JOHNS HOPKINS BLOOMBERG SCHOOL of PUBLIC HEALTH

NANOTOXICOLOGY: BIOENGINEERING BASED APPROACHES TO A NEW TECHNOLOGY

Ellen Silbergeld, PhD
Johns Hopkins University
Bloomberg School of Public Health
Collegium Ramazzini 2008

Looking as we leap…or before we leap?

Topics for this presentation

- Work at Hopkins on nanobiotechnology
- State of the technology
- Signals of concern
- A nanobiological approach to assessing nanotechnology

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UNIVERSITY

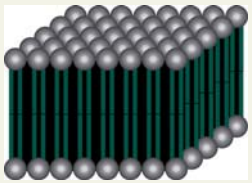
Institute for NanoBioTechnology

Johns Hopkins University

Why is nanotechnology relevant to biology and medicine

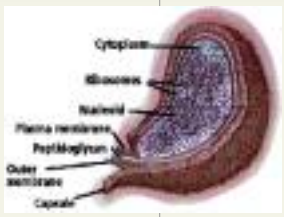
Au atom

H_2O molecule



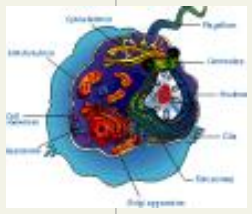
cell membrane:

Viruses:



prokaryotic cells:

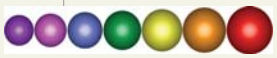
animal and plant cells



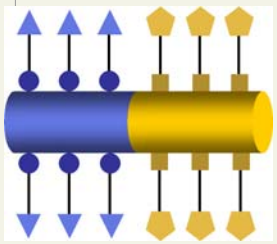
red blood cells:

DNA: 2.5 nm

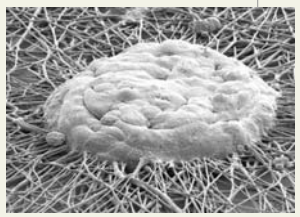
0.1 nm | 1 nm | 10 nm | 100 nm | 1 µm | 10 µm



Quantum Dots:



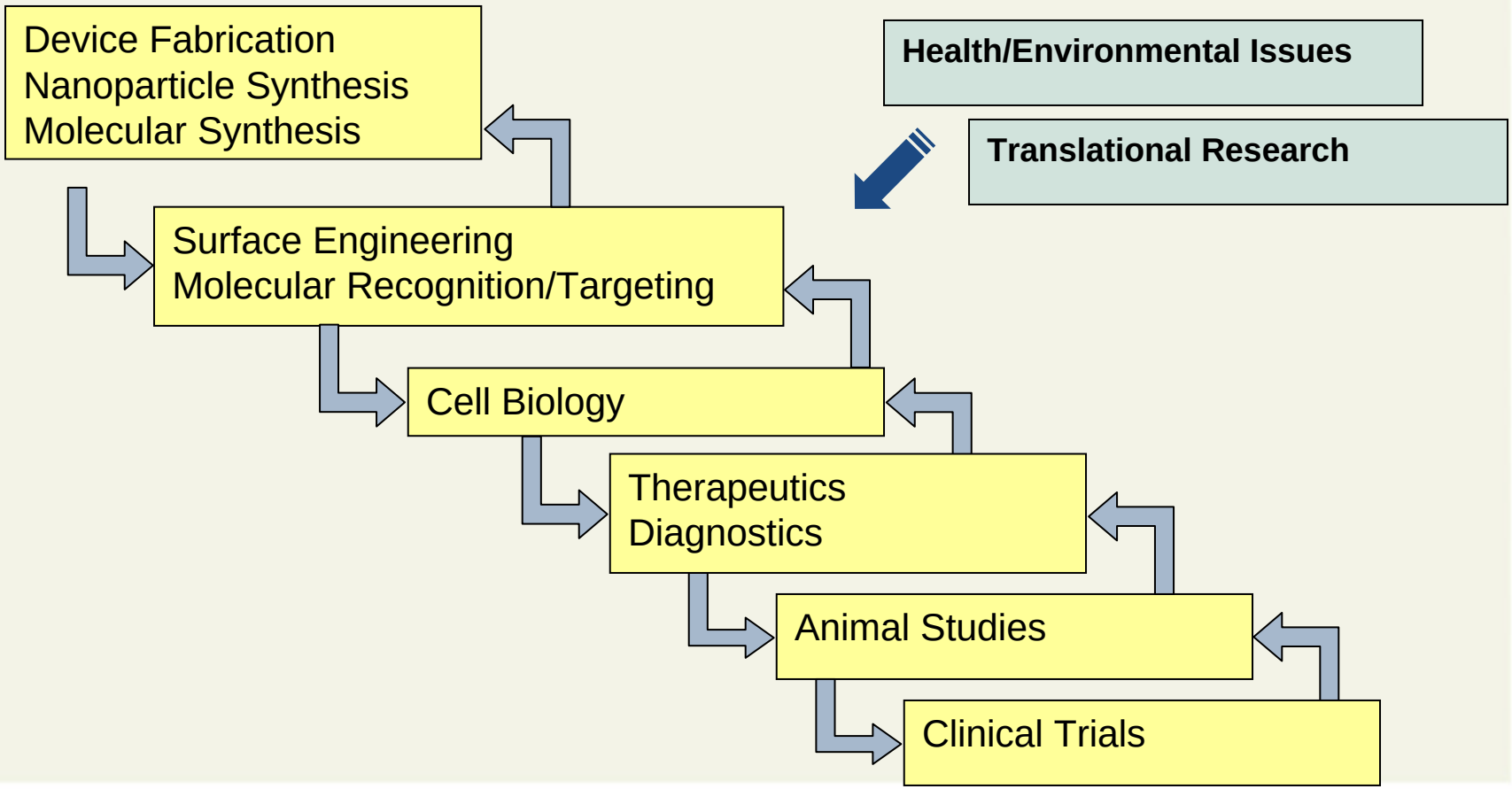
multifunctional nanoparticle



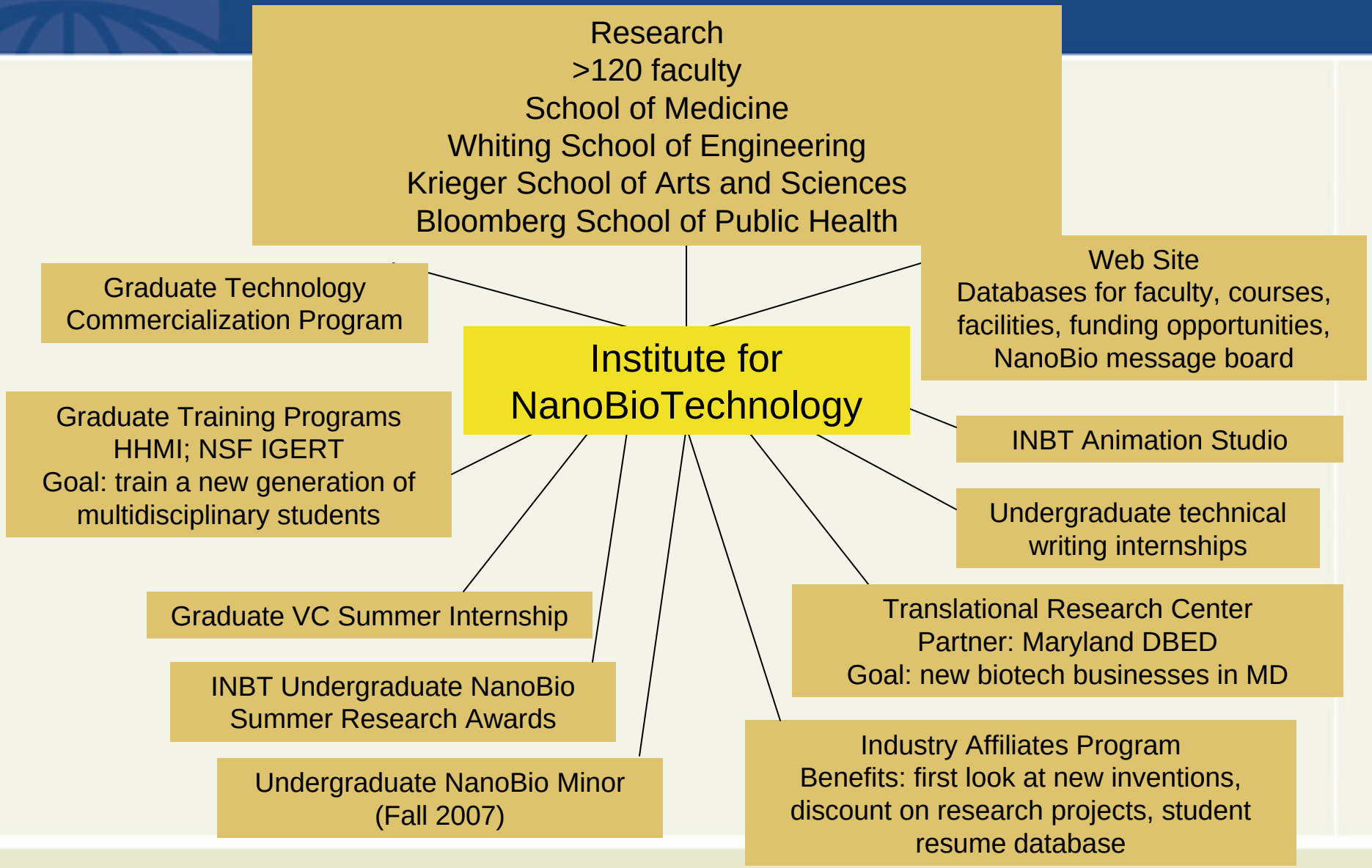
scaffolds for tissue engineering

lab-on-a-chip

Why an Institute?



INBT Activities



What is nanotechnology?

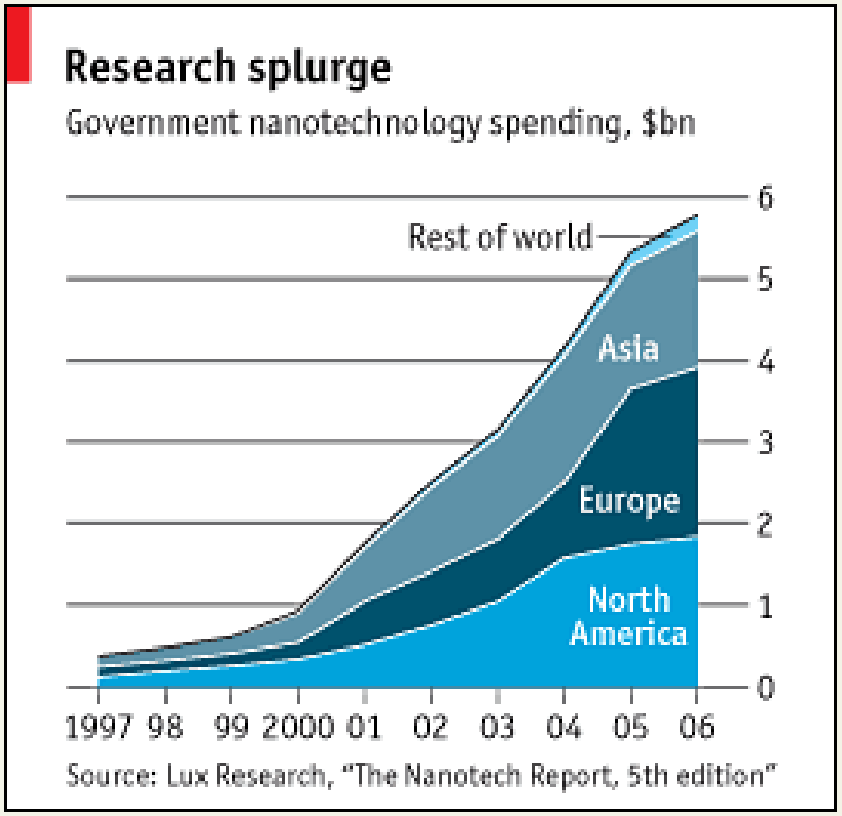
- *Engineered materials* with at least one dimension less than ≤ 100 nm
  - Nanodots, nanotubes, nanospheres
  - Approaching or at the subcellular scale
- Highly purified materials
  - Often metals
- High surface to mass ratio – high surface reactivity
  - Potential for chemical delivery/binding
- Multiple potential uses
  - Passive, active, systems

