UCCEIN Center for Environmental Implications of NanoTechnology

Establishing the New Science of Nano-Safety: Towards Safe Implementation of Nanotechnology

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University of California, Los Angeles
Department of Civil & Environmental Engineering and California NanoSystems Institute

Nanotech III, March 19, 2009, Sacramento, California

"NanoRegulation – Anticipating the Smallest Threats and the Largest Opportunities"

Outline of talk…

- Potential benefits/uses of nanotechnology
- Lessons learned from the past
  - One at a time *vs*. high throughput risk assessment
- Complicating factors and sources of uncertainty
  - A nanoparticle is not always a nanoparticle
- A new paradigm for assessing environmental risk
  - UC TSR&TP: Nanotoxicology + UC CEIN
- Case study…towards reduced uncertainty

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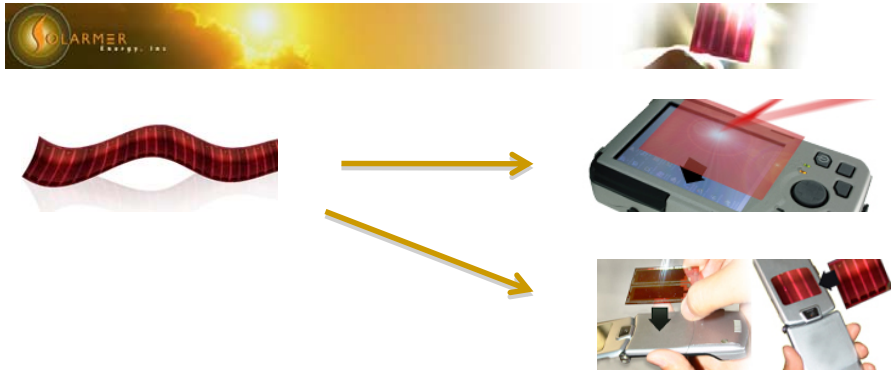
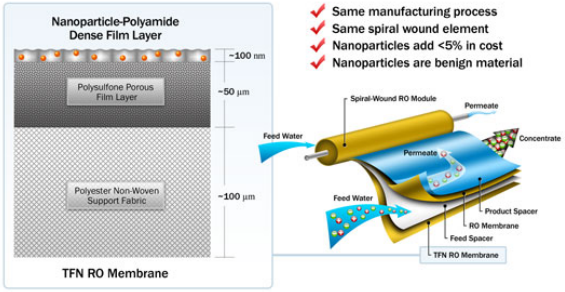
Potential benefits/uses of nanotechnology…

Renewable energy



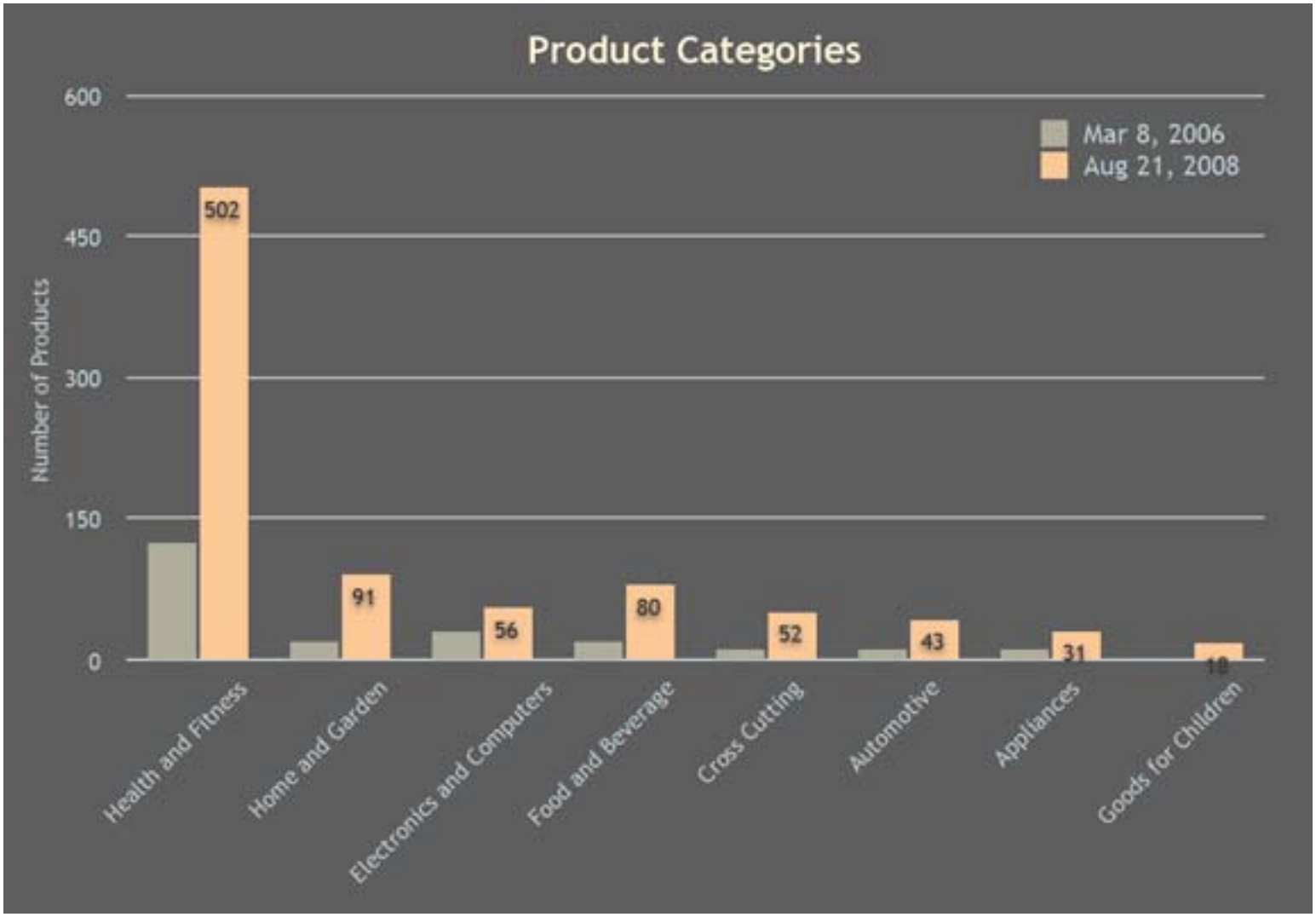
Better healthcare

Clean water



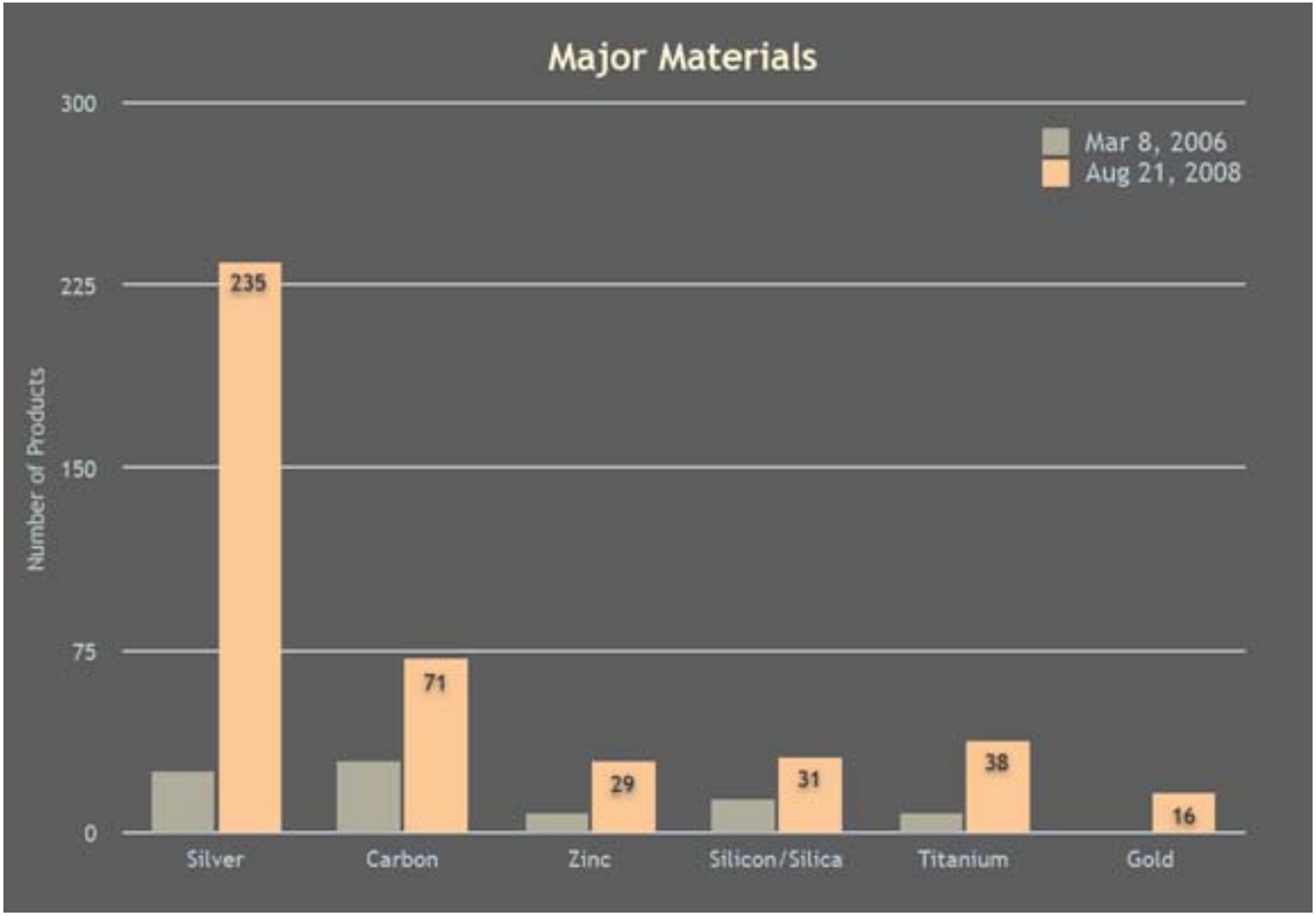
- Can we leverage these benefits while minimizing or eliminating the potential risks?

