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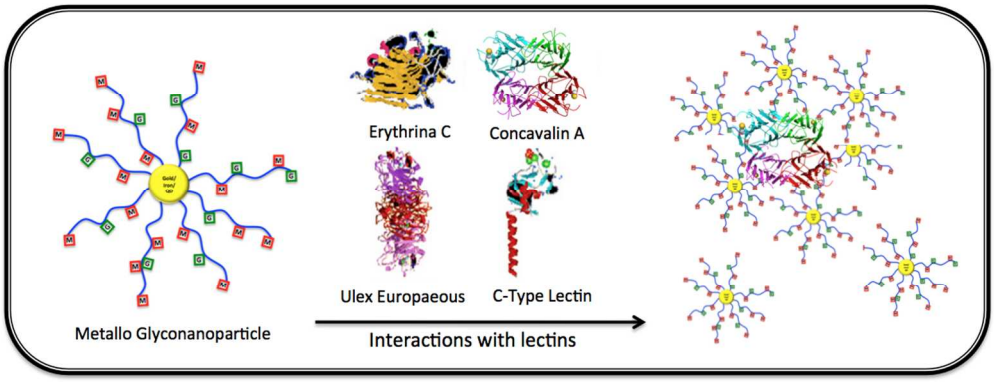

Glyconanoparticles and their interactions with lectins
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Glyconanoparticles and their interactions with lectins

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ABSTRACT

Recent progress in nanobiotechnology has allowed using glycans and their conjugates as biofunctional molecules for many biological and biomedical applications. Therefore, specific interactions of carbohydrate-binding proteins (lectins) are continuously been elucidated for a deep understanding of their functions and the precise mechanism of their association with specific ligands. New generation of glyconanoparticles with outstanding features that present carbohydrates in a multivalent manner and locally in high concentrations have been promising to establish the glycan code and to function as a glycan mimic. In the first part of this review article, different types of lectins have been summarised and their main properties are highlighted. Whereas, in the second part, recent successful examples of glyconanoparticles formed of synthetic polymers and in most cases conjugated with metals have been discussed in terms of their synthesis and interactions with lectins.

Keywords: Glyconanoparticles, lectins, glycopolymers, carbohydrate interaction, multivalency.

1. Introduction

During the last decade, there has been a great deal of interest in the integration of nanotechnology with carbohydrates. The advances in glyconanotechnology have allowed creating different bioactive glyconanostructures for various health related applications such as drug delivery, biomaterials, gene therapy, pathogen detection, inhibitors of toxins and lectin-based biosensors.[1-6] Nanoparticles functionalized with carbohydrates presented a highly multivalent way for interaction with lectins and allowed the obtainment of high local concentrations of ligands on a small surface. Carbohydrates have attracted much attention to insert their biological properties into nanostructured materials due to their use for bio-mimetic purposes, their crucial role in bio-recognition processes at molecular level and their functional role in living systems.[7-8] Their major function is to serve as recognition markers.[9] Although the binding between carbohydrates and lectins is weak, it could be greatly enhanced by the multivalent effect of densely packed carbohydrate molecules with unique functionalities, which is known as the "glycocluster effect".[10-11] Glyconanoparticles as carbohydrate-based systems provide in a similar manner to mimic the behavior of naturally existing glycocalyx. Therefore, the fabrication and engineering of highly innovative glyconanoparticles with unique physiochemical properties help to further enhance their specific recognition properties on multivalent scaffolds in glycoscience.

Glycopolymers are synthetic macromolecules with sugar moieties and have a great potential for mimicking the oligosaccharides.[12-15] Oligosaccharides play a key role for many biological processes such as signal transmission, intercellular recognition and fertilization.[16-19] Self-assembled/decided nano-objects and scaffolds functionalized with oligosaccharides were designed to study and evaluate carbohydrate–carbohydrate and carbohydrate–protein interactions.[20-21] The conjugation of glycopolymers with biomolecules such as proteins, oligopeptides, deoxyribonucleic acid plasmids, lipids,

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phospholipids, ligands was performed to yield a large family of neoglycoconjugates with desirable properties for the construction of self-assembled glyconanoparticles (Figure 1).

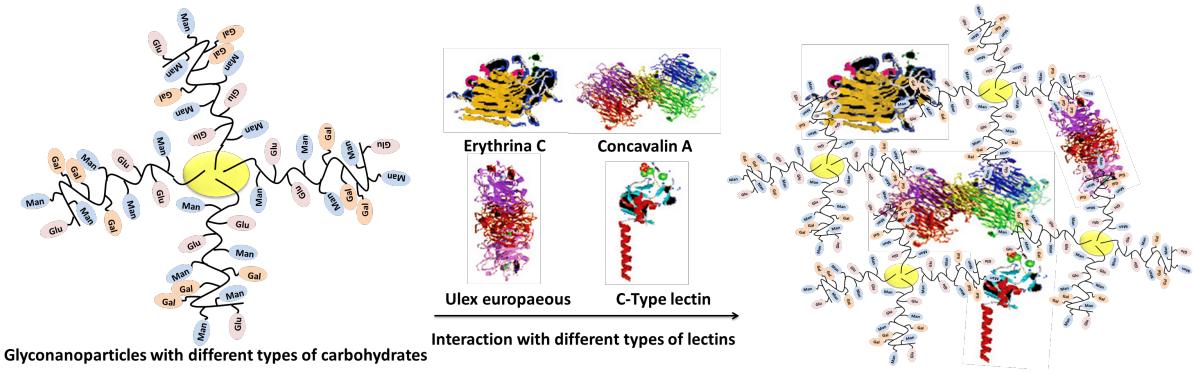

Figure 1. Schematic representation of metallic nanoparticles with different types of carbohydrates and their interaction with different types of lectins.

Moreover, glycopolymer nanocarriers have been prepared in different forms including vesicles, micelles, nanoparticles and nanofibers, for the delivery of bioactive molecules, such as folic acid, biotin–avidin, antibodies and DNA sequences. According to recent developments in the routine synthetic methods of complex glycoconjugates, it is nowadays possible to design new polyvalent systems providing multivalent carbohydrate−receptor interactions. Basically, there are several types of glyconanoparticles, namely, metallic, semiconductor glyco-quantum dots, magnetic and self-assembled glyconanoparticles. In this context, recent advanced and selected metallic and polymeric glyconanoparticles showing specific interactions with various lectins were highlighted, but firstly typical lectins utilized in glyconanoparticles interactions were summarized.

