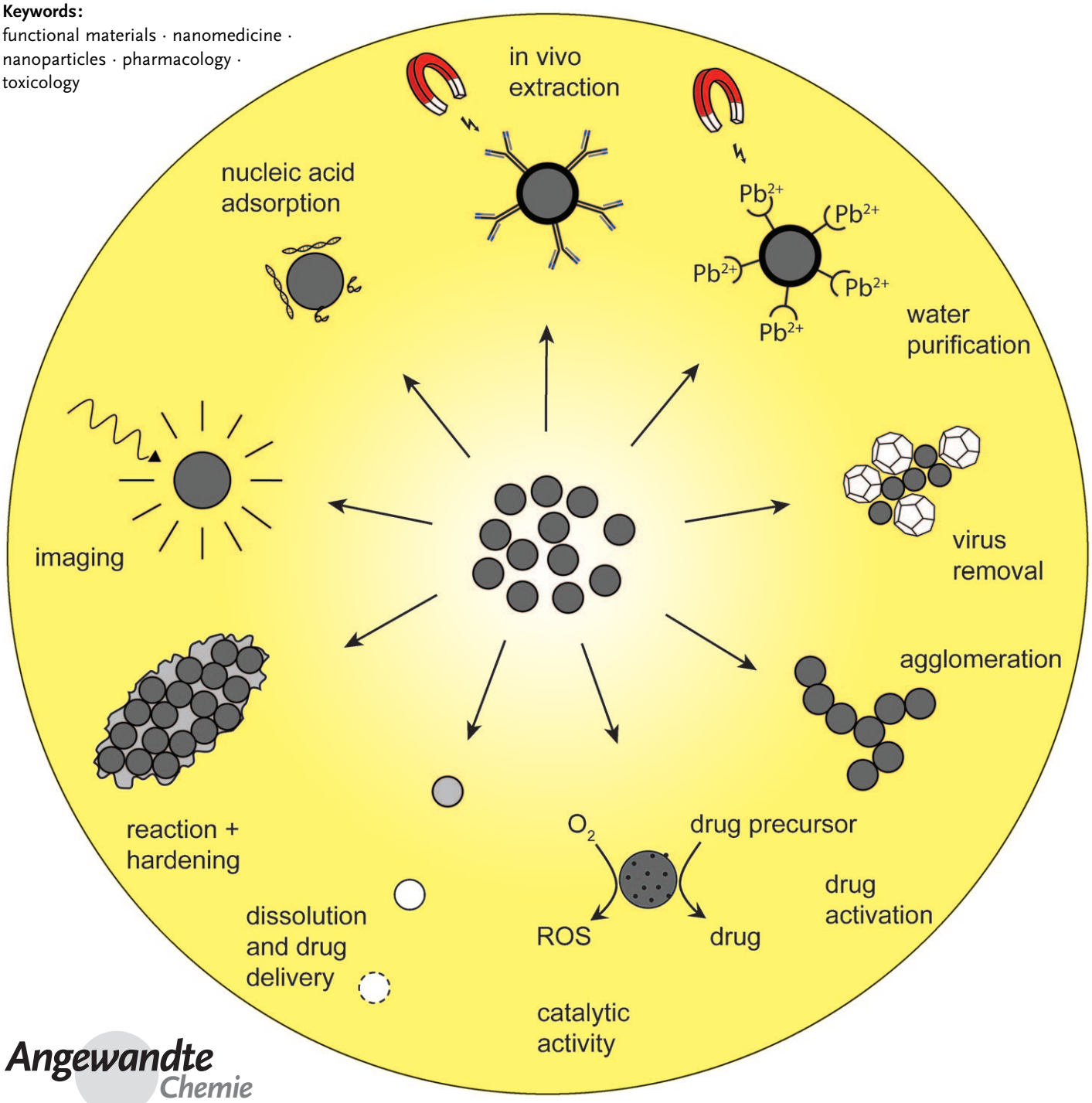
DOI: 10.1002/anie.200906684

Nanomedicine

Nanoparticles in Biological Systems

*Wendelin J. Stark**

Keywords:
functional materials · nanomedicine · nanoparticles · pharmacology · toxicology



Understanding the behavior of nanoparticles in biological systems opens up new directions for medical treatments and is essential for the development of safe nanotechnology. This Review discusses molecules and nanoparticles when in contact with cells or whole organisms, with a focus on inorganic materials. The interaction of particles with biology unravels a series of new mechanisms not found for molecules: altered biodistribution, chemically reactive interfaces, and the combination of solid-state properties and mobility. Externally guided movement of medicaments by using functional nanomagnets brings mechanics into drug design. In subsequent sections, the role of inertness and bioaccumulation is discussed in regard to the long-term safety of nanoparticles.

From the Contents

1. Introduction

The wealth of pharmaceuticals available today has largely been fueled by a detailed understanding of molecules within biological systems.[1–3] As a result, the majority of the widely used medicaments were originally identified on the basis of a toxic effect or through design towards a specific molecular target (for example, a key protein in a cascade).[4–7] Can this most successful concept be extended?[8] What happens if we start combining the properties of molecules (for example, optical, magnetic, or mechanical properties) with those of classical solids? Can we make solids so small that they start moving like classical molecules? A quick look at the diffusion behavior of particles and the relevant size range (Figure 1)

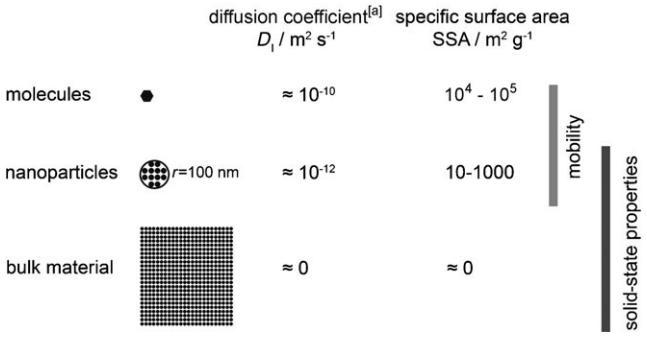


Figure 1. Nanoparticles resemble both molecules and solids: The combination of mobility (a property of classical molecules; high diffusion coefficient) with the specific properties of a solid material (for example, magnetism; response to light, radiation; catalytic/chemical activity, surface[31,40,161,162]) allows delivery of the physical properties of solids into an organism. [a] In water.

indeed shows that such a fascinating merger between solids and molecules appears to be feasible. How does that affect the way we think about solving a given (medical or industrial) problem "chemically"?

The objective of this Review is to show how functional nanoparticles can provide a logical extension beyond classical (molecular) design concepts in chemistry, where we arrange atoms in a way to reach a biochemical/medical/processing design target. The use of small and, therefore, transportable particles may make it possible to apply previously inaccessible physical effects (such as, the use of solid/mechanic properties and physical effects as part of a pharmaceutical formulation) to the development of pharmaceutical lead compounds and therapy design.

In addition to the introduction and conclusions, the Review is divided into three parts. In Section 2 we discuss nanoparticles in biological systems, with a focus on the fundamental differences between particles and molecules. Physical aspects relate to a size- and density-dependent mobility which affects the uptake, biodistribution, and clearing. This most crucial difference of mobility is highlighted with several examples currently encountered in preclinical or toxicological studies. In terms of chemical reactivity, solids have traditionally supported heterogeneous catalysis and were characterized through activity and surface properties. With small particles, such reactive surfaces can enter biological systems and interfere with existing (natural) chemical transformation pathways.

Section 3 discusses the most recently uncovered mechanistic insights from nanotoxicology[9–12] as a starting point for improved or new medical treatment concepts. The direct comparison of molecules and nanoparticles allows the metric and language to be defined to properly discuss the fundamental physical/chemical differences where relevant for biological systems. Selected key properties of nanoparticles are then analyzed in detail to illustrate the potential of this concept in applications.

Section 4 gives a long-term perspective that highlights emerging research areas at the interface between small molecular drugs and material science. This section includes catalysis inside cells, the extraction of noxious compounds from a human as an alternative to the addition of another

[*] Prof. Dr. W. J. Stark
Department of Chemistry and Applied Biosciences
ETH Zurich
Wolfgang Pauli Strasse 10, HCI E 107, Zürich (Switzerland)
Fax: (+41) 44-633-1083
E-mail: wstark@ethz.ch
Homepage: http://www.fml.ethz.ch

molecule (drug), and elaboration of ways to use the understanding gained for the development of safe nanoparticles for use in consumer goods.

The Review focuses on inorganic, metal-based nanoparticles, the largest class of small particles. Since such particles have defined properties[13] and follow rules for connecting amongst themselves and others, they have been suggested as building/design elements[14,15] in physics/electronics[16,17] and material science.[18–21] From a chemical perspective, we may consider the multifunctional building blocks as advanced molecules[22,23] (namely, the basic entities have the possibility to connect to others and form new entities) with properties traditionally associated with solid-state physics or mechanics (the core of the entities has properties of a solid). Given the considerable size of this topic, the present Review can only address individual aspects in an incomplete way and often refers to more specialized reviews where a better defined, selected number of application examples have been discussed.

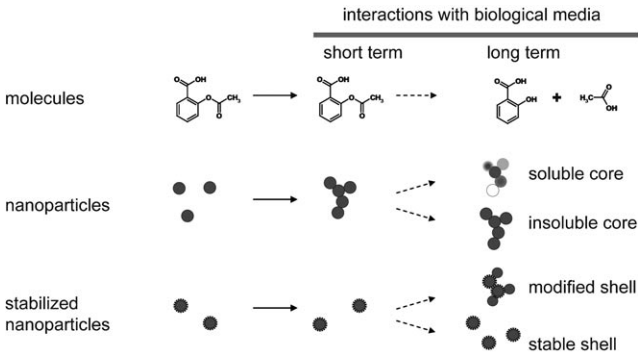


Figure 2. The chemical fate of molecules inside an organism can be described through degradation and metabolism. For nanoparticles, agglomeration complicates the in vivo behavior and significantly alters mobility, biodistribution, and clearance. Stabilized particles can become unstable through chemical modification or loss of surface functionality. Inside the organism, particles can have mass-related effects (dissolution and release) or catalytic effects (time- and mass-dependent).

2. Fundamentals

Molecules are defined by their atomic constituents, their connectivity, and by the spatial orientation of the subunits. The clear identity of molecules allows for accurate preparation and analysis, which results in well-defined physical and chemical properties. Application-oriented chemistry links desired target properties (for example, blocking of an enzyme) with chemical lead structures and uses the elaborate methods of synthetic chemistry to provide the compounds for performance testing (Figure 2).

Classical solids are considered as infinite repetitions of smaller subunits, where the properties of the material do not depend on the location within the material. In contrast to molecules, the identity of a solid is given by the local (repeated) arrangement of atomic or ionic constituents in an exact (crystalline) or averaged (amorphous solids) way. Solid-state physics provides a series of technically most relevant additional properties (optical, magnetic, thermodynamic, gradients in semiconductors, force anchoring, etc.). Chemistry has partially succeeded in providing molecules with such "solid" properties, but in most cases classical solids outperform their molecular counterparts (for example, organic dyes↔inorganic pigments, fluorescein↔quantum dots, silicon photovoltaic↔organic solar cells). From an application-oriented perspective, an ideal product (a set of properties) would, therefore, combine design elements from synthetic chemistry (function, connection, recognition), classical solids (force anchor, imaging by light conversion, contrast or magnetic response), while remaining mobile (diffusion and transport inside an organism or system).

If this fundamental distinction between molecules and classical solids is now considered in regard to biological systems, the mobility of molecules is indeed the most discriminative argument why classical pharma has relied on the use of molecules to convey action (for example, to block a specific enzyme) within an organism at the cellular level. From a (much more) abstract point of view, we may consider surgical instruments as specialized solids, which currently act on the size range of a meter (hip bone implant) down to a micrometer (ophthalmology). We may thus ask the question of where chemistry will ultimately meet mechanical engineering, particularly when dealing with a large number of identical objects (large molecules with multiple function, bioconjugates, highly functionalized particles).

Wendelin J. Stark (born in 1976) received his Master in Chemistry in 2000 from ETH Zurich, followed by a PhD in Mechanical Engineering in 2002, also from ETH Zurich. In 2004 he founded the Functional Materials Laboratory within the Departments of Chemistry and Applied Bioscience at the ETH Zurich. His research group pursues application-oriented research at the interface of chemistry with material science and medicine.

2.1. Between Molecules and Solids

2.1.1. Nanoparticles Are Mobile Solids

Nanoparticles combine the properties of solids (for example, fluorescence in the case of quantum dots) with the ability to move (a property of molecules).[24–26,40,161,162] As a result, the discussion of nanoparticle-related investigations requires a different language, as outlined in Figures 1–3. Furthermore, the small size of nanoparticles creates large surfaces that can interact with biological systems in non-classical ways (see Figure 4, right).[10–12,40,54] The failure of numerous early nanotoxicological investigations to yield reliable experimental data sets (effect versus dose) is directly related to a lack of physical understanding (of particles). To provide a chemical analogy, one may consider talking about a set of compounds in terms of color, density, water solubility,

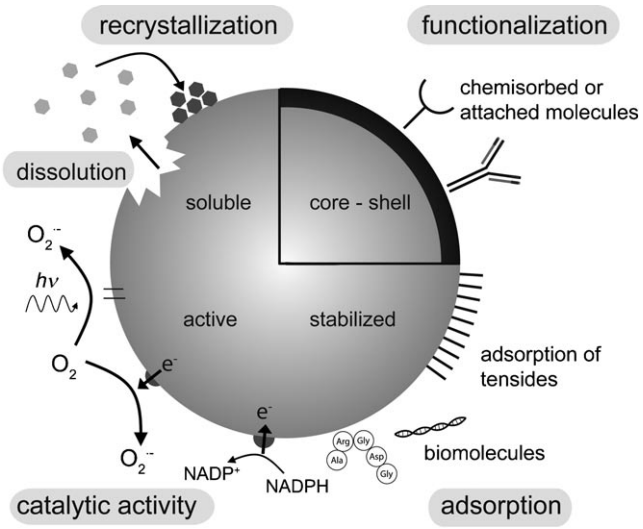


Figure 3.
Nanoparticles come in various shapes and modifications.[10] Surface functionalization allows controlled changes of the interface properties and alters the dissolution and degradation. Stabilization is routinely made through adsorption of macromolecules. Catalytic activity alters the interaction of the particles with their surroundings and may make use of oxygen, reducing agents, or even light. Reactive materials dissolve and may recrystallize, thereby offering additional surfaces where molecules may attach or react.

and boiling point (namely, observables) without knowing the structure. Clearly, such Edisonian trial and error methods would rarely result in an efficient drug design. An introduction to the concepts of mobility and agglomeration is therefore provided below.

