Monitoring of Fc Fusion Protein Sialylation during Cell Line and Upstream Process Development using Lectin-based Glycan Screening Assays

Gregory Keil[1], Jack Bruno[1], Sebastian Giehring[2], Yudi Mejia[1], Linda Hoshan[1], Mei Lin[1], Dan Huang[1], Anurag Khetan[1]

[1] Bristol Myers Squibb, Global Upstream & Cell Line Development, Summit, New Jersey, United States

[2] PAIA Biotech GmbH, Cologne, Germany

Introduction

- Cell line development (CLD) is responsible for the stable transfection of Chinese hamster ovary (CHO) cells with the transgene encoding the protein of interest. During this process, thousands of clones are produced with differing expression levels and product quality attributes of the protein. Extensive clone screening is necessary to select clones with high productivity and appropriate product quality, which can be a labor-intensive process. Clones will have heterogenous glycan profiles, making it desirable to screen for critical glycan species during clone development. Due to the large number of clones produced, using a robust, high-throughput, and reliable glycan screening tool would best fit the needs of CLD.

- Upstream process development (USP) involves the scale up of top production clones into larger bioreactors. Experiments are performed to identify bioprocess parameters that influence productivity and product quality attributes so that critical process parameters can be finetuned to match the desired product quality profile. Glycosylation screening at this stage can give an understanding as to how certain bioreactor conditions, feed supplements, and other process parameters can influence glycosylation.

- Similar to other biologics, glycosylation can have a profound effect on Fc fusion protein pharmacokinetics, safety, and efficacy. Sialic acid is critical to Fc fusion proteins, as sialylation levels can impact plasma half-life. During the process development of this Fc fusion protein, sialic acid was screened for with the high-throughput assay PA-201 from PAIA Biotech.

Methods

- The Fc fusion protein was stably expressed in a CHO cell line. Cells were grown in proprietary chemically defined medium, feeds and supplements. For fed-batch production studies, cells were inoculated in the ambr15 or ambr250 bioreactor systems [Sartorius]. Harvest was performed on day 14.

- Harvest supernatants were passed through a 0.2-micron filter followed by Protein A (ProA) purification. Purified material was run on both the PAIA glycan assay and HILIC chromatography for *N*-glycans.

- The PA-201 assay format is a sandwich immunoassay, in which Fc-specific capture beads bind the analyte and fluorescent labeled lectins specifically bind the glycans present on the bound analyte. The whole assay is performed in the specialized 384-well PAIAplate, which allows for separation of the beads and fluorescence measurement. This fluorescence measurement determines the amount of unbound lectin and the relative amount of lectin bound to the sample using a negative control without analyte (**Figure 1**).

- For the PAIA assay, samples were diluted in media to the same concentration and mixed at a 1:1 ratio with PAIA sample buffer solution. All analyzed samples were ProA purified excluding the ones in the crude supernatant experiment. Samples were run in triplicates, with lectin binding being calculated as the mean of the triplicates.

- Standard analytical methods for glycosylation analysis include mass spectrometry (MS) and liquid chromatography such as UPLC/HILIC. These methods require purified material, involving intensive sample preparation and lengthier processing times.

Table 1: Analytical methods for glycan analysis

	PAIA	HILIC	MS
Throughput	24 samples (4 lectins) 48 samples (2 lectins) 96 samples (1 lectin)	*24-48 samples	*12-24 samples
Time	^4-6 hours	*2-7 days	*2-7 days
Advantage	High-throughput	Accurate quantitation, glycan profiling	Site-specific glycan profiling
Limitation	Relative glycan quantitation	Throughput/time, complex data analysis, N-glycan only	Relative quantitation, complex data analysis

^Turnaround time dependent on number of samples to be prepared.

*Total samples, turnaround time are dependent on sample priority, analytical resources, and organizational structure.

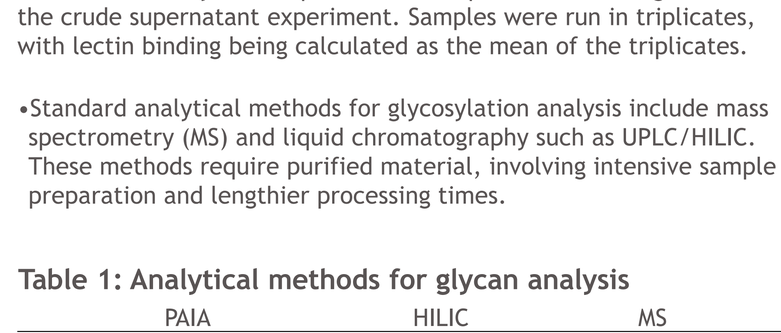

Figure 1: PAIA glycan assay workflow
A) Addition of labeled lectin and sample to the well.
B) Mixing of components to allow formation of bead-analyte-lectin complex.
C) Sedimentation of beads with bound analyte and marker.
D) Fluorescence measurement of unbound marker.

