Chapter 2
Structural Biology of Glycans

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2.1 Study of Glycan Structure and Its Recognition Mechanism by Cryo-electron Microscope Single Particle Analysis

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Keywords Cryo-electron microscopy, Single particle reconstruction, Drug design, Glycan-protein complex, Infectious diseases

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1. Significance in the field of glycoscience and its current situation
   Recent development in single particle reconstruction using cryo-electron microscopy has enabled structure determination of glycans [1], which has been difficult due to their structural variations and soft nature. Single particle reconstruction determines a structure by averaging, then only the core 3D structure necessary for its function can be reconstructed. This will enable drug design and further induce development of glycan-related industries.
2. Impact on the other fields of research
   Single particle reconstruction is suitable to determine molecular complexes, and can determine multiple structures, leading to understanding of molecular movement. Such information would be valuable for structure-oriented drug design and antibody production, and yield various applications in medicine, agriculture, food science and material science.
3. Significance as the fundamental research
   Structure determination of glycans using X-ray crystallography has been difficult due to their structural variations, which sometimes precludes crystal formation. Recent developments in single particle cryo-electron microscopy have a potential to overcome this difficulty by reaching the core structure determination by 3D averaging. Such approaches and results have a big impact in basic biology and biochemistry.

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4. Possible application for industry and medicine, if any
   Since single particle reconstruction might enable determination of soft glycan structures that are recognized by infectious bacteria and virus; the core 3D structure necessary for its function and recognition could be reconstructed using the glycoproteins docked with their receptors. This will provide missing information for the drug design and development of glycan related technologies.
5. Future perspectives
   Single particle reconstruction has been developed to determine large molecular complexes, and a big potential to determine multiple structures, that reveal molecular movements. It is advantageous in the study of receptor-glycoprotein complex study for cancer metastasis and embryonic development, in combination with other microscopies for glycoscience [2–5].
6. Problems to be solved
   The best resolution of single particle reconstruction for glycans is 4.2 Å by Lee et al. (Science 2016) [1]. This is a great milestone, but resolution should be improved because present resolution is not enough for drug design. For this purpose, single particle reconstruction microscopy focused on glycan should be intensively developed and enforced, including sample preparation methods and algorithms as a inter-national project (Fig. 2.1).

Fig. 2.1 Single particle reconstruction is a promising method for the structure determination of glycans. In this method, ice-embedded biological samples are imaged using cryo-electron microscope. An example of cryo-TEM is shown here. Further methodological development including biochemistry, electron microscopy and reconstruction software is required for high-throughput reconstructions of glycans at atomic resolution

2.2 Conformational Analysis of Oligosaccharides

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Keywords NMR spectroscopy, Molecular dynamics simulation, Conformational fluctuation, Molecular recognition

1. Significance in the field of glycoscience and its current situation
   The versatile functions of oligosaccharides are exerted primarily through their interactions with cognate proteins. To gain a deeper understanding of carbohydrate recognition by proteins, it is essential to elucidate the conformations of the oligosaccharides in detail. However, conformational fluctuations of the oligosaccharides due to their high degrees of freedom as to internal motion hamper detailed conformational analyses. To address this, NMR spectroscopy can provide atomic-level information regarding the dynamic structures of biomacromolecules in solution and therefore will play an indispensable role in conformational analyses of oligosaccharides [6]. In particular, the recently developed paramagnetism-assisted NMR method, when combined with molecular dynamics simulation, has enabled accurate description of conformational spaces occupied by dynamic oligosaccharides [7–9].
2. Impact on the other fields of research
   The methodology developed for conformational analysis of oligosaccharides will be applicable to structural studies of flexible biomacromolecules, including intrinsically disordered proteins, and therefore will promote biomolecular science in general.
3. Significance as the fundamental research
   Exploration of conformational spaces of oligosaccharides will contribute to a quantitative understanding of the energetics of carbohydrate-protein interactions [9]. Deeper insights into the physicochemical bases of molecular recognition involving oligosaccharides are essential for elucidating glycofunction mechanisms.
4. Possible application for industry and medicine, if any
   Needless to say, conformational analyses of oligosaccharides are important for developing drugs targeting carbohydrate recognition systems. Moreover, most biopharmaceuticals are modified with oligosaccharides and their functional roles are defined through conformational analyses [10].

5. Future perspectives
   It is expected that current analytical techniques will be developed to allow conformational analyses of more complicated glycoconjugates and dynamic supramolecular complexes exemplified by microdomains. This methodological development will provide molecular bases for a variety of biological processes and enable conformational analyses of artificial oligosaccharides, thereby contributing to drug discovery research.
6. Problems to be solved
   For further advancement of carbohydrate conformational analyses, it is necessary to develop experimental and theoretical approaches for observing the dynamics of water molecules surrounding glycans and for charactering the dynamic structures of glycans in heterogeneous environments (Fig. 2.2).

