REVIEW

Glycopolymer-Based Materials: Synthesis, Properties, and Biosensing Applications

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Abstract

Glycopolymer materials have emerged as a significant biopolymer class that has piqued the scientific community's attention due to their potential applications. Recently, they have been found to be a unique synthetic biomaterial; glycopolymer materials have also been used for various applications, including direct therapeutic methods, medical adhesives, drug/gene delivery systems, and biosensor applications. Therefore, for the next stage of biomaterial research, it is essential to understand current breakthroughs in glycopolymer-based materials research. This review discusses the most widely utilized synthetic methodologies for glycopolymer-based materials, their properties based on structure–function interactions, and the significance of these materials in biosensing applications, among other topics. When creating glycopolymer materials, contemporary polymerization methods allow precise control over molecular weight, molecular weight distribution, chemical activity, and polymer architecture. This review concludes with a discussion of the challenges and complexities of glycopolymer-based biosensors, in addition to their potential applications in the future.

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Graphical Abstract
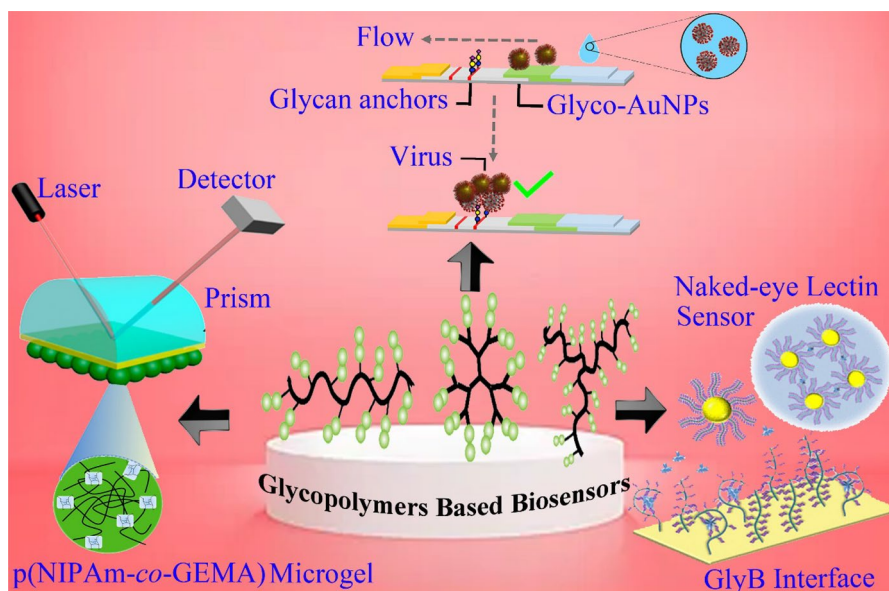
1 Introduction

Recently, several researchers have demonstrated considerable interest in using many synthetic polymers in diverse applications [1–5]. Synthetic polymers can look like natural polymers, and natural polymers can be designed to appear like synthetic polymers [3–5]. Polymer structures can now be more precisely controlled because of breakthroughs in newly found technologies, allowing researchers to develop custom multifunctional materials with exact topologies [6, 7]. Innovative functional materials with biological activity beyond natural polymers and bioinspired linear polymers with features comparable to natural products are now available in the early research stages. Although synthetic polymers have been used in a wide range of commercial and medical applications, it is expected that the next generation of functional materials, which combine the best properties of synthetic and natural systems, will discover a wealth of exciting applications such as drug delivery systems [8], tissue engineering [9], biosensing [10], and flexible energy storage devices [11]. Glycopolymers, which feature carbohydrates as pendent groups, are the most common synthetic polymers [12–15]. Carbohydrate-functionalized synthetic polymers can have a central sugar unit or insert sugar units inside polymer chains or the main chain. Glycopolymers have pendant sugar groups, such as poly(vinyl saccharide), as shown

in Fig. 1a [12, 16]. While synthetic polysaccharides mimic natural polysaccharides, glycopolymers are better mimics of the real thing [17–20]. Natural saccharides (such as carbohydrates) are particularly interesting as they are considered biomass, food, and raw material. It has recently been discovered that saccharides and their derivatives have various biological functions. Surfactants and cosmetics are only two examples of items made from saccharides that have been chemically altered [21].

Glycopolymers are natural polymers that have undergone chemical modification and are grafted onto synthetic polymers [23]. Each biological functional group is represented by a sugar moiety, analogous to the sugar moieties found in naturally occurring glycoconjugates. This means that sugar units are present as a pendant or terminal group in glycopolymers regardless of whether they are physiologically active. To this end, it is critical from the standpoint of polymer materials science that simple and acceptable synthetic methodologies for incorporating essential moieties of sugar, which are required for molecular recognition, into polymers are developed without compromising the fundamental properties of the polymers. The development of many glycopolymers with a focus on surface modification has allowed researchers to explore the effect of sugar presentation and its physicochemical characteristics on the evolution of sugar polymers over time. It has been possible to manufacture glycopolymers in various topologies [22], including liner glycopolymers, star-shaped glycopolymers, and brush glycopolymers, as illustrated in Fig. 1b.

Kobayashi et al. produced the first lactose-containing glycopolymer in the 1980s, while the Whitesides team researched sialic acid-containing glycopolymers and multivalent interactions in the 1990s [24]. Radical polymerization is the
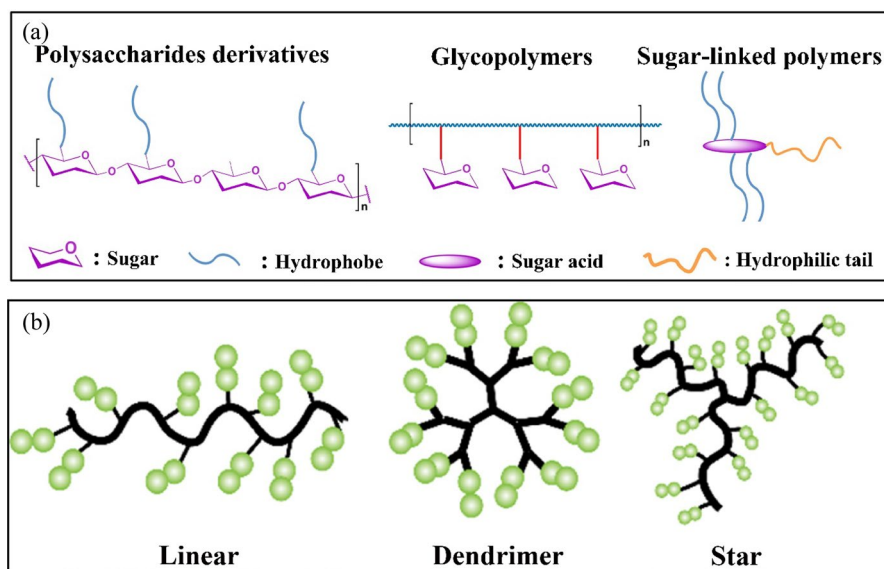

Fig. 1 Schematic structures of **a** sugar-based polymeric delivery systems (reproduced with permission from Ref. [16]) and **b** glycopolymers with linear, dendrimer, and star-shaped polymer structures (reproduced with permission from Ref. [22])

most common method in synthesizing glycopolymers because the radical reaction is orthogonal to the hydroxyl group on the saccharide side chain. However, protecting hydroxyl groups was often necessary to avoid side reactions. Other glycopolymers have been synthesized using a polymer reaction involving polymers with functional groups such as poly(*L*-glutamic acid) and carboxylic acid (alginic acid) [25]. The synthesized polymers displayed polydispersity with sufficient multivalent effects to serve as biomaterials [26]. The most noteworthy property of glycopolymers is their ability to interact with lectins and particular cells in various ways, depending on their sugar moieties [27]. The kind of sugar moiety, the linkage position, the density of sugars, and the degree of polymerization and branching influence the intensity of interactions [28]. Cell surface saccharides, such as glycolipids, glycoproteins, and glycans, are connected to various biological activities, including cellular contacts, adhesion, cancer cell metastasis, and pathogen infection, all mediated by protein-saccharide interactions [29]. Because the protein-saccharide interaction is weak, the multivalent impact of clustered saccharides, known as the glycocluster effect [27], can improve it. Because of their biorecognition characteristics, glycopolymers are promising materials for future biomedical applications such as drug or gene delivery and biosensing.

Rapid advancements in polymerization processes have enabled the production of macromolecules with precisely controlled structures that precisely correlate the functions of glycopolymers with their structures at the molecular level and provide glycopolymers with more complex functions that can outperform those of natural glycoconjugates in terms of functionality and complexity. Several significant review papers on glycoconjugates, such as glycopolymers, glycodendrimers, and glycoclusters, have been published [22, 30–32]. Compared to other published reviews, this review focuses on recent advances by broadening the literature studies by investigating glycopolymer properties based on structure–function interactions that require their immobilization onto surfaces, emphasizing standard techniques for their synthesis, and focusing on their applications in the biosensing field.

2 Glycopolymer Synthesis Methodologies

Following prior reports [30, 32, 33], many glycopolymers have been synthesized using many possible synthetic approaches. Despite this, sugar-containing monomer polymerizations and chemical modification of premade polymers using sugar-containing reagents [27] are the two most frequently used synthetic techniques for their fabrication. Because the synthesis of sugary monomers is sometimes a time-consuming operation comprising several stages, the alternative methodology is both simpler and more convenient than the traditional method. When steric hindrance occurs, irregular glycopolymer structures are formed instead of steric facilitation. The typical polymerization processes used in synthesizing glycopolymers are addressed in detail in this part of the review.

2.1 Polymerizations of Sugar-Containing Monomers

Researchers have created various glycopolymers using vinyl monomers that include sugar as building blocks. Living polymerization is defined as a method that does not involve the termination or transfer of chain segments during the polymerization process [34]. Controlled polymerization, on the other hand, refers to those in which the termination of the polymer is hindered but not entirely eliminated by the introduction of a dormant species into the system [35]. The main differences between living and controlled polymerases are that the first type has an uncontrolled molecular weight, and the second type has a well-defined structure, controlled molecular weight, and a low possible polydispersity [36].

The aim of glycopolymer chemistry is to develop an efficient technique that incorporates both the durability of radical polymerization and the control and accuracy of living polymerization. While living polymerization allows for more accurate molecular structures and significant control over polymers, it is less adaptable than radical polymerization since it is so intolerant of functionality and contaminants. Today, controlled/living radical polymerization provides control nearly as excellent as living anionic polymerization while maintaining the robustness and flexibility of a free radical process in terms of tolerance and adaptability. However, despite controlled/living polymerization techniques successfully employed for glycopolymer synthesis, their molecular weights and molecular weight distributions were not completely managed [16].

Several similar approaches, including cationic, anionic, and controlled radical phases, have been developed [37]. Overall, controlled polymerization is the most feasible and extensively utilized technique in polymerization. So, the first two preparative techniques will be briefly discussed before moving on to the most common and versatile ways of controlling radical polymerization.

2.1.1 Anionic and Cationic Polymerization

Anionic polymerization (Fig. 2a) is regarded as particularly desirable due to the inherent character of the process, which prevents chain termination from occurring. In addition to providing low dispersity, macromolecules with exact control over their molecular weight and chain-end fidelity are synthesized using this approach [38]. However, due to constraints including the need for aprotic solvents, the difficulty of polymerizing monomers with unprotected acid or electrophilic functionality, and the technology's vulnerability to oxygen and contaminants, the use of an alternate approach has been suggested. While cationic polymerization (Fig. 2a) offers performance characteristics equivalent to other methods, it has the drawback of being more prone to side reactions. Several approaches have been devised to allow the controlled/living polymerization of functional monomers [33, 39], particularly via the insertion of protecting groups, as seen in Fig. 2b.

