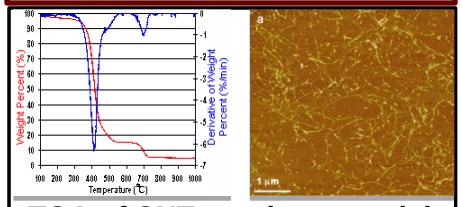
The University of Texas at Dallas BioNanosciences Group

Predicting, Testing, and Neutralizing Nanoparticle Toxicity

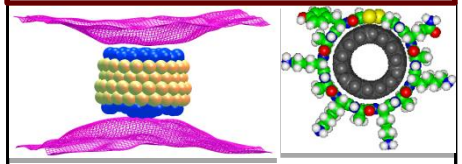
Nanoparticle material

Chemical & Physical Characterization



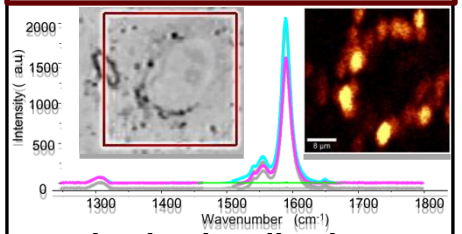
TGA of CNT starting material; AFM of biocompatible material

Computational Modeling & Predictions



CNT – bio interactions

Biological Testing



in vivo localization of CNTs using Raman

ESH Management

Task Title: (*Task Number: 425.027*)

Predicting, Testing, and Neutralizing Nanoparticle Toxicity

The University of Texas at Dallas

Departments of Chemistry and Molecular & Cell Biology; Alan G. MacDiarmid NanoTech Institute

PIs:

- **Steven O. Nielsen (PI)**
- **Rockford K. Draper (co-PI)**
- **Paul Pantano (co-PI)**
- **Inga H. Musselman (co-PI)**
- **Gregg R. Dieckmann (co-PI)**

Graduate Students:

- **Udayana Ranatunga (talk): PhD candidate, 100% funded**
- **David Bushdiecker (poster): PhD candidate, Not funded**
- **Nancy Jacobsen: MS candidate, Not funded**

Undergraduate Students:

- **Tyler Hughes (poster), Samee Vakil, Simon Beck, Dat Nguyen, Triet Nguyen**

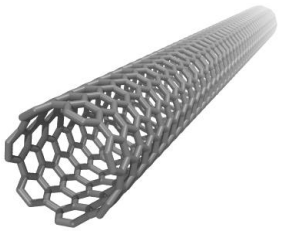
Senior Personnel:

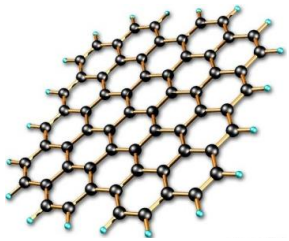
- **Ruhung Wang: Research Associate**
- **Bob Helms**

Deliverables & Objectives

- Obtain and validate data on the physical and chemical characteristics of carbon nanotubes (CNTs) and CMP nanoparticles correlated with structural modeling, interaction with model mammalian cells, toxicity, and bioactivity

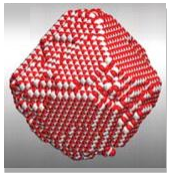
Year 1: single-walled carbon nanotubes



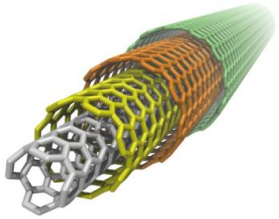


graphene oxide

Year 2: ceria

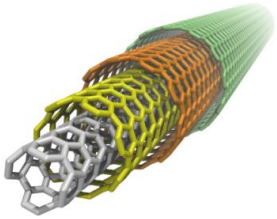


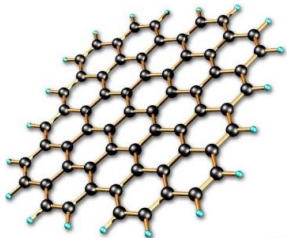
Science 312, 1504 (2006)



multi-walled carbon nanotubes

Year 3: (this year) multi-walled carbon nanotubes





graphene oxide

identification or prediction of inherent material ESH properties and any process by-products

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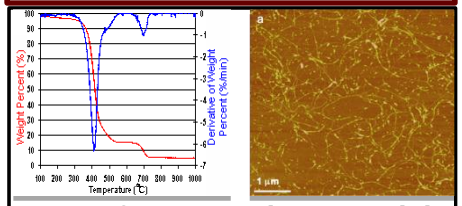

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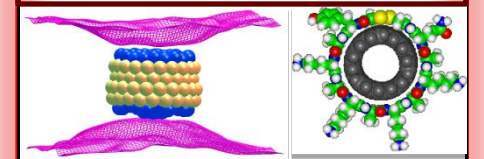

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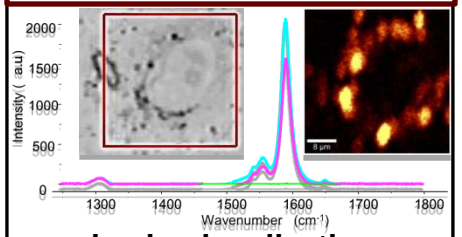
Biological Testing



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ESH Management

Dispersion of Carbon Nanotubes (CNTs)

- **Aggregation of nanomaterial has been implicated in toxicity**
- **Aggregation can be controlled through the use of dispersants**
- **Dispersants used in this study:**

Bovine Serum Albumin (BSA)

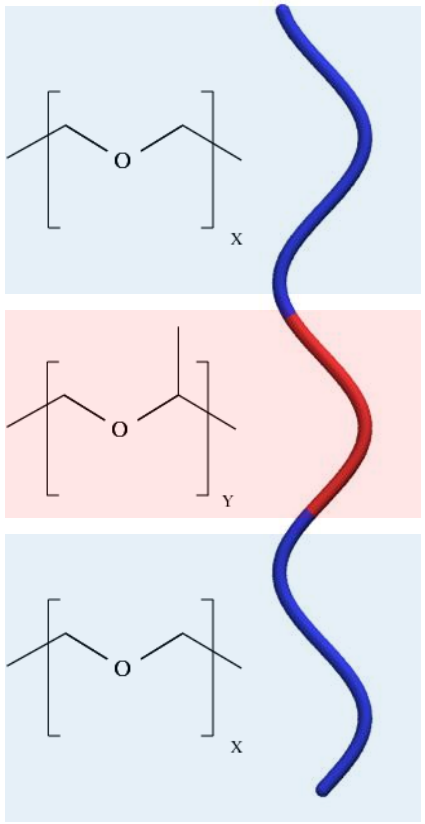
Natural protein

(~600 residues → 67 kDa)

Pluronics®

Tri-block copolymer

- **Commercially available**
- **Inexpensive**
- **FDA approved**
- **Block lengths vary**
- **Structure affects behavior**



