Inorganic colloidal nanoparticles are very small, nanoscale objects with inorganic cores that are dispersed in a solvent. Depending on the material they consist of, nanoparticles can possess a number of different properties such as high electron density and strong optical absorption (e.g. metal particles, in particular Au), photoluminescence in the form of fluorescence (semiconductor quantum dots, e.g. CdSe or CdTe) or phosphorescence (doped oxide materials, e.g. Y2O3), or magnetic moment (e.g. iron oxide or cobalt nanoparticles). Prerequisite for every possible application is the proper surface functionalization of such nanoparticles, which determines their interaction with the environment. These interactions ultimately affect the colloidal stability of the particles, and may yield to a controlled assembly or to the delivery of nanoparticles to a target, e.g. by appropriate functional molecules on the particle surface. This work aims to review different strategies of surface modification and functionalization of inorganic colloidal nanoparticles with a special focus on the material systems gold and semiconductor nanoparticles, such as CdSe/ZnS. However, the discussed strategies are often of general nature and apply in the same way to nanoparticles of other materials.

Keywords: inorganic chemistry; metal clusters; nanoparticles; colloids

1. Introduction

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in particular Au), photoluminescence in the form of fluorescence (semiconductor quantum dots, e.g. CdSe or CdTe) or phosphorescence (doped oxide materials, e.g. Y₂O₃), or magnetic moment (e.g. iron oxide or cobalt nanoparticles).

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(a) Particle synthesis

Nowadays nanoparticles of a large variety of different materials, differing in their elemental composition, size, shape and physical or chemical properties, can be synthesized (Murray et al. 2000; Masala & Seshadri 2004). Colloidal nanoparticles are dispersed in a solvent that can be either water-based or an organic solvent for hydrophilic or hydrophobic particles, respectively, while amphiphilic nanoparticles can be dispersed in both kinds of solvents. The term ‘nanoparticle’ usually applies to particles between 1 and 100 nm. Small particles consisting of only a few to some hundred atoms are often referred to as clusters, and owing to their small size, which is similar to molecules, the terms dispersion and solution are often equally used. Generally, the synthesis of nanoparticles involves surfactant molecules that bind to their surface, which stabilize the nuclei and larger nanoparticles against aggregation by a repulsive force, and which generally control the growth of the nanoparticles in terms of rate, final size or geometric shape. In some cases, depending on the material combination, the surfactant may be identical to the solvent or perform the function of a reduction agent.

The synthesis of nanoparticles of different materials is the subject of a number of dedicated reviews, such as for gold (Daniel & Astruc 2004; Schmid 2008) or silver nanoparticles, semiconductor quantum dots (Masala & Seshadri 2004; Embden et al. 2007; Rogach et al. 2007; Reiss et al. 2009) or magnetic nanoparticles (Hyeon 2003; Lin & Samia 2006).

Depending on the nanoparticle material and surfactant molecules used, shape-controlled growth is also possible, e.g. by stronger binding of the ligand to certain crystal facets, as discussed in greater detail and with more examples in a number of reviews (Peng & Peng 2001; Manna et al. 2002; Burda et al. 2005; Perez-Juste et al. 2005; Yin & Alivisatos 2005; Kumar & Nann 2006; Grzelczak et al. 2008; Kudera et al. 2008; Tao et al. 2008). Recently, composite particles with domains of different materials have also been demonstrated (Mokari et al. 2004, 2005; Kudera et al. 2005; Yu et al. 2005; Pellegrino et al. 2006; Zhang et al. 2006), e.g. heterodimers of CdS and FePt (Gu et al. 2004), Co/CdSe (Kim et al. 2005c) and others (Carpenter et al. 2000; Quarta et al. 2007; Zanella et al. 2008) that can possess e.g. both fluorescent and magnetic properties.
(b) Stabilization against aggregation

The ligand molecules bound to the nanoparticle surface not only control the growth of the particles during synthesis, but also prevent the aggregation of the nanoparticles. The repulsive force between particles can, in principle, be due to electrostatic repulsion, steric exclusion or a hydration layer on the surface. Depending on the particle system, i.e. the core material and the solvent in which the particles are dispersed, the choice of the right ligand may yield to stable particles. First, the ligand molecules have to be bound to the particle surface by some attractive interaction, either chemisorption, electrostatic attraction or hydrophobic interaction, most commonly provided by a head group of the ligand molecule. Various chemical functional groups possess a certain affinity to inorganic surfaces, the most famous example being thiol to gold. In many cases, this principle is already exploited during synthesis, as briefly pointed out earlier. As to the interaction of the ligand molecule with the solvent, polar or charged ligand molecules provide solubility in polar or aqueous solvents, while nanoparticles with apolar ligand molecules such as hydrocarbon chains are only soluble in apolar organic solvents, e.g. hexane, toluene or chloroform. Certain amphiphilic ligand molecules, e.g. poly(ethylene) glycol (PEG), possess amphiphilic properties, and nanoparticles with those or other ligand molecules can be soluble in a number of solvents, with intermediate polarity.

In organic solvents, the nanoparticle surface is covered by hydrophobic ligand molecules that prevent the aggregation of the particle cores. However, the bonds between the inorganic nanoparticle surface and, e.g. an electron-donating end group of a ligand molecule, such as thiol (Weisbecker et al. 1996; Lin et al. 2004a; Love et al. 2005), amine or phosphine (Leff et al. 1996), undergo dynamic binding and unbinding processes (Döllefeld et al. 2002; Ji et al. 2008). This yields to the important consequence that the ligand molecules can get off, e.g. by excessive washing or mass action by another incoming ligand, which might compromise the stability of the nanoparticles that might ultimately aggregate and precipitate. In particular, in the case of fluorescent quantum dots, irradiation with light can enhance oxidation of the inorganic particle surface (Clarke et al. 2006), and photo-oxidation may eventually result in aggregation caused by desorption of the stabilizing ligands (Aldana et al. 2001, 2005; Clarke et al. 2006). Figure 1 displays some commonly used hydrophobic ligand molecules drawn to scale along with a particle of 5 nm diameter.

In aqueous solutions, the ligand–nanoparticle interaction is basically the same, but a number of different effects that are important for stability arise. Most commonly, hydrophilic nanoparticles are stabilized by electrostatic repulsion by the equally charged ligand molecules on the particle surface. However, in the presence of high salt concentrations, the electric field is shielded, and the nanoparticles can come close to each other until the attractive forces, such as induced dipole interaction, i.e. van der Waals force, or hydrogen bonds, eventually cause the particles to agglomerate (Laaksonen et al. 2006). Depending on the isoelectric point (pI) and the pH of the solution, nanoparticles can also lose or change the sign of their charge. While this is, in principle, well understood and described by theory, nanoparticles are often quite complex objects with properties different from simple model systems: the particles are not spherical hard objects, but are covered with a soft organic ligand shell of which the charge...
Figure 1. A nanoparticle of 5 nm core diameter with different hydrophobic ligand molecules both drawn to scale. The particle is idealized as a smooth sphere; the schematic molecule structures above are not drawn to scale. Left to right: trioctylphosphine oxide (TOPO), triphenylphosphine (TPP), dodecanethiol (DDT), tetraoctylammonium bromide (TOAB) and oleic acid (OA). The spatial conformation of the molecules is only shown schematically as derived from their chemical structure and space-filling models.

distribution is, in most cases, not known (Kimura et al. 2002). Heterogeneity in the surface coverage can result e.g. in hydrophobic patches on the nanoparticle surface, or the ligand shell can undergo conformational changes depending on the external factors. Furthermore, bi- or multi-valent, oppositely charged ions or polyelectrolytes can bridge the particles by electrostatic attraction, again causing aggregation.

As already pointed out, the possible choice of ligand molecules can depend on the material of the nanoparticle core, the particle size and the solvent. Generally, it is found that strongly binding molecules forming a dense layer stabilize particles better than weakly binding ones, in particular, in further processing and purification steps after the particle synthesis. In aqueous solutions, strongly charged ligand molecules, containing e.g. carboxylic or sulphonic acid groups, are found to stabilize the particles for longer time and also at more elevated salt concentrations. Finally, ligand molecules providing steric stabilization are found to be much more resistant to high salt concentrations than electrostatically stabilized nanoparticles (Sakura et al. 2005), provided they are strongly bound to the nanoparticle surface, while in the few cases of nanoparticles that are not stabilized by ligand molecules, often poor stability to external factors is observed.
Also a combination of electrostatic and steric stabilization is found, e.g. in the case of certain polymers (Stenkamp et al. 2001; Fritz et al. 2002) or dendrimers (Wang et al. 2002; Zheng et al. 2002).

(c) Ligand exchange

In order to improve the stability of given nanoparticles, the ligand molecules on the surface can be exchanged by others that can possibly provide new properties or functionality to the particles. In most cases, the incoming ligand molecule binds more strongly to the inorganic nanoparticle surface.

A common example is Au nanoparticles in an aqueous solution synthesized by citrate reduction. The resulting nanoparticles have negatively charged citrate ions adsorbed on their surface and are thus stabilized by electrostatic repulsion. While such colloids may be stable for years in the as-synthesized solution, they cannot be concentrated well and aggregate irreversibly, e.g. in the presence of salts. The citrate layer can be replaced by ligands binding stronger to the particle surface; popular examples include sulphonated phosphines or mercaptocarboxylic acids, and common examples being mercaptoaetic acid (MAA) mercaptoacetic acid to mercaptaoacetic acid, mercaptopropionic acid (MPA) or mercaptoundecanoic acid (MUA) (Lin et al. 2004). Modifying the nanoparticles with phosphines already allows for achieving highly concentrated particle solutions; the particles can be precipitated by salt-induced aggregation and redissolved again as single particles in low-salt buffers. Then, thiol-containing ligand molecules can be added to again replace the phosphine, a strategy that is, for example, commonly employed for the attachment of thiol-modified DNA to nanoparticles. If the particles are not completely saturated with the new ligand molecule, the remaining phosphine molecules covering the surface help in stabilizing the nanoparticle.

