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New Research Approaches for Managing the Bio-Environmental Implications of Nanotechnology

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NSF Workshop on Nanotechnology and the Environment

Some Challenges for NanoSafety Research

Engineered nanomaterials:

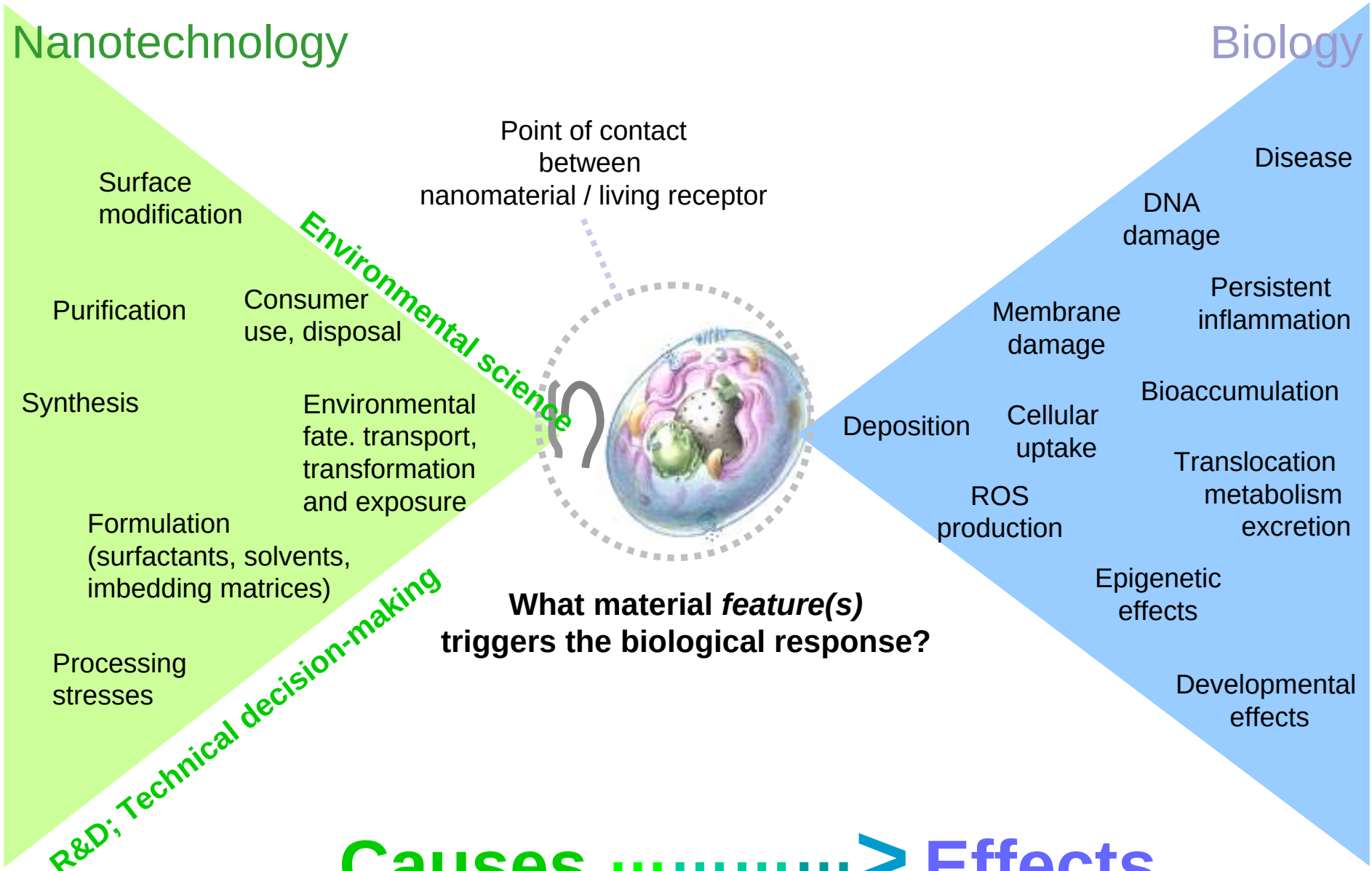
- are not yet found in the natural environment or tissue in quantities sufficient for field studies or molecular epidemiology

- are extremely diverse (compositions, morphologies, surface treatments)
  ---- analogy: chemicals

- are often complex, possessing multiple material *features* relevant to biological impacts (unreacted precursors, attached and free ligands, adsorbed species, catalytic residues, surface states, size/shape distributions)
  ---- biological responses can be quite sensitive to *details*

- can transform in the environment or in biological compartments

- are high-technology products subject to ongoing development. Engineered NP features are *created* and *controlled* by modern nanosynthesis tools. There is a direct cause-effect continuum linking synthesis/processing and bio-environmental impact.

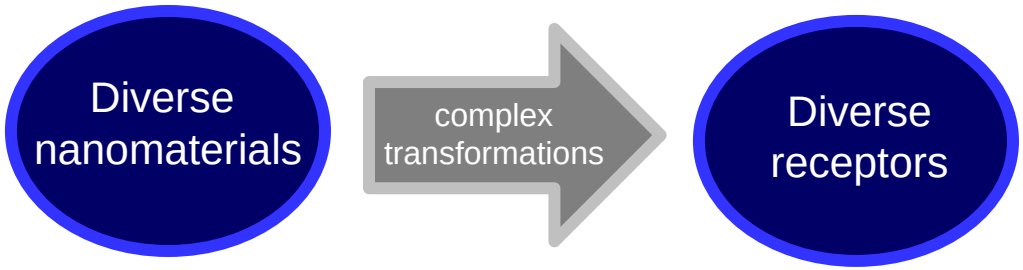

Nanotechnology
Biology
Point of contact between nanomaterial / living receptor
Surface modification
Purification
Consumer use, disposal
Synthesis
Environmental fate. transport, transformation and exposure
Formulation (surfactants, solvents, imbedding matrices)
Processing stresses
Environmental science
R&D; Technical decision-making
Disease
DNA damage
Persistent inflammation
Membrane damage
Bioaccumulation
Deposition
Cellular uptake
Translocation metabolism excretion
ROS production
Epigenetic effects
Developmental effects
What material feature(s) triggers the biological response?
Causes ..... > Effects

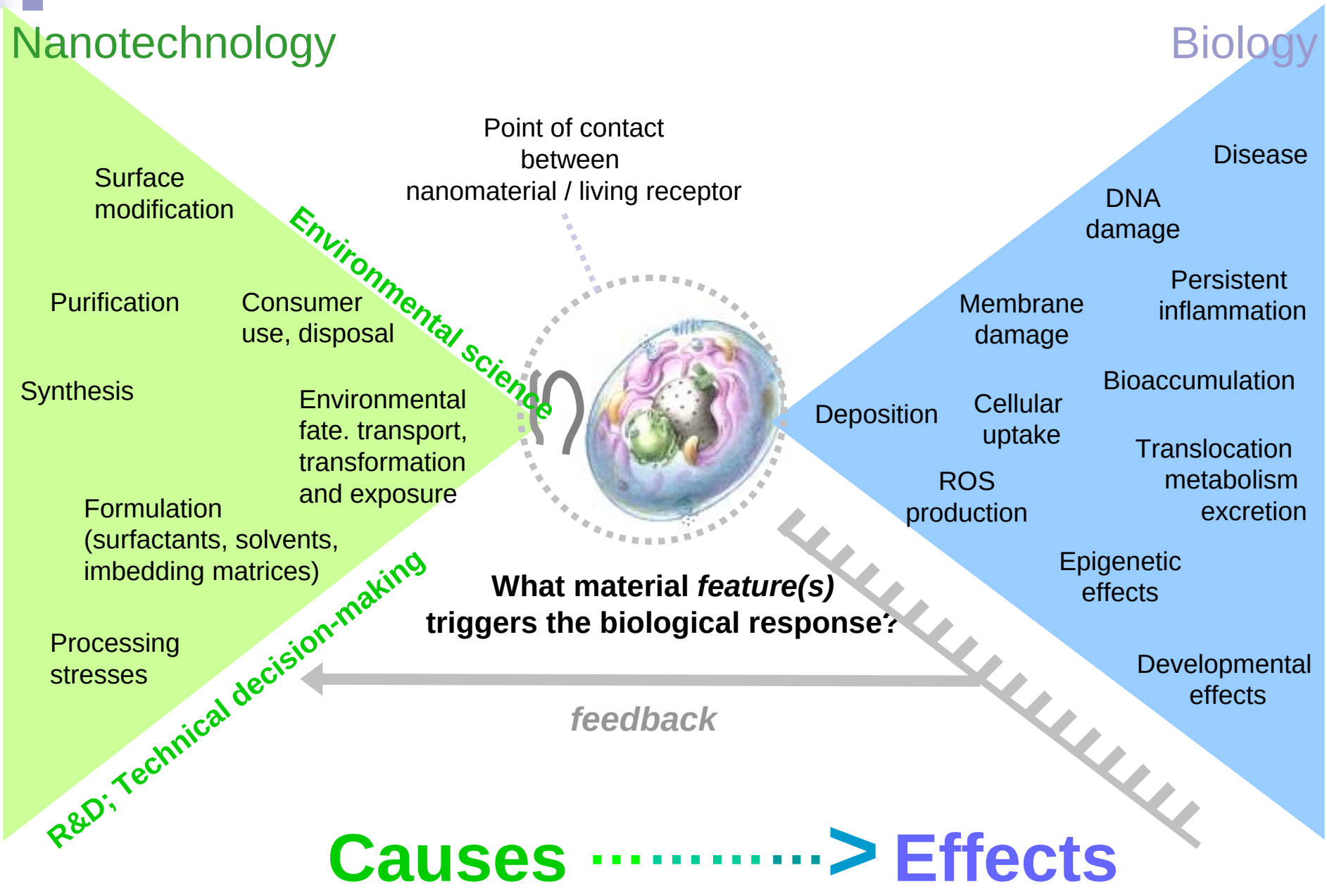
More Challenges for NanoSafety Research

- Engineered NPs are not yet implicated in environmental damage or disease, which could serve to rationally define model systems and suggest mechanistic *in vitro* studies.

