

Nanoparticle–Cell Interactions: Relevance for Public Health

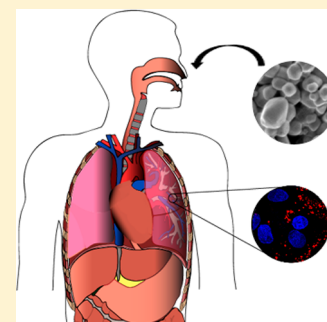
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ABSTRACT: Nanoparticles, especially metal oxide nanoparticles, are used in a wide range of commercial and industrial applications that result in direct human contact, such as titanium dioxide nanoparticles in paints, food colorings, and cosmetics, or indirectly through release of nanoparticle-containing materials into the environment. Workers who process nanoparticles for downstream applications are exposed to especially high concentrations of nanoparticles. For physical chemists, nanoparticles present an interesting area of study as the small size of nanoparticles changes the properties from that of the bulk material, leading to novel properties and reactivity. For the public health community, this reduction in particle size means that exposure limits and outcomes that were determined from bulk material properties are not necessarily valid. Informed determination of exposure limits requires a fundamental understanding of how nanoparticles interact with cells. This Feature Article highlights the areas of intersection between physical chemistry and public health in understanding nanoparticle–cell interactions, with a focus on titanium dioxide nanoparticles. It provides an overview of recent research examining the interaction of titanium dioxide nanoparticles with cells in the absence of UV light and provides recommendations for additional nanoparticle–cell research in which physical chemistry expertise could help to inform the public health community.



INTRODUCTION

The field of public health can be broadly defined as a multidisciplinary effort to identify, through statistical, epidemiologic, and molecular methods, and address trends in population health and then use health education and policy decisions to prevent disease and improve quality of human life.^{1–5} While national and local health departments are largely a modern innovation, sanitary practices, like the separation of drinking water and human waste, as well as cultural and religious mandates aimed at regulating food, have been observed for millennia. The first emergence of a national public health program was the smallpox vaccination program, introduced by Edward Jenner in 1798 and provided by the British government in 1840. The field of epidemiology, founded during John Snow's investigation of the 1854 cholera outbreak in London, established cohort investigation and statistical analysis as central tenets of public health research. In the 1900s, chemistry and biology broadened the understanding of disease etiology to incorporate pathogens, exposures, lifestyle, and heredity into the larger picture of health. On the basis of these advancements, public health today integrates environmental, occupational, and mental health, disease control, toxicology, and health economics into conducting ethical research on health outcomes and creating sustainable policies that improve human life.

In contrast with the population-level view that defines public health, physical chemists focus on individual nanoparticles, molecules, atoms, and electrons. The best known example of the intersection of physical chemistry and public health comes from atmospheric and ozone chemistry.^{6–8} The molecular-level, physical chemistry, understanding of ozone, pollution,

and climate has been instrumental in the establishment of government programs regulating not only chlorofluorocarbon production, but also modeling of climate conditions and air pollution to address health outcomes ranging from respiratory disease⁹ to skin cancer due to heightened UV radiation.^{10,11} In this sense, physical chemists helped construct the framework for climate-related disease study.

Exposure assessments for the increasingly complex nanomaterials that have emerged over the last few decades offer a new opportunity for interdisciplinary collaboration between public health scientists and physical chemists.^{12–14} For physical chemists, the small size of nanoparticles changes the properties from that of the bulk material, leading to novel properties and reactivity. For the public health community, this reduction in size means that exposure limits and outcomes that were determined from bulk material properties need to be re-evaluated. For example, most people are exposed to metal oxide NPs, especially titanium dioxide nanoparticles (TiO₂ NPs), on a daily basis through their use as pigments in paints, food, and cosmetics (Figure 1). Annual production is estimated at levels of >200,000 t.^{15–17} Exposure limits in food products and sunscreens are set by the FDA at 1 wt/wt % and 25 wt/wt %, respectively.^{18–21} In terms of food, the FDA declared TiO₂ “inert” in a 1969 report and therefore there was no need to regulate the percentage. The 25 wt/wt % for sunscreen was set by the FDA in 1978 based on analysis of data at the time including studies in rats and accidental human ingestion.²²

