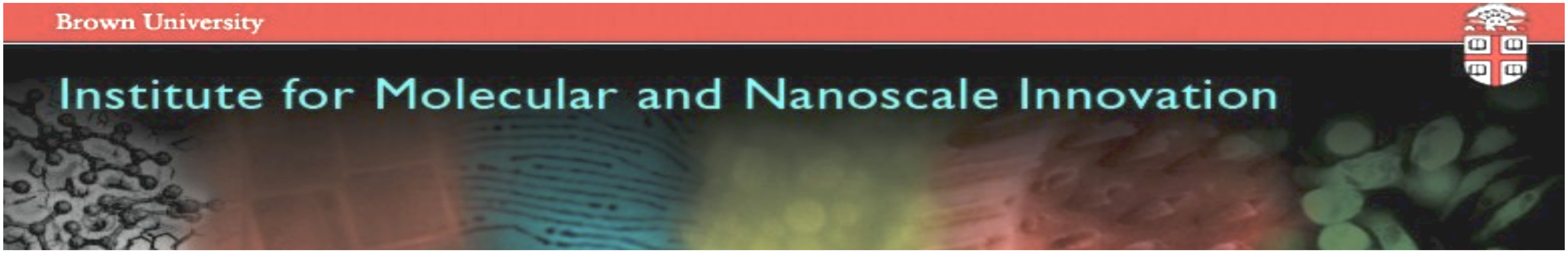


Which material properties / features determine the biological response to carbon nanotubes?

Robert Hurt
Brown University, Providence Rhode Island

Presented at NIST March 1st, 2011

Examples from literature on carbon nanotube health risks

- producing asbestos-like pathology in mice
  [Poland et al.]

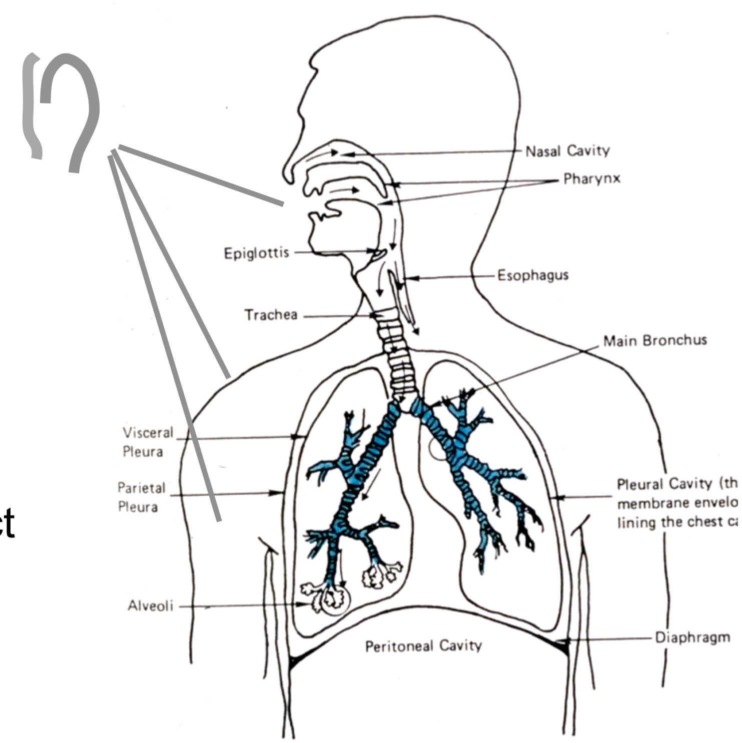
- promising biocompatible materials for drug delivery
  ….with no toxic side effects [Dai and coworkers]

- showing only "false" toxicity through an adsorptive artifact
  in a common toxicity assay [Worle-Knirsch et al.]

- causing oxidative damage to mouse lung
  and heart tissue [Lie et al.]

- toxic only if <u>not</u> purified; copepods [Templeton et al.] ; human lung cells [Krug et al.]

- only toxic if not functionalized [Sayes et al.]



The largest discrepancy is between biomedical studies and toxicological studies

Institute for **Molecular and Nanoscale Innovation**

Some Basics…..

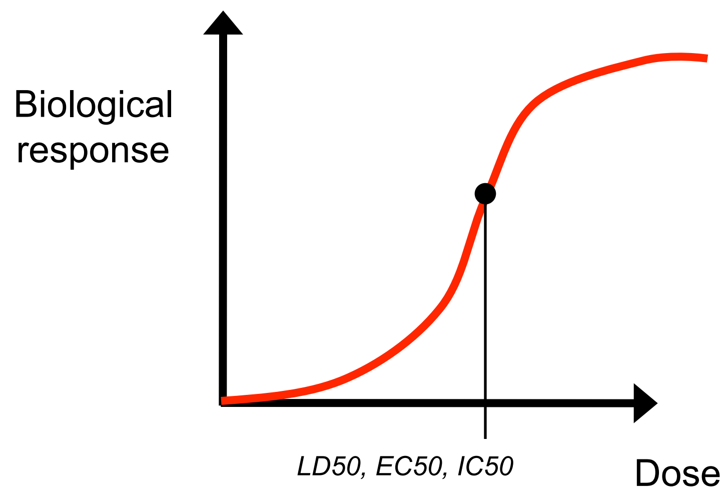
Risk = Hazard x Exposure

"All things are toxic…."
or "the dose makes the poison"
[only by defining and limiting dose
can something be regarded as
non-toxic, in that situation]

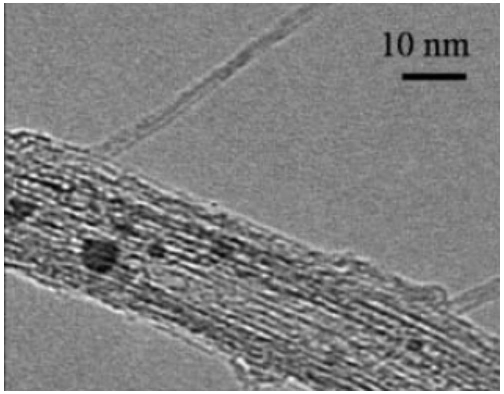
Paracelsus: 1493-1541
"father of toxicology"

Toxics affects have been documented for
ethanol, vitamin E, oxygen, water……

Scientific papers on nano-toxicity will,
by design, report and discuss toxic effects