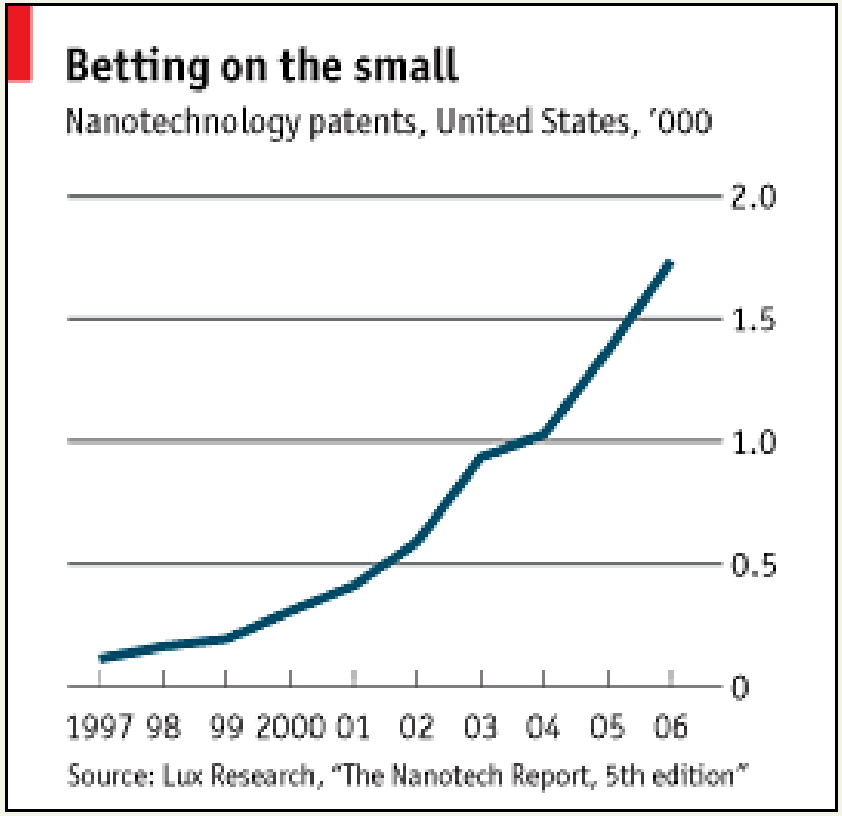
Nano Applications

- **Current uses**
  - Surface treatments – “nanowhiskers”
  - Water/stain repellent treatments
  - Cosmetics/UV protection
  - Fillers in plastics
  - Semiconductors
  - *Fuel additives*
  - Biomedical imaging materials
- **Near term introductions**
  - Drug delivery systems
  - Nanoenergy sources
  - Environmental remediation products
  - sensors

On the drawing board: intelligent systems, complex machines

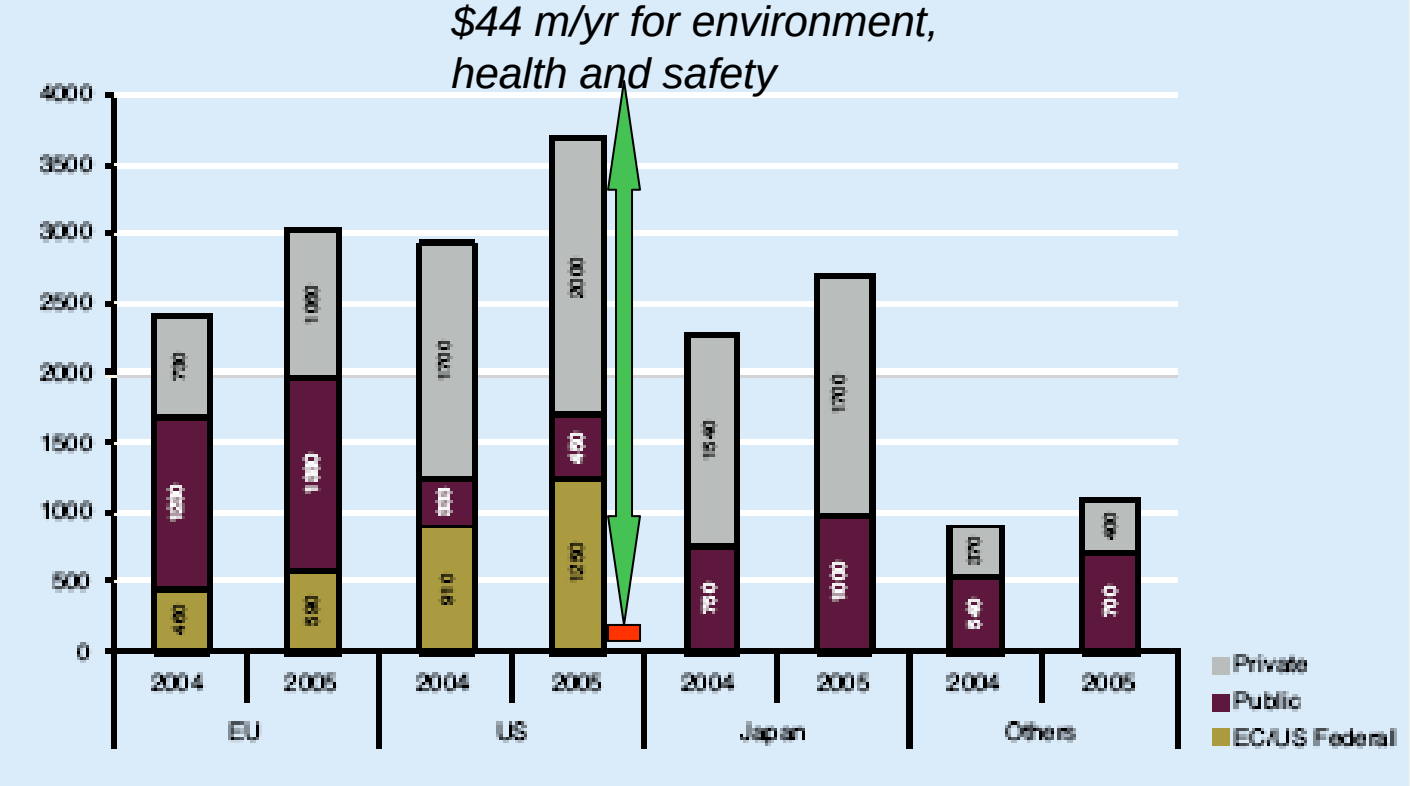
State of nanotechnology: precaution is no longer an option





The Economist 22/11/2007

Large government investments…in R&D



Source: UNEP (2007) Geo Yearbook

<2% of publications in 2006 on health and safety

Nanomaterials: Is there cause for concern?

- The existing literature on nanomaterials
- TSP → PM10 → PM5 → ultrafines
- What can happen at the nanoscale?
- The sorcerer’s apprentice…

Status of nanotoxicology

- Toxicological research
- Inferences from current knowledge
- Risk assessment ?
  - Something about hazard identification (toxicity testing)
  - Very little about dose
  - Uncertain exposure assessment

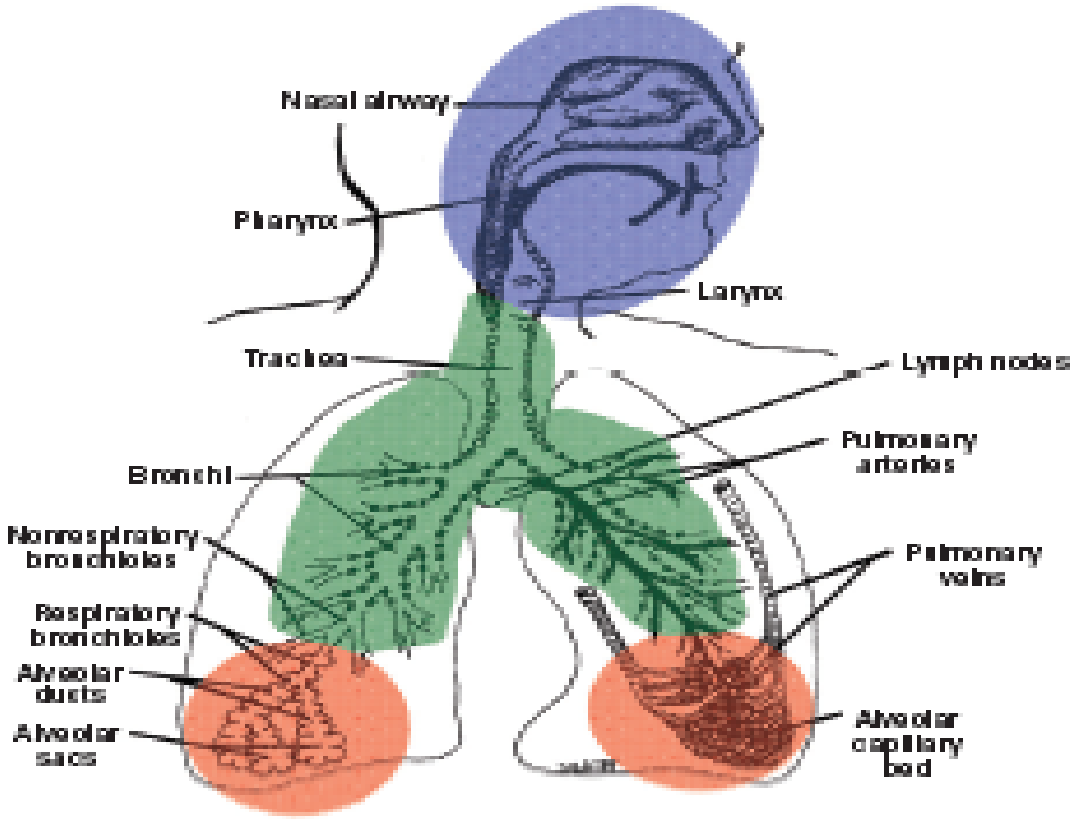
Reasons for concern: Metals form the base for nanomaterials development

