

## Chapter 5

# Ecotoxicological Aspects of Nanomaterials in the Aquatic Environment

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## 5.1 Introduction

Ecotoxicology thrives to understand how natural or synthetic chemical pollutants impact, alone or in combination with other stressors, on constituents of ecosystems in an integral manner (Truhaut, 1977). Nanoecotoxicology has recently emerged as a sub-discipline of ecotoxicology and specifically aims to identify and predict effects

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elicited by nano-sized materials on ecosystems. To achieve this aim, nanoecotoxicology needs to take into consideration the entry routes and fate of nanomaterials in the abiotic and biotic environment to define exposure. It moreover needs to identify those interactions of nanomaterials with biota that alter the proper function of cells comprising an organism, thus impacting populations, which in turn can lead to changes in community structure and function. Together, this information can be used to evaluate the risk that nanomaterials may have in a given environment.

An increasing number of ecotoxicity studies are reporting on the toxicity of various engineered nanomaterials, specifically nanoparticles (NP), to organisms living in the aquatic environment including plants, fungi, algae, invertebrates and fish (Baun *et al.*, 2008; Farre *et al.*, 2009; Handy *et al.*, 2008a; Navarro *et al.*, 2008a). In a first stage, most studies have been descriptive or “proof-of-principle” experiments that have tried to document toxic effects, and report the concentrations of NP that produce toxicity to individual organisms. Reported effect concentrations were often high, in the mg/L range, but were based on intended (i.e., nominal) exposure concentrations irrespective of behavior in aqueous media and bioavailability. More emphasis has recently been laid on proper characterization of the NP in single species experimental setups as well as in model ecosystems. Nevertheless, information on environmental NP bioavailability to different aquatic organisms, interaction with biomolecules in cells of organisms, and sensitivity of biotic communities and ecosystem processes to NP exposure is still scarce. It does not allow as yet to conclude on ecological implications; thus, the need of an ecological perspective in nanoecotoxicology has been emphasized (Bernhardt *et al.*, 2010; Klaine *et al.*, 2008).

Given the growing variety of NP, along with the diversity of aquatic species and environments, a key to promote sound risk assessment in nanoecotoxicology is to understand the mechanisms that govern the fate of NP in aquatic environments and their behavior at the NP-biota interface. The aim of this chapter is to provide an overview of those mechanisms known or anticipated to date. We focus on metal-based NP (MeNP) because, for the areas addressed by us, research is more advanced for these NP compared to other types of NP. However, the mechanistic view provided here can in principle be transferred to other types of NP as well.

## 5.2 Fate in the Aquatic Environment

The most important processes affecting the fate of nanoparticles in aquatic systems are agglomeration and aggregation, dissolution, redox reactions and transformation into new solid phases (Handy *et al.*, 2008b; Klaine *et al.*, 2008; Navarro *et al.*, 2008a; Nowack and Bucheli, 2007). Agglomeration or aggregation of nanoparticles lead to larger particles, which may be removed from the water column and transported to the sediments. Nanoparticles are only weakly bound by agglomeration, whereas chemical bonds between particles are formed upon aggregation (Jiang *et al.*, 2009). Bioavailability of NP and their dissolution behavior may also be different for larger agglomerated particles. Agglomeration is determined by the medium composition, mostly ionic strength, pH and the concentration of natural organic matter. To assess the effects of these various factors, the behavior of different types of NP needs to be considered, such as elemental MeNP (e.g., Ag(0), Au(0), Cu(0)), metal oxide NP (e.g., TiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub>, CeO<sub>2</sub>, ZnO), quantum dots and carbon-based NP (carbon nanotubes and fullerenes).

Elemental MeNP are maintained in suspension by their surface coating, which may be negatively or positively charged, or may be stabilizing based on steric effects. Examples are AgNP suspensions which are stabilized by carbonate, citrate, or charged polymers. Other polymeric coatings stabilize AgNP by steric effects (e.g., polyvinylpyrrolidone PVP). Effects of pH and ionic strength on the agglomeration of AgNP suspensions have been examined for various coatings. Agglomeration has been observed in particular at low pH, when the negative surface charge of the coating becomes neutralized, and at high ionic strength (Badawy *et al.*, 2010; Elzey and Grassian, 2010; Gao *et al.*, 2009; Huynh and Chen, 2011; Piccapietra *et al.*, 2012). Humic acids are expected to influence the suspension stability by interactions with the surfaces and by steric effects. In natural waters, the water composition with respect to pH, ionic strength, concentration of divalent cations and of fulvic and humic acids will determine the colloidal stability of AgNP suspensions (Piccapietra *et al.*, 2012). These factors have also to be considered in media used for biological experiments with AgNP.

