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RESEARCH ARTICLE

BIOTECHNOLOGY PROGRESS

Lectin bio-layer interferometry for assessing product quality of Fc- glycosylated immunoglobulin G

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Abstract

Glycosylation, as the most prominent posttranslational modification, is recognized as an important quality attribute of monoclonal antibodies affected by various bioprocess parameters and cellular physiology. A method of lectin-based bio-layer interferometry (LBLI) to relatively rank galactosylation and fucosylation levels was developed. For this purpose, Fc-glycosylated immunoglobulin G (IgG) was recombinantly produced with varying bioprocess conditions in 15 L bioreactor and accumulated IgG was harvested. The reliability, the robustness and the applicability of LBLI to different samples has been proven. Data obtained from LC–MS analysis served as reference and were compared to the LBLI results. The introduced method is based on non-fluidic bio-layer interferometry (BLI), which becomes recently a standard tool for determining biomolecular interactions in a label-free, real-time and high-throughput manner. For the intended purpose, biotinylated lectins were immobilized on disposable optical fiber streptavidin (SA) biosensor tips. *Aleuria aurantia* lectin (AAL) was used to detect the core fucose and *Ricinus communis* agglutinin 120 (RCA120) to determine galactosylation levels. In our case study it could be shown that fucosylation was not affected by variations in glucose feed concentration and cultivation temperature. However, the galactosylation could be correlated with the ratio of mean specific productivity (q_P) and ammonium (q_{NH4+}) but was unrelated to the ratio of mean q_P and the specific glucose consumption (q_{gluc}). This presented method strengthens the applicability of the BLI platform, which already enables measurement of several product related characteristics, such as product quantity as well as kinetic rates (k_d,k_{on}) and affinity constants (k_D) analysis.

KEYWORDS
bio-layer interferometry, CHO cell culture, fucosylation, galactosylation, glycosylation

1 | INTRODUCTION

Monoclonal antibodies (mAbs) continue to dominate biopharmaceutical approvals and constitute about 40% of biotherapeutics that are available on the market and thus form a major class of molecules produced and developed by the biopharmaceutical industry.[1] Product quality and quantity are important measures in antibody discovery and in process development. These requirements are driven by numerous influential factors such as process performance and cell physiology. The complexity of such a multifactorial system makes it

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difficult to identify the parameters with relevant influence on product quality. Thus, direct control of product quality in the individual bioprocess, a central goal of the quality by design approach, is severely limited.[2] Consequently, it is crucial to get a deeper understanding of the biological system, the process, the product and the interdependencies amongst each other.

In respect of product quality, the glycosylation is among others, a relevant characteristic, which can profoundly affect protein stability and the functions, which are relevant to their therapeutic application. Recently, the correlation between the glycoform profiles and the safety and efficacy of a drug, in particular, has achieved significant attention of researchers worldwide.[3] The glycan structure is affected by the enzymatic machinery of the host cell, transit time in the Golgi bodies, environmental factors and the availability within the sugar nucleotide pool.[4-6] With respect to process conditions, several publications have shown that the osmolality level (in combination with pH),[7] availability of glucose,[8,9] ammonium production rates[10] or dissolved CO2 level[7,11] can lead to alternations in glycosylation patterns. In order to study such mechanisms in more detail and to measure product quantity and relevant quality parameter, appropriate analytical assays are required.

Several techniques already exist to measure those quality specifications. The most commonly applied technique for the complete structural elucidation of glycoprotein oligosaccharides is the application of a combination of chemical, enzymatic, and chromatographic techniques combined with mass spectrometry.[12,13] Another promising approach is the application of lectin-binding assays.[14] Lectins are glycan-binding proteins that selectively recognize glycan epitopes of glycoproteins, which enables the specific monitoring of oligosaccharide structures. The interactions of lectins with glycan structures can be measured via the bio-layer interferometry (BLI), a well-established biosensor technology.[15] The BLI technology is performed in an open shaking micro-well plate format without any micro-fluidics and using disposable optical fiber biosensors. The physical principle of this technique is based on the correlation of the spectral shift $\Delta\lambda$ with a change in thickness (nm) on the biosensor surface. The platform also allows the measurement of biomolecular interactions, enabling full kinetic measurements and facilitates the quantitation of biomolecules.[16] Accordingly, several product quality characteristics and the product quantity can be determined on a single platform.

