Nanoscale

RSC Publishing

REVIEW

View Article Online
View Journal | View Issue

Cite this: *Nanoscale*, 2013, **5**, 10729

Recent advances in surface chemistry strategies for the fabrication of functional iron oxide based magnetic nanoparticles

Kostiantyn Turcheniuk,[ab] Arkadii V. Tarasevych,[b] Valeriy P. Kukhar,[b] Rabah Boukherroub[a] and Sabine Szunerits*[a]

The synthesis of superparamagnetic nanostructures, especially iron-oxide based nanoparticles (IONPs), with appropriate surface functional groups has been intensively researched for many high-technological applications, including high density data storage, biosensing and biomedicine. In medicine, IONPs are nowadays widely used as contrast agents for magnetic resonance imaging (MRI), in hyperthermia therapy, but are also exploited for drug and gene delivery, detoxification of biological fluids or immunoassays, as they are relatively non-toxic. The use of magnetic particles *in vivo* requires IONPs to have high magnetization values, diameters below 100 nm with overall narrow size distribution and long time stability in biological fluids. Due to the high surface energies of IONPs agglomeration over time is often encountered. It is thus of prime importance to modify their surface to prevent aggregation and to limit non-specific adsorption of biomolecules onto their surface. Such chemical modifications result in IONPs being well-dispersed and biocompatible, and allow for targeted delivery and specific interactions. The chemical nature of IONPs thus determines not only the overall size of the colloid, but also plays a significant role for *in vivo* and *in vitro* applications. This review discusses the different concepts currently used for the surface functionalization and coating of iron oxide nanoparticles. The diverse strategies for the covalent linking of drugs, proteins, enzymes, antibodies, and nucleotides will be discussed and the chemically relevant steps will be explained in detail.

Received 6th August 2013
Accepted 22nd August 2013

DOI: 10.1039/c3nr04131j

www.rsc.org/nanoscale

1. Introduction

Nanoparticle-based research has rapidly advanced over the last decade to a point where the research focus is shifting away from the mere synthesis and characterization of the nanostructures to the development and investigation of multifunctional nanomaterials, where materials science, chemistry and medical research are deriving tangible benefits from advances in the synthesis and functionalization of nanoparticle platforms.[1–3] Iron-oxide nanoparticles (IONPs) represent one class of inorganic materials that is strongly contributing to the current evolution and interest in nanostructured materials.[1–12] Their unique physical properties, including high surface-to-volume ratios and superparamagnetism, confer useful attributes to IONPs. Two distinct classes of superparamagnetic IONPs are currently used for medical applications: superparamagnetic iron-oxide nanoparticles, often referred to as SPION with a mean diameter of 50–100 nm and ultra-small superparamagnetic iron-oxide nanoparticles with a size below 50 nm. Both are composed of ferrite nanocrystals of magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3), with Fe_3O_4 based particles having a magnetic moment as high as 92–96 emu g^{-1} *versus* 60–80 emu g^{-1} for γ-Fe_2O_3.[13] Other materials commonly investigated are mixed oxides ($Fe_{(3-x)}O_{(4-y)}$; $1 \geq x, y \geq 0$), iron/iron-oxide core–shells ($Fe@Fe_{(3-x)}O_{(4-y)}$), $FePt@Fe_{(3-x)}O_{(4-y)}$ core shells or ferrites (MFe_2O_4 with $M = Mn^{2+}$, Co^{2+}, Ni^{2+}, *etc.*) with magnetic momentums varying between 52 and 110 emu g^{-1}.[14]

IONPs are mostly prepared by one of the following methods: co-precipitation, solvothermal/hydrothermal synthesis, flame spray pyrolysis, micro-emulsion and high-thermal decomposition. As this review focuses on the chemical modification of IONPs, for more detailed information on IONP synthesis readers are referred to other reviews.[1,2,4] However, the co-precipitation process is the simplest and most widely employed chemical route for the synthesis of IONPs. The technique is based on aging a stoichiometric mixture of ferrous and ferric salts under basic conditions, yielding magnetite in the absence of oxygen. This oxidation state is, however, unstable and is quickly transformed into maghemite in air. The main advantages of this approach is the possibility to produce a large amount of material in short time with a good control over shape

[a]*Institut de Recherche Interdisciplinaire (IRI, USR 3078 CNRS), Université Lille 1, Parc de la Haute Borne, 50 Avenue de Halley, BP 70478, 59658 Villeneuve d'Ascq, France. E-mail: sabine.szunerits@iri.univ-lille1.fr; Fax: +33 3 62 53 17 01; Tel: +33 3 62 53 17 25*

[b]*Department of Fine Organic Synthesis, Institute of Bioorganic Chemistry and Petrochemistry NAS of Ukraine, 1 Murmanska Str., 02660 Kiev, Ukraine*

and particle size (2–50 nm) by adjusting the pH, ionic strength and concentration of the growth solution. However, the IONPs produced using this method tend to agglomerate when exposed to physiological environment and aqueous solutions and are prone to degradation though oxidation. The vulnerability of IONPs towards oxidation becomes more pronounced for particles smaller than 20 nm. Most applications of IONPs require long term stability and non-toxicity over a large time scale, making surface stabilization of high importance. Two approaches can be used for stabilizing IONPs: one is ligand addition during (*in situ*) or after (post-coating) the synthesis of IONPs; the other is ligand exchange. For IONPs prepared by the co-precipitation method, additives such as sodium citrate, oleic acid, cetyltrimethylammonium bromide (CTAB), dopamine, and polymers (*e.g.* dextran, chitosan, polyvinyl pyrrolidone (PVP), polyvinyl alcohol (PVA), *etc.*) are used. IONPs produced by high-temperature decomposition of organometallic precursors (*e.g.* $Fe(CO)_5$) are often coated during the process with hydrophobic surfactant molecules such as oleic acid or oleylamine, facilitating their stabilization in organic solvents. However, this treatment reduced their stability in aqueous media. Ligand exchange with molecules having a stronger affinity for iron oxides such as dopamine, carboxylates, phosphonates or thiols is a widely used strategy for replacing these surface coatings and making IONPs water soluble.

This review is focusing on some of the most common strategies used for the protection and stabilization of IONPs which have proven to be of uttermost importance for the use of IONPs in biological applications and in nanomedicine.[15,16] The purpose of this review is to outline the surface strategies employed to make IONPs adaptable to *in vivo* and *in vitro* applications and to highlight some relevant studies in this fast growing research field. Apart from surface passivation with metal coating, surface modifications with polymers, inorganic shells and bi-functional ligands such as dopamine or phosphonates are the most prevailing methods. Advantages and drawbacks of the different approaches will be discussed and highlighted. It has to be kept in mind that there is not a single strategy that is the most adapted for all the different applications of IONPs. For biomedical applications, targeting agents and therapeutic drugs need to be additionally integrated onto the IONPs' surface. The development of different bio-conjugation strategies is thus mandatory. The review will summarize the most commonly investigated schemes for the incorporation of different ligands onto IONPs. Most approaches are based on the formation of a relatively stable, covalent linkage between the hydroxyl groups on the IONP surface and a suitable anchoring agent present in the specific position of the target molecule of interest.

2. Protection and stabilization of IONPs

The most important issue to be accomplished after the preparation of IONPs is to provide the nanoparticles with a sufficient degree of stability. The high chemical reactivity of the nanoparticles' surface could pose health risks to patients when IONPs are used for biomedical applications. This section will focus on some of the different strategies established for the protection and stabilization of IONPs (Fig. 1). All the surface modification routes used so far have resulted in a core–shell like structure, with IONPs being the core and some kind of inert organic or inorganic material being the protecting/stabilizing shell.

2.1. Silica shell

The advantage of using silica as a coating material is based on its exceptional stability in aqueous media and its compatibility with various bioconjugation reactions for the introduction of specific ligands. From a more technical point of view, the possibility of controlling the coating thickness and its porosity combined with the chemical inertness of the silica matrix, its low cost of production and optical transparency make silica-coated IONPs very promising nanostructures.[17–20] Alkaline hydrolysis of tetraethyl orthosilicate (TEOS) known as the Stöber synthesis is most widely employed for the formation of silica shell on IONPs (Fig. 2A).[21] It involves preliminary formation of an intermediate silica layer through deposition of TEOS on the IONP surface, which can be further silanized with bifunctional organosilanes. In order to coat silica onto IONPs while avoiding the formation of silica spheres, it is necessary to tune the competition between nucleation (hydrolysis) and growth (condensation) of silica. Silica tends to coat the IONPs as the condensation rate is much higher than the nucleation rate. Low temperature, pH and TEOS concentrations and a small amount of water are predominating factors in facilitating the condensation process.[22]

Further developments in this area concern the formation of a new family of nanomaterials: magnetic nanocomposites with ordered mesoporous silica structures. These hybrid materials are very useful containers for molecules because of their unique features such as stable mesoporous structure, large surface area, tunable pore size and volume for hosting molecules with various size, shape, functionality and especially well-defined magnetic property that is very desirable for site-specific delivery.[23–25] Magnetic mesoporous silica nanocomposites covalently functionalized with light-responsive azobenzene derivatives have been recently reported as novel target delivery

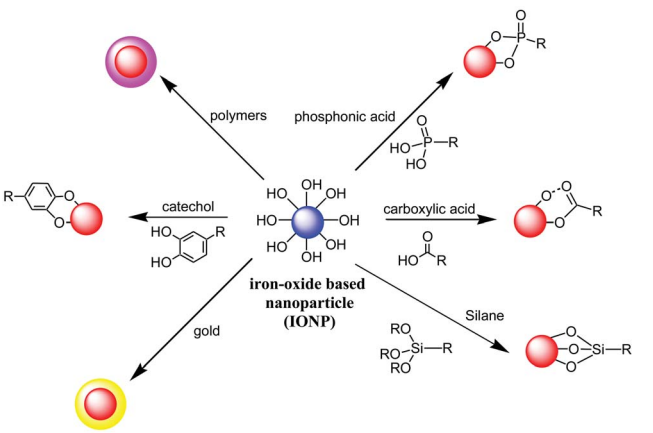

Fig. 1 Different strategies for the protection/stabilization of IONPs.

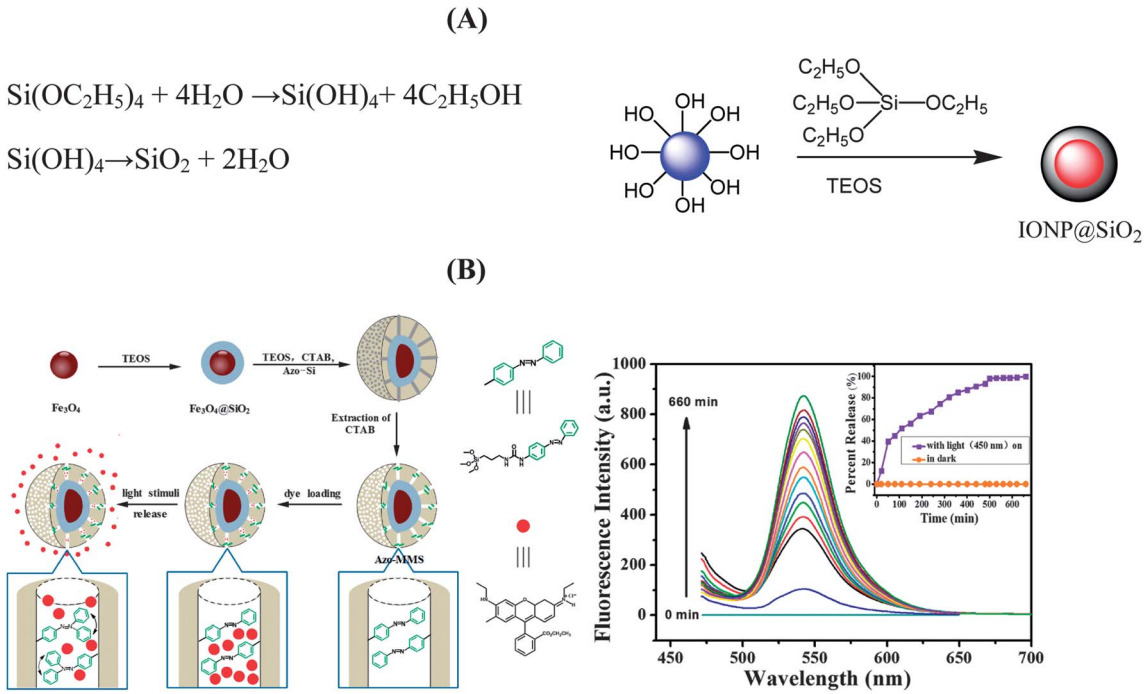

Fig. 2 (A) Formation of silica coated IONPs through hydrolysis and condensation of alkoxysilanes such as tetraethylorthosilicate (TEOS); (B) (left) Formation of a magnetic mesoporous silica nanocomposite with light-controlled release character, (right) emission spectra of rhodamine 6G release upon irradiation at 450 nm (reprinted with permission from ref. 23).

systems (Fig. 2B).[23] The material integrates magnetic targeting and stimuli-responsive release properties. Indeed, guest molecules hosted in the pores cannot diffuse out because of the blocking by high density azobenzene chains. Visible light irradiation causes a dynamic motion of azobenzene chains (*trans*/*cis* isomerization) opening pathways and expelling the guest molecules out of the pores.

Like most oxide surfaces, the presence of hydroxyl groups (Fe–OH) on the IONPs' surface provides a versatile synthetic handle allowing the attachment of different functionalities. One of the most successful approaches for the functionalization of oxide-based materials has been the chemical grafting of organic chains *via* a trichlorosilane ($-SiCl_3$) or trimethoxysilane ($-Si(OCH_3)_3$) end group. This reaction, termed silanization, was derived more than 70 years ago for chromatographic applications[26] and is now routinely used for the formation of various monolayers on different surfaces through silicon–oxygen bonds.[17] The use of bifunctional organosilanes (*e.g.* 3-aminopropyltrimethoxysilane (APTES), mercapto-propyltrimethoxysilane, cyanoethyltriethoxysilane, *etc.*) allows in addition the direct introduction of amine, thiol, cyano and other moieties required for post-conjugation with different ligands (Fig. 3). The versatility of silane organic functional groups makes these compounds ubiquitous for surface modification of IONPs. Amal and co-workers investigated the effect of differently functionalized (amine, aldehyde, carboxylic, epoxy, thiol, and maleimide) IONPs using organosilane linkers on the activity, kinetics, stability and reusability of trypsin modified IONPs (Fig. 3A).[27] This study revealed that the highest activity per gram of IONPs is obtained on HOOC- and HS-terminated surfaces, followed by $-NH_2$ and –CHO surfaces. The epoxy (–COC–) surface was found to have less affinity to trypsin as a result of the partial epoxy ring opening that slowed the kinetics of epoxy–amine reaction (Fig. 3B).[27]

The main drawback is that silane chemistry is not entirely well controlled and thus lacks reproducibility. In addition, Si–O–Si bonds are prone to hydrolysis when exposed to harsh conditions such as highly saline conditions encountered in biological media.[28–32] Other strategies have been developed and are outlined below.