Metal oxide NP such as TiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub>, and CeO<sub>2</sub> behave as oxides with a strongly pH-dependent surface charge, due to the protonation and deprotonation of the surface OH-groups, if they are not coated

by organic compounds or functionalized. Agglomeration of these metal oxides is expected at neutral surface charge in the pH range of the zero point of charge, which is a distinct characteristic of each of the oxides. For example, CeO<sub>2</sub> NP have a zero point of charge in the neutral pH-range 7–8 (De Faria and Trasatti, 1994; Nabavi *et al.*, 1993). Agglomeration of CeO<sub>2</sub> NP has been observed around pH 7, such as in media used for growth and toxicity testing of aquatic organisms (Rogers *et al.*, 2009; Van Hoecke *et al.*, 2009). TiO<sub>2</sub> behaves in a similar way, with a zero point of charge in the neutral pH-range. Stabilization of TiO<sub>2</sub> NP by humic acids has been demonstrated (Domingos *et al.*, 2009).

Dissolution reactions of NP are expected to play an important role in their toxicity, as toxic metal ions may be released from the NP, in particular Ag<sup>+</sup> from AgNP, Cu<sup>2+</sup> from elemental Cu or from CuO NP, Zn<sup>2+</sup> from ZnO, Cd<sup>2+</sup> from quantum dots (e.g., CdSe, CdTe). Toxicity of these ions to aquatic organisms is well known and strongly depends on speciation of these ions in solution (Campbell *et al.*, 2002; Hiriart-Baer *et al.*, 2006).

Dissolution reactions of AgNP lead under oxidation of Ag(0) to the release of free Ag<sup>+</sup> ions, which may be determining the toxic effects (Kittler *et al.*, 2010; Navarro *et al.*, 2008b). The rate and extent of dissolution is expected to be dependent on the solution conditions, such as pH, presence of oxygen and effects of ligands (Galloway *et al.*, 2010). Increased dissolution at low pH, as well as the role of O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub> as oxidants was shown (Galloway *et al.*, 2010). Increased dissolution of AgNP in the presence of algae has been postulated to influence their toxicity (Navarro *et al.*, 2008b). Various coatings on AgNP determine the extent and kinetics of the dissolution reactions (Odzak *et al.*, 2012). The release of Ag<sup>+</sup>-ions from AgNP in the presence of chloride or sulfide may lead to the formation of solid silver chloride or silver sulfide at the surface of the NP, as demonstrated upon aging of AgNP with chloride (Badawy *et al.*, 2010), and for the formation of silver sulfide upon exposure of AgNP in a pilot wastewater treatment plant (Kaegi *et al.*, 2011). Sulfide may form the very insoluble silver sulfide solid phase, but also dissolved silver complexes (e.g., AgHS<sup>0</sup>, Ag(HS)<sub>2</sub><sup>-</sup>) and small Ag<sub>x</sub>S<sub>y</sub> aggregates (Bell and Kramer, 1999; Luther and Rickard, 2005).

The solubility of metal oxide NP varies over a wide range, depending on their composition and on the crystal structures. For example, Ce(IV)O<sub>2</sub> has very low solubility at neutral pH, and soluble

Ce(IV) species occur only at  $\text{pH} < 4$  (Hayes *et al.*, 2002; Yu and O'Keefe, 2006). Free  $\text{Ce}^{4+}$  ions only occur in very acidic solutions. However, upon reduction to Ce(III), the Ce solubility is strongly increased (Hayes *et al.*, 2002). In a similar way, the solubility of  $\text{TiO}_2$  is very low at neutral pH (Knauss *et al.*, 2001; Ziemniak *et al.*, 1993). In contrast to these poorly soluble oxides, ZnO is readily soluble, and effects of ZnO have been shown to mostly depend on released  $\text{Zn}^{2+}$  (Franklin *et al.*, 2007).

