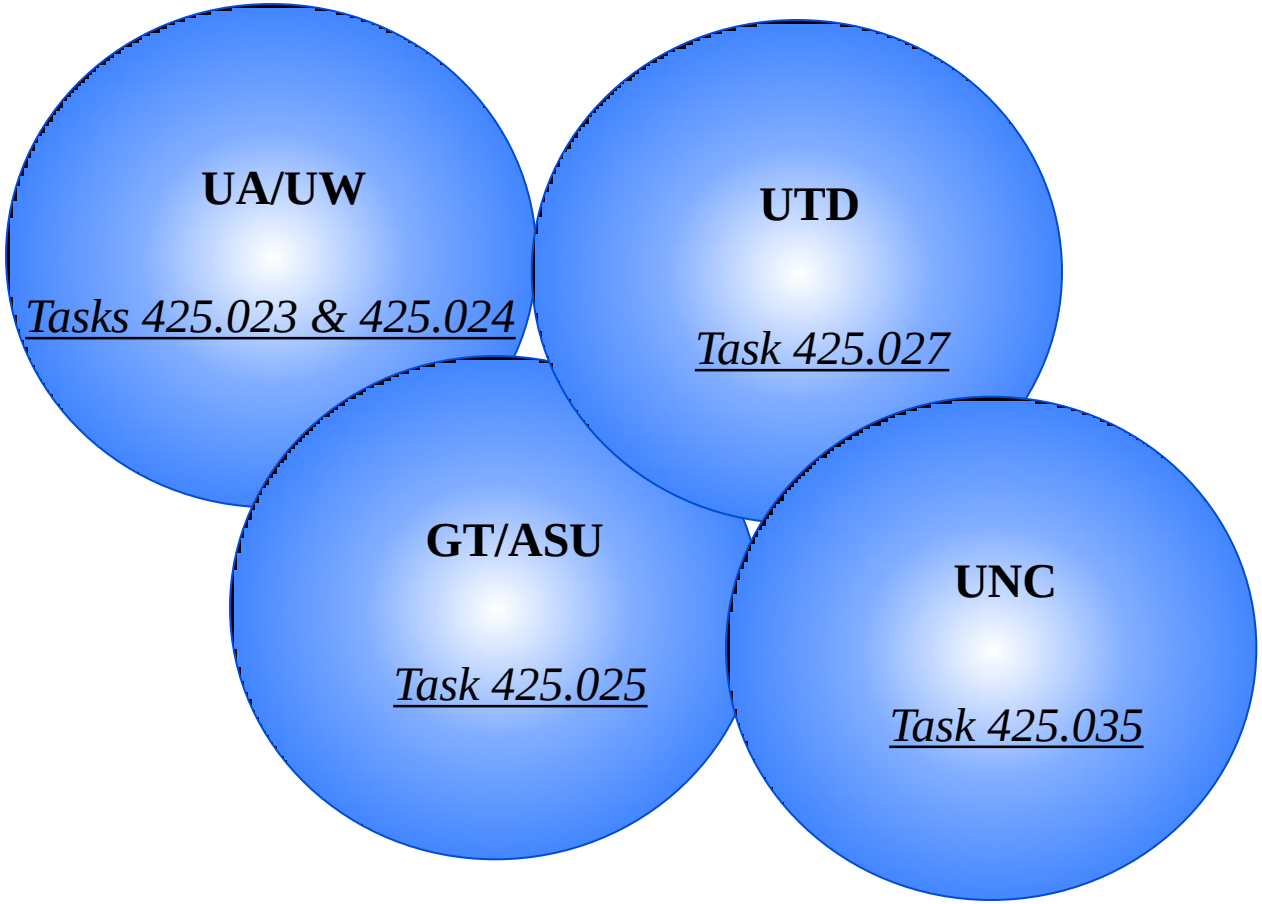
Summary (nanocluster):

Projects on ESH Aspects of Nanomaterials



Outline Summary Talk

Part 1. Round Robin

Impact nanoparticle aggregation on toxicity UA, UTD

Part 2. Summary of Nanocluster UA/UW, UTD, UNC, GT/ASU

Cytotoxicity

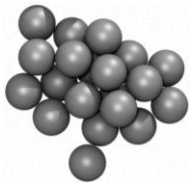
Mechanisms

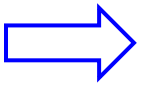
Cell Uptake

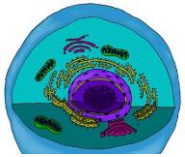
Fate

Methodologies

Risk Assessment







Round Robin: Aggregation – Toxicity

University of Texas – Dallas

- Tested correlation between toxicity of multi-walled carbon nanotubes (MWNT) with aggregation state
- NRK cells, cell proliferation assay
- Prepared protein (BSA) dispersed MWNT
- Removed protein with dialysis, creating conditions for aggregation

