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FEATURE ARTICLE

Advances in mass spectrometry-based glycomics: progress, applications, and perspectives

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As one of the most common post-translational modifications, glycosylation plays a vital role in various biological processes, including tumor-immune interactions. Aberrant glycosylation is associated with the onset and progression of numerous human diseases. Mass spectrometry-based glycomics is highly significant for elucidating the underlying the molecular mechanisms and exploring the novel potential diagnostic and prognostic biomarkers. In this feature article, we summarize the workflow of mass spectrometry-based glycomics, covering key experimental steps including glycan release, separation and purification, derivatization and labeling, mass spectrometry analysis, and quantification strategies. Besides,we also discuss the applicability and limitations of various techniques in glycomics research. This review aims to provide a systematic reference for glycomics research and promote the in-depth application of glycomics analysis in bio-medical studies and biomarker discovery.

Introduction

Glycosylation is one of the most prevalent and complex post-translational modifications (PTM) of proteins. In addition, glycosylation also occurs on lipids, primarily in sphingolipid molecules, forming glycosphingolipids. Recently, it has been reported that glycosylation can also occur on small non-coding RNA molecules, including snRNA, rRNA, and miRNA.[1, 2]

Glycosylation plays an important and unique role in biological systems, participating in processes such as cell signaling, protein folding and immune responses.[3, 4] The disruption of glycosylation may affect the normal functioning of cells, leading to the onset or exacerbation of various diseases.[5] Therefore, abnormal glycosylation is widely recognized as a hallmark of many diseases, including cancers,[6] cardiovascular diseases,[7] immune system disorders[8] and diabetes.[9] Many clinically approved biomarkers are glycoproteins,[10] including human epidermal growth factor receptor 2 (HER2) for breast cancer, alpha-fetoprotein (AFP) for liver cancer, carcinoembryonic antigen (CEA) for colorectal cancer, and prostate-specific antigen (PSA) for prostate cancer.

Glycosylation can be primarily classified into N-glycosylation and O-glycosylation according to the linkage between glycans and proteins. N-glycans are linked to the asparagine residue of proteins, which only occur within a specific amino acid sequence (asparagine−X−serine or threonine (Asn−Xxx−Ser/Thr or N−X−S/T, X can be any of the amino acids except for proline)). Besides, N-glycans have a conserved pentasaccharide core structure ($Man_3GlcNAc_2$). In contrast, O-glycans are linked to Ser or Thr residues without a specific amino acid sequence and lack a conserved core structure. Based on their structures, O-glycans can be further classified into various types, such as Core1, Core2, Core3, and Core4.[11] Distinct from the nucleic acid-mediated, template-directed biosynthesis of proteome and genome, glycome synthesis occurs independently of molecular templates and glycans are produced dynamically through the coordinated and competitive actions of a series of enzymes, including glycosyltransferases and glycosidases.

The evolution of mass spectrometry (MS) technologies has revolutionized glycomic profiling by enabling comprehensive characterization of complex biological specimens. This review critically evaluates cutting-edge innovations in sample processing methods, mass spectrometric approaches as well as data analysis techniques in MS-based glycomics research and discuss future prospects in this field.

Overview of MS-based glycomics

The workflow of MS-based glycomics research primarily consists of several key steps: sample collection, glycan release, separation and purification, derivatization and labeling, MS analysis, and data analysis. This section provides a systematic overview and discussion of advances in sample preparation and MS techniques within glycomics research.

Glycomics analysis of different sample types

Glycomics research encompasses diverse sample types with distinct biological properties, offering critical insights into glycosylation mechanisms across biological systems. Therefore, prior

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to detailing standardized analytical workflows, we outline the strategic applications of these specimens.

Contemporary glycomics research predominantly prioritizes biofluid analysis, with blood specimens representing the most characterized sample type. The non-invasive or minimally invasive acquisition of these specimens reduces procedural discomfort while preserving analytical validity for translational studies. Studies have shown that long term storage of frozen blood samples at -20°C or -80°C has only a minor influence on the glycome.[12] Blood samples not only exhibit excellent stability but also require an extremely low starting amount, making them an ideal sample type for glycomics research. Notably, as little as 10 µL of blood is sufficient to obtain a comprehensive glycomic profile, and in some studies, even less than 1 µL is adequate.[13] Additionally, other types of biofluid samples, such as saliva,[14] urine[15] and cerebrospinal fluid[16-18] are also applied in glycomics research.

Besides biofluid samples, cell samples are also widely used for studying biological processes. Glycome analysis of mammalian cells typically requires 10^5-10^7 cells.[19, 20] Nevertheless, population-averaged measurements from bulk analyses fail to capture cellular heterogeneity and inevitably dilute subtle glycomic variations across individual cells. Single-cell glycomics has emerged as a promising approach to overcome the limitations of bulk analyses, which may mask cellular heterogeneity and subtle intercellular variations. This technology enables precise characterization of distinct cellular subpopulations, rare cell types, and dynamic cell-state transitions. Recently, Marie et al. reported a method for the direct analysis and quantification of N-glycans from single cells by employing an integrated platform that combines online in-capillary sample processing with high-sensitivity label-free capillary electrophoresis-mass spectrometry, enabling the detection of up to 100 N-glycans per single HeLa or U87 cell.[21]

Extracellular vesicles (EVs) are lipid bilayer-enclosed particles released by various types of cells carrying various bioactive molecules, including nucleic acids, proteins, and metabolites, which play unique biological roles in tumor progression, immune responses, and intercellular communication. They are present in almost all body fluids, including urine, blood, saliva, and breast milk. In recent years, the study of EV glycomics has also become a hot research focus.[22] Our group successfully isolated and purified EVs from human serum and human urine samples and conducted an in-depth analysis of the N-glycome within them. We were the first to characterize the N-glycome of EVs from human serum, revealing their potential as a source for biomarker discovery.[23] Furthermore, based on 333 urine samples, we generated a comprehensive N-glycome landscape of urinary EVs and this study pioneers urinary EV N-glycomics for bladder cancer diagnosis.[24]

Glycan release

The efficient liberation of glycans from their carrier proteins or peptides is a prerequisite for comprehensive glycomic investigations. To facilitate subsequent MS analyses, the controlled cleavage of glycosidic linkages must be achieved through optimized enzymatic or chemical methodologies, ensuring complete glycan release while preserving their structural integrity.[25]

Enzymatic release of glycans

Enzymatic release methods are commonly applied to the release of N-glycans. Among various enzymes used for N-glycan release, PNGase F is the most commonly used for mammalian proteins. It cleaves the glycosidic bond between the asparagine side chain and the reducing end of the glycan under mild conditions, creating an aspartic acid residue and a free glycosylamine, which would be subsequently hydrolyzed to form a reducing N-glycan. While PNGase F exhibits high cleavage efficiency for many types of N-glycans, it cannot cleave N-glycans with core α-1,3-fucosylation, which obstruct its utilization in the N-glycome analysis of invertebrates and plants.[26, 27] To compensate for the limitations of PNGase F, PNGase A, which shares the same cleavage sites, has been applied to the release of N-glycans with core α-1,3-fucosylation. Notably, PNGase A exhibits lower efficiency in glycan release. PNGase H^+, derived from Terriglobus roseus, can also release N-glycans with core α-1,3-fucosylation. Additionally, compared to PNGase A, it exhibits higher efficiency in releasing N-glycans without core α-1,3-fucosylation.[28] However, it is important to note that both PNGase A and PNGase H^+ require acidic conditions for activity,[29, 30] which may lead to the loss of sialic acids. Therefore, further evaluation and optimization of digestion conditions are needed to balance enzymatic efficiency and the degradation of acid-sensitive glycans.

Besides the PNGase family, endoglycosidases (Endo) are also widely used in N-glycan analysis, including Endo F1-F3 and Endo H. Endo H and the Endo F enzymes primarily catalyze the hydrolysis of the β-1,4-glycosidic bond between the two N-acetylglucosamine (GlcNAc) units in the core structure of N-glycans and leave a single GlcNAc residue on the protein or peptide substrate. However, different endoglycosidases exhibit varying catalytic activities toward different types of N-glycans.[31] To ensure complete glycan release, a combination of multiple glycosidases can be utilized for obtaining comprehensive N-glycan profile.

