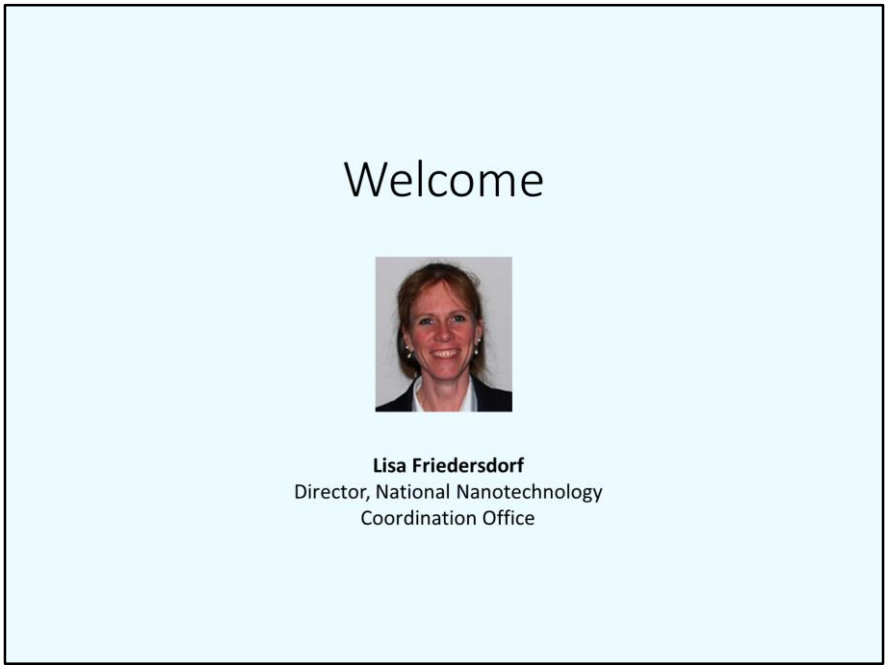
Key Takeaways: Nanotechnology is poised to revolutionize many technologies, including medicine.

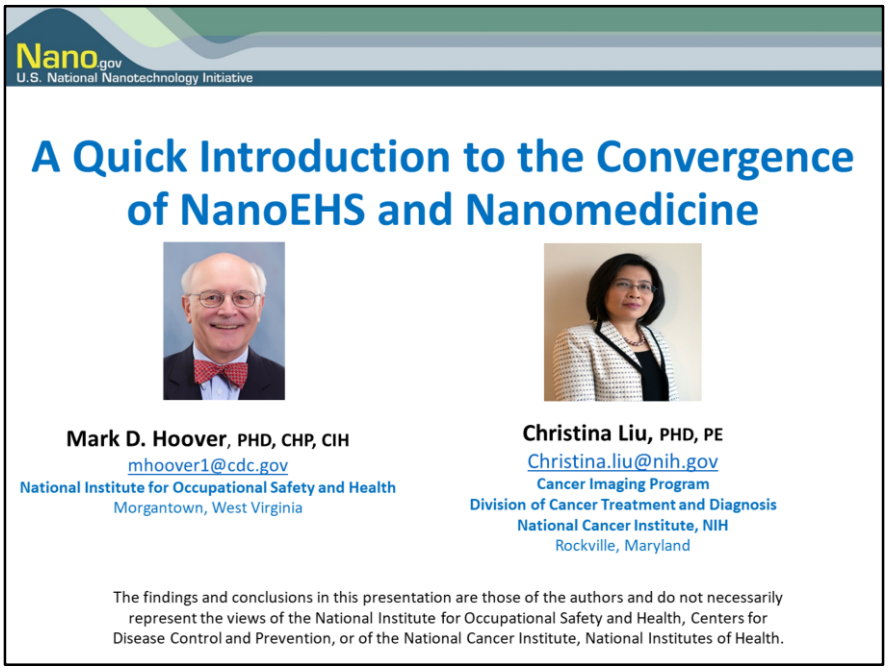
- As medical applications of nanotechnology often involve intentional exposure of engineered nanomaterials (ENMs) at relatively high doses, there are untapped synergies between nanotechnology-enabled medicine (nanomedicine) and research on the environmental, health, and safety effects of ENMs (nanoEHS).
- The proper experimental design and detailed ENM characterization are integral to the robust assessment of potential exposure and risk for both nanomedicine and nanomaterial-enabled products in the environment.
- While both communities have individually come to many similar conclusions, more work is need in areas such as understanding the effects of low-dose, chronic exposure to ENMs. There exists great opportunity to leverage efforts across both fields.



>> LISA FRIEDERSDORF: Good afternoon. My name is Lisa Friedersdorf. I am the director of the NNCO. Welcome to the webinar. This afternoon the speakers will explore the synergies between the research focused on nano environmental health and safety and nanomedicine, and how to leverage the advancements in each of these fields.

Before I turn it over to the moderators, I wanted to make sure you were aware of two upcoming workshops focused on NanoEHS, nanotechnology-related environmental, health, and safety issues, during the second week of October. The first event is the Quantifying Exposures to Engineered Nanomaterials, or QEEN II, and the second meeting is the U.S.-EU nanoEHS communities of research meeting. Both meetings will be held in D.C. this year. We would also like to make sure that you're aware that we are in the process of pulling together a community of research in nanomedicine. Please keep your eye on nano.gov and follow us on Twitter for more information.

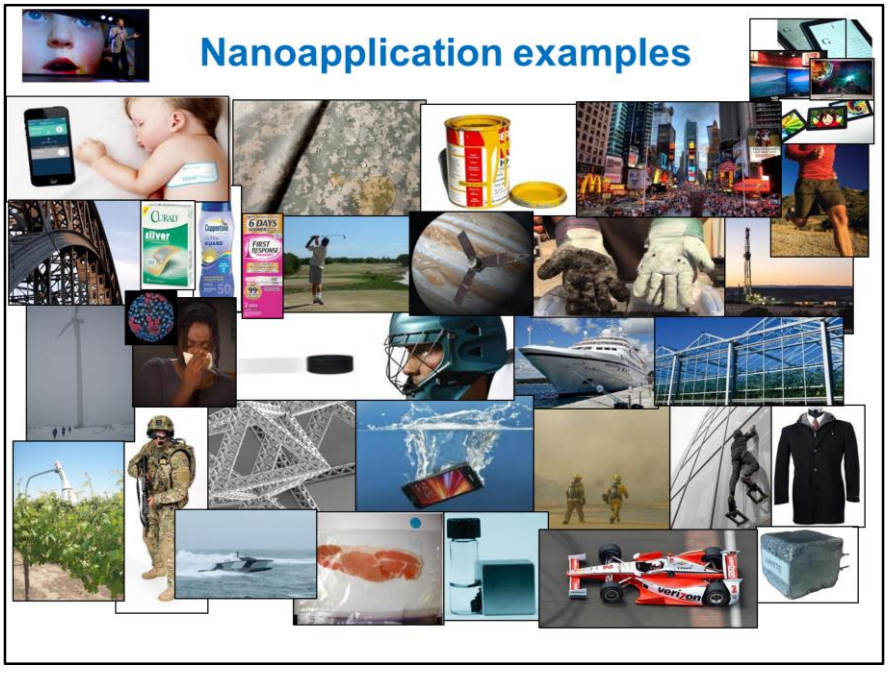
And with that brief announcement, I would like to turn it over to the moderators, Mark and Christina. Thank you very much for your time this afternoon, and I'll let you take it away.



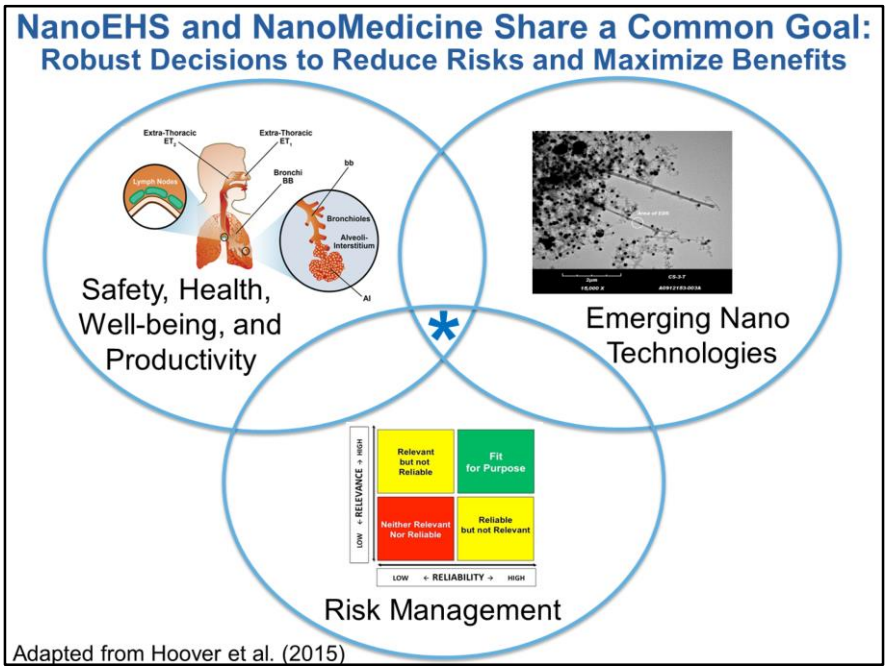
>> MARK HOOVER: Hello, everyone, I'm Mark Hoover.

>> CHRISTINA LIU: Hi, this is Christina Liu. We are very happy to have three very wonderful panelists: Dr. Hamid Ghandehari from the University of Utah, Dr. Christine Payne from Duke University, and Dr. Monika Mortimer from the Bren School of Environmental Science and Management, University of California, Santa Barbara.

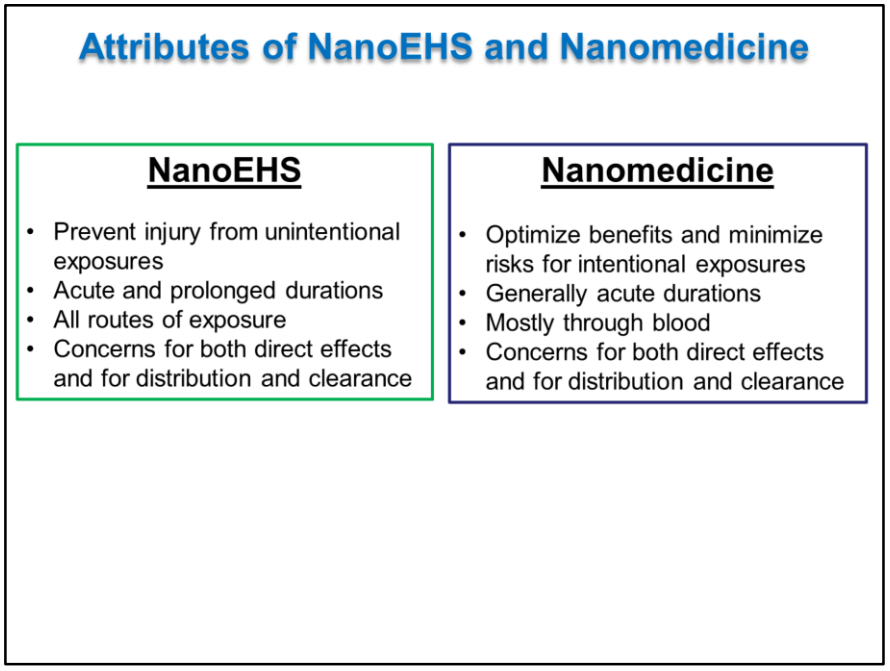
Before the panel presentation, we have a couple slides from the moderators.



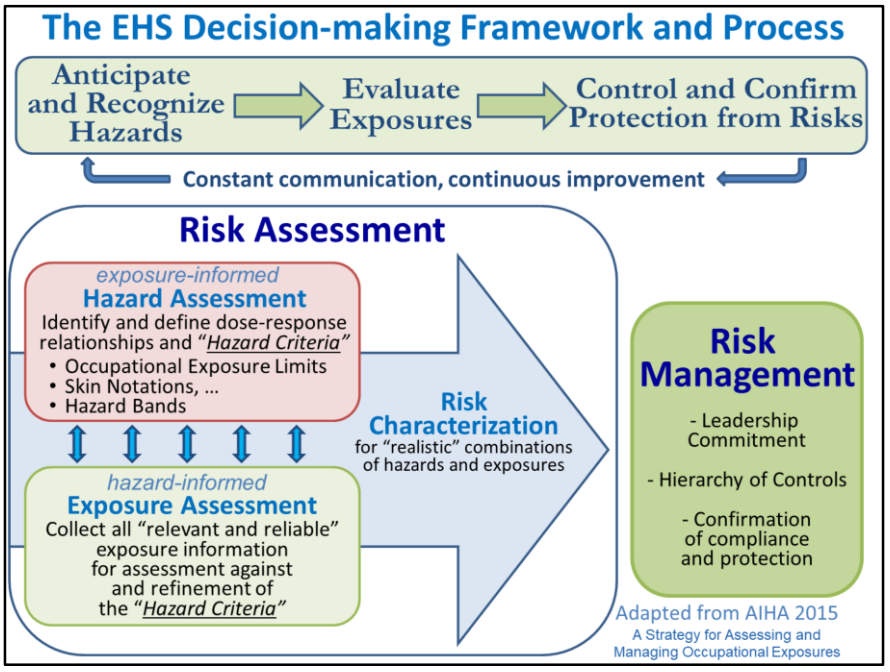
>> MARK HOOVER: So nano applications are everywhere, and as you can see from this collage of medicine, coatings, vehicles, smartphones, it's amazing how much nanotechnology is there, both with safety questions and in applications where we want to know more about how materials behave so we can make them more effective in applications.



>> MARK HOOVER: NanoEHS and nanomedicine share a common goal—robust decisions to reduce risks and maximize benefits.

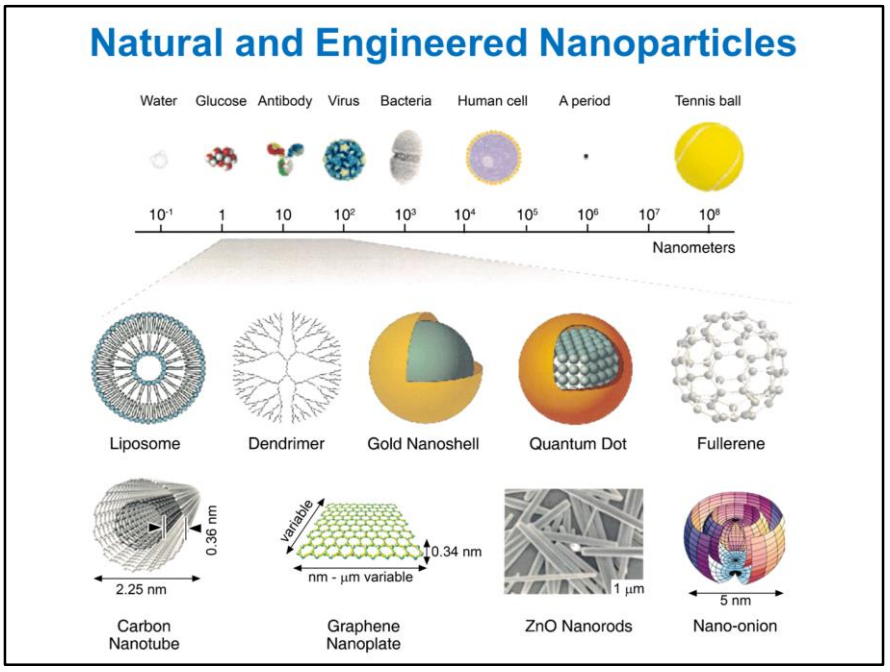


>> MARK HOOVER: And in the next slide, we listed briefly some of the similarities in the areas between health, safety, and well-being. We're seeing the overlap of nanoEHS and nanomedicine, and this is at the convergence of trying to make things safe, trying to apply things, and trying to manage risks- whether we're doing nanoEHS, where we're making sure that things are done safely or nanomedicine, where we want to make sure things are done effectively and safely.



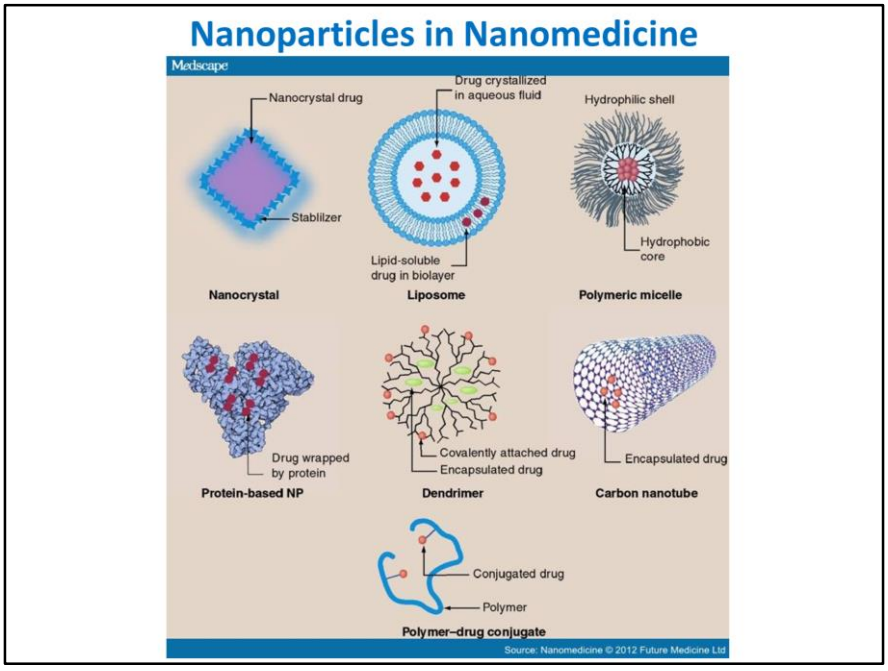
>> MARK HOOVER: And then on this slide, from the point of view of those of us who work in environmental safety and health, we have this figure, which brings together what we call the industrial hygiene occupational health decision making framework. It is designed to anticipate and recognize the hazards, evaluate the exposures, and control and confirm protection from risks. If we're doing this in nanomedicine, you have the same thought process. Anticipate and recognize the hazards, as well as the benefits, evaluate the exposures to the patient and to the staff that may be doing the medical application, and then control and confirm protection from unwanted risks and insure the desired outcomes.

Then we talk about hazard-informed exposure assessment and exposure-informed hazard assessment. So how much would people be exposed to? That would help us do a hazard assessment, and at the same time, a hazard- informed exposure assessment. We would want to know the complete composition of a nano-formulated material so that we could do a measurement for it, and then characterize the risk, and then manage that risk. We think that leadership commitment to doing this is especially important.



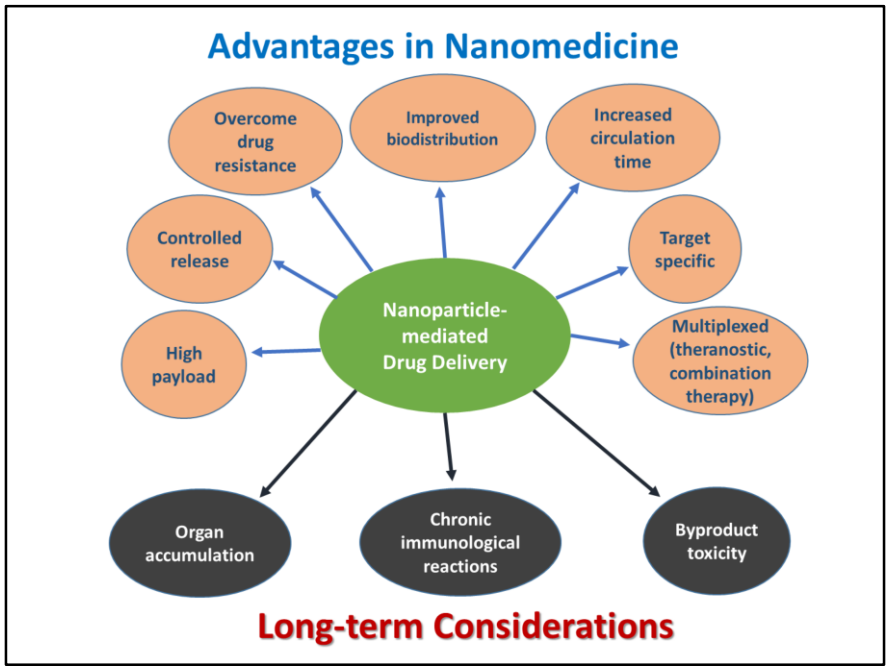
>> MARK HOOVER: In this slide, I'll turn it over to Christina. There's just this whole myriad of natural and engineered particles.

>> CHRISTINA LIU: You can see a beautiful description of all sorts of nanoparticles, which are natural and engineered. They vary in sizes on the nanometer scale.



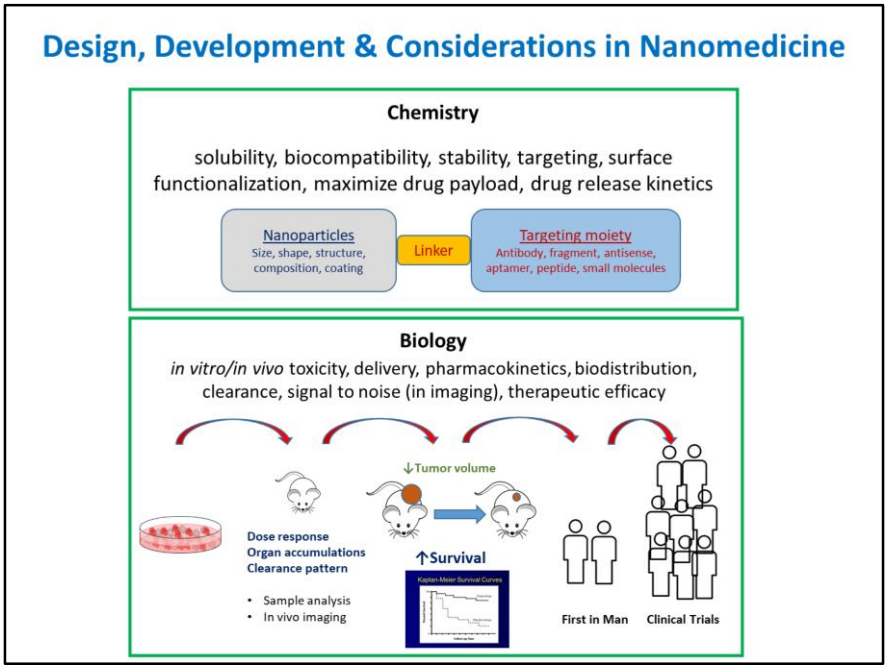
>> CHRISTINA LIU: Nanoparticles have many unique properties, which can make them appealing in the design and development of nanomedicine. This slide shows popular nanoparticles that are used to carry therapeutic agents to targeted disease sites.

Doxil and Abraxane are the only nanoparticle-based drug delivery vehicle which have been approved by the FDA to treat cancer, originally for breast cancer, but now they are used in conjunction with other chemotherapeutic drugs to treat other cancers. For your information, Doxil is a liposome based drug carrier, whereas Abraxane is an albumin-based drug carrier.



>> CHRISTINA LIU: On this slide, nanoparticle-mediated drug delivery has several advantages because of the flexibility in their design and manipulations. These advantages include increasing blood circulation time, carrying high doses of chemotherapeutic drugs to specific cancer sites, and controlled release under certain conditions. These properties can minimize the side effects and toxicity caused by the conventional small- molecule chemotheraputics.

As we are excited about the current progress in cancer nanomedicine, during or immediately after treatment, we tend to neglect the long-term effect of these nanocarriers in the body if they are not biodegradable.



>> CHRISTINA LIU: This slide briefly describes what is involved in nanomedicine design and development. On the chemistry side, one needs to take into account several factors associated with their physicochemical properties. Also, this nanoparticle can be linked to a targeting moiety, which is often biologically based. The safety of all three components should therefore be considered in developing nanomedicine.

