Review

Journal of
Biomedical Nanotechnology
Vol. 10, 2828–2851, 2014
www.aspbs.com/jbn

Molecular Toxicity of Nanomaterials

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With the rapid developments in the fields of nanoscience and nanotechnlogy, more and more nanomaterials and their based consumer products have been used into our daily life. The safety concerns of nanomaterials have been well recognized by the scientific community and the public. Molecular mechanism of interactions between nanomaterials and biosystems is the most essential topic and final core of the biosafety. In the last two decades, nanotoxicology developed very fast and toxicity phenomena of nanomaterials have been reported. To achieve better understanding and detoxication of nanomaterials, thorough studies of nanotoxicity at molecular level are important. The interactions between nanomaterials and biomolecules have been widely investigated as the first step toward the molecular nanotoxicology. The consequences of such interactions have been discussed in the literature. Besides this, the chemical mechanism of nanotoxicology is gaining more attention, which would lead to a better design of nontoxic nanomaterials. In this review, we focus on the molecular nanotoxicology and explore the toxicity of nanomaterials at molecular level. The molecular level studies of nanotoxicology are summarized and the published nanotoxicological data are revisited.

KEYWORDS: *Nanotoxicology, Molecular Level, Biomolecules, Surface Chemistry, Degradation, Toxicological Mechanism.*

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Received: 10 February 2014
Accepted: 18 February 2014

INTRODUCTION

In past decades, nanomaterials (NMs) have been extensively studied and applied in various areas, due to their nanostructures, nanosurface, and many unique physicochemical properties.[1–5] NMs have launched a new revolution in material sciences, and significantly influenced many other areas, such as electronics, information, energy, biomedicine, environments and so on. The massive production and wide applications of NMs render serious concerns on the potentially hazardous effects of nanomaterials.[6–14] Their safety evaluation has been regarded as the essential issue to be solved before large-scale applications.

A large number of investigations on the bio-effects and bio-safety of NMs have been reported.[15–18] Many NMs have been investigated with exposure to microorganisms, cells, animals and plants.[19–32] Thus, nanotoxicology, as a new multidisciplinary branch of toxicology, has been established and well acknowledged by the scientific community and the public.[33,34] NMs exhibit some unique biological or toxicological effects. For example,

 1550-7033/2014/10/2828/024 doi:10.1166/jbn.2014.1936

the size-dependent toxicity of various NMs was reported in literature.[35, 36] The nanonization of materials also lead to new safety problems, which depend on the physicochemical properties of NMs.[37, 38] Taking carbon as an example, carbon is usually regarded as nontoxic element, but different kinds of toxic effects were observed from carbon nanotubes (CNTs), fullerene and graphene.[39–42]

In the ongoing nanotoxicology studies, there is a growing recognition that understanding of the toxicity of NMs at molecular level is essential.[43–45] When NMs enter the environment or encounter with biosystems, their interactions at a molecular level determine their environmental or biological fate.[46] The first level events of NMs in biosystems should be the interactions between NMs and biomolecules *in vivo*, which results in a NM-biomolecule complex.[9, 47] Then, the NM-biomolecule complex might be partially biodegraded by some enzymes in biosystems. The bioavailability and transportation of NMs also involve many molecular mechanisms, in which the nanosurface and its surface chemistry play important roles.[48, 49] For the toxicity study, the toxicological mechanism requires molecular level explanations. Beyond that, to design biocompatible and safer NMs, the surface chemistry and chemical mechanisms are two key aspects to consider, which obviously demands the molecular understanding of the nano-bio processes.

In this review, we focus on the toxicity of NMs from the point view of their interactions with biosystem at molecular level. The published data related to this topic are summarized, and their implications to future nanotoxicology studies are extensively discussed.

INTERACTION BETWEEN NANOMATERIALS AND BIOMOLECULES

Interaction Between Nanomaterials and Proteins

Upon entering biosystem, the NM interaction with proteins is inevitable. Many papers have reported the interaction of NMs with proteins and how these interactions alter the protein conformation, protein function, or NM-protein

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complex and so on.[9] Typically, smaller nanoparticles (NPs), such as fullerene, might be fitted into the pores (usually the active site) of proteins, forming a combined structure.[50, 51] For larger NPs, the interaction mostly leads to the attachment of proteins on the surface of nanoparticles.[52, 53] The binding models of proteins on some nanomaterials such as fullerene and CNTs have been investigated in detail and published in literature.[9] This is probably due to the fact that the nanostructures of fullerene and CNTs are relatively simple and strictly defined. For example, Ge et al. investigated the binding of various proteins on CNTs.[54] The binding models were built by atomic force microscopy (AFM) and also molecular dynamics methods (Fig. 1). For other NMs, the binding models are somewhat vaguer so far and the protein corona is widely adopted to describe the situation.[9]

There are several forces contributed to the interaction between NMs and proteins. The major one is the van der Waals' interaction, in particular, when the topographies of both NMs and proteins match each other. When the NPs are surrounded by solvent protein molecules, only closely contacted atoms have net contribution to the binding, where the weak van der Waals' interactions become very strong because a large number of atoms are composed in both proteins and NPs. The second is hydrogen bond (H-bond), it is a much stronger (5 to 30 kJ/mol) interaction than van der Waals force, but the net contribution of H-bond to protein-NP interaction is usually small. The importance of H-bond is that the directional H-bond would provide specificity for the protein-NP interaction. The third is the electrostatic force between proteins and NPs, which is mostly determined by the surface charges and could be regulated by the pH. When electrostatic interaction dominates, the dispersion state of proteins or NMs might be changed. The fourth is hydrophobic interaction, this is the entropic effect originated from excluding the ordered water molecules from nonpolar surface. When NMs have large hydrophobic surface (domains), they would bind the partially exposed nonpolar residues of proteins, resulting in the unfolding of proteins. The fifth is the π–π stacking interaction, which is usually found between proteins and sp^2 carbon NMs, such as fullerene, CNTs and graphene.[51, 55, 56] The π–π stacking is an attractive interaction between aromatic rings. Only several amino acids contain aromatic rings, which are most possibly buried inside the hydrophobic core of proteins. Therefore, π–π

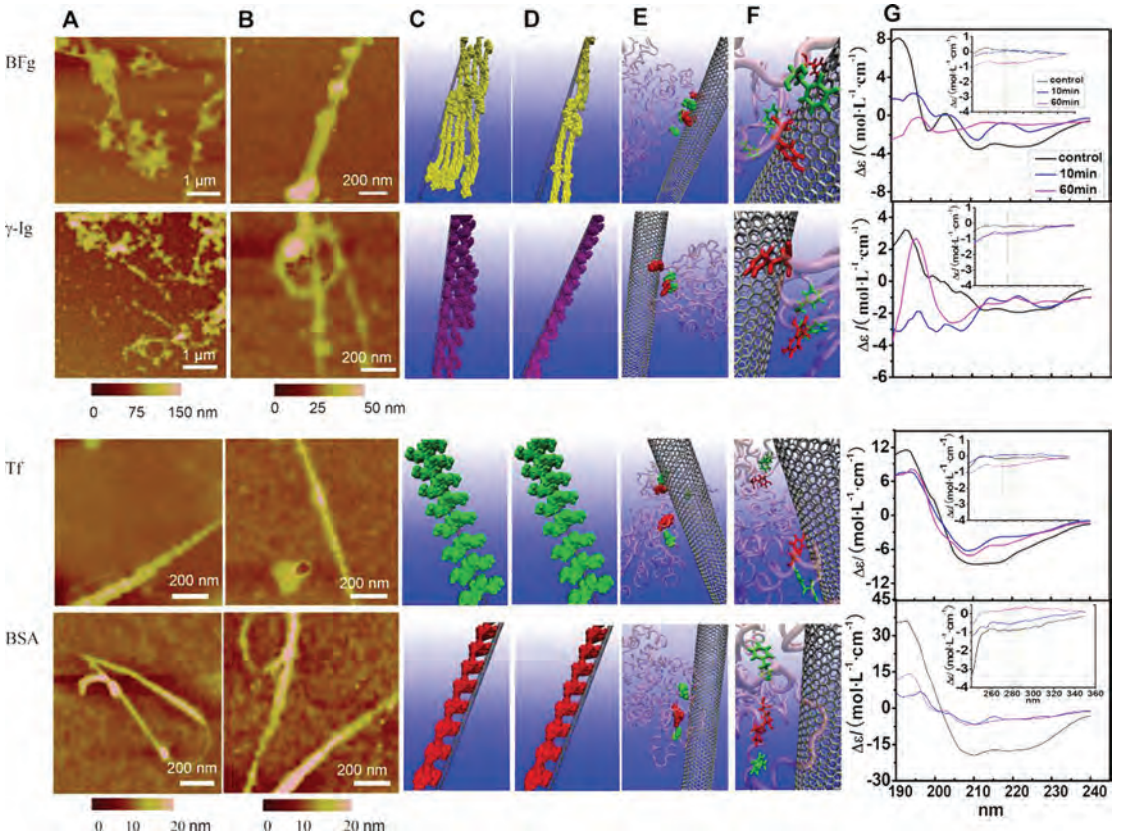

Figure 1. **Binding of proteins on CNTs. (A), (B) AFM images after incubation with CNTs for 10 min (A) and 5 h (B); (C)–(F) molecular modeling illustrations of the binding model after incubation for 10 min (C) and 5 h (D) with the binding sites (E) and the orientations of aromatic rings (F) indicated; (G) the far-UV CD spectra of proteins (insets: near-UV CD spectra). Reprinted with permission from [54], C. Ge, et al., Binding of blood proteins to carbon nanotubes reduces cytotoxicity. *Proc. Natl. Acad. Sci. USA* 108, 16968 (2011). © 2011, National Academic of Sciences.**

stacking could be regarded as another specific interaction. Anyhow, the total bind strength between NMs and proteins is regulated by many factors. There have been plenty of studies investigated the issue though some inconsistent phenomena were also reported.

Undoubtedly, the NM properties highly influence on the NM interactions with proteins. The components of NMs and their preparation methods could affect the capacity and kinetics of proteins adsorption on NMs. Au or Ag NPs showed much faster adsorption kinetics in forming protein corona than Fe_3O_4, CoO and CeO_2 did.[57] Nanoshell carbon (NSC) prepared by Fe catalysis and Co catalysis showed completely different adsorption capacity.[58] The physical properties of NMs, including size, shape and surface morphology, also directly influence these interactions. For instance, Wu et al. docked fullerenes of different carbon numbers to proteins and demonstrated the changes in binding energy.[51] The protein corona formed on silica NPs is significantly affected by the size of silica NPs.[59] Lysozyme and α-chymotrypsin (ChT) adsorbed much more on rod-like AuNPs (forming multi-layers) than on spherical AuNPs (only monolayer formed).[60] The binding constant for the interaction between rod-like AuNPs and BSA is about 2 orders of magnitude higher than that between spherical AuNPs and BSA.[61]

Surface chemistry of NMs deserves a separate highlight. Surface chemistry could alter the surface charge of NMs, thus directly regulate the electrostatic interaction. An elegant demonstration was performed with Al_2O_3 NPs.[62] For positively charged Al_2O_3 NPs and Al_2O_3–NH_2 NPs, negatively charged BSA dominated the adsorption on Al_2O_3 NPs and Al_2O_3–NH_2 NPs, which were positively charged. However, due to the electrostatic repulsion, lysozyme adsorbed very little. The situation could be reversed by changing the surface functionalities to negatively charged groups like –COOH, $–SO_3$ and $–PO_3H_2$, etc. Surface chemistry also regulates the hydrophilicity/hydrophobility of NMs. Wu et al. have showed that hydroxylation of fullerene decreased the binding strength of proteins on fullerene.[51] This could be assigned to the decrease of hydrophobic interaction. The influence of hydrophilicity/hydrophobility was also observed by Cedervall et al. where the number of protein molecule bound to NPs was positively related to the nanoparticle hydrophobicity.[63]

Here the properties of proteins should be considered, too. Different proteins usually interact with the same NM differently. For example, Wang et al. showed that immunoglobulin G (IgG) adsorbed three times more on oxidized CNTs than BSA did.[64] In a more complicated system, the blood proteins did not equally adsorbed on NPs, but a selective binding occurred and corona with unique components was formed.[65] Despite the difference of proteins, when increasing the protein concentration, the binding of proteins on NMs would be promoted. Interestingly, it was also reported that the protein concentration affected the binding kinetics and also the final composition of proteins on NMs.[65]

In addition, the biological microenvironment will also affect the interaction between proteins and NMs. The pH value is an important parameter of aqueous media, through which the charge state of proteins and NMs could be regulated. When pH is close to the pI of proteins, they become uncharged and would aggregate/precipitate without intermolecular repulsion. The maximum adsorption of lysozyme on NPs was observed at pH 11, quite close to the pI of lysozyme (11.1).[66] However, when the NMs are charged, the binding would be more efficient at a pH that enables opposite charges. Such phenomena were observed in the study of BSA on *N*-methyl-*d*-glucamine (NMDG) modified silica NPs.[67] Ionic strength of medium is another important parameter. High ionic strength can reduce the electrostatic interaction, but strengthen the hydrophobic interaction. High NaCl concentration was reported to inhibit the adsorption of lysozyme and horseradish peroxidase (HRP) on silicon nanowires.[68] When hydrophobic interaction contributes significantly, a dual effect might appear. The adsorption of BSA to NMDG modified silica NPs were enhanced at low NaCl concentrations, but inhibited at high NaCl concentrations.[67]

The interaction between NMs and proteins would change the properties of both NMs and proteins. The most obvious alteration of proteins is the conformational change which depends on many factors, such as concentration, size and shape of NMs. CNTs with larger diameter induced more conformational changes of BSA and carbonic anhydrase.[69] Rod-like AuNPs led to more helix loss than spherical AuNPs.[61] Some of the conformational changes are irreversible, while the rest are reversible.[70, 71] Interesting and useful phenomenon is that the partially changed conformation of proteins might be more stable than the original one, which has been adopted in enzyme loading.[72–74] Upon the conformational changes, the function of proteins can be disturbed. Particularly, the enzyme activities could be inhibited (e.g., active site was dominated/blocked by NMs) or promoted (e.g., active site was more accessible to substrate), which can be regulated by surface coverage, size and shape of NMs.[75–77] For the promoting effect, lipase was converted to open form by poly-*DL*-lactic acid NPs, resulting in an increase of the enzyme activity.[78] In a strict condition, the protein even could be fibrillated in the presence of NMs, where NMs shorten the nucleation lag-phase.[79] The protein fibrillation will lead to the complete lose of protein functions. Such fibrillation was proven in Skaat et al.'s study of F-γ-Fe_2O_3~HSA-PEG (polyethylene glycol)-Aβ(1-40) NPs. The F-γ-Fe_2O_3~HSA-PEG (polyethylene glycol)-Aβ(1-40) NPs accelerated the fibrillation of Aβ(1-40), while F-γ-Fe_2O_3 did not. Other studies also showed the inhibition of fibrillation by NMs.[79] Cabaleiro-Lago et al. reported the inhibition of amyloid

β protein Aβ(1-40) fibrillation by *N*-isopropylacrylamide (NIPAM)/*N*-tert-butylacrylamide (BAM) NPs.[80]

The binding of proteins alters the properties of NMs, too. The first change of NMs is the different surface chemistry. A protein shell might form on NMs, usually called as protein corona.[9] The hydrophobic pristine carbon NMs, such as fullerene, CNT, and nanodiamond (ND), became more hydrophilic and water dispersible after coating with proteins.[81, 82] If the proteins are charged, then the surface charge of NMs could be changed upon the binding of proteins.[62, 71, 83, 84] Consequently, the colloidal state of NMs is changed, leading to the dispersing/agglomerating state of NMs.[85–88] Since the dispersing/agglomerating has significant effects on the behaviors of NMs in biological systems or the environment, we would discuss this issue in the later sections in detail. Other changes of NMs, e.g. spectroscopic changes, have less impact on the biosafety of NMs, thus not concerned here.

Interaction Between Nanomaterials and Nucleic Acids

Nucleic acids, including DNA and RNA, are another category of important biomolecules interacting with NMs. However, when NMs entered into the cells or animals, they could first interact with proteins, directly resulting in the protein-coating of NMs surface.[9] Experimental data showed that NMs mostly could not enter the nucleus, thus, it is not very possible to interact with DNA.[37] To this regard, the interaction between NMs and nucleic acids is less concerned currently. Nevertheless, there are more literatures showing that NMs bind nucleic acids, interfere with the expression of nucleic acids and induce damage to them.

Generally, the interaction between NMs and nucleic acids is very similar to that between NMs and proteins. The van der Waals' interaction, H-bond, electrostatic interaction, π–π interaction and hydrophobic interaction also exist between NMs and nucleic acids. There are two distinct characteristics. The first one is that nucleic acids are usually negatively charged. The electrostatic interaction between positively charged NMs and nucleic acids is very strong. The strong electrostatic interaction has been widely used in preparing NM-nuclear acid composites for gene delivery. For example, polyetherimide (PEI) functionalized carbon dots interacted with DNA strongly to form a stable composite.[89] The second characteristic is that nucleic acids contain aromatic rings. The abundance of aromatic rings in nucleic acids makes them interact with sp^2 carbon NMs strongly via π–π interaction.[90] This phenomenon has been used for dispersing CNTs.[91] and preparing nucleic acid-CNT probes.[92]

Upon the interaction, nucleic acids might change their conformations. For instance, Pershina et al. reported that DNA interacted with $CoFe_2O_4$ NPs, which led to the destabilization of DNA and B conformation of polynucleotide.[93] Xu et al. computed the binding of DNA with fullerene C_{60}[90]. C_{60} bound with the minor grooves of double-stranded DNA and triggered the unwinding and disrupting of the DNA helix. On the other hand, C_{60} only bound to the major grooves of RNA helix, which stabilized the RNA structure or transformed the configuration from stretch to curl. The binding of C_{60} led to the unwinding of dsDNA, disrupt of G-quadruplex DNA and so on. In addition, Hekmat et al. found that Ag NPs induced DNA conformational changes to a more compact form, while Ag NPs and doxorubicin together induced different DNA conformational changes and inhibited the proliferation of cells.[94]

Interaction Between Nanomaterials and Other Biomolecules

Beyond the proteins and nucleic acids, interactions of NMs with other small molecules (not strictly biomolecules) produce also significant influence on the toxicity of NMs. For example, the adsorption of amino acids, folic acid and Ca^{2+} on NPs led to the depletion of nutrients, it is regarded as one of the toxicological mechanism of CNTs.[95]

The adsorption of biomolecules on NMs is widely acknowledged. Hurt and co-workers investigated the adsorption of micronutrients on CNTs, graphene and GO.[95, 96] It was found that the side-chain hydrophobicity index was positively correlated with the adsorption of amino acids on CNTs.[95] The presence of CNTs led to

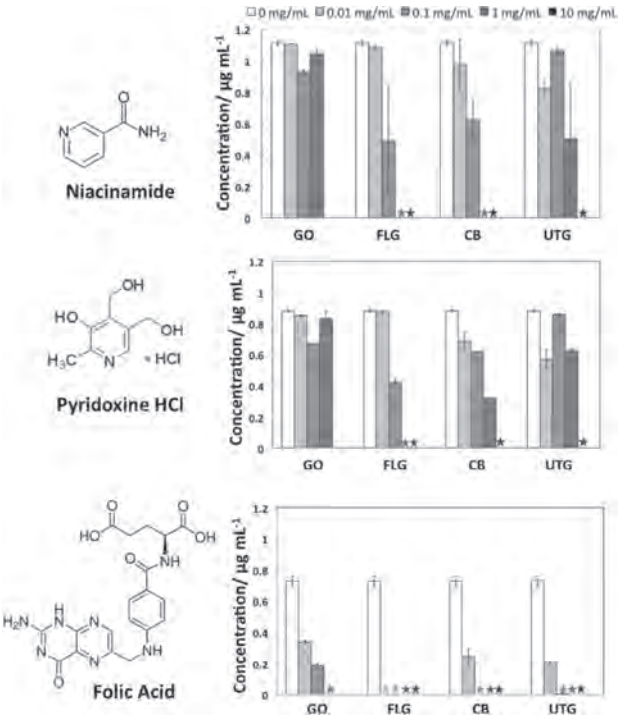

Figure 2. Interactions of graphene materials with essential micronutrients in RPMI cell culture media. Reprinted with permission from [96], M. A. Creighton, et al., Graphene-induced adsorptive and optical artifacts during *in vitro* toxicology assays. *Small* 9, 1921 (2013). © 2013, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

the depletion of amino acids. Not only amino acid can be adsorbed, but also other important biomolecules, such as riboflavin, biotin, pantothenic acid, folic acid, thiamine, pyridoxine and niacinamide. For example, graphene oxide (GO) showed much less adsorptive effect on niacinamide, pyridoxine and folic acid than few layered graphene (with much less oxygen containing groups) (Fig. 2).[96] Horie et al. found that the addition of NPs (TiO_2, CeO_2, SiO_2, NiO, ZnO and Fe_2O_3) could reduce the concentrations of Na, P, Ca and protein in the cell culture medium.[97]

NMs have strong affinity to many pollutant molecules, such as heavy metals, pesticides, dyes, organic molecules and antibiotics.[98] The interaction between NMs with these pollutants might lead to the combined effects to biosystems/environment. Therefore, it should be taking into account when considering molecular nanotoxicity. Anyhow, there are some published reviews summarizing the adsorption of pollutants on NMs.[98–100]

TRANSFORMATION/DEGRADATION OF NANOMATERIALS

Biofunctionalization/Biodefunctionalization of Nanomaterials

When NMs enter biosystems or the environment, the functionalization and/or defunctionalization of NMs are nearly inevitable, which changes the bio-behaviors or environmental behaviors of NMs, and consequently their toxicities.