Number of nano-products by category



http://www.nanotechproject.org/inventories/consumer/analysis_draft/

Most common nano-materials



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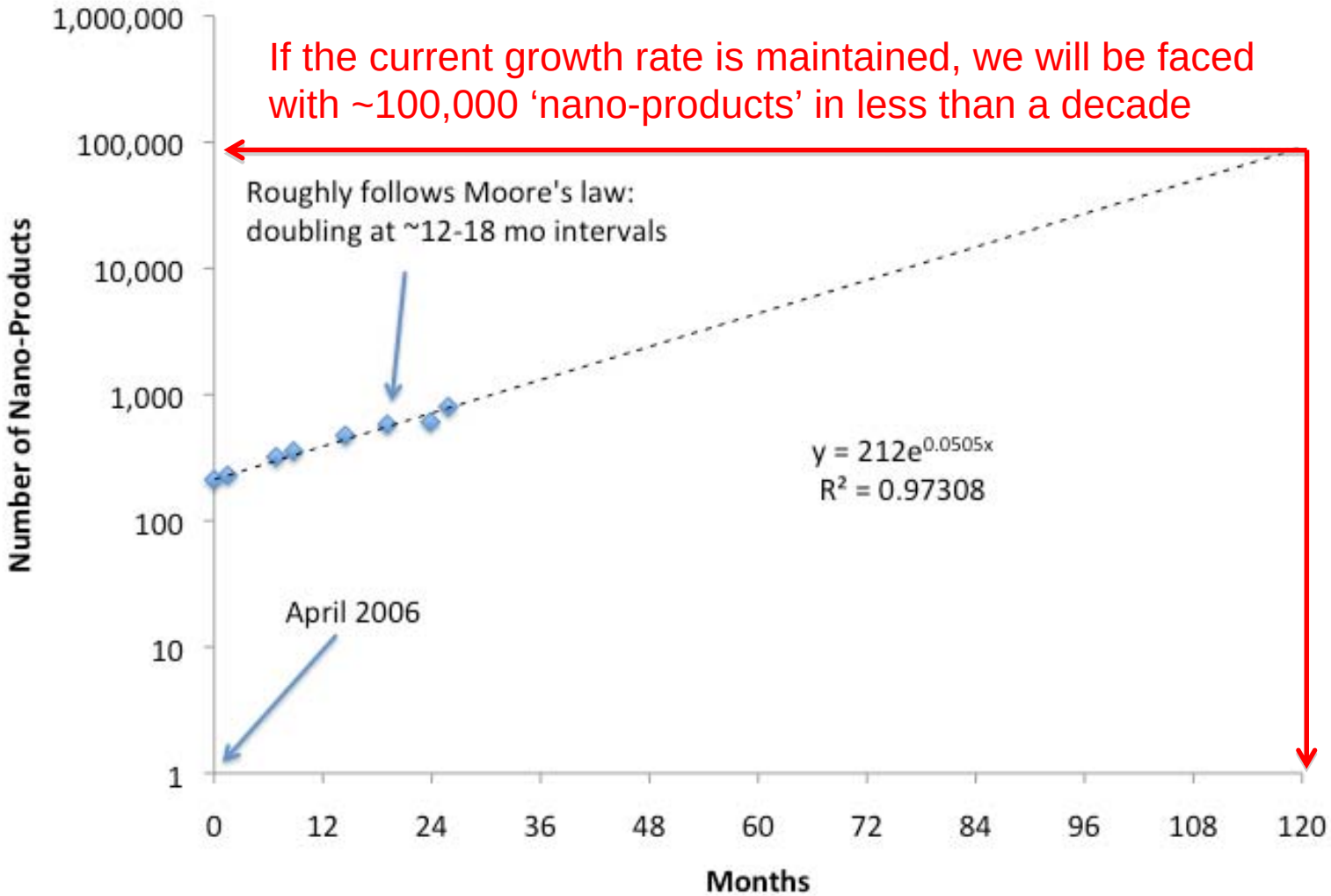
Growth in nano-products



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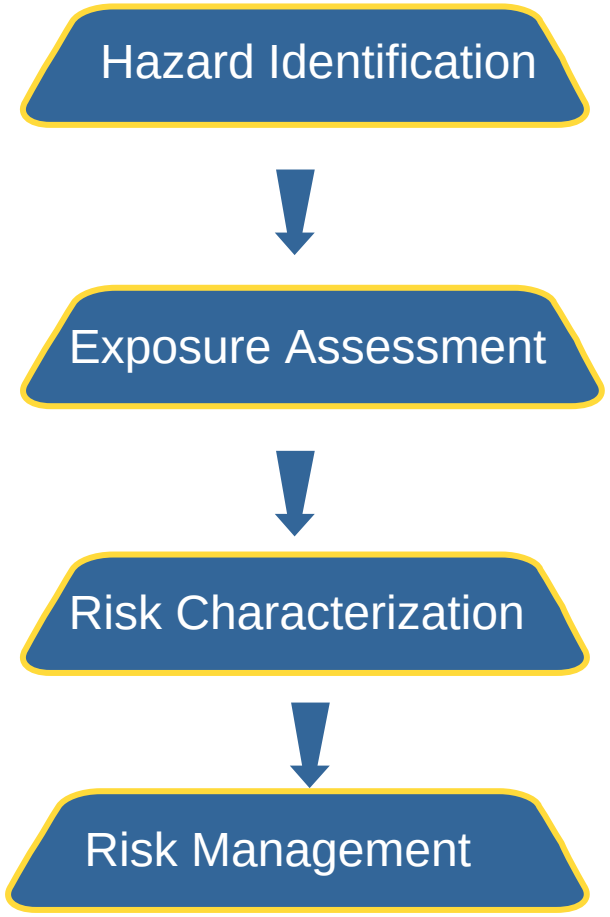
Traditional Toxicological Approach



Failure to address Industrial Chemical Toxicology at the scale of production

50,000 + chemicals registered for commercial use in the US

< 1,000 have undergone toxicity testing

Overwhelming of resources: each test
- $2-$4 million (for in vivo studies)
- > 3 years to complete

US National Academy of Science (NAS) Report (2007): *"Toxicity Testing in the 21st Century: A Vision and a Strategy"*

Current approach to toxicity relies primarily on an evaluation of observable outcomes in whole animals

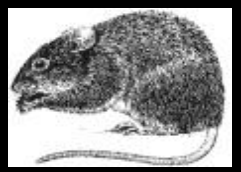
- Time consuming
- Resource intensive
- Paradigm shift is required

http://www.nap.edu/catalog.php?record_id=11970

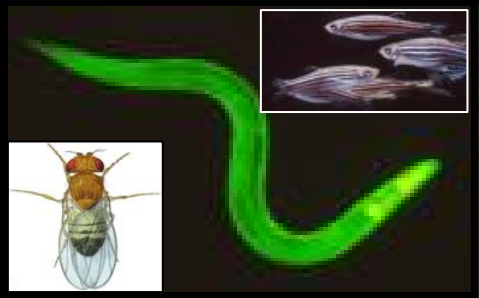
NAS's Recommended Transformative Approach

- Provide broad coverage of potential toxicants
- Use a robust scientific basis for EHS testing
- Comprehensive array of predictive *in vitro* tests that utilize toxicity pathways and mechanisms
- High content or high throughput screening to facilitate testing of large batches of materials
- *In vitro* hazard to be confirmed *in vivo*

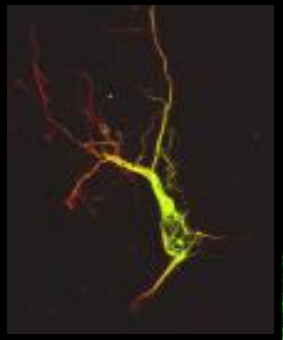
Toxicological testing is moving towards paradigm of high throughput screening and data-mining to establish QSAR relationships, rank risk and prioritize *in vivo* testing



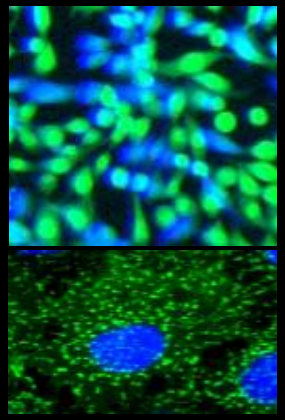
100's/year



1000's/year



10,000's/day



100,000's/day

Immediate Relevance

High Throughput Cellular or Molecular Screening

Prioritize *in vivo* testing

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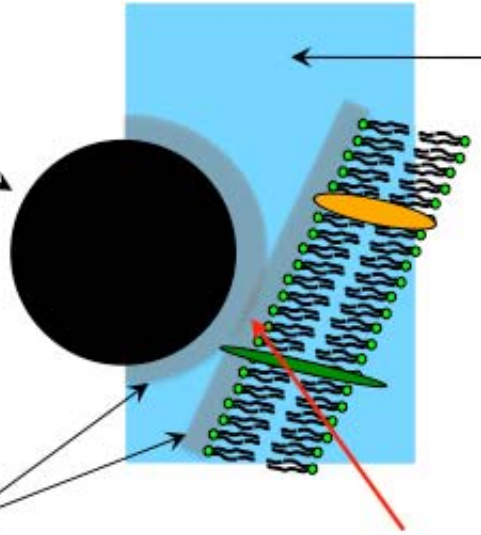
Intrinsic versus environmentally acquired properties and the nano-bio interface



Nanoparticle

- Size/shape/surface area
- Surface charge, energy, rugosity, & porosity
- Valence/conductance states
- Functional groups, ligands
- Crystallinity & defects

Suspending media influences

- H_2O molecules
- Acids & bases
- Salts & multivalent ions
- Natural organic matter (humics, proteins, lipids)
- Surfactants, polymers, & polyelectrolytes

