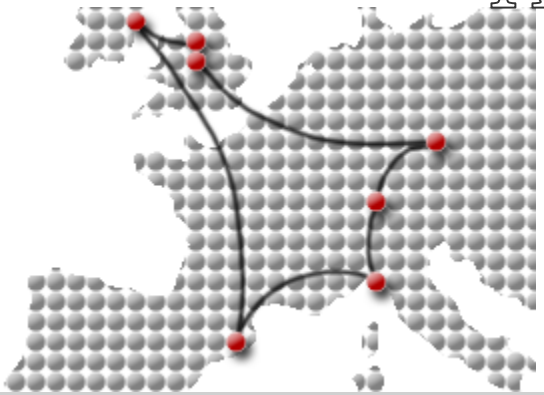
RESEARCH & INNOVATION
Marie Curie Actions

Kick off meeting

MagNETic FUN



Barcelona
23-24 February 2013

Nanotoxicology

Robert N. Grass
ETH Zurich, Switzerland
TurboBeads GmbH, Zurich, Switzerland

The problem with nanotoxicology - generalization

Penetration of Intact Skin by Quantum Dots with Diverse Physicochemical Properties

Jessica P. Ryman-Rasmussen, Jim E. Riviere, and Nancy A. Monteiro-Riviere[1]

Center for Chemical Toxicology Research and Pharmacokinetics, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina 27606

Test of 1 spherical QD (4.6 nm) and 1 ellipsoid QD (6x12 nm) with 3 surface coatings

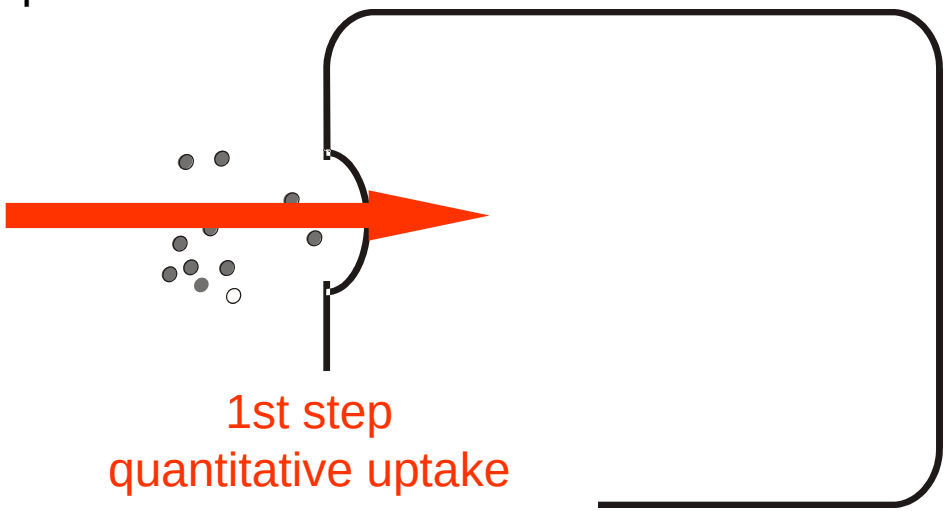
The problem with nanotoxicology

"The penetration of QD through skin was addressed by the Monteiro-Riviere research group employing an ex vivo porcine skin model. Their **initial results found that**, with 8 and 24 h of exposure to QD, porcine skin exhibited **QD penetration throughout the epidermis** and deep into the dermis in some cases. In a more **recent follow-up study, they** reported **contrasting results** in that minimal penetration of QD through ex vivo porcine skin was found, with the bulk of the QD remaining in the stratum corneum. Reasons for this discrepancy remain unclear, however, **other researchers** have examined the question of skin penetration employing different NP types (metals, polymers) using ex vivo skin models, **and again contrasting** results of both high and low levels of NP penetration are reported."

NANO LETTERS
2008 Vol. 8, No. 9 2779-2787

Why we are afraid: Nanoparticle in living cells?

