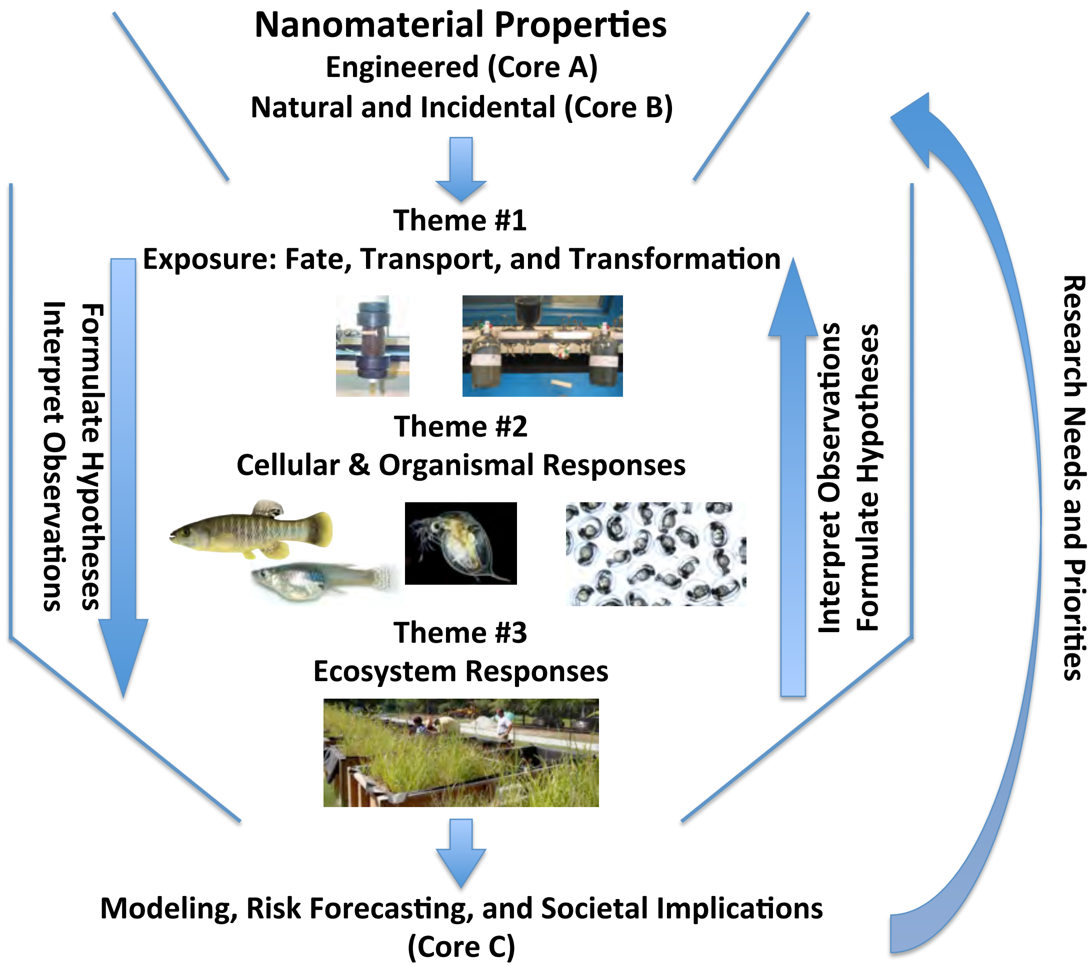
CEINT Organizational Structure and Research Roles

Center Leadership:
Director: Mark Wiesner (Duke)
Deputy Director: Greg Lowry (CMU)
Executive Director: Cole Matson (Duke)

Thrust Leaders:
Theme 1: Greg Lowry (CMU)
Theme 2: Richard Di Giulio (Duke)
Theme 3: Emily Bernhardt (Duke)
Core A: Jie Liu (Duke)
Core B: Michael Hochella (VT)
Core C: Elizabeth Casman (CMU)

Other Steering Committee Members:
Kim Jones (Howard)
Paul Bertsch (Kentucky)
Mélanie Auffan (CEREGE)
Glenda Kelly (Duke)



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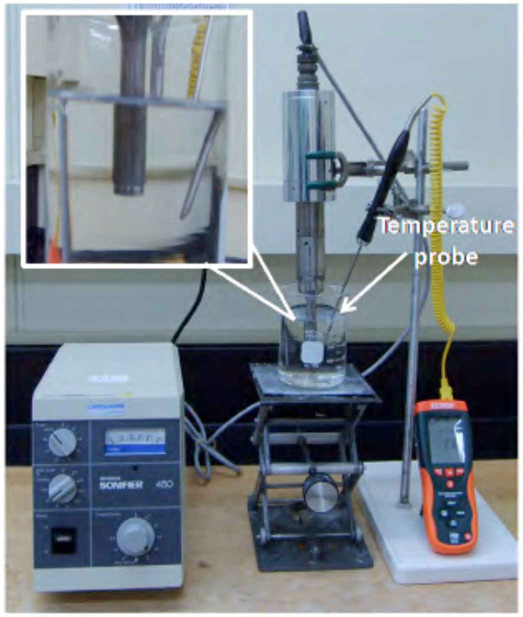
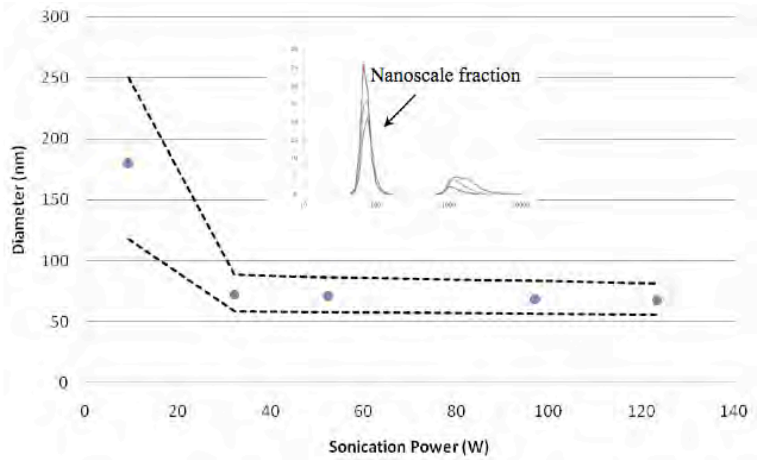
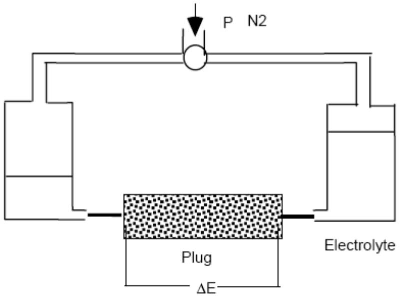
Protocol Development