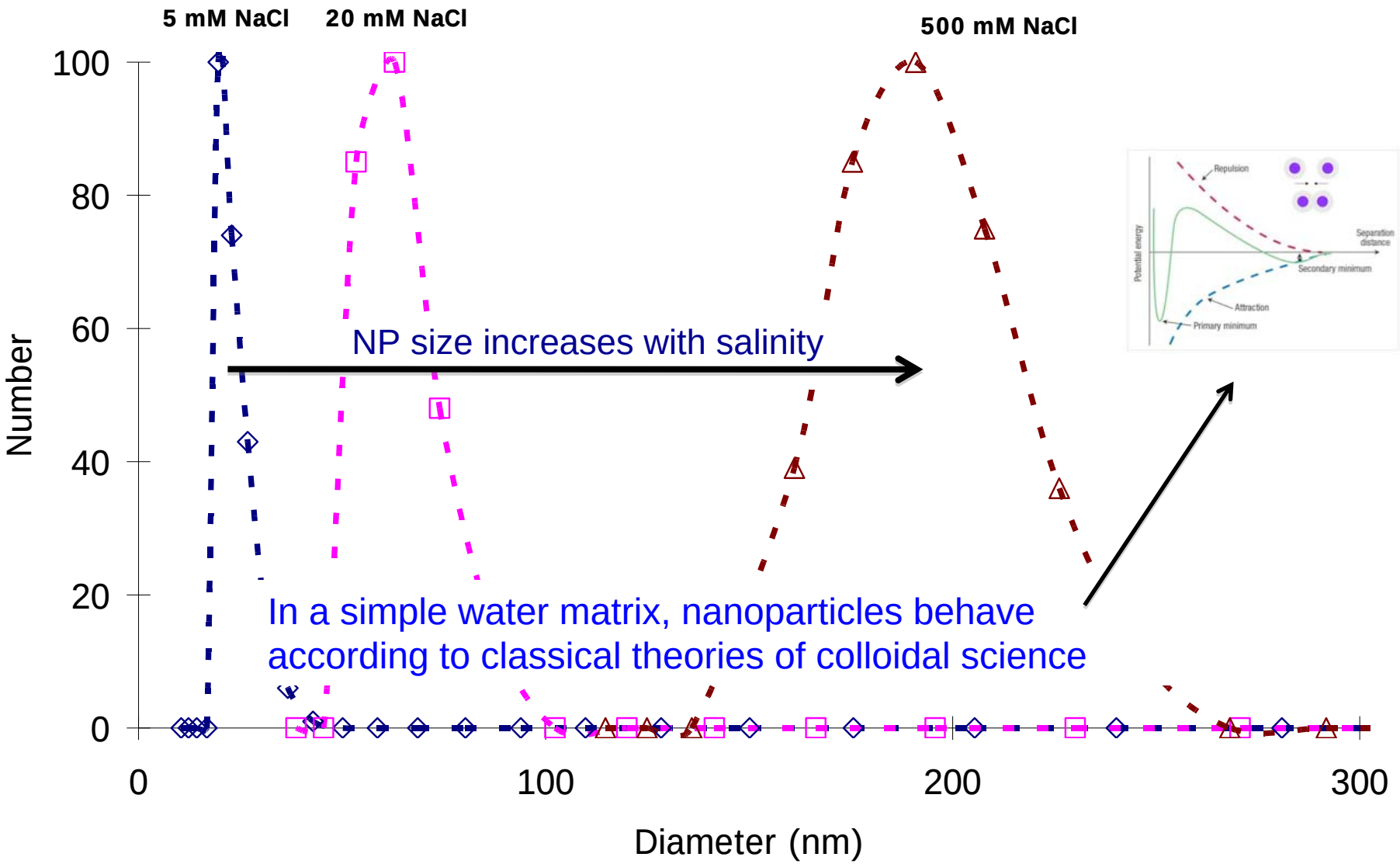
Solid-liquid interface

- Surface hydration-dehydration
- Surface reconstruction & release of free surface energy
- Ion adsorption & charge neutralization
- Electrical double layer formation, zeta potential, and iso-electric point
- Sorption of steric molecules & toxins
- Electrostatic, hydrophilic-hydrophobic, steric, and electro-steric interactions
- Aggregation, dispersion, & dissolution

Nano-bio interactions

- Membrane interactions (specific & non-specific)
- Receptor-ligand binding interactions
- Membrane wrapping (resistive/promotive forces)
- Biomolecule interactions (lipids/proteins/DNA) leading to structural and functional effects
- Free energy transfer to biomolecules
- Conformational change in biomolecules
- Oxidant injury to biomolecules
- Mitochondrial/lysosomal damage, ↓ATP

Variable Size of Silver "Nanoparticles"



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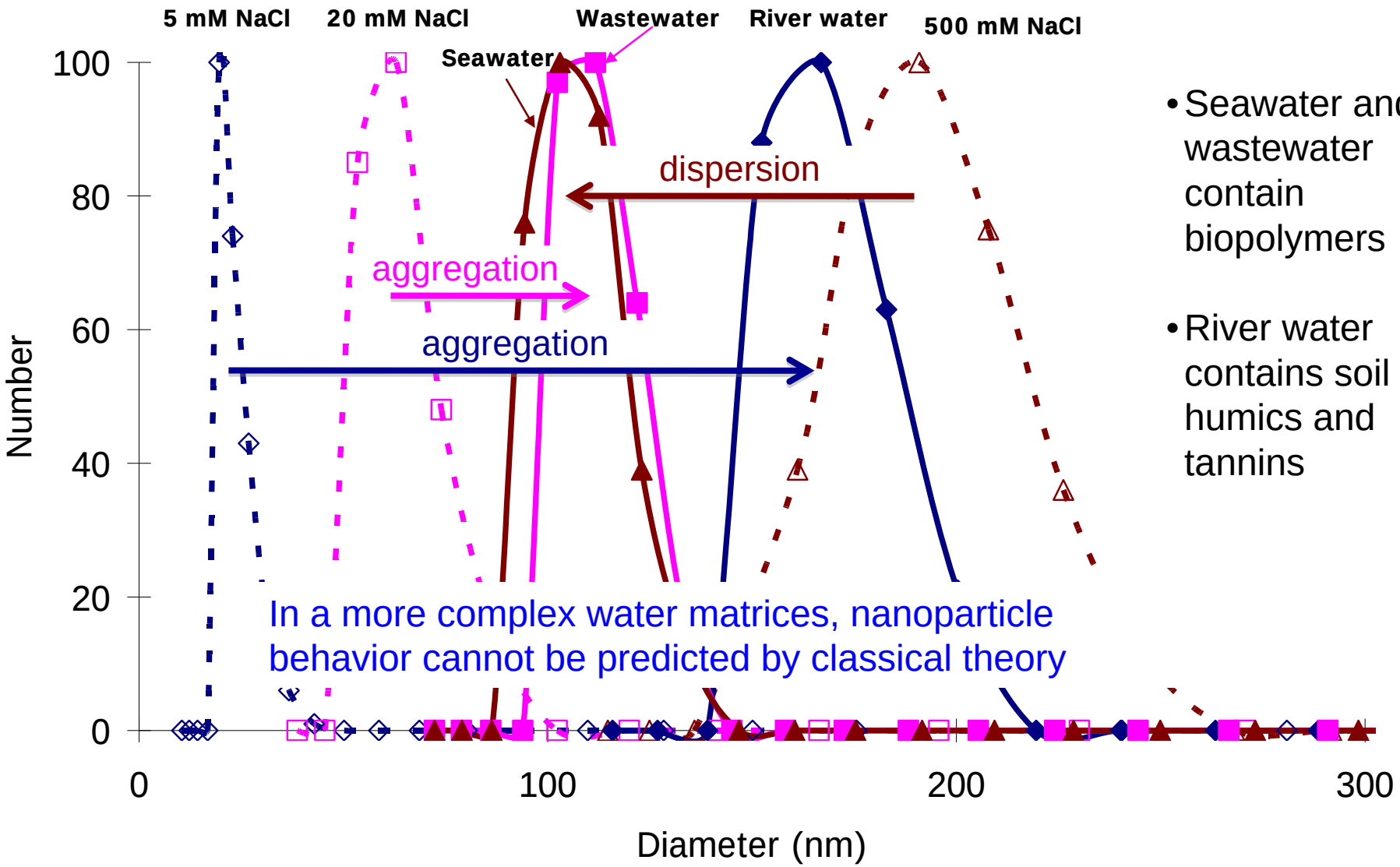
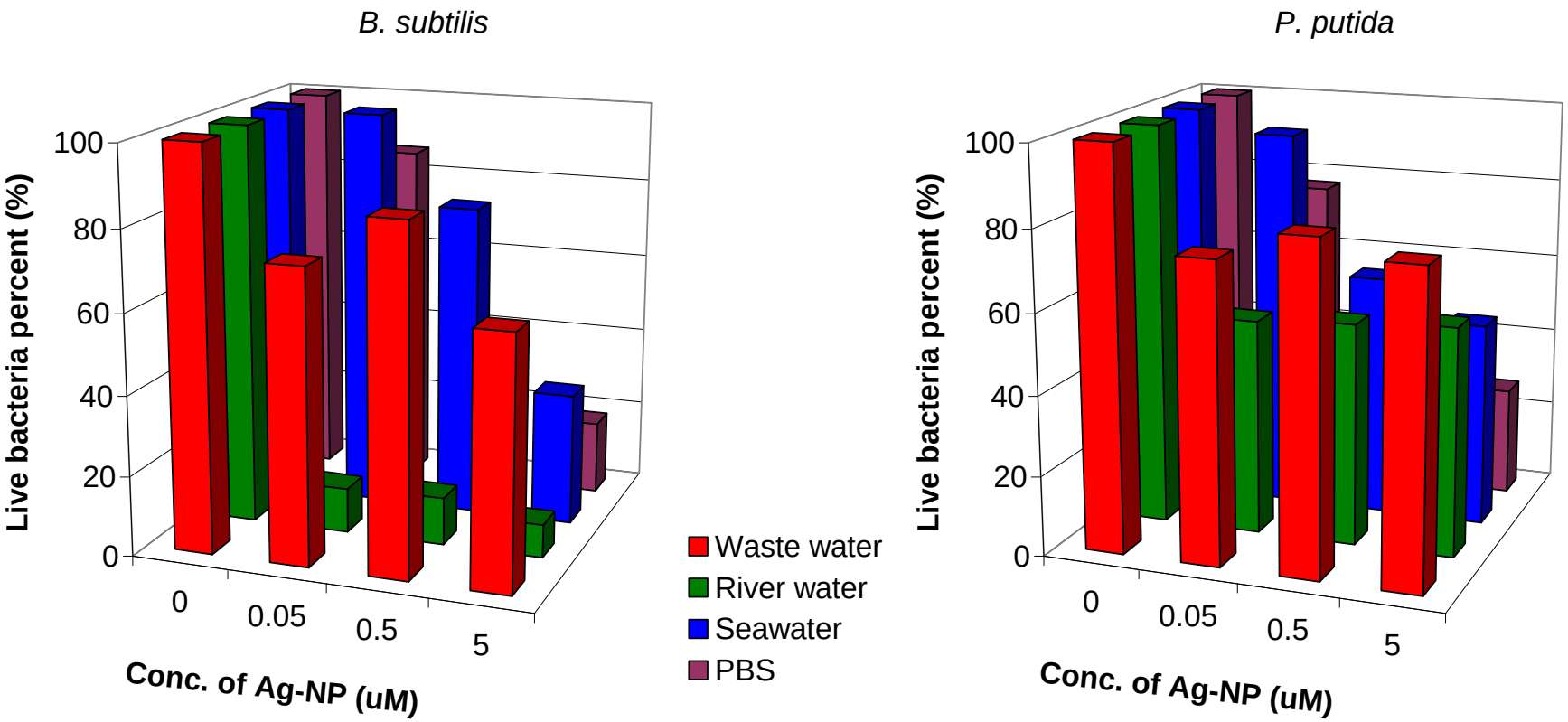


- Seawater and wastewater contain biopolymers
- River water contains soil humics and tannins

Variable Toxicity of Silver Nanoparticles



- Wastewater may protect bacteria against silver toxicity
- In freshwater, silver toxicity is virtually independent of silver nanoparticle concentration above 0.05 μM
- In seawater and PBS, toxicity increases with silver concentration

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UCCEIN Center for Environmental Implications of NanoTechnology

The **University of California Center for Environmental Implications of Nanotechnology (UC CEIN)** will explore the impact of libraries of engineered nanomaterials on a range of cellular lifeforms, organisms and plants in terrestrial, fresh water and sea water environments.