In organic solutions, Au nanoparticles are commonly synthesized by the Brust two-phase method employing tetraoctylammonium bromide and dodecanethiol as stabilizing ligands that also control the size of the resulting nanoparticles. Alternatively, the nanoparticles can also be synthesized in the absence of the thiol ligand, i.e. solely with the organic quaternary ammonium ion. In this way, synthesis and final capping of the nanoparticles become independent steps in the synthesis process, and the commonly used dodecanethiol can be substituted by a variety of other molecules (Ackerson et al. 2005) in order to vary the surface properties and functionalization of the nanoparticles generated by this synthetic route. Similarly, Au and Ag nanoparticles of different sizes are synthesized with oleylamine in toluene have been subsequently derivatized by thiols (Hiramatsu & Osterloh 2004).

Thiol groups are considered to show the highest affinity to noble metal surfaces, in particular to gold (approx. 200 kJ mol$^{-1}$; Love et al. 2005). While this binding is often termed ‘chemisorption’, sometimes also noted as covalent bond, the exact processes and the microscopic nature are still subject to research and discussion. Contrary to the case of self-assembled monolayers (SAMs) on a well-defined planar crystal face, the surface of a nanoparticle consists of not only a number of different crystal facets, but also a large part of edges, terraces and vertices (Hostetler et al. 1999), resulting in binding sites with different affinities for the ligand molecules. This complicates the characterization of the ligand shell compared with ‘classical’ SAMs, like the well-studied Au–alkanethiol system.
In addition, bound ligands appear to be mobile on the surface, i.e. able to diffuse to some extent on the particle surface after having bound (Hostetler et al. 1999). In the aqueous phase, the replacement of ligand molecules may be facilitated by additional detergents (Aslan & Pérez-Luna 2002), and the incoming, stronger binding ligand may render the particles more stable in regard to possible aggregation at high salt concentrations or acidic pH (Weisbecker et al. 1996; Mayya et al. 1997). Ligands with two thiol moieties have also been reported (Zhao et al. 2005).

The same principles hold for semiconductor quantum dots (Alivisatos 1996), CdSe and CdSe/ZnS among others, that are often stabilized by trioctylphosphine (TOP), or its oxide (trioctylphosphine; TOPO), binding preferentially to the Cd or Zn atoms of the nanocrystals. Also in these systems, binding dynamics and surface diffusion are found (Moreels et al. 2007); the surface coverage may depend on the particle size and geometric shape of the ligand molecules involved (Bowen-Katari et al. 1994). Besides TOP and/or TOPO, hexadecylamine or other amines are also commonly used ligands for CdSe quantum dots (Ji et al. 2008), which can also be replaced by stronger binding molecules, e.g. with one or more thiol groups (Döllefeld et al. 2002; Dubois et al. 2007). In the case of quantum dots, the ligand shell may influence the fluorescence properties of the particles (Wuister et al. 2004; Kalyuzhny & Murray 2005; Bullen & Mulvaney 2006; Smith et al. 2006; Ji et al. 2008), in particular the quantum yield, especially in the aqueous phase, as discussed in the following section.

Furthermore, ligand exchange has also been demonstrated with dendrimers (Wang et al. 2002; Kim et al. 2005d) and thiol-containing peptides in the case of gold nanoparticles (Levy et al. 2004; Nativo et al. 2008; Bastus et al. 2009), and as well with proteins binding to quantum dots by replacing MAA on the surface (Gao et al. 2002). The same principles also apply to Au rods, where the replacement of the cetyltrimethylammonium bromide (CTAB) double layer by thiol-containing PEG has been reported (Niidome et al. 2006; Pierrat et al. 2007).

These considerations imply that, for ligand exchange, the new ligand molecules should have an affinity as strong as possible to the inorganic core in order to quickly and effectively replace the original surfactant molecules. In addition, the molecular geometry of the ligands in relation to the particle diameter is a factor that influences how densely the molecules are packed around the particles, which in turn influences ultimately the colloidal stability of the particles.

However, ligand-coated nanoparticles differ from simple micelles consisting of the ligand molecules alone which are held together only by intramolecular forces, in that on nanoparticles, ligand molecules are additionally attached to the nanoparticles surface, in most cases via a chemical functional group. This bond can be electrostatic, e.g. for gold nanoparticles capped with tetraoctylammonium bromide or covalent-like as, for instance, the gold–thiol bond. Naturally, ligand molecules that are strongly bound to the nanoparticle surface or more tightly to each other will be less subject to get off the particles’ surface, as, for instance, shown for different peptide sequences (Levy et al. 2004; Fabris et al. 2006). In analogy to the concept of critical micelle concentration, such ligand molecules bind dynamically to the particle surface and are thus subject to mass action and may be washed off by continued purification of the nanoparticles (Lin et al. 2004a).
2. Phase transfer

Since many types of colloidal nanoparticles are synthesized in organic solvents, for certain possible applications they have to be transferred to aqueous solutions, e.g. to be compatible with biological systems. For other applications, hydrophobic nanoparticles are required to be compatible with a given solvent/or material system, e.g. for polymer blends (Kim & Bawendi 2003; Kim et al. 2005b), even though the particles may have been synthesized in aqueous solution. In these cases, phase transfer becomes necessary if the desired particle type cannot be synthesized with the corresponding ligand on the surface.

In general, for phase transfer in both directions, there exist three strategies: ligand exchange, ligand modification and additional layers of molecules that stabilize the particles in the desired phase. In addition to these approaches, silanization is also used for surface modification and phase transfer, representing a case in-between this classification.

(a) Ligand exchange

In the ligand-exchange strategy, the molecules stabilizing the particles in the original first phase are replaced by other, more strongly binding ligands that allow the transfer to the second phase and provide colloidal stability there, e.g. by exchanging hydrophobic by hydrophilic ligands. Commonly used ligand molecules include thiol groups that bind strongly to inorganic surfaces of nanoparticles, e.g. Au and Ag (Gittins & Caruso 2002) or CdSe, replacing the weaker bound ligands that the nanoparticles usually have from synthesis (figures 1 and 2).

Examples include the transfer of TOP/TOPO-coated CdSe/ZnS quantum dots to an aqueous solution by replacing the phosphine-based hydrophobic ligands with a hydrophilic thiol-based molecule, often mercaptocarboxylic acids (e.g. MPA, MUA, etc.). Variations include derivatives with multiple moieties, e.g. mercaptosuccinic acid (two carboxylic groups) (Clarke et al. 2006) and lipoic acid (or dihydrolipoic acid, respectively, with two sulphhydril groups; Mattoussi et al. 2000; Algar & Krull 2007). Optionally, the free end of the ligand molecules can also carry different residues (Dubois et al. 2007), e.g. PEG as demonstrated for Au nanoparticles (Kanaras et al. 2002) or quantum dots (Uyeda et al. 2005). Sometimes, a mixture of different ligand molecules is employed, e.g. to introduce additional functional groups to the particle surface (Liu et al. 2008).

For the transfer of hydrophilic particles to the organic phase, the same concept is applied: this time, one chemical group has to bind strongly to the nanoparticle surface in order to replace the original ligand molecules and the other end has to be of hydrophobic character. Examples include the phase transfer by linear hydrocarbon molecules with e.g. a single thiol or amino group (Kumar et al. 2003) or molecules with more than one hydrocarbon chain and also possibly multiple anchor groups (Zhao et al. 2005), such as dihydrolipoic acid (Mattoussi et al. 2000) or other ligands (Balasubramanian et al. 2001; Misra et al. 2006). Other examples include Au nanoparticles with resorcinarene (Misra et al. 2006), platinum nanoparticles transferred to the organic phase by dodecylamine (Yang et al. 2004), as well as CdS particles with octadecanethiol (Kumar et al. 2000).

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Molecules, such as mercaptocarboxylic acids, that are used to stabilize nanoparticles in the aqueous phase are often readily soluble in organic solvents, e.g. toluene or tetrahydrofuran (Simard et al. 2000). Thus, transfer from organic to the aqueous phase can occur spontaneously (Kanaras et al. 2002; Algar & Krull 2007), while the transfer of nanoparticles from the aqueous to the organic phase is often more difficult because ligands for the organic phase are often very poorly soluble in the aqueous phase.

In order to facilitate the contact of the nanoparticles with the phase boundary, additional components can be added, e.g. acetone (which decreases the surface tension at the interface; Gaponik et al. 2002) or a strong acid (Sarathy et al. 1997a,b; Zhao et al. 2002b) or base (Yang et al. 2004) that protonates the negatively charged groups (or deprotonates positively charged amino groups), rendering the particle less charged and thus less oleophobic. For a few weakly binding ligands, also spontaneous transfer without the help of additional agents has been reported (Griffin & Fitzmaurice 2007).

In addition, ligand exchange protocols with amphiphilic molecules have been reported that allows one to dissolve the same particles in both polar and apolar solvents. Examples include small molecules that can change their orientation depending on the surrounding solvent (Bala et al. 2005) or polymers that can be either non-ionic (Liz-Marzán & Lado-Touriño 1996) or ionic like poly(ethylene imine) (PEI; Nann 2005) and poly(acrylic acid) (Lin et al. 2008b), in which the positively or negatively charged moieties can bind to the inorganic particle surface and render the surface of the whole particle polar. In those approaches, ligand
exchange takes place in the same phase in which the nanoparticles are already present, but the new ligand molecules are then able to disperse the particles in other solvents.

An important issue in regard to ligand exchange and phase transfer is the stability of the optical properties in the case of fluorescent quantum dots. Especially when brought to the aqueous phase, both the particle surface and possibly the thiol group of the ligands are prone to oxidation. Often the fluorescence quantum yield is reduced and desorption of the ligand molecules can eventually yield to aggregation. This effect can be further enhanced by irradiation of the particles with light (Aldana et al. 2001, 2005).

Hydrophobic nanoparticles of different core materials have been transferred to the organic phase by different polyelectrolytes adsorbing to the inorganic particle surface (Zhang et al. 2007) stabilizing the particles by electrostatic repulsion or by ligand exchange with a PEI–PEG copolymer (Duan & Nie 2007).

(b) Ligand modification

An alternative approach to phase transfer is ligand modification: the ligand molecule stabilizing the nanoparticles in the original first phase is rendered hydrophilic or hydrophobic to transfer and stabilize the particles in the second phase. Hydrophilic nanoparticles stabilized by a mercaptocarboxylic acid can be modified, for instance, by a hydrophobic molecule that is chemically bound to its carboxylic terminal groups (e.g. dicyclohexylamine to MAA, McMahon & Emory 2007), or by modification with a compound that can change its polarity—e.g. by stripping off a capping agent (cyclodextrin rings complexing octadecanethiol, Lala et al. (2001) or by formation of a complex of cyclodextrin with oleic acid present on the nanoparticle surface, Wang et al. (2003)), or by covalent attachment of an amphiphilic, V-shaped ligand (Zubarev et al. 2006). The concept of ligand modification may provide efficient phase transfer because the particles are modified with a new ligand in the same phase they are already in. However, it is restricted to certain systems that are compatible with each other and for which the colloidal stability of the nanoparticles is maintained during the reaction.