Chemical release of glycans

Compared with enzymatic methods, chemical release methods can release not only N-glycans but also O-glycans, and are not significantly restricted by glycan structural variations. Especially, due to the lack of suitable enzymes for releasing O-glycans, chemical release methods are widely used in the analysis of O-glycans. Currently, the classic chemical methods mainly include hydrazinolysis and β-elimination. Hydrazinolysis employs hydrazine, typically anhydrous hydrazine, to cleave the amide bond between the glycan and the asparagine residue, releasing the glycan in the form of a glycosylamine. However, side reaction may occur and causes the loss of monosaccharides from the reducing terminus of the glycans (known as peeling).[32] In addition, anhydrous hydrazine poses significant safety hazards. Hydrazine monohydrate has been reported as a safer alternative with equivalent yields to the traditional method using anhydrous hydrazine.[33] Besides, buffer exchange with low concentrations of trifluoroacetic acid (TFA) or low-molarity ethylenediaminetetraacetic acid (EDTA) solutions prior to hydrazinolysis can significantly mitigate the impact of peeling.[34, 35] β-elimination is a more commonly used chemical method for glycan release. Reductive β-elimination generates alditols that lack a reducing end, making them unsuitable for subsequent reducing-end derivatization and thereby limiting their application in MS analysis. In contrast, non-reductive β-elimination retains the reducing end of the released glycans, allowing for subsequent reducing-end derivatization. However, under alkaline conditions, glycans released by nonreductive β-elimination are prone to undergo peeling.[36] Over the past decades, chemical release methods have made significant progress, keeping peeling to a minimum and introducing microwave-assisted methods to improve the efficiency of O-glycan release.[37, 38] In addition to the traditional hydrazinolysis and β-elimination methods, an oxidation-based release method using hypochlorite (NaClO) has been developed for glycan release.[39] It has been

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reported that this method effectively avoids peeling reactions during the release of O-glycans,[40] while also demonstrating high sensitivity and efficiency in N-glycan analysis.[41] Furthermore, release methods based on the organic superbase, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) have also been developed, providing a more efficient approach for the release of O-glycans.[42, 43] DBU demonstrates strong alkalinity in aqueous solutions and can rapidly and efficiently release O-glycans from proteins within 0.5 to 1 hour. Moreover, as an organic base, DBU can be easily removed through hydrophilic desalting methods. Building upon this, our group has further developed method by combining DBU-based release with light and heavy isotopic labeling, which will be discussed in detail in the quantification section.[36]

Separation and purification methods

Limited by the inherently low abundance of glycoproteins in biological samples, the released glycans also tend to be of low abundance. Moreover, the abundance of different glycans can vary by several orders of magnitude. Therefore, it is necessary to perform glycan separation and purification prior to MS analysis to enhance the detection sensitivity and analytical accuracy.

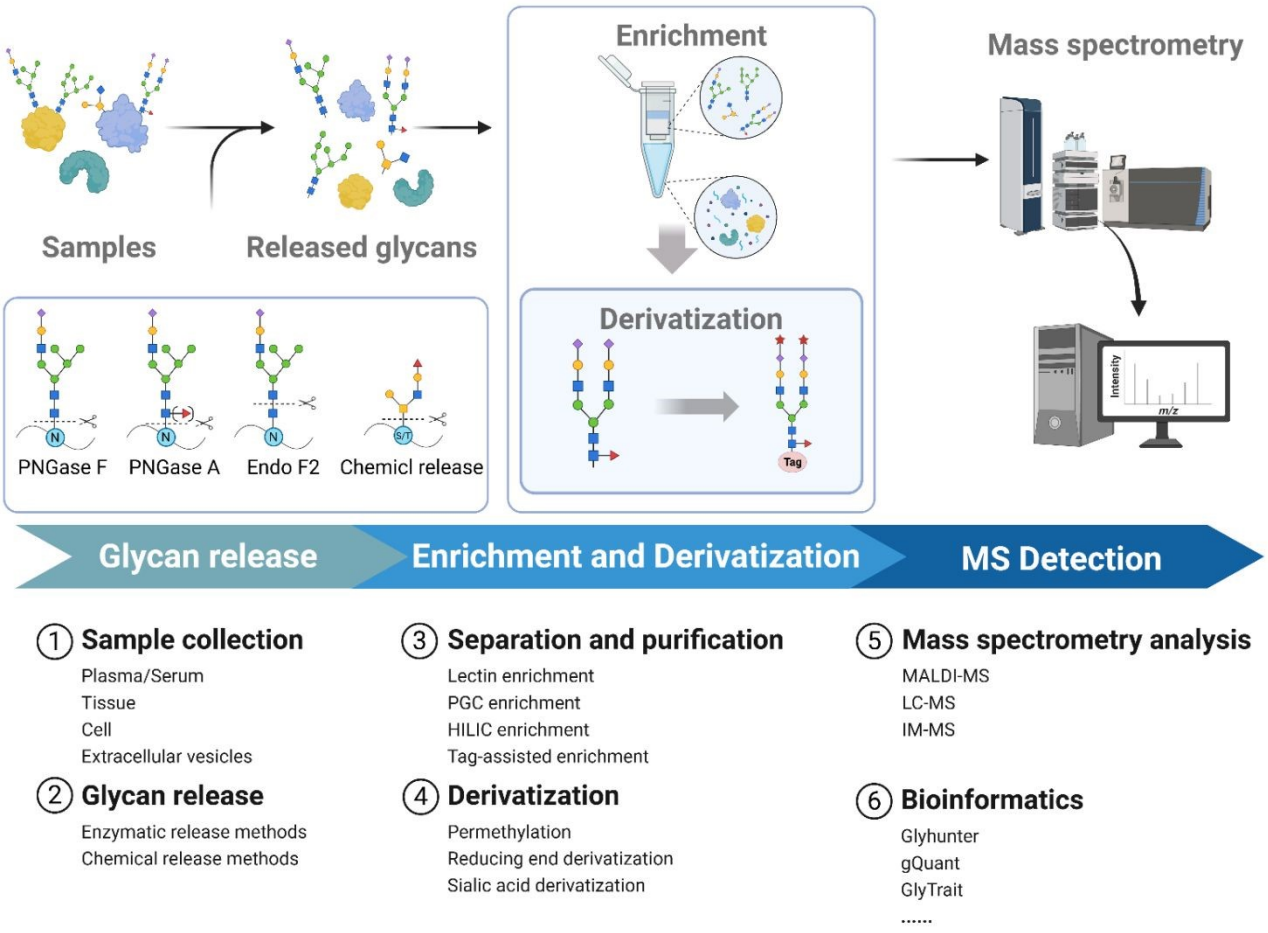

Figure 1. General workflow of MS-based glycomics analysis. Figure created with BioRender.com. Copyright 2025.

Lectin enrichment

Lectins are a group of proteins that can specifically and reversibly recognize and capture glycans with particular motifs. Different lectins exhibit significant differences in their affinity and specificity for glycans. For example, Concanavalin A (Con A) recognizes glycans with branched α-mannosidic structures; Wheat germ agglutinin (WGA) has specificity towards sialylated glycans; Sambucus nigra agglutinin (SNA) and Maackia amurensis lectin (MAL-II) exhibit high affinity for sialic acid residues with α-2,6-and α-2,3-linkages, respectively.[44, 45] Lectin enrichment methods offer relatively simple and convenient workflows. Nevertheless, these method are limited by their coverage, as only glycans with specific structures can be captured. To broaden the range of captured glycans, multiple lectins with different specificity are combined for more comprehensive enrichment.[46, 47] Given the unique properties of lectins, they can not only be used to enrich target glycans but also can be used to deplete high-abundance N-glycans in samples to enhance the identification of low-abundance glycans.[48]

Porous graphitized carbon (PGC) enrichment

PGC enrichment is also commonly used for glycan purification. Through multiple interactions between glycans and the graphitized carbon surface, it enables effective separation of glycan isomers, playing a crucial role in modern glycomics analysis. Moreover, this method utilizes mobile phases compatible with mass spectrometric analysis, making it widely applied in MS-based glycomics studies. With the increasing demand for enrichment sensitivity, traditional PGC-based methods have shown limitations in sensitivity. Therefore, the development of novel carbon-based materials to enhance detection sensitivity represents a promising direction for further

research in this field.[49] Lin et al. synthesized a structurally tunable 3D hierarchical porous carbon matrix (HPC-Ce/Fe) via a microemulsion-guided assembly strategy.[50] The unique hierarchical porous architecture of HPC-Ce/Fe facilitates ultrahigh-sensitivity enrichment of N-glycans. Additionally, PGC is also suitable for the separation and purification of O-glycans. Nevertheless, traditional PGC methods are limited in detecting smaller glycans consisting of one or two monosaccharides, such as the Tn antigen (a single GalNAc), which is a well-known tumor-associated carbohydrate antigen.[51] Zhang et al. developed a novel hybrid approach by combining PGC with boronic acid solid-phase extraction and employing new PGC particles with a narrow particle size distribution.[52] This strategy enhanced the binding capacity for truncated O-glycans and improved isomer separation across the entire glycan structural range.

