In vitro assessment methods for nanomaterials

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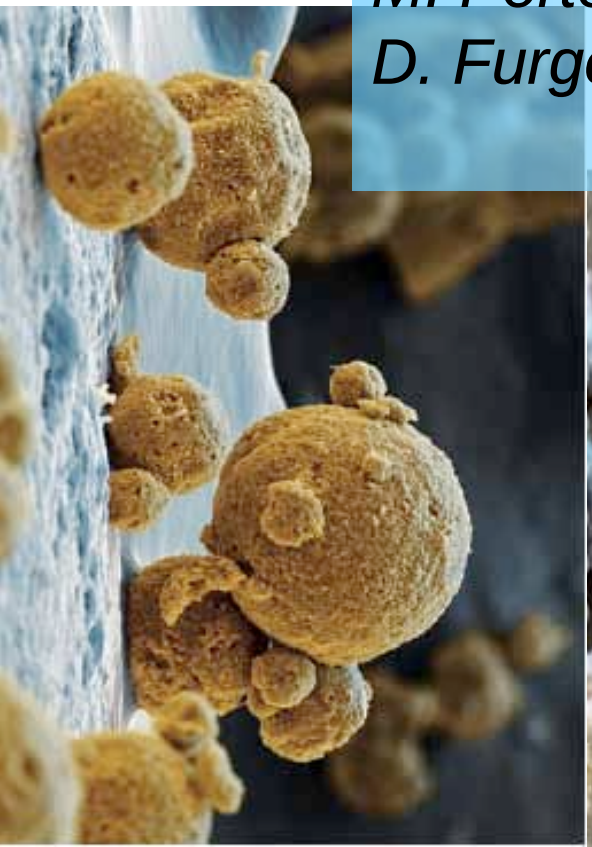
Images courtesy of NanoCare



Nanotechnology's "dirty little secret"? Paranoia or just small talk?

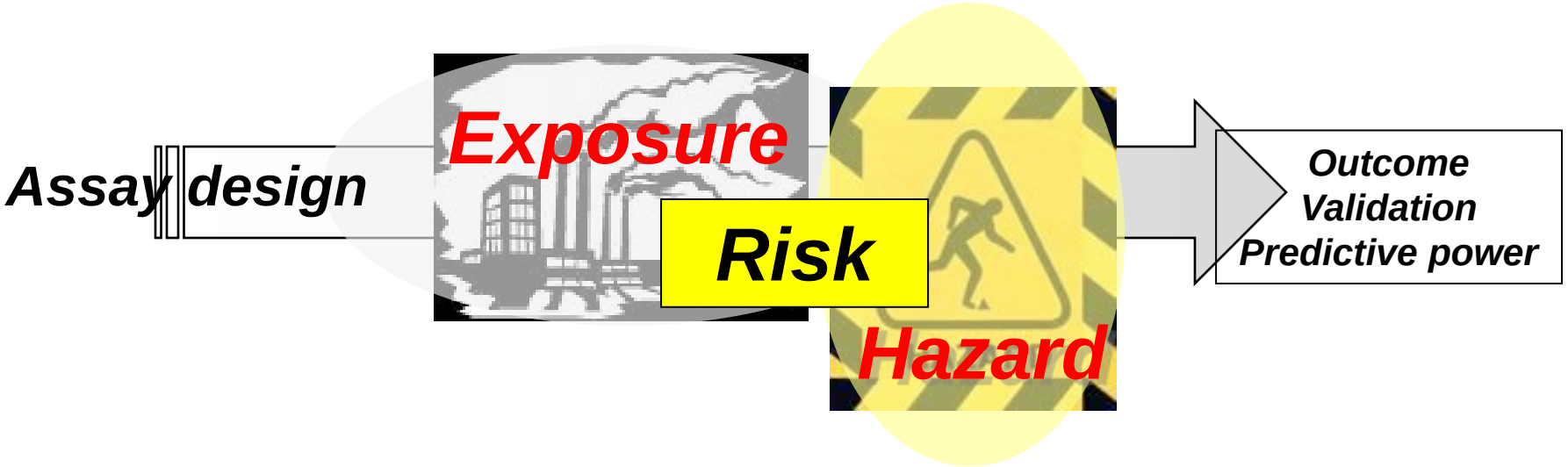
Control…… of toxicity: both in deliberate and non-deliberate exposures

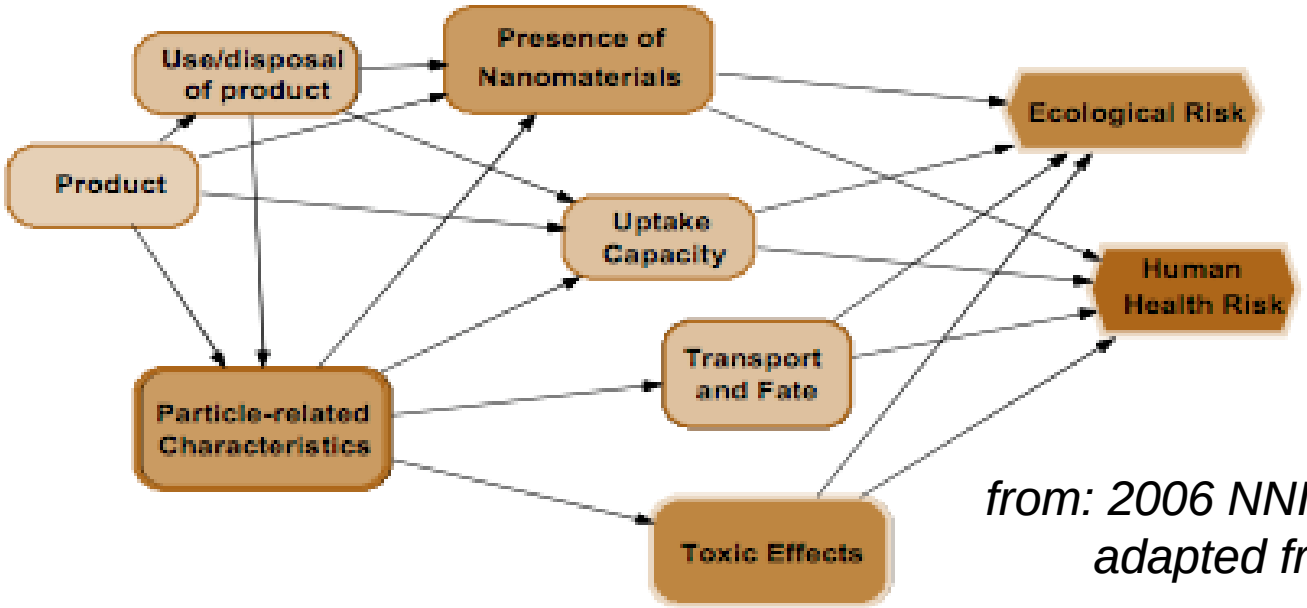
"The dose makes the poison….."

"Agent concentration at the site of action…."

<u>Paracelsus</u>: "all things are poison; there is nothing without poison; only the dose makes a thing non-poisonous…"

Risk ~ function(exposure, hazard)

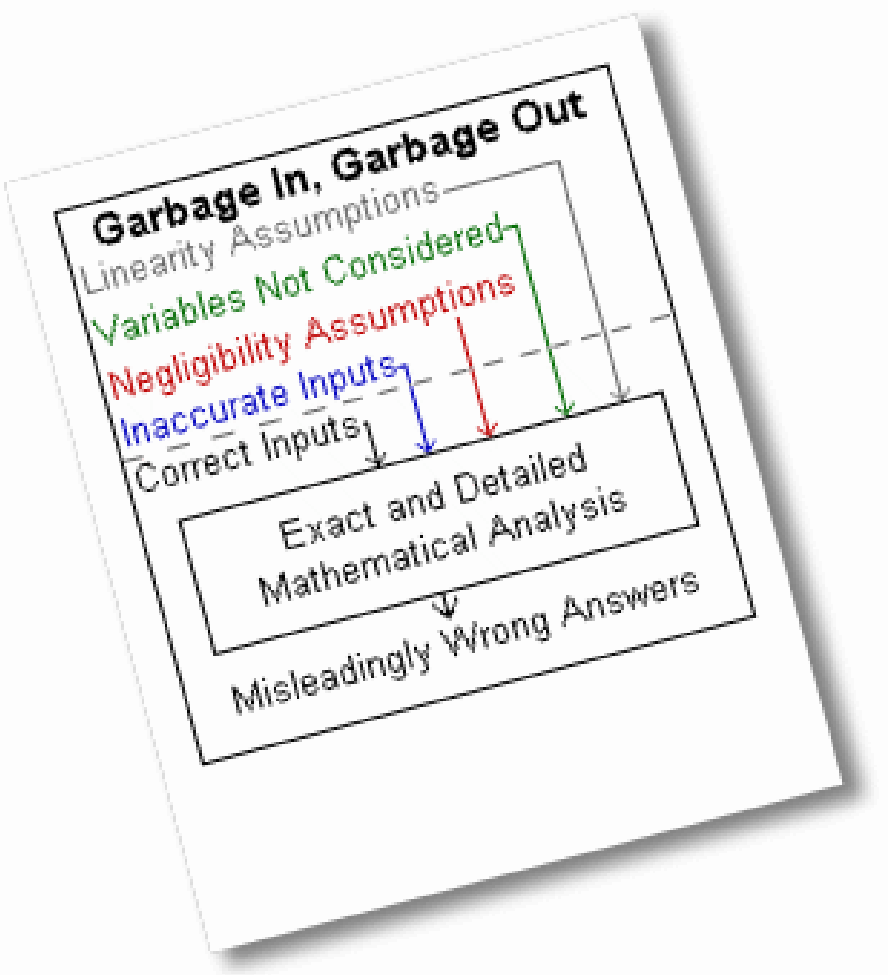




from: 2006 NNI EHS research needs, adapted from Morgan, 2005

Mueller, Nowack, Environ. Sci. Technol. 42 (2008) 4447

Caution about how we choose our course:



Assay continuum: In vitro-in vivo correlations

- *predictive, reliable, mechanistic*

Sayes CM et al., Toxicol Sci 2007, 97:163; Shaw et al., Schreiber et al., PNAS 105 7387 (2008)

Most commonly reported issue in nanotox comparisons:

"…The omission of physicochemical characterization data also complicates efforts to compare toxicity results between research studies. Meta-analyses of carbon nanomaterial toxicity draw upon studies with vastly different solution chemistry, sample purity, synthesis technique, and nano- material manufacturer (4,5,7,8).

The nanomaterials were often poorly characterized prior to experimentation, and the toxicity results provide little direction for green applications or green design of carbon-based nanomaterials…"

Kang et al., Environ. Sci. Technol. 2008, 42, 7528

A token example of materials characterization….for in vitro use

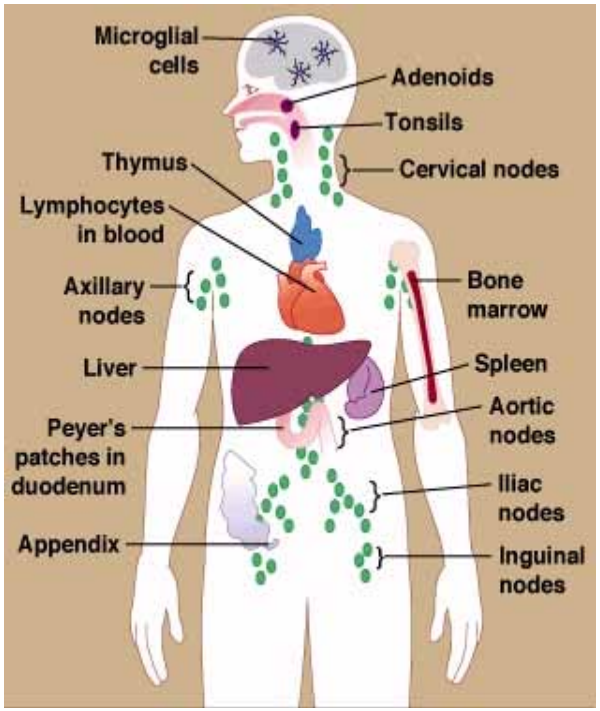
"…. SWCNTs were obtained in highly purified form (>90%) from [COMPANY X]. According to the certificate of analysis reported by the manufacturer, they have an average outside diameter of 1.1 nm, an average length of 50 µm and the following components content in percentage: C 96.30; Al 0.08; Cl 0.41; Co 2.91; S 0.29. They are water insoluble and were suspended at a concentration of 0.5 mg/ml in RPMI medium, sterilized by autoclavation and dispersed by 3 h treatment in an ultrasound bath prior to being administered to the cells."

