Dispersion, Bioaccumulation, and Mechanisms of Nanoparticle Toxicity

University of Texas at Dallas   Task Number: 425.042

PIs:

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- **Inga H. Musselman (co-PI), Dept. Chem. & Biochem.**
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- **Sarah Karimi, Blake Wilson, Elizabeth Braun, Dept. Chem. & Biochem.**

Undergraduate Students:

- **D. Deutsch, M. Lee, L. Miadzvedskaya, M. Chopra, A. Huang, C. Tusa**

Other Researchers:

- **Dr. Ruhung Wang, Senior Scientist, Dept. Chem. & Biochem. and Dept. Biol. Sci.**
- **Dr. Carole Mikoryak, Senior Scientist, Dept. Biol. Sci.**

Year 3 Cost Share (other than core ERC funding):

- **$11,700**

Objectives

- **Computational modeling and experimental validation of correlations between nanoparticle size, cellular uptake, toxicity, and dispersant effectiveness.**

- **Completing work on multi-walled carbon nanotubes (MWNTs), especially their accumulation and effects on zebrafish embryos.**

- **Main focus to characterize the chemical and physical properties of model CMP slurry nanoparticles and assess their biological effects.**

ESH Metrics and Impact

1. **Reduction in the use or replacement of ESH-problematic materials**
   - **Identification of problematic materials requires accurate toxicity tests.**

   - **Pristine and carboxylated multi-walled carbon nanotubes (MWNTs) and single-walled carbon nanotubes (SWNTs) suspended in Pluronic® F-108 surfactant were not very toxic to zebrafish embryos up to 200 µg/mL, even though it was demonstrated that the MWNTs and SWNTs were accumulated in a dose-dependent fashion by the embryos.**

   - **The toxicity of acidic silica, fumed silica, ceria, and alumina was assessed with human lung adenocarcinoma A549 cells and with RAW 264.7 macrophage cells after exposures of 1-3 days. None of the metal oxide slurries were very toxic to A549 cells. In contrast, both acidic and fumed silica were much more toxic to macrophages, with IC-50 values of 17 $\pm$ 4 µg/mL and 7.7 $\pm$ 1 µg/mL, respectively. Ceria and alumina were not very toxic to macrophages.**

2. **Reduction in emission of ESH-problematic material to environment**

3. **Reduction in the use of natural resources (water and energy)**

4. **Reduction in the use of chemicals**

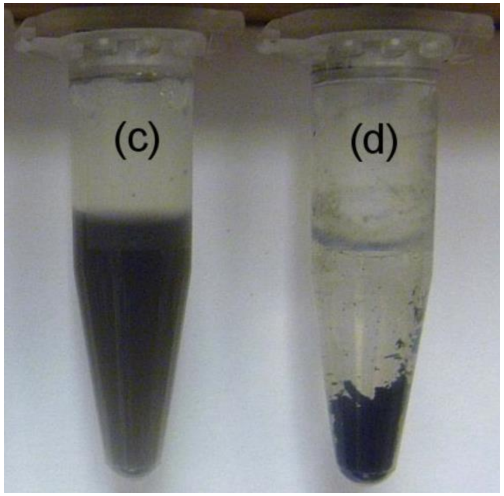
Overview

1. **Modeling of MWNT interactions with Pluronic® surfactants**
   - ➢Evaluation of thermodynamic stability of a wide range of Pluronics® interacting with pristine and carboxylated SWNTs and MWNTs.

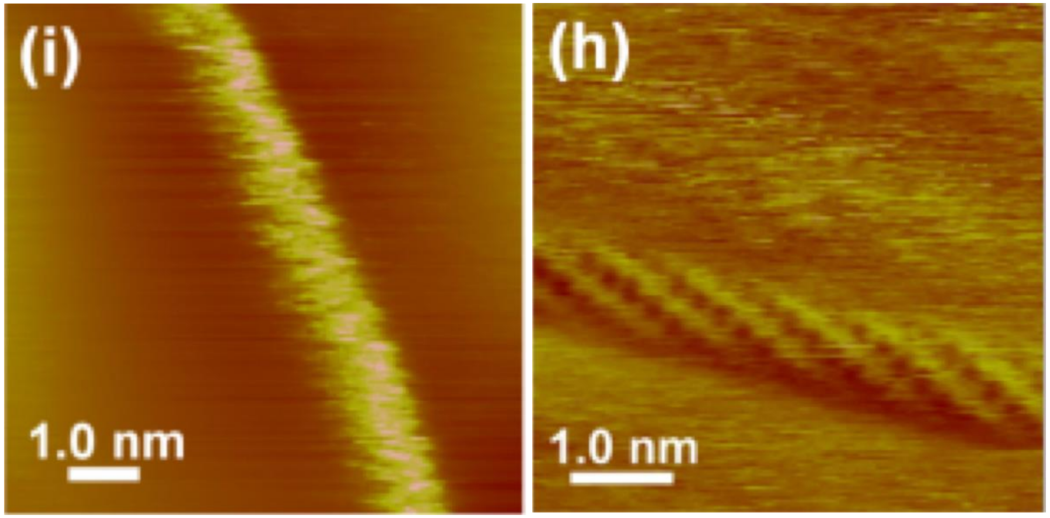
2. **Toxicity and bioaccumulation of CMP nanoparticles, acidic silica, fumed silica, ceria and alumina**
   - ➢Effect of CMP slurries on proliferation of A549 human lung adenocarcinoma cells and mouse RAW 264.7 macrophages.
   - ➢Label-free imaging of the bioaccumulation and subcellular distribution of nanoparticles by the cells.

3. **Toxicity and bioaccumulation of multi-walled carbon nanotubes (MWNTs) by zebrafish embryos**
   - ➢Preparation of MWNT suspensions by sonication with Pluronic® F-108 surfactant while controlling for toxic sonolysis products of the surfactant.
   - ➢Effects of MWNTs on embryo viability and development.
   - ➢Bioaccumulation of MWNTs by zebrafish embryos.