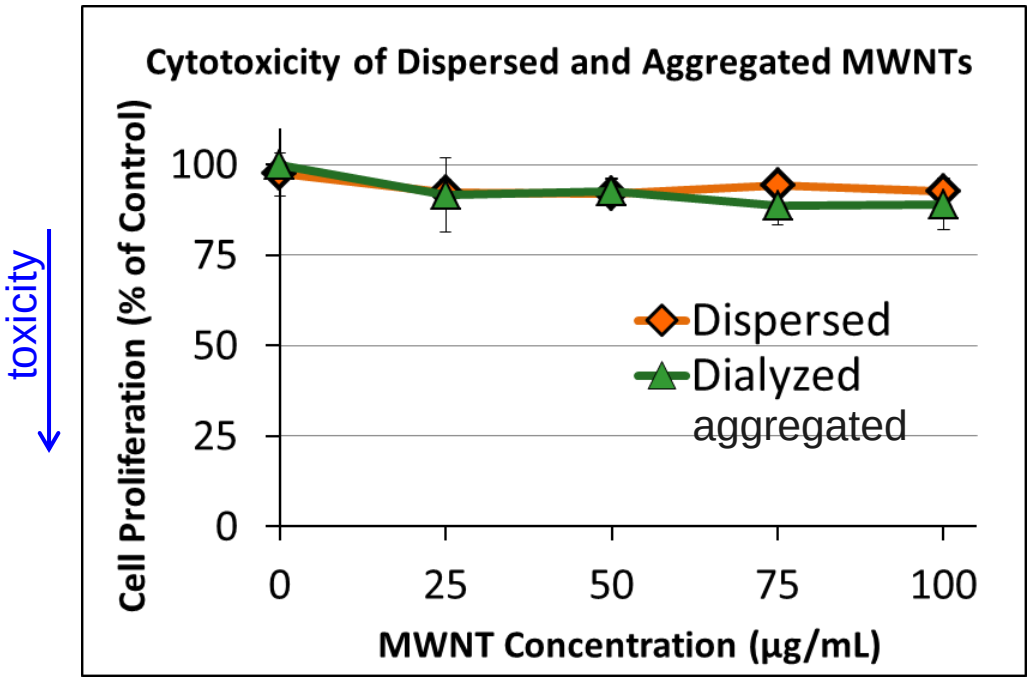
Aggregated

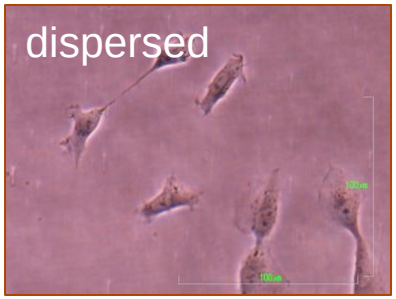
Dispersed

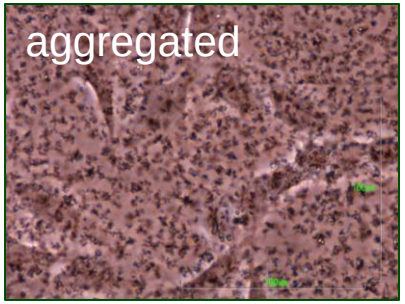
Round Robin: Aggregation – Toxicity

University of Texas – Dallas

- No evidence that aggregation changes toxicity







Round Robin: Aggregation – Toxicity

University of Arizona

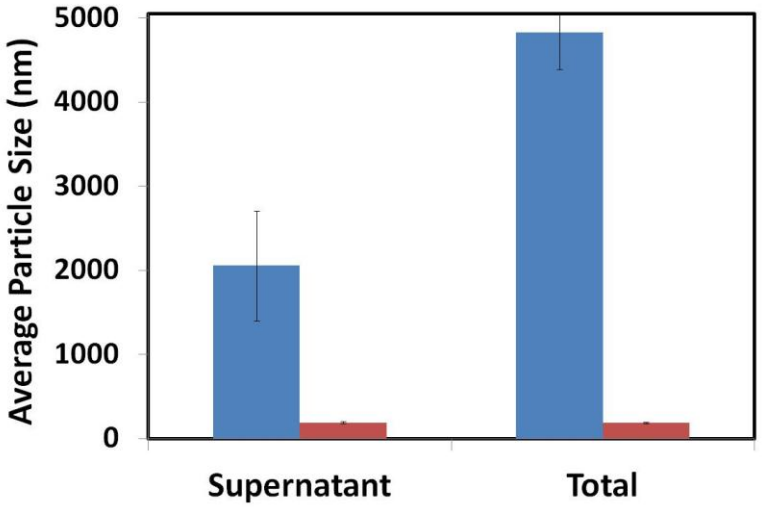
- Tested correlation between toxicity of nano CeO_2 and nano Al_2O_3 with aggregegation state
- Lung epithelial cells (16HBE14o-), RTCA and MTT
- Compared protein (FBS) dispersed and non-dispersed nanoparticles
- Compared shaken and non-shaken incubating conditions

