Dispersion, Bioaccumulation, and Mechanisms of Nanoparticle Toxicity

University of Texas at Dallas   Task Number: 425.042

PIs:

- **Steven O. Nielsen (PI), Chemistry**
- **Rockford K. Draper (co-PI), Chemistry and Mol. & Cell Biol.**
- **Paul Pantano (co-PI), Chemistry**
- **Inga H. Musselman (co-PI), Chemistry**
- **Gregg R. Dieckmann (co-PI), Chemistry**

Graduate Students:

- **Blake Wilson, Elizabeth Braun, Sarah Karimi, Chemistry**
- **Tyler Hughes, Mol. & Cell Biol.**

Undergraduate Students:

- **Dakota Deutsch, Samee Vakil, Michael Lee, Liza Miadzvedskaya, Mol. & Cell Biol.**
- **Michael Yukica, Chemistry**

Other Researchers:

- **Dr. Ruhung Wang, Senior Scientist, Chemistry and Mol. & Cell Biol.**
- **Dr. Carole Mikoryak, Senior Scientist, Mol. & Cell Biol.**

Cost Share (other than core ERC funding):

Objectives

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

- **Initial focus on multi-walled carbon nanotubes (MWNTs): Prepare MWNTs that differ only in length, dispersant, and agglomeration. Test effects of length, dispersant, and agglomeration on cytotoxicity. Provide material to other members of ERC/SRC nanotox consortium for testing.**

- **Midyear additional 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) were not cytotoxic to A549 cells but reduced proliferation of macrophages after a 3 day exposure to 100 µg/mL MWNTs. Surprisingly, carboxylated MWNTs were taken up by macrophages 100 times more than pristine MWNTs, yet their effect on proliferation was the same.**
   - **Acidic silica and fumed silica reduced growth of A549 cells in 24 hours with IC50's of 3.1 mg/mL and 3.3 mg/mL, respectively. Ceria and alumina were not toxic in a 24 hour exposure.**
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

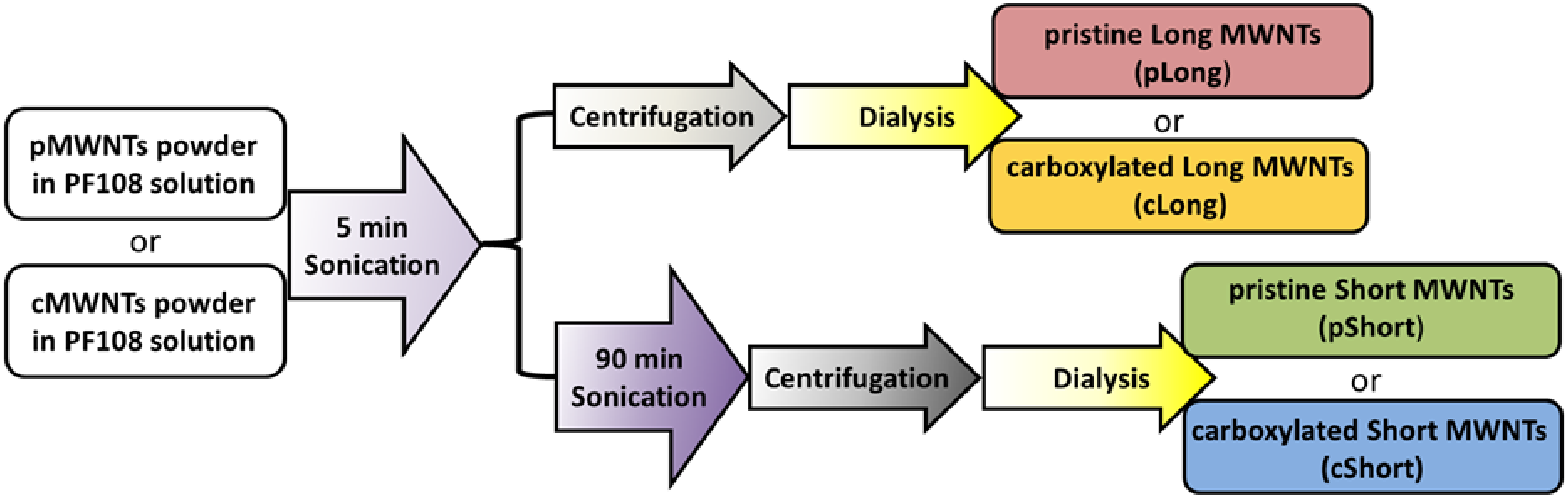
1. **Toxicity and bioaccumulation of multi-walled carbon nanotubes (MWNTs)**
   - ➢**Preparation of long and short pristine and carboxylated MWNTs.**
   - ➢**Length analysis of MWNTs.**
   - ➢**Effects on cell proliferation of A549 adenocarcinomic human alveolar basal epithelial cells and RAW 264.7 Mouse leukaemic monocyte macrophage cells.**
   - ➢**Bioaccumulation by cells of MWNTs.**

2. **Toxicity and bioaccumulation of CMP nanoparticles, acidic silica, fumed silica, ceria and alumina**
   - ➢**Effect of CMP slurry *supernatants* on proliferation of A549 cells.**
   - ➢**Effect of CMP slurry *nanoparticles* on proliferation of A549 cells.**
   - ➢**Future work: Bioaccumulation of nanoparticles by cells.**

3. **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.**

Preparation of MWNT-Pluronic F108 (PF108) suspensions by sonication and purified by centrifugation and dialysis

- Starting material is long pristine MWNT or long carboxylated MWNT powder.
- After sonication, suspensions were dialyzed against 0.2 mM PF108 to remove degradation products of PF108 that accumulate during sonication.



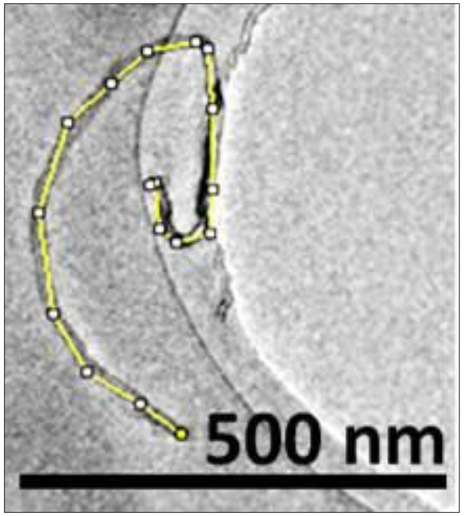
Two matched sets of pristine and carboxylated MWNT suspensions:

pristine MWNTs { **pLong**, **pShort**

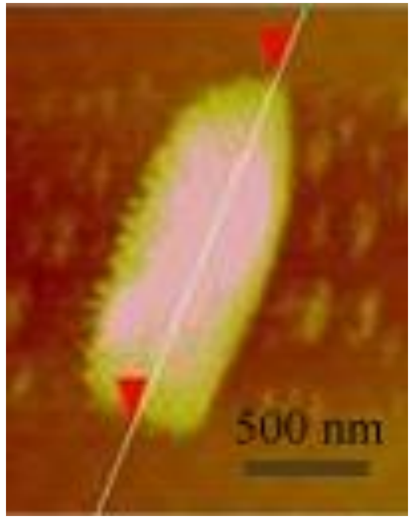
carboxylated MWNTs { **cLong**, **cShort**

Length of MWNTs in Pluronic F108 (PF108) suspensions

- Length analysis by transmission electron microscopy (TEM).
- Length analysis by atomic force microscopy (AFM).