2.1.2. *Homologous Series of Molecules and Nanoparticles*

It is currently difficult to provide an accurate, full description of a specific nanoparticle sample. In contrast, substances can be accurately described as mixtures of compounds. Today, only a few well-defined nanoparticles are available (for example, Au_{20},[27] Au_{102}[28]), and creative approaches are needed to define/describe nanoparticle samples. An example will illustrate this: The homologous series of linear alkenes is defined by the presence of a C=C bond within a linear hydrocarbon. A common set of chemical properties (for example, the ability to react with a specific reagent) defines this series from an experimentalist's viewpoint. In a similar way, nanoparticles of a given composition (for example, metallic gold) form a homologous set of materials of increasing size (or, more specifically: the number of Au atoms per particle).[29,30] Similar to alkenes, the defining properties change along this series (for example, band gap, catalytic reactivity[31]).

As in the case of molecules, the complexity of nanomaterials can become large: Substituted penicillins form a group of complex molecules, while the group of poly(ethylene oxide)-functionalized carbon nanotubes form a larger group of complex materials. An entry point into a more systematic nomenclature for nanomaterials was proposed most recently by Gentleman and Chan.[32] Looking back at the historical development of chemical nomenclature reveals a similar difficulty of a research community adapting to a specific way of naming and describing molecules.

2.2. *Dynamic Properties: Mobility and Agglomeration*

2.2.1. *Diffusion of Small Particles*

The mobility of molecules is high. When working in the laboratory, chemists (intuitively) make a valid approximation based on diffusion coefficients: For most chemical applications in a homogeneous phase we traditionally avoid lengthy discussions on diffusion effects (so called mass-transfer limitations). As a result, chemists only occasionally encounter mass transfer when carrying out a heterogeneous reaction. Drug development, therefore, traditionally relies on organ/tissue distribution models to simulate biodistribution and local metabolic effects.[33] The partition coefficient concept (for example, the octanol-water coefficient K_{OW}) provides a

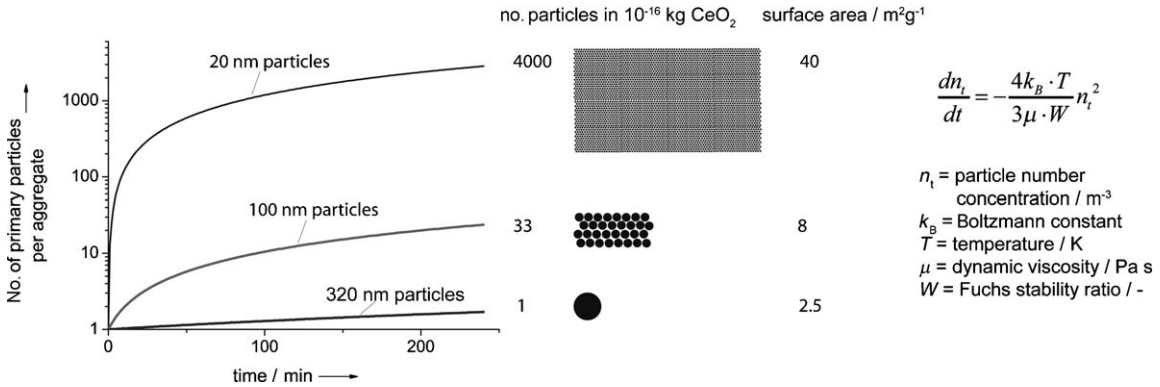


Figure 4. Agglomeration strongly depends on the nanoparticle concentration (particles per volume). Under the typical concentrations of biomedical or toxicological investigations (for example, 1 μg particles per mL), the bi-particle kinetics of agglomerations rapidly transform small particles (highly mobile) into large, basically immobile aggregates unless special precautions or surface treatments are used. This clearly shows how the form of the material (size) has a tremendous, but indirect effect on biodistribution. This often paradox-appearing behavior explains why very small nanoparticles are often less widely distributed in exposed organisms than particles of intermediate size.

well established tool to rapidly discuss hydrophilic/hydrophobic behavior.[34,35] Similarly, solubility defines the interaction between solids and dissolved species when in direct contact.

In sharp contrast to molecules, the mobility of nanoparticles is a dynamically changing property (Figure 4). The ability to form groups (so-called agglomerates) is a fundamental property of nanoparticles. As described by Einstein,[36] the diffusion coefficient of such larger objects rapidly drops with size according to Equation (1), where D is the diffusion coefficient, k_B the Boltzmann constant, T the temperature, μ the viscosity, and r the particle radius.

$$D = \frac{k_B T}{6\pi\mu r} \quad (1)$$

Most reviews[10,40,98,129,161] and recommendations on nanoparticle/biology interactions, however, describe single particle effects thoroughly (Figure 3), but mostly neglect the pronounced effects of the nontraditional mobility of these tiny particles on biological systems.

2.2.2. Particles can Form Groups: Agglomeration

The agglomeration process itself[37](Figures 2 and 4) is analogous to a bimolecular reaction and, therefore, strongly depends on the concentration of particles (chemical analogue: concentration of a reactant A in a bimolecular, elementary reaction $2A = B$). The concept of activation energy (the percentage of collisions that successfully lead to reaction) is analogously expressed using a stability ratio. This is a striking example of co-evolution in two different scientific disciplines. Measurement of zeta-potentials (see Figure 8 in Section 3.1.1) can provide an experimentally accessible measure to assess the relative electrostatic stability of a nanoparticle dispersion against rapid agglomeration.[38,39] In terms of the difference between molecules and nanoparticles, the zeta-potential and surface charge density play a similar role as the surface-charge of a molecule or protein.

It should be noted that steric repulsion (for example, through non-ionic dispersion agents, polymer or silica shells,[40a] or surfactants[40b–43]) can also contribute to stable, even nearly electrically neutral dispersions,[44] and strongly affects biodistribution.[45,54,161] In biological systems, however, highly charged proteins (for example, albumin in blood) or specific, charged adsorbates[46,47] result in predominantly charge-stabilization effects.[48–50]

The phase behavior and biodistribution of nanoparticles is complicated by the above-mentioned process of agglomeration (Figure 5), but the principal concept of phase-distribution coefficients appears to be applicable if particles are highly stabilized. Some authors even use the concept of "solubility" to describe a particle dispersion, but struggle with a precise definition since a rigid approach must rely on the above concepts of agglomeration and deagglomeration (chemical analogy: dissolution). In addition, interface effects (particle surface) complicate the above discussion and again underline why chemical concepts alone are of limited use when describing nanoparticles in complex systems.

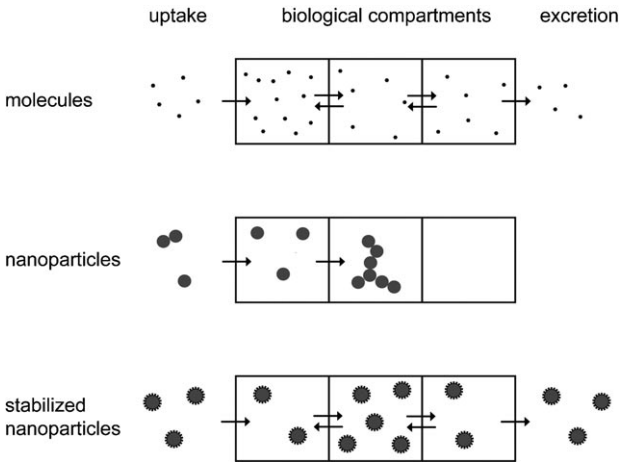


Figure 5. The biodistribution of molecules and nanoparticles depends on the mobility, size, and surface properties.[10–12,40,45,54,161,162] While molecules diffuse between different compartments of an organism, particles may undergo preferred agglomeration in a specific part (organ, tissue).[45] Since the formation of agglomerates results in a slow or even immobile form of the material, nanoparticles can accumulate locally. This, in particular, affects excretion,[45,55] and may result in long residence time inside an exposed organism if a material is persistent or of very low solubility.

3. Mechanisms

Biological systems are defined by compartment formation (for example, cells) and rely on lipid-based membranes as diffusion barriers. A discussion on particle behavior in biological systems, therefore, starts with the uptake of particles (that is, transfer through the first barrier between the outside world and an organism). In the case of classical pharmaceuticals, most organic molecules enter cells by diffusion (Figure 6) and the rate of cellular uptake is largely defined by the size and partition coefficient (K_{OW}) of a

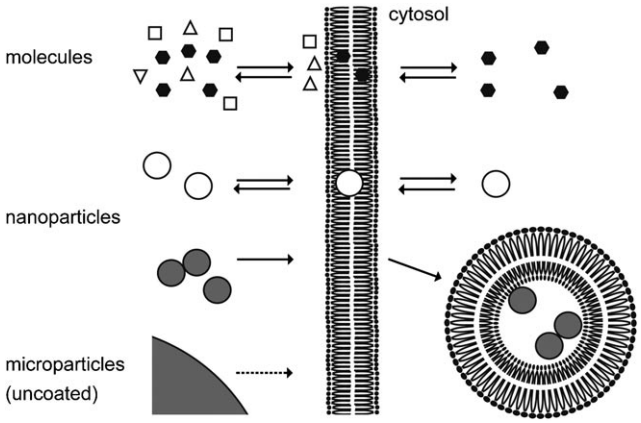


Figure 6. Biological membranes are the predominant barriers in all organisms. Lipophilic molecules, in particular, diffuse through classical lipid bilayers and their concentrations are a function of thermodynamics. Specific nanoparticles may enter membranes and diffuse inside a cell depending on their surface.[10–12,54,63,69,70] Most hydrophilic (oxides) nanoparticles are found bound to vesicles inside cells. The mechanism of uptake is under intense debate. Uncoated microparticles rarely enter non-phagocytotic cells.[70]

substance.[51,52] Well-established in vitro setups are widely used to measure uptake and resulting biological effects (for example, in vitro cytotoxicity).[33]

The rapidly emerging field of nanotoxicology attempts to translate concepts from chemical toxicology to nanoobjects. In view of the differences between molecules and nanoparticles highlighted above, it is not surprising that such a transfer of methods is difficult.[53] The following section presents some of the key mechanisms that are inherently new to nanoparticles and starts with particle uptake by cells, nontraditional interactions, and clearance from an organism.

3.1. Uptake of Nanoparticles into Cells and Organs

3.1.1. Mammalian Cells

Depending on the particle surface,[10–12,56–59] affinity to water (hydrophilic/hydrophobic), charge,[60–62] and protein adsorption,[63–67] nanoparticles enter cells either bound inside vesicles (most oxide nanoparticles) or free-floating in the cytosol (predominantly polymer and some derivatized metal nanoparticles).[68] The combined effects of diffusion and agglomeration make even simple in vitro experiments quite complicated.[39] Accurate control over the dispersion quality (temporal evolution of the hydrodynamic particle size distribution) and timing of exposure is a prerequisite for reliable experimentation, but can easily be modeled on the basis of the above concepts.

Figure 4 shows that the agglomeration process is rapid at typical physiologically relevant concentrations in the $\mu g\,mL^{-1}$ range. The cellular uptake after exposure to a nonstabilized nanomaterial (most technically relevant samples) strongly depends on the mean size of the particles—larger particles (for example, 320 nm diameter) are relatively heavy, diffuse slowly (see Equation (1)), and do not significantly agglomerate during the typical duration of standard in vitro experiments (several minutes to a few days). In contrast, if nanoparticles (100 or 20 nm diameter) are used, the equivalent mass of every single particle of 320 nm diameter now corresponds to 33 (100 nm diameter) or 4000 particles (20 nm diameter). Since the rate law concerning agglomeration depends on the square of the particle concentration (number of particles per volume of liquid), smaller particles agglomerate within seconds to minutes. This process is further accelerated through the higher diffusion coefficient of the smaller particles [Equation (1) and Figure 7].[39]

These findings have a direct impact on most medical/toxicological questions, as typical doses (drug delivery/diagnostic aid …) or accidentally ingested or inhaled particle samples (safety concerns, workplace exposure) are in the sub-mg to 100 mg range. As a result, relevant tissue concentrations are in the above mass and particle concentration regime (Figure 7). This fundamental physical aspect affected the majority of early toxicological investigations on nanomaterials[58] by typically reducing the uptake, as agglomerates are less mobile.[71] As a result, careful choice of the materials and methods is a prerequisite for carrying out useful nanotoxicological studies.[10–12,72] The mechanism of the uptake of nanoparticles is not known, and studies report indications of both active[73–75] and passive[39,70] (diffusion, sedimentation) mediated transport through the cell membrane. The delivery of nanoparticles directly into the cytosol requires specific surface properties[76] or rupture of the lysosome wall.[77]

The transport of nanoparticles within a cell culture or exposure chamber setup is often decisive for the uptake of nanoparticles into cells (the dose). Exposure to industrially used oxide nanoparticles (the largest class of engineered nanoparticles) has faced considerable experimental difficulty.[78a] This class of particles is generally not stabilized (see Figures 2 and 8) and rapidly agglomerates (Figures 4 and 7), which alters the mobility of the particles (diffusion to/inside the cells in a culture dish; Figure 7).[39] In the case of large agglomerates (concentrations above the ppm range; high density oxides; larger primary particles), the large weight can