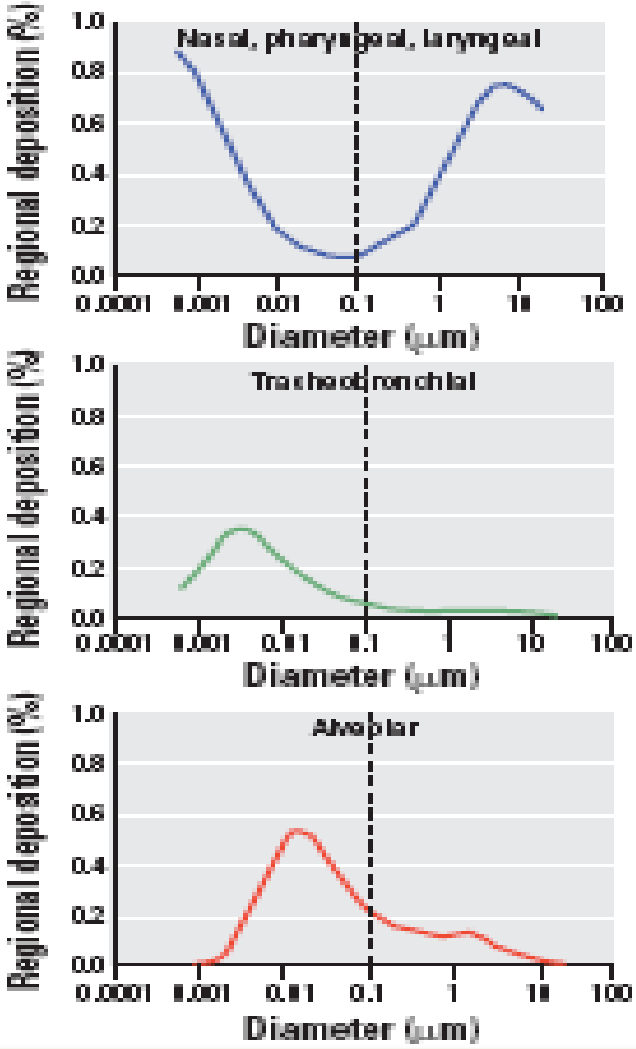
Major types of nanoparticles anticipated to be commercially available in 2006-14

Product	2006-07	2008-10
		Tonnes/year
Nickel (carbon-coated) (Ni-C) powders	3 500	7 500
Poly (L-lactic acid) (PLLA) nanofibres	500	2 500
Yttrium Oxide (Y_2O_3) nanopowders	2 500	7 000
Ceria (CeO_2) nanoparticles, coatings	N/A	10 000
Fullerenes	N/A	300
Graphite Particles	1 000 000	N/A
Silica (SiO_2) nanoparticles, coatings	100 000	100 000
Titania (TiO_2) nanopowders, thin layers	5 000	5 000
Zinc Oxide (ZnO) nanopowders, thin films	20	N/A
		USD/year
Carbon black	~ 8 billion	10 billion
Carbon nanotubes	700 million	3.6 billion

Source: UNEP (2007) Geo Yearbook

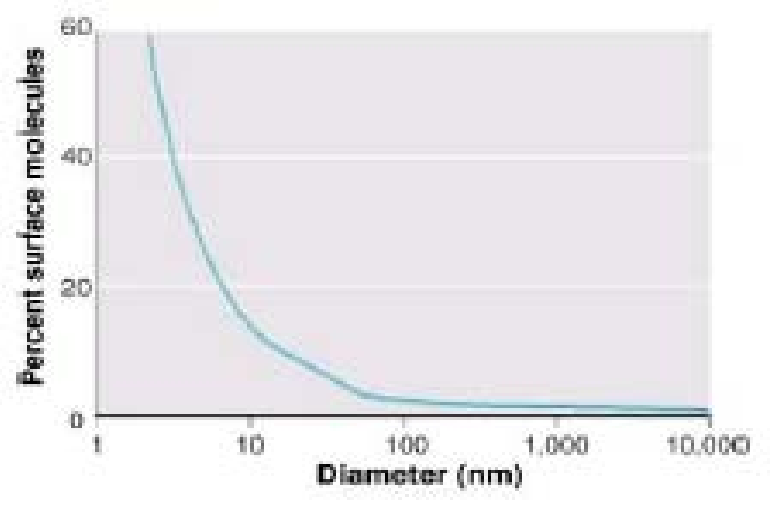
Smaller is worse: size determines exposure: size and lung deposition





Oberdorster et al (2005) EHP

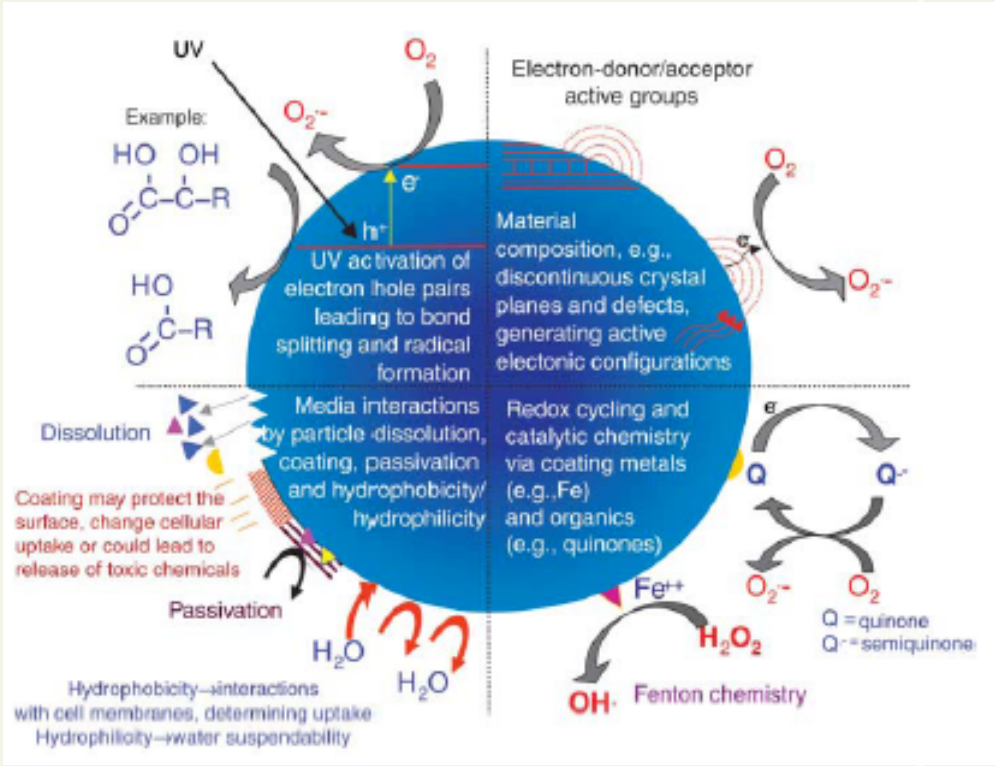
Structure determines activity and function



And toxicity?

Table 1. Particle number and particle surface area for 10 μg/m³ airborne particles (5).

Particle diameter (μm)	Particles/ml of air	Particle surface area (μm²/ml of air)
2	2	30
0.5	153	120
0.02	2,390,000	3000



Nel et al Science 2006 (Feb 3)

SIZE AND STRUCTURE

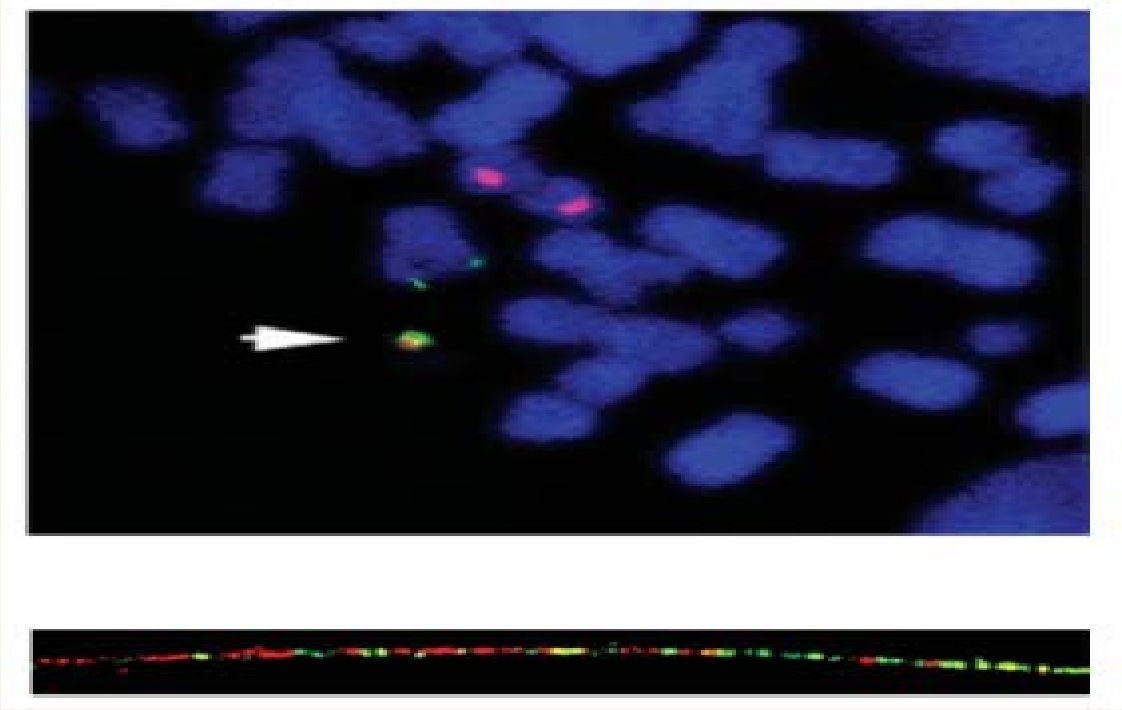
Hazard identification The dose makes the poison

Paracelsus, Father of Toxicology

How to prudently evaluate the safety/risks of nanomaterials … and can we do it in time?

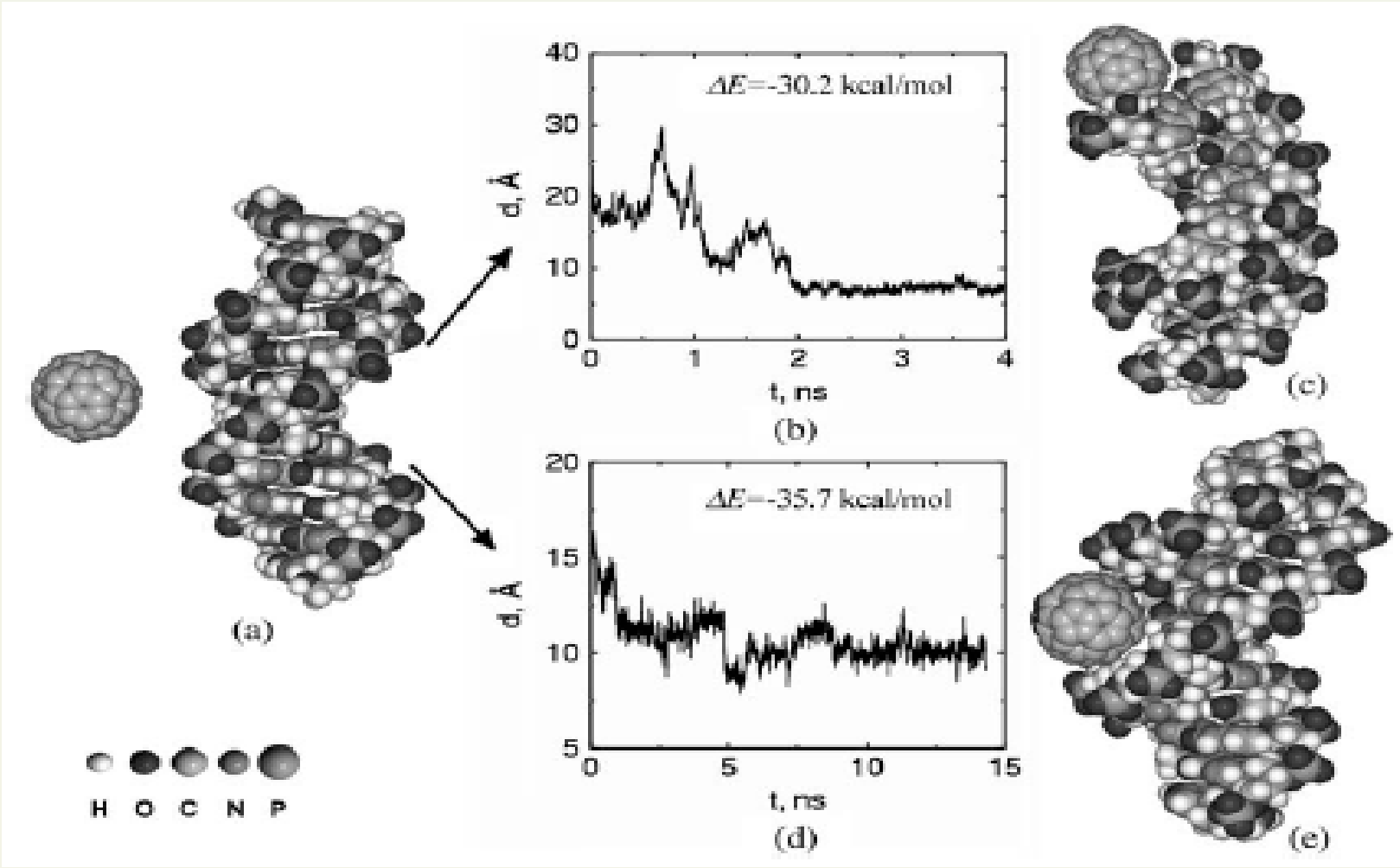
- Conventional toxicology
- Exposure assessment
- Fate and transport
- Predictive models
- *Let biology guide*
- *Alternatives: Nanoscale toxicology and modeling*