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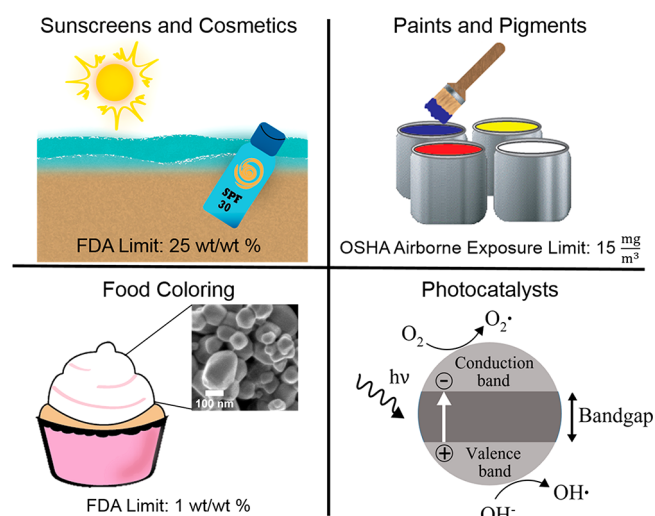


Figure 1. Humans are exposed to TiO_2 NPs through their daily activities. TiO_2 NPs have been of great interest to physical chemists for the photocatalytic properties.

The National Institute of Occupational Safety and Health (NIOSH) provides recommendations for TiO_2 particle exposure: 2.4 mg/m^3 for fine (primary particle diameter $1\text{--}10 \mu\text{m}$) and 0.3 mg/m^3 for ultrafine (primary particle diameter $<100 \text{ nm}$) particles in the air of the work environment as a time-weighted average over a 10 h day and a 40 h work week,²³ meaning that high exposure over a short interval could be balanced by reduced, or no, exposure for the rest of the work day. This exposure limit is based on a review of toxicology and epidemiologic literature carried out by NIOSH.²⁴ The legal limit for TiO_2 exposure is set by the Occupational Safety and Health Administration (OSHA) at 15 mg/m^3 , commonly listed on MSDS sheets (CAS Number: 13463-67-7).

TiO_2 NPs are familiar to physical chemists as photocatalysts with UV-light-initiated production of reactive oxygen species (ROS).^{25–27} However, the most important exposure pathways,

in the lung following inhalation or in the gut following ingestion, occur in the absence of light. TiO_2 NPs applied to the skin as sunscreen or cosmetics do not penetrate skin and are coated with alumina, silica, or silicone dioxide, or doped with metals to absorb ROS generated by UV exposure.^{21,28–31} Additionally, cellular-level interactions, following either inhalation or ingestion, do not involve an interaction with the bare NP but rather the “corona” of proteins that adsorb on the surface of the NP. This protein corona forms an interface between the NPs and the cell (Figure 2). Extensive previous work has shown that it is this protein corona that dictates the interaction of NPs with cells.^{32–37} Surface modification with neutral polymers, such as PEG, can reduce nonspecific binding, but complete inhibition remains a challenge.^{38–42} Understanding this NP–protein interface is a molecular-level question that can be addressed by the toolbox of the physical chemist, helping to inform public health research.

