Tracking Silver Nanoparticles Aging in the Environment

Brian Reinsch and Gregory Lowry (Carnegie Mellon University)

CEINT researchers in Dr. Lowry's lab have shown that AgNPs are particularly susceptible to weathering by inorganic or organic sulfur containing species in the environment like sulfide and cysteine. This weathering impacts the fate, transport, lifetime, and potential toxicity of Ag NPs in the environment. Silver nanoparticles are exposed to simulated environmental conditions and then characterized using X-rays from the Stanford Synchrotron Radiation Laboratory (SSRL), a DOE research facility. These analyses provide specific information about the mineralogical changes AgNPs will undergo in the environment. Figure A is a TEM image of a cluster of silver nanoparticles and Figure B shows a single Ag nanoparticle that has formed a Ag_2S shell from exposure to sulfide, indicated by different lattice fringes in the center and outside of the particle. X-ray absorption spectroscopy (EXAFS) shows a quantitative confirmation of this oxidation of the particle from ~90% Ag^0 to 50% Ag^0 and 38% Ag_2S, when exposed to sulfide (Figure C).

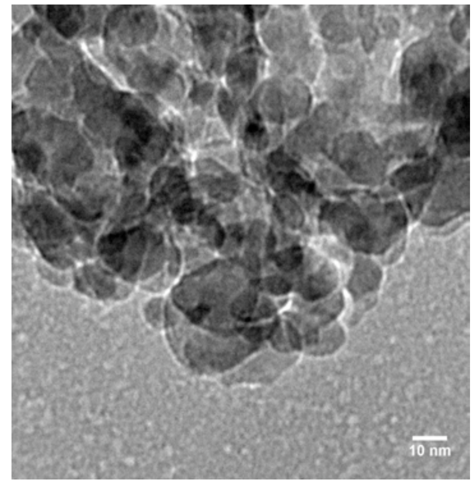

Figure A

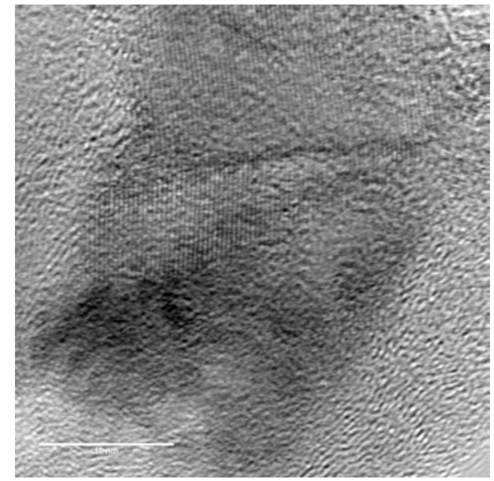

Figure B

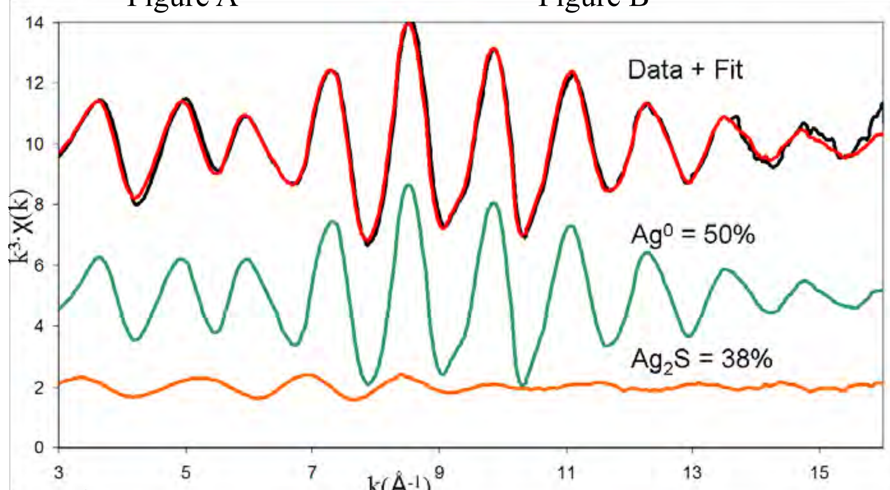

Figure C

NSF EF-0830093

Silver Nanoparticle Impact on Biofilm Viability: Effect of Adsorbed Natural Organic Matter

Caitlin Trombly, Stacy Pustulka and Robert D. Tilton (Carnegie Mellon University)

Students in the laboratory of Prof. Robert Tilton (CEINT investigator) are investing adsorption of macromolecules, representative of natural organic matter (NOM), to silver nanoparticles for the purpose of interpreting subsequent interactions with bacterial biofilms. The sequence of adsorption is of particular interest, as the composition and structure of adsorbed macromolecular layers often are trapped in persistent non-equilibrium states. Figure 1 shows that competitive adsorption of humic acid (HA) and bovine serum albumin (BSA), a model protein, is dominated by the protein. When humic acid is present in a mixture with the protein at a concentration that corresponds to saturation adsorption in the absence of protein, the humic acid has little effect on protein adsorption, while increasing protein concentrations decrease the humic acid adsorption.

Figure 2 shows that silver nanoparticles, without exposure to NOM, inhibit biofilm formation when the particles are introduced to a planktonic suspension. Results to date indicate that the minimum inhibitory concentration depends on the bacterial suspension cell density, *suggesting* direct nanoparticle/ cell contact plays a role. Work is currently underway to measure cell viability and biomass production after nanoparticle introduction to pre-formed biofilms, with particular attention to the effects of adsorbed NOM.

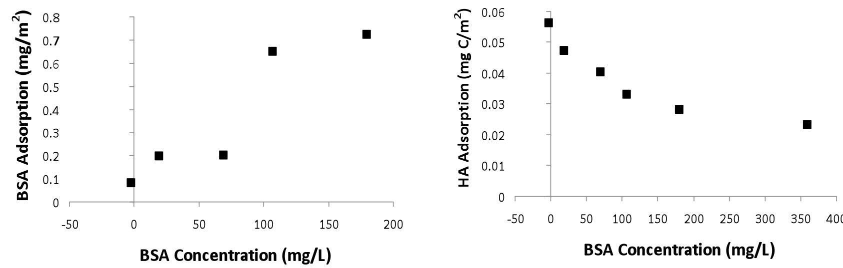

Figure 1. BSA adsorption isotherm on silver nanoparticles is unaffected by presence of HA in solution, while HA adsorption is inhibited by presence of BSA.

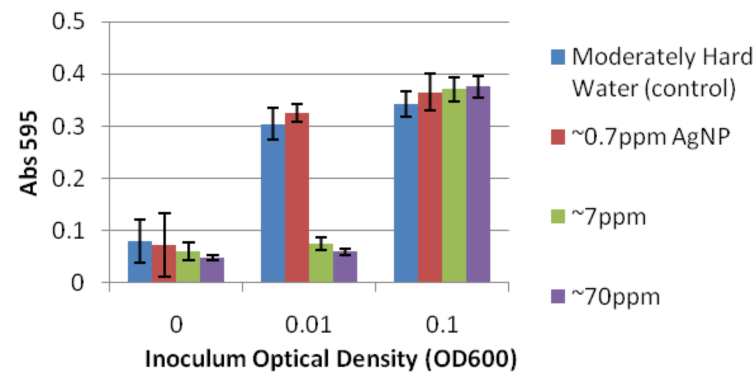

Figure 2. Crystal violet assay of biomass contained in biofilm shows that the inhibitory silver nanoparticle concentration depends on inoculum cell density.

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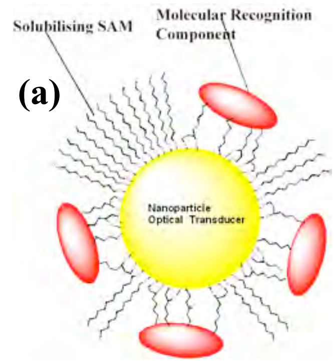
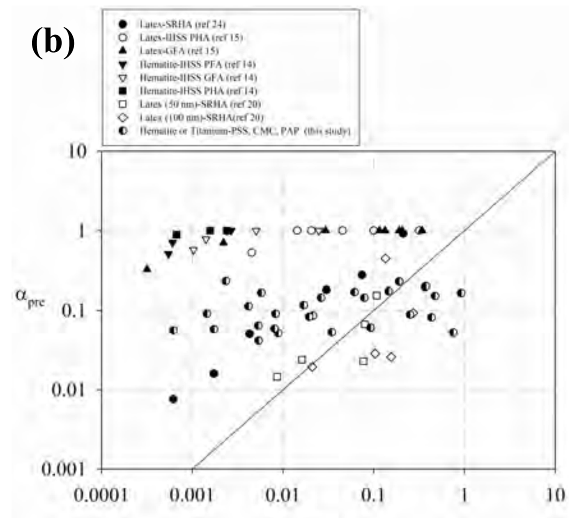
Predicting Attachment of Nanoparticles Coated with Organic Macromolecules

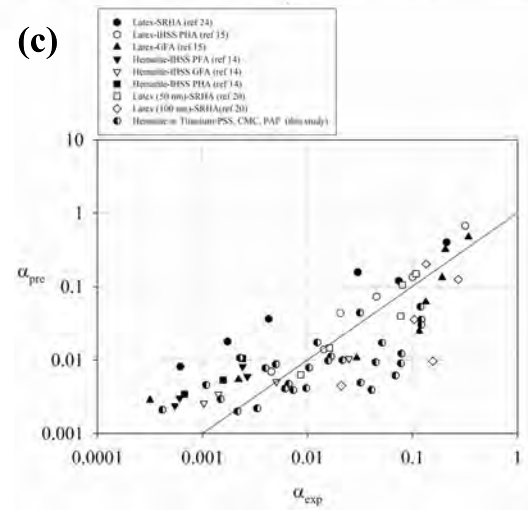
Tanapon Phenrat, Jee Eun Song, Charlotte M. Cisneros, Daniel P. Schoenfelder, Robert D. Tilton, and Gregory V. Lowry (Carnegie Mellon University)

CEINT researchers in Prof. Lowry's laboratory at CMU developed a model to estimate attachment, fate, and transport of nanoparticles (NPs) coated with macromolecules. This research is motivated by the fact that most NPs are either intentionally modified by polymer or polyelectrolyte during manufacturing step to provide specific functionality (Figure A) or unintentionally coated with natural organic matter (NOM) including humic and fulvic acid in the environment. Therefore it is important to understand the effect of organic macromolecule surface coatings on NP fate and transport.





The researchers gathered data of attachment efficiency (α_{exp}) of NPs coated with polyelectrolytes or NOM from literature (since 1998) and their own experiments. Existing correlations used to predict attachment of bare colloids could not predict the attachment of NPs coated with macromolecule (Figure B), in good agreement with their hypothesis that electrosteric repulsion afforded by adsorbed macromolecule layer substantially alters NP attachment. A model that incorporates the physics and chemistry of the coatings on the NPs significantly improves the ability to predict attachment of NPs coated with organic macromolecules and NOM (Figure C). This model also provides mechanistic understanding to design macromolecule surface coatings which minimizes unintended transport in the environment.



