PERSPECTIVE

Correlating Physico-Chemical with Toxicological Properties of Nanoparticles: The Present and the Future

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ABSTRACT Nanotoxicology is still a new discipline. In this Perspective, both its origins and its future trends are discussed. In particular, we note several issues we consider important for publications in this field.

In the past decade, there has been a dramatic increase in the use of nanoparticles (NPs) in research and in industrial production, which has raised questions about the potential toxicity of such materials.[1,2] Thus, the field of nanotoxicology was born, giving rise to conference series and specialized journals. Historically,[3] interest in the potential toxicity of very small particles goes back to studies of workers exposed to metal fumes[4] and—after recognizing the nano-sized nature of these fumes—inhalation studies with ultrafine particles.[5−8] With the expected increased intentional (*e.g.*, in the field of medical diagnostics[9−12]) and unintentional (*e.g.*, in occupational settings[13] and chemical waste streams) exposures to NPs, however, nanotoxicology has now become a critical element in safety assessments of nanomaterials. From the viewpoint of journals focused on the nanoscale perspective, such as *ACS Nano*, the question of whether the nanoparticulate state itself contributes to toxicity is most important. Whereas first investigations concerning the toxicity of NPs were based on *in vivo* experiments (*i.e.*, inhalation studies, *etc.*), the ability to design a large variety of different NPs led to a huge body of work based on *in vitro* studies. Due to their presumed lower complexity, *in vitro* studies with well-defined model NPs enable the identification of conceptual models for interactions of NPs with cells. *In vitro* high-dose toxicology and mechanistic studies should be viewed as proof-of-principle studies, though, that ultimately require validation *in vivo*. A major issue that needs to be carefully considered is the relevancy of the doses applied *in vitro* for predicting *in vivo* outcomes. With the necessity of *in vivo* validation kept in mind, several properties of NPs have been demonstrated to change the *in vitro* (and partly also *in vivo*) toxicity of NPs as compared to the bulk state.

First, in comparison with bulk materials, NPs possess a higher surface-to-volume ratio and thus an enhanced contact area with their surroundings than do bulk materials at the same mass. This is particularly true for porous NPs.[14] This could mean that catalytic or other active sites on the particle surface are exposed,[6,15−17] in some cases inducing the formation of reactive oxygen species (ROS). Increased surface area may also foster dissolution of the materials and thus lead to the release of potentially toxic ions, which would be very undesirable in the case of several types of heavy metals such as Cd or Ag.[12,18−22] However, it is well-known that some NPs have high surface energy, which promotes both interparticle agglomeration and adsorption of constituents from the environment, such as proteins.[23−25] NPs have also been demonstrated *in vitro* to themselves alter biological matter at the contact interface, as, for example, in the conformation of proteins.[26,27] All of these events at the NP surface could change the mode of interaction with the environment.[28,29]

Second, due to their small size, NPs are retained in many cells and organs to a larger extent than are larger particles.[30−33] Arguably, effects of a reactive material at the same concentration can be higher at the intracellular level than at the extracellular level[34] due to solubilization or degradation that takes place inside cells.[35] Toxic effects have also been demonstrated to depend on

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Published online October 26, 2010.
10.1021/nn1025687

the uptake mechanism,[36] presumably due to differences in fate, for example, being stored in intracellular vesicles.[37] *In vivo* experiments of NP retention on the order of years have been reported.[38] This opens the door for long-term effects that have to be considered in case of prolonged accumulation and retention in the organism, such as in the spleen and liver.[39]

Third, in comparison with unstructured bulk material, the shape of NPs can play a crucial role in a determining response.[40−43] Geometric effects have been highlighted by the example of needle-shaped carbon nanotubes, which impale entire cells.[44−47]