TEM example
(in collaboration with Drs. Y. Yang & P. Westerhoff, Arizona State)



AFM example

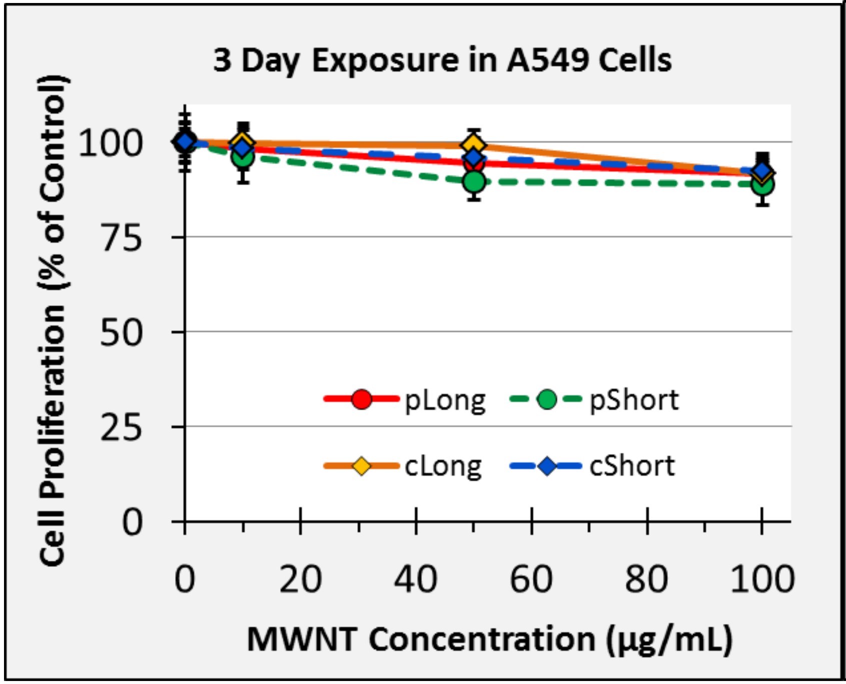
MWNT Type	Mean ± SD (nm)	
	TEM	AFM
pLong	1593 ± 1689	807 ± 707
cLong	495 ± 476	554 ± 299
pShort	167 ± 91	139 ± 57
cShort	137 ± 147	199 ± 56

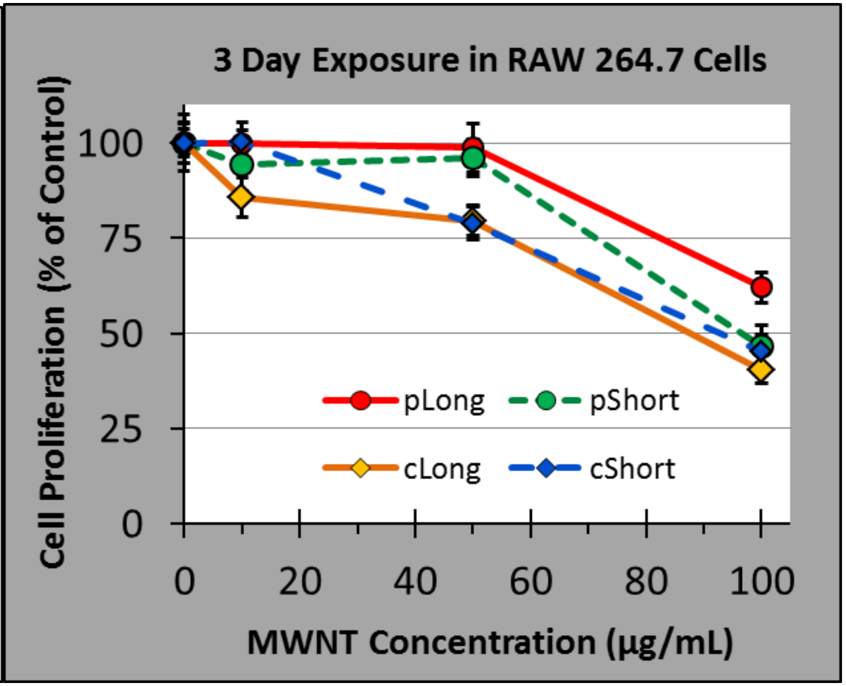
CONCLUSIONS

- **The lengths of the pLong MWNTs were similar to cLong MWNTs while the lengths of the pShort MWNTs were similar to cShort MWNTs.**
- **MWNT length analysis results obtained by TEM agreed well with results obtained by AFM analysis.**

Effect of Pristine and Carboxylated MWNTs on Proliferation of A549 and RAW 264.7 Cells

- Cells exposed to 0, 10, 50, and 100 µg/mL MWNTs for 1, 2, or 3 days
- Proliferation assessed by increase in cell numbers compared to controls