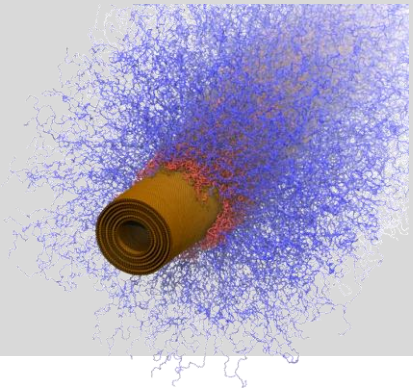
naming convention

F68

physical state

x10 = % hydrophilic

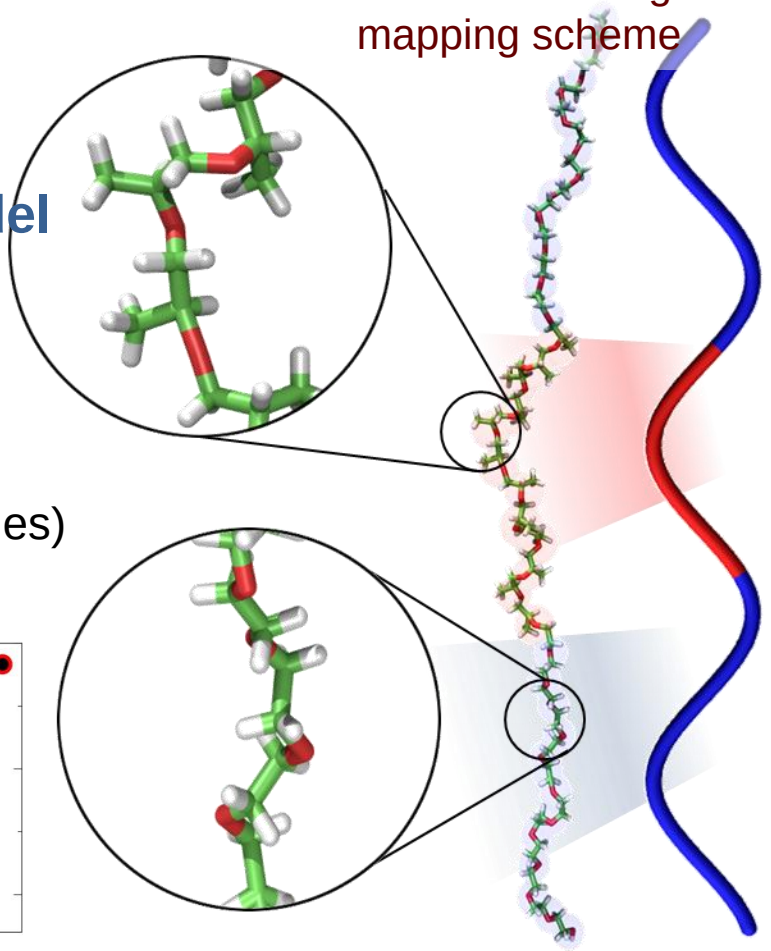
Hydrophobic block mass

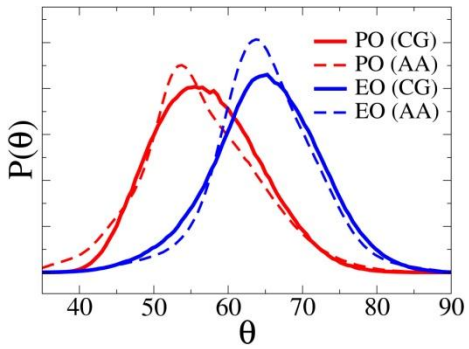


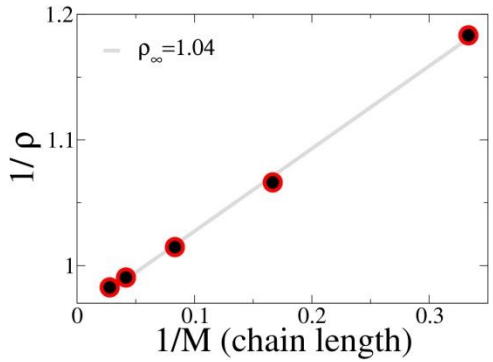
Modeling Pluronics

- **Existing models**
  - All atom representation
  - Cannot simulate adequate sizes
- **Our solution: a coarse grained model**
  - Reduce complexity while maintaining molecular structure
  - All atom simulations used to set bonded interactions
  - Experiment data (density, surface energies) used to tune non-bonded interactions

All atom to coarse grain mapping scheme







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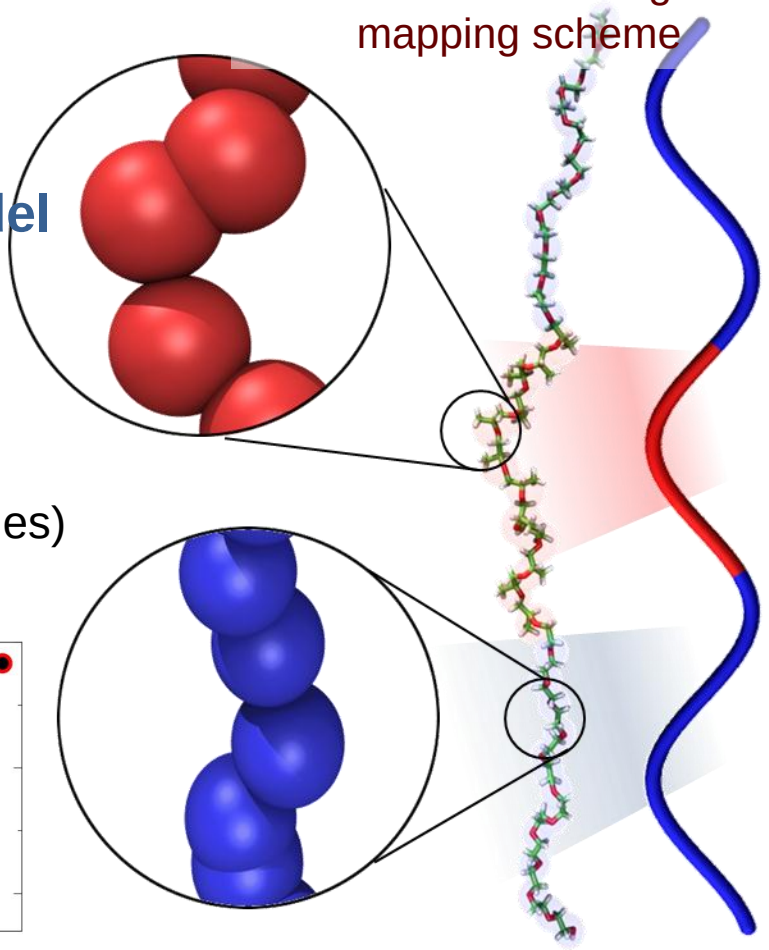
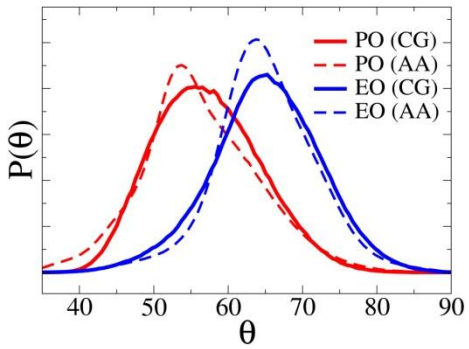
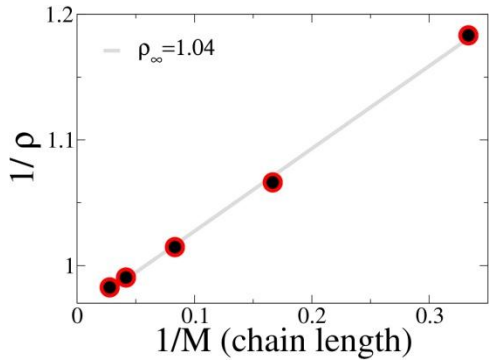
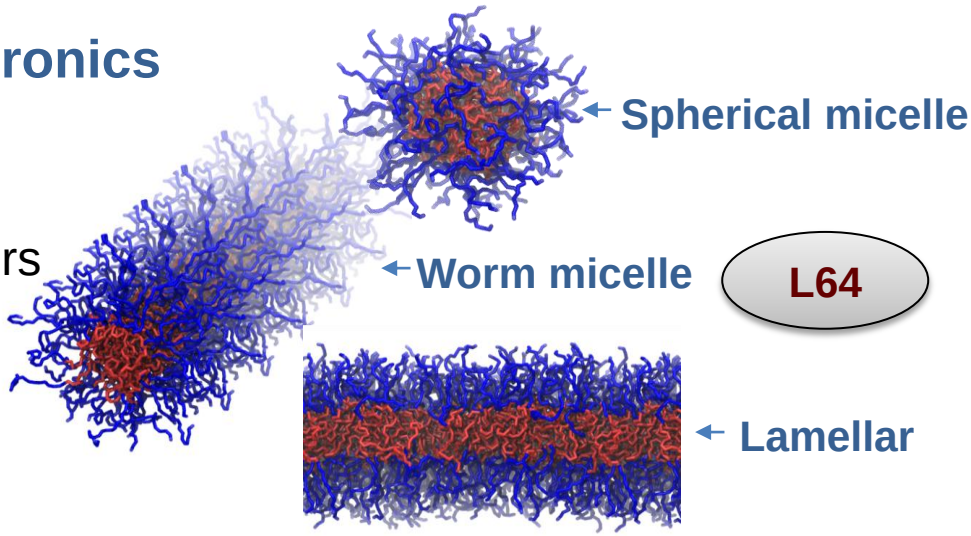




