Toxicologically Relevant Characterization of Carbon Nanomaterials

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Nanotoxicology

Some nanomaterial properties raise toxicity concerns

Small size

- small aerodynamic diameter
  → deep lung penetration
- high permeability in biological membranes
- enhanced cellular uptake
  (endocytosis, phagocytosis)

High surface area

- high surface activity
- facilitated transport – the “Trojan horse” effect

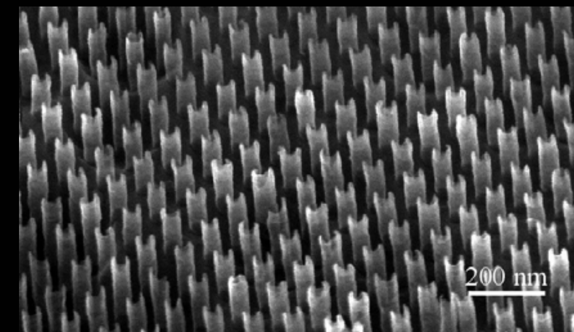
Fibrous morphology

- entanglement and airway blockage
- difficulty with macrophage clearance

carbon nanotubes: 100 gms!

Is there a Role for Materials Science in Nanotoxicology?

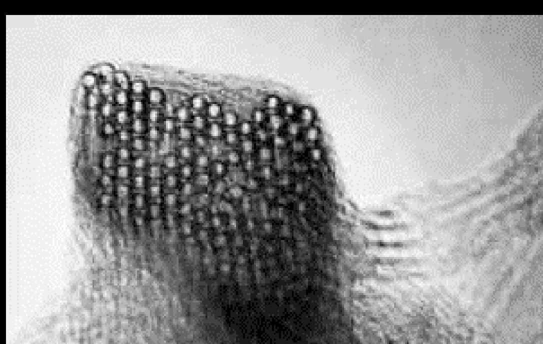
Carbon nanotubes are a *family* of complex materials



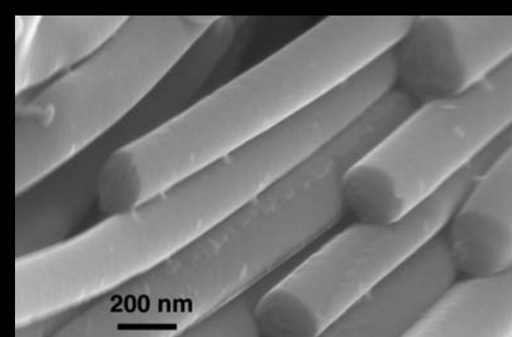
Xu et al. *Brown Univ.*

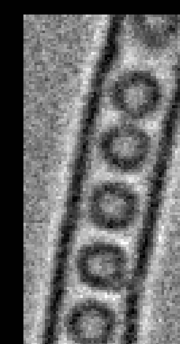
CNTs vary greatly in:

- diameter (0.4 nm – 200 nm) and length
- surface chemistry (hydrophilic / phobic)
- coatings (polymers, surfactants, proteins, DNA).
- aggregation state
- composition (Fe, Co, Y, Ni, amorphous carbon, graphite)



Thess et al. *Science,* 1996





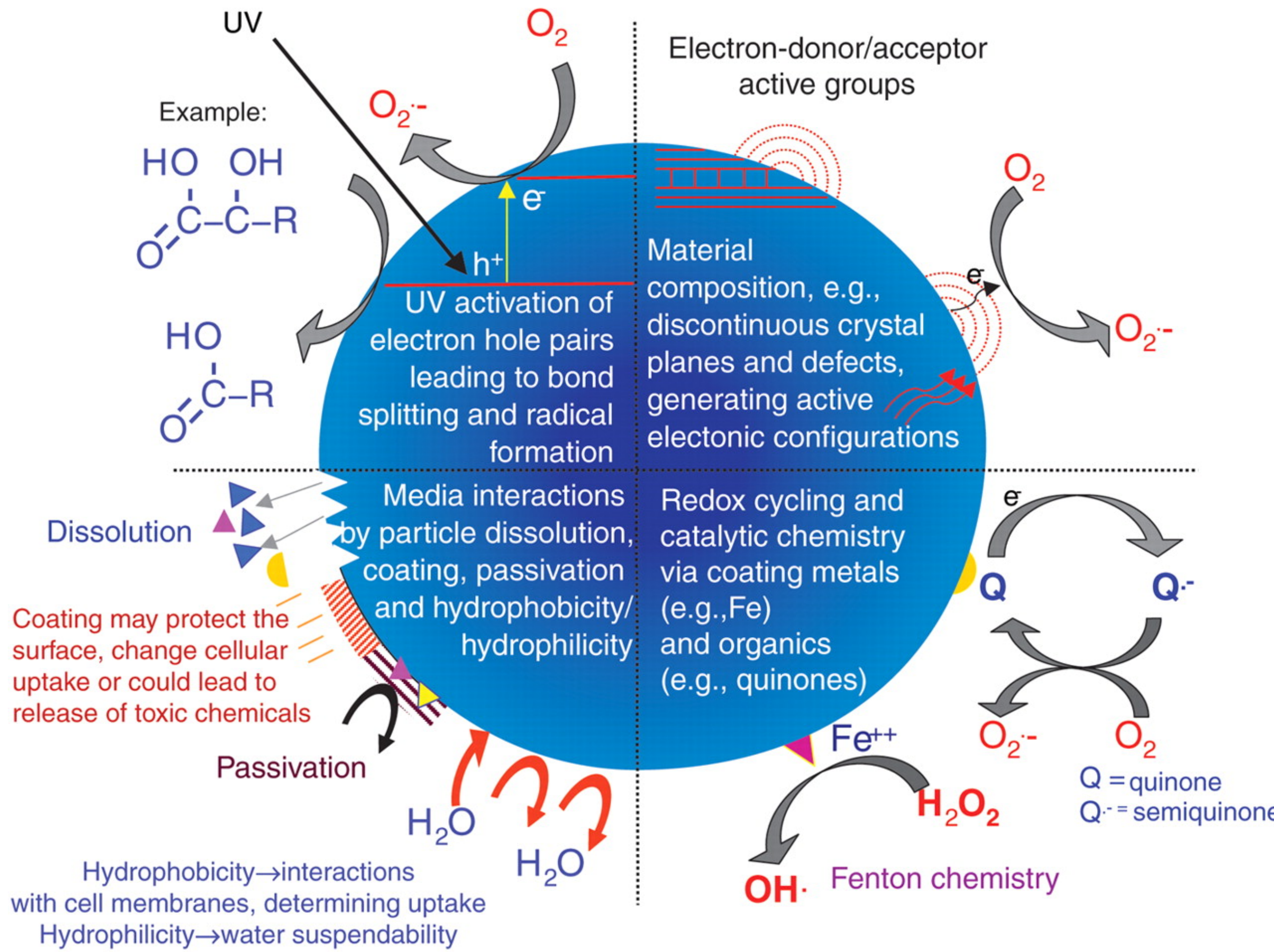
Basic nanomaterial properties relevant to toxicity

> Dose

> Size and shape

> Biopersistence

> Surface chemistry



Surface Reactivity of Nanoparticles

Nel et al. Science 311: 622-627, 2006

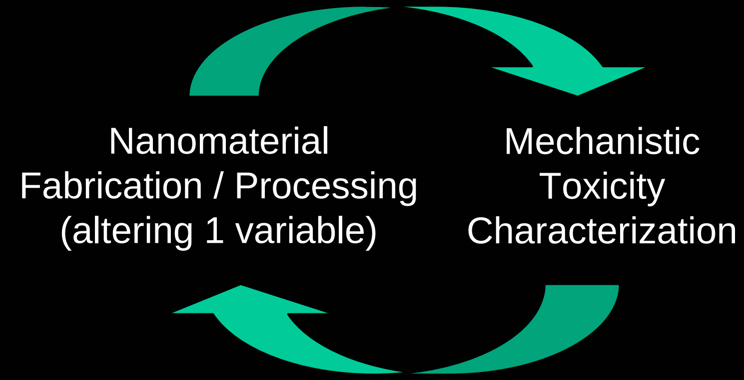
Suggested Roles for Materials Scientists

1. professional characterization of materials used in toxicity testing

Material names “MWNTs” or “SWNTs” are not enough !

Key issue: what characterization methods are most relevant to toxicity?