Hydrophilic interaction chromatography (HILIC) enrichment

HILIC is an enrichment method that employs a polar stationary phase and a high-percentage organic mobile phase. Hydrophilic glycans are selectively retained through strong hydrophilic interactions with the stationary phase. The packing materials used in commercial ZIC-HILIC columns are commonly employed as stationary phases for HILIC-based glycan enrichment.[53] However, the high cost of ZIC-HILIC columns increases the overall expense of glycan analysis. Therefore, low-cost and easily accessible materials may be more suitable for large-scale glycomics studies in the future. Wu et al. employed a natural hydrophilic material, bacterial cellulose (BC), as the stationary phase for HILIC enrichment.[54] This method enabled the purification of N-glycans from mixtures with the 10-minute enrichment process and the specificity of this method is over 94%. At the same time, our group also developed an N-glycan enrichment method using easily prepared and cost-effective sterilized cotton.[55] We chose sterilized cotton as the enrichment material for N-glycans because it is primarily composed of cellulose and features a rough surface and a loose, porous structure. The exposed hydroxyl groups allow for the highly selective capture of hydrophilic molecules, like glycans. This enrichment method enables high-selectivity and high-sensitivity N-glycan analysis of multiple samples within a total processing time of just 2.5 hours, and successfully identified 52 N-glycans from only 1 µL of human serum. Moreover, our group compared the HILIC enrichment method based on sterilized cotton with the PGC enrichment method in a study of the N-glycome of porcine sperm membrane.[56] The results showed that the sterilized cotton-based method identified a greater number of N-glycans and exhibited higher consistency in the analysis. Furthermore, our group integrated the sterilized cotton-based enrichment method with an automated platform to develop the GlycoPro platform. This platform seamlessly incorporates key steps including protein extraction, desalting, digestion, derivatization, and N-glycan enrichment. Utilizing a 96-well plate format, GlycoPro enables the high-throughput processing and enrichment of up to 384 samples within a single day, significantly enhancing the efficiency and scalability of glycomics research.[57]

Tagging-assisted enrichment

Another type of enrichment method involves introducing enrichable tags on glycans through derivatization, enabling selective enrichment based on these tags. Our group developed an N-glycan enrichment method based on phosphate derivatization combined with titanium ion (Ti^{4+}) immobilized solid-phase extraction (SPE).[58] The phosphorylated N-glycans are specifically enriched using an optimized Ti^{4+}-SPE protocol, which is commonly used for phosphopeptide enrichment. This strategy achieves nearly 100% derivatization efficiency and demonstrates excellent selectivity and high analytical sensitivity. Moreover, considering that fluorous solid-phase extraction (FSPE) can effectively separate highly fluorinated compounds from non-fluorinated ones, our group introduced a hydrophobic fluorocarbon tag at the reducing end of N-glycans to achieve efficient enrichment via FSPE.[59] Furthermore, we developed a strategy for simultaneous enrichment and quantification of N-glycans using light and heavy isotopic fluorous reagents, O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (PFBHA and PFBHA-d_2).[60]

Derivatization methods

The inherent hydrophilicity and poor ionization efficiency of glycans frequently compromise detection sensitivity in mass spectrometric analysis. Chemical derivatization (e.g., permethylation, reductive amination with hydrophobic tags, or hydrazide labeling) is therefore essential to enhance ionization efficiency by modulating their hydrophobicity and charge affinity. Furthermore, derivatization not only enhances ionization efficiency but also facilitates the enrichment and purification of glycans, as well as more accurate quantification, by leveraging the specific properties of certain derivatization tags.

Permethylation

Permethylation is a widely used derivatization method for N-glycans and O-glycans. Glycans react with methyl iodide (CH_3I) under alkaline conditions to convert all free hydroxyl groups into methyl ethers, while also esterifying the carboxyl groups of sialic acids or other carboxylic acids.[61-63] Permethylation offers several notable advantages. First, it increases the hydrophobicity of glycans, making them more easily ionized during MS analysis and enabling the acquisition of more sequence-informative fragment ions with high sensitivity. Second, permethylation effectively neutralizes the negative charge of sialic acids by esterifying their carboxyl groups, which stabilizes the glycan structure and enables comprehensive N-glycome analysis in the positive-ion mode of matrix-assisted laser desorption/ionization (MALDI) without sialic acid loss. Third, the use of isotopically labeled permethylation reagents can further enable the quantitative analysis of glycans. Traditional isotopic permethylation-based quantification strategies are typically limited to 2-plex.[64, 65] To enhance multiplexing capability while preserving the advantages of permethylation, Dong et al. developed an 8-plex permethylation quantification strategy using isotopically labeled CH_3I reagents.[66] This method enables the simultaneous quantification of up to eight samples in a single MS run. Despite its significant advantages, permethylation requires additional chemical reactions, which not only increase the complexity of the experimental workflow but also place higher demands on the precision of experimental procedures and the operator's chemical expertise. Moreover, the reaction may introduce side products, such as the loss of O-acetyl functions and incomplete permethylation of hydroxyl groups, resulting in more complex mass spectra and increased difficulty in data interpretation. Recent research efforts have prioritized developing automated, microscale, and high-throughput permethylation workflows, including the use of 96-well microplates, to minimize experimental heterogeneity caused by manual operations and to precisely control reaction time.[67-69]

Reducing end derivatization

Following enzymatic or chemical release, the reducing end of glycans exposes a reactive aldehyde group, which serves as an important site for chemical derivatization. Common reducing end derivatization reactions mainly include three types: reductive

amination, oximation formation, and hydrazone formation.

Reductive amination refers to the reaction in which the aldehyde group at the reducing end of glycans condenses with a reagent containing a primary amine under mildly acidic conditions to form an imine, which is subsequently reduced by a reducing agent to yield a stable secondary amine product. Reductive amination is widely employed to add fluorescent groups to glycans, which not only facilitates liquid chromatography detection but also significantly enhances the hydrophobicity and ionization efficiency, thereby improving the sensitivity of MS analysis. Common fluorescent derivatization reagents include 2-aminobenzoic acid (2-AA),[70] 2-aminobenzamide (2-AB),[71] and 1-aminopyrene-3,6,8-trisulfonic acid (APTS).[72] Traditional reductive amination methods typically require the removal of excess derivatization reagents, which may lead to potential sample loss. Hronowski et al. reported a nonreductive labeling approach in which 2-aminobenzoic acid (2-AA) reacts directly with the aldehyde group at the reducing end of glycans on a MALDI target plate, without the need for a reduction step.[73] In this method, 2-AA also serves as the matrix for MALDI analysis, thus eliminating the cumbersome step of removing excess derivatization reagents. Compared to the conventional use of 2,5-dihydroxybenzoic acid (2,5-DHB) as the matrix, this strategy significantly enhances the signal intensity of free oligosaccharides in the negative ion mode. Our group developed a method using aminopyrazine as a derivatization reagent. This approach takes advantage of aminopyrazine's co-matrix properties after nonreductive amination, allowing the derivatized products to be directly analyzed without cumbersome purification steps.[74] Our group also developed a method for enriching N-glycans by introducing phosphate groups at the reducing end of N-glycans via reductive amination, followed by enrichment using Ti^{4+}-SPE.[58] Besides, our group has also labeled the reducing ends of glycans using biotinylated arginine (GRACAT).[75] The strong affinity between biotin and streptavidin enables the highly specific enrichment of labeled glycans from mixtures of proteins and peptides. The derivatized glycans exhibit a nearly 50-fold increase in ionization efficiency, and the improvement in fragment signals in tandem mass spectrometry allows for the differentiation of glycan isomers. In addition to enrichment and purification, our group has also developed several derivatization strategies based on reductive amination for quantitative analysis. The GRIAL quantification strategy involves using isotope-coded arginine with an amino group (NH_2-Arg) as the derivatization reagent to label the reducing end of N-glycans. This strategy offers nearly 100% labeling efficiency, increases the signal-to-noise ratio of glycans by 3 to 5 times, and does not cause chromatographic isotope effects.[76] The MeCTL quantification strategy involves the derivatization of N-glycans using metal element chelated tags. This strategy not only exhibits high labeling efficiency and quantitative accuracy but also provides significant cross-ring fragmentation, enabling the differentiation of N-glycan isomers. Additionally, by chelating with several different rare earth elements, the MeCTL strategy allows for multiplex quantification.[77]