Effect of SWNTs on Liver Cells



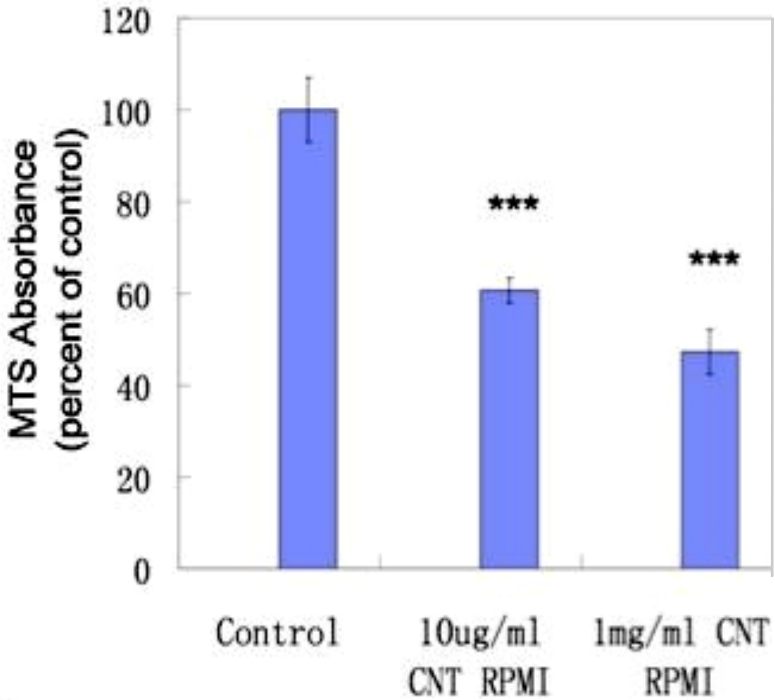
Simple Experiment

SWNTs + Cell culture medium

SWNT removal by centrifugal ultrafiltration

culture cells in “exposed” media

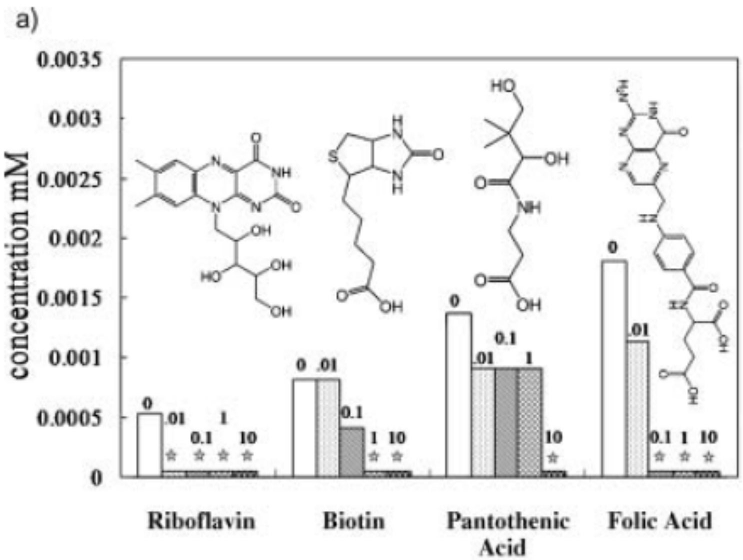
Viability of HepG2 liver cells



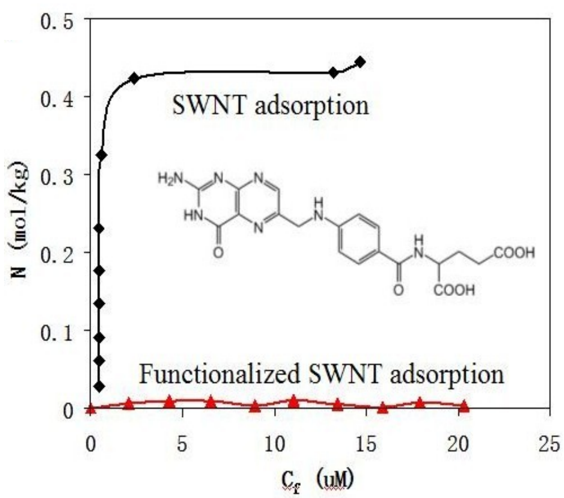
IMNI

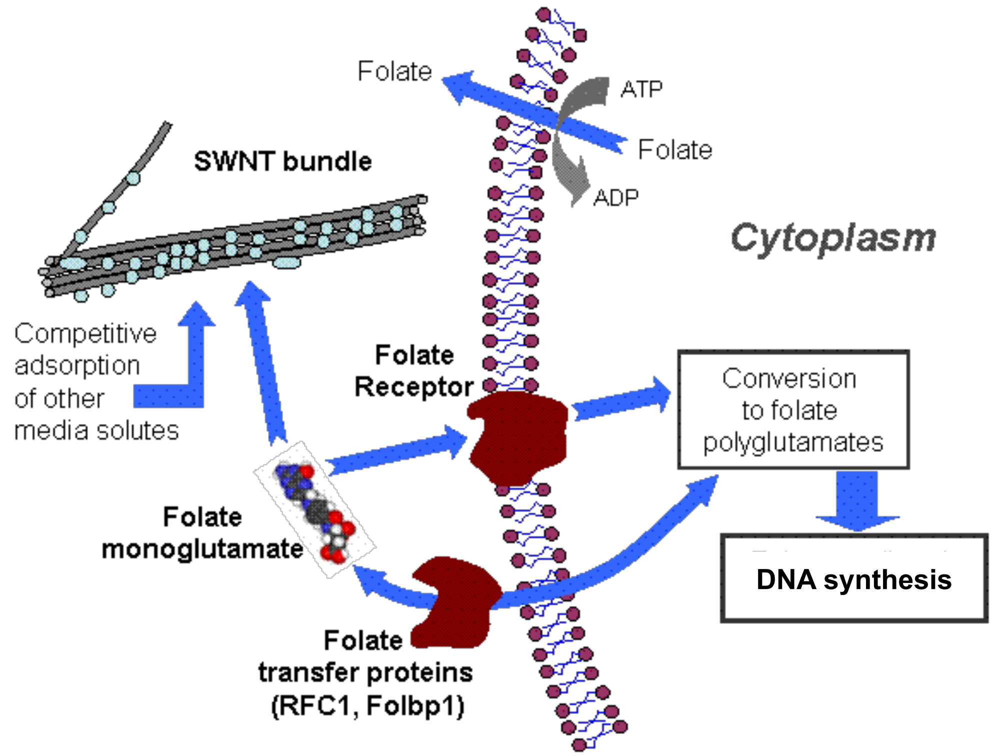
Nanotubes Inhibit Cell Proliferation by Hydrophobic Adsorption of Folic Acid

[Guo et al., *Small*, 4 (6) 721–727 2008]



