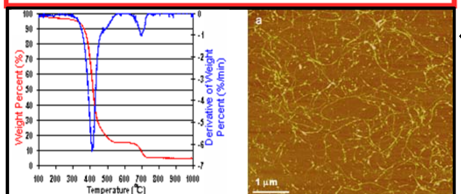
The University of Texas at Dallas BioNanosciences Group UTD

Predicting, Testing, and Neutralizing Nanoparticle Toxicity

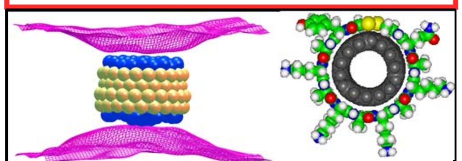
Nanoparticle material

Chemical & Physical Characterization



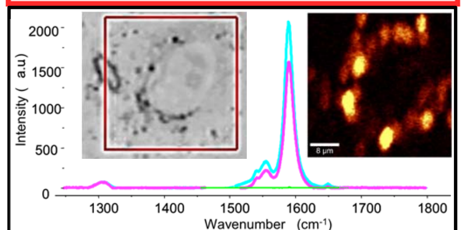
TGA of CNT starting material; AFM of biocompatible material

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)**
- **Inga H. Musselman (co-PI)**
- **Paul Pantano (co-PI)**
- **Gregg R. Dieckmann (co-PI)**

Graduate Students:

• Chi-cheng Chiu	Ph.D. defended 2010 (100% funded)
• Udayana Ranatunga:	PhD candidate (100% funded)
• David Bushdiecker:	PhD candidate (Not funded)
• Priya Richards	PhD candidate (Not funded)

Undergraduate Students:

- **Sri Magali, Nancy Jacobsen, Prashant Raghavendran, Simon Beck**

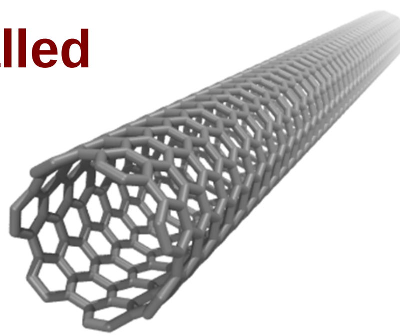
Senior Personnel:

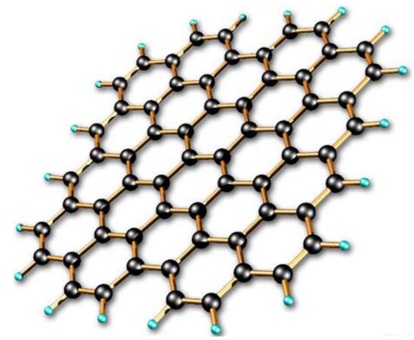
- **Ruhung Wang (poster presentation):** Research Associate
- **Bob Helms**

Year 2 Deliverables & Objectives

- **Obtain and validate data on the physical and chemical characteristics of carbon nanotubes (CNTs) and CMP nanoparticles with an initial attempt to correlate with structural modeling, interaction with model mammalian cells, toxicity, and bioactivity.**

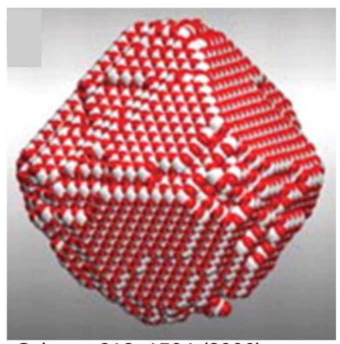
Last year: **single-walled carbon nanotube**



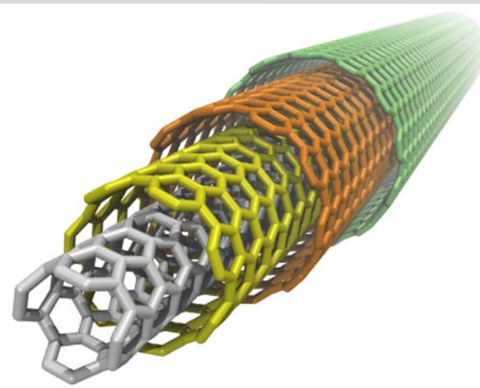


graphene oxide

This year: **ceria**



Science 312, 1504 (2006)