On the biological side, this nano construct could be evaluated in a biological system, beginning with the study in cell or tissue culture, followed by animal studies through sample analysis or *in vivo* imaging tools.

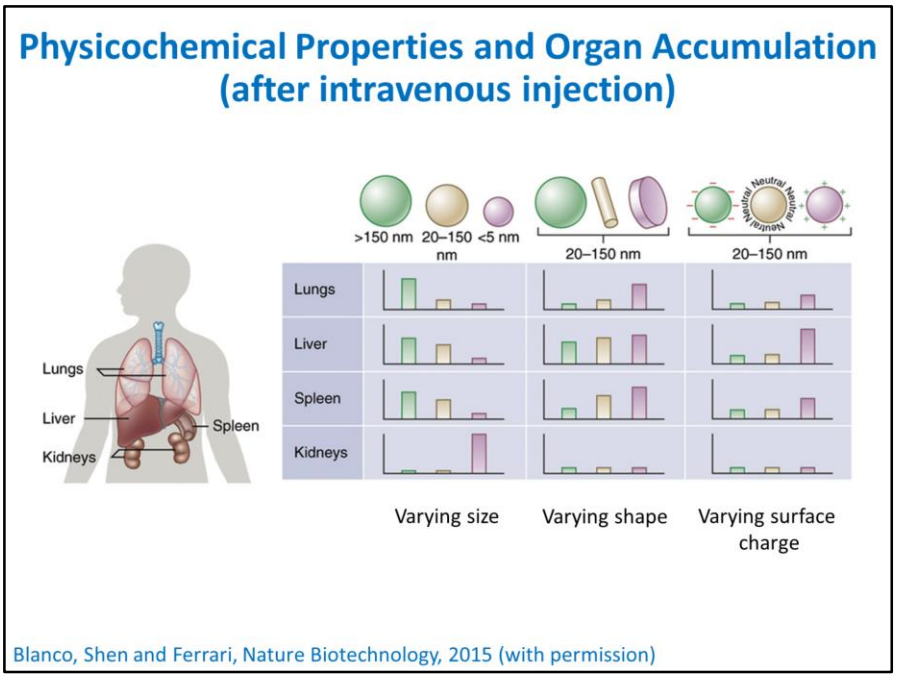
Later on, animal studies could include cancer models to evaluate the efficacy based on the criteria's such as reducing tumor volume as well as improving the survival rates. With sufficient safety efficacy data, the first human study could be conducted on healthy human subjects then moved to clinical trials.

It is worth noting that many of these tests are iterative; many may not succeed and could take years to bring to the clinic.

Delivery Routes for Nanomedicine

Intratumoral,
Intravenous,
Intraperitoneal,
Intracranial,
Intrathecal,
Transdermal,
Nasal,
Oral,
Ophthalmic,
Topical,
Implantable

>> CHRISTINA LIU: This slide lists the different delivery routes for nanomedicine: Direct injection into tumors, through the blood, lymphatics, skin, nose, mouth, eyes, or even through implantable devices .



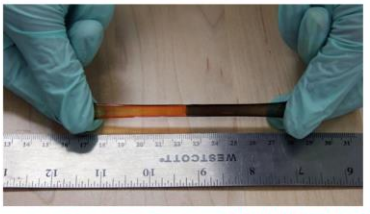
>> CHRISTINA LIU: As Mark mentioned before, cancer patients are subjected to acute, maybe multiple, and intentional delivery of nanomedicine at relatively high doses—compared to environmental exposure, which often involves lower doses but prolonged exposures. It's possible that the body can respond differently to these nanoparticles just by the way the body is exposed. Therefore, we cannot ignore the body's interaction and the response to the delivery carrier.

This slide summarizes the importance of the physicochemical properties in organ accumulation after intravenous injection of nanomedicine, which could have a long term effects if these particles remain in the body after they have done their job.

Other Nano Applications for Medicine

Will Nanotechnology Allow You to "Swallow the Doctor?"
http://edition.cnn.com/2015/01/29/tech/mci-nanobots-eth/index.html

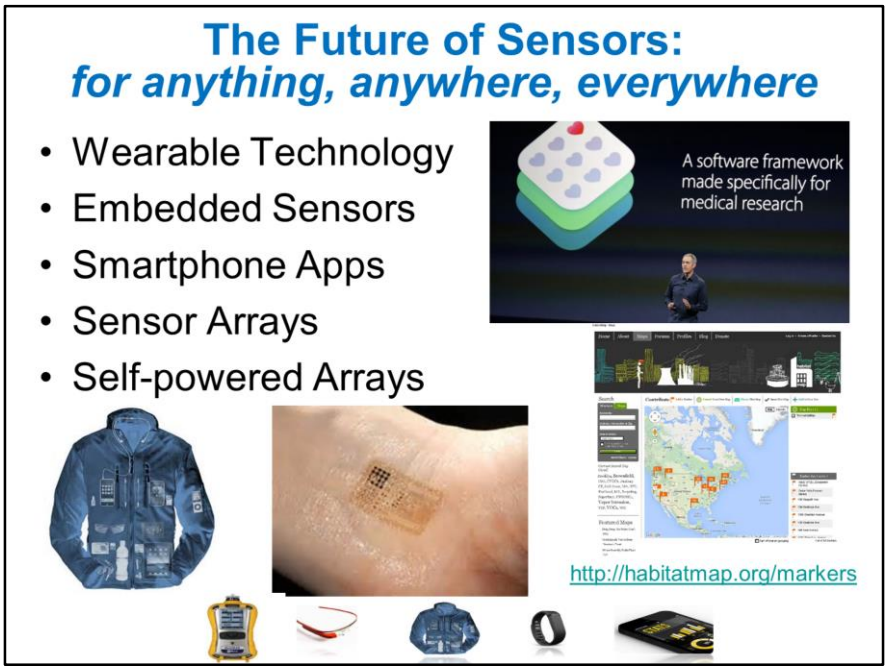
Nano-tattoo brain computer interface



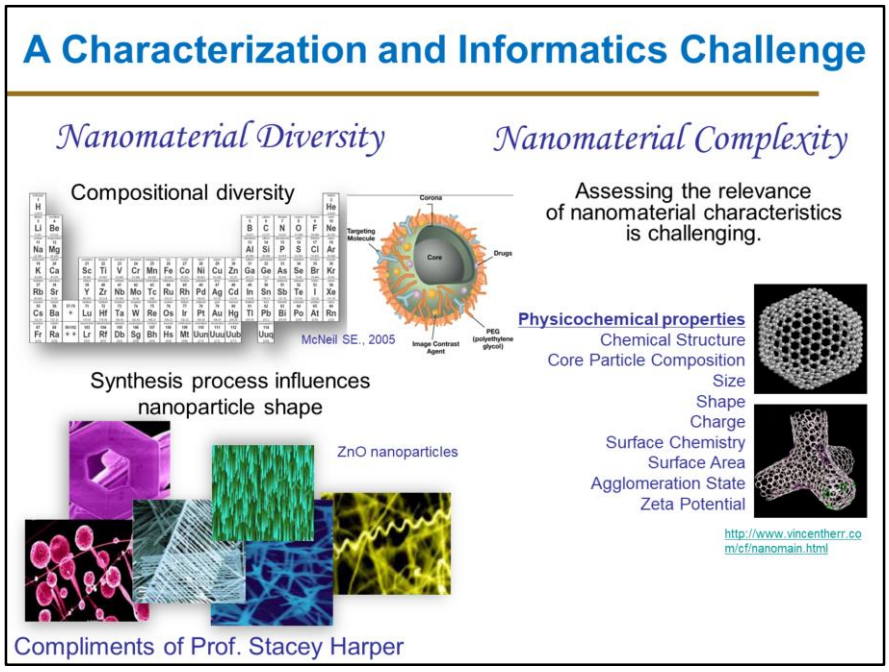
www.sciencemag.org/news/2016/04/artificial-muscle-can-heal-itself

http://ideastations.org/science-matters/hot-shots/hot-jobs/hot-jobs-biomechanical-engineering-nano-tattoo

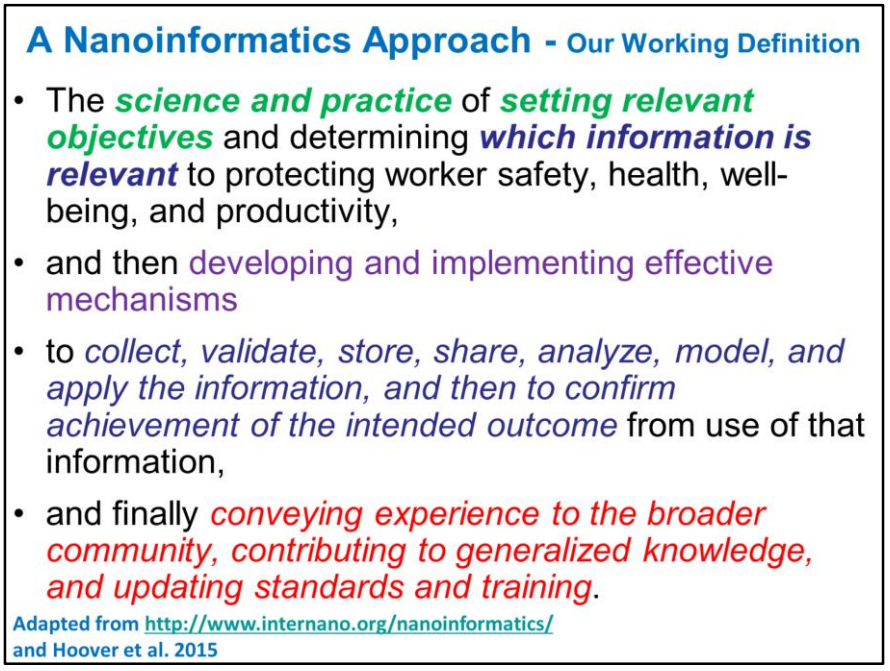
>> CHRISTINA LIU: This slide shows other nanotechnology applications in medicine. In the future, we might be able to swallow a nanodevice that can detect or diagnose disease. We might be able to implant artificial muscle that can heal itself, or get nano-tattoos that can interface with our brain activities.



>> CHRISTINA LIU: This slide shows the future of nanoparticle- or nanodevices-based sensors, which can continuously monitor an individual's health status.

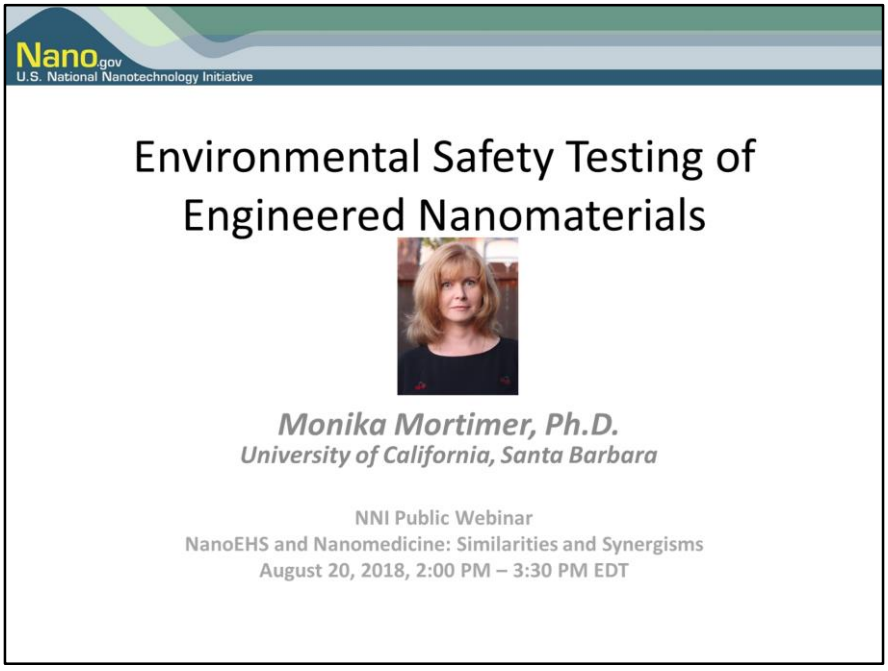


>> MARK HOOVER: One of the big challenges that we face—both in protecting people, workers, and the public and environment—and developing new medical applications of nanotechnology—is that it can be done with the entire periodic table and it can be done in an incredible array of different shapes and morphologies. The challenge for us is to understand the differences, and whether we can categorize things to say "this group behaves in a certain way" or not. In our next slide we have our definition of informatics.



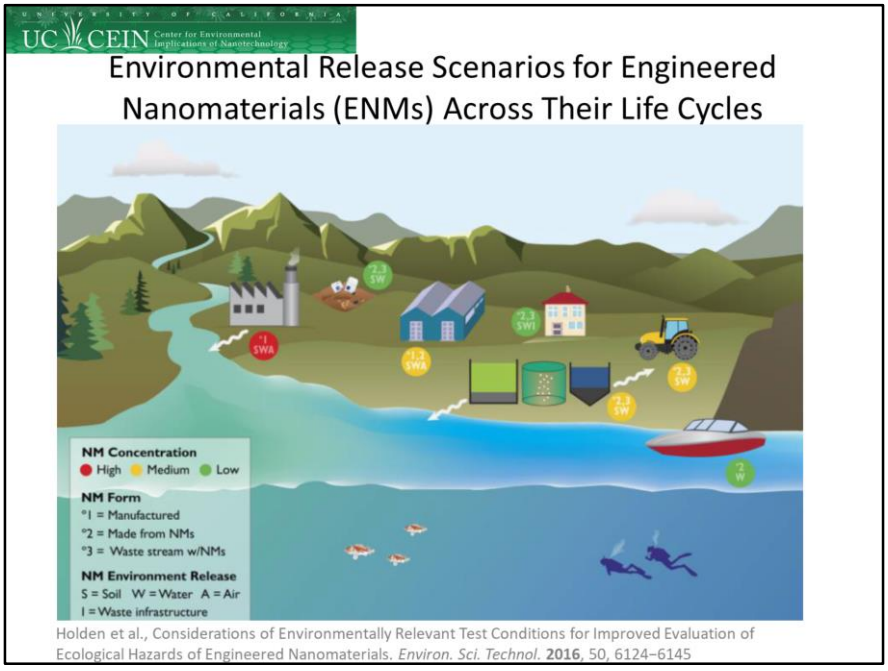
>> MARK HOOVER: This slide shows a working definition for our solution to this characterization and informatics challenge. We've worked on this definition for a very long time, refining it to have a number of steps. The science and practice of nanoinformatics begins with the setting of relevant objectives—that's the key part. What do you want to accomplish? Setting the relevant objectives and then determining which information is relevant to protecting workers' safety, health, wellbeing, & productivity, or in the case of medicine, the patient and the staff—and then developing and implementing effective mechanisms to collect, validate, store, share, analyze, model, and apply the information, and then to confirm the achievement of the intended outcome for the use of that information, and finally conveying the experience to the broader community, contributing to generalized knowledge and updating standards and training.

So part of what we're doing today is conveying experience to you, the broader community, and we hope there will be more in our series on the interface of nanomedicine and nanoEHS because it is an opportunity for us to learn. And as Christina noted, patients are often the ones that get very high exposures, and workers are also the earliest ones, with some receiving the highest exposure, so we have a lot to share. With that, I'll turn it over to our first presenter.



>> MONIKA MORTIMER: Thanks, Mark and Christina, for the introduction. And thank you, everyone, for logging in and listening to us today.

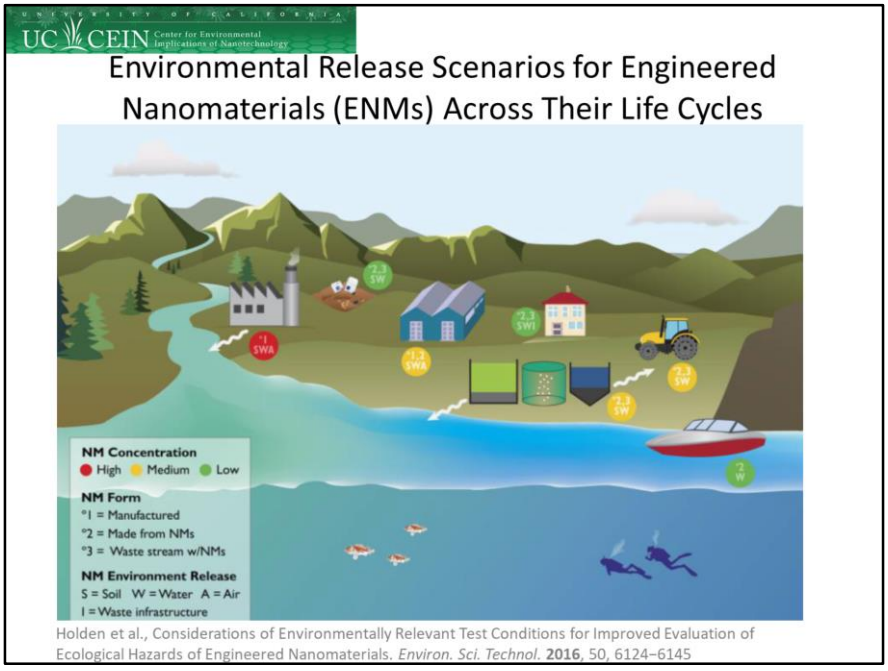
I will briefly talk about environmental safety testing of Engineered Nanomaterials (ENMs) and give some examples for consideration in ENM testing strategies which would be applicable in both environmental and biomedical fields.



>> MONIKA MORTIMER: I would like to begin with an overview of conceptual environmental release scenarios of ENMs, to give you an idea of where and how biota, but also humans, can be exposed to ENMs in the environment.

Based on models and predictions, the highest likelihood for human and environmental exposure is expected to be during the manufacturing process, which is depicted with the red circle in the scheme here. The mass flow analysis shows that most ENM-enabled products will be deposited into the landfills at the end of their lifecycles. However, environmental and human exposure to ENMs via landfills is expected to be low if landfills are well managed.

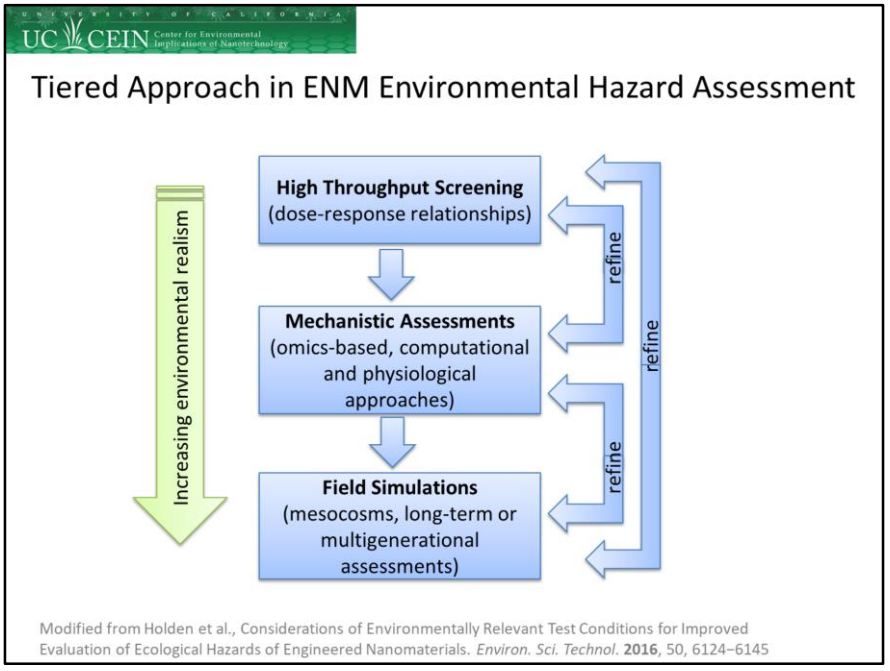
ENMs from secondary processing and household use, including personal care products, will be transported to waste water treatment plants and may be released via effluent if not removed during the waste water treatment process.



>> MONIKA MORTIMER: On the other hand, from certain applications, ENMs can be directly released into the environment. These pathways include ENM applications in agricultural products and surface coatings on ships as well as personal care products like sunscreens.

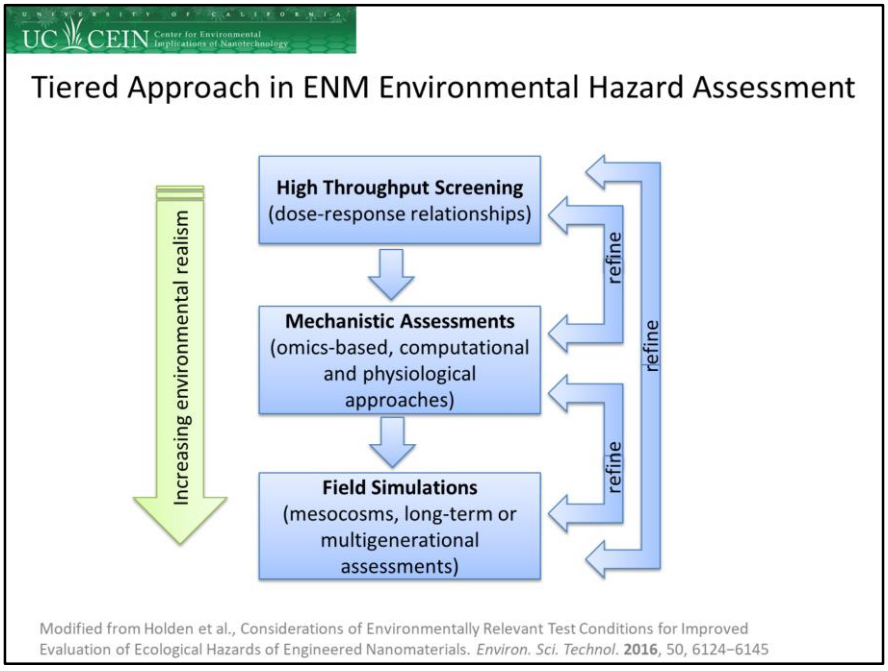
And regarding environmental compartments, relatively higher ENM concentrations are expected in soils and sediments, compared to water and air, based on the tendency of ENMs to agglomerate in the environment.

So possible routes for a human exposure include applications of ENMs in personal care products, agricultural, and marine based products, and studies have also shown that “trophic” transfer through seafood and plants is possible. The key connection between these environmental exposures and nanomedicine is intentional introduction of ENMs in both cases.



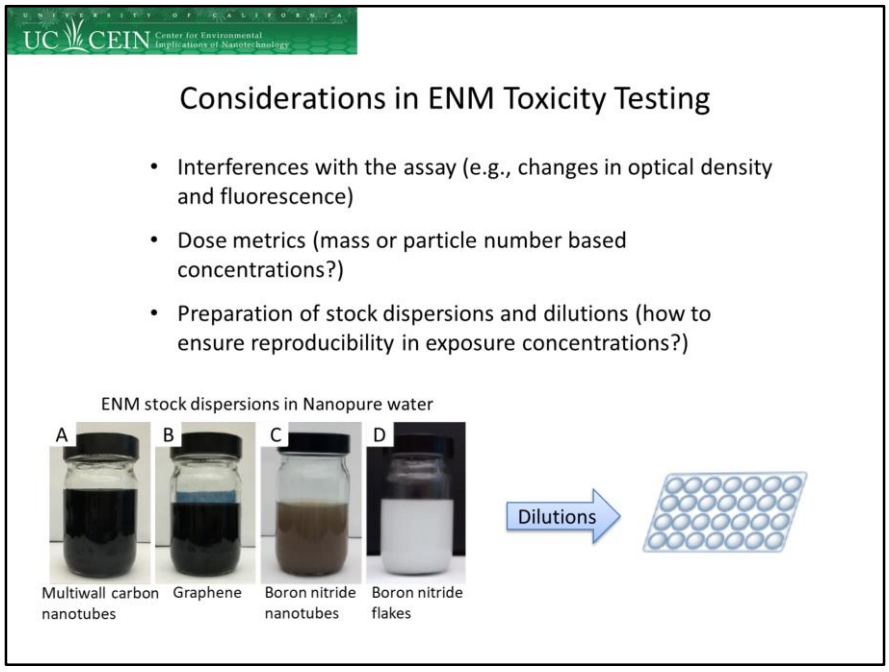
>> MONIKA MORTIMER: In my next slide, I am showing a scheme for a tiered approach in ENM environmental hazard assessment. This approach has been proposed due to a large number of diverse ENMs and challenges in their hazard assessment. It's not a new concept as it has been proposed for ENM human health hazard assessment previously and more recently for ENM environmental hazard assessment.