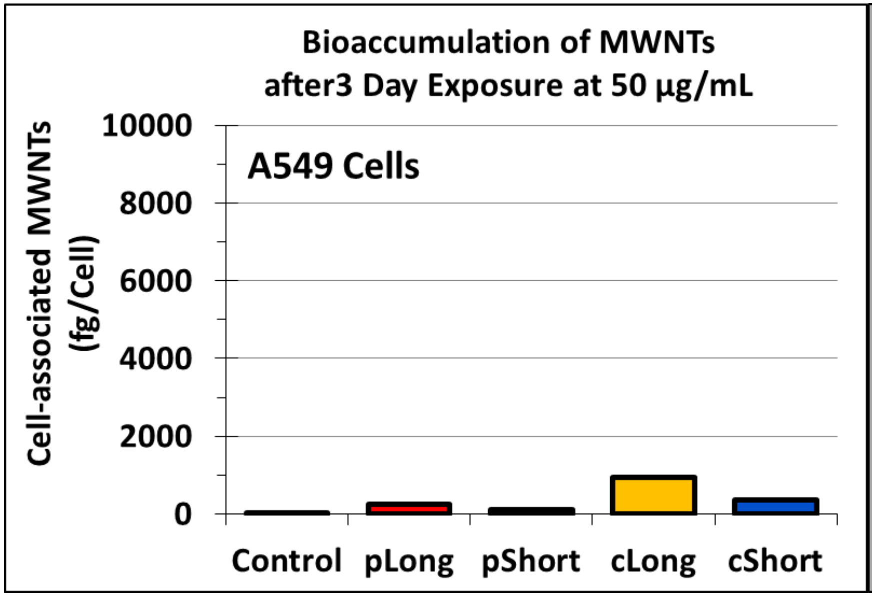


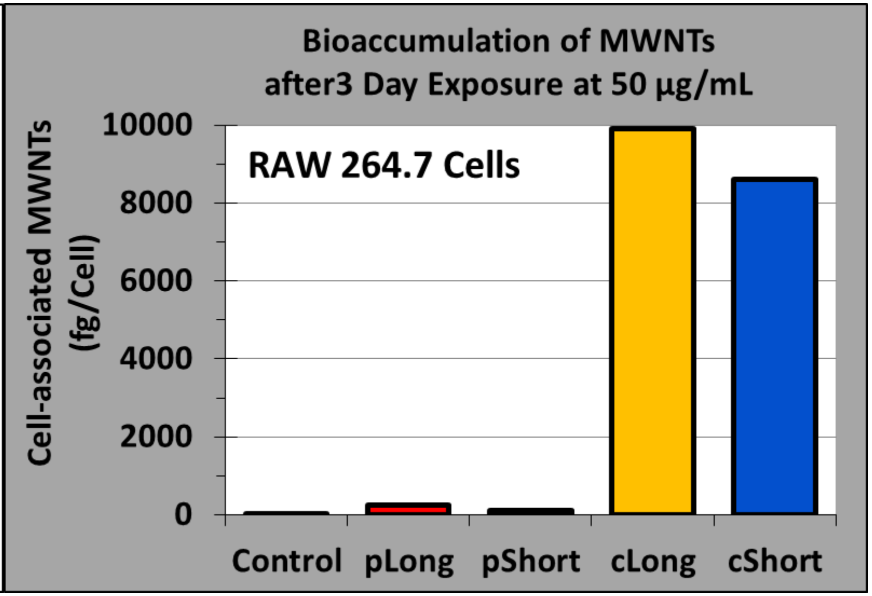
CONCLUSIONS

- No effect of MWNTs on A549 cell proliferation even after 3 day exposure at 100 µg/mL.
- Proliferation reduced with RAW 264.7 cells, unlike with A549 cells. Thus, not all cells respond the same way to MWNTs
- Little difference between pristine and carboxylated or long and short.

Bioaccumulation of Pristine and Carboxylated MWNTs by A549 and RAW 264.7 Cells

- Cells exposed to 50 µg/mL MWNTs for 1, 2, or 3 days
- MWNTs extracted from cells and quantified



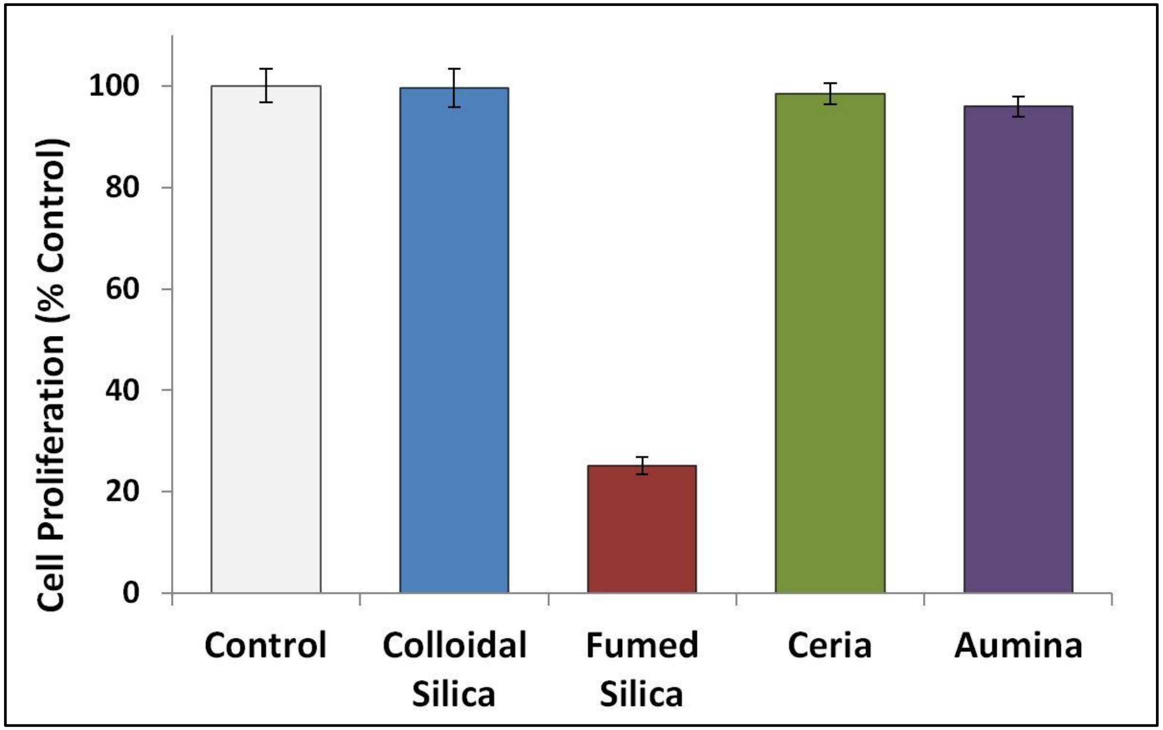


CONCLUSIONS

- Carboxylated MWNTs were taken up ~10 times more than pristine by A549 cells.
- In RAW 264.7 cells, carboxylated MWNTs were taken up ~100 times more than pristine MWNTs. The reason for this profound difference requires further investigation.
- Little difference between MWNTs of long and short length.