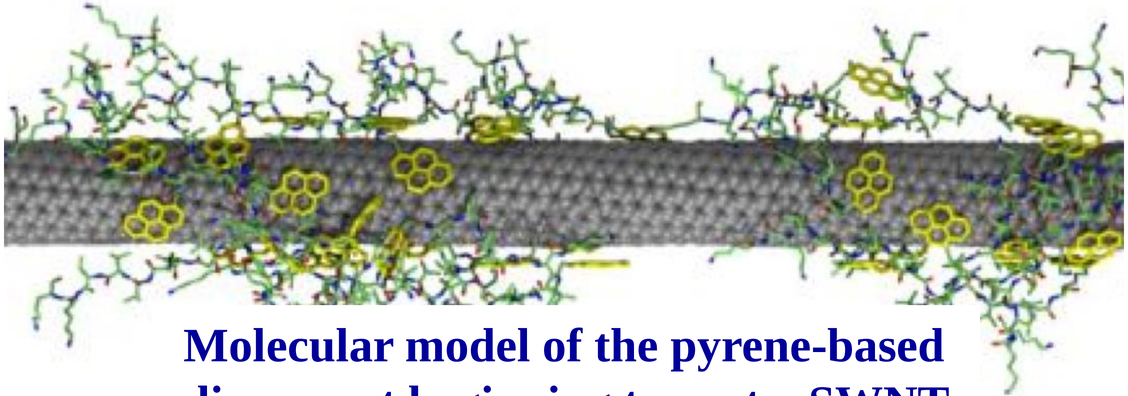
SWNT Dispersant Effectiveness



50,000 x g centrifugation of SWNT in water with (c) and without (d) dispersant.



Scanning tunneling microscope images of a dispersant-coated SWNT (i) and a bare SWNT (h).



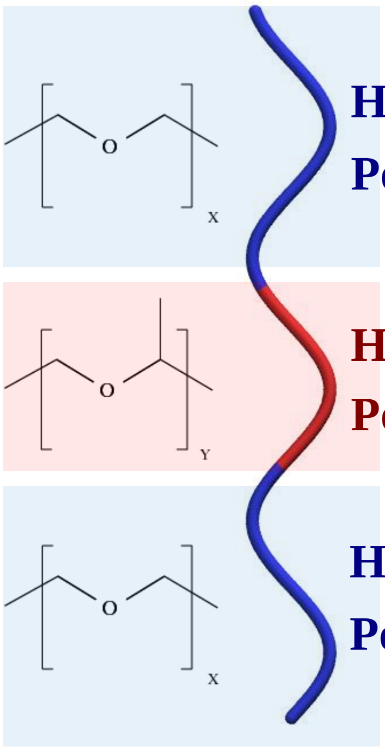
Molecular model of the pyrene-based dispersant beginning to coat a SWNT.

D.R. Samarajeewa, G.R. Dieckmann, S.O. Nielsen, I.H. Musselman, Carbon 57, 88-98 (2013).

Modeling Carbon Nanotube – Pluronic® Suspensions

Successfully developed two models for Pluronics®

- **coarse grained molecular model**
- **thermodynamic model**



Hydrophilic
Polyethylene Oxide (PEO)

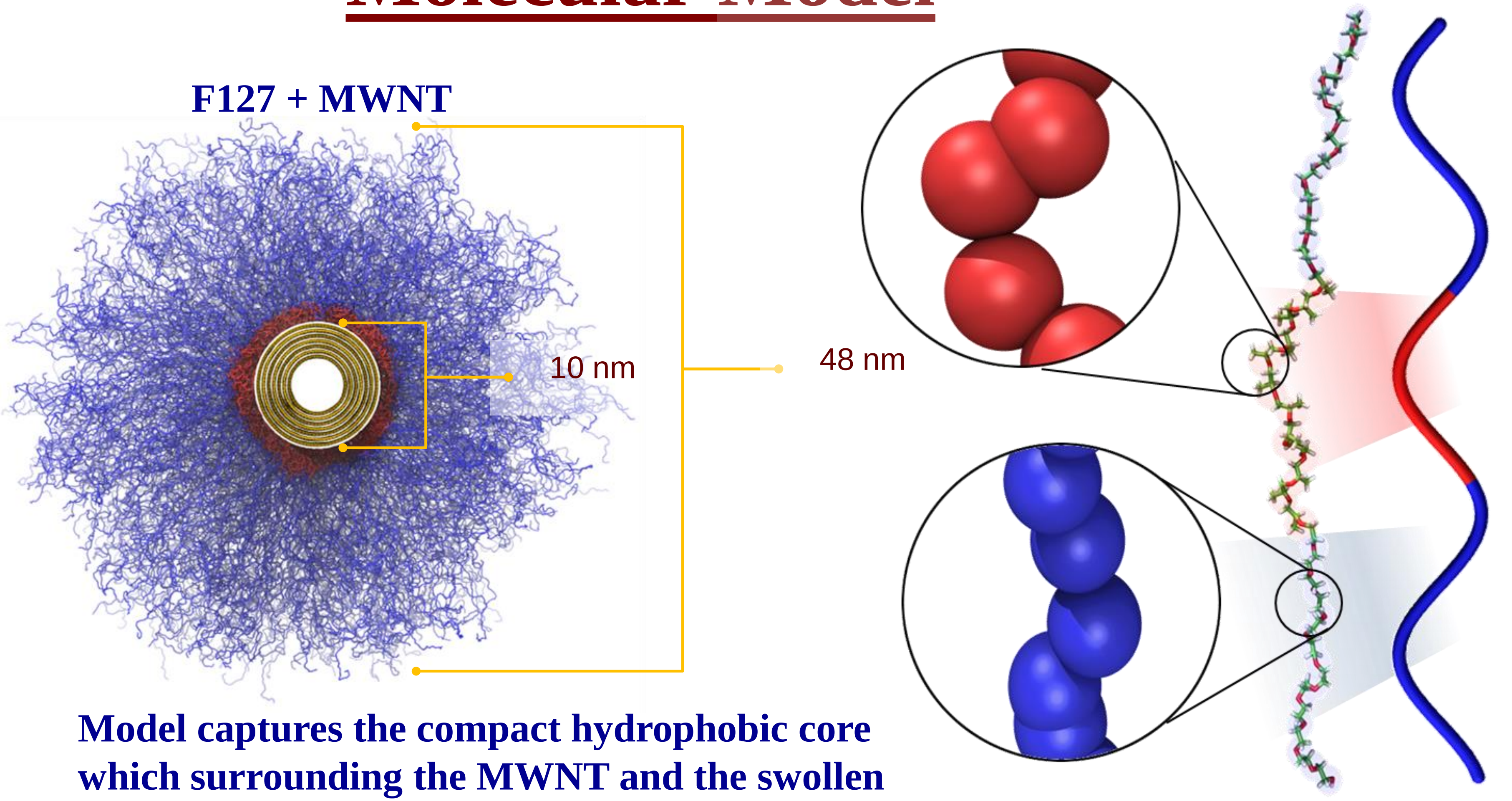
Hydrophobic
Polypropylene Oxide (PPO)