A recent study showed the applicability of the BLI platform as a high-throughput technique for determining the sialylation of mAbs.[17] This was done by measuring the binding rate of *Maackia amurensis* lectin II (MALII) to (a-2,3)-linked sialic acids of highly sialylated proteins bound in a native state to Protein A sensors. However, all oligosaccharides, except (a-2,3)-linked sialic acids are normally hidden located within the folded structure of immunoglobulin G (IgG) and are not accessible to related lectins. This induces the necessity for reduction of the disulfide bonds, which lead to the opening of the tertiary and quaternary protein structure of IgG, and enables lectins to bind to oligosaccharides attached in the Fc component.[18,19] In the present

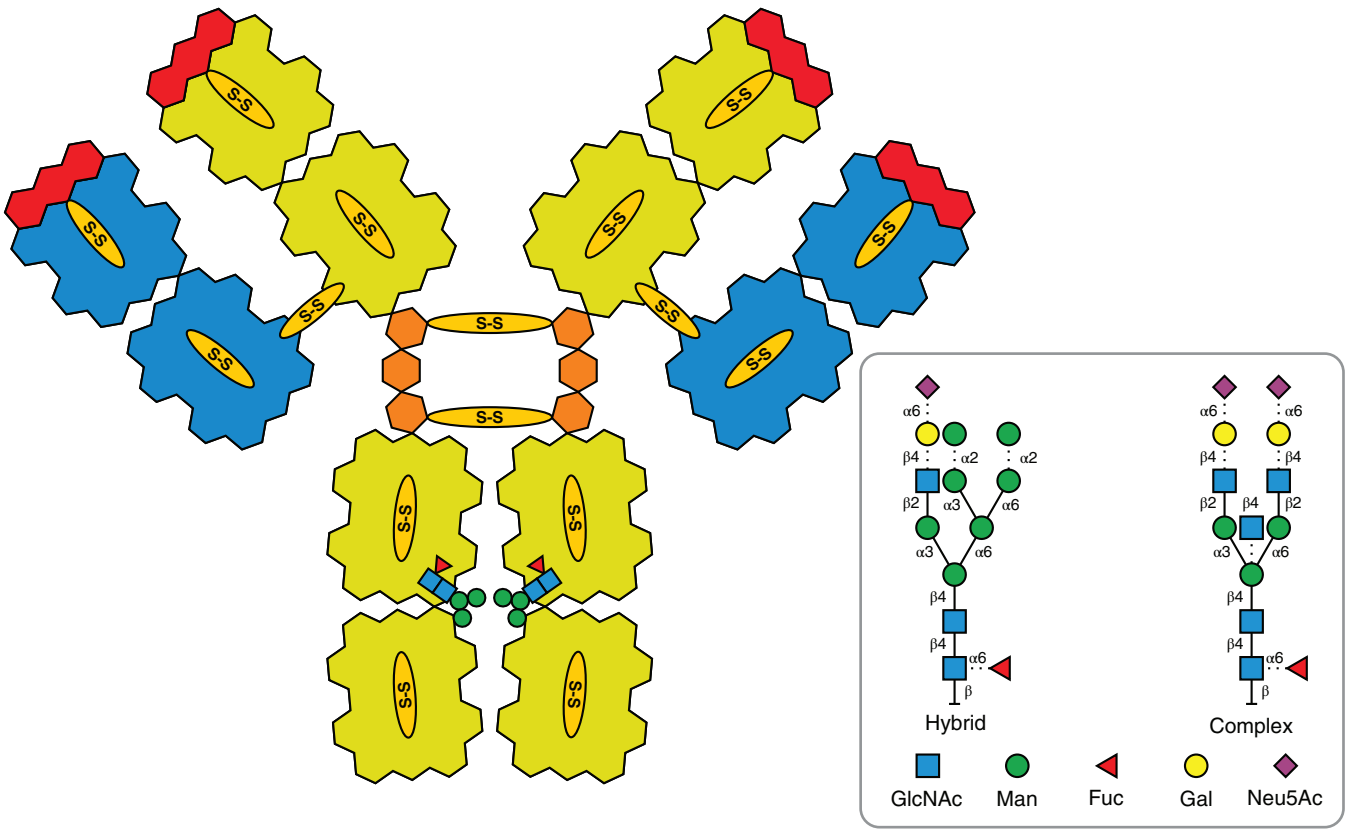

FIGURE 1 IgG antibody and N-glycan structures. Schematic representation of a glycoprotein IgG (mAbs contains only Fc glycosylation). The disulfide bonds stabilizing the tertiary and quaternary protein structure are also shown

study a non-sialylated antitumor necrosis factor (anti-TNF-α) IgG1 (Figure 1) recombinantly produced in Chinese hamster ovary (CHO) cells in a 15 L pilot scale fed-batch process under varying conditions was used as a model protein. For this study the mAB was purified from the harvest of 13 fed batch cultivation runs t with variations in feed media glucose concentration and cultivation temperature.

Ricinus communis agglutinin 120 (RCA120) and *Aleuria aurantia* lectin (AAL) were used to determine the terminal galactose and core fucose content of the pre-purified IgG.[14] The resulting galactosylation and fucosylation levels were compared with results obtained by the well-established analysis technique using MS detection.[20] Since the harvest samples represent the accumulated product of the entire bioprocess, the glycosylation was further related to the overall process performance. To the best of our knowledge this study presents for the first time application of the BLI platform to determine the galactosylation and fucosylation levels of CHO culture samples.