2.2. Gold shell

The other inorganic material, highly adequate to implement protection to IONPs is gold.[33–42] Gold is the material of choice because of its chemical stability and biocompatibility, as well as its established reactivity with thiolated compounds.[43] Core–shell IONP@Au particles have attracted enormous interest for bioassays as they combine the properties of gold nanoparticles with high catalytic efficiency, high conductivity and attractive optical properties known as localized surface plasmon resonance (LSPR)[44] with the magnetic functionality of IONPs. Initial attempts to coat IONPs with gold involved the use of reverse micelles as constrained reactors for both particle synthesis and gold coating, which resulted however in rather low yields.[45,46] Synthesis and coating in the aqueous phase as shown by Lyon *et al.* have shown to overcome this limitation.[47] The IONPs are synthesized by the precipitation of iron salts in an alkaline environment followed by the reduction of $HAuCl_4$ to form a gold coating by electroless gold deposition. This approach is simple,

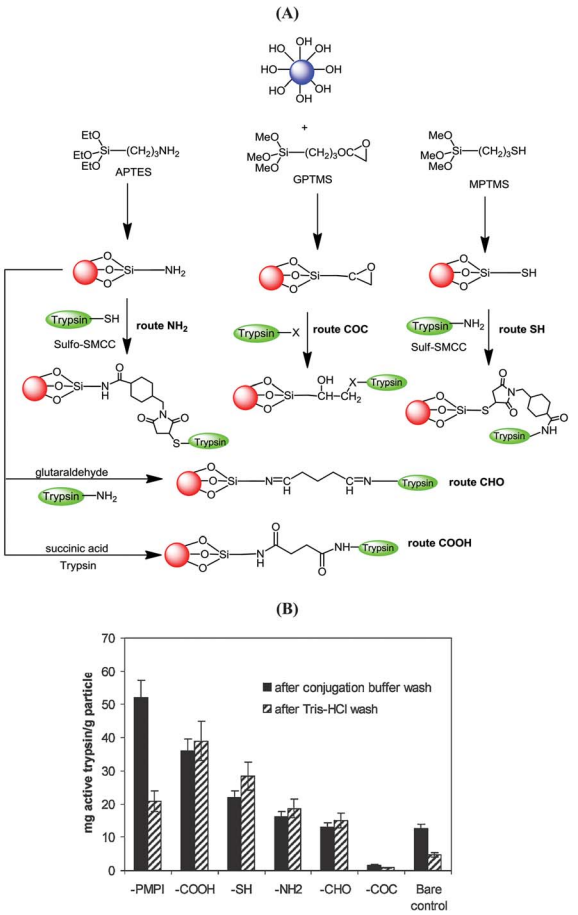

Fig. 3 (A) Schematic illustration of the functionalization of IONPs with different organosilane linkers; (B) activity of immobilized trypsin on differently functionalized IONPs (reprinted with permission from ref. 27).

fast and produces particles that are dispersible in water. However, the particle size and gold-shell thickness are difficult to control and the synthesized samples contain a mixture of coated and uncoated IONPs that has proven difficult to separate. The third category consists of IONP synthesis and gold coating in the organic phase resulting in particles of significantly enhanced particle size and shell thickness control with excellent resistance to aggregation in organic solvents.[48] To achieve size control and viability under aqueous conditions, another synthetic method has emerged involving a combination of organic synthesis of the IONP core followed by gold coating in aqueous solution.[49] Fig. 4 shows this concept schematically, starting with the coating of gold onto the surface of IONPs by reducing $HAuCl_4$ in a chloroform solution of oleylamine, which is used as a mild reduction agent for $HAuCl_4$ as well as a surfactant for IONPs. The resulting IONP@Au particles are transferred to water with cetyltrimethylammonium bromide (CTAB) and sodium citrate and they served as seeds for the formation of IONP@Au nanoparticles with thicker gold coating achieved by adding more $HAuCl_4$ under reductive conditions.[49] Similarly, starting from IONP@Au, silver coated particles (IONP@Au@Ag) can be synthesized by addition of $AgNO_3$ to the reaction medium.[49]

A fifth category of synthetic methods has been proposed by the group of Amal and is based on the use of the biocompatible polyelectrolyte polyethyleneimine (PEI) for the dual function of attaching gold seeds and preventing the formation of large aggregates (Fig. 5A)[42,50,51] Such particles were further transformed by modification of the gold shell with peptides, antibodies and organic molecules (Fig. 5B).[50,52,53]

Other approaches for the formation of IONP@Au nanostructures consist of attachment of preformed gold nanoparticles onto IONPs modified with thiols such as cysteamine[39] or 3-mercaptopropyltrimethoxysilanes.[54] Recently, the group of Hildebrandt reported on the synthesis of magnetic silver hybrid nanoparticles (Fig. 6A). The particles were deposited on silica-coated IONPs, which were subsequently functionalized with 3-aminopropyltrimethoxysilane (APTMS) to allow for electrostatic binding of silver seeds with the negatively charged surface, where silver particles were grown subsequently.[55] During the growth process, simultaneous coating with chitosan resulted in the formation of an additional chitosan protection layer, where the amine-groups of chitosan were used for the covalent linking of myoglobin (MB) using glutaraldehyde as a cross-linker.[55] Myoglobin allows capture of toxic targets (NO_2^-, CN^- or H_2O_2) and the decontamination process was followed by Surface-Enhanced Resonance Spectroscopy, SERS (Fig. 6B).

A separate class of magnetic-gold composites has been reported involving the attachment of discrete gold nanoparticles onto IONPs without however full coating and protection of IONPs.[33,40,56] The synthesis of such dumbbell-like Au–IONP nanoparticles is similar to the synthesis of core–shell nanoparticles with the difference that the nucleation and growth are anisotropically centered on the specific crystal plane around the seed nanoparticles, and not uniformly distributed over the seed nanostructures.

2.3. Catechol coordination

While simple alcohols bind weakly to IONPs,[57] limiting the applicability of this strategy for surface modification, catechol ligands form extremely stable complexes with IONPs.[58] A stability constant of $K_{stab} = 10^{44.9}$ has been determined for the octahedral complex $[Fe(cat)_3]^{3-}$ formed between Fe^{3+} and 3 dianionic catechol ligands. This metal catecholate bond is highly stable with both σ- and π-donor bonding contributing to the overall stability of the complex. Catechol-based functionalization of IONPs has consequently become one of the routine ways to stabilize IONPs and has been successfully utilized in a range of conjugation protocols.[59–71] Xu *et al.* were the first to describe a general strategy using dopamine as a stable anchor to graft functional molecules onto the surface of IONPs.[61] The approach is based on ligand exchange between oleic acid or oleylamine stabilized IONPs and dopamine–succinyl-nitriloacetic acid.[61] Our group[71] has recently shown that different catechol ligands can be simultaneously attached to IONPs allowing the formation of multifunctional nanostructures in an easy and reproducible way (Fig. 7).

While dopamine derivatives are the most extensively used linkers for the conjugation onto IONPs, the use of dopamine

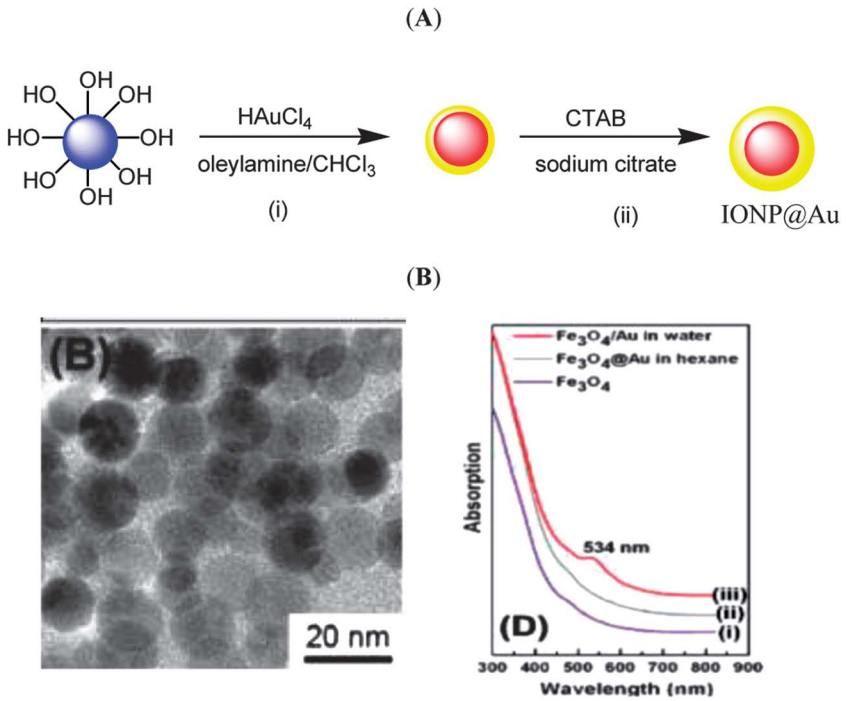

Fig. 4 (A) Schematic illustration of surface coating of IONPs with Au to form hydrophobic IONP@Au nanoparticles; (B) TEM image and UV/Vis absorption spectra of the IONP@Au particles (reprinted with permission from ref. 49).

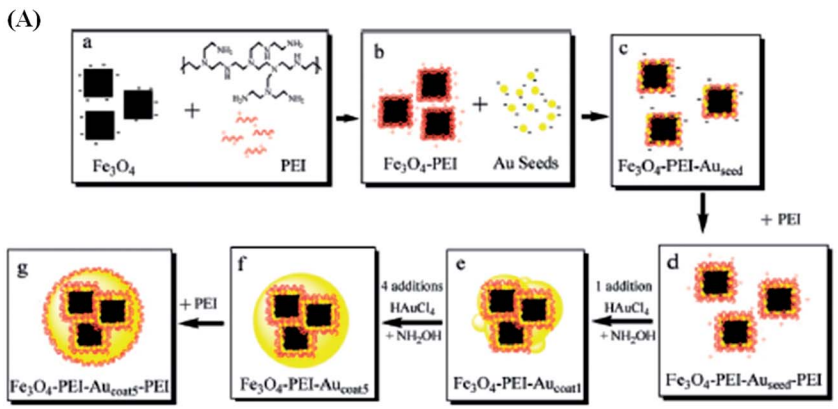

Fig. 5 Schematic representation of the synthesis of IONP@Au nanostructures: (a) cationic PEI self-assembled onto negatively charge IONPs, (b) IONP–PEI mixed with gold seeds to obtain (c) gold-seeded IONPs, after which PEI is again added to obtain (d) PEI-coated gold-seeded IONPs. (e) One addition of $HAuCl_4$ and NH_2OH results in an uneven gold shell. (f) Four subsequent addition of $HAuCl_4$ and NH_2OH form even gold coating. (g) PEI is added to increase the stability of IONP@Au nanostructures (reprinted with permission from ref. 42).

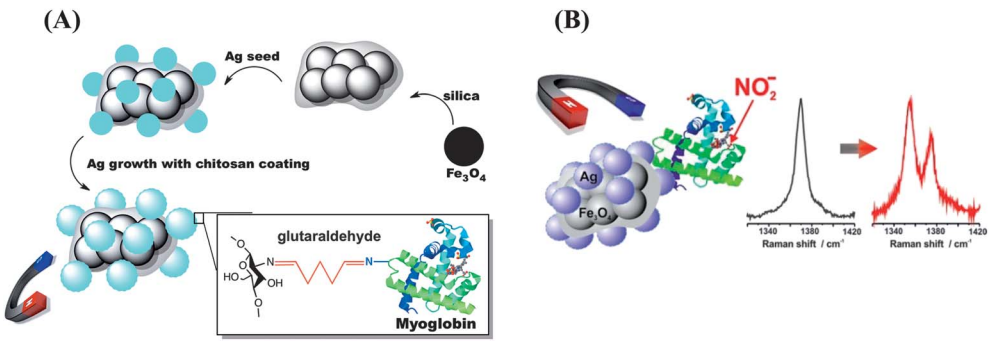

Fig. 6 (A) Schematic illustration of the formation of IONP@Ag nanoparticles with chitosan coating; (B) interaction of myoglobin with contaminants, their separation by magnetic force and detection by SERS (reprinted with permission from ref. 55).

has been questioned as the IONP–catechol complex has a tendency to dissociate at physiological pH and can be oxidized to a quinone-like structure in the presence of Fe^{3+} both at the surface and in solution (Fig. 8Aa).[72] Particle-etching and iron-semiquinone formation were also noted by some authors.[64,73–75] The amine-function of dopamine is also highly reactive, resulting in the formation of 5,6-dihydroxylindole as an intermediate and finally polydopamine (PDA).[69] Despite the wide application of PDA as a coating material it is only recently that the structure of PDA has been finally identified.[76] PDA is thus believed to be composed of dihydroxyindole, indoledione and non-cycled dopamine units, which are assumed to be covalently linked (Fig. 8Ab). Reimhult and coworkers demonstrated that this limitation can be overcome by attaching electronegative nitro-groups to the aromatic catechol system (Fig. 8B).[64] The introduction of nitro-groups renders the dopamine ligand electron deficient and increases the oxidation potential of the 2-nitrocatechol ligand, resulting in a high and irreversible binding affinity to IONPs.[64] The 2-nitrocatechol linker has the additional advantage of being less neurotoxic than other metabolites of dopamine, including 6-hydroxydopamine.[77]

The high stability of IONPs modified with nitrocatechol ligands was more recently used by the group of Textor for the construction of IONPs with dendritic polymer shell architectures with excellent colloidal stability (Fig. 9).[78] Moreover, these particles showed a reversible temperature-induced aggregation behavior in contrast to the essential irreversible aggregation and sedimentation observed for the linear PEG analogues. This new class of dendritically stabilized IONPs could have potential for future biomedical and other applications, in which the stability, resistance or reversible aggregation, ultrasmall size and high density of bioactive ligands are key parameters.

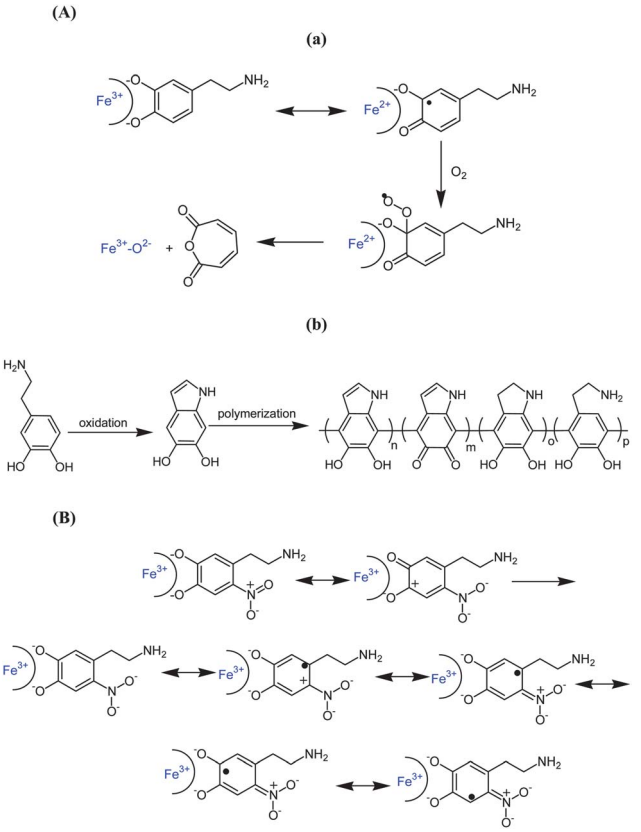

Fig. 8 (A) (a) Iron-catalyzed catechol degradation: Fe^{3+} complexed catechols are oxidized to semiquinones while Fe^{3+} is reduced to Fe^{2+} before semiquinones are further degraded through reactions with oxygen, (b) polymerization of dopamine; (B) suggested binding mechanism of 2-nitrocatechols with initial binding to Fe^{3+} ions (reprinted with permission from ref. 77).

2.4. Other monomeric stabilizers: carboxylates, phosphates, and phosphonates

As discussed previously, nucleophiles such as catechols have a good affinity to IONPs. The nucleophilic character of carboxylates and phosphates is also responsible for binding to electrophilic IONP surfaces through the interaction with iron empty orbitals, forming a combination of covalent and physisorptive bonds.[57] One of the simplest methods for the protection of the native IONP surface is through coordinative binding with conjugated bases such as phosphonates or carboxylates. Citric acid has been used commercially for the stabilization of

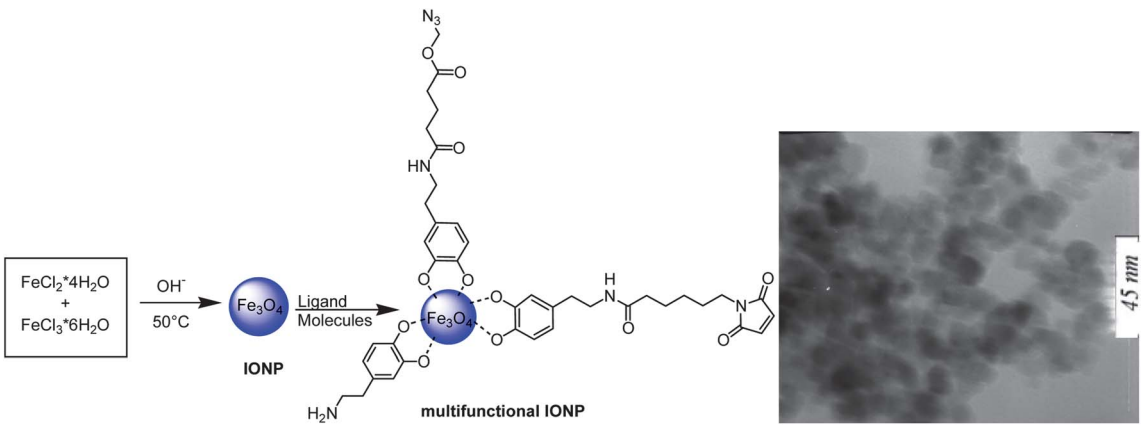

Fig. 7 Schematic illustration and TEM image of multifunctional IONPs formed by simultaneous modification of co-precipitated IONPs with differently functionalized dopamine derivatives.