Oxime formation refers to the reaction in which the aldehyde group at the reducing end of glycans reacts with hydroxylamine-containing reagents under mild conditions to form oxime derivatives. Our previous research has shown that fluorinated compounds with high hydrophobicity and chemical stability can significantly enhance the hydrophobicity of glycans, thereby improving their ionization efficiency in mass spectrometry analysis. Additionally, by combining FSPE, selective enrichment of N-glycans can be achieved.[59] On this foundation, we developed a strategy for the simultaneous enrichment and quantification of N-glycans based on the light and heavy fluorous reagents PFBHA and PFBHA-d_2 through oxime click chemistry.[60] This derivatization method not only exhibits high labeling efficiency but also shows compatibility with other derivatization strategies, offering broader application potential in multiplex labeling, structural analysis, and quantitative studies. To validate this, we further introduced isotopic methylamine labeling of sialic acids on the basis of PFBHA labeling, and proposed a combined strategy that enables selective enrichment, quantification, and structural identification of both neutral and sialylated glycans.[78] Additionally, our group also developed a strategy based on hydrazone formation by selecting hydrazinonicotinic acid (HYNIC) as both the derivatization reagent and co-matrix, which is applicable for the analysis of both neutral and sialylated N-glycans.[79]

Recent advances in reducing-end derivatization techniques have significantly advanced glycomics by improving ionization efficiency, enrichment capacity, and quantitative accuracy. Current methods typically involve complex procedures that restrict their utility in large-scale clinical investigations, highlighting the essential need for automated derivatization platforms to enable high-throughput clinical applications. The development of automated derivatization platforms is essential for enabling high-throughput clinical applications. Future optimization efforts should prioritize workflow simplification, minimization of manual operations, and elimination of purification steps through strategies like magnetic bead-based enrichment or on-target derivatization, thereby enhancing overall efficiency and practical applicability.[45]

Sialic acid derivatization

Sialic acids play a crucial role in many biological processes, especially in cancer development and immune responses, where changes in their structure have a significant impact on the occurrence and progression of diseases.[80-82] Sialylated glycans exhibit reduced ionization efficiency in positive ion mode mass spectrometry due to the negatively charged carboxyl group of sialic acid residues, leading to suppressed signal intensity and compromised detection sensitivity. Additionally, sialic acids have poor stability and are prone to loss or fragmentation during sample processing or mass spectrometry analysis.[83] It is necessary to derivatize sialic acids to neutralize their negative charge and enhance their stability.[84] Notably, sialic acids have linkage isomers, such as α-2,3 and α-2,6, and the derivatization of sialic acids not only improves signal intensity and quantification accuracy but also enables structural characterization in complex biological samples.

Methylamine is one of the most commonly used derivatization reagents for sialic acids, capable of achieving complete methylamidation while preserving O-acetyl groups. However, this method lacks specificity toward different sialic acid linkage isomers and cannot distinguish between α-2,3 and α-2,6-linked sialic acids.[62, 85] Currently, an increasing number of linkage-specific sialic acid derivatization methods have been developed and applied to distinguish linkage isomers. Nishikaze et al. developed a one-pot strategy termed sialic acid linkage-specific alkylamidation (SALSA) to selectively labels α-2,3 and α-2,6-linked sialic acids through a two-step sequential alkylamidation.[86] As a result of the reactions, α-2,3 and α-2,6-linked sialic acids are selectively amidated with different length of alkyl chains, allowing distinction of α-2,3/α-2,6-linkage isomers from given mass spectra. Hanamatsu et al. further reported a novel SALSA-based strategy focused on ring-opening aminolysis (aminolysis-SALSA).[87] This approach retains the advantage of linkage specificity while significantly reducing reaction time and simplifying the experimental procedure.

Our group has developed several derivatization methods for the

analysis of sialic acid linkage isomers. We developed a novel strategy based on the sequential derivatization for sialyl-linkage isomers using the different reactivity of α-2,3-and α-2,6-linked sialic acids toward nucleophiles. Generally, a weak activation at first would readily react α-2,6-linked sialic acids, while α-2,3-linked ones usually form intramolecular lactone or stay unchanged. Then a strong activation would further transform the remaining α-2,3-linked sialic acids.[88] This strategy demonstrated high reproducibility and quantitative accuracy over two orders of magnitude of dynamic range during both forward (light-heavy) and reverse (heavy-light) labeling. We also combined linkage-specific derivatization with charge-sensitive separation methods based on microfluidic chip-capillary electrophoresis-mass spectrometry (MCE-MS) for the specific analysis of α-2,3-linked sialic acid. The α-2,6 and α-2,3-linked sialic acids were selectively labeled with methylamine (MA) and N,N-dimethylethylenediamine (DMEN), respectively, which selectively makes α-2,3-sialylated N-glycans positively charged and realizes online purification, concentration, and discrimination of α-2,3-sialylated N-glycans from other N-glycans in microchip CE-MS.[89] Based on this, an integrated one-step sialic acid derivatization, cationic separation, and high-sensitivity detection method was developed for the rapid and specific analysis of α-2,3 and α-2,6-linked sialylated N-glycan isomers.[90] The easily charged long-chain amine compound N,N-Dimethyldipropylenetriamine (DMDT) was selected for the one-step derivatization of sialic acid. Due to the structural differences in the α-2,3 and α-2,6-linkage isomers, the derivatized glycans can be quickly and efficiently separated within 10 minutes in MCE.

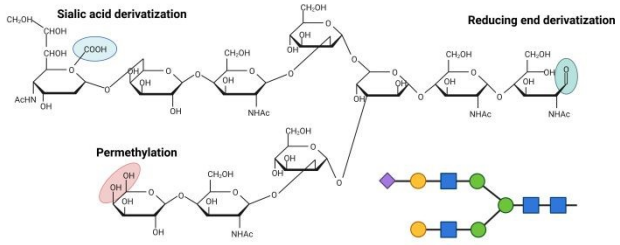

Figure 2. Commonly used derivatization methods in N-glycan. Figure created with BioRender.com. Copyright 2025.

