ACCOUNTS
of chemical research

Physicochemical Properties Determine Nanomaterial Cellular Uptake, Transport, and Fate

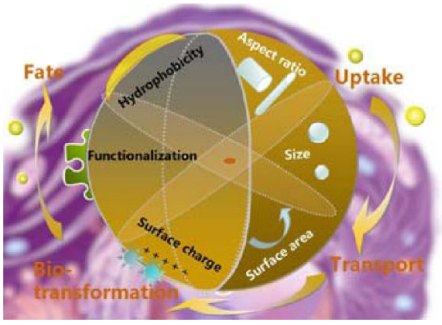
MOTAO ZHU,[†] GUANGJUN NIE,[†] HUAN MENG,[‡] TIAN XIA,[‡] ANDRE NEL,[*,‡] AND YULIANG ZHAO[*,†,§]

[†]*CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, National Center for Nanoscience and Technology of China, Beijing 100190, China,* [‡]*Division of NanoMedicine, David Geffen School of Medicine, UC Center for the Environmental Impact of Nanotechnology, UCLA Center for Nano Biology and Predictive toxicology, California Nanosystem Institute, University of California, Los Angeles, California 90095, United States, and* [§]*CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, Institute of High Energy Physics, Chinese Academy of Sciences (CAS), Beijing 100049, China*

RECEIVED ON JANUARY 29, 2012

CONSPECTUS

Although a growing number of innovations have emerged in the fields of nanobiotechnology and nanomedicine, new engineered nanomaterials (ENMs) with novel physicochemical properties are posing novel challenges to understand the full spectrum of interactions at the nano–bio interface. Because these could include potentially hazardous interactions, researchers need a comprehensive understanding of toxicological properties of nanomaterials and their safer design. In depth research is needed to understand how nanomaterial properties influence bioavailability, transport, fate, cellular uptake, and catalysis of injurious biological responses. Toxicity of ENMs differ with their size and surface properties, and those connections hold true across a spectrum of *in vitro* to *in vivo* nano–bio interfaces. In addition, the *in vitro* results provide a basis for modeling the biokinetics and *in vivo* behavior of ENMs. Nonetheless, we must use caution in interpreting *in vitro* toxicity results too literally because of dosimetry differences between *in vitro* and *in vivo* systems as well the increased complexity of an *in vivo* environment.



In this Account, we describe the impact of ENM physicochemical properties on cellular bioprocessing based on the research performed in our groups. Organic, inorganic, and hybrid ENMs can be produced in various sizes, shapes and surface modifications and a range of tunable compositions that can be dynamically modified under different biological and environmental conditions. Accordingly, we cover how ENM chemical properties such as hydrophobicity and hydrophilicity, material composition, surface functionalization and charge, dispersal state, and adsorption of proteins on the surface determine ENM cellular uptake, intracellular biotransformation, and bioelimination versus bioaccumulation.

We review how physical properties such as size, aspect ratio, and surface area of ENMs influence the interactions of these materials with biological systems, thereby affecting their hazard potential. We discuss our actual experimental findings and show how these properties can be tuned to control the uptake, biotransformation, fate, and hazard of ENMs. This Account provides specific information about ENM biological behavior and safety issues. This research also assists the development of safer nanotherapeutics and guides the design of new materials that can execute novel functions at the nano–bio interface.

Introduction

With the ability to manipulate structures at nanoscale, significant breakthroughs have been achieved in material design to impact industrial use of engineered nanomaterials (ENMs), as well as their application for nanomedicine.[1] However, the dramatic increase in the number of new ENMs and their novel physicochemical properties introduce the potential to generate adverse biological outcomes in humans and the environment.[2−4] In order to understand material hazard and develop safer ENMs, we need a

Published on the Web 08/14/2012 www.pubs.acs.org/accounts
10.1021/ar300031y

platform that allows rational exploration of the cellular nano–bio interface, including predictions for how ENM physicochemical properties relate to cellular bioavailability, uptake, and bioprocessing.

Numerous studies have attempted to address the role of physicochemical properties on ENM uptake, transport, and fate. These ENM physicochemical properties include (1) surface chemistry,[5−8] (2) physical properties (size, shape, and surface area),[7,9] (3) surface modifications under biological conditions (e.g., acquisition of a protein corona),[7,10,11] (4) dispersion, aggregation, and agglomeration of the ENMs,[12,13] and (5) stability in physiological conditions.[14−16] However, most published research on the bioprocessing and biological fate of ENMs lacks information to allow interpretation of quantitative property–activity relationships.[17] This lack of knowledge hampers a solid understanding of the biological behavior, beneficial use, and safety assessment of nanomaterials. For this field to further evolve, we need to develop a scientific approach to understand how ENM physicochemical properties relate to biological behavior and how designs of those properties could be used to optimize the utility of the ENMs for therapeutic use and safety.

In order to address the uptake, transport, and fate of ENMs, our understanding should transcend the knowledge of the biological behavior of traditional small molecules or micrometer scale particles. Generally, most organic and inorganic ENMs cannot be described only in terms of chemical composition but also have to take into consideration size, shape, and surface modification. Moreover, their tunable compositions and structural features lead ENMs to undergo dynamic and subtle changes under biological conditions. This leads to the emergence of a series of distinct ENM behaviors under biological conditions, including the impact on cells during the uptake, transport, and fate of ENMs. Most small drug molecules enter the cell through passive diffusion,[17] whereas most ENMs are taken up by active processes such as phagocytosis or pinocytosis depending on a dynamic series of physicochemical properties.[4,6,8,9,18] This introduces a range of biological response differences that could be used to therapeutic advantage or to understand and study hazard potential. Moreover, the intracellular fate and biotransformation of ENMs could differ from small molecules or larger particles due to the complex interaction of ENM compositions and physicochemical properties with cellular molecules and structures.[19] This could introduce additional biological variation. The intracellular fate and toxicity of biopersistant ENMs could be very complicated.[12,18]

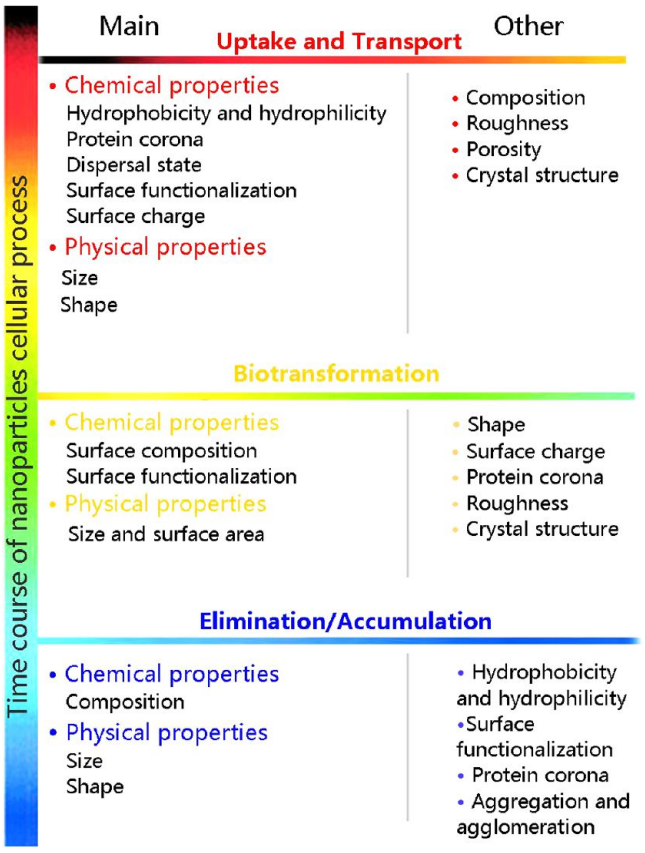


FIGURE 1. Scheme of the main physicochemical properties governing the cellular process of ENMs that will be introduced in this Account. Other properties that are not elucidated in this Account but are also involved in ENM cellular process are listed as other.

