

# Developing an Understanding of Nanotoxicology

2010 APVMA Science Fellows Symposium

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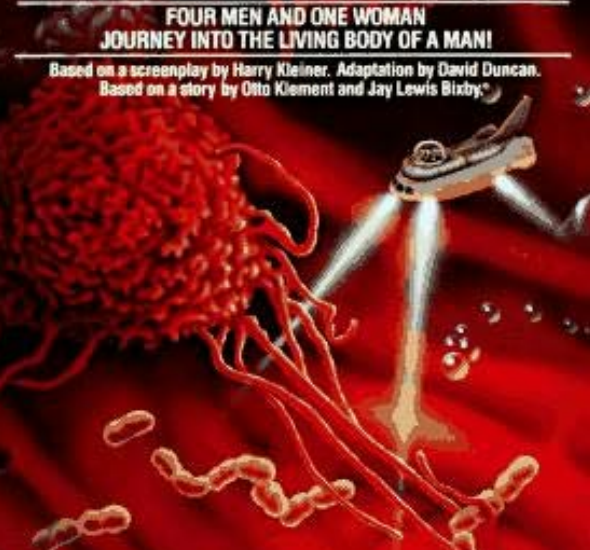
# Definitions

*“Toxicology is the study of the adverse effects of chemical, physical or biological agents on people, animals, and the environment.”*

(Society of Toxicology [SOT])

*“Nanotoxicology is the study of the adverse effects of engineered nanomaterials (ENMs) on living organisms and the ecosystems, including the prevention and amelioration of such adverse effects.”*

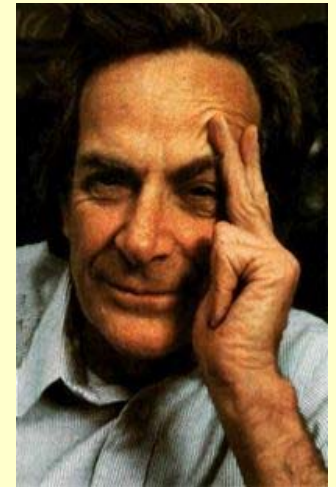
(G. Oberdorster, *J Internal Medicine* (2010) **267**, 89-105)



# Nanomaterials – *From Science Fiction to Actuality*

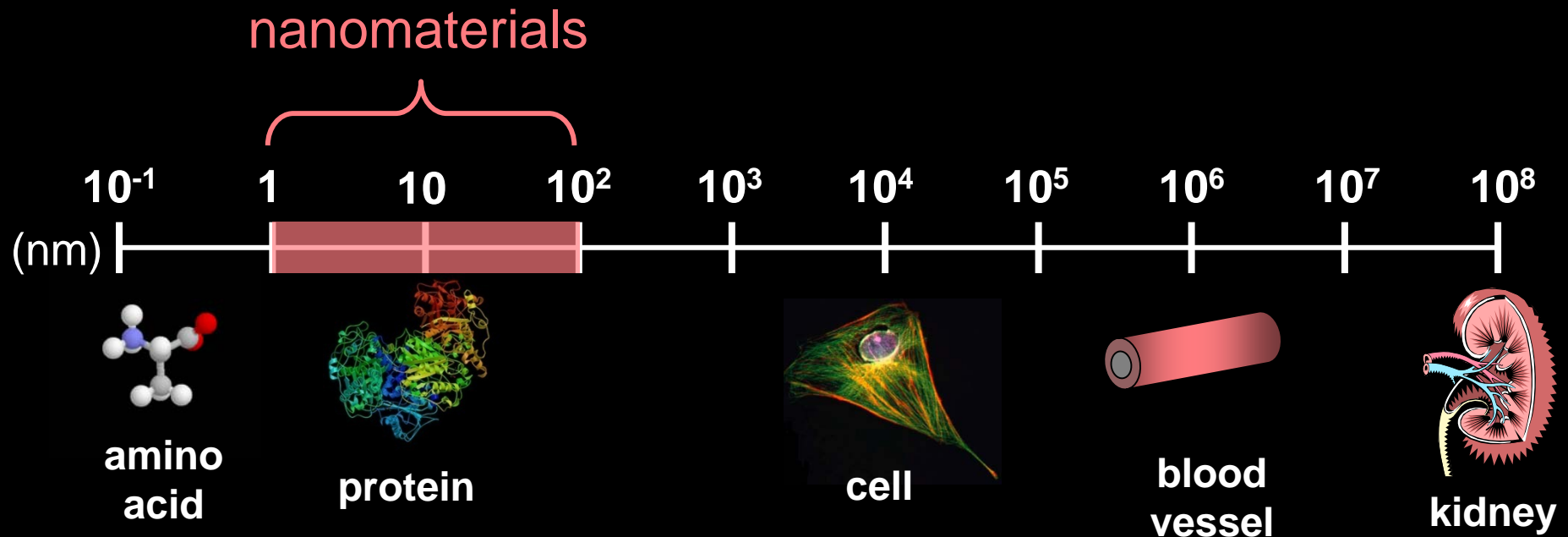
## Richard Feynman

- 1959, American Physical Society
- Lecture: *“There’s Plenty of Room at the Bottom”*
- Manipulating matter at near-atomic scale produces unique properties
- *“Swallowing a surgeon”*



# The Scale of Nanotechnology

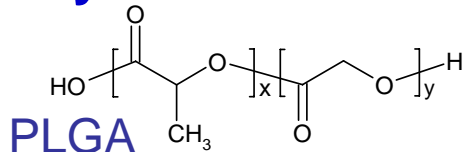
- By definition, a nanomaterial is an object with 1 or more dimensions  $< 100$  nm
- Comparable size to biological macromolecules



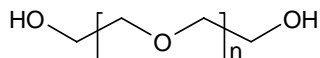
# Types of Nanoparticles (NPs)

(after AJ Gormley & H. Ghandehari [2009])

