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In Situ Cellular Glycan Analysis

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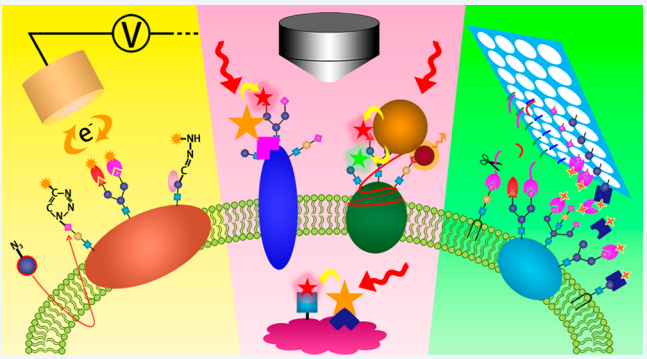
CONSPECTUS: Glycan decorates all mammalian cell surfaces through glycosylation, which is one of the most important post-modifications of proteins. Glycans mediate a wide variety of biological processes, including cell growth and differentiation, cell−cell communication, immune response, pathogen interaction, and intracellular signaling events. Besides, tumor cells aberrantly express distinct sets of glycans, which can indicate different tumor onsets and progression processes. Thus, analysis of cellular glycans may contribute to understanding of glycan-related biological processes and correlation of glycan patterns with disease states for clinical diagnosis and treatment. Although proteomics and glycomics have included great efforts for in vitro study of glycan structures and functions using lysis samples of cells or tissues, they cannot offer real-time qualitative or quantitative information, especially spatial distribution, of glycans on/in intact cells, which is important to the revelation of glycan-related biological events. Moreover, the complex lysis and separation procedures may bring unpredictable loss of glycan information. Focusing on the great urgency for in situ analysis of cellular glycans, our group developed a series of methods for in situ analysis of cellular glycans in the past 10 years. By construction of electrochemical glycan-recognizable probes, glycans on the cell surface can be quantified by direct or competitive electrochemical detection. Using multichannel electrodes or encoded lectin probes, multiple glycans on the cell surface can be dynamically monitored simultaneously. Through design of functional nanoprobes, the cell surface protein-specific glycans and intracellular glycan-related enzymes can be visualized by fluorescence or Raman imaging. Besides, some biological enzymes-based methods have been developed for remodeling or imaging of protein-specific glycans and other types of glycoconjugates, such as gangliosides. Through tracing the changes of glycan expression induced by drugs or gene interference, some glycan-related biological processes have been deduced or proved, demonstrating the reliability and practicability of the developed methods. This Account surveys the key technologies developed in this area, along with the discussion on the shortages of current methodology as well as the possible strategies to overcome those shortages. The future trend in this topic is also discussed. It is expected that this Account can provide a versatile arsenal for chemical and biological researchers to unravel the complex mechanisms involved in glycan-related biological processes and light new beacons in tumor diagnosis and treatment.



INTRODUCTION

Glycans on mammalian cell surfaces are composed of 10 kinds of monosaccharides:[1] glucose (Glc), galactose (Gal), mannose (Man), fucose (Fuc), *N*-acetylglucosamine (GlcNAc), *N*-acetylgalactosamine (GalNAc), xylose (Xyl), glucuronic acid (GlcA), iduronic acid (IdA), and sialic acid (Sia), which are connected through glycosyltransferase (GT) catalyzed substitution from nucleotide pyrophosphate. According to their different binding sites on proteins, they can be classified into N-linked glycans (binding on the Asn of glycoproteins), O-linked glycans (binding on the Ser or Thr of glycoproteins and predominate on mucins), glycosaminoglycans (binding on Ser residues of proteoglycan molecules), and glycolipids (binding on ceramide of lipids). Besides, the GlcNAc can be modified on Ser or Thr residues of intracellular proteins.[2] These connections make their structures vary from highly branched to linear, which leads to enormous information contents.

Glycans play important roles in intercellular interactions. Their expression depends on cellular conditions, as well as the onset of diseases, such as cancer[3] and inflammation.[4] For example, enhanced level of α2,6-sialylation is a significant alteration in cancer progression, which can be used as an important diagnostic basis.[5] The glycosylation patterns vary with different tumor phenotypes[3] and malignant transformation of cancer,[6] which can provide new aspects for the research of pathology. In addition, glycans can be used as markers for targeted therapy.[7−10] Therefore, in situ analysis of cellular glycans may provide information for the diagnosis and therapy of cancer-related diseases.

LABELING AND RECOGNITION OF GLYCANS

Three types of methods have been developed for glycan labeling and recognition: lectin, chemical covalent recognition, and metabolic labeling.

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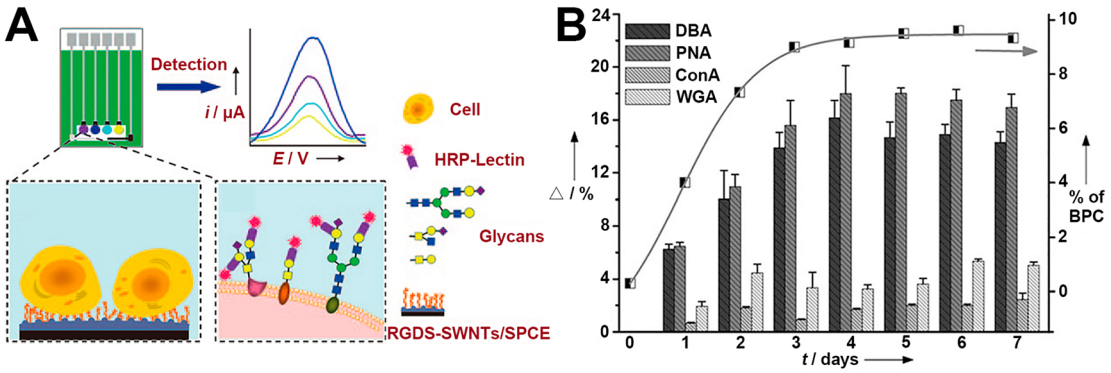

Figure 1. (A) Scheme of the electrochemical cytosensor for cell-surface glycan analysis. (B) Changes of cellular glycan expression after treatment with sodium butyrate. Reproduced with permission from ref 40. Copyright 2009 Wiley.