- CEINT has published 13 protocols on its website describing methods for preparing nanoparticle suspensions, measuring properties of nanoparticles, characterizing surfaces, and evaluating toxicological endpoints
- Two protocols, and one set of guidelines on preparation of nanoparticle dispersions have been developed in collaboration with the National Institute of Standards and Technology (NIST).
- Additional draft protocols related to evaluation of nanoparticle affinity for surfaces, bacteriological evaluations, mesocosm and characterization are undergoing internal evaluation by CEINT and our international partners (ICEINT). Draft protocols are accessible to all CEINT/ICEINT members including partners at NIST, EPA and DOE.







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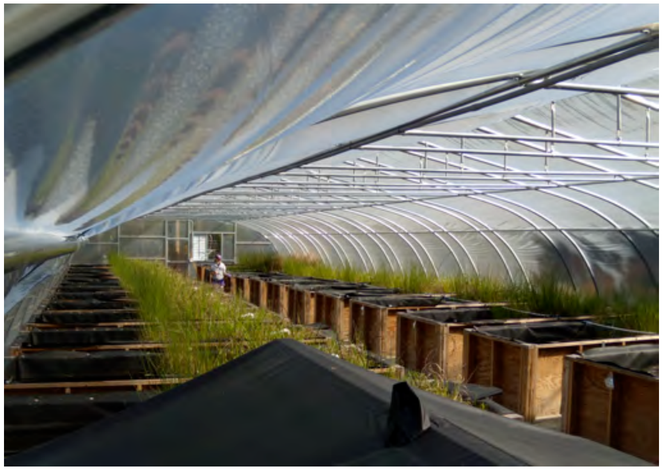
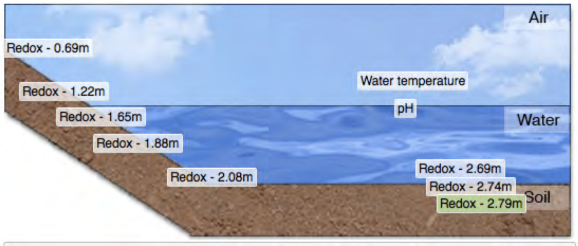
Mesocosm Shared Facility



- **Location / space distribution** – Located in the middle of Duke Forest with: one facility building (~1500 sq ft), 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** – 30 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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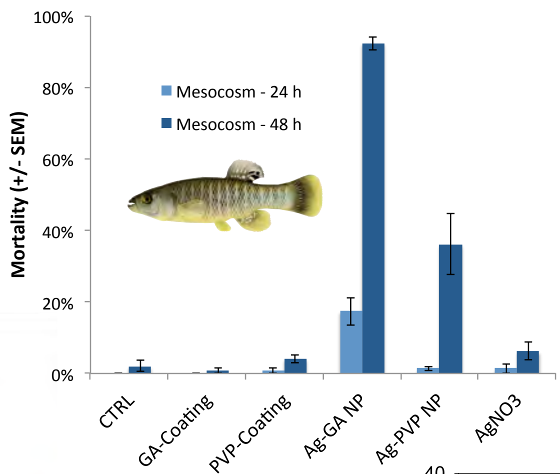
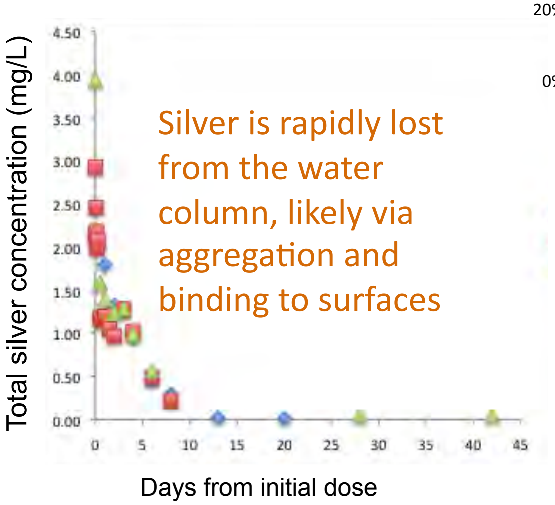
Preliminary Mesocosm Results

Treatment		# Replicates
Control (True Control)	+0.32 mg N L^{-1} as KNO_3	4
Gum Arabic Coated AgNPs	[Ag] = 2.5mg L^{-1}, +0.32 mg N L^{-1} as KNO_3	3
PVP Coated AgNPs	[Ag] = 2.5mg L^{-1}, +0.32 mg N L^{-1} as KNO_3	3
$AgNO_3$ (positive control)	[Ag] = 2.5mg L^{-1}	3
Gum Arabic (coating control)	GA +0.32 mg N L^{-1} as KNO_3	3
PVP (coating control)	PVP +0.32 mg N L^{-1} as KNO_3	3

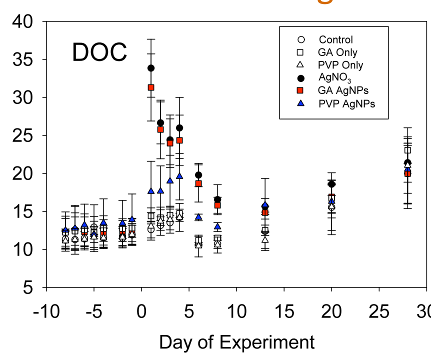
AgNPs are more toxic to killifish larvae on an equal Ag mass basis than $AgNO_3$

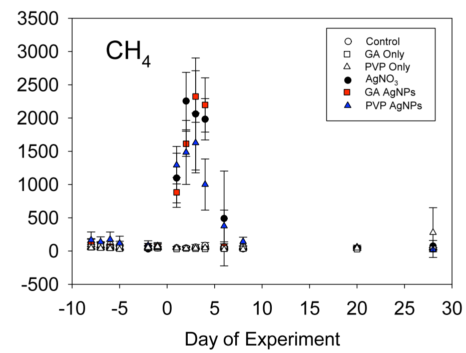




Silver is rapidly lost from the water column, likely via aggregation and binding to surfaces

There were rapid responses in dissolved organic carbon, likely from plant release, and in microbial activity, following treatment with Ag





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NanoDays 2011:CEINT Expands Outreach Nationally Museum Visitors Number over 4,800

CEINT has participated in NISE Net affiliated NanoDays since 2009.

