

MICROPLATE BASED GLYCAN MONITORING

to maximize quality of biotherapeutics

As the molecular complexity and number of biologics increase, challenges in product development increase as well. For many next-generation biologics like antibodies, product development often results in the production of different yet highly similar versions of the desired protein, making cell line management and process development complicated. From lead identification to full scale production, regulators are now requesting deeper knowledge about both the process and the product.

Productivity remains the key driver for process development and manufacturability, opening new challenges in product quality. In early development, product quality is most than often drastically altered because cell factories poorly adapt to high density culture and fail to deliver high titer production. Upscaling processes further introduce uncertainties which also impact product quality.

Cell line development is a lengthy and hazardous process which can deliver a wide array of variants. Go/no go decisions are to be made quite early in process development to maximize manufacturability. Among them, glycosylation is key to maintain product quality as it impacts product lifespan, immunogenicity and efficacy. A repertoire of >1000 N-glycans can be selected by each cell like CHO or HEK in less than 2hrs, delivering numerous variants of the protein over days of production and making product consistency difficult to achieve.

Glycosylation is protein-, cell- and process specific because it is synthesized by a host cell machinery constantly adapting to growth and nutrients during production.

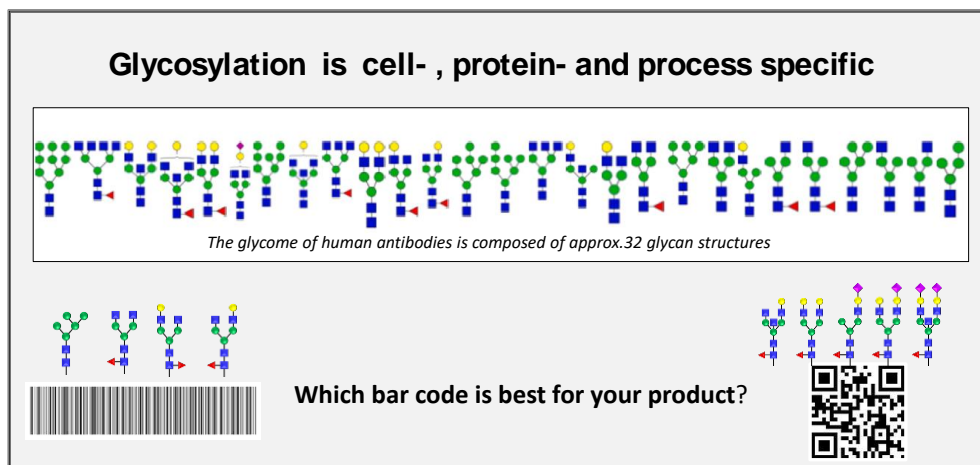


Figure 1: Barcoding antibody glycosylation to maximize product quality.

Glycan heterogeneity can be seen as a bar code which reveals product versatility at all steps. As suggested in Figure 1, it can be used to assess and maximize product quality throughout development. Glycan monitoring aims at making decision with confidence and without delay to produce high potency products

How can glycan monitoring advance product quality ?

A key step in quality control is the need to assess when and how cell metabolism and nutrient feeding impact product quality. To produce the desired product, there is a need to control the various variants produced during cell line development as well as during process optimization. Glycan monitoring applies to all biologics as well as biosimilars, serum and fusion glycoproteins i.e hundreds of candidates under current development. It is based on customizable testing which enable rapid, yet accurate, product development at each step.

More especially, glycan monitoring helps to readily maximize antibody glycans in newly designed candidates because in antibodies, glycans function as a physiological barcode which controls effector functions. A specific sugar switch targets the antibody to Fc and lectin receptors and activate/inhibit immune cells. As a result, antibody derived products can show variable efficacy and immunogenicity when their glycopattern does not match with the needed key sugar. It is highly desirable that product development can assess and even decide which glycan pattern is best for product efficacy. Glycan monitoring can thus be used to get this pattern reproducible throughout process development and production cycles.

1-What are glycotests and what are they used for?

Product glycans do not need to be fully characterized throughout bioprocesses. They rather need to be rapidly monitored to reduce undesired variants and obtain the desired glycan patten which maximize product efficacy and achieve product consistency.

Today, a glycan pattern cannot readily be traced by analytics because it requests glycan release followed by highly specialized analysis and therefore, is often outsourced. No data are readily available online. As a result, product glycosylation is often assessed post-production and found to be truncated , delivering a product with reduced efficacy. Also, expensive losses can be encountered during upstream and downstream processing to ensure product consistency. Glycotests offer new analytics to reduce product heterogeneity and maximize quality at all steps.