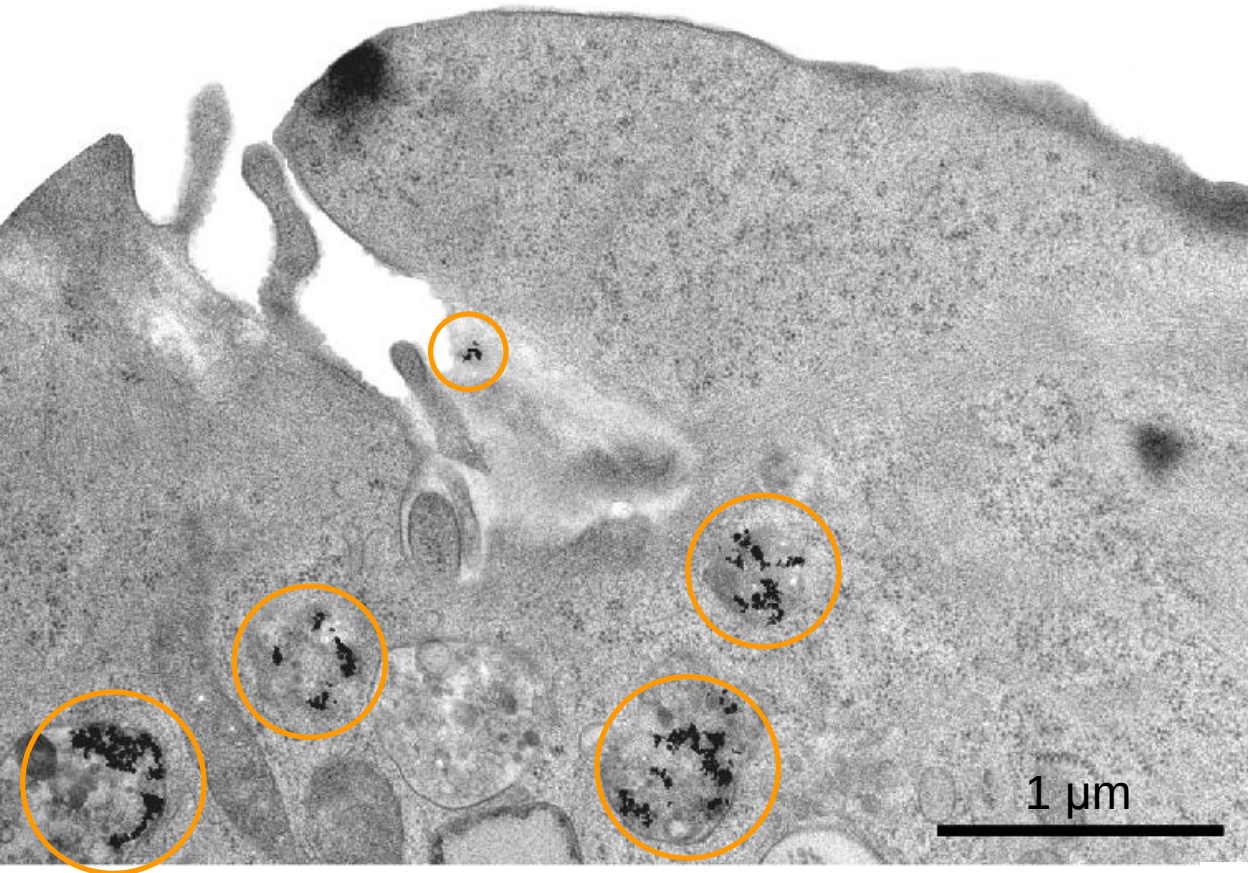
uptake of
nanoparticles



1st step
quantitative uptake

Limbach et al. ***Environ. Sci. Technol.*** 2005

quantitative uptake of nanoparticles in cells

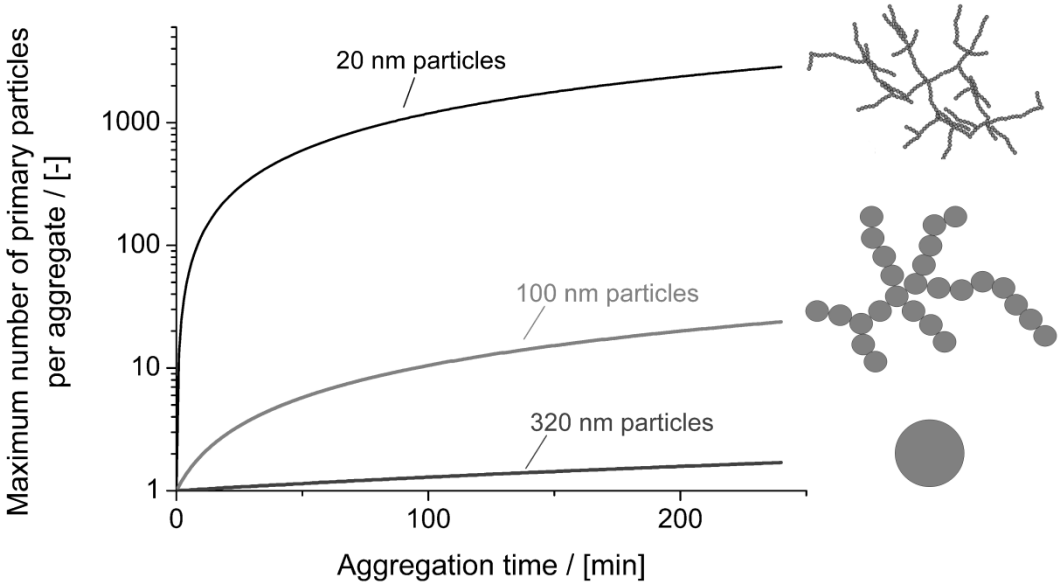