The tiered approach comprises an initial screening step where preferably high-throughput assays are used to establish if ENM induces dose dependent lethality or growth inhibition, for example.



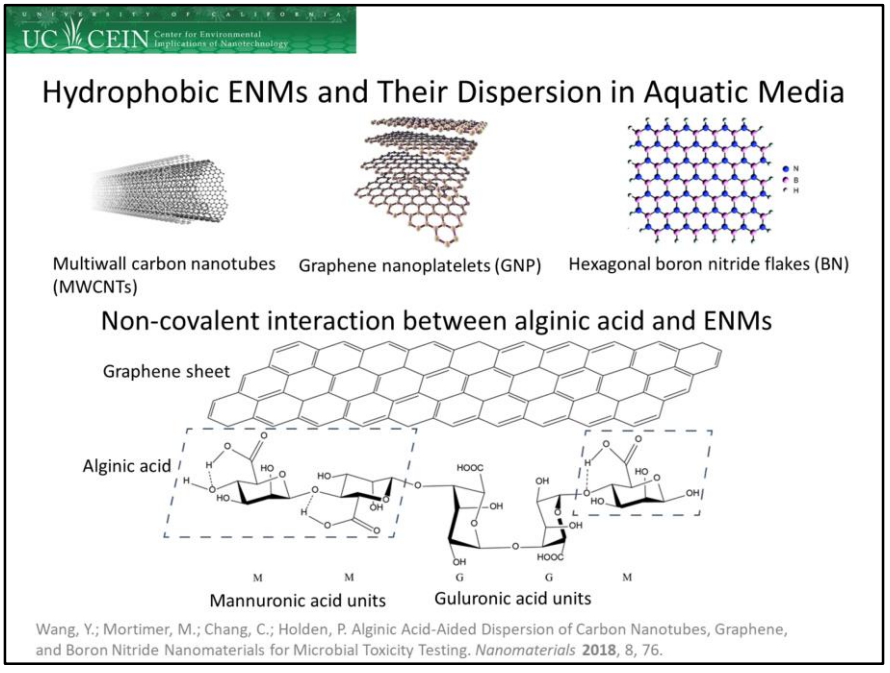
>> MONIKA MORTIMER: The next step is to use the information from screening assays for designing mechanistic studies. Mechanistic assessments could be based on emerging omics-based methods, computational methods, and physiological assays. For understanding if effects observed in simple *in vitro* tests would be translatable to population, communities, and ecosystem-levels, hazard assessment should be conducted in simulated field conditions.

Test results at different levels should be used to refine testing strategies both in lower and higher tiers depicted by these arrows here to the right.



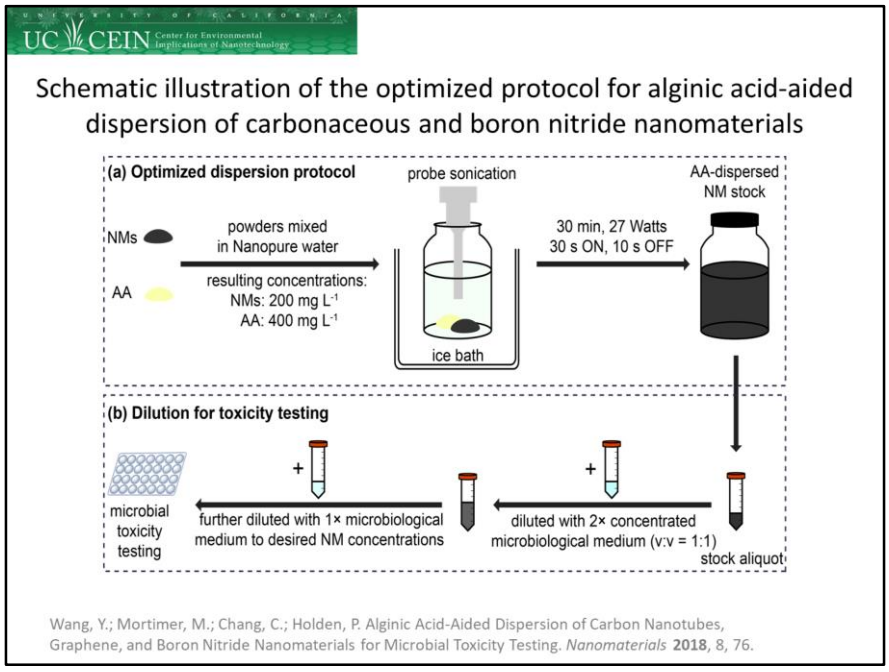
>> MONIKA MORTIMER: In nanomedicine, the field simulation level would translate into taking into consideration how the drug would behave at the whole organism level, as opposed to sub-cellular or cell culture level. So, for toxicity testing at any level of complexity, there are ENM-specific aspects that need to be considered. Different from soluble chemicals, for which most of the standard and commercially available assays have been developed, ENMs are particulate materials that may interfere, for example, with optical density and fluorescence-based measurements.

Another widely-debated issue in ENM testing, both in human health and environmental hazard assessment, is the metrics. For conventional pollutants, mass concentration is usually the relative metric for determining exposure. However, exposure potential of ENMs is affected by particle size. As such, number-based concentrations may be more relevant. However, it's difficult to determine in a case of, for example, non-spherical or agglomerated ENMs, because of lack of suitable methods.



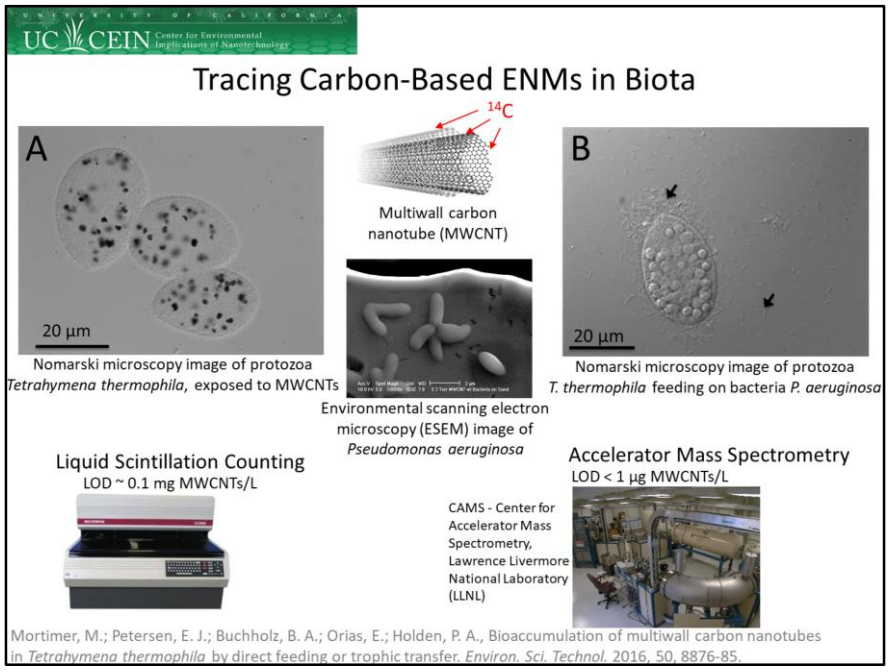
>> MONIKA MORTIMER: The third aspect of ENM toxicity testing I'd like to point out is the preparation of stock dispersions in a way that would ensure that these are reproducible across experiments. Dispersion of hydrophobic ENMs for aqueous toxicity testing is especially challenging and often requires the use of suitable dispersing agents. In choosing the dispersant, it is important to consider its relevance to the test system and biocompatibility with the organism or cell.

In addition, coating ENMs for dispersing purposes should preferably leave pristine ENM surfaces exposed for interactions with cells, if the purpose is to study the effects of the original ENM. As an example, I am presenting the use of alginic acid as an environmentally relevant dispersant of ENMs for the preparation of well dispersed ENM stock dispersions. Alginic acid is a natural polysaccharide, widely distributed in the cell walls of brown algae and secreted by certain environmental bacteria. As you can see from the scheme here, the mannuronic acid units of alginic acid noncovalently adsorb to ENM surfaces, while guluronic acid units, with their exposed carboxylic and hydrophilic groups, provide water dispersibility. As a result, ENM pristine surfaces are partly exposed for interaction with cells.

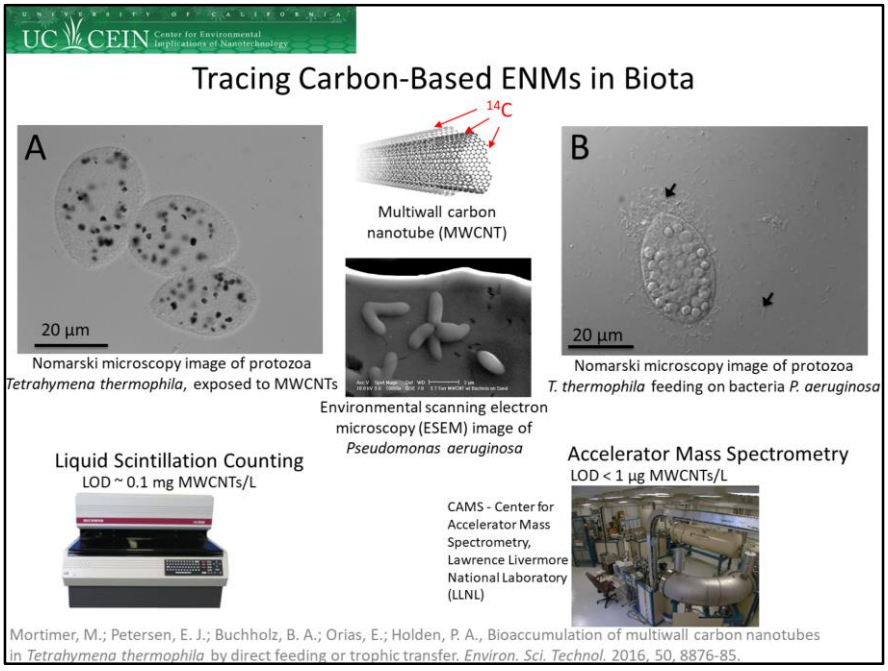


>> MONIKA MORTIMER: Probe sonication of ENMs and alginic acid in Nanopure water will lead to well dispersed ENM stock dispersions which could be used for preparing ENM dilutions for microbiological toxicity screening. Using dispersion for preparing ENM stocks for health-related assessments should follow similar principles of being biocompatible and relevant to the intended application of the ENM.

Another aspect in ENM safety testing I'd like to briefly talk about concerns the methodological considerations of ENM detection and quantification in complex environmental matrices and biota. This combination of environmental and health hazard assessment methods, such as single particle ICP-MS, have been recently advanced to detect low concentrations of metal based ENMs and also some carbon-based ENMs based on their metal impurities.

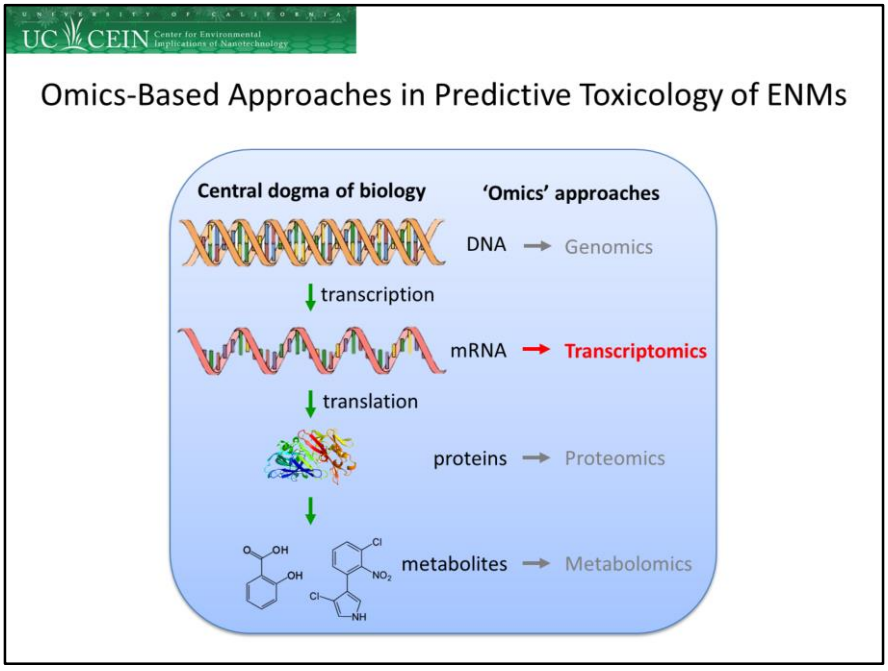


>> MONIKA MORTIMER: For organic ENMs, fluorescent scanning can be used for tracing ENMS in mechanistic studies but not for absolute quantification. For multi-walled carbon nanotubes (MWCNTs), we have shown, in collaboration with NIST and Lawrence Livermore National Lab, that employing ^{14}C labelled CNTs allowed CNT quantification in biota such as protozoa and bacteria, as shown in the optical microscopy and environmental scanning electron microscopy images, respectively, at a very low concentration. As a side mark, I want to say here that protozoa and bacteria can serve as excellent environmental and biomedical models.



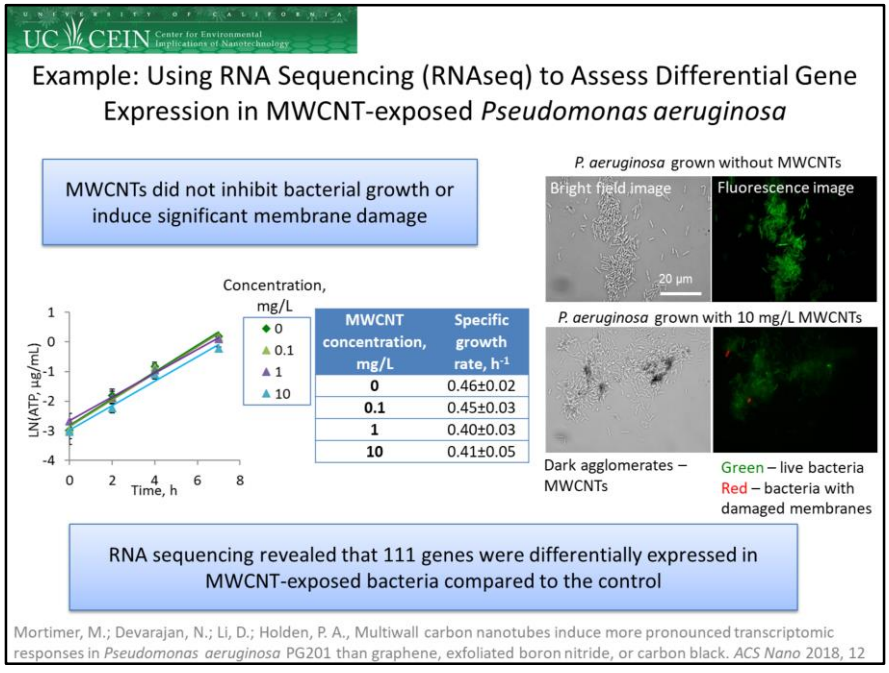
>> MONIKA MORTIMER: Low amounts of CNTs adsorbed to bacteria that were ingested by protozoa, depicted in the right image here, and these low CNT amounts were quantified using a novel approach for engineered nanomaterials, accelerator mass spectrometry. This method has actually been widely used in biomedical research but had not been used for quantifying CNTs before. The limit of detection is less than one microgram of CNTs per liter concentration, which is significantly lower compared to the limit of detection of conventional liquid scintillation counting method.

In summary, this slide illustrates that radioactive isotope-labelled ENMs allow for sensitive tracking and quantification for ENMs in *in vitro* and *in vivo* tests.



>> MONIKA MORTIMER: To advance mechanistic understanding of ENM toxicity and bioactivity, application of omics technology has been proposed. Omics approaches allow studying systemic genome responses to substances and can be considered as high throughput. The methods provide high content data sets for each single exposure condition and can be used for developing predictive models and biomarkers for ENM effects.

As an example of such application of transcriptomics, I will next describe a study we conducted, with *Pseudomonas aeruginosa*, which is clinically and environmentally important bacterial taxon.



>> MONIKA MORTIMER: When *Pseudomonas aeruginosa* was grown with MWCNTs, conventional toxicity end points such as growth and membrane damage did not indicate significant growth inhibition or significant membrane damage, as indicated by the fluorescent images.

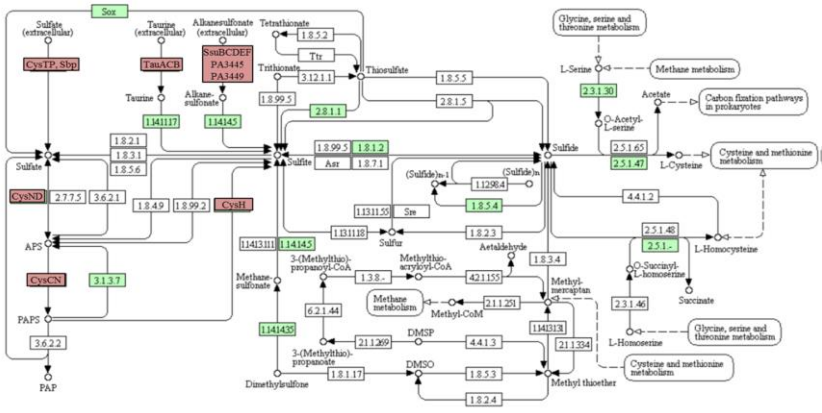
However, the whole genome sequencing using RNAseq revealed in total, 111 genes were differentially expressed in MWCNTs exposed bacteria, compared to the control bacteria not grown with CNTs. This indicated there was a biological response to CNTs which was not detectable when using less sensitive assays such as growth inhibition and membrane damage.

UC CEIN Center for Environmental Implications of Nanotechnology

Example cont'd

Mapping Differentially Regulated Gene Products in KEGG Pathways

SULFUR METABOLISM Organism: *P. aeruginosa* PAO1



Red - transcripts upregulated in *P. aeruginosa* PG201 upon 5-h growth in MWCNT-amended medium.

The map was retrieved from: http://www.genome.jp/kegg-bin/show_pathway?pae00920.

>> MONIKA MORTIMER: In the next slide, I'm showing an example how differentially regulated gene sets can be mapped in metabolic pathways to obtain knowledge about the relationship of genes, and which metabolic pathways are affected by the toxicant.

And as an example, here is the sulfur metabolism pathway in *Pseudomonas aeruginosa*, where transcripts highlighted in red were up-regulated in bacteria grown with MWCNTs.

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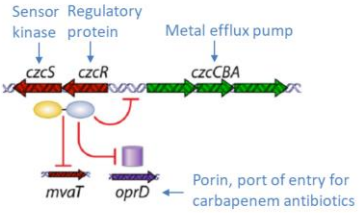
Example cont'd

Major functional groups of genes affected by MWCNTs in *P. aeruginosa*

KEGG module	Up- (↑) or down- (↓) regulated
Sulfur metabolism	↑
Nitrogen metabolism	↓
Central carbohydrate metabolism	↓
Amino acid metabolism	↓
Motility and virulence	↓

KEGG - Kyoto Encyclopedia of Genes and Genomes, http://www.kegg.jp

MWCNTs downregulated a two-component regulatory system (czcS/czcR) known to control heavy metal and antibiotic resistance

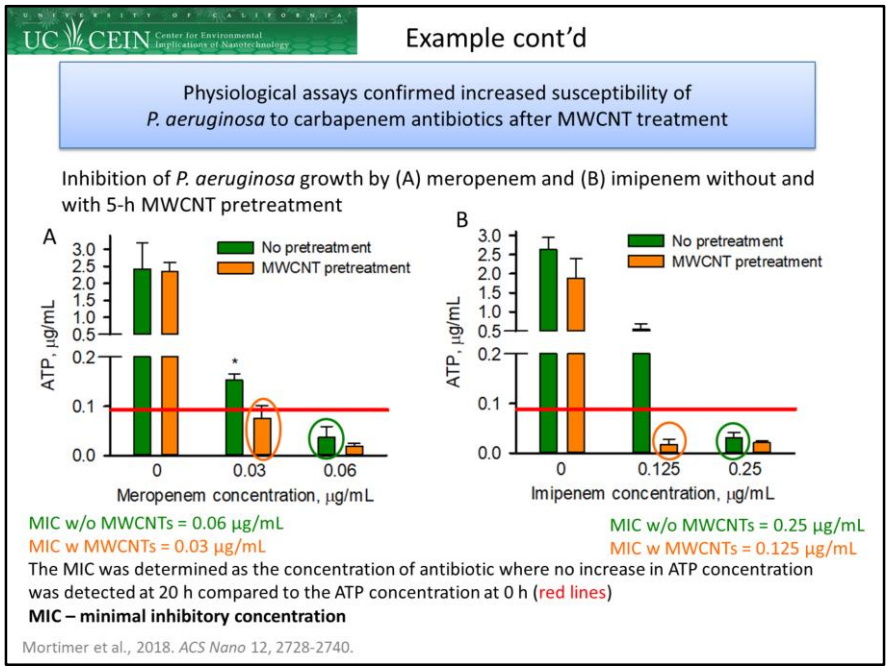


Li et al., *Clin. Microbiol. Rev.* **2015**, doi:10.1128/CMR.00117-14.

Mortimer, M.; Devarajan, N.; Li, D.; Holden, P. A., Multiwall carbon nanotubes induce more pronounced transcriptomic responses in *Pseudomonas aeruginosa* PG201 than graphene, exfoliated boron nitride, or carbon black. *ACS Nano* 2018, 12

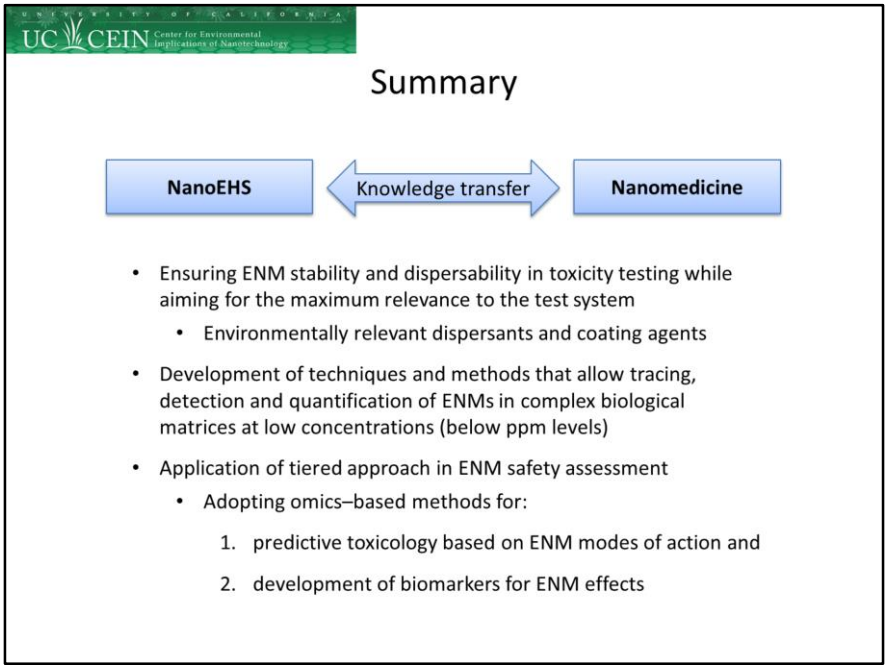
>> MONIKA MORTIMER: Transcript analysis revealed up- or down-regulation of other major functional groups of genes. In addition to up regulation of sulfur metabolism, CNTs down-regulated genes in nitrogen, carbohydrates, and amino acid metabolic pathways, and also some motility and virulence-related genes. Interestingly, the analysis indicated down-regulation of a two-component regulatory system that is known to control heavy metal and antibiotic resistance in *Pseudomonas aeruginosa*.