Quantum dots, such as CdSe, are usually coated with a zinc sulfide shell and with organic polymers. In spite of this coating, substantial release of  $\text{Cd}^{2+}$  from CdSe quantum dots has been observed in toxicity experiments (King-Heiden *et al.*, 2009; Klaine *et al.*, 2008).

### 5.3 Fate in Model Ecosystems

Ecotoxicity studies of appropriate ecological relevance include those involving communities where various species occur within a complex network and interact with each other and the abiotic environment to provide ecosystem processes. Considering the entrance of NP in the aquatic environment, most urgent questions are (1) where do NP partition in aquatic systems and in which physico-chemical state; (2) are the NP bioavailable; (3) do NP affect the structure and function of biotic communities, and (4) do community changes entail changes in ecological processes? Among a dozen of published studies that can be considered as ecologically relevant, all have considered MeNP and have examined their distribution in diverse model systems enclosed in microcosm or mesocosms. Examination of the fate of gold nanorods in an estuarine ecosystem containing sediments, microbial biofilms, primary producers, filter feeders, grazers and omnivores identified the filter feeders as the most effective sink for NP, followed closely by biofilms (Ferry *et al.*, 2009). While the question whether and how NP are taken up by aquatic organisms remains to be elucidated in detail (see Section 5.4), trophic transfer of NP from algae to daphnids (Bouldin *et al.*, 2008) and to clams (Croteau *et al.*, 2011), and as well as from daphnids to fish (Zhu *et al.*, 2010) was shown for metal-based quantum dots, ZnO and  $\text{TiO}_2$  NP, respectively, evidencing diet as one pathway through which daphnids, clams and fish accumulate the metals. In another study, quantum dots were transferred to higher trophic organisms (rotifers) through dietary uptake of

ciliated protozoans (Holbrook *et al.*, 2008). However, these studies did not resolve whether intact particles or the dissolved metals have been transferred. Similar as for dissolved metals, biomagnification, a process through which concentrations of chemicals increase in organisms from a lower to a higher trophic level within the same food web, was not observed to occur in these studies. However, even in absence of biomagnification, aquatic organisms can accumulate large amounts of metals and become a significant dietary source of metal to their predators (Reinfelder *et al.*, 1998).

One study has reported on the trophic transfer of intact particles in a system including bacteria previously exposed to quantum dots and protozoa that fed on the bacteria (Werlin *et al.*, 2011). In this system, quantum dots were shown to be biomagnified in the protozoa that also displayed inhibited digestion as shown by the presence of intact bacteria containing NP in the food vacuoles of the protozoa. Trophic transfer and biomagnification of NP was also demonstrated in a terrestrial system composed of plants dosed with AuNP that were fed to hornworm larvae (Judy *et al.*, 2011). The observation that NP may be biomagnified not only confirms the importance of dietary exposure for the transfer of NP, but also the fact that NP might accumulate up the food chain to concentrations eventually resulting in toxicity for predators. Clearly, more systematic work is necessary to understand the apparent contradictory information on NP biomagnification.

## **5.4 Routes and Mechanisms of Uptake into Aquatic Organisms**

Routes for uptake of NP are defined by the organism's physiology, with uptake consisting of two principal steps. The first is association and potential uptake of NP by the cells forming the environment-organism barrier. Damage at this level may impair barrier functions, e.g., regarding nutrient uptake or pathogen defense. The second step in NP uptake comprises the ability of NP to actually translocate through this barrier tissue into the organism from where internal distribution can prevail. Unicellular organisms are special in a way that the cell itself comprises the environment-organism barrier so that uptake by the cell is equivalent to organism uptake.

### 5.4.1 Uptake Routes

One route of uptake is through the respiratory system, e.g., the gills of fish or molluscs. Inasmuch as the gills' function is gas exchange ("breathing"), their large surface and direct exposure to water may render them sensitive targets of NP suspended in the water column and possible transfer points into organisms. Indeed, association with fish gill tissue of various MeNP has been found (Chen *et al.*, 2011; Federici *et al.*, 2007; Griffitt *et al.*, 2012; Griffitt *et al.*, 2009; Griffitt *et al.*, 2007; Johnston *et al.*, 2010; Li *et al.*, 2009; Scown *et al.*, 2010).

