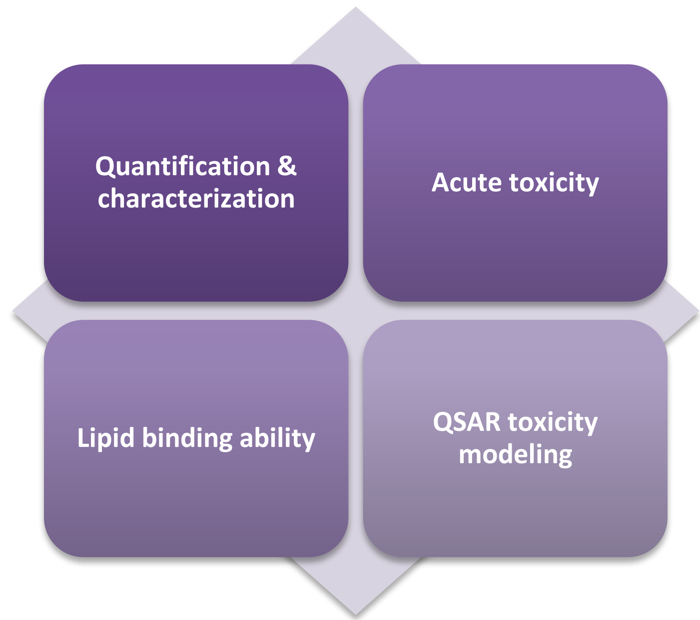
CONSORTIUM OF NANO-EH&S PROJECTS COORDINATED ACTIVITIES

Arizona State University
Colorado School of Mines
Johns Hopkins University
North Carolina A&T
University of Arizona
University of North Carolina
University of Texas - Dallas

Consortium of Nano-EH&S Projects Coordinated Activities

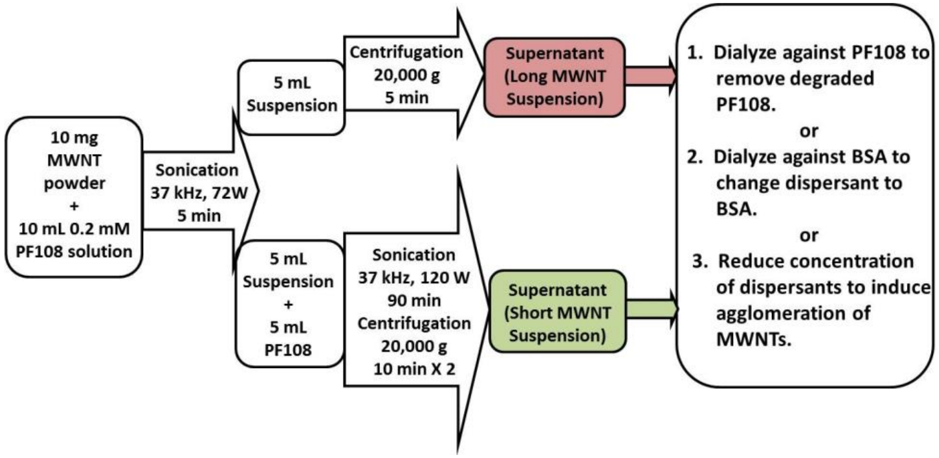
- **Goal:**
  - Identify and conduct experiments using a common set of nanomaterials across all platforms of five funded EH&S projects to demonstrate how each supports differentiation of nanomaterial impacts
- **Approach:**
  - As a group agree upon nanomaterials to study based upon the ability to address a fundamental question(s)
  - Conduct measurements in individual laboratories
  - Develop a framework for assessing and minimizing nanomaterial "hazard"
- **Results:**
  - Preliminary nanomaterial selection includse 2 sizes of CNTs
  - Working question: "Can each of the platforms funded under EH&S differentiate responses, impacts, or quantification of the four selected nanomaterials?"



