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Perspective

In Situ Glycan Analysis and Editing in Living Systems

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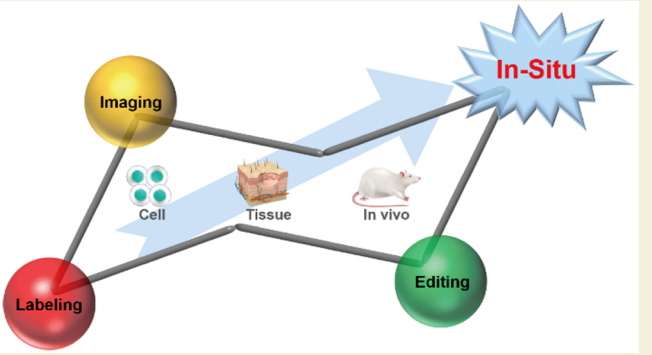
ABSTRACT: Besides proteins and nucleic acids, carbohydrates are also ubiquitous building blocks of living systems. Approximately 70% of mammalian proteins are glycosylated. Glycans not only provide structural support for living systems but also act as crucial regulators of cellular functions. As a result, they are considered essential pieces of the life science puzzle. However, research on glycans has lagged far behind that on proteins and nucleic acids. The main reason is that glycans are not direct products of gene coding, and their synthesis is nontemplated. In addition, the diversity of monosaccharide species and their linkage patterns contribute to the complexity of the glycan structures, which is the molecular basis for their diverse functions. Research in glycobiology is extremely challenging, especially for the in situ elucidation of glycan structures and functions. There is an urgent need to develop highly specific glycan labeling tools and imaging methods and devise glycan editing strategies. This Perspective focuses on the challenges of in situ analysis of glycans in living systems at three spatial levels (i.e., cell, tissue, and in vivo) and highlights recent advances and directions in glycan labeling, imaging, and editing tools. We believe that examining the current development landscape and the existing bottlenecks can drive the evolution of in situ glycan analysis and intervention strategies and provide glycan-based insights for clinical diagnosis and therapeutics.



KEYWORDS: *glycan, living system, in situ, glycan labeling, glycan imaging, glycan editing*

INTRODUCTION

Glycans are ubiquitous natural polymers composed of carbohydrates in various configurations.[1] In living systems, glycans are essential participants in biological recognition processes and critical directors of cellular behaviors,[2] including cell proliferation,[3] migration,[4] substance exchange,[5] and signal transduction.[6] Mammalian glycans are composed primarily of nine monosaccharide building blocks—glucose (Glc), mannose (Man), galactose (Gal), *N*-acetylgalactosamine (GalNAc), *N*-acetylglucosamine (GlcNAc), *N*-acetylneuraminic acid (Neu5Ac), fucose (Fuc), glucuronic acid (GlcA), and xylose (Xyl). In general, glycans can be covalently attached to proteins, lipids, or RNAs to form various glycoconjugates (e.g., glycoproteins, proteoglycans, glycolipids, glycosaminoglycans, and glyco-RNAs).[7,8] Glycoproteins make up an important class of glycoconjugates of which there are two main types, *N*-glycosylated and *O*-glycosylated. Those attached to asparagine are called *N*-glycans, and those attached to serine or threonine are called *O*-glycans. *N*-Glycans have a conserved pentasaccharide core structure ($GlcNAc_2Man_3$) and are divided into three major types: high-mannose, complex, and hybrid. Cell-surface *O*-glycans often start with GalNAc that is attached directly to amino acid residues of proteins. In addition, *O*-GlcNAcylation is a common type of glycosylation that occurs in the cytoplasm.[7] The diversity of glycosylation is reflected through several elements: (1) Glycosylation is a nontemplated process, meaning that glycan structures and functions are difficult to predict from the genome. (2) The glycan structures at a given glycosylation site can vary in both composition and sequence. (3) The glycosylation site and linkage format are diverse. (4) Environmental factors and disease conditions also affect glycosylation. Together, they pose a great challenge to the elucidation of glycan structures and functions. Current studies on glycans have lagged far behind those on nucleic acids and proteins, which has resulted in missing pieces of the life science puzzle.

Uncovering the regulatory mechanisms of glycosylation and the biological effects of glycans is at the core of glycobiology research, and the key is to develop analytical techniques for glycan structures and functions. With the rise of chemical biology, tools for glycan analysis have evolved rapidly. In 2022, Carolyn Bertozzi was awarded the Nobel Prize in Chemistry for her contribution to the field of click chemistry and

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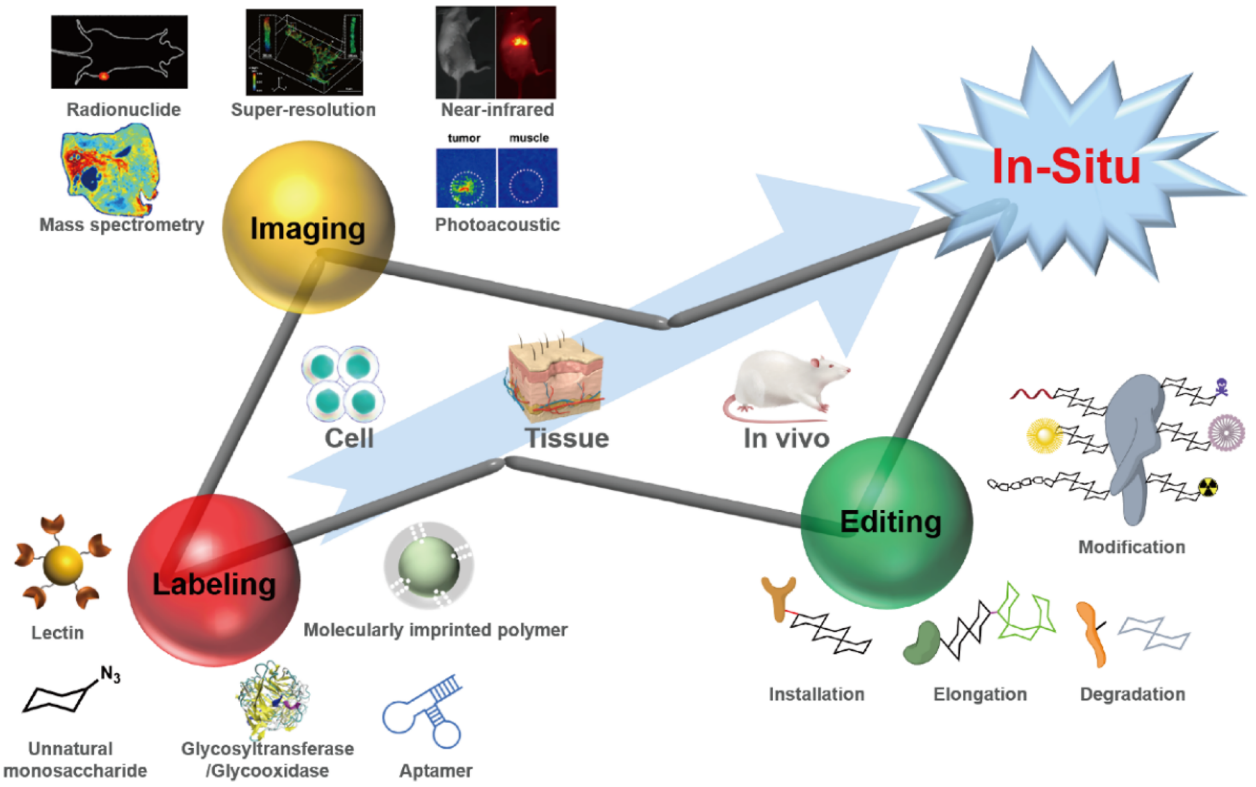
Scheme 1. In Situ Analytical Tools for Labeling, Imaging, and Editing Glycans in Living Systems[a]



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bioorthogonal labeling of glycans. The study of glycans can be carried out either ex situ or in situ. In a typical ex situ study, glycosidases are used to release glycans from glycoproteins or glycolipids, and the composition, structures, and interactions of glycans can be resolved using methods such as lectin arrays,[9,10] mass spectrometry (MS),[11,12] and surface plasmon resonance (SPR).[13] Among them, MS can provide high-throughput information on glycans and glycosylation sites and has become the most important tool in glycomics research. However, these ex situ methods are unable to profile the dynamic distribution of glycans in living systems. From the perspective of "understanding the functions of glycans," we believe that in situ labeling and detection of glycans in living systems and further integration of glycan editing technologies can contribute to the revelation of glycan functions in situ by tracking the biological effects of structural changes in glycan chains.