The most common ways for functionalization and/or defunctionalization of NMs in biosystems are noncovalent adsorption or desorption of other molecules. For example, CNTs could adsorb dissolved organic matters (DOM) in water.[101] The adsorption of DOM on CNTs increased the hydrophilicity, thus, the dispersion of CNTs was obtained.[102] In fact, such strategy was widely applied in preparing the dispersible NMs. Similarly, the desorption of functionalities on NMs was also reported in literature. A typical example comes from surfactant dispersed CNTs. Cherukuri et al. evidenced the replacement of adsorbed surfactants (Pluronic F108) on CNTs by serum proteins using NIR fluorescence.[103] Roberts et al. reported that lysophophatidylcholine (LPC) coated SWCNTs could be ingested by Daphnia magna and LPC would be taken from SWCNT surface as food source.[104] After desorption of LPC, the naked SWCNTs aggregated and were excreted.

The covalent functionalization and/or defunctionalization of NMs in biosystems are of particular importance and interest but much difficult to study. There are several well-designed experiments to reveal the *in vivo* functionalization and/or defunctionalization of carbon NMs. Fullerene C_{60} is of certain structure and could be detected by mass spectrometry. Moussa et al. reported that C_{60} formed retinyl palmitate adducts *in vivo* via Diels-Alder-like reactions.[105] It meant that pristine fullerene would react in biosystem and the biotranformation of NMs

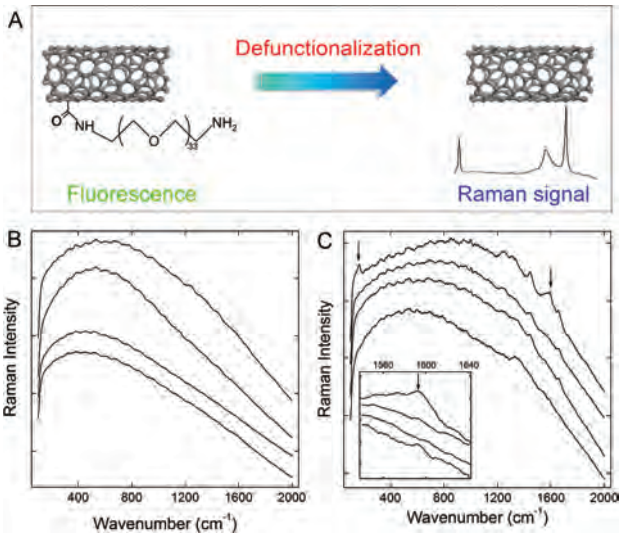

Figure 3. Defunctionalization of PEG-CNTs *in vivo*. (A) schematic illustration; (B) stability in spleen; (c) defunctionalization in liver. Reprinted with permission from [48], H. Wang, et al., Quantification of carbon nanomaterials *in vitro*. *Acc. Chem. Res.* 46, 750 (2013). © 2013, American Chemical Society.

in vivo was possible. Yang et al. used Raman spectroscopy to investigate the biodefunctionalization of polyethylene glycol (PEG)-CNTs.[106] While the well functionalized PEG-CNTs were fluorescent, the defunctionalized CNTs showed strong Raman signals. PEG-CNTs were defunctionalized in liver after 4 weeks post intravenous injection (Fig. 3). The simulated defunctionalization with H_2O_2 suggested that the defunctionalization of PEG-CNTs was the break of amide bonds through radical reaction.

Degradation of Nanomaterials

The degradation of NMs has been studied in the polymer based nanocarriers, which are aimed to develop biodegradable drug delivery systems.[107, 108] In the nanotoxicology, more attentions should be paid to those inorganic NMs. After two decades, there are more and more evidences accumulated that NMs could be degraded in biosystems.

CNTs are the most concerned NMs in the degradation study due to the unique tube structure. Generally, the skeleton is much more stable than the functional groups on CNTs. CNTs were very stable against *in vivo* metabolism in our 90 d observation as well as the acid treatment for preparing the transmission electron microscopy (TEM) samples.[39] Under very harsh conditions (HNO_3/H_2SO_4 and sonication), the carbon skeleton of CNTs only broke at the injured defects. Unlike most common results, Allen et al. reported the biodegradation of CNTs through enzymatic catalysis *in vitro*.[109] CNTs were incubated in phosphate buffer solution (PBS) with horseradish peroxidase and H_2O_2 in dark. After 16 weeks degradation, the loss of tube structure was observed. Similarly, Kagan et al. reported that the degradation of CNTs by neutrophil

myeloperoxidase.[110] Recently, Zhang et al. reported the degradation of CNTs by bacteria.[111] About 2%∼7% of CNTs were transformed into CO_2 after 7 d incubation with bacteria. Some intermediate products (small organic molecules) were also proved by liquid chromatography (LC)-mass spectrometer (MS)/MS.

Another concerned NM in the biodegradation study is quantum dots (QDs), because semiconductor QDs usually contain Cd, a very toxic element. In the initial studies of QDs, it was claimed that QDs were stable thus safe for biomedical applications.[112] However, current results indicated that QDs would be biodegradated *in vivo*. Li et al. found that CdS QDs induced the increase of intracellular Cd^{2+} concentration, which implied that QDs were degradated *in vitro*.[113] Kwon et al. observed the uptake of QDs by *Daphnia magna* and the degradation of QDs in the digestive tract.[114] QDs were degradated in the digestive tract and the release of Cd^{2+} led to the increase of Cd^{2+} level in water. Han et al. used radioactive tracing to quantify the Cd and Te levels *in vivo* after injection with CdTe QDs.[115] The Cd and Te exhibited completely different behaviors in blood circulation and biodistribution. This implied that CdTe QDs were degradable *in vivo*. Liu et al. measured the Cd and Te levels in mice after the exposure to CdTe/ZnS QDs.[116] The Cd:Te ratio was not changed in the first hour (about 3:1) and sharply increased in liver after that (29:1 at 28 d).

A simple degradation way is the dissolution of NMs in microenvionment of biosystems. Morelli et al. observed the dissolution of CdS QDs in seawater.[117] The encapsulation of CdS QDs by ZnS significantly inhibited the dissolution. The dissolution of ZnO NPs was widely reported in literature, where the release of Zn^{2+} was believed as the toxicological mechanism.[118] Yang et al. revealed the CO_2 could promote the dissolution of ZnO NPs in cell culture medium.[118] The much higher dissolution of ZnO NPs was attributed to the continuous CO_2 supply, which produced H^+ to react with ZnO. This mechanism could be expanded to other metal oxides, that metal oxides might dissolve more than expected. Zhang et al. observed the transformation of CeO_2 NPs in plants (Fig. 4).[119] CeO_2 NPs were partially dissolved with the assistance of the organic acids and reducing substances excreted by the roots and the released Ce (III) ions were precipitated on the root surfaces and in intercellular spaces with phosphate, or form complexes with carboxyl compounds during translocation to the shoots. Similar results were also observed in the study of La_2O_3 NPs and Yb_2O_3 NPs.[120, 121] Levy et al. reported the accumulation and degradation of iron oxide NPs in lysosome, where iron oxide NPs were likely dissolved and stored into ferritin proteins.[122]

Aggregation/Dispersion of Nanomaterials

The aggregation/dispersion of NMs will not change the chemical nature, but the physical properties of NMs. It could be regarded as some kind of transformation,

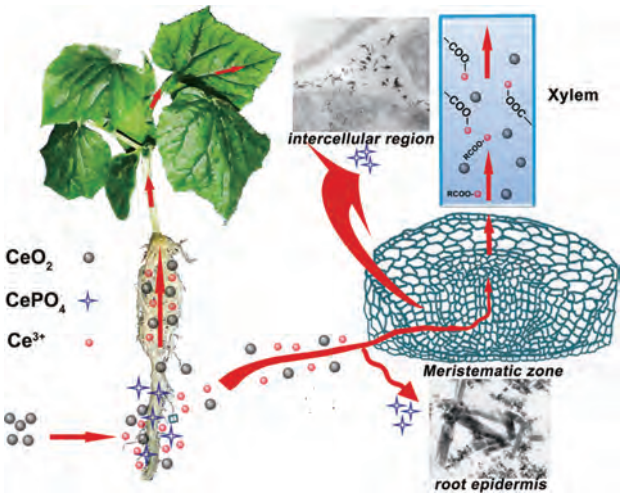

Figure 4. Biotransformation of CeO_2 NPs in cucumber plants. Reprinted with permission from [119], P. Zhang, et al., Biotransformation of ceria nanoparticles in cucumber plants. *ACS Nano* 6, 9943 (2012). © 2012, American Chemical Society.

because size is always the key parameter to consider in nanotoxicology. The aggregation/dispersion of NMs is closely determined by the molecular design and interaction of NMs.

The dispersion of NMs in biosystems is primarily influenced by the surface functionalities. When the surface is strongly charged, NMs are likely to disperse in biosystems. This could be due to the strong electrostatic repulsion. Attaching highly hydrophilic chains on the surface of NMs also lead to the dispersion of NMs in biosystems. In this case, similar structures to micelles are formed. When NMs enter biosystems or environment, there might be adsorption/desorption and functionalization/defunctionalization occur, which consequently alter the surface charge and hydrophilicity of NMs. Thus, the dispersion state of NMs might be changed. Many NMs could interact with proteins and nucleic acids. And the surface coating of proteins and nucleic acids lead to the dispersion of NMs. In fact, protein coating and nucleic acid wrapping have been used for dispersing those are hardly compatible to aqueous environment.[82, 91] NMs can interact with other molecules to reach better dispersion, too. DOM is usually negatively charged and would adsorb on many NMs. The attachment of DOM enables the dispersion of NMs in environment and changes the environmental behaviors of NMs.[101, 102] Among the studies of DOM with NMs, CNTs are the most concerned, because of the strong $\pi-\pi$ interaction. DOM facilitated the dispersion of CNTs and consequently the migration of CNTs in the environment was changed.

There might be "stickers" in the biosystems that make the NMs aggregate. The first sticker could be ions in the systems. Yang et al. showed that the oxidized CNTs aggregated with Ca^{2+}, where Ca^{2+} interacted with multiple carboxyl groups through electrostatic interaction.[123]

Such sticker effect was more obvious when Cu^{2+} encountered with GO.[124] Due to the chelation and electrostatic interaction, GO sheets folded and wrinkled in the presence of Cu^{2+}, which induced serious aggregation of GO sheets into large agglomerates. Another sticker could be proteins, which interact with multiple NPs and induce the agglomerating effect. It was widely observed that many NMs aggregated in cell culture medium. Liu et al. semi-quantitatively measured the aggregation of GO in serum.[88] GO of larger sheets agglomerated more seriously. Similarly, Orts-Gil et al. showed that 28 nm silica NPs agglomerated in the culture medium DMEM.[86] Zhou et al. found that CeO_2 NPs agglomerated in cell culture medium into larger particles.[87]

UNDERSTANDING THE UPTAKE AND TRANSLOCATION OF NANOMATERIALS AT MOLECULAR LEVEL

Cellular Uptake of Nanomaterials

The cellular uptakes of NMs have been widely studied.[37] The current studies have already deeply reached the molecular level to reveal the uptake mechanism and influencing factors. Several uptake pathways were confirmed (Fig. 5).

Endocytosis is the most common pathway for the cellular uptakes of NMs. Endocytosis is an active and energy-dependent transport of external matters by enclosing them in vesicles or vacuoles from the cytoplasmic membrane. There are two main types of endocytosis, namely phagocytosis and pinocytosis. When particles (or their aggregates) are larger than ~750 nm, the NMs are likely be swallowed via phagocytosis by cells, such as monocytes, macrophages, and neutrophils. Smaller NMs are internalized by pinocytosis, which is observed among nearly all cells. For very small particles, direct penetration is another choice. Wang et al. found that small gold NPs passively penetrated the cell membranes.[125] Prato and coworkers reported CNTs could penetrate cell membrane and the uptake of CNTs was not affected by temperature.[126]

Mechanically, the pinocytosis can be divided into macropinocytosis, clathrin-mediated endocytosis, caveolin-mediated endocytosis and clathrin/caveolin independent endocytosis. Macropinocytosis is very similar to phagocytosis, where macropinocytosis means that cells drink small volume of liquid by surface ruffling. There are many molecules involved in the macropinocytosis of NMs, e.g., phosphoinositide 3-kinase. Specific inhibitors, such as 5-(*N*,*N*-dimethyl)-amiloride, Ly294002, wortmannin, NaN_3, can be used to test whether the cellular uptake of NMs is macropinocytosis. For instance, Iversen et al. proved that ricinB-QDs were internalized by HeLa cells via macropinocytosis-like mechanism.[127]

For clathrin-mediated endocytosis and caveolin-mediated endocytosis, NMs are presented in small endocytic vesicles, and the vesicles fuse with early endosomes. In the clathrin-mediated pathway, the clathrin coated vehicles containing NMs are internalized. The process

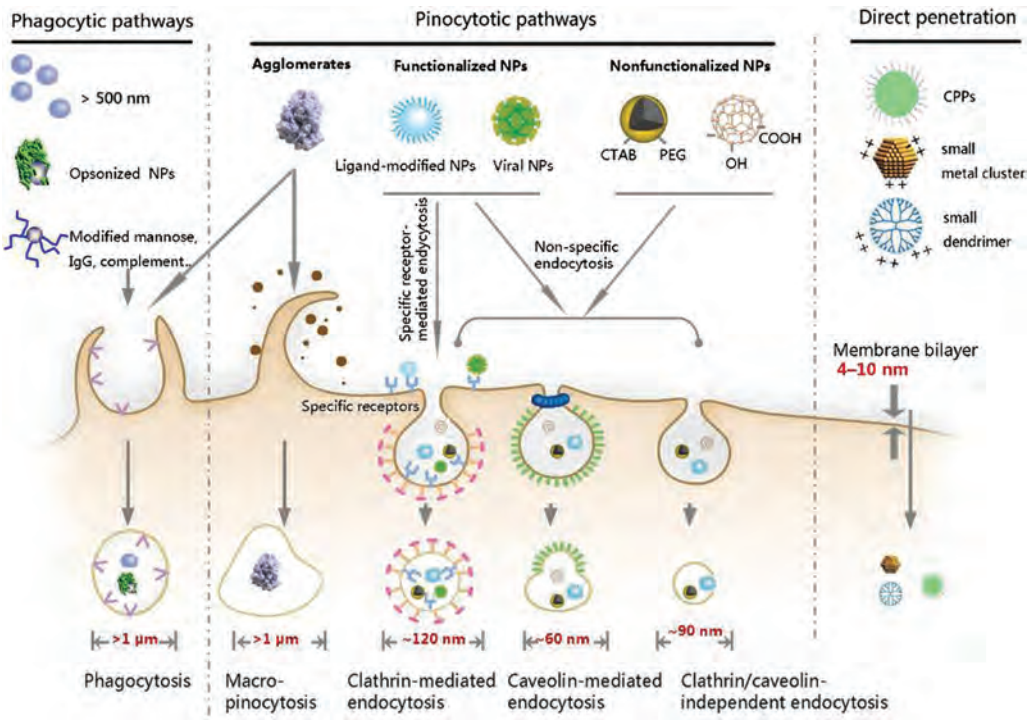

Figure 5. Cellular uptake of NPs via different pathways. Reprinted with permission from [37], M. Zhu, et al., Physicochemical properties determine nanomaterial cellular uptake, transport, and fate. *Acc. Chem. Res.* 46, 622 (2013). © 2013, American Chemical Society.

can be inhibited by several novel inhibitors. Sucrose and chlorpromazine showed inhibitory effects in the cellular uptake of PEG-poly(ε-caprolactone) (PCL) NPs (functionalized with/without Interleukin 13), which meant that the uptake of PEG-PCL NPs involved clathrin-mediated endocytosis.[128] The formation of caveolar endocytic vesicles is crucial for caveolin-mediated endocytosis. Similarly, caveolin-mediated endocytosis could be identified by specific inhibitors, too. Lee et al. revealed that the endocytosis of conjugated polymer NPs (CPNs) was caveolae-mediated.[129] Genistein inhibited the uptake of CPNs significantly, suggesting the endocytosis was caveolae-mediated. The mechanism was further confirmed by the co-localization of CPNs and caveolin-1.