2. synthesis / processing of model materials for *mechanistic* toxicology

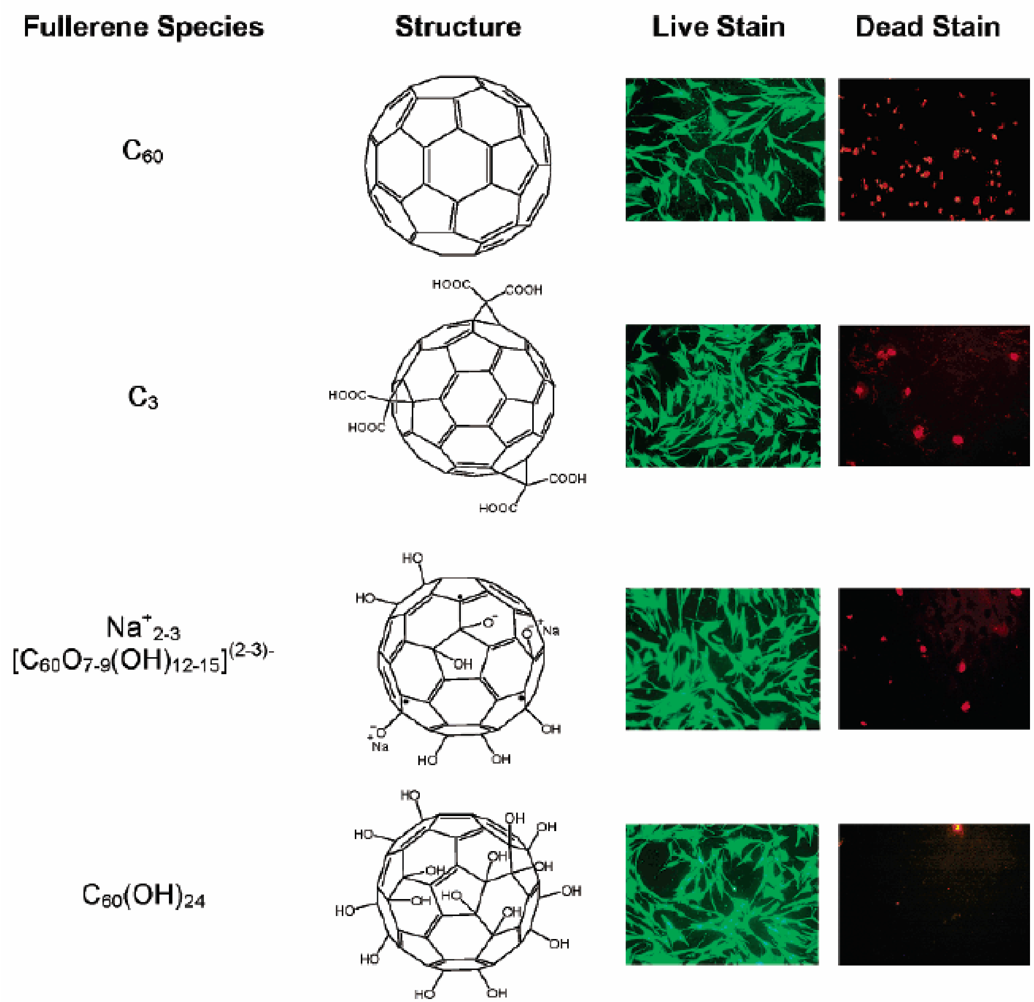


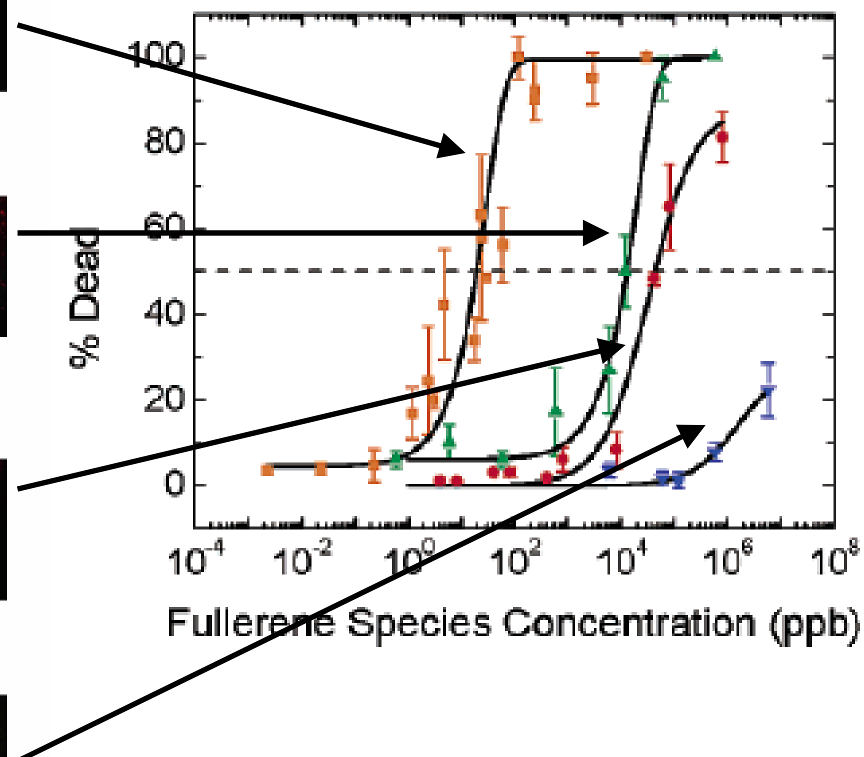
3. development of “green” nanomaterials or eco-nanomaterials

-- the co-optimization of materials for both function and safety *

* also known as “materials safety by design”

Toxicity of Fullerenes Depends on Surface State

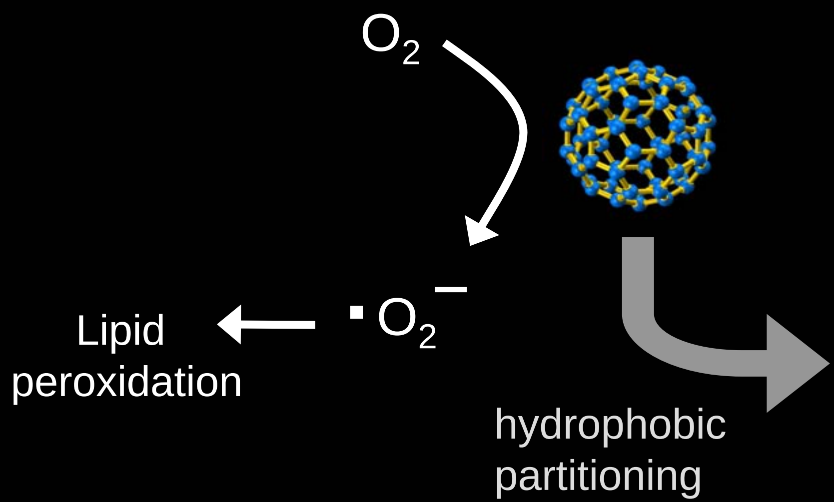




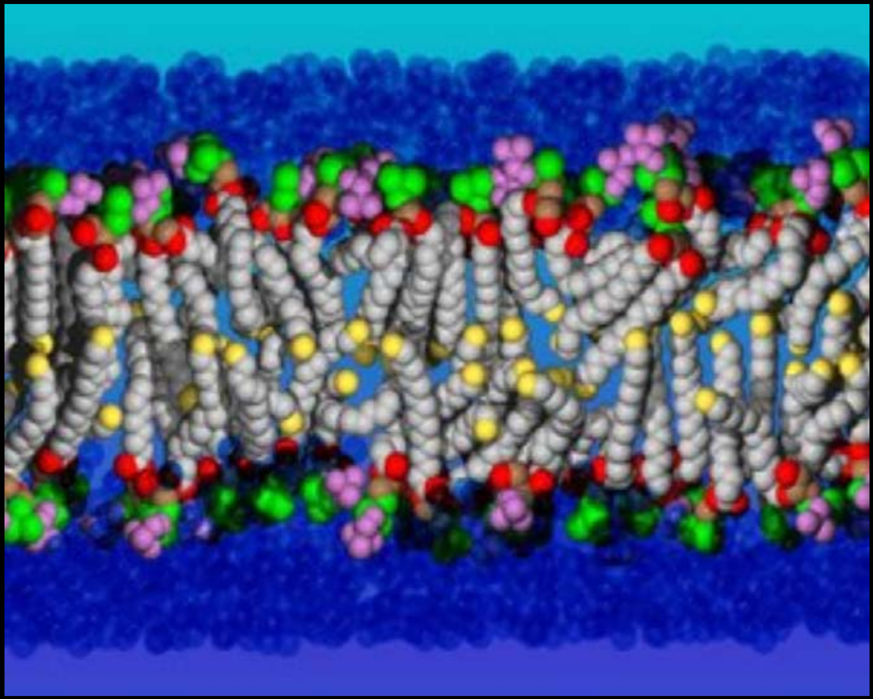
Sayes et al., NANO LETTERS
2004 Vol. 4, No. 10
1881-1887

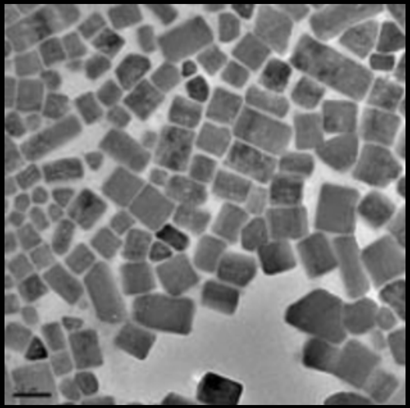
Possible Mechanisms of Fullerene Toxicity

based on Sayes et al., 2004, Oberdorster, 2004



Simulated lipid bilayer structure



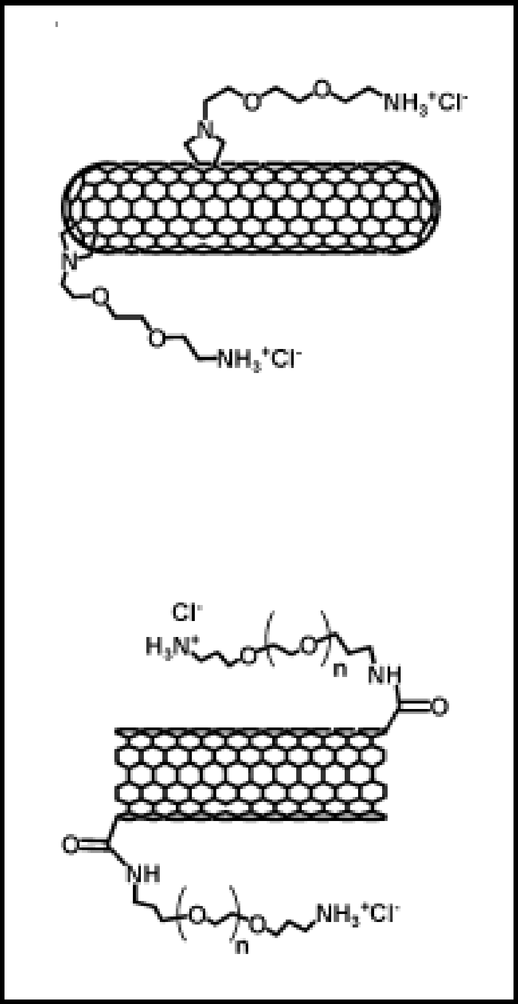


Unsubstituted fullerene aggregates in aqueous media to form “nano-C60”

Postulated mechanism:
Hydrophobic attachment to / incorporation in cell membranes with redox catalysis of lipid peroxidation

Toxicity of Functionalized Carbon Nanotubes

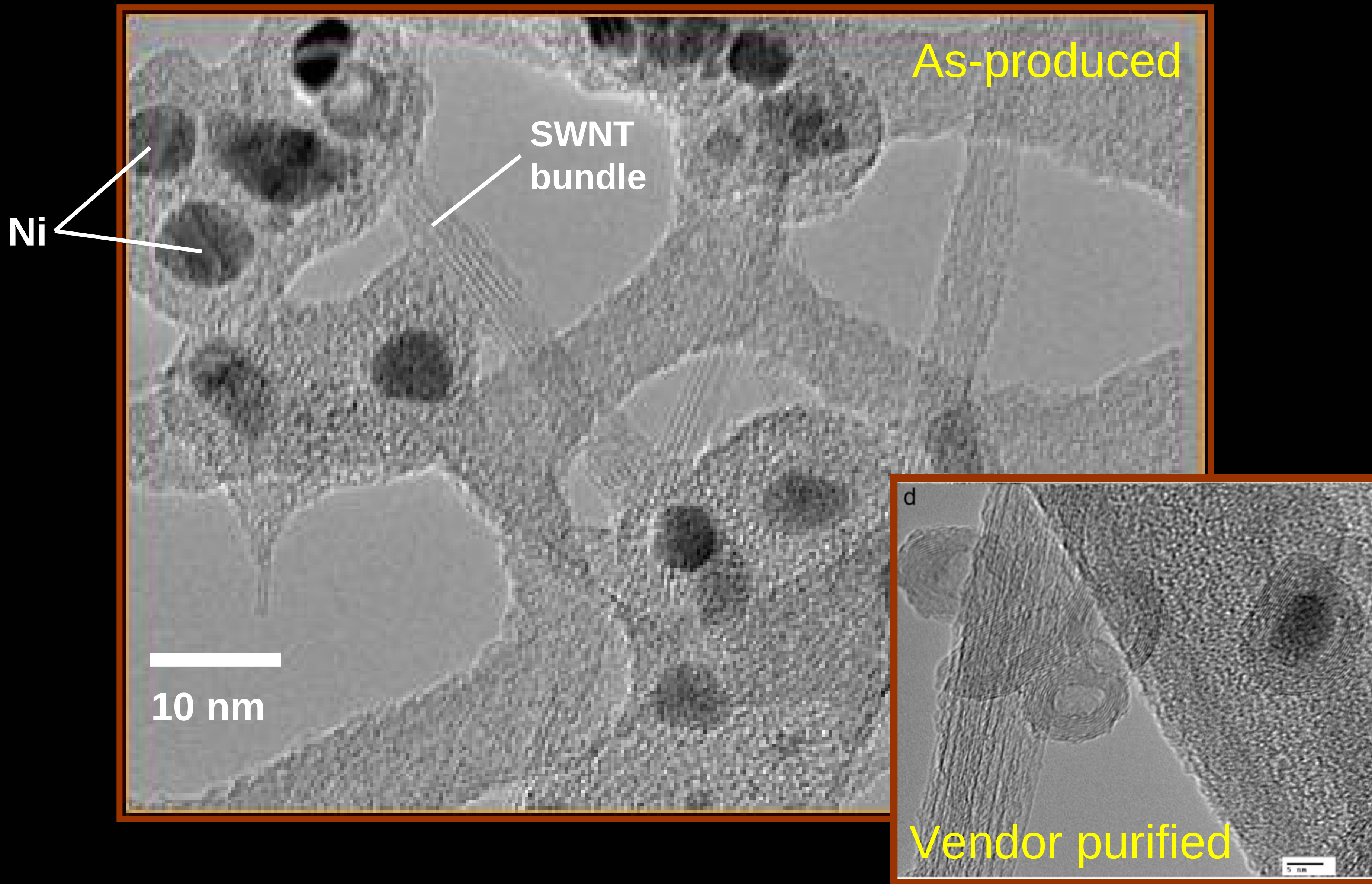
Dumortier et al., Nano Letters, 2006



- Both CNT types are taken up by immune cells (B, T lymphocytes, macrophages) and are *non-cytotoxic*