Round Robin: Aggregation – Toxicity

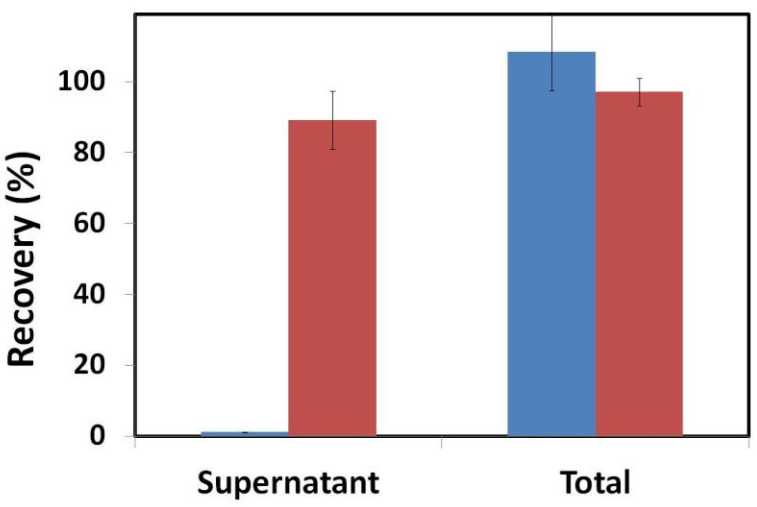
University of Arizona

- Impact FBS on particle properties (the data)

Aggregate Particle Size



Recovery of Cerium

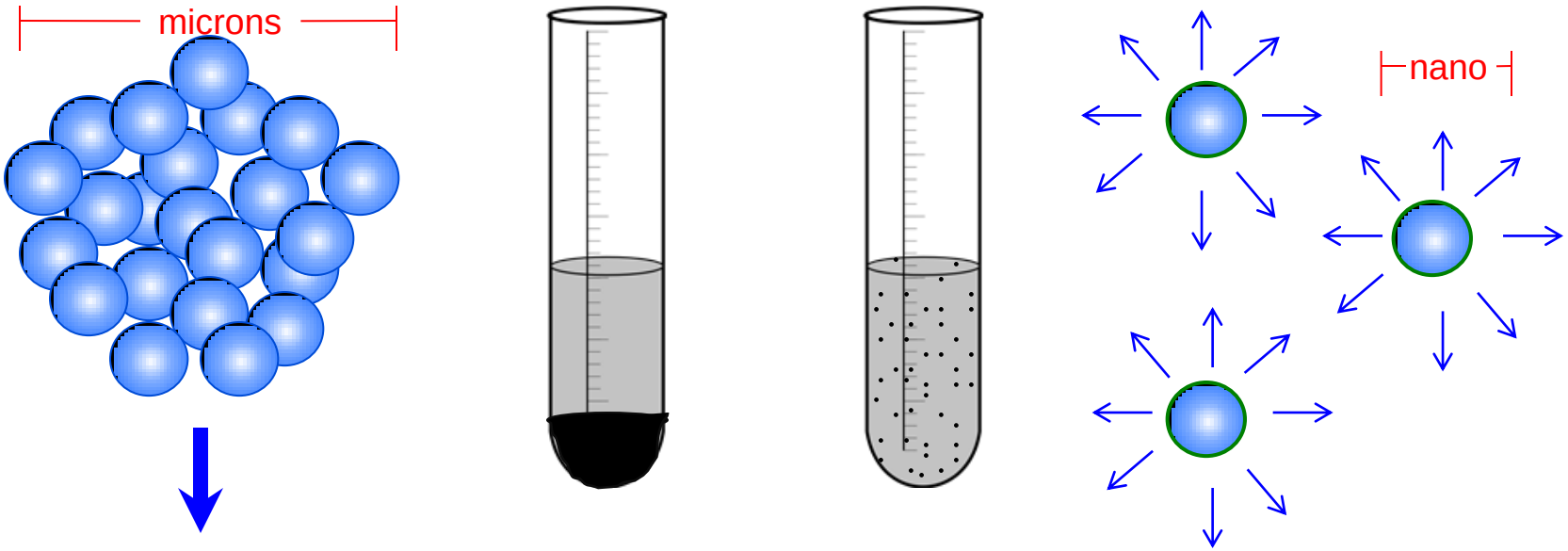


Medium without FBS Medium with FBS

Round Robin: Aggregation – Toxicity

University of Arizona

- Impact FBS on particle properties (pictogram interpretation of data)



