Task Title:

Predicting, Testing, and Neutralizing Nanoparticle Toxicity (*Task Number: 425.027)*

PIs:

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- **Paul Pantano (co-PI), Chemistry and NanoTech, UTD**
- **Inga H. Musselman (co-PI), Chemistry and NanoTech, UTD**
- **Gregg R. Dieckmann (co-PI), Chemistry and NanoTech, UTD**

Graduate Students:

- **Chi-cheng Chiu: PhD candidate, Department of Chemistry, UTD (100% funded)**
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Undergraduate Students:

- **Laura Lockwood, Kyle Bruner, Nancy Jacobsen, and Prashant Raghavendran, UTD**

Other Researchers:

- **Ruhung Wang: Postdoctoral Fellow, Department of Molecular and Cell Biology, UTD**

Year 1 Cost Share (other than core ERC funding):

- **$45K from UTD Dean of Science for a zeta potential analyzer**
- **$25K from UTD Engineering School for RA support**
- **$20K from UTD Vice President of Research for supplies**

Year 1 Deliverables & Objectives

- **Obtain and validate data on the characterization, fate, and toxicity (tested in model mammalian cells) of carbon nanotube (CNT) nanoparticles.**

ESH Metrics and Impact

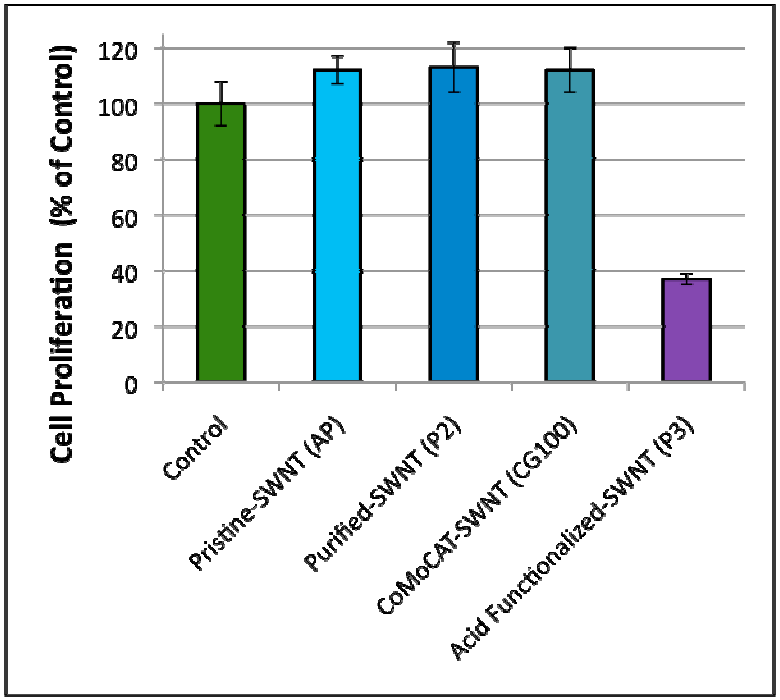
1. *Reduction in emission of ESH problematic material to environment:*
   - Reduce the toxic material associated with commercial preparations of a variety of CNT types to a level such that the final CNT material expresses very little toxicity to cells in a sensitive model cell culture system.
2. *Identification or prediction of inherent material ESH properties and any process by-products:*
   - Assess ESH properties of inherent CNTs and separable by-products.
   - Demonstrate that 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 for pure and oxidized CNTs:*
   - Conduct three day growth assays using cultured mammalian cells to compare any possible effects of CNTs on cell growth with untreated controls.
   - Conduct two-week plating efficiency experiments for a more stringent assay of toxicity.
   - Purified CNTs show little toxicity in either type of assay.
4. *Development of analytical tools to measure trace levels of CNT materials in process effluents:*
   - Detect as little as 5 micrograms of CNTs per milliliter of solution.
   - Develop methods to detect CNTs in process effluents, adhering to tools, or in the air.
   - Develop methods to detect graphene and graphene oxide.
5. *Development of molecular simulation parameters to enable nanoparticle-bio interaction studies:*
   - Develop mesoscopic models for C60 and CNT interactions with cellular membranes.

in vitro Cytotoxicity Comparison of Various SWNTs

Four different single-walled carbon nanotube (SWNT) samples were tested.

Pristine, purified, and CoMoCAT are various un-modified SWNTs from two vendors.

Acid functionalized SWNTs are carboxylated by the vendor to make them more water soluble.



Cultured NRK cells were incubated in media with various SWNT dispersions at the same concentration or in the absence of SWNTs for 3 days.

