MINIREVIEW

Engineering Nanomaterial Surfaces for Biomedical Applications

XIN WANG,* LI-HONG LIU,* OLOF RAMSTRÖM,*,† AND MINGDI YAN*,[1]

*Department of Chemistry, Portland State University, Portland, Oregon 97207-0751; and
†Department of Chemistry, KTH - Royal Institute of Technology, S-10044 Stockholm, Sweden

Nanomaterials, possessing unique physical and chemical properties, have attracted much interest and generated wide varieties of applications. Recent investigations of functionalized nanomaterials have expanded into the biological area, providing a versatile platform in biomedical applications such as biomolecular sensing, biological imaging, drug delivery and disease therapy. Bio-functions and bio-compatibility of nanomaterials are realized by introducing synthetic ligands or natural biomolecules onto nanomaterials, and combining ligand-receptor biological interactions with intrinsic nanomaterial properties. Common strategies of engineering nanomaterial surfaces involve physisorption or chemisorption of desired ligands. We developed a photochemically initiated surface coupling chemistry, bringing versatility and simplicity to nanomaterial functionalization. The method was applied to attach underivatized carbohydrates efficiently on gold and iron oxide nanoparticles, and the resulting glyconanoparticles were successfully used as a sensitive biosensing system probing specific interactions between carbohydrates and proteins as well as bacteria. Exp Biol Med 234:1128–1139, 2009

Key words: nanomaterials; surface functionalization; gold nanoparticles; iron oxide nanoparticles; carbohydrates; perfluorophenylazide

Introduction

Research on nanoscience and nanotechnology has increased exponentially over the past decade, with new investigations and discoveries appearing daily (1). Unique properties of optical, electronic, magnetic, mechanical, and chemical reactivities have been discovered and are associated with nanomaterials solely because of their nanoscale sizes and shapes. These materials serve as model systems providing fundamental understanding of structure-property relationships at the nanoscale. The investigations in turn guide the creation of new structures, systems, and devices with novel properties, functions, and utilities. The interdisciplinary research on nanomaterials merges the fields of synthetic and materials chemistry, condense-matter physics, and fabrication engineering, solving problems in materials synthesis and characterization, and providing core frameworks for biomedical functions (2). While challenges remain in improving the capabilities of characterization tools at the nanoscale, and the synthesis of nanomaterials of well-defined size, shape and composition, progress has already been made beyond fundamental research to the development of diverse and versatile nanomaterials-based biomedical devices adopting nanomaterials into current biomedical technologies (3, 4). For instance, chip-based microfluidic nanodevices enable high-throughput and exceptionally efficient analysis of gene sequences, greatly expanding the ability for the characterization of genetic makeup and revolutionizing the specificity of diagnostics and therapeutics. Nanomaterials, having at least one dimension smaller than 100 nm, are comparable in size to many biological molecules. The nanosize dimension allows them to incorporate into cells for *in vitro* and *in vivo* imaging, drug-delivery, and targeting tumor cells (5). Nanosensors aid early detection and prevention of diseases, and nanodevices, used remotely and *in vivo*, show high

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[1] To whom correspondence should be addressed at Portland State University, Department of Chemistry, P.O. Box 751, Portland, OR 97207. E-mail: yanm@pdx.edu

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Table 1. Properties of Typical Nanomaterials and Biomedical Applications

Nanomaterials			
Category	Examples	Intrinsic properties	Biomedical applications
Metallic	Au, Ag	SPR	Biosensing, drug delivery, bioimaging
Semiconductor	CdS, CdSe	Fluorescence, luminescence	Immunoassays, bioimaging, biosensing
Magnetic	Fe_3O_4	Magnetism	MRI, drug delivery
Carbon-based	CNTs, Fullerene	Electronic and mechanical properties, conductivity	Drug and gene delivery, therapy, biosensing

promise for effective and low-cost home-based health care, benefiting the well-being of the entire human society.

The field of nanomaterials is vastly diverse and is evolving rapidly. This mini-review focuses on the subject of surface functionalization of nanomaterials, limiting the scope to the biomedically important nanomaterials of metal, semiconductor, and carbon nanotubes. A great variety of nanomaterials are synthesized for biomedical applications, a considerable number of which are polymeric nanomaterials formed either by applying the techniques of nanofabrication or via molecular self-assembly. These polymers, carrying multiple or multifunctional ligands by synthesis or physical encapsulation, have been used in biomedical imaging, as vehicles for drug delivery, and as scaffolds in tissue engineering (6). The functionalities on these polymeric nanomaterials are in general built into the material synthesis rather than by surface functionalization, and this class of materials is not included in the current discussion. This mini-review is organized in the following manner. First, several key classes of nanomaterials will be surveyed with regard to their properties and utilities (Table 1). Methods for nanomaterial surface functionalization are summarized in the general category of non-covalent and covalent approaches. Subsequently the photocoupling method developed in our laboratory will be discussed together with the process for surface functionalization and the subsequent conjugation of carbohydrate ligands on iron oxide and gold nanoparticles. Applications of the prepared glyconanoparticles for the detection and sensing of proteins and bacteria will be presented. The final section includes a summary and the concluding remarks.

