

Chemical Basis of Interactions Between Engineered Nanoparticles and Biological Systems

Qingxin Mu,^{†,‡} Guibin Jiang,[§] Lingxin Chen,^{||} Hongyu Zhou,^{†,⊥} Denis Fournes, Alexander Tropsha,[#] and Bing Yan^{*,†}

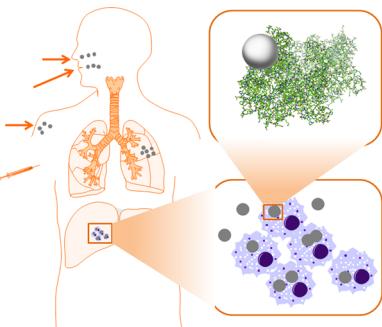
[†]School of Chemistry and Chemical Engineering, Shandong University, Jinan 250100, China

[§]State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, 100085, China

^{||}Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China

[⊥]Department of Surgery, Emory University School of Medicine, Atlanta, Georgia 30322, United States

[#]Laboratory for Molecular Modeling, UNC Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599, United States



CONTENTS

1. Introduction
2. Factors Affecting Nanoparticle–Biological System Interactions
 - 2.1. Nanoparticles
 - 2.2. Biological Factors
3. Nanoparticle–Biomolecule Interactions
 - 3.1. Nanoparticle–Phospholipid Interactions
 - 3.1.1. Interaction of Nanoparticles with Phospholipids in Pulmonary Surfactant Solutions
 - 3.1.2. Interactions Between Nanoparticles and Model Membranes
 - 3.2. Nanoparticle–Protein Interactions
 - 3.2.1. Determination of Nanoparticle–Protein Interactions
 - 3.2.2. Chemical Origin of Nanoparticle–Protein Interactions
 - 3.2.3. Interactions Between Nanoparticles and Proteomes
 - 3.3. Nanoparticle–DNA Interactions
 - 3.3.1. Determination of Nanoparticle–DNA Interactions
 - 3.3.2. Current Understanding of Nanoparticle–DNA Interactions
 - 3.4. Adsorption of Crucial Small Molecules by Nanoparticles
4. Nanoparticle–Cell Interactions
 - 4.1. Cellular Uptake and Intracellular Transport of Nanoparticles

4.1.1. Kinetics and Thermodynamics of the Cellular Uptake of Nanoparticles	O
4.1.2. Determinants of the Cellular Uptake of Nanoparticles	O
4.2. Cellular Substructural and Functional Alterations by Nanoparticles	P
4.2.1. Nanoparticle-Induced Cell Membrane Disruption	P
4.2.2. Ion Channel Inhibition by Nanoparticles	P
4.2.3. Cytoskeleton Alterations by Nanoparticles	Q
4.2.4. Mitochondrial Alterations by Nanoparticles	Q
4.2.5. Nuclear Alterations by Nanoparticles	R
4.3. Oxidative Stress and Nanoparticles	R
4.3.1. Oxidative Stress Induced by Nanoparticles	R
4.3.2. Reduction of Oxidative Stress by Nanoparticles	S
4.4. Nanoparticle-Induced Systematic Cellular Biochemical Perturbations	S
4.4.1. Perturbation of Cell Signaling Pathways by Nanoparticles	S
4.4.2. Global Biochemical Alterations by Nanoparticles	U
5. Chemical Approaches To Regulate and Understand Nanoparticle–Biological System Interactions	V
5.1. One-at-a-Time Nanoparticle Surface Chemistry Modification	V
5.1.1. Modulation of the Surface Chemistry of Nanoparticles	V
5.1.2. Modulation of the Surface Charge of Nanoparticles	V
5.1.3. Modulation of the Surface Hydrophobicity of Nanoparticles	W
5.1.4. Changes in the Interactions of Nanoparticles with Biological Systems	X

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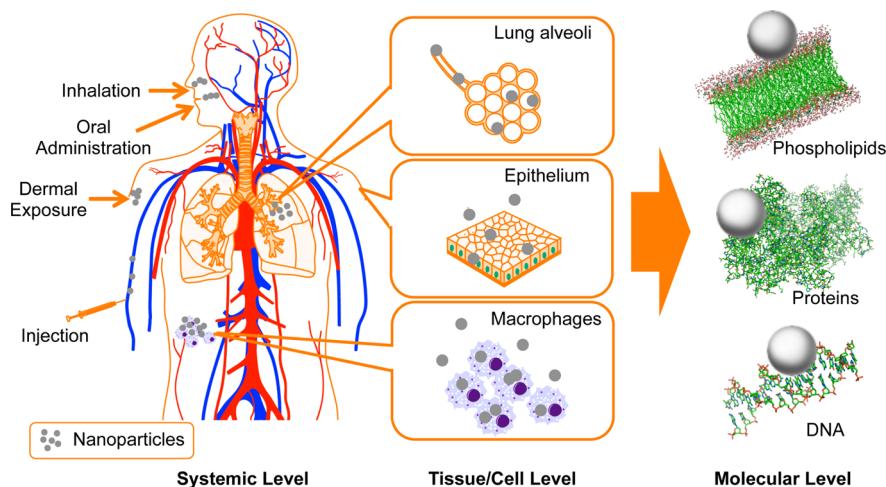


Figure 1. Interactions of nanoparticles with biological systems at different levels. Nanoparticles enter the human body through various pathways, reaching different organs and contacting tissues and cells. All of these interactions are based on nanoparticle–biomacromolecule associations.

5.2. Combinatorial Chemistry for Nanoparticle Modifications	X
5.2.1. Parallel Chemistry for Nanoparticle Modifications	X
5.2.2. Nanocombinatorial Chemistry To Modify Nanoparticles	AA
5.3. Computational Approaches To Elucidate Nano–Bio Interactions	AA
5.3.1. Quantitative Structure–Activity Relationships (QSAR)	AB
5.3.2. QNAR Modeling: Applications to the Rational Design of Nanoparticles with Desired Bioactivity Profiles	AD
6. Concluding Remarks	AE
Author Information	AF
Corresponding Author	AF
Present Address	AF
Notes	AF
Biographies	AG
Acknowledgments	AG
Abbreviations	AH
References	

1. INTRODUCTION

As defined by the European Commission, a nanomaterial is a natural, an incidental, or a manufactured material containing particles in an unbound state or as an aggregate or agglomerate in which $\geq 50\%$ of the particles in the number size distribution have one or more external dimensions in the size range from 1 to 100 nm. In specific cases and where warranted by concerns for the environment, health, safety, or competition, the number size distribution threshold of 50% may be replaced with a threshold between 1% and 50%.¹ Engineered nanomaterials (ENMs) refer to man-made nanomaterials. Materials in the nanometer range often possess unique physical, optical, electronic, and biological properties compared with larger particles, such as the strength of graphene,² the electronic properties of carbon nanotubes (CNTs),³ the antibacterial activity of silver nanoparticles,⁴ and the optical properties of quantum dots (QDs).⁵ The unique and advanced properties of ENMs have led to a rapid increase in their application. These applications include aerospace and airplanes, energy, architec-

ture, chemicals and coatings, catalysts, environmental protection, computer memory, biomedicine, and consumer products. Driven by these demands, the worldwide ENM production volume in 2016 is conservatively estimated in a market report by Future Markets to be 44 267 tons or $\geq \$5$ billion.⁶ As the production and applications of ENMs rapidly expand, their environmental impacts and effects on human health are becoming increasingly significant.⁷ Due to their small sizes, ENMs are easily made airborne.⁸ However, no accurate method to quantitatively measure their concentration in air currently exists. A recently reported incident of severe pulmonary fibrosis caused by inhaled polymer nanoparticles in seven female workers obtained much attention.⁹ In addition to the release of ENM waste from industrial sites, a major release of ENMs to environmental water occurs due to home and personal use of appliances, cosmetics, and personal products, such as shampoo and sunscreen.¹⁰ Airborne and aqueous ENMs pose immediate danger to the human respiratory and gastrointestinal systems. ENMs may enter other human organs after they are absorbed into the bloodstream through the gastrointestinal and respiratory systems.^{11,12} Furthermore, ENMs in cosmetics and personal care products, such as lotion, sunscreen, and shampoo, may enter human circulation through skin penetration.¹³ ENMs are very persistent in the environment and slowly degraded. The dissolved metal ions from ENMs can also revert back to nanoparticles under natural conditions.¹⁴ ENMs are stored in plants, microbes, and animal organs and can be transferred and accumulated through the food chain.^{15,16} In addition to accidental entry of ENMs into human and biological systems, ENMs are also purposefully injected into or enter humans for medicinal and diagnostic purposes.¹⁷ Therefore, interactions of ENMs with biological systems are inevitable.

In addition to engineered nanomaterials, there are also naturally existing nanomaterials such as proteins and DNA molecules, which are key components of biological systems. These materials, combined with lipids and organic and inorganic small molecules, form the basic units of living systems—cells.¹⁸ To elucidate how nanomaterials affect organs and physiological functions, a thorough understanding of how nanomaterials perturb cells and biological molecules is required (Figure 1). Rapidly accumulating evidence indicates that ENMs interact with the basic components of biological systems, such as proteins, DNA molecules, and cells.^{19–21} The driving forces

for such interactions are quite complex and include the size, shape, and surface properties (e.g., hydrophobicity, hydrogen-bonding capability, π bonds, and stereochemical interactions) of ENMs.^{22–25}

Evidence also indicates that chemical modifications on a nanoparticle's surface alter its interactions with biological systems.^{26–28} These observations not only support the hypothesis that basic nano–bio interactions are mainly physicochemical in nature but also provide a powerful approach to controlling the nature and strength of a nanoparticle's interactions with biological systems. Practically, a thorough understanding of the fundamental chemical interactions between nanoparticles and biological systems has two direct impacts. First, this knowledge will encourage and assist experimental approaches to chemically modify nanoparticle surfaces for various industrial or medicinal applications. Second, a range of chemical information can be combined with computational methods to investigate nanobiological properties and predict desired nanoparticle properties to direct experiments.^{29–31}

The literature regarding nanoparticle–biological system interactions has increased exponentially in the past decade (Figure 2). However, a mechanistic understanding of the

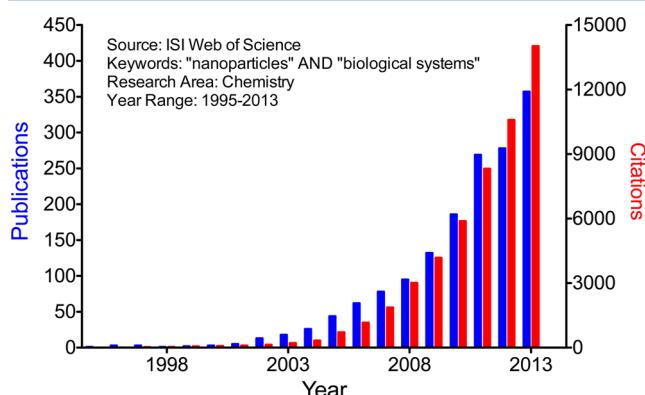


Figure 2. Analysis of literature statistics indicates growing concern for the topics that are the focus of this review. The number of publications and citations was obtained using the keywords “nanoparticles” and “biological systems” in the subject area of “Chemistry” when searching the Thomson Reuters (ISI) Web of Knowledge for the period 1995–2013.

chemical basis for such complex interactions is still lacking. This review intends to explore such an understanding in the context of recent publications.

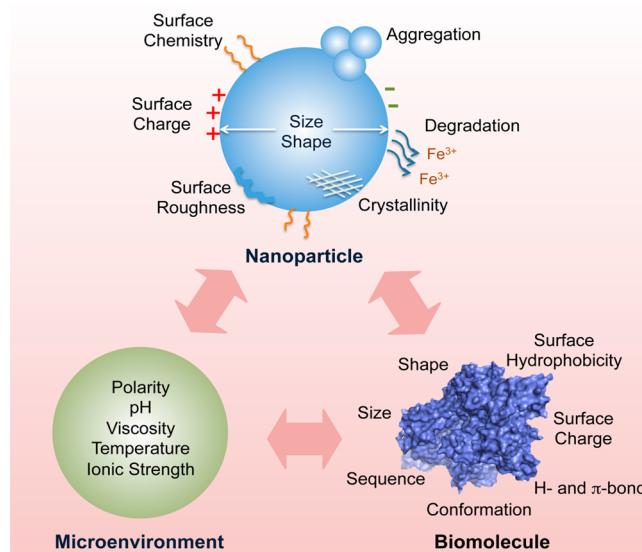
A breakthrough technology cannot prosper without wide acceptance from the public and society, that is, it must pose minimal harm to human health and the environment. Nanotechnology is now facing such a critical challenge. We must elucidate the effects of ENMs on biological systems (such as biological molecules, human cells, organs, and physiological systems). Accumulating experimental evidence suggests that nanoparticles interact with biological systems at nearly every level, often causing unwanted physiological consequences. Elucidating these interactions is the goal of this review. This endeavor will help regulate the proper application of ENMs in various products and their release into the environment. A more significant mission of this review is to direct development of “safe-by-design” ENMs, as their demands for and applications continue to increase.

2. FACTORS AFFECTING NANOPARTICLE–BIOLOGICAL SYSTEM INTERACTIONS

2.1. Nanoparticles

The extremely large surface area of nanoparticles results in strong adsorption of various molecules to reduce nanoparticle surface energy. Pristine nanoparticles are nearly nonexistent. Interactions between nanoparticles and biological systems (nano–bio interactions) are actually driven by modified nanoparticles in biological media, such as nanoparticle complexes or their aggregates.³² The real identity of nanoparticles in biological systems is determined by their intrinsic properties, such as their size, shape, surface charge, and hydrophobicity (Scheme 1). Therefore, a rigorous characterization of nanoparticles is essential prior to investigating nano–bio interactions.

Scheme 1. Factors Influencing Nanoparticle–Biomolecule Interactions



A very large collection of methods has been used to characterize nanoparticle physical and chemical properties. Transmission electron microscopy (TEM), scanning electron microscopy (SEM), and atomic force microscopy (AFM) provide information on nanoparticle morphology.³³ Dynamic light scattering (DLS) provides information on the hydrodynamic radii of nanoparticles in solution.³⁴ The surface charge properties of nanoparticles can be determined with ζ -potential measurements.³⁵ Auger electron spectroscopy, X-ray photoelectron spectroscopy (XPS), time-of-flight mass spectrometry (TOF MS), and elemental analyses reveal chemical composition details of nanoparticles.^{36,37} Surface ligands or adsorbed molecules can be identified with magic angle spinning nuclear magnetic resonance (MAS NMR), liquid chromatography mass spectroscopy (LC-MS), and Fourier-transform infrared spectroscopy (FTIR).^{38–40} Surface-enhanced Raman spectroscopy (SERS) is also among the most frequently applied analytical method for characterizing nanoparticles.⁴¹

2.2. Biological Factors

In biological systems, nanoparticles are surrounded by excessive amounts of biological molecules, which may result in the saturation of the nanoparticle surface. This phenomenon occurs

whether the nanoparticles are present in the body or in a cellular environment. Such biologically modified nanoparticles are the real participants in nano–bio interactions.³² These modifications also affect cell binding events and internalization of nanoparticles. Inside the cell, nanoparticles tend to localize in certain cellular organelles and affect specific cell regions.^{42–44}

In biological fluids, nanoparticles interact with phospholipids, proteins, DNA, and small metabolites. Phospholipids are the main components in the pulmonary system and cell membranes, consisting of a negatively charged phosphate group (head) and a hydrophobic carbon chain (tail).¹⁸ Nanoparticles may interact with the charged head and/or the hydrophobic tail depending on their surface hydrophobicity. Nanoparticles spontaneously adsorb proteins in the bloodstream or biological fluid. Proteins are large biomolecules of folded amino acid residues with different sizes, shapes, and net charges at physiological pH. Protein surfaces also exhibit varying hydrophobicity depending on the nature of the exposed amino acid residues.¹⁸ All of these properties may affect the stoichiometry and orientation of their bindings to nanoparticles. Nanoparticles may also bind DNA molecules in biological systems. DNA molecules carry negative charges from the phosphate groups and base π systems.¹⁸ Single- or double-strand DNA molecules can bind to nanoparticles through electrostatic, π–π stacking, and hydrophobic interactions (section 3.3). Therefore, molecular interactions of nanoparticles in biological environments involve interactions with proteins, DNA, phospholipids, small molecules, and inorganic ions. Such interactions are further complicated by the kinetics and thermodynamics of the nanoparticle associations with each component.

The organ from which a cell originates influences nanoparticle–cell interactions. First, different cell types have different shapes. Human erythrocytes exhibit a smooth, spherical shape, while ependymal cells demonstrate a long, slender shape.^{45,46} These differences produce different cellular responses when internalizing foreign matter, such as nanoparticles. Second, plasma membranes from different cell types have different lipid compositions, which influence the physical properties of membranes and make them function differently. Third, different types of cells express different membrane receptors. For example, macrophages express a large number of scavenger receptors that help internalize foreign matter nonspecifically.⁴⁷ Fourth, different cellular organelles have various impacts on nanoparticles. Lysosomes are acidic organelles, while the cytoplasm is nearly neutral. Nanoparticles in lysosomes may undergo degradation, which may result in the release of ions and a toxic state.⁴⁸ However, drug cargos may be preferably released in acidic environments for therapeutic purposes.⁴⁹

3. NANOPARTICLE–BIOMOLECULE INTERACTIONS

Nanoparticles enter the human body via multiple routes, encountering various biomolecules regardless of the pathway by which they enter the human body. Airborne nanoparticles may enter the human body through inhalation. In the lungs, nanoparticles exhibit strong interactions with the lung surfactant system, which consists of proteins and phospholipids.^{50,51} Engineered medical nanoparticles are generally administered into the circulation system through intravenous injection. Injected nanoparticles first interact with blood proteins before being distributed in various organs.⁵² Some nanoparticles may also enter the cell nucleus and interact with

nucleic acids, such as DNA.⁵³ Furthermore, nanoparticles in the blood or cells also adsorb small molecules, such as amino acids, biotin, and folic acid. Depletion of small metabolites may also contribute to the toxicity of nanoparticles.⁵⁴ In this section, we discuss interactions of nanoparticles with proteins, lipids, DNA, and small molecules. We focus on the characterization and mechanistic elucidation of these interactions.

3.1. Nanoparticle–Phospholipid Interactions

3.1.1. Interaction of Nanoparticles with Phospholipids in Pulmonary Surfactant Solutions. Phospholipids and proteins constitute a pulmonary surfactant system. Inhaled nanoparticles inevitably interact with these components. Interactions between nanoparticles and the pulmonary surfactant system determine nanoparticles' pulmonary toxicity or drug delivery efficacy. Therefore, understanding these interactions and effectively regulating these interactions are imperative for safe nanotechnology and better nanomedicine. Pulmonary surfactant solutions have been used to mimic the human pulmonary surfactant system for mechanistic studies. The nature of nanoparticle–phospholipid interactions can be investigated after isolating and identifying nanoparticle-bound surfactant molecules and characterizing the properties of nanoparticle–surfactant complexes. Nanoparticle-bound lipid molecules can be isolated by size exclusion chromatography⁵⁵ or thin layer chromatography⁵⁰ and structurally identified by NMR⁵⁵ or MS.⁵¹ Nanoparticle–phospholipid interactions can also be measured by identifying alterations in nanoparticle physicochemical properties. Surfactant molecule coatings alter nanoparticle sizes and dispersion states, which can be measured using DLS,⁵⁶ TEM,^{56,57} or SEM.⁵⁸ Surfactant molecules can also alter the surface charge status of nanoparticles, as determined with ζ-potential analysis.⁵⁶ Binding of surfactant molecules onto the surfaces of nanoparticles may alter their surface plasma resonance (SPR) absorption and can also be determined using UV–vis absorption spectroscopy.⁵⁶ The surface properties of pulmonary surfactant solutions, such as equilibrium and dynamic surface tension behavior under the pulsation of samples, are also used to evaluate the effects of nanoparticles.^{57,59}

Dipalmitoylphatidylcholine (DPPC, the most abundant surfactant molecule in a pulmonary surfactant mixture; also termed Survanta) is strongly adsorbed onto the surface of nanoparticles (e.g., carbon-based nanoparticles), forming a rigid monolayer. The binding of long aliphatic hydrocarbon chains to carbon nanoparticle surfaces results in increased nanoparticle surface charge, size, and stability. Adsorption onto a hydrophobic surface is also energetically favorable for DPPC.⁵⁶ Although proteins bind to multiwalled CNTs (MWNTs) with different binding patterns depending on the surface functionalization of the CNTs, the surfactant lipid may nonspecifically adsorb onto different MWNTs.⁵⁰ Adsorption of lipids onto the surface of carbon-based nanoparticles may affect their *in vitro* and *in vivo* behavior. When coated with DPPC, nanoscale carbon black and crocidolite asbestos increase reactive oxygen species (ROS) generation in primary bronchial epithelial cells and in A549 alveolar epithelial carcinoma cells, while fetal bovine serum protein-coated nanoparticles protect cells from oxidative insult.⁶⁰ However, in another study, DPPC-coated, single-walled CNTs (SWNTs) did not generate cytotoxic or fibrogenic effects *in vitro*. Lung fibrosis in mice can be induced by SWNTs with or without DPPC coating, indicating that DPPC does not significantly mask the bioactivity of SWNTs in

vivo.⁵⁸ Surfactants and the nanoparticle system can also affect each other. Phospholipids have been shown to stabilize colloidal gold nanoparticles (GNPs) by forming lipid bilayers on the particle surface, while the presence of GNPs reduces the surface activity of surfactants, such as their ability to form a surface film or the ability of this film to reach low surface tension during compression.⁵⁷ The size of particles also affects their interactions with lipids. TiO₂ nanoparticles but not microsized particles increase the adsorption surface tension of the surfactant in a dose-dependent manner. These phenomena are also observed during surface area cycling in the presence of nanoparticles. TEM observations have demonstrated that lamellar body-like structures are deformed and decreased in size, with formation of unilamellar vesicles.⁶¹ Nanoparticles enter the human body through respiratory, ingestion, and dermal systems and are distributed in many internal organs after absorption. Therefore, nanoparticles tend to interact with many cell types. Since DPPC is also a key membrane component of many cells, studies using DPPC as a model system have broad implications.

The binding events of proteins to nanoparticles most likely affect the surfactant interactions of nanoparticles. Interactions of polymeric nanoparticles with lipids in human plasma have been investigated using size exclusion chromatography, NMR, and enzymatic assays. Nanoparticles bind cholesterol, triglycerides, and phospholipids in human plasma. The binding events depend on the nanoparticle surface properties. Higher cholesterol content is present in the pellets of hydrophobic nanoparticles when compared to those of more hydrophilic nanoparticles.⁵⁵

3.1.2. Interactions Between Nanoparticles and Model Membranes. Model membranes are organized lipids that mimic the arrangement of lipids in natural cell membranes. Interactions between nanoparticles and model membranes have been investigated using biophysical approaches to evaluate the cellular translocation of nanoparticles and the potential effects of nanoparticles on the integrity of the cell membrane. Lipid monolayers, bilayers, and liposomes are three widely used model membranes.⁶²

The surface pressure–area (π –A) isotherm is an indicator of the monolayer properties of an amphiphilic material and obtained by measuring the surface pressure as a function of the water surface area available to each molecule.⁶³ The isotherm is recorded by compressing the film at a constant rate while continuously monitoring the surface pressure. Although π –A isotherm measurements have been used to study the properties of model lipid monolayers in the presence of nanoparticles, this approach is limited to monolayer membranes that lack anchoring membrane proteins.⁶⁴ AFM can be used to measure the effects of nanoparticles on morphological changes of Langmuir–Blodgett (LB) films. AFM measures subtle morphological details of soft matter at subnanometer levels, enabling observation of the formation of lipid bilayers and their disruption by nanoparticles.^{65–68} A liquid crystal-based system has been developed to study the molecular interactions between protein-coated nanoparticles and a liquid crystal-supported model membrane. The spatial and temporal organization of the lipid monolayer is coupled to the orientation of the liquid crystal, permitting direct observation of the dynamic characteristics of the lipid monolayer.^{69,70} Differential scanning calorimetry (DSC)^{68,71,72} and isothermal titration calorimetry (ITC)⁷³ can also be used to measure nanoparticle-induced thermodynamic changes in supported

membranes or liposomes. A quartz crystal microbalance (QCM) with dissipation monitoring can be used for real-time monitoring of nanoparticle–lipid membrane interactions.⁷⁴ Fluorescence microscopy,^{65,75} fluorescence spectroscopy,⁶⁸ fluorescence correlation spectroscopy (FCS),⁷³ Föster resonance energy transfer,^{73,76} NMR,⁷⁷ and liposomal leakage assay⁷⁸ have all been used to study the biophysical changes and disruptions of model lipid membranes or liposomes by nanoparticles.

Hydrophobic^{69,70} and electrostatic interactions^{74,78} may serve major roles in nanoparticle–lipid membrane interactions. The interactions of nanoparticles with the lipid membranes lead to changes in both components, such as disruption of the lipid membrane and modification of the nanoparticle surface properties. Guanidinylated dendrimers that carry a positive surface charge first adhere to the liposomal membrane through binding to negatively charged phosphate groups. The dendrimer surfaces then become hydrophobic due to charge neutralization. The dendrimers are consequently transported through the hydrophobic liposomal membrane.⁶⁸ Other polycationic polymers (i.e., polyamidoamine dendrimers, pentanol-core polyamidoamine dendrons, polyethylenimine, and diethylaminoethyl dextran) also induce the disruption of supported bilayers, including formation of holes, membrane thinning, and membrane erosion.⁷⁹ Polycationic polymers disrupt the liquid phase but not the gel phase of dimyristoylphosphatidylcholine bilayers.⁷⁷ Electrostatic interactions can also occur between protein-coated CNTs and liposomes, leading to liposome leakage.⁷⁸ Surface-charged polystyrene nanoparticles bind to liposomes and induce surface reconstruction of the phospholipid membranes, indicating a potential structural perturbation of the cellular membrane by nanoparticles (Figure 3).⁷³ Nanoparticle-induced lipid bilayer rearrangements are limited to bubble-like liposomes not supported bilayers.⁷⁶ QDs can insert into lipid membranes and induce a current burst under an electric field, indicating their potential to perturb neuronal and muscle cells.^{80,81} Cationic phosphorus dendrimers can alter the thermotropic behavior of lipid bilayers by reducing the cooperativity of the phospholipids; this effect is membrane surface charge dependent.⁷¹ A model has been proposed to describe the behavior of nanoparticles at the air–liquid interface of alveoli (Figure 4). Nanoparticles inhaled into the alveolar space during breathing may interact with continuous surfactant films. The nanoparticles then become wetted and lined with phospholipid bilayers.⁵⁷

In addition to the lipid membranes properties, factors that influence nanoparticle–lipid interactions include nanoparticle dose, composition, size, and surface chemistry. Hydrophobic polyorganosiloxane nanoparticles do not substantially affect the structural organization of a model pulmonary surfactant film at low concentrations (e.g., 10 μ g/mL) but significantly affect such properties at high concentrations (e.g., 3000 μ g/mL).⁸² Polystyrene nanoparticles with a diameter of 20 nm demonstrate greater interactions with the endothelial model membrane than those with a diameter of >60 nm.⁶⁴ Perturbations from nanoparticles may cause pore formation in lipid bilayers.^{65,66,79} Studies of polycationic polyamidoamine dendrimers and supported dimyristoylphosphatidylcholine bilayers have demonstrated that G7 dendrimers (8.0–8.2 nm) but not G3 dendrimers (3.5–4.1 nm) induce formation of holes in lipid bilayers. G5 dendrimers (4.3–6.6 nm) have a reduced effect when compared to G7 dendrimers but had no

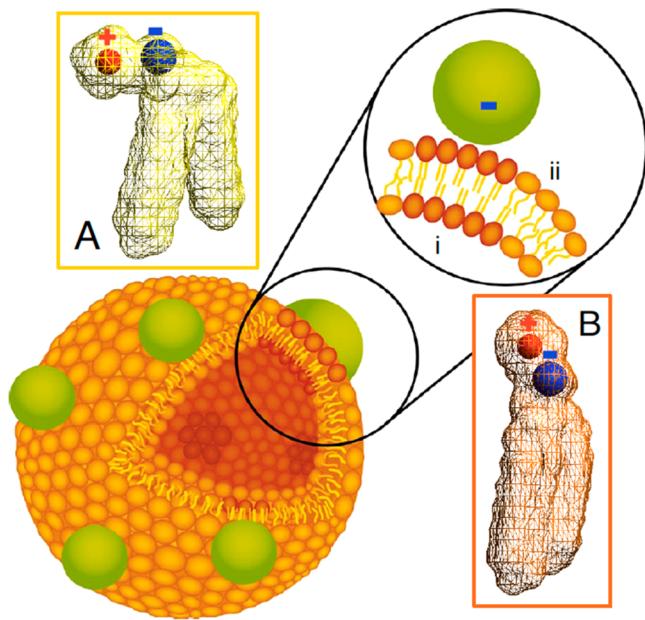


Figure 3. Nanoparticle-induced surface reconstruction of phospholipid membranes. Phospholipid bilayer vesicle with bound nanoparticles is shown. Binding of anionic nanoparticles to the lipid bilayer in the fluid phase causes a transition to gel phase at the site at which the nanoparticle binds. Binding-induced reorientation of the phosphocholine (PC) headgroup causes lipids in the fluid phase to exhibit a lower density (A) than in the gel phase (B). In the PC headgroup, P^- and N^+ are denoted by blue and red, respectively. Reprinted with permission from ref 73. Copyright 2011 National Academy of Sciences, USA.

effect when their amine end groups were replaced with acetamide groups, indicating the role of surface chemistry.⁶⁷ The density of the functional groups also affects the interactions. Lightly guanidinylated dendrimeric derivatives act as “molecular glue” by adhering to liposomes, while highly guanidinylated dendrimeric derivatives induce liposome fusion.⁶⁸ The crucial role of nanoparticle surface hydrophobicity has also been demonstrated. Hydrophobic, cholesterol-coated GNPs interact with DPPC vesicles and decrease their melting point, while hydrophilic nanoparticles increase the melting point of DPPC vesicles.⁷² The properties of model membranes, such as membrane surface charge⁷¹ and cholesterol content, also affect these interactions. A high cholesterol content in the phospholipid/cholesterol monolayer makes the lipids more susceptible to disruption by protein-coated GNPs.⁶⁹

Development of efficient nanocarrier systems based on model membrane studies requires a good correlation between model systems and cellular studies. The effects of the size and surface chemistry of polymeric nanoparticles in model membranes correlate well with cell membrane disruption and cellular uptake.^{83–86}

The interactions of nanoparticles with model membranes have also been investigated by molecular simulation approaches. Although computational methods cannot fully mimic complex biological systems, they facilitate an enhanced mechanistic understanding of their interactions and improved property predictions. The interactions of carbon nanoparticles, polymers, and nanocrystals with lipid bilayers have been simulated by all-atom and coarse-grained approaches.^{87–94} The influences of nanoparticle size, surface chemistry, hydrophobicity, shape, and bilayer properties on the interaction

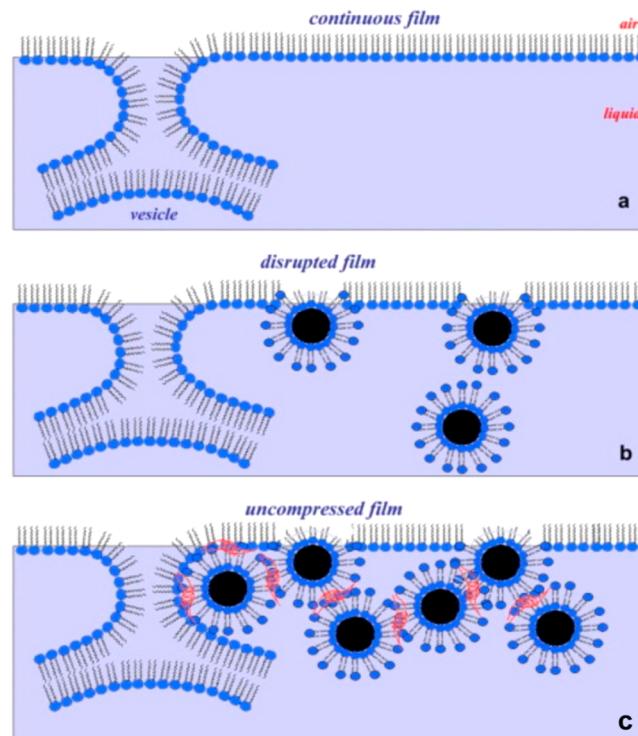


Figure 4. Potential inhibitory effects of GNPs on pulmonary surfactants in the alveolar space. (a) Continuous surfactant film (monolayer and underlying multilayer) forms after the rupture of pulmonary surfactant vesicles at the air–liquid interface as the hypothetical rate-limiting intermediate structure between bilayer vesicles and the interfacial monolayer. (b) Disrupted interfacial surfactant film due to entrapment of GNPs (from the air phase as pollutants) by pulmonary surfactant is depicted. (c) Self-aggregation of lipid-capped GNPs in the presence of surfactant protein B is shown in red. Reprinted with permission from ref 57. Copyright 2008 Cell Press.

mechanisms have been studied, and the results have yielded explanations for some experimental outcomes.^{87,88,90,93,95–98}

In summary, investigation of nanoparticle–lipid membrane interactions through molecular simulations, biophysical characterization, and cellular studies may collectively unravel the mechanisms of these processes and elucidate regulation strategies. Such developments will benefit nanotechnology-based drug delivery and rational reduction of nanotoxicity.

3.2. Nanoparticle–Protein Interactions

The interactions between nanoparticles and proteins are complex. Nanoparticles are generally surrounded by plasma proteins upon entering the circulatory system. As nanoparticles enter cells and are distributed into different organs, they are also in close contact with cellular proteins. Protein binding-induced changes in the surface properties of nanoparticles affect their biodistribution and clearance.⁹⁹ Moreover, conformational changes in bound proteins may expose unexpected epitopes to activate cell signaling.¹⁰⁰ The interactions of nanoparticles with proteins are dynamic processes and governed by properties of both components. The physicochemical changes in nanoparticles or proteins also allow characterization of the thermodynamic and kinetic processes of their interactions.

3.2.1. Determination of Nanoparticle–Protein Interactions. Similar to drug–protein or protein–protein interactions, nanoparticle–protein interactions are also characterized by binding affinity, stoichiometry, and kinetic properties.

Determining these properties helps us understand the chemical and dynamic nature of these interactions. Modulating these properties by modified nanoparticles may prevent unwanted interactions or enhance specific binding events.

3.2.1.1. Determination of Binding Affinity and Stoichiometry and Identification of Binding Sites. Steady-state and time-resolved fluorescence spectroscopy are used to study nanoparticle–protein binding affinities, complex formation, and binding-induced protein conformational changes.¹⁹ The dependence of protein fluorescence intensity or lifetime on quencher (nanoparticle) concentration follows the Stern–Volmer equation, eq 1

$$F_0/F = \tau_0/\tau = 1 + K_{sv}[Q] \quad (1)$$

where F_0 , F , τ_0 , and τ represent the initial or modified fluorescence intensity or lifetime, respectively. K_{sv} is the Stern–Volmer constant, and $[Q]$ is the quencher (nanoparticle) concentration.¹⁰¹

Fluorescence polarization permits the detection of interacting macromolecules in homogeneous solutions and can quantify nanoparticle–protein interactions based on the change in rotational freedom of the fluorescent molecule upon complex formation.^{23,101} An advantage of fluorescence polarization is that it avoids complex separation, which may induce denaturation of proteins or interrupt reaction equilibria. The binding ratio can be determined on the basis of fluorescence polarization measurements.