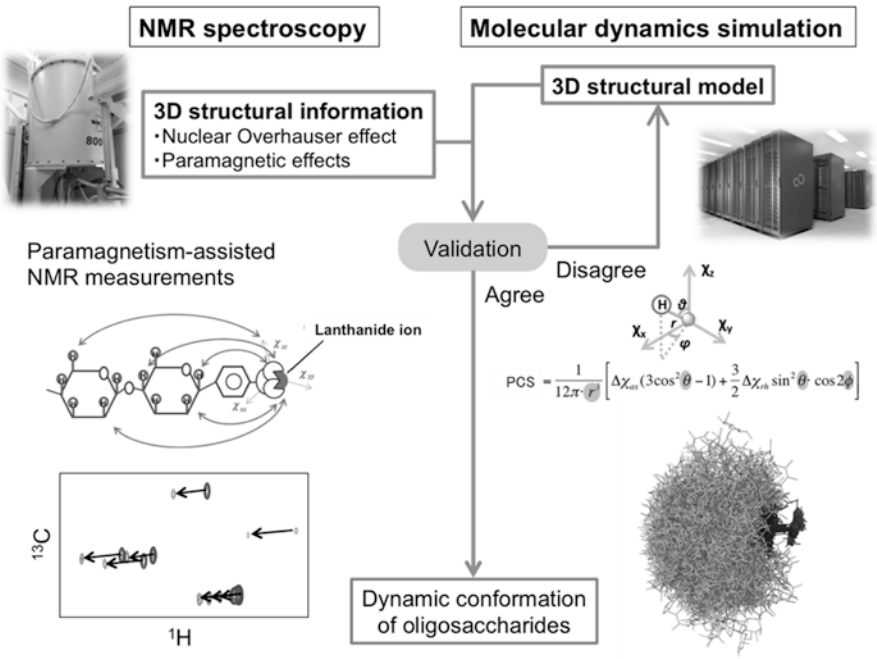

Fig. 2.2 Conformational analysis of oligosaccharides by a hybrid approach combining NMR spectroscopy and molecular dynamics simulation. Part of the figure was adapted from Kato et al. (2018) [Modern Magnetic Resonance, Webb G. (eds), 2018, pp 737–754 with the permission of Springer

2.3 Conformational Analysis of Glycans

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Keywords NMR, Molecular dynamics simulation, Lectin, Dynamics, Affinity

1. Significance in the field of glycoscience and its current situation
   Glycans attached to proteins contribute to the maintenance of protein structure and protein stability through intramolecular interactions [11]. Glycans are also involved in signal transduction via intermolecular interactions with lectin receptors. In order to understand these biological events, it is essential to analyze the conformations and interactions of glycans. Currently, conformational analysis of glycans is being performed by means of experimental (e.g. NMR or X-ray) [12] or theoretical (e.g. molecular dynamics simulation) [13, 14] methods. However, it is often difficult to define the conformations experimentally because glycans are inherently flexible. Theoretical approaches have an advantage in considering the dynamics, but the output is highly dependent on the force field and theory applied.
2. Impact on the other fields of research
   The techniques used in glycan analysis will be applicable to other molecules such as polysaccharides and glycoproteins. Similarities and differences will be discussed by comparison with other flexible biomolecules such as intrinsically disordered proteins.
3. Significance as the fundamental research
   It is currently impossible to describe the conformations and dynamics of glycans based solely on experimental data. Further efforts are necessary to develop experimental methods. Theoretical approaches need improvement of the force field and its parameters by considering experimental data.
4. Possible application for industry and medicine, if any
   Conformational analysis of glycans will play a significant role when a lectin receptor is a target of drug development. Furthermore, it is essential to analyze the structure-function relationships of biopharmaceuticals such as antibody therapeutics and to develop an inhibitor of glycosidases.
5. Future perspectives
   Through accumulation of knowledge on the conformations and dynamics of glycans, it will be possible to predict the binding affinities between glycans and proteins. Currently, qualitative estimation of binding free energy is being successfully performed.
6. Problems to be solved
   It is challenging to accurately estimate the binding free energies of lectin-glycan complexes. It is important to develop a suitable method to predict the effect of a "non-epitopic" glycan region on the binding to lectin molecules [15] (Fig. 2.3).

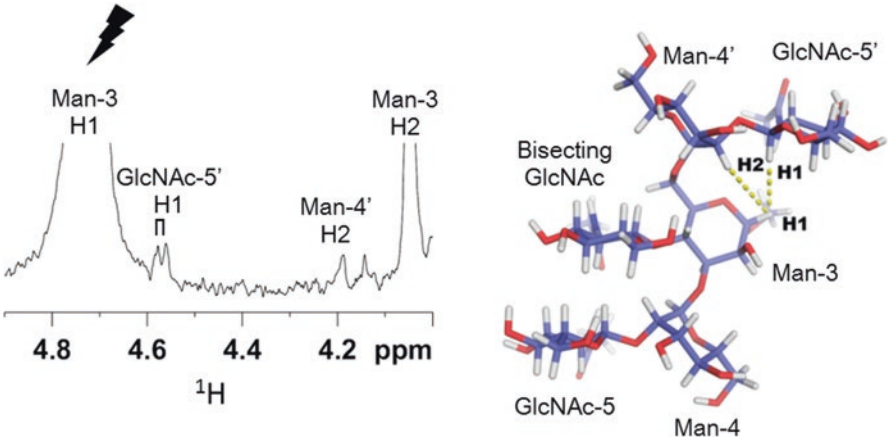

Fig. 2.3 Analysis of lectin-bound glycan conformation by solution NMR spectroscopy. A 1D selective NOESY spectrum was obtained for the glycan in the presence Calsepa lectin, inverting the Man-3 H1 signal. Inter-residue TR-NOE signals were detected to define a flipping-back conformation