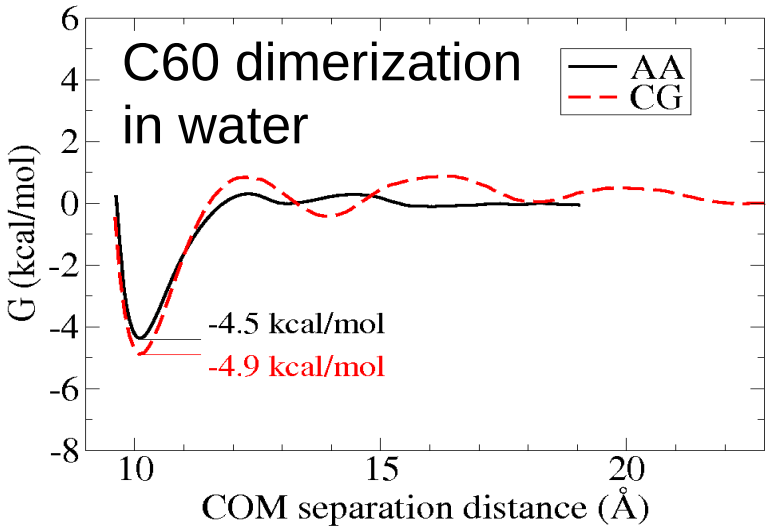
Cytotoxicity was determined by measuring the ability of the cells to proliferate, quantified by comparing the number of cells after 3 days in the presence or absence of the SWNTs.

Significant toxicity was detected only in cells treated with P3 material, carboxylated SWNTs.

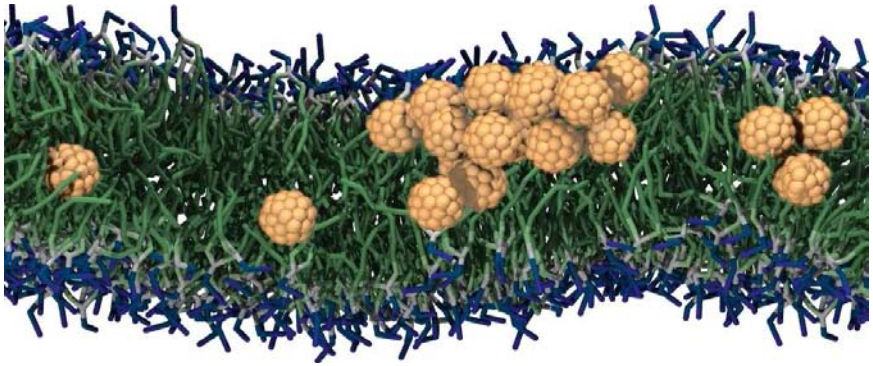
Only the more water-soluble carboxylated P3 SWNTs were cytotoxic. This is surprising given our molecular simulation data (next 4 slides). But the last 3 slides demonstrate that the toxic component could be separated from the SWNTs themselves.

Study the interaction between SWNTs and a cellular membrane

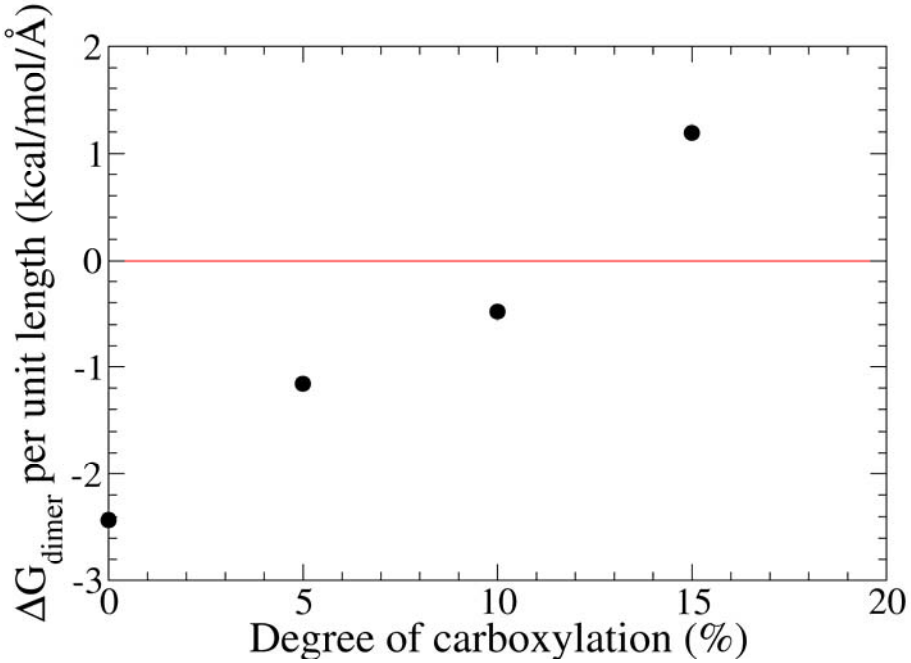
- To interact with living organisms, SWNTs may cross a membrane barrier.
- Molecular simulations can examine this nano-bio interaction.
- Coarse grain (CG) model needed due to required time and size scales.
- We have developed and validated a CG model for C60 and SWNTs: Chiu, C.-c., Nielsen, S. O. *et al*., submitted to J. Phys. Chem. B
- Validated against surface tension data, transfer free energy data, and aggregation behavior.