Current in situ methods mainly include the following categories: (1) Interference with the expression of glycosidases[14,15] and glycosyltransferases[16,17] or mutation of glycosylation sites by genetic manipulation. This category represents one of the most classical approaches for studying glycan functions. However, it is difficult to confine the glycosylation changes to specific proteins, the means of glycan editing are limited, the changes in glycoforms cannot be detected directly, and genetic methods require sophisticated manipulation and are time-consuming. (2) Use of inhibitors or antagonists[18,19] to affect the activities of glycan-modifying enzymes and, thus, the expression of glycans. Again, this category lacks specificity and detection module. (3) Labeling of glycans by recognition,[20,21] metabolic glycoengineering,[22,23] or chemoenzymatic modification[24,25] to convert the glycan information into detectable signals for real-time in situ reporting of glycans in living systems. These labeling techniques can further enable glycan editing through the formation of natural glycosidic linkages and the integration of click chemistry, thereby revolutionizing the way we study the biological functions of glycans. The ability to characterize and reshape glycans can provide new dimensions for clinical medicine—development of glycan-based diagnostic markers and therapeutic strategies. In this Perspective, we systematically summarize the development of tools for in situ glycan labeling via extracellular routes (i.e., genetic technologies are not included), focus on the breakthroughs in glycan imaging and editing, and outline future prospects for in situ glycan analysis and intervention methods (Scheme 1).

IN SITU GLYCAN LABELING TOOLS

Since glycan biosynthesis is nontemplated, in situ labeling of glycans with high specificity and affinity in biological systems is a prerequisite for exploring the specific spatiotemporal variation of glycans. The current toolbox for in situ glycan labeling is mainly based on (1) recognition (e.g., lectins, antibodies, aptamers, and molecularly imprinted polymers) and (2) covalent linkage [e.g., metabolic or chemoenzymatic glycan tagging for further bioorthogonal labeling and direct chemical labeling of sialic acid (Sia) with phenylboronic acid (PBA)]. With these methods, glycan information can be converted to fluorescence, mass spectrometry, electrochemistry, nuclear magnetic resonance, Raman, photoacoustic, and other signals for the in situ imaging and quantification of glycans. In this section, we will comment on novel in situ

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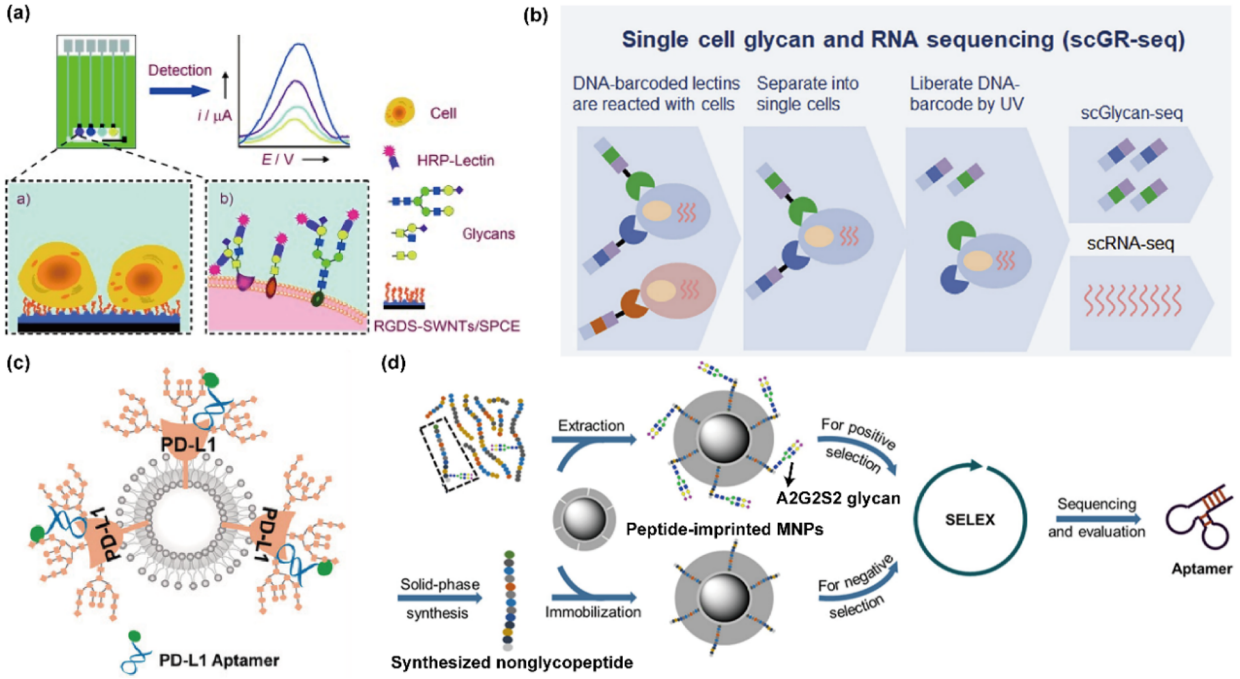

Figure 1. Natural and artificial glycan-binding molecules for cell labeling. (a) Horseradish peroxidase (HRP)-modified lectins were utilized to label various glycoforms present on the surface of cells. Adapted with permission from ref 32. Copyright 2009 Wiley-VCH GmbH. (b) DNA-barcoded lectins were employed for single-cell glycan sequencing. Adapted with permission from ref 34. Copyright 2021 Elsevier. (c) Screened aptamer for identification of highly glycosylated PD-L1. Adapted with permission from ref 37. Copyright 2020 Wiley-VCH GmbH. (d) Screening of the aptamer for the A2G2S2 glycan by combining molecular imprinting with systematic evolution of ligands by exponential enrichment (SELEX). Adapted with permission from ref 38. Copyright 2021 American Chemical Society.

glycan labeling tools that have been applied in cell, tissue, and in vivo scenarios.

In Situ Labeling of Cellular Glycans

The labeling of cellular glycans is the basis of all glycobiological research. All of the methods described above can be applied to cell samples. However, there is currently no reliable standardized strategy that can be applied to all types of glycans. This is because of the complexity of the glycan structures and the limited application scenarios of each method.

In situ lectin staining is often used to validate glycoforms on the cell surface. The well-established disadvantages of lectins are their weak binding to monosaccharides and generally low specificity.[26−28] In addition, the recognition events usually alter cell signaling pathways.[26] The artificial construction of tandem repeat lectins as novel multivalent receptors can greatly enhance the affinity for glycan ligands.[29−31] Given the extensive repertoire of lectins (more than a hundred have been commercialized), their advantages in the simultaneous detection of multiple glycans are unmatched by other methods (Figure 1a).[32,33] Recently, lectin−glycan recognition has been integrated into new single-cell sequencing technologies (Figure 1b)[34−36] to elucidate the functions of glycans in cell−cell and cell−microbe communication, reveal cellular heterogeneity in glycans, and facilitate cell typing according to glycan levels.

Aptamers and molecularly imprinted polymers (MIPs) are excellent artificial antibodies that expand the range of glycan-binding molecules. They overcome the difficulty of setting up control experiments for lectin recognition and have shown great potential for application in glycan labeling. Yang's group screened an aptamer against highly glycosylated PD-L1 with a higher binding affinity (K_d = 91 ± 12 nM) than the antibody (K_d = 295 ± 44 nM), thereby highlighting the importance of glycosylation in the screening of recognition molecules (Figure 1c).[37] This potentially implies the possibility of differentiating glycoproteins with different glycoforms, which would provide a new dimension and research paradigm for protein-based disease typing and facilitate personalized precision therapy. Liu's group used MIPs to capture the target glycopeptide from cell lysates and then screened an aptamer that binds to the glycoform of the glycopeptide using systematic evolution of ligands by exponential enrichment (SELEX). This aptamer was successfully applied to imaging biantennary digalactosylated disialylated *N*-glycan on the cell surface (Figure 1d).[38]

Liu's group has done a series of outstanding works on the design of MIPs for glycan recognition.[39−41] PBA, as a small molecule that can specifically recognize *cis*-diol, has been widely used for glycan labeling.[2,42−45] On this basis, the group used a series of PBA-functionalized quantum dots with different emission wavelengths to imprint different monosaccharides and realized in situ labeling and multiplexed glycan imaging on the cell surface.[39] They also cleaved polysialic acids from the surface of neuroblastoma for imprinting and loaded indocyanine green (ICG) into MIPs to integrate neuroblastoma-targeted glycan labeling and photothermal therapy.[40] Future research in this direction will require a detailed characterization of the retention of MIPs on the cell surface and their effects on cells.

