

Biological Responses to Engineered Nanomaterials: Needs for the Next Decade

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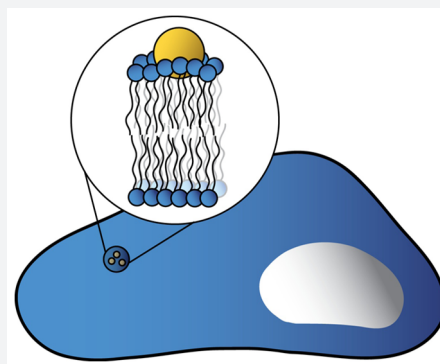
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ABSTRACT: The interaction of nanomaterials with biomolecules, cells, and organisms is an enormously vital area of current research, with applications in nanoenabled diagnostics, imaging agents, therapeutics, and contaminant removal technologies. Yet the potential for adverse biological and environmental impacts of nanomaterial exposure is considerable and needs to be addressed to ensure sustainable development of nanomaterials. In this Outlook four research needs for the next decade are outlined: (i) measurement of the chemical nature of nanomaterials in dynamic, complex aqueous environments; (ii) real-time measurements of nanomaterial–biological interactions with chemical specificity; (iii) delineation of molecular modes of action for nanomaterial effects on living systems as functions of nanomaterial properties; and (iv) an integrated systems approach that includes computation and simulation across orders of magnitude in time and space.



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Engineered nanomaterials began to be created in earnest by chemists and materials scientists at the dawn of the 21st century due to government investments such as the U.S. National Nanotechnology Initiative (NNI).¹ Most fundamentally interesting are materials with quantitatively and qualitatively unique behaviors that emerge at the 1–100 nm length scales. For instance, semiconductors exhibit quantum confinement effects in the ~1–10 nm range, and metals display plasmon resonances at optical frequencies on the ~10–100 nm scale.^{2–4} Even small organic molecules, when formulated into nanoscale rather than micrometer-scale particles, possess remarkable properties. For example, nanoscale pharmaceutical formulations have faster dissolution rates than earlier micrometer-scale technologies, enhancing bioavailability.⁵

The physical properties of nanoscale materials are fascinating on many levels. The nexus of these materials with biology is currently an area of intense study, driven largely by the promise in biomedical applications (Figure 1): diagnostic chemical

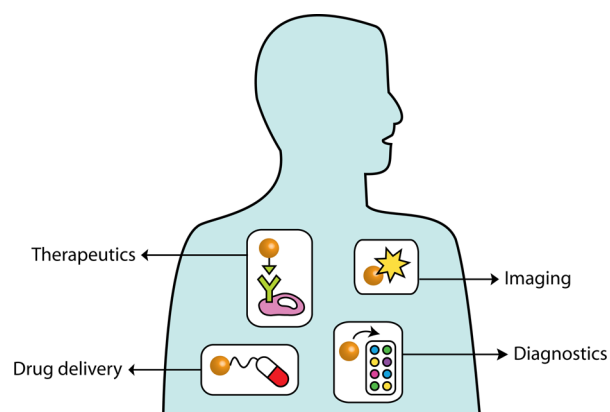


Figure 1. The promise of nanotechnology to improve human health includes diagnostics, drug delivery, imaging, and therapy.

sensing, cellular imaging, drug delivery, therapeutics, and tissue engineering. Yet, notions of peril temper this promise with concerns about biological or environmental exposures that may lead to unintended adverse consequences. The managers of the NNI have been aware of these environmental, health, and safety (EHS) concerns regarding nanotechnology since its inception and have supported programs to study and address EHS issues.^{1,6} This is a welcome development in the chemical sciences compared to the past, in which new chemicals or materials entered commerce with little thought for their potential EHS impacts (e.g., asbestos, DDT, CFCs, PCBs).

