Glycosidases as an analytical tool in glycomics assays

Rebello, O.D.

Chapter 1

Introduction

Analytical assay development, particularly pertaining to glycomics, is an exciting amalgam of biology, chemistry and engineering. Besides academic research in natural and medical sciences, glycomics assays have immense importance in industrial applications such as in quality control (QC) and quality assurance (QA) of glycoproteins. An up-coming industrial and clinical application is the high-throughput glycan profiling of clinical samples, such as plasma, for identifying disease associations. These glycomics assays are often based on chromatographic and mass spectrometric instrumentation. Thus, they create a requirement of instrumentation infrastructure as well as technical skills which are both not always readily available. This creates a demand in industry for the development of glycomics assays that have a low infrastructure cost as well as minimal training requirements and that are user-friendly. With these objectives in focus, this thesis develops novel exoglycosidase-based high-throughput glycomics assays for use in industrial glycan profiling. In doing so, this thesis also contributes to the development of potential products, such as glycomics kits.

Glycosylation in human health

Protein glycosylation is a post-translational modification that can be characterised as either *N*-type or *O*-type, depending on glycan structures and their attachment to the protein backbone [1, 2]. Although *N*-glycans have diverse molecular structures, owing to variations in their composition and linkage, they have a fundamental pentasaccharide core structure composing of two *N*-acetylglucosamines (GlcNAcs) and three mannoses (Man). The core asparagine-linked GlcNAc may or may not be decorated with a fucose (Fuc) residue [3]. Variations in the outer branches, extending from the core's Man triose arms, allow for further categorising of the glycans as either, oligomannose, complex or hybrid type. Complex glycans contribute to about 97% of total plasma protein *N*-glycosylation (TPNG) [4]. These complex glycans can be di-, tri- or tetraantennary depending on the branching GlcNAc motifs which can be β(1-2/4/6) linked [1]. These antennary GlcNAcs can be further decorated in an orderly fashion with galactose (Gal) and sialic acid (Sia) residues, and to lesser extent Fuc residues [5]. As far as humans are concerned, these Gal residues are β(1-3/4) linked while the Sia residues are exclusively 5-*N*-acetylneuraminic acid (Neu5Ac) of α(2-3/6) linkage [6]. Antennary fucosylation can be either α(1-3/4) linked to the antennary GlcNAcs or α(1-2) linked to Gal [7].

Glycosylation plays an important role in protein folding and structural maintenance [8]. Because glycans have multiple hydroxyl groups, they can influence the hydrophilicity of the protein surface which is an important physical property for protein stability [8-10]. These glycans can also form hydrogen bonds to the peptide residues which may aid in maintaining protein conformation. Besides intra-molecular functionality, glycans may also serve as ligands for inter-molecular interaction or modify protein-protein interactions and thus contribute to inter-molecular crosstalk. A previous review has described the glycosylation on a selection of 24 plasma proteins which contribute to nearly half of the plasma protein concentration [11]. Alterations in plasma protein glycosylation may influence protein function. For example, changes in IgG glycosylation during an inflammatory response are associated with the immunoregulatory functionality of the IgG molecule [12, 13].

A large repertoire of medical and clinical research has extensively demonstrated the ability to correlate plasma glycosylation to the pathophysiological state of patient populations [1]. For example, in TPNG, changes in antennary branching, sialylation and antennary fucosylation of glycans have been associated with inflammatory diseases such as diabetes [14, 15], cancers [16-18], rheumatoid arthritis [19] and inflammatory bowel disease [20]. TPNG changes have also been corelated to mental conditions such as ADHD in children [21] and Down syndrome [22]. Interestingly, TPNG changes can also be correlated to age [23, 24] and standard of living of a population [25]. These associations can be explained by external factors [25] or genetic factors [7] that cause a change to the patient's physiological state. These physiological alterations may further lead to changes in the glycosylation processes and hence a shift in the glycosylation pattern on proteins and lipids. Furthermore, since glycosylation on proteins can influence their stability or is involved in inter-molecular interactions, these glycosylation changes could sometimes further escalate the disease state [26].

In addition to the importance of glycosylation in medical and clinical sciences, it also has a significant importance in biopharmaceutical production. Glycosylation does not only influence the stability of a therapeutic protein, but can also influence the immune response of a recipient of a biopharmaceutical [27, 28]. For example, Cetuximab is a recombinant IgG1 antibody which targets the epidermal growth factor receptor and was approved for treatment of colorectal cancer. However, this antibody was associated with a high incidence of hypersensitivity among patients which was routed to the presence of a non-human Gal-α(1-3)-Gal motif [29]. Hence, knowing and monitoring the precise glycosylation profile of biopharmaceuticals is vital. In fact, glycosylation is a critical quality attribute (CQA) which must be monitored and engineered to ensure safety for many biopharmaceutical products [30, 31].