Metabolic oligosaccharide engineering (MOE),[22] now more commonly known as metabolic glycoengineering (MGE),[46] has been developed for nearly three decades and remains a powerful and popular tool for in situ labeling and analysis of glycans in living systems. As the studies have progressed, however, researchers have identified problems with the commonly used MGE techniques, including a long metabolic

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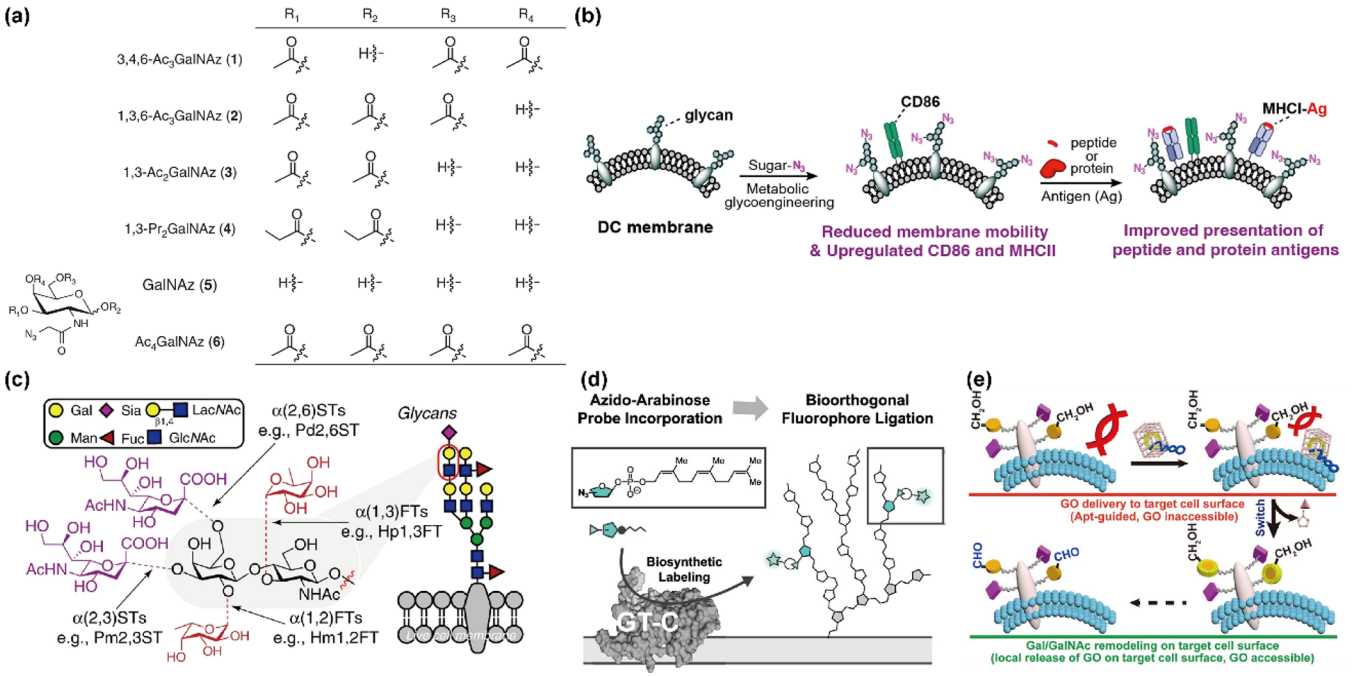

Figure 2. Covalent labeling strategies for cellular glycans. (a) Screening of galactosamine metabolic labeling reagents for low nonenzymatic *S*-glyco-modification and high membrane permeability. Adapted with permission from ref 49. Copyright 2019 Springer Nature. (b) MGE resulted in upregulation of CD86 and MHCII in mouse bone-marrow-derived dendritic cells. Adapted with permission from ref 50. Copyright 2023 Springer Nature. (c) Recombinant bacterial glycosyltransferases for labeling sialic acid or fucose of LacNAc-containing glycans, LacNAc (*N*-Acetyl-D-lactosamine). Adapted with permission from ref 56. Copyright 2019 Springer Nature. (d) Incorporation of azido-arabinose into the cell wall of mycobacteria using arabinofuranosyltransferase (GT-C). Adapted with permission from ref 57. Copyright 2021 American Chemical Society. (e) Cell-specific glycan labeling was achieved by caging galactose oxidase (GAO) with metal−organic framework (MOF) and then activating the enzyme for remodeling. Adapted with permission from ref 64. Copyright 2019 Wiley-VCH GmbH.

time and bottlenecks in metabolic efficiency. In particular, Chen's group found that per-*O*-acetylated monosaccharides produce nonenzymatic *S*-glyco-modification of cysteine residues in various proteins, and *N*-azidoacetylgalactosamine (GalNAz), an unacetylated unnatural sugar, can effectively avoid this artifact.[47] However, the purification and mass production of unacetylated unnatural sugars are challenging. To improve the yield, they developed a per-*O*-tetramethylsilane (TMS) protection strategy for easy and efficient synthesis of unprotected and 1,6-di-*O*-acylated unnatural sugars.[48] They also developed a new generation probe, 1,3-di-*O*-propionylated GalNAz (1,3-Pr_2GalNAz), to enhance metabolic labeling efficiency (Figure 2a).[49] Meanwhile, a recent study found that the commercial glycan metabolic labeling reagent Ac_4ManNAz can upregulate the expression of membrane proteins CD86 and MHCII on mouse bone-marrow-derived dendritic cells (BMDCs), and this upregulation shows a positive correlation with the concentration of Ac_4ManNAz (Figure 2b).[50] This finding emphasizes the necessity of considering the effects of MGE on proteins and may further revolutionize MGE.

In contrast to MGE, chemoenzymatic glycan labeling (CeGL) does not rely on intracellular biosynthetic pathways and can directly attach biotin-, fluorescent-, or even protein-tagged sugars to specific substrate glycans via a one-step chemoenzymatic reaction.[51,52] In addition to specifically forming natural glycosidic linkages, CeGL also allows for labeling of higher-order glycan structures and glycan chain elongation. This is extremely important for researchers to identify glycan structures, elucidate the biological effects of specific glycan chains, and intervene in glycosylation. Wu's group developed a robust CeGL method for Tn-associated antigens that allows efficient and selective labeling of Tn (GalNAcα-*O*-Ser/Thr), Thomsen−Friedenreich (Galβ1−3GalNAcα-*O*-Ser/Thr, TF), and STF (Neu5Acα2−3Galβ1−3GalNAcα-*O*-Ser/Thr) antigens in whole blood.[53] In recent years, in addition to the already widely used glycosyltransferases (Figure 2c),[54−56] Kiessling's group introduced azide-modified D-arabinofuran (D-Ara*f*) into polysaccharides using a membrane glycosyltransferase (Figure 2d).[57] This strategy can selectively modify cell wall glycans with glycolipid donors. Wen's group innovatively applied two mutant endoglycosidases to efficiently and specifically recognize core Fuc and *O*-GlcNAc and to mediate the labeling of a biantennary *N*-glycan probe bearing azido and oxazoline groups.[58] This strategy expands the range of enzymes suitable for the CeGL method. CeGL is undoubtedly a powerful class of glycan labeling methods, but how to ensure the labeling efficiency of the chemoenzymes in complex systems in vivo, how to develop more efficient chemoenzymes, and how to expand the range of labeled glycan substrates are the next steps worthy of in-depth research. Meanwhile, for Sia at the end of the glycan chains, CeGL methods need to be combined with other methods (e.g., mild chemical oxidation by $NaIO_4$) to adapt to more glycan labeling scenarios.

Glycooxidases belong to a rather special class of enzymes used for CeGL. The popular galactose oxidase (GAO) specifically oxidizes the C6-OH of terminal Gal or GalNAc at the end of the glycan chains on the cell surface to generate bioorthogonal aldehyde groups that allow glycan labeling via hydrazone/oxime formation. To alter its specificity or activity, directed evolution is a promising direction.[59,60] For example, Li

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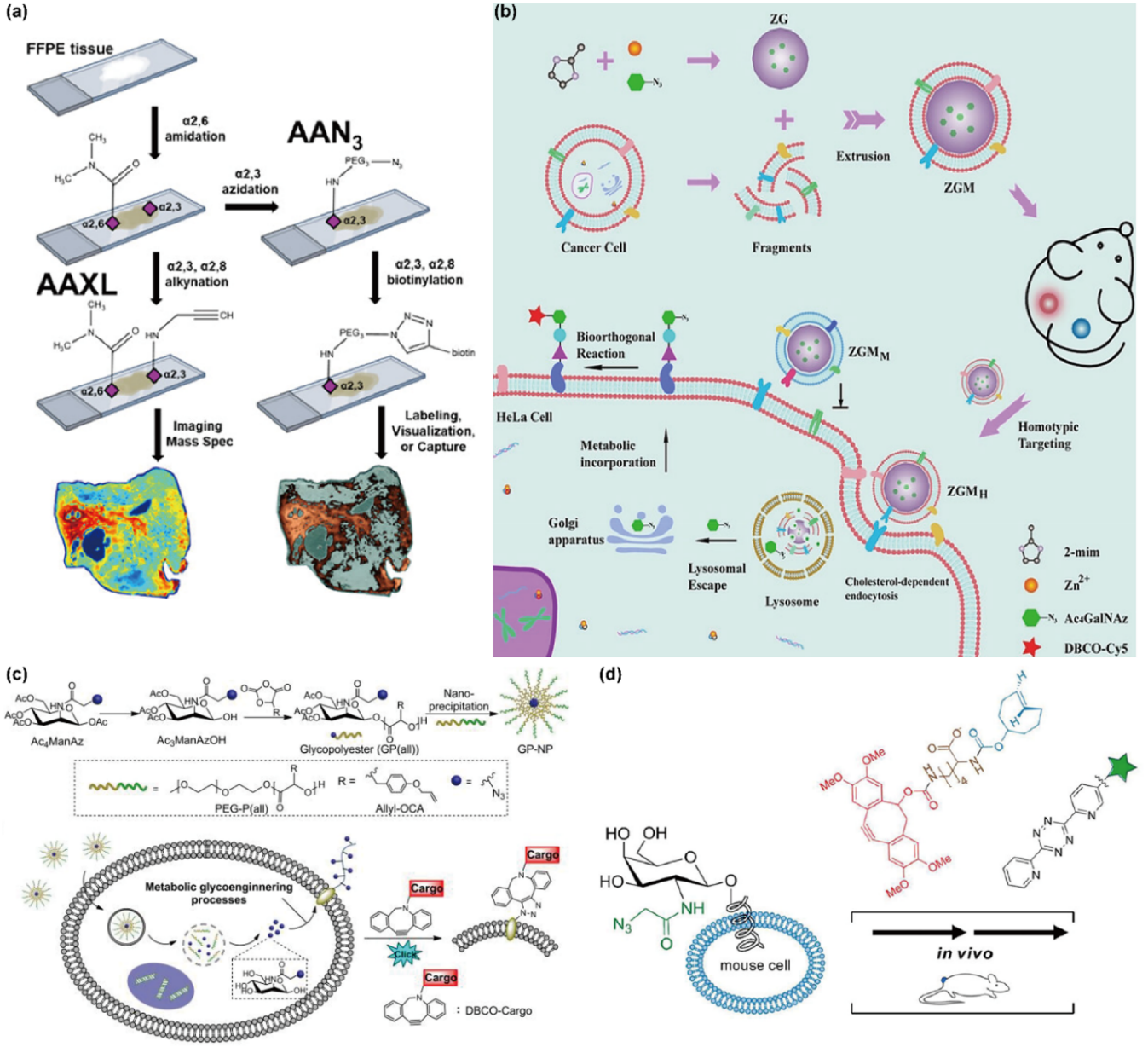