(c) Additional coating layers

The third strategy for phase transfer is an additional molecular layer on the particles that adsorbs on the original ligand molecules and changes the surface properties accordingly. In this way, a ligand bilayer is formed that allows one to transfer hydrophilic particles from the aqueous phase to organic solvents (Liu et al. 2001; Mayya & Caruso 2003) and as well hydrophobic nanoparticles to water (Wooding et al. 1991; Shen et al. 1999; Gittins & Caruso 2001). The molecules acting as phase-transfer agents have to be amphiphilic, comprise a hydrophobic and a hydrophilic part, commonly one or more aliphatic chains and a polar, often charged, end group. This approach is sketched in figure 3 (centre) for small molecules and for oligomeric or polymeric molecules (right).

One common class of such surfactants for the transfer from aqueous to organic phase are quaternary ammonium salts in which four hydrocarbon chains are bound to a nitrogen atom that is thus positively charged, the counter ions usually being chloride or bromide. Those molecules are known as classical phase-transfer agents and are also used in colloidal science not only for the phase
transfer of ionic precursors, e.g. AuCl$_4^-$, to the organic phase (two-phase synthesis, see §1a), but also for the transfer of hydrophilic nanoparticles to the organic phase. The ammonium salt is dissolved in the organic phase and can adsorb electrostatically onto the negatively charged surface of the nanoparticles. The actual kind of hydrophilic ligand molecule is not important, the phase transfer can work well with nanoparticles coated with e.g. citrate, sulphonated phosphines or mercaptocarboxylic acids. The quaternary ammonium salts employed for phase transfer include tetrachloeammonium bromide (Yao et al. 2000; Cheng & Wang 2004), cetyltrimethylammonium chloride (Tian & Fendler 1996) and others. After phase transfer, the ligand shell can again be replaced by strongly binding ligands, as, for instance, dodecanethiol in the case of Au nanoparticles. In another example, octadecyl-$p$-vinyl-benzylidemethylammonium chloride has been used, of which the vinyl moiety can be employed for the covalent embedding of particles in a polymer matrix (Zhang et al. 2003).

A similar procedure can be applied for the transfer of hydrophobic particles to the aqueous phase, for example with CTAB and dodecylamine-capped Au (Swami et al. 2003), in which the hydrocarbon chains of the CTAB adsorb on the octadecylamine layer by hydrophobic interaction, while the positively charged ammonium moiety points outwards into solution.

The same approach utilizing an additional ligand layer for phase transfer also applies to lipids that have been used for the coating of hydrophobic nanoparticles (Dubertret et al. 2002; Stroh et al. 2005; Srinivasan et al. 2006; Carion et al. 2007;
Erogbogbo et al. 2008). The additional layer conserves the native environment of the inorganic nanoparticles because the original ligand molecules are not replaced. This may be beneficial e.g. to prevent sensitive core materials from oxidation. Owing to the additional layer bound by hydrophobic interactions, this coating strategy can be applied regardless of the material of the inorganic particle core. Variations include the embedding of hydrophobic quantum dots into the lipid bilayer of vesicles and liposomes (Gopalakrishnan et al. 2006) and paramagnetic lipids that yield fluorescent nanoparticles with additional magnetic properties (Mulder et al. 2006a, b).

Naturally, by any additional layer, the nanoparticle size is increased, and owing to the nature of organic molecules, these organic shells appear to be rather soft. In the following, the coating of nanoparticles with amphiphilic and other polymers will be pointed out in more detail.

(d) Polymer coatings

Although there are a number of well-established variations of phase transfer by ligand exchange, in particular mercaptocarboxylic acid-based ligands for the transfer of nanoparticles from the organic to the aqueous phase, this approach suffers from several drawbacks: (i) small ligands with one head group binding to the nanoparticle surface can easily desorb and impair the stabilization of the particles, especially in solutions free of excess unbound ligands, and (ii) although thiol-containing ligands bind relatively strong to various metal particles and quantum dots, in general the ligand molecule has to be carefully chosen to the given core material, which is reflected in the large variety of reported protocols.

In contrast, an additional amphiphilic coating layer that adsorbs by hydrophobic interaction to the hydrophobic ligand molecules of the nanoparticles has the advantage that it does not depend on the inorganic core material (and possibly not even on the exact type of ligand molecules) since the adsorption is predominantly based on hydrophobic interaction of hydrocarbon chains and van der Waals forces between the molecules. In the case of amphiphilic polymers, many contact points between the ligand molecules and the polymer prevent facile desorption of the polymer molecule from the particle, e.g. by thermal fluctuations. Finally, the coated particles have the same physical and chemical surface properties independent of their core material. On the right-hand side of figure 3, the approach of an additional shell of polymer molecules is sketched.

One common example includes a poly(acrylic acid)-based polymer with hydrophilic side chains. Poly(acrylic acid) is a highly charged linear polyelectrolyte, its carboxylic groups can be modified with aliphatic amines via an amide bond (Wang et al. 1988). For the phase transfer of nanoparticles, poly(acrylic acid) with a molecular weight of 2000 g mol\(^{-1}\) modified with 40 per cent octylamine (in respect to the number of carboxylic groups) has been used by Bruchez and coworkers (Wu et al. 2003), and is probably also used for commercial water-soluble quantum dots. The comb-like polymer is soluble in organic solvent and can be added to hydrophobic nanoparticles, e.g. quantum dots with TOP/TOPO ligands. After evaporation of the solvent, the solid can be dissolved in an aqueous buffer, yielding stable, single nanoparticles. In order to increase the stability, the polymer shell has been further cross-linked with lysine by 1-ethyl-3-(3-dimethylaminopropyl)carboiimide (EDC) chemistry.
The same kind of polymer has been used to disperse quantum dots in ethanol (Petruska et al. 2004). A longer poly(acrylic acid) backbone has been modified with a mixture of octylamine and isopropylamine (Luccardini et al. 2006). Recently, the grafting density and length of the hydrophobic side chains has been studied in great detail (Anderson & Chan 2008), as well as the modification with aminopentanol (Snee et al. 2006), or crosslinking of the shell with diamino propanol (Kairdolf et al. 2008a), which resulted in a large number of hydroxyl groups on the particle surface.

Interestingly, also the synthesis of CdTe/CdSe quantum dots has been carried out in the presence of poly(acrylic acid) with 40 per cent modification with dodecylamine (Kairdolf et al. 2008b), yielding amphiphilic nanoparticles that were soluble in a number of organic solvents, as well as in water, where the polymer is assumed to form a double layer around the particles.

Another closely related class of amphiphilic polymers is based on poly(maleic anhydride) copolymers that are synthesized by copolymerization of maleic anhydride with olefins, resulting in alternating copolymers. When coming into contact with water, the maleic anhydride rings hydrolyse and open, forming two carboxylic groups each. Compared with modified poly(acrylic acid), the hydrophobic side chains are not randomly grafted and the density of carboxylic groups is higher. The phase transfer of hydrophobic nanoparticles of a variety of different core materials has been demonstrated by Pellegrino et al. (2004), using commercial poly(maleic anhydride-alt-1-tetradecene), which is no longer available. The still available analogue poly(maleic anhydride-alt-1-octadecene) can be used with an adopted procedure (Di Corato et al. 2008). A similar commercial derivative with tertiary amino groups has also been used for nanoparticle coating and phase transfer (Qi & Gao 2008), saving the step of post-modification with dimethylethylenediamine and EDC (Yezhelyev et al. 2008).

A very useful property of the maleic anhydride moieties is their spontaneous reactivity towards primary amines (and to alcohols under acidic conditions), which can be exploited for a pre-modification of the polymer before it is used for particle coating (Lin et al. 2008a). Each maleic anhydride ring yields a free carboxylic group after reaction with the amine. This has been demonstrated for PEG (Yu et al. 2006b), resulting in nanoparticles with increased stability, e.g. in a biological environment (Yu et al. 2007).

Recently, another design for amphiphilic polymer has been presented, combining the advantage of maleic anhydride moieties for pre-modification and custom modification with side chains (Lin et al. 2008a). Hydrophobic side chains consisting of dodecylamine are grafted to a poly(maleic anhydride)-based backbone, leaving a part of the anhydride rings intact. If desired, additional functional molecules like fluorescent dyes, sugars, biotin or PEG can be covalently grafted to the polymer if they exhibit an amine function. This again saves the additional steps and purification of post-modification with additional crosslinkers.

The discussed comb-like amphiphilic polymers used for the synthesis of nanoparticles are made up of either an alternating or random sequence of building blocks that consist commonly of aliphatic chains as hydrophobic elements and charged groups as hydrophilic parts. Hydrophobic side chains cover or intercalate the hydrophobic ligand molecules of the nanoparticles, while the hydrophilic backbone is exposed to the outside aqueous environment. Even though the attraction between the polymer and the particle is due to rather weak van der
Waals forces between the aliphatic chains, the hydrophobic interaction and the large number of contact points by the several side chains of the polymer result in a very stable coating. By this, the thickness of the shell is increased by only one additional monolayer of polymer and, owing to the nature of the interaction, this coating approach works in principle for any hydrophobic nanoparticles, regardless of the inorganic core material (Pellegrino et al. 2004; Tromsdorf et al. 2007; Lin et al. 2008a).

Nanoparticles can also be directly synthesized in the presence of such polymers (Kairdolf et al. 2008b), or other weakly adsorbing species like poly(vinyl pyrilidone) (Guo et al. 2000). Furthermore, the particle modification by adsorption of polyelectrolytes has been reported, either as additional layer (Sukhanova et al. 2004) or replacing the original surfactant (Zhang et al. 2007; Lin et al. 2008b) of different core materials. As shown by Nann, quantum dots coated with branched PEI could be dispersed in both aqueous and organic solution, the primary amino groups either binding to the particle surface or pointing outwards to solution (Nann 2005).

