Modulating *N*-glycan fucosylation of IgG1 using GlymaxX® technology and monitoring of fucosylation with a high throughput lectin-based glycan screening assay

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Abstract

Fucose is a monosaccharide and naturally present in glycoproteins like antibodies. In therapeutic monoclonal antibodies fucosylation is exclusively found as core fucosylation in *N*-glycans on the Fc domain. The absence of core fucosylation has shown significant potential for increasing the antibody-dependent cellular cytotoxicity (ADCC) and may therefore increase the clinical efficacy of antibodies. Here, we describe how a GlymaxX® producer cell line (from ProBioGen AG) generates IgG1 with varying degrees of core fucosylation and how these levels can be easily assessed with a high throughput lectin-based glycan screening assay (PA-201, PAIA Biotech GmbH). This assay can analyze cell-free supernatants directly and measures up to 96 samples in less than two hours total handling time. We also demonstrate that the results of the supernatant samples correlate very well with standard hydrophobic interaction liquid chromatography (HILIC) after Protein A purification. The lectin-based glycan screening assay represents a much faster and less labor-intensive alternative for the determination of core fucosylation levels in monoclonal antibodies.

Principle of PAIA glycan screening assay

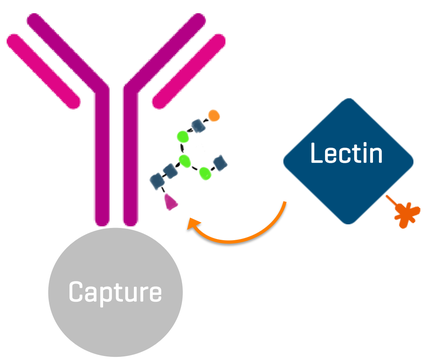


Figure 1. Principle of the PA-201 glycan screening assay for Fc containing proteins
The PA-201 glycan screening assay is a sandwich immunoassay, in which capture beads bind the Fc domain of the analyte and fluorescence labelled lectins bind the glycans present on the antibody. The whole assay is performed in the 384-well PAIA*plates*.

Results

Fig. 2.: Correlation between the amount of fucose added to the cultivation medium in a 14-day fed batch and binding of core fucose-specific lectin to Trastuzumab.
Lectin binding to supernatant samples, supplemented with increasing amounts of fucose. Two independent experiments with the PA-201 glycan assay (mean of technical triplicates per sample and experiment) show high reproducibility of the assay with typical CVs of 2%.

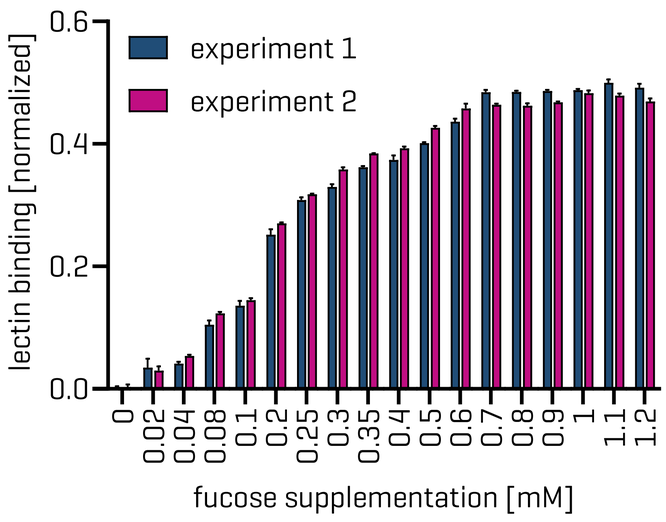


Fig. 3: Increase of fucose addition leads to increased core fucosylation of Trastuzumab.
Relative core fucose content of purified samples were determined by HILIC/UHPLC and plotted against the concentration of fucose added to the cultivation media in a 14-day fed batch.