Hydrophilic
Polyethylene Oxide (PEO)

Pluronic® F-108:
PEO blocks (x): 133
PPO blocks (y): 50
MW: 12700 to 17400

Pluronic® Coarse Grained Molecular Model

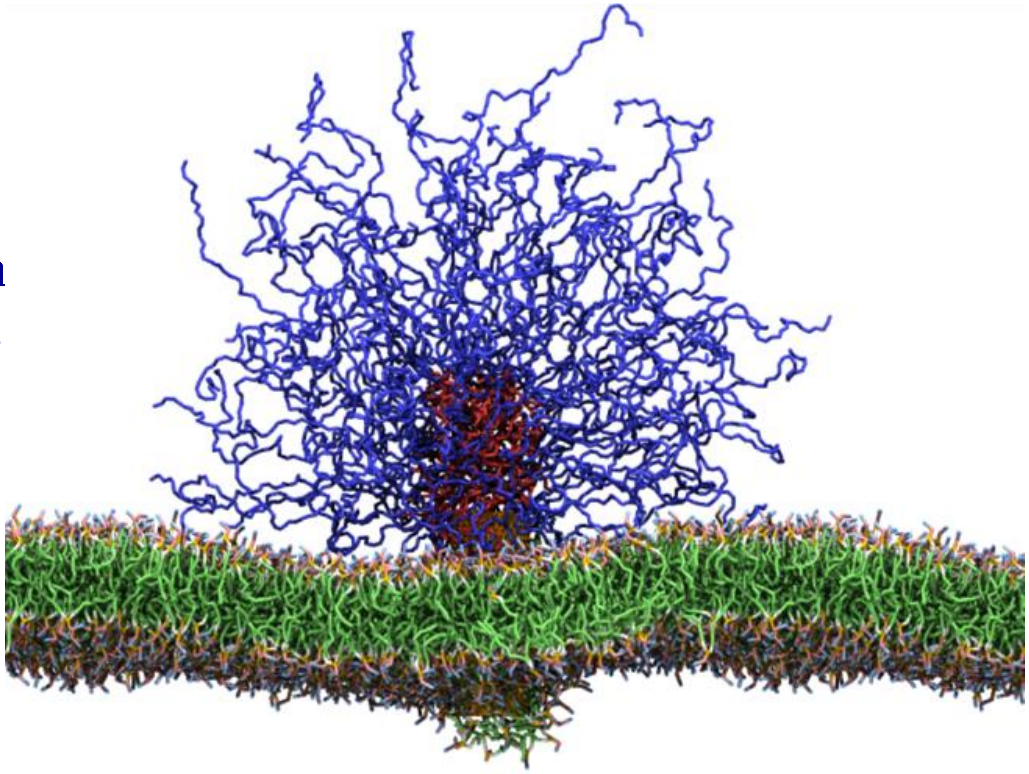
F127 + MWNT



Model captures the compact hydrophobic core which surrounding the MWNT and the swollen hydrophilic corona which provides steric stability

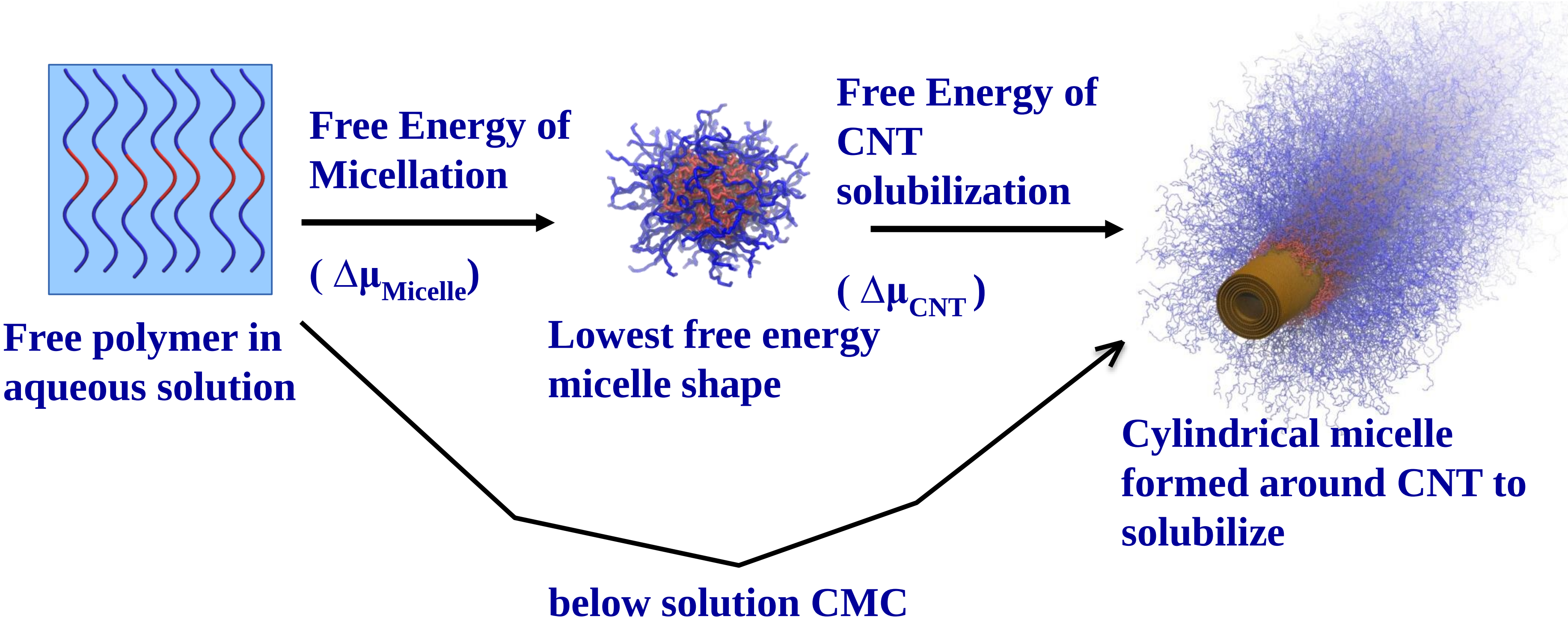
Pluronic® Coarse Grained Molecular Model

