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Functionalization of Inorganic Nanoparticles for Bioimaging Applications

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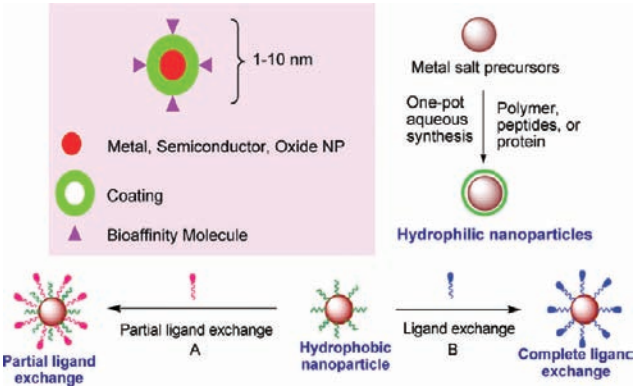

Modern biomedical imaging technologies have led to significant advances in diagnosis and therapy. Because most disease processes occur at the molecular and cellular levels, researchers continue to face challenges in viewing and understanding these processes precisely and in real time. The ideal imaging resolution would be in nanometers, because most biological processes take place on this length scale. Therefore, the functionalization of nanoparticles (NPs) and their use in therapeutic and diagnostic applications are of great interest. Molecular and cellular imaging agents made from inorganic NPs have been developed to probe such biological events noninvasively. The conjugation of tiny NPs with specific biomolecules allows researchers to target the desired location, reduce overall toxicity, and boost the efficiency of the imaging probes. In this Account, we review recent research on the functionalization of NPs for bioimaging applications.

Several types of NPs have been employed for bioimaging applications, including metal (Au, Ag), metal oxide (Fe_3O_4), and semiconductor nanocrystals (e.g. quantum dots (QDs) and magnetic quantum dots (MQDs)). The preparation of NPs for bioimaging applications can include a variety of steps: synthesis, coating, surface functionalization, and bioconjugation. The most common strategies of engineering NP surfaces involve physical adsorption or chemisorption of the desired ligands onto the surface. Chemisorption or covalent linkages are preferred, and the coated NPs should possess high colloidal stability, biocompatibility, water solubility, and functional groups for further bioconjugation.

Many of the functionalization techniques that have been reported in the literature suffer from limitations such as complex synthesis steps, poor biocompatibility, low stability, and hydrophobic products. Coating strategies based on chemisorption and ligand exchange often provide a better way to tailor the surface properties of NPs. After conjugation with the appropriate targeting ligands, antibodies, or proteins, the NPs may exhibit highly selective binding, making them useful for fluorescence imaging, magnetic resonance imaging (MRI), positron emission tomography (PET) imaging, and multimodal imaging.

Introduction

The synthesis of NPs has received a great deal of interest because of their potential biomedical applications in imaging and drug targeting and delivery. Besides their size-dependent properties, NP applications are affected by their surface modification. Most surface modifications of NPs for bioimaging applications are based on chemisorption since it offers a stronger and more robust bond and a more stable surface ligand, compared with physisorption (Table 1). The successful conjugation of biomolecules onto NPs depends on the proper surface modification.

Synthesis and surface functionalization of NPs have been extensively investigated in the past decade.[1] Semiconductor, noble metal, heterometallic, and metal oxide

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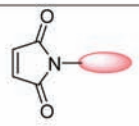
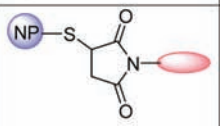
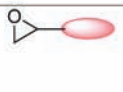
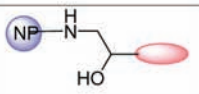
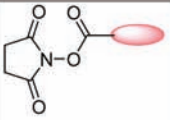
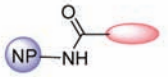
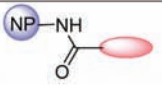
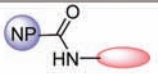
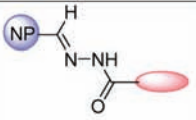
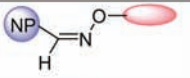
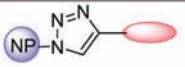
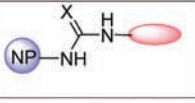
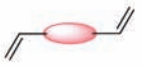
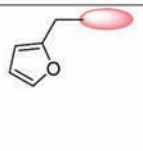
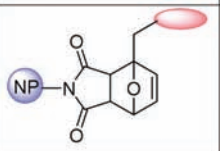
TABLE 1. Functionalization Chemistry of NPs

Ligand	Substrate	Ligand Attached to Substrate	Reaction
NP—SH			Michael Addition
NP—NH_2			Epoxide Opening
NP—NH_2			Amidation
NP—NH_2	HOOC—		Amide Bond Formation
NP—COOH	H_2N—		Amide Bond Formation
NP—CHO	H_2NHN—		Imine Formation
NP—CHO	H_2NO—		Imine Formation
NP—N_3			Click Chemistry
NP—NH_2	XCN— x = O, S		Addition of Amine to Cyanates
NP		NP	Ring-Closing or Ring-Opening Metathesis
NP			Diels-Alder Reaction

NPs of 1−100 nm have unique size-dependent properties and are useful in various imaging applications leading to medical diagnosis, therapy, or combination therapy. Fluorescence imaging can greatly benefit from the use of QDs owing to their remarkable optical properties.[2] Compared with fluorescent dyes, QDs do not suffer from photobleaching and their emissions are tunable from the visible to the near-infrared (NIR) region by varying the size or composition of QDs. A wide variety of contrast agents and optical labels is required for different types of imaging and detection modalities, such as MRI, PET, single-photon emission computed tomography (SPECT), and fluorescence-based imaging.[3] MRI, PET, and SPECT are very attractive for *in vivo* imaging, whereas fluorescence-based imaging is the most widely employed for *in vitro* imaging.