Biomedically Important Nanomaterials—A Brief Survey

Metal nanoparticles (NPs) such as Au and Ag NPs are excellent nanomaterials providing a powerful platform in biomedical applications of biomolecular recognition and sensing, drug delivery, and imaging (7, 8). Au NPs are among the most used and studied nanomaterials owing to their ease of preparation, stability, well-established surface functionalization chemistry, and their unique optoelectronic properties. The so-called surface plasmon resonance (SPR) absorption, produced by the collective oscillation of conducting electrons in the metal NP core upon interacting with the incident light, is dependent on the NP size and shape, the dielectric property of the media, and the distance between particles. This provides a unique and convenient platform for monitoring the molecular recognition event occurring at close to the surface of the nanoparticles. Colorimetric bioassays have thus been achieved based on the SPR shift when molecular interactions take place at the surface of the nanoparticles, and have been employed to study fundamental biorecognition processes including cell-cell communication, enzymatic activity, protein-protein interaction, and DNA hybridization. When the ligand-receptor interaction causes additional aggregation of nanoparticles, very large SPR shifts occur producing intense color changes visible to the naked eyes (9). These optical properties, induced by single particles or interactions between particles, allow the highly sensitive detection of molecular binding events. In addition, the SPR absorptions are not subject to quenching/photobleaching that are frequently associated with organic fluorophores, or blinking that occurs in quantum dots. An early example was demonstrated by Mirkin and coworkers using oligonucleotide-capped Au NPs (10). Hybridization of the complementary oligonucleotide strands induced the aggregation of Au NPs leading to a distinct solution color change easily visualized by naked eyes. Numerous examples can be found in the literature where bioconjugated Au NPs are used as colorimetric biosensors detecting proteins, viruses, and bacteria at an extremely sensitive level. An additional advantage of nanoparticles is that the multiple ligands presented on the nanoparticle surface could drastically enhance affinities of specific monovalent interactions via the multivalent binding between NPs and the biological target. Lin *et al.* reported that the observed binding affinity of mannose-encapsulated Au NPs with Concanavalin A (*Con A*) was several orders of magnitude higher in comparison with that of mannose with *Con A* in solution (11). In the study of Melander *et al.*, SDC-1721, which is a structural fragment of the HIV inhibitor TAK-77 and displays no inhibition activity in solution, became a potent inhibitor when coupled to 2-nm Au NPs (12). The authors attributed the enhanced activity to the multivalency effect where multiple ligands presented on the nanoparticle surface greatly enhanced the overall binding affinity with the protein.

Quantum dots (QDs) are zero-dimension materials exhibiting quantum confinement in all three spatial

dimensions. They are semiconductor nanocrystals whose bandgap depends on the size of the QDs. The energy gap increases with decreasing particle size, and therefore smaller QDs emit light at higher energy, i.e., lower wavelength and blue-shift, whereas larger QDs absorb and fluoresce at longer wavelengths and red shift. QDs have broad excitation spectra yet narrow and tunable emissions, and have thus been widely used as optical labels in a wide range of biomedical applications including immunoassays for proteins, nucleic acids, bacteria, and toxin analysis (13, 14). Compared with the organic fluorescent dyes, QDs have additional advantages of high quantum yields and high photochemical stability, and offer improved detection sensitivity and application lifetime (15). Early work by Nie and coworkers clearly demonstrated that ZnS-capped CdSe QDs as the luminescent label are much brighter than organic dyes, and immunomolecule-labeled QDs specifically recognized antibodies or antigens with ultrahigh sensitivity (16). QDs are in addition used to probe and track single biomolecules in live cells (17, 18) owing to their high photostability and strong luminescence. Because QDs have broad absorption bands and narrow emission spectra, they can be excited with the same excitation wavelength while allowing the fluorescence emission of each QD to be measured simultaneously in parallel. Therefore QDs of different sizes and composition can be used as the coding labels to simultaneously track multiple target molecules in a multiplexed fashion (19, 20). Luminescent QDs were also used as photosensitizers to generate singlet oxygen in photodynamic cancer therapy, as well as radiosensitizers in radiotherapy (21).

Magnetic nanoparticles of iron oxides have a long history of investigation and have shown remarkable potentials in biomedical research. A unique characteristic of magnetic particles is their ability to move simply by the influence of an external magnetic field. Magnetic nanoparticles with the appropriate surface chemistry have thus attracted increasing interests and have been widely used in the life sciences (22–30) including magnetic resonance imaging (MRI) contrast enhancement (31–33), drug delivery (34–37), hyperthermia (38–40), cell separation (41–44), and tissue repair (45). Both inherent properties of magnetic nanoparticles (magnetic, non-porous, controllable size, and high stability) and modification of their surfaces play crucial roles in such applications. Superparamagnetic iron oxide nanoparticles can furthermore improve the diagnostic value by enhancing the MRI contrast on surrounding healthy and pathological tissues, increasing the MRI resolution at the microscopic level (46). Weissleder *et al.* demonstrated that attaching tat peptides to superparamagnetic iron oxide nanoparticles induced the intracellular accumulation of iron oxide and made cells highly detectable by MRI (47). Compared with untagged particles, tat peptides-coated iron oxide nanoparticles show over 100-fold concentration enhancement in lymphocytes. Similar strategy has been applied to modify magnetic nanoparticles with peptides and proteins (48, 49), RNA and DNA (50), providing recognition elements on nanoparticles capable of binding specific target molecules. Recently, the distinctive magnetic relaxation switching (MRS) phenomenon has been reported (51). When magnetic nanoparticles self-assemble into larger nano-assemblies in biosystems, the superparamagnetic nanoparticles become more efficient at dephasing the surrounding water protons leading to enhanced spin-spin relaxation times. This strategy has been used to detect different molecular interactions with high sensitivity and selectivity in biological samples with minimal or no sample preparation. It also can be conducted in turbid samples, suspensions, and whole cell lysates without the need of protein purification. Applications include the detection of oligonucleotide sequences (51, 52), proteins, enzyme activity (53), pathogens (54), and enantiomeric impurities (55).

