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Synthesis of Glyconanomaterials: A Review

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Abstract: Glyconanomaterials broadly defined as carbohydrate presenting structures below 100nm in size, exhibit remarkable chemical and physical properties with high potential for modern biomedical applications. The results demonstrate that this approach to carbohydrate presentation at nonmaterial surfaces leads to efficient and selective binding to cognate proteins, enabling new applications in carbohydrate-lectin recognition, profiting, biosensing, screening, cell imaging and bacteria detection.

Keywords: Glyconanomaterials, Carbohydrates, Imaging, Therapy, biomolecules.

1 Introduction

Carbohydrates are the most abundant biomolecules in nature and essential elements in a wide range of processes in living systems. [1-5] Besides their uses as structural materials and energy sources, they are to large extent mediating recognition events through their interactions with proteins and other biological entities.[6-8] Complex carbohydrate structures are thus involved in, for example, cell communication and trafficking, tumor genesis and progression, immune responses, fertilization, apoptosis, and infection.[9-11] The field has recently experienced a dramatic upsurge, much on account of the very strong developments in carbohydrate synthesis, glycan analysis methods, and nanotechnology[12-15]

Nanomaterials as scaffolds for carbohydrate ligand display have recently emerged, and glyconanomaterials have thus been synthesized, demonstrating great potential in biomedical imaging, diagnostics, and therapeutics.[16-18] Compared with molecular scaffolds, nanomaterials as ligand carriers offer a number of attractive features. Nanomaterials, being small in size, have high specific surface areas and can therefore accommodate high density ligands promoting multivalent interactions with their binding partners.[19] The ligand density can be modulated by the size and shape of the nanomaterial, and multiple epitopes of the same ligand can be exposed and presented in a three-dimensional format. Nanomaterials possess unique optical, electronic, magnetic, and mechanical properties as well as chemical reactivities [20-21] These properties, together with their nanosized dimensions, allow for their incorporation into cells for in vitro and in vivo imaging, drug-delivery, and targeting tumor cells. This opens up a wide range of possibilities, the potential of which is just emerging. [22]

Nanomaterials constitute a class of structures that have unique physiochemical properties and are excellent scaffolds for presenting carbohydrates, important biomolecules that mediate a wide variety of important biological events.[23-25] The fabrication of carbohydrate-presenting nanomaterials, glyconanomaterials, is of high interest and utility, combining the features of nanoscale objects with biomolecular recognition.[26] The structures can also produce strong multivalent effects, where the nanomaterial scaffold greatly enhances the relatively weak affinities of single carbohydrate ligands to the corresponding receptors, and effectively amplifies the carbohydrate-mediated interactions. Glyconanomaterials are thus an appealing platform for biosensing applications.[27-30] In this review, we discuss the chemistry for conjugation of carbohydrates to nanomaterials, summarize strategies, the limitations and future perspectives of these emerging glyconanomaterials sensing systems are furthermore discussed.

Nanoparticles are the subject of numerous papers and reports and are full of promises for electronic, optical, magnetic and biomedical applications. [31-3] Although metallic nanoparticles have been functionalized with peptides, proteins and DNA during the last 20 years, carbohydrates has not been used with this purpose until 2001. Since the first synthesis of gold nanoparticles functionalized with carbohydrates (glyconanoparticles) was

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reported, the number of published articles has considerably increased. [33] This review article is the progress in the development of nanoparticles functionalized with biological relevant oligosaccharides.

Nanomaterials have unique optical, electronic, or magnetic properties, thus explaining their potential applications in complex biosystems when coupled with biomolecules, such as DNA, peptides, proteins, or carbohydrates. [34] With a large surface-to-volume ratio and homogeneity in aqueous solutions, various biomolecule conjugated nanomaterials are exploited for elucidating biological interactions. During the past decade, biomolecule-conjugated nanoparticles (NPs) have been prepared and used in diagnostics, creative therapeutics, biomolecular interactions, and in vivo cell imaging.[35] For example, Mirkin et al. developed an ultrasensitive bio-barcode detection method based on oligonucleotide-conjugated gold NP (AuNP) for biomarkers in small amounts in complex biofluids. [36] (Fig.1)

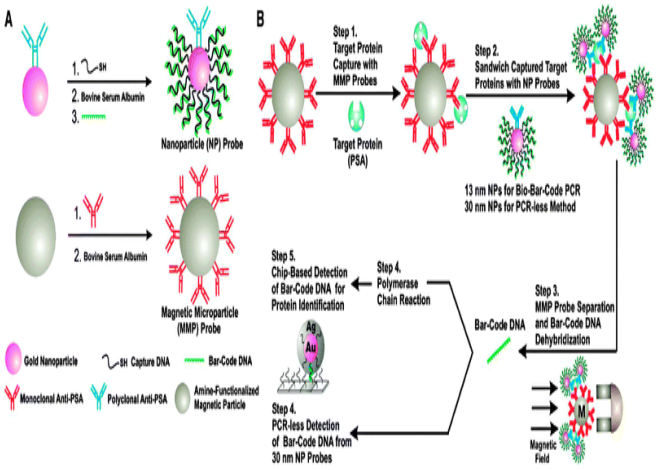

Figure 1. Bio-barcode detection method based on oligonucleotide-conjugated gold NP (AuNP)

Weissleder et al. fabricated antibody-conjugated iron oxide NPs and used them to enhance T2 signals in magnetic resonance imaging. [37] Since they have unique magnetic properties, diverse functionalized magnetic nanoparticles (MNPs) have been designed and prepared to purify target proteins from crude cell lysate by simple magnetic separation. Recently, the authors combined antibody-conjugated MNP with matrix-assisted laser desorption/ionization–time of flight (MALDI–TOF) mass spectrometry (MS) as a rapid and cost-effective detection method for diagnosing disease markers in human sera. [38] Biomolecule-modified quantum dots (QDs) have also been demonstrated as having promising applications in in vivo imaging, including cell trafficking and targeting. Besides metallic NPs, carbon nanotubes (CNTs) have also been demonstrated to be powerful carriers and to be useful in biological systems because of their high surface utilization efficiency and good size uniformity. [39-40].

2 Preparation of Glyconanomaterials

A critical step in the preparation of glyconanomaterials is the surface coupling chemistry for attaching carbohydrates to the nanomaterial. Nanomaterials come in different forms, sizes, and shapes. Conjugation chemistry should therefore be designed by taking into consideration the chemical nature of the nanomaterial to afford efficient ligand coupling and to provide optimal ligand presentation. [41-42]

2.1 Conjugation of Carbohydrates to Nanomaterials

Two general strategies for nanomaterial functionalization can be discerned, based on either noncovalent or covalent protocols. Both approaches are associated with advantages and drawbacks, although covalent protocols are generally preferred due to the considerably higher stabilities of the constructs. [43]

2.2 Noncovalent Attachment

A variety of glyconanomaterials based on physisorption of carbohydrate ligands to the material surface has been reported.[44-45] The attachment relies on noncovalent interactions, including, for example, hydrogen bonding, Coulombic interactions, and hydrophobic effects. A method for producing metallic glyconanoparticles through electrostatic adsorption was reported by Yang and co-workers, in which metal/chitosan nanocomposites were prepared on a range of different metals including Au, Ag, Pt, and Pd. [46] The nanoparticles were synthesized by reducing metal salts in the presence of chitosan, resulting in simultaneous ligand adsorption. Rosenzweig et al. synthesized dextran-coated quantum dots (QDs) where negatively charged carboxymethyldextran was adsorbed onto QDs by mixing with positively charged polylysine via electrostatic interactions.[47] Khiar et al. functionalized carbon nanotubes (CNTs) with pyrene-modified neoglycolipids.[48] Carbohydrate-conjugated, self-assembled CNT bundles could be exfoliated, yielding individual functionalized nanotubes. As noticed from these examples, a notable advantage of the physisorption strategy is that the reaction conditions are relatively mild, and minimal chemical derivatization is required for the nanomaterial substrates and the carbohydrate ligands. Nevertheless, the physical adsorption is relatively random and disordered compared to covalent linkages. In addition, the association is not sufficiently strong, which may lead to potential bond breakage during interactions, as well as increased nonspecific or unexpected interactions with the target molecules. This can significantly affect the specificity and sensitivity in applications such as biological sensing and recognition. However, as demonstrated in the mentioned examples, oligomer/polymer-based ligands can to some extent circumvent the stability problems.[49-50]

3 Covalent Attachment

The most commonly used method for conjugating carbohydrate structures to nanomaterials is based on covalent attachment. Among the various nanomaterials, gold nanoparticles (Au NPs) are the most extensively used scaffold materials especially in fundamental studies due to their ease of preparation, exceptional stability, and high reproducibility.[51] Au NPs of different sizes, shapes, and controlled dispersity can now be synthesized using simple solution-based methods. The well-established thiol– and disulfide–Au chemistry, first applied to nanoparticles using a two-phase system by Brust et al., allows the preparation of Au NPs with well-defined surfaces. [52] These surface ligands serve as a protective layer to provide high stability for the nanomaterials in media ranging from organic solvents to biological milieus. The chemistry has been widely adopted to prepare Au NPs modified with various functional groups, and biological molecules including DNA, proteins, peptides, and carbohydrates have all been successfully introduced into the system. Penadés and co-workers reported the first synthesis of carbohydrate-functionalized Au NPs. [53] (Fig.2)

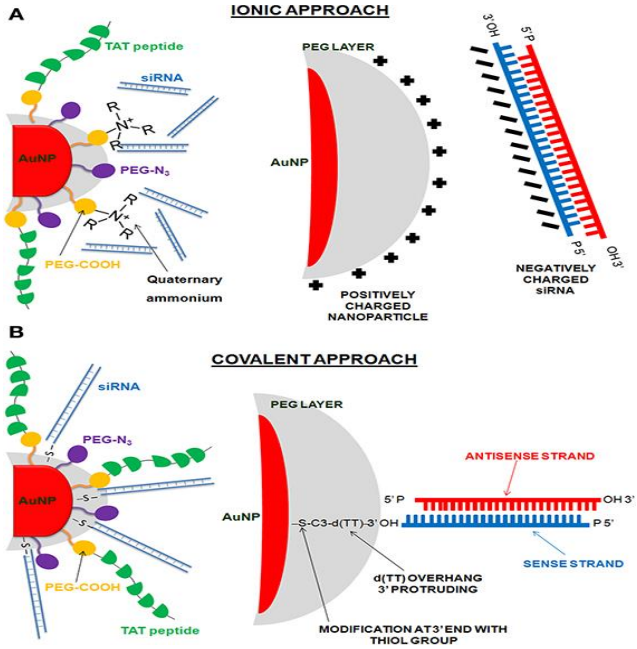

Figure 2. First synthesis of carbohydrate-functionalized Au NPs.