- In such cases, it can take many years to identify risks, understand biological mechanisms. Effects depend on dose, exposure route and frequency, susceptible populations, etc. and may involve long latency periods

- Nanotechnology development is proceeding on a faster time scale and suffers from the corresponding uncertainty, even when most products are likely of low risk

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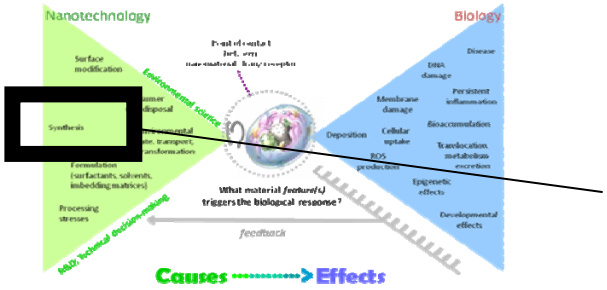
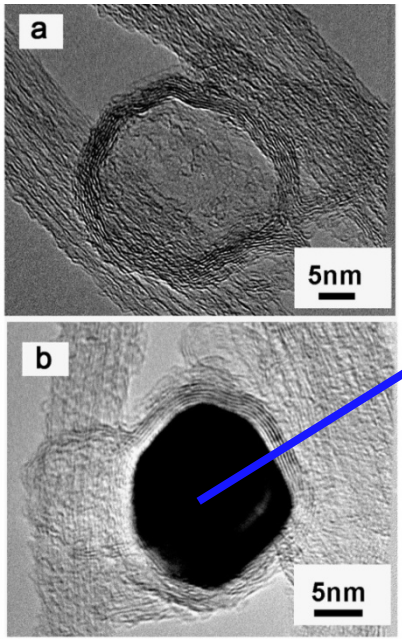


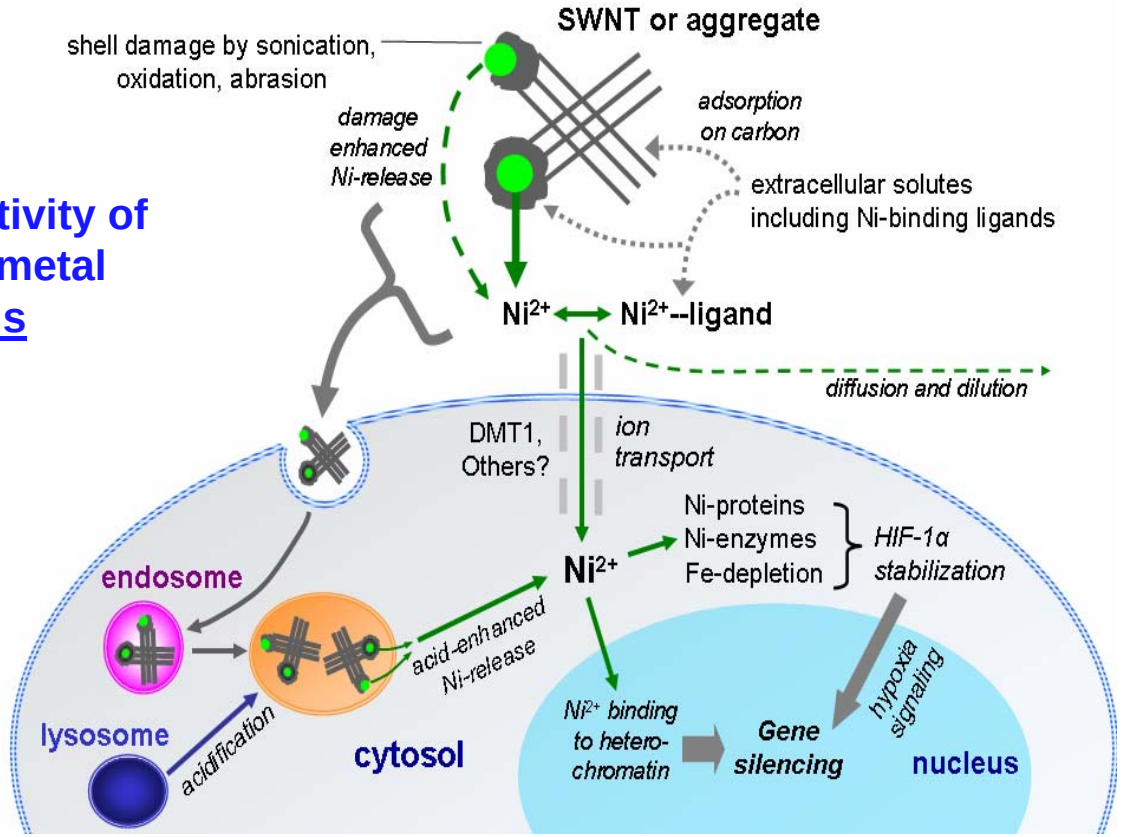
Synthesis:

Bioavailability of Nickel in Single-Wall Carbon Nanotubes

Liu, Gurel, Morris, Murray, Zhitkovich, Kane, Hurt
Advanced Materials, 19 2790 (2007)



biological activity of C-imbedded metal is not obvious



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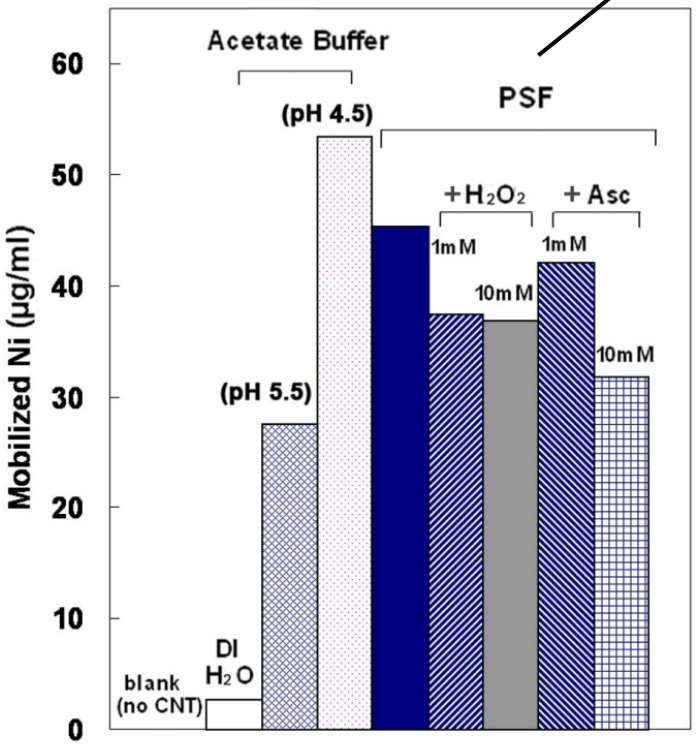
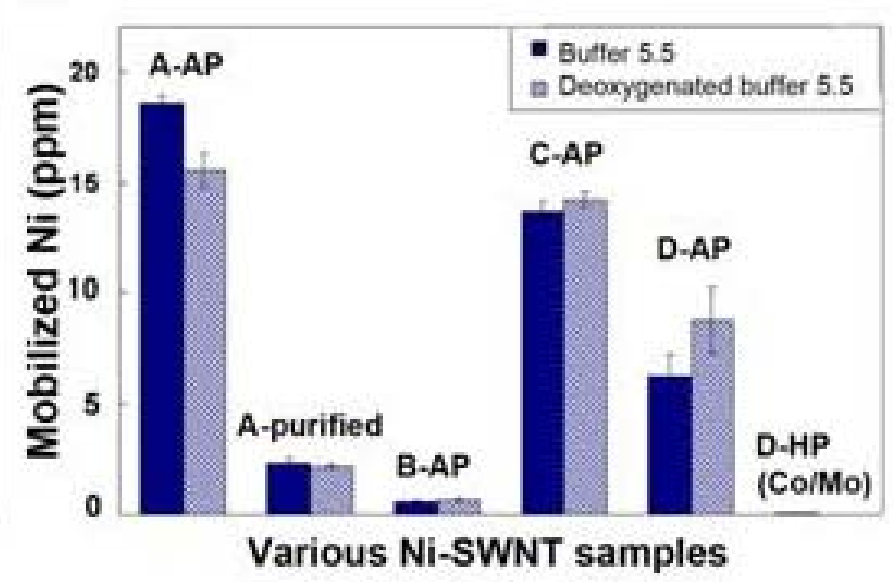
Nickel Release from SWNTs