In this Account, the major physicochemical properties (Figure 1) of ENMs that impact biological interactions at the cellular level, including uptake, fate, accumulation, and biotransformation, are discussed. We will endeavor to explain the principal chemical and physical properties of ENMs that impact bioprocessing by providing examples of the biological events at the nano–bio interface and nanotoxicology emerging from our laboratories.

Impact of Chemical Properties on Nanomaterial Cellular Uptake, Transport, and Accumulation

When nanomaterials encounter cells, what do the cells see? And how do the cells respond? The chemical properties at the nanomaterial surface play an important role in determining interactions at the nano–bio interface.[4,19] The composition, coating, charge, placement of ligands, and wettability of the material surface play roles in the adsorption of biomolecules in cellular fate and uptake.[11−13] These surface properties also determine interactions with membranes, ions, organelles,

nucleic acids, etc. and thus are capable of influencing the structure and function of biomolecules and cells to affect homeostasis or induction of toxicity. Surface composition also determines the stability and fate of ENMs in biology.[12,14,16,20,21] Here, we will focus on the impact of the above properties in cellular uptake, biotransformation, fate, and safety and illustrate some successful approaches that improve ENM biocompatibility and safety by adjusting the surface properties.

Impact of Surface Hydrophobicity and Hydrophilicity. Hydrophobic nanoparticles are generally not stable and are poorly dispersed in biological fluids and culture medium.[11−13] Hydrophobic interactions promote hydrophobic nanoparticles forming aggregates or interact with hydrophobic residues of blood proteins or peptides to enhance their dispersion.[10,22] ENMs taken up as aggregates or agglomerates also tend to be less avidly cleared by the host. The residual nanoparticles in macrophages or stromal cells could last for one up to several months, thus leading to cumulative toxicity.[12,15] It also seems that increased hydrophobicity is favored for blood protein binding.[10,11,22] According to our recent findings, when nanoparticles enter a biological milieu, their original surface will have contact with proteins and other biomolecules that form a dynamic protein corona whose composition varies over time due to continuous protein association and dissociation as well as changes in the environment.[10] The composition of the protein corona depends chiefly on particle surface chemistry (primarily hydrophobicity or charge) and compositions.[10,22] Our recent research indicates that serum proteins could competitively bind on the single-walled carbon nanotube (SWCNT) hydrophobic surface. The $\pi-\pi$ stacking interactions between SWCNTs and hydrophobic residues tyrosine, phenylalanine, and tryptophan play key roles in determining their absorption capacity on the SWCNT surface.[11] The formation of the protein corona is one of the most significant alterations of ENMs' surface chemical properties, and may, in turn, strongly influence the uptake, biotransformation, and biocompatibility of these particles.[10,11] For instance, we found that by preincubating CNTs with serum protein, CNTs can be individually dispersed and be taken up at higher concentrations into human mesenchymal stem cells, HeLa cells, monocytes/macrophages, and bronchial epithelial and endothelial cells.[11,12] It is worth noting that the high dosage of intracellular SWCNTs did not cause any apparent acute cytotoxicity.[23] This also implies that looking at the chronic toxicity *in vivo* is most important. In contrast, noncoated and agglomerated CNTs were less bioavailable and did not induce profibrogenic cellular responses and pulmonary fibrosis to the same extent as dispersed tubes.[12]

An additional effect of protein adsorption to the surface of CNTs is opsonization and the removal by phagocytic cells such as monocytes and macrophages in the liver and spleen within minutes.[24] Opsonization of therapeutic nanoparticles could lead to significant removal by the cells of the reticuloendothelial (RES), leading to a decrease of circulating ENMs and reduced bioavailability at the intended delivery site.[24,25] Thus, with the view to improve the bioavailability and decrease toxicity, modification of poly(ethylene glycol) (PEG) onto the nanoparticle surface is frequently used to improve ENM dispersibility and decrease subsequent opsonization.[25] If combined with polyethyleneimine (PEI) in a PEI−PEG copolymer in mesoporous silica nanoparticles (MSNP), the particle dispersal became better by electrostatic repulsion, which reduced opsonization and increased both the circulatory time and passive drug delivery to a tumor site.[26]

Impact of Surface Functionalization and Surface Charge. Use of ENMs for therapeutic or diagnostic purposes often involves functionalization of nanomaterials with specific biomolecules (e.g., peptides, ligands) or chemical groups to achieve drug, nucleic acid, or dual drug−nucleic acid delivery to cells and targeted disease sites. The interaction strength between nanoparticle surface groups and membrane receptors can be controlled by the type of biomolecules/chemicals (e.g., affinity) or by changing the density of surface biomolecules/chemicals (e.g., avidity).[5,8,19]

The cell membrane consists of an anionic hydrophilic outer surface. In contrast to neutral or anionic nanoparticles, cationic particles attach more readily to the cell surface, from where they may also be taken up more avidly if size permits.[7] Therefore, cationic surface is frequently used to promote cellular entry for drug and gene delivery applications.[6,8] We showed that cellular uptake of cationic PEI-coated MSNP is considerably enhanced compared with unmodified MSNP (silanol surface) or particles coated with phosphonate or PEG groups.[6] Both the rate and abundance of cellular uptake are enhanced by a positive surface charge.[7] In the case of PEI, this effect is tunable by the attachment of longer length polymers that display a higher density of cationic surface groups that are asymmetrically displayed and more amenable to attach to negatively charged membrane phospholipids than shorter length polymers.[6] However, this comes at the expense of increased toxicity, because high cationic density could lead to physical membrane damage that is associated with increased intracellular calcium flux and cytotoxicity.[5,6] Besides the generation of surface membrane damage, cationic particles coated with unsaturated amines can also initiate intracellular

