SMART4FABRY

Smart Functional GLA—nanoformulation for Fabry disease

Therapeutic enzymes: how to produce them

Lysosomal storage disorders (LSDs) are inherited rare diseases caused by the lack or malfunction of proteins involved in lysosomal activity. Enzyme replacement therapies (ERTs, based on the administration of a functional version of the defective enzyme) have gained clinical relevance in LSD health care. Therefore, production of therapeutic enzymes is a key aspect in the development of therapies for many rare diseases.

Proteins are essential macromolecules in life that combine structural and biological functionalities. Enzymes are proteins that act as biological catalysts, accelerating chemical reactions.

For thousands of years, humans have used enzymes in industries related to dairy, bakery, brewing, and wine, beer or cheese making. While they are still employed for those purposes, protein science has greatly evolved, raising opportunities for new applications in many industries and fields, like nanomedicine.

Partner in Smart-4-Fabry project addressing human GLA enzyme production:

NanoBioTechnology

PRODUCING THERAPEUTIC PROTEINS

- Recombinant proteins have become a priceless tool in the field of nanomedicine.
- Expression system chosen to fabricate such recombinant proteins will influence yield, characteristics and properties of the protein obtained.
- Production procedures must be optimized and scaled-up.
- Once synthesized, target protein must be purified to the desired level of purity.
- Purified protein must fulfill both therapeutic and regulatory requirements in order to be validated as a therapeutic molecule.

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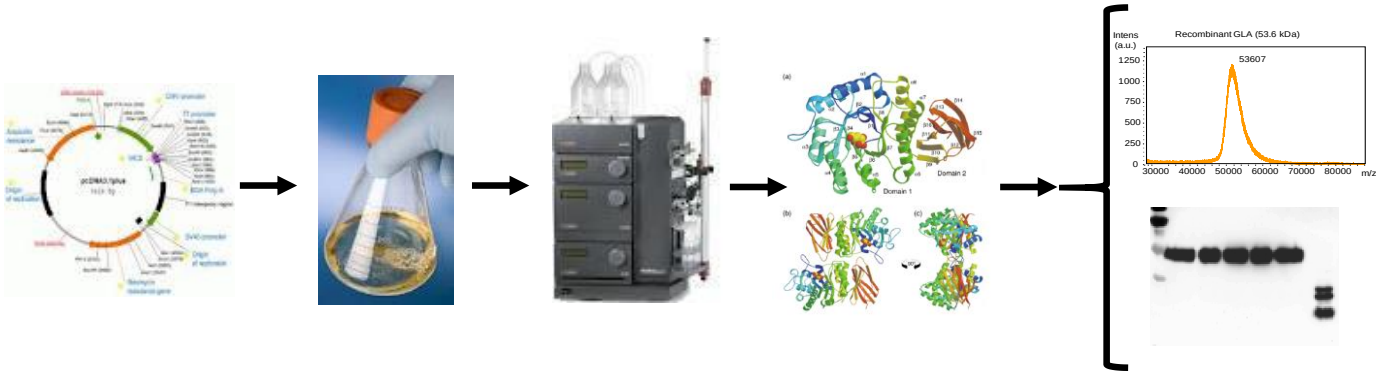
European Commission | Horizon 2020 European Union funding for Research & Innovation

Cell metabolism needs enzymes to occur at rates fast enough to sustain life. Therefore, any defect (mutation, overproduction, underproduction or deletion) in a single enzyme can lead to a genetic disease. Such diseases can have dramatic consequences that require lifelong treatments. Often, the best available therapy is to replace the deficient or absent enzyme in a process known as **Enzyme Replacement Therapy** (ERT). ERT consists in the periodic intravenous infusion of the enzyme and is currently available for some lysosomal storage diseases (like Gaucher or Fabry diseases). Thus, in the pharma industry, enzymes play a major role as drugs for the treatment of rare genetic disorders related to enzyme deficiencies. However, enzymes needed for ERT cannot always be obtained from their natural sources in amounts adequate for medical use, resulting in a growing interest in new and efficient methodologies for the economic and high-yield production of recombinant enzymes.