Figure 3. Tissue and in vivo glycan labeling. (a) Terminal sialic acids of tissue sections were labeled and analyzed by using matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). Adapted with permission from ref 72. Copyright 2023 American Chemical Society. (b) Encapsulation of MOF-coated unnatural monosaccharides with tumor cell membranes for in vivo glycan labeling. Adapted with permission from ref 76. Copyright 2021 Wiley-VCH GmbH. (c) Self-assembly of unnatural monosaccharide into glycopolyester nanoparticles for in vivo labeling and cancer therapy in mice. Adapted with permission from ref 78. Copyright 2019 Elsevier. (d) Two-step glycan labeling in vivo with reduced background signal. Adapted with permission from ref 82. Copyright 2013 American Chemical Society.

et al. found that the incorporation of F_2-Tyr or Cl_2-Tyr into the Tyr272 site of GAO induced carbon−halogen bond cleavage[61] and led to a decrease in catalytic activity and efficiency. Birmingham et al. improved the activity and specificity of GAO to oxidize 5-hydroxymethylfurfural, an important renewable feedstock.[62] Also recently, Feng et al. developed a directed evolution screening platform on the basis of flow cytometry and screened for a mutant GAO variant (T521A) with a K_m value 4.4-fold lower than that of the wild-type GAO.[63] In addition to regulating enzyme specificity and activity, spatiotemporal control of enzyme activity is important for improving the specificity of glycan labeling. Ding and Ju et al. has developed a series of strategies to modulate GAO activity via metal−organic framework (MOF) (Figure 2e)[64] or polymer[65,66] caging. In these works, physical or chemical triggers were used to deactivate the caging materials or molecules and initiate the labeling process. However, the application of these methods in vivo is limited. Future in vivo spatiotemporal control of enzymatic processes may consider the integration of an off/on switch based on specific responsible mechanisms toward the disease microenvironment or a light-based control mechanism.

Glycan Labeling on Tissue Sections

Glycan labeling on tissue sections mainly suffers from poor permeability and low sensitivity. Therefore, labeling with small probes is often more effective. Detection sensitivity can be improved by chemically derivatizing glycans[67] with strategies

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such as dimethylation, reductive amination, Michael addition, and hydrazone formation.[68] These derivatization reagents have received much attention in chromatography- and MS-based glycan detection and have been extended to in situ tissue labeling.[69,70] For example, Han et al. developed a derivatization reagent 1-naphthaleneacethydrazide (NAH) for on-tissue derivatization of monosaccharides and quantified the expression of aldose and ketose monosaccharide isomers.[71] Drake's group introduced alkyne moieties into α-2,3-linked Sia with an amidation derivatization reagent and labeled glycans via click chemistry to analyze the number and distribution of alkyne-tagging glycoproteins on cancer tissue sections (Figure 3a).[72] Wu's group achieved one-step labeling of glycans on tissue sections using bacterial glycosyltransferases.[56] In addition to these methods, Song and Yang's group labeled the target glycan and protein with fluorescent lectin and aptamer, respectively, and visualized protein-specific glycoforms on tissue sections via a Förster resonance energy transfer (FRET) mechanism.[73]

In Vivo Glycan Labeling

In situ labeling of glycans in vivo has always been a critical challenge in glycan labeling. The main scientific questions are (1) how to achieve cell/organ selectivity and (2) how to reduce the background signal to improve the signal-to-background ratio (SBR).

Wilson's group synthesized [^{18}F]-labeled disaccharides from the readily available precursor 2-deoxy-[^{18}F]-fluoro-D-glucose and introduced them into microorganisms to achieve in vivo glycan labeling.[74] To label Lewis A/C/X glycoforms in vivo, Sier's group used chimeric human/mouse variant CH88.2 conjugated with a near-infrared probe IRDye800CW to visualize subcutaneous colon and pancreatic tumors.[75] To endow MGE with tumor cell selectivity in vivo, Qu's group used cancer cell membranes to encapsulate unnatural sugar-functionalized MOF (Figure 3b).[76] Using a similar strategy, they also achieved separate targeting of different tumor models in vivo.[77] The implementation of selective labeling in vivo relies on the recognition modules of the probes. Cheng's group prepared glycopolyesters by ring-opening polymerization of *O*-carboxyanhydrides to form nanoparticles (Figure 3c).[78] By exploiting the enhanced permeability and retention (EPR) effect, they achieved in vivo labeling of tumor glycans.

Covalent labeling is more commonly used for in vivo scenarios because covalent bonds are more stable and less susceptible to direct clearance by the liver and kidneys. To achieve selective labeling of specific cell types, Bertozzi's group developed a monosaccharide probe, *N*-(*S*)-azidopropionylgalactosamine (GalNAzMe), which is specific for cancer-relevant Ser/Thr(*O*)-linked GalNAc glycosylation.[79] Chen's group reported a genetically encoded metabolic glycan labeling (GeMGL) method and demonstrated the selective incorporation of 1,3-di-*O*-propionylated *N*-pentynylacetylglucosamine (1,3-Pr$_2$GlcNAl) into cardiomyocyte cells expressing AGX2F^{383G}.[80] This idea is highly creative because it allows organ selectivity to be resolved by genetic modification of a rate-limiting enzyme in glycan metabolic pathways, although the modification, itself, requires certain experimental skills in molecular biology.

In general, covalent labeling requires higher concentrations of reagents than recognition-based labeling. For in vivo experiments, the concentrations of reagents, such as azide and cyclooctyne, are typically in the mM range,[81] and high concentrations of imaging reagents tend to produce high background (Figure 3d).[82] Brindle's group developed a two-step labeling strategy ("double-click") in which the second click reaction is 10^4−10^5 times faster than the first, thereby enhancing the SBR and reducing the dose of imaging reagents.[82] Another solution is to design fluorescent or radiative switches based on the tetrazine click chemistry. Prescher's[83] and Wu's groups[84] have made considerable progress in developing a fluorescence "off−on" switching mechanism for 1,2,4,5-tetrazine. Bertozzi's group also developed a 1,2,4,5-tetrazine-based fluorogenic probe that allowed visualization of sialoglycoconjugates during zebrafish embryogenesis.[85]

Each labeling method has its own characteristics. These labeling methods should be selected according to the nature of the objects and research goals and should be further refined and innovated from the perspective of specificity, affinity, stability, convenience, and so on. The progress and enrichment of the glycan labeling toolbox not only contribute to the revelation of the structures and functions of glycans but also lay the foundation for the development of glycan-targeted intervention and regulation technologies.

RECENT PROGRESS IN GLYCAN IMAGING

In the previous section, we discussed different labeling techniques and their pros and cons. We will now focus on how they can address the challenges and needs in cell surface, tissue, and in vivo scenarios.

Cell-Surface Glycan Imaging

The recent developments in cell-surface glycan imaging can be summarized into two directions: (1) advancing glycan imaging from the whole-cell level to specific glycoconjugates by designing a closed-loop signaling topology, thus promoting the development of the field of glycoconjugate-specific glycan imaging, and (2) improving the spatial resolution of glycan imaging by introducing super-resolution microscopy and expansion microscopy.

