



Effects and Regulation of Glycosylation Modifications on G Protein-coupled Receptor Function*

WU Fang-Ming^{1)**}, LING Sheng-Long²⁾, SHI Pan³⁾, SUN Yu^{1,4)}, TIAN Chang-Lin⁵⁾

⁽¹⁾High Magnetic Field Laboratory, Hefei Institutes of Physical Science, Chinese Academy of Sciences, Hefei 230031, China;

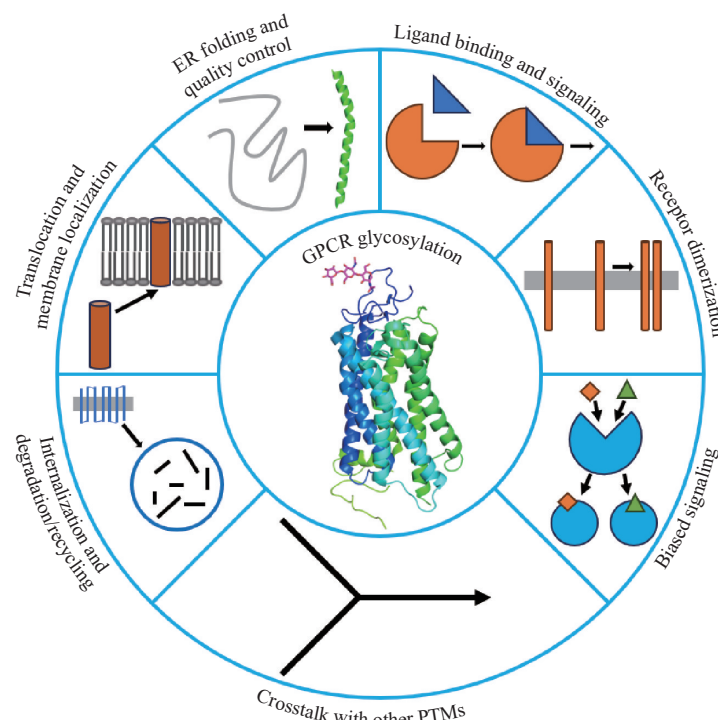
²⁾School of Biomedical Engineering, Suzhou Institute for Advanced Research, University of Science and Technology of China, Suzhou 215123, China;

³⁾Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei 230026, China;

⁴⁾School of Life Sciences and Medical Engineering, Anhui University, Hefei 230601, China;

⁵⁾Zhangjiang Institute for Advanced Studies, Shanghai Jiao Tong University, Shanghai 201203, China)

Graphical abstract



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** Corresponding author.

Tel: 86-13866138467, E-mail: fmwu@hmf.ac.cn

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Abstract Glycosylation constitutes a critical, highly intricate, and diverse co- and post-translational modification characterized by the covalent attachment of sugar moieties to proteins, lipids, or small RNAs. Complex glycans are ubiquitously present across nearly all living organisms. Despite their prevalence, our comprehension of glycan diversity remains limited, likely due to the inherent challenges associated with elucidating their structural complexity. Among post-translational modifications, protein glycosylation is notably prevalent, involving the enzymatic transfer of oligosaccharides to specific amino acid residues by glycosyltransferases. This modification plays a pivotal role in modulating protein function, including participation in various biological and biochemical recognition processes. G protein-coupled receptors (GPCRs), encoded by approximately one thousand genes, share a conserved architecture comprising seven transmembrane helices interconnected by three intracellular and three extracellular loops. Representing the largest family of human membrane proteins, GPCRs regulate a vast array of physiological and pathological processes, thereby constituting the most extensive class of therapeutic targets. In mammalian systems, the majority of GPCRs undergo glycosylation predominantly at their extracellular N-terminus or extracellular loops, primarily through N-linked and O-linked glycosylation. These covalent carbohydrate modifications exert profound effects on multiple facets of GPCR biology, including endoplasmic reticulum folding and quality control, membrane trafficking and surface expression, receptor internalization and recycling or degradation, ligand binding and signal transduction, biased signaling, and receptor dimerization. Notably, glycosylation can exert both positive and negative regulatory influences on these processes. Dysregulation of GPCR glycosylation has been implicated in a range of pathological conditions, such as cancer, diabetes, and neurological disorders, underscoring its physiological and pathological significance. Aberrant glycosylation patterns may lead to dysfunctional receptor signaling, thereby contributing to disease progression. Consequently, elucidating the precise roles of glycosylation in GPCR function not only enhances fundamental understanding of receptor biology but also facilitates the development of novel therapeutic strategies targeting glycosylation pathways or specific GPCR glycoforms. Although the functional consequences of glycosylation have been investigated for several decades, delineating their exact structural underpinnings has remained challenging due to the intrinsic flexibility and heterogeneity of carbohydrate structures. Recent advances in structural biology, particularly single-particle cryo-electron microscopy (cryo-EM) and X-ray crystallography, have catalyzed a paradigm shift in our understanding of GPCR glycosylation. The integration of these sophisticated structural techniques with biochemical approaches has redefined glycosylation as a complex structural element integral to receptor function. These developments provide a molecular framework for comprehending how glycans influence receptor pharmacology and open new avenues for the rational design of glyco-engineered biologics and allosteric modulators targeting specific extracellular motifs. This mini-review concentrates on glycosylation within the GPCR superfamily, summarizing the impact of glycosylation modifications on receptor function and regulatory mechanisms, recent progress in the structural biology of GPCR glycosylation, and prospective directions for future research in this field.