2. Lectins

Lectins (latin: legere (to select)) that have first been described at the end of the 19th century are defined as carbohydrate-binding proteins.[22] Lectins are fundamental to many important biological process and living organisms.[23-24] They are ubiquitously spread in nature and also they are major components of the outer surface of mammalian cells. Lectins have a specific and reversible interaction with carbohydrates non-covalently, that is known as

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cell agglutination. Furthermore, many bacteria have the ability to agglutinate erythrocytes by own lectins. The major attribute of the activity of lectins is obtained by using the agglutination and precipitation processes. There are a wide variety of lectins in terms of their different structure and size. Sharon and co-workers have pioneered to study the binding between lectins and carbohydrates and also did a classification based on the monosaccharide ligand toward which they exhibit the highest affinity.[23] This classification is losing its utility slowly due to marked differences in the fine specificities of lectins within a single category. Moreover, several different types of lectins that have now been isolated from organisms such as plants, animals and microorganisms do not show sufficient binding affinity toward simple saccharides. There is another way to make a classification according to their molecular structure. First group named simple lectins consist of a small number of subunits with molecular weights typically below 40 kDa. Secondly, mosaic lectins those are very diverse in structure from different sources: viral hemagglutinins, animal lectins of C-, P- and I-types consist of different types of protein domains. Last group is macromolecular assembly lectins, which are filamentous and heteropolymeric organelles. They commonly exist in bacteria. However, their structure has a minor component site for the interaction with carbohydrate.

Bacterial adhesion that is an important mechanism for the attachment of bacteria onto cell is based on molecular interactions between cell surface carbohydrates of the host and specialised carbohydrate-specific bacterial lectins.[25] This adhesion process has to be accomplished by bacteria for the arrangement onto cell surfaces of the host because of withstands natural defence mechanisms and mechanical shear stress. Stable adhesion can lead to the formation of bacterial biofilms, which is accompanied by vital advantages for the microbial colonies but disadvantages for the host. Fimbriae are particularly efficient adhesion tools of bacteria to mediate colonization of various biotic and abiotic surfaces.

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Fimbriae are a hair-like lectin that is present on bacteria. Fimbriae-mediated bacterial adhesion is so important because α-D-mannosides with an aromatic aglycone moiety exhibit an improved affinity to the bacterial lectin and an enhanced potency as inhibitors of type 1 fimbriae-mediated bacterial adhesion to surfaces. As depicted in Figure 2, the carbohydrate-binding site of FimH (type 1 fimbrial lectin) has a specific interaction with two tyrosine residues (Y48 and Y137) due to π-π interactions with an aromatic aglycone of an α-D-mannoside ligand. [26]

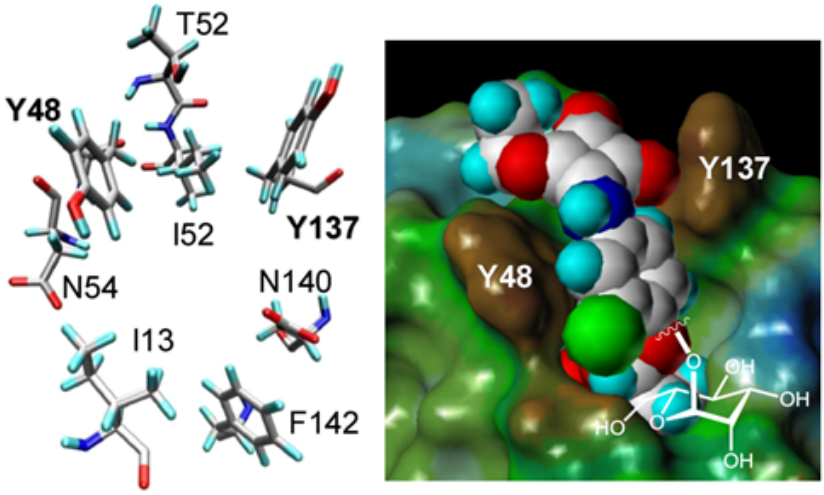

Figure 2. Spatial orientation of the amino acid residues at the entrance of the carbohydrate binding site of the bacterial lectin FimH as revealed by crystallography (*left*). Fit of top scoring conformations of designed mannoside ligands. The FimH carbohydrate binding site depicted as Connolly surface and complexed with the squaric acid derivative of α-D-mannoside (*right*). Reproduced with permission from Royal Society of Chemistry. [26]

2.1. Plant Lectins

Plant lectins are used very commonly for the examination of the multivalent binding of carbohydrates. Even though many different biological functions of lectins in plants have still not been investigated in great details, it is known that they play important roles in external

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and internal biological activities of plants such as protect the plant from fungal attack and herbivorous animals, storage of proteins, modulation of enzymatic activities, and adjustment to altered environmental conditions.[27] Legume lectins are the biggest family and the most well-studied among basic plant lectins. More than 100 lectins have been isolated and characterized structurally from the seeds of the plants in which they are present in. Concanavalin A (Con A) was the first member of this family since its discovery in 1919.[28] Con A is the most popular and low-cost plant lectin as well as it has high binding affinity with mannose and glucose. Therefore, it is usually used as a model lectin to investigate the multivalent binding of glycopolymers. Con A was originally extracted and isolated from Jack bean and its molecular weight is around 104-112 KDa. As in C-type lectins, Con A binds metallic atoms (Mn^{+2} and Ca^{+2}) and also these cations enhance significantly the binding ability of Con A with carbohydrates. Moreover, Con A is a tetramer at neutral pH with four subunits in a tetrahedral orientation where the binding sites are 72 Å apart from each other.

Peanut agglutinin (PNA) is another legume that binds to galactose, preferably to galactosyl (β,1-3) N-acetylgalactosamine, and has wide range of applications.[29] PNA shows an unusual "open" quaternary structure, where the homotetramer possesses neither 222 (D_2) nor 4-fold (C_4) symmetry.[29] This structure can be stabilised mainly by hydrophobic, hydrogen-bonded and water-mediated interactions. Moreover, a partially unfolded intermediate of PNA retains carbohydrate binding ability with affinities that are 75–85% of those of native PNA.[29] Although not always directly necessary for binding, the divalent cations help their carbohydrate binding. *Triticum vulgare* (wheat germ agglutinin, WGA) is in Gramineae family and a dimeric lectin with eight binding sites for GlcNAc that are separated by distances of 14 Å.[30] It exists in three isoforms, WGA1, WGA2 and WGA3 with a high specify to *N*-acetylglucosamine and *N*-acetylneuraminic acid. WGA has the ability to recognise specifically the pathogen for plant defence mechanisms. Ricinus

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communis (Ricin) is isolated from castor bean and binds selectively to galactose.[31] Ricin is a ribosome-inactivating protein and also used for generating immunotoxins. The two lectins, ricin and Ricinus communis agglutinin (RCA) are closely related to be one of the most toxic lectins and can cause rapid death.[32] Jacalin from the Moraceae family that extracted from the Jackfruit (Artocarpus integrifolia) can recognize to galactose (β,1-3) *N*-acetylgalactosamine and Immunoglobulin A (IgA).

2.2. Animal Lectins

Animal lectins were originally listed in 12 families, which are C-, I-, P, S-type lectins, pentraxins, trout lectins, discoidins, calnexin and calreticulin, ERGIC-53 and VIP-36, fucolectins, annexin lectins and fibrinogen-type lectins.[33] Despite of increased knowledge about their structures and functionalities, animal lectins are still more complicated in comparison to plant lectins. C-type lectins are the most commonly used animal lectins that need Ca^{+2} ions for binding with carbohydrates. The large family of C-type lectins includes collectins, selectins, endocytic receptors, and proteoglycans.[34] They differ significantly in the types of glycans that they recognize with high affinities due to their carbohydrate-recognition domains. C-type lectins serve many different functions in animal, such as cell-cell adhesion, immune response to pathogens, the control of protein levels in the blood and apoptosis. Proteins use the C-type lectin fold to bind other proteins, lipids, inorganic molecules. C-Type lectins can have a variable number of subunits with 1–8 binding sites per subunit. The C-type lectin folding is unique and a compact domain of 110–130 amino acid residues with a double-looped, two-stranded antiparallel β-sheet formed by the amino-and carboxy-terminal residues connected by two α-helices and a three-stranded antiparallel β-sheet. As depicted in Figure 3, Ca^{+2} cations in the carbohydrate-recognition domains often coordinated to amino acid residues with carbonyl side chains and these residues directly bind to sugars when Ca^{+2} is bound in site 2.