Due to their surface plasmon resonance properties, noble metal NPs such as gold (Au) and silver (Ag) are considered to be promising materials and alternatives to semiconductor nanocrystals,[4] but their application in bioimaging is largely limited. Recent advances in synthesis have made it possible to explore a variety of metallic nanostructures as optical contrast agents.[3−5] Superparamagnetic NPs are emerging as versatile probes, especially in MRI, and iron oxide magnetic particles have been widely used as T_2 (negative) contrast agents. Recently, manganese oxide magnetic particles have been described as promising contrast agents due to their size-dependent magnetic properties.[6] Hybrid inorganic NPs (e.g., Fe_3O_4−Au, CdSe−Au, CoPt−Au, PbSe−Au−Fe_3O_4) are useful probes for magnetic-based targeting, delivery, cell separation, MRI, and fluorescence-based biolabeling applications.[7]

Our laboratory has developed silica coating methods for CdSe@ZnS and PbSe QDs and bifunctional NPs consisting of CdSe@ZnS QDs and γ-Fe_2O_3 magnetic particles.[8] In another approach, we have designed synthetic polymers containing multiple thiol groups for coating Au, Ag, and QD NPs in cellular imaging.[9,10] To bypass the problems associated with high-temperature organic synthesis, an aqueous synthesis of QDs has also been established with glutathione capping.[11,12] This Account reviews the functionalization of hydrophobic and hydrophilic NPs reported in recent years.

Functionalization of NPs

Synthesis of NPs can be divided into two major categories, involving (i) hydrophobic conditions and (ii) direct synthesis in aqueous phase, resulting in hydrophobic and hydrophilic NPs, respectively. Due to the properties of the surfactants used, many of the existing synthetic methods available for various NPs are hydrophobic in nature, causing insolubility in water and preventing further functionalization. Thus, water solubilization and functionalization are the key steps prior to the applications of NPs, and coating chemistry is critical toward deriving colloidally stable, water-soluble, robust NPs with flexible surface chemistry. The common functionalization strategies include (i) direct encapsulation of the hydrophobic NPs by hydrophilic polymers,[1−4] (ii) ligand exchange of the original surfactant[13] with hydrophilic ligands, such as thiols, and (iii) the formation of an interdigitated bilayer between amphiphilic molecules or polymers and the passivating surfactant layer

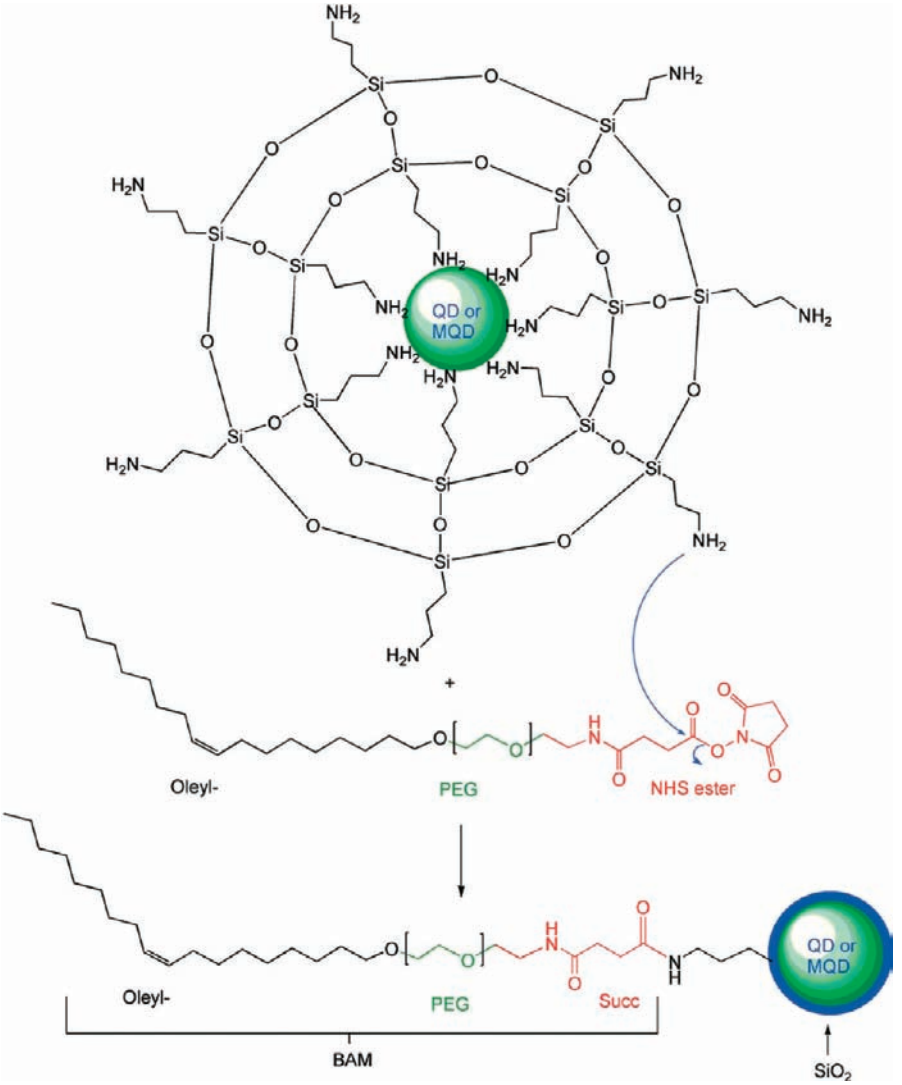


FIGURE 1. Schematic of a CdSe QD or Fe_2O_3–CdSe MQD and its silanization in a reverse microemulsion.[8d] The surface NH_2 groups were then treated with bioanchored membrane (BAM) to form a covalent amide bond. The oleyl groups were used to anchor the QDs to the cell membrane during biolabeling. PEG = poly(ethylene glycol); NHS = *N*-hydroxysuccinimide; Succ = succinimide. Reprinted with permission from reference 8d. Copyright 2007 Wiley Interscience.

on the NP surface.[14] These approaches have been successfully applied to noble metal NPs. Several methods exist in the literature on the design of water-soluble QDs using coating of polymers, micelles, thiols,[15] and silica.[1] Ideally, a good coating material should provide multiple and optimal binding sites to the NPs, water solubility, chemical functionality for further functionalization, and biocompatibility and involve a facile or green coating process.