2 | MATERIAL AND METHODS

2.1 | Bioprocess set up

As a model protein Fc-glycosylated anti-TNF-α IgG1 was used, produced by a recombinant monoclonal CHO cell line (Antibody Lab GmbH, Austria). Generation of the cell line was conducted by applying the Rosa26 bacterial artificial chromosome (BAC) expression strategy to a serum-free adapted host cell line derived from CHO-K1 (ATCC CCL-61).[21]

A vial of the working cell bank (5×10^6 cells) was thawed in a chemically defined culture medium (Dynamis AGT, A26175, Thermo Fisher Scientific) supplemented with 8 mM L-glutamine (25030081, Sigma Aldrich, Germany), 3 mL/L phenol red solution (RNBD642, Sigma Aldrich, Germany), 1:1000 Anti Clumping Agent (0010057DG, Thermo Fisher Scientific) and 1 mg/mL G418 (G8168, Sigma Aldrich, Germany).

Every three to 4 days the cells were passaged with the aforementioned media but without anti-clumping agent and G418. Cultivation was performed in a humidified incubator (HeracellTM VIOs 160i, Thermo Scientific) at 37°C, 5% vol/vol CO_2 and 200 rpm (MaxQ 2000 CO_2 Plus, Thermo Scientific).

With the fourth passage the cells were transferred into the 15 L bioreactor (LabQube, Bilfinger Industrietechnik GmbH, Austria) with a seeding concentration of 2.5×10^5 cells/mL and a starting volume of 10 L. The batch phase was kept identical for every experiment. The experimental setup included the variation of temperature, at 31, 34, and 37°C, in addition to variation in the amount of glucose (G7021, Sigma Aldrich, Germany), with the addition of 10, 20, or 30 g/L of glucose, and aspartate concentration with additions of 0 or 7 g/L aspartate, in the feed phase (CHO CD EfficientFeed A, A1442001, Thermo Fisher Scientific). Additionally, the feed medium was also supplemented with 0.1% antifoam (A8011, Sigma Aldrich, Germany) to maintain a constant antifoam concentration during the process. A constant feed rate of 418 g/d was used during the feed-phase.

The process was controlled via process air mass flow (PA) and stirrer speed to maintain the dissolved oxygen level (DO) above 30% and CO_2 mass flow to keep the pH constant at 7.0. The total gas-flow range was kept within 0.01–0.1 vvm.

The experimental setup used is displayed in Table 1.

TABLE 1 Experimental design of the fed-batch processes

Run number	Shift 1	Shift 2	Shift 3	Shift 4
1	36.3°C/F3 (72 hr)			
2	36.3°C/F3 (72 hr)			
3	37°C/F3 (72 hr)	37°C/F1 (192 hr)		
4	34°C/F1 (72 hr)			
5	34°C/F2 (72 hr)			
6	34°C/F2 (72 hr)			
7	34°C/F2 (72 hr)			
8	34°C/F2 (72 hr)			
9	34°C/F2 (72 hr)	37°C/F2 (120 hr)	34°C/F1 (192 hr)	31°C/F1 (240 hr)
10	31°C/F2 (72 hr)	34°C/F2 (120 hr)	37°C/F3 (192 hr)	34°C/F3 (240 hr)
11	34°C/F1 (72 hr)r	31°C/F1 (120 hr)	31°C/F2 (192 hr)	34°C/F2 (240 hr)
12	37°C/F2 (72 hr)	34°C/F3 (120 hr)	31°C/F2 (192 hr)	34°C/F1 (240 hr)
13	34°C/F3 (72 hr)	37°C/F2 (120 hr)	31°C/F2 (192 hr)	37°C/F3 (240 hr)

Note: Experiments are presented by the time point (hours [hr]), type of shift, temperature or feed change. F1, F2, and F3 represent the additional glucose concentration in the feed, consisting of 10, 20, and 30 g/L, respectively.

2.2 | Off-line analyses

The total cell concentration (TCC) was determined by counting the cell nuclei using a Z2 particle counter (Beckman Coulter). Therefore, an appropriate amount of cell suspension was centrifuged at 180g for 10 min. The cell pellet was subsequently resuspended in a 0.1 M citric acid monohydrate and 2% (vol/vol) Triton X-100 buffer to lyse the cells for a minimum of 1 hr before measurement. Sample dilution was performed using a 0.9% NaCl solution.

Culture viability was assessed using a haemocytometer and trypan blue exclusion. The viable cell concentration (VCC) was determined by multiplying viability with the TCC.