Core assessment or measurement capabilities of the funded EH&S consortium researchers

Preparation of MWCNTs (UT-D)

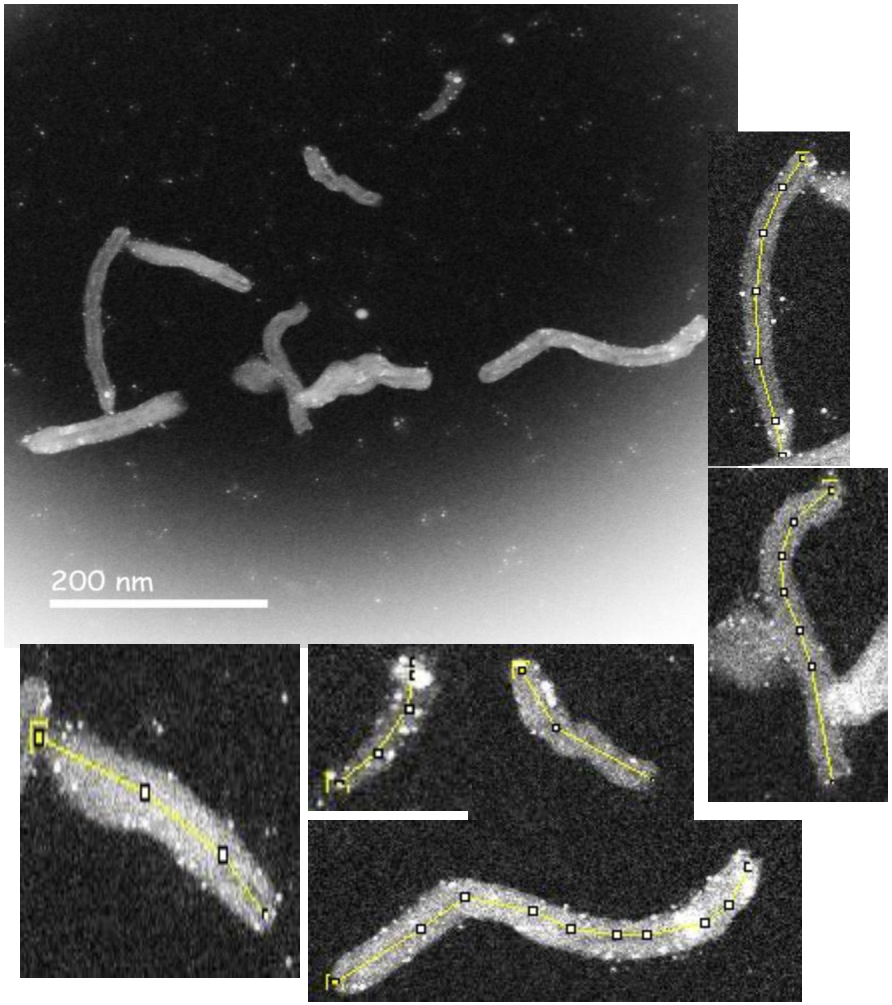
- Two sizes of MWCNTs were prepared by sonication and separation
- Entire procedure done under sterile conditions so final MWCNTs can be used with in vitro cell culture assays.
- Coated with pluronics to make stable in water
- Provided supplies to rest of consortium for testing