Silica Coating

One of the most widely used methods for the surface functionalization of NPs is silica coating, which has a number of advantages over organic coating.[16] Silica-coated NPs are robust, water-soluble, colloidally stable, and photostable and have a low nonspecific interaction with biosystems.[17]

Yang et al. have encapsulated hydrophobic QDs (such as CdSe) and magnetic NPs (MPs) (such as Fe_2O_3 or both QDs and MPs) within silica shell using reverse microemulsion.[18] The as-synthesized hydrophobic TOPO-capped CdSe QDs could be coated with silica in a direct one-pot reverse microemulsion method, without ZnS capping.[8a] The mechanism of incorporating hydrophobic QDs into silica spheres was clearly elucidated by Koole et al.[19] The reverse microemulsion method provided good control over particle size and required no prior ligand exchange.

In another approach, poly(ethylene glycol) (PEG)-modified silica-coated core–shell NPs have been synthesized by a similar reverse microemulsion method (Figure 1).[8d] The silica coating provided a robust barrier against oxidation of the QD core and reduced nonspecific adsorption on the NPs.

Various functionalized PEGs could be incorporated onto silica for bioconjugation applications (Figure 1). The *in vitro* cytotoxicity studies conducted on different cell lines showed that silica-coated QDs were much less toxic compared with other water-soluble QDs coated with organic thiols.

Bifunctional NPs consisting of QDs and MPs, termed magnetic quantum dots (MQDs), are multifunctional materials tailored for both fluorescence and magnetic applications. Our laboratory has developed a seed-mediated synthesis of MQDs by growing CdSe QDs on Fe_2O_3 cores, yielding either heterodimers or a homogeneous dispersion of QDs around Fe_2O_3 (Figure 1).[8d]

A variety of water-soluble, functionalized NPs could be synthesized using a thin silica coating procedure.[20] This thin silica coating was conducted in the toluene phase using triethoxysilane or trihydroxysilane, which has the advantage of terminating silica polymerization and thus restricting the thickness of the silica shell, inhibiting the particle–particle cross-linking, and producing finer water-soluble NPs. The thin silica coating was highly reproducible and could be applied to a variety of hydrophobic NPs, such as Au, Ag, Fe_3O_4, and QDs. The resulting silanized particles were nearly monodisperse and had high water solubility and colloidal stability. Recently, PEG–silane-coated MPs have been developed as contrast agents to visualize tumors by MRI.[21] We have also functionalized thin silica-coated gold NPs with aptamers and antibodies and used them for the detection of proteins by the naked eye.[22]

Functionalization of Silica-Coated NPs with Biomolecules

Two distinct surface modifications to enhance the biocompatibility of silica nanospheres have been reported to date, both of which depend on the use of silane coupling agents.[1] In the first method, the silica spheres were terminated by an amine or thiol group using aminopropylsilane (APS) or mercaptopropylsilane (MPS) to which biofunctional groups could be covalently linked. The second method involved the modification of the silica surface by molecules that already have a silane group integrated within the molecule. There were several serious drawbacks for these methods. First, due to steric hindrance and differences in reactivity with coupling agents, it was not clear to what extent and in what ratio the silica surface was covered by the PEGylated and biofunctional molecules. Second, the density of the PEGylated coating around the silica spheres was not well-defined, and unfavorably affected the particle stability under physiological conditions. Third, the flexibility of these methods was limited to molecules with reactive groups for the covalent linking step.

Koole et al. reported a novel strategy to coat silica particles with a dense monolayer of lipids without the use of coupling agents. In the first step, the highly monodisperse silica particles that have a single core–shell QD incorporated in their center and Gd–diethylene triamine pentaacetic acid (DTPA)–bistearyl amide (DSA) in the lipid coating were rendered hydrophobic.[23] They were coated with both paramagnetic and PEGylated lipids in the second step and made target-specific by the conjugation of multiple $\alpha_v\beta_3$-integrin-specific RGD peptides to enable their detection with both fluorescence techniques and MRI. An extensive study by Mulder and co-workers of bare and lipid-coated silica NPs in mice using a variety of imaging techniques showed that the lipid coating enabled direct functionalization and introduction of multiple properties to enhance bioapplicability and pharmacokinetics.[23] Silica-coated particles were functionalized in sequence with fluorescent contrast agent ATTO647N, drug photosensitizer Pd-porphyrin payload for therapeutic intervention (photodynamic therapy), and biomolecular ligands cRGDyK peptides on the outermost surface for targeting the $\alpha_v\beta_3$ integrins of cancer cells (Figure 2).[24] A membrane composed of bis-sorbylphosphatidylcholine, a synthetic polymerizable lipid that was chemically cross-linked, has been used to improve the environmental and chemical stability of the coating.[25] This system successfully reduced nonspecific interactions and permitted functionalization of the particles.