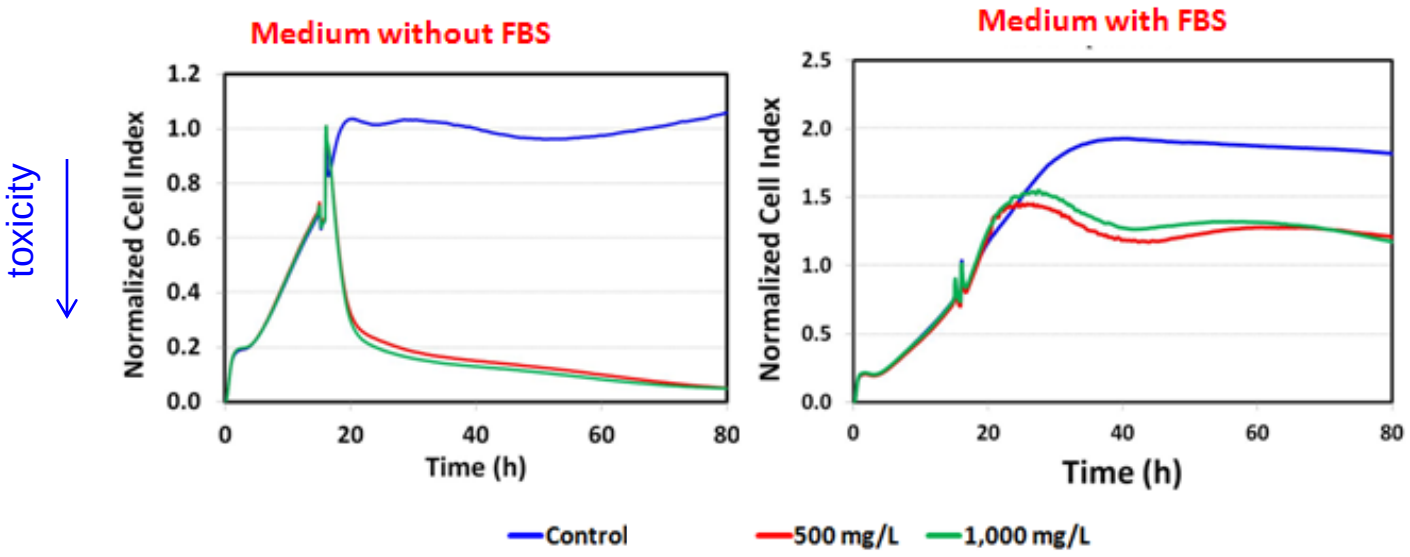
No FBS, sedimentation

FBS, dispersed

Round Robin: Aggregation – Toxicity

University of Arizona

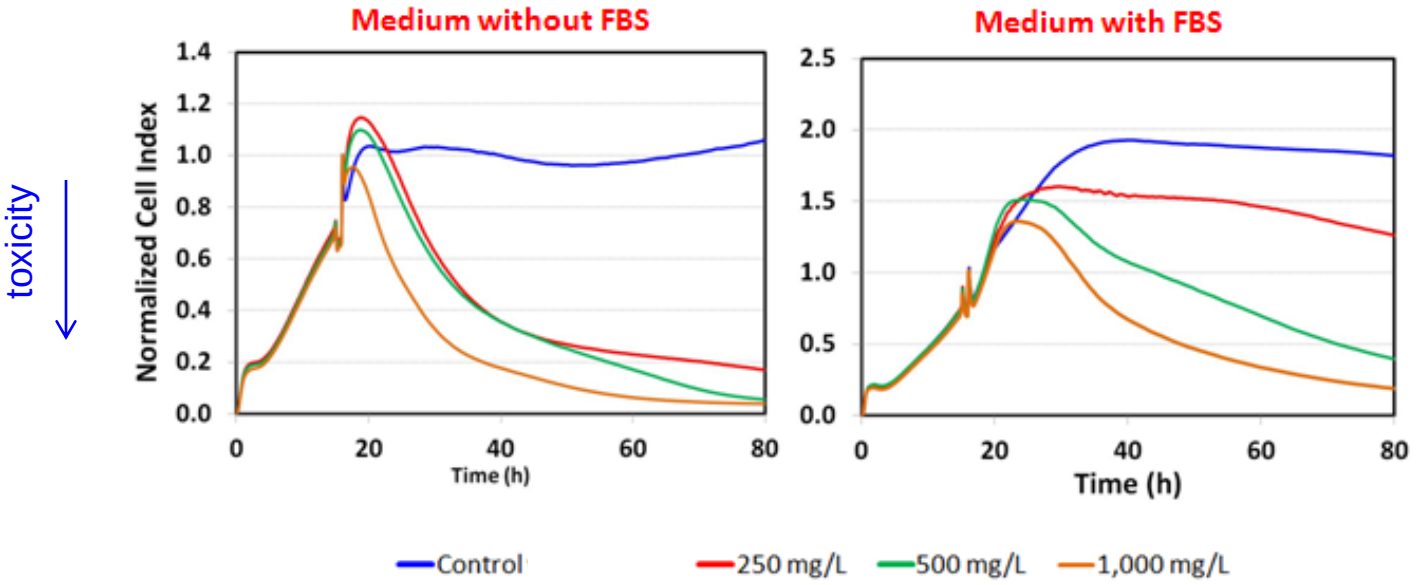
- Impact FBS on RTCA toxicity of **nano CeO_2**
- FBS-dispersed NPs are less toxic



Round Robin: Aggregation – Toxicity

University of Arizona

- Impact FBS on RTCA toxicity of **nano Al_2O_3**
- FBS-dispersed NPs are less toxic



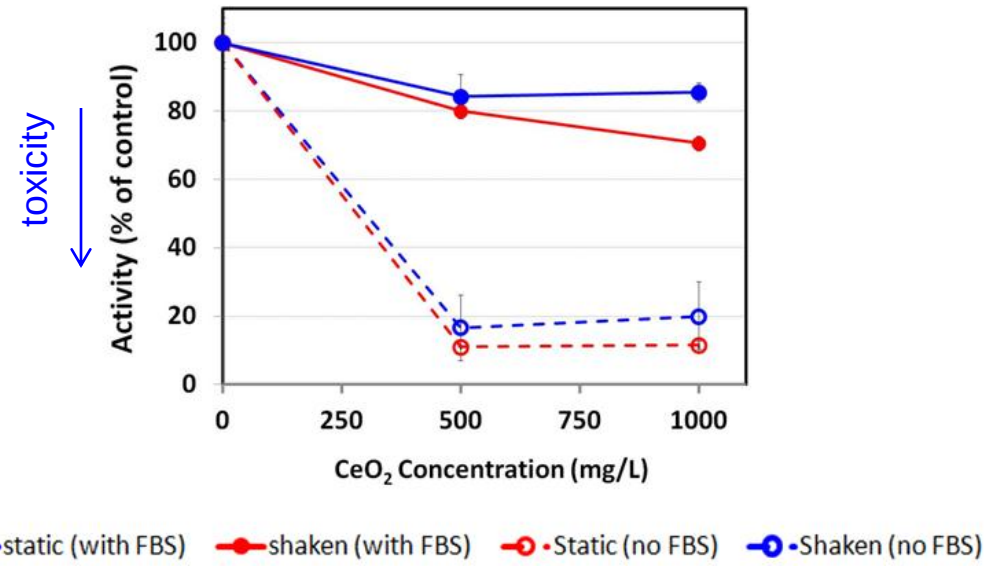
Round Robin: Aggregation – Toxicity

University of Arizona

- Impact Shaking on MTT of **nano CeO_2**

  Testing whether localized concentration of NPs sedimented on top of cells is key issue

- Shaking doesn't significantly change toxicity



Round Robin: Aggregation – Toxicity

Conclusions Round Robin

- Preliminary results suggest there is no evidence that localized concentration due to aggregation and sedimentation of NPs on top of cells has impacted toxicity

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- MWNTs need to be tested at a higher (cytotoxic) concentration
- Several possible roles of why FBS has an impact on CeO_2 & Al_2O_3 toxicity