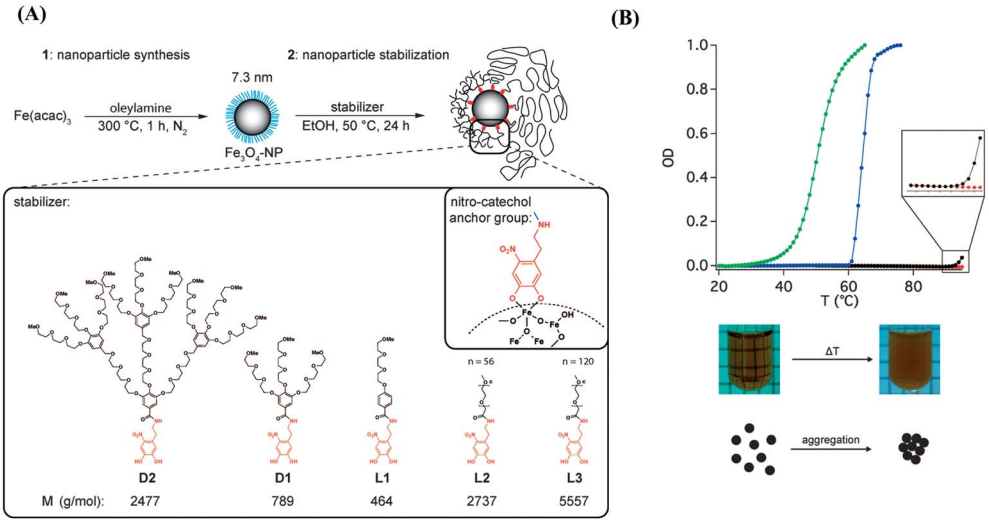

Fig. 9 (A) Illustration of the formation of PEG-stabilized IONPs using linear and dendrite 2-nitrodopamine based structures. A single 2-nitrocatechol linker serves as an anchor; (B) cloud-point determination of L3-IONP (red), L2-IONP (black), D2-IONP (blue), and D1-IONP (green) in 0.1 M HEPES solution. The optical density was recorded *via* UV/Vis spectroscopy as 600 nm as a function of temperature (reprinted with permission from ref. 78).

IONPs, such as in the MRI contrast agent VSOP C184.[79] This acid adsorbs on IONPs by coordinating *via* one or two of the carboxylate functionalities (Fig. 10A).[80,81] This leaves at least one carboxylic acid group exposed to the solvent, making the particle hydrophilic and negatively charged.[80,82] Citrate-coated IONPs have shown to be efficient intracellular magnetic labels for *in vivo* stem cell tracking by MRI.[83] Internalization of citrate-coated IONPs was increased by more than one order of magnitude compared to the uptake of commercial Resovist and Endorem IONPs as clearly indicated by the highest iron content per cell and most intense Prussian blue staining at the same iron labeling concentration (Fig. 10B). Besides citric acid, other

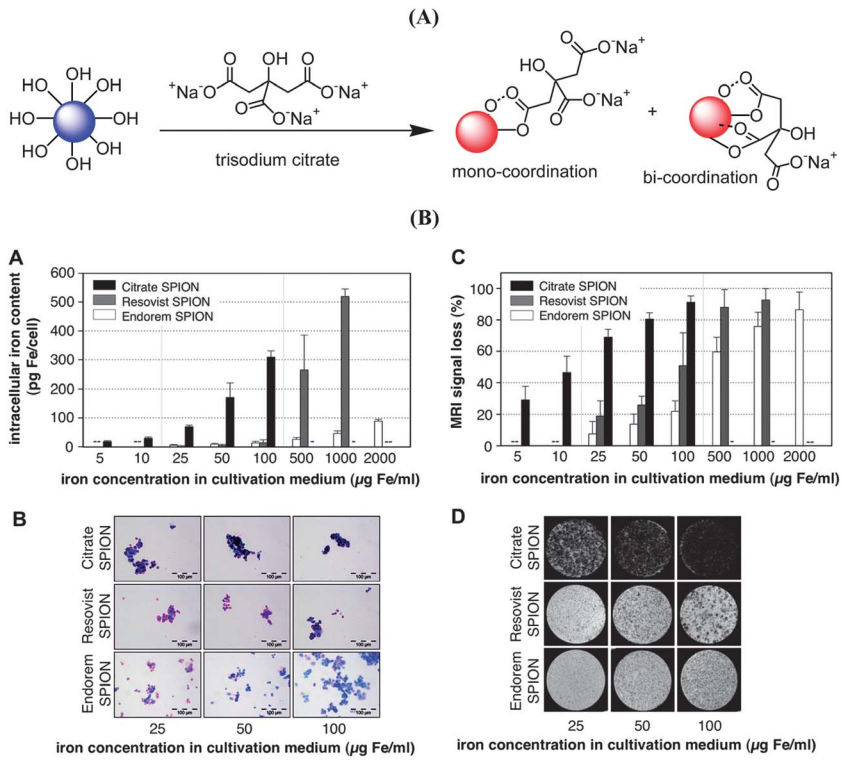

Fig. 10 (A) Citrate-modified IONPs; (B) dose- and type-dependent human MSC cell labeling with IONPs. Adherent MSCs were incubated for 24 h with increasing particle concentration in the cultivation medium. Magnetic labeling of human MSCs was investigated as a function of the IONPs iron concentration in the cultivation medium and of the IONPs type by quantification of the intracellular iron content using ICP–OES (A), Prussian blue staining (B) and visualization by *in vitro* MRI (C and D). As comparison dextran (Endorem) and carboxydextran (Resovist) commercially available IONPs were used (reprinted with permission from ref. 83).

carboxylic acid based molecules (*e.g.* dimercaptosuccinic acid,[84] and 3-allylacetylacetonate[85]) have been investigated. However, the IONP/COOH coordination bond is very labile and can be easily broken by increasing the temperature or by exchange with other ligand molecules. This has limited their general use in IONPs' surface modification.

Although much less widespread than citric acid, phosphonic acid has shown strong affinity to IONPs through the formation

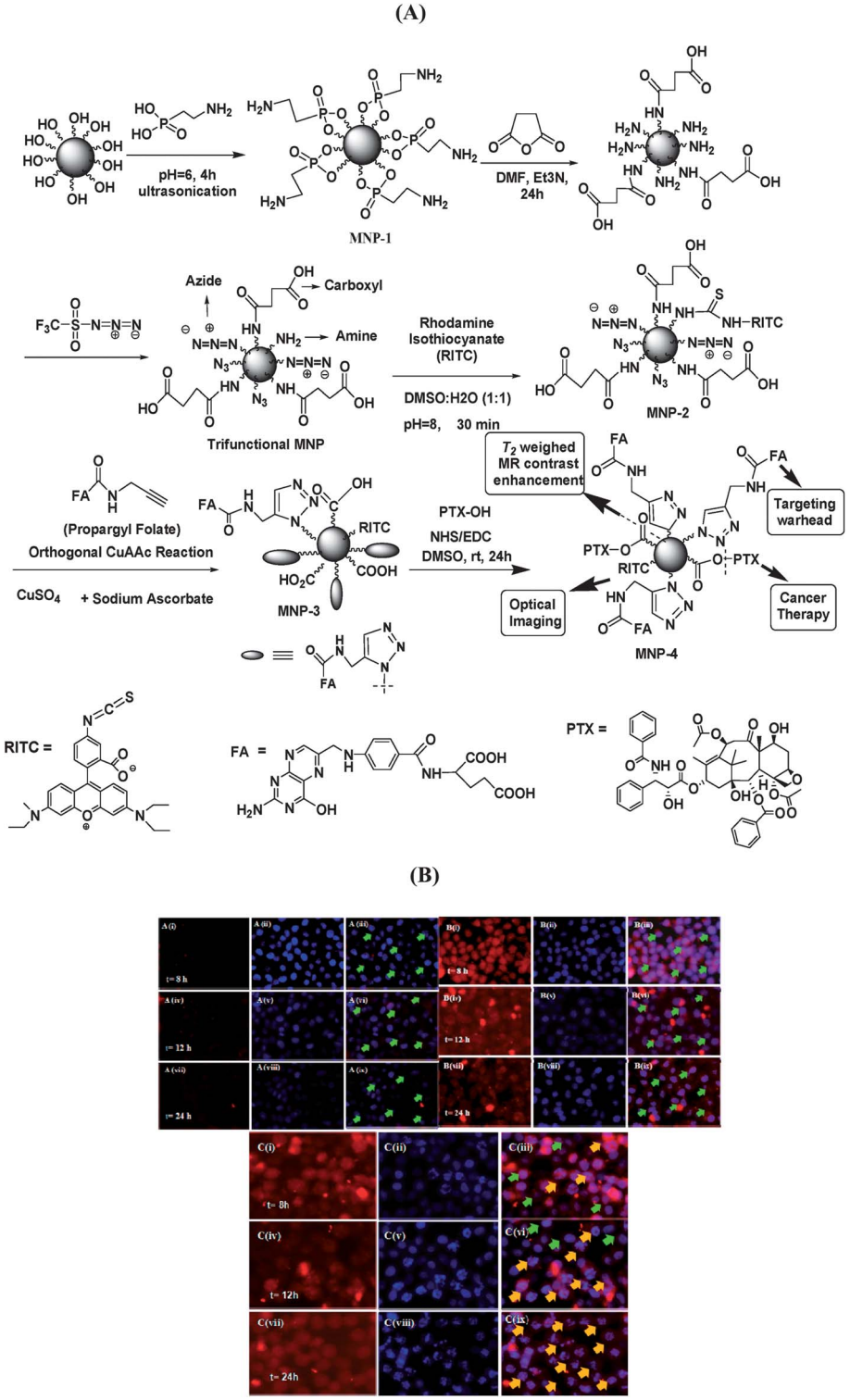

Fig. 11 (A) Formation of bidentate complexes between IONPs and 2-aminoethylphosphonic acid and further functionalization with rhodamine B isothiocyanate, propargyl folate and paclitaxel; (B) fluorescence microscopy of HeLa cells incubated with trifunctional IONPs for 8, 12 and 24 h. A: MNP1, B: MNP3, and C: MNP4. The green and yellow arrows denote healthy and apoptotic nuclei (reprinted with permission from ref. 92).

of strong bidentate Fe–O–P bonds (Fig. 11).[86–88] The bonds are stronger than those formed by carboxylic acid molecules with a good stability over several weeks at neutral pH. The grafting densities are in addition enhanced over the ones using carboxylic acid groups.[86] In comparison to silane-based protection strategies (see Section 2.1.), phosphonic acid monolayers are formed independent of the amount of water used, making these ligands of high interest for controlled surface functionalization.[89–92] Das and co-workers have demonstrated that this approach allows for the multi-functionalization of IONPs for combined cancer-targeted therapy and multimodal imaging (Fig. 11).[92] Simultaneous functionalization with amine, carboxylic, and azide groups was achieved through a sequence of stoichiometrically controlled partial succinylation and Cu(II) catalyzed diazo transfer in the reactive amine termini of 2-aminoethylphosphonate modified IONPs (Fig. 11A). Chemoselective conjugation with rhodamine B isothiocyanate, propargyl folate and paclitaxel *via* tandem nucleophilic addition of amine to isothiocyanates, Cu(I) catalyzed azide–alkyne click chemistry and carbodiimide-promoted esterification resulted in trifunctional IONPs. These particles could selectively target and induce apoptosis to folate-receptor over-expressing cancer cells with higher efficacy when compared to the free drug (Fig. 11B).[92]

2.5. Polymeric ligands

For many applications in medicine, a polymer coating is preferred over simple functionalization with small organic molecules.[12,93,94] Polymer attachment to the IONP surface can be achieved *via* three alternative approaches: (1) grafting "to" during the IONP synthesis, (2) through ligand exchange or (3) grafting "from" by immobilizing an initiator onto the surface. The grafting "from" approach has the advantage of producing higher grafting densities, while the grafting "to" approach allows a better control over the polymer architecture and functionality, and is therefore more versatile than the grafting "from" method.

The first generation of polymers used for IONP stabilization was based on polymers such as dextran, chitosan, poly(ethylene oxide) (PEO), poly(imine), poly(acrylic acid), poly(vinyl alcohol), *etc.* (Fig. 12A). Currently, all the commercially available MRI agents (beside VOP 345) are coated with natural carbohydrate polymers (*e.g.* Ferrumoxtran-10, Feraheme[95]). A limitation for using physically adsorbed polymers is the lack of stability; attempts to address this issue have involved cross-linking to form cross-linked iron oxide (CLIO) particles (Fig. 12B). CLIO particles are based on dextran-coated IONPs, formed by co-precipitation of iron salts in the presence of an excess of

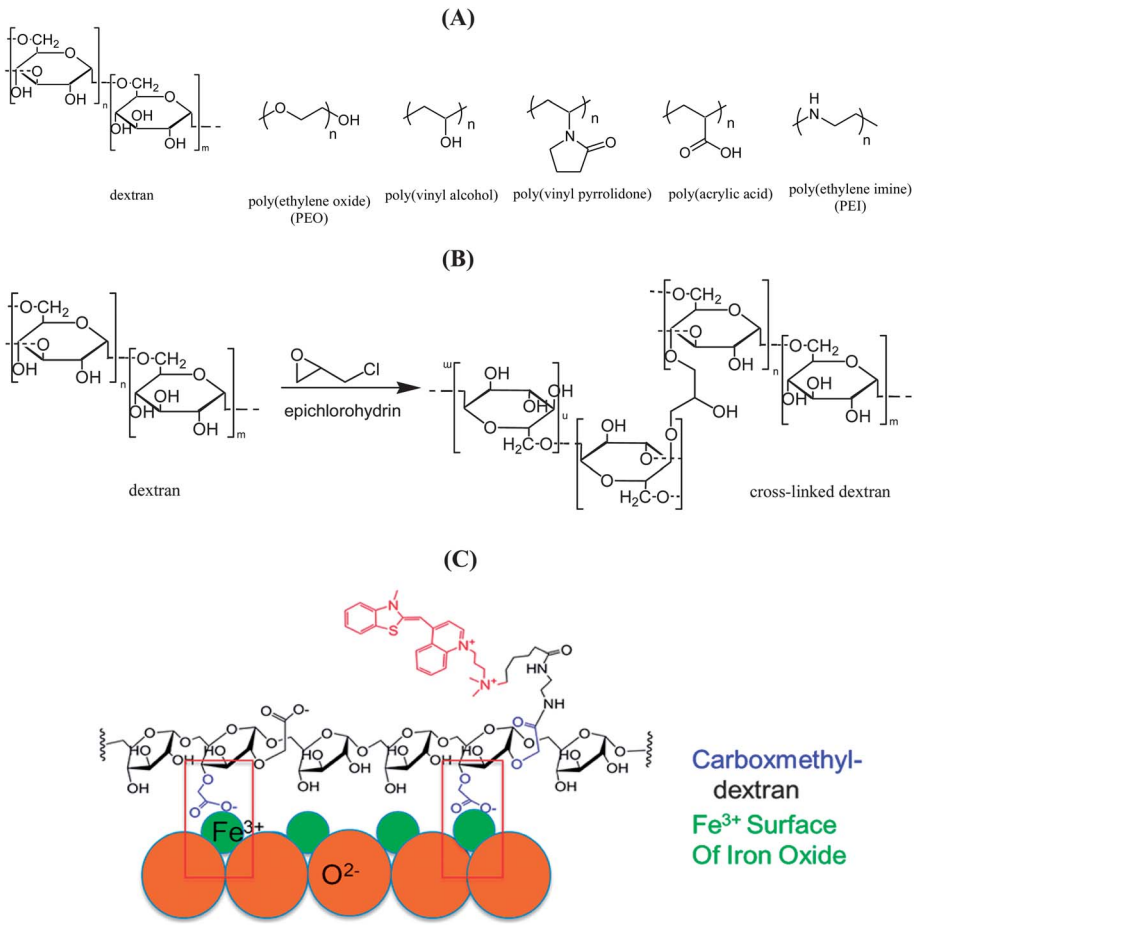

Fig. 12 (A) Structures of polymers used for stabilization; (B) formation of cross-linked dextran on CLIO particles; (C) surface chemistry for fluorochrome functionalized NPs. The carboxymethyldextran provides carboxyl groups for fluorochrome attachment and binds to the iron oxide, generating high thermal stability (reprinted with permission from ref. 95).

dextran.[96] The molecular weight of dextran and the ratio of dextran to Fe^{2+} and Fe^{3+} define the size distribution and stability of the resulting colloidal suspensions. Dextran complexes with IONPs through weak interactions between the hydroxyl groups of dextran and Fe, resulting in a relatively stable coating that can, however, be displaced over time in the presence of stronger surface binders. The introduction of carboxymethyl groups improved their stability; however a stronger attachment was still required and can be achieved by cross-linking dextran with epichlorohydrin, resulting in stable CLIO particles whose size is not altered even under harsh conditions.[97] Feridex and Combiex are products of this approach that have been marketed and are in clinical trials. In another example, carboxymethyl groups of dextran coated nanoparticles (Feraheme) have been reacted with ethylenediamine by means of EDC to yield amino-species. The amine-modified nanoparticles were successfully coupled with a fluorochrome to yield fluorochrome-functionalized Feraheme nanoparticles. These particles are able to enhance the fluorescence upon intercalation with DNA and thus used in DNA recognition.[95]

Besides dextran, poly(ethylene oxide) (PEO) otherwise known as polyethylene glycol (PEG) or poly(oxyethylene) (PEO) is certainly the most extensively investigated polymer. This water soluble polymer is commonly used to enhance the aqueous solubility of hydrophobic molecules such as drugs, and it minimizes non-specific uptake in the body, shows increased circulation time in the blood without any immune interaction and acts as a good spacer for the attachment of different biomolecules.[98,99,100] PEO molecules are bound to IONPs almost exclusively by the grafting "to" approach.[78,101,102] As stated above, this approach often leads to low grafting density and low film thickness, as the polymer molecules must diffuse through the existing polymer film to reach the reactive sites of the surface. Furthermore, each surface-immobilized PEO macromolecule has only one site available for ligand coupling limiting their utility for further functionalization. The grafting "from" method is thus more suitable for achieving highly stable polymer layers and high grafting density.[103] Progress in polymer synthesis techniques such as atom-transfer radical polymerization (ATRP) and reversible addition-fragmentation chain transfer (RAFT)-mediated polymerization makes it possible to produce polymer chains with controllable length. Grafting "from" of PEO chains by surface-initiated atom-transfer radical polymerization (SI-ATRP) is thus an interesting alternative.[104,105] Lattuada *et al.* grafted several polymers (PEO, poly(methacrylic acid)), poly(hydroxyethylmethacrylate), polystyrene sulfonate, poly(*N*-isopropylacrylamide), "on" and "from" IONPs using amine-functionalized PEO chains or living radical polymerization with an atom transfer radical polymerization (ATRP) initiator (Fig. 13).

