Inorganic–Organic Hybrid Nanoparticles for Medical Applications

Cristina I. Olariu, Humphrey H. P. Yiu and Laurent Bouffier

Abstract Recent advancement in the synthesis of inorganic nanoparticles provides scientists a wide range of nanomaterials for their research. Biomedical applications of inorganic nanoparticles have received much attention because of their potential to carry out specific tasks inside our body. To make these small particles compatible and functional inside a human body, designed organic groups are usually attached onto the surface of these particles. In this chapter, we focus on three most commonly used functional inorganic nanoparticles (gold, iron oxides, and quantum dots) for biomedical applications. Gold nanoparticles can be used as a contrast agent for CT scans. Iron oxides nanoparticles have already been used in clinical trials as a contrast agent for MRI scans. Quantum dots can provide strong luminescence for labeling cells and other biological species. Synthesis, functionalization and applications of these inorganic nanoparticles will be discussed.

1 Introduction

Particles with a diameter less than 100 nm are classified as nanoparticles. On the other hand, the identity of particles with diameter between 100 nm and 1 μm is somehow debatable. In biological terms, the major difference between these two

C. I. Olariu · L. Bouffier
Department of Chemistry, University of Liverpool, Liverpool, L69 7ZD, UK
e-mail: cristina.olariu@liv.ac.uk

L. Bouffier
e-mail: Laurent.Bouffier@enscbp.fr

H. H. P. Yiu (✉)
Chemical Engineering, School of Engineering and Physical Sciences,
Heriot-Watt University, Edinburgh, EH14 4AS, UK
e-mail: h.h.yiu@hw.ac.uk

Adv Struct Mater (2013) 4: 85–133
DOI: 10.1007/8611_2010_30

Published Online: 7 July 2010

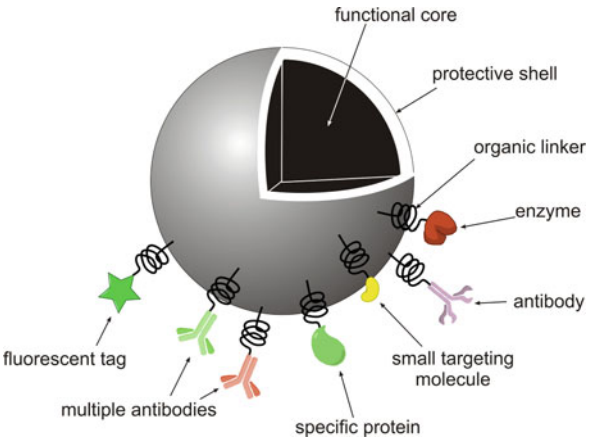

Fig. 1 A typical design of an inorganic nanoparticles functionalized with biomolecules for biomedical applications

types of particles is on their ability to penetrate the cell membranes. Particles of size smaller than 150 nm are thought to be internalized into cells via endocytosis processes. Therefore, nanoparticles should have no size problem while internalization of larger "sub-micron" particles may not be so straightforward.

Due to their small size, which is hundred or thousand times smaller than red blood cells, nanoparticles can travel through our circulation system and access many organs. Such property in penetration across our body makes them possible to carry out some specific tasks. This can well be developed into advanced therapy for the next generation, in particular to those diseases with ineffective treatment at the moment.

Recently, nanoparticles have been on the spotlight of scientific community. With the advancement in nanotechnology, nanoparticles of many materials, in particular inorganic nanoparticles, have been successfully prepared with various methodologies. This made inorganic nanoparticles widely available for scientists to choose for their research interests.

However, for medical applications, use of inorganic nanoparticles still has many obstacles. One major issue is their biocompatibility and toxicity. This is especially important when the final products are to be used in vivo. Therefore, not many inorganic nanomaterials are suitable for medical use. In order to improve their biocompatibility, inorganic nanoparticles are usually coated with protective coating. The coating material needs to be non-toxic in nature. Natural polymer (carbohydrate, peptides), synthetic polymer [polyethylene glycol (PEG), polyvinyl alcohol (PVA), and polyglycolic acid (PGA)], gold and silica are commonly used for coating nanoparticles because these materials are found to be non-toxic and relatively inert inside human body. Such protective coating is only unnecessary if the core material is non-toxic, such as gold nanoparticles. Also, biospecific entities are normally tagged onto the surface of these coated nanoparticles in order to provide specific targeting towards certain tissues, or certain cell types, or even particular compartments inside a cell. Figure 1 illustrates a typical design of a functionalized inorganic nanoparticle for biomedical applications.

Nonetheless, inorganic nanoparticles exhibit some physical properties that their organic counterparts lack. In this chapter, we focus on three types of inorganic

nanoparticles: gold, iron oxides and quantum dots. This includes their preparation procedures, physical and chemical properties, coating and functionalization methodologies, and their applications in advanced medicine.

2 Gold (Au)

2.1 Introduction

Gold is the most fascinating metal and has attracted constant attention from mankind since its very first discovery. Nowadays, gold is still playing a central role in art, jewellery and particularly in the finance world as a strong value indicator [1, 2]. It is also interesting to stress that the price of gold has never been as high as today and gradually increased new records were set up during the autumn 2009 with an all-time high above $1,217 an ounce on the 2nd of December 2009 (gold is traditionally weighed in Troy ounces, i.e. 31.1035 g).

Chemically, gold is extraordinary stable. It does neither tarnish nor oxidize on exposure to the atmosphere and retains its shiny bright aspect over very long time. But the strong stability of gold does result in a proportional lack of reactivity as a bulk metal. And this is essentially why gold had not attracted the attention of chemist until recently simply because it belongs to the family of noble metals which implies that it is very inert under normal pressure and temperature conditions.

This state of fact has changed in the 1980s with the discovery of Haruta that small aggregates of gold atoms can generate a fantastic catalytically activity [3–6]. Indeed this major breakthrough has opened a new era for modern gold chemistry at the nanoscale. Since that reports, gold is a hot topic of research in almost all fields from chemistry, biology, physics and engineering. This new nanochemistry of gold has found highly useful applications in synthetic chemistry thanks to the catalytic activity [7–10] but also in biology and medicine because these tiny clusters of gold could be employing as smart addressable scaffolds to promote multiple applications like for examples: drug delivery, specific targeting, local in vitro or in vivo imaging [11, 12]. It is noteworthy that all these applications in biology or medicine have raised issues about gold nanoparticles toxicity but non-relevant harmful effect to human cells has been noticed by Wyatt and co-workers with 4, 12 and 18 nm in diameter particles [13].

2.2 Synthesis of Gold Nanoparticles

The preparation of monodisperse nanoparticles with controlled size and shape is a key challenge in the area of biotechnology. Realistic applications in that field of research require a fairly good colloidal stability over hours or days and indeed the

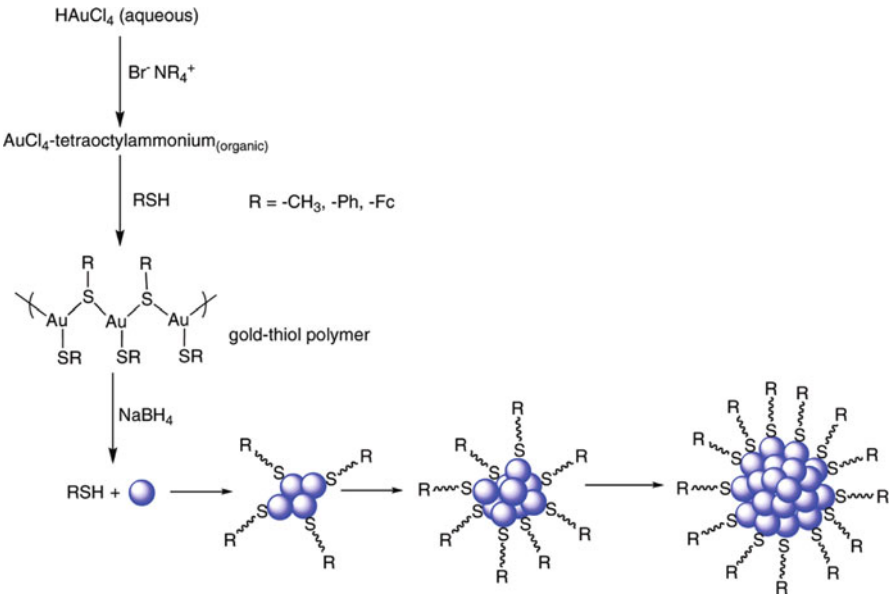

Fig. 2 Scheme for Au nanoparticle synthesis by the Brust–Schiffrin two-phase approach. Reprinted with permission from Ref. [16]. Copyright 2009 American Chemical Society

smaller nanoparticles have a strong tendency to aggregate and settle down in order to minimize the free energy of the system. The most common strategy developed to overcome that thermodynamic constraint consists in coating the surface of the corresponding particles to increase inter-particles repulsion and stabilize the dispersion within an adequate solvent.

2.2.1 Thiolate-Capped Gold Nanoparticles

The most effective way to make noble metal nanoparticles is the so-called bottom-up approach based on the chemical reduction of metal ions under conditions that allow the control of both nucleation and growing thanks to protective capping ligands. The resulting nanoparticles size distribution is directly determined and controlled by the molar *ratio* between the metal ions and the coating agent.

A real breakthrough synthesis has been reported in 1994 by Brust et al. at the University of Liverpool [14]. This work which has more than 2200 citations on SciFinder (December 2009) 15 years after its initial publication is summarized in Fig. 2. $HAuCl_4$ salt initially dissolved in an aqueous phase is transfer into an organic solvent by using tetraoctylammonium bromide as a phase transfer agent while the addition of a thiol R−SH (with R = Me, Ph, Fc) and the subsequent reduction with an excess of sodium borohydride leads to the perfectly controlled growth of thiolate-protected gold nanoparticles. The so-called Brust–Schiffrin

method has been thoroughly studied by several groups and in particular the versatility of this approach has been highlighted by Murray and colleagues [15].

The main advantage of this methodology arises from the precise control of the average size of the Au nanoparticles by the reduction condition as well as the stoichiometry of the available gold salt with respect to the concentration of capping thiol molecules.

2.2.2 Dendrimer-Protected Gold Nanoparticles

Several years after the design of thiolate-protected particles, dendrimer molecules have also been reported to thermodynamically stabilize Au nanoparticles [17]. In 2005, Crooks and co-authors rationalized the improved stabilization of the nanoparticles within the dendrimer matrix and therefore opened the door to substantial new applications. Their work reported in details the preparation of alloy as well as core/shell particles by applying three different strategies (co-complexation method, sequential method and partial displacement method according to their own terminology). This innovative approach is now a relevant alternative to prepare Au nanoparticles thanks to several inerrant advantages. First, the dendrimer architecture strongly protects the resulting nanoparticles from aggregation (site isolation). Secondly, the functionality of the dendrimers on the periphery of the structure can be adjusted in order to tune the solubility or colloidal stability of the nanoparticles in an organic or aqueous medium depending on the targeted application.

On the other hand, extremely small gold clusters (Au_8 nanodots) have been stabilized in hydroxyl-terminated dendrimers earlier in 2003 [18] and the resulting material exhibited highly tunable fluorescent properties that correlate with the size of the Au nanoparticles. This unprecedented radiative emission at short wavelengths (blue emission at 450 nm with excitation at 384 nm) was not observed with thiolate-protected nanoparticles which do emit at near-infrared wavelengths.

2.2.3 Immobilization Through Monolayer Films

Immobilization of these capped-nanoparticles has been intensively investigated thanks to a major significance at the fundamental level as well as potential applications in synthetic chemistry (supported heterogeneous catalysis) or nanotechnology (optical or electrochemical biosensors). In that context, the first report of citrate and alkanethiol-stabilized gold colloid monolayers electrophoretically deposited onto carbon-coated copper grids was published in 1993 by Mulvaney [19]. This approach could be applied to the specific modification of electrodes for generating electrochemical sensors and Murray extensively reviewed the state-of-the-art in a recent issue of *Chemical Reviews* [20].

More complexity and potential sensitivity has been reached with the formation of multilayers of thiolate-protected nanoparticles instead of a monolayer. Several strategies could be identified such as non-specific electrostatic interactions,

coupling *via* dithiol linker or controlled covalent grafting using either amide or ester chemical bonding. Multilayer films of organic compounds deposited on solid surfaces have been studied for many years. An alternative approach to classical Langmuir–Blodgett technique or chemisorption consists in the fabrication of multilayers by consecutive adsorption of polyanions and polycations [21]. The ligand shell of the capped-Au nanoparticles which could be either cationic or anionic acts as a polyelectrolyte and drives the layer by layer deposition. This latter approach allows precise fabrication of multilayer architectures by precisely controlling the number of layers and the subsequent properties of the polyelectrolyte film.

Stellacci and co-workers at the Massachusetts Institute of Technology described an original control of the Au-nanoparticle shell by preparing mixed self-assembled monolayers from a binary mixture of 1-nonanethiol and 4-methylbenzenethiol [22]. The strong disparities between both molecules generate a phase separation at the surface of the nanoparticles which appeared under the form of striped surface ordering in highly contrasted STM imaging. This interesting work was shown in August 2009 during the 42nd IUPAC congress in Glasgow (Adaptive Nanomaterials symposium).

2.2.4 Post-modification of Gold Nanoparticles

The capping ligand plays a central role in the control of the average size and the colloidal stability of the Au nanoparticles but could also increase the functionality level after a post-modification step. This has been done either by exchanging the coated molecules of the shell with another capping ligand (so-called ligand exchange) or by direct reaction on the initial ligand when bearing a reactive functional group (post-functionalisation) [23, 24].

The most popular example is the exchange of the weakly attached citrate ligand against a thiolate. One can assume that this fairly simple exchange reaction procedure drives a complete ligands exchange as the thiolate exhibits a stronger binding to gold atoms than citrate ligands. This strategy was successfully used by Mirkin and co-workers in 1996 to functionalize 13 nm in diameter Au nanoparticles with a single-stranded synthetic DNA. The self-assembly of a 12-mer oligonucleotide with its complementary strand *via* specific hybridization through Watson–Crick base-pairing interactions controls the resulting assembly (Fig. 3) [25]. More recently, the same group also highlighted how this initial work on DNA and the fundamental understanding of the aggregation *versus* colloidal stability process had successful applications in the area of bioanalysis with the objective of improving both sensitivity and speed (Fig. 4) [26].

The exchange of thiolate ligands on Au-nanoparticles is also possible but matrix dissolution rather than full exchange tends to take place. One should keep in mind than the whole exchange mechanism is still subject to discussion. First of all, the capping of thiol on gold substrate is still being investigated and the direct evidence that hydrogen release occurs during the binding was only reported

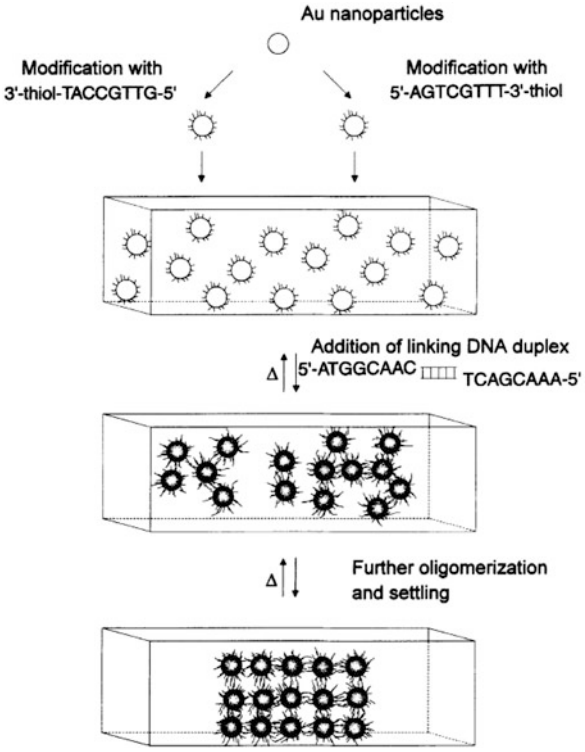

Fig. 3 Scheme illustrating the DNA-based colloidal nanoparticle assembly strategy (the hybridized 12-base pair portion of the linking duplex is represented by ladder). Reprinted with permission from Ref. [25]. Copyright 1996 Nature Publishing Group

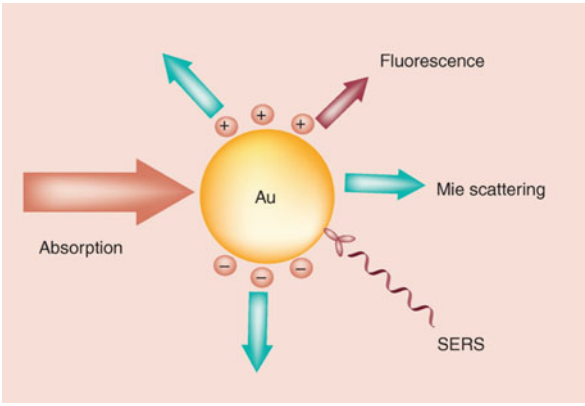

Fig. 4 Optical processes resulting from the interaction of the light with a gold nanoparticle. Reprinted with permission from Ref. [30]. Copyright 2007 Future Medicine Limited

recently [27]. Murray and Mulvaney discussed the mechanism and kinetics of the exchange in a perspective article published in *Langmuir* [16]. The exchange of a molecule of thiol ligand by another could be described as a serial process and the number of exchanged ligands on an individual nanoparticle was found to vary statistically following a Poisson distribution.

Moreover, the kinetics of the ligand exchange has been investigated in details by the same group and others [28, 29]. While Murray and co-workers reported a second-order kinetics during the early stages of the exchange with average constant rates between 1.6×10^{-3} and 1.01×10^{-2} M^{-1} s^{-1} depending on the nature of the incoming ligand; Lennox et al. fitted long time reaction data to Langmuir diffusion kinetics. It is noteworthy that these apparently contradictory results are in fact compatible as in the latter case; the system reached the thermodynamic equilibrium. The reaction was then described by a single rate constant of 1.37×10^{-2} $s^{-1/2}$ which was independent in incoming ligand concentration (i.e. zero-order kinetics).