The trisaccharide determinant of the Lewisx (Lex) antigen was derivatized with an alkylthiol, and Lex-coated Au NPs were prepared by reducing HAuCl4 with NaBH4 in presence of the thiol-derivatized Lex. Based on this strategy, Au NPs functionalized with monosaccharide's (glucose), disaccharides (maltose), and tetrasaccharides (Ley) were prepared and applied to the studies of various biological interactions.[54] Later, several other research groups utilized a similar strategy to produce Au and Ag glyconanoparticles using thiolated carbohydrates.[55-57] Furthermore, thiolated carbohydrate derivatives have been adopted in the preparation of glyco-quantum dots (GQDs). Additional coupling methods based on the reaction of complementary functional groups have also been developed to facilitate the conjugation of carbohydrates other than the thiolated derivatives. Examples include coupling *N*-hydroxysuccinimide (NHS)-functionalized dextran to amine-functionalized Ag NPs and amine-derivatized carbohydrates to aldehyde-functionalized Au NPs.

Current methods for the preparation of carbohydrate-conjugated nanomaterials generally require the use of derivatized carbohydrates, amenable to coupling to the chosen nanomaterial surface. Un-derivatized carbohydrate structures present a considerable challenge. A few reported examples apply to flat substrates in microarray construction. One approach used hydrazide-modified gold films, where the hydrazide reacted with the terminal aldehyde group of the carbohydrates.[58] A similar approach employed amine-functionalized surfaces and the coupling of the carbohydrates took place by reductive amination. In both cases, reducing carbohydrates are necessary and, for monosaccharide's, the coupled products often became acyclic and lost their binding affinities. [59-60]

A simple method for attaching un-derivatized carbohydrates to gold and iron oxide nanoparticles is based on the well-established procedure for the covalent attachment of molecules and materials to solid substrates using functionalized perfluorophenylazides (PFPAs).The azide moiety on PFPAs can be activated by UV light, converting into the highly active nitrene that undergoes insertion reaction into diverse CH bonds and addition reaction to C=C bonds. Polymers carbon nanotubes, graphene and small organic molecules have been successfully immobilized onto PFPA-modified flat substrates and nanoparticles, providing highly robust and stable linkages. [61-62] Carbohydrates are another category of substances that are well-suited for this photo initiated immobilization chemistry. Carbohydrates have a number of CH bonds that can be used for the insertion reaction with PFPA, while leaving OH groups intact for the binding interactions with lectins. [63] More importantly, the coupling chemistry does not require chemical derivatization of the carbohydrates. This is especially attractive for higher carbohydrate structures, the synthesis of which are often complex and time-consuming due to the stereochemistry control and multiple protection/deprotection steps involved in the site-specific glycosylation and derivatization reactions.[64] The photocoupling reaction is also facile and efficient, taking place in a few minutes at room temperature in the ambient environment. Photochemical methods for carbohydrate attachment have been explored and reported in the literature.[65-66] Sprenger and co-workers employed carbenes to attach glycans and glycoconjugates in the fabrication of microarrays.[67] (Fig.3)

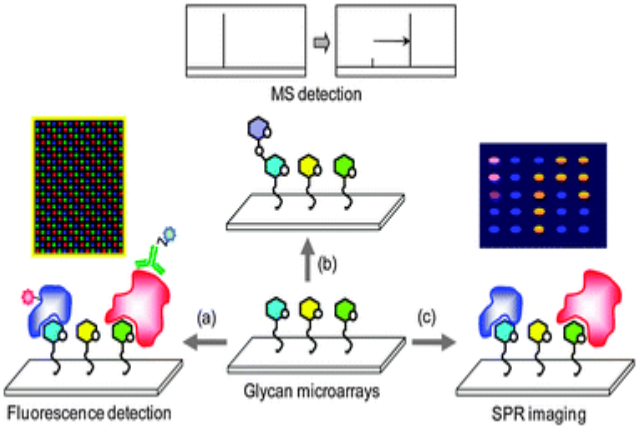

Figure 3. Carbenes to attach glycans and glycoconjugates in the fabrication of microarrays

The photoactive aryltrifluoromethyldiazirine was conjugated to dextran and was then applied to glass slides to form a photoactive coating. Activation by UV light produced highly reactive carbenes, which attached glycans via insertion reactions. In the method of Wang et al., the photoactive species was a phthalimide chromophore that induces H abstraction and subsequent recombination reaction with neighboring molecules.[68] This photochemistry was adopted to covalently attach unmodified mono-, oligo-, and polysaccharides on glass slides. PFPA as the photocoupling agent was used by Addadi and co-workers in the preparation of hyaluronan-coated polystyrene beads.[69] PFPA was first coupled onto amino-capped polystyrene beads, and hyaluronan was subsequently immobilized by UV irradiation.(Fig.4)

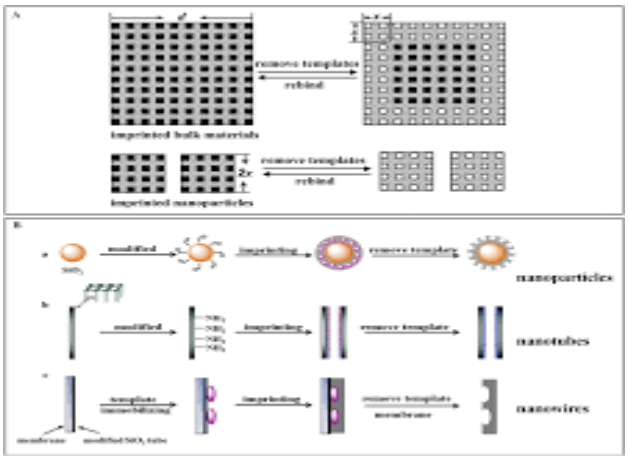

Figure 4. PFPA was first coupled onto amino-capped polystyrene beads, and hyaluronan was subsequently immobilized by UV irradiation.

PFPAs can be employed to conjugate monosaccharides and oligosaccharides to nanomaterials. Compared with polysaccharides, mono- and oligo-saccharides are smaller carbohydrate structures and more challenging for this coupling chemistry. In principle, only one covalent bond is needed to attach the entire molecule to the surface. The probability of bond formation increases with the number of CH bonds, or the size of the carbohydrate structure. Indeed, our results showed that the coupling yield increased from 57% for d-mannose, to 74% and 81% for 2-*O*-α-d-mannopyranosyl-d-mannopyranose (Man2) and 3, 6-di-*O*-(α-d-mannopyranosyl)-d-mannopyranose (Man3), respectively.

The presence of PFPA on the NPs was confirmed by NMR, FTIR, and X-ray photoelectron spectroscopy (XPS). UV–vis spectroscopy and transmission electron microscopy (TEM) images of PFPA/Au NPs showed excellent dispersibility and stability of these nanoparticles in organic solvents. To couple carbohydrates to the NPs, a solution of PFPA NPs mixed with the carbohydrate ligand was irradiated with 280-nm UV light for 5 min (Fig. 1) to yield nanoparticles that were well-dispersed and readily soluble in water. (Fig.5)

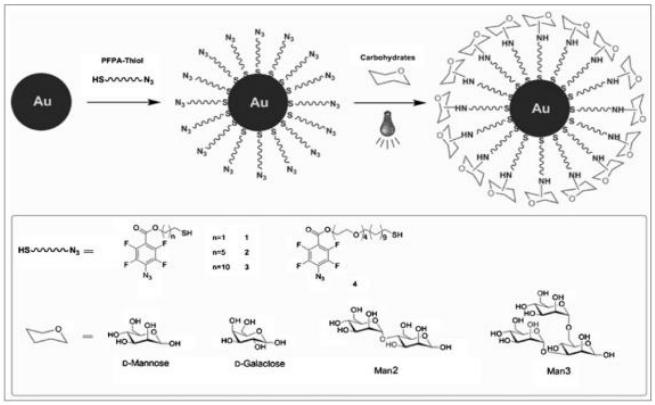

Figure5. Synthesis of Au NPs functionalized with PFPA-thiol and subsequent photoinitiated coupling of carbohydrates.

Carbohydrates constitute the most abundant organic matter in nature, serving as structural components and energy sources, and mediating a wide range of cellular activities. The emergence of nanomaterials with distinct optical, magnetic, and electronic properties has witnessed a rapid adoption of these materials for biomedical research and applications.[70-72] Nanomaterials of various shapes and sizes having large specific areas can be used as multivalent scaffolds to present carbohydrate ligands. (Fig.6) The resulting glyconanomaterials effectively amplify the glycan-mediated interactions, making it possible to use these materials for sensing, imaging, diagnosis and therapy.

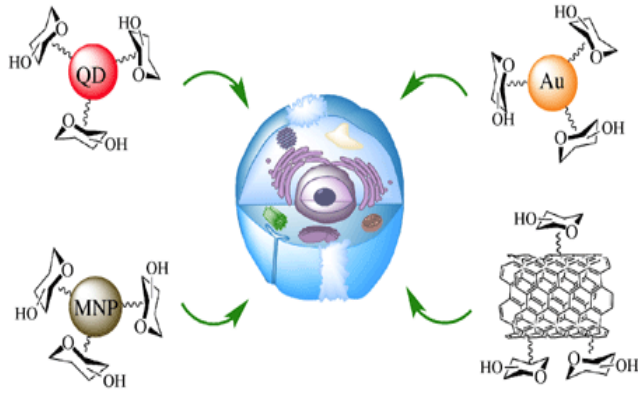

Figure6. Various types of glyconanomaterials have been de veloped and used in imaging, diagnosis, and therapeutics.