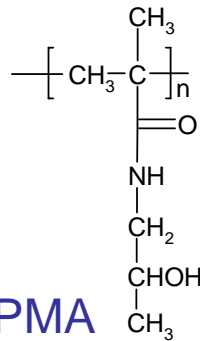
## Polymeric



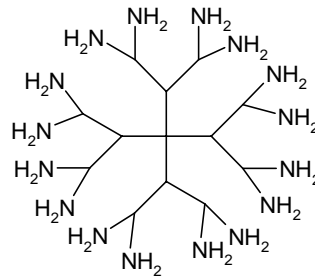
PLGA



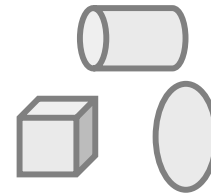
PEG



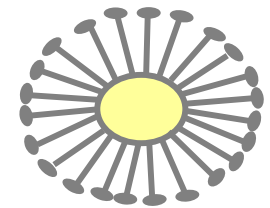
HPMA



Dendrimer

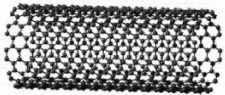


Particulate



Liposome

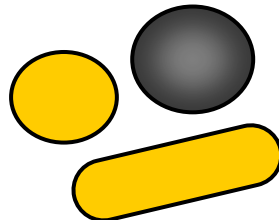
## Inorganic



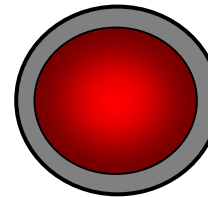
Carbon nanotubes



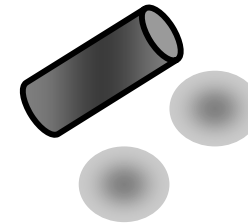
Fullerene



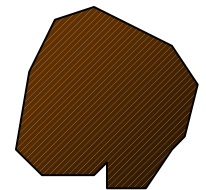
Gold and silver



Quantum dots

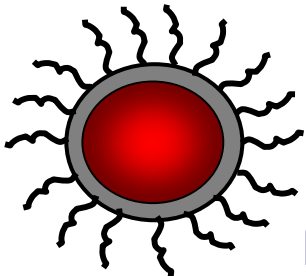


Silica

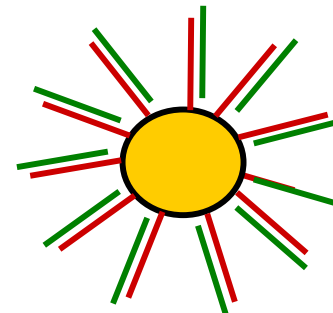
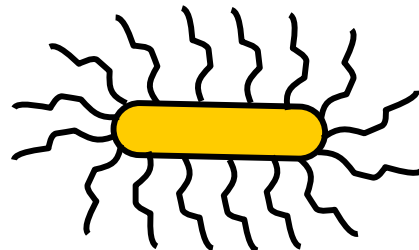


Metal oxides

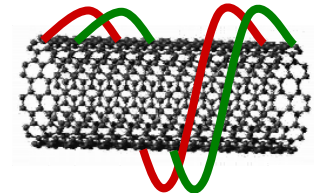
## Hybrid



PEGylated NPs



DNA NPs



# The Premises, Promises & Problems of Nanotechnology

- Unforeseen properties will emerge from nanoscale-aggregates of atoms and molecules
- Not predicted by quantum mechanics (individual atoms) or classical physics (bulk materials)
- Novel physicochemical properties will open door to novel commercial applications
- Physical laws underlying behaviour of nanoscale objects poorly characterised (esp. in “real-world” applications)

# Growth of Nanotechnology Sector

- 2001, National Nanotechnology Initiative (USA)
- 2002, Nanotechnology Network (Australia)
- 2005, \$32 billion globally
- 2007, \$147 billion
- 2010, ~ 1,015 products (24 nations)
- 2015: \$2.6 trillion (projected)

**Project on Emerging Technologies**

[www.nanotechproject.org](http://www.nanotechproject.org)



Image source: [www.eagleone.com](http://www.eagleone.com)

# Nanotechnology Products

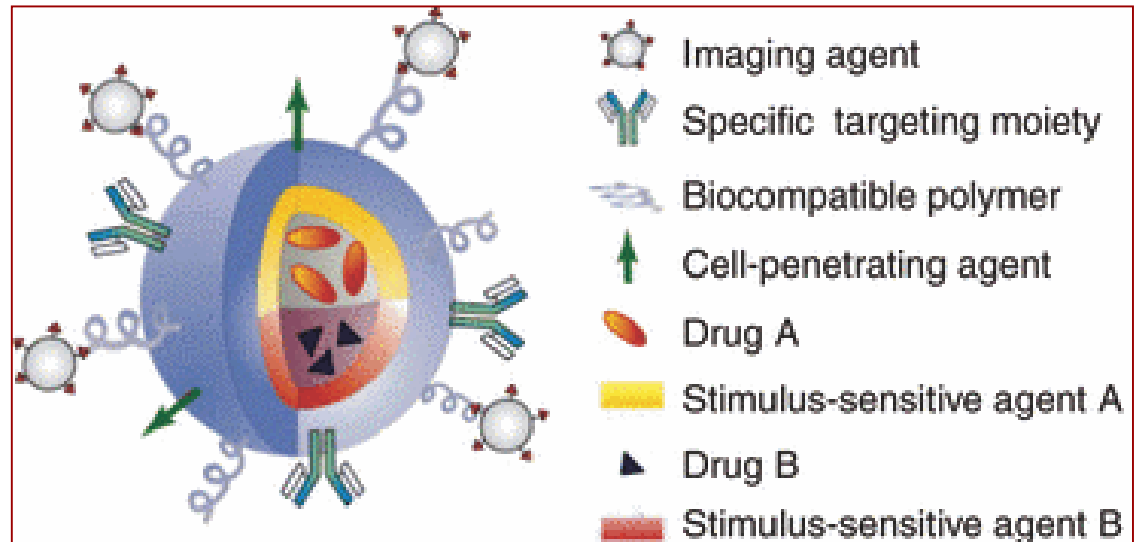
- sporting goods
- smart apparel & fabrics
- food packaging & additives
- cosmetics & sunscreen lotions
  - automotive coatings
- cleaning/self-cleaning agents
  - green adhesives
  - solar cells & batteries
  - hard drives, *etc.*