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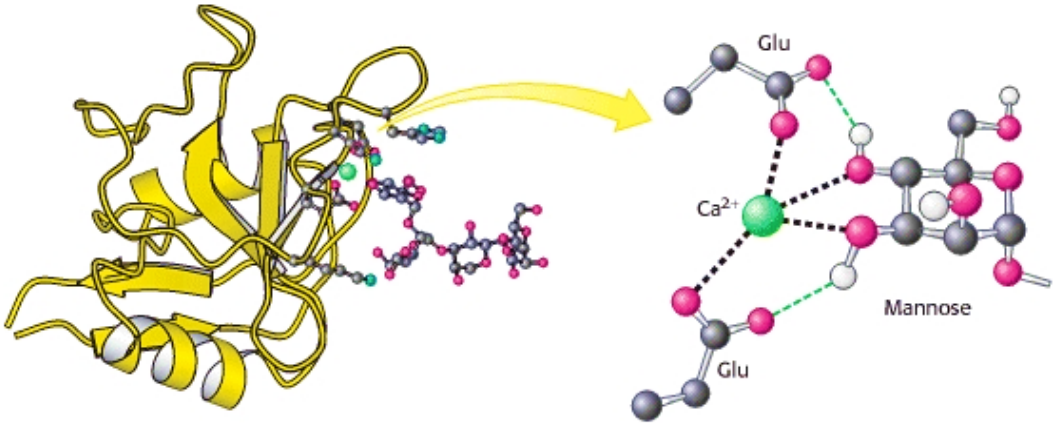

Figure 3. Structure of a C-Type Carbohydrate-Binding Domain from an Animal Lectin. Reproduced with permission from W. H. Freeman and Company, Copyright 2002.[34]

C-type lectins are the endocytic lectins as the hepatic galactose/*N*-acetylglucosamine receptor. Moreover, many C-type lectins exist as trimers, including the trimeric rat mannose-binding protein-A in complex with α-methyl mannoside. Interestingly, some C-type lectins are extremely small, e.g. hepatic intestinal pancreatic protein and pancreatic stone protein.[35] The selectins are the best characterized family of C-type lectins in terms of their extensively documented roles as cell-adhesion molecules.[35] Specific interaction between the selectins and cell-surface glycoconjugate ligands plays a key role in adhesive interactions among these cells. Selectins are classified into four subtypes: E-selectin (in endothelial cells), PSGL-1-selectin (in hematopoietic cells), L-selectin (in leukocytes) and Pselectin (in platelets and endothelial cells). They show a specific affinity to mannose, but also fucose and *N*-acetylglucosamine.

I-type lectins are from the immunoglobulin superfamily, excluding antibodies and T-cell receptors. The Siglec family of sialic acid–binding lectins is the only well-characterized group of I-type lectins regarding both structurally and functionally.[36] P-type lectins play an important role in the generation of functional lysosomes within the cells of higher eukaryotes

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by directing newly synthesized lysosomal enzymes bearing the mannose 6-phosphate signal to lysosomes.[37] The only two members of the P-type lectin family, the cation-dependent mannose 6-phosphate receptor and the insulin-like growth factor II/mannose 6-phosphate receptor, have the great ability to recognize phosphorylated mannose residues.

Pentraxins that are a family of evolutionarily conserved pattern-recognition proteins are divided into two groups: short pentraxins and long pentraxins.[38] The two short pentraxins are C-reactive protein (CRP) and serum amyloid P-component (SAP). The prototype protein of the long pentraxin group is pentraxin 3 (PTX3). CRP and SAP are produced primarily in the liver while PTX3 is produced in a variety of tissues during inflammation.[39] The short pentraxins perform several functions to recognize a variety of pathogenic agents and then to either eliminate them or neutralize their harmful effects by utilizing the complement pathways and macrophages in the host. CRP has a specific interaction with small nuclear ribonucleoproteins while SAP has recognition ability with DNA, two nuclear autoantigens. CRP participates in the resolution of cardiovascular, infectious, and autoimmune diseases. However, SAP recognizes carbohydrates, nuclear substances, and amyloid fibrils and thus participates in the resolution of infectious diseases, autoimmunity, and amyloidosis. PTX3 can interact with several ligands, including growth factors, extracellular matrix component and selected pathogens, playing a role in complement activation and facilitating pathogen recognition by phagocytes.

Galectin first appeared in 1976 and was isolated from chick muscle.[40] After its purification, the calf heart/lung galectin is approximately 15 kDa in size and occurs as a noncovalent dimer. Many galectins have now been identified in animals based on the conserved galectin carbohydrate-recognition domains. Generally, galectins have the binding ability with β-galactosides, preferably as lactose and *N*-acetyl lactosamine, and a significant sequence similarity in the carbohydrate-binding site. Galectins can contribute to cell–cell and

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cell–matrix interactions and regulate immune and inflammatory responses. Some galectins have shown the importance and necessity in cancer progression.

3. Glyconanoparticles

3.1. Metallic Glyconanoparticles

A metallic core (noble metals, magnetic elements, semiconductors) can be functionalized with different types of glycopolymers to make a carbohydrate shell. Conjugation of sugar derivatives onto these metal-based nanoclusters present interesting properties which include a wide array of assembling model and size-related electronic, magnetic and optical properties. Moreover, metallic glyconanoparticles (GNPs) opened avenues to develop the carbohydrate-based multivalent systems due to their easy modification in size and composition. In this way, multifunctional behaviour can be directly inserted in the organic shell. The interaction with suitable functionalized biological ligands onto metallic GNPs can offer an important contribution for the development of diagnostic tools and innovative therapies. GNPs can create a glycocalyx-like shell with globular shape and chemically defined composition to understand better the main functions of the carbohydrates at cell surface.