$$\frac{dn_t}{dt} = -\frac{1}{2}\frac{\beta}{W} n_t^2$$

Friedlander (1953), Smoke, Dust and Haze

Limbach et al. ***Environ. Sci. Technol.*** 2005

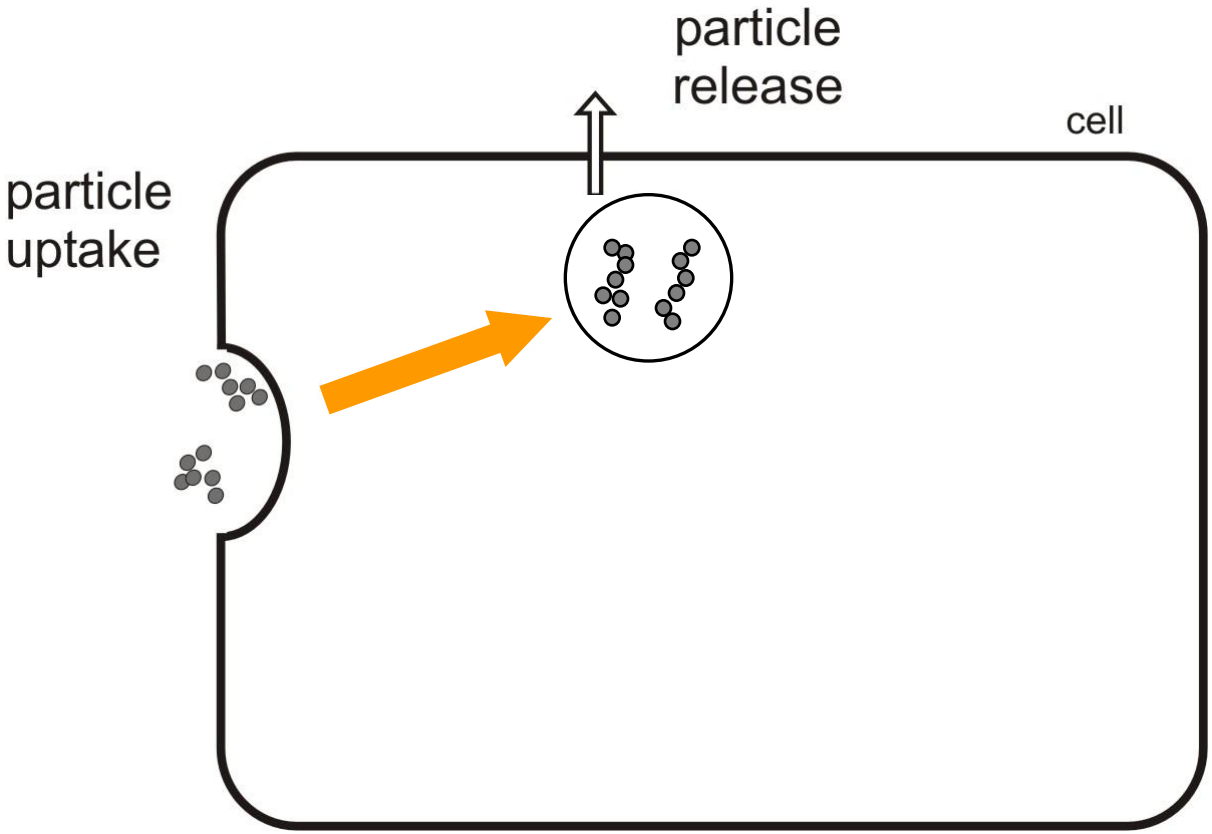
agglomeration – same mass concentration different size