The attachment of polymer chains onto IONPs can also be achieved using polymers with functionalities capable of binding to IONPs such as phosphonic acid,[107] carboxylic acid groups,[108] cystein,[109] triethoxylsilyl groups[110] or dopamine.[111] Amstadt *et al.* linked dopamine-terminated PEG to IONPs to stabilize the particles in biological fluids.[111] Sandiford *et al.* described the stabilization of IONPs using biphosphonate-anchored PEGylation of IONPs (Fig. 14A).[112] Using this method, colloidally stable PEGylated IONPs were formed that could be stored as a dispersion in water or saline solutions for at least 7 months

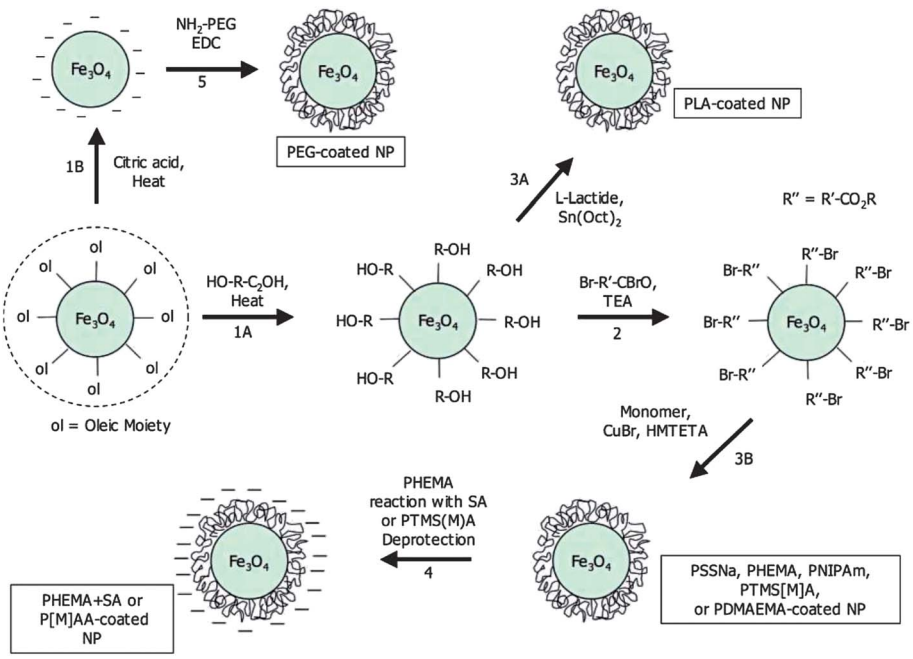

Fig. 13 Formation of polymer-functionalized IONPs. Steps 1A and 1B: ligand exchange reaction. Step 2: acylation of hydroxyl groups to prepare ATRP surface initiators. Step 3A: surface-initiated ring opening polymerization of L-lactide. Step 3B: surface-initiated ATRP. Step 4: deprotection or additional reaction after polymerization. Step 5: grafting "on" end-functionalized PEO chains onto IONPs using amidation reaction (reprinted with permission from ref. 106).

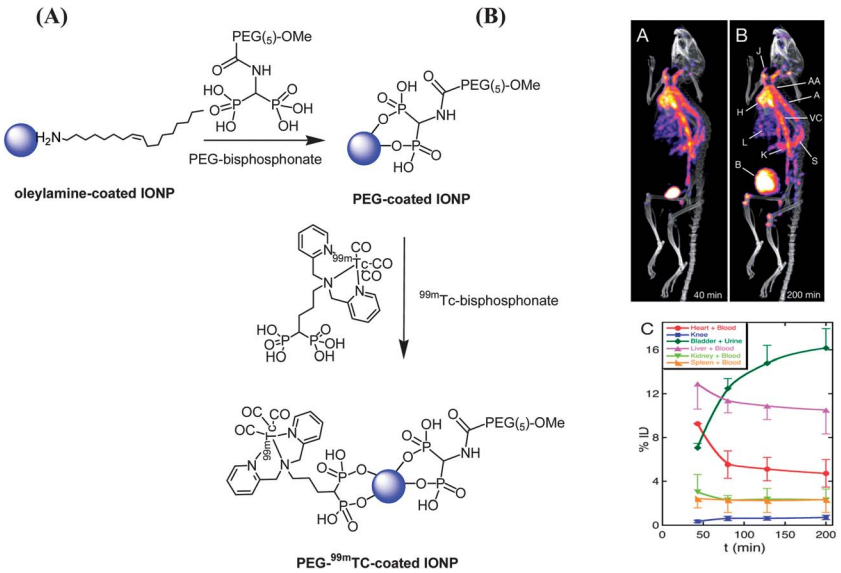

Fig. 14 (A) Formation of multifunctional IONPs (PEG and gamma-emitting ^{99m}Tc isotope) using biphosphonate ligands; (B) *in vivo* single photon emission computed tomography (SPECT) studies with these IONPs at the first 40 min (A) and last 200 min (B) in the heart (H), jugular vein (J), aortic arch (AA), aorta (A), vena cava (CA), liver (L), kidney (K), spleen (S), and bladder (B) (reprinted with permission from ref. 112).

without change in the hydrodynamic diameter. Such particles were used as efficient contrast agent (Fig. 14B) for T1-weighted imaging.

Besides dextran and PEO, other polymers have been investigated for the coating of IONPs. Turro *et al.* described for example the stabilization of IONPs using poly(*tert*-butyl acrylate) in a two-step process. First, azide-terminated organophosphate groups were introduced onto the IONPs' surface through ligand exchange with oleic acid modified particles. The copper(I) catalyzed azide–alkyne cycloaddition (CuAAC) was subsequently applied to covalently immobilize the polymeric ligand, α-acetylene-poly(*tert*-butyl acrylate).[113] Polyethyleneimine (PEI) modified IONPs have been employed for magnetotransfection experiments.[114,115] Injectable, *in situ*-gelling IONPs functionalized with poly(*N*-isopropylacrylamide) were fabricated by the group of Hoare and they showed potential for an "on-demand" drug release.[116] Poly(lactide-*co*-glycolide) (PLGA) coated IOPNs loaded with paclitaxel were used for cancer therapy and MRI,[117] and for nucleic acid detection.[118] IOPNs modified with polymeric coatings bearing folic acid ligands were also reported and investigated for targeting and detection of cancer cells.[119,120] The examples of polymer-coated IONPs are countless and readers are referred to a recent review paper by Muthiah *et al.*[12]

3. Post-functionalization of chemically stable IONPs: bioconjugation strategies and others

Protection and stabilization of IONPs is mostly not sufficient for active targeting of the particles. Simple electrostatic interactions have been exploited to bind enzymes and proteins onto the IONPs' surface. This approach is however rarely used as it requires freshly synthesized IONPs and yields surfaces with variable stabilities. The direct conjugation of carboxylic acid (–COOH) terminated molecules to the hydroxyl groups present on freshly formed IONPs is also not a very common procedure as it relies on the very limited reactivity of the native surface. The addition of targeting functionality is thus commonly achieved using numerous synthetic strategies, including carboxylic acid–amine reaction, click chemistry, thiol–ene, *etc.* (Fig. 15). These approaches play important roles in particle targeting applications in theragnostics with the main goals being the preservation of the activity of the immobilized molecule and the overall stability of the IONPs. This part of the review will focus more closely on the individual coupling strategies used and Table 1 summarizes some of the most important examples.

3.1. Carbodiimide-mediated chemistry

The carbodiimide protocol, based on the interaction between amine and carboxylic acid functions, remains one of the most popular immobilization strategies for IONPs (Fig. 16A).[69,121–127] One of the main advantages of the carbodiimide protocol is that the coupling is performed in water at room temperature or even at lower temperatures (does not require harsh reaction conditions). It is a two step reaction, involving first the activation of the carboxylic function by carbodiimide (EDC, cyanamide) giving rise to an *O*-acylurea ester, which is rather unstable and usually transformed immediately in the presence of *N*-hydroxysuccinimide (NHS) to the activated ester. Carbodiimide coupling without NHS has also been performed on IONPs.[101,24,128] However, as pointed out in the original paper by Starso, Wright and Single, carbodiimide coupling without NHS results in lower coupling yields because of the rapid hydrolysis of the carbodiimide intermediate, being the main reason for using NHS.[129]

Important for the success of the reaction is that equivalent amounts of carbodiimide to carboxylic groups are used, as excess carbodiimide causes formation of multilayers and

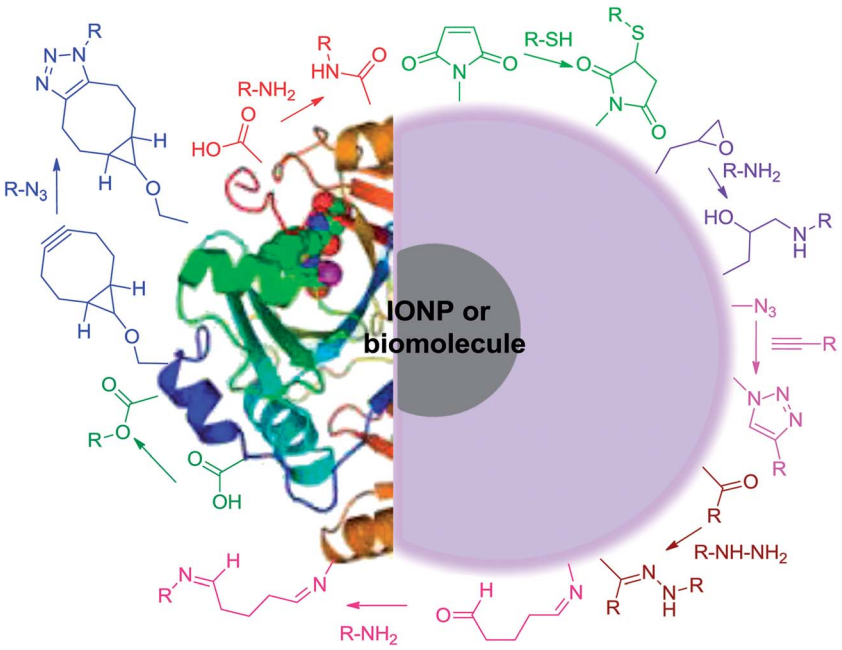

Fig. 15 Bioconjugation strategies employed on IONPs.

aggregates due to direct cross-linking of biomolecules. In an ideal situation, excess carbodiimide should be removed (*e.g.* in the case of EDC when quenching with 2-mercaptoethanol)[130] before the addition of amino-terminated species takes place, preventing biomolecule activation and inter-biomolecular linking.[131] EDC/NHS coupling has been employed recently by Sung *et al.* for the formation of novel antibody/gold NPs/IONP composites for immunomagnetic separation and rapid colorimetric detection of *Staphylococcus aureus* in milk (Fig. 16B).[121] The nanocomposites were synthesized by covalent linkage of anti-*Staphylococcus aureus* to carboxylated IONPs or by first linking bovine serum albumin to carboxylated IONPs by the EDC/NHS protocol followed by adsorption of gold NPs and anti-*Staphylococcus aureus*. The capture efficiency for the IONPs/Au NPs/antibody was as high as 96% for *Staphylococcus aureus* in PBS and 78% in the milk sample (Fig. 16B).[121] The detection limits of these colorimetric sensors were 1.5×10^3 CFU (PBS) and 1.5×10^5 CFU (milk) for *Staphylococcus aureus.*[121] Carbodiimide mediated reaction was also one of the three post-functionalization strategies employed on IONPs modified with amine, azide and maleimido functionalized dopamine derivatives for the linking of horseradish peroxidase.[71] Several other enzymes such as lipase[132] or lactase[130] have been effectively immobilized by means of EDC, and sulfo-NHS onto IONPs showing high enzymatic activity. While mostly the EDC/NHS protocol is employed, cyanamide (NH = C=NH or H_2N–CN) can be used as a coupling reagent as shown in the case of concanavalin A-modified IONPs.[128]

To limit the cytotoxicity of anticancer drugs against healthy cells, an appropriate carrier should deliver the drug specifically to the tumor tissue. The anchoring of anticancer drugs such as doxorubicin (DOX), paclitaxel or a mixture of both to IONPs using a properly directed external magnetic field is thus a highly appealing approach. The effectiveness of the drug delivery was found to depend strongly on the bonding type between the drug and the IONPs. While an amide bond is barely cleavable inside the cell,[133,134] ester and hydrazone bonds[135] can be easily cleaved upon pH variation. Chemotherapeutics such as gemcitabine could be covalently linked to IONPs by means of the carbodiimide protocol.[136]

3.2. Use of heterobifunctional cross-linkers: glutaraldehyde and others

Among the different heterobifunctional cross-linkers, glutaraldehyde, disuccinimidyl suberate (DSS), NHS–PEG–maleimide and NHS–propargyl have been employed for conjugation with IONPs (Fig. 17A).[122,127,137,138] The disadvantage of the glutaraldehyde linker is its high toxicity but covalent immobilization of *Bacillus licheniformis* γ-glutamyl transpeptidase onto IONPs could be achieved.[137] The immobilized enzyme could be recycled ten times with 36.2% retention of the initial activity and had a comparable stability with respect to the free enzyme during the storage period of 30 days. NHS–PEG–maleimide cross-linkers were used for creating linkage between IONPs and imunoglobulins[127] while NHS-propargyl linked glycoproteins to IONPs.[138] The use of suberic acid bis-*N*-hydroxysuccinimide ester (DSS) grafted onto amine-terminated IONPs allowed the subsequent covalent immobilization of various tag molecules (chitin, amylase, heparin, *etc.*).[122] In a cell recognition study, mannose-functionalized IONPs allowed specific purification of *Escherichia coli* with FimH adhesion (*E. coli* ORN178) on the surface. Fig. 17B shows fluorescence and optical images of two strains of *E. coli* (ORN178 and ORN208) cells captured by mannose-modified IONPs. In the case of *E. coli* ORN178 expressing FimH on its pili, clear red fluorescence was

Table 1 Summary of the most important surface modification strategies for IONPs and their target application

Immobilized molecule	Modification strategy	IONP coating	Target	Ref.
Anti-cTnI antibody	EDC/sulfo-NHS	HOOC-terminated poly(maleic anhydride-alt-1-octadecene)	Cardiac troponin I	123
NH_2–ctxAB-F	EDC	Carboxyl-terminated poly(*N*-isopropylacrylamide)	*Vibrio cholera*	101
R11 peptide	EDC	Poly(*N*-isopropylacrylamide-acrylamide-allylamine)-	Prostate cancer cells	126
Holo-Transferrin	EDC/NHS	APTES	Transferrin receptor-1	125
Anti-*C. Sakazakii* – antibody	EDC/NHS	NH_2-modified silica-coated	*Cronobacter sakazakii*	179
Anti-*S. Aureus* antibody	EDC/NHS	Carboxymethyl-dextran	*Staphylococcus aureus*	121
Anti-prostate specific antigen	EDC/NHS	Gold-shell	Prostate-specific antigen	50
Exendin-4 peptide	EDC/NHS	NH_2- PEG	Glucagon-like peptide 1 receptor expressed in B-cells and insulinoma	69
Anti-CD20 antibody-streptavidin fusion protein	Biotin-avidin fusion	Chitosan-grafted PEG	CD20 B-cell marker	180
Lactase	EDC/sulfo-NHS	Oleic acid	—	130
Lipase	EDC/sulfo-NHS	NH_2-chitosan, poly[*N*-benzyl-2-(methacryloxy)-*N*,*N*-dimethylethanaminium bromide]	Racemic Ibuprofen	132
DOX	EDC/NHS	Dodecanethiol–polymethacrylic acid	HepG2 cells	134
Horse radish peroxidase	EDC/NHS	Dopamine	—	71
Rabbit anti-goat IgG or streptavidin	EDC/NHS	HCOOC-PEG starch	—	127
Concanvalin A	Cyanamide	Citrate	Lactoferrin	128
Bacillus licheniformis γ-glutamyl transpeptidase	Glutaraldehyde	APTES	Transfer of the γ-glutamyl moiety to the Gly–Gly peptide	137
Pseudomonas fluorescens Lp1 lipase	Glutaraldehyde	APTES	—	181
β-glucosidase	Glutaraldehyde	—	Cellobiose hydrolysis	182
Sialic acid-rich glycoproteins	CuAAc	Alkynyl-modified poly(vinyl alcohol)	Sialic acid-rich glycoprotein on the surface of A549 cells	138
BSA	CuAAc	Poly(acrylic acid-*co*-propargyl acrylate) polymer coated	—	183
HER2/neu targeting affibodies	Copper-free click reaction	Pristine	—	145
Rabbit anti-goat IgG or streptavidin	Copper free click reaction	HOOC-PEG starch	—	127
Rabbit anti-goat IgG or streptavidin	Thiol–ene reaction	NH_2-PEG starch	—	127
Anti-epidermal growth factor receptor (EGFR) affibody proteins	Thiol–ene reaction	Gold	A431 tumor-bearing mice	184
Methotrexate	Thiol–ene reaction	—	—	152
Proteins from *E. coli*	Epichlorhydrin	Polystyrene	—	154
Trypsin	Epichlorhydrin	Poly (glycidylmethacrylate)	Cytochrome C	153
Chlorin	Epichlorhydrin	Dextran T10	B16 mouse melanoma cell line (B16F10 and B16G4F)	155
CBD-fused CMP–sialic acid synthetase (CSS)	DSS	Pristine	—	122
Starch-binding domain (SBD) fusion EGFP	DSS	Pristine	—	122
Heparin binding protein (A27L)	DSS	Pristine	—	122
Shiga-like toxin B subunit	DSS	APTMS	—	122
His-tag fused recombinant Chi-A protein	DSS	APTMS	—	122
Human IgG antibodies	DSS	APTMS	—	122