*Project on Emerging Technologies*  
[www.nanotechproject.org](http://www.nanotechproject.org)



# Nanotechnology Innovation in Human and Veterinary Medicine

- Regenerative medicine/tissue engineering
  - tissue repair (e.g. cell sheets, nanofibres)
- Imaging and “point of care” diagnostics
- Biosensors (toxins, microbes, pollutants)
- Drug delivery
- Gene delivery
- Vaccine delivery
  - (nanoneedles)



# But Are They Safe? (Nanotoxicology)

- *Precautionary Principle*: Novel properties of NP might confer unusual toxic actions

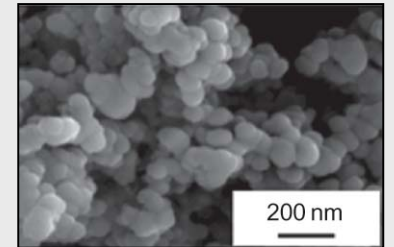
cedent for toxicity from ambient and  
thropogenic nanoparticles

ilar size to cell machinery (proteins,  
ceptors, pumps, DNA, etc)

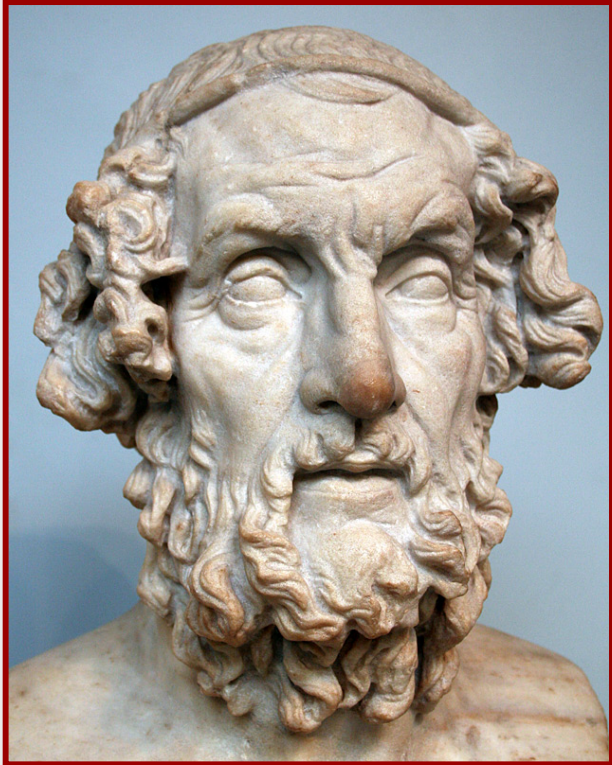
essential for exposure:  
ing manufacture



Blue asbestos  
(crocidolite)



Airborne soot  
(vehicular)



“Once harm has  
been done, even a  
fool understands  
it.”

Homer

(The Iliad, ca. 8th century BC)