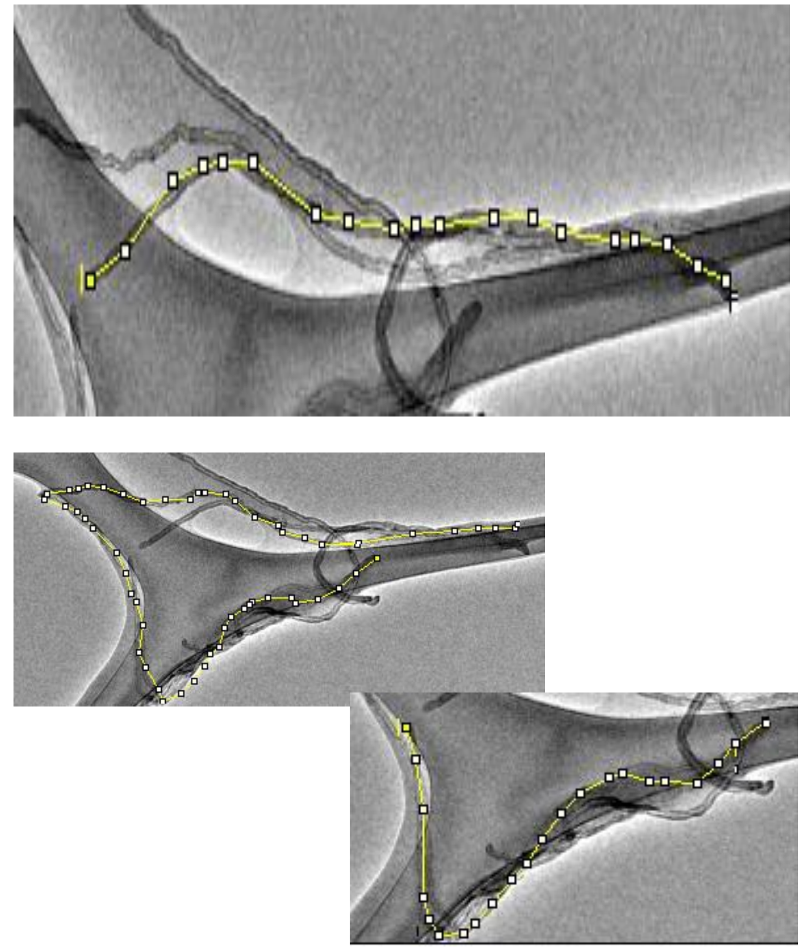
Sonication, Centrifugation, and Dialysis

Characterization of CNTs (ASU)

Shorter CNTs = 159 ± 72 nm

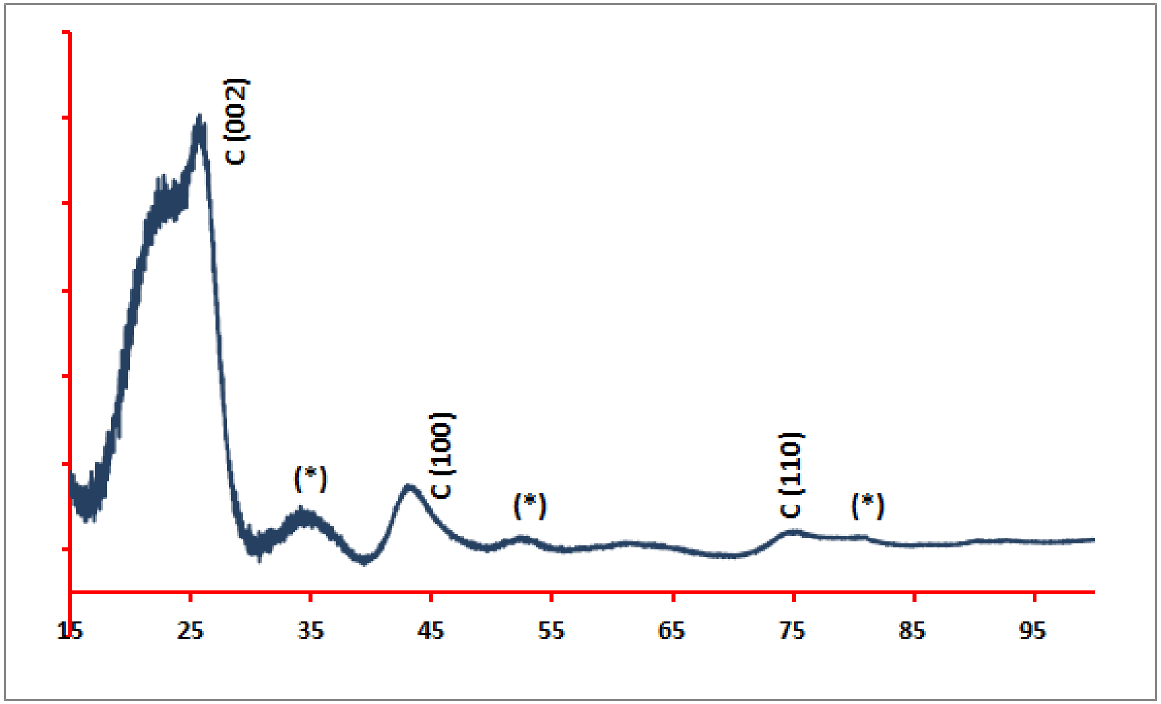


Longer CNTs = 2,180 ± 1,225 nm



Characterization of MWCNTs (NC A&T / UNC-G)