Although a lot of research has been dedicated to these issues,[47−50] involving new high-throughput assays for toxicological screening and new cell culture models,[22,27,51,52] the general questions of nanotoxicology are far from being resolved. The examples given above should be viewed as snapshots of specialized conditions involving high doses or concentrations. Thus, there is a consequent demand for interlaboratory comparisons, given the different results based on varying methods of outcome measurement, pretest particle preparation, and analyses of results. Interlaboratory and international standardization and harmonization of methods are certainly desirable goals for the future. In the same vein, we again have to point out that the above model on specific interactions between NPs and cells focuses heavily on *in vitro* findings, and extrapolation to *in vivo* is not straightforward. In the following, we thus provide several points that we feel are important for nanotoxicological screening approaches in the future. Pretesting criteria that should be kept in mind include (1) the need for defined and well-characterized NPs as model systems; (2) knowledge of NP properties and potential for exposure during all stages of their life cycle; (3) the need for knowledge about biokinetics; (4) the need for validated *in vitro* models that are predictive of outcomes following *in vivo* exposures; (5) the need for evidence that *in vitro* outcomes are NP-specific *via* appropriate benchmarking (*i.e.*, does a solute produce the same response? Do larger particles of the same composition produce the same response?); and (6) the need for ranking new NPs against well-validated benchmark NPs.

Starting with the NPs themselves: What happens to NPs once they are synthesized? Phenomena concerning NPs at the nanoscale are complex, and the characterization techniques that are available are still limited, as are the descriptions of NP behavior during their full life cycle. Physico-chemical characterization of NPs is paramount in order to correlate biological/toxicological responses with these properties.[53] For example, when investigating size-dependent effects,[54−57] the size distribution of the NPs in relevant biological media needs to be described.[58] Agglomeration of NPs has been demonstrated to have a profound impact on their toxicity *in vitro*.[41] Although standard procedures for the synthesis of NPs now exist, we have to be critical about how precisely the properties of these NPs are defined. First, NPs can be synthesized in different ways (*e.g.*, by laser ablation or wet synthesis), possibly leading to different behavior when exposed to biological media. In fact, although normal aging (*e.g.*, Ostwald ripening, corrosion, aggregation, surface state modification) and physical interactions may already affect NP properties, biomolecules can also substantially modify NPs—and hence their reactivity. NPs must be suspended in biocompatible aqueous culture media to allow interaction with cells in culture, tissue, or organisms. As a result, phenomena of aggregation and agglomeration can occur. Also, depending on the composition and exposure time to the biological environment, NPs can be corroded or dissolve into their constituent atoms, which modifies the chemical composition of the mixture and may affect redox and other process. Finally, unreacted precursors could themselves affect biological responses unless proper purification after synthesis is performed. As previously mentioned, proteins present in biological media can readily associate with the NP surface (protein corona), which changes the features of their interaction with cells.[23,59] Thus, key NP characteristics including, but not limited to, shape, size, number, chemical composition, and surface properties such as charge and coating need to be assessed before use, at the moment of delivery, and during interaction with biological environments. Respective methods should be developed and standardized to facilitate interlaboratory comparisons.

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An important question in nanotoxicology relates to the selection of doses, both for *in vitro* and *in vivo* studies.[60,61] Many studies are driven by a desire to demonstrate an effect and to determine underlying mechanisms, which is most easily achieved with high NP doses. These doses may never be reached under realistic exposure conditions at the organ of entry or in secondary organs, however, considering the expected low translocation rates of NP distribution within and between organ systems (Figure 1).[62] Any NP ad-