Effect of CMP Slurry Supernatants on Proliferation of A549 Cells

- **Do CMP slurries have soluble toxic material in them?**
- **Remove nanoparticles by centrifugation, then test the effect of slurry supernatants (1:1 diluted in culture medium) on proliferation of A549 cells after a 24 hour exposure.**

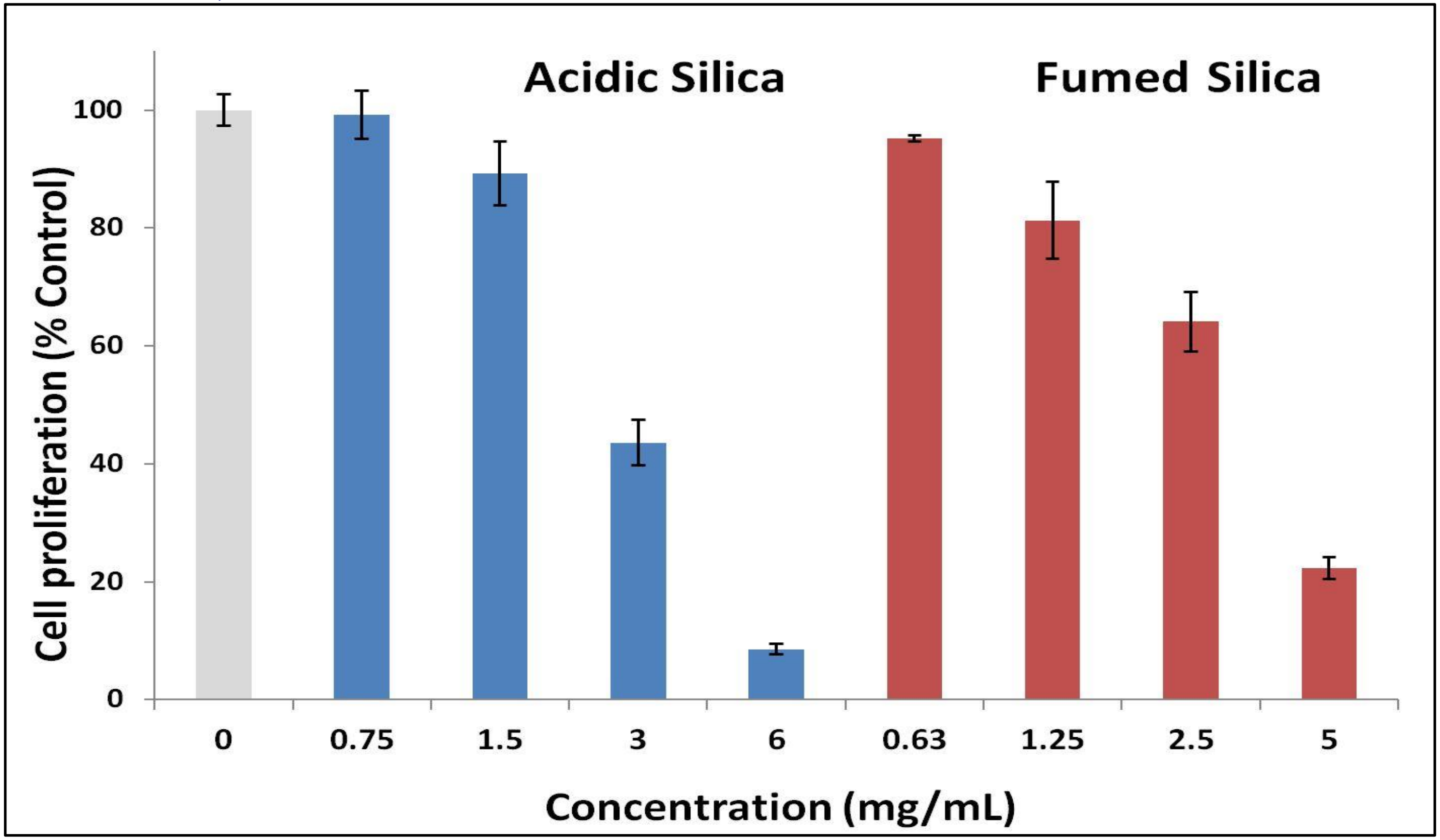


CONCLUSION

- **Fumed silica CMP slurry contains at least one substance other than fumed silica that inhibits cell proliferation.**
- **Elemental analysis (Arizona State) did not find any elements present at obviously toxic amounts. Suggests possible organic toxicant?**

Effect of CMP Slurries on Proliferation of A549 Cells

- **Slurries were adjusted to pH 7, mixed with cell culture medium at the indicated concentrations, and added to cells for 24 hours.**