A novel delivery system termed a "nanoshuttle" has been reported with a nanoscale PEGylated-phospholipid coating and a 13-(chlorodimethylsilylmethyl)heptacosane-derived mesoporous silica NP.[26] Therapeutic or imaging agent fluorescein isothiocyanate was trapped in the coating, and folate-assisted targeted delivery was achieved through surface functionalization of the phospholipids. In Yoon et al.'s work, the silica shell of the mesoporous nanoparticle (MNP) incorporated with commonly used organic dyes was modified with various functional organosilicon compounds.[27] The surface of the core–shell NPs presented two key functional groups, a PEG moiety that enhanced biocompatibility *in vivo* and *in vitro*, and an amine moiety to which desired molecules could be added. Folate-functionalized fluorescent mesoporous silica NPs have also been reported.[28] They were biocompatible, preferentially accumulated in tumors, and effectively delivered drugs to the tumors and suppressed tumor growth. Fluorescein-functionalized silica NPs have also been prepared by sol–gel reaction by Seo et al., and their optical

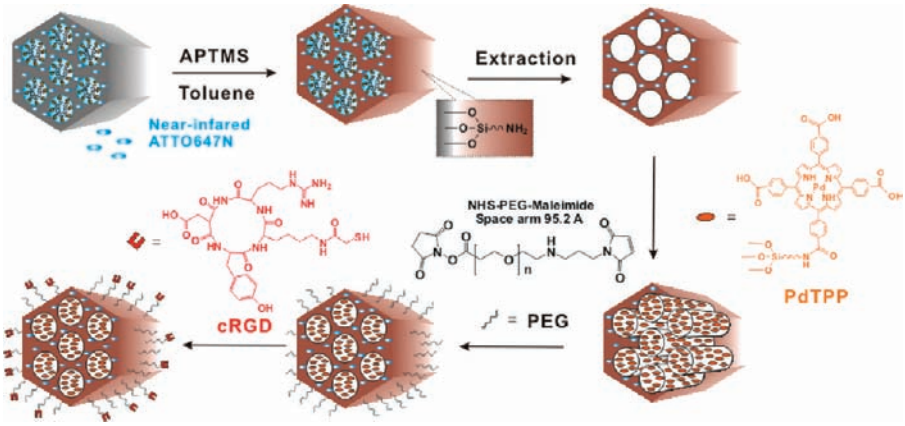


FIGURE 2. Synthesis of trifunctionalized mesoporous silica nanoparticles (MSNs), A647@MSN-RGD-PdTPP.[24] MSNs were functionalized first with the contrast agent (ATTO647N), then with the photodynamic therapy (PDT) and photosensitizer (PS) agent (3-aminopropyltrimethoxysilane (APTMS)–PdTPP), and last with the tumor-targeting ligand (cRGD). Reprinted with permission from reference 24. Copyright 2010 Royal Society of Chemistry.

sensing abilities were studied as a new type of fluorogenic chemosensor for imaging Cu^{2+} ions in living cells.[29]

Kumar et al. have synthesized theranostic multifunctional organically modified silica NPs having a covalently incorporated fluorophore with a variety of functional groups on the surface that were readily conjugated to bioactive molecules. Their selective targeting *in vitro* was demonstrated without any indication of cytotoxicity.[30] Patel et al. have designed a series of multifunctional, iron oxide-containing mesoporous silica NPs with tunable surface charges, which could modulate the ability of stem cells to endocytose them.[31] Amino-functionalized $NaYF_4$:Yb,Er upconversion NPs (UCNPs) with a thin and uniform silica coating on their surface have been prepared and further linked to the rabbit anti-CEA8 antibody to form antibody–UCNP conjugates by a simple route. The antibody–UCNP conjugates were used as fluorescent biolabels for the effective and time-efficient immunolabeling and imaging of HeLa cells.[32]

Functionalization of Hydrophobic NPs by Polymers

NPs have been functionalized with oligonucleotides, peptides, antibodies, and other molecules and used in cellular and *in vivo* imaging.[2] Several methods have been reported for core–shell nanocrystal synthesis with the shells comprising dendrons, oligomeric phosphines, polymers, and other small molecules.[2,33] In these methods, the original surfactants were exchanged with the new ligands or polymer precursors, which were then cross-linked or polymerized on the nanocrystal surface to produce the shells.[20] They involved complex procedures and often suffered from aggregation of NPs. The most widely used approach was the

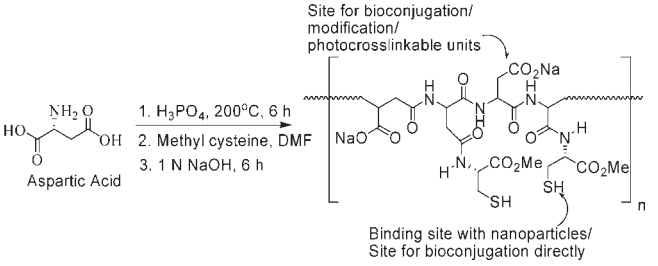


FIGURE 3. Synthesis of cysteine-functionalized polyaspartate.[10] Reprinted with permission from reference 10. Copyright 2010 American Chemical Society.

small molecule based ligand-exchange method.[34] Ligands or polymers with multiple functional groups that could form direct multiple bindings with the NP surface could improve the colloidal stability.[34,35] A cysteine–styrene block copolymer based coating has emerged as an interesting alternative to producing core–shell NPs via polymer self-assembly on the NP surface by simple ligand exchange.[9]

We have successfully synthesized cysteine[36]-functionalized polyaspartic acid by a nucleophilic opening of polyaspartimide by methyl cysteine (Figure 3).[10] The polymer has been designed in such a way that a simple ligand-exchange could be used to coat this polymer on Au, Ag, and ZnS-capped CdSe nanocrystals without producing particle aggregates. The multiple thiol groups on the polymer backbone provided strong chemisorption to the NP surface, and the carboxyl group imparted water solubility and provided for further functionalization. The anti-m-EGFR-functionalized QDs were successfully employed in cell labeling studies to image mouse breast cancer cells.