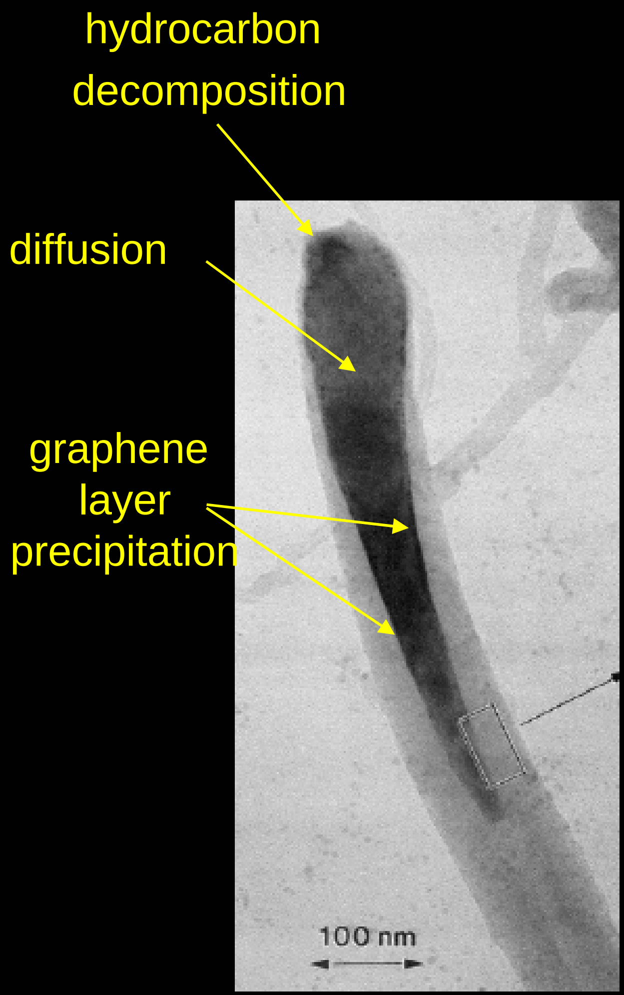
- The less soluble type (below) does elicit release of pro-inflammatory cytokines from macrophages; the more soluble formulation (above) does not

Catalyst Residues in Carbon Nanotube Samples



Catalytic Synthesis of Carbon Nanotubes

Vendor A	AP SWNT	Ni-Y (35%)
B	AP SWNT	Ni-Co (25%)
C	AP SWNT High purity (HP)	Ni-Y (25%) Co-Mo (<2%)
D	AP SWNT Purified SWNT	Ni-Y (30%) Ni-Y (15%)
E	Unpurified MWNT Purified MWNT	Fe (4.2%) Fe (0.1%)
F	Unpurified MWNT Purified MWNT	Fe (4.25%) Fe (3.29%)
G	Unpurified SWNT Purified SWNT	Fe (22.2%) Fe (10.9%)



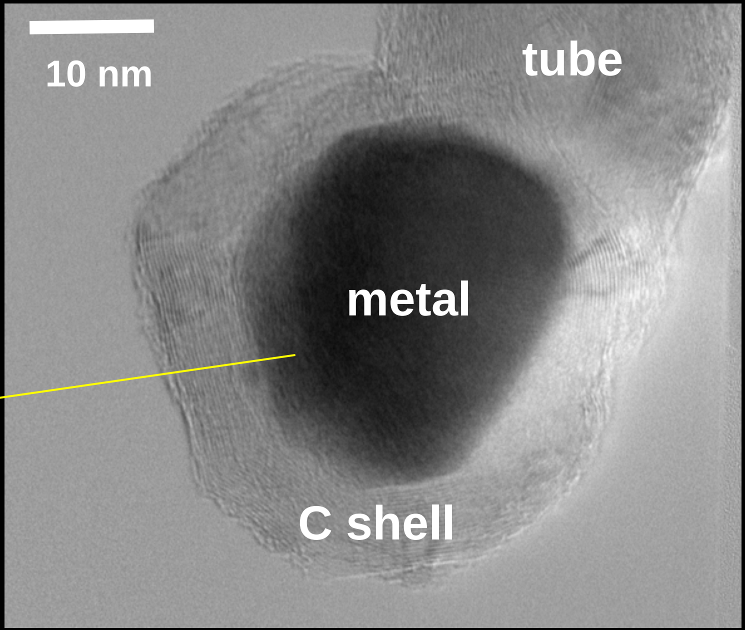
Can metals cause or contribute to CNT toxicity?

- Catalytic growth methods:
  -- now dominant for synthesis of multiwall nanotubes (esp. large scale)
  -- only route for single-wall nanotube synthesis

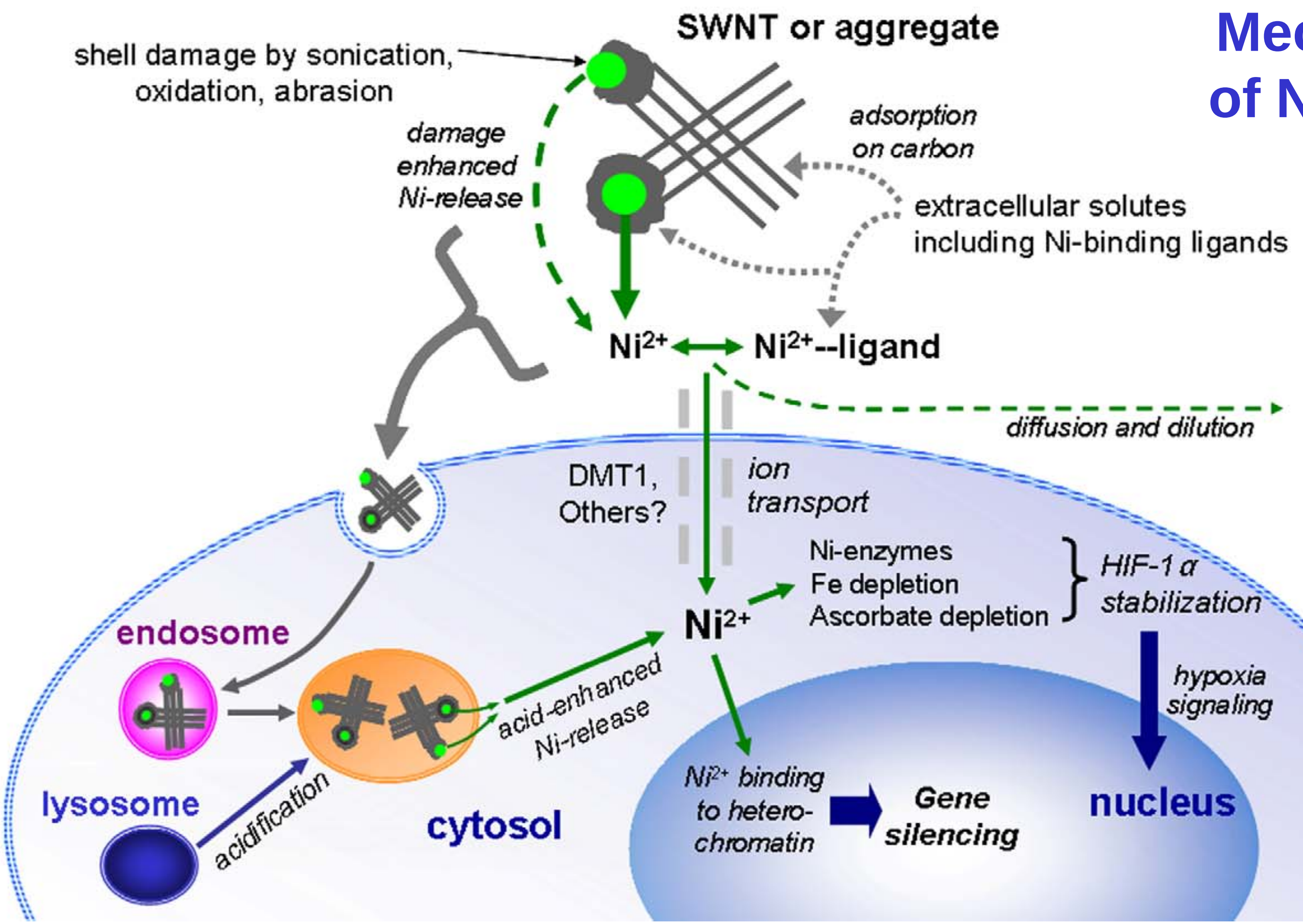
- Over 20 metals have been used to synthesize carbon nanotubes.
  Most common elements in CNT catalyst formulations are Fe, Ni, Y, Co, Mo

- Ultrafine metals pose documented inhalation health risks depending on form, exposure route, dose

Bioavailability?
(two viewpoints)