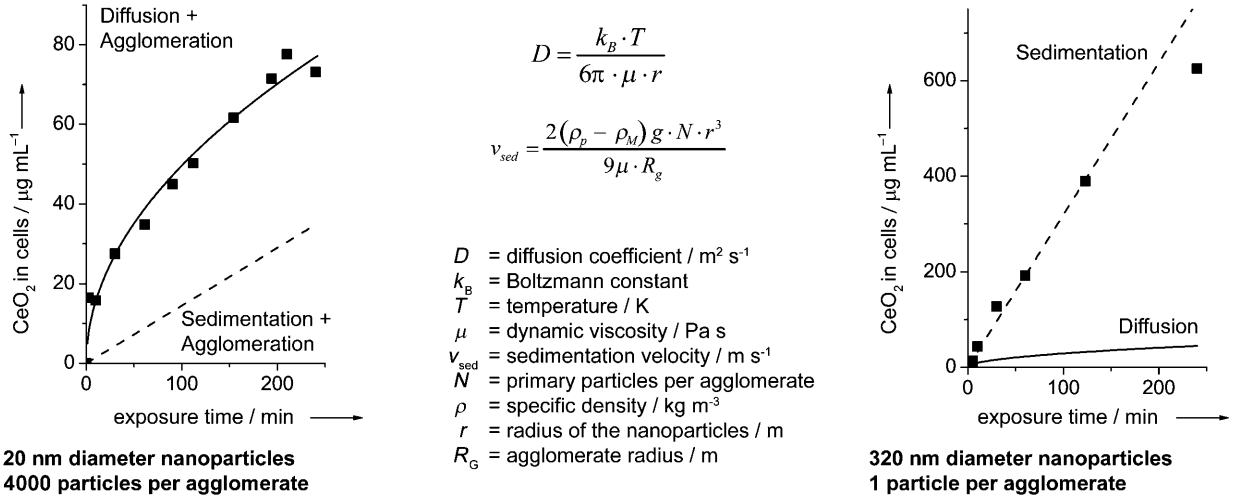


Figure 7.
A quantitative understanding of agglomeration is crucial for reliable experimentation in nanomedicine and toxicology. In vitro exposure of human lung fibroblasts to CeO_2 nanoparticles is governed by either sedimentation (right, 320 nm particles) or simultaneous diffusion and agglomeration (left, 20 nm particles). Squares: experimental data; lines: calculated model behavior.

even result in sedimentation being the major uptake process. Such a test has little "nano" relevance, as an in vitro cell culture is then just exposed to "precipitating" agglomerated particles. We may now consider these observations again in the context of molecules versus nanoparticles: While chemical toxicology and/or drug development routinely use well established in vitro tests, the additional complexity of small particles altering the mobility makes the intended transfer of existing testing protocols to the new area of nanotoxicology difficult. If molecules require physicochemical characterization (solubility, K_{OW}, and concentration), small particles additionally require that the time-dependent changes in mobility are taken into consideration. Simple everyday operations such as making a stock solution or sterilization[78b] therefore become highly critical (time-dependent particle agglomerate size) and demand additional caution during experimental design (Figure 8).

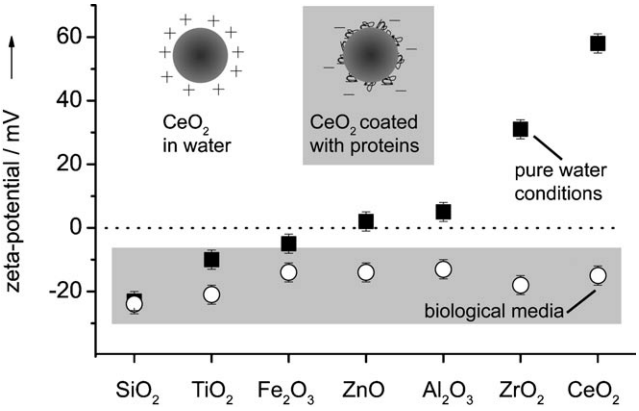


Figure 8. Charge effects strongly affect the stability of colloids and dispersions.[39] Most technically used oxide nanoparticles have high zeta-potentials in pure water and can form stable dispersions. Upon contact with biological media (cell cultures or body fluid), rapid protein adsorption changes the surface charge distribution significantly and most materials have a similarly low colloidal stability. The formation of a so-called protein corona significantly alters the interaction of an organism with the nanoparticles (for example, immune reactions).

3.1.2. *Plants and Microorganisms*

Plant cells and most microorganisms have a very different membrane/wall structure than mammalian cells. Figure 9 summarizes the current understanding on the limited uptake by plants[79] and bacteria/yeasts[80] with complex, thick cell walls. This fundamentally different behavior (little or no uptake through complex walls) again suggests a rather passive nature of particle uptake into mammalian cells (ballistic diffusion through the membrane), but this topic requires more in-depth mechanistic studies.

The pores of a plant cell wall have a diameter of a few nanometers[81] and are therefore inaccessible for most nanoparticles.[82,83] As a result, insoluble particles have a limited effect on plants. Soluble ones, such as ZnO release toxic, ionic zinc at high concentrations and result in the inhibition of growth.[79,84] Most bacteria have similarly well-engineered cell

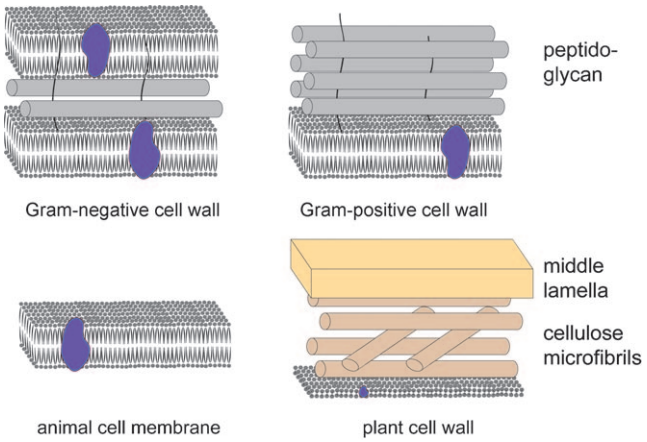


Figure 9. Depending on the configuration of the cell wall, nanoparticles have little chance to get through intact cell walls of bacteria or plants. Since the animal cell walls consist only of a lipid bilayer, they offer little resistance to particle entry. If particles release toxic intermediates (for example, reactive oxygen species), the cell wall may be damaged and ultimately result in loss of integrity and cell death.

walls, with little permeability above a few nanometers.[85,86] The entry of particles has been suggested as a result of previous damage of the cell wall.[87]

3.1.3. *Solubility often Determines Mean Residence Time Inside an Organism*

Next to agglomeration and mobility, another physicochemical factor of decisive influence is the solubility or degradation behavior of a material in typical biological compartments (Figures 2, 5).[55,88] Whilst most organisms live in rather neutral pH ranges, intracellular compartments (vesicles, phagosomes) and specialized organs (stomach) significantly extend the possibility for degradation or chemical modification of a material.

It is clear that degrading nanoparticles show distinctly different behavior than persistent (inert) ones. In most cases, soluble materials rapidly release their constituents and can result in acute effects which, in most cases, are easy to detect.[88,89] In contrast, persistent materials remain inside the organism for months to years. Here, a clear prediction of risk is difficult, and proactive identification of harmful materials is challenging. These fundamental physical aspects of persistence/solubility will be discussed in more detail below, particularly with an eye on analogous problems associated with the release of persistent molecules into the biosphere.

3.2. *Soluble Nanoparticles: Trojan-Horse-Type Toxin Uptake*

3.2.1. *Mammalian Cells*

Figure 10 illustrates the fate of a partially degradable nanoparticle, which is the case for the majority of (inorganic) main-group and transition-metal oxides, salts, and numerous organic nanoparticles.[88] Once inside vesicles, the cell's internal digestion system (lysosomes and autophagosomes) lowers the pH value of the compartment and secretes a

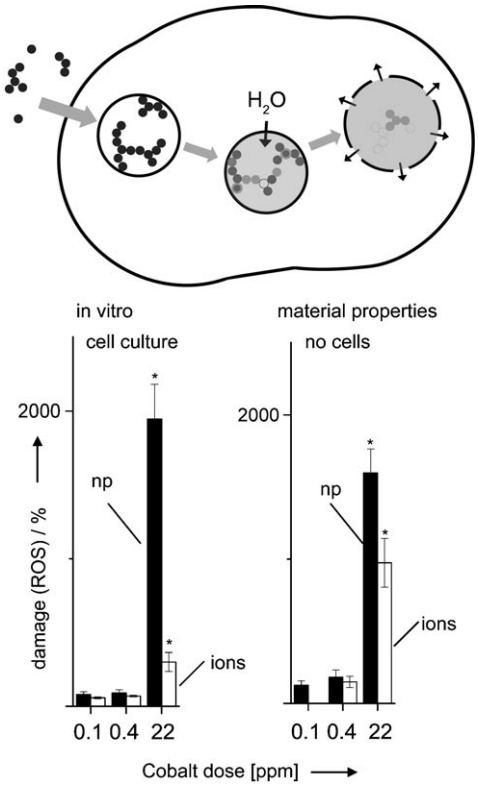


Figure 10. Nonclassical interactions: Trojan-horse-type toxin uptake. Uptake of heavy-metal oxide nanoparticles (for example, cobalt oxide) results in vesicle formation, where the lowered pH value of the lysosomes promotes dissolution of the nanoparticles. The rapidly increasing osmotic pressure may result in water uptake, swelling, and eventual bursting of the vesicle with release of heavy-metal ions. This nonclassical uptake is responsible for the over 10 times higher toxicity of cobalt on a mass basis if comparing nanoparticles and ionic cobalt (right).

number of degradation enzymes. As a result of their chemical properties, many technically important oxides and metal nanoparticles dissolve in dilute acids. As a consequence, the nanoparticles release considerable local concentrations of ions, often containing heavy metal ions, as has been shown for ZnO, WC_x, Co/WC_x,[90] and copper.[91] The resulting increase in osmotic pressure may lead to rupture of the vesicle (currently still a hypothesis) and release of the ions into the cytosol. There, heavy-metal ions in particular cause considerable stress and often affect the production/degradation/activation of reactive oxygen species (ROS) inside the cell. This behavior can be partially controlled through the addition of polymer or silica shells or suitable capping agents.[40a]

The Trojan-horse-type heavy-metal-uptake mechanism is particularly apparent for oxides of zinc, iron, manganese, and cobalt.[88] Figure 10 gives the relative concentration of (toxic) ROS inside human lung fibroblasts following exposure to cobalt oxide nanoparticles or the equivalent dose of ionic cobalt (aqueous solution). While the natural cell membrane offers good protection against ion uptake or loss (osmotic pressure, particularly important for water-dwelling microorganisms), the lipid membrane allows particle entry. In analogy to Greek mythology, this mechanism has been denoted Trojan-horse-type toxin uptake as it circumvents an evolutionary grown diffusion barrier by packaging the toxin as small particles which sneak inside the cells. Comparing this behavior to molecules, we immediately realize that the Trojan-horse-type nanoparticle uptake does not exist for molecules, as this mode of entry is indeed a direct result of the particulate nature.

3.2.2. Whole Organisms

The Trojan-horse-type entry/dissolution mechanism[88] dominates the behavior of numerous technically important nanoparticles in biological systems such as nematodes,[92] microalgae,[93] zebrafish,[94,95] and rats,[96] and may result in toxic compounds being smuggled inside cells.[97,98] It also offers possibilities to actively shuttle a compound inside a cell or even an organism, as proposed by Soppimath et al.,[99] by using an acid-triggered release mechanism. This more beneficial, therapy-oriented aspect is discussed further in Section 4.1. On a whole-organism basis, similar particle-facilitated uptake concepts[69] have most successfully allowed nonclassical drugs (for example, proteins) to shuttle inside the human body[100] and allow oral administration.[101,102]

3.3. Chemically Active Nanoparticles Inside Cells

One of the key promises of nanoparticles has been their improved chemical activity, particularly for catalysis, where the most prominent example is undoubtedly the high activity of gold nanoparticles.[31] What happens in regard to chemical activity inside a living organism? Figure 11 gives a schematic view of particles entering a cell and interacting with its components. The most important catalytic mechanism is related to the generation of ROS.[10–12,103] Here, the particles assist in reducing an oxygen molecule (thus, activating it as ROS: $O_2 + e^- = O_2^{\bullet -}$) which then reacts with the internal structure of the cell. This mechanism is already active stoichiometrically if reduced iron oxide or even iron particles get inside a cell or close to a (micro-)organism.[95,104–108] In the case of catalytic nanoparticles, the oxidized site (for example, Fe^{3+} in silica, or redox-active rare-earth oxides[109]) is embedded in the cytosol, where reducing agents (for example, NADH) are abundant and can re-establish the active form of the heavy-metal center through reduction ($Fe^{3+} + e^- \rightarrow Fe^{2+}$).[103]

It is apparent that a broad variety of more-complex catalytic cycles is imaginable that contribute to nonclassical interaction mechanisms of toxins. Jia et al. most recently reported on the gold-catalyzed formation of NO in blood plasma.[110] Since the catalytic particle is not degraded during the process, the effect of exposure to such materials does not scale with the mass, and even small amounts of catalytically active, yet persistent (non-biodegrading), particles may result in chronic effects, or it may assist a therapy (see Section 4). It is known that algae, daphnids,[111] and trout are affected by (photoactive) titania.[112] Here, the toxicity is again linked to a nonclassical activity of the material. In contrast to this negative impact, Chen et al. and Karakoti et al. reported on

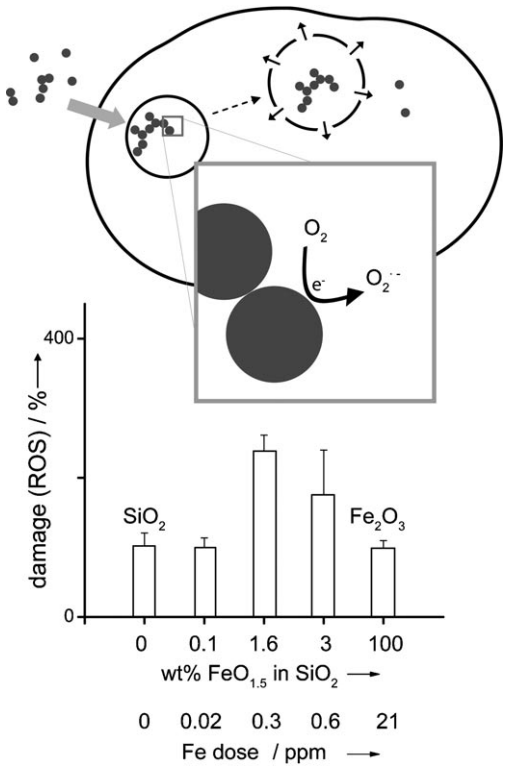


Figure 11. Nonclassic interactions: Heterogeneous catalysis inside a living cell.[103] If nanoparticles of a catalytically suitable material are ingested, they can interfere with reactions inside the cytosol. As an example, activation of oxygen through single-electron transfer affords highly noxious radicals that damage the vesicles wall and release the reactive cargo inside the cytosol (top). Bottom: Proof of the mechanism. If iron is exposed as a salt or pure oxide, it creates limited oxidative stress. A much smaller dose of iron in the form of iron-doped silica (a heterogeneous catalyst for oxidations) provokes high oxidative stress and demonstrates a nonclassical toxin behavior with an apparently inverse dose-effect relationship.

the beneficial effects of ceria nanoparticles, as evident from a catalytically reduced oxidative stress level in retinal tissue.[113,114] In contrast, most molecules have a mass-/dose-dependent effect inside an organism and long-term catalytic effects are rare.