Glycan profiling is heavily dependent on chromatographic and spectrometric techniques. This will be elaborated in the next section. The use of exoglycosidases for glycan sequencing was and continues to be a vital approach that complements these analytical instrumentation techniques for glycan profiling [32]. Exoglycosidases are enzymes that can hydrolyse specific glycan residues from the non-reducing end of glycans, with varying degrees of monosaccharide and linkage specificity [33]. Although various workflows are published, the principle remains the same. Released glycans are treated by an assortment of exoglycosidases, followed by a liquid chromatography (LC) - fluorescence detection (FLD) analysis of the cleaved products [34, 35]. By correlating chromatographic peak shifts between exoglycosidase-treated and non-treated samples, one can sequence a glycan by consecutively removing residues. The substrate specificity of exoglycosidases, in terms of glycan residues and their linkages, can range from narrow to broad, thus creating the need to source exoglycosidase of high substrate specificity for the purpose of glycan profiling [35]. To answer this demand, one approach is bio-engineering well-characterised exoglycosidases of a certain activity to obtain an enhancement of its activity or selectivity [33, 36, 37]. Another, more widely used approach, is meta-genomic screening of complex glycan environments such as gut microbiota, for discovering exoglycosidases of desired specificities [38-40]. This approach is based on an organism's need to consume the available nutrients, in this case the need to use glycosidases to consume the glycans in their environment. This approach has proven

valuable in discovering glycosidases [40, 41] that have potential in glycan profiling [34, 35] and glycoengineering [42].

Glycomics assays

Glycan variations are owed to compositional and linkage differences [1, 43]. Most glycomics assays are based on identifying both these compositional and linkage variations, usually in complex samples containing multiple glycan structures [44-48]. Although some glycomics assays are microarray-based binding assays that can identify glycan epitopes [49, 50], most glycomics assays rely on chromatography-, electrophoresis- and/or mass spectrometry (MS)-based techniques [51-55]. These glycomics assays have co-advanced with the development of instrumental analytical techniques. Advances in liquid chromatography (LC) column chemistry and silanol neutralisation have proven vital in chromatographic and capillary electrophoresis separations of glycan isomers [56, 57]. Additionally, advances in mass analysers and ion optics in components such as ion traps, quadrupoles, collision induced dissociation (CID) cells and ion mobility mass spectrometry (IMS) drift tubes have aided glycan structural identification by MS [58-62]. Furthermore, development of chemical derivatisation techniques such as sialic acid esterification [63], hydroxyl group methylation [64-66] and reducing-end tag conjugations [67] have complemented these instrumentational techniques for glycan analysis. Finally, advances in liquid handling robotic platforms have contributed to high-throughput sample processing which is important in clinical glycomics [51, 68].

Microarray-based binding assays

The microarray-based binding assay is a versatile tool in glycobiochemistry research, and it principally relies on glycan-ligand interactions [50]. Generally, this technique, when established in a laboratory, can be used for performing assays in a high-throughput manner with limited technical skills required. These microarray-based assays can be widely classified as either glycan arrays or lectin arrays. The former involves glycans / glycoconjugates immobilised onto a solid surface such as a microarray with the lectins or antibodies introduced in solvent phase, while the latter array is reversed in format [69]. By using known glycan structures, the assay could be used to screen for lectins or antibodies with certain specificities. In contrast, by using immobilized lectins or antibodies with known binding specificities, unknown glycan profiles in a sample can be screened. Furthermore, sandwich style lectin arrays, which use two or more lectins or antibodies of known binding specificities, have allowed for the identification of both glycan motifs as well as the associated protein or lipid on the glycoconjugates [70].

The measurements are usually performed by fluorescence spectroscopy. The solution phase components which may be either glycans or lectins are usually conjugated to a fluorophore or a molecular ligand probe such as biotin. The latter serves to bind an enzyme that could further catalyse the detectable fluorescence or chemiluminescent reaction. Label free assays have also been developed which use surface plasmon resonance (SPR) for real-time detection

of binding [71, 72]. Similar assays with MS based detection have provided a second dimension, *m/z* analysis, for identifying glycan structures [50].

Lectin microarrays have proven valuable in a number of research fields in glycobiochemistry [73], but of relevance here is their use in TPNG analysis for disease association. These assays are usually applied for in-depth profiling of glycoforms for biomarker discovery [70, 74, 75]. The approach often involves identifying glycoforms on specific target proteins which require several processing steps limiting the throughput of the assay as compared to other glycomics techniques. Since the assay depends on glycan-lectin/antibody interactions, the glycan identification specificity of the assay is limited to the specificity of the detecting lectin/antibody. As mentioned above, when these assays are established in a laboratory, they may not require extensive technical skills. However, preparation and adaptation of these assays for new applications is not trivial. Adapting the assay to identify a new target glycan motif requires much effort in lectin/antibody discovery and optimisation of binding conditions.