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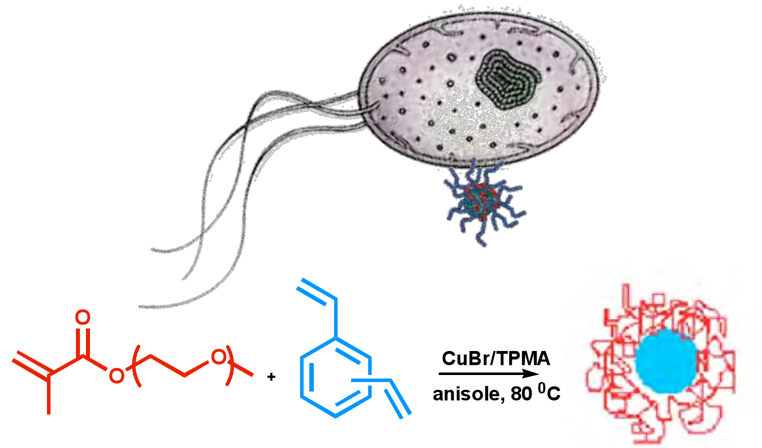
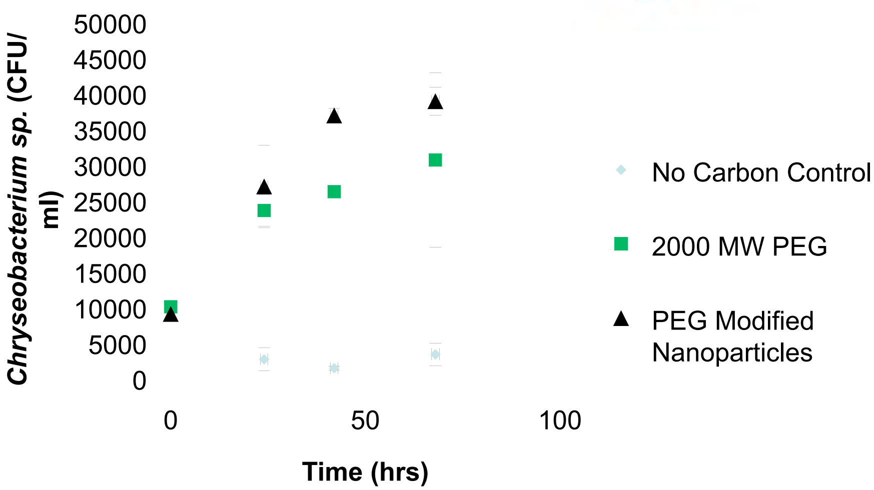
Biodegradation of Nanoparticle Surface Coatings

Teresa Kirschling, Patricia Golas, Kelvin Gregory, Robert Tilton, Gregory Lowry (CMU)





Coatings dictate how particles transport in the environment. An important and yet unanswered question is whether or not polymers that are attached to nanoparticles are bioavailable. Novel nanoparticles with covalently bound polyethylene glycol (PEG) arms were synthesized and fed to bacteria isolates which grow with PEG as the sole carbon source. Growth of the isolates was not inhibited by the presence of the particles, indicating that the polymer coatings are bioavailable. Thus, coatings on NPs will likely be altered upon release to the environment, affecting their fate and transport.

NSF EF-0830093

Adsorbed Polymer and NOM Limits Adhesion and Toxicity of Nano Scale Zero-Valent Iron (NZVI) to *E. coli*.

Zhiqiang Li, Karl Greden, Pedro J. J. Alvarez, Kelvin Gregory, and Gregory V. Lowry
(Carnegie Mellon University)

CEINT researchers in Lowry's and Gregory's lab have found that adsorbed polymer and NOM limits toxicity of NZVI to *E. coli*. Exposure to 100 mg/L of bare NZVI with 28% Fe^0 content resulted were toxic to *E. Coli*. Adsorbed poly(styrene sulfonate) (PSS), poly(aspartate) (PAP), or NOM on NZVI with the same Fe^0 content significantly decreased its toxicity, (Figure A). TEM images (Figure B) and heteroaggregation studies indicate that bare NZVI adheres significantly to cells. Adsorbed polyelectrolyte or NOM prevents adhesion, thereby decreasing NZVI toxicity. This study indicates that polyelectrolyte coatings and NOM will mitigate the toxicity of NZVI for exposure concentrations below 0.1 to 0.5 g/L depending in the coating.

Figure A

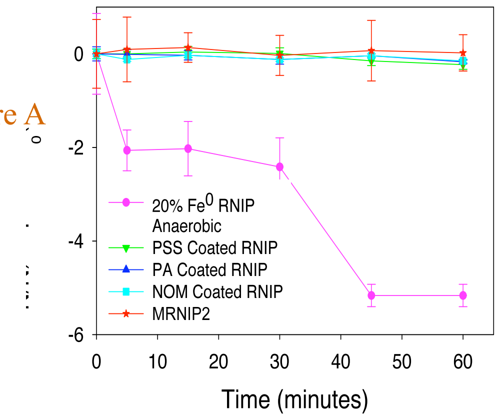
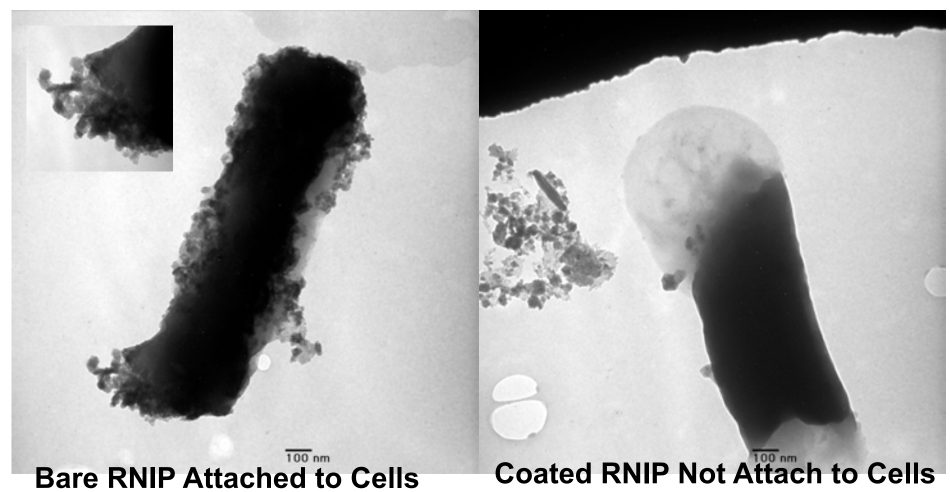


Figure B

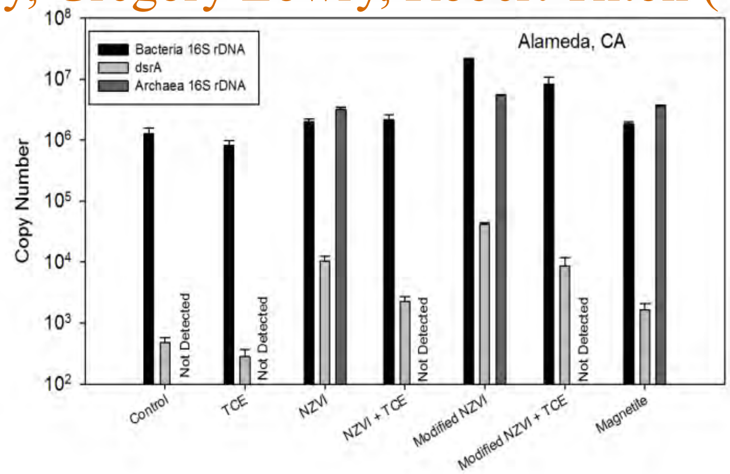


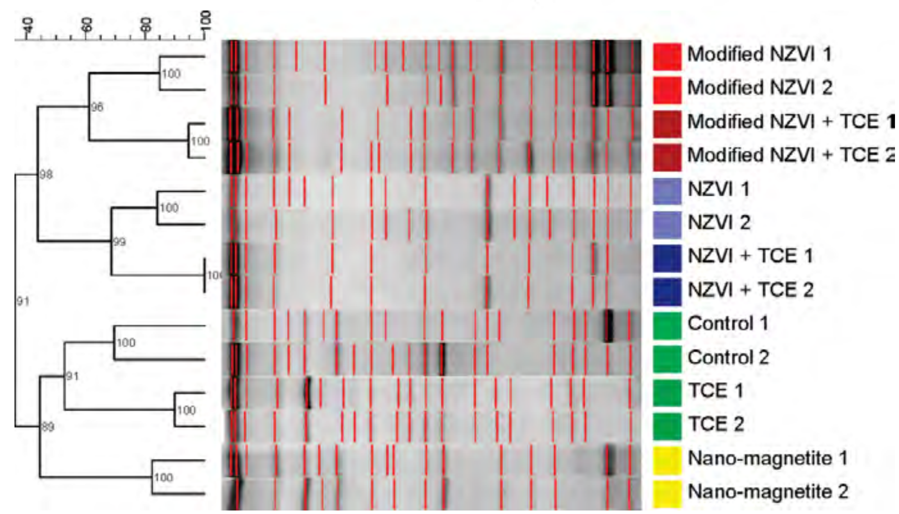
NSF EF-0830093, U.S. EPA R833326, NSF BES-0608646

Coated Fe(0) Nanoparticles Shifts Microbial Communities and *Increases* Biomass

Teresa Kirschling, Kelvin Gregory, Ned Minkley, Gregory Lowry, Robert Tilton (CMU)

Interdisciplinary research at CMU has demonstrated that nanoscale Fe(0) particles create reducing conditions in aquifer environments which lead to significant changes in the microbial community. Sulfate reducing and (in some systems) methanogen populations are stimulated by nanoparticle addition. Despite demonstrated toxicity of NZVI in *E. coli* cultures, in aquifer materials there are observed increases in Eubacterial populations.





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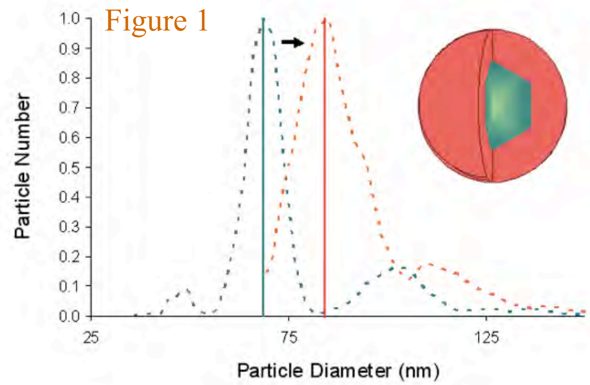
Nanoparticles Grow via Condensation of Sulfate and Organics

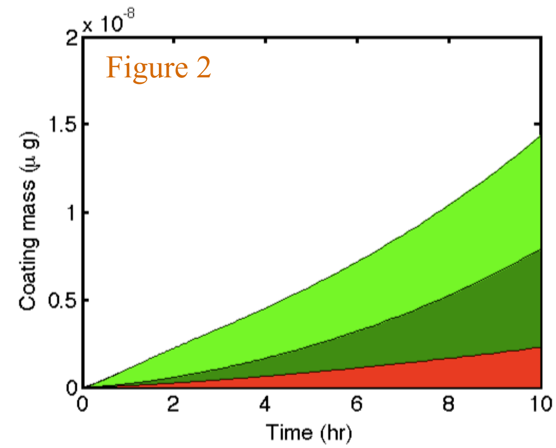
Neil M. Donahue and Allen L. Robinson (Carnegie Mellon University)

Carnegie Mellon University researchers have shown via a combination of laboratory measurements and modeling that nanoparticles emitted into the atmosphere will become coated with a combination of sulfate (ammonium sulfate) and various organics within hours of release.