In another approach, we have synthesized two polymer-derived low molecular weight natural chitosan

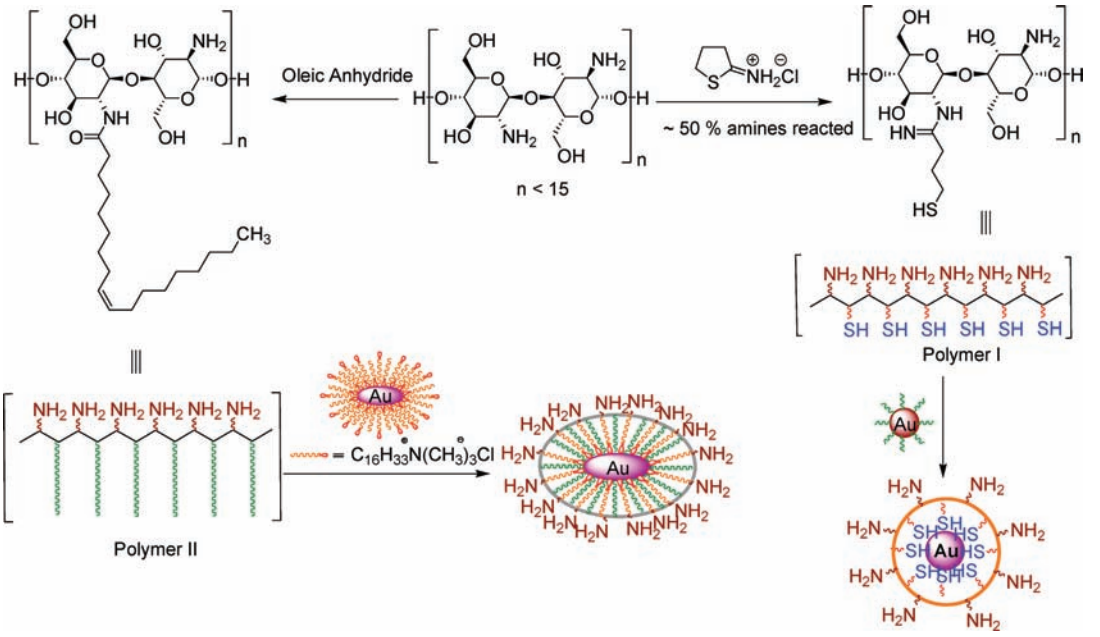


FIGURE 4. Chitosan oligosaccharide modification and coating steps via ligand exchange and interdigitated bilayer formation.[37] Reprinted with permission from reference 37. Copyright 2008 Wiley Interscience.

oligosaccharides for the surface modification of gold nanospheres and nanorods (Figure 4).[37] These biopolymers have multiple thiols or oleic esters and primary amine groups for bioconjugation. Hydrophobic gold nanospheres were converted to hydrophilic NPs via ligand exchange with the thiol polymer. The oleyl polymer was employed to functionalize gold nanorods via interdigitated bilayer formation with cetyl trimethylammonium bromide. Multiple thiol/oleyl groups on the polymer backbone introduced multiple anchoring points to the NP surface and improved the colloidal stability.

Hyaluronic acid–QD conjugates (Figure 5)[38] have been successfully developed for bioimaging. Mattoussi et al. reported a new set of PEG-based ligands, whereby the PEG segment appended with two thioctic acid (TA) or two dihydrolipoic acid (DHLA) anchoring groups, bis(TA)–PEG–OCH_3 or bis(DHLA)–PEG–OCH_3, was synthesized by Michael addition.[39]

A noble metal (Ag) nanocrystal based fluorescence indicator combined with a magnetic NP (Fe_3O_4) has been developed by us as a new class of bifunctional nanocomposites.[40] To render the Fe_3O_4–Ag heterodimer NPs water-soluble, tetramethylammonium hydroxide and glutathione (GSH) were ligand-exchanged with the oleic acid and oleylamine on the surface of Fe_3O_4 and Ag, respectively. The resulting NPs could be magnetically manipulated and were used for two-photon fluorescence imaging of macrophage cells.

Dumbbell-like Au–Fe_3O_4 NPs have been functionalized by a surfactant exchange reaction using epidermal growth factor receptor antibody through PEG and dopamine by Xu et al.[41] The Au particles were protected with HS–PEG-NH_2 with the thiol attaching to Au.

Several new PEG-grafted branched polymers have been synthesized for functionalizing nanomaterials such as carbon nanotubes, gold NPs, and gold nanorods.[11] The functionalized materials demonstrated high aqueous solubility and stability. Functionalized Au@MnO nanocomposites have also been developed.[12] Their gold core was anchored with thiols, and their metal oxide petals were anchored with multidentate catechol.

Azide–alkyne click chemistry has been developed and validated for the rapid site-specific modification of NPs with small molecules.[42] A copper-free click chemistry between strained cyclooctyne functionalized QD and azido-biomolecules led to highly luminescent conjugates (Figure 6).[43] Alkyne functionalized analog of bombesin was conjugated to dye-functionalized dextran-coated superparamagnetic iron oxide (SPIO) using click chemistry.[44]

Pentapeptide cyclo(-RGDfK-) has been employed to specifically label membrane integrins in living osteoblast cells with biofunctionalized QDs.[45] QD-capped magnetite nanorings were derived as a new class of magnetofluorescent nanoprobes by grafting cationic polyethyleneimine-capped QDs onto negatively charged magnetite nanorings modified with citric acid surface.[46]

Iron oxide NPs have been loaded onto bombesin-conjugated *N*-acetylhistidine-glycol chitosan NPs for MRI