A variety of other multi-dentate polymers has been demonstrated to coat nanoparticles by direct binding to the inorganic particle surface: hydrophilic and hydrophobic oligomers with phosphine anchor groups (Kim & Bawendi 2003) and a PEG-modified polymeric variant with phosphine oxide that can be used for phase transfer of different core materials to an aqueous solution (Kim et al. 2005e).

Other comb-like polymers contain a mixture of simple aliphatic side chains and others with primary amines at their ends. The polymer can bind to the nanoparticle surface via the amino groups. Additionally, it was modified by fluorescent dye molecules (Potapova et al. 2005). A similar polymer was used to transfer negatively charged quantum dots to organic solution, after electrostatic adsorption of the polymer by its amino groups (Potapova et al. 2003). Also a polymer with tertiary amines and pyrene as a fluorescence marker (Wang et al. 2006) has been shown to stabilize quantum dots in organic solution. In addition, poly(acrylic acid) modified with free thiol and amino groups at the ends of the side chains has been demonstrated as coating for quantum dots, resulting in a relatively thin shell with apparently little effect on the quantum yield of the particles after transfer to the aqueous phase (Smith & Nie 2008).

Alternative to coating particles with amphiphilic or other polymers, nanoparticles with a polymer shell can be obtained by lateral crosslinking or polymerization of the small molecules forming the ligand shell (Mandal et al. 2002; Jiang et al. 2006; Alloisio et al. 2008; Zheng et al. 2008), or in an alternative approach by growing a polymer off the particle surface from the attached ligands (Watson et al. 1999).

Again, yet another class of amphiphilic polymers used for the coating of nanoparticles is block-copolymers, consisting, in general, of a hydrophobic and a hydrophilic part, the latter possibly a polyelectrolyte. These polymers form micellar structures with their hydrophilic or hydrophobic part inside, in contrast to the respective solvent in which they are dispersed. Such structures can be used for the synthesis of nanoparticles (Möller et al. 1996; Stevenson et al. 2001; Rutnakornpituk et al. 2002), for coating (Berret et al. 2006) and for phase transfer. Certain copolymers have also been laterally cross linked (Kim et al. 2005a,b; Cheng et al. 2008). The thickness of the polymer shell can be adjusted.
by the choice of polymers with appropriate block lengths (Kang & Taton 2005). In a number of cases, the coating by block-copolymer micelles does not yield individual particles but from a few to many particles inside the same micelle (Euliss et al. 2003), which in turn can be exploited for the generation of multifunctional objects in the case of nanoparticles of different materials (Kim & Taton 2007). Besides the few examples given here, a much more detailed discussion of block-copolymers, the grafting-from approach and other polymers used for nanoparticle coatings and composite materials can be found in a recent review (Grubbs 2007).

Finally, dextran-coated nanoparticles, in particular iron oxide, are readily obtained by the synthesis carried out in the presence of this polymer, as already mentioned in a previous section, as well as nanoparticles with dendrimer shells. Nanoparticles coated with PEG will be discussed in §3b about chemical surface modification, if not already mentioned previously in the case of PEG-containing small ligands.

(e) Silanization

Nanoparticles of different core materials can also be modified with a silica shell, which can be considered as an inorganic polymer. The method comprises first a ligand exchange procedure in which a first layer of silanes is bound to the nanoparticle surface. Then, using this first layer, a polymeric, cross-linked inorganic silica shell is deposited on the particles, which can be further derivatized. Nanoparticles of different materials (Mulvaney et al. 2000; Graf et al. 2003; Jana et al. 2007), in particular noble metals (Au, Liz-Marzán et al. 1996; Buining et al. 1997; Ag, Hardikar & Matijevic 2000; Han et al. 2008), fluorescent quantum dots (CdSe/ZnS, Gerion et al. 2001; Nann & Mulvaney 2004; Darbandi et al. 2005), phosphorescent (Ehlert et al. 2008) and magnetic nanoparticles (e.g. Fe, Ohmori & Matijevic 1993; Co, Kobayashi et al. 2003; CoFe2O4, Wagner et al. 2002) and particles of different shapes have been coated with silica shells.

This technique is outside the focus of this work; however, in the following sections, many concepts and techniques may also equally apply to silica-coated nanoparticles, since the inner material composition is, in principle, not important for further conjugation steps. More detailed descriptions of successfully applied silanization techniques can be found in the work of the different groups cited above.

(f) Comparison and remarks

The described methods for phase transfer reflect general strategies that, however, may not be applicable to any particular or given particle system. The critical issue of the phase transfer of colloidal nanoparticles is their colloidal stability. Nanoparticles are stabilized in an original phase and shall be transferred to another, non-miscible phase, where the particles are, at first, not able to disperse. In the process of the phase transfer, e.g. by ligand exchange, modification or additional coating, the particles might partially lose their surface properties to be stably dispersed in the original phase, while not yet being ‘compatible’ with the new phase. This is the moment in which aggregation and precipitation of particles can occur, possibly in an irreversible manner.
These considerations imply that, for phase transfer, as well as for ligand exchange in §1c, the binding affinity of the ligand molecules and their geometric shape in relation to the particle diameter has a strong influence on how rapidly the particle surface is changed and how densely the ligand molecules are packed on the particle surface, respectively. Both factors ultimately determine how efficient the phase transfer works and how stable the nanoparticles will be in the new phase.

3. Particle functionalization

(a) Chemical functional groups

Ligand particles stabilizing the nanoparticles against aggregation can simply consist of an inert molecular chain (hydrocarbon chain or PEG) or have functional groups that are, in most cases, terminating linear molecules (see above). In the case of water-soluble nanoparticles, these functional groups are often carboxylic acids stabilizing the nanoparticles by electrostatic repulsion, and can be exploited for the conjugation of other molecules to the particles. Common examples include Au nanoparticles or quantum dots stabilized with mercaptocarboxylic acids.

In the same way, other functional groups can be introduced to the nanoparticle by their ligand molecules or a mixture of different ligands. Already in the organic phase, for instance, the dodecanethiol monolayer of Au nanoparticles can be modified with bifunctional ligands by place-exchange reactions to introduce e.g. bromide, ferrocene, hydroxyl and carboxyl functional groups (Ingram et al. 1997; Templeton et al. 2000).

This strategy can also be applied for phase transfer by ligand exchange, when the incoming ligand already contains the desired functional group, as demonstrated for hydrophobic CdSe/ZnS quantum dots and ligands comprising e.g. –COOH, –OH, –NH2 (Hoshino et al. 2004; Susumu et al. 2007; Howarth et al. 2008), or CdTe and, for instance, a mixture of MPA and aminoethylthiol (Wuister et al. 2003).

Quantum dots in aqueous solution stabilized with MAA have been modified by co-adsorption of thiol-containing PEG and short peptides (Akerman et al. 2002). In the case of lipid-coated quantum dots, a part of the lipids can carry e.g. amino groups or PEG (Dubertret et al. 2002) in order to add additional functionality and steric stabilization.

Iron oxide nanoparticles with (di)mercaptosuccinic acid have been shown to exhibit both carboxylic and thiol functional groups (Fauconnier et al. 1997; Wilhelm et al. 2003).

Another interesting case is quantum dots stabilized with MAA to which a protein (bovine serum albumin; BSA) was adsorbed. The amino groups of the protein could then be exploited for further conjugation chemistry (Gao et al. 2002).

Alternatively, functional groups present on the nanoparticle surface can be converted to other functional groups by bifunctional molecules. Especially in the case of nanoparticles dispersed in an aqueous solution, the reaction conditions may harm the stability of the nanoparticles; so, often, rather mild reactions have to be chosen like the ones applied for the chemical modification of biomolecules (bioconjugation chemistry), and a large number of bifunctional molecules are commercially available (Hermanson 2008). Commonly found carboxylic groups
can be reacted with primary amines by means of a condensation reaction to yield amide bonds. For this, a water-soluble carbodiimide (e.g. EDC) is commonly used, cf. figure 4. After forming an intermediate compound with the carboxylic moiety, the activated group is reactive towards primary amines. In the case of primary amines present on the particle surface, active ester compounds (N-hydroxy-succinimide; NHS) can be used to equally form amide bonds, one example is succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) containing an NHS group reacting with primary amines, converting them to maleimides that are reactive towards thiols (Kuhn et al. 2007), as sketched in figure 4a. There are a number of other crosslinker molecules and different derivatives available, for instance, with sulpho-NHS functionality and/or hydrophilic spacer arms of different length to improve water solubility.

The majority of ligand molecules or additional amphiphilic coatings stabilize the nanoparticles by negatively charged carboxylic groups. These can be converted e.g. to hydroxyl groups (Kairdolf et al. 2008a) or tertiary amines (Yezhelyev et al. 2008). Primary amines are commonly introduced by partial conversation of functional groups, for instance carboxylic acids, by (partial) ligand exchange or adsorption of polyelectrolytes (Wuister et al. 2003; Hoshino et al. 2004; Schellenberger et al. 2004; Sukhanova et al. 2004; Sun et al. 2006b; Susumu et al. 2007; Zhang et al. 2007; Liu et al. 2008). At pH values below the $pK_a$ value of the amines, the particles are positively charged and can be prone to aggregation during the modification steps, e.g. by ligand exchange. Silica coating, however, seems to yield very robust nanoparticles functionalized with primary amines (Buining et al. 1997; Li et al. 2007).
In the last few years, click-chemistry has attracted much attention (Binder & Sachsenhafer 2008; Meldal & Tornøe 2008), and has also been carried out for nanoparticle derivatization in both organic (Binder et al. 2006; Fleming et al. 2006) and aqueous solution (Brennan 2006; Sun et al. 2006a; Polito et al. 2008), as well as to polymer nanoparticles (O’Reilly et al. 2005) and carbon nanotubes (Voggu et al. 2007). The concept of click-chemistry consists of ‘spring-load’-like chemical reactions that occur spontaneously and with high yield and selectivity between stable functional groups under mild conditions (Kolb et al. 2001), perhaps the most common example is between alkyne and azide moieties in the presence of a catalyst (Rostovtsev et al. 2002).

Common to all chemical surface modification schemes involving functional groups that are present on the nanoparticle surface is that they predominantly depend on the ligand shell or surface coating, not on the actual inorganic core material. Therefore, provided that the nanoparticles are stable under the reaction condition and subsequent purification, the same chemical routes for functionalization apply for Au nanoparticles, quantum dots or magnetic particles (Schellenberger et al. 2004; Sun et al. 2006b; Kuhn et al. 2007), as well as for silica nanoparticles (Schiestel et al. 2004).