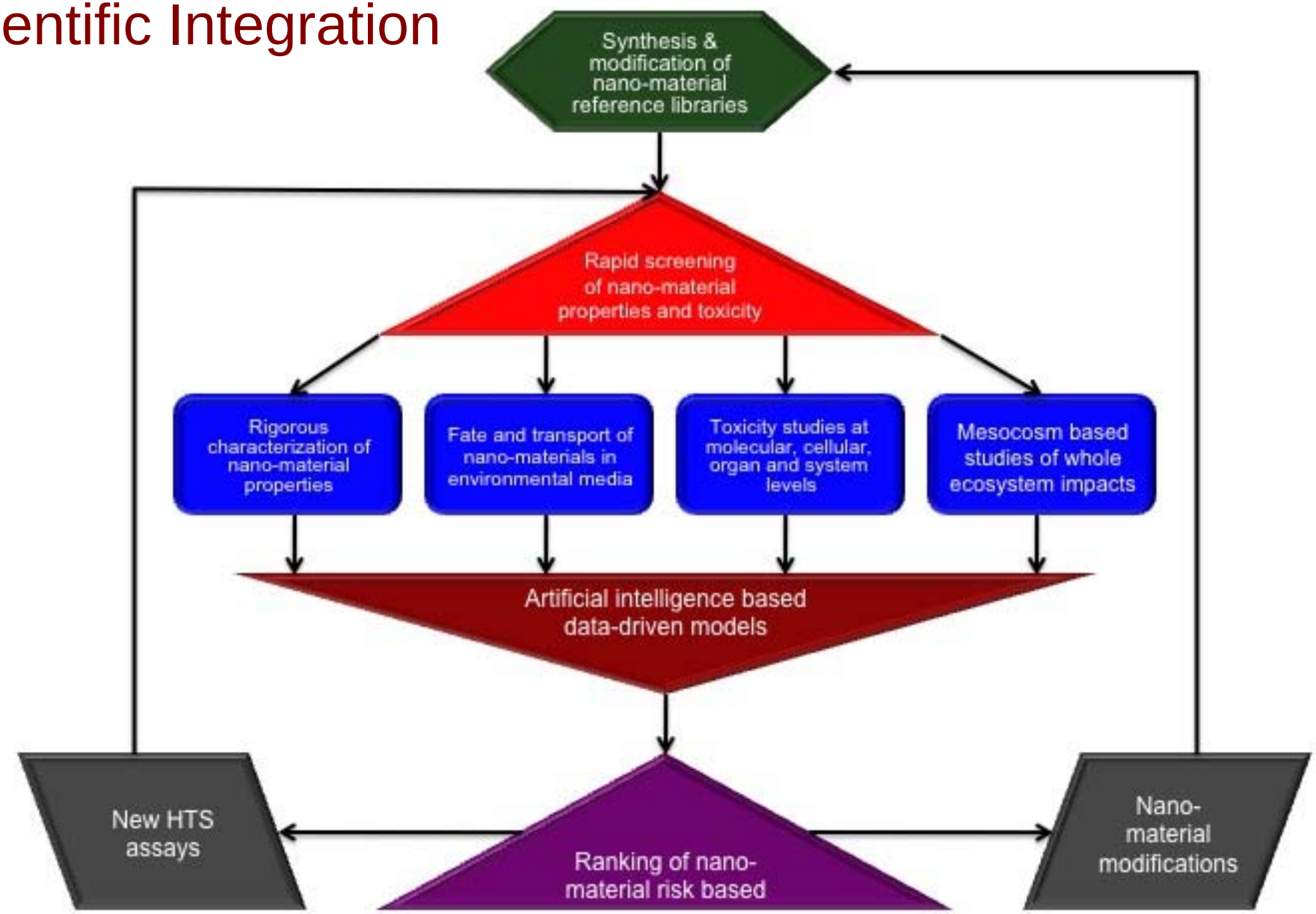
By being able to predict which nanomaterial physicochemical properties are potentially hazardous, the CEIN will be able to provide advice on the safe design of engineered nanomaterials from an environmental perspective.

University of California Los Angeles, Santa Barbara, Davis, Riverside

Columbia University, University of Texas, University of New Mexico, Lawrence Livermore National Laboratory, Lawrence Berkeley National Laboratory

University College Dublin, Nanyang Techonological University, Cardiff University Wales, Unversity of British Columbia, Universitat Rovira i Virgili, Foundation Institute for Materials Science

Scientific Integration

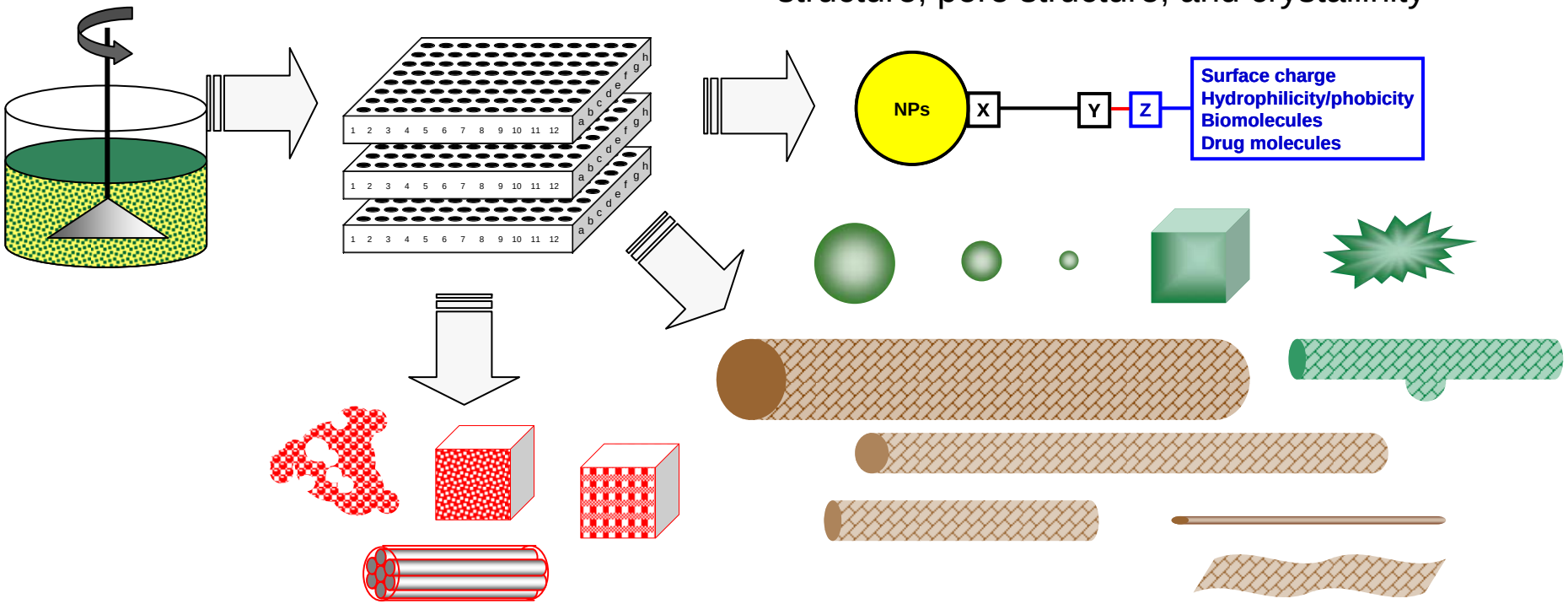


Standard Reference Material Production
-- Creating a Combinatorial Library

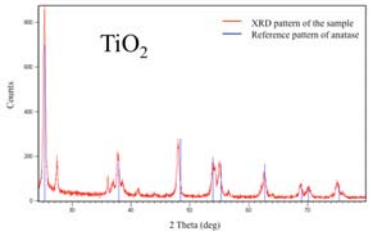
Conventional NM synthesis

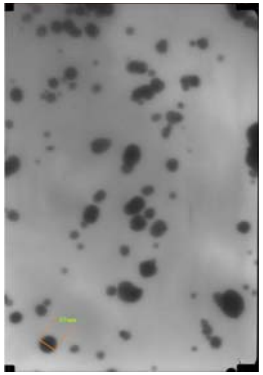
High-throughput NM synthesis & modification

Combinatorial library designed to probe NM surface chemistry, size, shape, roughness, aspect ratio, aggregate structure, pore structure, and crystallinity

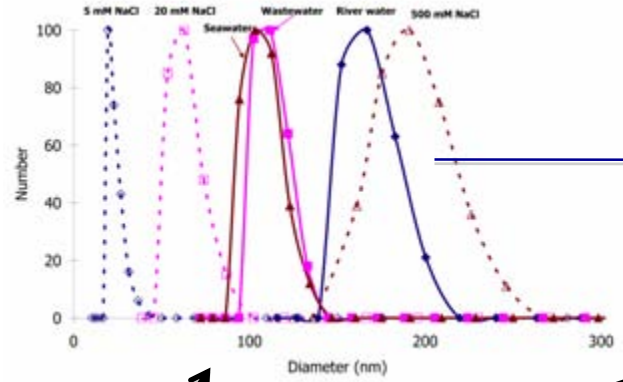


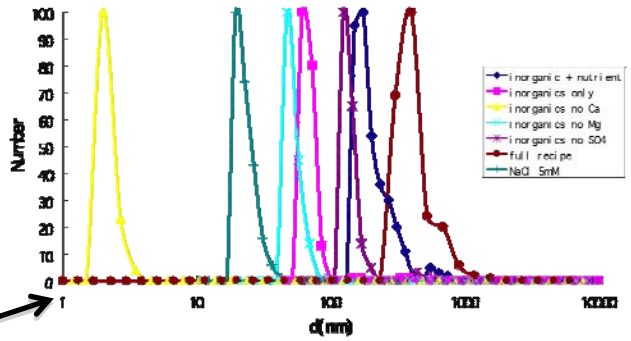
Nano-material characterization



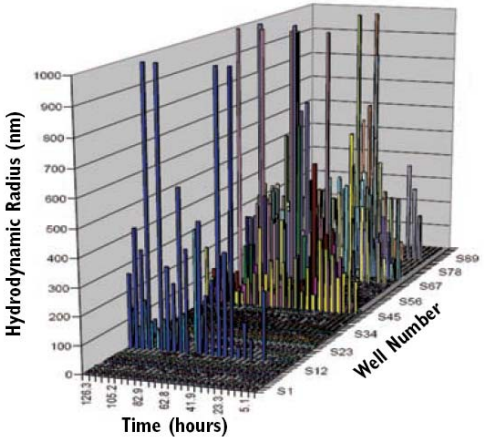


Adapting standard analytical methods to enable high throughput characterizations



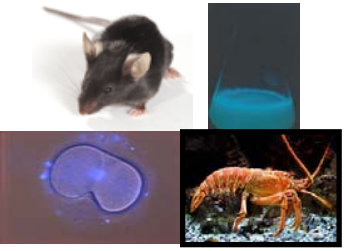


- Several weeks
- Several hours



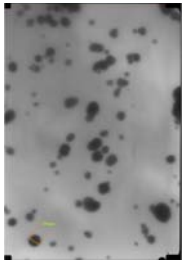
High throughput synthesis, characterization, fate, transport, and toxicity screening