2.3 Gold Nanoparticles for Bioimaging

2.3.1 Interaction with Light and Surface Plasmon Resonance

Au nanoparticles are offering a significant contribution in the field of labeling and biomedical visualization. Essentially, the particles are used as passive markers which concentration could be either naturally or artificially enriched within the region of interest. In that case, the inerrant physical properties of Au-nanoparticles are used to carry out and/or enhance the imaging by using a large variety of techniques.

Gold particles strongly absorb and scatter visible light and therefore most of the detection methods are based on the interaction between gold particles and light [30]. When Au particles are illuminated, the light energy excites the free electrons of the metal which generates an electromagnetic surface oscillation (surface plasmon) [31]. At the resonance frequency, the excited electron plasmon relaxes thermally by transferring the energy to the crystal lattice of the gold atoms which leads to heating of the particles.

20 nm in diameter gold nanoparticles modified with nuclear localization peptides were imaged by several techniques and their subcellular distribution in cells evaluated by enhanced differential interference contrast microscopy and transmission electron microscopy [32]. Pasqualini and colleagues reported an approach for fabrication of spontaneous, biologically active molecular networks consisting of bacteriophage directly assembled with larger Au nanoparticles (44 ± 9 nm). The spontaneous organization of these targeted networks has been manipulated further by post-functionalization with an imidazole moiety, which induces changes in near-infrared optical properties. The resulting networks were successfully used as labels for enhanced fluorescence and dark-field microscopy, surface-enhanced Raman scattering detection, and near-infrared photon-to-heat conversion [33].

2.3.2 Photothermal Imaging and Luminescence

As previously mentioned, absorbed light eventually leads to heating of the particles which tend to transfer this energy to the surrounding environment. The spatially localized heating can be directly exploit for imaging as reported in 2002

by Lounis and co-workers from the University of Bordeaux [34]. They demonstrated that photothermal method that combines high-frequency modulation and polarization interference contrast allows far-field optical detection of gold colloids down to diameters of 2.5 nm.

This method has been also used to give a dynamic picture of the movement of receptors selectively labeled with 5 nm Au nanoparticles [35]. Small gold particles (size < 2 nm) with high surface to volume *ratio* do emit fluorescence upon photoexcitation and thus can be potentially visualized with fluorescence microscopy. Murray and co-workers reported visible-near-IR luminescence in the range from 700 to 1300 nm depending on the nature of the protecting monolayer and claimed that the emission results from localized electronic surface states [36].

One can note that these aforementioned methods involving photoexcitation (interference contrast microscopy, dark field microscopy, photothermal imaging, or fluorescence microscopy) are now sensitive enough to detect and image mater at the single particle level. As an example, 20–30 nm in diameter Au nanoparticles that scattered light individually can be visualized with high-resolution dark field microscopy [37].

2.3.3 Contrast Agent in X-ray Imaging

The intrinsic physical properties of gold and notably the high atomic weight provide high contrast in transmission electron microscopy (TEM), on the other hand gold nanoparticles scatter X-rays very efficiently and thus provides opportunities in X-ray imaging.

Current computed tomography contrast agents such as iodine-based compounds have several limitations, including short imaging times due to rapid renal clearance, renal toxicity, and vascular permeation. In that context, new contrast agents based on 30 nm gold nanoparticles that overcome these limitations were described in 2007 by Jon and co-workers [38]. Measurement of the X-ray absorption coefficient in vitro revealed that the attenuation of PEG-coated Au nanoparticles is 5.7 times higher than that of the current iodine-based contrast agent. Furthermore, colloidal gold nanoparticles are likely to cause less cytotoxic damage than the last generation iodine-based agents.

2.4 Local Heat Induced by Gold Nanoparticles

Parak and co-workers published a state-of-the-art review entitled “Biological applications of gold nanoparticles” in the special issue of *Chemical Society Review* dedicated to gold (guest editors: Hutchings, Brust and Schmidbaur) [2, 12]. The authors focused on gold nanoparticles as a heat source in a whole section of this extensive review.

As mentioned previously, the heat from the gold particles which results from the localized electronic excitation (Plasmon) is dissipated into the surrounding media. And this can not only be useful for imaging techniques, but also for anti-cancer therapy (hyperthermia).

2.4.1 Hyperthermia

Cells are extremely sensitive to changes in temperature because this affects metabolism kinetics as well as adhesion properties. Even temperature rises of only a few degrees can be lethal to cells.

The general idea here is to deliver colloidal nanoparticles to the cancerous tissue. This can be achieving for instance by functionalizing the particle surface with targeted molecules (such as specific ligands or monoclonal antibody) that are specifically recognized by receptors over-expressed at the surface of cancer cells and could therefore locally concentrate/accumulate close to tumor cells. One possible strategy here is to take advantage of the specific binding between the ubiquitous triad sequence Arg-Gly-Asp (RGD) and $\alpha_v\beta_3$ integrin receptor [39, 40].

If the particles can be heated up by an external stimulus; then the cells in the vicinity of the particles can be selectively killed thanks to a local raised in temperature. Wei and co-workers demonstrated that gold nanorods coated with a cationic surfactant (cetyltrimethylammonium bromide) can be internalized within hours into KB cells by a non-specific pathway [41]. The nanorods render the tumor cells highly susceptible to photothermal damage when irradiated, generating extensive blending of the cell membrane at laser intensity as low as 30 J/cm^2.

Xia and co-workers prepared 45 nm in edge length gold nanocages which can achieve strong absorption in the near-infrared (NIR) region for photothermal cancer treatment [42]. Numerical calculations show that the nanocages have a large absorption cross section, facilitating conversion of NIR irradiation into heat. The gold nanocages were successfully conjugated with monoclonal antibodies (anti-HER2) to target epidermal growth factor receptors (EGFR) that are over-expressed on the surface of breast cancer cells (SK-BR-3).

2.4.2 Heat-Induced Molecular Motion and Delivery

Photo-induced heating of Au particles can also be used for the cleavage of weak chemical and for the opening of synthetic vehicles or macromolecular “cargo” designed to specific targeting.

Puntes and co-workers shown that the local heat delivered by Au nanoparticles (10 nm in diameter) capped with hexa-peptide (Cys-Leu-Pro-Phe-Phe-Asp) can be used as a molecular surgery to safely remove toxic aggregates [43]. This strategy was applied to protein aggregates [amyloid beta (Aβ) protein] involved in Alzheimer’s disease. The authors reported the possibility to redissolve the protein deposits and to interfere with their growth, using the local heat generated with a

low gigahertz range electromagnetic field and subsequently dissipated by gold nanoparticles selectively attached to the aggregates. A letter to *Nature* published in 2002 by Jacobson and co-workers demonstrated remote electronic control over the hybridization of DNA molecules, by inductive coupling of a radio-frequency magnetic field to a 1.4 nm gold nanocrystal covalently linked to a 15-mer oligonucleotide [44]. Inductive coupling to the gold-based assembly increases the local temperature of the bound DNA, triggering efficient thermal denaturation. This is a clear example of specific control of hybridization and biological function at the nanoscale; and moreover, the switch is fully reversible thanks to the extremely fast heat dissipation ($\sim$50 ps timescale).

More recently but based on the same principle, Feldmann and colleagues reported that double-stranded DNA assembly that melts (i.e. denatures) within microseconds could induces the irreversible desaggregation of gold nanoparticle on a millisecond timescale [45]. This concept was exploited to distinguish a single point-mutated DNA from a perfectly matching sequence even in a 1 to 1 mixture of both targets.

The work of Caruso and co-workers published in 2005 did report the preparation and characterization of light-responsive delivery microcapsules composed of multiple polyelectrolyte layers and Au nanoparticles assembly [46]. Further encapsulated material was released from the nanostructured capsule on demand upon irradiation with 10 ns laser pulses in the near-infrared region (1064 nm). Finally, the release of encapsulated material from polyelectrolyte-multilayer capsules has been demonstrated inside living cells [47]. Metal nanoparticles were incorporated inside the walls of the capsules, and served as energy absorbing centers for illumination by laser light. Even if this was done with larger than 20 nm Ag nanoparticles, this strategy could clearly be extended to Au-based nanomaterials.

2.5 Gold Nanoparticles-Assisted Drug Delivery

Inorganic/organic hybrid nanomaterials exhibit a wide range of attributes that make them highly promising candidates for successful drug delivery. The inorganic moiety which is generally the centre core of these materials provides unique and tunable properties to the resulting assembly. Moreover, the surface of the inorganic-based structures also generates scaffolds for the controlled presentation or encapsulation within an addressable vehicle of drugs, imaging or contrast agents.

Gold nanoparticles are not only capable of delivering small drugs but also much larger biomolecules like synthetic peptides, natural or modified proteins, and nucleic acids (i.e. DNA for gene delivery or RNA in case of RNA interference strategy).

2.5.1 Small Drugs Delivery

In the context of photodynamic therapy of cancer, Au nanoparticles could be employed in localized delivery of diatomic therapeutic agents like nitrogen

monoxide (NO) or singlet oxygen ($^1O_2$) that are cytotoxic because they generate oxidative stress and/or triggered oxidative chain reactions. Moreover, nitric oxide plays a key regulating role in several cellular processes like angiogenesis or immune system response. The therapeutic opportunities and the potential application in cancer biology have been reviewed in 2007 by Mocellin and colleagues [48].

Russell et al. stabilized Au nanoparticles (2–4 nm in diameter) with zinc porphyrine-like phthalocyanine photosensitizer elongated with a –SH terminated spacer [49]. Energy-dispersive X-ray analysis confirmed the presence of the drug and the resulting nanocomposite was shown to generate singlet oxygen with enhanced quantum yields from 45 to 65% as compared to the free phthalocyanine. The group of Schoenfisch reported that NO can be reversibly immobilized at the surface of gold nanoparticles by covalent linking with a polyamine-based anchoring group *via* formation of *N*-diazeniumdiolate [50]. They demonstrated efficient and controlled acido-labile release of nitric oxide at pH $\sim$ 3 which highlight the feasibility of realistic applications thanks to slightly acidic media localized in lysosomes or endosomes.

Another advantage of engineering delivery systems is to improve the intrinsic properties of the "free" drug (solubility and in vivo stability or even biodistribution). The control of the drug loading combines to efficient vectorization and release of the active form of a prodrug could result in decreasing the side-effect.

Zubarev and co-workers described in 2007 the first example of gold nanoparticles covalently functionalized with paclitaxel which is one of the most effective chemotherapeutic drug against a large range of cancers [51]. The immobilization method involves the attachment of a short flexible polyethylene glycol linker on the paclitaxel pharmacophore followed by conjugation to 2 nm in diameter gold nanocrystals under mild esterification conditions. Thermogravimetric analysis (TGA) evidenced a high content of organic shell (67 weight percents) which corresponds to a well-defined number of molecules of paclitaxel (73 $\pm$ 4 per nanoparticle).

Another step toward a greater control of the release of the drug has been achieved with glutathione (GSH)-mediated process. GSH is the most abundant thiol in the cytoplasm and the major reducing agent in biochemical processes, providing a potential in situ releasing source in living cells [52]. This methodology relies on the significant difference in intracellular GSH concentration (1–10 mM) *versus* extracellular levels (2 μM in blood plasma). Methods are based on disulfide linkages between the drugs and the carriers that can be displaced in presence of the competitive GSH thiol (see Ref. [53] for review). But an uncontrolled parameter in the release process as well as bioavailability relies on thiol–disulfide exchange which can occur with cysteine residues exposed at the surface of blood proteins. In that context, Rotello and co-workers published a proof of principle when reporting in 2006 the cellular delivery and subsequent GSH-mediated release of a thiolated dye (BODIPY) mimicking hydrophobic drugs [54]. Au nanoparticles with a core dimension of about 2 nm were functionalised with short PEG cationic ligand and fluorophore *via* SAM methodology. The authors explained that the cationic nature of the shell facilitates the crossing of cell membrane barrier

whereas the steric shielding of the gold–thiol interface enhances the resistance to exchange with competitive proteins.

2.5.2 Gold Nanoparticles for Biomolecules Delivery

For gene therapy and therefore DNA delivery, two different strategies can be distinguished; involving either non-covalent or covalent (but usually cleavable) binding between the nanoparticle and the DNA material.

Viral vectors are generally used in gene therapy because they provide highly efficient biocompatible carrier. In that case, the corrective genes have been packaged into modified retroviruses, which can incorporate themselves into a host cell's DNA. Yet, viruses have already raised many health and safety issues concerning non-predictable toxicity and/or unexpected immune responses. The biomedical correspondent of *Nature* reported a tragic case in 2002 when a French gene therapy patient had unfortunately developed a form of cancer while being treated for severe combined immune deficiency [55].

Therefore, non-viral gene delivery using inorganic-based nanocarriers presents a promising and relevant alternative to the aforementioned side-effect. Yet, a remaining problem that should be addressed concerns limitations in both transfection efficiency and associated pharmacokinetics.

In 2003, Klibanov and Thomas did report an enhance transfection while using polycation-mediated DNA delivery with gold nanoparticles [56, 57]. For this approach, 2 kDa branched polyethylenimine (PEI) chains have been covalently attached to gold nanoparticles (between 2.3 and 4.1 nm in size), and the authors evaluated the potency of the resulting conjugates as vectors for the delivery of plasmid DNA into kidney cells. The transfection efficiency was found to vary as a function of the PEI to gold molar *ratio* with the best candidate being about 12 times more potent than the unmodified polycation. Moreover, the intracellular trafficking of the DNA complexes of these vectors, monitored by transmission electron microscopy (TEM) was detected in the nucleus within 1 h timescale after transfection.

Rotello and co-workers investigated a mixed monolayer protected gold clusters functionalized with quaternary ammonium chains for transfection in mammalian cell cultures [58]. The success of these method assemblies depended on several variables, including the *ratio* of DNA to nanoparticle during the incubation period, the number of charged substituents in the monolayer core (several candidates with cationic coverage percentage found between 58 and 100%), and the hydrophobic packing surrounding these amines. The most efficient nanoparticle studied was eight fold more effective than 60 kDa PEI used as a positive control transfection agent. Later in 2006, the same group reported another positively charged gold nanocarrier bearing a photoactive ester linkage, which allows temporal and spatial release of DNA by light. These cationic photocleavable nanoparticles are initially associated with DNA through electrostatic interaction [59]. Irradiation at $\lambda = 350$ nm cleaves the *o*-nitrobenzyl linkage, releasing the DNA from the nanoparticle, and resulting in a high level of recovery of in vitro DNA transcription. Furthermore, effective DNA

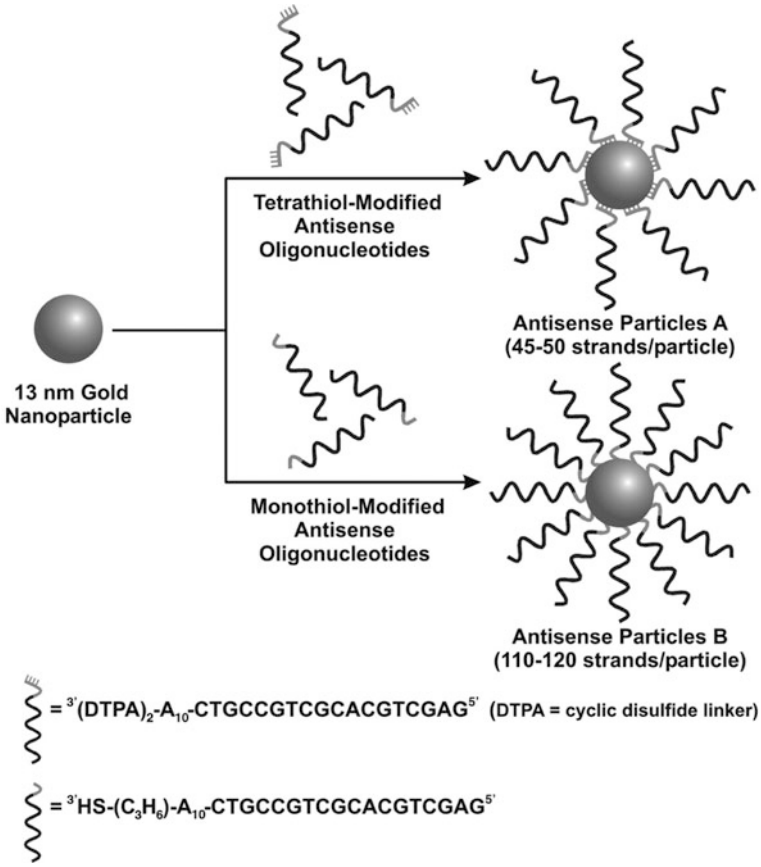

Fig. 5 Preparation of antisense citrate-stabilized gold nanoparticles with tether or single alkylthiol anchoring groups respectively. Reprinted with permission from Ref. [60]. Copyright 2006 Science Magazine

delivery and release in living cells with significant nuclear localization of the DNA were obtained with this system, thus providing the first proof of concept for the development of light-regulated drug-delivery systems.

Mirkin and co-workers described in 2006 the use of Au nanoparticle/oligonucleotide assemblies as intracellular gene regulation agents for the control of protein expression in cells (Fig. 5) [60]. These highly tunable gene transporters (with precise control of the DNA loading) are less susceptible to degradation by nuclease activity and exhibit as high as 99% cellular uptake. This was a significant breakthrough to introduce oligonucleotides at a higher effective concentration than conventional transfection agents. The following year, the same group demonstrated the whole mechanism of cellular uptake of these DNA/nanoparticles conjugates by endocytosis process which is initiated by adsorption of serum proteins onto the surface of the particles [61].

To conclude this section and to demonstrate that gold nanoparticles can also carry larger biomolecules (i.e. proteins), a recent study by Pokharkar et al. has reported functionalized gold nanoparticles as carriers of insulin [62]. Their strategy relies on the loading of gold nanoparticles with chitosan, a widely available biocompatible polymer. As expected, chitosan improved the surface properties for binding of biomolecules and zeta potential measurements have shown an increase from +4.23 to +62.7 mV depending on the chitosan loading (study between 0.01 and 1%). Finally, the pharmacodynamic activity was also improved after oral and nasal administration of insulin loaded samples.

2.5.3 In Vivo Limitations and Targeting

Efficient in vivo applications where the inorganic/organic delivery system is directed to his target following administration into circulatory system is the next step to achieve. For that, two approaches for targeting have been developed: passive and active targeting [63–65].