Key words glycosylation, protein modification, G-protein coupled receptor

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Protein glycosylation is an important co- and post-translational modification process of proteins involving the covalent linkage of sugar molecules to proteins^[1]. This modification not only increases the complexity and diversity of proteins but also plays a key role in protein functions, stability, localization, signal transduction, and other cellular processes. Glycosylated proteins (glycoproteins) are found in almost all living organisms, including eukaryotes, eubacteria, and archaea^[2]. Glycosylation mainly takes place in the endoplasmic reticulum (ER) and Golgi apparatus^[3], and can also occur in the nucleus, cytoplasm, and mitochondria^[4].

Depending on the glycopeptide chain, protein glycosylation mainly includes four different types: N-glycosylation to Asn, O-glycosylation to Ser, Thr, or Tyr, C-glycosylation to Trp, and glypiation (GPI-anchoring)^[3]. Among them, there are 11 types of O-glycosylation, as they can be linked to different sugars, including GalNAc, Fuc, GlcNAc, Man, Glc, Xyl, and Gal^[3]. N-glycosylation and GalNAc-type

O-glycosylation are the most well-studied^[3, 5]. N-glycosylation occurs in the ER in a co-translational manner, and connects a sugar moiety to the side-chain NH₂ group of Asn residue at the consensus Asn-X-Ser/Thr sequon, where X can be any amino acid except proline^[6]. Very recently, non-canonical N-glycan was identified on the Ala-Asn-Asp (AND) motif^[7]. N-linked glycan structures often contain a highly conserved oligosaccharide core structure (Glc₃Man₉GlcNAc₂)^[8] (Figure 1). N-glycosylation plays important roles in protein folding, stability, and ER quality control^[8]. GalNAc-type O-glycosylation is one of the most abundant and diverse protein modifications^[9]. It occurs in the Golgi apparatus after protein folding as well as in the nucleus and is initiated by the covalent linkage of a single monosaccharide (generally α -GalNAc residues) to the hydroxyl group of serine, threonine, and occasionally tyrosine residues (Figure 1), which is catalyzed by a polypeptide GalNAc-transferase^[3, 10]. O-glycans vary in size and structure, and are normally capped with terminal, negatively charged sialic acids^[11].

O-GalNAc glycans account for a significant proportion of the glycocalyx, and play important roles in hemostasis, the gastrointestinal system, the respiratory system, and host-microbe interactions^[12].

1 GPCR glycosylation

G protein-coupled receptors (GPCRs) constitute the largest family of cell surface proteins and play a crucial role in cellular signal transduction^[13-14]. GPCRs recognize signaling molecules outside the cell and translate these signals into intracellular responses^[15]. These signaling molecules include photons, ions, neurotransmitters, hormones, taste molecules, and others^[16]. The typical structure of a GPCR consists of seven transmembrane α -helices that span the cell membrane, forming three intracellular

and three extracellular loops^[17]. The C-terminus of a GPCR usually interacts to the β and γ subunits of the G protein, while the N-terminus is located extracellularly. GPCRs are classified into different families based on sequence similarity and function: the rhodopsin-like family (Class A), the secretin/adhesion family (Class B), the metabotropic family (Class C), the smoothened/frizzled family (Class F), and the taste2 family (Class T)^[17].

Post-translational modifications (PTMs) can take place at different regions of GPCRs including the N-terminus, extracellular loops (ECLs), intracellular loops (ICLs), and C-terminus, since these regions are much more accessible to corresponding enzymes than transmembrane domains^[18]. N- and O-linked glycosylation are prevalently present at the N-terminal and/or ECL domains of GPCRs (Figure 1)^[18].

