Rapid glycan screening assays for monitoring product quality in bioreactor runs

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Abstract

Background and novelty: Glycosylation of therapeutic antibodies is a critical quality attribute (CQA) which affects stability, aggregation, serum half-life and immunogenicity of the drug substance. The control of glycosylation during cell line development receives increasing attention because different studies have shown that small-scale fed-batch cultures are not only predictive for product titers but also for glycosylation at larger scales. Traditional analytical methods are not able to cope with the high numbers of samples that are generated during this process, because they are too labor-intensive and slow. We developed the PA-201 glycan screening assay to detect differences in product glycosylation for many samples in a matter of hours rather than weeks. This allows to screen more clones and cell culture conditions for glycosylation and include this important CQA into the selection process.
Experimental approach: The PA-201 assay utilizes affinity capture beads and fluorescence labeled lectins that detect different types of glycosylation. Fluorescence read-out is performed on common plate readers.
Results and discussion: We present case study data examining the differences in glycosylation of different molecules that were produced in different cell culture conditions. We highlight data that show how product quality changes over time during bioreactor runs and how this information can be used to select optimal process conditions. Furthermore, we benchmark the PA-201 assay against analytical standard techniques such as UPLC or MS peptide mapping.

The PA-201 glycan assay provides a fast method to determine relevant differences in product quality very early in the cell line selection process, e.g. 96 DWPs. We believe that this technology will close an important analytical gap in cell line development.

Assay principle and workflow

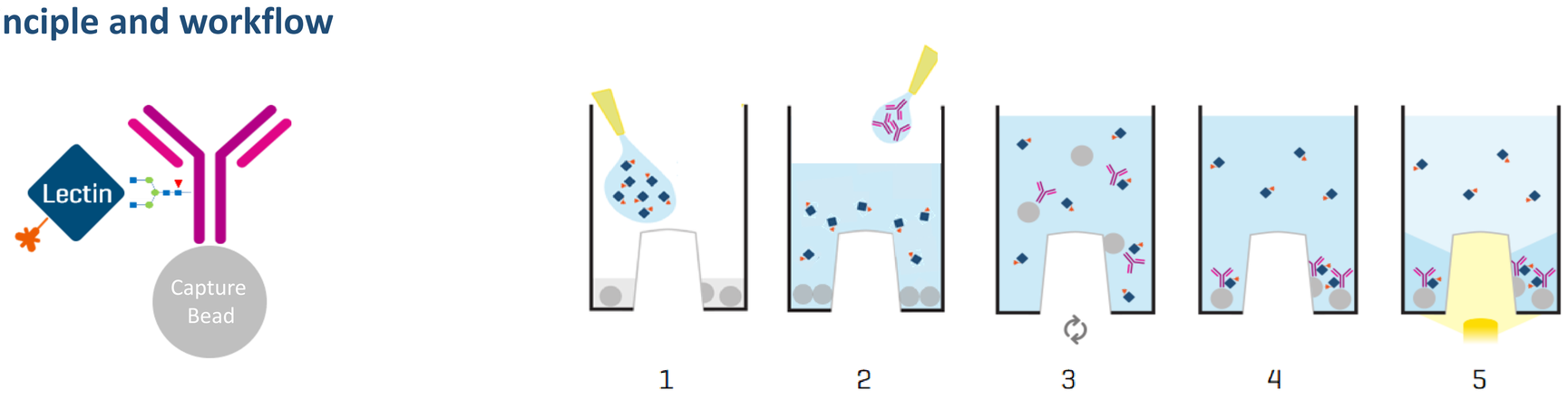

Figure 1. Workflow for glycosylation analysis with the PA-201 glycan assay kit
The complete assay is performed in the 384 well PAIA*plates*. At first, the different lectins (e.g. for detecting sialic acid, galactose and high mannose) are added into separate wells that are already pre-loaded with capture beads [1]. The samples are added to the wells (typically 5 - 10 µL @ 100-200 µg/mL) [2]. After shaking for 45 min [3] and bead settling [4], the plates can be measured on a plate reader [5]. The whole process can be easily automated and takes roughly 60 minutes.

