Nanometallomics: an emerging field studying the biological effects of metal-related nanomaterials

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Metallomics, focusing on the global and systematic understanding of the metal uptake, trafficking, role and excretion in biological systems, has attracted more and more attention. Metal-related nanomaterials, including metallic and metal-containing nanomaterials, have unique properties compared to their micro-scaled counterparts and therefore require special attention. The small size effect, surface effect, and quantum size effect directly influence the physicochemical properties of nanostructured materials and their fate and behavior in biota. However, to our knowledge, the metallomics itself did not touch this special category of materials yet. Therefore, the term “nanometallomics” is proposed and the systematic study on the absorption, distribution, metabolism, excretion (ADME) behavior of metal-related nanomaterials in biological systems and their interactions with genes, proteins and other biomolecules will be reviewed. The ADME behavior of metal-related nanomaterials in the biological systems is influenced by their physicochemical properties, the exposure route, and the microenvironment of the deposition site. Nanomaterials may not only interact directly or indirectly with genes, proteins and other molecules to cause DNA damage, genotoxicity, immunotoxicity, and cytotoxicity, but also stimulate the immune responses, circumvent tumor resistance and inhibit tumor metastasis. Nanometallomics needs to be integrated with other omics sciences, such as genomics, proteomics and metabolomics, to explore the biomedical data and obtain the overall knowledge of underlying mechanisms, and therefore to improve the application performance and to reduce the potential risk of metal-related nanomaterials.

1. Introduction

Nanomaterials are widely used in many fields due to their fascinating properties. However, safety concerns on the potential risk of nanomaterials when entering into biological systems directly during manufacturing processes or indirectly via the environment and food chains are raised. 1–5 For example,
some studies showed that nanoparticles, after deposition in the lungs, largely escape alveolar macrophage surveillance and gain access to the pulmonary interstitium with a greater inflammatory effect than larger particles.\(^6\)\(^7\) A fast translocation of nanoparticles from pulmonary and gastrointestinal epithelium into the systemic circulation through animal studies was also observed.\(^8\) Therefore, the study of the toxicological effect of nanomaterials is necessary and a new research field known as nanotoxicology has emerged in recent years.\(^9\)\(^-\)\(^11\) Besides the toxicological effect of nanomaterials, the elucidation of the total biological effects (including both toxicological and beneficial effects) of the nanomaterials is important, not only for the protection of human health and environmental integrity but also for aiding industry and regulatory bodies in maximizing the application of nanomaterials.

In recent decades, omics studies, especially genomics, proteomics or metabolomics (metabonomics), aiming at the collective characterization and quantification of pools of biological molecules that translate into the structure, function, and dynamics of an organism, have received great attention.\(^12\)-\(^14\) Similarly, metallomics, aiming to provide a global and systematic understanding of the metal uptake, trafficking, role and excretion in biological systems, and potentially able to predict all of these \textit{in silico} using bioinformatics, is attracting more and more attention.\(^15\)\(^-\)\(^16\) It focuses on the systematic study of metallomes and the interactions and functional connections of metal ions and their species with genes, proteins, metabolites and other biomolecules within organisms and ecosystems.\(^17\) Four successful meetings for metallomics have been held since 2007 and the journal “Metallomics” has been published since 2009 by the Royal Society of Chemistry.\(^18\)\(^,\)\(^19\)

However, to our knowledge, the metals studied in metallomics generally refer to naturally occurring micro-scaled (bulk) ones, without consideration of nano-scaled metal-related engineered materials. As mentioned above, materials at the nano-scale may exhibit totally different properties compared with those at the micro-scale. Therefore, the terminology “nanometallomics” was initiated as a branch of metallomics and the application of advanced nuclear analytical techniques to the study of nanometallomics was also summarized previously.\(^20\) Besides, a session for nanometallomics was organized at the 4th international symposium on metallomics in Spain in July 2013.\(^21\)

In this paper, we will systematically review the absorption, distribution, metabolism, and excretion (ADME) behavior of nanomaterials and their interactions with genes, proteins and other biomolecules of metal-related nanomaterials in biological systems. Although a lot of related studies have been published recently, we are not trying to cover all these publications, and only selective and representative work is cited here. This minireview is aimed to help improve the application performance and reduce the potential hazard of metal-related nanomaterials.