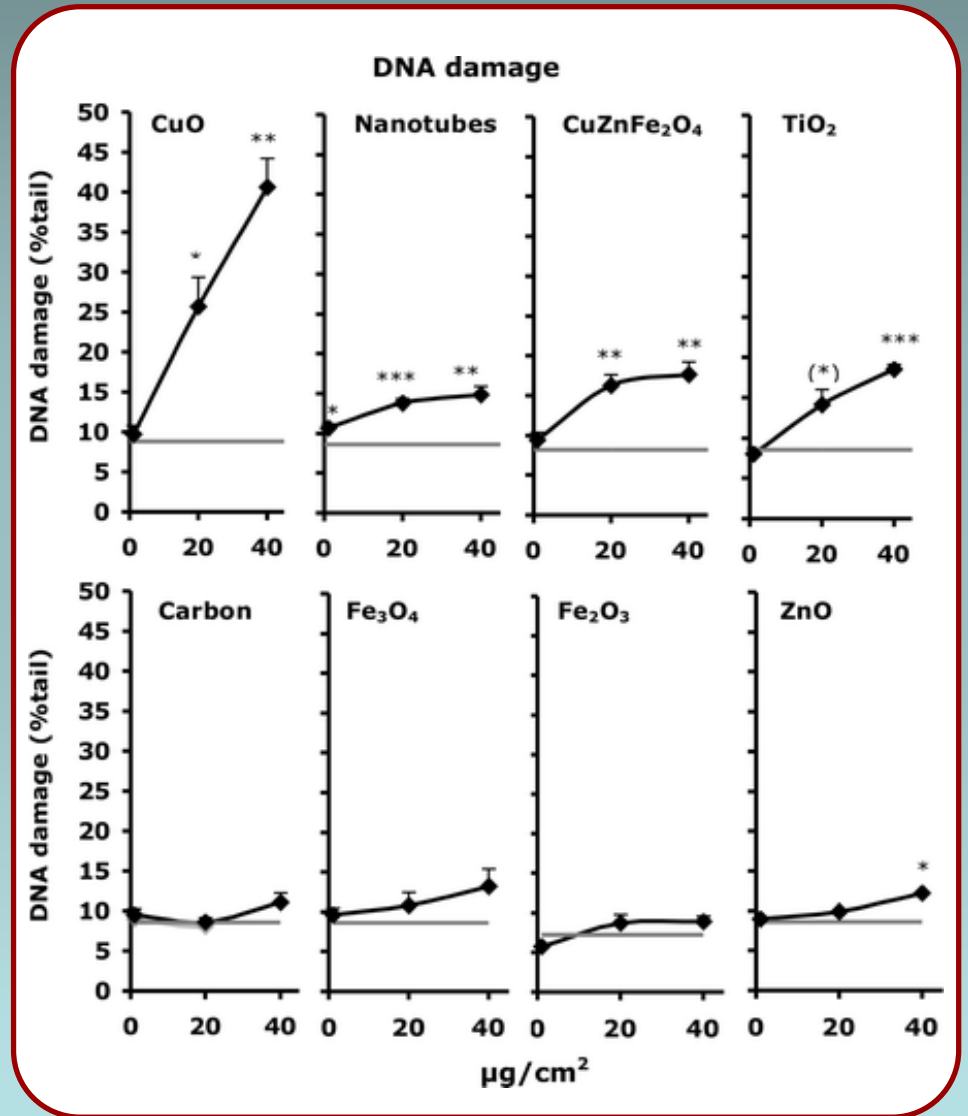
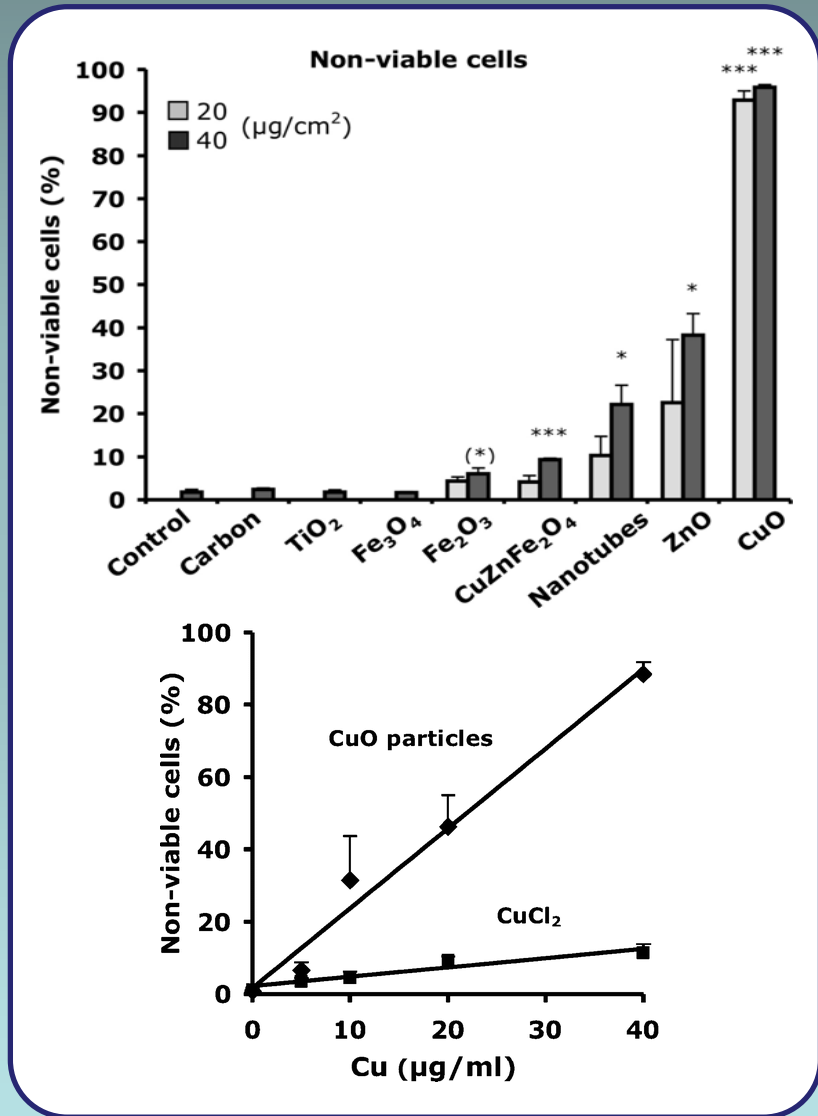
# Nanomaterial Diversity & the Task Ahead

- Different shapes (e.g., spheres, tubes, rods)
- Different chemistries (e.g., metals, semiconductors, carbon)
- Different surface characteristics (coating, functionalisation, charge, porosity)
- Such diversity precludes blanket assessments

“One of the greatest challenges facing the toxicology community is the prioritization of nanomaterials to evaluate and the depth of toxicological evaluation that should be conducted.”

Walker, NJ and Bucher, JR (2009) A 21st century paradigm for evaluating the health hazards of nanoscale materials? *Toxicol Sci* 110: 251-254

