Development of Simple, Rapid & High-Throughput Glycoanalytics for Biopharmaceuticals

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Introduction

Over two thirds of biotherapeutic products are glycosylated proteins. A biotherapeutic's glycosylation profile is a critical product quality attribute that impacts on the efficacy and safety of these therapeutic molecules. The glycosylation profile must be characterised and monitored throughout product development and manufacturing processes. Lectins are bioaffinity proteins that can recognize and bind to specific glycan structures on intact glycosylated biomolecules and can therefore be used for the analysis of these molecules. Currently available lectins are predominantly plant based. These, however, lack specificity, are structurally complex (glycosylated) and difficult to produce recombinantly. GlycoSelect's Recombinant Prokaryotic Lectins (RPLs) are superior glycoselective molecules that facilitate simple and effective analysis and isolation of intact glycoproteins. RPLs are much more specific, consistent and scalable than their plan counter parts. This project aims to develop a novel analytical platform to advance the rapid analysis of the glycosylation profiles of biotherapeutics. Using GlycoSeLect RPLs integrated into ForteBio's biosensor platform we will analyse biotherapeutics provided by Allergan Biologics.

Methods

Two biopharmaceutical samples provided by Allergan were analysed using the Octet platform: FSH (follicle stimulating hormone) and Eylea (Fc fusion protein). Samples were tested for terminal β1-4 galactose and sialic acid structures using RPL-Gal1 and RPL-Sia1, respectively. The robustness of the Octet quantitation assay developed at GlycoSeLect for each RPL-Biopharmaceutical pair was assessed at CPI by verifying the linearity, repeatability and percentage spike recovery of each assay.

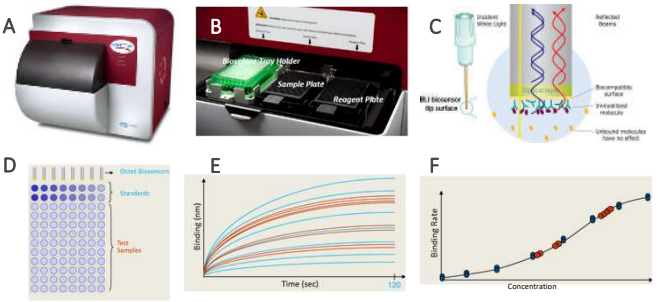
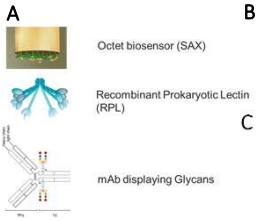


Figure 1 (A) – Octet red 384; (B) – Octet red 384 stage; (C) – Octet biosensor tip; (D) 96 well sample plate; (E)Binding over time graph; (F)Binding rate over concentration graph



B

Step	Buffer	Duration	Agitation
Baseline	TBST	60sec	1000rpm
Loading	RPLs (at 20µg/ml)	600sec	1000rpm
Wash	TBST	180sec	1000rpm

C

RPL- Biopharmaceutical pair	Linearity range (µg/mL)	Spike recovery concentration (µg/mL)	Repeatability concentration (µg/mL)
Gal1-FSH	500-7.8	50, 25, 12.5	200
Gal1- Eylea	500-15.6 (≥15.6)	N/A	200
Sia1- FSH	150 - 1.2	15, 7.5, 1.9	50
Sia1- Eylea	250-7.8	N/A	100

Figure 2 (A) – Method Overview; (B) – Biotinylated RPL's on-line immobilisation method; (C) – Concentration ranges tested for each RPL- Biopharmaceutical pair.

Results

The linearity of FSH (β1-4 galactose) analysis was determined by the binding rate of two replicates (n=2) at 8 concentration levels: 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.3 µg/ml, 15.6 µg/ml, 7.8 µg/ml and 0 µg/ml. The acceptable range of the assay was 7.8 to 500 µg/ml (%CV <20%).

