High Throughput Glycosylation Assays For Glycoproteins Using Lectins

in collaboration with cevec

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Abstract

Background and novelty: Glycosylation of therapeutic glycoproteins is a critical quality attribute (CQA) which affects different properties of the drug such as stability, aggregation and serum half-life. It is critical to control glycosylation during cell line and bioprocess development because culture conditions have a great impact on product glycosylation and because they allow optimization of glycosylation properties, e.g. sialylation. Non-Mab glycoproteins can have very complex glycosylation patterns, making the analysis of many samples in parallel a difficult and time-consuming task. In addition to that, purification of glycoproteins is necessary for analytical methods using released glycans. We present a bead-based assay that does not require the release of glycans and uses lectins to detect differences in glycosylation.

Experimental approach: The PAIA assay uses capture beads carrying a lectin that binds a fluorescent marker for the detection of different types of glycosylation. The glycoproteins in the sample release the marker from the beads depending on its degree of glycosylation. The release of fluorescent marker is measured directly in an entirely plate-based no-wash assay in 384-well plates.

Results and discussion: We present data for assays testing sialylation with different glycoproteins, discuss the influence of cell culture media and supernatants on the assay and the applicability of the assays in supernatant screenings.

Workflow

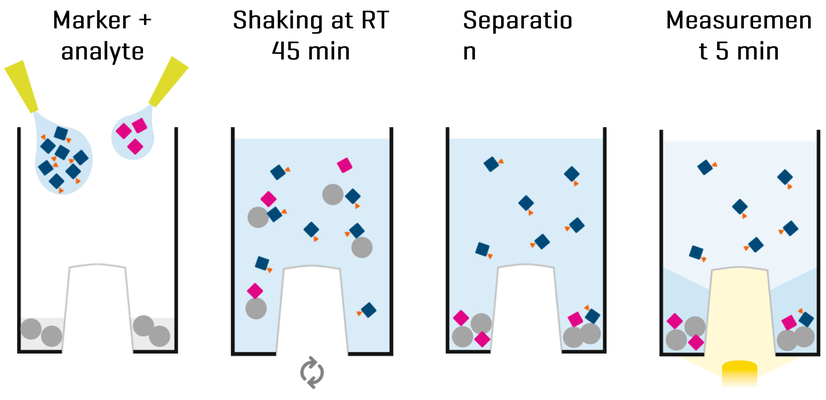
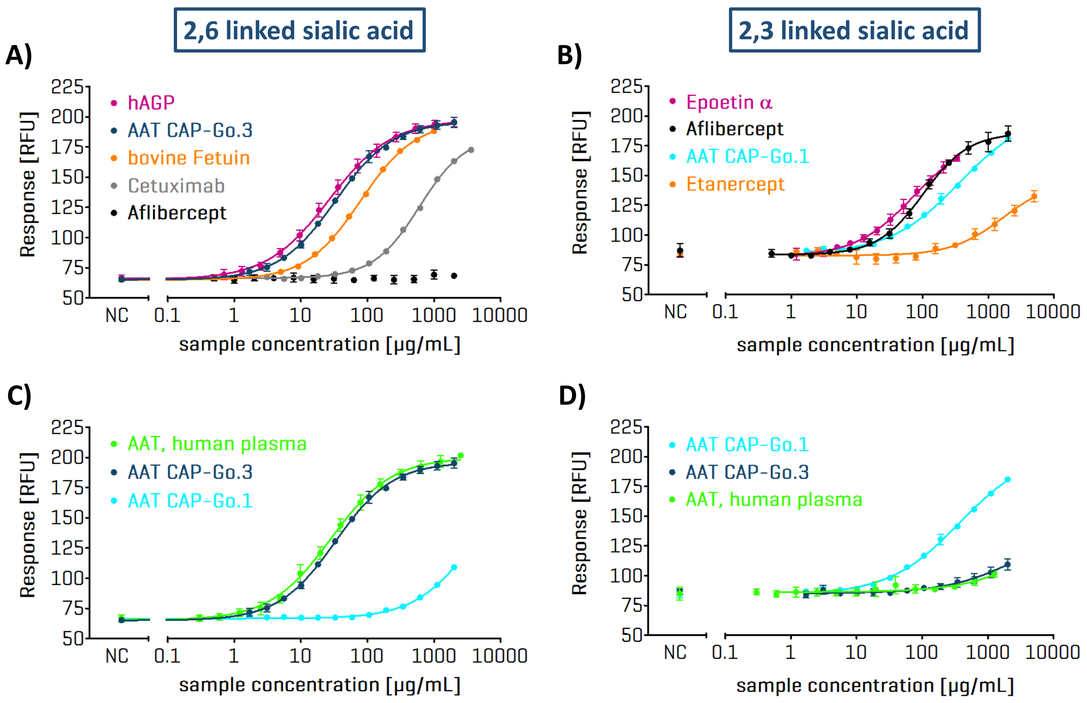