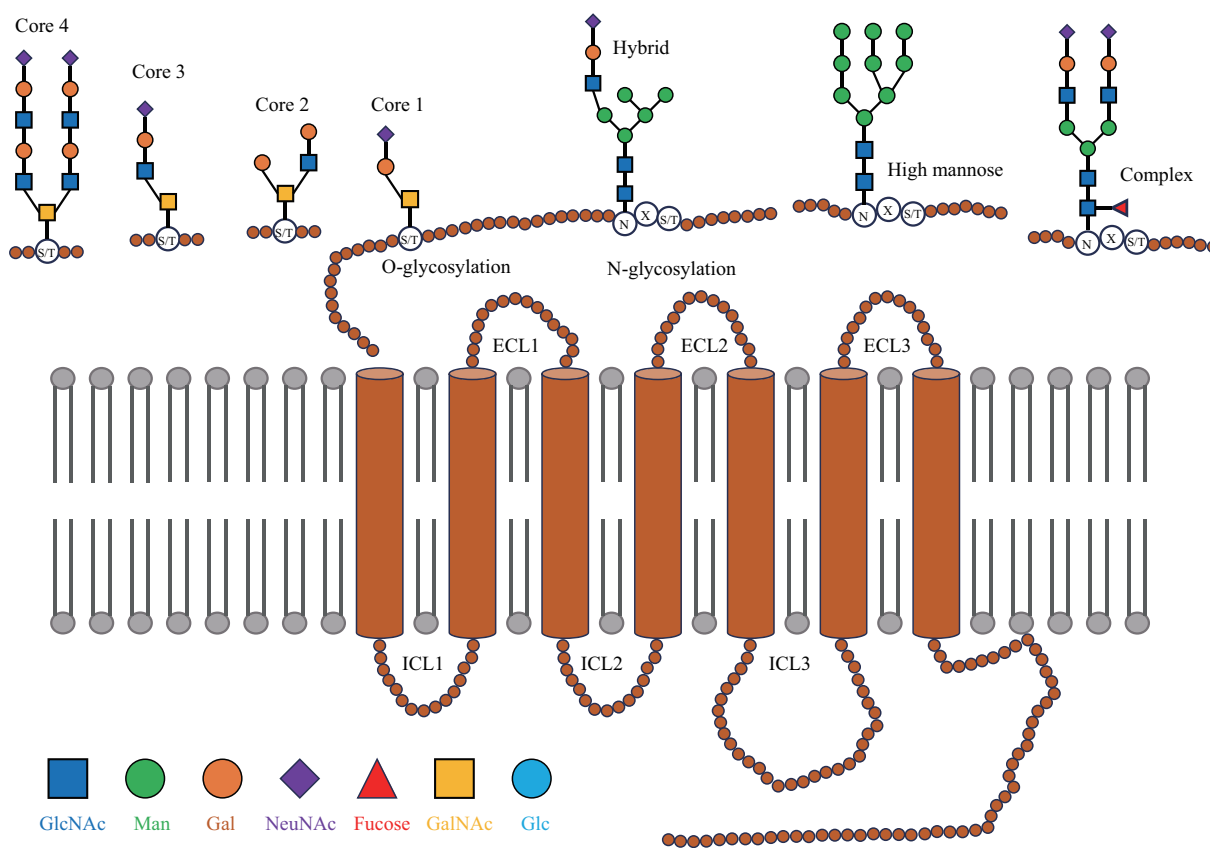


Fig. 1 N- and O-linked glycosylation of GPCR at the N-terminal and/or ECL domains

N-glycosylation is the major form of glycosylation found in GPCRs^[18]. The N-termini of the vast majority of mammalian GPCRs contain at least one N-X-S/T sequence^[11], while the presence of N-X-S/T sequence is much less in the ECL1 (14%), ECL2 (66%), and ECL3 (20%) regions^[19-20]. Zielinska

et al.^[21] identified 6 367 N-glycosites from 2 352 proteins in four mouse (C57BL/6) organs (brain, liver, kidney, and heart) and blood plasma using a “filter aided sample preparation” (FASP) -based method, while 180 N-glycosites can be mapped to 84 GPCRs. Later, the study of Liu *et al.*^[22] generated a dataset of

1 988 N-glycosites from 955 proteins in five mouse (C57BL/6) tissues (brain, heart, kidney, liver, and lung), wherein 51 N-glycosites can be mapped to 26 GPCRs. It was found that 8 GPCRs, such as metabotropic glutamate receptor 3 (mGluR3), G protein-coupled receptor 158 (Gpr158), and sphingosine 1-phosphate receptor 1 (S1PR1), showed dysregulated N-glycosylation occupancy on specific sites in the Alzheimer's disease (AD) mouse brain relative to the wild-type (WT) mouse brain^[23]. The N-glycosites of 28 GPCRs in mouse pancreatic β -cells or human islets were identified by Danzer and colleagues^[24].

In contrast to N-glycosylation, O-glycosylation is much less discovered in GPCRs, which is possibly due to the lack of a consensus sequence, enzymatic tools, and their highly complex and heterogeneous glycan structures^[25]. By blocking the elongation process, truncated and homogeneous O-linked glycopeptides were produced and analyzed by MS in 12 human cancer cell lines, and 35 O-glycosites were discovered from 14 GPCRs^[26]. Yang *et al.*^[27] developed a new method called extraction of O-linked glycopeptides (EXoO) to analyze human kidney

tissue, T cells, and serum, and they found 23 GPCRs with 39 O-glycosites. They also identified aberrant expression of O-linked glycoproteins in kidney tumor tissue compared to normal tissue^[27]. Among the 56 identified O-linked glycoproteins that exhibit significant change between normal and tumor samples, only one of them is a GPCR (EGF-like module-containing mucin-like hormone receptor-like 2)^[27].

2 The roles of glycosylation in the GPCR life cycle

Biological functions of glycans can roughly be divided into three categories: structural contributions, energy metabolism, and information carriers^[10]. The roles of glycosylation in regulating GPCR biology are expansively studied, and it has been revealed to contribute to the GPCR life cycle (including ER folding and quality control, translocation and cell surface localization, internalization and degradation/recycling) and GPCR signaling (including ligand binding and signaling, biased signaling, and receptor dimerization) (Figure 2).