Based on this finding, we hypothesized that CNT exposure makes *Pseudomonas aeruginosa* more susceptible to certain class of antibiotics.



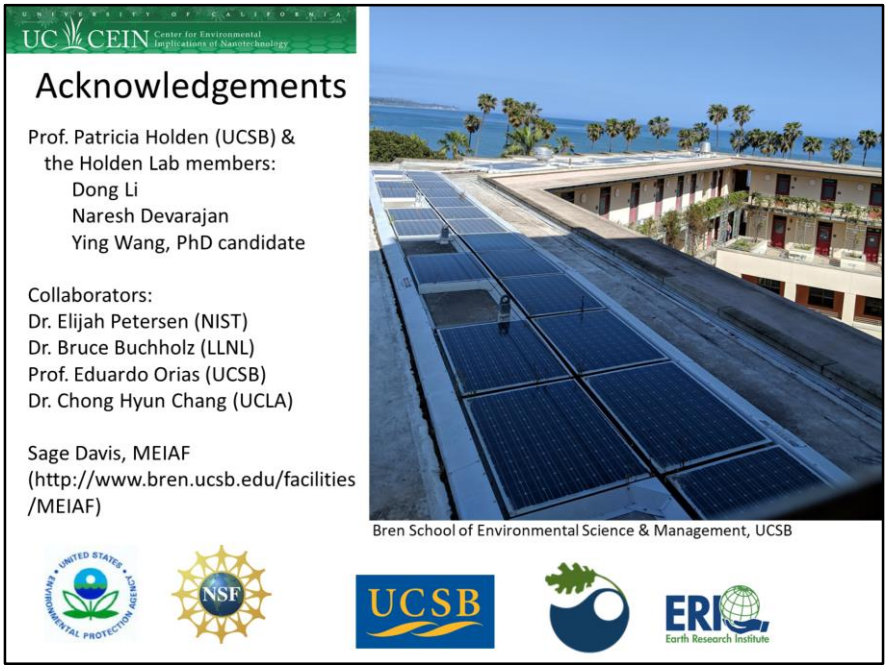
>> MONIKA MORTIMER: We tested this hypothesis by performing bacterial growth assays with antibiotics, with and without CNT pre-treatment. The results confirmed that the minimal inhibitory concentrations of antibiotics—meropenem in the left graph and imipenem in the right—were lower when bacteria was pretreated with CNTs, which indicated increased susceptibility to antibiotics.

This example illustrates the potential of using transcriptomics for understanding modes of action of ENMs at the genome regulation level, at sub-lethal or sub-growth inhibitory concentrations of ENMs. This approach can be adapted for nanomedicine, especially for anti-microbial applications, which are one of the major focus areas of the latter field.



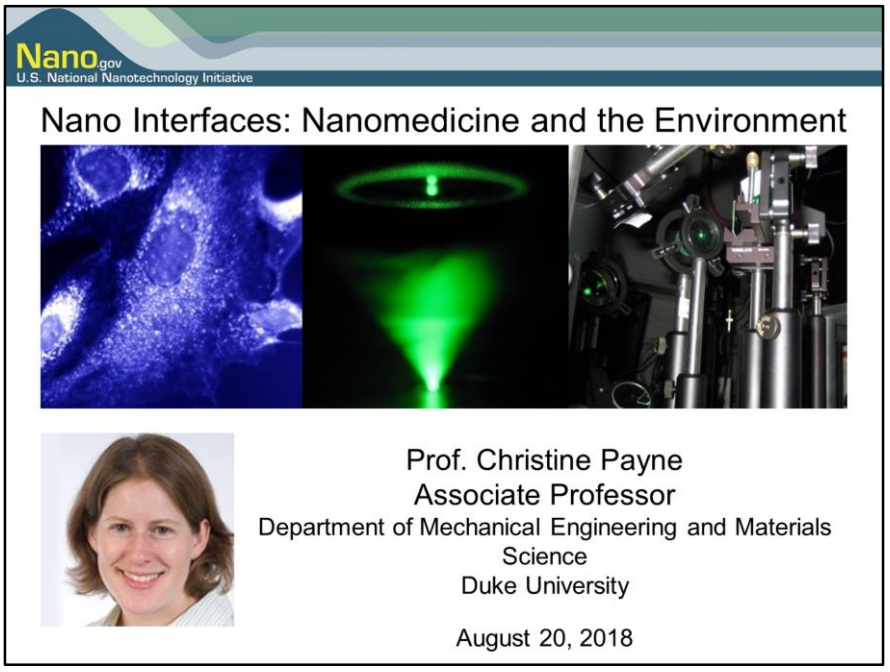
>> MONIKA MORTIMER: And in summary, I would like to highlight some of the aspects where knowledge gained in nanoEHS is transferrable to nanomedicine, but also vice versa, as illustrated by the examples presented. First, considering the importance of reproducibility and standardizability of high-throughput toxicity screening of ENMs, I feel that attention should be paid to preparing well-dispersed ENMs stock dispersions to deliver known and controllable doses to cells. In choosing the conditions for dispersions, the test should be designed as similar to actual exposure scenarios as possible.

Secondly, analytical techniques shown to successfully work in environmentally relevant organisms could likely be applied in biomedical research and safety assessment, since the complexity of the biological matrix is similar. And thirdly, a tiered approach is applicable both in the environmental and health safety assessment of ENMs. To advance and make the tiered approach more efficient, omics approaches are emerging as promising methods for advancing predictive toxicology and finding new biomarkers for ENMs.

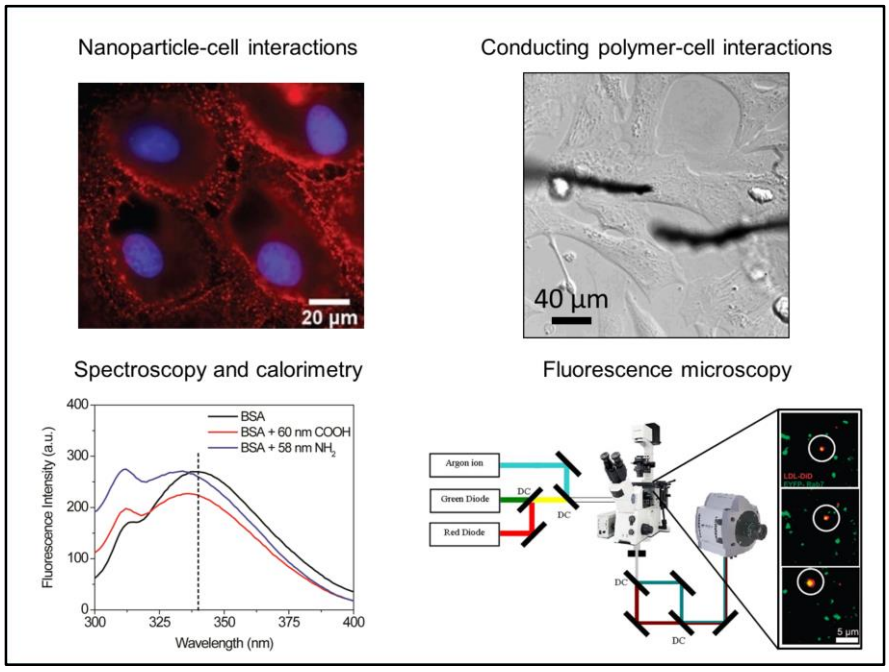


>> MONIKA MORTIMER: And lastly, I would like to acknowledge all the people who contributed to the results I have just presented.

And with this, I will turn it over to the next speaker.

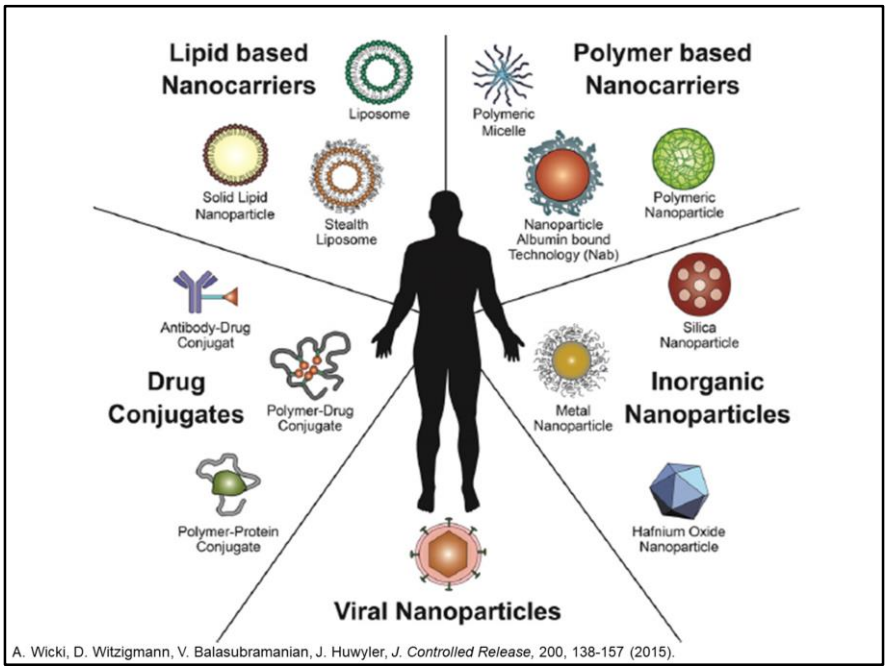


>> CHRISTINE PAYNE: I'm Christine Payne from Duke. I moved there from Georgia Tech at the end of December. What I wanted to do today was to give an overview of both the nanomedicine and environmental nanoparticle research in my group, and then I have a few slides outlining the overlapping themes and challenges we can use for discussions later.



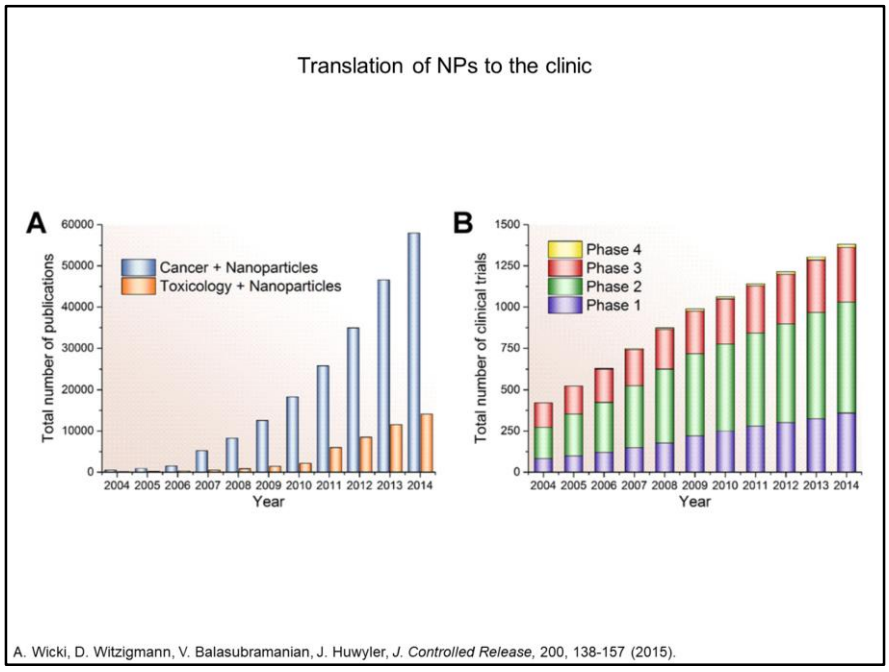
>> CHRISTINE PAYNE: My lab is interested in how cells interact with materials and how materials can be used to control cellular properties. And what you see on the left is an example of our work with Nanoparticle cell interaction. You see four nuclei stained blue and you see red nanoparticles bound to the surface of the cells; I'll focus both on the nanomedicine and environmental nanoparticles.

The other half of my lab works on conducting polymer-cell interactions for applications in neuroengineering and regenerative medicine. What you see here is a monolayer of cardiomyocytes and two conducting polymer wires. We do a lot of fluorescence microscopy to look at the underlying biophysics of these interactions, specifically single particle tracking and then spectroscopy and calorimetry to get at the molecular level details.



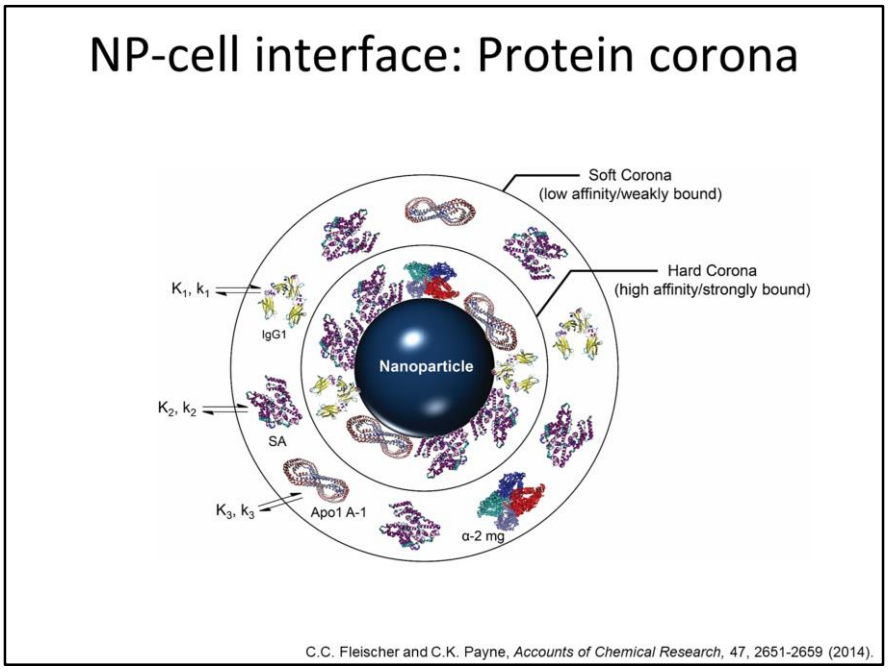
>> CHRISTINE PAYNE: My lab got interested in this question, and we entered it through the field of nanomedicine. This slide is an example of all of the different types of nanoparticles that have been proposed as cancer therapeutics.

My lab is specifically interested in what we would call hard nanoparticles (NPs), so metal nanoparticles, silicon nanoparticles, gold, iron oxide, and nanoparticles that have certain theranostics applications. Christina had a slide on this, the benefits of nanoparticles, the ability to have high payloads and multiple functionalities is unique to the hard NPs in comparison to the protein or lipid nanoparticles that are used as well.



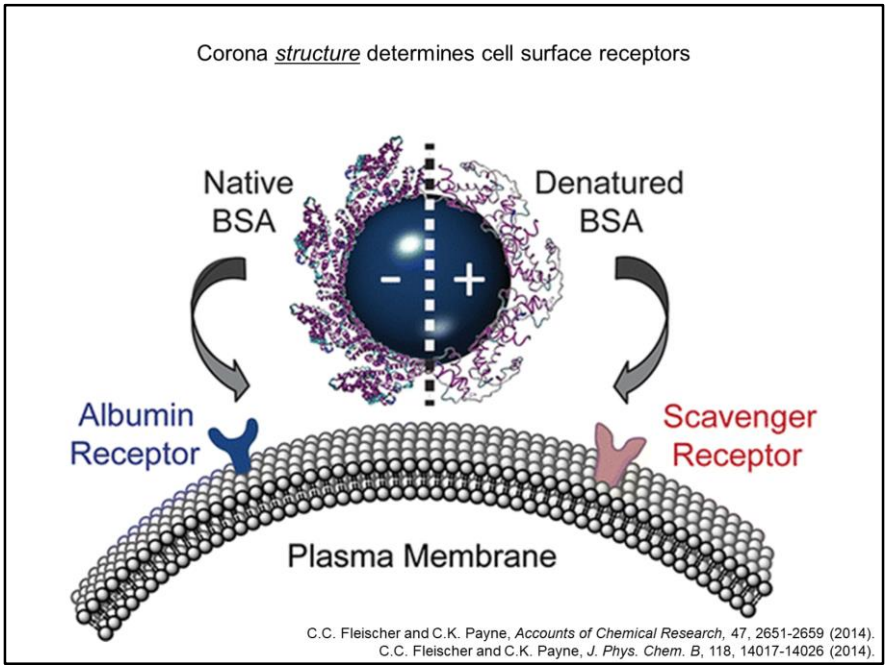
>> CHRISTINE PAYNE: The challenge has been that, despite a lot of advances in the chemistry and design of these particles, that very few types of nanoparticles—excluding liposomal formulations, have even made it into clinical trials. I think three overall—metal and inorganic nanoparticles only. And one group shown here did a quantified disconnect between fundamental research and the translation. So, in figure A, you can see the number of publications that look at, first of all, cancer in nanoparticles in blue, and then the next step, which would have been nanoparticles in toxicology, in orange. Then in comparison, in B what you see are the total number of clinical trials by phase. And this includes all types of nanoparticles. This would also include protein- and lipid-based delivery methods. So, for example, antibodies conjugated to drugs, which we might not think of as nanoparticles but are in the nano-regime.

What is important to look at here is the difference in the Y axis between the two plots. So in terms of total number of publications in these areas, you're seeing 60,000, but in terms of total number of clinical trials, you're seeing 1500. And this is from 2015. So our big question has been why the difference? Why are so few nanoparticles making it to the clinic?



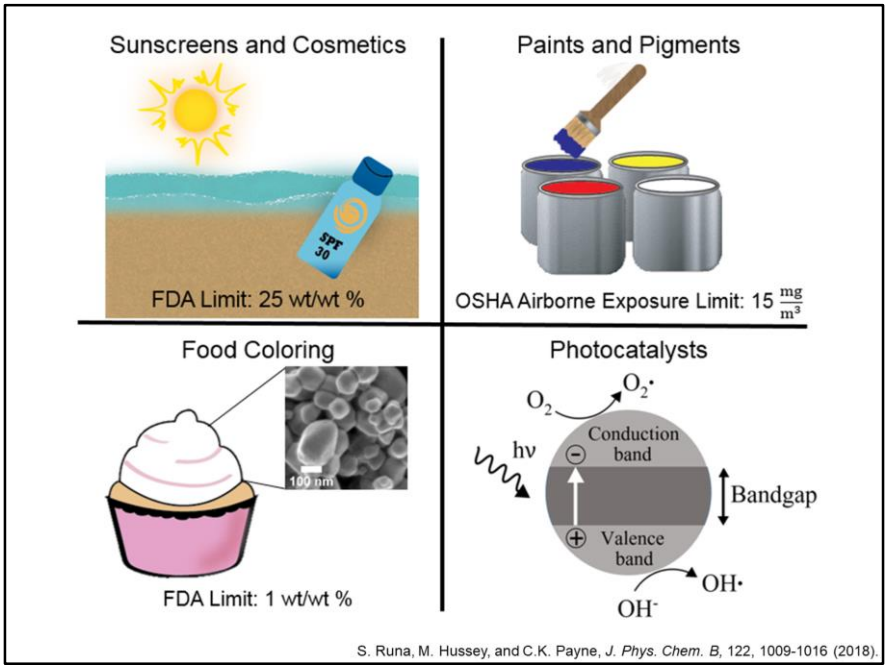
>> CHRISTINE PAYNE: And a major reason: there is clearance from circulation and accumulation in the liver. I think a lot of people in the nanomedicine are familiar with this problem. You have a well-designed nanoparticle, but when you actually try it *in vivo*, most of it just ends up in the liver. The first step in that process is the nanoparticle is recognized by the immune system and cleared.

What's being recognized, though, isn't the bare nanoparticle, but rather the serum proteins that adsorb onto the surface of the nanoparticles. That protein layer is called the protein corona, and what you see here, from an *Accounts of Chemical Research* paper from my lab, is a hard corona of tightly-bound serum proteins that adsorb on the surface and stay there the whole time, and the soft corona of protein that exchange on and off the nanoparticle surface. So for example, the macrophages will recognize the proteins in the corona and engulf those particles for clearance.



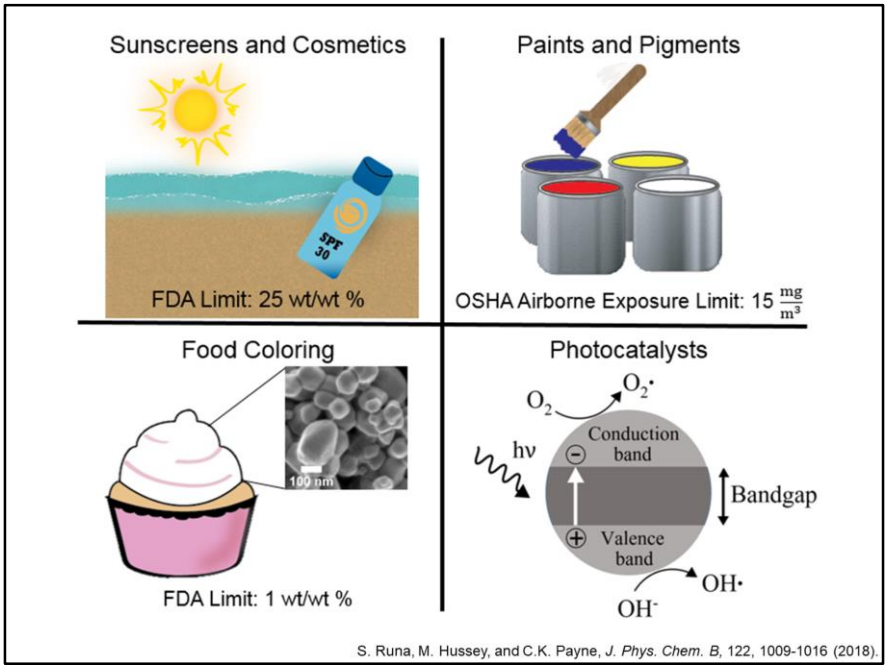
>> CHRISTINE PAYNE: To summarize from my lab, I think our major contribution here had been determining, using a combination of microscopy and spectroscopy, that the nanoparticle surface itself could alter the structure of the adsorbed protein. That was well known for planar surfaces, but it was new to the nanoparticle community.

We looked at a model system of polystyrene nanoparticles that were anionic, so you see the negatively charged particles, and what we found was that for albumin, and albumin is 55% of the serum protein composition, that albumin would adsorb on the surface of these particles but would maintain its native conformation. And then those protein NP complexes would bind to albumin receptors on the cell surface and follow an albumin uptake pathway. On the other hand, cationic nanoparticles, and these were specific surface coatings, albumin on the surface of those particles would undergo a partial denaturation that we could see with CD, i.e., circular dichroism, and fluorescence spectroscopy. Those protein nanoparticle complexes would bind to scavenger receptors which recognize denatured albumin and they would follow a scavenger uptake and transport pathway.



>> CHRISTINE PAYNE: Through this work in the protein corona area, and for us this is the three-body interaction of how proteins interact with nanoparticles and how that interaction affects their downstream interaction with cell surface receptors, we got interested in the question of how this applies to what we think of as an environmental nanoparticle, specifically titanium dioxide, TiO_2 which is produced industrially at huge levels for use in commercial products, and so we encounter it every day, in sunscreens and cosmetics.