Refinement of Signal Generation to Specific Glycoconjugates

In nature, the glycan patterns (glycoforms) of glycoconjugates are dynamically regulated and are often heterogeneous.[86] These glycans dictate the structures and functions of the underlying biomolecules and also reflect changes in physiological conditions (e.g., during cancer progression).[87] As a result, visualization of glycans at the glycoconjugate-specific level can provide a valuable perspective for understanding the glycosylation mechanism.

Developing protein-specific glycan imaging techniques constitutes the primary axis of this development trend and provides a paradigm for visualizing glycans on other glycoconjugates, such as glycoRNA.[88] For the interrogation of protein-specific glycans, the key question is how to establish a link between the proteins and glycans. One approach is to separately label glycans of interest (GOIs) and proteins of interest (POIs) and then measure the signals generated by energy transfer or spatial proximity between the labels on GOIs and POIs, which represent the glycan information on the POI. As summarized by Ding and Ju et al., the formation of a closed-loop signaling topology between the glycoconjugate and the labeling elements is the prerequisite for ensuring protein-specific glycan imaging.[89] The protein/glycan identity signal transmission along the loop, composed of the POI, the label on

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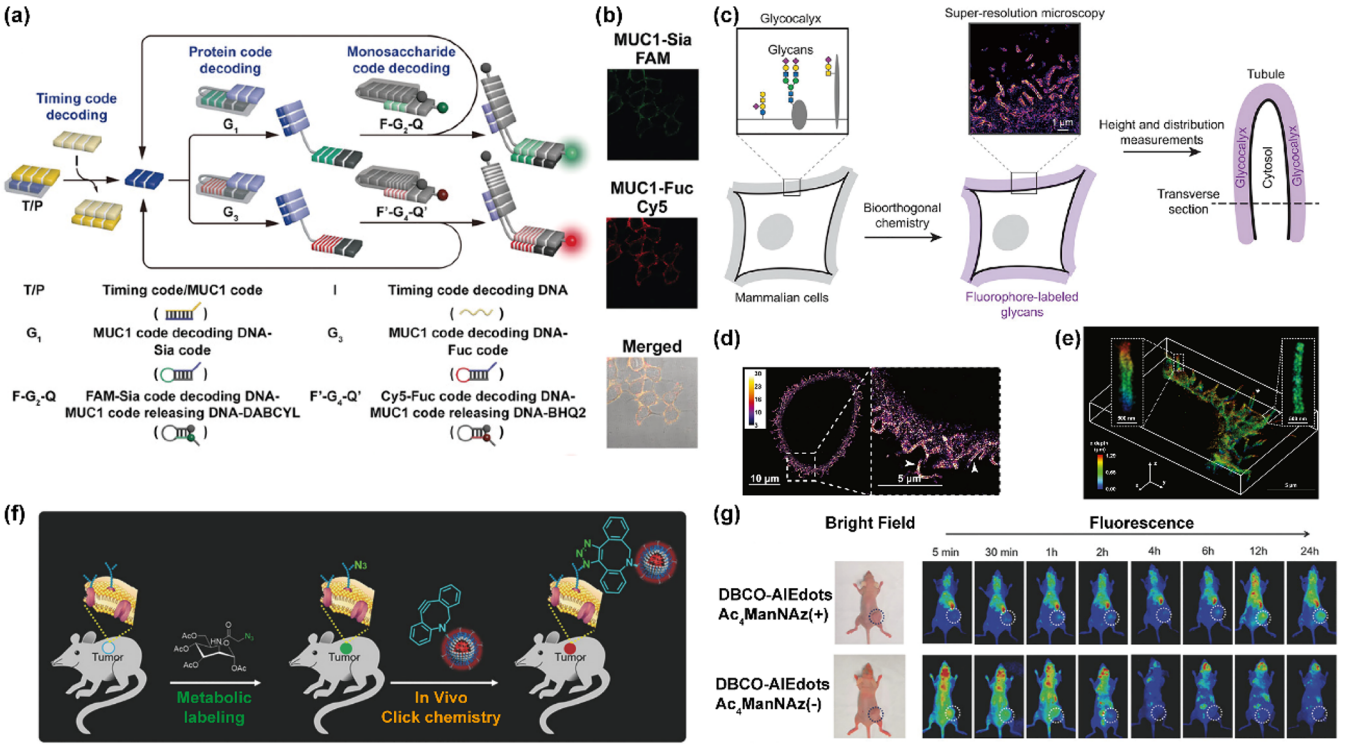

Figure 4. Advances in glycan imaging. (a) A hierarchical DNA coding (HieCo) strategy for simultaneous imaging of Sia and Fuc on specific proteins. (b) Confocal laser scanning microscope (CLSM) images of MUC1-specific Sia and Fuc. (a,b) Adapted with permission from ref 109. Copyright 2018 Wiley-VCH GmbH. (c) Schematic of super-resolution imaging of cellular glycocalyx. (d,e) 2D (d) and 3D (e) images of the glycocalyx. (c–e) Adapted with permission from ref 130. Copyright 2019 Elsevier. (f) Schematic of using aggregation-induced emission dots (AIEdots) for in vivo imaging of glycans. (g) In vivo images showing the labeled glycans. (f,g) Adapted with permission from ref 171. Copyright 2018 Wiley-VCH GmbH.

the POI, the label on the GOI, and the GOI, can ensure that the detectable signal is not triggered by nontargeted proteins or glycans.

In the early literature,[90−92] GOIs and POIs were separately labeled with FRET acceptors and donors, and the FRET signal could indicate GOIs in the proximity (<10 nm) of a POI. This strategy has recently been applied to exosomes,[93] tissue sections,[73] and living animals.[94] However, FRET efficiency is relatively low for conventional fluorescent acceptor−donor pairs, which creates a bottleneck for sensitivity improvement. Increasing the number of donors[95]/acceptors[96] labeled on one POI/GOI (or vice versa) sounds like a straightforward way to enhance FRET efficiency but poses problems in the context of protein-specific glycan detection. A large-sized label on a POI adds uncertainty to its position, which undermines the reliability of the distance-dependent FRET signals, and a large-sized label on a GOI may cause nonspecific signals on neighboring proteins and false-positive signals (e.g., cross-excitation, bleed-through).[97] Therefore, a high resonance energy transfer (RET) efficiency and small label size are both important criteria in the selection of donor−acceptor pairs. Lanthanide-based probes are ideal donors for luminescence resonance energy transfer (LRET) because of their large Stokes shifts, photostability, sharp emissions, and multiplexing capability.[98] In a pioneering work by Ding and Ju et al.,[99] two types of protein-specific glycans were simultaneously imaged by LRET between an upconversion nanoparticle (UCNP) donor on POI and two fluorescent dye acceptors on each type of glycan. Recent progress in improving the LRET efficiency of UCNPs[100−102] and the invention of ultrasmall luminescent probes[103] seem to promise novel donor−acceptor pairs and may find applications in protein-specific glycan imaging. Nevertheless, multiplex glycan imaging is difficult because of the limitations in multiplex RET, and the distance-dependent RET signals are unable to quantify the number of GOIs.

In contrast to RET, DNA circuits require a physical interaction between the labels on GOIs and POIs (i.e., spatial overlap). In a typical strategy, two DNA modules are separately labeled on POIs (POI-DNA) and GOIs (GOI-DNA), and when POI-DNA and GOI-DNA spatially overlap (i.e., in the case of protein-specific glycans), they interact to either expose a sequence[104] or form a complete sequence[105−107] to initiate DNA circuits. In most studies, DNA assemblies are output to allow signal amplification, which is crucial for nanoscale exosomes[105] or in vivo[104,108] scenarios. However, the signals on DNA assemblies cannot represent the localization of glycans, and such DNA circuits output only one glycan signal on each POI, which neglects the natural heterogeneity of glycoproteins. To reflect the true abundance of glycans on POIs, Ding and Ju et al. developed a series of strategies capable of recycling the formation of DNA circuits between a POI-DNA and multiple GOI-DNAs on the basis of nicking endonuclease cleavage[89] and hierarchical DNA coding (HieCo) (Figure 4a,b).[109] Notably, two orthogonal DNA circuits were used at the same time for the visualization of MUC1-specific terminal Sia and Fuc, thereby highlighting the multiplexing capacity of the DNA circuits. Nevertheless, there is currently a limited repertoire of glycan labeling techniques that are bioorthogonal to each other for conjunction with

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multiplex DNA circuits, which highlights the need for further expansion and development.

Another way to form a closed-loop signaling topology is to first target the POIs and then specifically label the GOIs on the POIs. Ding and Ju et al. first proposed a localized chemical remodeling (LCM) strategy[110] in which the GAO−aptamer conjugate can oxidize protein-specific terminal Gal/GalNAc to yield aldehyde tags via $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ regulation of enzyme activity. Later, MOF caging[64] and poly(ethylene glycol) (PEG) cloaking[66] were used to modulate the accessibility of the GAO activity site, as an alternative for GAO activity regulation. Compared with the abovementioned methods, the LCM strategy has two advantages, including that (1) the probe design is simple, and only one probe is needed, and (2) a bioorthogonal warhead is generated on glycans to facilitate covalent labeling or editing. To advance the idea, we believe that new glycoprotein targets will be unlocked with the help of small protein-binding probes. The innovation of the labeling machinery, although more difficult, may be inspired by two aspects: (1) glycan-specific reactions (e.g., chemoenzymatic labeling by glycosyltransferases) and (2) proximity-induced reactions.