multi-walled carbon nanotube

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

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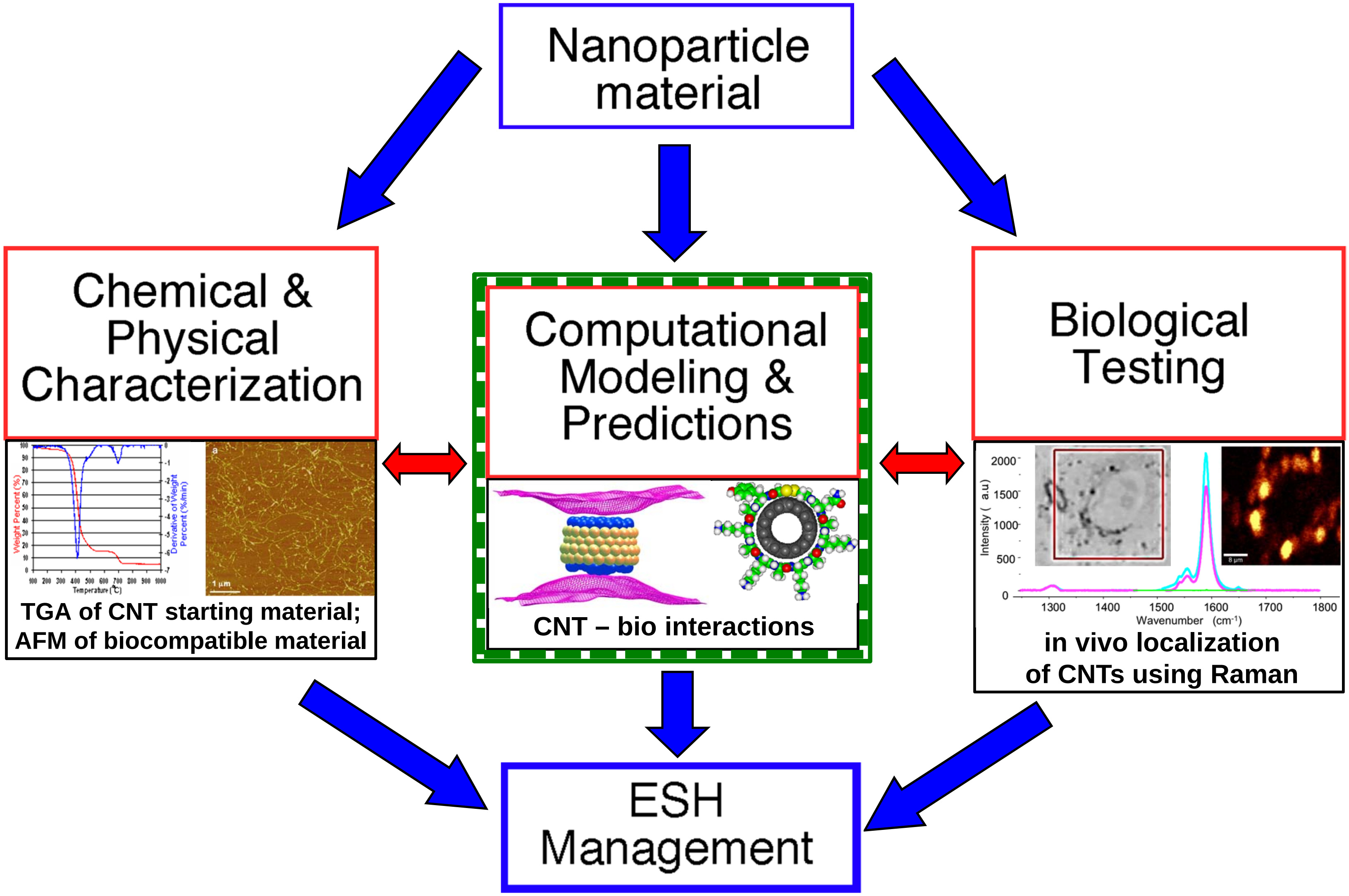


Nanoparticle Toxicity often linked to Aggregation

NANO LETTERS *Nano Lett.* **2010,** *10,* 1664–1670

Biocompatible Nanoscale Dispersion of Single-Walled Carbon Nanotubes Minimizes in vivo Pulmonary Toxicity

Marc Hersam *et al*, Northwestern University Feinberg School of Medicine,
†Department of Medicine, Division of Pulmonary and Critical Care Medicine,

Thirty days after lung exposure:

- **granuloma-like structures observed in mice treated with aggregated SWNTs**
- **absent in mice treated with nanoscale dispersed SWNTs**

Conclusion: toxicity of SWNTs *in vivo* is attributable to <u>aggregation</u> of the nanomaterial rather than the large aspect ratio of the individual nanotubes.

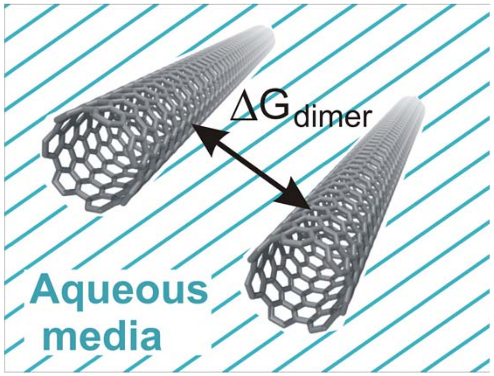
Nanoparticle Aggregation

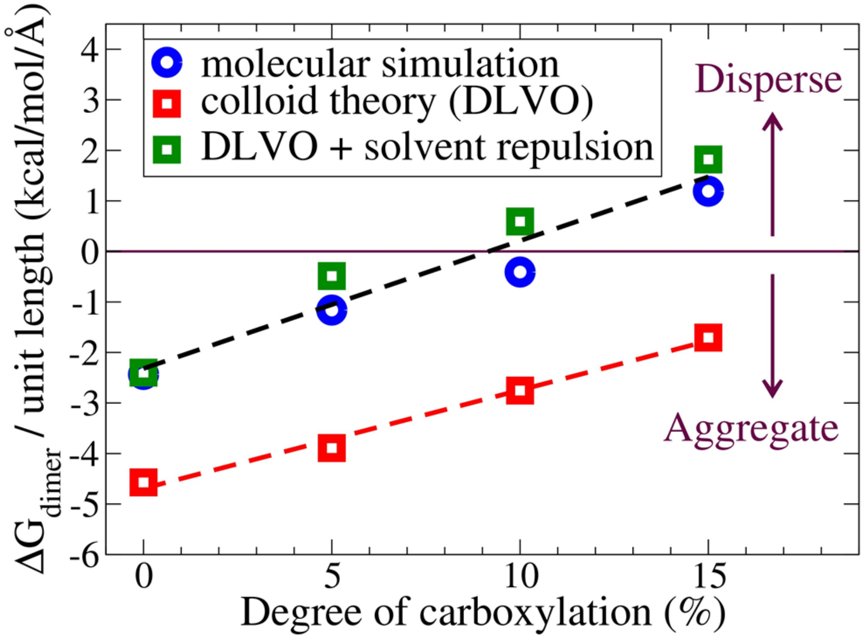
Experimental measure of the propensity to aggregate: zeta-potential