Loykulnant et al. were the first to successfully apply the anionic polymerization strategy to manufacture glycopolymers [40, 41], which extended their previous work on the anionic polymerization monomers containing protected functional groups. The synthesis of six styrene derivatives (Fig. 3), including meta
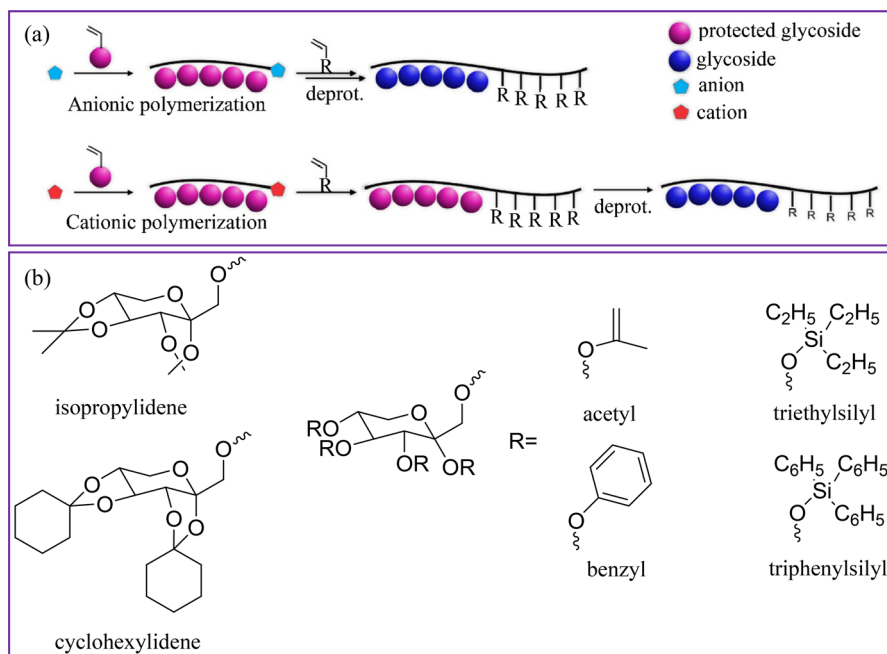
Fig. 2 a Anionic and cationic polymerization strategies for synthesizing block-type glycopolymers [33]. **b** Protective groups are frequently added to saccharides to prevent side reactions and/or tailor their solubility [33]

(m)-substituted with acetal-protected glucofuranose (**1**) and (**2**), galactopyranose (**3**), fructopyranose (**4**), sorbofuranose (**5**), and para (p)-substituted with acetal-protected glucofuranose (**6**), was accomplished through Williamson reactions of m- or p-(chloromethyl) styrene with the corresponding protected monosaccharides in DMF, followed by polymerization with *sec*-BuLi at −78 °C.

Chain transfer events are often seen when polymerizing vinyl ethers and styrenes, making it challenging to form polymers with regulated molecular weights. The developing chain-end was stabilized using hydrogen iodide/iodine (HI/I$_2$) or hydrogen iodide/weak Lewis acid initiator systems [42]. A varied spectrum of dispersed macromolecules was produced by Minoda et al. via the consecutive cationic polymerization of vinyl ether-functionalized glycomonomers followed by the polymerization of isobutyl vinyl ether [43]. Thin-film deposition of amphiphilic block copolymers results in microphase separation into spheres, cylinders, and lamellae, depending on the composition and molecular weight of the block copolymers. Motoyanagi et al. [44] have described an innovative technique for producing glycopolymers with a polymerizable end-group that combines cationic polymerization and the click reaction. According to Fig. 4, they successfully polymerized cationically of alkynyl-functionalized vinyl ether to prepare glycopolymer-type macromonomers and investigate their dispersion copolymerization with styrene obtain carbohydrate-decorated polymer particles using this strategy.
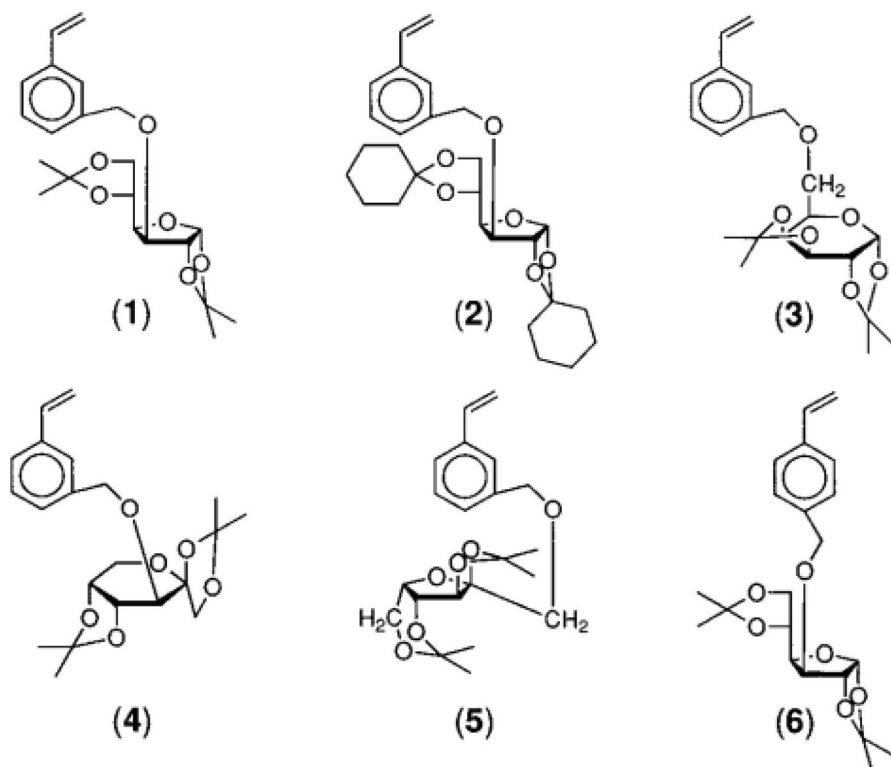
Fig. 3 Styrene derivatives with a sugar moiety that has been protected by acetal (reproduced with permission from Ref. [40])

2.1.2 Radical Polymerization

Radical polymerization is used to create well-defined polymeric materials [45]. It has the benefit of inherent robustness, it does not require high monomer purity, and it is tolerant of a wide range of reaction conditions and monomer functionality. As a result of the widespread commercialization of radical polymerization, initiators are very affordable. One of the downsides of the current technology is that it is difficult to regulate the molecular weight of the final polymer without the use of a large number of potentially hazardous initiators and chain transfer agents, which is one of the limitations of the technology. Some of the methods for achieving radical polymerization include stable free-radical polymerization (SFRP), atom transfer radical polymerization (ATRP), and reversible addition-fragmentation chain transfer (RAFT) [46]. As a result of their compatibility with most vinyl monomers and the relatively mild polymerization conditions under which they may occur, ATRP and RAFT are the most extensively utilized procedures in this application. The glycopolymers were synthesized using free radical initiation, in which they generated a variety of polymers from unprotected vinylic monosaccharides [47].
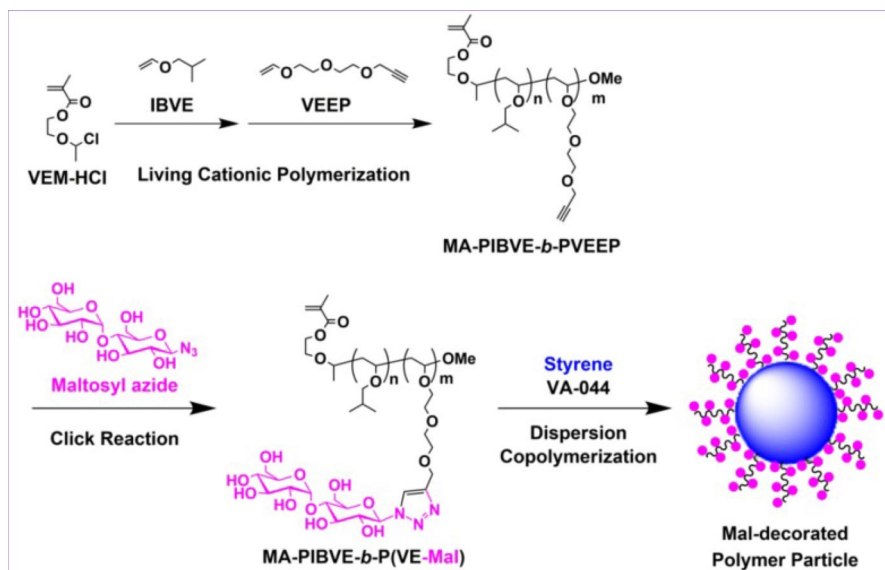
Fig. 4 Synthesis of glycopolymer-type amphiphilic macromonomers and their application to prepare maltose-decorated polymer particles by dispersion polymerization [44]

It is possible to safeguard radical propagating in the SFRP by using a stable free radical such as 2,2,6,6-trimethyl-1-piperidinyloxy in the nitroxide-mediated radical polymerization [48] which is reversibly protective of radical propagating. This is accomplished by the reversible covering of propagating radicals with a stoichiometric quantity of stable free radicals, allowing precise control over the molecular weights and molecular weight distributions. The SFRP approach [49] is shown in Fig. 5a. A weak link in a covalent species breaks down thermally and homiletically, releasing free radicals that reversibly produce a growing radical and a less reactive radical. Although this technique relies on maintaining a dynamic balance between low concentrations of active propagating chains and a majority of dormant chains that cannot multiply or terminate, it has the additional benefit of extending the life of the propagating chains. The equilibrium is pushed to the left side (deactivated, k_{deact}), resulting in more dormant species due to the persistent radical effect. Grande and colleagues [50] employed cyanoxyl-mediated polymerization to produce a variety of biomimetic glycopolymer species (*N*-acetyl-D-glucosamine-containing glycomonomers) from alkenyl, acryloyl, and acrylamido glycomonomers, which were often sulfated. Free-radical polymerization (both sulfated and non-sulfated) was used to create a fucoidan mimic glycopolymer using cyanoxyl-mediated free radical polymerization [51]. According to the results, when tested against the herpes virus (infection with HSV-1), the synthetic glycopolymer displayed anti-viral activity comparable to that of the natural polymer.

