Wheat germ cell-free expression system: An alternative method for the production of functional membrane and toxic proteins

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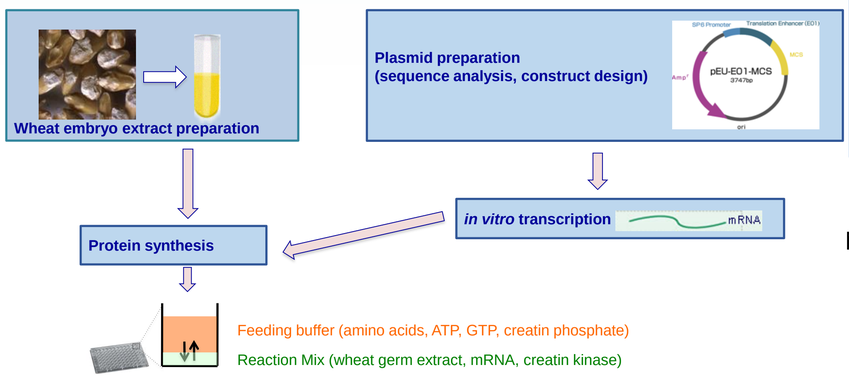
While several systems are available for protein production, recombinant protein synthesis often faces to difficulties to express and purify them and preserving their biological activity. It is especially the case for membrane proteins (MPs) which are notoriously difficult to express in a soluble form. MPs represent 20-30% of proteins encoded by genomes and more than half of the targets of pharmaceutical drugs.
Here, we use wheat germ cell-free expression in the presence of various detergents to produce several membrane proteins. Indeed, we screened various types of detergents to determine translation conditions that can yield essentially soluble membrane proteins at detergent concentrations that do not inhibit the cell-free reaction.

Wheat Germ cell free system

Wheat Germ Cell-free system (WGE)

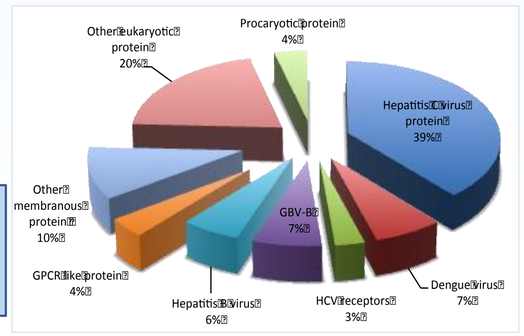
Main advantages over classical cell-based methods

- Expression of proteins that are toxic to the cells
- Translation is 10 times slower than in bacteria → better protein folding!!
- Proteins can be expressed in a large temperature range (4° C-30° C)
- No need for codon optimisation
- Open system: supplementation with detergents or phospholipids possible
- Effective and specific incorporation of labeled amino acids (NMR studies)
- No need for cell harvesting and cell lysis



System efficiency

- More than 100 contructs tested up to now

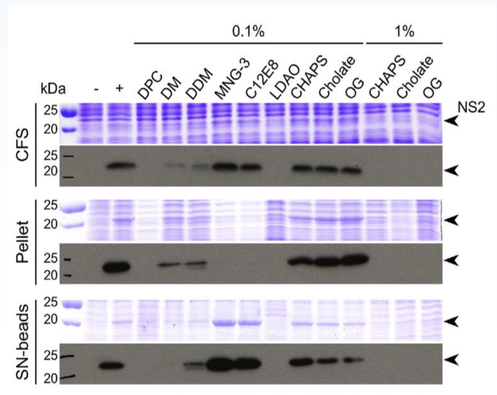


More than 90% of proteins were successfully expressed

Membrane protein production

Membrane proteins are kept in soluble form by insertion into provided detergent micelles

(Protein Expr Purif. Fogeron *et al.*, 2015)



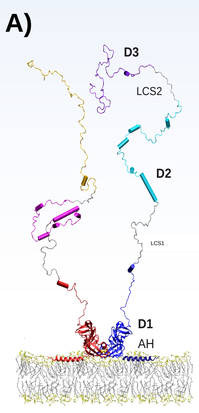
MNG-3 and C12E8, detergents with low critical micelle concentration (CMC) can yield essentially **soluble membrane proteins** at detergent concentrations that **do not inhibit the cell-free reaction.**

Results indicate that **low CMC detergents** keep the **monomer concentrations low** while**,** at the same time**,** providing the **necessary excess of detergent** concentration above CMC required **for full target protein solubilization**.