The promise of nanotechnology to improve human health is clear. Nanoparticles are of the right size to circulate through the body, be taken up by living cells, or passively accumulate near tumors (according to many, but not all, studies).^{7–10} Therefore, drug delivery has emerged as a potential application for many organic and inorganic nanoparticles. If nanoparticles can be made active—optically, thermally, or magnetically—then they can be employed as bioimaging and contrast agents that go beyond the current standards in biology, for instance, to enable both diagnosis and therapy from a single engineered nanoparticle platform.^{11–16} These formulations include examples such as near-infrared-absorbing inorganic nanoparticles that upon illumination at the proper wavelengths produce enough heat to kill cancer cells^{11,12} and silver nanoparticles embedded in wound dressings that slowly oxidize to produce a steady stream of

antimicrobial silver ions.¹⁵ However, nanomaterials that have been approved by the U.S. Food and Drug Administration for drug delivery consist largely of organic nanoformulations: liposomes, lipoplexes, polyethylene glycol–drug nanocomplexes, or albumin nanosphere conjugates.^{16–19} Superparamagnetic iron oxide nanoparticles have been the only clinically approved metal oxide nanoparticles,²⁰ and of the metal nanoparticles, gold nanoparticles have entered clinical trials as drug delivery vehicles or as photothermal therapeutics for light-induced ablation of tumors.²¹

Beyond direct use of engineered nanomaterials for applications in health and medicine, large quantities (tons, in some cases) of nanomaterials are now constituents of mass consumer products, with more coming online every day. Many of these nanomaterials are components in emerging clean energy and clean water systems that benefit both human and environmental health.^{22,23} For example, an electric vehicle (e.g., the 2015/2016 Nissan Leaf) will contain approximately 50 kg of nanostructured metal oxide electrodes in its cathodes;²² as electric vehicles replace conventional vehicles, automobile emissions will decline. Nanomaterials can adsorb environmental contaminants and are being actively studied for drinking water treatment.²⁴ Nanoscale semiconductor quantum dots are now being integrated into plastic films to enhance color saturation in displays for consumer electronics, including laptop computers, electronic readers, and cell phones.²⁵ Concerns regarding the biological, environmental, and ultimately human health consequences of inadvertent release of such engineered nanomaterials into the environment through a variety of pathways need to be taken seriously, given our past history and experience with introducing supposedly benign materials into the environment. From a fundamental chemistry perspective, then, we need to ask: what will we need to know about new nanomaterials that are to be produced on a large scale if we wish to avoid negative outcomes, while at the same time take advantage of what these new materials offer?

The EHS aspect of nanomaterials research has led to a number of studies aimed at correlating the physicochemical characteristics of nanomaterials with biological or environmental outcomes. For example, substantial evidence exists that cationic nanoparticles lead to more deleterious effects than their anionic counterparts at the cellular level.²⁶ Existing large-scale efforts to screen nanomaterials for biological effects, while valuable, focus on identifying exposure levels that cause death or induce physiological changes or on the development of structure–toxicity relationships.²⁷ Such empirical studies provide few mechanistic details about the chemical and physical processes occurring where a nanoparticle interfaces with its environment: its “skin,” the nanoparticle surface. Molecular-scale insight into the nanomaterial interactions with biological systems (Figure 2) is essential for effective design of nanomaterials with minimal detrimental biological effects while maintaining their function. Here, we argue that establishing causality—rather than correlations—in how nanomaterial properties and behavior impact biological outcomes is a key challenge that can be addressed by specifically drawing on recent advances and developments in the chemical sciences. Previous work in the nano-EHS area has generally focused on single-component nanoparticles such as TiO₂, Au, and carbon nanotubes. Looking ahead, it becomes apparent that the nanoparticles used in technology will often be multicomponent systems or nanocomposites; thus, increasingly complex and technologically relevant nanomaterial products need to be examined in as