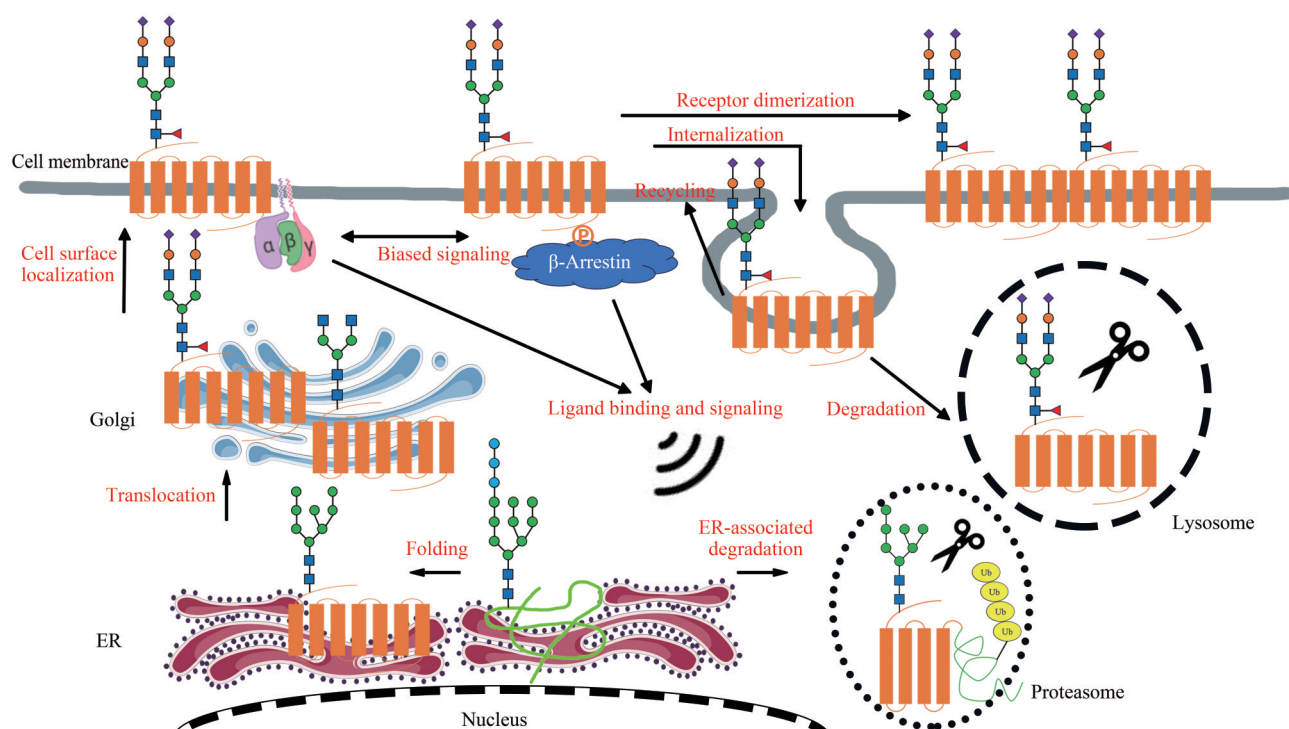


Fig. 2 The roles of glycosylation in the GPCR life cycle

2.1 ER folding and quality control

N-glycan, and its ability to interact with the lectin-based chaperone system, play a crucial role in

facilitating the proper folding and quality control of glycoproteins in the ER, a process known as the "calnexin/calreticulin cycle"^[28-29]. Several studies

have shown that correct glycosylation is necessary for GPCR folding and its quality control^[11]. In follicle-stimulating hormone receptor (FSHR), a glycan moiety at either N174 or N276 is required for the efficient folding of the nascent receptor protein into a conformation that allows high-affinity binding of ligand^[30]. Moreover, glycosylation at N58 and N69 of the vasoactive intestinal peptide-1 receptor (VIPR1) is involved in calnexin-dependent and -independent mechanisms of protein folding and quality control, respectively^[31]. N336 is likely the site of an essential glycan modification that contributes to *Drosophila* Smo (protein smoothened) folding and ER exit, and the N-linked glycan on N213 can partially compensate for its loss^[32]. On the other hand, loss of glycosylation does not negatively impact mouse Smo folding^[32]. Loss of glycosylation at N15 but not N2 of rhodopsin caused poor folding^[33]. In the human thyrotropin receptor (THSR), glycosylation of at least four out of six sites appears necessary for its folding^[34-35]. The modified carbohydrate is important in the correct folding or stabilization of the vasopressin v1a receptor (V1aR)^[36]. It was shown that non-glycosylated rhodopsin is unstable and is regulated *via* ubiquitin-mediated proteasomal degradation^[37].