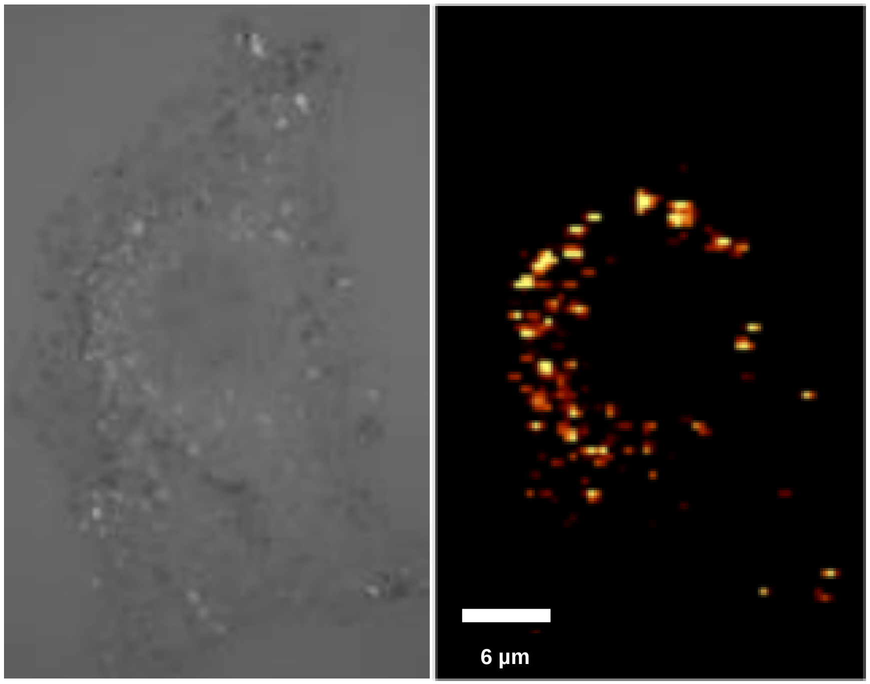


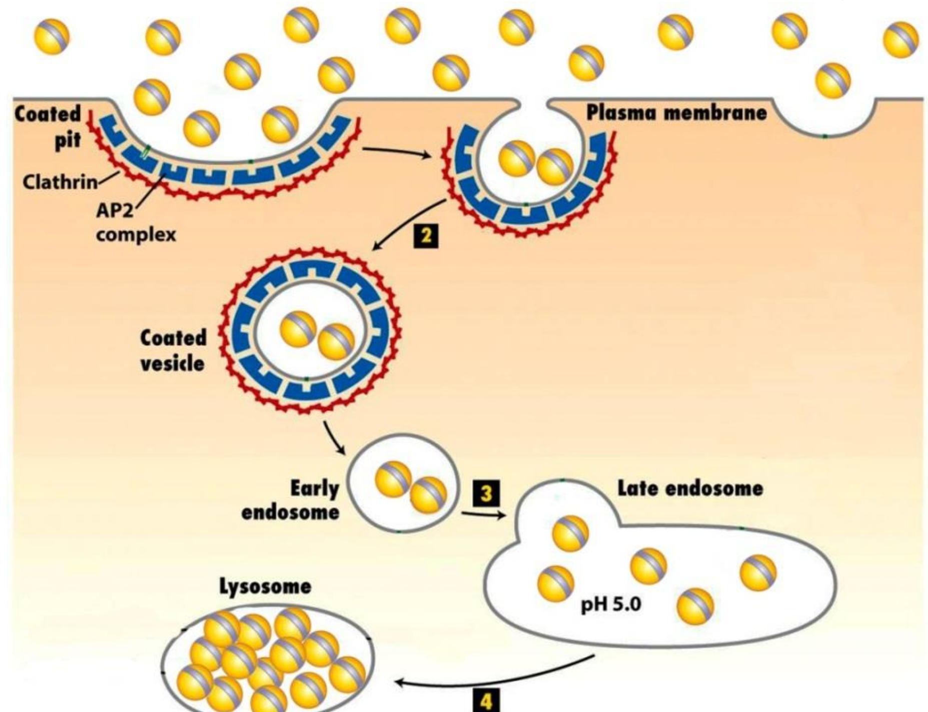
CONCLUSION

- **Colloidal and fumed silica reduce proliferation of A549 cells. Not clear whether material other than fumed silica is active material for that slurry because the slurry supernatant affected cell growth (previous slide). Ceria and alumina had no effect on cell growth.**

Laser Scanning Confocal Raman Mapping of CMP Ceria Bioaccumulation Within A549 Cells

- **A549 cells were incubated with 0.05 mg/mL CMP ceria in culture medium for 48 hours and the location of ceria in the cells was determined with Raman mapping.**





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

CONCLUSION

- **Ceria at concentrations that were not overtly toxic in this time frame nevertheless accumulated within cells, probably inside lysosomes. Continued accumulation of foreign material in lysosomes over the long term may be damaging.**

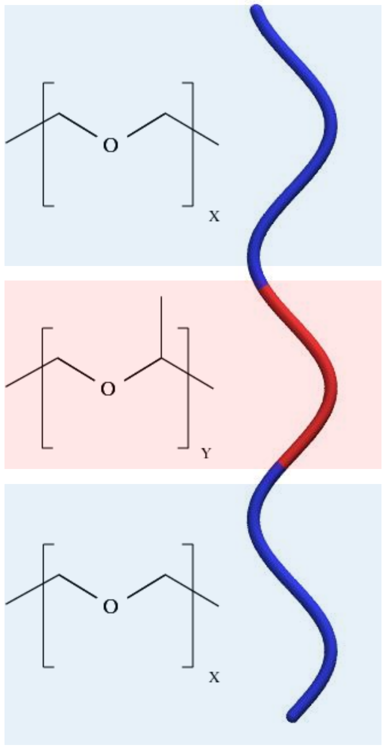
Modeling of Pluronics and Carbon Nanotubes (CNTs)

- **Structure of Pluronics: Amphiphilic Tri-block copolymers**

Polyethylene Oxide (PEO, E) — **Hydrophilic**

Polypropylene Oxide (PPO, P) — **Hydrophobic**

Polyethylene Oxide (PEO, E) — **Hydrophilic**



naming convention

F68

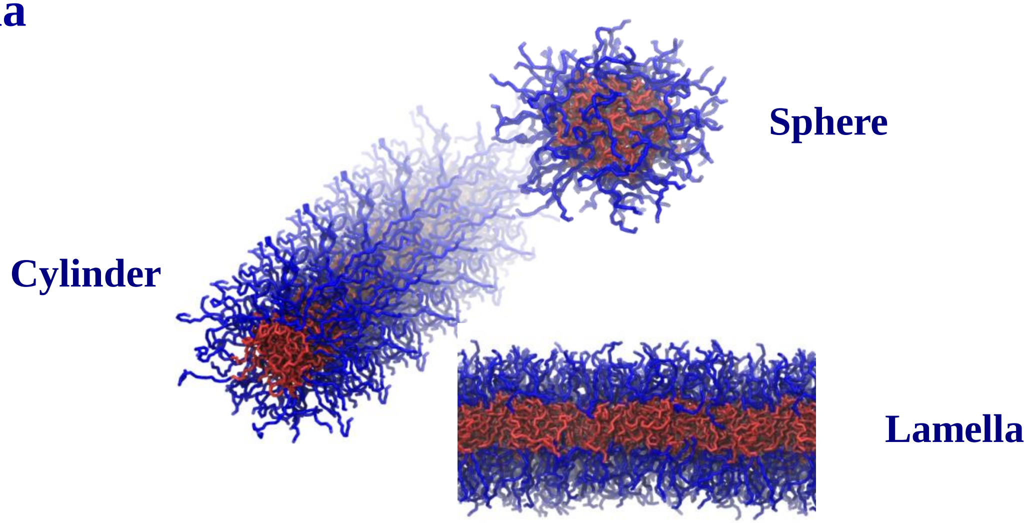
Single letter; physical state (F=Flake, P=Paste, L=Liquid)

one-two digits; x300 =hydrophobic block mass

One digit; x10 = % hydrophilic

Pluronics in Aqueous Environment

- **Aggregate to form micelles of 3 primary shapes**
  - **Sphere**
  - **Cylinder**
  - **Lamella**



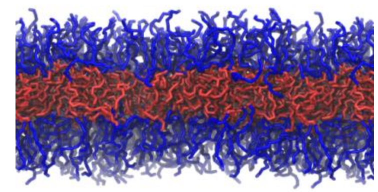
- **The micelle shape that a Pluronic preferentially adopts in aqueous solution depends on its composition**

Theoretical Molecular Model of Pluronic Aggregates

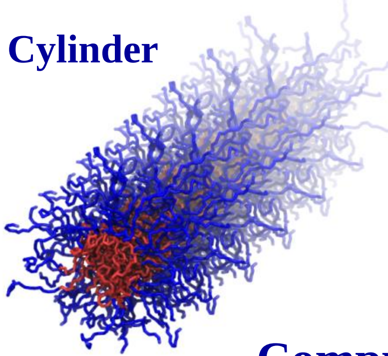
- **Proposed by Nagarajan *et al* (Colloids and Surfaces B, 1999)**