Chromatography- and mass spectrometry-based assays

The techniques of chromatography, capillary electrophoresis (CE) and MS have been around for many decades, and they have always played central roles in profiling glycan structures [60]. Table 1 compares some of the common techniques used for glycomics applications in academia and industry. Glycan profiling by LC-FLD is widely accepted in industrial laboratories for quality control (QC) and quality assurance (QA) purposes because conclusive information on glycan profiles can be obtained whilst having a relatively low infrastructural cost of instrumentation [76]. More importantly, among analytical chemists, chromatograms are easily comprehensible and can even be processed rapidly in a semi-automated manner [77]. The LC techniques commonly used are hydrophilic interaction liquid chromatography (HILIC) for glycans [56, 67] and reverse phase (RP) chromatography for glycopeptides, methylated glycans or some reducing-end conjugated glycans [78]. Additionally, analyses on some mixed-mode column-chemistries have been reported [79-81]. The molecular identification is based on retention time. In some applications this information level can be limiting. However for QC, QA and other routine applications, this may not be an issue as the profiles of the samples are usually well known. LC-FLD has also been taken up with success in some industrial service laboratories for the analysis of patient samples, for examples, TPNG [7] and IgG glycomes [82, 83]. However, for the former, the achievable information is limited by the glycome complexity of these samples. Glycan structures may be unresolved and low abundance structures could be overshadowed by co-eluting structures of higher abundance. For example, information on antennary fucosylation in TPNG [5] is usually difficult to obtain due its low abundance and overlapping peaks. However, antennary fucosylation is an important motif in association with many inflammatory diseases [7, 17, 46]. Furthermore, it is particularly challenging to identify the triantennary and tetraantennary structures of TPNG by LC-FLD owing to their low relative abundances and their structural complexity resulting in several isomers with highly similar retention times. Capillary electrophoresis (CE) - laser induced fluorescence (LIF) is an electrophoretic separation technique that has been around for several decades but is now

gaining popularity in glycomics analysis [84]. Capillary zone electrophoresis (CZE) is a CE technique which separates molecules according to their electrophoretic mobility. This mobility depends on the analyte's hydrodynamic radius and charge state in solution [85]. The separation resolution obtained with these separation methods is usually superior to that of LC methods. Capillary gel electrophoresis (CGE)-LIF is another CE-based technique that is particularly promising for industrial laboratories. The ability to multiplex sample analysis makes CGE-LIF a high-throughput electrophoretic technique unlike CZE [86, 87].

The use of MS in glycomics has been increasing over the decades, because it analytically complements LC and CE techniques for identifying and confirming glycan structures. In glycomics, since it is usually important to maintain the intact structure of the glycan during ionisation, soft ionisation techniques such as electrospray ionisation (ESI) and matrix-assisted laser desorption ionisation (MALDI), are mostly adopted [88-90]. Advances in mass analysers have led to high resolution MS which was fuelled by the need for molecular identification and has indeed also improved glycomics analysis. The mass analysers used are mostly Time of flight (TOF) [59, 91] and ion traps [92] and to a lesser extent (triple-)quadrupoles [93]. The use of some ultrahigh resolution analysers, such Orbitraps [94] and ion cyclotron resonance (ICR) analysers [95] has further allowed for differentiating certain isobaric glycan/glycopeptide structures in the human *N*-glycome. The inclusion of scanning quadrupoles and IMS drift tubes [96, 97] in addition to fragmentation associated ion optics, such as CID cells [98] and electron capture dissociation (ECD) cells [99] have enhanced MS/MS and MS^n analysis of glycans to deduce structural isomers [100]. Interestingly, in-source fragmentation, mainly on MALDI systems, has also been successful in MS^n analysis [101].

Due to the linkage variants of glycan residues, fragmentation of glycans by MS is vital in determining their isomeric structure. Method of choice is usually CID which typically breaks the glycosidic bonds of the glycan in positive ion mode. In negative ion mode, this fragmentation technique can induce cross--ring C-C bond breaks, thus providing detailed structure information [96, 102]. However, these fragmentation patterns are not always sufficiently informative or abundant to identify the glycan structure. This is especially complicated with co-eluting structures from a coupled LC/CE platform. Additionally, some motifs, such as antennary fucosylation, can undergo molecular rearrangement in the gas phase especially during CID. This can provide overlapping fragmentation patterns which complicates precise structural identification [103]. Hence, many MS-based assays rely on a two-dimensional approach of coupling separation techniques, such as LC or CE, to MS for improved structural resolution. Advantageously, these separation techniques can be easily coupled to an ESI source [83, 98], whilst additionally a few customised in-line MALDI based instruments have been reported [104]. CE-MS, in particular, is becoming popular in glycomics assays, because of its often-higher separation power compared to LC and the low amounts of sample required [84, 98]. Furthermore, the low nanoliter per minute flow rates can benefit sensitivity when using nano-ESI sources [105, 106].