NISE Net is the largest network of informal science educators/researchers for fostering awareness of nanoscale science/engineering in U.S.

NC Partner Museums 2011: Museum of Life and Science, Durham, NC
Marbles Kids Museum, Raleigh, NC

NanoDays 2011: CEINT partnered with Children's Museum of Pittsburgh

- CEINT Deputy Director Greg Lowry and CMU graduate student team engaged museum visitors with hands-on nanoscience activities.

Visitors to CEINT partner museums for NanoDays 2009-11 have numbered over 8,500.

Activities Children's Museum of Pittsburgh *

1. **Scented Balloons.** Demo: "nano" is very small and size matters.
2. **Antacid Tablets.** Demo: nanomaterials have higher surface area than bulk, macro-sized material, which increases reactivity.
3. **Nano-sand / Nano-pants.** Demo: nano-scale coatings and surface structures can change properties of a material.
4. **NanoToss:** Surface Coatings (Developed by CEINT team). Demo: surface coatings change properties of nanomaterials (focus on environmental implications)

*Activities 1-3 from the NISE Network website: http://www.nisenet.org

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CEINT Expands Education Programs

New Center-wide Research Experiences for Undergraduate (REU) Program funded to begin summer 2011

REU Research Sites: Duke, Virginia Tech, Carnegie Mellon and the European Center for Research and Education in Geosciences and the Environment (CEREGE) in Aix-en-Provence, France

Summer research opportunities will span the six CEINT research themes designed to link fundamental physical and chemical properties of nano-scale materials with their observed biological and ecosystem effects.

Students will learn how risk assessment provides feedback to guide future research and about the importance of developing nanotechnologies in an environmentally responsible manner

11 students were recruited for Summer 2011 from across the U.S. and Puerto Rico

Interested applicants for summer 2012 CEINT REU can download application at:

http://www.ceint.duke.edu/content/reu

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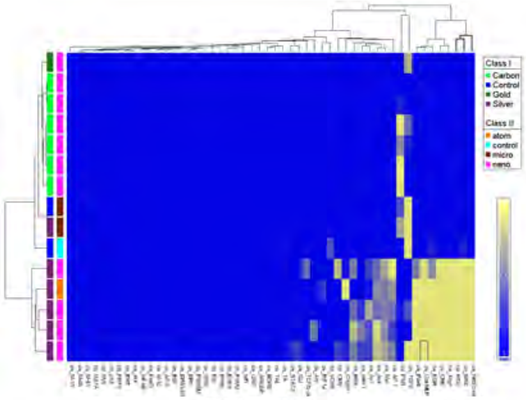
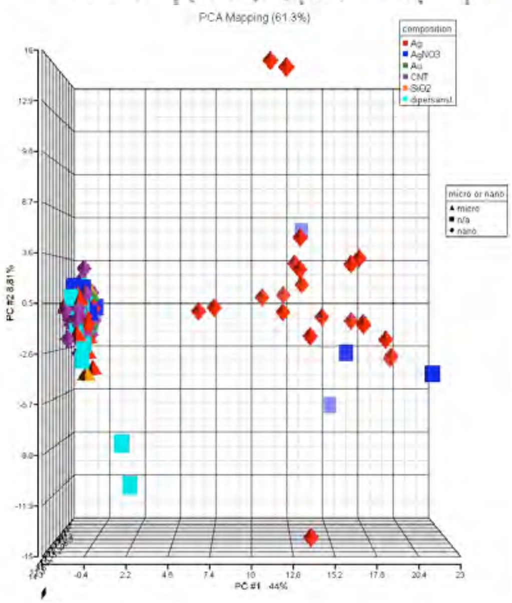
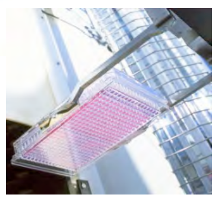
ToxCast High-throughput screening (HTS) of nanomaterial bioactivites in cultured cells and zebrafish embryos

National Center for Computational Toxicology (NCCT), U.S. EPA In Collaboration with CEINT







Preliminary analysis of CNT and nano-Ag results suggested that

- Cell-based HTS assays can be used for nanomaterials
- HTS we used did not detect CNT toxicity
    - Cytotoxicity : $AgNO_3$ > nano-Ag of various coating (relative ranking vary by cells) >>> CNT
    - Nano-Ag and $AgNO_3$ affected similar pathways/ functions, while CNT affected different pathways/ functions

- CNT and nano-Ag did not increase malformation in zebrafish embryos
    - Decreasing viability: $AgNO_3$ > nano-Ag-gum arabic > nano-Ag_M300 (from ENPRA) >>> CNT, nano-Ag –citrate, nano-Ag-PVP, or micro-Ag

This slide does not necessarily reflect U.S. EPA policy.

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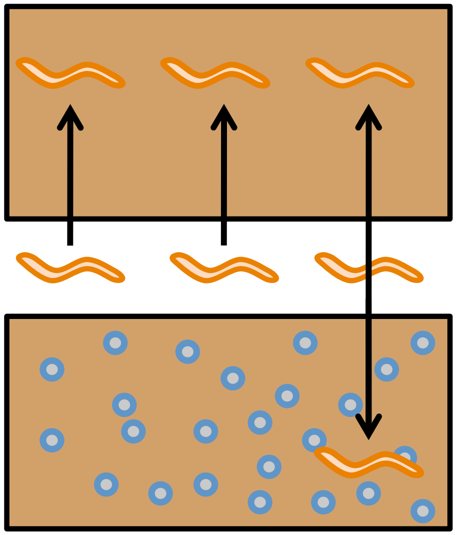
Avoidance of Ag NPs at environmentally relevant concentrations