Glucose was determined via ion exclusion chromatography (HPX 87H, 300 × 7.8 mm, #1250140, BioRad) using an Agilent 1,200 series (Agilent) at 25°C. The mobile phase consisted of 5 mM sulphuric acid

and the flowrate was set to 0.45 mL/min and measured via a Refractive Index Detector (35°C). The calibration range for D(+)-glucose was 100–2000 mg/L. The chromatograms were evaluated using Chemstation software (revision B.04.01, Agilent).

Ammonium was measured via an ion selective electrode (ISE, GZ-27512-00, Cole-Parmer). Therefore, the cell suspension was centrifuged at 180 rpm for 10 min and two times 3 mL of the supernatant were stored at −20°C. Before each measurement the ISE was filled with the reference solution (0.1 M NH_4Cl, GZ-27503-71, Cole Palmer) and calibrated (from 1 to 100 mM, GZ-27503-00, Cole Palmer). The 60 µL of 10 M sodium hydroxide solution (221,465, Merck, Germany) were added to each sample and the solution was vortexed, shortly before measurement.

Osmolality was measured by using a freezing point osmometer (Osmomat 030-D, Gonotec, Germany). Thawed samples of the cell broth supernatant were measured in duplicates.

The product titre was determined by BLI using Protein A tips (Octet System, QK, ForteBio).[22]

2.3 | Lectin assay

2.3.1 | Immunoglobulin G purification

The mAb was purified from clarified supernatants using Protein A affinity chromatography. The experiments were performed on an Äkta Pure system (GE Healthcare). A POROS A 20, 2.1 × 30 mm column was used for the stationary phase (Thermo Scientific). The column was equilibrated with phosphate buffered saline (PBS), pH 7.4 for 25 column volumes (CV). The column was loaded with 2 mL clarified supernatant at a residence time of 0.1 min (except for sample ID 160714 and 180,202 where only 1.5 mL was available). After sample application the column was washed with PBS for 20 CV. The mAb was eluted with 100 mM glycine pH 3.0 in a 10 CV step gradient elution and the collected fractions were immediately neutralized with 1 M Tris HCl pH 8.0. The column was cleaned with 6 M guanidine HCl, 50 mM Tris, pH 8.0 solution for two CV and immediately re-equilibrated with PBS. The outlet was monitored by measuring UV absorbance at 280 nm to detect eluting antibody. Due to tailing of the elution, only the main portion of the peak was collected, resulting in a loss of ~5% in the tail.

2.3.2 | Reduction of disulphide bonds

Purified IgG was diluted with PBS to a concentration of 100 µg/mL (± 5 µg/mL), as determined by absorbance at 280 nm, using ε 1.43.[23] Nine volumes of the diluted IgG sample were mixed with one volume of 2-mercaptoethanol (1 M in PBS) Sigma (Vienna, Austria) and incubated for 2 hr at 37°C. To block thiol-groups, one volume of 0.2 M iodoacetamide Sigma (Vienna, Austria) was added to the reduced sample (final concentration 0.1 M). Incubation was performed overnight, in the dark and at room temperature.[19]

2.3.3 | Bio-layer interferometry for analysis of galactose and fucose content on IgG

Octet Red96e (ForteBio, Menlo Park, CA) was used for lectin/carbohydrate binding studies. Typical assay performance is shown in Figure 2a. Samples were diluted in black 96-well plates (Nunc F96 MicroWellTMPlates, ThermoFisher Scientific, Langenselbold, Germany). Puffer (Sample diluent) contained PBS, 0,005% P20, Sigma (Vienna, Austria) and 0.1 mg/mL BSA. The total working volume for each step was 210 µL per well and the rpm setting for each baseline, loading, and association was 1,000 rpm. The test was performed at 25°C. Prior to each assay, streptavidin (SA) biosensor tips (ForteBio, Menlo Park, CA) were pre-wetted in 210 µL sample diluent for at least 10 min followed by equilibration with sample diluent for 60 s. Afterward, SA biosensor tips were non-covalently loaded with biotinylated *Ricinus communis* agglutinin I (RCA I) or biotinylated AAL, both obtained from Vector Labs, UK, in a sample diluent concentration of 0.83 µg/ for 120 s, followed by an additional equilibration step (60 s) with sample diluent. Prior to analysis, reduced samples were diluted three times in sample diluent, yielding an IgG concentration of 15 µg/mL (± 5 µg/mL). Association was carried out for 600 s. All measurements were performed in triplicate. Raw data,