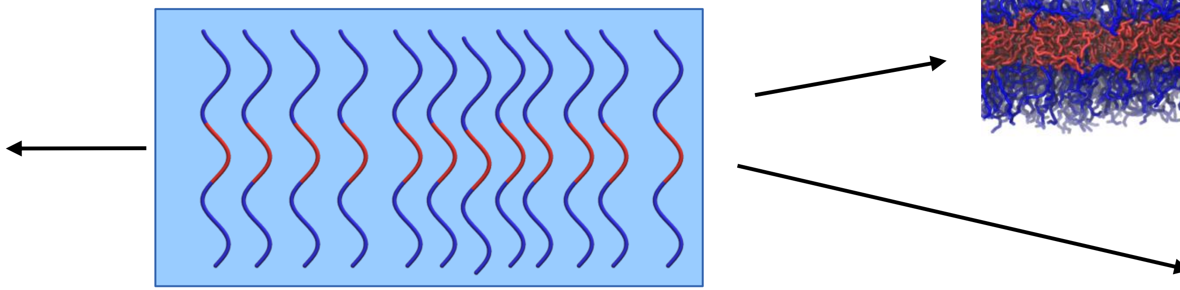
  - **Consider free polymer in aqueous solution**

Lamella

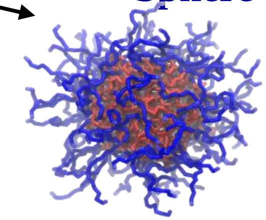


Cylinder





Sphere



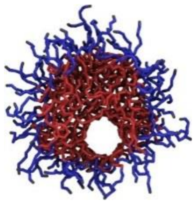
  - **Compute the free energy of formation of each micelle shape accounting for these interactions thermodynamically:**

    - **dilution state of the two polymer blocks**
    - **deformation state of the two polymer blocks**
    - **copolymer localization into micellar structure**
    - **core solvent interface in micelle**
    - **backfolding of triblock copolymer in micelle**

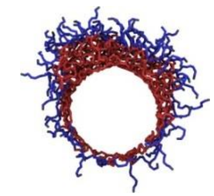
  - **The micelle shape that yields lowest free energy is the preferred shape of aggregates of that polymer in aqueous solution**

Theoretical Molecular Model of Pluronic Solubilization of CNTs

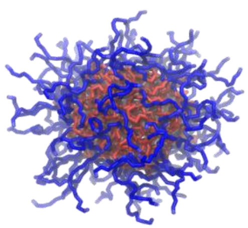
- Proposed by Nagarajan *et al* (J. Chem. Phys. 2009)
- Consider same molecular interaction terms (as in CNT free model) which are indirectly affected by geometrical constraints of CNT, e.g.:



Block deformation to make space for CNT →

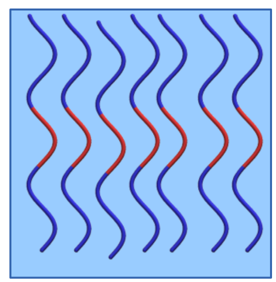


- But also include additional term:
  - CNT – hydrophobic block (PPO) interaction



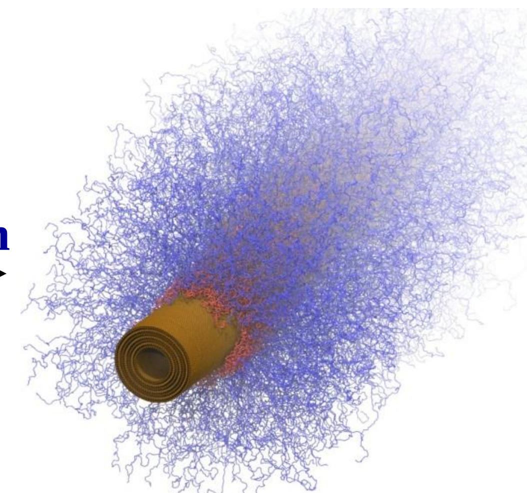
Lowest free energy micelle shape

← Free Energy of Micellation ($\Delta\mu_{Micelle}$)



Free polymer in aqueous solution

Free Energy of CNT Solubilization → ($\Delta\mu_{CNT}$)

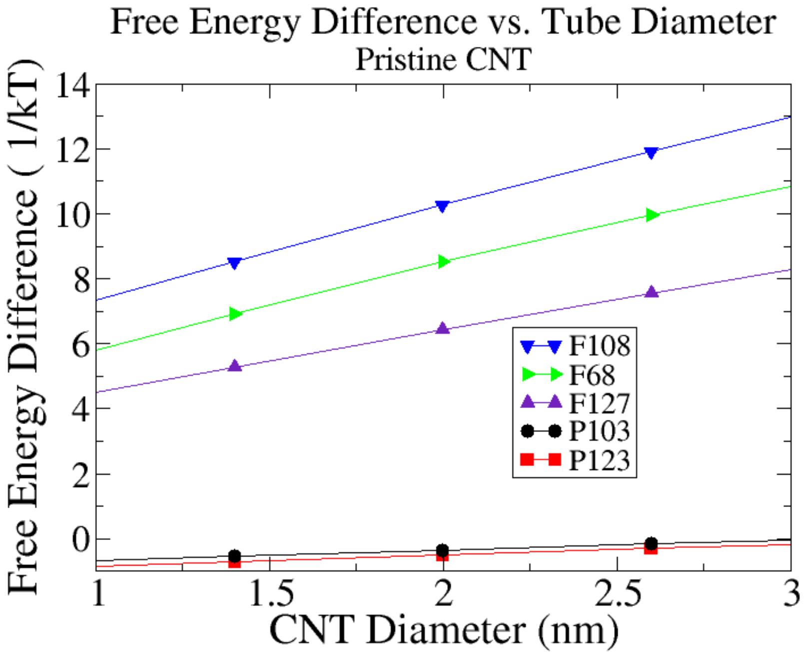


Cylindrical micelle formed around CNT to solubilize

- Free Energy Difference ($\Delta\mu_{CNT}$ - $\Delta\mu_{Micelle}$) less than zero denotes CNT solvation is thermodynamically favorable

Results and Model Predictions

- **15 Pluronics were examined; Results from 5 (P103, P123, F68, F108, and F127) are shown**
- **A wide range of CNT diameters were modeled**
- **Can predict and compare relative stability of CNT solvation for the various Pluronics at fixed CNT diameter**
- **Can tune model parameters to model oxidized CNTs**