Mass spectrometry analysis

Mass spectrometry has become a cornerstone in glycomics research owing to its exceptional sensitivity, high resolution, and ability to provide detailed structural information. MALDI-MS demonstrates outstanding performance in rapidly generating glycan profiles from complex samples and has thus emerged as a preferred strategy for N-glycan analysis. Furthermore, its compatibility with high-throughput sample preparation formats, such as 96-well plates, significantly enhances its utility in high-throughput and rapid analysis. ESI-MS, typically coupled with separation techniques like HPLC, offers superior resolution and sensitivity, making it well-suited for the analysis of more complex samples; however, its throughput is relatively limited. Glycans exhibit a high degree of isomeric diversity, and the emergence of ion mobility mass spectrometry (IM-MS) has introduced a new dimension for their separation.[91]

Development of matrix for MALDI-MS analysis

Currently, the most commonly used matrix for glycomics analysis is 2,5-dihydroxybenzoic acid (DHB). However, DHB tends to form uneven needle-shaped crystals around the spot edges upon drying, leading to poor reproducibility of mass spectra. Ionic liquid matrices, composed of conventional solid matrices and organic bases, can maintain relative fluidity under vacuum conditions, enabling highly uniform mixing between the matrix and the analyte, thereby significantly improving the reproducibility of the analysis.[92] Kaneshiro et al. developed a method using a liquid matrix composed of 3-aminoquinoline (3-AQ)/α-cyano-4-hydroxycinnamic acid (CHCA) for the direct labeling of free oligosaccharides on MALDI targets, enabling high-sensitivity detection.[93, 94] Subsequently, they introduced a similar liquid matrix, 3-aminoquinoline/coumaric acid (3-AQ/CA), for glycan analysis.[95] Compared with 3-AQ/CHCA or DHB, 3-AQ/CA exhibited higher sensitivity, effectively suppressed the dissociation of sialic acids, and improved the fragmentation of neutral glycans. To achieve efficient ionization of sialylated glycans without chemically modifying sialic acids, Hinou et al. developed solid ionic matrices based on O-benzylhydroxylamine (BOA)/DHB/Na and N-methylaniline (NMA)/DHB/Na.[96] The DHB salts formed amorphous and homogeneous solid surfaces with aniline derivatives, which were suitable for automated analysis together with analytes. More recently, they reported a new solid ionic matrix system by introducing BOA instead of aniline, further improving the ionization efficiency of glycans.[97] Although these novel matrices have, to some extent, improved the ionization efficiency of glycans and enhanced glycan signal intensity in mass spectrometry analysis, 2,5-DHB is still widely regarded as the gold standard in glycomics research. Emerging ionic liquid matrices and solid ionic matrices have demonstrated superior performance in certain studies, particularly in improving signal reproducibility and reducing fragmentation. However, current investigations using these new matrices are mostly limited to a small number of analytes, often focusing on model systems or standard samples, and there is still a lack of systematic validation in large-scale and complex biological samples.

Ion dissociation methods

In glycomics research, structural elucidation is crucial for gaining a deeper understanding of the biological functions of glycans. Glycan structures encompass not only monosaccharide composition but also complex features such as branching patterns. Accurate structural characterization helps deciphering the roles of glycans in disease development and is essential for identifying potential biomarkers. To obtain a comprehensive structural insights, glycans are typically subjected to fragmentation by mass spectrometry, generating characteristic fragment ions. Analyzing the m/z of these fragments allows the deduction of the specific glycan structures. Different fragmentation patterns and ion dissociation methods provide complementary structural insights, enabling comprehensive glycan analysis.[98] In principle, two different types of cleavage can occur in oligo- and polysaccharides: glycosidic cleavage and cross-ring cleavage. The resulting fragments are commonly designated using the Domon–Costello nomenclature.[99]

Collision-induced dissociation (CID) is the most widely used dissociation method in mass spectrometry. In CID, precursor ions are accelerated in an electric field and collide with neutral gas, converting kinetic energy into internal energy of the precursor ions. With continuous collisions, the internal energy gradually increases, eventually causing the weakest bonds (usually glycosidic bonds) in

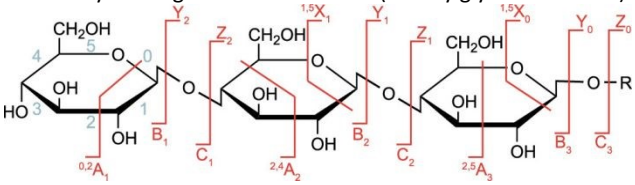

Figure 3. Domon-Costello nomenclature of carbohydrate fragmentation.[99] Reproduced from reference 99 with permission

the glycan to break, producing B/Y ions. This method is widely employed in mass spectrometric platforms such as quadrupole and fourier transform ion cyclotron resonance (FTICR) instruments.[100] Nevertheless, CID often leads to the loss of labile monosaccharide residues, particularly fucose and sialic acid. In addition, CID suffers from a significant issue known as the "migration phenomenon." The most prominent example is fucose migration, where under positive ionization mode, fucose residues may migrate from the non-reducing terminus to the reducing end of the glycan chain, leading to misidentification or loss of internal sugar residues[101]. The primary requirement for fucose migration is the presence of mobile protons or, at least, poor charge fixation, which is most commonly observed in protonated glycans ($[M + xH]^{x+}$). Similarly, glycans adducted with ammonium ions (NH_4^+) are also prone to fucose migration, whereas those adducted with alkylated ammonium salts are not, as they lack mobile protons.[102] Importantly, derivatization strategies have been shown to effectively mitigate such migration, thereby improving the accuracy of glycan structural analysis. High-energy collision dissociation (HCD) refers to a fragmentation technique involving keV-level collision energies, capable of generating extensive and rich molecular fragments. It is primarily applied in Orbitrap mass spectrometers, enabling the acquisition of high-resolution and high-accuracy data, which greatly enhances identification accuracy.[103]

In addition to collision-induced dissociation (CID)-based strategies, electron-based ion dissociation methods (ExD) play a critical role in the structural characterization of glycans. ExD encompasses a series of fragmentation techniques that induce highly specific bond cleavages, including electron capture dissociation (ECD),[104] electron transfer dissociation (ETD),[105] electron detachment dissociation (EDD),[100] electron-induced dissociation (EID)[106] and electron excitation dissociation (EED).[107] Compared with traditional collision-based approaches, ETD and ECD provide richer cross-ring cleavage information essential for determining glycosidic linkages. Furthermore, owing to their gentle fragmentation nature, ExD techniques are particularly advantageous in preserving glycan moieties during glycopeptide analysis, thereby facilitating more accurate structural elucidation.[108, 109] EED is also recently emerged as a powerful tool in structural glycomics. EED is capable of providing detailed structural information in a single stage of MS/MS analysis and has been successfully integrated with online liquid chromatography (LC) and ion mobility separation for the efficient characterization of glycan mixtures. The application of EED MS/MS for the structural analysis of free oligosaccharides has thus far been primarily demonstrated on FTICR MS instruments. The limited accessibility and high operational costs of FTICR MS have hindered the widespread adoption of EED MS/MS in glycoscience research. Recently, Wei et al. validated the performance of EED MS/MS on a hybrid Orbitrap-Omnitrap QE-HF instrument, demonstrating high sensitivity, fragmentation efficiency, and analytical speed.[110]

In addition to the aforementioned dissociation methods, ultraviolet photodissociation (UVPD) is an emerging fragmentation technique that delivers higher-energy UV photons to excite ions to elevated energy states, thereby enabling new fragmentation pathways. For glycans, UVPD induces both glycosidic bond cleavages and cross-ring cleavages, resulting in the production of more A-type and X-type ions, which facilitates the elucidation of glycan branching patterns.[100, 111] For example, Helms et al. employed UVPD to perform structural analysis of O-glycans in mucin. They presented a representative UVPD spectrum, which generated a rich array of fragment ions, including two key cross-ring cleavage products that enabled the localization of Gal and Fuc to the third and fourth carbon atoms of the GlcNAc residue, respectively.[112] UVPD can generate a wide variety of fragment ions, which provides abundant structural information for glycans but also increases the complexity of spectral interpretation. With the continuous advancement of artificial intelligence technologies, particularly the widespread application of machine learning algorithms in data analysis, it is anticipated that these approaches can be integrated into glycomics data interpretation, not only improving the accuracy and efficiency of fragment ion identification but also facilitating the automated annotation and deeper understanding of complex glycan structures.