PA-201 glycan assay in a nutshell

Assay type	No-wash, bead-based, sandwich assay Working with intact glycoproteins
Detection	Fluorescence read-out using labeled lectins
Glycan features	Fucose, Galactose, High Mannose, Sialic acid
Sample requirements	1 µg of protein per well (e.g. 5 µL @ 200 µg/mL)
Sample throughput	Up to 96 samples per 60 minutes (1 lectin in triplicates)
Instruments required	Orbital shaker, Fluorescence plate reader

Rapid glycan profiling in media optimization

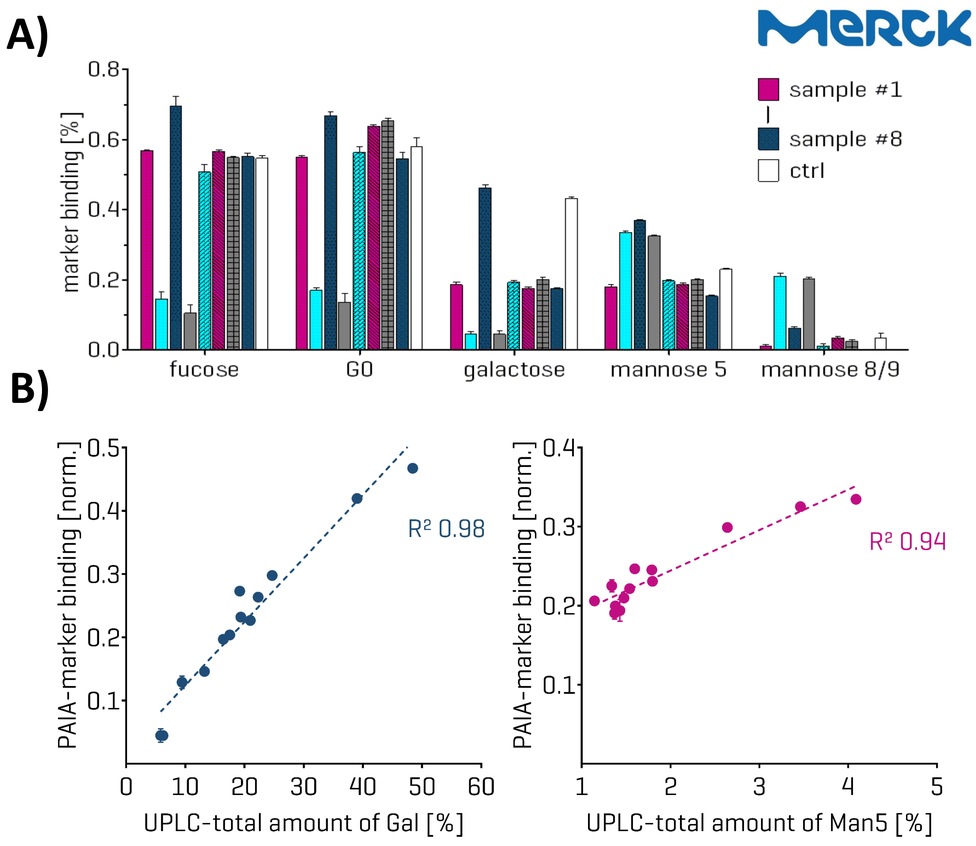

Figure 2. Media optimization study
A) Rapid profiling of selected samples from different wells of a 96-DWP media screening campaign. Differences in glycosylation can be detected and favorable culture conditions and media compositions can be identified.
B) Correlation of PA-201 glycan assays results with 2-AB UPLC (data courtesy of Merck).