3.4. Solubility Determines Environmental Long-Term Fate

Similar to the above mechanistic arguments for nanoparticles inside a living organism, particles in the environment can be distinguished between persistent and degradable ones.[88,115,229] Dispersal naturally depends on mobility—here, the above concepts of colloid stability and agglomeration kinetics provide the basis for detailed investigations. Van Hoecke et al. reported a significant influence of agglomeration on the effects of ceria nanoparticles in a number of aquatic organisms,[116] while Wiench et al. reported on the agglomeration of titania nanoparticles (a nonsoluble material) on exposure of Daphnia to the material.[117] A comparison to ZnO nanoparticles (a partially soluble material) within the same study confirmed that the toxicity of the material mainly occurred through dissolved ionic zinc.[117] This finding clearly shows how very basic, physicochemical concepts from the single cell level (in vitro) can at least be used as a guiding principle[88] when designing ecotoxicological studies.

Solubility and degradation tests for chemicals in individual compartments of ecosystems have been developed. These tests now require coupling to the above-mentioned physical aspects before they are adapted to deduce the long-term fate of specific nanomaterials. This is indeed of some urgency, as initial studies have shown considerable concentrations of titania nanoparticles in urban run-off water[118,119] and limited retention of ceria (model) nanoparticles in biological wastewater treatment units.[120] This latter effect was most recently also confirmed for titania.[121] Such early investigations urge for more detailed and mechanistic investigations on the interactions of nanoparticles with the environment.

4. Applications

There are numerous applications of nanoparticles in biological systems, and several detailed reviews provide an excellent entry into more specific areas.[40,122–129,161]

4.1. Adsorption of Molecules on Nanoparticles

4.1.1. Delivery of Drugs and Viruses, Transfection

The ability of mobile nanoparticles to carry a cargo has enabled a broad range of applications. Their use for enhanced drug delivery[129] has concentrated on the application of biopolymers (for example, polylactic acids and others[129–132]), dendrimers,[133] porous particles,[134,135] nanogels,[136] and even metals[137,138] as carriers for highly potent drugs[139] of otherwise low bioavailability.[140–142] Several research groups[129a,b,143,144] have reviewed particle-based drug delivery and demonstrated how the previously discussed tendency of mammalian cells to ingest small particles can be used therapeutically. Recent concepts have focused on investigating the ability of nanoparticles to shuttle a drug through the mucosa as an alternative drug-delivery pathway.[145] Most recent concepts go further than passive delivery (nanoparticle carries the drug inside a cell) and actively shuttle[146,147] and precisely release a cargo through an external trigger.[148–153]

The adsorption of macromolecules onto nanoparticles[154] can also be used to facilitate gene delivery or virus transfection, as shown for calcium phosphate (CaP) and cationic nucleic acid carriers[155,156] (Figure 12; see the review in Ref. [157]. Both adsorption and subsequent uptake are size- and charge-dependent.[158] A typical procedure consists of two steps: A) Adsorption of the corresponding nucleic acid or whole virus onto the carrier (CaP or biopolymer) nanoparticle. B) Exposure of the colloid to the cell culture. Figure 12 shows the enhanced transfection of a number of cell lines using a green fluorescence protein (GFP) encoding sequence when compared to classical protocols. The preferred use of (intracellular) rapidly dissolving particles as carriers enables cargo delivery within minutes.[159] This range of applications directly uses an inherent property of the particles

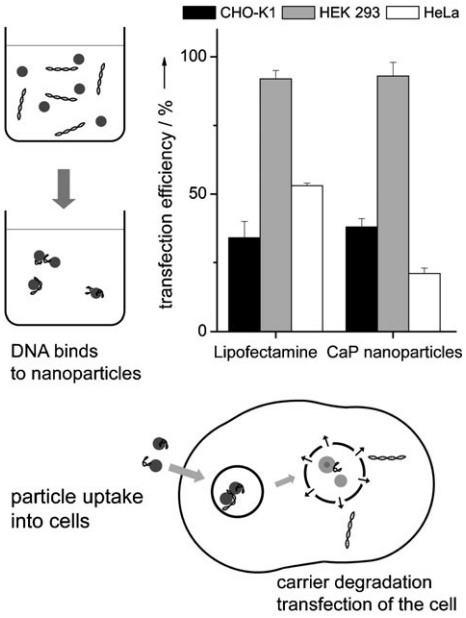


Figure 12. Macromolecules adsorb on nanoparticles and can be shuttled inside cells. This can be used for transfection, where a gene is brought into cells on purpose and alters the expression profile (for example, for biotechnological protein production). The efficiency of calcium phosphate is comparable to the best currently used protocols based on cationic polymers (right).[159]

(uptake and adsorption of cargo) and provides another example of a "nanospecific" set of applications, which are difficult to realize with small, individual molecules.

4.1.2. *Clearance of Viruses from Water*

As a logical extension of these adsorption-based concepts (one organism→larger system), reactive nanoparticles can be used to remove viruses from contaminated (drinking or processing) water (Figure 13).[159] The tendency of large biomacromolecules (for example, viruses) to adsorb on to the reactive CaP surface is sufficiently high so as to reduce the number of viruses in cleared solutions below the detection level.[159] If a nontoxic, but reactive system is used, the supernatant is directly ready for consumption, with the precipitated aggregate consisting of inactivated viruses, biopolymers, and the inorganic precipitate. Here, amorphous, glassy calcium phosphate nanoparticles[160] are advantageous as they provide mineral constituents if used at an excess concentration. The reactive system can be described as a simple hydration and yields calcium deficient hydroxyapatite [Eq. (2)].

$$3\,Ca_3(PO_4)_2 + H_2O \rightarrow Ca_9(PO_4)_5HPO_4(OH) \qquad (2)$$

A mechanism involving recrystallization and hydration of the particles alters the properties of a material without involving mass transfer. From a thermodynamic point of view, the initial phase is a carrier of energy, which is released during recrystallization. Molecules can behave similarly, for example, by hydrolysis of an ester bond.

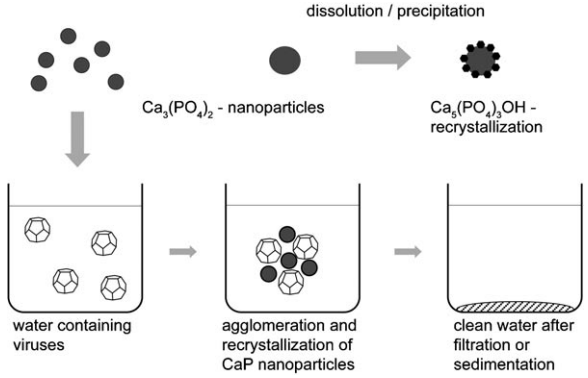


Figure 13. Removal of viruses from drinking or processing water. The ability of reactive nanoparticles to adsorb and irreversibly agglomerate/bind numerous viruses allows clearance of contaminated water within minutes. The reactive $Ca_3(PO_4)_2$ nanoparticles recrystallize concomitantly with virus adsorption and bind them in an easy separable precipitate (right).

4.1.3. *Imaging*

Medical imaging has strongly profited from the development of bioconjugated nanocrystals (Figure 14).[123,161] So-called quantum dots today serve as imaging tools and markers

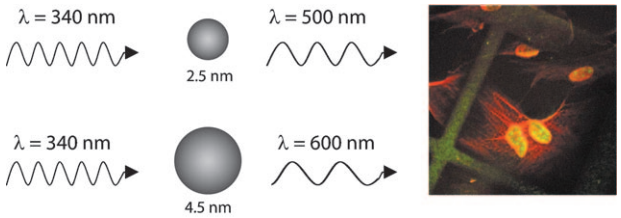


Figure 14. Combining optical properties and molecular mobility. In contrast to organic dyes, semiconductor quantum dots are highly photostable. As bioconjugates, they assist the imaging of life processes inside cells and significantly improve the sensitivity of numerous assays in research and clinics. Here, a typical solid-state property (fluorescence; quantum confinement) has been rendered mobile (size) and selective (bioconjugate). Adapted from Ref. [162b].

in molecular biology and even clinical medicine.[162,163] Freeman et al. most recently even reported the imaging of intracellular metabolic transport, thus enabling a new level of hierarchical investigations[164] and targeting.[165] This line of application again demonstrates well the advantage of a combination of molecular properties (movement, chemical specificity) and solid-state properties (photon conversion; size-dependent fluorescence spectra). Magnetic nanoparticles for magnetic resonance imaging[166] enhance the contrast[167] and allow selective imaging of a target cell group or tissue.[163] Here, a suitable design[168] is also based on molecular (selective connection,[169] mobility) and solid-state (superparamagnetic) properties. The same combination of properties is used as a basis for hyperthermia, with radiofrequency electromagnetic fields selectively heating a tissue containing magnetic nanoparticles.[170] The use of surface-enhanced Raman scattering (SERS) as a much more sensitive analytical

method is similarly based on an interface effect of (traditionally) solid-state physics.[171] If SERS is used as an imaging method in biology, the carrier/effector (the metal particle) again requires mobility (the molecular property) to find and map a specific property (for example, an actin filament inside a cell).

From a long-term perspective, biologically used nanoparticles must be robust and reliable,[172] which can either be achieved through strong adsorption of surface-protecting agents[173] or even covalent C–C bonding to carbon-encaged particles.[174]

4.2. Bulk Applications of Reactive Nanoparticles

The enhanced reactivity of nanoparticulate, calcium-based biomaterials have opened up fascinating possibilities, particularly for dental and bone surgery.[175,176] The reaction shown in Equation (2) allows the preparation of self-setting, injectable bone cements[177] or can be used together with a polymer[178] to convey bioactivity[179] (bonding to existing tissue; Figure 15). A particular advantage of decreasing

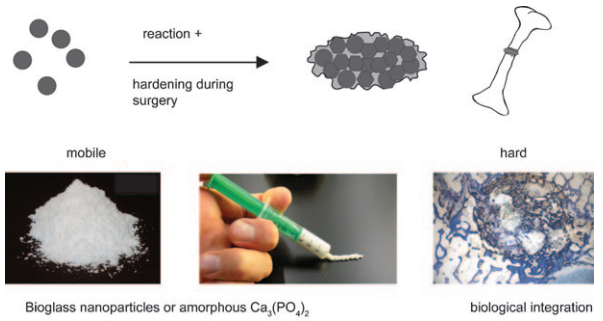


Figure 15. The reactivity of nanoparticles allows a controlled transition from mobile to immobile. Amorphous calcium phosphate can be transformed into a toothpaste-like injectable bone cement and hardens within minutes inside a living organism. Possible applications to assist bone healing or osteoporosis are under preclinical investigation.

particle size has been demonstrated for bioglass, one of the most prominent biomaterials besides calcium phosphate. The complex glass (Na_2O, SiO_2, CaO, P_2O_5, optional F, Mg) has traditionally been manufactured by melt processing and yielded micrometer-sized granulates of limited reactivity.[180] If manufactured as nanoparticles,[88] the nanoglass has high antimicrobial activity,[177] thus suggesting use in dental root canal treatments,[177] where cement hardening and disinfection can be combined in a single material.[177] Strong physicochemical differences can be seen between nanoglass and its ionic constituents. Indeed, the difference between window glass, nanoglass particles, and a solution containing Na^+ and $H_xSiO_4^{(4-x)-}$ ions is chemically difficult to describe.

The use of zero-valent iron nanoparticles for the removal of halogenated organic soil and water contamination has recently attracted significant interest. Early studies on the mobility of iron particles in soil[40,41,181] have demonstrated the possibility of reducing otherwise rather inert chlorohydrocarbon contaminants in soil or an aquifer without excessive (energy intense) pumping or earth moving. Here, the reducing reagent must be transported through the soil. In this case, the nanoparticle allows transport of the reducing power to another medium. Undoubtedly the most established bulk application is the use of anatase titania particles as photocatalysts for the oxidation of organic compounds in waste water.[182,183]

4.3. Functional Nanomagnets

4.3.1. Stable Nanomagnets

Since specific nanoparticles combine properties of molecules (mobility) and solids (for example, response to electromagnetic fields), magnetic nanoparticles have attracted tremendous interest[172,184,185] for targeted drug delivery (magnetic accumulation of a drug-loaded particle within a specific tissue or magnetophoresis for enhanced uptake[186]), cell sorting and manipulation,[187] hyperthermia (external heating of particles inside a cancerous tissue to kill cells locally[188]), and diagnostics (separate a biomarker from a complex mixture).