Improvement of Spatial Resolution of Imaging

Glycocalyx is a dense sweet shell on the cell surface and is mainly composed of glycans and glycoconjugates.[111] From a horizontal perspective, glycans spread out with nanoscale variability, thereby dictating the mechanical and functional information on the cell membrane.[112] Moreover, the vertical depth of the glycocalyx, which varies from tens to hundreds of nanometers, is below or near the diffraction limit of light. Therefore, deciphering the spatial information on glycocalyx is rather difficult for conventional optical microscopy techniques. Super-resolution (SR) imaging, with a spatial resolution below the diffraction limit, has shown its unique power and position in dissecting the nanoscale spatial information on glycocalyx on the cell surface. Stimulated emission depletion (STED) microscopy, direct stochastic optical reconstruction microscopy (dSTORM), and structured illumination microscopy (SIM) are well-established SR techniques and have been widely used for imaging cell-surface glycans, but for simplicity, we categorize them here according to different labeling methods.

First, glycans can be noncovalently labeled with fluorescent dyes. Since 2015, Wang's group has conducted in-depth studies on the dSTORM imaging of glycans via lectin[113−116] and aptamer[117,118] recognition. Their results provide solid evidence of glycan clustering. SIM has also been widely used to visualize glycans after lectin[119−122] or antibody[123] recognition. However, lectins suffer from low affinity and low specificity, and their relatively large size and inherent multivalency can lead to mislocalization and a quantitative discrepancy of glycans. Antibodies are also large and are often restricted to binding complex glycoforms. Therefore, small and highly specific glycan recognition counterparts (e.g., aptamers and nanobodies) are urgently needed for SR imaging of cell surface glycans.

Covalent glycan labeling with small fluorescent probes is well suited to the characteristics of SR microscopy. After glycans are tagged with bioorthogonal groups, fluorescent dyes can be introduced to these glycans through highly efficient bioorthogonal reactions to provide nearly "precise" localization. This type of strategy has been universally demonstrated by combination with dSTORM,[124−126] SIM,[121,122,126,127] STED,[128,129] and others.[79] Recently, Möckl et al.[130] visualized three components on the cell surface—the nonreducing end (Sia) and reducing end (GalNAc) of cell-surface glycan chains and lipid membrane (Figure 4c−e)—with the help of 2D and 3D single-molecule SR microscopy[131] to provide quantitative vertical details of the glycocalyx. Future progress in this direction will be inevitable with the improvement of glycan tagging methods and bioorthogonal reactions.

Although SR imaging has fostered a multifaceted understanding of the distribution and organization of various glycans, the spatial dynamics of the glycocalyx depends not only on glycans, per se, but also on their underlying conjugates (e.g., proteins and lipids). Thus hierarchical colocalization of glycans and glycoconjugates and cell-surface-localized imaging are important. However, the role of glycans in biomolecular interactions and related processes is largely unclear. Proximity labeling technology should be one of the solutions because it has recently been revolutionized with the advancement of SR imaging.[132] The conditions of SR imaging should also become more biocompatible and less phototoxic to collect live-cell and dynamic information.

Unlike traditional SR microscopy, which relies on specialized optical and computational techniques, expansion microscopy (ExM) offers a chemical approach to achieve super-resolution by isotropically expanding the distance between biomolecules in three dimensions. By introducing fluorescent labeling of abundant reactive entities (FLARE), Vaughan's group achieved the first mapping of glycans with ExM,[133] but glycans were labeled without specificity and not directly anchored to hydrogels. Later, Chen's group proposed click-expansion microscopy (click-ExM) on the basis of click chemistry and glycan labeling methods, including MGE and CeGL.[128] More recently, Chen's group labeled glycans with Yb^{3+} complex,[134] which extended ExM-based imaging to near-infrared regions. Together, these techniques significantly leverage the advantages of the existing labeling toolbox and broaden the choice of glycan targets. Although, in principle, in situ information on living cells is lost during sample preparation, ExM-based imaging can achieve a spatial resolution similar to that of SR imaging but at a lower cost. In addition, the generality of ExM for many types of biomolecules may encourage research on hierarchical imaging, such as glycan imaging on lipid rafts or specific proteins.

Tissue Section Imaging

Lots of cell surface glycan imaging strategies can be adapted to tissue section samples, as represented by conventional histochemistry based on lectin recognition[135] and chemoenzymatic labeling.[56,136] ExM-based imaging, which has only recently entered the field, skillfully breaks through the spatial resolution bottleneck at the tissue level.[133]

In contrast to those optical approaches, mass spectrometry imaging (MSI) can determine glycan structures with high throughput and visualize the spatial distribution of the structures. Although MSI has comparatively lower spatial resolution, recent collisions between ExM and MS[134,137] have led us to wonder whether MSI at the single-glycan level will be achievable in the future.

Matrix-assisted laser desorption/ionization (MALDI) is the most popular ionization technique for MSI, and MALDI-MSI has become a necessity for laboratories to investigate glycans.

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In a typical workflow,[138−140] the key step is to spray a thin layer of peptide *N*-glycosidase F (PNGase F) enzyme on the tissue to release *N*-glycans from their protein carrier. After matrix incorporation, these glycans are separated and imaged. However, the cleavage efficiency of PNGase F may be overestimated and it varies with glycoforms[141] and hindrance on tissue samples, which is unfavorable for glycan quantification or cross-comparison between different glycans. Also, whether the cleaved glycans remain in their original positions depends on the time of cleavage, the thickness of the spray layer, and the manipulation technique. Similar questions will also apply to alternative enzymes, including endoglycosidase F3 for core fucosylated *N*-glycans[142,143] and endo-α-*N*-acetylgalactosaminidase for specific *O*-glycan subsets.[144] Another bottleneck in MALDI-MSI is the poor ionization efficiency of the glycans, which is attributed to their hydrophilic nature and low proton affinity. To overcome this, several reagents have recently been developed to derivatize reducing glycans and also act as a matrix.[145−148] Furthermore, Sia in glycans, which plays a significant role in glycobiology, can decompose in MALDI and complicate mass spectra.[149,150] Drake and Wuhrer's group tackled this by Sia derivatization, even with the ability to differentiate Sia isomers.[72,151]

Ongoing advances in MSI in sample preparation, ion sources, and mass analyzers have enabled broader and more diverse research in glycoscience. As these have been reviewed recently,[152−156] we focus on a special topic: mass-tag-based strategies for MSI of glycans. A typical probe contains a labeling module targeting known GOIs and a mass tag (or several mass tags) that can be easily released for MALDI detection. Mass-tag-based MSI is essentially a means of indirectly sensing and mapping GOIs through the efficient ionization of mass tags, thereby facilitating spectral analysis and enhancing signals. From the few studies on this topic, we notice that lectins are the most common labeling modules, and the mass tag could be a cleavable organic molecule, a PEG-based tag, or a single-strand DNA tag on a rolling circle amplification (RCA) product.[157−159] Considering the complex environment of tissue sections, a labeling method with monovalency, higher specificity, and affinity/reactivity is needed, and two-step labeling may be better. In addition, the choice of mass tags and possible nanocarriers[160,161] must be carefully considered, as they would greatly affect ionization efficiency and spatial resolution.

In Vivo Imaging

Since 2008, Bertozzi's group has pioneered efforts to image glycans in living zebrafish[162−164] and *Caenorhabditis elegans*[165] using a chemical reporter strategy in which GOIs are metabolically labeled and then conjugated to fluorescent reporters via bioorthogonal chemistry. The efficiency of the covalent reaction for glycan labeling is one of the key factors that affect signal-to-noise ratio (SBR). Given the high dilution factor and complexity in vivo, only a narrow range of reactions are suitable, such as strain-promoted azide−alkyne cycloaddition (SPAAC) reaction[76] and inverse electron-demand Diels−Alder (iEDDA) reaction.[82] The current trend is gradually moving toward the much faster iEDDA reactions. As demonstrated by Brindle's group, the use of iEDDA reaction as the second click reaction to generate fluorescence signal can effectively reduce background and enhance SBR.[82] The future direction will be to expand the types of reactions to facilitate multiplex glycan imaging and perhaps incorporate spatiotemporal control (e.g., photocontrollable reactions[166]).

Compared with glycan imaging methods for cells or tissue sections, in vivo glycan imaging has higher requirements for signal penetration depth. Solutions include two main ideas: (1) the use of near-infrared fluorescent dyes and (2) the introduction of other imaging modalities. When using other imaging modalities, one must fully evaluate the resolution and sensitivity of the technique. Despite the widespread use of near-infrared organic dyes for in vivo glycan imaging, their structural properties may result in nonspecific binding,[167] which may hinder glycan studies at the organ-specific level. Alternatives, such as zwitterionic cyanine dyes[168,169] and "turn-on" probes,[84] may be considered. In addition, lanthanide complexes[170] and aggregation-induced emission (AIE) luminogens (Figure 4f,g)[171] have recently been adopted for in vivo glycan imaging.