- X-ray diffraction (XRD) patterns were measured with a Oxford Gemini X-Ray Diffractometer with Cu Kα radiation (l = 1.5418 Å)
- A graphite like peak (002) is observed along with a family of carbon peaks due to honeycomb lattice of single graphene sheet



XRD of MWNT containing powder. (*) are metal oxides

Characterization of MWCNTs (NC A&T / UNC-G)

- North Carolina A&T/UT-Dallas consortium work
- Varian 710 ES ICP Axial Spectrometer - Inductively coupled plasma optical emission spectrometry (ICP-OES) was used to measure metal impurity content in the as-obtained samples
- DI Water used as control showed no impurities

% impurity in MWVNT containing powder as measured in ICP-OES

Element	% content
Ni	0.03-0.15
Fe	0.01
Si	0.01-0.07
Al	Nil

- We will continue to characterize the starting material to understand its size, distribution, structure and composition using techniques such <u>Raman Spectroscopy, Nanoparticle Tracking Analysis and Thermogravimetric Analysis</u>

Characterization of CNTs (CSM)

- Catalytic elements used in CNT growth and are typical metallic contaminants are shown above.

- CNT can be detected and counted using spICPMS
  - Metallic impurities are detected by the MS
  - Pulses of ions from the CNTs are counted

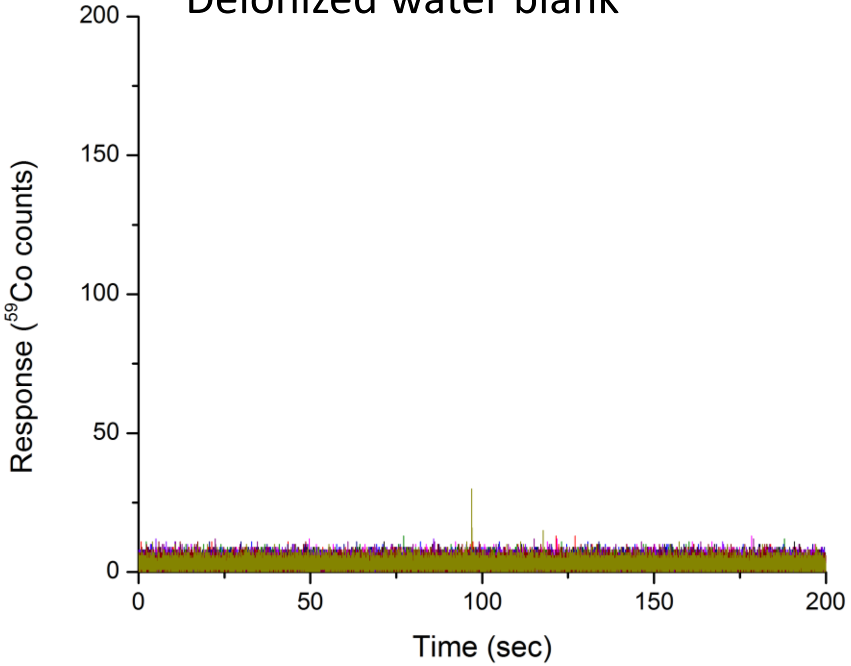
Eleme nt	Short CNT conc. (ppb)	Long CNT conc. (ppb)
Y	0.033	0.0167
Mo	1.717	1.363
Co	0.090	0.076
Ni	1.152	1.145