Since the surface of nanoparticles is covered by a large number of ligand molecules ranging from some tens for small clusters to hundreds or thousands, the number of functional groups per particle generally exceeds a few, is given by a statistic distribution and not always easily accessible by analytic methods. Owing to the multi-valency of nanoparticles, only rather simple structures like particles surrounded by molecules or other particles can be easily generated, or inter-particle crosslinking can occur in experiments with other multi-valent objects.

For a number of applications, monovalent particles or particles with a defined number of functional groups are desirable, and several strategies to prepare such particles have been found in the last years. The principle difficulty is that nanoparticles consist of many identical atoms and a number of equal surface facets; so, in the first instance, all particle modification will be subject to statistics involving competing equivalent binding sites. One obvious approach is, therefore, dilution or stoichiometric control, i.e. to adjust the experimental conditions in a way that only a minor fraction of nanoparticles reacts at all to a certain molecule with the desired function, leaving the other particles unmodified and eventually only a negligible fraction that has two or more modifications. One way is then to separate the modified particles from the unmodified majority, as shown e.g. by Levy et al., where Au nanoparticles modified with a polypeptide could be immobilized on an affinity column by an oligohistidine domain present in the peptide (Levy et al. 2006). After washing out all unbound particles, the immobilized particles could be eluted by addition of imidazole yielding particles modified with a single peptide with a single amino group at its terminal end.

Another way uses methods from solid-phase peptide synthesis where the solid phase (a resin-packed column) is modified with a cleavable, thiol-terminated molecule at very low grafting density (Shaffer et al. 2004; Sung et al. 2004; Worden et al. 2004). When the distance between two thiol-terminated molecules is (on average) orders of magnitudes larger than the particle diameter, every gold nanoparticle binding to the resin via a thiol-gold linkage is only bound by one single molecule. After washing out all unbound particles, the linker arm is cleaved...
and the nanoparticles can be eluted from the column, each particle carrying one single fragment of the linker arm exhibiting a single free functional group. A variation of this concept includes ligand molecules electrostatically adsorbed on the solid phase (Liu et al. 2006; Zou et al. 2007).

Alternatively, there are a number of systems and separation methods that allow the statistical binding of a few ligand molecules to the nanoparticles and the subsequent separation according to the number of attached molecules. For this, each attached molecule has to change at least one property of the particle sufficiently in order to allow separation, e.g. by size or electric charge (Surugua & Urban 2009). This concept has probably been first developed for small gold clusters of which fractions with a different number of e.g. amine-containing ligands could be separated by ion-exchange chromatography (Safer et al. 1986). Monofunctional gold clusters prepared by this method have already been commercially available for several years.

As shown for larger gold nanoparticles modified by single-stranded DNA oligomers with a thiol function, particles with exactly 0, 1, 2, 3, ... attached DNA molecules could be separated by gel electrophoresis (Zanchet et al. 2001). The attachment of each additional DNA molecule renders the Au nanoparticle significantly larger, so that the fractions appear as discrete bands on the gel. Depending on the nanoparticle size, this effect appears above a certain molecular weight or number of base pairs of the DNA, respectively. Dependent on the agarose concentration, separation is observed, for instance, with 43 bases in the case of 10 nm, but not of 20 nm nanoparticles (Pellegrino et al. 2007), or by means of a long extension strand hybridized to the short first (Aldaye & Sleiman 2007). The fractions of particles can be extracted from the gel and the defined number of functional groups (DNA molecules here) has been probed by the controlled assembly of particle groupings (Zanchet et al. 2002; Aldaye & Sleiman 2006, 2007). The restriction to DNA molecules of a certain length that allow the complete separation of the different fractions has recently been elegantly overcome by ion-exchange chromatography (Claridge et al. 2008), demonstrating the separation of 20 nm Au nanoparticles with a defined number of short DNA molecules (15 bases).

Separation by gel electrophoresis has been carried out for polymer-coated nanoparticles that have been modified with PEG (Sperling et al. 2006). When the PEG had a molecular weight more than or equal to 5000 g mol\(^{-1}\), discrete bands could be separated by gel electrophoresis, and by employing bifunctional PEG, nanoparticles with a defined number of chemical reactive groups could be prepared (cf. figure 5). Based on the same principle, quantum dots with a defined number of maltose binding protein (Pons et al. 2006b) and a single monovalent streptavidin molecule have been demonstrated recently (Howarth et al. 2008), which allowed the preparation of quantum dots conjugated with single antibodies.

Finally, it is worth mentioning two other concepts that have been recently developed. One is exploiting a general topological feature for the attachment of two functional groups by exchanging two ligands at singular positions of the nanoparticle surface (DeVries et al. 2007; Nakata et al. 2008). The other one is based on polymerizing the vinyl-thiophenol ligand molecules attached to the nanoparticle surface, resulting in nanoparticles with only one single carboxylic group left, and only smaller fractions or particles with two or more groups (Krüger et al. 2008).
Figure 5. (a) Gel electrophoresis of polymer-coated, PEG-modified Au and CdSe/ZnS conjugates. Stoichiometric ratio of EDC/nanoparticle (NP) was increased from top to bottom, yielding nanoparticles with an increased number of covalently bound PEG molecules. With a molecular weight of more than 5000 g mol⁻¹, discrete bands of particles with exactly 0, 1, 2, 3, ... PEG molecules are resolved. At very high EDC concentrations, the nanoparticles are saturated with PEG and migrate towards the negative electrode. (b) With bifunctional PEG molecules, this allows for the preparation of nanoparticles with controlled valency, i.e. a defined number of functional groups. Nanoparticles modified with H₂N-PEG-NH₂ yield nanoparticles with exactly one or two amino groups, separated by gel electrophoresis (Sperling et al. 2006). MW is molecular weight.

(b) Poly(ethylene glycol)

PEG is a linear polymer consisting of repeated units of –CH₂–CH₂–O--; depending on the molecular weight, the same molecular structure is also termed poly(ethylene oxide) or polyoxyethylene. The polymer is well soluble in a number of organic polar and apolar solvents, as well as in water where it is heavily hydrated, forming random coils with diameters much larger than proteins of the corresponding molecular weight. Owing to its simple structure and chemical stability, it is a prototype of an inert, biocompatible polymer. The inertness and non-toxic properties of PEG give rise to a number of applications in medicine, chemistry or biotechnology. PEG is used for non-ionic surfactants and as an additive in cosmetics, pharmaceuticals and food. When bound to surfaces (Zareie et al. 2008), PEG repels other molecules by steric effects; the incoming molecule is not attracted by e.g. electrostatic force and cannot penetrate the hydrated PEG layer. This results in inert hydrophilic surfaces with less ‘stickyness’. The same applies for molecules or particles: PEG-modified proteins (Veronese 2001) or drugs show increased water solubility and decreased immunogenicity in organisms, antibodies bind to a much lesser extent to the drug or protein, resulting e.g. in an increased half-life in the blood stream. PEG-modified nanoparticles are more stable at high salt concentrations and in biological environments; they show less non-specific binding to proteins and cells (Gref et al. 2000; Ballou et al. 2004; Liu et al. 2007b, 2008; van Vlerken et al. 2007; Daou et al. 2009).

The modification of other molecules with PEG is often referred to as ‘PEGylation’; for proteins, PEG with functional groups is coupled to certain amino acids, most commonly lysine and cysteine, exhibiting amino and cysteine groups, respectively. An increasing variety of mono-, homo- and heterobifunctional PEG reagents is commercially available, with different functional groups,
molecular weights and multiple arms (Roberts et al. 2002). Those reagents can be used for the PEGylation of synthetic nanoparticles in the same way as for proteins and coupled to available functional groups on the nanoparticle surface, provided by the ligands or additional shells (Ballou et al. 2004; Gao et al. 2004).

While monofunctional PEG molecules, e.g. with a methoxy group at the free end, yield basically more stable and inert particles (Hirsch et al. 2003; Niidome et al. 2006), bifunctional PEG molecules can be used to introduce new functional groups on the surface, like with bifunctional crosslinkers in conjugation chemistry (Jayagopal et al. 2007). In particular, mixed PEG layers of mono- and bifunctional molecules can be useful because they stabilize the particles sterically by PEG molecules without free functional end and provide a number of new functional groups by the bifunctional PEG molecules (Sperling et al. 2006; Pierrat et al. 2007; Liu et al. 2008). Again, the conjugation chemistry used for PEGylation and the further modification does not depend any more on the material of the particle cores and applies in the same way for other particle species (Butterworth et al. 2001; Zillies et al. 2007).

Apart from the post-modification approach by covalent chemistry, PEG-modified nanoparticles can also be obtained by ligand molecules that contain a block of PEG (Uyeda et al. 2005; Susumu et al. 2007; Howarth et al. 2008) or that consist entirely of PEG with a functional group that can bind to the nanoparticle surface (Kanaras et al. 2002; Liu et al. 2007b; Qi et al. 2008). Again, particle synthesis can already be carried out in the presence of these ligands (Wuelfing et al. 1998; Sakura et al. 2005); new PEG-containing ligands can be introduced by place-exchange reactions or added as additional molecules such as lipids (Dubertret et al. 2002; Srinivasan et al. 2006) or polymers (Duan & Nie 2007) that have been modified with PEG before used for the coating process (Lin et al. 2008a). As already discussed, such PEG-containing ligands can be used for phase transfer of nanoparticles (Kanaras et al. 2002; Skaff & Emrick 2003) and owing to the solubility of PEG itself, PEG-coated nanoparticles can also be dispersed in polar organic solvents such as chloroform, methanol, dimethyl sulphoxide and dimethylformamide (Kim et al. 2005d).

As for any additional shell, the overall particle diameter is increased by PEG modification. Increasing grafting density and molecular weight of the employed PEG molecules yields thicker shells that can be found to be of the order of a few to tens of nanometers (Sperling et al. 2007), the thickness of about the order of the hydrodynamic diameter of a free PEG molecule forming a random coil (Fee & Alstine 2004; Fee 2007). In figure 6 (left), PEG with a molecular weight of 2000 and 5000 g mol$^{-1}$ is drawn to scale with a 10 nm nanoparticle (core diameter: 5 nm).