■ NANOPARTICLE–CELL INTERACTIONS

Research within the Payne Lab in the School of Chemistry and Biochemistry at Georgia Tech has focused on understanding the interaction of proteins, NPs, and cells with a specific interest in determining how these NP–protein interactions affect cellular outcomes.³² The interaction of TiO_2 NPs with proteins and cells presents an especially interesting and important question due to the high levels of human exposure. TiO_2 NPs, like NPs used for biomedical applications,^{43,44} form a protein corona when incubated with serum proteins. Mass spectrometry shows that the five most abundant proteins in the TiO_2 NP protein corona formed from fetal bovine serum (FBS) are, in order of abundance, complement C3, plasminogen, albumin, complement factor H, and complement C7 (Figure 2).³⁷ This corona is similar to the corona observed previously following incubation of TiO_2 NPs in plasma proteins,⁴⁵ which includes the clotting factors (fibrinogen and kininogen) that are removed from plasma to produce serum. Interestingly, although serum albumin is the most abundant protein serum protein by a

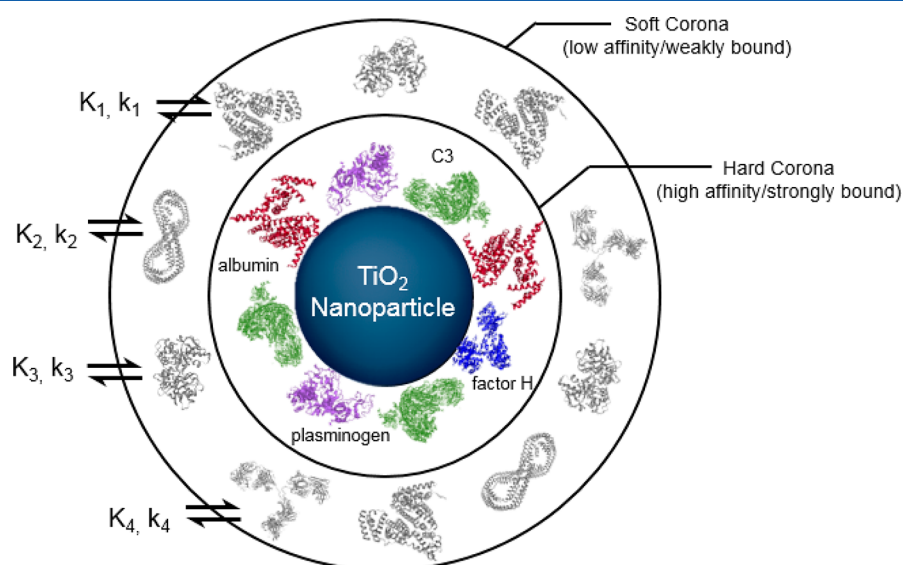


Figure 2. Schematic of the protein corona formed following incubation of TiO_2 NPs with fetal bovine serum (FBS), a commonly used nutrient source for cells grown in culture. Proteins shown in the hard corona were the four most abundant proteins in the corona identified using mass spectrometry.³⁷ Soft corona proteins were not characterized in our experiments. Common serum proteins are shown in the schematic as an example. Protein structures were obtained from the Protein Data Bank. Adapted with permission from ref 32. Copyright 2014 American Chemical Society.