Conceptual understanding from Colloid Theory (DLVO):

- **electrostatic double layer repulsion**
- **van der Waals attraction**
- **balance determines propensity to aggregate**

Let's apply this theory to carboxylated (acid-functionalized) SWNTs

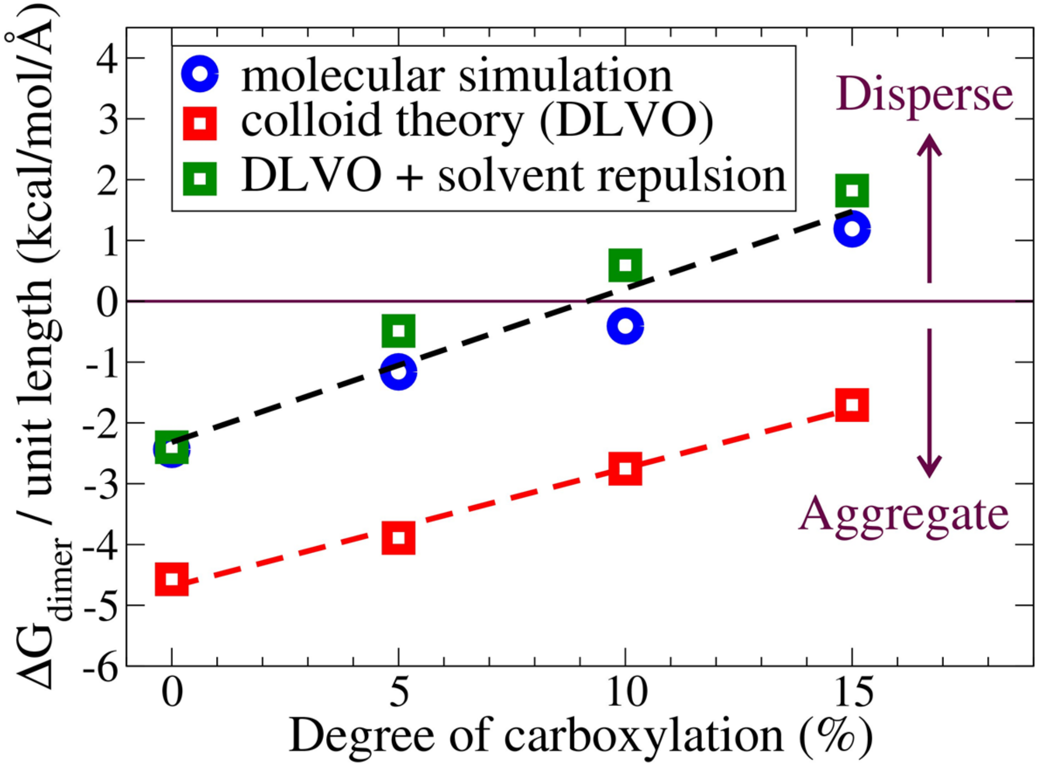




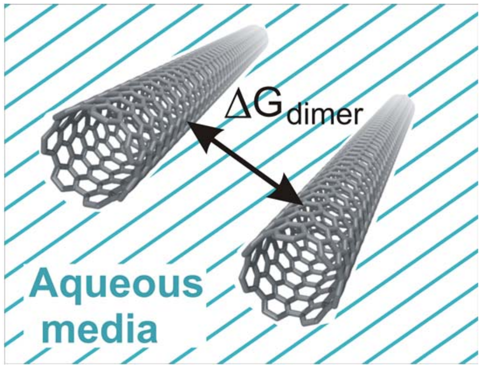
Nanoparticle Aggregation

What we found:

- colloid theory fails for carbon nanotubes
- it misses the solvent repulsion term
- adding this term brings colloid theory into agreement with molecular simulation data