2. Metal-related nanomaterials

Metal-related nanomaterials, including metallic and metal-containing nanomaterials, are frequently used as catalysts, sensors or probes due to their unique crystalline forms and superior mechanical, electrical, magnetic, optical and catalytic properties.\(^22\) Among them, metal, metal crystals, metal nanoclusters, metal oxide, and other metal multi-component core-shell nanomaterials, intermetallic or alloyed nanomaterials, metal fluorescent nanoclusters and metal nanoparticles-based hybrid nanomaterials can be categorized as metallic nanomaterials.
Nobel metal nanomaterials with interesting physical and chemical properties via controllable synthesis are ideal building blocks for enhanced functions and application potentials, e.g. single-component Pt, Pd, Ag and Au nanomaterials. Quantum dots (QDs) are semiconductor nanomaterials (~2–100 nm), with their cores consisting of a variety of metal complexes such as semiconductors, noble metals, and magnetic transition metals, which can also be regarded as metallic nanomaterials. As a typical metal oxide, iron-oxide nanoparticles are widely used in different medical applications. Metallofullerenes (fullerenes with metal atom(s) encapsulated) are novel forms of fullerene-based materials showing attractive applications in biomedicines, which can be regarded as metal-containing nanomaterials. The endohedral metallofullerenes so far produced are centered on group 2 or 3 metallofullerenes such as Sc, Y, La, Ca, Sr and Ba as well as lanthanide metallofullerenes (Ce–Lu). These metal atoms have been encapsulated in higher fullerenes, especially in C_{62}. Besides metallofullerenes, metal-decorated multi-wall carbon nanotubes (MWCNTs) and metal filled single-walled carbon nanotubes (SWCNTs) can also be regarded as metal-containing nanomaterials. In this paper, all these metallic or metal-containing nanomaterials are categorized as metal-related nanomaterials (Fig. 1).

3. Absorption, distribution, metabolism and excretion of metal-related nanomaterials in biological systems

Animals can be exposed to metal-related nanomaterials through different routes, mainly ingestion, inhalation, and dermal absorption. The absorption, distribution, metabolism and excretion of these metal-related nanomaterials in the biological systems can be influenced by their physicochemical properties, the exposure route, and the bioenvironment of the deposition site. Integrated analytical methods can be applied for characterization of metal-related nanomaterials and the study of absorption, distribution, metabolism and excretion of nanoparticles in vivo (for example, Fig. 2A).26

3.1 Absorption and excretion of metal-related nanomaterials

Exposure through ingestion may lead to extended retaining of the nanomaterials in the body. Oughton et al.27 studied the dietary absorption (uptake) and excretion of a cobalt nanopowder (average particle size, 4 nm; surface area, 59 m² g⁻¹) in earthworm E. fetida. It was found that cobalt nanoparticles were taken up to a high extent during 7 d of exposure (concentration ratios of 0.16–0.20 relative to the nanoparticles concentration in horse manure) and were largely retained within the worms for eight weeks, with less than 20% of absorbed nanoparticles being excreted. Similarly, Meng et al.28 studied the copper content in mice after oral gavage of nano-copper particles at the dose of 70 mg kg⁻¹ body weight. They found massive copper enrichment in renal tissue 24 h after the mice were exposed to nano- and ion-copper (Fig. 2B and C). The copper content in renal tissue drops from 12.6 ± 2.2 µg g⁻¹ to 6.5 ± 1.3 µg g⁻¹ in the ion-copper group at 72 h, however, in the nano-copper group, a high copper content level in kidneys is still maintained (11.5 ± 2.5 µg g⁻¹). This implies that the rate of elimination of nano-copper is very low in kidneys, with only 1.5 µg g⁻¹ reduction within 48 h.