2.4 Computational Science (Supercomputer) and AI (Artificial Intelligence)

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Keywords Supercomputer, Computational science, AI, Machine learning, Black box

1. Significance in the field of glycoscience and its current situation
   Computational sciences using supercomputers is now prevailing in all scientific fields. In life science, supercomputers are used for molecular dynamics simulation of macromolecules in a cell [16, 17], rapid sequencing of whole genome data [18], and drug-receptor interaction analysis [19]. RIKEN's K computer belongs to the highest level of supercomputers in the world and is utilized in every area from basic science to industrial application. Meanwhile, artificial intelligence (AI) is highly dependent on the performance of computers and is now attracting the attention of the public [20]. Computational science including AI will play a significant role in the glycoscience field, however, the applications are currently limited.
2. Impact on the other fields of research
   In order to solve the issues in glycoscience using computational science or AI, a database must be organized with a proper ontology format. In particular, glycan structures are often ambiguous and its incorporation into a database is not straightforward. However, once this is resolved, the glycoscience field will be totally open to many other scientists.
3. Significance as the fundamental research
   AI is now mostly utilized for industrial applications by IT companies. The use of AI for basic research is rather limited. Some of the important issues in basic science might be solved by AI.
4. Possible application for industry and medicine, if any
   By utilizing computational science and AI, many advances will be possible such as the development of glycan-related drug (glycomimetics) or finding of some correlations between glycan structures and a certain disease (glycan biomarker).
5. Future perspectives
   AI can facilitate early diagnosis and personalized care of particular diseases by analyzing the patterns of glycan structures.
6. Problems to be solved
   The computational thinking is a black box to us. One critical issue is to open the black box. Another important issue is to collect high-quality, well-annotated data for machine learning (Fig. 2.4).

Fig. 2.4 A supercomputer with a massively parallel computing system

2.5 Structural Study of Proteins in the Glycoscience Field

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Keywords Structural biology, X-ray crystallography, Nuclear magnetic resonance, Cryo-electron microscopy, Single particle analysis

1. Significance in the field of glycoscience and its current situation
   Molecular insights into protein structures are essential for understanding the functions of biomolecules in glycoscience, as in other life science fields. The targets of structure determination are diverse, including biosynthetic and degrading enzymes for glycans, and proteins that bind to and transport glycans. Whole-genome analysis will reveal many candidate genes for glycosyltransferases and glycosidases, but their precise biological roles cannot be logically inferred from the amino acid sequences. The three-dimensional structures could provide critical clues as to their biological functions. Usually, proteins from eukaryotic organisms are chosen as targets, but ones from prokaryotic organisms are also useful as model systems of eukaryotic proteins, since eubacteria and archaea also utilize glycans as an essential component of their cells.
2. Impact on the other fields of research
   The three-dimensional structures of monosaccharides and oligosaccharides have similar chemical properties and only differ in stereochemistry. Elucidation of the recognition mechanisms of monosaccharides and oligosaccharides by proteins is a very difficult task, but it will be very useful for clinical and industrial applications.
3. Significance as the fundamental research
   The atomic coordinates of proteins are very useful information in basic sciences. Almost all are deposited in the Protein Data Bank and available for public use.
4. Possible application for industry and medicine, if any
   For commercial application of glycoproteins, such as erythropoietin, blood-clotting factors, anti-coagulants, immunoglobulins, gonadotropins and interferons, fully occupied glycosylation sites and homogeneous glycan structures are crucial. Structural studies of glycoproteins of interest and related enzymes will provide useful hints for the quality improvement of glycoprotein production.
5. Future perspectives
   X-ray crystallography and cryoEM single particle analysis are the methods of choice for structural determination of proteins. However, due to the intrinsic flexibility of glycans, crystallization of glycoproteins is difficult and cryoEM single particle analysis only provides information on protein portions, i.e., not on glycan portions. NMR spectroscopy is indispensable for the structural and dynamical analyses of glycans themselves. Technical developments for in-depth characterization of glycoproteins will be beneficial in the glycoscience field.

6. Problems to be solved
   Low-cost and efficient large-scale preparation of glycans and glycoproteins is required for structural biology [21]. In particular, a low-cost and efficient stable isotope labeling method for glycans is essential for NMR spectroscopy [22, 23]. Molecular simulation calculation of oligosaccharide structures should be developed [24] (Fig. 2.5).

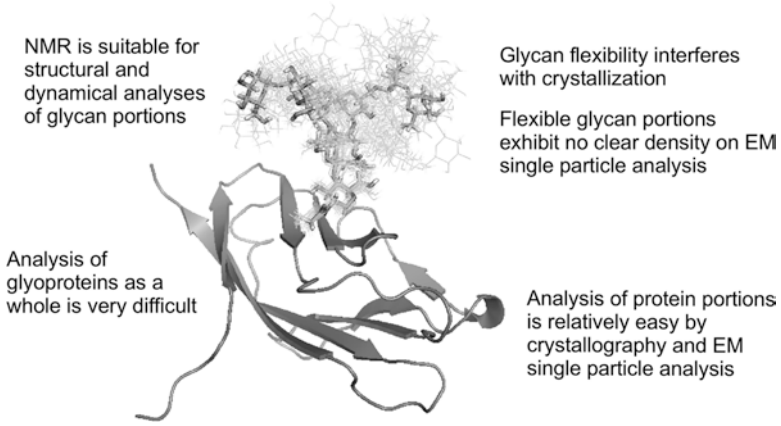

Fig. 2.5 The flexibility of glycan portions makes structural analysis of glycoproteins very difficult. Frequently, the trimming or removal of glycans is performed prior to structural determination of protein portions. NMR is suitable for the structural and dynamical analyses of glycan portions