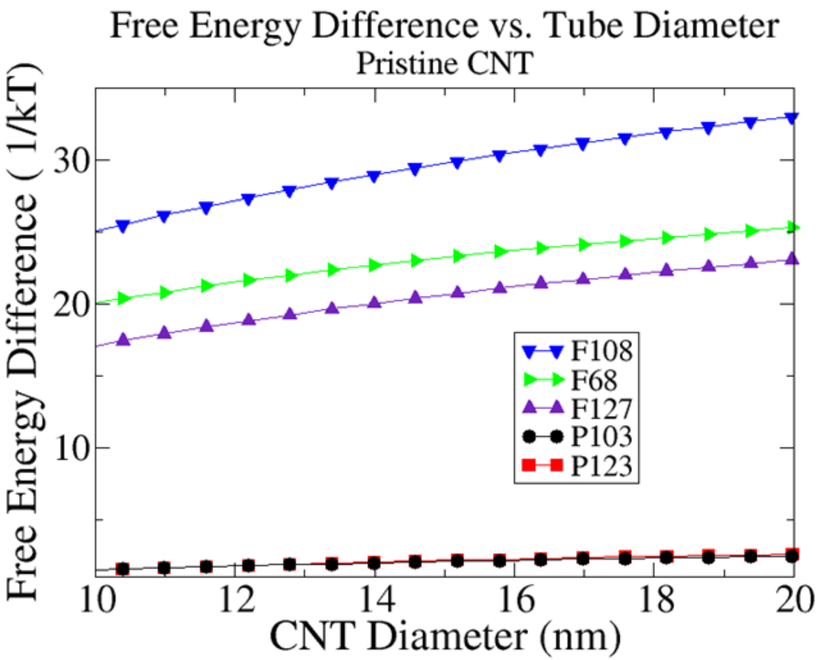
Pluronics and Small Diameter Pristine CNTs

- Smaller tube diameters are more representative of solubilizing SWNTs

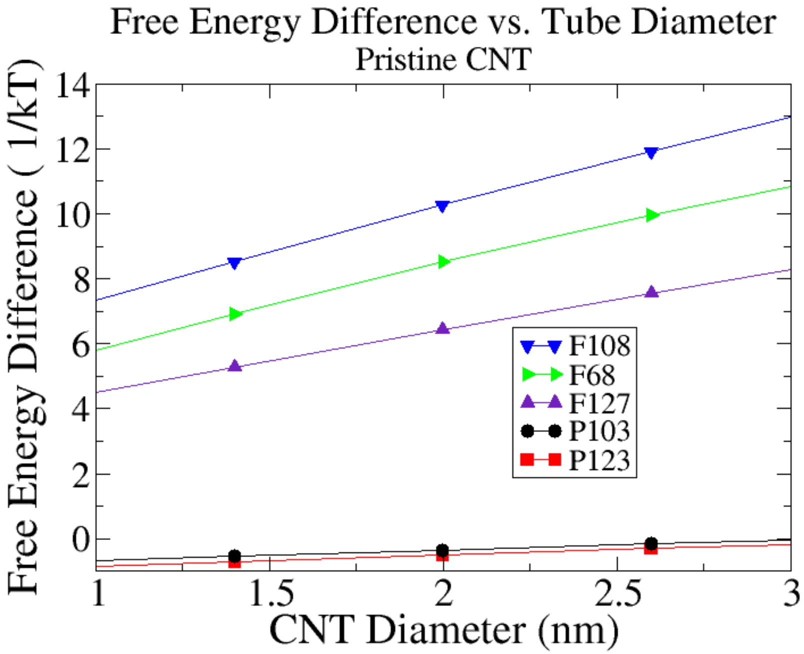
Relative Ranking at CNT diameter of 1.4 nm	Trade Name	Free Energy Difference	Number of PEO units per block	Number of PPO units per block	Preferred Shape of CNT Free Aggregate
1	P123	-0.704	20	70	Cylinder
2	P103	-0.542	17	60	Cylinder
3	F127	5.277	100	64	Sphere
4	F68	6.934	77	29	Sphere
5	F108	8.527	133	50	Sphere

Pluronics and Large Diameter Pristine CNTs

- **Larger tube diameters are more representative of solubilizing MWNTs**



Relative Ranking at CNT diameter of 15.2 nm	Trade Name	Free Energy Difference	Number of PEO units per block	Number of PPO units per block	Preferred Shape of CNT Free Aggregate
1	P103	2.103	17	60	Cylinder
2	P123	2.176	20	70	Cylinder
3	F127	20.725	100	64	Sphere
4	F68	23.310	77	29	Sphere
5	F108	29.902	133	50	Sphere



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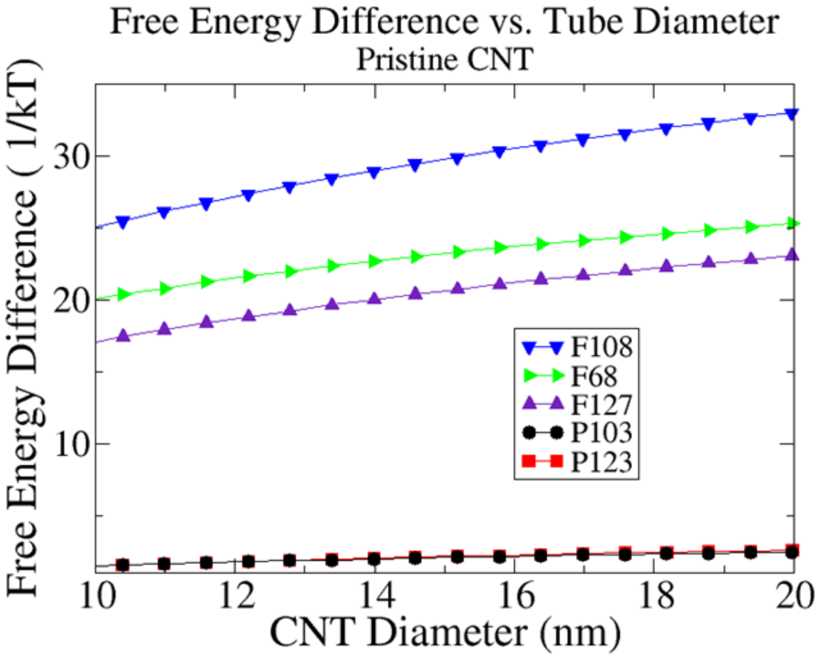