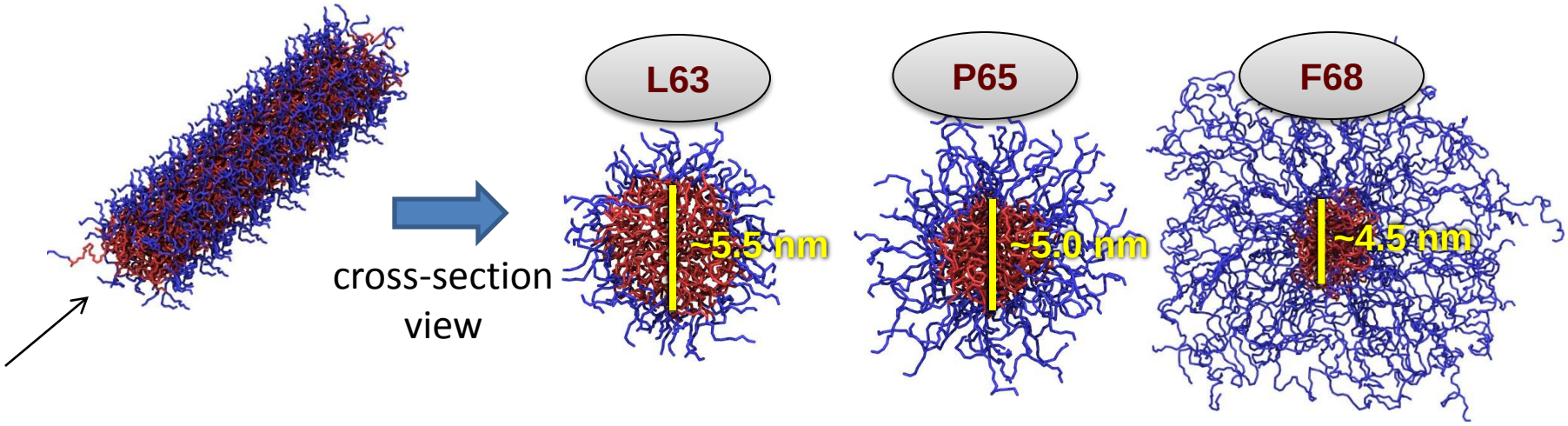


Polymer Self Assembly

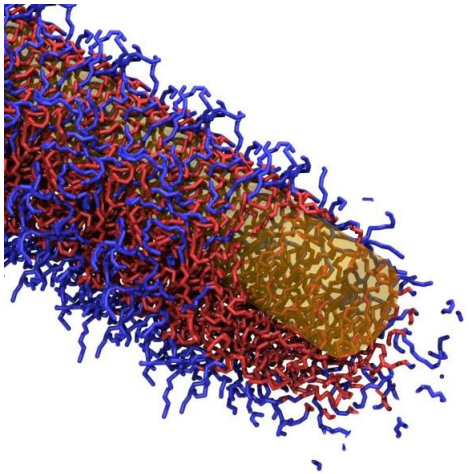
- **Aggregate shape of Pluronics**
  - Depends on structure & concentration
  - Balance of several factors
    - Packing of A
    - Solvation of B



- **Metastable worm-micelles**

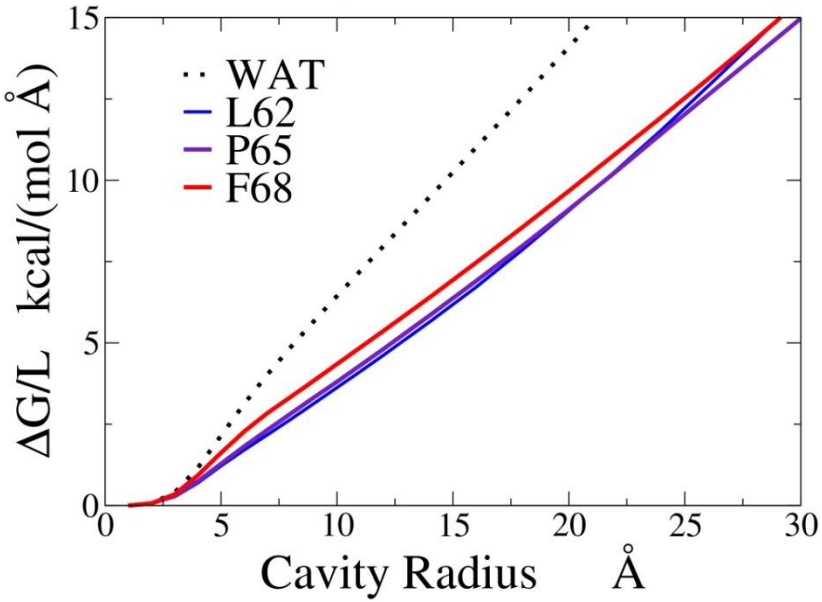


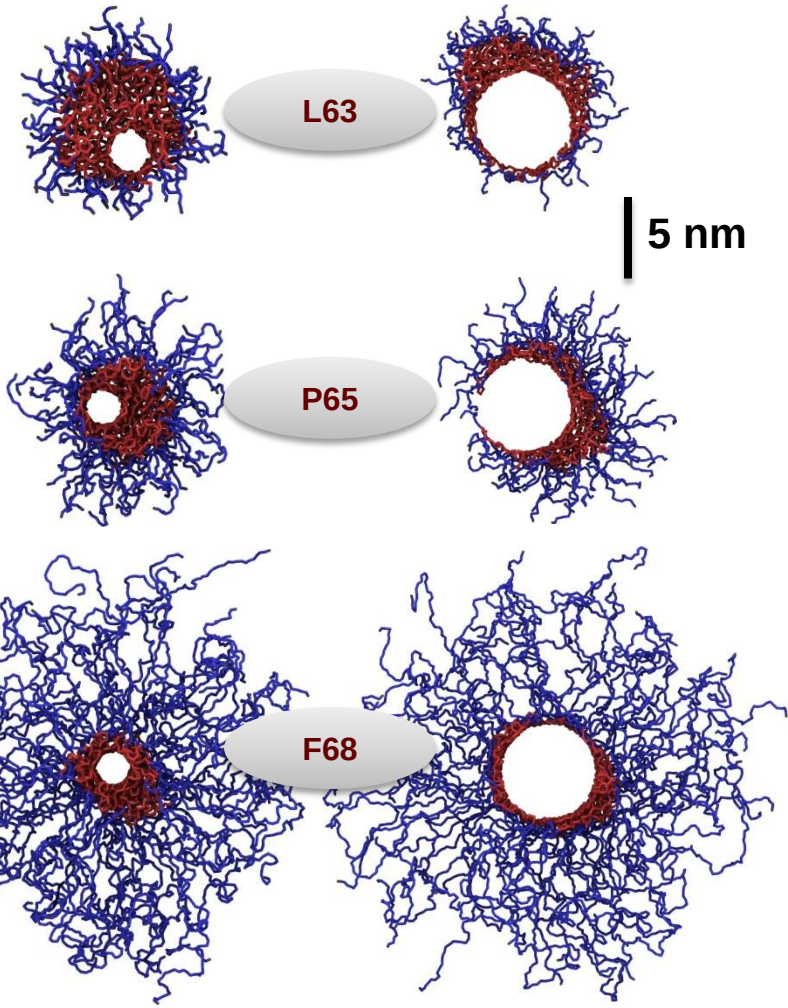
Polymer Assembly Around CNTs



The free energy of growing a hydrophobic cylinder can be measured using our novel method

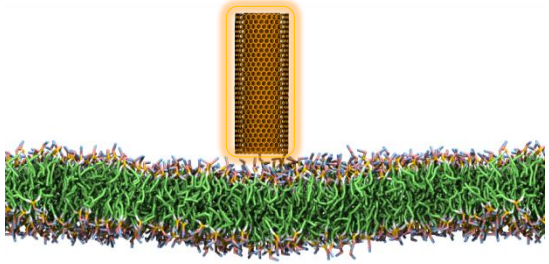
J. Chem. Phys. **132**, 054706 (2010)

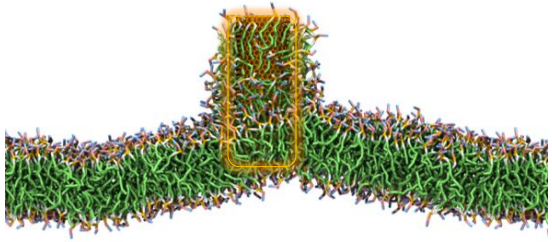


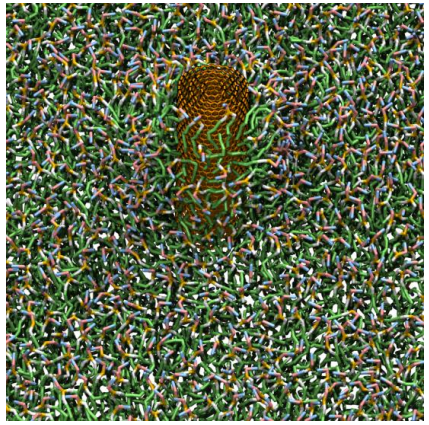


Nanotube – Lipid Interaction

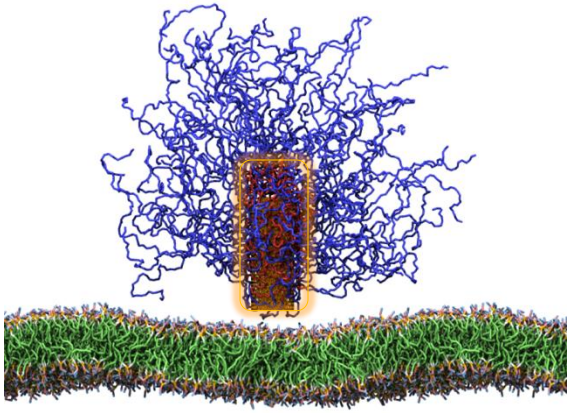
- **Bare MWNT spontaneously penetrates into a lipid membrane**