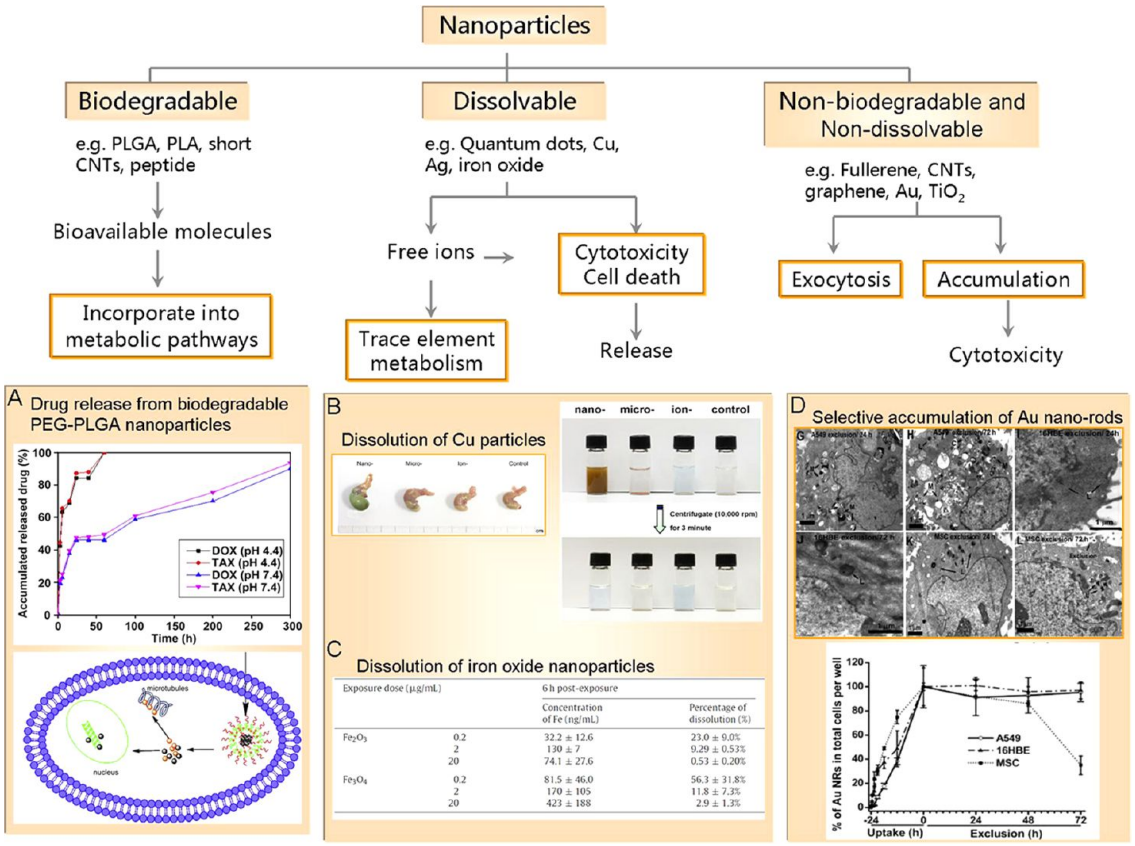


FIGURE 2. Biotransformation and fate of biodegradable, dissolvable and nondissolved and nonbiodegradable nanomaterials: (A) modulating drugs release by PLGA nanoparticles (Reproduced with permission from ref 31. Copyright 2011 Elsevier); (B) dissolution difference between small size (23.5 nm) and big size (17 μm) copper nanoparticles in murine stomach and in artificial acidic stomach fluid (Reproduced with permission from ref 16. Copyright 2007 Elsevier); (C) dissolution of iron oxide nanoparticles by human monocytes (Reproduced with permission from ref 14. Copyright 2011 Elsevier); (D) selective accumulation of Au nanorods in cancer and normal cells result in distinct cytotoxicity (Reproduced from ref 18. Copyright 2011 American Chemical Society). PLGA, poly(D,L-lactide-*co*-glycolide); PLA, polylactide; Cu, copper; CNTs, carbon nanotubes; TiO_2, titanium dioxide.

damage when taken up into the lysosomal compartment. According to the proton sponge hypothesis,[19] polyamine groups with high proton binding affinity could lead to buffering and exaggerated proton pump activity. This toxicity results from chloride influx to maintain charge neutrality, thereby leading to osmotic swelling and lysosomal rupture.[19] For instance, we have shown that cationic polystyrene (PS) nanoparticles with amine-functionalized surfaces are associated with a high rate of macrophage cell death following lysosomal rupture, intracellular calcium flux, and mitochondrial injury.[5,27]

In order to achieve a therapeutically beneficial cationic nanoparticle, it is necessary to control cationic density. We evaluated PEI polymer sizes ranging from 0.6 to 25 kD MW to balance the efficiency of intracellular delivery and cytotoxicity.[6] We demonstrated that the reduction of the polymer size was able to scale back the cytotoxic effect of higher MW PEI. Particles coated with PEI polymers of 10 kD or less maintained the feature of facilitated cellular uptake due to high membrane binding avidity and ability to be efficiently wrapped by the surface membrane. Additionally, MSNP particles coated with PEI polymers $\leq$10 kD in length can efficiently bind and deliver siRNA, with significant gene knock down and without provoking cytotoxicity.[5,8] Therefore, careful selection and control of surface cationic groups can achieve the goal of constructing cationic ENMs capable of enhanced intracellular siRNA delivery with minimal or no cytotoxicity.

Most of the delivered nanoparticles may get entrapped in endomembrane compartments, such as late endosome or lysosome.[28] To escape from endosomal pathways into the cytoplasm, cationic groups such as reducible polyethylenimine (PEI) or cell-penetrating peptides (CPPs) are frequently applied. For this out-of-endosomal target delivery, the transportation must minimally satisfy the following requirements: (i) avoid or escape from endosomal/lysosomal pathways, (ii) possess an organelle localization signal (sorting signals), such as nuclear localization signal (NLS) or mitochondrial leader peptides to interact with the nuclear pore complex or mitochondria,[29] and (iii) if the target is in