Figure 1. Workflow of the PAIA sialylation assay. Marker and analyte are added to the wells of the PAIA*plate* containing capture beads. The plate is incubated for 45min on an orbital shaker. After incubation and bead settlement, unbound fluorescent marker is measured through the transparent protrusions. Marker and capture beads are either specific for 2,3 or 2,6 sialylation.

Assessment of sialic acids on glycoproteins



E)

name	type	derived from	expected sialylation
Aflibercept (Zaltrap®)	Fc fusion protein	CHO cell line	2,3-SA
hAGP (human alpha-1-acid glycoprotein)	glycoprotein	human plasma	2,6-SA
AAT (alpha 1-antitrypsin) CAP-Go.1	glycoprotein	glyco-engineered human cell line	2,3-SA
AAT CAP-Go.3	glycoprotein	glyco-engineered human cell line	2,6-SA
AAT (Prolastin®)	glycoprotein	human plasma	2,6-SA
Cetuximab (Erbitux®)	IgG	murine myeloma cell line	2,6-SA
Etanercept (Enbrel®)	Fc fusion protein	CHO cell line	2,3-SA
Epoetin α (Epoetin alfa HEXAL®)	glycoprotein	CHO cell line	2,3-SA
bovine Fetuin	glycoprotein	fetal calf serum	2,6-SA

Figure 2. Quantification of 2,3- and 2,6-linked sialic acid (SA) on glycoproteins.
Glycosylation assay to investigate 2,6 sialic acid **(A,C)** and 2,3 sialic acid **(B,D)** content on glycoproteins, IgGs and Fc-fusion proteins. The calibration curve of each protein depends on the amount and the availability of the terminal sialic acid.
All analytes are described in table **E**.
CAP-Go cell lines are glyco-engineered cell lines displaying different defined glycosylation patterns. As expected, AAT derived from CAP-Go.3 and human plasma show high levels of 2,6-SA **(C)** while AAT from CAP-Go.1 possesses more 2,3-SA **(D)**.
Presented data were obtained in two independent experiments with technical triplicates.