To summarize, passive targeting tries to take advantage of differential properties of specific unhealthy tissues or hyper-vascularized cells while active targeting relies on presenting ligands on the nanocarrier surface for at least selective but possibly specific recognition by cell surface receptors. The ligands could be short peptidic sequences for vectorization, proteins or specific substrates. Potentially, the combination of both types of targeting will further improve the properties of an optimized carrier for in vivo delivery. Nanocarriers efficiency is limited by non-specific uptake and potential degradation in macrophages (immune response). Therefore, proper targeting is essential for maximizing drug efficacy but others physical parameters of the inorganic moiety need to be considered such as average size, monodispersity, morphology and shape anisotropy. In recent works, Chan and colleagues investigated both size and shape dependence of Au nanoparticles on mammalian cell uptake. They showed that kinetics and saturation concentrations are highly dependent upon the physical dimensions of the nanoparticles by measuring uptake half-life of 14, 50, and 74 nm nanoparticles (2 h 06 min, 1 h 54 min and 2 h 15 min respectively at a rate of 622, 1,294, and 417 nanoparticles per hour respectively) [66]. They have also elucidated the mechanism by which transferrin-coated gold nanoparticles of different sizes and shapes entered mammalian cells *via* clathrin-mediated endocytosis pathway. The nanoparticles exocytosis process was also found to correlate linearly with size which was a different conclusion from their previous work on cell uptake [67]. Furthermore, they developed a model to predict the relationship of size *versus* exocytosis for different cell lines which has major implications in determining nanotoxicity.

2.6 Gold Nanoparticles-Based Biosensors

The function of a biosensor is to detect target molecules (or analytes) and to generate a recordable signal which will possibly allow a fully-quantitative

measurement [68]. Two main aspects are governing the efficiency of such sensors: the molecular recognition step which plays a major role in the selectivity/specificity and the transduction process responsible for the sensitivity. Researches in nanotechnology are growing exponentially since the past 10 years and the integration of biosensoring systems is still a key stake. At the nanoscale level, gold nanoparticle-based biosensors could therefore have a significant impact in modern diagnostics due to their small adaptative size and high degree of functionality.

2.6.1 Nanoscale Surface Plasmon

The intrinsic Plasmon resonance frequency in gold nanoparticles (diameter up to 40 nm) offers a typical "signature" with wavelengths centered around 520 nm and that can be directly exploited for sensing applications. Indeed, the interaction and/or binding of molecules to the particle surface can be responsible for shift in the Plasmon resonance frequency. Raschke and co-workers reported a method for biomolecular recognition using light scattering of a single gold nanocrystal functionalized with biotin [69]. Addition of streptavidin and subsequent specific binding events modify the dielectric properties at the interface between the nanoparticle and the surrounding environment, resulting in a spectral shift of the particle Plasmon resonance.

By analogy to the well-known H and J aggregation in organic chromophores, El-Sayed et al. observed that nanorods exhibiting shape anisotropy can be assembled in two distinct orientational modes (i.e., end-to-end *versus* side-by-side) [70]. A red-shift of the longitudinal Plasmon band for the end-to-end linkage of nanorods resulting from the Plasmon coupling between neighboring nanoparticles was already reported. But here, the authors did observe a blue-shift of the longitudinal Plasmon band of side-by-side linkage with a concomitant red-shift of the transverse Plasmon band.

The pioneering work by Mirkin and co-workers used Plasmon coupling shift to design colorimetric assay method which is nowadays probably the most cited example of a gold-based sensor [25, 71]. In the original assay developed for DNA sensoring, Au nanoparticles and oligonucleotides that are complementary to the specific target sequence are conjugated. The colloidal solution initially appears red because the nanoparticles are well-dispersed without the specific target sequence in solution. Then, the presence of the DNA complementary strands drives the molecular recognition process (hybridization) which results in the formation of small aggregates of Au nanoparticles. The subsequent violet/blue coloration originates from a change in the Plasmon resonance. Several DNA assays are based on this concept and the perfecting of this method has allowed quantitative detection of DNA sequences of concentrations as low as 5 fM [72].

The same concept was eventually extended to non-DNA analytes. Brust and colleagues reported the use of specifically designed, peptide-stabilized gold nanoparticles as artificial substrates for kinases [73, 74]. A very simple colorimetric protocol for the evaluation of kinase activity and inhibition was then

developed by using aptamers-based strategy (i.e. nucleic acid or peptide sequences that change their conformation upon specific binding). The inter-particle distance which is the key parameter is modulated and thus the color of the gold colloids changes from red to violet/blue. Lu and Liu demonstrated in a work published in *Chemical Communication* the possibility of designing nanoparticle-based colorimetric sensors using Cu^{2+}-dependent DNAzyme catalyzed ligation reactions [75]. Compared to DNA or RNA cleaving enzymes, ligation enzymes are intrinsically more sensitive due to the lack of non-specific background.

Interestingly, Li and co-workers investigated the effect of the aptamer folding on the colloidal stability of two different aptamers-tethered Au nanoparticles (adenosine and K^+-dependent aptamers). After the folding process, the particles were in fact found to be more stable toward salt induced aggregation than those tethered to unfolded aptamers. The local conformation adopted on the surface appears to be a key factor that determines the relative stability of the nanoparticles. Finally, on the basis of this unique phenomenon, colorimetric biosensors have been developed for the detection of adenosine, K^+, adenosine deaminase, and its inhibitors [76].

2.6.2 Surface-Enhanced Raman Scattering (SERS)

Most of molecules can be detected by Raman scattering and unequivocally identified by their characteristic spectra. Theory of Raman scattering are well-known and basically, the inelastic scattering process could statistically happen either at lower (Stokes) or higher (anti-Stokes) energy than those of the incident light. However, typical Raman signals are very weak and as a consequence, high surface concentration of the analyte is compulsory in order to provide a recordable signal. SERS provides an efficient circumventing of this limitation by taking advantage of the enhance response when the molecules are close enough to a gold surface with very high curvature, such as nanoscale Au particles [77, 78]. As a result, a much higher scatter probability is achieved and the Raman-scattered light intensity that is detected increases dramatically by several orders of magnitude. The use of SERS allows a major breakthrough in sensitivity and spectroscopic detection of single molecules and single nanoparticles have since been achieved. For single rhodamine 6G molecules adsorbed on the selected nanoparticles, the intrinsic Raman enhancement factors were on the order of 10^{14} to 10^{15} much larger than the ensemble-averaged values derived from conventional measurements. This enormous enhancement leads to vibrational Raman signals that are even more intense and more stable than single-molecule fluorescence [79, 80].

Mirkin et al. performed the multiplexed detection of oligonucleotide targets with labeled Au nanoparticle probes. The gold particles facilitate the formation of a silver coating that acts as a surface enhanced Raman scattering promoter for the dye-labeled particles that have been immobilized onto an underlying chip in microarray format. The strategy provides equally-high sensitivity and selectivity and adds multiplexing capability because a very large number of probes can be addressed based on the concept of using a Raman spectroscopic fingerprint [81]. The current unoptimized detection limit of this method is 20 fM.

Kneipp and colleagues improved further more this concept by the mean of surface-enhanced hyper-Raman scattering (SEHRS) which exploits the incoherent inelastic scattering of two photons on the vibrational quantum states [82]. The authors reported effective two-photon cross-sections for SEHRS on the order of 10^{-46} to 10^{-45} cm^4/s which made the method an extremely promising spectroscopic tool for ultrasensitive bioanalytical applications.

2.6.3 Fluorescence Quenching

Most of fluorophores are quenched when they are close enough to gold surfaces. This phenomenon was evidenced with lissamine dye molecules chemically attached to different size gold nanoparticles [83]. A pronounced fluorescence quenching was observed already for nanoparticles as small as 1 nm radius. The quenching is caused by an increased nonradiative rate as well as a drastic decrease in the dye's radiative rate. Assuming resonant energy transfer to be responsible for the nonradiative decay channel, the experimental finding was compared with theoretical results derived from the Gersten–Nitzan model. This effect can be used for several sensor strategies and the most common example relies on competitive displacement assay which have been formalized by Mc Ghee and von Hippel [84]. For that, gold particles are conjugated with ligands that specifically bind to the targeted analyte. The binding sites of the ligands should be fully blocked by saturating them with analyte-like molecules modified with fluorophores. As the fluorophores are in close proximity to the Au particles they do not emit light (quenching). Then, if these particles are now immersed in a solution containing the specific analyte molecules, they will progressively displace the fluorophore-labeled competitor and therefore enhance the light emission. For thermodynamic reason, the corresponding luminescence correlates to the concentration of analyte molecules in solution and a calibration curve allows a quantitative sensoring.

Kim and co-workers reported in 2005 an inhibition assay method based on the modulation in FRET efficiency between quantum dots (QDs) and gold nanoparticles conjugated with biomolecules [85]. In their strategy, QDs are conjugated with streptavidin (rod shape with a length of 10–15 nm and a diameter of 5 nm) while Au particles are coupled with biotin (spherical shape with 2–3 nm in diameter). By employing the most popular molecular recognition process as a model system, this sensing system allows determining the avidin concentration in sample solution by direct monitoring of the changes in the photoluminescence quenching. Parak and colleagues measured the fluorescence quantum yield of Cy5 dyes attached to gold nanoparticles *via* modulated single-stranded DNA spacers [86]. The distance between the core particle and Cy5 was tuned from 2 to 16 nm. The change in both radiative and nonradiative molecular decay rates with distance was determined using time-resolved photoluminescence spectroscopy. Remarkably, the distance dependent quantum efficiency was almost exclusively governed by the radiative rate.

Brust and co-workers developed "The Peptide Route to Multifunctional Gold Nanoparticles" which used cysteine-containing peptides as capping agents [87]. Based on the fluorescence quenching principle, they designed highly stable peptide-capped gold nanoparticles functionalized with two different biomolecular recognition motifs and measured their binding to DNA and protein microarrays.

2.6.4 Electrochemical Sensors

Joseph Wang authored a review in *Electroanalysis* in 2007 about nanoparticle-based electrochemical bioassays of proteins in order to emphasize the input of electrochemistry in recent sensoring progresses [88]. This work pointed out a variety of new nanoparticle/biomolecule assemblies for advanced electrical detection of proteins. Thanks to electrochemical transduction, ultrasensitive monitoring of biomolecular interactions particularly in the field of DNA can be achieved without any time-consuming PCR-like amplification step.

A pioneering contribution from Mirkin et al. reported a DNA array detection method in which the binding of oligonucleotides functionalized with gold nanoparticles leads to conductivity changes associated with target-probe binding events [89]. Silver deposition improved measurable conductivity changes and an unusual salt concentration-dependent hybridization behavior associated with these nanocomposite probes was exploited to achieve high selectivity. 50 fM in DNA detection was achieved with this method, with a point mutation selectivity factor of 1×10^5 to 1. The very next year, Willner and co-workers described the reconstitution of potent apo-proteins on a functionalized 1.4-nm Au nanocrystal integrated into a conductive film [90]. This construction (Fig. 6) yields to a bioelectrocatalytic system with a turnover rate of $\sim$5000 per second, which is seven times faster to the corresponding rate at which the natural co-substrate accepts electrons. The gold nanoparticle acts as an electron relay or "electrical nanoplug" for the alignment of the enzyme on the conductive support and for the electrical wiring of its redox-active center.

In parallel, Caruso reported another electrochemical sensors based on 4-(dimethylamino)pyridine-stabilized gold nanoparticle hybrid films prepared by infiltration into polyelectrolyte multilayers preassembled on indium tin oxide (ITO) electrodes [91]. Quartz crystal microgravimetry (QCM) and UV–Vis spectroscopy showed that composite films achieved high density. Electrochemical experiments revealed that the presence of gold nanoparticles in the multilayers significantly improves the electron-transfer characteristics of the films, which showed high electrocatalytic activity to the oxidation of nitric oxide (NO). The sensitivity of the composite films for measuring NO could be eventually further tailored by controlling the gold nanoparticle loading within the film.

More recently, Holzinger and co-authors at the University of Grenoble developed a concept of three-dimensional biostructures immobilized on electrode surface *via* affinity reactions [92]. The 3D architectures based on single-walled carbon nanotubes (SWCNTs) frameworks interconnected with gold nanoparticles

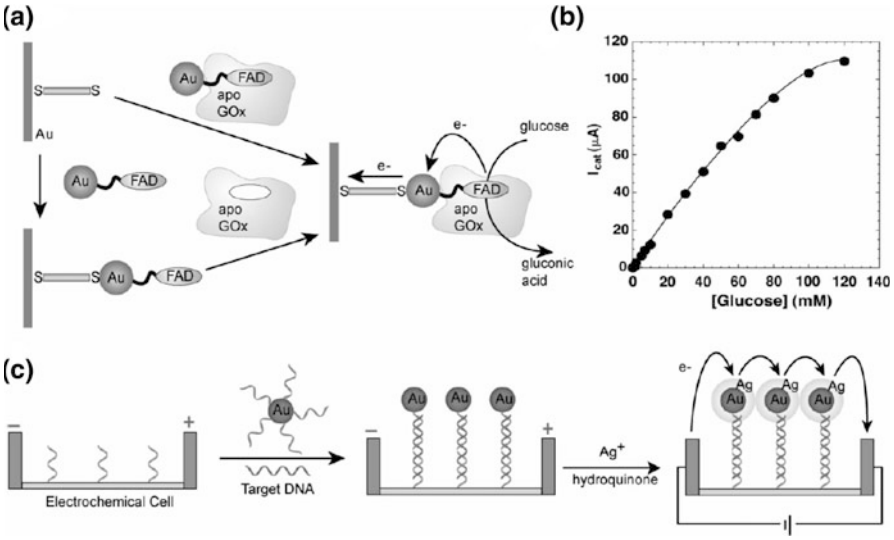

Fig. 6 **a** Fabrication of a GOx electrode by the reconstitution of apo-enzyme on a FAD-functionalized gold nanoparticle and **b** plot of the current developed by the reconstituted GOx electrode in the presence of different concentrations of glucose. **c** Electrical detection of DNA based on the ''sandwich'' hybridization with DNA-functionalized AuNPs followed by silver deposition. Reprinted with permission from Ref. [11]. Copyright 2008 Wiley-VCH

relays were applied to glucose sensoring *via* glucose oxidase (GOx) anchoring. This electrocatalytical model process was used as a proof of principle and the authors highlighted a combined synergetic effect of SWCNTs and gold nanoparticles that provides a higher amount of immobilized enzyme as well as improved electrochemical performances.

3 Iron Oxides (FeO_x)

3.1 Introduction

Magnetic nanoparticles have long been of technological and scientific interest as they behave like small magnets having either ferromagnetic or superparamagnetic character which makes them attractive for a broad range of applications like electronic devices, information storage, catalysis, magnetic inks, etc. [93, 94]. Furthermore, magnetic nanoparticles have also attracted much attention in biomedicine in applications such as magnetic drug delivery, biosensing, magnetic hyperthermia, regenerative medicine and magnetic resonance imaging (MRI) as contrast enhancers [95–97]. Each of these applications requires that the magnetic nanoparticles have specific properties. For example, the most important properties

required for biomedical use are: biocompatibility, superparamagnetic character at room temperature (no magnetic remanence), stable at physiological pH and salinity, long blood half-life and efficient internalisation [98, 99]. Colloidal properties of the magnetic fluid are also essential and depend firstly on the particles dimensions and secondly on surface chemistry and charge.

3.2 Synthesis Methods for Magnetic Nanoparticles

Considering the wide range of possible applications for this type of nanoparticles, it is no surprise that there are numerous chemical methods for synthesis: coprecipitation [100, 101], microemulsion [102], high temperature decomposition of precursors [103], polyol methods [104], sonolysis [105].

3.2.1 Coprecipitation Methods

The precipitation from iron salts is probably the most extensively utilized route of preparation. Typically, iron oxides are prepared by addition of a basic solution to a mixture of ferric and ferrous salts. Generally the reaction for the formation of Fe_3O_4 can be written as:

$$Fe^{2+} + 2Fe^{3+} + 8OH^- \rightarrow Fe_3O_4 + 4H_2O$$

This reaction is taking place at a pH ranging between 8 and 14 and in an oxygen free environment [106], otherwise the formed magnetite is not very stable and it undergoes oxidation into Fe_2O_3:

$$4Fe_3O_4 + O_2 \rightarrow 6Fe_2O_3$$

The process of coprecipitation is characterized by a nucleation phase followed by a growth phase which is responsible for the particle's shape, size and dispersity. To produce monodisperse nanoparticles, the two phases must be separated as shown in the LaMer diagram in Fig. 7 [107].

Other parameters which can influence the size, shape, magnetic properties are pH, temperature, oxygen presence, nature of salts (chlorides, sulfates, nitrates) or Fe^{2+}/Fe^{3+}*ratio* [109].

Massart was the first who synthesized superparamagnetic iron oxide nanoparticles using alkaline precipitation of ferric and ferrous chloride [110]. The original method was varied by many groups to achieve smaller size particles (from 8 to 3 nm) or to allow coating with different groups like hydroxyacids (citric, gluconic acids), dimercaptosuccinic acid, silica [111–113]. Several researchers have studied the influence of mixing rate upon particle size and showed that increasing this parameter the size and polydispersity decreases [110]. Other investigations show that rising the temperature of the reaction reduces the nanoparticles formation

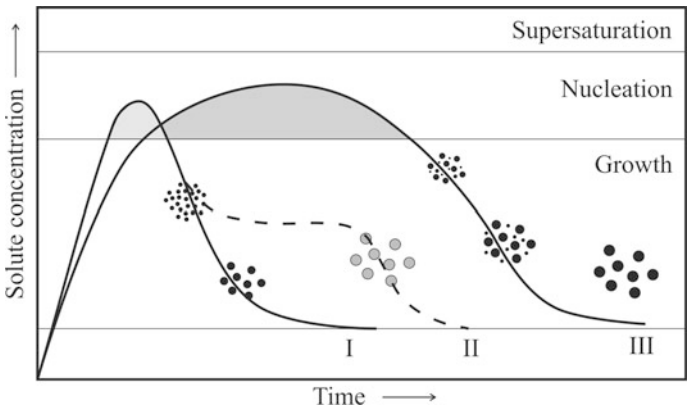

Fig. 7 Schematic representation of proposed mechanisms of formation of uniform particles: *curve I*. Single nucleation and uniform growth: *curve II*. Nucleation, growth and aggregation of small particles: *curve III*. Multiple nucleation events. Adapted with permission from Ref. [108]

[109]. This method offers the advantage that it is easy to follow and produces large quantities of nanoparticles.