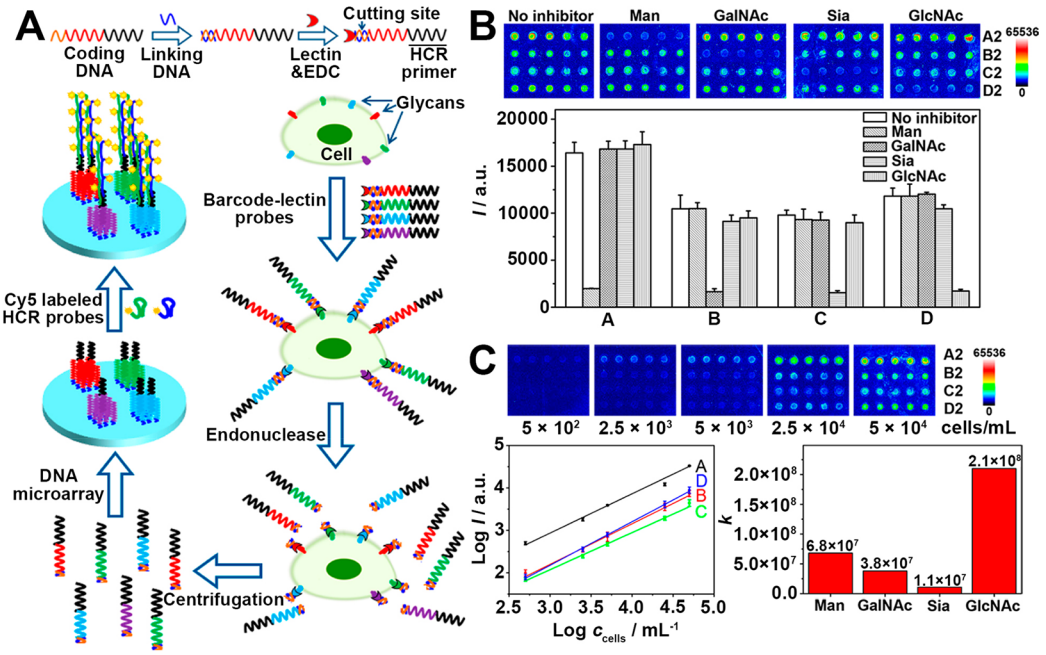

Figure 2. (A) Scheme of probe preparation and assay procedure. (B) Images of DNA microarray and corresponding average spot intensity from monosaccharide inhibition test. (C) DNA microarray images for quantification of cell surface glycan. Reproduced with permission from ref 41. Copyright 2013 American Chemical Society.

Lectins are a kind of proteins that can specifically bind to saccharides through a nonimmune response.[11] They can be conjugated with different molecules, such as fluorescein, biotin, and horseradish peroxidase (HRP), for fluorescence and electrochemical analysis of cellular glycan.[12,13] Using lectins to functionalize nanoparticles, such as gold nanoparticles (AuNPs),[14] silver nanoparticles,[15] upconversion nanoparticles,[16] and quantum dots[17] through electrostatic adsorption or covalent cross-linking, different nanoprobes can be prepared to recognize saccharides for analysis of related glycans by the optical, electrochemical, or catalytic properties of nanoparticles.

Chemical covalent recognition is based on chemical binding of the labeling molecule to glycan, which can be classified into two categories. The first one is based on the mild oxidization of polyhydroxyls of carbohydrates into aldehydes, which are ligated with probes containing aminooxy or hydrazine groups to achieve labeling and recognition of glycans.[18] For example, Han et al. used periodate and Gal oxidase (GO) to oxidize the hydroxyls of cell surface Sia and Gal into aldehydes, respectively. The aldehydes were then step-by-step ligated with biotin-labeled hydrazide, avidin and biotin/HRP comodified nanoprobe to achieve signal amplified chemiluminescence imaging of Sia and Gal on cell surface.[19]

Another category is based on the covalent connection between carbohydrate and boric acid. Boric acid can conjugate with 1,2- and 1,3-substituted Lewis base donors to form tight and reversible complexes, such as hydroxyl and diol groups that are rich in carbohydrates.[20] The binding affinity depends on the pK_a value of boronic acid and the spatial structure of hydroxyl or diol. Thus, boronic acids with different main molecular structures show distinct preference to different carbohydrates. Especially, phenylboronic acid (PBA) exhibits a strong preference to bind Sia with an equilibrium constant of 37.6 under physiological pH,[21] which makes it a very important targeting molecule for Sia. By modifying PBA onto nanostructures such as gold nanoflowers[22] or nanoclusters,[23] cell surface Sia can be sensitively detected by SERS or fluorescence. In addition, Sia-contained glycolipids, i.e., gangliosides,[24] can also be quantitated with a PBA functionalized silica bubble.