Gold and silver metallic nanoplatforms are the most common used as a metallic surface due to their high stabilization and unexpected size-related magnetic and electronic properties. The group of Penadés firstly presented gold glyconanoparticles in 2001.[41] The thiol-ended disaccharide lactose (Galβ1-4Glcβ1) or the trisaccharide Lewis X (Galβ1-4[Fucα1-2]GlcNAcβ1, Le^X) were immobilized on gold nanoparticle surfaces in the presence of reducing agent ($NaBH_4$). In another work, a simple and versatile approach succeeded to prepare gold glyconanoparticles by reducing a gold salt ($HAuCl_4$) in the presence of an excess of thiol-armed glycoconjugates.[42] Basically, there are two synthetic approaches to prepare metallic core GPNs. First one is the direct (one-step) preparation in which gold/silver salts ($AuCl_4^-/Ag^+$) are reduced by $NaBH_4$ in the presence of the desired thiol-functionalized

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glycopolymers or gold/silver surface are introduced by noncovalent interaction.[43-44] Second approach is the multi-step (generally three steps) procedure in which suitable carbohydrate derivatives are conjugated to previously prepared nanoparticles.[45]

Biotinylated gold GPNs were prepared from well-defined biotinylated glycopolymers, poly(N-isopropylacrylamide), poly(ethyleneglycol), and $HAuCl_4$ via the *in-situ* photochemical reduction of $HAuCl_4$ and glycopolymers in the presence of Irgagure-2959, a water-soluble benzoin. These biotinylated gold GPNs with a high colloidal stability showed high affinity for bioconjugation to streptavidin.[46-47] (Figure 4)

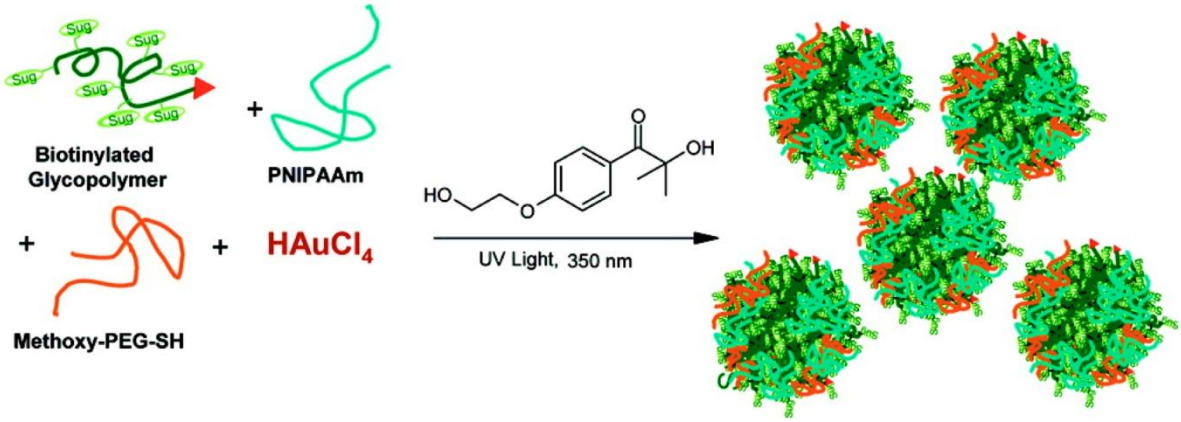

Figure 4. Schematic representation of the preparation of Au-GPNs via a photochemical process. Reproduced with permission from American Chemical Society, Copyright 2007.[47]

Multivalent manno-glyconanoparticles succeeded to inhibit DC-SIGN-mediated HIV trans-infection of human activated peripheral blood mononuclear cells.[48] These manno-GNPs showed an ability to intervene in vitro HIV direct infection of T-lymphocytes in the nanomolar range. Gibson *et al.* developed a versatile method to produce silver GPNs by reducing glycopolymers to thiol-terminal and then grafting them onto the citrate-stabilized AuNPs surface.[49]

Chen and co-workers have recently been able to prepare glycopolymer-functionalized Ag nanoclusters that showed the fluorescent and cytotoxity ability.[50] The synthesis of

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sugar- and acid-containing polymers was carried out via RAFT polymerization. Then, these glycopolymers were mixed with $AgNO_3$ and then placed into the CEM instruments. After microwave irradiation treatment, silver nanoclusters decorated glycopolymers were fabricated without using reducing agents. Silver nanoclusters were chosen to decorate the glycopolymers due to their fluorescent and cytotoxic properties providing advantages in both cancer imaging and therapy. These nanoclusters showed significant binding ability and cytotoxicity against GLUT over-expressing cancer cells K562. Furthermore, they inhibited their viability possibly through the enhancement of reactive oxygen species (ROS) production.

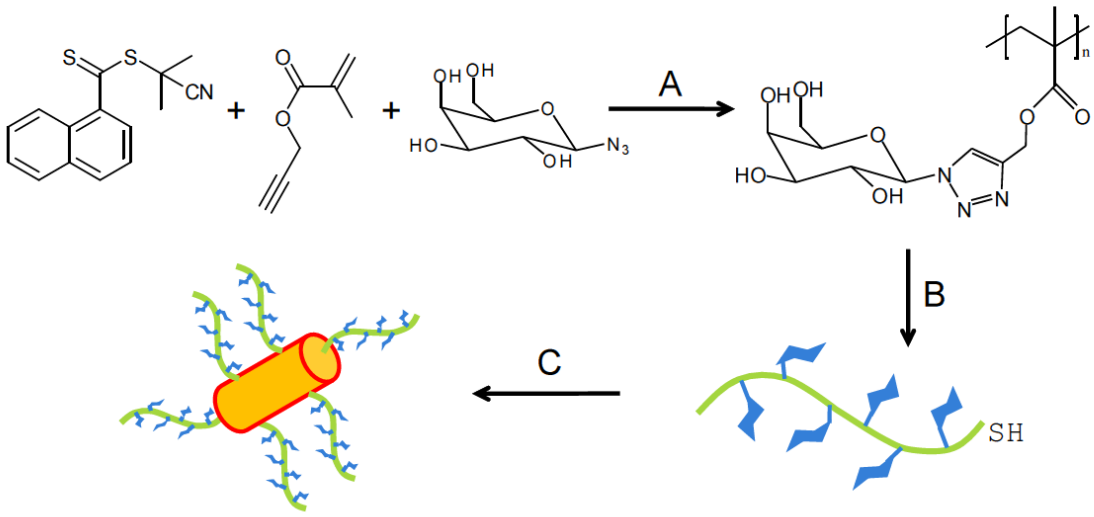

Figure 5. Synthesis of Polymer-Coated Gold Nanoparticle. Conditions: (A) copper powder, methyl 2-bromopropionate, DMSO, 25 °C; (B) 2 equiv of R-NH_2 /NEt_3, DMF, 50 C, 24h; (C) gold nanorods, H_2O, 12h. Reproduced with permission from Polymer Chemistry, Copyright 2014.[51]

Recently, the Cu(0)-catalyzed one-pot reaction for the synthesis of glycopolymers was developed to prepare gold GNPs for the first time.[51] The end-group reduction was performed to make the glycopolymers being grafted to gold nanorods. Lastly, these thiol-terminated glycopolymers covered the surface of gold nanorods to form a self-assembled monolayer on the GNPs surface due to the interaction of Au-S bond. The obtained glyco-

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nanorods were examined via Transmission Electron Microscopy (TEM) and DLS. These glycopolymer substituted gold nanoparticles showed have a great binding affinity with PNA due to the sufficient numbers of galactose groups on the surface of the nanoparticles. (Fig. 5).