Carbon nanotubes (CNTs) are well-ordered, hexagonal lattice networks of carbon atoms, which can be viewed as one or more layers of graphene sheets rolled up into a cylinder. The diameter of CNTs varies depending on the number of layers, and CNTs of high aspect ratios can be fabricated (56, 57). CNTs have been the subject of intense interest due to their superb electrical and thermal conductivities, exceptional mechanical strength, and excellent chemical and thermal stability. Biomedical applications of CNTs require the functionalization of the materials with appropriate ligands rendering them bioactive and at the same time compatible with the biological environment (58–60). A number of methods have been developed to conjugate carbohydrates, peptides, and proteins on CNTs (61–64). Sun *et al.* reported that the monosaccharide-functionalized single wall carbon nanotubes (SWNTs) could effectively bind and aggregate anthrax spores in the presence of Ca^{2+} (65). This is in contrast with monosaccharide-functionalized polystyrene beads which did not induce binding and aggregation of the spores under the same conditions, suggesting that SWNTs as the scaffold promoted the multivalent display of the monosaccharides and greatly enhanced the binding affinity of the bound ligands towards the anthrax spore. The field effect transistor (FET) fabricated employing the semiconducting property of SWNTs is another highlighted area that has been studied extensively (66–68). A typical SWNT-FET device involves anchoring a biological receptor such as nucleotides, aptamers, or antibodies on SWNTs, providing recognition elements for the target analytes. Dai *et al.* reported that protein adsorption on SWNT led to appreciable changes in the electrical conductance of the FET devices, which can be exploited for label-free detection of biomolecules with a high potential for miniaturization (69). It was concluded that the biosensing signal was generated primarily due to the electronic effect occurring at the metal-nanotube contacts upon protein adsorption, rather than the adsorption along the exposed surface of the nanotubes. The detection limit for

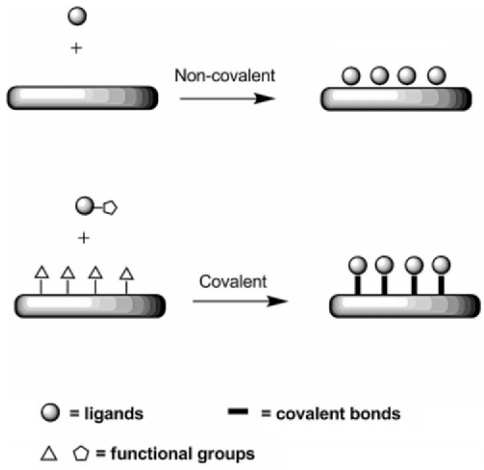


Figure 1. Modification of nanomaterials surface by non-covalent and covalent approaches.

proteins or protein-protein interactions was in range of 100 pM to 100 nM.

An inherent feature of nanomaterials is their high surface areas, i.e., high surface-to-volume ratio in comparison with their bulk material counterpart. For instance, for a CdSe QD of ~2 nm in diameter, ~90% of the atoms are located on the surface (70). Nanomaterials thus have high surface energy resulting from increased surface curvature and a greater percentage of dangling bonds that lack nearest bonding neighbors. To minimize the surface energy, nanomaterials tend to adopt a spherical shape, and in addition, to agglomerate into large particles reducing the surface area and thus lowering the surface energy. Surface modification/passivation of nanomaterials is highly necessary where the surface layer serves to reduce the surface energy and at the same time acts as the protective coating preventing nanoparticles from agglomerating thus increasing their long-term stability (71). The capping layer can be further derivatized with additional ligands or functional groups introducing diverse functions and properties to the nanomaterials (72).

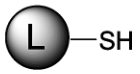
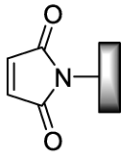
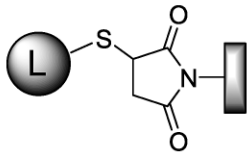
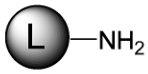
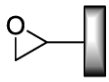
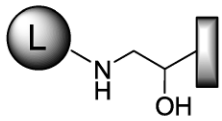
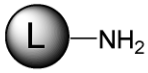
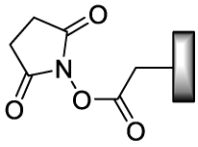
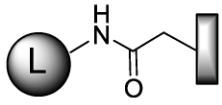
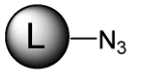
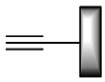
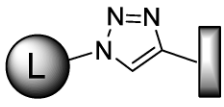
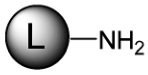
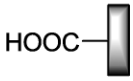
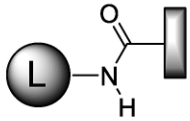
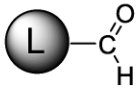
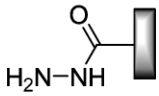
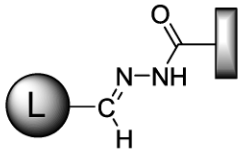
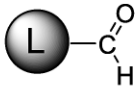
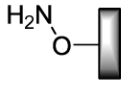
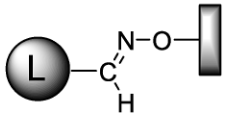
Nanomaterial Surface Functionalization