Model can also examine MWNT-Pluronic® interaction with lipid bilayer membranes



F68 + MWNT + lipid bilayer

Pluronic® Thermodynamic Model

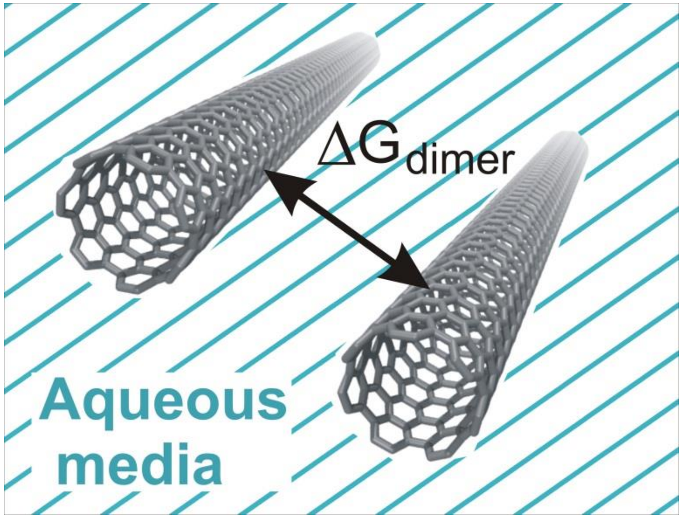


- A wide variety of Pluronics® and CNT diameters were examined
- Can tune model parameters to model oxidized CNTs

Carboxylated (acid-functionalized) SWNTs: Validation of Molecular Simulation Results Against Experimental Data

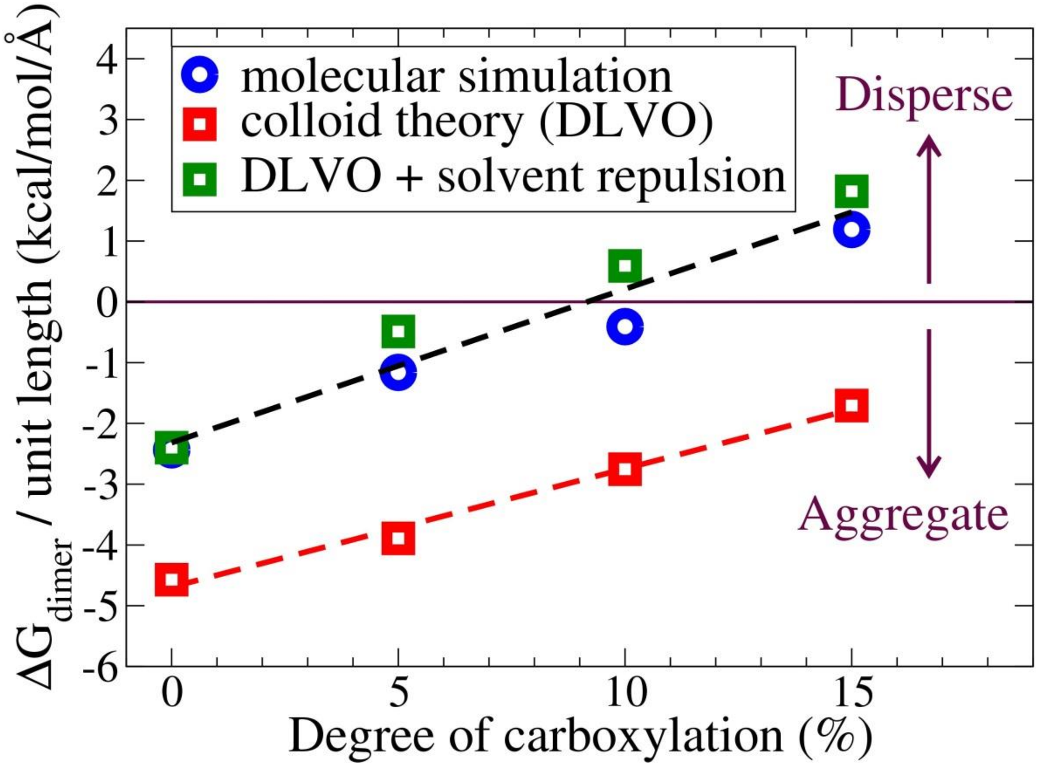
Experimental Data: I.H. Musselman's Lab

- **SWNTs were dispersed via sonication in an aqueous solution of Triton X-100 and then oxidized by nitric acid reflux.**
- **Aliquots of SWNTs were removed following reflux times ranging from 30 min to 48 h and the extent of carboxylation was quantified by XPS.**
- **SWNTs with more than 12% carboxylation are mostly debundled and dispersed in aqueous solvent**