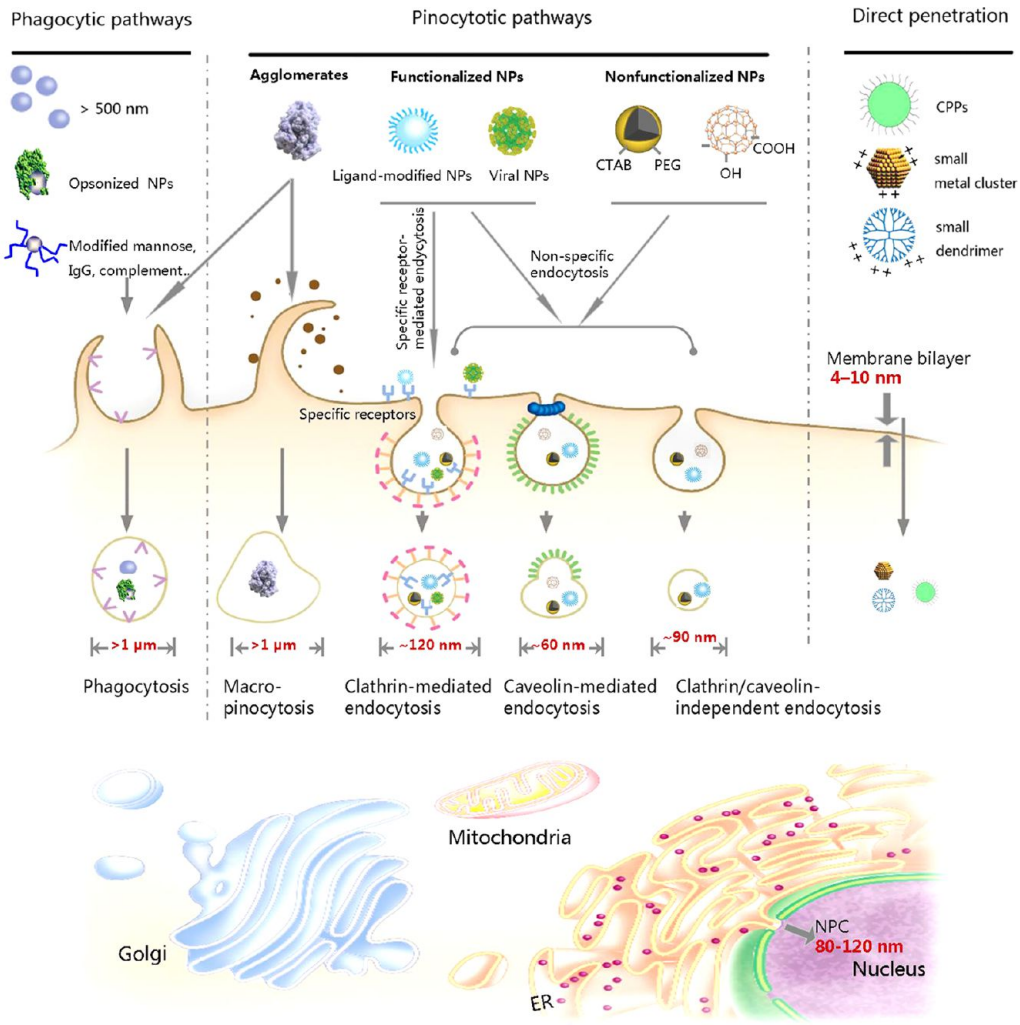


FIGURE 3. Natural size rules and gatekeepers within a mammalian cell. The thickness of membrane bilayer is typically 4−10 nm. The nuclear pore complex (NPC) is approximately 80−120 nm in diameter.[17] The sizes of endocytic vesicles in both phagocytosis and pinocytosis pathways for nanoparticle internalization were also introduced.[24] Phagocytes could take up large particles (or nanoparticle aggragates), opsonized nanoparticles, or nanoparticles with certain liagnd modification via phagocytosis. Nanoparticle internalization in a nonphagocytic mammalian cell is mainly through pinocytosis or direct penetration. With different surface modifications, nanoparticles may be taken up via specific (receptor-mediated) endocytosis or nonspecific endocytosis. The heterogeneity of nanoparticle surfaces and dispersion always requires multiple uptake pathways to be involved. These natural size-restricted structures execute their barrier functions when nanoparticle comes in and out. Therefore, the convergence of spatial sizes indicates that the behaviors (uptake, transport, and accumulation) of ENMs are restricted by the innate rules of biology. MR, mannose receptor; PRRs, pattern-recognition receptors; FcγR, immunoglobulin Fcγ receptor; CR, complement receptor; CPPs, cell-penetrating peptides; IgG, immunoglobulin G; ER, endoplasmic reticulum; Golgi, Golgi apparatus.

nucleus be small enough (<30 nm) to cross the nuclear membrane.[30]

Impact of Material and Surface Composition. ENMs entering the cell by endocytosis are directed to a series of early and late endosomes.[24] Some of these ensomes undergo acidification that could vary from a slightly acidic environment (pH 6.2−6.5) in early endosomes to more pronounced acidity (pH ≈ 4.5 and 5.5) in late endosomes and lysosomes. This process is also accompanied by enzyme recruitment to these compartments to digest vesicular content. In the case of nanoparticles, the material composition and surface coatings are important in determining the intracellular fate and biopersistance in this destructive environment. From the perspective of the material and surface stability, nanoparticles may be regarded as (i) biodegradable (e.g., biodegradable polymer, peptide),[31,32] (ii) dissolvable (e.g., quantum dots, zinc oxide, copper, silver, iron oxide),[14−16,21,33] or (iii) nonbiodegradable and nondissolvable nanomaterials (e.g., CNTs, graphene, gold) (Figure 2). For biodegradable polymers, such as poly(D,L-lactide-*co*-glycolide) (PLGA) and polylactide (PLA), their hydrolytic degradation is accelerated in low pH endosomal or lysosomal

environments.[31,32] The metabolic products that form such as lactic acid and glycolic acid, could become incorporated into biocompatible metabolic pathways.[31] The biodegradation rate and release kinetics of the encapsulated guest molecules are controlled by particle size, composition, and molecular weight of the shell polymer (Figure 2A).[31,32]

The dissolution of metallic nanoparticles, such as quantum dots, copper nanoparticles, and magnetic iron oxide nanoparticles, is a dynamic process under biological conditions (Figure 2B,C).[14,16,34] Material solubility depends on solvent properties (e.g., pH, ionic strength, and concentration) and may therefore vary from one to another cellular compartment (e.g., early endosome, lysosome, and cytosol). Dissolution of metallic nanomaterials might persist over periods of weeks to months to get rid of nanoscale materials.[34] In case of exhaustion of enzymes or proton pump activity, nanomaterial overload may perturb cellular homeostasis or induce cell death, leading to the release of undigested material that could start a vicious cycle.[14] Dissolution of hybrid nanomaterials with a core−shell structure may proceed layer by layer. Thus, contents shielded by the shell can be shielded from being degraded or biotransformed until at a more advanced stage of biotransformation. Cadmium (Cd)-containing quantum dots (QDs) are somewhat cytotoxic due to the presence of free Cd (QD core degradation) or interaction of QDs with intracellular components. By manipulation of the outer coating (capping material, functional groups), reduction of interfacial exposure of QDs could minimize cytotoxicity.[20,21] Our recent study showed that different chirality of biomolecules (e.g., D- and L-glutathione, GSH) on the QD surface determines the ligand exchange between QD surface group and the intrinsic homochiral glutathione. This ultimately determined the shell degradation of QDs and their toxicity.[20]

Impact of Physical Properties on Nanomaterial Cellular Uptake, Transport, and Accumulation

Impact of Nanoscale Size. The most important physical property of a nanomaterial in determining cellular uptake, transport and accumulation is its nanoscale size. Organisms have highly tuned and precise function of regulating the uptake and transportation of nanosize biological components. There also exist some scale rules within the cell. For example, most membrane bilayers exhibit a thickness of 4−10 nm. The vertebrate nuclear pore complex is approximately 80−120 nm in diameter.[17] These natural size-restricted structures execute their barrier functions when nanoparticles enter and exit. Figure 3 illustrates the most crucial sizes involved in different ways in cellular uptake, transport, and accumulation. Therefore, the convergence of spatial sizes indicates that behaviors such as uptake, transport, and ENM accumulation are restricted by the innate rules of biology that include regulation at the nanoscale level. In the following discussions, the role of ENMs physical properties such as size (for zero-dimensional ENMs), aspect ratio (for one-dimentional ENMs), and surface area will be discussed in terms of impact on cellular uptake, transport, and bioaccumulation.