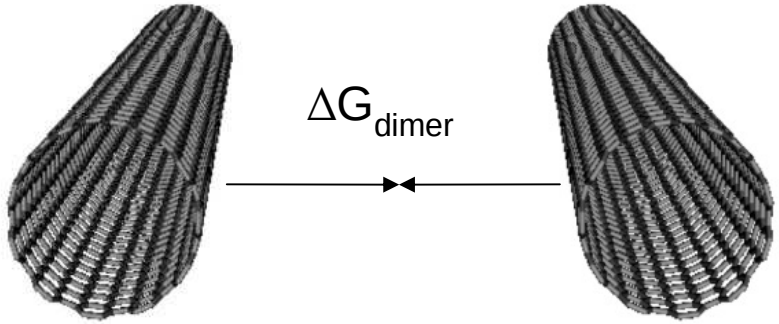


Li, L. *et al*. Physical Review E 2005, 71, 011502
Li, L. *et al.* J. Phys. Chem. B 2007, 111, 4067



Carboxylated (acid-functionalized) SWNTs: validation of molecular simulation results against experimental data

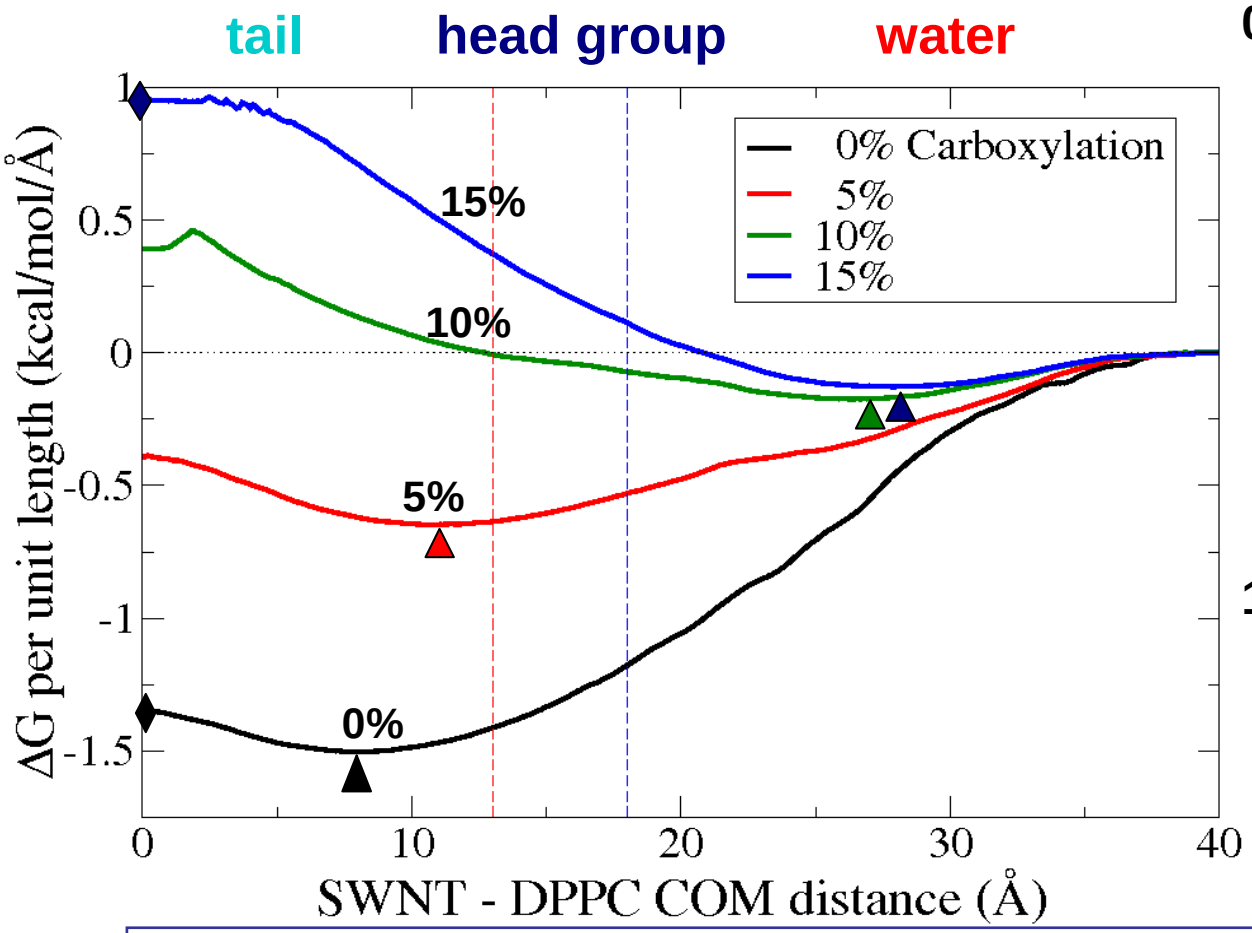


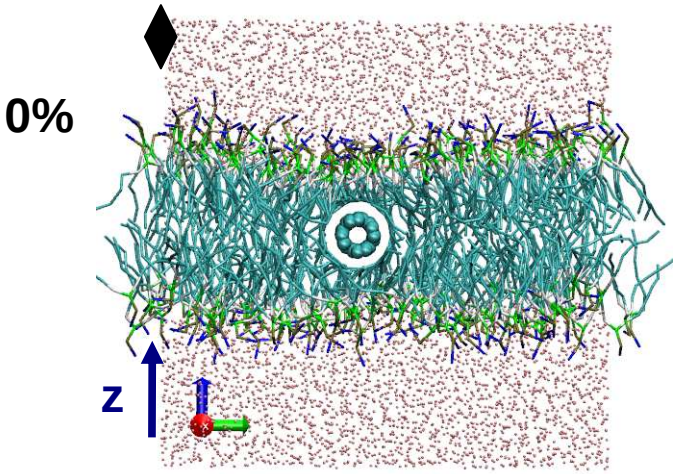


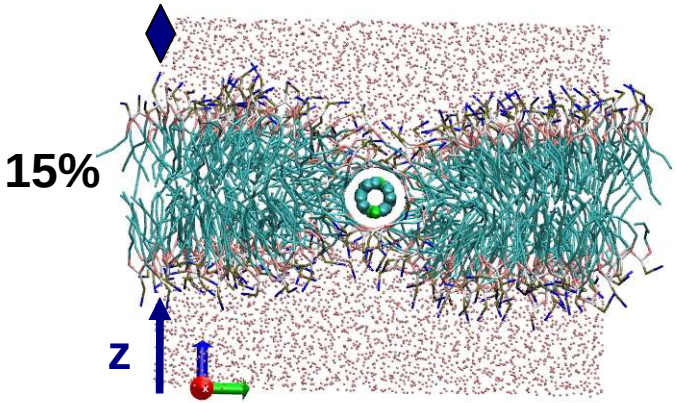
- SWNT dimerization free energy in water computed from molecular simulation as a function of SWNT % carboxylation.
- $\Delta G_{dimer} < 0$ indicates that SWNTs will bundle (aggregate) in water.
- $\Delta G_{dimer} > 0$ indicates that SWNTs will disperse individually in water.
- Crossover % carboxylation agrees with our experimental data: Bajaj, P. M.S. Thesis, The University of Texas at Dallas, Richardson, TX, 2008

SWNT/Lipid Bilayer Membrane

- Pristine SWNTs spontaneously diffuse into bilayer membrane.
- Above 10% carboxylation, it is no longer favorable for SWNTs to penetrate the bilayer membrane.