PSF: Phagolysosomal Simulant Fluid



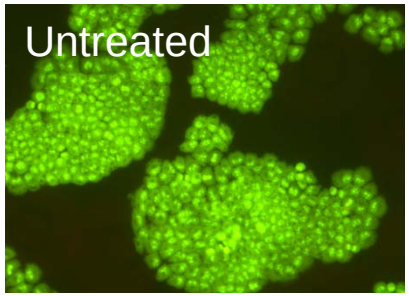
Effect of fluid media

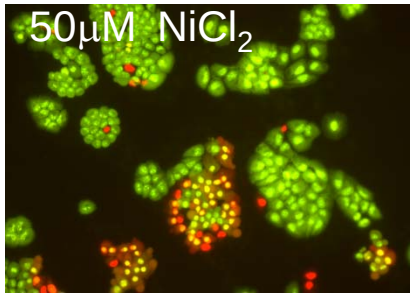


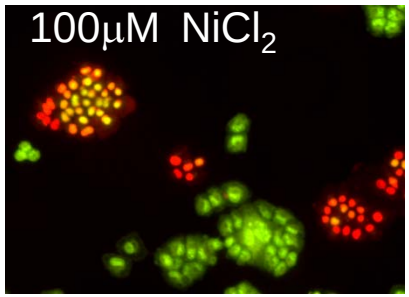
Effect of CNT source / type

Cytotoxicity and Cellular Uptake of Mobilized Ionic Nickel

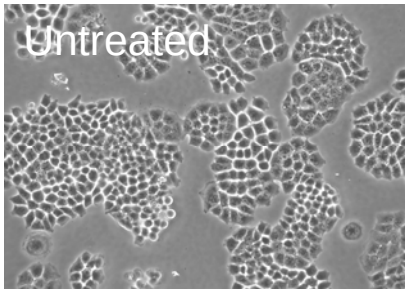
Syto-10/Ethidium Homodimer Viability Assay – 48 hours (100x)

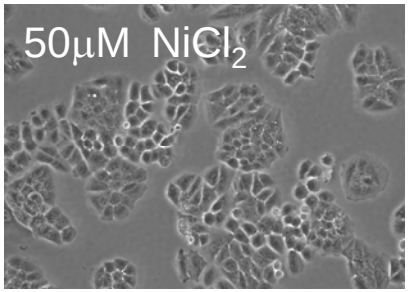


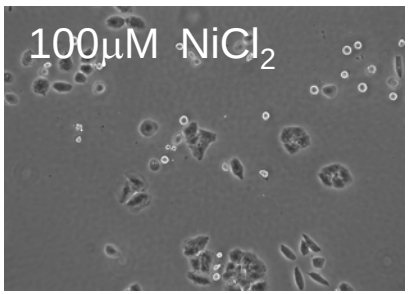


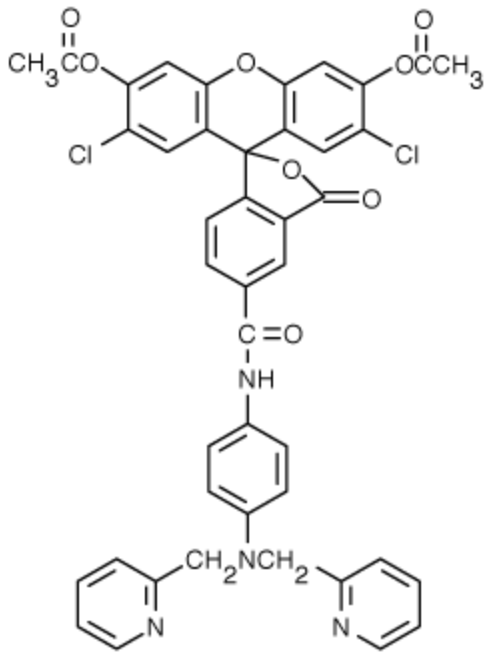


Phase Contrast Microscopy – 48 hours (100x)



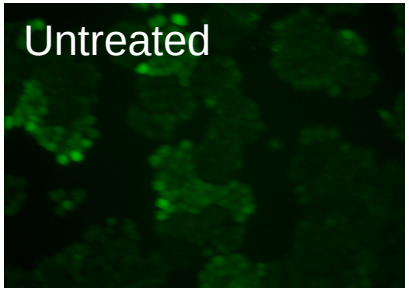


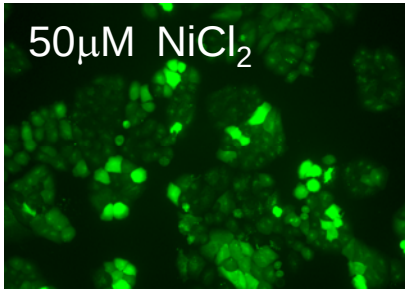


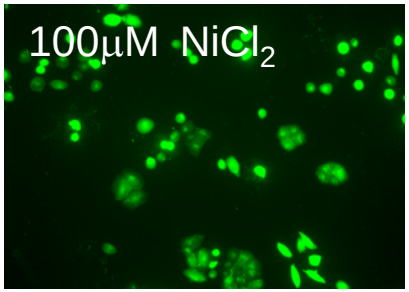


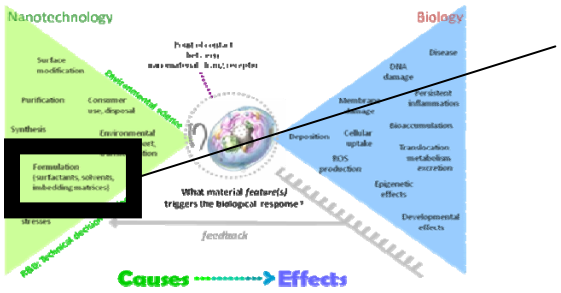
Newport Green DCF

Newport Green Fluorescence – 48 hours (200x)









Formulation

Effect of CNT hydrophobicity and surface functionalization

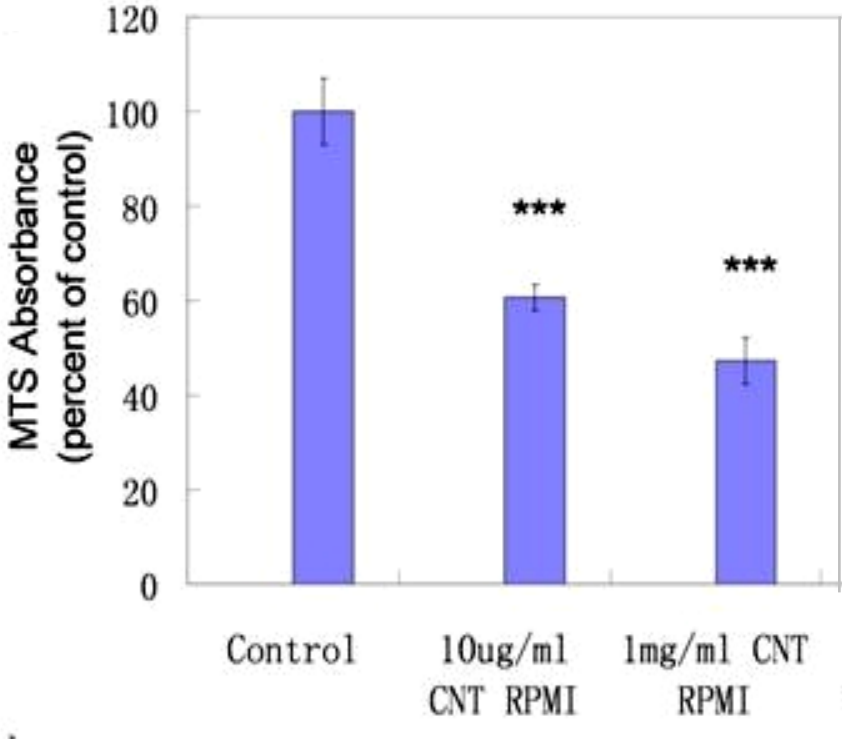
<u>Simple Experiment</u>

SWNTs + Cell culture medium

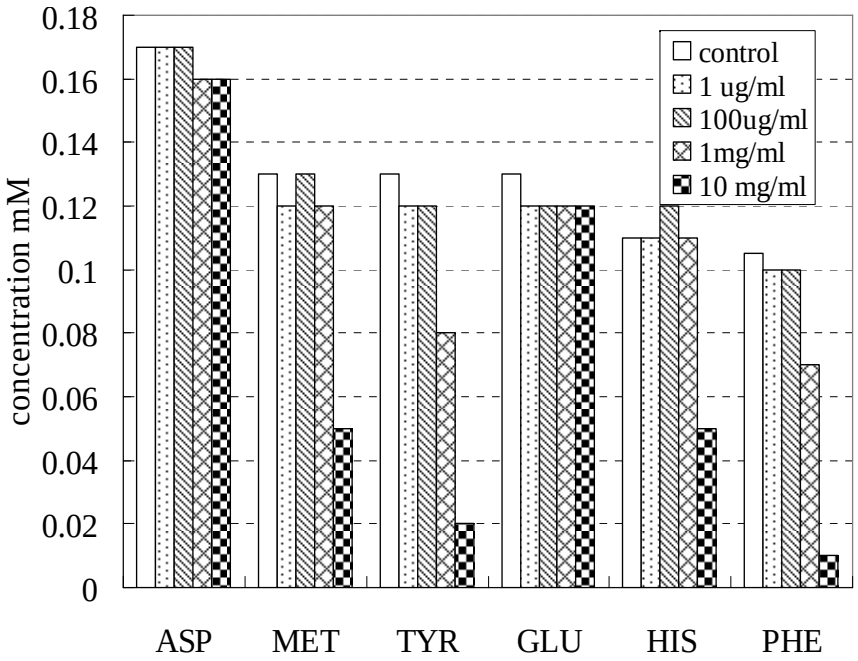
SWNT removal by centrifugal ultrafiltration