Cellular uptake of NP is difficult to confirm *in vivo*. Uptake was, however, demonstrated for AgNP in rainbow trout (*Oncorhynchus mykiss*) primary gill cell preparations (Farkas *et al.*, 2011) and for WC-Co NP in the rainbow trout gill cell line, RTgill-W1 (Kuhnel *et al.*, 2009). The latter study explicitly showed that NP can also be taken up by the cells when present in the exposure medium in agglomerated form. Indication for a possible translocation of NP through the gill epithelium comes from the study by (Farkas *et al.*, 2011) where total silver transport through a primary gill cell monolayer was higher in the presence of AgNP compared to Ag<sup>+</sup> ions.

Another route of NP exposure is through diet. Filter feeders, i.e., animals that feed by straining suspended matter and food particles from water, such as many forage fishes, crustaceans and aquatic molluscs, may be particularly exposed to NP dispersed in the water column, especially if NP are agglomerated/aggregated or aggregate-bound (Ward and Kach, 2009). Indeed, NP have been found in association with intestinal structures upon aqueous exposure of the water flea, *Daphnia magna*, to AuNP (Lovern *et al.*, 2008) and CuO NP (Heinlaan *et al.*, 2011). Damage to digestive cells by MeNP has been demonstrated, e.g., in the freshwater bivalves *Elliption complanata* (Gagne *et al.*, 2008) and *Mytilus edulis* (Koehler *et al.*, 2008; Tedesco *et al.*, 2008). Moreover, considering the common phenomenon of NP agglomeration/aggregation and sedimentation in natural waters, organisms living in sediments or feeding on biofilms may be particularly prone to NP exposure and thus uptake ((Ferry *et al.*, 2009), see Section 5.3). Along these lines, incorporation of TiO<sub>2</sub> into the intestinal lumen was observed for the sediment dwelling marine polychaete, *Arenicola marina* (Galloway *et al.*, 2010). Uptake into intestinal structures was also found in rainbow trout upon dietary TiO<sub>2</sub> exposure (Johnston *et al.*, 2010; Ramsden *et al.*, 2009) and

through drinking water containing dispersed TiO<sub>2</sub> NP (Federici *et al.*, 2007).

Despite MeNP reaching, and interacting with, gill and intestinal tissue, it has not yet been shown conclusively that NP also cross these barriers to translocate into the body. The main reason for this lack of knowledge is instrumental limitations because it is difficult to track and quantify NP in complex biological matrices and to distinguish NP from ions dissolving from it. Thus, a biological response detected in a certain tissue or organ upon MeNP exposure could be an indirect effect of metal ions rather than particles reaching the target site; or it could result from a chain of events initiated at the organism-environment barrier and then being propagated to other tissues or organs without direct exposure to NP. Nevertheless, reports about the detection, e.g., in fish, of total metal content in brain, kidney, liver, ovary, and heart (Chen *et al.*, 2011; Federici *et al.*, 2007; Johnston *et al.*, 2010; Scown *et al.*, 2010) upon MeNP exposure are strong indications that uptake via environment-organism barriers does take place. Support also comes from a study with see-through medaka (*Oryzias latipes*): Kashiwada (Kashiwada, 2006) exposed adult medaka to 39.4 nm fluorescent latex particles for seven days after which he was able to quantify the fluorescence in many organs, including gills, intestine, liver, gallbladder and kidney. In the future, perfused organ cultures or *in vitro* models mimicking the gill and intestinal epithelial barriers could help to clarify if MeNP translocation indeed takes place (Handy *et al.*, 2011). One aspect that should be considered though is the role of the mucosa, which could act in two opposing ways. Either, by enclosing the NP, it may prevent uptake by cells; or, by affording a biological coating, may facilitate NP uptake by epithelial cells. Clearly, these mechanisms need to be elucidated in order to understand bioavailability and thus uptake and internal distribution of NP.

An interesting observation is that NP can enter *ex utero* fish embryo. This was first demonstrated by Kashiwada (Kashiwada, 2006) in see-through medaka with the fluorescent latex particles (see above), which were first found in the envelop, yolk area and oil droplet and then shifted to yolk and gall bladder during embryo development. AgNP were subsequently demonstrated in zebrafish (*Danio rerio*) embryos to diffuse through chorion pore channels (Lee *et al.*, 2007). Incorporation of AgNP was also found for fathead minnow (*Pimephales promelas*) embryos (Laban *et al.*, 2010). Aside

from this route of entry being an ecologically relevant exposure path for egg-laying species, it also indicates potential uptake by diffusion via pores in cell membrane containing organisms, such as aquatic algae and plants.