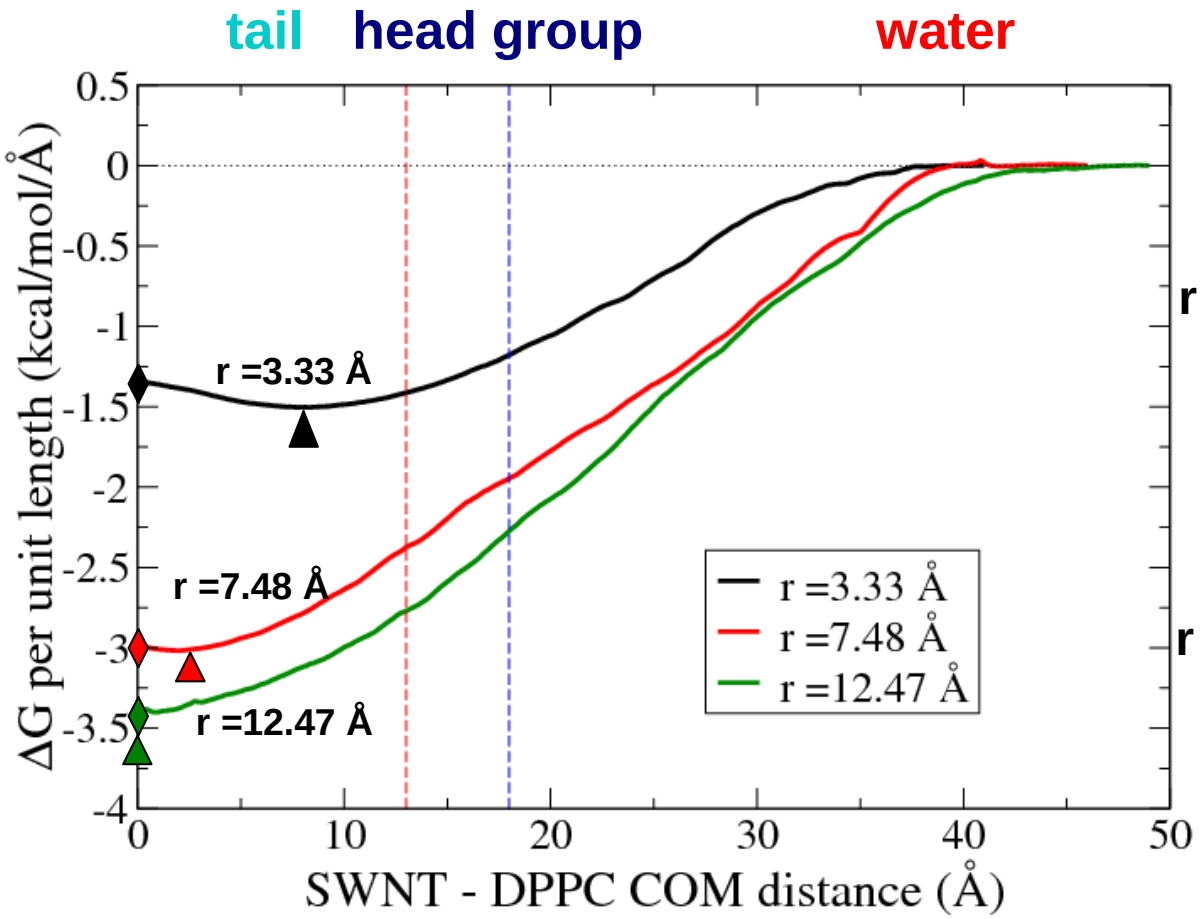


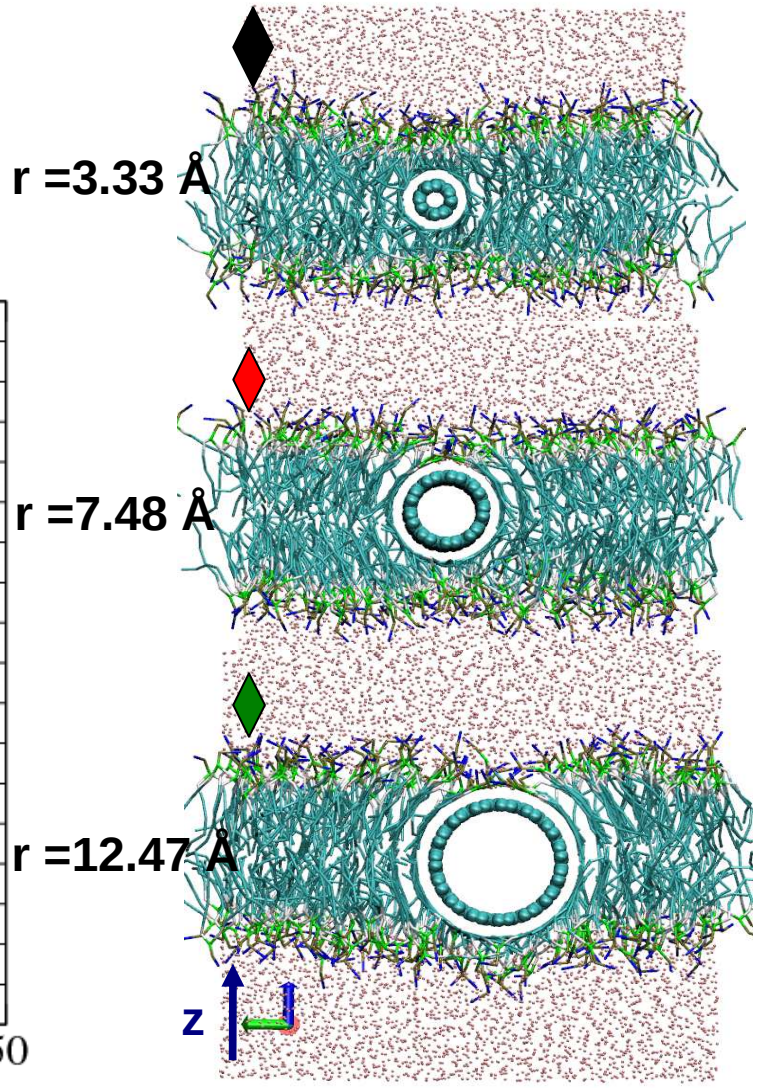


r =3.33 Å

SWNT/Lipid Bilayer Membrane

- Pristine SWNTs show expected diameter dependence on bilayer membrane interaction.





What we learn from comparing the experimental and simulation data

- Simulation studies predict that carboxylated SWNTs should not interact with cellular membranes, suggesting that they would not be cytotoxic.

- However, experimental data in slide 4 showed evidence of a cytotoxic effect.

