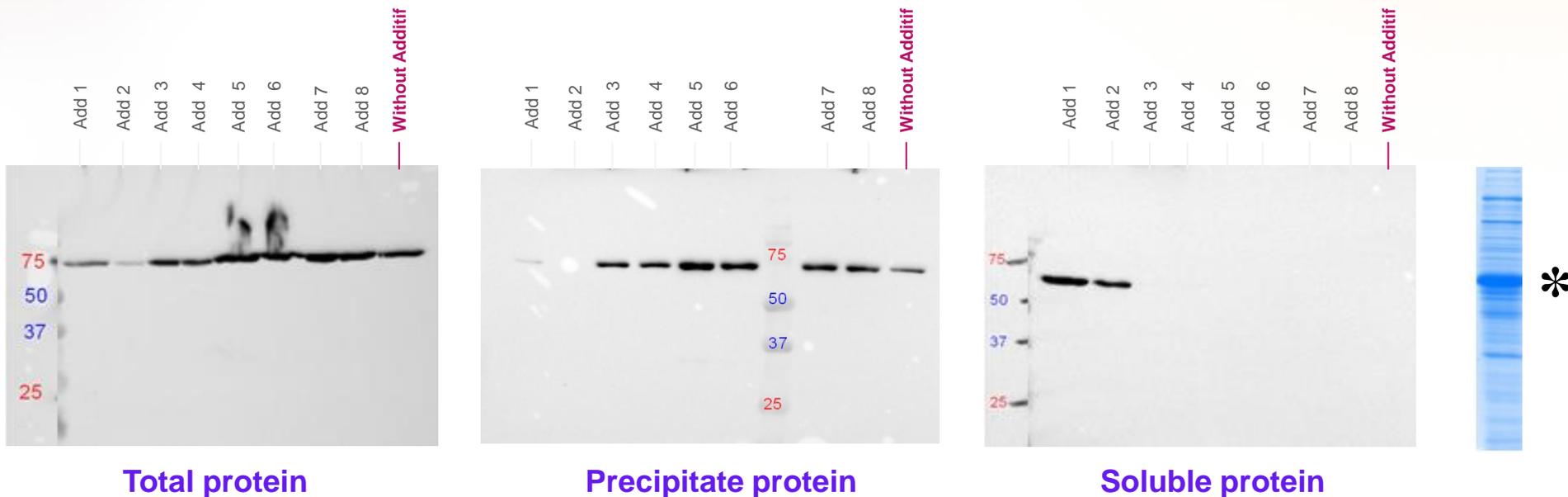
* Expression yield in cell based system : 0,1 µg/mL (data from Hui Ren, PLOS ONE, Feb. 2009 | Volume 4 | Issue 2 |)

Case study 3 : Inclusion bodies

Problematic : Inclusion bodies with conventional *in vivo* methods

Strategy : Express soluble protein by cell-free expression system using different additives directly into reaction mix and different expression vectors

Structure & Protein function : No indication



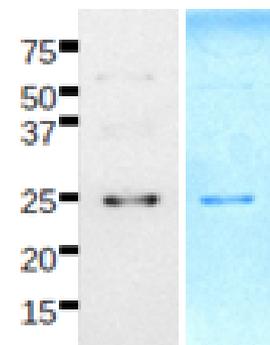
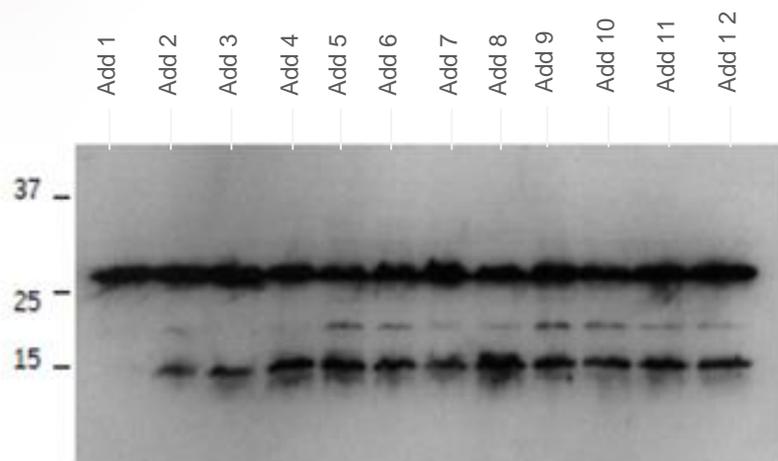
Results: Expression Yield of soluble fraction : 425 µg/mL

Case study 4 : Truncated forms

Problematic : Low expression yield & Truncated forms

Strategy : Express soluble protein by cell-free expression system using different additives directly into reaction mix.

Structure & Protein function : No indication



WB Coomassie Blue

Results: Full size protein expressed with expression Yield of **50 µg/mL** after purification step.

Conclusion

CFES, a promising alternative to classical cell-based protein production

- CFES provides a **rapid, robust, scalable** and efficient way to produce common proteins and **difficult-to-express proteins** (membrane proteins, cytotoxic proteins, instable proteins...). As it is an *in vitro* system, expression is separated from the limitations of the host cell
- CFES allows a **high degree of control** on the parameters that influence protein expression (yield, quality)
- CFES in **robotic platforms** allow for **high-throughput screening** of conditions/constructs /additive conditions /environment parameters / mutants ...
- CFES produces **correctly folded**, active protein difficult to produce
- CFES allows **cost effective** especially for labeling of protein for structural work

THANK YOU FOR YOUR ATTENTION !

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