Table 1. Comparison of common chromatographic, electrophoretic and mass spectrometric techniques used for glycomics applications in for academia and industry.

Analytical separation	Detection	Conjugation and derivatization	Application	Isomeric differentiation	Ease of use and adaptability	Throughput	Infrastructure requirements
LC	FLD	Fluorophore conjugation	Industry and academia	+	+++	++	+
	ESI-qTOF-MS/MS	Reducing end conjugation	Industry and academia	++	++	++	+++
	ESI-iontrap-MS^n	Reducing end conjugation	Mostly academia	+++	++	++	+++
CE	LIF	Fluorophore conjugation	Mostly academica	++	+	+	+
	ESI-qTOF-MS/MS	Reducing end conjugation	Mostly academica	+++	+	+	+++
CGE	LIF	Fluorophore conjugation	Industry and academia	++	++	+++	++
-	MALDI-qTOF-MS	Sialic acid stabilization, Hydroxyl group methylation	Mostly academia	Possible only for sialic acid linkages	++	+++	+++

The use of exoglycosidases in glycan profiling

Glycosidases are enzymes that hydrolyse the glycosidic bonds within glycans and are broadly categorised as either exo- or endo glycosidases, depending on their site of hydrolysis within a glycan. Exoglycosidases can hydrolyse the glycosidic bonds at the non-reducing terminals whilst endoglycosidases hydrolyse the glycosidic bonds closer to the reducing end [33]. Owing to the substrate specificity of glycosidases, they have become a valuable tool in glycobiochemistry research as well as for industrial applications such as glycan profiling and glycoengineering (Figure 1). The use of exoglycosidases in glycan profiling is of especial interest to this thesis.

