

# GlycoEngineering – a Technology for Production of Glycoproteins

Human proteins are produced in large amounts and the number of products increases year by year. The demand for these recombinantly produced proteins is mainly governed by their medical need (e.g. growth factors, cytokines, antibodies, see table page 13). The *in vivo* counterparts of most of the therapeutic proteins are glycoproteins with single carbohydrates or large carbohydrate moieties attached to the protein. This glycosylation affects the bioactivity of a protein, such that physical features, the tertiary structure and stability are changed. The type of glycosylation is crucial e.g. for serum-half-life and immunogenicity of a glycoprotein, receptor binding, antibody mediated cell cytotoxicity, cell activation and cell-cell communication, and targeting to specific organs and organelles. Therefore, for the new generation of therapeutic proteins an optimized glycosylation is and will be of great impact.

## Current Systems for the Production of Therapeutic Proteins

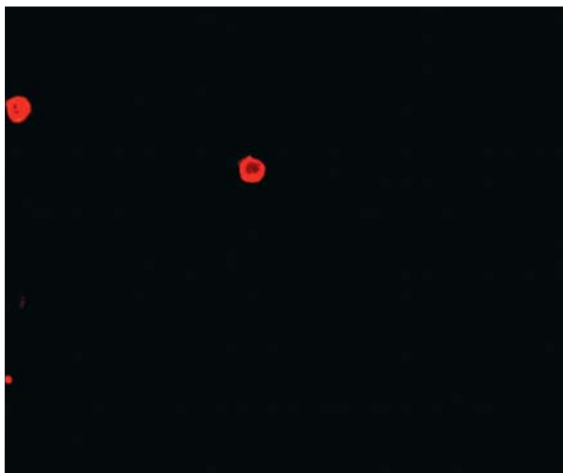
To produce therapeutic proteins mainly three production systems are currently in use. The first generation of therapeutic proteins was and still is produced mainly in bacteria (*Escherichia coli*) and some in yeast cells (*Pichia pastoris*, *Saccharomyces cerevisiae*), because of its relatively easy and cheap production process with very high yields of recombinant proteins. Besides the hurdles of purification and refolding the recombinant proteins, the major drawback of the two systems is that bacteria don't glycosylate as mammalian cells do and yeast cells are very restricted in their ability to glycosylate. However, these two systems are still in use for production of several proteins (e.g. insulin and some cytokines), although reduced bioactivities of not- or very differentially glycosylated proteins have to be taken into account.

Chinese hamster ovary (CHO) cells are mostly chosen when a mammalian production system is necessary for proper glycosylation. The main reason for CHO is that these cells are widely established as production cell line for therapeutic proteins. But typically for established systems they are not necessarily the best. In case of CHO cells they are capable of glycosylati-

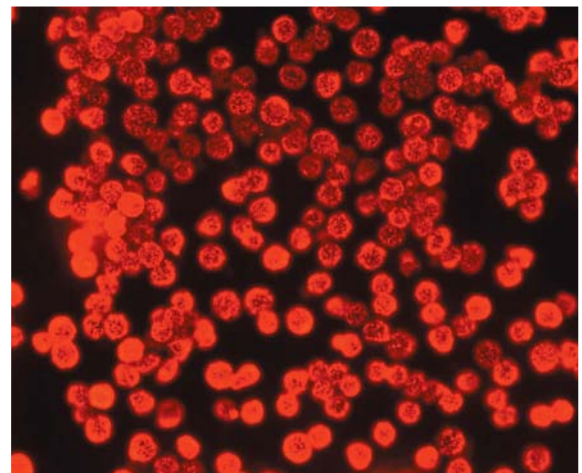
Dr. Hans Baumeister  
hans.baumeister@glycotope.com  
GLYCOTOPE GmbH

A human cell line glycoengineered for expression of a sialic acid free glycotope

wild-type cells after mutagenesis



glycoengineered cells



Immunocytochemical staining for a sialic acid free glycotope (Thomsen-Friedenreich): A human cell line glycoengineered for expression of a sialic acid free glycotope. Cells were visualized by immunocytochemical staining for the sialic acid free Thomsen-Friedenreich disaccharide. The left and right panel shows cells before and after Glycoengineering, respectively.

on in the complex manner of mammalian cells. However, the complex mammalian glycosylation machinery made up by more than 200 enzymes and transporters differs from cell type to cell type of one organism and even more from species to species. Since CHO cells are not of human origin, glycoproteins produced in CHO are differentially glycosylated compared to their native human counterparts.