Stepwise photobleaching has been used to characterize nanoparticle–protein interactions. Both protein and nanoparticles are labeled with fluorescence dyes. The protein–nanoparticle binding ratio is estimated by counting the steps of the nanoparticle photobleaching. The distribution of photobleaching steps is quantified to obtain the average number of proteins per nanoparticle. The photobleaching counts can be obtained by ensemble or single-molecule measurements.¹⁰²

FCS measures the fluctuations of fluorescence intensity due to the Brownian motion of fluorescent particles in a small space. The hydrodynamic particle radii (R_H) can be calculated through the average diffusion time (τ_D), the diffusion coefficient (D), and the Stokes–Einstein relationship. Using this approach, the K_D of human serum albumin–nanoparticle binding ($\sim 5.1 \mu\text{M}$) and the protein–nanoparticle thickness ($\sim 3.3 \text{ nm}$) were estimated.¹⁰³

The binding sites of nanoparticles on a protein can be identified using NMR. 2D [$^{15}\text{N}-^1\text{H}$]-Heteronuclear single-quantum coherence (HSQC) NMR was used to identify a specific binding domain of GNPs on the surface of human ubiquitin. Addition of GNPs changed the positions of certain peaks, while others were unaffected (Figure 5), indicating specific interactions. In the preassigned peak map, the largest chemical shift changes were from the NH backbone of Q2, L15, and E18. On the basis of a combination of the chemical shift changes within the protein crystal structure, a domain containing Q2–I3 and L15–E18 was proposed to be the specific binding site for GNPs in GNP–ubiquitin interactions.¹⁰⁴

The binding sites of nanoparticles on a protein can also be identified using cross-linking chemistry and MS. As shown in Figure 6, the peptides located very close to poly(acrylic acid)-coated Fe_3O_4 nanoparticles in the binding site of human serum albumin (HSA) were cross-linked onto the nanoparticle surface with 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride. The attached peptides were identified by protein

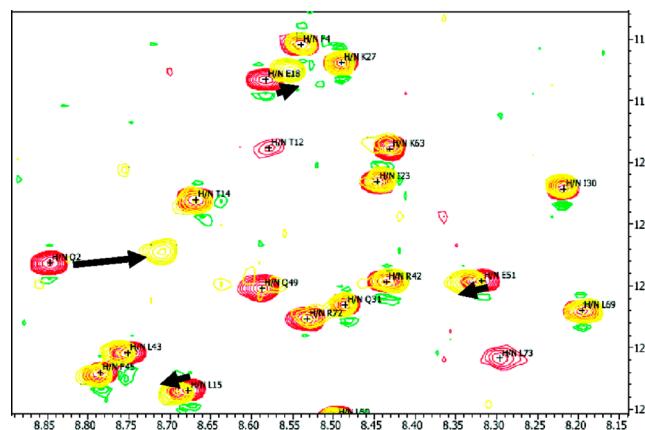


Figure 5. Two-dimensional (2D) [$^{15}\text{N}-^1\text{H}$]-HSQC NMR spectra of free human ubiquitin (red) and human ubiquitin plus GNPs (yellow) at pH 7.7, showing chemical shift perturbation for some NH groups upon interaction with GNPs. Black arrows indicate the NH backbone groups with the largest chemical shift perturbations (Q2, L15, and E18) in this spectral window. Reprinted with permission from ref 104. Copyright 2010 American Chemical Society.

cleavage followed by matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI TOF-MS). The nanoparticle binding site on HSA was therefore identified. Identification of binding sites helps determine the binding specificity and predict possible biological consequences of nanoparticle–protein interactions.¹⁰⁵

DLS can be used to estimate nanoparticle–protein binding stoichiometry. Adsorption of proteins causes a small increase in the dynamic radius of nanoparticles. In one study, adsorption of hexahistidine-tagged cytochrome P450_{BSβ} molecules to the surface of QDs was investigated by DLS. The measurements revealed that six P450_{BSβ} molecules bind to the surface of each QD.¹⁰⁶

3.2.1.2. Measuring Nanoparticle–Protein Binding Kinetics. SPR technology is based on the change in the oscillation of surface plasmon waves caused by adsorption of molecules onto a metal surface.¹⁰⁷ Using this approach, polymeric nanoparticle–plasma protein binding kinetics have been investigated. Nanoparticles are anchored on the gold surface of the sensor chip, and proteins are injected to flow over the nanoparticle-modified surface.¹⁰⁸ A QCM can be used to measure the resonant frequency shift, which can be correlated with a mass change at the oscillating quartz surface. Either proteins or nanoparticles can be immobilized onto the gold surface on the quartz crystal. The binding partner (nanoparticles or protein) is injected and flowed through a flow chamber over the quartz surface, and the frequency is monitored.^{109,110} Using either SPR or QCM, the real-time and quantitative binding profile can be obtained, and the association and dissociation constants can be determined by fitting to the Langmuir adsorption isotherm or Scatchard equation.

3.2.2. Chemical Origin of Nanoparticle–Protein Interactions. Interactions of nanoparticles with proteins are dominated by many chemical processes. The properties of the nanomaterials significantly influence these processes. Next, we discuss the mechanisms of interactions between proteins and different nanomaterials.

3.2.2.1. Carbon Nanoparticles. Carbon nanoparticles, such as CNTs, fullerene, and graphene, contain only sp^2 carbons,

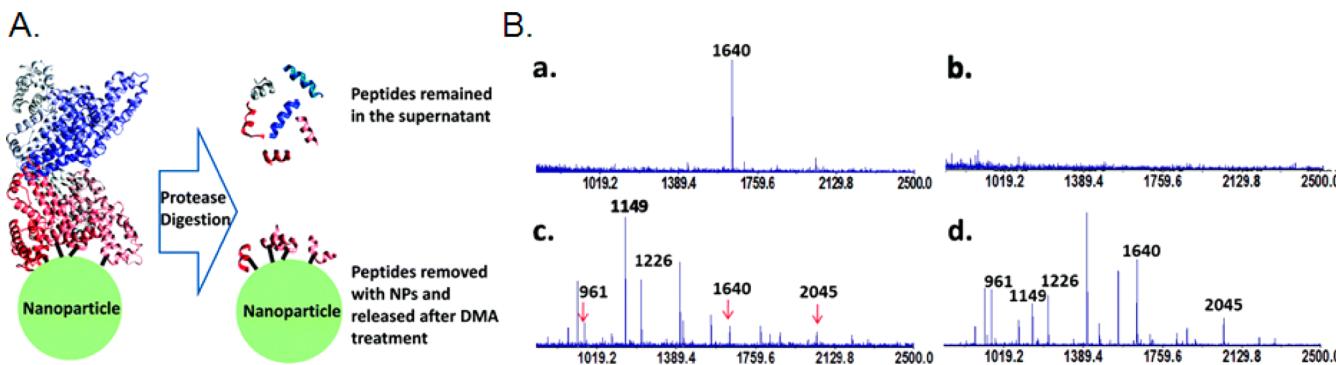


Figure 6. Identification of peptides associated with nanoparticle–protein interactions by cross-linking and MS. (A) Schematic illustration of the detection principle. (B) MALDI-MS spectra of released peptides when human serum albumin was (a) cross-linked and (b) adsorbed to the poly(acrylic acid)– Fe_3O_4 nanoparticles. Analysis of the supernatant from the cross-linked and non-cross-linked samples is shown in c and d, respectively. A peak of m/z 1640 in Ba indicates a peptide cross-linked onto the nanoparticle surface; in the supernatant, the peak intensity is significantly reduced (Bc). Arrows in Bc indicate 3 of 13 identified peptides with consistently reduced peak intensity in supernatant samples. Reprinted with permission from ref 105. Copyright 2011 American Chemical Society.

while nanodiamonds contain exclusively sp^3 carbons.^{111–114} The uniqueness of sp^2 carbon nanomaterials is due to their overwhelming π – π systems.¹¹⁴ Strong interactions between these materials and proteins are derived from π – π stacking, hydrophobic, and electrostatic interactions.^{19,115,116} Interactions between aromatic groups on carbon nanomaterials and aromatic or amine residues in proteins play a vital role in binding.¹¹⁷ Therefore, nanoparticle–protein binding events depend on the carbon nanoparticles, the nature of proteins, and microenvironmental factors, such as pH and ionic strength.^{19,115,118–122} Charge transfer between CNTs and streptavidin has been observed, indicating interactions between the amine groups of the protein and the aromatic surface of the CNTs.¹¹⁷ TEM and AFM observations have indicated that the bound proteins were found to form well-organized structures on the nanotube surface depending on the nanotube's diameter and the bound proteins (Figure 7).^{52,123} CNT-bound proteins generally form multiple layers on the nanotube surface.¹²⁴ This property can be used for nanotube debundling, stabilization, separation, and protein sensing.^{115,120,125–127} The bound proteins often undergo changes in structure and activity. Molecular simulation studies have demonstrated that proteins undergo stepwise conformational changes after binding to CNTs.^{128–130} For example, both soybean peroxidase and α -chymotrypsin bind to SWNTs; soybean peroxidase retains 30% activity, while α -chymotrypsin retains only 1% activity. This difference in activity occurs because soybean peroxidase partially maintains its secondary structure after binding, while α -chymotrypsin completely loses its secondary structure.¹¹⁸ CNTs enhance the rate of protein fibrillation by decreasing the lag time preceding nucleation, indicating the potential of CNTs to induce amyloid formation, as well as assembly of novel nanoparticles.¹³¹

The interactions of C60 with proteins have been studied using biochemical, structural, and molecular simulation approaches.^{132–137} The possible chemical interactions responsible for C60–protein binding events include hydrophobicity, surface curvature, π – π stacking, uneven charge distribution, site fit, and solvent displacement.¹³⁵ Molecular dynamic studies have demonstrated that interactions such as hydrophobicity and π – π stacking represent the dominant factors.¹³⁸ The binding site of C60 on its antibody has been identified using X-ray crystallography. C60 binds to the interface of the antibody light

and heavy chains.¹³⁹ The binding sites of C60 in human and bovine serum albumin (BSA) and HIV–protease are quite similar but different from that in the C60 antibody.¹³² Amino acid-modified C60s can penetrate the lipid membrane, bind to the hydrophobic domains of proteins, and alter the function of membrane-bound enzymes.¹⁴⁰

Graphene is another hexagonal carbon nanostructure. In contrast to CNTs and fullerenes, graphene consists of planar atomic thin layers.^{2,141} Because of the sp^2 carbon structure, the driving forces for interactions between proteins and graphenes are mainly hydrophobicity and π – π stacking. Molecular dynamics simulation predicted that human insulin can be adsorbed onto graphene surfaces and that some proteins would be destroyed or partially destroyed.¹⁴² Water-soluble graphene oxide nanosheets (GO) are oxidation products of graphene nanosheets. GOs have a stronger serum protein adsorption capability than SWNT or MWNT. The thickness of the GO layer increased from ~1.0 to 4.0–18.0 nm after protein binding.¹⁴³ The function of proteins adsorbed on the GO surface can be inhibited (e.g., the enzymatic activity of chymotrypsin¹⁴⁴). The GO–protein binding equilibrium can be reached in 30 min, and protein adsorption mitigates the cytotoxicity of GO.¹⁴³

In contrast to sp^2 nanostructures, diamond nanoparticles consist exclusively of sp^3 bonds. These nanoparticles bind protein molecules through hydrophobic interactions and physical adsorption and are used in tissue engineering (e.g., immobilization of bone morphogenetic protein 2 (BMP-2) to promote bone formation¹⁴⁵) or as mass spectrometric matrices.¹⁴⁶

3.2.2.2. GNPs. GNPs most likely interact with proteins through electrostatic, hydrophobic, and sulfur–gold interactions.^{23,147–151} GNPs with citrate as a stabilizing agent can bind to proteins according to the Langmuir isotherm.^{151,152} The size of nanoparticles influences the mechanism of protein binding. One study demonstrated that electrostatic interaction is the major binding force for nanoparticles with a diameter of 16 nm, while hydrophobic interaction is the major force for nanoparticles with a diameter of 2–4 nm.¹⁴⁸ Because the gold surface forms strong bonds with thiol groups in cysteine residues, the GNPs bind BSA and heparin-binding growth factors.^{151,153} The latter activity may be used to inhibit tumor angiogenesis.¹⁵⁴ Perturbation of the conformation and activity

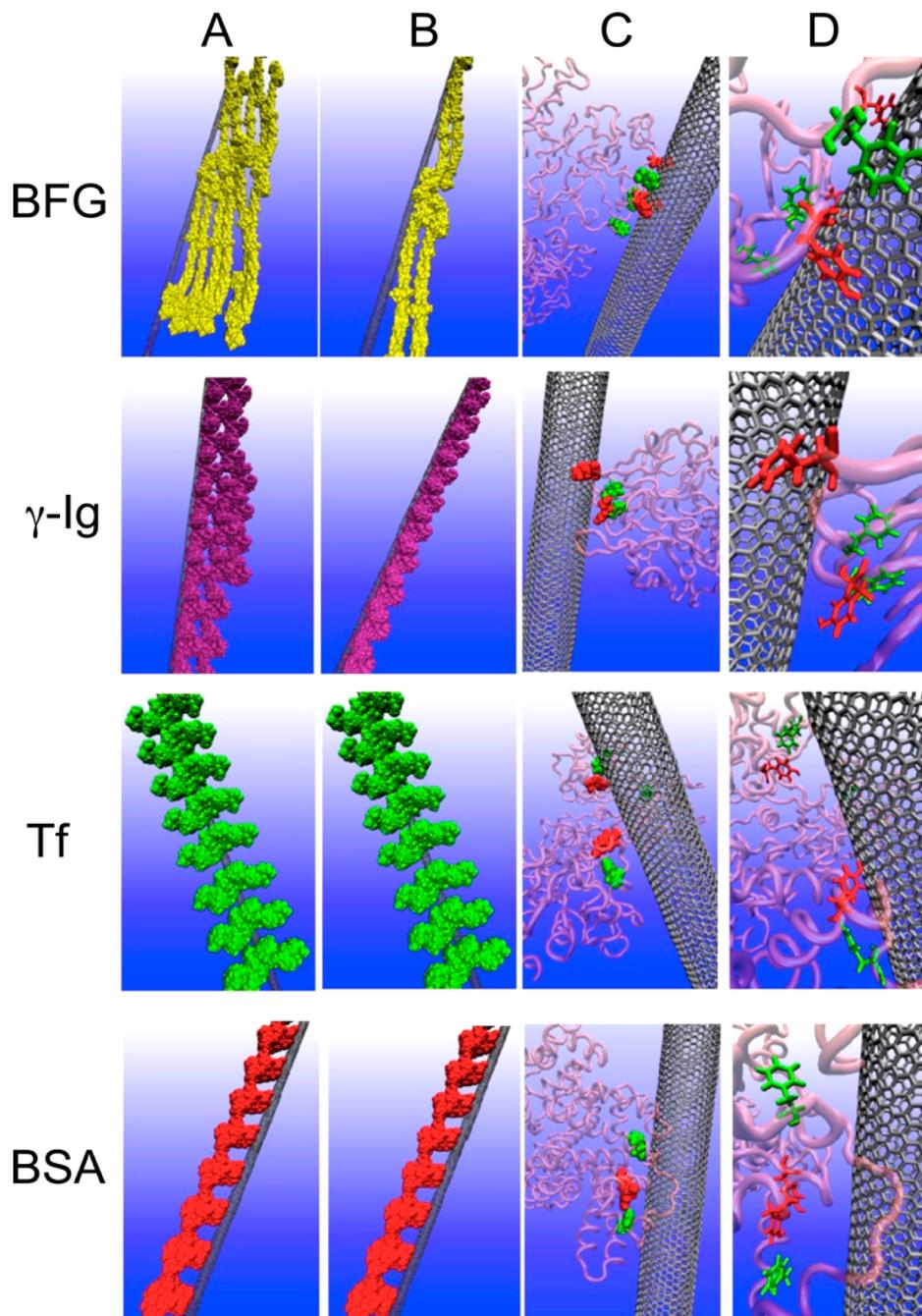


Figure 7. Interactions between bovine fibrinogen, immunoglobulin, transferrin, bovine serum albumin, and SWNTs. Molecular modeling illustrations of proteins (in bead representation) binding to SWNTs after incubation for 10 min (A) and 5 h (B). (C) Locations of the most preferred binding sites on proteins for SWNTs. Residues highlighted in the van der Waals representation correspond to tyrosine (red) and phenylalanine (green). Other regions of the protein are presented in pink. (D) Detailed orientations of the aromatic rings of the tyrosine and phenylalanine residues interacting with the six-membered rings of SWNTs are represented in silver. Tyrosine residues are rendered as licorice representations (red), while phenylalanine residues are shown in green. Adapted from ref 52 with permission. Copyright 2011 National Academy of Sciences, USA.

of bound proteins is influenced by the size and surface properties of the nanoparticles, the characteristics of the protein, and the ionic strength and pH of the solution.^{148,155–158} Cytochrome *c* is adsorbed onto anionic nanoparticles followed by conformational changes and proteolysis.¹⁴⁷ Chymotrypsin binds to GNPs and undergoes a two-stage change: fast, reversible inhibition followed by slow, irreversible inactivation.¹⁵⁰ The nanoparticle surface chemistry can regulate GNP–protein interactions.^{155,159} Amino acid-

modified GNPs can also tune the enzymatic activity of bound α -chymotrypsin.¹⁵⁹ The nanoparticle size influences the denaturation kinetics of BSA on GNPs.¹⁵⁷ Negatively charged poly(acrylic acid)-conjugated GNPs bind to and induce the unfolding of fibrinogen. The unfolded fibrinogen promotes MAC-1 receptor activation and inflammation.¹⁶⁰

3.2.2.3. Magnetic Iron Oxide Nanoparticles (MNPs). Adsorption of BSA molecules onto MNPs fits well to the Langmuir isotherm.¹⁶¹ The adsorption kinetics fit to a linear

driving force mass-transfer model. Similar to other nanoparticles, iron oxide nanoparticles also induce protein conformational changes and alter protein activity. Human transferrin molecules are adsorbed onto the surface of MNPs and undergo conformational changes from a compact to an open structure. This transformation induces the release of iron.¹⁶²

3.2.2.4. Quantum Dots (QDs). Electrostatic interactions, sulfur–metal bond formation, and spatial effects are believed to be major driving forces for QD–protein binding.^{163–165} CdS nanoparticles are trapped in the cylindrical cavity of chaperonin proteins and become thermally stable and tolerant to electrolytes.¹⁶³ The binding between QDs and HSA occurs near a protein pocket centered at Lys199.¹⁶⁴ Calculations reveal negative enthalpy (ΔH) and positive entropy (ΔS) values for this interaction, indicating that electrostatic interactions play a major role in the binding reaction. The stoichiometry of bound protein molecules on each QD is approximately 6. The bound proteins undergo substantial secondary and tertiary structural changes.¹⁶⁴ In another study, the electrostatic adsorption of hemoglobin (Hb) to CdS nanoparticles was shown to be energetically favorable. Raman spectroscopic results indicated that the sulfur atoms of the cysteine residues form direct chemical bonds with the CdS QD surface. Although higher structures are disturbed, the spin state of the heme iron is not affected.¹⁶⁶ In addition to native proteins, denatured BSA molecules also bind to the surface of CdTe QDs and form a shell-like complex.¹⁶⁷ The protein shell structure results in the removal of Te atoms from the CdTe surface and the formation of a thermodynamically stable nanostructure. Similar to CNTs, QDs also enhance protein fibrillation.¹³¹

3.2.2.5. Silica Nanoparticles. Electrostatic interactions may also be a driving force for protein adsorption by silica nanoparticles, as determined by DSC.¹⁶⁸ A decrease in ionic strength is accompanied by a reduction in protein binding enthalpy, indicating that there is a strong electrostatic attraction in silica nanoparticle/lysozyme and silica nanoparticle/RNase binding.¹⁶⁸ Hydrophobic interactions and hydrogen bonding are also driving forces in silica nanoparticle–peptide binding.²² The bound proteins on silica nanoparticles assume a specific orientation. Adsorption of human carbonic anhydrase II onto negatively charged silica nanoparticles appears to be specific to limited regions at the N-terminal domain of the protein.¹⁶⁹ In this study, the orientation of the bound proteins is also pH dependent. At pH 6.3, a histidine-rich area around residue 10 is the dominant binding region. At higher pH values (e.g., pH 9.3), the protein is adsorbed near a region close to residue 37, which contains several lysine and arginine residues. This absorption behavior indicates that specific binding may be a result of electrostatic interactions between the positively charged areas on the protein surface and the negatively charged silica surface. The binding of protein onto nanoparticles occurs in two steps: rapid formation of a protein–particle complex followed by an irreversible protein conformational change.¹⁷⁰ Nanoparticle-induced protein conformational changes vary depending on the nature of proteins. Unlike the “hard” (very stable) protein carbonic anhydrase II, the “soft” (less stable) protein carbonic anhydrase I undergoes a larger structural rearrangement when adsorbed onto silica nanoparticles, as shown by NMR.¹⁷¹ The size of silica nanoparticles determines the surface curvature, which influences protein adsorption and the stability of the adsorbed proteins. For instance, larger nanoparticles cause a greater loss of α helicity and enzymatic

activity in bound lysozyme¹⁷² and induce a greater decrease in the thermodynamic stability of RNase A.¹⁷³ In another study, silica nanoparticles were shown to promote α -helical structures in a designed peptide of low helical content in solution.¹⁷⁴ Consequently, the protein conformational changes altered their cell binding capability and reduced proliferation of human cells.¹⁷⁵

3.2.2.6. Polymeric Nanoparticles. Adsorption of proteins onto polymeric nanoparticles also depends on the hydrophobicity and surface curvature of nanoparticles. Interactions of proteins with *N*-isopropylacrylamide/*N*-*tert*-butylacrylamide copolymer nanoparticles with different monomer ratios and particle sizes demonstrate that proteins tend to bind to more hydrophobic nanoparticles by forming a single layer on the surface.¹⁷⁶ Surface chemistry also influences protein binding. For instance, in dextran phenoxy/poly(ethylene oxide) copolymer-modified polystyrene latex nanoparticles, BSA can deform and penetrate the poly(ethylene oxide) layer to bind to the nanoparticle surface. In contrast, a densely packed dextran phenoxy layer prevents nonspecific adsorption.¹⁷⁷ Similar to CNTs and QDs, polymer nanoparticles also promote protein fibrillation by enhancing the nucleation of protein fibrils from human β_2 -microglobulin.¹³¹ Promotion of fibrillation may be a common property for a large number of nanoparticles. Another study concluded that the first layer of bound proteins is much more stable than the secondary layer on the nanoparticle surface. The first layer was hence called the “hard corona”, indicating nearly irreversible binding, and the secondary layer was termed the “soft corona”, indicating greater dynamic exchange with the surrounding milieu.¹⁷⁸

The above studies demonstrate that protein binding is a nature of all nanoparticles, regardless of material. The material origin, size, shape, and surface chemistry of nanoparticles determine the chemical or physical forces involved in the interactions of nanoparticles with proteins. These interactions can be modified through adjusting the physicochemical properties of the nanoparticles.

3.2.3. Interactions Between Nanoparticles and Proteomes. Although the elucidation of the interactions between nanoparticles and single proteins facilitates the understanding of the chemical nature of such interactions, in biological systems, nanoparticles encounter proteomes, not a single protein. Blood and cellular proteomes consist of thousands of proteins with diverse functions, sequences, molecular weights, isoelectric points, surface hydrophobicities, and concentrations. The protein corona composition is determined by the thermodynamic and kinetic properties of the interactions between the nanoparticles and proteins.¹⁰⁸ The selectivity in protein binding is influenced by factors such as the size and surface chemistry of the nanoparticles and the properties of the proteins.^{179–182}

Binding between polymer nanoparticles and plasma proteins is highly dynamic. Abundant yet low-affinity HSA molecules quickly bind to the surface of nanoparticles and are soon replaced by the high-affinity and slow-exchanging apolipoproteins AI, AII, AIV, and E. These proteins remain associated with the particles.^{108,182,183} Although formation of a protein monolayer is an acceptable model, nanoparticles can also attract additional layers of proteins. By analyzing the adsorption of fetal bovine serum proteins onto iron oxide nanoparticles using gel electrophoresis and LC-MS/MS, proteins that strongly bind to the surface of nanoparticles were identified. These proteins include complement factor H, antithrombin,

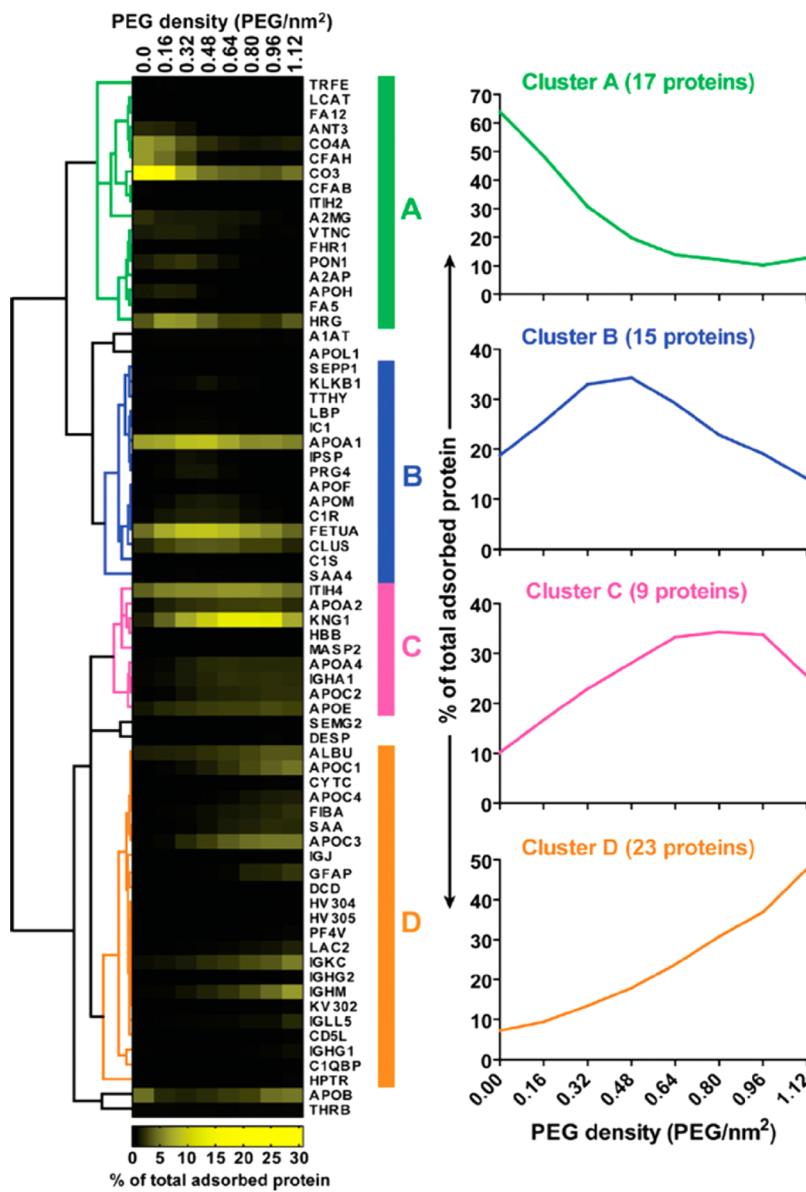


Figure 8. PEG density-based determination of the composition of the adsorbed serum protein corona on the surface of GNPs. Adsorbed serum proteins were isolated from 15 nm GNPs grafted with PEG at varying densities, purified, digested with trypsin, and characterized by LC-MS/MS. Proteins were clustered into one of four groups, A, B, C, or D (represented by the colored bars), based on the correlation of their relative abundance across PEG densities. PEG/nm^2 : number of PEG molecules per square nanometer. Reprinted from ref 181 with permission. Copyright 2012 American Chemical Society.

complement factor I, α -1-antiproteinase, and apolipoprotein E. These surface-bound proteins serve as linkers to further bind other serum proteins, such as BSA molecules, which adsorb with lower affinity onto iron oxide nanoparticles.¹⁸⁴

Like single-protein bindings, proteome binding by nanoparticles is strongly affected by the surface chemistry of nanoparticles. Low-density lipoprotein, very-low-density lipoprotein, and high-density lipoprotein bind strongly to QDs with cholesterol and atheronal-B modifications when compared to amine-modified QDs.¹⁷⁹ Poly(ethylene glycol) (PEG)-modified nanoparticles reduce protein binding. PEG chain length and density on polymeric nanoparticle surfaces regulate the amount of human plasma protein adsorption but not the binding pattern. The main bound proteins identified by 2D polyacrylamide gel electrophoresis analyses are albumin, fibrinogen, IgG, Ig light chains, and the apolipoproteins AI

and E. The reduced protein binding on nanoparticles coincides with decreased nanoparticle internalization by cells.¹⁸⁰ Stepwise increases in PEG density on nanoparticle surfaces shift the serum protein binding pattern in addition to the binding amount (Figure 8). An increase in PEG density reduces the internalization of GNPs into cells, similar to polymeric nanoparticles.¹⁸¹ Nanocapsules with longer PEG chains and higher PEG density also reduce complement system activation and uptake into J774A1 macrophage-like cells.¹⁸⁵ Polysaccharide modifications on the surface of poly(isobutylcyanoacrylate) nanoparticles reduce plasma protein binding and complement system activation.¹⁸⁶ MNPs with different sizes and surface chemistries bind different serum proteins without changing the protein secondary structure.¹⁸⁷ The PEG coating on these nanoparticles reduces both cell uptake and cytotoxicity. Smaller PEG-coated nanoparticles bind relatively more serum proteins

and display reduced cellular uptake. The composition of cell culture media also affects protein adsorption and cellular effects. Using GNPs as a model, Dulbecco's modified Eagle's medium elicited formation of a protein corona, while use of Roswell Park Memorial Institute medium resulted in different dynamics with reduced protein coating. Protein–nanoparticle complexes formed in the latter medium are more abundantly internalized by cells and cause greater cytotoxicity than those formed in the former medium.¹⁸⁸ Heat inactivation of serum affects protein corona formation and cellular uptake of protein–nanoparticles complexes because heating induces changes in serum composition and protein conformation.¹⁸⁹

3.3. Nanoparticle–DNA Interactions

Nanoparticles bind nucleic acids (e.g., DNA) in biological systems. The features of intermolecular forces involved in DNA binding are similar to those involved in nanoparticle–protein binding. Such interactions can be exploited for biomedical applications, including DNA loading on nanoparticles for gene transfer and siRNA delivery. Nanoparticle–DNA interactions depend on the properties of both the DNA molecules and the nanoparticles.

3.3.1. Determination of Nanoparticle–DNA Interactions. Nanoparticle–DNA interactions can be detected through determining complex formation, binding-induced DNA conformational changes, and DNA degradation.

The binding of DNA molecules to nanoparticles alters the properties of both components. For example, binding alters nanoparticle dispersibility and surface charge. Such changes can be detected with DLS and ζ -potential measurements.^{53,190,191} The nanoparticle–DNA complexes have altered electron absorption spectroscopic patterns, which can also be measured using UV–vis spectroscopy.^{192–194} DNA binding may increase the near-IR fluorescence of some nanoparticles. This increase can be used to monitor the binding process.¹⁹⁰ DNA molecules, when bound to nanoparticles, can be detected using XPS.^{20,195} By enhancing adenine and guanine ring breathing vibrations, surface-enhanced Raman spectroscopy (SERS) has been used to monitor DNA binding onto GNPs.¹⁹⁶ AFM detects subnanometer-scale phenomena and is used for the ultrastructural observation of nanoparticle–DNA complexes.^{190,193,197–199} Linear dichroism monitors the differential absorption of polarized light parallel and perpendicular to the orientation direction and enables determination of the approximate orientations of the DNA molecules on CNTs.^{200,201}

Fluorescence spectroscopy can be employed to determine DNA/nanoparticle binding affinity. Because of the weak intrinsic fluorescence emission of DNA, ethidium bromide (EB) or the low toxicity dye SYBR Green is used to label DNA molecules.^{53,202} The resonance light-scattering method has been used to detect metal ions and biomolecules.^{203,204} Resonance light scattering from nanoparticles and DNA molecules is weak, but the scattering intensity is enhanced dramatically when nanoparticles and DNA bind to each other, permitting quantification of nanoparticle–DNA interactions.^{191,205} Real-time polymerase chain reaction (RT-PCR) can be used to amplify short DNA fragments attached to nanoparticles to quantify their surface coverage.²⁰⁶ The adsorption and desorption processes of nanoparticle–DNA complexes can also be measured using electrochemical approaches, such as cyclic voltammetry.²⁰⁷ In some cases, the

nano particle–DNA complexes can be isolated by anion exchange chromatography.¹⁹³

Circular dichroism (CD) can be used to detect DNA conformational changes induced by nanoparticles.^{53,191,197,202,208–213} B–A and B–Z transitions can be identified by the appearance of characteristic CD bands.^{197,211} NMR provides more detailed structural information than CD.²⁰⁸ Conformational information can also be obtained by surface-enhanced infrared spectroscopy, which measures the vibrational modes of DNA molecules.²¹⁴ The structural stability of DNA can also be measured by its melting curve.^{53,197,206,208,210,212} DNA degradation can be evaluated by gel electrophoresis.^{198,201,208,209,215–217} In addition to experimental approaches, molecular simulations can predict nanoparticle–DNA binding patterns and nanoparticle-induced DNA conformational changes.^{218,219}

3.3.2. Current Understanding of Nanoparticle–DNA Interactions

3.3.2.1. Carbon Nanoparticles. Due to the large surface area of CNTs, DNA molecules bind to the surfaces of nanoparticles almost spontaneously.²¹⁸ This binding enhances the aqueous solubility of CNTs.^{193,220} Due to the unique shape and electronic properties of CNTs, the interaction modes between DNA and CNTs are different than those between DNA and other nanoparticles. DNA molecules can adsorb and wrap onto the nanotube through π – π stacking and hydrophobic interactions or even insertion into the central cavity.^{190,199,218,219} Such interactions also cause conformational changes in DNA molecules.^{190,197,208,211,214}

Both experimental evidence and molecular simulation indicate that DNA molecules form a helical structure before wrapping up CNTs.^{193,199} Optical absorbance measurements have revealed that anisotropic hypochromicity results from single-strand DNA/CNT interactions through π – π stacking interactions.¹⁹⁴ Due to π – π stacking interactions, DNA bases tend to orient themselves (Figure 9) facing the tube, as

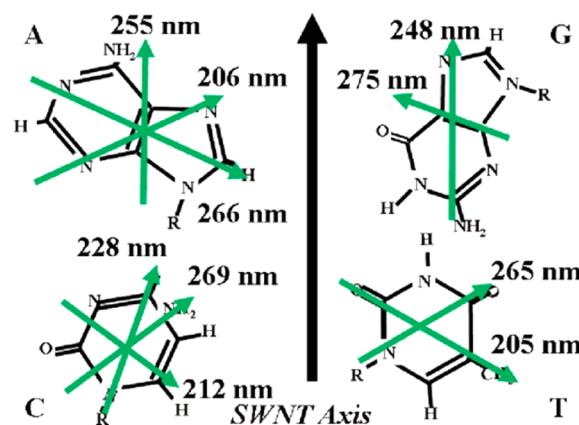


Figure 9. Orientation of the bound DNA bases relative to the SWNT axis. Green arrows and wavelengths indicate the directions and wavelengths of optical dipole transitions, respectively. Reprinted with permission from ref 194. Copyright 2007 American Chemical Society.

determined by investigating the interactions between CNTs and DNA homopolymers.¹⁹⁴ Although one study demonstrated that binding of CNTs with DNA is independent of DNA sequence,¹⁹⁸ the sorting and separation of CNTs by DNA has been shown to be affected by DNA sequence.¹⁹⁹ The separation of CNTs by $d(GT)_n$ ($n = 10–45$) is more efficient than separation by other sequences present in a single-strand

DNA library. DNA molecules with GT-rich sequences wrap individual nanotubes more effectively and form a helical structure.¹⁹⁹ Linear dichroism revealed that the DNA molecules lay flat on the nanotube surface with the backbone wrapping around the nanotube at an oblique angle of 45°.²⁰⁰

DNA/CNT binding induces DNA conformational changes. Binding of SWNTs to DNA induces B–A and B–Z (Figure 10) transitions.^{197,211,214,218} The B–Z transition modulates the

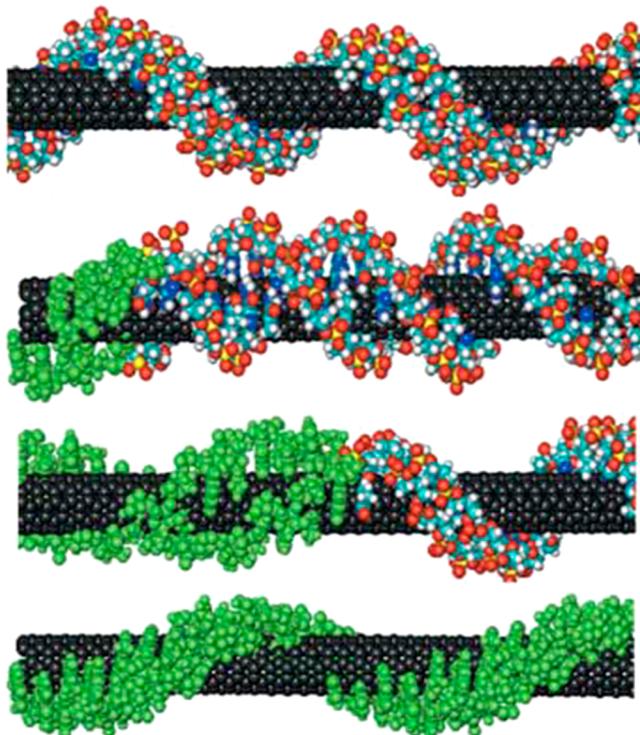


Figure 10. Illustration of DNA undergoing a conformational transition from B form (top) to Z form (bottom) on a carbon nanotube. Reprinted with permission from ref 211. Copyright 2006 American Association for the Advancement of Science.

dielectric environment of the SWNTs and decreases their near-IR emission. These changes have been detected in whole blood, tissue, and living cells.²¹¹ SWNTs inhibit DNA duplex association and induce telomeric i-motif formation by binding to the 5'-end major groove.²⁰⁸ In addition to CNT-induced DNA conformational changes, the binding of SWNTs affects DNA hybridization. DNA molecules adsorbed to SWNTs display much slower hybridization kinetics than free DNA.¹⁹⁰ Preadsorption on the SWNT surface causes a decrease in the free energy of the DNA, which increases the energy required for conversion of the DNA strands into the transition state. The process can be fitted well with a two-step Langmuir model.¹⁹⁰ The binding of DNA to CNTs can also alter the optical properties of SWNTs. Racemic SWNTs exhibit circular dichroism only when wrapped by DNA molecules. This induced circular dichroism occurs when transition dipole moments of optically active electronic transitions in the DNA molecule are coupled to transition dipole moments of the SWNTs.²¹³ The altered CD spectra can be used to characterize DNA–SWNT wrapping.