(c) Biomolecules

Bioconjugation of colloidal nanoparticles is the ‘natural’ extension of the described concepts of ligand exchange and chemical functionalization to biomolecules. Nature offers a large variety of organic molecules of different composition, size and complexity that serve to provide structure and function to biological process and organisms. Examples include, on the one hand, small molecules like lipids, vitamins, peptides, sugars and larger ones such as natural polymers including proteins, enzymes, DNA and RNA.

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Conjugation of inorganic nanoparticles to biomolecules generates hybrid materials that can be used to let the nanoparticles interact specifically with biological systems. On the other hand, biomolecules can be seen as ordinary, though sometimes complex, molecules or polymers that can be exploited for the functionalization or spatial assembly of nanoparticles. Nanoparticle–biomolecule conjugates bring together the unique properties and functionality of both materials, e.g. fluorescence or magnetic moment of the inorganic particles and e.g. the ability of biomolecules for highly specific binding by molecular recognition.

The strategy for the conjugation of biomolecules to nanoparticles generally falls into four classes:

- ligand-like binding to the surface of the inorganic particle core, commonly by chemisorption of e.g. thiol groups,
- electrostatic adsorption of positively charged biomolecules to negatively charged nanoparticles or vice versa,
Figure 7. (a) (i) Structure of biotin and (ii) streptavidin. Streptavidin is an approximately 53 kgg mol\(^{-1}\) protein of four identical barrel-like subunits, each tetrameric protein has four binding sites for biotin. (b) Schematic drawing of nitrioltriacetic acid (NTA)–Ni\(^{2+}\) attached to nanoparticles forming a complex with a protein with two terminal histidine residues (image of NTA-histidine complex adopted from Hainfeld et al. 1999). All objects are not drawn to scale with respect to each other.

— covalent binding by conjugation chemistry, exploiting functional groups on both particle and biomolecules, and

— non-covalent, affinity-based receptor-ligand systems, as discussed in the following.

Besides the first three approaches, affinity-based systems found in nature have attracted increasing attention during past years. Maybe the most well-known example in the last several decades is the avidin–biotin system (Green 1975; Wilchek & Bayer, 1988, 1989, 1990). Based on molecular recognition, the system consists of a ligand, the small molecule biotin (vitamin H), and a receptor, the protein avidin that is present e.g. in egg white. The globular protein avidin is made up by four identical subunits, yielding four binding pockets that specifically recognize and bind to biotin (figure 7a). The dissociation constant is of the order of 10\(^{15}\) M and the bond, though not covalent, is found to be extremely stable, resisting harsh chemical conditions and elevated temperature. Besides the natural glycoprotein avidin (pI \(\sim\) 10), analogue proteins expressed in bacteria or recombinant proteins without carbohydrates and a near-neutral PI are available, the most common being streptavidin and neutravidin. Also monomeric streptavidin with, however, reduced affinity to biotin has been reported (Wu & Wong 2005). By genetic modification resulting in deactivated binding pockets, a tetrameric but monovalent streptavidin derivative with only one single binding site for biotin has also been recently demonstrated (Howarth & Ting 2008).

The strong bond and specificity of the biotin–avidin system has allowed researchers to employ it for a large number of applications in bio(nano)technology, and a large variety of biotinylation reagents and biomolecules like DNA oligomers, peptides, antibodies and fluorescent dyes readily modified with biotin or avidin (or one of its derivatives) are commercially available. Nanoparticles modified with biotin or avidin enable these materials to be used within the technologically already well-established conjugation platform.
So-called protein tags are another class of such affinity-based systems and commonly used for protein purification: proteins can be expressed with additional fusion proteins or short amino acid sequences by genetic engineering in order to manipulate the desired protein by this handle-like tag. One example is the protein O\(^6\)-alkylguanine-DNA-alkyltransferase (hAGT) that binds specifically and eventually by a covalent bond to benzylguanine (Keppeler et al. 2003; Kufer et al. 2005). Another well-known system is polyhistidine, commonly introduced by six or more histidine residues to one of the terminal ends of a protein. Polyhistidine is found to bind strongly and specifically to nitrilotriacetic acid (NTA) via a chelation complex with Ni\(^{2+}\) or other bivalent metal ions, as sketched in figure 7b (Schmitt et al. 2000). This system is widely used for affinity purification of proteins that have been expressed with this polyhistidine tag: these proteins are specifically immobilized on a column with Ni–NTA. After washing out other unmodified molecules, the desired protein is eluted with an excess of imidazole that competes with polyhistidine in binding to the Ni–NTA complex. Systematic studies with two, three or more NTA groups (Lata et al. 2005) revealed that three NTA moieties can bind to a strand of six histidines, resulting in a stable bond of higher binding energy compared with other stoichiometries.

A more detailed review about the NTA-polyhistidine system can be found in the seminal work of Hainfeld et al. who modified small Au clusters with NTA-terminated ligands (Hainfeld et al. 1999), which were then used to label proteins by their polyhistidine tag for electron microscopy. In the following, larger Au nanoparticles (Brinas et al. 2008) and quantum dots (Gupta et al. 2008; Kim et al. 2008b) have also been modified with NTA-containing ligands, as well as silanized iron oxide nanoparticles (Li et al. 2007) by conjugation of amine-containing NTA to carboxylic groups on the particle surface. After loading the NTA groups with bivalent cations, the modified particles could be used for conjugation to proteins with polyhistidine residues. It remains to be noted that in many of the systems mentioned here, in fact several single NTA moieties were present that may bind together to the same oligohistidine residues of the proteins.

Mattoussi et al. reported the specific and strong binding of polyhistidine-containing proteins to CdSe/ZnS nanoparticles with a dihydrolipid acid (DHLA) ligand layer without NTA or bivalent ions present (Mattoussi et al. 2000). Recently, it was shown that the polyhistidine moiety can directly bind to the inorganic particle, apparently to Zn atoms present in the ZnS shell, as demonstrated by control experiments with different target molecules and ligands without NTA or free carboxylic groups (Sapsford et al. 2007). In this seemingly robust self-assembly approach, no conjugation chemistry and only few purification steps are necessary, which led to the conjugation of quantum dots with a number of different proteins for a variety of applications (Pons et al. 2006a; Liu et al. 2008).

(i) Biotin, avidin and derivatives

The biotin–avidin system consists of a small molecule (biotin) and a protein (avidin), either with or without carbohydrates (streptavidin, neutravidin and other derivatives). Here, in order to avoid later redundancy, both species serve as examples for the discussion of different conjugation strategies of small and large biomolecules to nanoparticles, which apply accordingly to a number of
different biomolecules such as DNA, antibodies, peptides or generic proteins. Afterwards, the linking of those different classes of biomolecules to nanoparticles by the same strategies, including the biotin–avidin system itself in addition to NTA-polyhistidine, will be reviewed.

Biotin as a small molecule with one free carboxylic group, also readily available with a number of modifications such as –NH₂ or –NHS, is covalently bound to the nanoparticle by conjugation chemistry (Skaff & Emrick 2003; Aslan et al. 2004) or directly to the inorganic particle surface by accordingly modified ligand molecules (Pinaud et al. 2004), as discussed previously. For the conjugation, only physicochemical properties such as solubility, charge or functional groups of the biotin molecule are of importance, not necessarily the biological functionality or related consequences.

Avidin, streptavidin and other variants are regular proteins characterized by their molecular weight, isoelectric point, degree of hydrophobicity and available functional amino acid residues commonly consisting of carboxylic acids, amino groups and thiols.

As zwitterionic molecules, proteins are positively charged at pH values below their isoelectric point and negatively above. This offers the possibility to adsorb avidin electrostatically to negatively charged nanoparticles, while streptavidin and neutravidin with a lower pI were reported to bind non-specifically to nanoparticles to a significantly lesser extent (Goldman et al. 2002; Lin et al. 2004b). To improve the stability of the nanoparticle–protein complex, an additional covalent crosslinking has been performed on a similar system (Herr et al. 2006).

By exploiting the functional groups on the protein surface, streptavidin has been covalently linked to quantum dots with carboxylic groups (Kim & Bawendi 2003; Wu et al. 2003; Meiser et al. 2004) or primary amines (Liu et al. 2008), commonly by EDC/NHS. Alternatively, streptavidin has been bound by direct adsorption to the inorganic quantum dot surface via a polyhistidine tag (Liu et al. 2008), also in the case of monovalent streptavidin (Howarth et al. 2008).

Naturally, biotin-modified nanoparticles can be decorated with an additional shell of streptavidin (Skaff & Emrick 2003; Pinaud et al. 2004) when added in excess. As demonstrated by the authors, nanoparticles modified with larger numbers of biotin molecules can form large aggregates with streptavidin by inter-particle crosslinking, owing to the multi-valency of both the nanoparticles and streptavidin. This effect is not only found for nanoparticles with biotin–avidin, but presents a general problem in the case of two or more all multi-valent binding partners, as well as for covalent conjugation, where often both nanoparticles and target molecules have a larger number of reactive functional groups, e.g. carboxylic acids and amines, as also in the case of proteins.

(ii) DNA

DNA is a linear polymer containing the genetic information of organisms in the form of the sequence of the four oligonucleotides being the monomeric building blocks, as analogously for RNA. Besides its biological function, DNA and RNA can be employed as generic polymeric molecules of which the most prominent feature, duplex formation with a strand of complementary sequence, presents a very specific mechanism of molecular recognition. As this mechanism depends on
the simple linear sequence of nucleotides, DNA can be used as a ‘programmable’ object with a very large number of possible sequences and conformations, and exploited e.g. as a building block and structural element for the assembly of artificial structures.

Synthetic DNA oligomers of arbitrary sequences and with a large variety of functional end groups, which are commercially available, can be conveniently attached in an aqueous solution to gold nanoparticles by a thiol–gold bond, in the same way as a place-exchange reaction already discussed for other ligand molecules (Mirkin et al. 1996; Demers et al. 2000; He et al. 2000; Jin et al. 2003). DNA oligomers are usually added in quite large excess to the gold particles and spontaneously bind to the nanocrystal surface. Variations include DNA oligomers modified by a cyclic steroid derivative with two thiols groups (Letsinger et al. 2000) or by up to three mercaptohexyl linkers (Li et al. 2002).

The same strategy of ligand exchange has also been applied to Au rods (Dujardin et al. 2001), silver nanoparticles (Thompson et al. 2008), MPA-stabilized CdSe/ZnS quantum dots (Mitchell et al. 1999; Gill et al. 2005) and Pt nanoparticles decorated with amino-modified DNA (Yang et al. 2004).