ER chaperone proteins play crucial roles in the folding and quality control processes of the GPCR life cycle^[38]. Calnexin and calreticulin bind to properly glycosylated and processed GPCRs in the ER, preventing their aggregation or degradation^[39]. Properly folded GPCRs then exit the ER, while misfolded GPCRs re-enter the quality control cycle or are targeted for ER-associated degradation^[40].

2.2 Translocation and membrane localization

It has been observed that N-glycans influence the transport and sorting of glycoproteins within the Golgi apparatus^[28]. In the case of GPCR, most require at least one N-glycan for efficient translocation to the cell surface. Examples include FSHR^[30], VIPR1^[31], dSmo (but not mSmo)^[32], THSR^[34-35], thyrotropin receptor^[41], Gastrin-releasing peptide receptor (GRP-R)^[42], dopamine D5 receptor (DRD5)^[43], angiotensin II type-1 receptor (AT1 receptor)^[44], bradykinin B2 receptor (B2R)^[45], hamster beta-2 adrenergic receptor (β_2 AR)^[46], calcium receptor (CaR)^[47], protease-activated receptor 1 (PAR-1)^[48], glucose-dependent insulinotropic polypeptide receptor (GIPR) and glucagon-like peptide 1 receptor (GLP-1R)^[49], prostaglandin E₂ receptor EP3 subtype^[50], melanocortin receptor 1 (MC1R)^[51], G protein-coupled receptor 176 (Gpr176)^[52], P2Y purinoceptor 2 (P2Y₂R)^[53], α_{1D} -adrenergic receptor (α_{1D} AR)^[54],

prokineticin receptor 2 (PKR2)^[55], G protein-coupled receptor family C group 6 member A (GPC6A)^[56], Opsin-3 (OPN3)^[57], dopamine D2 and D3 receptors (D2R and D3R)^[58], and among others. Similarly, O-glycosylation of the N-terminal domain of human δ OR (δ -opioid receptor) (at residues S6, S25, and S29) has been shown to modulate receptor cell surface expression^[59]. However, exceptions do exist. For instance, N-glycosylation is not essential for effective plasma membrane expression of rhodopsin^[33], V1aR^[36], dopamine D1 receptor (DRD1)^[43], muscarinic acetylcholine receptor M2 (M2 mAChR)^[60], canine histamine H2 receptor (H2R)^[61], orphan G protein-coupled receptor 61 (GPR61)^[62], vasopressin V2 receptor (V2R)^[63], human parathyroid hormone/parathyroid hormone-related peptide receptor (PTH/PTHrP receptor)^[64], pyroglutamylated RF-amide peptide receptor (QRFPR)^[65], and human β_2 AR^[66]. Conversely, the succinate receptor 1 (SUCNR1) predominantly resides in the ER, a localization dependent on N-glycosylation at position N8; the nonglycosylated mutant receptor, however, localizes to the plasma membrane^[67].

Today, our knowledge about GPCR trafficking from the ER to the plasma membrane, known as anterograde trafficking, remains insufficient^[38, 68]. This process is regulated and mediated by multiple pathways, in a cell type- and receptor-specific manner^[68]. A number of factors are involved in this process, other than modified glycans. These factors can be divided into three groups: general chaperone system (such as calnexin, calreticulin, and BiP), receptor-specific proteins (such as DRiP78, Homer proteins, and NinaA), and several conserved motifs (such as E(X)₃LL, F/Y(X)₃F(X)₃F, FN(X)₂LL(X)₃L, F(X)₆LL, di-acidic, and di-basic motifs) in the C-terminal and N-terminal regions of GPCRs^[38, 40, 68]. The experimental approach used in the aforementioned observation typically involves site-directed mutagenesis or incubation of cells with tunicamycin. It is possible that some of the reported observations are actually the result of compensatory adaptations rather than a direct consequence of the absence of glycan chains.

2.3 Internalization and degradation/recycling

Furthermore, glycosylation also plays a role in GPCR internalization of D2R and D3R *via* clathrin-mediated and caveolae-dependent pathways^[58]. Similarly, in C-C chemokine receptor type 7 (CCR7), the N-terminal glycans but not the ECL3 one, are contributing to receptor internalization^[69]. The N-glycosylation of S1PR1 is required for both caveolae association and agonist-induced

internalization^[70]. Activated proteinase-activated receptor 1 (PAR1) internalization was impaired in the mutant lacking glycosylation at ECL2, while the constitutive internalization of inactivated PAR1 was unaffected^[48]. Receptor internalization of β_2 AR was severely impaired by mutants N6Q, N15Q, and N6Q/N15Q, while the impact of N187Q was negligible^[66]. In contrast, N-glycosylation of neurokinin 1 receptor (NK1R) might reduce the internalization^[71].

Glycosylation regulates GPCR internalization by enhancing the interaction between the glycosylated GPCR and clathrin or caveolae^[72].