Surface modification of nanomaterials follows the general strategies of non-covalent and covalent approaches (Fig. 1). The non-covalent approach is a physisorption process where the ligand is adsorbed to the nanomaterials via the non-covalent forces, including electrostatic interactions, hydrogen bonding, and hydrophobic interactions. A popular method of non-covalent surface functionalization is the so-called steric stabilization that involves polymers or surfactants as the capping layer. The surface coating stabilizes individual nanoparticles, and at the same time, the steric repulsion inhibits agglomeration by keeping the nanoparticle dispersion intact. An added benefit of this process is that monodisperse nanoparticles can be synthesized. The polymer layer adsorbed on the surface of nanoparticles serves as a diffusion barrier to the growing species, resulting in a diffusion-limited growth in the subsequent growth of nuclei. Diffusion-limited growth would reduce the size distribution of the initial nuclei, leading to monodisperse nanoparticles. Furthermore, polymers as the coating materials provide high-density functional groups that can be subsequently derivatized with appropriate ligands for bioconjugation. In the example by Star *et al.*, a FET device was constructed using polymer-coated CNTs for the detection of protein binding (73). CNTs were coated with a mixture of poly(ethylene imine) (PEI) and poly(ethylene glycol) (PEG). PEI provided the functional groups, i.e., $-NH_2$, for the covalent immobilization of the ligand, biotin, to CNTs. PEG, on the other hand, served as a non-fouling coating preventing the non-specific adsorption of proteins on the device, thus giving much increased sensitivity.

The majority of surface functionalization methods are based on the covalent bond formation, which offers the advantage of robust linkage and the stability of the surface ligand. If the ligand possesses a functional group that is reactive towards the substrate materials, it can chemisorb to the nanomaterial surface and yield self-assembled structures. Typical examples of chemisorption include thiol/disulfide on metals (Au, Ag, Cu) and semiconductors (CdS, CdSe, ZnS), silanes on oxides (SiO_2, TiO_2), and phosphates on metal oxides (iron oxide, TiO_2). Depending on the nature of the substrate material, ligands possessing the corresponding functional groups are chosen and synthesized. Of the chemisorbed self-assembly systems, thiol/Au is the most studied and used. The process is well-established and it produces well-behaved self-assembled monolayers that are stable, reproducible, and thoroughly characterized. The system is therefore widely used especially for proof-of-principle studies. The surface functionalization generally follows a simple process where the nanomaterial is immersed in a solution containing the ligand. The reaction occurs readily at room temperature. Excess ligands are then removed by rinsing with the solvent, leaving behind nanomaterials that are surface-functionalized with the ligand. A highly effective surface engineering strategy is the so-called ligand exchange technique where the ligand of interest displaces the stabilizing capping layer on the nanomaterial. In this case the ligand should have at least equal or higher affinity than the capping molecule towards the nanomaterial in order to partially or fully displace them. In the synthesis of gold nanoparticles by the classic citrate reduction reaction of auric acid, the as-prepared Au NPs bear the citrate capping layer that can be subsequently replaced by a thiol or disulfide ligand (74). The same strategy is frequently applied in QD synthesis where a capping agent is used to stabilize the CdSe or CdSe/ZnS core-shell nanoparticles. Subsequent treatment with thiol-functionalized ligand introduces the desired ligand shell to the QDs (75).

Covalent bond formation is also accomplished by

Table 2. Typical Complementary Functional Groups of Covalent Coupling Chemistries[a]

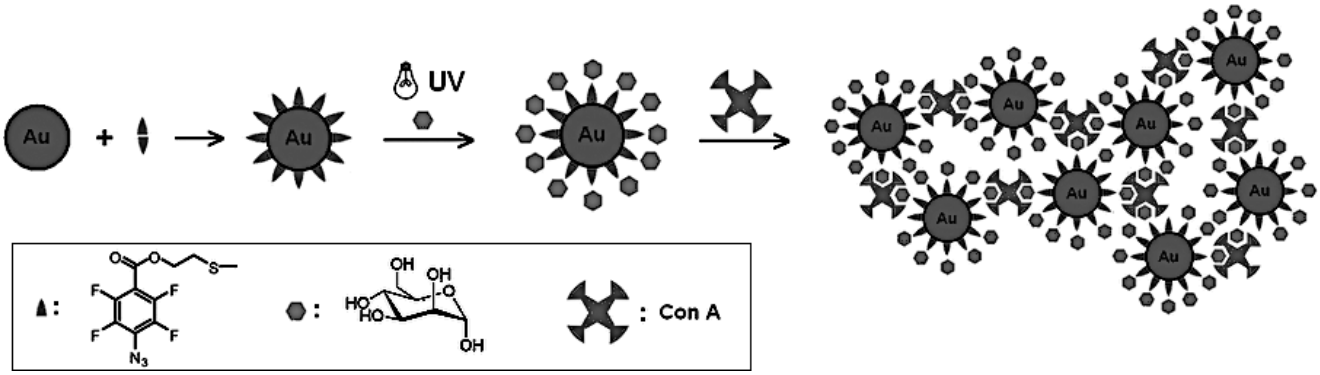
Ligand	Substrate	Ligand decorated surface	Ref.
			(76, 77)
			(78, 79)
			(80)
			(81, 82)
			(83)
			(84)
			(84, 85)