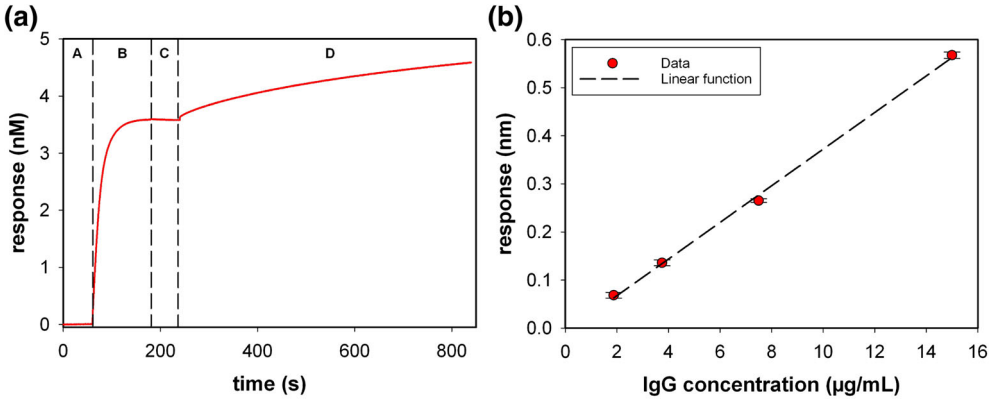

FIGURE 2 (a) Sensorgram of a typical test performance, including baseline steps (A, C), lectin loading (B) and association of reduced immunoglobulin G (D). (b) Dose–response curve of IgG by serial dilution of one reduced sample (run 12), diluted to 15, 7.5, 3.75, and 1.875 µg/mL in sample diluent. RCA120 lectin was immobilized on streptavidin sensor tips prior IgG association. The linear signal curve resulted in an equation of y = 0.0381 × −0.0084 and in a correlation coefficient of 0.9986

obtained with the Octet Software (Version 11.0, Menlo Park, CA) were exported to Excel spreadsheets (Version 2003, Microsoft, Redmond, WA). Raw data of the IgG association response were aligned to the individual association step. The individual specific response R at a defined time t (Rt) for each concentration was calculated as an average of three independent measurements. The reproducibility of (Rt) was determined with $n = 8$. The responses at 600 s (Rt) of the harvest 13 fed-batch experiments were compared with the outcomes of mass spectrometry measurements, using linear regression analysis. The computations were performed using Mathematica (Version 11.3 of 2018, Wolfram Research Inc., Urbana-Champaign, IL). The statistical analysis was based on 95% significance. The quantitation limit (LOQ) for IgG association was assumed by measuring the minimum concentration at which the analyte can be reliably quantified. A typical signal-to-noise ratio is 10:1. The baseline noise was determined during the initial 60 s PBS buffer step ($n = 8$).

2.3.4 | Monosaccharide analysis performed with mass spectrometry

The samples were digested in gel. The proteins were S-alkylated with iodoacetamide and digested with trypsin (Promega). The digested samples were loaded on a BioBasic C18 column (BioBasic-18, 150 × 0.32 mm, 5 μm, Thermo Scientific) using 65 mM ammonium formiate buffer as the aqueous solvent. A gradient from 5% B (B: 100% ACCN) to 32% B in 35 min was applied, followed by a 15-min gradient from 32 to 75% B that facilitates the elution of large peptides, at a flow rate of 6 μL/min. Detection was performed with QTOF MS (Bruker maXis 4G) equipped with the standard ESI source in positive ion, DDA mode (i.e., switching to MSMS mode for eluting peaks). MS-scans were recorded (range: 150–2,200 Da) and the three highest peaks were selected for fragmentation. Instrument calibration was performed using ESI calibration mixture (Agilent). Manual glycopeptide searches were made using DataAnalysis 4.0 (Bruker). For the quantification of the different glycoforms the peak areas of the extracted ion chromatograms (EICs) of the first four isotopic peaks were summed, using the quantification software Quant Analysis (Bruker). Note that MS of glycopetides only allows identifying the composition of the glycan and as a consequence only one possible isomer is annotated.[20,24]

3 | RESULTS AND DISCUSSION

3.1 | Development of an appropriate bio-layer interferometry method for the determination of galactose and fucose