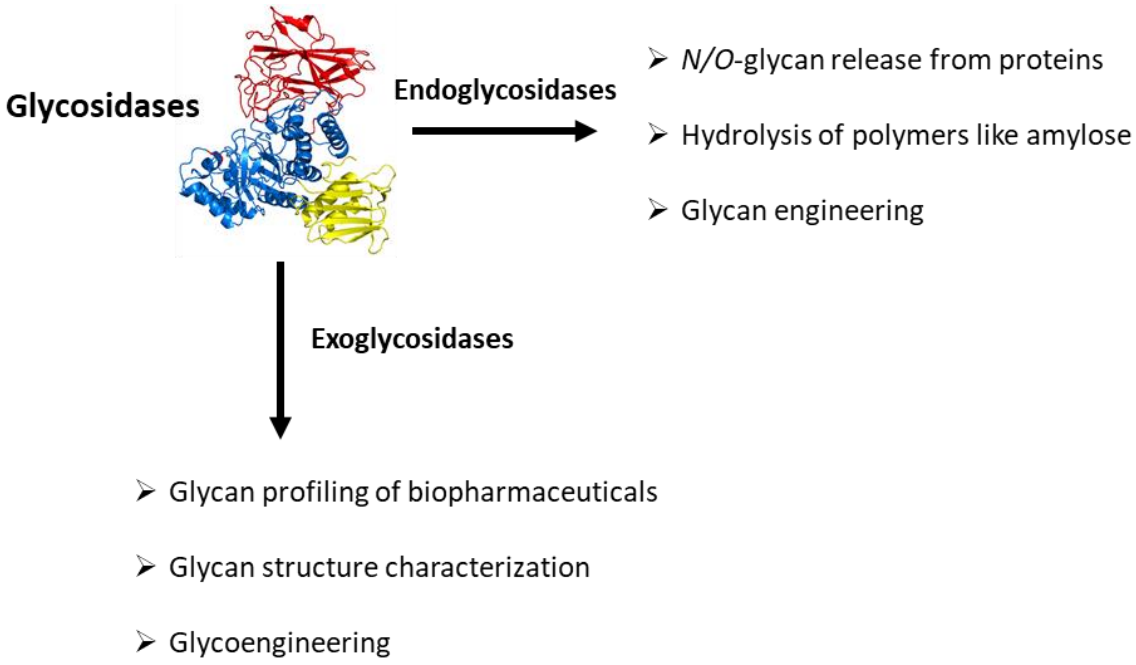
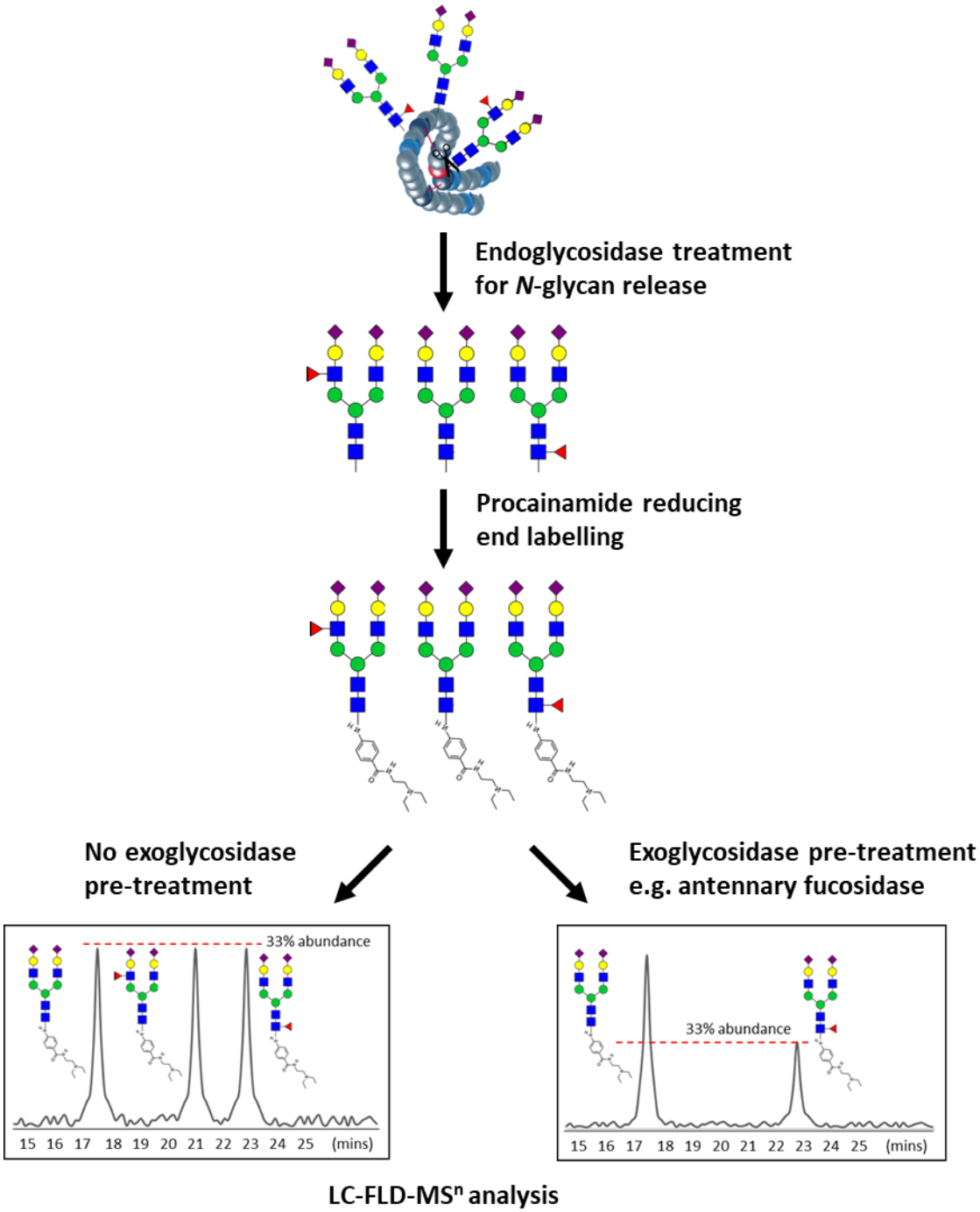


Figure 1. Common uses of glycosidases in industrial applications.