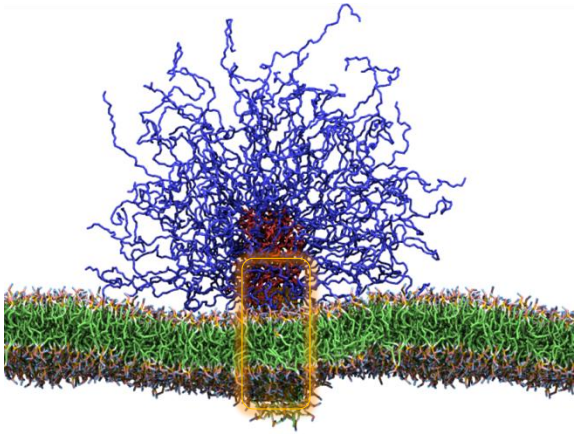


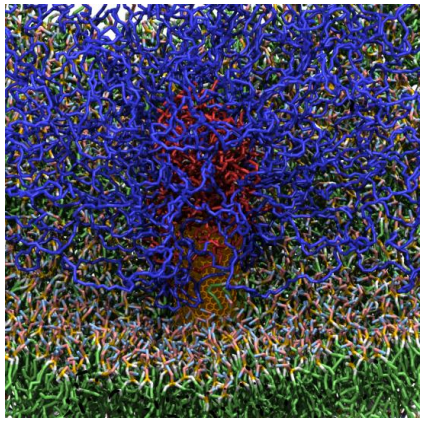




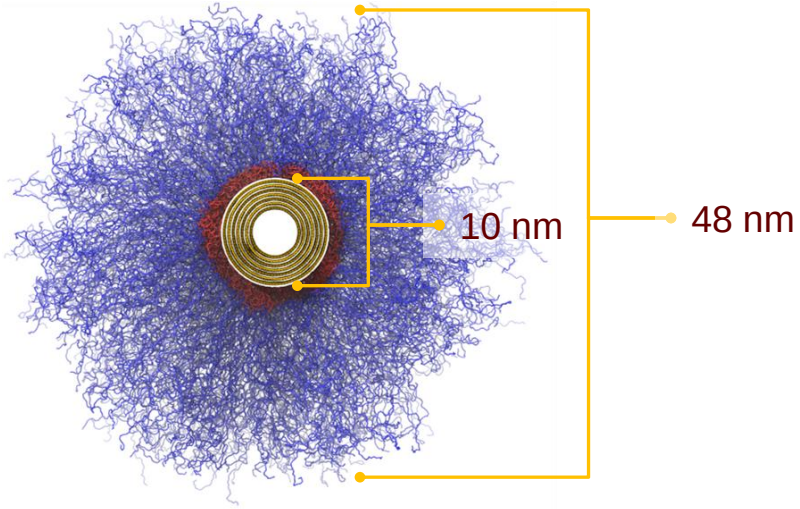
- **F68 wrapped MWNT: activation barrier to insert into lipid membrane**

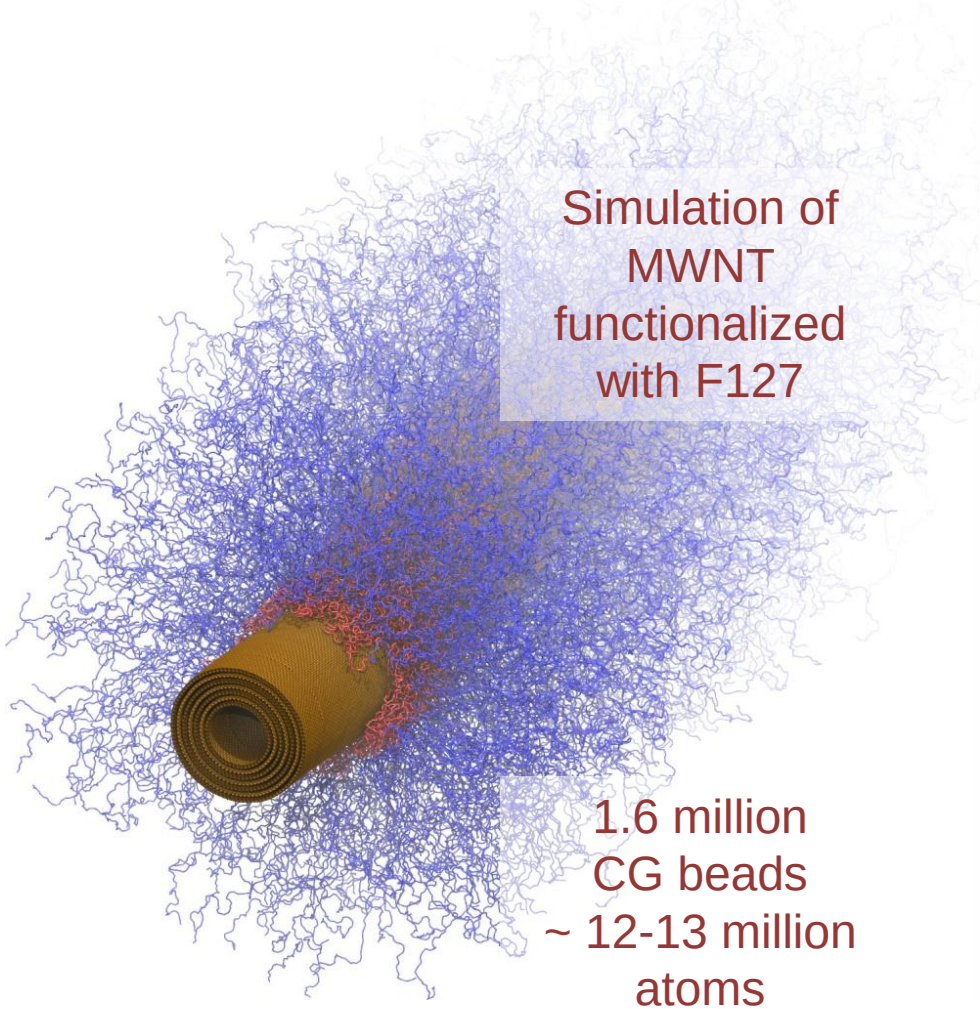






Simulating Pluronic-MWNTs





Simulation of MWNT functionalized with F127

1.6 million CG beads
~ 12-13 million atoms

- **Simulations are too CPU intensive**
  - Diameter of experimentally used MWNT ~10-20 nm
  - Too many atoms to simulate!
  - Need a more efficient approach

Conclusion from Theory / Simulation Studies

- **Successfully constructed a Coarse Grained model of Pluronics**
- **Pluronics with higher % hydrophilic mass are suitable for dispersing larger hydrophobes**
- **Pluronic-functionalized MWNTs interact differently with lipid bilayer membranes**
- **Simulating experimentally relevant Pluronic-functionalized MWNTs leads to the development of new computational methodology**

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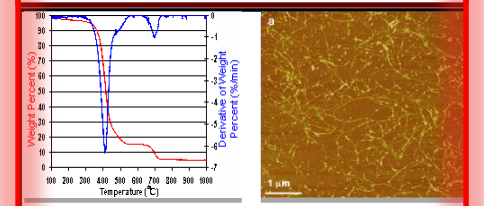

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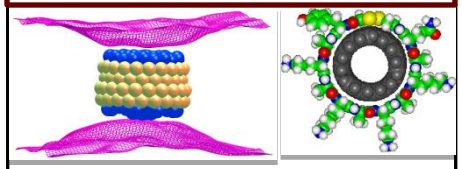

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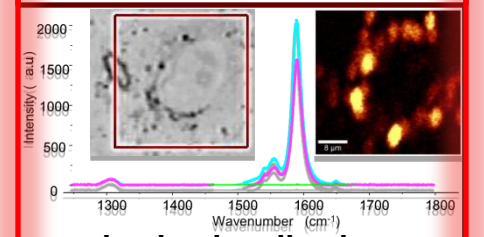

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Overview: Nanoparticle Characterization and Biological Testing

Review work on multi-walled carbon nanotubes:

1. **Control MWNT aggregation -**

   testing correlation between toxicity and aggregation

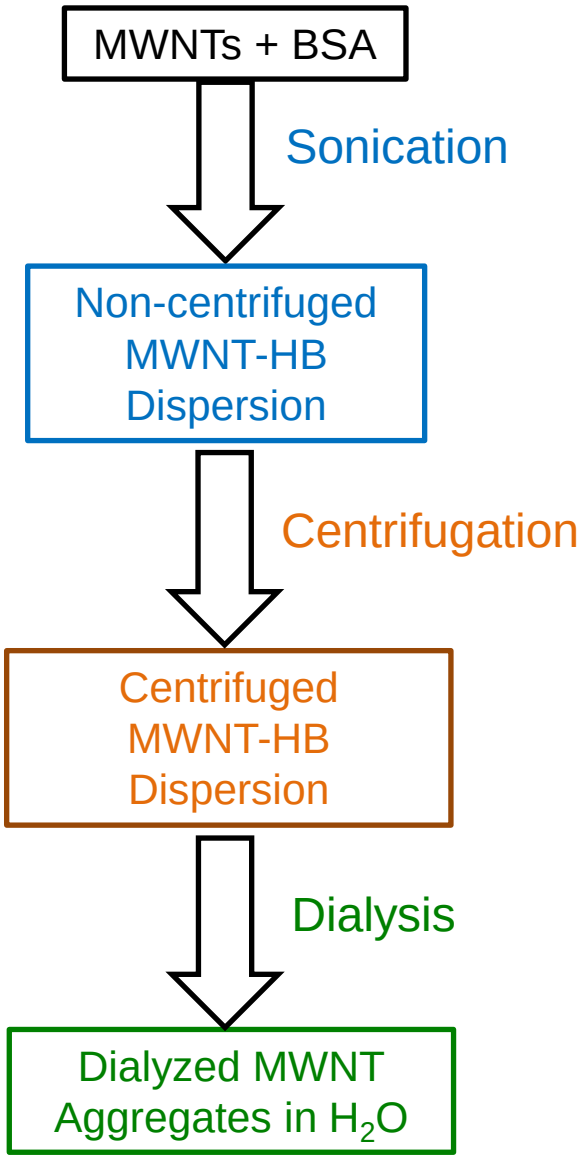
2. **Optimizing MWNT dispersions -**

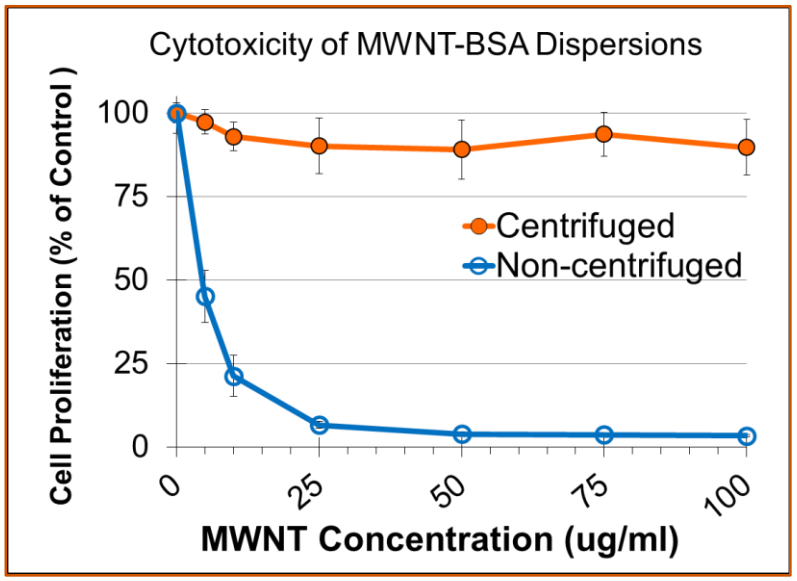
   testing the effectiveness and toxicity of Pluronic F-68 as a dispersant for MWNTs

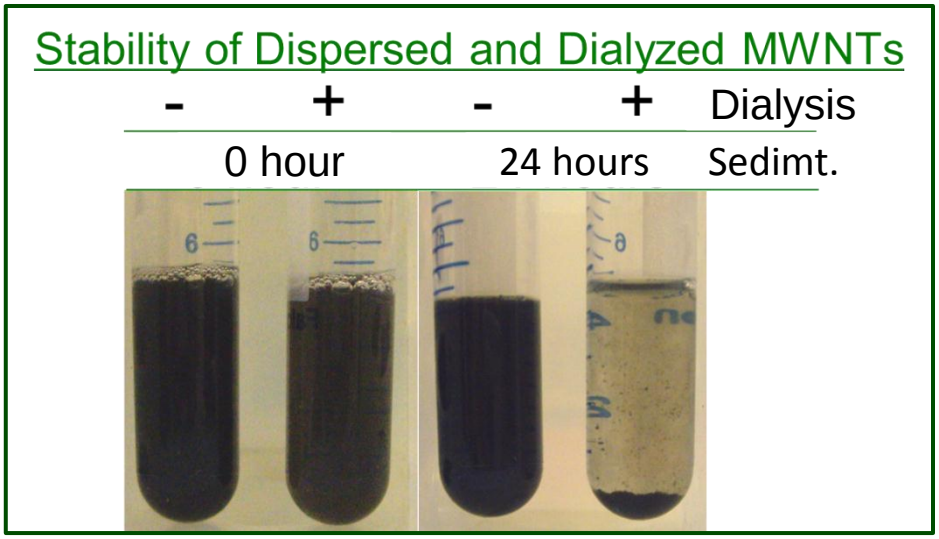
*** See poster by Hughes et al. for further details.**

*** Poster by Bushdiecker et al. on carboxylated SWNT toxicity.**

Preparation of Dispersed and Aggregated MWNTs



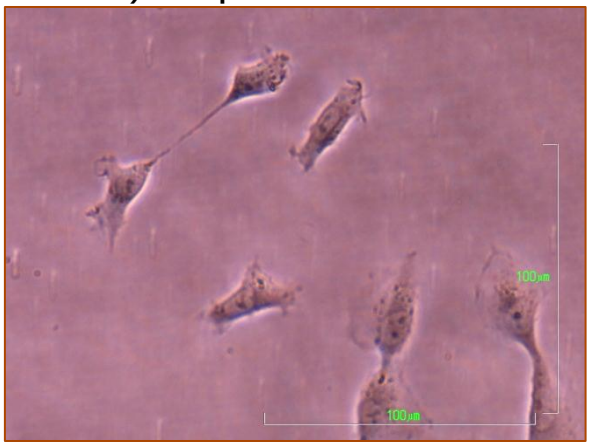




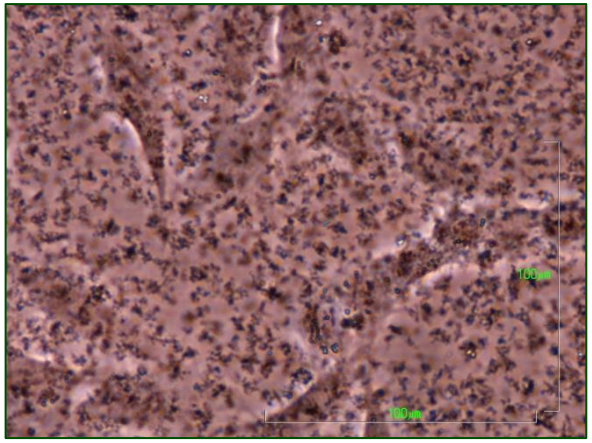
Cytotoxicity of Dispersed and Aggregated MWNTs

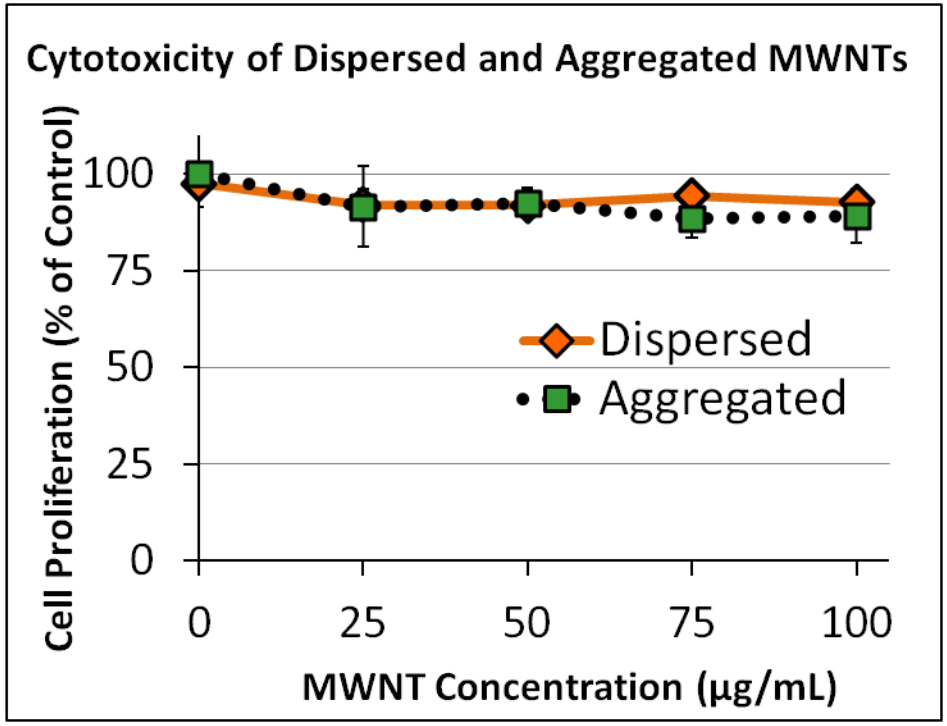
NRK Cells in Culture Media

A) Dispersed MWNTs



B) Aggregated MWNTs

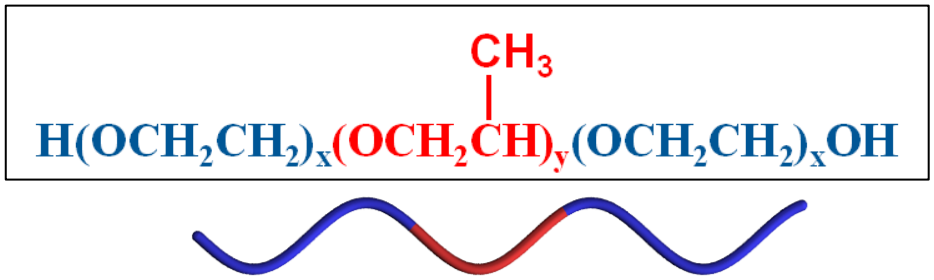




Removal of BSA induces MWNT aggregation.
MWNT aggregation has no effect on cell proliferation.