<u>No information on</u>:

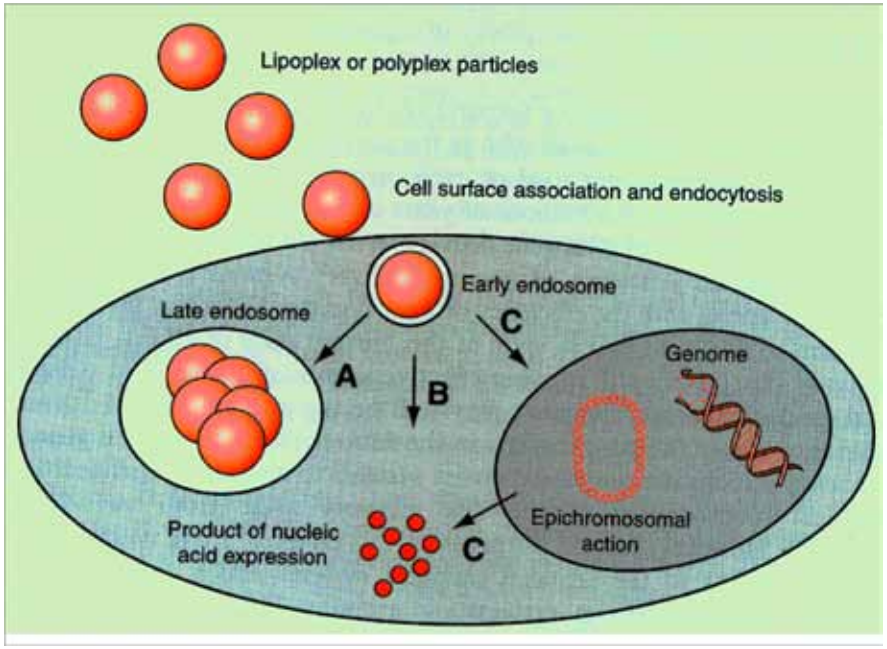
- ***materials aggregation state (water-insoluble)***
- ***confirmation of purity***
- ***SWCNT length or polydispersity***
- ***conditions of cell culture (serum-free?)***

Drug delivery provides experience:

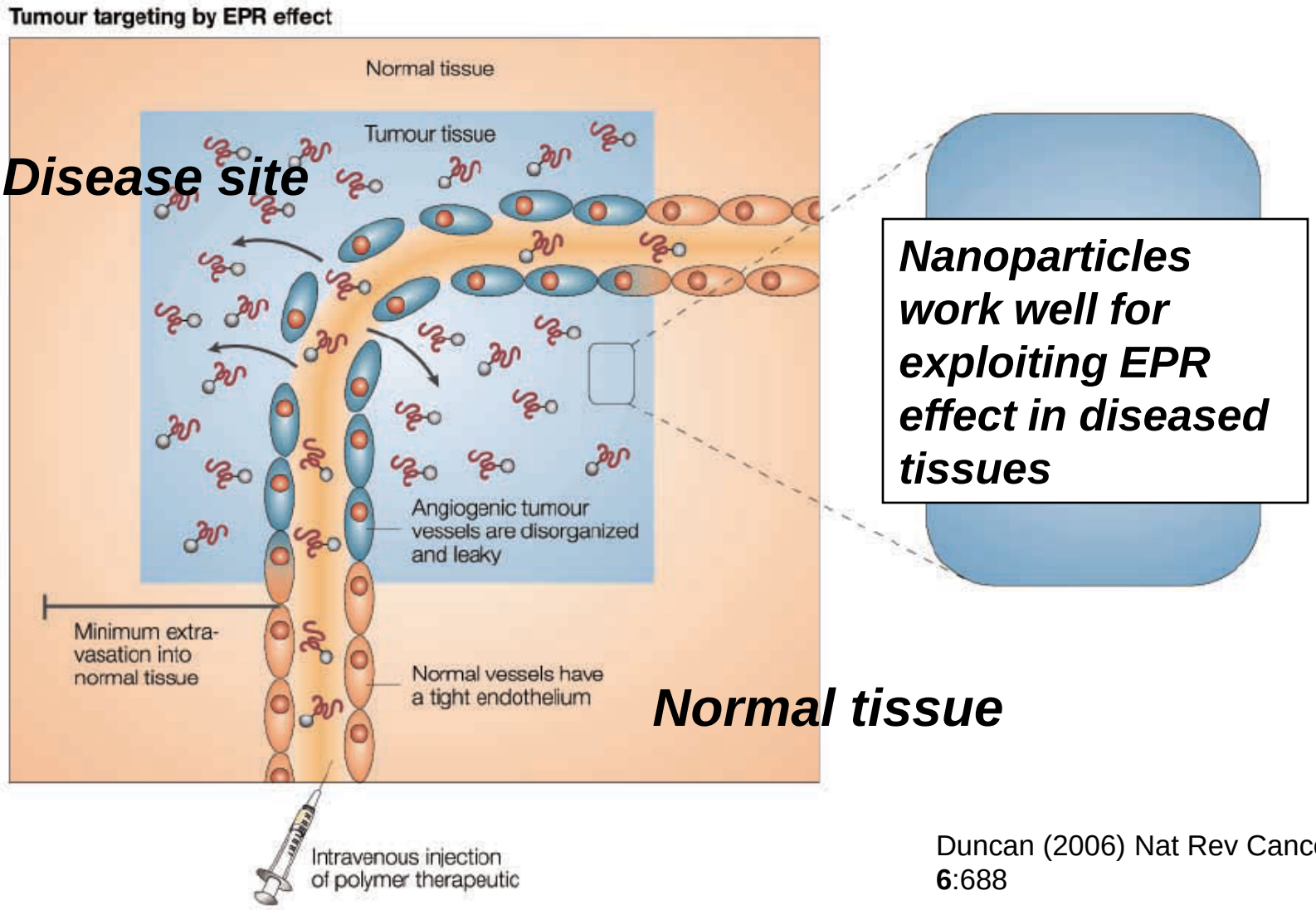
Decades of research with particles interacting with physiological systems at molecular, cellular, tissue and organismal levels



- Extracellular: organ-level
- Epithelial barriers
  - Circulation, Blood Components
  - Reticuloendothelial System (RES)
- Intra-cellular
  - Cell Membrane
  - Endosomal and Nuclear Membrane
  - DNA gene release and integration



Nanomedicine targeting: EPR effect theory



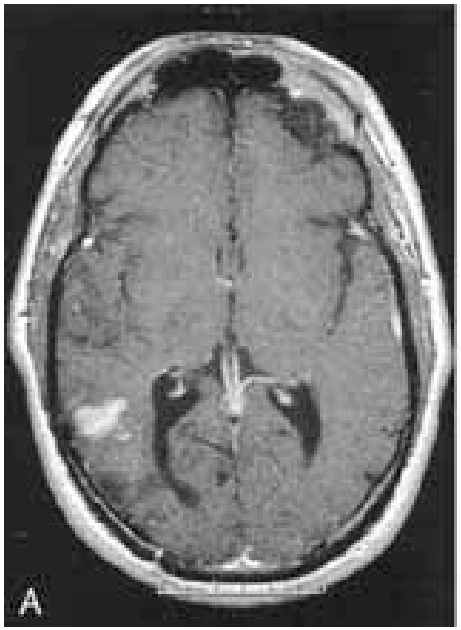
Duncan (2006) Nat Rev Cancer **6**:688

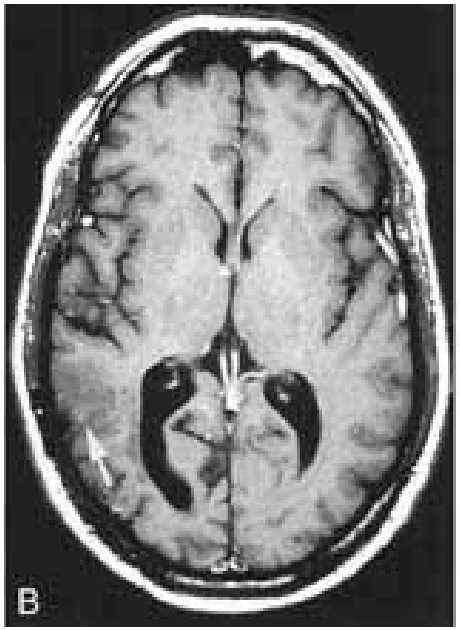
Figure 3 | **Biological rationale for the design of polymeric anticancer therapeutics (part 1).** Tumour targeting of long-circulating polymer therapeutics occurs passively by the 'enhanced permeability and retention' (EPR) effect. Hyperpermeable angiogenic tumour vasculature allows preferential extravasation of circulating macromolecules and polymeric micelles. Once present in the tumour interstitium, polymer therapeutics act either after endocytic internalization or extracellularly (FIG. 4).

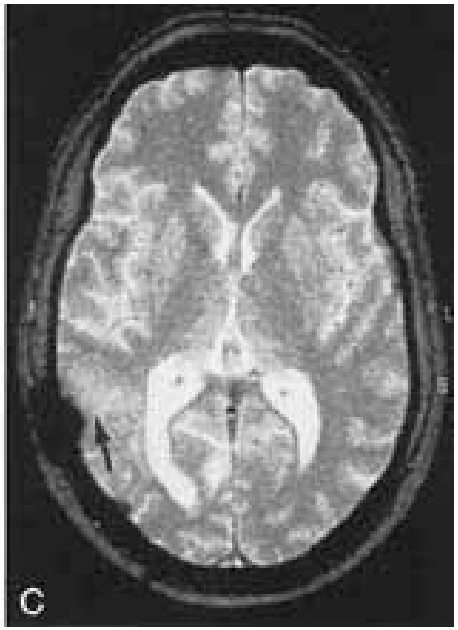
EPR of Nanoparticles in Humans: BBB

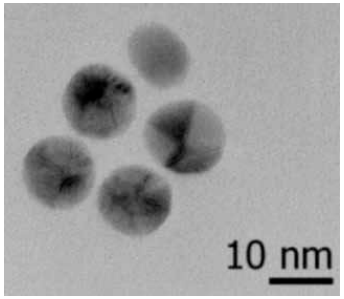
Human Brain MRI:

Magnetic nanoparticle-based imaging through blood-brain barrier









Iron oxide particles

Dextran-coated magnetic iron oxide nanoparticles administered i.v. : passively penetrate the inflamed blood-brain barrier to image lesions (Oregon Health Sciences University, 2004; also R. Weissleder et al.)