So far the experimental evidence showed that most NMs cannot enter the nucleus. A popular way to facilitate the nucleus uptake is to modify NMs with nucleus targeting moieties. There are also other reports showing NMs entering nucleus, where the size of NMs has to be smaller than the nucleus pore (80–120 nm). Cheng et al. reported the accumulation of PEGylated CNTs in cell nucleus.[130] Liu et al. found that different sized polystyrene NPs entered the nucleus of HeLa cells and NIH/3T3 cells.[131]

Since the cellular uptake of NMs is mostly biomolecule regulated, there are many factors influencing the cellular uptake, including size, shape, surface charge/functionalization, and chemical composition. For example, Liu et al. investigated the cellular uptake of gold NPs of different sizes and charges by phagocytic and nonphagocytic cells.[132] Positively charged gold NPs were internalized by HepG2 cells much more than negatively charged gold NPs. For RAW 264.7 cells, the surface charge seemed not crucial, indicating a cell-type dependent uptake of NMs. For negatively charged gold NPs, cellular uptake of NMs shows a size-dependent nature, a diameter of about 40 nm was most preferable for above both cell lines. For positively charged ones, a clear size-dependent uptake was less reported. A most effective way to enhance the cellular uptake of NMs is to modify them with specific ligands, which enhance specific receptor-mediated internalization. Other possibility is adding promoting reagents to enhance the cellular uptake of NMs.[133] The cellular uptake processes of NMs have been recently summarized in our previous reviews.[15, 37]

Uptake and Biodistribution of Nanomaterials *In Vivo*

The bioavailability and biodistribution of NMs are important issues to consider, which have significant influence on the toxicity of NMs.[10, 48, 134] The evidences accumulated these years collectively indicate that the properties of NMs at molecular level affect their uptake and biodistribution *in vivo*.

The particulates larger than 2 μm in diameter will be trapped in lungs via the pulmonary capillary filtration. When NMs enter blood circulation, the cross-interaction of NMs and proteins might lead to agglomeration of NMs and consequently induce the pulmonary uptake. Liu et al. found that GO agglomerated in blood circulation in the presence of serum proteins, and serious pulmonary trap was observed.[88] The aggregation induced by serum proteins of NMs can be easily avoided via surface functionalization of protein-resistant polymers (e.g., PEG).[135] Other functionalities, which are stable enough against agglomeration, are also efficient in avoiding pulmonary uptake.[136]

Then, the recognition of NMs by opsonins is the next step after the NMs escaping from the pulmonary capillary. Opsonins are a big family of complement proteins and immunoglobulin G (IgG). However, the concept of opsonin can be extended to any blood serum component, which aids the process of phagocytic recognition. Once the NMs are labeled by opsonins, the reticuloendothelial system (RES) uptake will be triggered. The RES system, including liver, spleen and lungs, will largely trap those of high protein affinity. Indeed, there are several studies concerned the adsorption of opsonins on NMs and showed the protein corona contained complement proteins and immunoglobulins after NMs were introduced into serum.[53, 59, 137, 138]

The data in literature suggest that the molecular design of NMs surface influences their biodistribution significantly. The most studied NMs in biodistribution are CNTs.[10] CNTs functionalized with $-SO_3H$, –COOH and other negatively charged groups were cleared from blood circulation quickly and accumulated in liver and spleen largely.[136, 139] By changing the surface functionalities to protein-resistant PEG, the blood circulation could be prolonged and the RES uptake could be reduced. Among the PEGylated methods, Yang et al. found that covalent PEGylation was the most effective one.[140] Upon the covalent PEGylation with PEG_{1500}, the blood circulation half-life of CNTs was prolonged to 15.3 h and the RES uptake reduced, comparing to that of the tween 80 dispersed CNTs.[141] The advantage of PEGylation also includes the high tumor uptake via EPR effect. Similar to the results of CNTs, the PEGylated QDs also showed long blood circulation, low RES uptake and tumor targeting.[142]

In addition, there were studies showed that the shape and size of NMs affected the biodistribution, too. Rod-like structure was believed to circulate longer in blood circulation. Geng et al. reported that particles with higher length/diameter ratio circulated longer in blood circulation.[143] Huang et al. found that SiO_2 NPs with higher aspect ratio accumulated less in liver and spleen.[144] After PEGylation, the pulmonary uptake of SiO_2 NPs increased. Akiyama et al. reported that the rod-like AuNPs accumulated less in liver, but higher in spleen.[145] Liao et al. found the size effect of polystyrene NPs on their biodistribution.[146] Larger NPs accumulated more in RES system and distributed less in skine, muscle and fat. Very recently, Chang et al. reported a different biodistribution

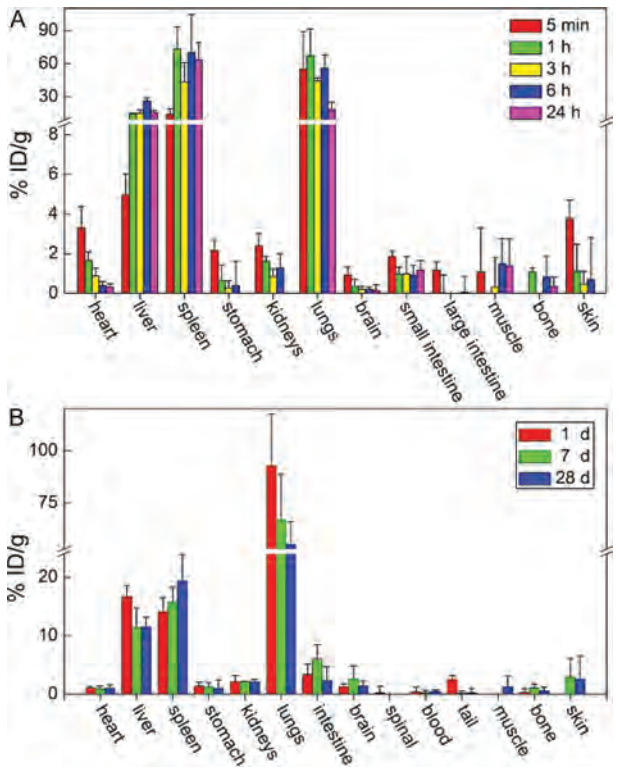

Figure 6. **Biodistribution of pristine fullerene C_{60} (A) and CNTs (B). Reprinted with permission from [147], X.-L. Chang, et al., Quantification of carbon nanomaterials *in vivo*: Direct stable isotope labeling on the skeleton of fullerene C_{60}. *Environ. Sci.: Nano* 1, 64 (2014). © 2014, The Royal Society of Chemistry; From [141], S.-T. Yang, et al., Biodistribution of pristine single-walled carbon nanotubes *in vivo*. *J. Phys. Chem. C* 111, 17761 (2007). © 2007, American Chemical Society.**

of C_{60} to that of CNTs (Fig. 6),[141, 147] where both were dispersed with tween 80, thus sharing the same surface chemistry. This definitely confirmed that the shape of NMs affects their biodistribution *in vivo*.

Moreover, the excretion of NMs is very important in nanotoxicology. The study of QDs suggested that QDs with diameters less than 5 nm would be excreted via urine, while the larger ones not.[148] The excretion via urine was also evidenced in the study of carbon dots, which were about 5 nm in diameters. However, other reports showed that larger particles were excreted via urine. Liao et al. found that polystyrene NPs (20–500 nm) could be excreted via urine, while the smaller particles were excreted more.[146] Similarly, in the study of CNTs, although the lengths of CNTs were usually longer than 5 nm (usually in the range of 50∼500 nm), well PEGylated CNTs were found to be excreted via urine.[140, 149] So did hydroxylated CNTs.[150] Anyway, according to Huang et al.'s study of SiO_2 NPs, short-rod NPs were excreted more than long-rod NPs.[144] Again, the molecular design might be useful in accelerating the excretion of NMs and reducing the potential exposure level.

MOLECULAR MECHANISM OF NANOTOXICITY

Released Chemical Components from Nanomaterials

In the very beginning of nanotoxicity studies, researches have already realized that chemical components released from NMs might lead to toxicity. This could be easily recognized in the studies of Ag NPs,[151, 152] CdSe QDs[153] and so on, which contains novel toxic ions. Generally, there are two categories of situations.

The first category is that the chemical components of NMs are toxic. Ag NPs, CdSe QDs, CuO NPs and ZnO NPs could be identified as containing toxic elements. Despite the debates on toxicity from NPs and/or ions, the heavy metal ions are indeed toxic. Current results clearly demonstrate the contribution of released ions to the total toxicity. Taking ZnO NPs as an example, Brunner et al. reported the toxicity of ZnO NPs to MSTO-211H cells and NIH/3T3 cells and speculated the origin of toxicity is Zn^{2+} from the solubilization of ZnO NPs.[154] Similar speculation was also made by Deng et al. in their study of neural stem cells, where Zn^{2+} showed even higher toxicity than ZnO did.[155] Yang et al. proposed the chemical dissolution mechanism of ZnO NPs in cell culture condition and proved the toxicity of ZnO NPs was induced by Zn^{2+} (Fig. 7).[118] The similar toxicity of ZnO NPs and Zn^{2+} was observed and a meaningful and fast increase of intracellular Zn^{2+} was identified by FluoZin-3^{AM} (fluorescent Zn^{2+} indicator). As discussed aforementioned, the solubilization of ZnO NPs was promoted by the environmental CO_2 during the cell culture. It should be stated that the toxicity of ZnO NPs might also come from the oxidative damage. As a compromise, Xia et al. suggested that both released

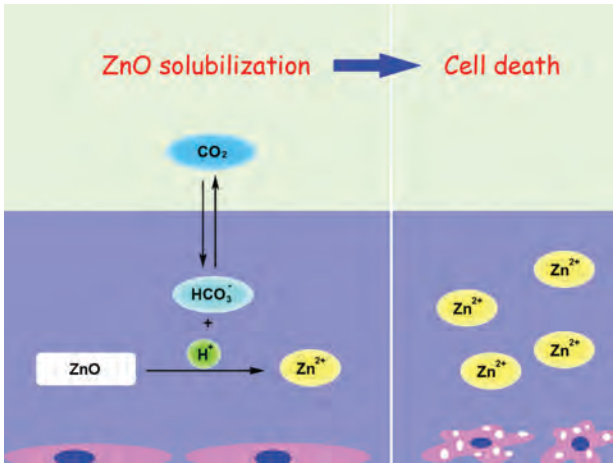

Figure 7. **Dissolution of ZnO NPs under cell culture atmosphere and the consequence of cytotoxicity. Reprinted with permission from [118], S.-T. Yang, et al., Cytotoxicity of zinc oxide nanoparticles: importance of microenvironment. *J. Nanosci. Nanotechnol.* 10, 8638 (2010). © 2010, American Scientific Publishers.**

Zn^{2+} and oxidative damage contributed to the toxicity of ZnO NPs.[156]

The second category is that the impurities of NMs are toxic, while the chemical components of NMs themselves are nontoxic. A typical example comes from carbon NMs. Hull et al. evaluated the aquatic toxicity of the leachates of metallofullerene waste and as-produced C_{60}.[157] The metal released from metallofullerene waste and as-produced C_{60} induced meaningful toxicity to Pimephales promelas and Ceriodaphnia dubia. The toxicity could be eliminated by adding ethylene diamine tetraacetic acid (EDTA), a typical chelator. Aldieri et al. compared the toxicity of Fe-rich CNTs and Fe-free CNTs to murine alveolar macrophages.[158] Fe-rich CNTs induced significantly cytotoxicity, genotoxicity and potent cellular oxidative stress, while Fe-free CNTs did not. Ge et al. have quantified the contribution of metal impurities to the toxicity of CNTs.[159] The cell viability loss was positively correlated to the metal impurity content. Fe showed a critical role in generating hydroxyl radicals, which reduced the cell viability and promoted the intracellular reactive oxidative species. They concluded that metal impurities had dominant role in the toxicity of CNTs, while the effect of CNTs was very limited. To address this impurity issue, Liu et al. developed a protocol to purify CNTs and the bioavailability of metals was reduced.[160] In addition, Gavello et al. found that metals had minor effect on the secretion in chromaffin cells.[161] The presence of metal impurities depressed the exocytosis milder, while the CNT structure and aggregation were more important.

As the downstream effect, many studies have observed the generation of reactive oxygen species (ROS).[44] The released metal ions (e.g., Fe^{3+}/Fe^{2+}) can generate radicals via electron transfer reactions. The metal ions can also interact with intracellular biomolecules, disrupted redox homeostasis and induce ROS generation.

Charge of Nanomaterials

Typically, positively charged nanomaterials are more toxic than neutralized or negatively charged ones in blood circulation, because positive charges induce hemolysis and platelet deposition. As discussed aforementioned, charge influences the aggregation/dispersion of NMs, which further regulates the uptake and transportation of NMs in biosystems. There are also reports showing that the nanosurface charge plays important role in the toxicity of NMs.

The positive charges of NMs make them favorable to the negatively charged cell membrane, and the disruption of cell membrane causes toxicity. The most accepted phenomenon is the hemolysis induced by positively charged NMs. Goodman et al. reported that cationic NPs induced hemolysis and viability loss to cells, where anionic NPs were nearly nontoxic.[162] Mai et al. found that only cationic NPs penetrated red blood cells.[163] The anionic NPs were unable to do so due to the electrostatic repulsion. The possible mechanism of membrane damages might be that the cationic NMs produce nanosized pores in the cell membranes of red blood cells, causing an influx of small solutes into the cells and leading to colloid-osmotic lysis.[164] It should be noted that the highly negatively charged NMs might induce hemolysis, too. For example, the negatively charged silicon NPs, Ag NPs and CNTs were found to have hemolytic activity.[165–167]

The positively charged NMs also induce damages to the membrane of other cells. Chen et al. reported the induction of nanoscaled disruption of cell membrane by cationic NPs.[168] The formation of nano-holes took 1–100 ms, while the resealing needed tens of seconds (Fig. 8). Ruenraroengsak et al. verified the holes on cell membrane with microscopy after the incubation of TT1 cells with amine-NPs.[169] The membrane damage led to cell detachment, cytotoxicity and apoptotic cell death. When NPs were functionalized with carboxyl groups or unfunctionalized, the membrane holes were not formed and the cytotoxicity was much lower.[169] Grandinetti et al. reported that cationic polymeric pDNA vehicles could induce permeability of nuclear membrane.[170] This finding implied that cationic NMs could not only cause cell membrane damage, but also induce nuclear membrane damage. De Planque et al. found that all the silica NPs permeabilized the lipid bilayers already at femtomolar concentrations, below the cytotoxic values.[171] Higher concentrations of NPs led to an increased surface coverage and a concomitant decrease

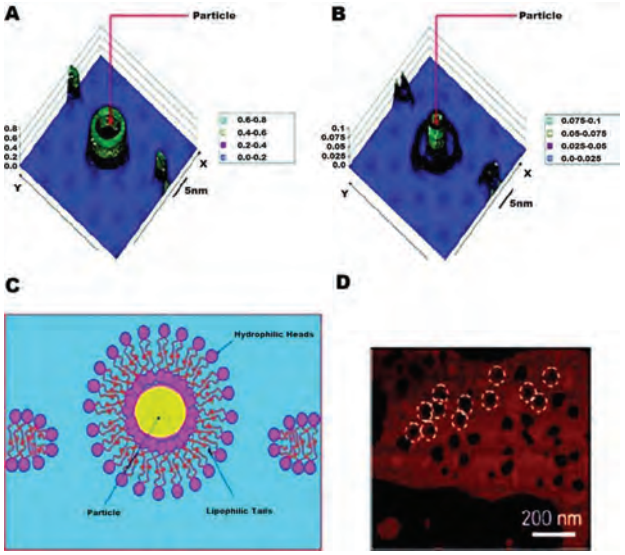

Figure 8. Model of PAMAM dendrimer causing hole formation analogous. (A) simulated density map of the lipophilic part of the bilayer; (B) simulated density map of the hydrophilic part of the bilayer; (C) the formation of a NP-bilayer complex and corresponding breakage of the original membrane. (D) AFM image of lipid bilayers with holes. Reprinted with permission from [168], J. Chen, et al., Cationic nanoparticles induce nanoscale disruption in living cell plasma membranes. *J. Phys. Chem. B* 113, 11179 (2009). © 2009, American Chemical Society.

in bilayer stability, which contributed to the plasma membrane damage. Highly charged NMs were even reported to enhance the permeability of blood-brain barrier.[172]

The charged NMs show different uptake and translocation behaviors, which alter the toxicity of NMs. Geys et al. reported that carboxyl-QDs targeted lungs and amine-QDs targeted liver.[173] Carboxyl-QDs induced pulmonary vascular thrombosis more, because negatively charged QDs activated the coagulation cascade via contact activation. Greish et al. found that amine-terminated poly(amidoamine) (PAMAM) NPs were trapped in liver.[174] Carboxyl-terminated and hydroxyl-terminated PAMAM NPs retained in blood stream and were excreted via urine. Amine-terminated NPs induced blood coagulation, hemolysis and haemorrhage of liver. The maximum tolerated doses of amine-terminated PAMAM NPs were much lower than those of carboxyl-terminated and hydroxyl-terminated PAMAM NPs. Schaeublin et al. reported that positively charged Au NPs induced cell death through apoptosis and neutral NPs led to necrosis.[175] Thus, the charge of NPs is one of the key factors that could determine the toxic mechanism.

Oxidative Stress Induced by Nanomaterials

Oxidative damage is a widely observed end point for nanotoxicity.[44] Associating with the oxidative damage, ROS species are detected as markers of oxidative damage.[176–179] The generation of ROS has been explored at a molecular level.

Metal NMs and the release components can generate radicals, which belong to ROS. The representative NMs are iron oxide NPs. Three typical reactions occur when H_2O_2 reach Fe_2O_3 NPs. The first one is Fenton reaction between H_2O_2 and Fe^{2+} released from Fe_2O_3 NPs. The Fe^{3+} from Fenton reaction can be reduced by H_2O_2.

$$Fe^{2+} + H_2O_2 = Fe^{3+} + OH^- + {}^{\bullet}OH \quad (1)$$

$$Fe^{3+} + H_2O_2 = Fe^{2+} + H^+ + HO_2^{\bullet} \quad (2)$$

The second type of reaction is Fenton-like reaction between H_2O_2 and Fe_2O_3 NPs, where the surface Fe^{2+} and Fe^{3+} of Fe_2O_3 NPs catalyze the generation of radicals.

$$Fe^{3+} + H_2O_2 = FeOOH^{2+} + H^+ \quad (3)$$

$$FeOOH^{2+} = Fe^{2+} + HO_2^{\bullet} \quad (4)$$

$$Fe^{2+} + H_2O_2 = Fe^{3+} + OH^- + {}^{\bullet}OH \quad (5)$$

The third type of reaction is Haber-Weiss reaction. Hydroxyl radicals are generated from ${}^{\bullet}O_2^-$ and H_2O_2. The net reaction is shown in Eq. (8).

$$Fe^{3+} + {}^{\bullet}O_2^- = Fe^{2+} + O_2 \quad (6)$$

$$Fe^{2+} + H_2O_2 = Fe^{3+} + OH^- + {}^{\bullet}OH \quad (7)$$

$${}^{\bullet}O_2^- + H_2O_2 = {}^{\bullet}OH + OH^- + O_2 \quad (8)$$

Wang et al. found that pH determined the mechanism of reaction (Fig. 9).[180] At pH higher than 4.2, heterogeneous Fenton and Fenton-like reaction occurred on the surface of α-Fe_2O_3 and γ-Fe_2O_3 NPs. At lower pH than 4.2, the radical generation was more attributed to the released ions from NPs. The presence of reduction reagents in biosystems, e.g., L-cystine and NADPH, would reduce Fe^{3+} on the NP surface to Fe^{2+}, which enhanced the radical generation. Considering that Fe_2O_3 NPs release Fe^{2+} and Fe^{3+} under acidic environment, Fe_2O_3 NPs can generate more radicals after they are trapped in acidic organelles. Huang et al. found that Fe_2O_3 NPs dissolved partially in lysosome, and the released ions enhanced the ROS generation. According to the studies of Fenton-like catalysis, the biosystem might be ideal for Fenton-like reaction, because the biomolecules will adsorb on Fe_2O_3 NPs and the surface coating is very important to enhance the catalytic activity.[181] Moreover, other valence variable metals and their chemicals can also serve as ROS generator. Fe NPs, Fe_3O_4 NPs, CeO_2 NPs and Cu_2O NPs are typical catalyzers to generate radicals.[182–186] Thus, oxidative stress was observed in the toxicological studies of aforementioned NMs.