A detailed review on the synthesis and application of magnetic nanoparticles up to 2007 has been provided by Lu et al.[172] The ideal magnetic carrier has to combine high magnetic saturation (preferably metals instead of weaker oxides), biocompatibility, chemical stability, and reliable linker with (mostly expensive) selectivity-giving agents (for example, antibodies).[189] The most recent development of carbon/metal nanomagnets has resulted in stable magnetic metal nanoparticles for complex medical applications that extend the pioneering work on iron oxides.[168,172,190–193] Figure 16 provides an overview on some of the direct applications resulting from the availability of functionalized metal nanomagnets.[172,194] The availability of stable nanoparticles allows new applications. For example, magnetic chelating agents (magnetic EDTA (ethylenediaminetetraacetate), a heavy metal ion catching reagent) have been suggested to enable removal of toxic metal ions directly from water or blood streams.[195] Since stability conveys long lifetime or minimal metal loss, sensitive applications (blood

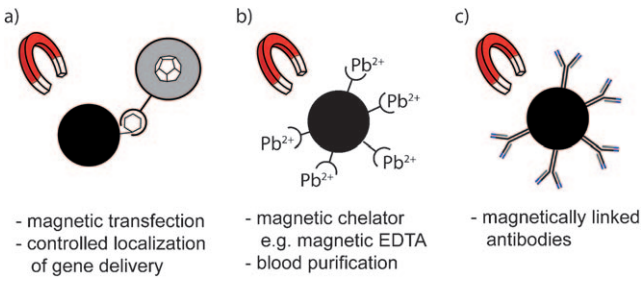


Figure 16. Combining force control and molecular mobility. Nanomagnets can act as a tool to control the delivery of drugs and genes, or give access to the controlled removal of a toxin from an organism (in vivo extraction). Magnetically bound antibodies are a key reagent in most diagnostic protocols.

cleaning) and long-term residence in a body can be investigated (see Section 4.3.2). The possibility to covalently structure the surface gives access to chemically functional nanomagnets,[174,196] or, if viewed from the other side, the structure can be seen as a magnetic molecule. Covalent functionalization of the surface in principle bridges the discipline of solid physics (the core of the particle) with chemistry (the ability to define the structure with atom by atom precision).

4.3.2. *In Vivo Extraction of Noxious Compounds*

The application of strong, stable nanomagnets is of particular interest if small concentrations of valuable (or toxic) compounds must be reliably removed from a liquid or living organism. The physical aspect of this so called magnetic filtration is not new and dates back to the mid-20th century.[197] The on-going advances in reagent properties, however, have opened up magnetic filtration to medicine.[194] Here, in vivo extraction of toxins or metabolites provides a novel concept for treatment: Whilst the traditional chemical approach of curing a disease (classical small molecular drug) provides a "drug in–disease out" concept, in vivo extraction ultimately allows selective removal of a noxious or damaging component out of a tissue. Since we have already seen that viruses can be easily and reliably attached to nanomagnets,[198] direct extraction and collection of an infectious agent will ultimately provide an alternative to a chemical treatment. In a first step, this concept is particularly interesting for blood-born diseases (sepsis, numerous viral infections, chronic inflammations, etc.).[199] From an engineering perspective, the use of functionalized nanomagnets is efficient as it only moves the compound of interest instead of the whole bulk of a liquid.[200]

4.4. Interactions with Microorganisms

The use of nanoparticulate antimicrobial agents has developed rapidly over the last decade,[201] and numerous consumer products[119] today contain silver nanoparticles as a substitute to classical compounds.[202–204] This demonstrates how a fundamental chemical element (Ag) can be used in a wide range of products once it is in another form. The focus, therefore, shifts away from a purely substance-/structure-oriented design (for example, make a novel complex molecule) to an integrated understanding of the process (delivery and transport to the organism, action, fate after use).[178,205] Such nontraditional improvements of classical antibiotics (Ag has been used for a very long time) have most recently been developed for "nano-silver" coupled to a nutrient. The principle consists of the growing microorganism dissolving the nutrient, which triggers release of the coupled 1–5 nm "nano-silver" and kills the bacteria.[178] Silver is chemically primitive—one may think about the tremendous possibilities of modifying more complex antibiotics, or coupling antibiotics to more advanced delivery systems. Choi and Hu compared silver particles of different size and found pronounced chemical activity for particles below 5 nm,[206] a behavior that shows some similarity to the altered activity of small gold particles.[31]

The above example of the nano-bioglass with antimicrobial properties,[177] the pronounced antimicrobial activity of certain magnesium oxide,[207] cerium oxide,[208] and iron nanoparticles under de-aerated conditions,[104] or even photoactive particles[209,210] again demonstrates how classical materials of limited reactivity gain novel functions if in a suitable size range. In a more complex study, Gunawan et al. coupled antimicrobial action (availability of silver) to photochemistry to provide a material with on/off-switchable antimicrobial activity.[211]

For chemistry, these trends clearly suggest the need for more integrated designs beyond the focus on "molecular properties" and requires strong integration of downstream components (for example, accurate end use) into creative, chemical thinking.

4.5. Long-Term Perspective: Catalytic Activation of Prodrugs

The treatment of chronic diseases mostly requires prolonged (months to years) drug treatment. The lack of site specificity of classical drugs results in it being distributed over the whole body, with other sensitive tissues being typically responsible for so-called adverse side effects. This is of particular importance when applying highly potent drugs for chemotherapy. An alternative to "targeted drug delivery" may be based on the above-discussed chemical activity of (rather) persistent nanoparticles immobilized in the target tissue: Here, a prodrug with ideally little to no biological activity (precursor) is given repeatedly to the patient (Figure 17). The target tissue has already been seeded with catalytically active nanoparticles, and their activity converts the precursor into an active drug that diffuses and reacts locally. If the active drug has limited stability, slow diffusion confines the action to a small area of the body, and as a result side effects can be minimized.

While present pharma development focuses on rather stable compounds (storage, transport, limited number of applications per day for a patient), in the proposed case of locally activated drugs, we want unstable, but highly active compounds. Since diffusion around a point source (the immobilized cloud of active nanoparticles in the target tissue) defines the dwell time, an active drug with limited stability would allow the action to be concentrated around the target tissue. This two-level treatment may enable the use of numerous "old" lead compounds of either limited stability or unsuitable cytotoxicity to be re-evaluated for joint use with immobilized catalytic nanoparticles. Tissue-specific catalytic activation of drug precursors will strongly profit from the now extensive understanding of long-circulating nanoparticles.[212]

4.6. Long-Term Perspective: Safe Nanoparticles

The undisputable benefits of nanoparticles have brought this material class rapidly into consumer products.[119] The question remains of the long-term safety/sustainability of such

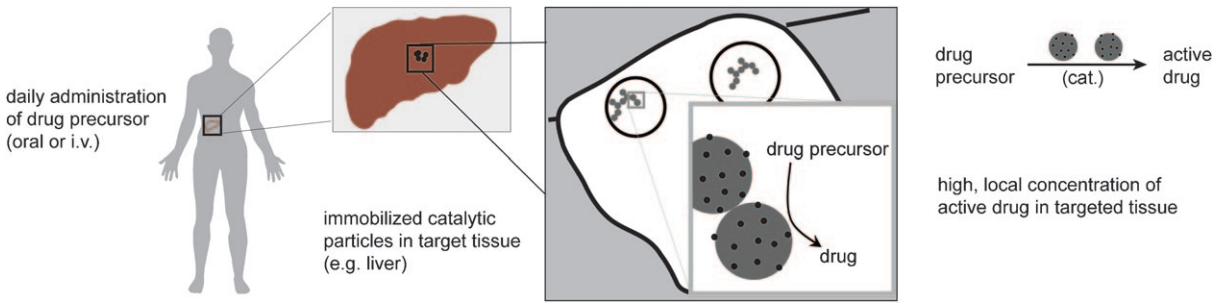


Figure 17. Vision of future pharmaceutical strategies that rely on the catalytic, local activation of a drug precursor at the target. In a first step, catalytically active nanoparticles are administered into a specific target organ or tissue. The drug precursor is administered traditionally (oral or by injection) and transformed at the target area into an active drug. In contrast to classical drugs, where stability is required to bridge the time between two administrations, an ideal active pharmaceutical ingredient within the present strategy has limited stability. This strategy allows localization around the source (the immobilized nanoparticles) and suggests significantly reduced side effects.

materials because of past "chemical" problems with other novel technology.[213] Table 1 lists the most prominent classes of chemical compounds with no acute toxicity, but they cause significant (environmental or/and health) problems because of their inertness. In retrospect, this list teaches us that any persistent compound will eventually create problems somewhere in the ecosystem or in a specific organism.

Table 1: Environmental large-scale incidents with industrial chemicals.

	Lifetime in years	Effect[a]
fluorochlorohydrocarbons (Freon)	>50	destruction of the ozone layer [214]
polychlorinated biphenyls[215,216]	>20	accumulation in the food web[217,218]
DDT[b]	>20	accumulation in the food web,[217,219] reduced bird fertility[220]
plastic (PE, PP)[c]	>10	waste accumulation in the open ocean[221–224]
radioactivity	up to 10^9	unspecific damage[225]
asbestos	>50	mesothelioma cancer[226,227]

[a] Action on a biological system. [b] 2,2-Dichlorodiphenyltrichloroethane. [c] Polyolefins, polyethylene, and polypropylene.

This argumentation and the observation of compartment-specific agglomeration and/or limited excretion of many engineered (technically used) nanoparticles[228] therefore suggest a big caveat when dealing with persistent materials.[229] The argument can also be turned around, to pose the question of the inherent safety of a specific nanomaterial: It is clear that biodegradable particles consisting of nontoxic elements are preferred. The best known example of this class is nano-calcium phosphate,[160] which is currently being tested in vivo for use as injectable bone cement (bulk application, several gram per person) or as a composite.[230]

A second observation refers to the mainly lipophilic behavior of most ecotoxicologically problematic chemicals.[215,216,219] Here, lipophilic nanoparticles also appear to be taken up better[70] than hydrophilic ones by model organisms: Rosenkranz et al. even reported a rapid uptake of (lipophilic) polystyrene particles through the gut of *Daphnia Magna*, a model organism for ecotoxicological investigations.[231]

From a long-term perspective, an integrated and responsible application of nanotechnology therefore requires a focus on nonpersistent material even at an early state of development (also in research). The clear challenge lies in the combination of desired product properties with a limited set of available design elements.

5. Summary

The combination of solid properties and mobility, as found in nanoparticles, allows fascinating interactions with living organisms and opens up numerous research directions for medicine, industrial product design, and environmental remediation. From a functional point of view, chemically well-defined nanoparticles are an extension of the classical concept of the molecule. While presently investigated shapes are mostly symmetric, the future development of highly anisotropic, functional building blocks strongly underlines the similarity between molecules and functional nanoparticles. At present, we must be modest as today's nanoparticles are still of limited complexity, with only one or two types of "functional groups/attachment sites". However, even this early state of development has resulted in a colorful array of valuable diagnostic methods for molecular biology, clinical medicine, and a rapidly growing number of consumer products.

The concept of safe nanoparticles is a result of a more-refined understanding of nanoparticle/biology interactions. An integrated product design makes use of biodegradable materials containing a limited amount of toxic species. This challenge is now passed on to researchers and engineers even at an early state of development, but ensures that large-scale applications of nanoparticles will have a sustainable future.

Financial support by ETH Zurich, the Swiss Department of Public Health (BAG), the Swiss National Science Foundation, the Gebert-Rüf, ResOrtho Foundation, and the Swiss Commission for Technology and Innovation is kindly acknowl-

edged. I would like to thank L. Limbach for assistance with the preparation of the graphics.