Reed et al, 2013

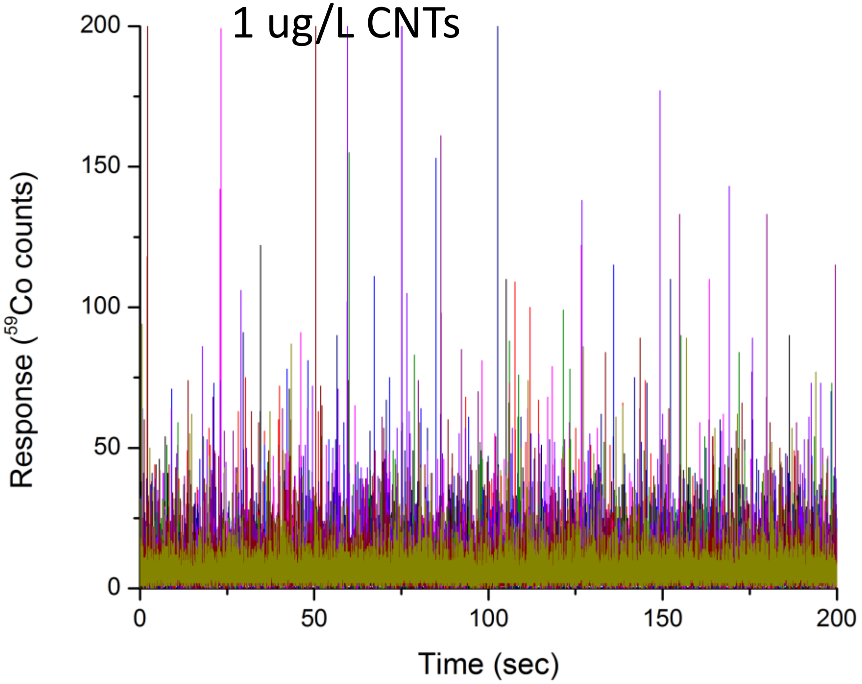
Characterization of CNTs (CSM)

- Preliminary Data collected using ^{59}Co in Southwest Nanotechnologies CNTs
- Work with consortium CNTs just beginning

Deionized water blank



1 ug/L CNTs



Most frequent literature terms associated with carbon nanotubes retrieved by Chemotext (UNC)

Terms	Number of assertions	Terms	Number of assertions
Cell Survival	**112**	Lymph Nodes	9
Lung	**102**	T-Lymphocytes	9
Particle Size	**62**	Biological Transport	8
Oxidative Stress	**58**	Chromosome Aberrations	8
Cell Proliferation	**46**	Neutrophils	8
Apoptosis	**45**		
Time Factors	**35**	Signal Transduction	8
Macrophages @	**29**	Skin	8
Surface Properties	**29**	Kidney	7
DNA Damage	**28**	Light	7
Tissue Distribution	**25**	Lymphocytes	7
Oxidation-Reduction	24	Molecular Structure	7
Liver	**23**	Organ Size	7
Macrophages, Alveolar @	**14**	Pleura	7
pH	**13**	Hydrophobic and Hydrophilic Interactions	6
Lipid Peroxidation	**12**	Lethal Dose 50	6
Phagocytosis @	**12**	Organ Specificity	6
Solubility	12	Pregnancy	6
Gene Expression	11	Protein Binding	6
Respiratory Mucosa	11	Trachea	6
Adsorption	10	Brain	5
Body Weight	10	Membrane Potential, Mitochondrial	5
Kinetics	10	Metabolic Clearance Rate	5
[illegible]	[illegible]	[illegible]	[illegible]

Cytotoxicity and immune toxicity assays are widely used when assessing ESH for carbon nanotubes. DNA damage impacting cell survival or inducing cell proliferation is clearly noticed in literature.