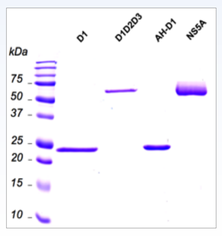
Production of full-length HCV NS2 protein using the wheat germ cell-free expression system in the presence of nine different detergents. SDS–PAGE analysis followed by Coomassie blue staining (upper panels) and western-blotting (lower panels). **CFS**, cell-free sample; **pellet**, pellet obtained after centrifugation of CFS; **SN-beads**, supernatant obtained after centrifugation of CFS and incubated with Strep-Tactin magnetic beads to capture Strep-tag II-tagged NS2 protein; **-**, negative control (no NS2)**; +**, positive control (NS2 expressed in the absence of detergent)

Some examples of membrane proteins among many others…

HCV Membrane protein production: example with NS5A

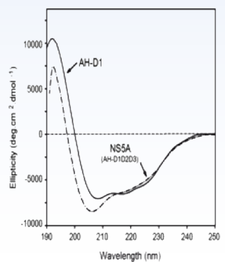


B) SDS-PAGE of purified proteins

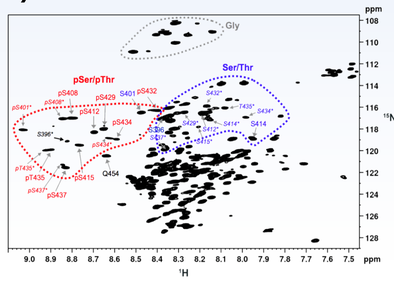


Yield: ~ 0,2 mg/ml WGE

C) Circular dichroism



D) NMR in solution



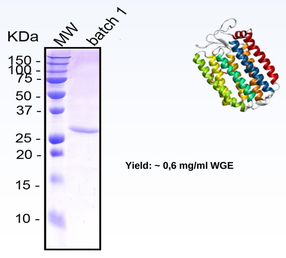
(A) Tentative model of the full length_NS5A dimer associated to a phospholipid POPC membrane (Badillo *et al*. submitted). **(B)** SDS-PAGE of purified NS5A and domains (in fusion with a C-terminal strep-tag) expressed in wheat germ cell-free system and purified on Strep-Tactin affinity column. **(C)** Circular dichroism spectra of purified NS5A and AH-D1 domain. AH-D1 secondary structure content is consistent with AH and D1 NMR and RX structures while NS5A exhibits additional coil structures due to the instrinsically disordered nature of D2 and D3 domains. **(D)** $^{1}H,^{15}N$-HSQC NMR spectrum of NS5A-D1D2D3 at 900MHz (Badillo *et al.* submitted).

- Up to now, tentatives to produce the **NS5A-D1D2D3 protein in other system led to a poor protein yield**
- By using **WGE system**, this full-length protein is **expressed and purified** in **sufficient amount for structural analysis**: Circular dichroism, NMR in solution…

Membrane proteins belonging to GPCRs family structure

Gensight Biologics Property (www.gensight-biologics.com)

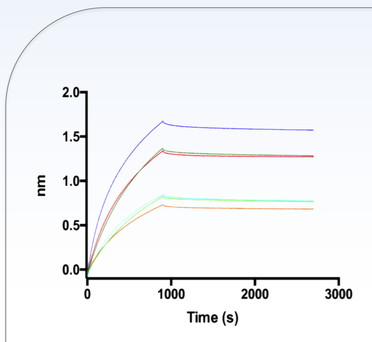
Halorhodopsin



SDS-PAGE of Halorhodopsin purified protein (in fusion with a C-terminal Strep-Tag) expressed in wheat germ cell-free system and purified on Strep-Tactin affinity column

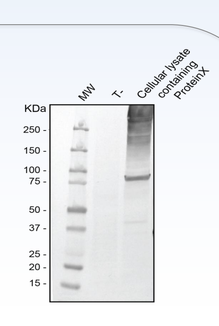
✓ Production of Halorhodopsin **in milligram range**

Protein X



Interferometry Binding assays of ProteinX to monoclonal antibodies GD6, JB6 and BD5. Sensorgrams resulting from the interaction of ProteinX and the three mAbs covalently immobilized by amine coupling

✓ **No dissociation** observed, which means a **high affinity** of the mAbs against ProteinX (nanomolar range)



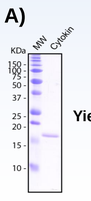
Western-Blotting of cellular lysate containing ProteinX detected with mAb GD6 produced from recombinant ProteinX