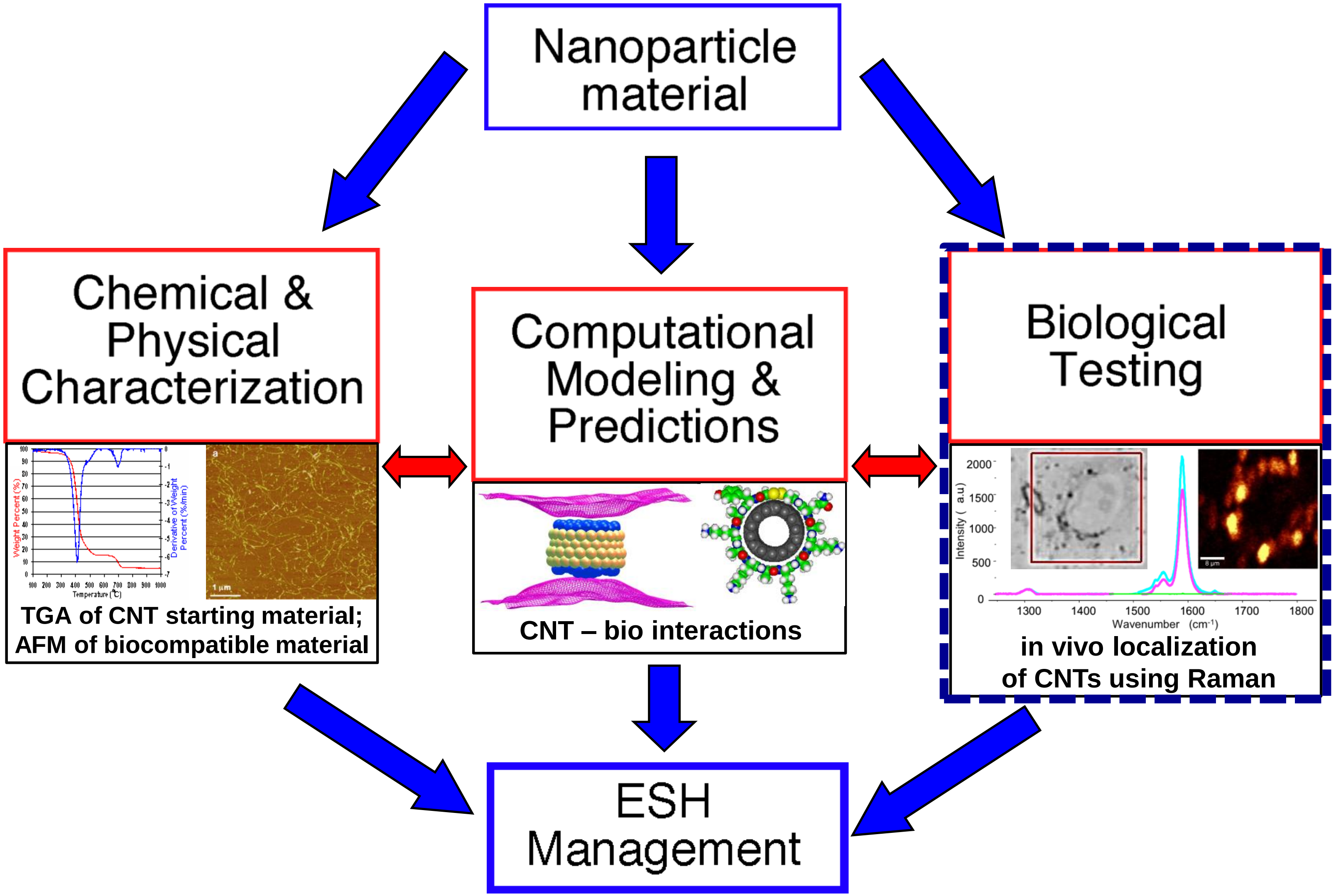
Carboxylated (acid-functionalized) SWNTs: Validation of Molecular Simulation Results Against Experimental Data

- **Colloid theory fails for carbon nanotubes because of their anomalously strong dispersion interaction with water**
- **Adding this term brings colloid theory into agreement with our molecular simulation data**
- **Crossover % carboxylation agrees with experimental data from I.H. Musselman's Lab**



C. Chiu, R.H. DeVane, M.L. Klein, W. Shinoda, P.B. Moore, S.O. Nielsen; J. Phys. Chem. C 116, 23102-23106 (2012).

Biological Testing



SRC Engineering Research Center for Environmentally Benign Semiconductor Manufacturing

Embryonic Zebrafish & Nanotoxicity

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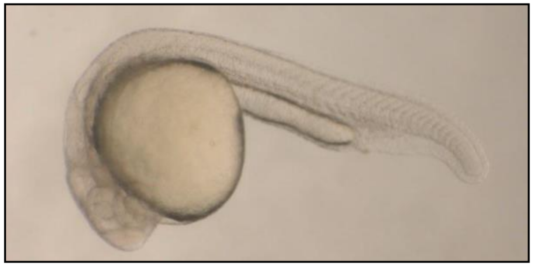
Dept. of Environmental and Molecular Toxicology, Oregon State University

Ruhung Wang, Paul Pantano, Rockford Draper

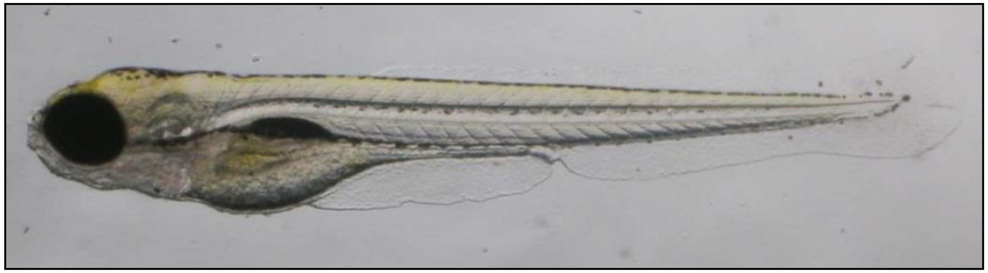
Depts. of Chem. & Biochem. and Biological Sciences, University of Texas at Dallas

Zebrafish (*Danio rerio*)



Zebrafish embryo 24 hours post fertilization (hpf)

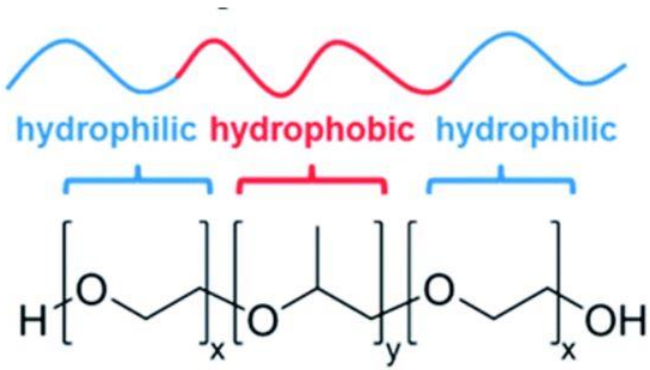
Evaluations: mortality, movement, development, etc.