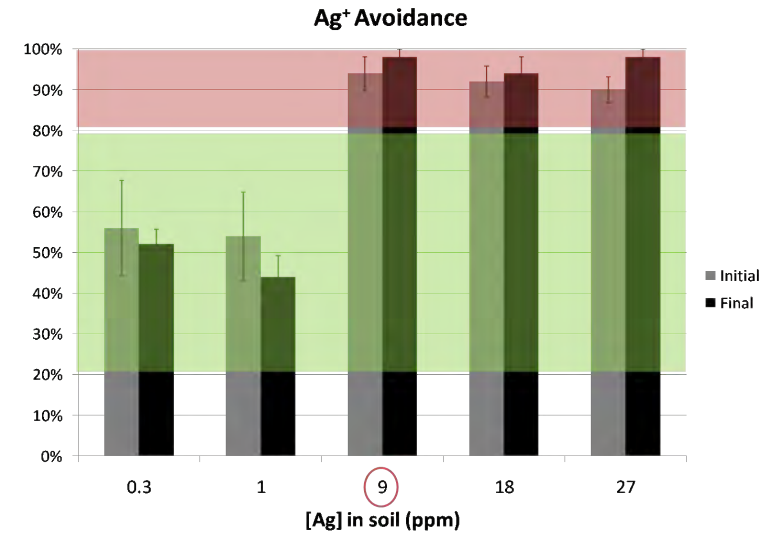
Avoidance:

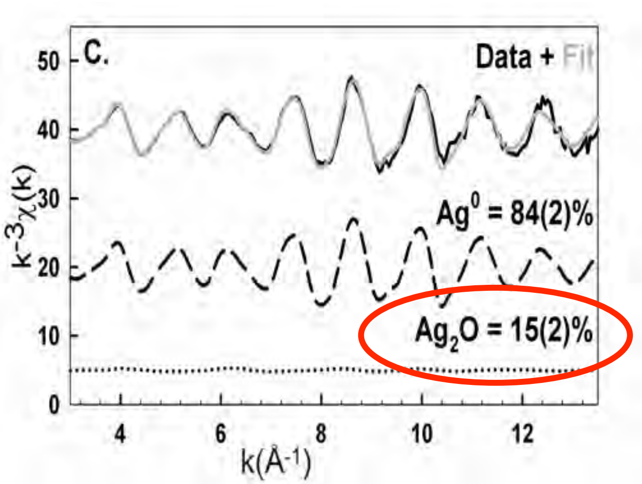
6.76–7.42 mg kg-1

vs.

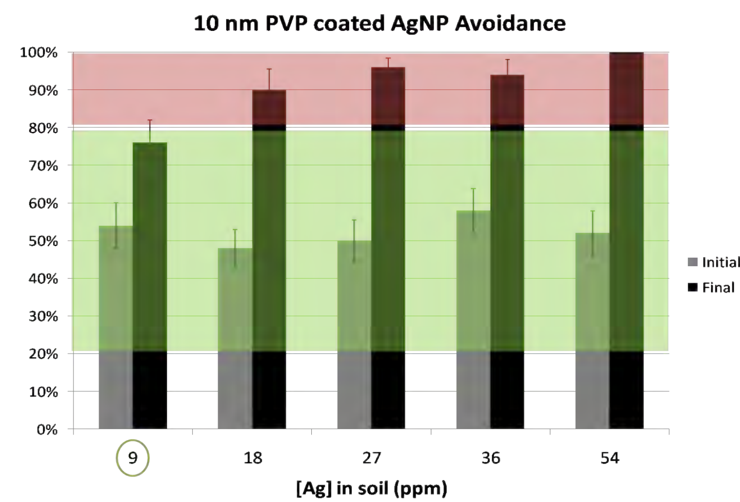
Growth, mortality, reproduction:

773.3–801 mg kg-1





Based on % of NPs oxidized: Max conc. Ag^+ ~1.35 mg kg^{-1}



Shoults-Wilson et al. 2010 & 2011

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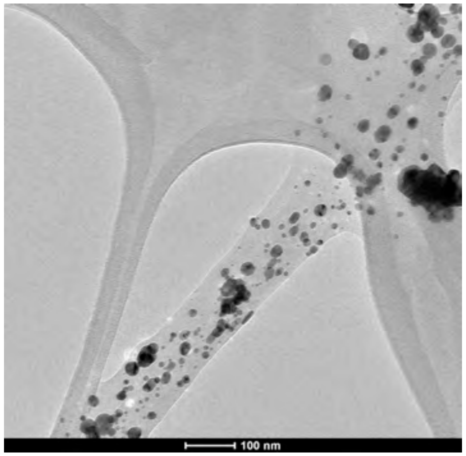
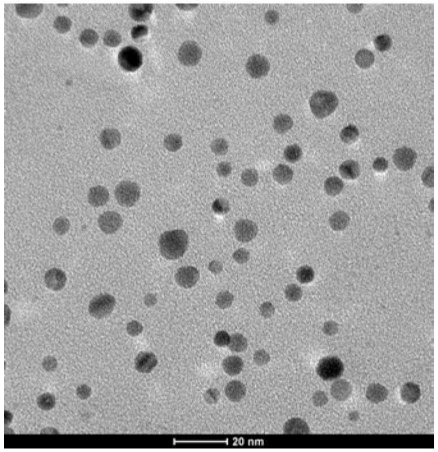
Commercial vs. Home-Made Materials

Commercial materials are not uniform



TEM of 10 nm Commercial Nano Ag



TEM of 8 nm home made Nano Ag

Home made samples offer more flexibility in size and coating for detailed study; Commercial and home-made materials show different toxicity

- Commercial (NanoAmor) PVP-AgNPs do not completely sulfidize and toxicity is not decreased until sulfide concentration is 15 mM after 24 hours.
- Lab Synthesized AgNP completely sulfidize and toxicity is decreased for sulfide concentrations of 2 mM after 24 hours.