# Karlsson *et al* [2008] Comparably-Sized Metal Oxides Differ in Cytotoxic and Genotoxic Potency in A549 Lung Cells



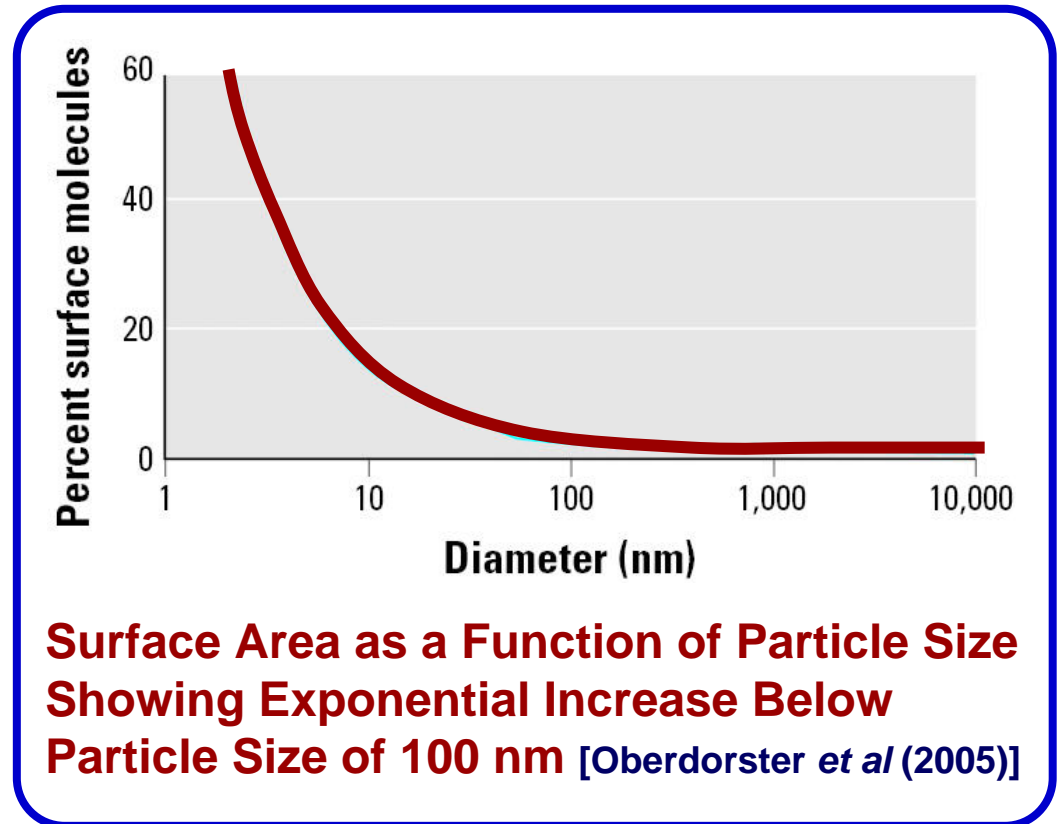
# Key Issues in Nanotoxicology

## 1) Physicochemical Characterisation

*“the forms nanomaterials adopt in vitro/in vivo”*

# Physicochemical Characterisation of Nanoparticles (NP) - *Agglomeration*

- High surface area confers distinctive behaviours
- May render NP prone to contaminants (metals, endotoxin, etc)
- Also, in test systems NP prone to clumping (liquid and gas phases)
- Reduces surface area
- *In vitro*, clumps interfere with bioassays
- *In vivo*, protein “corona”



# Key Issues in Nanotoxicology

## 1) Physicochemical Characterisation

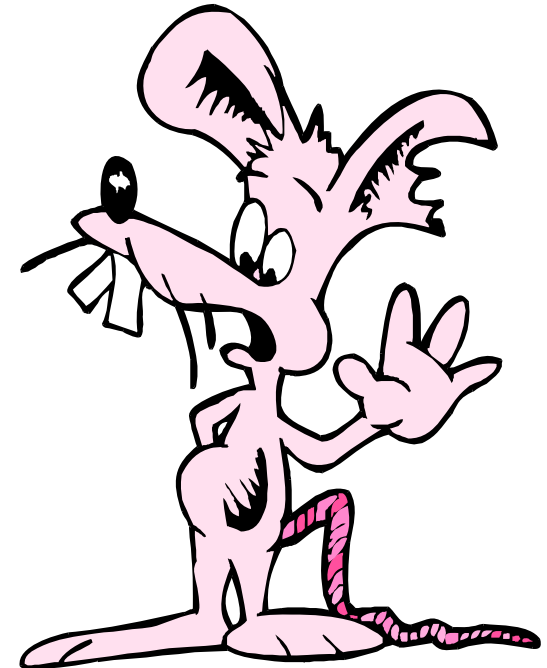