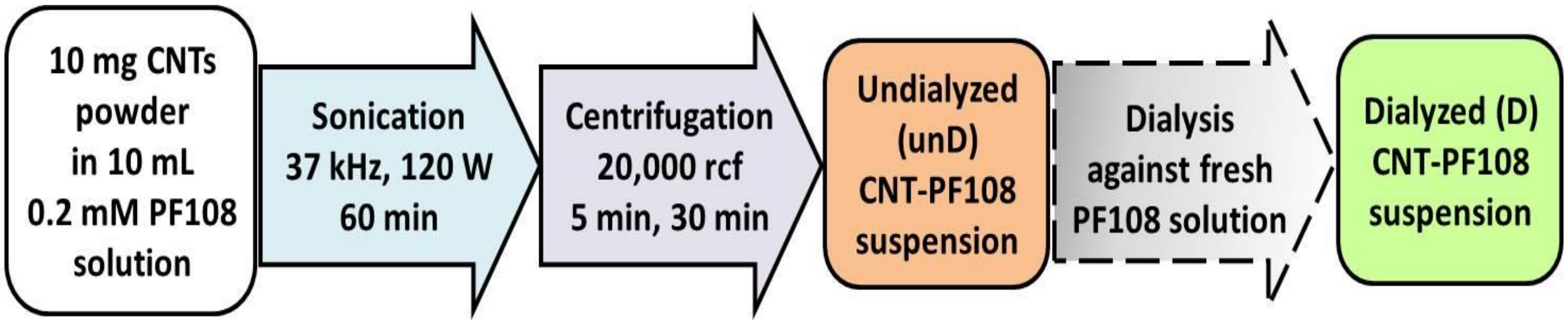
Zebrafish embryo 120 hpf

Evaluations: mortality, eye, snout, brain, fins, bladder, motility, etc.

Preparation of Carbon Nanotubes (CNTs) suspended in Pluronic® F-108 Surfactant



Pluronic® F-108:
PEO blocks (x): 133
PPO blocks (y): 50
MW: 12700 to 17400

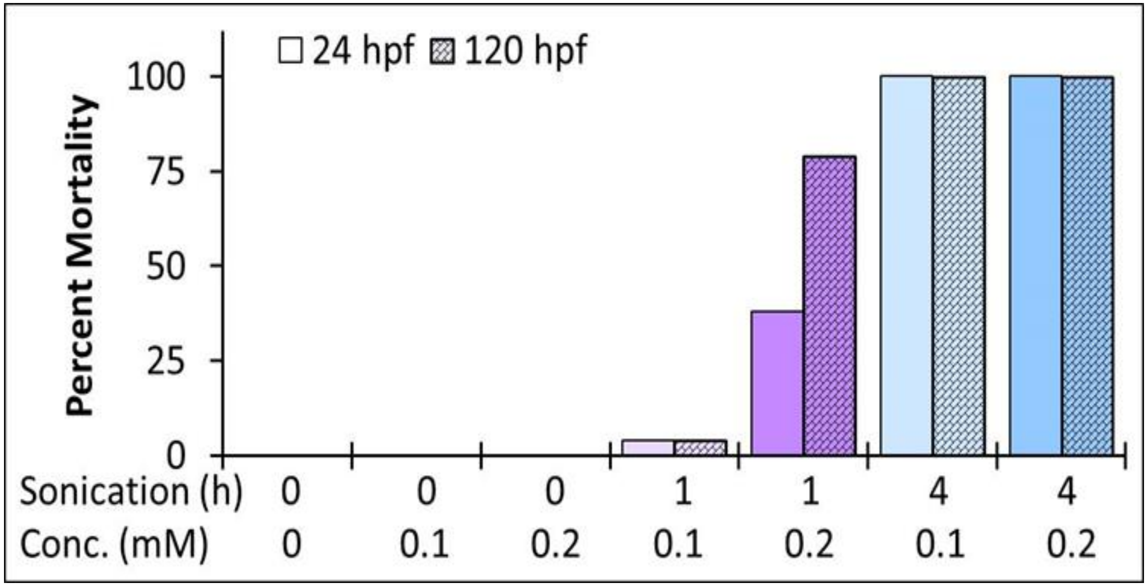


Problem: Sonication degrades Pluronics®. We previously noted that the degradation products are toxic to mammalian cells (Wang, R. et al., 2013. Nanotoxicology 7, 1272-1281). Degraded Pluronic® fragments are removed by the dialysis step.

Question: Is sonicated Pluronic® toxic to zebrafish?

Effect of Sonicated Pluronic® F-108 (in the absence of CNTs) on Embryos

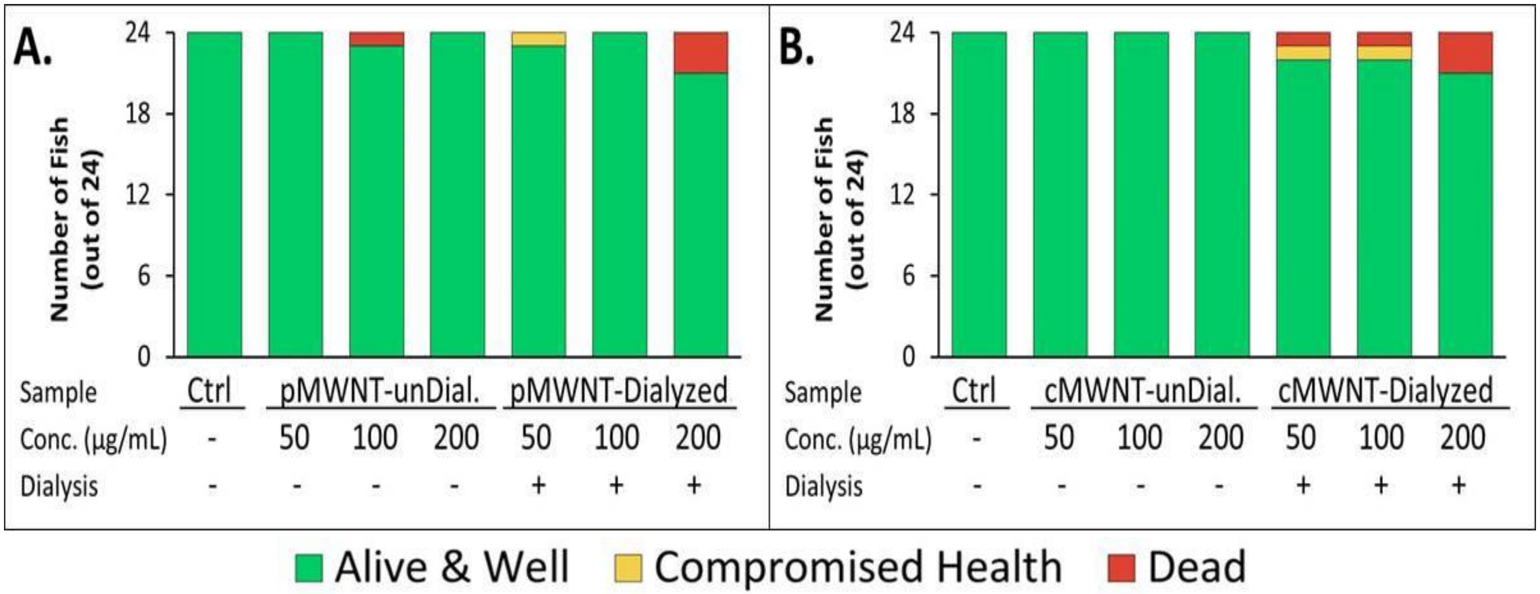
Method: **Sonicate 0. 2 mM Pluronic® F-108 for 0, 1, or 4 h (37 kHz, 120W) and add to embryos at 8 hpf. Evaluate embryos at 24 and 120 hpf.**