Nanostructure and function of human artificial chromosomes



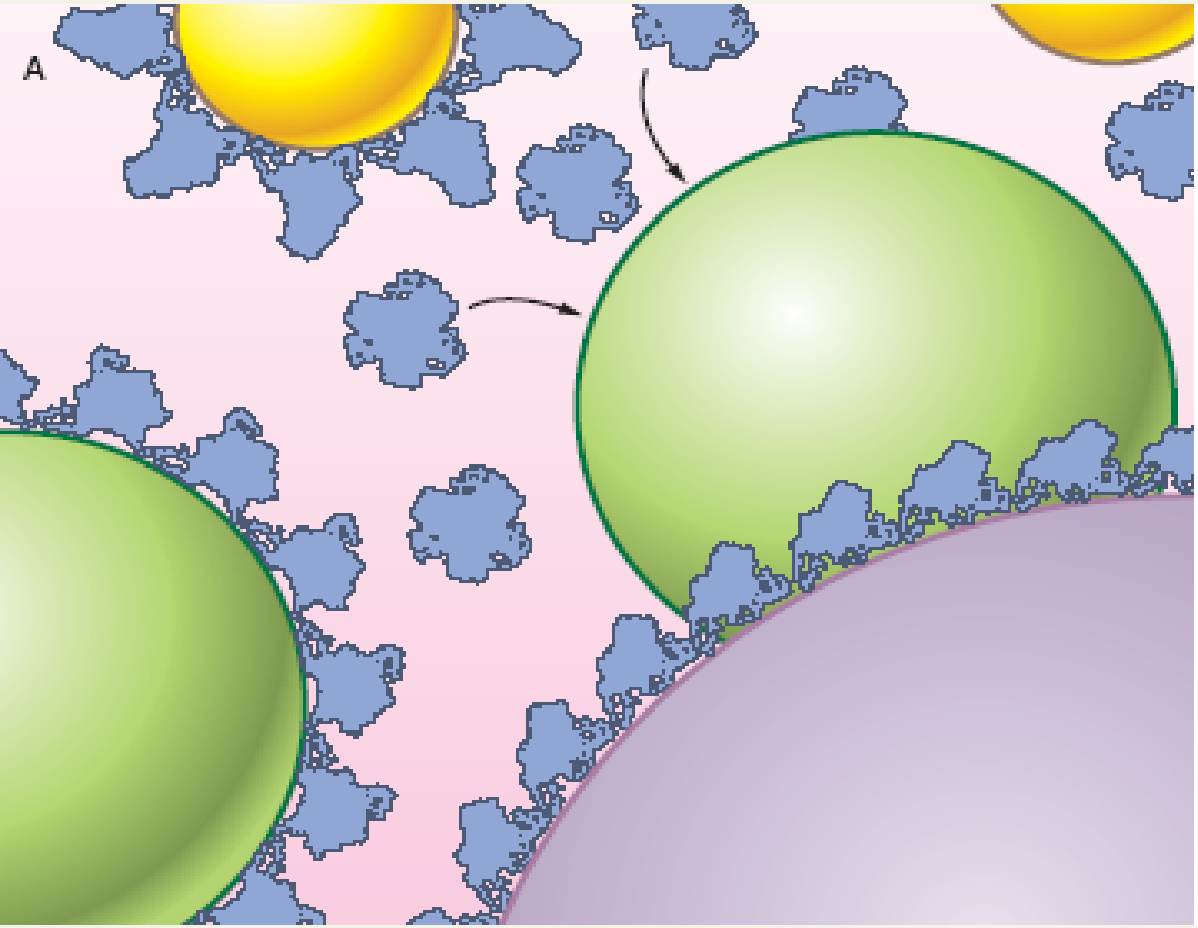
Monaco and Moralli (2006) Biochem Soc Trans 34: 324.

Toxicity at the nanoscale: nanospheres and DNA



Zhao et al (2005) Biophys J

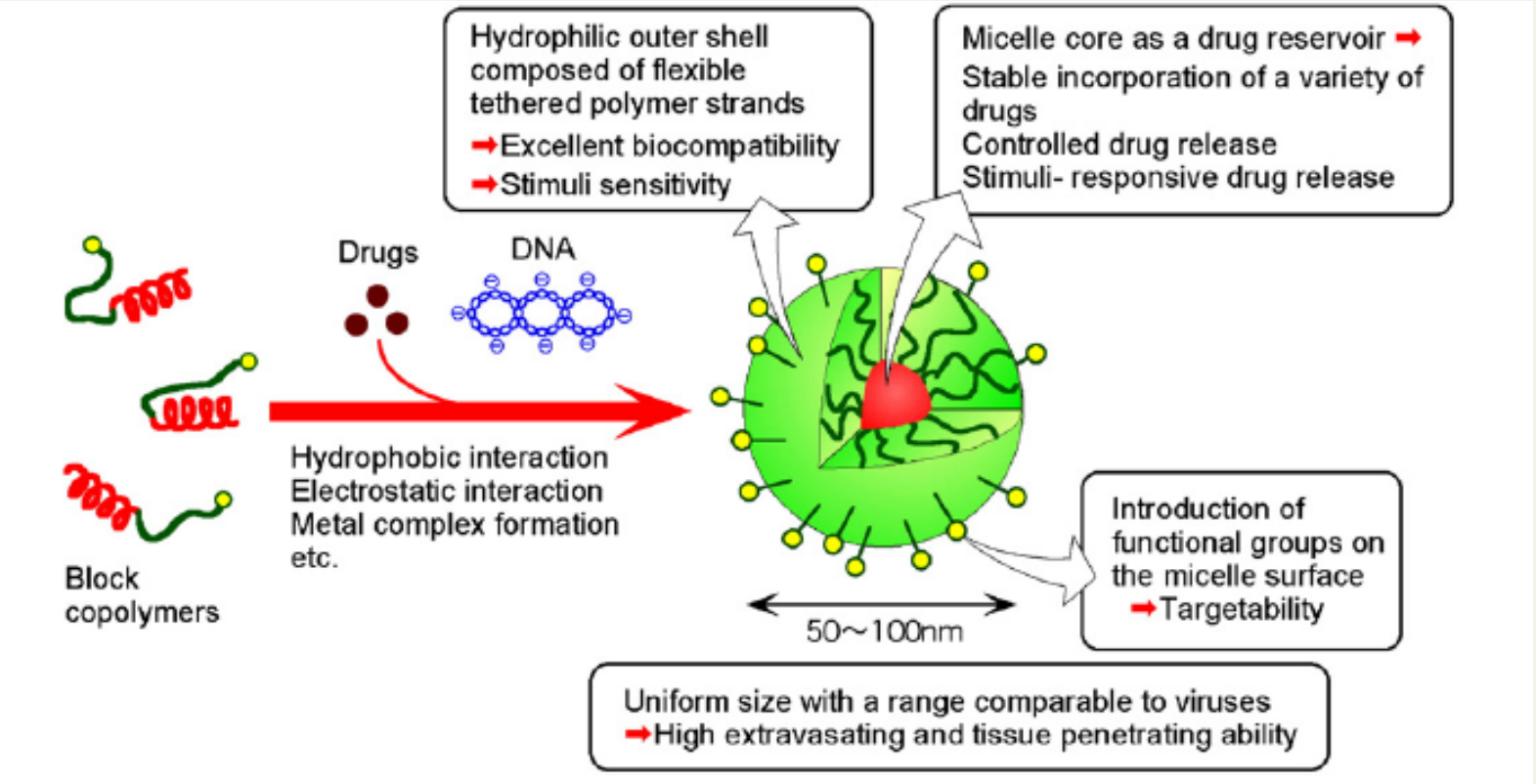
Immunotoxicity at the nanoscale: uncovering cryptic epitopes



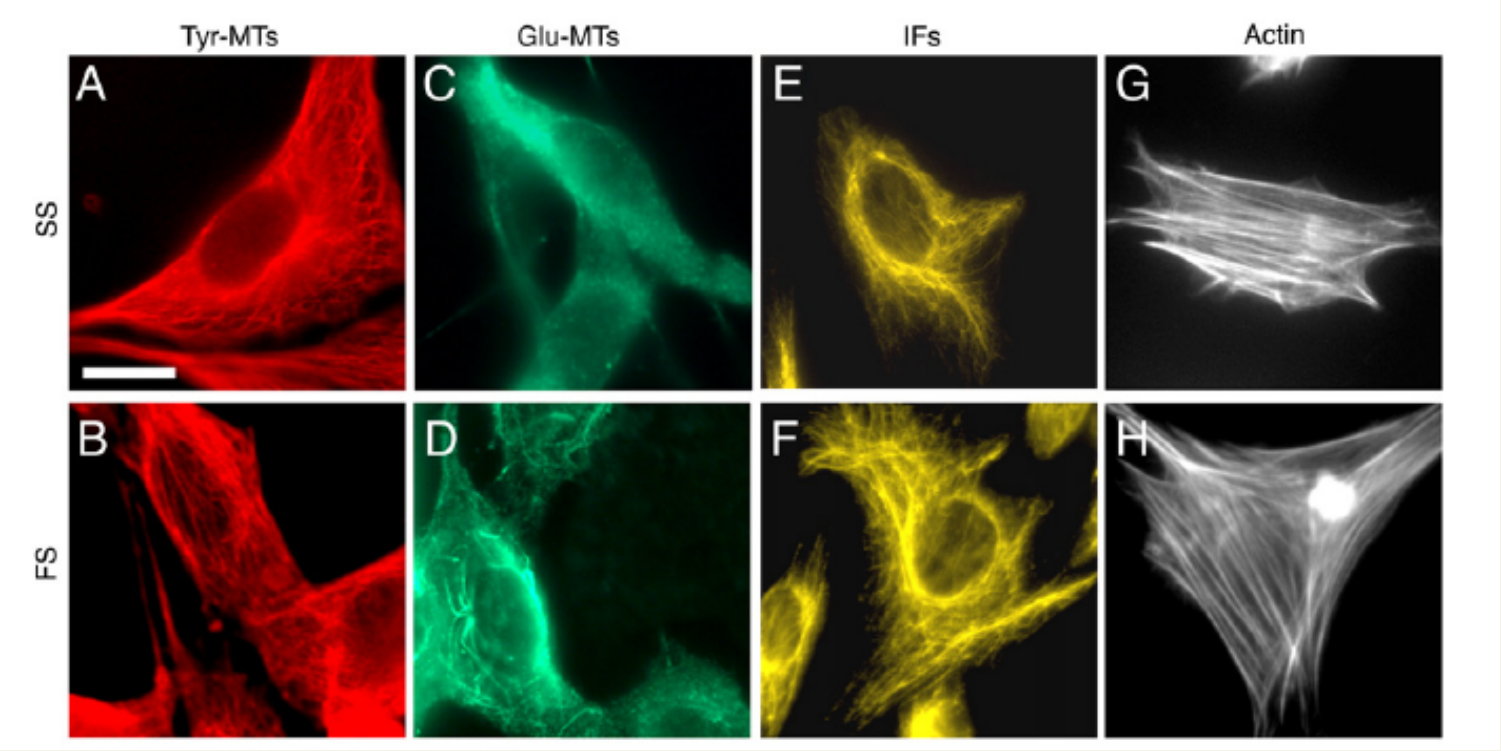
Nanoparticles (large spheres) cause proteins to change shape, revealing regions that trigger antibody-mediated immune response