Currently, ATRP is the most extensively used approach for controlled live polymerization, and it is also the most expensive. Protected glycomonomers, on the other hand, have been employed in the majority of glycopolymer syntheses [31, 52].
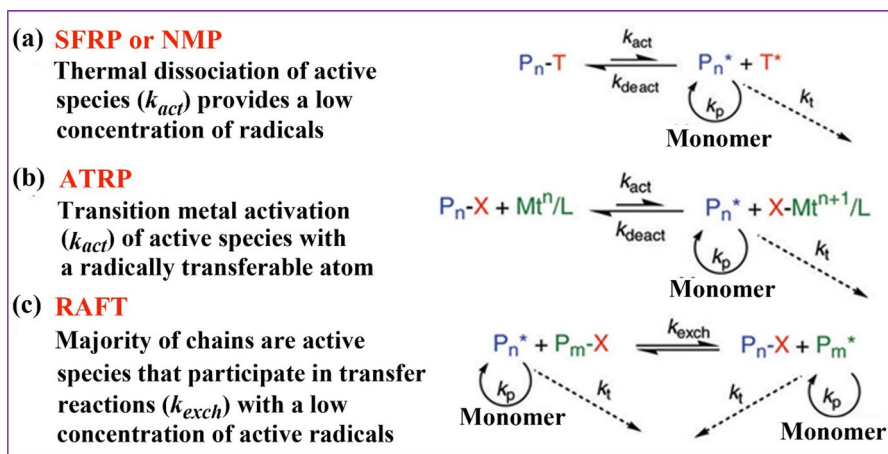
(a) SFRP or NMP
Thermal dissociation of active species (k_{act}) provides a low concentration of radicals

(b) ATRP
Transition metal activation (k_{act}) of active species with a radically transferable atom

(c) RAFT
Majority of chains are active species that participate in transfer reactions (k_{exch}) with a low concentration of active radicals

Fig. 5 The three primary radical polymerization processes [49]

Figure 5b depicts the operation of the ATRP mechanism. ATRP can polymerize most vinyl monomers [53]. At the heart of the ATRP mechanism is the homolytic cleavage of the carbon–halogen (R–X) bond in the alkyl halide, which is catalyzed by a metal complex with a lower oxidation state (Mt^n/L), which results in the formation of a metal complex with a higher oxidation state and active species [54]. The ATRP mechanism is described as follows: the active radicals continue to add monomers until they are deactivated, resulting in the active species (P_n*) and the renewal of the metal complex in the lower oxidation state of the metal complex.

On the other hand, it is possible to limit, if not prevent, the end of ATRP by maintaining a very low concentration of the radicals forming [55, 56] throughout the reaction. Consequently, the equilibrium is shifted almost entirely towards active species. The active (propagating) radical concentration is low enough to reduce the contribution of radical–radical termination, which can occur through radical coupling or disproportionation due to this shift. At the same time, quick initiation and deactivation with low stationary radical concentrations are essential for extending the life span of polymer chains, resulting in improved control over the final polymer [57]. Zhao et al., for example, presented the ATRP approach for the synthesis of dextran-*g*-poly (2-dimethylaminoethyl methacrylate-*co*-2-lactobionamidoethyl methacrylate (DDrLs)/PEI complexes [58], which is seen schematically in Fig. 6. The strong DNA-binding feature of the complex made it an ideal carrier for gene delivery. The decreased cytotoxicity of the DDrLs/PEI/plasmid (p) DNA mixture compared to that of PEI was because DDrLs can mitigate and shield PEI's cytotoxic effects. As a result, both the excellent transfection efficiency of PEI and the serum tolerance of DDrLs were achieved with the DDrLs/PEI combo. In addition, hepatocyte transfection efficiency was improved using galactosylated DDrLs with asialoglycoprotein receptors (ASGPr). In the presence of 30% serum, most other polycation gene vectors have a DDrLs/PEI that is two orders of magnitude more efficient than PEI alone. This makes DDrLs/PEI different from most other polycation gene
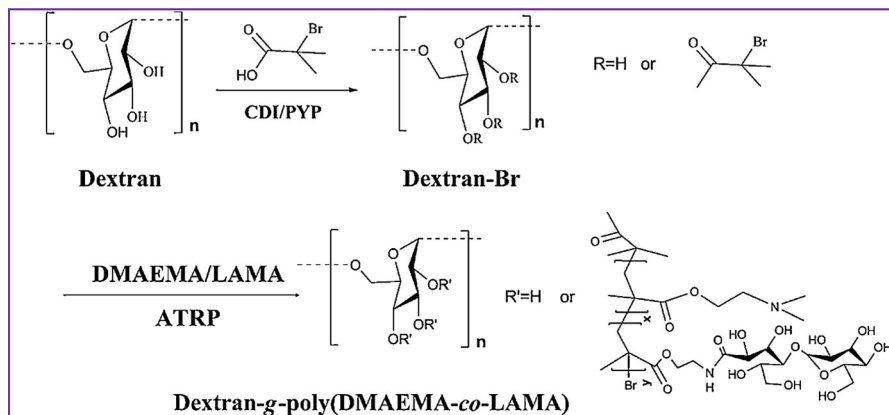
Fig. 6 Atom transfer radical polymerization method for producing DDrLs [58]

vectors. Pasparakis et al. [59] described poly(di(ethylene glycol) methyl ether methacrylate) (PDEGMA) as a glycopolymer block. The thermo-responsive characteristics of the PDEGMA segment allowed the macromolecules to stay unimers at temperatures below 15 °C while assembling into vesicles at temperatures above 15 °C. Depending on the temperature, the mean nanostructure size can also be controlled reversibly between 300 nm at 20 °C and 500 nm at 37 °C.

By modifying the ATRP approach, block copolymers with distinct properties such as properly controlled molecular weights, minimal disparities, and a wide range of functions can be synthesized [60]. A radical ring-opening polymerization can combine it with other monomers, such as butyl acrylate, styrene, methacrylic acid, and *N*-isopropylacrylamide with 5,6-benzo-2-methylene-1,3-dioxepane. Incorporating disulfide bonds into ATRP to make copolymers is another intriguing approach. For example, Fedorczyk et al. [61] synthesized poly(*N*-isopropylacrylamide block-styrene) PNIPAM-b-PS using ATRP. 2-Chloro-*N*-(2-hydroxyethyl) propanamide (NCPAE) is used as an initiator for ATRP of two monomers that vary in activity and polarity into two phases. The polymer chains in the synthesized copolymer molecules are linked together by a linker that is a derivative of the suggested initiator. PNIPAMs with well-planned molecular weights, minimal polydispersity, and hydroxyl functionality were created using NCPAE. Styrene polymerization was started using α-bromoisobutyryl bromide as an activator. The synthesized homopolymers served as macroinitiators in ATRP after such a modification, and a well-defined polystyrene block was successfully generated because of this modification. With specified lower critical solution temperatures, the synthesized PNIPAM and PNIPAM-b-PS demonstrated thermo-responsive behavior.

RAFT polymerization is the most recent controlled/living radical polymerization method that has gained widespread acceptance due to its tolerance to a wide range of reaction conditions and functionality, making it ideal for polymerizing an almost infinite number of monomers [62, 63]. RAFT was used to produce 2-methacryloxyethyl glucoside (glycopolymer) [64]. When the propagating radical (P_n^*) is

introduced to the P_m-X compound, an adduct radical is generated, which is fragmented to create a polymeric (P_n-X) molecule and a new (P_m*) radical, as seen in Fig. 5c [65]. The radical (P_m*) then starts the polymerization process repeatedly, resulting in the propagation of more radicals. As a result of addition-fragmentation, a balance is established between proliferating radicals and inactive polymeric molecules. Most chains maintain the end group after the polymerization process is finished or paused, allowing them to be extracted as stable components. Spain et al. [66] used an enzymatic lipase-catalyzed synthesis to make methacryloyl monomers from 1-*O*-methyl-D-gluco- and mannopyranosides. As a result, the methacrylate was linked to the sugar's 6-position. Polymerizations were carried out using the identical chain transfer agent/initiator combination. However, the chain transfer agent and initiator were dissolved in EtOH before addition. The addition of EtOH improved the solubility of the chain transfer agent and initiator without a base, thereby reducing the hydrolysis rate of the chain transfer agent. After a brief inhibitory phase, it was observed that the polymerizations proceeded with pseudo-first-order kinetics and linearly increasing molecular weights, but at lower values than expected, in which the polydispersity was consistently low. Bernard et al. used the RAFT approach to synthesize poly(acryloyl glucosamine) chains before chain extension in water/ethanol using a water-insoluble 3-arm chain transfer agent [67]. The same group created multiple glycopolymer-block-poly(methyl methacrylate) by chain-extension of a fructose-based macro-RAFT agent. After removing the isopropylidene groups, self-assembly into micelles and glycopolymer vesicles was effective [68, 69].

2.1.3 Ring-Opening Polymerizations

The ring-opening metathesis polymerization (ROMP) method [70, 71] has produced a diverse spectrum of glycopolymers. Many factors [72] make the use of ROMP in glycopolymer synthesis appealing, including the discovery of ROMP catalysts capable of tolerating monomers having unprotected polar functionality. This is essential because many physiologically relevant saccharide determinants include sulfated groups that are difficult to conceal in natural environments. It is possible to construct block copolymers using a similar polymerization approach [73, 74], which may modulate a material's immunogenicity or target it to a certain cell type. Mortell and colleagues [75] used the ROMP approach to produce glycopolymers. The researchers created several glycopolymers (e.g., D-mannose-carrying polymer, Fig. 7a) [76] with various molecular weights and densities, then tested for their ability to recognize proteins, cells, and bacteria. They also investigated molecular recognition and cell function regulation aspects with these glycopolymers. Additionally, it has been reported that the addition of saccharides to polymers by ROMP using succinimide norbornene may be used to synthesize well-defined glycopolymers.

Figure 7b shows the multivalent architecture of glycosaminoglycans, which was discovered in another study by Yang et al. [77]. In addition to chemically synthesizing the sulfated galactosamine derivatives, the ROMP method produced six glycosaminoglycan-mimetic glycopolymers. These glycopolymer conjugates were used to
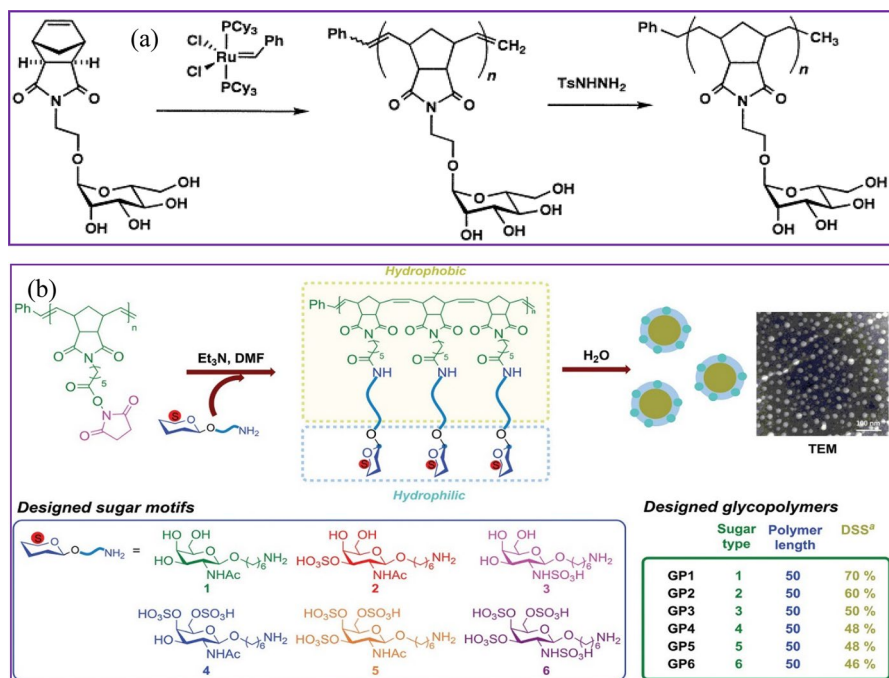
Fig. 7 Ring-opening metathesis polymerization approach for the synthesis of **a** D-mannose-carrying polymer [76], and **b** various glycopolymers with galactosamine derivatives (DSS is the degree of sugar substitution) [77]

target tumor cells differently. This study shows that glycopolymers strongly inhibited heparanase with varying galactosamine sulfation patterns.