3.2.2 Microemulsion Method

The water-in-oil (W/O) microemulsions (inverse micelles) are systems of fine nanodroplets of aqueous phase trapped by surfactant molecules dispersed in an oil phase. This method can also be used for the preparation of iron oxide nanoparticles as illustrated in Fig. 8. Compared with coprecipitation this method has certain advantages due to the presence of surfactant stabilized cavities which act as a constraining environment for the particles growth and agglomeration [114, 115].

W/O microemulsion method offers the opportunity to modulate the size of the nanoparticles by varying the size of the nanodroplets, which is the major advantage. Lee et al. have prepared nanoparticles with an adjustable diameter between 2 and 10 nm by varying the solvent, surfactant or concentration of iron salt [117]. Also this method was used by Carpenter to synthesize thin layer gold-coated iron oxide particles. Gold protects the core from oxidation, providing biocompatibility and functionality at the same time [118].

Vidal-Vidal et al. reported a one-pot microemulsion preparation for coated and uncoated monodisperse magnetic nanoparticles [119]. Numerous types of surfactants can be employed in the preparation procedure like ionic or non-ionic surfactants. The main disadvantage of using the ionic ones is that the produced nanoparticles are less crystalline [120] than the ones formed in the presence of non-ionic surfactants [121]. However, this technique has major drawbacks as it is very difficult to be scaled up and the surfactant molecules adhered to the nanoparticles are hard to be removed. The surfactant traces on the surface are an impediment to be used in biological applications.

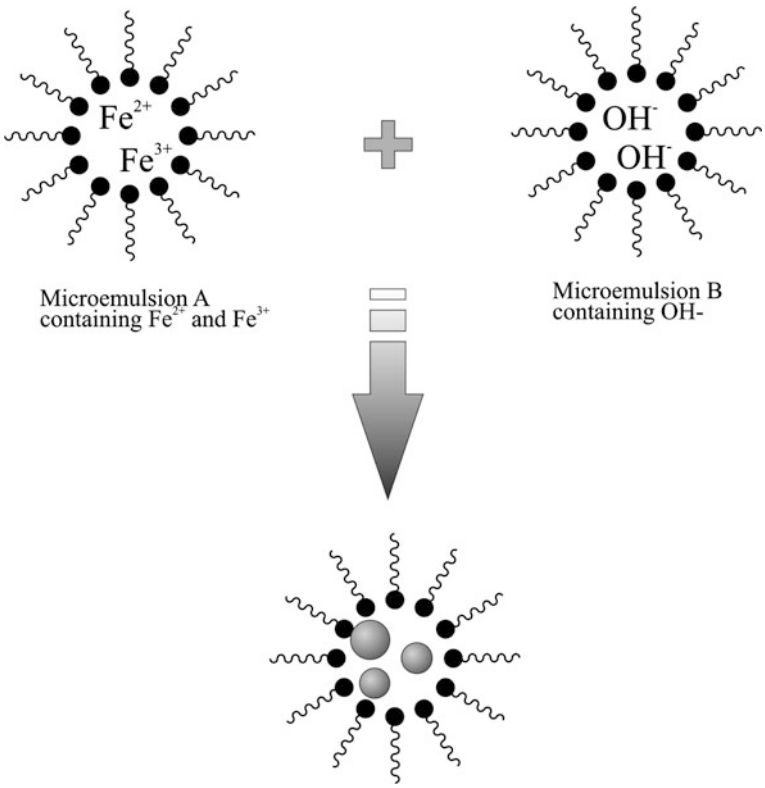

Fig. 8 Schematic representation of formation of magnetite nanoparticles using W/O microemulsion. Adapted with permission from Ref. [116]

3.2.3 High Thermal Decomposition

This method is based on the decomposition of different types of iron precursors like iron oleate, iron acetylacetonate [$Fe(acac)_3$] or iron pentacarbonyl [$Fe(CO)_5$], at a high temperature in the presence of organic solvents and stabilizing molecules like oleic acid, oleylamine or steric acids. The presence of surfactants clearly improves size control, narrow size distribution and crystallinity of the individual iron oxide nanoparticles. For example, highly monodispersed maghemite nanoparticles were produced by Hyeon et al. at 100°C by thermally decomposing $Fe(CO)_5$ in the presence of oleic acid, followed by a second step consisting in aging the iron oleic acid complexes at 300°C. With this procedure particles with controllable sizes between 4 and 16 nm were prepared [122]. More recently Sun et al. have reported the preparation of monodisperse iron oxide nanoparticles with sizes from 4 to 20 nm using the high temperature decomposition reaction of $Fe(acac)_3$ in phenyl ether in presence of stabilizing oleic acid and oleylamine

[103]. To obtain nanoparticles with diameters up to 20 nm a seed-mediated growth method was necessary. The parameters which can be varied during the process to produce different size particles are reaction time, nature of solvents, stabilizing agents, addition of seeds, precursors. However, this method produces hydrophobic magnetic nanoparticles and following reactions to transform them into hydrophilic ones are required. For instance, after the thermal decomposition of $Fe(acac)_3$ in high boiling point solvent the oleic acid nanoparticles have been coated with dimercaptosuccinic acid using a ligand exchange reaction to disperse the nanoparticles in water without aggregation [123]. Another example of successful phase transfer from hydrophobic to hydrophilic character was reported using the Pluronic block copolymer on oleic acid capped magnetite particles [96]. Also Li and co-workers recently reported that thermal decomposition of ferric triacetylacetonate in 2-pyrrolidone in presence of mPEG-COOH could produce crystalline PEGylated nanoparticles [124].

3.2.4 Synthesis Using Liquid Polyols

The polyol process is a chemical method of nanoparticle synthesis which uses the liquid polyol to reduce the metal precursors to particles with improved properties, crystallinity, monodispersity, higher magnetization [125, 126]. The polyols (for example, polyethylene glycol) act as solvents which are able to dissolve the inorganic compounds and at the same time are stabilizing the in situ formed nanoparticles. Cai and Wan [104] have reported a facile route of preparing directly non-aggregated superparamagnetic magnetite nanoparticles *via* polyol reaction. They investigated the influence of four types of polyol (mono-, di-, tri-, and tetraethylene glycol) in the direct reaction with $Fe(acac)_3$ and the results showed that only triethylene glycol produces non-aggregated nanoparticles due to suitable number of coordinating groups of the polymer with the particles surface. In comparison with the precipitation method, this route has the advantage to produce much narrower particle sizes and the surface will be coated with the hydrophilic polyol ligands.

3.3 Surface Modification of Iron Oxide Nanoparticles

Although there has been important progress regarding synthesis methods for magnetic nanoparticles, maintaining their stability for a long period of time without aggregation or precipitation is still an issue [127]. The agglomeration of nanoparticles may cause problems when they are to be used for in vivo applications because of the possibility of causing capillary blockage. As a result, it is very important to coat the surface of the magnetic nanoparticles with inorganic or organic layers and to form stable colloids. Also the protective coating may not only be used against degradation but also for further functionalization with drugs,

targeting molecules, or other functional groups. The coating is also a key factor for the biocompatibility properties of the nanoparticles. The colloidal stability can be achieved either by electrostatic or by steric repulsion as exemplified in Fig. 9.

The key parameter to generate good magnetic colloids is to control the strength of these two forces. The steric repulsion can be controlled by using polymers and it depends mostly on the molecular weight and density of the polymer [129]. The electrostatic repulsion can be generated with charged molecules bound to the surface of nanoparticles [130, 131]. The surface of iron oxide is rather inactive compared to other kind of nanoparticles (quantum dots, gold) and there is only limited number of functional groups that are able to anchor on it. It is known that carboxylates and phosphates are functional groups that bind to the surface of magnetite [132]. Citric acid is a one type of stabilizing agent which is commonly used to make magnetite surface negatively charged and hydrophilic at the same time. It has been used by many groups during the coprecipitation process to control the growth phase and magnetic properties of nanoparticles and because of these characteristics, citric acid stabilized iron oxide nanoparticles (VSOP C184) are now under clinical trials [133, 134]. Cheon and co-workers were able to prepare water-dispersible iron oxide nanoparticles using 2,3-dimercaptosuccinic acid, which has a bidentate carboxyl group [123]. Alkanephosphonic acids are also suitable ligands for magnetite stabilization and coating. Yee et al. reported that phosphonate ions are able to anchor on iron oxide surface through bidentate or monodentate bonding [135].

Silica is the most used inorganic coating for iron oxide nanoparticles. Generally, the silica shell prevents the aggregation phenomenon of magnetite nanoparticles and improves their chemical stability [136]. Although the toxicity of silica shells is still uncertain, there have been promising reports on the use of silica encapsulated nanoparticles for in vitro and in vivo use [137]. Ying and co-workers reported on silica-protected iron oxide nanoparticles synthesized using an inverse microemulsion method. The thickness of the silica layer can be varied from 2 to 100 nm depending on the synthesis method and the concentration of precursors used [138].

Functional organosilane compounds also form strong interactions with the metal oxide and for this reason can be successfully used as stabilization materials and at the same time to give functionality to the magnetic nanoparticles [139, 140].

Gold is another inorganic type of coating material which can be used in case of iron oxide nanoparticles because it offers functionality for the surface of magnetic particles and also improves their stability in aqueous dispersions. Superparamagnetic nanoparticles with a gold nanoshell have been reported only recently by Kim et al. [141].

Polymers have as well been used for magnetic nanoparticles surface modification on their own or in conjunction with other elements. The polymer encapsulation can be achieved during the particles formation or post-synthesis. Various natural and synthetic polymers have been used as a coating for iron oxide nanoparticles but the most common ones are dextran, carboxymethyl dextran, polyethylene glycol (PEG), polyvinyl alcohol (PVA), starch and chitosan.

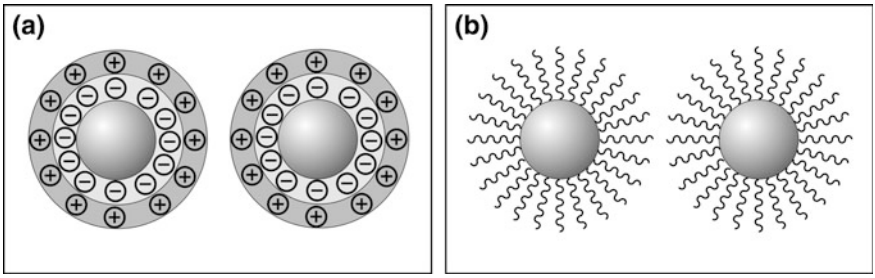

Fig. 9 Stabilisation techniques for magnetic nanoparticles: **a** electrostatic stabilisation; **b** steric stabilisation. Adapted with permission from Ref. [128]

Dextran is a natural polysaccharide and has been used for coating iron oxide particles due to its biocompatibility properties. For example, Feridex, Resovist and Combidex are all MRI contrast agents based on dextran-coated iron oxide nanoparticles. These particles show a long blood resistance time which allows their uptake by the macrophages in kidney, brain, osteoarticular tissues. A detailed structural and magnetic study on iron oxide formed in presence of dextran (MW = 40,000) has been elaborated by Pardoe et al. Their results show that dextran has an important role in particle size [142]. Dextran-coated magnetic nanoparticles based on physical absorption are unstable as dextran tends to dissociate from the magnetic cores [116]. The usual method to avoid desorption is to use a cross-linking agent such as epichlorhydrin which makes tighter association with the superparamagnetic iron oxide nanoparticles (commonly termed as SPIONs) [143]. Covalently bound dextran-SPIONs can be prepared using functional aminopropyl silane to modify the surface of the particles and then covalently conjugate them with partially oxidized dextran [144].

Polyvinyl alcohol (PVA)-coated magnetic nanoparticles are also of great interest as PVA prevents nanoparticles from aggregation. Recently Lin et al. have synthesized PVA encapsulated iron oxide particles according to the Massart method of coprecipitation [145]. The results showed that the polymer restricts the growth in size of the particle and that it binds irreversibly to the surface.

Polyethylene glycol (PEG) is a representative synthetic, hydrophilic, biocompatible polymer. The surface coverage with PEG minimizes the protein absorption onto magnetic nanoparticles surface increasing the blood time circulation. The unique property of PEG of non-specific absorption events is still in debate but is believed to be related with the large hydration volume, osmotic repulsion by the PEG chains and its unique interaction with water [146]. PEG-coated magnetic nanoparticles are prepared using different surface anchoring materials like phospholipids, copolymers, and silica because PEG itself is very inert. LaConte et al. reported the preparation of water-dispersible PEG-phospholipids block-copolymers magnetic nanoparticles which are based on the hydrophobic interaction between the hydrophobic tail group of surfactants and phospholipids parts [147]. Functional silane coupling agents such as 3-aminopropyltriethoxysilane are

commonly employed to immobilize PEG onto the SPIONs surface. Because PEG molecules have only a hydroxyl at the end, the polymer was further modified to generate amine or carboxyl groups [139].

Chitosan with its biodegradable, bioadhesive and biocompatible properties has been used in the pharmaceutical industry for a long time and nowadays have started to attract attention as a coating material for iron oxide nanoparticles. Chitosan-coated SPIONs prepared by a sonochemical method have been reported to have superior properties as contrast agents for MRI [148]. Other examples of polymers which have been employed in the stabilization of magnetic nanoparticles include poly(caprolactone), poly(lactic)acid, poly(acrylic acid), arabinogalactan or polyethyleneimine [149–151].

3.4 Biomedical Applications of Magnetic Nanoparticles

3.4.1 Magnetic Nanoparticles as Contrast Agents in MRI

At present MRI (magnetic resonance imaging) is one of the most powerful imaging techniques in medicine but often the difference in the relaxation times between abnormal and normal tissue times is very small and the image might provide a false diagnostic. To allow a better interpretation of the MRI image, contrast agents like iron oxide nanoparticles or gadolinium chelates have been introduced. Since their first use as MRI contrasting agents 20 years ago, nanoscale iron oxides have received enormous attention due to their ability to shorten T_2 relaxation time in liver, spleen, bone marrow because of their selective uptake by the cells of the reticuloendothelial system (RES). The mechanism for contrast in MRI is quite complicated and it mostly results from the dipolar interactions between the water proton spins and the magnetic moment of the iron oxide nanoparticles (Fig. 10) [152].

Several different formulations are currently used clinically for MRI and are based on superparamagnetic cores and a polymeric coating (dextran). Table 1 summarizes these types of contrast agents.

These types of contrast agents can be applied only for imaging the mentioned organs based on the biological distribution of nanoparticles. Recently iron oxide nanoparticles have attracted interest for targeted molecular imaging because of their large surface area to which targeting probes can be coupled. These include aptamers, oligonucleotides, peptides, antibodies, small molecules (folic acid) or other imaging molecules for improving the detectability of nanoparticles. At the beginning, monoclonal antibodies (mAbs) were the first to be used for targeting the magnetic nanoparticles. For example, Suzuki et al. have prepared an MRI contrast agent based on the covalent bonding between polyethylene glycol-coated iron oxide and a surface antibody specific for human glioma [153]. Recently, research is focused on using only fragments of the antibody which are still functional to direct the magnetic nanoparticles to the area of interest [154].

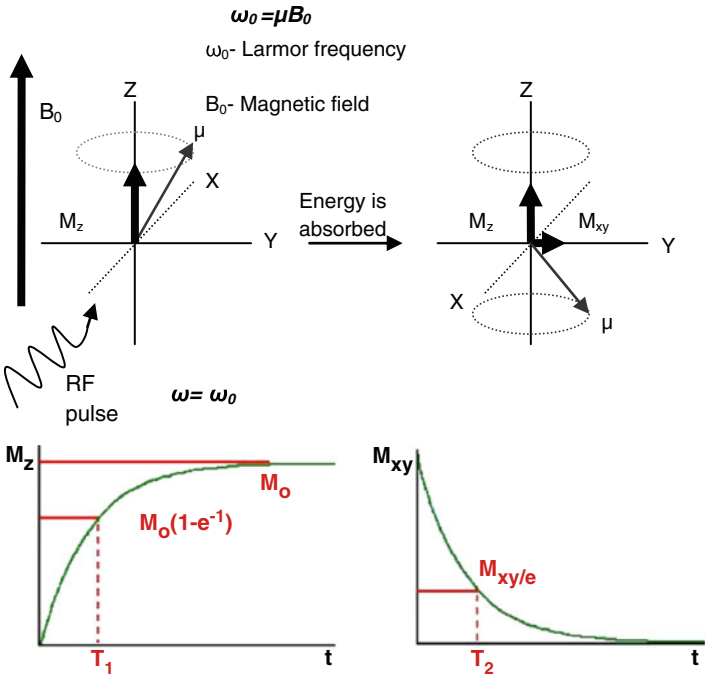

Fig. 10 Longitudinal and transverse magnetization mechanism (above); T_1 and T_2 relaxation time curves (below). Adapted with permission from Ref. [152]

Sun et al. have developed an imaging/drug delivery dual system that include PEG-coated SPIONs with chlorotoxin (peptide which binds to brain cancer cells) and methotrexate (a chemotherapeutic drug). This system has demonstrated promising results for in vivo applications [155]. There is no doubt that the current advances in the area of multifunctional magnetic nanoparticles will improve the sensitivity and quality of MRI.

3.4.2 Magnetic Hyperthermia

Hyperthermia, which is based on the fact that cancerous cells are more sensitive to temperature than normal cells, is a very promising treatment for cancer. The major technical difficulty with the application of hyperthermia is to heat only abnormal cells without affecting the normal surrounding tissue. Magnetic nanoparticles are good candidates for an effective treatment since they can be directed to and concentrated at the tumor site (due to the leaky hypervasculature) and can generate heat reaching 42–45°C under an applied alternating magnetic field [156]. The key qualities of SPIONs as mediators of hyperthermia are: (1) higher specific absorption rate and (2) the high cellular selectivity via surface functionalisation.