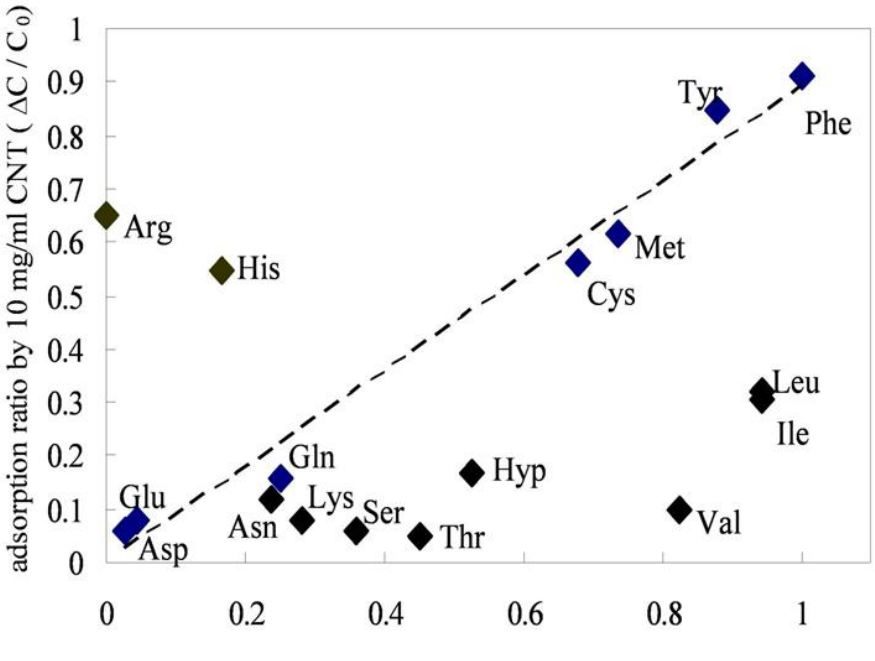
solute profiling and cell culture in “exposed” media

Viability of HepG2 liver cells



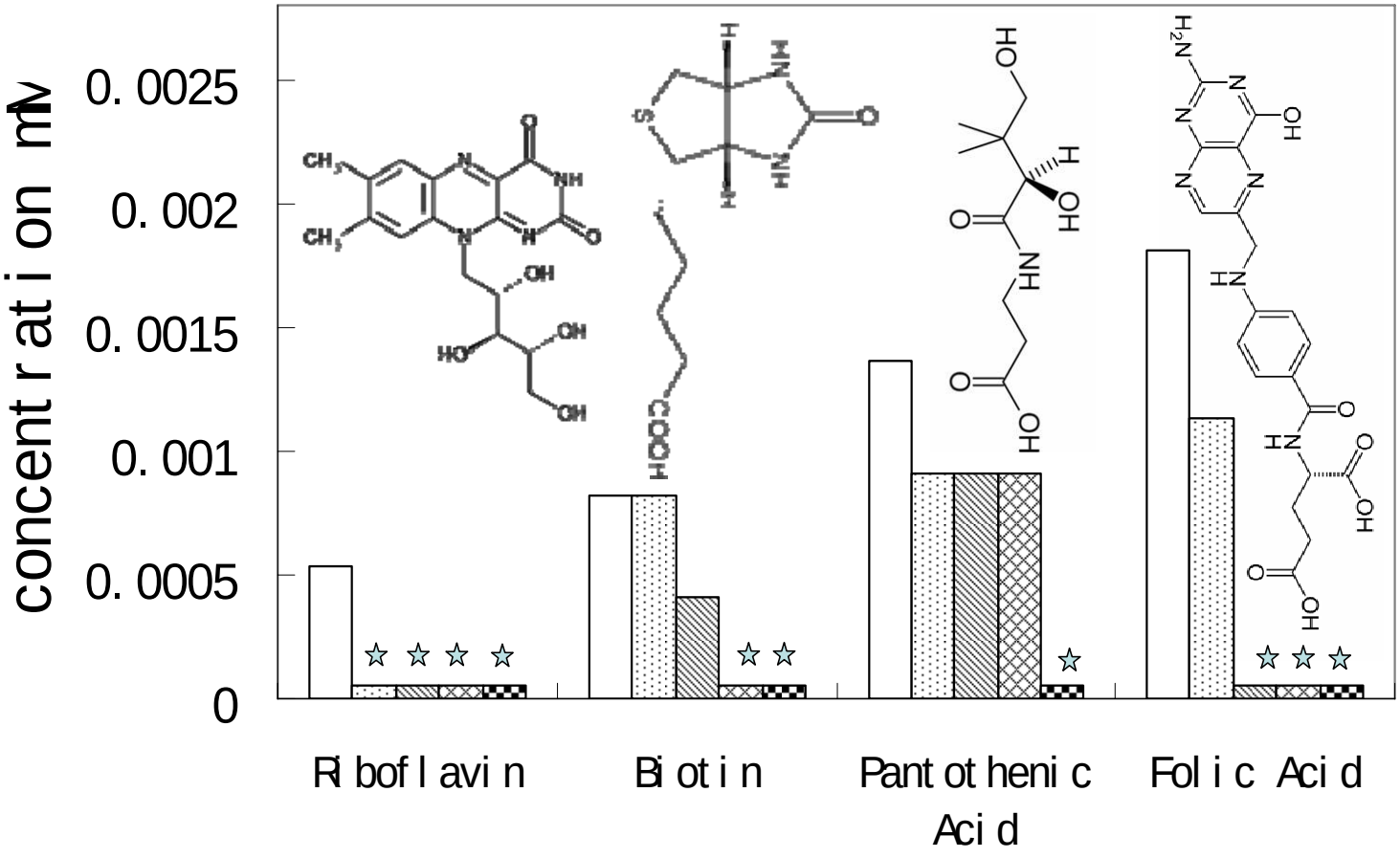
Amino acid profiling after dose-dependent SWNT exposure





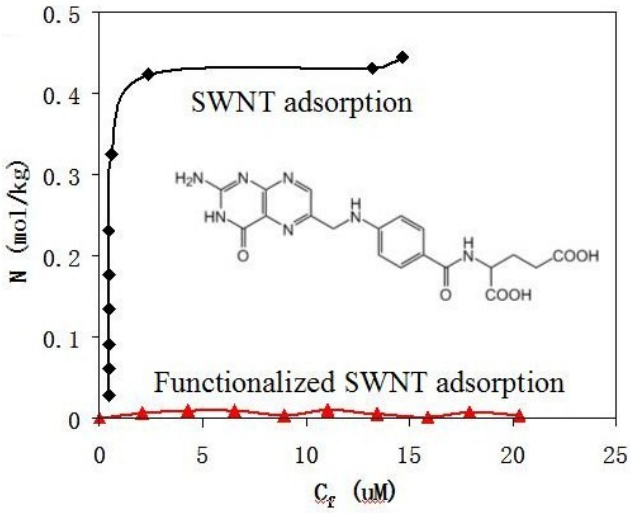
Hydrophobicity index
of Black and Mould, 1991

Some vitamins are depleted at CNT doses as low as 10 ug/ml !

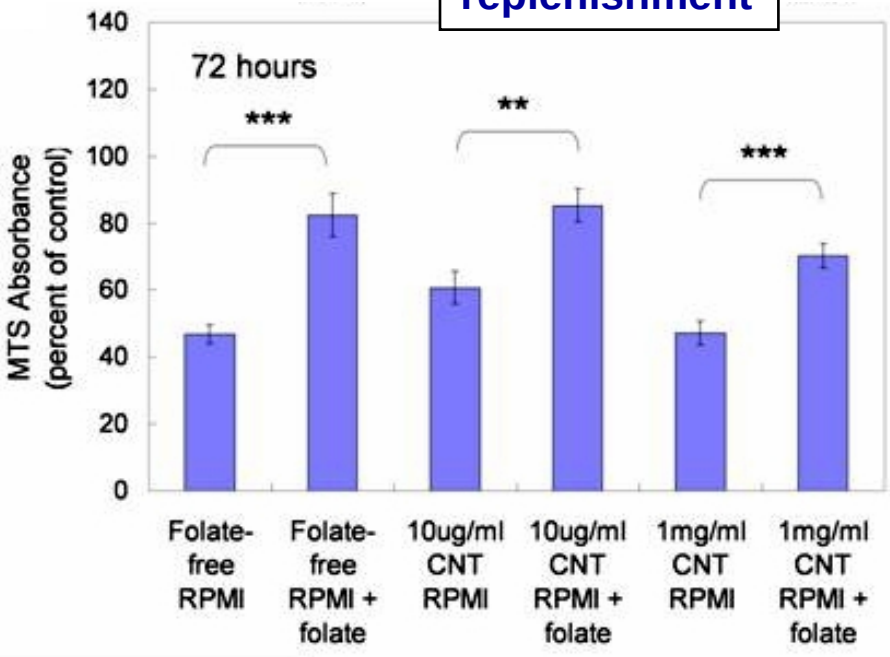


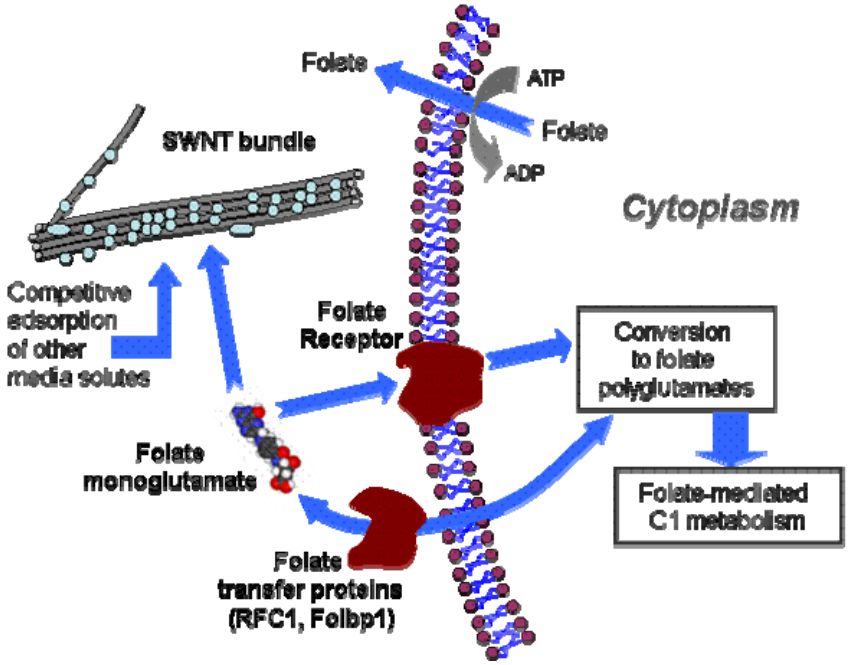
Result: A new "starvation mechanism" driven by hydrophobic depletion of essential micronutrients