All particles are bombarded with sulfuric acid (formed from oxidation of SO_2 gas by OH radicals) and condensible organics known as secondary organic aerosol (SOA) vapors (formed from oxidation of more volatile organics, again by OH radicals). Figure 1 shows laboratory data in which 70 nm size-selected nanoparticles are coated with SOA formed by oxidation of alpha-pinene in an aerosol flow reactor being developed to coat model nanoparticles for subsequent use in CEINT exposure studies. Sulfuric acid is rapidly neutralized by ammonia vapors to produce non-volatile ammonium sulfate, and will thus coat any particle in the atmosphere; organics coat many particles tested so far, including ammonium sulfate.

Figure 2 shows model results reproducing observed atmospheric growth rates and illustrating a major feature of organic coatings. Organics form solutions with constituents spanning a huge volatility range. Sulfate coating is shown in red, while coating of low-volatility organics is shown in dark green. The light green wedge is more volatile organics from background particles. The reverse case, with fresh condensable organics more volatile than background particle organics, leads to negligible organic growth on the nanoparticles.





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Properties and Environmental Fate of Airborne Particles Released During Nanomaterial Production, Use, and Disposal

Amara Holder, Marina Eller Quadros, Andrea Tiwari , & Linsey Marr (Virginia Tech)

Researchers in the laboratory of Dr. Linsey Marr (CEINT Co-Investigator at Virginia Tech) have shown that nanotechnology-based products can be a source of inhalation exposure to nanomaterials. They estimate that ~14% of silver nanotechnology products that have been inventoried could potentially release particles into the air during use. When one nano-silver product was sprayed in a chamber, concentrations of nanoscale particles (< 100 nm) increased significantly; those in the range of 10-20 nm rose by 3000 cm^{-3}. TEM analysis revealed particles spanning a wide size range from ~10 nm (Figure A) up to several micrometers.

Researchers are also characterizing the effects that atmospheric processing of nanomaterials may have on their physical and chemical properties. Exposure of powdered C_{60} fullerenes to ozone results in increased UV-VIS absorbance (Figure B), suggesting enhanced aqueous solubility, and the formation of smaller colloids.

In addition to these questions, Marr's group is continuing to investigate the toxicity of engineered nanoparticles at the air-liquid interface of lung epithelial cells (Figure C).

Figure A

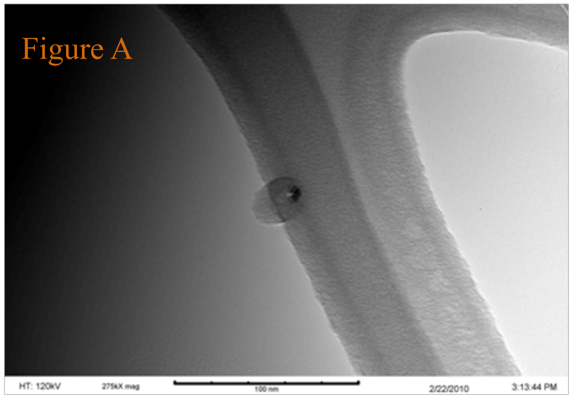


Figure B

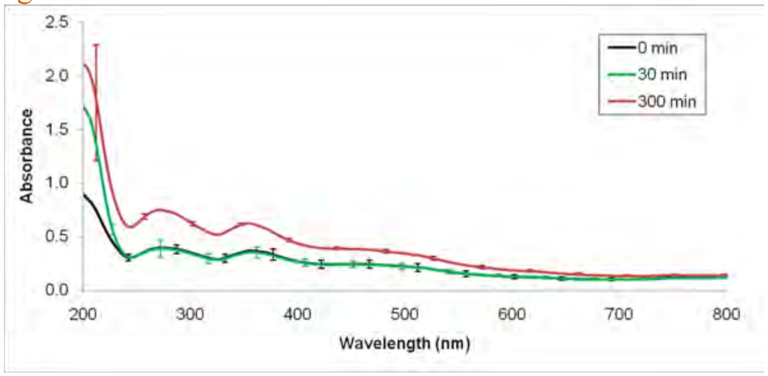
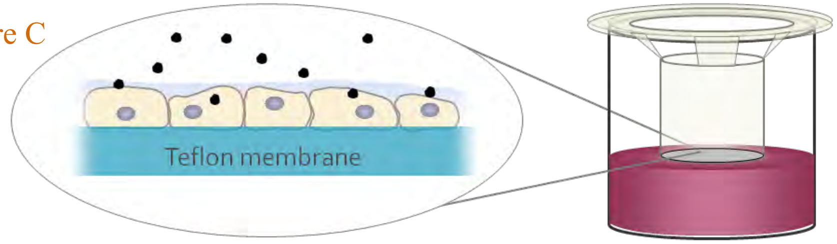


Figure C



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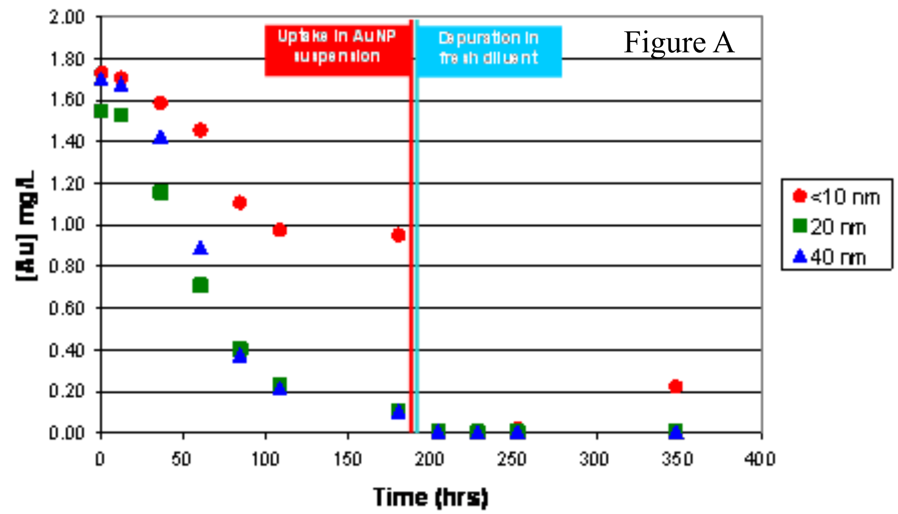
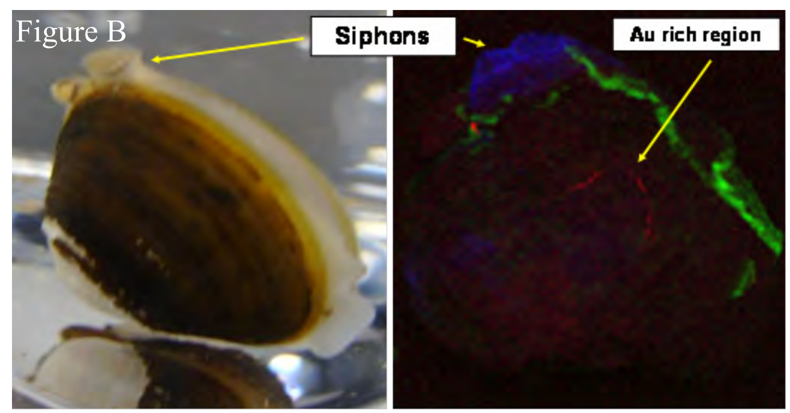
Size-dependent Uptake of Gold Nanoparticles by a Freshwater Clam

Matthew Hull & Peter Vikesland (Virginia Tech)

Suspensions (2 mg•L^{-1} as [Au]) of three different sizes of bovine serum albumin-stabilized gold nanoparticles (BSA-AuNP) were used in biological uptake experiments with the Asian clam, *Corbicula fluminea*. Figure A shows the water column gold concentration (mg/L as [Au]) as measured throughout exposures by inductively coupled plasma emission mass spectroscopy (ICP-MS). These data show that the majority of AuNP are removed from the water column within the first 100 h of exposure. It is also evident that relative to the intermediate and largest size classes of BSA-AuNP, the uptake rate for the smallest particles (<10 nm) was markedly reduced. In conjunction with the removal of water column samples, individual *C. fluminea* were removed throughout the exposure and analyzed by X-ray Fluorescence Micro-spectroscopy (Micro-XRF). These results indicate that the uptake of Au into *C. fluminea* tissue coincides well with observed decreases in water column [Au] as measured by ICP-MS. Further, and consistent with ICP-MS results, within the first 100 h of uptake, levels of Au measured in clams exposed to the intermediate and largest sizes of BSA-AuNP are greater than Au levels measured in clams exposed to the smallest BSA-AuNP. These results suggest that in *C. fluminea*, uptake of BSA-AuNP occurs as a function of size, with smaller particles (<10 nm) being accumulated less rapidly than larger particles. Finally, based on whole-body images generated from Micro-XRF scans (Figure B; collected in collaboration with Chaurand and Rose, *CEREGE*), it appears that BSA-AuNP accumulate primarily in regions of the digestive gland, digestive track, and feeding groove of *C. fluminea* (Au-rich regions are colored red in the right-hand panel of Figure B).