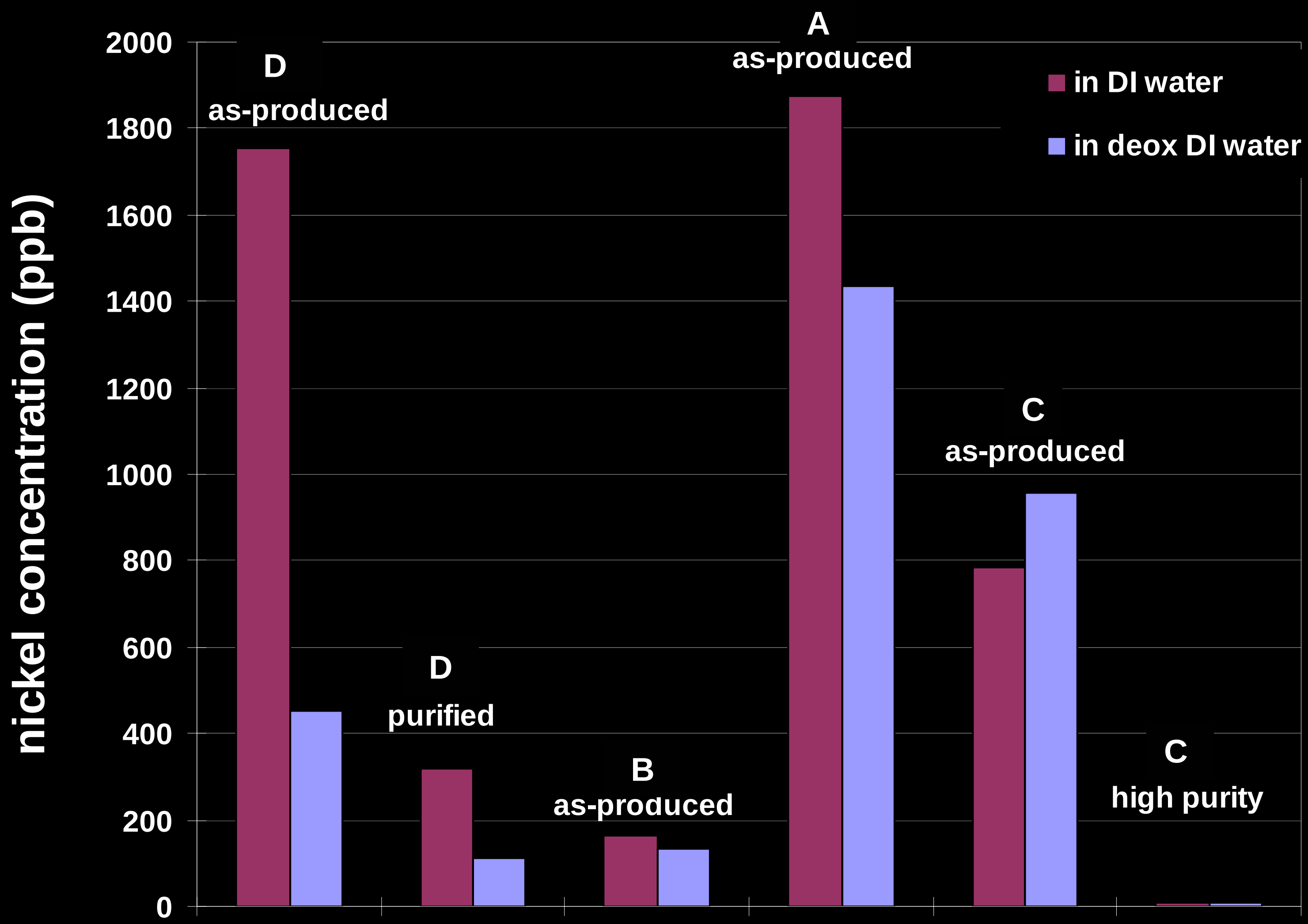
Molecular Mechanisms of Ni Toxicity



The nickel-ion hypothesis: nickel toxicity, carcinogenesis mainly depend on intracellular nickel(II) ion concentrations, independent of the original nickel compound [Snow, 1998].

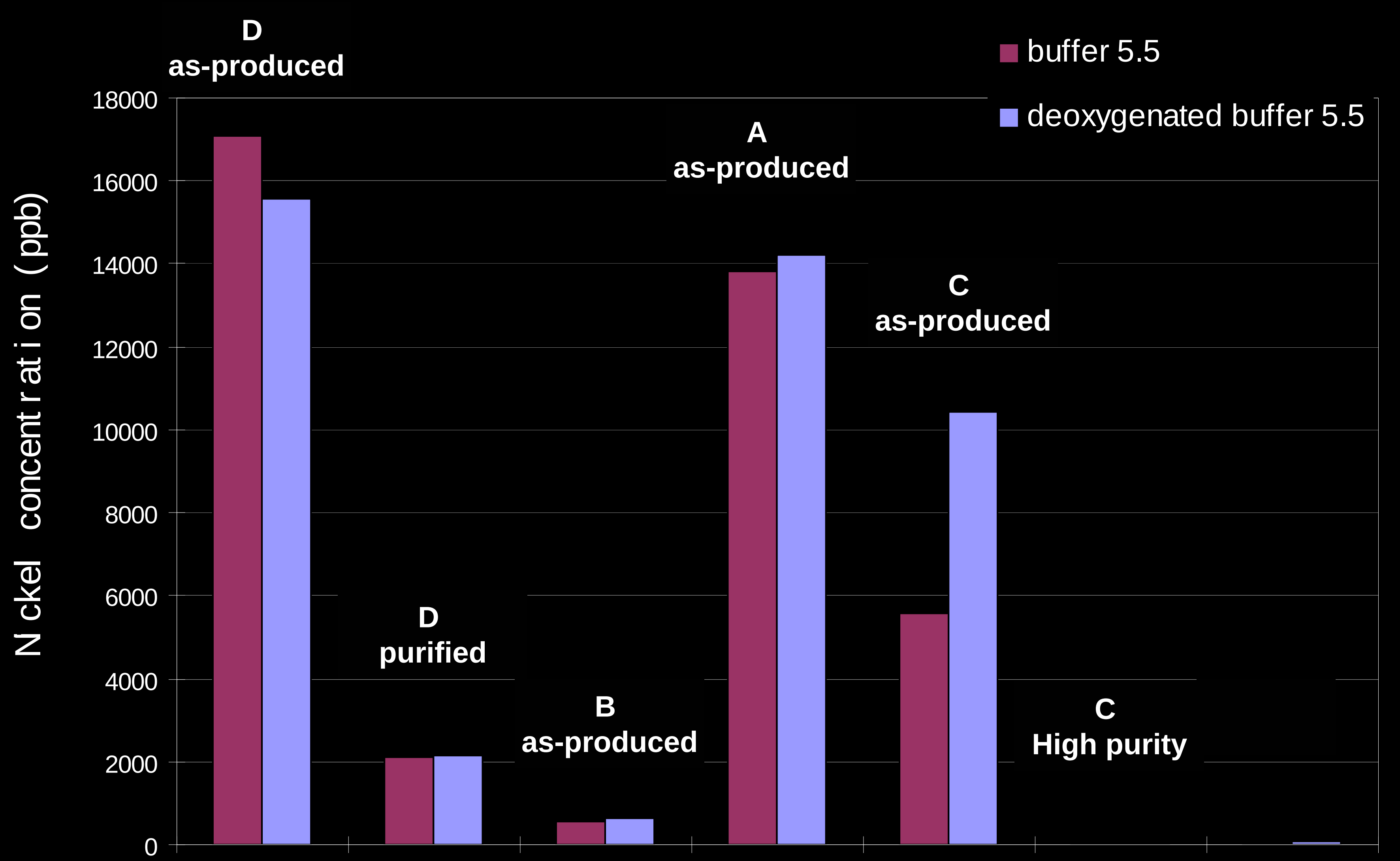
Ni mobilized at extracellular pH (7)

Various commercial samples

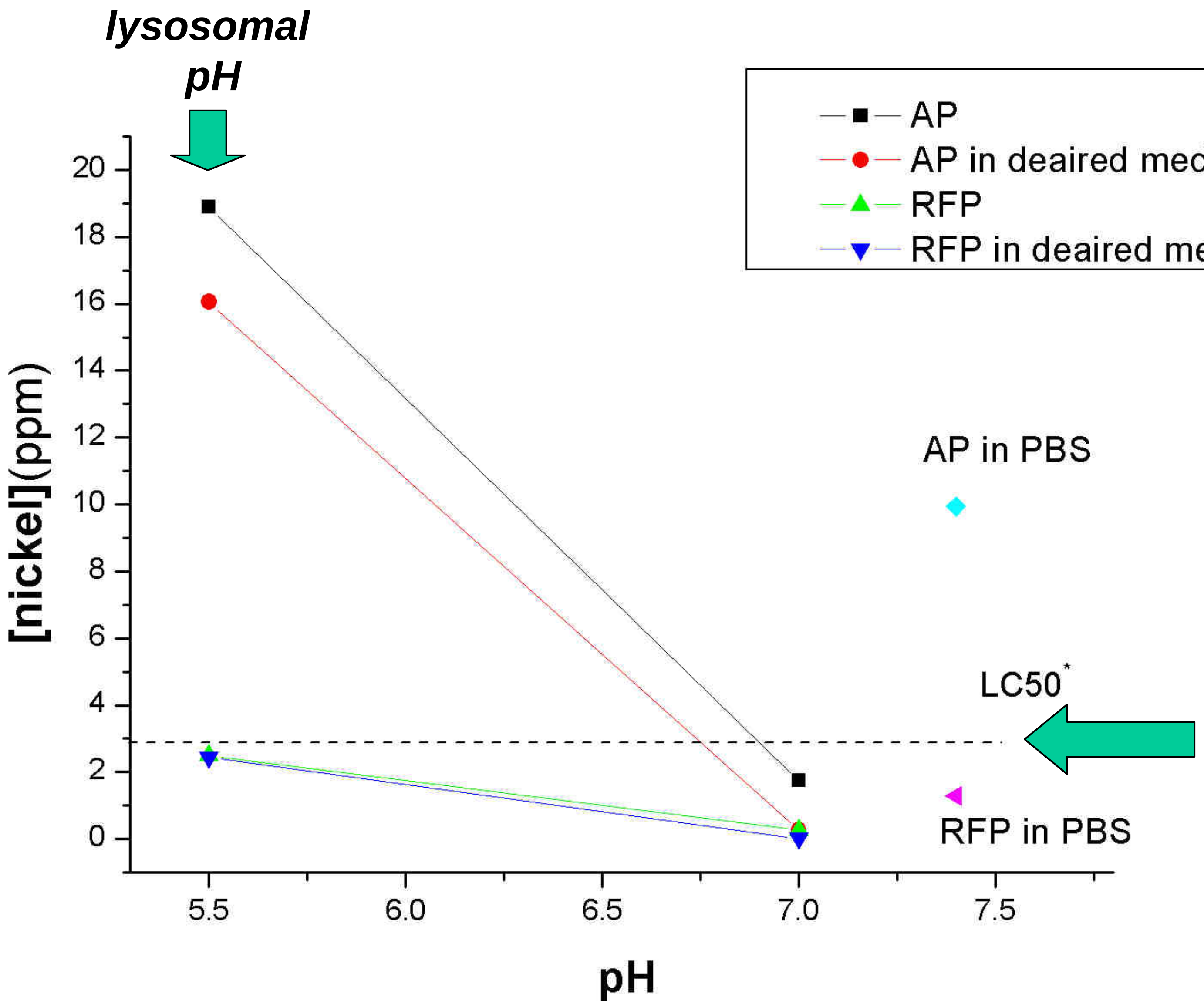


Ni mobilized at lysosomal pH (5.5)