MWNTs: Improving the Dispersant

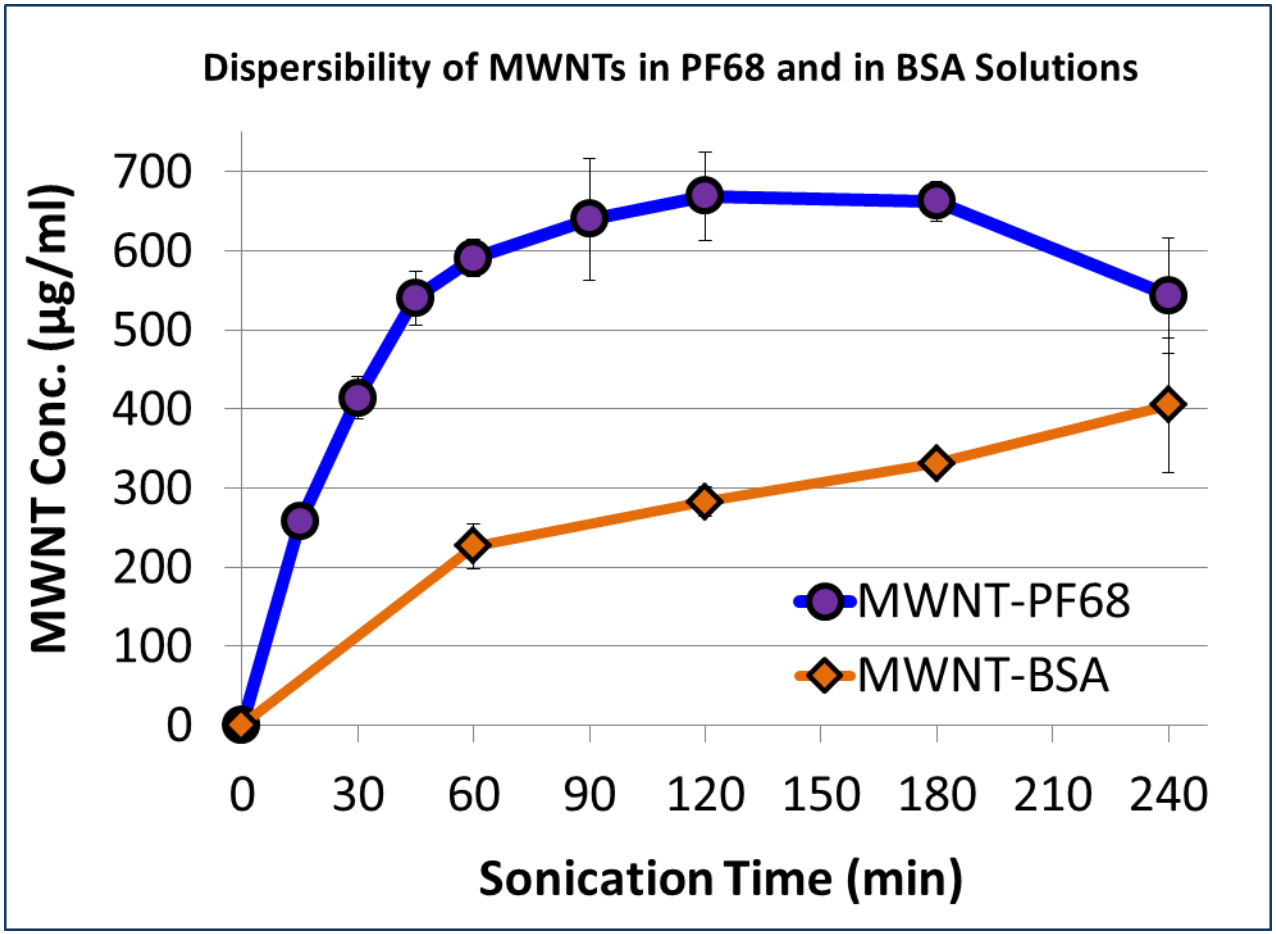
- We have been using the protein BSA
- Focused on Pluronic F-68 block copolymer



x ~80
y ~27
Molecular Weight ~ 8,400

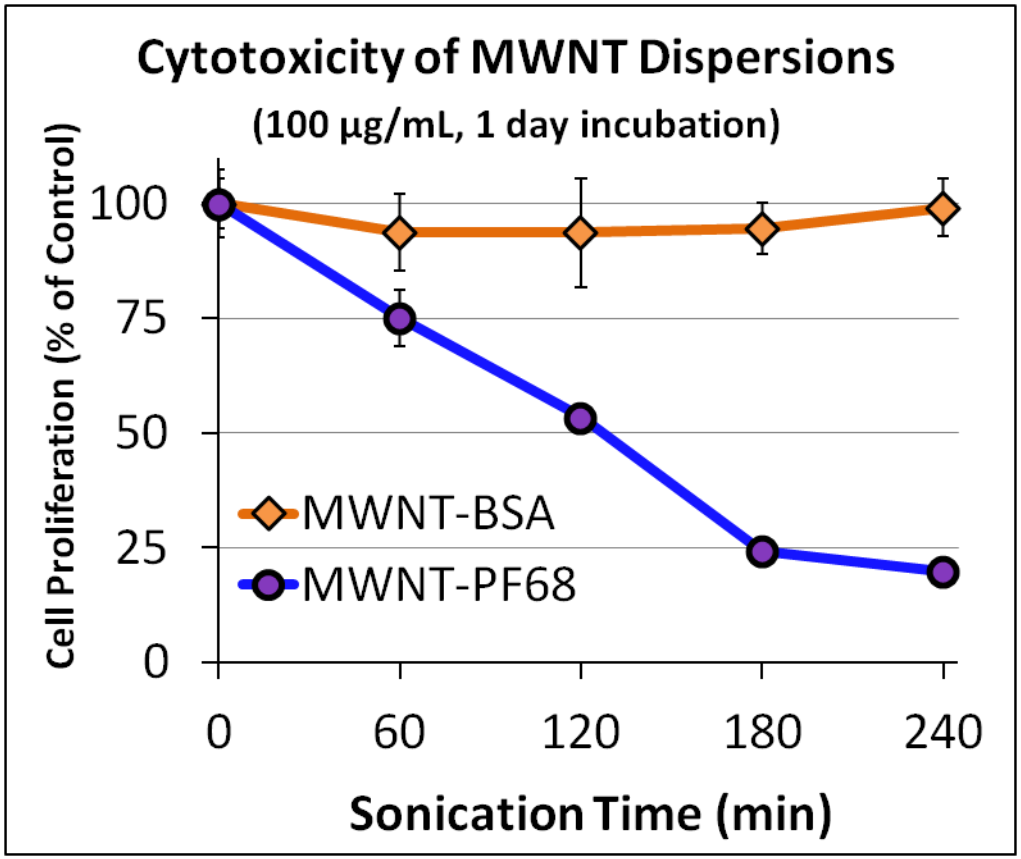
- ✓ Defined structure for modeling studies
- ✓ Inexpensive: BSA ~$3,000/kg vs. Pluronic ~$120/kg
- ✓ Amenable to cleaning spills and instruments
- ✓ Effective
- ✓ Biocompatible: Oral LD50 => 15,000 mg/kg (Rat)
  Dermal LD => 5,000 mg/kg (Rabbit)
  FDA approved as a component of intravenous injections