Exposure through inhalation showed different absorption and excretion behavior. Kreiling et al. studied the absorption and excretion of 192Ir nanoparticles (15 and 80 nm) after inhalation exposure by young adult rats.29 They found that particles were predominantly cleared via airways into the gastrointestinal tract and feces during week 1 after inhalation. Additionally, minute particle translocation of <1% of the deposited particles into secondary organs such as liver, spleen, heart, and brain was found after systemic uptake from the lungs. The translocated fraction of the 80 nm particles was about an order of magnitude less than that of 15 nm particles.

The coating of nanomaterials was also found to influence their absorption and excretion behavior in rats after intravenous injection. Fischer et al.30 found that QDs coated with mercaptoundecanoic acid and crosslinked with lysine (denoted as QD-BSA) were cleared from plasma with a clearance of 0.59 ± 0.16 mL min⁻¹ kg⁻¹. A higher clearance (1.23 ± 0.22 mL min⁻¹ kg⁻¹) exists when the QDs are conjugated to bovine serum albumin (denoted as QD-BSA, p < 0.05). QDs are not detected in feces or urine for up to ten days after intravenous dosing. In contrast, Chen et al. found that 33.3% and 23.8% of the intravenously given silica coated CdSe QDs in mice were cleared via feces and urine.31 This was attributed to the difference in coating materials of these CdSe QDs.

In general, vertebrate organisms tend to recognize nanoparticles as foreign objects, with elimination of the materials through the primary excretory organs/systems. It was found that the particles with sizes over 100 nm will be caught by the reticuloendothelial systems (RES) while particles with sizes below 5 nm can be removed by the kidneys.32

3.2 Distribution of metal-related nanomaterials in biological systems

It is necessary to find out where the nanoparticles are accumulated after absorption for better understanding the biological
effects of metal-related nanomaterials in cells, tissues, animals and ecosystems.

Whole body quantification of exposed nanoparticles was generally achieved after sacrificing the animals. Liu et al. evaluated the distribution of nasal instilled copper nanoparticles (23.5 nm) in mice (Fig. 3A). After instillation of copper nanoparticles for one week, the copper level was significantly higher in the liver, kidneys, the olfactory bulb and blood in the high-dose (40 mg kg\(^{-1}\) body weight instilled for three times) H-Nano group than in the control, which is in agreement with the damage to the liver, kidneys and olfactory bulb clearly seen upon pathological examination. Therefore, the nasal inhaled copper particles at very high dosage can translocate to other organs and tissues and induce certain lesions.

Wang et al. studied the whole body distribution of 20 and 120 nm ZnO at doses of 1, 2, 3, 4, 5 g kg\(^{-1}\) body weight for healthy adult mice after oral intake. The accumulation of Zn in the tissues and serum of the mice in 5 g kg\(^{-1}\) body weight dose

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**Fig. 2** (A) Integrated analytical techniques for biodistribution and characterization of metal nanomaterials in vivo. The characterization of nanomaterials can be achieved by high resolution TEM, dynamic light scattering (DLS). The quantification, identification and structural characterization of nanomaterials can be achieved through ICP-MS, EDX-TEM and XAFS. (B) The level of Cu in the renal tissues of mice which are treated using nano-, micro- and ion-copper at the dose of 70 mg kg\(^{-1}\). The asterisk indicates that the copper content is significantly higher versus control (*p < 0.05; **p < 0.01). Both nano- and ion-copper lead to higher Cu content in renal tissue, by contrast, no difference is observed between the micro-group versus control. In the ion-copper group, the Cu content in kidneys sharply declines 72 h after the oral gavage (Δnano), however, the Cu content remains stable when the mice were treated with nano-copper even 72 h after the exposure (Δnano). (C) The appearance of the stomach of mice after single oral gavage for 24 h. The stomach swells up after nano-copper treatment and there appear significant changes in color. (Adapted from the data of the cited papers by permission from Elsevier. Ref. 26 and 28.)
Among the observed organs, the highest Zn content was found in the kidneys, pancreas and bone. The authors concluded that the liver, spleen, heart, pancreas and bone are the target organs for 20 and 120 nm ZnO oral exposure. The studies on the distribution of many other metal-related nanomaterials like gold and silver nanoparticles were also conducted and target organs like the liver and spleen were identified.35,36