2.6 Structural Analysis of Sugar Related Proteins

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Keywords Glycosyltransferase, Lectin, Three dimensional structure, Molecular recognition

1. Significance in the field of glycoscience and its current situation
   Since sugar chains are extremely diverse, research on how they are biosynthesized, and how they are recognized, etc. has not progressed much. However, along with the progress of research in the field, it is being revealed that protein groups recognize only a part of sugar chains and consequently bind multiple sugar chains, and that other protein groups specifically recognize specific sugar chains. These proteins range from ones showing simple sugar-binding activity to ones showing glycosyltransferase activity. Also, their physiological activities are also being analyzed. As an example, analysis of the three dimensional structure of the glycosyltransferase POMGnT1 and glycopeptide complexes revealed the molecular mechanism of onset of muscular dystrophy [25]. In summary, in order to clarify the molecular recognition mechanism between sugar chains and proteins, three-dimensional structure information in these complexes is indispensable.
2. Impact on the other fields of research
   Most tumor markers are sugar-binding proteins [26], and some of the targets of antiviral drugs are glycosylases [27]. In addition, a genetic disease, muscular dystrophy, is a glycosyltransferase abnormality [28]. As in these examples, research on sugar chain-related proteins is directly linked to the medical and medicine fields.
3. Significance as the fundamental research
   Recently, a sugar that has not been found in mammals was found in sugar chains involved in muscular dystrophy [29]. Research on biosynthetic enzymes for the sugar and elucidation of the molecular function is awaited. As in this example, fundamental scientific research in this field remains to performed.
4. Possible application for industry and medicine, if any
   Improvements and new development of tumor markers, development of anticancer drugs expected to develop from those, etc. are expected. In addition, sugar-related proteins could be widely used as a research tool for sugar chains themselves.

5. Future perspectives
   Sugar chains and sugar-related proteins remain unknown. As research progresses, not only contribution to fundamental science but also possibilities for application are expected.
6. Problems to be solved
   It is necessary to clarify the molecular mechanisms of how various sugar chains are synthesized and how they are recognized by proteins. It is also important to clarify the relationship between the mechanisms and physiological functions (Fig. 2.6).

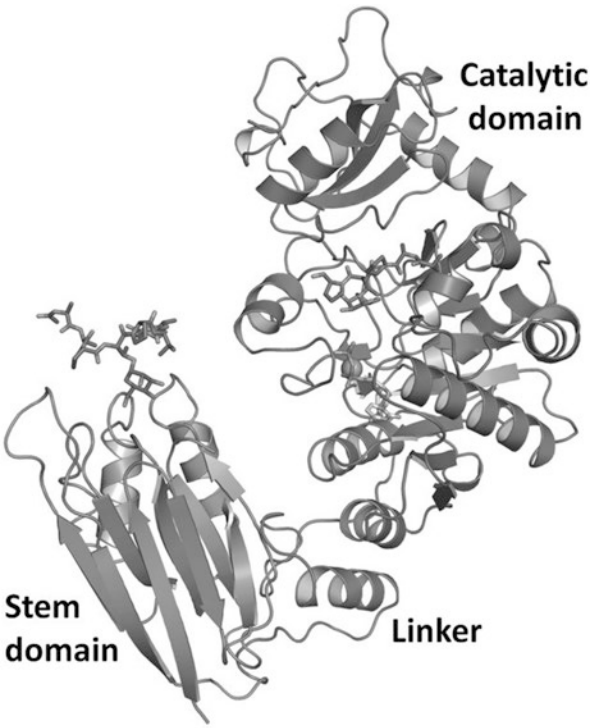

Fig. 2.6 Structure of a complex of POMGnT1 and a glycopeptide. The glycopeptide shown by the rod model binds not only to the catalytic domain but also to the stem domain. This explains well the molecular mechanism of biosynthesis of the core M1 and core M3 sugar chains, which are related to muscular dystrophy

2.7 Simulation and Imaging of Membranes

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Keywords Fluorescence labeling, Click reaction, Efficient synthesis of glycan, Fluorescence resonance energy transfer, Single-molecule imaging