Received: November 26, 2009
Revised: February 23, 2010
Published online: January 10, 2011

[1] G. M. Cragg, D. J. Newman, K. M. Snader, *J. Nat. Prod.* **1997**, *60*, 52.
[2] J. Drews, *Science* **2000**, *287*, 1960.
[3] R. Hirschmann, *Angew. Chem.* **1991**, *103*, 1305.
[4] F. E. Koehn, G. T. Carter, *Nat. Rev. Drug Discovery* **2005**, *4*, 206.
[5] D. J. Newman, G. M. Cragg, *J. Nat. Prod.* **2007**, *70*, 461.
[6] D. J. Newman, G. M. Cragg, K. M. Snader, *Nat. Prod. Rep.* **2000**, *17*, 215.
[7] K. C. Nicolaou, J. A. Pfefferkorn, A. J. Roecker, G. Q. Cao, S. Barluenga, H. J. Mitchell, *J. Am. Chem. Soc.* **2000**, *122*, 9939.
[8] K. H. Bleicher, H. J. Böhm, K. Müller, A. I. Alanine, *Nat. Rev. Drug Discovery* **2003**, *2*, 369.
[9] M. R. Wiesner, G. V. Lowry, P. Alvarez, D. Dionysiou, P. Biswas, *Environ. Sci. Technol.* **2006**, *40*, 4336.
[10] a) A. Nel, T. Xia, L. Madler, N. Li, *Science* **2006**, *311*, 622; b) A. E. Nel, L. Madler, D. Velegol, T. Xia, E. M. V. Hoek, P. Somasundaran, F. Klaessig, V. Castranova, M. Thompson, *Nat. Mater.* **2009**, *8*, 543.
[11] a) W. G. Kreyling, M. Semmler-Behnke, W. Möller, *J. Nanopart. Res.* **2006**, *8*, 543; b) W. G. Kreyling, M. Semmler, F. Erbe, P. Mayer, S. Takenaka, H. Schulz, G. Oberdörster, A. Ziesenis, *J. Toxicol. Environ. Health Part A* **2002**, *65*, 1513.
[12] a) G. Oberdörster, E. Oberdörster, J. Oberdörster, *Environ. Health Perspect.* **2005**, *113*, 823; b) G. Oberdörster, Z. Sharp, V. Atudorei, A. Elder, R. Gelein, W. Kreyling, C. Cox, *Inhalation Toxicol.* **2004**, *16*, 437; c) G. Oberdörster, Z. Sharp, V. Atudorei, A. Elder, R. Gelein, A. Lunts, W. Kreyling, C. Cox, *J. Toxicol. Environ. Health Part A* **2002**, *65*, 1531; d) M. Semmler, J. Seitz, F. Erbe, P. Mayer, J. Heyder, G. Oberdörster, W. G. Kreyling, *Inhalation Toxicol.* **2004**, *16*, 453.
[13] H. Wang, D. W. Brandl, P. Nordlander, N. J. Halas, *Acc. Chem. Res.* **2007**, *40*, 53.
[14] R. Shenhar, V. M. Rotello, *Acc. Chem. Res.* **2003**, *36*, 549.
[15] T. Pellegrino, S. Kudera, T. Liedl, A. M. Javier, L. Manna, W. J. Parak, *Small* **2005**, *1*, 48.
[16] J. H. Fendler, *Chem. Mater.* **2001**, *13*, 3196.
[17] R. J. Schoelkopf, S. M. Girvin, *Nature* **2008**, *451*, 664.
[18] S. A. Claridge, A. W. Castleman, S. N. Khanna, C. B. Murray, A. Sen, P. S. Weiss, *ACS Nano* **2009**, *3*, 244.
[19] C. N. R. Rao, G. U. Kulkarni, P. J. Thomas, P. P. Edwards, *Chem. Soc. Rev.* **2000**, *29*, 27.
[20] C. A. Mirkin, R. L. Letsinger, R. C. Mucic, J. J. Storhoff, *Nature* **1996**, *382*, 607.
[21] N. C. Seeman, A. M. Belcher, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 6451.
[22] T. Fink, J. L. Reymond, *J. Chem. Inf. Model.* **2007**, *47*, 342.
[23] F. Remacle, R. D. Levine, *ChemPhysChem* **2001**, *2*, 20.
[24] M. E. Akerman, W. C. W. Chan, P. Laakkonen, S. N. Bhatia, E. Ruoslahti, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 12617.
[25] A. P. Alivisatos, W. W. Gu, C. Larabell, *Annu. Rev. Biomed. Eng.* **2005**, *7*, 55.
[26] P. Alivisatos, *Nat. Biotechnol.* **2004**, *22*, 47.
[27] J. Li, X. Li, H. J. Zhai, L. S. Wang, *Science* **2003**, *299*, 864.
[28] P. D. Jadzinsky, G. Calero, C. J. Ackerson, D. A. Bushnell, R. D. Kornberg, *Science* **2007**, *318*, 430.
[29] M. C. Daniel, D. Astruc, *Chem. Rev.* **2004**, *104*, 293.
[30] M. J. Hostetler, J. E. Wingate, C. J. Zhong, J. E. Harris, R. W. Vachet, M. R. Clark, J. D. Londono, S. J. Green, J. J. Stokes, G. D. Wignall, G. L. Glish, M. D. Porter, N. D. Evans, R. W. Murray, *Langmuir* **1998**, *14*, 17.
[31] M. Haruta, *Catal. Today* **1997**, *36*, 153.
[32] D. J. Gentleman, W. C. W. Chan, *Small* **2009**, *5*, 426.
[33] C. A. Lipinski, F. Lombardo, B. W. Dominy, P. J. Feeney, *Adv. Drug Delivery Rev.* **1997**, *23*, 3.
[34] R. Mannhold, G. I. Poda, C. Ostermann, I. V. Tetko, *J. Pharm. Sci.* **2009**, *98*, 861.
[35] P. Buchwald, N. Bodor, *Curr. Med. Chem.* **1998**, *5*, 353.
[36] A. Einstein, *Ann. Phys.* **1906**, *324*, 371.
[37] S. E. Pratsinis, *Prog. Energy Combust. Sci.* **1998**, *24*, 197.
[38] K. A. Dunphy Guzman, M. P. Finnegan, J. F. Banfield, *Environ. Sci. Technol.* **2006**, *40*, 7688.
[39] L. K. Limbach, Y. C. Li, R. N. Grass, T. J. Brunner, M. A. Hintermann, M. Muller, D. Gunther, W. J. Stark, *Environ. Sci. Technol.* **2005**, *39*, 9370.
[40] a) F. Caruso, *Adv. Mater.* **2001**, *13*, 11; b) F. He, D. Y. Zhao, *Environ. Sci. Technol.* **2007**, *41*, 6216.
[41] B. W. Hydutsky, E. J. Mack, B. B. Beckerman, J. M. Skluzacek, T. E. Mallouk, *Environ. Sci. Technol.* **2007**, *41*, 6418.
[42] H. Hyung, J. D. Fortner, J. B. Hughes, J. H. Kim, *Environ. Sci. Technol.* **2007**, *41*, 179.
[43] T. Phenrat, N. Saleh, K. Sirk, R. D. Tilton, G. V. Lowry, *Environ. Sci. Technol.* **2007**, *41*, 284.
[44] M. Madlova, S. A. Jones, I. Zwerschke, Y. Ma, R. C. Hider, B. Forbes, *Eur. J. Pharm. Sci.* **2009**, *72*, 438.
[45] a) K. F. Pirollo, E. H. Chang, *Trends Biotechnol.* **2008**, *26*, 552; b) M. L. Schipper, G. Iyer, A. L. Koh, Z. Chen, Y. Ebenstein, A. Aharoni, S. Keren, L. A. Bentolila, J. Li, J. Rao, X. Chen, U. Banin, A. M. Wu, R. Sinclair, S. Weiss, S. S. Gambhir, *Small* **2009**, *5*, 126–134; c) H. S. Choi, B. I. Ipe, P. Misra, J. H. Lee, M. G. Bawendi, J. V. Frangioni, *Nano Lett.* **2009**, *9*, 2354–2359; d) H. C. Fischer, L. Liu, S. Pang, W. C. W. Chan, *Adv. Funct. Mater.* **2006**, *16*, 1299–1305.
[46] Y. Zhang, Y. S. Chen, P. Westerhoff, J. C. Crittenden, *Environ. Sci. Technol.* **2008**, *42*, 321.
[47] M. Auffan, L. Decome, J. Rose, T. Orsiere, M. De Meo, V. Briois, C. Chaneac, L. Olivi, J. L. Berge-Lefranc, A. Botta, M. R. Wiesner, J. Y. Bottero, *Environ. Sci. Technol.* **2006**, *40*, 4367.
[48] T. Cedervall, I. Lynch, M. Foy, T. Berggad, S. C. Donnelly, G. Cagney, S. Linse, K. A. Dawson, *Angew. Chem.* **2007**, *119*, 5856; *Angew. Chem. Int. Ed.* **2007**, *46*, 5754.
[49] R. A. French, A. R. Jacobson, B. Kim, S. L. Isley, R. L. Penn, P. C. Baveye, *Environ. Sci. Technol.* **2009**, *43*, 1354.
[50] B. Chanteau, J. Fresnais, J. F. Berret, *Langmuir* **2009**, *25*, 9064.
[51] A. Leo, C. Hansch, D. Elkins, *Chem. Rev.* **1971**, *71*, 525.
[52] D. F. Veber, S. R. Johnson, H. Y. Cheng, B. R. Smith, K. W. Ward, K. D. Kopple, *J. Med. Chem.* **2002**, *45*, 2615.
[53] S. H. Doak, S. M. Griffiths, B. Manshian, N. Singh, P. M. Williams, A. P. Brown, G. J. S. Jenkins, *Mutagenesis* **2009**, *24*, 285.
[54] a) M. Semmler-Behnke, W. G. Kreyling, J. Lipka, S. Fertsch, A. Wenk, S. Takenaka, G. Schmid, W. Brandau, *Small* **2008**, *4*, 2108; b) S. W. Root, G. A. Andrews, R. M. Kniseley, M. P. Tyor, *Cancer* **1954**, *7*, 856; c) D. B. Zilversmit, G. A. Boyd, M. Brucer, *J. Lab. Clin. Med.* **1952**, *40*, 255; d) R. Gref, M. Luck, P. Quellec, M. Marchand, E. Dellacherie, S. Harnisch, T. Blunk, R. H. Muller, *Colloids Surf. B* **2000**, *18*, 301; e) G. Storm, S. O. Belliot, T. Daemen, D. D. Lasic, *Adv. Drug Delivery Rev.* **1995**, *17*, 31; f) R. M. Urban, J. J. Jacobs, M. J. Tomlinson, J. Gavrilovic, J. Black, M. Peoc'h, *J. Bone Jt. Surg. Am. Vol.* **2000**, *82A*, 457; g) S. E. A. Gratton, P. A. Ropp, P. D. Pohlhaus, J. C. Luft, V. J. Madden, M. E. Napier, J. M. DeSimone, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 11613; h) S. Mitragotri, J. Lahann, *Nat. Mater.* **2009**, *8*, 15; i) J. Rejman, V. Oberle, I. S. Zuhorn, D. Hoekstra,