Noninvasive study of the biodistribution of nanomaterials was also possible with specific tools. For example, the whole body distribution of silver labeled with 125I after intravenous injection in Balb/c mice was studied using computed tomography coregistered with single-photon emission computerized tomography (CT-SPECT). Predominant accumulation of the silver nanoparticles in the spleen (41.5% ID g⁻¹) and liver (24.5% ID g⁻¹) at 24 h was found.37 In vivo positron emission tomography (PET) imaging showed that 64Cu²⁺-labeled QDs (15.1 ± 7.6 nm) in mice were excreted via renal filtration shortly post-injection and accumulated in the liver.38

Zhu and coworkers39 found that the intratracheal-instilled nano-59Fe₂O₃ could pass through the alveolar-capillary barrier into systemic circulation within 10 min after intratracheal instillation into the male SD rats at a dose of 4 mg per rat using isotopic tracing techniques. The nano-59Fe₂O₃ in the lung was distributed to the organs rich in mononuclear phagocytes, including the liver, spleen, kidneys and testicle. The plasma elimination half-life of nano-59Fe₂O₃ was 22.8 days and the lung clearance rate was 3.06 μg per day, indicating systemic accumulation and lung retention. The extrapulmonary transported 59Fe₂O₃ was redistributed in many organs, which indicates that 59Fe₂O₃ can easily pass through a number of tissue compartments and accumulate in the extrapulmonary organs. The highest extrapulmonary 59Fe levels were found in the liver, followed by in the decreasing order: spleen, heart, kidneys, pancreas, testicles and brain. The 59Fe in the liver and heart showed time-response of accumulation from post instillation day 1 to day 21, and then decreased at day 50. The 59Fe showed persistent high levels till post instillation day 50 in spleen.

All these studies suggested an extensive distribution of metal-related nanomaterials in the tissues of the reticuloendothelial system, which requires further investigation of the interaction of nanoparticles with hepatic and splenic tissues at the cellular level. This is critical for evaluation of the in vivo effects and potential toxicity of metal-related nanomaterials.

For studying the distribution of metal-related nanomaterials in specific animal organs or tissues, or in small-size animal models, even in cells, dedicated tools are necessary. Wang et al.40 studied the accumulation of two crystalline phases of TiO₂ nanoparticles (80 nm, rutile and 155 nm, anatase; purity >99%) in murine brain using synchrotron radiation-based X-ray fluorescence (SRXRF) after intranasal instillation with 500 μg of TiO₂ nanoparticles suspension every other day for 30 days. It was found that titanium accumulated mainly in the cerebral cortex, thalamus and hippocampus, especially in the CA1 and CA3 regions of hippocampus at 30 days (Fig. 4). The significantly increased Ti contents in the hippocampus result in the obviously irregular arrangement and loss of neurons in the hippocampus. Intranasal instillation of either rutile or anatase TiO₂ nanoparticles produced sustained accumulation in brain tissues especially in the hippocampus, which indicates that the TiO₂ nanoparticles can enter the brain via the olfactory bulb.40,41
SRXRF mapping was applied to investigate the distribution of Cu nanoparticles in the whole body of *C. elegans*. It was found that the exposure to Cu nanoparticles can result in an obvious elevation of Cu and K levels, and a change of biodistribution of Cu in nematodes. Accumulation of Cu occurs in the head and at a location 1/3 of the way up the body from the tail compared to the unexposed control. In contrast, a higher amount of Cu was detected in other parts of the worm body, especially in its excretory cells and intestine when exposed to Cu<sup>2+</sup>. The nondestructive and multi-elemental SRXRF provides an important tool for mapping the elemental distribution in the whole body of a single tiny nematode at low levels.