Monitoring sialylation of a Fc Fusion protein in bioreactor runs

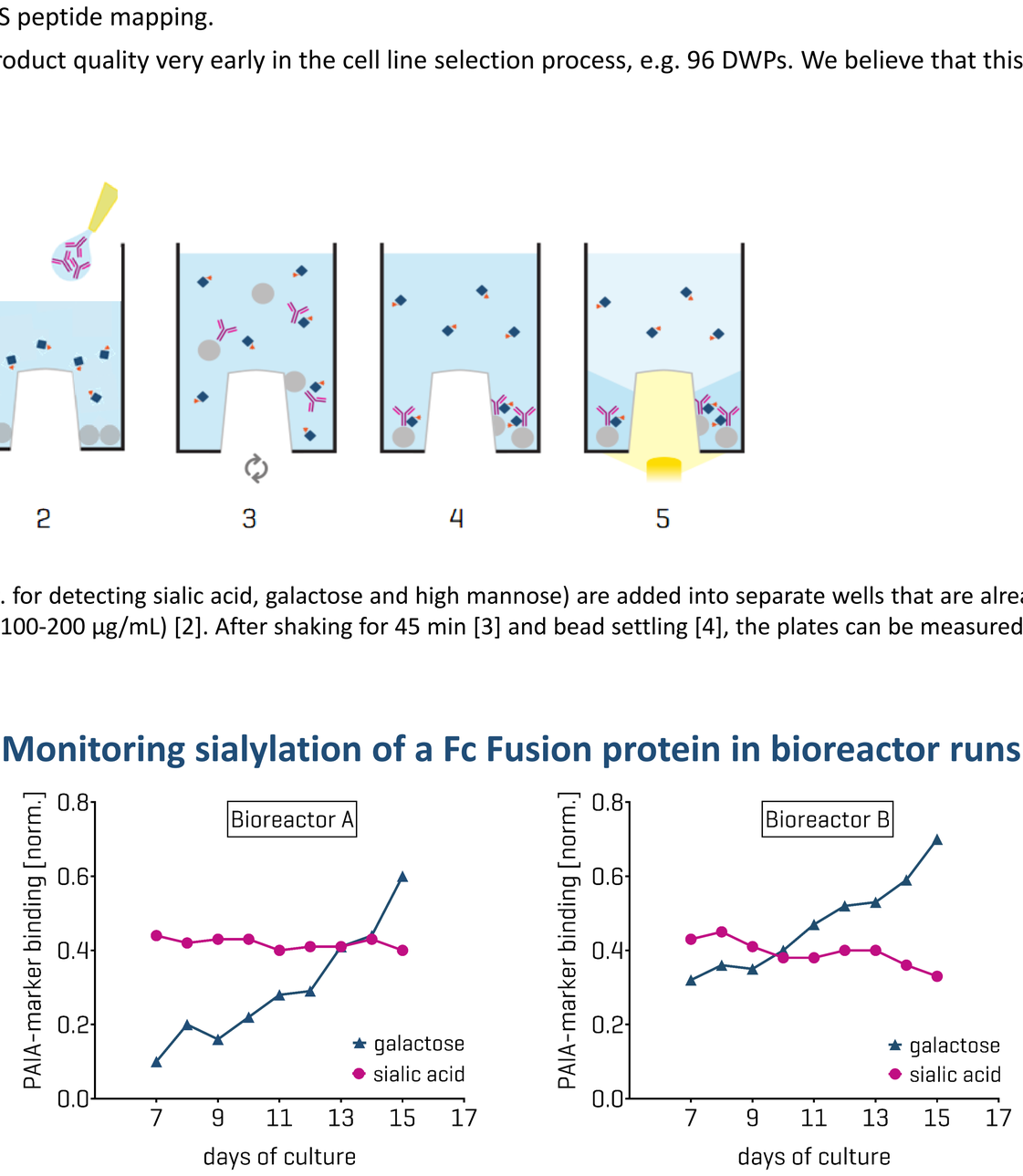

Figure 3. Sialylation and galactosylation monitoring in parallel bioreactors
Sialylation is an important quality parameter for Fc-fusion proteins which can change over the course of a bioreactor run. Screening for galactosylation at the same time provides important complementary information. Using the PA-201 glycan assays, these trends can be analyzed quickly and on a high number of samples.

High Mannose monitoring during bioreactor runs

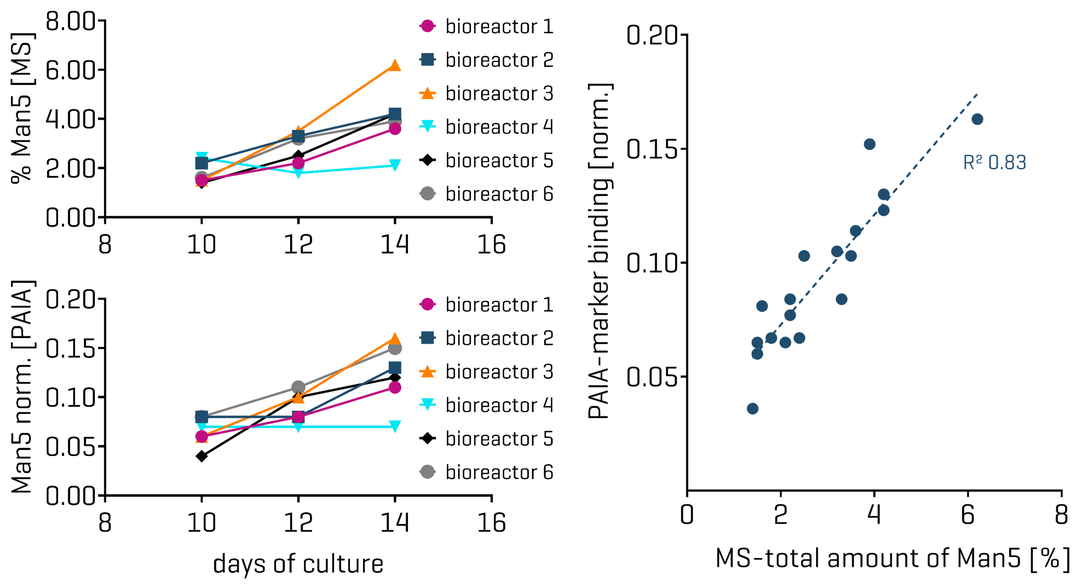

Figure 4. Results from a High Mannose monitoring campaign in 3 L-Bioreactors
Samples from days 10, 12 and 14 of six parallel bioreactors were analyzed using the PA-201. Differences in highly mannosylated IgG over time could be detected and were shown to correlate well with peptide mapping data from MS. The PA-201 can be used to quickly identify clones and cell culture conditions that result in highly mannosylated product.