IM-MS in glycan Isomer separation

Glycan isomerism occurs on different levels, including glycan composition, connectivity, configuration, and branching.[98] In the derivatization section in this review, we provide a detailed description of the separation of the linkage isomers of sialic acids. In addition to these chemical strategies, ion mobility spectrometry (IMS) has also been applied to the separation of glycan isomers. IMS is a technique that separates ions based on differences in their mobility through a gas phase. Ion mobility is influenced by multiple factors, including the ion's mass, charge, size, and shape. A key parameter in IMS is the collision cross section (CCS), also known as the rotationally averaged collision integral, which reflects the effective area of an ion during collisions with gas molecules and is directly related to the ion's size and shape. Therefore, CCS serves as an important indicator for the separation of glycan isomers. Notably, the CCS of an ion is instrument independent and exhibits good comparability across different IM-MS platforms. Moreover, CCS can be theoretically calculated, providing a powerful basis for the standardized analysis and structural identification of glycan isomers.[113]

O-glycans in mucins exhibit extensive isomeric diversity, which poses significant challenges for differentiation using conventional MS techniques. Jin et al. evaluated the capability of IM-MS in separating O-glycan isomers, and their study demonstrated that IM-MS has the potential to assist in identifying structurally complex O-glycan isomers via CCS values. However, traveling wave ion mobility spectrometry (TWIMS) suffers from limited resolution, making it difficult to separate most intact glycan ions and accurately estimate their CCS values.[114] Bechtella et al. proposed a rapid separation and identification approach based on high-resolution trapped ion mobility spectrometry (TIMS) for analyzing O-glycans released from mucins. In porcine gastric mucin (PGM), a total of 49 structures were identified, with an average of two isomers per composition. Notably, the TIMS-based method provided conclusions comparable to a one-hour LC-MS analysis within just a few minutes, highlighting its great potential as a powerful alternative to conventional LC-MS workflows.[115]

Quantitative glycomics

To comprehensively elucidate the roles of glycans in biological processes, it is imperative to establish glycan quantification strategies that can reveal expression differences across various biological samples and physiological conditions. Next, we will discuss the quantification strategies of glycomics. Quantification methods mainly include relative and absolute quantification. MS-based relative quantification of glycans primarily relies on the stable isotopic reagents which introduce a mass difference for the same glycan derived from different samples while maintaining similar chromatographic and mass spectrometric behaviors. Common glycan isotope labeling quantification strategies can be classified into

three categories: metabolic labeling, enzymatic-assisted labeling, and chemical labeling (Fig. 4).

Metabolic labeling

Metabolic labeling refers to the process of introducing labeled reagents during cell culture, enabling glycans to carry isotope labels through the cells' own metabolic processes. The advantage of this type of quantification strategy is that different isotope-labeled cell lysates are mixed immediately after cell lysis, ensuring that all samples are under identical experimental conditions. Jiang et al. developed a metabolic isotope labeling strategy called GlyProSILC (Glycoprotein Stable Isotope Labeling in Cell Culture), which can simultaneously label both glycans and peptides in cells.[116] However, metabolic labeling method is limited to cell samples and is not applicable to clinical tissue or body fluid samples.

Enzymatic labeling

The enzymatic labeling quantitative method mainly involves introducing heavy or light atoms during the enzymatic cleavage process, creating a mass difference. For example, Zhang et al. developed an enzymatic labeling method called glycan reducing-end ^{18}O labeling (GREOL).[117] This method utilizes the phenomenon that the Endo H enzyme can incorporate ^{18}O from $H_2{}^{18}O$ during the release of N-glycans. By performing enzymatic cleavage in $H_2{}^{16}O$ and $H_2{}^{18}O$, a 2 Da mass difference is generated for quantification. However, the 2 Da mass difference can be affected by isotope effects, requiring an additional deconvolution step for accurate quantification. To address this issue, they further developed the glycan-reducing end dual isotopic labeling (GREDIL) method, using PNGase F and adding an extra reduction step with $NaBH_4$/$NaBD_4$ during deglycosylation. This increases the mass difference to 3 Da, thereby avoiding isotope overlap.[118] Due to the availability of specific glycan-releasing enzymes for N-glycans, such as PNGase F, enzymatic labeling is more commonly used in N-glycan analysis. In contrast, the application of enzymatic labeling in O-glycan analysis is limited by the scarcity of enzymes capable of releasing O-glycans.

Chemical labeling

Chemical labeling is the most commonly used method for quantitative glycan analysis, not only because isotopic labels can be efficiently introduced through chemical reactions but also because it can increase the ionization efficiency of glycans and facilitate their enrichment and purification as described in the derivatization section. Our group has developed several strategies for the quantitative analysis of N-glycans using isotopic labeling reagents. For example, we established the GRIAL strategy using commercially available isotopically labeled amino acids.[76] We also developed the MeCTL strategy based on metal-chelating tags.[77] MeCTL enables detection in the negative ion mode, providing a clean background and rich MS/MS fragmentation, which is particularly advantageous for sialic acid analysis. Furthermore, we developed the DuSIL strategy specifically for sialic acid quantification.[84] This method employs dual isotopic labeling reagents, allowing simultaneous identification and quantification of both neutral and sialylated N-glycans.

Currently, most of the above quantitative methods are focused on N-glycans. Quantifying O-glycans faces significant challenges due to their low abundance, the lack of a universal enzyme, and the difficulties in sample preparation. To address these challenges, our group developed O-GlycoIsoQuant, a novel O-glycome quantitative approach utilizing superbase release and isotopic Girard's P labeling.[36] This method uses rapid and efficient nonreducing β-elimination to dissociate O-glycans from proteins using DBU, an organic superbase, combined with light and heavy isotopic Girard's reagent P (GP) labeling for relative quantification of O-glycans by MS. Using this method, the labeled O-glycans exhibit a double peak with a 5 Da mass difference, which is suitable for stable relative quantification.

Absolute quantification

The relative quantification method is well-suited for comparative studies involving large sample sizes, as it does not rely on glycan standards, making it both efficient and cost-effective. When it comes to clinical diagnostics or drug development, the absolute quantification method can provide more rigorous and precise quantitative data.[119] Zhao et al. proposed a novel strategy that utilizes two external standards of glycans to measure the absolute abundance of aging-associated IgG glycans and established a predictive model for biological aging—abGlycoAge based on their absolute concentration changes.[120] However, many natural glycans have complex structures, making it difficult to obtain high-purity standards, which are often expensive and result in high overall experimental costs. In recent years, with the continuous advancement of glycan synthesis technologies, the efficiency of both chemical and biosynthetic approaches has significantly improved.[121, 122] It is believed that in the near future, driven by these technological developments, absolute quantification methods for glycans will achieve major breakthroughs in practical applications.

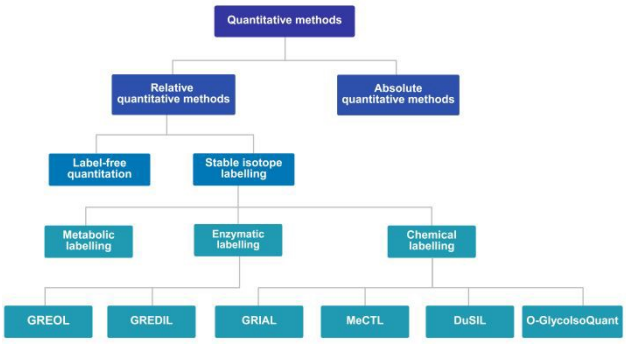

Figure 4. Quantification methods of glycomics mentioned in this review.[36, 76, 77, 84, 117, 118] Figure created with BioRender.com. Copyright 2025.

Bioinformatics

In MS-based glycomics research, the high structural complexity of glycans makes the interpretation of glycan structures from mass spectrometry data a persistent and significant challenge. To date, many tools and software have been developed to interpret glycan structures from mass spectrometry data. Currently, a semi-automated annotation tool, GlycoWorkbench,[123] developed by EUROCardDB in 2008, is in common use. The main function of GlycoWorkbench is to evaluate a set of structures proposed by the user by matching a list of corresponding theoretical fragment masses with a list of peaks derived from the spectra. Additionally, the developers integrated GlycanBulider,[124] a glycan visualisation editor, into GlycoWorkbench, which allows for the rapid assembly of structural models using a comprehensive set of constructs. In recent years, artificial intelligence, particularly deep learning, has played an increasingly important role in glycan composition and structure annotation. Urban et al. developed a scalable and accurate workflow centered around a deep learning model named CandyCrunch for predicting glycan structures from LC-MS/MS data, which employs Convolutional Neural Network (CNN) with dilations and residual

connections to classify spectra into glycan classes, followed by post-processing filters to refine structural assignments. They compiled a large database of nearly 500,000 MS/MS spectra from more than 2,000 glycomics experiments to train the model. In about 90% of cases CandyCrunch correctly predicted glycan structures and, more importantly, completed the analysis of the entire dataset within seconds.[125] Additionally, in recent years, researchers have applied Transformer architectures to MS data analysis in the fields of proteomics.[126] Transformers utilize self-attention mechanisms to effectively model complex, long-range dependencies within the data. These attributes make them particularly well-suited for analyzing mass spectra, where the structural information encoded in the fragmentation patterns often involves relationship between peaks that are non-adjacent. Abtheen et al developed two glycomics-specific models based on the Transformer framework: GlycoBERT and GlycoBART, which are built upon the BERT (Bidirectional Encoder Representation from Transformers) and BART (Bidirectional and Auto-Regressive Transformers) architectures, respectively. GlycoBERT is trained as a sequence classifier that can accurately classify mass spectra into specific glycan structures. GlycoBART is a generative model capable of de novo glycan structure inference, allowing for the prediction of novel glycans beyond the scope of existing databases.[127]