Carbon NMs can also generate ROS. Most CNTs generate ROS because there are metal impurities[187] and amorphous carbon.[188] Fullerene and its derivatives are also capable in generating ROS, where fullerene and its derivatives serve as electron shuttles between electron donors and O_2 to form ${}^{\bullet}O_2^-$.[189] Recent reports indicated that graphene could induce ROS generation. Zhang et al. revealed the mechanism of radical generation by GO functionalized with PEGylated poly-*L*-lysine.[190] H_2O_2 firstly added two hydroxyl groups on graphene sheets, and then the detachment of hydroxyl groups generated two hydroxyl radicals. GO could not only catalyze the formation of hydroxyl groups, but also facilitated electron transfer from cytochrome *c* to H_2O_2 to generate active free radicals.

In addition, NMs with photocatalytic activity also generate radicals under irradiation. QDs, carbon dots, TiO_2 NPs and ZnO NPs belong to this category.[191–194] Under light irradiation, electron–hole pairs are generated, which cause the formation of radicals. This mechanism has been observed in the phototoxicity studies and photodynamic therapies.

There are indirect pathways to increase the ROS level. The disruption of the electron-transport chain in mitochondria results in the indirect production of ROS in cells. Under normal physiological conditions, excess ROS will be depleted by antioxidative defense systems. If NMs block the electron-transfer chain, or transfer electrons, the generation of ROS would increase. NMs adsorb enzymes and alter their structures and activities, which might slow down the decomposition of H_2O_2 and resulted into the accumulation of radicals. For example, Ag NPs deposited in mitochondria and disrupted their electron-transfer chains.[195] The increased ROS population led to oxidative damage and cytotoxicity. Ag could also bind to

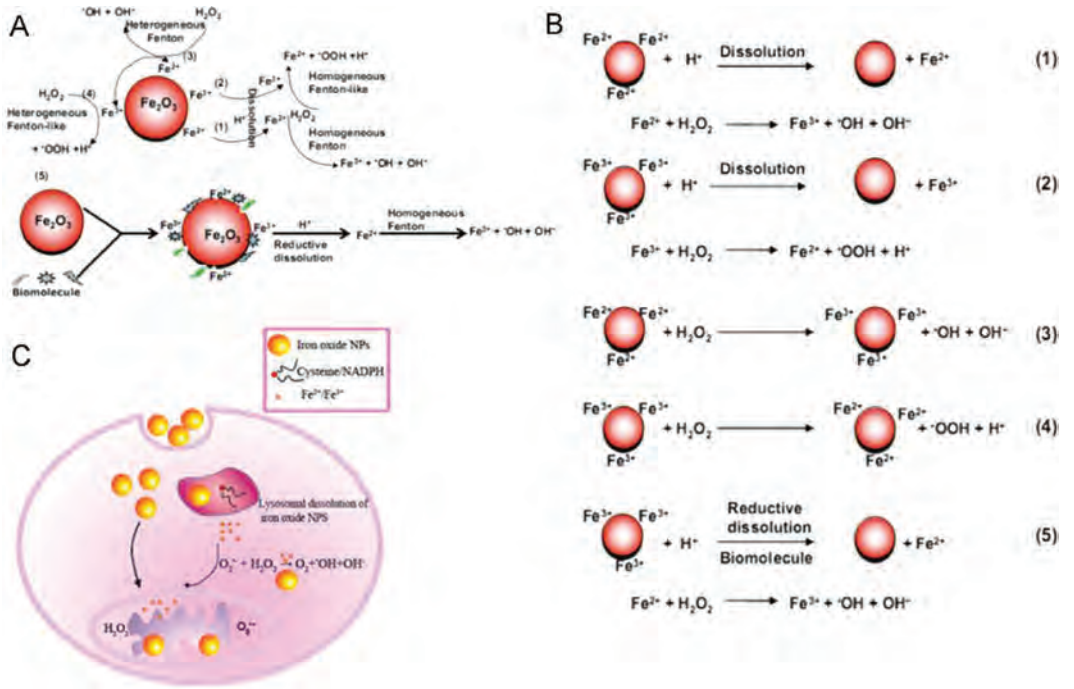

Figure 9. **The generation of ˙OH free radical by iron oxide NPs in biomicroenvironment. (A) ˙OH generation at the nanobio interface of Fe_2O_3 NPs; (B) chemical processes of ˙OH generation; (C) intracellular ˙OH free radical generation. Reprinted with permission from [180], B. Wang, et al., Physicochemical origin for free radical generation of iron oxide nanoparticles in biomicroenvironment: Catalytic activities mediated by surface chemical states. *J. Phys. Chem. C* 117, 383 (2013). © 2013, American Chemical Society.**

NADH dehydrogenases in *E. coli*, and led to an inefficient passage of electrons to oxygen at the terminal oxidase to cause the generation of large quantities of ROS.[196]

Another indirect pathway of generating radicals is the activation of ROS-related enzymes and receptors. NMs are internalized by cells, thus possibly interact with those ROS-related enzymes and receptors, which might lead to changes in protein function and chemical fragmentation. Many NMs can oxidize NADPH to NADP and cytochrome *c*-Fe^{2+} into cytochrome *c*-Fe^{3+}.[197] Asbestos particles and cationic polystyrene NPs have been reported to induce the assembly and activation of NADPH oxidase in human macrophages.[198, 199] More recent examples are CeO_2 NPs, SiO_2 NPs and CNTs.[182, 200, 201] For example, Culcasi et al. reported that CeO_2 NPs induced radical generation of human fibroblasts by Fenton-like reactions, which further induced the activation of mitochondrial source and NADPH oxidase.[182] Nabeshi et al. showed that NADPH oxidase inhibitor could significantly reduce the ROS generation of HaCaT cells after the exposure to amorphous silica NPs.[201] NADPH oxidase inhibitor also reduced the DNA damages, which were associated with oxidative stress.

Disturbed Signaling Pathway Induced by Nanomaterials

The biological functions are usually controlled or regulated by signaling pathways, which involve specific signal molecules. After NMs enter the biosystems, they might disrupt and even block the signaling pathways, which definitely result in unwanted bioeffects. Thus, the disturbed signaling pathways have received more attentions in nanotoxicology recent years.[202–205]

The most commonly disturbed signaling pathways are those associated with ROS. Among the ROS-mediated signaling pathways, MAPKs pathways are the important and frequently referred category. Li et al. reported that graphene could trigger the apoptosis of macrophage through MAPK and TGF-β signaling pathways (Fig. 10).[206] The upstream affairs were the depletion of the mitochondrial membrane potential and the increase of intracellular ROS. The apoptosis was triggered by the activation of the mitochondrial pathway. MAPKs (including JNK, ERK and p38) and TGF-β-related signaling pathways were activated in the pristine graphene treated cells. Then, two pro-apoptotic member of Bcl-2 protein family (Bim and Bax), caspase 3 and its downstream effector proteins such as PARP were activated and the execution of apoptosis was initiated. Roh et al. reported MAPK signaling pathways were involved in the defense of Ag NP exposure in *C. elegans*.[207] The ROS initiated the over expression of genes (nsy-1, sek-1, pmk-1, jkk-1, jnk-1 and mpk-2) in MAPKs signaling pathways. Eom et al. reported that the oxidative stress of CeO_2 NPs to human bronchial epithelial cell (Beas-2B) was via p38-Nrf-2 signaling pathway.[208] ERK1/2 and JNK showed much less

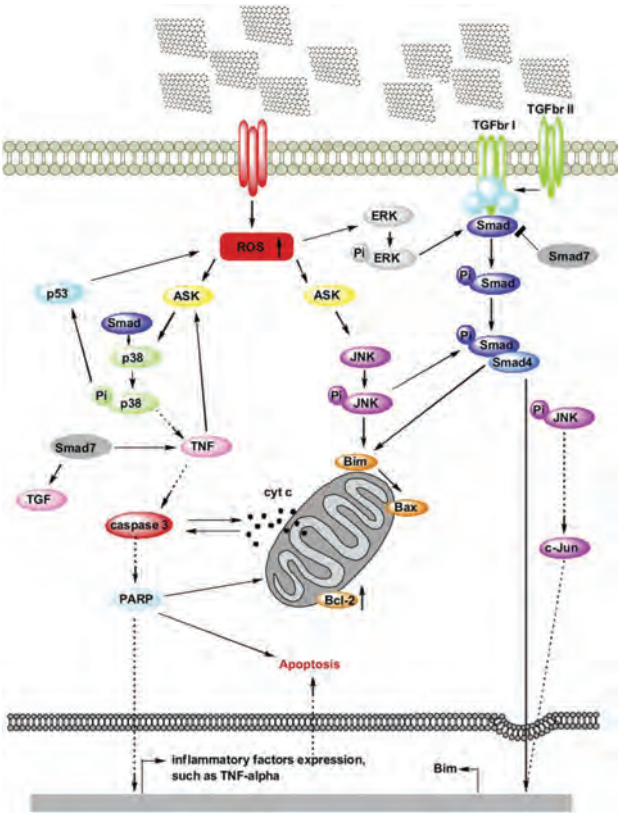

Figure 10. Signaling pathways involved in pristine graphene-induced cell apoptosis. Reprinted with permission from [206], Y. Li, et al., The triggering of apoptosis in macrophages by pristine graphene through the MAPK and TGF-beta signaling pathways. *Biomaterials* 33, 402 (2012). © 2012, Elsevier Ltd.

increases than p38. The activation of p38-Nrf-2 signaling pathway led to the induction of HO-1 and consequently the apoptosis. Cheng et al. reported that CeO_2 NPs induced the generation of ROS in hepatoma SMMC-7721 cells and activated MAPKs signaling pathways.[209] When the cells were treated with antioxidant *N*-acetylcysteine, the ROS level decreased, which led to the suppression of phosphorylation levels of ERK1/2, JNK and p38 in MAPKs signaling pathways. Wang et al. found that cuprous oxide NPs could induce the apoptosis of tumor cells by mitochondrion-mediated apoptosis pathway, where ROS increase was observed as the sign of mitochondrion disruption.[210] Ding et al. found that WC-Co NPs generated higher level of hydroxyl radicals and greater oxidative stress comparing to fine WC-Co particles.[211] WC-Co NPs activated AP-1 and NF-κB more efficiently in $JB6^{+/+}$ cells. Both particles stimulated MAPKs with significantly higher potency of WC-Co NPs. The inhibition of oxidative stress by N-acetylcysteine decreased the AP-1 activation and phosphorylation of MAPKs, indicating the involvement of oxidative stress in the signaling activation.

Other ROS-mediated signaling pathways contribute to the nanotoxicity, too. Nishanth et al. reported that Ag NPs induced more serious inflammation in RAW264.7 cells comparing to Al NPs, Au NPs and carbon black NPs, which was mediated by ROS and NF-κB signaling pathways.[212] The activation of NF-κB signaling pathways led to the induction of COX-2, TNF-α and IL-6. Akhtar et al. reported that PAMAM NPs stimulated the EGFR-ERK1/2 signal transduction pathway via oxidative stress in HEK 293 cells.[213] The stimulations of EGFR and ERK1/2 phosphorylations were time and dose-dependent. The selective EGFR tyrosine kinase antagonist (AG1478) inhibited EGFR-ERK1/2 signaling. The stimulation of EGFR-ERK1/2 signaling could be attenuated by the antioxidants apocynin, catalase and tempol, suggesting the oxidative stress was involved. Ge NPs induced the necrotic cell death of CHO K1 cells.[214] Intracellular calcium and ROS levels increased after the exposure to Ge NPs. The intracellular ROS led to the reduction of mitochondrial membrane potential (MMP), and consequentially resulted in necrotic cell death. The toxicity of Ge NPs could be blocked by the transduction of necrotic signaling pathway with inhibitors.

Beyond the ROS-mediated signaling pathways, various ROS-independent ones also widely reported in nanotoxicology. Meng et al. reported that CNTs stimulated the differentiation of PC12 cells via up-regulation of the neurotrophin signaling pathway.[215] CNTs stimulated the expression of neurotrophin signaling pathway associated TrkA/p75 receptors and Pincher/Gap43/TH proteins. Yang et al. reported that $[Gd@C_{82}(OH)_{22}]_n$ NPs facilitated the differentiation of bone marrow stromal cells (MSCs) toward osteoblasts through BMP signaling pathway. Phosphorylated Smad1/5 was significantly up-regulated, but the total Smad and α-tubulin was not changed upon NP treatment.[216] Noggin, the inhibitor of the BMP signaling pathway, could inhibit the effects. Wang et al. found that long CNTs significantly activated macrophages and increased the production of TGF-β1, which induced the phosphorylation of Smad2 and then the expression of collagen I/III and extracellular matrix (ECM) protease inhibitors in lung tissues.[217] The TGF-β/Smad signaling pathway was necessary for the expression of collagen III in fibroblast cells. The activation of TGF-β/Smad signaling pathway resulted in pulmonary fibrosis. Zhu et al. revealed the mechanism of iron oxide NPs initiated Th1-type immune activation, where the formation of exosomes as extrapulmonary signaling conveyors was the key step.[218] Other interesting examples are also included here. Khan et al. reported that Fe_3O_4 NPs specifically interfered with TGF-β signaling by inhibiting the expression of ID and SMAD genes.[219] Matsumoto et al. reported CNTs activated ERK through the phospholipase C signaling pathway.[220] Guidetti et al. Found that NMs induced platelet activation *in vitro* through stimulation of canonical signalling pathways, such as the stimulation of phospholipase C and Rap1b.[221] Li et al. reported that PAMAM triggered the autophagic cell death by deregulating the

Akt-TSC2-mTOR signaling pathway, and the acute lung injury was promoted.[222]

Membrane Damage Induced by Nanomaterials

Membrane damage is a widely monitored endpoint in nanotoxicology. In some specific cases, membrane damage is the origin of nanotoxicity. After NP permeation, the water penetration, ion transport and lipid flip-flop will be triggered.[223] There are several studies on understanding the mechanism of membrane damage at the molecular level.

NMs might cut the cell membrane to induce toxicity. Liu et al. reported that individual CNTs were more toxic to bacteria than CNT aggregates.[224] CNTs were visualized as nano dart here to degrade the cell integrity. By dispersing CNTs well and shaking CNTs faster, the CNT dart caused more toxicity to bacteria. Similarly, graphene materials, which have sharp sheets, can cause membrane damage to bacteria.[225]

When NMs are functionalized with hydrophobic or amphiphilic chains, NMs can damage the membrane by diffusion effects. Yang et al. found that PEGylated carbon dots were toxic to cells, because PEG acted as fusogen in cell membrane diffusion.[226] Dubavik et al. functionalized CdTe QDs with thiolated methoxypolyethylene glycol penetrated cell membrane easily.[227] Gkeka et al. modeled the permeation of NPs through lipid membrane.[228] There was a tendency that homogeneous pattern of hydrophobic-hydrophilic surface chains was formed to facilitate the permeation. Not toxicological relevant but inspiring, the sealing of cell membrane has been widely reported. For instance, Cho et al. found that chitosan could act as fusogen to cell membrane, and chitosan NPs could be used for membrane sealing.[229]

Computation is very powerful in revealing the mechanism of membrane damage. Fiedler et al. investigated the permeation of C_{60}, open-C_{60} and nano C through lipid membrane.[230] Phan et al. calculated the interaction of lipid membrane and graphene, and the contribution of van der waals interaction was the major binding force.[231] Alexeev et al. found that NPs created controllable pores on membrane by external force, where the pores persisted after the force was released.[232] Jing et al. revealed that the hydrophobic interaction underlied the envelopment of NPs by lipids, which were attracted from lipid layers to the surface of NPs.[233] The formation and growth of lipid-poor regions (referred as pores or holes) were controlled by NP concentration, size, and surface hydrophobicity. Recently, Tu et al. reported the molecular mechanism of membrane damage caused by graphene (Fig. 11).[234] First, graphene penetrated into the cell membrane of E. coli. Then, phospholipids were extracted by graphene layers. The TEM and simulation results together suggested that the two steps resulted in destructive extraction of phospholipids, which was the molecular basis of graphene toxicity.

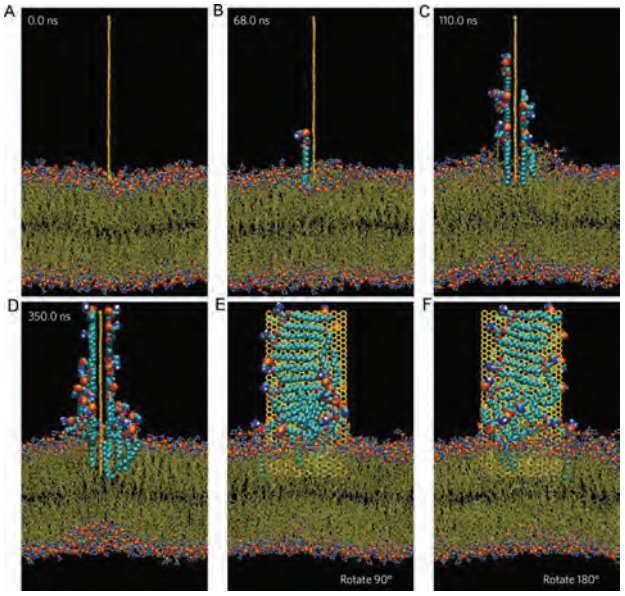

Figure 11. Docking simulations of the lipid extraction by graphene. Reprinted with permission from [234], Y. Tu, et al., Destructive extraction of phospholipids from *Escherichia coli* membranes by graphene nanosheets. *Nat. Nanotechnol.* 8, 594 (2013). © 2013, Macmillan Publishers Limited.

Synergistic Toxicity of Nanomaterials with Other Toxicants

The synergistic toxicity of NMs with other toxicants is attracting more attention nowadays. The enhanced toxicity or reduced toxicity of toxicants in presence of NMs are reported by different groups.