Results

ambr250 USP media/feed study (PAIA assay optimization)

- **Goal:** find optimal assay sample concentration and make first comparisons between PAIA lectin assay and HILIC total sialylation %
- Tested sample concentrations of 25, 50, 100 and 200 ug/mL
- Samples included a commercially available Fc fusion (external control), the molecule reference standard (RS, internal control), and purified material from D12 and D14 of the ambr250 study (two media/feed conditions)

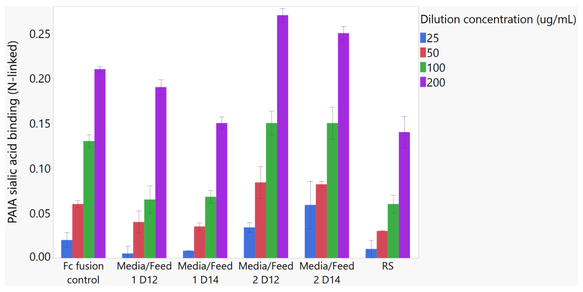

Figure 2: Sample concentration optimization for PAIA assay. Optimized concentration of 200 ug/mL.

- The PAIA lectin binding exhibited a gradual increase in signal from 50 to 200 ug/mL, with only the 200 ug/mL producing lectin binding greater than 0.1 for all samples. Based on lectin binding and signal:noise ratio, 200 ug/mL was chosen as the sample concentration moving forward.
- Media/feed 2 resulted in higher PAIA lectin binding

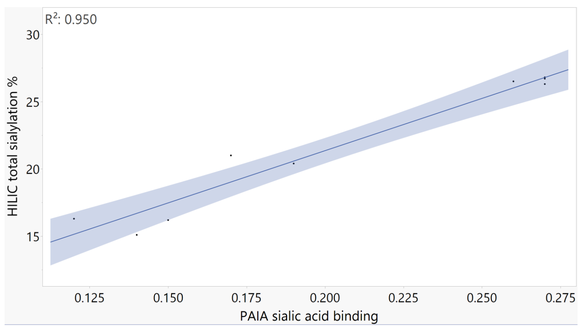

Figure 3: Correlation of PAIA assay with HILIC results of total sialylation % for samples at 200 ug/mL. R^2 = 0.95

ambr15 CLD clone/feed experiment

- **Goal:** identify potential clone and feed combinations with similar sialylation to the RS
- 6 clones in 3 feed conditions to analyze sialic acid heterogeneity between clones and the effect of different feeds on sialic acid levels

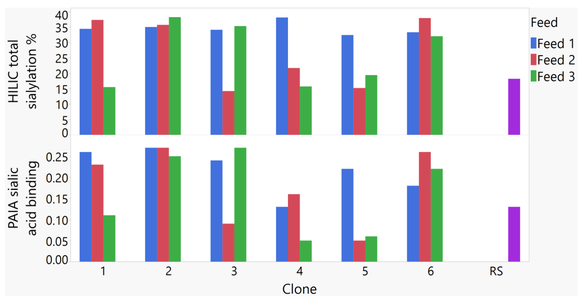

Figure 4: Comparison of PAIA assay and total sialylation % from HILIC in the ambr15 clone-feed experiment

- The following clones were identified as having sialic acid levels close to that of the RS: clone 1-feed 3, clone 3-feed 2, clone 4-feed 2, clone 4-feed 3, clone 5-feed 2, and clone 5-feed 3.
- Clone 2 and 6 sialic acid levels were relatively constant across the feeds, whereas the rest of the clones showed fluctuations in sialic acid for the different feeds.
- R^2 = 0.742 (without outlier clone4-feed 1: R^2 =0.889)

Ambr250 USP DOE and process condition study

- **Goal:** identify bioreactor and media conditions that push the process towards RS sialic acid levels for one selected clone
- Evaluate the effect of additional media/feed supplements and bioreactor conditions on sialylation
- Supplement DOE
  - S1/S2 – supplement 1 and 2
  - C1-C5 – concentration 1-5

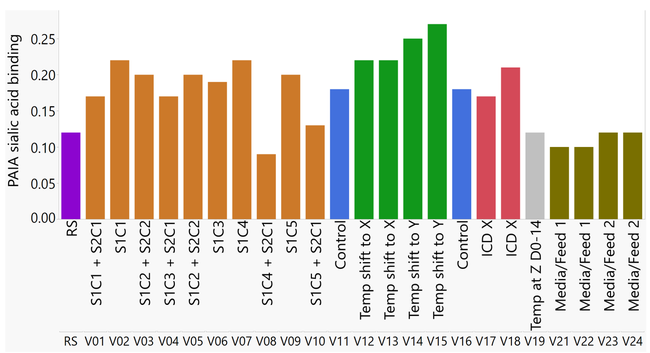

Figure 5: ambr250 process condition DOE with PAIA assay

- Multiple conditions produced sialic acid levels similar to the RS (V08, V10, V19, V21-24). Identified the following trends:
- Sialic acid decrease with increasing supplement 1 concentration
- Sialic acid increase was observed from temperature downshift, which is consistent with literature research