4 Preparation of Glyconanomaterials: Carbon nanomaterials

Carbon-based nanomaterials have a long history, from the oldest nanomaterial of amorphous carbon, to the newly discovered fullerenes, carbon nanotubes (CNTs) and graphene. [73-75] These materials continue to break records of material and physical properties, and hold promise to impact a wide range of fields, including electronics, sensing, imaging and therapeutics. However, several disadvantages limit their biological applications, such as poor water solubility, lack of reactive functionality, and potentially high cytotoxicity. An effective way to overcome these limitations is to introduce an organic coating on the carbon materials. Carbohydrates are in these sense suitable candidates that not only increase the biocompatibility and solubility, but also introduce molecular recognition features to the carbon materials, which can impact cellular interactions and uptake of these entities.

One approach to carbohydrate conjugation is through non-covalent interactions between carbon materials and modified carbohydrates. In this case, carbohydrates are chemically derivatized with non polar moieties such as lipids polyaromatic hydrocarbons, or porphyrins which are capable of interacting with the hydrophobic carbon materials. The resulting carbon materials are not chemically functionalized, and their properties are thus preserved. Bertozzi and co-workers coated single-wall CNTs (SWCNTs) with poly (methyl vinyl ketone) having a 18 lipid tail that could self-assemble on the SWCNTs through hydrophobic effects. [76] (Fig.7).

The polymer was functionalized with α-D-*N*-acetylgalactosamine (α-GalNAc) as pendant groups. The resulting mucin mimic-modified SWCNTs resisted non-specific protein adsorption, and could also recognize the *Helix pomatia* agglutinin (HPA) and α-GalNAc-binding lectin.

Surface functionalization of nanomaterials is an area of current investigation that supports the development of new biomaterials for applications in biology and medicine. [77-78] The synthesis, characterization, and antibacterial properties of the first examples of antibiotic-labeled graphitic carbon nanofibers (GCNFs) covalently functionalized with amino glycoside and quinolone antibiotics were described. Ruthenium tetroxide oxidation of herringbone GCNFs gave higher amounts of surface carboxyl groups than previous methods. These carboxyl groups served as sites of attachment for antibiotics by acyl substitution. Bioassay of this novel, functionalized GCNFs using serial dilution and optical density methods demonstrated that antibiotic-labeled GCNFs possess significant antibacterial activity against Pseudomonas aeruginosa.

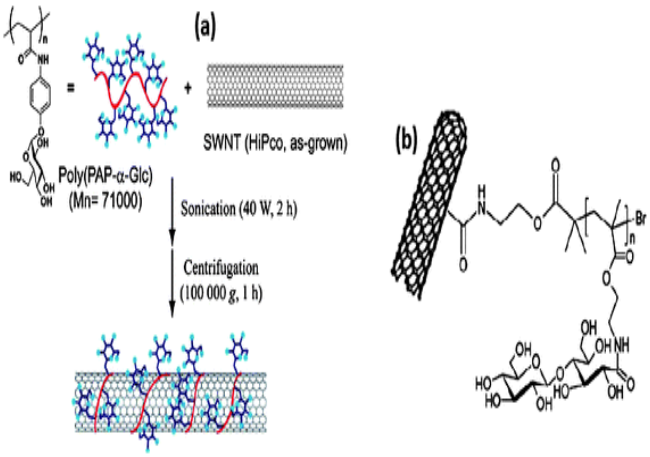

Figure 7. single-wall CNTs (SWCNTs) coated with poly (methyl vinyl ketone) having a 18 lipid tail that could self-assemble on the SWCNTs through hydrophobic effects

In another study, the group functionalized monosaccharides with pyrene, which were subsequently adsorbed on SWCNTs. The modified SWCNTs were used to promote cell adhesion and study dynamic cellular activities. The same concept was used in a work by Lin et al. where pristine graphene was functionalized with pyrene-modified maltose.[79] Upon adsorption; graphene quenched the pyrene fluorescence, which was then recovered by addition of the lectin Concanavalin A (Con A). This displacement-type assay provided a means for lectin sensing, where the detection limit was estimated to be 0.8 nM in the case of Con A.

Covalent modification requires a chemical reaction between the carbon nanomaterial and the carbohydrate. [80] In this case, either the carbon nanomaterial or the carbohydrate, or both, need to be chemically functionalized. Carbon nanomaterials are relatively inert chemically, and therefore, methods for the chemical functionalization of carbon materials often involve the use of reactive intermediates such as azomethineylides, radicals, carbenes, and nitrenes.

Among the carbon materials, fullerenes, especially buckminsterfullerene C60, have the richest and the most

established functionalization chemistry. Well-defined fullerene derivatives can be synthesized, and the number of functional groups can be precisely controlled. [81] For CNTs and graphene, the most common way to achieve covalent functionalization is to use the oxidized forms. Oxidation generates oxygen-containing functional groups, such as epoxy and carboxylic acid moieties, which can then be used to react with, *e.g.*, amine-functionalized carbohydrates. In the case of graphene, the vast majority of the literature uses the oxidized form, which can be prepared inexpensively from graphite to produce single-layer graphene oxide in large quantities. Single-layer pristine graphene, on the other hand, is still difficult to obtain, especially on a larger scale. Aryl diazonium salts are among the most used reagents to functionalize pristine CNTs and graphene. The reaction has been suggested to proceed via aryl radicals that are generated by electron transfer from the CNTs or graphene to the aryl diazonium ions after elimination of N2.[82] This chemistry was for example used by Torres et al., who prepared SWCNTs and graphene coated with α-D-mannosyl (Man) dendrons following a sequential functionalization approach.[83] A diazonium salt, prepared from 4-[(trimethylsilyl)ethynyl)]aniline with isoamylnitrite, was activated under microwave irradiation and reacted with SWCNTs and graphene. The carbohydrates were subsequently conjugated to the material via a copper-catalyzed alkyne-azide cycloaddition (CuAAC) reaction using azide-functionalized-α-D-mannosyl dendrons. Another useful functionalization method relies on azomethineylides, which undergo 1,3-dipolarcycloaddition with CNTs to form pyrrolidine derivatives.[84] This method can also be applied to indirect carbohydrate functionalization as exemplified in a study by Hong et al.[85-86] The azomethineylides were in this case generated from α-amino acids and an aldehyde, and carboxy-functionalized D-*N*-acetyl glucosamine(GlcNAc) structures were then conjugated to the resulting pyrrolidinyl CNTs .Nitrenes constitute another widely used reactive intermediate to introduce functional groups directly on pristine carbon nanomaterials. Generated by thermal or light activation, these reactive intermediates are perceived to undergo cheletropic cycloaddition reactions with alkenes to form aziridines Azide-functionalized carbohydrates was used to functionalize CNTs in refluxing chlorobenzene to render CNTs water soluble. This chemistry is very efficient, and perfluorophenylnitrene formation was reported to functionalize different pristine carbon nanomaterials. Unlike the singlet phenyl nitrene that primarily ring expands to form the dehydroazepine, the singlet perfluorophenylnitrene can undergo efficient C=C addition reactions due to its longer lifetime and higher activation energy barrier for the ring expansion reaction. Therefore, reaction with perfluorophenylazide (PFPA) resulted in covalent functionalization of fullerenes, CNTs and graphene. The properties of the materials can be tailored by the PFPA functionality. For instance, pristine graphene can be made soluble in water or common organic solvents, and CNTs can be derivatized with polymer brushes through the conjugation of an atom-transfer radical-polymerization (ATRP) initiator to PFPA.[87-88] Using PFPA-NHS to functionalize CNTs or graphene, the material can be further conjugated with amine-functionalized carbohydrates. The resulting materials selectively recognized carbohydrate-binding lectins.(Fig. 8) Methods for direct functionalization of pristine CNTs or graphene using (a), aryl diazonium salt, (b) azomethineylide,(c) alkyl azide, and (d) PFPA.

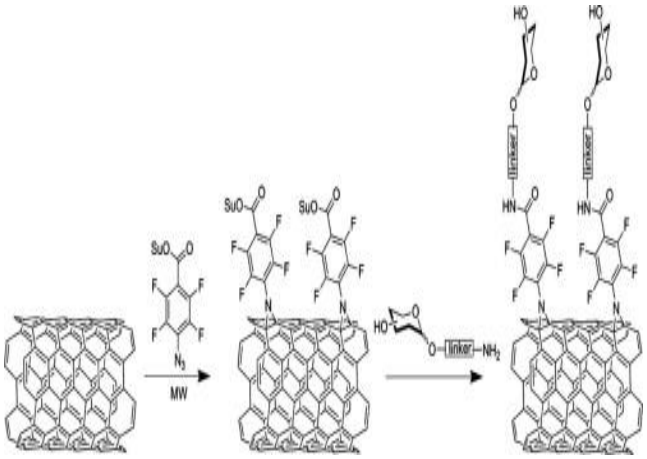

Figure 8. Carbohydrate conjugation through microwave-assisted functionalization of single-walled carbon nanotubes using perfluorophenyl azides

To take full advantage of the remarkable applications of carbon nanotubes in different fields, there is a need to develop effective methods to improve their water dispersion and biocompatibility while maintaining their physical properties. In this sense, current approaches suffer from serious drawbacks such as loss of electronic structure together with low surface coverage in the case of covalent functionalizations, or instability of the dynamic hybrids obtained by non-covalent functionalizations. Thus, the molecular basis of an original strategy was examined that combines the advantages of both functionalizations without their main drawbacks. [89-90] The hierarchical self-assembly of diacetylenic-based neoglycolipids into highly organized and compacted rings around the nanotubes, followed by photo polymerization leads to the formation of nanotubes covered with glyconano rings with a shish kebab-type topology exposing the carbohydrate ligands to the water phase in a multivalent fashion. The glyco nanotubes obtained are fully functional, and able to establish specific interactions with their cognate receptors. [91-92] In fact, by taking advantage of this selective binding, an easy method to sense lectins as a working model of toxin detection was developed based on a simple analysis of TEM images. (Fig.9). Remarkably, different experimental settings to assess cell membrane integrity, cell growth kinetics and cell cycle demonstrated the cellular biocompatibility of the sugar-coated carbon nanotubes compared to pristine single-walled carbon nanotubes.