Nanomaterials in Biological Systems:

Synthesis: chemistry and clean-up
(stabilization, additives, contaminants)

Surface chemistry, shape, functionalization
(analysis, incomplete and competing reactions)

Biological/Environmental Context and Function
(tracking in complex environments)

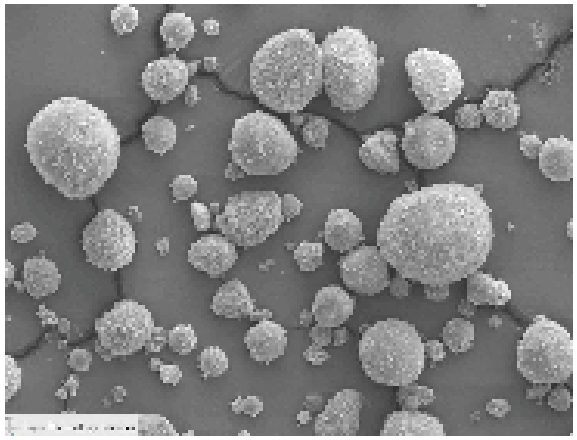
In vitro	*Ex vivo*	*In vivo*

Powers et al., *Nanotoxicol.*, **1** (2007) 42.
Warheit, *Toxicol. Sci.* **101** (2008) 183.
Ott & Finke, *Coord. Chem. Rev.* **251** (2007) 1075.
Grainger & Castner, *Adv. Mater.* **20** (2008) 867.

In vitro methods for assessing exposure, risk, toxicity

1. ***Cell-based assays with nanomaterials***
    - ***Cell adhesion, cell entry***
    - ***Cell processing/trafficking***
    - ***Cell signal production: cytokines, ROS***
    - ***Cell toxicity/viability***
    - ***Cell phenotype influence/stress***

2. ***Protein interactions with nanomaterials***
    - ***Adsorption***
    - ***Aggregation***
    - ***Opsonization***
    - ***Kinetic and chemistry effects***



C. Jones, D.W. Grainger, Adv Drug Del Rev. 61 (2009) 438–456

Observation #1: Nanomaterials interfere with assays

Table 1
Nanoparticle interference with cytotoxicity assays

A. Kroll et al. / Eur. J. Pharmaceut Biopharm. 72 (2009) 370

Cytotoxicity assay	Detection principle	NP interference	Altered readout	Particle/Reference
Cell viability				
MTT	Colorimetric detection of mitochondrial activity	Adsorption of substrate	Reduced indication of cell viability	Carbon nanoparticles [42,44,79]
LDH	Colorimetric detection of LDH release	Inhibition of LDH	Reduced indication of necrosis	Trace metal-containing nanoparticles [59,98]
Annexin V/ propidium iodide	Fluorimetric detection of Phosphatidylserine exposure (apoptosis marker)	Ca^{2+}-depletion	Reduced indication of apoptosis	Chitosan nanoparticles [61,114]
	Propidium iodide-staining of DNA (necrosis marker)	Dye adsorption	Reduced indication of necrosis	Carbon nanoparticles [71]
Neutral red	Colorimetric detection of intact lysosomes	Dye adsorption	Reduced indication of cell viability	Carbon nanoparticles [45]
Caspase	Fluorimetric detection of Caspase-3 activity (apoptosis marker)	Inhibition of Caspase-3	Reduced indication of apoptosis	Trace metal-containing nanoparticles, especially Zn^{2+} [92,93,98,115]
Stress response				
DCF	Fluorimetric detection of ROS production	Fluorescence quenching	Reduced indication of oxidative stress	Carbon nanoparticles [104]
Inflammatory response				
ELISA	Colorimetric detection of cytokine secretion	Cytokine adsorption	Reduced indication of cytokine concentration	Carbon nanoparticles [79] Metal oxide nanoparticles [112]

Au NP false positive

- ***SWNTs interfere with MTT in cell-free systems:*** *Belyanskaya et al., Carbon 45 (2007) 2643; Worle-Knirsch et al, Nano Lett., 6 (2006) 1261.*
- ***Reporting dyes are pH-dependent and reside in multiple different pH compartments***
  - *pH tumor tissue ~6.5, pH late endosome 5.5, etc.*
- ***MTT assay depends upon phagocyte activation state*** *(THP-1: Leuk. Res. 29 (2005) 863; RAW: Anal. Biochem. 313 (2003) 338)*
- ***Some metal NPs (ceria) are redox-active and interfere with redox dyes***
- ***LPS LAL endotoxin*** *(Dobrovolskaia et al., Nat. Nanotechnol 4 411 (2009)*

Observation #2: Nanomaterials change physical state in biological milieu *(Schulze et al., Nanotoxicol. 2008 2 51)*

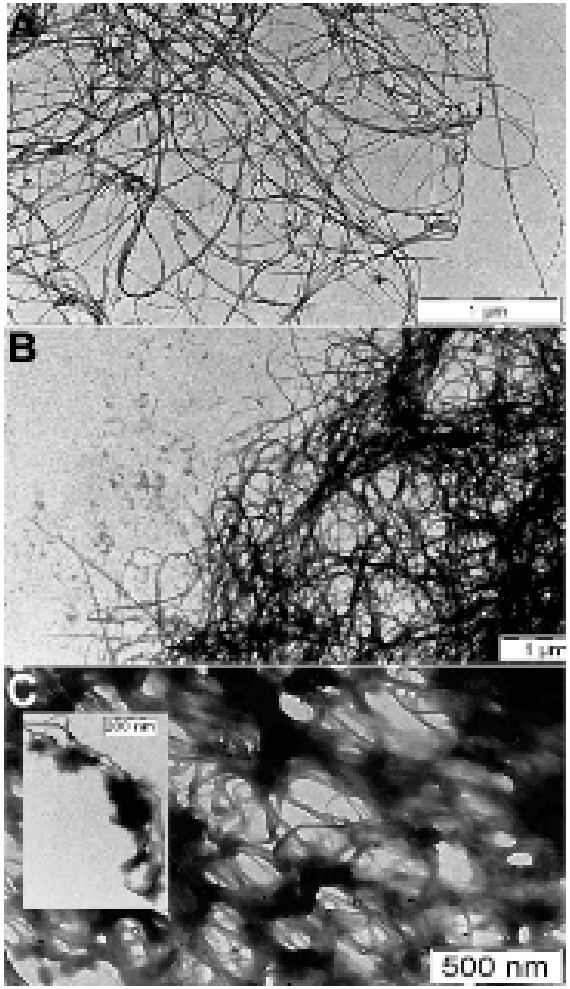


Figure 7. TEM preparations of SWCNTs after incubation with human A549 cells for 24 h in complete media and extraction with 2-propanol/HCl. Crude preparations of SWCNTs after incubation in media (without MTT) and cells for 24 h and after 2-propanol/HCl extraction can be seen in A. This picture shows the regular and well-known appearance of SWCNTs without any structural particularities. In B WST-1 was added 2 h prior to extraction; full recovery of WST-1 formazan leaves no crystals behind. However, in C a structural change in the SWCNTs can be seen with strong distinctions all over the SWCNT meshwork, which might be formazan crystals that cannot be resolved.

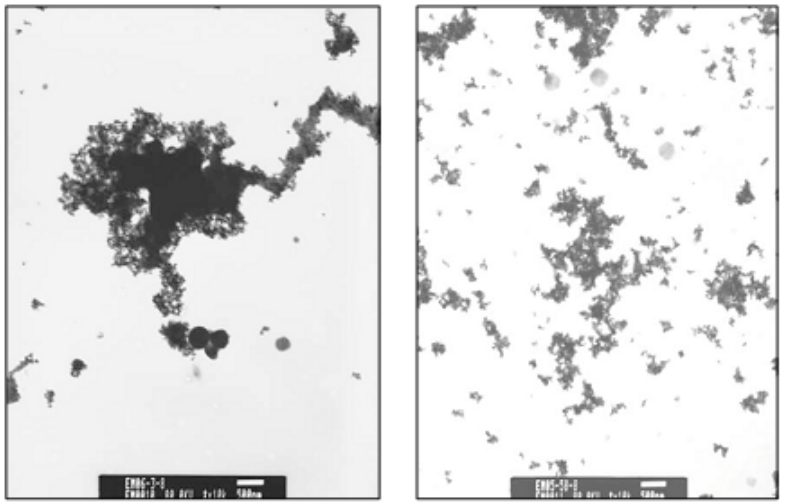
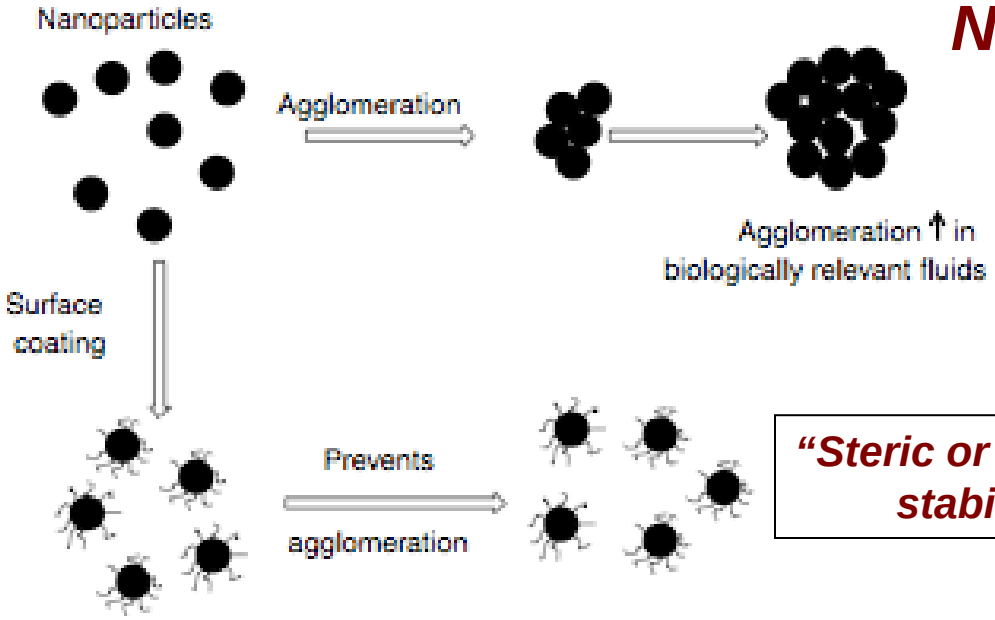


Figure 4. Transmission electron microscopy evidence for improved dispersion of ultrafine titanium dioxide ($UFTiO_2$) by bronchoalveolar lavage fluid (BALF). *Panel A*: 3.47 mg/ml of $UFTiO_2$ suspended in PBS. *Panel B*: 3.47 mg/ml of $UFTiO_2$ suspended in BALF. Samples were aerosolized as described in the methods and viewed with a transmission electron microscope. The $UFTiO_2$ suspended in the BALF (*panel B*) had noticeably smaller agglomerates and a more even and uniform dispersion pattern than the $UFTiO_2$ suspended in the PBS (*panel A*). TEM micrographs of $UFTiO_2$ suspensions were taken at a magnification of 30,000 ×.