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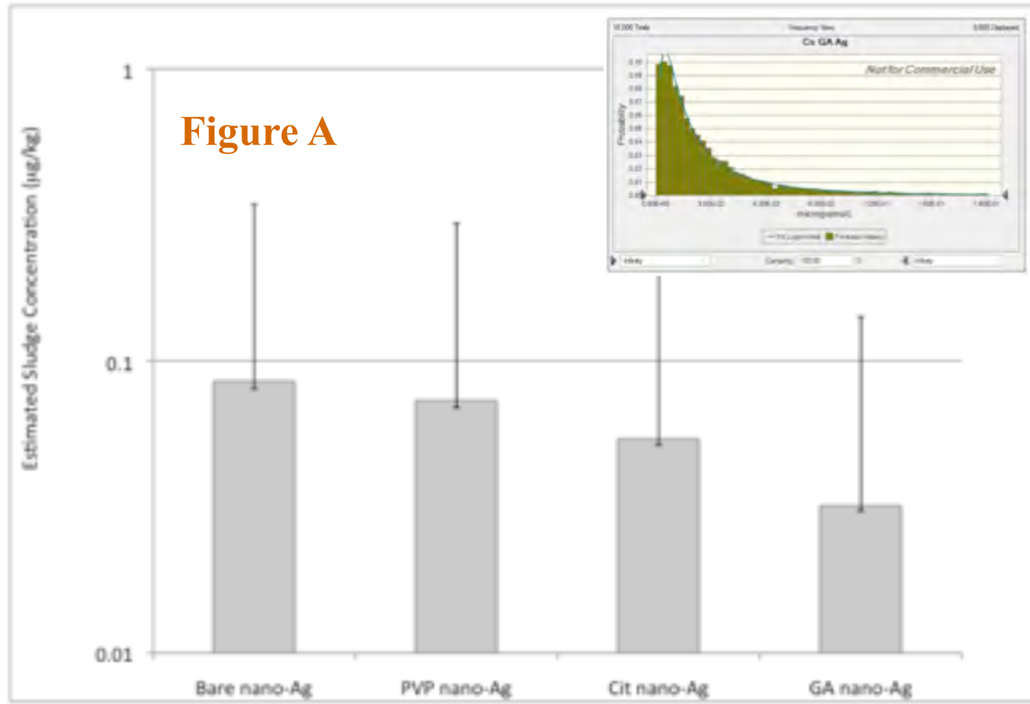
Forecasts and measurements of nanosilver in wastewater

Christine Hendren, Mark Wiesner, Elizabeth Casman, Bojeong Kim, Kelly Plathe, and Mike Hochella

CEINT researchers at Duke, CMU and Virginia Tech are collaborating to determine possible and measured levels of nano-scale silver particles in wastewater.

Products containing nanomaterials may be disposed of via municipal sewage and thus encounter wastewater treatment plants as their first gateway into the environment. Estimates of the quantities of sillver nanoparticles that may enter wastewater and the be subsequently removed to biosolids or remain in treated water are calculated by first obtaining estimates for the quantity of U.S. production and combining these with laboratory data on the affinity of nanoparticles for biosolids in Monte Carlo simulations of the treatment plant. The resulting probability distributions of silver nanoparticles in biosolids and treated water (Figure A) show that the fate of these particles during treatment will vary as a function of the coatings engineered on the nanoparticles.

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. The question remains as to whether or not nano-scale particles of silver found in waste water originate from engineeered sources or are formed from discharged dissolved silver.



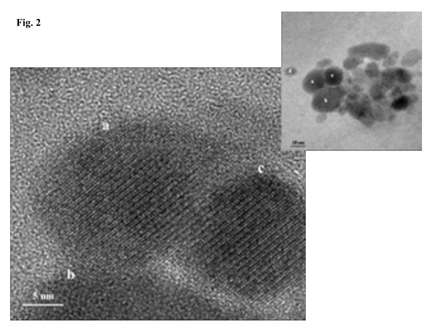


Figure B

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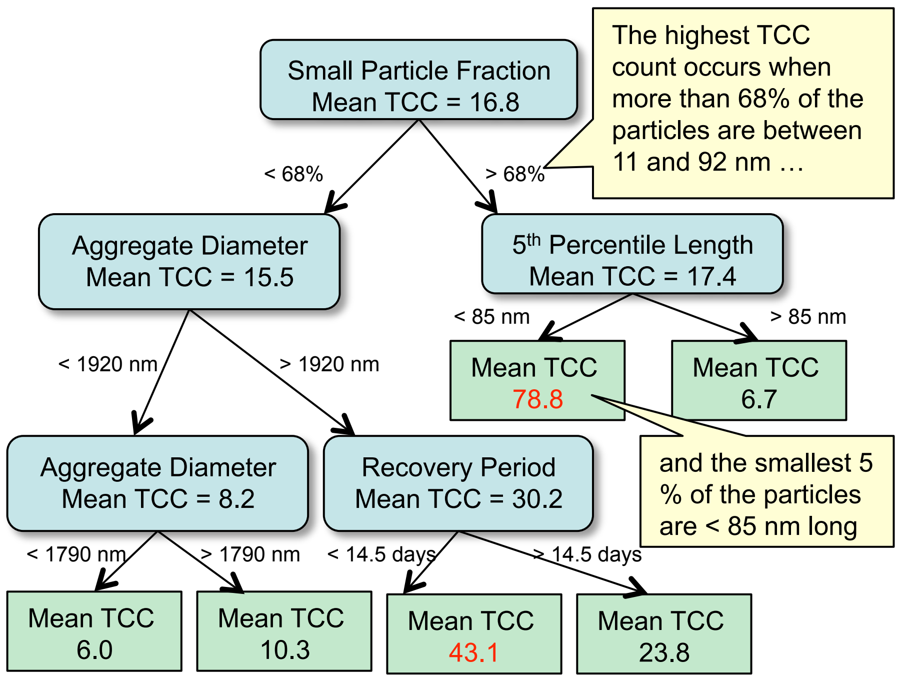
Mapping Nanoparticle Properties to Toxicity

Jeremy Gernand and Elizabeth Casman (CMU)

Regression Tree Analysis was used to perform a meta-analysis of 8 rodent inhalational toxicology studies of carbon nanotubes (CNT) to identify and rank the important predictive variables.

Regression Tree for Total Cell Count (TCC) in BAL Fluid



Major findings:

- Different CNT properties were associated with different toxicological endpoints measured in BAL fluid (e.g., immune response, inflammation, cell damage).
- For Total Cell Count, there are two size-related responses, the more severe associated with high doses of short CNT fragments, the other one associated with large aggregate sizes > 2μm.
- For Total Protein concentration, the impurities in the CNTs are the most important variables.
- The mode of exposure (instillation vs. inhalation) has very little effect on toxicity outcomes relative to the other variables like dose and CNT geometry.
- The most information-rich measure of dose for CNTs is by mass, however limitations in particle count and surface area measurement methods may explain this.
- Mouse and rat studies are comparable for outcomes measured on a relative-change-from-control basis.