- In the following 3 slides, we investigate whether the cytotoxic activity was inherent to carboxylated SWNTs, or due to a separable by-product.

Separation of the Toxic Components from P3 Carboxylated SWNTs

Step 1:

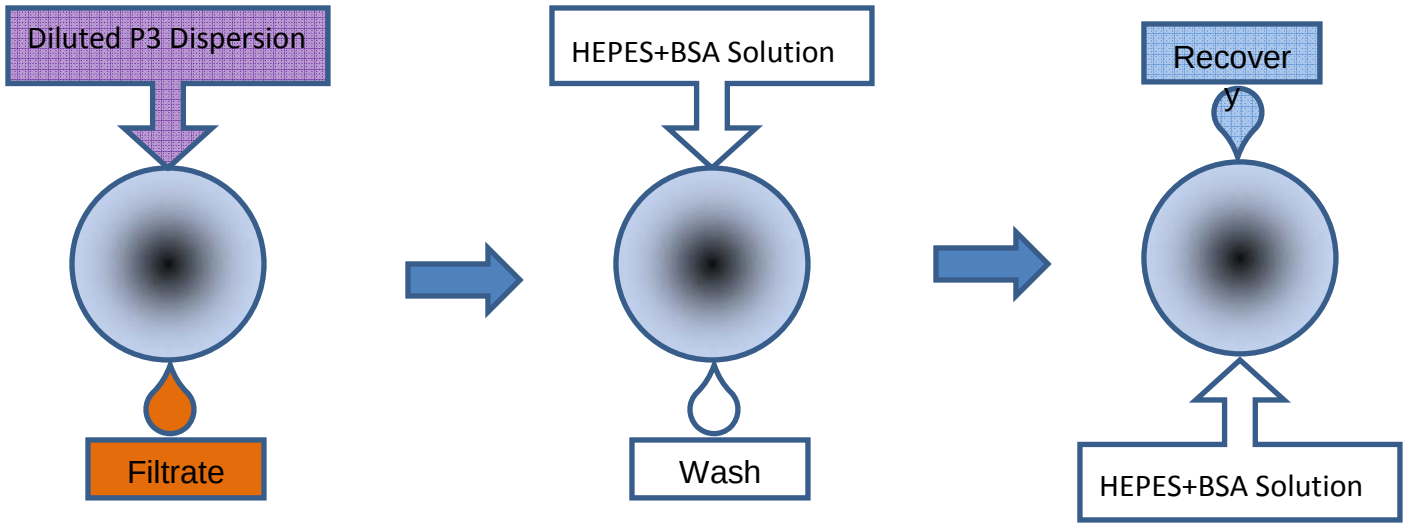
Filter 5 mL diluted P3 dispersion (in HEPES+BSA solution) through a 0.22 µm membrane and collect the Filtrate.

Step 2:

Pass 5 mL HEPES+BSA solution through the same membrane and collect the solution passed through.