ministered at high enough doses will induce "toxicity"; as Paracelsus' rule states, the dose alone defines the poison. *In vivo* extrapolation of results from high-dose *in vitro* studies and the interpretation of results of high-dose *in vivo* studies need to be undertaken with great caution. Using the lung as an example, predictive NP deposition models show that the amount of inhaled NPs depositing per unit surface area of the alveolar region is up to several orders of magnitude lower than the doses that are routinely used in *in vitro* studies with alveolar epithelial cells.[63] Likewise, the relevance of *in vivo* bolus-type instillation studies (nasal, intratracheal) comes into question when researchers use doses that far exceed those achieved under realistic human exposure scenarios. Results of such studies may be considered as proof-of-principle or as hypothesis-forming, but verification by appropriately designed inhalation studies will be necessary. Proper inhalation studies will also inform about the biokinetics of NPs and provide quantification of the amounts that reach secondary organs; these are only very small fractions of the doses depositing in the lung.[64] Such data will be important not only for identifying specific target organs but also for designing *in vitro* studies so that relevant doses can be selected for assessing NP-induced effects and underlying mechanisms.

Which cells or tissues or organisms should be used for screening? Naturally, the choice of model is critical to a more thorough understanding of NP hazards. The serious limitations of *in vitro* studies, including the use of single cell types in culture and the methods of exposing cells, should be considered. Physiological relevance comes into question in the first case, as an *in vivo* scenario in which only a single cell type interacts with the NPs is unrealistic. Not only are organ systems diverse in their cellular makeup, but different cell types often participate in coordinated

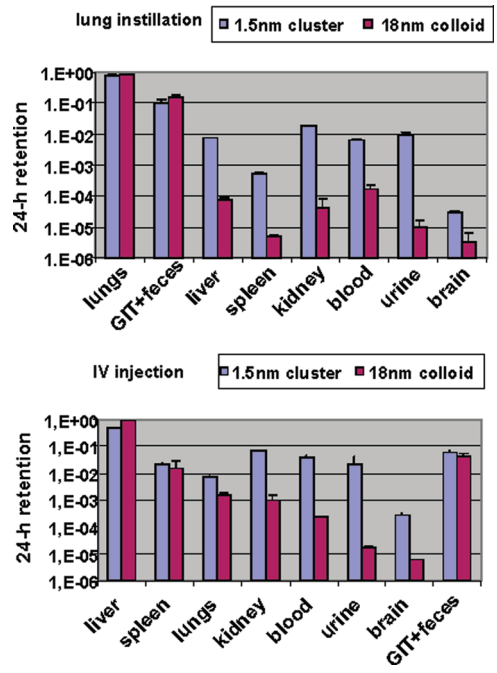

Figure 1. Biodistribution of 18 (red) and 1.4 nm (blue) gold particles 24 h after instillation (% instilled dose/organ). Note the (1) extremely low translocation efficiency of <10% after IV injections and even <1% after intratracheal instillation and (2) the significant differences between small and larger particles. Adopted from Semmler-Behnke *et al.*[62]

responses. Recent work focusing on the lung, for example, has demonstrated how cultured epithelial cells, macrophages, and dendritic cells cooperate in nanoparticle trafficking, and that uptake into the cells is enhanced in coculture in comparison with monoculture.[65,66] Furthermore, the delivery of particles in a bolus of culture medium does not accurately reflect real-world dosimetry, where NPs are likely to accumulate gradually. Thus, *in vitro* studies have to be critically challenged and results cautiously interpreted regarding their biological relevance.[67]

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Nanotoxicology is a relatively new field, and the concepts of dosimetry, dose metrics, exposure assessment, hazard identification, and risk characterization are still being assessed. The fact that we are still far away from the state in which a conclusive overall picture exists makes it an exciting, open area of modern research.

REFERENCES AND NOTES

1. Lewinski, N.; Colvin, V.; Drezek, R. Cytotoxicity of Nanoparticles. *Small* **2008**, *4*, 26–49.
2. Meng, H.; Xia, T.; George, S.; Nel, A. E. A Predictive Toxicological Paradigm for the Safety Assessment of Nanomaterials. *ACS Nano* **2009**, *3*, 1620–1627.
3. Oberdörster, G.; Stone, V.; Donaldson, K. Toxicology of Nanoparticles: A Historical Perspective. *Nanotoxicology* **2007**, *1*, 2–25.
4. Drinker, P.; Thomson, R. M.; Finn, J. L. Metal Fume Fever: II. Resistance Acquired by Inhalation of Zinc Oxide on Two Successive Days. *J. Ind. Hyg.* **1927**, *9*, 98–105.