Table 1 Iron oxide-based MRI contrast agents

Name	Formulation	Particle size (NM)	Target organs
Feridex®/Endorem® (Ferumoxide)	SPION/dextran	160	Liver Spleen
Resovist® (Ferucarbotran)	SPION/carboxydextran	60	Liver Spleen
Combidex®/Sinerem® (Ferumoxtran)	SPION/dextran	20–40	Lymph nodes
Lumirem®/Gastromark® (Ferumoxsil)	SPION/silane	300	Bowel marker

Recent reports have highlighted that 10–30 nm magnetic nanoparticles have been effectively heated on human breast cancer models, but the control of the distribution of nanoparticles is still an issue [157]. A clear progress in hyperthermia research is the start of human clinical trials of magnetic hyperthermia treatment for brain and prostate cancer conducted by Jordan and co-workers at the Charité Hospital in Berlin. Preliminary results are encouraging because of the direct injection in multiple sites in the tumor rather than the use of targeted magnetic nanoparticles [158].

3.4.3 Magnetic Drug Targeting (MDT)

Conventional chemotherapy and radiotherapy are the current options for treating the majority of cancers but side effects are a common consequence of the systemic delivery of drugs. The principle of magnetic drug delivery is based on the use of an external magnetic field targeting and accumulating the magnetic carriers loaded with drugs at a specific location in the body (solid tumour). Afterwards the drug is released from the magnetic complexes *via* enzymatic cleavage, change in pH or temperature [116]. For instance, Alexiou et al. have reported the use of magnetic ferrofluids loaded with mitoxantrone for treatment of squamous cells carcinoma in rabbits [159]. Recently, a new MDT technique has begun to be the focus of the research community. This new technique is based on two steps: first, intravenous injection of the magnetic drug carriers and second, localization of the magnetic nanoparticles at the target site using an implanted magnetizable stent [160]. Although the MDT results are promising on animal models, the human trials still remain as a challenge.

3.4.4 Stem Cell Tracking

In recent years, magnetic nanoparticles have also been emerging as an attractive system for labeling and tracking stem cells in vivo. Magnetic nanoparticles offer the possibility to non-invasively monitor the stem cells migration and

differentiation after transplantation using MRI. There are an increasing number of stem cell tracking studies using magnetic nanoparticles with MRI, particularly in the area of brain and spinal cord regenerative medicine [161]. Sometimes the MRI results can be confirmed by using fluorescence imaging of the animal which means that bifunctional magnetic nanoparticles are needed to label the stem cells. The study reported by Frank et al. shows how is possible to follow the migration of neuronal restricted precursors labeled with superparamagnetic iron oxide contrast agents such as Feridex© using ex vivo MRI and histological methods [162]. Although many research studies demonstrate the advantages of using such systems, there are still many challenges that need more focus such as the lack of a rapid cell delivery system that does not use a toxic transfecting agent or suitable coating materials for the magnetic nanoparticles to enable the survival of these in the cellular environment.

3.4.5 In Vitro Separation and Purification of Targets

Another important type of application of the magnetic nanoparticles is the functionalization for in vitro separation of cells and proteins. In this procedure the iron oxide nanoparticles are added to the solution or suspension which contains the cells/proteins of interest. The cell or the protein reacts with the specifically tagged magnetic nanoparticles and then is separated from the mixture using a magnetic separator. This approach offers clear advantage over classical chromatography methods: simplicity, time efficiency and possibility to be carried out in a small scale. Commercially available products can be found for a variety of applications such as DNA, RNA or protein separation and purification [163, 164]. Interesting results have been reported in this area by Xu et al. who used dopamine functionalized magnetic nanoparticles to separate histidine-tagged proteins from a cell lysate with high efficiency. Another good example is the immobilization of vancomycin to the magnetic cores using the amino group of the antibiotic. This system was able to capture vancomycin-resistant enterococci or Gram-positive bacteria even at low concentration [165].

4 Quantum Dots (QDs)

Quantum dots (QDs) are inorganic colloidal semiconductors with a nanocrystal structure. These inorganic nanoparticles are luminescent at a particular range of wavelength. The first report for preparing these semiconducting nanocrystals was published in 1993 [166]. In 1994, Alivasatos reported a material with CdSe particles dispersed in a conducting polymer and a fluorescent blue color was observed [167]. This paper has been widely regarded as the major breakthrough in the research of quantum dots and rapid development has been observed in the next 15 years.

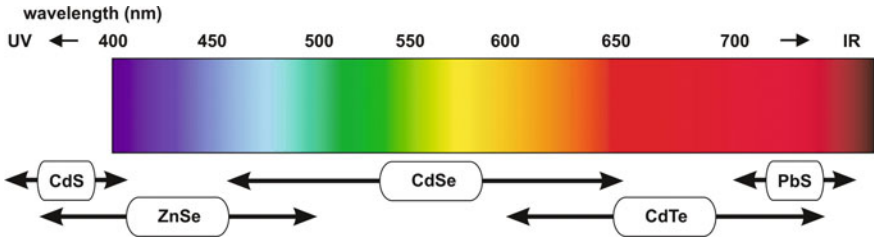

Fig. 11 Emission spectra of common quantum dots

Generally, when the crystal size of a semiconductor material decreases to a level of several hundreds to several thousands of atoms, the quantum-confinement effect becomes observable. Such an effect is defined by an increase in band gap together with the quantization of energy levels to discrete values. This effect is usually observed with semiconductor particles of size around a few nanometers and the extent of this effect is directly related to the size of the particle. The wavelength of their emission radiation increases as their particle size increases. For example, CdSe nanoparticles of 2.6 nm in diameter emit greenish-blue color (510 nm) while a red color (610 nm) is emitted from larger particles of 4.8 nm [168]. However, the equation governs this luminescent property is rather complicated and varies from one semiconducting material to another. It is rather impossible to predict the emission wavelength of a new quantum dots material.

There are many nanosized semiconducting materials show such quantum-confinement effects. Commonly used materials include ZnS, ZnSe, CdS, CdSe (types II–VI), GaN, GaP, InN, InP (types III–V) and PbS (types IV–VI). These materials all have their characteristic emission spectrum (see Fig. 11). Among all these semiconducting materials, CdSe covers the widest range of color or wavelength in the visible spectrum. For biomedical labeling, visual effect is important and, therefore, CdSe quantum dots are the most popular choice for biologists.

Although use of organic fluorescent molecules (e.g. fluorescein) for labeling biological species has been well documented, scientists continue to find new materials to replace these organic molecules. This is because organic fluorescent molecules usually have problems of bleaching, i.e. losing fluorescent intensity over long exposure to light, which may temporarily alter their structure. In contrast to fluorescent molecules, the emission wavelength of quantum dots is not only determined by their chemical formulation, but also their particle size. Also, bleaching is much less likely to quantum dots, due to their inorganic nature. Quantum dots present scientists a new opportunity to be used as an alternative for fluorescent labeling in biology.

However, the problem of using quantum dots for labeling biological samples is not associated with their optical property. Many semiconducting materials are highly toxic with notorious elements such as Cd, Se and Te [169]. Hence, the primary task for scientists to develop quantum dots for in vivo use is to protect them from exposure directly to our body system and a very robust coating is undoubtedly essential.

4.1 Preparation of QDs

Quantum dots are now commercially available but they are not difficult to synthesize in a standard laboratory with equipments for synthetic chemistry. Similar to nanoparticles of inorganic oxides, quantum dots are usually prepared with decomposition of organometallic precursors at high temperature (usually about 250–300°C) in the presence of ligands. The combination of trioctylphosphine and trioctylphosphine oxide (TOP/TOPO) as a high temperature solvent and ligand has been widely used in the preparation of CdSe quantum dots. In the original paper, trioctylphosphineselenide $(C_8H_{17})_3PSe$ and dimethylcadmium $Cd(CH_3)_2$ were used as precursors [166]. Since then, there are many modifications of this method were reported including the use of a single precursor diselenocarbamates and CdSe quantum dots of high crystallinity was prepared with this precursor [170]. Other ligands used for preparing quantum dots include hexadecylamine $C_{16}H_{33}NH_2$, replacing the highly toxic phosphine [171].

One of the tricky parts of the synthesis of quantum dots is the transfer of nanoparticles from the oil phase to an aqueous medium. Since the quantum dots were prepared in these "oily" ligands and they are stabilizing the quantum dots by assembling a hydrophobic shell, as-prepared quantum dots are only oil-soluble. This is particular troublesome to many biomedical applications, especially in vivo, as the reagents for such applications have to be water soluble or dispersible in aqueous phase. One relatively simple way on tackling this problem is to coat these ligand-stabilized quantum dots with amphiphilic polymers with both hydrophobic and hydrophilic segments. In such these multilayered quantum dots will have a hydrophilic surface (usually PEG or –COOH) and be readily transferable to an aqueous phase [172].

4.2 Coating the QDs

As mentioned previously, most of the materials for quantum dots are highly toxic. In order to use in biomedicines, particularly for in vivo applications, these quantum dots have to be sealed with an inert and robust coating. Because of their similarity in crystal structure, ZnO and ZnS are popular choices for coating CdSe quantum dots. In the first report on these core–shell quantum dots, a ZnS shell was coated over CdSe quantum dots [173]. It was found that such a ZnS shell not only protected the core materials from chemical reactions but also stabilized it against photobleaching.

At the beginning, precursors for preparing the ZnS shell are dimethylzinc and hexamethyldisilanthiane. However, these compounds are difficult to handle and scaling up becomes problematic. Zinc carboxylates and elemental sulfur then become the preferred precursors. Also, monomolecular precursor (both Zn and S in one compound) such as zinc xanthates and zinc dithiocarbamates are also used for coating CdSe quantum dots [174].

There are a number of methods for coating quantum dots but the two-step method (synthesis of CdSe core, purification, and coating with ZnS) is usually the preferred procedure. In general, up to five monolayers of the ZnS are coated onto the cores in order to preserve the optical property of the core material. To avoid nucleation between cores, the temperature of coating needs to be lower than that during the synthesis of cores. Slow addition, possibly aided by an automatic syringe, is required to ensure homogeneous coating. Controlling the thickness of coating is simply carried out by stoichiometric calculation of the core and shell materials. Detailed experimental procedures are widely available in the literature [175].

4.3 Surface Modification of QDs

As discussed previously, CdSe coated with ZnS or ZnO are the most commonly used quantum dots for biomedical applications due to their optical property. Therefore, we focus on the surface functionalization based on the ZnS shell. There are many different routes for functionalization but we can group them into several mechanisms according to the chemical interaction between the quantum dots and the designed functional molecules.

4.3.1 Thiols

Thiol groups are not only suitable for functionalizing gold surface, several other transition metals such as Cd, Pt, Pd and Hg also interact strongly with thiol groups. Besides, thiol also forms –S–S– interaction with the sulfur atoms on the ZnS coating. With a ZnS shell, quantum dots can be functionalized in a similar way as gold nanoparticles.

One of the early examples of using thiol as a functionalization agent for quantum dots was demonstrated by Chan et al. [176]. In this report, the thiol-carboxylic acid analogue has been used to functionalize CdSe/ZnS quantum dots (Fig. 12a). With the thiol groups strongly bound to the ZnS shell, the carboxylic acid groups become available for further functionalization, through ester or amide linkage. The carboxylic acid groups also make the quantum dots water soluble, which is essential for most of the biomedical applications. Coupling of carboxylic acids on nanoparticle surface with the amine groups on a protein molecules using coupling agents such as EDC, 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide is commonly used in binding proteins onto nanoparticles. This protocol is easily applicable to –COOH functionalized quantum dots. In fact, this method is used to manufacture streptavidin-tagged quantum dots. This material has about 20 streptavidin molecules per particle and retains its high quantum yield. With streptavidin on the surface, these quantum dots bind biotinylated species, including antibodies and proteins, and the product can be used in targeted imaging [177]. Using the same principle, alkylthiol terminated DNA has also been tagged onto CdSe/ZnS quantum dots [178].

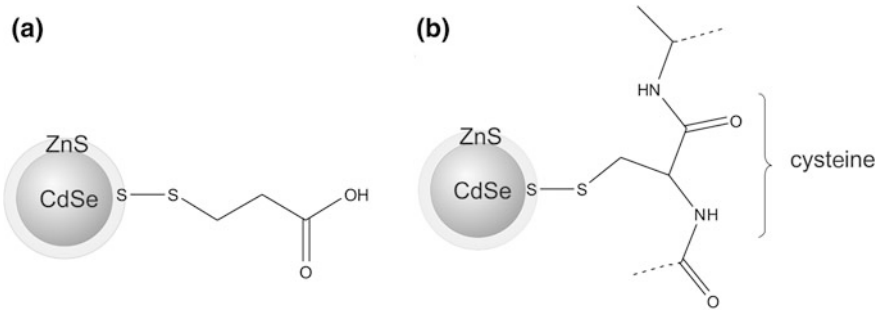

Fig. 12 Schematic showing the –S–S– interaction between a thiol group and the ZnS surface. **a** A carboxylic acid group is hence attached onto the quantum dot, and **b** a peptide with a cysteine unit in the amino acid sequence can bind to a ZnS-coated quantum dot with the same interaction

Similar to gold, ZnS-coated quantum dots has strong affinity to cysteine due to its –SH group. Therefore, these quantum dots can be functionalized by binding peptides with cysteine in the amino acid sequence. Logically, the more cystine units are in the sequence, the stronger its interaction to the ZnS coating (Fig. 12b). For example, Pinaud et al. used Biotin-G**C**E**C**GG**C**E**C**G-Cha-C_2H_4-Cha-Cmd (**C** = cysteine, Cha = 3-cyclohexylalamine, Cmd = carboxamide) and other similar sequences to functionalize quantum dots for efficiently binding to streptavidin and are specifically targeted to GPI-anchored avidin-CD14 chimeric proteins expressed on the membranes of live HeLa cells [179].

4.3.2 Phosphine and Phosphine Oxide

The success of preparation of CdSe quantum dots was due to the discovery of strong interactions of Cd–O=P and Se–P. These specific interactions can also be used in surface functionalization. However, it would still be better to use a ZnS-coated CdSe in order to minimize any concern about toxicity of CdSe. Fortunately, the equivalent Zn–O=P and S–P interactions are just as strong and exploitable for surface functionalization (see Fig. 13). Oligomeric phosphines with carboxylic acids on branches have been used to functionalize CdSe/ZnS quantum dots and the material was used for binding streptavidin and these quantum dots were shown to be able to conjugate with biotinlyated quantum dots [180]. However, the main concern regarding use of phosphine or phosphine oxide to functionalize quantum dots is that these are toxic chemical and leaching can cause serious health issues. Unlike thiol, there is no natural analogue to substitutes for either phosphine or phosphine oxide.

4.3.3 Histidine

Histidine is an amino acid with an imidazole group. When two histidines adjacent to each other in a peptide sequence, a strongly chelating site to transition metals

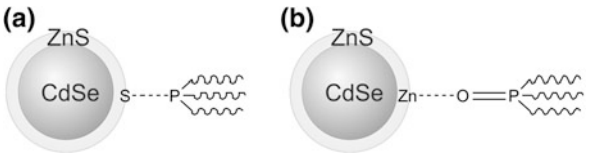

Fig. 13 Interaction between ZnS-coated quantum dots and **a** phosphine, or **b** phosphine oxide ligands

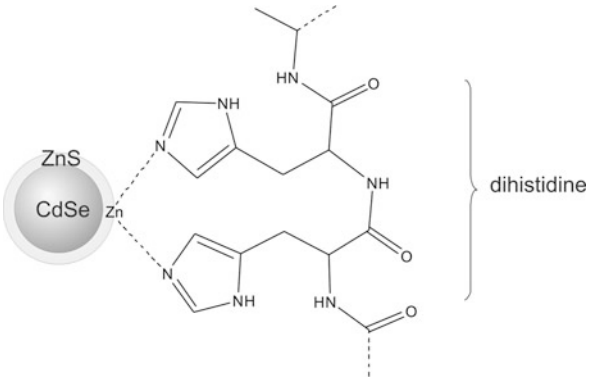

Fig. 14 Binding of dihistidine to Zn on ZnS-coated quantum dots

is created. Such chemistry has been exploited in biochemistry. For example, supported Ni^{2+}–NTA groups (a Ni organometallic complex) have been wildly used to chelate dihistidine unit of peptides as such interaction is measured to be a lot stronger than that of antibody binding [181]. Dihistidine has been found to chelate several transition metals strongly, including Ni, Zn, Fe, Cu, Co, and Mn. As a result, peptides with dihistidine group can be used to functionalize the CdSe/ZnS quantum dots by binding directly onto the ZnS shell (see Fig. 14).

Despite their harmless nature, use of dihistine to functionalize is still relatively rare. This is because not many natural peptides have a dihistidine unit in their sequence and many of these dihistidine ligands are synthetic or genetically engineered. As a result, the cost of using dihistidine ligands is high. Another difficulty of using dihistidine ligands for functionalizing quantum dots is the drawback of their own harmless nature. Because these ligands are mainly peptides, it is difficult to bind functional units other than amino acids or peptides onto the ligands. For example, binding a carbohydrate molecule onto a dihistidine ligand may not be straightforward.

4.3.4 Aliphatic Chains

Use of aliphatic chains to functionalize quantum dots is different from using other ligands because there is no direct interaction with these ligands to the quantum dot surface. Instead, they interact with the phosphine/phosphine oxide shell with strong hydrophobic interaction. And because of such hydrophobic interaction, unlike other mechanism, this functionalization retained the phosphine/phosphine oxide shell. In other words, this is an indirect method to functionalize quantum dots. The advantage about this strategy is that a hydrophobic shell is self-assembled round

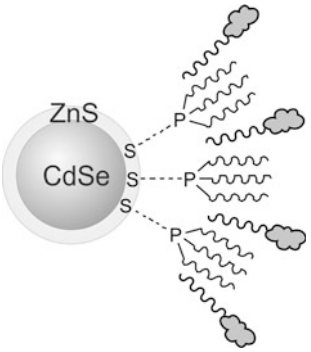

Fig. 15 Self-assembly of functional molecules with a hydrophobic, aliphatic tail onto the phosphine shell of quantum dots

the quantum dot and this can serve as an extra protecting layer against any hydrophilic reactants (see Fig. 15).