✓ **mAbs produced from recombinant proteinX are specific those produced by the cells**

Other protein productions

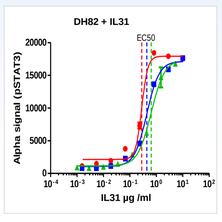
Cytokine production

A)



Yield : ~ 0,3 mg/ml WGE

B)

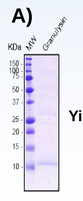


✓ EC50 similar to IL31 produced in cells

✓ **Cytokines produced are active**

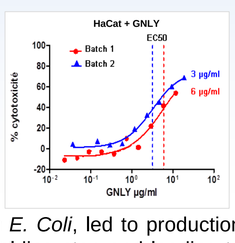
Toxic protein: *i.e* Granulysin

A)



Yield : ~ 0,1 mg/ml WGE

B)



✓ Attempts to produce Granulysin in *E. Coli*, led to production of inclusion bodies, generating refolding step and leading to a **very poor protein yield**

✓ **Granulysin produced in WGE doesn't need refolding and is active**

For both protein: (A) SDS-PAGE of purified protein (in fusion with a C-terminal strep-tag) expressed in wheat germ cell-free system and purified on Strep-Tactin affinity column; **(B)** Bio-assay

References

• Wheat germ cell-free expression: Two detergents with a low critical micelle concentration allow for production of soluble HCV membrane proteins.
Fogeron ML, Badillo A, Jirasko V, Gouttenoire J, Paul D, Lancien L, Moradpour D, Bartenschlager R, Meier BH, Penin F, Böckmann A. Protein Expr Purif. 2015 Jan;105:39-46. doi: 10.1016/j.pep.2014.10.003.

• Functional expression, purification, characterization, and membrane reconstitution of non-structural protein 2 from hepatitis C virus. Fogeron ML, Paul D, Jirasko V, Montserret R, Lacabanne D, Molle J, Badillo A, Boukadida **C**, Georgeault S, Roingeard P, Martin A, Bartenschlager R, Penin F, Böckmann A. Protein Expr Purif. 2015 Dec;116:1-6. doi: 10.1016/j.pep.2015.08.027.

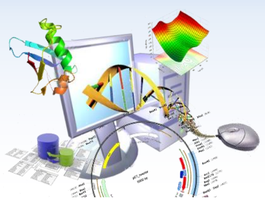
• Cell-free expression, purification, and membrane reconstitution for NMR studies of the nonstructural protein 4B from hepatitis C virus. Fogeron ML, Jirasko V, Penzel S, Paul D, Montserret R, Danis C, Lacabanne D, Badillo A, Gouttenoire J, Moradpour D, Bartenschlager R, Penin F, Meier BH, Böckmann A. J Biomol NMR. 2016 Jun;65(2):87-98. doi: 10.1007/s10858-016-0040-2.

• Overall Structural Model of NS5A Protein from Hepatitis C Virus and Modulation by Mutations Confering Resistance of Virus Replication to Cyclosporin A . Badillo A, Receveur-Brechot V, Sarrazin S, Cantrelle FX, Delolme F, Fogeron ML, Molle J, Montserret R, Böckmann A, Bartenschlager R, Lohmann V, Lippens G, Ricard-Blum S, Hanoulle X, Penin F. Biochem 2016 submitted.

A group of skills!

Products & Services in Immunology	CRO, custom services	Products & Services in Immunology
CD markers *Cytokines* *Chemokines* *Apoptosis* *Cytokines receptors…*	*Immunology* *Biochemistry* *Molecular biology* *Cell culture*	mAbs development platform (Rat, Guinea pig)
•Monoclonal antibodies •ELISA kits •ELISpots kits •Multiplex kits •Bioessais platform	•Custom bioproduction •Research contracts •Analytical services •FastELISA kits	•Secondary monoclonal antibodies •Anti-species mAbs, •Isoptypes controls, …)

Recombinant Protein platform: From Gene to Protein



Bioinformatic analysis, determination of the most relevant expression system to express the target protein

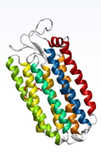
Gene synthesis and cloning

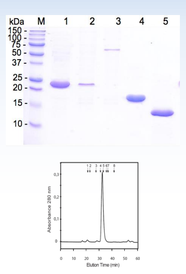
E. Coli

Mammalian cells, HEK and CHO (transient, stable)

Wheat germ cell-free system

Protein expression and purification





QC measurements

Delivery