Organisms

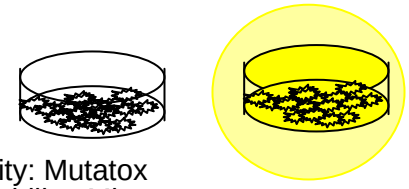


+

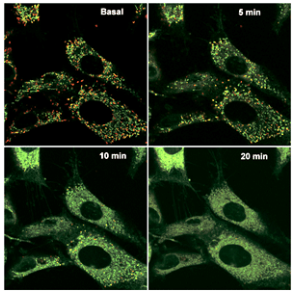
Nano-Materials



Luminescence

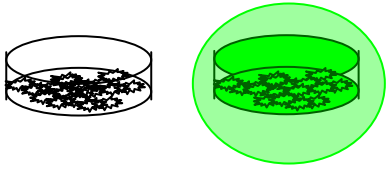


- Genotoxicity: Mutatox
- Cellular viability: Microtox
- ATP levels: ATPlite
- Luminescent reporter genes



Mitochondrial damage, ROS generation, stress response, cellular apoptosis

Fluorescence microscopy

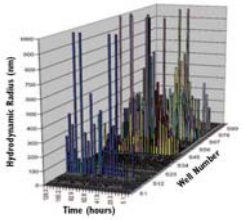


- Fluorescent Reporter Genes (GFP)
- Mitochondrial damage (Mitotox)
- ROS generation
- Cellular apoptosis

UV/Vis spectroscopy

- NADP (Mitoscan)
- Growth/viability/proliferation via culture optical density

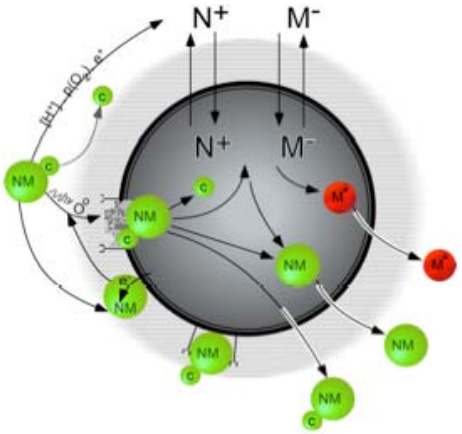
Light scattering + ???



- Aggregation & dissolution
- Transport in aquatic environments
- Protein interactions in biological media

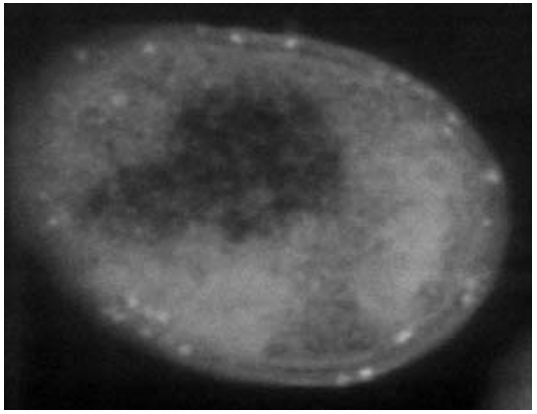
Interactions and responses at molecular, cellular, organ and systemic levels

Interactions



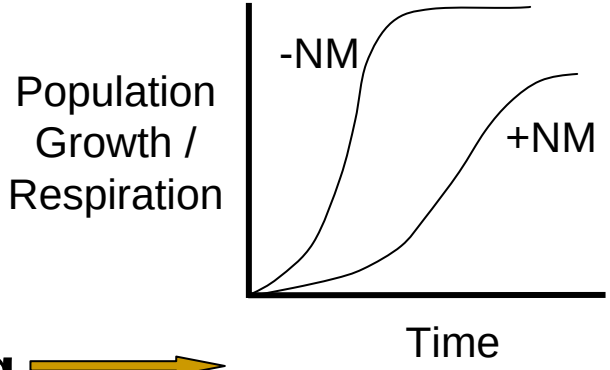
Responses

Cellular



Organismal

Population



DEB Modeling

Mesocosms: model ecological systems

Terrestrial

Micro arthropods

Protozoa

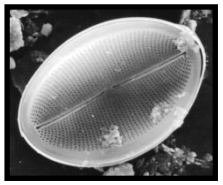
Bacteria

Freshwater

Predatory fish

Invertebrate grazers

Benthic algae

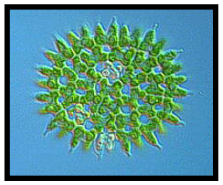


Marine

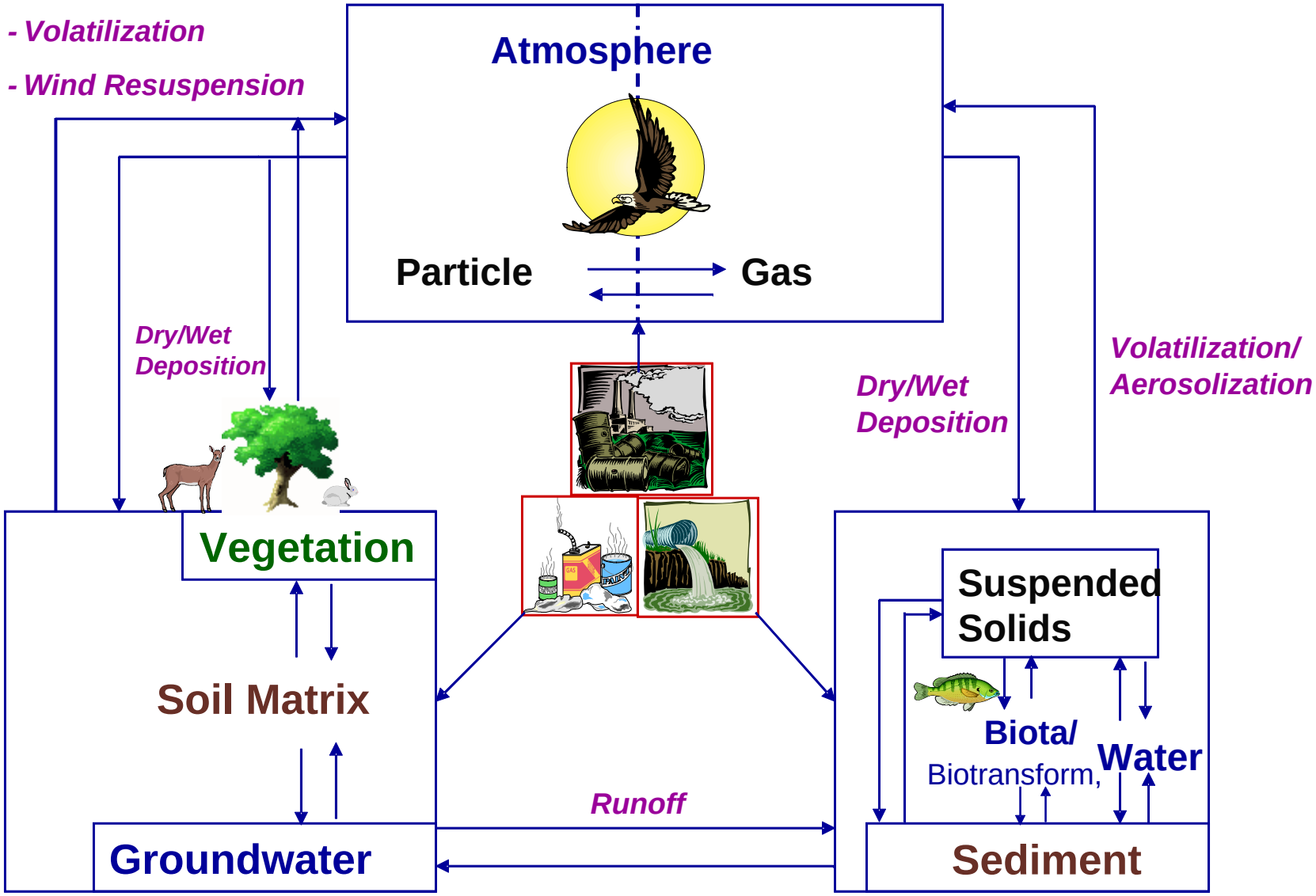
Predatory invertebrate

Invertebrate filter-feeders

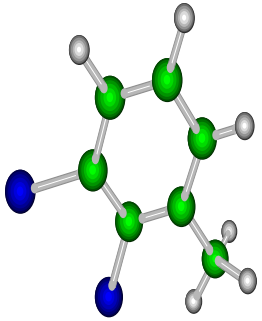
Planktonic algae

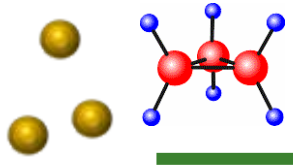


Multimedia Environmental Fate & Transport

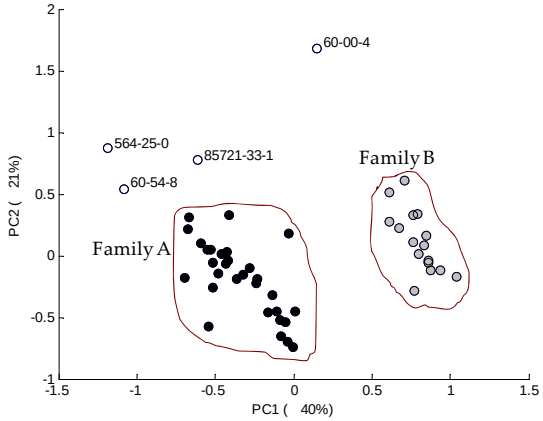


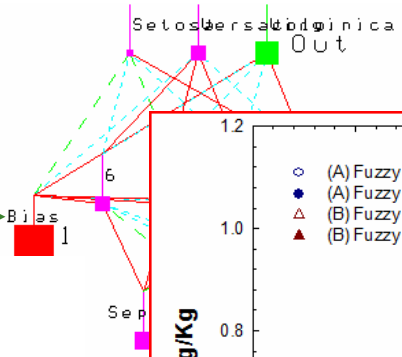
Nanomaterial risk ranking with data-driven models…Putting it all together