Microbial Inhibition by MWCNTs (UofA)

Testing methodology:

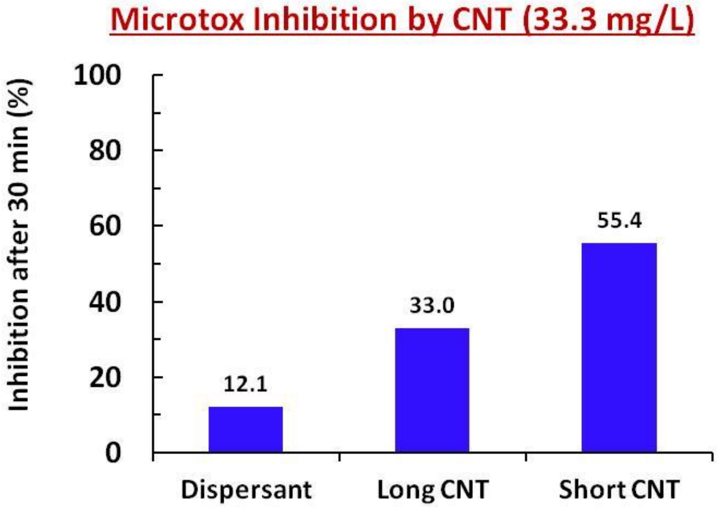
Microtox assay

Marine bioluminescent *bacterium Vibrio fischeri*

NPs dosing

Inhibition leads to decrease in bioluminescence

- Widely used assay; large database of Microtox values facilitates comparison with other chemicals.
- Microtox inhibition generally shows good correlation with aquatic toxicity (algae, fish).

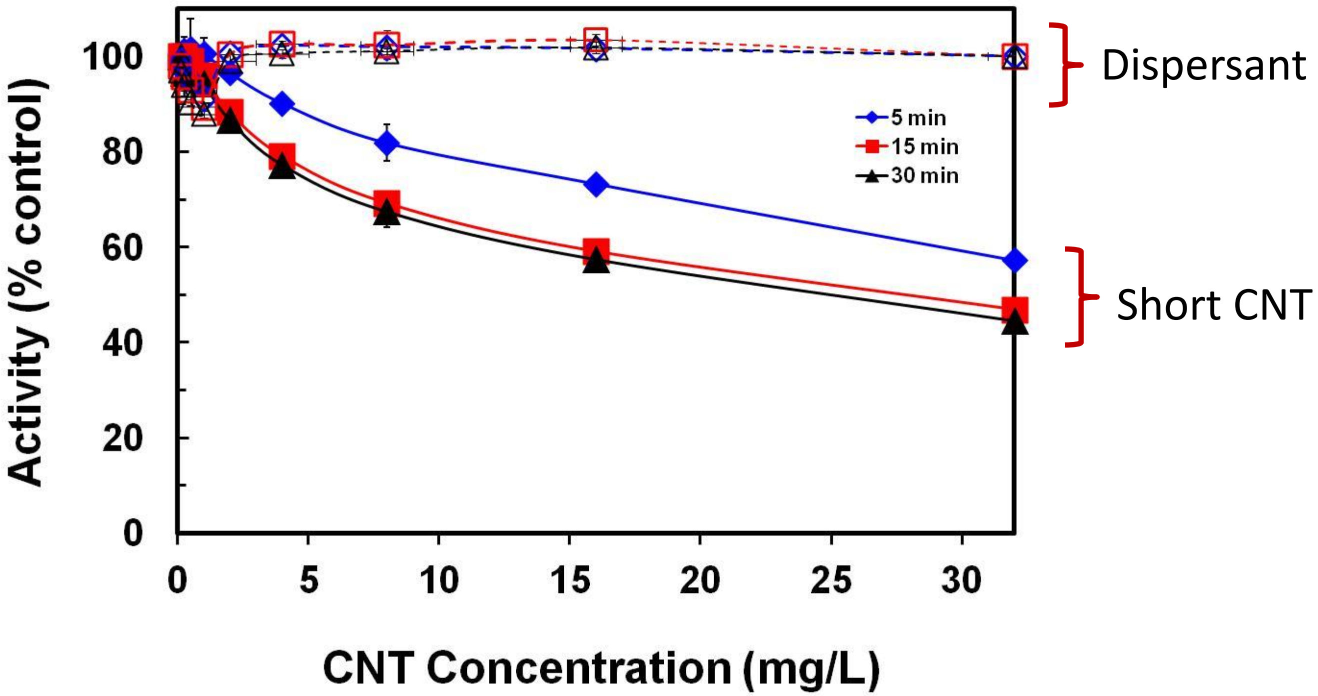


- Short CNT more inhibitory to microbial respiration activity compared to long CNT. Long CNT IC_{50}= 27. 9 mg/L.
- The dispersant used to stabilize the MWCNT caused very low inhibition.