Various commercial samples

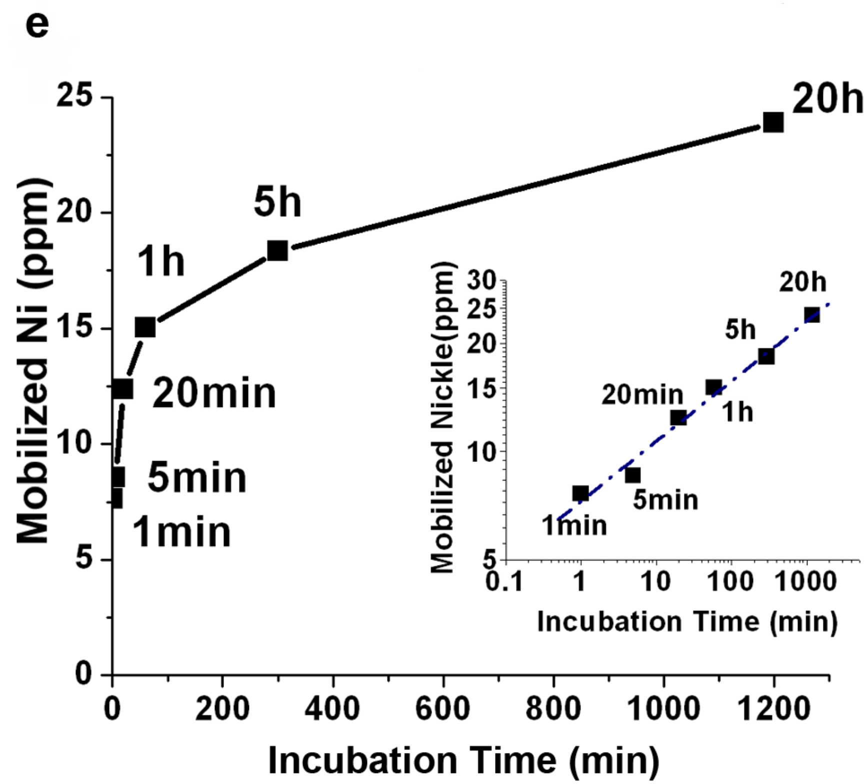


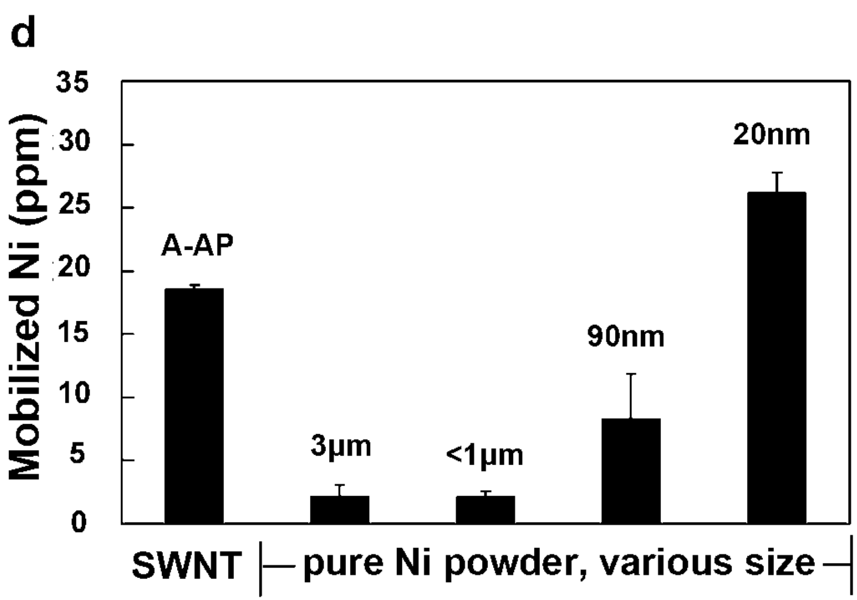
Nickel Mobilization from SWNTs: Effect of pH and Media



Lethal dose reported for [Ni^{+2}] added to culture medium for fibroblast Balb/3T3 clone A31 cells. [Taira et al, 2003]

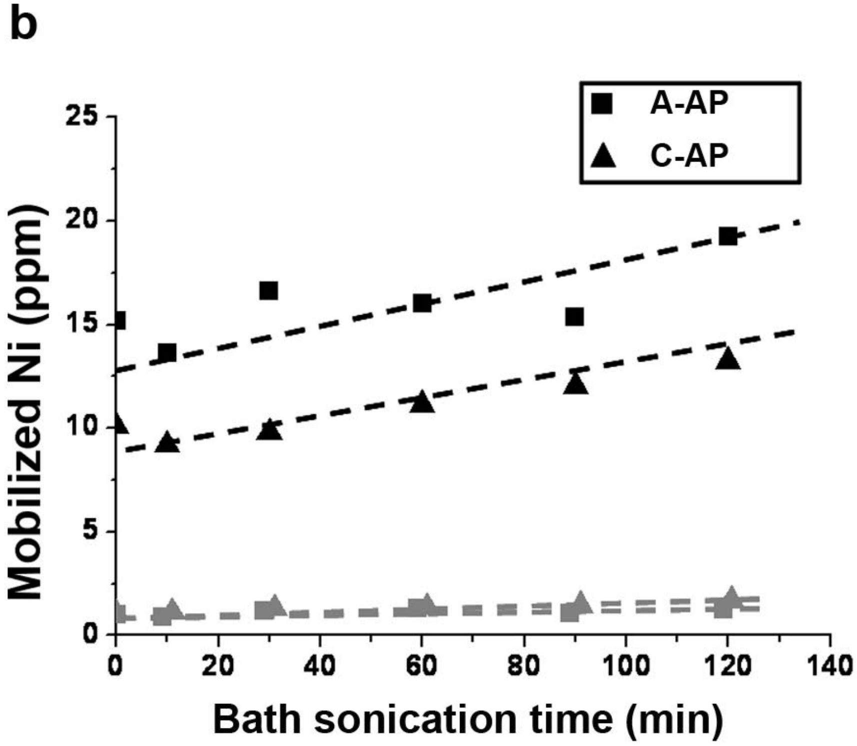
Ni Release: Dynamics and Encapsulation Effects



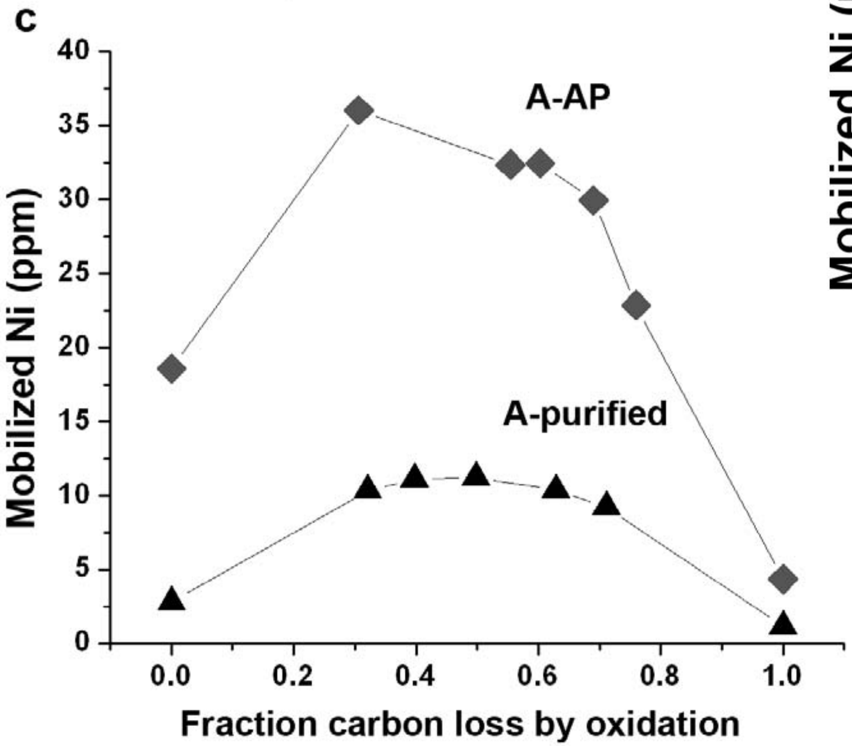


Can Material Stresses Enhance Ni Release?