Table 1 (*Contd.*)

Immobilized molecule	Modification strategy	IONP coating	Target	Ref.
Plasma membrane proteins of HeLa cells	DSS	APTMS	—	122
E. coli ORN178	DSS	APTMS	—	122
DOX	Hydrazone formation	Pristine	HeLa cells	135
DOX	Adipoyl chloride	Pristine	Human urinary bladder carcinoma cells UM-UC-3	133

observed. Specific separation of ORN178 (Fig. 17B, image F) was also achieved showing the high specificity of the IONP probes.[122]

3.3. Click chemistry

One chemical reaction that has gained increasing attention over the last decade is "click" chemistry.[139–141] The concept of click chemistry was born from the realization that in order for chemical reactions to be most efficient reactions should be simple, efficient, highly chemoselective, regioselective, and stereoselective, of high yield with no release of byproducts and be amendable to occur at ambient temperature and atmospheric conditions, and in benign solvents.[142] In the original article by Kolb *et al.* a range of reactions were considered such

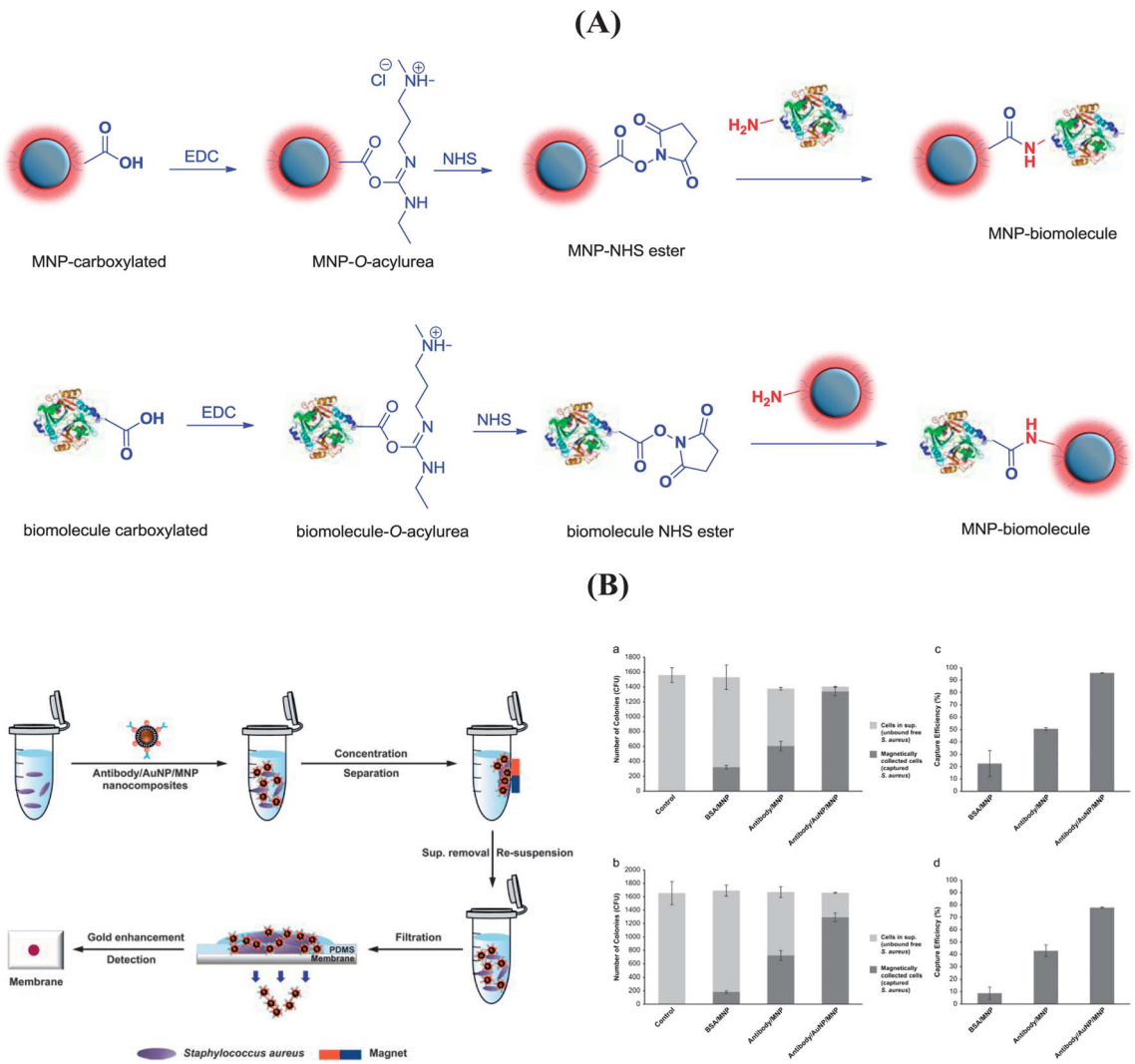

Fig. 16 (A) Covalent binding of biomolecules through amine or carboxylic acids to IONPs *via* EDC/NHS coupling; (B) (left) immunomagnetic separation (IMS) and colorimetric detection process for the *S. aureus* sensing using the antibody/AuNP/MNPs and the selective filtration system. (right) The number of colonies formed by the nanoparticle-bound *S. aureus* in the magnetically collected pellet or by the unbound cells remaining in the supernatant, enumerated by surface plating and colony counting for (a) the pure sample and (b) the milk sample. The percentages of the captured cells out of the total cells (*i.e.* capture efficiency) for the BSA/MNPs, the antibody/MNPs, the antibody/AuNP/MNPs in (c) the pure culture and (d) the milk sample. (Reprinted with permission from ref. 121).

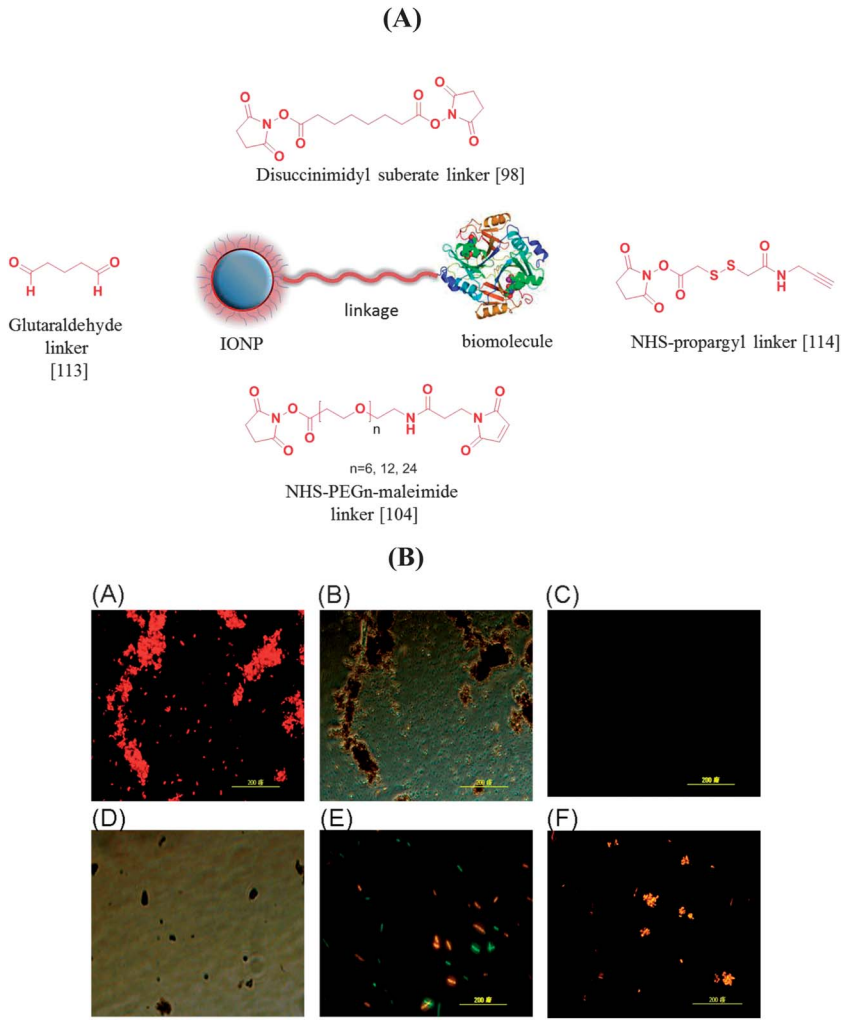

Fig. 17 (A) Bioconjugation of IONPs *via* heterobifuncitonal cross-linkers, (B) images of ORN178 cells captured by mannose-modified IONPs (A) fluorescence image and (B) optical image; images of ORN208 cells captured by mannose-modified IONPs (C) fluorescence image and (D); mixing pool of ORN178 and ORN208 cells, and (F) fluorescent image of bacteria extracted by mannose-modified IONPs from a mixed pool. (Reprinted permission from ref. 122).

as nucleophilic ring opening reactions of epoxides, aziridines, *etc.*, non-aldol carbonyl chemistry resulting in the formation of ureas, oximes and hydrazones, additions to carbon–carbon multiple bonds and cycloaddition reactions such as Diels–Alder and 1,3-dipolar cycloaddition. Although a myriad of reactions fulfill the "click" criteria, in view of their fast, complete transformability, the Cu(I) catalyzed Huisgen 1,3-dipolar cycloaddition of azides with terminal alkynes (CuAAc reaction) has emerged as the frontrunner under this category[143,144] (Fig. 18A). We have shown recently that this reaction is adaptable for the modification of IONPs with alkyne-modified glycans such as mannose.[71] This reaction was also recently used to link azido modified glycoproteins present on the surface of A549 cells to alkyne-modified IONPs by means of [3 + 2] cycloaddition.[138]

One of the disadvantages of the CuAAC is that catalyst traces of Cu(I) might remain on the particle surface. Cyclooctyne derivatives have garnered particular attention due to the strained triple bond allowing copper-free click reaction conditions (Fig. 18B). In recent work the development of a novel bioconjugation strategy that combines expressed protein ligation (EPL) and "click" chemistry to produce a highly efficient and site-specific bioconjugation reaction that ensures that all

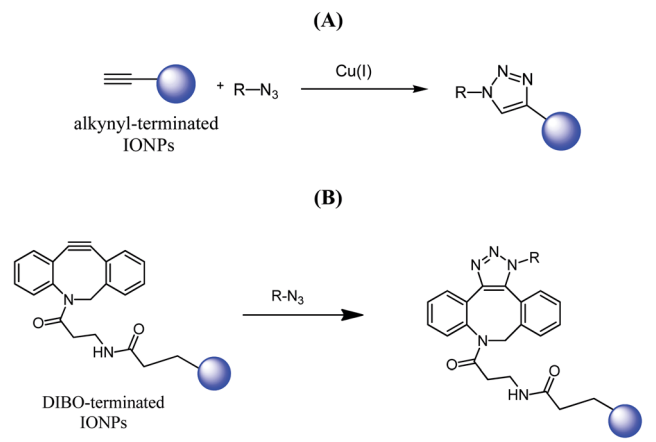

Fig. 18 "Click" chemistry between an azide function and (A) alkynyl-terminated IONPs; (B) dibenzocyclooctyne (DIBO)-terminated IONPs.

target ligands have the same orientation has been proposed.[145] By using copper-free EPL-"click" on dibenzocyclooctyne (DIBO) modified IONPs a better control of the ligand density on IONPs was achieved.[145]

Another conjugation route with "click chemistry" character is the radical addition reaction of thiols to alkenes and alkynes (Fig. 19A).[146–149] The versatility of the reaction is enhanced by the fact that it does not require any catalyst as it is initiated thermally or photochemically. The main difference between the use of alkenes and alkynes is that alkynes can react with two thiol molecules to form a double addition product. Less investigated than the thiol–ene reaction, thiol–yne chemistry has been successfully employed for example for the construction of a DNA array.[150] Surprisingly, both reactions have not been widely employed until now on IONPs.[85,91,151,152] Recently, the reaction of thiolated hyper-branched polyglycerol (HPG) and alkene bonds on IONPs under UV irradiation allowed the formation of HPG modified IONPs where methotrexate (MTX), one of the most used chemotherapeutic drugs, was further coupled *via* the formation of ester bonds (Fig. 19B).[152] The evaluation of the targeting specificity uptake of the MTX-modified IONPs by normal TS fibroblasts, RAW macrophages and KB cancer cells was assessed (Fig. 19C). The uptake of MTX-modified IONPs in KB cells is significantly higher compared to the other cells and increases with increased MTX level.

3.4. Other chemistries to conjugate biomolecules/drugs

Besides the above mentioned conjugation strategies, the ring opening reaction of epoxy groups *via* nucleophilic attack by amines is an interesting alternative (Fig. 20A). Although this type of reaction usually proceed under mild conditions, the use of epoxide such as epichlorhydrin is rather unacceptable due to its cancerous nature. However in most recent studies trypsin was immobilized on IONPs through opening of the epoxy ring of poly(glycidylmethacrylate)-coated IONPs.[153] In another interesting example, modified lipase with both hydrophobic–hydrophilic properties (Janus character) acted as an anchor for

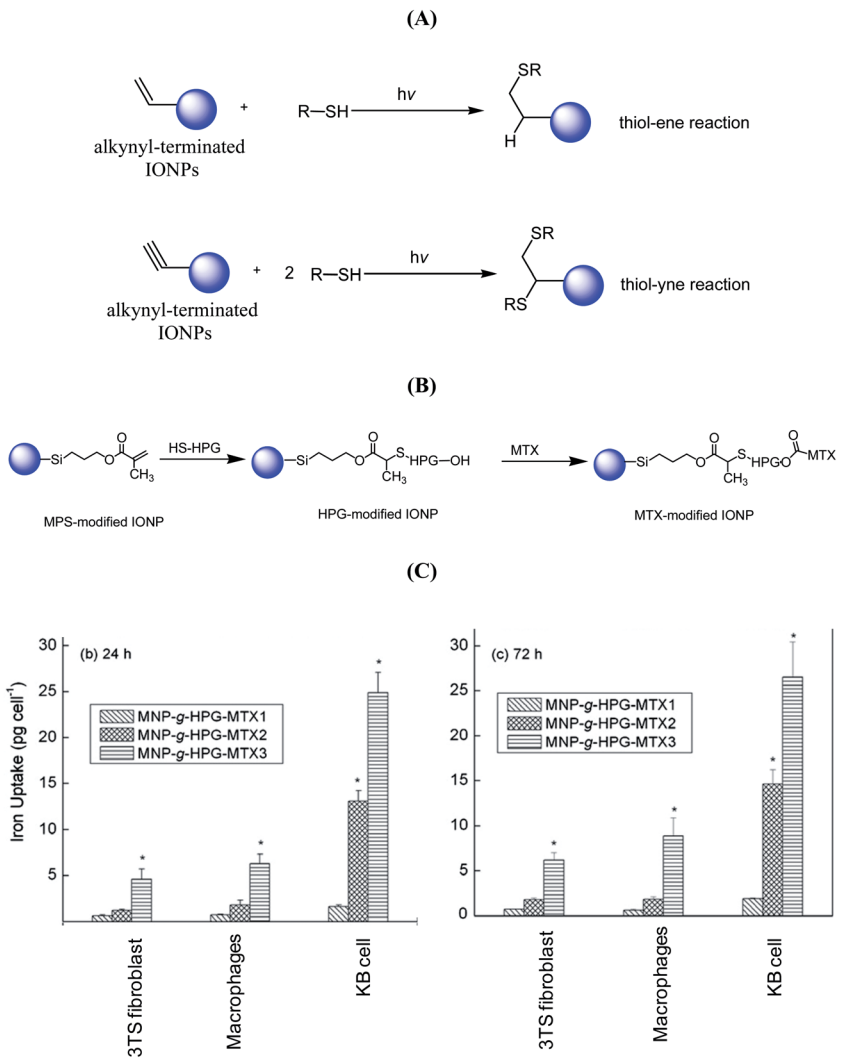

Fig. 19 (A) Concept between thiol–ene and thiol–yne reaction, (B) formation of methotrexate (MTX) modified IONPs: MPS (3-(trimethoxysilyl)propylmethacrylate) modified IONPs react in thiol–ene reaction with thiolated HPG (hyper branched polyglycerol), esterification reaction with MXT; (C) uptake of MTX-modified IONPs (0.2 mg mL^{-1}) by three different cell lines after 24 h and 72 h (reprinted with permission from ref. 152).