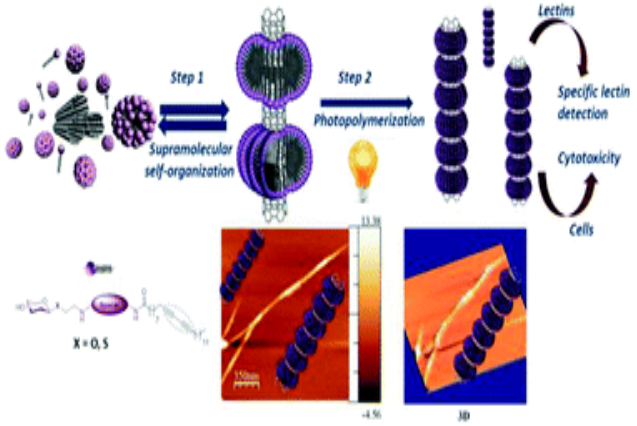

Figure 9. Self-assembly of diacetylenic-based neoglycolipids into highly organized and compacted rings around the nanotubes, followed by photo polymerization leads to the formation of nanotubes covered with glyconano rings.

A new approach was developed for the non-covalent functionalization of multiwalled carbon nanotubes (MWNTs), which allows a presentation of carbohydrate on their surface by hydrophobic interactions.[93-95] The approach is based on the self-assembly of a sugar-based amphiphile on MWNTs in alcohol/water mixtures, which has been investigated by means of ultraviolet (UV), Raman spectra, Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), X-ray diffraction (XRD), and high-resolution transmission electron microscopy (HRTEM). It was demonstrated that alcohols not only can promote the self-assembly of the amphiphile on MWNTs but also can regulate the amount and conformation of the assembled amphiphile. The adsorption of bovine serum albumin (BSA) onto functionalized MWNTs was studied and characterized with circular dichroism (CD) spectra. The results showed that the conformation of adsorbed BSA has been well preserved. It has been demonstrated that, the functionalized MWNTs with disaccharide groups on their exterior surface have a good dispersibility in water and are biocompatible, may have a potential application of molecular recognition.

The utilization of a nanomaterial wrapped in biologically relevant molecules to study and solve biomedical problems is a new and stimulating field of research.[96-97] One of the most salient features of using nanomaterials, such as nanoparticles, nanorods, nanowires and carbon nanotubes in biology is their ability to carry multiple copies of a single drug or various active principles with different, ideally synergistic, modes of action.[98] Consequently, those diseases or biological processes whose biological targets require a multivalent display of the active epitope, are expected to benefit from the application of a nanometric platform. Illustrative examples of such events are those mediated by carbohydrates, which include cell adhesion, inflammation, tumor cell metastasis, and pathogen infections. It has been shown that the weak interaction between an individual ligand and the corresponding specific lectin is compensated by the multivalent display of carbohydrates through the so called cluster effect.[99] On the other hand, single-walled carbon nanotubes (SWCNTs) as interesting 1D nanomaterials, are actually being actively investigated as vehicles for the in vivo smart delivery of biologically relevant cargoes including drugs, proteins, and nucleic acids, as nanometric sensors and for cancer treatment. However, concerns about their potential toxicity have reduced much of the original enthusiasm about their promising clinical applications. Nevertheless, recent investigations, including a pilot study, on the in vivo behavior of SWCNTs, have concluded that conveniently functionalized water soluble SWCNTs are completely cleared from the body via the biliary and renal pathway, and are non toxic.[100]

While covalent, and non-covalent, approaches have been followed for the dispersion of SWCNTs in aqueous media, the latter one is highly desirable as it conserves the nanotubes structure, while the former one has been shown to disrupt their p-network, leading to possible losses in their mechanical, electrical, and bio- sensing properties.(Fig.10).

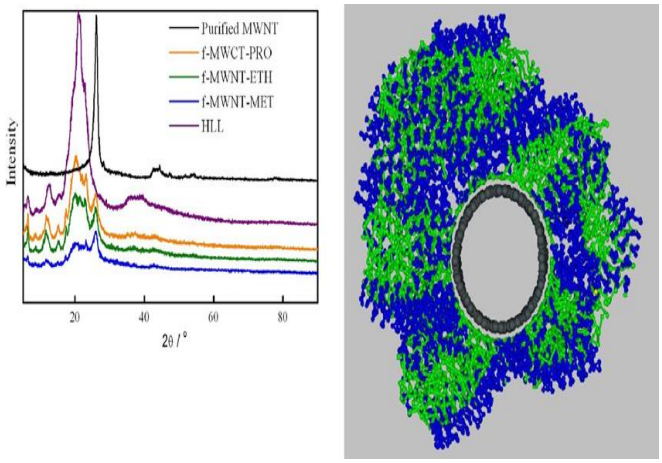

Figure 10. Dispersion of SWCNTs in aqueous media

In this review, we discuss the utilization of carbon nanotubes as molecular platforms for a multivalent presentation of biologically relevant saccharide epitopes.[101-102] The strategy is based on the utilization of neutral pyrene functionalized neoglycolipids that interact with a CNT's surface giving rise to a nanometric material with a multivalent display of carbohydrates, much like the glycocalyx on the cell surface (Fig.11). Between the pyrene tail and the glycoligand, the designed amphiphilic compound I exhibits an advantageous variable spacer derived from tetraethylene glycol for the fine-tuning of the hydrophilic-hydrophobic balance of the pyrene-polyethlyene glycol-sugar (Py-PEG-Sugar) I.

A selection of glycosylated polyacrylate nanoparticles has been reported by radical-initiated emulsion polymerization in aqueous media. Using ethyl acrylate as a co-monomer, carbohydrate acrylates were incorporated into the poly

(ethyl acrylate) framework to give stable emulsions of glyconanoparticles with an average particle size of around 40 nm.

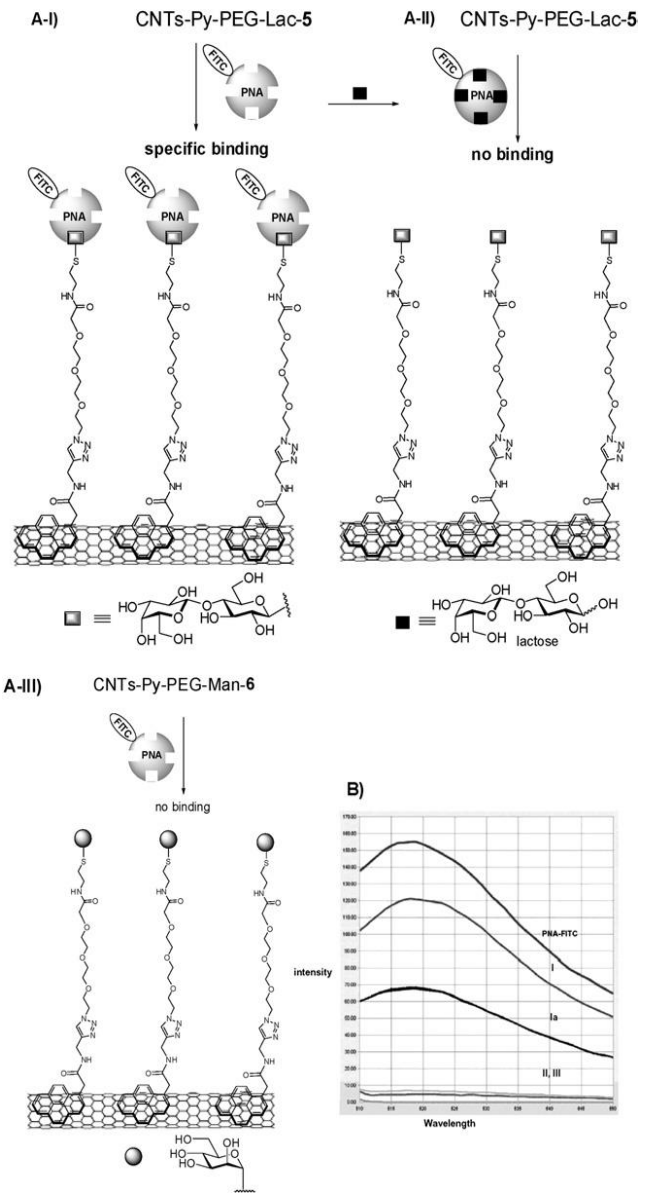

Figure 11. Specificity acquired by the aggregates toward specific receptors.(A). Schematic representation of: selective recognition of CNT- Py-PEG- Lac-5 (I), inhibition of lectin binding by adding monovalent lactose (II), absence of selective interaction of PNA-FITC with CNT-Py-PEG-Man-6 displaying an a-mannose epitope on their surface (III). (B) Fluorescence spectra of I: PNA-FITC with MWCNTs-Py-PEG-Lac-5, I-a: PNA-FITC with SWCNTs-Py-PEG-Lac-5, II: MWCNTs-Py-PEG-Lac-5 with PNA-FITC, previously incubated with lactose, III, PNA-FITC with MWCNTs-Py-PEG-Man-6. [PNA-FITC] ¼ 0.82 mM.