2.2 Synthesis of Glycopolymers via Post-Polymerization Modification

Even though sugar-containing monomer polymerization is effective, modifying pre-formed polymers with saccharide-containing chemicals provides a superior alternative synthetic technique [78]. It aids in the production of glycopolymers with the same macromolecular architecture because it connects diverse sugar moieties to pre-formed polymer scaffolds [16, 27]. Aside from that, it provides a more straightforward approach since certain sugar-containing monomers prefer to self-polymerize during the purification process. In part because of its flexibility, versatility, and excellent chemical performance under mild conditions, the "Click" synthesis strategy, which includes reactions such as copper-catalyzed azide-alkyne-cycloaddition (CuAAC), Michael thiol addition, and radical thiol-ene reaction, is one of the most commonly used organic reactions for post-modification to prepare responsive glycopolymers [3, 79]. Huang et al. synthesized a diverse spectrum of block-type polypeptides by anionic ring-opening polymerization using a tertiary amine/LiBr starting system. CuAAC was then utilized to install azide-functionalized galactose and

lactose [80]. Amphiphilic glycopolypeptides can self-assemble into polymerases or micellar structures.

3 The Properties of Glycopolymers: Structure–Function Interactions

Polysaccharides are an excellent demonstration of a dynamic interplay between structure and function, even though many biopolymers perform by adopting spatial configurations that are less ordered than their core sequences. Polysaccharide structure–function interactions are challenging to characterize because they are dependent on sophisticated molecular interactions between primary and higher-order structures and their functional properties. Protein and nucleic acid studies on structure–function interactions and the consequences of functional properties are more advanced than those of glycopolymers. For this reason, this section was broken into four parts to highlight different aspects of the structure–function interactions of glycopolymers.

3.1 Surface Properties

Surface-interacting glycopolymers have a high binding affinity for carbohydrate-binding lectins, making them useful for many applications. Because of the apparent impact of cluster glycosides, the binding of this interaction is substantially greater than that of monosaccharides [81]. As a result, immobilized glycopolymers have been employed to increase the chances of proteins adhering together [82]. Immobilized glycopolymers are used to bind certain lectins. This interaction can be influenced based on the carbohydrate and the binding mechanism, as summarized in Table 1. Particularly, the position of the sugar carbon atom that has been substituted is crucial. To look at it another way, the sugar ring exposed to the outside of the polymeric structure has a greater influence on disaccharide lectin interactions. However, it is not always possible to foresee the expected encounter favorably. For

Table 1 Examples of lectin types that interact with the immobilized-glycopolymers

Type of sugar	Type of lectins	References
N-Acetylglucosamine	Wheat germ agglutinin (WGA)	[87]
	Griffonia simplicifolia (GS II)	[88]
	Concanavalin A (ConA)	[89]
α-*N*-acetylgalactosamine	*Helix pomatia* agglutinin (HPA)	[90]
	Soybean agglutinin (SBA)	[91]
β-Galactose	*Peanut* Agglutinin (PNA)	[92]
	Erythrina cristagalli lectin (ECL)	[88]
	Ricinus communis agglutinin (RCA$_{120}$)	[83]
	Ricinus communis I (RCA I)	[93]
		[94]
α- and β-Glucose	ConA	[95, 96]
Lactose	RCA$_{120}$, PNA	[97, 98]

example, polymerizable group attachment to mannose at the 6-position inhibits Con A binding, although galactose modified at the 6-position may bind to the (RCA$_{120}$) lectin [83]. Interestingly, poly(D-gluconamidoethyl methacrylate), a glycopolymer that has been studied extensively, has shown inconsistent results [84–86].

3.2 Biological Properties

The biological functions of glycopolymers are based on saccharides' unique molecular and cellular recognition abilities, which are the most distinctive and vital properties. Most of the studies in the molecular design of glycopolymers have been devoted to translating the advanced biological capabilities of sugars to synthetic polymer backbones [99]. Because saccharides are natural ligands for cells and pathogens, glycopolymers interact with many pathogen and cell targets [99]. To evaluate the molecular recognition capabilities of glycopolymers, it is necessary to quantify the strength of their interactions with relevant lectins (saccharide recognition proteins). For instance, it was found that the wheat germ agglutinin (WGA), which recognizes GlcNAc and its oligomers, had come into contact with a polyvinyl ether-poly(isobutyl vinyl ether) block copolymer containing GlcNAc. This interaction was demonstrated by Yamada et al. [100]. Kobayashi et al. studied the binding of polystyrene-type glycopolymers to N-linked banomeric oligosaccharides (*N*-acetyllactosamine, *N*-acetylisolactosamine, and 4′-galactosyllactose) and specific lectins by using hemagglutination inhibition experiment [101]. The inhibitory effect of the *N*-acetyllactosamine-carrying homopolymer on lectin-induced hemagglutination was about 103-fold greater than the inhibitory effect of the oligosaccharide alone.

The higher binding capability of the lectin to the polymer chain may be explained by a multivalent or cluster effect occurring along the chain. The use of copolymers reduced hemagglutination more effectively than the use of homopolymers in several lectin-polymer combinations. In copolymers, acrylamide units serve to separate the pendant oligosaccharide moieties. Many lectins can bind to the glycopolymer with much greater force when the oligosaccharide units are far apart. The anticoagulant activity of poly(glucosyloxyethyl methacrylate)-sulfate was examined [102]. It was observed that the polymer formed an insoluble combination with fibrinogen, limiting the synthesis of fibrin and, therefore, impeding coagulation. According to the findings, sulfated saccharide residues are essential for producing anticoagulant activity in the body. As a result of this research, it was shown that heparin had far more activity than these heparinoid polymers. Polymethacrylates, including sulfated pendant oligosaccharides, were synthesized for anti-human immunodeficiency virus (HIV) activity [103]. The relationship between the architectures of polymethacrylates and their biological properties were investigated. It was discovered that reducing the amount of sulfated oligosaccharide moiety enhances anti-HIV activity.

Additionally, when the number of sulfated maltoheptaose units was decreased, the blood anticoagulant activity increased from 9 to 18 units mg^{-1}. The findings indicate that the spatial distance between sulfated oligosaccharide substituents in the polymethacrylate main chain significantly affects biological activities. Moreover, the differentiation and form of the oligosaccharide side chains were critical.

3.3 Self-Assembly Properties

Glycopolymers are amphiphilic due to the hydrophilicity of the saccharides and the hydrophobicity of the polymer backbone [22]. Amphiphilic glycopolymers can form well-defined nanostructures such as micelles, attracting increasing attention in several applications such as drug delivery and release mechanisms. Another structure is polymeric vesicles, also called polymersomes, which are bilayered membrane capsules and are considered an intriguing example of self-assembled morphology. For example, glycopolymers substituted with polystyrene produce helical rod-like structures due to the amphiphilicity and chirality of the glycopolymers. Poly(*N*-p-vinylbenzyl-D-lactonamide) (PVLA) glycopolymers containing polystyrene showed high circular dichroism due to the amphiphilicity and chirality of the polymers [104]. PVLA polymers crystallized into helical rod-like structures with a hydrophobic cavity. The Yoshiko group showed that PVLA glycopolymer can self-assemble into a cylindrical shape due to its amphiphilicity [105]. The hydrophobic cavity of the glycopolymer incorporated π-conjugated polymers of polythiophene to produce the polymer nanowire, a process analogous to natural polysaccharide production.

Lipids form various supramolecular structures, including micelles, rod-shaped micelles, vesicles, and hexagonal inverted micelles [106]. The Armes group has reported various self-assembled glycopolymers, as shown in Fig. 8 [107]. They synthesized well-defined amphiphilic glycopolymers and carefully studied the lipid-like self-assembly properties of the glycopolymers using block polymers containing galactose and hydrophobic methacrylate. They found that nanospheres, micelles, and vesicle structures interact strongly with galactose-specific lectins in vitro, which shows that they are very specific and can be used for targeted drug delivery.
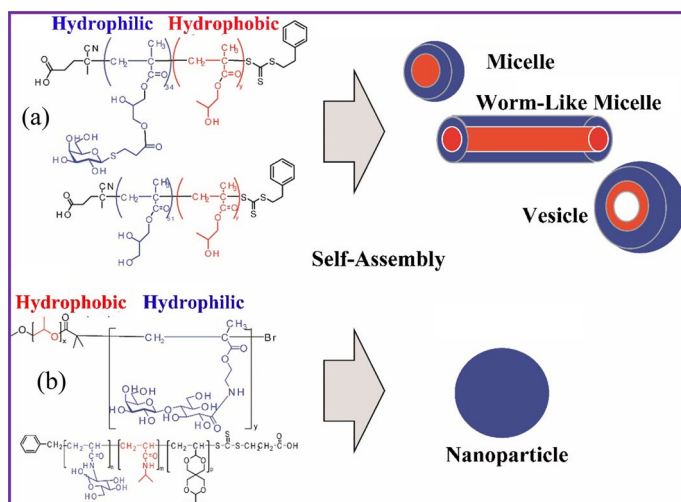

Fig. 8 Self-assembly structures based on the glycopolymers **a** molecular packing parameter, and **b** amphiphilicity formed nanoparticles [22]

3.4 Hydrogel Properties

The biocompatibility and hydrophilicity of sugar-based hydrogels have attracted the attention of researchers [108, 109]. Sugar-based hydrogels are expected to provide cells with a novel growth substrate. Using hydrogels containing sugar instead of agar in culture plates can increase the adhesion and proliferation of both microbial and mammalian cells. Zhou et al. [110] synthesized a hydrogel containing acrylamidolactamine (LAM) in the presence of the crosslinking reagent *N, N*'-methylenebis-acrylamide (1.2 mol%). When the LAM concentration exceeded 47 mol%, the equilibrium water absorption of the hydrogels increased, and when the LAM concentration reached 100 mol%, the equilibrium water absorption increased by up to 44-fold. It was found that, depending on composition, the gels showed rapid swelling transitions. This study used differential scanning calorimetry (DSC) to investigate the swelling transition and water organization in copolymer hydrogels using differential scanning calorimetry (DSC). There was no difference in the water fraction that could be frozen in these hydrogels, regardless of the sugar monomer present. Cai et al. [111] developed carbohydrate-containing responsive hydrogel sensing materials for the selective detection of lectin proteins, namely Con A, ricin, and jacalin. Copolymerization of a vinyl-linked carbohydrate monomer with acrylamide and acrylic acid results in the formation of a carbohydrate hydrogel with specific multivalent binding to lectin proteins. To detect lectins, sensors integrating acrylamide and acrylic acid hydrogels with pendant β-lactoside, α- or β-galactoside, and α-mannoside moieties were utilized. Glycopolymer-hydrogels were attached to two-dimensional photonic crystals, which diffracted visible light substantially. This diffraction yields an optical readout that accurately measures the hydrogel volume. According to the findings, these effects can be explained by increasing diffraction. Blue shifts caused by increasing lactose concentration in hydrogels occur because higher lactose concentration enhances the binding between lactose and lectin proteins, resulting in more crosslinks and, thus, more hydrogel shrinkage. The hydrogel sensors had detection limits of 7.5×10^{-8} M for ricin, 2.3×10^{-7} M for jacalin, and 3.8×10^{-8} M for Con A.