[a] L, ligand.

reacting the complementary functional groups on the ligand and the surface of the nanomaterial. In this case, the nanomaterial is derivatized with a functional group, which then reacts with the ligand that either possesses the complementary functional group in its native form or is derivatized by chemical synthesis. Table 2 shows typical complementary functional groups used for coupling ligands to nanomaterials (76–85).

Photoinitiated Coupling Chemistry

We have developed a photochemically initiated coupling chemistry that employs functionalized perfluorophenyl azides (PFPAs) to attach molecules and materials to solid substrate surfaces (86–88). Upon light activation, the azide moiety converts to a highly reactive nitrene that, notably, inserts into CH and NH bonds, creating highly robust covalent linkages. The photochemistry of PFPAs is well-established and PFPA derivatives have been widely used as efficient photoaffinity labels in elucidating enzyme active site structures (89). We have successfully employed PFPAs in surface modification, targeting polymeric materials that lack reactive functional groups for surface coupling (90–94). Carbohydrates, being complex in structure and challenging to chemically derivatize, are another class of compounds that are well-suited for the PFPA photocoupling chemistry. The design of our approach is to prepare PFPA-functionalized nanoparticles that can be subsequently used



Scheme 1. Schematic illustration of the interaction of mannose-coupled Au NPs with *Con A*, and the formation of Au NP aggregates.

to covalently couple, in principle, any carbohydrate structures by way of the insertion reactions of photochemically activated nitrene species. Furthermore, the coupling reaction is fast, taking place in minutes instead of hours, which is needed in most thermally initiated conjugation reactions.

Thiol-, phosphate-, and silane-functionalized PFPA photocoupling agents were synthesized for gold, iron oxide, and silica nanoparticles, respectively (Fig. 2). Spacer linkages of different chain lengths, structure, and property were incorporated into the coupling agents. The ethylene oxide units are polar and hydrophilic, whereas the methylene linkages are nonpolar and hydrophobic. One utility of the spacer is to modulate the surface property, polarity, and solubility of the nanomaterials. For example, we found that iron oxide nanoparticles functionalized with PFPA-phosphate 1 were miscible with protic solvents of water and ethanol, whereas nanoparticles functionalized with PFPA-phosphate 2 were miscible with aprotic solvents such as acetonitrile (95, 96). The ethylene oxide linkage has the additional property of being protein resistant, and thus has been widely used to create biocompatible and antifouling surfaces (97, 98). Nanoparticles were functionalized with the photocoupling agent by chemisorption via a simple solution incubation process. In the case of gold and silica, a one-pot process was developed whereby the PFPA-functionalized nanoparticles were prepared by adding the corresponding PFPA directly to the solution in which the nanoparticles were synthesized.

The photoactive nanoparticles were then tested for their abilities to attach ligands to the nanoparticles. The coupling reaction was accomplished by irradiating the solution containing the ligand and the nanoparticles. We have successfully attached monosaccharides and oligosaccharides on Au and iron oxide nanoparticles, and polymers such as polystyrene, pole(2-ethyloxazoline), and poly(vinyl pyrrolidone) on silica nanoparticles. The coated nanoparticles are stable in solutions, and the carbohydrate-coated NPs were readily dispersed in water to give homogeneous solutions. The amount of polymers or carbohydrate ligands bound to the nanoparticles can be determined by the thermogravi-