hydrophobic NPs. Once lipase was immobilized on the particles' surface, its hydrophilic part consisting of epoxy groups was attacked by amino groups of FITC-labeled proteins from *E. coli* (Fig. 20B).[154] Independently, a new therapeutic platform based on porphyrin for melanoma treatment was synthesized from epichlorhydrin–dextran coated IONPs and polyaminated chlorin *p6*.[155]

3.5. Immobilization of metal complexes and ligands

While a tremendous amount of work has been carried out to link biomolecules and biological active composites to IONPs, metal complexes immobilized onto IONPs are a category of relatively new promising materials for catalysis, analytical chemistry and magnetic manipulation.[156] Transition-metal catalyzed reactions are widely used in organic synthesis but raise the question for industry of how to remove the toxic metal ions from the final product. According to the recommendations of the European Agency for the Evaluation of Medicinal Products, the combined content of 6 noble metals (Ru, Os, Rh, Ir, Pd, Pt) in a drug substance should not be higher than 5 ppm. Taking into account the price of the catalysts and ligands, the problem of recovery is not only of pharmaceutical importance. On the other hand, asymmetric synthesis is one of the most widely used approaches to prepare pure enantiomeric products, where highly expensive chiral ligands and their noble metal complexes are the usual sources of asymmetry. The question of their recovery and removal can be solved *via* immobilization of these ligands onto the surface of IONPs.[157] Alongside classical methodologies for obtaining chiral substances in the enantiopure or enantio-enriched state, magnetic separation of enantiomers *via* enantioselective adsorption on the surface of asymmetrically decorated IONPs has been developed.[158,159] The asymmetric surface of IONPs can act as a support for the formation of enantio-pure domains in the "racemate–asymmetric IONPs–solvent" multicomponent system. It is critical that only ~5% of all known racemates are conglomerates,[160] what in all other cases makes impossible their direct resolution through the spontaneous separation *via* crystallization, entrainment[160] and *via* the asymmetric transformation of the second order involving complete deracemization.[161,162] From this point of view, asymmetrically decorated IONPs open broad perspectives for direct magnetic resolution of racemates. Several standard methods are used for the decoration of the surface of IONPs with the target molecule containing either a coordinated metal or a fragment capable of forming a coordination bond. Typically silane-modified IONPs are used for the grafting of metal complexes (Fig. 21).

The oxygen sensitive Ru^{I}-complex I was for example grafted onto IONPs using 3-(triethoxysilyl)propyl isocyanate as the starting material.[163] 3-Mercaptopropyltrimethoxysilane was reported for immobilization of cinchonidine by means of thiol–ene reaction.[164] The obtained cinchonidine-modified nanoparticles II have appeared to be effective chemosensors for Eu^{3+} ion detection. Another use of 3-mercaptopyltrimethoxysilane is in the synthesis of Pd^{0} decorated nano-support III and it has found application in highly selective conversion of aryl iodides and arylboronic acids into the corresponding ketones (with minimum formation of biaryls due to the competitive standard Suzuki coupling), and in the hydrogenation reactions of aromatic nitro- and vinyl compounds.[165] Immobilization of palladium NPs for the synthesis of Suzuki reaction catalyst IV

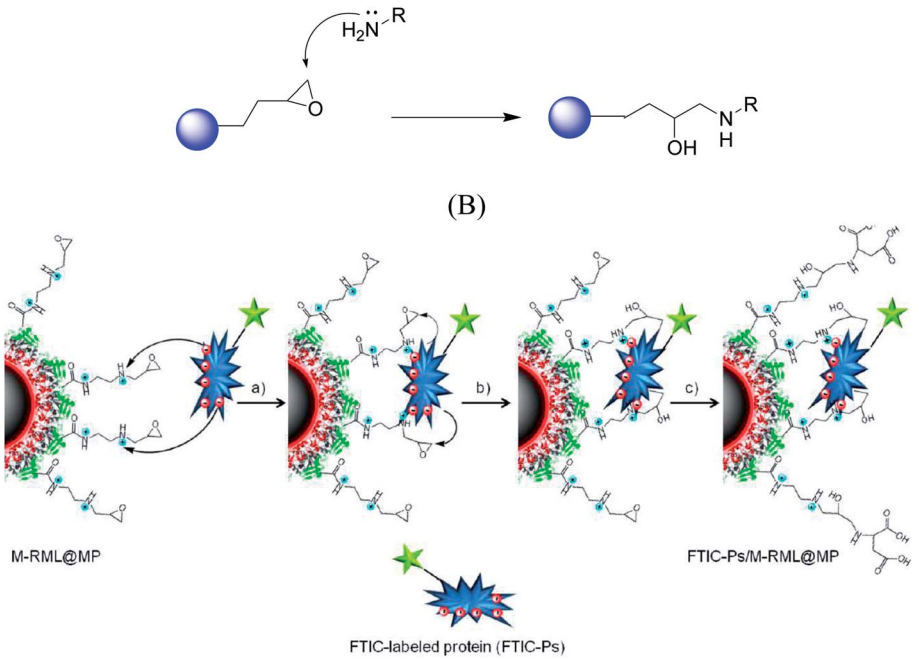

Fig. 20 (A) Nucleophilic attack of amino-terminated species on glycidyl-modified IONPs; (B) covalent immobilization of FTIC-labeled proteins on amino epoxidate lipase modified IONPs by a two-step mechanism: (a) ionic adsorption, (b) intramolecular covalent immobilization, (c) unreacted epoxy groups blocking (reprinted with permission from ref. 154).

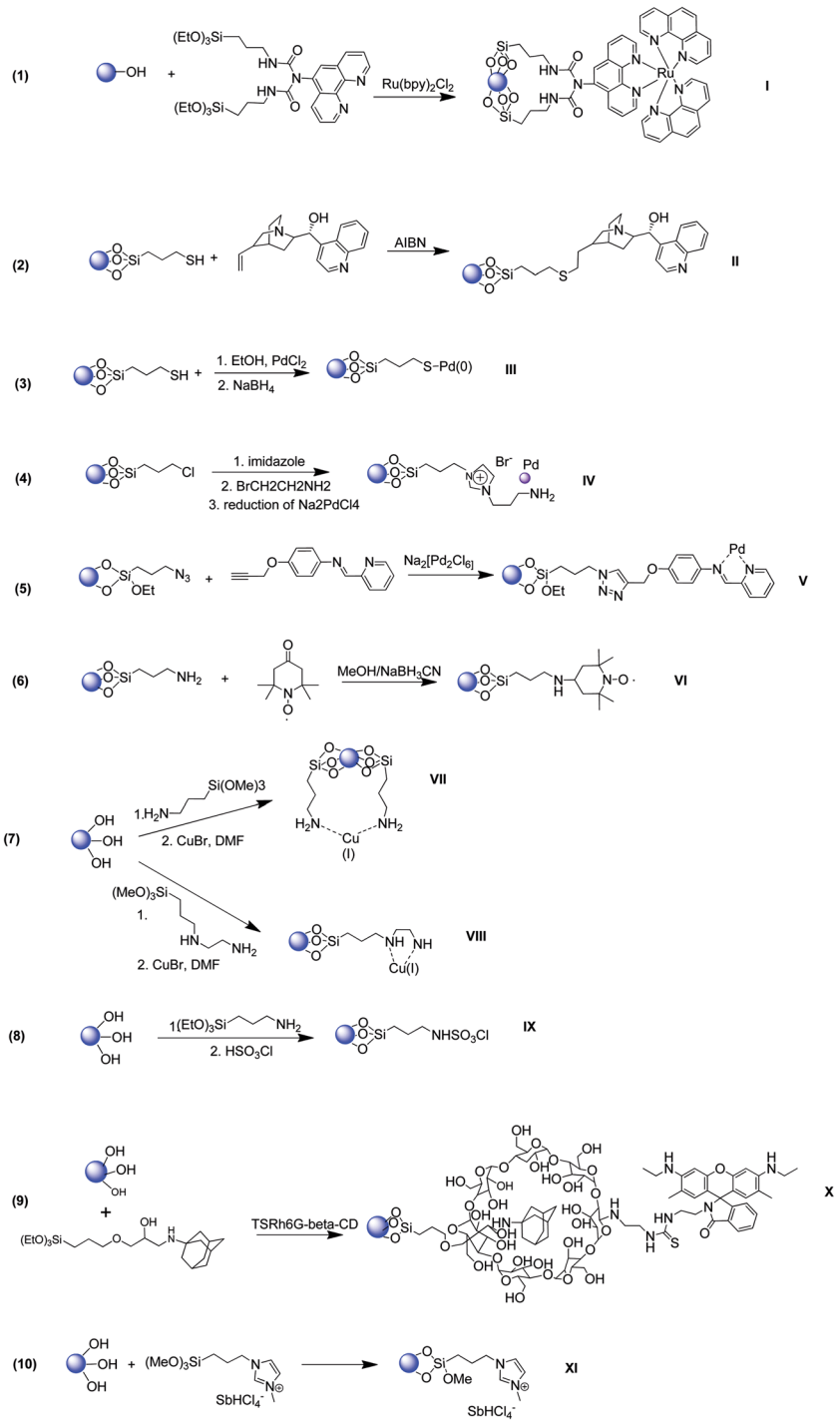

Fig. 21 Silane-modified IONPs for the anchoring of metal atoms.

was performed through reduction of $PdCl_4^{2-}$ coordinated to an ionic liquid fragment anchored onto the NP surface *via* siloxy groups.[166] Modification of IONPs surface V with 3-azidopropyltriethoxysilane permitted introduction of the azido functionality on the nanoparticle to be further exploited in one-pot multicomponent click synthesis of 1,4-substituted 1,2,3-triazoles by 1,3-dipolar cycloaddition of azide anions to alkynes with an imino-pyridine pre-catalyst substrate. Coordination with palladium gave rise to a catalyst for Suzuki–Miyaura coupling reactions. The estimation of Pd-leaching, magnetic recyclability, and stability of the novel nanocatalyst revealed its excellent properties.[167] 3-Aminopropyltriethoxysilane found its use in the synthesis of TEMPO decorated IONPs VI as nanocatalysts in the domino oxidative Passerini three-component reaction between primary alcohol, carboxylic acid and isocyanide. The catalyst revealed very good magnetic recyclability without considerable loss of the catalytic activity up to 14 runs.[168] Similarly, immobilization of TEMPO onto IONPs VI

has found its application in consequent aerial oxidative cyclization of bisnaphthols to spirodienones (10 substrates).[169] In another example, 3-aminopropyltriethoxysilane was exploited for grafting copper(I) complexes onto the IONPs surface to attain highly active, easy recoverable and eco-friendly catalysts VII and VIII for the 1,3-dipolar cycloaddition of azide anions to alkynes.[170] Rostamy *et al.* used the triethoxypropyl linker for anchoring sulfamic acid onto the IONP surface.[171] The nanocatalyst IX revealed excellent activity in the condensation reaction of anthranilamide with aldehydes and ketones providing 2,3-dihydroquinazolin-4(1H)-ones in good yields (16 examples). Detection and removal of one of the most toxic pollutants, namely mercury, has been recently reported by Wang and colleagues.[172] They designed an elegant method for immobilization of the fluorophore through the host–guest interaction. The adamantyl (Ad) groups were anchored on the surface *via* standard silylation chemistry providing adamantane-functionalized magnetic silica spheres (IONP@SiO_2–Ad). The self-assembly of Fe_3O_4@SiO_2–Ad IONPs with the rhodamine–cyclodextrin fluorophore moiety led to the desired magnetically recyclable nanomaterial X, which showed high sensitivity to target Hg^{2+} ions. In another example, an antimony-containing ionic liquid was successfully immobilized onto the IONP surface *via* silanization of 1-methyl-3-(trimethoxysilylpropyl)-imidazolium tetrachloridoantimonate (XI). The resulting nanomaterial XI showed excellent catalytic activity in the Clauson–Kaas reaction (42 examples) providing *N*-substituted pyrroles in high yields. The catalyst was recovered up to six times retaining almost identical catalytic efficiency.[173]

Besides silane-based chemistry, other approaches have been reported for the integration of metal oxides onto IONPs (Fig. 22). Bronstein and co-workers[174] studied the possibility of immobilization of catalytically active palladium species onto IONPs formed from a number of long-chain double-bond containing acids and substituted pyridine carboxylic acids. The formation of Pd π-complexes XII involving double bonds or aromatic rings of the acids led to the introduction of $PdCl_2$ into the shell. In another example ruthenium noble metal was immobilized onto the IONP surface *via* the phosphonate linkage methodology. The myriad of Ru-modified IONPs have been synthesized and showed excellent catalytic activity for highly stereospecific epoxidation of olefins.[175] Polyvinylbenzyl chloride coated NPs XIV for immobilization of Pt have been synthesized by Darwish *et al.*[176] The catalytic activity of the novel nanomaterial was tested in the chemoselective reduction of cinnamaldehyde (PhCH = CH–CHO) to cynnamyl alcohol (PhCH = $CHCH_2OH$) without affecting the double bond.

The promising magnetically recoverable catalysts XV were tested in a model reaction of selective hydrogenation. Rhodamine 6G derivatives have been used for highly selective

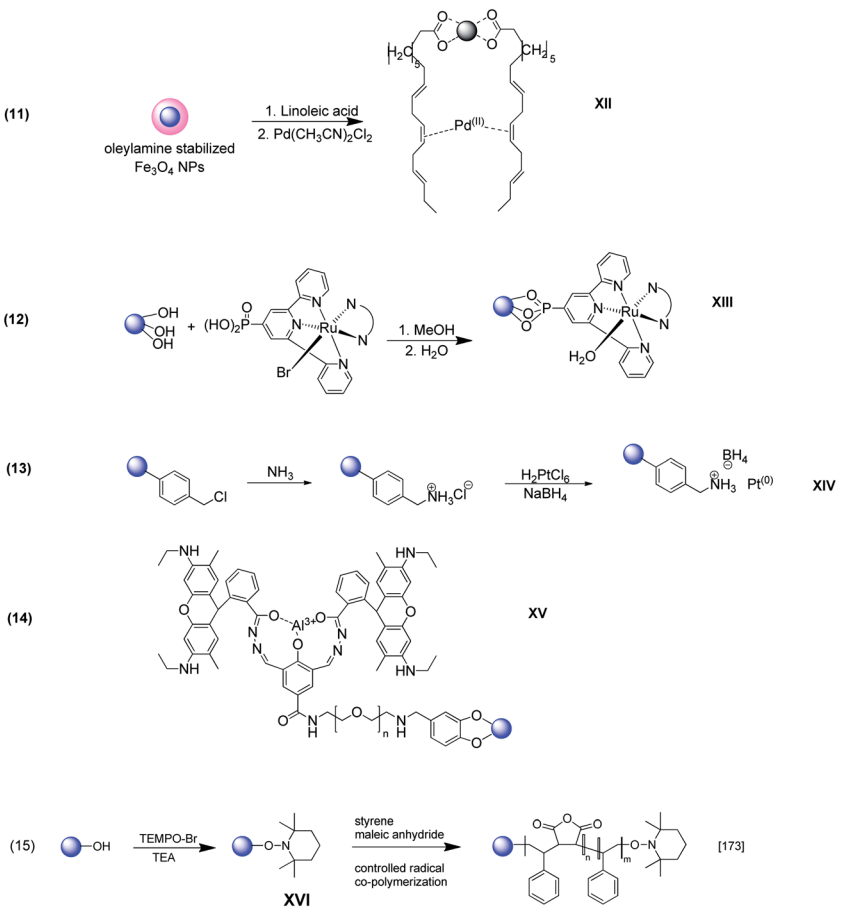

Fig. 22 Metal oxide modified IONPs based on other dispersants.

detection of metal ions at extremely low concentrations. Trying to overcome the main disadvantage of these chemosensors – low water solubility – a group of Chinese researchers developed a strategy for immobilization of rhodamine 6G Schiff base onto the surface of magnetic NPs thus making the dye useful not just for analytical purposes.[177] The surface of IONPs was decorated with the rhodamine dye using the standard dopamine anchor through the polyethylene glycol linker ($M_w = 4000$). Magnetic properties of the substrate XV can potentially find an application in enriching or removing toxic metal ions present in the environment and living organisms. An interesting strategy for the immobilization of TEMPO and its application in polymer grafting has been developed by Chen *et al.*[178] The pristine IONPs functionalized with TEMPO XVI were investigated for controlled/"living" radical co-polymerization of styrene and maleic anhydride. The surface-initiated nitroxide-mediated radical polymerization led to the growth of poly(styrene-*co*-maleic anhydride) brushes (PSMA) from the magnetic core.