The first effect of NMs on toxicants is the regulation on bioavailability and retention. Henry et al. showed that fullerene C_{60} aggregated and sedimented in the presence of Hg^{2+}, which led to the reduce of Hg^{2+} uptake by larval zebrafish *Danio rerio*.[235] However, for zebrafish residing at the bottom, the Hg^{2+} bioavailability was elevated. Tan et al. showed that the uptake and retention of Cd and Zn in *Daphnia magna* was enhanced when they were adsorbed on TiO_2 NPs.[236] Guo et al. revealed the mechanism of SiNP enhanced Cd bioavailability in mice.[237] BSA adsorption on SiNPs was a great enhancer of Cd loading, which led to the high Cd contents in liver and kidneys (statistically higher than $CdCl_2$ alone). Yu et al. found that oxidized CNTs enhanced the uptake of Cd and Zn in *Daphnia magna*, while the non-oxidized CNTS reduced the uptake.[238] The difference might come from the surface oxygen containing groups, which adsorbed Cd and Zn effectively.

The enhanced bioavailability of toxicant increases the real concentration of toxicant, thus, an enhanced toxicity is expected. Zhu et al. suggested that phenol red became toxic after carbon NMs delivered it into cells.[239] Zheng et al. reported that TiO_2 NPs aggregated in presence of bisphenol A, and TiO_2 NPs deliverd bisphenol A to the nuclei area of L-02 cells.[240] The delivery enhanced

the oxidative stress and DNA damage, which were more serious than the toxicity of bisphenol A or TiO_2 NPs alone. Guo et al. showed the high Cd uptake in liver and kidneys in the presence of SiNPs and proteins.[237] The synergistic toxicity, including body weight loss, organ index increase, serum biochemistry, histological damage and oxidative stress, was more serious than the toxicity of $CdCl_2$. Fan et al. reported that TiO_2 NPs adsorbed Cu^{2+}, enhanced the ingesting of Cu, and consequently resulted in higher toxicity.[241] The LC_{50} for *Daphnia magna* was reduced from 111 μg/L to 42 μg/L, and the metallothionein decreased from 135 μg/g wet weights to 99 μg/g. Hu et al. reported the adsorption on TiO_2 NPs and CeO_2 NPs enhanced the toxicity of Pb^{2+}, since NPs were ingested in the gastrointestinal tract of *Ceriodaphnia dubia*.[242] Reducing the pH value would enhance the toxicity of Pb^{2+} further. Zhang et al. reported the enhanced Cu^{2+} burden in zebrafish by CdTe QDs.[243] The mortality, un-hatch rate and malformation induced by Cu^{2+} were all increased when CdTe QDs presented. Kim et al. reported a special mechanism of CNT enhanced Cu^{2+} toxicity.[244] Since Cu^{2+} was bound with natural organic matter (NOM), the free Cu^{2+} concentration was reduced and the toxicity of Cu^{2+} was relieved. CNTs competitively bound to NOM, which caused the release of free Cu^{2+}. The mortality of *Daphnia magna* increased when Cu^{2+} was co-exposed with CNTs.

There are some interesting results different from the sole enhancement. Wang et al. reported that Al_2O_3 NPs could adsorb As (V) and be ingested by *Ceriodaphnia dubia* to increase the bioavailability and toxicity at low Al_2O_3 concentrations.[245] When very high Al_2O_3 concentrations were adopted, the Al_2O_3 NPs blocked the release of As (V) and a relieved toxicity was found. Similar results were reported in their study of TiO_2 NPs and As (V).[246] Zhang et al. found that TiO_2 NPs increased the Pb level after the co-administration of TiO_2 NPs and $Pb(Ac)_2$.[247] However, the TiO_2 NPs $Pb(Ac)_2$ group and $Pb(Ac)_2$ group showed very similar toxicity to liver and kidneys, although both were significantly more toxic than TiO_2 NPs.

Other Mechanisms of Nanotoxicity

Despite the aforementioned novel mechanisms, there are other less focused but still important mechanisms of nanotoxicology. Taking these mechanisms into consideration would help us understand nanotoxicology better.

The depletion of nutrients is an unusual nanotoxicological mechanism. Hurt and coworkers revealed the depletion of amino acids and vitamins from cell culture medium by CNTs and graphene.[95,96] CNTs induced cytotoxicity via this pathway, where the toxicity could be reduced by supplying additional folate. Horie et al. showed the adsorption of Ca^{2+} and proteins on TiO_2 NPs, which induced toxicity to cells.[97] The pretreatment of TiO_2 NPs with FBS blocked the adsorption effect, thus their toxicity was diminished.

The misfolding of protein or nucleic acids has been observed in the presence of NMs. The conformation loss of proteins or nucleic acids leads to the false or reduced functions. The protein misfolding diseases (e.g., Alzheimer's disease) are well acknowledged. Therefore, logically we speculated that the misfolding of biomolecules would induce toxicity to biosystems. Unfortunately, no such investigation is available nowadays. We prefer to propose this unproven mechanism here to encourage the related studies. Combinations of structural studies and toxicological studies are highly appreciated.

SUMMARY AND OUTLOOK

In summary, after the rapid developments of nanotoxicology, the studies of nanotoxicity should be pushed to a new stage that not only accumulates toxicological data, but also revealing the underneath mechanisms. We summarized here the toxicity explorations of NMs at the molecular level. NMs interact with biomolecules after entering the biosystems, which lead to the transformation and/or degradation of NMs. The interactions between NMs and biomolecules help to understand the uptake and translocation of NMs in biosystems, where the molecular recognition seems very important in this stage. Then, taking the NM-biomolecule interactions and the chemical nature of NMs into consideration, the nanotoxicological mechanisms could be partially understand at molecular level. However, due to the complexity of NMs and biosystems, the available data are not systematic and consistent with each other yet. There are several major issues to consider in future molecular nanotoxicity studies.

The first one is the reproducible production and full characterization of NMs. Since the physicochemical properties, including charge and charge distribution, surface funtioncalization and functionality pattern, size and shape, etc. directly affect the toxicity of NMs. The reproducibility of NMs preparation is also crucial for toxicologists to reach consistent and reliable conclusions. To ensure the reproducibility, standard characterization techniques are highly demanded. In fact, the characterization is also important for the transformation/degradation studies of NMs. After the transformation/degradation, the properties of NMs will change, so for the toxicity. Unlike direct characterization of as-received samples, the characterization of NMs in nanotoxicology requires capability of *in situ* characterization in biosystems, in particular for the low contents and minor modifications. After obtaining the full information of NMs in biosystems, we can then possibly reach some conclusive points of molecular toxicity of NMs.

Secondly, building the structure-toxicity relationship should receive much more attentions. The quantitative structure-activity relationship (QSAR) is widely used in the pharmacology studies. There are accumulating evidences showing that the structure (including surface functionalities) regulates the toxicity of NMs. It would help

the future study a lot if the structure-toxicity relationship is clearly clarified. The building of QSAR like structure-toxicity relationship in nanotoxicology obviously requires tremendous efforts at molecular level. The computational assisted researches are extremely welcome in building the structure-toxicity relationship. Beyond that, the re-visiting of published data is also very valuable for this purpose.

Thirdly, how to link the molecular outcome with diseases with hazards exposure is another important issue. Currently, many molecular results, in particular the studies in solution, have not been associated with the diseases or toxicity observation. In many cases, the observed changes are referred to have impact on the health. A direct connection between the molecular changes and toxicity/disease is worthy. The representative example could be that NMs alter the protein fibrillation.[79] Would the accelerated fibrillation lead to Alzheimer's disease? Another recent example was the broad-spectrum antibacterial activity of CNTs to human gut bacteria.[248] Would the disturbance of gut bacteria affect the health of human beings? Answering such questions will largely deeps our understanding of nanotoxicity.

The last but not the least, designing biocompatible NMs and developing curing strategy of nanotoxicity are always the most attractive pearls of nanotoxicology. As we discussed aforementioned, many factors regulated the toxicity of NMs. We need to consider these factors in future design of biocompatible NMs. For instance, Ge et al. reported that many proteins could bind to CNTs, resulting in a full coverage of CNT surface, which decreased the toxicity of CNTs to cells.[54] Thus, one can pre-coating CNTs with proteins to mitigate their toxicity. Li et al. showed that D-GSH coating reduced the toxicity of QDs more than L-GSH coating.[249] This finding suggested that the chirality should be considered in designing biocompatible NMs. Another issue has been even less concerned, that how can we cure nanotoxicity after the toxic hazards are induced. This hold great importance, since we cannot achive the complete elimination of nanotoxicity before the use. The potential strategies could be designed from the molecular nanotoxicity results. Oxidative stress is the common pathway of nanotoxicity, we may treat the patients with antioxidants to inhibit the oxidative damage. Such strategy has been proven by co-exposure of antioxidants and NMs. The performance of antioxidants after the injury occurs need to be evaluated.

Acknowledgments: We thank the financial support from the NSFC (Nos. 11005116 and 21307101), and MOST 973 programs (2012CB932601 and 2013CB932703).

REFERENCES

1. S. Guo and S. Dong, Graphene nanosheet: Synthesis, molecular engineering, thin film, hybrids, and energy and analytical applications. *Chem. Soc. Rev.* 40, 2644 **(2011)**.
2. C. Sanchez, P. Belleville, M. Popall, and L. Nicole, Applications of advanced hybrid organic–inorganic nanomaterials: From laboratory to market. *Chem. Soc. Rev.* 40, 696 **(2011)**.
3. J. A. Barreto, W. O'malley, M. Kubeil, B. Graham, H. Stephan, and L. Spiccia, Nanomaterials: Applications in cancer imaging and therapy. *Adv. Mater.* 23, H18 **(2011)**.
4. H. S. Nalwa (ed.), Encyclopedia of Nanoscience and Nanotechnology, American Scientific Publishers, Los Angeles, CA **(2004/2011)**, Vols. 1–25.
5. H. S. Nalwa (ed.), Handbook of Nanostructured Materials and Nanotechnology, Academic Press, San Diego, CA **(2000)**, Vols. 1–5.
6. A. Nel, Y. Zhao, and L. Mädler, Environmental health and safety considerations for nanotechnology. *Acc. Chem. Res.* 46, 605 **(2013)**.
7. C. Chen, Y.-F. Li, Y. Qu, Z. Chai, and Y. Zhao, Advanced nuclear analytical and related techniques for the growing challenges in nanotoxicology. *Chem. Soc. Rev.* 42, 8266 **(2013)**.
8. B. Pelaz, G. Charron, C. Pfeiffer, Y. Zhao, J. M. De La Fuente, X. J. Liang, W. J. Parak, and P. Del Pino, Interfacing engineered nanoparticles with biological systems: Anticipating adverse nano–bio interactions. *Small* 9, 1573 **(2012)**.
9. S.-T. Yang, Y. Liu, Y. W. Wang, and A. Cao, Biosafety and bioapplication of nanomaterials by designing protein–nanoparticle interactions. *Small* 9, 1635 **(2013)**.
10. S.-T. Yang, J. Luo, Q. Zhou, and H. Wang, Pharmacokinetics, metabolism and toxicity of carbon nanotubes for biomedical purposes. *Theranostics* 2, 271 **(2012)**.
11. S. W. Y. Wong, K. M. Y. Leung, and A. B. Djurišic, A comprehensive review on the aquatic toxicity of engineered nanomaterials. *Rev. Nanosci. Nanotechnol.* 2, 79 **(2013)**.
12. G. J. Horbach, S. V. D. Brule, C. Magkoufopoulou, G. Vietti, D. Papoutsi, L. Leyns, and M. Kirsch-Volders, The safety of nanomaterials: Translation from academic research to industrial and regulatory applications. *J. Transl. Toxicol.* 1, 40 **(2014)**.
13. S. Singh and H. S. Nalwa, Nanotechnology and health safety–toxicity and risk assessments of nanostructured materials on human health. *J. Nanosci. Nanotechnol.* 7, 3048 **(2007)**.
14. Y. L. Zhao and H. S. Nalwa (eds.), Nanotoxicology: Interactions of Nanomaterials with Biological Systems, American Scientific Publishers, Los Angeles, CA **(2007)**.
15. L.-C. Cheng, X. Jiang, J. Wang, C. Chen, and R.-S. Liu, Nano-bio effects: Interaction of nanomaterials with cells. *Nanoscale* 5, 3547 **(2013)**.
16. S. Eduok, B. Martin, R. Villa, A. Nocker, B. Jefferson, and F. Coulon, Evaluation of engineered nanoparticle toxic effect on wastewater microorganisms: Current status and challenges. *Ecotox. Environ. Safe.* 95, 1 **(2013)**.
17. H. Wang, L.-J. Du, Z.-M. Song, and X.-X. Chen, Progress in the characterization and safety evaluation of engineered inorganic nanomaterials in food. *Nanomedicine* 8, 2007 **(2013)**.
18. A. Kahru and A. Ivask, Mapping the dawn of nanoecotoxicological research. *Acc. Chem. Res.* 46, 823 **(2013)**.
19. P. Cortes, S. Deng, and G. B. Smith, The toxic effects of single wall carbon nanotubes on E. coli and a spore-forming bacillus species. *Nanosci. Nanotechnol. Lett.* 6, 26 **(2014)**.
20. R. K. Basniwal, R. P. S. Chauhan, V. Bhatia, and V. K. Jain, Toxicity study of multiwalled carbon nanotubes on freshwater aquatic algae. *J. Bionanosci.* 7, 597 **(2013)**.
21. M. Carrière, S. Pigeot-Rémy, A. Casanova, A. Dhawan, J.-C. Lazzaroni, C. Guillard, and N. Herlin-Boime, Impact of titanium dioxide nanoparticle dispersion state and dispersion method on their toxicity towards A549 lung cells and escherichia coli bacteria. *J. Transl. Toxicol.* 1, 10 **(2014)**.
22. A. Kumar, S. Khan, and A. Dhawan, Comprehensive molecular analysis of the responses induced by titanium dioxide nanoparticles in human keratinocyte cells. *J. Transl. Toxicol.* 1, 28 **(2014)**.
23. A. B. More, M. D. Patel, V. C. Malshe, P. V. Devarajan, and G. R. Vanage, Genotoxicity and mutagenicity evaluation of polyethylene sebacate nanoparticles. *J. Nanopharmaceutics Drug Delivery* 1, 301 **(2013)**.

24. T. Chen, J. Hu, C. Chen, J. Pu, X. Cui, and G. Jia, Cardiovascular effects of pulmonary exposure to titanium dioxide nanoparticles in ApoE knockout mice. *J. Nanosci. Nanotechnol.* 13, 3214 **(2013)**.

25. S. Hackenberg, A. Scherzed, A. Technau, K. Froelich, R. Hagen, and N. Kleinsasser, Functional responses of human adipose tissue-derived mesenchymal stem cells to metal oxide nanoparticles *in vitro*. *J. Biomed. Nanotechnol.* 9, 86 **(2013)**.

26. A. R. Kim, F. R. Ahmed, G. Y. Jung, S.-W. Cho, D.-I. Kim, and S. H. Um, Hepatocyte cytotoxicity evaluation with zinc oxide nanoparticles. *J. Biomed. Nanotechnol.* 9, 926 **(2013)**.

27. E. M. Kim, P. Palmer, V. Howard, A. Elsaesser, A. Taylor, G. Staats, and E. O'hare, Effect of intracerebroventricular injection of TiO_2 nanoparticles on complex behaviour in the rat. *J. Nanosci. Nanotechnol.* 13, 8325 **(2013)**.

28. J. Li, Y. Song, X. Liu, M. Zhang, R. He, Y. Chang, J. Jin, G.-M. Xing, and J. Zhang, The effects of $C_{60}(C(COOH)_2)_2$-FITC on proliferation and differentiation of human mesenchymal stem cells *in vitro*. *J. Nanosci. Nanotechnol.* 13, 6517 **(2013)**.

29. X. Li, L. Xu, A. Shao, G. Wu, and N. Hanagata, Cytotoxic and genotoxic effects of silver nanoparticles on primary Syrian Hamster Embryo (SHE) cells. *J. Nanosci. Nanotechnol.* 13, 161 **(2013)**.

30. N. V. Pinto, N. F. De Andrade, D. S. Teodoro Martinez, O. L. Alves, A. G. Souza Filho, M. R. Lima Mota, K. S. Nascimento, B. S. Cavada, and A. M. S. Assreuy, Inflammatory and hyperalgesic effects of oxidized multi-walled carbon nanotubes in rats. *J. Nanosci. Nanotechnol.* 13, 5276 **(2013)**.

31. J. Sengupta, P. Datta, H. K. Patra, A. K. Dasgupta, and A. Gomes, *In vivo* interaction of gold nanoparticles after acute and chronic exposures in experimental animal models. *J. Nanosci. Nanotechnol.* 13, 1660 **(2013)**.

32. L. Xue, X. He, Y. Li, M. Qu, and Z. Zhang, Pulmonary toxicity of ceria nanoparticles in mice after intratracheal instillation. *J. Nanosci. Nanotechnol.* 13, 6575 **(2013)**.

33. G. Oberdörster, V. Stone, and K. Donaldson, Toxicology of nanoparticles: A historical perspective. *Nanotoxicology* 1, 2 **(2007)**.

34. G. Oberdörster, E. Oberdörster, and J. Oberdörster, Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. *Environ. Health Persp.* 113, 823 **(2005)**.

35. M.-T. Zhu, W.-Y. Feng, B. Wang, T.-C. Wang, Y.-Q. Gu, M. Wang, Y. Wang, H. Ouyang, Y.-L. Zhao, and Z.-F. Chai, Comparative study of pulmonary responses to nano- and submicron-sized ferric oxide in rats. *Toxicology* 247, 102 **(2008)**.

36. B. Wang, W.-Y. Feng, T.-C. Wang, G. Jia, M. Wang, J.-W. Shi, F. Zhang, Y.-L. Zhao, and Z.-F. Chai, Acute toxicity of nano- and micro-scale zinc powder in healthy adult mice. *Toxicol. Lett.* 161, 115 **(2006)**.

37. M. Zhu, G. Nie, H. Meng, T. Xia, A. Nel, and Y. Zhao, Physicochemical properties determine nanomaterial cellular uptake, transport, and fate. *Acc. Chem. Res.* 46, 622 **(2013)**.

38. H. Jaganathan and B. Godin, Biocompatibility assessment of Si-based nano-and micro-particles. *Adv. Drug Deliver. Rev.* 64, 1800 **(2012)**.

39. S.-T. Yang, X. Wang, G. Jia, Y. Gu, T. Wang, H. Nie, C. Ge, H. Wang, and Y. Liu, Long-term accumulation and low toxicity of single-walled carbon nanotubes in intravenously exposed mice. *Toxicol. Lett.* 181, 182 **(2008)**.

40. G. Jia, H. Wang, L. Yan, X. Wang, R. Pei, T. Yan, Y. Zhao, and X. Guo, Cytotoxicity of carbon nanomaterials: Single-wall nanotube, multi-wall nanotube, and fullerene. *Environ. Sci. Technol.* 39, 1378 **(2005)**.

41. O. Akhavan and E. Ghaderi, Toxicity of graphene and graphene oxide nanowalls against bacteria. *ACS Nano* 4, 5731 **(2010)**.

42. Y. Liu, Y. Zhao, B. Sun, and C. Chen, Understanding the toxicity of carbon nanotubes. *Acc. Chem. Res.* 46, 702 **(2013)**.

43. M. Pumera, Nanotoxicology: The molecular science point of view. *Chem. Asian J.* 6, 340 **(2011)**.

44. L. Yan, Z. Gu, and Y. Zhao, Chemical mechanisms of the toxicological properties of nanomaterials: Generation of intracellular reactive oxygen species. *Chem. Asian J.* 8, 2342 **(2013)**.