The cell wall of algae, plants and bacteria comprises an additional barrier to the entry of NP compared to animal cells. Only few studies have unequivocally shown uptake of NP, such as the uptake of carbon-based NP, into plant cells (Lin *et al.*, 2009; Liu *et al.*, 2009). In our own work we have seen uptake of AgNP in cells of the unicellular green algae, *Chlamydomonas reinhardtii*, but only in the presence of Ag<sup>+</sup> ions, which may be due to altered permeability of the cell wall (Behra *et al.*, unpublished). Conceivably, the composition and state of the cell wall likely plays a role in NP uptake. For example, permeability may increase during cell division (Navarro *et al.*, 2008b). Complicating the issue of unequivocal proof of MeNP uptake by microbial cells and plant material is biogenesis of NP from metal ions, a process which is also biotechnologically exploited (Kannan and Subbalaxmi, 2011). Whether or not MeNP are taken up by cell-wall containing aquatic organisms, it has repeatedly been pointed out that damage to cells can proceed as long as NP are closely associated with the cell wall, e.g., (Rodea-Palomares *et al.*, 2011). However, once the cell wall is damaged and the cell membrane reached, internalization into cells may proceed via fluid phase endocytic uptake (Etxeberria *et al.*, 2006; see Section 5.4.2).

### 5.4.2 Mechanisms of Uptake

As pointed out above, uptake of NP by organisms takes place by incorporation into cells; thus, uptake mechanisms need to be viewed on the cellular level. Understanding processes leading to particle uptake are important not only for linking particle exposure and toxicological effects but also because it connects to issues such as particle–biomolecule interactions (see Section 5.5), particle accumulation and transfer between tissues or along the food chain (see Section 5.3).

Initially, particle incorporation into animal cells was attributed mostly to cells able to perform phagocytosis, an actin-filament dependent process. However, Rejman *et al.* (Rejman *et al.*, 2004) showed internalization of 500 nm and smaller fluorescent beads via endocytic paths into non-phagocytic cells (which did not take up

beads of 1000 nm size). The ability by non-phagocytic cells to take up NP has now been unequivocally shown in many studies. Examples are the incorporation of a variety of MeNP into human-derived cell cultures originating from different organs (Busch *et al.*, 2011) and into fish gill cells (Farkas *et al.*, 2011; Kuhnel *et al.*, 2009). Two main paths of endocytosis can be distinguished. In caveolae-mediated endocytosis, cell surface lipid rafts lead to transfer of particles into the endoplasmic reticulum, the Golgi or through the cell by transcytosis. This path is also exploited by many viral pathogens (which often have a net-negative surface charge). In contrast, conventional endocytosis proceeds via uncoated (fluid-phase endocytosis) or clathrin-coated pits into the lysosomal degradative compartment (Moore, 2006). Which of these energy-dependent pathway(s) is more relevant for which type of particle is still very much debated and studies largely limited to polymer-based NP but multiple pathways often seem to simultaneously play a role (Hillaireau and Couvreur, 2009). In fact, in addition to active uptake mechanisms, passive diffusion has been suggested as a possible path of entry of NP into cells (Yacobi *et al.*, 2010). Even though these insights largely stem from studies with vertebrate (and mostly mammalian) cells, endocytosis is an evolutionarily conserved pathway and cell membrane structures are universal. Therefore, similar mechanisms of uptake are likely to occur in other organisms as well.

Finally, the question arises as to whether coating of NP with biomolecules will influence particle uptake and cell-internal trafficking (see also Section 5.5). Previously, serum and bovine serum albumin have been shown to stabilize tungsten-based NP in suspension (Bastian *et al.*, 2009; Kuhnel *et al.*, 2009; Meissner *et al.*, 2010). A recent study focusing on serum-coated polystyrene particles showed that NP uptake rate into porcine aortic endothelial cells was drastically reduced in serum-containing exposure medium. However, intracellular trafficking was unaffected by the presence of serum (Guarnieri *et al.*, 2011).

## 5.5 Nanoparticle–Biomolecule Interactions

When NP come into contact with biological fluids they immediately interact with the biomolecules present. This changes the physico-chemical properties of the NP, by affecting their zeta potential, their size and the functional groups exposed to the bulk solution, but also

affects the biological functioning of the bound biomolecules, for instance through conformational changes in proteins.