Biochem. J. **2004**, *377*, 159; j) W. Zauner, N. A. Farrow, A. M. R. Haines, *J. Controlled Release* **2001**, *71*, 39.
[55] A. P. Roberts, A. S. Mount, B. Seda, J. Souther, R. Qiao, S. J. Lin, P. C. Ke, A. M. Rao, S. J. Klaine, *Environ. Sci. Technol.* **2007**, *41*, 3025.
[56] M. Schwalbe, N. Buske, M. Vetterlein, K. Hoffken, K. Pachmann, J. H. Clement, *Z. Phys. Chem.* **2006**, *220*, 125.
[57] M. J. D. Clift, B. Rothen-Rutishauser, D. M. Brown, R. Duffin, K. Donaldson, L. Proudfoot, K. Guy, V. Stone, *Toxicol. Appl. Pharmacol.* **2008**, *232*, 418.
[58] B. D. Chithrani, A. A. Ghazani, W. C. W. Chan, *Nano Lett.* **2006**, *6*, 662.
[59] K. Y. Win, S. S. Feng, *Biomaterials* **2005**, *26*, 2713.
[60] S. J. Kemp, A. J. Thorley, J. Gorelik, M. J. Seckl, M. J. O'Hare, A. Arcaro, Y. Korchev, P. Goldstraw, T. D. Tetley, *Am. J. Respir. Cell Mol. Biol.* **2008**, *39*, 591.
[61] M. Feng, D. Lee, P. Li, *Int. J. Pharm.* **2006**, *311*, 209.
[62] C. M. Goodman, C. D. McCusker, T. Yilmaz, V. M. Rotello, *Bioconjugate Chem.* **2004**, *15*, 897.
[63] C. Wilhelm, C. Billotey, J. Roger, J. N. Pons, J. C. Bacri, F. Gazeau, *Biomaterials* **2003**, *24*, 1001.
[64] S. Patil, A. Sandberg, E. Heckert, W. Self, S. Seal, *Biomaterials* **2007**, *28*, 4600.
[65] I. Lynch, T. Cedervall, M. Lundqvist, C. Cabaleiro-Lago, S. Linse, K. A. Dawson, *Adv. Colloid Interface Sci.* **2007**, *134–135*, 167.
[66] C. Lemarchand, R. Gref, C. Passirani, E. Garcion, B. Petri, R. Müller, D. Costantini, P. Couvreur, *Biomaterials* **2006**, *27*, 108.
[67] M. Horie, K. Nishio, K. Fujita, S. Endoh, A. Miyauchi, Y. Saito, H. Iwahashi, K. Yamamoto, H. Murayama, H. Nakano, N. Nanashima, E. Niki, Y. Yoshida, *Chem. Res. Toxicol.* **2009**, *22*, 543.
[68] R. Shukla, V. Bansal, M. Chaudhary, A. Basu, R. R. Bhonde, M. Sastry, *Langmuir* **2005**, *21*, 10644.
[69] N. W. Thomas, P. G. Jenkins, K. A. Howard, M. W. Smith, E. C. Lavelle, J. Holland, S. S. Davis, *J. Anat.* **1996**, *189*, 487.
[70] a) M. Geiser, B. Rothen-Rutishauser, N. Kapp, S. Schurch, W. Kreyling, H. Schulz, M. Semmler, V. I. Hof, J. Heyder, P. Gehr, *Environ. Health Perspect.* **2005**, *113*, 1555; b) B. M. Rothen-Rutishauser, S. Schurch, B. Haenni, N. Kapp, P. Gehr, *Environ. Sci. Technol.* **2006**, *40*, 4353.
[71] O. Schipper, *Environ. Sci. Technol.* **2005**, *39*, 473 A.
[72] K. W. Powers, S. C. Brown, V. B. Krishna, S. C. Wasdo, B. M. Moudgil, S. M. Roberts, *Toxicol. Sci.* **2006**, *90*, 296.
[73] L. W. Zhang, N. A. Monteiro-Riviere, *Toxicol. Sci.* **2009**, *110*, 138.
[74] H. L. Wong, R. Bendayan, A. M. Rauth, H. Y. Xue, K. Babakhanian, X. Y. Wu, *J. Pharmacol. Exp. Ther.* **2006**, *317*, 1372.
[75] J. S. Kim, T. J. Yoon, K. N. Yu, M. S. Noh, M. Woo, B. G. Kim, K. H. Lee, B. H. Sohn, S. B. Park, J. K. Lee, M. H. Cho, *J. Vet. Sci. U. A. R.* **2006**, *7*, 321.
[76] A. Verma, O. Uzun, Y. H. Hu, Y. Hu, H. S. Han, N. Watson, S. L. Chen, D. J. Irvine, F. Stellacci, *Nat. Mater.* **2008**, *7*, 588.
[77] H. W. Duan, S. M. Nie, *J. Am. Chem. Soc.* **2007**, *129*, 3333.
[78] a) Y. Q. Ge, Y. Zhang, J. G. Xia, M. Ma, S. Y. He, F. Nie, N. Gu, *Colloids Surf. B* **2009**, *73*, 294; b) A. França, B. Pelaz, M. Moros, C. Sanchez-Espinel, A. Hernandez, C. Fernandez-Lopez, V. Grazu, J. M. de La Fuente, I. Pastoriza-Santos, L. M. Liz-Marzan, A. Gonzalez-Fernandez, *Small* **2010**, *6*, 89.
[79] D. H. Lin, B. S. Xing, *Environ. Sci. Technol.* **2008**, *42*, 5580.
[80] K. Kasemets, A. Ivask, H. C. Dubourguier, A. Kahru, *Toxicol. in Vitro* **2009**, *23*, 1116.
[81] N. Carpita, D. Sabularse, D. Montezinos, D. P. Delmer, *Science* **1979**, *205*, 1144.
[82] S. J. Lin, J. Reppert, Q. Hu, J. S. Hudson, M. L. Reid, T. A. Ratnikova, A. M. Rao, H. Luo, P. C. Ke, *Small* **2009**, *5*, 72.
[83] E. Navarro, A. Baun, R. Behra, N. B. Hartmann, J. Filser, A. J. Miao, A. Quigg, P. H. Santschi, L. Sigg, *Ecotoxicology* **2008**, *17*, 372.
[84] D. H. Lin, B. S. Xing, *Environ. Pollut.* **2007**, *150*, 243.
[85] P. Demchick, A. L. Koch, *J. Bacteriol.* **1996**, *178*, 768.
[86] J. A. Kloepfer, R. E. Mielke, J. L. Nadeau, *Appl. Environ. Microbiol.* **2005**, *71*, 2548.
[87] V. I. Slaveykova, K. Startchev, J. Roberts, *Environ. Sci. Technol.* **2009**, *43*, 5117.
[88] T. J. Brunner, P. Wick, P. Manser, P. Spohn, R. N. Grass, L. K. Limbach, A. Bruinink, W. J. Stark, *Environ. Sci. Technol.* **2006**, *40*, 4374.
[89] M. Auffan, J. Rose, M. R. Wiesner, J. Y. Bottero, *Environ. Pollut.* **2009**, *157*, 1127.
[90] W. Busch, D. Kuhnel, A. Springer, T. Meissner, M. Gelinsky, A. Potthoff, S. Scholz, V. Richter, K. Schirmer, *Toxicol. Lett.* **2009**, *189*, S185.
[91] M. Midander, P. Cronholm, H. L. Karlsson, K. Elihn, L. Moller, C. Leygraf, I. O. Wallinder, *Small* **2009**, *5*, 389.
[92] H. H. Wang, R. L. Wick, B. S. Xing, *Environ. Pollut.* **2009**, *157*, 1171.
[93] N. M. Franklin, N. J. Rogers, S. C. Apte, G. E. Batley, G. E. Gadd, P. S. Casey, *Environ. Sci. Technol.* **2007**, *41*, 8484.
[94] R. J. Griffitt, R. Weil, K. A. Hyndman, N. D. Denslow, K. Powers, D. Taylor, D. S. Barber, *Environ. Sci. Technol.* **2007**, *41*, 8178.
[95] N. Lubick, *Environ. Sci. Technol.* **2007**, *41*, 7958.
[96] E. Okon, D. Pouliquen, P. Okon, Z. V. Kovaleva, T. P. Stepanova, S. G. Lavit, B. N. Kudryavtsev, P. Jallet, *Lab. Invest.* **1994**, *71*, 895.
[97] S. Mahendra, H. G. Zhu, V. L. Colvin, P. J. Alvarez, *Environ. Sci. Technol.* **2008**, *42*, 9424.
[98] M. Kovochich, B. Espinasse, M. Auffan, E. M. Hotze, L. Wessel, T. Xia, A. E. Nel, M. R. Wiesner, *Environ. Sci. Technol.* **2009**, *43*, 6378.
[99] K. S. Soppimath, D. C. W. Tan, Y. Y. Yang, *Adv. Mater.* **2005**, *17*, 318.
[100] A. T. Florence, *J. Drug Targeting* **2004**, *12*, 65.
[101] P. Jani, G. W. Halbert, J. Langridge, A. T. Florence, *J. Pharm. Pharmacol.* **1990**, *42*, 821.
[102] E. Allemann, J. C. Leroux, R. Gurny, *Adv. Drug Delivery Rev.* **1998**, *34*, 171.
[103] L. K. Limbach, P. Wick, P. Manser, R. N. Grass, A. Bruinink, W. J. Stark, *Environ. Sci. Technol.* **2007**, *41*, 4158.
[104] C. Lee, J. Y. Kim, W. Il Lee, K. L. Nelson, J. Yoon, D. L. Sedlak, *Environ. Sci. Technol.* **2008**, *42*, 4927.
[105] C. R. Keenan, R. Goth-Goldstein, D. Lucas, D. L. Sedlak, *Environ. Sci. Technol.* **2009**, *43*, 4555.
[106] M. Auffan, W. Achouak, J. Rose, M. A. Roncato, C. Chaneac, D. T. Waite, A. Masion, J. C. Woicik, M. R. Wiesner, J. Y. Bottero, *Environ. Sci. Technol.* **2008**, *42*, 6730.
[107] C. Lee, D. L. Sedlak, *Environ. Sci. Technol.* **2008**, *42*, 8528.
[108] T. Phenrat, T. C. Long, G. V. Lowry, B. Veronesi, *Environ. Sci. Technol.* **2009**, *43*, 195.
[109] E. G. Heckert, S. Seal, W. T. Self, *Environ. Sci. Technol.* **2008**, *42*, 5014.
[110] H. Y. Jia, Y. Liu, X. J. Zhang, L. Han, L. B. Du, Q. Tian, Y. C. Xut, *J. Am. Chem. Soc.* **2009**, *131*, 40.
[111] K. Hund-Rinke, M. Simon, *Environ. Sci. Pollut. Res.* **2006**, *13*, 225.
[112] G. Federici, B. J. Shaw, R. D. Handy, *Aquat. Toxicol.* **2007**, *84*, 415.
[113] J. P. Chen, S. Patil, S. Seal, J. F. McGinnis, *Nat. Nanotechnol.* **2006**, *1*, 142.
[114] A. S. Karakoti, N. A. Monteiro-Riviere, R. Aggarwal, J. P. Davis, R. J. Narayan, W. T. Self, J. McGinnis, S. Seal, *JOM* **2008**, *60*, 33.

[115] L. K. Adams, D. Y. Lyon, P. J. J. Alvarez, *Water Res.* **2006**, *40*, 3527.
[116] K. Van Hoecke, J. T. K. Quik, J. Mankiewicz-Boczek, K. A. C. De Schamphelaere, A. Elsaesser, P. Van der Meeren, C. Barnes, G. McKerr, C. V. Howard, D. Van De Meent, K. Rydzynski, K. A. Dawson, A. Salvati, A. Lesniak, I. Lynch, G. Silversmit, B. De Samber, L. Vincze, C. R. Janssen, *Environ. Sci. Technol.* **2009**, *43*, 4537.
[117] K. Wiench, W. Wohlleben, V. Hisgen, K. Radke, E. Salinas, S. Zok, R. Landsiedel, *Chemosphere* **2009**, *76*, 1356.
[118] B. Nowack, *Environ. Pollut.* **2009**, *157*, 1063.
[119] B. Nowack, T. D. Bucheli, *Environ. Pollut.* **2007**, *150*, 5.
[120] L. K. Limbach, R. Bereiter, E. Mueller, R. Krebs, R. Gaelli, W. J. Stark, *Environ. Sci. Technol.* **2008**, *42*, 5828.
[121] M. A. Kiser, P. Westerhoff, T. Benn, Y. Wang, J. Perez-Rivera, K. Hristovski, *Environ. Sci. Technol.* **2009**, *43*, 6757.
[122] K. Riehemann, S. W. Schneider, T. A. Luger, B. Godin, M. Ferrari, H. Fuchs, *Angew. Chem.* **2009**, *121*, 886; *Angew. Chem. Int. Ed.* **2009**, *48*, 872.
[123] J. M. Klostranec, W. C. W. Chan, *Adv. Mater.* **2006**, *18*, 1953.
[124] R. A. Sperling, P. Rivera Gil, F. Zhang, M. Zanella, W. J. Parak, *Chem. Soc. Rev.* **2008**, *37*, 1896.
[125] A. Burns, H. Ow, U. Wiesner, *Chem. Soc. Rev.* **2006**, *35*, 1028.
[126] M. De, P. S. Ghosh, V. M. Rotello, *Adv. Mater.* **2008**, *20*, 4225.
[127] J. W. Card, D. C. Zeldin, J. C. Bonner, E. R. Nestmann, *Am. J. Physiol. Lung Cell. Mol. Physiol.* **2008**, *295*, L400.
[128] E. Katz, I. Willner, *Angew. Chem.* **2004**, *116*, 6166; *Angew. Chem. Int. Ed.* **2004**, *43*, 6042.
[129] a) J. Kreuter, *J. Controlled Release* **1991**, *16*, 169; b) R. Langer, *Science* **1990**, *249*, 1527; c) S. E. Dunn, A. G. A. Coombes, M. C. Garnett, S. S. Davis, M. C. Davies, L. Illum, *J. Controlled Release* **1997**, *44*, 65.
[130] C. Fonseca, S. Simoes, R. E. Gaspar, *J. Controlled Release* **2002**, *83*, 273.
[131] D. Gao, H. Xu, M. A. Philbert, R. Kopelman, *Angew. Chem.* **2007**, *119*, 2274; *Angew. Chem. Int. Ed.* **2007**, *46*, 2224.
[132] G. B. Jacobson, R. Shinde, C. H. Contag, R. N. Zare, *Angew. Chem.* **2008**, *120*, 7998; *Angew. Chem. Int. Ed.* **2008**, *47*, 7880.
[133] C. Dufes, I. F. Uchegbu, A. G. Schatzlein, *Adv. Drug Delivery Rev.* **2005**, *57*, 2177.
[134] M. Vallet-Regí, F. Balas, D. Arcos, *Angew. Chem.* **2007**, *119*, 7692; *Angew. Chem. Int. Ed.* **2007**, *46*, 7548.
[135] M. Vallet-Regí, *Chem. Eur. J.* **2006**, *12*, 5934.
[136] A. V. Kabanov, S. V. Vinogradov, *Angew. Chem.* **2009**, *121*, 5524; *Angew. Chem. Int. Ed.* **2009**, *48*, 5418.
[137] K. K. Sandhu, C. M. McIntosh, J. M. Simard, S. W. Smith, V. M. Rotello, *Bioconjugate Chem.* **2002**, *13*, 3.
[138] J. D. Gibson, B. P. Khanal, E. R. Zubarev, *J. Am. Chem. Soc.* **2007**, *129*, 11653.
[139] T. Musumeci, C. A. Ventura, I. Giannone, B. Ruozi, L. Montenegro, R. Pignatello, G. Puglisi, *Int. J. Pharm.* **2006**, *325*, 172.
[140] S. S. Feng, L. Mu, K. Y. Win, G. F. Huang, *Curr. Med. Chem.* **2004**, *11*, 413.
[141] S. S. Davis, *Trends Biotechnol.* **1997**, *15*, 217.
[142] T. K. Jain, M. A. Morales, S. K. Sahoo, D. L. Leslie-Pelecky, V. Labhasetwar, *Mol. Pharm.* **2005**, *2*, 194.
[143] I. Brigger, C. Dubernet, P. Couvreur, *Adv. Drug Delivery Rev.* **2002**, *54*, 631.
[144] E. Soussan, S. Cassel, M. Blanzat, I. Rico-Lattes, *Angew. Chem.* **2009**, *121*, 280; *Angew. Chem. Int. Ed.* **2009**, *48*, 274.
[145] Y. Y. Wang, S. K. Lai, J. S. Suk, A. Pace, R. Cone, J. Hanes, *Angew. Chem.* **2008**, *120*, 9872; *Angew. Chem. Int. Ed.* **2008**, *47*, 9726.
[146] G. Gopalakrishnan, C. Danelon, P. Izewska, M. Prummer, P. Y. Bolinger, I. Geissbuhler, D. Demurtas, J. Dubochet, H. Vogel, *Angew. Chem.* **2006**, *118*, 5604; *Angew. Chem. Int. Ed.* **2006**, *45*, 5478.
[147] J. H. Lee, K. Lee, S. H. Moon, Y. Lee, T. G. Park, J. Cheon, *Angew. Chem.* **2009**, *121*, 4238; *Angew. Chem. Int. Ed.* **2009**, *48*, 4174.
[148] A. P. Griset, J. Walpole, R. Liu, A. Gaffey, Y. L. Colson, M. W. Grinstaff, *J. Am. Chem. Soc.* **2009**, *131*, 2469.
[149] E. Aznar, M. D. Marcos, R. Martinez-Manez, F. Sancenon, J. Soto, P. Amoros, C. Guillem, *J. Am. Chem. Soc.* **2009**, *131*, 6833.
[150] A. Corma, U. Diaz, M. Arrrica, E. Fernandez, I. Ortega, *Angew. Chem.* **2009**, *121*, 6365; *Angew. Chem. Int. Ed.* **2009**, *48*, 6247.
[151] A. G. Skirtach, A. M. Javier, O. Kreft, K. Köhler, A. P. Alberola, H. Möhwald, W. J. Parak, G. B. Sukhorukov, *Angew. Chem.* **2006**, *118*, 4728; *Angew. Chem. Int. Ed.* **2006**, *45*, 4612.
[152] G. Han, C. C. You, B. J. Kim, R. S. Turingan, N. S. Forbes, C. T. Martin, V. M. Rotello, *Angew. Chem.* **2006**, *118*, 3237; *Angew. Chem. Int. Ed.* **2006**, *45*, 3165.
[153] D. V. Volodkin, A. G. Skirtach, H. Möhwald, *Angew. Chem.* **2009**, *121*, 1839; *Angew. Chem. Int. Ed.* **2009**, *48*, 1807.
[154] K. A. Janes, P. Calvo, M. J. Alonso, *Adv. Drug Delivery Rev.* **2001**, *47*, 83.
[155] R. M. Schiffelers, A. Ansari, J. Xu, Q. Zhou, Q. Q. Tang, G. Storm, G. Molema, P. Y. Lu, P. V. Scaria, M. C. Woodle, *Nucleic Acids Res.* **2004**, *32*, e149.
[156] D. W. Bartlett, M. E. Davis, *Bioconjugate Chem.* **2007**, *18*, 456.
[157] V. Sokolova, M. Epple, *Angew. Chem.* **2008**, *120*, 1402; *Angew. Chem. Int. Ed.* **2008**, *47*, 1382.
[158] A. M. De Campos, A. Sanchez, R. Gref, P. Calvo, M. J. Alonso, *Eur. J. Pharm. Sci.* **2003**, *20*, 73.
[159] I. A. J. Dreesen, N. A. Luchinger, W. J. Stark, M. Fussenegger, *Biotechnol. Bioeng.* **2009**, *102*, 1197.
[160] S. Loher, W. J. Stark, M. Maciejewski, A. Baiker, S. E. Pratsinis, D. Reichardt, F. Maspero, F. Krumeich, D. Gunther, *Chem. Mater.* **2005**, *17*, 36.
[161] a) S. T. Selvan, P. K. Patra, C. Y. Ang, J. Y. Ying, *Angew. Chem.* **2007**, *119*, 2500; *Angew. Chem. Int. Ed.* **2007**, *46*, 2448; b) Ref. [26]; c) M. Bruchez, Jr., M. Moronne, P. Gin, S. Weiss, A. P. Alivisatos, *Science* **1998**, *281*, 2013; d) I. L. Medintz, H. T. Uyeda, E. R. Goldman, H. Mattoussi, *Nat. Mater.* **2005**, *4*, 435; e) N. L. Rosi, C. A. Mirkin, *Chem. Rev.* **2005**, *105*, 1547.
[162] a) X. Michalet, F. F. Pinaud, L. A. Bentolila, J. M. Tsay, S. Doose, J. J. Li, G. Sundaresan, A. M. Wu, S. S. Gambhir, S. Weiss, *Science* **2005**, *307*, 538; b) X. Michalet, F. F. Pinaud, T. D. Lacoste, M. Dahan, M. P. Bruchez, A. P. Alivisatos, S. Weiss, *Single Mol.* **2001**, *2*, 261.
[163] C. C. Berry, A. S. G. Curtis, *J. Phys. D* **2003**, *36*, R198.
[164] R. Freeman, R. Gill, I. Shweky, M. Kotler, U. Banin, I. Willner, *Angew. Chem.* **2009**, *121*, 315; *Angew. Chem. Int. Ed.* **2009**, *48*, 309.
[165] J. Conroy, S. J. Byrne, Y. K. Gun′ko, Y. P. Rakovich, J. F. Donegan, A. Davies, D. Kelleher, Y. Volkov, *Small* **2008**, *4*, 2006.
[166] J. W. M. Bulte, D. L. Kraitchman, *NMR Biomed.* **2004**, *17*, 484.
[167] C. H. Cunningham, T. Arai, P. C. Yang, M. V. McConnell, J. M. Pauly, S. M. Conolly, *Magn. Reson. Med.* **2005**, *53*, 999.
[168] A. K. Gupta, M. Gupta, *Biomaterials* **2005**, *26*, 3995.
[169] Y. M. Huh, Y. W. Jun, H. T. Song, S. Kim, J. S. Choi, J. H. Lee, S. Yoon, K. S. Kim, J. S. Shin, J. S. Suh, J. Cheon, *J. Am. Chem. Soc.* **2005**, *127*, 12387.
[170] A. Jordan, R. Scholz, P. Wust, H. Fahling, R. Felix, *J. Magn. Magn. Mater.* **1999**, *201*, 413.
[171] K. Kneipp, H. Kneipp, J. Kneipp, *Acc. Chem. Res.* **2006**, *39*, 443.
[172] A. H. Lu, E. L. Salabas, F. Schüth, *Angew. Chem.* **2007**, *119*, 1242; *Angew. Chem. Int. Ed.* **2007**, *46*, 1222.