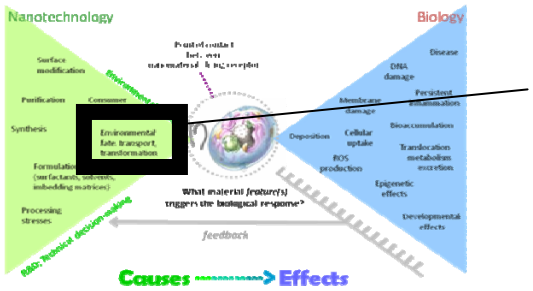
Adsorption of Essential Micronutrients by Carbon Nanotubes and Its Implications for Nanotoxicity Testing, Guo, Von Dem Bussche, Buechner, Kane, Hurt



Effect of folate replenishment

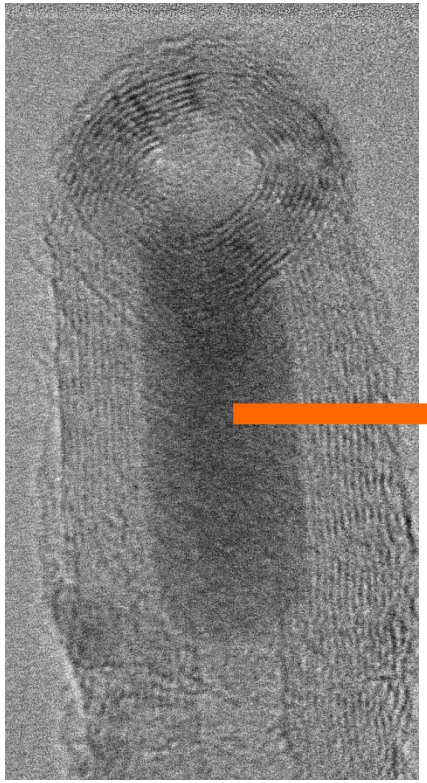






Environmental transformation → Example: CNT metals

Iron bioavailability and redox-activity in diverse CNT samples
[Guo, Morris, Liu, Vaslet, Hurt, Kane, *Chemistry of Materials,* 2007]



Fe-containing CNTs

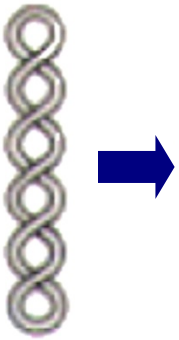
ascor-bate

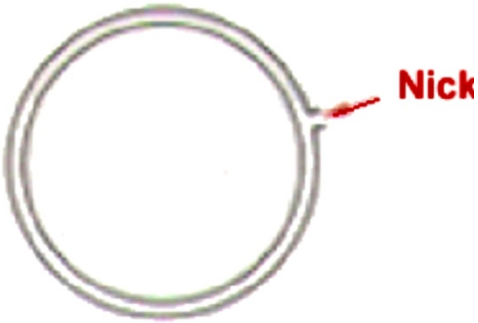
Fe^{2+}

O_2

Fe^{3+}

ROS +





Supercoiled **Open Circular**

Plasmid DNA assay
Single-strand break
Induces uncoiling event
detectable by
gel electrophoresis

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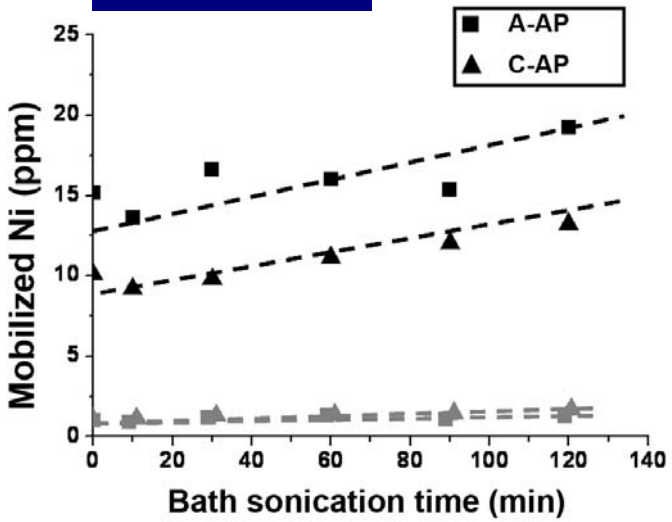
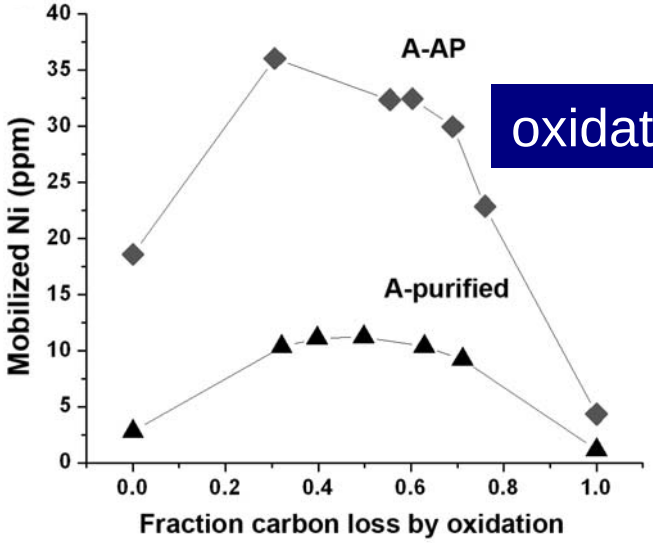
Environmental Transformation of CNT metals

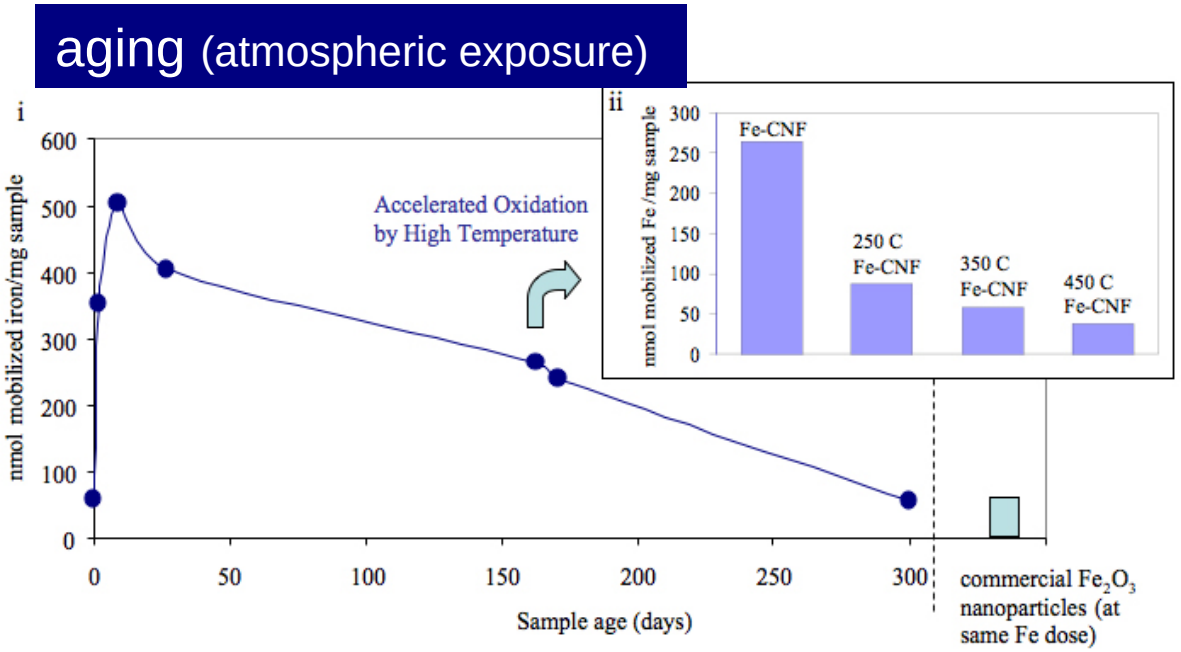
sonication



oxidation



aging (atmospheric exposure)



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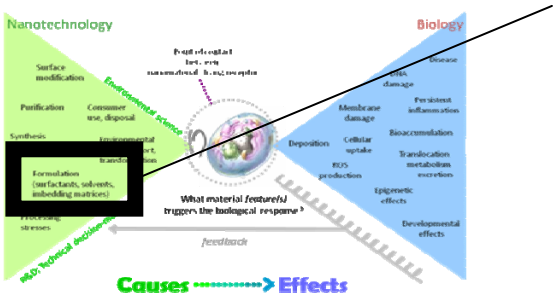