### 5.5.1 Biologically Induced Transformation of Nanoparticles

Nanoparticles that come into contact with biological systems, e.g., blood plasma, immediately get coated with biomolecules, forming a corona where amount and type of bound biomolecules is determined by the NP size and surface properties (Cedervall *et al.*, 2007; Lundqvist *et al.*, 2008; Ruh *et al.*, 2012), and also by the interaction of the biomolecules among themselves (Jiang *et al.*, 2010). Suresh *et al.*, e.g., showed that cytotoxicity induced by AgNP depends on surface coatings and cell types (Suresh *et al.*, 2012). Other biomolecules that have been shown to adsorb to NP at environmentally relevant pH and salt concentrations are bacterial extracellular proteins and polysaccharides present in biofilms (Khan *et al.*, 2011a; Khan *et al.*, 2011b). Various techniques have been used to analyze the structure of the resulting nanomaterial bio-conjugates, ranging from separation techniques, to scattering techniques, microscopy and spectroscopy (Sapsford *et al.*, 2011).

It has been proposed that the particle's "corona" of adsorbed biomolecules is the driving force in the interaction with biological systems, and not the NP itself (Lynch *et al.*, 2007; Lynch and Dawson, 2008; Nel *et al.*, 2009). This has been supported by the fact that blood plasma-derived particle–biomolecule complexes are sufficiently long-lived that they can be extracted from the plasma and still show the same properties as in their native environment, as long as their tertiary structure is not affected (Walczyk *et al.*, 2010). While the structure and size of the NP will determine the nature of the corona formed in a given matrix, the coating will define its surface charge, its stability and its hydrodynamic size, and thus its interaction with a cell (Colvin and Kulinowski, 2007). There is for instance evidence that suggests cellular uptake of NP is reduced for NP with a protein corona (Jiang *et al.*, 2010). Furthermore, the corona is a dynamic system exchanging with the surrounding matrix, leading to an enrichment of proteins with high affinity for the NP surface after an initial coating by highly abundant proteins (Cedervall *et al.*, 2007).

In blood plasma, the corona on citrate-stabilized AuNP has been shown to grow with incubation time (Dobrovolskaia *et al.*, 2009).

Various plasma proteins were found bound to the surface of these AuNP and it is well possible that their biological functioning has been affected by the binding due to altered protein conformation, exposure of novel epitopes, and avidity effects caused by the close spatial repetition of identical proteins (Cedervall *et al.*, 2007). The dynamics of the corona is reflected by measured residence times in the order of 100 s for human serum albumin bound to polymer-coated NP (FePt, CdSe/ZnS) (Jiang *et al.*, 2010; Röcker *et al.*, 2009).

### 5.5.2 Nanoparticle-Induced Changes to Biological Target Sites

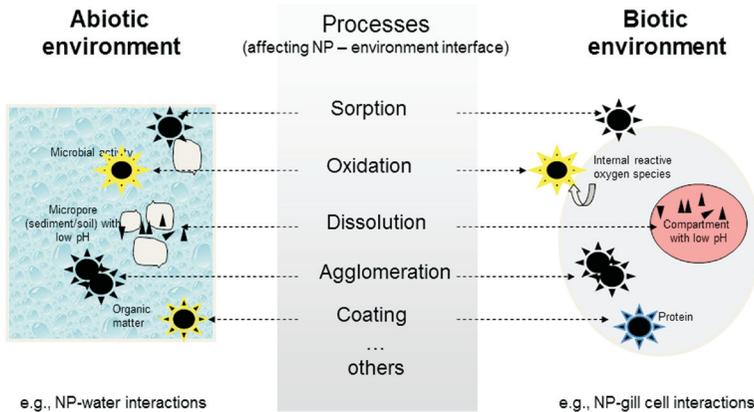
The binding of proteins and other biomolecules to NP is strongly driven by electrostatic forces but can also be based on direct interaction of functional groups with the metal surface. This specific interaction can affect cellular functioning by blocking access to binding domains of ligands, cofactors or DNA, or by inducing conformational changes in proteins and protein complexes. Altered protein conformation could be shown by Bellezza *et al.* (2009). They found that the tertiary structure of myoglobin bound to nano-sized nickel aluminum hydrotalcite was disturbed (Bellezza *et al.*, 2009). The native conformation was re-established upon desorption from the particle. Wigginton *et al.* showed that AgNP specifically bind enzymes such as bacterial tryptophanase (TNase), as well as membrane proteins (Wigginton *et al.*, 2010). They found two tryptic fragments of TNase that specifically bound uncoated AgNP but did not bind carbonate coated AgNP. It was hypothesized that these two peptides, located very close to the active site of TNase, were responsible for the strongly suppressed enzymatic activity when the enzyme was exposed to bare AgNP compared to the coated particles.