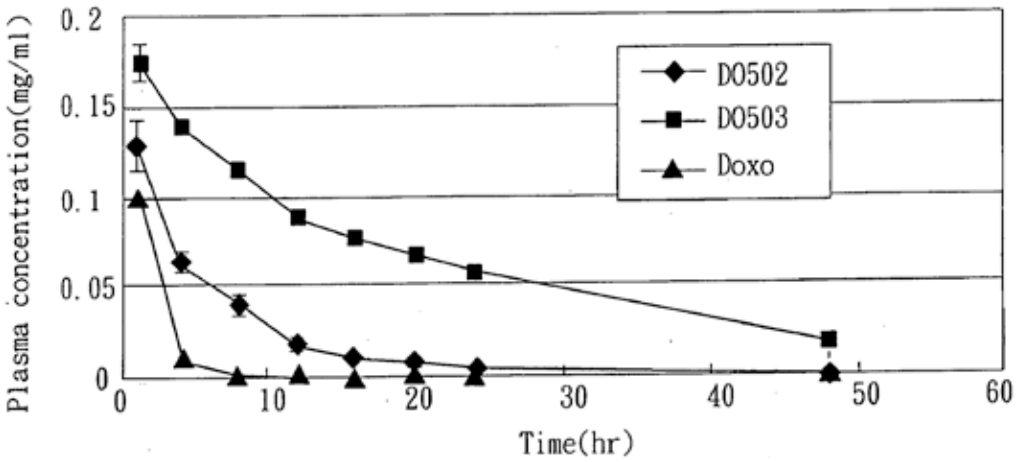
- Skebo et al., Assessment of metal nanoparticle agglomeration, uptake, and interaction using high-illuminating system. Int J Toxicol *2007 26:135.*
- Soto et al., Cytotoxic effects of aggregated nanomaterials. Acta Biomater 2007, 3:351.
- Sager et al: Improved method to disperse nanoparticles for in vitro and in vivo investigation of toxicity. Nanotoxicol 2007, 1:118.
- Mackay et al., General strategies for nanoparticle dispersion, Science 2006, 311:1740.
- Monteiro-Riviere et al., Surfactant effects on carbon nanotube interactions with human keratinocytes. Nanomed 2005, 1:293-9.

Nanomaterial physical state

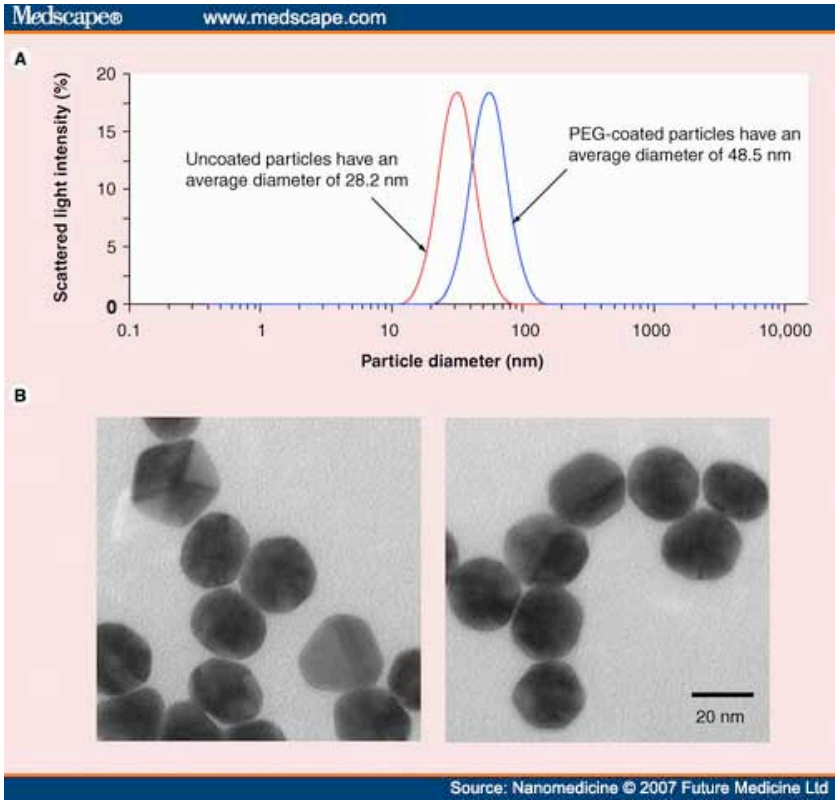


"Steric or electrostatic stabilization" → ***PEGylation polyions***

PEGylated liposomes: plasma clearance



Niidome et al. 2006



and this aggregation affects their toxicity….

Wick et al., The degree and kind of agglomeration affect carbon nanotube cytotoxicity. Toxicol Lett . (2007) 168:121

- ***cytotoxic effects of well-dispersed CNTs compared with conventionally purified rope-like agglomerated CNTs and asbestos***

- ***concluded that CNT agglomerates evoke similar effects on cell morphology and cell performance as the asbestos reference.***

- ***CNT interaction with the mesothelioma cell line (MSTO- 211H) caused morphological changes in the cell lines.***

- ***CNT agglomerates showed negative effects on cell proliferation and cell viability.***

- ***acidic and oxidative treatments of CNTs modify the surface of CNTs leading to CNT agglomeration (Johnston et al. Nat. Mater. 4:589 2005).***

- ***degree of dispersion and agglomeration modifies CNT toxicity***

Observation #3: Different labs get different answers

Papageorgiou et al., Biomaterials 2007, 28:2946; Weyermann et al., Int J Pharm 2005, 288:369; Worle-Knirsch et al., Nano Lett 2006, 6:1261; Davoren et al., Toxicol In Vitro 2007, 21:438.

Table 2: Cell viability after 24 hours incubation on THP-1 cells, measured with MTT assay

Particle Name		IC50 (μg/ml)	IC75 (μg/ml)	IC25 (μg/ml)
Nickel oxide	Lab. B	23.31 (17.14–31.69)	6.44	84.42
	Lab. C	1613 (694.6–3745)	628.18	>3300
Zirconia	Lab. A	570.6 (170.2–1913)	22.84	>3300
	Lab. C	171.9 (87.95–336.1)	60.07	491.87
Yttria doped Zirconia	Lab. B	107.9 (30.5–381.9)	49.45	235.44
	Lab. C	NA		
Stainless steel	Lab. B	62.42 (33.99–114.6)	23.32	167.05
	Lab. C	NT		
Alumina	Lab. A	866 (291.9–2569)	48.08	>3300
	Lab. B	82.19 (10.28–67.4)	13.54	498.9
Tin oxide	Lab. A	174.1 (40.48–748.3)	4	>3300
	Lab. B	3.39 (1.79–6.44)	1.08	10.66

From Lanone et al., Particle Fibre Toxicol 6 (2009) 14

Observation #4: Increasing evidence that classic cytotoxicity assays may not be appropriate for nanotoxicity testing

*Oberdörster et al., Environ. Health Perspect., **113** (2005) 832*
*Oberdörster et al., Part. Fibre Toxicol. **5** (2005) 2*
Lewinski et al., Small 4 (2008) 26
*Kroll et al., Eur. J. Pharm Biopharm **72** (2009) 370*

1. ***Cultured cells do not represent in vivo phenotype***
2. ***Monocultures cannot replicate in vivo response***
3. ***Short-term cultures cannot reliably duplicate aspects of either acute or chronic exposure***
4. ***Cell culture conditions (e.g., media) confound the state of material-cell interactions***
5. ***Super-dosing of nanomaterials used to obtain effects***
6. ***Induction of pro-inflammatory markers very dependent on culture conditions; greatest in LPS-stimulated co-cultures.***

- ***Cell culture staging affects proliferation, endo/phagocytosis rates***

- ***Some cell lines are contact inhibited, others not (affects phenotype and mass transport, cell trafficking)***

- ***Some epithelial cell lines are mucinylated, others not***

- ***Transformed secondary cells have a higher intrinsic uptake and proliferation rate than primary cells***

- ***Different cell lines have intrinsically different reporting sensitivities to the same assays and exposure: from differential penetration, generation of oxidative stress, inflammation responses: rat alveolar macrophages (NR8383 cell line) were most sensitive to metals by nearly one order of magnitude in metal concentration, followed by the two alveolar epithelial cell lines studies: rat RLE- 6TN and human A549 cells (***Riley et al., Toxicol In Vitro, 2005, 19:411***)***

- ***How to model acute versus chronic exposure?***

1. ***Passage number effects uncontrolled: Caco-2 example below; RAWs***
2. ***Over half of cell lines are obtained from colleagues and never verified for phenotype or purity; over 20% of cell lines tested are contaminated (e.g., by HeLa cells or mycoplasma) or mis-identified.***
3. ***Significant genotypic drift with sub-culture passaging (e.g., MCF-7 cells)***

(See: BioTechniques 43 (2007) 575)

Table 1: Summary of Reports Detailing the Effects of Cell Passage Level on Caco-2 Cells

Characteristic	Cell Response After Passaging	Reference
TEER	Increase in TEER from passage 29 to passage 198. Increase in TEER from passage 35–47 to passage 87–112. Decrease in TEER from passage 36 to 86. www.mfbprog.org.uk/themes/themes.asp Decrease in TEER from passage 20 to 72.	6 13 14
Cell density	Increase in cell density from passage 29 to passage 198.	6
Proliferation rates	Growth rates lower in passage 35–47 to passage 87–112. Growth rates lower in passage 36 to passage 86. www.mfbprog.org.uk/themes/themes.asp Growth rates higher in passage 20 compared with passage 72.	13 14
Paracellular permeability	Decrease in paracellular permeability from passage 29 to passage 198. Increase in paracellular permeability from passage 36 to passage 86. www.mfbprog.org.uk/themes/themes.asp No significant difference in permeability from passage 35–47 to passage 87–112.	6 8 13
Transcellular permeability	Increase in transcellular permeability from passage 29 to passage 198. No significant difference in permeability from passage 35–47 to passage 87–112.	6 13
Carrier-mediated transport	Decrease in transcellular permeability from passage 29 to passage 198. No significant difference in permeability from passage 35–47 to passage 87–112.	6 13
Alkaline phosphatase	Higher expression of AP in passage 29 compared with passage 198. Higher expression of AP in passage 20 compared with passage 72.	6 14

TEER, transepithelial electrical resistance.

Cell line contamination profoundly affects:

- ***Cell growth and function***
- ***Transfection***
- ***Morphology and differentiation state***
- ***Gene expression***

High passage cell lines have altered:

- ***Cell growth rates***
- ***Responses to stimuli***
- ***Morphology***
- ***Protein and gene expression***

The data demonstrate differences in proliferation and secretion in low- and high-passage LNCaP cells.

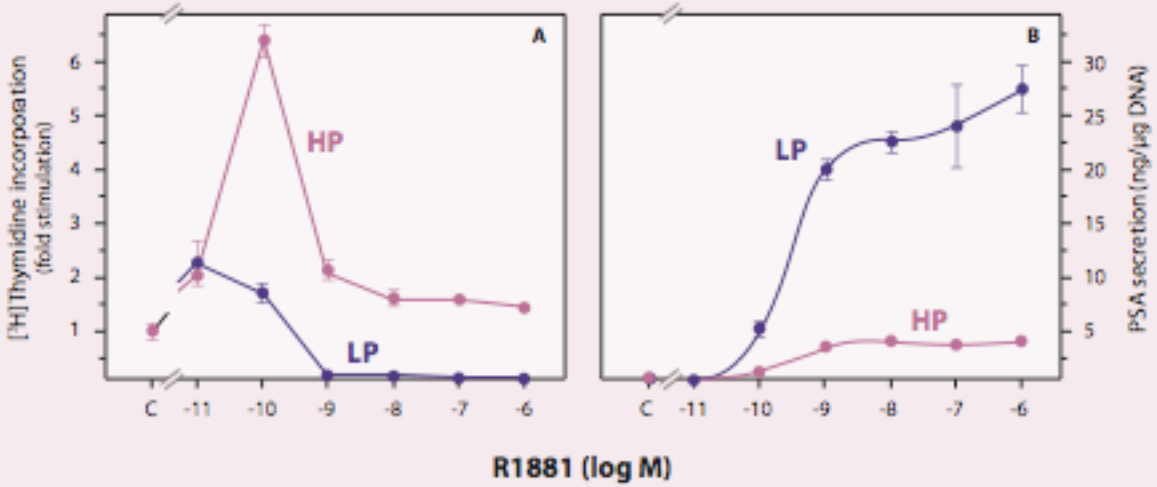


Figure 10 ▪ Two samples of LNCaP prostatic adenocarninoma cells were obtained from ATCC. One sample was passaged 24 times (low passage, LP) and a second sample was passaged approximately 80 times (high passage, HP). [³H]Thymidine incorporation (A) and PSA secretion (B) were measured after three days of incubation with increasing concentrations of the synthetic androgen R1881, as described in Esquenet et al. 1997. With this and other data, the authors concluded: "Low passage and high passage LNCaP cells display markedly divergent responses not only to androgens but also to retinoids."