Other carbon nanoparticles also exhibit strong interactions with DNA molecules. For example, graphene oxide nanosheets and fullerene can cleave DNA strands when copper ions or

visible light is present.^{209,217} The magnitudes of the binding energy of bases with graphene are similar to those found in SWNTs. Interactions are also driven by hydrophobic interactions and π–π stacking.^{221,222} The binding of a DNA duplex to graphene oxide causes a partial deformation of the double helix.²²³ Therefore, a high binding affinity to DNA molecules may be a common feature of carbon-based nanomaterials.

3.3.2.2. GNPs. Interactions between GNPs and DNA molecules are mainly driven by electrostatic interactions.^{192,216,224} Formation of a complex between DNA and tiopronin-modified GNPs occurs in three steps:⁵³ the first step is diffusion-controlled formation of an external adduct; the second step is formation of DNA–GPN complex I based on their binding affinity; and the third step is formation of a more compact DNA–GPN complex II after DNA conformational changes. DNA–GPN interactions are influenced by the properties of the DNA and nanoparticles and the microenvironment.^{202,225} Small Au₅₅ clusters (size ≈ 1.8 nm) attach to the major grooves of B–DNA and cause a B–A transition. Au₅₅ nanoclusters are then degraded to 13-atom nanoclusters due to shrinkage of the major grooves. Au₁₃ nanoclusters and A–DNA ultimately form a wire-like structure.²⁴ In another study, GNPs with a diameter of 4.4 nm and coated with trimethyl(mercaptoundecyl)ammonium monolayer assembled into a one-dimensional (1D) chain along a DNA strand through electrostatic interactions between the cationic nanoparticle surface and the anionic DNA bases.¹⁹² The surface coverage of thiol-capped oligonucleotides bound to GNPs decreases from 30 to 13 pmol/cm³ when the nanoparticle diameter is increased from 13 to 30 nm.²⁰⁶ In addition to nanoparticle size, nanoparticle surface chemistry, salt concentration, and sonication affect DNA/GNP interactions.²²⁵

In addition to GNP-induced DNA conformational changes, nanoparticles also induce other changes in DNA molecules. Nanoparticles promote relaxation of supercoiled DNA under X-ray radiation,²²⁶ inhibit hybridization and transcription,^{20,227} induce double-strand separation,²²⁸ and enhance Raman signals from double-stranded DNA molecules.¹⁹⁶ These altered properties can be employed for DNA detection,²²⁹ protection of DNA from DNase I digestion,²³⁰ inhibition of the amplification of mismatched primer–template pairs, and enhancement of the specificity of allele-specific PCR.²³¹ Interactions between DNA and GNP can also be used to build novel nanostructures. For example, hybridization of complementary DNA is used to construct discrete GNP–QD nanostructures.²³² Organized DNA motifs are used to make ordered GNP arrays.²³³

3.3.2.3. Other Nanoparticles. Other nanoparticles also interact with DNA molecules through electrostatic and hydrophobic interactions. Linear QD chains are formed along double-stranded DNA through electrostatic interactions.²³⁴ Plasmid DNA bound to the QD surface can be released by intracellular glutathione (GSH) during transfection.¹⁹⁵ Calf thymus DNA molecules are adsorbed onto zirconia nanoparticles.²⁰⁷ Titanium oxide nanoparticles are widely used in sunscreen products, and their potential toxicity has received considerable attention.²³⁵ These nanoparticles bind to DNA molecules and cause DNA degradation due to generation of hydroxyl radicals on the nanoparticle surfaces.^{236,237} This DNA damages induce oxidative stress in living cells and animals.²³⁸ Similar to titanium oxide nanoparticles, copper nanoparticles cause DNA degradation through generation of singlet oxy-

gen.²³⁹ Ag and Pt nanoparticles inhibit DNA hybridization by disrupting hydrogen bonding between DNA double strands. This inhibition is weakened as the nanoparticle size increases.²⁰ Binding of calf thymus DNA to Ag nanoparticles induces conformational changes in DNA molecules.²¹⁰

Both cationic and anionic polymer nanoparticles bind DNA molecules and cause DNA conformational changes.^{191,212} Cationic poly-L-lysine-modified silica nanoparticles inhibit DNA transcription, and this inhibition is size dependent (Figure 11). DNA chains are adsorbed irreversibly to the

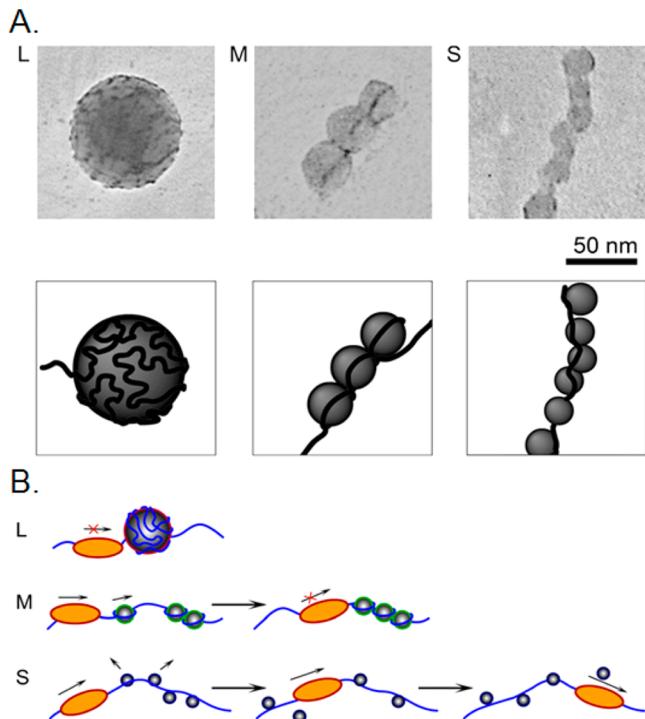


Figure 11. TEM of T4 DNA complexes with L (large, 40 nm), M (medium, 15 nm), and S (small, 10 nm) nanoparticles, and schematic representations of the complexes. Reprinted with permission from ref 240. Copyright 2007 Cell Press.

surface of nanoparticles (40 nm), inhibiting polymerase function. However, smaller nanoparticles (10 nm) form chains along DNA strands and might eventually dissociate from the DNA strand. This allows the DNA molecules to become accessible to the polymerase, and transcription proceeds.²⁴⁰

Interactions between nanoparticles and DNA molecules share some common features with nanoparticle–protein interactions. In both cases, biomolecules are adsorbed to the nanoparticle surface and binding induces conformational changes in these molecules. Nanoparticle–DNA interactions can also be used to develop advanced nanoparticle–DNA hybrids to control cellular processes for biomedical and biosensing purposes.

3.4. Adsorption of Crucial Small Molecules by Nanoparticles

Due to their high surface energy, nanoparticles also adsorb various small molecules in biological systems. These molecules include but are not limited to carbohydrates, vitamins, hormones, amino acids, and nucleic acid bases.^{54,241–245} These small molecules normally play essential roles in cell signaling and cell physiology. A sudden depletion of these

molecules may influence proper cellular functions and cause toxicity.^{54,244,246} Small molecules are adsorbed onto nanoparticles through molecular interactions, such as hydrophobic, π – π stacking, and electrostatic interactions.^{54,247} Although the chemical bases of nanoparticle/protein interactions and nanoparticle/amino acids interactions are similar, the latter is simpler and faster and does not involve conformational alterations.

SWNTs adsorb various amino acids (e.g., Arg, His, Met, Phe, and Tyr), vitamins (folate, riboflavin, and thiamine), and phenol red in cell culture media.⁵⁴ An analysis of the structures of the adsorbed molecules indicates that π – π interactions and electrostatic interactions are likely the driving forces for adsorption. The SWNT-induced depletion of amino acids and vitamins in cell culture medium reduces cell viability. Spectroscopic studies have revealed that both SWNTs and MWNTs affect the cell culture medium in this manner.^{244,248} A study of the adsorption of folic acid and vitamin B1 by carbon fibers with modified nanopores revealed that the pore structure and surface modifications influenced the adsorption capacity and adsorption rate.²⁴⁵

Computational chemistry reveals that the curvature of nanoparticles and the polarizability of amino acids determine binding strength.²⁴⁷ Doping the carbon surface with calcium atoms results in a dramatic enhancement of binding of collagen amino acids (Gly, Pro, and Hyp) to graphene. Electronic charge transfers from the calcium atoms (donor) to graphene (acceptor), and the carboxyl group of the amino acid (acceptor) may contribute to this enhancement.²⁴⁹

4. NANOPARTICLE–CELL INTERACTIONS

The cell is the elemental unit of all living organisms. Nanoparticles perturb living systems by interacting and altering live cells. Such perturbations occur at various levels. Nanoparticles are first adsorbed to the cell surface and then internalized. As the nanoparticles are endocytosed and transported within cells, the cells undergo structural and functional changes. Some crucial processes, such as oxidative balance, are perturbed. Eventually, global alterations occur that involve cell signaling, genomic, proteomic, and metabolomic processes.

4.1. Cellular Uptake and Intracellular Transport of Nanoparticles

Because of their sizes and surface properties, nanoparticles can enter various organisms and cells, including mammalian cells, plant cells, bacteria, fungi, and viruses.^{250–254} These processes can be energy-dependent intake or energy-independent insertion.^{255,256} The former is also referred to as endocytosis, a process by which cells take up molecules or particles by engulfing them.²⁵⁷ Nanoparticles enter cells by taking advantage of various known endocytic pathways, such as clathrin-mediated endocytosis, caveolae-mediated endocytosis, phagocytosis, and macropinocytosis.^{256,258–261} Direct penetrations occur for 1D nanostructures or strongly positively charged nanoparticles.^{255,262} After entering cells, nanoparticles may localize in endosomes, lysosomes, the cytoplasm, mitochondria, the endoplasmic reticulum, or the nucleus, depending on the nature of the nanoparticle.^{256,258–261,263–266} Various imaging techniques have been developed to monitor the cell uptake process, in addition to conventional TEM and confocal laser scanning microscopy (CLSM) analyses.^{267,268} For example, X-ray fluorescence microscopy is used to determine chemical

element distribution of nanoparticles in cell.²⁶⁹ Magneto-photoacoustic imaging permits the differentiation of membrane-adhered or endocytosed nanoparticles.²⁷⁰ Dynamic colocalization microscopy permits the spatiotemporal characterization of internalized nanoparticles.²⁷¹ Raman spectral imaging maps vibrational bands of nanoparticles in live cells in the absence of an external label.²⁷² Atomic force microscopy (AFM) measures the force between nanoparticles and the cell surface, indicating receptor binding and binding strength.^{273–275}

4.1.1. Kinetics and Thermodynamics of the Cellular Uptake of Nanoparticles. Endocytosis of nanoparticles is a dynamic process, and endocytosis and exocytosis processes eventually reach thermodynamic equilibrium. Endocytosis likely involves at least two steps: binding of the nanoparticles to the cell surface followed by internalization of the nanoparticles into cells (Figure 12).²⁷⁶ Binding parameters

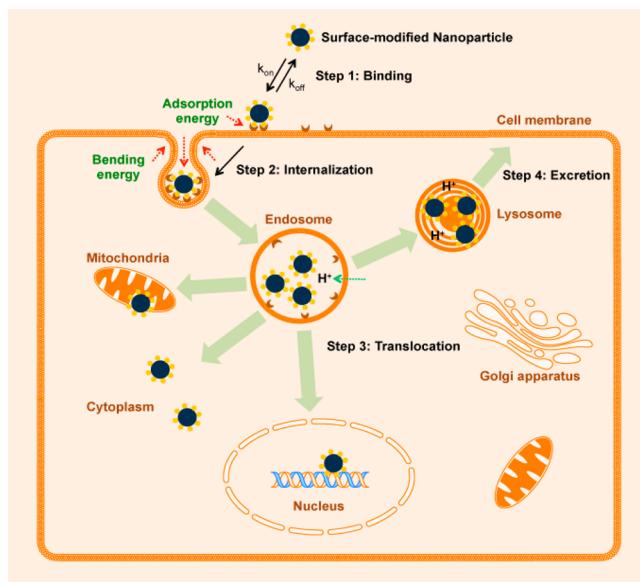


Figure 12. Receptor-mediated cellular uptake and translocation of nanoparticles.

derived from the binding of anionic MNPs to RAW macrophages and HeLa cells reveal that macrophages and tumor cells behave equally with respect to cell surface binding, although they differ with respect to internalization capability. Adsorption onto the cell surface is a slow step, and internalization is fast.^{277–279} Nanoparticles are eventually exocytosed from living cells. The exocytosis rate of SWNTs is reported to closely match their endocytosis rate with negligible temporal offset.²⁸⁰ However, the uptake of dye-labeled polystyrene nanoparticles is reported to increase with time and be essentially irreversible. The fluorescence signal decreases when cells divide.²⁶⁸ This controversy suggests that cell uptake kinetics may depend on both nanoparticle properties and cell type. Endocytosis of nanoparticles is also under thermodynamic control. Thermodynamic studies have revealed that adhesion strength governs receptor-mediated cell uptake. Endocytosis is determined by the enthalpic and entropic limits of the adhesion strength.²⁸¹ On the basis of the energy requirements for a receptor-mediated uptake process, the optimal size of nanoparticles is 25–30 nm.²⁸² When the nanoparticles are too small (less than 20 nm),

endocytosis is limited because the adhesion energy is too low to compensate for the bending energy of the cell membrane. When the nanoparticles are too large (greater than 60 nm), the free receptors on the cell membrane are depleted and nanoparticles may be only partially wrapped without endocytosis. Here, size refers to the apparent size after aggregation. A further understanding of the kinetics and thermodynamics of the cellular uptake of nanoparticles will assist future nanoparticle design and applications.

4.1.2. Determinants of the Cellular Uptake of Nanoparticles.

Many physicochemical and biological factors govern the cellular uptake of nanoparticles. These factors include the size, shape, surface chemistry, surface charge, and mechanical properties of nanoparticles as well as cell type. The combination of these factors affects the cell surface binding strength,^{273,275} cellular entrance route,^{283,284} uptake amount,^{278,285–290} and intracellular distribution.^{283,291,292} SWNTs with lengths of 100–200 nm can be internalized into cells through clathrin-coated pits. SWNTs with lengths of 50–100 nm enter cells through caveolae-mediated endocytosis, and some can enter the nucleus. The shortest (less than 50 nm) SWNTs can enter cells through direct insertion, which is energy independent.²⁸³ Studies of GNPs (4–17 nm) in HeLa cells have revealed that the uptake force increases with the size of the nanoparticles.²⁷⁵ The shape of the nanoparticle may also play a role. Gold nanospheres enter fibroblasts and tumor cells more efficiently than nanorods,^{293–295} while nanorods are internalized more efficiently than nanospheres in blood phagocytes.²⁹⁶ Particle replication in nonwetting template (PRINT) nanoparticles with a higher aspect ratio enter HeLa cells more readily than those with a lower aspect ratio.²⁹⁷ The surface charge on nanoparticles can play a significant role in cellular uptake. Due to the presence of phosphate groups on lipids, cell membranes are negatively charged. Nanoparticles with positive charges display strong interactions with cells compared with those with negatively charged or neutral surfaces.^{292,298}

Furthermore, the structure, density and distribution of surface chemical groups all play significant roles in determining the cellular uptake of nanoparticles. For peptide-functionalized GNPs, an aromatic structure at the end of the peptide enhances their cellular uptake.²⁹⁹ A higher density of oligonucleotide loading on the surface of GNPs generates higher cellular uptake, while a higher density of PEG reduces GNP uptake.^{181,287} GNPs that have striations on the surface with alternating anionic and hydrophobic groups can penetrate the plasma membrane without disrupting it.²⁹⁹ The nanoparticle surface ligand induces protein corona misfolding and therefore indirectly enhances cellular uptake.³⁰⁰ A molecular simulation study has proposed that the effective surface charge density determines cell uptake.³⁰¹ An experimental study of a surface-modified GNP array indicated that the effective surface charge density determines the electrostatic interactions between the positively charged nanoparticles and the negatively charged cell membrane (Figure 13).³⁰²

Shape is another significant factor in the cellular uptake process. For 1D nanotubes, the uptake process is spontaneous when the orientation favors insertion, while 2D nanosheets prefer to adhere to the cell surface.^{255,303} A nanosphere and nanodisk cell uptake comparison study demonstrated that nanodisks mainly adhere to the cell surface, while nanospheres are easily internalized into cells (Figure 14).²⁵ A similar conclusion was reached in a subsequent study. Nonspherical

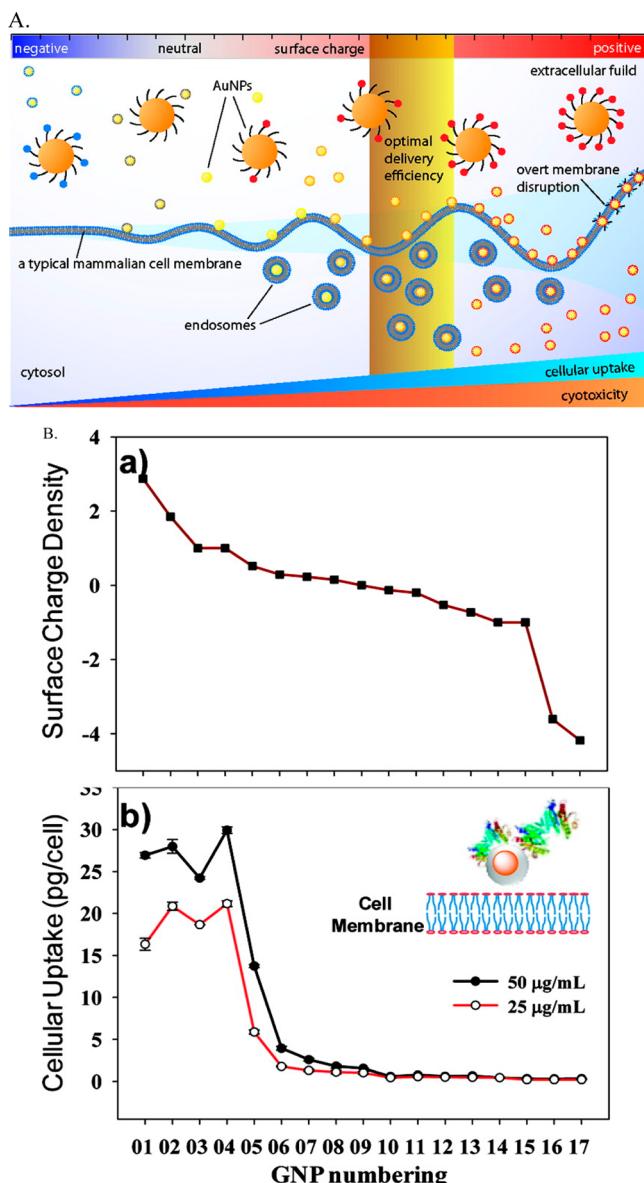


Figure 13. (A) Schematic illustration of the effect of GNP surface charge on their cellular uptake by a typical mammalian cell. (B) Electrostatic attraction between nanoparticles and cells. (a) Surface charge density of a GNP library. (b) Cellular uptake of these GNPs in HeLa cells, as determined by ICP-MS. Reprinted with permission from refs 301 and 302. Copyright 2010 and 2012, respectively, American Chemical Society.

polymeric nanoparticles exhibit reduced cellular uptake compared to their spherical counterparts. The larger average curvature radius of adsorbed nonspherical particles may be responsible for this difference.³⁰⁴ However, 1D superparamagnetic iron oxide nanoworms showed enhanced tumor-targeting efficiency compared to spherical nanoworms.³⁰⁵ This controversy indicates effects of nanoparticle shape on cell uptake are material dependent.

The mechanical properties of nanoparticles also influence the rate, route, and amount of cellular uptake.^{306,307} Soft nanoparticles enter cells through macropinocytosis, while stiff nanoparticles enter through clathrin-mediated endocytosis. Nanoparticles with an intermediate stiffness have multiple internalization routes.³⁰⁶ The aggregation and sedimentation of

nanoparticles in physiological environments also impacts cellular uptake. More nanoparticles are taken up in an upright cell culture configuration than in an inverted one, and nanoparticles with faster sedimentation rates display greater differences in uptake between the two configurations, suggesting that sedimentation needs to be considered for cellular uptake studies of heavy or large nanoparticles.^{278,308,309} The different protein binding properties of nanoparticles often indicate different nanoparticle–cell interactions.^{287,293,309} The pericellular matrix enhances the retention and cellular uptake of nanoparticles due to the restriction and deceleration of diffusion by the entrapment and accumulation of nanoparticles.³¹⁰ In summary, multiple factors collectively determine the cellular uptake of nanoparticles and their subsequent bioactivity in cells.

4.2. Cellular Substructural and Functional Alterations by Nanoparticles

Nanoparticles are translocated to different compartments in live cells and may interact with relevant subcellular structures and affect their function. Cell membranes, ion channels, the cytoskeleton, mitochondria, and the nucleus have all been reported to be affected by nanoparticles.

4.2.1. Nanoparticle-Induced Cell Membrane Disruption. The cellular uptake of nanoparticles results in disruption of the plasma membrane. Membrane disruption likely involves several physicochemical interactions. Electrostatic interactions of positively charged nanoparticles with negatively charged groups on the membrane surface alter cell membranes. Membrane penetration of positively charged nanoparticles into the cytoplasm causes formation of holes within 1–100 ms, followed by a slow resealing in tens of seconds.³¹¹ Interactions between positively charged nanoparticles with negatively charged membrane also causes membrane depolarization and perturbation of membrane potential. Furthermore, an increase in $[Ca^{2+}]$ influx inhibits cellular proliferation.³¹² Membrane disruption of red blood cells and perturbation of their hemolytic activity by amorphous and mesoporous silica nanoparticles have been attributed to silanol groups that are accessible to the membrane.³¹³ Nanoparticle-induced generation of ROS may also cause membrane damage. A water-soluble fullerene suspension disrupts cellular functions through lipid peroxidation by ROS. Upon addition of an antioxidant, the oxidative damage can be completely prevented.³¹⁴ Nanoparticle shape may also serve a role in membrane perturbation. Needle-shaped nanoparticles, rather than sphere or flat nanoparticles, cause a transient disruption of the endothelial cell membrane.³¹⁵ Stimulation such as near-infrared (NIR) radiation cause cavitation of the membrane, followed by an increased influx of $[Ca^{2+}]$ and a degradation of the actin network.³¹⁶ By expanding our understanding of the various mechanisms of interactions between nanoparticles and the cell membrane, reduction of membrane disruption will be achieved through the regulation of size, shape, surface geometry, surface charge, and surface chemistry.^{46,313,317,318}

4.2.2. Ion Channel Inhibition by Nanoparticles. Ion channels consist of pore-forming proteins that help establish and control a small voltage gradient across the cell membrane.³¹⁹ When nanoparticles reach the cell surface, they may affect ion channel function by interacting with the extracellular domains of ion channel proteins. SWNTs reversibly block the K^+ channel in a diameter-dependent manner, most likely by interacting with extracellular domains of

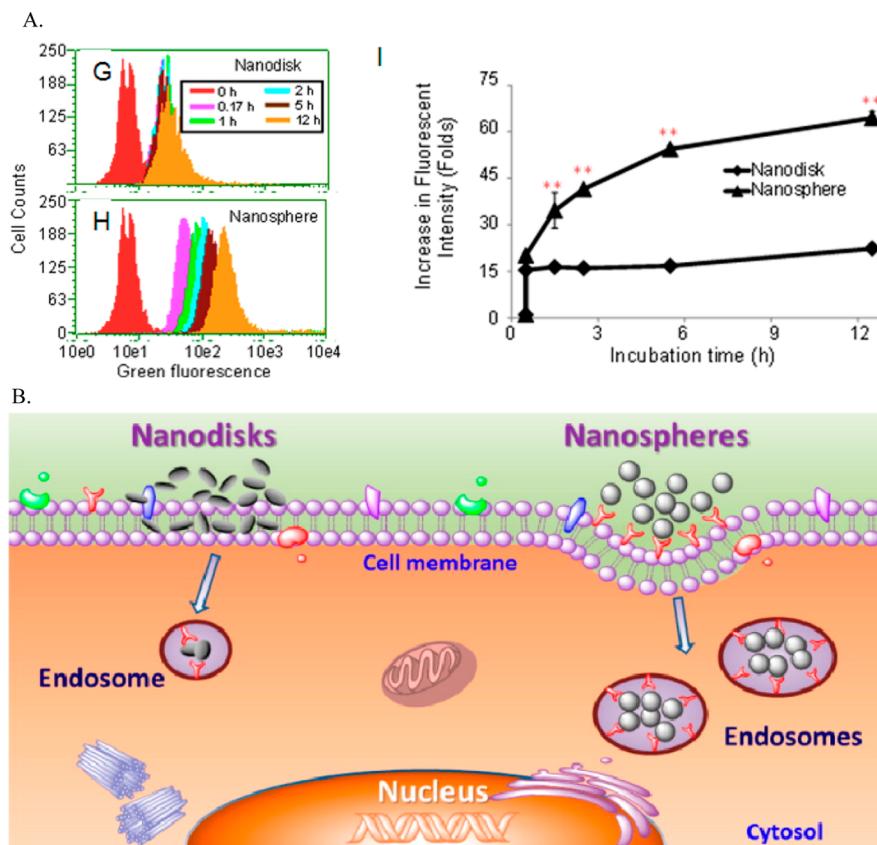


Figure 14. Changing polystyrene nanoparticles from a three-dimensional (3D) spherical shape to a 2D disk shape promotes their cell surface binding, with significant reduction of cell uptake. (A) Experimental evidence of a nanodisk reducing cellular uptake. (B) Model for reduced cellular uptake but enhanced cell surface binding by nanodisks. Reprinted with permission from ref 25. Copyright 2012 American Chemical Society.

the channel.³²⁰ In contrast to pristine SWNTs, SWNTs modified with 2-aminoethylmethane thiosulfonate inhibit the K⁺ channel irreversibly, suggesting possible bonding between functional groups on SWNTs and the cysteine groups in ion channels.³²¹ Carboxylated MWNTs suppress the K⁺ channel in PC12 cells without induction of ROS.³²² Molecular simulations indicate that there are multiple C60 binding sites on K⁺ channels. The energetically favorable bindings between C60 and K⁺ channels cause ion channel inhibition and cytotoxicity.³²³ Although direct interactions between nanoparticles and ion channels are observed, impurities may also perturb channel proteins. Contamination with yttrium, which is used as a catalyst in the production of CNTs, has been shown to inhibit ion channels.³²⁴ Thus, nanoparticle–ion channel interactions must be studied with care.

4.2.3. Cytoskeleton Alterations by Nanoparticles. The cytoskeleton maintains the cell shape and serves significant roles in intracellular transport, force generation, cell motility, and division.¹⁸ The internalization of nanoparticles results in direct or indirect cytoskeletal alterations. The loss of proper cytoskeletal function further leads to reduced cell motility, division, and proliferation.^{325–329} SWNTs induce actin bundling in both isolated actin filaments and HeLa cells. This induces actin-related cell division defects and retards cell proliferation. A weak binding enthalpy between SWNTs and actin filaments has been speculated to arise because both structures are anisotropic and similar in size. Such interactions induce dramatic anisotropic actin bundle formation both in cells and *ex vivo*.³²⁵ Nanoparticles may also affect the cytoskeleton through indirect interactions. Surface-modified

iron oxide nanoparticles and gelatin nanoparticles cause a reorganization of the F-actin and β-tubulin cytoskeletons in human fibroblasts after endocytosis. Because the actin cytoskeleton plays important roles in receptor-mediated endocytosis, the different nanoparticle-induced alterations in cytoskeleton organization may indicate different mechanisms of internalization.^{327,328} Gold nanorods coated with hexadecyltrimethylammonium bromide cause actin aggregation and β-tubulin redistribution in the cytosol. Such effects reduce micromotion (fluctuations in cell shape) in MDCK-II kidney cells. Coating of nanorods with PEG diminishes such perturbations.³²⁶ High concentrations of surface-modified iron oxide nanoparticles affect cytoskeleton and focal adhesion formation and maturation. The reduced expression and decreased activity of focal adhesion kinase in turn alters the actin cytoskeleton. Large endosomes that trap nanoparticles may also affect the normal localization of actin fibers and disrupt the actin cytoskeleton through steric hindrance.³²⁹

4.2.4. Mitochondrial Alterations by Nanoparticles. Mitochondria serve as “cellular power plants” by supplying chemical energy (ATP) to cells.¹⁸ They are also involved in a range of other processes, such as signaling and cell differentiation. Nanoparticles enter the mitochondria and may affect their functions.^{330–333} SWNTs have been identified inside mitochondria.³³³ GNPs (3 nm) enter mitochondrial intermembrane spaces through voltage-dependent anion channels.³³⁴ The direct interaction of nanoparticles with mitochondria has been detected using SERS. SERS revealed that GNPs (13 nm) may bind to proteins, lipids, and carbohydrates present on the mitochondrial membrane in live cells and in isolated

mitochondria. The bands at 886, 875, and 1070 cm⁻¹ are due to C–C and PO₂⁻ vibrations. Changes in these bands indicate interactions between nanoparticles and membrane lipids. However, these interactions do not cause damage to the mitochondria.³³¹ GNPs with a much smaller size (1.4 nm) cause indirect damage by increasing the permeability of the inner mitochondrial membrane through generation of oxidative stress.³³⁰

4.2.5. Nuclear Alterations by Nanoparticles. The nucleus maintains the integrity of genes and controls cellular activity cells by regulating gene expression. The nuclear structures include the nuclear envelope, which consists of a double lipid bilayer, the nuclear lumina, which consists of intermediate filaments and membrane-associated proteins, the chromosome, which consists of DNA and histones, and the nucleolus, which consists of rRNA, rDNA, and proteins.⁴⁹ Because the nuclear envelope keeps many macromolecules from entering the nucleus, nanoparticles entry into the nucleus is limited unless the nanoparticles are extremely small (<5 nm) or engineered with nuclear targeting molecules.^{335–338} Unmodified QDs (3.2 nm) are able to accumulate in the nucleus and exhibit strong affinity to core histones and histone-rich cell organelles. Histone binding is driven by electrostatic interactions between negatively charged QDs and positively charged core histone proteins. The interaction causes a red shift of the steady-state photoluminescent emission of the QDs and a decrease in the fluorescence lifetime.³³⁵ GNPs (2.5 nm) may disturb the decondensation of nuclear chromatin in mouse sperm, indicating their potential spermatotoxicity. Such effects are likely driven by interactions between GNPs with DNA in the nucleus.³³⁷ Elucidation of nanoparticle–nucleus interactions provides crucial insight into the biological activity of nanoparticles; however, further investigation is still warranted.

4.3. Oxidative Stress and Nanoparticles

Cells maintain a homeostatic oxidative state and counterbalance external stimuli-induced oxidative stress through several regulation mechanisms.³³⁹ When these regulating mechanisms are overwhelmed, the excessive ROS or reactive nitrogen species (RNS) can damage cellular components, affect biomolecule function, and ultimately induce cell death through apoptosis or necrosis. Nanoparticles may affect the cellular oxidative state in various ways. Generation of ROS in cells is considered a major factor in nanoparticle-induced cytotoxicity.³⁴⁰ However, nanoparticles with reductive properties may act as free radical scavengers to remedy oxidative damage in cells and tissues.³⁴¹

4.3.1. Oxidative Stress Induced by Nanoparticles. Production of nanoparticle-induced ROS is triggered by electronically active nanoparticle surfaces, photoactivation, impurities, dissolution of metal ions, and interactions of nanoparticles with biomolecules.^{342,343} Nonfluorescent dichlorofluorescin can be oxidized to fluorescent dichlorofluorescein inside the cell by various oxidants, and this reaction can be used to detect the total oxidative level in live cells.³⁴⁴ ROS generation can also be measured by free radical content, GSH level, mitochondrial membrane potential, stress-related gene/protein expression, microarray, and stress-specific reporter assays.^{330,340,345–349}

Most nanoparticles cause oxidative stress. Although the outcome is similar, the mechanisms may be material dependent. First, GNPs with a diameter of 1.4 nm induce oxidative stress and reduce intracellular antioxidant levels. The oxidative stress

causes mitochondrial damage, an upregulation of stress-related genes, and cell necrosis.³³⁰ Generation of ROS by GNPs upon short-term (4 h) exposure can be counterbalanced by cells; however, longer exposure (48 h) can overwhelm antioxidant defenses.³⁵⁰ GNPs also catalyze nitrogen monoxide (NO) production from endogenous S-nitrosothiols in serum through formation of Au–thiolate on the surface of nanoparticles.³⁴² Second, TiO₂ nanoparticles, which are widely used in cosmetics, cause oxidative stress with or without light activation. TiO₂ nanoparticles absorb UV light and catalyze DNA damage in human cells.²³⁸ In the dark, TiO₂ nanoparticles induce ROS generation, a decrease in GSH, upregulation of stress-related genes, activation of JNK and p53, and apoptosis.^{351,352} TiO₂ nanoparticles generate O₂[•] in sunlight and HO[•] free radicals in the dark.³⁵³ Third, carbon black (CB) nanoparticles induce oxidative stress by producing highly diffusible 4-hydroxyonenal. This species mediates cytotoxicity through formation of 4-hydroxyonenal–protein adducts and GSH modification.³⁵⁴ CB nanoparticles enhance the oxidative stress induced by Fe₂O₃ nanoparticles in cell culture through coexposure. However, surface-oxidized CB nanoparticles have no such effects, indicating that the oxidative stress is mediated by the surface reactivity of CB.³⁴⁸ Fourth, CNTs induce ROS-related inflammasome activation.³⁵⁵ Metallic impurities from the CNT synthesis process, if present, may be responsible for generation of oxidative stress.³⁵⁶ Surface chemistry modifications by PEG reduce ROS and cytotoxicity.³⁵⁷ Fifth, fullerene (C60) causes membrane damage and cytotoxicity through ROS generation and lipid peroxidation. An antioxidant, L-ascorbic acid, completely prevents such oxidative damages.³¹⁴ Sixth, other nanoparticles, including ZnO, silica, polystyrene, nickel ferrite, silver, and nano-Co, have also been reported to induce oxidative stress, mitochondrial damage, and apoptosis.^{346,347,358–361}

A nanoparticle's potential to generate oxidative stress depends on the nature of the materials. At the same dose, different nanomaterials cause different levels of oxidative stress. For example, ZnO, CuO, and CdS nanoparticles generate a higher level of ROS than that of TiO₂, MgO, SiO₂, CNTs, and Fe₃O₄ nanoparticles.^{362–366} CeO₂ can even protect cells from oxidative damage.³⁶² Due to the significance of nanomaterials' intrinsic properties, a predictive paradigm for oxidative stress has been developed based on metal oxide nanoparticle band gaps.³⁶⁷ Critical physicochemical effectors are discussed below.