SRXRF mapping with the nano-sized spatial resolution (nanoXRF) has been applied to study the distribution of metal-related nanomaterials in cells. For example, Corezzi et al. studied the distribution of a commercially available QD-secondary antibody conjugate in SKOV3 cancer cells. Pixel-by-pixel analysis of the elements present in the core–shell of QDs (S, Zn, Cd, and Se) was performed to retrieve a topographical map of their intracellular distribution (Fig. 5). It was found that the emission intensity of Zn and S, the elements that also constitute the shell of the QDs, is located mainly in the nuclear region, although a clear signal can also be detected in the cytoplasm. The relatively high concentration of these elements and their presence in both the test and control samples suggest that the main contribution to the detected signal comes from the elements naturally present in the cell. On the other hand, the Cd signal is almost undetectable, whereas the Se signal is clearly detected only in the cytoplasm of the labeled sample and is not present in either the cytoplasm or the nucleus of the control sample.

The SRXRF technique has also been applied to study cell–carbon nanotubes interactions. Bussy and coworkers studied the distribution of unpurified and purified single-walled and multiwalled carbon nanotubes (CNTs) in macrophages by monitoring the catalyst metal particle employed in most synthesis techniques, which was attached to or contained in nanotubes. The SRXRF technique allows CNT localization at the single-cell level with simultaneous analysis of the biological response through observation of changes in cell elemental composition (calcium in the present study).

Since the availability of synchrotron radiation based bioimaging tools like SRXRF is limited, commercially available laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is a convenient alternative. LA-ICP-MS provides easy sample preparation, multielemental detection with high sensitivity, and high spatial resolution. The distribution of gold and silver nanoparticles in individual fibroblast cells was studied using LA-ICP-MS. Nanoparticles were visualized with respect to cellular substructures and were found to accumulate in the perinuclear region with increasing incubation time.

### 3.3 Metabolic responses of metal-related nanomaterials in biological systems

After entering the body, metal-related nanomaterials may dissolve, be decomposed, be oxidized or reduced and this may lead to the electronic and/or ionic transfer either within the nanoparticles lattice or to the release into culture medium. The metal ions from dissolved, decomposed, oxidized or reduced metal-related nanomaterials may behave like the "traditional metallomes" in the body; however, the remaining unchanged nanoparticles may still show their unique nano-features.

Nanomaterials may dissolve in physiologically relevant media to form partially soluble metal ions or metal–ligand complexes. Franklin *et al.* compared the dissolution of both nano- and bulk ZnO in a freshwater system and found rapid dissolution of ZnO nanoparticles with a saturation solubility in the milligram per liter range, similar to that of bulk ZnO.
Further toxicity tests confirmed the comparable toxicity to *P. subcapitata* for nanoparticulate ZnO, bulk ZnO and ZnCl₂, suggesting that the toxicity aroused solely from the dissolved zinc. Further, an enhanced dissolution of iron oxide nanoparticles under the acidic conditions of lysosomes or in a microenvironment containing ligands with a strong affinity was also observed.47,48

The toxicity of nanomaterials may occur due to their decomposition. Derfus *et al.*49 observed the liberation of free Cd²⁺ from CdSe QDs and found that the cytotoxicity of CdSe QDs correlated with the free Cd²⁺ concentration in air-oxidized and UV-exposed samples. On the other hand, Qu *et al.*50 found that CdSe@ZnS core–shell QDs were degraded in *C. elegans* and Se₂⁻ in the CdSe core was oxidized to Se⁴⁺. Both results

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**Fig. 5** NanoXRF elemental maps of thin (60 nm) sections of adherent human SKOV3 cells included in epoxy resin and mounted on Si₃N₄ grids. Left: β-tubulin labeled by CdSe QDs. Right: control sample. The elemental concentrations are given in g cm⁻³. (Adapted from the data of the cited papers by permission from Elsevier. Ref. 43.)
confirmed that QDs could be decomposed in the body and lead to the nanotoxicity of QDs.