Nanopart
physicocl
+ toxicity





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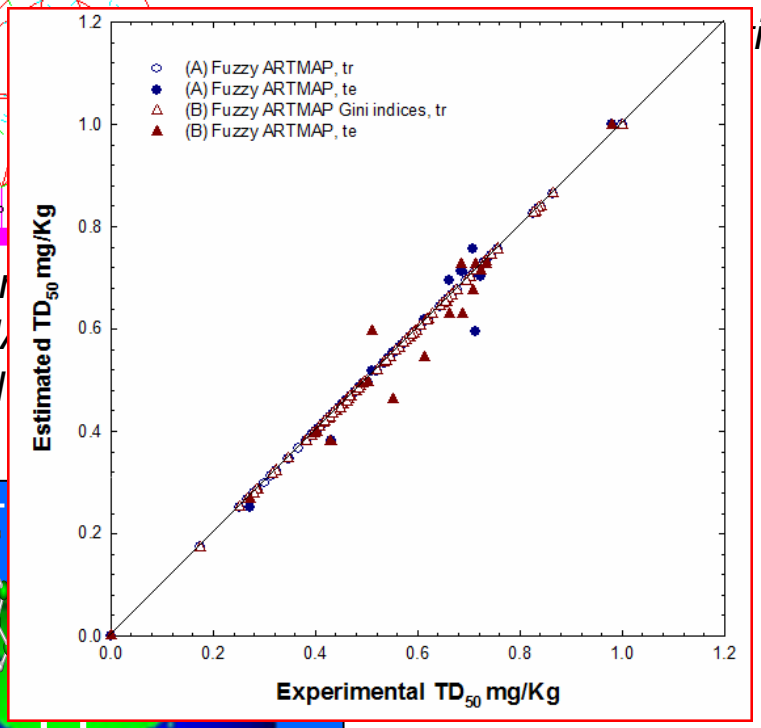

Physical-
chemical
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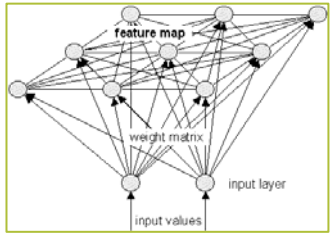
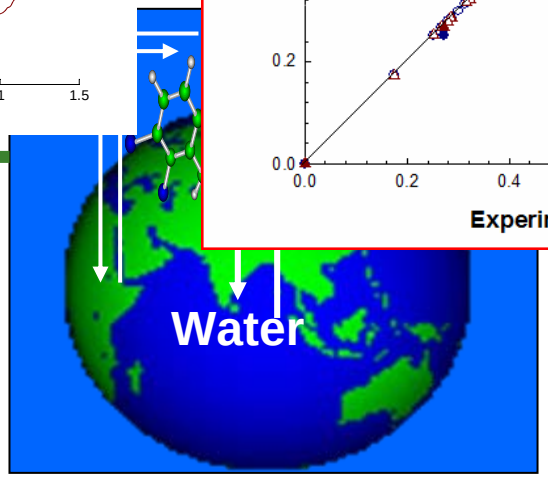
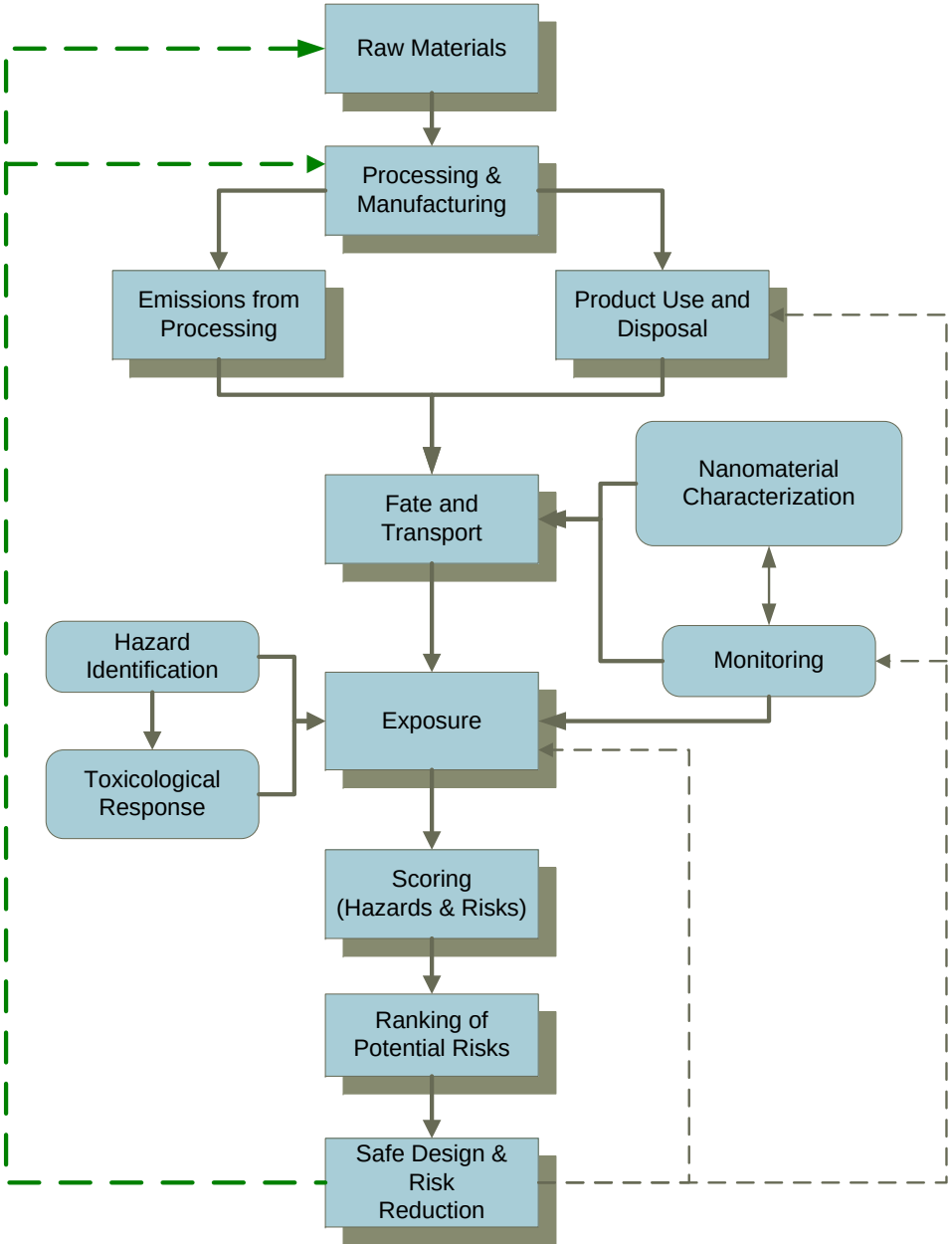
- Environn
- Exposur
- Risk Evaluation
  - Scoring
  - Ranking
  - QRA





Multimedia Analysis

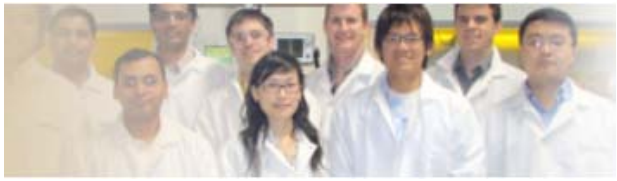
Safe Design of NM



Risk evaluation as a basis for NM safe design

- Emissions
- Hazard identification
- Toxic response
- Exposure assessment
- Scoring analysis
- Risk ranking
  - Quantitative risk scoring
  - Expert opinion
  - Risk perception
  - Integrated weighted scoring
- Safe design tools

Education and Outreach



Objectives

- Train a diverse cohort of new scientists to handle complex issues related to nanomaterials in the environment
- Train researchers to use safe design and appropriate safeguards when handling or disposing of nanomaterials
- Build a cohesive network of stakeholders with interests at the interface of nanoscience, human health, and the environment
- Accurately communicate to the public the implications of nanotechnology in the environment

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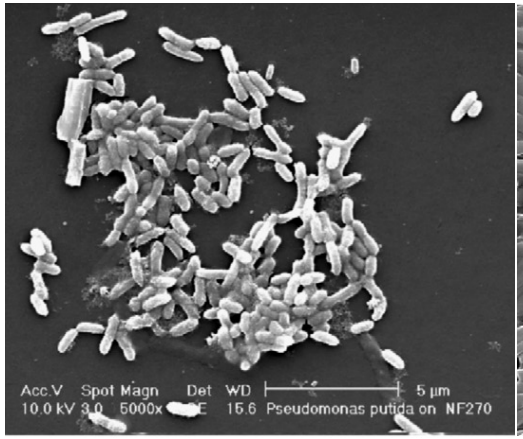
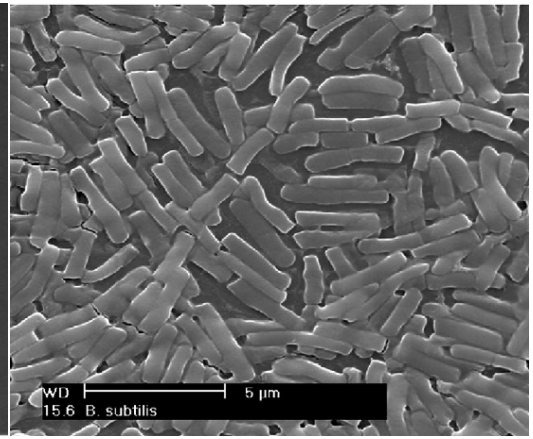
Case Study: Bacterial Toxicity of Silver Nanoparticles in Different Water Chemistries