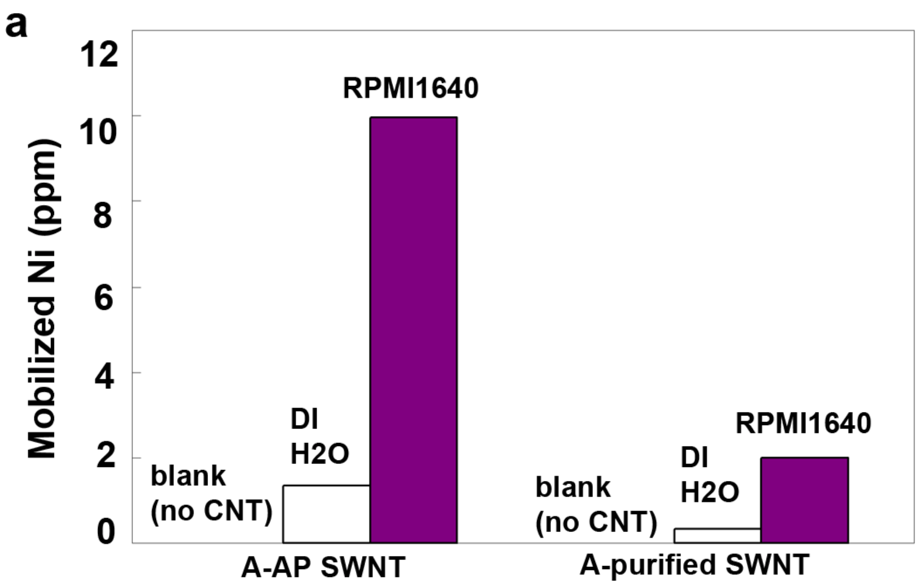
sonication



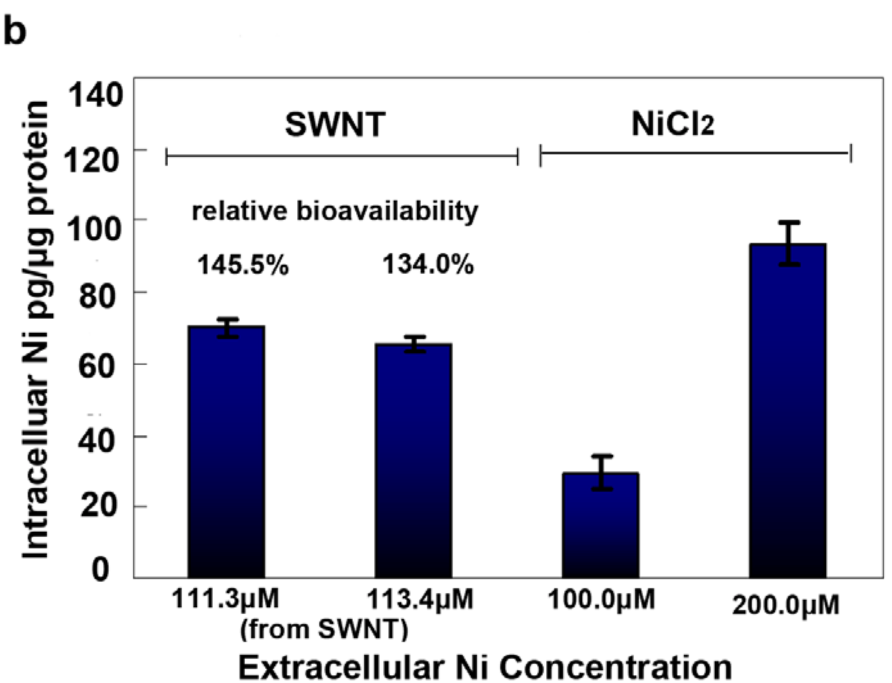
oxidation



Ni Release into Cell Culture Medium and Uptake by Human Lung Epithelial Cells

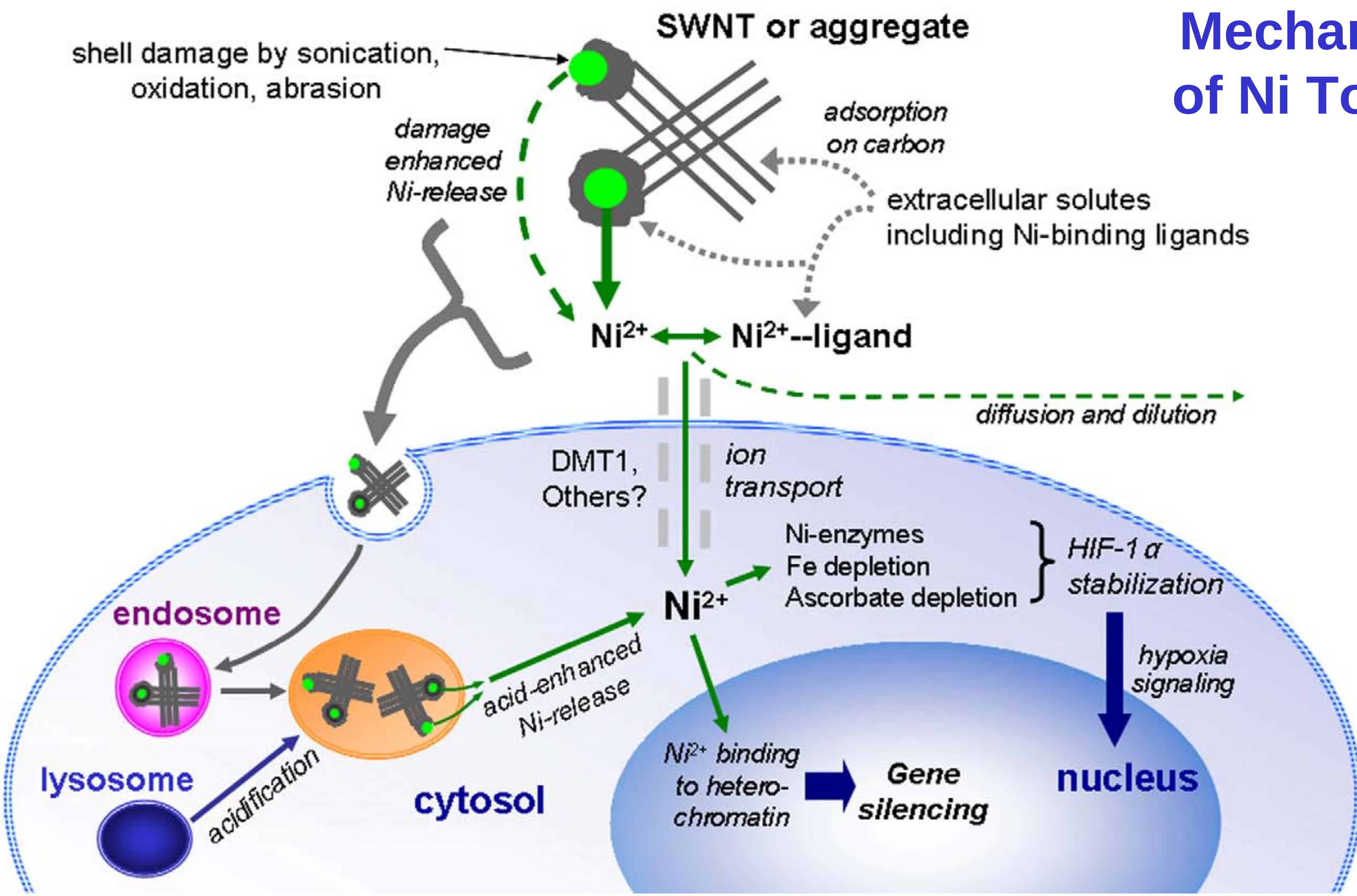


media solutes enhance Ni mobilization



CNT-Ni is more bioavailable than $NiCl_2$

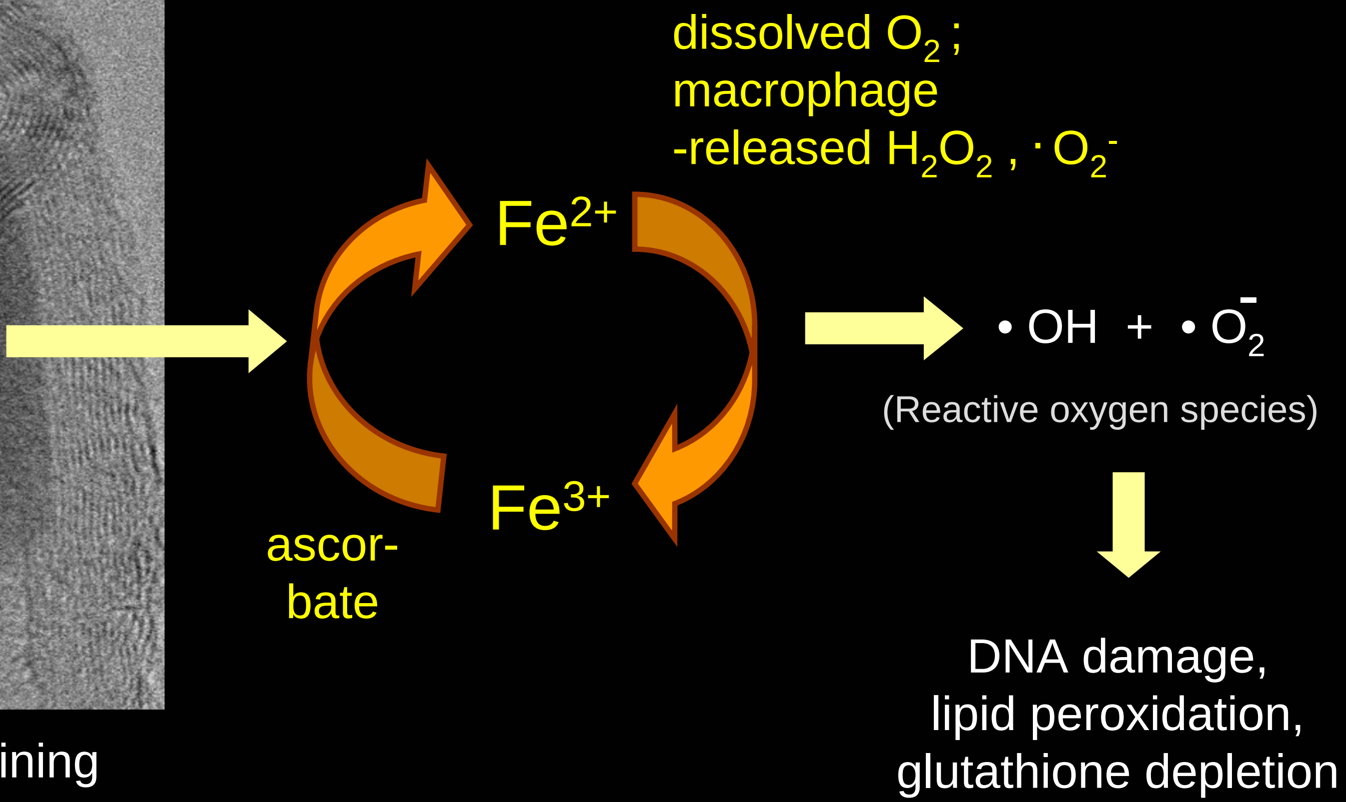
Molecular Mechanisms of Ni Toxicity



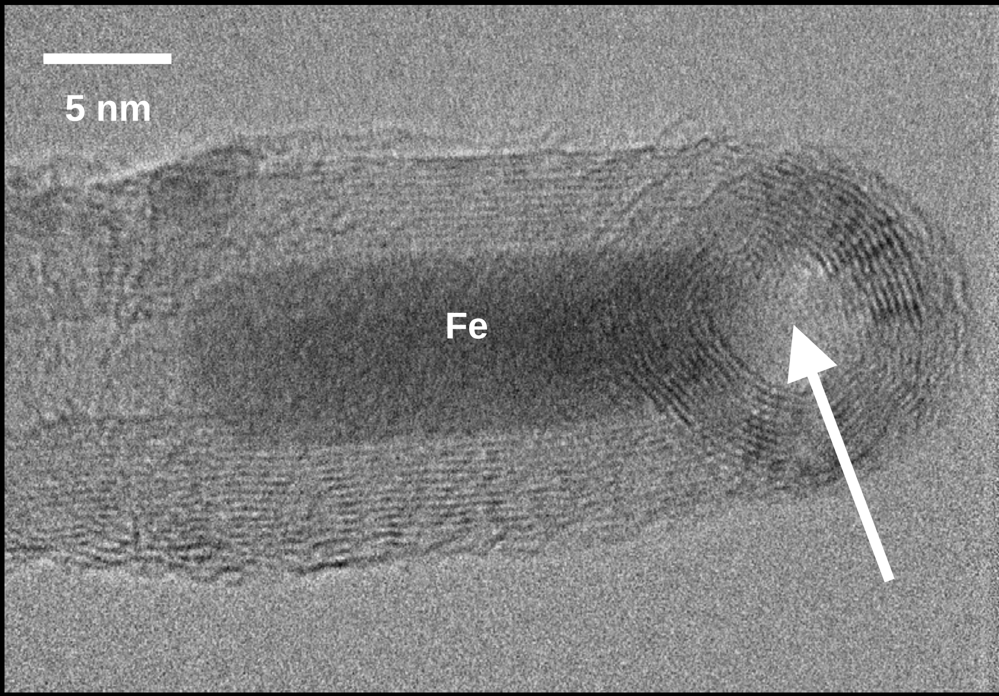
The nickel-ion hypothesis: nickel toxicity, carcinogenesis mainly depend on intracellular nickel(II) ion concentrations, independent of the original nickel compound [Snow, 1998].

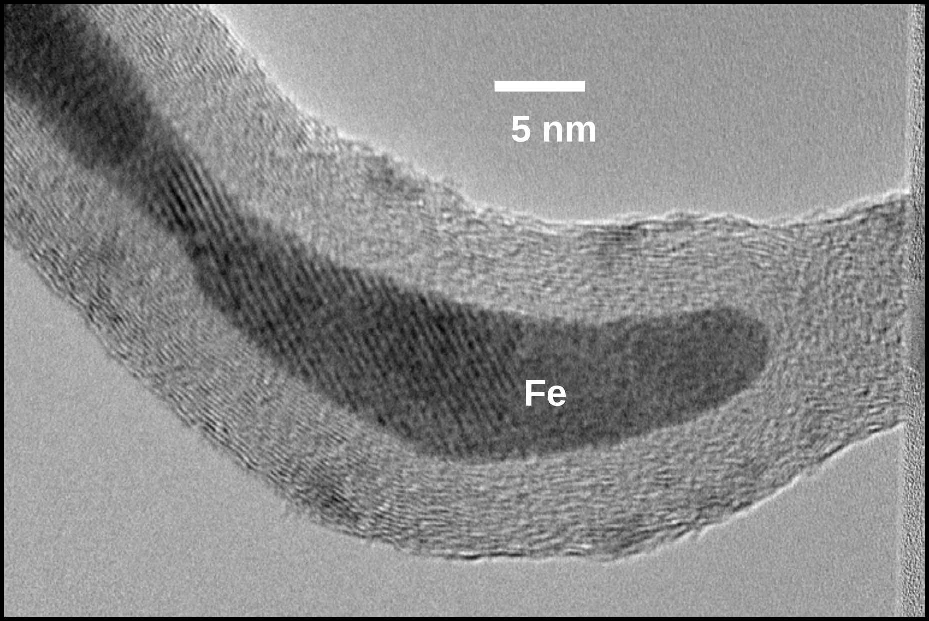
Free iron can catalyze radical generation and oxidative stress