Lynch et al (2006) Science STKE March 21

Nanowire delivery systems in nanomedicine

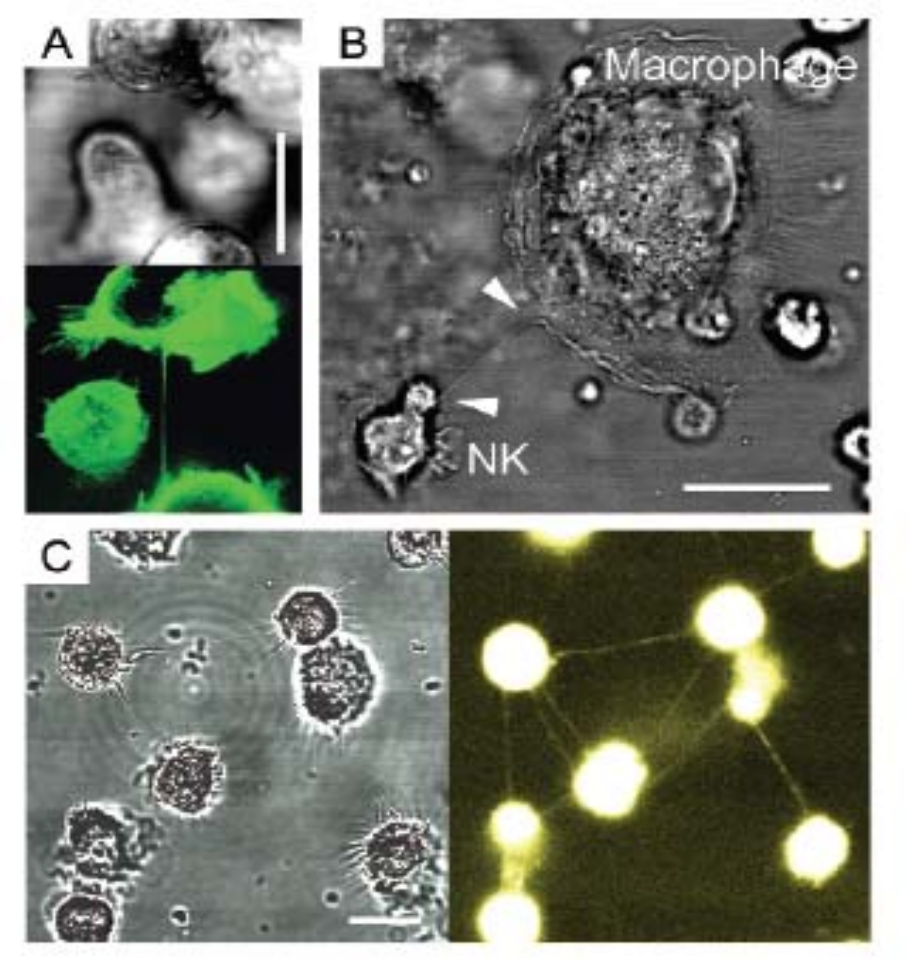


Start with what we know about biology at the nanoscale: cellular and subcellular structures



Pelling et al (2007) Nanomedicine 3: 43

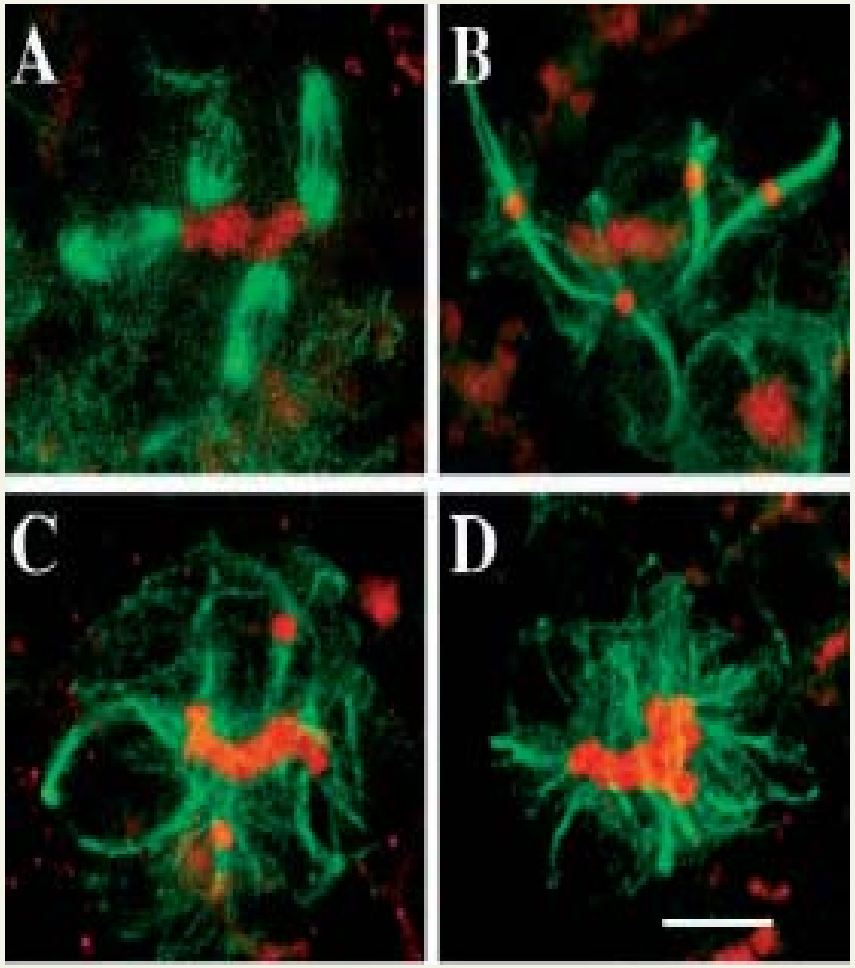
Nanoscale events in intercellular communication



Nanotubes connecting macrophages, NK cells, B cells

Onfelt B et al (2005) STKE Science

Nanostructural events during early development

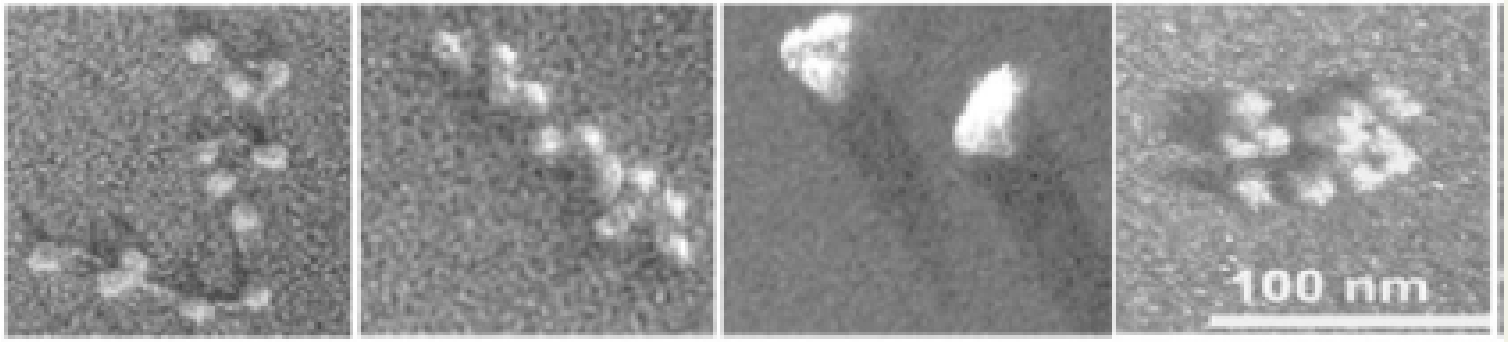


Role of tubular structures throughout early development in *Drosophila*

Grieder N et al (2000) Development

Nanoscale regulation of gene expression through chromatin binding molecules

Property	Histone H1	MeCP2	MENT
Transcriptional repression	Rarely *in vivo*	Yes	Yes
Preferred binding site	Near linker entry–exit	Methylated DNA	Linker DNA
Chromatin compaction	Yes, zigzag conformation	Yes, high compaction	Yes
Interarray interactions	Yes	Yes	Yes



12-nucleosome array | +H1 | +MeCP2 | +MENT

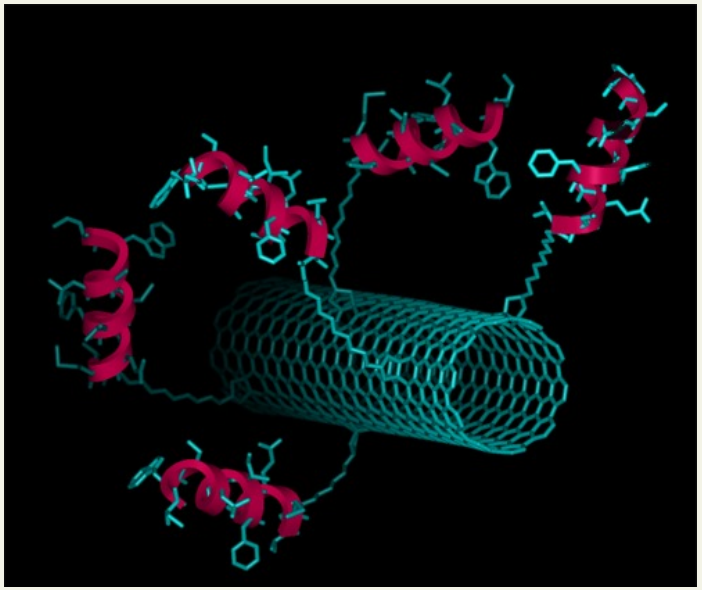
Woodcock (2006) Curr Opin Struct Biol March 13 epub