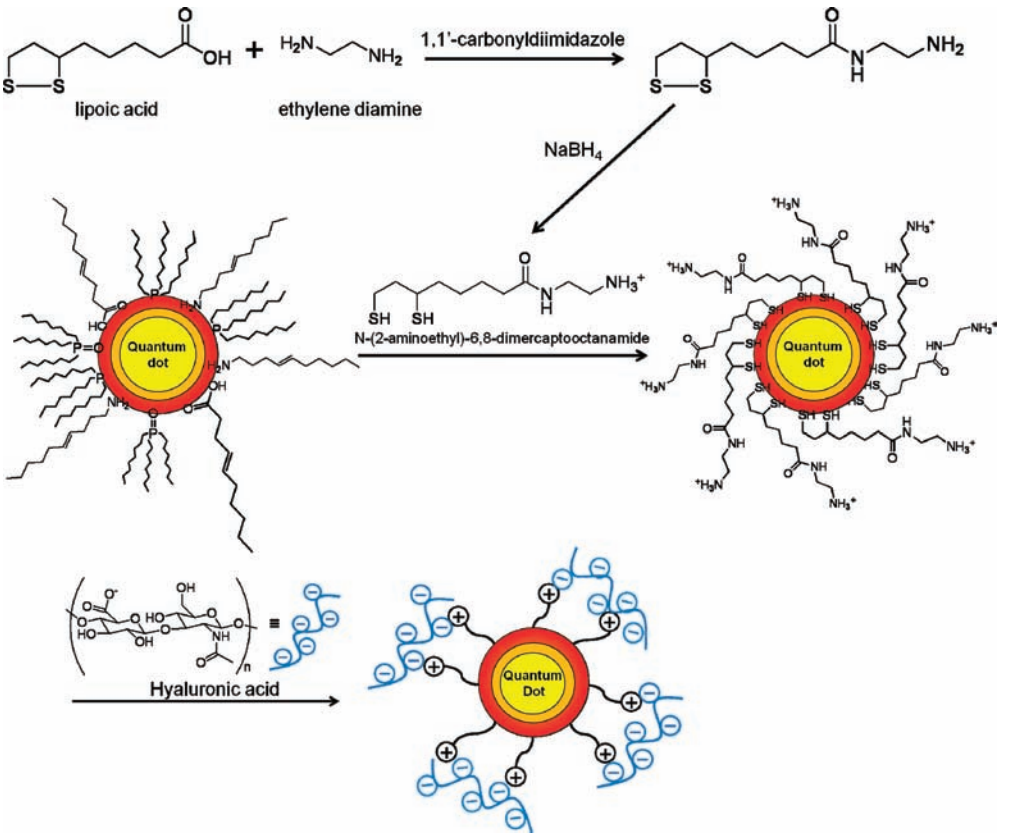


FIGURE 5. Synthesis of hyaluronic acid–QD conjugate.[38] Reprinted with permission from reference 38. Copyright 2009 American Chemical Society.

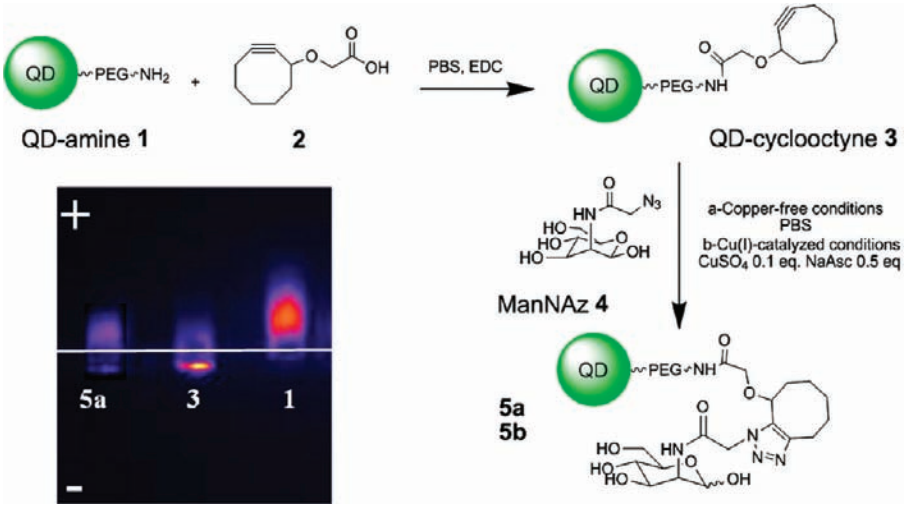


FIGURE 6. Functionalization of QD surface by (a) copper-free and (b) Cu(I)-catalyzed click chemistries.[43] Reprinted with permission from reference 43. Copyright 2010 American Chemical Society.

application.[47] SPIO NPs were surface functionalized with a lung cancer targeting peptide containing a PEG-tethered cysteine residue through ligand exchange (Figure 7).[48] A multifunctional micelle that was encoded with a lung cancer-targeting peptide NH_2-RGDLATLRQL–PEG11–Cys has been encapsulated with SPIO and doxorubicin for MRI and therapeutic delivery.[49]

Despite the simplicity of ligand exchange, this method often compromises the fluorescence efficiency and photochemical stability because the ligands tend to detach from the surface of the QDs, leading to the aggregation of NPs. In general, cross-linking of the ligands improves long-term stability but results in larger particle size.

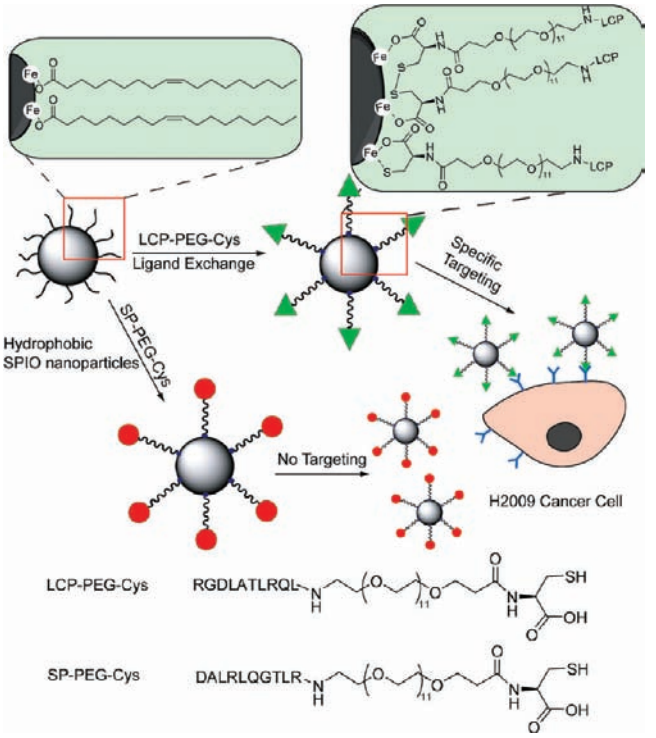


FIGURE 7. Surface modification of SPIO NPs with LCP–PEG–Cys for lung cancer targeting.[48] A scrambled peptide control (SP–PEG–Cys) is included to examine the $\alpha_v\beta_6$ specificity for cell targeting. Reprinted with permission from reference 48. Copyright 2009 Royal Society of Chemistry.