University of Arizona

Microbial Inhibition by MWCNTs

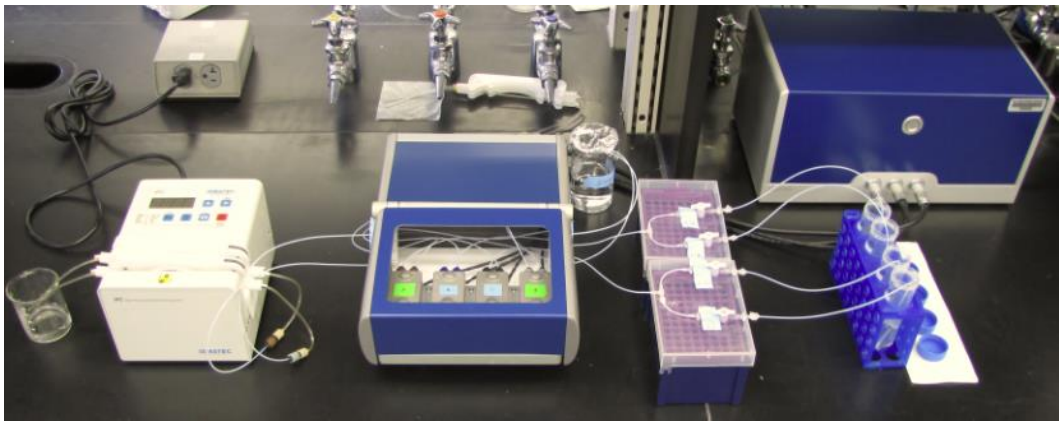
Microtox Inhibition Assay with Short MWCNT



- The inhibitory impact of the short MWCNT increased with exposure time (up to 15 min)

Binding of CNTs to Model Cell Membranes (JHU)

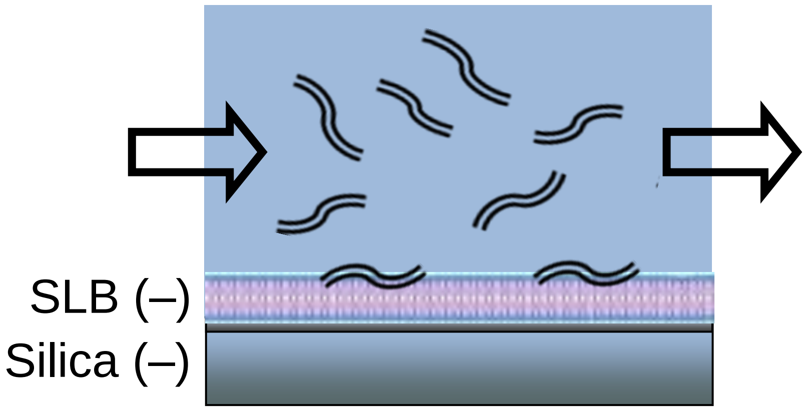
Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D)