To obtain direct bilayer penetration independent of endocytosis, the ENM size must be small (only a few nanometers) and its surface properties well designed to facilitate cellular entry.[35,36] Larger particles or particles with high-density cationic surfaces may lead to generation of holes in the membrane, thereby generating cytotoxicity.[35] ENMs taken up via endocytosis-mediated internalization are restricted by the size of each endocytotic portal (Figure 3). Mammalian cells exhibit five endocytic pathways for nanoparticle endocytosis: phagocytosis, macropinocytosis, and clathrin-mediated, caveolin-mediated, and clathrin/caveolin-independent endocytosis (Figure 3).[24] Each of these portals has its own dynamics and size rules. For example, ligand-modified nanoparticles are typically taken up by clathrin-coated vesicles, which are ∼120 nm in diameter (Figure 3). Ligand-modified nanoparticles larger than 120 nm are less facilely endocytosed via clathrin-mediated pathway.[37]

Impact of the Aspect Ratio. When nanoparticle size falls within the restricted size range of 120 nm, the aspect ratio of the material could make an additional independent impact on uptake and transport. ENM uptake typically proceeds through a four-step process, namely, nano−bio recognition, membrane binding, membrane wrapping, and pinching off.[37] Both ENM size and aspect ratio impact membrane wrapping (Figure 4).[9,38−40] To investigate how aspect ratio impacts cellular uptake, we constructed an ENM library in which a series of MSNPs with different aspect ratios were synthesized. This library included spheres and different nanorods with aspect ratios of 1 to 4.5. MSNP spheres have 110 nm diameter. Rod-shaped MSNP cylinders have dimensions of 110−130/60−80 nm (AR from 1.5 to 1.7), 160−190/60−90 nm (AR from 2.1 to 2.5), and 260−300/50−70 nm (AR from 4 to 4.5). We demonstrated that rod-shaped particles are preferentially taken up in HeLa and A549 cells. Particles exhibiting an aspect ratio of 2.1−2.5 were taken up faster and in larger quantities compared with spheres as well as shorter and longer length rods. We further showed that the intermediate length rods can be taken up via a macropinocytosis process. The rods with intermediary

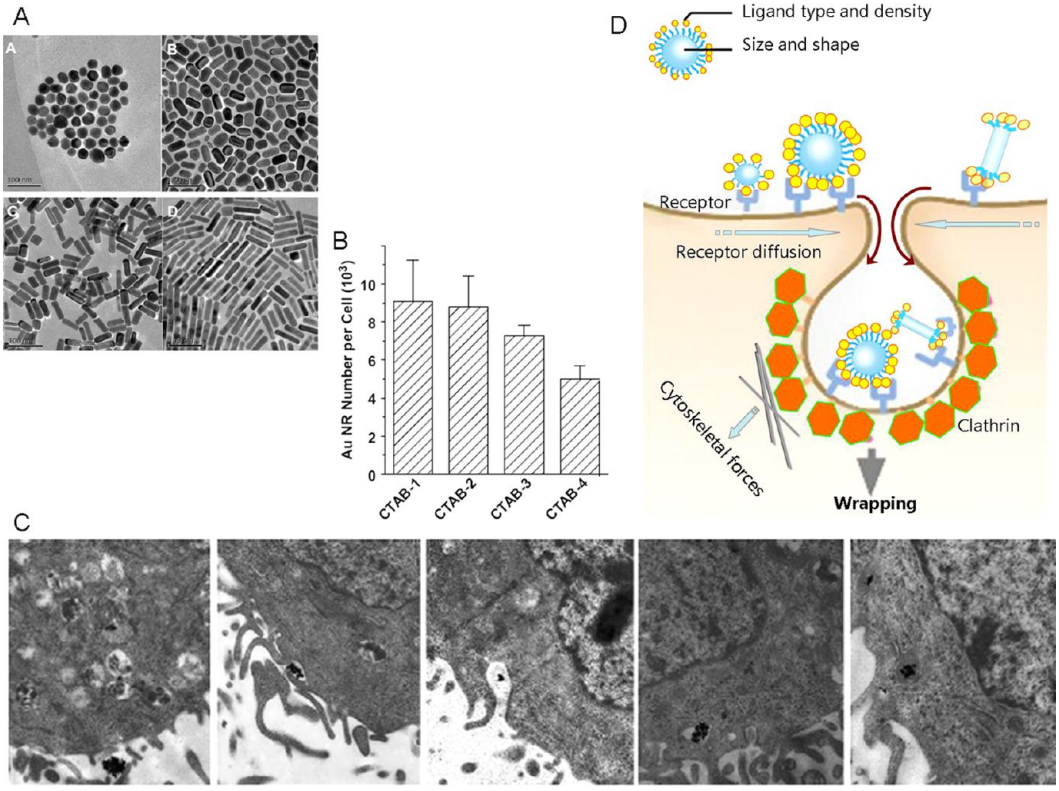


FIGURE 4. Impact of size and aspect ratio on ENM cellular uptake: (A) Au nanorods of different aspect ratio of 1.0 (CTAB-1), 2.0 (CTAB-2), 2.9 (CTAB-3), and 4.2 (CTAB-4) (CTAB, cetyltrimethylammonium bromide);[7] (B) numbers of Au nanorods within human breast adenocarcinoma (MCF-7) cells;[7] (C) TEM image showing the process of cellular uptake of Au nanorods, the Au nanorods wrapping into vesicle and further getting into the lysosome;[7] (D) sketch map for how size and shape affect membrane wrapping kenetics in cell endocytosis. Changes in nanoparticle size may affect the surface ligand density, ligand conformation, surface curvature, and relative orientation during nanoparticle membrane docking. Changes in nanoparticle aspect ratio may affect the position of surface ligand and wrapping time. Figures A, B and C are reproduced with permission from ref 7. Copyright 2010 Elsevier.

aspect ratio induced the maximal number of filopodia, actin polymerization, and activation of small GTP-binding proteins involved in the assembly of the actin cytoskeleton and filopodia formation.[9] In another study, we demonstrated that Au nanorods of longer aspect ratio [aspect ratio ranges from 1 to 4, with the sizes about 33 × 30, 40 × 21, 50 × 17, and 55 × 14, (length × diameter, nm), respectively] are internalized slower than shorter Au nanorods. We believe that this is mainly attributed to the longer membrane wrapping time required for the longer rod-shaped particles (Figure 4).[7,39] Comparing spherical nanoparticles with rod-shaped nanoparticles, the cellular uptake of spherical Au was 5−7 times faster than that of rod-shaped Au particles.[37]