Gernand J. and E. Casman (In preparation) “Identification of information-rich attributes for carbon nanotube pulmonary toxicological risk assessment." *Risk Analysis*.

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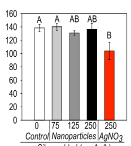
Research at Multiple Scales and Levels of Complexity

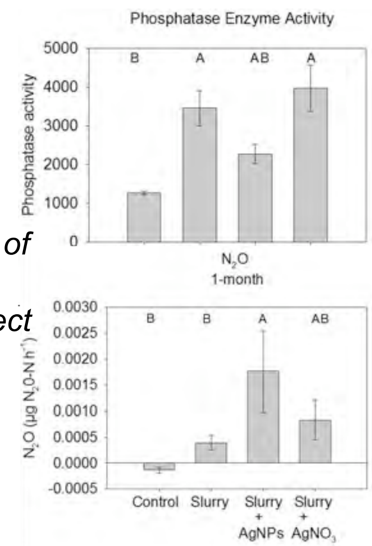
To predict the fate and effect of nanomaterials in the environment researchers typically scale up from highly controlled
lab experiments

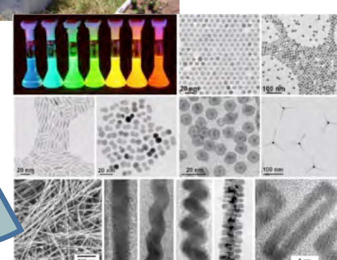


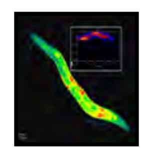
Central to **CEINT** is our commitment to testing emerging models and assumptions under field conditions to identify critical uncertainties

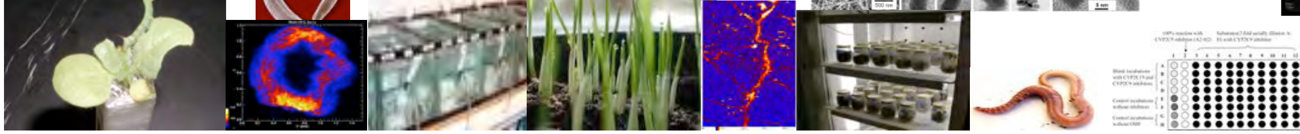
In 7 day lab assays AgNPs added to floodplain sediments had no effect on microbial composition or activity

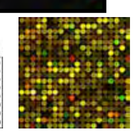
Nanocosm differed from laboratory experiments: lower concentration of AgNPs had significant effect on microbial activity











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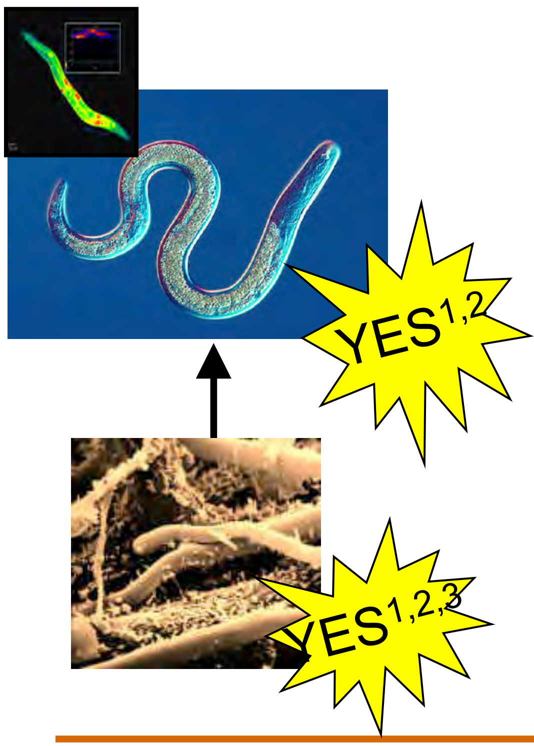
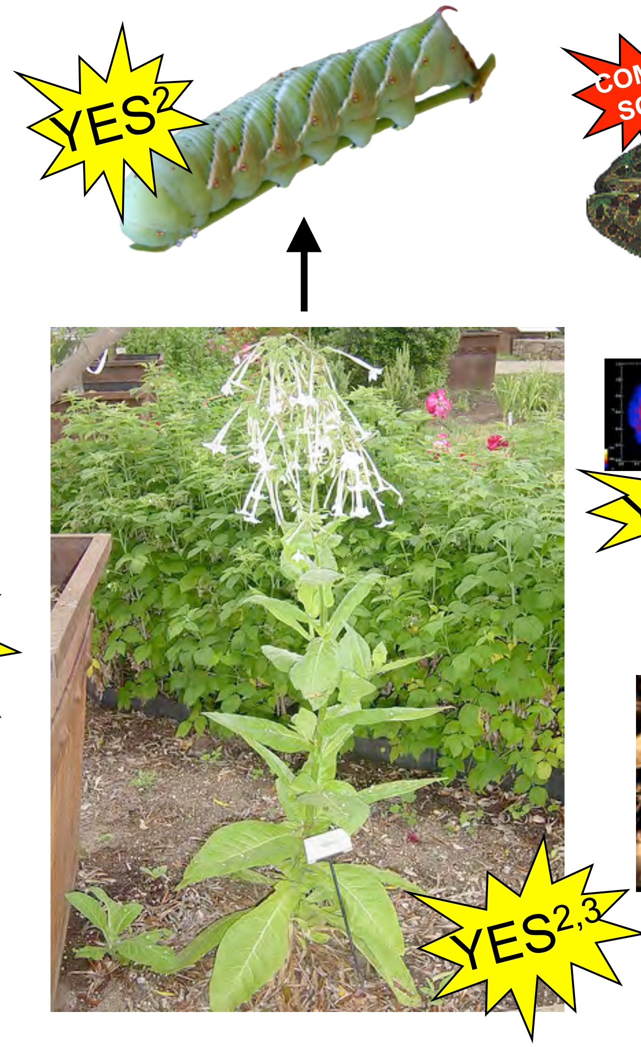
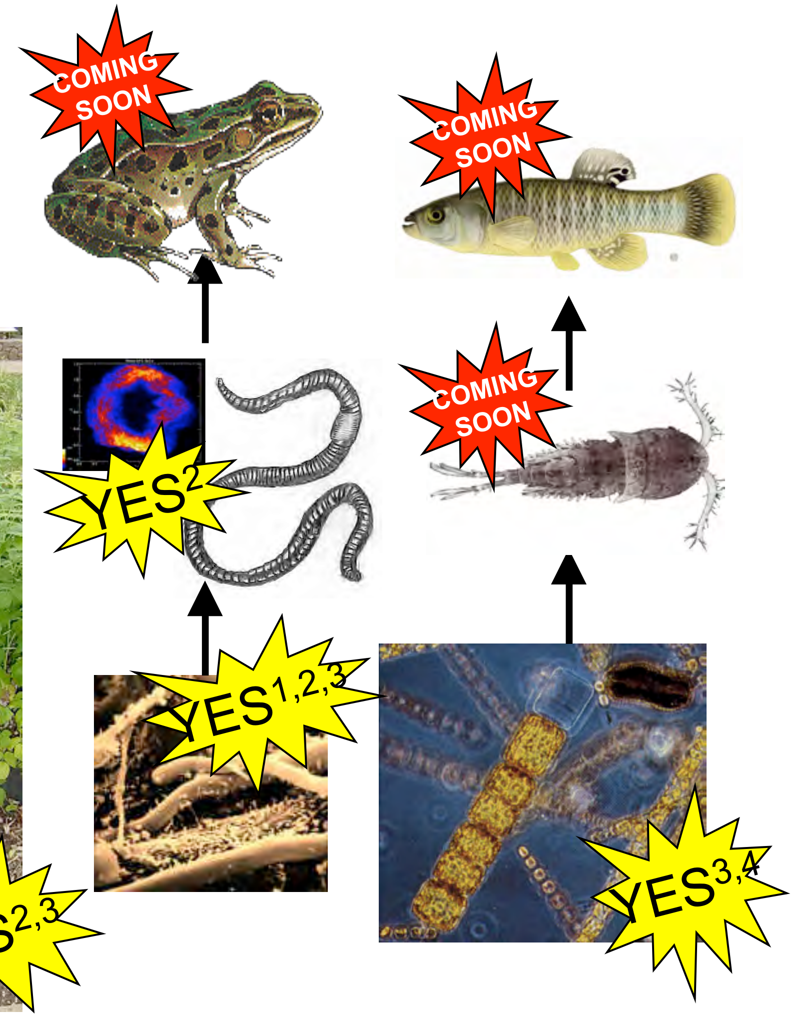
Trophic Transfer of Nanomaterials