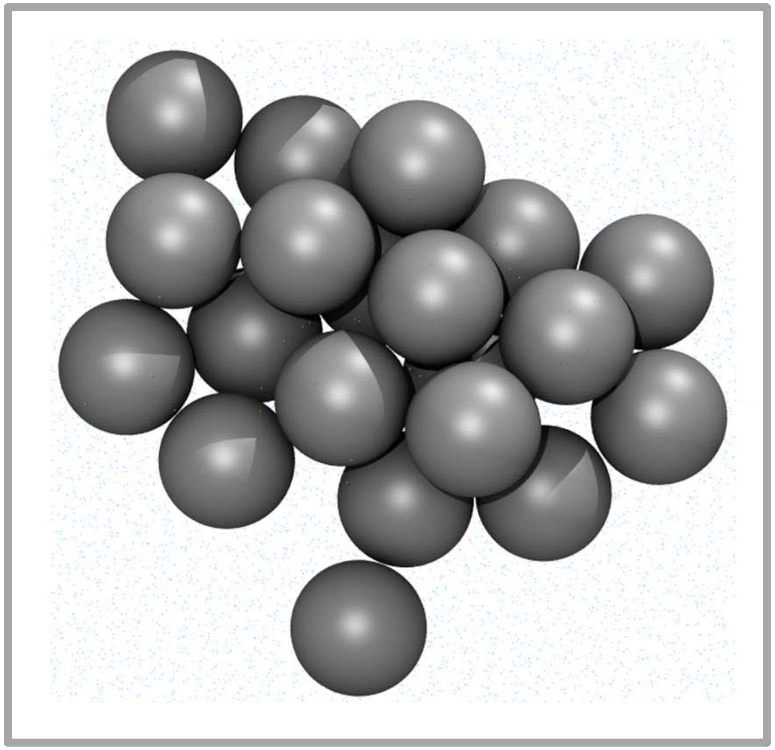


We are producing SWNTs with varying degrees of surface carboxylic acid groups and quantifying their physical properties and toxicity (experiments in progress)

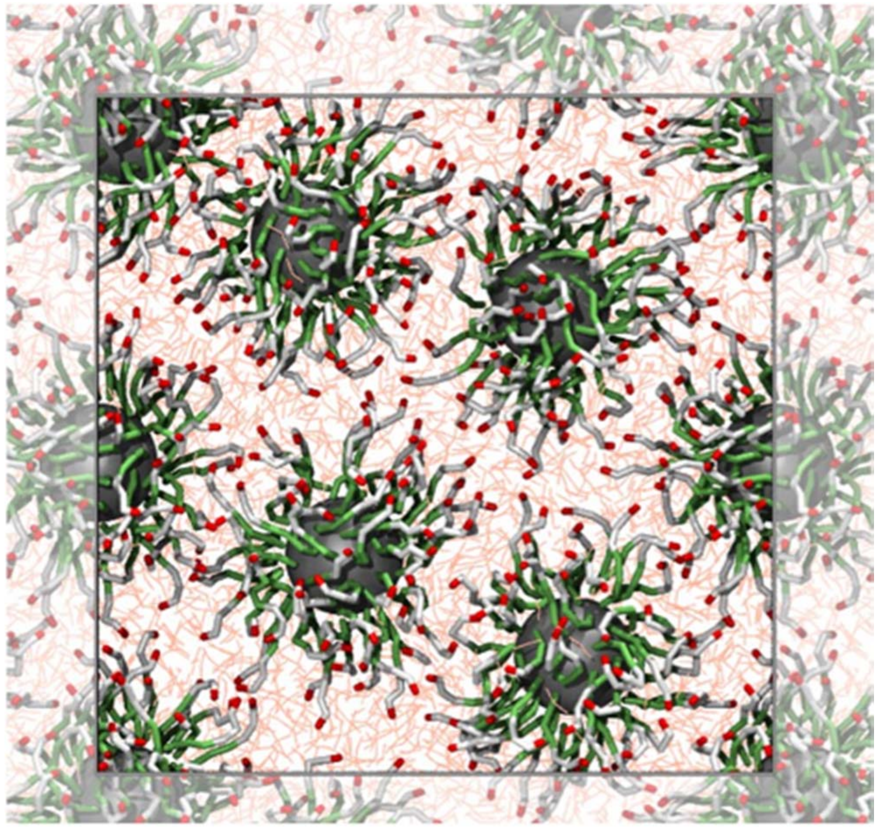


Nanoparticle Aggregation

use of dispersal agents can alter aggregation behavior



NPs in aqueous media aggregate



addition of a block copolymer dispersal agent changes NP behavior

Conclusion from Theory / Simulation Studies

Biocompatible nanoparticle dispersions typically show minimal toxicity, thus allowing them to be used safely in commercial applications.

- **direct modification of NP structure (carboxylation of carbon nanotubes) can lead to stable dispersions**
- **use of dispersal agents can drastically affect the NP aggregation state**

To understand these effects

- **for nanoscale objects, colloid theory misses key interactions but these can be captured with the aid of molecular simulation to yield a correct physical understanding of aggregation behavior**

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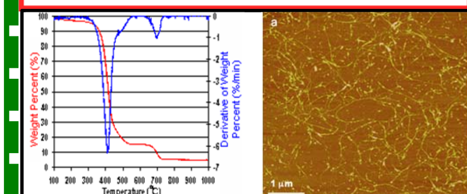

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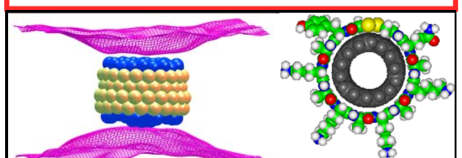
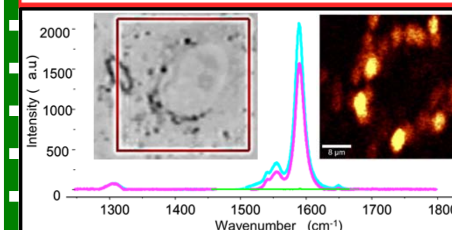