*“the forms nanomaterials adopt in vitro/in vivo”*

## 2) Dose-Selection

*“human relevance of toxicological models”*

# Issues in Nanotoxicology: *Dose Selection*

- Rapidly growing in vitro toxicity database
- Temptation to use high NP levels with questionable relevance for “primary” or “secondary” target cells
- In vivo studies – frequently use bolus, high dose exposures
- e.g. intranasal 7.5 mg TiO<sub>2</sub> in rats to elicit CNS injury (Wang *et al*, [2008])
- Need for greater attention to quantifying human exposures prior to experimental design



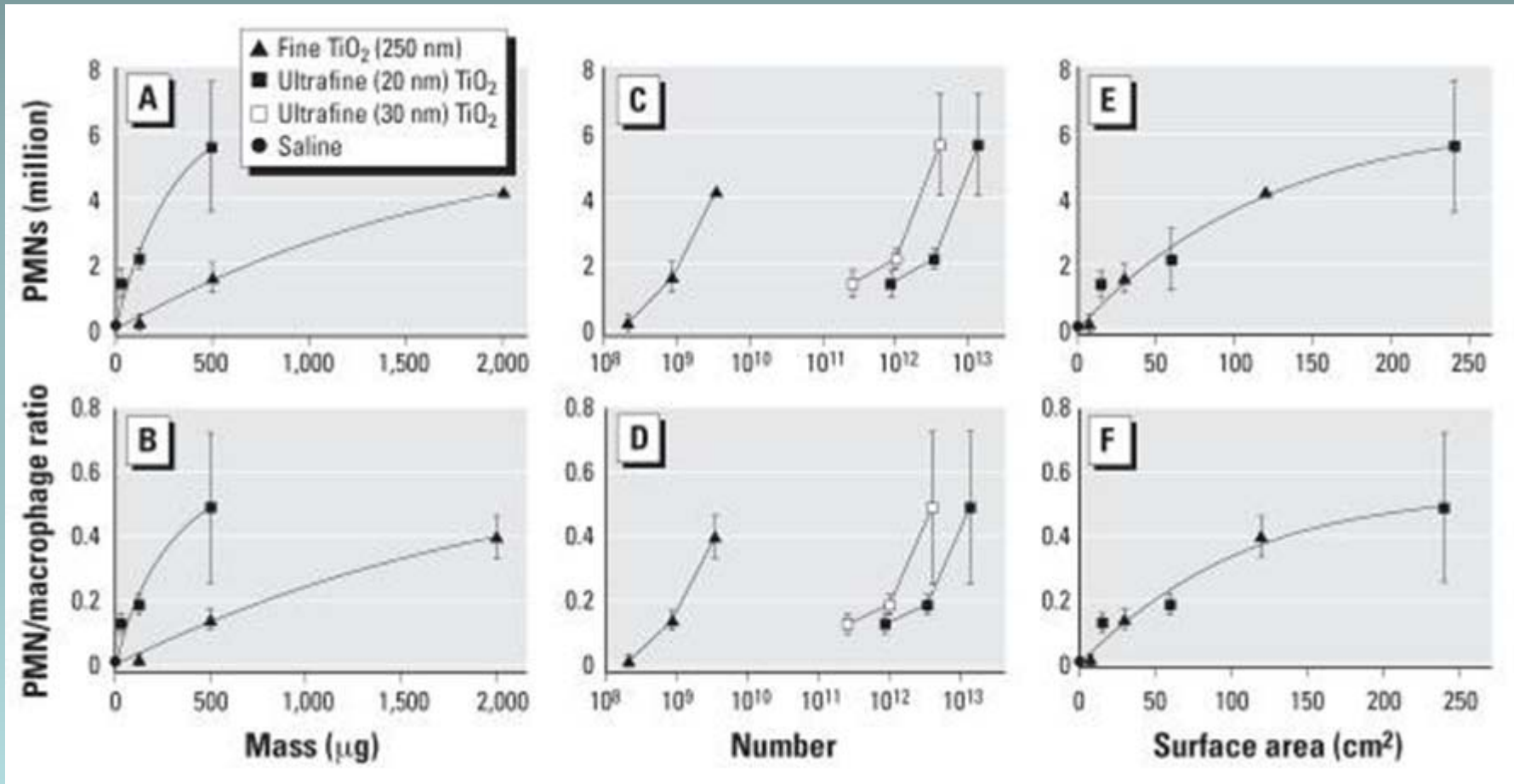
# Issues in Nanotoxicology - *Dosing Metrics*