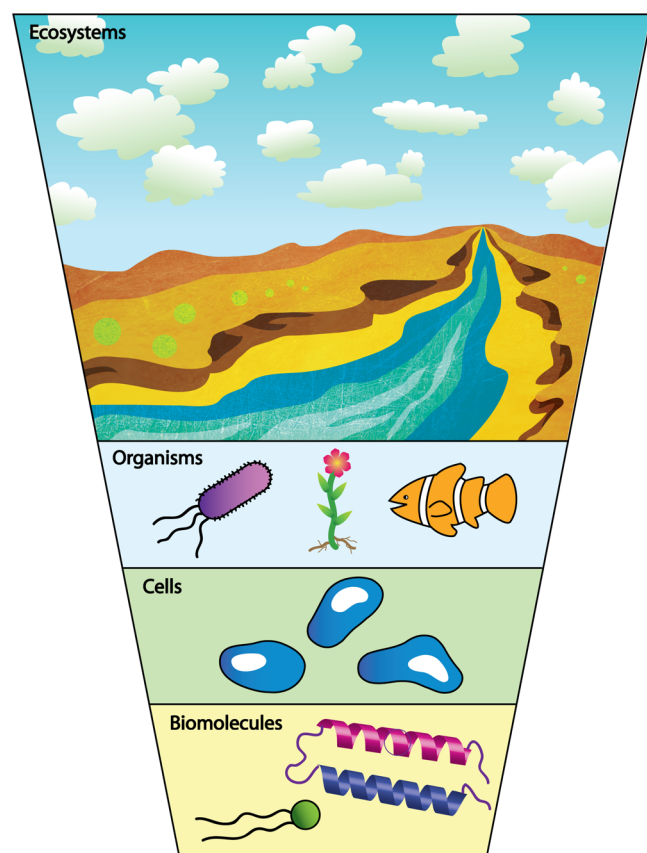


Figure 2. Nanoparticles interact with biological systems at the molecular, cellular, organismal, and ecosystem levels.

great detail, if not greater, than the constituent nanomaterials for EHS concerns.

Given the wide variety of nanomaterial–biological studies with so many variables, some broad needs clearly exist at the basic science level:

1. Chemically Driven Understanding of the Molecular Nature of Engineered Nanoparticles in Complex, Realistic Environments. The problem is rooted in complexity: nanomaterials come in a variety of sizes, shapes, and initial surface coatings, all of which affect their bioactivity. Many commercial nanomaterials are delivered to users as agglomerates of smaller primary particles; determination of the actual particle size under relevant conditions can be very challenging. Well-documented cases in which apparent adverse nanomaterial effects were due to leftover reagents from the synthesis^{28,29} highlight the importance in this field for careful controls and attention to nanoparticle purification methods. Similarly, the low quality of nanomaterials in some studies (e.g., mixtures of different sizes and shapes of particles) complicates the extraction of nanosize-specific information in terms of biological impact. New methods, therefore, are needed to assess nanomaterial quality during synthesis and when in use. In the U.S., the Nanotechnology Characterization Laboratory (NCL) of the National Cancer Institute currently performs a well-regarded set of biological assays on submitted nanomaterials, at no charge; but the NCL rejects samples that are deemed too variable in their physical and chemical parameters such as size, shape, composition, and solubility. The U.S. National Institute of Standards and Technology (NIST) has only a few standard reference nanomaterials available, including 10, 30, and 60 nm diameter gold

nanoparticles (SRM 8011, 8012, 8013 respectively) and, as of March 2015, polymer-coated silver nanoparticles with a nominal diameter of 75 nm (SRM 8017). The fact that the main U.S. agency charged with providing chemical and materials standards to the scientific community has taken so long to produce very basic nanomaterials speaks to the difficulty of developing reproducible and scalable syntheses. The current “best” colloidal nanomaterials with respect to polydispersity have dimensions within 5% of the mean length across one or more axes.

The initial surface chemistry of the nanomaterials clearly influences their fate and distribution in biological systems, despite the well-known biomolecular “coronas” that overcoat the nanomaterials upon immersion into biological fluids.³⁰ It is not yet clear what combination of initial nanomaterial properties (e.g., charge, size, surface functionalization, aggregation state) can be used to predict the composition of the biomolecular coronas they acquire, how this corona changes over time, or the ultimate biological outcome of nanoparticles bearing biomolecular coronas; yet all of these initial nanomaterial properties have been reported to generally affect biological outcomes in one form or another. In situ measurements of the dynamic transformations of nanomaterials and their surfaces, in biological environments, represents a key need for the next decade.