3.2.Quantum dots and magnetic glyconanoparticles

Fluorescent semiconductor nanocrystals that are termed as quantum dots (QDs) have attracted great interest in the last decade due to their unique size-dependent optical properties. Quantum mechanical and electronic properties of these materials show significant differences from those of the bulk solid. Even though GNPs that contain semiconductor nanocrystals can be used in optical imaging, the initial hydrophobic QDs showed poor solubility in biological system and high cytotoxicity. Therefore, the protection process should be performed to conjugate with proteins, antibodies, DNA and other small ligands to confer stability and water solubility. QDs capped with sugars (glyco-QDs) have been used for labelling cells and optical imaging in vivo at the same time. The first study was undertaken by Fang and co-workers to use glyco-QDs as in vitro bio-labels.[52] The optical properties of mannose-encapsulated CdSe/ZnS core/shells QDs were performed in confocal microscope imaging to stain living cells. According to the results, despite of spreading of mannose-QDs over the whole sperm body, GlcNAc-QDs concentrated at the sperm heads due to the different distribution of the GlcNAc and Man receptors on the sperm surface. In another work, chitosan-QD (CS-QD) hybrid nanospheres were used for bioimaging and biolabeling.[53] The hybrid nanospheres were prepared by a non-solvent-aided counterion complexation method, in which the as-prepared QDs were added to the CS aqueous solution and crosslinked by the addition of glutaraldehyde aqueous solution. Both works revealed that QDs suitable systems can make the strong fluorescence emission and long stability in comparison to classical organic fluorescent dyes for imaging applications.

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The coupling of commercially available QDs-streptavidin with a biotin end-terminated lactose glycopolymer was reported by Chaikof *et al.*[54] Confocal microscopy confirmed fluorescent staining of Ricinus communis agglutinin (RCA_{120})-immobilized agarose beads due to the glycopolymer–lectin interaction. Chen and collaborators demonstrated a strategy that directly functionalized ZnS and ZnS: Mn^{2+} QDs with chitosan (CS-ZnS and CS-ZnS:Mn^{2+} QDs). The chitosan functionalized QDs with the mean size of 4.5 nm were obtained when a mixture of chitosan, $Zn(Ac)_2$, $Mn(Ac)_2$, and $Na_2S_2O_3$ aqueous solution was irradiated with a 1.1×10^{15} Bq ^{60}Co γ-ray source at room temperature and atmospheric pressure.[55]

Surolia *et al.* succeeded to demonstrate that melibiose-QDs selectively bound to soybean agglutinin (SBA) and were specifically deagglutinated using α-galactose.[56] Moreover, the binding ability of maltotriose-QDs with Con A was analysed via monitoring light scattered at 600 nm. (Figure 6)

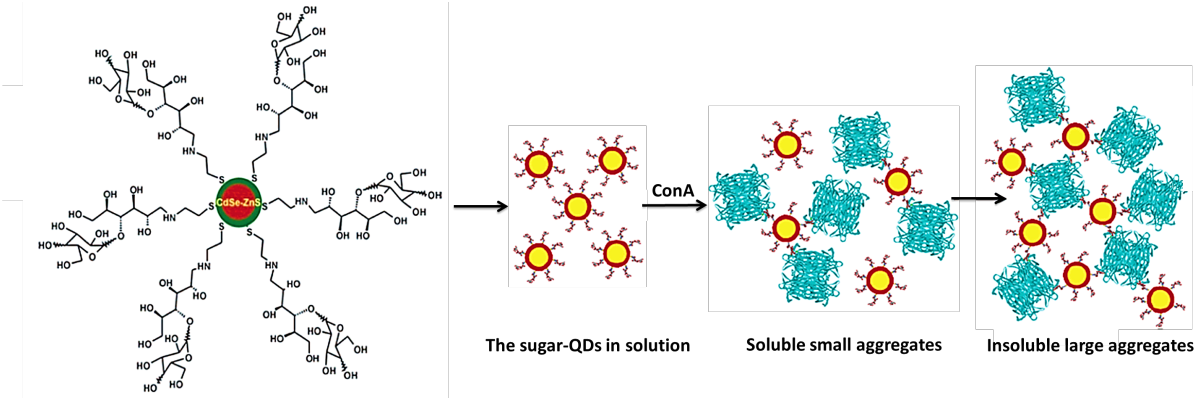

Figure 6. Schematic representation of agglutination of the sugar-QDs by ConA tetramer. Reproduced with permission from American Chemical Society, Copyright 2007.[56]

The results confirmed the specific and multivalent carbohydrate–protein interaction. Recently, biotinylated glycopolymer was functionalized with QDs by coupling RAFT synthesized biotinyl-glycopolymer to QDs-COOH in the presence of 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride. These glyco-QDs can be used as suitable

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fluorescent probes in biological systems due to their easy aqueous dispersion and potential low cytotoxicity.[57]

Magnetic nanoparticles (MNPs) that can be manipulated by an external magnetic field offer new opportunities including the improvement of the quality of magnetic resonance imaging (MRI), hyperthermic treatment for malignant cells, site-specific drug delivery and also the recent research interest of manipulating cell membranes. Moreover, they have a potential to be used in clinical diagnosis and therapies due to their unique physical properties. Magnetic cores (such as iron oxide, manganese, nickel, and cobalt ferrites) can be functionalised with suitable glycoconjugates. In general, the most common used methods of preparing magnetic nanoparticles are co-precipitation, thermal decomposition, and microemulsion. Kasteren and co-workers used cross-linked iron oxide (CLIO) amine-functionalized dextran-coated nanoparticles as a platform for the incorporation of multiple copies of sialyl Lewis^X.[58] Metal oxide surfaces can be easily modified through alkoxy-, chloro-, aminoalkyl-silanes for further coupling of biomolecules. For example, silica-coated magnetite nanoparticles functionalized with α-D-mannosides were performed through a triazole (Huisgen-type reaction) or an amide (peptidic coupling) linkage. Terminal alkynyl or carboxy functional groups were introduced onto silica oxide-coated magnetite and azidopropyl- or aminopropyl-armed α-D-mannosides were used to uniformly orientate the carbohydrates at nanoparticles' surface. The binding efficiency with mannose-MGNPs was higher in comparison with other magnetic particles functionalized with lectins.[59]

Recently, Pieters *et al.* investigated that the magnetic nanoparticles conjugated to monovalent galabiose conjuagtes and tetravalent galabiose-linked dendrimers via biotin-streptavidin coupling could be used in detecting Streptococcus suis.[60] The amount of bacteria was determined by a standard luminescence-based adenosine triphosphate detection assay after magnetic separation. According to these results, the magnetic particles

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functionalized with monovalent galabiose conjugates showed higher interaction than the tetravalent ones. The group of Davis used iron oxide nanoparticles (IONPs) to functionalize with three different glycopolymers (α-D-mannose, α-D-glucose and β-D-glucose bearing glycopolymers) via grafting to IONPs.[61]

In another work, photochemically-induced coupling of unmodified monosaccharides onto activated spherical magnetite and spindle-type hematite nanoparticles has been performed.[62] Firstly**,** 4-Azido-2,3,5,6-tetrafluorobenzamido derivatives functionalized the iron oxide nanoparticles by sonication and terminal phosphate groups were coupled to the metal oxide surface of nanoparticles creating Fe-O-P structures. Then, D-Mannose was coupled to these functionalized nanoparticles by photochemically-induced CH without chemical derivatization of the carbohydrates.(Figure 7) These synthesised mannose conjugated magnetic nanoparticles showed high recognition ability towards Con A.