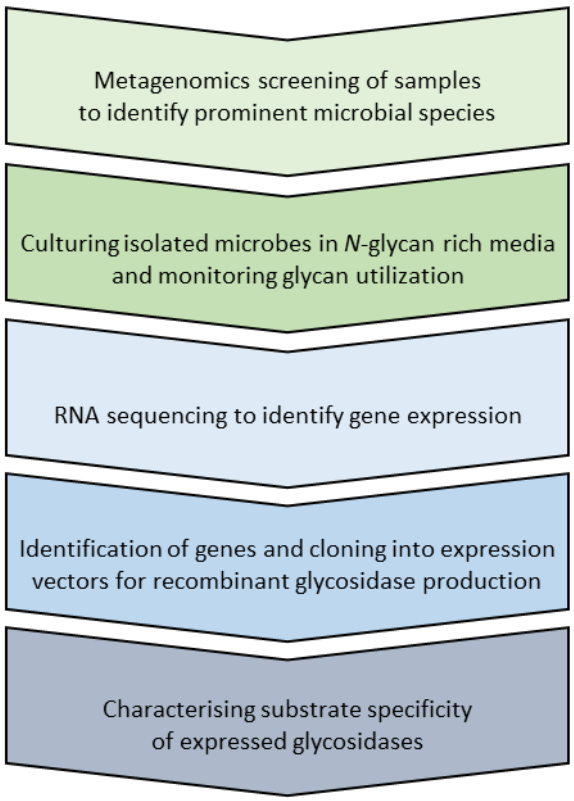
Glycan profiling is heavily dependent on chromatographic and spectrometric techniques, as mentioned above. The use of exoglycosidases for glycan sequencing was and continues to be vital in complementing these analytical instrumentation techniques for glycan profiling [32]. The principle of exoglycosidase-based glycan profiling is as follows. Released glycans or glycopeptide samples are treated consecutively with an array of exoglycosidases of known specificity for identifying glycan residues as well as linkages. Such treated samples are then subjected to LC-FLD/MS analysis for the identification of the cleavage products [34, 35]. By correlating chromatographic peak shifts between exoglycosidase treated and non-treated samples, one can follow the sequential removal of residues from the non-reducing termini. This profiling approach has vital importance in biopharmaceutical characterisation and biomarker discovery for identifying glycan structures. It also serves as an alternative in confirming glycan structures that are not always conclusively identified by MS-based techniques.



Scheme 1. Substrate specificity characterisation of potential exoglycosidases of interest, for example an antennary fucosidase.

The value of exoglycosidases as a profiling tool lies in the fact that they are enzymes and can have specificity for certain residues and for certain linkages. However, in practice, specificity of exoglycosidases range from narrow to broad, thus creating the need to source and characterise exoglycosidases of high substrate specificity for the purpose of glycan profiling [35]. Furthermore, owing to glycan conformation and composition complexity, the activities of some exoglycosidases can be restricted by co-occurring motifs, thus creating an additional

demand for exoglycosidases with novel activity. The latter can be exemplified by the fact that until the work of this thesis, all known antennary fucosidases had their activities restricted by steric hinderances of sialyation on the antennary fucose arm of the *N*-glycans. Scheme 1 depicts a general analytical procedure used in screening exoglycosidases for their substrate specificity.



Scheme 2. General workflow of glycosidase discovery from metagenomics data.

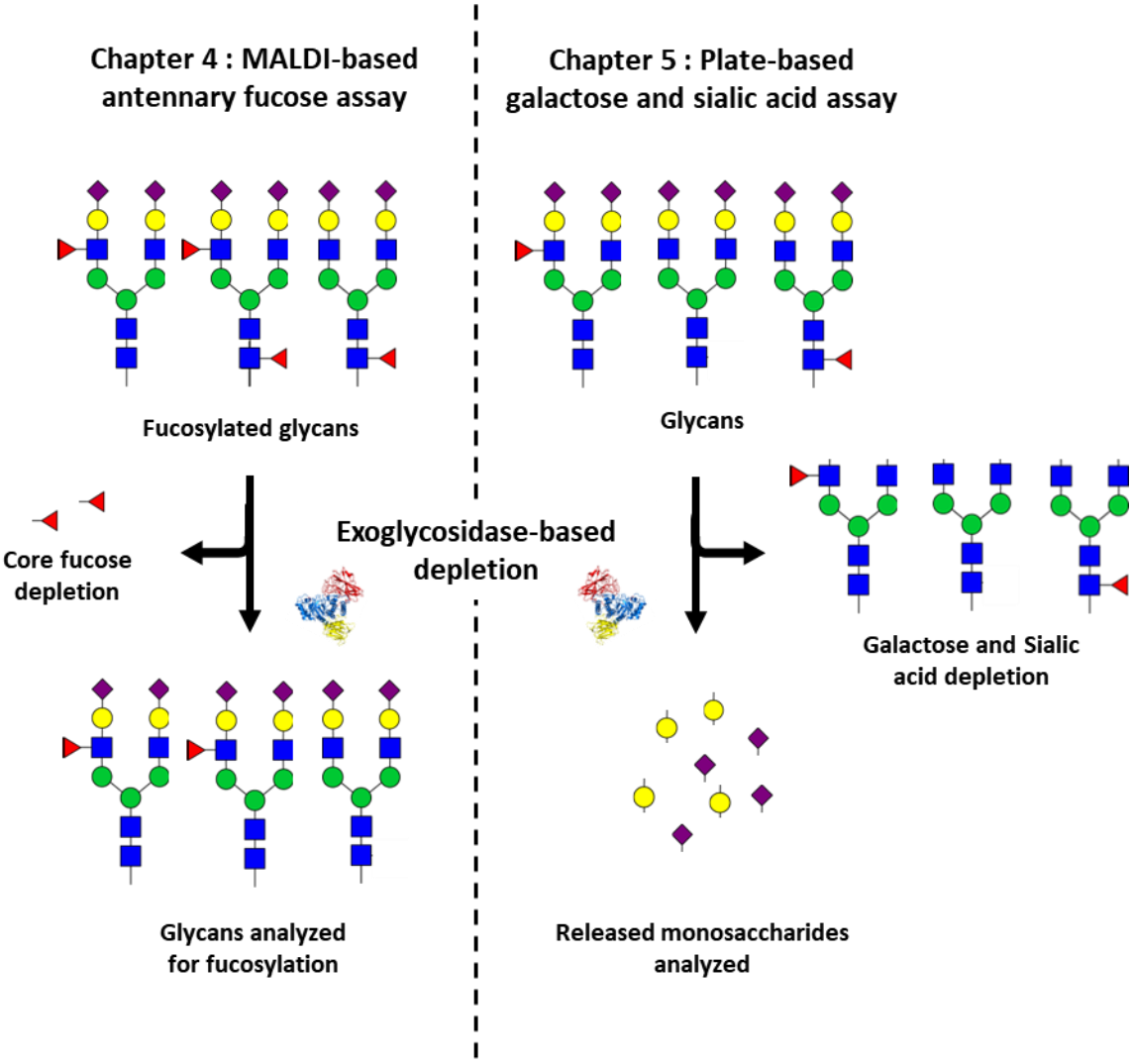
A common approach of sourcing exoglycosidases is metagenomic screening of complex glycan environments such as gut microbiota, for discovering exoglycosidases of desired specificities, as shown in Scheme 2 [38-40]. The prominent microbes in these environments are identified and their gene expression screened for potential glycosidases of interest. Basically, a successfully propagating living organism, will produce the required components to consume the available nutrients, in this case exoglycosidases to consume the glycans in their environment. Metagenomic screening for discovering novel glycosidases has proven valuable [40, 41] for identifying potential exoglycosidases for glycan profiling [34, 35] and glycoengineering [42]. A more targeted approach is bio-engineering well-characterised exoglycosidases to obtain an enhancement of activity, for example an improvement of linkage specificity [33, 36, 37]. Usually, exoglycosidases of well understood enzymatic mechanism are subjected to genetic manipulation to obtain recombinant exoglycosidases of enhanced activity and/or specificity.