The physicochemical properties that regulate the exocytosis of nanoparticles are still not clear, but it appears to be largely impacted by size and aspect ratio. For instance, transferrin-coated spherical-shaped Au nanoparticles (Tf-Au) are exocytosed in a linear relationship to size.[39] Smaller Tf-Au appeared to exocytose at a faster rate and at a higher percentage than large Tf-Au. The fraction of spherical-shaped Tf-Au exocytosed (F_{exo}) could be written as $F_{exo} = \alpha N_0/S$; here, α is a constant that depends on the cell type and its value is determined experimentally; N_0 is the number of Tf-Au internalized at the beginning of the exocytosis process; S is the surface area of each Tf-Au. Rod-shaped Tf-Au exocytosed was higher than spherical-shaped nanoparticles.[39] However, in case of much longer and more rigid multiwalled CNTs (MWCNTs), the clearance of these high aspect ratio carbon nanomaterials proceed by an extremely slow rate *in vivo*.[12] Inability to efficiently clear the aggregated MWCNTs that form rigid and fiber-like stacks could lead to toxicity by initiating frustrated phagocytosis.[12]

Impact of the Surface Area, Dissolvability and Degradability. Beyond cellular uptake, a key question becomes which ENM properties determine the materials' elimination kinetics or cellular retention, biotransformation, biodegradability, and metabolic pathways. Under certain conditions, the dissolution rate constant (k) of dissolvable ENMs

depends on the surface area of particles (A) as shown: $k = A(D/V)h$, (D, diffusion coefficient of solute molecule; V, volume of solution; h, thickness of diffusion layer).[34] Therefore, nanosized materials are often expected to dissolve more quickly and to a greater extent than large particles of the same material. The free ions released by dissolvable nanomaterials may be utilized as trace element or induce heavy metal toxicity (Figure 2). We experimentally determined that there is a major difference in the dissolution of small (23.5 nm) or big (17 μm) copper particles. The released copper ions lead to the accumulation of excessive alkaline substances *in vivo* and overload of heavy metal ions (copper ions) (Figure 2B).[16] Our most recent research also indicates that inhaled magnetic iron oxide nanoparticles (MIONs) might be excreted from the cells in the form of breakdown products or ions via extracellular secreted membrane vesicles (named exosomes).[41]

It is also very important to understand the fate of ENMs that are not readily dissolved or biodegraded such as CNTs, graphene, Au, titanium dioxide nanomaterials, etc. These materials may either be cleared from or accumulate inside the cell. The limited literature on clearance of nondegradable nanomaterials suggested that it mainly occurs by exocytosis that depends on the endomembrane system.[9,18,39] Furthermore, besides the physicochemical properties, the cellular trafficking and intracellular fate of nanoparticles are also cell-type and cell-phase dependent.[18,39] For instance, the Au nanoparticles show different exocytosis processes in Hela cells, SNB19, and STO cells, which could influence the cellular accumulation and clearance rates of the particles.[39] Also, the Au nanorods in cancer and normal cells show selective accumulation (Figure 2D).[18] Au nanorods within tumor cells could translocate to mitochondria, inducing decreased mitochondrial membrane potentials, increased oxidation stress, and finally reduced cell viability. This is an innate character in development of tumor cell targeted nanomedicines with low toxicity to normal cells. Recent studies also indicated that internalization of nanoparticles by cells could be ranked according to the different phases: G2/M > S > G0/G1.[42] Partitioning of nanoparticles in cell division is random and asymmetric uptake of nanoparticles by cells is also influenced by their cell cycle phase.[43]

Conclusion and Perspectives

Although ENMs have had numerous brilliant applications over the past decade, understanding of their bioprocesses is still on the way. Among these correlations between cellular trafficking and intracellular fate of ENMs and their physicochemical properties are the underlying fundaments. A thorough understanding of biological behavior and safety issues of ENMs requires further knowledge of how nanoparticles interact with biological membranes, organelles, and biomolecules and what their biological consequences are;[11] these generally lack systematic investigation so far. Up to the present, experimental findings can provide us with very useful information but are still limited to help prediction of certain physicochemical properties on the cellular behavior of ENMs, especially on the processes of biotransformation and elimination of ENMs. A major challenge of identifying the causative relationships between physicochemical properties of ENMs and their toxicity responses from the viewpoint of cellular trafficking is the lack of better probing techniques or methodology, in particular, the real time, *in situ*, rapid, and quantitative analysis methodology for characterizing the cellular behavior of ENMs, in which areaa breakthrough is urgently needed in the future. Because of the large number of variables in nanomaterials, experimental exploration requires a long time and great cost to clarify the cellular uptake, transport, and fate of each ENM. Thus, modeling from *in vitro* data to *in vivo* metabolism using computer simulation becomes a great challenge but is urgently needed to be developed to assist design of biologically safer nanomaterials or nanoplatforms. The knowledge we gain from the dynamic processes of ENMs in biological systems like living cells would feed back to the rational design of safer ENMs. In general, the *in vitro* results at the cellular level are more useful for understanding the mechanism of biokinetics (ADME) of ENMs *in vivo* and for predicting the possibly potential toxic responses at a whole body level when a living body is exposed to a given ENM. For example, the results obtained *in vitro* can be gathered to predict *in vivo* ADME/Tox (absorption, distribution, metabolism, excretion, and toxicity) of the ENMs through systematic information on (a) the most effective cellular uptake and bioavailability at target sites, (b) cellular metabolism and organ toxicity, and (c) cellular excretion and tissue accumulation and long-term risks. All these are essential knowledge for us toward the development of a sustainable nanotechnology.

This work was supported by the National Basic Research Program of China (Grants 2011CB933403, 2012CB934000, and 2012CB932504), National Natural Science Foundation of China (Grant 31100721), National Science Foundation and the Environmental Protection Agency (Cooperative Agreement Number DBI 0830117), and US Public Health Service Grants U19 ES019528 and RO1 ES016746.

BIOGRAPHICAL INFORMATION

Motao Zhu is an Assistant Professor in National Center for Nanoscience and Technology of China. She obtained her Ph.D. in Bioinorganic Chemistry from Institute of High Energy Physics, Chinese Academy of Sciences (CAS), in 2009. Her research interests focus on the biological trafficking of nanomaterials, nanotoxicology, and drug delivery using natural membrane vesicles.

Guangjun Nie obtained his Ph.D. in Biochemistry and Biophysics at Institute of Biophysics, CAS, in 2002. He is currently CAS 100 Talents Scientist and Chief Scientist of National Basic Research Programs. His research interests mainly foucus on cancer biology, blood physiology, and design of bioinspired biomaterials to overcome the current barriers in tumor therapy.

Huan Meng, Researcher in the Division of NanoMedicine at UCLA, received his Ph.D. in Bioinorganic Chemistry from Institute of High Energy Physics, CAS, in 2008. His research interests include nanotoxicology and nanomedicine.

Tian Xia is an Adjunct Assistant Professor in the Division of NanoMedicine at UCLA and Project Leader of UCLA CNPT. He received his Ph.D. in Biophysics from Peking University in 2001. His research interests are nanobiology, nanotoxicology, and nanomedicine.