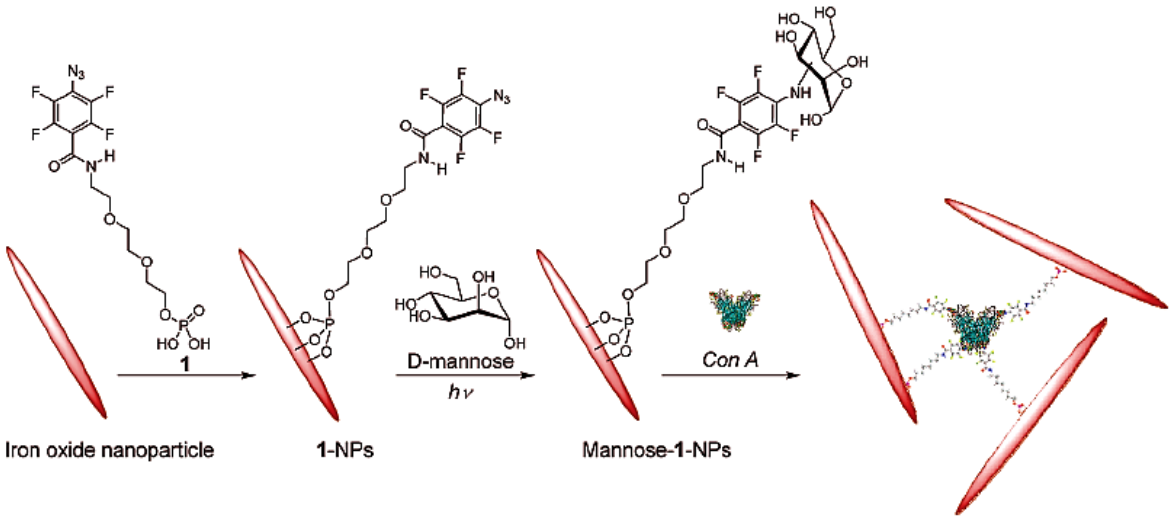

Figure 7. Functionalization of Hematite Nanoparticles with 1 Followed by Coupling of D-Mannose and Subsequent Binding with Con A. Reproduced with permission from American Chemical Society, Copyright 2009.[62]

Recently, a novel strategy was developed to produce glycopolymer modified magnetic nanoparticles by combination of a polymer "grafting from" approach with

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glycosylation by click chemistry.[63-64] Poly(N-carboxyanhydrides) was synthesised in the presence of 3-aminopropyl-triethoxysilane (APTS)-functionalized magnetic nanoparticles via ring-opening polymerization and afforded *clickable* alkyne groups. The azide-functionalized galactose was then attached on these nanoparticles to fabricate glycol-MNPs by the Huisgen click reaction. Such particles exhibited excellent water dispersion property, optimal T_1-weighting and selective binding to lectins.

3.3. Polymeric glyconanoparticles

A wide range of polymeric glyconanoparticles that were prepared in the last years exhibited excellent and significant recognition properties towards lectins. Very recent and elegant synthetic routes have allowed polymer chemists to prepare polymeric glyconanoparticles with different chemical functional groups and a broad variety of morphologies. These polymeric glyconanoparticles are promising on the creation of different bioactive glycopolymer structures for various health related applications such as drug delivery, biomaterials, bio- and nanotechnologies, and gene therapy. Chen *et al.* developed a strategy to synthesise self-assembled porphyrin-glycopolymer conjugates via the combination of RAFT polymerization and one-pot conjugation reaction as an one-pot of multi-reactions.[65] These porphyrin-PMAG conjugates showed the self-assembly behaviour to form micelles in the water due to hydrophobic porphyrin in the middle and hydrophilic glycopolymer at both ends. The binding ability of the synthesised glyconanoparticles was tested with Con A and anti-cancer effect for cancer cells (K562). They showed high and specific binding ability with Con A. Moreover, *in vitro* studies, the cytotoxic test of the glycomicelles against K562 cells in dose revealed that these self-assembled micelles killed these cancer cells under light irradiation and light treatment length dependent manners. Therefore, this report is very important for the development of applications for cancer imaging and therapy.[65] (Figure 8)

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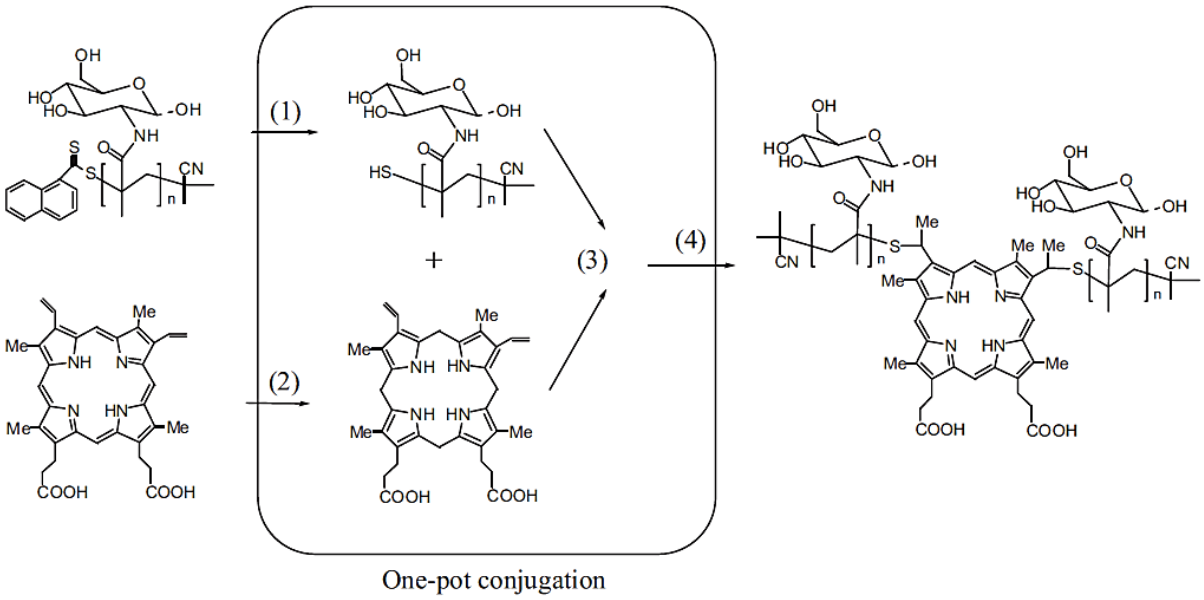

Figure 8. Schematic representation of one-pot synthesis of glycopolymer-porphyrin conjugate: (1) reduction of RAFT end group to thiols, (2) reduction of PpIX to Propoporphyrinogen, (3) thiol–ene reaction of Protoporphyrinogen with thiolterminal glycopolymer and (4) the oxidation of Protoporphyrinogen to afford the porphyrin-glycopolymer conjugate. Reproduced with permission from Macromolecular Bioscience, Copyright 2012.[65]