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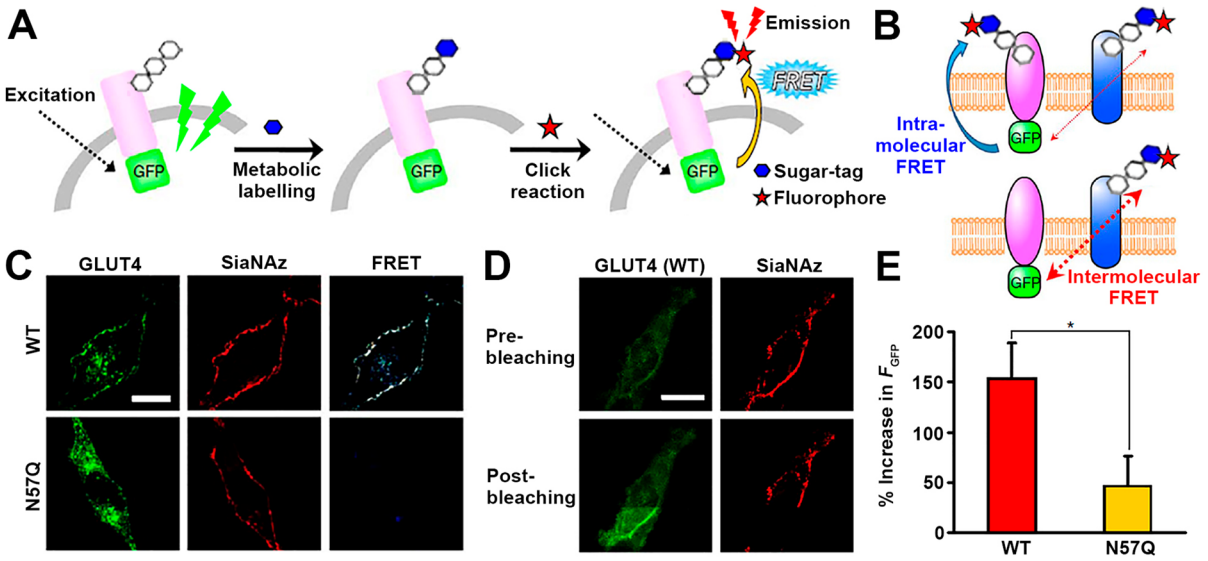

Figure 3. (A) Scheme of visualization of a specific sugar on a target glycoprotein. (B) Scheme of intramolecular and intermolecular FRET. (C) Fluorescence images of intramolecular and intermolecular FRET on HeLa cells. (D) Acceptor photobleaching. (E) Average increases of the donor fluorescence after photobleaching. Reproduced with permission from ref 45. Copyright 2012 Nature Publishing Group.

Metabolic labeling is based on incorporation of a bioorthogonal chemical reporter into a target biomolecule using the biosynthetic machinery of cells,[25] which does not produce significant structural perturbation and can be tolerated by promiscuous enzymes involved in the installation process. It usually makes use of copper-catalyzed azide−alkyne cycloaddition (CuAAC)[26] to induce fluorescence dye with low background and high sensitivity for glycan imaging. Comparing to alkynyl-sugars,[27] azido-sugars exhibit drawbacks of certain cellular toxicity and poor or different acceptance as substrates for different GTs. Besides, the CuAAC process often needs cell fixing due to cellular toxicity, and the dibenzocyclooctyne based copper-free process is actually too slow to reflect the fast kinetics of the glycosylation process. However, through a biocompatible CuAAC,[28] dynamic single-molecule tracking and super-resolution imaging of Sia and GalNAc on living cells have been achieved by controlling the bleaching of fluorescent probes conjugated on the metabolically labeled glycans.[29] Other reporters such as cyano and deuterated methyl have also been used for metabolic incorporation,[30] which exhibit Raman signals in the Raman-silent region of a cell (approximately 1800−2800 cm^{-1}) for SERS detection.

The cell-specificity of the metabolic labeling process has been improved by encapsulating the metabolic reagents with ligand-targeted liposomes,[31] or converting the anomeric acetyl group of the metabolic reagent to a caged ether bond, which can be selectively cleaved by cancer-overexpressed enzymes.[32] The metabolically labeled glycans on cell surface can be further engineered with different groups to study the interactions between glycans and the glycan binding immunoglobulin-like lectins,[33] or chemically remodeled to achieve manipulation of the cell-surface charge for regenerative medicine,[34] as well as applied to tissue slice cultures for clinical application.[35]

In comparison of these methods, lectins can recognize various kinds of glycans, which is suitable for identification of glycan types. Nevertheless, their application are limited due to their large molecular volume and certain cellular toxicity. Chemical covalent recognition processes the advantages of small molecular volume and low cellular toxicity, but it needs cell pretreatment, and the types of recognizable glycan are very limited. Metabolic labeling can provide a high binding affinity between glycan and the labeling component through click chemistry, which can be applied on both cell surface and intracellular glycan. However, this technology needs a long-time cell preincubation, and the variety of substrates and labeling efficiency of the target glycan need further improvement. Thus, novel methods for glycan labeling and recognition are still of great urgency.

MULTIPLE ANALYSIS OF CELL SURFACE GLYCANS

Tumor cells aberrantly express distinct sets of glycans, which can indicate different tumor onsets and progression. Thus, it is important to achieve multiple analysis of cell surface glycans. Mass spectrometric[36] and lectin array[37] are two main tools for multiple analysis of glycans, but are unsuitable for application on intact cells. Recently, methods based on electrochemistry and barcoding technology have been successfully used for multiple analysis of glycans on intact cell surface.

Electrochemical Analysis

Electrochemical methods exhibit attractive features of high sensitivity, easy and fast operation, which have been applied in nondestructive analysis of glycans of living cells.[38] To obtain electrochemical signal, lectins are often labeled with HRP or quantum dots.[39] Cheng et al. used peptide functionalized carbon nanotubes to modify screen-printed carbon electrode array for capturing cells, and then used four HRP-labeled lectins to recognize four kinds of carbohydrates to produce electrochemical signals of glycans via enzymatic catalysis (Figure 1A).[40] This method was used to accurately evaluate glycan expression on cancer cell surface and analyze their dynamic change during erythroid differentiation induced by sodium butyrate (Figure 1B). However, this method requires the immobilization of cells on the electrodes, which might bring certain biological influence to cells. Besides, the glycans on the immobilized side of cells have poor accessibility to the lectin probes, which causes certain deviation of the results.