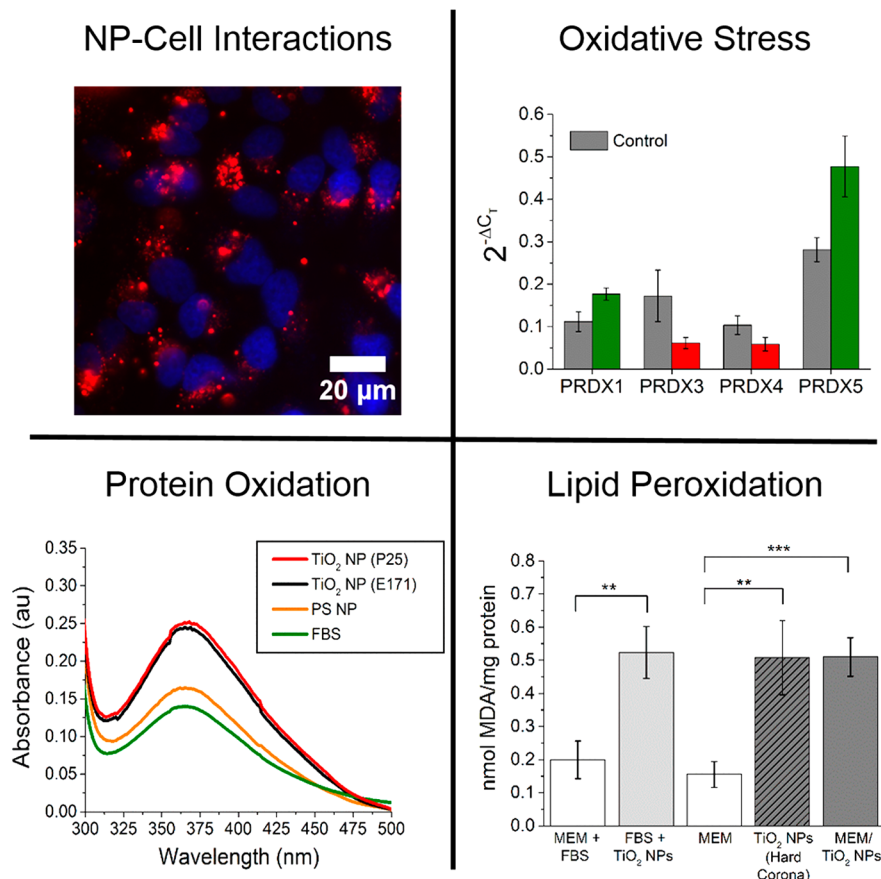


Figure 3. Summary of TiO₂ NP–protein–cell interactions. Top right reprinted with permission from ref 37. Copyright 2016 American Chemical Society. Bottom right reprinted with permission from ref 50. Copyright 2017 American Chemical Society. Bottom left reprinted with permission from ref 61. Copyright 2017 Royal Society of Chemistry.

significant margin (55%),^{46–48} it is only the third most abundant protein in the TiO₂ NP corona. In comparison, complement C3, an immune system protein that is the 28th most abundant protein in human serum,⁴⁹ is highly enriched on the NP surface. Super-resolution fluorescence microscopy was used to image the distribution of corona proteins on the surface of the TiO₂ NPs.⁵⁰ Albumin was used as a representative serum protein and labeled with AlexaFluor647 (excite: 650 nm/emit: 668 nm) for imaging. While conventional, diffraction-limited, fluorescence microscopy shows a diffuse layer of protein on the NP surface, super-resolution (STORM) imaging shows that albumin is localized in distinct clusters on the NP surface, at odds with the familiar schematic of a protein layer. Protein clusters were observed at all ratios of albumin to TiO₂ tested (0.01 mg of albumin/mg of TiO₂ to 10 mg of albumin/mg of TiO₂).

These experiments with FBS proteins are identical to the conditions used for cell culture and serve as a model for NPs in the bloodstream. In comparison, inhaled TiO₂ NPs are expected to interact with lung surfactants before crossing epithelial cells to gain entry to the bloodstream.⁵¹ In lung fluid, a corona of lung surfactant protein A is formed on the surface of NPs,^{52–54} including TiO₂ NPs.⁵⁴ Future work will need to examine protein coronas as a function of exposure pathway with initial exposure to lung fluid or gut followed by transport to the bloodstream. This is essentially a question of thermodynamics and kinetics, determining if lung or gut

proteins are displaced by blood serum proteins as a function of binding affinity, relative abundance, and exposure time.