Selected IgG samples of defined bioprocesses were purified using Protein A affinity chromatography to remove potential inferring host cell proteins. For this approach, in principle also other procedures (e.g., immunoprecipitation, etc.) could be applied as long as the glycosylation of IgGs is not affected. After the purification procedure, IgG was diluted to a concentration of 100 μg/mL determined by absorbance at 280 nm. In a first step lectin binding to non-reduced IgG was tested. As expected, binding of non-reduced IgG to the immobilized lectins could not be achieved (see Figure S1). These results are in excellent agreement with published data and provide additional evidence that reduction of IgG is a prerequisite to obtain freely accessible carbohydrate structures attached to the Fc part of the IgG.[18,19,25] Consequently, IgG was reduced with β-mercaptoethanol followed by carboxymethylation of cysteines with iodoacetamide to avoid reformation of the disulphide linkages.[26] For the BLI assay, the lectin and the IgG concentration was adjusted to gain an optimized test performance as described in the materials and methods section. Finally, SA biosensor tips were captured to saturation with biotinylated lectins (data not shown). An additional equilibration step with buffer was applied to remove the excess of biotinylated lectins and to obtain a constant loading baseline. The equilibration time was 60 s to achieve a sufficient baseline signal. Moreover, the lectin-coated SA biosensor tips were incubated with the purified, reduced IgG to measure the corresponding association profiles. In preliminary experiments, different IgG concentrations were tested and finally optimized. After reduction the samples contained 0.1 M β-mercaptoethanol and 0.1 M iodoacidamid. During the test optimization it became evident that these concentrations are already too high to accurately associate the IgG to the lectins. A simple threefold dilution step with sample diluent was sufficient to obtain reliable signals. Nonspecific interactions of the reduced IgG with the biosensor were eliminated by introducing 0.005% P20 and 0.1 mg/mL BSA to the sample diluent. No unspecific interaction of samples with uncoated SA biosensor tips occurred, which confirms the selectivity of the procedure. Finally, a standardized IgG concentration of 15 μg/mL was used. Dose–response linearity was demonstrated with a serial dilution experiment (Figure 2b). Therefore, one sample (No 12) was diluted to 15, 7.5, 3.75, and 1.875 μg/mL in sample diluent, attached to the pre-coated AAL lectin sensor and measured. A linear signal curve resulted in a correlation coefficient of 0.9986. The coefficient of variation for triplicate determinations of each concentration was <10%. The assay sequence begins with the equilibration of the SA biosensor tips with the PBS buffer in order to measure the baseline signal for calculation of the LOQ, which is defined as the lowest concentration at which the analyte can not only be reliably detected but at which some predefined goals for bias and imprecision are met. To obtain LOQ datasets for the evaluation of significant/reliable measurements, the average baseline noise of SA biosensor tips in PBS was determined and the LOQ assumed to be a signal-to-noise ratio of 10:1.[27] The baseline noise of the initial 60 s was 0.004 nm ($n = 8$). Thus, the LOQ was calculated as 0.04 nm. From the dose–response linearity measurements, as explained above, it was found that the mean R_t of the lowest used IgG concentrations (1.875 μg/mL) toward the pre-coated RCA120 lectin sensor was 0.068. Prior reduction IgG was diluted to a concentration of 100 μg/mL. However, dose–response linearity experiments and estimation of LOQ demonstrate that significant lower initial IgG concentration can be applied. For both, galactose and fucose, it could be demonstrated that the lectin-BLI (LBLI) is a promising tool when the accessibility of the protected sugar moieties can be achieved.

3.2 | Determination of fucosylation of IgG in different mammalian cell culture processes

In a next step the LBLI method was compared to a widely used standard approach for determining glycan structures. Therefore, the selected 13 harvest samples were purified and glycosylation levels were measured via LC–MS and the LBLI method as described above (data is shown in Table S1). In general, the fucosylated forms of the harvest product were constant within the experimental setup. With both methods a similar fucosylation level (Figure 3a) was determined. The level range was found to be 82.3 ± 1.2% with respect to the LC–MS analysis. The new established LBLI method obtained a mean value of R_T 1.11 ± 0.09 nm. A regression analysis in order to compare both methods was not feasible due to the constant output.

3.3 | Determination of galactosylation of immunoglobulin G in different mammalian cell culture processes

Significant variations were observed for the galactosylation of the mAb. They ranged from approximately 16.5–33.1% calculated by LC–MS and 0.24–0.70 nm RT for the LBLI analysis (Table S1 and Figure 3b). A linear relationship between the LC–MS and the LBLI responses was observed. Specifically, a function of r = a + b*p with a = −0.277 nm (confidence limits: −0.428–0.126) and a significantly positive parameter b = 0.031 nm (confidence limits: 0.023–0.038) was obtained. Hence, higher LBLI-responses corresponded to higher proportions of galactosylation (Figure 3b). It follows that with the LBLI tool the galactosylation levels can be successfully measured and data obtained from LC–MS analysis were equal compared to the LBLI results.

3.4 | Process parameters affecting glycosylation

The product is continuously secreted into the supernatant. The finally determined glycosylation pattern, represents the accumulated profile from the entire production process. Connecting the overall process performance with the glycosylation can already provide an appropriate picture about certain impacts. However, for a comprehensive understanding of the impact of process and cellular dynamics on glycosylation, straightforward techniques that enable the analysis of samples throughout the process, will be beneficial.

Since, fucosylation levels stayed constant in all samples of the test case we merely focused on the evaluation of the galactosylation. According to recent studies[10,28-30] glucose and ammonium can be environmental key factors in context of mAb galactosylation.