Researchers are also investigating pH-sensitive hydrogels to control the stem cell environment. When the pH of the environment changes, these hydrogels swell/collapse depending on the acidity or basicity [112]. The hydrogel's swelling properties are controlled by varying degrees of polymer crosslinking. Therapeutic peptides and proteins are often delivered orally using pH-sensitive hydrogels. Free-radical photopolymerization of methacrylic acid and 2-methacryloxyethyl glucoside has led to the synthesis of pH-sensitive glycopolymer hydrogels. These hydrogels can be used to treat insulin insufficiency [113]. In acidic conditions, polyanions, like these glycopolymers, stay collapsed. However, they swell in basic or neutral conditions. Even when the hydrogels have collapsed, the drugs remain safe and secure. The drugs are released into the environment as they swell. The glycopolymers are converted at a pH of 5 [113]. Swelling is negligible at neutral pH, and drug release occurs in acidic environments such as the stomach with polycationic hydrogels. Using this design, antibiotics may be delivered more effectively. Changing how drugs are released from pH-sensitive hydrogels in different body parts [114].

4 Glycopolymer-Based Biosensing Application

Biosensors are tiny analytical instruments that include biological or biomimetic sensing components [115]. They are used to detect and monitor many analytes or pathogens significant for the environment, health, and food sectors. Biosensors must fulfill stringent sensitivity, response accuracy, repeatability, high specificity toward the chosen target element, non-toxicity, and cost-effectiveness [116]. They may be classified into several categories, including optical, piezoelectric, and electrochemical sensors. Figure 9 depicts a high-level overview of the most common ways of incorporating transducers into biosensors. The most basic type of biosensor is an optical transducer (Fig. 9a), which can detect analytes or pathogens by measuring changes in the sensing material's fluorescence, absorption, or reflectance performance [117]. This sensor can detect analytes or pathogens by measuring changes in the sensing material's fluorescence, absorption, or reflectance performance. The micromechanical transducer is the second kind of biosensor, and it is based on monitoring changes in the resonance frequency [118] to function as a biosensor. Using a quartz crystal microbalance (QCM) with piezoelectric capabilities, such as those shown in Fig. 9b, may benefit from frequency shifts or deflections of the sensor material. The electrochemical biosensor (Fig. 9c) is the third kind of biosensor and may be categorized into four types: amperometric, potentiometric, impedimetric, and conductometric (Fig. 9d) [119, 120].

As a result of their unique features, low cost of manufacture, and on-field accessibility, electrochemical biosensors are now considered the most popular sensors for human lifestyle and healthcare management, particularly for point-of-care (POC) diagnostic applications [122]. Figure 9c depicts a schematic representation of an electrochemical sensor, and the responses of this sensor were measured using square-wave voltammetry at concentrations of 0, 4, 6, 8, and 10 ng mL^{-1} of total prostate-specific antigen (PSA). The surface of the working electrode is coated with a mixture of graphene oxide, gold nanoparticles, and an anti-total PSA antibody for antigen collection. To create a sandwich-like system, the antigen/antibody-modified electrode is mixed with the generated graphene oxide/Au nanoparticles/anti-free PSA antibody mixture before being treated with the final product. The enormous specific surface area of graphene oxide and the strong electrochemical current of Au in this biosensor result in a detection limit for this biosensor that is difficult to exceed. Saccharides can recognize molecules in their environment, which may be employed as a chemical recognition tool. If the contact between the saccharide and the protein is strong enough, the interactions may be exploited to create biodevices that work similarly to antibodies. Biosensing and diagnosis are two key uses of antibodies; as a result, the utilization of the saccharide protein interaction in glycopolymers as a possible alternative to antibodies for use in biosensing and diagnostics [123] has been proposed. In addition, saccharides are often hydrophilic, which prevents the non-specific adsorption of proteins [124], which is beneficial.

An example of this is a glucose sensor made of poly(*N*-isopropylacrylamide-*co*-glycosyloxyethyl methacrylate) microgel, which was produced by free-radical
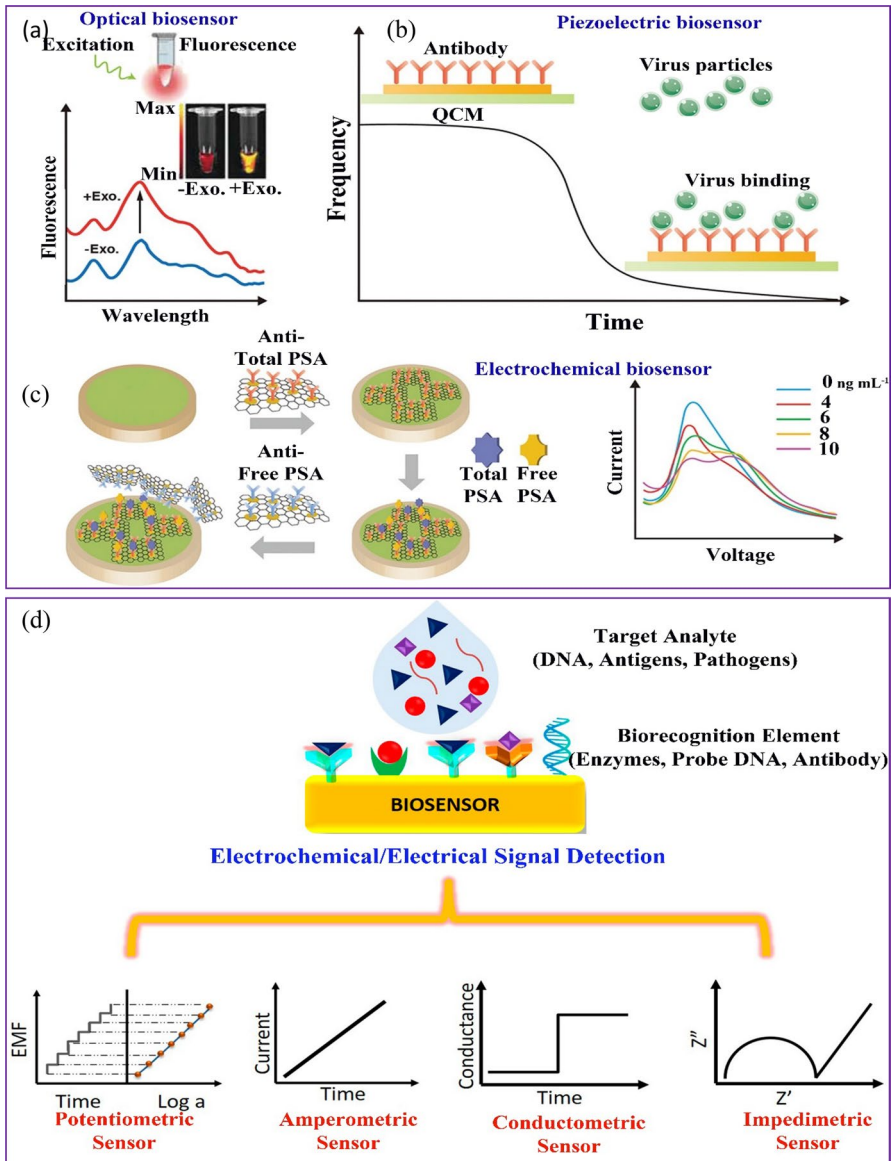
Fig. 9 Schematic representation of the three major biosensor approaches **a** an optical biosensor, **b** a piezoelectric biosensor, and **c** an electrochemical biosensor [121]. **d** Schematic shows the development of a biosensor interface and various electrochemical techniques used to detect specific biochemical interactions [120]

polymerization in the presence of a crosslinker, *N,N′*-methylenebisacrylamide (BIS) (Fig. 10a) [27], which included a glucose sensor. A significant number of glucose pendants enables the glycopolymer to form constricted microgels by interacting with Concanavalin A (Con A) on many different levels. As soon as the
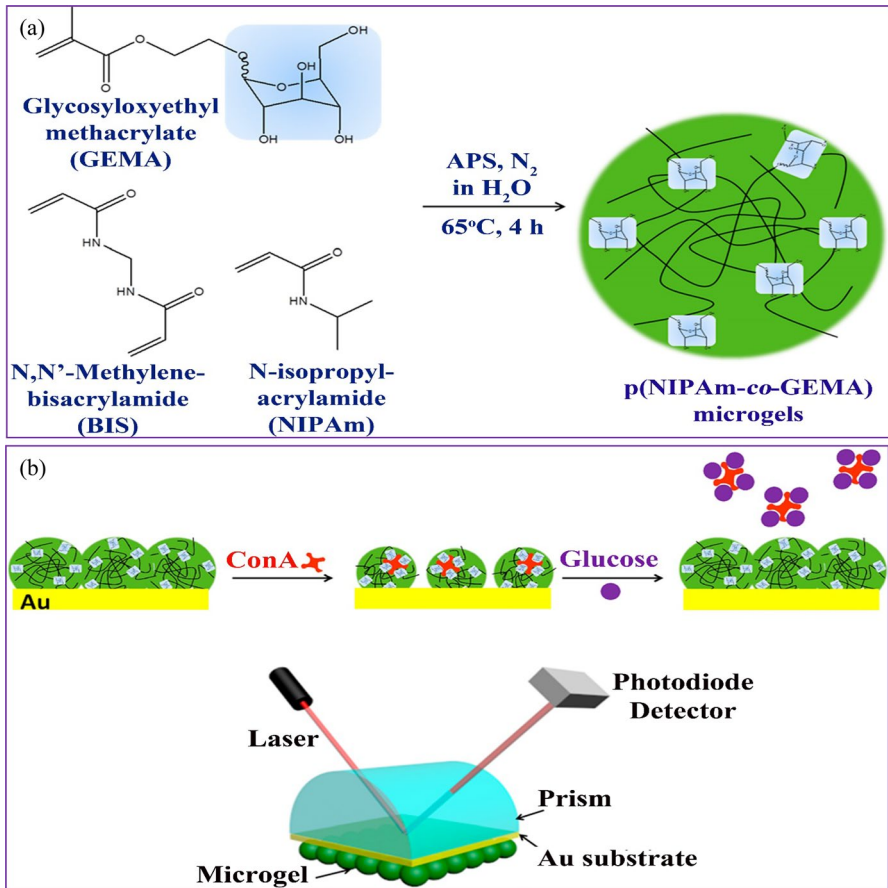
Fig. 10 **a** Synthetic scheme for p(NIPAm-*co*-GEMA) microgels. **b** A schematic of the SPR spectrometer set up on the 50 nm Au sensor surface for the p(NIPAm-*co*-GEMA) microgel-based sensing mechanism for glucose detection [125]

constricted microgel is exposed to glucose, it instantly expands. Surface plasmon resonance (SPR) may be used to quantitatively monitor glucose concentration in a solution by using these processes (Fig. 10b). It is found that when the glyco-polymer microgels are swelled as a result of glucose, the reflected light intensity of the SPR drops as a result of the lower refractive index of the swollen microgel, and vice versa.