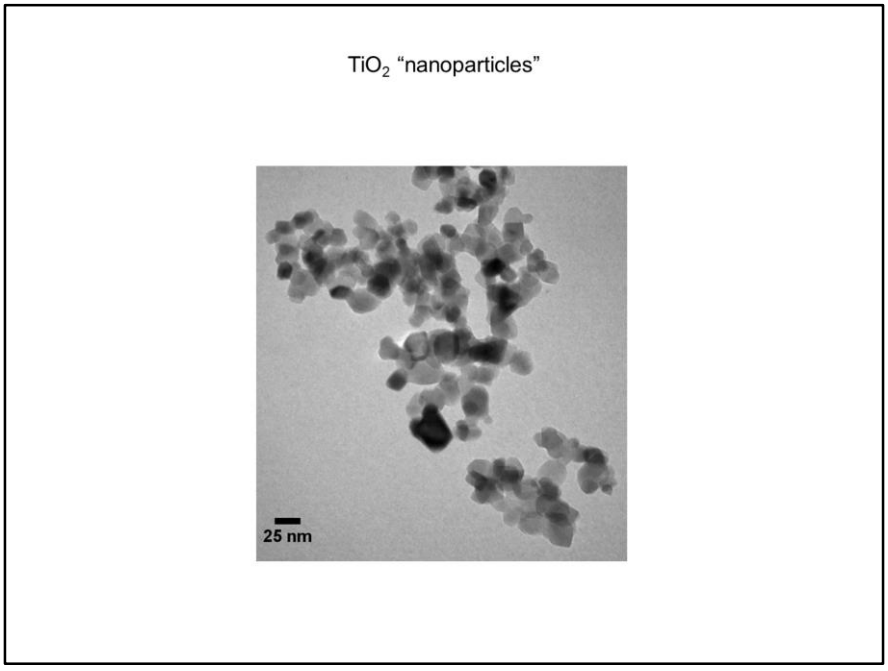
TiO_2 NPs are the white pigment in paints. They're also the white color in frostings and coatings on gum and different candy, they are the white food coloring.



>> CHRISTINE PAYNE: As chemists, we're interested in TiO_2 NPs as photo-catalysts, so these particles exposed to UV light generate reactive oxygen species, and there's a little bit of a disconnect between the chemistry community and the more applied TiO_2 community in terms of applications. The application that really matters in terms of human exposure turns out to be paints and pigments.

Sunscreens are safe, those nanoparticles don't penetrate your skin, food colorants aren't an issue, but for workers in factories that are processing paints and pigments, for example, these workers can inhale the TiO_2 particles and they will accumulate in the lungs, so OSHA sets a daily work limit for these workers.

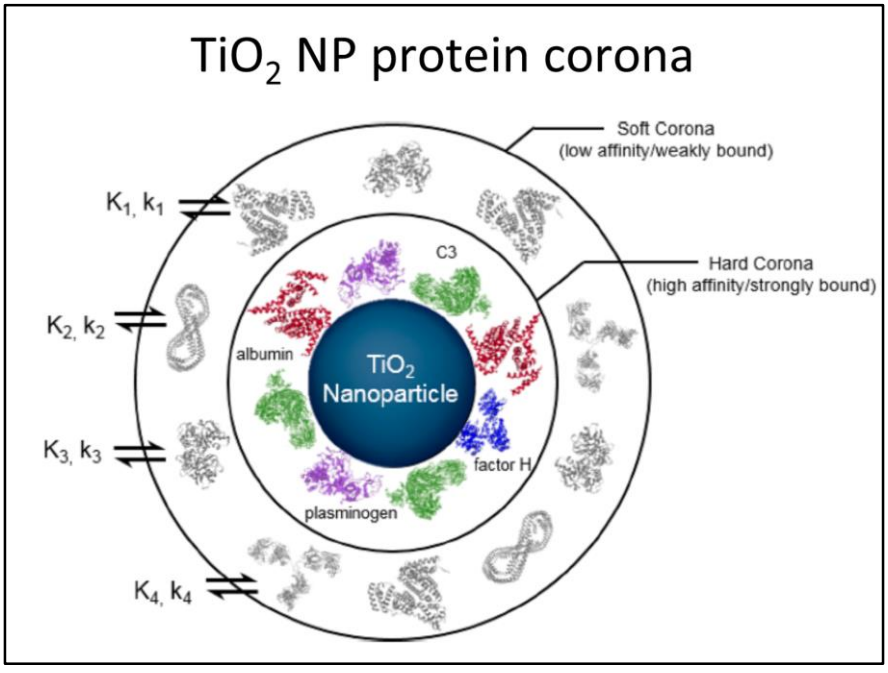
And there are quite a few toxicology experiments done on TiO_2 that set these limits for food coloring and sunscreens as well.



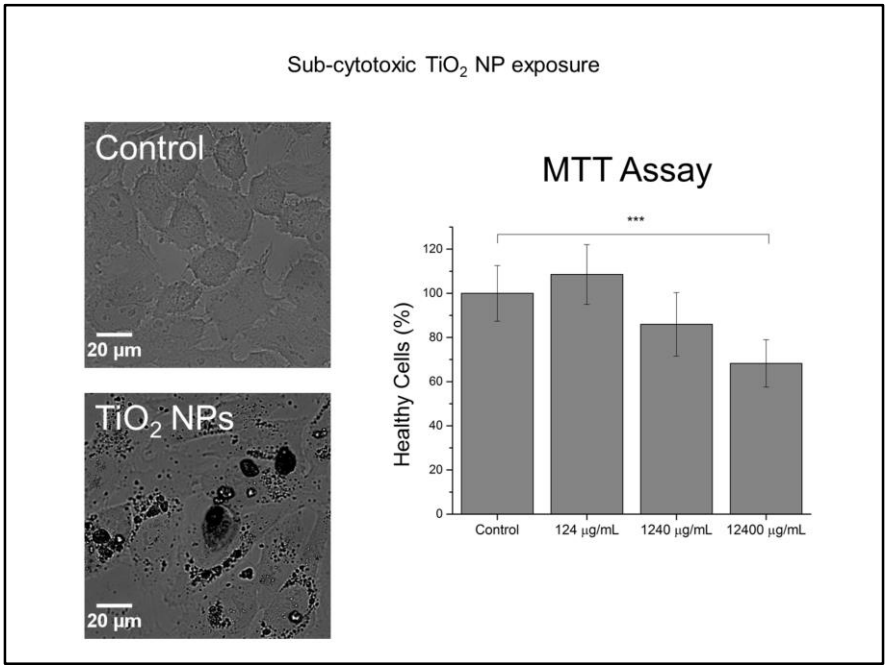
>> CHRISTINE PAYNE: What most chemists have looked at was actually cells incubated with these TiO_2 particles, and then exposed to UV light because they were interested in the photocatalytic properties, but that turns out not to be especially relevant to lung inhalation, since there's no UV light in your lungs. In this case, what matters is really doing an experiment with the nanoparticles kept in the dark, so you're not photoactivating them.

Nanoparticles, I'll point out from a material science point of view, are really interesting because they're complex materials and this gets back, actually, to some of Monika's slides about how to think of dispersibility and suspension of these materials.

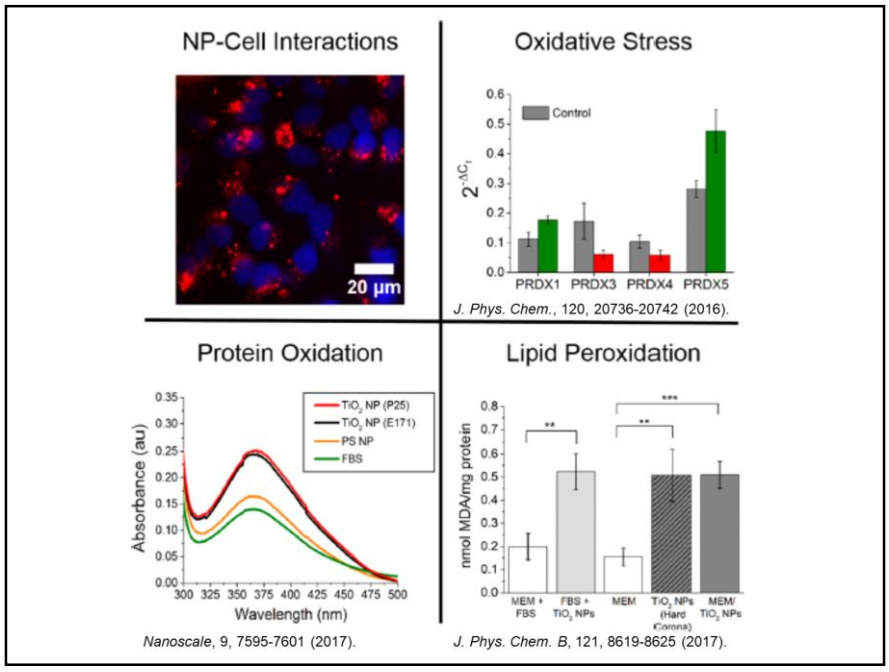
This slide shows an electron microscopy image of industrial TiO_2 NPs that are used as photocatalysts, and these would be labeled as 22 nanometer nanoparticles. But what you can see is that the individual grains are really just 22 nanometers; these are fused aggregates that cannot be separated physically. This is after sonication, for example, and we see that the aggregate size will range from 100 nanometers to one micron. They're very complex materials.



>> CHRISTINE PAYNE: So TiO_2 NPs, like any nanoparticle, will form a protein corona. These are some of the proteins that we had identified with proteomics as forming a corona on these NPs, they have a hard and soft corona as well. This again relates to some of Monika's slides, but we're interested not in doing toxicity experiments—those have been done before—but looking at more subtle effects. What do TiO_2 NPs do to cells at very low concentrations over a lifetime of exposures?

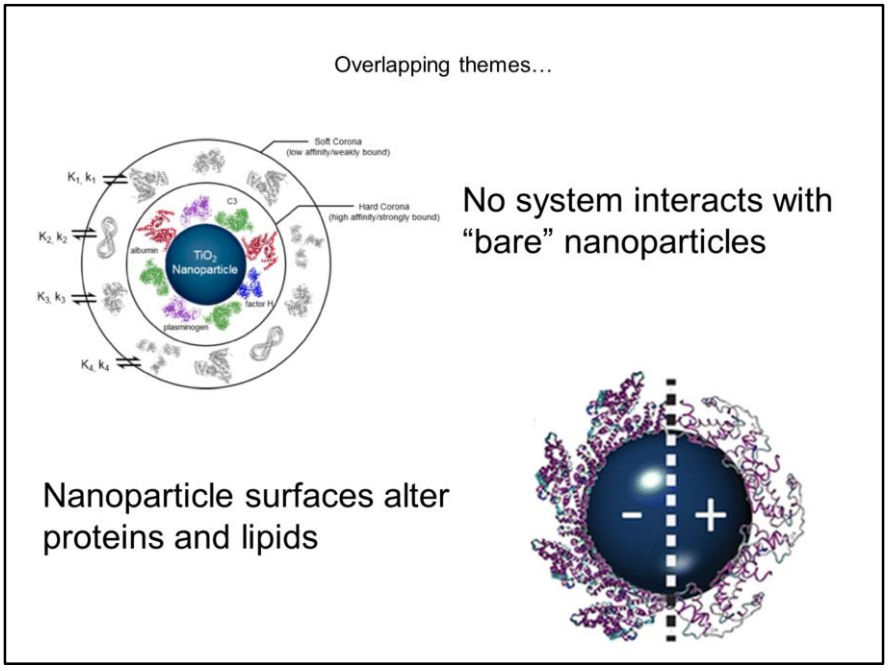


>> CHRISTINE PAYNE: So we've set this in a somewhat arbitrary way, but to say we use an MTT assay of mitochondrial enzyme activity: we find a NP concentration in which we see about 30% cell death. Then for our experiments we back off by a factor of 100 where we don't see toxicity, and then ask, what are the more subtle effects from the particles?



>> CHRISTINE PAYNE: I'm going to summarize all of our work here in one slide. In terms of TiO_2 (shown here in red), these experiments were all done in the dark, so it is not a photocatalytic experiment. We saw these NPs themselves caused oxidative stress in cells, and there is a change in the expression of the peroxiredoxin, and that change is actually due to an oxidation of the corona proteins

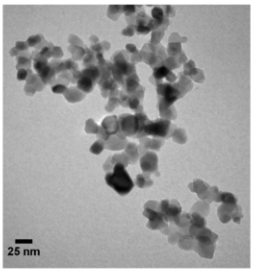
If you put those oxidized proteins onto non-oxidizing inert nanoparticles, you will see the same oxidative stress response; in the absence of a protein corona, these NPs are actually much more toxic, and part of the reason for that is because they oxidize the lipids of the plasma membrane directly.



>> CHRISTINE PAYNE: So in terms of overlapping themes between nanomedicine and nanoEHS, I just wanted to point out for all of these systems, the system of interest is never interacting with a bare nanoparticle. It's almost always interacting with either a protein corona, a lipid corona, or a corona that has been formed environmentally. The nanosurface itself will alter anything that interacts with that surface.

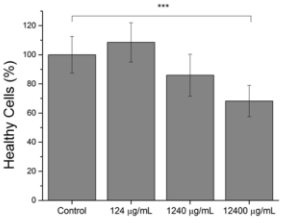
That's important because if you think about the protein corona, the first step of the experiment is to do proteomics and get a list of proteins absorbed onto the nanoparticle surface: It's not the list of proteins that matters, but rather how their structure is altered by the interaction with the nanoparticle surface.

…and challenges.



How to characterize these highly complex systems?

What constitutes a realistic model system for laboratory experiments?



>> CHRISTINE PAYNE: And the challenge, which is a little bit of an overlap with what Monika discussed, is how to characterize these complex systems. These are said to be 22 nanometer NPs when you buy them. Obviously for any of us looking at them, that's not how they should be classified.

And how do we think about realistic systems for laboratory experiments where we want mechanistic details and connecting those up to real in vivo systems? So in our case, we use the 30% cell death backed off by 100-fold, but is that the best model for what's happening in an animal?

Acknowledgments

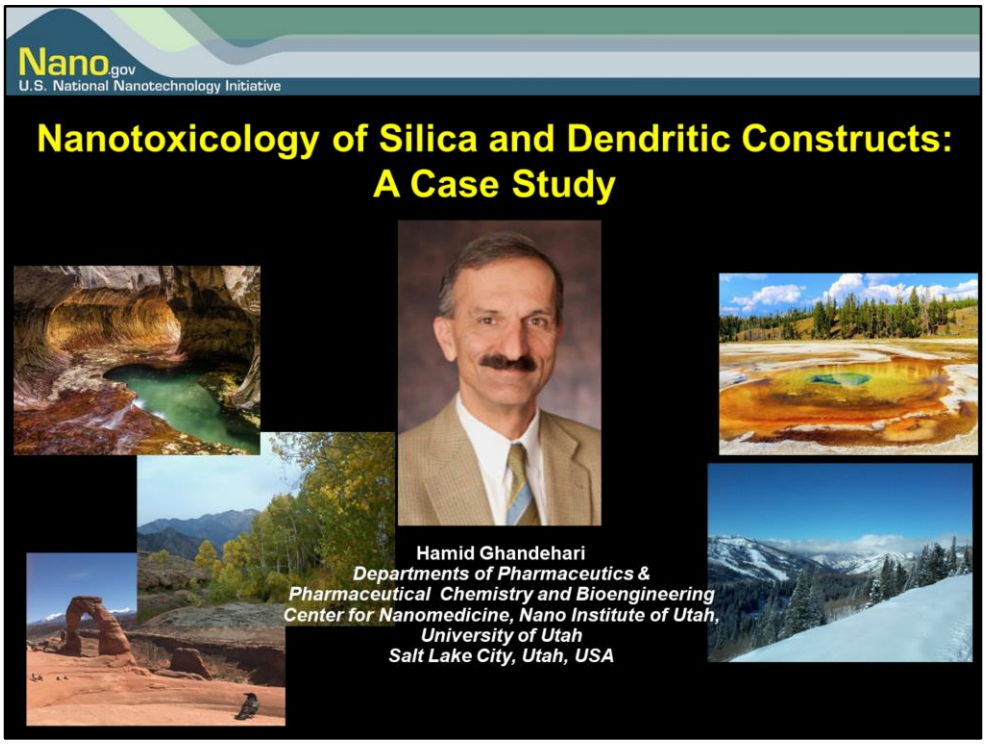
Nick Cariello (Physics, UNC)
Dhanya Jayaram
Kosa Johnson (BME, NC A&T)
Zeqing Shen
Quinton Tran (Physics, Duke)

Recruiting PhD students and postdocs!

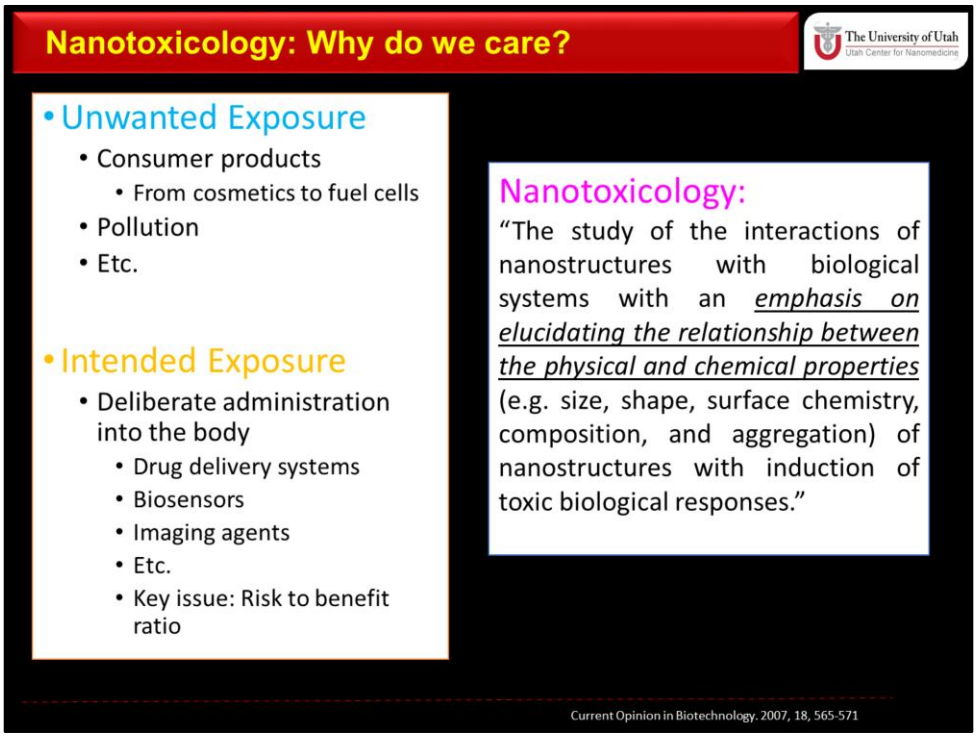
HERCULES Exposome Center;
Rollins School of Public Health at Emory University
NIH-NIEHS
NIH (BRAIN Initiative)

>> CHRISTINE PAYNE: Acknowledgements: I have a group of summer students who have done a great job getting my lab started at Duke, a post doc, Dhanya Jayaram who has done most of this research, and I'm looking for Ph.D. students and post-docs for my new lab. Funding came through the Hercules center at the Rollins School for Public Health at Emory University and NIEHS.

And thanks to all of you for logging into the webinar.

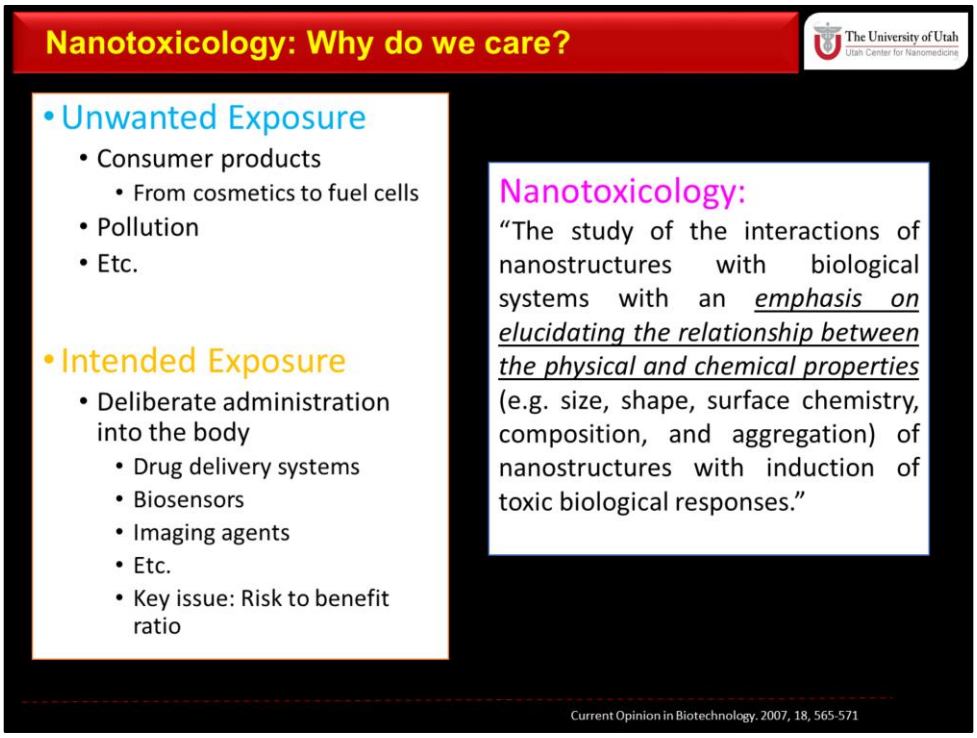


>> HAMID GHANDEHARI: I'm Hamid Ghandehari from University of Utah, and I will talk more about the medicine side of nanoEHS things and mostly on nanotoxicology.



>> HAMID GHANDEHARI: In the second slide, we ask why do we care about nanotoxicology? You could have unwanted exposure in consumer products or pollution, or in the case of nanomedicine, when we're primarily dealing with intended exposure, how do we use these nanoparticles as drug delivery systems, use them as biosensors, imaging agents?

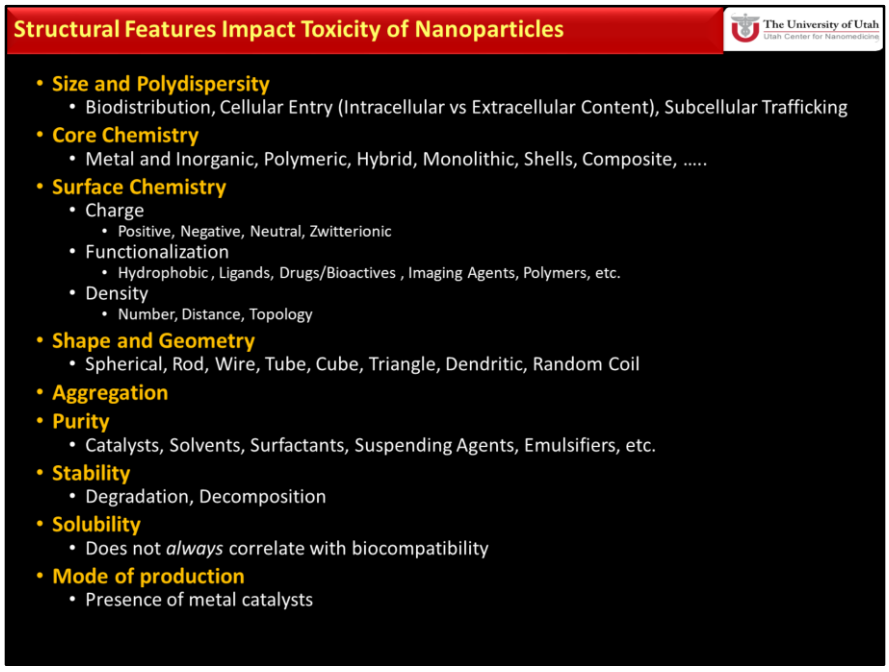
The key point here is there's no such a thing as a non-toxic material. Everything is relative. At the end of the day, we have to worry about the risk-to-benefit ratio. It's the same thing as drug molecules. Aspirin is safe, yet it can be toxic if you take a lot of aspirin for long periods of time. It's really a matter of risk-to-benefit ratio.