Although GlycoWorkbench performs well in glycan structure annotation and visualization, it lacks a quantification module, making it incapable of directly processing quantitative mass spectrometry data or comparing glycan abundances across different samples. As a result, researchers often have to rely on additional tools for data integration and subsequent analysis. Tools such as GlycoReSoft,[128] MassyTools,[129] and MultiGlycan[130] have been developed to support the identification and auxiliary quantification of free glycans, but their quantification capabilities remain limited—particularly in the context of stable isotope-based relative quantification of glycans. To address this issue, Huang et al. developed gQuant, a MALDI-MS-based data processing tool for the relative quantitation of free glycans.[131] gQuant can automatically and efficiently process quantitative glycan mass spectrometry data and report all matched glycans along with their quantitation ratios. Besides, Kalmar et al. reported GlycoHunter, a user-friendly open-source software developed in MATLAB, which is designed to identify and perform the relative quantitation of N-linked glycans using the isotopic labeling-based INLIGHT strategy.[132] Unlike proteomics, which treats proteins as independent variables to distinguish different disease states and elucidate underlying mechanisms, glycosylation is a more complex biological process regulated by multiple coordinated factors. As a result, direct comparison of individual glycans often provides limited biological insight. Moreover, glycomics data are typically sparse and heterogeneous, further hindering direct comparisons of samples. Using glycosylation-derived traits is a method to gain deeper insights into biological processes. Therefore, our group developed GlyTrait, a Python-based framework aimed at enhancing glycomics analysis by innovatively computing and interpreting derived traits in N-glycome data.[133] GlyTrait automates the derivation of biologically significant features, shifting the focus from simply glycan abundance to functional glycan characteristics, such as branching and fucosylation. The GlyTrait workflow mainly comprises three steps. First, it constructs derived traits that are more biologically relevant than glycan abundances. Second, the newly generated glycan traits are pruned to reduce information redundancy and further enhance the interpretability of the variables. Finally, supported by robust statistical methods and interpretable machine learning techniques, the refined dimension is is scrutinized to unravel the association of glycosylation with research questions (the “statistical analysis” and “interpretable machine learning” modules).

As glycomics continues to evolve, there is an increasing demand for the large-scale, high-throughput processing of mass spectrometry data. To address this need, a free software called "GRITS Toolbox"[134] was developed in 2019 to process, annotate and archive glycomics mass spectrometry data. The core function of the software is to reveal the structure of glycans based on mass spectrometry data. In addition, it supports a number of features not available in other software, including the prediction of ions generated during neutral loss and the ability to specify custom ion structures. The GRITS toolbox also marks an advancement in handling complex data and supporting high-throughput experiments. With more and more experimental and analytical data being uploaded, Rojas-Macias et al. developed the concept of an informatics infrastructure that allows the MIRAGE (Minimum Information Required for A Glycomics Experiment) guidelines to be better implemented.[135] Their group established the UniCarb-DR repository, a MS characterisation glycan repository for storing peak lists and the GlycoPOST repository for storing raw data. Based on these two repositories and the MIRAGE they proposed a standardised process for recording glycomics data to ensure the reproducibility and sustainability of scientific research.

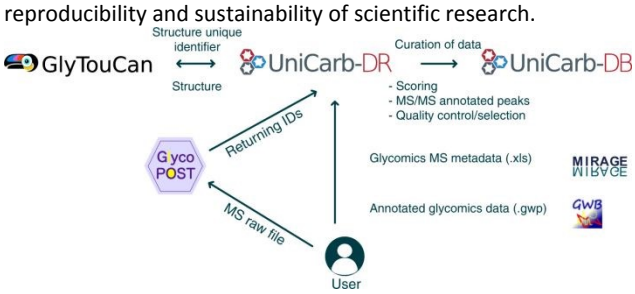

Figure 5. Workflow for MIRAGE data submission to UniCarb-DR.[135] Reproduced from reference 135 with permission from Springer Nature, copyright 2019.

Clinical glycomics

Abnormal expression levels of glycans are often closely associated with the onset and progression of various diseases, especially cancer.[136, 137] Therefore, by precisely analyzing the changes in glycan expression levels in patients, glycomics can not only assist in the early diagnosis of disease but also monitor disease progression and even predict a patient's response to treatment. As a rapidly evolving field, glycomics is increasingly demonstrating its translational potential in clinical applications. In the following sections, we will focus on glycomics research in several types of cancer, highlighting the critical role of glycan biomarkers in cancer diagnosis and treatment, and further underscoring their broad potential in precision medicine.

Liver cancer

Liver cancer is a highly prevalent malignant tumor originating in liver tissue, often triggered by chronic hepatitis or cirrhosis, and is associated with high incidence and mortality rates worldwide. Among its subtypes, hepatocellular carcinoma (HCC) is the most common, accounting for approximately 75% of all liver cancer cases. However, AFP, the classical biomarker for HCC, shows significant limitations in both sensitivity and specificity, making it unreliable as a standalone diagnostic or screening tool.[138] In recent years, serum N-glycans have emerged as highly promising biomarkers for the diagnosis and detection of HCC.[139, 140] Li et al. integrated transcriptomics, glycomics, and glycoproteomics to systematically investigate the dynamic changes of glyco-genes, N-glycans, and

glycoproteins during the progression of hepatitis B virus (HBV)-associated HCC. Gene expression analysis revealed dysregulation of fucosyltransferases (FUTs) in liver tissues from HCC patients. Glycomic analysis showed elevated levels of fucosylated N-glycans, and glycoproteomic analysis further demonstrated a progressive increase in fucosylation levels on IgA1 and IgG2. These findings collectively indicate that aberrant fucosylation plays a key role in the progression of HBV-related HCC.[141] Our group combined isotopic fluorous labeling with FSPE to analyze serum N-glycan alterations associated with HCC. Using only 0.5 µL of human serum, we identified 50 fluorinated N-glycans and quantified 30 of them. Among these, 15 N-glycans showed significant differences in abundance between HCC patients and healthy controls. These differentially expressed N-glycans were predominantly characterized by bisecting GlcNAc, sialic acid, and core fucosylation. However, further experimental validation is required to confirm their potential as clinical biomarkers.[60]

Gastric cancer

Gastric cancer (GC) is a malignant tumor originating from the epithelial lining of the stomach and is one of the most common and deadly cancers worldwide, with particularly high incidence in East Asian countries such as China and Japan. Due to its non-specific early symptoms and insidious progression, most patients are diagnosed at an advanced stage, making treatment challenging and prognosis poor. Early detection and treatment are critical to improving survival rates. Currently, endoscopy is the gold standard for diagnosing gastric cancer, but its invasive nature leads to poor patient compliance. As a result, the development of non-invasive, convenient, and sensitive diagnostic methods has become a research focus. Among these, glycomics-based biomarker studies offer new insights and approaches for the early detection and precision treatment of gastric cancer. Wang et al. labeled the serum of healthy controls and GC patients with light and heavy isotope reagents to analyze N-glycans.[78] A total of 35 glycans were successfully quantified, and it was found that hybrid and branched N-glycans were upregulated in GC, while levels of mono-antennary, bisected, and core fucosylated glycan types were reduced. Notably, bisected GlcNAc-type glycans with core fucose were upregulated in serum associated with colorectal cancer and hepatocellular carcinoma, but downregulated in GC, suggesting that this glycan may be a potential specific biomarker for the early diagnosis of GC. In addition, Qin et al. conducted a serum IgG glycomics study to compare the glycosylation differences between responders and non-responders to neoadjuvant chemotherapy (NACT) in patients with locally advanced gastric cancer (LAGC), aiming to identify potential biomarkers for predicting NACT efficacy. They constructed a predictive model that integrated patients' age, histological type, chemotherapy regimen, and three significantly different IgG glycoforms: GP4 (H3N4F1), GP6 (H3N5F1), and GP18 (H5N4F1S1). Receiver operating characteristic (ROC) analysis showed that the model had high predictive accuracy for NACT response, with an area under the curve (AUC) of 0.840, a sensitivity of 64.00%, and a specificity of 100%.[142]