Summary of CEINT investigations into trophic transfer in different systems







[1]Meyer, [2]Paul Bertsch & Unrine, [3]Bernhardt, Richardson & Gunsch, [4]Hunt

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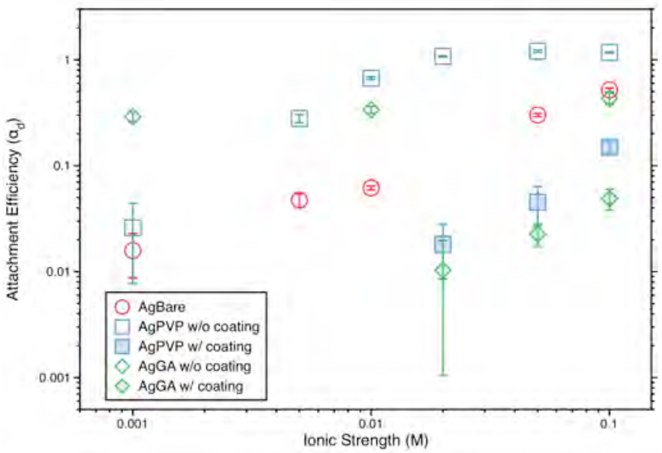
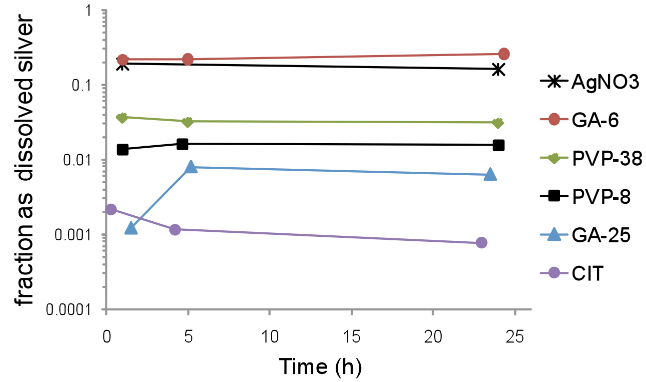
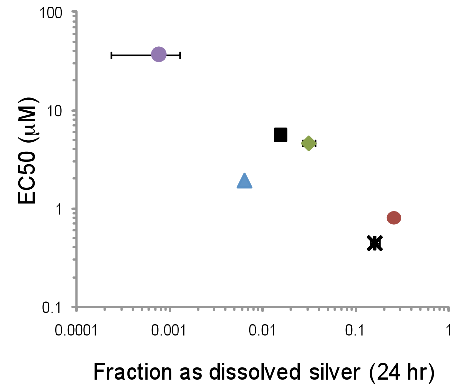
Nanoparticle Coatings Matter…or not

Shihong Lin, Mark Wiesner, Stella Marinakos, Jie Liu, Gregory Lowry, Joel Meyer, Helen Hsu-Kim, Cole Matson



Polymeric surface coating may stabilize AgNPs against deposition only if the coatings are also present in the AgNPs suspension or on the collector surface. Differences between coatings are greater at high ionic strength. In comparison with uncoated AgNPs, coated AgNPs may have a higher affinity for uncoated silica surfaces, contrary to common understanding.





AgNPs order of toxicity to *C. elegans* (by molarity):

$AgNO_3 > GA_6 > GA_{25} > PVP_8 = PVP_{38} > CIT_{10} > PVP_S > PVP_L$

Coating does not predict toxicity, except where they alter dissolution rates. Interplay between coating and particle size affects dissolution and subsequently toxicity.