3 The roles of glycosylation in GPCR signaling

3.1 Ligand binding and signaling

Glycosylation also plays important roles in ligand binding and signaling of GPCR proteins. Perturbation of N-glycosylation of GPCRs may cause severe deficits in ligand binding and/or signaling, as demonstrated by studies on rhodopsin^[33], the thyrotropin receptor^[41], PKR2^[55], QRFP^[65], luteinizing hormone receptor (LHR)^[73], prostaglandin D₂ and E₂ receptors^[74], somatostatin receptor subtype 3^[75], C-X-C chemokine receptor type 4 (CXCR4)^[76], G protein-coupled receptor 30 (GPR30)^[77], and FSHR^[78]. In the case of the calcitonin receptor (CTR), N-glycosylation enhances ligand affinity by altering receptor dynamics^[79-80]. Moreover, β_2 AR signaling is triggered by direct mechanical traction forces *via* two N-glycans during meningococcal infection^[81]. The mechanosensitive response of β_2 AR is affected by site-directed mutagenesis of extracellular N-glycosylation sites in the N-terminus and second extracellular loop^[82]. In contrast, loss of N-glycosylation of PAR1 and CCR7 causes a marked increase in signaling^[48, 69], possibly because glycosylation hinders ligand access or stabilization of the active conformation. In the N-terminal fragment of adhesion G protein-coupled receptor G6 (ADGRG6), N-glycosylation in the CUB, SEA, and HrmR domains regulates basal cAMP signaling, whereas N-glycosylation in the PTX and GAIN domains is necessary for trafficking^[83]. Furthermore, *Drosophila* Smo signaling is impaired by loss of N-linked glycosylation at N336 and is further compromised by introduction of a second mutation at N213^[32]. N-glycosylation of mouse Smo is dispensable for ligand binding and canonical signaling to Gli transcriptional effectors but is essential for its ability to signal through G α i^[32].

O-glycosylation of the prostaglandin D₂ and E₂

receptors is also important for high-affinity ligand-binding activity^[74]. In the C-C chemokine receptor type 5 (CCR5), the O-linked glycans—particularly sialic acid moieties—together with tyrosine sulfates significantly enhance chemokine ligand binding^[84]. Studies show that O-glycosylation co-regulates beta-1 adrenergic receptor (β_1 AR) proteolytic cleavage and downstream signaling^[85-87]. Disruption of O-glycosylation—or simply removal of capped sialic acids from the O-glycans of G protein-coupled receptor 15 (GPR15)—enhances its binding to protein GPR15 ligand (GPR15L)^[88].

Polysialylation of N- and/or O-linked glycans on CCR7 is essential for recognition and binding of the CCR7 ligand CCL21^[89]. Furthermore, unusual glycosylations—such as C-mannosylation and O-fucosylation of brain-specific angiogenesis inhibitor (BAI)—directly participate in its binding to the Reticulon-4 (RTN4) receptor and contribute to subsequent synaptic circuit development^[90].

3.2 Receptor dimerization

In many cases, GPCRs form homodimers or heterodimers to modulate receptor function^[91]. PTMs such as glycosylation likely affect GPCR dimerization. Glycosylation-deficient mutants of β_1 AR and β_2 AR both show decreased homodimerization^[66, 92]. N-glycans have also been reported to negatively regulate heterodimerization between β_1 AR and α_{2A} -adrenergic receptors (α_{2A} -AR)^[93] as well as between gamma-aminobutyric acid type B (GABA_B) receptor subunits 1 and 2^[94]. Similarly, sialylation and N-glycosylation of human bradykinin B2 receptor participate in surface receptor dimerization^[95]. Agwuegbo *et al.*^[96] reported that differential FSH glycosylation modulates FSHR oligomerization. In 2001, Max *et al.*^[97] predicted that N-glycosylation of N58 in the mouse taste receptor taste receptor type 1 member 3 (T1R3) would preclude formation of receptor homodimers or heterodimers. However, this prediction remains unconfirmed as of 2026. In contrast, loss of N-linked glycosylation does not affect mSmo dimerization^[32].

3.3 Biased signaling

Glycosylation plays an important role in biased signaling by tuning receptor conformation and ligand-binding affinity, thereby influencing biased signaling outcomes^[98]. ECL2-glycosylated PAR1 preferentially activates G_{12/13}-dependent signaling, whereas its glycosylation-deficient mutant favors G_q signaling^[99]. Meanwhile, glycosylated and non-glycosylated PAR1 show equal efficacy in coupling to G_i signaling and β -arrestin recruitment^[99]. Similarly, wild-type mSmo signals to Gli transcriptional effectors in a G protein-

independent manner as well as a $G_{\alpha i}$ -dependent manner, whereas unglycosylated Smo fails to induce signaling through $G_{\alpha i}$ ^[32]. O-glycosylation of β_1 AR disrupts the balance between G_s -mediated and extracellular regulated protein kinases (ERK) signaling, favoring G_s -mediated signaling^[100]. More recently, it was discovered that N-glycosylation at the N-terminus (N2, N13, and N20) confers constitutive high G_s signaling activity and low β -arrestin recruitment activity in G protein-coupled receptor 52 (GPR52)^[101]. Interestingly, differential glycosylation of ligands such as FSH affects receptor signaling bias^[102].