5. Heinrich, U.; Fuhst, R.; Rittinghausen, S.; Creutzenberg, O.; Bellmann, B.; Koch, W.; Levsen, K. Chronic Inhalation Exposure of Wistar Rats and 2 Different Strains of Mice to Diesel-Engine Exhaust, Carbon-Black, and Titanium-Dioxide. *Inhal. Toxicol.* **1995**, *7*, 533–556.
6. Donaldson, K.; Beswick, P. H.; Gilmour, P. S. Free Radical Activity Associated with the Surface of Particles: A Unifying Factor in Determining Biological Activity. *Toxicol. Lett.* **1996**, *88*, 293–298.
7. Donaldson, K.; Li, X. Y.; MacNee, W. Ultrafine (Nanometre) Particle Mediated Lung Injury. *J. Aerosol Sci.* **1998**, *29*, 553–560.
8. Oberdörster, G.; Ferin, J.; Soderholm, S.; Gelein, R.; Cox, C.; Baggs, R.; Morrow, P. E. Increased Pulmonary Toxicity of Inhaled Ultrafine Particles: Due to Lung Overload Alone? *Ann. Occup. Hyg.* **1994**, *38*, 295–302.
9. McCarthy, J.; Weissleder, R. Multifunctional Magnetic Nanoparticles for Targeted Imaging and Therapy. *Adv. Drug Delivery Rev.* **2008**, *60*, 1241–1251.
10. Bruns, O. T.; Ittrich, H.; Peldschus, K.; Kaul, M. G.; Tromsdorf, U. I.; Lauterwasser, J.; Nikolic, M. S.; Mollwitz, B.; Merkel, M.; Bigall, N. C.; *et al.* Real-Time Magnetic Resonance Imaging and Quantification of Lipoprotein Metabolism *In Vivo* Using Nanocrystals. *Nat. Nanotechnol.* **2009**, *4*, 193–201.
11. Kim, S.; Lim, Y. T.; Soltesz, E. G.; De Grand, A. M.; Lee, J.; Nakayama, A.; Parker, J. A.; Mihaljevic, T.; Laurence, R. G.; Dor, D. M.; *et al.* Near-Infrared Fluorescent Type II Quantum Dots for Sentinel Lymph Node Mapping. *Nat. Biotechnol.* **2004**, *22*, 93–97.
12. Pons, T.; Pic, E.; Lequeux, N.; Cassette, E.; Bezdetnaya, L.; Guillemin, F.; Marchal, F.; Dubertret, B. Cadmium-Free $CuInS_2$/ZnS Quantum Dots for Sentinel Lymph Node Imaging with Reduced Toxicity. *ACS Nano* **2010**, *4*, 2531–2538.
13. Lee, J.; Mahendra, S.; Alvarez, P. J. J. Nanomaterials in the Construction Industry: A Review of Their Applications and Environmental Health and Safety Considerations. *ACS Nano* **2010**, *4*, 3580–3590.
14. Maurer-Jones, M. A.; Lin, Y. S.; Haynes, C. L. Functional Assessment of Metal Oxide Nanoparticle Toxicity in Immune Cells. *ACS Nano* **2010**, *4*, 3363–3373.
15. Barnard, A. S.; Xu, H. F. An Environmentally Sensitive Phase Map of Titania Nanocrystals. *ACS Nano* **2008**, *2*, 2237–2242.
16. Colvin, V. L.; Kulinowski, K. M. Nanoparticles as Catalysts for Protein Fibrillation. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 8679–8680.
