Early stage development of biologics: Monitoring glycosylation to generate the best quality profile

Protein quality is a topic of increasing importance in bioprocessing as several quality factors proved to have a substantial impact on the clinical behavior of biologics. Over the past decades, glycosylation appeared to be a key quality attribute for many products and should be optimized to provide high potency biotherapeutics.

Key features

It has been well established that the glycome of biologics is driven by the host system and largely dependent on multiple parameters including cell line, cultivation mode and processes. So far however, it has been challenging to predict, achieve and maintain preferred glycosylation profiles from clone isolation to bioproduction. This is not merely incidental but proved to be crucial for product lifespan, immunogenicity and therapeutic action. There is thus a need to select drug candidates with the most appropriate glycoprofile, optimize production processes and satisfy increasingly stringent regulation.

Improvment in cell line development largely include a stepwise control over post translational modifications to :

- optimize and increase bioprocess efficiency to boost productivity
- generate high yielding clones and produce biosimilars as well as new biologics with the desired glycosylation
- in antibodies more particularly, govern glycosylation and switch functionality from superagonistic (low fucose) to antagonistic activities (6-linked sialic acid).

Glycomonitoring

Glycosylation patterns of both product and cells offer an accurate assessment of cell metabolic changes during bioprocessing because they reflect the coordinated action of numerous glycosylation enzymes during protein biosynthesis. Changes in glycan structure can occur within hours and lead to a variable pattern for a given cell line throughout passages and/or days of production. Accordingly, fast track glycomonitoring aims at selecting the desired product glycoprofile during early stages of product development. It is a customizable method which can be run daily at line. It helps at reducing product heterogeneity at all steps and ultimately improve product safety and efficacy.

Glycotests

Glycans are variable because they are both protein-, cell- and process specific. Biologics can receive a quite distinct glycosylation pattern when expressed in insect, plant, mammalian or human cell lines (Figure 1). In all cases, the presence of antigenic sugars should be tested and avoided.

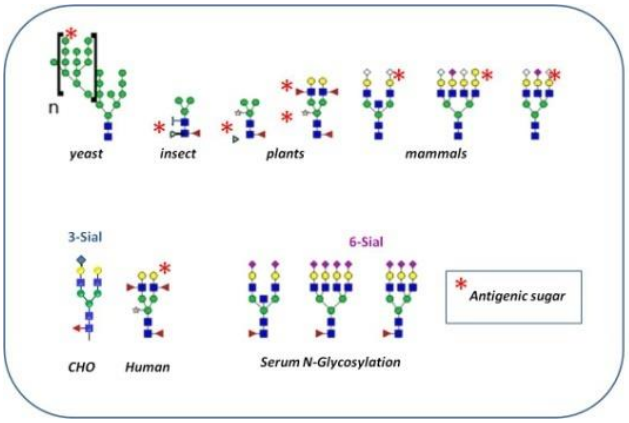

Figure 1 : Typical glycosylation in expression systems

Glycotests can thus be applied to:

- assess changes in glycosylation in upstream and downstream processes
- prevent loss of sialic acid throughout bioprocessing
- compare products from different origins
- accelerate cell line optimization
- speed up product development

N-linked glycans cover a repertoire of about a thousand of different structures which can be recognized by lectins according to both their sugar and glycosidic linkage: assays have thus been designed to map the variable heterogeneity in branching and terminal glycosylation .

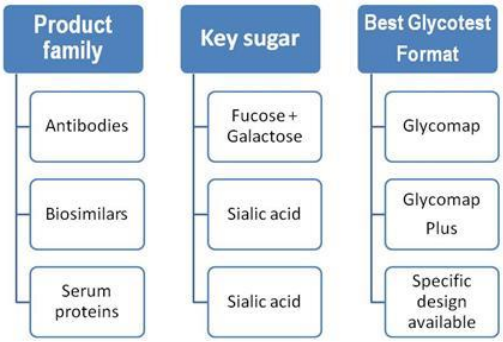

Figure 2: Glycotests cover all families of biologics

Glycotests are customizable multi-lectin assays which identify changes in product and/or cell glycoprofile. They are constructed as two formats :

1-Glycomap format : the lectin panel accomodates for the various classes of N-linked glycans. As shown in Figure 2, glycotests can be applied to all families of biologics and allow to focus on key sugar(s) of special biological interest.