According to another work, Chia et al. [126] created polyaniline (PANI)-contain-ing glycopolymer gold nanoparticles (Au@PGlyco NPs) by in situ polymerization of ortho-nitrophenyl-β-galactoside with the assistance of Au nucleation. An electro-magnetic boost in surface-enhanced Raman scattering (SERS) was seen when the nano-Au-carrying polyaniline block was used. Au NPs during the polymerization of ortho-nitrophenyl compounds were used to examine the underlying polymerization process. The Au@PGlyco NP-mediated SERS biosensor was capable of detecting

low levels of bacteria (1×10^2 CFU mL^{-1}) depending on how the galactoside moiety interacted with a bacterial generated-β-galactosidase. In addition to the fact that they include SERS signals through the PANI backbone on the Au surface, they also provide homo-multivalent targeting capacity using a galactose-rich surface without post-immobilized SERS probes or capping layer protection. The enzymatic reaction with Au@PGlyco NPs was carried out at room temperature for 60 min using the Raman system at 671 nm to assess the biological activity of the galactoside moiety at Au@PGlyco NPs. The presence of β-gal resulted in an obvious drop in the intensity of the SERS throughout the incubation time, demonstrating that the enzyme reactivity of Au@PGlyco NPs was activated by the presence of the compound (Fig. 11a). When detecting measurable quantities ranging from 10^{-7} to 10^{-12} M β-gal, the limit of detection (LOD) was 1.84×10^{-12} M. According to the findings of this work, Au@PGlyco NPs may be used as a biosensing platform because of their labeling potential for galactose-binding receptors that are expressed in cells and immune cells.

Figure 11b shows the immobilization of an *O*-cyanate chain-end functionalized glycopolymer containing multivalent lactose units synthesized by cyanoxyl-mediated free radical polymerization (CMFRP) followed by enzymatic sialylation on amine-functionalized glass slides via *O*-cyanate-based isourea bond formation for glycoarray application [127], which the Sun group reported. Additionally, they similarly immobilized sialyllactose-containing glycopolymer produced by chemoenzymatic synthesis for glycoarray and surface plasmon resonance (SPR)-based glyco-biosensor applications. Lectins and influenza virus hemagglutinins (HA) were detected by the SPR-based glyco-biosensor, which demonstrated specific binding activity. While this biosensor is not quite accurate in sialyloligosaccharide presentation in three dimensions, it will provide valuable high-throughput tools for virus diagnostics and possible antiviral drug candidate screening applications.

An *N*-acetyl-galactosamine glycopolymer was grafted onto a surface-enhanced Raman spectroscopy (SERS) substrate [128]. These brush-like glycopolymer ligands have the potential to grab targets that are proximal to the SERS sensing interface, allowing for the detection of ricin's intrinsic SERS signal. The findings
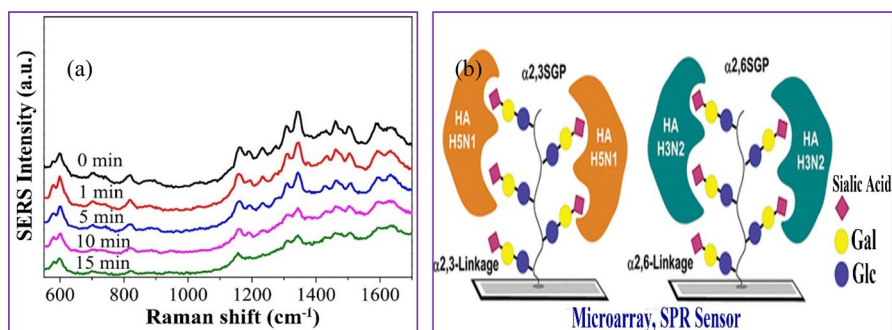

Fig. 11 a Au@PGlyco SERS spectra [126]. **b** orientation and immobilization of *O*-cyanate chain-end functionalized sialyllactose-containing glycopolymers for microarray and SPR applications via isourea bond formation [127]

of this study provide a broad framework for the use of polymer ligands to accomplish target-specific SERS detection. A simple label-free electrochemical biosensor for measuring anti-glycan IgG antibodies in serum from toxoplasmosis seropositive patients was described for the first time by Echeverria et al. [129]. SPAuEs were screen-printed gold electrodes immobilized with a synthetic GPI phosphoglycan bioreceptor using a linear alkanethiol phosphodiester to create the biosensor. As seen in Fig. 12a, the electrochemical impedance spectroscopy (EIS) technique was used to detect and quantify the antigen–antibody interaction. The resulting device demonstrated a linear dynamic range of anti-GPI antibodies in serum ranging from 1.0 to 10.0 IU mL^{-1}, with LOD of 0.31 IU mL^{-1}, a limit of quantification of 1.00 IU mL^{-1}, and less than a 4% deviation in quantification when compared to the IFAT assay, among other characteristics. The glycobiosensor was specific for *Toxoplasma gondii*, and it was capable of detecting and measuring anti-GPI antibodies from individuals who were seropositive for toxoplasma. After being treated
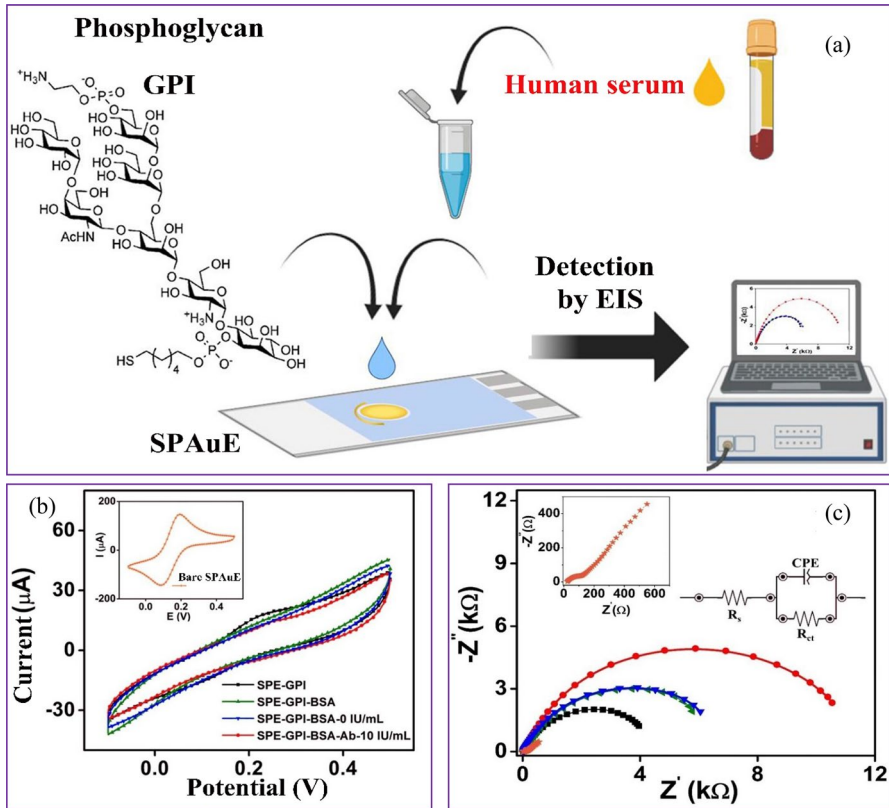

Fig. 12　a A schematic representation of developing a functional phosphoglycan-sensitized platform. **b** CVs for each modification step at 50 mV s^{-1} scan rate with the bare electrode inset. **c** Nyquist plot with a frequency range of 50 kHz to 0.01 Hz, and an amplitude of 10 mV (the bare electrode and the equivalent Randles circuit are in the inset). The redox probe is 5 mM [Fe (CN)$_6$]$^{3-/4-}$/PBS 1X, pH 7.4 [129]

with phosphoglycan and BSA, the activated Au electrode was characterized using CV and EIS measurements, among other methods (Fig. 12b, c). After the SPAuE alteration, only minor modifications in the electrochemical behavior, cyclic voltammograms (CVs), and somewhat decreased current intensity were found, all of which were minor.

On the other hand, changes in electrochemical behavior were more visible when utilizing EIS, which revealed an increase in charge transfer resistance following each alteration stage. This approach was successful in the identification of toxoplasmosis. It may have significant promise for quantifying anti-glycan antibodies using other biomarkers and creating new glycan-based diagnostic tests that are more convenient for patients to use.

Recent research by Baker et al. [130] has resulted in developing a point-of-care lateral flow immunoassay to detect the spike protein of coronavirus (SARS-CoV-2) within 30 min on paper. Figure 13 illustrates this method. In this research, they used a glycopolymer functionalized Au nanoparticles-based biosensor platform to investigate whether the functional chain of *N*-acetylneuraminic acid is effective in capturing the spike protein of the SARS-CoV-2 infection. Also investigated was whether or not there was any cross-reactivity between the SARS-CoV spike protein and the clinical sample. The LOD of this LFIA was 5.0 g mL^{-1}, which was considered high.

A dilution series of SARS-CoV-2, S1 was used to test the NeuNAc (positive) and galactose (negative) nanoparticles, revealing the detection limits and specificity of this approach. Specifically, they discovered that galactose particles had very poor binding to SARS-CoV-2, S1 at the highest dose tested (0.5 mg mL^{-1}). However, NeuNAc particles exhibited substantially greater binding, with an apparent limit of detection below 8 µg mL^{-1} or 8 nM in the case of the NeuNAc particles [130]. Erofeev et al. [131] reported label-free fast detection of influenza. The virus was created using PZT piezoelectric disks with a 100 µm thickness and a 2 µm radius coated with Ag.

Floating viral suspensions were introduced into the disks, modified with synthetic sialylglycopolymers based on a polymer matrix that biospecifically binds the influenza virus's hemagglutinin proteins. Fabrication of the PZT disk sensor is shown in Fig. 14a–f. The disk radial mode resonance frequency change was used to identify the virus, allowing label-free detection. At concentrations below 10^5 virions mL^{-1}, it has been established that influenza A viruses may be detected without using a label. The frequency shift caused by viral adsorption was also related to the surface tension caused by the virus.