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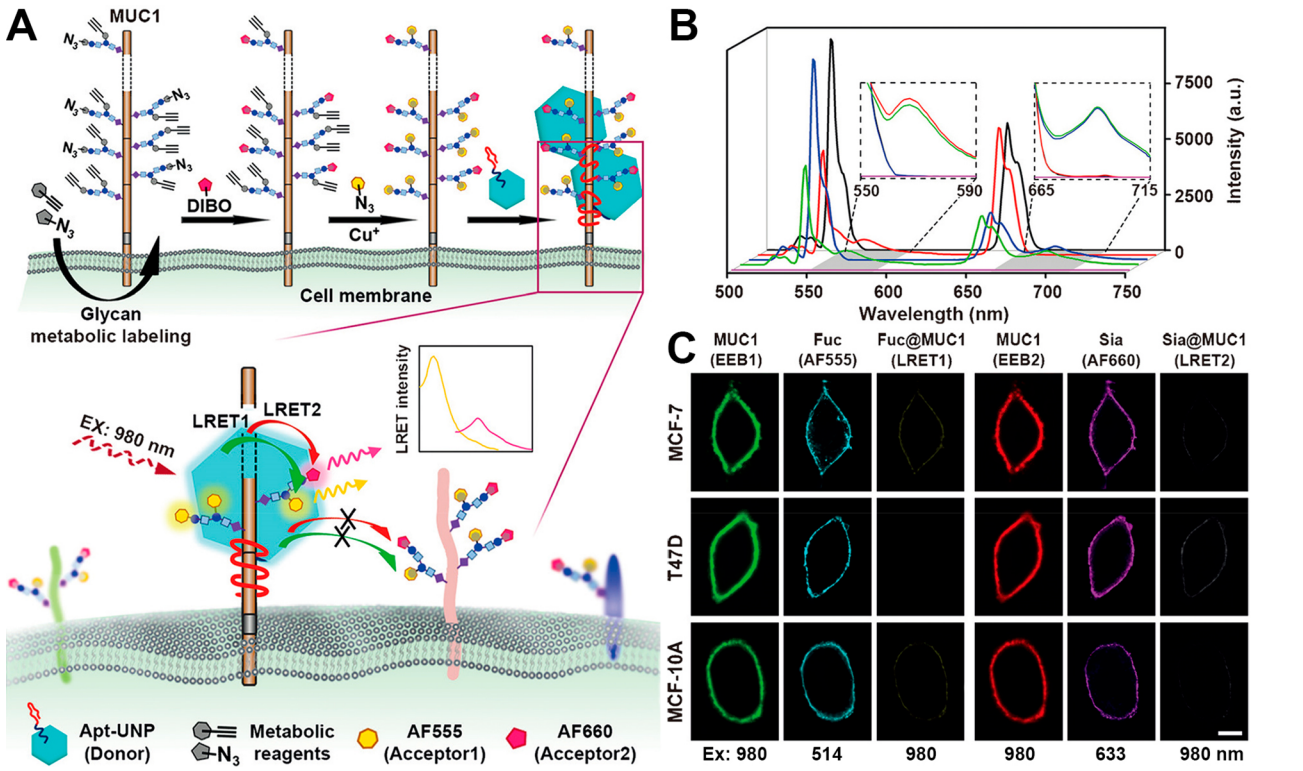

Figure 4. (A) Scheme of site-specific D-LRET for duplexed imaging on cell surface. (B) Emission spectra of the UNP probes. (C) Duplexed protein-specific imaging of metabolically labeled Fuc and Sia of MUC1 on different cells. Reproduced with permission from ref 49. Copyright 2016 Wiley.

Barcoding Analysis

To achieve whole-surface accessibility, higher sensitivity and broader applicability, some barcoding methods have been developed for multiple analysis of glycans by encoding different lectins with different barcodes. DNA is an ideal choice for barcoding because of its abundant sequences and various DNA detection techniques. Chen et al. encoded four kinds of lectins with four DNA barcodes containing a common cutting site, which could be cleaved with endonuclease (Figure 2A).[41] After the DNA-encoded lectins bound to cell surface glycans, they were cleaved and captured by a DNA microarray. With a further HCR amplification step, the amount of different glycans on cells could be simultaneously quantified. The designed DNA microarray exhibited good selectivity to DNA barcodes (Figure 2B) and excellent quantification of four different kinds of glycans on BGC-823 cells (Figure 2C). He et al. also encoded lectins with DNA primers to produce long single-strand DNA by rolling circle amplification and hybridized with short DNA probes to obtain multiple glycan information by MS.[42]

Raman signal molecules are also good choice for barcoding due to their distinguishable characteristic peaks. The signal amplification can be achieved by SERS. By using of these two advantages, Chen et al. constructed Raman signal molecule and lectin dual-coded AuNPs to analyze multiple glycans on cell surface through a microcompetition system between polysaccharide functionalized silica bubbles and cells.[43] This design originated from a "two surface-one molecule competition" concept developed by Ding et al.,[14,17] and the amounts of three different glycans on cell surfaces could be accurately reflected by the amounts of nanoprobes selectively bound on the artificial glycan surfaces through sensitive SERS detection.

■ ANALYSIS OF PROTEIN-SPECIFIC GLYCANS

Glycans on specific protein play important roles in regulating the dynamics and functions of the proteins, which further influence the biological capabilities and physiological state of the cells.[44] Thus, analysis of protein-specific glycans can provide great promotion for revealing glycan related biological process. Förster resonance energy transfer (FRET) is the main technology for analysis of protein-specific glycans. Besides, some luminescence resonance energy transfer (LRET), SERS, and enzyme based methods have also been developed.