X-ray absorption spectroscopy (XAS) is a powerful tool to investigate the electronic and/or ionic transfers of metal-related nanomaterials in biological systems and can provide structural details of the metabolites. The metabolism of gold nanorods in SD rats was studied using XAS and it was found that long-term retention of gold nanorods in the liver and spleen did not induce changes in the oxidation states of gold, suggesting that gold nanorods are inert in the body. For other elements, however, the oxidation state was found to have changed in the body. Auffan et al. investigated the metabolism of three kinds of Fe nanoparticles in E. coli. According to Fe K-edge XAS spectra, three compounds, including Fe₂O₃, Fe₃O₄, and γ-FeOOH had different pre-edge intensities, positions of pre-edge and main edge, ramped absorption positions. The pre-edge information showed that FeII in nMagnetite could be oxidized to Fe III in water and in contact with E. coli. All the Fe0 atoms in nZVI were highly active to oxygen atoms under the same conditions and transformed into γ-FeOOH and Fe₂O₃ supported by X-ray diffraction (XRD) and XAS results. The process of Fe0 and FeII oxidation resulted in ionic or electronic transfer on the nanoparticle surface that might intervene with the bacterial metabolism when the cell membrane and its components came into contact with them. Consequently, the authors concluded that the toxicity of Fe nanoparticles containing ferrous and zerovalent iron resulted from the generation of reactive oxygen species or interference in electron/ion transport chains. This work suggested that surface properties of metal nanomaterials may affect their toxicity heavily by reactive oxygen species (ROS) generation and suitable surface modification needs consideration in order to make them more biocompatible and healthy.

The nanomaterials may induce other metabolic changes even if they are inert. The Au NRs-induced time-dependent metabolic changes in A549 and 16HBE cells were studied by ¹H nuclear magnetic resonance (NMR) spectroscopy as shown in Fig. 6. The dominant metabolites present in the cell extracts include a range of amino acids, organic acids, such as lactate, creatine, and citrate, membrane metabolites including choline, phosphocholine, glycerophosphocholine, and a number of nucleosides and nucleotides, such as inosine, adenosine, uracil, inosine-5'-monophosphate, and nicotinamide adenine dinucleotide. A significant reduction in the levels of lactate in both 16HBE and A549 cells suggests that protein-coated Au NRs exposure inhibits the intracellular anaerobic glycolysis process.
4. Interactions with genes, proteins and other biomolecules of metal-related nanomaterials in biological systems

The metal-related nanomaterials and their metabolites can interact with genes, proteins and other biomolecules in biological systems, which may result in different biological responses.

Nanomaterials are engineered to have various unique properties, which will affect their possible direct or indirect interaction with the DNA. Landsiedel et al.\textsuperscript{53} did not observe the genotoxicity of titanium dioxide and zinc oxide nanomaterials in vitro (Ames' Salmonella gene mutation test and the V79 micronucleus chromosome mutation test) or in vivo (mouse bone marrow micronucleus test and Comet DNA damage assay in lung cells of rats exposed by inhalation). However, the study on silver nanoparticles in \textit{A. cepa} found that they could penetrate the plant system and may impair stages of cell division causing chromatin bridge, stickiness, the disturbed metaphase, multiple chromosomal breaks and cell disintegration.\textsuperscript{54} For most nanomaterials it is still unknown whether they directly interact with DNA or whether indirect effects such as inflammation-mediated oxidative stress may infer a threshold for the genotoxicity of some nanomaterials.\textsuperscript{55}

As mentioned above, QDs have been used as fluorescent markers in biological applications. However, it is essential to know that QDs themselves do not induce adverse effects when they are used in \textit{in vivo} studies or DNA based assays. Green and Howman\textsuperscript{56} observed the DNA damage in plasmid nicking assays with water-soluble CdSe QDs by comparing the electrophoresis bands. Assays with DNA that had been incubated with quantum dots and exposed to UV light showed damage (Fig. 7). Samples of DNA incubated with quantum dots in the dark also showed evidence of a damaged band not observed in assays using DNA stored alone in the dark. It is worth noting that the intensity of the bands in assays carried out with DNA that was incubated with QDs is significantly weaker than experiments run without QDs present. This was attributed to the DNA coordinating to the dots during incubation, resulting in smaller yields of DNA when isolated. Up to 70% of the DNA coordinated to the dots non-specifically and was therefore unavailable for assay analysis. These results suggest that there may be serious issues to address concerning the use of such materials in DNA based assays or in \textit{in vivo} applications, as well as highlighting potential toxicological and environmental implications.