45. Y. Qu, Y. Huang, and X. Lu, Proteomic analysis of molecular biocompatibility of gold nanoparticles to human dermal fibroblasts-fetal. *J. Biomed. Nanotechnol.* 9, 40 **(2013)**.

46. P. Westerhoff and B. Nowack, Searching for global descriptors of engineered nanomaterial fate and transport in the environment. *Acc. Chem. Res.* 46, 844 **(2013)**.

47. G. Zuo, S.-G. Kang, P. Xiu, Y. Zhao, and R. Zhou, Interactions between proteins and carbon-based nanoparticles: Exploring the origin of nanotoxicity at the molecular level. *Small* 9, 1546 **(2013)**.

48. H. Wang, S.-T. Yang, A. Cao, and Y. Liu, Quantification of carbon nanomaterials *in vitro*. *Acc. Chem. Res.* 46, 750 **(2013)**.

49. B. Wang, X. He, Z. Zhang, Y. Zhao, and W. Feng, Metabolism of nanomaterials *in vivo*: Blood circulation and organ clearance. *Acc. Chem. Res.* 46, 761 **(2013)**.

50. S.-T. Yang, H. Wang, L. Guo, Y. Gao, Y. Liu, and A. Cao, Interaction of fullerenol with lysozyme investigated by experimental and computational approaches. *Nanotechnology* 19, 395101 **(2008)**.

51. X. Wu, S.-T. Yang, H. Wang, L. Wang, W. Hu, A. Cao, and Y. Liu, Influences of the size and hydroxyl number of fullerenes/fullerenols on their interactions with proteins. *J. Nanosci. Nanotechnol.* 10, 6298 **(2010)**.

52. L. Wang, J. Li, J. Pan, X. Jiang, Y. Ji, Y. Li, Y. Qu, Y. Zhao, X. Wu, and C. Chen, Revealing the binding structure of the protein corona on gold nanorods using synchrotron radiation-based techniques: Understanding the reduced damage in cell membranes. *J. Am. Chem. Soc.* 135, 17359 **(2013)**.

53. M. P. Monopoli, D. Walczyk, A. Campbell, G. Elia, I. Lynch, F. B. Bombelli, and K. A. Dawson, Physical-chemical aspects of protein corona: Relevance to *in vitro* and *in vivo* biological impacts of nanoparticles. *J. Am. Chem. Soc.* 133, 2525 **(2011)**.

54. C. Ge, J. Du, L. Zhao, L. Wang, Y. Liu, D. Li, Y. Yang, R. Zhou, Y. Zhao, and Z. Chai, Binding of blood proteins to carbon nanotubes reduces cytotoxicity. *Proc. Natl. Acad. Sci. USA* 108, 16968 **(2011)**.

55. V. Sanz, H. Coley, S. R. Silva, and J. Mcfadden, Modeling the binding of peptides on carbon nanotubes and their use as protein and DNA carriers. *J. Nanopart. Res.* 14, 1 **(2012)**.

56. L. Baweja, K. Balamurugan, V. Subramanian, and A. Dhawan, Hydration Patterns of graphene-based nanomaterials (GBNMs) play a major role in the stability of a helical protein: A molecular dynamics simulation study. *Langmuir* 29, 14230 **(2013)**.

57. E. Casals, T. Pfaller, A. Duschl, G. J. Oostingh, and V. F. Puntes, Hardening of the nanoparticle–protein corona in metal (Au, Ag) and oxide (Fe_3O_4, CoO, and CeO_2) nanoparticles. *Small* 7, 3479 **(2011)**.

58. M. Matsui, N. Takahashi, and J.-I. Ozaki, Adsorption of cytochrome *c* on nanoshell carbon. *Carbon* 49, 4505 **(2011)**.

59. S. Tenzer, D. Docter, S. Rosfa, A. Wlodarski, J. R. Kuharev, A. Rekik, S. K. Knauer, C. Bantz, T. Nawroth, and C. Bier, Nanoparticle size is a critical physicochemical determinant of the human blood plasma corona: A comprehensive quantitative proteomic analysis. *ACS Nano* 5, 7155 **(2011)**.

60. J. E. Gagner, M. D. Lopez, J. S. Dordick, and R. W. Siegel, Effect of gold nanoparticle morphology on adsorbed protein structure and function. *Biomaterials* 32, 7241 **(2011)**.

61. S. Chakraborty, P. Joshi, V. Shanker, Z. Ansari, S. P. Singh, and P. Chakrabarti, Contrasting effect of gold nanoparticles and nanorods with different surface modifications on the structure and activity of bovine serum albumin. *Langmuir* 27, 7722 **(2011)**.

62. F. Meder, T. Daberkow, L. Treccani, M. Wilhelm, M. Schowalter, A. Rosenauer, L. Mädler, and K. Rezwan, Protein adsorption on colloidal alumina particles functionalized with amino, carboxyl, sulfonate and phosphate groups. *Acta Biomater.* 8, 1221 **(2012)**.

63. T. Cedervall, I. Lynch, S. Lindman, T. Berggard, E. Thulin, H. Nilsson, K. A. Dawson, and S. Linse, Understanding the nanoparticle–protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles. *Proc. Natl. Acad. Sci. USA* 104, 2050 **(2007)**.

64. Y. Wang, S.-T. Yang, Y. Wang, Y. Liu, and H. Wang, Adsorption and desorption of doxorubicin on oxidized carbon nanotubes. *Colloid Surf. B* 97, 62 **(2012)**.

65. A. L. Barrán-Berdón, D. Pozzi, G. Caracciolo, A. L. Capriotti, G. Caruso, C. Cavaliere, A. Riccioli, S. Palchetti, and A. Laganà, Time evolution of nanoparticle–protein corona in human plasma: Relevance for targeted drug delivery. *Langmuir* 29, 6485 **(2013)**.

66. T. Nguyen, H.-C. Chang, and V. W.-K. Wu, Adsorption and hydrolytic activity of lysozyme on diamond nanocrystallites. *Diam. Relat. Mater.* 16, 872 **(2007)**.

67. J. Han, P. Silcock, A. J. Mcquillan, and P. Bremer, Bovine serum albumin adsorption on *N*-methyl-*d*-glucamine modified colloidal silica. *Colloid Surf. A* 349, 207 **(2009)**.

68. L. Wang, H. Wang, L. Yuan, W. Yang, Z. Wu, and H. Chen, Stepwise control of protein adsorption and bacterial attachment on a nanowire array surface: Tuning surface wettability by salt concentration. *J. Mater. Chem.* 21, 13920 **(2011)**.

69. Q. Mu, W. Liu, Y. Xing, H. Zhou, Z. Li, Y. Zhang, L. Ji, F. Wang, Z. Si, and B. Zhang, Protein binding by functionalized multiwalled carbon nanotubes is governed by the surface chemistry of both parties and the nanotube diameter. *J. Phys. Chem. C* 112, 3300 **(2008)**.

70. Z. Peng, K. Hidajat, and M. Uddin, Adsorption of bovine serum albumin on nanosized magnetic particles. *J. Colloid Interface Sci.* 271, 277 **(2004)**.

71. M. Mahmoudi, M. A. Shokrgozar, S. Sardari, M. K. Moghadam, H. Vali, S. Laurent, and P. Stroeve, Irreversible changes in protein conformation due to interaction with superparamagnetic iron oxide nanoparticles. *Nanoscale* 3, 1127 **(2011)**.

72. P. Asuri, S. S. Karajanagi, A. A. Vertegel, J. S. Dordick, and R. S. Kane, Enhanced stability of enzymes adsorbed onto nanoparticles. *J. Nanosci. Nanotechnol.* 7, 4 **(2007)**.

73. M. Lv, E. Zhu, Y. Su, Q. Li, W. Li, Y. Zhao, and Q. Huang, Trypsin-gold nanoparticle conjugates: Binding, enzymatic activity, and stability. *Prep. Biochem. Biotechnol.* 39, 429 **(2009)**.

74. Y. Zhang, Z. Tang, J. Wang, H. Wu, C.-T. Lin, and Y. Lin, Apoferritin nanoparticle: A novel and biocompatible carrier for enzyme immobilization with enhanced activity and stability. *J. Mater. Chem.* 21, 17468 **(2011)**.

75. A. A. Vertegel, R. W. Siegel, and J. S. Dordick, Silica nanoparticle size influences the structure and enzymatic activity of adsorbed lysozyme. *Langmuir* 20, 6800 **(2004)**.

76. C. Palocci, L. Chronopoulou, I. Venditti, E. Cernia, M. Diociaiuti, I. Fratoddi, and M. V. Russo, Lipolytic enzymes with improved activity and selectivity upon adsorption on polymeric nanoparticles. *Biomacromolecules* 8, 3047 **(2007)**.

77. C. Yi, C.-C. Fong, Q. Zhang, S.-T. Lee, and M. Yang, The structure and function of ribonuclease A upon interacting with carbon nanotubes. *Nanotechnology* 19, 095102 **(2008)**.

78. L. Chronopoulou, G. Kamel, C. Sparago, F. Bordi, S. Lupi, M. Diociaiuti, and C. Palocci, Structure-activity relationships of Candida rugosa lipase immobilized on polylactic acid nanoparticles. *Soft Matter* 7, 2653 **(2011)**.

79. S. Linse, C. Cabaleiro-Lago, W.-F. Xue, I. Lynch, S. Lindman, E. Thulin, S. E. Radford, and K. A. Dawson, Nucleation of protein fibrillation by nanoparticles. *Proc. Natl. Acad. Sci. USA* 104, 8691 **(2007)**.

80. C. Cabaleiro-Lago, F. Quinlan-Pluck, I. Lynch, S. Lindman, A. M. Minogue, E. Thulin, D. M. Walsh, K. A. Dawson, and S. Linse, Inhibition of amyloid β protein fibrillation by polymeric nanoparticles. *J. Am. Chem. Soc.* 130, 15437 **(2008)**.

81. S. Deguchi, T. Yamazaki, S.-A. Mukai, R. Usami, and K. Horikoshi, Stabilization of C_{60} nanoparticles by protein adsorption and its implications for toxicity studies. *Chem. Res. Toxicol.* 20, 854 **(2007)**.

82. H. Nie, H. Wang, A. Cao, Z. Shi, S.-T. Yang, Y. Yuan, and Y. Liu, Diameter-selective dispersion of double-walled carbon nanotubes by lysozyme. *Nanoscale* 3, 970 **(2011)**.

83. F. Turci, E. Ghibaudi, M. Colonna, B. Boscolo, I. Fenoglio, and B. Fubini, An integrated approach to the study of the interaction between proteins and nanoparticles. *Langmuir* 26, 8336 **(2010)**.

84. E. Perevedentseva, P.-J. Cai, Y.-C. Chiu, and C.-L. Cheng, Characterizing protein activities on the lysozyme and nanodiamond complex prepared for bio applications. *Langmuir* 27, 1085 **(2010)**.

85. Y. Chang, S.-T. Yang, J.-H. Liu, E. Dong, Y. Wang, A. Cao, Y. Liu, and H. Wang, *In Vitro* toxicity evaluation of graphene oxide on A549 cells. *Toxicol. Lett.* 200, 201 **(2011)**.

86. G. Orts-Gil, K. Natte, D. Drescher, H. Bresch, A. Mantion, J. Kneipp, and W. sterle, Characterisation of silica nanoparticles prior to *in vitro* studies: From primary particles to agglomerates. *J. Nanopart. Res.* 13, 1593 **(2011)**.

87. X. Zhou, B. Wang, Y. Chen, Z. Mao, and C. Gao, Uptake of cerium oxide nanoparticles and their influences on functions of A549 cells. *J. Nanosci. Nanotechnol.* 13, 204 **(2013)**.

88. J.-H. Liu, S.-T. Yang, H. Wang, Y. Chang, A. Cao, and Y. Liu, Effect of size and dose on the biodistribution of graphene oxide in mice. *Nanomedicine* 7, 1801 **(2012)**.

89. J. Kim, J. Park, H. Kim, K. Singha, and W. J. Kim, Transfection and intracellular trafficking properties of carbon dot-gold nanoparticle molecular assembly conjugated with PEI-pDNA. *Biomaterials* 34, 7168 **(2013)**.

90. X. Xu, X. Wang, Y. Li, Y. Wang, and L. Yang, A large-scale association study for nanoparticle C_{60} uncovers mechanisms of nanotoxicity disrupting the native conformations of DNA/RNA. *Nucleic. Acids. Res.* 40, 7622 **(2012)**.

91. M. Zheng, A. Jagota, E. D. Semke, B. A. Diner, R. S. Mclean, S. R. Lustig, R. E. Richardson, and N. G. Tassi, DNA-assisted dispersion and separation of carbon nanotubes. *Nat. Mater.* 2, 338 **(2003)**.

92. Z. Wu, Z. Zhen, J.-H. Jiang, G.-L. Shen, and R.-Q. Yu, Terminal protection of small-molecule-linked DNA for sensitive electrochemical detection of protein binding via selective carbon nanotube assembly. *J. Am. Chem. Soc.* 131, 12325 **(2009)**.

93. A. Pershina, A. Sazonov, L. Ogorodova, Investigation of the interaction between DNA and cobalt ferrite nanoparticles by FTIR spectroscopy. *Russ. J. Bioorg. Chem.* 35, 607 **(2009)**.

94. A. Hekmat, A. A. Saboury, and A. Divsalar, The effects of silver nanoparticles and doxorubicin combination on DNA structure and its antiproliferative effect against T47D and MCF7 cell lines. *J. Biomed. Nanotechnol.* 8, 968 **(2012)**.

95. L. Guo, A. Von Dem Bussche, M. Buechner, A. Yan, A. B. Kane, and R. H. Hurt, Adsorption of essential micronutrients by carbon nanotubes and the implications for nanotoxicity testing. *Small* 4, 721 **(2008)**.

96. M. A. Creighton, J. R. Rangel-Mendez, J. Huang, A. B. Kane, and R. H. Hurt, Graphene-induced adsorptive and optical artifacts during *in vitro* toxicology assays. *Small* 9, 1921 **(2013)**.

97. M. Horie, K. Nishio, K. Fujita, S. Endoh, A. Miyauchi, Y. Saito, H. Iwahashi, K. Yamamoto, H. Murayama, H. Nakano, N. Nanashima, E. Niki, and Y. Yoshida, Protein adsorption of ultrafine metal oxide and its influence on cytotoxicity toward cultured cells. *Chem. Res. Toxicol.* 22, 543 **(2009)**.

98. J. Xu, H. Lv, S.-T. Yang, and J. Luo, Preparation of graphene adsorbents and their applications in water purification. *Rev. Inorg. Chem.* 33, 139 **(2013)**.

99. G. Zhao, T. Wen, C. Chen, and X. Wang, Synthesis of graphene-based nanomaterials and their application in energy-related and environmental-related areas. *RSC Adv.* 2, 9286 **(2012)**.

100. M. S. Mauter and M. Elimelech, Environmental applications of carbon-based nanomaterials. *Environ. Sci. Technol.* 42, 5843 **(2008)**.

101. D. Lin and B. Xing, Tannic acid adsorption and its role for stabilizing carbon nanotube suspensions. *Environ. Sci. Technol.* 42, 5917 **(2008)**.

102. D. Lin, N. Liu, K. Yang, L. Zhu, Y. Xu, and B. Xing, The effect of ionic strength and pH on the stability of tannic acid-facilitated carbon nanotube suspensions. *Carbon* 47, 2875 **(2009)**.

103. P. Cherukuri, C. J. Gannon, T. K. Leeuw, H. K. Schmidt, R. E. Smalley, S. A. Curley, and R. B. Weisman, Mammalian pharmacokinetics of carbon nanotubes using intrinsic near-infrared fluorescence. *Proc. Natl. Acad. Sci. USA* 103, 18882 **(2006)**.

104. A. P. Roberts, A. S. Mount, B. Seda, J. Souther, R. Qiao, S. Lin, P. C. Ke, A. M. Rao, and S. J. Klaine, *In vivo* biomodification of lipid-coated carbon nanotubes by *Daphnia magna*. *Environ. Sci. Technol.* 41, 3025 **(2007)**.

105. F. Moussa, M. Pressac, E. Ge, M. Hadchouel, F. Trivin, and H. Szwarc, *In vivo* reaction between [60] fullerene and vitamin A in mouse liver. *New J. Chem.* 22, 989 **(1998)**.

106. S.-T. Yang, H. Wang, M. J. Meziani, Y. Liu, X. Wang, and Y.-P. Sun, Biodefunctionalization of functionalized single-walled carbon nanotubes in mice. *Biomacromolecules* 10, 2009 **(2009)**.

107. A. Mahapatro and D. K. Singh, Biodegradable nanoparticles are excellent vehicle for site directed *in-vivo* delivery of drugs and vaccines. *J. Nanobiotechnol.* 9, 55 **(2011)**.

108. J. Panyam and V. Labhasetwar, Biodegradable nanoparticles for drug and gene delivery to cells and tissue. *Adv. Drug Deliver. Rev.* 64, 61 **(2012)**.

109. B. L. Allen, P. D. Kichambare, P. Gou, I. I. Vlasova, A. A. Kapralov, N. Konduru, V. E. Kagan, and A. Star, Biodegradation of single-walled carbon nanotubes through enzymatic catalysis. *Nano Lett.* 8, 3899 **(2008)**.

110. V. E. Kagan, N. V. Konduru, W. Feng, B. L. Allen, J. Conroy, Y. Volkov, I. I. Vlasova, N. A. Belikova, N. Yanamala, and A. Kapralov, Carbon nanotubes degraded by neutrophil myeloperoxidase induce less pulmonary inflammation. *Nat. Nanotechnol.* 5, 354 **(2010)**.

111. L. Zhang, E. J. Petersen, M. Y. Habteselassie, L. Mao, and Q. Huang, Degradation of multiwall carbon nanotubes by bacteria. *Environ. Pollut.* 181, 335 **(2013)**.

112. W. C. Chan and S. Nie, Quantum dot bioconjugates for ultrasensitive nonisotopic detection. *Science* 281, 2016 **(1998)**.

113. K. G. Li, J. T. Chen, S. S. Bai, X. Wen, S. Y. Song, Q. Yu, J. Li, and Y. Q. Wang, Intracellular oxidative stress and cadmium ions release induce cytotoxicity of unmodified cadmium sulfide quantum dots. *Toxicol. Vitro* 23, 1007 **(2009)**.

114. D. Kwon, M. J. Kim, C. Park, J. Park, K. Choi, and T. H. Yoon, *in vivo* biodegradation of colloidal quantum dots by a freshwater invertebrate, *Daphnia Magna*. *Aquat. Toxicol.* 114, 217 **(2012)**.

115. Y. Han, G. Xie, Z. Sun, Y. Mu, S. Han, Y. Xiao, N. Liu, H. Wang, C. Guo, and Z. Shi, Plasma kinetics and biodistribution of water-soluble CdTe quantum dots in mice: A comparison between Cd and Te. *J. Nanopart. Res.* 13, 5373 **(2011)**.

116. N. Liu, Y. Mu, Y. Chen, H. Sun, S. Han, M. Wang, H. Wang, Y. Li, Q. Xu, and P. Huang, Degradation of aqueous synthesized CdTe/ZnS quantum dots in mice: Differential blood kinetics and biodistribution of cadmium and tellurium. *Part. Fibre Toxicol.* 10, 37 **(2013)**.