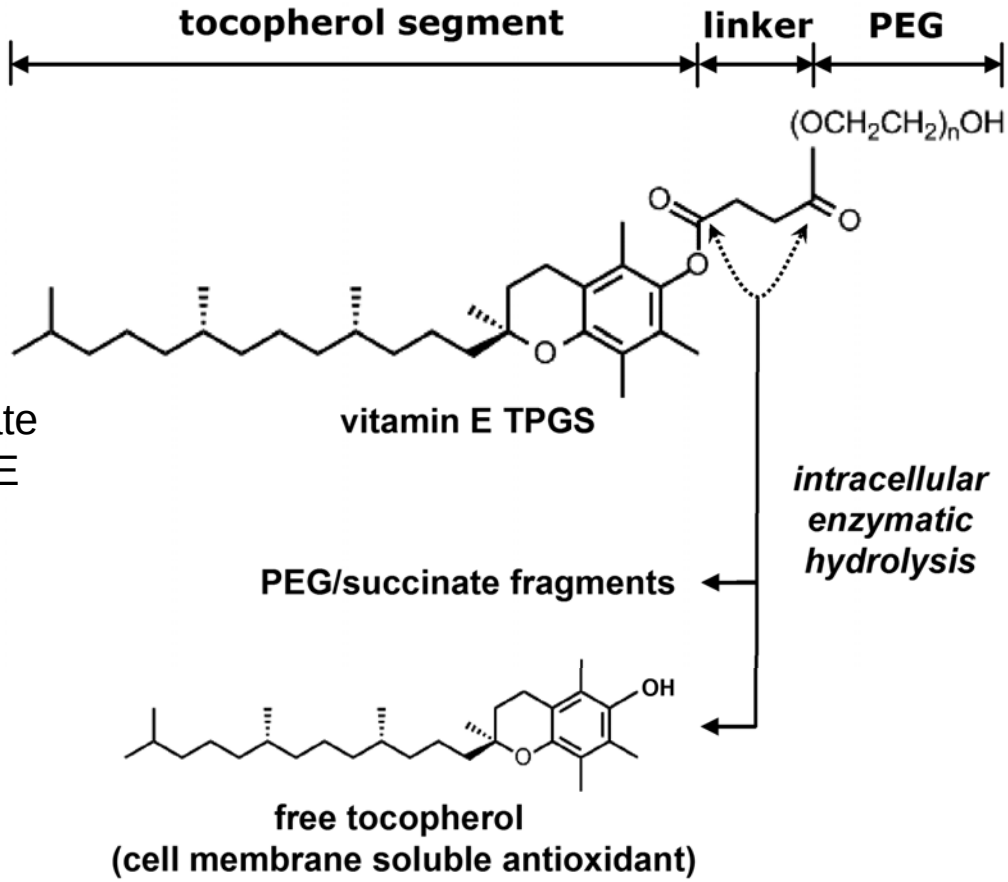
Formulation/Coating

"TPGS as a safe, antioxidant surfactant
for processing nanotubes and fullerenes"
[Yan, Von Dem Bussche, Kane, Hurt, *Carbon,* 2007]

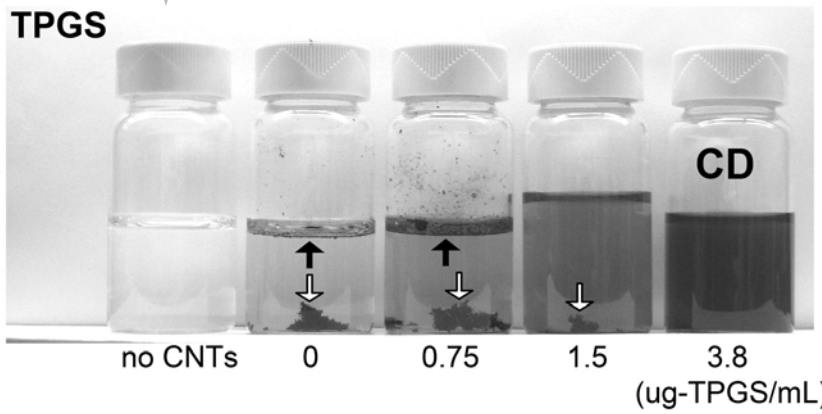
The most common toxicity mechanism for (nano)particles is oxidative stress

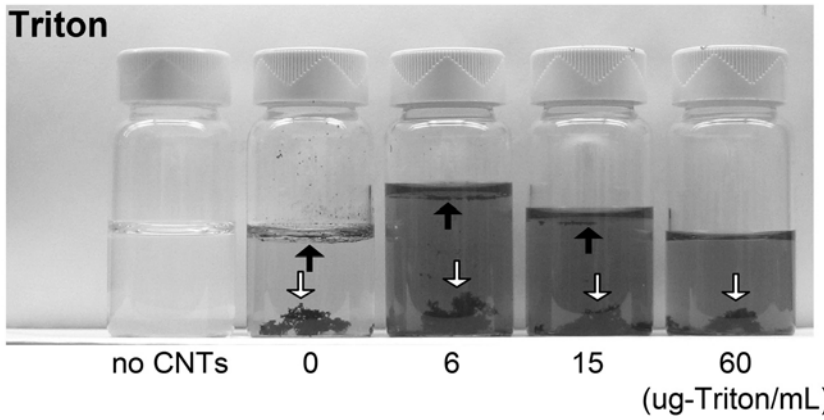
α-Tocopheryl Polyethylene Glycol Succinate (TPGS) is a water soluble form of vitamin E used as a dietary supplement and drug delivery vehicle

TPGS cleaves by enzymatic hydrolysis to deliver the lipophilic α-tocopherol (Vitamin E) to cell membranes, where it Serves as an antioxidant

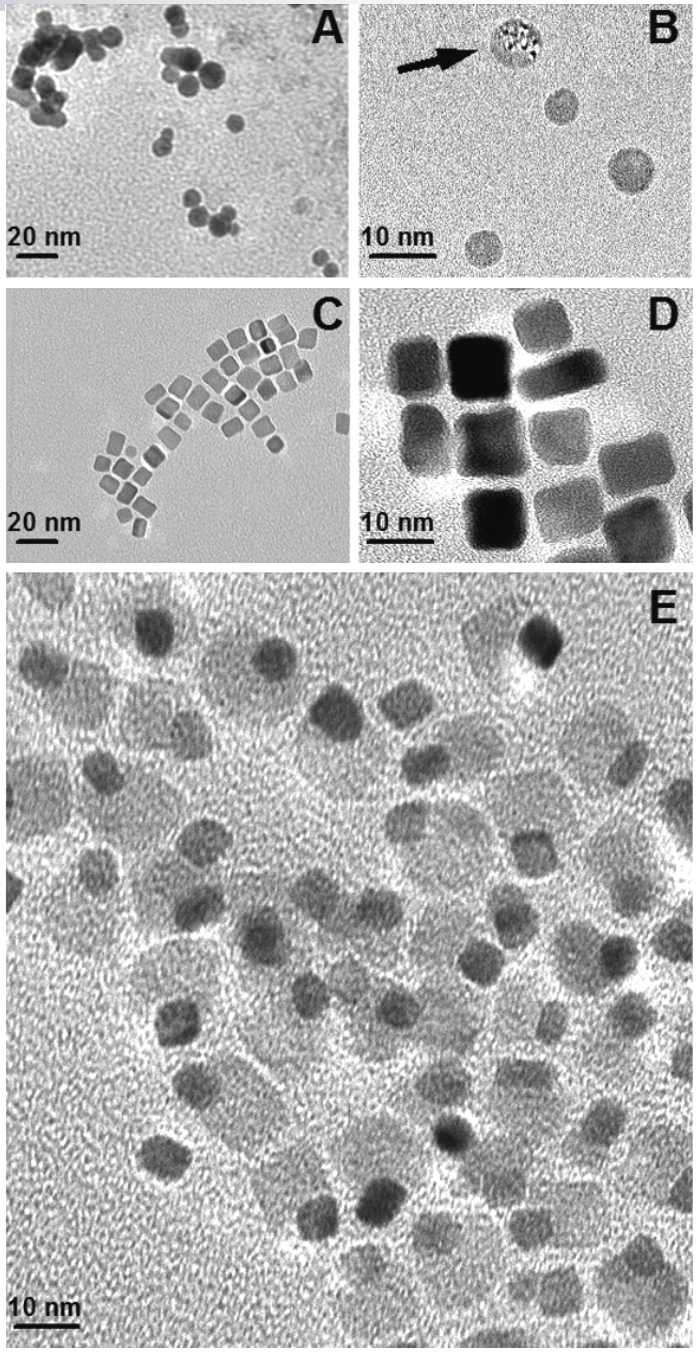


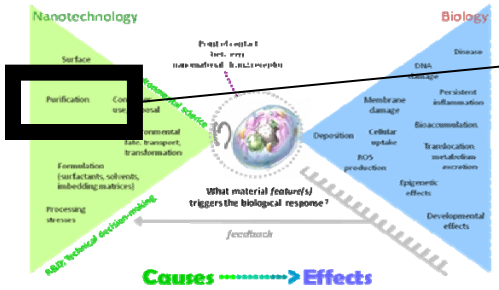
TPGS is an effective dispersant for MWNTs and shows a unique co-self-assembly with C60