17. Xia, T.; Kovochich, M.; Liong, M.; Madler, L.; Gilbert, B.; Shi, H.; Yeh, J. I.; Zink, J. I.; Nel, A. E. Comparison of the Mechanism of Toxicity of Zinc Oxide and Cerium Oxide Nanoparticles Based on Dissolution and Oxidative Stress Properties. *ACS Nano* **2008**, *2*, 2121–2134.
18. Derfus, A. M.; Chan, W. C. W.; Bhatia, S. N. Probing the Cytotoxicity of Semiconductor Quantum Dots. *Nano Lett.* **2004**, *4*, 11–18.
19. Kirchner, C.; Liedl, T.; Kudera, S.; Pellegrino, T.; Javier, A. M.; Gaub, H. E.; Stölzle, S.; Fertig, N.; Parak, W. J. Cytotoxicity of Colloidal CdSe and CdSe/ZnS Nanoparticles. *Nano Lett.* **2005**, *5*, 331–338.
20. Kittler, S.; Greulich, C.; Diendorf, J.; Köller, M.; Epple, M. Toxicity of Silver Nanoparticles Increases during Storage Because of Slow Dissolution under Release of Silver Ions. *Chem. Mater.* **2010**, *22*, 4548–4554.
21. AshaRani, P. V.; Low Kah Mun, G.; Hande, M. P.; Valiyaveettil, S. Cytotoxicity and Genotoxicity of Silver Nanoparticles in Human Cells. *ACS Nano* **2009**, *3*, 279–290.
22. George, S.; Pokhrel, S.; Xia, T.; Gilbert, B.; Ji, Z.; Schowalter, M.; Rosenauer, A.; Damoiseaux, R.; Bradley, K. A.; Madler, L.; *et al.* Use of a Rapid Cytotoxicity Screening Approach To Engineer a Safer Zinc Oxide Nanoparticle through Iron Doping. *ACS Nano* **2010**, *4*, 15–29.
23. Walczyk, D.; Bombelli, F. B.; Monopoli, M. P.; Lynch, I.; Dawson, K. A. What the Cell "Sees" in Bionanoscience. *J. Am. Chem. Soc.* **2010**, *132*, 5761–5768.
24. Röcker, C.; Pötzl, M.; Zhang, F.; Parak, W. J.; Nienhaus, G. U. A Quantitative Fluorescence Study of Protein Monolayer Formation on Colloidal Nanoparticles. *Nat. Nanotechnol.* **2009**, *4*, 577–580.
25. Lacerda, S. H.; Park, J. J.; Meuse, C.; Pristinski, D.; Becker, M. L.; Karim, A.; Douglas, J. F. Interaction of Gold Nanoparticles with Common Human Blood Proteins. *ACS Nano* **2010**, *4*, 365–379.
26. Bastus, N. G.; Sanchez-Tillo, E.; Pujals, S.; Farrera, C.; Lopez, C.; Giralt, E.; Celada, A.; Lloberas, J.; Puntes, V. Homogeneous Conjugation of Peptides onto Gold Nanoparticles Enhances Macrophage Response. *ACS Nano* **2009**, *3*, 1335–1344.
27. Tarantola, M.; Schneider, D.; Sunnick, E.; Adam, H.; Pierrat, S.; Rosman, C.; Breus, V.; Sonnichsen, C.; Basche, T.; Wegener, J.; *et al.* Cytotoxicity of Metal and Semiconductor Nanoparticles Indicated by Cellular Micromotility. *ACS Nano* **2009**, *3*, 213–222.