1. Significance in the field of glycoscience and its current situation
   It is necessary to carry out molecular imaging of glycans in living cell membranes to determine their functions. To fluorescently label glycans in cell membranes, a method with which modified glycans are taken into cells and fluorescent dyes are conjugated with the modified glycans in cell membranes has been used [30]. However, this method is not suitable for function analysis because all the modified glycans are detected. To avoid loss of the function of glycolipids after labeling with fluorophores, a highly efficient synthesis technique is required. For these reasons, molecular imaging of glycans has not been necessarily performed in a variety of systems. Coarse grained and all atom molecular dynamics simulation of glycans in membranes has been recently reported [31].
2. Impact on the other fields of research
   Improvement of the imaging (fluorescent labeling) technique for glycans will allow us to detect glycan-protein interactions by the fluorescence resonance energy transfer (FRET) method [32], and increase understanding of the interaction mechanisms.
3. Significance as the fundamental research
   It has been proposed that lipid rafts are the platform for signal transduction, and enriched in glycolipids such as gangliosides. Raft mechanisms may be unraveled by molecular imaging and simulation of glycans.
4. Possible application for industry and medicine, if any
   Interactions between glycans and proteins are involved in invasion of pathogens into cells, and carcinoma cell metastasis. It may be possible to unravel the mechanisms by the methods mentioned above. Elucidation of the mechanisms may also facilitate drug discovery.
5. Future perspectives
   As mentioned above, the method involving labeling of modified glycans with fluorophores is not appropriate for functional analysis of glycans. However, specific glycans can be visualized by observing FRET between GFP fused with the proteins and the fluorescently labeled modified glycans [32]. Furthermore, improvement of the techniques of glycan synthesis and single-molecule imaging may allow us to detect weak interactions of glycans.

6. Problems to be solved
   As mentioned in 1), it is impossible to specifically label glycans with fluorophores by labeling modified glycans that are taken up by cells. Furthermore, highly efficient synthesis techniques are required for fluorescence labeling of glycolipids such as gangliosides [33, 34]. These issues should be solved (Fig. 2.7).

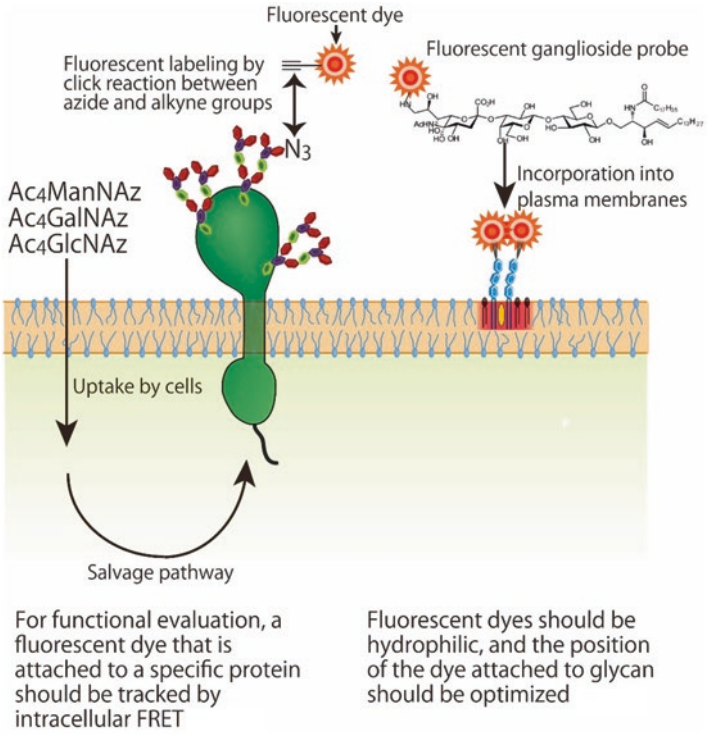

Fig. 2.7 Metabolic labeling of glycans of membrane proteins with fluorescent dyes and synthesis of fluorescent ganglioside probes that behave like the parental molecules. In both cases, we need to devise a way to avoid losing the glycan functions

2.8 Simulation/Imaging of Membranes

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Keywords Imaging, Membrane protein, Lipid, Trafficking, Labeling

1. Significance in the field of glycoscience and its current situation
   Imaging techniques involving fluorescence and electron microscopes provide valuable information to elucidate the roles of saccharides in the functions and trafficking of membrane proteins and lipids. To date, it has been verified that glycan addition to membrane proteins is significant for endocytosis or localization of the proteins [35, 36]. In addition, previous studies have shown that some lipids with glycans accumulate in lipid rafts that are involved in regulation of signal transduction [37]. Disorders of glycoconjugates cause various diseases including cancer and diabetes [37, 38]. Thus, imaging analyses of saccharide function will contribute to elucidation of biological phenomena or pathogenic mechanisms related to trafficking of membrane proteins and lipids, and thus become increasingly important.
2. Impact on the other fields of research
   Combination of imaging analyses with various techniques in the field of chemical biology, omics, informatics and so on will lead to identification of molecules in biological membranes whose functions are regulated by saccharides. It is presumed that saccharides are involved in a wide range of biological phenomena and pathogeneses, since there are a lot of molecules modified with saccharides in biological membranes.
3. Significance as the fundamental research
   It is becoming clear that saccharides control various biological phenomena in biological membranes. It is thus important to detect the interactions of saccharides with membrane proteins and lipids, and image them in real time. Information on these interactions is meaningful for understanding the regulation mechanisms of life.
4. Possible application for industry and medicine, if any
   Much research has shown that saccharide-bound molecules on biological membranes are involved in cancer, diabetes, and neurological disorders. Findings obtained on imaging research will be useful for the diagnosis and treatment of these diseases.
5. Future perspectives
   Imaging-based screening analyses will promote development of molecules that target interactions between saccharides and other biomolecules in membranes. These molecules will be useful for treatment of various diseases involving saccharides or related factors [39].