TiO₂ NPs are well-characterized as photocatalysts,^{25–27} but as described above, relevant exposure pathways occur in the dark. Previous luminol assays have shown that anatase TiO₂ NPs produced reactive oxygen species (ROS) in the absence of light.⁵⁵ Although the mechanism underlying this “dark” production of ROS is not well-understood, it is likely linked to surface defects, such as oxygen vacancies.^{56–60} ROS measurements in our lab used a combination of colorimetric and fluorescence assays and EPR spectroscopy. Experiments were carried out in the dark, in aqueous (water or PBS) solutions, in the absence of cells. The assays detected hydroxyl radicals and superoxide generation by TiO₂ NPs, both the photocatalytic (equivalent to Degussa P25) and food-grade (designated E171 by the E.U.) NPs.⁶¹ No hydrogen peroxide was detected. Surface passivation of the TiO₂ NPs with an alumina–silica shell blocked ROS production, as did the presence of isopropanol and superoxide dismutase, used as scavengers for hydroxyl radicals and superoxide, respectively.⁶¹ In addition, serum proteins incubated with TiO₂ NPs were oxidized, with a greater carbonyl content (77 ± 5 and 73 ± 15% increase for P25 and E171, respectively, DNPH assay) than serum proteins in the absence of TiO₂ NPs.⁶¹ Similarly, the direct incubation of “bare” TiO₂ NPs, lacking a protein corona, with cells led to oxidation of plasma membrane lipids (156 ± 5% increase, MDA assay).⁵⁰

A cellular response to these oxidized serum proteins was detected with a PCR array (Human Oxidative Stress Plus, Qiagen) that allows researchers to screen 84 different oxidative-stress-related genes simultaneously. Incubation of cells with TiO₂ NPs and serum proteins for 24 h under standard cell culture conditions (dark, 37 °C, 5% CO₂) led to changes in expression of six genes, including four members of the six-membered peroxiredoxin family of antioxidant enzymes. Western blotting of peroxiredoxin 1 was used to validate the PCR experiments. These enzymes degrade peroxides within the relevant organelle, making them important for the oxidative stress response of the cell.^{62–66} Of the four peroxiredoxins identified in our PCR screen, peroxiredoxin 1 is mostly localized in the cytosol, peroxiredoxin 3 is mostly localized in the mitochondria, and peroxiredoxin 4 is extracellular, secreted from the cell. Peroxiredoxin 5 is delocalized, found in the cytosol, nucleus, mitochondria, and peroxisomes.^{65,67} It is important to note that at the very low concentration of TiO₂ NPs used for these experiments no changes in cell health were observed.³⁷ To examine the effects of these oxidized corona proteins on the oxidative stress response of the cells, we used TiO₂ NPs to oxidize serum proteins and then removed the TiO₂ NPs from the mixture by centrifugation. We then used these now-oxidized proteins to form a corona on polystyrene NPs. Polystyrene NPs had been used as a negative control throughout previous experiments; no ROS was detected, and no oxidative stress response was observed.^{37,61} With an oxidized protein corona, the nonoxidizing polystyrene NPs led to oxidative stress response in cells characterized by PCR (changes in expression of peroxiredoxins 3,4,5) and Western blotting of peroxiredoxin 4. The oxidized serum proteins, adsorbed on the surface of NPs, appear to send an oxidative stress signal to the cells resulting in changes in the peroxiredoxin family of antioxidant enzymes.^{37,61} Previous work had found that TiO₂ NPs, in the absence of light, led to DNA damage, lipid peroxidation, and micronuclei formation,⁶⁸ additional reporters of oxidative stress.

■ FUTURE DIRECTIONS FOR PHYSICAL CHEMISTS

Taken together, our experiments show that TiO₂ NPs, even in the dark, produce low levels of hydroxyl radicals and superoxide and these ROS oxidize corona proteins.⁶¹ Oxidized serum proteins trigger an oxidative stress response in cells, specifically changing the expression of the peroxiredoxins (Figure 3).^{37,61} While these results start to address the question of TiO₂-NP-induced oxidative stress under relevant physiological conditions, they also raise many more questions that require the expertise of physical chemists. For example, previous researchers have observed ROS generation, directly or indirectly, from TiO₂ NPs in the dark.^{55,68} This research area is certainly small in comparison to photocatalytic studies of TiO₂ NPs^{25–27} but, considering the biological relevance, may be worth a closer look, especially in terms of the relationship between crystal structure and NP-induced toxicity.⁵⁵ Fundamentally, this is a question of quantum mechanics and surface science.

