Institute of Chemistry and Biological Chemistry
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Transient Gene Expression for Protein Therapeutics

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Recombinant proteins are currently used for a multitude of diseases as part of the standard of care and generate a global turnover of more than 80 billion dollars. The project, in cooperation with ExcellGene SA and a laboratory at the EPFL, involves a novel method to produce proteins in GMP quality for clinical trials: the proteins are to be manufactured by transient gene expression (TGE) instead of from stable cell lines.

State of the Art: Stable Cell Lines

Recombinant protein therapeutics are mainly produced from mammalian cells, since only these cells allow post-translational modifications, such as glycosylations, which are often required for activity. For this purpose, «immortal» cell lines, such as the chinese hamster ovary cell line, are used, in which the genetic information of the target protein is stably integrated by gene transfer into the genome of the cells. However, the production of stable cell lines is very time consuming.

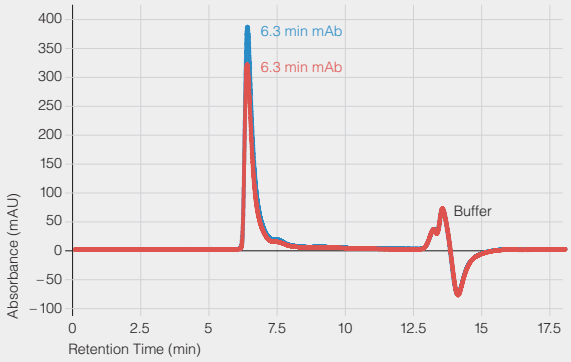
Transient Gene Expression as an Alternative Method

Transient gene expression is an alternative to protein production from stable cell lines. In this process, expression vectors are introduced in large quantities into cells cultured in bioreactors. The vector DNA is then transcribed in the cell into the corresponding mRNA, from which the protein is produced. Protein expression occurs until the expression vectors are degraded, inactivated or diluted by cell division (approx. 2 weeks). Unlike production with stable cell lines, this method requires no selection of individual clones and no subsequent proliferation of the cells (which is time consuming). It therefore allows a much faster and cheaper production of target proteins, especially if this can be carried out in larger bioreactors.

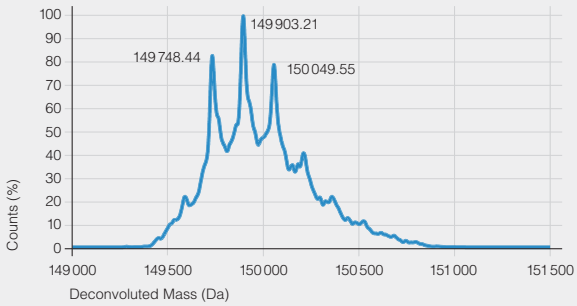
Protein Expression, Downstream Processing and Analysis

The aim of the project is to establish a platform for TGE of recombinant proteins for pre-clinical and early clinical studies. To enable direct comparison of the two methods, a model protein is being produced by the project partners using a stable cell line and by TGE. The model protein used was a monoclonal antibody (mAb) against Rhesus D. Anti-Rhesus D antibodies are used for prophylaxis in Rhesus D-negative women during pregnancy.

The proteins from both processes are being purified at the ZHAW with chromatographic methods. The purification processes are characterised in terms of the depletion of impurities such as DNA, host cell proteins and endotoxins. The target molecules of the two processes are investigated in detail for their physicochemical properties. Methods such as isoelectric focusing, size exclusion HPLC, reversed-phase HPLC and mass determination by MS are used.



SEC-HPLC chromatogram of purified anti-Rhesus D mAb after stable (red) and transient (blue) expression. The retention times and the profiles of the two mAbs are not different.



Mass spectrum (ESI-Q-TOF) of purified anti-Rhesus D mAb after transient expression. The three main peaks are due to various post-translational modifications.

Research project

Establishment of a Robust GMP-ready Technology Platform for Large-Scale Transient Gene Expression for the Manufacture of Clinical Grade Protein Pharmaceuticals

Leadership ZHAW:	Prof. Dr. Christiane Zaborosch
Project duration:	2 years
Partners:	ExcellGene S.A., Monthey; Laboratory of Cellular Biotechnology, EPFL, Prof. Dr. F. Wurm
Sponsorship:	Commission for Technology and Innovation CTI, Bern
Project volume:	CHF 1 115 000.–