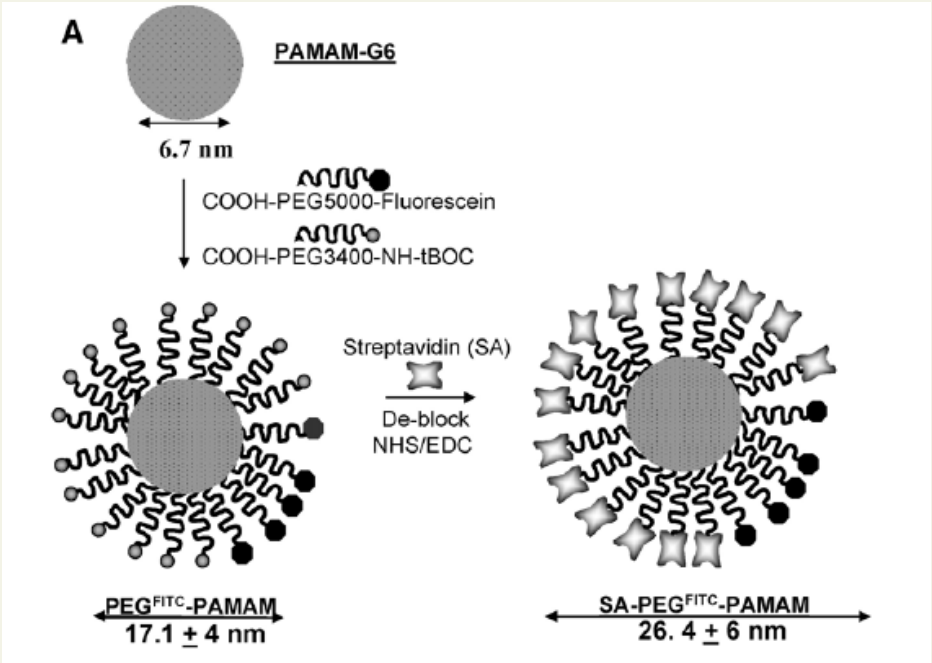
Think about unintended consequences of engineered functions of nanomaterials

- Nanomaterials as drug delivery systems…

“The Sorceror’s Apprentice” – Walt Disney

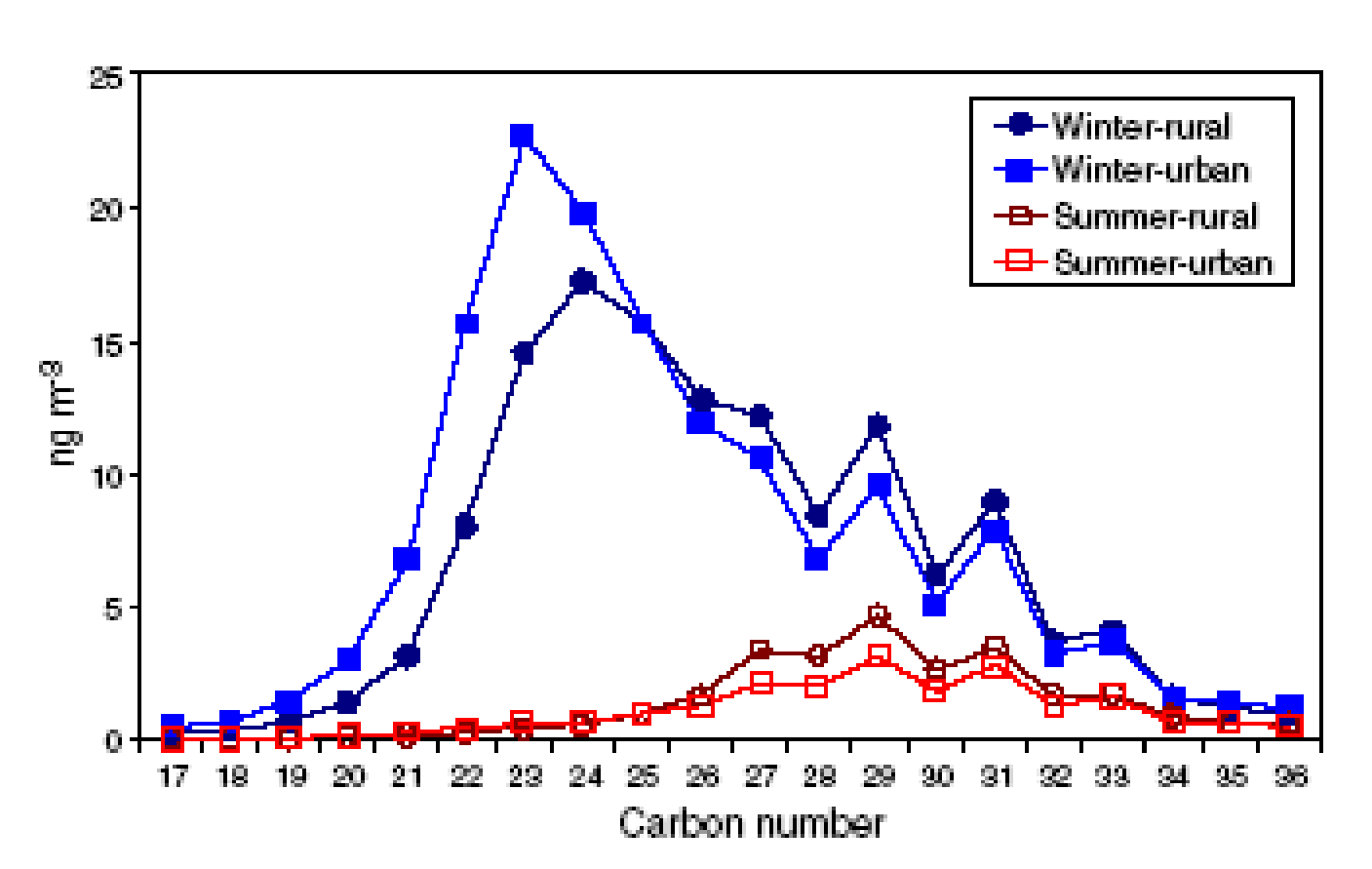
Developing nanomaterials as drug delivery nanosystems





Falmy et al (2007) Nanomedicine 3: 75

What if nanomaterials pick up environmental contaminants?



Feng et al (2006) Chemosphere epub

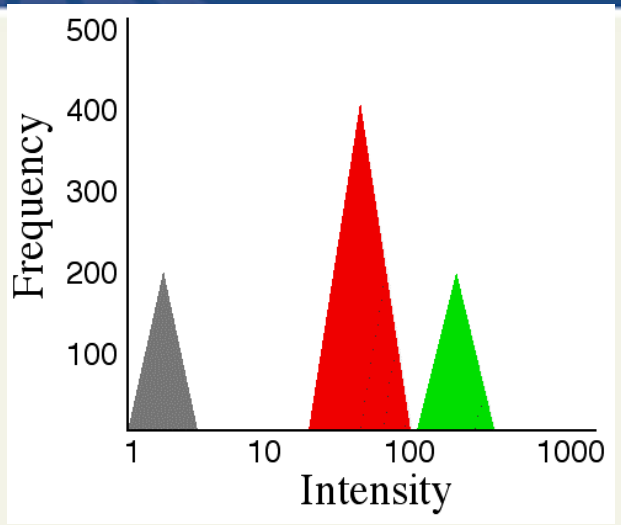
Try to think like a nanobiologist and a nanotechnologist

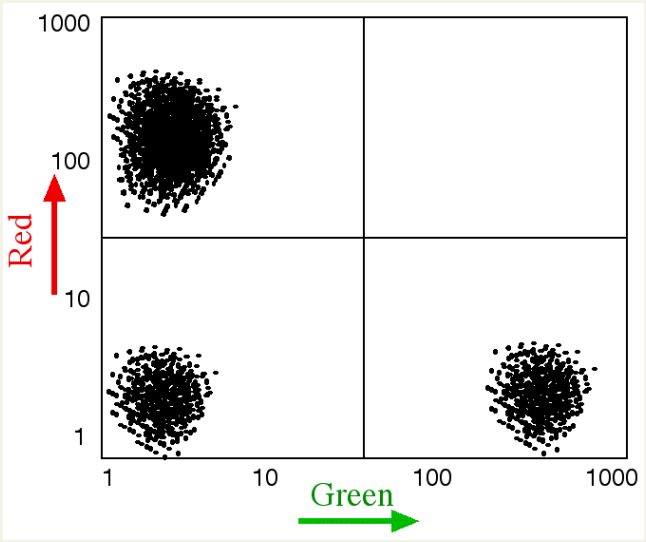
- Use biological methods to visualize nanoparticles
  - In vitro assessment of cellular interactions with flow cytometry
  - NPs tagged with embedded dyes
- Study cellular reactions to nanoparticles in real time
  - FACS real time assessment of population responses
  - Biological markers of cellular response

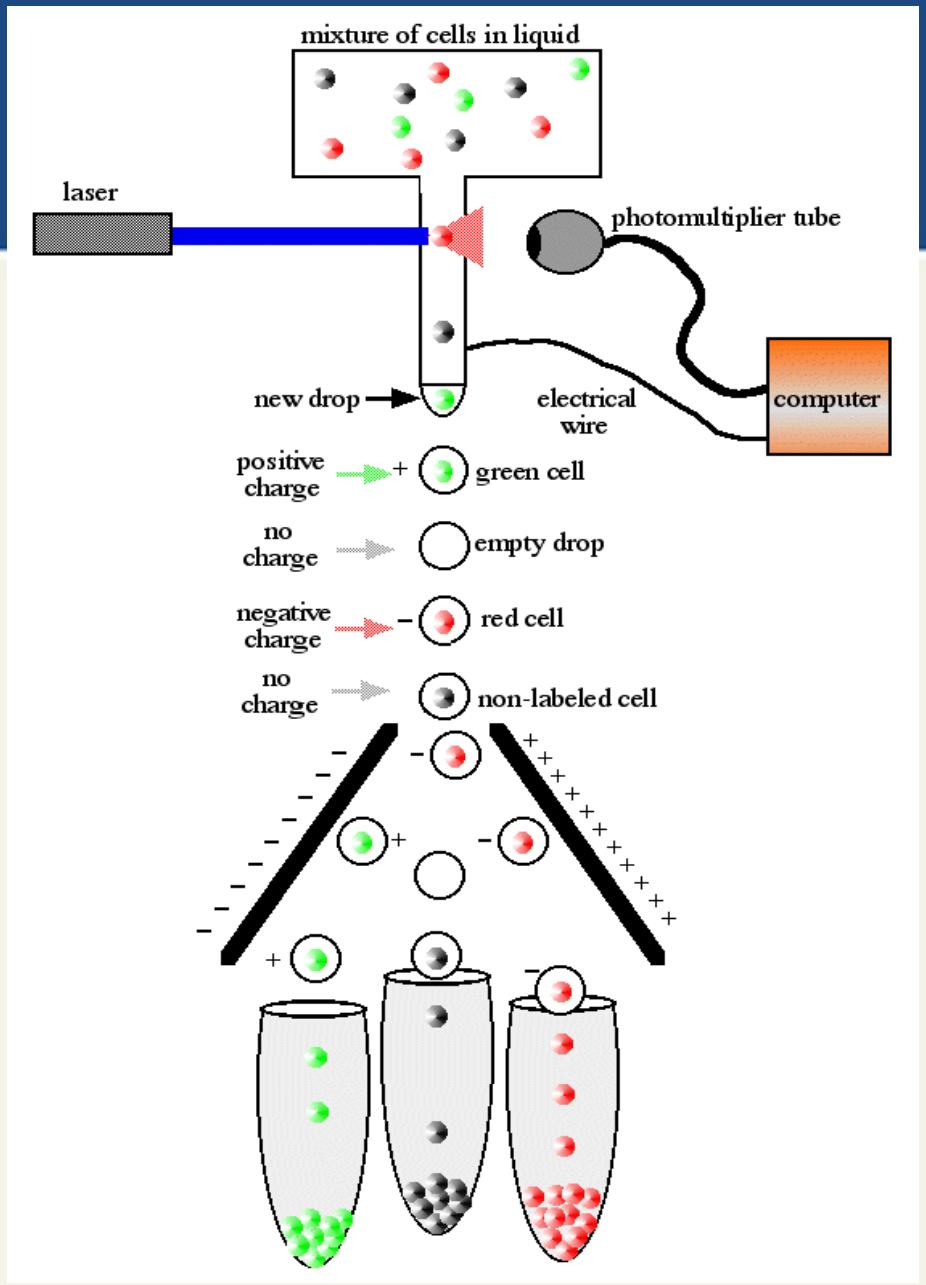
Toxicology at the nanoscale: A collaboration between materials science and toxicology