Since the IgG is accumulated during the process in the supernatant, the average production ($NH4^+$) and consumption (glucose) rate for the entire process should, presupposed that there is an impact, may reflect the overall variance of the glycosylation pattern. The rates $\bar{q}_n$ were calculated according to Equation (1). However, in the experimental runs different product titres were achieved, which means that different amounts of protein were available and susceptible to post-translational modification. Accordingly, the rates $\bar{q}_{NH4^+}$ and $\bar{q}_{gluc}$ were set into a relationship with the specific protein production rate $\bar{q}_p$, which was also calculated according to Equation (1).

$$\bar{q}_n = \frac{\sum_{t_0}^{t_{harvest}} n_{t+1} - n_t + n_{feed}}{\sum_{t_0}^{t_{harvest}} \frac{X_{v,t+1} - X_{v,t}}{\mu_t}}, \quad (1)$$

n represents the glucose, ammonium or mAb, respectively, X_v the amount of viable cells and μ the growth rate to a given time point. The variable n_{feed} represents the amount of substrate feed into the system. Hence, for mAb and ammonium this term becomes zero.

The results obtained from different experimental setups indicate that the average specific glucose uptake rate does not correlate with the galactosylation content (Figure 4a). This finding may be attributed to the fact that glucose concentrations were never limiting throughout the processes. Reduced glucose level can negatively influence the galactosylation index due to a reduced availability of uridindiphsophate

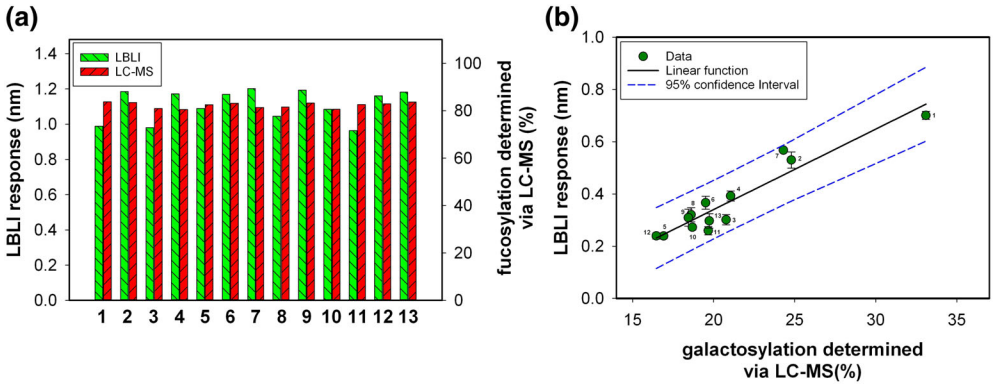

FIGURE 3 (a) The proportion of fucosylated glycoforms determined via LC–MS and the responses of the lectin based BLI (LBLI) assay in nm depicted for all runs. (b) Linear regression analysis of all 13 CHO cell culture fed batch runs performed in 15 L pilot scale. The responses in [nm] of LBLI assay were compared with obtained galactosylated glycoforms in (%) of LC–MS measurements. Dots and error bars represent the mean Rt values and standard deviation of LBLI triplicate measurements, and the corresponding results from a single LC–MS run (without *SD*). The regression line and its single confidence band (dashed line) are plotted. The linear regression line resulted in the equation of y = 0.0309 × −0.277 and a correlation coefficient of 0.8925