Essential micronutrient (vitamin) profiling after SWNT exposure



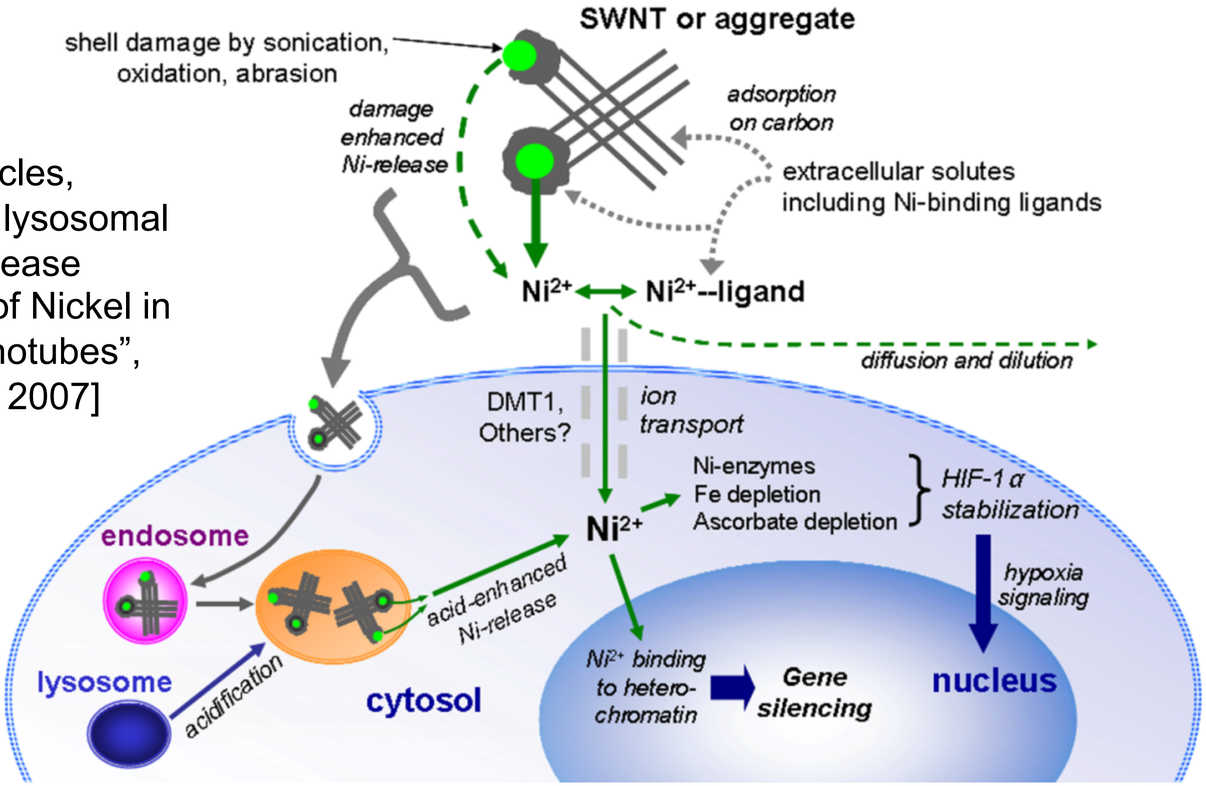


Hydrophobic surfaces can also adsorb molecular probe dyes to give false positives in toxicity assays [Worle-Knirsch et al., 2006]

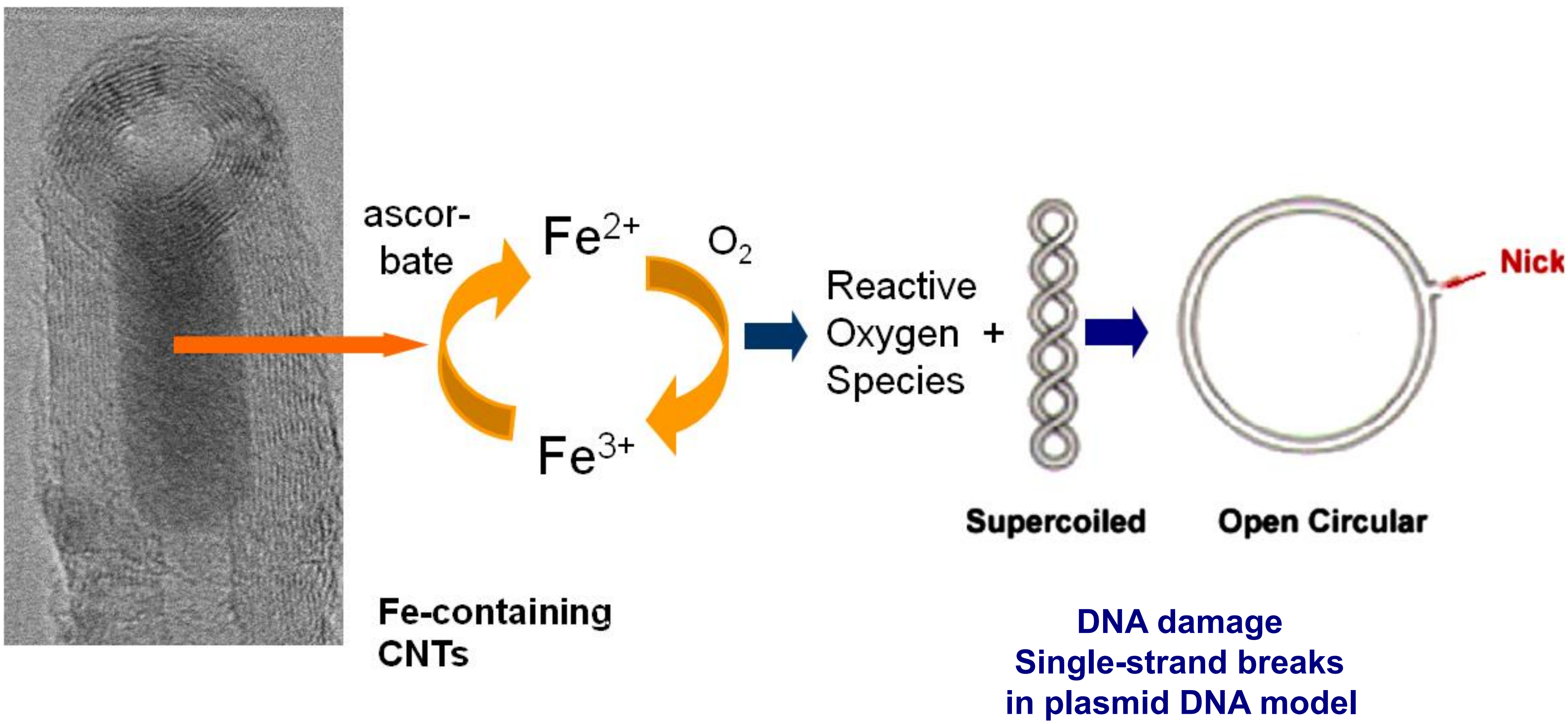
Metal Catalyst Residues in Carbon Nanotubes

"Trojan horse" mechanism

For metal nanoparticles, H^+-mediated corrosion at lysosomal pH enhances ion release [Liu et al. "Bioavailability of Nickel in Single-Wall Carbon Nanotubes", *Advanced* Materials, 2007]