The synthesis of cationic poly(methacrylamidotrehalose) (poly(trehalose)) was reported by Reineke *et al.* via RAFT polymerization.[66] Nanocomplexes of these cationic polymers with siRNA were fabricated in order to develop the delivery system of targeting system to glioblastoma cells. Another versatile strategy to synthesize glycopolymers through condensation polymerization of esterified carbohydrates and diamines has also been reported by the same group. Those glycopolymers formed nanocomplex with nucleic acid and were used for gene delivery purpose.[67-68] Zhou and collaborators investigated a strategy for gene delivery system based on the synthesis of glycopolymers grafted onto Poly(l-lysine) (PLL).[69] Despite of the significant condensation capacity with plasmid DNA due to positively charged hydrophilic amino group in water, PLL is not generally chosen as gene

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delivery vectors because of high cytotoxicity and low transfection efficiency. However, in here, PLL was modified by well-defined saccharide-containing polymers in order to reduce the cytotoxicity and increase the transfection efficiency of PLL. The glycopolymer modified PLL showed lower cytotoxicity than PLL in according to the MTT assays with the mouse embryonic fibroblast cell line (NIH3T3) and human hepatoma cell line (HepG2). The pDNA condensation of the glycopolymer modified PLL complexes was investigated by agarose gel electrophoresis. The results showed that low SD (substitution degree) on PLL increased the pDNA condensation capacity. This work allowed PLL to be more useful and applicable for gene delivery with enhanced biological properties.

Chen and co-workers have performed three self-assembled nanoparticles from triblock copolymers with the same polymeric backbone but different sugar regioisomers as pendant groups.[70] Firstly, rod-block of poly(9,9-dioctylfluorene) macroinitiator was conjugated with the bromine-functionalized polyfluorene (PF) initiator from the two ends of it to exist in the middle block of the glycopolymers. Triblock copolymer (PGMA-b-PF-b-PGMA) was synthesised and reacted with NaN_3 for the sugars modified with alkynes click targeting. 1-(2'-Propargyl)-D-galactose (α:β = 10:3) and 1-(2'-propargyl)-D-mannose were prepared and used for the click chemistry that gave PF-1-Gal, PF-6-Gal and PF-1-Man. (Figure 9) Well-dispersed spheres were obtained from the polymer self-assembled into nanoparticles with the glyco block as the shell and the rod block as the core. The bioactivity of these formed nano-objects was analysed with PNA and Erythrina cristagalli agglutinin (ECA) via a quartz crystal microbalance (QCM).

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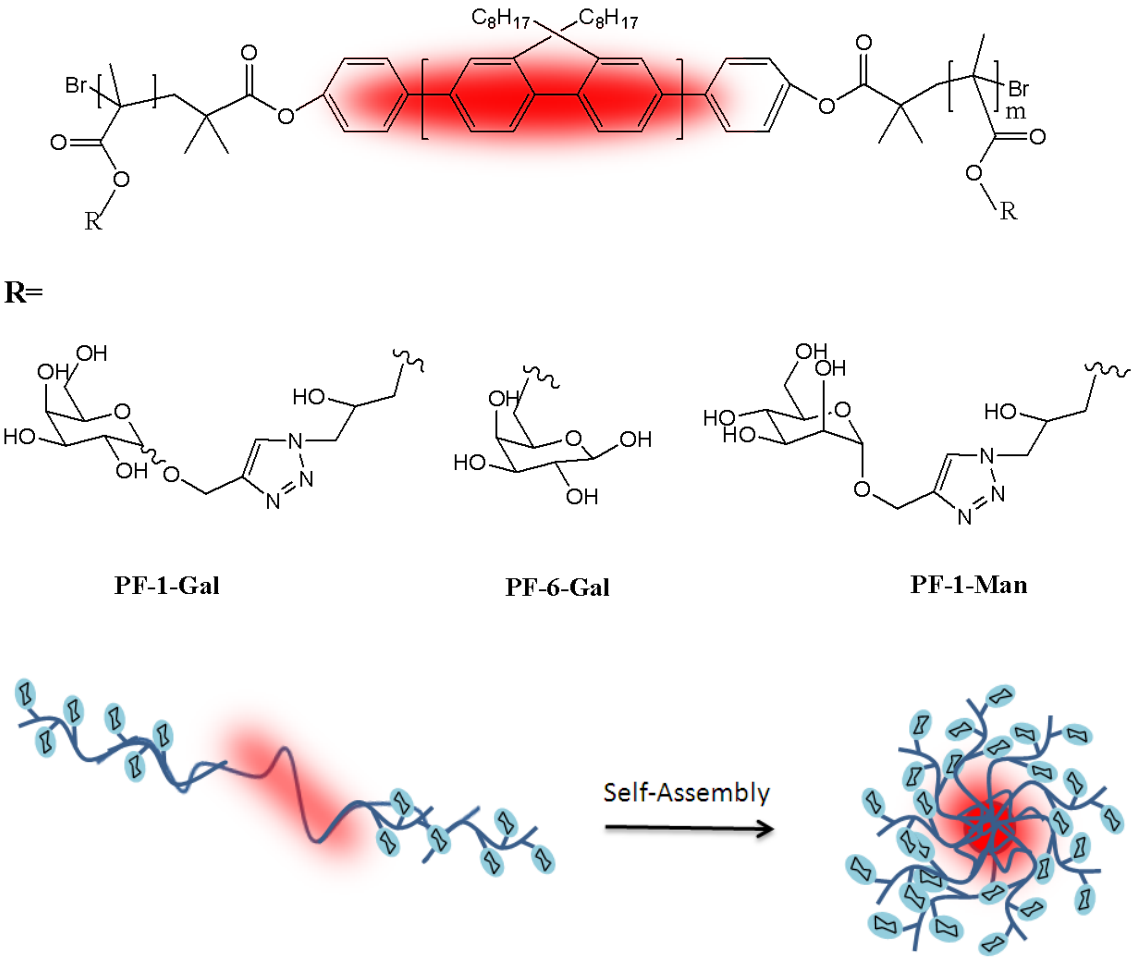

Figure 9. Chemical structures of PF-1-Gal, PF-6-Gal, and PF-1- Man and schematic representation of the self-assembled nanoparticles.

Thermoresponsive micelles were prepared from 'coil-comb-coil' triblock self-assembly glycopolymers.[71] These triblock glycopolymers were synthesized via the combination of RAFT and ring-opening polymerization. The triblock glycopolymers were grafted with ε-caprolactone at the middle block (HEMA section) to yield poly(3-O-methacryloyl-1,2:5,6-di-O-isopropylidiene-D-glucofuranose)-*block*-poly(2-hydroxyethyl methacrylate-*graft*-poly(ε-caprolactone))-*block*-poly(N-isopropylacrylamide) (PMAIpGlc-*b*-P(HEMA-*g*-PCL)-*b*-PNIPAM). Another elegant method was developed to synthesize the thermoresponsive glycopolymers poly(*N*-isopropyl-acrylamide-*co*-6-O-vinyladipoyl-D-glucose) (poly-NIPAM-*co*-OVDG; PND) and poly(*N*-isopropylacrylamide-*co*-6-O-vinylazelaicoyl-D-glucose) (poly-NIPAM-*co*-OVZG; PNZ) via free radical polymerization

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process.[72] Electrospinning process was employed to prepare nanofibers comprising blends of poly-NIPAM-*co*-OVDG/ poly-NIPAM-*co*-OVZG with poly-L-lactide-*co*-ε-caprolactone (PLCL). An electrical potential of 12 kV was applied while the spinning solution was flowing through a stainless steel capillary needle to a collector plate coated in Al foil. The obtained nanofibers were characterized by using Scanning Electron Microscopy (SEM). Additionally, MTT assay confirmed that these nanofibers have generally good biocompatibility with HeLa cells and minimum cytotoxicity as well. Even though these nanofibers did not have sufficient ability to inhibit non-specific adsorption of bovine serum albumin onto their surfaces, they showed significant interaction with ConA. This work can be utilized easily into other glycosylated polymers given their temperature sensitive properties and have the sensitive and specific recognition with lectins.