TPGS is a more effective dispersant for MWNTs than Triton

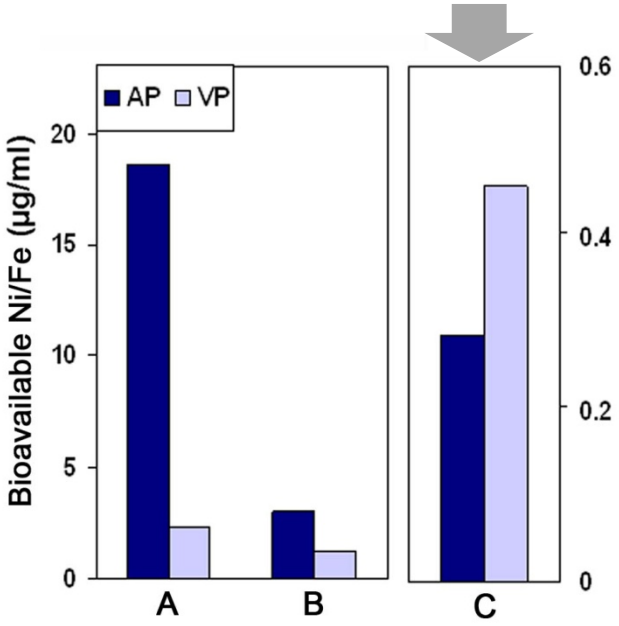


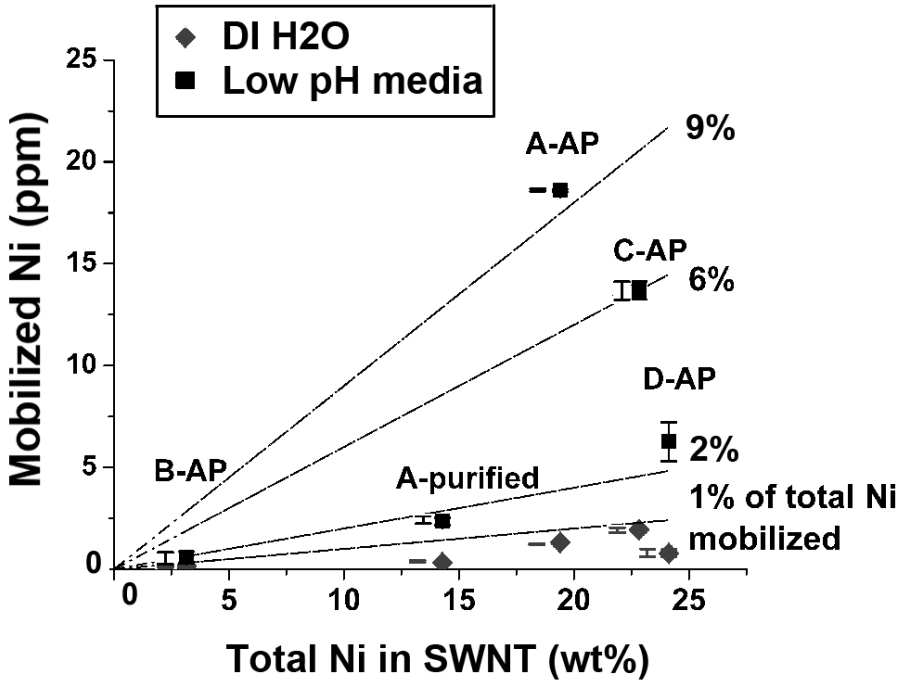


Purification

Example: targeted removal of *bioavailable* metal from CNTs

purification ***increases*** bioavailable metal !

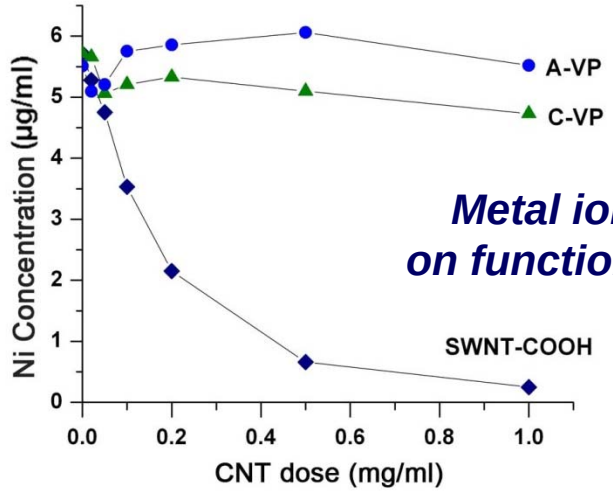




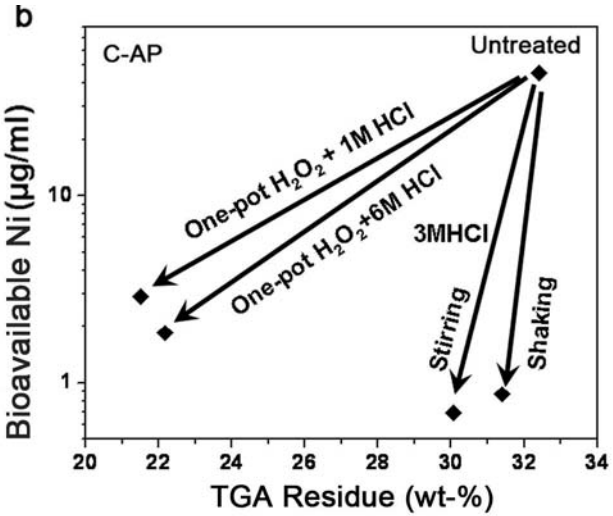
Question 1:
What is origin of bioavailable metal in “purified” CNTs ?
(and why does “purification” sometimes ***increase*** it?)

Question 2:
How can we target the bioactive portion of the metal for removal (and detoxification)

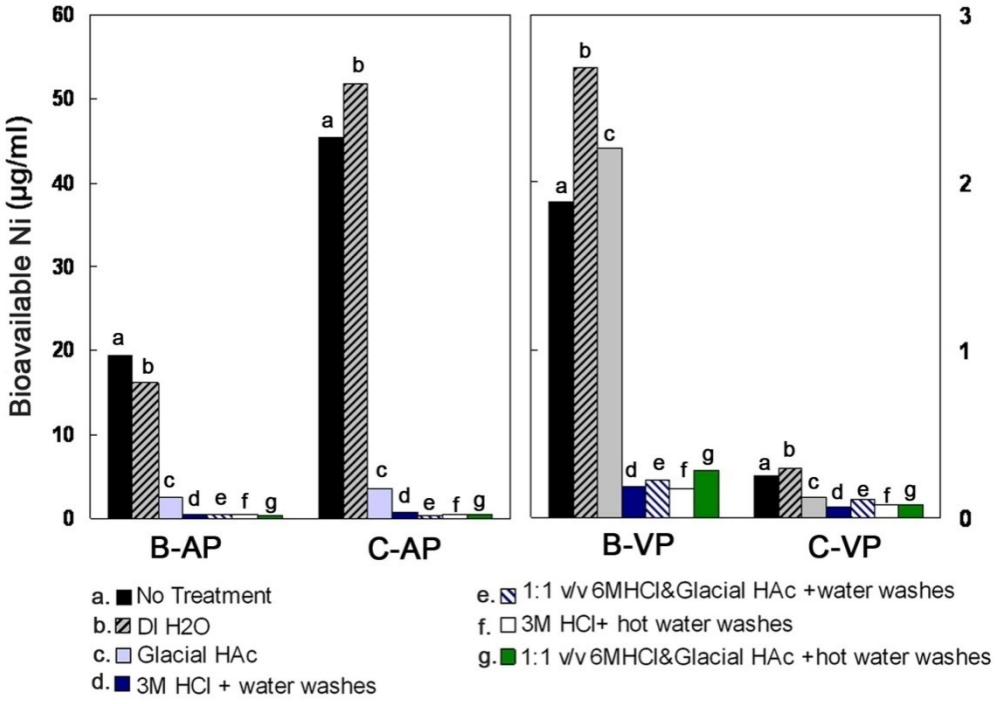
Purification do's and don'ts



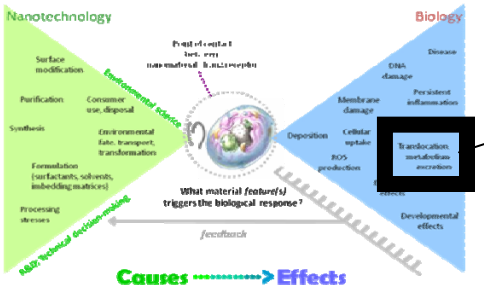
Metal ion re-deposition on functional groups (don't)



Oxidation during or after acid wash (don't)

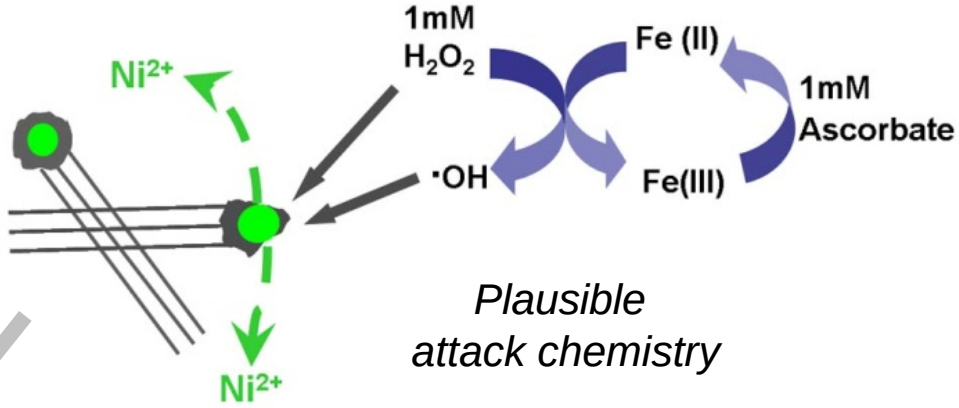


Last step wash with non-oxidizing acid (do)