Screening of purified and crude supernatant samples from ambr15 experiment

- **Goal:** test accuracy of PAIA assay for crude supernatant samples
- The ability to use crude supernatant samples would further add to the time advantage of the PAIA assay, requiring no need for ProA purification
- Compared purified and crude supernatant duplicates and correlated both against HILIC total sialylation %

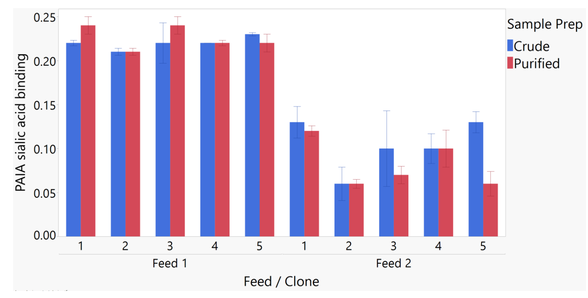

Figure 6: PAIA lectin binding comparison of crude and purified samples

- There was minimal difference in PAIA lectin binding between the crude and purified samples, outside of clone 5-feed 2, which had about two-fold higher binding for the crude sample (purified sample CV = 22%).
- The sample set also demonstrated that feed 2 produced lower PAIA lectin binding for all clones, regardless of sample preparation.
- The R^2 between purified and HILIC was 0.93, and the R^2 between crude and HILIC was 0.974.

PAIA assay consistency – molecule reference standard

For all sample sets, the molecule reference standard (RS) was included in the PAIA assay to serve as an internal control. Including data sets not mentioned in this poster, the RS was used in nine assays throughout the study (Table 2).

Table 2: Summary statistics for RS PAIA lectin binding

Total samples	Range	Mean	Standard deviation	CV
9	0.12 - 0.14	0.1278	0.007	5.5%

Discussion

- With the PAIA results for the ambr15 clone screening and feed experiment, CLD was able to quickly identify potential clone and feed combinations for USP to scale-up and develop the process further.
- Inclusion of the reference standard in all PAIA assays allowed for direct comparison of sialic acid levels with the reference sample RS we were trying to match.
- With initial assay development showing clones producing higher sialic acid binding than the RS, it was decided that it was desirable to shorten this gap.
- Through PAIA screening of feed experiments of certain clones and a process condition DOE, it was possible to identify preferred clones and conditions. The binding consistency of the RS also proved that the PAIA assay was reliable and precise.
- When crude supernatant samples were tested against ProA purified samples we did not observe a change in the PAIA lectin binding of the samples. This showed that the crude supernatant did not interfere with the assay. This would allow for an even faster turnaround, as we do not need to wait for downstream purification.

Conclusions

- The PAIA glycan assay is a versatile tool for rapid screening of sialic acid levels at different stages of the development process. With the availability of this tool, timely measurements of sialic acid could be taken for many samples, allowing for faster decision making and collaboration between CLD and USP groups.
- An orthogonal method, HILIC *N*-glycan, was used throughout the process to verify the sialylation readings of this novel assay.
- Strong correlation between both methods was observed for the Fc fusion protein
- Future work will evaluate this assay for other types of molecules and/or other glycan species.

References

1. Czajkowsky, D. M., Hu, J., Shao, Z., & Pleass, R. J. (2012). Fc-fusion proteins: new developments and future perspectives. EMBO molecular medicine, 4(10), 1015–1028. https://doi.org/10.1002/emmm.201201379
2. Chen, X., Liu, X., Xiao, Z. et al. (2019). Insights into the loss of protein sialylation in an fc-fusion protein-producing CHO cell bioprocess. Appl Microbiol Biotechnol 103, 4753–4765. https://doi.org/10.1007/s00253-019-09850-8
3. Nam, J. H., Zhang, F., Ermonval, M., Linhardt, R. J., & Sharfstein, S. T. (2008). The effects of culture conditions on the glycosylation of secreted human placental alkaline phosphatase produced in Chinese hamster ovary cells. Biotechnology and bioengineering, 100(6), 1178–1192. https://doi.org/10.1002/bit.21853
4. Yoon, S. K., Song, J. Y., & Lee, G. M. (2003). Effect of low culture temperature on specific productivity, transcription level, and heterogeneity of erythropoietin in Chinese hamster ovary cells. Biotechnology and bioengineering, 82(3), 289–298. https://doi.org/10.1002/bit.10566

Acknowledgments

•CLD: Jack Bruno, Yudi Mejia, Dan Huang, Anurag Khetan
•USP: Greg Keil, Linda Hoshan
•DSP: Tom Tahan, Christ Furcht, Andrew Garret
•PDA: Mei Lin, Tianyin Yu

Presented at the ESACT, June 26th - 29th, 2022

Email: gregory.keil@bms.com