2. Real-Time Measurements of Nanomaterial Interaction with Living Cells and Organisms That Provide Chemical Information at Nanometer Length Scales To Yield Invaluable Mechanistic Insight and Improve Predictive Understanding of the Nano–Bio Interface. While nanomaterials can be large enough to visualize in many different microscopy experiments, doing so in aquo and in real time while simultaneously acquiring molecular information (as opposed to the localization of fluorescent spots) represents a major challenge. In many cases, the first biological interface nanomaterials encounter is a cell membrane. The lipid bilayer–nanomaterial interface has therefore emerged as an important focus of experimental and theoretical interest over the last several years.^{31–34} Our recent paper demonstrated that a large assembly of physical and analytical measurements was needed to quantitatively assess the thermodynamics and electrostatics of nanoparticle–membrane interactions.³⁵ To date, most such model membrane systems are quite simple, consisting of single phospholipids or binary or ternary mixtures of lipids. Intact cellular membranes contain a large variety of components in addition to phospholipids (e.g., peripheral and transmembrane proteins, proteoglycans, glycolipids) that may also influence nanomaterial interaction with cell surfaces. From an imaging perspective, tracking nanomaterials as they interact with living cells or tissues^{36,37} using a combination of standard and super-resolution fluorescence microscopies,³⁸ as well as nonlinear optical microscopies with video-rate tracking,^{39,40} is a burgeoning area of interest. Super-resolution vibrational imaging would provide nanoscale spatial *and* chemical information. Additional innovative approaches combining existing and novel instrumentation to access the nano–bio interface need to be developed to enable these fascinating molecular interactions to be understood, controlled, and predicted.

3. Delineation of Molecular Modes of Action for Nanomaterial Effects on Living Systems as Functions of Nanomaterial Properties. Over 50,000 studies have now been published on the interaction of nanomaterials, both organic and inorganic, with cultured cells and whole organisms (Web of Science, searching topic of (nanomaterials OR nanoparticles) AND biol* AND (cell* OR organ*), accessed May 22, 2015).



Figure 3. Interactions between nanoparticles that are chemically complex and biological “receptors” need to be understood at a molecular level.

These studies provide a plethora of data that inform us of potential mechanistic interactions of nanoparticles with cells.^{41–45} However, these studies have covered dozens of different cell lines, either established or primary; the time of nanomaterial exposure ranges from minutes to days; the nanomaterial doses applied can differ by 6 orders of magnitude, which can dramatically impact the molecular responses instigated in a system and the ultimate consequence of the exposure. Therefore, stating general conclusions about chemical—let alone nanomaterial—effects on living cells becomes difficult, complicating attempts to extrapolate from cellular data to whole organisms.⁴⁶ Indeed, it is rare that a clear molecular pathway from nanomaterial to cellular response can be constructed. Yet, such molecular pathways, if properly understood, could serve as a means to predict the future impact of nanomaterials on living systems, as evidenced by the groundswell of papers that tell us that cells and organisms can up- or downregulate the expression of specific genes upon exposure to nanomaterials.^{44,47–51} Other molecular signals have been found: nearly 7000 papers point to reactive oxygen species as a “smoking gun” in cell–nanomaterial toxicity experiments (although the ability of cells and organisms to adapt to oxidative stress is well-known). Moreover, nanomaterial interference with specific enzymes has been observed.⁵² Even nanomaterial alteration of the extracellular matrix that surrounds cells can lead, indirectly, to nano–bio interactions.^{47,53} Given this context, the chemical nature of the biological “receptor” (cell membrane, tissue lining, etc.) needs to be understood at the molecular level in time and space, as it is an equal partner in nano–bio interactions (Figure 3).

Compounding the problems associated with the delineation of molecular modes of action for nanomaterial effects on living systems is that many of the available studies used acute (short-term) exposures, and we know from other classes of chemicals (e.g., pesticides, endocrine disruptors) that extending measures of biological response beyond mortality and short time points is needed to fully assess the potential unintended consequences of exposure. Numerous investigations have shown that aspects of the chemical nature of nanomaterials (e.g., initial surface charge, ligand composition, potential for dissolution) influence their organism-level impact.^{54–57} However, studies are difficult to compare in that different organisms respond differently to a given chemical, let alone nanomaterial.^{41–46,58} The difficulty of understanding and predicting bioactivity of nanomaterials is exacerbated when one considers that very small metallic

nanomaterials (≤ 3 nm) are catalytically active in a size-dependent manner.^{59,60} Thus, biological impacts from size alone can be convolved with new intrinsic reactivity if the studies include samples that span the “unreactive” and “reactive” regimes.