Since the production of recombinant insulin in the late 70s, emergence of molecular biology and biotechnology has enabled the biological fabrication of a long list of therapeutic proteins. Today, recombinant DNA and cell technologies are main-stream platforms to obtain most of the currently marketed protein drugs such as monoclonal antibodies, hormones, cytokines, growth factors or enzymes.



Design **Production** **Purification** **Purified enzyme** **Characterization**

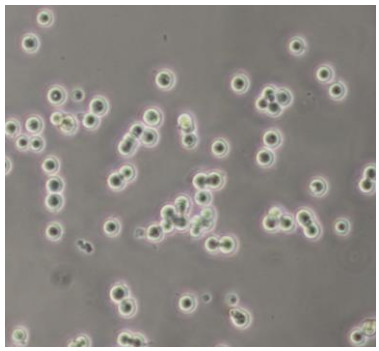
General procedure to obtain a recombinant protein

Most of therapeutic proteins used in clinics are recombinant versions, produced by heterologous organisms. In this sense, there is not such a thing as an “universal host” for recombinant protein expression. Microorganisms as *Escherichia coli* or yeast cells (still considered the first choices to this purpose) have intrinsic limitations restricting their use. Production of heterologous proteins in *E. coli* is limited by cytotoxicity, incorrect folding, aggregation and/or lack of secretion, while recombinant protein production in yeasts is often associated with hyperglycosylation and product retention within the periplasmic space. Many other expression systems (like insect cells, microalgae, plant cells, or even transgenic plants or animals) have been used and developed to express recombinant proteins. However, for proteins intended for therapeutic use in humans, **mammalian cells** are preferred over other cell factories.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N° 720942.

MAMMALIAN CELLS TO PRODUCE RECOMBINANT THERAPEUTIC ENZYMES

Due to their ability to perform proper modifications (as glycosylation), like those naturally occurring in human cells, mammalian cells are nowadays the preferred system to produce biopharmaceuticals, becoming a routine source of monoclonal antibodies, hormones or enzymes. Thus, mammalian cells dominate as expression system among all approved recombinant protein-based biopharmaceuticals in the last years.



Culture of human cells (growing in suspension) used to produce recombinant enzymes.

HOW TO PRODUCE A RECOMBINANT PROTEIN IN MAMMALIAN CELLS

Mammalian cells continuously producing recombinant proteins ("**stable clones**") are established by insertion of the recombinant gene into host genome and selection of stable clones. For large scale production of therapeutic proteins, stable clones are the preferred option as it allows greater process consistency, yields and control of the final product quality. However, this approach (costly and time-consuming) may be useless if our protein is not suitable or approved for its therapeutic use.

Mammalian cells dominate as expression system for recombinant proteins intended for therapeutic purposes.

- Between 2014 and 2018, 62 out of 71 new biopharmaceuticals reaching the market were recombinant proteins.
- Of those 62 proteins, 84% were expressed in mammalian cell lines.

<15 LSDs

Nowadays, Enzyme Replacement Therapy using recombinant proteins is available for less than 15 LSDs.