- Splenic lymphocytes isolated from BALB/c mice
- Cell cultures (10^6) established
- NPs tagged by embedded dansylamide dye
- FACS staining for activation markers for lymphocyte subsets and key events: iNOS, TLR4, CTLA-4, Tim-3; apoptosis (annexin V and 7-AAD)

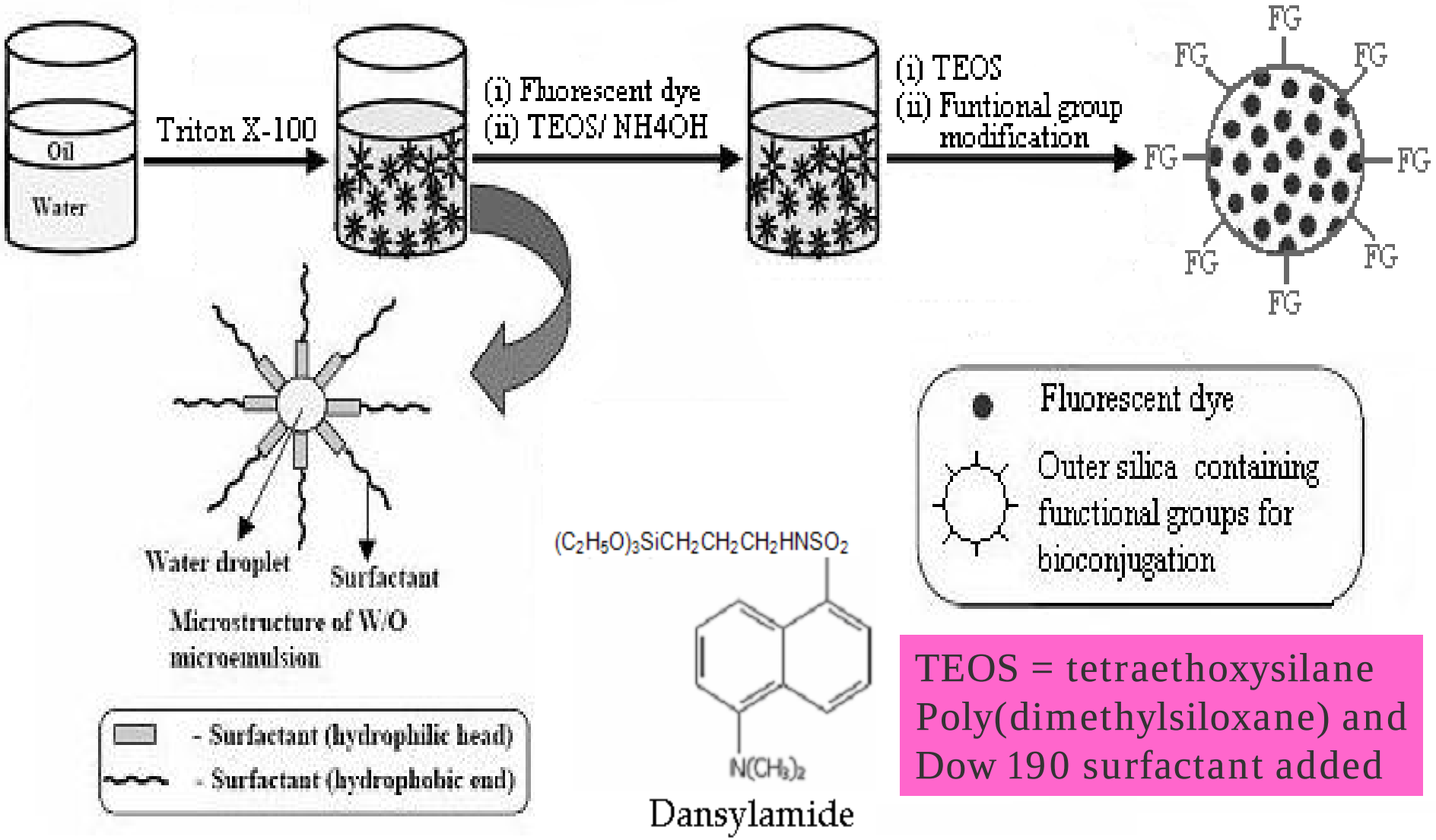
How FACS works





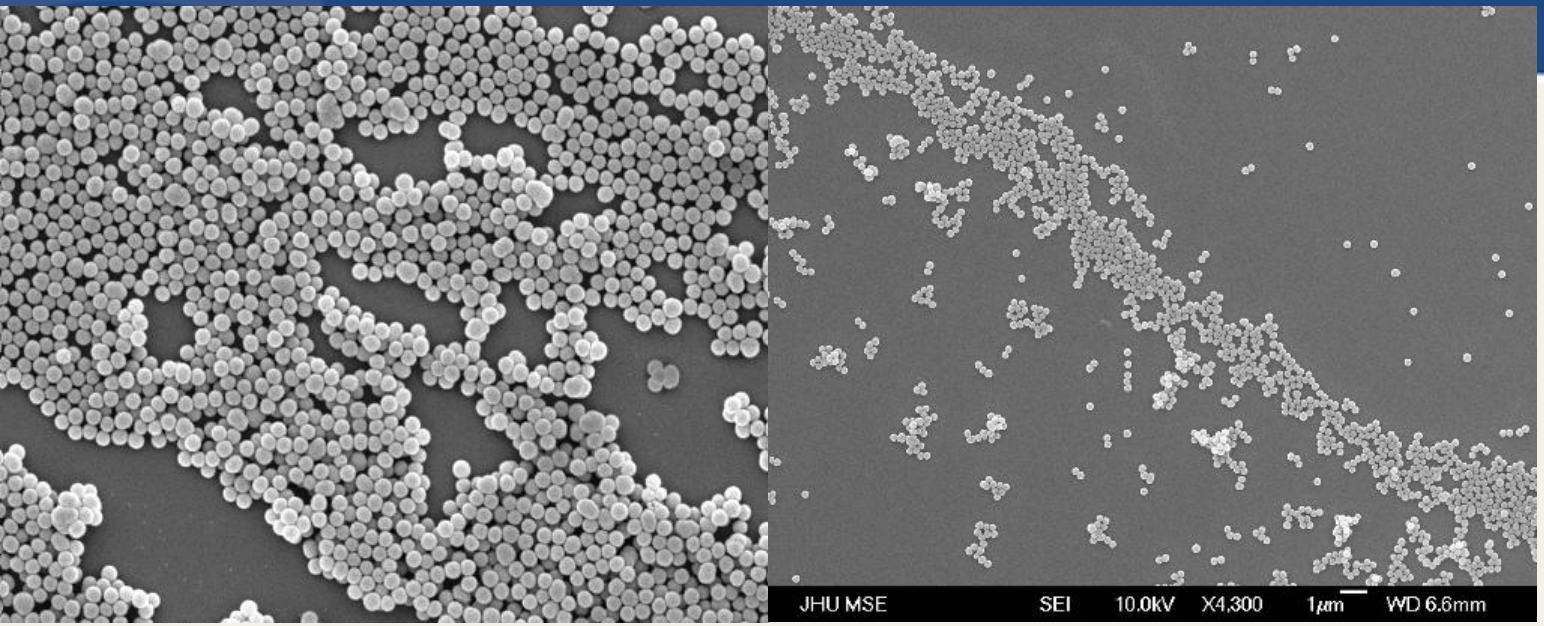


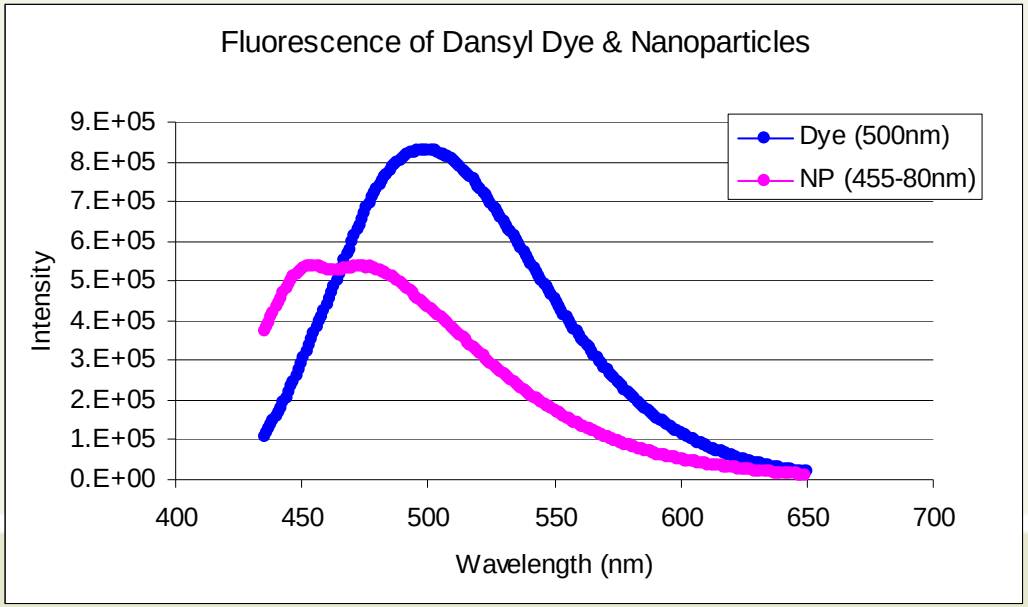
Synthesis of Dansylamide-containing Silica Nanoparticles



TEOS = tetraethoxysilane
Poly(dimethylsiloxane) and Dow 190 surfactant added

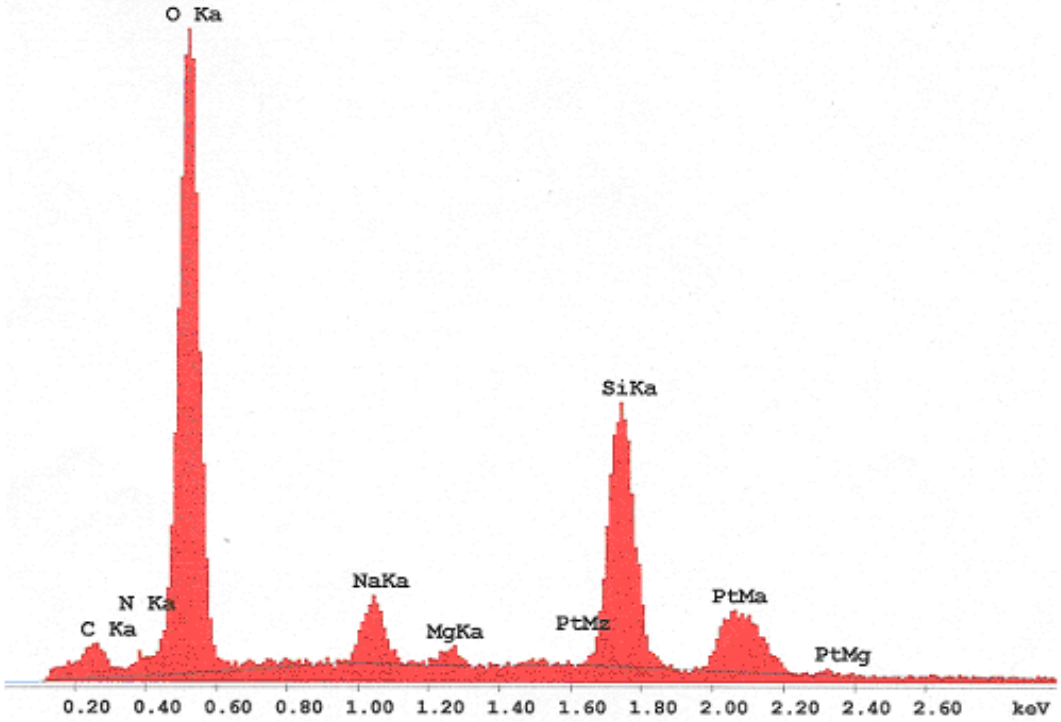
Properties of Dansylamide-embedded Nanoparticles