Siphoning Clam

Clam MicroXRF Profile

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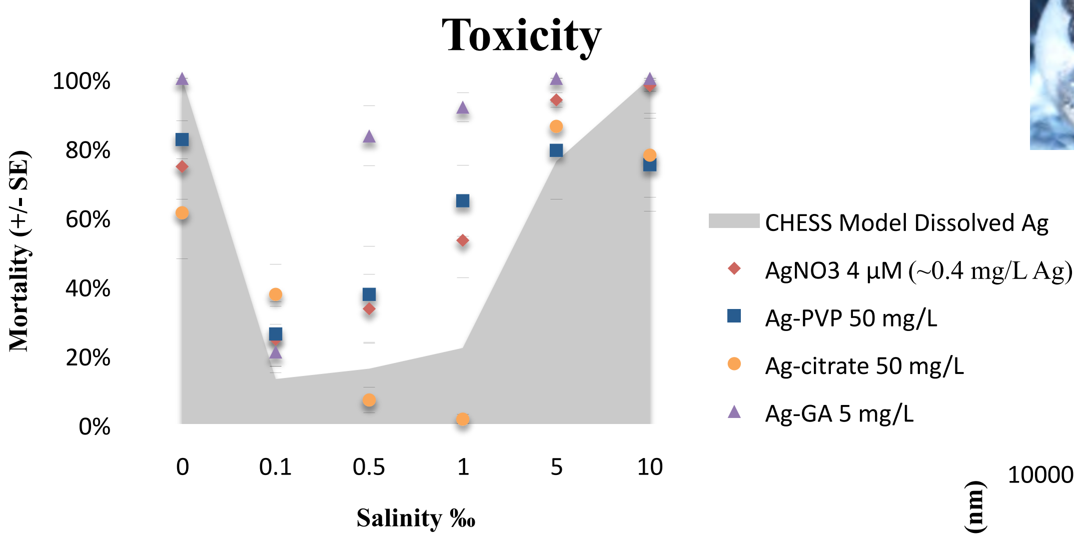
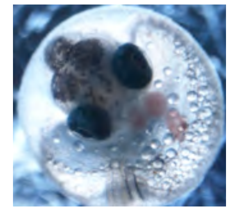
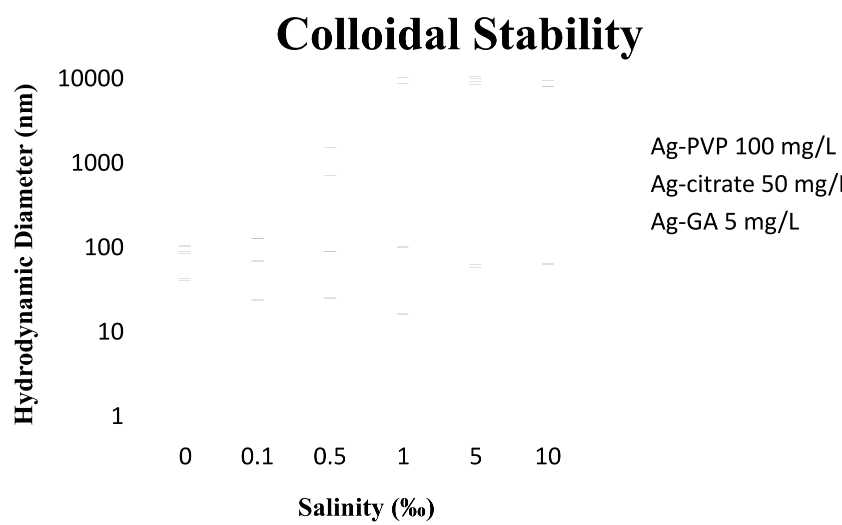
Ag NP Embryotoxicity across a Salinity Gradient – The Role of Coatings and Dissolved Silver

Di Giulio Lab, Duke University





Atlantic killifish
Fundulus heteroclitus



- Ag NP coatings significantly affect particle behavior
    - Stability/Aggregation (Ag-gum arabic most stable)
    - Toxicity (Ag-gum arabic most toxic)
- Dissolved silver and silver speciation play a significant role in toxicity
    - Toxicity curve shape related to silver speciation (total dissolved Ag, not Ag^+)

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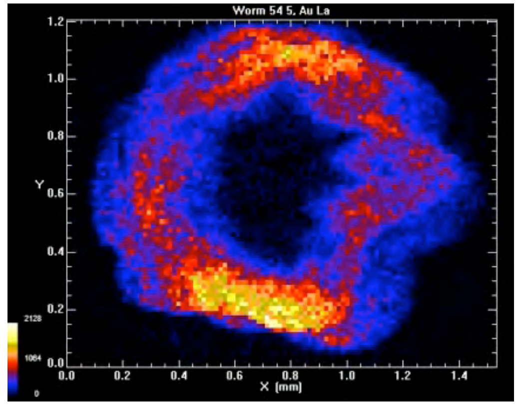
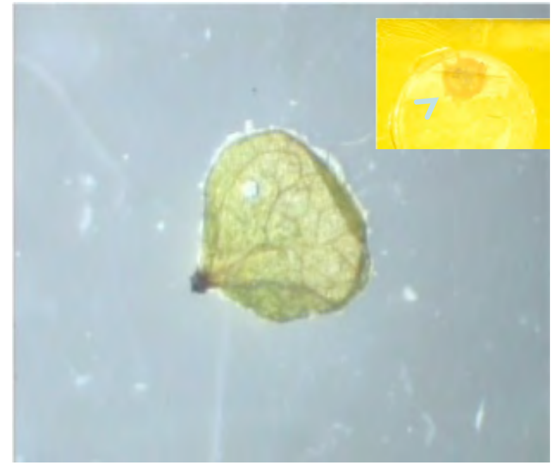
Trophic Transfer and Bioaccumulation of Gold NPs

Bertsch Lab,
University of Kentucky

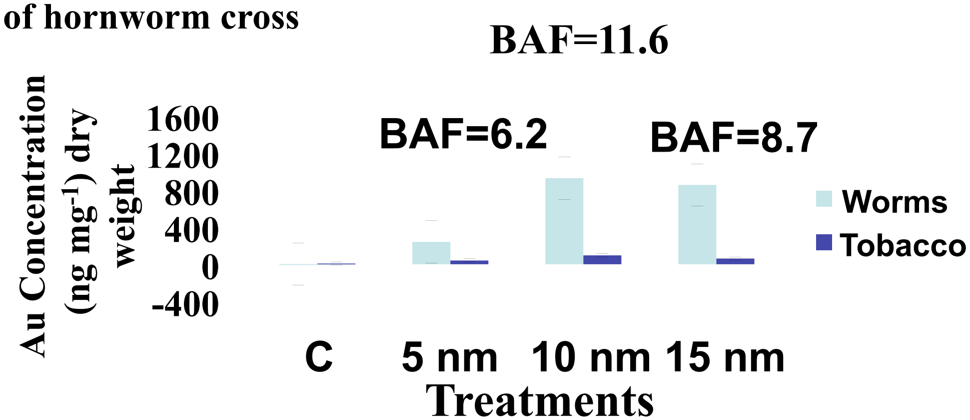
- Au NPs are taken up by tobacco plants and transferred to hornworms
- Significant bioaccumulation factors, which vary by particle size



Au La x-ray microprobe analysis of hornworm cross section



LA-ICPMS analyses of Au NP exposed leaf tissue



Total Au analyses of plant and hornworm tissues following exposure/consumption to three Au NP size classes. The biological accumulation factors (BAF) are also shown.

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Intracellular uptake, intergenerational transfer, and toxicity of silver nanoparticles in *Caenorhabditis elegans*

Meyer Laboratory (Duke University)

Researchers in the laboratory of Dr. Joel Meyer, in collaboration with the Wiesner and Chilkoti laboratories, have shown that silver nanoparticles are taken up into the cells of the nematode *C. elegans*, and transferred to the next generation (Fig. A).

Exposure causes growth inhibition in the developing nematode (Fig. B). This effect is observed at the low mg/L levels in high-chloride medium and the low µg/L levels in low-chloride medium (not shown)

Surprisingly, oxidative stress mutants including a mitochondrial superoxide dismutase knockout were not more sensitive to this toxicity, but a metallothionein knockout was (Fig. C).

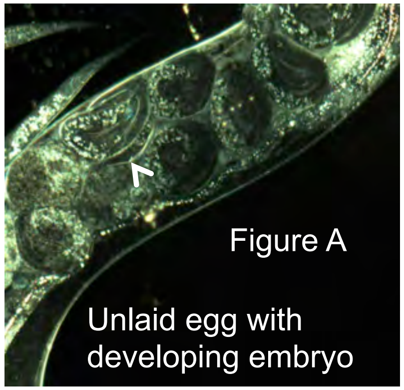

Figure A

Unlaid egg with developing embryo

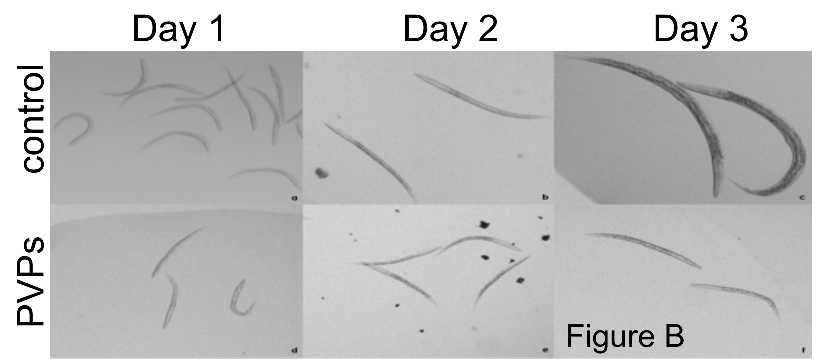

Figure B

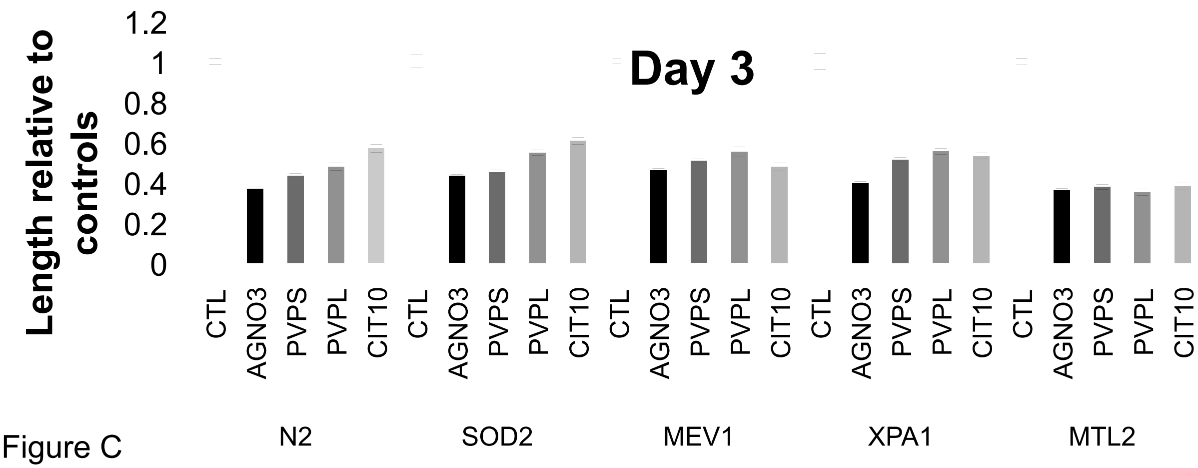

Figure C

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Silver nanoparticles: Influence on physiology and behavior when administered from early development

Duttaroy Lab, Howard University

Ag-citrate interferes with body pigmentation

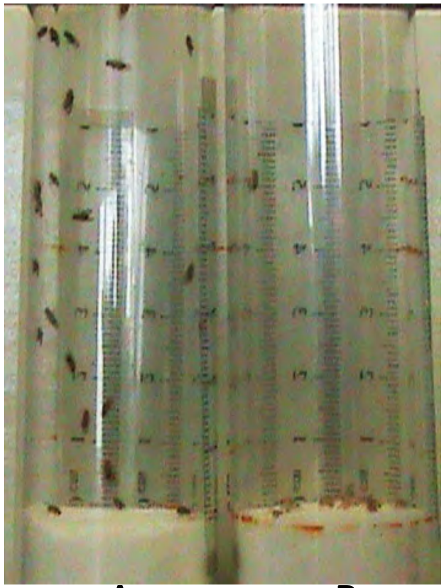
Ag-citrate was fed at a concentration of 100 ppm to wild type flies starting from early development.

(A) Control flies fed with Na-citrate have normal pigmentation.

(B) Silver citrate fed (100 ppm) adults flies appeared completely bleached. Note only the body pigments are affected by silver, while the eye pigments remain unaffected.