CNT – bio interactions

Biological Testing



in vivo localization of CNTs using Raman

ESH Management

Overview: Nanoparticle Characterizations and Biological Testing

- **Review and update of work on single-walled carbon nanotubes (SWNTs) since last year.**

- **Current work on multi-walled carbon nanotubes (MWNTs).**
  - **Industrial advisors expressed interest**
  - **Optimizing MWNT dispersions**
  - **Biological testing**

- **Current work on ceria nanoparticles.**
  - **Characterizations**
  - **Interactions with mammalian cells**

Characterization, Cytotoxicity, and Uptake of SWNTs by Mammalian Cultured Cells

- **9 different SWNT samples from 5 vendors**
- **Dispersed all with a standard protocol that uses bovine serum albumin as the dispersant**
- **Detailed characterization of chemical and physical properties**
- **Of the 9 samples, only 2 were significantly toxic, and both were functionalized by carboxylation**
- **However, the toxic activity could be removed from the SWNT samples**
- **The toxic material co-purified with small fragments of amorphous carbon.**
- **SEE POSTER BY WANG ET AL. FOR FURTHER DETAILS**

MWNTs: Improving the Dispersant

- We have been using the protein bovine serum albumin (BSA)

- Dispersant should be:
  - Effective (aggregates may be toxic)
  - Biocompatible (not toxic itself)
  - Defined structure for modeling studies
  - Amenable to cleaning spills and instruments
  - Inexpensive

- Surveyed a number of dispersants to compare with BSA

- Focused on Pluronic F-127 block copolymer

Pluronic F-127

POLYOXYPROPYLENE-POLYOXYETHYLENE TRIBLOCK COPOLYMER

$$H(OCH_2CH_2)_x(OCH_2\underset{}{\overset{CH_3}{\overset{|}{C}}}H)_y(OCH_2CH_2)_zOH$$

x, z ~101; y ~56
Molecular Weight ~ 12,600

Oral LD50 = > 15,000 mg/kg (Rat)
Dermal LD50 = > 5,000 mg/kg (Rabbit)
FDA approved as a component of i.v. injections

- **BSA: ~$3,000/Kg**
- **Pluronic F-127: ~$120/Kg**

- **MWNT dimensions: Outer diameter ~ 30-50 nm, length ~ 500-2000 nm**
- **Two surface chemistries are being studied: pristine MWNTs and carboxylated (cMWNTs)**

Comparing BSA and Pluronic F-127 in dispersing MWNTs and cMWNTs

10 mg CNT (MWNT or cMWNT) + 10 mL Solution (H_2O, BSA, or Pluronic)

Sonication

Dispersion A (Not Centrifuged)

1:10 Dilution

24 h

Centrifugation

Dispersion B (Centrifuged)