6. Problems to be solved
   It is highly necessary to develop novel techniques for specific labeling and sensitive detection of membrane proteins and lipids modified with saccharides. Moreover, in addition to the use of imaging techniques such as super-resolution imaging and correlative light electron microscopy (CLEM), a technological breakthrough in the imaging field will be required to analyze the functions and trafficking of biomolecules in membranes with higher temporal and spatial resolution (Fig. 2.8).

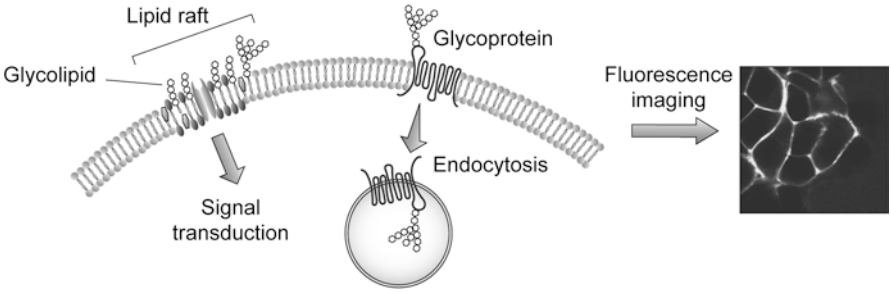

Fig. 2.8 Functions and trafficking of glycolipids and glycoproteins in biological membranes and imaging

2.9 Molecular Imaging of Cells and Organisms Using Labeled Glycans

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Keywords Metabolic labeling, Bioorthogonal reactions, Protein modification, Molecular imaging, Glycosyltransferases

1. Significance in the field of glycoscience and its current situation
   Molecular imaging of glycans is an important topic in glyco-science. The key to this technique is the preparation of labeled glyco-conjugates. This approach can be roughly categorized into two methods: analysis of the localization and behavior of labeled glyco-conjugates analogues prepared by organic synthesis [40, 41], and visualization of the expression of target glycans prepared by insertion of labeled monosaccharide analogues into glyco-conjugates through metabolic processes [42, 43]. These methods continue to evolve, with new chemical tools being developed for *in vivo* imaging [44, 45] in a model system mimicking the heterogeneity of glyco-conjugates [40, 41] or of sialic acid analogues capable of passing through the blood brain barrier [46]. Recent advances include the detection of a specific glycosylation pattern on a specific protein using FRET analysis [47, 48].
2. Impact on the other fields of research
   Since the progress of this field is highly relevant to the development of protein modification methods such as the RIKEN click reaction, and bioorthogonal organic reactions such as the copper-catalyzed azide-alkyne click reaction or the Bertozzi ligation, methods for site-specific/selective chemical modification of biomolecules applicable in cells or *in vivo* are being actively developed.
3. Significance as the fundamental research
   Unlike the molecular imaging of proteins, glycans are difficult to visualize by genetic engineering procedures. Although this field has dramatically advanced after the breakthrough reports of bioorthogonal organic reactions, it should be mentioned that it is still in the process of development. Thus, further fundamental research will be highly significant.
4. Possible application for industry and medicine, if any
   Molecular imaging of a specific glyco-conjugate related to a certain disorder will be applicable as a diagnostic method. Application to drug delivery is expected by using artificial glyco-conjugate analogues with high accumulation properties as to specific tissues/tumors.
5. Future perspectives
   Development of imaging technologies for a specific glycosylation pattern on a specific protein (especially intracellular proteins) as well as *in vivo* imaging,

which is currently limited to several organisms, will enable the determination of the precise functions of glyco-conjugates more spatiotemporally and in real time. Utilization of recently developed imaging technologies (such as Raman microscopy and photo-acoustic effect) is considered to be effective.

6. Problems to be solved
   Preparation of glyco-conjugates by metabolic labeling methods is still limited, and chemical synthesis of these molecules is a rather time-consuming and complicated process. Modification of glycan chains via the degradation of prepared labeled glyco-conjugates by glycohydrolases needs to be taken into account, and could be overcome by the development of metabolically stable glyco-conjugate analogues (Fig. 2.9).

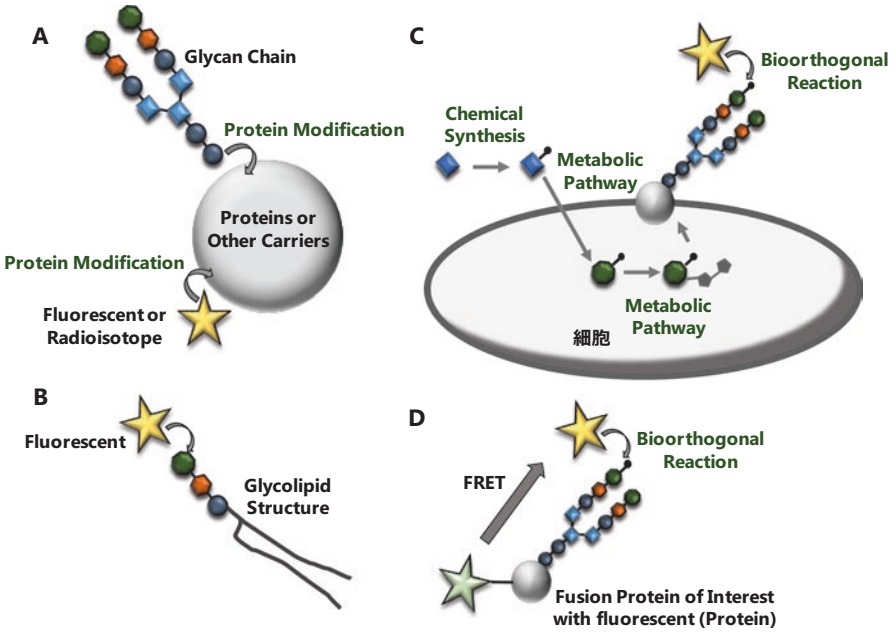

Fig. 2.9 (**A**) Preparation of labeled glyco-conjugates; (**B**) Preparation of labeled glycolipids by chemical synthesis; (**C**) Comprehensive labeling of glyco-conjugates through metabolic processes; (**D**) FRET imaging for the detection of a specific glycosylation pattern on a specific protein