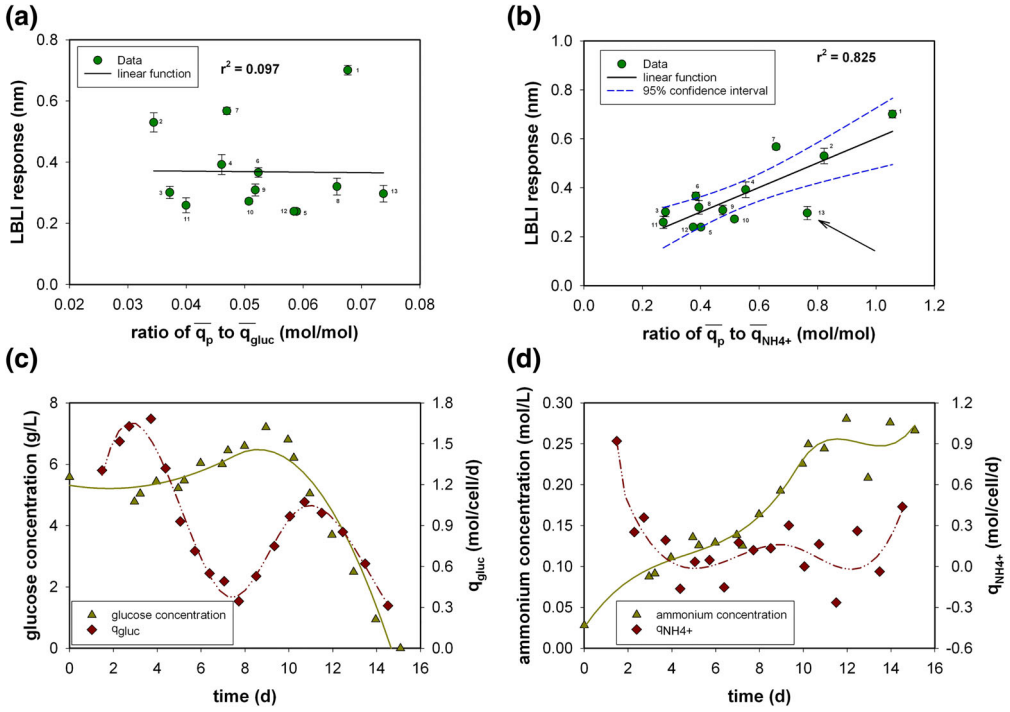

FIGURE 4 The impact of the ratios q_P to q_{gluc} (a) and to q_{NH4+} (b) on the LBLI response or respectively the galactosylation proportion of the mAb. The regression lines and coefficients are depicted. (b) Regression line depicted was performed without the outlier. The arrow indicates the outlier. The confidence band (dashed line) are plotted. Example trends of process run 12 for (c) glucose concentration and the respective specific rate and (d) ammonium concentration and the respective specific rate

(UDP) sugars and decrease the galactosylation index.[8,9] This observation points to the fact that other additional factors might influence the galactosylation.

In this respect, the ratio of mean q_p to q_{NH4+} was calculated (Figure 4b). A linear relationship with the galactosylation levels of the mAb could be determined. The less ammonium per mAb produced, the more galactosylation occured. A regression analysis performed with all runs resulted in the equation of $y = 0.502 \times x + 0.100$ with a regression coefficient of 0.660. In conclusion our results indicate that the ammonium production impacts the galactosylation profile. This finding is in agreement with the already known fact that high ammonium concentrations can increase the internal pH of the Golgi bodies and subsequently promote the inhibition of the enzymes required for the oligosaccharide processing. Galactosylation and sialylation of mAbs are mainly influenced by this regulation.[3,29] This result suggests that the production of ammonium per product is a crucial parameter.

In this study we assumed that the rates were constant for an entire process run. Due to this simplification certain dynamics and correlations might be undetectable. For instance, the availability of glucose, due to glucose depletion or a low glucose uptake rate, might have had an influence on the galactosylation level (Figure 4c). It also remains unclear if the ammonium production rate or the ammonium concentration in the supernatant was the real cause for the determined glycosylation pattern (Figure 4d). A clear understanding of the dynamics can only be achieved if the whole process progression is taken into consideration. In principle the presented LBLI method is capable of high throughput analysis. In this respect, the protein purification step still remains a bottleneck for this analysis. Therefore, future research will focus on different high throughput (HT) protein purification procedures to reduce the work load and to be capable to identify such process dynamics.

4 | CONCLUSION

The glycosylation is a relevant key quality attribute for monoclonal antibodies. It can be affected by many different factors, such as the expression system, process conditions, or media composition and feed protocols and thereof vary from batch to batch.[3] In this study we showed that the developed LBLI method, verified with data resulted from LC–MS, is feasible to determine the Fc fucosylation and galactosylation of an anti-TNF alpha antibody. Samples containing mAbs with varying glycosylation profiles were used to show the method applicability. By both applied analytical techniques it could be shown that fucosylation remained constant within the experimental design, while galactose varied. Based on the monitored glucose and ammonium levels it could deduce that a low ratio of q_p to q_{NH4+} resulted finally in a reduced galactosylation level in harvest samples. To gain more insight into the very complex process dynamics individual, additional in-process measurements and intra-cellular analysis would be necessary.

The application of HTX-BLI-based instruments in the 32-channel mode and 384 well plates enables 32 individual glycoanalytic measurements to be performed in less than 15 min. Antibodies either captured by Protein A or complementary methods prior reduction under standardized conditions, allows the identification of protected glycan structures within the protein in an efficient manner. This is a huge advantage compared to conventional techniques, where determination of glycosylation patterns are still accompanied with a high

workload, expenditures costs and usually requires an advantage expertise.[31] For the future, the LBLI method and the in general the BLI platform offer a simple and inexpensive high throughput (HT) technique for the analysis of several important product related parameters such as the product titer, the antibody antigen binding kinetics and the glycosylation pattern. Although, in research very often different techniques and equipment are used, in R&D and production the use of only one machinery is definitely advantageous to fulfill all the regulatory needs and the future goals of automatization. The presented technique will accelerate cell line, media and process development but also will be important as a process monitoring tool. To transfer the proposed platform to industrial application, automatization of protein capture need to be established to overcome this bottle neck for future analysis. Currently, different HT purification systems are under examination with the aim to complete the proposed platform.

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CONFLICT OF INTEREST

The authors declare no commercial or financial conflict of interest.

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