Esquenet M et al. LNCaP prostatic adenocarcinoma cells derived from high and low passage numbers display divergent responses not only to androgens but also to retinoids. Journal of Steroid Biochemistry and Molecular Biology. 62: 391-399. 1997.

Langeler EG et al. Effect of culture conditions on androgen sensitivity of the human prostate cancer cell line LNCaP. Prostate. 23(3): 213-223. 1993.

Mucus, epithelial cell lines, and uptake

- **Mycoplasma-free** Caco-2 cells at passage numbers 42-50
  - Immortalized line of heterogeneous human epithelial colorectal adnocarcinoma cells.
  - Size-, passage-, concentration-, and temperature–dependent uptake
  - Non-mucinylated

- HT29-MTX clone E12 at passage numbers 35-37
  - Mucus-secreting monolayers. Secreted mucus consisted of glycoprotein mucin that potentially decreases the diffusion of particles
  - Physical barrier for NP transport

The effect of mucus on particle binding

Table I. Physico-Chemical Properties, TEER-Values (Determined During CLSM Studies), and LDH-Release of Polystyrene, Chitosan, and PLA-PEG NP

	Polystyrene	Chitosan	PLA-PEG
Size (nm)	213 ± 8	290 ± 7	196 ± 20
ζ-potenial (mV) in PBS	+1.1 ± 1	+17.5 ± 3.6	−23.9 ± 1.2
Encapsulation efficiency (%)	covalently bound	92.3 ± 0.7	56.0 ± 2.07
TEER (% of initial)	110 ± 0.6	119 ± 4.7	121 ± 1.6
LDH-release after 120 min (%)	2.01 ± 0.5	2.86 ± 0.85	1.97 ± 0.52

Cellular association of NP with Caco-2 and MTX-E12

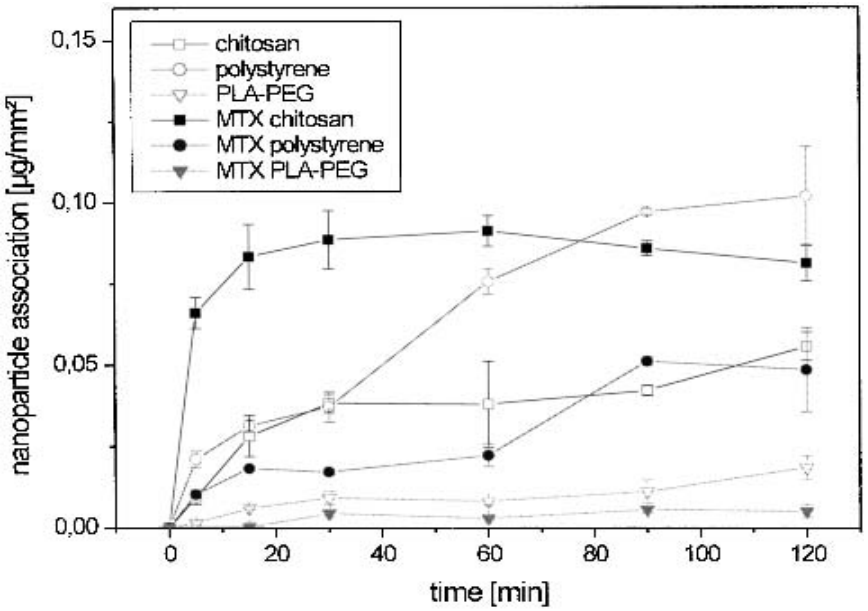


Fig. 1 Time course of chitosan (●), polystyrene (■), and PLA-PEG (▼) NP association in enterocyte-like Caco-2 (open symbols) and mucus-secreting MTX-E12 cells (closed symbols). (n = 3)

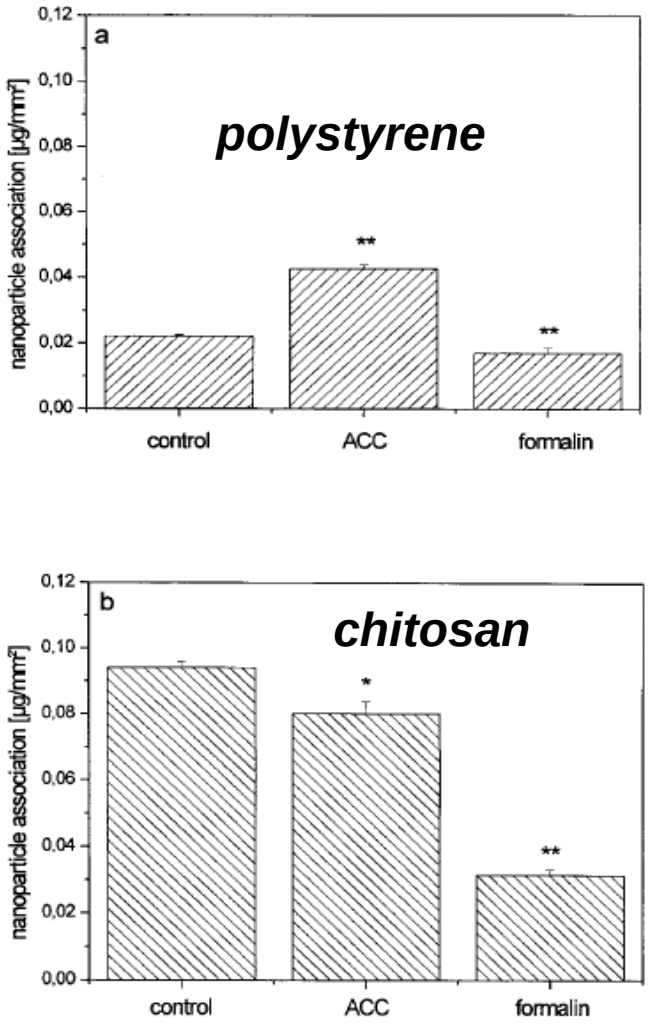


Fig. 2. Cellular association of fluorescence of polystyrene (a) and chitosan (b) NP after 60 min with untreated mucus-secreting MTX-E12 (control) and after the removal of the mucus layer before the experiment by acetylcysteine treatment (ACC) and after the experiment by formalin treatment. (n = 3)

Influence of Cell Culture Media on Cell-Particle Phagocytosis

F99 and SFM Serum-free Media

F99 and SFM with 5% Serum

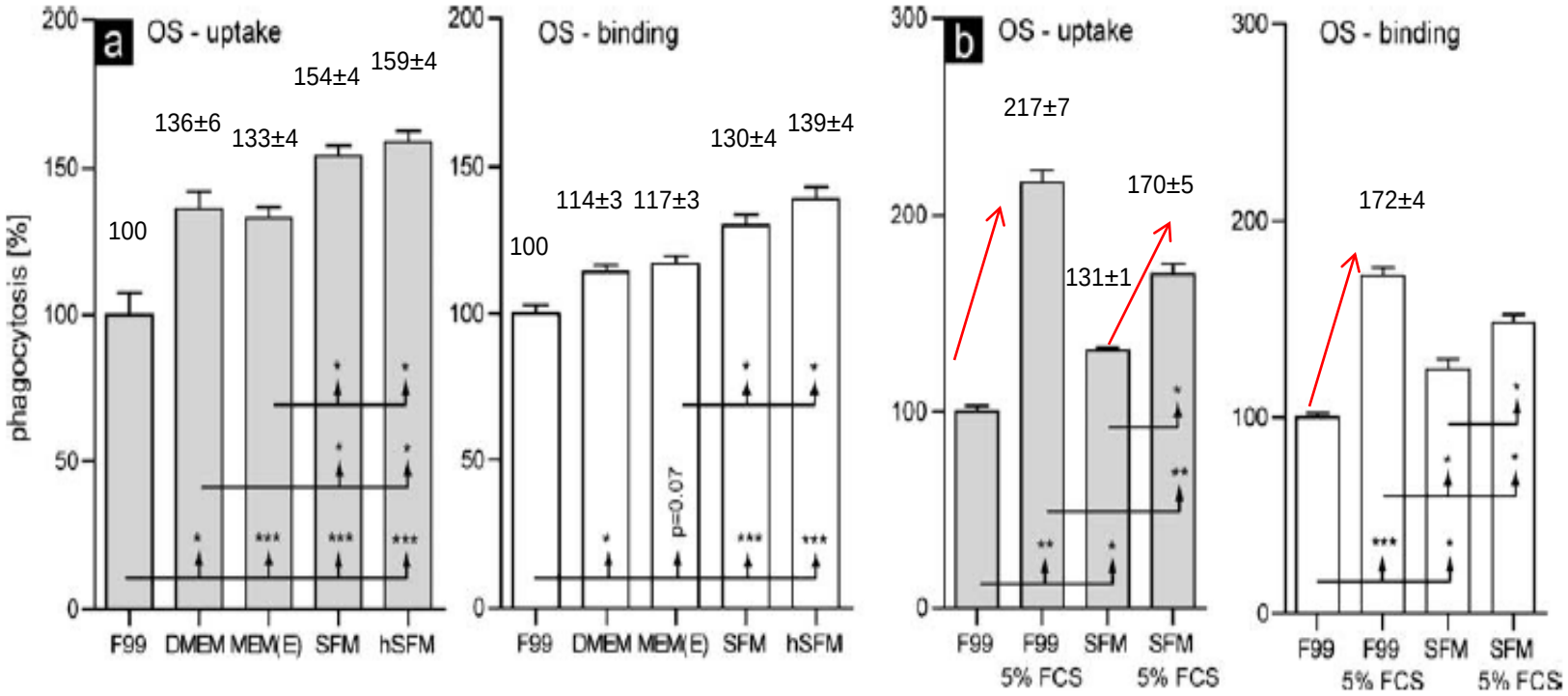
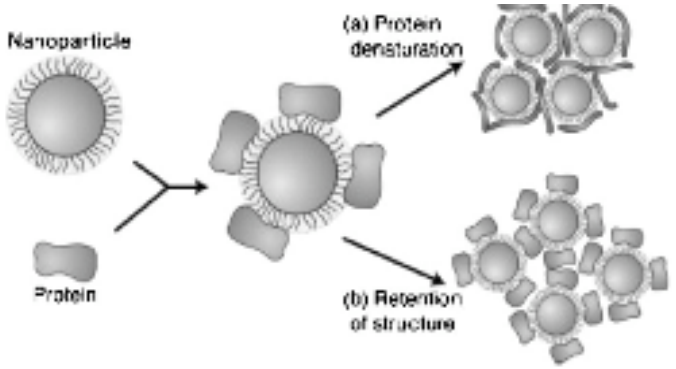


Fig. 3 Phagocytosis & cell culture medium relationship. a Influence of different basic media without serum. SV40-RPE cells exhibit differences in phagocytosis depending on basic cell culture media. A 12-h preincubation allowed cells to adapt to the media. Under these conditions the phagocytosis capacity was lowest in F99, and slightly but significantly increased in MEM(E) and DMEM in uptake and binding respectively. SFM and hSFM exhibited highest uptake and binding (n=4). b Basic media supplemented with serum. Based on results presented in Fig. 3a we asked whether or not these differences would also be apparent if the media were supplemented with serum. As shown in this figure, the stimulation of uptake with 5% serum is not as large in SFM as in F99, and therefore the behaviour of the media is different once more under serum stimulation compared to serum-free conditions (n=3)

Karl et al., Graefes Arch Clin Exp Ophthalmol. 2007 245:981

Observation #5: Protein interactions with colloidal systems determines biological destiny

e.g., *Blunk et al. 1993, Cedervall et al. 2007, Labarre et al. 2005; Dutta et al., Toxicol. Sci., (2007) 10 303*