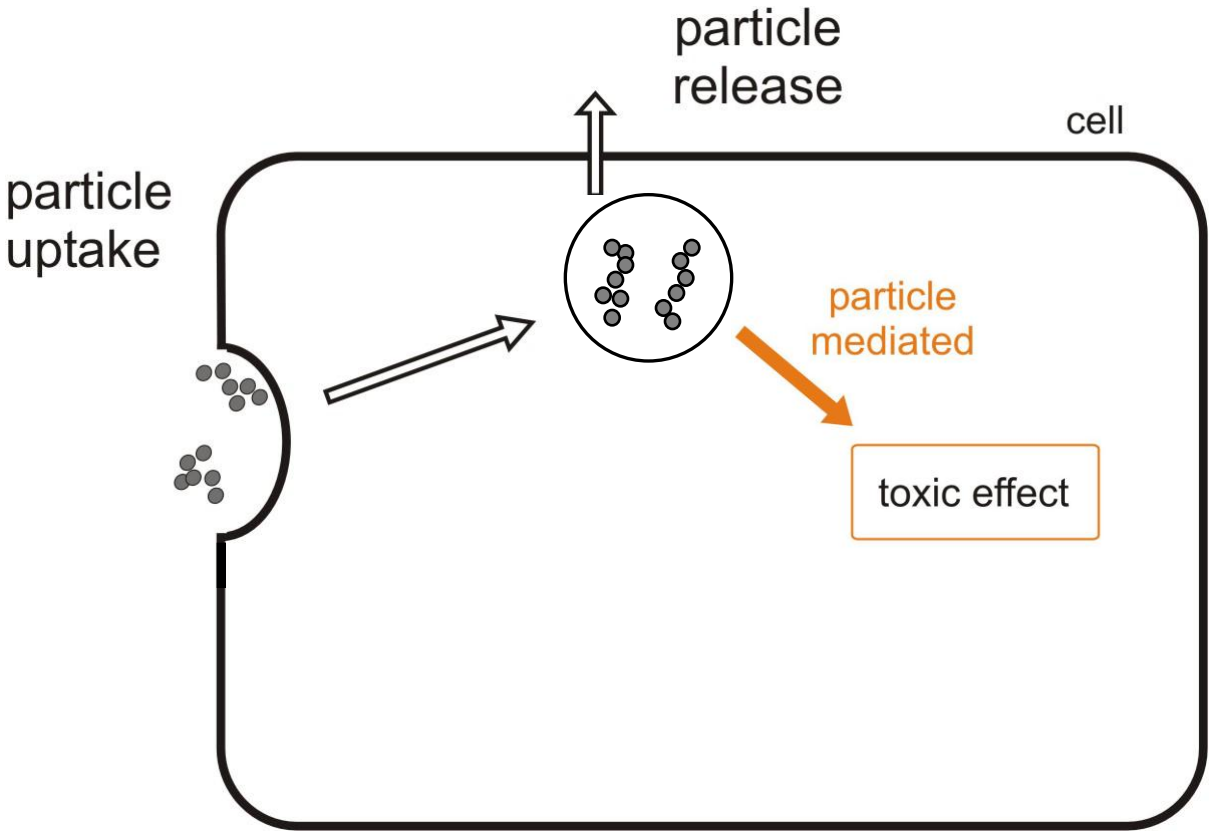
Biodistribution = f (time, concentration)