Using this technique a variety of glyconanoparticles were prepared from 3-O-acryloyl-1,2:5,6-di-O-isopropylidene-a-D-glucofuranose, 1-O-acryloyl-2,3:5,6-di-O-isopropylidene-a-D-mannofuranose, 6-O-acryloyl-1,2:3,4-di-O-isopropylidene-a-D-galactopyranose, 2-N-acryloyl-1,3,4,6-tetra-O-acetyl-b-D-glucosamine, 5-O-acryloyl-2,3-isopropylidene-1-methoxy-b-D-ribofuranose and 4-N-acetyl-5^0-O-acryloyl-2^0,3^0-O-isopropylidene cytidine. Scanning electron microscopy, dynamic light scattering and proton NMR analysis of the emulsions indicated essentially 100% incorporation of the carbohydrate acrylate monomer into the polymer with the exception of O-benzyl- and O-benzoyl-protected carbohydrate acrylates, which gave incomplete incorporation.[103-104] Formation of larger glyconanoparticles of 80 nm with (unprotected) 3-O-acryloyl-D-glucose and 5-O-acryloyl-1-methoxy-b-D-ribofuranose revealed the influence of free hydroxyl groups in the monomer on the particle size during polymerization, a feature which is also apparently dependent on the amount of carbohydrate in the matrix. This methodology allows for a new, simple route to the synthesis of polymeric glyconanoparticles with potential applications in targeted drug delivery and materials development.

5 Metal Nanoparticles

Metal nanoparticles, for example Au, Ag, and Cu nanoparticles, show distinct optical properties that are different from bulk materials due to the quantum confinement effect resulting from the reduction of the particle size.[105-107] The collective oscillation of electrons in the metal nanoparticles, generated by light illumination, is highly sensitive to the dielectric environment close to the nanoparticle surface. The so-called localized surface plasmon resonance (LSPR), provides a powerful means to monitor the molecular events occurring at the particle surface . Many studies have taken advantage of the change in LSPR, often resulting in a color change visible by naked eyes, as means to study carbohydrate-mediated interactions or as a detection mechanism for sensing carbohydrates. Gold glyconanoparticles as elements of the nano world belong to a group of particles with diameters not exceeding 100 nm.[108-109]. This size scale makes them conformable to common biomolecules. A gold glyconanoparticles consists of three different parts: the gold core, the linkers, and saccharide ligands. The glycocalyx-like surface of these particles mimics the presentation of carbohydrate epitopes of cell surface glycoconjugates. As a consequence, gold glyconanoparticles provide inimitable tools for probing and manipulating the mechanisms of biological processes based on carbohydrate interactions. Each component of the gold glyconanoparticles has a profound effect on the nanoparticles properties. Carbohydrate-conjugated Au nanoparticles (AuNPs) were employed to differentiate plant-legume lectins. Various AuNPs were treated lectins, and changes in LSPR were subjected to linear discriminant analysis to successfully differentiate all lectins.(Fig. 12).

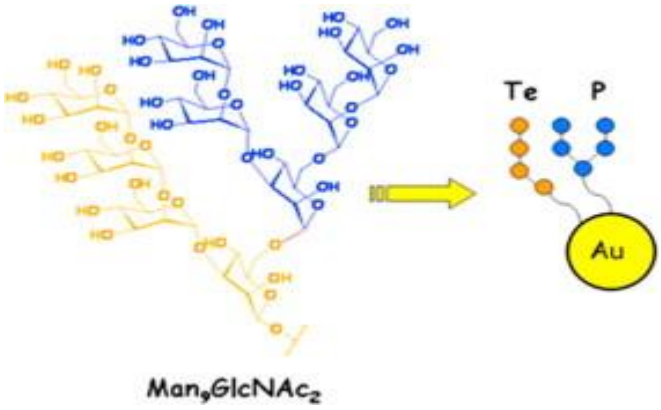

Figure 12. Assembling different antennas of the gp120 high mannose-type glycans on gold nanoparticles provides superior binding to the anti-HIV antibody 2G12 than the individual antennas.

Other studies in chemical and bio-sensing used Au and Ag nanoparticles in surface-enhanced Raman spectroscopy (SERS).[110-112] For example, lactose-functionalized Ag nanoparticles were used by Graham et al. to probe the interaction with Con A, where the SERS intensity could be enhanced for lectin detection at pico molar level .[113] Among the metal nanoparticles, AuNPs are most widely used due to their relative inert nature and ease of preparation in comparison to other metal nanoparticles. In most cases, the carbohydrates were derivatized with a thiol or a disulfide structure, and the carbohydrate conjugation was accomplished by either a one-pot protocol or a two-step process. Early examples by Penadés and coworkers demonstrated the one-pot synthetic method, where disulfide-derivatized oligosaccharides were dissolved in methanol and then mixed with an aqueous tetrachloroauric acid solution.[114] The carbohydrate-conjugated AuNPs were subsequently obtained following addition of NaBH4 under vigorous stirring. Similarly, Iyer and coworkers thiolated the trisaccharide portion of globotriaosylceramide Gb3, which was then directly added into HAuCl4 solution followed by NaBH4 reduction. The trisaccharide-conjugated AuNPs showed selective inhibition towards Shiga toxins 1 and 2. The two-step process involves the synthesis of AuNPs followed by the addition of thiolated carbohydrates. A ligand exchange reaction occurs in the second step, where the ligand such as citric acid on the as-prepared AuNPs is replaced by the thiolated carbohydrate as a result of the higher bond strength between thiols and Au than carboxylic acid. For example, thiolated lactose and glucose derivatives prepared by Russell et al. were bound to citrate-coated AuNPs by ligand replacement. Apart from these examples, a large number of other studies have involved the synthesis of Au glyconanoparticles following either of these two methods.[115-117] Post-modification of nanomaterials with carbohydrate structures is another method to conjugate carbohydrates on metal nanoparticles. In this case, a functional group is introduced on the metal nanoparticle surfaces, and carbohydrates, either derivatized or underivatized, are then conjugated to the nanoparticles through a coupling reaction. This method was, for example, used by Kataoka et al. who synthesized AuNPs with an acetal-terminated PEG-SH ligand. The acetal was then converted to aldehyde, which in turn was used to attach *p*-amino phenyl-lactose and *p*-amino phenyl-α–D-mannose by reductive amination. In a two-step process, AuNPs were first treated with a thiol- or disulfide-functionalized PFPA. Light activation of the PFPA then resulted in the covalent conjugation of carbohydrates to the AuNPs. In this case, the carbohydrates were used in their native form and no chemical derivatization was needed. (Fig.13). Using this methodology, various carbohydrates including monosaccharide's, oligosaccharides and polysaccharides were conjugated, as well as reducing- or non-reducing carbohydrates to AuNPs without affecting their binding affinities.

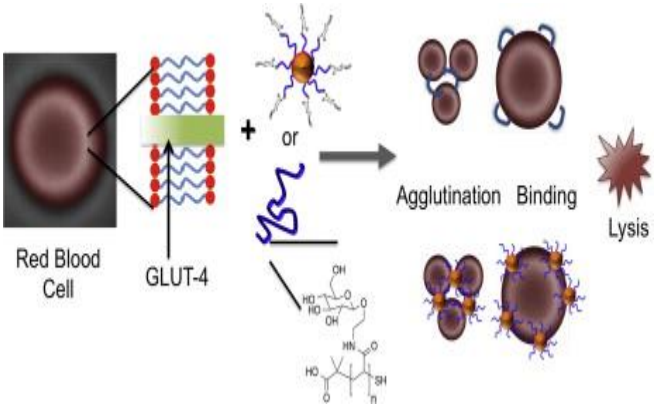

Figure 13. Synthesis and characterization of glucose-functional glycopolymers and gold nanoparticles: study of their potential interactions with ovine red blood cell

A method to measure the binding affinity of glyco nanoparticle (GNP)-protein interactions was reported which was based on a fluorescent competition binding assay, which yielded the apparent dissociation constant (K_d) of GNPs with the interacting protein. Au nanoparticles conjugated with underivatized mono-, oligo-, poly-saccharides were synthesized using photocoupling chemistry.[118-119] The affinities of these GNPs with lectins were measured and were several orders of magnitude higher than the corresponding free ligands with lectins. The effect of ligand display on the binding affinity of GNPs was furthermore studied where GNPs of varying linker type, spacer length, ligand density, and nanoparticle size were prepared and K_d values determined. The long spacer linker containing hydrocarbon and ethylene oxide units gave the highest binding affinity as well as assay sensitivity. The binding affinity increased with ligand density in general, showing a drastic increase in affinity at low ligand density. In addition, the affinity enhancement was more pronounced on smaller NPs than the larger ones. These results not only demonstrate that the binding affinity of GNPs is highly influenced by how the ligands are presented on the nanoparticles, but also pave the way for tailor-made glyconanomaterials with tunable affinity by way of ligand display.

The ability to produce monomolecular coatings with well-defined structural and functional properties is of key importance in biosensing, drug delivery, and many recently developed applications of nano-technology.[120-122] Organic chemistry has proven to be a powerful tool to achieve this in many research areas. Thus, three oligo (lactosides) were glycosylated in a (1 → 3) manner, and which are further functionalized with amide-linked short alkanethiol spacers. The oligosaccharides (di-, tetra-, and hexasaccharide) originate from the inexpensive and readily available lactose disaccharide. These thiolated derivatives were immobilized onto gold surfaces, and the thus formed self-assembled monolayer's (SAMs) on planar gold were characterized by wettability, ellipsometry and infrared reflection–absorption spectroscopy. Further, the ability of these SAMs to stabilize gold nanoparticles in saline solutions was also demonstrated, indicating that the oligosaccharides may be used as stabilizing agents in gold nanoparticle-based assays.(Fig. 14).

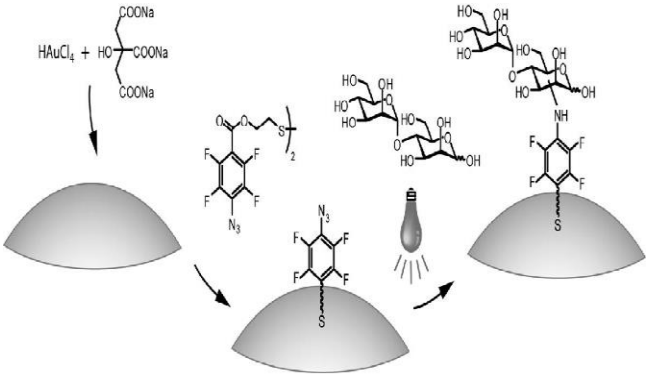

Figure 14. Synthesis of PFPA-Au NPs and subsequent coupling of α-1,4-mannobiose.