>> HAMID GHANDEHARI: If you look at the earlier literature in nanotoxicology, maybe 10, 12, 15 years ago, you have a lot of interesting, novel materials that are simply put on cells, and people have inferred whether they're toxic or non-toxic, but the key point really is the detailed characterization of nanomaterials. I really cannot emphasize more on that topic.

People have been working on understanding the toxicology or to put it a different way, biocompatibility of nanomaterials, for many years, you have liposome that have been around for about 40-50 years; linear water-soluble polymers such as polyethylene glycol have been used extensively; people have had to worry about the biocompatibility of these materials for biomedical applications, including drug delivery, over the past ten to twelve years. This whole field of nanotoxicology has evolved because now you have also the ability, because of the advances in fabrication technologies (whether it's bottom up or top down), to make materials with very well defined features.

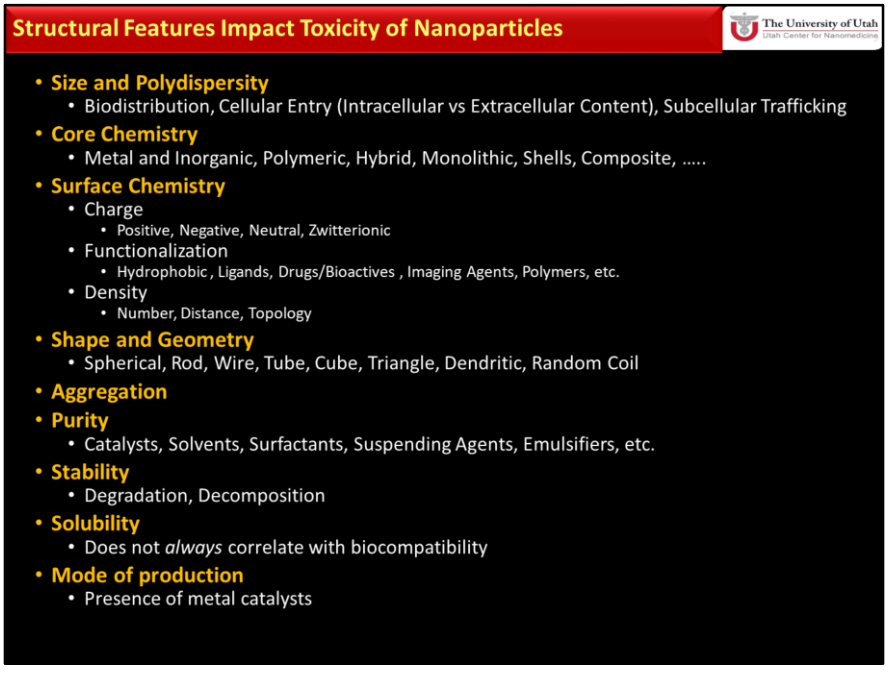
I would like to emphasize in this definition of nanotoxicology, on really elucidating the relationship between the physical and chemical properties with the induction of toxic biological responses.



>> HAMID GHANDEHARI: So in this next slide—the type of structural features that impact toxicity of nanoparticles—I've listed a few attributes, and I think they were already discussed, but I will go over them really quickly.

Not only size matters, but polydispersity matters because if you have a very polydisperse material, you will have a distribution of size, and as a consequence, the biodistribution of these materials in the body will be different. Because now you are dealing with a distribution of different sizes. So, the size, as well as polydispersity matters.

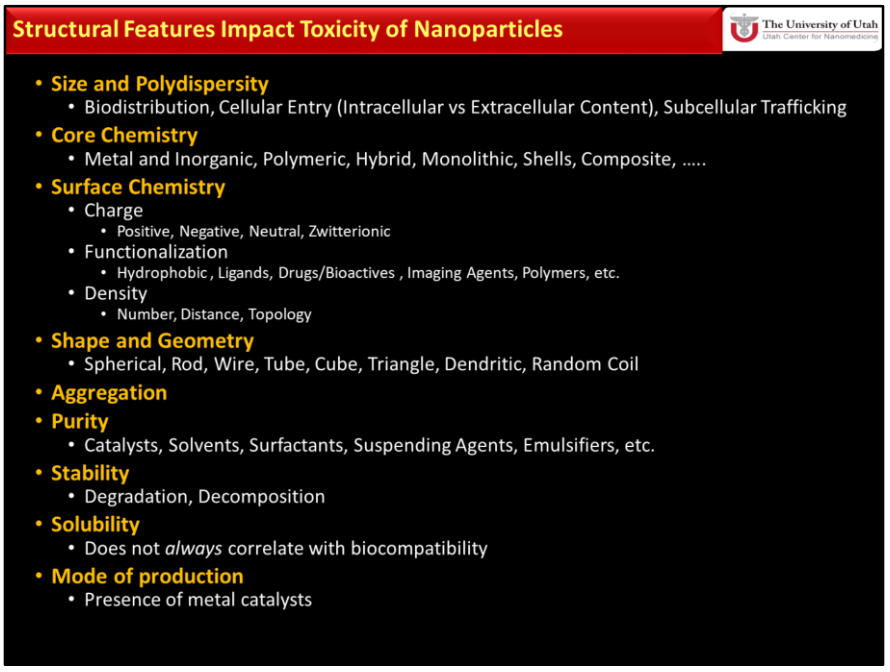
Core chemistry matters. As Christine pointed out, you can have metal inorganic, you can have polymeric, you can have a hybrid of these materials. For example pegylated gold NP (AuNP): that surface is totally different from just gold NP that does not have any polymer on its surface in terms of protein adsorption, in terms of compatibility, cellular uptake, and so forth.



>> HAMID GHANDEHARI: Surface chemistry has a huge effect, I will give some examples of some of these structural features and how they impact toxicology.

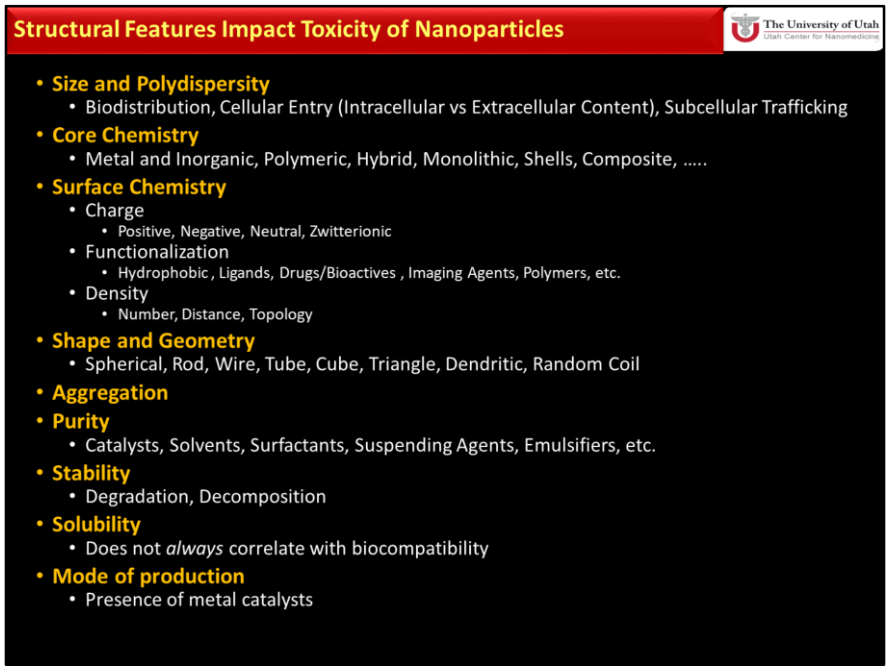
Charge has a huge effect on NMs. Functionalization: a lot of times, for example, in the drug delivery field, we have to functionalize the nanoparticles with ligands that target specific receptors. The moment that you put these ligands (or even if you put them with a spacer) on the surface of these NPs, the physicochemical properties of these NPs change; you may get them aggregated or you are introducing hydrophobic surfaces that then behave differently with each other, with the solvent, as well as when you put them on the cells with the media. Functionalization has a huge effect.

Interestingly, the density of NPs matters. You could have a nanoparticle with similar sizes and very similar surface properties, but variations in density will have an influence on how fast and to what extent they're going to sediment, let's say, in your subculture media. And at the end of the day, the toxicity of these materials is determined by how much of these particles are taken up by the cell.



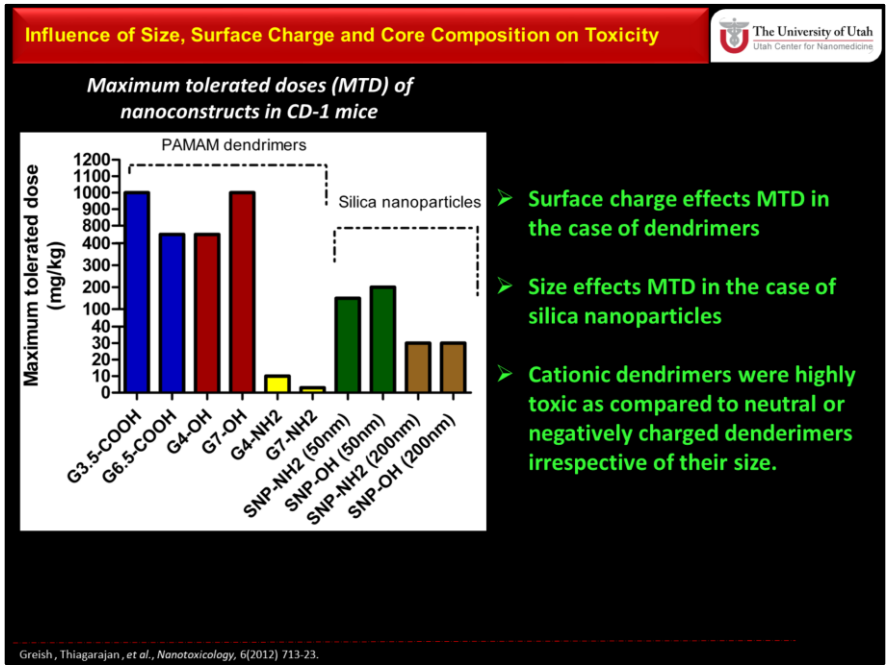
>> HAMID GHANDEHARI: Another attribute that my colleagues earlier discussed: shape and geometry have influence, and I'll talk about that. You've got this proliferation of what I call beautiful nanomaterials, crescents, triangles, circles, rods, worm-like, and so forth, and the question is how would this geometry influence the distribution in the body and how would the geometry influence the uptake by the cells, the mechanisms of uptake by the cells, and ultimately toxicity?

I'll go over very briefly these other points, which I think are self-explanatory. Christine showed a slide on aggregation, which would have a huge effect. Obviously, the purity of the materials: you have to make sure your nanomaterial at the end of the day is free of these catalysts, solvents, surfactants, what you put in initially to make these NMs, and you have to do sophisticated analytics to make sure you don't have them in the pores of these particles.



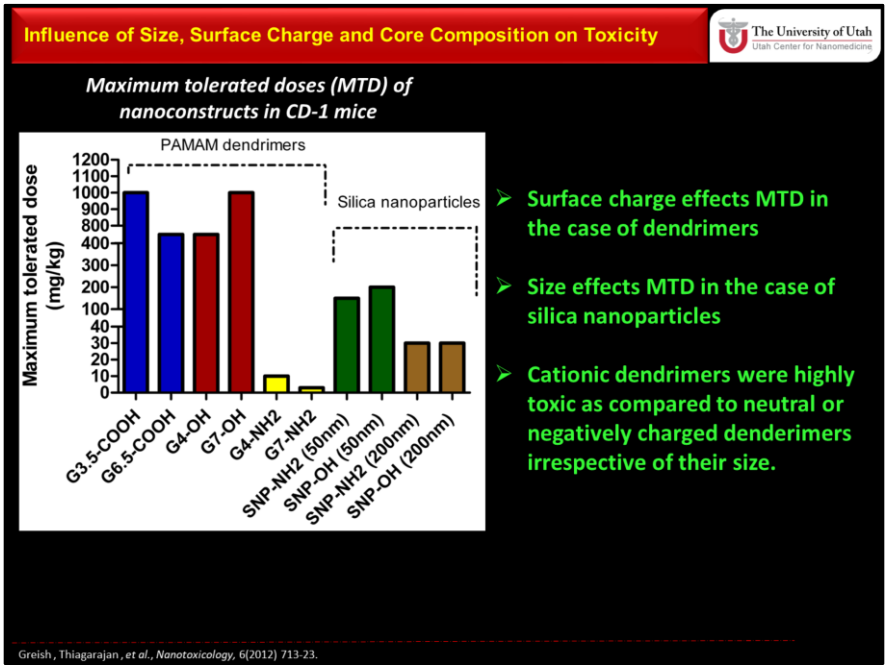
>> HAMID GHANDEHARI: Some materials are degradable, and some of these materials are not degradable, so if you're dealing with understanding the biological fate of degradable materials, you also have to know what is the degradation fate of these materials: how fast do they degrade, what are the degradation products, and how would those degradation products by themselves over a period of time influence toxicity?
Solubility, which is really suspendability, but it doesn't necessarily directly correlate with biocompatibility. And finally, mode of production.

Based on those key parameters, I'll give a few examples of studies we have done in our lab.



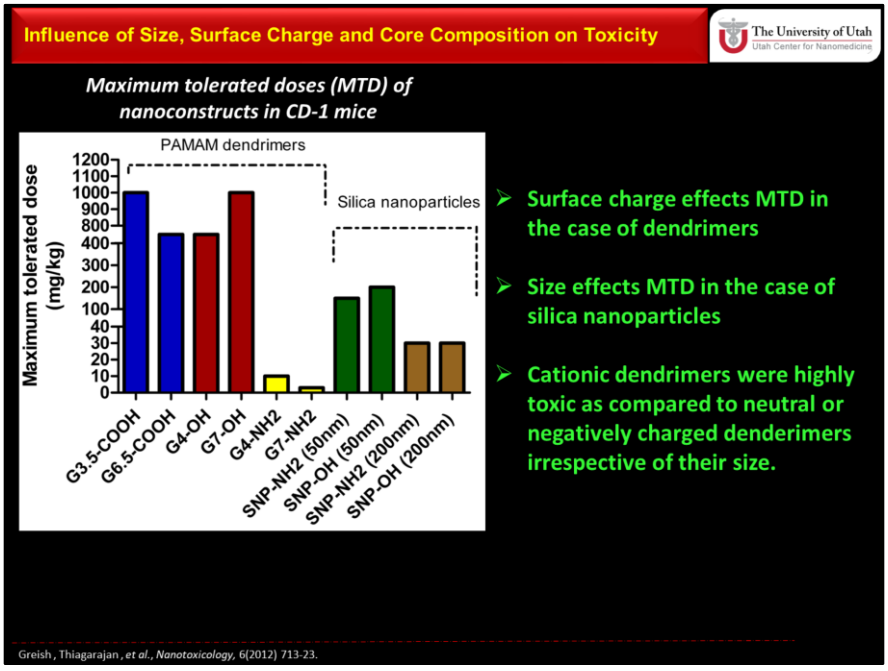
>> HAMID GHANDEHARI: The next slide, titled “influence of size, surface charge, and core composition” really just shows the maximum tolerated dose in CD-1 mice. In other words, to what extent can the mice tolerate the dose after which about half of the mice die?

On the left-hand side, these are poly (amido amide) dendrimers. Many of you are familiar with these polymeric structures. They are branched structures, and the diameter ranges in these particular cases that we have studied are anywhere from 3-13.5 nanometers. If you look at it, the surface charge in this case has a profound effect on the maximum tolerated dose. The anionic dendrimers, which are labeled G3.5 carboxyl and G6.5 carboxyl, as well as G4 hydroxyl and G7 hydroxyl, these are two different sizes; with one carboxyl terminated one hydroxyl terminated the maximum tolerated dose in terms of milligram per kg is somewhere around 400-1000 mg/kg.



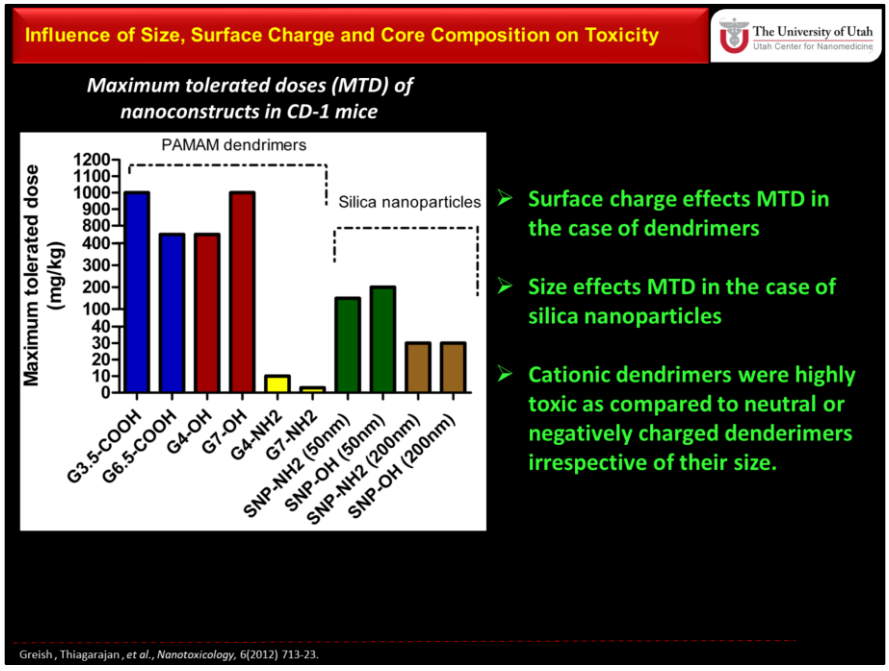
>> HAMID GHANDEHARI: With the very same PAMAM (polyamidoamine) dendrimers, except now that the surface is now amine-terminated, G4-NH2 and G7-NH2, you get a drastic decrease in maximum tolerated dose, and in the subsequent slides, we have shown that they cause blood clots. So in our community of drug delivery, you see people raise their hands—PAMAM dendrimers are toxic or not toxic? I think that's an irrelevant question. You have to look at the concentration of the material, the generation, surface charge, and how long you're going to incubate them with what type of material.

These are all different factors, which then we can conclude that there's a window of opportunity in terms of how much of the material that you can use them for specific biological applications.



>> HAMID GHANDEHARI: For example, if you want to use a PAMAM dendrimer that is carboxyl terminated for a specific imaging agent, for which you need to just deliver trace amounts, that won't be toxic for all practical purposes, but if you want to use the same material, let's say for delivery of drugs, and you can only attach five drug molecules to the surface of these dendrimers, then you have to deliver a lot of these carboxylated materials, and that could potentially cause toxic effects. Likewise, the surface charge.

Now, if you go to the right-hand side of that same slide, we did within the same animal model, everything is the same, the same lab, we looked at the maximum tolerated dose of much larger particles, in this case particles of SiO_2 (silica) NP that are either amine or hydroxyl terminated at 50 and 200 nanometers.



>> HAMID GHANDEHARI: Very interestingly, for amine-terminated silica particles, we didn't see the same toxicity profile as we saw on the PAMAM dendrimer, so we cannot generalize that the moment you have this cationic surface, you are going to have toxic effect., It really depends on the particle. And secondly, for the larger silica nanoparticle, 200 nm, you see an increase in toxicity; in our various studies we have shown that's basically an obstruction effect in these different tissues.

A lot of times you hear that gold is non-toxic, silica is non toxic; and again, that's a matter of relativity. In other words, how much of the particle do you have to inject into the animal, let's say, for a specific drug delivery application? In this case, for example, If you have a 50 nanometer SiO_2 particle, lets say you're going to inject 100 mg/kg or less, you are pretty much in the safe range as far as acute toxicity is concerned. Another important issue in our lab and other people are studying, is what will happen long-term, once these particles are in the body for, let's say, six months, a year, and beyond: I don't think I have a slide today to share, but I'll be happy offline to speak to anyone who's interested.

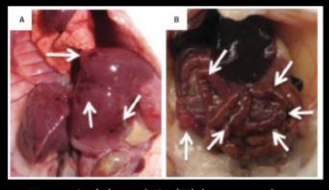
Blood Hemocompatibility of PAMAM Dendrimers

The University of Utah
Utah Center for Nanomedicine

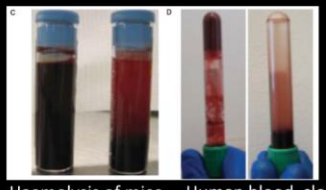
Table III. Levels of blood coagulation indicators *in vivo* after i.v. administration of nanoconstructs.

Probe (dosed at 30 mg/kg)		Platelets ($\times 10^3/\mu l$) ± S.D.	Haemolysis	Clotting	Fibrinogen level (mg/dl)	Fibrin degradation products (μg/ml)
Animal [a]	Control	453 ± 163	-	-	235 ± 38	<10
	G4-NH_{22}	149 ± 135*	+	++	82 ± 90	>40
	G7-NH_2	129 ± 53*	+	+++	[b]	>40
Human	Control	60 ± 15	–	–	185 ± 30	<10
	G4-NH_2	19.5 ± 0.5*	+	[NA]	<30	>40
	G7-NH_2	25 ± 5*	+	[NA]	[b]	>40

Haemolysis: (+) present, (–) absent; Clotting was scored as following: (++) the ability to withdraw >0.3 ml and <4 ml, (+++) >0.3 ml of blood immediately after euthanasia. [a]Data is for SD rats, as enough samples for the test could not be collected from CD-1 mice. [b]Could not be measured. [NA]Not applicable; however, clotting developed in both G4-NH_2 and G7-NH_2 after the addition of dendrimers to the collected blood from human volunteers, with G7 exhibiting relatively more clotting. *Values are statistically significant compared with the control group.