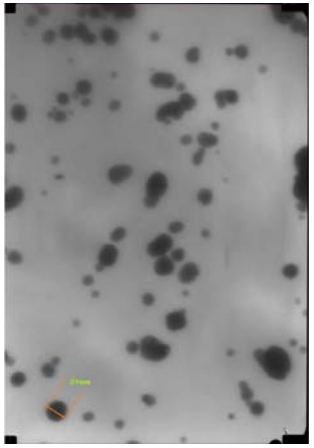
P. putida



B. subtilis



Silver NPs



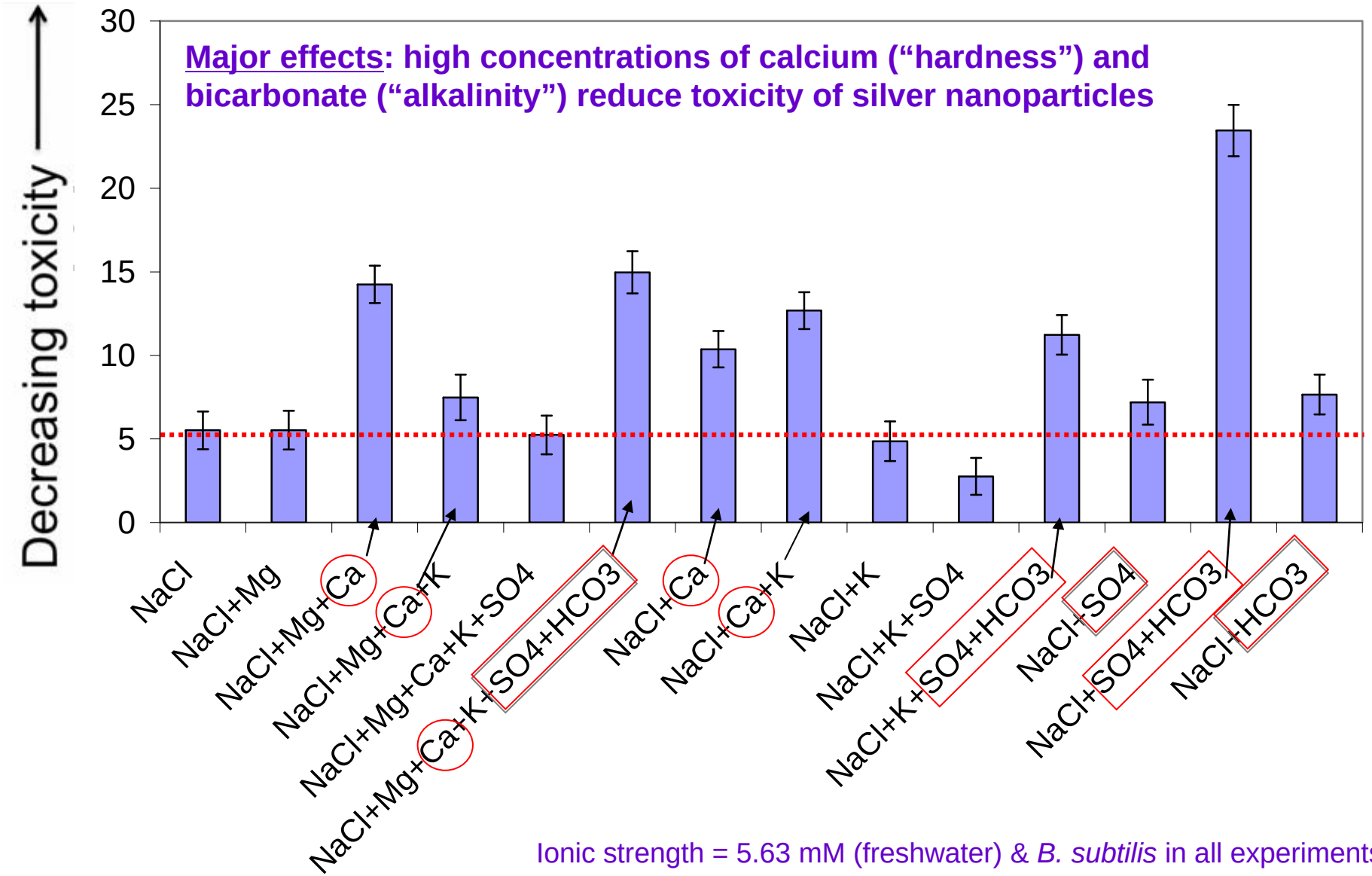
Seawater

Wastewater

Freshwater

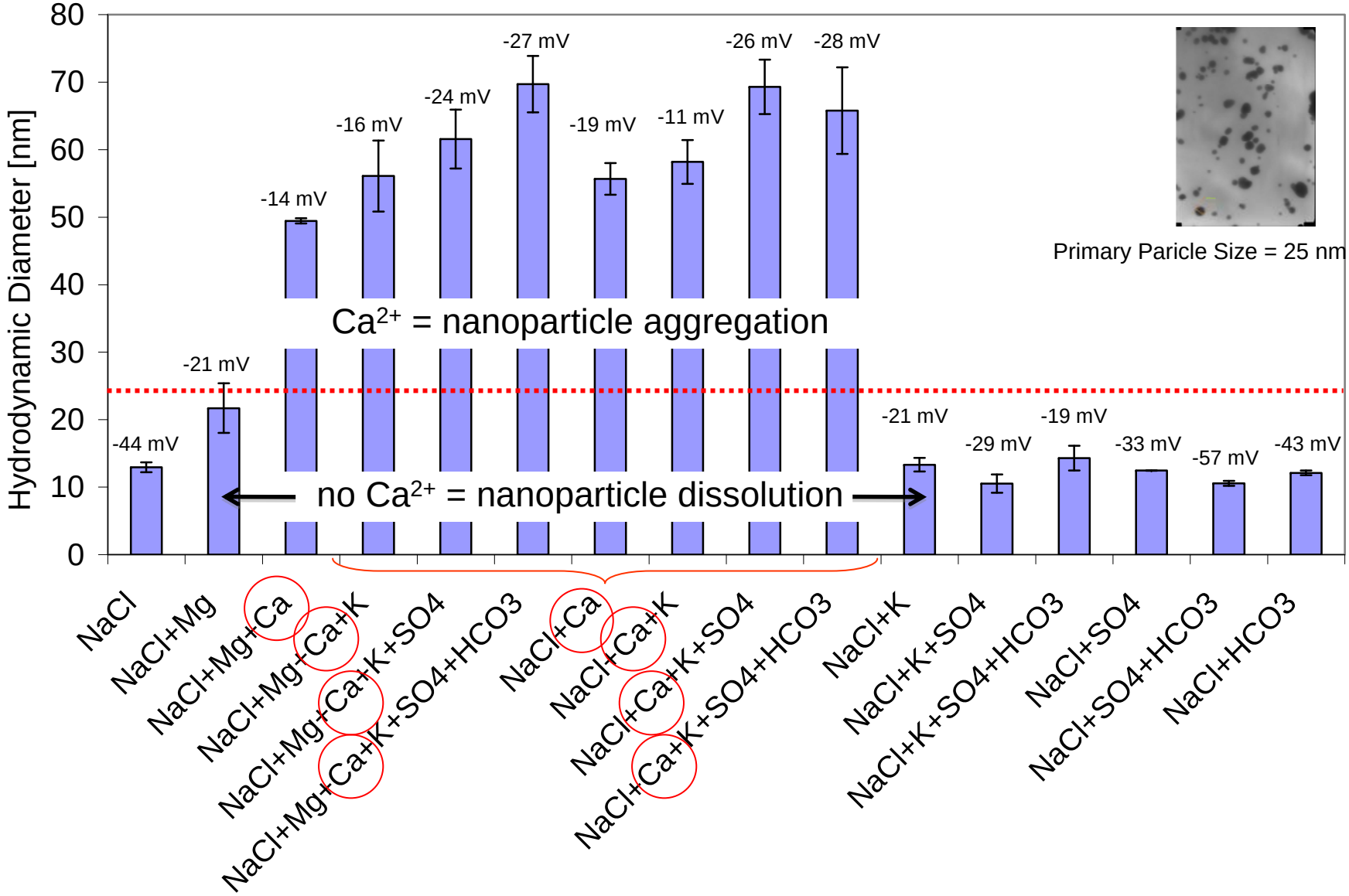
Courtesy: Dr. Xue Jin, UCLA Civil and Environmental Engineering Department and California NanoSystems Institute

Water Chemistry Dependent Toxicity of Silver Nanoparticles



Ionic strength = 5.63 mM (freshwater) & *B. subtilis* in all experiments

Hardness controls Ag-NP aggregation and dissolution



Concluding Remarks

- Nanotechnology is neither intrinsically BAD nor GOOD, but it offers tremendous opportunities to improve the world we live in; hence, we must learn how to implement nanotechnology safely

- The UC TSR&TP/CEIN programs uniquely positioned to establish the new science of nano-safety by:
  - Identifying risk in a probabilistic framework using HTS & AI-models
  - Reducing uncertainty by elucidating fundamental mechanisms governing nanomaterial risk (= exposure x toxicity x s.f.)
  - Developing robust solutions for safe design of nanomaterials
  - Actively engaging public, private, and government stakeholders
  - Training the next generation of nano-scientists

- Much credit belongs with the State of California, University of California, UC TSR&TP, and US NSF/EPA for making these visionary programs possible

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Reference material production

- example using metal oxides

Jeffrey I. Zink, UCLA

- Derivatize titania, ceria and zinc oxide particles with functionalities and fluorescent tags to probe transport, uptake, and toxicity outcomes
- Explore speciality coatings for multifunctional properties, including coatings that passivate and reduce material toxicity

C. Jeffrey Brinker, UNM/Sandia

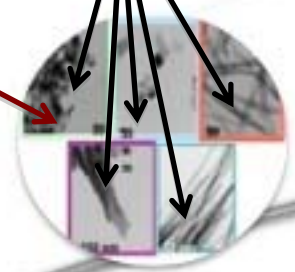
- Non-porous and nano-porous TiO_2 nanoparticle library with systematic variation of particle size, shape, aspect ratio as well as pore size, surface area, and extent of crystallinity

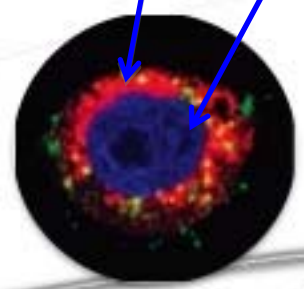
Galen Stucky, UCSB

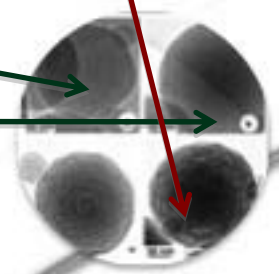
- Non-porous, microporous, and mesoporous TiO2 nanospheres to probe effects of crystallinity, porosity, and surface area
- Transition metal doped TiO_2 NPs (< 130 nm) to systemically probe reactive properties (i.e., electronic, photonic, catalytic, magnetic, etc.)