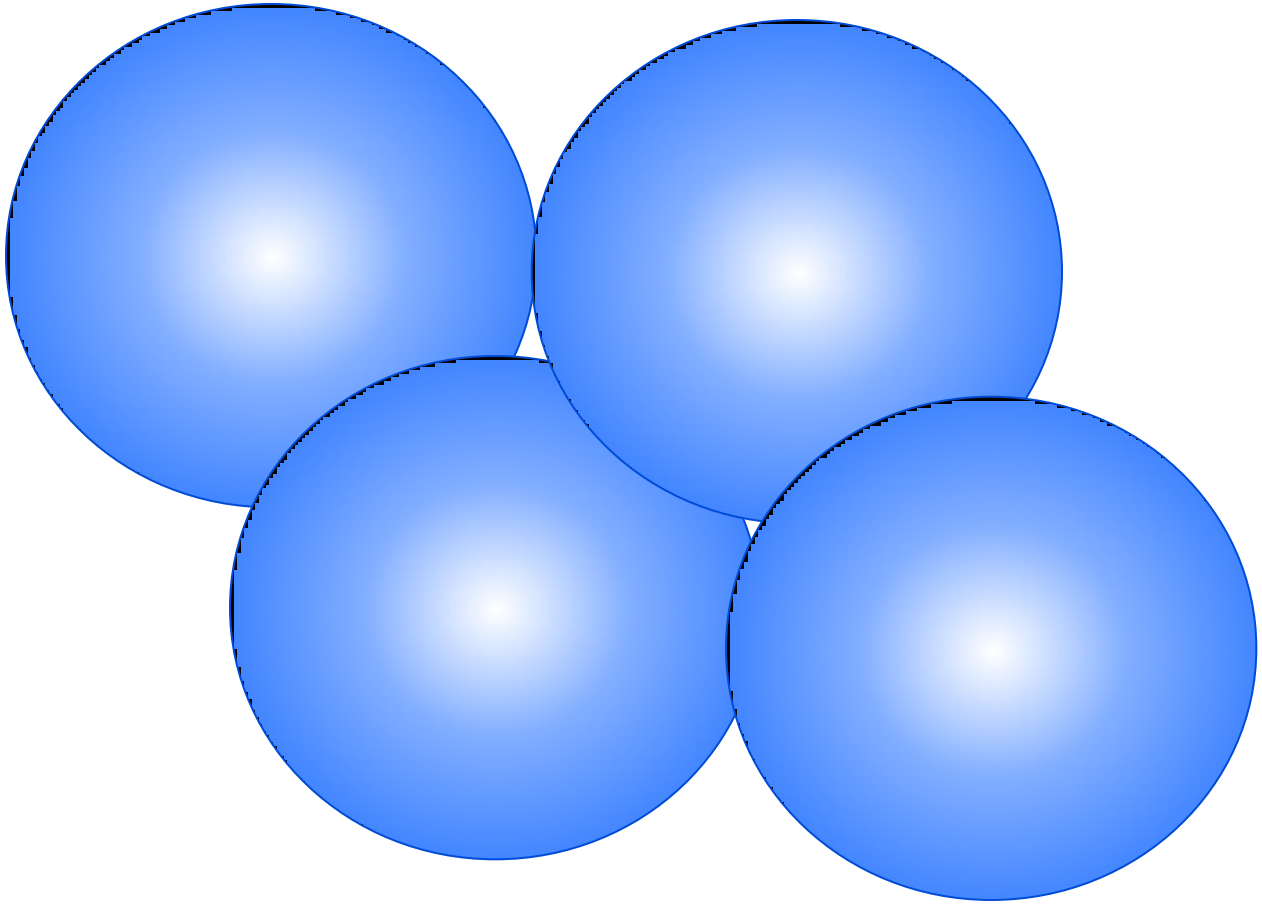
⇨ **Medium Matters:** media composition impacts toxicity (supported by findings in literature)

⇨ **Stress:** media without FBS causes stress, and NPs are more toxic to stressed cells

⇨ **Protein coating:** protein coating on NP surfaces makes NP more biocompatible to cells

3 year Summary

Projects on ESH Aspects of Nanomaterials



Acute Cytotoxicity NP

- High Toxicity (large impact at < 100 mg/L)

  Examples: Cu^0, CuO, Mn_2O_3, Ag^0, ZnO, (Fe_2O_3 to some cells)

- Moderate Toxicity (large impact at 100 - 1000 mg/L)

  Examples: SiO_2, Al_2O_3, CeO_2 (no protein medium)

- Low to No Toxicity (impact at >1000 mg/L)

  Examples: CeO_2 (protein medium), C-nanotubes (clean), HfO_2 (clean), ZrO_2, Fe_2O_3, Fe^0, TiO_2 (dark)