The crystal structure of nanoparticles appears to play a role in ROS generation. Rutile TiO₂ nanoparticles cause generation of ROS and cell apoptosis, while anatase TiO₂ nanoparticles induce necrosis with reduced ROS generation.³⁶⁸ The size and surface area of nanoparticles affect ROS generation. Smaller nanoparticles with larger surface areas generate much more ROS than larger nanoparticles.^{369–371} Surface chemistry can regulate oxidative stress generation. Cellular ROS generation by surface-modified silicon nanoparticles occurs in the following order: positively charged > neutral > negatively charged.³⁷² Surface-modified ZnO nanoparticles generate ROS in the following order: uncoated > oleic acid coated > poly(methacrylic acid) coated > protein coated.³⁷³ Nanoparticles with hydrophobic molecules on the surface cause oxidative stress in the cell. GNPs coated with longer hydrophobic alkyl chains cause much more ROS generation and cytotoxicity than those with shorter hydrophobic chains.³⁷⁴ PEG modification on the surface reduces oxidative stress caused by SWNTs.³⁵⁷ Some environmental factors may impact cellular ROS production. For

instance, exposure to light enhances generation of ROS in cells incubated with TiO_2 nanoparticles.³⁶⁴ Fe_2O_3 or CB nanoparticles alone do not cause cellular oxidative stress. However, coapplication of these nanoparticles induced a 2-fold increase in the oxidation of cellular proteins. Fe_2O_3 nanoparticles in endosomes or lysosomes are acidified to release Fe^{3+} ions, which do not generate hydroxyl radicals in the presence of H_2O_2 . However, CB nanoparticles reduce Fe^{3+} ions to Fe^{2+} ions. Fe^{2+} ions can permeate the vesicle membrane, take part in the Fenton reaction, and induce protein oxidation.³⁷⁵ In addition to these physicochemical factors, cells from different organs have different capabilities of internalizing nanoparticles and resisting oxidative stress.³⁷⁶

4.3.2. Reduction of Oxidative Stress by Nanoparticles.

Several nanoparticles have free radical scavenging capabilities, which originate from their chemical structures. These effects may be potentially applied in antioxidative therapy. Cerium oxide (ceria) nanoparticles, fullerene and its derivatives, CNTs, and heavy metal (Au and Pt) nanoparticles have been reported to reduce ROS through various mechanisms.^{341,377–386}

Ceria nanoparticles inhibit inflammatory mediator production and reduce cell apoptosis. These nanoparticles protect rats against oxidative damage by scavenging free radicals.^{378,381,384} When ceria nanoparticles are doped with samarium, which decreases Ce^{3+} without affecting the oxygen vacancy (defect in oxides) contents, their antioxidant and antiapoptotic abilities are reduced. This decrease indicates that the $\text{Ce}^{3+}/\text{Ce}^{4+}$ redox reaction and not oxygen vacancy is responsible for the antioxidant activity of ceria nanoparticles.³⁸¹ Fullerene and its derivatives have the ability to quench superoxide radicals.^{377,379} CNTs are able to scavenge free radicals and retard the oxidation reaction.^{380,385} Doping of boron on CNTs enhances the efficiency of radical scavenging, indicating that electron affinity may be responsible for the effect. Au and Pt nanostructures have also been reported to display antioxidant activities.^{382,383,386} Pt nanoparticles exhibit antioxidant activity by scavenging $\text{O}_2^{\cdot-}$ and HO^{\cdot} with a second-order reaction rate.³⁸² Au- and Pt-supported nanodiamonds exhibit a high antioxidant activity, as indicated by their peroxidase activity and the ability to trap carbon radicals.³⁸³ Apoferritin-coated Pt nanoparticles enter cells through receptor-mediated endocytosis, decrease hydrogen peroxide-induced oxidative stress, and reduce cytotoxicity.³⁸⁶

4.4. Nanoparticle-Induced Systematic Cellular Biochemical Perturbations

Cells have complex biochemical networks that maintain homeostasis during proliferation, differentiation, and apoptosis. Any extrinsic factors, such as nanoparticles, may interfere with these processes. Therefore, understanding nanoparticle-induced cellular biochemical perturbations to elucidate the chemical mechanisms of nanoparticle–biological system interactions is imperative.

4.4.1. Perturbation of Cell Signaling Pathways by Nanoparticles. Nanoparticles perturb many cellular signaling pathways. These pathways are involved in important biological processes that determine cell fate. The delineation of these mechanisms will facilitate an understanding of the cytotoxicity of nanoparticles and development of safe nanomedicines and nanomaterials.

4.4.1.1. Mitogen-Activated Protein Kinase (MAPK) Signaling. MAPK signaling is a significant regulatory mechanism in eukaryotic cells. The MAPKs are serine/threonine-specific

protein kinases that respond to external stimuli, such as mitogen, heat shock, and oxidative stress. The MAPK signaling pathway regulates cell proliferation, apoptosis, morphogenesis, cell cycle, and immunity.³⁸⁷

There are three groups of kinases in MAPK signaling. The first group, extracellular signal-related kinase (ERK), is activated by growth factors, mitogen, and G-protein-coupled receptors (GPCRs). Carbon nanoparticles activate Erk1/2 in multiple cell lines.³⁸⁸ ERK is also activated by silica and iron oxide nanoparticles.^{389,390} Activation of Erk1/2 by iron oxide nanoparticles promotes neurite outgrowth in PC12 cells.³⁹⁰ The second group, p38 MAPK, and the third group, JNK, are activated by stress, GPCRs, and inflammatory cytokines. GNPs can promote osteogenic differentiation through activation of p38 MAPK.²¹ TiO_2 nanoparticles activate JNK and p53 in neuronal cells and cause G2/M cell cycle arrest and apoptosis.³⁵¹ Silica nanoparticles activate p38 and JNK and reduce cell viability.³⁹¹ Not all nanoparticles activate MAPK signaling. Carboxyl-modified fullerene nanoparticles enter oxidative stress-damaged cerebral microvessel endothelial cells, maintain cell integrity, and inhibit apoptosis. Such effects are the result of inhibiting the JNK pathway by nanoparticles.³⁹²

4.4.1.2. Nuclear Factor Kappa B (NF- κ B) Pathway. The NF- κ B pathway is an important cellular signaling pathway in response to external stimuli. Under normal circumstances, NF- κ B forms a stable complex with I κ B in the cytoplasm. When cells are exposed to stimuli, such as stress, free radicals, or irradiation, the kinase IKK is phosphorylated (activated) by receptors. The activated IKK subsequently phosphorylates I κ B. Phosphorylated I κ B undergoes ubiquitous degradation, and NF- κ B is released and translocated to the nucleus to initiate transcription of inflammatory factors.³⁹³ Silver nanoparticles, CNTs, silica nanoparticles, TiO_2 , and double hydroxide ($\text{Mg(OH)}_2:\text{Al(OH)}_3$ 1:1) nanoparticles have been reported to activate NF- κ B signaling and induce generation of pro-inflammatory cytokines including COX-2, TNF- α , IL-6, IL-12, and inducible nitric oxide synthase.^{47,389,394–398} The chemical basis for the activation of NF- κ B signaling by different nanoparticles is that all of these nanoparticles generate ROS in cells. Activation of NF- κ B finally leads to a reduction of cell proliferation, inflammation, and apoptosis. Surface modifications on MWNTs direct the receptor recognition from mainly mannose receptors to scavenger receptors. Activation of NF- κ B through the mannose receptor is alleviated, and the immunotoxicity of MWNTs is reduced.⁴⁷ This study demonstrates that activation of cell signaling by nanoparticles can be regulated through modulating receptor recognition events by surface chemistry modifications.

4.4.1.3. DNA Damage and Repair. The cellular ROS generated by nanoparticles may cause DNA damage and activate DNA repair signals.³⁹⁹ Such damage may induce G2/M cell cycle arrest, apoptosis, mutagenesis, and tumorigenesis.^{400–404} Therefore, an understanding of nanoparticle-induced DNA damage and its repair is critical. MWNTs increase the expression of the base excision repair protein OGG1, the double-strand break repair protein Rad 51, the phosphorylation of p53 by the checkpoint protein kinase ChK2, the phosphorylation of histone H2AX at serine 139, and SUMO modification of XRCC4 (in response to DNA double-strand breakage) in mouse embryonic stem cells.⁴⁰² Similarly, nanodiamonds and silver nanoparticles also induce DNA

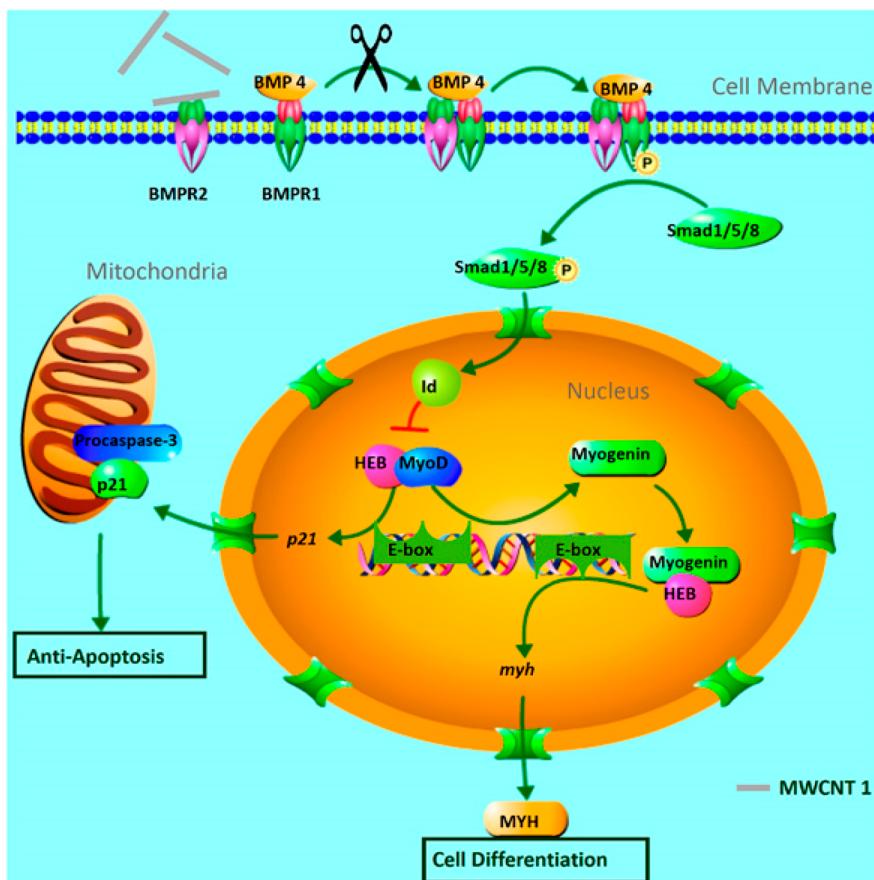


Figure 15. Schematic diagram showing the molecular interactions involved in MWNT 1-enhanced cell differentiation and apoptosis inhibition. Reprinted with permission from ref 406. Copyright 2012 Nature Publishing Group.

damage, as demonstrated by increased expression of OGG1 and Rad51 and phosphorylation of p53 and H2AX.^{400,401,403}

4.4.1.4. Bone Morphogenetic Protein (BMP) and Transforming Growth Factor beta (TGF- β) Signaling. BMP receptors belong to the TGF- β receptor superfamily.⁴⁰⁵ CNTs suppress BMP signaling pathways and affect cell function (Figure 15).^{406–408} Suppression of BMP signaling reveals a nonapoptotic mechanism for the perturbation of cell proliferation by CNTs. A global examination of gene expression and Western blotting revealed that the Id (inhibitor of DNA binding) genes and proteins are suppressed by SWNTs and MWNTs. Id genes are direct targets of Smad-dependent BMP receptor signaling through activation of Smad 1/5/8 proteins. The activated Smad proteins translocate into the nucleus to initiate transcription. The phosphorylation and nuclear translocation of Smad 1/5/8 are inhibited by CNTs. Id genes and proteins are subsequently inhibited. Inhibition of the Id protein negatively regulates the cell cycle transition from G1 to S phase and cell proliferation.⁴⁰⁸ A further study revealed that suppression of BMP signaling is due to binding of CNTs to BMP receptor type II (BMPR2) but not BMP receptor type I or the BMP ligand.⁴⁰⁶

BMP regulates a class of bHLH transcription factor proteins. Id proteins are also bHLH proteins but lack the DNA binding domain. Id proteins function as negative regulators of transcription. This regulation is significant in cell differentiation, tumorigenesis, and apoptosis. Suppression of BMP signaling in mesenchymal stem cells leads to inhibition of proliferation, myogenic differentiation, and reduced apoptosis. Surface

chemical modifications of CNTs can tune cell differentiation to different levels through the regulation of CNT/BMPR2 binding and BMP signaling.⁴⁰⁶

Graphene nanosheets activate MAPK and TGF- β signaling pathways in murine RAW 264.7 macrophages. Graphenes cause an increase in cellular ROS and activation of phosphorylation of ERK, JNK, and P38, indicating activation of MAPK signaling. Furthermore, expression of TGF- β receptor mRNA and phosphorylation of Smad2 are also enhanced, indicating activation of TGF- β signaling. The ROS and activated TGF- β signaling pathways further induce cellular apoptosis.⁴⁰⁹

4.4.1.5. Other Signaling Pathways. GNPs bind to heparin-binding growth factors but not to nonheparin-binding growth factors and selectively inhibit heparin-binding growth factor-induced cell proliferation. This interaction is due to direct binding of gold surfaces with sulfur or amine groups present in the heparin-binding domain. Inhibition of heparin-binding growth factors causes a decrease in vascular endothelial growth factor (VEGF)-165-induced cell proliferation in vitro and angiogenesis in vivo.^{153,154} GNPs with a poly(acrylic acid) coating selectively bind fibrinogen and induce protein unfolding to expose the C terminus of the γ chain. The newly exposed epitope interacts with the Mac-1 receptor and activates its signaling. Mac-1 activation induces the release of inflammatory cytokines, which is an alternative mechanism for the inflammatory response of cells to nanoparticles.¹⁶⁰ Silver nanoparticles suppress glial cell line-derived neurotrophic factor signaling in spermatogonial stem cells and reduce cell proliferation. This inhibition may be due to interactions

between nanoparticles and Fyn protein, a kinase in glial cell line-derived neurotrophic factor signaling.⁴¹⁰ Graphene oxide nanosheets activate the toll-like receptor signaling cascades and trigger ensuing cytokine responses, which subsequently induce autophagy.⁴¹¹

The above reports indicate that nanoparticles may affect oxidative stress-dependent and -independent pathways. BMP, growth factors, and Mac-1 cascades are affected by direct interactions between signaling proteins and nanoparticles (BMPR-CNT, VEGF-GNP, Mac-1 receptor-GNP, respectively). These interactions do not necessarily induce oxidative stress and apoptosis but may affect proliferation or differentiation. Other signaling pathways such as MAPK, NF- κ B, and DNA damage are responsible for nanoparticle-induced oxidative stress. These events eventually cause cell death. All of these events are determined by both the nature of the nanoparticles and their surface chemistry. Control of nanoparticle-induced signaling events and related cellular outcomes are crucial subjects for future investigations.

4.4.2. Global Biochemical Alterations by Nanoparticles. In contrast to small molecule drugs, for which the intracellular targets and mechanisms of action have been at least partially elucidated, knowledge of the molecular events involved in nanoparticle–biological system interactions is limited. Nanoparticle-induced global biochemical alterations may provide useful insights into the molecular mechanisms and chemical basis of nanoparticle–biological systems interactions. The replication, transcription, and translation of genes and synthesis of bioactive small molecules are biochemical processes that are controlled by tens of thousands of signals in response of intracellular demands or extracellular stresses. Mapping these global profiles will facilitate identification of nanoparticle-generated biological outcomes and eventual regulation of these outcomes through modulation of the physicochemical properties of nanoparticles.

4.4.2.1. Genomic Transcriptional Profile. Unlike small molecules, which may significantly regulate >5000 genes in living cells, most nanoparticles usually affect much fewer (<500) genes, indicating that they may be less active and more selective toward cells.^{330,408,412–415} The biological activity of nanoparticles can be investigated via genomic transcriptional profiling. Carboxylated SWNTs inhibit Id genes in HEK293 cells in a whole genome background. BMP signaling, which targets Id genes, was identified through multiple assays.⁴⁰⁸ Pristine SWNTs or MWNTs affect a large number of genes related to the cell cycle, signaling transduction, apoptosis, oxidative stress, metabolism, transport, and immune responses in human cells.^{398,414,416} By contrast, multiwalled carbon nanonions affect genes that fall into the category of external stimuli other than the immune response.⁴¹⁶ PEG-modified SWNTs affect fewer genes, indicating a reduced biological activity.³⁵⁷ PEG-modified, silica-coated QDs have been reported to affect 38 genes at low doses (8 nM) and 12 genes at high doses (80 nM), with four shared genes. These genes are related to carbohydrate binding, intracellular vesicle localization, and vesicular proteins involved in stress responses.⁴¹² Fe₃O₄ MNPs alter 711, 545, and 434 genes in mouse macrophage cells 4, 24, and 48 h after treatment. MAPK, TLR, and JAK-STAT signaling pathways are also affected by Fe₃O₄ nanoparticles. Bioinformatic analyses have revealed that these nanoparticles activate inflammatory and immune responses and inhibit biosynthesis and metabolism.⁴¹⁵ In the absence of illumination, anatase TiO₂ nanoparticles with diameters of 7, 20, and 200 nm

upregulate the inflammatory response and cell adhesion genes but not oxidative stress genes in human keratinocytes.⁴¹⁷ These effects indicate that TiO₂ nanoparticles do not cause oxidative damage in the dark. Genomic expression profiles reveal that GNPs of smaller sizes (1.4 nm) upregulate heat shock and stress-related genes and lead to cell necrosis. However, larger GNPs (15 or 18 nm) affect fewer genes, indicating their superior biocompatibility.^{330,418} The toxicity of some metallic nanoparticles may be due to the release of the metal ions. Genomic transcriptional profiling reveals that silver nanoparticles affect 128 genes in human epithelial cells, similar to the response of cells exposed to silver ions.⁴¹³ Treatment of epithelial cells with copper oxide nanoparticles upregulates genes in MAPK and downregulates cell cycle genes, also similar to cells treated with copper ions.⁴¹⁹ These discoveries suggest that the release of metal ions from nanoparticles must be considered when evaluating the bioactivity of metallic nanoparticles.

4.4.2.2. Proteomic Expression. Protein synthesis is downstream of gene transcription and controlled by a number of regulatory factors. Evaluation of proteomic expression provides more relevant information than genomic transcriptome analyses. The global protein expression profile is generally examined using 2D gel electrophoresis combined with MS.^{420–422} Generally, total proteins extracted from cells are separated by 2D gel electrophoresis, and the protein spots on the gel are excised and digested, followed by MS analysis. MS data are analyzed by searching databases to identify proteins. Another approach is the isobaric tags for relative and absolute quantitation (iTRAQ) technique.⁴²³ iTRAQ uses liquid chromatography to separate isotopically labeled peptides and MS/MS analysis to identify and quantitate peptides. Protein microarrays can be used for high-throughput evaluation of protein expression/phosphorylation.⁴²⁰ Antibodies against protein targets can be spotted on nitrocellulose membranes, enabling detection of multiple proteins in a single experiment. Using the above technologies, proteomic expression/phosphorylation profiles can be obtained. A total of 51 proteins in human hepatic carcinoma HepG2 cells are significantly affected by SWNTs, as detected by iTRAQ-coupled LC-MS/MS analyses. These proteins are involved in metabolic pathways, redox regulation, signaling pathways, cytoskeleton formation, and cell growth.⁴²³ Using 2D gel electrophoresis with MALDI-TOF MS, 45 altered proteins were identified in human monoblastic leukemia U937 cells in response to MWNTs. These proteins participate in metabolism, biosynthesis, stress response, and differentiation processes.⁴²² Additionally, 2D gel electrophoresis/MS analyses and protein phosphorylation antibody array analyses revealed that GNPs activate endoplasmic reticulum (ER) stress response in human chronic myelogenous leukemia K562 cells.⁴²⁰ A similar analysis indicated that TiO₂ nanoparticles significantly alter 46 proteins in human bronchial epithelial BEAS-2B cells. These proteins are involved in the stress response, metabolism, adhesion, cytoskeletal dynamics, cell growth, cell death, and signaling pathways. Nanoparticles perturb approximately 20 proteomic pathways, consistent with pathways from genomic data analyses.⁴²¹

4.4.2.3. Metabonomic Alterations. Nanoparticles cause physiological changes in living systems and generate altered metabolic profiles. Therefore, investigation of global metabonomic profiles provides insight into the physiological processes that are perturbed by nanoparticles. Nanoparticle-induced metabonomic alterations are studied in live animals or

cells.^{424–427} Analytical methods that include ¹H NMR, MS, HPLC-MS, and gas chromatography–mass spectrometry (GC-MS) have been used in metabonomic analyses.⁴²⁴ A global metabolism study in live cells using high-resolution MAS NMR spectroscopy demonstrated that silver nanoparticles decrease the levels of GSH, lactate, taurine, and glycine and increase the levels of most amino acids, choline analogues, and pyruvate. These results indicate that the depletion of GSH by nanoparticles may induce conversion of lactate and taurine to pyruvate, providing a biochemical mechanism for oxidative stress.⁴²⁵ HR-MAS ¹H NMR was also used to study the metabonomic changes in RAW264.7 mouse macrophages following treatment with MNPs. The nanoparticles caused a decrease in the levels of triglycerides, essential amino acids, and choline metabolites while increasing glycerophospholipids, tyrosine, phenylalanine, lysine, glycine, and glutamate. These changes reflect phagocytosis and cell membrane perturbation.⁴²⁷

In general, interactions between nanoparticles and biological systems include direct and indirect effects. Direct effects, such as the binding of nanoparticles to membrane phospholipids, receptors, cytosol proteins, and DNA molecules, are modulated by the physicochemical properties of the biomacromolecules and nanoparticles and their microenvironment. Direct interactions are much more specific and material dependent compared to indirect interactions and may have therapeutic potential. However, investigation of direct interactions is challenging because of the complex cellular background. Indirect effects, such as signaling pathway activation by nanoparticle-induced ROS, cause cytotoxicity. Future investigations will enhance and regulate direct interactions while minimizing indirect interactions. In this endeavor, chemical technology may be very powerful for modulating the biological activities of nanomaterials, as discussed below.

5. CHEMICAL APPROACHES TO REGULATE AND UNDERSTAND NANOPARTICLE–BIOLOGICAL SYSTEM INTERACTIONS

Interactions between nanoparticles and biological systems are complex. The biological activities of nanoparticles depend on their size, shape, structure, and surface properties. As nanoparticle sizes fall into the nanometer range, the surface-exposed atoms increase exponentially. Therefore, changing the properties of the surface molecules may significantly alter the biological activities of nanoparticles. In this section, we explore various surface chemistry modification methods, the biological impacts of such modifications, and computational models to predict the relationships between nanoparticle surface chemistries and their biological activities.

5.1. One-at-a-Time Nanoparticle Surface Chemistry Modification

5.1.1. Modulation of the Surface Chemistry of Nanoparticles. Various molecules have been used to modify nanomaterial surfaces via coating chemistry and, consequently, their biological activity. Surface modification strategies include covalently or noncovalently linking small organic ligands or biomolecules to nanoparticles or encapsulating nanoparticles in surface coatings, such as polymers, micelles, liposomes,⁴²⁸ graphene oxide,¹⁴¹ and silica shells.^{412,429} The ligand exchange method is effective for transferring nanoparticles synthesized in organic solvents to aqueous media by displacing hydrophobic ligands on nanoparticles with water-soluble ligands. For

example, dithiocarbamate moieties are designed to replace trioctylphosphine oxide capped on CdSe/ZnS QDs,⁴³⁰ while (3-carboxypropyl)trimethylammonium chloride is used to replace lauric acid on Fe₃O₄ nanocrystals.⁴³¹ This ligand exchange step is essential to apply these nanoparticles in biological systems. The covalent linkage strategy requires the aid of coupling reagents, such as 1-ethyl-3-(dimethylamino)-propyl carbodiimide and *N*-hydroxysuccinimides, or Cu²⁺-mediated click chemistry⁴³² to react with functional groups on nanoparticles. Some nanoparticles with an aliphatic shell can be encapsulated in polymer coatings or micelles through an intercalating effect with amphiphilic polymers.⁴³³ In addition, the polymer coating can be achieved through a layer-by-layer technique by sequentially trapping nanoparticles in layers of polymers of alternating charge.⁴³⁴

5.1.2. Modulation of the Surface Charge of Nanoparticles. An acidic group is often used to generate nanoparticles with negative charges on their surfaces. Similarly, an amine group is used to generate nanoparticles with a positively charged surface. Surface-charged nanoparticles differ greatly in their electrostatic and electrodynamic properties, which can be experimentally determined by ζ -potential measurements. Polystyrene nanoparticles with different surface functional groups, such as carboxylic acid, PEG, amine, and amidine groups, have ζ -potential values ranging from –50 to 40 mV. The binding of serum proteins alters their ζ -potential values and narrows them to a range from –40 to –20 mV.⁴³⁵ BSA, myoglobin, and cytochrome *c* are adsorbed to mercaptoundecanoic acid-protected GNPs, as shown by a quartz crystal microbalance study. All three proteins form adsorption layers consisting of an irreversibly adsorbed layer and a reversibly adsorbed layer.¹⁰⁹ These data suggest that the charged surface of a nanoparticle is quite complicated in solution. Multiple layers of molecules are adsorbed and dissociated constantly. The charged surface of nanoparticles also affects the conformation of the adsorbed proteins. Cytochrome *c* maintains its conformation when adsorbed to neutral GNPs, and it is denatured on the surface of charged GNPs.⁴³⁶ GNPs surface modified with charged and neutral amino acids affect the conformation and activity of α -chymotrypsin differently. Charged GNPs exhibit more effects than neutral particles.⁴³⁷ To understand the influence of surface charge density on protein binding, a series of polystyrene latex nanoparticles with the same size were prepared using different amounts of Na-styrenesulfonate comonomer. As the charge density increased, there was no change in the bound protein species, while the amount of adsorbed protein increased.⁴³⁸

The different electrostatic and electrodynamic properties of nanoparticles also modulate their interactions with cells. Such alterations include effects on cell internalization, cell function, and cell death. A better understanding of these effects will not only help us design nanomaterials for biomedical applications but will also elucidate mechanisms of nanotoxicity. Due to the presence of phosphate groups from lipids, cell membranes are negatively charged. Positively charged nanoparticles generally exhibit higher cell binding and cellular uptake than negatively charged nanoparticles. To understand the electrostatic and electrodynamic interactions between nanoparticles and cells with minimal secondary interactions, a nanoparticle library with a continuous change in the surface charge density was synthesized and investigated. The nanoparticle/cell interaction, however, did not exhibit a continuous change. Instead, a sharp increase in cell binding was observed as the positive surface

charge density increased. The effective surface charge density on the outermost layer of the nanoparticle determined the electrostatic attraction between the nanoparticles and the cells.⁴³⁹ Nanoparticles with different charges induce different perturbations of the cell membrane potential. Positively charged nanoparticles depolarize the membrane, and negatively charged nanoparticles have a negligible effect. Membrane potential perturbations result in an increase in $[Ca^{2+}]$ influx, which in turn inhibits the proliferation of cells (Figure 16).³¹²

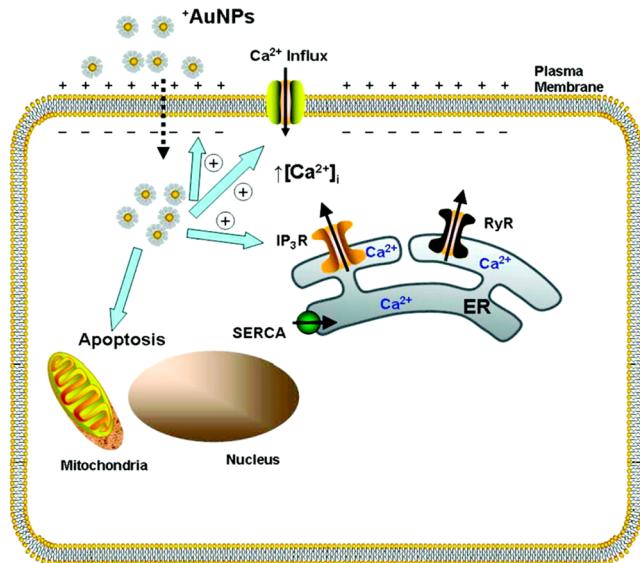


Figure 16. Schematic depicting the surface charge effects of GNPs on cellular function. Reprinted with permission from ref 312. Copyright 2010 American Chemical Society.

method has been developed to differentiate GNPs bound to the cell surface from internalized GNPs. Neutral and negatively charged GNPs displayed lower cell membrane binding and cell internalization compared to positively charged nanoparticles.⁴⁴⁰

The surface charge effect seems applicable to a variety of nanomaterials. Positively charged GNPs, MNPs, silica nanoparticles, and QDs all exhibit higher cellular uptake efficiency (Figure 17).⁴⁴⁰ The internalization of negatively charged nanoparticles also occurs and is likely due to other interactions and subsequent endocytosis.^{441,442} QDs with a carboxylic acid surface coating are recognized by lipid rafts but not by clathrin or caveolae in human epidermal keratinocytes. QDs are internalized into early endosomes and then transferred to late endosomes or lysosomes. In addition, endocytic interfering agents have been used to investigate the mechanism by which QDs enter cells. The cellular uptake of QDs with negative surface charges is primarily regulated by pathways associated with G-protein-coupled receptors, low-density lipoprotein receptors, and scavenger receptors.⁴⁴³

In a kinetics study, positively charged nanoparticles displayed a higher rate of endocytosis than negative charged materials.⁴⁴⁴ Positively charged core–shell silica nanoparticles coated with polyethylenimine have been shown to escape from endosomes and enter the cytoplasm and nucleus.⁴⁴⁵ Positively charged nanoparticles destabilize cell membranes and form holes or expand holes at pre-existing defects.⁴⁴⁶ The holes led to internalization of nanoparticles into cells as well as diffusion of cytosolic proteins out of cells. Further studies have demonstrated that formation of holes in lipid bilayers by positively

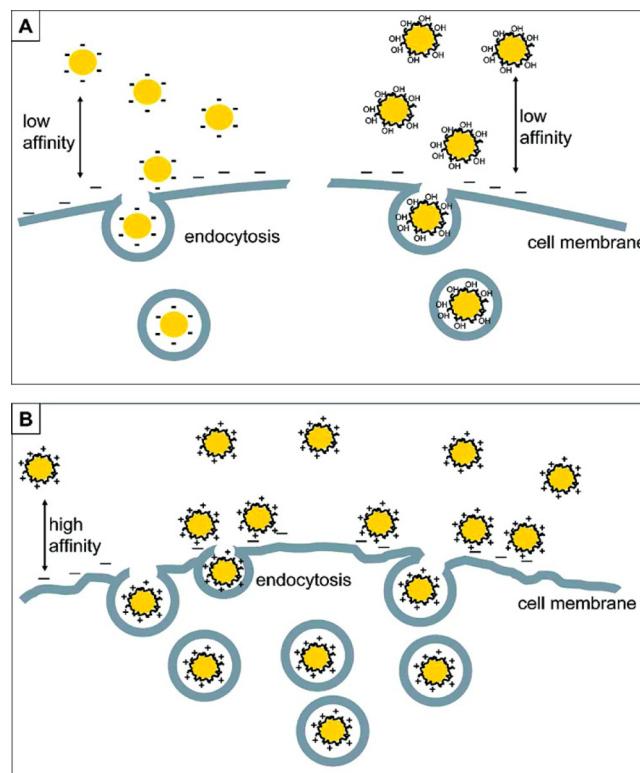


Figure 17. Schematics illustrating the interactions between cells and nanoparticles with different types of surface charges: (A) negatively and neutrally charged nanoparticles. (B) Positively charged nanoparticles. Reprinted with permission from ref 440. Copyright 2009 American Chemical Society.

charged nanoparticles is a common property regardless of the shape, size, and chemical composition of the nanoparticles.⁴⁴⁷

5.1.3. Modulation of the Surface Hydrophobicity of Nanoparticles. The hydrophobicity of nanoparticles influences their ability to interact with biomolecules and cells. To investigate the correlation between plasma protein adsorption and the surface hydrophobicity of nanoparticles, latex nanoparticles with varied surface hydrophobicity were synthesized and examined. The number of bound proteins increased with increasing particle hydrophobicity, as determined by 2D polyacrylamide gel electrophoresis analyses and ITC. A clear difference in protein affinity for polymeric particles of different hydrophobicity has been observed.^{108,448}

A set of polymethacrylate nanoparticles (140 nm) with fluorescence labels was investigated to reveal the relationship between particle hydrophobicity and cellular uptake. The monomer chain length controls the hydrophobicity of the modified nanoparticles, as evaluated by measuring the interfacial tensions between the monomers and water. The length of the monomer chains influences the cellular uptake of nanoparticles. Increasing the side chain length increases cellular uptake significantly. This phenomenon can be explained by the increased hydrophobicity and softness of the particles.⁴⁴⁹

The surface hydrophobicity of nanoparticles is also modified by peptide–GNP hybrids. By altering the end amino acids of the peptide ligands, the uptake of GNPs can be dramatically increased or suppressed. Nanoparticles with tryptophan end groups enhance cellular uptake due to the membrane anchoring and permeation effects of the aromatic group.⁴⁵⁰

5.1.4. Changes in the Interactions of Nanoparticles with Biological Systems. *5.1.4.1. Making Toxic Nanoparticles More Biocompatible.* Nanoparticles may induce toxicity in biological systems because of their specific properties.^{451,452} First, the large surface area to volume ratio leads to very strong nonspecific interactions with biomolecules. These interactions will alter the conformation of proteins or DNA, resulting in their malfunction.⁴⁵³ Second, some nanoparticles are composed of heavy metals, such as CdTe and CdHgTe QDs. Under some conditions, the free metal ions may be released into biological systems and induce toxicity.⁴⁵⁴ Third, nanoparticles may induce ROS, such as free radicals (hydroxyl radical, $\cdot\text{OH}$; superoxide, $\cdot\text{O}_2^-$) and singlet oxygen ($^1\text{O}_2$) (e.g., QDs⁴⁵⁵ and Ag nanoparticles⁴⁵⁶). ROS are known to cause irreversible damage to nucleic acids, enzymes, and cellular components that included the mitochondria, plasma, and nuclear membrane. Fourth, some capping ligands derived from the nanoparticle synthesis system may induce cytotoxicity.⁴⁵⁷ Surface chemistry modifications may diminish the above side effects and greatly improve the biocompatibility of potentially toxic nanoparticles. For example, the biocompatibility of QDs has been improved after surface modification with dendrimers,^{458,459} PEGylated dihydroliopic acid,^{460,461} triblock copolymers,⁴⁶² cyclodextran,⁴⁶³ and alkyl-modified poly(acrylic acid).⁴⁶⁴ The coating of SWNTs with polyvinylpyrrolidone polymers,⁴⁶⁵ phospholipid derivatives⁴⁶⁶ on silver nanoparticles, or human serum protein⁴⁶⁷ also prevents their toxicity. Hexadecyltrimethylammonium bromide (CTAB) released from CTAB-coated gold nanorods is highly cytotoxic at a nanoparticle concentration as low as 0.05 mM (20% cell viability). However, when toxic CTAB was replaced by PEG-SH, the cell viability when subjected to nanoparticles was 95%, even at a concentration of 0.5 mM.⁴⁵⁷ Similar effects have also been reported after replacement or overcoating of CTAB with poly(sodium 4-styrenesulfonate),⁴⁶⁸ copolymer poly(diallyldimethylammonium chloride)/polystyrene sulfonic acid,⁴⁶⁹ or poly(allylamine hydrochloride).⁴⁷⁰

5.1.4.2. Regulating Interactions with Cells. The interaction of nanoparticles with cells is affected by the surrounding biomolecules in physiological media. When bare nanomaterials (particularly inorganic nanoparticles) encounter biological macromolecules, they may form a nanoparticle–macromolecule (mainly protein) corona hybrid nanostructure.⁴⁵³ Moreover, a secondary protein corona on the hybrid nanoparticles may be further formed via protein–protein interactions. Some of these interactions do not occur in the native system in the absence of the nanoparticles, and an immunological response may be triggered. As a result, the nanohybrids are detected as foreign materials by the immune system. The nanohybrids are rapidly cleared from the bloodstream and translocated to the liver and spleen,⁴⁷¹ potentially limiting the biological applications of nanoparticles. Fortunately, the fate of nanoparticles in biosystems can be regulated. Two main requirements, cell targeting and in vivo stealth properties, can be modulated by proper nanoparticle surface chemical modifications.

When a nanoparticle is presented to a cell, the cell first recognizes the coating molecules at the outermost layer of the nanoparticle. For targeting purposes, linking specific ligands to nanoparticles is necessary. Certain molecules, such as antibodies, aptamers, or small-molecule ligands, can bind to receptors overexpressed in cells in a specific “lock–key” manner.^{428,472–475} After modification, nanohybrids are able to target specific cancer cells. This targeting strategy lays the

foundation for designing nanomedicines for cancer diagnosis and therapy. Detection of circulating breast cancer cells in the blood was achieved using a combination of an epithelial cell-specific, antibody-conjugated magnetic nanoprobes and anti-HER2 antibody-conjugated SERS tags.⁴⁷⁶ Because the HER2 receptors are highly expressed on the breast cancer cell membrane, the SERS tags recognized these tumor cells. Thus, the cancer cells were detected rapidly with good sensitivity. An antiprostate antigen was conjugated to a multifunctional nanomedicine composed of loaded drugs, NIR QDs, and a polystyrene matrix with internally embedded superparamagnetic Fe_3O_4 nanoparticles. Using this multifunctional nanoparticle, specific detection of LNCaP prostate cancer cells was achieved.⁴⁷⁷

After being administered into the blood, most unmodified nanomaterials are taken up by the mononuclear phagocyte system within minutes or hours.⁴⁷⁸ Surface modification of nanomaterials is generally regarded as a practical strategy to prevent protein adsorption and, consequently, avoid triggering immune responses.⁴⁷⁴ Various biocompatible polymer surface coatings, such as PEG,⁴⁷⁹ hyaluronic acid,^{480,481} cyclodextran,^{482,483} and polymers based on phosphorylcholine,⁴⁸⁴ provide effective barriers against protein bindings and therefore reduce both protein adsorption and cellular uptake. These modifications are widely used to impart stealth properties to nanoparticles in biomedical applications. These surface modifications also change the *in vivo* pharmacokinetics of nanoparticles.⁴⁸⁵ QDs modified with mPEG 5000 display a significantly prolonged *in vivo* circulation time in addition to reduced nonspecific accumulation in various organs.⁴⁸⁶ After surface PEGylation, the blood half-life of gold nanorods is increased to 24 h.^{457,487} PEG-terminated phospholipid was used to encapsulate QDs⁴⁸⁸ and iron oxide nanocrystals⁴⁸⁹ for a longer blood circulation time *in vivo*. A mPEG-alkyl-thiol polymer with an alkyl linker between the PEG and the thiol moiety yielded a much stronger GNP surface protection compared to commonly used methoxy-PEG-thiol in the presence of cysteine and cystine molecules at the physiological concentrations.⁴⁹⁰ Surface coating of lipid molecules has also improved the pharmacokinetics of the silica nanoparticles.⁴⁹¹

5.2. Combinatorial Chemistry for Nanoparticle Modifications

The discovery and optimization of novel nanoparticles with unique properties by conventional methods has always been challenging and time consuming. High-throughput approaches, including parallel and combinatorial synthesis, are more efficient than conventional linear synthesis, and in recent years, these techniques have shown an increasing impact in nanotechnology.