Breast cancer

Breast cancer (BC) is a malignant tumor originating from the epithelial cells of the breast and is the most commonly diagnosed cancer among women worldwide. It typically arises in the lobules or ducts of the breast and often presents no obvious symptoms in the early stages. It is usually detected through mammography or self-examination as a painless lump. Therefore, identifying biomarkers for early diagnosis is crucial for improving patient survival and prognosis. Gebrehiwot et al. analyzed serum N-glycans from Ethiopian women with breast cancer (BC) across stages I–IV, as well as from normal controls (NC).[143] They identified 17 complex-type N-glycans that exhibited strong diagnostic potential in early-stage (stage I and II) BC patients (AUC=0.8-1). These glycans were predominantly characterized by core fucosylation, high branching, and sialylation—features that are closely associated with greater cancer invasiveness and metastatic potential. Our group developed a high-throughput and efficient glycomics analysis platform, GlycoPro, and applied it to serum samples from breast cancer patients, including 88 healthy controls, 88 infiltrating carcinoma (IC) patients, and IC patients with lymph node metastasis (LNM), aiming to identify breast cancer-related biomarkers. Using logistic regression analysis, five N-glycans were selected as a biomarker panel, which demonstrated a sensitivity of 88.24% for correctly identifying true positives and a specificity of 78.95% for correctly identifying true negatives. The model showed strong discriminative power with an AUC of 0.89, indicating a significant ability to distinguish between healthy/benign controls and breast cancer samples.[57]

Bladder cancer

Bladder cancer (BC) is one of the most common malignant tumors of the urinary system, with a high risk of recurrence—50% to 70% of patients experience recurrence within five years, and the disease may progress to muscle-invasive bladder cancer (MIBC). Cystoscopy is currently the gold standard for BC diagnosis, but it is an inherently invasive procedure that often causes significant discomfort. Our group aims to advance the discovery of diagnostic biomarkers for BC through N-glycomics analysis of urinary EVs. By analyzing the N-glycome of EVs from 333 urine samples, we identified eight potential glycan biomarkers. During the validation phase, we developed a logistic regression-based machine learning model that calculates a predictive probability score—EVGScores—to effectively distinguish BC patients from healthy individuals. The model demonstrated robust diagnostic performance in two independent validation cohorts, achieving ROC AUC values of 0.88 and 0.86, respectively. Furthermore, it successfully differentiated between non-muscle-invasive BC (NMIBC) and MIBC, highlighting its superior diagnostic capability.[24]

Conclusion and perspective

Glycomics is a relatively new field compared to genomics and proteomics, but it is developing at an astonishing pace. The advancement of glycomics has provided a novel perspective for diseases. It not only facilitates the elucidation of molecular mechanisms underlying disease onset and progression but also enables the discovery of potential biomarkers and therapeutic targets, thereby promoting the development of precision medicine.

Glycomics research involves a wide variety of sample types, and each sample type may provide different insights into the same disease. For example, distinct biomarkers may be identified from different sample types such as serum, urine, or EVs, suggesting that glycans may exert specific biological functions under diverse microenvironmental conditions. Integrative analyses across multiple sample types facilitate the identification of disease-associated multidimensional glycosylation alterations, which may further deepen our understanding of disease pathogenesis. This diversity in biological sample sources underscores the necessity for effective sample preparation and purification techniques that can specifically and selectively isolate glycan structures. Many laboratories, including ours, have developed highly efficient glycan enrichment

and purification techniques, which are not only highly efficiency but also time-saving. These advancements lay the foundation for the rapid development of glycomics. Additionally, many highly efficient derivatization methods have been developed to improve the ionization efficiency of glycans, enabling the acquisition of richer fragment ions. By incorporating multiple isotopic labeling reagents, glycan derivatization can significantly enhance the accuracy and throughput of mass spectrometric quantification, thereby facilitating reliable relative quantification of distinct glycans or different biological samples.

In the field of mass spectrometry data processing, many advanced tools and methodologies have been developed to address the limitations in glycomics data analysis. Currently, glycomics still lacks a standardized data analysis pipeline. In contrast, other-omics fields such as proteomics[144] and metabolomics[145] have already established relatively comprehensive data processing frameworks that enable standardized analysis, differential analysis, and downstream functional interpretation across different platforms. Due to the high structural complexity and heterogeneity of glycans, glycomics still faces significant technical bottlenecks and inconsistencies in key steps such as data preprocessing, spectral interpretation, structural annotation, quantification, and database matching. A universally accepted and standardized analytical workflow for glycomics remains to be developed. Therefore, there is an urgent need to establish a unified and scalable data analysis framework for glycomics, which would enhance data standardization and cross-study compatibility, thereby laying a solid foundation for the advancement of glycomics and its broader applications. Additionally, single-omics studies offer only a limited perspective, focusing on specific molecules and their functions, and may lack the systemic information needed to understand dynamic biological processes. Integrative multi-omics research plays a central role in uncovering the relationships and interactions among various biomolecules, helping to construct a comprehensive view of biological systems. Glycomics faces several challenges in multi-omics integration. First, glycan molecules are structurally complex and highly heterogeneous, involving not only monosaccharide composition but also linkage types, configurations, and branching patterns. This complexity makes glycan data formats and annotation standards difficult to align with those of other -omics fields, such as proteomics or transcriptomics. Second, glycan expression is not directly regulated at the transcriptional level, but instead influenced by a variety of enzymes, substrates, and cellular environments. As a result, its regulatory mechanisms do not directly correspond to gene or protein expression levels, adding further complexity to integrative analyses. Therefore, to enable the integration of glycomics into systems biology and precision medicine, it is essential to develop data models and analytical platforms capable of handling the unique complexity of glycomic data, and to promote the establishment of cross-disciplinary standards. This will not only enhance the accuracy and depth of multi-omics integration but also advance our understanding of glycosylation regulation, disease associations, and individual variability. Moreover, the clinical application of glycomics is still in its early stages.

Although numerous studies have shown that glycomics-based biomarkers hold significant potential in disease prevention and monitoring, these biomarkers still require further validation in large-scale cohorts to ensure their clinical applicability and reliability. In 2025, the U.S. Food and Drug Administration (FDA) released the Bioanalytical Method Validation for Biomarkers guidance document, aiming to provide a clear framework for validating analytical methods used in biomarker studies and to ensure the reliability and reproducibility of the resulting data. The guidance emphasizes several key validation parameters, including specificity, accuracy, precision, sensitivity, reproducibility, stability, and procedures for sample collection, handling, and storage. Glycomics-based biomarkers should be comprehensively validated according to these key principles to ensure their reliability and utility as diagnostic or prognostic indicators. Specifically, glycomic biomarkers should not only demonstrate strong specificity and sensitivity in distinguishing disease from healthy states but also exhibit high reproducibility and stability across different experimental conditions, operators, and instrument platforms. Moreover, according to the MIRAGE guidelines, the standardization of sample collection, storage, and analytical procedures is urgently needed to reduce batch-to-batch variability and enhance data consistency.[146-148] The incorporation of automated high-throughput platforms into glycomics research can significantly improve experimental efficiency and analytical throughput while minimizing human errors, thereby accelerating the clinical translation of glycomics and its application in large-scale precision medicine studies.

In conclusion, while glycomics is rapidly developing and offers tremendous potential to deepen our understanding of diseases, much work remains to be done, including further improving analytical techniques and integrating multi-omics data. This will enable us to better understand the role of glycosylation in biology and drive the development of precision medicine.

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

Conflicts of interest

There are no conflicts to declare.

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Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.