FRET and LRET Based Methods

A FRET system is usually composed with an energy donor and an energy acceptor. By labeling the target protein with the energy donor and the target glycan with the energy acceptor, the corresponding protein-specific glycans can be identified by monitoring the FRET events. The key factor in this process is the distance between donor and acceptor, which determines the protein specificity of the glycan analysis. Haga et al. investigated the distance between the intracellular labeled proteins and the extracellular sialylated sites by green fluorescent protein (GFP) and metabolic labeling (Figure 3A).[45] By respectively expressing wild-type GLUT4-GFP and mutant GLUT4-GFP in HeLa cells and comparing their FRET signals to the Sia (Figure 3B), they found intramolecular FRET, even when the protein and the glycan were segregated by the lipid bilayer (Figure 3C–E). Meanwhile, the intermolecular FRET of glycans with adjacent protein was not observed (Figure 3C, E), which demonstrated that intramolecular FRET could be used for detection of protein-specific glycan.

However, GFP labeling can only be applied to the limited kinds of proteins and may hinder the efficiency of energy transfer in the FRET system. Other protein labeling

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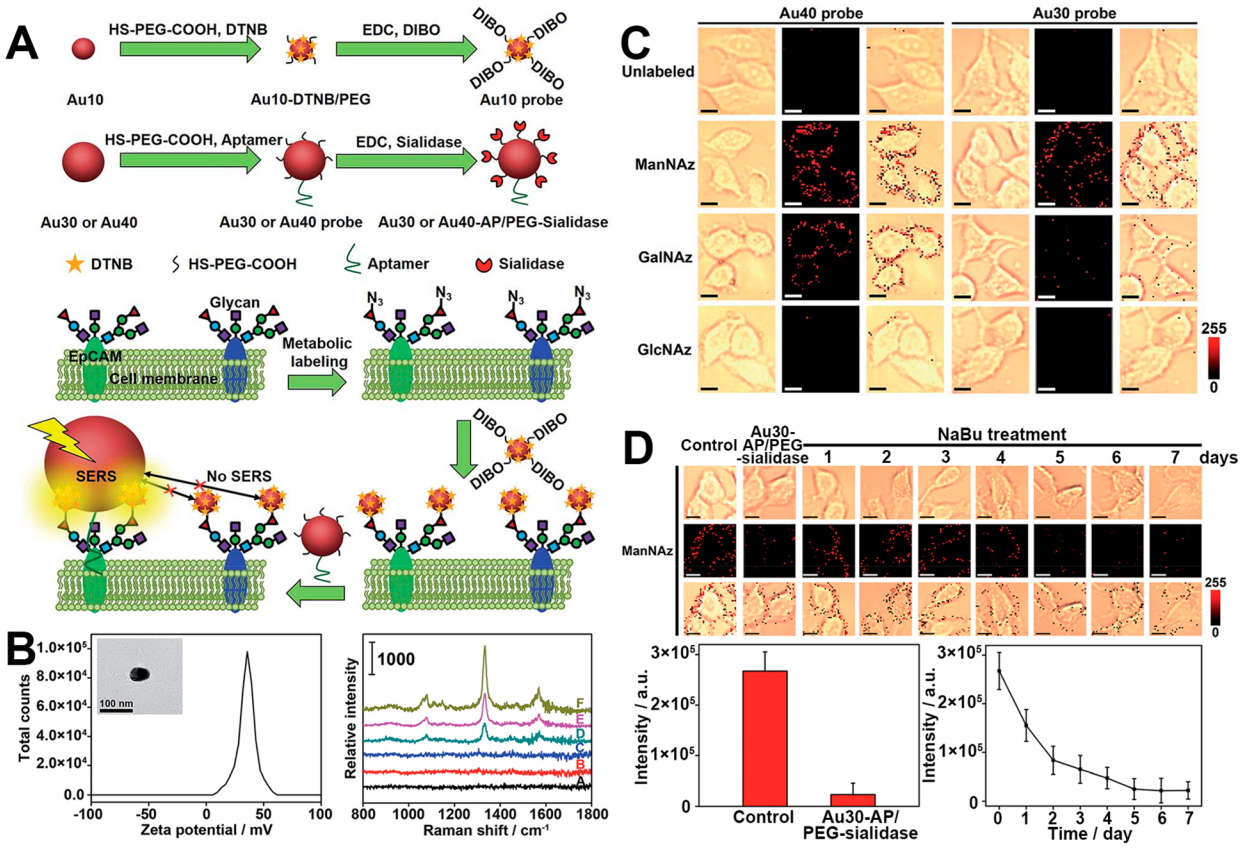

Figure 5. (A) Scheme of the synthesis of two types of Au nanoprobes and the zone-controllable SERS effect for imaging of protein-specific glycans on cell surface. (B) Characterization and Raman spectra of the probes. (C) Raman imaging of different glycans on cells. (D) Raman imaging of cells treated with sialidase-modified AuNPs or sodium butyrate for different times. Reproduced with permission from ref 51. Copyright 2016 Royal Society of Chemistry.

technologies, such as antigen-binding fragment,[46] probe incorporation mediated by enzyme,[47] and aptamer,[48] have also been applied for protein-specific analysis of glycans through FRET in combining with the metabolic labeling of glycan.

To achieve simultaneous imaging of two different protein-specific glycans, upconversion nanoparticles (UNPs), which exhibit unique polychromatic light emitting properties, have been used as the common donor for construction of a single excitation-dual LRET (D-LRET) system on cell surface (Figure 4A).[49] The UCNPs functionalized with aptamer can specifically bind the target protein mucin 1 (MUC1). Two target monosaccharides, Sia and Fuc, are stepwise labeled with two different fluorescence acceptors, Alexa Fluor 555 and Alexa Fluor 660, which can be simultaneously excited by the UCNPs through D-LRET (Figure 4B). This D-LRET system achieves the imaging of MUC1-specific Sia and Fuc on different cell types (Figure 4C). By using O-GalNAc as the native reference, this strategy has been be used for relative quantification of MUC1-specific Sia and Fuc.