Effect of Ag-nanoparticles on fertility, and life span also measured.

Loss of vertical climbing ability



A B

Ag-citrate fed (100 ppm) flies lose vertical climbing ability, which requires neuromuscular coordination.

(A) Control flies fed with Na-citrate climb up as a natural response.

(B) Silver citrate fed flies stay at the bottom

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Silver Nanoparticles Affect Microbial Diversity in Wastewater and Soils Receiving Biosolids

Christina L. Arnaout and Claudia K. Gunsch (Duke University)

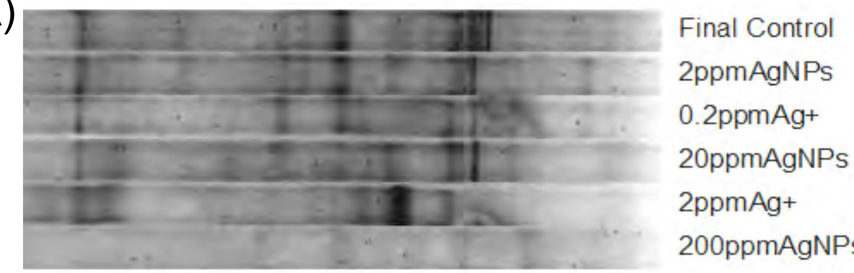
Researchers in the laboratory of Dr. Claudia Gunsch (CEINT co-Investigator at Duke University) have determined that silver nanoparticles (AgNPs) impact microbial community structure in soils treated with biosolids as well as wastewater.

AgNP concentrations greater than 20 ppm led to a significant decrease in microbial growth and diversity in batch reactors inoculated with wastewater activated sludge (Figures A and B). Data suggest that the effect is correlated to ionic silver concentrations in the samples.

Microbial diversity was also affected in soils treated with AgNP containing biosolids (Figures C and D). After 8 weeks, significant shifts in total and denitrifying bacteria were observed. Population shifts in soils exposed to AgNP appear to be more significant than those receiving ionic silver.

The Gunsch group is presently identifying the specific microorganisms which were impacted as well as evaluating the effects in terms of functional efficiency. Experiments are underway to determine if AgNPs affect wastewater treatment efficiency (i.e., organic carbon and nitrogen removal). Finally, the propagation of silver resistant microorganisms is also being investigated.

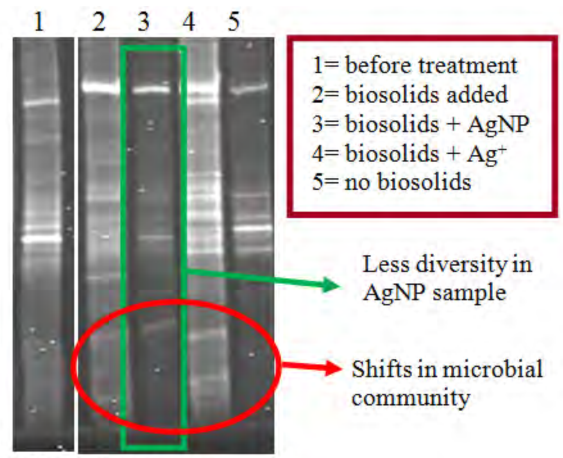
A)



B)

C)

D)



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CEINT

Sunlight Reduced the Toxicity of Polymer-Stablized Silver Nanoparticles

Yingwen Cheng & Jie Liu (Duke University)

Researchers in the Dr. Jie Liu group at Duke University have found that the UV-content of sunlight induced the aggregation of both PVP and GA coated Ag nanoparticles (NPs) (Figure A). Toxicity studies based on the germination of a wetland plant *lolium multiflorum* shows that the root grown in sunlight irradiated NPs have normal root hair and is much longer in length than in pristine NPs (Figure B & C), which don't have normal root hair and is shorter (Figure B & C), indicating the nanoparticle toxicity is reduced by sunlight.

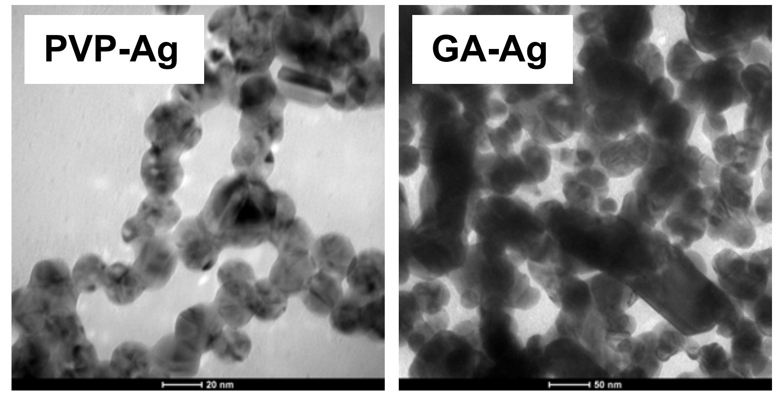

Figure A: Ag NPs after sunlight irradiation

The study on GA-Ag NPs with different sizes shows similar results, indicating the effect of sunlight might be size-insensitive. Current research at the Liu group aims at examining other factors that would affect the NPs aggregation, including salinity and light intensity.

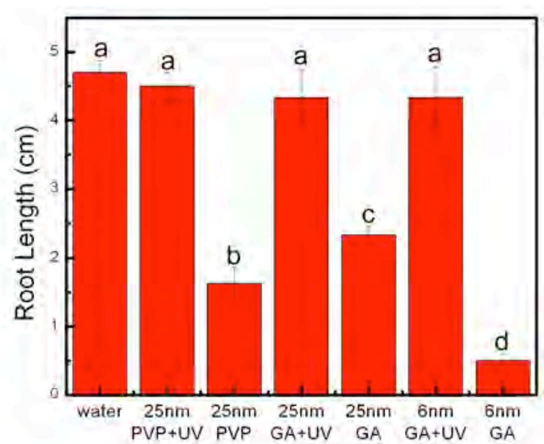

Figure C: root length grown in different media

Figure B: Root grown in sunlight irradiated NPs (up) and pristine NPs (down)

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Synthesis and Characterization of Silver Nanoparticles

Stella M. Marinakos and Ashutosh Chilkoti (Duke University)

Researchers in the laboratory of Dr. Ashutosh Chilkoti have been working on the synthesis of citrate-stabilized silver nanoparticles, which are typically difficult to synthesize with a narrow size dispersity. The Chilkoti Lab has been able to size-separate small particles from a polydisperse sample by centrifugation (Figure 1). The narrower dispersity is reflected in the narrower UV-VIS peak of the small particles as compared to the original sample. Alternatively, adding tannic acid during synthesis produces much more monodisperse particles (Figure 2).

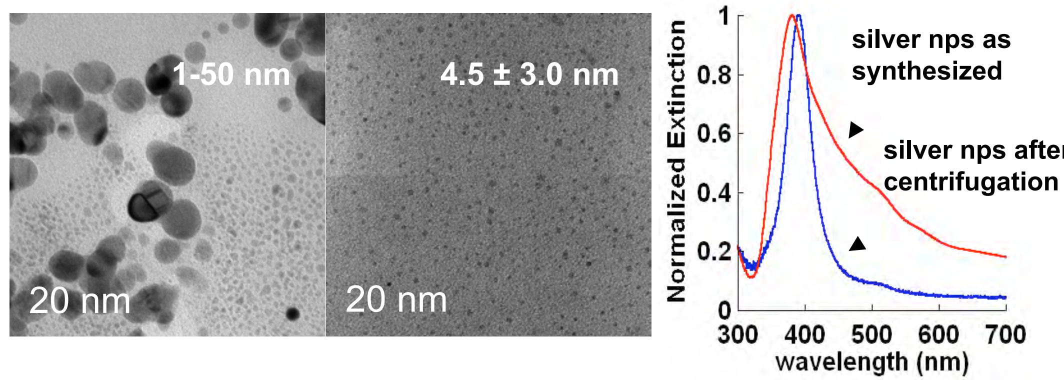

Figure 1. TEM images and UV-VIS spectra of citrate-stabilized silver nanoparticles before and after size-separation.

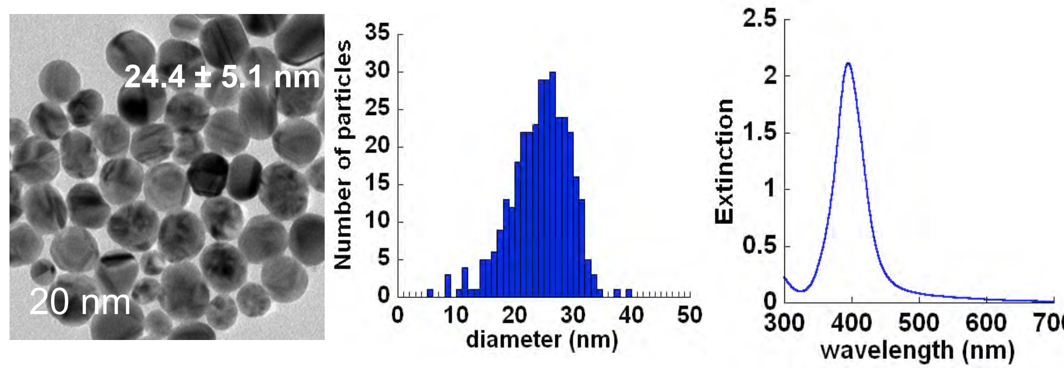

Figure 2. TEM image, size histogram, and UV-VIS spectrum of citrate/tannic acid-stabilized silver nanoparticles.

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CEINT

Newly Discovered Natural and Incidental Nanoparticles

Bojeong Kim, Kelly Plathe, and Mike Hochella (Virginia Tech)

Researchers in CEINT Co-PI Mike Hochella's group have discovered new natural and incidental nanoparticle processes.

Analytical transmission electron microscopy and flow field flow fractionation were utilized to elucidate relationships between toxic heavy metals and nanoparticles in contaminated sediment from the Clark Fork River (Montana, USA) Superfund Complex, the largest in the United States. It was discovered that the majority of toxic metals are moved hundreds of kilometers in this system by metal-oxide mineral nanoparticles. Figure A shows nanogoethite as a carrier of arsenic.

We also studied the sludge samples that were collected for the Targeted National Sewage Sludge Survey (TNSSS) by the U.S. EPA in an effort to determine the fate of Ag NPs in waste water treatment plants. The Ag NPs found in the sludge material are small aggregates of individual 15-20 nm NPs (Figure B). EDX and HR-TEM show this material to be α-Ag_2S. Therefore, sludge disposal, or sludge used as a soil amendment, results in the introduction of Ag_2S into the environment.