Limbach et al. ***Environ. Sci. Technol.*** 2005

Schematic diagram



Schematic diagram



Trojan horse mechanism

ZnO-nanoparticle

Zn^{2+}-ions

ZnO-microparticle

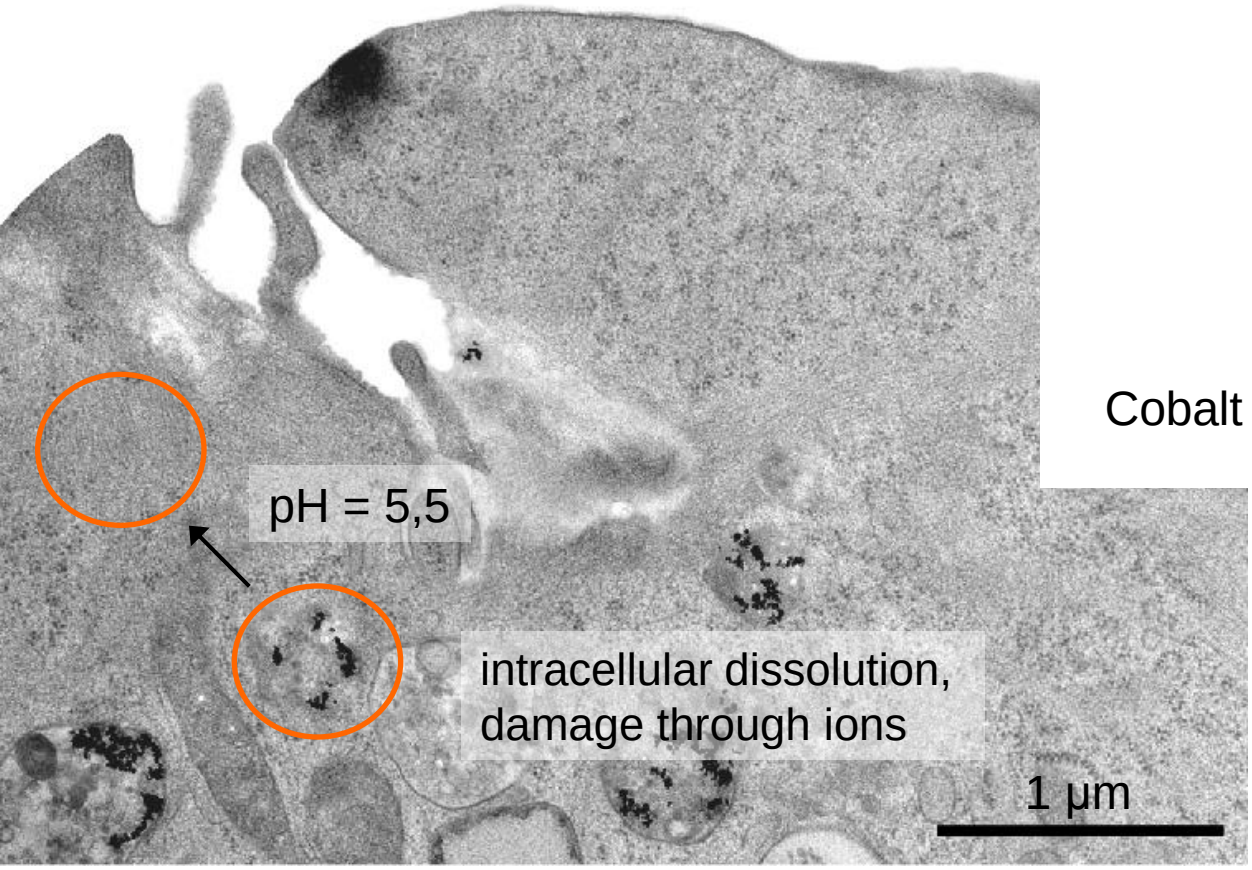
cell

pH = 5.5

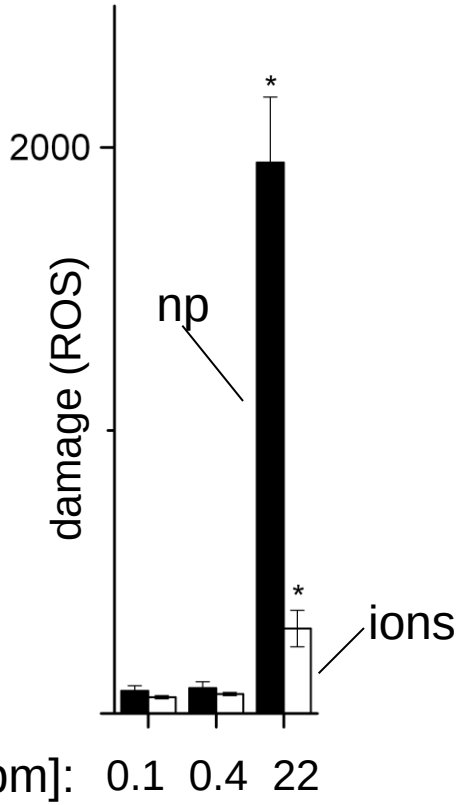
Intracellular dissolution
additional damage through ions