2-Glycomap Plus format : glycoproteins may contain multiple glycosylation sites, multi-antennary glycans as well as O-glycans. O-glycans are highly heterogeneous. Accordingly, the Glycomap Plus format may be complemented by additional lectins on a case-to-case basis.

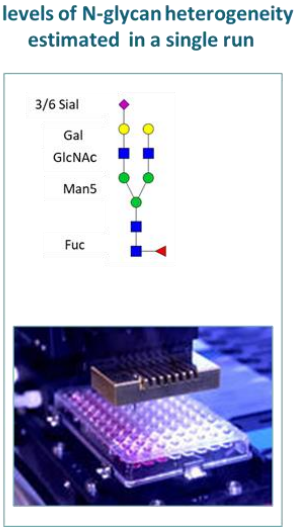

Figure 3 : Glycotests are tailored to map glycan polymorphism

Glycotests are performed on the intact protein : no release nor labeling of the glycans is needed. Each sample (<10µg) is analyzed with the best suited panel of lectins within hours. All the formats may be specifically tailored for the product and process (Figure 3). These assays thus allow fast screening and accelerate development timelines.

Antibodies

Recent advances in antibody glycosylation revealed the presence of a specific sugar switch which governs antibody conformation towards a close or an open conformation (Figure 4). Fc-linked glycans govern binding to activating or inhibitory receptors as well as to lectin receptors.

Fucose, galactose and sialic acid, specifically in the 6-linkage, are the key sugars for antibody targeting to immune cells. Glycotests therefore assess the glycoform content at all steps and in turn helps to optimize antibody glycoprofiles

and maximize efficiency throughout bioprocessing.

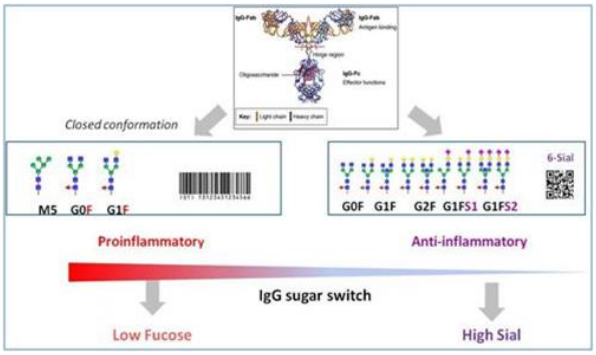

Figure 4: Glycosylation barcode in IgG biopotency

Customizing antibody glycosylation during early stage development

Tailored glycotests offer a case-to-case analysis of the protein glycoprofile from cell line management to batch delivery. This is to select the best samples which will enter further analytical and biological characterization to comply with regulation.

Glyco-optimization can proceed throughout cell line development without impacting growth rate, cell viability and productivity. Figure 5 provides an example of screening 4 media/feed conditions of a high producing clone. It can be seen that both the fucose and galactose content of the antibody can be significantly altered by culture conditions. Glycomonitoring thus provides a quality testing to maximize productivity and product consistency.

Glycoprofile customization of an antibody-based product allows to select very early the most appropriate array of glycans adapted to a pro-inflammatory or anti-inflammatory glycoprofile.It therefore advantageously combines quality to quantity to increase product performances.

Customization of mab glycoprofile can be achieved very early in process development. It can simultaneously speed up the selection of clones and identify the optimal quality of the product: glycotests, data interpretation and further analysis are fully integrated to identify the best glycoprofile which improve product performances. This aims at matching productivity, analytical characterization (MS, LC-MS, CE..), immuno monitoring and biotesting.

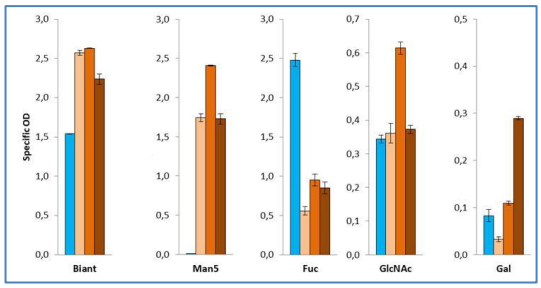

Figure 5 : Customization of an antibody glycosylation during cell line optimization

Summary : using glycosylation as a barcode for quality testing can accelerate product development to define the best tolerated product, improve kinetic parameters and drug efficacy.

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