Strain Sensitivity	N2	mtl-2	pcs-1	mev-1	sod-3
$AgNO_3$	+	++	++	+	+
PVP_8	+	++	++	+	+
PVP_{38}	+	++	++	++	++
CitrateAg	+	++	++	++	++
GA_6	+	++	++	+	+
GA_{25}	+	++	++	++	++

Mutant strain sensitivity in *C. elegans* does not clearly track coating, but does seem to relate to NP size.

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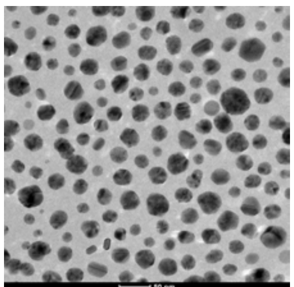
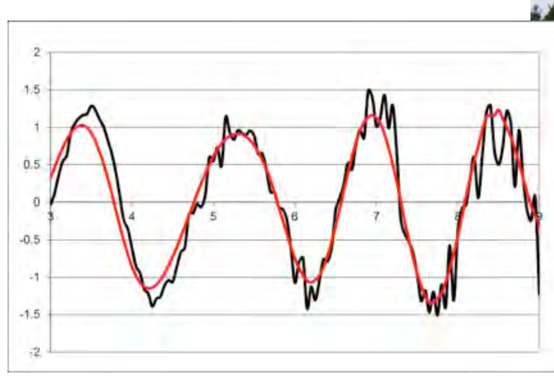
Ag NPs rapidly transform to Ag_2S and Ag-S (organic) in natural and engineered systems

Brian Reinsch, Clement Levard, Ben Colman, Nadine Kabengi, Stella Marinakos, Jie Liu, Gordon Brown, Gregory Lowry

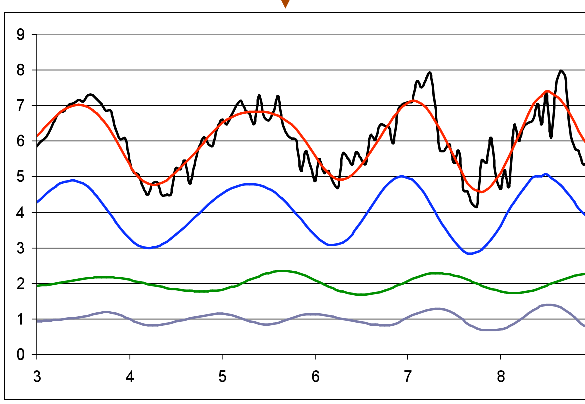
Ag PVP-25 nm



2 weeks



3 months



Biosolids (anaerobic)
100% Ag_2S

Simulated Wetland Sediment
85% Ag_2S
15% Ag-S (organic)
5% Ag(0)

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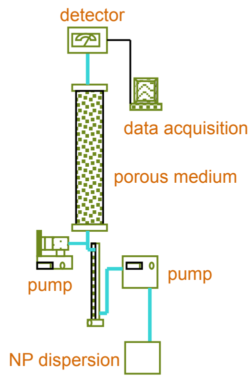
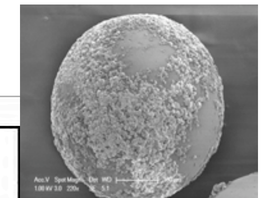
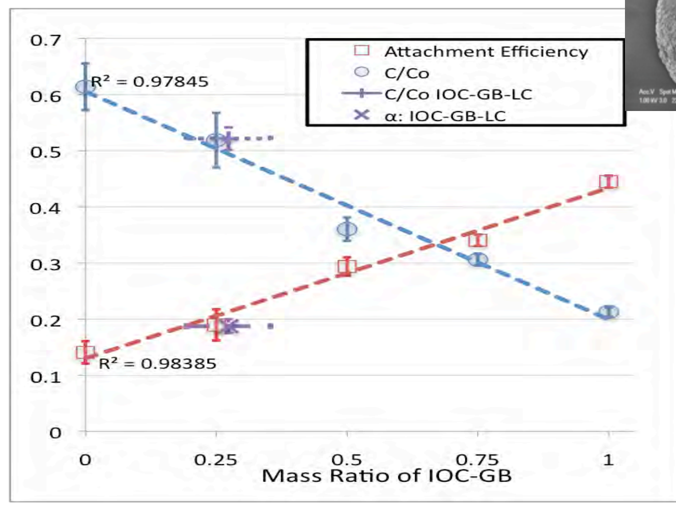
Predicting deposition of NPs Coated with organic macromolecules

Shihong Lin, Jee Eun Song, Malai Ramamoorthy, Stella Marinakos, Mark Wiesner, Gregory Lowry, Kim Jones, Jie Liu

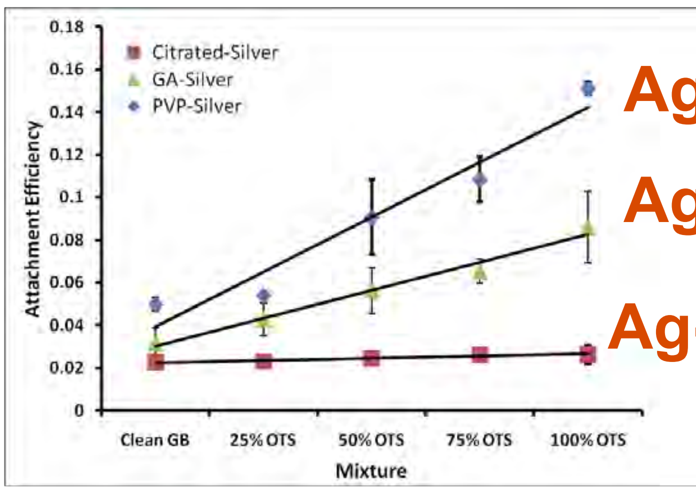






NP Hydrophobicity
Ag-PVP>Ag-GA>>Ag-citrate

OTS (hydrophobic) sand



Ag-PVP

Ag-GA

Ag-citrate

- Deposition of PVP-coated Ag NPs to Fe-oxide surfaces and hydrophobic surfaces increases linearly with increasing amount of that surface
- More hydrophobic coatings deposit greater onto hydrophobic surfaces
- Models using linear combinations of environmental surfaces may be possible to describe distribution of ENPs in the environment.

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