28. Calvaresi, M.; Zerbetto, F. Baiting Proteins with C60. *ACS Nano* **2010**, *4*, 2283–2299.
29. Linse, S.; Cabaleiro-Lago, C.; Xue, W.-F.; Lynch, I.; Lindman, S.; Thulin, E.; Radford, S. E.; Dawson, K. A. Nucleation of Protein Fibrillation by Nanoparticles. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 8691–8696.
30. Nativo, P.; Prior, I. A.; Brust, M. Uptake and Intracellular Fate of Surface-Modified Gold Nanoparticles. *ACS Nano* **2008**, *2*, 1639–1644.
31. Hess, H.; Tseng, Y. Active Intracellular Transport of Nanoparticles: Opportunity or Threat. *ACS Nano* **2007**, *1*, 390–392.
32. Orr, G.; Panther, D. J.; Phillips, J. L.; Tarasevich, B. J.; Dohnalkova, A.; Hu, D.; Teeguarden, J. G.; Pounds, J. G. Submicrometer and Nanoscale Inorganic Particles Exploit the Actin Machinery to be Propelled Along Microvilli-like Structures into Alveolar Cells. *ACS Nano* **2007**, *1*, 463–475.
33. Porter, A. E.; Gass, M.; Bendall, J. S.; Muller, K.; Goode, A.; Skepper, J. N.; Midgley, P. A.; Welland, M. Uptake of Noncytotoxic Acid-Treated Single-Walled Carbon Nanotubes into the Cytoplasm of Human Macrophage Cells. *ACS Nano* **2009**, *3*, 1485–1492.
34. Chang, E.; Thekkek, N.; Yu, W. W.; Colvin, V. L.; Drezek, R. Evaluation of Quantum Dot Cytotoxicity Based on Intracellular Uptake. *Small* **2006**, *2*, 1412–1417.
35. Thubagere, A.; Reinhard, B. M. Nanoparticle-Induced Apoptosis Propagates through Hydrogen-Peroxide-Mediated Bystander Killing: Insights from a Human Intestinal Epithelium *In Vitro* Model. *ACS Nano* **2010**, *4*, 3611–3622.
36. Xia, T.; Kovochich, M.; Liong, M.; Zink, J. I.; Nel, A. E. Cationic Polystyrene Nanosphere Toxicity Depends on Cell-Specific Endocytic and Mitochondrial Injury Pathways. *ACS Nano* **2008**, *2*, 85–96.
37. Parak, W. J.; Boudreau, R.; Gros, M. L.; Gerion, D.; Zanchet, D.; Micheel, C. M.; Williams, S. C.; Alivisatos, A. P.; Larabell, C. A. Cell Motility and Metastatic Potential Studies Based on Quantum Dot Imaging of Phagokinetic Tracks. *Adv. Mater.* **2002**, *14*, 882–885.
38. Ballou, B.; Lagerholm, B. C.; Ernst, L. A.; Bruchez, M. P.; Waggoner, A. S. Noninvasive Imaging of Quantum Dots in Mice. *Bioconjugate Chem.* **2004**, *15*, 79–86.
39. Casals, E.; Vazquez-Campos, S.; Bastus, N. G.; Puntes, V. Distribution and Potential Toxicity of Engineered Inorganic Nanoparticles and Carbon Nanostructures in Biological Systems. *TrAC, Trend Anal. Chem.* **2008**, *27*, 672–683.
40. Albanese, A.; Sykes, E. A.; Chan,