117. E. Morelli, P. Cioni, M. Posarelli, and E. Gabellieri, Chemical stability of CdSe quantum dots in seawater and their effects on a marine microalga. *Aquat. Toxicol.* 122–123, 153 **(2012)**.

118. S.-T. Yang, J.-H. Liu, J. Wang, Y. Yuan, A. Cao, H. Wang, Y. Liu, and Y. Zhao, Cytotoxicity of zinc oxide nanoparticles: Importance of microenvironment. *J. Nanosci. Nanotechnol.* 10, 8638 **(2010)**.

119. P. Zhang, Y. Ma, Z. Zhang, X. He, J. Zhang, Z. Guo, R. Tai, Y. Zhao, and Z. Chai, Biotransformation of ceria nanoparticles in cucumber plants. *ACS Nano* 6, 9943 **(2012)**.

120. Y. Ma, X. He, P. Zhang, Z. Zhang, Z. Guo, R. Tai, Z. Xu, L. Zhang, Y. Ding, and Y. Zhao, Phytotoxicity and biotransformation of La_2O_3 nanoparticles in a terrestrial plant cucumber (*Cucumis sativus*). *Nanotoxicology* 5, 743 **(2011)**.

121. P. Zhang, Y. Ma, Z. Zhang, X. He, Z. Guo, R. Tai, Y. Ding, Y. Zhao, Z. Chai, Comparative toxicity of nanoparticulate/bulk Yb_2O_3 and $YbCl_3$ to cucumber (*Cucumis sativus*). *Environ. Sci. Technol.* 46, 1834 **(2012)**.

122. M. Levy, N. Luciani, D. Alloyeau, D. Elgrabli, V. Deveaux, C. Pechoux, S. Chat, G. Wang, N. Vats, and F. Gendron, Long term *in vivo* biotransformation of iron oxide nanoparticles. *Biomaterials* 32, 3988 **(2011)**.

123. S.-T. Yang, H. Wang, Y. Wang, Y. Wang, H. Nie, and Y. Liu, Removal of carbon nanotubes from aqueous environment with filter paper. *Chemosphere* 82, 621 **(2011)**.

124. S.-T. Yang, Y. Chang, H. Wang, G. Liu, S. Chen, Y. Wang, Y. Liu, and A. Cao, Folding/aggregation of graphene oxide and its application in Cu^{2+} removal. *J. Colloid Interface Sci.* 351, 122 **(2010)**.

125. T. Wang, J. Bai, X. Jiang, and G. U. Nienhaus, Cellular uptake of nanoparticles by membrane penetration: A study combining confocal microscopy with FTIR spectroelectrochemistry. *ACS Nano* 6, 1251 **(2012)**.

126. D. Pantarotto, J.-P. Briand, M. Prato, and A. Bianco, Translocation of bioactive peptides across cell membranes by carbon nanotubes. *Chem. Commun.* 16 **(2004)**.

127. T. G. Iversen, N. Frerker, and K. Sandvig, Uptake of ricinB-quantum dot nanoparticles by a macropinocytosis-like mechanism. *J. Nanobiotechnol.* 10, 1 **(2012)**.

128. H. Gao, Z. Yang, S. Zhang, S. Cao, S. Shen, Z. Pang, and X. Jiang, Ligand modified nanoparticles increases cell uptake, alters endocytosis and elevates glioma distribution and internalization. *Sci. Rep.* 3, 2534 **(2013)**.

129. J. Lee, M. Twomey, C. Machado, G. Gomez, M. Doshi, A. J. Gesquiere, and J. H. Moon, Caveolae-mediated endocytosis of conjugated polymer nanoparticles. *Macromol. Biosci.* 13, 913 **(2013)**.

130. J. Cheng, K. S. Fernando, L. M. Veca, Y.-P. Sun, A. I. Lamond, Y. W. Lam, and S. H. Cheng, Reversible accumulation of PEGylated single-walled carbon nanotubes in the mammalian nucleus. *ACS Nano* 2, 2085 **(2008)**.

131. Y. Liu, W. Li, F. Lao, Y. Liu, L. Wang, R. Bai, Y. Zhao, and C. Chen, Intracellular dynamics of cationic and anionic polystyrene nanoparticles without direct interaction with mitotic spindle and chromosomes. *Biomaterials* 32, 8291 **(2011)**.

132. X. Liu, N. Huang, H. Li, Q. Jin, and J. Ji, Surface and size effects on cell interaction of gold nanoparticles with both phagocytic and nonphagocytic cells. *Langmuir* 29, 9138 **(2013)**.

133. J. Wang, Z. Teng, Y. Tian, T. Fang, J. Ma, J. Sun, F. Zhu, J. Wu, X. Wang, N. Yang, X. Zhou, S. Yun, and G. Lu, Increasing cellular uptake of mesoporous silica nanoparticles in human embryonic kidney cell line 293T cells by using lipofectamine 2000. *J. Biomed. Nanotechnol.* 9, 1882 **(2013)**.

134. Z. Wang, S. Zhang, G. Qu, and S. Liu, The capability of quantum dots in crossing the placental barrier and the potential influence on erythrocytes. *J. Nanosci. Nanotechnol.* 13, 6529 **(2013)**.

135. K. Yang, J. Wan, S. Zhang, Y. Zhang, S.-T. Lee, and Z. Liu, *In vivo* pharmacokinetics, long-term biodistribution, and toxicology of PEGylated graphene in mice. *ACS Nano* 5, 516 **(2010)**.

136. X. Deng, G. Jia, H. Wang, H. Sun, X. Wang, S. Yang, T. Wang, and Y. Liu, Translocation and fate of multi-walled carbon nanotubes *in vivo*. *Carbon* 45, 1419 **(2007)**.

137. M. Lundqvist, J. Stigler, T. Cedervall, T. Berggard, M. B. Flanagan, I. Lynch, G. Elia, and K. Dawson, The evolution of the protein corona around nanoparticles: A test study. *ACS Nano* 5, 7503 **(2011)**.

138. M. Lundqvist, J. Stigler, G. Elia, I. Lynch, T. Cedervall, and K. A. Dawson, Nanoparticle size and surface properties determine the protein corona with possible implications for biological impacts. *Proc. Natl. Acad. Sci. USA* 105, 14265 **(2008)**.

139. X. Deng, S. Yang, H. Nie, H. Wang, and Y. Liu, A generally adoptable radiotracing method for tracking carbon nanotubes in animals. *Nanotechnology* 19, 075101 **(2008)**.

140. S.-T. Yang, K. Fernando, J. H. Liu, J. Wang, H. F. Sun, Y. Liu, M. Chen, Y. Huang, X. Wang, and H. Wang, Covalently PEGylated carbon nanotubes with stealth character *in vivo*. *Small* 4, 940 **(2008)**.

141. S.-T. Yang, W. Guo, Y. Lin, X.-Y. Deng, H.-F. Wang, H.-F. Sun, Y.-F. Liu, X. Wang, W. Wang, and M. Chen, Biodistribution of pristine single-walled carbon nanotubes *in vivo*. *J. Phys. Chem. C* 111, 17761 **(2007)**.

142. M. L. Schipper, G. Iyer, A. L. Koh, Z. Cheng, Y. Ebenstein, A. Aharoni, S. Keren, L. A. Bentolila, J. Li, J. Rao, X. Chen, U. Banin, A. M. Wu, R. Sinclair, S. Weiss, and S. S. Gambhir, Particle size, surface coating, and PEGylation influence the biodistribution of quantum dots in living mice. *Small* 5, 126 **(2009)**.

143. Y. Geng, P. Dalhaimer, S. Cai, R. Tsai, M. Tewari, T. Minko, and D. E. Discher, Shape effects of filaments versus spherical particles in flow and drug delivery. *Nat. Nanotechnol.* 2, 249 **(2007)**.

144. X. Huang, L. Li, T. Liu, N. Hao, H. Liu, D. Chen, and F. Tang, The shape effect of mesoporous silica nanoparticles on biodistribution, clearance, and biocompatibility *in vivo*. *ACS Nano* 5, 5390 **(2011)**.

145. Y. Akiyama, T. Mori, Y. Katayama, and T. Niidome, Conversion of rod-shaped gold nanoparticles to spherical forms and their effect on biodistribution in tumor-bearing mice. *Nanoscale Res. Lett.* 7, 1 **(2012)**.

146. W.-Y. Liao, H.-J. Li, M.-Y. Chang, A. C. Tang, A. S. Hoffman, and P. C. Hsieh, Comprehensive characterizations of nanoparticle biodistribution following systemic injection in mice. *Nanoscale* 5, 11079 **(2013)**.

147. X.-L. Chang, L. Ruan, S.-T. Yang, B. Sun, C. Guo, L. Zhou, J. Dong, H. Yuan, G. Xing, and Y. Zhao, Quantification of carbon nanomaterials *in vivo*: Direct stable isotope labeling on the skeleton of fullerene C_{60}. *Environ. Sci.: Nano* 1, 64 **(2014)**.

148. H. S. Choi, W. Liu, P. Misra, E. Tanaka, J. P. Zimmer, B. I. Ipe, M. G. Bawendi, and J. V. Frangioni, Renal clearance of quantum dots. *Nat. Biotechnol.* 25, 1165 **(2007)**.

149. Z. Liu, C. Davis, W. Cai, L. He, X. Chen, and H. Dai, Circulation and long-term fate of functionalized, biocompatible single-walled carbon nanotubes in mice probed by Raman spectroscopy. *Proc. Natl. Acad. Sci. USA* 105, 1410 **(2008)**.

150. H. Wang, J. Wang, X. Deng, H. Sun, Z. Shi, Z. Gu, Y. Liu, and Y. Zhao, Biodistribution of carbon single-wall carbon nanotubes in mice. *J. Nanosci. Nanotechnol.* 4, 1019 **(2004)**.

151. S. Kittler, C. Greulich, J. Diendorf, M. Koller, and M. Epple, Toxicity of silver nanoparticles increases during storage because of slow dissolution under release of silver ions. *Chem. Mater.* 22, 4548 **(2010)**.

152. M. S. Park, J. Park, S. K. Jeon, and T. H. Yoon, The effects of sedimentation and dissolution on the cytotoxicity of ag nanoparticles. *J. Nanosci. Nanotechnol.* 13, 7264 **(2013)**.

153. K.-T. Yong, W.-C. Law, R. Hu, L. Ye, L. Liu, M. T. Swihart, and P. N. Prasad, Nanotoxicity assessment of quantum dots: From cellular to primate studies. *Chem. Soc. Rev.* 42, 1236 **(2013)**.

154. T. J. Brunner, P. Wick, P. Manser, P. Spohn, R. N. Grass, L. K. Limbach, A. Bruinink, and W. J. Stark, *In vitro* cytotoxicity of oxide nanoparticles: Comparison to asbestos, silica, and the effect of particle solubility. *Environ. Sci. Technol.* 40, 4374 **(2006)**.

155. X. Deng, Q. Luan, W. Chen, Y. Wang, M. Wu, H. Zhang, and Z. Jiao, Nanosized zinc oxide particles induce neural stem cell apoptosis. *Nanotechnology* 20, 115101 **(2009)**.

156. T. Xia, M. Kovochich, M. Liong, L. MäDler, B. Gilbert, H. Shi, J. I. Yeh, J. I. Zink, and A. E. Nel, Comparison of the mechanism of toxicity of zinc oxide and cerium oxide nanoparticles based on dissolution and oxidative stress properties. *ACS Nano* 2, 2121 **(2008)**.

157. M. S. Hull, A. J. Kennedy, J. A. Steevens, A. J. Bednar, J. Weiss, Charles A, and P. J. Vikesland, Release of metal impurities from carbon nanomaterials influences aquatic toxicity. *Environ. Sci. Technol.* 43, 4169 **(2009)**.

158. E. Aldieri, I. Fenoglio, F. Cesano, E. Gazzano, G. Gulino, D. Scarano, A. Attanasio, G. Mazzucco, D. Ghigo, and B. Fubini, The role of iron impurities in the toxic effects exerted by short multiwalled carbon nanotubes (MWCNT) in murine alveolar macrophages. *J. Toxicol. Environ. Health A* 76, 1056 **(2013)**.

159. C. Ge, Y. Li, J.-J. Yin, Y. Liu, L. Wang, Y. Zhao, and C. Chen, The contributions of metal impurities and tube structure to the toxicity of carbon nanotube materials. *NPG Asia Mater.* 4, e32 **(2012)**.

160. X. Liu, L. Guo, D. Morris, A. B. Kane, and R. H. Hurt, Targeted removal of bioavailable metal as a detoxification strategy for carbon nanotubes. *Carbon* 46, 489 **(2008)**.

161. D. Gavello, I. Fenoglio, B. Fubini, F. Cesano, F. Premoselli, A. Renna, E. Carbone, and V. Carabelli, Inhibition of catecholamine secretion by iron-rich and iron-deprived multiwalled carbon nanotubes in chromaffin cells. *Neurotoxicology* 39, 84 **(2013)**.

162. C. M. Goodman, C. D. Mccusker, T. Yilmaz, and V. M. Rotello, Toxicity of gold nanoparticles functionalized with cationic and anionic side chains. *Bioconjugate Chem.* 15, 897 **(2004)**.

163. T. D. Mai, F. D'orlye, C. Menager, A. Varenne, and J.-M. Siaugue, Red blood cells decorated with functionalized core-shell magnetic nanoparticles: Elucidation of the adsorption mechanism. *Chem. Commun.* 49, 5393 **(2013)**.

164. I. Sovadinova, E. F. Palermo, R. Huang, L. M. Thoma, and K. Kuroda, Mechanism of polymer-induced hemolysis: Nanosized pore formation and osmotic lysis. *Biomacromolecules* 12, 260 **(2010)**.

165. M. L. Zhao, D. J. Li, L. Yuan, Y. C. Yue, H. Liu, and X. Sun, Differences in cytocompatibility and hemocompatibility between carbon nanotubes and nitrogen-doped carbon nanotubes. *Carbon* 49, 3125 **(2011)**.

166. A. Yildirim, E. Ozgur, and M. Bayindir, Impact of mesoporous silica nanoparticle surface functionality on hemolytic activity, thrombogenicity and non-specific protein adsorption. *J. Mater. Chem. B* 1, 1909 **(2013)**.

167. J. Choi, V. Reipa, V. M. Hitchins, P. L. Goering, and R. A. Malinauskas, Physicochemical characterization and *in vitro* hemolysis evaluation of silver nanoparticles. *Toxicol. Sci.* 123, 133 **(2011)**.

168. J. Chen, J. A. Hessler, K. Putchakayala, B. K. Panama, D. P. Khan, S. Hong, D. G. Mullen, S. C. Dimaggio, A. Som, G. N. Tew, A. N. Lopatin, J. R. Baker, M. M. B. Holl, and B. G. Orr, Cationic nanoparticles induce nanoscale disruption in living cell plasma membranes. *J. Phys. Chem. B* 113, 11179 **(2009)**.

169. P. Ruenraroengsak, P. Novak, D. Berhanu, A. J. Thorley, E. Valsami-Jones, J. Gorelik, Y. E. Korchev, and T. D. Tetley, Respiratory epithelial cytotoxicity and membrane damage (holes) caused by amine-modified nanoparticles. *Nanotoxicology* 6, 94 **(2012)**.

170. G. Grandinetti, A. E. Smith, and T. M. Reineke, Membrane and nuclear permeabilization by polymeric pDNA vehicles: Efficient method for gene delivery or mechanism of cytotoxicity? *Mol. Pharm.* 9, 523 **(2012)**.

171. M. R. De Planque, S. Aghdaei, T. Roose, and H. Morgan, Electrophysiological characterization of membrane disruption by nanoparticles. *ACS Nano* 5, 3599 **(2011)**.

172. P. R. Lockman, J. M. Koziara, R. J. Mumper, and D. D. Allen, Nanoparticle surface charges alter blood-brain barrier integrity and permeability. *J. Drug Target.* 12, 635 **(2004)**.

173. J. Geys, A. Nemmar, E. Verbeken, E. Smolders, M. Ratoi, M. F. Hoylaerts, B. Nemery, and P. H. Hoet, Acute toxicity and prothrombotic effects of quantum dots: Impact of surface charge. *Environ. Health Persp.* 116, 1607 (**2008**).

174. K. Greish, G. Thiagarajan, H. Herd, R. Price, H. Bauer, D. Hubbard, A. Burckle, S. Sadekar, T. Yu, A. Anwar, A. Ray, and H. Ghandehari, Size and surface charge significantly influence the toxicity of silica and dendritic nanoparticles. *Nanotoxicology* 6, 713 (**2012**).

175. N. M. Schaeublin, L. K. Braydich-Stolle, A. M. Schrand, J. M. Miller, J. Hutchison, J. J. Schlager, and S. M. Hussain, Surface charge of gold nanoparticles mediates mechanism of toxicity. *Nanoscale* 3, 410 (**2011**).

176. D. Bhattacharya, C. R. Santra, A. N. Ghosh, and P. Karmakar, Differential toxicity of rod and spherical zinc oxide nanoparticles on human peripheral blood mononuclear cells. *J. Biomed. Nanotechnol.* 10, 707 (**2014**).

177. D. Guo, Y. Zhao, Y. Zhang, Q. Wang, Z. Huang, Q. Ding, Z. Guo, X. Zhou, L. Zhu, and N. Gu, The cellular uptake and cytotoxic effect of silver nanoparticles on chronic myeloid leukemia cells. *J. Biomed. Nanotechnol.* 10, 669 (**2014**).

178. R. Wahab, S. Dwivedi, A. Umar, S. Singh, I. H. Hwang, H.-S. Shin, J. Musarrat, A. A. Al-Khedhairy, and Y.-S. Kim, ZnO nanoparticles induce oxidative stress in cloudman S91 melanoma cancer cells. *J. Biomed. Nanotechnol.* 9, 441 (**2013**).

179. J. Wang, J. Ma, L. Dong, Y. Hou, X. Jia, X. Niu, and Y. Fan, Effect of anatase TiO_2 nanoparticles on the growth of RSC-364 rat synovial cell. *J. Nanosci. Nanotechnol.* 13, 3874 (**2013**).

180. B. Wang, J.-J. Yin, X. Zhou, I. Kurash, Z. Chai, Y. Zhao, and W. Feng, Physicochemical origin for free radical generation of iron oxide nanoparticles in biomicroenvironment: Catalytic activities mediated by surface chemical states. *J. Phys. Chem. C* 117, 383 (**2013**).

181. A. L. Rose, Effect of dissolved natural organic matter on the kinetics of ferrous iron oxygenation in seawater. *Environ. Sci. Technol.* 37, 4877 (**2003**).

182. M. Culcasi, L. Benameur, A. Mercier, C. Lucchesi, H. Rahmouni, A. Asteian, G. Casano, A. Botta, H. Kovacic, and S. Pietri, EPR spin trapping evaluation of ROS production in human fibroblasts exposed to cerium oxide nanoparticles: Evidence for NADPH oxidase and mitochondrial stimulation. *Chem. Biol. Interact.* 199, 161 (**2012**).