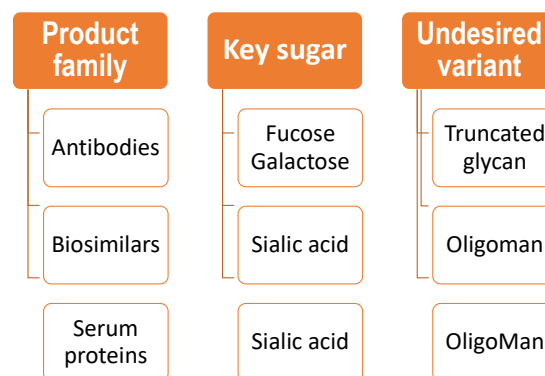


Figure 2: Tailored microplate-based assays to reduce glycan versatility and increase product quality

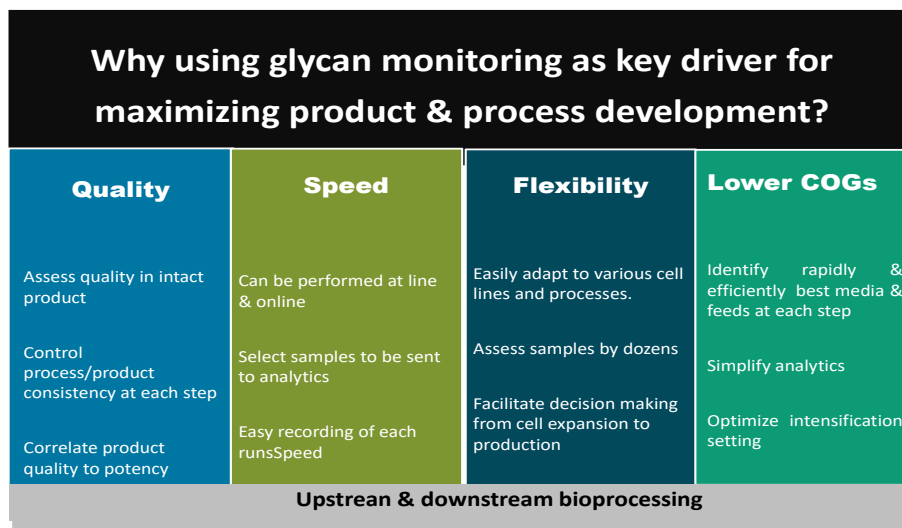
Glycotests present the best solution to provide rapid evaluation of glycan versatility and quantitative data. Various formats are designed for antibodies, biosimilars and fusion proteins. They can be run by hundreds on the intact protein within hours at a reduced cost. The assays utilize lectins to bind native intact glycoproteins in an ELISA format. Optimal glycans as well as undesired variants can be identified and measured in the same run. Basically 3 levels of glycan heterogeneity can be monitored using intact proteins (Figure 2). Most than often, glycan completion fails and mannose-rich glycans

accumulate, reducing product lifespan. Alternatively, altered glycosylation may increase product immunogenicity. The assays can be performed at all steps to sustain high productivity of the desired variants. Overall, they facilitate quick decision making, maximize product quality and accelerate development timelines.

2- Challenges and solutions : mapping a path for quality

Microplate lectin-based assays enable hundreds of samples to be measured in the same run, with the ability to analyze multiple cell lines, media formulations and/or production batches simultaneously. Miniaturizing glycan screening throughout bioprocessing reduces handling, saves on consumables and increases efficiency (Figure.3). Glycan monitoring has potential for use in a wide range of processes and offer key features to maximize product quality daily in the most flexible way and store data to meet regulatory compliance.

Figure 3: Glycan monitoring offers a new control strategy to produce better and safer product



Conclusion

Glycosylation is now well recognized as a key quality attribute for many biologics. Glycan monitoring provides a customizable screening which can govern and control product versatility at all steps of production to reduce variants and improve product potency. Process optimization can be looked at in two ways: (1) improving product quality by selecting desired variants in real time and (2) increasing manufacturability by simplifying protocols and process conditions. To this end, tailored assays aim at improving biological performances of the product and keep them reproducible during process development. Such control strategy greatly facilitate decision making before entering further analytical and biological characterization as it derisks and accelerates product development to deliver better and safer bioproducts.

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Find out more about the assays at www.siamed-tech.com

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