A strategy based on the utilization of neutral pyrene functionalized neoglycolipids I that interact with a CNT's surface giving rise to biocompatible nanomaterials which are able to engage specific ligand-lectin interactions similar to glycoconjugates on the cell membrane is reported.[123]

The utilization of a nanomaterial wrapped in biologically relevant molecules to study and solve biomedical problems is a new and stimulating field of research. [124]One of the most salient features of using nanomaterials, such as nanoparticles, nanorods, nanowires and carbon nanotubes in biology is their ability to carry multiple copies of a single drug or various active principles with different, ideally synergistic, modes of action. Consequently, those diseases or biological processes whose biological targets require a multivalent display of the active epitope are expected to benefit from the application of a nanometric platform. Illustrative examples of such events are those mediated by carbohydrates, which include cell adhesion, inflammation, tumor cell metastasis, and pathogen infections.[125] It has been shown that the weak interaction between an individual ligand and the corresponding specific lectin is compensated by the multivalent display of carbohydrates through the so called cluster effect.[126] On the other hand, single-walled carbon nanotubes (SWCNTs) as interesting 1D nanomaterials, are actually being actively investigated as vehicles for the in vivo smart delivery of biologically relevant cargoes including drugs, proteins, and nucleic acids, as nanometric sensors, and for cancer treatment. However, concerns about their potential toxicity have reduced much of the original enthusiasm about their promising clinical applications. Nevertheless, recent investigations, including a pilot study, on the in vivo behavior of SWCNTs, have concluded that conveniently functionalized water soluble SWCNTs are completely cleared from the body via the biliary and renal pathway, and are non toxic. While covalent, and non-covalent, approaches have been followed for the dispersion of SWCNTs in aqueous media, the latter one is highly desirable as it conserves the nanotubes structure, while the former one has been shown to disrupt their p-network, leading to possible losses in their mechanical, electrical, and biosensing properties.[127]

The ability to produce monomolecular coatings with well-defined structural and functional properties is of key importance in biosensing, drug delivery, and many recently developed applications of nano-technology.[128] Organic chemistry has proven to be a powerful tool to achieve this in many research areas. The synthesis of three oligo (lactosides) glycosylated in a (1 → 3) manner is reported and which are further functionalized with amide-linked short alkanethiol spacers. [129]The oligosaccharides (di-, tetra-, and hexasaccharide) originate from the inexpensive and readily available lactose disaccharide. These thiolated derivatives were immobilized onto gold surfaces, and the thus formed self-assembled monolayer's (SAMs) on planar gold were characterized by wettability, ellipsometry and infrared reflection–absorption spectroscopy. Further, the ability of these SAMs to stabilize gold nanoparticles in saline solutions was also demonstrated, indicating that the oligosaccharides may be used as stabilizing agents in gold nanoparticle-based assays. [130]

QDs such as CdSe, CdTe, CdS and ZnS are crystalline semiconductor nanoparticles display unique electronic properties resulting from the size-dependent quantum confinement. Broad absorption, tunable and narrow emission made them promising nanomaterials for imaging and sensing. When functionalized with carbohydrates, the water solubility and biocompatibility of the modified QDs is enhanced, in addition to exerting molecular recognition abilities. Similar to metal nanoparticles, carbohydrate conjugation to QDs can be accomplished by one-pot synthesis, ligand exchange reaction, or post-modification. Examples of the one-pot synthesis involved mixing disulfide-functionalized carbohydrate structures with Cd(NO3)2.4H2O at pH10, followed by the drop wise addition of Na2S. As QDs were formed via the combination

of Cd^{2+} andS^{2-} in basic solution, the carbohydrate disulfide ligands were directly attached to the QDs forming stabilizing layers .In an example of the ligand exchange protocol, Surolia and coworkers synthesized carbohydrate-conjugated CdSe-ZnS core-shell QDs by treating as-prepared QDs with thiol-functionalized lactose, melibiose and maltotriose.[131] Seeberger and coworkers further developed a controllable glyco-QDs synthesis method by using a continuous-flow microreactor.Cd and Se precursors were injected into a heating chamber followed by coating with Zn and S precursors in a second chamber.[132] The size and fluorescence emission of the QDs could be controlled by the precursor concentrations, temperature and flow rate. Carbohydrate conjugation was accomplished in the last step in a ligand exchange chamber using thiol-functionalized carbohydrate ligands such as α-D-mannosides or β-D-galactosides. In the post-modification method, a functional group is introduced to QDs, which is then coupled to carbohydrates. For example, Wang and coworkers modified CdS QDs with carboxy-terminated alkylthiol, which were then used to conjugate amine-derivatized carbohydrates using standard coupling reagents for amidation.[133] Other coupling methods such as thiol-ene and reductive amination have also been demonstrated to introduce carbohydrates onto QDs. For instance,Seeberger's group synthesized a series of carbohydrate-capped CdSe/ZnS core-shell QDs using the thiol-ene reaction .[134] To introduce double bonds on the nanoparticles, the QDs were first functionalized through ligand exchange with an amino-terminated PEG2000-linked dihydrolipoic acid structure, followed by conjugation with amaleimide moiety. Thiol-functionalized α-D-mannosides-D-galactosides and β-D-galactosamine derivatives were subsequently conjugated to the QDs through a thiol-ene reaction. In another example, Jana et al. synthesized glyco-QDs by reductive amination. The amine-functionalized QDs were prepared by encapsulating hydrophobic QDs into a polymer prepared by reverse micelle polymerization of acrylates containing *N*-(3-aminopropyl)methacrylamide. Carbohydrate immobilization was subsequently achieved by adding maltose, lactose and dextran to the amino-functionalized QDs in the presence of Na(CN)BH3 at pH 9 in borate buffer followed by dialysis.

6 MNPs

MNPs constitute an important class of nanomaterials suitable for biomedical imaging such as magnetic resonance imaging (MRI), and therapeutics such as hyperthermia treatment. The most frequently used MNPs in these applications are iron oxide nanoparticles including magnetite (Fe_3O_4) and maghemite (α-Fe_2O_3).[128] Iron oxide nanoparticles can be readily prepared using simple protocols to give particles in the size range of 5-20 nm. These nanoparticles can be readily dispersed in aqueous solutions to form homogeneous and stable suspensions. Iron oxide nanoparticles have excellent biocompatibility and are highly desirable for *in vivo* studies. In fact, Feraheme, a product based on carbohydrate-coated magnetite nanoparticles, have already been in clinical use for the treatment of iron deficiency anaemia.[135]

To prepare carbohydrate-conjugated MNPs, similar strategies to those of QDs can be adopted.For example, Horák et al. reported a one-pot protocol where D-mannose was directly added to a reaction mixture with $FeCl_3$and $FeCl_2$in the presence of NH_4OH.[130] In this case, D-mannose was thought to act as a metal-coordinating ligand that bound to the nanoparticles by chelation to Fe(II)/Fe(III). In this context, carboxylic acid- and phosphate-functionalized carbohydrates are also effective in binding to iron oxide nanoparticles. For example, lactobionic acid, D-gluconic acid, Ficolland carboxy-terminated glycolipids were used by Kekkonen et al. and Baccile et al. to stabilize MNPs.[136-137] These MNPs showed increasing stability with increasing carbohydrate ligand size. In the case of ligand exchange reactions, phosphate-functionalized, per acetylated mannose, rhamnose and ribose derivatives were used by Lartigue et al. to replace the original ligands on their on oxide nanoparticles such as oleic acid/oleylamine.[138] Removal of the acetyl protection groups resulted in significant increase in water solubility of the resulting nanoparticles .Other routes include initial nanoparticle functionalization to introduce a functional group, followed by carbohydrate conjugation. For example, Huang and coworkers prepared magnetite nanoparticles coated with sialic acid (Sia) using amide coupling.[139] The magnetite nanoparticles were synthesized by NH_4OH-induced co-precipitation of $FeCl_3$ and $FeSO_4$ in the presence of dextran as a coating ligand. Amino groups were subsequently introduced by treating the dextran layer with epichlorohydrin/NaOH followed by ammonia. The partially protected sialic acid derivative having a carboxylic acid end group was then conjugated to amine-MNPs by amide coupling, and the final glyco-MNPs were obtained after removing the protecting groups in aqueous NaOH. Other examples, from our group, involved the preparation of PFPA-functionalized iron oxide nanoparticles by treating the particles with PFPA-phosphate. Carbohydrate conjugation was then achieved photochemically by irradiating the dispersion of iron oxide nanoparticles and carbohydrate followed by dialysis.

7 SNPs

SNPs are widely used in biochemistry due to their outstanding biocompatibility, water dispersability, stability and functionality . Among different SNPs formats, mesoporous SNPs have over the last 20 years been developed to possess unique and advantageous properties such as tunable particle size, pore size and shape. These properties have enabled their use as drug delivery systems in for example anti-cancer therapy. Mesoporous SNPs were in this case loaded with multiple drugs and functionalized

with active targeting ligands, including carbohydrates.[140] The resulting SNPs were able to selectively target tumor cells, including multi-drug resistance (MDR) cells, leading to cancer cell death without damaging normal tissue (Table 1).

Table 1: Properties of typical nanomaterials and their biomedical applications.