Fe-containing CNTs

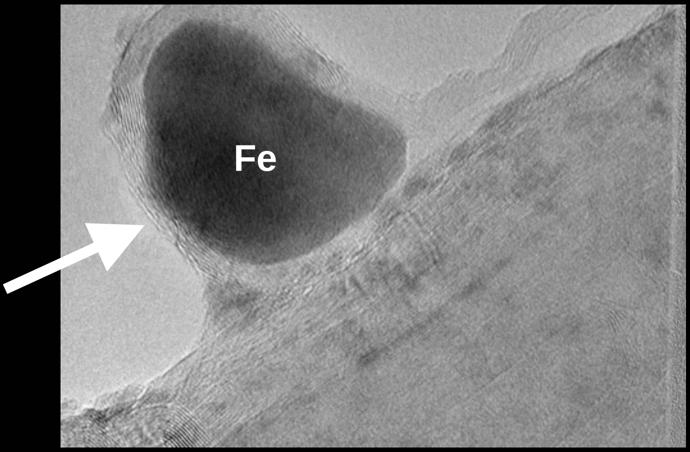


Fe/C Association Morphologies

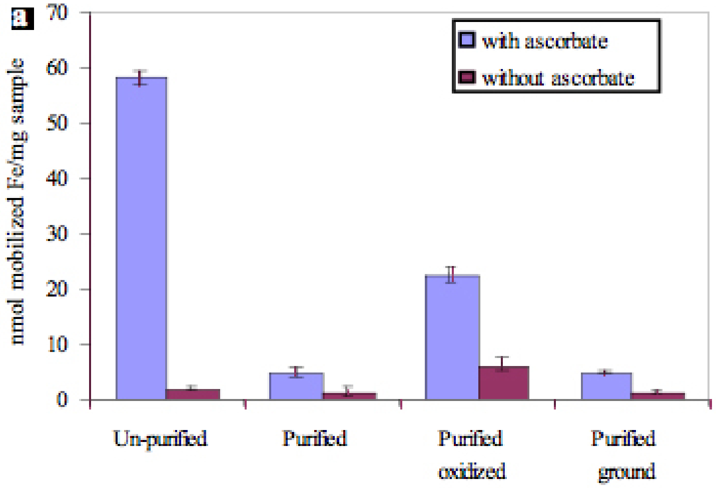


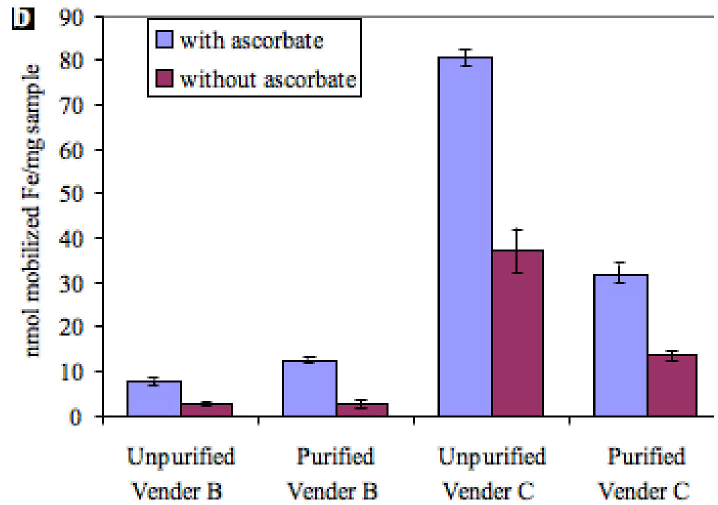


Fe-C morphologies in commercial CNTs

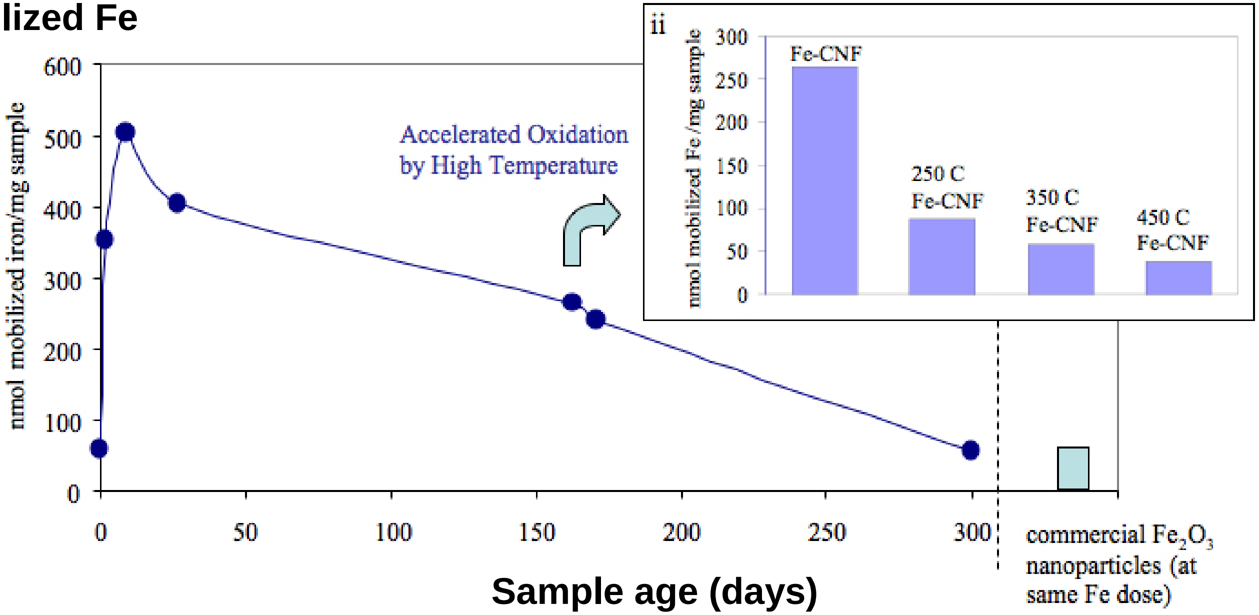


Fe Release From CNTs





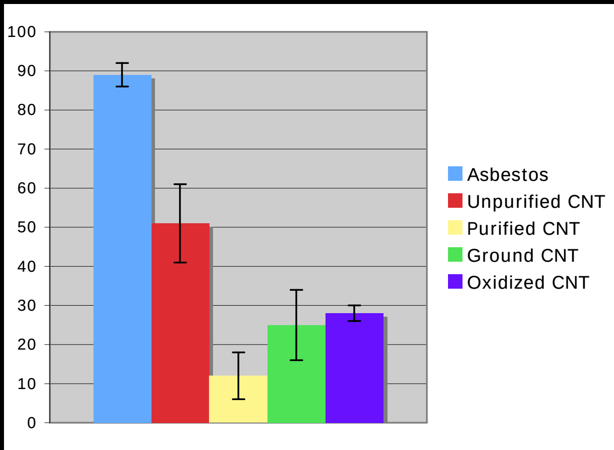
Mobilized Fe



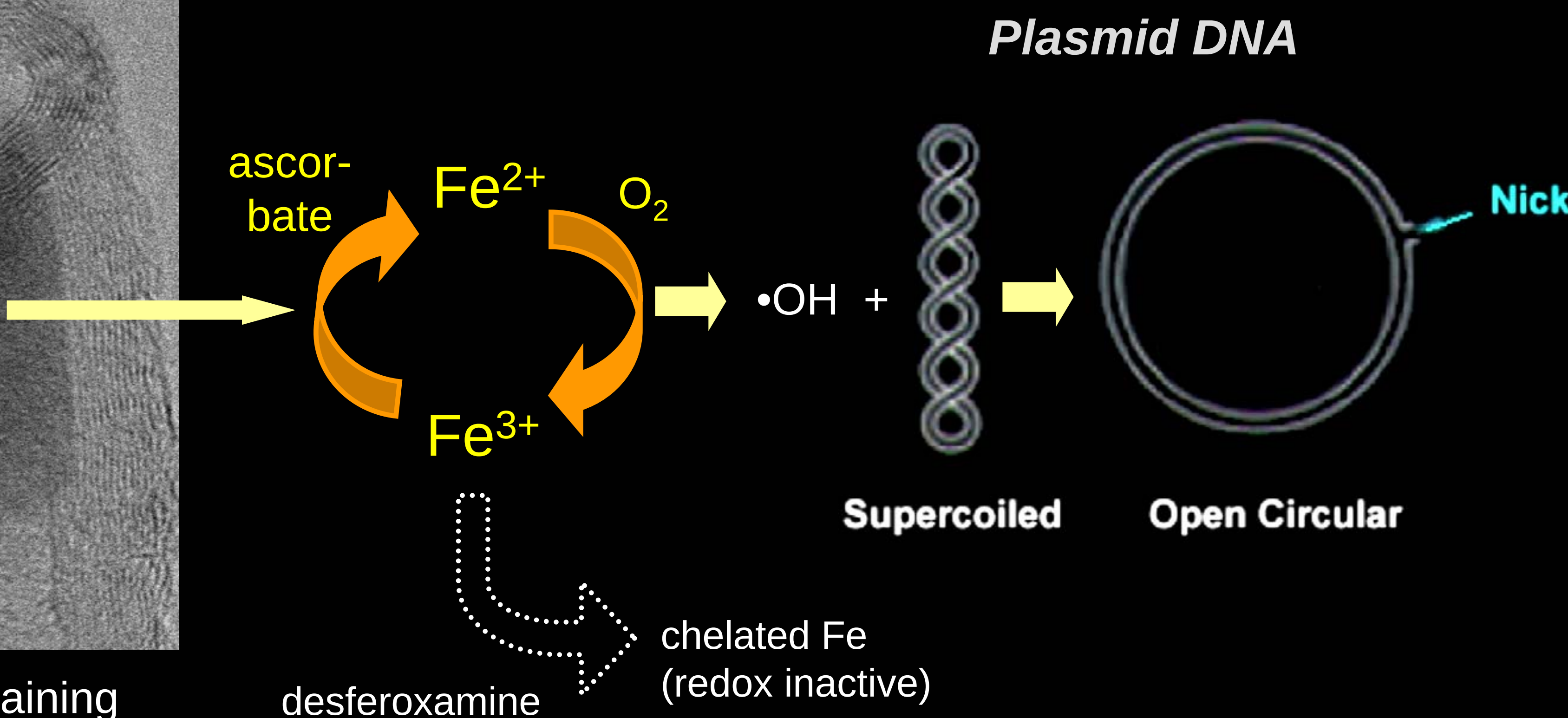
Are "fresh" nanotubes more toxic??