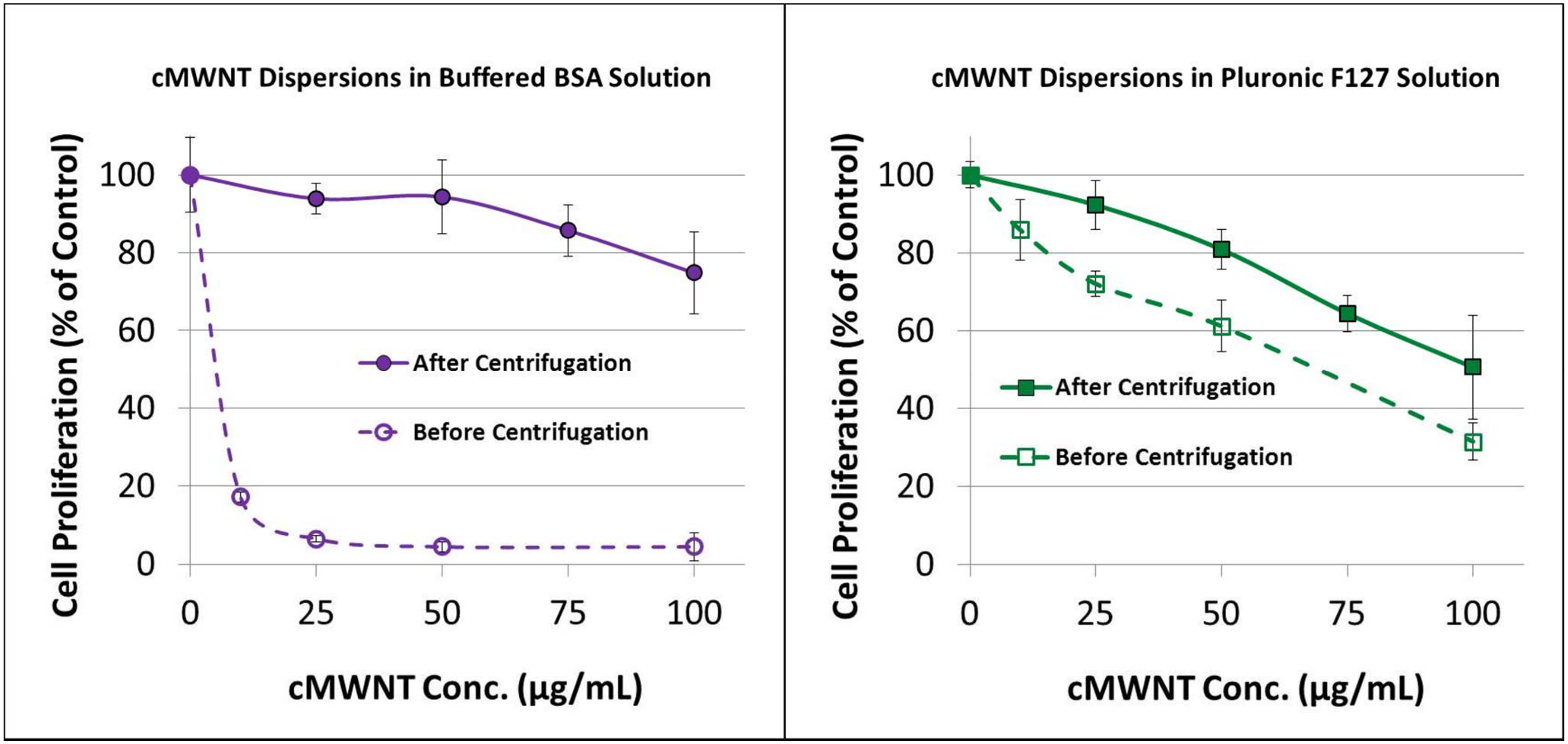
1:10 Dilution

24 h

CNT	MWNT			cMWNT		
Solution	H2O	BSA	Pluronic	H2O	BSA	Pluronic

Percent Dispersed = (B/A)*100%	NA	12%	68%	16%	22%	60%

Cytotoxicity of C-MWNT Dispersions in BSA and Pluronic F-127



In-Progress and Future Work with MWNT Dispersions

- Continue chemical and physical characterizations of MWNT dispersions
  - Metal content and surface functionalization
  - Particle analysis by dynamic light scattering and AFM
  - Correlate chemical & physical properties with theoretical studies

- Expand studies to include MWNTs from more vendors to verify properties
  - Identify products from different vendors that meet standards

- Cytotoxicity analyses
  - Understand what causes toxicity (correlation between toxicity and aggregation?)

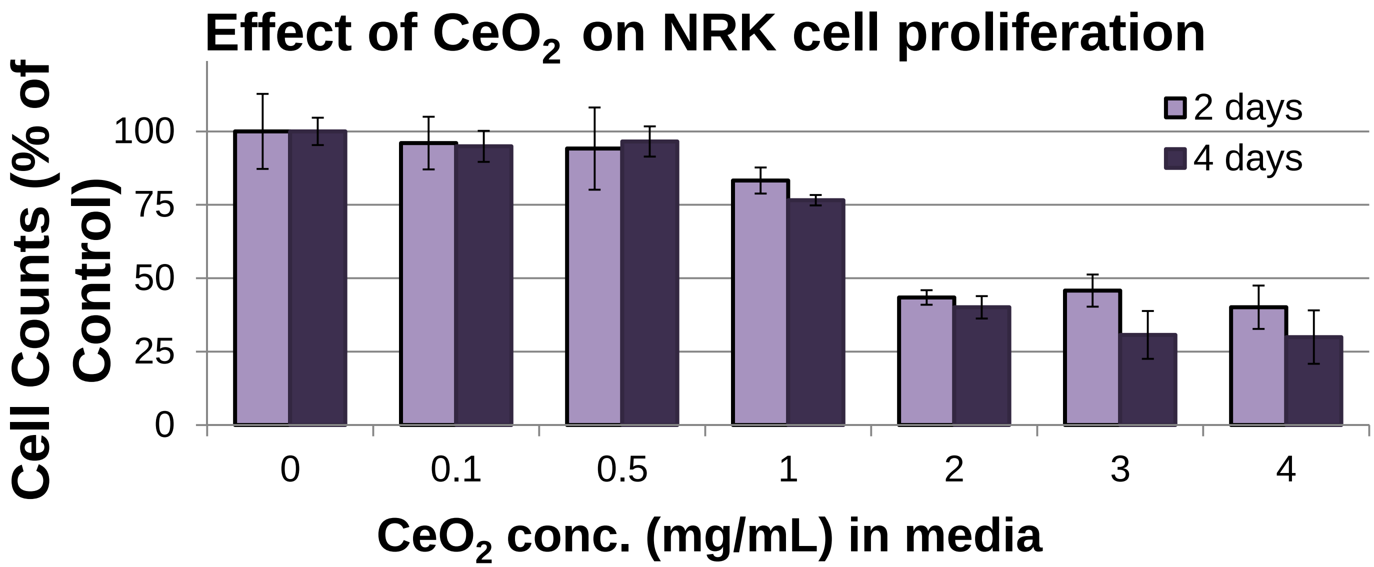
NOTE: The UTD group has leveraged our SRC experience to receive a National Institute of Environmental Health & Safety (NIH) phase I STTR grant for:

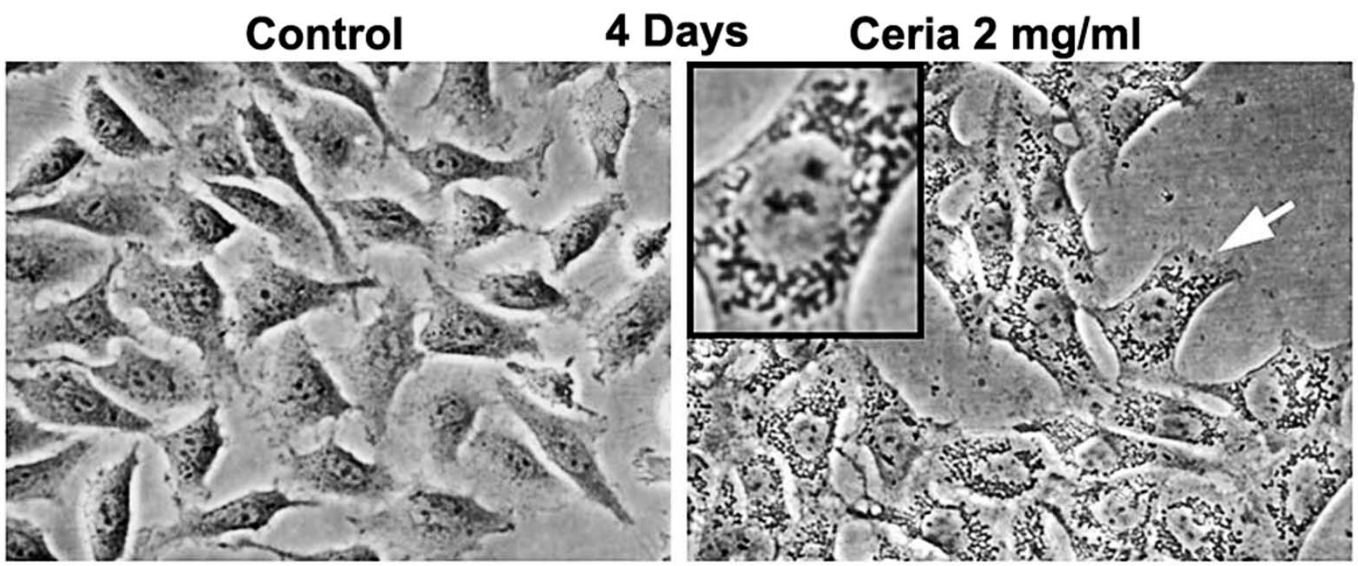
- Quality control verification of commercially purchased SWNT and MWNT material

- Cytotoxicity and biological tests to better understand and control potential toxicity

- In the future, workplace testing/decontamination procedures for SWNTs, MWNTs, and graphene oxide