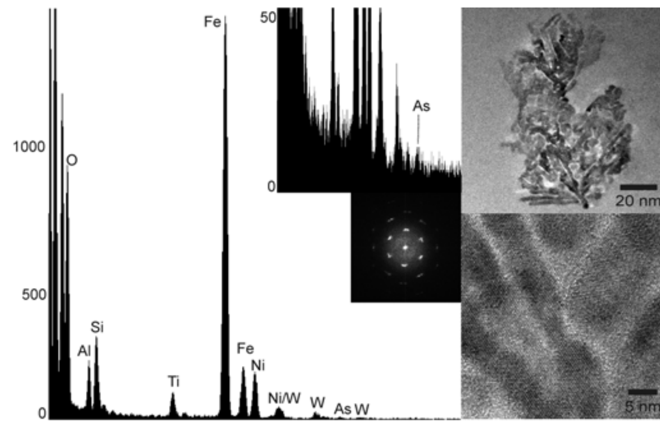

Figure A

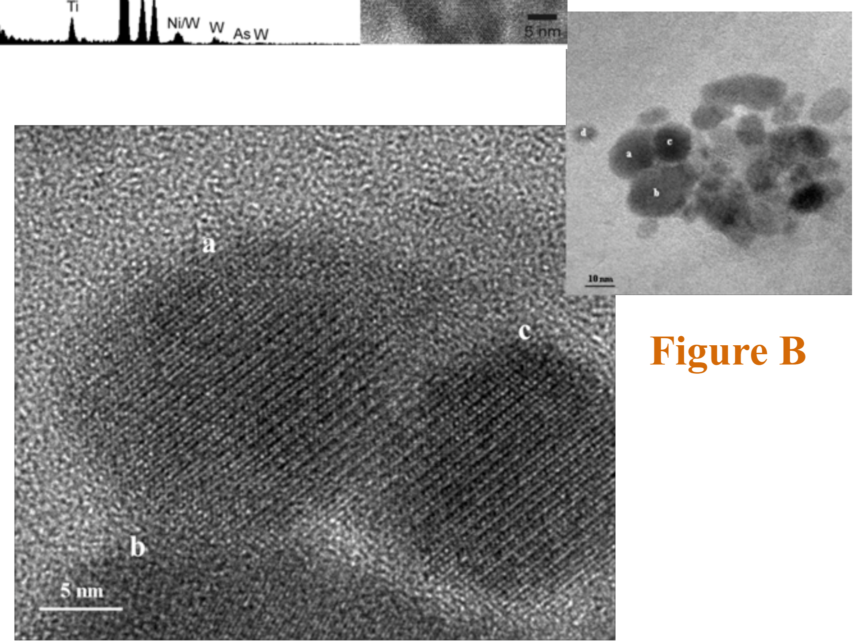

Figure B

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Role of NOM for precipitation, growth, and aggregation of metal sulfide and silver nanoparticles

Heileen Hsu-Kim, Andreas Gondikas, and Amrika Deonarine (Duke University)

Key findings by the Hsu-Kim group:

- Natural organic matter plays a critical role in stabilizing ZnS and HgS nanoparticles when they form naturally in the environment (HgS NPs on the right). (Figure A)
- Surface association of NOM may occur through with thiol-containing ligands such as cysteine (ZnS aggregation below). (Figure B)
- Cysteine was unexpectedly found to increase the aggregation of silver nanoparticles. The extent of destabilization depended on the type of surface coating used to manufacture the Ag NPs. (Figure C)

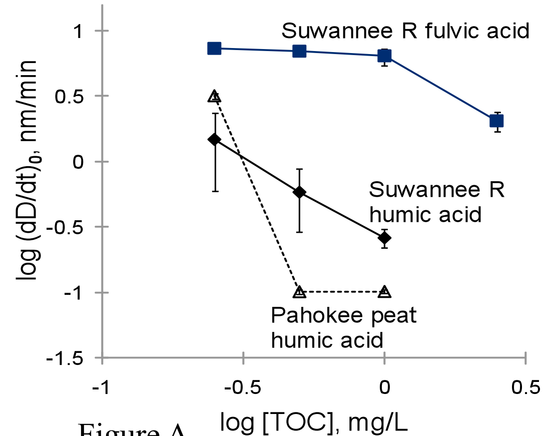
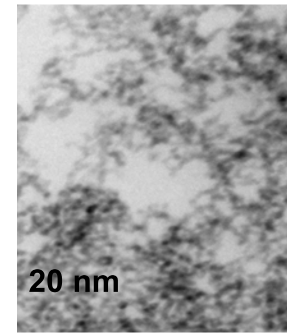


Figure A



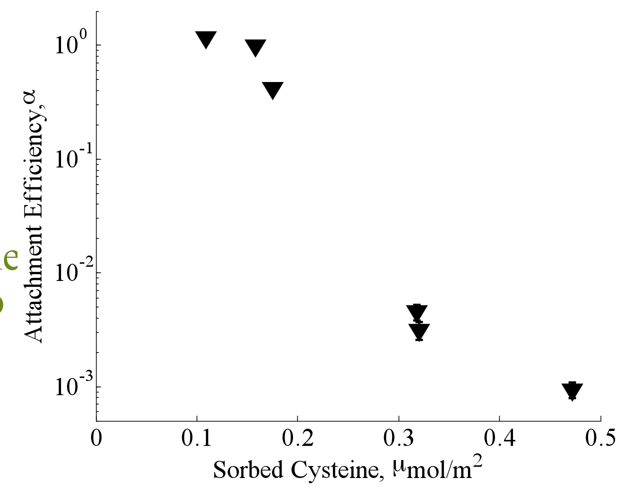


Figure B

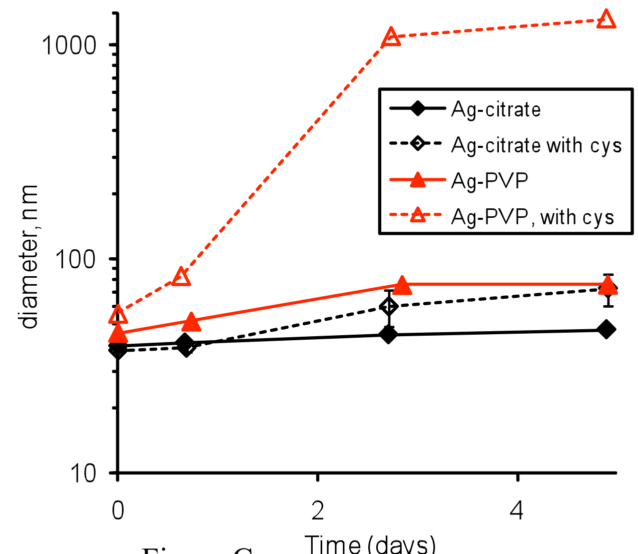


Figure C

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Relationship Between Nanoparticle Production and Emission

Christine O. Robichaud, Mark Wiesner (Duke), Elizabeth Casman (CMU)

Environmental risk assessment requires a good estimate of the amount of nanomaterial emitted to the environment. While significant progress has been made by CEINT in estimating global production, the relationship between production and emission is a complex function of emissions from the various life cycle stage of each nano-enabled product. (Figure 1) For nano-enabled products that enter the wastewater stream, Robichaud and others have developed a simple method to scale these environmental releases to the nanomaterial's annual global production. This method hinges on measurement of how nanomaterials partition between sewage sludge and sewage effluent, and uses this information to back-calculate a scaling factor that relates annual nanomaterial production to environmental emission. To supplement partitioning coefficients found in the literature, and to extend this technique to a range of nanomaterials, the Wiesner lab is determining partitioning coefficients for a number of nanomaterials. (Figure 2)

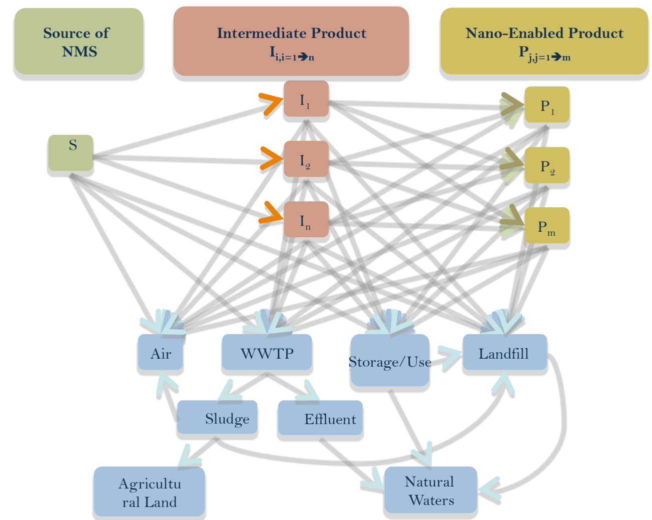

Figure 1

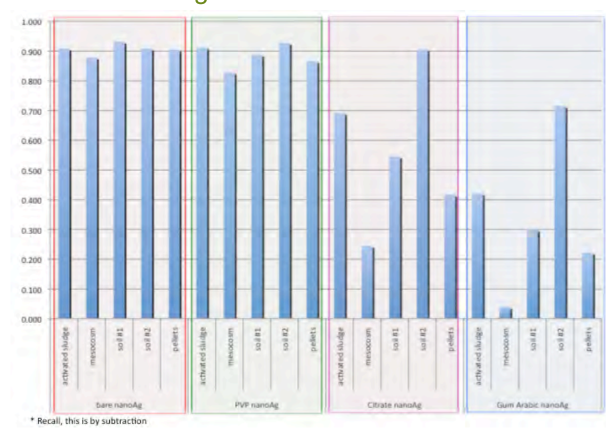

Figure 2- Partitioning of nanoAg (4 types) into sludges (5 types)

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Modeling the Environmental Risks from Nanoparticles

Eric Money & Ken Reckhow (Duke University)

Researchers in the laboratory of CEINT Co-investigator Dr. Kenneth Reckhow are developing a comprehensive model of the potential enviromental risks of nanomaterials, linking particle characterization, fate, transport, uptake, and ecological effects into a probabilistic framework (Bayesian Network) to not only assess ecological risks, but also highlight the uncertainty that currently surrounds many of these issues.

Recent research efforts have focused on the structural development of a general model framework (Figure A, top) that serves as the basis for the more complex components of the model, such as particle characterization and fate (Figure A - expansion). These models are a result of a collaborative effort with more than a dozen CEINT researchers across a variety of disciplines.

Work is beginning to shift towards the probabilistic assessment of these models to create a predictive and diagnostic model for scenario testing that can be formally updated as our scientific knowledge changes. This model will not only inform stakeholders of environmental risks from nanomaterials, but will also inform researchers of areas where experimental efforts could be targeted to reduce future uncertainty.

Figure A

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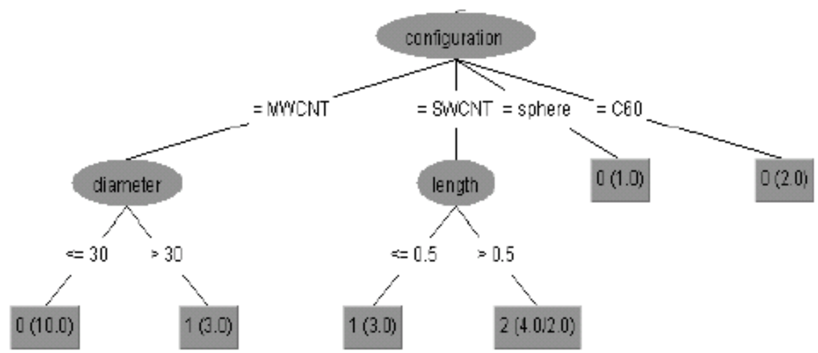
Deciding What Properties Matter

Jeremy Gernand & Elizabeth Casman (Carnegie Mellon University)

One of the basic and most pressing questions in environmental risk assessment for nanomaterials is which specifically nano-scale properties are relevant and which properties can safely be ignored. Jeremy Gernand has begun exploratory work on methods to answer this question. Gernand has assembled a data base of physical properties associated with the inhalation toxicity of C_{60}, SWCNT, and MWCNT nanoparticles, and analyzed these data using a machine learning algorithm (C4.5/J48) to identify groups of properties most strongly associated with toxicity. The preliminary results seem to indicate that this may be a useful approach to the problem. Gernand is expanding this data base and also assembling one on nanoparticle aggregation.