5.2.1. Parallel Chemistry for Nanoparticle Modifications. A parallel chemistry approach provides the opportunity to rapidly synthesize a large amount of nanoparticles with similar surface chemistries. However, this approach has limitations, mostly based on the lack of a general method to modify nanoparticles rapidly and the availability of small molecules that can be used.

5.2.1.1. Parallel Nanoparticle Libraries for Regulating Gene Delivery. Numerous biomaterials have been studied as potential nonviral gene delivery vectors to improve DNA stability and the efficiency of cellular uptake. The tested materials include inorganic surfactants, cationic lipids, polysaccharides, cationic polymers, and dendrimers. These materials

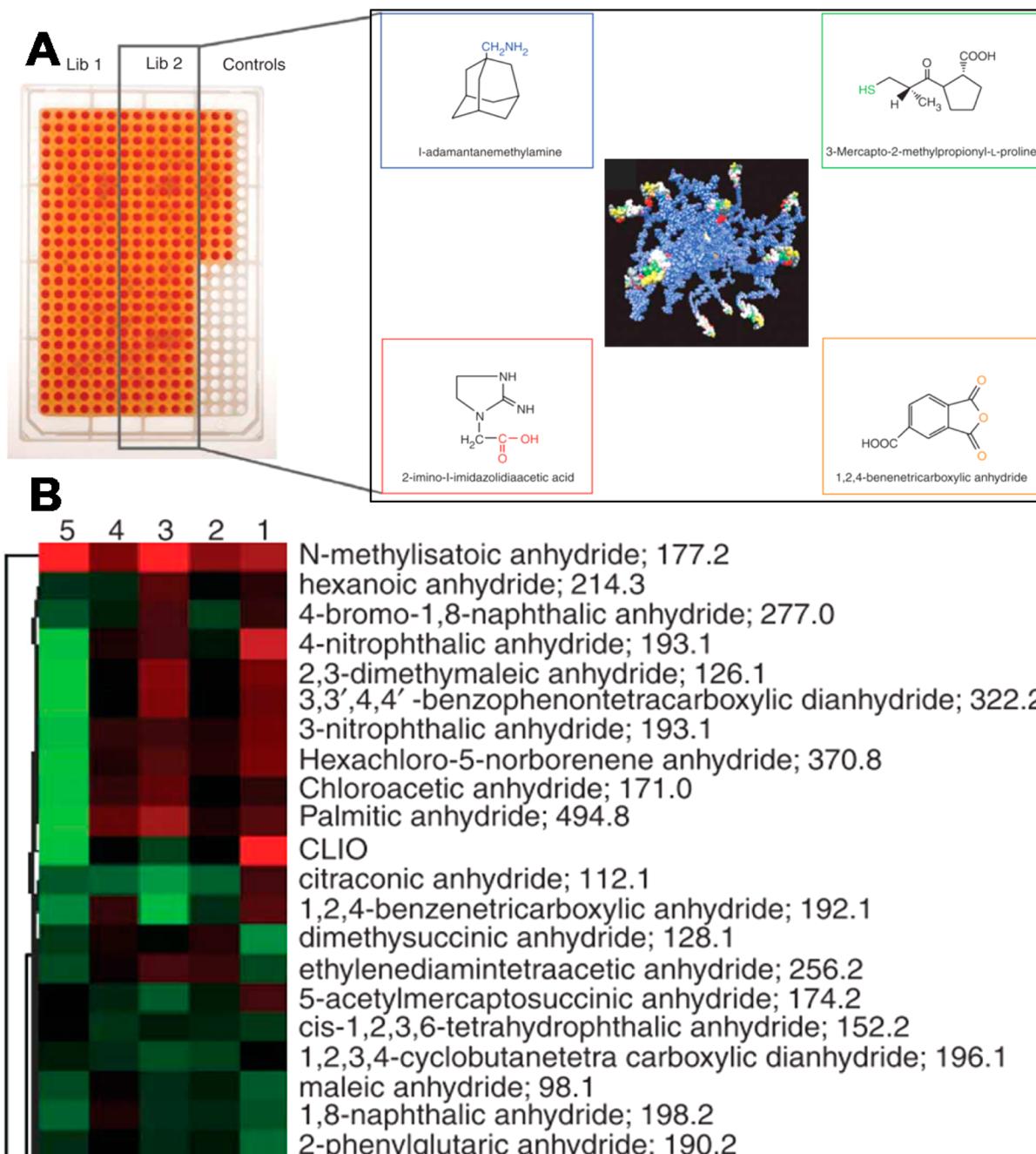


Figure 18. (A) Model of the cross-linked dextran coating modified with small molecules. Different classes of small molecules with amino, sulphydryl, carboxyl, or anhydride functionalities were anchored onto nanoparticles and stored in multiwell plates for testing. (B) Part of a heat map representing the cellular uptake of different nanoparticle preparations. Columns from right to left: 1, pancreatic cancer cells (PaCa-2); 2, macrophage cell line (U937); 3, resting primary human macrophages; 4, activated primary human macrophages; 5, human umbilical vein endothelial cells (HUVEC). Each column represents mean values from six different experiments. Red indicates the lowest accumulation, and green indicates the highest accumulation. Reprinted with permission from ref 28. Copyright 2005 Nature Publishing Group.

display small cargo capacity, inefficient uptake, poor biostability, and low safety. Using high-throughput synthesis and screening techniques, libraries of poly- β -amino esters (PBAEs) of >2000 members were synthesized to evaluate their gene delivery efficiency.^{492–494} A total of 46 PBAEs were superior to polyethylenimine. A second-generation library of 486 polymers was synthesized to optimize the polymers and investigate their structure–function relationships.⁴⁹⁵ The best-performing polymer nanoparticles were <150 nm and had positive surface charges. PBAEs have been used to deliver DNA to primary

human endothelial cells.⁴⁹⁶ To investigate a larger PBAE chemical space, libraries of end-modified PBAEs were also synthesized using various end-capping reagents.^{497,498} Two modified polymers with greatly improved transfection efficiency both *in vitro* and *in vivo* were discovered.

5.2.1.2. Parallel Nanoparticle Libraries for Regulating Cell Targeting. MNPs that target tissues of interest have been developed by conjugating antibodies, peptides,⁴⁹⁹ or small molecules to the surface of MNPs.^{26,28} Small molecules with anhydride, amine, hydroxyl carboxyl, thiol, and epoxy handles

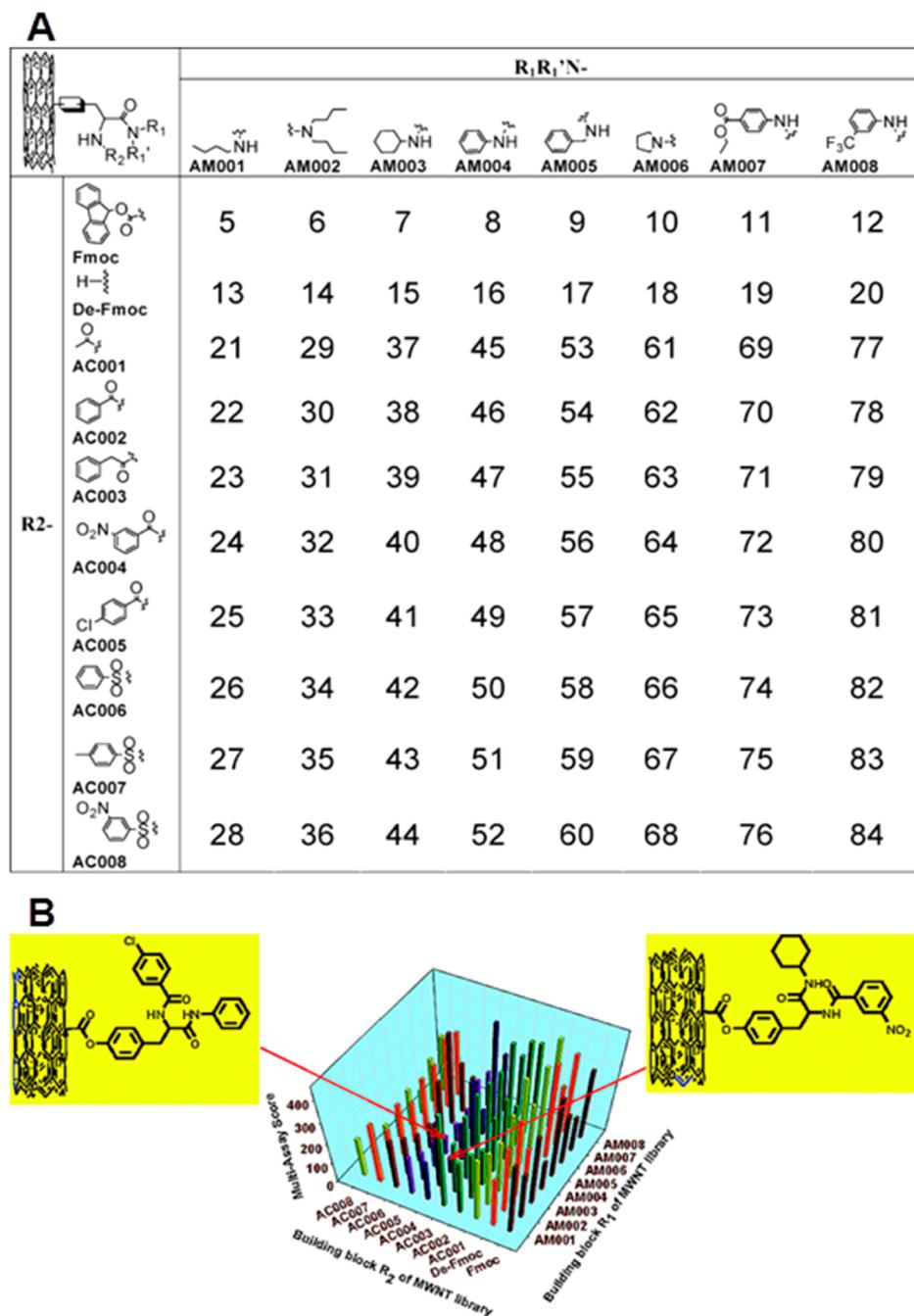


Figure 19. (A) Surface molecular compositions of combinatorial MWNT library members. (B) Multiassay score of the f-MWNT library from four protein binding assays, cytotoxicity, and immune response assays (at 50 $\mu\text{g}/\text{mL}$) were ranked for all library members. Sum of their ranks was designated the multiassay score and is shown as vertical bars in the graph. Structures of the optimal hits are shown. Reprinted with permission from ref 27. Copyright 2008 American Chemical Society.

were coupled to the MNPs via different synthetic routes. The nanoparticles were printed on glass slides and showed different binding properties toward different targeting proteins using fluorescence detection. A collection of MNPs comprising 146 nanoparticles modified by different synthetic small molecules was synthesized and screened against different cell lines (Figure 18). In this screen, nanoparticles with high specificity for endothelial cells, pancreatic cancer cells, or activated human macrophages were identified.²⁸

5.2.1.3. Parallel Nanoparticle Libraries for Imaging. Weissleder et al. developed fluorescent nanoparticles derivatized with small molecules using a parallel chemistry approach.

Cellular uptake was rapidly profiled in vitro via binding of those nanoparticle imaging probes in primary cell isolates. This approach selects nanoparticles that display the desired targeting effects across all tested members of a class of cells and decreases the likelihood that an idiosyncratic cell line will unduly skew the screening results. The identified nanoparticle imaging probes were validated in vivo via intravital microscopy using pancreatic cancer mouse model.⁵⁰⁰ Although the molecular target of the probes remains to be discovered, the specific localization of these probes validates their respective use as in vivo neural tracers and cell-discriminating agents.

5.2.1.4. Parallel Nanoparticle Libraries for Biosensors. Six functionalized poly(*p*-phenyleneethynylene)s were used to construct a protein biosensor array containing six nanoprobe. These highly fluorescent polymer nanoparticles possessed various charge characteristics and molecular weights. In a proof-of-principle experiment, 17 protein analytes were successfully discriminated by their different fluorescence properties after nanoparticle binding. However, those probes were not tested in real samples, such as blood serum, containing complex mixtures of proteins with potentially interfering species.⁵⁰¹ Parallel modifications of magnetic nanoparticles aided in designing biomimetic nanoparticle biosensors with selectivity for specific cell types. Attachment of fluorescent molecules to magnetic nanoparticles permits the sorting or targeting of cells using both fluorescence-based technologies and MR imaging.^{26,499} The conjugation of a lipoic acid-containing NIR active tricarbocyanine library to GNPs yielded probes with a strong SERS enhancement with 12-fold higher sensitivity than the current standard, 3,3'-diethylthia-tricarbocyanine iodide. By further conjugating the probe to a scFv anti-HER2 antibody, this ultrasensitive SERS bioprobe was used for *in vivo* cancer imaging with potential for use as a noninvasive diagnostic tool for HER-2-positive cancers.⁵⁰²

5.2.2. Nanocombinatorial Chemistry To Modify Nanoparticles. Compared with a parallel chemistry approach, combinatorial chemistry can provide a large number of different but structurally related molecules or materials. A series of nanoparticles with different surface coatings but the same core structure and different diversity points can be synthesized rapidly using nanocombinatorial chemistry. The number of compounds synthesized increases exponentially with the number of diversity points. Furthermore, because the nanoparticles obtained by nanocombinatorial chemistry have surface coatings with similar structures, structure–activity relationships can be obtained, which is critical for computer-aided design for predicting nano–bio interactions.

5.2.2.1. Combinatorial Surface Modifications To Regulate Biocompatibility. Drug-loaded nanoparticles with targeting ligands can grant them therapeutic, targeting, or imaging functions. However, achieving a low blood clearance rate, low acute toxicity, and a low immune response remains challenging. To discover biocompatible MWNTs without a priori knowledge of the related targets, our laboratory developed a computer-assisted, nanocombinatorial synthetic library strategy. Combinatorial modifications of nanotube surfaces allowed us to map the unknown chemical space more effectively and rapidly. Computer-aided design was used to select surface molecules to present the most diverse structural and physicochemical properties. As a result of multiple biological screenings, we identified nanomaterials with the most optimal biocompatibility and preliminary structure–activity relationship (Figure 19).²⁷ Furthermore, to test the improved molecular recognition after surface modifications on MWNTs, the library was screened against α -chymotrypsin to compare the MWNT interactions with the enzyme catalytic site and other binding sites. Four of 80 MWNTs were found to significantly affect enzymatic catalysis with much less nonspecific bindings.⁵⁰³ The combinatorial chemistry modifications on MWNTs have also regulated immune perturbations in mice and macrophages. In another example, a modified nanotube deterred the mannose surface receptor from binding to the scavenger receptor and reduced immunotoxicity.⁵⁰⁴

5.2.2.2. Combinatorial Modifications To Regulate Cell Targeting. Recently, we reported a dual-ligand GNP library to discern the slight difference between cells that have different surface receptor profiles surrounding a common primary receptor.⁵⁰⁵ This library used folic acid as the primary targeting ligand. The secondary ligand was one of the ligands from a combinatorial library of 42 molecules. Cell recognition was investigated in four cell lines with various expressions of folate receptor (FR) and totally unknown secondary receptor profile. Cell binding and uptake in different cells were quite different, suggesting different receptor profiles surrounding the FRs. Furthermore, multivalent bindings of dual ligands can differentiate cells with high and low expression of FRs and enhance the contrast between these cells in radiation-induced cell death. This enhanced cell recognition and discrimination of cells with seemingly a common receptor provide an opportunity to treat patients at a personalized medicine level with a much better safety profile.⁵⁰⁵

5.2.2.3. Combinatorial Modifications To Regulate Cell Differentiation. Biomaterials can drive stem cells to an appropriate differentiation level and decrease the apoptosis of transplanted cells. We observed that carboxylated MWNTs promote myogenic differentiation of mouse myoblast cells C2C12 and inhibit cell apoptosis under differentiation conditions.⁵⁰⁶ We explored the tuning of cell differentiation using combinatorial surface modifications on MWNTs. Different surface modifications conferred different modulations of binding to BMP receptor type II and caused different levels of suppression of the BMP signaling pathway. Modified MWNTs fine tuned the downstream signaling through a series of bHLH proteins and myogenic differentiation. These results demonstrated control of biological activities through surface-modified nanomaterials and opened an avenue for potential application of nanomaterials in regenerative medicine.⁵⁰⁷

5.3. Computational Approaches To Elucidate Nano–Bio Interactions

The recent emergence and proliferation of experimental studies on the biological and toxicological activities of nanoparticles has created new challenges for computational scientists interested in rationalizing and exploiting these data by generating predictive models. Similar to data-rich areas of chemistry and biology, where development of high-throughput technologies and accumulation of large amounts of data have spurred the establishment of cheminformatics and bioinformatics, we are beginning to witness the appearance of nanoinformatics, which naturally complements nanotechnology. For instance, a group of academic, governmental, and industrial scientists interested in informatics opportunities in nanotechnology met at the first Nanoinformatics conference in 2010 (<http://nanotechinformatics.org/2010/overview>). Presentations and idea exchanges at the conference led to creation of the Nanoinformatics 2020 Roadmap (<http://eprints.internano.org/607/>) as the “first broad-based community effort to articulate the comprehensive needs and goals in nanoinformatics”.

One of the most important components of nanoinformatics, which resonates with similar components of bio- and cheminformatics, concerns the understanding of the relationships between the structure and the physical and/or biological properties of nanoparticles. To this end, given that sufficient and organized data collections are available, well-established methods of quantitative structure–activity relationship (QSAR) modeling, widely used in cheminformatics,⁵⁰⁸ could be

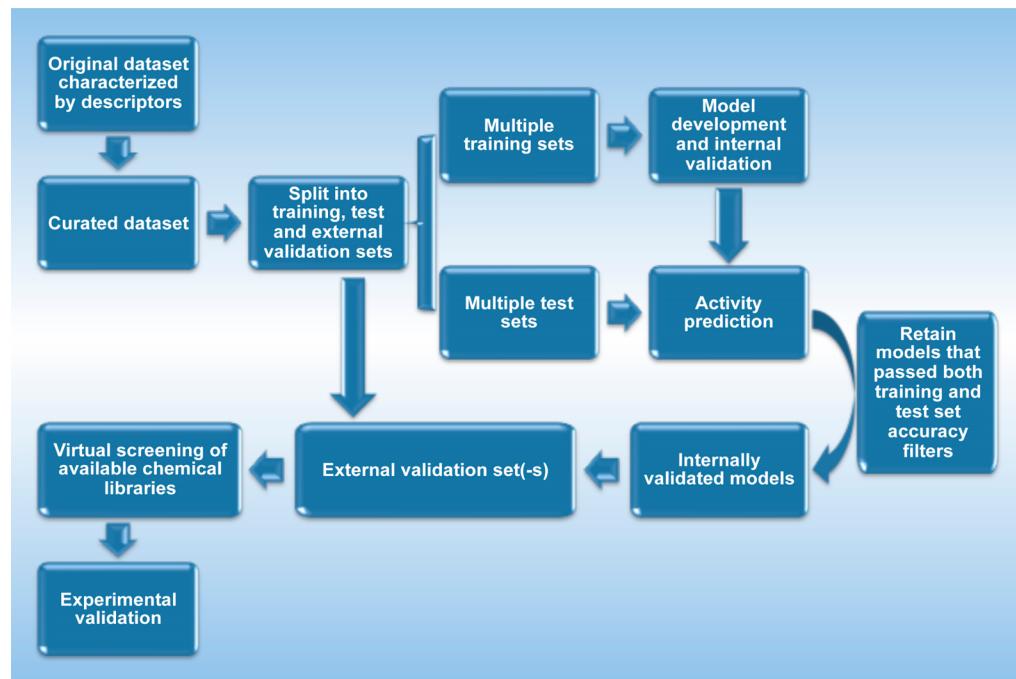


Figure 20. General workflow for development of validated and externally predictive QSAR models.

expanded toward nanoparticles, as we have begun to advocate for recently.^{29,31} Herein, we provide a brief overview of the quantitative nanostructure–activity relationships (QNAR) modeling approach, which should help experimental scientists studying nanoparticle–biological systems interactions rationalize their data as well as employ QNAR models to enable rational design of novel nanoparticles with the desired properties. We describe major principles and best practices in QSAR modeling, which serves as a basis for QNAR, and present a few recent examples of QNAR studies to illustrate the concept and its applicability to the computational modeling of nanoparticles.

5.3.1. Quantitative Structure–Activity Relationships (QSAR). QSAR modeling has been a widely and successfully used approach in medicinal chemistry and drug discovery for nearly 50 years. The QSAR approach can be generally described as an application of data analysis methods and statistics to developing models for accurate prediction of biological activities or properties of compounds based on their structures. Any QSAR method can be generally defined as an application of mathematical and statistical methods to the problem of finding empirical relationships (QSAR models) of the form $P_i = \hat{k}(D_1, D_2, \dots, D_n)$, where P_i are the biological activities (or other properties of interest) of the molecules, D_1, D_2, \dots, D_n are calculated (occasionally experimentally measured) structural properties (molecular descriptors) of the compounds, and \hat{k} is some empirically established mathematical transformation that should be applied to descriptors to calculate the property values for all molecules. The goal of QSAR modeling is to develop a computational means of predicting compound target properties (e.g., specific bioactivities or toxicities) from their chemical structure, as characterized with computed chemical descriptors or, in some cases, by experimental physical chemical characteristics. All QSAR approaches directly or indirectly imply simple similarity principles, which for some time have provided a foundation for experimental medicinal chemistry: compounds with similar structures are expected to

have similar biological activities. The detailed description of major tenets of QSAR modeling is beyond the scope of this paper; the overview of many popular QSAR modeling techniques, including statistical and data mining techniques and descriptor calculation approaches, can be found in many reviews and monographs.^{509,510}

For brevity, we shall comment on the most critical general aspects of model development and, most importantly, validation, which are especially significant in the context of using QSAR models for identifying novel hit compounds by virtual screening. Additional essential information concerning both common errors and established practices in the QSAR modeling field can be found in other critical essays on the subject.^{511,512} We emphasize such aspects because they are generic for any QSAR-based approach application (i.e., QNAR) to enable rigorous model development.

The strategy for developing extensively validated and externally predictive QSAR models is summarized in Figure 20 (described in more detail elsewhere⁵¹³). We begin by carefully curating chemical structures and, if possible, associated biological activities to prepare the data set for subsequent calculations. Then, a fraction of compounds (generally 10–20%) is selected randomly as an external evaluation set. A more rigorous n -fold external validation protocol can be employed when the data set is randomly divided into n nearly equal parts. The $n - 1$ parts are then systematically used for model development, and the remaining fraction of compounds is used for model evaluation. The remaining subset of compounds (the modeling set) is divided into multiple training and test sets. The training sets are used to establish correlations between values of chemical descriptors and those of biological activities or other target properties using different machine learning techniques; the models are validated internally for their ability to accurately predict the target properties of compounds in the test sets. All of the models that display high prediction accuracy for both the training and the test sets form an ensemble that is applied to predict target properties for compounds in the

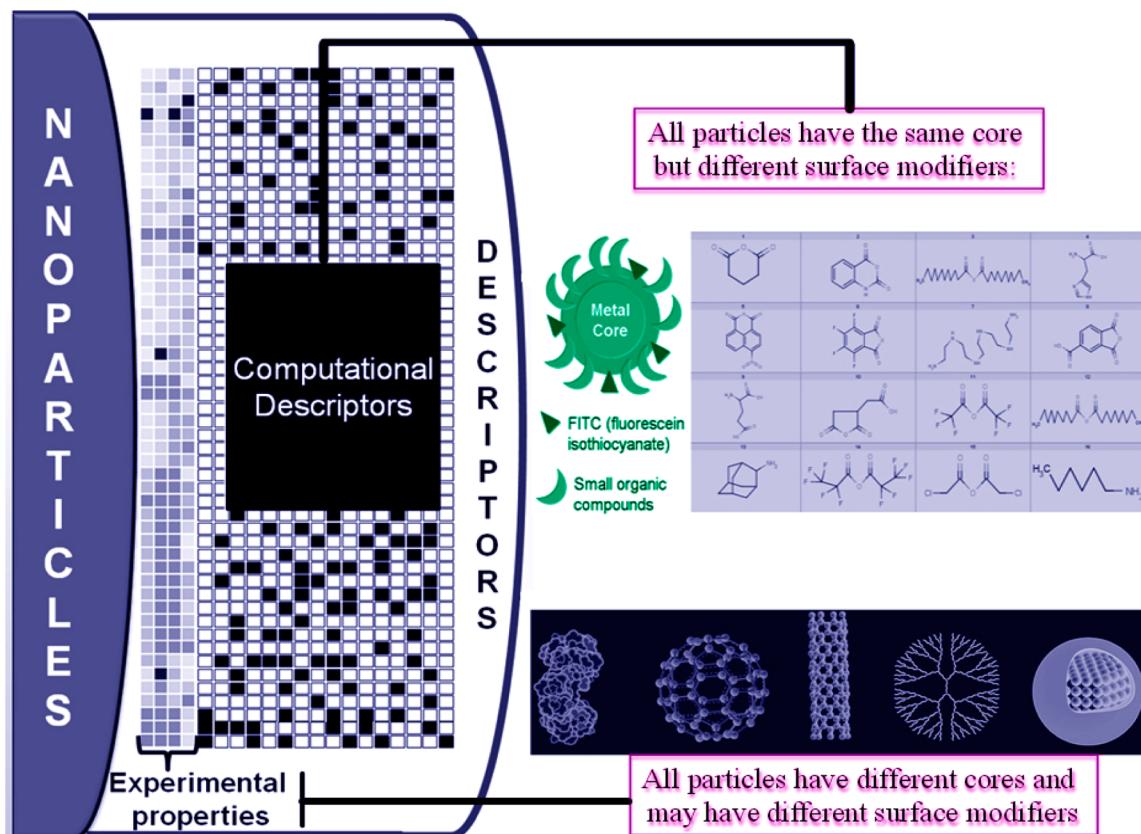


Figure 21. Applications of QNAR methods to nanomaterials with either diverse cores (characterized by experimental properties) or similar cores but diverse surface modifiers (characterized by computed chemical descriptors of modifying organic molecules). Adapted with permission from ref 31. Copyright 2010 American Chemical Society.

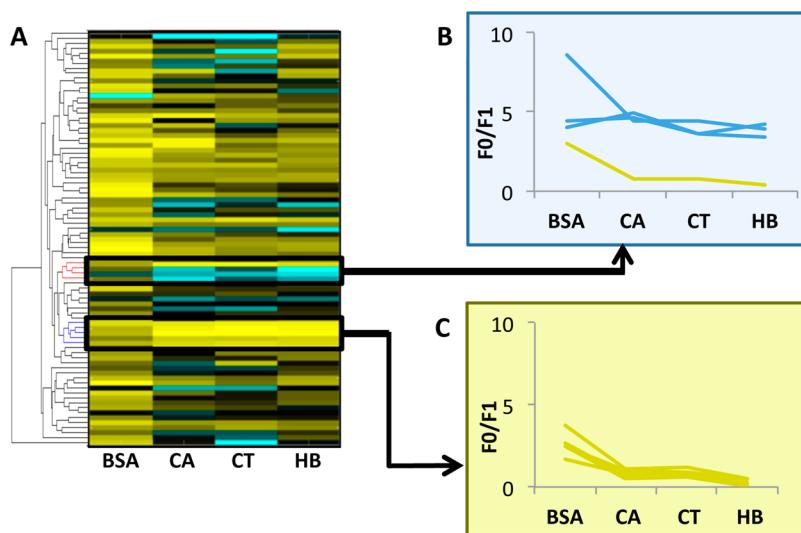


Figure 22. Unsupervised hierarchical clustering of CNTs uncovered common subgroups for both protein binders and nonbinders. (A) Heat map represents protein binding activities of the CNTs, which are sorted according to the dendrogram. Cyan color indicates high activity, while yellow represents low activity. (B) Cyan cluster represents four compounds consisting of a common substituent causing similar (high) protein binding responses as measured in four different assays. (C) Yellow cluster represents five compounds consisting of another common substructure highlighted in the yellow square. These compounds have similar (low) activity in four protein binding assays. BSA, bovine serum albumin; CA, carbonic anhydrase; CT, α -chymotrypsin; HB, hemoglobin.

external evaluation set. The critical step of the external validation is related to the use of the applicability domain,⁵¹⁴ which is defined uniquely for each model used in the consensus (ensemble) prediction of the external set. If external validation demonstrates the significant predictive power of the models, we

employ the models for virtual screening of available chemical databases (e.g., ZINC⁵¹⁵) to identify putative active compounds and work with collaborators who could experimentally validate such hits.

We shall emphasize two of the most important aspects of the QSAR modeling workflow that directly pertain to QNAR modeling. First, the most critical difference between any chemical data sets that the QSAR modeling strategy is applied to is the type of descriptors used to characterize the compounds. Thus, selection of the most appropriate descriptors of nanoparticles is the main challenge for developing QNAR strategies. Second, models resulting from the predictive QSAR modeling workflow (Figure 1) can be used in virtual screening to prioritize selection of chemicals for experimental validation. In fact, it is increasingly critical to include experimental validation as the ultimate assertion of the model-based prediction. This paradigm fully applies to the use of QNAR modeling in nanotechnology as described in the next section.

5.3.2. QNAR Modeling: Applications to the Rational Design of Nanoparticles with Desired Bioactivity Profiles. Evaluation of both the desired and the undesired biological effects of nanoparticles is of critical importance for the future of nanotechnology. Predictive QNAR modeling can be used, by analogy with QSAR, to establish statistically significant relationships between the measured biological effects of nanoparticles and their physical, chemical, and geometrical properties. As follows from the previous section, modeling of the biological effects of nanoparticles is possible if they can be characterized by descriptors. Figure 21 illustrates two different categories of nanoparticle data sets: those comprising nanoparticles with diverse metal cores and organic decorations for which only experimentally measured properties (i.e., size, shape, zeta potential, morphology, surface area, chemical reactivity, chemical composition, and aspect ratio) can be used as descriptors and those involving nanoparticles possessing the same core (e.g., CNTs) but different surface-modifying organic molecules for which descriptors can be calculated for a single representative of the surface-modifying molecule. Several commercially available software packages, such as Dragon,⁵¹⁶ MOE,⁵¹⁷ and ISIDA,⁵¹⁸ could compute hundreds to thousands of theoretical descriptors for any single compound directly from its molecular structure. To illustrate the possible applications of the QNAR method, we describe several case studies for which we constructed and validated models (Figure 22).

5.3.2.1. Modeling of Cellular Effects Induced by Diverse Nanoparticles Using Experimentally Measured Properties as Descriptors. In a study,⁵¹ diverse nanoparticles were tested in various cell-based assays.³⁰ All nanoparticles were tested in vitro against four cell lines in four different assays at four different concentrations, resulting in a 51 × 64 biological data matrix of experimental results. Each cell of this matrix reported the biological activity profile induced by a given nanoparticle at a certain concentration in a particular assay for a given cell line. Assay response values were expressed in units of standard deviations of the distribution obtained when control cells were treated with phosphate buffered saline (PBS) alone: $Z_{NP} = (\mu_{NP} - \mu_{PBS})/\sigma_{PBS}$, where μ_{PBS} is the mean of the control tests with PBS and σ_{PBS} is its standard deviation. The authors also reported four experimentally measured descriptors for 44 of 51 tested nanoparticles: size, two types of relaxivities, and zeta potential.

We demonstrated the feasibility of deriving robust QNAR models using the following four experimental descriptors: size, two types of relaxivities, and zeta potential. Four of such structural descriptors were available for 44 of the 51 nanoparticles. To capture nanoparticles' activity across a

broader swath of biology, the Shaw group measured the effects of nanoparticles in four diverse cell line at four doses and using four assays that interrogate different aspects of cellular physiology. Thus, the biological effects of each nanomaterial can be described as a 64-feature vector (4 cell lines × 4 assays × 4 doses).

In theory, 64 independent QNAR models could be developed with each model attempting to reproduce the biological response induced by 44 nanoparticles for a given assay in a particular cell line at a given dose; however, we were unable to develop statistically significant models. Therefore, we combined the entire 64-dimensional vector for each nanoparticle into a single averaged response. We then defined two binary classes using an arbitrary threshold at $Z_{mean} = -0.40$ to enable a binary classification of nanoparticles. Two groups, each containing the same number of nanoparticles, were thus defined: 22 nanoparticles belonged to class 1 ($Z_{mean} \geq -0.40$), and the remaining 22 were placed in class 0 ($Z_{mean} < -0.40$).³¹

The WinSVM program (a Windows implementation of a support vector machine) developed in house was used to derive QNAR classification models. A rigorous 5-fold external cross-validation was followed to validate the models; under this procedure, the program splits the entire data set five times into a modeling set including 80% of nanoparticles and the external validation set, comprising the remaining 20% of nanoparticles. Only the modeling set (which is divided additionally into multiple training and test sets) was used to build and validate models, and models with reasonable training and test set prediction power were selected for predicting the class membership of the external set compounds. Importantly, each nanoparticle was included in a validation set only once, allowing us to calculate the overall external prediction accuracy for the whole set.

The results demonstrated that the SVM models yielded an external prediction as high as 73% for the entire set of 44 nanoparticles. Within five independent external folds, prediction performances led from 56% to 88% accuracy. Apparently, the combination of biological profiles into one single binary classification scheme allowed us to detect the overall biological signal from noise and thus obtain QNAR models with both good internal fitness and external predictive power.

5.3.2.2. Modeling of Nanoparticle Uptake in PaCa2 Cancer Cells Using Computational Descriptors. Whether the multivalent attachment of small organic molecules to the same nanoparticles can increase their specific binding affinity to certain cells without a priori knowledge of the cell target was investigated.⁵¹⁹ A combinatorial library of 109 nanoparticles was synthesized in which a superparamagnetic nanoparticle (cross-linked iron oxide with amine groups, CLIO-NH₂) was decorated with different synthetic small molecules. Nanoparticles were screened against different cell lines, including PaCa2 human pancreatic cancer cells, U937 macrophage cell lines, resting and activated primary human macrophages, and human umbilical vein endothelial cells (HUVECs). In contrast to the other cell lines, the uptake of the nanoparticles in PaCa2 pancreatic cancer cells was diverse and highly dependent on surface modification, enabling application of the QSAR modeling approach to these data. We represented each individual nanoparticle by the structure of a single, organic molecule decorating its surface. Thus, each nanoparticle was represented by a unique set of descriptor values determined for the conjugated small molecule.

Using this representation, we developed statistically significant models by relying on chemical descriptors and nanoparticle cellular uptake. The models' external prediction power was shown to be as high as $R^2 = 0.72$ and a mean absolute error of 0.18 under a 5-fold external validation procedure.³¹ This study suggests that the cellular behavior of a series of nanoparticles sharing a common core can be reasonably well predicted using QNAR models built with the descriptors of surface modifying ligands only.

5.3.2.3. QNAR Models as Tools for Designing CNTs with Desired Biological Properties. CNTs are among the most well-known nanoparticles, particularly in the area of drug delivery.^{520–523} We utilized a combinatorial synthesis technique to decorate the CNTs' large surface area with a series of congeneric organic molecules and investigated the overall toxicity of this series of nanoparticles. A total of 84 CNTs were tested in several in vitro toxicological assays, including protein binding assays (BSA, carbonic anhydrase, chymotrypsin, and hemoglobin), acute toxicity assays (survival percentage), and immune toxicity assays (secretion of nitric oxide), all at two different doses (7.5 and 15 $\mu\text{g}/\text{mL}$). All of the CNTs shared the same core structure, and thus, the variability of their biological/toxicological behavior could be interpreted only based on the surface modifications.²⁷

Each CNT was represented with a unique organic molecule from its coating surface, similar to the study described above, and chemical descriptors were calculated for each molecule. We assigned a binary toxicity class (1 as toxic and 0 as nontoxic) to each CNT based on the acute toxicity assay and the toxicity measured for pristine CNTs (CNTs with no surface modifiers). Only strong and weak CNT toxicants were considered for the modeling: 38 strong toxicants (class 1) vs 35 weak toxicants (class 0) were subjected to QNAR modeling using the Random Forest approach. Similarly to other case studies, we realized a 5-fold external cross-validation. The results were surprisingly good with an overall external prediction accuracy as high as 74% ($n = 73$, sensitivity = 79%, specificity = 69%). We also developed QNAR models using protein-binding profiles that yielded 75% external prediction accuracy for classifying strong vs weak CNT protein binders.