Synthesis and Functionalization of Hydrophilic NPs in Aqueous Phase

The size of QD-based bioprobes is considered to be the major limitation for their applications in biological imaging. Multilayers of polymer or silica are typically coated on QDs, giving rise to an overall particle size of at least 10 nm. Another approach is to replace the original surfactants on QDs with bifunctional thiol-containing ligands,[50] such as thioacetic acid.[51] Recently, QDs were first ligand-exchanged with mercaptoundecanoic acid, and then cross-linked by either lysine or diaminopimelic acid.[52] Such methods gave rise to QDs with an overall size of 22–30 nm. Our laboratory has reported a simple and effective strategy to derive a cross-linked peptide coating on the surface of QDs while keeping the resulting particles as small as possible (Figure 8).[53] We made use of GSH, which is a tripeptide that consists of glutamic acid, cysteine, and glycine having thiol, amine, and two carboxylate groups. Employing thiol groups as the capping agents, highly fluorescent GSH-capped QDs (GSH-QDs)[54] were synthesized directly in aqueous solution (Figure 9).[54a] The coating or "phytochelatin" formed by polymerized GSH stabilized the heavy metal nanoclusters against leaching. GSH–ZnS–CdS–CdSe QDs have been conjugated with doxorubicin for successful delivery into the nuclei of live cells.

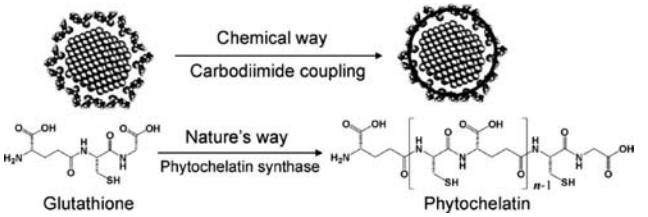


FIGURE 8. Mimicking nature's way in cross-linking GSH to form phytochelatin-like coating on QDs.[53] Reprinted with permission from reference 53. Copyright 2008 Wiley Interscience.

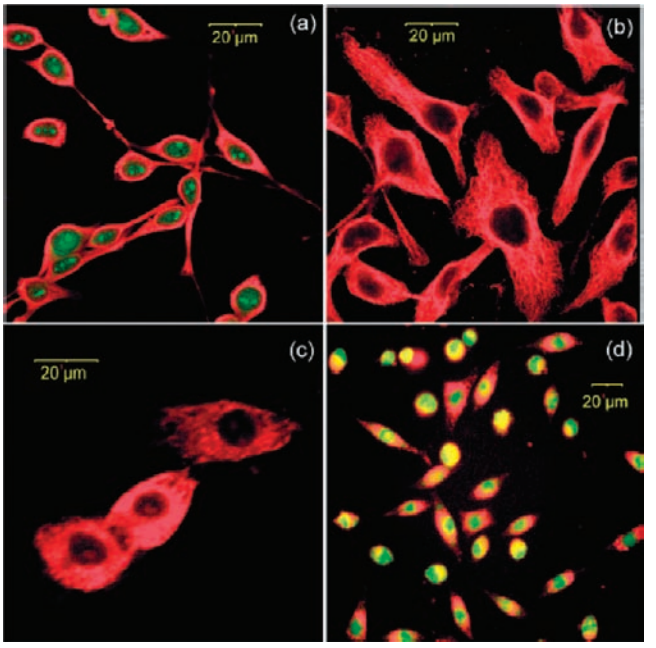


FIGURE 9. Confocal fluorescence images of cells stained with QDs: (a) fixed HepG2 cells with nucleoli and cytoplasm stained by GSH–CdTe-517 QDs (green) and GSH–CdTe618 QDs (red); (b) fixed NIH 3T3 cells with actin immunostained using biotin-labeled GSH–CdTe618 QDs; (c) live MDA-MB-435 cells incubated with F3-labeled GSH–CdTe618 QDs (red); (d) live macrophage RAW264.7 cells incubated with GSH–CdTe618 QDs (red) and cell viability calcein dye (green).[54a] Reprinted with permission from reference 54a. Copyright 2007 Wiley Interscience.

A simple, one-pot synthesis of water-stabilized, monodisperse gold NPs that were coated with multifunctional peptides was accomplished.[55] The A3 parent dodecapeptide AYSSGAPPMPPF, identified from the phage display library, was found to bind to both gold and silver surfaces. In another study, a simple, one-pot, protein-directed synthesis of Au nanoclusters was achieved using bovine serum albumin (BSA) as the coating material.[56] The BSA–Au nanoclusters were highly stable both in solutions and in the solid form. The Au nanoclusters consisted of 25 gold atoms and exhibited a red emission.