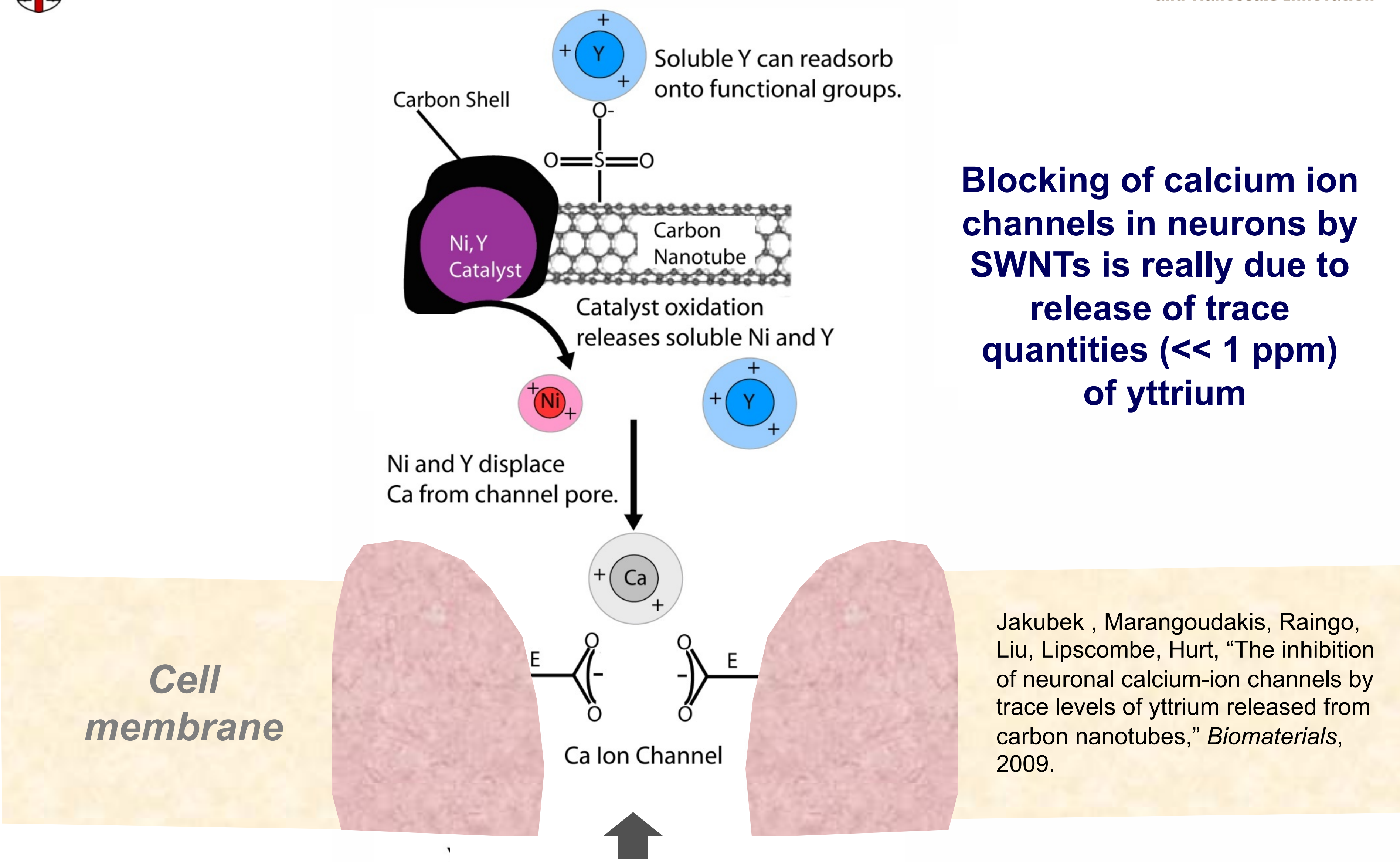
CNT iron catalyzes reactive oxygen species (ROS) generation and inflammatory reactions



Studies on Fe effects in CNT biological response:

Kagan et al., 2006; Guo et al., *Chemistry of Materials*, 2007; Koyama et al. *Carbon*, 2009

Blocking of calcium ion channels in neurons by SWNTs is really due to release of trace quantities (<< 1 ppm) of yttrium

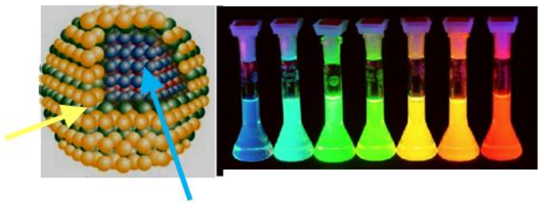


Selectivity filter in neuronal voltage-gated calcium-ion channel

Jakubek , Marangoudakis, Raingo, Liu, Lipscombe, Hurt, "The inhibition of neuronal calcium-ion channels by trace levels of yttrium released from carbon nanotubes," *Biomaterials*, 2009.

Leaching, or ion-particle partitioning,
Is a major theme in nanomaterial safety

Quantum dots

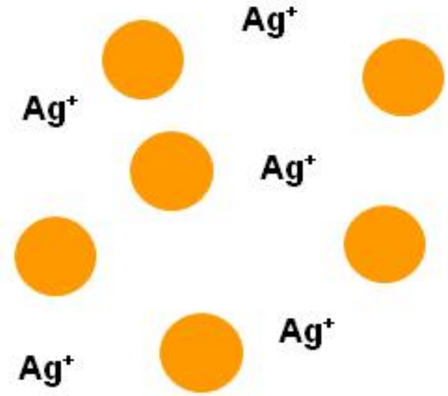


ZnS

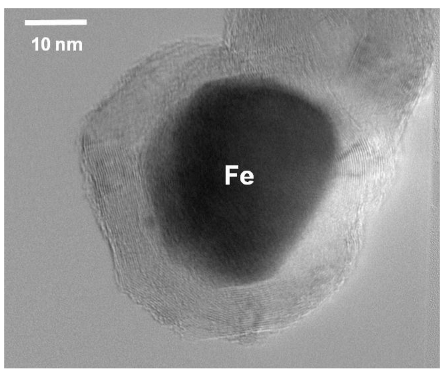
CdSe

Cd^{2+} , Se^{2-} , Zn^{2+}

Antibacterial nanosilver



Carbon nanotubes



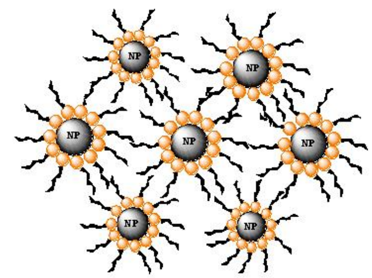
Fe^{2+}, Ni^{2+}, Y^{3+}, Co^{3+}….