An acellular assay for redox-activity in iron-containing CNTs

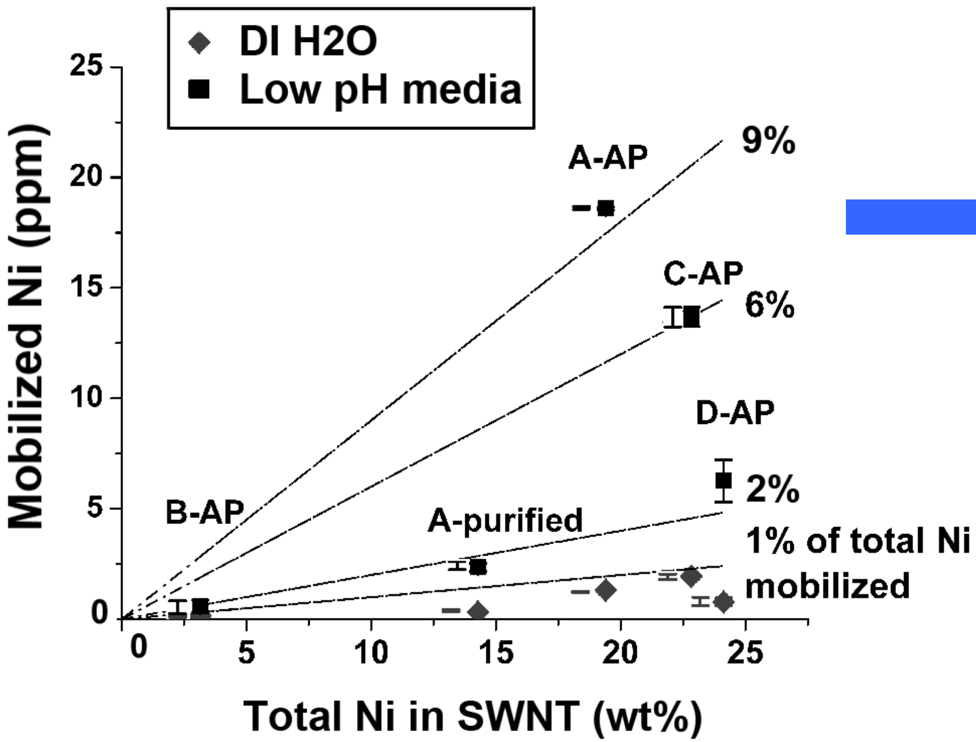
DNA single-strand breaks



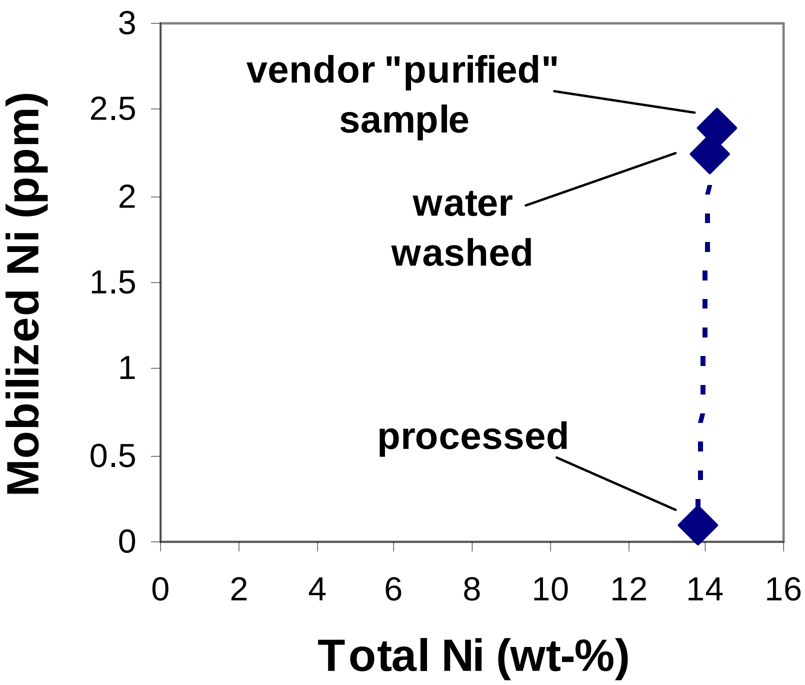
Fe-containing CNTs



Toward Carbon Nanotube Detoxification

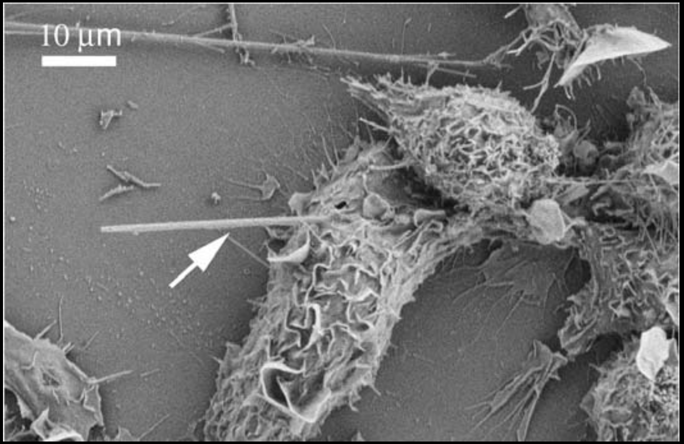


Targeted removal of bioavailable metal in carbon nanotubes



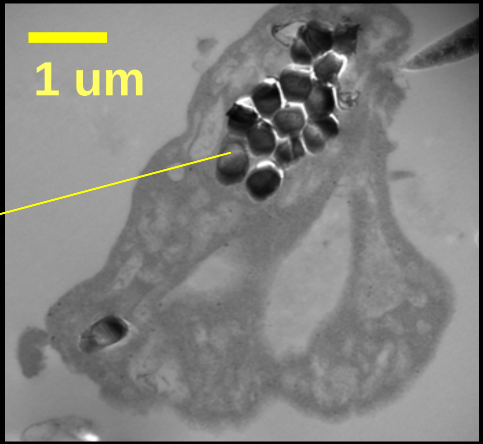
CELLULAR UPTAKE

Macrophages actively engulf (phagocytose) foreign bodies and transport them up the mucocilliary escalator out of the lung

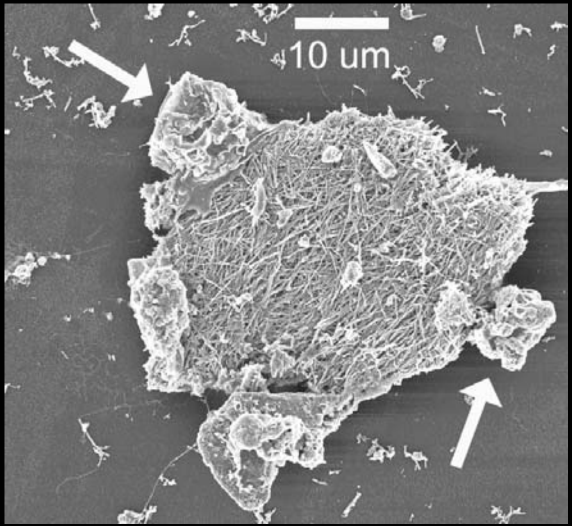


frustrated phagocytosis of asbestos fiber

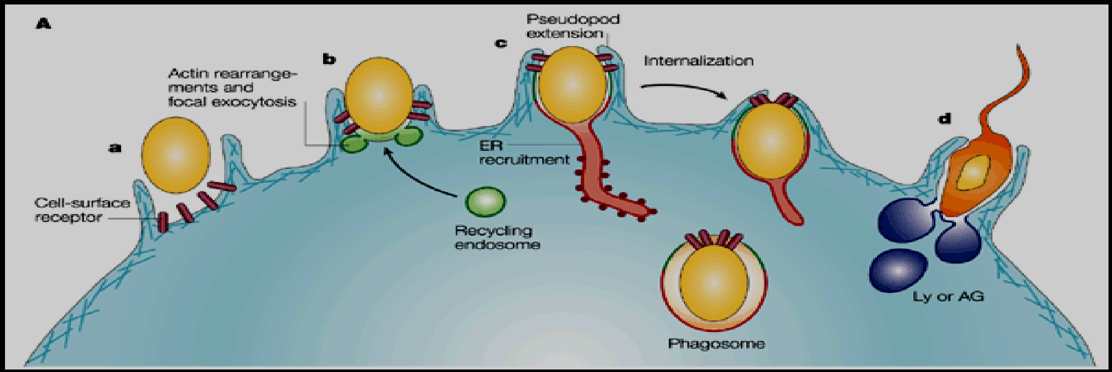
Can macrophages phagocytose nanomaterials completely, and without injury or inflammatory signaling?



complete phagocytosis of carbon nanofiber bundle



Macrophages adhering to large aggregate of carbon nanofibers



M. Desjardins, Nature Reviews/Immunology, 3:280, 2003

Summary

Materials names / labels (SWNTs, as-produced, “purified”) are of limited use. We need materials characterization and it should be toxicologically-relevant

Likely key variables:

- size, shape, aggregation state, and surface chemistry (hydrophobicity, redox activity)
- metals content, phase, location as contributors to bioavailability

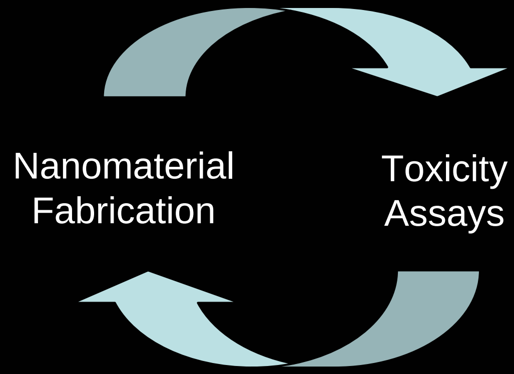
Because most nanomaterials are:

- fabricated, not natural
- developmental, not commercial

→ often no large barriers to reformulation

The Opportunity

Understand relationships between toxicity and specific material features (size, shape, metals content, surface chemistry) to guide the development of intrinsically safe nanomaterials

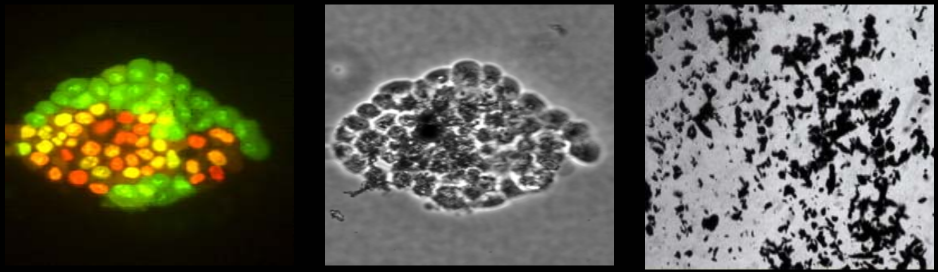


BACKUPS

TECHNICAL PROBLEMS WITH NANOTOXICITY ASSAYS

MONTEIRO-RIVIERE AND INMAN, CARBON 44: 1070-1078, 2006.

- Dose metrics - mass, particle number, surface area, dose delivered to target cell or tissue
- Acute vs. chronic toxicity endpoints
- Particle interference with fluorescent or colorimetric assays
- Adsorption of serum or cellular proteins
- Nanoparticle aggregation or agglomeration



SWNTs form large aggregates in cell culture medium containing 10% serum and induce toxicity in human lung epithelial cells. Live/Dead viability cytotoxicity assay (Molecular Probes, 200X)

Ashley Smith, research assistant

Attempts to disperse nanoparticles:

Sonication

Detergents

DMSO

Lung surfactant

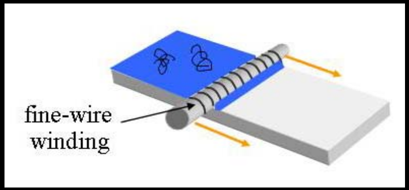
Serum

PEG

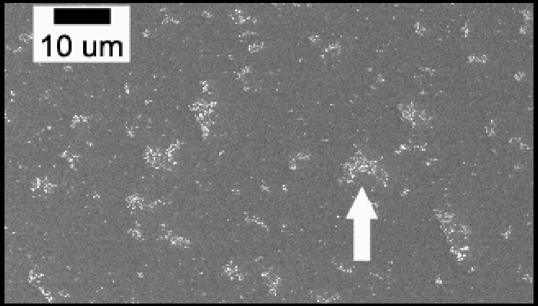
Organic solvents

V. Sanchez, Pathobiology graduate student

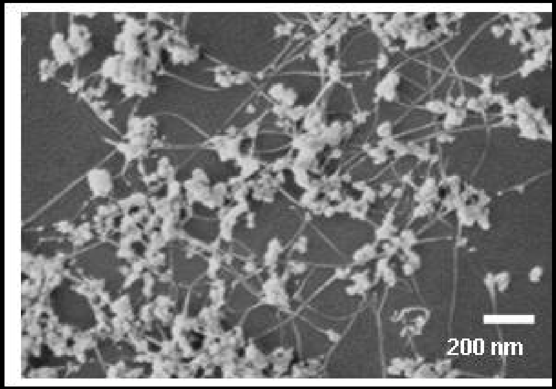
ENGINEERING SOLUTIONS FOR NANOTOXICOLOGY ASSAYS



Mayer-bar-coating technique for formation of controlled carbon nanotube aggregates on substrates



SEM micrographs of 1-5 um SWNT aggregates bar-coated on to a quartz substrate from ethanol suspension



Bundles of SWNTs linked to aggregates of carbon-coated catalyst nanoparticles

Robert Hurt and Lorin Jakubek, Biomedical engineering graduate student