Comparing the Effectiveness of Pluronic and BSA in Dispersing MWNTs



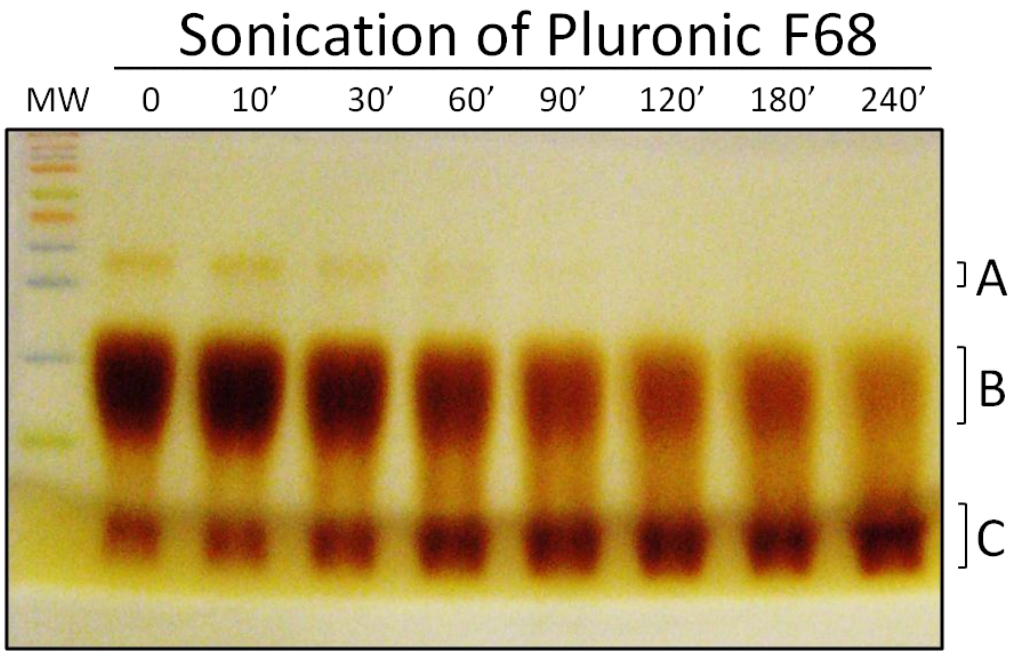
Pluronic F-68 disperses MWNTs faster and better than BSA solution.

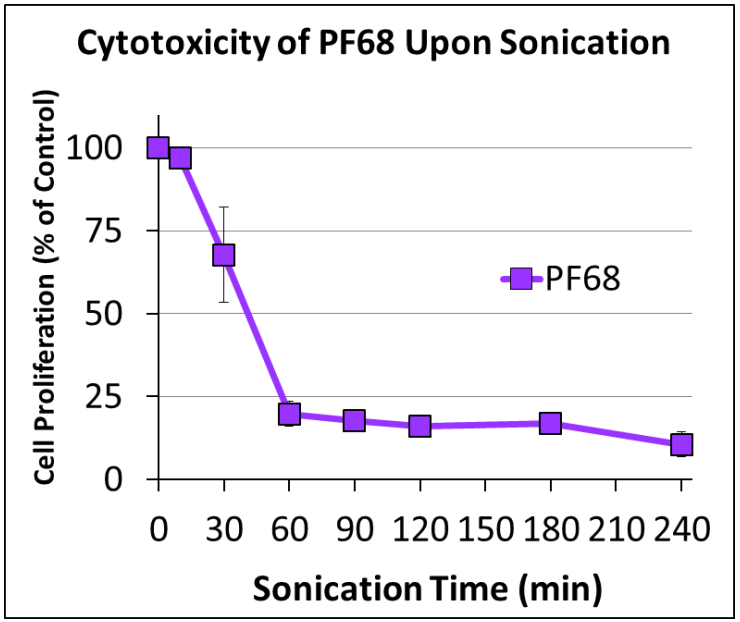
Comparing the Cytotoxicity of MWNT-BSA and MWNT-PF68 Dispersions



MWNT-BSA dispersions are not toxic.
MWNT-PF68 dispersions become toxic upon sonication.

Degradation and Cytotoxicity of PF68 upon Sonication





Increasing Sonication Time

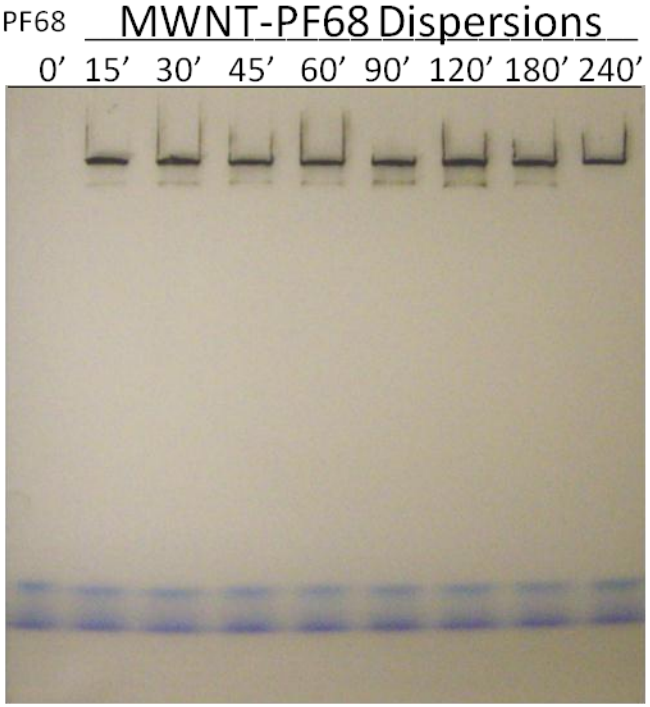
- Less high MW components (A & B)
- More low MW components (C)

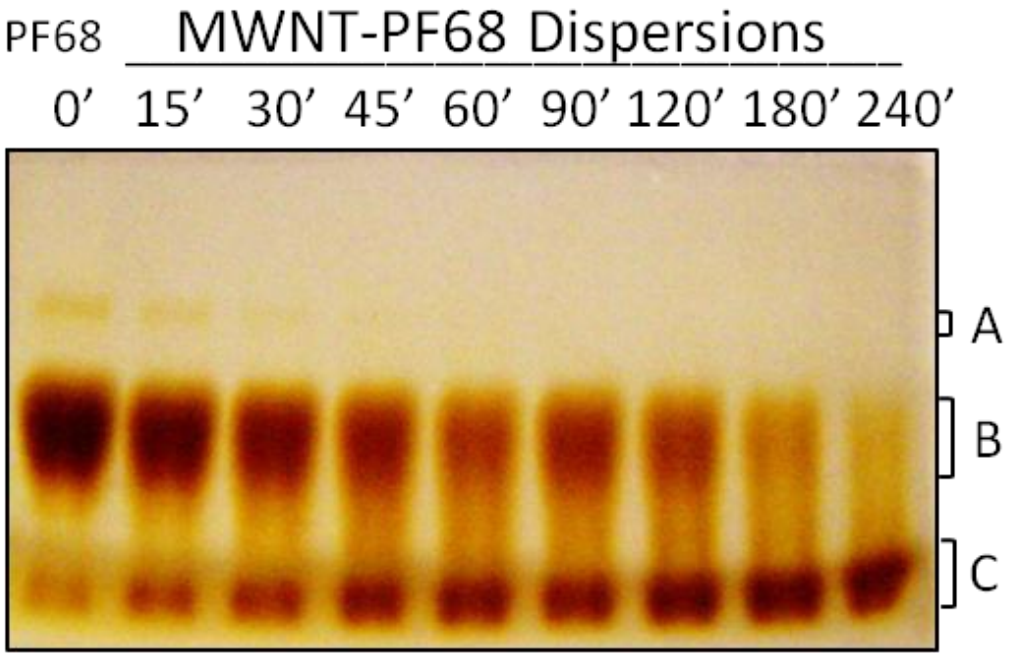
Increasing Sonication Time

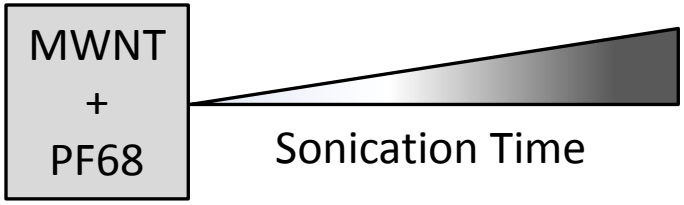
- Reduced cell proliferation

Sonication of Pluronic Polymers =➔ Degradation =➔ Toxicity

Degradation of PF68 in MWNT-PF68 Dispersions







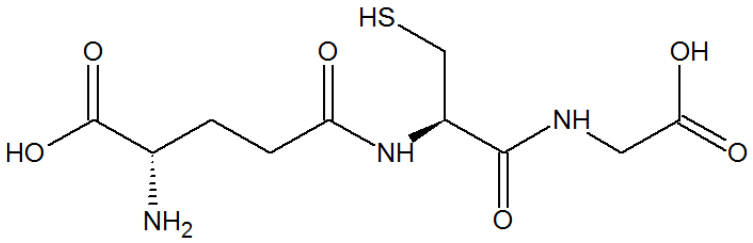
↑**Dispersibility** – higher MWNT concentration