Nanomaterials		Intrinsic properties	Biomedical applications
Category	Examples		
Metallic	Au, Ag	SPR	Biosensing, drug delivery, bioimagin
Semiconductor	CdS, CdSe	Fluorescence, Luminescence	Immunoassays, bioimaging, biosensing
Magnetic	Fe3O4	Magnetism	MRI, drug delivery
Carbon-Based	CNTs, Fullerene	Electronic and mechanical properties, conductivity	Drug and gene delivery, therapy, biosensing

SNPs surfaces can be efficiently functionalized with carbohydrates by post-modification methods, generally involving initial functionalization of the SNPs, and subsequent conjugation of derivatized or underivatized carbohydrates.[136] Several different conjugation chemistries have here been used, including CuAAC, amide coupling, nucleophilic substitution, and photocoupling. For example, Basuet al. synthesized azide-functionalized SNPs using ((azidomethyl) phenylethyl)-trimethoxysilane, and conjugated alkyne-functionalized carbohydrate derivatives in the presence of CuSO4/sodium ascorbate or CuI/diisopropylethylamine, while heating to 70°C in a microwave reactor.[141-143] Liu et. al. utilized amide coupling to conjugate galactose (Gal) derivatives onto SNPs.[144] The SNPs surface wasfirstfunctionalizedwith*N*-(β-ethylenamine)-γ-propylaminetriethyloxylsilane and lactobionic acid was then coupled to the amino-functionalized surface in the presence of amide coupling reagents. Gary-Bobo et al. prepared mannose-functionalized mesoporous SNPs by coupling aminopropyl-functionalized SNPs to squarate ester-derivatized α-mannose.[145] For the photo initiated carbohydrate conjugation to SNPs, a method developed was the nanoparticles were first functionalized with PFPA-silane, after which the carbohydrates were conjugated by irradiation in the presence of carbohydrates.[146] Using this methodology, carbohydrate-functionalized SNPs were prepared from different mono- and oligosaccharides.

Over the past decade, diagnostics and therapeutics have changed gradually towards the use of more specific and targeted approaches.[146-147] The most profound impact has been in the nanotechnology sectors, where an explosion in directing biomolecules to specific biomarkers has illustrated great potentials not only in detection but also in targeted therapy. Increased knowledge of the diseases at the molecular level catalyzed a shift towards identifying new biological indicators. In particular, carbohydrate-mediated molecular recognitions using nano-vehicles are likely to increasingly affect medicine opening a new area of biomedical applications. This article provides an overview of the recent progress made in recruiting the "*sugar code"* functionalized on various nano-platforms to decipher cellular information for both *in vitro* and *in vivo* applications.[148-149] Today's glyco-technologies are enabling better detection with great therapeutic potentials.(Table 1). Tomorrow they are likely to bring a full understanding of the "cell-glyconanomaterial bio-conversation" where major biomedical problems will be overcome translating insights from the "glyco-nanoworld" into clinical practice.[150-152]

Creative Glyconanoparticles provides a simple and environmental friendly method to prepare glyconanoparticles (GNPs). Nanoparticles functionalized with glycans can be applied as powerful solid-phase chemical tools for the study of protein–carbohydrate interactions using nanoscale properties for detection of binding events. This method allows the easy synthesis of stable glyconanoparticles with reduced dispersion and controlled size.[149-150] Creative Glyconanoparticles has been reported that boost up the study and applications of carbohydrates in glycobiology, biomedicine and material science. (Fig.15)

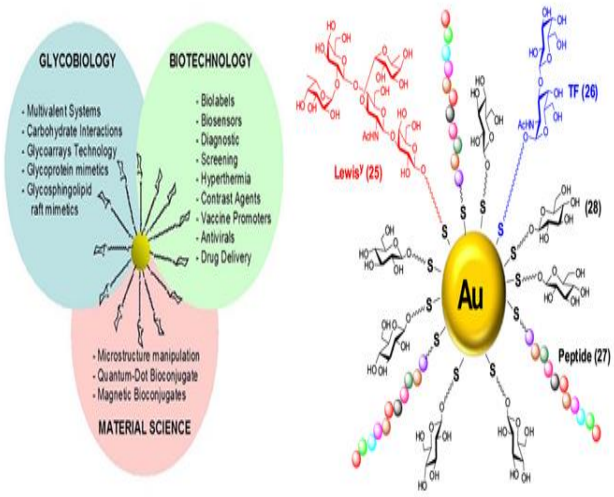

Figure 15. Applications of Glyconanoparticle

8 Characterization of Glyconanomaterials

Glyconanomaterials are synthesized under various conditions using specific chemistry and reagents. These materials must therefore be carefully evaluated to fully characterize the structure, composition, density of surface ligands, and biological activities in order to obtain proper correlation with their performances.[151-153] Conventional chemical analytic techniques that are insensitive to flat

substrates can be readily adopted to the significantly increased specific surface areas of nanomaterials for nanomaterial characterization.[154-155] In the paper by Brust et al. on the preparation of thiol-capped Au NPs, the products were characterized by FTIR showing the presence of alkanethiol and TEM revealing the size and shape of the nanoparticles.[156] With the rapid development of advanced analytical tools, especially sensitive surface characterization techniques, nanomaterials can now be analyzed more accurately, providing in-depth understanding of the chemical and physical properties of glyconanomaterials. NMR, FTIR, and surface-enhanced Raman spectroscopy (SERS) offer detailed structural analysis of nanomaterials and surface ligands. Thermo gravimetric analysis (TGA) yields the amount of organic components on the nanomaterials, from which the ligand densities can be derived.[157] Elemental analysis and XPS provide information on the elemental composition and chemical state of the bulk nanomaterials and the surface ligands. A combination of microscopy techniques, scanning probe techniques (STM, AFM), TEM, and small-angle X-ray scattering (SAXS) reveals the physical characteristics of size, shape, and assembly behavior of the nanomaterials.[158-160] Caution should be used when analyzing the results as the experimental conditions applied to each technique (vacuum, ambient, solution) can significantly impact the outcome. Microscopic techniques can also be used to directly visualize the interactions of glyconanomaterials with their binding partners. When d-mannose-functionalized iron oxide nanoparticles were treated with *Escherichia coli* strain ORN178, the nanoparticles selectively bound to the FimH lectin on the bacteria, which was clearly shown by TEM. The surfaces can be further characterized by taking advantage of the unique properties offered by the nanomaterials.[161-162] Classic examples are metal nanoparticles, which exhibit plasmon resonance that is highly sensitive to the surface constituents and can be conveniently monitored colorimetrically, as the molecular recognition event occurs at, or close to, the surface of the nanoparticles.[163-165]

Carbohydrate–lectin interactions of free ligands in solution have been studied by many biochemical and biophysical methods including NMR spectroscopy, surface plasmon resonance (SPR), X-ray crystallography, isothermal titration calorimetry (ITC), and fluorescence spectroscopy.[166-168] Quantitative analysis of glyconanomaterials is investigated to a lesser extent and only a few protocols were reported to determine the binding affinity of glyconanoparticles.[169-170] Lin and coworkers used SPR to analyze the multivalent interactions between mannose-, glucose-, or galactose-encapsulated gold nanoparticles with Con A. A competition binding study was carried out where equilibria were established between mannopyranoside attached on the SPR sensor, Con A, and varied concentrations of mannose-encapsulated Au NPs.[171] The dissociation constant K_d of mannose/Au NPs with Con A was determined to be 2.3 nM, representing a binding affinity over 5 orders of magnitude higher than that of the free d-mannopyranoside with Con A in solution (K_d = 470 μm measured by ITC. In the system developed by Wu and co-workers, magnetite/gold core/shell nanoparticles coated with proteins were allowed to interact with carbohydrate ligands on a glycan array. A magnetic field was applied to amplify the protein–carbohydrate interactions and the signals were visualized and quantified using a silver enhancement reagent. Apparent K_d values of 66 nm, 61 nm, and 57 nm were determined for Man, Man4, and Man9 ligands with Con A, respectively. [172]

Binding Affinity of Glyconanomaterials Biomedical imaging, therapeutics, medical diagnosis, and drug delivery are among the many areas glyconanomaterials have the potential to impact. The interaction of glyconanomaterials with biological receptors and targets is a critical process involved in these applications and the binding affinity is thus an important parameter for evaluating the performance of glyconanomaterials.[173-175] (Fig.16).

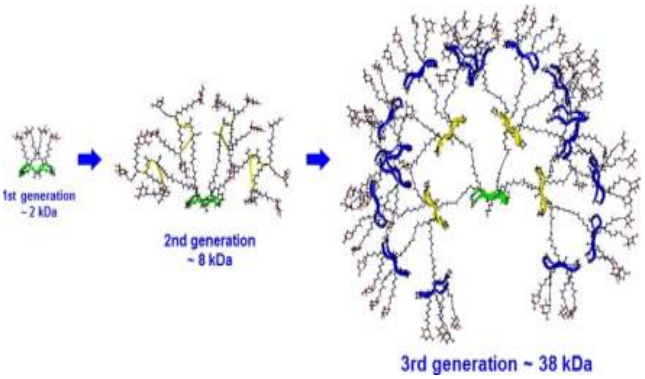

Figure 16: Multivalent glycocyclopeptides: toward nano-sized glycostructures

Fluorescence-based competition assay was developed to determine the binding affinity of glyconanoparticles with lectins.[176-177] In the assay, a fixed concentration of a free ligand (for example, d-mannose) and varying amounts of ligands bound to Au NPs were incubated with fluorescein isothiocyanate (FITC)-labeled Con A. The solution was then centrifuged and the fluorescence intensity of the supernatant was measured. Two equilibria co-exist in the system: FITC-Con A with free d-mannose and FITC-Con A with d-mannose bound on nanoparticles (Fig. 17). Since very low concentrations of Con A and free d-mannose were used, it was assumed that no agglomeration occurred. Both interactions are reversible, and steady equilibria are reached rapidly.