Preliminary regression tree analysis of factors affecting toxicity

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Research Prioritization for Nanomaterial Risk Assessment

Alan Masinter, Mitch Small, Elizabeth Casman (Carnegie Mellon University)

Environmental risk analysis involves a sequence of causally linked phenomena, starting with pollutant emissions and ending with effects on target organisms. Because of these linkages, information on any phenomenon in this sequence has implications for the understanding of the other domains. Does this structure tell us anything interesting about how research should be prioritized? Carnegie Mellon University Ph.D. student, Alan Masinter, is exploring this question with a probabilistic network model of environmental risk assessment for optimally allocating research resources according to the value of information each research investment contributes to the understanding of the entire system. For the pilot study, Masinter has borrowed the structure of a simple nano-silver risk assessment model from the literature (Blaser, et al. (2008). *Science of The Total Environment* **390**(2-3): 396-409), Figure A. Linkages between different compartments in this model are considered to be potential research fields, where knowledge in those fields is represented probabilistically. A given level of funding can then be distributed optimally according to single or multiple research objectives (Figure B).

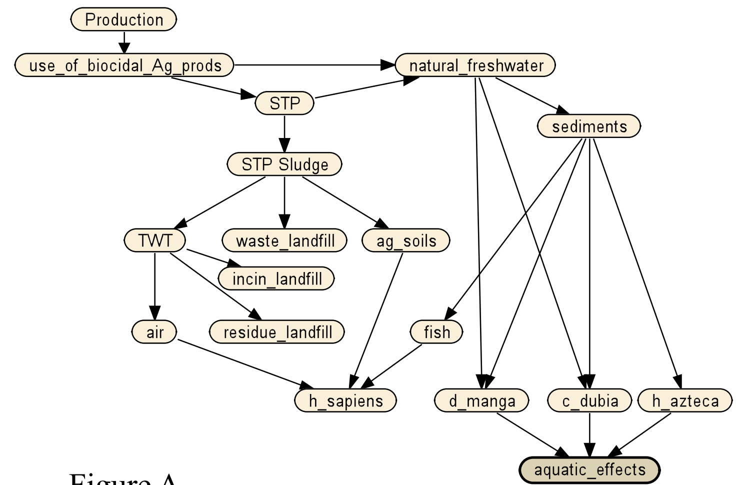

Figure A

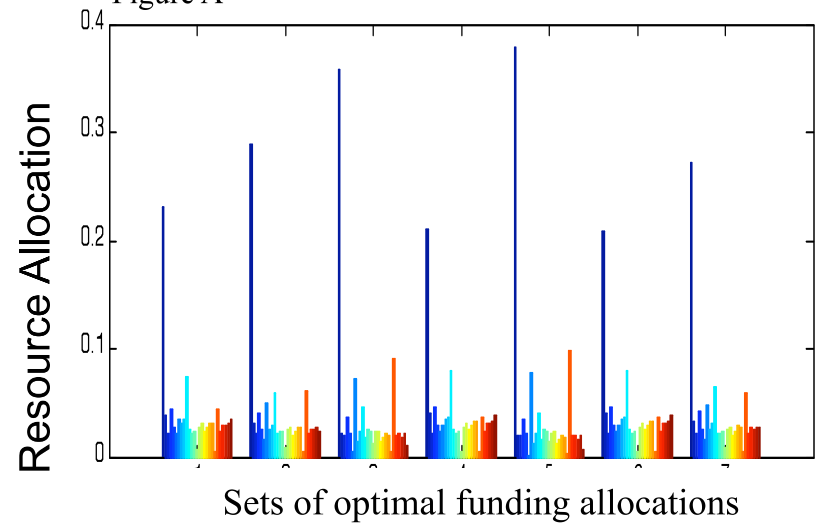

Figure B

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Nanosilver Toxicity to the Medaka Eleutheroembryo

Kevin Kwok, Melanie Auffan, Appala Raju Badireddy, Benjamin Espinasse, Mark R Wiesner and David Hinton (Duke University)

Researchers in the laboratory of Dr. David Hinton (CEINT Co-Investigator at Duke University) have found that nanosilver toxicity is dependent on the coating material; and, less importantly on the ~1% that is dissolved. By use of ultra-centrifugation (40k rpm for 1h), the resultant supernatant contains dissolved nanosilver (Sn; **Fig A** red bars) provides a way to contrast and understand the nanoparticle-specific toxicity. In 21d exposures, supernatants of all nanosilvers exhibited toxicity significantly different from corresponding nanoparticle suspensions (NP; **Fig. A** blue bars). Differences were in magnitude (**Fig. A**) and in time required to cause mortality (data not shown). Exposure to the NP, but not the Sn, led to increased spinal malformation (**Fig B**).

Ongoing experiments are designed to demonstrate uptake of nanosilver particles by medaka. In these, we deploy hyperspectral imaging via Cytoviva® microscopy on 5 μm paraffin sections of exposed fish.

Fig. A

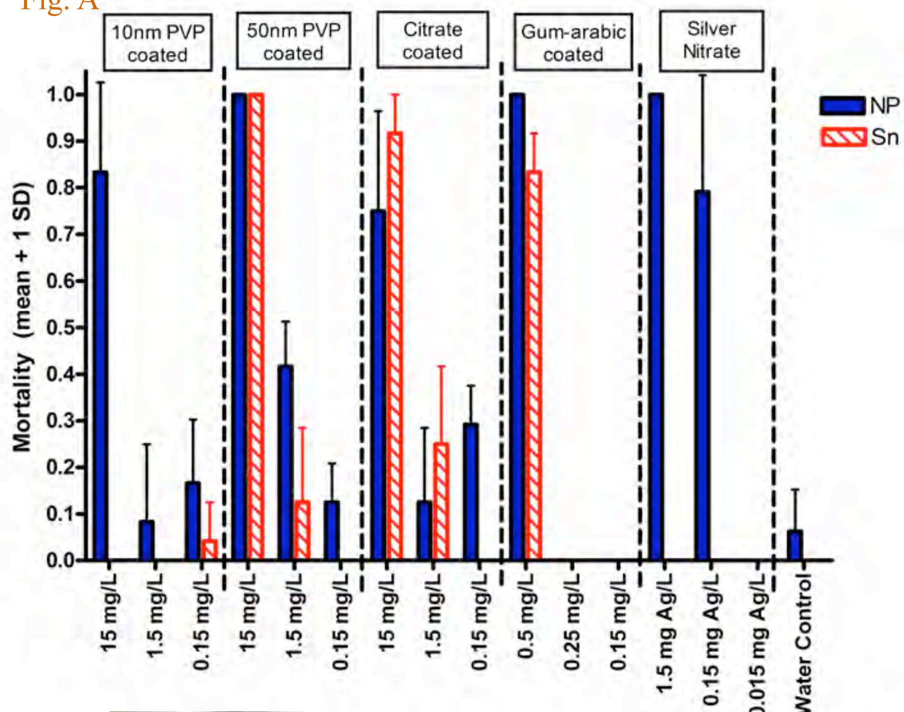
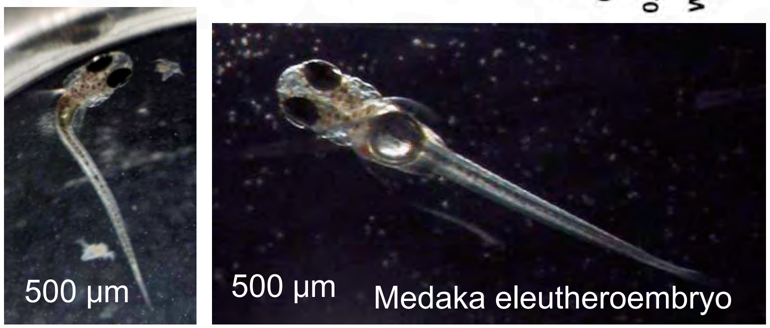


Fig. B



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NanoDays 2009

CEINT Partners with NC Museum of Life and Science and NISE Network

CEINT participated in the NISE Net affiliated NanoDays 2009 at the NC Museum of Life and Science in Durham NC with museum visitors numbering over 1700. CEINT faculty, graduate students and post-docs from Duke created engaging, hands-on activities involving nano-science for this public education initiative.

• **Dr. David Hinton &** researcher **Clay Nelson** displayed an aquarium of Medaka fish and visuals on impacts of nanoparticles on embryonic development of Medaka.

•**Dr. Emily Bernhardt** and student team added nanosilver from 15 commercial products to plated bacteria cultures and allowed visitors to examine colony clearing rates.

•**Dr. Ben Colman** demonsrated the many roles microorganisms play in natural ecosystems and showed examples of how the antimicrobial nature of silver nanoparticles could influence those roles

•**CEINT Director Dr. Mark Wiesner** gave an engaging talk on the many current applications of nano-materials and the importance of risk assessment and sustainable development to packed audience of museum visitors.

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CEINT

NISE Net sponsored NanoDays 2010

CEINT partners with NC Museum of Life and Science and Marbles Kids Museum

Museum visitors number over 2000 for NanoDays 2010 for 2 museums:

➢**NC Museum of Life & Science, Durham NC:**

•**Dr. David Hinton** & **Dr. Cole Matson** displayed Medaka and Killifish & visuals from research on impacts of nanosilver on these fish while visitors viewed these species at different developmental stages under microscopes

•**Dr. Claudia Gunsch** & graduate student **Christina Aranout** demonstrated their research on impacts of commercial products treated with nanosilver on bacteria & engaged visitors in discovering whether these materials were effective

•**Dr. Ben Colman** demonstrated the many helpful roles microorganisms play in natural ecosystems & showed examples of how silver nanoparticles could influence those roles

➢**Marbles Kids Museum, Raleigh NC**

•Graduate student **Chai Hoon Quek** demonstrated quantum dot color changes with UV light exposure & biomedical applications of quantum dots.