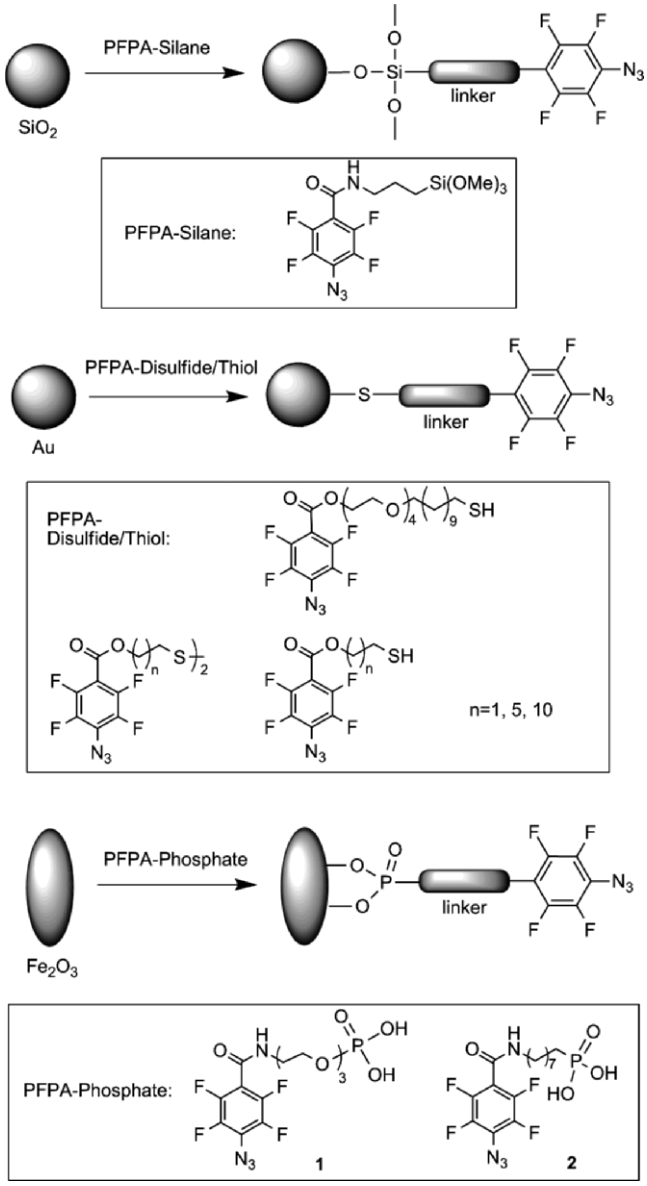


Figure 2. Functionalization of nanoparticles with PFPA.

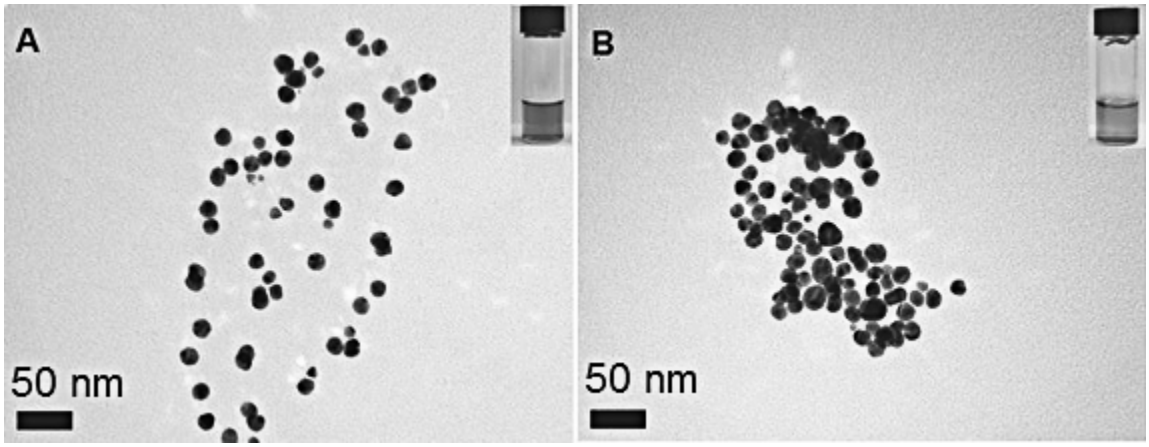


Figure 3. TEM images of D-mannose-functionalized Au nanoparticles (A), and subsequent binding with *Con A* (B).

metric analysis (TGA) (99, 100). TGA measures the mass loss of the organic materials as they decompose upon heating. Carbohydrate ligands can in addition be analyzed by a colorimetry assay using anthrone and sulfuric acid[1].

Here we give two specific examples where iron oxide and Au glyconanoparticles (GNPs) were successfully prepared using the PFPA photocoupling chemistry (Scheme 1). We target carbohydrates for additional reasons that they are an important class of biomolecules. Naturally occurring carbohydrates, glycoproteins and glycolipids present at the surface of nearly every cell in living systems play crucial roles in biological events as recognition sites between cells and factors. They can trigger various phenomena such as cell growth, inflammatory responses or viral infections. Carbohydrate-mediated interactions at the cell surface range from hormones, enzymes and antibodies to bacteria, viruses, and toxins. Surface-exposed carbohydrate moieties that are characteristic of a given microbe may serve as key biomarkers for bacteria and pathogen identification, diagnosis, and vaccine development. Carbohydrates as a detection platform have already demonstrated tremendous potential to achieve superior sensitivity and selectivity (102). Carbohydrate-functionalized glyconanomaterials are finding important applications in elucidating carbohydrate-protein interactions, imaging, and sensing.

Monosaccharides were chosen in our proof-of-principle experiments. These are the smallest carbohydrate structures and the most challenging for the photocoupling chemistry. The probability of attaching the ligand by CH insertion reaction increases as the size of the carbohydrate structure, or the number of CH bonds, increases. Additionally, monosaccharides are the lowest-affinity ligands lacking the multivalency effect that often occurs in their oligosaccharide counterparts (102). Thus, if these monosaccharide ligands can be successfully coupled to NPs, and the resulting surface-tethered ligands retain their recognition abilities, the methodology developed can be readily applied to other higher carbohydrate structures.