Step 3:

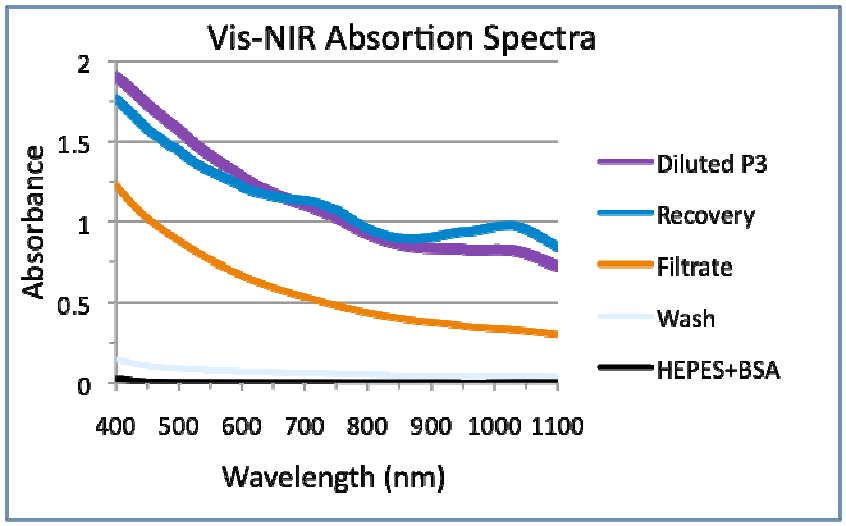
Extract material retained on the membrane with 2 mL HEPES+BSA solution, termed Recovery.

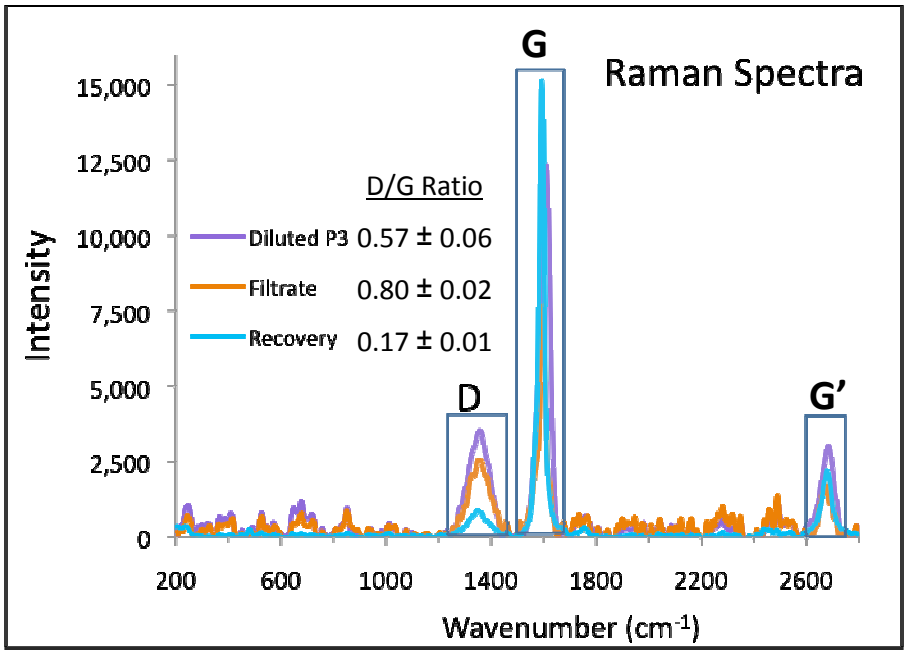


P3 dispersion and samples collected along the filtration process were tested for:

- **Presence of C-SWNTs and amorphous carbons – by Vis-NIR absorption spectroscopy and confocal Raman spectroscopy**
- **Cytotoxicity – by measuring cell proliferation over 3 days**

Majority of P3 Carboxylated SWNTs are Recovered





✓**The non-filtered P3 dispersion has two broad peaks, ~750 and ~1050 nm.**

✓**The Filtrate has relatively high background but no peaks.**

✓**The recovered material has two peaks of higher intensities.**

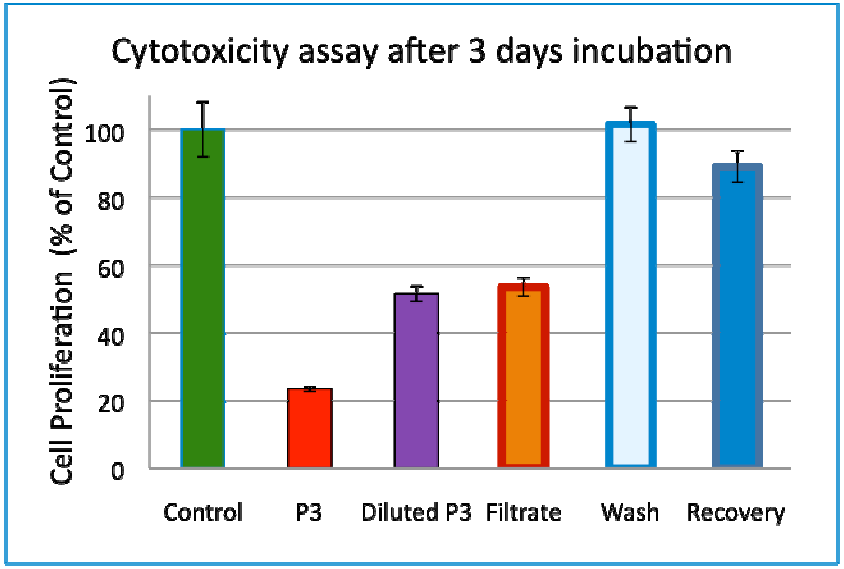
➢**The filtration membrane retains most SWNTs that can be recovered.**