Even under optimized conditions (Hurst et al. 2006), the binding of DNA to nanoparticles does not occur quantitatively; however, the DNA density can be influenced by adjusting the excess ratio or by dilution with other ligands, e.g. short ‘spacer’ oligomers (Peña et al. 2002). In the case of rather long DNA strands, attachment of a single DNA molecule increases the size of the Au–DNA conjugate sufficiently to separate discrete bands by gel electrophoresis, consisting of nanoparticles with exactly 0, 1, 2, 3, . . . DNA molecules per particle (Aldaye & Sleiman 2007). Recently, the separation of such conjugates has been demonstrated by ion-exchange chromatography, enabling the separation of nanoparticles with a defined number of shorter DNA oligomers.

While double-stranded DNA forms its characteristic double-helix structure by Watson–Crick basepairing, single-stranded DNA is more flexible (Steel et al. 2000), and can take a curved or coiled conformation. In figure 6 (bottom left), a 20mer ssDNA strand is displayed in scale with a 5 or 10 nm nanoparticle, respectively. Single-stranded DNA attached to nanoparticles is found to undergo a stretching when an increased surface coverage induces steric pressure, or when it is hybridized with a complementary strand, which results in a stiffer double helix (Peña et al. 2002; Parak et al. 2003; Pellegrino et al. 2007).

Owing to the large numbers of different possible sequences for a DNA or RNA strand of given length, there also exists an even larger variety of possible conformations (in terms of secondary and tertiary structure) of these linear molecules. It has been found that certain sequences can strongly bind to a target molecule by molecular recognition, determined by geometric matching of the surfaces of the two molecules. This interaction is mediated by electrostatic, hydrophobic van der Waals forces or hydrogen bonds. This can be exploited to generate the so-called aptamer sequences to a given target molecule by molecular evolution, technically realized by multiple randomization, selection and amplification of strongly binding sequences, resulting in an optimized strand of DNA, RNA or peptide for the target molecule with affinities comparable to antibodies (Bunka & Stockley 2006; Lu & Liu 2006; Mairal et al. 2008). Aptamers have been attached to gold nanoparticles via a thiol function (Liu et al. 2007a; Zhao et al. 2008), to quantum dots or silica-coated Au particles.
by covalent conjugation chemistry (Bagalkot et al. 2007; Jana & Ying 2008), to avidin-modified magnetic nanoparticles (Herr et al. 2006), as well as biotinylated DNA aptamers to quantum dots with streptavidin (Levy et al. 2005).

Owing to its phosphate backbone, DNA is negatively charged and essentially a polyelectrolyte molecule. Naturally, this results in electrostatic adsorption of DNA to positively charged surfaces, such as nanoparticles with quaternary amines (McIntosh et al. 2001; Sandhu et al. 2002; Wang & Murray 2004), as that of RNA to nanoparticles with tertiary amines (Qi & Gao 2008; Yezhelyev et al. 2008) as shown. However, non-specific, i.e. generally unwanted, adsorption of DNA to nanoparticles has also been found, especially when incubated at high stoichiometric excess (Parak et al. 2003; Pellegrino et al. 2007) and also in the case of single nucleotides (Storhoff et al. 2002).

Besides the ligand-like direct binding of DNA to the nanoparticle surface, conjugation chemistry can also be employed to covalently bind the DNA to functional groups available on the nanoparticle surface. This has been carried out with EDC chemistry to bind amino-functionalized DNA to nanoparticles with carboxylic groups, and thiol-modified DNA to maleimide groups (Alivisatos et al. 1996; Srinivasan et al. 2006). Alternatively, nanoparticle derivatization can be carried out in organic solution (Pathak et al. 2001) prior to the coupling to amino-modified DNA oligomers, or the 5′ end phosphate group of DNA can be reacted with EDC and imidazole to primary amino groups (Hermanson 2008) present on the particle surface (Skaff & Emrick 2003). Apart from covalent conjugation chemistry, avidin–biotin has also been used as the non-covalent receptor-ligand system for the binding of DNA to nanoparticles, both with biotin-modified DNA (Levy et al. 2005) and biotin-modified nanoparticles (Niemeyer et al. 1998).

(iii) Peptides, proteins, enzymes and antibodies

Peptides and proteins are polymeric compounds of amino acids, linked to linear sequences by amide bonds. Short sequences, peptides usually consisting of up to 50–100 amino acids, are commercially available by custom synthesis, while proteins are usually found in the form of larger poly-amino acids exhibiting a tertiary and possibly quaternary structure. However, there are also rather small proteins and there is no general and well-defined differentiation between those terms. Special classes of proteins comprise enzymes and antibodies. Enzymes are highly specialized molecules with reactive centres that catalyse biochemical reactions and are responsible for metabolism. Antibodies, also known as immunoglobulins, are large Y-shaped proteins with important functions in the immune system. They have the ability to specifically bind, using their active region, to antigens, in principle arbitrary target molecules, mediated by molecular recognition. Other proteins are used by the cell, e.g. for signalling or structure formation.

Proteins are generally made up by a sequence of 20 different standard amino acids (in addition to other naturally occurring or synthetic amino acids) that are linked together by amide bonds and possess different side-chain residues. Naturally, each peptide or protein has one carboxylic and one primary amino group at its ends, while the amino acid side chains introduce additional functional groups or other properties, depending on their molecular structure. The amino
acid sequence determines the unique properties of each of a large number of possible structures, i.e. \(20^n\) for a sequence of \(n\) amino acids, in terms of charge, polarity and hydrophobicity. These, in turn, determine the secondary and tertiary structure that a protein is folding into and that ultimately results in the functional biomolecule. In many cases, the specific function of a protein (enzyme, antibody) is determined by its geometric and physicochemical properties of the outer surface, given by the almost arbitrary motifs of the folded amino acid sequence. Often the inside of a protein is hydrophobic, while hydrophilic amino acid side chains tend to point outwards into solution, while for instance membrane proteins generally have a partially hydrophobic surface. Cysteine residues, even if far apart in the sequence, can come spatially close to each other in folded proteins and form stabilizing disulphide bonds. The thiol group of a terminal cysteine residue can also be exploited as the anchor group for the attachment of a peptide to the surface of nanoparticles.

This clearly makes peptides and proteins interesting objects to be combined with inorganic nanoparticles, both for basic research and applications that make use of the specific functions of these biomolecules.

Peptides as rather small molecules with a ‘programmable’ sequence of amino acids allow the rational design of ligand molecules that are optimized to stabilize nanoparticles (Levy et al. 2004) or introduce various functional groups (Wang et al. 2005; Medintz et al. 2006; Garanger et al. 2008). In addition, examples for particle synthesis (Slocik et al. 2005) and phase transfer (Euliss et al. 2003) with the help of peptides have been reported. Making use of the biological functionality of certain peptides, the specific uptake of nanoparticles by cells can be optimized by conjugation of nanoparticles with the corresponding peptide, as reported for Au nanoparticles (de la Fuente & Berry 2005; Liu et al. 2007b; Nativo et al. 2008) or quantum dots (Pinaud et al. 2004) by ligand exchange with cysteine-containing peptides, as well as the reaction of the immune system towards nanoparticles may be modulated by the peptide coating (Bastus et al. 2009). Also, coating of quantum dots with lipids pre-modified with e.g. trans-activator of transcription (Tat) peptide has been reported (Stroh et al. 2005). Peptide modification can also be carried by covalent conjugation chemistry, as demonstrated for quantum dots with amino groups (Cai et al. 2006) or magnetic iron oxide nanoparticles (Josephson et al. 1999; Zhao et al. 2002a; Schellenberger et al. 2004). In addition, quantum dots modified with streptavidin (which are also commercially available) have been conjugated to different biotinylated peptides (Chen & Gerion 2004; Lagerholm et al. 2004; Vu et al. 2005; Kim et al. 2008c). Small monofunctional Au cluster have been modified by conjugation chemistry with a peptide containing a polyhistidine tag that bounds to the inorganic surface of CdSe/ZnS quantum dots (Pons et al. 2007; Sapsford et al. 2007), or alternatively to quantum dots modified with NTA (Li et al. 2007). Figure 2 illustrates the relative size of a short peptide (five amino acids) in respect to a 5 nm nanoparticle; figure 6 shows a number of different proteins in comparison with a 5 or 10 nm nanoparticle.

Several strategies are available for conjugation of proteins to nanoparticles, including enzymes or antibodies. First, ‘non-specific’ adsorption can be employed: the nanoparticles are incubated with the protein, which adsorbs to the particles by electrostatic attraction if both partners are oppositely charged, by van der Waals forces, hydrogen bridges, gold–thiol bonds (from cysteine residues) or
by hydrophobic interaction, e.g. when the pH is close to the pI of the protein or the nanoparticle so that the electrostatic repulsion is reduced. After adsorption, the protein can be irreversibly immobilized by those forces or a combination of them (Gole et al. 2001). Potentially, the protein can get into intimate contact with the particle surface by partial or complete denaturation (Gao et al. 2002), giving rise e.g. to hydrophobic interaction of the inner part of the protein and/or an increased contact area between the binding partners. Electrostatic binding has been demonstrated e.g. for protease to MPA-modified quantum dots (Lin et al. 2003), and desorption of proteins can be triggered by increasing the electrolyte concentration that effectively shields the attractive electrostatic interaction (Bucak et al. 2003). Traditionally, these effects have been exploited for the preparation of the so-called immunogold (Geoghegan 1988), and small Au nanoparticles conjugated with antibodies that have been used as labels for immunostaining in electron microscopy (Faulk & Taylor 1971; Putman et al. 1993; Hermann et al. 1996; Roth 1996; Ni et al. 1999; He et al. 2008) and have already been commercially available for many years. A related issue is surface passivation or ‘blocking’, carried out with proteins that are inert and do not interfere with the assay aimed-at, which bind to ‘sticky’ surface spots and thus prevent undesired binding of functional proteins. Common examples are serum albumins (bovine or from other sources Hanaki et al. 2003), which have also been used to functionalize nanoparticles with amino groups originating from their lysine residues (Gao et al. 2002). Besides intended protein conjugation, nanoparticles entering organisms are found to be spontaneously coated by serum proteins (Cedervall et al. 2007; Lees et al. 2008; Lundqvist et al. 2008; Röcker et al. 2009).