- In conventional toxicology, mass most common dosing metric (mg/kg, nmol/L, etc)
- Mass used in setting exposures for airborne fine particles
- Less suitable for NP?
- ↓ radius ↑ no. particles per unit mass
- No. particles(?) or surface area may be better dosimeters

Particle number and particle surface area per 10 $\mu\text{g}/\text{m}^3$ airborne particles)		
Particle diameter ( $\mu\text{m}$ )	Particle No. ( $\text{cm}^{-3}$ )	Particle surface area ( $\mu\text{m}^2/\text{cm}^2$ )
5	153,000,000	12,000
20	2,400,000	3,016
250	1,200	240
5000	0.15	12

Table redrawn from Oberdorster et al (2005) *Envir. Hlth Persp.*, **113**, 825-839.

# TiO<sub>2</sub>-Induced Pulmonary Inflammation is Best Described by Surface Area Dose Metrics



Oberdorster et al (2007) *Env Hlth Persp*, 115, A290.

# Key Issues in Nanotoxicology

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*“the forms nanomaterials adopt in vitro/in vivo”*

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## 3) Toxicokinetics

*“what the body does to nanomaterials”*

# Toxicokinetics of Nanoparticles (ADME) - I

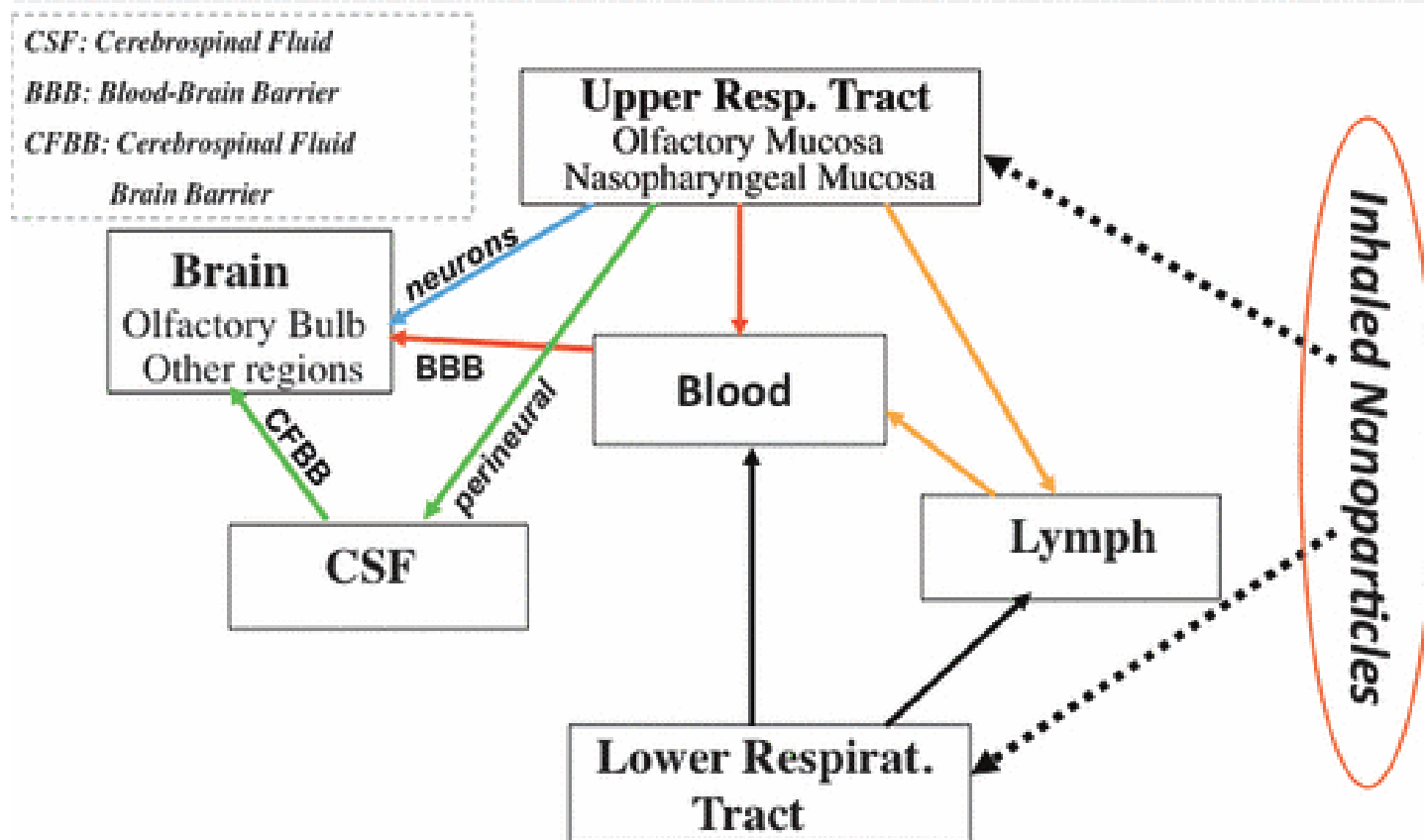
## Absorption

- Routes of entry include lung (inhalation), GIT, dermal, *i.v.*, *s.c.*, etc
- Variable transport across epithelial barriers (influenced by size, shape, surface chemistry)

## Distribution

- NP can be cleared – translocated - from blood by secondary tissues (esp. liver, kidney, spleen, etc)
- NP size & charge important (*c.f.* size of fenestrae in epithelial cell barriers)
- Dispersal by lymphatic system also for some NP

# Multiple Routes for Translocation of Inhaled (Pulmonary) NP to the CNS



# Toxicokinetics of Nanoparticles (ADME) - II

## Metabolism

- Likely minimal for most NP (c.f. polymeric forms?)
- Chemical stability a determinant of persistence for some NP classes (e.g. leaching of ions)

## Excretion

- Clearance by renal filtration for many NP
- NP size & charge important (glomerular pore diameters approx. 5 nm, favours +ve particles)
- Hepatic (biliary) export also likely
- Clearance by immune system (macrophages, reticuloendothelial system [RES])

# Key Issues in Nanotoxicology

## 1) Physicochemical Characterisation

*“the forms nanomaterials adopt in vitro/in vivo”*

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*“what the body does to nanomaterials”*

## 4) Toxicodynamics

*“what nanomaterials do to the body”*

# Two Toxicity Concerns with Nanomaterials

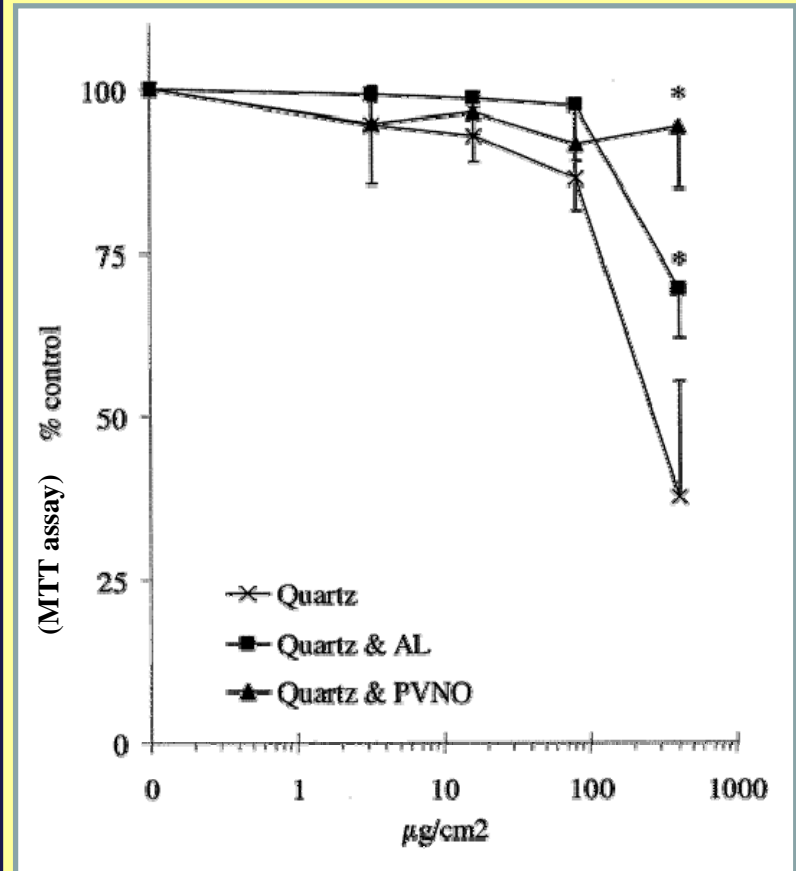
- Very little human data concerning NP toxicity
- In test systems, toxicity influenced by NP formulation, surface chemistry
- In vitro and in vivo animal studies highlight two concerns:

**1) Immunotoxicity**

**2) Oxidative stress**

Shvedova *et al* (2010) *Ann Rev Pharmacol Toxicol* 50: 63-88

E.g. Surface modification of silica NP (quartz) suppresses toxicity towards A549 Lung Cells.