Conclusion: **There is minimal effect on embryos after 1 h of sonication at 0.1 mM Pluronic® F-108, but there is significant mortality at higher concentrations and longer sonication times. Avoid sonication times over 1 h and exposure at Pluronic® concentrations above 0.1 mM. Also, use dialyzed MWNT suspensions to ensure the absence of toxic Pluronic® sonolysis products.**

Effect of MWNT Suspensions on Embryos

Method: **Expose embryos at 8 hpf to different concentrations of dialyzed or undialyzed MWNTs suspensions (0.1 mM Pluronic® F-108) and evaluate embryos for multiple traits at 120 hpf.**



What the embryos experience:

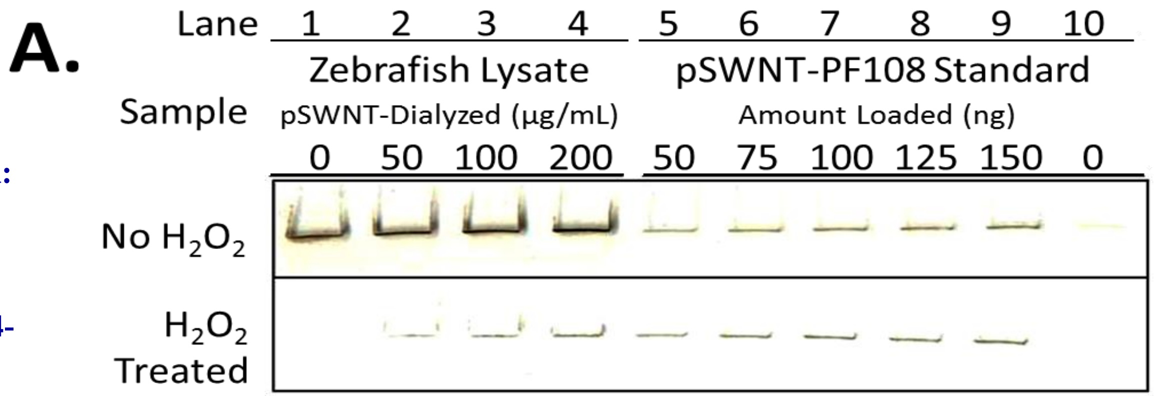
No MWNTs **200 µg/mL MWNTs**

Conclusion: **There is minimal effect of MWNTs on embryo development even at high concentrations (200 µg/mL).**

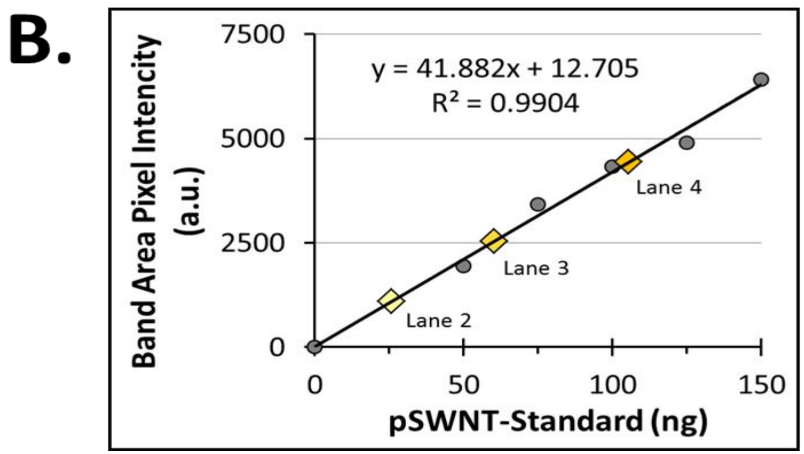
Question: **Do the embryos even take up the MWNTs? We developed a method to answer this question.**

SDS-PAGE for Quantifying CNTs in Embryo Extracts

Method: Lyse embryos with SDS to extract CNTs, run SDS-PAGE (along with standards), treat gel with H_2O_2 to bleach fish pigments, quantify CNTs by optical flat-bed scanner.