These models have been employed for the virtual screening of a large library of 240,000 potential CNT surface modifiers in order to identify those with desired properties (low toxicity, low binding affinity for carbonic anhydrase). Ten compounds that were predicted to be toxic and ten compounds that were predicted to be nontoxic were selected for the experimental validation. The preliminary results suggest that all predictions for nontoxic compounds were accurate, and for 6 out of 10 compounds predicted to be toxic, the experimental results were in agreement with the computational annotation.

We conclude that analogous to conventional applications of QSAR modeling for analysis of bioactive organic molecules, QNAR models can be useful for predicting activity profiles of novel nanoparticles from either their experimental or their computationally derived descriptors and for designing and manufacturing safer nanoparticles with desired properties. However, a shortage of systematic experimental data that characterize the constitutional, physical, and chemical properties of nanoparticles remains. In addition, no standard set of biological assays has been clearly defined to objectively and relevantly evaluate the *in vitro* (and *in vivo*) effects of tested CNTs. Development of systematized data repositories through collective efforts of experimental and computational scientists

will enable the development of new and highly valuable QNAR models capable of rationally guiding further experimental studies toward safer and more effective nanoparticles.

6. CONCLUDING REMARKS

A double-edged sword, nanotechnology provides us with enormous opportunities while posing a potential threat to human health. Therefore, understanding how engineered nanoparticles interact with biological systems is urgent and critical. Nanoparticles are known to cause organ damage in experimental animals and even in humans. Such damage is the direct or indirect result of perturbations of cells and biological molecules such as proteins, lipids, DNA, and small molecules by nanoparticles. The nature and magnitude of nanoparticle interactions with biological molecules are determined by both the biological molecules and the engineered nanoparticles themselves.

The fundamental physicochemical properties of nanoparticles, such as their surface charge, hydrophobicity, and chemical bonding interactions, play significant roles in nano–bio interactions. Nanoparticles with different physicochemical properties exhibit different interactions with biological systems. For example, they may be surrounded by protein coronas of different nature, bind to different cell membrane proteins, enter or perturb cells differently, or exhibit diverse compatibility with physiological systems. A systematic understanding of such chemical interactions will help elucidate the QNAR of nano–bio interactions. Such an understanding also facilitates the generation of nanomaterials through safe-by-design processes.

On the basis of these considerations, intentional surface modification of nanomaterials by one-at-a-time, parallel, or combinatorial synthesis has been shown to alter nano–bio interactions. Such effects have encouraged the development of biocompatible or biotargeting nanoparticles. Another significant development is that nano–bio interactions can now be predicted by computational chemistry. These experimental and theoretical approaches have strengthened our understanding of the chemical basis of nano–bio interactions and provide unprecedented methodologies to proactively design and manufacture safe nanomaterials for a sustainable nanotechnology era.

However, investigation of nano–bio interactions is by no means complete. Many challenges remain. First, we must understand the mechanisms of nano–bio interactions with greater clarity via better characterization of experimental systems, such as nanoparticles. Due to their chemical nature and their interactions with microenvironmental molecules, the chemical identity, biological identity, or cellular identity of nanoparticles may differ. Comprehensive characterization will improve our understanding of such interaction mechanisms, and QNAR studies can be conducted with better accuracy. Moreover, diverse findings from different laboratories can be compared to form a systematic knowledge base. Second, at the cellular level, an interaction with a receptor protein leads to a network of cellular signaling and release of numerous molecules for signal propagation, feedback, and adaptation. Therefore, an in-depth understanding of nano–bio interactions at the cellular level relies on future development of systems biology and related knowledge bases. Third and most significant, at the physiological level, the impact of nanoparticles on human health is an end result of complicated interplays of complex interactions. For example, nanoparticles always adsorb environmental pollutants and perturb cellular nutrient molecules and

redox balance, yielding multipronged effects. Furthermore, such toxicant exposures are due to various sources and last a lifetime, beginning with a fertilized egg. Therefore, research aimed at understanding nano–bio interactions is an integral part of exploration in chemical biology and toxicology, having unique features. Therefore, it relies on the future progress of the chemical and biological sciences.

AUTHOR INFORMATION

Corresponding Author

*Phone: +86-531-88380019. Fax: +86-531-88380029. E-mail: drbingyan@yahoo.com.

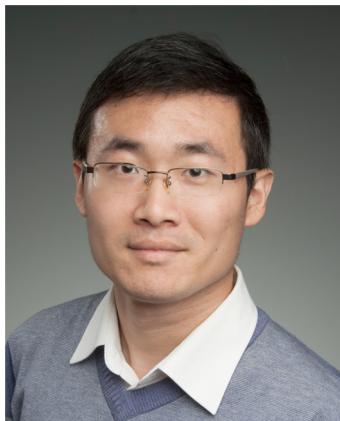
Present Address

[†]Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, and Department of Materials Science & Engineering, University of Washington, Seattle, WA 98195, United States.

Notes

The authors declare no competing financial interest.

Biographies



Qingxin Mu received his Ph.D. degree from Shandong University, China, in 2010. From 2007 to 2013, he worked at St. Jude Children's Research Hospital and University of Kansas as a research scholar and postdoctoral fellow, respectively. He is currently an NIH T32 postdoctoral research fellow at Clinical Research Division, Fred Hutchinson Cancer Research Center and Department of Materials Science & Engineering, University of Washington. His research interests include surface engineering of nanoparticles, bioactivity investigation of engineered nanoparticles and their applications in targeted drug delivery and tumor imaging.



Guibin Jiang received his Ph.D. degree from the Chinese Academy of Sciences in 1991 and carried out postdoctoral research at University of Antwerp, Belgium, from 1994 to 1996. He is now a professor at the Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, and Associate Editor for the *Journal of Environmental Sciences and Technology* published by the American Chemical Society. He is interested in understanding the environmental processing and toxicology of persistent toxic substances, environmental applications, and impacts of nanomaterials.



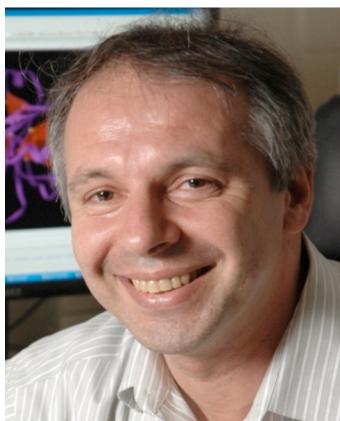
Lingxin Chen received his Ph.D. degree from the Dalian Institute of Chemical Physics, Chinese Academy of Sciences, China, in 2003. After 2 years of postdoctoral experience at the Department of Chemistry, Tsinghua University, China, he joined first as a BK21 researcher and then as a research professor at the Department of Applied Chemistry, Hanyang University, Ansan, Korea, in 2006. Now he is a professor in the Yantai Institute of Coastal Zone Research under the program so-called One Hundred Outstanding Young Chinese Scientists, Chinese Academy of Sciences, China. His research interests include the study of novel properties of materials such as functionalization nanoparticles for developing nanometer biochemical analysis methods, molecular imprinting-based sample pretreatment technology—chromatography (including microfluidic chip) combined technology, and the microbial degradation pollutant mechanism.



Hongyu Zhou received his Ph.D. degree in Medicinal Chemistry from Shandong University, China, in 2009. He was a postdoctoral researcher from 2009 to 2012 under the supervision of Dr. Bing Yan in the Department of Chemical Biology & Therapeutics at St. Jude Children's Research Hospital in Memphis, TN. He is currently a postdoctoral fellow in the Department of Surgery at Emory University. His research focuses on development of novel surface-modified nanoparticle combinatorial libraries to regulate the biological properties of nanoparticles and nanoparticle-based drug delivery systems and their applications in targeted cancer therapy and imaging.



Denis Fourches is Research Assistant Professor at the UNC Eshelman School of Pharmacy, UNC—Chapel Hill. He received his Ph.D. degree in Chemistry in 2007 from the University of Strasbourg, France. He came to UNC—Chapel Hill in 2008 as a postdoctoral fellow and became an assistant professor in 2010. His research interests are in the areas of cheminformatics, computer-assisted drug design, computational toxicology, and structural bioinformatics.



Alexander Tropsha is K.H. Lee Distinguished Professor and Associate Dean for Research at the UNC Eshelman School of Pharmacy, UNC—Chapel Hill. He received his Ph.D. degree in Chemical Enzymology in 1986 from Moscow State University, Russia. He came to UNC—Chapel Hill in 1989 as a postdoctoral fellow and became an assistant professor in 1991. His research interests are in the areas of computer-assisted drug design, computational toxicology, cheminformatics, and structural bioinformatics. He has authored or coauthored more than 160 peer-reviewed research papers, reviews, and book chapters and coedited two monographs. His research is supported by multiple grants from the NIH, NSF, EPA, and private companies. He is a member of editorial boards of several scientific journals and an elected member of the Board and vice-chair of the international Cheminformatics and QSAR Society.



Bing Yan received his Ph.D. degree from Columbia University in 1990 and carried out postdoctoral research at the University of Cambridge, U.K., and University of Texas Medical School in Houston from 1990 to 1993. From 1993 to 2005 he worked at Novartis, Discovery Partners International, and Bristol-Myers Squibb. He has been a full faculty member at the Department of Chemical Biology and Therapeutics, St. Jude Children's Research Hospital, in Memphis, TN, from 2007 to 2012, and Professor at Shandong University, China, from 2005 to present. He served as Associate Editor for the *Journal of Combinatorial Chemistry* published by the American Chemical Society from 2005 to 2012 and was book series editor for *Critical Reviews in Combinatorial Chemistry* published by the Francis & Taylor Group. He is interested in understanding the biological activities of nanoparticles, regulation of such activities by nanoparticle modifications, and medicinal applications of nanotechnology.

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ABBREVIATIONS

AFM	atomic force microscopy
ATP	adenosine-5'-triphosphate
BMP	bone morphogenetic protein
BMPR2	bone morphogenetic protein receptor type II
BSA	bovine serum albumin
CB	carbon black
CD	circular dichroism
CLSM	confocal laser scanning microscopy
CNTs	carbon nanotubes
CTAB	hexadecyltrimethylammonium bromide
DLS	dynamic light scattering
DPPC	dipalmitoylphosphatidylcholine
DSC	differential scanning calorimetry
EB	ethidium bromide
ENMs	engineered nanomaterials
ER	endoplasmic reticulum
FCS	fluorescence correlation spectroscopy
FTIR	Fourier-transform infrared spectroscopy
GC-MS	gas chromatography–mass spectrometry
GNNPs	gold nanoparticles
GO	graphene oxide
GPCRs	G-protein-coupled receptors
GSH	glutathione
Hb	hemoglobin
HSA	human serum albumin

HSQC NMR	heteronuclear single quantum coherence nuclear magnetic resonance
HUVECs	human umbilical vein endothelial cells
ITC	isothermal titration calorimetry
iTRAQ	isobaric tags for relative and absolute quantitation
LB	Langmuir–Blodgett
LC-MS	liquid chromatography mass spectroscopy
MALDI TOF-MS	matrix-assisted laser desorption-ionization time-of-flight mass spectrometry
MAPK	mitogen-activated protein kinase
MAS NMR	magic angle spinning nuclear magnetic resonance
MNPs	magnetic nanoparticles
MS	mass spectrometry
MWNTs	multiwalled carbon nanotubes
NF-κB	nuclear factor kappa B
NIR	near-infrared
NMR	nuclear magnetic resonance
PBAEs	poly-β-amino esters
PBS	phosphate-buffered saline
PEG	poly(ethylene glycol)
PRINT	particle replication in nonwetting template
QCM	quartz crystal microbalance
QDs	quantum dots
QNAR	quantitative nanostructure activity relationships
QSAR	quantitative structure–activity relationships
RNA	ribonucleic acid
RNS	reactive nitrogen species
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
SEM	scanning electron microscopy
SERS	surface-enhanced Raman spectroscopy
SPR	surface plasma resonance
SVM	support vector machine
SWNTs	single-walled carbon nanotubes
TEM	transmission electron microscopy
TGF-β	transforming growth factor beta
TLR	Toll-like receptor
TOF MS	time-of-flight mass spectrometry
UV-vis	ultraviolet–visible
VEGF	vascular endothelial growth factor
XPS	X-ray photoelectron spectroscopy

REFERENCES

- (1) European Union Law 32011H0696. <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32011H0696:EN:NOT> (accessed Sept 12, 2013).
- (2) Stankovich, S.; Dikin, D. A.; Dommett, G. H. B.; Kohlhaas, K. M.; Zimney, E. J.; Stach, E. A.; Piner, R. D.; Nguyen, S. T.; Ruoff, R. S. *Nature* **2006**, *442*, 282.
- (3) Tans, S. J.; Verschueren, A. R. M.; Dekker, C. *Nature* **1998**, *393*, 49.
- (4) Morones, J. R.; Elechiguerra, J. L.; Camacho, A.; Holt, K.; Kouri, J. B.; Ramirez, J. T.; Yacaman, M. J. *Nanotechnology* **2005**, *16*, 2346.
- (5) Medintz, I. L.; Uyeda, H. T.; Goldman, E. R.; Mattoussi, H. *Nat. Mater.* **2005**, *4*, 435.
- (6) Nanomaterials Production 2002–2016: Production Volumes, Revenues and End User Market Demand. http://www.researchandmarkets.com/research/97chkk/nanomaterials_prod (accessed Sept 12, 2013).
- (7) Colvin, V. L. *Nat. Biotechnol.* **2003**, *21*, 1166.

- (8) Maynard, A. D.; Kuempel, E. D. *J. Nanopart. Res.* **2005**, *7*, 587.
- (9) Song, Y.; Li, X.; Du, X. *Eur. Respir. J.* **2009**, *34*, 559.
- (10) Gottschalk, F.; Nowack, B. *J. Environ. Monit.* **2011**, *13*, 1145.
- (11) Kendall, M.; Holgate, S. *Respirology* **2012**, *17*, 743.
- (12) Wiecinski, P. N.; Metz, K. M.; Mangham, A. N.; Jacobson, K. H.; Hamers, R. J.; Pedersen, J. A. *Nanotoxicology* **2009**, *3*, 202.
- (13) Pietrojasti, A. *Nanoscale* **2012**, *4*, 1231.
- (14) Chao, J.-b.; Liu, J.-f.; Yu, S.-j.; Feng, Y.-d.; Tan, Z.-q.; Liu, R.; Yin, Y.-g. *Anal. Chem.* **2011**, *83*, 6875.
- (15) Judy, J. D.; Unrine, J. M.; Bertsch, P. M. *Environ. Sci. Technol.* **2011**, *45*, 776.
- (16) Werlin, R.; Priester, J. H.; Mielke, R. E.; Kramer, S.; Jackson, S.; Stoimenov, P. K.; Stucky, G. D.; Cherr, G. N.; Orias, E.; Holden, P. A. *Nat. Nanotechnol.* **2011**, *6*, 65.
- (17) De Jong, W. H.; Borm, P. J. A. *Int. J. Nanomed.* **2008**, *3*, 133.
- (18) Alberts, B.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. *Molecular Biology of the Cell*, 5th ed.; Garland Science: New York, 2007.
- (19) Mu, Q.; Liu, W.; Xing, Y.; Zhou, H.; Li, Z.; Zhang, Y.; Ji, L.; Wang, F.; Si, Z.; Zhang, B.; Yan, B. *J. Phys. Chem. C* **2008**, *112*, 3300.
- (20) Yang, J.; Lee, J. Y.; Too, H. P.; Chow, G. M. *Biophys. Chem.* **2006**, *120*, 87.
- (21) Yi, C. Q.; Liu, D. D.; Fong, C. C.; Zhang, J. C.; Yang, M. S. *ACS Nano* **2010**, *4*, 6439.
- (22) Puddu, V.; Perry, C. C. *ACS Nano* **2012**, *6*, 6356.
- (23) Fischer, N. O.; Verma, A.; Goodman, C. M.; Simard, J. M.; Rotello, V. M. *J. Am. Chem. Soc.* **2003**, *125*, 13387.
- (24) Liu, Y. P.; Meyer-Zaika, W.; Franzka, S.; Schmid, G.; Tsoli, M.; Kuhn, H. *Angew. Chem., Int. Ed.* **2003**, *42*, 2853.
- (25) Zhang, Y.; Tekobo, S.; Tu, Y.; Zhou, Q. F.; Jin, X. L.; Dergunov, S. A.; Pinkhassik, E.; Yan, B. *ACS Appl. Mater. Interfaces* **2012**, *4*, 4099.
- (26) Sun, E. Y.; Josephson, L.; Kelly, K. A.; Weissleder, R. *Bioconjugate Chem.* **2005**, *17*, 109.
- (27) Zhou, H.; Mu, Q.; Gao, N.; Liu, A.; Xing, Y.; Gao, S.; Zhang, Q.; Qu, G.; Chen, Y.; Liu, G.; Zhang, B.; Yan, B. *Nano Lett.* **2008**, *8*, 859.
- (28) Weissleder, R.; Kelly, K.; Sun, E. Y.; Shtatland, T.; Josephson, L. *Nat. Biotechnol.* **2005**, *23*, 1418.
- (29) Fourches, D.; Pu, D. Q. Y.; Tropsha, A. *Comb. Chem. High Throughput Screening* **2011**, *14*, 217.
- (30) Shaw, S. Y.; Westly, E. C.; Pittet, M. J.; Subramanian, A.; Schreiber, S. L.; Weissleder, R. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 7387.
- (31) Fourches, D.; Pu, D. Q. Y.; Tassia, C.; Weissleder, R.; Shaw, S. Y.; Mumper, R. J.; Tropsha, A. *ACS Nano* **2010**, *4*, 5703.
- (32) Walczyk, D.; Bombelli, F. B.; Monopoli, M. P.; Lynch, I.; Dawson, K. A. *J. Am. Chem. Soc.* **2010**, *132*, 5761.
- (33) Rao, C. N. R.; Biswas, K. *Annu. Rev. Anal. Chem.* **2009**, *2*, 435.
- (34) Zimbone, M.; Calcagno, L.; Messina, G.; Baeri, P.; Compagnini, G. *Mater. Lett.* **2011**, *65*, 2906.
- (35) Patil, S.; Sandberg, A.; Heckert, E.; Self, W.; Seal, S. *Biomaterials* **2007**, *28*, 4600.
- (36) Elzey, S.; Tsai, D. H.; Yu, L. L.; Winchester, M. R.; Kelley, M. E.; Hackley, V. A. *Anal. Bioanal. Chem.* **2013**, *405*, 2279.
- (37) Baer, D. R.; Gaspar, D. J.; Nachimuthu, P.; Techane, S. D.; Castner, D. G. *Anal. Bioanal. Chem.* **2010**, *396*, 983.
- (38) Zhou, H.; Li, X.; Lemoff, A.; Zhang, B.; Li, W.; Yan, B. *Analyst* **2010**, *135*, 1210.
- (39) Zhou, H. Y.; Du, F. F.; Li, X.; Zhang, B.; Li, W.; Yan, B. *J. Phys. Chem. C* **2008**, *112*, 19360.
- (40) Zhang, B.; Yan, B. *Anal. Bioanal. Chem.* **2010**, *396*, 973.
- (41) Wang, Y.; Yan, B.; Chen, L. *Chem. Rev.* **2012**, *113*, 1391.
- (42) Kumari, A.; Yadav, S. K. *Expert Opin. Drug Delivery* **2011**, *8*, 141.
- (43) Zhao, F.; Zhao, Y.; Liu, Y.; Chang, X. L.; Chen, C. Y.; Zhao, Y. *L. Small* **2011**, *7*, 1322.
- (44) Treuel, L.; Jiang, X. E.; Nienhaus, G. U. *J. R. Soc., Interface* **2013**, *10*, No. 20120939.
- (45) Fadaee-Shohada, M. J.; Hirst, R. A.; Rutman, A.; Roberts, I. S.; O'Callaghan, C.; Andrew, P. W. *PLoS One* **2010**, *5*, e10450.

- (46) Zhao, Y. N.; Sun, X. X.; Zhang, G. N.; Trewyn, B. G.; Slowing, I. I.; Lin, V. S. Y. *ACS Nano* **2011**, *5*, 1366.
- (47) Gao, N. N.; Zhang, Q.; Mu, Q. X.; Bai, Y. H.; Li, L. W.; Zhou, H. Y.; Butch, E. R.; Powell, T. B.; Snyder, S. E.; Jiang, G. B.; Yan, B. *ACS Nano* **2011**, *5*, 4581.
- (48) Corazzari, I.; Gilardino, A.; Dalmazzo, S.; Fubini, B.; Lovisolo, D. *Toxicol. In Vitro* **2013**, *27*, 752.
- (49) Seib, F. P.; Jones, G. T.; Rnjak-Kovacina, J.; Lin, Y.; Kaplan, D. L. *Adv. Healthcare Mater.* **2013**, *2*, 1606.
- (50) Gasser, M.; Rothen-Rutishauser, B.; Krug, H. F.; Gehr, P.; Nelle, M.; Yan, B.; Wick, P. *J. Nanobiotechnol.* **2010**, *8*, 31.
- (51) Kapralov, A. A.; Feng, W. H.; Amoscato, A. A.; Yanamala, N.; Balasubramanian, K.; Winnica, D. E.; Kisin, E. R.; Kotchey, G. P.; Gou, P. P.; Sparvero, L. J.; Ray, P.; Mallampalli, R. K.; Klein-Seetharaman, J.; Fadeel, B.; Star, A.; Shvedova, A. A.; Kagan, V. E. *ACS Nano* **2012**, *6*, 4147.
- (52) Ge, C. C.; Du, J. F.; Zhao, L. N.; Wang, L. M.; Liu, Y.; Li, D. H.; Yang, Y. L.; Zhou, R. H.; Zhao, Y. L.; Chai, Z. F.; Chen, C. Y. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 16968.
- (53) Prado-Gotor, R.; Grueso, E. *Phys. Chem. Chem. Phys.* **2011**, *13*, 1479.
- (54) Guo, L.; Bussche, A. V.; Buechner, M.; Yan, A. H.; Kane, A. B.; Hurt, R. H. *Small* **2008**, *4*, 721.
- (55) Hellstrand, E.; Lynch, I.; Andersson, A.; Drakenberg, T.; Dahlback, B.; Dawson, K. A.; Linse, S.; Cedervall, T. *FEBS J.* **2009**, *276*, 3372.
- (56) Kumar, P.; Bohidar, H. B. *Colloids Surf., A* **2010**, *361*, 13.
- (57) Bakshi, M. S.; Zhao, L.; Smith, R.; Possmayer, F.; Petersen, N. O. *Biophys. J.* **2008**, *94*, 855.
- (58) Wang, L. Y.; Castranova, V.; Mishra, A.; Chen, B.; Mercer, R. R.; Schwegler-Berry, D.; Rojanasakul, Y. *Part. Fibre Toxicol.* **2010**, *7*, 31.
- (59) Beck-Broichsitter, M.; Ruppert, C.; Schmehl, T.; Guenther, A.; Betz, T.; Bakowsky, U.; Seeger, W.; Kissel, T.; Gessler, T. *Nanomed.-Nanotechnol. Biol. Med.* **2011**, *7*, 341.
- (60) Herzog, E.; Byrne, H. J.; Davoren, M.; Casey, A.; Duschl, A.; Oostingh, G. J. *Toxicol. Appl. Pharmacol.* **2009**, *236*, 276.
- (61) Schleh, C.; Muhlfeld, C.; Pulskamp, K.; Schmiedl, A.; Nassimi, M.; Lauenstein, H. D.; Braun, A.; Krug, N.; Erpenbeck, V. J.; Hohlfeld, J. M. *Respir. Res.* **2009**, *10*, 90.
- (62) Peetla, C.; Stine, A.; Labhsetwar, V. *Mol. Pharmaceutics* **2009**, *6*, 1264.
- (63) Baoukina, S.; Monticelli, L.; Marrink, S. J.; Tielemans, D. P. *Langmuir* **2007**, *23*, 12617.
- (64) Peetla, C.; Labhsetwar, V. *Mol. Pharmaceutics* **2008**, *5*, 418.
- (65) Roiter, Y.; Ornatska, M.; Rammohan, A. R.; Balakrishnan, J.; Heine, D. R.; Minko, S. *Langmuir* **2009**, *25*, 6287.
- (66) Roiter, Y.; Ornatska, M.; Rammohan, A. R.; Balakrishnan, J.; Heine, D. R.; Minko, S. *Nano Lett.* **2008**, *8*, 941.
- (67) Mecke, A.; Majoros, I. J.; Patri, A. K.; Baker, J. R.; Holl, M. M. B.; Orr, B. G. *Langmuir* **2005**, *21*, 10348.
- (68) Tsogas, I.; Tsiorvas, D.; Nounesis, G.; Paleos, C. M. *Langmuir* **2006**, *22*, 11322.
- (69) Hartono, D.; Hody, Yang, K. L.; Yung, L. Y. L. *Biomaterials* **2010**, *31*, 3008.
- (70) Hartono, D.; Qin, W. J.; Yang, K. L.; Yung, L. Y. L. *Biomaterials* **2009**, *30*, 843.
- (71) Ionov, M.; Gardikis, K.; Wrobel, D.; Hatziantoniou, S.; Mourelatou, H.; Majoral, J. P.; Klajnert, B.; Bryszewska, M.; Demetzos, C. *Colloids Surf., B* **2011**, *82*, 8.
- (72) Bhattacharya, S.; Srivastava, A. *Langmuir* **2003**, *19*, 4439.
- (73) Wang, B.; Zhang, L. F.; Bae, S. C.; Granick, S. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 18171.
- (74) Zhang, X. F.; Yang, S. H. *Langmuir* **2011**, *27*, 2528.
- (75) Harishchandra, R. K.; Saleem, M.; Galla, H. J. *J. R. Soc., Interface* **2010**, *7*, S15.
- (76) Goertz, M. P.; Goyal, N.; Bunker, B. C.; Montano, G. A. *J. Colloid Interface Sci.* **2011**, *358*, 635.
- (77) Mecke, A.; Lee, D. K.; Ramamoorthy, A.; Orr, B. G.; Holl, M. M. B. *Langmuir* **2005**, *21*, 8588.
- (78) Hirano, A.; Uda, K.; Maeda, Y.; Akasaka, T.; Shiraki, K. *Langmuir* **2010**, *26*, 17256.
- (79) Leroueil, P. R.; Berry, S. A.; Duthie, K.; Han, G.; Rotello, V. M.; McNerny, D. Q.; Baker, J. R.; Orr, B. G.; Holl, M. M. B. *Nano Lett.* **2008**, *8*, 420.
- (80) Ramachandran, S.; Merrill, N. E.; Blick, R. H.; van der Weide, D. W. *Biosens. Bioelectron.* **2005**, *20*, 2173.
- (81) Ramachandran, S.; Kumar, G. L.; Blick, R. H.; van der Weide, D. W. *Appl. Phys. Lett.* **2005**, *86*, 083901.
- (82) Sachan, A. K.; Harishchandra, R. K.; Bantz, C.; Maskos, M.; Reichelt, R.; Galla, H. J. *ACS Nano* **2012**, *6*, 1677.
- (83) Peetla, C.; Labhsetwar, V. *Langmuir* **2009**, *25*, 2369.
- (84) Hong, S. P.; Bielinska, A. U.; Mecke, A.; Keszler, B.; Beals, J. L.; Shi, X. Y.; Balogh, L.; Orr, B. G.; Baker, J. R.; Holl, M. M. B. *Bioconjugate Chem.* **2004**, *15*, 774.
- (85) Hong, S. P.; Leroueil, P. R.; Janus, E. K.; Peters, J. L.; Kober, M. M.; Islam, M. T.; Orr, B. G.; Baker, J. R.; Holl, M. M. B. *Bioconjugate Chem.* **2006**, *17*, 728.
- (86) Peetla, C.; Rao, K. S.; Labhsetwar, V. *Mol. Pharmaceutics* **2009**, *6*, 1311.
- (87) Li, Y.; Chen, X.; Gu, N. *J. Phys. Chem. B* **2008**, *112*, 16647.
- (88) Yang, K.; Ma, Y. Q. *Nat. Nanotechnol.* **2010**, *5*, 579.
- (89) Monticelli, L.; Salonen, E.; Ke, P. C.; Vattulainen, I. *Soft Matter* **2009**, *5*, 4433.
- (90) Park, J.; Lu, W. *Phys. Rev. E* **2009**, *80*, No. 021607.
- (91) Choe, S.; Chang, R.; Jeon, J.; Violi, A. *Biophys. J.* **2008**, *95*, 4102.
- (92) Jusufi, A.; DeVane, R. H.; Shinoda, W.; Klein, M. L. *Soft Matter* **2011**, *7*, 1139.
- (93) Song, B.; Yuan, H. J.; Jameson, C. J.; Murad, S. *Mol. Phys.* **2011**, *109*, 1511.
- (94) Fiedler, S. L.; Violi, A. *Biophys. J.* **2010**, *99*, 144.
- (95) Ding, H. M.; Ma, Y. Q. *Nanoscale* **2012**, *4*, 1116.
- (96) Ginzburg, V. V.; Balijepailli, S. *Nano Lett.* **2007**, *7*, 3716.
- (97) Kelly, C. V.; Leroueil, P. R.; Orr, B. G.; Holl, M. M. B.; Andricioaei, I. *J. Phys. Chem. B* **2008**, *112*, 9346.
- (98) Qiao, R.; Roberts, A. P.; Mount, A. S.; Klaine, S. J.; Ke, P. C. *Nano Lett.* **2007**, *7*, 614.
- (99) Aggarwal, P.; Hall, J. B.; McLeland, C. B.; Dobrovolskaia, M. A.; McNeil, S. E. *Adv. Drug Delivery Rev.* **2009**, *61*, 428.
- (100) Lynch, I.; Dawson, K. A.; Linse, S. *Sci. STKE* **2006**, *2006*, pe14.
- (101) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*, 3rd ed.; Springer: Berlin, Germany, 2009.
- (102) Casanova, D.; Giaume, D.; Moreau, M.; Martin, J. L.; Gacoin, T.; Boilot, J. P.; Alexandrou, A. *J. Am. Chem. Soc.* **2007**, *129*, 12592.
- (103) Rocker, C.; Potzl, M.; Zhang, F.; Parak, W. J.; Nienhaus, G. U. *Nat. Nanotechnol.* **2009**, *4*, 577.
- (104) Calzolai, L.; Franchini, F.; Gilliland, D.; Rossi, F. *Nano Lett.* **2010**, *10*, 3101.
- (105) Li, N.; Zeng, S.; He, L.; Zhong, W. W. *Anal. Chem.* **2011**, *83*, 6929.
- (106) Ipe, B. I.; Shukla, A.; Lu, H.; Zou, B.; Rehage, H.; Niemeyer, C. M. *ChemPhysChem* **2006**, *7*, 1112.
- (107) Rich, R. L.; Myszka, D. G. *Curr. Opin. Biotechnol.* **2000**, *11*, 54.
- (108) Cedervall, T.; Lynch, I.; Lindman, S.; Berggard, T.; Thulin, E.; Nilsson, H.; Dawson, K. A.; Linse, S. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 2050.
- (109) Kaufman, E. D.; Belyea, J.; Johnson, M. C.; Nicholson, Z. M.; Ricks, J. L.; Shah, P. K.; Bayless, M.; Pettersson, T.; Feldtö, Z.; Blomberg, E.; Claesson, P.; Franzen, S. *Langmuir* **2007**, *23*, 6053.
- (110) Gerdon, A. E.; Wright, D. W.; Cliffel, D. E. *Anal. Chem.* **2004**, *77*, 304.
- (111) Shenderova, O. A.; Zhirnov, V. V.; Brenner, D. W. *Crit. Rev. Solid State Mater. Sci.* **2002**, *27*, 227.
- (112) Kroto, H. W. *J. Chem. Soc., Faraday Trans.* **1990**, *86*, 2465.
- (113) Geim, A. K.; Novoselov, K. S. *Nat. Mater.* **2007**, *6*, 183.
- (114) Iijima, S.; Ichihashi, T. *Nature* **1993**, *363*, 603.
- (115) Nepal, D.; Geckeler, K. E. *Small* **2006**, *2*, 406.
- (116) Li, X. J.; Chen, W.; Zhan, Q. W.; Dai, L. M.; Sowards, L.; Pender, M.; Naik, R. R. *Phys. Chem. B* **2006**, *110*, 12621.