A growing number of imaging modalities have deepened our understanding of the spatial information on glycans, thereby providing distinct complements to optical methods. Radionuclide imaging methods based on single-photon emission computed tomography (SPECT) and positron emission tomography (PET) provide high sensitivity for the visualization of deep tissue glycans.[172,173] However, their practical application in research has progressed slowly, probably because of low resolution and possible radiation damage.

Magnetic resonance imaging (MRI) also excels in deep tissue visualization but provides higher spatial resolution. Brindle's group used a gadolinium-based probe for in vivo glycan ^{1}H MRI to show significant tetra-acetylated *N*-azidoacetylgalactosamine (Ac_4GalNAz)-dependent T1 contrast.[174] In contrast, ^{19}F MRI has high sensitivity and a negligible biological background. Gao and Lin's group treated mice with tetra-acetylated *N*-azidoacetylmannosamine (Ac_4ManAz) and then a ^{19}F-containing cyclooctyne probe for glycan imaging.[175] Later, this group designed two ^{19}F-containing monosaccharide probes with different ^{19}F chemical shifts to visualize two glycans in vivo, thereby demonstrating the multiplexing potential of ^{19}F MRI.[176]

GLYCAN EDITING IN LIVING SYSTEMS

Glycan editing refers to the modification of glycans, including cleavage, elongation, and chemical modification of the structure. This operation not only affects the properties of the modified proteins or lipids but can also affect the functions of the whole cell, tissue, or even organism. The establishment of in situ glycan editing techniques is particularly significant for glycobiology research. The modification of specific glycans can reveal their biological effects, regulate the functions of the biomolecules they modify, and offer promising avenues for the development of glycan-targeted disease intervention technologies. Current glycan editing techniques fall into two main categories: genetic engineering strategies and nongenetic engineering strategies. The main drawbacks of the former include limited editing functions, difficulty in multistep editing, complex molecular biology operation, potential biosafety risks, and limited application to some cell types. The latter also includes two main categories: noncovalent hydrophobic insertion methods and covalent linkage methods. Chen's group constructed a tumor microenvironment-triggered degradable hydrogel that contained liposome-presenting natural killer (NK)-activating Lewis X trisaccharide (Lewis X). Following tumor-microenvironment-triggered degradation

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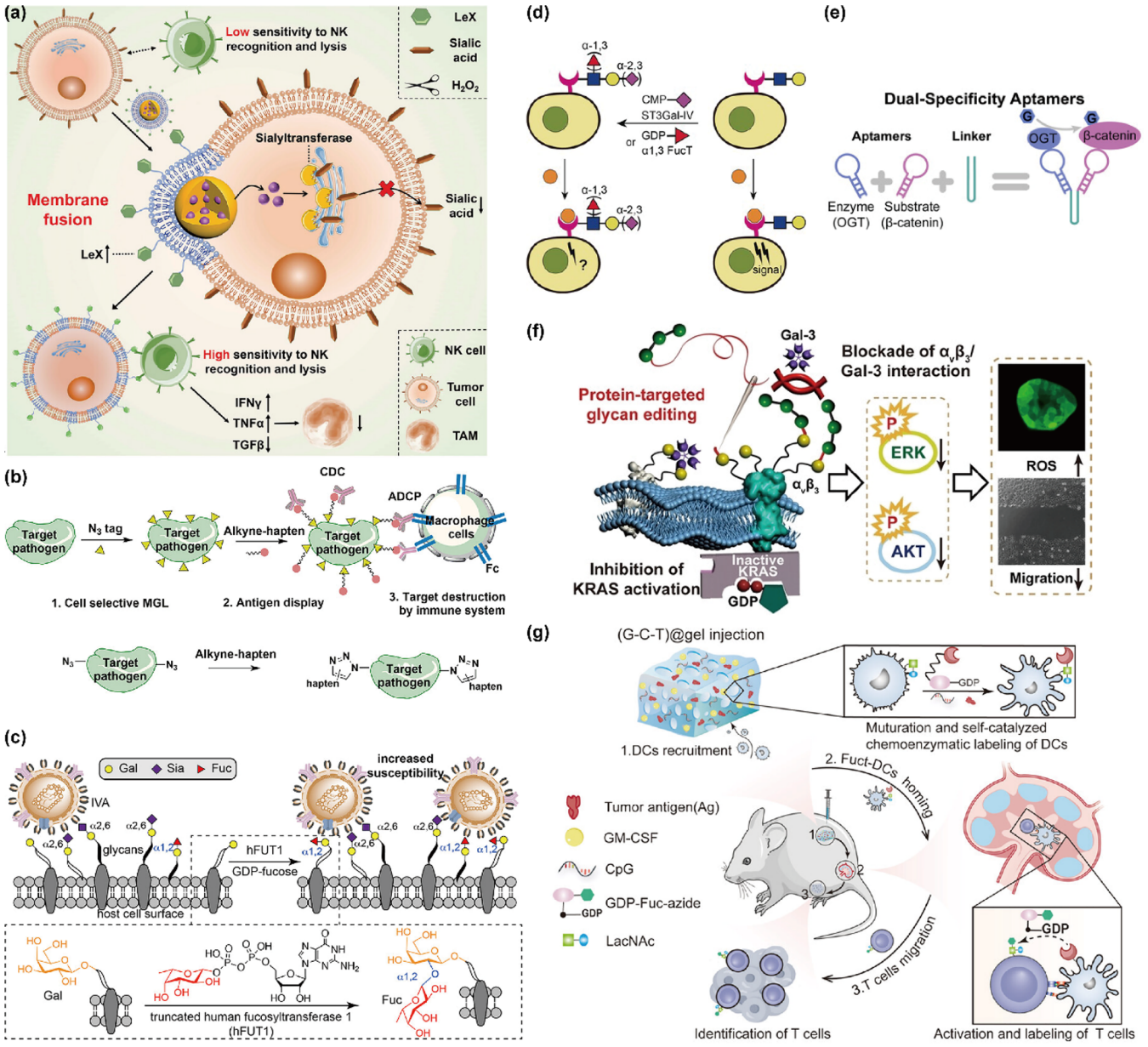

Figure 5. Application of glycan editing strategies in living systems. (a) Installation of Lewis X on the tumor cell membrane surface via membrane fusion induces NK cell killing. Adapted with permission from ref 177. Copyright 2023 Wiley-VCH GmbH. (b) Introduction of rhamnose on tumor cells by metabolic labeling for triggering antibody-dependent cytotoxicity and complement-dependent cytotoxicity. Adapted with permission from ref 181. Copyright 2018 American Chemical Society. (c) Installation of Fuc to the surface of host cells by chemoenzymatic editing to enhance virus infection. Adapted with permission from ref 186. Copyright 2020 American Chemical Society. (d) Regulation of the cell signaling by chemoenzymatic glycan editing of the epidermal growth factor receptor (EGFR). Adapted with permission from ref 189. Copyright 2018 Wiley-VCH GmbH. (e) Editing of *O*-GlcNAc on *β*-catenin using dual-specificity aptamers. Adapted with permission from ref 197. Copyright 2023 Elsevier. (f) Protein-targeted installation of mannotriose to the glycan chains of integrins disrupts the KRAS downstream pathway. Adapted with permission from ref 198. Copyright 2023 Wiley-VCH GmbH. (g) Glycosyltransferase-modified dendritic cells (DCs) are used to label and activate T cells to induce an immune response against tumors in vivo. Adapted with permission from ref 201. Copyright 2023 Wiley-VCH GmbH.

and membrane fusion, Lewis X was anchored to the tumor cell membrane to trigger NK cell recognition and activation (Figure 5a).[177] This is a promising way to install sugars on the target cell membrane in a noncovalent manner. However, covalent glycan editing may provide a more robust linkage that facilitates prolonging the residence time of introduced glycans in the cell membrane. For example, sugars with bioorthogonal groups can be "clicked" onto cellular glycans following MGE, and donor sugars can be transferred to acceptor glycans via CeGL. The "sugar ligation" step is carried out extracellularly and is highly efficient for both click and chemoenzymatic reactions, thus, providing flexibility in tailoring the editing protocol and enabling multiplexed editing.