André Nel, Professor of Medicine and Chief of the Division of NanoMedicine at UCLA, directs the UC CEIN and UCLA Center for Nanobiology and Predictive Toxicology (UCLA CNPT). He obtained his M.D. and Doctorate degrees in Cape Town, South Africa. His chief research interests are nano-EHS, nanobiology, and nanotherapeutics.

Yuliang Zhao received his Ph.D. at Tokyo Metropolitan University and worked at RIKEN (Japan). He moved to Chinese Academy of Sciences and became the full professor as a Hundred Elite Professorship in 2001. His research interests include nanochemistry (nanotoxicology, nanobioanalytical chemistry, and cancer nanotechnology) and radiochemistry. He has published more than 240 papers and authored or edited 10 books. *Nanotoxicology* published in U.S.A. (2007) is the first textbook worldwide in the field. He has delivered 147 invited lectures and is serving as Associate Editor or Editorial Board Member for eight international SCI journals in the U.S.A., U.K., and Germany.

FOOTNOTES

*To whom correspondence should be addressed. E-mail addresses: zhaoyuliang@ihep.ac.cn; anel@mednet.ucla.edu.

The authors declare no competing financial interest.

REFERENCES

1 Yan, L.; Zheng, Y. B.; Zhao, F.; Li, S.; Gao, X.; Xu, B.; Weiss, P. S.; Zhao, Y. Chemistry and physics of a single atomic layer: Strategies and challenges for functionalization of graphene and graphene-based materials. *Chem. Soc. Rev.* **2011**, *41*, 97–114.

2 Gilbert, N. Nanoparticle safety in doubt. *Nature* **2009**, *460*, 937.

3 Zhao, Y. L.; Xing, G. M.; Chai, Z. F. Nanotoxicology: Are carbon nanotubes safe? *Nat. Nanotechnol.* **2008**, *3*, 191–192.

4 Zhao, F.; Zhao, Y.; Liu, Y.; Chang, X.; Chen, C. Cellular uptake, intracellular trafficking, and cytotoxicity of nanomaterials. *Small* **2011**, *7*, 1322–1337.

5 Xia, T.; Kovochich, M.; Liong, M.; Zink, J. I.; Nel, A. E. Cationic polystyrene nanosphere toxicity depends on cell-specific endocytic and mitochondrial injury pathways. *ACS Nano* **2008**, *2*, 85–96.

6 Xia, T.; Kovochich, M.; Liong, M.; Meng, H.; Kabehie, S.; George, S.; Zink, J. I.; Nel, A. E. Polyethyleneimine coating enhances the cellular uptake of mesoporous silica nanoparticles and allows safe delivery of siRNA and DNA constructs. *ACS Nano* **2009**, *3*, 3273–3286.

7 Qiu, Y.; Liu, Y.; Wang, L.; Xu, L.; Bai, R.; Ji, Y.; Wu, X.; Zhao, Y.; Li, Y.; Chen, C. Surface chemistry and aspect ratio mediated cellular uptake of Au nanorods. *Biomaterials* **2010**, *31*, 7606–7619.

8 Meng, H.; Liong, M.; Xia, T.; Li, Z.; Ji, Z.; Zink, J. I.; Nel, A. E. Engineered design of mesoporous silica nanoparticles to deliver doxorubicin and P-glycoprotein siRNA to overcome drug resistance in a cancer cell line. *ACS Nano* **2010**, *4*, 4539–4550.

9 Meng, H.; Yang, S.; Li, Z.; Xia, T.; Chen, J.; Ji, Z.; Zhang, H.; Wang, X.; Lin, S.; Huang, C.; Zhou, Z. H.; Zink, J. I.; Nel, A. E. Aspect ratio determines the quantity of mesoporous silica nanoparticle uptake by a small GTPase-dependent macropinocytosis mechanism. *ACS Nano* **2011**, *5*, 4434–4447.

10 Cedervall, T.; Lynch, I.; Lindman, S.; Berggard, T.; Thulin, E.; Nilsson, H.; Dawson, K. A.; Linse, S. Understanding the nanoparticle-protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 2050–2055.

11 Ge, C.; Du, J.; Zhao, L.; Wang, L.; Liu, Y.; Li, D.; Yang, Y.; Zhou, R.; Zhao, Y.; Chai, Z.; Chen, C. Binding of blood proteins to carbon nanotubes reduces cytotoxicity. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 16968–16973.

12 Wang, X.; Xia, T.; Addo Ntim, S.; Ji, Z.; Lin, S.; Meng, H.; Chung, C. H.; George, S.; Zhang, H.; Wang, M.; Li, N.; Yang, Y.; Castranova, V.; Mitra, S.; Bonner, J. C.; Nel, A. E. The dispersal state of multi-walled carbon nanotubes elicits pro-fibrogenic cellular responses that correlate with fibrogenesis biomarkers and fibrosis in the murine lung. *ACS Nano* **2011**, *5*, 9772–9787.

13 Wang, X.; Xia, T.; Ntim, S. A.; Ji, Z.; George, S.; Meng, H.; Zhang, H.; Castranova, V.; Mitra, S.; Nel, A. E. Quantitative techniques for assessing and controlling the dispersion and biological effects of multiwalled carbon nanotubes in mammalian tissue culture cells. *ACS Nano* **2010**, *4*, 7241–7252.

14 Zhu, M. T.; Wang, B.; Wang, Y.; Yuan, L.; Wang, H. J.; Wang, M.; Ouyang, H.; Chai, Z. F.; Feng, W. Y.; Zhao, Y. L. Endothelial dysfunction and inflammation induced by iron oxide nanoparticle exposure: Risk factors for early atherosclerosis. *Toxicol. Lett.* **2011**, *203*, 162–171.

15 Zhu, M. T.; Feng, W. Y.; Wang, Y.; Wang, B.; Wang, M.; Ouyang, H.; Zhao, Y. L.; Chai, Z. F. Particokinetics and extrapulmonary translocation of intratracheally instilled ferric oxide nanoparticles in rats and the potential health risk assessment. *Toxicol. Sci.* **2009**, *107*, 342–351.

16 Meng, H.; Chen, Z.; Xing, G.; Yuan, H.; Chen, C.; Zhao, F.; Zhang, C.; Zhao, Y. Ultrahigh reactivity provokes nanotoxicity: Explanation of oral toxicity of nano-copper particles. *Toxicol. Lett.* **2007**, *175*, 102–110.

17 Alber, F.; Dokudovskaya, S.; Veenhoff, L. M.; Zhang, W. H.; Kipper, J.; Devos, D.; Suprapto, A.; Karni-Schmidt, O.; Williams, R.; Chait, B. T.; Sali, A.; Rout, M. P. The molecular architecture of the nuclear pore complex. *Nature* **2007**, *450*, 695–701.

18 Wang, L.; Liu, Y.; Li, W.; Jiang, X.; Ji, Y.; Wu, X.; Xu, L.; Qiu, Y.; Zhao, K.; Wei, T.; Li, Y.; Zhao, Y.; Chen, C. Selective targeting of gold nanorods at the mitochondria of cancer cells: Implications for cancer therapy. *Nano Lett.* **2011**, *11*, 772–780.