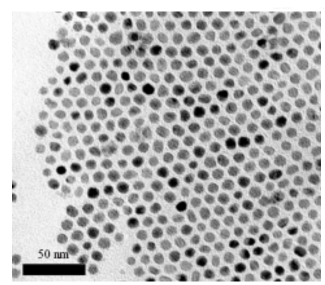
ZnO

Zn^{2+}

Ni

NiO

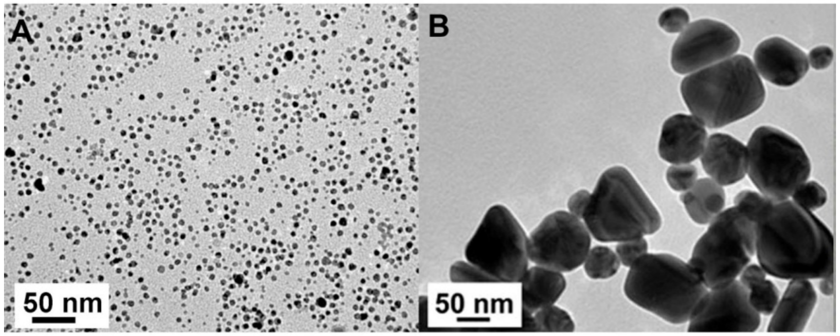




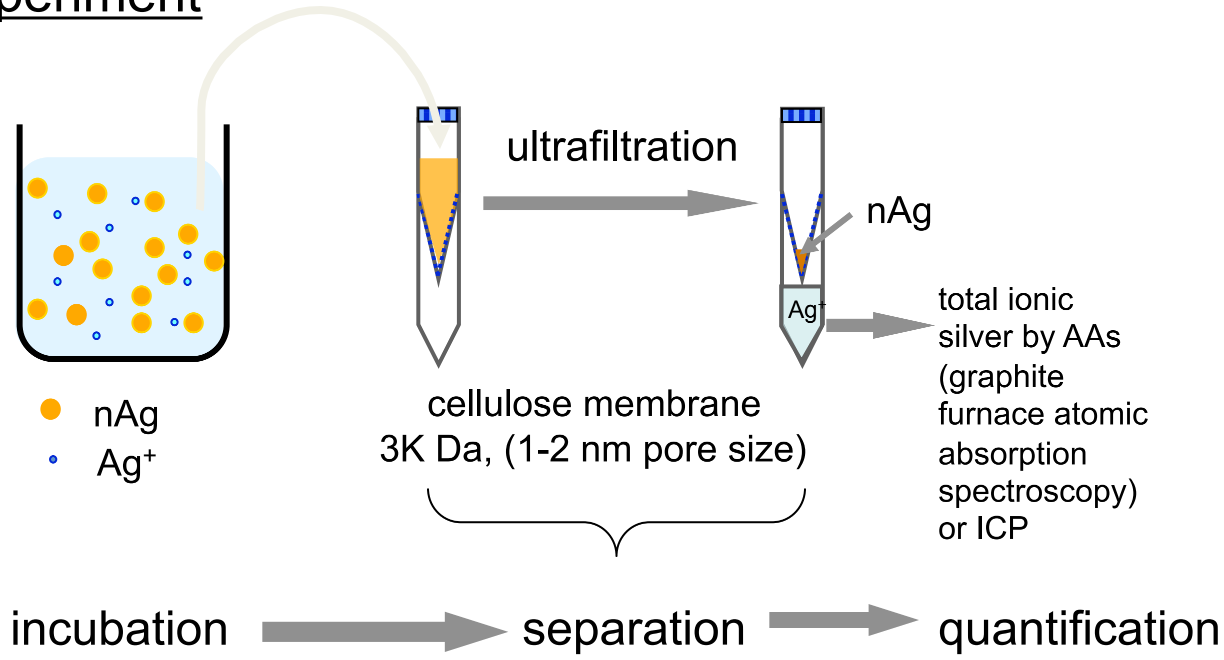
Ni^{2+}

A general experimental method to measure metal bioavailability or ion-nanoparticle partitioning

[Liu et al. *Env. Sci. Tech.*, 44:6 2169–2175 2010]



<u>Basic Experiment</u>



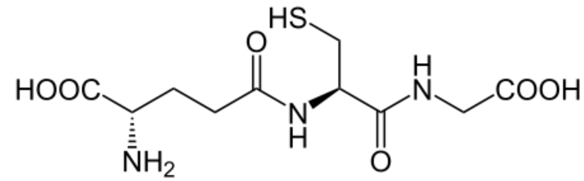
BROWN

Institute for Molecular and Nanoscale Innovation

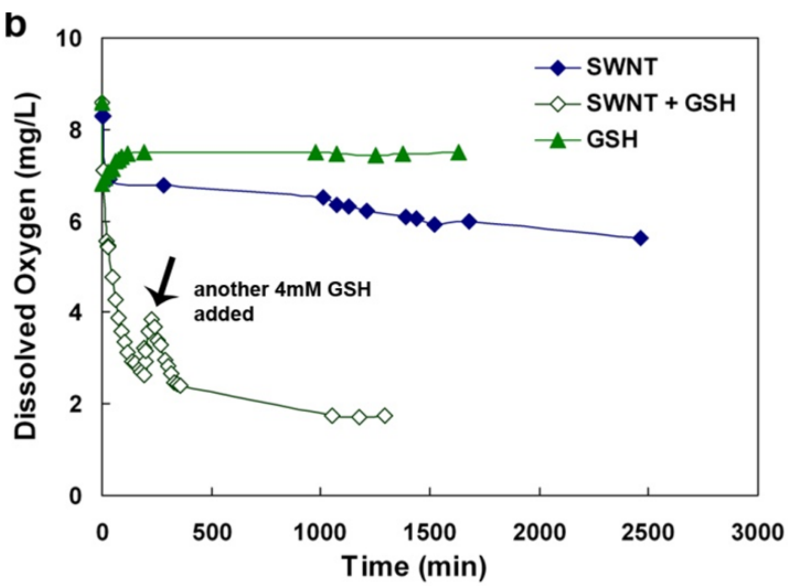
Biological surface reactivity of CNTs

Dr. Xinyuan Liu

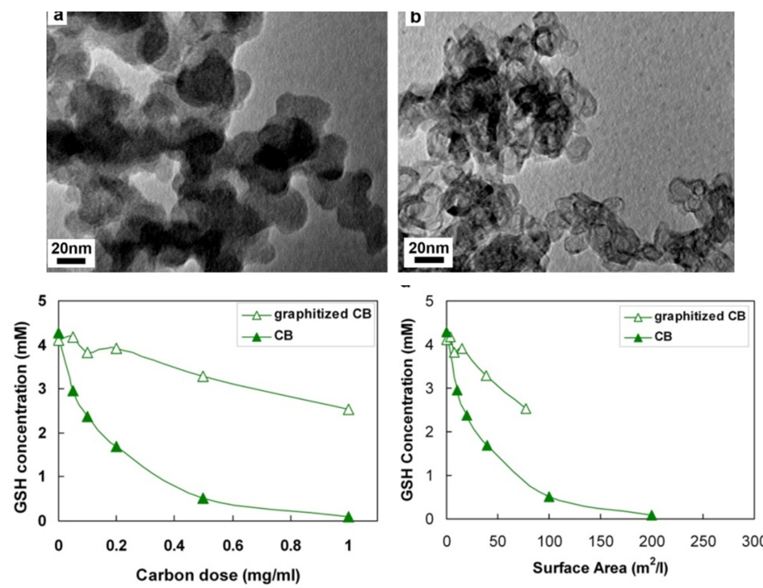
Glutathione (GSH):
the key intracellular antioxidant:



Dissolved O_2 consumption

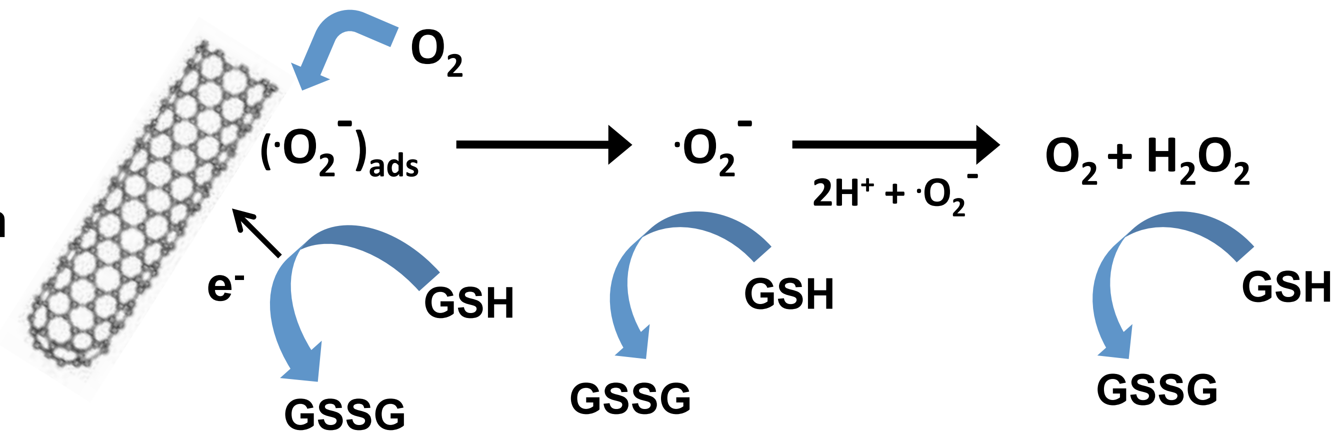


Annealing

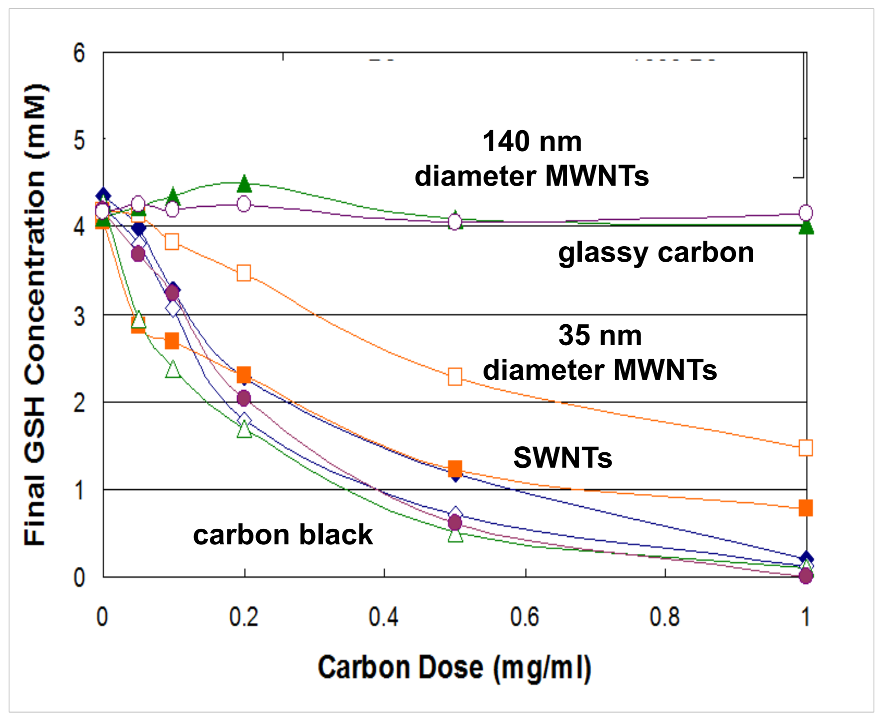


Biological surface reactivity

Proposed mechanism



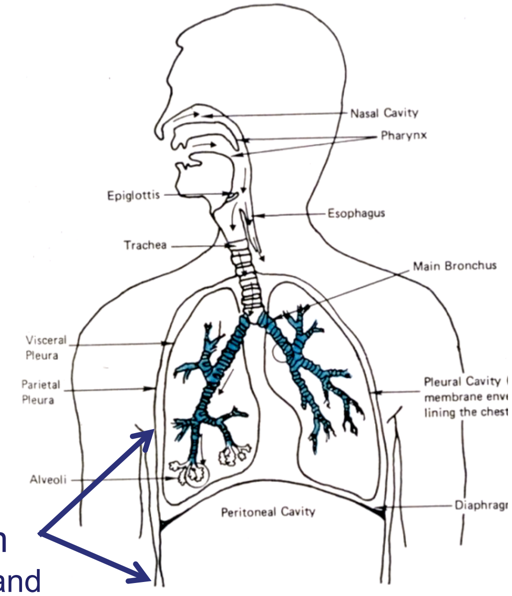
Reactivity of other "graphenic" carbons



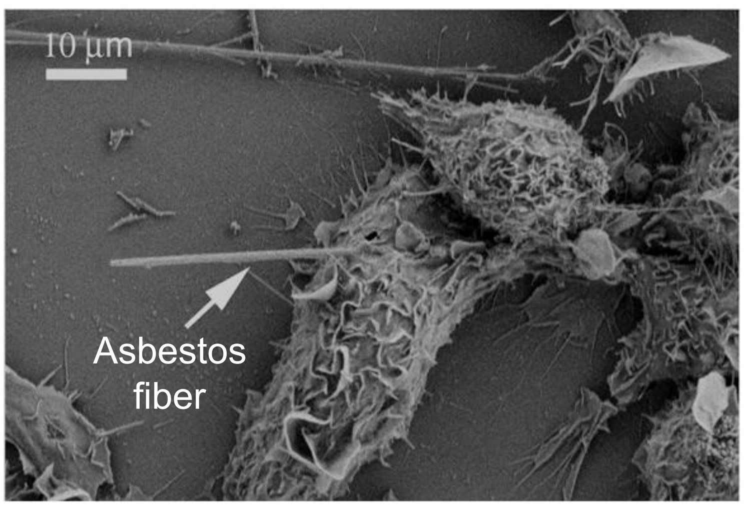
The Effect of CNT Geometry

The Carbon Nanotube / Asbestos Analogy
A. Kane and R. Hurt, Nature Nanotechnology, 2008