To some extent, MGE manipulates cellular metabolism while interfering with glycosylation.[178] The bioorthogonal groups introduced to cell surface glycans by MGE can serve as anchors for various molecules, including nonsugars and sugars. In an early effort, Bertozzi's group coupled hydrazide-biotin to keto-bearing Sia generated by MGE and further combined it with ricin toxin A chain (RTA)-avidin conjugate to achieve

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selective drug delivery and cell killing.[179] Yarema's group attached thiols to cell-surface Sia by MGE to modulate adhesion and stem cell biology.[180] Recently, Wang's group covalently attached L-rhamnose to Sia via click reaction to enhance the immune response of macrophages (Figure 5b).[181] Chen's group covalently attached bulky glycan chains to the cell surface using MGE. They then injected the glycopolymer-functionalized dendritic cells (DCs) into mice and achieved inhibition of B16-OVA (expressing OVA antigen) tumor.[182] However, the lack of temporal or spatial selectivity in glycan editing is a major drawback for such strategies. Fukase's group recently introduced temporal control by installing a photocleavable cage on rhamnose, which is a practical strategy for editing the cell-surface glycocalyx under promiscuous conditions.[183]

The use of glycosyltransferases is also common for in situ glycan editing. For example, α-1,3-fucosyltransferase can convert the native CD44 glycoform on mesenchymal stromal cells to hematopoietic cell E-selectin/L-selectin ligand (HCELL), which results in osteoid generation in mice.[184,185] This type of global glycan editing has also recently been used to modulate microbial invasion. Using a truncated human fucosyltransferase 1 (hFUT1), Wu's group anchored α1−2-fucosides to host cells, thereby enhancing influenza A virus infection (Figure 5c).[186] In contrast, Heaton's group found that beta-glucuronyltransferase 1 (B3GAT1) could outcompete sialyltransferase to prevent the expression of Sia on the cell surface and, thus, resist influenza virus infection.[187] Friscourt's group found that the covalent ligation of sydnone-modified unnatural sugars to the cell surface with sialyltransferase could resist cleavage by bacterial sialidases.[188] Furthermore, to precisely elucidate the functions of different glycoforms on different glycoconjugates, Wu's group used glycosyltransferases to install Fuc and Sia to the glycans of epidermal growth factor receptor (EGFR) and examined the changes in the signaling pathway after epidermal growth factor (EGF) binding (Figure 5d).[189]

Recently, a pivotal direction in glycan editing has been the pursuit of specificity at the glycan, protein, or cellular level. To achieve selective editing of *N*-glycans, Huang's group performed subtype-selective "delete" and "insert" operations on cell-surface glycans on the basis of the substrate selectivity of different endoglycosidases and their mutants.[190] Withers's group discovered that endo-*O*-glycan hydrolases can selectively cleave *O*-glycans at the cellular and protein level.[191] Protein- and cell-specific glycan editing helps us to better understand and intervene in the functions of glycans on glycoconjugates and cell conditions. To selectively edit tumor cells, Bertozzi's group employed antibody−sialidase conjugates to achieve selective desialylation of tumor cells.[192,193] Ding and Ju et al. achieved selective desialylation of cells[194] and protein[66] by regulating sialidase activity through polymer blocking. Woo's group first designed a nanobody-fused split *O*-GlcNAcase to achieve selective deletion of *O*-GlcNAc on target proteins.[195] They also developed a nanobody-fused *O*-GlcNAc transferase (OGT) to facilitate selective *O*-GlcNAc glycosylation on target proteins.[196] Hart's group designed a dual-specific RNA aptamer probe to simultaneously bind OGT and β-catenin and achieved glycosylation on β-catenin (Figure 5e).[197] Moreover, Ding and Ju et al. elongated glycans on a specific protein via a "localized oxidation-coupling" strategy, and they discovered that attaching mannotriose to the terminal Gal/GalNAc of integrin $\alpha_v\beta_3$ could block its interaction with galectin-3 and inhibit the KRAS activation pathway (Figure 5f).[198] The most unique feature of this strategy compared with other glycosyltransferase-based editing strategies is that it allows the installation of arbitrary glycan structures on the target proteins, thereby making it a powerful tool for manipulating the glycosylation of specific proteins.

Glycan editing can interfere with the functions of glycosylation in living systems and has immense application potential in disease therapeutics, especially cancer-related therapies. Wu and Paulson's group attached high-affinity and specific CD22 ligands to the surface of natural killer (NK) cells via glycosyltransferases so as to target tumor-specific CD22.[199] Bertozzi's group conjugated a genetically modified sialidase with low recognition capability to an antibody for targeted desialylation and immune checkpoint activation of tumor cells in vivo. Wang's group used a similar strategy in which tumor-targeted molecule−sialidase conjugates can efficiently and selectively cleave Sia from a variety of cancer cells.[200] In their study, they found that targeted desialylation could enhance the infiltration and activation of induced pluripotent stem cell (iPSC)-derived CAR-macrophages (CAR-iMacs), thereby achieving a synergistic effect of glycoimmune checkpoint blockade and CAR-based cellular immunotherapy in solid tumors. Xie's group conducted chemoenzymatic proximity labeling of DCs to obtain fucosyltransferase-labeled DCs (Fuct-DCs), which underwent homing and performing CeGL of T cells and triggered their transfer to the tumor tissue for immunotherapy (Figure 5g).[201] The application of chemoenzymatic proximity labeling strategy in vivo can realize a tumor-specific immune response cascade. It is also an inspiring strategy that can be applied to various immune cells and has the potential to be used to study cell−cell interactions.

Recently, some new chemical installation approaches have also energized the field of glycan editing. Ding and Ju et al. developed an aptamer-enabled proximity catalytic covalent labeling platform. By coupling horseradish peroxidase to a cell-selective aptamer, the probe formed can bind selectively to the target cell and catalyze the attachment of phenol derivatives to adjacent electron-rich amino acid sites. They synthesized phenol-modified lactose and achieved rapid covalent attachment of lactose to the target cells.[202] This can be considered as a neo-glycosylation method. Chen's group exploited the property that HaloTag forms irreversible covalent bonds with chloroalkane ligands and achieved covalent installation of glycopolymers on the surface of cell membranes under physiological conditions by expressing HaloTagged proteins on cells and modifying glycopolymers with chloroalkane ligands.[203−205] Using T cells as a model, Ding and Ju et al. introduced azide groups for anchoring chain transfer agents at the Sia sites of the cell membrane using MGE. By triggering Fenton-reversible addition−fragmentation chain transfer (Fenton-RAFT) polymerization, they creatively realized the in situ polymerization of sugar-modified monomers at the glycan site on the surface of living cells.[206] The polymer growth process can be viewed as a chemical mimicry of the growth of glycan chains on cells in situ, and the synthesized glycopolymer presents an entirely new form of glycan distribution as a bionic glycocalyx to regulate cell recognition behavior.

SUMMARY AND OUTLOOK

As essential building blocks of cells, glycans possess unique intrinsic characteristics, such as the regularity of glycosylation sites, the flexibility of the glycan length and type, and the

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configurational diversity of glycoforms. These together confer a molecular basis for glycans to perform different physiological functions. Undoubtedly, the analysis of glycosylation types, the elucidation of glycoform structures, and the development of glycan-related biomarkers and therapeutics have become hot topics nowadays. Although the current research on glycan labeling, imaging, and editing is developing rapidly, there is still a considerable distance to the goal of elucidating the regulatory mechanism and biological effects of glycosylation. Here, we summarize some promising directions for this path: (1) The classical MGE and CeGL methods need to be further extended to cover a wider range of unnatural glycan probes and enzymes and to improve the labeling efficiency. In particular, the impact of these methods on living systems should be carefully investigated and distinguished and, more importantly, should be skillfully exploited. (2) There is an urgent need to accelerate the development of aptamers, molecularly imprinted polymers, and other artificial antibodies that can target glycoforms. Efforts can be made to enrich the abundance and improve the binding affinity and specificity. The development of new recognition molecules will bring new ideas for the development of in vivo glycan research tools. (3) High-throughput glycan labeling and detection on tissue samples is imperative for clinical disease diagnosis because it adds a new dimension to the traditional immunohistochemical information so that comprehensive "molecular pathological information" can be obtained. The collection of bulk data for disease-specific glycans requires the development of mass spectrometric methods based on multiplexed glycan labeling and the cooperation of bioinformatics analysis. (4) It is still difficult to perform glycan labeling, imaging, and editing in vivo, and we cannot simply transform methods at the cellular level to in vivo scenarios. We should take advantage of recent developments in the field of in vivo drug delivery for targeted delivery of glycan labeling and editing probes and explore the feasibility of responsive release of probes. In vivo stability and clearance of the probes are also the main concerns for in vivo scenarios. In addition, the development of imaging modalities, such as photoacoustic imaging, magnetic resonance imaging, and near-infrared imaging, can help to better characterize and optimize the in vivo glycan labeling/editing process. (5) How to build an analytical platform for dynamic tracking of glycans at a specific spatial level remains to be solved. Such tools are crucial for the understanding of cell−environment interactions. Techniques such as electrochemiluminescence imaging, super-resolution imaging, and high-content imaging may revolutionize the field in the future. The above directions cannot be explored without in-depth interdisciplinary integration of multiple disciplines, including nanoscience, biomedicine, physics, chemistry, mechanics, computation, and artificial intelligence (AI). For example, it is particularly necessary to advance the construction and application of databases related to glycan structures and functions. As glycan labeling and editing technologies are directly related to clinical medicine, efforts should be made to promote the translation of glycobiological technologies and discoveries into the clinical field and to provide glycan-related markers, targets, and intervention technologies for disease diagnosis and therapeutics.

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Notes

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