Iron Oxide Nanoparticles

A number of methods have been reported for functionalizing iron oxide nanoparticles with organic compounds (103, 104) such as silanes (105, 106) and phosphoric acids (107–113). Silanes form robust siloxane bonds with surface -OH groups; the drawback is the self-condensation reaction leading to multilayer formation or deposition of aggregates on the material surface. Phosphates, on the other hand, do not undergo self-condensation reactions. These agents couple with surface -OH groups creating stable Fe-O-P structures (103, 107). We synthesized a number of phosphate-functionalized PFPAs, which were used to functionalize iron oxide nanoparticles introducing PFPA to the nanoparticle (Fig. 2). The functionalization reaction was carried out by treating the nanoparticles with a chloroform solution of PFPA, and the process can be conveniently monitored by IR evidenced by the appearance of the characteristic azide ($-N_3$) antisymmetrical stretch at 2126 cm^{-1} in the PFPA-functionalized iron oxide nanoparticles. The reaction is applicable to both hematite α-Fe_2O_3 and magnetite Fe_3O_4 nanoparticles (100).

The coupling reaction was carried out by irradiating a mixture of PFPA-functionalized NPs and the monosaccharide in water/ethanol. The magnetic glyconanoparticles were readily dispersed in water to yield homogeneous solutions. The amount of carbohydrate ligands bound to the nanoparticles was determined by TGA, which yielded the ligand density of 128 D-mannose molecules per Fe_3O_4 nanoparticle of 5 nm in average diameter. The TGA results also showed that there were roughly the same numbers of PFPA and coupled D-mannose molecules on the nanoparticles, indicating that the coupling reaction was fairly efficient.

Gold Nanoparticles

A one-pot process was developed to simultaneously synthesize and functionalize Au NPs with PFPA. Au NPs of

[1] Wang X, Ramström O, Yan M. A photochemically initiated chemistry for coupling underivatized carbohydrates to gold nanoparticles. 2009, submitted.

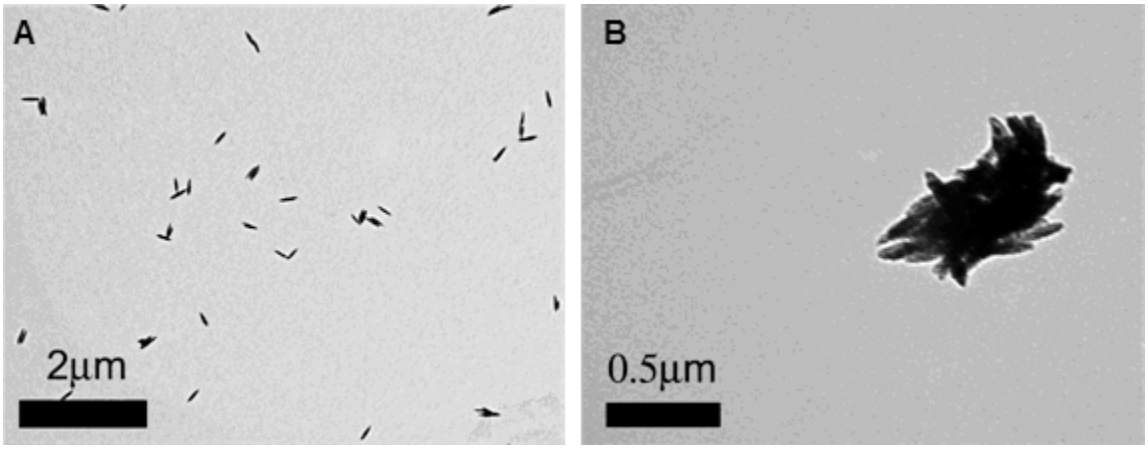


Figure 4. TEM images of iron oxide NPs with surface-coupled D-mannose (A), and after treating with *Con A* (B).

20 nm in diameters were prepared following the widely adopted protocol using auric acid and sodium citrate, and were then directly functionalized with disulfide-functionalized PFPA (Fig. 2) using a modified phase-transfer procedure[2]. The PFPA-functionalized Au NPs were subsequently activated with UV light in the presence of the carbohydrate ligand, resulting in the covalent attachment of the ligand to Au NPs. The density of the carbohydrate ligand on the nanoparticles was determined quantitatively by an anthrone-sulfuric acid colorimetry assay, which showed the coupling yield of over 50%, and the ligand density of 58 nmol/mg NPs for D-mannose. This corresponds to a surface coverage of 80% in comparison to the theoretically calculated ligand density of closely-packed D-mannose on the Au NP (72 nmol/mg NPs).

The surface-tethered carbohydrates were then tested to see if they retain the recognition abilities. When D-mannose-functionalized iron oxide or gold nanoparticles were treated with *Con A*, a plant lectin that specifically binds α-D-mannopyranosides and, to a lesser extent, α-D-glucopyranosides, they assembled into bundled or agglomerated clusters, respectively (Figs. 3 and 4). The aggregation is the result of *Con A* serving as an equivalent of a tetrafunctional crosslinker, which, upon interacting with D-mannose on NPs, brings together the nanoparticles forming a crosslinked network structure (114). The recognition ability of D-mannose bound to the hematite NPs was further evaluated in a bacterial binding study by treating D-mannose-functionalized NPs with ORN 178, an *E. coli* strain having a mannose specific binding domain. Hematite nanoparticles were observed attaching themselves at the lateral ends and along the pili of the bacteria (Fig. 5). The strong binding between the nanoparticles and the pili of ORN178 can be attributed to the multivalent interactions between the surface-confined D-mannose with the FimH lectin domain on type 1 pili. When the D-mannose-functionalized nanoparticles were treated with ORN 208, an *E. coli* strain deficient of mannose-binding FimH protein, no nanoparticles were observed on the bacteria surface, demonstrating the high recognition specificity of these surface-tethered D-mannose ligands.