Scope

The above sections briefly describe the major analytical techniques used in glycan profiling. Many of these techniques are common to proteomics, metabolomics and to a lesser extent genomics. However, these omics fields are also complemented by biochemical assays which have a targeted approach to quantification of certain analytes. For example, enzyme linked immuno sorbent assay (ELISA) is widely used in proteomic research to identify and even quantify certain proteins of interest in a sample, while qPCR is used for quantification of certain gene sequences in a genomic sample. With the exception of lectin microarrays, glycomics assays based on the directed quantification of certain glycan epitopes have not been investigated extensively, especially in human health. Such targeted assays can meet demands in industrial applications such as QC, QA and glycome profiling where usually routine analytical queries are addressed. Additionally, focusing on quantification of certain glycan analytes of interest may simplify complicated data which could further make such assays user-friendly. The work of this thesis involves the development of novel glycomics assays that quantify glycan features of interest. A re-fashioned approach to exoglycosidase-based glycan profiling by LC-FLD/MS is taken for the development of these assays (Scheme 3). This approach is coined here as *destructive enhancement*. In short, exoglycosidases are used for cleaving certain glycan residues of certain linkages in glycome samples of TPNG or IgG. This is followed by quantifying the released desired glycan residues from the sample or profiling the remaining glycan structures that still contains the desired epitopes. Thus, by using exoglycosidases to degrade certain glycan epitopes, a destructive process, the possibility opens to the analyse of the same or different epitopes in a simple manner which would normally be tedious, leading to an enhancement of analysis (Scheme 3).

The assays developed in this thesis are heavily dependent on commercially available exoglycosidases. In addition, new exoglycosidases were characterized in this thesis to aid glycan profiling by LC-FLD-MS^n. Some of the exoglycosidase activities desired for such profiling were not commercially available or mentioned in literature. The analytical chemistry work of this thesis aided metagenomic screening efforts for sourcing these desired exoglycosidases. In Chapter 2 and 3, the importance of analytical chemistry in complementing the sourcing of exoglycosidases of novel specificity is demonstrated.

Chapter 2 characterises the substrate specificity and structure of a fucosidase that is specific to antennary fucose of linkage α(1-3/4). This fucosidase, from Ruminococcus gnavus, was discovered in a metagenomic screening of the human gut microbiome. To our knowledge, this is the first reported antennary fucosidase which is not inhibited by sialyation such as in sialyl lewis X epitopes. This enzyme demonstrated potential in complementing the profiling of antennary fucosylation in complex glycome samples of TPNG on a LC-FLD-MS platform [80]. The analytical task of antennary fucose quantification in TPNG is especially challenging due to the low abundance of this epitope in TPNG and the phenomenon of fucose rearrangement in the gas phase. The work of this chapter has contributed to exoglycosidase tools required for enhancing this analytical task.