Biomolecules might also be modified by reactive oxygen species (ROS) which can be formed directly or through released metal ions, which then induce DNA damage, lipid peroxidation and oxidative protein damage (Braconi *et al.*, 2011; Nele *et al.*, 2009). One very specific modification of proteins caused by oxidative stress is carbonylation of amino acids, but cleavage of the protein backbone or amino acid side chains is also observed (Madian and Regnier, 2010). Protein carbonylation together with ubiquitination was for instance shown to be induced by AuNP in the blue mussle *Mytilus edulis* (Tedesco *et*

*al.*, 2008). The authors also found increased levels of thiol oxidation, which they also attributed to oxidation (Tedesco *et al.*, 2010). TiO<sub>2</sub> NP also significantly increased the antioxidant enzyme activity in *Daphnia magna* leading to a dose-dependent increase of catalase, glutathione peroxidase and glutathione-S-transferase (Kim *et al.*, 2010). Similar effects were seen in *Danio rerio* upon exposure to ZnO NP (Xiong *et al.*, 2011). A consequence of protein oxidation is the thermodynamic instability of the tertiary structure, which leads to exposed hydrophobic amino acids, resulting in aggregation and loss of enzymatic activity (Squire, 2001). This was confirmed by Linse *et al.* who showed that NP (copolymer particles, CeO<sub>2</sub>, quantum dots, and carbon nanotubes) catalyzes aggregation of proteins, and by this potentially increases the risk of amyloidosis and other protein-misfolding diseases (Linse *et al.*, 2007).

## 5.6 Research Needs

A proper evaluation of risks of NP to the aquatic environment requires synthesis of knowledge covering physical, chemical and biological aspects of NP behavior and NP–biota interaction. A useful framework is to acknowledge the similarity of processes taking place in either the abiotic or biotic environment (Fig. 5.1).



**Figure 5.1** NP can undergo a variety of processes which determine their fate in the abiotic environment and interaction with biota. These processes are of relevance to both the abiotic and biotic environment; therefore, understanding of processes in either environment can inform about impact in the other.

For example, knowledge on the pH-dependence of MeNP dissolution is of relevance to predict the role of metal ion vs. MeNP exposure and at the same time helps understand intracellular fate, such as potential dissolution processes in the lysosome, a cellular compartment with low pH. In fact, given the substantial knowledge on impact of metal ions on aquatic organisms, examination of the distinct contribution of the particulate and dissolved form in both the abiotic environment and in biota is of high importance because for regulatory purposes, risk assessment based on the dissolved form may suffice. To this end, uptake mechanisms and organism- and cell-internal processes need attention to allow to truly link NP exposure and effects. In the same vein, understanding the role of intended (such as citrate) or unintended (such as humic acids or proteins) coating of NP is required to properly account for bioavailability and potential effects. Effects studies should refer to concentrations that can reasonably be expected in the aquatic environment based on knowledge on the use, physico-chemical properties of the NP or also evolving fate models (e.g., Mueller and Nowack, 2008). Concentration should also be seen in the context of the natural background, such as in the case of  $\text{TiO}_2$ . The published distribution studies in model ecosystems do inform on exposure pathways and allow to identify environmental sinks of NP. However, the question of whether bio-accumulated NP result in a diminished performance of ecosystem processes is still open to investigation. Because NP display a high diversity in chemical composition and physico-chemical characteristics and the fact that ecological experiments are time-consuming and complex, research efforts should put priority on those NP predicted to occur in the environment in larger quantities. Having said this, one should not lose sight of advancements in knowledge of NP behavior and effects in other contexts, such as from the medical field, because these studies can inform about NP–biota interaction for aquatic organisms as well.

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