In order to calculate the binding affinity constant, the concentration of the carbohydrate ligand on Au NPs must be determined.[178-180] The colorimetric assay of anthrone-sulfuric acid was adopted to measure the ligand density on the nanoparticles. A calibration curve was first established using the corresponding free carbohydrate, and the amount of surface-bound ligand on the Au NPs was subsequently determined. The fluorescence intensity

measured from the competition studies was plotted against the concentration of d-mannose on the Au NPs (Fig. 4).The result was a typical concentration-response curve for ligand–receptor binding, validating the assumptions made for the system. The concentration of ligands displaying 50% of specific binding (IC_{50}) value was subsequently derived and the apparent dissociation constant (K_{d2}) calculated using the Cheng–Prusoff equation (Eq.1).

$$Kd2=IC50/1+[M]/Kd1$$

Equation 1: where [M] is the concentration of free ligand, i.e., d-mannose, K_{d1} is dissociation constant of free ligand to Con A, and K_{d2} is the apparent dissociation constant of surface bound d-mannose to Con A.

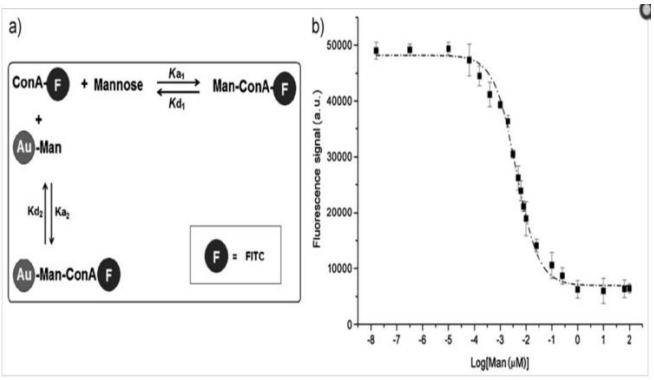

Figure 17: Equilibria involved in the competition binding assay. b) Concentration dependent fluorescence intensity curve (right). [Man] is the concentration of d-mannose on NPs determined using the anthrone/H_2SO_4 colorimetry assay.

9 Ligand Presentation and Binding Affinity

Nanomaterials, being three-dimensional in shape and small in size, are capable of hosting ligands in higher densities in comparison to their flat counterparts due to greatly increased specific surface areas.[181-182] This has significant implication for glyconanomaterials where the substrate configuration could dictate the ligand presentation and cooperativity and thus impact the interactions of the glyconanomaterials with their binding partners. The result is markedly enhanced affinities of these glyconanomaterials with the relevant biological targets. Data showed that the apparent K_d of d-mannose tethered on Au NPs with Con A can be as low as 0.43 nm (Table 2), representing a binding affinity of over six orders of magnitude higher than for free d-mannopyranoside with Con A. These results demonstrate that nanoparticles are excellent scaffolds for amplifying the weak affinities of carbohydrate ligands with lectins. Similar observations were reported by Sun and co-workers where single-walled carbon nanotubes functionalized with mannose and galactose selectively bound anthrax spores, inducing aggregation of the spores in the presence of Ca^{2+}.[183] In contrast, carbohydrate-conjugated polystyrene beads did not exhibit the observed affinity towards anthrax spores. This was attributed to the ability of nanotubes to promote multivalent interactions of the carbohydrate ligands with receptors on the spores.[184]

Table 2: Binding affinity of D-mannose on Au NPs with Con A. Au NPs were functionalized with PFPA–thiol before d-mannose was coupled.

PFPA-thiol	Spacer	K_d [nM]
1.	CH_2CH_2	19± 2.2
2.	$CH_2(CH_2)_4CH_2$	15± 2.0
3.	$CH_2(CH_2)_9CH_2$	5.3± 0.72
4.	$CH_2(CH_2)_9CH_2(OCH_2CH_2)_4$	0.43± 0.044

Unlike the free ligand that has the translational and rotational freedom in solution, the surface-bound ligand is no longer an unrestricted entity. Each ligand becomes a member of the nanomaterial carrier and can act cooperatively when interacting with their binding partners.[185-186] The efficiency of the ligand association with the binding site, i.e., the binding affinity, is sensitive to a number of factors: how the ligand is attached, i.e., the coupling chemistry, the type and length of the spacer connecting the ligand and the nanomaterial, the flexibility/rigidity of the spacer, the density of ligands, and the distance between them.

10 Ligand Density

Ligand density is another important parameter affecting the binding of surface-tethered carbohydrates.[187-188] A few studies report the impact of ligand density on the binding affinity of carbohydrates immobilized on a flat surface, but the topic has not been extensively investigated for glyconanomaterials. Wong and co-workers used a fluorescence assay to analyze mannose–Con A interaction on glycan microarrays. K_d decreased from 214 nM to 76.8 nM when the mannose printing concentration increased from 0.6 μm to 80 μm.[189] However, K_d increased to 80.4 nm when the mannose printing concentration increased to 100 μm. Corn and co-workers employed the technique of SPR imaging to study carbohydrate–protein interactions using carbohydrate microarrays.[190] The binding affinity, measured by the adsorption coefficient (K_{ADS}), increased slightly from $5.0 \times 10^6\ M^{-1}$ to $5.6 \times 10^6\ M^{-1}$ when the surface mannose concentration increased from 10% to 50%, but remained unchanged up to 100% of the surface Using the photocoupling chemistry, glyconanoparticles were synthesized and the relationship between ligand density and the binding affinity of the resulting glyconanoparticles was studied.[191-192] The ligand density was controlled by adding varying amounts of 1-hexanethiol to PFPA–thiol 3, and treating Au NPs with the mixed thiols before d-mannose was coupled. The ligand density was measured by the anthrone-sulfuric acid assay, and the apparent K_d values were determined by the fluorescence competition assay.

Results show that the binding affinity increased with the ligand density (Table 2). Interestingly, the binding affinity of the glyconanoparticles with only 2.8% of surface coverage was over 3,800 times higher than that for free d-mannopyranoside with Con A in solution. The binding affinity in123±16creased 5.7 times from 123 nm to 21.4 nm when the ligand density increased only 2.6 times from 2.0 to 5.3 nmol mg^{-1} NPs. In both cases, multivalency could well be in play, demonstrating the enormous power of the ligand cooperativity.(Table 3).

Table 3: Binding affinity versus ligand density. The Au NPs were functionalized with mixed thiols of 1-hexanethiol and PFPA–thiol 3.

% of PFPA-Thiol 3	D-Mannose density [nmol mg^{-1} Au NPs]	Surface Coverage [%]	K_d [nM]
10%	2.0±0.54	2.8	123±16
30%	5.3±0.83	7.4	21.4±7.3
50%	11.2±2.32	16	16.3±5.4
70%	24.3±2.78	34	14.4±2.4
90%%	35.2±1.29	49	12.7±4.0
95%	42.6±3.81	59	9.4±2.3
98%	50.3±4.17	70	6.7±1.4
100%	57.4±3.21	80	5.3±1.6

mannose density.

11 Conclusions

Combining nanotechnology with glycobiology has triggered an exponential growth of research activities for the design of novel functional bionanomaterials (glyconanotechnology). More specifically, recent advances in the tailored and versatile synthesis of glycosylated nanoparticles (glyconanoparticles), considered as synthetic mimetics of natural glycoconjugates, have paved the way towards diverse biomedical applications. The accessibility of a wide variety of these structured nanosystems, in terms of shape, size, and organization around stable nanoparticles, has readily contributed to their development and application in nanomedicine. In this context, glycosylated gold nanoparticles, glycosylated quantum dots, fullerenes, single-wall nanotubes, and self-assembled glyconanoparticles using amphiphilic glycopolymers or glycodendrimers have received considerable attention for their application in powerful imaging, therapeutic, and biodiagnostic devices.

Together with sensitive detection devices, inhibitors of bacterial adhesion to host tissue, and cancer vaccines in therapeutic systems (including photo sensitizers for photodynamic therapies), these novel bionanomaterials are finding widespread relevance.

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List of Abbreviations

- DNA: Deoxyribo Nucleic Acid
- NPs: nanoparticles
- AuNP: Gold : nanoparticle
- MNPs: magnetic nanoparticles
- MALDI–TOF: matrix assisted laser desorption ionization-time of flight
- MS: mass spectrometry
- QDs: quantum dots
- CNTs: carbon nanotubes
- $HAuCl_4$: Chloroauric acid
- $NaBH_4$: sodium borohydride
- GQDs: glyco-quantum dots
- NHS: *N*-hydroxysuccinimide
- UV: Ultraviolet
- PFPA: perfluorophenyl azide
- SWCNTs: single-wall carbon nanotubes
- α-GalNAc: α-D-*N*-acetylgalactosamine
- HPA: Helix pomatia agglutinin
- GCNFs: graphitic carbon nanofibers
- Con A: Concanavalin A
- C60: buckminsterfullerene
- GlcNAc: D-*N*-acetylglucosamine
- ATRP: atom-transfer radical-polymerization
- PFPA-NHS: perfluorophenyl azide- N-Hydroxysuccinimide
- MWNTs: multiwalled carbon nanotubes
- XRD: X-ray diffraction
- HRTEM: high-resolution transmission electron microscopy
- BSA: bovine serum albumin
- CD: circular dichroism
- Py-PEG: Pyrene-polyethylene glycol
- PNA-FITC: Fluorescein isothiocyanate- Peptide nucleic acid
- LSPR: Localized surface plasmon resonance spectroscopy
- SERS: surface-enhanced Raman spectroscopy
- Gb3: globotriaosylceramide
- GNP: : glyco nanoparticle
- K_d: dissociation constant
- SAMs: self-assembled monolayer's
- p-network: Polymer network
- MRI: magnetic resonance imaging
- Sia: sialic acid
- SNP: Single-nucleotide Polymorphism.
- CuAAC : Copper-Catalyzed Azide-Alkyne Cycloaddition
- MDR: multi-drug resistance
- NMR: Nuclear magnetic resonance
- FTIR: Fourier transform Infrared spectroscopy
- TGA: Thermo gravimetric analysis
- SAXS: small-angle X-ray scattering

- STM: Scanning tunneling spectroscopy
- AFM: AFM
- TEM: Transmission electron microscopy
- SPR: surface plasmon resonance
- ITC: isothermal titration calorimetry
- FITC: fluorescein isothiocyanate
- K_{ADS} : adsorption coefficient

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