SERS Based Methods

Raman imaging is a nondestructive and nonphotobleaching imaging technique,[50] which possesses high sensitivity by introduction of SERS. The implement of SERS effect needs the positioning of signal molecules in the enhance zone of the substrate. Inspired by this property, Chen et al. designed a zone-controllable SERS effect by controlling the size of the substrate to match the expression zone of the protein-specific glycans for Raman imaging (Figure 5A).[51] The target protein EpCAM on the MCF-7 cell surface was labeled with aptamer functionalized AuNPs with a diameter of 30 or 40 nm (Au30 or Au40), which served as SERS substrates. The target glycan Sia was metabolically labeled with azide groups and then labeled with Raman signal reporting molecule and dibenzocyclooctyne-amine dual-functionalized AuNPs with a diameter of 10 nm (Au10), which served as the signal probe. Au10 could not exhibited SERS signal itself, but generate SERS signal after approaching to Au30 or Au40 (Figure 5B). Thus, the zone controllable SERS strategy achieved Raman imaging of EpCAM-specific Sia (Figure 5C). By using the O-GalNAc as the negative control, which was absence on the EpCAM, the distribution zone of glycans on EpCAM could be estimated. This zone-controllable Raman imaging strategy could be used to monitor the variation of protein-specific glycosylation during drug treatment (Figure 5D).

Enzyme Based Methods

Enzymes such as GTs and glycosyl-oxidase, which can introduce or reform the glycans on proteins, are powerful tools for glycan research. Robinson et al. used a permissive galactosyltransferase to append an azide-functionalized GalNAc onto the O-GlcNAc residue, and further labeled the residue with an alkyne−biotin probe and antibody−DNA conjugate to achieve in vitro analysis of O-GlcNAcylated proteins.[52] Hui et al. located galactose oxidase (GO) to cell surface MUC1 protein with an aptamer (Figure 6A).[53] The GO could be switched between the off and on states with an inactivator

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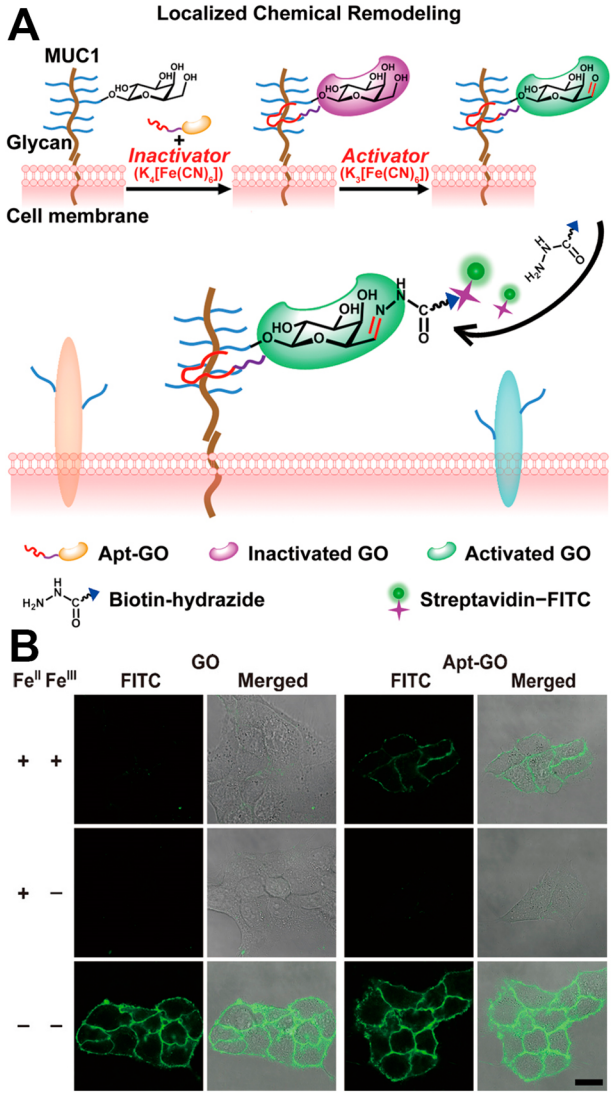

Figure 6. (A) Scheme of the localized chemical remodeling strategy. (B) Demonstration of the feasibility for live cell imaging of MUC1-bound terminal Gal/GalNAc. Reproduced with permission from ref 53. Copyright 2017 Wiley.

$(K_4Fe(CN)_6)$ and an activator $(K_3Fe(CN)_6)$ through redox, respectively. After the off-stated GO was delivered to cell surface MUC1, it was switched to on state for catalyzing the MUC1-bound Gal/GalNAc termini to generate aldehyde group, which was further conjugated with biotin through hydrazine ligation and then labeled with streptavidin-FITC to generate fluorescence signal of MUC1- specific Gal/GalNAc (Figure 6B). The protein specificity could be proved by D-Gal blocking of GO activity and Western blot analysis of biotin labeling on MUC1 protein. This strategy provided a localized chemical remodeling handle for in situ analysis of protein-specific glycans on living cells.

Despite of glycan-related enzymes, Chen et al. developed an exonuclease III-aided recycling hybridization strategy to liberate protein-specific glycan information.[54] They designed two kinds of DNA probes to target the proteins and glycans, respectively. Upon sequential binding to their targets, the complementary sequences of two probes approached enough for hybridization, which led to the cleavage of hybridized glycan-bound probe by exonuclease III. The "hybridization and cleavage" process could be recycled by protein-bound probe and other adjacent glycan-bound probes to release the fluorescent information on protein-specific glycosylation, which could be measured by the standard curves to obtain quantitative results.