Schins *et al* (2002) *Chem Res Toxicol* 15: 1166-1173

# Immunotoxicity of Nanomaterials

- Song *et al* (2009) *Eur Resp J* paper - fatal pulmonary inflammatory disorders in Beijing NP workers)
- Shown in animals for diverse nanomaterial classes (e.g. metal oxides, carbon nanotubes, fullerenes, etc)
- Inflammation can be consequence of excessive doses
- E.g. inhalation studies with high dose  $\text{TiO}_2$ , “macrophage overload,” chronic inflammation, fibrosis, tumours, etc)
- Recent induction of mesothelioma with MWCNTs in rats (c.f. asbestos) cause for concern (Sakamoto *et al* [2009] *Tox Sci*)

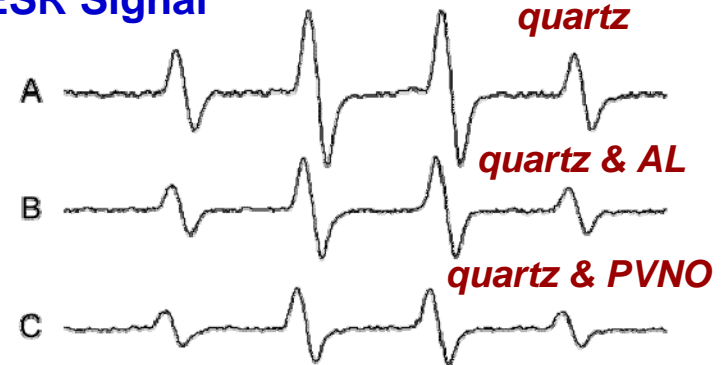
# Oxidative Stress

## *Surface Properties Matter*

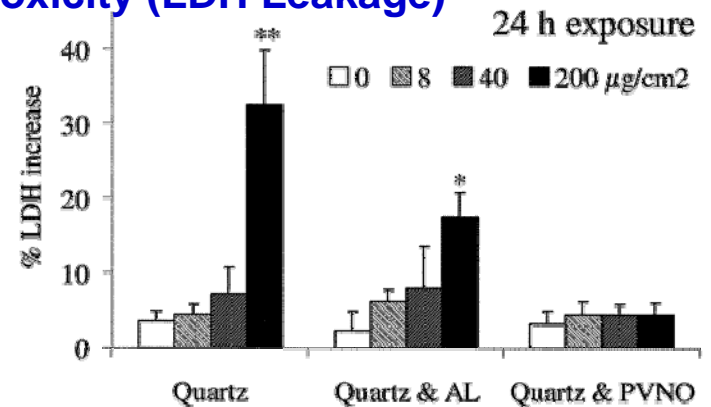
- Production of reactive oxygen species (ROS) a common NP property
- Can reflect NP surface contamination with metals
- Used for *in vitro* NP screens
- In vivo, oxidative damage to lipids, protein, DNA by NP
- ROS may be phagocyte-derived (e.g. NOX)

E.g. Surface modification of silica NP (quartz) suppresses free radical production and toxicity in A549 cells.

### ESR Signal



### Toxicity (LDH Leakage)



Schins *et al* (2002) *Chem Res Toxicol* 15: 1166-1173

# Key Issues in Nanotoxicology

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*“what the body does to nanomaterials”*

## 4) Toxicodynamics

*“what nanomaterials do to the body”*

## 5) Toxicity Prediction in the “Omics Era”

*“bringing scientific rigour to nanomaterial assessment”*

# New Paradigms in Toxicology

- 2007, “*Toxicity Testing in the 21<sup>st</sup> Century*,” NRC (USA)
- Advances in toxicogenomics, bioinformatics, systems biology, epigenetics, computational toxicology, *etc*
- Identification of common “toxicity pathways” in exposed cells
- Allow toxicity testing to move from whole-animals to primarily human cells in vitro
- Nanotoxicology likely to benefit

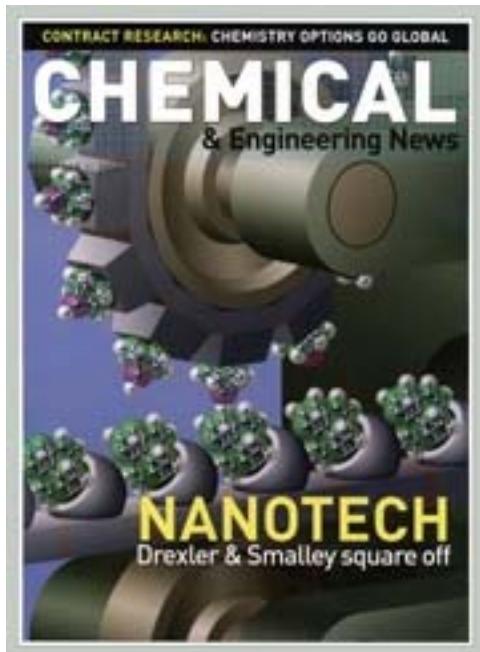


# Nanotoxicology

- 1) Nanoengineered materials hold great economic promise on account of novel properties
- 2) Most appear safe under common conditions of use, but vigilance needed to detect rogue toxicities
- 3) Toxicological evaluation of nanoparticles needs to be informed by modern scientific concepts

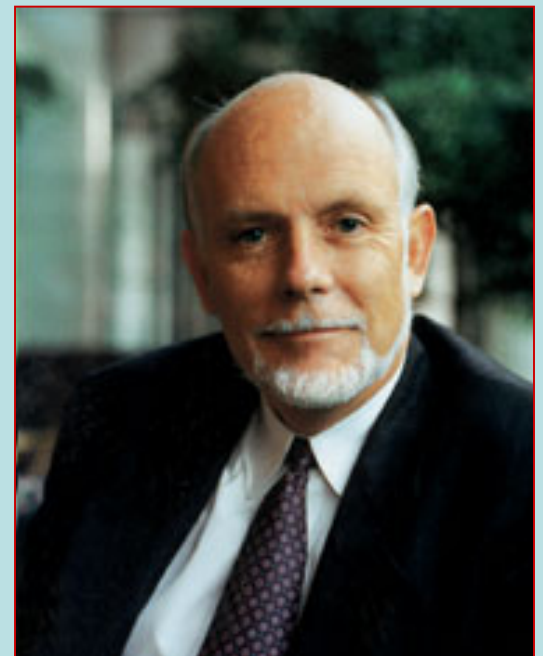
# The Future Prospects for Nanotechnology

A Public Spat



**Eric Drexler**  
*Foresight Institute*

*"Engines of Creation:  
The Coming Era of  
Nanotechnology"*  
(1986)



**Rick Smalley**  
*Rice University*

*Nobel Laureate  
(Chemistry, 1996)  
Buckyball Co-  
discover*