Several examples have been reported using this functionalization mechanism. For example, modified acrylic acid polymer has been coated onto quantum dot surface by hydrophobic interaction with the TOP/TOPO shell [182]. These polymer-coated particles have been used in imaging in vivo [183]. Other examples using this mechanism include quantum dots coated with amphiphilic triblock copolymer [184] and amphiphilic saccharides [185].

4.4 Medical Applications of Surface Functionalized QDs

4.4.1 Bioassays Using QDs

Bioassays using labeled quantum dots can detect specific DNA, proteins and other biomolecules. For example, DNA-labeled quantum dots have been used as probes for human chromosomes, single-nucleotide and multi-allele DNA detections [186]. Usually, this is carried out using a specific single-stranded DNA labeled quantum dots for binding their complement DNA molecules. Because of their high quantum yield, these DNA-labeled quantum dots show a high sensitivity compared with fluorescent molecules.

In addition to DNA, antibody-tagged quantum dots have been used to detect specific proteins. The principle is similar to those detecting DNA. A multi-colored system using antibody-tagged quantum dots have shown to be able to analyze biomolecules including toxins [187]. Because these bioassays are all ex vivo analysis, there is no problem associated with the toxicity of quantum dots.

4.4.2 Cell Labeling Using QDs

Recently, rapid development has been seen on using quantum dots to label cells, thanks to the improvement in controlling their cytotoxicity and internalizing into cells. Use of quantum dots for cell labeling has experienced significant

advancement in the past few years. One of the major areas of development was on a multicolor labeling system for cells [188]. The advantages of using quantum dots over traditional fluorophores have been discussed earlier.

Quantum dots can be used to label both live and fixed cells. Labeling fixed cells are relatively straightforward because these cells are dead and can withstand rather harsh conditions. To label live cells, a certain amount of functionalized quantum dots are needed to be internalized into the cells unless the specific receptors are on the cell membrane. There are several strategies for the internalization of quantum dots into cells for internal labeling.

1. *Endocytosis*. Functionalized quantum dots are being taken up by the cells non-specifically through endocytosis and the quantum dots are normally localized in the cytoplasm initially.
2. *Transfection*. Similar to endocytosis, functionalized quantum dots are being non-specifically internalized but with the assistance of a transfecting reagent, e.g. DOTAP or lipofectamine. These reagents are normally used for gene delivery but can be used for transporting other negatively charged species. A much higher uptake efficiency is usually observed using commercial transfecting reagent than uptake through normal endocytosis.
3. *Microinjection*. Functionalized quantum dots are being injected into the cells using micro-needles. This method only allows a small number of cells to be labeled.
4. *Electroporation*. In this method, functionalized quantum dots are delivered into the cells using charge. By applying an external electrical field, the electrical conductivity of the cell membrane is increased, hence its permeability. Quantum dots can therefore be internalized easily. This method has also been used in gene delivery.

After internalization, these functionalized quantum dots need to find their way to the target compartments of the cell. Usually specific peptides or antibody will be tagged onto these quantum dots for this purpose. There are many examples of using targeting peptides and antibody for labeling particles. For example, Wu et al. tagged monoclonal anti-Her2 antibody onto CdSe/ZnS core–shell QDs (both green QD-535 and red QD-630) and successfully labeled the Her2 receptors on the surface of breast cancer SK-BR-3 cells [189]. In contrast, unlabelled quantum dots were scattered around the cells.

4.4.3 In Vivo Imaging Using QDs

For in vivo tissue imaging, quantum dots emitting near-infrared (NIR) radiations (e.g. 840 nm) have been used to map lymph nodes for cancer surgery in an animal model [190]. Use of NIR quantum dots can avoid the problem of auto-fluorescence from tissues. Compared with the use of NIR dyes, emissions from quantum dots can be detected from deeper locations. For example, lymph nodes in 1 cm deep in tissue have been imaged with the associated lymphatic vessels clearly seen.

Moreover, the fluorescence of quantum dots in tissues can still be detectable in vivo after 4 months. This provides an advantageous possibility for using quantum dots for a long-term experiments or monitoring. Such observation can also be used in tumor imaging as tumors normally have a poor lymphatic drainage system and quantum dots tend to accumulate inside.

Tumor imaging can also be achieved by active targeting using quantum dots tagged with specific antibodies. For example, prostate-specific membrane antigen (PSMA) has been tagged onto quantum dots as a marker for diagnosis of prostate cancer in a mouse model [191].

The major drawback in using quantum dots for in vivo diagnosis is, without a doubt, their toxicity. As discussed earlier, scientists have been making progress in this area by coating the CdSe core with less toxic ZnS shell [192]. However, this is still not enough to prevent the release of highly toxic Cd^{2+} ions into the body. In particular, these quantum dots are highly toxic to cells under UV radiation for an extended period of time because Cd^{2+} ions were thought to be released during photolysis under UV. Unfortunately, many of the in vivo imaging experiments using quantum dots require UV or visible radiation of high energy for excitation. Therefore, there is an urgent need for a robust coating for quantum dots. Many studies showed polymer-coated quantum dots are non-toxic in vitro. Nonetheless, these are only short-term studies on cultured cell lines. The long-term cytotoxicity effect of these polymer-coated quantum dots is still to be tested in vivo. Under an extended time period, the polymer coating is likely to disintegrate due to enzymatic digestion and the cores of quantum dots become exposed to the body fluid. It is still unknown if such an effect can be eliminated completely. As a result, use of quantum dots in vivo still needs detailed studies on their long-term effects in animal bodies.

5 Bimodal Core–Shell Nanoparticles

There are a lot of concerns about the accuracy of diagnosis using one single imaging technique. Hence, a combination of more than one imaging techniques was developed in order to enhance the resolution of scans. For example, the combined CT–MRI scans have been regarded as a diagnostic technique with much improved results. Whilst physicists and engineers are trying to develop the latest machines for combined imaging and mathematicians are working out the digital merging of these images, chemists are given the task to assemble a single contrast reagent for this combined technique. Unfortunately, there is no such a universal compound as a contrast agent for multiple imaging. As a result, scientists have been working hard in developing bimodal contrast agents. A core/shell nanostructure of two compounds would be the ideal materials for this task.

For example, gold-coated iron oxide nanoparticles can be used as a contrast agent for a combined CT–MRI scan [193]. With the gold coating, simple modification using a thiolated compound, such as thiolated PEG, can be carried out for

biocompatibility and biospecificity. However, a homogeneous gold coating on iron oxide particles is still difficult to achieve. Silver is another possible coating material and has some similar chemical property as gold. Silver-coated iron oxide nanoparticles has also been developed recently [194] but their biomedical applications are still to be explored. Platinum, despite its high cost, is another possible coating material for such use.

On the other hand, bimodal core–shell nanoparticles with quantum dots are rear. This is mainly because the fluorescence property of quantum dots is sensitive to coatings. Therefore, it is likely that the semiconducting materials are to be the coating rather than the core. In spite of this limitation, gold–CdS nanocomposite material has been reported [195]. This material was constructed using silica-coated CdS quantum dots coupling with Au nanoparticles. Again, their applications are not yet studied. Recently, a layer-by-layer (LbL) method was published for preparing iron oxide nanoparticles coated with Au nanoparticles or CdTe quantum dots [196]. A silica layer is also used as an intermediate for coupling these materials together. Nonetheless, in-depth investigation on their medical applications are still needed, particularly their cytotoxicity.

6 Future Developments

Development of inorganic nanoparticles with delicate structures has experienced significant advancement in the past decade. Nowadays, nanoparticles of a wide range of sizes can be prepared and sometimes at a large scale. Preparation of bimodal and multimodal nanoparticles is still a challenge but, for medical applications, the major concerns are on their localization and their specification. Although we have seen many reports on nanoparticles targeting certain cell types with antibodies or specific ligands, many of these research works are still carried out in vitro or ex vivo. In vivo studies are still limited and less successful. This is due to the complexity of the cell systems. A precise, robust, reliable targeting system is still to be developed.

In addition, multifunctional nanoparticles are also very much in the agenda. A small body capable of multi-tasking, including imaging, diagnosis, and therapy, will be the holy grail for the medical community. So far, magnetic nanoparticles with drug storage and delivery capability have been reported and the delivery of anticancer agents has also been tested [197]. Another area to be developed is a multiple targeting system for the precise delivery of drugs. A designed nanoporous iron oxide–silica composite material tagged with antibody has been developed for drug storage and delivery [198]. Such a complex material opens up a research direction for magnetic-antibody dual targeting drug delivery. The iron oxide particles in the composite can also act as a contrast agent for MRI scans. Multiple targeting systems for localization of particles with a high accuracy may well be available soon. Clinical trials of these complex nanomaterials are yet to be carried out but an entirely new therapeutic system based on nanoparticles may be commonly used in medicine in a few decades time.

References

1. Hutchings, G.: A golden future. Nat. Chem. **1**, 584 (2009)
2. Hutchings, G.J., Brust, M., Schmidbaur, H.: Gold—an introductory perspective. Chem. Soc. Rev. **37**, 1759–1765 (2008)
3. Haruta, M.: When gold is not noble: catalysis by nanoparticles. Chem. Rec. (N.Y.) **3**, 75–87 (2003)
4. Sinha, A.K., Seelan, S., Tsubota, S., Haruta, M.: A three-dimensional mesoporous titanosilicate support for gold nanoparticles: vapor–phase epoxidation of propene with high conversion. Angew. Chem. Int. Ed. **43**, 1546–1548 (2004)
5. Daté, M., Okumura, M., Tsubota, S., Haruta, M.: Vital role of moisture in the catalytic activity of supported gold nanoparticles. Angew. Chem. Int. Ed. **43**, 2129–2132 (2004)
6. Haruta, M.: Catalysis: gold rush. Nature **437**, 1098–1099 (2005)
7. Hashmi, A.S.K., Rudolph, M.: Gold catalysis in total synthesis. Chem. Soc. Rev. **37**, 1766–1775 (2008)
8. Marion, N., Nolan, S.P.: N-Heterocyclic carbenes in gold catalysis. Chem. Soc. Rev. **37**, 1776–1782 (2008)
9. Della Pina, C., Falletta, E., Prati, L., Rossi, M.: Selective oxidation using gold. Chem. Soc. Rev. **37**, 2077–2095 (2008)
10. Corma, A., Garcia, H.: Supported gold nanoparticles as catalysts for organic reactions. Chem. Soc. Rev. **37**, 2096–2126 (2008)
11. De, M., Ghosh, P.S., Rotello, V.M.: Applications of nanoparticles in biology. Adv. Mater. **20**, 4225–4241 (2008)
12. Sperling, R.A., Rivera Gil, P., Zhang, F., Zanella, M., Parak, W.J.: Biological applications of gold nanoparticles. Chem. Soc. Rev. **37**, 1896–1908 (2008)
13. Connor, E.E., Mwamuka, J., Gole, A., Murphy, C.J., Wyatt, M.D.: Gold nanoparticles are taken up by human cells but do not cause acute cytotoxicity. Small **1**, 325–327 (2005)
14. Brust, M., Walker, M., Bethell, D., Schiffrin, D.J., Whyman, R.J.: Synthesis of thiol-derivatised gold nanoparticles in a two-phase liquid–liquid system. J. Chem. Soc. Chem. Commun. 801–802 (1994)
15. Hostetler, M.J., Wingate, J.E., Zhong, C.-J., Harris, J.E., Vachet, R.W., Clark, M.R., Londono, J.D., Green, S.J., Stokes, J.J., Wignall, G.D., Glish, G.L., Porter, M.D., Evans, N.D., Murray, R.W.: Alkanethiolate gold cluster molecules with core diameters from 1.5 to 5.2 nm: core and monolayer properties as a function of core size. Langmuir **14**, 17–30 (1998)
16. Sardar, R., Funston, A.M., Mulvenay, P., Murray, R.W.: Gold nanopartciles: past, present, and future. Langmuir **25**, 13840–13851 (2009)
17. Scott, R.W.J., Wilson, O.M., Crooks, R.M.: Synthesis, characterization, and applications of dendrimer-encapsulated nanoparticles. J. Phys. Chem. B **109**, 692–704 (2005)
18. Zheng, J., Petty, J.T., Dickson, R.M.: High quantum yield blue emission from water-soluble Au8 nanodots. J. Am. Chem. Soc. **125**, 7780–7781 (2003)
19. Giersig, M., Mulvaney, P.: Preparation of ordered colloid monolayers by electrophoretic deposition. Langmuir **9**, 3408–3413 (1993)
20. Murray, R.W.: Nanoelectrochemistry: metal nanoparticles, nanoelectrodes, and nanopores. Chem. Rev. **108**, 2688–2720 (2008)
21. Decher, G.: Fuzzy nanoassemblies: toward layered polymeric multicomposites. Science **277**, 1232–1237 (1997)
22. DeVries, G.A., Brunnbauer, M., Hu, Y., Jackson, A.M., Long, B., Neltner, B.T., Uzun, O., Wunsch, B.H., Stellacci, F.: Divalent metal nanoparticles. Science **315**, 358–361 (2007)
23. Templeton, A.C., Wuelfing, W.P., Murray, R.W.: Monolayer-protected cluster molecules. Acc. Chem. Res. **33**, 27–36 (2000)
24. Daniel, M.C., Astruc, D.: Gold nanoparticles: Assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. Chem. Rev. **104**, 293–346 (2004)

25. Mirkin, C.A., Letsinger, R.L., Mucic, R.C., Storhoff, J.J.: A DNA-based method for rationally assembling nanoparticles into macroscopic materials. Nature **382**, 607–609 (1996)
26. Rosi, N.L., Mirkin, C.A.: Nanostructures in biodiagnostics. Chem. Rev. **105**, 1547–1562 (2005)
27. Kankate, L., Turchanin, A., Gölzhäuser, A.: On the release of hydrogen from the S–H groups in the formation of self-assembled monolayers of thiols. Langmuir **25**, 10435–10438 (2009)
28. Guo, R., Song, Y., Wang, G., Murray, R.W.: Does core size matter in the kinetics of ligand exchanges of monolayer-protected Au clusters? J. Am. Chem. Soc. **127**, 2752–2757 (2005)
29. Kassam, A., Bremner, G., Clark, B., Ulibarri, G., Lennox, R.B.: Place exchange reactions of alkyl thiols on gold nanoparticles. J. Am. Chem. Soc. **128**, 3476–3477 (2006)
30. Huang, X.H., Jain, P.K., El-Sayed, I.H., El-Sayed, M.A.: Gold nanoparticles: Interesting optical properties and recent applications in cancer diagnostics and therapy. Nanomedicine **2**, 681–693 (2007)
31. Sonnichsen, C., Franzl, T., Wilk, T., von Plessen, G., Feldmann, J.: Plasmon resonances in large noble–metal clusters. New J. Phys. **4**, 93.1–93.8 (2002)
32. Tkachenko, A.G., Xie, H., Liu, Y., Coleman, D., Ryan, J., Glomm, W.R., Shipton, M.K., Franzen, S., Feldheim, D.L.: Cellular trajectories of peptide-modified gold particle complexes: comparison of nuclear localization signals and peptide transduction domains. Bioconjug. Chem. **15**, 482–490 (2004)
33. Souza, G.R., Christianson, D.R., Staquicini, F.I., Ozawa, M.G., Snyder, E.Y., Sidman, R.L., Miller, J.H., Arap, W., Pasqualini, R.: Networks of gold nanoparticles and bacteriophage as biological sensors and cell-targeting agents. Proc. Natl. Acad. Sci. U.S.A. **103**, 1215–1220 (2006)
34. Boyer, D., Tamarat, P., Maali, A., Lounis, B., Orrit, M.: Photothermal imaging of nanometer-sized metal particles among scatterers. Science **297**, 1160–1163 (2002)
35. Lasne, D., Blab, G.A., Berciaud, S., Heine, M., Groc, L., Choquet, D., Cognet, L., Lounis, B.: Single nanoparticle photothermal tracking (SNaPT) of 5-nm gold beads in live cells. Biophys. J. **91**, 4598–4604 (2006)
36. Wang, G.L., Huang, T., Murray, R.W., Menard, L., Nuzzo, R.G.: Near-IR luminescence of monolayer-protected metal clusters. J. Am. Chem. Soc. **127**, 812–813 (2005)
37. Cang, H., Wong, C.M., Xu, C.S., Rizvi, A.H., Yang, H.: Confocal three dimensional tracking of a single nanoparticle with concurrent spectroscopic readouts. Appl. Phys. Lett. **88**, 223901 (2006)
38. Kim, D., Park, S., Lee, J.H., Jeong, Y.Y., Jon, S.: Antibiofouling polymer-coated gold nanoparticles as a contrast agent for in vivo X-ray computed tomography imaging. J. Am. Chem. Soc. **129**, 7661–7665 (2007)
39. Garanger, E., Boturyn, D., Jin, Z., Dumy, P., Favrot, M.-C., Coll, J.-L.: New multifunctional molecular conjugate vector for targeting, imaging, and therapy of tumors. Mol. Ther. **12**, 1168–1175 (2005)
40. Foillard, S., Jin, Z.-h., Garanger, E., Boturyn, D., Favrot, M.-C., Coll, J.-L., Dumy, P.: Synthesis and biological characterisation of targeted pro-apoptotic peptide. ChemBioChem **9**, 2326–2332 (2008)
41. Huff, T.B., Tong, L., Zhao, Y., Hansen, M.N., Cheng, J.X., Wei, A.: Hyperthermic effects of gold nanoreods on tumor cells. Nanomedicine **2**, 125–132 (2007)
42. Chen, J., Wang, D., Xi, J., Au, L., Siekkinen, A., Warsen, A., Li, Z.Y., Zhang, H., Xia, Y., Li, X.: Immuno gold nanocages with tailored optical properties for targeted photothermal destruction of cancer cells. Nano Lett. **7**, 1318–1322 (2007)
43. Kogan, M.J., Bastus, N.G., Amigo, R., Grillo-Bosch, D., Arraya, E., Turiel, A., Labarta, A., Giralt, E., Puntes, V.F.: Nanoparticle-mediated local and remote manipulation of protein aggregation. Nano Lett. **6**, 110–115 (2006)
44. Hamad-Schifferli, K., Schwartz, J.J., Santos, A.T., Zhang, S., Jacobson, J.M.: Remote electronic control of DNA hybridization through inductive coupling to an attached metal nanocrystal antenna. Nature **415**, 152–155 (2002)