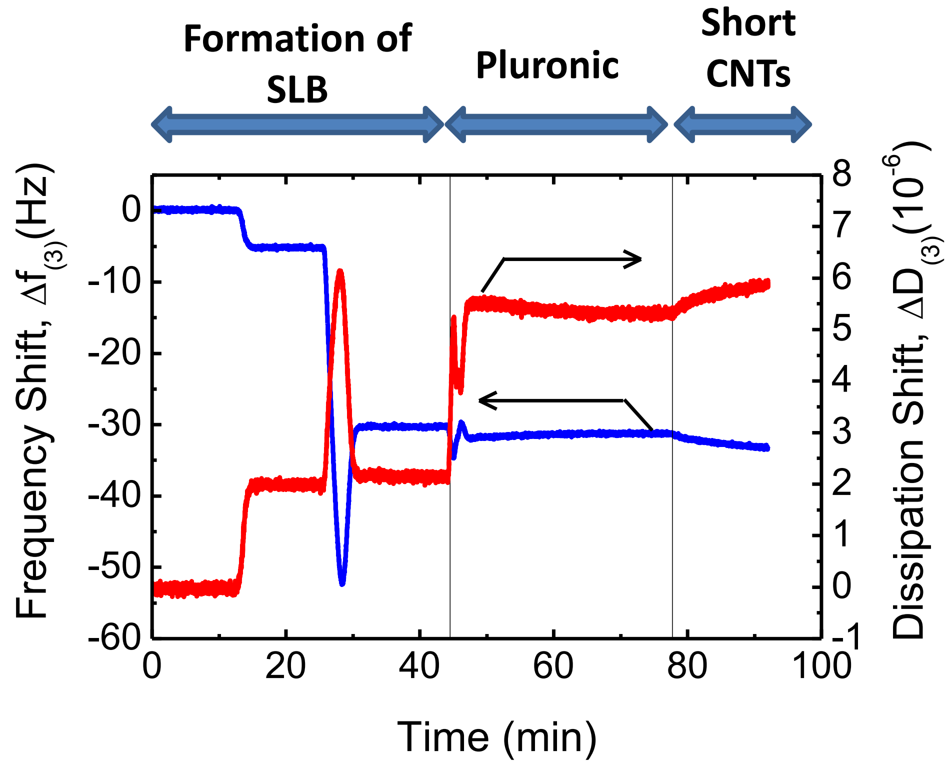


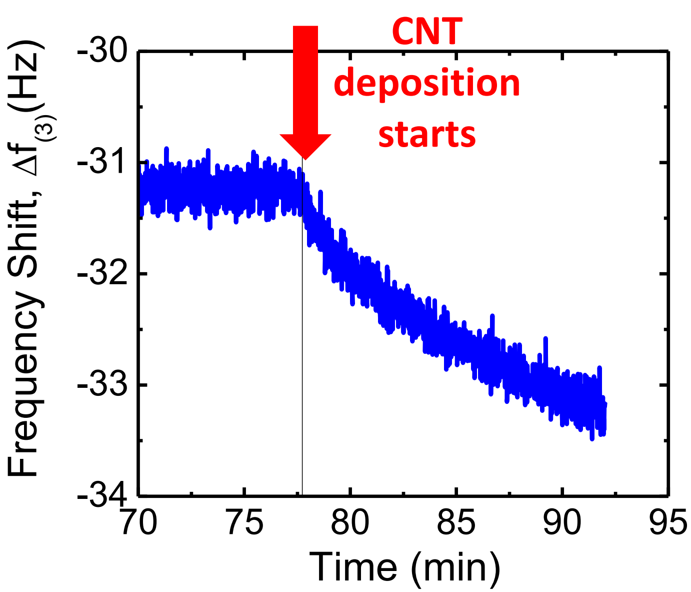
	Short CNTs	Long CNTs
Hydrodynamic Diameter (nm)	72	134
Electrophoretic Mobility (10^{-8} m^2/Vs)	–0.68	–1.00

- Propensity of CNTs to bind to model cell membranes evaluated with QCM-D
- Both short and long CNTs were determined to be negatively charged in Pluronic F108 solution

Binding of CNTs to Model Cell Membranes







- Both short and long CNTs were found to bind to zwitterionic DOPC supported lipid bilayers (SLBs)

Conclusions

- Consortium has monthly conference calls
- Selected MWCNT (short-/long-) to answer question – can our groups detect differences associated with length?
- Succeeded with material exchange between universities; addressed impurity concerns
- Built confidence in working with each other
- Provided cross-validated analytical data on MWCNTs
- Will complete MWCNT (short-/long-) work
- Wants consortium to approach nanoparticles in CMP & use “real” NP solutions representing CMP fluids