INTRACELLULAR GLYCANS ANALYSIS

Comparing to cell surface glycans, research of intracellular glycans is a great challenge due to the complicated intracellular

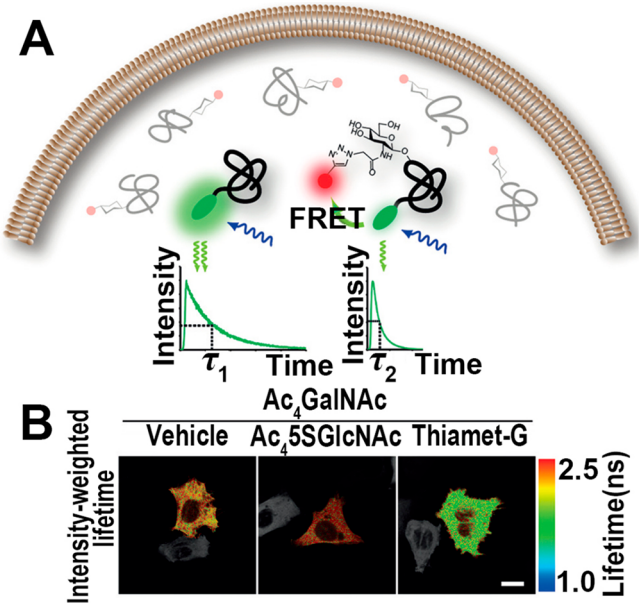

Figure 7. (A) Scheme of protein-specific imaging of O-GlcNAcylation by FLIM−FRET microscopy in single cells. (B) Imaging of the changes of tau-specific O-GlcNAc induced by OGT and OGA inhibitors. Reproduced with permission from ref 56. Copyright 2015 Wiley.

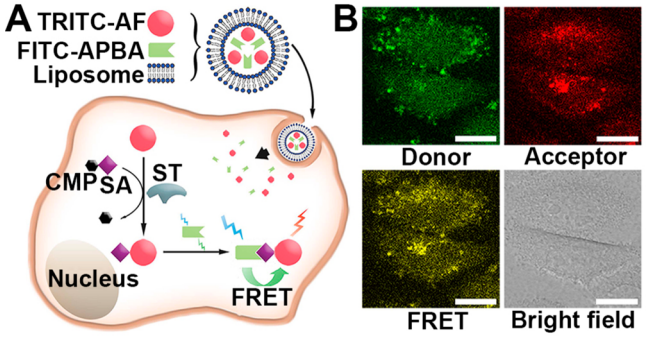

Figure 8. (A) Scheme of FRET-based noninvasive imaging of intracellular ST activity. (B) Assessment of intracellular ST activity of living cells using TRITC-protein. Reproduced with permission from ref 60. Copyright 2015 Nature Publishing Group.

environment. Current researches on intracellular glycans are mainly focused on intracellular O-GlcNAcylation and Sia-related enzymes.

O-GlcNAcylation

O-GlcNAcylation, the attachment of a single monosaccharide GlcNAc to serine or threonine residues, is the main glycosylation form inside cells. O-GlcNAcylation is associated with various biological processes such as transcriptional regulation, protein trafficking, and stress response.[55] Similar

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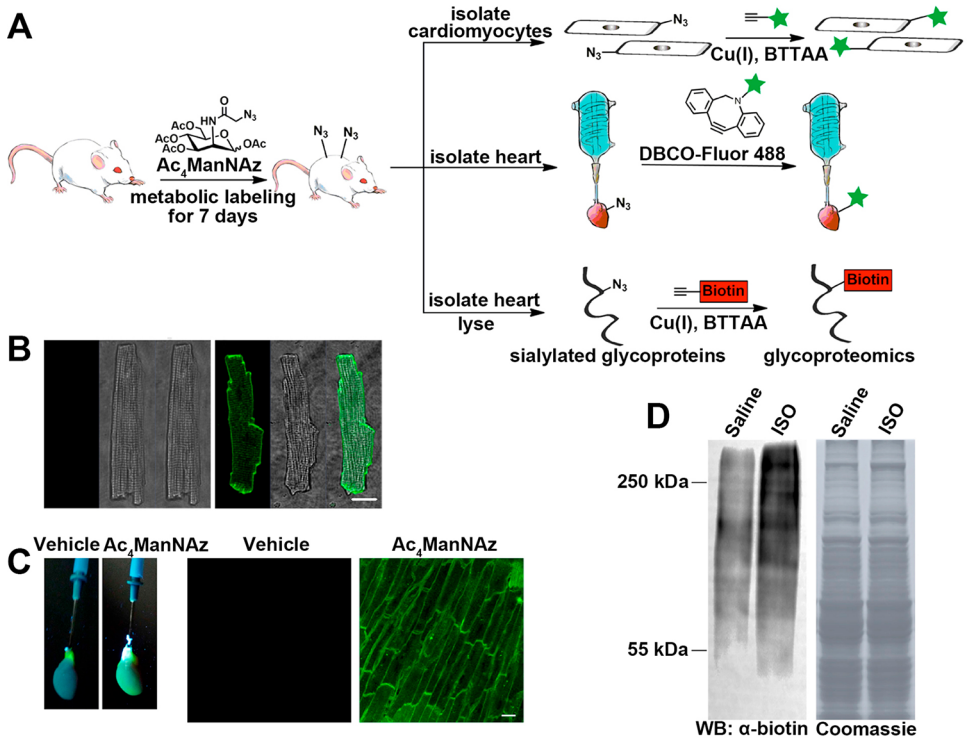