Examples of modification of nanoparticles with proteins by covalent conjugation chemistry include transferrin on quantum dots (Chan & Nie 1998; Kloepfer et al. 2003; Jiang et al. 2006) or rods (Yong et al. 2007), bungarotoxin (Casanova et al. 2007) and also magnetic nanoparticles possessing amino groups that have been modified by enzymes (Kuhn et al. 2007). By click-chemistry, lipase has been conjugated to Au nanoparticles (Brennan 2006), as well as peptides to gold rods (Oyelere et al. 2007).

Streptavidin-functionalized nanoparticles have been used to label the biotinylated motor protein kinesin with quantum dots (Courty et al. 2006), or biotinylated epidermal growth factor (Lidke et al. 2004).

Proteins with a polyhistidine modification have been bound to nanoparticles modified by NTA, e.g. adenovirus knob protein to small Au clusters (Hainfeld et al. 1999), the enzyme glutathione S-transferase (Gupta et al. 2008) to quantum dots or by direct binding of the polyhistidine tag to the Zn-containing inorganic surface of CdSe/ZnS quantum dots as discussed before, e.g. for maltose binding protein (Mattoussi et al. 2000; Pons et al. 2006a) or fluorescent proteins (Dennis & Bao 2008).

Antibodies, besides adsorbed to gold nanoparticles as mentioned above, have been conjugated to quantum dots covalently by EDC (Sukhanova et al. 2002; Wu et al. 2003), as well as to magnetic-fluorescent composites (Wang et al. 2004) and by bifunctional crosslinkers to thiol-containing, silanized CdTe quantum dots (Wolcott et al. 2006). Antibody fragments possessing free thiol groups have been bound to free amino groups of quantum dots by means of a heterobifunctional crosslinker (Jayagopal et al. 2007), and biotinylated antibodies

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to streptavidin-modified quantum dots (Dahan et al. 2003). More protocols and a comparison of the different approaches have been carried out recently (Xing et al. 2007).

Silica-coated nanoparticles of different core materials with amino groups have been modified with antibodies by bis-NHS reagents (Jana et al. 2007). As shown by Weissleder et al. (Perez et al. 2004), dextran-coated magnetic iron oxide nanoparticles can be covalently conjugated with peptides (Josephson et al. 1999), oligonucleotides (Josephson et al. 2001), proteins and antibodies (Perez et al. 2003).

Gold nanorods have been modified first with methoxy-PEG-thiol and thiolated antibodies (Liao & Hafner 2005), or PEG with free biotin or thiol groups to which then streptavidin or other gold spheres could be bound (Pierrat et al. 2007).

Small biomolecules include quantum dots modified with dopamine via EDC chemistry (Clarke et al. 2006, 2008), with serotonin via a PEG spacer (Rosenthal et al. 2002) or gold nanoparticles decorated with sugar molecules (de la Fuente et al. 2005).

Further examples can be found in a number of reviews about bioconjugation of gold nanoparticles (Niemeyer 2001), quantum dots (Gao et al. 2005) or nanoparticle-enzyme conjugates (Ghadiali & Stevens 2008).

(d) Fluorescent dyes and other functions, multi-functional particles

Apart from chemical functional groups or biomolecules, nanoparticles have also been modified with a number of other functionalities. Common examples include fluorescent dyes (Templeton et al. 1999; Gill et al. 2005; Nikiforov & Beechem 2006; Fernández-Argüelles et al. 2007; Ren et al. 2008) or fluorescent proteins (Dennis & Bao 2008) that can be used for fluorescence labelling of non-fluorescent particles (Schellenberger et al. 2004; Garanger et al. 2008) or to generate systems exhibiting energy transfer. Common examples include fluorescent dyes that are quenched by gold nanoparticles (Dulkeith et al. 2002, 2005) or that can be excited by fluorescence resonant energy transfer via a quantum dot serving as donor (Funston et al. 2008; Liu et al. 2008), for instance for biosensors. Other functions include paramagnetic ligand molecules (Mulder et al. 2006a) or chelator molecules for radionuclides (Schipper et al. 2007; Shokeen et al. 2008).

In summary, nanoparticle synthesis, phase transfer, functionalization and bioconjugation all have some common aspects and overlap, e.g. with regards to the binding of organic ligand molecules to the inorganic particle surface, and often there, they depend on each other since those ligand molecules introduce chemical functionality, as finally do complex molecules, which again will determine the physico-chemical properties of the resulting particles.

The motivation for particle modification is the control over the interaction of the particles with their environment, which is naturally taking place at the particle surface. By appropriate modification, phase transfer, specific and non-specific binding to target molecules or surfaces, biomolecules or cells can be tuned, e.g. for the controlled targeting or assembly of nanoparticles. Furthermore, more functionality can be added to the properties the particle inherently has from its core material, e.g. for biological impact (for drug delivery or therapy) or fluorescence emission for non-luminescent materials. For multi-functional
particles, three approaches can be identified: (i) composite materials generated *in situ* during synthesis, e.g. by growing nanocrystals with domains of different functional materials (Gu *et al.* 2004; Kim *et al.* 2005c; Zanella *et al.* 2008), (ii) post-modification of particles with functional molecules, e.g. fluorescent quantum dots with paramagnetic organic molecules (Mulder *et al.* 2006a; Bakalova *et al.* 2007) or non-fluorescent nanoparticles with fluorescent dyes (Bertorelle *et al.* 2006; Bagalkot *et al.* 2007; Kim *et al.* 2008a; Liong *et al.* 2008), bio-functional molecules (Schellenberger *et al.* 2004; Herr *et al.* 2006; Garanger *et al.* 2008), or (iii) assembly of composite materials e.g. by a combination of different nanoparticles with different functionality (Wang *et al.* 2004), or e.g. microbeads or capsules loaded with a mixture of different classes of nanoparticles or functional molecules (Xie *et al.* 2005; Sukhorukov *et al.* 2007).

While, in the first approach, certain material properties, e.g. differences in lattice parameters, might be limiting for the crystal growth, the second offers great flexibility for the price of finding robust systems and the appropriate conditions for the nanoparticle modification, the third usually results in objects rather large compared with single nanoparticles.

*(e) Characterization*

Nanoparticle surface modification often directly affects other physical properties of the particle that can be used for characterization of the modification. As nanoparticle characterization could serve as the topic for its own dedicated review, only a few examples will be given in the following.

Maybe the most basic property of a nanoparticle, besides its material, is its size, i.e. diameter. By modification of the nanoparticle surface, the size of the particle is often prone to change. The size of nanoparticles is classically characterized by transmission electron microscopy (TEM); however, this technique is rather suitable for inorganic particles but less for organic molecules that originate from the surfactant layer or that provide other functionalization, as in the case of any nanoparticle–biomolecule conjugates. Owing to their smaller electron density, organic molecules provide only poor contrast in TEM and may be stained with heavy elements (Dubertret *et al.* 2002); in addition, samples have to be prepared in a dry state for observation under vacuum and will thus differ from the natural colloidal state in solution, e.g. by interdigitated ligand molecules (Fink *et al.* 1998).

(Sperling et al. 2007). A number of methods are limited to certain material classes as fluorescence particles, may be not compatible with them or their application may be hindered by the limited colloidal stability of the particles. Engineered nanoparticles consist many times of core-shell systems, and surface modification in terms of functionalization or bioconjugation adds more complexity to their physicochemical properties. Thus, quantitative size characterization of such particles may be more difficult compared with homogenous particles of different sizes, which only consist of one material. In any case, control experiments and the critical comparison of different methods can yield reliable results, even though precise absolute numbers may be difficult to obtain for particles with diameters of only a few nanometers.

As to chemical functional groups, there are a number of colorimetric assays reported that allow for the quantification of amine groups, thiols and others (Ballou et al. 2004; Jayagopal et al. 2007). Possibly, a chromophore released by the reaction with the target analyte (Zhao et al. 2002a; Maus et al. 2009) has to be separated from the nanoparticles because of overlapping absorption spectra. Furthermore, binding of ligand molecules to metal nanoparticles can be probed by surface-enhanced Raman scattering (Oyelere et al. 2007).

Other functional molecules, in particular biomolecules, present on the particle surface can be assayed by their specific function, e.g. binding capability to a substrate, as demonstrated for target molecules immobilized on flat surfaces or microbeads in solution. Again, the attachment of larger biomolecules can be observed by the increase of the total particle size as discussed before. In combination with organic fluorophores conjugated to the molecule of interest, the presence of those molecules on the particle surface can be accessed by the absorption or fluorescence emission (Demers et al. 2000; Meiser et al. 2004; Sari et al. 2004; Hurst et al. 2006), or by stepwise photobleaching of the organic dye (Casanova et al. 2007).

4. Applications, outlook

Applications of colloidal nanoparticles, in particular of those discussed in the present review, can be categorized into three classes:

— labelling, tracing and imaging,
— sensing and detection, and
— active elements, e.g. for heat mediation, optical sensitizing or delivery vehicles.

Naturally, the material of the particles often plays the key role in applications by providing unique inherent properties like strong optical absorption or scattering, fluorescence emission or magnetic moment. Second, the particle surface and its modification determine particle stability, interaction with the particle environment, in particular in biological systems (Nel et al. 2009), and possibly controlled assembly or targeting.

General reviews about the application of nanoparticles in biological systems can be found in a number of articles (Riu et al. 2006; De et al. 2008) focusing on analytics (Rosi & Mirkin 2005; Wilson 2008), gold nanoparticles
(Jennings & Strouse 2007; Sperling et al. 2008; Boisselier & Astruc 2009) and quantum dots (Hotz 2005; Medintz et al. 2005; Michalet et al. 2005; Klostranec & Chan 2006; Yu et al. 2006a; Delehanty et al. 2009; Medintz & Mattoussi 2009). Both surface modification and applications of magnetic iron oxide nanoparticles have been reviewed recently (Mosqueira et al. 2001; Berry & Curtis 2003; Ueno & Sekino 2006; Lu et al. 2007; Xu & Sun 2007; Laurent et al. 2008; Sun et al. 2008).

In the self-assembly of nanoparticles, surface properties determining the interactions between particles play a fundamental role (Niemeyer 2001; Shenhar et al. 2005; Srinivasan et al. 2006; Ofir et al. 2008; Talapin 2008; Lim & Zhong 2009). Finally, owing to the extremely high surface-to-volume ratio of nanoparticles, they may be ideally suited for catalytic purposes, as recently summarized in a review (Narayanan & El-Sayed 2008).

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References


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