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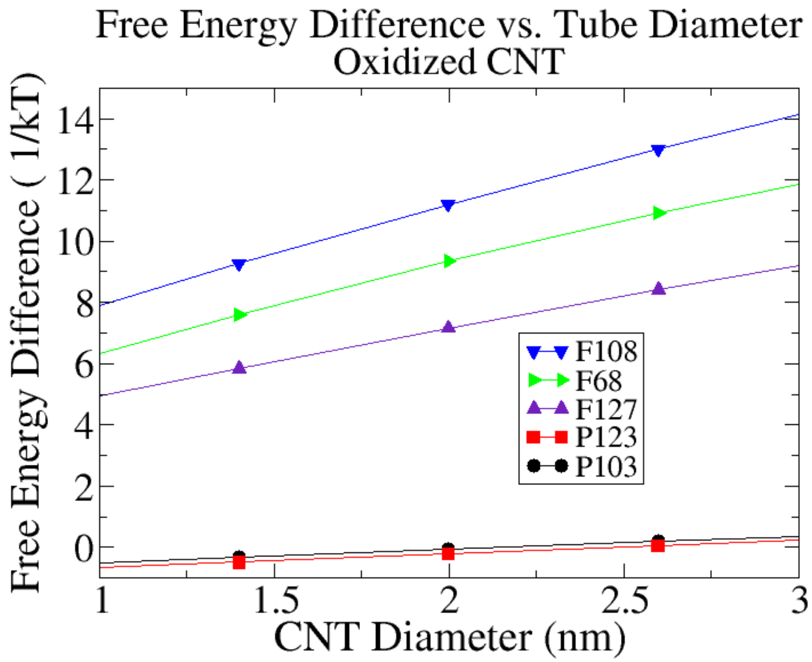


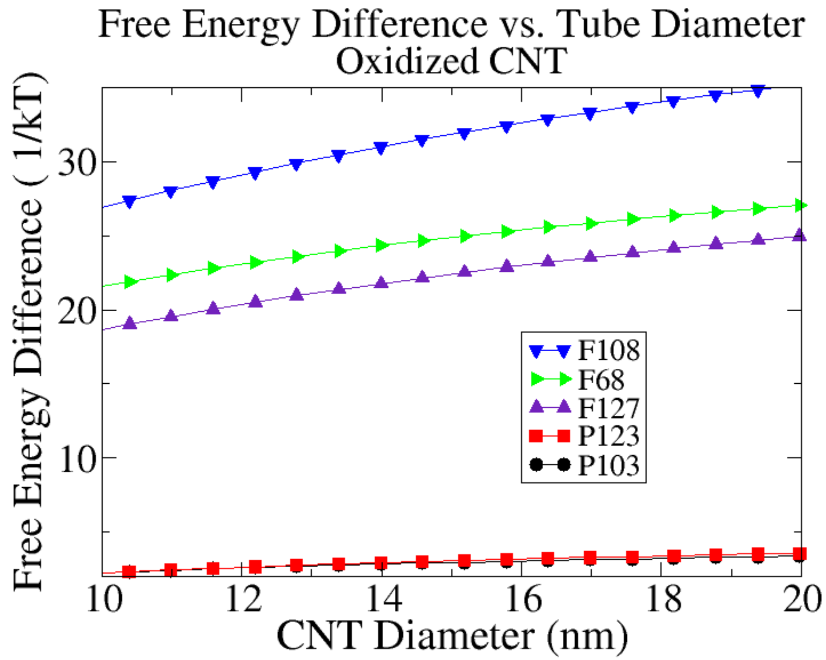
Pluronics and Small Diameter Oxidized CNTs

- **Smaller tube diameters are more representative of solubilizing SWNTs**

Relative Ranking at CNT diameter of 1.4 nm	Trade Name	Free Energy Difference	Number of PEO units per block	Number of PPO units per block	Preferred Shape of CNT Free Aggregate
1	P123	-0.485	20	70	Cylinder
2	P103	-0.321	17	60	Cylinder
3	F127	5.837	100	64	Sphere
4	F68	7.604	77	29	Sphere
5	F108	9.262	133	50	Sphere

Pluronics and Large Diameter Oxidized CNTs

- **Larger tube diameters are more representative of solubilizing MWNTs**



Relative Ranking at CNT diameter of 15.2 nm	Trade Name	Free Energy Difference	Number of PEO units per block	Number of PPO units per block	Preferred Shape of CNT Free Aggregate
1	P103	2.971	17	60	Cylinder
2	P123	3.082	20	70	Cylinder
3	F127	22.151	100	64	Sphere
4	F68	24.987	77	29	Sphere
5	F108	31.975	133	50	Sphere

Comparison to Experimental Dispersion Data

- Pluronic F108 ($E_{133}P_{50}E_{133}$) has been used most recently while F68 ($E_{77}P_{29}E_{77}$) and F127 ($E_{100}P_{64}E_{100}$) have been used in the past for experimental dispersions of CNTs

- However, this model predicts certain Pluronics to be better at solubilizing CNTs at various tube dimensions. (e.g. P103 and P123)

- In the future we can
  - experimentally determine the dispersion efficiency of various Pluronics using the model predictions as a guide for Pluronic selection
  - compare experimental dispersion results to model predictions
  - refine the theoretical model to better account for realistic dispersion conditions (e.g. energetic perturbation by sonication)

Industrial Interactions and Technology Transfer

- **Participated in 8 teleconferences in 2013 with consortia members and industrial liaisons.**
- **Attended 9 ERC/SRC teleseminars**
- **Presented at the Annual SEMI Strategic Materials Conference**
- **Presented two ERC/SRC teleseminars**
- **Hosted two ERC/SRC teleseminars**
- **Continue to interact with TI World Wide Environmental & Safety**

Future Plans

Next Year Plans

- Evaluate dispersion efficiency of Pluronics predicted to be optimal by the thermodynamic model
- Write up MWNT work for publication.
- Focus on assessing potential toxicity and bioaccumulation of CMP slurry nanoparticles on mammalian cells.

Long-Term Plans

- Assess effects of CMP nanoparticle bioaccumulation on specific pathways of potential toxicity.

Publications, Presentations, and Recognitions/Awards

- **Publications: 2 peer reviewed publications (Nanotoxicology, Analytical Chem.)**

- **Submitted manuscripts: 2 (Carbon, Analyst)**

- **Submitted grant proposals: 4 (1 NIH, 2 NSF, 1 Welch Foundation)**

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

- **Presentations at meetings and invited seminars: 9**

- **Awards: Student Dakota Deutsch won an Undergraduate Research Award at UT Dallas**

For More Detailed Information

- UTD Group is presenting 4 posters:
  1. Theoretical Modeling and Predictions of Pluronics/CNT Aggregate Stability
  2. Toxicity and Bioaccumulation of Multi-walled Carbon Nanotubes and CMP Slurry Nanoparticles on Cultured Epithelial and Macrophage Cell Lines
  3. The Importance of a Thorough Elemental Analysis of Carbon Nanotube Soot
  4. Differentiation of Carbon Nanotube and Particulate Matter Contamination on Workplace Surfaces using microProbe Raman Spectroscopy

- Include information on:
  - Industrial Interactions and Technology Transfer
  - Future Plans
  - Publications, Presentations, and Recognitions/Awards

Thank You!

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