The D/G ratio is proportional to the amorphous carbon content relative to intact SWNT content.

✓**The Filtrate has the highest D/G ratio indicating that it contains mostly amorphous carbon fragments.**

✓**The recovered material contains intact SWNTs as indicated by its low D/G ratio.**

Filter Purified P3 Carboxylated SWNTs are Not Cytotoxic



✓ **The Filtrate is as toxic as the diluted but not filtered P3 dispersion.**

✓**The recovered material shows almost no toxicity.**

➢**The filtration process efficiently removes toxic component from the P3 carboxylated SWNT dispersion.**

Conclusions:

➢ **Commercially purchased carboxylated SWNTs appear to contain carboxylated amorphous carbon fragments.**

➢ **These impurities are toxic but can be removed by filtration.**

➢ **The carboxylated SWNTs recovered are not toxic, in agreement with predictions of simulation studies, and can be used for various applications with reduced cytotoxic ESH complications.**

Industrial Interactions and Technology Transfer

- **Industrial interaction with Marc Heyns, IMEC.**
  - **Analysis of pads used for CMP of CNT's.**
- **Industrial interaction with Don Hooper, Intel.**
  - **ESH of CMP slurries.**
- **Industrial interaction with Mario Bolanos-Avila, TI.**
  - **ESH of packaging.**

Future Plans

Next Year Plans

- **Obtain data on physical and chemical characteristics of CNT and CMP nanoparticles with an initial attempt to correlate with structural modeling, interaction with model mammalian cells, toxicity, and bioactivity. (Deliverables Report, 2011)**
- **Acid-functionalize CNTs using our nitric acid reflux protocol to assess CNT toxicity vs. percent carboxylation.**
- **Preliminary ESH investigation of graphene oxide.**

Long-Term Plans

- **Obtain data on physical and chemical characteristics of CNT and CMP nanoparticles correlated with structural modeling, interaction with model mammalian cells, toxicity, and bioactivity. (Deliverables Final Report, 2012)**
- **Obtain ESH data on graphene and graphene oxide.**
- **ESH of packaging. (Mario Bolanos-Avila, TI: Strategic Packaging Research Manager)**

Presentations

- **ERC Teleseminar, April 16, 2009: "Challenges in Assessing the Potential Cytotoxicity of Carbon Nanotubes" by P. Pantano**
- **ERC Teleseminar, Nov. 12, 2009: "Computer Simulations of the Interaction between Carbon Based Nanoparticles and Biological Systems" by C.-c. Chiu**
- **AIST (Japan) seminar, 2009: "Nanoparticles from a Soft Matter Viewpoint" by S. Nielsen**
- **UT Arlington and TCU seminars, 2009: "Accurately Assessing the Potential Toxicity of Carbon Nanotubes and the Use of Carbon Nanotubes as Cancer Theranostic Agents" by P. Pantano**
- **UTD Institute for Innovation & Entrepreneurship: Conference on "Nanomedicine: Enterprise and Society", Jan 22, 2010**

Publications

- **Draper, R.K.; Wang, R.; Mikoryak, C.; Chen, E.; Li, S. and Pantano, P. "Gel electrophoresis method to measure the concentration of single-walled carbon nanotubes extracted from biological tissue". Anal. Chem. 81: 2944-2952 (2009).**
- **Katari, S.C.; Wallack, M.; Hubenscmidt, M.; Pantano, P. and Garner, H.R. "Fabrication and evaluation of a near-infrared hyperspectral imaging system", J. Microscopy 236: 11-17 (2009).**
- **Marches, R.; Chakravarty, P.; Bajaj, P.; Musselman, I.H.; Pantano, P.; Draper, R.K. and Vitetta, E.S. "Specific thermal ablation of tumor cells using a monoclonal antibody covalently coupled to a single-walled carbon nanotube", Int. J. Cancer 125: 2970-2977 (2009).**

Awards

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