4. Computation and Simulation of the Nano–Bio Interface. A need exists to develop multiscale algorithms and models that provide direct and correct structure and dynamics of nanoparticles in complex environments including those within organisms. At the smallest scale, such multiscale computational tools must address molecular-scale interactions that can be specifically tailored through chemical control of a nanoparticle in the initial design and are modified through its life cycle in products and in the environment. At the largest scales, such tools must address the changing behavior and transport of nanoparticles inside organisms. The span of these scales, illustrated in Figure 4, is on the order of 10^{10} . Establishing explicit connections to experimental observables at all these scales is essential for validation and refinement of computational methodologies.

To this end, computational frameworks that enable prediction of molecular-level interactions between nanoparticles and their environment are vitally needed. These frameworks may integrate state-of-the-art computational methods spanning multiple length scales, from angstrom using atomistic quantum-mechanical calculations through particle and continuum levels using coarse-grained modeling. Reliable atomistic force fields need to be developed based on accurate quantum chemistry computations, including advanced density functional theory^{61,62} and embedding methods that integrate correlated *ab initio* and DFT calculations. To efficiently characterize the binding of biomolecules to nanoparticles under complex environmental conditions,⁶³ novel equilibrium^{64,65} and nonequilibrium⁶⁶ sampling techniques are also required. Effective coarse-grained (CG)^{67–70} and dynamic models⁷¹ guided by atomistic simulations to model nanoparticle assembly and their interaction with complex membranes⁷² are likely to play increasingly important roles as well because they can deal with longer time scales and larger length scales than their atomistic counterparts. Ideally, computational frameworks that feature intimate coupling of CG dynamics, kinetic Monte Carlo, and local atomistic computations will be outcomes from such endeavors, along with the ability to use such new modeling frameworks for computationally driven, rationally designed nanomaterials that are also sustainable. A validated multiscale framework providing a

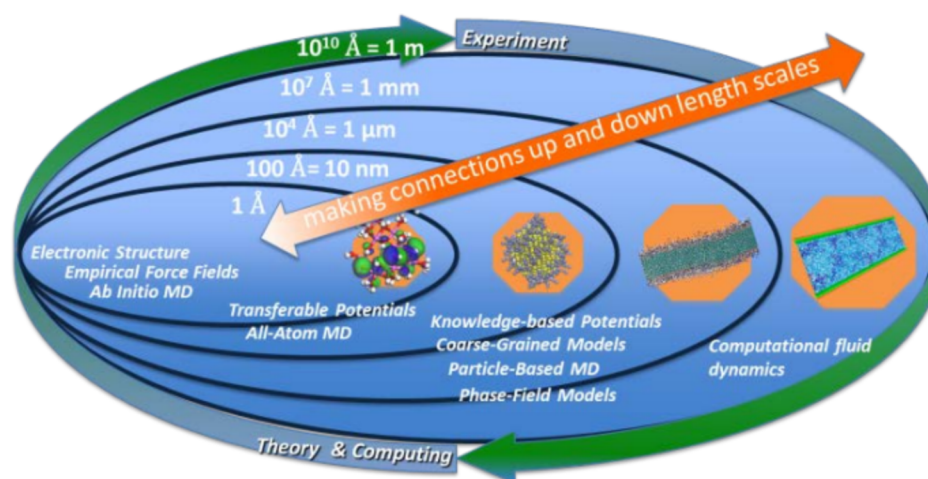


Figure 4. Computational approaches to understanding the nano–bio interface span orders of magnitude in time and space.

connection between molecular-scale tunability and meso- to macroscale structure and function would be useful beyond the applications of interest to studying nanomaterials, potentially including emergent catalytic behavior and transport of various species within organisms.

Overall, this is an exciting time to be studying the nanomaterial–biological interface. Nanomaterial syntheses have improved to the point where very monodisperse and well-characterized samples can be prepared at reasonably large scales; computation and simulation have improved to the point where an entire virus can be simulated with all-atom molecular dynamics;⁷³ super-resolution fluorescence microscopy approaches have been developed in the past 10 years that enable 10–20 nm resolution imaging in intact hydrated cells;^{74,75} and genomics, proteomics, and metabolomics greatly speed the measurement of biological end points and uncovering of pathways associated with cellular response and toxicity.^{76,77} Taken together, this confluence of scientific advances will enable true molecular-level understanding of the nanomaterial–biology interface in the next decade and beyond.

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Notes

The authors declare no competing financial interest.

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