Prof. A. Kane
Pathology and Laboratory Medicine



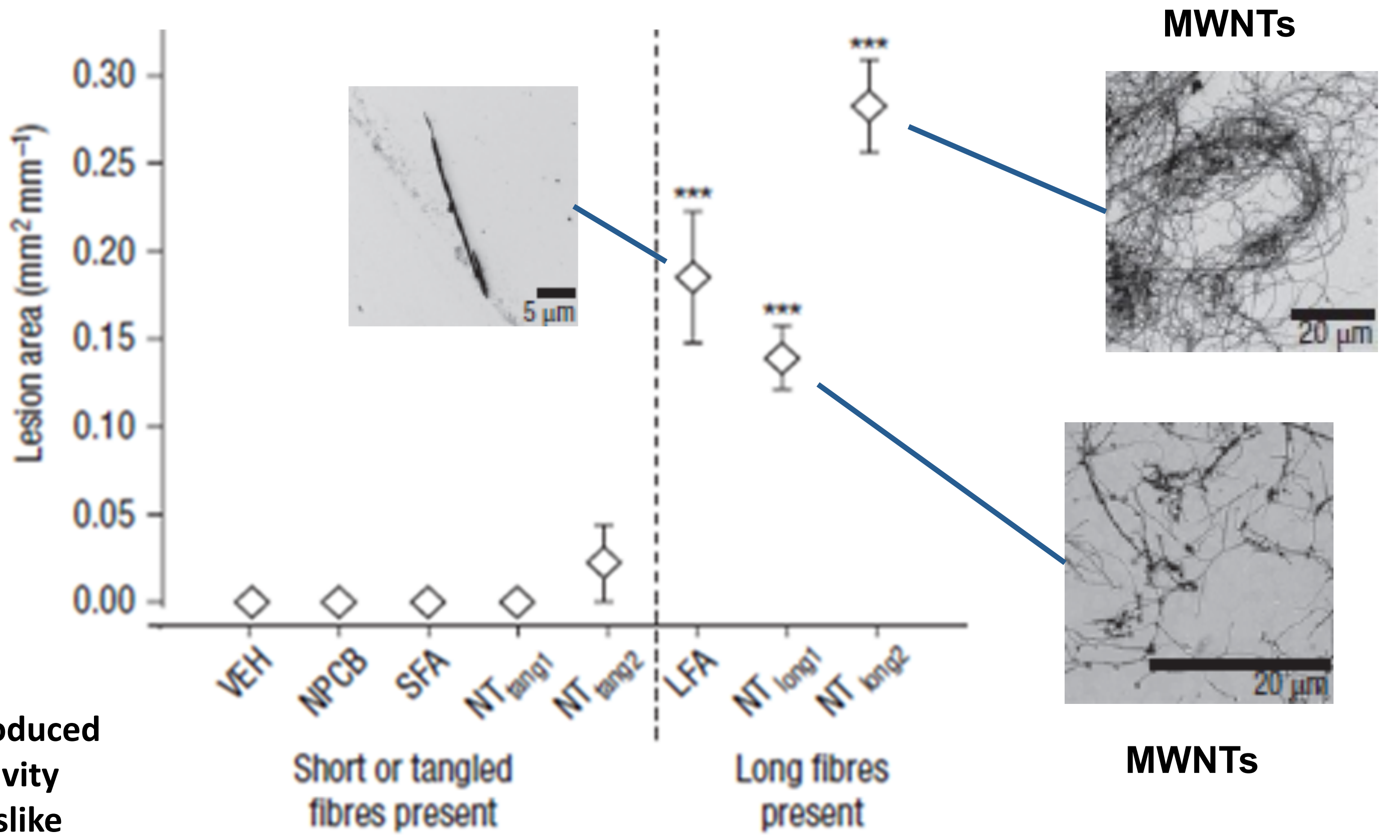
Mesothelium
(linings of lung and abdominal cavity)



The Fiber Pathogenicity Paradigm

Asbestos-like mechanisms
may arise for fibers with :
d <~ 3 um
(for inhalation into the deep lung)
and
L > 10-20 um
(for impaired clearance by macrophages)
and
biopersistence
(for long-term effects)

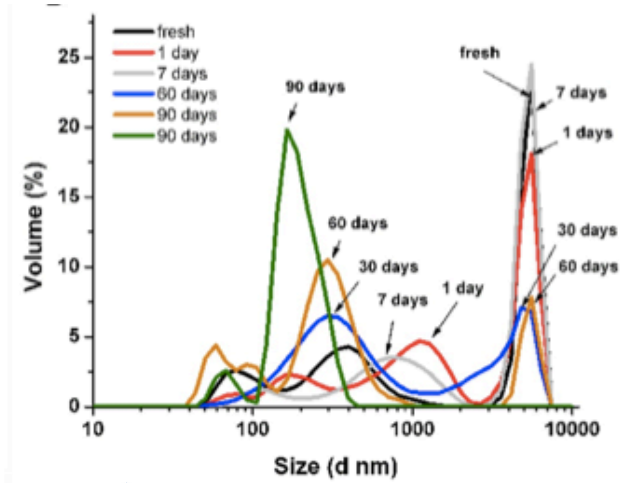
There is evidence that long length (> 10-20 um) impairs lung clearance and also causes cytotoxicity



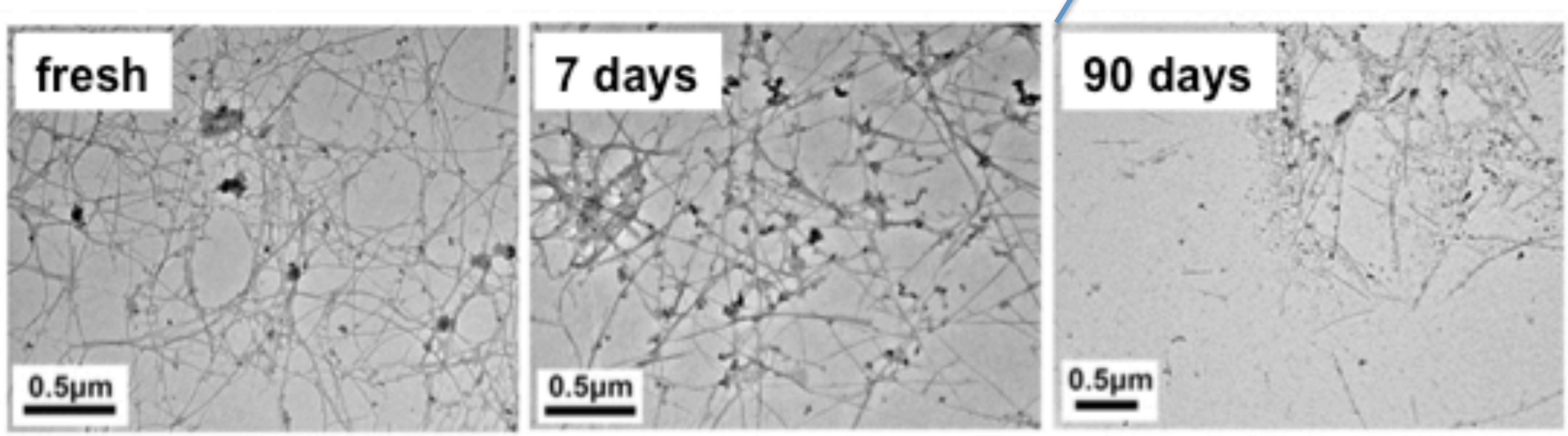
"Carbon nanotubes introduced into the abdominal cavity of mice show asbestoslike pathogenicity in a pilot study", Poland et al., *Nature Nanotechnology*, 2008

Biopersistence / biodegradability

- Biopersistence is the single most important material property influencing toxicity of respirable fibers.
- Environmental persistence greatly increases the potential adverse impact of a toxicant
- Carbon does not dissolve, but can, in principle, be oxidized



Carboxylate-functionalized SWNTs <u>do</u> degrade during 90 days in phagolysosomal simulant fluid



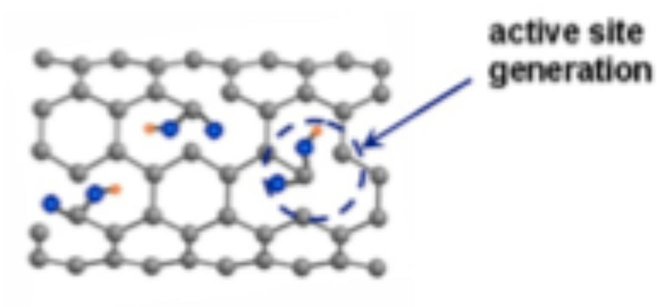
Biodegradation of Single-Walled Carbon Nanotubes through Enzymatic Catalysis