4. Conclusion and perspectives

This review highlighted the recent applications of traditional methods for the coating and functionalization of IONPs. These methods advance the degree of control over the properties of IONPs, reproducibility, bioconjugation valence, *etc.* Control over these properties is paramount in optimizing the use of IONPs in applications such as diagnostic imaging, sensing, drug delivery, and catalysis. While traditional conjugation methods (*e.g.* EDC/NHS) are still widely employed for the functionalization of IONPs, they are poorly suited for a controlled, efficient and multifunctional integration of ligands. Efficient and bioorthogonal cycloaddition and ligation reactions enable a greater level of control and allow the integration of one or more molecules in a controlled manner onto the surface of IONPs. "Click" chemistry based reactions have in particular shown to be of great use in this respect. This highlights also the point that these non-traditional methods are not meant to completely replace standard surface functionalization strategies, but rather to supplement them. Azide or alkynyl functions are indeed introduced onto IONPs using amidation and esterification reactions. These novel strategies, well-developed on flat surfaces and more recently applied to IONPs, proved to provide new levels of control over the properties of IONPs in a simple manner. Such strategies also allowed tuning of the ligand density on the IONPs, which is an important issue for their application in cell targeting, as capture probes or in sensing. They also allowed the formation of IONPs with increased complexity. The development of "toolkits" such as dopamine-based ligands is thus of utter importance and will allow enlarging fast the application of these IONPs by different communities, less familiar with the different aspects of surface chemistry. While multifunctional IONPs are important emerging platforms, before these IONPs can be used practically as probes for diagnostic and therapeutic applications, numerous challenges need to be met. Biocompatibility, biodistribution issues as well as long term stability have to be investigated in detail. The elimination of such particles by biological systems without any other side effects has to be achieved. Extensive and multidisciplinary research efforts are still needed to enhance medical, therapeutic and other abilities of IONPs. The future of IONPs is thus bright.

Acknowledgements

Financial support from the Centre National de Recherche Scientifique (CNRS), the Université Lille 1, the Nord Pas de Calais region and the Institut Universitaire de France (IUF) is gratefully acknowledged. Support from the European Union through a FP7-PEOPLE-IRSES (no. 269009) is acknowledged. Funding support was also received from the State Agency on Science, Innovation and Informatization of Ukraine (project Dnipro M/7-2013).

References

1 T. D. Schladt, K. Schneider, H. Schild and W. Tremel, *Dalton Trans.*, 2011, **40**, 6315.
2 S. Laurent, D. Forge, M. Port, A. Roch, C. Robic, L. Vander Elst and R. N. Muller, *Chem. Rev.*, 2008, **108**, 2064.
3 K. E. Sapsford, W. R. Algar, L. Berti, K. Boeneman Gemmill, B. J. Casey, E. Oh, M. H. Stewart and I. L. Medintz, *Chem. Rev.*, 2013, **113**, 1904–2074.
4 A. K. Gupta and M. Gupta, *Biomaterials*, 2005, **26**, 3995.
5 D.-E. Lee, H. Koo, I.-C. Sun, J. H. Ryu, K. Kim and I. C. Kwon, *Chem. Soc. Rev.*, 2012, **41**, 2656.
6 S. A. Corr, Y. P. Rakovich and Y. K. Gun'ko, *Nanoscale Res. Lett.*, 2008, **3**, 87.
7 G. Mistlberger, K. Koran, E. Scheucher, D. Aigner, S. M. Borisov, A. Zankel, P. Polt and I. Klimant, *Adv. Funct. Mater.*, 2010, **20**, 1842.
8 M. Colombo, S. Carregal-Romero, M. F. Casula, L. Gutiérrez, M. P. Morales, I. B. Bohm, J. T. Heverhagen, D. Prisperi and W. J. Parak, *Chem. Soc. Rev.*, 2012, **41**, 4306.
9 F. M. Kievit and M. Zhang, *Acc. Chem. Res.*, 2011, **44**, 853.
10 D. Ho, X. Sun and S. Sun, *Acc. Chem. Res.*, 2011, **44**, 875.
11 J. Gao, H. Gu and B. Xu, *Acc. Chem. Res.*, 2009, **42**, 1097.
12 M. Muthiah, I.-K. Park and C.-S. Cho, *Biotechnol. Adv.*, 2013, DOI: 10.1016/j.biotechadv.2013.03.005.
13 R. M. Cornell and U. Schwertmann, *The iron oxides: structures, properties, reactions, occurences, and use*, Wiley-VCH Verlag GmbH, 2003.
14 K. H. J. Buschow, *Handbook of Magnetic Materials*, North Holland, 1995.
15 M. Colombo, S. Carregal-Romero, M. F. Casula, L. Gutiérrez, M. P. Morales, I. B. Bohm, J. T. Heverhagen, D. Prisperi and W. J. Parak, *Chem. Soc. Rev.*, 2012, **41**, 4306.
16 Y. Cohen and S. Y. Shoushan, *Curr. Opin. Biotechnol.*, 2013, **24**, 1.
17 H. Lee, M. K. Yu, S. Park, S. Moon, J. J. Min, Y. Y. Jeong, H.-W. Kang and S. Jon, *J. Am. Chem. Soc.*, 2007, **129**, 12739.
18 S. T. Selvan, T. T. Y. Tan, D. K. Yi and N. R. Jana, *Langmuir*, 2010, **26**, 11631.
19 Y. Piao, A. Burns, J. Kim, U. Wiesner and T. Hyeon, *Adv. Funct. Mater.*, 2008, **18**, 3745.

20 I. K. Herrmann, R. N. Grass, D. Mazunin and W. J. Stark, *Chem. Mater.*, 2009, **21**, 3275.
21 W. Stöbern, A. Fink and E. Bohn, *J. Colloid Interface Sci.*, 1968, **26**, 62.
22 C. Hui, C. Shen, J. Tian, L. Bao, C. Li, Y. Tian, X. Shi and H.-J. Gao, *Nanoscale*, 2011, **3**, 701.
23 Y. Wang, B. Li, L. Zhang, H. Song and L. Zhang, *ACS Appl. Mater. Interfaces*, 2013, **5**, 11.
24 J. Liu, S. Z. Qiao, Q. H. Hu and G. Q. Lu, *Small*, 2011, **7**, 425.
25 P. Liu, M. Chen, C. C. Chen, X. Fang, X. Chen and N. Zheng, *J. Mater. Chem. B*, 2013, **1**, 2837.
26 W. C. Bigelow, D. L. Pickett and W. A. Zisman, *J. Colloid Sci.*, 1946, **1**, 513.
27 D. Li, W. Y. Teoh, J. J. Gooding, C. Selomulya and R. Amal, *Adv. Funct. Mater.*, 2010, **20**, 1767.
28 M. Cypryk and Y. Apeloig, *Organometalics*, 2002, **21**, 2165.
29 R. Boukherroub, *Curr. Opin. Solid State Mater. Sci*, 2005, **9**, 66.
30 M. J. Sailor and E. J. Lee, *Adv. Mater.*, 1997, **9**, 783.
31 J. M. Buriak, *Chem. Rev.*, 2002, **102**, 1271.
32 S. Ciampi, J. B. Harper and J. J. Gooding, *Chem. Soc. Rev.*, 2010, **39**, 2158–2218.
33 Z. Xu, Y. Hou and S. Sun, *J. Am. Chem. Soc.*, 2007, **129**, 8698.
34 L. Lingyan, J. Luo, Q. Fan, M. Suzuki, I. S. Suzuki, M. H. Engelhard, Y. H. Lin, N. Kim, J. Q. Wang and C.-J. Zhong, *J. Phys. Chem. B*, 2005, **109**, 21593.
35 C.-H. Liang, C.-C. Wang, Y.-C. Lin, C.-H. Chen, C.-H. Wong and C.-Y. Wu, *Anal. Chem.*, 2009, **81**, 7750.
36 M. Mandal, S. Kundu, S. K. Ghosh, S. Panigrahi, T. K. Sau, S. M. Yusuf and T. Pal, *J. Colloid Interface Sci.*, 2005, **286**, 187.
37 J. L. Lyon, D. A. Flemming, M. B. Stone, P. Schiffer and M. E. Williams, *Nano Lett.*, 2004, **4**, 719.
38 A. Muller, A. Goette, C. Perings, H. Nagele, T. Konorza, W. Spitzer, S. S. Schulz, C. von Bary, M. Hoffmann, M. Albani, S. Sack, A. Niederlohner and T. Lewalter, *Telemed J E Health*, 2013, **19**, 460.
39 Y. Zhao, W. Zhang, Y. H. Lin and D. Du, *Nanoscale*, 2013, **5**, 1121.
40 C. J. Xu, B. D. Wang and S. H. Sun, *J. Am. Chem. Soc.*, 2009, **131**, 4216.
41 R. Hao, R. Xing, Z. Xu, Y. Hou, S. Gao and S. Sun, *Adv. Mater.*, 2010, **22**, 2729.
42 I. Y. Goon, L. M. H. Lai, M. Lim, P. Munroe, J. J. Gooding and R. Amal, *Chem. Mater.*, 2009, **21**, 673.
43 R. Shukla, V. Bansal, M. Chaudhary, A. Basu, R. R. Bhonde and M. Sastry, *Langmuir*, 2005, **21**, 10644.
44 S. Szunerits and R. Boukherroub, *Chem. Commun.*, 2012, **48**, 8999.
45 M. Mandal, S. Kundu, S. K. Ghosh, S. Panigrahi, T. K. Sau, S. M. Yusuf and T. Pal, *J. Colloid Interface Sci.*, 2005, **286**, 187.
46 Y. Lee, J. Lee, C. J. Bae, J. G. Park, H. J. Noh, J. H. Park and T. Hyeon, *Adv. Mater.*, 2005, **15**, 503.
47 J. L. Lyon, D. A. Flemming, M. B. Stone, P. Schiffer and M. E. Williams, *Nano Lett.*, 2004, **4**, 719.
48 L. Y. Wang, J. Luo, Q. FAn, M. Sizuki, I. Suzuki, M. H. Engelhard, Y. H. Lin, N. Kim, J. Q. Wang and C. J. Zhong, *J. Phys. Chem. B*, 2005, **109**, 21593.
49 Z. Xu, Y. Hou and S. Sun, *J. Am. Chem. Soc.*, 2007, **129**, 8698.
50 K. Chuah, L. M. H. Lai, I. Y. Goon, S. G. Parker, R. Amal and J. J. Gooding, *Chem. Commun.*, 2012, **48**, 3503.
51 M. Arsianti, M. Lim, S. N. Leou, I. Y. Goon, C. P. Marquis and R. Amal, *J. Colloid Interface Sci.*, 2011, **354**, 536.
52 I. Y. Goon, L. M. H. Lai, M. Lim, R. Amal and J. J. Gooding, *Chem. Commun.*, 2010, **46**, 8821.
53 L. M. H. Lai, I. Y. Goon, K. Chuah, M. Lim, F. Braet, R. Amal and J. J. Gooding, *Angew. Chem., Int. Ed.*, 2012, **51**, 6456.
54 H.-X. Shen, J.-L. Yao and R.-A. Gu, *Trans. Nanoferrous Met. Soc. China*, 2009, vol. 19, p. 652.
55 X. X. Han, A. M. Schmidt, G. Marten, A. Fischer, I. M. Weidonger and P. Hildebrandt, *ACS Nano*, 2013, **7**, 3212.
56 H. Yu, M. Chen, P. M. Rice, S. X. Wang, R. L. White and S. Sun, *Nano Lett.*, 2005, **5**, 379.
57 N. J. Turro, P. H. Lakshminarasimhan, S. Jockuscj, S. P. O'Brien, S. G. Grancharov and F. X. Redl, *Nano Lett.*, 2002, **2**, 325.
58 A. K. L. Yuen, G. A. Hutton, A. F. Masters and T. Maschmeyer, *Dalton Trans.*, 2012, **41**, 2545.
59 J. Xie, C. Xu, N. Kohler, Y. Hou and S. Sun, *Adv. Mater.*, 2007, **19**, 3163.
60 J. Xie, K. Chen, H.-Y. Lee, C. Xu, A. R. Hsu, S. Peng, X. Chen and S. Sun, *J. Am. Chem. Soc.*, 2008, **130**, 7542.
61 C. Xu, K. W. Xu, H. Gu, R. Zhend, H. Liu, X. Zhang, Z. Guo and B. Wu, *J. Am. Chem. Soc.*, 2004, **126**, 9938.
62 Y. Lee, H.-Y. Lee, Y. B. Kim, J. Kim, T. Hyeon, H. Park, P. B. Messerschmith and T. G. Park, *Adv. Mater.*, 2008, **20**, 4154.
63 B. Wang, C. Xu, J. Xie, Z. Yang and S. Sun, *J. Am. Chem. Soc.*, 2008, **130**, 14436.
64 E. Amstad, A. U. Gehring, H. Fischer, v. V. Nagaiyanallur, G. Hahner, M. Textor and E. Reimhult, *J. Phys. Chem. C*, 2011, **115**, 683.
65 R. Hong, N. O. Fischer, E. Tood and V. M. Rotello, *Chem. Mater.*, 2005, **17**, 4617.
66 J. Xie, K. Chen, J. Huang, S. Lee, J. Wang, J. Gao and X. Li, *Biomaterials*, 2010, **31**, 3016.
67 C. Xu, J. Xie, D. Ho, C.-C. Wang, N. Kohler, E. G. Walsh, J. R. Morgan, Y. E. Chin and S. Sun, *Angew. Chem., Int. Ed.*, 2008, **47**, 173.
68 L. Lartigue, D. Alloyesu, J. Kolosnjaj-Tabi, Y. Javed, P. Guardia, A. Ridinger, C. Péchoux, T. Pellegrino, C. Wilhelm and F. Gaeau, *ACS Nano*, 2013, **7**, 3939.
69 C. Lei, F. Han, D. Li, W.-C. Li, Q. Sun, X.-Q. Zhang and A.-H. Lu, *Nanoscale*, 2013, **5**, 1168.
70 C. E. Hoyle and C. N. Bowman, *Angew. Chem., Int. Ed.*, 2010, **49**, 1540.
71 M. Mazur, A. Barras, V. Kuncser, A. Galantanu, V. Zaitzev, K. Turcheniuk, P. Woisel, J. Lyskawa, W. Laure, A. Siriwardena, R. Boukherroub and S. Szunerits, *Nanoscale*, 2013, **5**, 2692.