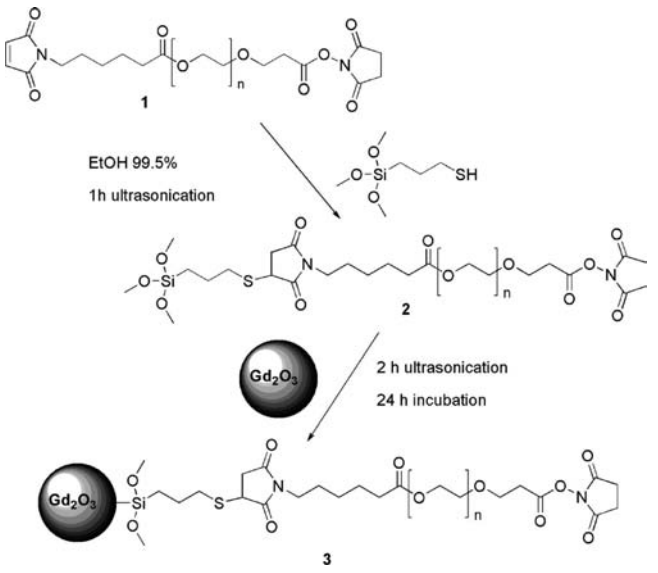


FIGURE 10. Reaction scheme showing the functionalization strategy for capping Gd_2O_3 NPs with (3-mercaptopropyl)trimethoxysilane (MPTS) and α-maleinimido-ω-carboxysuccinimidyl ester poly(ethylene glycol) (Mal-PEG-NHS(1)). The NP solution was added to the intermediate molecule (2), and the functionalized NPs (3) were denoted as GMP.[57] Reprinted with permission from reference 57. Copyright 2010 American Chemical Society.

Gd_2O_3 NPs have been prepared by the polyol method and functionalized with a bifunctional PEG for imaging applications (Figure 10).[57] A targeted contrast agent, a conjugate of cyclic decapeptide CGLIIQKNEC (CLT1) and Gd-DTPA, has been successfully employed in the MRI of fibrin−fibronectin complexes in tumor tissues.[58]

A simple, general strategy for the preparation of a range of functionalized, hydrophilic, monodisperse, and biocompatible magnetic polymer particles has been developed by selecting different polymers as surfactants in a one-pot synthesis scheme.[59] Biomolecules were attached to these magnetic particles and used for intracellular imaging. Multifunctional hollow manganese oxide (MnO) NPs have also been produced using 3,4-dihydroxy-L-phenylalanine.[60] Maghemite NPs have been coated by chitosan for use as MRI probes (Figure 11).[61] A synthetically diverse linker molecule consisting of both a terminal epoxide and a terminal amine has been reacted with dextran-coated iron NPs, converting the surface alcohols to amines.[62]

Peptide and protein coatings are not widely used because of the relatively high complexity of the techniques and the difficulty in characterization. Appropriate encapsulation of NPs results in better stability and preservation of optical properties. However, deposition of several organic or inorganic layers typically increases the particle size significantly.

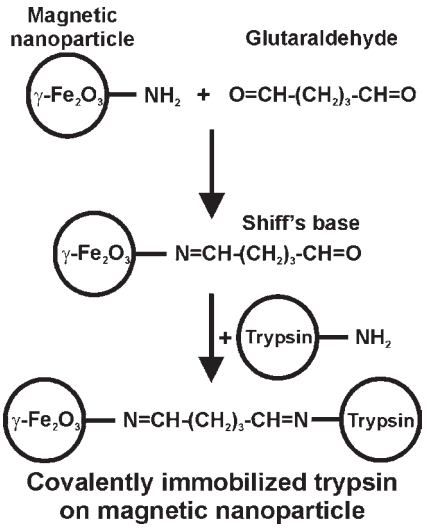


FIGURE 11. A general scheme of trypsin immobilization on chitosan-modified maghemite.[61] Reprinted with permission from reference 61. Copyright 2009 Elsevier.

While ligand-exchange approaches do not impact the particle size substantially, the resulting probes often suffer from reduced stability and fluorescence efficiency. Thus, although a variety of water solubilization and functionalization methods have been developed for NPs, it remains a major challenge to satisfy all the design criteria required in biological applications.

Conclusions and Outlook

Over the past decade, NPs have been developed toward biological and biomedical applications as novel imaging probes and targeting agents. They are powerful tools for studying biomolecular pathways within cells, diagnosis of diseases, and delivery of therapeutics. This Account reviews a selection of advanced coating and functionalization techniques to derive functionalized metallic, heterometallic, metal oxide, and QD NPs with interesting physicochemical properties. The challenge remains in the clinical translation of NP probes, and issues such as biocompatibility, toxicity, *in vivo* and *in vitro* targeting efficiency, and long-term stability of the functionalized NPs need to be addressed. There is a great demand for effective surface functionalization chemistry that is efficient, preserves bioaffinity, provides for ligand diversity, and offers bioactive and stable interfaces. The successful engineering of multifunctional NPs would be of particular interest for the development of theranostic nanomedicine.

BIOGRAPHICAL INFORMATION

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Jackie Y. Ying received her Ph.D. from Princeton University. She was Professor of Chemical Engineering at Massachusetts Institute of Technology. She has been the Executive Director of the Institute of Bioengineering and Nanotechnology in Singapore since 2003. For her research on nanostructured materials, Prof. Ying has been recognized with the American Ceramic Society Ross C. Purdy Award, David and Lucile Packard Fellowship, Office of Naval Research and National Science Foundation Young Investigator Awards, Camille Dreyfus Teacher-Scholar Award, American Chemical Society Faculty Fellowship Award in Solid-State Chemistry, Technology Review TR100 Young Innovator Award, American Institute of Chemical Engineers (AIChE) Allan P. Colburn Award, and Singapore National Institute of Chemistry-BASF Award in Materials Chemistry. Prof. Ying was elected a World Economic Forum Young Global Leader and a member of the German National Academy of Sciences, Leopoldina. She was named one of the "One Hundred Engineers of the Modern Era" by AIChE in its Centennial Celebration. She is the Editor-in-Chief of *Nano Today*.

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FOOTNOTES

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