45. Stehr, J., Hrelescu, C., Sperling, R.A., Raschke, G., Wunderlich, M., Nichtl, A., Heindl, D., Kürzinger, K., Parak, W.J., Klar, T.A., Feldmann, J.: Gold nanostoves for microsecond DNA melting analysis. Nano Lett. **8**, 619–623 (2008)
46. Angelatos, A.S., Radt, B., Caruso, F.: Light-responsive polyelectrolyte/gold nanoparticle microcapsules. J. Phys. Chem. B **109**, 3071–3076 (2005)
47. Skirtach, A.G., Javier, A.M., Kreft, O., Köhler, K., Alberola, A.P., Möhwald, H., Parak, W.J., Sukhorukov, G.B.: Laser-induced release of encapsulated materials inside living cells. Angew. Chem. Int. Ed. **45**, 4612–4617 (2006)
48. Mocellin, S., Bronte, V., Nitti, D.: Nitric oxide, a double edged sword in cancer biology: searching for therapeutic opportunities. Med. Res. Rev. **27**, 317–352 (2007)
49. Hone, D.C., Walker, P.I., Evans-Gowing, R., FitzGerald, S., Beeby, A., Chambrier, I., Cook, M.J., Russell, D.A.: Generation of cytotoxic singlet oxygen via phthalocyanine stabilized gold nanoparticles: a potential delivery vehicle for photodynamic therapy. Langmuir **18**, 2985–2987 (2002)
50. Polizzi, M.A., Stasko, N.A., Schoenfisch, M.H.: Water-soluble nitric oxide-releasing gold nanoparticles. Langmuir **23**, 4938–4943 (2007)
51. Gibson, J.D., Khanal, B.P., Zubarev, E.R.: Paclitaxel-functionalized gold nanoparticles. J. Am. Chem. Soc. **129**, 11653–11661 (2007)
52. Meister, A., Anderson, M.E.: Glutathione. Annu. Rev. Biochem. **52**, 711–760 (1983)
53. Saito, G., Swanson, J.A., Lee, K.D.: Drug delivery strategy utilizing conjugation via reversible disulfide linkages: role and site of cellular reducing activities. Adv. Drug Deliv. Rev. **55**, 199–215 (2003)
54. Hong, R., Han, G., Fernandez, J.M., Kim, B.J., Forbes, N.S., Rotello, V.M.: Glutathione-mediated delivery and release using monolayer protected nanoparticle carriers. J. Am. Chem. Soc. **128**, 1078–1079 (2006)
55. Check, E.: Gene therapy: a tragic setback. Nature **420**, 116–118 (2002)
56. Thomas, M., Klibanov, A.M.: Conjugation to gold nanoparticles enhances polyethylenimine's transfer of plasmid DNA into mammalian cells. Proc. Natl. Acad. Sci. U.S.A. **100**, 9138–9143 (2003)
57. Thomas, M., Klibanov, A.M.: Non-viral gene therapy: polycation-mediated DNA delivery. Appl. Microbiol. Biotechnol. **62**, 27–34 (2003)
58. Sandhu, K.K., McIntosh, C.M., Simard, J.M., Smith, S.W., Rotello, V.M.: Gold nanoparticle-mediated transfection of mammalian cells. Bioconjug. Chem **13**, 3–6 (2002)
59. Han, G., You, C.C., Kim, B.J., Turingan, R.S., Forbes, N.S., Martin, C.T., Rotello, V.M.: Light-regulated release of DNA and its delivery to nuclei by means of photolabile gold nanoparticles. Angew. Chem. Int. Ed. **45**, 3165–3169 (2006)
60. Rosi, N.L., Giljohann, D.A., Thaxton, C.S., Lytton-Jean, A.K.R., Han, M.S., Mirkin, C.A.: Oligonucleotide-modified gold nanoparticles for intracellular gene regulation, Science **312**, 1027–1030 (2006)
61. Giljohann, D.A., Seferos, D.S., Patel, P.C., Millstone, J.E., Rosi, N.L., Mirkin, C.A.: Oligonucleotide loading determines cellular uptake of DNA-modified gold nanoparticles. Nano Lett. **7**, 3818–3821 (2007)
62. Bhumkar, D.R., Joshi, H.M., Sastry, M., Pokharkar, V.B.: Chitosan reduced gold nanoparticles as novel carriers for transmucosal delivery of insulin. Pharm. Res. **24**, 1415–1426 (2007)
63. Brannon-Peppas, L., Blanchette, J.O.: Nanoparticle and targeted systems for cancer therapy. Adv. Drug Deliv. Rev. **56**, 1649–1659 (2004)
64. Brigger, I., Dubernet, C., Couvreur, P.: Nanoparticles in cancer therapy and diagnosis. Adv. Drug Deliv. Rev. **54**, 631–651 (2002)
65. Ghosh, P., Han, G., De, M., Kim, C.K., Rotello, V.M.: Gold nanoparticles in delivery applications. Adv. Drug Deliv. Rev. **60**, 1307–1315 (2008)
66. Chithrani, B.D., Ghazani, A.A., Chan, W.C.W.: Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. Nano Lett. **6**, 662–668 (2006)

67. Chithrani, B.D., Chan, W.C.W.: Elucidating the mechanism of cellular uptake and removal of protein-coated gold nanoparticles of different sizes and shapes. Nano Lett. **7**, 1542–1550 (2007)
68. Mohanty, S.P., Kougianos, E.: Biosensors: a tutorial review. IEEE Potentials **25**, 35–40 (2006)
69. Raschke, G., Kowarik, S., Franzl, T., Sonnichsen, C., Klar, T.A., Feldmann, J., Nichtl, A., Kurzinger, K.: Biomolecular recognition based on single gold nanoparticle light scattering. Nano Lett. **3**, 935–938 (2003)
70. Jain, P.K., Eustis, S., El-Sayed, M.A.: Plasmon coupling in nanorod assemblies: optical absorption, discrete dipole approximation simulation, and exciton–coupling model. J. Phys. Chem. B **110**, 18243–18253 (2006)
71. Elghanian, R., Storhoff, J.J., Mucic, R.C., Letsinger, R.L., Mirkin, C.A.: Selective colorimetric detection of polynucleotides based on the distance-dependent optical properties of gold nanoparticles. Science **277**, 1078–1081 (1997)
72. Nam, J.M., Stoeva, S.I., Mirkin, C.A.: Bio-bar-code-based DNA detection with PCR-like sensitivity. J. Am. Chem. Soc. **126**, 5932–5933 (2004)
73. Wang, Z., Lévy, R., Fernig, D.G., Brust, M.: Kinase-catalyzed modification of gold nanoparticles: a new approach to colorimetric kinase activity screening. J. Am. Chem. Soc. **128**, 2214–2215 (2006)
74. Wang, Z., Lee, J., Cossins, A.R., Brust, M.: Microarray-based detection of protein binding and functionality by gold nanoparticle probes. Anal. Chem. **77**, 5770–5774 (2005)
75. Liu, J., Lu, Y.: Colorimetric Cu^{2+} detection with a ligation DNAzyme and nanoparticles. Chem. Commun. 4872–4874 (2007)
76. Zhao, W., Chiuman, W., Lam, J.C.F., McManus, S.A., Chen, W., Cui, Y., Pelton, R., Brook, M.A., Li, Y.: DNA aptamer folding on gold nanoparticles: from colloid chemistry to biosensors. J. Am. Chem. Soc. **130**, 3610–3618 (2008)
77. Campion, A., Kambhampati, P.: Surface-enhanced Raman scattering. Chem. Soc. Rev. **27**, 241–250 (1998)
78. Kneipp, K., Kneipp, H., Kneipp, J.: Surface-enhanced Raman scattering in local optical fields of silver and gold nanoaggregates—from single-molecule Raman spectroscopy to ultrasensitive probing in live cells. Acc. Chem. Res. **39**, 443–450 (2006)
79. Nie, S., Emory, S.R.: Probing single molecules and single nanoparticles by surface-enhanced Raman scattering. Science **275**, 1102–1106 (1997)
80. Krug, J.T., Wang, G.D., Emory, S.R., Nie, S.: Efficient Raman enhancement and intermittent light emission observed in single gold nanocrystals. J. Am. Chem. Soc. **121**, 9208–9214 (1999)
81. Cao, Y.C., Jin, R., Mirkin, C.A.: Nanoparticles with Raman spectroscopic fingerprints for DNA and RNA detection. Science **297**, 1536–1540 (2002)
82. Kneipp, J., Kneipp, H., Kneipp, K.: Two-photon vibrational spectroscopy for biosciences based on surface-enhanced hyper-Raman scattering. Proc. Natl. Acad. Sci. U.S.A. **103**, 17149–17153 (2006)
83. Dulkeith, E., Morteani, A.C., Niedereichholz, T., Klar, T.A., Feldmann, J., Levi, S.A., van Veggel, F.C.J.M., Reinhoudt, D.N., Möller, M., Gittins, D.I.: Fluorescence quenching of dye molecules near gold nanoparticles: radiative and nonradiative effects. Phys. Rev. Lett. **89**, 203002-1–203002-4 (2002)
84. McGhee, J.D., von Hippel, P.H.: Theoretical aspects of DNA-protein interactions: co-operative and non-co-operative binding of large ligands to a one-dimensional homogeneous lattice. J. Mol. Biol. **86**, 469–489 [*Erratum* (1976) J. Mol. Biol. **103**, 679] (1974)
85. Oh, E., Hong, M.-H., Lee, D., Nam, S.-H., Yoon, H.C., Kim, H.-S.: Inhibition assay of biomolecules based on fluorescence resonance energy transfer (FRET) between quantum dots and gold nanoparticles. J. Am. Chem. Soc. **127**, 3270–3271 (2005)
86. Dulkeith, E., Ringler, M., Klar, T.A., Feldmann, J., Javier, A.M., Parak, W.J.: Gold nanoparticles quench fluorescence by phase induced radiative rate suppression. Nano Lett. **5**, 585–589 (2005)

87. Wang, Z., Lévy, R., Fernig, D.G., Brust, M.: The Peptide route to multifunctional gold nanoparticles. Bioconjug. Chem **16**, 497–500 (2005)
88. Wang, J.: Nanoparticle-based electrochemical bioassays of proteins. Electroanalysis **19**, 769–776 (2007)
89. Park, S.-J., Taton, T.A., Mirkin, C.A.: Array-based electrical detection of DNA with nanoparticle probes. Science **282**, 1503–1506 (2002)
90. Xiao, Y., Patolsky, F., Katz, E., Hainfeld, J.F., Willner, I.: "Plugging into enzymes": nanowiring of redox enzymes by a gold nanoparticle. Science **299**, 1877–1881 (2003)
91. Yu, A., Liang, Z., Cho, J., Caruso, F.: Nanostructured electrochemical sensor based on dense gold nanoparticle films. Nano Lett. **3**, 1203–1207 (2003)
92. Holzinger, M., Bouffier, L., Villalonga, R., Cosnier, S.: Adamantane/β-cyclodextrin affinity biosensors based on single-walled carbon nanotubes. Biosens. Bioelectron. **24**, 1128–1134 (2009)
93. Sun, S., Murray, C.B., Weller, D., Folks, L., Moser, A.: Monodisperse FePt nanoparticles and ferromagnetic FePt nanocrystal superlattices. Science **287**, 1989–1992 (2000)
94. Yi, D.K., Lee, S.S., Ying, J.Y.: Synthesis and applications of magnetic nanocomposite catalysts. Chem. Mater. **18**, 2459–2461 (2006)
95. Miller, M.M., Prinz, G.A. Cheng, S.F., Bounnak, S.: Detection of a micron-sized magnetic sphere using a ring-shaped anisotropic magnetoresistance-based sensor: a model for a magnetoresistance-based biosensor. Appl. Phys. Lett. **81**, 2211–2213 (2002)
96. Jain, T.K., Morales, M.A., Sahoo, S.K., Leslie-Pelecky, D.L., Labhasetwar, V.: Iron oxide nanoparticles for sustained delivery of anticancer agents. Mol. Pharm. **2**, 194–205 (2005)
97. Chourpa, I., Douziech, E.L., Ngaboni-Okassa, L., Fouquenet, J.F., Cohen, J.S., Souce, M., Marchais, H., Dubois, P.: Molecular composition of iron oxide nanoparticles, precursors for magnetic drug targeting, as characterized by confocal Raman microspectroscopy. Analyst **130**, 1395–1403 (2005)
98. Weissleder, R., Bogdanov, A., Neuwelt, E.A., Papisov, M.: Long-circulating iron oxides for MR imaging. Adv. Drug Deliv. Rev. **16**, 321–334 (1995)
99. Stella, B., Arpicco, S., Peracchia, M.T., Desmaele, D., Hoebeke, J., Renoir, M., D'Angelo, J., Cattel, L., Couvreur, P.: Design of folic acid conjugated nanoparticles for drug targeting. J. Pharm. Sci. **89**, 1452–1464 (2000)
100. Sun, Y.K., Ma, M., Zhang, Y., Gu, N.: Synthesis of nanometer-size maghemite particles from magnetite. Colloids Surf. A **245**, 15–19 (2004)
101. Lee, S.J., Jeong, J.R., Shin, S.C., Kim, J.C., Kim, J.D.: Synthesis and characterization of superparamagnetic maghemite nanoparticles prepared by coprecipitation technique. J. Magn. Magn. Mat. **282**, 147–150 (2004)
102. Park, J., Lee, E., Hwang, N.M., Kang, M., Kim, S.K., Hwang, S., Park, J.-G., Noh, H.-J., Kim, J.-Y., Park, J.-H., Hyeon, T.: One-nanometer scale size-controlled synthesis of monodisperse magetic iron oxide nanoparticles. Angew. Chem. Int. Ed. **44**, 2872–2877 (2005)
103. Sun, S., Zeng, H., Robinson, D.B., Raoux, S., Rice, P.M., Wang, S.X., Li, G.: Monodisperse MFe_2O_4 (M = Fe, Co, Mn) nanoparticles. J. Am. Chem. Soc. **126**, 173–279 (2004)
104. Cai, W., Wan, J.: Facile synthesis of superparamagnetic magnetite nanoparticles in liquid polyols. J. Coll. Inter. Sci. **305**, 366–370 (2007)
105. Charles, S.W., Popplewell, J.: Properties and applications of magnetic liquids. Endeavour **6**, 153–61 (1982)
106. Jolivet, J.P., Chaneac, C., Tronc, E.: Iron oxide chemistry. From molecular clusters to extended solid networks. Chem. Commun. **5**, 481–487 (2004)
107. LaMer, V.K., Dinegar, R.H.: Theory, production and mechanism of formation of monodispersed hydrosols. J. Am. Chem. Soc. **72**, 4847–4854 (1950)
108. Tartaj, P., Morales, M.P., Veintemillas-Verdaguer, S., Gonzales-Carreno, T., Serna, C.J.: The preparation of magnetic nanoparticles for applications in biomedicine. J. Phys. D **36**, R182–R197 (3003)

109. Babes, L., Denizot, B., Tanguy, G., Le Jeune, J.J., Jallet, P.: Synthesis of iron oxide nanoparticles used as mri contrast agents: a parametric study. J. Colloid Interface Sci. **212**, 474–482 (1999)
110. Massart, R., Cabuil, V.: Monodisperse magentic nanoparticles: preparation and dispersion in water and oils. J. Chim. Phys. **84**, 2975–2981 (1987)
111. Bee, A., Massart, R., Neveu, S.: Synthesis of very fine maghemite particles. J. Magn. Magn. Mater. **149**, 6–9 (1995)
112. Fauconnier, N., Pons, J.N., Roger, J., Bee, A.: Thiolation of maghemite nanoparticles by dimercaptosuccinic acid. J. Colloid Interface Sci. **194**, 427–433 (1997)
113. Liu, X., Xing, J., Guan, Y., Shan, G., Liu, H.: Synthesis of amino-silane modified superparamagnetic silica supports and their use for protein immobilization. Colloids Surf. A **238**, 127–131 (2004)
114. Heath, J.R.: The chemistry of size and order on a nanometer scale. Science **270**, 1315–1316 (1995)
115. Neru, M., Tapas, K.D., Amarnath, M.: Size modulation of polymeric nanoparticles under controlled dynamics of microemulsion droplets. J. Colloid Interface Sci. **109**, 387–391 (1997)
116. Lin, M.M., Kim, D.K., Haj, A.J., Dobson, J.: Development of superparamagnetic iron oxide nanoparticles for translation to clinical applications. IEEE Nanobiosci. **7**, 298–305 (2008)
117. Lee, Y., Lee, J., Bae, C.J., Park, J.-G., Noh, H.-J., Park, J.-H., Hyeon, T.: Large-scale synthesis of uniform and crystalline magnetite nanoparticles using reverse micelles as nanoreactors under reflux conditions. Adv. Funct. Mater. **15**, 503–509 (2005)
118. Carpenter, E.E.: Iron nanoparticles as potential magnetic carriers. J. Magn. Magn. Mater. **225**, 17–20 (2001)
119. Vidal-Vidal, J., Rivas, J., Lopez-Quintela, M.A.: Synthesis of monodisperse maghemite nanoparticles by the microemulsion method. Colloid Surf. A **288**, 44–51 (2006)
120. Kandori, K., Fukuoka, M., Ishikawa, T.: Effects of citrate ions on the formation of ferric oxide hydroxide particles. J. Mater. Sci. **26**, 3313–3319 (1991)
121. Dimitrova, G.T., Tadros, T.F., Luckham, P.F., Kipps, M.R.: Investigations into the phase behavior of nonionic ethoxylated surfactants using 2H NMR spectroscopy. Langmuir **12**, 315–318 (1996)
122. Hyeon, T., Lee, S.S.: Synthesis of highly crystalline and monodisperse maghemite nanocrystallites without a size-selection process. J. Am. Chem. Soc. **123**, 12798–12801 (2001)
123. Jun, Y.W., Huh, Y.M., Choi, J.: Nanoscale size effect of magnetic nanocrystals and their utilization for cancer diagnosis via magnetic resonance imaging. J. Am. Chem. Soc. **127**, 5732–5733 (2005)
124. Li, Z., Chen, H., Bao, H., Gao, M.: One-pot reaction to synthesize water-soluble magnetite nanocrystals. Chem. Mater. **16**, 1391–1393 (2004)
125. Viau, G., Fievet-Vincent, F., Fievet, F.: Monodisperse iron-based particles: precipitation in liquid polyols. J. Mater. Chem. **6**, 1047–1053 (1996)
126. Toneguzzo, P., Viau, G., Acher, O., Fievet-Vincent, F., Fievet, F.: Monodisperse ferromagnetic particles for microwave applications. Adv. Mater. **10**, 1032–1035 (1998)
127. Lu, A.H., Salabas, E.L., Schuth, F.: Magnetic nanoparticles: synthesis, protection, functionalization and application. Angew. Chem. Int. Ed. **46**, 1222–1244 (2007)
128. Xu, C., Sun, S.: Monodisperse magnetic nanoparticles for biomedical applications. Polym. Int. **56**, 821–826 (2007)
129. Fritz, G., Schaedler, V., Willenbacher, N., Wagner, N.J.: Electrosteric stabilization of colloidal dispersions. Langmuir **18**, 6381–6390 (2002)
130. Holthoff, H., Egelhaaf, S.U., Borkovec, M., Schurtenberger, P., Sticher, H.: Coagulation rate measurements of colloidal particles by simultaneous static and dynamic light scattering. Langmuir **12**, 5541–5549 (1996)
131. Lattuada, M., Sandkuhler, P., Wu, H., Sefcik, J., Morbidelli, M.: Aggregation kinetics of polymer colloids in reaction limited regime: experiments and simulations. Adv. Colloid Interface Sci. **103**, 33–56 (2003)