183. M. T. Zhu, B. Wang, Y. Wang, L. Yuan, H. J. Wang, M. Wang, H. Ouyang, Z. F. Chai, W. Y. Feng, and Y. L. Zhao, Endothelial dysfunction and inflammation induced by iron oxide nanoparticle exposure: Risk factors for early atherosclerosis. *Toxicol. Lett.* 203, 162 (**2011**).

184. W. Fan, X. Wang, M. Cui, D. Zhang, Y. Zhang, T. Yu, and L. Guo, Differential oxidative stress of octahedral and cubic Cu_2O micro/nanocrystals to *Daphnia magna*. *Environ. Sci. Technol.* 46, 10255 (**2012**).

185. P.-J. Chen, W.-L. Wu, and K. C.-W. Wu, The zerovalent iron nanoparticle causes higher developmental toxicity than its oxidation products in early life stages of medaka fish. *Water Res.* 47, 3899 (**2013**).

186. A. Sarkar, M. Ghosh, and P. C. Sil, Nanotoxicity: Oxidative stress mediated toxicity of metal and metal oxide nanoparticles. *J. Nanosci. Nanotechnol.* 14, 730 (**2014**).

187. A. Ambrosi, M. Pumera, Regulatory peptides are susceptible to oxidation by metallic impurities within carbon nanotubes. *Chem. Eur. J.* 16, 1786 (**2010**).

188. W. Xu, K. E. Dana, and W. A. Mitch, Black carbon-mediated destruction of nitroglycerin and rdx by hydrogen sulfide. *Environ. Sci. Technol.* 44, 6409 (**2010**).

189. L. Kong and R. G. Zepp, Production and consumption of reactive oxygen species by fullerenes. *Environ. Toxicol. Chem.* 31, 136 (**2012**).

190. W. Zhang, C. Wang, Z. Li, Z. Lu, Y. Li, J.-J. Yin, Y.-T. Zhou, X. Gao, Y. Fang, G. Nie, and Y. Zhao, Unraveling stress-induced toxicity properties of graphene oxide and the underlying mechanism. *Adv. Mater.* 24, 5391 (**2012**).

191. J. Kim, Y. Park, T. H. Yoon, C. S. Yoon, and K. Choi, Phototoxicity of CdSe/ZnSe quantum dots with surface coatings of 3-mercaptopropionic acid or tri-*n*-octylphosphine oxide/gum arabic in *Daphnia magna* under environmentally relevant UV-B light. *Aquat. Toxicol.* 97, 116 (**2010**).

192. I. L. Christensen, Y.-P. Sun, and P. Juzenas, Carbon dots as antioxidants and prooxidants. *J. Biomed. Nanotechnol.* 7, 667 (**2011**).

193. T. Xia, M. Kovochich, J. Brant, M. Hotze, J. Sempf, T. Oberley, C. Sioutas, J. I. Yeh, M. R. Wiesner, and A. E. Nel, Comparison of the abilities of ambient and manufactured nanoparticles to induce cellular toxicity according to an oxidative stress paradigm. *Nano Lett.* 6, 1794 (**2006**).

194. C.-C. Wang, S. Wang, Q. Xia, W. He, J.-J. Yin, P. P. Fu, and J.-H. Li, Phototoxicity of zinc oxide nanoparticles in HaCaT keratinocytes-generation of oxidative DNA damage during UVA and visible light irradiation. *J. Nanosci. Nanotechnol.* 13, 3880 (**2013**).

195. P. V. Asharani, G. Low Kah Mun, M. P. Hande, and S. Valiyaveettil, Cytotoxicity and genotoxicity of silver nanoparticles in human cells. *ACS Nano* 3, 279 (**2008**).

196. K. B. Holt and A. J. Bard, Interaction of silver(I) ions with the respiratory chain of *Escherichia coli*:? An electrochemical and scanning electrochemical microscopy study of the antimicrobial mechanism of micromolar Ag^+. *Biochemistry* 44, 13214 (**2005**).

197. H. Zhang, Z. Ji, T. Xia, H. Meng, C. Low-Kam, R. Liu, S. Pokhrel, S. Lin, X. Wang, Y.-P. Liao, M. Wang, L. Li, R. Rallo, R. Damoiseaux, D. Telesca, L. Mädler, Y. Cohen, J. I. Zink, and A. E. Nel, Use of metal oxide nanoparticle band gap to develop a predictive paradigm for oxidative stress and acute pulmonary inflammation. *ACS Nano* 6, 4349 (**2012**).

198. C. Dostert, V. Pétrilli, R. Van Bruggen, C. Steele, B. T. Mossman, and J. Tschopp, Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. *Science* 320, 674 (**2008**).

199. H. Mohan, D. K. Palit, J. P. Mittal, L. Y. Chiang, K.-D. Asmus, and D. M. Guldi, Excited states and electron transfer reactions of $C_{60}(OH)_{18}$in aqueous solution. *J. Chem. Soc., Faraday Trans.* 94, 359 (**1998**).

200. S. Ye, Y. Wang, F. Jiao, H. Zhang, C. Lin, Y. Wu, and Q. Zhang, The role of NADPH oxidase in multi-walled carbon nanotubes-induced oxidative stress and cytotoxicity in human macrophages. *J. Nanosci. Nanotechnol.* 11, 3773 (**2011**).

201. H. Nabeshi, T. Yoshikawa, K. Matsuyama, Y. Nakazato, S. Tochigi, S. Kondoh, T. Hirai, T. Akase, K. Nagano, Y. Abe, Y. Yoshioka, H. Kamada, N. Itoh, S. Tsunoda, and Y. Tsutsumi, Amorphous nanosilica induce endocytosis-dependent ROS generation and DNA damage in human keratinocytes. *Part. Fibre Toxicol.* 8, 1 (**2011**).

202. J. Rauch, W. Kolch, S. Laurent, and M. Mahmoudi, Big signals from small particles: Regulation of cell signaling pathways by nanoparticles. *Chem. Rev.* 113, 3391 (**2013**).

203. D. Guo, H. Bi, Q. Wu, D. Wang, and Y. Cui, Zinc oxide nanoparticles induce rat retinal ganglion cell damage through Bcl-2, Caspase-9 and Caspase-12 pathways. *J. Nanosci. Nanotechnol.* 13, 3769 (**2013**).

204. V. Karabanovas, Z. Zitkus, D. Kuciauskas, R. Rotomskis, and M. Valius, Surface properties of quantum dots define their cellular endocytic routes, mitogenic stimulation and suppression of cell migration. *J. Biomed. Nanotechnol.* 10, 775 (**2014**).

205. Y. Pan, H. Ding, L. Qin, X. Zhao, J. Cai, and B. Du, Gold nanoparticles induce nanostructural reorganization of VEGFR2 to repress angiogenesis. *J. Biomed. Nanotechnol.* 9, 1746 (**2013**).

206. Y. Li, Y. Liu, Y. Fu, T. Wei, L. Le Guyader, G. Gao, R. S. Liu, Y. Z. Chang, and C. Chen, The triggering of apoptosis in macrophages

by pristine graphene through the MAPK and TGF-beta signaling pathways. *Biomaterials* 33, 402 **(2012)**.

207. J. Y. Roh, H. J. Eom, and J. Choi, Involvement of *Caenohabditis elegans* MAPK signaling pathways in oxidative stress response induced by silver nanoparticles exposure. *Toxicol. Res.* 28, 19 **(2012)**.

208. H. J. Eom and J. Choi, Oxidative stress of CeO_2 nanoparticles via p38-Nrf-2 signaling pathway in human bronchial epithelial cell, Beas-2B. *Toxicol. Lett.* 187, 77 **(2009)**.

209. G. Cheng, W. Guo, L. Han, E. Chen, L. Kong, L. Wang, W. Ai, N. Song, H. Li, and H. Chen, Cerium oxide nanoparticles induce cytotoxicity in human hepatoma SMMC-7721 cells via oxidative stress and the activation of MAPK signaling pathways. *Toxicol. Vitro* 27, 1082 **(2013)**.

210. Y. Wang, F. Yang, H. X. Zhang, X. Y. Zi, X. H. Pan, F. Chen, W. D. Luo, J. X. Li, H. Y. Zhu, and Y. P. Hu, Cuprous oxide nanoparticles inhibit the growth and metastasis of melanoma by targeting mitochondria. *Cell Death Dis.* 4, e783 **(2013)**.

211. M. Ding, E. R. Kisin, J. Zhao, L. Bowman, Y. Lu, B. Jiang, S. Leonard, V. Vallyathan, V. Castranova, A. R. Murray, B. Fadeel, and A. A. Shvedova, Size-dependent effects of tungsten carbide-cobalt particles on oxygen radical production and activation of cell signaling pathways in murine epidermal cells. *Toxicol. Appl. Pharm.* 241, 260 **(2009)**.

212. R. P. Nishanth, R. G. Jyotsna, J. J. Schlager, S. M. Hussain, and P. Reddanna, Inflammatory responses of RAW 264.7 macrophages upon exposure to nanoparticles: Role of ROS-NFkappaB signaling pathway. *Nanotoxicology* 5, 502 **(2011)**.

213. S. Akhtar, B. Chandrasekhar, S. Attur, M. H. Yousif, and I. F. Benter, On the nanotoxicity of PAMAM dendrimers: Superfect stimulates the EGFR-ERK1/2 signal transduction pathway via an oxidative stress-dependent mechanism in HEK 293 cells. *Int. J. Pharm.* 448, 239 **(2013)**.

214. Y. H. Ma, C. P. Huang, J. S. Tsai, M. Y. Shen, Y. K. Li, and L. Y. Lin, Water-soluble germanium nanoparticles cause necrotic cell death and the damage can be attenuated by blocking the transduction of necrotic signaling pathway. *Toxicol. Lett.* 207, 258 **(2011)**.

215. L. Meng, R. Chen, A. Jiang, L. Wang, P. Wang, C. Z. Li, R. Bai, Y. Zhao, H. Autrup, and C. Chen, Short multiwall carbon nanotubes promote neuronal differentiation of PC12 cells via up-regulation of the neurotrophin signaling pathway. *Small* 9, 1786 **(2013)**.

216. K. Yang, W. Cao, X. Hao, X. Xue, J. Zhao, J. Liu, Y. Zhao, J. Meng, B. Sun, J. Zhang, and X. J. Liang, Metallofullerene nanoparticles promote osteogenic differentiation of bone marrow stromal cells through BMP signaling pathway. *Nanoscale* 5, 1205 **(2013)**.

217. P. Wang, X. Nie, Y. Wang, Y. Li, C. Ge, L. Zhang, L. Wang, R. Bai, Z. Chen, Y. Zhao, and C. Chen, Multiwall carbon nanotubes mediate macrophage activation and promote pulmonary fibrosis through TGF-beta/Smad signaling pathway. *Small* 9, 3799 **(2013)**.

218. M. Zhu, X. Tian, X. Song, Y. Li, Y. Tian, Y. Zhao, and G. Nie, Nanoparticle-induced exosomes target antigen-presenting cells to initiate Th1-type immune activation. *Small* 8, 2841 **(2012)**.

219. J. A. Khan, T. K. Mandal, T. K. Das, Y. Singh, B. Pillai, and S. Maiti, Magnetite (Fe_3O_4) nanocrystals affect the expression of genes involved in the TGF-beta signalling pathway. *Mol. Biosyst.* 7, 1481 **(2011)**.

220. K. Matsumoto and N. Shimizu, Activation of the phospholipase C signaling pathway in nerve growth factor-treated neurons by carbon nanotubes. *Biomaterials* 34, 5988 **(2013)**.

221. G. F. Guidetti, A. Consonni, L. Cipolla, P. Mustarelli, C. Balduini, and M. Torti, Nanoparticles induce platelet activation *in vitro* through stimulation of canonical signalling pathways. *Nanomedicine* 8, 1329 **(2012)**.

222. C. Li, H. Liu, Y. Sun, H. Wang, F. Guo, S. Rao, J. Deng, Y. Zhang, Y. Miao, C. Guo, J. Meng, X. Chen, L. Li, D. Li, H. Xu, H. Wang, B. Li, and C. Jiang, PAMAM nanoparticles promote acute lung injury by inducing autophagic cell death through the Akt-TSC2-mTOR signaling pathway. *J. Mol. Cell Biol.* 1, 37 **(2009)**.

223. B. Song, H. Yuan, S. V. Pham, C. J. Jameson, and S. Murad, Nanoparticle permeation induces water penetration, ion transport, and lipid flip-flop. *Langmuir* 28, 16989 **(2012)**.

224. S. Liu, L. Wei, L. Hao, N. Fang, M. W. Chang, R. Xu, Y. Yang, and Y. Chen, Sharper and faster "nano darts" kill more bacteria: A study of antibacterial activity of individually dispersed pristine single-walled carbon nanotube. *ACS Nano* 3, 3891 **(2009)**.

225. S. Liu, T. H. Zeng, M. Hofmann, E. Burcombe, J. Wei, R. Jiang, J. Kong, and Y. Chen, Antibacterial activity of graphite, graphite oxide, graphene oxide, and reduced graphene oxide: Membrane and oxidative stress. *ACS Nano* 5, 6971 **(2011)**.

226. S.-T. Yang, X. Wang, H. Wang, F. Lu, P. G. Luo, L. Cao, M. J. Meziani, J.-H. Liu, Y. Liu, M. Chen, Y. Huang, and Y.-P. Sun, Carbon dots as nontoxic and high-performance fluorescence imaging agents. *J. Phys. Chem. C* 113, 18110 **(2009)**.

227. A. Dubavik, E. Sezgin, V. Lesnyak, N. Gaponik, P. Schwille, and A. Eychmüller, Penetration of amphiphilic quantum dots through model and cellular plasma membranes. *ACS Nano* 6, 2150 **(2012)**.

228. P. Gkeka, L. Sarkisov, and P. Angelikopoulos, Homogeneous hydrophobic–hydrophilic surface patterns enhance permeation of nanoparticles through lipid membranes. *J. Phys. Chem. Lett.* 4, 1907 **(2013)**.

229. Y. Cho, R. Shi, and R. Ben Borgens, Chitosan nanoparticle-based neuronal membrane sealing and neuroprotection following acrolein-induced cell injury. *J. Biol. Eng.* 4, 1 **(2010)**.

230. S. L. Fiedler and A. Violi, Simulation of nanoparticle permeation through a lipid membrane. *Biophys. J.* 99, 144 **(2010)**.

231. A. D. Phan, T. X. Hoang, T. L. Phan, and L. M. Woods, Repulsive interactions of a lipid membrane with graphene in composite materials. *J. Chem. Phys.* 139, 184703 **(2013)**.

232. A. Alexeev, W. E. Uspal, and A. C. Balazs, Harnessing janus nanoparticles to create controllable pores in membranes. *ACS Nano* 2, 1117 **(2008)**.

233. B. Jing and Y. Zhu, Disruption of supported lipid bilayers by semi-hydrophobic nanoparticles. *J. Am. Chem. Soc.* 133, 10983 **(2011)**.

234. Y. Tu, M. Lv, P. Xiu, T. Huynh, M. Zhang, M. Castelli, Z. Liu, Q. Huang, C. Fan, H. Fang, and R. Zhou, Destructive extraction of phospholipids from *Escherichia coli* membranes by graphene nanosheets. *Nat. Nanotechnol.* 8, 594 **(2013)**.

235. T. B. Henry, S. J. Wileman, H. Boran, and P. Sutton, Association of Hg^{2+} with aqueous $(C_{60})n$ aggregates facilitates increased bioavailability of Hg^{2+} in zebrafish (*Danio rerio*). *Environ. Sci. Technol.* 47, 9997 **(2013)**.

236. C. Tan, W.-H. Fan, and W.-X. Wang, Role of Titanium dioxide nanoparticles in the elevated uptake and retention of cadmium and zinc in *Daphnia magna*. *Environ. Sci. Technol.* 46, 469 **(2011)**.

237. M. Guo, X. Xu, X. Yan, S. Wang, S. Gao, and S. Zhu, *In vivo* biodistribution and synergistic toxicity of silica nanoparticles and cadmium chloride in mice. *J. Hazard. Mater.* 260, 780 **(2013)**.

238. Z. G. Yu, W. X. Wang, Influences of ambient carbon nanotubes on toxic metals accumulation in *Daphnia magna*. *Water Res.* 47, 4179 **(2013)**.

239. Y. Zhu, X. Zhang, J. Zhu, Q. Zhao, Y. Li, W. Li, C. Fan, and Q. Huang, Cytotoxicity of phenol red in toxicity assays for carbon nanoparticles. *Int. J. Mol. Sci.* 13, 12336 **(2012)**.

240. D. Zheng, N. Wang, X. Wang, Y. Tang, L. Zhu, Z. Huang, H. Tang, Y. Shi, Y. Wu, M. Zhang, and B. Lu, Effects of the interaction of TiO_2 nanoparticles with bisphenol A on their physicochemical properties and *in vitro* toxicity. *J. Hazard. Mater.* 199–200, 426 **(2012)**.

241. W. Fan, M. Cui, H. Liu, C. Wang, Z. Shi, C. Tan, and X. Yang, Nano-TiO_2 enhances the toxicity of copper in natural water to *Daphnia magna*. *Environ. Pollut.* 159, 729 **(2011)**.

242. J. Hu, D. Wang, J. Wang, and J. Wang, Toxicity of lead on *Ceriodaphnia dubia* in the presence of nano-CeO_2 and nano-TiO_2. *Chemosphere* 89, 536 **(2012)**.

243. W. Zhang, Y. Miao, K. Lin, L. Chen, Q. Dong, and C. Huang, Toxic effects of copper ion in zebrafish in the joint presence of CdTe QDs. *Environ. Pollut.* 176, 158 **(2013)**.

244. K. T. Kim, A. J. Edgington, S. J. Klaine, J. W. Cho, and S. D. Kim, Influence of multiwalled carbon nanotubes dispersed in natural organic matter on speciation and bioavailability of copper. *Environ. Sci. Technol.* 43, 8979 **(2009)**.

245. D. Wang, J. Hu, B. E. Forthaus, and J. Wang, Synergistic toxic effect of nano-Al_2O_3 and As(V) on *Ceriodaphnia dubia. Environ. Pollut.* 159, 3003 **(2011)**.

246. D. Wang, J. Hu, D. R. Irons, and J. Wang, Synergistic toxic effect of nano-TiO_2 and As(V) on *Ceriodaphnia dubia*. *Sci. Total. Environ.* 409, 1351 **(2011)**.

247. R. Zhang, Y. Niu, Y. Li, C. Zhao, B. Song, Y. Li, and Y. Zhou, Acute toxicity study of the interaction between titanium dioxide nanoparticles and lead acetate in mice. *Environ. Toxicol. Phar.* 30, 52 **(2010)**.

248. H. Chen, B. Wang, D. Gao, M. Guan, L. Zheng, H. Ouyang, Z. Chai, Y. Zhao, and W. Feng, Broad-spectrum antibacterial activity of carbon nanotubes to human gut bacteria. *Small* 9, 2735 **(2013)**.

249. Y. Li, Y. Zhou, H.-Y. Wang, S. Perrett, Y. Zhao, Z. Tang, and G. Nie, Chirality of glutathione surface coating affects the cytotoxicity of quantum dots. *Angew. Chem. Int. Edit.* 50, 5860 **(2011)**.