3.4 Crosstalk with other PTMs

GPCRs undergo various types of PTMs at different times and locations. Different PTMs can influence each other in a positive or negative way^[11]. For example, polysialylation and tyrosine sulfation of CCR7 are both critical for CCL21 ligand binding: polysialic acid relieves the autoinhibition conformation of CCL21, while sulfated tyrosine increases receptor-ligand affinity^[89]. We hypothesize that these two modifications act synergistically to enhance CCR7 ligand binding and subsequent signaling—though experimental confirmation or refutation is still needed. The S1PR1 can be sulfated and O-glycosylated on the same Y22 residue^[103-104]. Loss of S1PR1 sulfation impairs ligand binding and downstream signaling, whereas the functional role of its O-glycan(s) remains unclear. In GPR15, O-glycosylation and tyrosine sulfation oppositely regulate ligand binding: sulfation enhances, whereas O-glycosylation inhibits, GPR15L binding^[88]. In GPR52, N-terminal N-glycosylation promotes constitutive G_s signaling, while phosphorylation in helix 8 suppresses β -arrestin recruitment and attenuates receptor internalization^[101].

Despite these findings, current knowledge about crosstalk between GPCR glycosylation and other PTMs remains limited. Further in-depth investigation is warranted to expand understanding in this field.

Based on the above, we conclude that glycosylation participates in regulating GPCR folding and stability, cell surface localization, internalization, ligand binding and signaling, biased signaling, and receptor dimerization (Table S1). Both positive and negative regulation exist in these processes.

4 Structural studies of GPCR glycosylation

It was estimated that approximately 35% of Food and Drug Administration (FDA)-approved drugs target GPCRs^[105]. Protein glycosylation abnormalities

lead to various diseases, and glycosylation could be a potential therapeutic target^[8]. Therefore, resolving the 3D structures of disease-implicated glycans is important and necessary. In the last decade, technological advances — especially electron microscopy (cryo-EM) — have enabled the determination of over 700 structures of GPCRs and their complexes (data from gpcrdb.org), facilitating the elucidation of molecular mechanisms underlying numerous GPCR-related biological processes. Nevertheless, most PTM moieties—especially large glycans—are typically invisible in high-resolution structures. For example, in the cryo-EM structure of the calcium-sensing receptor (CaSR) solved by our group, N-linked glycans are observed in six different residues (Figure 3a), yet the most well-defined glycan contains only a trisaccharide, indicating that most of the glycan mass remains unresolved^[106]. Similarly, in the crystal structure of the extracellular domains of G protein-coupled receptor 110 (GPR110), six linked carbohydrate units were clearly identified at residue N389 (Figure 3b)^[107]. Although more monosaccharide units were resolved than in the CaSR case, the glycan structure is still likely incomplete, consistent with the known complexity of full protein glycosylation. Similar limitations are also observed in other GPCR structural studies, such as those of the human sweet taste receptor^[108], the medaka fish taste receptor^[109], the fungal GPCR Ste2^[110], and metabotropic glutamate receptor 3^[111].

5 Summary and future perspectives

From the above-mentioned information, we can see that GPCR glycosylation studies have mainly occurred in the fields of biochemistry and functional biology. Most of these studies focused on elucidating the function of specific glycosylation site(s) in a particular GPCR overexpressed in certain cell lines. Meanwhile, structural studies of GPCR glycosylation remain insufficient. Although we can map glycan attachment sites, profile glycan structural variations, and determine detailed fine structures of glycans—when glycans are removed from the glycoprotein or attached to a short peptide other than the full-length protein—their native context is lost. With the continuous development of cryo-EM, we are now able to obtain atomic-resolution images of glyco-GPCRs with glycans still attached. Nevertheless, substantial information about the glycans is missing in these cryo-EM images, as evident from the studies cited above^[106-111]. This is very likely hindered by the high flexibility of both the glycosylation sites and glycan

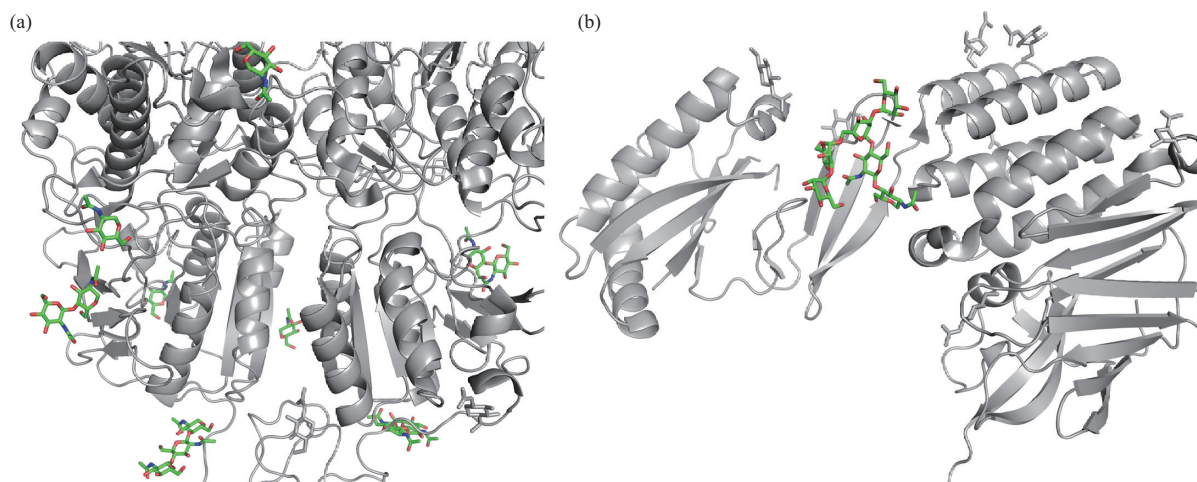


Fig. 3 The N-linked glycans found in the cryo-EM structure of CaSR (a) and crystal structure of extracellular domains of GPR110 (b)

