1 Chapter One: Introduction

Regarding the global pharma market, it is remarkable that the expected market-share for biologicals gets up to 11% in 2005, producing sales higher than 50 billion US$ [1, 2], tendency increasing (Figure 1). Most of them are glycoproteins, produced in mammalian expression systems like Chinese Hamster Ovary (CHO), Baby Hamster Kidney (BHK) and Myeloma (NS0, Sp2/0) cells.

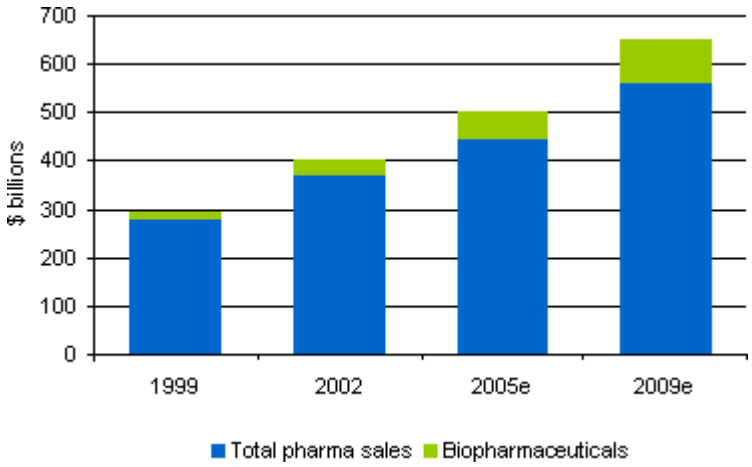

Figure 1: IMS Health - Biopharmaceuticals' share of global prescription sales

In the past, much research has been focused on how to increase productivity of those cells (product quantity). However, general strategies to ensure and to control product quality even in early phases of biotechnological process development are the main challenging issues nowadays. To be able to select the clone with the optimal product quality during cell line development reduces costs and avoids the commitment of unnecessary process development resources.

Characteristic for mammalian expression systems is their ability to modify proteins post-translational. One of the most important post-translational modifications is protein glycosylation because of its influence on pharmacodynamics, pharmacokinetics and immunogenicity of the product [3]. Glycosylation is the attachment and modification of oligosaccharide structures to the polypeptide chain during the process of protein formation

inside the endoplasmic reticulum (ER) and Golgi vesicles. It is initiated by the transfer of an oligosaccharide precursor onto a nascent polypeptide chain inside the ER, followed by multi-step tailoring and modification of the oligosaccharide structure inside both the ER and the Golgi vesicles.

Because of their glycosylation, glycoproteins are heterogeneous. They generally exist as a set of different glycoforms in terms of the fraction of potential glycosylation sites, that are occupied, as well as the oligosaccharide profile at each site. Due to its sensitivity, glycosylation is subjected to variations within the biotechnological production process [4]. For example, the heterogeneity of glycoproteins can be influenced by cell culture conditions.

The selection of a specific expression system as well as specific developed process parameters are often derived from a predefined product glycosylation of the manufactured glycoprotein.

To guarantee a reliable product quality, glycosylation analysis has to be part of the regular quality control. Because of the complexity of the glycan structures, their analysis contains a broad spectrum of methods like High Performance Liquid Chromatography (HPLC) and Mass Spectrometry (MS).

Another important issue in biopharmaceutical manufacturing today is the improvement of the effectiveness of the biotechnological production processes. The majority of biotechnological products today are monoclonal antibodies which are manufactured in batch or fed batch processes. This is due to an optimal space-time-yield for this kind of glycoprotein that is robust and less sensitive to culture variations.

However, a lot of new developed products are more complex glycoproteins which tend to be instable in the culture medium (factor VIII) or incline self-inhibition (interferon-β) during the upstream process. Production processes for this kind of glycoproteins require continuous product harvest with simultaneous long term fermentation. Compared to batch processes, continuous production processes take longer than batch processes (on average: 1 to 2 weeks vs. several months). Regarding comparability control, a batch consistency analysis has to be transferred into an "intra"-batch

consistency analysis that also needs acceptance criteria for process management.

1.1 Thesis Objective

This work has two objectives:

- The establishment and optimization of glycoanalytical methods which enable very precise quantification of relative differences in N-glycosylation patterns, independently from a detailed glycan structure characterization. The combination of several profiling methods with high resolution and sensitivity on the one hand and the normalization of different, often unknown, glycan structures to the same detection response factors on the other hand, seems to be the optimal strategy to solve this problem. These techniques shall not only be able to profile microheterogeneities in N-glycan structures, but also to detect even minor differences in apparently "consistent" production processes.
- The established methods shall be applied to different model proteins and the limits of the methods shall be statistically evaluated and proved within a validation procedure. Furthermore, the applicability of these methods shall be demonstrated within the clone selection procedure and during process development, especially to understand the influence of cultivation time and production system on glycosylation.

1.2 Thesis Organization

This thesis consists of seven chapters in total. Following a brief introduction in Chapter One, a literature review of protein glycosylation is presented in Chapter Two. Chapter Three gives a short description of the clone creation, the upstream and the downstream procedure of the recombinant produced model proteins for this study. Chapter Four describes the evaluation of the analytical methods used for glycosylation analysis in this thesis. The validation approach of the analytical techniques is described in Chapter Five. In Chapter Six follows a description of the applications of the above methods at ProBioGen. Three different studies that were tailored to the characteristics

of ProBioGen's production technology were performed to examine the influence of several upstream parameters on the glycosylation of the two model proteins. In detail, the influence of long-time cultivation, the exploration of different clones and the effect of different cell densities were investigated. Finally, conclusions as well as suggestions for future work are offered in Chapter Seven.

1.3 Role of ProBioGen

This doctorate study was an external graduation and most of the analytical work was accomplished in the laboratories of ProBioGen AG (www.probiogen.de) in Berlin.

The company ProBioGen is a specialist in mammalian cell engineering and cell culture. It offers their customers a complete service spectrum: from gene to market supply, providing every step from protein expression to GMP manufacturing, including full project management support. These services are fully integrated to ensure that each individual development step, starting from the vector construction, will contribute towards obtaining the most efficient manufacturing process at the end.

Among other things, ProBioGen produces API according to GMP-guidelines as a contract manufacturing organization (CMO). The manufacturing facilities have a Quality Management System according to ISO-DIN 9001:2000 and are certified according the EU-Guidelines for the manufacturing of investigational medicinal products.

As ProBioGen possesses a multi-product facility and GMP production capacities up to 600L scale, the company is able to manufacture glycosylated proteins for pre-clinical and clinical studies I and II.

The two model-glycoproteins are not described in detail in this work. This is due to the fact that both proteins were part of development projects for customers and the publication of product details would infringe their intellectual properties.