Dansylamide nanoparticles (particle size: 150 ~200nm)

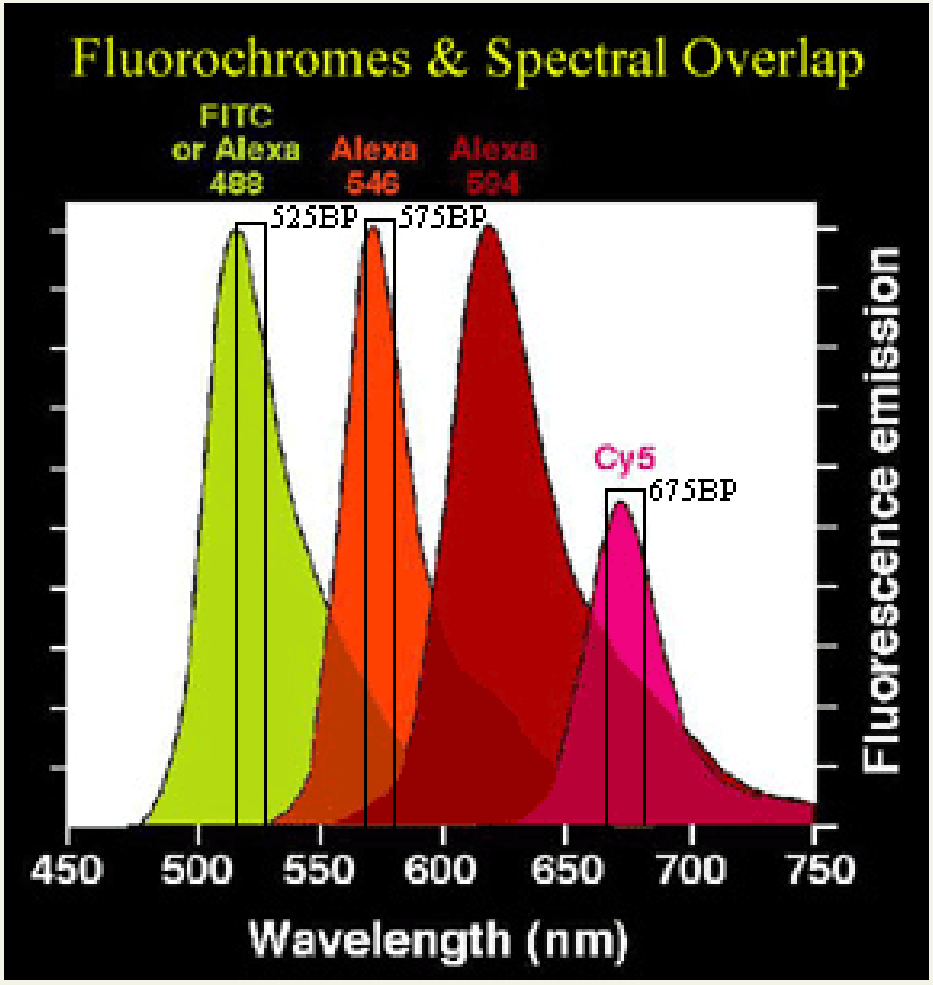
These NPs are pure



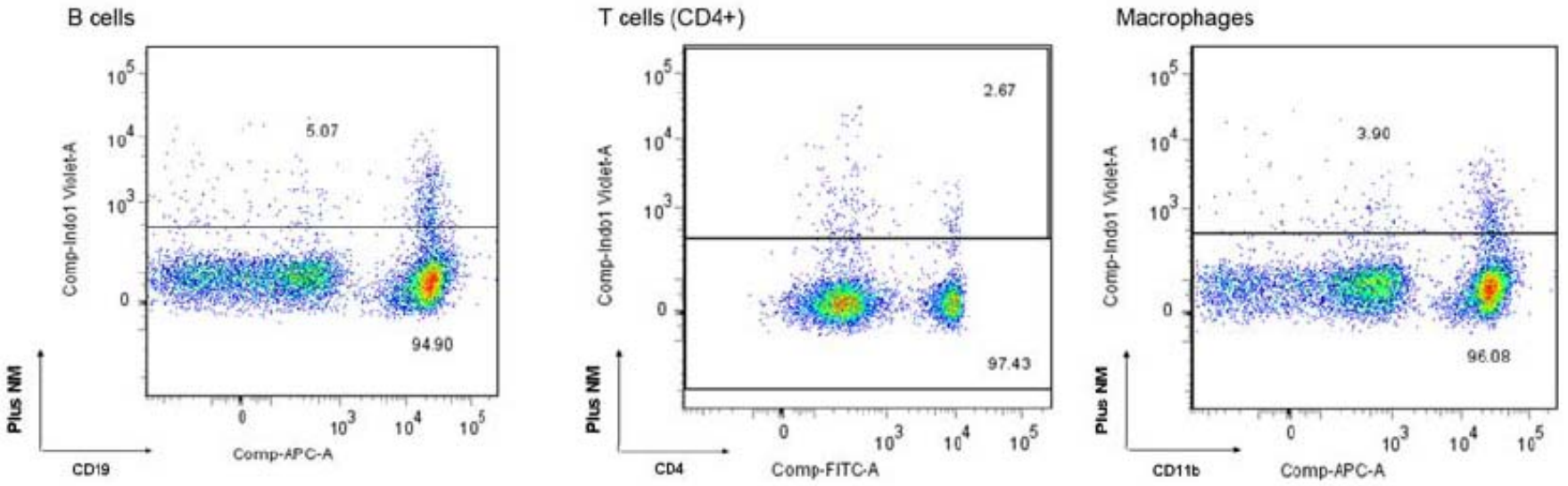
EDAX ZAF Quantification (Standardless)
Element Normalized
SEC Table : Default

Element	Wt %	At %	K-Ratio	Z	A	F
C K	5.67	9.36	0.0272	1.0711	0.4475	1.0002
N K	2.67	3.78	0.0181	1.0540	0.6415	1.0007
O K	39.56	49.00	0.3276	1.0407	0.7955	1.0002
NaK	5.89	5.07	0.0511	0.9535	0.9093	1.0022
MgK	1.69	1.38	0.0155	0.9711	0.9382	1.0047
SiK	44.51	31.41	0.4179	0.9574	0.9806	1.0000
Total	100.00	100.00				

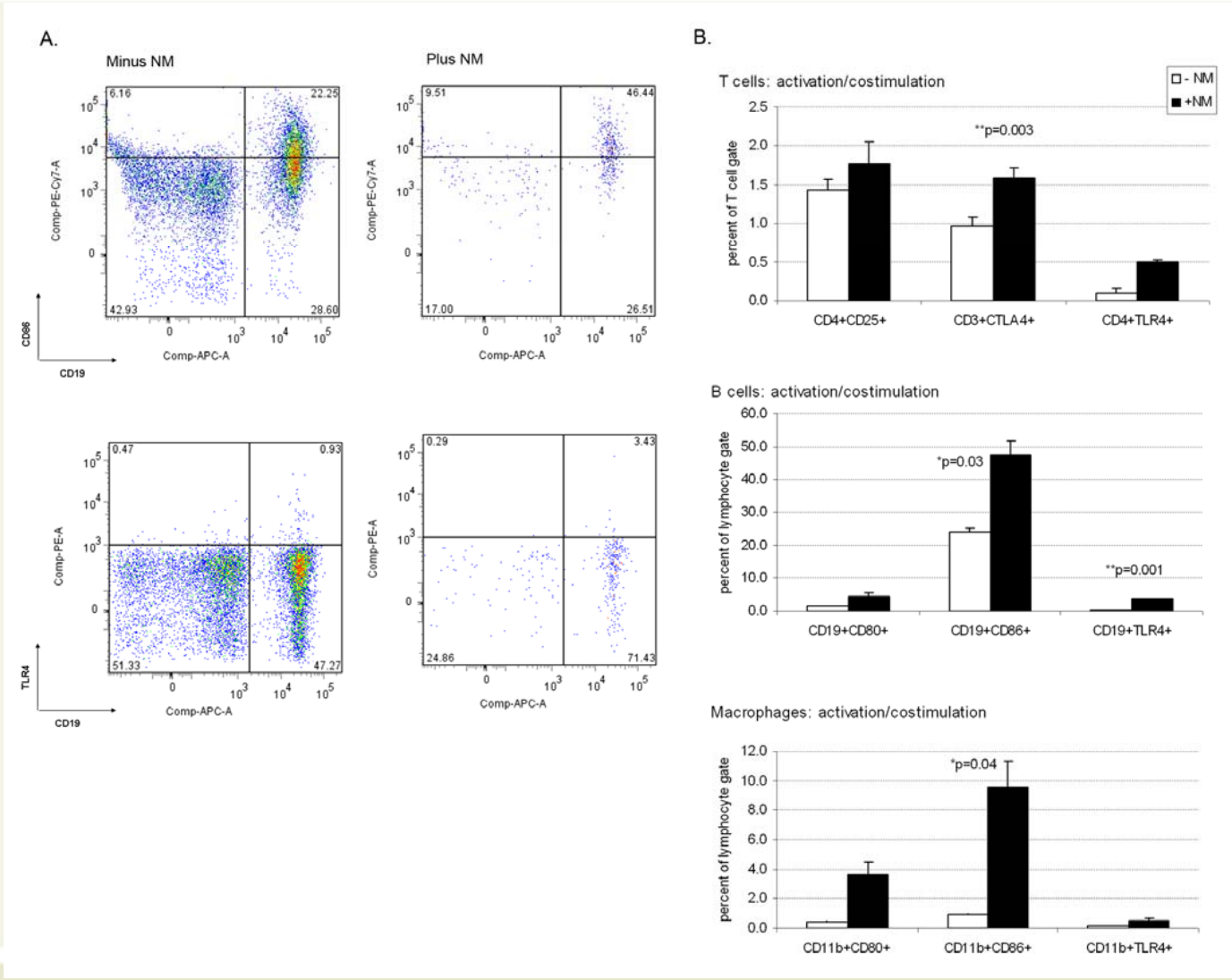
Spectral overlap of common FACS dyes



NPs enter B cells, T cells, and macrophages



Activation of cell subsets by nanoparticles: quantifiable results from real time FACS analyses



Biological effects of NPs in vitro

- NPs can readily enter cells
- No increase in apoptosis or cell loss
- NPs affect immune function at much lower levels than immunoactive chemicals in solution (LPS; lead; mercury)
- NPs increase expression of CTLA-4, marker of T regulatory cell activation
- NPs increased expression of CD86, marker of B cell activation
- NPs increase CD86 and TLR-4, markers of macrophage activation

Current studies

- Time course
- Effects of size and functionalization
- Other markers and signals of cell response
  - Sort cells to examine specific population response
  - Cytokine/chemokine production
  - Gene expression

Some reflections on experience in nanotoxicology

- Of course these are new materials – that's why they are being made!
- "Old toxicology" will not meet the challenge and is probably often irrelevant to the mechanisms of toxicity
- Close collaborations between nanotechnologists and nanotoxicologists are essential
- Think about the intended activity of a nanomaterial and then consider how this might be a hazard

Looking as we leap…or before we leap?