Hepatic (L) and GI (R) haemorrhage



Haemolysis of mice RBC (L), control (R)

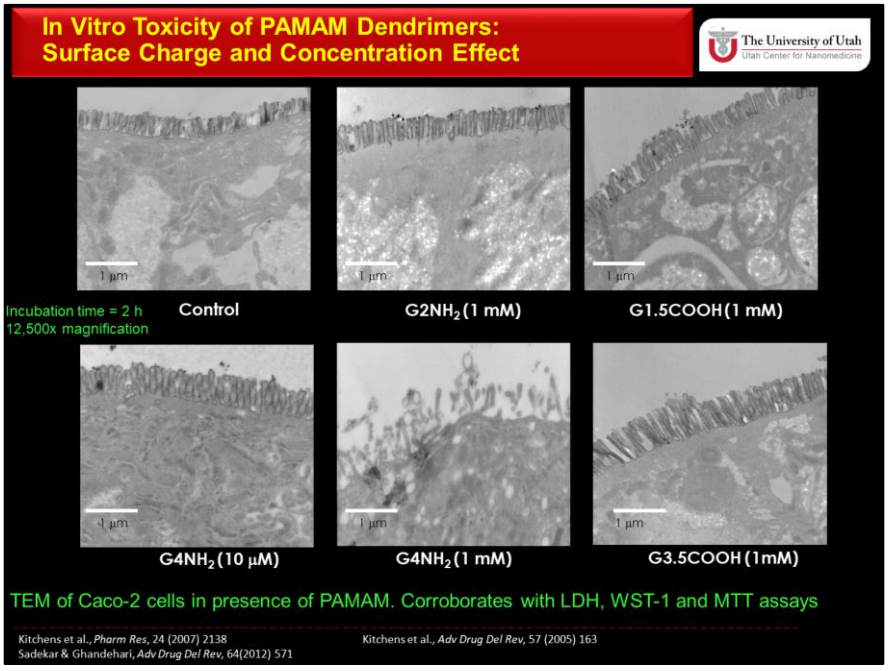
Human blood clotting (L), control (R)

- **Amine-terminated dendrimers cause DIC (Disseminated Intravascular Coagulopathy)**

Greish K., *et al.* Nanotoxicology, 2012;6(7):713-723

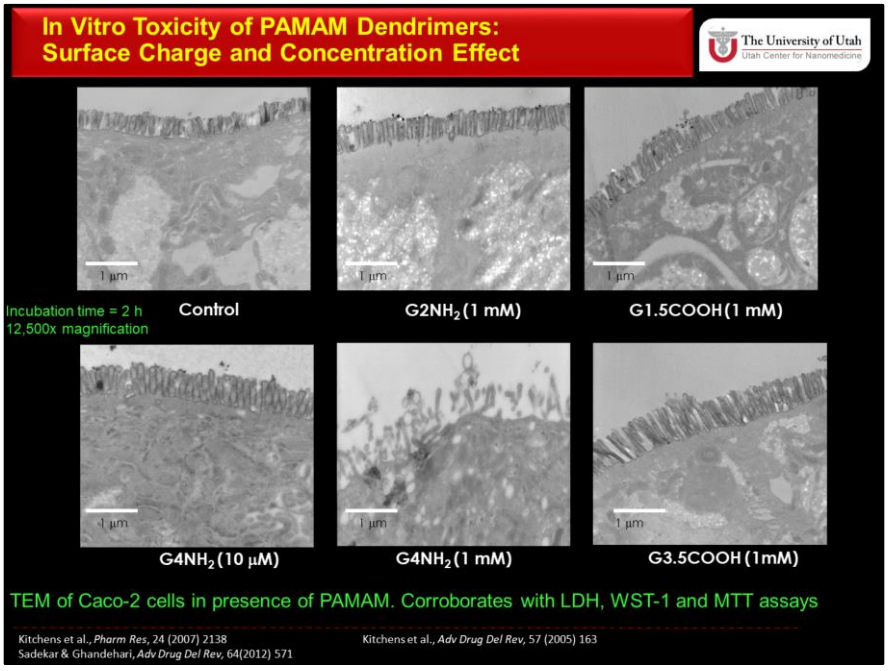
>> HAMID GHANDEHARI: The next slide, blood hemocompatibility with PAMAM dendrimers, we have clearly shown that cationic PAMAM dendrimer G4 and G7, both in human blood as well as animal blood, cause these blood clots and basically, because of their size, these small particles, as I mentioned, I am talking about 3, 4, all the way to 10 to 12 nm, because of their size and flexibility and all these high-density cationic residues, are able to cross link fibrinogen. We have published a couple of papers on how they cause blood clots. So it's very clear that, similar to environmental effects, in medicine investigating the biocompatibility of the materials is extremely important.

At the end of the day, we don't want a cancer patient to have blood clots while you're trying to deliver, let's say doxorubicin with cationic dendrimers.



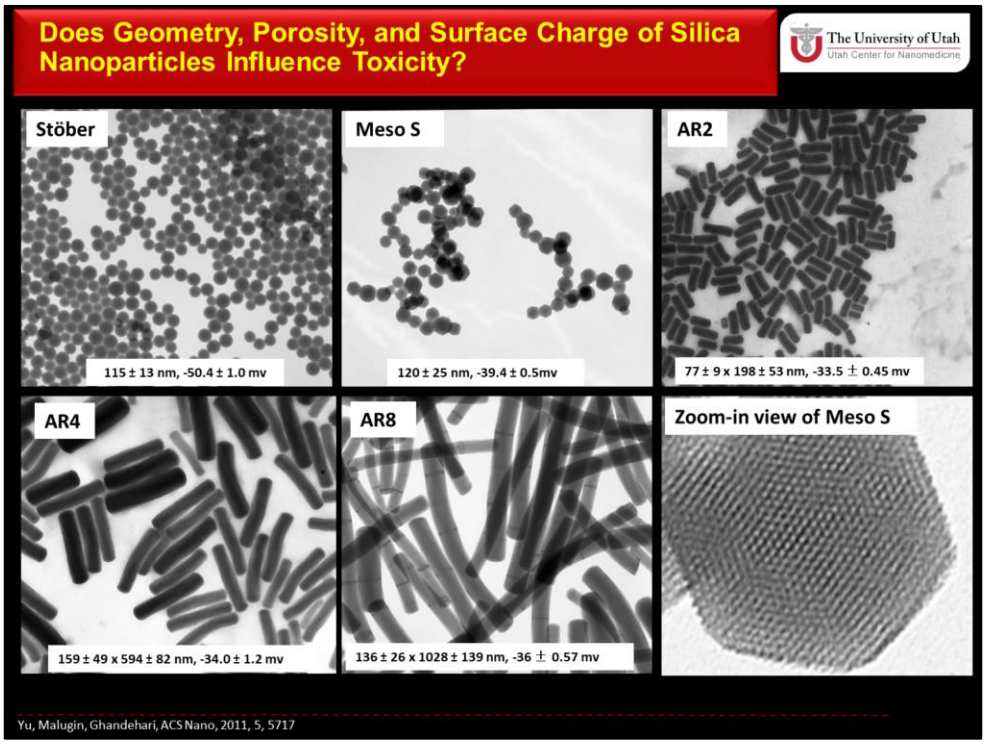
>> HAMID GHANDEHARI: This slide shows our efforts on trying to understand how would dendrimers of various charges and generations influence epithelial barrier of the gut? The gut is lined with mucous and underneath that you have the epithelial cells that have a lot of microvilli which are responsible for absorption and transport and a whole bunch of other things.

Again, this is just an example of a whole series of studies that we did that demonstrated that PAMAM dendrimers opened the tight junction. This is not shown here; also they enhance their own endocytosis, but what is shown here is TEM images of Caco-2 cells that resemble the epithelial cells commonly used for transport of drugs across the epithelial barrier of the gut. On the top left you see the control: there's no dendrimer, just buffer; the microvilli are intact.



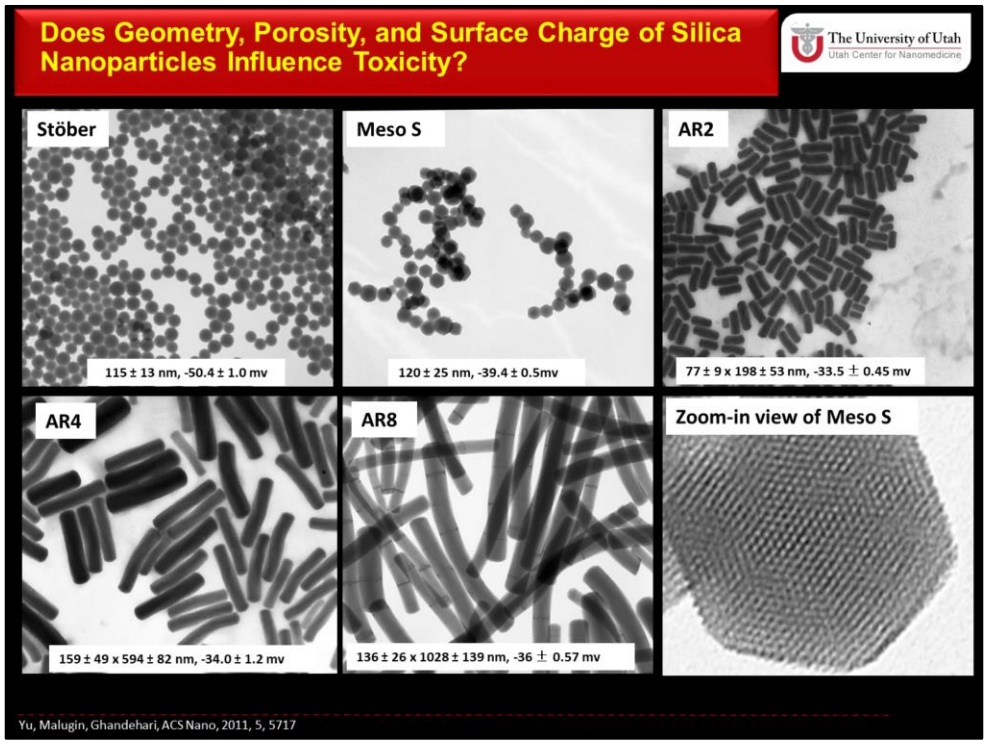
>> HAMID GHANDEHARI: You go to the right, G2 is a smaller amine-terminated dendrimer; still the microvilli are intact. Further to the right, G1.5 which is the same size as G2 but carboxyl-terminated, does not show any changes in the microvilli. But at the bottom of the slide on the left-hand side, G4, which is the larger dendrimer amine-terminated at 10 micromolar, you don't see an effect, but at 1 mM, the moment that you increase the concentration, you see a sloughing of the microvilli. We have also corroborated these results with other assays such as LDH, MTT and other conventional assays.

What I'm trying to say in this slide is that toxicity is concentration- and generation-dependent, and in other works we have shown it's also incubation time-dependent. In other words, generation for NH_2 at a lower concentration during that period of time is not toxic, but the moment you increase the concentration, or in some cases, where you incubate for a long time with the cells, you will have a toxic effect.



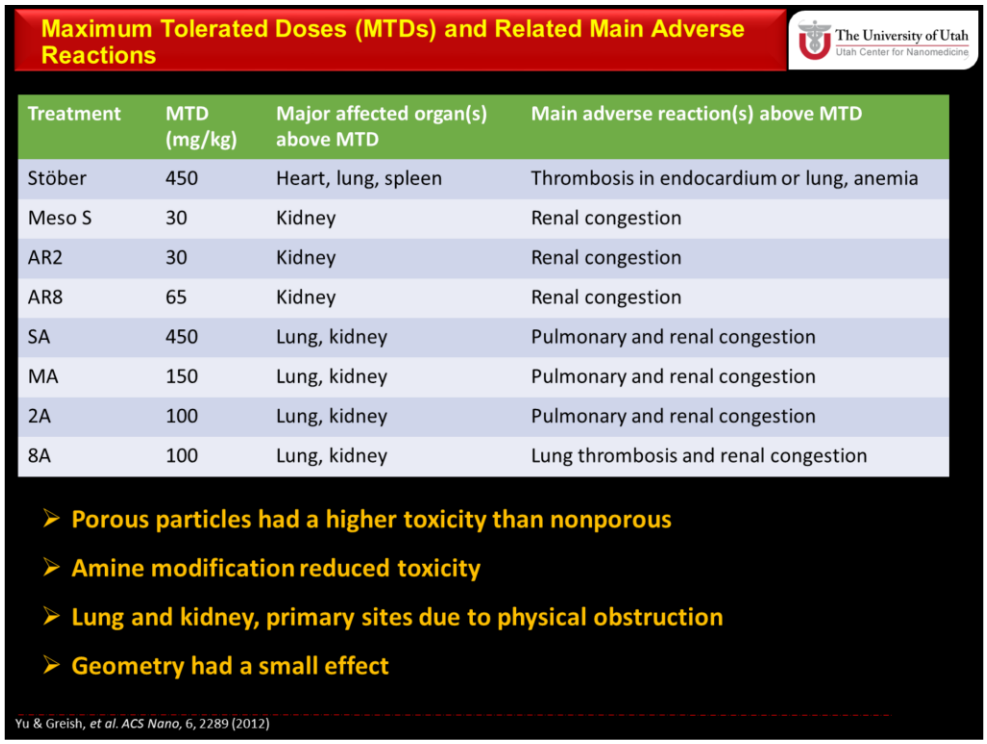
>> HAMID GHANDEHARI: The next slide shows some examples of how geometry may or may not have an effect. This slide is titled "does geometry, porosity, and surface charge of silica nanoparticles influence toxicity?" We synthesize a series of different silica NPs. At the top left is Stöber; these are nonporous materials. The top middle shows mesoporous materials; if you look at the larger zoom-in view of that mesoporous material on the lower right hand side, you see that there's the porosity. So we synthesize non-porous, porous, and three different aspect ratios of these SiO_2 NPs. The third one at the top is aspect ratio 2, AR2, which means that basically you divide the length by the diameter and that's aspect ratio 2, 4, 8.

These ones with the rod shaped structures, they are porous materials so the only non-porous materials we studied were the Stöber particles. We did our best to keep one dimension similar. In other words, for Stöber, you could call it 100 nm Stöber, that's 115 nm plus or minus 30 nanometer, the mesoporous 120 nm or so, and the other ones, we tried to keep this to the best of our ability one dimension constant, and measured zeta potential. They're very similar, and then we looked back at the maximum tolerated dose and some of the related adverse reactions, which is the next slide, in mice, and I think these are also the CD-1 mice.



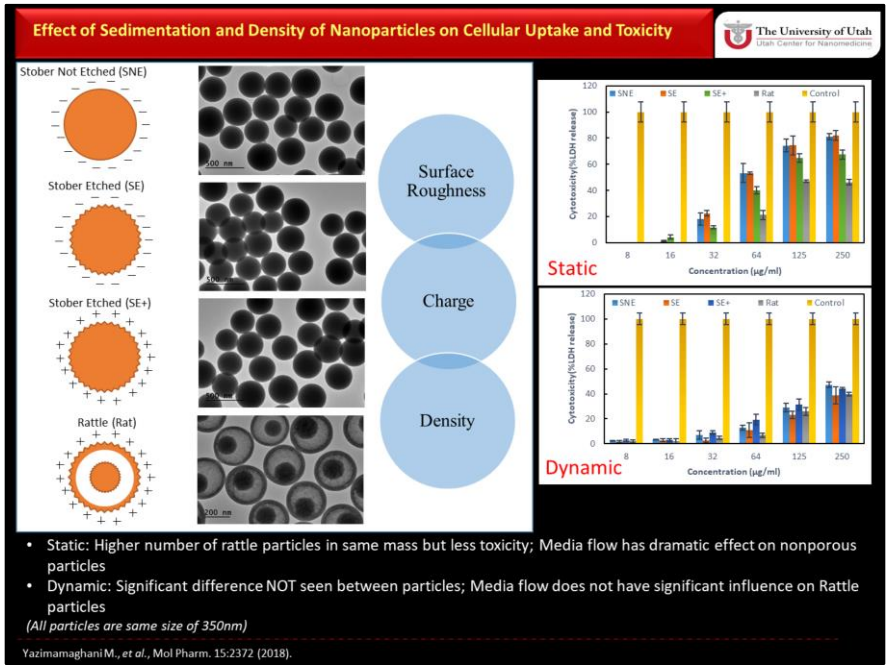
>> HAMID GHANDEHARI: Interesting results that we found: We don't have the answers for the reasons for all of these, but we see this consistently and we're trying to investigate some of those mechanisms. So for Stöber particles, those are the non-porous small surface particles, we see that the maximum tolerated dose is about 450 mg/kg and above that you see thrombosis in the endocardium, lung, and some anemia. Basically when we look at the histology, which again I don't have the time to show but I will be happy to share it and it's listed in the publications listed in the very last slide), we see that when you deliver a lot of these materials it's going to just get stuck in these different tissues; that's related directly to the toxicity of that tissue.

Interestingly, with mesoporous materials of similar features except porosity, *in vitro* cell culture, we see in fact that they're less toxic, but *in vivo* consistently we see that they're more toxic compared to the Stöber particles. We don't have a good reason for that yet; we believe it has to do in part with aggregation of the materials. We have carefully purified this characterization, and it's not the effect of the CTAB (cetyl trimethyl ammonium bromide), but consistently we see that mesoporous materials are less tolerated by mice compared to Stöber particles.



>> HAMID GHANDEHARI: Geometry in this case did not have a huge effect in terms of their toxicity profile. Even though longer particles in this case had higher maximum tolerated doses (MTD) and when we amine modified, this is SAMA2A8A, when we amine modified these systems, we saw they're less toxic even though with a lot of the polymer materials it is commonly known that cationic polymers have more toxic effect. For some reason we see consistently when we amine modified these SiO_2 nanoparticles, we see this toxicity reduced, which I think has to do with the amount of uptake by macrophages of the reticulum endothelial systems. And in the liver, spleen and lungs, the amine modified systems are taken up to a lower extent.

Again, we have to carefully investigate the reasons. One potential reason, as Christine pointed out, is protein absorption. We have investigated the protein absorption *in vitro* and we don't see a lot of different types of proteins. Depending on the surface charge, there are very similar types of proteins adsorbed. Again, I don't have the slide here but I'm happy to discuss it later.

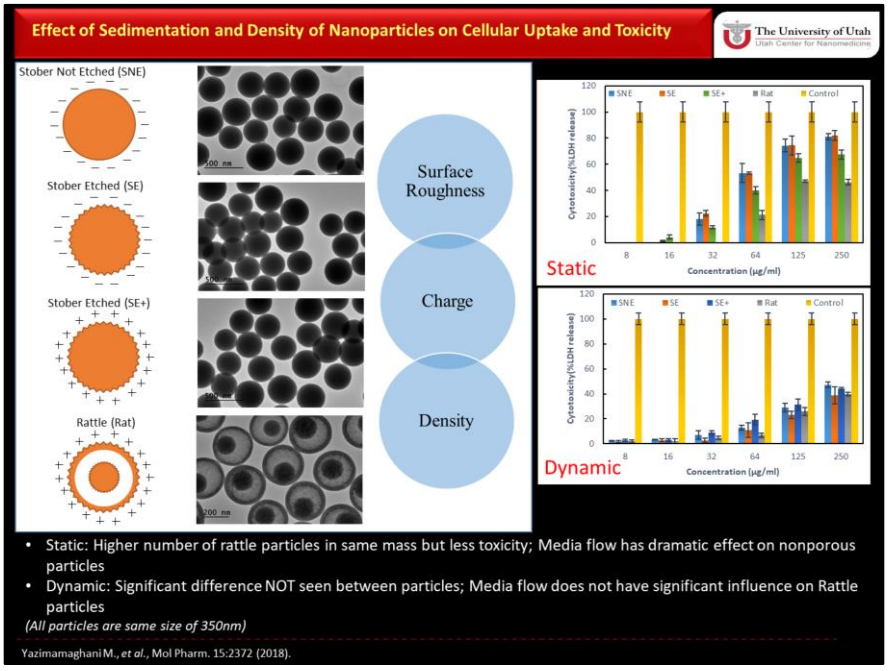


>> HAMID GHANDEHARI: The next slide is an illustration of how density and flow conditions can have an effect.

Often times when we do nanotoxicology experiments, in majority of the cases, you have a simple 96-well plate. The cells are there and you have the media on the top, and then we just administer our nanomaterial, hopefully homogeneous material, at the right dosimetry as Monika mentioned earlier.

Covering for all of that, still a lot of times in the body, for example in the bloodstream, you're dealing with these materials that are under flow. So we ask a question: would flow have an influence on these materials and whether density would have an effect?

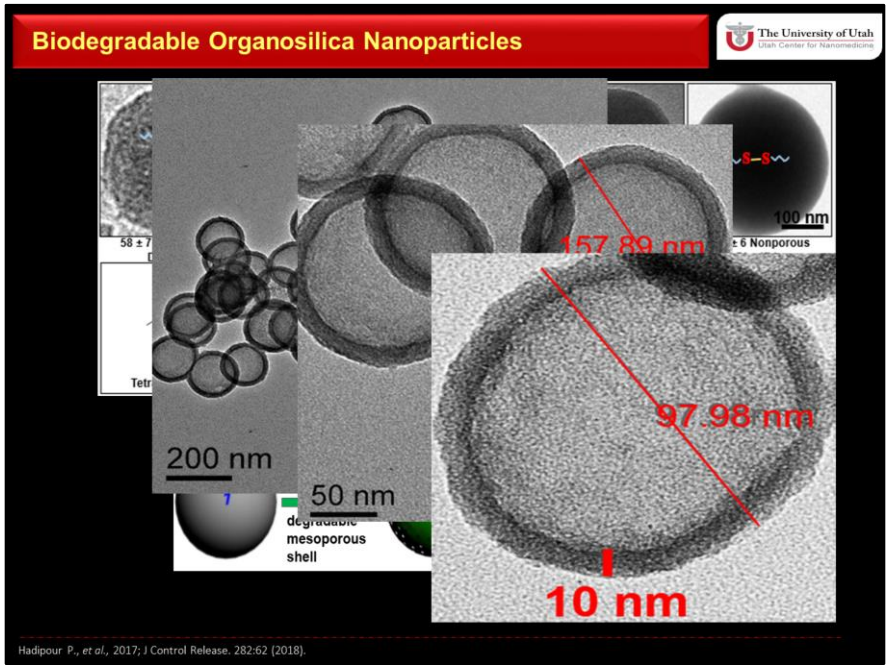
So in this slide, what you see here is from the very same templates of silica nanoparticles, we have the negative surface due to the siloxanes. Then we etch the surface to make it rougher, and then we have a surface that is positively charged. Then finally we have a nanoparticle that was etched inside so it has lower density.



>> HAMID GHANDEHARI: So in this slide, the key point is that we tried to vary surface charge and vary the density and surface roughness, and on the right-hand side we looked at the toxicity based on just a simple LDH assa: cytotoxicity of these materials as a function of concentration and depending on which particles we used. Under static or dynamic conditions, and in this case dynamic was simply tilting with a shaker, we didn't do anything more sophisticated, lets' say microfluidics or what not, but simply shaking the suspension of particles.

What you see on the top side, if you look at, for example, the gray bar, this is the rattle particle which has lower density. You control the rattle particle, in this case under static conditions, and you have a very different toxicity profile compared to dynamic conditions.

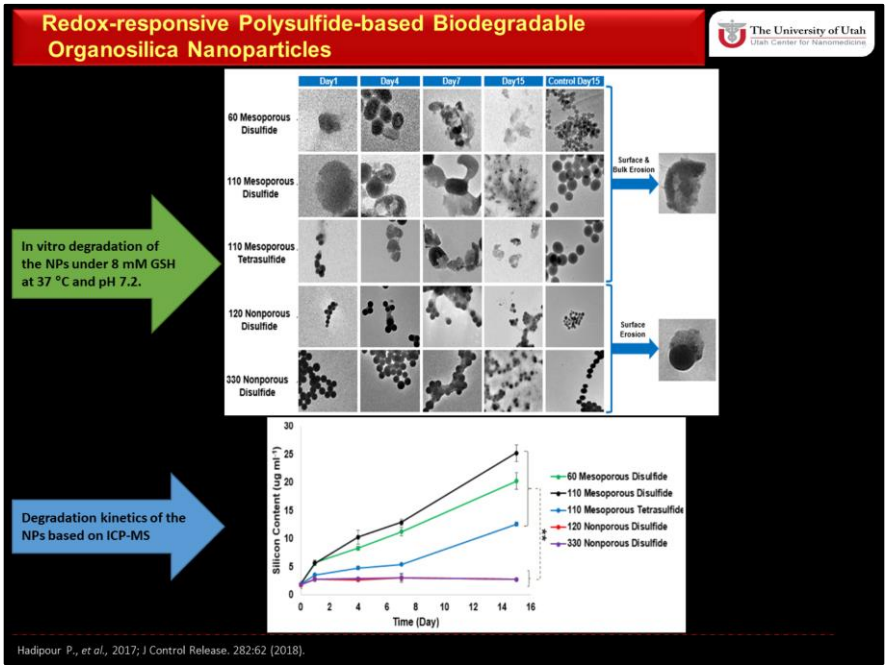
Under dynamic conditions, the toxicity profile of all these systems are the same because simply you don't have that effect of sedimentation present under static conditions. So flow matters; density of the materials also matters.