2.10 Diagnosis and Imaging Using Labeled Glycans in Cells and Organisms

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Keywords Click chemistry, Sugar analog, Imaging, Biomarker

1. Significance in the field of glycoscience and its current situation
   As particular changes in glycan structure were revealed to cause the development and progression of diseases by altering the functions of proteins and cells, visualization (imaging) of the particular glycans will allow us to clarify the functions and states of proteins, cells and organisms, and to diagnose diseases. Currently, two methods are commonly used; one is metabolic glycan labeling in combination with sugar analog and click chemistry [49–52], and the other is incorporation of modified glycolipids and glycoproteins having chemically labeled glycans [53].
2. Impact on the other fields of research
   Visualizing glycans is critically important for both neuroscience and medical science, which favor visual and spatial information of molecules and easy analysis without complex steps and expertise, respectively. Development of easy imaging tools for glycans would contribute to the spread of glycobiology to these fields.
3. Significance as the fundamental research
   To understand the functions, localization and metabolic pathways of a given glycan, it is indispensable to label and trace it in living cells and organisms. Currently, the lack of good imaging tools hampers our examination of the detailed spatial information of glycans, and thus development of novel tools is strongly desired.
4. Possible application for industry and medicine, if any
   Advance in this technology would lead to development of novel glycan biomarkers and methods for detection of those markers. In particular, development of new ways to detect glycan markers is expected, which will be useful for the diagnosis and prognosis of diseases including cancer.
5. Future perspectives
   Visualizing specific glycans and glycoforms can contribute to elucidation of the genuine functions of glycans that have so far been obscured by the term “heterogeneity”. In addition, visualization of specific glycoforms would lead to development of novel glycan biomarkers that have been overlooked and buried in the vast glycoform forest.

6. Problems to be solved
   The three major challenges to be tackled; first is the development of methods to label and detect a specific glycan on a specific glycoprotein. Second is development of methods to detect sugar analogs in labeled glycans without using click chemistry. Third is development of novel ways to visualize sugars to which current labeling methods are not applicable, such as glucuronic acid, xylose and mannose (Fig. 2.10).

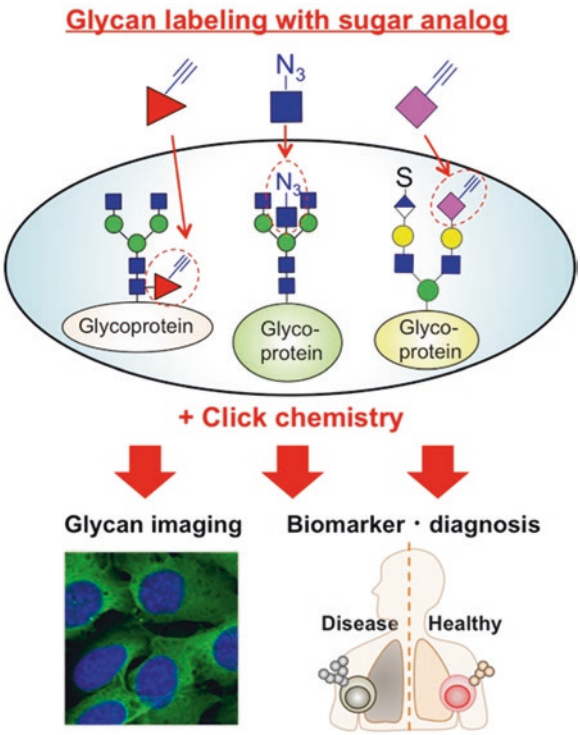

Fig. 2.10 Living cells are treated with sugar analogs, which are incorporated into glycans. The glycans can be easily labeled by click chemistry. This approach can be applied to glycan imaging and biomarker discovery

2.11 Glycoconjugates and Glycoclusters as New Drug Delivery Molecules for In Vivo Molecular Imaging and Theranostics

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Keywords Glycoconjugate, Glycocluster, Heterogeneity, Pattern recognition, Molecular imaging