- (117) Bradley, K.; Briman, M.; Star, A.; Gruner, G. *Nano Lett.* **2004**, *4*, 253.
- (118) Karajanagi, S. S.; Vertegel, A. A.; Kane, R. S.; Dordick, J. S. *Langmuir* **2004**, *20*, 11594.
- (119) Han, Z. J.; Ostrikov, K.; Tan, C. M.; Tay, B. K.; Peel, S. A. F. *Nanotechnology* **2011**, *22*, 295712.
- (120) Matsuuwa, K.; Saito, T.; Okazaki, T.; Ohshima, S.; Yumura, M.; Iijima, S. *Chem. Phys. Lett.* **2006**, *429*, 497.
- (121) Lin, Y.; Allard, L. F.; Sun, Y. P. *J. Phys. Chem. B* **2004**, *108*, 3760.
- (122) Su, Z. D.; Leung, T.; Honek, J. F. *J. Phys. Chem. B* **2006**, *110*, 23623.
- (123) Balavoine, F.; Schultz, P.; Richard, C.; Mallouh, V.; Ebbesen, T. W.; Mioskowski, C. *Angew. Chem., Int. Ed.* **1999**, *38*, 1912.
- (124) Ling, W. L.; Biro, A.; Bally, I.; Tacnet, P.; Deniaud, A.; Doris, E.; Frachet, P.; Schoehn, G.; Pebay-Peyroula, E.; Arlaud, G. J. *ACS Nano* **2011**, *5*, 730.
- (125) Nepal, D.; Geckeler, K. E. *Small* **2007**, *3*, 1259.
- (126) Zorbas, V.; Ortiz-Acevedo, A.; Dalton, A. B.; Yoshida, M. M.; Dieckmann, G. R.; Draper, R. K.; Baughman, R. H.; Jose-Yacaman, M.; Musselman, I. H. *J. Am. Chem. Soc.* **2004**, *126*, 7222.
- (127) Karajanagi, S. S.; Yang, H. C.; Asuri, P.; Sellitto, E.; Dordick, J. S.; Kane, R. S. *Langmuir* **2006**, *22*, 1392.
- (128) Shen, J. W.; Wu, T.; Wang, Q.; Kang, Y. *Biomaterials* **2008**, *29*, 3847.
- (129) Fu, Z. M.; Luo, Y.; Derreumaux, P.; Wei, G. H. *Biophys. J.* **2009**, *97*, 1795.
- (130) Kang, Y.; Liu, Y. C.; Wang, Q.; Shen, J. W.; Wu, T.; Guan, W. *J. Biomaterials* **2009**, *30*, 2807.
- (131) Linse, S.; Cabaleiro-Lago, C.; Xue, W. F.; Lynch, I.; Lindman, S.; Thulin, E.; Radford, S. E.; Dawson, K. A. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 8691.
- (132) Benyamin, H.; Shulman-Peleg, A.; Wolfson, H. J.; Belgorodsky, B.; Fadeev, L.; Gozin, M. *Bioconjugate Chem.* **2006**, *17*, 378.
- (133) Belgorodsky, B.; Fadeev, L.; Kolsenik, J.; Gozin, M. *ChemBioChem* **2006**, *7*, 1783.
- (134) Nakamura, E.; Isobe, H. *Acc. Chem. Res.* **2003**, *36*, 807.
- (135) Chen, B. X.; Wilson, S. R.; Das, M.; Coughlin, D. J.; Erlanger, B. F. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 10809.
- (136) Zhu, Z. W.; Schuster, D. I.; Tuckerman, M. E. *Biochemistry* **2003**, *42*, 1326.
- (137) Sijbesma, R.; Srdanov, G.; Wudl, F.; Castoro, J. A.; Wilkins, C.; Friedman, S. H.; Decamp, D. L.; Kenyon, G. L. *J. Am. Chem. Soc.* **1993**, *115*, 6510.
- (138) Noon, W. H.; Kong, Y. F.; Ma, J. P. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 6466.
- (139) Braden, B. C.; Goldbaum, F. A.; Chen, B. X.; Kirschner, A. N.; Wilson, S. R.; Erlanger, B. F. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 12193.
- (140) Kotelnikova, R. A.; Bogdanov, G. N.; Frog, E. C.; Kotelnikov, A. I.; Shtolko, V. N.; Romanova, V. S.; Andreev, S. M.; Kushch, A. A.; Fedorova, N. E.; Medzhidova, A. A.; Miller, G. G. *J. Nanopart. Res.* **2003**, *5*, 561.
- (141) Sreejith, S.; Ma, X.; Zhao, Y. *J. Am. Chem. Soc.* **2012**, *134*, 17346.
- (142) Liang, L. J.; Wang, Q.; Wu, T.; Shen, J. W.; Kang, Y. *Chin. J. Chem. Phys.* **2009**, *22*, 627.
- (143) Hu, W. B.; Peng, C.; Lv, M.; Li, X. M.; Zhang, Y. J.; Chen, N.; Fan, C. H.; Huang, Q. *ACS Nano* **2011**, *5*, 3693.
- (144) De, M.; Chou, S. S.; Dravid, V. P. *J. Am. Chem. Soc.* **2011**, *133*, 17524.
- (145) Steinmuller-Nethl, D.; Kloss, F. R.; Najam-U-Haq, M.; Rainer, M.; Larsson, K.; Linsmeier, C.; Koehler, G.; Fehrer, C.; Lepperdinger, G.; Liu, X.; Memmel, N.; Bertel, E.; Huck, C. W.; Gassner, R.; Bonn, G. *Biomaterials* **2006**, *27*, 4547.
- (146) Kong, X. L.; Huang, L. C. L.; Hsu, C. M.; Chen, W. H.; Han, C. C.; Chang, H. C. *Anal. Chem.* **2004**, *77*, 259.
- (147) Worrall, J. W. E.; Verma, A.; Yan, H. H.; Rotello, V. M. *Chem. Commun.* **2006**, 2338.
- (148) Jiang, X.; Jiang, U. G.; Jin, Y. D.; Wang, E. K.; Dong, S. J. *Biomacromolecules* **2005**, *6*, 46.
- (149) Chah, S.; Hammond, M. R.; Zare, R. N. *Chem. Biol.* **2005**, *12*, 323.
- (150) Fischer, N. O.; McIntosh, C. M.; Simard, J. M.; Rotello, V. M. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5018.
- (151) Brewer, S. H.; Glomm, W. R.; Johnson, M. C.; Knag, M. K.; Franzen, S. *Langmuir* **2005**, *21*, 9303.
- (152) Dominguez-Medina, S.; McDonough, S.; Swanglap, P.; Landes, C. F.; Link, S. *Langmuir* **2012**, *28*, 9131.
- (153) Bhattacharya, R.; Mukherjee, P.; Xiong, Z.; Atala, A.; Soker, S.; Mukhopadhyay, D. *Nano Lett.* **2004**, *4*, 2479.
- (154) Mukherjee, P.; Bhattacharya, R.; Wang, P.; Wang, L.; Basu, S.; Nagy, J. A.; Atala, A.; Mukhopadhyay, D.; Soker, S. *Clin. Cancer. Res.* **2005**, *11*, 3530.
- (155) Hong, R.; Fischer, N. O.; Verma, A.; Goodman, C. M.; Emrick, T.; Rotello, V. M. *J. Am. Chem. Soc.* **2004**, *126*, 739.
- (156) Verma, A.; Simard, J. M.; Rotello, V. M. *Langmuir* **2004**, *20*, 4178.
- (157) Teichroeb, J. H.; Forrest, J. A.; Jones, L. W. *Eur. Phys. J. E: Soft Matter Biol. Phys.* **2008**, *26*, 411.
- (158) Pramanik, S.; Banerjee, P.; Sarkar, A.; Bhattacharya, S. C. *J. Lumin.* **2008**, *128*, 1969.
- (159) You, C. C.; De, M.; Han, G.; Rotello, V. M. *J. Am. Chem. Soc.* **2005**, *127*, 12873.
- (160) Deng, Z. J.; Liang, M. T.; Monteiro, M.; Toth, I.; Minchin, R. F. *Nat. Nanotechnol.* **2011**, *6*, 39.
- (161) Peng, Z. G.; Hidajat, K.; Uddin, M. S. *J. Colloid Interface Sci.* **2004**, *271*, 277.
- (162) Mahmoudi, M.; Shokrgozar, M. A.; Sardari, S.; Moghadam, M. K.; Vali, H.; Laurent, S.; Stroeve, P. *Nanoscale* **2011**, *3*, 1127.
- (163) Ishii, D.; Kinbara, K.; Ishida, Y.; Ishii, N.; Okochi, M.; Yohda, M.; Aida, T. *Nature* **2003**, *423*, 628.
- (164) Xiao, Q.; Huang, S.; Qi, Z. D.; Zhou, B.; He, Z. K.; Liu, Y. *Biochim. Biophys. Acta, Proteins Proteomics* **2008**, *1784*, 1020.
- (165) Pompa, P. P.; Chiuri, R.; Manna, L.; Pellegrino, T.; del Mercato, L. L.; Parak, W. J.; Calabi, F.; Cingolani, R.; Rinaldi, R. *Chem. Phys. Lett.* **2006**, *417*, 351.
- (166) Shen, X. C.; Liou, X. Y.; Ye, L. P.; Liang, H.; Wang, Z. Y. *J. Colloid Interface Sci.* **2007**, *311*, 400.
- (167) Wang, Q.; Kuo, Y. C.; Wang, Y. W.; Shin, G.; Ruengruglikit, C.; Huang, Q. *R. J. Phys. Chem. B* **2006**, *110*, 16860.
- (168) Larsericsdotter, H.; Oscarsson, S.; Buijs, J. *J. Colloid Interface Sci.* **2001**, *237*, 98.
- (169) Karlsson, M.; Carlsson, U. *Biophys. J.* **2005**, *88*, 3536.
- (170) Karlsson, M.; Martensson, L. G.; Jonsson, B. H.; Carlsson, U. *Langmuir* **2000**, *16*, 8470.
- (171) Lundqvist, M.; Sethson, I.; Jonsson, B.-H. *Langmuir* **2005**, *21*, 5974.
- (172) Vertegel, A. A.; Siegel, R. W.; Dordick, J. S. *Langmuir* **2004**, *20*, 6800.
- (173) Shang, W.; Nuffer, J. H.; Dordick, J. S.; Siegel, R. W. *Nano Lett.* **2007**, *7*, 1991.
- (174) Nygren, P.; Lundqvist, M.; Broo, K.; Jonsson, B.-H. *Nano Lett.* **2008**, *8*, 1844.
- (175) Lord, M. S.; Cousins, B. G.; Doherty, P. J.; Whitelock, J. M.; Simmons, A.; Williams, R. L.; Milthorpe, B. K. *Biomaterials* **2006**, *27*, 4856.
- (176) Lindman, S.; Lynch, I.; Thulin, E.; Nilsson, H.; Dawson, K. A.; Linse, S. *Nano Lett.* **2007**, *7*, 914.
- (177) Delgado, A. D.; Leonard, M.; Dellacherie, E. *Langmuir* **2001**, *17*, 4386.
- (178) Milani, S.; Bombelli, F. B.; Pitek, A. S.; Dawson, K. A.; Radler, J. *ACS Nano* **2012**, *6*, 2532.
- (179) Prapainop, K.; Wentworth, P. *Eur. J. Pharm. Biopharm.* **2011**, *77*, 353.

- (180) Gref, R.; Luck, M.; Quellec, P.; Marchand, M.; Dellacherie, E.; Harnisch, S.; Blunk, T.; Muller, R. H. *Colloids Surf, B* **2000**, *18*, 301.
- (181) Walkey, C. D.; Olsen, J. B.; Guo, H. B.; Emili, A.; Chan, W. C. W. *J. Am. Chem. Soc.* **2012**, *134*, 2139.
- (182) Lundqvist, M.; Stigler, J.; Elia, G.; Lynch, I.; Cedervall, T.; Dawson, K. A. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 14265.
- (183) Cedervall, T.; Lynch, I.; Foy, M.; Berggård, T.; Donnelly, S. C.; Cagney, G.; Linse, S.; Dawson, K. A. *Angew. Chem., Int. Ed.* **2007**, *46*, 5754.
- (184) Wiogo, H. T. R.; Lim, M.; Bulmus, V.; Yun, J.; Amal, R. *Langmuir* **2010**, *27*, 843.
- (185) Mosqueira, V. C. F.; Legrand, P.; Gulik, A.; Bourdon, O.; Gref, R.; Labarre, D.; Barratt, G. *Biomaterials* **2001**, *22*, 2967.
- (186) Labarre, D.; Vauthier, C.; Chauvierre, C.; Petri, B.; Muller, R.; Chehimi, M. M. *Biomaterials* **2005**, *26*, 5075.
- (187) Mu, Q. X.; Li, Z. W.; Li, X.; Mishra, S. R.; Zhang, B.; Si, Z. K.; Yang, L.; Jiang, W.; Yan, B. *J. Phys. Chem. C* **2009**, *113*, 5390.
- (188) Maiorano, G.; Sabella, S.; Sorce, B.; Brunetti, V.; Malvindi, M. A.; Cingolani, R.; Pompa, P. P. *ACS Nano* **2010**, *4*, 7481.
- (189) Lesniak, A.; Campbell, A.; Monopoli, M. P.; Lynch, I.; Salvati, A.; Dawson, K. A. *Biomaterials* **2010**, *31*, 9511.
- (190) Jeng, E. S.; Barone, P. W.; Nelson, J. D.; Strano, M. S. *Small* **2007**, *3*, 1602.
- (191) Chen, H.; Zou, Q. C.; Yu, H.; Peng, M.; Song, G. W.; Zhang, J. Z.; Chai, S. G.; Zhang, Y. H.; Yan, C. E. *Microchim. Acta* **2010**, *168*, 331.
- (192) Wang, G. L.; Murray, R. W. *Nano Lett.* **2004**, *4*, 95.
- (193) Zheng, M.; Jagota, A.; Semke, E. D.; Diner, B. A.; McLean, R. S.; Lustig, S. R.; Richardson, R. E.; Tassi, N. G. *Nat. Mater.* **2003**, *2*, 338.
- (194) Hughes, M. E.; Brandin, E.; Golovchenko, J. A. *Nano Lett.* **2007**, *7*, 1191.
- (195) Li, D.; Li, G. P.; Guo, W. W.; Li, P. C.; Wang, E. K.; Wang, J. *Biomaterials* **2008**, *29*, 2776.
- (196) Gearheart, L. A.; Ploehn, H. J.; Murphy, C. J. *J. Phys. Chem. B* **2001**, *105*, 12609.
- (197) Li, X.; Peng, Y. H.; Qu, X. G. *Nucleic Acids Res.* **2006**, *34*, 3670.
- (198) Gigliotti, B.; Sakizie, B.; Bethune, D. S.; Shelby, R. M.; Cha, J. N. *Nano Lett.* **2006**, *6*, 159.
- (199) Zheng, M.; Jagota, A.; Strano, M. S.; Santos, A. P.; Barone, P.; Chou, S. G.; Diner, B. A.; Dresselhaus, M. S.; McLean, R. S.; Onoa, G. B.; Samsonidze, G. G.; Semke, E. D.; Usrey, M.; Walls, D. J. *Science* **2003**, *302*, 1545.
- (200) Rajendra, J.; Rodger, A. *Chem.—Eur. J.* **2005**, *11*, 4841.
- (201) Rajendra, J.; Baxendale, M.; Rap, L. G. D.; Rodger, A. *J. Am. Chem. Soc.* **2004**, *126*, 11182.
- (202) Goodman, C. M.; Chari, N. S.; Han, G.; Hong, R.; Ghosh, P.; Rotello, V. M. *Chem. Biol. Drug Des.* **2006**, *67*, 297.
- (203) Dong, L.; Li, Y.; Zhang, Y.; Chen, X.; Hu, Z. *Microchim. Acta* **2007**, *159*, 49.
- (204) Xiao, J.; Chen, J.; Ren, F.; Chen, Y.; Xu, M. *Microchim. Acta* **2007**, *159*, 287.
- (205) Zheng, J. H.; Wu, X.; Wang, M. Q.; Ran, D. H.; Xu, W.; Yang, J. H. *Talanta* **2008**, *74*, 526.
- (206) Kim, E. Y.; Stanton, J.; Vega, R. A.; Kunstman, K. J.; Mirkin, C. A.; Wolinsky, S. M. *Nucleic Acids Res.* **2006**, *34*, S4.
- (207) Liu, S. Q.; Xu, J. J.; Chen, H. Y. *Colloids Surf, B* **2004**, *36*, 155.
- (208) Li, X.; Peng, Y. H.; Ren, J. S.; Qu, X. G. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 19658.
- (209) Ren, H. L.; Wang, C.; Zhang, J. L.; Zhou, X. J.; Xu, D. F.; Zheng, J.; Guo, S. W.; Zhang, J. Y. *ACS Nano* **2010**, *4*, 7169.
- (210) Rahban, M.; Divsalar, A.; Saboury, A. A.; Golestani, A. *J. Phys. Chem. C* **2010**, *114*, 5798.
- (211) Heller, D. A.; Jeng, E. S.; Yeung, T. K.; Martinez, B. M.; Moll, A. E.; Gastala, J. B.; Strano, M. S. *Science* **2006**, *311*, 508.
- (212) Bhattacharyya, S. S.; Paul, S.; De, A.; Das, D.; Samadder, A.; Boujedaini, N.; Khuda-Bukhsh, A. R. *Toxicol. Appl. Pharmacol.* **2011**, *253*, 270.
- (213) Dukovic, G.; Balaz, M.; Doak, P.; Berova, N. D.; Zheng, M.; McLean, R. S.; Brus, L. E. *J. Am. Chem. Soc.* **2006**, *128*, 9004.
- (214) Dovbeshko, G. I.; Repnytska, O. P.; Obraztsova, E. D.; Shtogun, Y. V. *Chem. Phys. Lett.* **2003**, *372*, 432.
- (215) Wu, Y. R.; Phillips, J. A.; Liu, H. P.; Yang, R. H.; Tan, W. H. *ACS Nano* **2008**, *2*, 2023.
- (216) Han, G.; Chari, N. S.; Verma, A.; Hong, R.; Martin, C. T.; Rotello, V. M. *Bioconjugate Chem.* **2005**, *16*, 1356.
- (217) Tokuyama, H.; Yamago, S.; Nakamura, E.; Shiraki, T.; Sugiura, Y. *J. Am. Chem. Soc.* **1993**, *115*, 7918.
- (218) Zhao, X.; Johnson, J. K. *J. Am. Chem. Soc.* **2007**, *129*, 10438.
- (219) Gao, H. J.; Kong, Y.; Cui, D. X.; Ozkan, C. S. *Nano Lett.* **2003**, *3*, 471.
- (220) Moulton, S. E.; Minett, A. I.; Murphy, R.; Ryan, K. P.; McCarthy, D.; Coleman, J. N.; Blau, W. J.; Wallace, G. G. *Carbon* **2005**, *43*, 1879.
- (221) Varghese, N.; Mogera, U.; Govindaraj, A.; Das, A.; Maiti, P. K.; Sood, A. K.; Rao, C. N. R. *ChemPhysChem* **2009**, *10*, 206.
- (222) Liu, M.; Zhao, H. M.; Chen, S.; Yu, H. T.; Quan, X. *Chem. Commun.* **2012**, *48*, 564.
- (223) Tang, L. H.; Chang, H. X.; Liu, Y.; Li, J. H. *Adv. Funct. Mater.* **2012**, *22*, 3083.
- (224) Zhang, X.; Servos, M. R.; Liu, J. W. *Langmuir* **2012**, *28*, 3896.
- (225) Hurst, S. J.; Lytton-Jean, A. K. R.; Mirkin, C. A. *Anal. Chem.* **2006**, *78*, 8313.
- (226) Foley, E. A.; Carter, J. D.; Shan, F.; Guo, T. *Chem. Commun.* **2005**, *3192*.
- (227) McIntosh, C. M.; Esposito, E. A.; Boal, A. K.; Simard, J. M.; Martin, C. T.; Rotello, V. M. *J. Am. Chem. Soc.* **2001**, *123*, 7626.
- (228) Railsback, J. G.; Singh, A.; Pearce, R. C.; McKnight, T. E.; Collazo, R.; Sitar, Z.; Yingling, Y. G.; Melechko, A. V. *Adv. Mater.* **2012**, *24*, 4261.
- (229) Cao, Y. W. C.; Jin, R. C.; Mirkin, C. A. *Science* **2002**, *297*, 1536.
- (230) Han, G.; Martin, C. T.; Rotello, V. M. *Chem. Biol. Drug Des.* **2006**, *67*, 78.
- (231) Chen, P.; Pan, D.; Fan, C. H.; Chen, J. H.; Huang, K.; Wang, D. F.; Zhang, H. L.; Li, Y.; Feng, G. Y.; Liang, P. J.; He, L.; Shi, Y. Y. *Nat. Nanotechnol.* **2011**, *6*, 639.
- (232) Fu, A. H.; Micheel, C. M.; Cha, J.; Chang, H.; Yang, H.; Alivisatos, A. P. *J. Am. Chem. Soc.* **2004**, *126*, 10832.
- (233) Zheng, J. W.; Constantinou, P. E.; Micheel, C.; Alivisatos, A. P.; Kiehl, R. A.; Seeman, N. C. *Nano Lett.* **2006**, *6*, 1502.
- (234) Torimoto, T.; Yamashita, M.; Kuwabata, S.; Sakata, T.; Mori, H.; Yoneyama, H. *J. Phys. Chem. B* **1999**, *103*, 8799.
- (235) Zhu, R. R.; Wang, S. L.; Zhang, R.; Sun, X. Y.; Yao, S. D. *Chin. J. Chem.* **2007**, *25*, 958.
- (236) Ashikaga, T.; Wada, M.; Kobayashi, H.; Mori, M.; Katsumura, Y.; Fukui, H.; Kato, S.; Yamaguchi, M.; Takamatsu, T. *Mutat. Res.-Genet. Toxicol. Environ. Mutag.* **2000**, *466*, 1.
- (237) Donaldson, K.; Beswick, P. H.; Gilmour, P. S. *Toxicol. Lett.* **1996**, *88*, 293.
- (238) Dunford, R.; Salinaro, A.; Cai, L. Z.; Serpone, N.; Horikoshi, S.; Hidaka, H.; Knowland, J. *FEBS Lett.* **1997**, *418*, 87.
- (239) Jose, G. P.; Santra, S.; Mandal, S. K.; Sengupta, T. K. J. *Nanobiotechnol.* **2011**, *9*, 9.
- (240) Zinchenko, A. A.; Luckel, F.; Yoshikawa, K. *Biophys. J.* **2007**, *92*, 1318.
- (241) Acosta, E. *Curr. Opin. Colloid Interface Sci.* **2009**, *14*, 3.
- (242) Velikov, K. P.; Pelan, E. *Soft Matter* **2008**, *4*, 1964.
- (243) Shan, C. S.; Yang, H. F.; Song, J. F.; Han, D. X.; Ivaska, A.; Niu, L. *Anal. Chem.* **2009**, *81*, 2378.
- (244) Casey, A.; Davoren, M.; Herzog, E.; Lyng, F. M.; Byrne, H. J.; Chambers, G. *Carbon* **2007**, *45*, 34.
- (245) Shen, W. Z.; Wang, H.; Guan, R. G.; Li, Z. J. *Colloids Surf, A* **2008**, *331*, 263.
- (246) Casey, A.; Herzog, E.; Lyng, F. M.; Byrne, H. J.; Chambers, G.; Davoren, M. *Toxicol. Lett.* **2008**, *179*, 78.
- (247) Rajesh, C.; Majumder, C.; Mizuseki, H.; Kawazoe, Y. *J. Chem. Phys.* **2009**, *130*, No. 124911.

- (248) Rafeeqi, T.; Kaul, G. *Adv. Sci. Lett.* **2011**, *4*, 536.
- (249) Cazorla, C. *Thin Solid Films* **2010**, *518*, 6951.
- (250) Kloepfer, J. A.; Mielke, R. E.; Nadeau, J. L. *Appl. Environ. Microbiol.* **2005**, *71*, 2548.
- (251) Lam, C. W.; James, J. T.; McCluskey, R.; Hunter, R. L. *Toxicol. Sci.* **2004**, *77*, 126.
- (252) Lin, D. H.; Xing, B. S. *Environ. Sci. Technol.* **2008**, *42*, 5580.
- (253) Lu, L.; Sun, R. W. Y.; Chen, R.; Hui, C. K.; Ho, C. M.; Luk, J. M.; Lau, G. K. K.; Che, C. M. *Antiviral Ther.* **2008**, *13*, 253.
- (254) Navarro, E.; Baun, A.; Behra, R.; Hartmann, N. B.; Filser, J.; Miao, A. J.; Quigg, A.; Santschi, P. H.; Sigg, L. *Ecotoxicology* **2008**, *17*, 372.
- (255) Shi, X. H.; von dem Bussche, A.; Hurt, R. H.; Kane, A. B.; Gao, H. J. *Nat. Nanotechnol.* **2011**, *6*, 714.
- (256) Jiang, X. E.; Rocker, C.; Hafner, M.; Brandholt, S.; Dorlich, R. M.; Nienhaus, G. U. *ACS Nano* **2010**, *4*, 6787.
- (257) Goldstein, J. L.; Anderson, R. G. W.; Brown, M. S. *Nature* **1979**, *279*, 679.
- (258) Krpetic, Z.; Porta, F.; Caneva, E.; Dal Santo, V.; Scari, G. *Langmuir* **2010**, *26*, 14799.
- (259) Faklaris, O.; Joshi, V.; Irinopoulou, T.; Tauc, P.; Sennour, M.; Girard, H.; Gasset, C.; Arnault, J. C.; Thorel, A.; Boudou, J. P.; Curmi, P. A.; Treussart, F. *ACS Nano* **2009**, *3*, 3955.
- (260) Schrand, A. M.; Lin, J. B.; Hens, S. C.; Hussain, S. M. *Nanoscale* **2011**, *3*, 435.
- (261) Singh, S.; Kumar, A.; Karakoti, A.; Seal, S.; Self, W. T. *Mol. BioSyst.* **2010**, *6*, 1813.
- (262) Taylor, U.; Klein, S.; Petersen, S.; Kues, W.; Barcikowski, S.; Rath, D. *Cytometry, Part A* **2010**, *77A*, 439.
- (263) Geiser, M.; Rothen-Rutishauser, B.; Kapp, N.; Schurch, S.; Kreyling, W.; Schulz, H.; Semmler, M.; Hof, V. I.; Heyder, J.; Gehr, P. *Environ. Health Perspect.* **2005**, *113*, 1555.
- (264) Nativo, P.; Prior, I. A.; Brust, M. *ACS Nano* **2008**, *2*, 1639.
- (265) Porter, A. E.; Gass, M.; Bendall, J. S.; Muller, K.; Goode, A.; Skepper, J. N.; Midgley, P. A.; Welland, M. *ACS Nano* **2009**, *3*, 1485.
- (266) Pantarotto, D.; Singh, R.; McCarthy, D.; Erhardt, M.; Briand, J. P.; Prato, M.; Kostarelos, K.; Bianco, A. *Angew. Chem., Int. Ed.* **2004**, *43*, 5242.
- (267) Mu, Q.; Broughton, D. L.; Yan, B. *Nano Lett.* **2009**, *9*, 4370.
- (268) Salvati, A.; Aberg, C.; dos Santos, T.; Varela, J.; Pinto, P.; Lynch, I.; Dawson, K. A. *Nanomed.-Nanotechnol. Biol. Med.* **2011**, *7*, 818.
- (269) Bussy, C.; Cambedouzou, J.; Lanone, S.; Leccia, E.; Heresanu, V.; Pinault, M.; Mayne-I'Hermitte, M.; Brun, N.; Mory, C.; Cotte, M.; Doucet, J.; Boczkowski, J.; Launoist, P. *Nano Lett.* **2008**, *8*, 2659.
- (270) Qu, M.; Mehrmohammadi, M.; Emelianov, S. *Small* **2011**, *7*, 2858.
- (271) Vercauteran, D.; Deschout, H.; Remaut, K.; Engbersen, J. F. J.; Jones, A. T.; Demester, J.; De Smedt, S. C.; Braeckmans, K. *ACS Nano* **2011**, *5*, 7874.
- (272) Chernenko, T.; Matthaus, C.; Milane, L.; Quintero, L.; Amiji, M.; Diem, M. *ACS Nano* **2009**, *3*, 3552.
- (273) Vasir, J. K.; Labhsetwar, V. *Biomaterials* **2008**, *29*, 4244.
- (274) Shan, Y. P.; Hao, X. A.; Shang, X.; Cai, M. J.; Jiang, J. G.; Tang, Z. Y.; Wang, H. D. *Chem. Commun.* **2011**, *47*, 3377.
- (275) Shan, Y. P.; Ma, S. Y.; Nie, L. Y.; Shang, X.; Hao, X.; Tang, Z. Y.; Wang, H. D. *Chem. Commun.* **2011**, *47*, 8091.
- (276) Wilhelm, C.; Gazeau, F.; Roger, J.; Pons, J. N.; Bacri, J. C. *Langmuir* **2002**, *18*, 8148.
- (277) Raemy, D. O.; Limbach, L. K.; Rothen-Rutishauser, B.; Grass, R. N.; Gehr, P.; Birbaum, K.; Brandenberger, C.; Gunther, D.; Stark, W. J. *Eur. J. Pharm. Biopharm.* **2011**, *77*, 368.
- (278) Limbach, L. K.; Li, Y. C.; Grass, R. N.; Brunner, T. J.; Hintermann, M. A.; Muller, M.; Gunther, D.; Stark, W. J. *Environ. Sci. Technol.* **2005**, *39*, 9370.
- (279) Cho, E. C.; Xie, J. W.; Wurm, P. A.; Xia, Y. N. *Nano Lett.* **2009**, *9*, 1080.
- (280) Jin, H.; Heller, D. A.; Strano, M. S. *Nano Lett.* **2008**, *8*, 1577.
- (281) Yuan, H. Y.; Li, J.; Bao, G.; Zhang, S. L. *Phys. Rev. Lett.* **2010**, *105*, No. 138101.
- (282) Zhang, S. L.; Li, J.; Lykotrafitis, G.; Bao, G.; Suresh, S. *Adv. Mater.* **2009**, *21*, 419.
- (283) Kang, B.; Chang, S. Q.; Dai, Y. D.; Yu, D. C.; Chen, D. *Small* **2010**, *6*, 2362.
- (284) Lunov, O.; Syrovets, T.; Loos, C.; Beil, J.; Delecher, M.; Tron, K.; Nienhaus, G. U.; Musyanovich, A.; Mailander, V.; Landfester, K.; Simmet, T. *ACS Nano* **2011**, *5*, 1657.
- (285) Becker, M. L.; Fagan, J. A.; Gallant, N. D.; Bauer, B. J.; Bajpai, V.; Hobbie, E. K.; Lacerda, S. H.; Migler, K. B.; Jakupciak, J. P. *Adv. Mater.* **2007**, *19*, 939.
- (286) Zahr, A. S.; Davis, C. A.; Pishko, M. V. *Langmuir* **2006**, *22*, 8178.
- (287) Giljohann, D. A.; Seferos, D. S.; Patel, P. C.; Millstone, J. E.; Rosi, N. L.; Mirkin, C. A. *Nano Lett.* **2007**, *7*, 3818.
- (288) Mok, H.; Bae, K. H.; Ahn, C. H.; Park, T. G. *Langmuir* **2009**, *25*, 1645.
- (289) Babic, M.; Horak, D.; Jendelova, P.; Glogarova, K.; Herynek, V.; Trchova, M.; Likavcanova, K.; Lesny, P.; Pollert, E.; Hajek, M.; Sykova, E. *Bioconjugate Chem.* **2009**, *20*, 283.
- (290) Yang, H.; Fung, S. Y.; Liu, M. Y. *Angew. Chem., Int. Ed.* **2011**, *50*, 9643.
- (291) Oh, E.; Delehanty, J. B.; Sapsford, K. E.; Susumu, K.; Goswami, R.; Blanco-Canosa, J. B.; Dawson, P. E.; Granek, J.; Shoff, M.; Zhang, Q.; Goering, P. L.; Huston, A.; Medintz, I. L. *ACS Nano* **2011**, *5*, 6434.
- (292) Asati, A.; Santra, S.; Kaittanis, C.; Perez, J. M. *ACS Nano* **2010**, *4*, 5321.
- (293) Chithrani, B. D.; Ghazani, A. A.; Chan, W. C. W. *Nano Lett.* **2006**, *6*, 662.
- (294) Chithrani, B. D.; Chan, W. C. W. *Nano Lett.* **2007**, *7*, 1542.
- (295) Qiu, Y.; Liu, Y.; Wang, L. M.; Xu, L. G.; Bai, R.; Ji, Y. L.; Wu, X. C.; Zhao, Y. L.; Li, Y. F.; Chen, C. Y. *Biomaterials* **2010**, *31*, 7606.
- (296) Bartneck, M.; Keul, H. A.; Singh, S.; Czaja, K.; Bornemann, J.; Bockstaller, M.; Moeller, M.; Zwadlo-Klarwasser, G.; Groll, J. *ACS Nano* **2010**, *4*, 3073.
- (297) Gratton, S. E. A.; Ropp, P. A.; Pohlhaus, P. D.; Luft, J. C.; Madden, V. J.; Napier, M. E.; DeSimone, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 11613.
- (298) Liang, M.; Lin, I. C.; Whittaker, M. R.; Minchin, R. F.; Monteiro, M. J.; Toth, I. *ACS Nano* **2010**, *4*, 403.
- (299) Verma, A.; Uzun, O.; Hu, Y. H.; Hu, Y.; Han, H. S.; Watson, N.; Chen, S. L.; Irvine, D. J.; Stellacci, F. *Nat. Mater.* **2008**, *7*, 588.
- (300) Prapainop, K.; Witter, D. P.; Wentworth, P. *J. Am. Chem. Soc.* **2012**, *134*, 4100.
- (301) Lin, J. Q.; Zhang, H. W.; Chen, Z.; Zheng, Y. G. *ACS Nano* **2010**, *4*, 5421.
- (302) Su, G. X.; Zhou, H. Y.; Mu, Q. X.; Zhang, Y.; Li, L. W.; Jiao, P. F.; Jiang, G. B.; Yan, B. *J. Phys. Chem. C* **2012**, *116*, 4993.
- (303) Mu, Q.; Su, G.; Li, L.; Gilbertson, B. O.; Yu, L. H.; Zhang, Q.; Sun, Y.-P.; Yan, B. *ACS Appl. Mater. Interfaces* **2012**, *4*, 2259.
- (304) Florez, L.; Herrmann, C.; Cramer, J. M.; Hauser, C. P.; Koynov, K.; Landfester, K.; Crespy, D.; Mailander, V. *Small* **2012**, *8*, 2222.
- (305) Park, J.-H.; von Maltzahn, G.; Zhang, L.; Derfus, A. M.; Simberg, D.; Harris, T. J.; Ruoslahti, E.; Bhatia, S. N.; Sailor, M. J. *Small* **2009**, *5*, 694.
- (306) Banquy, X.; Suarez, F.; Argaw, A.; Rabanel, J. M.; Grutter, P.; Bouchard, J. F.; Hildgen, P.; Giasson, S. *Soft Matter* **2009**, *5*, 3984.
- (307) Liu, W. J.; Zhou, X. Y.; Mao, Z. W.; Yu, D. H.; Wang, B.; Gao, C. Y. *Soft Matter* **2012**, *8*, 9235.
- (308) Cho, E. C.; Zhang, Q.; Xia, Y. N. *Nat. Nanotechnol.* **2011**, *6*, 385.
- (309) Safi, M.; Courtois, J.; Seigneuret, M.; Conjeaud, H.; Berret, J. F. *Biomaterials* **2011**, *32*, 9353.
- (310) Zhou, R.; Zhou, H. Y.; Xiong, B.; He, Y.; Yeung, E. S. *J. Am. Chem. Soc.* **2012**, *134*, 13404.
- (311) Chen, J. M.; Hessler, J. A.; Putchakayala, K.; Panama, B. K.; Khan, D. P.; Hong, S.; Mullen, D. G.; DiMaggio, S. C.; Som, A.; Tew,