Research shows that nanoparticles can stimulate or suppress the immune responses. Liu et al. studied the effect of Gd@C$_{82}$(OH)$_{22}$ nanoparticles on the release of Th1/Th2 cytokines and found markedly enhanced immune responses and stimulated immune cells to release more cytokines, helping eliminate abnormal cells.\textsuperscript{57} Exosomes are extracellularly secreted membrane vesicles which act as Trojan horses for the dissemination and intercellular communication of natural nano-sized particles (like viruses). Upon exposure to magnetic iron oxide nanoparticles, maturation of dendritic cells and activation of splenic T cells were significantly induced by these exosomes. Furthermore, exosome-induced T-cell activation was more efficient toward sensitized T cells and in ovalbumin (OVA)-sensitized mice than in the unsensitized counterparts. The studies suggested that exosomes may act as conveyors of extrapulmonary signal transduction in nanoparticle-induced immune systemic responses.\textsuperscript{47}

Since nanomaterials are foreign to the host, the penetration of nanomaterials into the organism results from the crossing of barriers like skin, lungs, the gastrointestinal tract, which are all patrolled and controlled by the immune system and this will trigger the formation of a series of proteolytic enzymes and may cause immunotoxicity.\textsuperscript{58} For example, the viability study of dendritic cells incubating in gold nanoparticles (NPs) showed that these NPs were not cytotoxic even at high concentration but significant amounts of gold NPs amassing in endocytic compartments were observed. Furthermore, the secretion of cytokines was significantly modified after such internalization which suggests that a potential perturbation of the immune response may occur.\textsuperscript{59}

![Fig. 7](image-url) Plasmid nicking assays of supercoiled DNA. From left to right are shown aliquots taken after every 15 min (0–60 min). (A) DNA after UV excitation/dark. All show supercoiled DNA undamaged; (B) DNA incubated with a known plasmid nicking agent. Shows change from undamaged (0 min) to almost complete damage (60 min); (C) DNA after incubation with quantum dots and UV excitation, shows an almost constant level of damage; (D) DNA after incubation with QDs in the dark, shows a slightly weaker damaged band. (Adapted from the data of the cited papers by permission from RSC Publishing. Ref. 56.)
When NPs enter a physiological environment, they can rapidly adsorb a layer of proteins to form the “protein corona”60–62 (Fig. 8A). Researchers found that characteristics of NPs, such as size, shape, and surface, could affect protein adsorption and also the structure of the adsorbed proteins. This will further affect the reactivity of NPs with cells and determine the route and efficiency of NP uptake. For example, NPs were found to accelerate the fibrillation of proteins and peptides, a process

![Fig. 8](image_url)
that is associated with several diseases like Alzheimer's disease. The adsorbed proteins may also promote translocation of the NP across cellular barriers, and clearance or accumulation in vital organs. Understanding the dynamics of this complex interaction can thus provide useful insights into cytotoxicity, inflammatory potential and other key properties of these novel materials that can be explored for developing safer and value added nanomaterials for future applications. On the other hand, proteins in the corona were found to be conserved and unique irrespective of the nanoparticles types and both size and surface properties of nanomaterials played a very significant role in determining the nanoparticles' coronas on different nanoparticles composed of identical materials. The “protein corona” has the potential to alter the size and interfacial composition of nanomaterials, thus inducing changes between their biological identity and their synthetic identity. For example, the adsorption of proteins onto NPs was found to modify their melting point in a composition- and size-dependent manner. Thorough understanding of NP–protein interactions might lead to strategic manipulation of NP surfaces to adsorb specific functional proteins or small drug molecules intended for delivery in vivo. Furthermore this knowledge might also prove to be useful in predicting nanotoxicity related safety concerns.