Scheme 3. The destructive enhancement approach used in assays developed in Chapters 4 and 5.

Chapter 3 is a follow-up study on a previously discovered GlcNAcase from Bacteroides thetaiotaomicron [40]. To our knowledge this is the first reported GlcNAcase that is specific to the bisecting β(1-4) linked GlcNAc on *N*-glycans. However, its activity is hindered by galactosylation and sialylation on the antennary arms. Investigating the structure of the enzyme may help us understand the basis of its substrate specificity, and thus aid in future bio-engineering efforts for enhancement of its activity. In Chapter 3, a structural characterisation of the GlcNAcase by X-ray crystallography is carried out. However, the study only provided limited insights regarding the mechanism of the enzyme due to the lack of glycan co-crystallisation. Although the catalytic residues in the active site are conserved among related GlcNAcases, the specificity to a GlcNAc linkage on a *N*-glycan is regulated by certain defining features around the entrance to the active site [40, 107]. The GlcNAcase studied here lacked such defining features and hence computer modelling of the glycan in the active site was not conclusive in determining the structural basis of its specificity.

Interestingly, a possible glycan binding activity of the C-terminal domain was proposed which has contributed to furthering the understanding of this enzyme.

The development of novel glycosidase-based assays for the quantification of certain target epitopes is carried out in Chapters 4 and 5. Chapter 4 describes the development of a novel glycosidase-based MALDI-MS assay for quantification of antennary fucosylation in TPNG. Within this chapter, quantitative superiority of this assay over a HILIC-MS^n method for industrial applications is demonstrated. Quoting the *destructive enhancement* approach, a core fucosidase pre-treatment was used to destructively deplete the core fucose residues in TPNG samples before analysis on MALDI FT-ICR or MALDI-TOF platforms. This assay was able to quantify up to 19 antennary fucosylated glycans in TPNG which was a great enhancement in quantification abilities as compared to an industrial HILIC-MS^n method which has long been considered the gold-standard technique for antennary fucose quantification. Furthermore, since this assay is based on MALDI-MS analysis, this makes the assay especially attractive in terms of high-throughput applications as compared to a much more lengthy HILIC-MS^n analysis.

Chapter 5 introduces a novel plate-based exoglycosidase assay and demonstrates its analytical abilities for the quantification of galactosylation and sialylation in human IgG glycome. Unlike Chapter 4, Chapter 5 utilises exoglycosidases to release monosaccharide residues that are subsequently quantified rather than removing interfering linkages on a glycan. Treatments with galactosidase and galactosidase + sialidase were used in a destructive manner for releasing exposed galactose and sialic acid residues from intact IgGs purified from human plasma. The released galactose monomers where subjected to an enzyme-based redox-reaction to produce a fluorescent signal which was measured on a spectrophotometric plate reader. The signal was proportional to the galactosylation and indirectly allowed to infer sialylation of the glycans. Since the assay is also able to measure IgG amounts in the sample, absolute quantification of galactose or sialic acid residues per IgG molecule is possible. Moreover, this also helps to normalise variations in IgG amounts between patient samples. The measured readouts are available as single values with minimal data processing thus enhancing an otherwise relatively complex measurement of IgG galactosylation by an industrial HILIC-MS^n method. Although, the assay provides a lower level of information as compared to LC-FLD/MS based assays, it is expected to detect associations to inflammatory diseases. In fact, the assay was successful in drawing disease association in inflammatory bowel disease (IBD) as identified previously by a LC-FLD assay [108]. Inflammatory diseases are usually associated with a change in abundance of total galactosylation and sialylation [109]. Often in LC-FLD/MS analysis of a glycome, a chromatogram is simplified to calculated derived traits which, in the case of inflammatory diseases, are total galactosylation and sialylation. The plate assay directly measures these monosaccharide units and thus serves its analytical purpose successfully. Furthermore, since this assay is based on a spectrophotometric plate reader, a relatively low infrastructure investment is required, unlike for chromatographic and/or MS based assays. This makes the assay especially attractive to laboratories that are not experienced in glycomics analysis provided they are interested in IgG galactosylation.

Chapter 6 is a detailed discussion on the current widely used techniques of chromatography, electrophoresis and mass spectrometry used in glycomics, as well as the on the analytical approaches, workflows and contributions of this thesis towards glycomics assay development. This discussion is especially pertaining to development of proof-of-concept assays that have potential of being transformed into commercial kits. The most important and critical characteristics of glycomics assays for industrial applications are discussed in great detail. Chapter 6 will also touch on outlooks for future assay development.

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