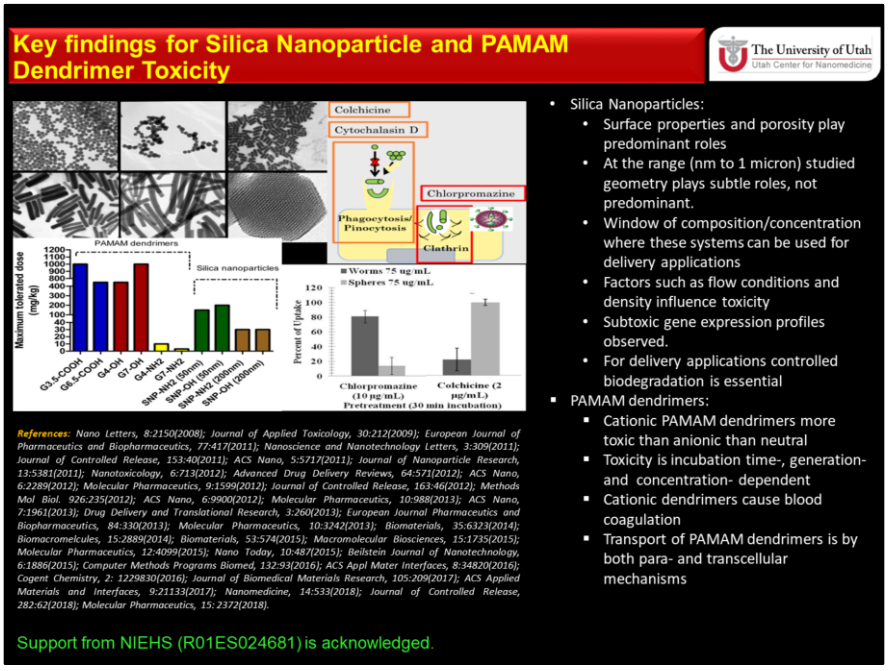
>> HAMID GHANDEHARI: One of the challenges with a lot of these inorganic materials, including silica NMs is you don't have a controlled biodegradation process. Those of us who have worked in the drug delivery field for many years know that it's really important to be able to have a material with a controlled degradation elimination process.

But for silica, you have the dissolution, but dissolution varies from particle to particle. So recently we have synthesized some SiO_2 particles, this is the slide on biodegradable organosilica particles that contain di or tetrasulfide bonds that will degrade with intracellular glutathione. I animated these slides and you can see that we have made some hollow silica nanoparticles. We are able to incorporate a lot of bioactive agents within these materials because one of the challenges with mesoporous silica particles is that you can load them but the loading is passive and easily the material will come out.

If you make hollow nanoparticles, the drug within the material would be better protected and release will be controlled over a period of time.



>> HAMID GHANDEHARI: In the next slide, I've shown the basically degradation kinetics of these materials. There's two take-home messages: size matters and porosity matters. In the case of this mesoporous disulfide, lets say for example that's a 60 nm mesoporous disulfide, these materials degrade much faster compared to much larger and nonporous disulfide systems. Because of the porosity, you have increased solvent penetration, and as a consequence, you have basically faster degradation.



>> HAMID GHANDEHARI: And in the final slide, I have summarized some of our findings. One thing I didn't get to talk about: if you look at this main slide on the right-hand side, we made some worm-like silica nanoparticles, which were about 1 micron or so in length and about 50 nm in diameter, and we looked at their uptake in various types of cells, epithelial macrophages, and bottom line, with the worm-like structures, which are long, high aspect ratio structures, we saw they are primarily being taken up by phagocytosis, where as some of the other structures made, which were spherical or with much lower aspect ratios, were taken up by clathrin-mediated endocytosis.

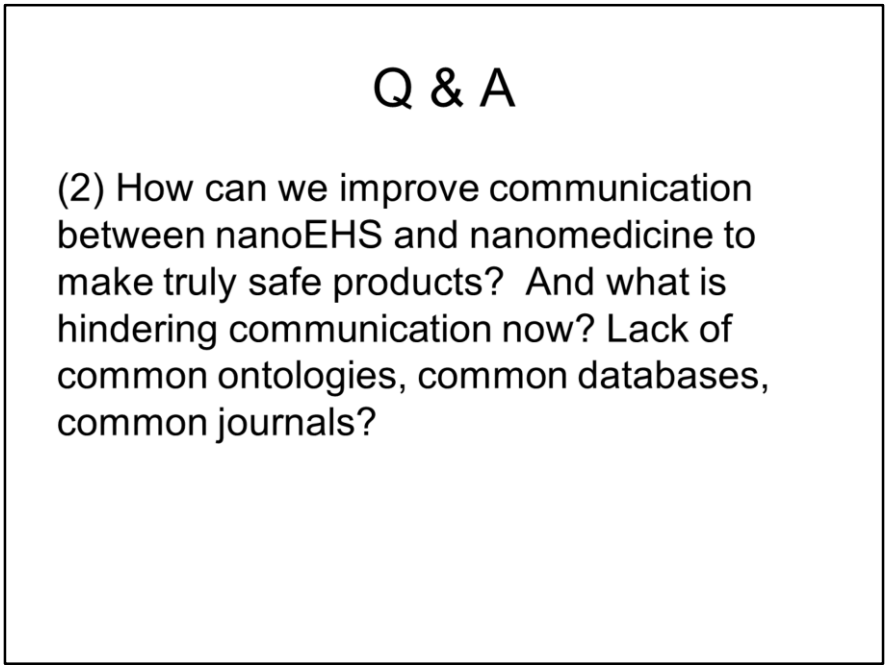
So again, that slide demonstrates that aspect ratio and geometry can influence not only the extent of cellular uptake and bio-distribution, but also mechanism of uptake. In this slide I tried to summarize some of these things and listed some of those publications if anyone is interested in further reading, and I would be happy to answer any questions anyone might have.

Q & A

(1) Is there a concern that the Nanomedicine can cross the blood brain barriers?

>> CHRISTINA LIU: We have questions from the attendees. The first one: Is there a concern that the nanomedicine can cross the blood brain barrier?

>> HAMID GHANDEHARI: Maybe I can address that. I wouldn't say we would be concerned or not: at the end of the day, what do you want to use it for? If this is a material that you don't want to go across a blood brain barrier and you want it to be in the systemic circulation, but not past the blood brain barrier, you have to design it as such. But there are cases where you want to actually have nanomaterials cross the blood brain barrier, specifically to the CNS (central nervous system) tissue.



>> CHRISTINA LIU: Next question: How can we improve communication between nanoEHS and nanomedicine to make truly safe products? And what is hindering communication now? Lack of common ontologies, common databases, common journals?

>> CHRISTINE PAYNE: This is Christine, I think that is a good and hard question, because these communities don't overlap very often. I guess we could ask NIH or NIEHS to organize a conference or a workshop that could bring the groups together so we could have a more personal connection, or have some overlap in the Gordon conferences that both groups organize.

Likewise, for nanoEHS, there's so many interesting methodologies that colleagues are using, and Monika talked about a couple of them; people in the drug delivery community and nanomedicine community can really try to use these technologies for better characterization of the material.

There's no question there is a significant overlap that we can all benefit by having these types of joint symposia and what not.

Q & A

(2) How can we improve communication between nanoEHS and nanomedicine to make truly safe products?

And what is hindering communication now? Lack of common ontologies, common databases, common journals?

>> HAMID GHANDEHARI: I completely agree. For example, I know for a fact that both communities can really benefit from each other.

For years and years, for three, four, five maybe, five decades or so, before even the word "nanomedicine" was articulated in the early 2000s, for example the liposomal polymeric drug delivery community have been working on these problems of trying to understand—they called it biocompatibility, but that's the other side of the coin for nanotoxicology.

And I noticed in the mid-2000's, where there was this surge of interest in nanotoxicology, the nano and toxicology communities working on this subject really didn't make direct connections with all the observations that, let's say, the drug delivery community had about these materials. So I think the two communities can learn from each other.

Q & A

(3) What are typical exposure threats we can expect from the Nanomedicines? How can they be reduced and what impact do tox findings normally have on formulation and development of Nanomedicines?

>> CHRISTINA LIU: Let's go for the next question.
What are typical exposure threats we can expect from the nanomedicines? How can they be reduced and what impact do tox findings normally have on formulation and development of Nanomedicines?

>> HAMID GHANDEHARI: Maybe I can comment on that. If I understood the question correctly, what are the typical exposures? And what would the formulation component impact the toxicity? I believe that's what the question was. And to me, the answer to that is really case specific. Nanomedicine is really broad. If you're trying to develop a trace imaging agent, the tox profile for that system, where you have to deliver small amounts to the body, is much different than, for example, when you're trying to deliver a drug that's not so potent with a nanoparticle, because then you have to deliver a lot of that nanoparticle. So that's one aspect that we have to worry about. What is the specific application?

Q & A

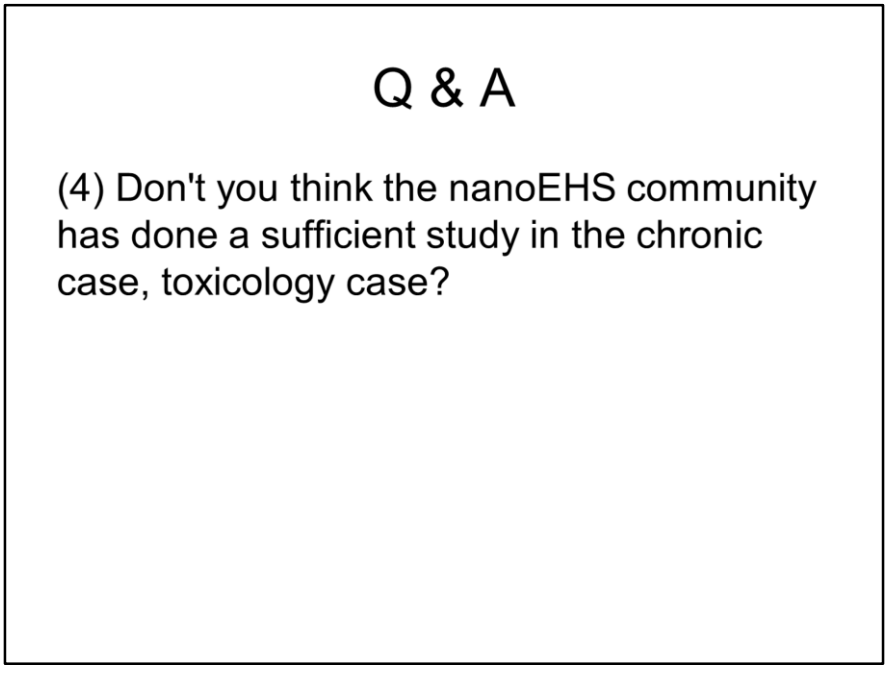
(3) What are typical exposure threats we can expect from the Nanomedicines?

How can they be reduced and what impact do tox findings normally have on formulation and development of Nanomedicines?

>> HAMID GHANDEHARI: Say, for example, for a solid tumor delivery, you have to deliver a lot or a little of it to get there, maybe 5% maximum or 10% injected dose, whereas if you're trying to treat infections in the liver, for example, because the nanoparticles are taken up by the liver, so that dosimetry is going to be very different.

That's one issue. I think another issue that hasn't been sufficiently studied and I think we have to pay more attention to, is to do chronic toxicology studies. There's very little information out there to tell us that this silica, or gold or CNTs or whatever they are exposed to, and they reside in a lysosomal compartment, what is that going to do to people?

So I think this chronic toxicology situation has to be closely examined. Those are some comments. But I think again it depends on the specific case.

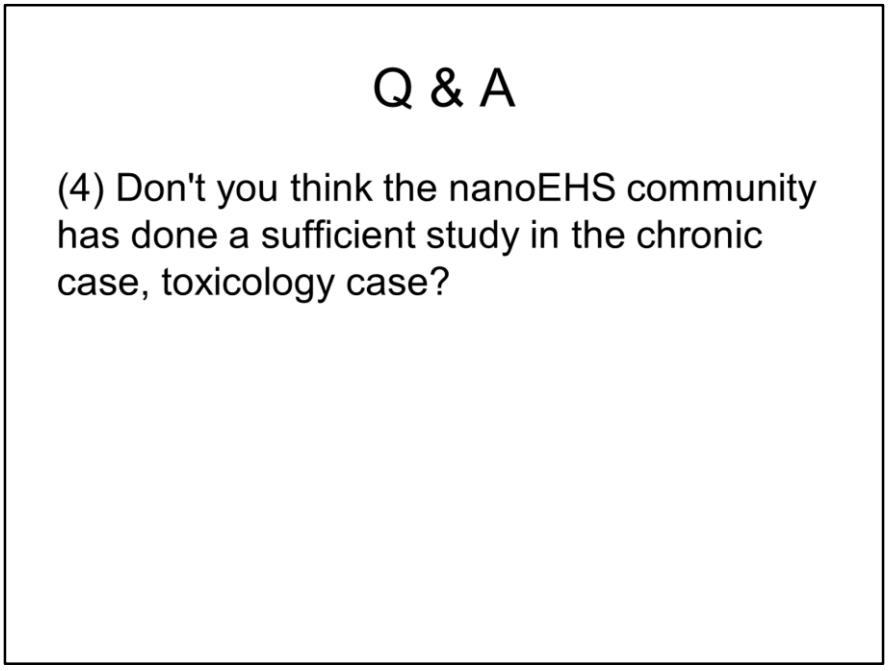


>> CHRISTINA LIU: Don't you think the nanoEHS community has done sufficient study in the chronic case, toxicology case?

>> HAMID GHANDEHARI: I personally don't think so. We just recently did a search on chronic evaluation of inorganic nanomaterials and there were literally a handful of papers. By chronic, I mean exposure over a year, and testing these animals and seeing what's happening. That's to the best of my knowledge.

>> CHRISTINA LIU: Do we have any comments from the other panelists?

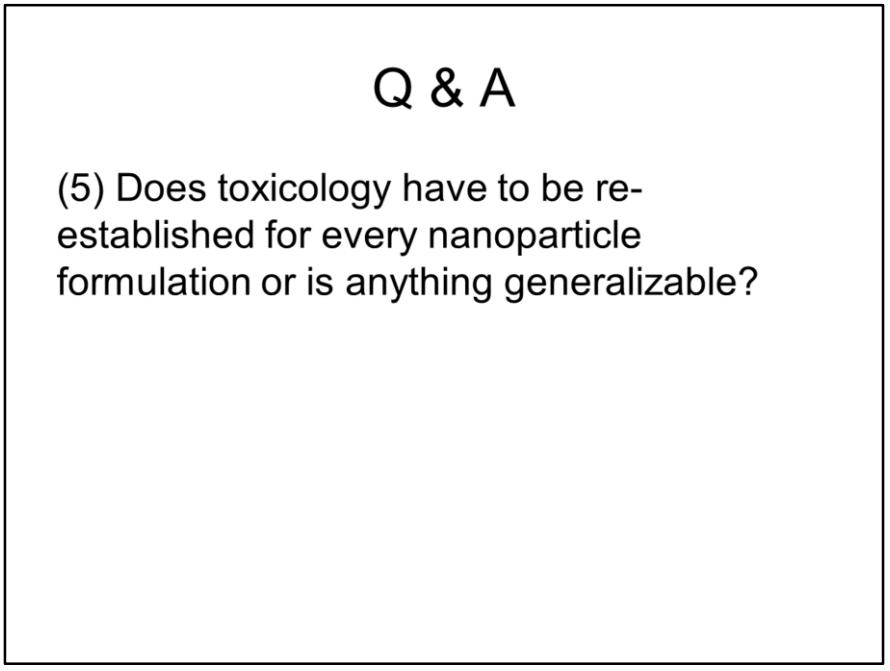
>> MARK HOOVER: I think this is an area, looking at chronic exposures, where we have an opportunity to be better informed about likely patient and workplace exposure scenarios so that there can be a proper selection of dose and dose rate for the chronic exposures.



>> MARK HOOVER: And when we look back at the experiments that have been done on materials, just for general toxicity, you see thousands and thousands of papers on toxicity studies in laboratory animals and cells, and you see just hands full of papers that actually document the concentrations and forms, the details of the concentrations and forms that would actually be used, and likely to be seen by real people.

So, if we're going to, as we should, enter into studies of chronic exposures, we really need to have the exposure-informed hazard assessment followed carefully, because these experiments are expensive, and they should be done in ways that inform us for dose-response curves that are relevant to situations that we really may encounter with real people.

That's my little aside and challenge and opportunity to the community.



>> CHRISTINA LIU: I have more questions on the screen right now. Does toxicology have to be re-established for every nanoparticle formulation, or is anything generalizable?

>> HAMID GHANDEHARI: That is a really good question, and my colleagues can pitch in. I have a hard time saying that for everything —some things are generalizable. There are certain things, like, for example, surface chemistry that really consistently has a predominant effect. Size matters.

So those are a couple of things that I think are, if you will, generalizable, but everything else has to be studied in detail, because the moment that you change the properties of nanoparticles, you're changing the whole set of things, such as aggregation, protein adsorption, potentially degradation, and things like that. But I think that maybe surface properties and size are two kind of key parameters to look for at the very beginning, in my view.

Q & A

(5) Does toxicology have to be re-established for every nanoparticle formulation or is anything generalizable?

>> MONIKA MORTIMER: So I wanted to add to that question about the generalizability of the nanotoxicity. I think the field in environmental or even human health risk assessment is moving in the direction of having methods in place that can be used for screening of toxicity for a large amounts of different nanomaterials.

So, this could not be directly applicable to nanomedicine field, where it might be important to test each single drug or medical application before it can be applied to humans, of course, but I think both fields, at least in the screening stage, should move forward to high-throughput testing, and as I mentioned omics technologies, computational methods, and read across just to facilitate and speed up the risk assessment in the first stage, where a large amount of different nanomaterials are synthesized.

When these methods improve over time, I think we can actually conclude in the screening step which materials should be tested more thoroughly in the next step. I think it's becoming more realistic over time as the methods improve.

Q & A

(6) Are these silica nanoparticles coated with PEG to prevent agglomeration and trapping in the lung?

When you say silica NP do you mean amorphous or crystalline SiO_2?

>> CHRISTINA LIU: The next two questions are more specific to silica nanoparticles: are these silica nanoparticles coated with PEG to prevent agglomeration and trapping in the lung? When you say silica NP do you mean amorphous or crystalline SiO_2?

>> HAMID GHANDEHARI: That's a very good question, the ones I showed in the slide were not PEGylated but we've also made PEGylated systems in the lab. These are amorphous silica, and of course the mesoporous systems are made with CTAB, but in these cases for which I've shown, they are not PEGylated.

Q & A

(6) Are these silica nanoparticles coated with PEG to prevent agglomeration and trapping in the lung?

When you say silica NP do you mean amorphous or crystalline SiO_2?

>> CHRISTINA LIU: And about the silica NPs, are they crystalline or amorphous?

>> HAMID GHANDEHARI: That's what I was just referring to. They're amorphous SiO_2 NPs, and the mesoporous systems were made with CTAB. But generally speaking the Stöber particles are amorphous particles and not PEGylated. But there were other works where we have PEGylated these systems as well.

Q & A

(7) Do you think there will ever be a unified framework, or will it always come down to the details of each nanomaterial in each experimental setting

>> CHRISTINA LIU: One of the themes from all of the talks seems to be that all of the details matter in assessing nanotoxicology. Do you think there will ever be a unified framework, or will it always come down to the details of each nanomaterial in each experimental setting

>> CHRISTINE PAYNE: I would really like to think that there will be a grand unified theory of nanotox, but it is hard, and right now we do, in general as a community, look at small sets of nanoparticles—maybe five or ten different functionalization schemes—and we compare some outcome.

Monika talked about an omics approach; that will be useful for developing larger trends, and there's a handful of groups that are working on automating functionalization and just doing much higher-throughput experiments. You can imagine doing 100 nanoparticles with different functionalizations instead of 10, and then we could have the ability to identify trends.

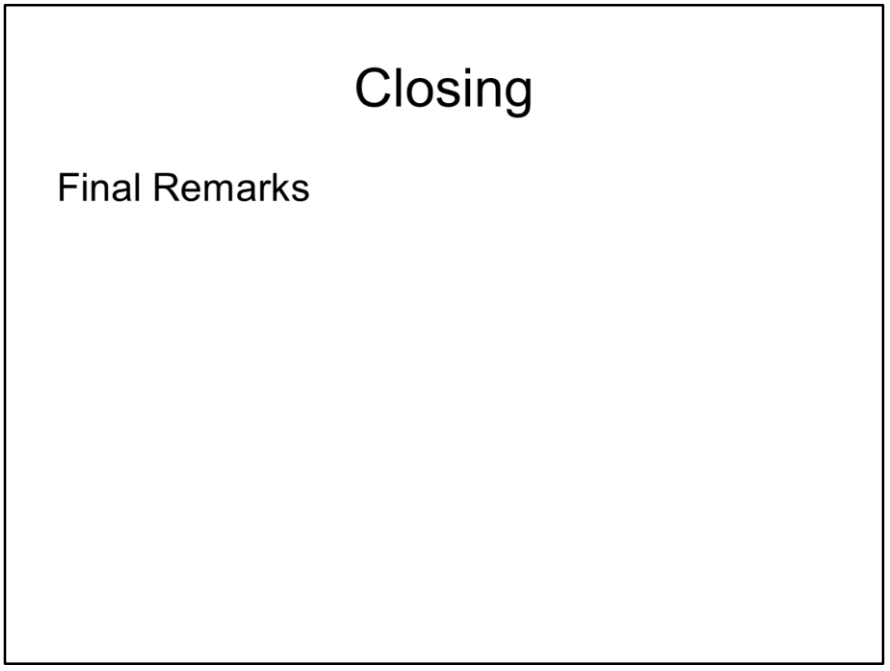
Q & A

(8) Do you think there will ever be a unified framework, or will it always come down to the details of each nanomaterial in each experimental setting

>> CHRISTINA LIU: The last question: Would you agree the role of functionalization in nanomaterial effects is in an area where both disciplines have seen cross-fertilization?

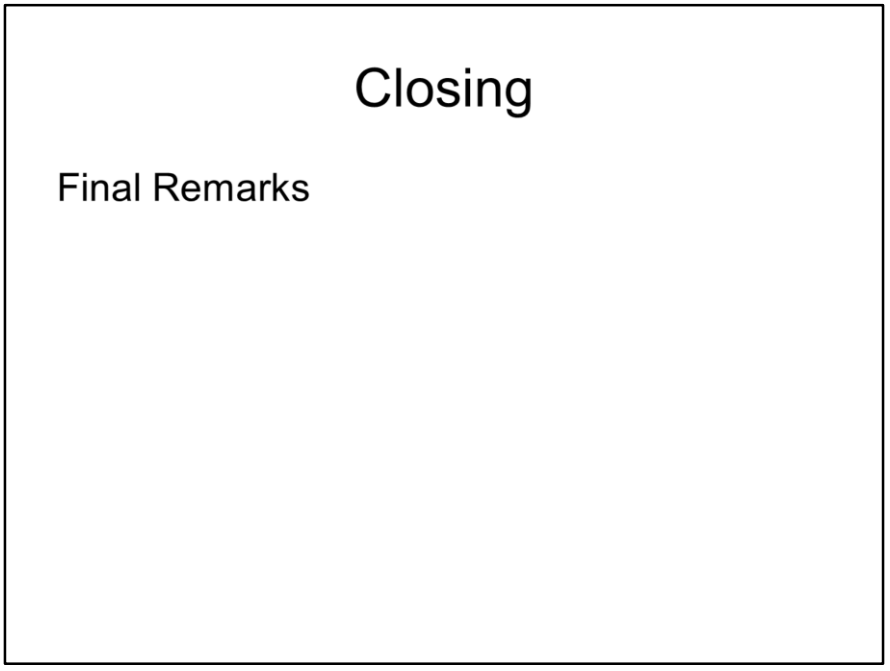
>> HAMID GHANDEHARI: I think the answer is yes.

>> CHRISTINE PAYNE: My concern is they've seen the same results but without knowing the other community was working on it.



>> MARK HOOVER: I certainly think that's a great note to end on —the fact that our communities are doing such terrific work, and that we have this opportunity to share what we're learning and how we're learning it and to have some common protocols and sharing, and “post-docing” and sharing scientists.

So, on behalf of the NNCO, and Christina and myself as moderators, thank you to the presenters and all of you who logged in today. I saw a lot of very familiar names, and also some that I haven't seen before, so thanks to everyone, especially as we continue to engage in this and as we create a new group on nanomedicine.



>> CHRISTINA LIU: Thank you for your participation. And we thank this wonderful panel for their informative presentation and very intelligent advice. Thank you.

>> MARK HOOVER: Yes, and to anyone who would like to participate in the future or has ideas for what we should include in the next webinars, please do let us know. So, with that, we'll close. Thanks to everyone.