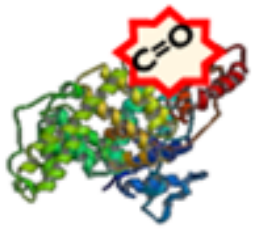
Mechanisms

Reactive Oxygen Species

Chemical ROS, NP-Biomolecule Interaction	UA
Photochemical ROS	GT
With cells present	UA, UNC
Caused by dispersant	UTD

Oxidation of Biomolecules

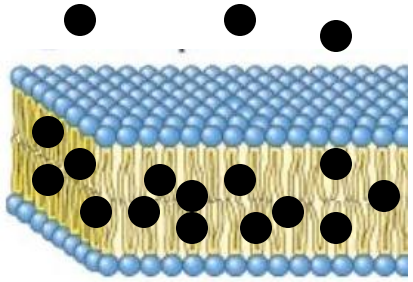
Oxidation of protein	UA



Mechanisms

Membrane

Partitioning into membrane



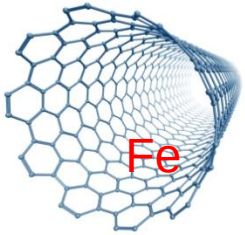
ASU, UTD

Membrane integrity compromised

UA, UNC

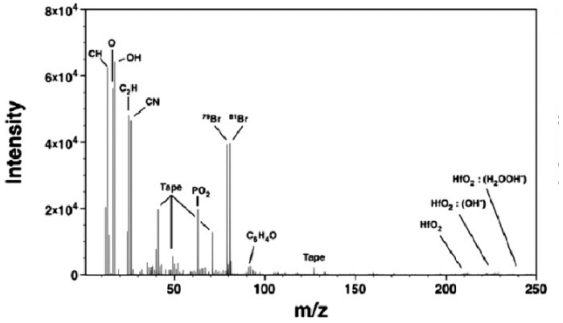
Impurities

Carbon NT



UTD

HfO_2



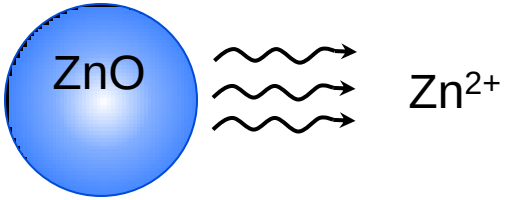
UW/UA

Mechanisms

Dissolution/Corrosion

UA, UNC, GT

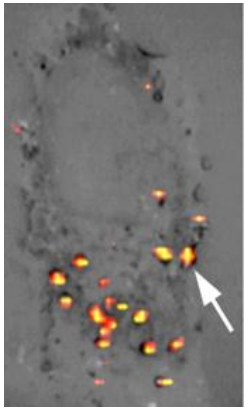
ZnO, Cu^0, Ag^0



The most toxic nanoparticle were associated with this mechanism

NP Uptake into Cells

- Evidence based on TEM

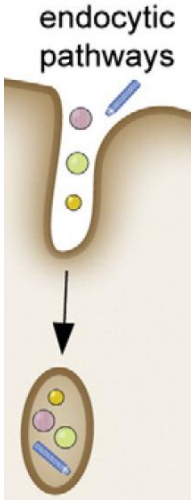


UNC

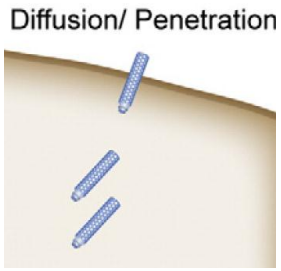
- Evidence based on LSCRM†

UTD

- Hypotheses for uptake



lysosome



partitioning into membrane

Endocytosis — UTD

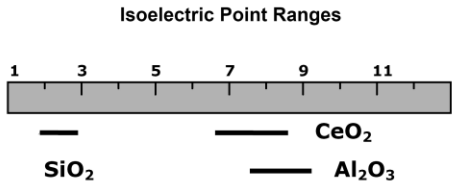
Membrane partitioning — ASU

† Laser scanning confocal Raman microscopy

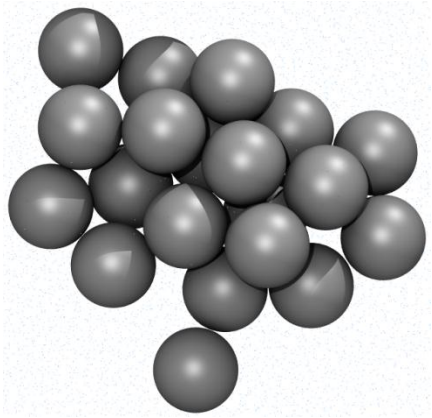
Fate in Medium and Environment

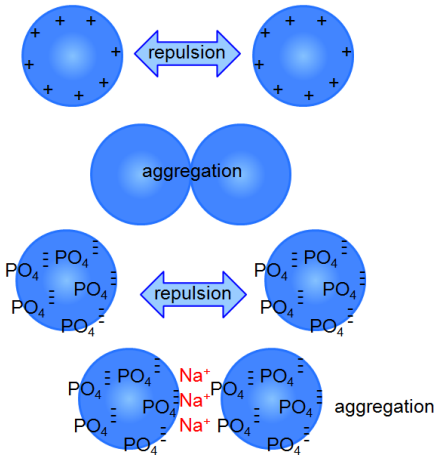
Aggregation

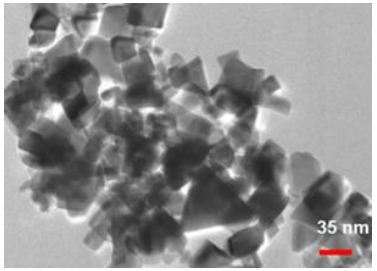
Biological Media causes aggregation of nanoparticles



UA/UW, UTD, UNC, GT/ASU







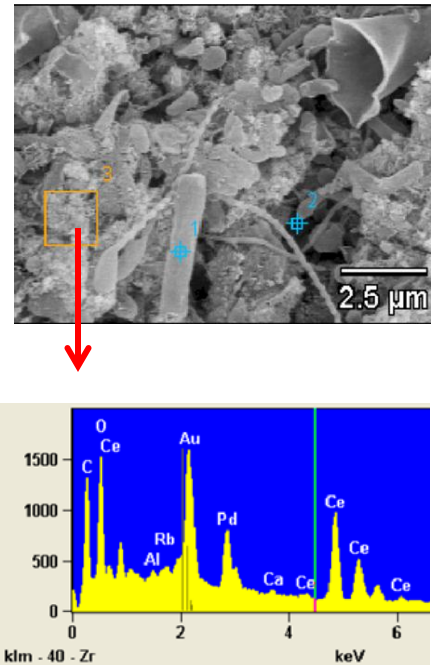
Dispersion

Proteins, surfactants (pluronic acid, dispex)

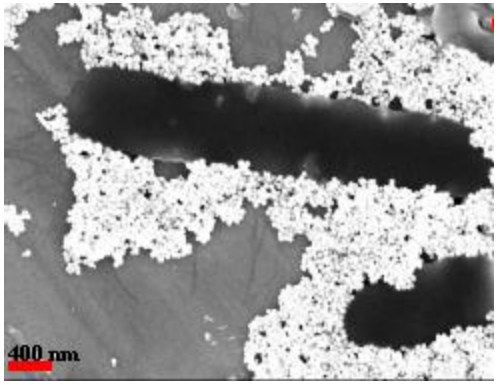
Fate in Medium and Environment

Adsorption

Nanoparticles sorbed by cell surfaces UA, GT/ASU