72 U. El-Ayaan, E. Herlinger, R. F. Jameson and W. Linert, *J. Chem. Soc., Dalton Trans.*, 1997, 2813.
73 J. Xie, C. Xu, Y. Xu, K. L. Young, S. X. Wang, N. Pourland and S. Sun, *Chem. Mater.*, 2006, **18**, 5401.
74 E. Amstad, T. Gillich, I. Bilecka, M. Textor and E. Reimhult, *Nano Lett.*, 2009, **9**, 4042.
75 M. D. Schultz, J. U. Reveles, S. N. Khanna and E. E. Carpenter, *J. Am. Chem. Soc.*, 2007, **129**, 2482.
76 J. Liebscher, R. Mrowczynski, H. A. Scheidt, C. Filip, N. D. Hadade, R. Turcu, A. Bende and S. Beck, *Langmuir*, 2013, **29**, 10539.
77 A. Palumbo, A. Napolitano, P. Barano and M. d'Ischia, *Chem. Res. Toxicol.*, 1999, **12**, 1213.
78 T. Gillich, C. Acikoz, A. D. Schluter, N. D. Spencer and M. Textor, *ACS Nano*, 2013, **7**, 316.
79 S. Srivastava, R. Awasthi, N. S. Gajbhiye, V. Agarwal, A. Singh, A. Yadav and R. K. Gupta, *J. Colloid Interface Sci.*, 2011, **359**, 104.
80 A. Bee, R. Massart and S. Neveu, *J. Magn. Magn. Mater.*, 1995, **149**, 6.
81 K. Kandori, Y. Kawashima and T. Ishikawa, *J. Colloid Interface Sci.*, 1992, **125**, 284.
82 C. Liu and P. M. Huang, *Soil Sci. Soc. Am. J.*, 1999, **63**, 65.
83 K. Andreas, R. Georgieva, M. Ladwig, S. Mueller, M. Notter, M. Sittinger and J. Ringe, *Biomaterials*, 2012, **33**, 4515.
84 N. Fauconnier, A. Bee, J. Roger and J. N. Pons, *J. Mol. Liq.*, 1999, **83**, 233.
85 K. Hayashi, K. Ono, H. Suzuki, M. Sawada, M. Moriya, W. Sakamoto and T. Yogo, *Chem. Mater.*, 2010, **22**, 3768.
86 Y. Sahoo, H. Pizem, T. Fried, D. Golodnitsky, L. Burstein, C. N. Sukenik and G. Markovich, *Langmuir*, 2001, **17**, 7907.
87 P. Hubert Mutin, G. Guerrero and A. Vioux, *J. Mater. Chem.*, 2005, **15**, 3761.
88 C. Yee, G. Kataby, G. Ulman, T. Prozorov, H. White, A. King, M. Rafailovich, J. Sokolov and A. Gedanken, *Langmuir*, 1999, **15**, 7111.
89 D. Portet, B. Denizot, E. Rump, F. Hindre, J. J. Le Jeune and P. Jallet, *Drug Dev. Res.*, 2001, **54**, 173.
90 M. Das, D. Mishra, T. K. Maiti, A. Basak and P. Pramanik, *Nanotechnology*, 2008, **19**, 415101.
91 R. D. Rutledge, C. L. Warner, J. W. Pittman, R. S. Addleman, M. Engelhard, W. Chouyyok and M. G. Warner, *Langmuir*, 2010, **26**, 12285.
92 M. Das, D. Bandyopadhyay, D. Mishra, S. Datir, P. Dhak, S. Jain, T. Kumar Maiti, A. Basak and P. Pramanik, *Bioconjugate Chem.*, 2011, **22**, 1181.
93 L. M. Bronstein and Z. B. Shifrina, *Chem. Rev.*, 2011, **111**, 5301.
94 C. Boyer, M. R. Whittaker, V. Bulmus, J. Liu and T. P. Davis, *NPG Asia Mater.*, 2010, **2**, 23.
95 H. Cho, D. Alcantara, H. Yuan, R. A. Sheth, H. H. Chen, P. Huang, S. B. Andersson, D. E. Sosnovik, U. Mahmood and L. Josephson, *ACS Nano*, 2013, **7**, 2032.
96 R. S. Molday and D. Mackenzie, *J. Immunol. Methods*, 1982, **52**, 353.
97 R. Weissleder, A. S. Lee, A. J. Fischman, P. Reimer, T. Shen, R. Wilkinson, R. J. Callahan and T. J. Brady, *Radiology*, 1991, **181**, 245.
98 J. V. Jokerst, T. Lobovkina, R. N. Zare and S. S. Gambhir, *Nanomedicine*, 2011, **6**, 715.
99 A. S. Karakoti, S. Das, S. Thevuthasan and S. Seal, *Angew. Chem., Int. Ed.*, 2011, **50**, 1980.
100 H. Otsuka, Y. Nagasaki and K. Kataoka, *Adv. Drug Delivery Rev.*, 2003, **55**, 403.
101 N. Kohler, G. E. Fryxell and M. Zhang, *J. Am. Chem. Soc.*, 2004, **126**, 7206.
102 Y. Zhang, N. Kohler and M. Q. Zhang, *Biomaterials*, 2002, **23**, 1553.
103 R. P. Quirk, R. T. Matherse, T. Cregger and M. D. Foster, *Macromolecules*, 2002, **35**, 9964.
104 F. Hu, K. G. Neoh, L. Cen and E.-T. Kang, *Biomacromolecules*, 2006, **7**, 809.
105 M. Lattuada and T. A. Hatton, *Langmuir*, 2007, **23**, 2158.
106 M. Lattuada and T. A. Hatton, *Langmuir*, 2007, **23**, 2158.
107 C. Boyer, V. Bulmus, P. Priyanto, W. Y. Teoh, R. Amal and T. P. Davis, *J. Mater. Chem.*, 2009, **19**, 111.
108 R. Narain, M. Gonzales, A. S. Hoffman, P. S. Stayton and K. M. Krishnan, *Langmuir*, 2007, **23**, 6299.
109 X. Li, Y. Liu, Z. Xu and H. Yan, *Eur. Polym. J.*, 2011, **47**, 1877.
110 N. Kohler, G. E. Fryxell and M. Zhang, *J. Am. Chem. Soc.*, 2004, **126**, 7206.
111 E. Amstad, S. Zurcher, A. Mashaghi, J. Y. Wong, M. Textor and E. Reimhult, *Small*, 2009, **5**, 1334.
112 L. Sandiford, A. Phinikaridou, A. Protti, L. K. Meszaros, X. Cui, Y. Yan, G. Frosham, P. A. Williamson, N. Gaddum, R. M. Botnar, P. J. Blower, M. A. Green and R. T. M. de Rosales, *ACS Nano*, 2013, **7**, 500.
113 M. A. White, J. A. Johnson, J. T. Koberstein and N. J. Turro, *J. Am. Chem. Soc.*, 2006, **128**, 11356.
114 M. Arsianti, M. Lim, C. P. Marquis and R. Amal, *Langmuir*, 2010, **26**, 7314.
115 H. Cai, X. An, J. Cui, J. Li, S. Wen, K. Li, M. Shen, L. Zheng, G. Zhang and X. Shi, *ACS Appl. Mater. Interfaces*, 2013, **5**, 1722.
116 S. B. Cambell, M. Patenaude and T. Hoare, *Biomacromolecules*, 2012, **14**, 644.
117 N. Schleich, P. Sibret, P. Danhier, B. Ulcakar, S. Laurent, R. N. Muller, C. Jerome, B. Gallez, V. Preat and F. Danhier, *Int. J. Pharm.*, 2013, **447**, 94.
118 C. M. Pandey, A. Sharma, G. Sumana, I. Tiwari and B. D. Malhotra, *Nanoscale*, 2013, **5**, 3800.
119 S. Mahajan, V. Koul, V. Choudhary, G. Shishodia and A. C. Bharti, *Nanotechnology*, 2013, **24**, 015603.
120 H.-M. Yang, C.-W. Park, P. K. Bae, T. Ahn, B.-K. Seo, B. H. Chung and J.-D. Kim, *J. Mater. Chem. B*, 2013, **1**, 3035.
121 Y. J. Sung, H. Y. Suk, T. Sung, T. Li, H. Poo and M. G. Kim, *Biosens. Bioelectron.*, 2013, **43**, 432.
122 E. L. Rudashevskaya, F. P. Breitwieser, M. L. Huber, J. Colinge, A. C. Muller and K. L. Bennett, *Anal. Chem.*, 2013, **85**, 1454.
123 L. Tang, J. Casas and M. Venkataramasubramani, *Anal. Chem.*, 2013, **85**, 1431.
124 R. Thiramanas, K. Jangpatarapongsa, P. Tangboriboonrat and D. Polpanich, *Anal. Chem.*, 2013, **85**, 5996.

125 H. Piraux, J. Hai, P. Verbeke, N. Serradji, S. Ammar, R. Losno, N. T. Ha-Dyong, M. Hemadi and J. M. El Hage Chahine, *Biochim. Biophys. Acta*, 2013, **1830**, 4254.
126 A. S. Wadajkar, J. U. Menon, Y. S. Tsai, C. Gore, T. Dobin, L. Gandee, K. Kangasniemi, M. Takahashi, B. Manandhar, J. M. Ahn, J. T. Hsieh and K. T. Nguyen, *Biomaterials*, 2013, **34**, 3618.
127 C. Gruttner, K. Muller and J. Teller, *IEEE Transactions*, 2013, **49**, 172.
128 B.-H. Lai, C.-H. Chang, C.-C. Yeh and D.-H. Chen, *Sep. Purif. Technol.*, 2013, **108**, 83.
129 J. V. Staros, R. W. Wright and D. M. Swingle, *Anal. Biochem.*, 1986, **156**, 220.
130 J. N. Talbert and J. M. Goddard, *Process Biochem.*, 2013, **48**, 656.
131 S. D. Xiang, K. Wilson, S. Day, M. Fuchsberger and M. Plebanski, *Methods*, 2013, **60**, 232.
132 T. Siódmiak, M. Ziegler-Borowska and M. P. Marszałł, *J. Mol. Catal. B: Enzym.*, 2013, **94**, 7.
133 A. M. Nowicka, A. Kowalczyk, A. Jarzebinska, M. Donten, P. Krysinski, Z. Stojek, E. Augustin and Z. Mazerska, *Biomacromolecules*, 2013, **14**, 828.
134 M. I. Majeed, Q. Lu, W. Yan, Z. Li, I. Hussain, M. N. Tahir, W. Tremel and B. Tan, *J. Mater. Chem. B*, 2013, **1**, 2874.
135 L. Zhu, D. Wang, X. Wei, X. Zhu, J. Li, C. Tu, Y. Su, J. Wu, B. Zhu and D. Yan, *J. Controlled Release*, 2013, **169**, 228.
136 G. Y. Lee, W. P. Qian, L. Wang, Y. A. Wang, C. A. Staley, M. Satpathy, S. Nie, H. Mao and L. Yang, *ACS Nano*, 2013, **7**, 2078.
137 Y. Y. Chen, M. G. Tsai, M. C. Chi, T. F. Wang and L. L. Lin, *Int. J. Mol. Sci.*, 2013, **14**, 4613.
138 Q. Cui, Y. Hou, J. Hou, P. Pan, L. Y. Li, G. Bai and G. Luo, *Biomacromolecules*, 2013, **14**, 124.
139 H. C. Kolb, M. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2001, **40**, 2004.
140 H. C. Kolb and K. B. Sharpless, *Drug Discovery Today*, 2003, **8**, 1128.
141 W. H. Binder and R. Sachsenhofer, *Macromol. Rapid Commun.*, 2007, **28**, 15.
142 H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2001, **40**, 2004.
143 Q. Wang, T. R. Chan, R. Hilgraf, V. V. Fokin, K. B. Sharpless and M. Finn, *J. Am. Chem. Soc.*, 2003, **125**, 3192.
144 V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem.*, 2002, **114**, 2708.
145 D. R. Elias, A. Poloukhtine, V. Popik and A. Tsourkas, *Nanomedicine*, 2013, **9**, 194.
146 G. L. Li, L. Q. Xu, X. Tang, K. G. Neoh and E. Kang, *Macromolecules*, 2010, **43**, 5797.
147 C. E. Hoyle and C. N. Bowman, *Angew. Chem., Int. Ed.*, 2010, **49**, 1540.
148 N. Maalouli, A. C. Gouget-Laemmel, B. Pinchemel, M. Bouazaoui, J. N. Chazalviel, F. Ozanam, Y. Yang, P. Burkhard, R. Boukherroub and S. Szunerits, *Langmuir*, 2011, **27**, 5498.
149 S. Szunerits, J. Niedziółka-Jönsson, R. Boukherroub, P. Woisel, J.-S. Baumann and A. Siriwardena, *Anal. Chem.*, 2010, **82**, 8203–8210.
150 D. Meziane, A. Barras, A. Kromka, J. Houdkova, R. Boukherroub and S. Szunerits, *Anal. Chem.*, 2011, **84**, 194.
151 A. Pfaff, A. Schallon, T. M. Ruhland, A. P. Majewski, H. Schmalz, R. Freitag and A. H. Muller, *Biomacromolecules*, 2011, **12**, 3805.
152 M. Li, K. G. Neoh, R. Wang, B. Y. Zong, J. Y. Tan and E. T. Kang, *Eur. J. Pharm. Sci.*, 2013, **48**, 111.
153 Y. Shen, W. Guo, L. Qi, J. Qiao, F. Wang and L. Mao, *J. Mater. Chem. B*, 2013, **1**, 2260.
154 M. Marciello, J. M. Bolivar, M. Filice, C. Mateo and J. M. Guisan, *Biomacromolecules*, 2013, **14**, 602.
155 J. P. Mbakidi, N. Drogat, R. Granet, T.-S. Ouk, M.-H. Ratinaud, E. Rivière, M. Verdier and V. Sol, *Bioorg. Med. Chem. Lett.*, 2013, **23**, 2486.
156 M. B. Gawande, P. S. Branco and R. S. Varma, *Chem. Soc. Rev.*, 2013, **42**, 3371.
157 A. Hu, G. T. Yee and W. Lin, *J. Am. Chem. Soc.*, 2005, **127**, 12486.
158 D. Liu, L. Zhang, M. Li, W. Yang and J. Deng, *Macromol. Rapid Commun.*, 2012, **33**, 672.
159 Y. Fu, T. Huang, B. Chen, J. Shen, X. Duan, J. Zhang and W. Li, *Sep. Purif. Technol.*, 2013, **107**, 11.
160 J. Jacques, A. Collet and S. H. Wilen, *Enantiomers, racemates, and resolutions*, Wiley, New York, 1981.
161 J. E. Hein, B. Huynh Cao, C. Viedma, R. M. Kellogg and D. G. Blackmond, *J. Am. Chem. Soc.*, 2012, **134**, 12629.
162 S. Wei, M. Mauksch and S. B. Tsogoeva, *Chem.–Eur. J.*, 2009, **15**, 10255.
163 Y. Wang, B. Li, L. Zhang and H. Song, *Langmuir*, 2013, **29**, 1273.
164 M. R. Ganjali, M. Hosseini, M. Khobi, S. Farahani, M. Shaban, F. Faridbod, A. Shafiee and P. Norouzi, *Talanta*, 2013, **115**, 271.
165 J. R. Niu, X. Huo, F. W. Zhang, H. B. Wang, P. Zhao, W. Q. Hu, J. Ma and R. Li, *ChemCatChem*, 2013, **5**, 349.
166 J. Wang, B. Xu, H. Sun and G. Song, *Tetrahedron Lett.*, 2013, **54**, 238.
167 Q. Zhang, H. Su, J. Luo and Y. Wei, *Catal. Sci. Technol.*, 2013, **3**, 235.
168 B. Karimi and E. Farhangi, *Adv. Synth. Catal.*, 2013, **355**, 508.
169 A. Khorramabadi-zad, S. Daliran and A. R. Oveisi, *C. R. Chim.*, 2013, DOI: 10.1016/j.crci.2013.02.002.
170 X. Xiong and L. Cai, *Catal. Sci. Technol.*, 2013, **3**, 1301.
171 A. Rostami, B. Tahmasbi, H. Gholami and H. Taymorian, *Chin. Chem. Lett.*, 2013, **24**, 211.
172 W. Wang, Y. Zhang, Q. Yang, M. Sun, X. Fei, Y. Song, Y. Zhang and Y. Li, *Nanoscale*, 2013, **5**, 4958.
173 F.-P. Ma, P.-H. Li, B.-L. Li, L.-P. Mo, N. Liu, H.-J. Kang, Y.-N. Liu and Z.-H. Zhang, *Appl. Catal., A*, 2013, **457**, 34.
174 S. H. Gage, B. D. Stein, L. Nikoshvili, V. G. Matveeva, M. G. Sulman, E. M. Sulman, D. G. Morgan, E. Y. Yuzik-Klimova, W. E. Mahmoud and L. M. Bronstein, *Langmuir*, 2013, **29**, 466.

175 L. Vaquer, P. Riente, X. Sala, S. Jansat, J. Benet-Buchholz, A. Llobet and M. A. Pericàs, *Catal. Sci. Technol.*, 2013, **3**, 706.
176 M. S. A. Darwish, U. Kunz and U. Peuker, *J. Appl. Polym. Sci.*, 2012, **129**, 1806.
177 L. Zhi, J. Liu, Y. Wang, W. Zhang, B. Wang, Z. Xu, Z. Yang, X. Huo and G. Li, *Nanoscale*, 2013, **5**, 1552.
178 F. Chen, Z. Cai, Y. Huang, W. Luo and J. Chen, *Polym. Eng. Sci.*, 2012, **53**, 956.
179 Y. Zhao, Y. Yao, M. Xiao, Y. Chen, C. C. Lee, L. Zhang, K. X. Zhang, S. Yang and M. Gu, *Food Control*, 2013, **34**, 436.
180 T. Wang, F. M. Kievit, O. Veiseh, H. Arami, Z. R. Stephen, C. Fang, Y. Liu, R. G. Ellenbogen and M. Zhang, *World Neurosurg.*, 2013, **80**, 134.
181 S. Kanimozhi and K. Perinbam, *Mater. Res. Bull.*, 2013, **48**, 1830.
182 M. L. Verma, R. Chaudhary, T. Tsuzuki, C. J. Barrow and M. Puri, *Bioresour. Technol.*, 2013, **135**, 2.
183 P. Rungta, Y. P. Bandera, R. D. Roeder, Y. Li, W. S. Baldwin, D. Sharma, M. G. Sehorn, I. Luzinov and S. H. Foulger, *Macromol. Biosci.*, 2011, **11**, 927.
184 M. Yang, K. Cheng, S. Qi, H. Liu, Y. Jiang, H. Jiang, J. Li, K. Chen, H. Zhang and Z. Cheng, *Biomaterials*, 2013, **34**, 2796.