The Au GNPs were furthermore developed into a colorimetric biosensor for probing carbohydrate-protein interactions. The as-prepared 20-nm Au NPs exhibit the characteristic wine-red color with a surface plasmon absorption (SPR) peak at 520 nm. Upon surface functionalization, the NPs undergo red-shift in its SPR absorption resulting in color change of the solution. The extent of the color change is related to the extent of surface functionalization and the particle agglomeration. Proof-of-principle experiments were carried out using *Con A* as the target protein. When D-mannose-functionalized Au NPs were treated with *Con A*, cross-linked aggregates were formed (Fig. 3B). Concurrently, rapid color change occurred while a large red-shift in the SPR and a broadening of the surface plasmon absorption peak were observed (Fig. 6). The

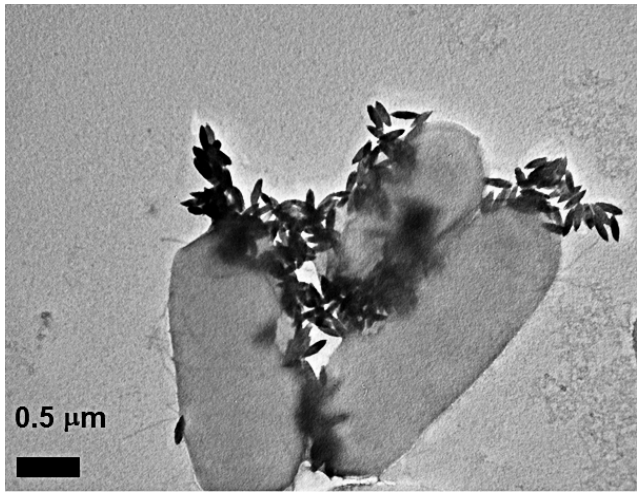


Figure 5. TEM image of *E. coli* ORN178 strain after treating with mannose-functionalized iron oxide nanoparticles.

[2] Wang X, Ramström O, Yan M. A photochemically initiated chemistry for coupling underivatized carbohydrates to gold nanoparticles. 2009, submitted.

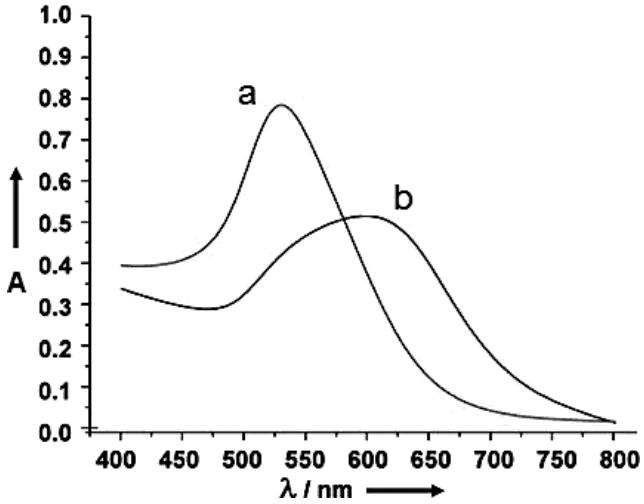


Figure 6. UV-vis spectra of (a) Au NPs with surface-coupled D-mannose; (b) after treating with *Con A*.

specificity of the biosensing system was tested by treating D-mannose-functionalized Au NPs with several other lectins including *Griffonia simplicifolia* lectin II (GS II), peanut agglutinin (PNA), and soybean agglutinin (SBA), The color of the resulting solutions remained unchanged and no notable red-shifts of the SPR peak were observed, demonstrating the high selectivity of the glyconanoparticle-based sensing system.

Concluding Remarks

Nanomaterials, having dimensions matching those of the biological molecules and entities, have demonstrated great potential in a wide range of biomedical applications, including analysis, imaging, sensing, diagnostics, and drug delivery. Surfaces as the outmost boundary of a material serve as the interface communicating with the physical phase surrounding the material, and play a critical role in the functions of nanomaterials. The need of surface engineering for nanomaterials is both inherent, i.e., high surface energy at the nanoscale, and application driven where ligands presented on the surface act as points of contact communicating with external receptors. An intricate balance must be achieved in order to provide the multifaceted functions of the surface ligands in preventing nanomaterials from agglomerating, presenting the necessary molecular recognition functions, and at the same time preserving the physical properties of the nanomaterials needed for translating the molecular recognition events into reliable readouts. Nanomaterials come in different compositions, structures, sizes, and shapes. Surface chemistry therefore should be designed taking into consideration the chemical nature of the nanomaterials and at the same time affording efficient ligand coupling and providing optimal ligand presentation. There is a great demand for effective surface coupling chemistry that is general, efficient, can accommodate ligand diversity, maintain ligand bioaffinity, and give bioactive and stable interfaces. There is enormous room at the surface awaiting exploration.

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