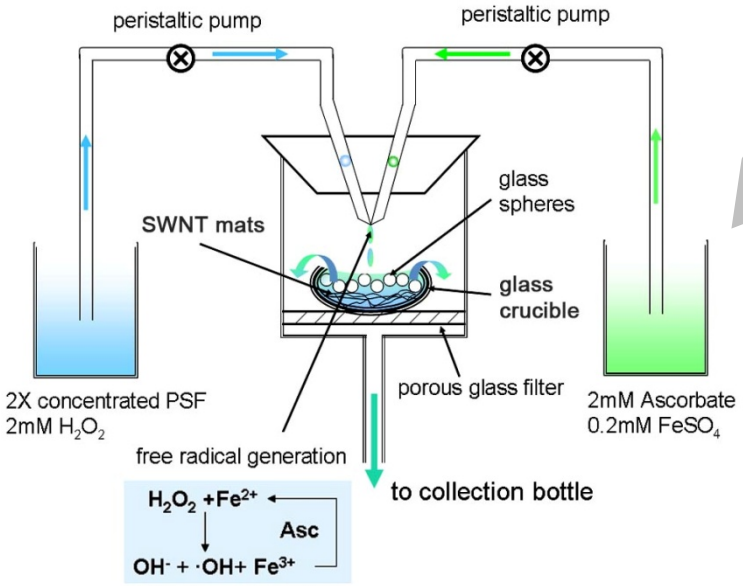


Biological transformation

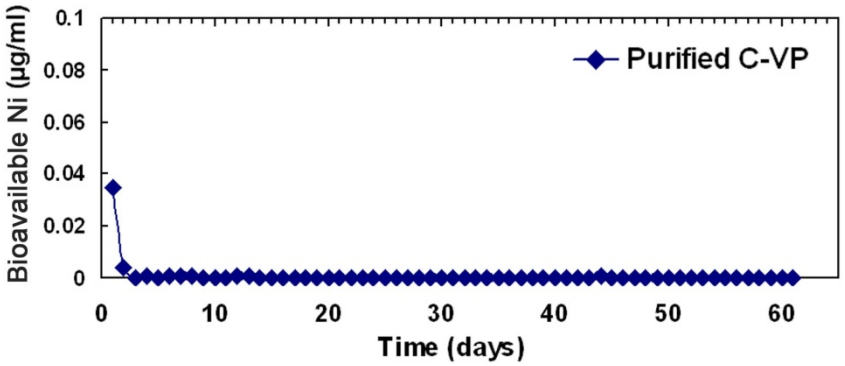
Example: stability of carbon shells in phagolysosomes



Plausible attack chemistry



Long-term flow assay



Final thoughts

- It is difficult to understand origin of biological impacts without expert knowledge of the nanomaterials themselves
  - materials are complex and exhibit unexpected behaviors
- Work needed on commercial (complex) materials with feedback to suppliers (critical for short/mid-term product safety)
  - many safety problems with nano-products are solvable
  - some safety solutions possible w/o waiting for complete knowledge of biomolecular mechanisms
- Work also needed on highly controlled, idealized materials to understand fundamental bio-environmental interactions of defined nanostructures (important for long term safety)
- Both goals require active engagement of nanomaterials scientists

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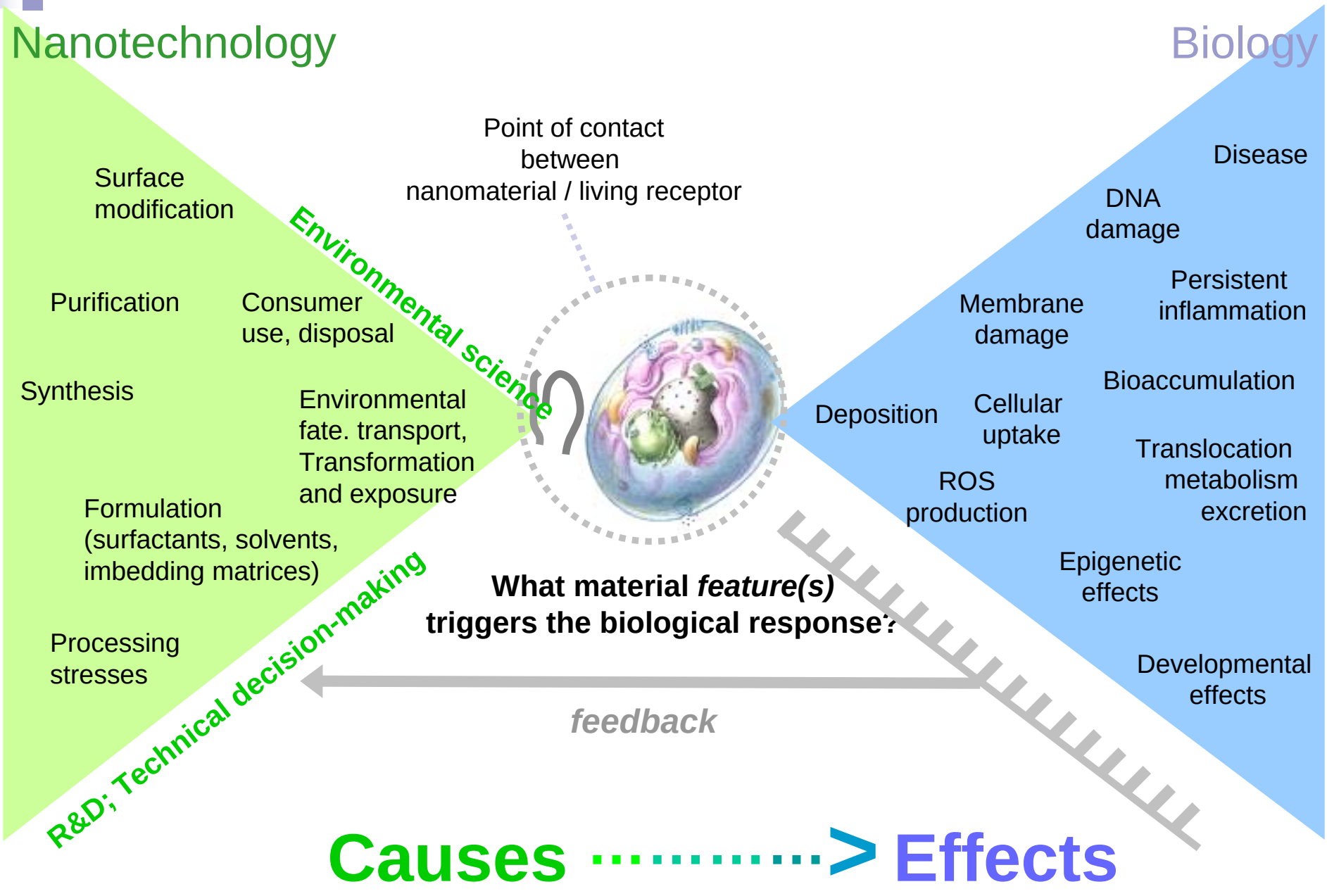
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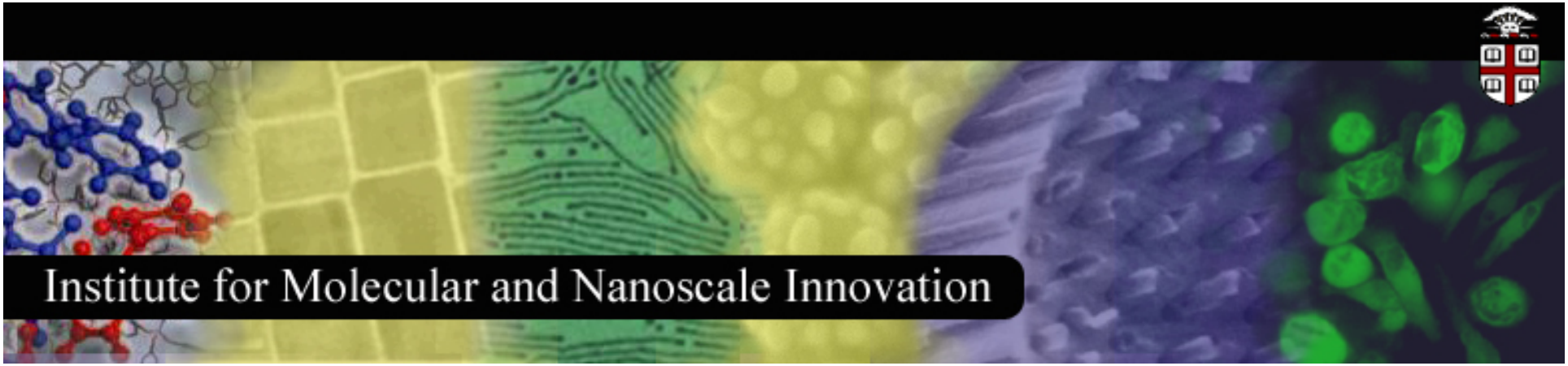
Causes
Effects



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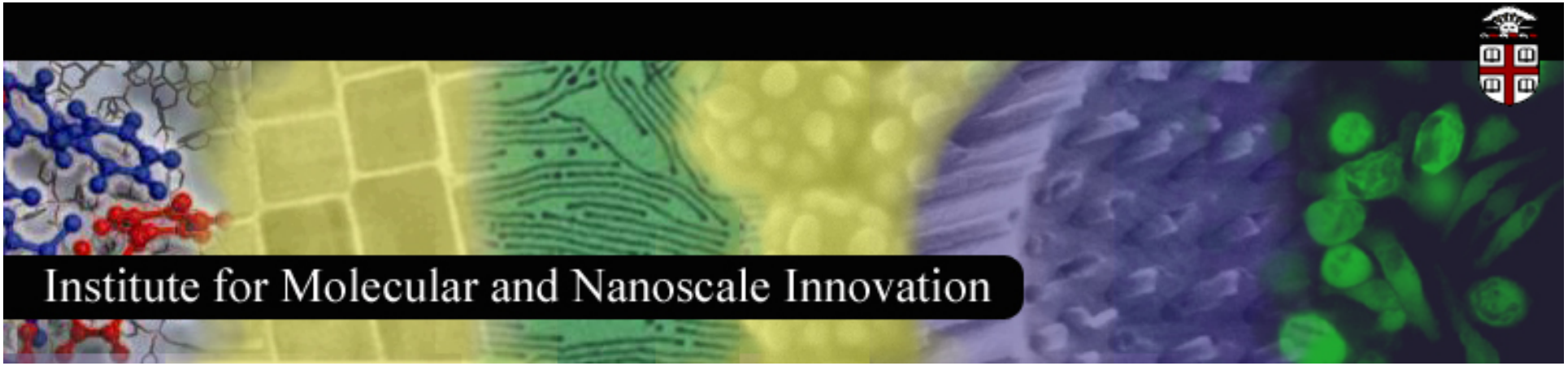

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