Glycans are shown in colored sticks, while the rest of the protein is shown in grey.

molecules themselves. Recently, Nieng Yan's group developed a strategy called CryoSeek and successfully resolved several glycan-protein complexes^[7, 112-114]. It seems they are currently focusing on glycofibrils, possibly due to its high symmetry and relatively low flexibility. We hope this strategy will continue to evolve and eventually be applicable to the analysis of typical glycosylated GPCRs. On the other hand, nuclear magnetic resonance (NMR) is a powerful tool for studying the conformations and dynamics of intrinsically flexible saccharides. A variety of NMR strategies—as well as other technical tools such as high resolution mass spectrometry, liquid chromatography, X-ray crystallography, and molecular dynamic simulation—can be employed combinatorically to investigate glycan geometry and dynamics of glycoproteins or glycopeptides, and their interactions with receptors^[115-117]. Hopefully, with the advent of new methods and technologies, we will be able to “see” this previously invisible component more clearly in the near future, thereby advancing the structural study of GPCR glycosylation.

Alterations in glycosylation patterns are increasingly recognized as fundamental drivers of tumorigenesis, and these abnormal glycosylation signatures serve as valuable diagnostic markers and potential therapeutic targets for various cancers^[118]. Dysregulation of GPCR signaling contributes to obesity, diabetes, depression, Alzheimer's disease and multiple types of cancer^[119-121], and glycosylation plays important roles in GPCR signaling.

Nevertheless, direct descriptions linking GPCR glycosylation to disease remain scarce in the literature. Therefore, we may aim to identify disease-specific GPCR glycosylation patterns through glycoproteomics or other approaches in the near future. A new method called DEED-GL-Seq was recently developed for glycosidic bond linkage determination, and successfully identified four novel oligosaccharide biomarkers in the urine of patients with glycogen storage disease type II^[122]. This would provide entirely new biomarker tools for early diagnosis, prognosis assessment, and patient stratification. With future advances in cryo-EM and artificial intelligence-based modeling, it will become possible to achieve atomic-resolution structural insights into glyco-GPCRs, thereby providing strong support for the precise design of GPCR-targeted therapeutics.

Supplementary Available at paper online (<http://www.pibb.ac.cn>, <http://www.cnki.net>): PIBB_20260124_Table_S1.pdf

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糖基化修饰对G蛋白偶联受体功能的影响和调控*

吴芳明^{1)**} 凌盛龙²⁾ 石攀³⁾ 孙玉^{1,4)} 田长麟⁵⁾

⁽¹⁾ 中国科学院合肥物质科学研究院强磁场科学中心, 合肥 230031;

⁽²⁾ 中国科学技术大学苏州高等研究院生物医学工程学院, 苏州 215123;

⁽³⁾ 中国科学技术大学生命科学与医学部, 合肥 230026;

⁽⁴⁾ 安徽大学生命科学与医学工程学院, 合肥 230601; ⁽⁵⁾ 上海交通大学张江高等研究院, 上海 201203)

摘要 糖基化是一种关键的翻译中和翻译后修饰, 通过共价键将糖分子连接到蛋白质、脂质或小RNA上, 形式高度复杂且多样。蛋白质糖基化是最普遍的修饰之一, 参与调控蛋白质功能。G蛋白偶联受体 (GPCR) 作为人体最大的膜蛋白家族, 几乎调控所有生理及病理过程, 因而也是目前药物研发中占比最高的靶点类别。绝大多数哺乳动物GPCR会发生糖基化修饰, 并深度参与其多个生物学环节: 内质网中的折叠与质量控制、向细胞膜的转运与定位、内吞及降解/再循环、配体结合与信号转导、偏向性信号传导, 以及受体二聚化。其中既有正向调控, 也有负向调控。近年来, 单颗粒冷冻电镜 (cryo-EM) 与X射线晶体学等结构生物学技术的飞跃, 正在重塑对GPCR糖基化的认知。如今, 高分辨结构揭示了糖链如何从原子层面影响受体的药理学特性。本综述聚焦GPCR超家族中的糖基化现象, 系统梳理其对受体功能的影响与调控机制, 并评述结构生物学领域的最新进展及未来发展方向。

关键词 糖基化, 蛋白质修饰, G蛋白偶联受体

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** 通讯联系人。

Tel: 13866138467, E-mail: fmwu@hmfl.ac.cn

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