↑**Cytotoxicity** – reduced cell proliferation

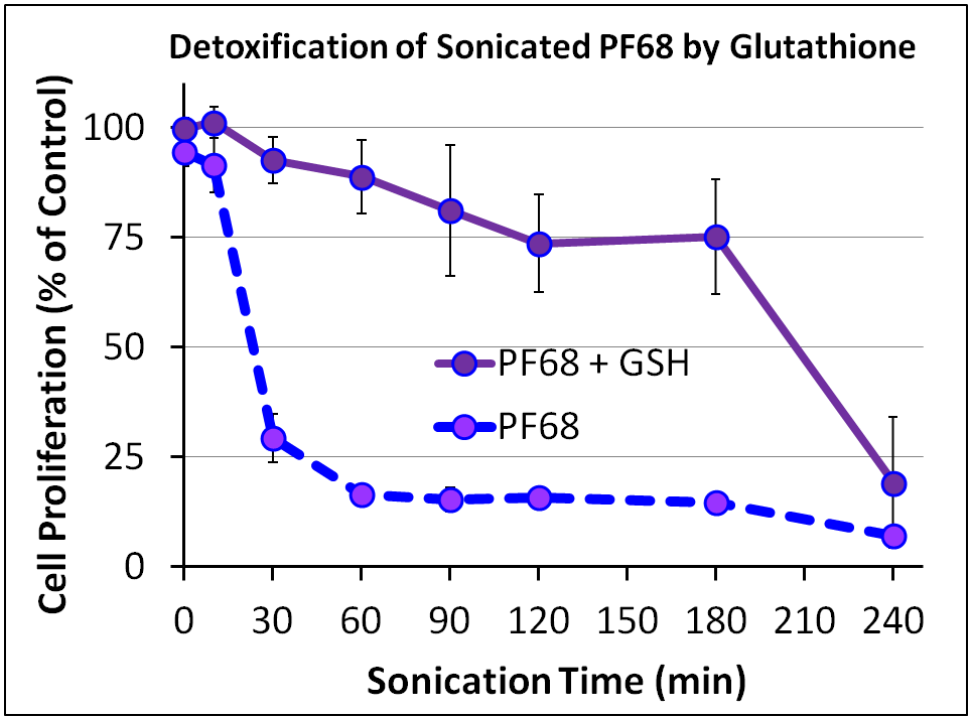
↑**Pluronic Degradation** – detected small fragments

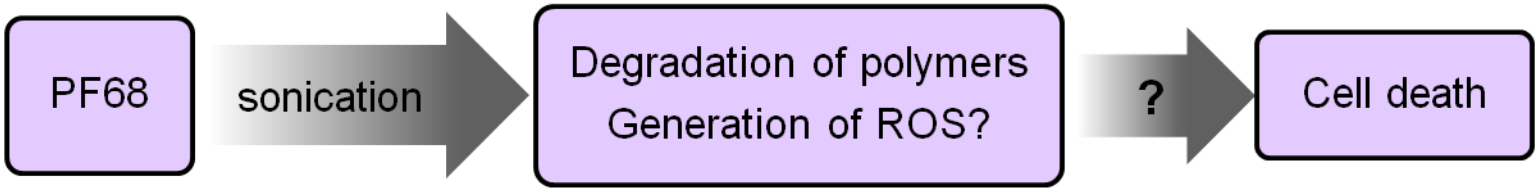
Detoxification of Sonicated PF68 by Glutathione

GSH – glutathione (reduced)
major endogenous antioxidant



Glutathione prevents damage to cells caused by reactive oxygen species (free radicals and peroxides)





Summary and Future Work

Summary:

1. **Induce MWNT aggregation –**
   - ✓ Aggregation of MWNTs did not induce toxicity
2. **Optimizing MWNT dispersions –**
   - ✓ Pluronic F-68 outperformed BSA in dispersing MWNTs
   - ✓ PF68 breakdown upon sonication, becomes toxic

Future Work:

1. **Chemical and physical characterization of MWNT dispersions –**
   - Control MWNT aggregation in various solutions
   - Particle size analysis by dynamic light scattering
   - Surface modification and impurities
2. **Survey biocompatible dispersants for various MWNTs –**
   - Effectiveness of Pluronic polymers in dispersing MWNT of various sizes
3. **Cytotoxicity analysis –**
   - ROS scavengers in preventing polymer toxicity upon sonication
   - Compare bio-accumulation of dispersed and aggregated MWNTs in cells

ESH Metrics and Impact

1. *Reduction in emission of ESH problematic material to environment:*
   - Reduced the toxic material associated with commercial preparations of a variety of single-wall and multiwall CNT types to a level such that the final CNT materials displayed minimal toxicity to cells in a sensitive model cell culture system.
2. *Identification of inherent material ESH properties and any process by-products:*
   - Assessed inherent ESH properties of single-wall CNTs, multiwall CNTs, graphene oxide, CMP ceria, and separable by-products.
   - Demonstrated that single-wall CNTs and multiwall CNTs themselves have little inherent toxicity in cell culture models, and that observed toxicity is due to by-products that can be separated from the CNTs or due to dispersant breakdown upon sonication.
3. *Establish dose metrics:*
   - Dose metrics established using cultured mammalian cells for single-wall CNTs, multiwall CNTs, graphene oxide, and CMP ceria.
4. *Develop analytical tools to measure trace levels of materials in process effluents:*
   - Developed sensitive methods to detect CNTs, functionalized CNTs, graphene oxide, and CMP ceria using their unique Raman signatures.
   - Improved the accuracy of the UV-VIS spectroscopy method to quantify the percentages of semi-conducting and metallic CNTs
5. *Predictive materials modeling and development of nanoparticle-bio interaction studies:*
   - Developed predictive DLVO theory for CNTs and computer models for CNT-bio and C60-bio interactions; developed coarse grained model of Pluronic dispersants.

Industrial Interactions and Technology Transfer

- Leveraged core ERC funding to obtain Small Business Technology Transfer (STTR) grant from the NIH.
  - CNT toxicity.
- Initiated MWNT studies due to industrial interest by Intel, TI, and others.
  - ESH of MWNTs.
- Submitted “round robin” report to ERC nanotox researchers and industrial affiliates.
  - ESH of ceria CMP.

Publications

- SRC Publication P058919 P. Pantano, N. Jacobsen, “Determining the Percentages of Semi-conducting and Metallic Single-walled Carbon Nanotubes in Bulk Soot”, Carbon 49, 1998-2006 (2011).
- SRC Publication P058918 R. DeVane, M. Klein, W. Shinoda, P. Moore, S. Nielsen, C. Chiu, “Effect of Carboxylation on Carbon Nanotube Aqueous Dispersibility – A Predictive Coarse Grained Molecular Dynamics Approach”, 24-Jan-2011.
- SRC Publication P057837 S. Nielsen, C. Chiu, R. DeVane, A. Jusufi, P. Moore, M. Klein, W. Shinoda, “Parametrization and Application of a Coarse Grained Forcefield for Benzene/Fullerene Interactions with Lipids”, J. Phys. Chem. B 114, 16364-16372 (2010).
- SRC Publication P058949 R. Wang, C. Mikoryak, S. Li, R. Draper, D. Bushdiecker, I. Musselman, P. Pantano, “Cytotoxicity Screening of Single-Walled Carbon Nanotubes: Detection and Removal of Cytotoxic Contaminants from Carboxylated Carbon Nanotubes”, 31-Jan-2011.
- SRC Publication P060355 U. Ranatunga, T. Nguyen, S. Nielsen, “The Effect of Size on the Behavior of Fullerenes in Lipid Bilayers”, 22-Jun-2011.
- P. Pantano, R. K. Draper, C. Mikoryak, R. Wang, “Electrophoretic Methods to Quantify Carbon Nanotubes in Biological Cells”, in Handbook of Carbon Nanomaterials; F. D’Souza, K. M. Kadish, Eds. (2012) in press.
- N. Jacobsen, “A Density Gradient Ultracentrifugation Analysis of Single-Walled Carbon Nanotube Soot”, M. S. Thesis (UT Dallas, 2012)

Presentations

- C. Chiu, “Computer Simulations of the Interaction between Carbon-Based Nanoparticles and Biological Membranes”, SRC TECHCON 2010, September 14, 2010.
- R. Wang, “Physical Characterization and In Vitro Toxicity Testing of Commercially Purchased Single-walled Carbon Nanotubes”, ERC Teleseminar, April 22, 2010.
- P. Pantano, “Confocal Raman Imaging of Carbon Nanomaterials Taken-up by Living Cells”, 66th SouthWest Regional ACS Meeting, Dec 2010.
- S. Beck, “Toxicity Assessments and Biomedical Applications of Single-Walled Carbon Nanotubes”, DFW MetroPlex Days, March 4th 2011.
- P. Pantano, “A Gel Electrophoresis Method to Improve the Accuracy of Carbon Nanotube Cytotoxicity Assessments”, Wichita State University.
- U. Ranatunga, R. Wang, “Nanoparticle aggregation and toxicity”, ERC Teleseminar, April 7th, 2011.
- U. Ranatunga, “Spherical Fullerenes in Lipid Membranes”, SRC TECHCON 2011, September 13, 2011.

Awards

- *2010 Simon Karecki Award*: graduate student Chi-cheng Chiu.