Figure 9. (A) Scheme of in vivo metabolic labeling enables visualization of cardiac sialome and proteomic identification of cardiac sialylated proteins. (B) Metabolic labeling of sialylated glycoconjugates on primary rat cardiomyocetes. (C) Imaging of cardiac glycans in intact rat hearts. (D) Proteomic analysis of sialylated glycoproteins in healthy and hypertrophic hearts. Reproduced with permission from ref 62. Copyright 2014 American Chemical Society.

to analysis of protein-specific glycans on cell surface, O-GlcNAcylation of a specific protein inside cells can also be achieved by FERT. Lin et al. employed the enhanced green fluorescence protein (EGFP) to tag the protein with fluorescence donor and the metabolic labeling to tag the O-GlcNAc moieties with fluorescence acceptor through copper-assisted click chemistry,[56] which would shorten the fluorescence lifetime of EGFP when the protein of interest was O-GlcNAcylated (Figure 7A). After blocking the O-GlcNAc transferase (OGT) or inhibiting the O-GlcNAcase (OGA), the fluorescence lifetime of EGFP exhibited corresponding increase or decrease (Figure 7B), demonstrating the feasibility of the designed strategy. This method suffered from the cytotoxicity during the copper-assisted click chemistry, and thus could not be applied for imaging of living cells. Doll et al. improved the metabolic labeling with a peracetylated methylcyclopropene-tagged GlcNAc,[57] which enabled the tag of the intracellular O-GlcNAc with methylcyclopropen group and further conjugation with Cy3-tetrazine as the FRET acceptor through Diels–Alder reaction. Similarly, by genetically fusing the protein with EGFP, this method has been used for fluorescence lifetime imaging of the O-GlcNAcylation on OGT, Foxo1, p53, and Akt1 proteins inside living cells. In fact, these FRET based methods are unsuitable to proteins with large volume due to the limited effective-distance for energy transfer between donor and acceptor, and the sensitivity is unsatisfactory for proteins of low abundance without signal amplification. Thus, novel methods for analysis of intracellular O-GlcNAcylation are still of great urgency.

Sia-Related Enzymes

Sialyltransferases (STs) and sialidases (Neus) are two important Sia-related enzymes, which can introduce Sia to the terminal position of glycan chains or remove α-glycosidically linked Sia residues from glycoconjugates.[58] They are located at different cellular compartments such as lysosomes, cytosol, plasma membrane and mitochondria, and play central roles in regulating cell-surface Sia expression and the biological process of cells.[59] Bao et al. encapsulated tetramethylrhodamine isothiocyanate labeled asialofetuin (TRITC-AF) as a ST substrate and fluorescein isothiocyanate labeled 3-aminophenylboronic acid (FITC-APBA) as the chemoselective recognition probe in a liposome vesicle for cellular delivery (Figure 8A).[60] The recognition of FITC-APBA to sialylated TRITC-AF led to the generation of FRET signal, which reflected the ST activity (Figure 8B). This strategy could be applied to monitor the ST activity during drug treatment and discriminate tumor and normal cells. To detect intracellular Neus, they delivered a lysosomal Neu-specific substrate 4-methylumbelliferyl-*N*-acetylneuraminic acid into lysosomes, which could release the cleavage product of 4-methylumbelliferone into the cytosol for neutral pH-enhanced fluorescence detection of lysosomal Neu activity in living cells.[61] The designed strategy enabled the monitor of the upregulation of lysosomal Neu induced by sodium butyrate, and the dynamic tracking of the variation of lysosomal Neu activity in living cells during the apoptosis process and drug treatment.

GLYCANS IN VIVO

Different from in vivo labeling proteins by genetically fusing, glycans in vivo are difficult to label because they are not genetically encoded. Fortunately, the metabolic labeling can also be used in vivo. The metabolic labeling precursor can passively diffuse into the cells and be metabolically converted to the corresponding azido Sia and then incorporated into the

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sialylated glycans after being directly injected into the mice (Figure 9A),[62] which can be analyzed by further reacting with alkyne conjugated fluorescent dyes in isolated cardiomyocytes (Figure 9B), intact hearts (Figure 9C), and lysed hearts (Figure 9D). The selectivity of the metabolic labeling for specific tissue in vivo can be improved by encapsulating the precursor with ligand- targeted liposomes,[63] which can even break the blood−brain barrier to metabolically label glycans inside brain.[64] Besides, the metabolic labeling has also been applied to plants. Using *Arabidopsis thaliana* as the plant model,[65] *N*-azidoacetylglucosamine can be metabolized through the salvage pathway of GlcNAc and incorporated into N-linked glycans, and possibly intracellular O-GlcNAc. The synthesized azide-labeled N-linked glycans can be detected with alkyne conjugated fluorescent dyes by fluorescence imaging.

SUMMARY AND OUTLOOK

During the past 10 years, the analysis of cellular glycans has expanded from cell surface to intracellular regions, single saccharide type to multiple glycans, cell specificity to protein specificity, cell to tissue and in vivo. These progresses are mainly attributed to the novel labeling and detecting technologies, which contribute to the research of glycan-related biological processes, and further promote the development of diagnosis and treatment of cancer. However, the labeling efficiency and detection sensitivity of current methods need to be improved. Future developments should be focused on design of targeted biomimetic molecular probes with novel recognition mechanisms, such as derivatives of functional carbohydrates, analogues of glycan-related enzymatic substrate, to achieve time-space resolved analysis of cellular glycans. Besides, glycans in lipids and cellular organelles need more attention, such as mitochondria, Golgi apparatus, and nucleus. Meanwhile, it is necessary to develop new principles and methods with highly efficient recognition capability for in situ tracking of the dynamic and interactional processes of glycosylation, which is an urgent demand for the research of life science and clinical medicine.

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Notes

The authors declare no competing financial interest.

Biographies

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