- G. N.; Lopatin, A. N.; Baker, J. R.; Holl, M. M. B.; Orr, B. G. *J. Phys. Chem. B* **2009**, *113*, 11179.
- (312) Arvizo, R. R.; Miranda, O. R.; Thompson, M. A.; Pabelick, C. M.; Bhattacharya, R.; Robertson, J. D.; Rotello, V. M.; Prakash, Y. S.; Mukherjee, P. *Nano Lett.* **2010**, *10*, 2543.
- (313) Slowing, I. I.; Wu, C. W.; Vivero-Escoto, J. L.; Lin, V. S. Y. *Small* **2009**, *5*, 57.
- (314) Sayes, C. M.; Gobin, A. M.; Ausman, K. D.; Mendez, J.; West, J. L.; Colvin, V. L. *Biomaterials* **2005**, *26*, 7587.
- (315) Doshi, N.; Mitragotri, S. *J. R. Soc., Interface* **2010**, *7*, S403.
- (316) Tong, L.; Zhao, Y.; Huff, T. B.; Hansen, M. N.; Wei, A.; Cheng, J. X. *Adv. Mater.* **2007**, *19*, 3136.
- (317) Zhang, W.; Kalive, M.; Capco, D. G.; Chen, Y. S. *Nanotechnology* **2010**, *21*, 355103.
- (318) Lin, Y. S.; Haynes, C. L. *J. Am. Chem. Soc.* **2010**, *132*, 4834.
- (319) Lauger, P. *J. Membr. Biol.* **1980**, *57*, 163.
- (320) Park, K. H.; Chhowalla, M.; Iqbal, Z.; Sesti, F. *J. Biol. Chem.* **2003**, *278*, 50212.
- (321) Chhowalla, M.; Unalan, H. E.; Wang, Y. B.; Iqbal, Z.; Park, K.; Sesti, F. *Nanotechnology* **2005**, *16*, 2982.
- (322) Xu, H. F.; Bai, J.; Meng, J.; Hao, W.; Xu, H. Y.; Cao, J. M. *Nanotechnology* **2009**, *20*, 285102.
- (323) Kraszewski, S.; Tarek, M.; Treptow, W.; Ramseyer, C. *ACS Nano* **2010**, *4*, 4158.
- (324) Jakubek, L. M.; Marangoudakis, S.; Raingo, J.; Liu, X. Y.; Lipscombe, D.; Hurt, R. H. *Biomaterials* **2009**, *30*, 6351.
- (325) Holt, B. D.; Short, P. A.; Rape, A. D.; Wang, Y. L.; Islam, M. F.; Dahl, K. N. *ACS Nano* **2010**, *4*, 4872.
- (326) Tarantola, M.; Schneider, D.; Sunnick, E.; Adam, H.; Pierrat, S.; Rosman, C.; Breus, V.; Sonnichsen, C.; Basche, T.; Wegener, J.; Janshoff, A. *ACS Nano* **2009**, *3*, 213.
- (327) Gupta, A. K.; Gupta, M. *Biomaterials* **2005**, *26*, 1565.
- (328) Gupta, A. K.; Gupta, M.; Yarwood, S. J.; Curtis, A. S. G. *J. Controlled Release* **2004**, *95*, 197.
- (329) Soenen, S. J. H.; Nuyttten, N.; De Meyer, S. F.; De Smedt, S. C.; De Cuyper, M. *Small* **2010**, *6*, 832.
- (330) Pan, Y.; Leifert, A.; Ruau, D.; Neuss, S.; Bornemann, J.; Schmid, G.; Brandau, W.; Simon, U.; Jahnens-Dechent, W. *Small* **2009**, *5*, 2067.
- (331) Karatas, O. F.; Sezgin, E.; Aydin, O.; Culha, M. *Colloids Surf. B* **2009**, *71*, 315.
- (332) Paunesku, T.; Vogt, S.; Lai, B.; Maser, J.; Stojicevic, N.; Thurn, K. T.; Osipo, C.; Liu, H.; Legnini, D.; Wang, Z.; Lee, C.; Woloschak, G. E. *Nano Lett.* **2007**, *7*, 596.
- (333) Zhou, F. F.; Xing, D.; Wu, B. Y.; Wu, S. N.; Ou, Z. M.; Chen, W. R. *Nano Lett.* **2010**, *10*, 1677.
- (334) Salnikov, V.; Lukyanenko, Y. O.; Frederick, C. A.; Lederer, W. J.; Lukyanenko, V. *Biophys. J.* **2007**, *92*, 1058.
- (335) Conroy, J.; Byrne, S. J.; Gun'ko, Y. K.; Rakovich, Y. P.; Donegan, J. F.; Davies, A.; Kelleher, D.; Volkov, Y. *Small* **2008**, *4*, 2006.
- (336) Chen, F. Q.; Gerion, D. *Nano Lett.* **2004**, *4*, 1827.
- (337) Zakhidov, S. T.; Marshak, T. L.; Malolina, E. A.; Kulibin, A. Y.; Zelenina, I. A.; Pavluchenkova, S. M.; Rudoy, V. M.; Dement'eva, O. V.; Skuridin, S. G.; Evdokimov, Y. M. *Biol. Membr.* **2010**, *27*, 349.
- (338) Akita, H.; Kudo, A.; Minoura, A.; Yamaguti, M.; Khalil, I. A.; Moriguchi, R.; Masuda, T.; Daney, R.; Nagayama, K.; Kogure, K.; Harashima, H. *Biomaterials* **2009**, *30*, 2940.
- (339) Martindale, J. L.; Holbrook, N. J. *J. Cell. Physiol.* **2002**, *192*, 1.
- (340) Xia, T.; Kovochich, M.; Brant, J.; Hotze, M.; Sempf, J.; Oberley, T.; Sioutas, C.; Yeh, J. I.; Wiesner, M. R.; Nel, A. E. *Nano Lett.* **2006**, *6*, 1794.
- (341) Karakoti, A.; Singh, S.; Dowding, J. M.; Seal, S.; Self, W. T. *Chem. Soc. Rev.* **2010**, *39*, 4422.
- (342) Jia, H. Y.; Liu, Y.; Zhang, X. J.; Han, L.; Du, L. B.; Tian, Q.; Xut, Y. C. *J. Am. Chem. Soc.* **2009**, *131*, 40.
- (343) George, S.; Pokhrel, S.; Xia, T.; Gilbert, B.; Ji, Z. X.; Schowalter, M.; Rosenauer, A.; Damoiseaux, R.; Bradley, K. A.; Madler, L.; Nel, A. E. *ACS Nano* **2010**, *4*, 15.
- (344) Wang, H.; Joseph, J. A. *Free Radical Biol. Med.* **1999**, *27*, 612.
- (345) Hwang, E. T.; Lee, J. H.; Chae, Y. J.; Kim, Y. S.; Kim, B. C.; Sang, B. I.; Gu, M. B. *Small* **2008**, *4*, 746.
- (346) De Berardis, B.; Civitelli, G.; Condello, M.; Lista, P.; Pozzi, R.; Arancia, G.; Meschini, S. *Toxicol. Appl. Pharmacol.* **2010**, *246*, 116.
- (347) Lu, X.; Qian, J. C.; Zhou, H. J.; Gan, Q.; Tang, W.; Lu, J. X.; Yuan, Y.; Liu, C. S. *Int. J. Nanomed.* **2011**, *6*, 1889.
- (348) Berg, J. M.; Ho, S.; Hwang, W.; Zebda, R.; Cummins, K.; Soriaga, M. P.; Taylor, R.; Guo, B.; Sayes, C. M. *Chem. Res. Toxicol.* **2010**, *23*, 1874.
- (349) Burello, E.; Worth, A. P. *Nanotoxicology* **2011**, *5*, 228.
- (350) Thakor, A. S.; Paulmurugan, R.; Kempen, P.; Zavaleta, C.; Sinclair, R.; Massoud, T. F.; Gambhir, S. S. *Small* **2011**, *7*, 126.
- (351) Wu, J.; Sun, J. A.; Xue, Y. *Toxicol. Lett.* **2010**, *199*, 269.
- (352) Park, E. J.; Yi, J.; Chung, Y. H.; Ryu, D. Y.; Choi, J.; Park, K. *Toxicol. Lett.* **2008**, *180*, 222.
- (353) Fenoglio, I.; Greco, G.; Livraghi, S.; Fubini, B. *Chem.—Eur. J.* **2009**, *15*, 4614.
- (354) Foucaud, L.; Goulaouic, S.; Bennasroune, A.; Laval-Gilly, P.; Brown, D.; Stone, V.; Falla, J. *Toxicol. In Vitro* **2010**, *24*, 1512.
- (355) Palomaki, J.; Valimaki, E.; Sund, J.; Vippola, M.; Clausen, P. A.; Jensen, K. A.; Savolainen, K.; Matikainen, S.; Alenius, H. *ACS Nano* **2011**, *5*, 6861.
- (356) Ambrosi, A.; Pumera, M. *Chem.—Eur. J.* **2010**, *16*, 1786.
- (357) Zhang, Y. B.; Xu, Y.; Li, Z. G.; Chen, T.; Lantz, S. M.; Howard, P. C.; Paule, M. G.; Slikker, W.; Watanabe, F.; Mustafa, T.; Biris, A. S.; Ali, S. F. *ACS Nano* **2011**, *5*, 7020.
- (358) Wan, R.; Mo, Y. Q.; Zhang, X.; Chien, S. F.; Tollerud, D. J.; Zhang, Q. W. *Toxicol. Appl. Pharmacol.* **2008**, *233*, 276.
- (359) Thubagere, A.; Reinhard, B. M. *ACS Nano* **2010**, *4*, 3611.
- (360) Ahamed, M.; Akhtar, M. J.; Siddiqui, M. A.; Ahmad, J.; Musarrat, J.; Al-Khedhairy, A. A.; AlSalhi, M. S.; Alrokayan, S. A. *Toxicology* **2011**, *283*, 101.
- (361) Piao, M. J.; Kang, K. A.; Lee, I. K.; Kim, H. S.; Kim, S.; Choi, J. Y.; Choi, J.; Hyun, J. W. *Toxicol. Lett.* **2011**, *201*, 92.
- (362) Xia, T.; Kovochich, M.; Liang, M.; Madler, L.; Gilbert, B.; Shi, H. B.; Yeh, J. I.; Zink, J. I.; Nel, A. E. *ACS Nano* **2008**, *2*, 2121.
- (363) Karlsson, H. L.; Cronholm, P.; Gustafsson, J.; Moller, L. *Chem. Res. Toxicol.* **2008**, *21*, 1726.
- (364) Gerloff, K.; Albrecht, C.; Boots, A. W.; Forster, I.; Schins, R. P. F. *Nanotoxicology* **2009**, *3*, 355.
- (365) Pujalte, I.; Passagne, I.; Brouillaud, B.; Treguer, M.; Durand, E.; Ohayon-Courtes, C.; L'Azou, B. *Part. Fibre Toxicol.* **2011**, *8*, 10.
- (366) Kocbek, P.; Teskac, K.; Kreft, M. E.; Kristl, J. *Small* **2010**, *6*, 1908.
- (367) Zhang, H. Y.; Ji, Z. X.; Xia, T.; Meng, H.; Low-Kam, C.; Liu, R.; Pokhrel, S.; Lin, S. J.; Wang, X.; Liao, Y. P.; Wang, M. Y.; Li, L. J.; Rallo, R.; Damoiseaux, R.; Telesca, D.; Madler, L.; Cohen, Y.; Zink, J. I.; Nel, A. E. *ACS Nano* **2012**, *6*, 4349.
- (368) Braydich-Stolle, L. K.; Schaeublin, N. M.; Murdock, R. C.; Jiang, J.; Biswas, P.; Schlager, J. J.; Hussain, S. M. *J. Nanopart. Res.* **2009**, *11*, 1361.
- (369) Liu, W.; Wu, Y. A.; Wang, C.; Li, H. C.; Wang, T.; Liao, C. Y.; Cui, L.; Zhou, Q. F.; Yan, B.; Jiang, G. B. *Nanotoxicology* **2010**, *4*, 319.
- (370) Hussain, S.; Boland, S.; Baeza-Squiban, A.; Hamel, R.; Thomassen, L. C. J.; Martens, J. A.; Billon-Galland, M. A.; Fleury-Feith, J.; Moisan, F.; Pairon, J. C.; Marano, F. *Toxicology* **2009**, *260*, 142.
- (371) Yu, K. O.; Grabinski, C. M.; Schrand, A. M.; Murdock, R. C.; Wang, W.; Gu, B. H.; Schlager, J. J.; Hussain, S. M. *J. Nanopart. Res.* **2009**, *11*, 15.
- (372) Bhattacharjee, S.; de Haan, L. H. J.; Evers, N. M.; Jiang, X.; Marcelis, A. T. M.; Zuilhof, H.; Rietjens, I.; Alink, G. M. *Part. Fibre Toxicol.* **2010**, *7*, 25.
- (373) Yin, H.; Casey, P. S.; McCall, M. J.; Fenech, M. *Langmuir* **2010**, *26*, 15399.
- (374) Chompoosor, A.; Saha, K.; Ghosh, P. S.; Macarthy, D. J.; Miranda, O. R.; Zhu, Z. J.; Arcaro, K. F.; Rotello, V. M. *Small* **2010**, *6*, 2246.

- (375) Guo, B.; Zebda, R.; Drake, S. J.; Sayes, C. M. *Part. Fibre Toxicol.* **2009**, *6*, 4.
- (376) Liu, Y. X.; Chen, Z. P.; Wang, J. K. *J. Nanopart. Res.* **2011**, *13*, 199.
- (377) Gharbi, N.; Pressac, M.; Hadchouel, M.; Szwarc, H.; Wilson, S. R.; Moussa, F. *Nano Lett.* **2005**, *5*, 2578.
- (378) Hirst, S. M.; Karakoti, A. S.; Tyler, R. D.; Sriranganathan, N.; Seal, S.; Reilly, C. M. *Small* **2009**, *5*, 2848.
- (379) Ali, S. S.; Hardt, J. I.; Quick, K. L.; Kim-Han, J. S.; Erlanger, B. F.; Huang, T. T.; Epstein, C. J.; Dugan, L. L. *Free Radical Biol. Med.* **2004**, *37*, 1191.
- (380) Watts, P. C. P.; Fearon, P. K.; Hsu, W. K.; Billingham, N. C.; Kroto, H. W.; Walton, D. R. M. *J. Mater. Chem.* **2003**, *13*, 491.
- (381) Celardo, I.; De Nicola, M.; Mandoli, C.; Pedersen, J. Z.; Traversa, E.; Ghibelli, L. *ACS Nano* **2011**, *5*, 4537.
- (382) Hamasaki, T.; Kashiwagi, T.; Imada, T.; Nakamichi, N.; Aramaki, S.; Toh, K.; Morisawa, S.; Shimakoshi, H.; Hisaeda, Y.; Shirahata, S. *Langmuir* **2008**, *24*, 7354.
- (383) Martin, R.; Menchon, C.; Apostolova, N.; Victor, V. M.; Alvaro, M.; Herance, J. R.; Garcia, H. *ACS Nano* **2010**, *4*, 6957.
- (384) Amin, K. A.; Hassan, M. S.; Awad, E. T.; Hashem, K. S. *Int. J. Nanomed.* **2011**, *6*, 143.
- (385) Fenoglio, I.; Tomatis, M.; Lison, D.; Muller, J.; Fonseca, A.; Nagy, J. B.; Fibini, B. *Free Radical Biol. Med.* **2006**, *40*, 1227.
- (386) Zhang, L. B.; Laug, L.; Munchgesang, W.; Pippel, E.; Gosele, U.; Brandsch, M.; Knez, M. *Nano Lett.* **2010**, *10*, 219.
- (387) Kyriakis, J. M.; Avruch, J. *Physiol. Rev.* **2012**, *92*, 689.
- (388) Peuschel, H.; Sydlik, U.; Haendeler, J.; Buchner, N.; Stockmann, D.; Kroker, M.; Wirth, R.; Brock, W.; Unfried, K. *Biol. Chem.* **2010**, *391*, 1327.
- (389) Liu, X.; Sun, J. A. *Biomaterials* **2010**, *31*, 8198.
- (390) Kim, J. A.; Lee, N. H.; Kim, B. H.; Rhee, W. J.; Yoon, S.; Hyeon, T.; Park, T. H. *Biomaterials* **2011**, *32*, 2871.
- (391) Mohamed, B. M.; Verma, N. K.; Prina-Mello, A.; Williams, Y.; Davies, A. M.; Bakos, G.; Tormey, L.; Edwards, C.; Hanrahan, J.; Salvati, A.; Lynch, I.; Dawson, K.; Kelleher, D.; Volkov, Y. J. *Nanobiotechnol.* **2011**, *9*, 29.
- (392) Lao, F.; Chen, L.; Li, W.; Ge, C. C.; Qu, Y.; Sun, Q. M.; Zhao, Y. L.; Han, D.; Chen, C. Y. *ACS Nano* **2009**, *3*, 3358.
- (393) Hayden, M. S.; Ghosh, S. *Cell* **2008**, *132*, 344.
- (394) Nishanth, R. P.; Jyotsna, R. G.; Schlager, J. J.; Hussain, S. M.; Reddanna, P. *Nanotoxicology* **2011**, *5*, 502.
- (395) Cui, Y. L.; Liu, H. T.; Zhou, M.; Duan, Y. M.; Li, N.; Gong, X.; Hu, R. P.; Hong, M. M.; Hong, F. S. *J. Biomed. Mater. Res., Part A* **2011**, *96A*, 221.
- (396) Li, A.; Qin, L. L.; Zhu, D.; Zhu, R. R.; Sun, J.; Wang, S. L. *Biomaterials* **2010**, *31*, 748.
- (397) Manna, S. K.; Sarkar, S.; Barr, J.; Wise, K.; Barrera, E. V.; Jejelowo, O.; Rice-Ficht, A. C.; Ramesh, G. T. *Nano Lett.* **2005**, *5*, 1676.
- (398) Chou, C. C.; Hsiao, H. Y.; Hong, Q. S.; Chen, C. H.; Peng, Y. W.; Chen, H. W.; Yang, P. C. *Nano Lett.* **2008**, *8*, 437.
- (399) Petersen, E. J.; Nelson, B. C. *Anal. Bioanal. Chem.* **2010**, *398*, 613.
- (400) AshaRani, P. V.; Mun, G. L. K.; Hande, M. P.; Valiyaveettil, S. *ACS Nano* **2009**, *3*, 279.
- (401) Xing, Y.; Xiong, W.; Zhu, L.; Osawa, E.; Hussin, S.; Dai, L. M. *ACS Nano* **2011**, *5*, 2376.
- (402) Zhu, L.; Chang, D. W.; Dai, L. M.; Hong, Y. L. *Nano Lett.* **2007**, *7*, 3592.
- (403) Ahamed, M.; Karns, M.; Goodson, M.; Rowe, J.; Hussain, S. M.; Schlager, J. J.; Hong, Y. L. *Toxicol. Appl. Pharmacol.* **2008**, *233*, 404.
- (404) Ljungman, M. *Environ. Mol. Mutag.* **2010**, *51*, 879.
- (405) Waite, K. A.; Eng, C. *Nat. Rev. Genet.* **2003**, *4*, 763.
- (406) Zhang, Y.; Mu, Q.; Zhou, H.; Vrijens, K.; Roussel, M. F.; Jiang, G.; Yan, B. *Cell Death Dis.* **2012**, *3*, e308.
- (407) Liu, D. D.; Yi, C. Q.; Zhang, D. W.; Zhang, J. C.; Yang, M. S. *ACS Nano* **2010**, *4*, 2185.
- (408) Mu, Q. X.; Du, G. Q.; Chen, T. S.; Zhang, B.; Yan, B. *ACS Nano* **2009**, *3*, 1139.
- (409) Li, Y.; Liu, Y.; Fu, Y. J.; Wei, T. T.; Le Guyader, L.; Gao, G.; Liu, R. S.; Chang, Y. Z.; Chen, C. Y. *Biomaterials* **2012**, *33*, 402.
- (410) Braydich-Stolle, L. K.; Lucas, B.; Schrand, A.; Murdock, R. C.; Lee, T.; Schlager, J. J.; Hussain, S. M.; Hofmann, M. C. *Toxicol. Sci.* **2010**, *116*, 577.
- (411) Chen, G. Y.; Yang, H. J.; Lu, C. H.; Chao, Y. C.; Hwang, S. M.; Chen, C. L.; Lo, K. W.; Sung, L. Y.; Luo, W. Y.; Tuan, H. Y.; Hu, Y. C. *Biomaterials* **2012**, *33*, 6559.
- (412) Zhang, T. T.; Stilwell, J. L.; Gerion, D.; Ding, L. H.; Elboudwarej, O.; Cooke, P. A.; Gray, J. W.; Alivisatos, A. P.; Chen, F. F. *Nano Lett.* **2006**, *6*, 800.
- (413) Bouwmeester, H.; Poortman, J.; Peters, R. J.; Wijma, E.; Kramer, E.; Makama, S.; Puspitaninganindita, K.; Marvin, H. J. P.; Peijnenburg, A.; Hendriksen, P. J. M. *ACS Nano* **2011**, *5*, 4091.
- (414) Cui, D. X.; Tian, F. R.; Ozkan, C. S.; Wang, M.; Gao, H. J. *Toxicol. Lett.* **2005**, *155*, 73.
- (415) Liu, Y. X.; Chen, Z. P.; Gu, N.; Wang, J. K. *Toxicol. Lett.* **2011**, *205*, 130.
- (416) Ding, L. H.; Stilwell, J.; Zhang, T. T.; Elboudwarej, O.; Jiang, H. J.; Selegue, J. P.; Cooke, P. A.; Gray, J. W.; Chen, F. Q. F. *Nano Lett.* **2005**, *5*, 2448.
- (417) Fujita, K.; Horie, M.; Kato, H.; Endoh, S.; Suzuki, M.; Nakamura, A.; Miyauchi, A.; Yamamoto, K.; Kinugasa, S.; Nishio, K.; Yoshida, Y.; Iwahashi, H.; Nakanishi, J. *Toxicol. Lett.* **2009**, *191*, 109.
- (418) Khan, J. A.; Pillai, B.; Das, T. K.; Singh, Y.; Maiti, S. *ChemBioChem* **2007**, *8*, 1237.
- (419) Hanagata, N.; Zhuang, F.; Connolly, S.; Li, J.; Ogawa, N.; Xu, M. S. *ACS Nano* **2011**, *5*, 9326.
- (420) Tsai, Y. Y.; Huang, Y. H.; Chao, Y. L.; Hu, K. Y.; Chin, L. T.; Chou, S. H.; Hour, A. L.; Yao, Y. D.; Tu, C. S.; Liang, Y. J.; Tsai, C. Y.; Wu, H. Y.; Tan, S. W.; Chen, H. M. *ACS Nano* **2011**, *5*, 9354.
- (421) Ge, Y.; Bruno, M.; Wallace, K.; Winnik, W.; Prasad, R. Y. *Proteomics* **2011**, *11*, 2406.
- (422) Haniu, H.; Matsuda, Y.; Takeuchi, K.; Kim, Y. A.; Hayashi, T.; Endo, M. *Toxicol. Appl. Pharmacol.* **2010**, *242*, 256.
- (423) Yuan, J. F.; Gao, H. C.; Sui, J. J.; Chen, W. N.; Ching, C. B. *Toxicol. In Vitro* **2011**, *25*, 1820.
- (424) Lenz, E. M.; Wilson, I. D. *J. Proteome Res.* **2007**, *6*, 443.
- (425) Kim, S.; Kim, S.; Lee, S.; Kwon, B.; Choi, J.; Hyun, J. W.; Kim, S. *Bull. Korean Chem. Soc.* **2011**, *32*, 2021.
- (426) Feng, J. H.; Liu, H. L.; Bhakoo, K. K.; Lu, L. H.; Chen, Z. *Biomaterials* **2011**, *32*, 6558.
- (427) Feng, J. H.; Zhao, J.; Hao, F. H.; Chen, C.; Bhakoo, K.; Tang, H. R. *J. Nanopart. Res.* **2011**, *13*, 2049.
- (428) Wang, Y.; Chen, L. *Nanomed.-Nanotechnol. Biol. Med.* **2011**, *7*, 385.
- (429) Wang, Y.; Chen, L.; Liu, P. *Chem.—Eur. J.* **2012**, *18*, 5935.
- (430) Dubois, F.; Mahler, B.; Dubertret, B.; Doris, E.; Mioskowski, C. *J. Am. Chem. Soc.* **2007**, *129*, 482.
- (431) Song, H. T.; Choi, J. S.; Huh, Y. M.; Kim, S.; Jun, Y. W.; Suh, J. S.; Cheon, J. *J. Am. Chem. Soc.* **2005**, *127*, 9992.
- (432) Li, N.; Binder, W. H. *J. Mater. Chem.* **2011**, *21*, 16717.
- (433) Pellegrino, T.; Manna, L.; Kudera, S.; Liedl, T.; Koktysh, D.; Rogach, A. L.; Keller, S.; Radler, J.; Natile, G.; Parak, W. J. *Nano Lett.* **2004**, *4*, 703.
- (434) Gole, A.; Murphy, C. J. *Chem. Mater.* **2005**, *17*, 1325.
- (435) Ehrenberg, M. S.; Friedman, A. E.; Finkelstein, J. N.; Oberdörster, G.; McGrath, J. L. *Biomaterials* **2009**, *30*, 603.
- (436) Aubin-Tam, M.-E.; Hamad-Schifferli, K. *Langmuir* **2005**, *21*, 12080.
- (437) You, C.-C.; De, M.; Han, G.; Rotello, V. M. *J. Am. Chem. Soc.* **2005**, *127*, 12873.
- (438) Gessner, A.; Lieske, A.; Paulke, B. R.; Muller, R. H. *Eur. J. Pharm. Biopharm.* **2002**, *54*, 165.
- (439) Su, G.; Zhou, H.; Mu, Q.; Zhang, Y.; Li, L.; Jiao, P.; Jiang, G.; Yan, B. *J. Phys. Chem. C* **2012**, *116*, 4993.

- (440) Cho, E. C.; Xie, J.; Wurm, P. A.; Xia, Y. *Nano Lett.* **2009**, *9*, 1080.
- (441) Wilhelm, C.; Billotey, C.; Roger, J.; Pons, J. N.; Bacri, J. C.; Gazeau, F. *Biomaterials* **2003**, *24*, 1001.
- (442) Mailaender, V.; Lorenz, M. R.; Holzapfel, V.; Musyanovich, A.; Fuchs, K.; Wiesneth, M.; Walther, P.; Landfester, K.; Schrezenmeier, H. *Mol. Imag. Biol.* **2008**, *10*, 138.
- (443) Zhang, L. W.; Monteiro-Riviere, N. A. *Toxicol. Sci.* **2009**, *110*, 138.
- (444) Fernandes, C. F.; Godoy, J. R.; Döring, B.; Cavalcanti, M. C. O.; Bergmann, M.; Petzinger, E.; Geyer, J. *Biochem. Biophys. Res. Commun.* **2007**, *361*, 26.
- (445) Fuller, J. E.; Zugates, G. T.; Ferreira, L. S.; Ow, H. S.; Nguyen, N. N.; Wiesner, U. B.; Langer, R. S. *Biomaterials* **2008**, *29*, 1526.
- (446) Leroueil, P. R.; Hong, S.; Mecke, A.; Baker, J. R., Jr.; Orr, B. G.; Holl, M. M. B. *Acc. Chem. Res.* **2007**, *40*, 335.
- (447) Leroueil, P. R.; Berry, S. A.; Duthie, K.; Han, G.; Rotello, V. M.; McNerny, D. Q.; Baker, J. R.; Orr, B. G.; Banaszak Holl, M. M. *Nano Lett.* **2008**, *8*, 420.
- (448) Gessner, A.; Waicz, R.; Lieske, A.; Paulke, B. R.; Mäder, K.; Müller, R. H. *Int. J. Pharm.* **2000**, *196*, 245.
- (449) Lorenz, S.; Hauser, C. P.; Autenrieth, B.; Weiss, C. K.; Landfester, K.; Mailaender, V. *Macromol. Biosci.* **2010**, *10*, 1034.
- (450) Yang, H.; Fung, S.-Y.; Liu, M. *Angew. Chem., Int. Ed.* **2011**, *50*, 9643.
- (451) Nel, A. E.; Maedler, L.; Velegol, D.; Xia, T.; Hoek, E. M. V.; Somasundaran, P.; Klaessig, F.; Castranova, V.; Thompson, M. *Nat. Mater.* **2009**, *8*, 543.
- (452) Richards, D.; Ivanisevic, A. *Chem. Soc. Rev.* **2012**, *41*, 2052.
- (453) Pelaz, B.; Charron, G.; Pfeiffer, C.; Zhao, Y.; de la Fuente, J. M.; Liang, X.-J.; Parak, W. J.; del Pino, P. *Small* **2012**, DOI: 10.1002/smll.201201229.
- (454) Wang, Y.; Ye, C.; Wu, L.; Hu, Y. *J. Pharm. Biomed. Anal.* **2010**, *53*, 235.
- (455) Lovric, J.; Cho, S. J.; Winnik, F. M.; Maysinger, D. *Chem. Biol.* **2005**, *12*, 1227.
- (456) Schrand, A. M.; Rahman, M. F.; Hussain, S. M.; Schlager, J. J.; Smith, D. A.; Syed, A. F. *Wiley Interdiscip. Rev.: Nanomed. Nanobiotechnol.* **2010**, *2*, 544.
- (457) Niidome, T.; Yamagata, M.; Okamoto, Y.; Akiyama, Y.; Takahashi, H.; Kawano, T.; Katayama, Y.; Niidome, Y. *J. Controlled Release* **2006**, *114*, 343.
- (458) Lemon, B. I.; Crooks, R. M. *J. Am. Chem. Soc.* **2000**, *122*, 12886.
- (459) Liu, J. a.; Li, H.; Wang, W.; Xu, H.; Yang, X.; Liang, J.; He, Z. *Small* **2006**, *2*, 999.
- (460) Susumu, K.; Mei, B. C.; Mattossi, H. *Nat. Protoc.* **2009**, *4*, 424.
- (461) Tan, S. J.; Jana, N. R.; Gao, S.; Patra, P. K.; Ying, J. Y. *Chem. Mater.* **2010**, *22*, 2239.
- (462) Gao, X. H.; Cui, Y. Y.; Levenson, R. M.; Chung, L. W. K.; Nie, S. M. *Nat. Biotechnol.* **2004**, *22*, 969.
- (463) Akiyoshi, K.; Sasaki, Y.; Sunamoto, J. *Bioconjugate Chem.* **1999**, *10*, 321.
- (464) Yu, W. W.; Chang, E.; Falkner, J. C.; Zhang, J.; Al-Somali, A. M.; Sayes, C. M.; Johns, J.; Drezek, R.; Colvin, V. L. *J. Am. Chem. Soc.* **2007**, *129*, 2871.
- (465) Lu, W.; Senapati, D.; Wang, S.; Tovmachenko, O.; Singh, A. K.; Yu, H.; Ray, P. C. *Chem. Phys. Lett.* **2010**, *487*, 92.
- (466) Chung, Y.-C.; Chen, I. H.; Chen, C.-J. *Biomaterials* **2008**, *29*, 1807.
- (467) Ge, C.; Du, J.; Zhao, L.; Wang, L.; Liu, Y.; Li, D.; Yang, Y.; Zhou, R.; Zhao, Y.; Chai, Z.; Chen, C. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 16968.
- (468) Leonov, A. P.; Zheng, J.; Clogston, J. D.; Stern, S. T.; Patri, A. K.; Wei, A. *ACS Nano* **2008**, *2*, 2481.
- (469) Hauck, T. S.; Ghazani, A. A.; Chan, W. C. W. *Small* **2008**, *4*, 153.
- (470) Alkilany, A. M.; Nagaria, P. K.; Hexel, C. R.; Shaw, T. J.; Murphy, C. J.; Wyatt, M. D. *Small* **2009**, *5*, 701.
- (471) Aillon, K. L.; Xie, Y.; El-Gendy, N.; Berkland, C. J.; Forrest, M. L. *Adv. Drug Delivery Rev.* **2009**, *61*, 457.
- (472) Barreto, J. A.; O'Malley, W.; Kubeil, M.; Graham, B.; Stephan, H.; Spiccia, L. *Adv. Mater.* **2011**, *23*, H18.
- (473) Petros, R. A.; DeSimone, J. M. *Nat. Rev. Drug Discovery* **2010**, *9*, 615.
- (474) Wang, M.; Thanou, M. *Pharmacol. Res.* **2010**, *62*, 90.
- (475) Xie, J.; Liu, G.; Eden, H. S.; Ai, H.; Chen, X. *Acc. Chem. Res.* **2011**, *44*, 883.
- (476) Sha, M. Y.; Xu, H.; Natan, M. J.; Cromer, R. *J. Am. Chem. Soc.* **2008**, *130*, 17214.
- (477) Cho, H.-S.; Dong, Z.; Pauletti, G. M.; Zhang, J.; Xu, H.; Gu, H.; Wang, L.; Ewing, R. C.; Huth, C.; Wang, F.; Shi, D. *ACS Nano* **2010**, *4*, 5398.
- (478) Albanese, A.; Tang, P. S.; Chan, W. C. W. *Annu. Rev. Biomed. Eng.* **2012**, *14*, 1.
- (479) Jokerst, J. V.; Lobovkina, T.; Zare, R. N.; Gambhir, S. S. *Nanomedicine* **2011**, *6*, 715.
- (480) Pasquì, D.; Golini, L.; Della Giovampaola, C.; Atrei, A.; Barbucci, R. *Biomacromolecules* **2011**, *12*, 1243.
- (481) Di Guglielmo, C.; De Lapuente, J.; Porredon, C.; Ramos-Lopez, D.; Sendra, J.; Borrás, M. J. *Nanosci. Nanotechnol.* **2012**, *12*, 6185.
- (482) Li, J.; Ni, X.; Leong, K. W. *J. Biomed. Mater. Res., Part A* **2003**, *65A*, 196.
- (483) Zhang, J.; Ma, P. X. *Nano Today* **2010**, *5*, 337.
- (484) Zhang, L.; Chan, J. M.; Gu, F. X.; Rhee, J.-W.; Wang, A. Z.; Radovic-Moreno, A. F.; Alexis, F.; Langer, R.; Farokhzad, O. C. *ACS Nano* **2008**, *2*, 1696.
- (485) Romberg, B.; Hennink, W. E.; Storm, G. *Pharm. Res.* **2008**, *25*, 55.
- (486) Ballou, B.; Lagerholm, B. C.; Ernst, L. A.; Bruchez, M. P.; Waggoner, A. S. *Bioconjugate Chem.* **2004**, *15*, 79.
- (487) Akiyama, Y.; Mori, T.; Katayama, Y.; Niidome, T. *J. Controlled Release* **2009**, *139*, 81.
- (488) Dubertret, B.; Skourides, P.; Norris, D. J.; Noireaux, V.; Brivanlou, A. H.; Libchaber, A. *Science* **2002**, *298*, 1759.
- (489) Gu, L.; Fang, R. H.; Sailor, M. J.; Park, J.-H. *ACS Nano* **2012**, *6*, 4947.
- (490) Larson, T. A.; Joshi, P. R.; Sokolov, K. *ACS Nano* **2012**, *6*, 9182.
- (491) van Schooneveld, M. M.; Vucic, E.; Koole, R.; Zhou, Y.; Stockx, J.; Cormode, D. P.; Tang, C. Y.; Gordon, R. E.; Nicolay, K.; Meijerink, A.; Fayad, Z. A.; Mulder, W. J. M. *Nano Lett.* **2008**, *8*, 2517.
- (492) Lynn, D. M.; Anderson, D. G.; Putnam, D.; Langer, R. *J. Am. Chem. Soc.* **2001**, *123*, 8155.
- (493) Akinc, A.; Lynn, D. M.; Anderson, D. G.; Langer, R. *J. Am. Chem. Soc.* **2003**, *125*, 5316.
- (494) Anderson, D. G.; Lynn, D. M.; Langer, R. *Angew. Chem., Int. Ed.* **2003**, *42*, 3153.
- (495) Anderson, D. G.; Akinc, A.; Hossain, N.; Langer, R. *Mol. Ther.* **2005**, *11*, 426.
- (496) Green, J. J.; Shi, J.; Chiu, E.; Leshchiner, E. S.; Langer, R.; Anderson, D. G. *Bioconjugate Chem.* **2006**, *17*, 1162.
- (497) Zugates, G. T.; Tedford, N. C.; Zumbuehl, A.; Jhunjhunwala, S.; Kang, C. S.; Griffith, L. G.; Lauffenburger, D. A.; Langer, R.; Anderson, D. G. *Bioconjugate Chem.* **2007**, *18*, 1887.
- (498) Green, J. J.; Zugates, G. T.; Tedford, N. C.; Huang, Y. H.; Griffith, L. G.; Lauffenburger, D. A.; Sawicki, J. A.; Langer, R.; Anderson, D. G. *Adv. Mater.* **2007**, *19*, 2836.
- (499) Schellenberger, E. A.; Reynolds, F.; Weissleder, R.; Josephson, L. *ChemBioChem* **2004**, *5*, 275.
- (500) Kelly, K. A.; Shaw, S. Y.; Nahrendorf, M.; Kristoff, K.; Aikawa, E.; Schreiber, S. L.; Clemons, P. A.; Weissleder, R. *Integr. Biol.* **2009**, *1*, 311.
- (501) Miranda, O. R.; You, C.-C.; Phillips, R.; Kim, I.-B.; Ghosh, P. S.; Bunz, U. H. F.; Rotello, V. M. *J. Am. Chem. Soc.* **2007**, *129*, 9856.
- (502) Samanta, A.; Maiti, K. K.; Soh, K.-S.; Liao, X.; Vendrell, M.; Dinish, U. S.; Yun, S.-W.; Bhuvaneswari, R.; Kim, H.; Rautela, S.;

- Chung, J.; Olivo, M.; Chang, Y.-T. *Angew. Chem., Int. Ed.* **2011**, *50*, 6089.
- (503) Zhang, B.; Xing, Y.; Li, Z.; Zhou, H.; Mu, Q.; Yan, B. *Nano Lett.* **2009**, *9*, 2280.
- (504) Gao, N.; Zhang, Q.; Mu, Q.; Bai, Y.; Li, L.; Zhou, H.; Butch, E. R.; Powell, T. B.; Snyder, S. E.; Jiang, G.; Yan, B. *ACS Nano* **2011**, *5*, 4581.
- (505) Zhou, H.; Jiao, P.; Yang, L.; Li, X.; Yan, B. *J. Am. Chem. Soc.* **2011**, *133*, 680.
- (506) Zhang, Y.; Yan, B. *Chem. Res. Toxicol.* **2012**, *25*, 1212.
- (507) Zhang, Y.; Mu, Q.; Zhou, H.; Vrijens, K.; Roussel, M. F.; Jiang, G.; Yan, B. *Cell Death Dis.* **2012**, *3*, e308.
- (508) Tropsha, A. *Mol. Inf.* **2010**, *29*, 476.
- (509) Tropsha, A. Predictive Quantitative Structure-Activity Relationship Modeling. In *Comprehensive Medicinal Chemistry II*; John, B. T., David, J. T., Eds.; Elsevier: Oxford, 2007; pp 149–165.
- (510) Todeschini, R.; Consonni, V. *Handbook of Molecular Descriptors*; Wiley-VCH: Weinheim, Germany, 2000.
- (511) Dearden, J. C.; Cronin, M. T. D.; Kaiser, K. L. E. *SAR QSAR Environ. Res.* **2009**, *20*, 241.
- (512) Stouch, T. R.; Kenyon, J. R.; Johnson, S. R.; Chen, X. Q.; Doweyko, A.; Li, Y. *J. Comput. Aided Mol. Des.* **2003**, *17*, 83.
- (513) Tropsha, A.; Golbraikh, A. *Curr. Pharm. Des.* **2007**, *13*, 3494.
- (514) Netzeva, T. I.; Worth, A. P.; Aldenberg, T.; Benigni, R.; Cronin, M. T. D.; Gramatica, P.; Jaworska, J. S.; Kahn, S.; Klopmann, G.; Marchant, C. A.; Myatt, G.; Nikolova-Jeliazkova, N.; Patlewicz, G. Y.; Perkins, R.; Roberts, D. W.; Schultz, T. W.; Stanton, D. T.; van de Sandt, J. J. M.; Tong, W. D.; Veith, G.; Yang, C. H. *ATLA, Altern. Lab. Anim.* **2005**, *33*, 155.
- (515) Irwin, J. J.; Shoichet, B. K. *J. Chem. Inf. Model.* **2005**, *45*, 177.
- (516) Dragon, Version 6.0; TALETE s.r.l.: Italy, 2013.
- (517) MOE, Version 2012.10; *Chemical Computing Group*: Canada, 2012.
- (518) Varnek, A.; Fourches, D.; Horvath, D.; Klimchuk, O.; Gaudin, C.; Vayer, P.; Solov'ev, V.; Hoonakker, F.; Tetko, I. V.; Marcou, G. *Curr. Comput.-Aided Drug Des.* **2008**, *4*, 191.
- (519) Weissleder, R.; Kelly, K.; Sun, E. Y.; Shtatland, T.; Josephson, L. *Nat. Biotechnol.* **2005**, *23*, 1418.
- (520) Tasis, D.; Tagmatarchis, N.; Bianco, A.; Prato, M. *Chem. Rev.* **2006**, *106*, 1105.
- (521) Menard-Moyon, C.; Kostarelos, K.; Prato, M.; Bianco, A. *Chem. Biol.* **2010**, *17*, 107.
- (522) Prato, M.; Kostarelos, K.; Bianco, A. *Acc. Chem. Res.* **2008**, *41*, 60.
- (523) Heister, E.; Lamprecht, C.; Neves, V.; Tilmaci, C.; Datas, L.; Flahaut, E.; Soula, B.; Hinterdorfer, P.; Coley, H. M.; Silva, S. R. P.; McFadden, J. *ACS Nano* **2010**, *4*, 2615.