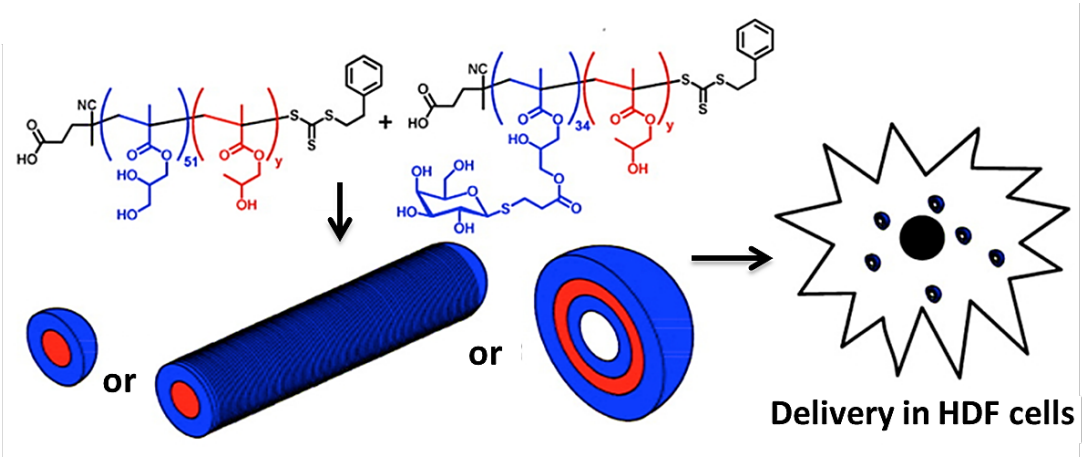

Figure 10. The preparation of self-assembled block copolymer nanoobjects (spheres, worms or vesicles). Reproduced with permission from American Chemical Society, Copyright 2013.[74]

Glycosylated peptide-based block copolymer was synthesised via the ring-opening polymerization of N-carboxyanhydrides to form well-defined different morphologies in aqueous solution.[73] Recently, Armes and co-workers presented the synthesis of galactose-functionalized diblock copolymer spheres, worm-like micelles and vesicles by polymerization-induced self-assembly approach in concentrated aqueous solution and

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exploited the relationship between the interaction of galactose-specific lectins with different morphologies of the nanoparticles.[74] (Figure 10).

Fernàndez-Garcìa and collaborators reported to prepare a various amphiphilic block glycopolymers based on 2-{[(D-glucosamin-2-*N*-yl)carbonyl]oxy}ethylacrylate (HEAGI) and *n*-butyl acrylate (BA) and methyl methacrylate (MMA) via ATRP.[75] A small library of the well-defined amphiphilic block glycopolymers having di- and triblock glycopolymers with different hydrophobic blocks and varying the hydrophilic block lengths was demonstrated. The self-assembly ability of the obtained amphiphilic block glycopolymers allowed to form micelles in aqueous solution. The Dynamic Light Scattering measurements revealed that the average diameters values are between 150 and 160 nm due to strong hydrogen bond interactions between hydroxyl groups and these polymers. Unexpectedly, the architecture of the block, diblock, or triblock glycopolymers did not show any significant influence on their interaction with Con A. In another study, the same group have proposed to synthesise different amphiphilic glycopolymers via free radical polymerization.[76] Poly(ethylene glycol) methacrylate (PEGMA) was prepared and copolymerised with methyl acrylate (MA). The free radical copolymerization of the glycomonomer and MA was undertaken using AIBN as an initiator in DMSO at 70 °C. The different amphiphilic glycopolymers with the different ratio of the monomers were prepared by using this approach. The binding affinity of these polymer coated particles with ConA was analysed via Fuorescence Microscopy. It was found that the number of glycounits in the glycopolymer stabilizers affected the binding ability directly and significantly. The synthesis of biodegradable polycarbonate displaying either glucose or galactose surface moieties was performed via controlled ring-opening polymerization to fabricate micelles in aqueous media.[77]

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4. Conclusion and future perspective

The integration of nanotechnology with carbohydrate-based systems opened a new avenue to create more complex scaffolds that exhibit excellent and significant recognition properties towards lectins. Very recent and elegant synthetic routes have allowed scientists to prepare a wide range of glyconanomaterials with different biofunctionalities. Conjugation of sugar derivatives onto metallic nanoplatforms present interesting properties which include a wide array of assembling model and size-related electronic, magnetic and optical properties. They are good biomimetic models of carbohydrate presentation at the cell surface. Glyco-quantum dots have attracted increasing attention due to their use as biological markers. Magnetic GNPs offer new opportunities including the improvement of the quality of MRI, hyperthermic treatment for malignant cells, site-specific drug delivery and also clinical diagnosis and therapies due to their unique physical properties. All these studies showed that multivalent lectin–carbohydrate interactions can be succeeded by the integration of biologically significant carbohydrates into nanosystems to develop versatile functions and the medical or pharmaceutical applications. Moreover, these GPNs can mimic the behavior of naturally existing glycocalyx.

The recent developments in carbohydrate-based nanomaterials allow the preparation of the broad diversity of glyconanoparticles that present outstanding physical, chemical and biological properties to improve their utility in biomedicine and materials science. It is now possible to insert different types of glycans in the nanosystems due to enhancement of multifunctionality. QDs can be used as a model system in biosensor applications due to its relatively small size and versatile surface chemistry functionalization, which make them useful for designing and engineering nanocarrier platforms.[78-79] Multivalent glycopolymer-stabilized AuNPs that offer to be used as a potential synthetic cancer vaccine generate a significant immune response upon binding to mucin-1 glycoproteins expressed on

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breast cancer cell lines.[80] The broad diversity of polymeric nanoparticles has been developed for their targeted delivery efficacies as non-viral vectors for gene and drug delivery applications.[81-83]

Even though considerable work has been done so far, there are still some questions that are waiting to be answered regarding the investigation of carbohydrate-based interactions. However, the limitations in the preparation of glyconanoparticles are still the major challenge. When the control of glyconanoparticle composition using different carbohydrate and non-carbohydrate ligands is achieved in extreme precision, it will open new avenues for the development of precision glyconanoparticles for *in vitro* and *in vivo* applications in the next few decades.

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