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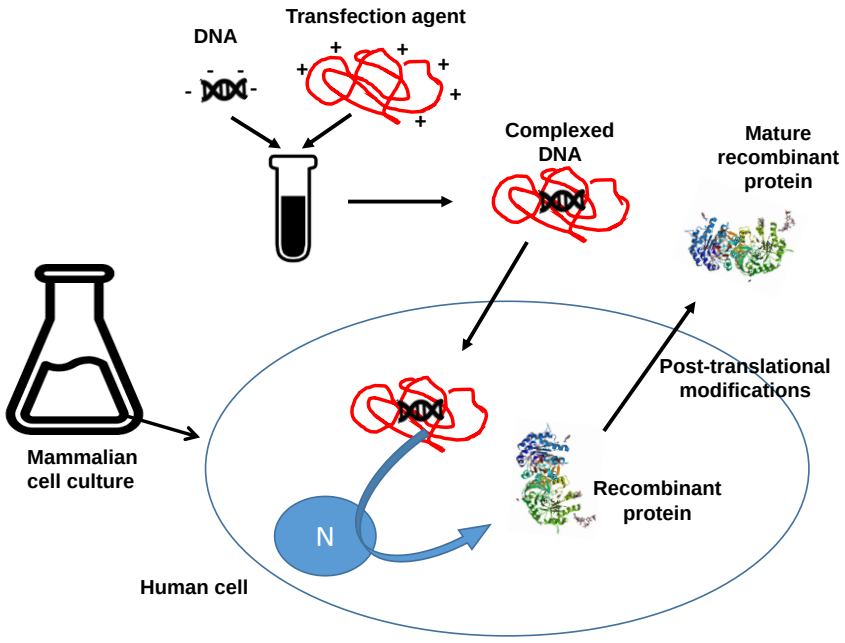
HOW TO OBTAIN THERAPEUTIC ENZYMES IN JUST A FEW DAYS

To avoid drawbacks of stable clones, faster and cheaper approaches for protein production are needed, especially if many proteins (or several variants of a single protein) must be rapidly evaluated as biopharmaceuticals. In this context, **transient gene expression (TGE)** is a well-suited strategy. TGE is defined as the production of a recombinant protein over a short period (1–14 days) after DNA transfer into producer cells. The recombinant gene is introduced into cells with a chemical agent, and gene expression and protein production occur just for a few days. The main cell lines used for TGE are CHO (from hamster) and HEK (human origin), since they are relatively easy to transfect, grow in single-cell suspension, and have been approved for the production of therapeutic proteins. Key features of TGE are simplicity, short times (days) for protein production, allowing the simultaneous study of many genes or their variants. By TGE approach, it is possible to produce milligram to gram quantities of protein within days or weeks.



General procedure for transfection and transient gene expression

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QUALITY OF THERAPEUTIC ENZYMES: PURIFICATION PROCESSES

Recombinant proteins are produced in host cells together with a great variety of molecules that cell naturally contains. Thus, our recombinant protein must be isolated and purified from the rest of cell molecules. Purification is always clearly necessary, but it is a “must” if your protein is intended for therapeutic applications. Then we need to ensure high purity levels of the therapeutic protein, in order to avoid unwanted side-effect effects due to contaminants. Factors receiving particular attention include the possible presence of contaminating oncogenic cell DNA, as well as the presence of viruses and other pathogens derived from transformed mammalian cells.

Purification processes are usually composed by various steps, being the final goal to obtain the protein of interest with the highest possible level of purity.

ENZYMES IN THE SMART-4-FABRY PROJECT

Human alpha-galactosidase (GLA) is the enzyme needed to treat Fabry disease. Therefore, in the frame of Smart-4-Fabry project, we have developed several procedures and optimized protocols in order to obtain enough amounts of recombinant GLA with the desired physico-chemical requirements.

For that, we have produced recombinant GLA by means of transfection and transient gene expression in human cells. We also established stable clones in mammalian (from Chinese hamster ovary) cells continuously producing recombinant GLA. Production and purification procedures, together with analytical techniques, have been set-up and optimized.

In this respect, GLA production for Smart-4-Fabry project has been possible thanks to the efforts and involvement of the Nanobiotechnology group (from CIBER-BBN and from Institute of Biotechnology and Biomedicine, UAB), the Platform for Protein Production (Unit 1 from NanBiosis, IBB, UAB) and LeanBio company (a market oriented CDMO that develops and manufactures biopharmaceutical products as new biological entities and biosimilars).

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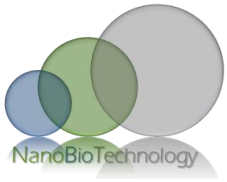

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