A second aspect of protein corona research that would benefit from physical chemists is the definition of the protein corona. Currently, the classification of the corona is “hard” or “soft”, which is a qualitative view of binding affinity and protein exchange. In practice, most researchers classify the corona on the basis of an experimental parameter—the hard corona requires detergent for removal from the NP surface, while the

soft corona can be removed through a “washing” process of repeated centrifugation and resuspension. The hard corona is expected to have a greater impact on the cellular interaction, as it is continuously present. The soft corona may only be important for specific events or at specific times. Quantifying the thermodynamics of the protein corona has generally relied on isothermal titration calorimetry and fluorescence spectroscopy, generating Stern–Volmer plots of protein quenching.^{69–74} The development of analytical ultracentrifugation to measure dissociation constant, stoichiometry, and Hill coefficient for albumin adsorbed to gold NPs⁷⁵ provides a new method for corona quantification.

The third question that requires the assistance of physical chemists is the mechanism of the oxidative stress pathway.^{76–80} While this is fundamentally a question of cell biology, imaging techniques and redox-sensitive probes informed by physical chemistry are necessary to map the signaling pathways used by the cell. Understanding these signaling pathways requires both spatial and temporal information, which requires advanced fluorescence microscopy techniques. The key cellular signaling molecule for redox biology is hydrogen peroxide. The recent development of protein, molecular, and nanoparticle sensors of hydrogen peroxide for intracellular applications provides new tools for mapping of these biological pathways.^{81–89}

There are many aspects of nanoparticle–protein–cell interactions that require better quantification. This system presents a challenging, highly complex, environment for measurements and mechanistic understanding. Even in cell-free systems, serum proteins present a complex mixture consisting of thousands of different proteins.^{46–49} Most quantitative methods are relatively low throughput and limited to single proteins. With the extension of NMR for characterization, the protein corona does not require the removal of unbound proteins,^{90,91} suggesting it may be useful for the mixtures of serum proteins. Simulations would help inform this array of experimental methods but are challenging in terms of system size. Recent research has used coarse-grained simulations,^{92–95} with promising results able to predict changes in protein structure that match with experimental CD spectroscopy.⁹⁵ Moving forward, for both experiments and simulations, it is important to consider even more realistic systems.

Because of the interest in nanomedicine applications in which NPs will be injected intravenously, much of the protein corona community has focused on blood serum proteins. In comparison, inhalation and ingestion, with their associated biomolecular coronas, may be the more important systems in terms of overall exposure. Similarly, a broader view of exposure may also be necessary. The extensive industrial use of TiO₂ NPs is associated with high levels of TiO₂ NP release into the environment. Although 96% of titanium is removed in wastewater treatment plants, concentrations of 1–100 µg/L are measured in the effluent in the form of aggregated nanoparticles.^{96–98} The Mesocosm Facility run by the NSF/EPA Center for the Environmental Implications of Nanotechnology (CEINT) provides a simulated wetland environment that would mimic environmental release prior to human exposure.

In addition to the interesting physical chemistry questions presented by the protein corona, it is also important to keep in mind the relevance of this research to the public health community. For example, a key parameter in human exposure assessment is NP aggregation. Bulk methods generally rely on

light scattering, measuring a hydrodynamic diameter and polydispersity index, although this is an imperfect measure of typically nonspherical aggregates. Beyond characterization, it is important to understand what leads to aggregation in order to control it. Elegant single molecule studies addressed this question for gold nanoparticles as a function of particle shape (sphere, rod, wire) and surface modification.⁹⁹ They found albumin adsorbed on the surface of the gold nanoparticles and its subsequent unfolding led to nanoparticle aggregation.