[173] R. C. Jin, *Angew. Chem.* **2008**, *120*, 6852; *Angew. Chem. Int. Ed.* **2008**, *47*, 6750.
[174] R. N. Grass, E. K. Athanassiou, W. J. Stark, *Angew. Chem.* **2007**, *119*, 4996; *Angew. Chem. Int. Ed.* **2007**, *46*, 4909.
[175] M. Bohner, T. J. Brunner, W. J. Stark, *J. Mater. Chem.* **2008**, *18*, 5669.
[176] S. S. Kim, M. S. Park, O. Jeon, C. Y. Choi, B. S. Kim, *Biomaterials* **2006**, *27*, 1399.
[177] T. Waltimo, T. J. Brunner, M. Vollenweider, W. J. Stark, M. Zehnder, *J. Dent. Res.* **2007**, *86*, 754.
[178] S. Loher, O. D. Schneider, T. Maienfisch, S. Bokorny, W. J. Stark, *Small* **2008**, *4*, 824.
[179] L. L. Hench, J. Wilson, *Science* **1984**, *226*, 630.
[180] L. L. Hench, *J. Am. Ceram. Soc.* **1991**, *74*, 1487.
[181] H. J. Kim, T. Phenrat, R. D. Tilton, G. V. Lowry, *Environ. Sci. Technol.* **2009**, *43*, 3824.
[182] I. K. Konstantinou, T. A. Albanis, *Appl. Catal. B* **2004**, *49*, 1.
[183] A. Fujishima, K. Honda, *Nature* **1972**, *238*, 37.
[184] J. Yang, C. H. Lee, H. J. Ko, J. S. Suh, H. G. Yoon, K. Lee, Y. M. Huh, S. Haam, *Angew. Chem.* **2007**, *119*, 8992; *Angew. Chem. Int. Ed.* **2007**, *46*, 8836.
[185] S. J. Son, J. Reichel, B. He, M. Schuchman, S. B. Lee, *J. Am. Chem. Soc.* **2005**, *127*, 7316.
[186] Y. Jing, N. Mal, P. S. Williams, M. Mayorga, M. S. Penn, J. J. Chalmers, M. Zborowski, *FASEB J.* **2008**, *22*, 4239.
[187] K. E. Scarberry, E. B. Dickerson, J. F. McDonald, Z. J. Zhang, *J. Am. Chem. Soc.* **2008**, *130*, 10258.
[188] M. Kettering, J. Winter, M. Zeisberger, C. Alexiou, S. Bremer-Streck, C. Bergemann, W. A. Kaiser, I. Hilger, *Rofo-Fortschr. Gebiet Rontgenstrahlen Bildgeb. Verfahr.* **2006**, *178*, 1255.
[189] H. W. Gu, K. M. Xu, C. J. Xu, B. Xu, *Chem. Commun.* **2006**, 941.
[190] Q. A. Pankhurst, J. Connolly, S. K. Jones, J. Dobson, *J. Phys. D* **2003**, *36*, R167.
[191] T. Neuberger, B. Schopf, H. Hofmann, M. Hofmann, B. von Rechenberg, *J. Magn. Magn. Mater.* **2005**, *293*, 483.
[192] S. M. Moghimi, A. C. Hunter, J. C. Murray, *Faseb J.* **2005**, *19*, 311.
[193] J. H. Lee, Y. M. Huh, Y. Jun, J. Seo, J. Jang, H. T. Song, S. Kim, E. J. Cho, H. G. Yoon, J. S. Suh, J. Cheon, *Nat. Med.* **2007**, *13*, 95.
[194] I. K. Herrmann, R. N. Grass, W. J. Stark, *Nanomedicine* **2009**, *4*, 787.
[195] F. M. Koehler, M. Rossier, M. Waelle, E. K. Athanassiou, L. K. Limbach, R. N. Grass, D. Gunther, W. J. Stark, *Chem. Commun.* **2009**, 4862.
[196] I. K. Herrmann, R. N. Grass, D. Mazunin, W. J. Stark, *Chem. Mater.* **2009**, *21*, 3275.
[197] J. H. P. Watson, *J. Appl. Phys.* **1973**, *44*, 4209.
[198] W. Weber, C. Lienhart, M. D. E. Baba, R. N. Grass, T. Kohler, R. Müller, W. J. Stark, M. Fussenegger, *J. Biotechnol.* **2009**, *141*, 118.
[199] I. K. Herrmann, M. Urner, F. M. Koehler, M. Hasler, B. Roth-Z'Graggen, R. N. Grass, U. Ziegler, B. Beck-Schimmer, W. J. Stark, *Small* **2009**, *6*, 1388 – 1392.
[200] M. Rossier, F. M. Koehler, E. K. Athanassiou, R. N. Grass, W. J. Stark, *J. Mater. Chem.* **2009**, *19*, 8239.
[201] I. Sondi, B. Salopek-Sondi, *J. Colloid Interface Sci.* **2004**, *275*, 177.
[202] J. R. Morones, J. L. Elechiguerra, A. Camacho, K. Holt, J. B. Kouri, J. T. Ramirez, M. J. Yacaman, *Nanotechnology* **2005**, *16*, 2346.
[203] A. Panáček, L. Kvitek, R. Prucek, M. Kolar, R. Vecerova, N. Pizurova, V. K. Sharma, T. Nevecna, R. Zboril, *J. Phys. Chem. B* **2006**, *110*, 16248.
[204] X. Chen, H. J. Schluesener, *Toxicol. Lett.* **2008**, *176*, 1.
[205] O. Choi, K. K. Deng, N. J. Kim, L. Ross, R. Y. Surampalli, Z. Q. Hu, *Water Res.* **2008**, *42*, 3066.
[206] O. Choi, Z. Q. Hu, *Environ. Sci. Technol.* **2008**, *42*, 4583.
[207] P. K. Stoimenov, R. L. Klinger, G. L. Marchin, K. J. Klabunde, *Langmuir* **2002**, *18*, 6679.
[208] A. Thill, O. Zeyons, O. Spalla, F. Chauvat, J. Rose, M. Auffan, A. M. Flank, *Environ. Sci. Technol.* **2006**, *40*, 6151.
[209] V. Sambhy, M. M. MacBride, B. R. Peterson, A. Sen, *J. Am. Chem. Soc.* **2006**, *128*, 9798.
[210] M. R. Elahifard, S. Rahimnejad, S. Haghighi, M. R. Gholami, *J. Am. Chem. Soc.* **2007**, *129*, 9552.
[211] C. Gunawan, W. Y. Teoh, C. P. Marquis, J. Lifia, R. Amal, *Small* **2009**, *5*, 341.
[212] S. M. Moghimi, A. C. Hunter, J. C. Murray, *Pharmacol. Rev.* **2001**, *53*, 283.
[213] R. D. Handy, R. Owen, E. Valsami-Jones, *Ecotoxicology* **2008**, *17*, 315.
[214] M. J. Molina, F. S. Rowland, *Nature* **1974**, *249*, 810.
[215] R. W. Risebrough, P. Rieche, D. B. Peakall, S. G. Herman, M. N. Kirven, *Nature* **1968**, *220*, 1098.
[216] S. H. Safe, *Crit. Rev. Toxicol.* **1994**, *24*, 87.
[217] J. Bitman, H. C. Cecil, *J. Agric. Food Chem.* **1970**, *18*, 1108.
[218] S. Jensen, A. G. Johnels, M. Olsson, G. Otterlin, *Nature* **1969**, *224*, 247.
[219] G. M. Woodwell, C. F. Wurster, P. A. Isaacson, *Science* **1967**, *156*, 821.
[220] C. F. Wurster, D. B. Wingate, *Science* **1968**, *159*, 979.
[221] J. G. B. Derraik, *Mar. Pollut. Bull.* **2002**, *44*, 842.
[222] A. T. Pruter, *Mar. Pollut. Bull.* **1987**, *18*, 305.
[223] R. C. Thompson, Y. Olsen, R. P. Mitchell, A. Davis, S. J. Rowland, A. W. G. John, D. McGonigle, A. E. Russell, *Science* **2004**, *304*, 838.
[224] C. S. Wong, D. R. Green, W. J. Cretney, *Nature* **1974**, *247*, 30.
[225] B. Jonsson, T. Forseth, O. Ugedal, *Nature* **1999**, *400*, 417.
[226] R. Doll, *Br. J. Ind. Med.* **1955**, *12*, 81.
[227] B. T. Mossman, J. Bignon, M. Corn, A. Seaton, J. B. L. Gee, *Science* **1990**, *247*, 294.
[228] A. M. Koch, F. Reynolds, M. F. Kircher, H. P. Merkle, R. Weissleder, L. Josephson, *Bioconjugate Chem.* **2003**, *14*, 1115.
[229] L. K. Limbach, R. N. Grass, W. J. Stark, *Chimia* **2009**, *63*, 38.
[230] O. D. Schneider, F. Weber, T. J. Brunner, S. Loher, M. Ehrbar, P. R. Schmidlin, W. J. Stark, *Acta Biomater.* **2009**, *5*, 1775.
[231] P. Rosenkranz, Q. Chaudhry, V. Stone, T. F. Fernandes, *Environ. Toxicol. Chem.* **2009**, *28*, 2142.