132. Sahoo, Y., Pizem, H., Fried, T., Golodnitsky, D., Burstein, L., Sukenik, C.N., Markovich, G.: Alkyl phosphonate/phosphate coating on magnetite nanoparticles: a comparison with fatty acids. Langmuir **17**, 7907–7911 (2001)
133. Sahoo, Y., Goodarzi, A., Swihart, M.T., Ohulchanskyy, T.Y., Kaur, N., Furlani, E.P., Prasad, P.N.: Aqueous ferrofluid of magnetite nanoparticles: fluorescence labeling and magnetophoretic control. J. Phys. Chem. B **109**, 3879–3885 (2005)
134. Taupitz, M., Wagner, S., Schnorr, J., Kravec, I., Pilgrimm, H., Bergmann-Fritsch, H., Hamm, B.: Phase I clinical evaluation of citrate-coated monocrystalline very small superparamagnetic iron oxide particles as a new contrast medium for magnetic resonance imaging.Invest. Radiol. **39**, 394–405 (2004)
135. Yee, C., Kataby, G., Ulman, A., Prozorov, T., White, H., King, A., Rafailovich, M., Sokolov, J., Gedanken, A.: Self-assembled monolayers of alkanesulfonic and -phosphonic acids on amorphous iron oxide nanoparticles. Langmuir **15**, 7111–7115 (1999)
136. Ma, D., Guan, J., Normandin, F., Denommee, S., Enright, G., Veres, T., Simard, B.: Multifunctional nano-architecture for biomedical applications. Chem. Mater. **18**, 1920–1927 (2006)
137. Bulte, J.W.M., Modo, M.M.J.: Nanoparticles in Biomedical Imaging-Emerging Technologies and Applications. Springer, New York (2008)
138. Lu, Y., Yin, Y., Mayers, B.T.: Modifying the surface properties of superparamagnetic iron oxide nanoparticles through a sol-gel approach. Nano Lett. **2**, 183–186 (2001)
139. Kohler, N., Fryxell, G.E., Zhang, M.: A bifunctional poly(ethylene glycol) silane immobilized on metallic oxide-based nanoparticles for conjugation with cell targeting agents. J. Am. Chem. Soc. **126**, 7206–7211 (2004)
140. Hirsch, R., Katz, E., Willner, I.: Magneto-switchable. J. Am. Chem. Soc. **122**, 12053–12054 (2000)
141. Kim, J., Park, S. et al.: Designed fabrication of multifunctional magnetic gold nanoshells and their application to magnetic resonance imaging and photothermal therapy. Angew. Chem. Int. Ed. **45**, 7754–7758 (2006)
142. Pardoe, H., Chua-anusorn, W., St. Pierre, T.G., Dobson, J.: Structural and magnetic properties of nanoscale iron oxide particles synthesized in the presence of dextran or polyvinyl alcohol. J. Magn. Magn. Mater. **225**, 41–46 (2001)
143. Fournier, C., Leonard, M., Le Coq, L.I., Dellacherie, E.: Coating polystyrene particles by adsorption of hydrophobically modified dextran. Langmuir **11**, 2344–2347 (1995)
144. Mornet, S., Portier, J., Duguet, E.: A method for synthesis and functionalization of ultrasmall superparamagnetic covalent carriers based on maghemite and dextran. J. Magn. Magn. Mater. **293**, 127–134 (2005)
145. Lin, H., Watanabe, M., Kimura, M., Hanabusa, K., Shirai, H.: Preparation of magnetic poly(vinyl alcohol) (PVA) materials by in situ synthesis of magnetite in a PVA matrix. J. Appl. Polym. Sci. **87**, 1239–1247 (2003)
146. Majewski, P., Thierry, B.: Functionalized magnetite nanoparticles—synthesis, properties and bio-applications. Crit. Rev. Solid State Mater. Sci. **32**, 203–215 (2007)
147. LaConte, L.E., Nitin, N., Zurkiya, O., Caruntu, D., O'Connor, C.J., Hu, X., Bao, G.: Coating thickness of magnetic iron oxide nanoparticles affects R2 relaxivity. Magn. Reson. Imaging **27**, 1634–1641 (2007)
148. Albornoz, C., Jacobo, S.E.: Preparation of a biocompatible magnetic film from an aqueous ferrofluids. J. Magn. Magn. Mater. **305**, 12–15 (2006)
149. Kim, D.K., Mikhaylova, M., Wang, F.H., Kehr, J., Bjelke, B., Zhang, Y., Tsakalakos, T., Muhammed, M.: Starch-coated superparamagnetic nanoparticles as MRI contrast agents. Chem. Mater. **15**, 4343–4351 (2003)
150. Gomez-Lopera, S.A., Arias, J.L., Gallardo, V., Delgado, A.V.: Colloidal stability of magnetite/poly(lactic acid) core/shell nanoparticles. Langmuir **22**, 2816–2821 (2006)
151. Flesch, C., Bourgeat-Lami, E., Mornet, S., Duquet, E., Delaite, C., Dumas, P.: Synthesis of colloidal superparamagnetic nanocomposites by grafting poly(epsilon-caprolactone) from the surface of organosilane-modified maghemite nanoparticles. J. Polym. Sci. A **43**, 3221–3231 (2005)

152. Hyon, B.N., In, C.S., Taeghwan, H.: Inorganic nanoparticles for MRI contrast agents. Adv. Mater. **21**, 2133–2148 (2009)
153. Suzuki, M., Honda, H., Kobayashi, T., Wakabayashi, T., Yoshida, J., Takahashi, M.: Development of a target directed magnetic resonance contrast agent using monoclonal antibody-conjugated magnetic nanoparticles. Brain Tumor Pathol. **13**, 127–132 (1996)
154. Yang, L., Mao, H., Wang, Y.A., Cao, Z., Peng, X., Wang, X., Duan, H., Ni, C., Yuan, Q., Adams, G., Smith, M.Q., Wood, W.C., Gao, X., Nie, S.: Single chain epidermal growth factor receptor antibody conjugatednanoparticles for in vivo tumor targeting and imaging. Small **2,** 235–243 (2009)
155. Sun, C., Fang, C., Stephen, Z., Veiseh, O., Hansen, S., Lee, D., Ellenbogen, R.G., Olson, J., Zhang, M.: Tumor-targeted drug delivery and MRI contrast enhancement by chlorotoxin-conjugated iron oxide nanoparticles. Nanomedicine **3**, 495–505 (2008)
156. Fortin, J.P., Gaxeau, G., Wilhelm, C.: Intracellular heating of living cells through Neel relaxation of magnetic nanoparticles. Eur. Biophys. J. **37**, 223–228 (2008)
157. Salloum, M., Ma, R.H., Weeks, D., Zhu, L.: Controlling nanoparticle delivery in magnetic nanoparticle hyperthermia for cancer treatment: experimental study in agarose gel. Int. J. Hyperthermia **24**, 337–345 (2008)
158. Berry, C.C.: Progress in functionalisation of magnetic nanoparticles for applications in biomedicine. J. Phys. D **42**, 224003 (2009)
159. Lubbe, A.S., Alexiou, C., Bergemann, C.: Clinical applications of magnetic drug targeting. J. Surg. Res. **95**, 200–206 (2000)
160. Rosengart, A.J., Kaminski, M.D., Chen, H., Caviness, P.L., Ebner, A.D., Ritter, J.A.: Magnetizable implants and functionalised magnetic carriers: a novel approach for noninvasive yet targeted drug delivery. J. Magn. Magn. Mater. **293**, 633–638 (2005)
161. Muzzarelli, R.A.A., Muzzarelli, C.: Chitosan chemistry: relevance to the biomedical sciences. Adv. Ploym. Sci. **186**, 151–209 (2005)
162. Frank, J.A., Miller, B.R., Arbab, A.S., Zywicke, H.A., Jordan, E.K., Lewis, B.K., Bryant, L.H., Bulte, J.W.M.: Clinically applicable labeling of mammalian and stem cells by combining superparamagnetic iron oxides and trasfection agents. Radiology **228**, 480–487 (2003)
163. Franzreb, M., Hobley, T.J., Siemann-Herzberg, M., Thomas, O.R.T.: Protein purification using magnetic adsorbent particles. Appl. Microbiol. Biotechnol. **70**, 505–516 (2006)
164. Berensmeier, S.: Magnetic particles for the separation and purification of nucleic acids. Appl. Microbiol. Biotechnol. **73**, 495–504 (2006)
165. Gu, H., Ho, P.L., Tsang, K.W.T., Wang, L., Xu, B.: Using biofunctional magnetic nanoparticles to capture vancomycin-resistant enterococci and other gram-positive bacteria at ultralow concentration. J. Am. Chem. Soc. **125**, 15702–15703 (2003)
166. Murray, C.B., Norris, D.J., Bawendi, M.G.: Synthesis and characterization of nearly monodisperse CdE (E = S, Se. Te) semiconductor nanocrystallites. J. Am. Chem. Soc. **115**, 8706–8715 (1993)
167. Colvin, V.L., Schlamp, M.C., Alivisatos, A.P.: Light-emitting-diodes made from cadmium selenide nanocrystals and a semiconducting polymer. Nature **370**, 354–357 (1994)
168. Medintz, I.L., Clapp, A.R., Mattoussi, H., Goldman, E.R., Fisher, B., Mauro, J.M.: Self-assembled nanoscale biosensors based on quantum dot FRET donors. Nat. Mater. **2**, 630–638 (2003)
169. Kirchner, C., Liedl, T., Kudera, S., Pellegrino, T., Javier, A.M., Gaub, H.E., Stolzle, S., Fertig, N., Parak, W.J.: Cytotoxicity of colloidal CdSe and CdSe/ZnS nanoparticles. Nano Lett. **5**, 331–338 (2005)
170. Ludolph, B., Malik, M.A., O'Brien, P., Revaprasadu, N.: Novel single molecule precursor routes for the direct synthesis of highly monodispersed quantum dots of cadmium or zinc sulfide or selenide. Chem. Commun. **17**, 1849–1850 (1998)
171. Hines, M.A., Guyot-Sionnest, P.: Bright UV-blue luminescent colloidal ZnSe nanocrystals. J. Phys. Chem. B **102**, 3655–3657 (1998)
172. Susumu, K., Uyeda, H.T., Medintz, I.L., Pons, T., Delehanty, J.B., Mattoussi, H.: Enhancing the stability and biological functionalities of quantum dots via compact multifunctional ligands. J. Am. Chem. Soc. **129**, 13987–13996 (2007)

173. Dabbousi, B.O., Rodriguez Viejo, J., Mikulec, F.V., Heine, J.R., Mattoussi, H., Ober, R., Jensen, K.F., Bawendi, M.G.: (CdSe)ZnS core-shell quantum dots: synthesis and optical and structural characterization of a size series of highly luminescent materials. J. Phys. Chem. B **101**, 9463–9475 (1997)
174. Pradhan, N., Katz, B., Efrima, S.: Synthesis of high-quality metal sulfide nanoparticles from alkyl xanthate single precursors in alkylamine solvents. J. Phys. Chem. B **107**, 13843–13854 (2003)
175. Reiss, P., Protière, M., Li, L.: Core/shell semiconductor nanocrystals. Small **5**, 154–168 (2009)
176. Chan, W.C.W., Nie, S.M.: Quantum dot bioconjugates for ultrasensitive nonisotopic detection. Science **281**, 2016–2018 (1998)
177. Medintz, I.L., Uyeda, H.T., Goldman, E.R., Mattoussi, H.: Quantum dot bioconjugates for imaging, labelling and sensing. Nat. Mater **4**, 435–446 (2005)
178. Mitchell, G.P., Mirkin, C.A., Letsinger, R.L.: Programmed assembly of DNA functionalized quantum dots. J. Am. Chem. Soc. **121**, 8122–8123 (1999)
179. Pinaud, F., King, D., Moore, H.P., Weiss, S.: Bioactivation and cell targeting of semiconductor CdSe/ZnS nanocrystals with phytochelatin-related peptides. J. Am. Chem. Soc. **126**, 6115–6123 (2004)
180. Kim, S., Bawendi, M.G.: Oligomeric ligands for luminescent and stable nanocrystal quantum dots. J. Am. Chem. Soc. **125**, 14652–14653 (2003)
181. Hainfeld, J.F., Liu, W., Halsey, C.M.R., Freimuth, P., Powell, R.D.: Ni- NTA gold clusters target His-tagged proteins. J. Stuct. Biol. **127**, 185–198 (1999)
182. Dubertret, B., Skourides, P., Norris, D.J., Noireaux, V., Brivanlou, A.H., Libchaber, A.: In vivo imaging of quantum dots encapsulated in phospholipids micelles. Science **298**, 1759–1762 (2002)
183. Ballou, B., Lagerholm, B.C., Ernst, L.A., Bruchez, M.P., Waggoner, A.S.: Noninvasive imaging of quantum dots in mice. Bioconj. Chem. **15**, 79–86 (2004)
184. Gao, X., Cui, Y., Levenson, R.M., Chung, L.W.K., Nie, S.: In vivo cancer targeting and imaging with semiconductor quantum dots. Nat. Biotechnol. **22**, 969–976 (2004)
185. Osaki, F., Kanamori, T., Sando, S., Sera, T., Aoyama, Y.: A quantum dot conjugated sugar ball and its cellular uptake on the size effects of endocytosis in the subviral region. J. Am. Chem. Soc. **126**, 6520–6521 (2004)
186. Kricka, L.J.: Source. Ann. Clin. Biochem. **39**, 114–129 (2002)
187. Goldman, E.R., Clapp, A.R., Anderson, G.P., Uyeda, H.T., Mauro, J.M., Medintz, I.L., Mattoussi, H.: Multiplexed toxin analysis using four colors of quantum dot fluororeagents. Anal. Chem.**76**, 684–688 (2004)
188. Jaiswal, J.K., Mattoussi, H., Mauro, J.M., Simon, S.M.: Long-term multiple color imaging of live cells using quantum dot bioconjugates. Nat. Biotechnol. **21**, 47–51 (2003)
189. Wu, X.Y., Liu, H.J., Liu, J.Q., Haley, K.N., Treadway, J.A., Larson, J.P., Ge, N.F., Peale, F., Bruchez, M.P.: Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots. Nat. Biotechnol. **21**, 41–46 (2003)
190. Kim, S., Lim, Y.T., Soltesz, E.G., De Grand, A.M., Lee, J., Nakayama, A., Parker, J.A., Mihaljevic, T., Laurence, R.G., Dor, D.M., Cohn, L.H., Bawendi, M.G., Frangioni, J.V.: Near-infrared fluorescent type II quantum dots for sentinel lymph node mapping. Nat. Biotechnol. **22**, 93–97 (2004)
191. Shi, C.M., Zhu, Y., Xie, Z.H., Qian, W.P., Hsieh, C.L., Nie, S.M., Su, Y.P., Zhau, H.E., Chung, L.W.K.: Visualizing human prostate cancer cells in mouse skeleton using bioconjugated near-infrared fluorescent quantum dots. Urology **74**, 446–451 (2009)
192. Derfus, A.M., Chan, W.C.W., Bhatia, S.N.: Probing the cytotoxicity of semiconductor quantum dots. Nano Lett. **4**, 11–18 (2004)
193. Mikhaylova, M., Kim, D.K., Bobrysheva, N., Osmolowsky, M., Semenov, V., Tsakalakos, T., Muhammed, M.: Superparamagnetism of magnetite nanoparticles: dependence on surface modification. Langmuir **20**, 2472–2477 (2004)
194. Garza-Navarro, M., Torres-Castro, A., Gonzalez, V., Ortiz, U., De la Rosa, E.: Magnetite and magnetite/silver core/shell nanoparticles with diluted magnet-like behavior. J. Solid State Chem. **183**, 99–104 (2010)

195. Pal, B., Torimoto, T., Iwasaki, K., Shibayama, T., Takahashi, H., Ohtani, B.: Synthesis of metal-cadmium sulfide nanocomposites using jingle-bell-shaped core-shell photocatalyst particles. J. Appl. Electrochem. **35**, 751–756 (2005)
196. Salgueirino-Maceira, V., Correa-Duarte, M.A., Lopez-Quintela, M.A., Rivas, J.: Advanced hybrid nanoparticles. J. Nanosci. Nanotechnol. **9**, 3684–3688 (2009)
197. Kohler, N., Sun, C., Wang, J., Zhang, M.Q.: Methotrexate-modified superparamagnetic nanoparticles and their intracellular uptake into human cancer cells. Langmuir **21**, 8858–8864 (2005)
198. Yiu, H.H.P., Niu, H.J., Biermans, E., van Tendeloo, G., Rosseinsky, M.J.: Designed multifunctional nanocomposites for biomedical applications. Adv. Funct. Mater. **20**, 1599–1609 (2010)