NPs can also interfere with the biological process of cancer cells and this may help in designing drugs for cancer therapy. For example, it was found that metallofullerene nanoparticles, formulated as [Gd@C_{82}(OH)_{22}]_n, could penetrate the plasma membrane of tumor cells and lead to the shrinkage of solid tumors and to a decrease in the activities of those enzymes responsible for catalyzing the production of reactive oxygen species in vivo. Besides, pretreatment of the cisplatin-resistant human prostate cancer (CP-r) cells with [Gd@C_{82}(OH)_{22}]_n enhanced intracellular accumulation of cisplatin and formation of cisplatin–DNA adducts by restoring the defective endocytosis of the CP-r cancer cells. The results suggested that [Gd@C_{82}(OH)_{22}]_n nanoparticles could overcome tumor resistance to cisplatin by increasing its intracellular accumulation through the mechanism of restoring defective endocytosis. More importantly, a recent study found that [Gd@C_{82}(OH)_{22}]_n could inhibit the metastasis of pancreatic tumor through the inhibition of matrix metalloproteinases (MMP-9) activity mainly via an excite interaction by interfering with the binding of the incoming ligands by remotely modulating the S1’ loop (Fig. 8B). Through molecular-dynamics simulations, detailed inhibition dynamics and the molecular mechanism of the [Gd@C_{82}(OH)_{22}]_n–MMP-9 interaction were revealed, which provided insights for the de novo design of nanomedicine for fatal diseases such as pancreatic cancer. Bovine serum proteins with negative charges are readily adsorbed onto the surface of the Au nanorods (NRs) when incubated at pH 7.4 as seen by a 6 nm thick gray shell around the rod surface in the TEM image. Serum protein-coated Au NRs maintained their stability, which played an important role in mediating cellular uptake, intracellular trafficking, and lysosome susceptibility resulting in selective accumulation of Au NRs in the mitochondria of cancer cells.

The interaction of DNA with nanomaterials is promising in medical applications. Hydroxylated C_{60} is actually a polyacid-like molecule, and each proton of the hydroxyl group (C–OH) can dissociate in an aqueous solution, thus yielding a conjugated base C–O-. Fullerences can encapsulate some Env plasmid DNA during the self-assembly by which the nanoadjuvant realizes dual functions as a plasmid DNA carrier and an activator of host immunity. Poly(diallyldimethylammonium chloride) (PDDAC) and polyethyleneimine (PEI) are adsorbed on the surface of the nanorod. These PDDAC- or PEI-modified Au NRs can significantly promote cellular and humoral immunity as well as T cell proliferation through activating antigen-presenting cells if compared to naked HIV-1 Env plasmid DNA treatment in vivo. Artificially synthesized CpG oligodeoxynucleotides (CpG ODNs) can imitate bacterial DNA and effectively stimulate the mammalian immune system. Self-assembled polyvalent CpG–AuNP conjugates enhance the efficiency of cellular uptake and stimulate secretion of cytokines. These findings have shed light on the fact that the intentional design of the interaction of low-toxic nanomaterials with biomolecules can be used as a versatile platform for drug and gene delivery systems and biomedical applications.

5. Conclusions

Nanometallomics is proposed as a branch of metallomics for the study of the biological effects of metal-related nanomaterials (Fig. 9). This includes the systematic study of the absorption, distribution, metabolism and excretion of metal-related materials in biological systems. Further, it also involves the systematic study of the interactions and functional connections of metal-related nanomaterials with genes, proteins, metabolites and other biomolecules within organisms. The absorption, distribution, metabolism and excretion of metal-related nanomaterials in the biological systems are influenced by their physicochemical properties, the exposure route, and the microenvironment of the deposition site. Nanomaterials not only may interact directly or indirectly with genes, proteins and other molecules to cause DNA damage, genotoxicity,
immunotoxicity, and/or cytotoxicity, but may also stimulate the immune responses, circumvent tumor resistance and inhibit tumor metastasis.

Since nanometallomics is such a young research field, publications on risk evaluation and biological behavior of these metal-related nanomaterials are sometimes contradictory. Therefore, a standardized procedure for nanometallomics study should be developed. Besides, dedicated analytical tools are necessary in this field together with the nuclear related techniques as summarized in our previous papers. Analytical tools used in metallomics may also be applicable for nanometallomics but again care should be taken considering the fascinating and unique properties of nanomaterials. Further, nanometallomics together with metallomics need to be developed with other -omics like genomics, proteomics and metabolomics to improve the application performance and reduce the potential hazard of metal-related nanomaterials.

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