Discrimination of 2,3 and 2,6 sialylation in cell culture supernatant

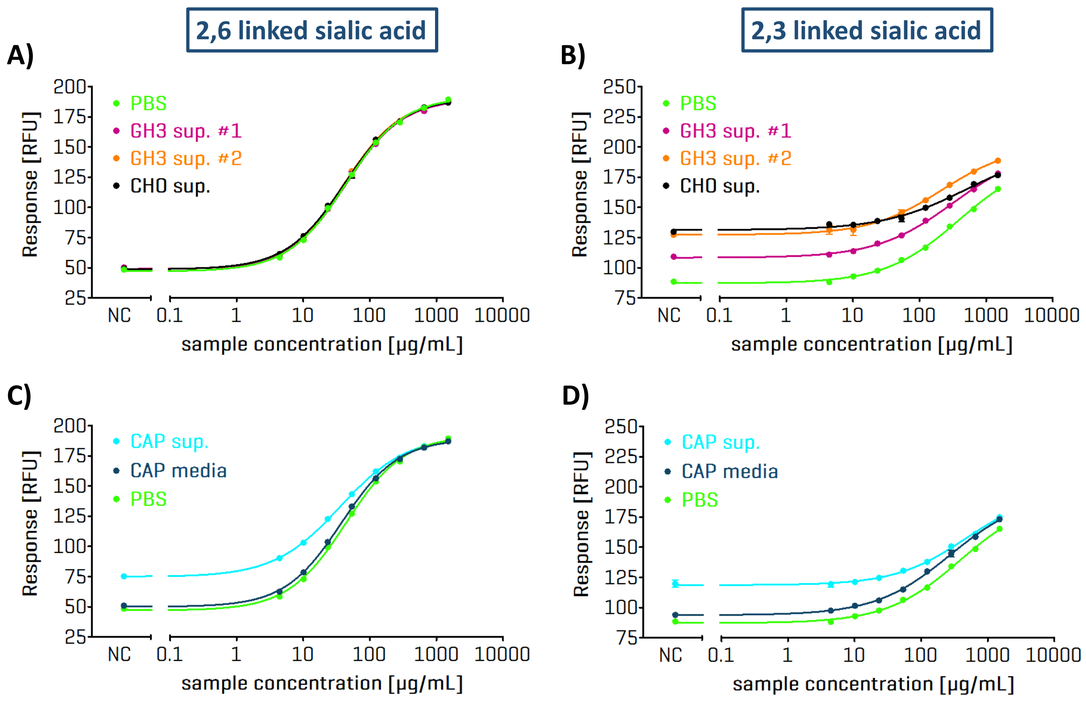


Figure 3. Quantification of AAT spiked into several cell culture supernatants and media.
Glycosylation assay to investigate 2,6 sialic acid **(A,C)** and 2,3 sialic acid **(B,D)** content on AAT from CAP-Go.1 and CAP-Go.3. AAT CAP-Go.3 calibration curves are mostly unaffected by presence of cell culture supernatant, except CAP supernatant where baseline is slightly elevated **(C)**. PAIA assay for 2,3 sialic acid is more sensitive towards cell culture supernatant but discrimination between different amounts of sialylation is still possible.
Presented data were obtained in two independent experiments with technical triplicates.

Screening of samples

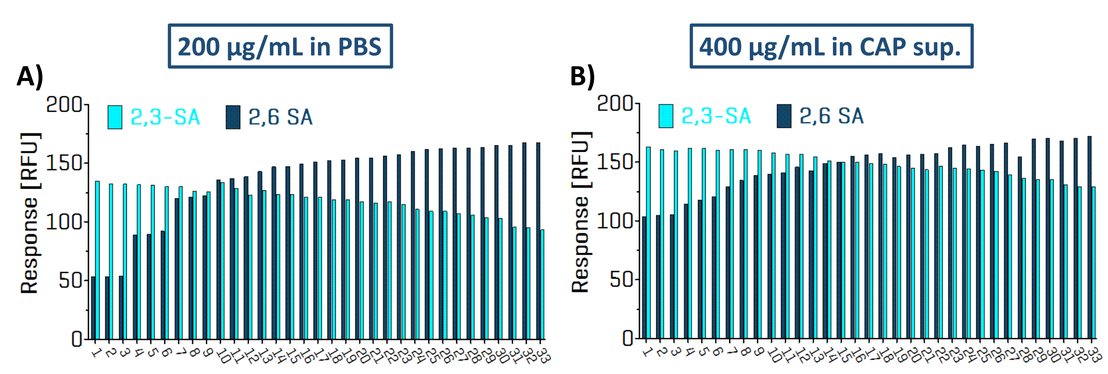


Figure 4. Screening of AAT mixtures from CAP-Go.1 and CAP-Go.3
Samples of AAT derived from CAP-Go.1 and CAP-Go.3 were mixed at differing ratios to the final concentration of either 200 µg/mL in PBS **(A)** or 400 µg/mL in CAP cell supernatant **(B)** thereby creating samples with differing sialylation content. These samples were measured with the PAIA glycosylation assay. As anticipated, samples yielding higher signals of 2,3 sialylation contain little amounts 2,6 sialic acids and vice versa.

Summary

- Glycosylation assays allow discrimination of 2,3 and 2,6 N-sialylation patterns
- Detection of sialylation is possible in cell culture supernatant
- Cell culture samples can be screened for 2,3 of 2,6 N-sialylation

Acknowledgement

- AAT CAP-Go.1, AAT CAP-Go.3, Prolastin®, cell culture media and supernatants were kindly provided by **CEVEC**.

PAIA Assay Applications:

clone screening | glycoengineering | media optimization