Time- and condition- dependent changes in nanoparticle behaviors

Naiive nanomaterial

Time-dependent changes in chemistry

Aggregation and inactivation in storage

Alterations in biological media (e.g. aggregation)

Alterations from biological interactions (e.g., protein adsorption)

Modified behavior with biological ligands and targets

***Formidable milieu*: plasma proteome: 70 mg/ml soluble proteins, >20,000 proteins, 10^{11} range in concentrations (most abundant; serum albumin)**

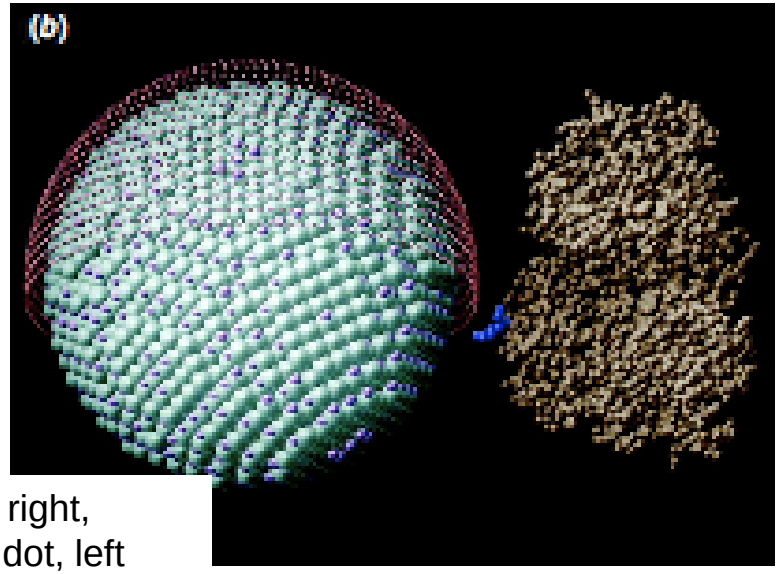
- ***Protein-surface exchange (Vroman effect) is observed: high abundance proteins are off-competed by slower, less abundant but high affinity proteins: forms a stable "protein corona" (Cedervall, et al., 2007)***

- ***NP surface ligand exchange with biologics: glutathione, lipids, proteins***

- ***Nanoparticles: promote protein assembly into amyloid fibrils in vitro by assisting with protein nucleation process (Linse et al. 2007).***

***Methods*:**

- ***Ultracentrifugation***
- ***SPR***
- ***Isothermal calorimetry***
- ***SDS-PAGE***
- ***PCS***
- ***DLS***
- ***Radiometry***



maltose binding protein right, interacts with a quantum dot, left

Effect of particle surface curvature on protein adsorption

M-E Aubin-Tam, K Hamad-Schifferli, Biomed. Mater. 3 (2008) 034001

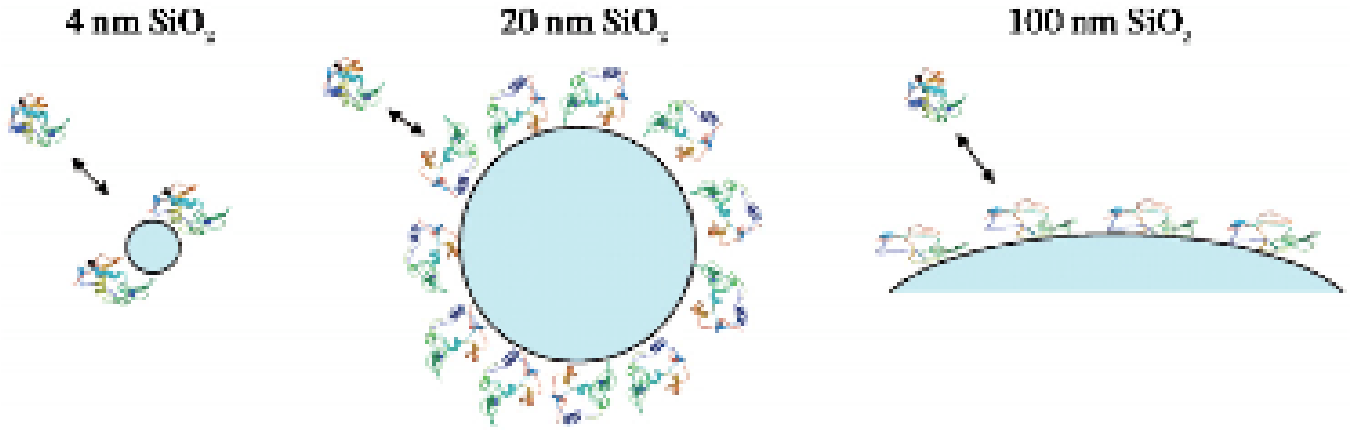


Figure 14. Effect of the NP size on the behavior of adsorbed lysozyme, from [72].

Protein corona determined by materials, proteins, curvature, time

Interparticle protein-protein interactions cause aggregation

Little control over 20,000 different protein-surface interactions

Röcker et al., Nat. Nanotechnol, 4 577 (2009)

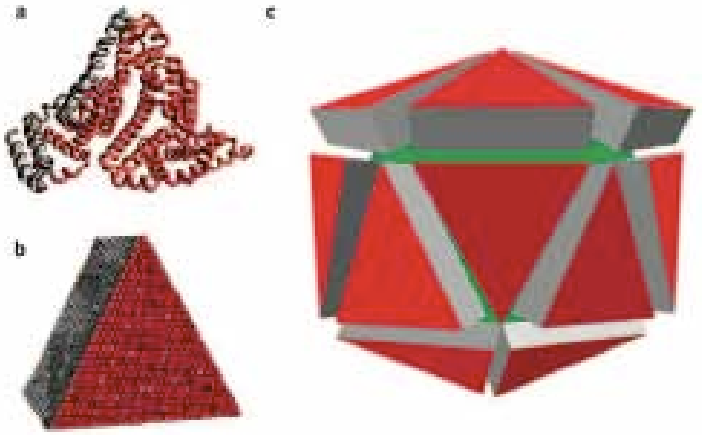


Figure 1 | Structure of HSA and the protein corona. a,b, Representation of the HSA polypeptide chain (**a**), which can be approximated by an equilateral triangular prism (**b**). **c**, At concentrations of HSA typically found in serum, the surface of the polymer-coated FePt nanoparticles (green) is covered by a monolayer of about 20 HSA molecules (red triangular prisms).

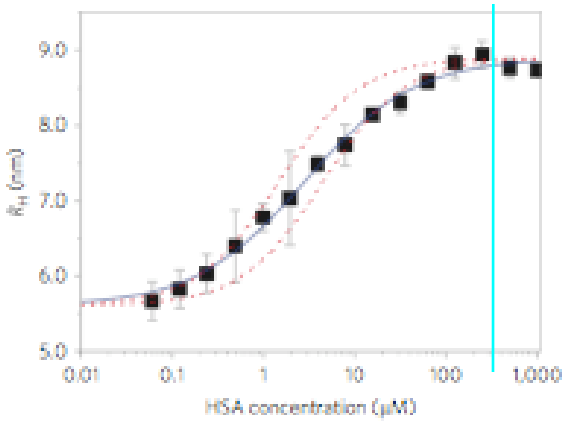


Figure 3 | Association of HSA to polymer-coated FePt nanoparticles measured by FCS. The hydrodynamic radii of the particles are plotted as a function of HSA concentration. The data points are averages from three independent series of measurements. The blue solid line represents a fit of an anti-cooperative binding model (equations (1) and (2)) to the data, and the red dashed lines are Langmuir binding isotherms fitted to the first and last 20% of the transition.

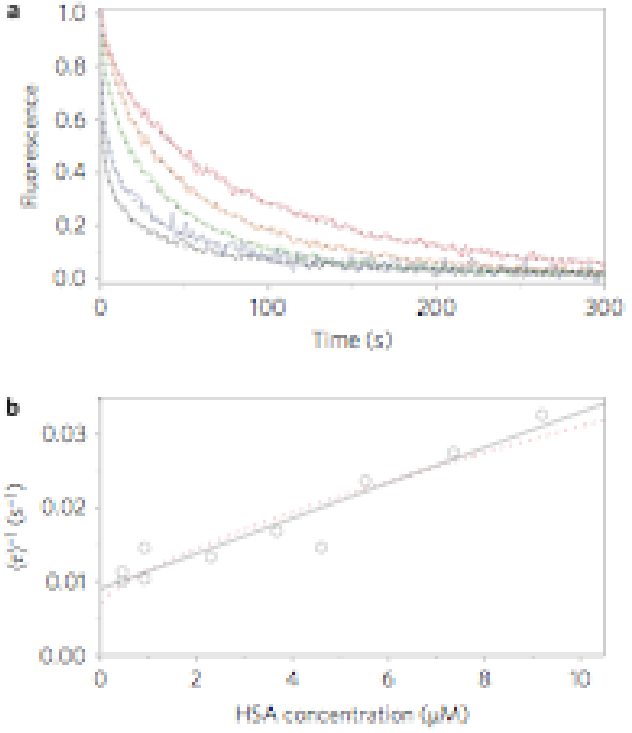
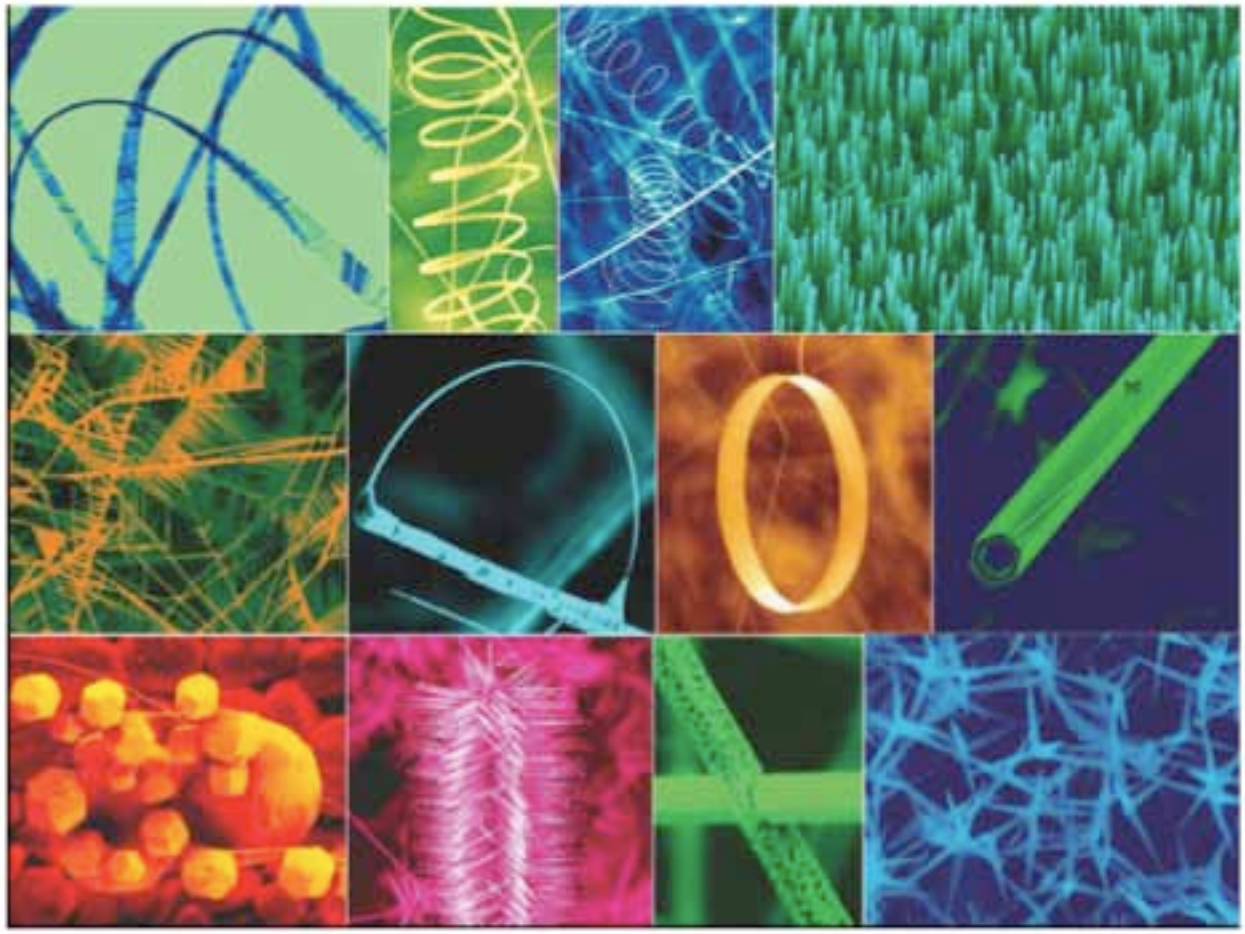