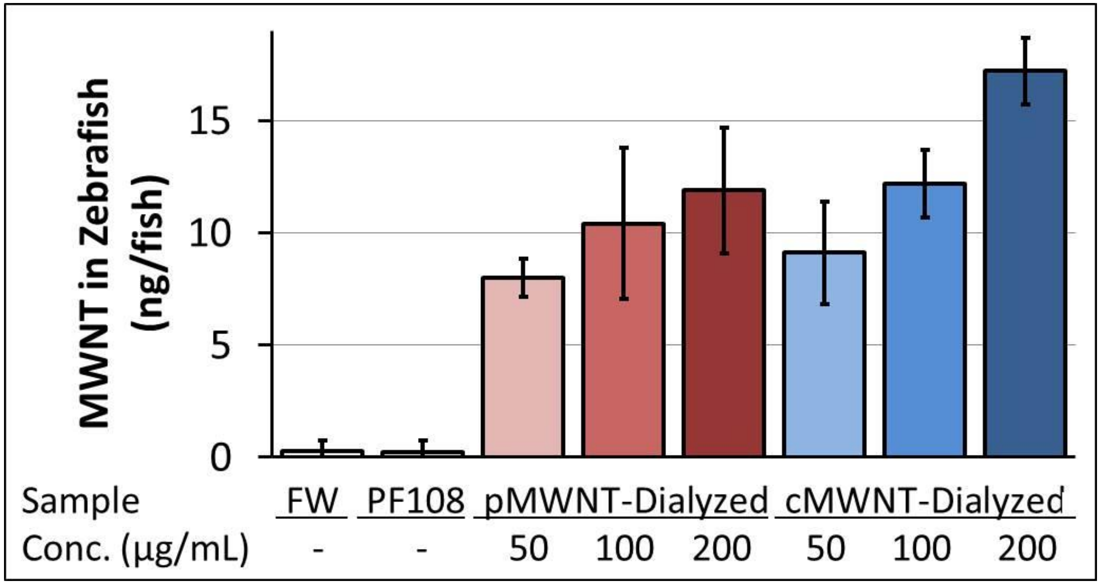
Based on a method we previously published: Wang, R. et al., (2009) Gel electrophoresis method to measure the concentration of single-walled carbon nanotubes extracted from biological tissue. Anal. Chem. 81, 2944-2952.



Conclusion: CNTs can be extracted from embryos and quantified by this method. Results with MWNTs on next slide.

Accumulation of MWNTs in Embryos

Method: **Lyse embryos to extract MWNTs and run SDS-PAGE gels along with known MWNT standards. MWNTs accumulate at gel interface. Quantitate MWNTs with an optical flat-bed scanner.**

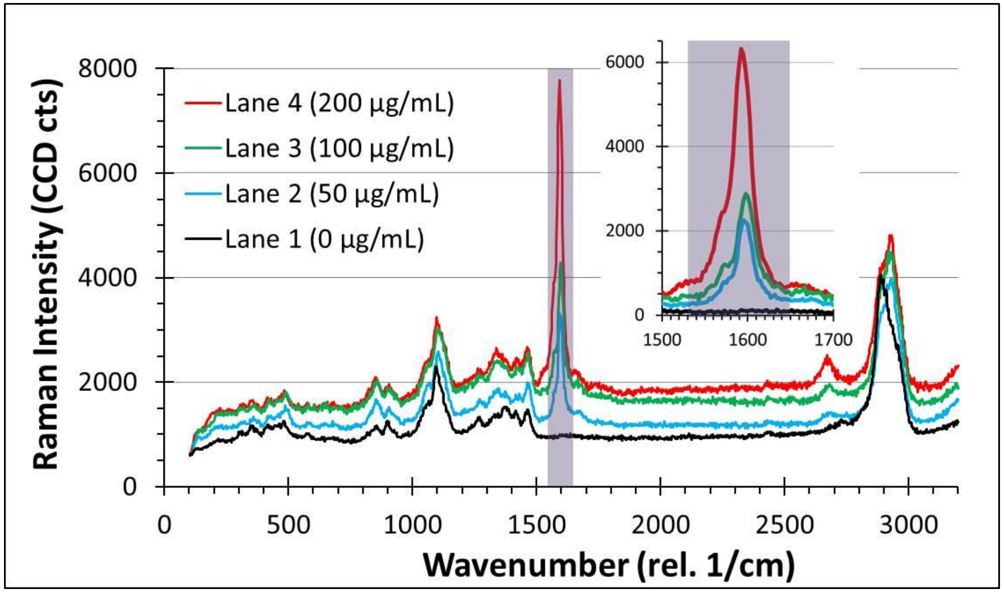


Conclusion: **Embryo-associated MWNTs increase as function of dose (although we do not know yet where in the fish the MWNTs accumulate).**

Question: **The optical scanner provides no structural information on the material being quantitated. How do we know the material is MWNTs? Also analyzed MWNTs in gel by Raman spectroscopy, which can both identify and quantify MWNTs (for details, see poster).**

Identification and Quantitation of MWNTs from Embryos by Raman Spectroscopy

Method: Collect Raman spectra from bands in SDS-PAGE gels using a WITec laser scanning confocal Raman microscope. Look for characteristic G-mode scattering peak (1595 cm^{-1}) that results from tangential vibrations of the carbon lattice.



Conclusion: The signature G-mode peak of the carbon lattice is present, evidence that the carbon nanotubes are present. In addition, the area under this peak should be proportional to the amount. Using standards we quantified the material and the results were within a factor of two compared with data from optical scanner. This provides confidence in the quantitative results.

Industrial Interactions and Technology Transfer

- **Participated in 2 teleconferences in 2014 with consortia members and industrial liaisons.**

- **Attended 7 ERC/SRC teleseminars**

- **Presented 1 ERC/SRC teleseminar**

- **Hosted 1 ERC/SRC teleseminar**

- **Continue to interact with TI World Wide Environmental & Safety**

- **One patent awarded in 2014: Method for Measuring Carbon Nanotubes Taken-up by a Plurality of Living Cells. Draper, R.K.; Pantano, P.; Wang, R.; Mikoryak, C.**
U. S. Patent No. 8,632,671

Publications, Presentations, and Recognitions/Awards

- **Publications: 4 peer reviewed publications (Carbon, Anal. Chem., Analyst, Experimental Biol. & Med.)**

- **Submitted manuscripts: 1 (Environmental Sci. Nano.)**

- **Submitted grant proposals: 3 (2 NSF, 1 Welch Foundation)**

- **Awarded grant proposals: (NIH: Multi-walled Carbon Nanotube Properties and Macrophage Proinflammatory Responses)**

- **Presentations at meetings and invited seminars: 4**

- **Awards: Students Dakota Deutsch and An Huang won Undergraduate Research Awards at UT Dallas.**

- **Professor P. Pantano received the UT Dallas Provost's Faculty Award for Excellence in Undergraduate Research Mentoring and the UT Dallas School of NS&M Outstanding Faculty Teacher Award.**