Brett L. Allen,† Padmakar D. Kichambare,† Pingping Gou,† Irina I. Vlasova,‡ Alexander A. Kapralov,‡ Nagarjun Konduru,‡ Valerian E. Kagan,‡ and Alexander Star*,†

Departments of Chemistry, and Environmental & Occupational Health, University of Pittsburgh, Pittsburgh, Pennsylvania 15260

NANO LETTERS

2008
Vol. 8, No. 11
3899-3903



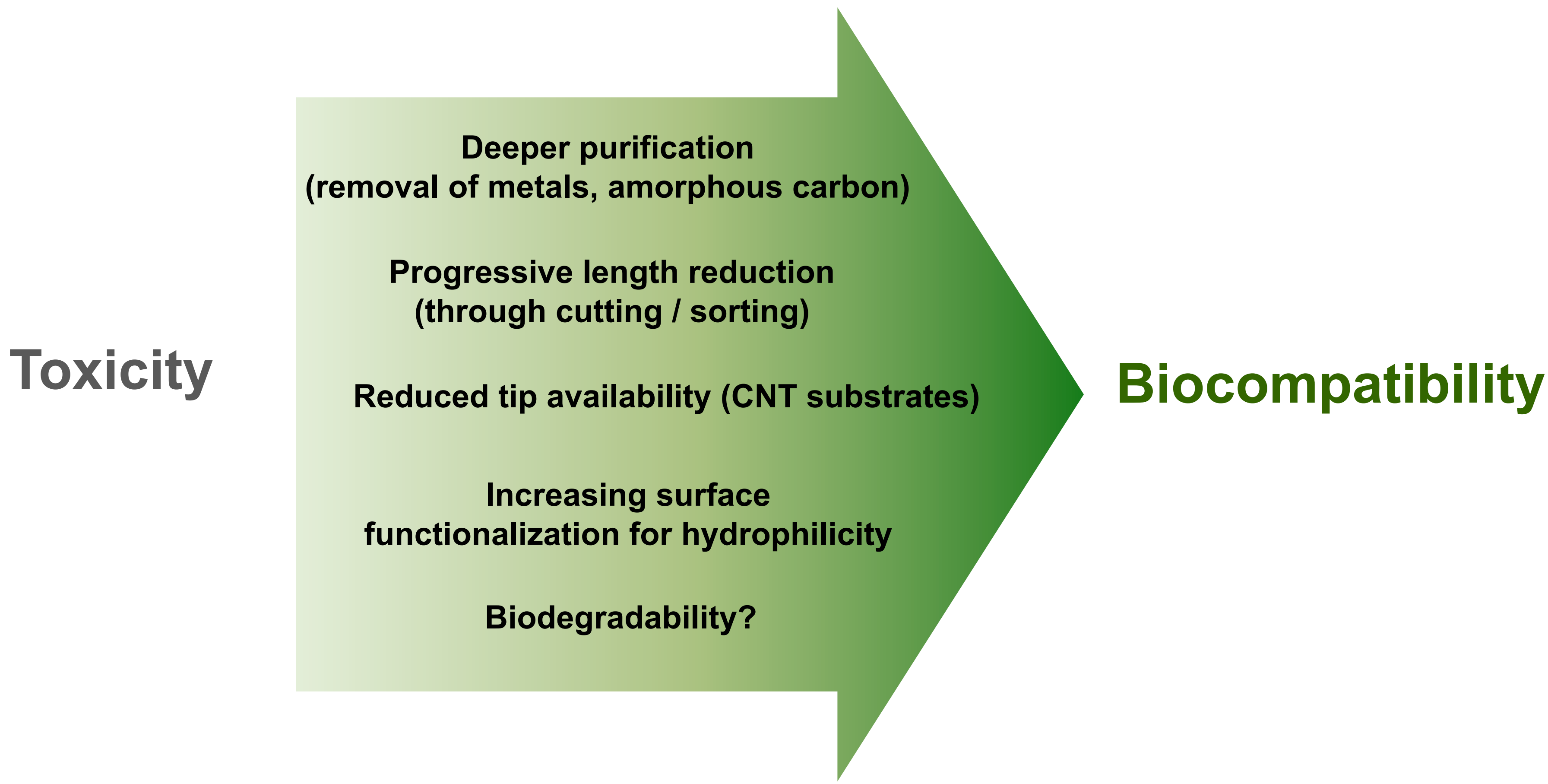
Only a few nanotube types show visible biodegradation [Liu et al., "Biodurability….", Carbon, 2010]

Sample	Functionalization	Observation
SWNTs (d:1-2nm)	Non-functionalized	No significant change
	Aryl sulfonate	No significant change
	Ozonate	No significant change
	1000ºC treated	No significant change
	Carboxylate	No significant change
	Commercial (nitric acid treated)	Visible degradation
	In-house functionalized (mixed acid treated)	
	15mi	No significant change
	1hr	Visible degradation
	3hr	Visible degradation
MWNTs (d: 35±10nm)	Non-functionalized	No significant change
	Aryl-sulfonate	No significant change
	CNFs (d: 200nm)	
	Non-functionalized	No significant change
	Aryl sulfonate	No significant change
Reference Materials	Wollasonite	Dissolved
	Crocidite Asbestos (d: 30-150nm)	No significant change

The only degradable nanotubes are SWNTs treated with oxidizing acids (HNO_3 / H_2SO_4), which are known to produce COOH groups

A hypothesis for carbon nanotubes:

Toxicity / biocompatibility is determined by multiple material features



CNTs are a good case study in that *material features and formulation* appear to matter

What can we do with this information?

Case I: Engineered CNTs in end applications

Data suggest real opportunities to design for safety, through:

- Binding to substrates or in matrices (no free tips)
- Shortening
- Deep purification
- Surface functionalization for hydrophilicity
- Biodegradability (?)

Can/should we define different hazard categories?

Case II: Primary manufacture

Exposures likely occur before opportunity to engineer properties

So: control exposure

- process release / respirators / air filtration

Case III: Misuse, Accident Scenarios, and End-of-Life

- Manufacturers lose control of their products after sale

- We need to envision, assess, and manage risk is multiple scenarios by managing hazard or exposure

Financial support is acknowledged from the NIEHS R01 and Superfund basic research program, NSF NIRT program, EPA STAR grants

Contributors

Materials Chemistry
Prof. Robert Hurt
Xinyuan Liu
Lorin Jakubek
Aihui Yan
Lin Guo
Dr. Indrek Kulaots
Daniel Morris

Pathobiology
Prof. Agnes Kane
Prof. Charles A. Vaslet
Ashley Smith
Dr. Annette Von Dem Bussche
Kevin McNeil
Vanesa Sanchez
Jody Pietruska

Neuroscience
Prof. Diane Lipscombe
Jessica Raingo
Spiro Marangoudakis

This research is funded by
U.S. EPA - Science To Achieve Results (STAR) Program
Grant # RD-83171901-0