W. C. Rough around the Edges: The Inflammatory Response of Microglial Cells to Spiky Nanoparticles. *ACS Nano* **2010**, *4*, 2490–2493.
41. Liu, S.; Wei, L.; Hao, L.; Fang, N.; Chang, M. W.; Xu, R.; Yang, Y.; Chen, Y. Sharper and Faster "Nano Darts" Kill More Bacteria: A Study of Antibacterial Activity of Individually Dispersed Pristine Single-Walled Carbon Nanotube. *ACS Nano* **2009**, *3*, 3891–3902.
42. Hutter, E.; Boridy, S.; Labrecque, S.; Lalancette-Hebert, M.; Kriz, J.; Winnik, F. M.; Maysinger, D. Microglial Response to Gold Nanoparticles. *ACS Nano* **2010**, *4*, 2595–2606.
43. Zhang, Y.; Ali, S. F.; Dervishi, E.; Xu, Y.; Li, Z.; Casciano, D.; Biris, A. S. Cytotoxicity Effects of Graphene and Single-Wall Carbon Nanotubes in Neural Phaeochromocytoma-Derived PC12 Cells. *ACS Nano* **2010**, *4*, 3181–3186.
44. Donaldson, K.; Aitken, R.; Tran, L.; Stone, V.; Duffin, R.; Forrest, G.; Alexander, A. Carbon Nanotubes: A Review of Their Properties in Relation to Pulmonary Toxicology and Workplace Safety. *Toxicol. Sci.* **2006**, *92*, 5–22.
45. Poland, C. A.; Duffin, R.; Kinloch, I.; Maynard, A.; Wallace, W. A. H.; Seaton, A.; Stone, V.; Brown, S.; MacNee, W.; Donaldson, K. Carbon Nanotubes Introduced into the Abdominal Cavity of Mice Show Asbestos-like Pathogenicity in a Pilot Study. *Nat. Nanotechnol.* **2008**, *3*, 423–428.
46. Warheit, D. B.; Laurence, B. R.; Reed, K. L.; Roach, D. H.; Reynolds, G. A. M.; Webb, T. R. Comparative Pulmonary Toxicity Assessment of Single-Wall Carbon Nanotubes in Rats. *Toxicol. Sci.* **2004**, *77*, 117–125.
47. Miyawaki, J.; Yudasaka, M.; Azami, T.; Kubo, Y.; Iijima, S. Toxicity of Single-Walled Carbon Nanohorns. *ACS Nano* **2008**, *2*, 213–226.
48. Magrez, A.; Horvath, L.; Smajda, R.; Salicio, V.; Pasquier, N.; Forro, L.; Schwaller, B. Cellular Toxicity of TiO_2-Based Nanofilaments. *ACS Nano* **2009**, *3*, 2274–2280.
49. Schanen, B. C.; Karakoti, A. S.; Seal, S.; Drake, D. R., III; Warren, W. L.; Self, W. T. Exposure to Titanium Dioxide Nanomaterials Provokes Inflammation of an *In Vitro* Human Immune Construct. *ACS Nano* **2009**, *3*, 2523–2532.
50. Wang, Y.; Seebald, J. L.; Szeto, D. P.; Irudayaraj, J. Biocompatibility and Biodistribution of Surface-Enhanced Raman Scattering Nanoprobes in Zebrafish Embryos: *In Vivo* and Multiplex Imaging. *ACS Nano* **2010**, *4*, 4039–4053.
51. Jan, E.; Byrne, S. J.; Cuddihy, M.; Davies, A. M.; Volkov, Y.; Gun'ko, Y. K.; Kotov, N. A. High-Content Screening as a Universal Tool for Fingerprinting of Cytotoxicity of Nanoparticles. *ACS Nano* **2008**, *2*, 928–938.
52. Braydich-Stolle, L. K.; Speshock, J. L.; Castle, A.; Smith, M.; Murdock, R. C.; Hussain, S. M. Nanosized Aluminum Altered Immune Function. *ACS Nano* **2010**, *4*, 3661–3670.
53. Warheit, D. B. How Meaningful are the Results of Nanotoxicity Studies in the Absence of Adequate Material Characterization. *Toxicol. Sci.* **2008**, *101*, 183–185.
54. Chithrani, B. D.; Chan, W. C. W. Elucidating the Mechanism of Cellular Uptake and Removal of Protein-Coated Gold Nanoparticles of Different Sizes and Shapes. *Nano Lett.* **2007**, *7*, 1542–1550.