•Graduate student **Jeff Farner-Budarz**, showed where nanomaterials are found in the environment & engaged visitors with 1 way CEINT tests nanosilver products for bacterial clearing

•Postdoc **Ariette Schierz,** CEINT Director **Wiesner, &** Assoc. Director **Kelly** demonstrated how size can impact chemical reactions using crumbled vs large alka-seltzer tablets

•**David Jassby,** graduate student, demonstrated differing properties of iron-from nano to macro-scale & engaged younger visitors with exciting hands-on activities involving states of matter

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Effects of silver nanoparticles on plants and microbes

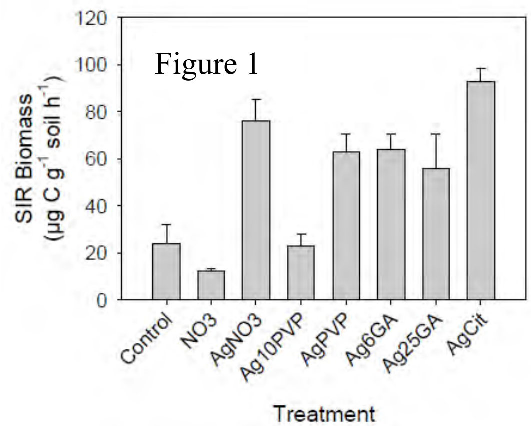
Emily S. Bernhardt, Ben P. Colman and Liyan Yin (Duke University

Key findings by the Bernhardt group:

- Silver nanoparticles, regardless of their size and coating, were found to have no or positive effects on sediment microbial biomass and activity in laboratory experiments, even at extremely high concentrations (Figure A).

•Silver nanoparticles, especially gum arabic coated 10nm AgNPs, dramatically reduced the growth of Lolium perenne seedlings (Figure 2). Exposing seeds to AgNPs led to reduced or no root elongation, branching or the development of root hairs (Figure 3). Effects of AgNPs were significantly greater than for exposure to similar concentrations of Ag+ and were not affected by the addition of cysteine (Figure B).

•In a risk assessment based field experiment where AgNPs were added together with biosolids at realistic concentrations to outdoor terrestrial mesocosms, we observed significant reductions in both plant and microbial biomass. These results suggest that either a) synergistic interactions between AgNPs and coocccuring contaminants generate toxicity OR b) environmental variation alters the fate and toxicity of AgNPs.



All Ag^+ and AgNP solutions contained 250mg Ag L^{-1}

Figure 2

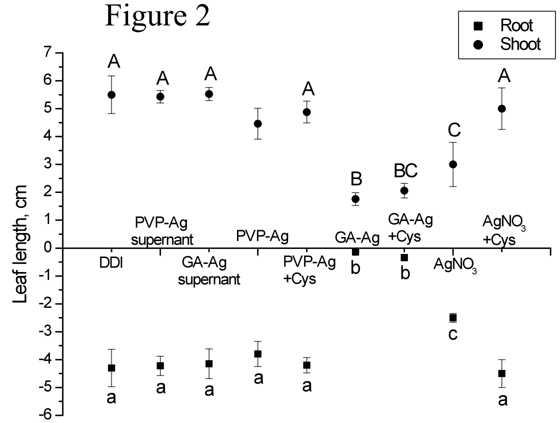
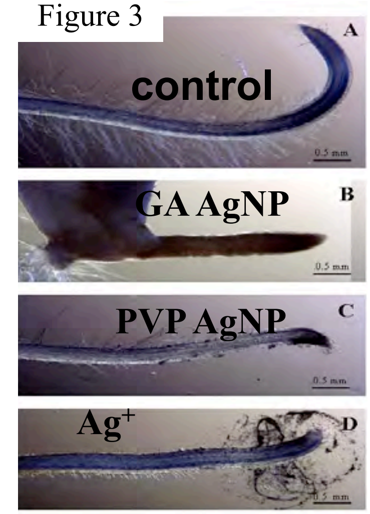


Figure 3



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Nanoparticle Detection and Abundance of PVP-Ag 10 nm in Mesocosm Water Using CytoViva Hyperspectral Image Analysis

CEINT Shared Resource

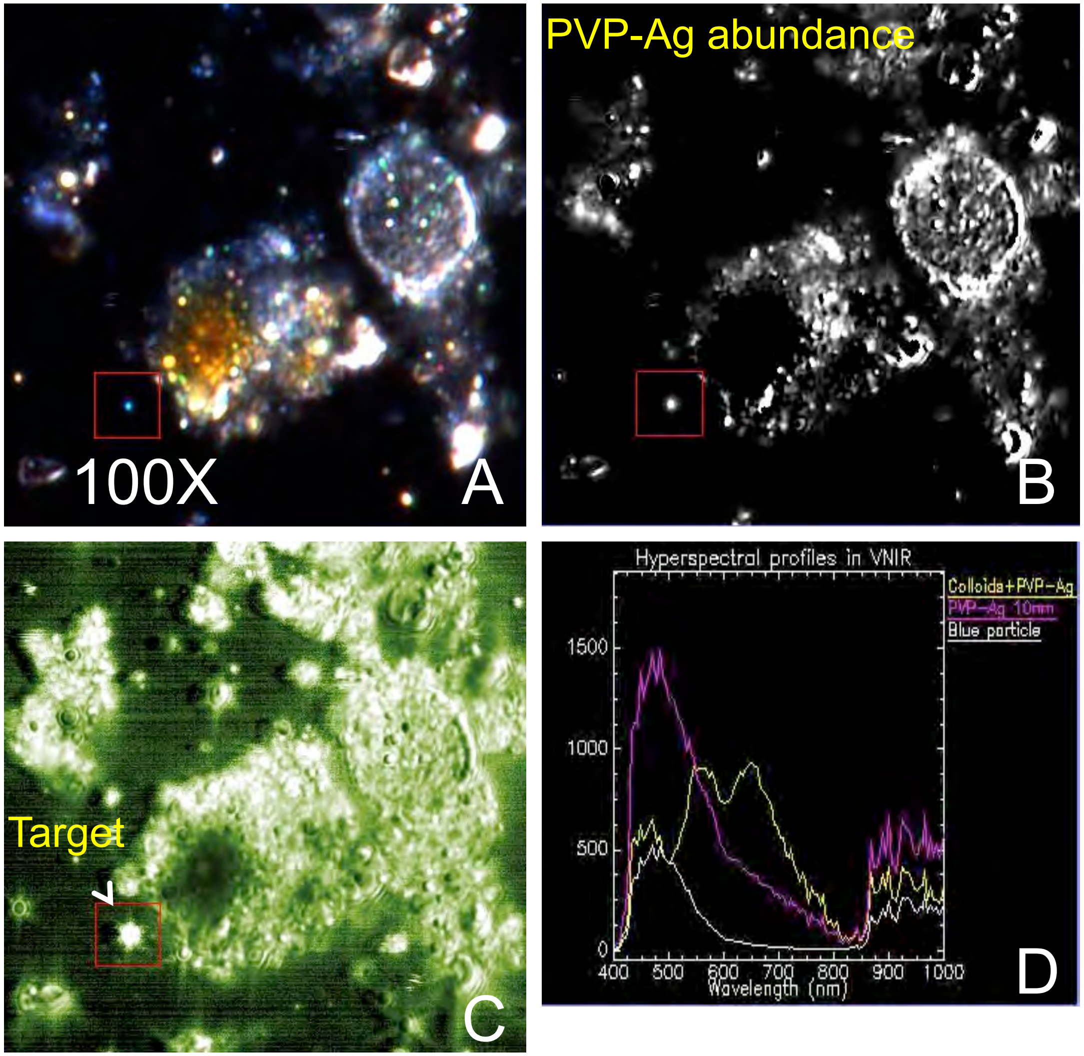
Hyperspectral images were acquired under dark-field illumination in visible and near infrared wavelengths (400 -1000 nm).

Figure A. Hyperspectral image of colloids from mesocosm water exposed to 25 ppm PVP-Ag 10 nm.

Figure B. Abundance of PVP-Ag 10 nm particles in the image field-of-view. All the white spots in the image shows the location of PVP-Ag in the vicinity- and on- the colloids.

Figure C. A target particle (in the red color rectangle) is successfully identified using spectral angle mapper and band-max technique. All the white spots, which are not clearly visible in Fig. A (blue colored particles) are visible in this image.

Figure D. A positive match of spectral features with library spectrum of pure PVP-Ag (magenta) shows the evidence of PVP-Ag in the vicinity- and on- the colloids.



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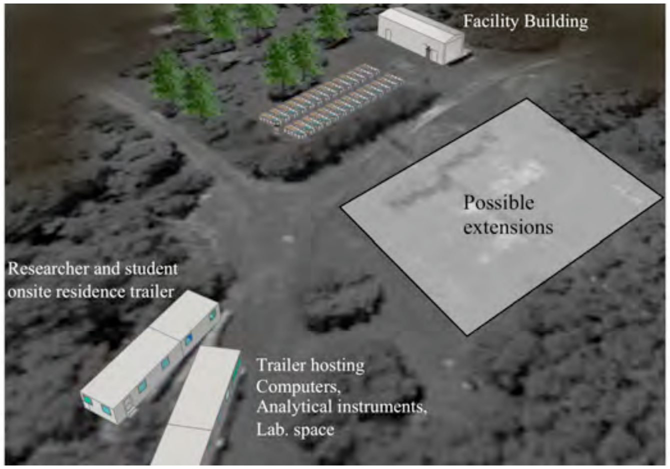
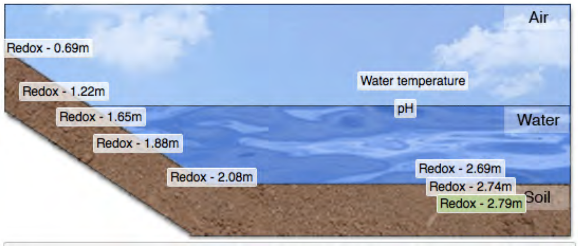
Mesocosm Shared Facility

- **Location / space distribution** – Located in the middle of Duke forest with : One facility building (~1500 sqft), One research trailer with lab space, computer (linked to the live Database), analytical instruments… One trailer for onsite residence of researchers and students. ~0.6 Acre of open space dedicated to mesocosms and future developments
- **Designs / conception** – 28 fully functional mesocosms were built based on the best prototype chosen among 5 designs.
- **Slant-board concept** : Water compartment and soil compartment with different soil redox conditions along the slantboard (from anoxic to oxic)
- **Monitoring** – Datalogers integrated in a wireless network accessible remotely around the world collecting data from various probes (Redox, Temperature, pH…)
- **Data Acquisition / distribution** – Data constantly saved in the Ceint/Duke Databases, distributed via an online web platform.
- **Research** – central platform to study Ecology, Toxicology, Transport and Transformations of the NP in a natural complex environment.





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Innovations in CEINT's Website

- **Materials distribution** – software tool enables participants to manage requests, distribution and documentation of mesocosm and nanoparticle samples
- **Webcam** – live feed of mesocosm site located in Duke Forest
- **Integrated work spaces** – centralized repository for managing working documents associated with individual research themes
- **Research protocol postings** – internal central repository for working drafts of protocols for CEINT researchers and collaborators; post-completion and approval, protocols will be made available online as disseminated resource
- **Publications** – central repository for documenting CEINT publications: journals articles, book chapters, conference proceedings, etc.)
- **Seminars** – web enabled tool for inviting, scheduling and advertising speakers
- **Live web-streaming & archived recording** of CEINT Seminars for real time and later viewing across multiple CEINT research and collaborative sites
- **Outreach Programs** – resource for individuals interested in CEINT's educational and K-PhD outreach programs
- **News** – repository for CEINT related articles, and news releases for general public
- **Events-** advertises CEINT sponsored Seminars, Brown-bag Colloquia, major conferences and scientific meetings
- **People directory** – searchable directory for CEINT faculty, staff & students

www.ceint.duke.edu

Live webcam for viewing mesocosm site

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