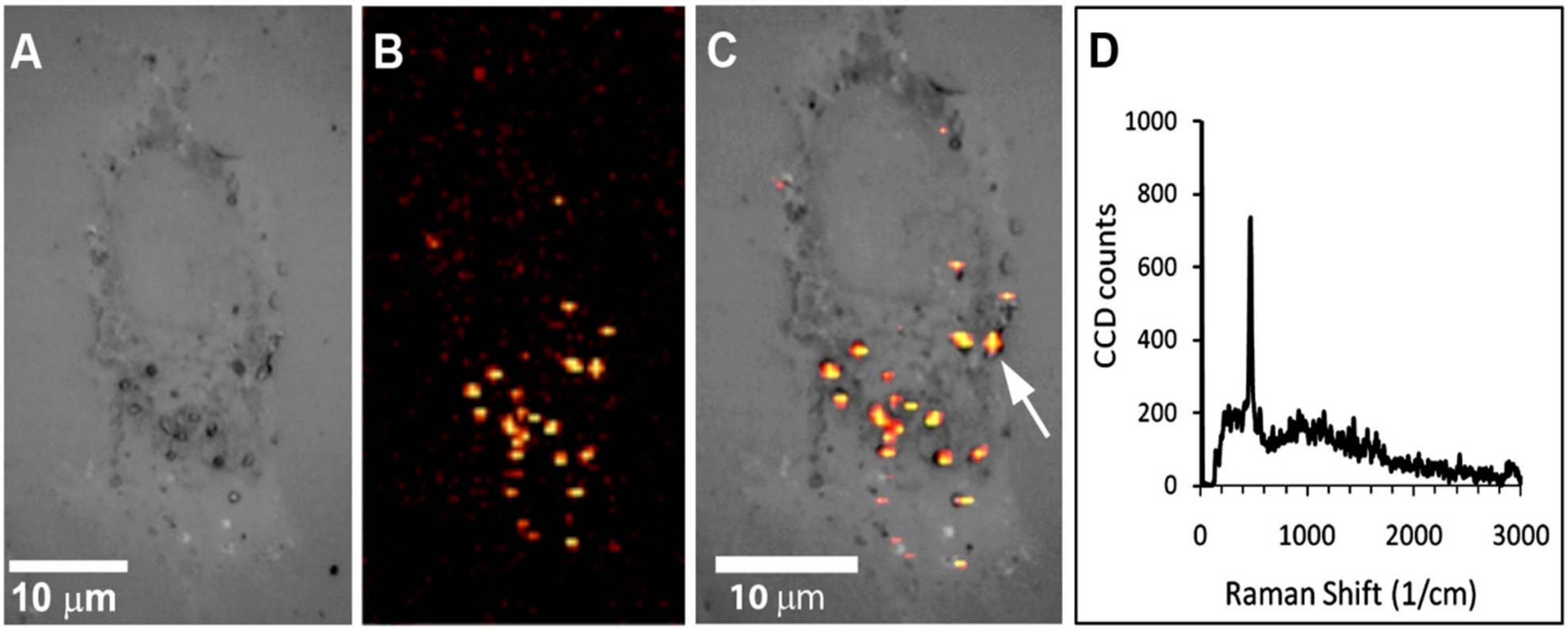
Ceria: Toxicity



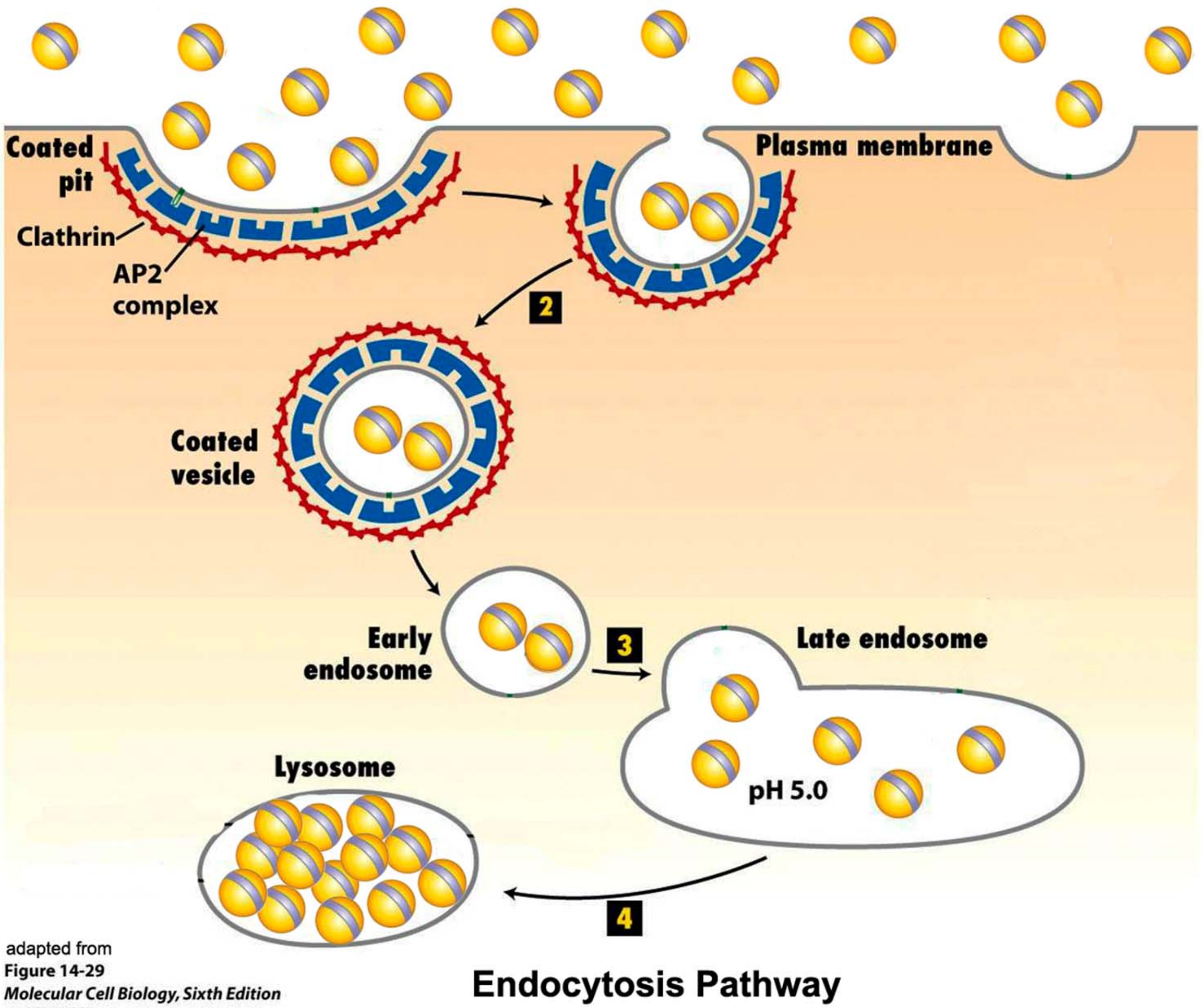


The Accumulation of Ceria Inside Cells

Laser Scanning Confocal Raman Microscopy



Endocytosis Pathway



adapted from
Figure 14-29
Molecular Cell Biology, Sixth Edition

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.
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.

Industrial Interactions and Technology Transfer

- **Leveraged core ERC funding to obtain Small Business Technology Transfer (STTR) grant from the NIH (Dan Herr, SRC wrote a key supporting letter).**
  - **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 P056398 C. Chiu, G.R. Dieckmann, P. Pantano , I. Musselman, R. Draper, S. Nielsen, “Computer Simulations of the Interaction between Carbon-Based Nanoparticles and Biological Membranes”, 11-Jun-2010.
- SRC Publication P055218 C. Chiu, R. DeVane, M. Klein, W. Shinoda, P. Moore, S. Nielsen, “Coarse Grained Potential Models for Phenyl Based Molecules: II. Application to Fullerenes”, J. Phys. Chem. B 114, 6394-6400 (2010).
- SRC Publication P058919 P. Pantano, N. Jacobsen, “Determining the Percentages of Semi-conducting and Metallic Single-walled Carbon Nanotubes in Bulk Soot”, Carbon (in press).
- 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 P056717 C. Chiu, “Molecular Dynamics Simulations of Nanoscale Interfaces: From Simple Liquids to Biological Systems”, Ph.D. Thesis (UT Dallas, 2010)
- 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.

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.**

Awards

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