55. Jamison, J. A.; Krueger, K. M.; Yavuz, C. T.; Mayo, J. T.; LeCrone, D.; Redden, J. J.; Colvin, V. L. Size-Dependent Sedimentation Properties of Nanocrystals. *ACS Nano* **2008**, *2*, 311–319.
56. Liang, M.; Lin, I. C.; Whittaker, M. R.; Minchin, R. F.; Monteiro, M. J.; Toth, I. Cellular Uptake of Densely Packed Polymer Coatings on Gold Nanoparticles. *ACS Nano* **2010**, *4*, 403–413.
57. Jin, H.; Heller, D. A.; Sharma, R.; Strano, M. S. Size-Dependent Cellular Uptake and Expulsion of Single-Walled Carbon Nanotubes: Single Particle Tracking and a Generic Uptake Model for Nanoparticles. *ACS Nano* **2009**, *3*, 149–158.
58. Heister, E.; Lamprecht, C.; Neves, V.; Tilmaciu, C.; Datas, L.; Flahaut, E.; Soula, B.; Hinterdorfer, P.; Coley, H. M.; Silva, S. R.; *et al.* Higher Dispersion Efficacy of Functionalized Carbon Nanotubes in Chemical and Biological Environments. *ACS Nano* **2010**, *4*, 2615–2626.
59. Casals, E.; Pfaller, T.; Duschl, A.; Oostingh, G. J.; Puntes, V. Time Evolution of the Nanoparticle Protein Corona. *ACS Nano* **2010**, *4*, 3623–3632.
60. Oberdörster, G.; Oberdörster, E.; Oberdörster, J. Nanotoxicology: An Emerging Discipline Evolving from Studies of Ultrafine Particles. *Environ. Health Persp.* **2005**, *113*, 823–839.
61. Kolosnjaj-Tabi, J.; Hartman, K. B.; Boudjemaa, S.; Ananta, J. S.; Morgant, G.; Szwarc, H.; Wilson, L. J.; Moussa, F. *In Vivo* Behavior of Large Doses of Ultrashort and Full-Length Single-Walled Carbon Nanotubes after Oral and Intraperitoneal Administration to Swiss Mice. *ACS Nano* **2010**, *4*, 1481–1492.
62. Semmler-Behnke, M.; Kreyling, W. G.; Lipka, J.; Fertsch, S.; Wenk, A.; Takenaka, S.; Schmid, G.; Brandau, W. Biodistribution of 1.4- and 18-nm Gold Particles in Rats. *Small* **2008**, *4*, 2108–2111.
63. Teeguarden, J. G.; Hinderliter, P. M.; Orr, G.; Thrall, B. D.; Pounds, J. G. Particokinetics *In Vitro*: Dosimetry Considerations for *In Vitro* Nanoparticle Toxicity Assessments. *Toxicol. Sci.* **2007**, *95*, 300–312.
64. Kreyling, W. G.; Semmler, M.; Erbe, F.; Mayer, P.; Takenaka, S.; Schulz, H.; Oberdörster, G.; Ziesenis, A. Translocation of Ultrafine Insoluble Iridium Particles from Lung Epithelium to Extrapulmonary Organs is Size Dependent but Very Low. *J. Toxicol. Environ. Health A* **2002**, *65*, 1513–1530.
65. Blank, F.; Wehrli, M.; Lehmann, A.; Baum, O.; Gehr, P.; von Garnier, C.; Rothen-Rutishauser, B. M. Macrophages and Dendritic Cells Express Tight Junction Proteins and Exchange Particles in an *In Vitro* Model of the Human Airway Wall. *Immunobiology* **2010**, in press.
66. Rothen-Rutishauser, B.; Mühlfeld, C.; Blank, F.; Musso, C.; Gehr, P. Translocation of Particles and Inflammatory Responses after Exposure to Fine Particles and Nanoparticles in an Epithelial Airway Model. *Part. Fibre Toxicol.* **2007**, *4*, 9.
67. Jones, C. F.; Grainger, D. W. *In Vitro* Assessments of Nanomaterial Toxicity. *Adv. Drug Delivery Rev.* **2009**, *61*, 438–456.