There is the desire in the public health community for a more thorough and uniform characterization of nanomaterials based on material properties, size and aggregation, and biological interactions. The EPA has compiled a list of parameters that should be characterized for nanotoxicity experiments from the initial materials, as supplied, as administered, and after administration.^{21,100} These parameters, including size, shape, surface area, porosity, crystal structure, and surface charge, are familiar to physical chemists with expertise in electron microscopy, dynamic light scattering, and BET measurements. In addition, research from the protein corona community suggests that nanomaterial characterization should include characterization of the nanomaterial with relevant proteins. This interaction and the resulting changes to the protein structure can lead to unexpected physiological outcomes.^{101–108} For example, lysozyme and chymotrypsin lose secondary structure and activity upon adsorption to 10 nm gold NPs and the secondary structure of cytochrome *c* is disrupted upon binding to sulfonated polystyrene NPs and magnetic NPs.^{109–111} In the Payne lab, we have observed that NP–protein interactions can lead to a change in protein secondary structure, detected with CD spectroscopy, that redirects the protein–NP complex to an off-target receptor on the cell surface.⁶⁹

SUMMARY

Historically, physical chemists have been counted among toxicologists, molecular biologists, and environmental scientists as the main contributors in the creation of today's public health paradigm. Physical chemists are in a unique position to provide insight into the underlying physical mechanisms that link NP properties to biological and environmental outcomes. A current example is seen with the NSF Center for Sustainable Nanotechnology, which examines the underlying mechanisms of NP toxicity on the scale of molecules to daphnia.¹² And while this Feature Article focuses on TiO₂ NPs, it is important to note that the increasing consumer uses of other NPs will lead to similar public health questions. For example, the quantum-dot-based LEDs popular in television monitors now raise a set of concerns including direct consumer contact, worker exposure, and environmental release, identical to those of TiO₂ NPs. Public health research addresses a vast array of outcomes, from the efficacy of intervention programs to stratified differences in disease risk, approached primarily through cohort studies, case-control studies, clinical trials, or other study designs. Constructing predictive risk assessments or modeling exposures, however, frequently requires mechanistic, molecular information, leading public health investigators to look for molecular-level insight and collaborations.

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Notes

The authors declare no competing financial interest.

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Sabiha Runa graduated from the University of Alabama in Huntsville with a B.S. in Chemistry and a B.S.E. in Chemical Engineering with *magna cum laude* distinction. A strong believer in “science for all”, she has dedicated much of her time outside of the lab to volunteering for programs that provide STEM education and opportunities for a K-12 audience. She is the creator of the Science Haiku Contest for the Atlanta Science Festival, and has worked as part of the communications team at the Center for Education Integrating Science, Mathematics, and Computing at Georgia Tech. She earned a Ph.D. in Chemistry from Georgia Tech in 2017 and is currently a postdoctoral researcher at National Taiwan University in Taipei.

Michael Hussey earned his B.S. in Biology and History from Boston College and is currently pursuing an MSPH in Environmental Health and Epidemiology at Emory University's Rollins School of Public Health. His current research under Dr. Carmen Marsit focuses on the epigenetic expression of placental long noncoding RNA among members of a Rhode Island birth cohort in relation to environmental exposures to lead, cadmium and mercury, and whether these expression differences relate to the outcome of birth weight. His interests include epidemiologic risk assessment, toxicology, and epigenetics.

Christine K. Payne is an Associate Professor in the School of Chemistry and Biochemistry at Georgia Tech. Her research focuses on understanding how cells interact with materials including nanoparticles and conducting polymers. Prof. Payne has received many honors including an NIH Director's New Innovator Award in 2009 and a DARPA Young Faculty Award in 2011. She earned a B.S. in Chemistry from the University of Chicago (1998) and a Ph.D. in Chemistry from the University of California, Berkeley (2003). Prof. Payne spent 2003–2006 as an NIH NRSA Postdoctoral Fellow at Harvard University. She joined the faculty of the School of Chemistry and Biochemistry at Georgia Tech in 2007.

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