A

FSH (µg/ml)	Conc. Avg. (µg/ml)	%CV
500.0	500.2	3.65
250.0	250.0	2.55
125.0	125.1	2.44
62.5	62.4	3.00
31.3	31.3	0.53
15.6	15.7	1.71
7.8	7.8	1.07
0	0.0	0.00

B

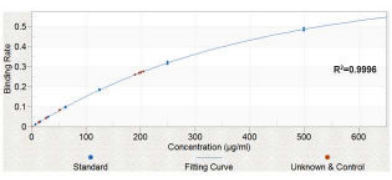


Figure 3 (A) – Linearity determination for FSH (β1-4 galactose); (B) – Analysis of FSH (β1-4 galactose) by Octet showing the standard curve (blue dots=standard curve and red dots= 6 replicates of FSH at 200µg/ml + experimental points).

Six replicates of FSH at 200 µg/ml were measured to determine the repeatability and precision of the Octet method. The concentration results in µg/ml for the six replicates are shown in Figure 4. The standard curve and results for the 6 replicates are shown in Figure 3. The assay demonstrated high levels of repeatability with a %CV of less than 10% for quantitation of the FSH (β1-4 galactose) at 200 µg/ml.

Octet analysis of FSH (β1-4 galactose)	
Repetition	FSH at 200 µg/ml
1	190.4
2	197.7
3	198.4
4	200.9
5	205.8
6	197.6
Conc. Avg.	198.5
%CV	2.53

Figure 4 – Results for the 6 replicates of FSH (β1-4 galactose) at 200 µg/ml analysed by Octet.

Percentage spike recovery tests were performed to investigate the extent of matrix interference in the quantitation of FSH (β1-4 galactose). The recovery of FSH (β1-4 galactose) at 12.5, 25 and 50 µg/ml from the specific matrix (a mix of FSH formulation buffer and assay buffer) was between 80-120%, with a %CV < 10% (see Figure 5).

Known Concentration (µg/ml)	Well 1 Conc. (µg/ml)	Well 2 Conc. (µg/ml)	Avg. Conc. (µg/ml)	%CV	% Spike Recovery
12.5	13.8	14.1	14.0	1.52	112%
25.0	27.5	27.0	27.3	1.30	109%
50.0	52.1	52.0	52.1	0.14	104%

Figure 5 – % Spike recovery determination for FSH (β1-4 galactose) at 12.5, 25 and 50 µg/ml.

Furthermore, the Octet method demonstrated percentage spike recoveries of 80-120%, high intra-assay linearity and repeatability for both the supplied FSH and Eylea samples with Gal1 and Sia1 RPLs-SAX sensors. These sensors where used in the Octet system to detect terminal β1-4 galactose and sialic acid structures in the FSH and Eylea samples. Figure 6 shows the standard curve obtained for these samples.

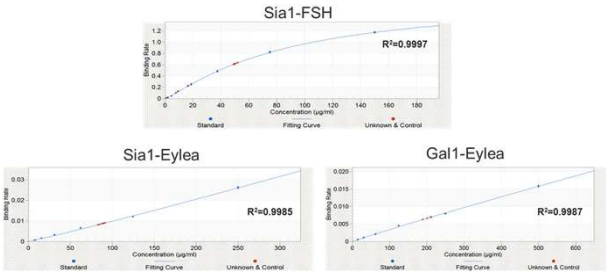


Figure 6 – Analysis of FSH and Eylea by Octet showing the standard curve obtained with with Gal1 and Sia1 RPLs-SAX sensors (blue dots=standard curve and red dots= 6 replicates of middle of standard curve + experimental points).

Conclusion/Summary

The suitability of a novel high-throughput method for the detection of glycans in purified biopharmaceutical samples has been assessed. The method, which uses the Octet system with Gal1 and Sia1 RPLs-SAX sensors to detect terminal β1-4 galactose and sialic acid structures, demonstrates high intra-assay repeatability and linearity for the supplied FSH and Eylea samples. Percentage spike recoveries of 80-120% indicate that glycan detection is not compromised by the presence of the sample buffer.

This project consortium brought together the expertise and resources of CPI, GlycoSeLect UK Ltd, ForteBio Pall Life Science and Allergan Biologics Ltd, to address the need for new glycoanalytical approaches to meet the needs of the growing biotherapeutics market.

Acknowledgements

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