nano-CeO_2 sorption activated sludge



nano-Fe_2O_3 sorption *E. coli*

Methodologies

- **Importance of controls** — UTD
  Pluronic acid toxicity by sonification
- **Importance of method validation** — UA
  RTCA correlation with MTT
- **Importance of Characterization** — UA/UW, UTD, UNC, GT/ASU
  Size, ZP, aggregation, purity *etc*.
- **Methods for Sub-Lethal Cellular Effects**
  Going beyond acute cytotoxicity measurements

- **High Throughput & Predictive**
  Depends on understanding important mechanisms
  Depends on data not being tainted with methodological errors

Health Risk = Exposure × Hazard

Still need evidence of attenuation of aerosols

Just started to develop advanced tissue and organ cultures to address this question. Also need for animal studies.

Good evidence that most NPs (except SiO_2) will be signifcantly attenuated in environmental liquid medium (e.g. during WWT)

Impact aggregation on mucociliary clearance of NPs from lung?

Acid in stomach and lysosomes might increase release of metal ions

Excellent evidence that certain NPs are cytotoxic to cells to varying degrees. Most toxic (ZnO, Cu^0, Ag^0, Mn_2O_3). Moderate toxicity (SiO_2, Al_2O_3). Also important mechanisms elucidated (*e.g.* ROS).

Just started to develop high throughput methods of detecting sub-lethal cellular effects

Proteins help to disperse, but saline causes aggregation. Need to test NP stability in real body fluids

Acknowledgement

- Thanks to Industrial Partners

  Helped guide the planning of round robin experiments

  Helped with selection of relevant nanoparticle materials

  Asking the challenging questions