1. Significance in the field of glycoscience and its current situation
   New drug delivery molecules, which target diseased cells or control excretion pathways in vivo, are desirable to develop an efficient theranostic (diagnosis & therapy) strategy. The interaction of a single molecule of glycan to lectin is generally weak, but can be significantly enhanced when the glycan molecules are conjugated to other biomolecules or arranged to construct a glycocluster environment [54–56]. In particular, heterogeneous glycoclusters could interact with specifically arranged molecules on the cell surface through "pattern recognition mechanisms" [55, 57], and hence result in a strong and selective interaction with target cells in vivo.
2. Impact on the other fields of research
   Glycoconjugates or glycoclusters could be used as new drug delivery molecules for a diagnostic [54, 56] and therapeutic [58] strategy (theranostics). These molecules could be more efficient than conventional antibodies or peptides.
3. Significance as the fundamental research
   Studying "heterogeneous glycan pattern recognition" [55, 57] could lead to new mechanisms of biological significance through glycan interactions.
4. Possible application for industry and medicine, if any
   Glycoconjugates or glycoclusters could be applied to PET (positron emission tomography) [54, 56] and MRI (magnetic resonance imaging), as well as to various carrier-based therapies with RI and anti-tumor drugs.
5. Future perspectives
   Innovative chemical and biological "molecular technologies" for (1) supplying various *N*- and *O*-glycans, and (2) preparing "structurally well-defined" hererogeneous glycococlusters [57] (Fig. 2.11).

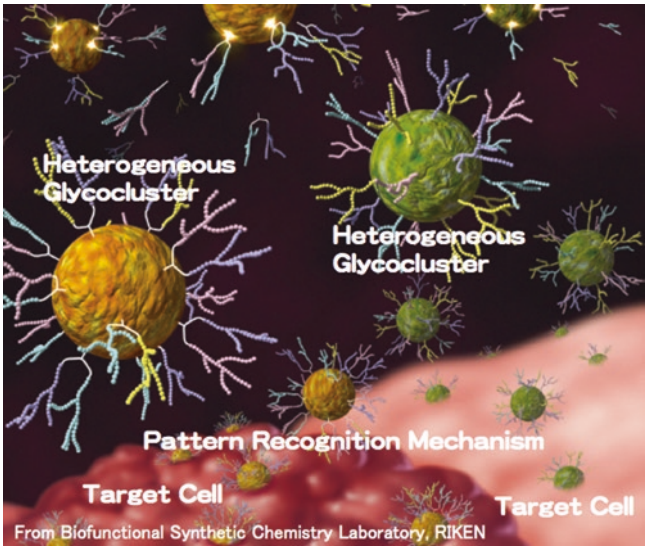

Fig. 2.11 Heterogeneous glycoclusters selectively interact with several molecules on the target cell surface through a "pattern recognition mechanism"

2.12 Imaging Mass Spectrometry (IMS)

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Keywords Imaging mass spectrometry (IMS), Glycolipids, Glycotherapeutics

1. Significance in the field of glycoscience and its current situation
   Imaging mass spectrometry (IMS) is a two-dimensional mass spectrometric method for visualizing the spatial distribution of biomolecules. The method does not require separation or purification of target molecules, and it can be applied, not only to the identification of unknown molecules, but also the localization of numerous molecules simultaneously. Japanese groups have already reported on numerous studies of glycolipids using IMS [59–63]. For example, in ganglioside studies, molecules of the same class that contain fatty acids with different lengths such as GM1 (d18: 1/18: 0) and GM1 (d20: 1/18: 0) can be analyzed simultaneously by IMS although it is difficult when other methods are used [60, 63]. IMS will contribute to the future development of the glycoscience.
2. Significance as the fundamental research
   IMS can distinguish different glycolipid molecular species by simultaneously measuring the difference in mass-to-charge ratio (m / z). In addition, the use of tandem mass spectrometry (MS^n) to examine the tissue surface permits the visualized molecule to be identified and further provides detailed information on its structure.
3. Possible application for industry and medicine, if any
   It is important to determine how candidate drugs such as glycotherapeutics are distributed and metabolized in the body at an early stage of drug discovery. IMS is gaining great interest in monitoring drug delivery and metabolism. Since this emerging technology allows for the simultaneous imaging of many types of metabolite molecules, IMS can be used to visualize and distinguish parent drugs and their metabolites.
4. Future perspectives
   The fundamental contribution of IMS to science makes it a powerful tool for use in the early detection and characterization of cellular processes, both in health and disease states, to understand and treat diseases very effectively. Hopefully we can expect that this approach will lead to the development of glycotherapeutics.

5. Problems to be solved
   Many significant advances have been made in IMS to characterize a variety of molecular species in various types of biological samples, but there is still room for improvements in the areas of sample preparation, ionization and instrumentation (Fig. 2.12).

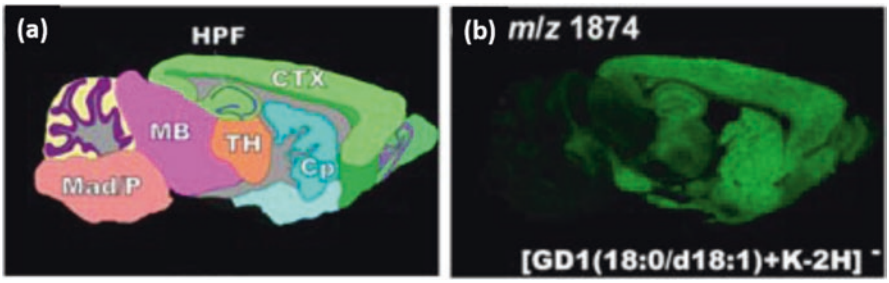

Fig. 2.12 Schematic diagram of the brain section (**a**). A representative result of the brain ganglioside obtained by IMS (**b**). These data were cited with modifications from Ref. [60]

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