The sensitivity was related in inverse proportion to the thickness of the resonator. Because of this, it is simple to enhance the sensitivity by many orders of magnitude by utilizing a thinner PZT substrate. Eventually, this PZT disk sensor technology for influenza A virus detection may be adapted for use in a personal computer. A human sweat-based wearable biosensor for measuring glucose concentrations was developed by Xuan et al. [132] and is shown in Fig. 14g. Chitosan-glucose oxidase composites were combined with micropatterned reduced graphene oxide using standard photolithography to form a new material. As part of the detection process, the enzyme glucose oxidase catalyzed the oxidation of glucose to produce hydrogen peroxide. Then the hydrogen peroxide generated at
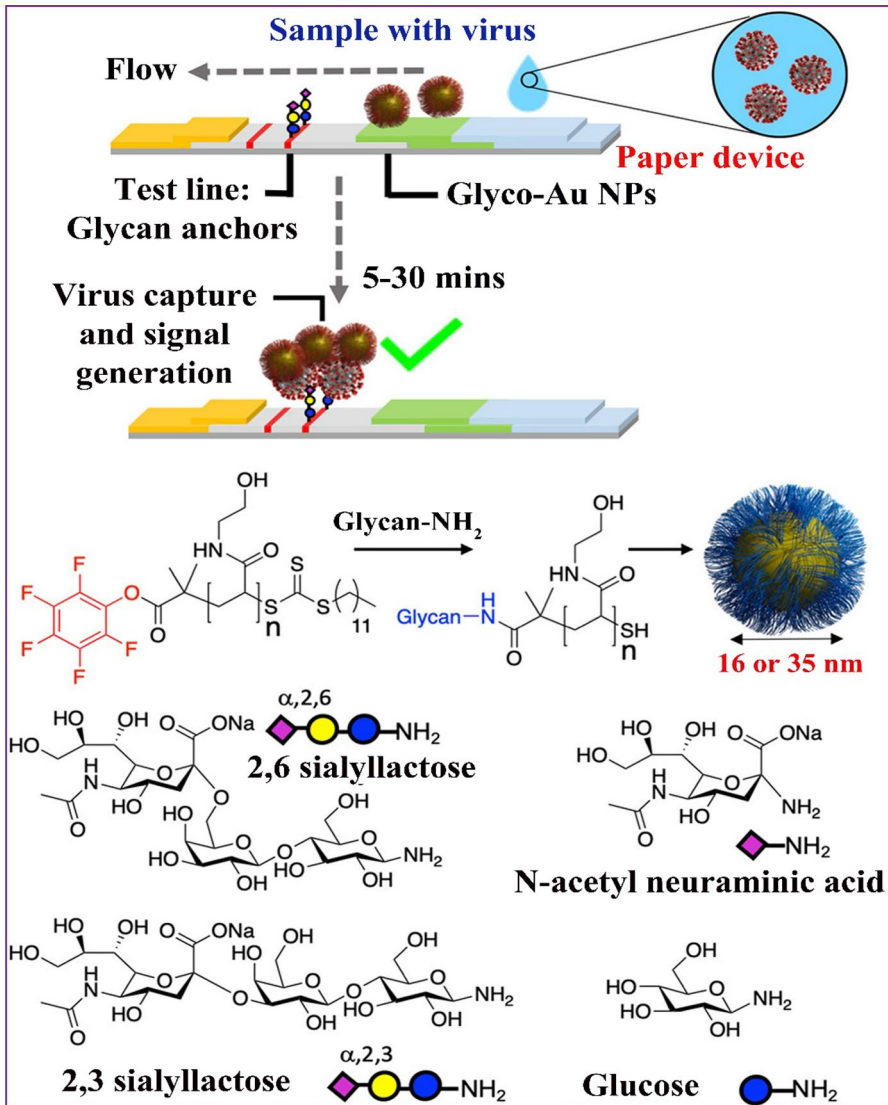
Fig. 13 Design concept for glyco-lateral flow devices; **a** a virus lateral flow assay using glycan capture units; **b** a glyconanoparticle synthesis procedure [130]

the working electrode underwent a redox reaction to change the current, thereby establishing a relationship between current and glucose concentration. Because of the unique nanostructure and strong catalytic performance of the reduced graphene oxide, the biosensor that was developed may be utilized to detect low glucose concentrations in athletes' sweat. Results indicated that the biosensor had high analytical activity, repeatability, and stability and was also inexpensive.
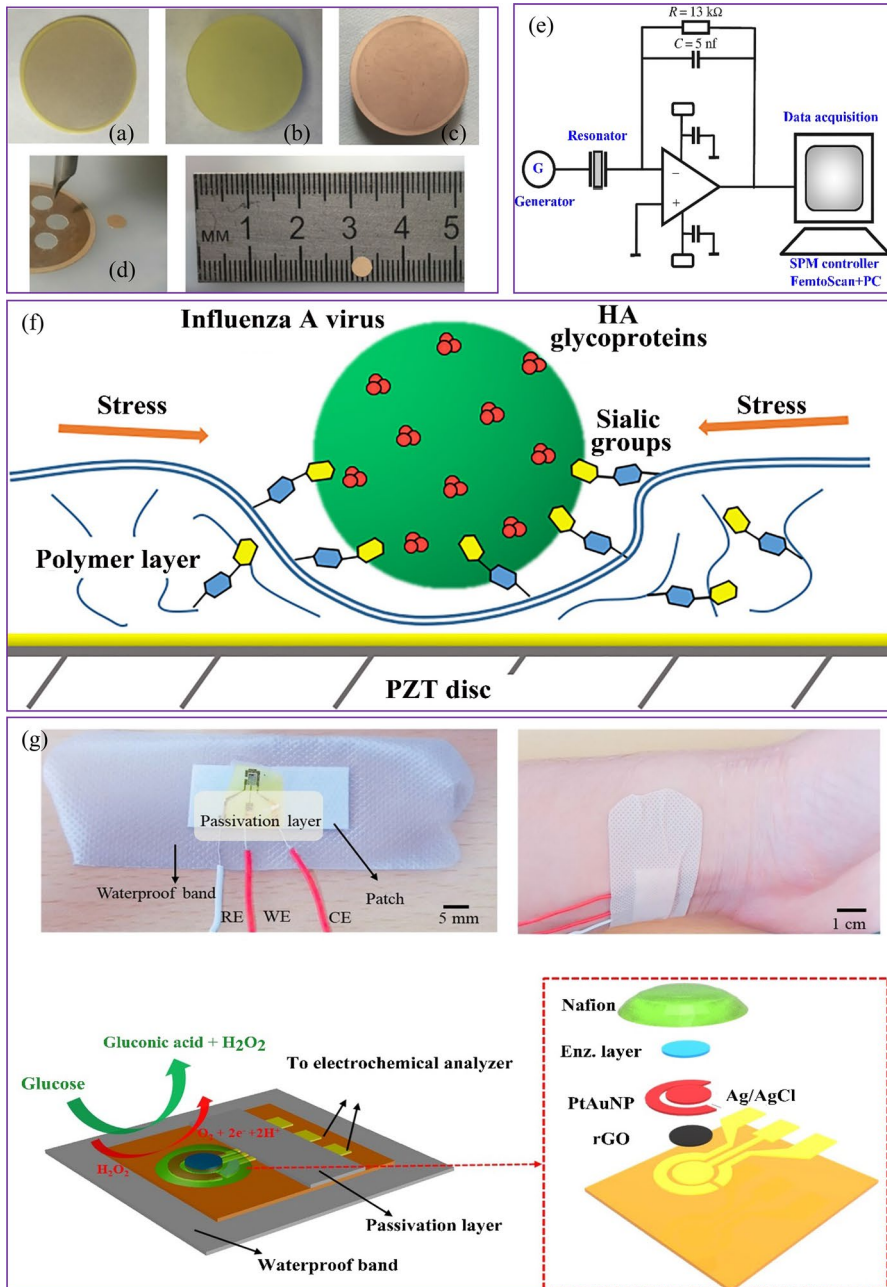
Fig. 14 PZT disc fabrication process: **a** 100-m-thick silver-coated piezo plate; **b** plate after incubation in 98% nitric acid solution; **c** gold (50 nm)-coated plate; and **d** 4-mm disc cut with chopped carbide grade from the aminated gold-coated plate. **e** Designed a board to measure the resonance frequency shift of PZT discs. **f** There is an increase in lateral stress within the polymer receptor layer with sialyloligosaccharide groups following virus particle binding [131]. **g** Photographs and schematics of the constructed sweat-based glucose biosensor [132]

    With the help of in situ reductions and stabilization using a D-glucose-based bisacrylamide solution (glycobisacrylamide (Glc-bis)-stabilized Au nanoparticles), Ajish and colleagues [133] have manufactured gold nanoparticles with a shelf life of more than 6 months at 25 °C. While undergoing this procedure, the intrinsic emission of self-assembled Glc-bis was quenched by AuNPs, and the resulting system (Glcbis@AuNPs) can function as a turn-on sensor, in which case the quenched emission is restored with the addition of the lectin, Concanavalin A (Con A). The Glc-bis@AuNPs system was shown to be stable up to 1 M NaCl concentration, an important aspect in biosensing applications since it meets the criteria for stability. The binding constant of Glc-bis@AuNPs towards Con A was 9.8×10^4 M^{-1}, and the LOD was 1.6 nM. Glc-bis@AuNPs was found to have a binding constant of 9.8×10^4 M^{-1}. In trials with the FimH-positive, pathogenic K-12 strain of *Escherichia coli* (*E. coli*), the interaction with Glc-bis@AuNPs resulted in the restoration of emission; however, in experiments with the FimH negative TOP 10 strain, there was no restoration of emission. Takara et al. [134] used RAFT polymerization and polymer-modified AuNPs in conjunction with polymer-modified AuNPs to synthesize poly(acrylamidophenyl α-mannose-*co*-acrylamide) glycopolymers (Fig. 15a). As an acrylamidophenyl mannose unit, the mannose unit was integrated into the polymer, which altered the material's stiffness. Using lectin, they looked at the physical and chemical characteristics and the molecular recognition capacities of the protein. An immunochromatographic experiment was used to evaluate the biosensing
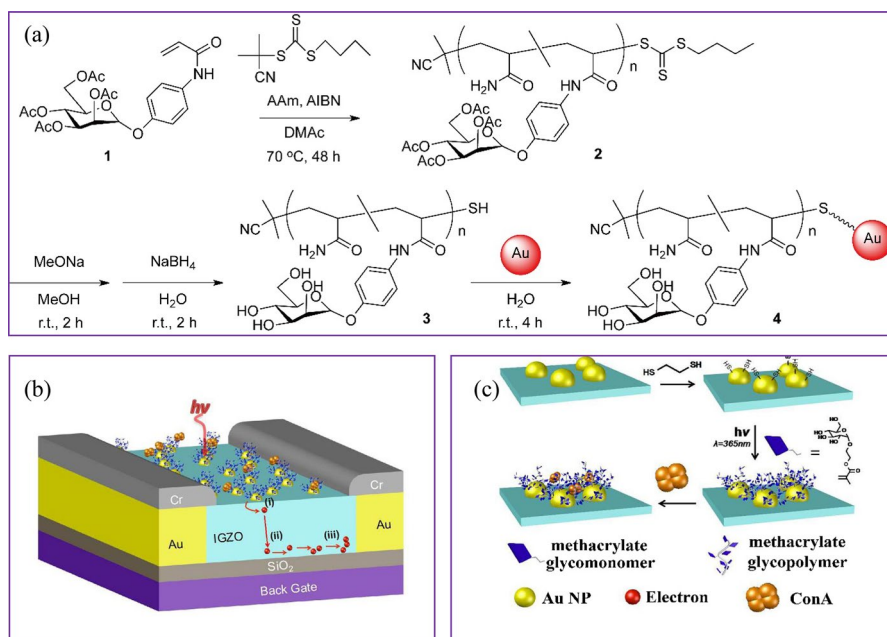

Fig. 15 **a** Synthesis of the glycopolymer and the preparation of the polymer-immobilized gold nanoparticles [134]. **b** A plasmonic field-effect transistor (FET) for sensing lectins is coated with Au NP-glycopolymer conjugates. **c** The construction of the lectin-binding plasmon FET involved microfabrication of the device followed by surface functionalization with synthetic glycopolymers [135]

properties of the AuNPs that had been treated with glycopolymers. The presence of higher sugar concentrations resulted in more sensitive detection. The sensitivity of the immunochromatographic test was shown to be dependent on not only the polymer type but also the sugar density on the AuNP surface, indicating that the assay was very sensitive. It is possible to detect the recognition between lectins and surface-immobilized glycopolymers over a wide dynamic range (from 10^{-10} to 10^{-4} M) in an environment that closely resembles the glycocalyx by immobilizing glycopolymers onto the surface of a recently developed plasmonic field-effect transistor (FET) (Fig. 15b, c) [135]. This study investigated the binding of various lectins to the sensor surface. The results showed that the selectivity and relative binding affinity trends observed in the solution were maintained on the sensor surface and that the significantly higher avidity observed on the surface was due to cluster-glycoside effects. According to the literature, polymer surface chemistry with optoelectronic output in this device design results in one of the greatest reported detection sensitivities for Con A. This work demonstrated the advantages of combining emerging device architectures with soft-matter systems to create cutting-edge nanotechnologies that are well suited for fundamental biological studies and integration into point-of-use diagnostics and sensors for the healthcare industry.