The main differences which are known and biotechnologically approached are that CHO cells

- 1) sialylate at a lower degree
- 2) lack a complete sialylation pathway
- 3) integrate additionally to the common sialic acid (NeuAc) another sialic acid (NeuGc)
- 4) contain terminally bound  $\alpha$ -1-3 galactose which is absent in human cells
- 5) produce a CHO-specific glycosylation pattern

All these differences have tremendous impact on the efficiency of most therapeutic proteins, with regard to serum-half-life, immunogenicity and bioactivity of most glycoproteins and heavily affect dosages and side-effects. For example a low level sialylation leads to a short serum-half-life of therapeutic proteins and non-human types of glycosylation (e.g. NeuGc and  $\alpha$ -1-3 bound galactose) are recognized by natural human antibodies leading to degradation and autoimmune symptoms.

### Optimised glycosylation of therapeutic proteins by glycoengineering

Glycoprotein-biotherapeutics are produced in cellular systems from bacteria to mammalian cells which are unable to glycosylate or glycosylate not human-like. To develop new products with higher activity, longer serum-half-life and lower immunogenicity glycoengineering technologies are used. Glycoengineering not only strives for a correct human glycosylation but especially for an optimised glycosylation with highest therapeutic efficiency. Such Glycoengineering technology is developed by GLYCOTOPE GmbH allowing the production of recombinant proteins with defined and optimised glycosylation pattern.

At GLYCOTOPE, we developed a Glycoengineering technology to generate new cell lines with defined glycosylation pattern. Based on this technology we engineered a human cell line (GlycoExpress™) that is capable to produce glycoproteins with fully human glycosylation and optimized sialylation. The cell line is characterized by optimal biotechnological properties (suspension cell line, very high proliferation rate, easy cell cloning and molecular biological modification, serum free and virus free, production of high amounts of glycoproteins). Based on a certain genetic defect glycoprotein production by

GlycoExpress™ could be achieved with a defined sialylation degree from virtually 0% to 100% by special media supplementation (metabolic engineering). Thus, 1) GlycoExpress™ is ideal for screening for the optimally sialylated form of a therapeutic protein, optimal for serum-half-life, activity, immunogenicity and stability of the protein. 2) The optimal sialylation conditions are then relatively easy transferred to a production process. Exactly this 2-step strategy was pursued by expressing a human growth factor in GlycoExpress™. Due to an optimized sialylation degree (high but not the highest) this factor was found to be manifold more active compared with the commercial equivalent and expecting good serum-half-life.

Some of the bestsellers among therapeutic proteins will soon lose patent protection. Therefore, a market for off-patent biotherapeutics is expected to open up \$30 billion in worth and with a growth rate of 10 per cent a year (see table page 13). Glycoengineering technologies are ideal for production of the next generation of therapeutic proteins, such as Erythropoietin, G-CSF, GM-CSF and FSH, especially as the IP situation allows a new patent protection of the product and the process. Of special interest is a new expression system for recombinant human antibodies to obtain functional antibodies mediating cytotoxicity. Another application of Glycoengineering is to generate new cell lines expressing carbohydrate antigens that are highly specific for tumor cells. Such cell lines are successfully developed at GLYCOTOPE for use as cellular vaccine against cancer and to generate therapeutic antibodies against these tumor antigens. ●●●

### Imprint BioTOPics 24

Publisher:	BioTOP Berlin-Brandenburg Fasanenstrasse 3   D-10623 Berlin Phone: +49 (0)30.318622-11 Fax: +49 (0)30.318622-22
Editor:	Christina Puhan   Dr. Gesche Harms
Design, Production	
Advertisements:	tnw. the networks. Ltd. Berlin
Translation:	All-Lingua Ltd. Bad Tölz
Figures:	Page 4: Sami-Al-Rawi   page 5: According to Sharon, N. and Lis, H.: Lectins: from hemagglutinins to biological recognition molecules. A historical overview. Glycobiology (2004) published online. All other figures supplied by the authors or BioTOP Berlin-Brandenburg © November 2004