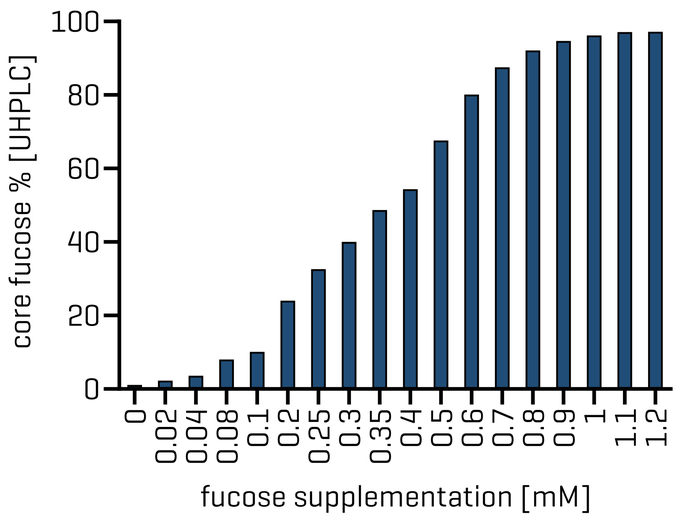
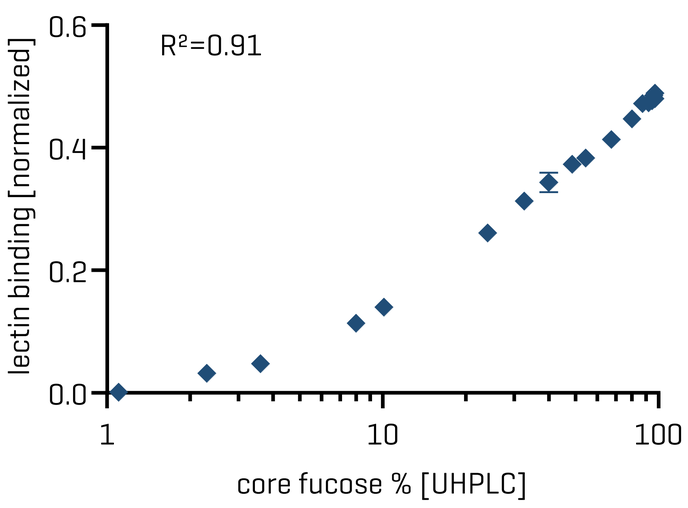


Fig. 4. : Correlation of lectin binding and % of core fucose (UHPLC).
Lectin binding to cell-free supernatant samples with PAIA glycan assay correlates very well with UHPLC data from purified samples (R^2=0.91). Lectin binding data: mean and standard deviation from two independent experiments, technical triplicates per experiment.



Materials and Methods

Cell culture: ProBioGen's CHO.RiGHT® Cell Line Development Platform was used to generate stable IgG1 expressing GlymaxX® cell lines. ProBioGen's chemically defined CD-C6 culture medium with chemically defined feeds, glucose, and fucose ranging from 0-1.2 mM was used. Cells were seeded at 0.4x10^6 viable cells/mL in TubeSpin® Bioreactor 50 (TPP Techno Plastic Products AG) and incubated at 180 rpm, 37°C and 8% CO_2 in a Multitron incubation shaker (Infors HT). The 14-day fed-batch included a temperature shift to 34°C.

Purification of material and determination of fucosylation by HILIC: Purification of harvest supernatants was performed by using Protein A HP SpinTrap™ columns (Cytiva). Samples were desalted and further processed using the "Gly-X™ *N*-Glycan Rapid Release and Labelling with instantPC"-Kit (Prozyme). Labeled glycans were analyzed by HILIC using an AdvanceBio Glycan Mapping column (2.7 µm, 2.1 x 150 mm, Agilent).

PAIA assay workflow: A core fucose-specific lectin was labeled with a fluorophore and used as marker molecule. The PAIA glycan assay for Fc containing proteins (Art.-No. PA-201) was performed according to the manufacturer´s protocol.

Supernatant samples were diluted in a 1:10 ratio to final concentrations between 0.44 and 0.47 mg/mL using Tris-buffered saline with Tween20. The diluted samples were mixed with PAIA sample preparation buffer in a 1:1 ratio and shaken on a Thermomixer comfort (Eppendorf) for 10 min, 700 rpm at room temperature. 50 µL of lectin reagent were added to each well of the 384-well PAIAplate and 10 µL of the treated sample were added, workflow see Fig. 5.

Figure 5. Workflow of the PA-201 glycan screening assay for Fc containing proteins
The whole assay is performed in the 384 well PAIA*plates.* Each well is pre-loaded with capture beads [1]. The lectin and the samples are added to the wells [2]. After shaking for 45 min [3] and bead settling [4], the plates can be measured on a fluorescence plate reader with bottom reading [5]. The whole process can be easily automated and takes roughly 60 minutes.

Conclusions

- The GlymaxX® technology enables flexible adjustment of the Trastuzumab fucosylation level – from afucosylated to fully fucosylated.
- The PA-201 glycosylation assay was successfully used to detect the different fucosylation levels and proved to be very reproducible.
- The PA-201 assay was performed on diluted supernatant samples directly, which significantly reduces sample preparation time and increases the throughput.
- The coefficient of determination [R^2] between the PAIA lectin-binding rates and the results of conventional HILIC/UHPLC is 0.91.

Acknowledgements
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