Figure 4 | Kinetic studies of HSA association to polymer-coated FePt nanoparticles. a, Kinetic traces of fluorescence quenching are shown for five different concentrations (red, 0.5 μM; orange, 4 μM; green, 6 μM; blue, 8 μM; black, 10 μM) of QSY 21-labelled HSA (solid lines) together with stretched exponential model functions (dashed lines). **b**, Concentration dependence of the average relaxation rate and the best fit using linear ($\propto$ [HSA], black solid line) and sublinear ($\propto$ $[HSA]^{0.74}$, red dashed line) relations.

Zinc oxide: many morphologies, not so simple

How to assess protein adsorption?



Wang 2004

Powers et al., Characterization of the size, shape, and state of dispersion of nanoparticles for toxicological studies. Nanotoxicology 1 (2007) 42

Deliberate protein interactions: biofunctionalized gold NPs:

Biomed. Mater. 3 (2008) 034001

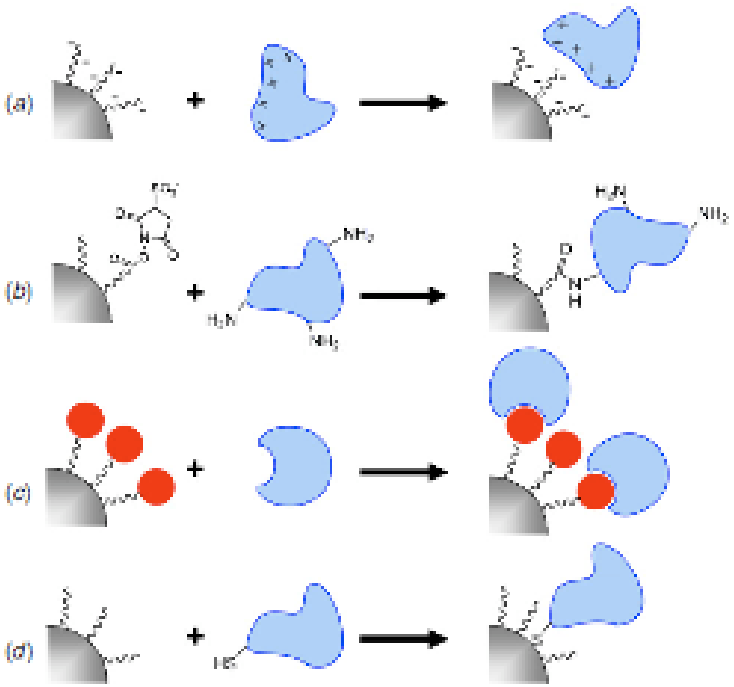
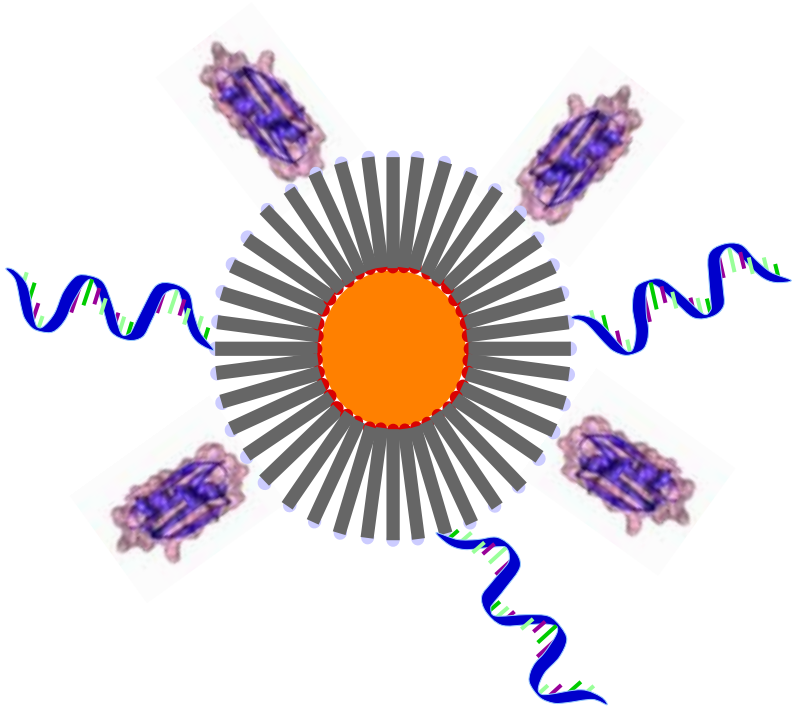


Figure 3. NP–protein labeling strategies: (*a*) electrostatic attachment of protein, (*b*) covalent attachment to the NP ligand, (*c*) attachment of a protein cofactor on NP, and (*d*) direct linkage of amino acid on the NP core.



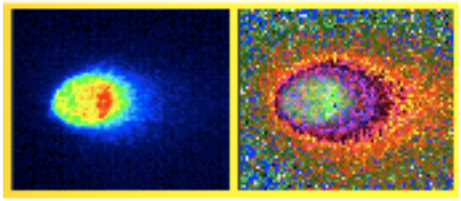
DNA-Gold NPs are FDA-approved for in vitro warfarin testing

Gold NPs for imaging and tumor thermal ablation are in Phase I trials

Genotoxic assay

Some nanoparticles asserted to be genotoxic (cause DNA damage, see Gonzalez et al. 2008, Landsiedel et al. 2008)

1. Comet assay: most popular to demonstrate DNA damage.



Genotoxicity: observed for C60 fullerene, SWCNTs, CoCr alloy, TiO_2, nanosized metal oxide V_2O_3, Carbon Black (CB), and nanosized diesel exhaust particles.

2. Micronucleus assay: presence of micronuclei in dividing cells reflects chromosomal aberrations

Genotoxic positive results for: TiO_2, SiO_2, CoCr, ZnO and multi-walled carbon nanotubes (MWCNT).

3. Gene mutation assays: positive results for nano-FePt, SiO_2, TiO_2, MWCNT, and CB.

In vitro in vivo correlations: what's expected?

A reductionist approach in cell lines has limitations

Observed trends are not "mechanisms"

In vitro-in vivo and species-species translation issues (Cell 134 559 2008)

Short-term cell in vitro studies (24 hours) provides what meaning for in vivo work?

Validation of new models will be critical for in vitro significance

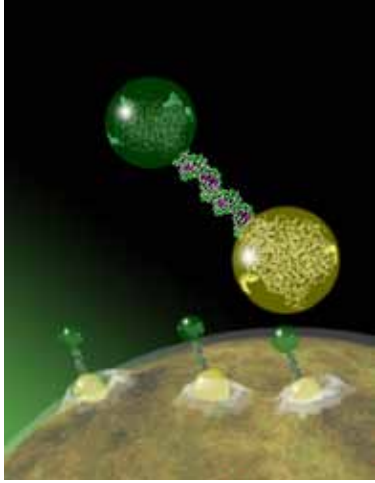
Summary: in vitro methods

1. *Nanotoxicity studies: relevance?*

- *transformed cell lines + low serum ≠ human host*
- *Particles aggregate in biological fluids --> stability unknown in serum*
  - *low-protein or serum-free assays irrelevant*
- *super-physiological acute exposure ≠ actual human exposures:*
  - *Example: Assuming all particles as uniform 50 nm-sized spheres, 0.1 µg TiO_2/cm^2 is equivalent to approximately 80 fg/cell under confluent cell culture conditions. Assuming that the material is 100% bound to or taken up, a cell might contain 300 nanoparticles.*

2. *Highly conflicting cell-based data sets to date*
   - *Particle dosing remains problem: high doses, low local effects*
3. *Protein adsorption is virtually unstudied: tools lacking*
4. *Few actual environmental exposures are highly refined, size-controlled monodisperse materials*
5. *Few studies start with known materials with stated properties*

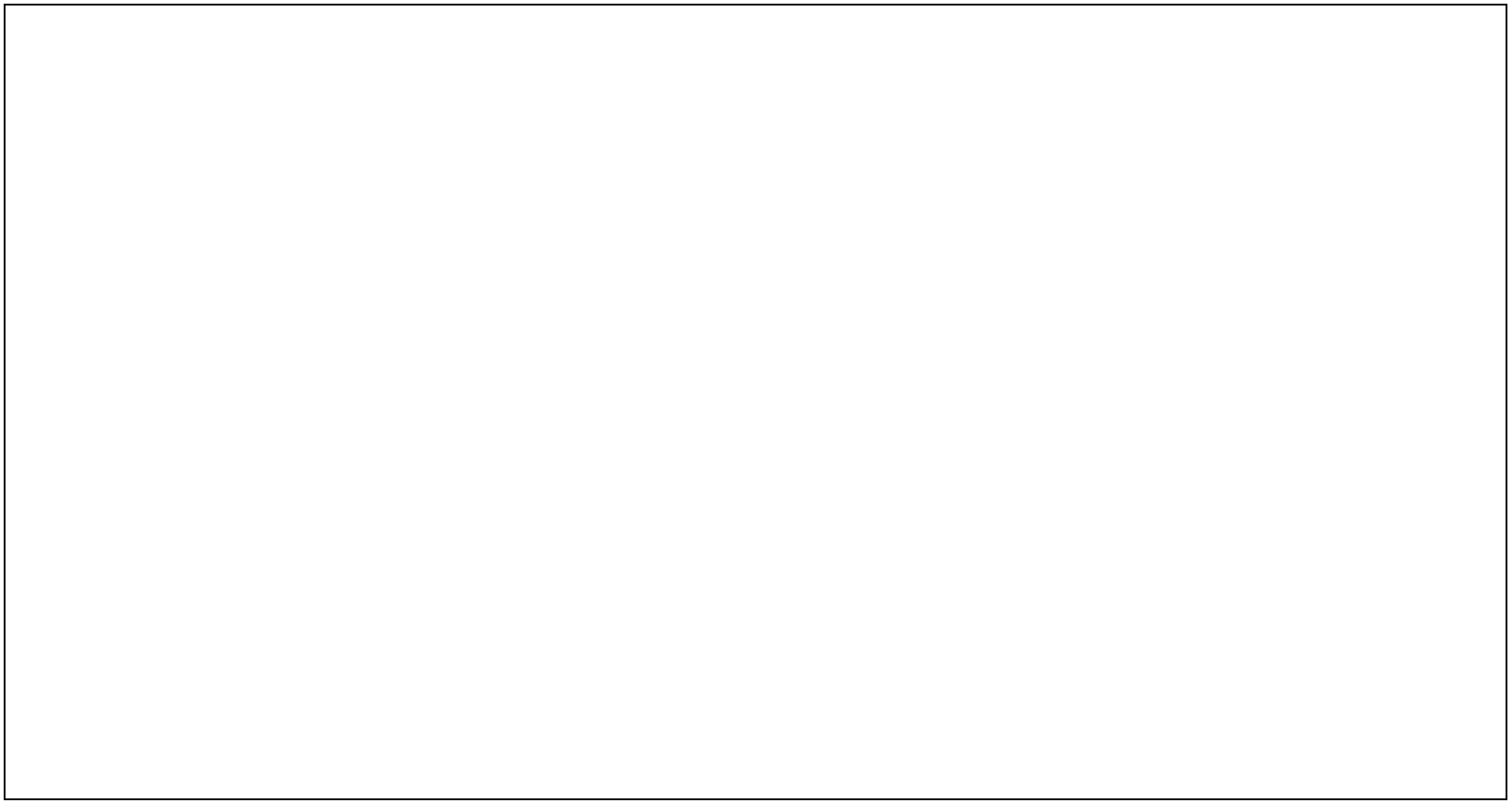
General needs:



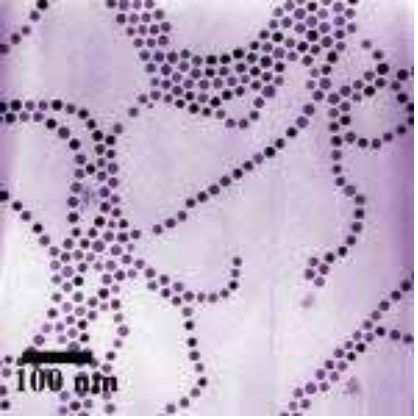
- *Evaluate studies for validity and relevance to human exposure*
- *Compare in vitro and in vivo outcomes in well-controlled studies*
- *Understand realistic dosing, exposure for materials for in vitro use*
- *Understand what differences in reported effects are due to dosing, route of administrations, cell line fidelity and control issues, materials chemistry, contamination, shape, or aspects of testing system*
- *Validate dispersion procedures, and confirm aggregation behaviors*
- *Mandate comparisons to standard reference materials*
- *Improve tools to detect and characterize nanomaterials in complex milieu*

"If you increase the magnification another million times you can see the safety regulations."

…and continue to use good science to characterize these systems to ascertain reasonable risks and eliminate paranoia



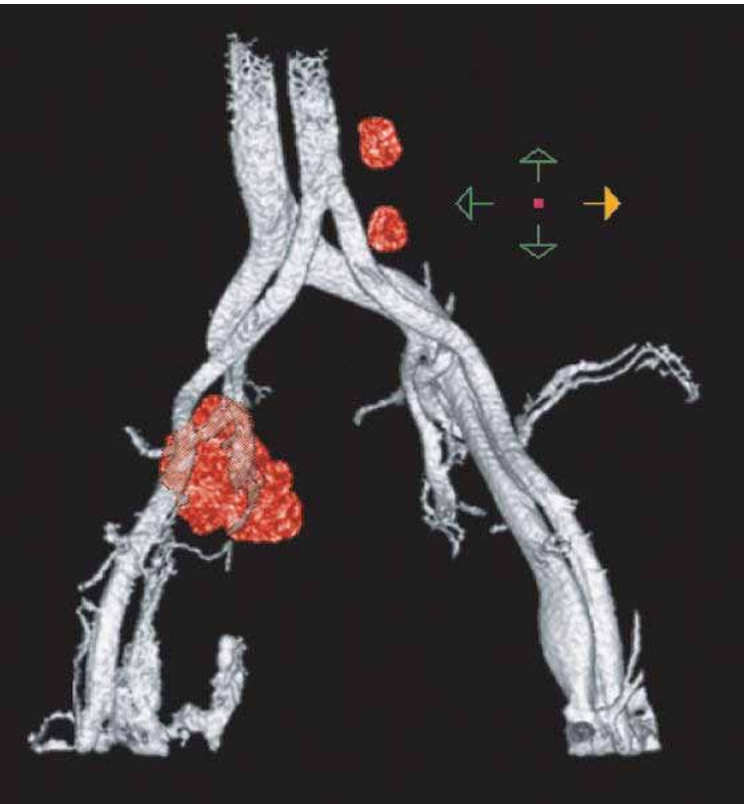
Nanoparticles in biotechnology



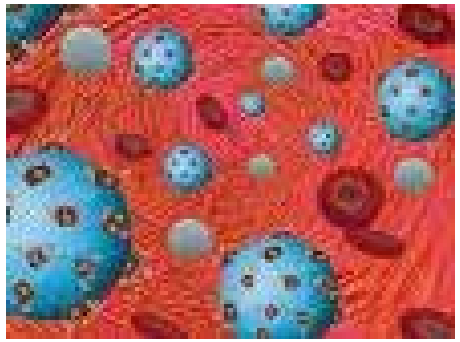
Separations - magnetic

- *World market for nanoparticles ~ $1.7 billion in 2012 (30% biomedical)*
- *biomedical, pharmaceutical, and cosmetic applications increasing at growth rate of 19% annually*
- *gold, titanium dioxide, zinc oxide and calcium phosphate accounted for more than 90% of 2006 market*
- *Sun-protection and cosmetic markets have greatest near-term potential*
- *Diagnostics and Imaging technologies now emerging*
- *Drug-carrier applications: slowest to market.*

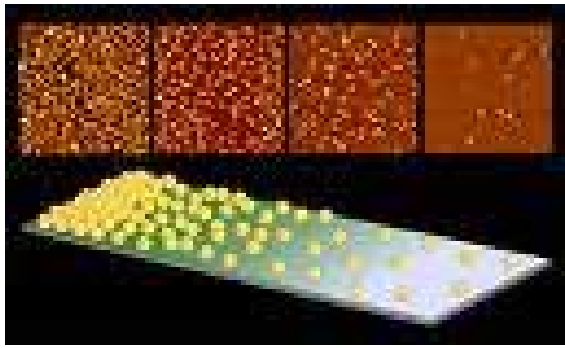
Bio-imaging in vivo

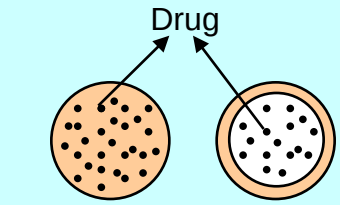


Drug delivery

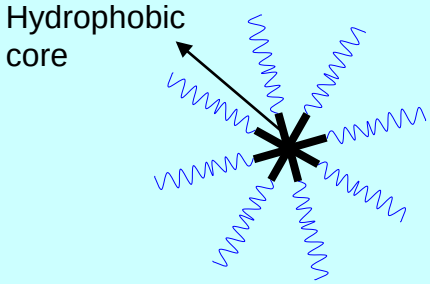


Sensing and diagnostics

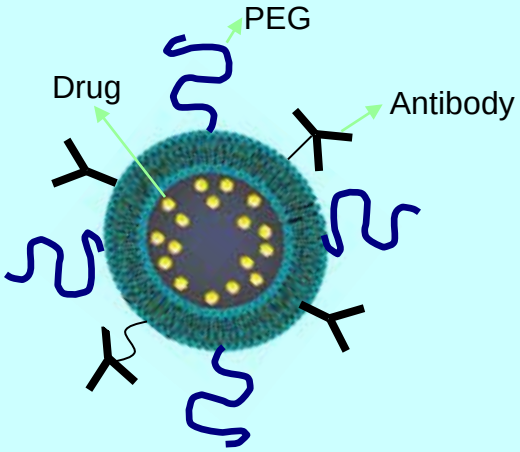




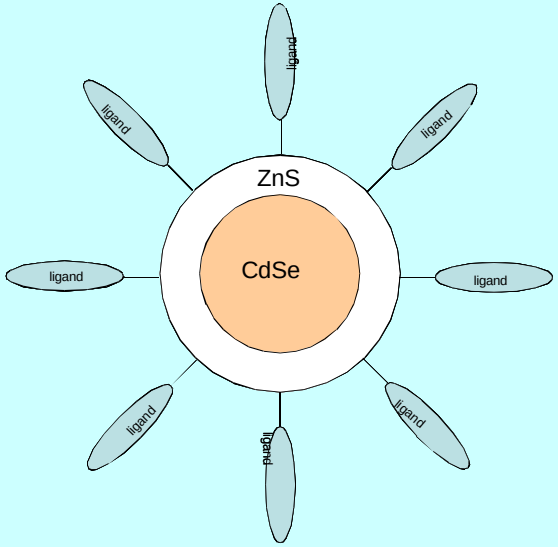
Nanosphere Nanocapsule



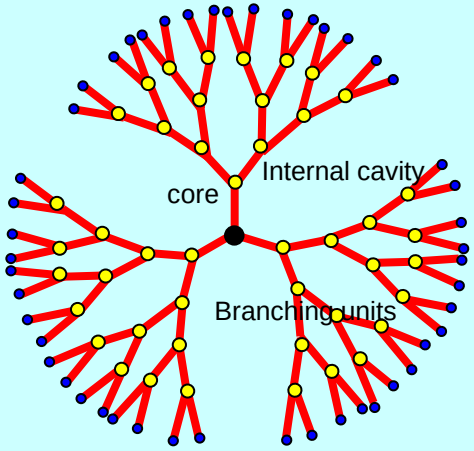
Polymeric Micelles



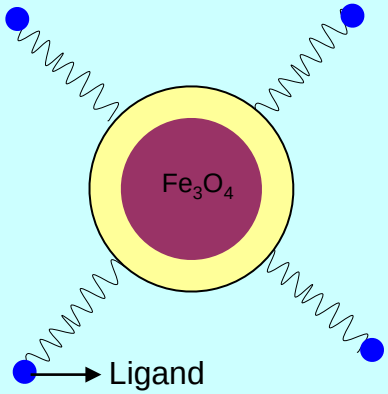
Liposome



Ligand Conjugated
ZnS-capped CdSe QD



Fourth Generation
Dendrimer



Ferrofluid conjugated to ligand

Sahoo S, Labhasetwar V.: Nanotech approaches to drug delivery and imaging. *Drug Discovery Today* 8, 1112-1120, 2003

	CNT (instillation)			
Author	Huczko et al., 2001 (guinea pigs) 0-25 mg/kg	Lam et al., 2004 (mice) 0-17 mg/kg	Warheit et al., 2004 (rats) 0-5 mg/kg dose	Shvedova et al., 2005 (mice) 0-2 mg/kg
Conclusion	High CNT content soot does not alter pulmonary function or induce inflammation Health risks unlikely	Dose-dependent, CNT-focused lesion formation Intrinsic CNT toxicity	Dose independent lesion formation which "may not have physiologic relevance"	Dose-dependent, CNT-focused lesion formation Intrinsic CNT toxicity
Modulation of cytokines & proteins (in BALF)	No change in cell differentials or total protein conc.	Severe peribronchial and interstitial inflammation, fibrosis, and necrosis that had extended into the alveolar septa	Transient increase (day 1 only) of inflammatory markers (LDH & total protein conc.)	Cell death, decreased lung function. Dose-dependent increases in TGF-*β,* LDH, total protein conc.
	TiO_2 particles			
Author	Hext, 1994; ILSI, 2000;Warheit and Frame, 2006 (rats) 250 mg/kg	Bermudez et al., 2002, 2004; Lee et al., 1985; Heinrich et al., 1995	Warheit et al., 2007	
Conclusion	Benign tumor formation from particle overload (100% rutile pigment-grade particles)	Anatase/rutile mixture 5x more potent in tumor formation	Rutile nanoparticles produced no lasting adverse pulmonary effects	80/20 anatase/rutile samples produced prolonged adverse inflammatory and cytotoxicity

Species-species differences and instillation vs. inhalation differences in NP response