Using an oxaborole and a glycopolymer conjugated polymer, Jiang et al. [136] reported the production of a conjugated polymer that was utilized to achieve very high selectivity in dopamine (DA) detection, which is a catecholamine neurotransmitter found in the nervous system that plays an essential function in learning and memory formation [137]. A displacement process is activated by the optimal binding affinity between the polymers, which increases the selectivity for DA while being unaffected by other structurally similar analogs and saccharide derivatives. By immobilizing polymer conjugates on surface plasmon resonance (SPR) and microcantilever (MCL) sensor systems, it has been established that real-time detection of DA can be accomplished with very high selectivity and sensitivity. The SPR biosensor could detect DA in the concentration range of 1×10^{-9} to 1×10^{-4} mol L^{-1} when the conjugated polymer sensing layer was used, but the MCL sensor had LOD of 5×10^{-11} mol L^{-1}. They discovered that the detecting process is based on DA-induced reversible swelling of the conjugated polymer layer, enabling the sensor to be regenerated and reused several times after its first usage. The researchers also concluded that SPR was a viable sensor platform for DA in-line detection at the clinical level, taking into account the detection time and stability, whereas MCL could only reach a considerably lower detection limit. With the use of poly(ethylene glycol) (PEG) with galactose and mannose-ended approaches, Richard et al. [138] have developed a system that is capable of distinguishing between various strains of *Escherichia coli*. The introduction of PEG chains improves the saline stability while maintaining the biorecognition capabilities of the compounds. When nanoparticles are attached to protein FimH-positive bacteria, the optical characteristics of the nanoparticles change, allowing the identification of specific bacterium strains. Using this approach, FimH-positive bacteria were found at a roughly 1.5×10^7 colony-forming units per milliliter of solution.

Tanaka et al. [139] have successfully synthesized glycopolymers that contain pendant thioglycosides by RAFT and then immobilized them on gold nanoparticles

and QCM. The gold nanoparticles and gold-coated quartz crystal microbalance sensor immobilized with the thiol terminated glycopolymers exhibited high affinity for the corresponding lectins due to multivalent interaction between saccharides and protein in an aqueous solution. They showed strong and specific binding with their corresponding lectins, with estimated association constant (Ka) values of 10^7 M^{-1}. This value is reached with only 10% carbohydrate units, which gives an idea of the strong glycocluster effect (the interaction between lectin and free saccharide molecule is around 10^3 M^{-1}). The RAFT process was used to create amphiphilic block glycopolymers based on 2-O-methacryloyloxyethyl-(β-D-lactose) and 4-pyridilmethyl methacrylate (PyMA) [140]. Through the pyridine groups, those can be chemo-adsorbed onto the gold surface of QCM. The constant value for the specific binding with ricinus communis agglutinin (RCA$_{120}$) was 6.26×10^6 M^{-1}. This approach was also employed to modify gold nanorods. A very small amount of lectin (100 pg mL^{-1} = 8.3×10^{-13} M) was detected by the aggregation of these glycogold nanorods, which gives an idea of the potential of these systems [141].

In Fig. 16a, QCM with dissipation was used to investigate bacterial adhesion to glycopolymers of poly(2-lactobionamidoethyl methacrylamide) and cationic 2-aminoethyl methacrylamide hydrochloride monomers produced by RAFT polymerization [142]. The QCM bacterial adhesion was tested using the galactose-specific lectin RCA$_{120}$. Then, as compared to *E. coli* K-12, more *Pseudomonas aeruginosa* (PAO1) attaches to the glycopolymer surface with strong contact point stiffness since *P. aeruginosa* has galactose-specific binding while *E. coli* has mannose-specific binding. In addition, RAFT glycopolymers of acrylamide and glycomonomers, including triazole-linked sialyloligosaccharides have been immobilized on a gold-coated sensor of QCM for further study and application. Several studies [143, 144] have shown that these glycopolymers bind to influenza A viruses in humans and birds. The strong binding observed between the glycocluster effect of the glycopolymer and the biantennary structure of the *N*-linked oligosaccharide, which were identified using the hemagglutination inhibition experiment, was attributable to the strong binding. In fabricating glycochips, Uzawa et al. [144] used polyanionic polymers and an adsorption technology called layer-by-layer (LbL) adsorption. Using surface plasmon resonance, the researchers created three glycochips that included globobioside (Gb$_2$), β-lactoside (β-Lac), or α-D-mannoside (α-Man) residues for the detection of Shiga toxins, namely Stx1 and Stx2 (SPR). For the Gb$_2$ glycochip, the toxicity of Stx1 and Stx2 toxins is highly selective, whereas their binding affinity for the β-Lac glycochip is minimal. So, they produce distinct SPR responses, which allows for the differentiation of the two toxicants. It was possible to perform a very sensitive and straightforward analysis because of the maximum affinity constants of Stx-1 and Stx-2 (Ka = 10^8–10^9 M^{-1}; 10 ng mL^{-1}, 30 min).

Li et al. [145] functionalized a layer of glycopolymer brush (GlyB) interface on the surface of gold substrates using a glycopolymer brush (GlyB) interface. Using a combination methodology of Cu(0)-living radical polymerization followed by the "CuAAC" click reaction, a series of new glycopolymer precursors with adjustable scaffold structure and pyranose ligands were synthesized and tested (Fig. 16b). The resultant glycopolymer had a finely adjusted molecular
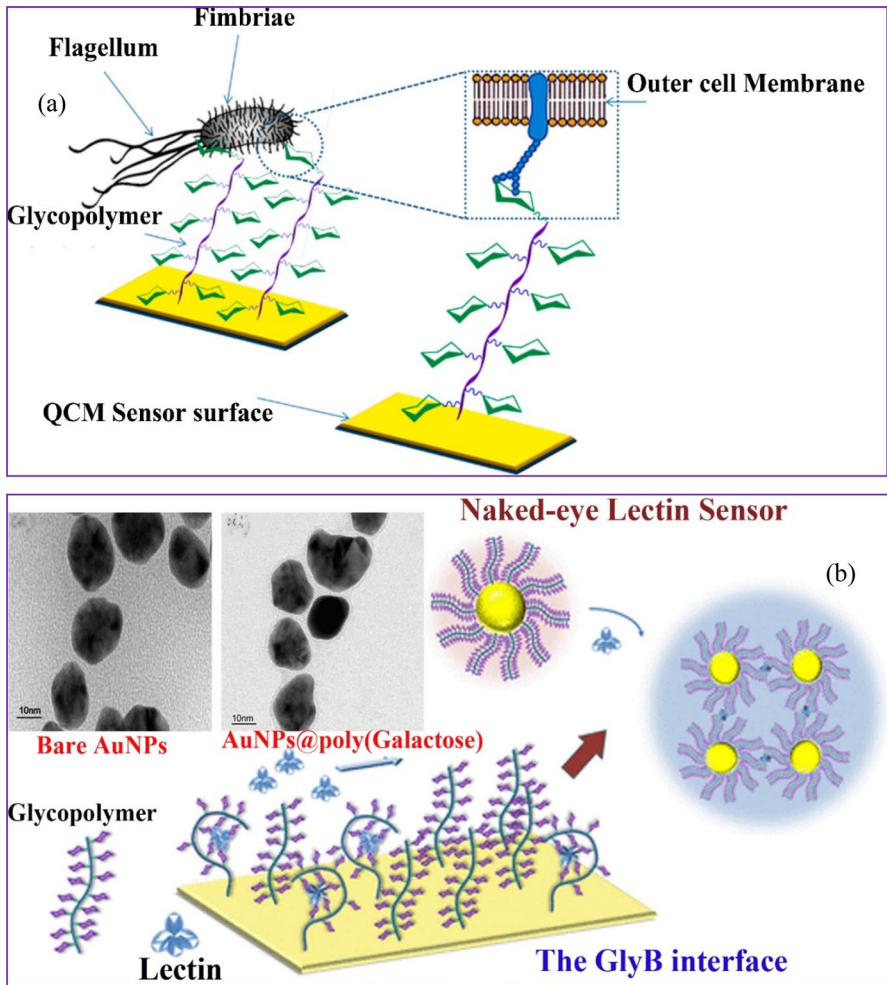
Fig. 16 Schematic representation of the **a** specific interactions of bacteria to glycopolymers immobilized on the QCM-D surface [142] and **b** GlyB interface and the sensing mechanism [145]

weight and a minor dispersion of 1.27, indicating that it was well tuned. The high affinity between poly(galactose) and peanut agglutinin (0.18 ng mm^{-2}) was used to develop a sensing system, which was accomplished by precisely identifying an internal binding site in the lectin structure in the poly(galactose). The transmission electron microscopy pictures and distinct borders show that plain AuNPs had rather regular and uniform forms (20 nm in diameter). Observations showing a unique shell layer of 2–3 nm in diameter surrounding each nanoparticle were made after the glycopolymer implantation. This ultra-minimal naked-eye sensor, composed of just two components, namely the AuNPs and the glycopolymers, was described and evaluated for potential use in measuring protein concentration.

5 Conclusion and Future Perspectives

This review presents a broad overview of the glycopolymers and their current biosensing applications, focusing on recent breakthroughs in several biodetection applications. Given the high volume of research being conducted in this area, it is reasonable to expect significant improvements in glycopolymer-based biosensor sensitivity, specificity, and practicality for real-world applications. Improvements in functionalization and immobilization methods are predicted to correspond with these breakthroughs, which is good. The diversity and translational capabilities of these technologies will continue to develop, owing to the advantages of glycopolymers, including biocompatibility and biological recognition, such as cell growth regulation and cancer cell metastasis, through specific interactions with lectins. Furthermore, many of the tactics that have been mentioned have only been used in the detection of a small number of targets. Because there are few reports showing the efficacy of the above-discussed techniques in a variety of detection situations, they will likely be used more widely in the future. Surface stability, the shelf life of immobilized substrates, compatibility with several target environments (e.g., water, serum, or food), and the minimization of non-specific binding should all be considered in the future, as these are the challenges in achieving extremely low limits of detection required in many applications. Detecting whether biomolecules interact with glycopolymer-based materials is also a difficult challenge. The biosensing performance of glycopolymer-based materials is strongly influenced by the amount and arrangement of biomolecules on the surface. The development of biosensors with excellent stability and reproducibility is another challenge that severely limits the practical use of biosensors based on glycopolymer materials.

Efforts are being made to better understand glycopolymer-based materials regarding their physical and chemical properties. By further investigating the unique properties of the glycopolymer-based material, researchers hope to develop biosensing applications. Medical translation of glycopolymer-based materials for biosensing applications can begin once technical and device-specific regulatory testing is complete. While some biosensors have already been tested and are now being used in healthcare, their design needs to be improved in other fields such as environmental monitoring and food safety. In addition, rigorous tests for sensitivity, specificity, response time, repeatability, and durability must be performed to ensure accurate and reliable results. These vital investigations have not yet been carried out sufficiently and must be further studied. Characteristics such as biocompatibility, toxicity, and extractable components should be considered when approving in vitro and, much more so, in vivo applications.

Finally, the effectiveness of glycopolymer-based biosensors is dependent on their immobilization, their activity after immobilization, and their ability to transmit signals effectively. This highlights the importance of developments in robust and stable immobilization procedures. Additional considerations should be given to the manufacturing cost to allow for possible adoption in large-scale applications.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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