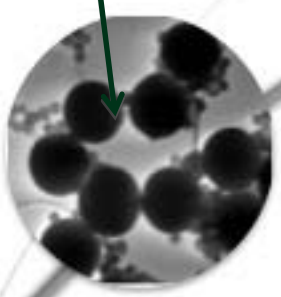
Lutz Mädler, Bremen

- Iron-doped ZnO to control dissolution & mitigate toxicity
- Iron-doped TiO_2 to study photo-induced toxicity in visible light
- Production of ZnO, TiO_2 and CeO_2 standard NMs 10, 17, & 25 nm









Surface Chemistry (6-18 mo)

- Fluorescent labels for cell assays
- Surface functionality (-COOH, -OH, $-NH_2$, PEG, etc.)
- Capping agents (PEI, PAH, PLL, etc.)

Physical Morphology (12-18 mo)

- Size, shape, crystallinity, and porosity to control transport, uptake, & engulfment

Secondary Composition (6-12 mo)

- Doping to control dissolution, redox reactivity and electronic states

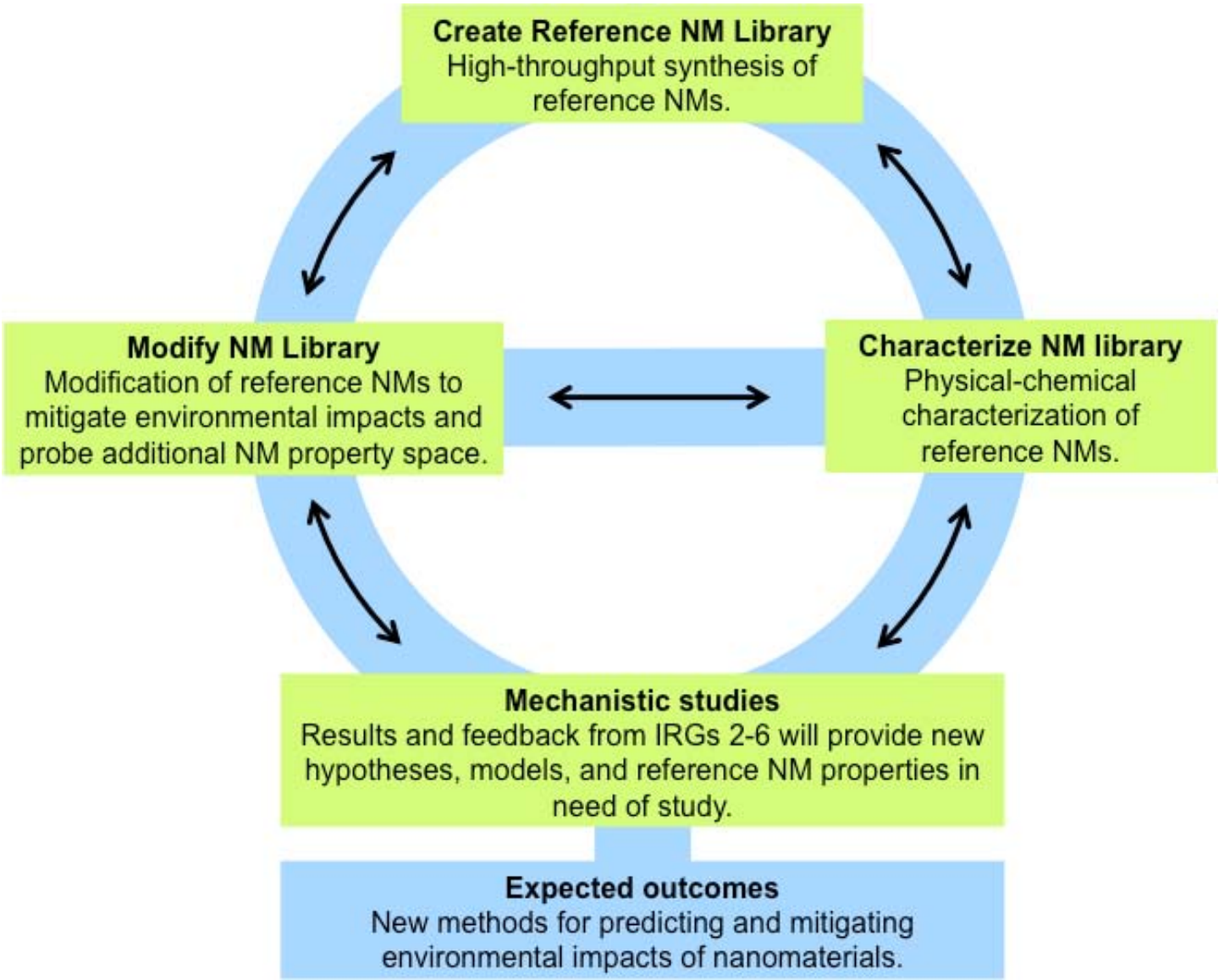
Primary Composition (0-6 mo)

- TiO_2 (inert), CeO_2 (protective), ZnO (toxic)

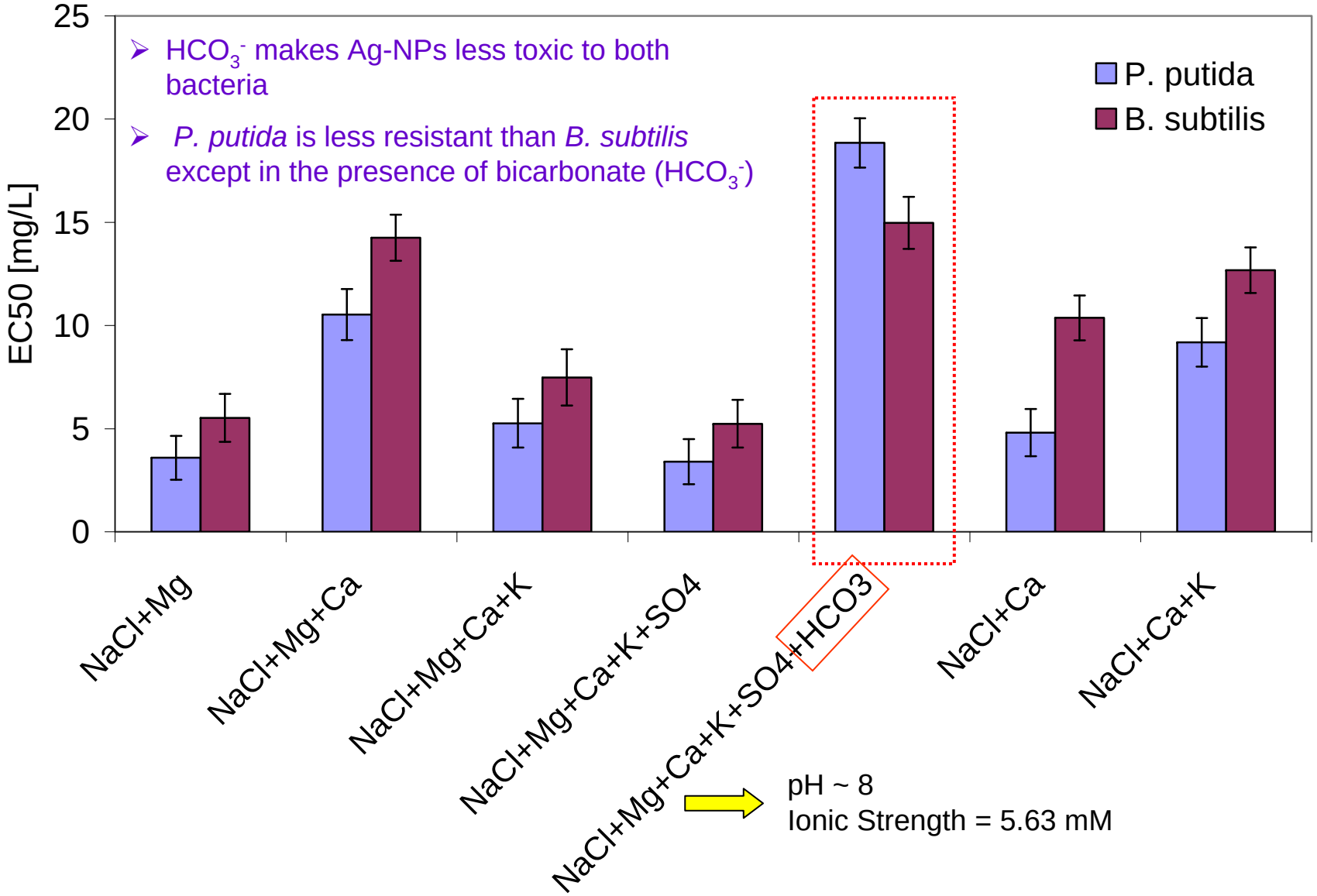
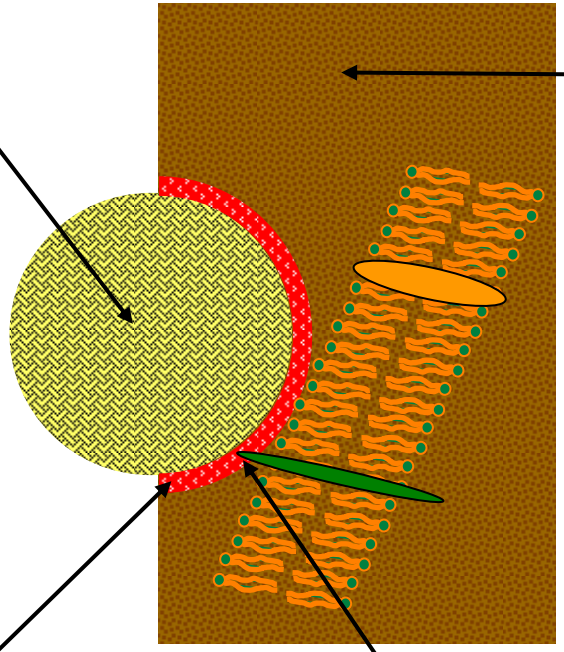
Hoek/Ji/Nel et al. (6-18 mo)

- High throughput dynamic light scattering in UCLA-CEIN HTS lab
- Extending the range of size, morphology, functionality via HT synthesis at LBNL Molecular Foundry

Scientific Integration



Species Dependent Toxicity of Silver Nanoparticles



Intrinsic versus environmentally acquired properties at the nano-bio interface