19 Nel, A. E.; Madler, L.; Velegol, D.; Xia, T.; Hoek, E. M.; Somasundaran, P.; Klaessig, F.; Castranova, V.; Thompson, M. Understanding biophysicochemical interactions at the nano-bio interface. *Nat. Mater.* **2009**, *8*, 543–557.

20 Li, Y.; Zhou, Y.; Wang, H. Y.; Perrett, S.; Zhao, Y.; Tang, Z.; Nie, G. Chirality of glutathione surface coating affects the cytotoxicity of quantum dots. *Angew. Chem., Int. Ed.* **2011**, *50*, 5860–5864.

21 Chen, Z.; Chen, H.; Meng, H.; Xing, G.; Gao, X.; Sun, B.; Shi, X.; Yuan, H.; Zhang, C.; Liu, R.; Zhao, F.; Zhao, Y.; Fang, X. Bio-distribution and metabolic paths of silica coated CdSeS quantum dots. *Toxicol. Appl. Pharmacol.* **2008**, *230*, 364–371.

22 Cedervall, T.; Lynch, I.; Foy, M.; Berggard, T.; Donnelly, S. C.; Cagney, G.; Linse, S.; Dawson, K. A. Detailed identification of plasma proteins adsorbed on copolymer nanoparticles. *Angew. Chem., Int. Ed.* **2007**, *46*, 5754–5756.

23 Holt, B. D.; Dahl, K. N.; Islam, M. F. Quantification of uptake and localization of bovine serum albumin-stabilized single-wall carbon nanotubes in different human cell types. *Small* **2011**, *7*, 2348–2355.

24 Dobrovolskaia, M. A.; McNeil, S. E. Immunological properties of engineered nanomaterials. *Nat. Nanotechnol.* **2007**, *2*, 469–478.

25 Gref, R.; Minamitake, Y.; Peracchia, M. T.; Trubetskoy, V.; Torchilin, V.; Langer, R. Biodegradable long-circulating polymeric nanospheres. *Science* **1994**, *263*, 1600–1603.

26 Meng, H.; Xue, M.; Xia, T.; Ji, Z.; Tarn, D. Y.; Zink, J. I.; Nel, A. E. Use of size and a copolymer design feature to improve the biodistribution and the enhanced permeability and retention effect of doxorubicin-loaded mesoporous silica nanoparticles in a murine xenograft tumor model. *ACS Nano* **2011**, *5*, 4131–4144.

27 Xia, T.; Kovochich, M.; Brant, J.; Hotze, M.; Sempf, J.; Oberley, T.; Sioutas, C.; Yeh, J. I.; Wiesner, M. R.; Nel, A. E. Comparison of the abilities of ambient and manufactured

nanoparticles to induce cellular toxicity according to an oxidative stress paradigm. *Nano Lett.* **2006**, *6*, 1794–1807.

28 Chou, L. Y.; Ming, K.; Chan, W. C. Strategies for the intracellular delivery of nanoparticles. *Chem. Soc. Rev.* **2011**, *40*, 233–245.

29 Entelis, N. S.; Kolesnikova, O. A.; Martin, R. P.; Tarassov, I. A. RNA delivery into mitochondria. *Adv. Drug Delivery Rev.* **2001**, *49*, 199–215.

30 Tkachenko, A. G.; Xie, H.; Coleman, D.; Glomm, W.; Ryan, J.; Anderson, M. F.; Franzen, S.; Feldheim, D. L. Multifunctional gold nanoparticle−peptide complexes for nuclear targeting. *J. Am. Chem. Soc.* **2003**, *125*, 4700–4701.

31 Wang, H.; Zhao, Y.; Wu, Y.; Hu, Y. L.; Nan, K.; Nie, G.; Chen, H. Enhanced anti-tumor efficacy by co-delivery of doxorubicin and paclitaxel with amphiphilic methoxy PEG-PLGA copolymer nanoparticles. *Biomaterials* **2011**, *32*, 8281–8290.

32 Miao, Q. H.; Xu, D. X.; Wang, Z.; Xu, L.; Wang, T. W.; Wu, Y.; Lovejoy, D. B.; Kalinowski, D. S.; Richardson, D. R.; Nie, G. J.; Zhao, Y. L. Amphiphilic hyper-branched co-polymer nanoparticles for the controlled delivery of anti-tumor agents. *Biomaterials* **2010**, *31*, 7364–7375.

33 Zhu, M. T.; Feng, W. Y.; Wang, B.; Wang, T. C.; Gu, Y. Q.; Wang, M.; Wang, Y.; Ouyang, H.; Zhao, Y. L.; Chai, Z. F. Comparative study of pulmonary responses to nano- and submicron-sized ferric oxide in rats. *Toxicology* **2008**, *247*, 102–111.

34 Borm, P.; Klaessig, F. C.; Landry, T. D.; Moudgil, B.; Pauluhn, J.; Thomas, K.; Trottier, R.; Wood, S. Research strategies for safety evaluation of nanomaterials, part V: role of dissolution in biological fate and effects of nanoscale particles. *Toxicol. Sci.* **2006**, *90*, 23–32.

35 Verma, A.; Uzun, O.; Hu, Y.; Han, H. S.; Watson, N.; Chen, S.; Irvine, D. J.; Stellacci, F. Surface-structure-regulated cell-membrane penetration by monolayer-protected nanoparticles. *Nat. Mater.* **2008**, *7*, 588–595.

36 Won, Y. W.; Lim, K. S.; Kim, Y. H. Intracellular organelle-targeted non-viral gene delivery systems. *J. Controlled Release* **2011**, *152*, 99–109.

37 Gao, H.; Shi, W.; Freund, L. B. Mechanics of receptor-mediated endocytosis. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 9469–9474.

38 Jiang, W.; Kim, B. Y.; Rutka, J. T.; Chan, W. C. Nanoparticle-mediated cellular response is size-dependent. *Nat. Nanotechnol.* **2008**, *3*, 145–150.

39 Chithrani, B. D.; Chan, W. C. Elucidating the mechanism of cellular uptake and removal of protein-coated gold nanoparticles of different sizes and shapes. *Nano Lett.* **2007**, *7*, 1542–1550.

40 Chithrani, B. D.; Ghazani, A. A.; Chan, W. C. Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. *Nano Lett.* **2006**, *6*, 662–668.

41 Zhu, M.; Li, Y.; Shi, J.; Feng, W.; Nie, G.; Zhao, Y. Exosomes as extrapulmonary signaling conveyors for nanoparticle-induced systemic immune activation. *Small* **2011**, *8*, 404–412.

42 Kim, J. A.; Aberg, C.; Salvati, A.; Dawson, K. A. Role of cell cycle on the cellular uptake and dilution of nanoparticles in a cell population. *Nat. Nanotechnol.* **2012**, *7*, 62–68.

43 Summers, H. D.; Rees, P.; Holton, M. D.; Brown, M. R.; Chappell, S. C.; Smith, P. J.; Errington, R. J. Statistical analysis of nanoparticle dosing in a dynamic cellular system. *Nat. Nanotechnol.* **2011**, *6*, 170–174.