Brunner et al. **Environ. Sci. Tech.** 41 (11), 2007, 4158-4163,

trojan horse-type enhanced heavy metal uptake

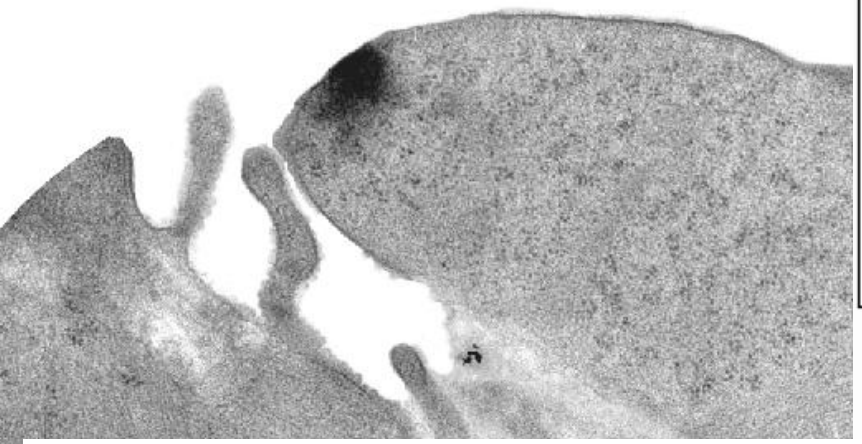


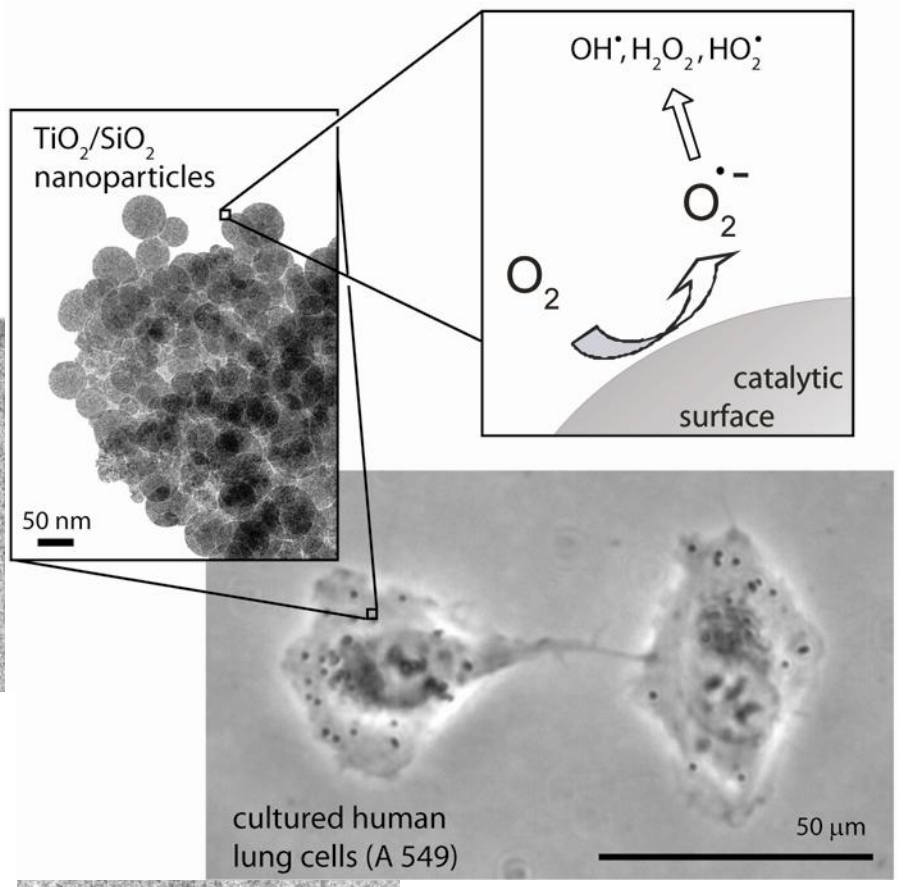
in vitro

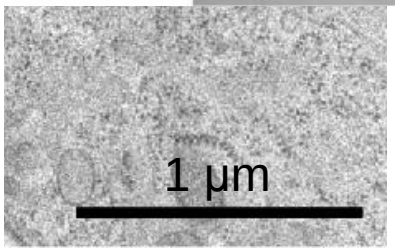


Limbach et al. ***Environ. Sci. Tech.*** 41, 4158-63, 2007

heterogeneous Catalysis inside living cells







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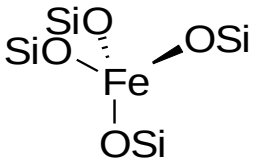
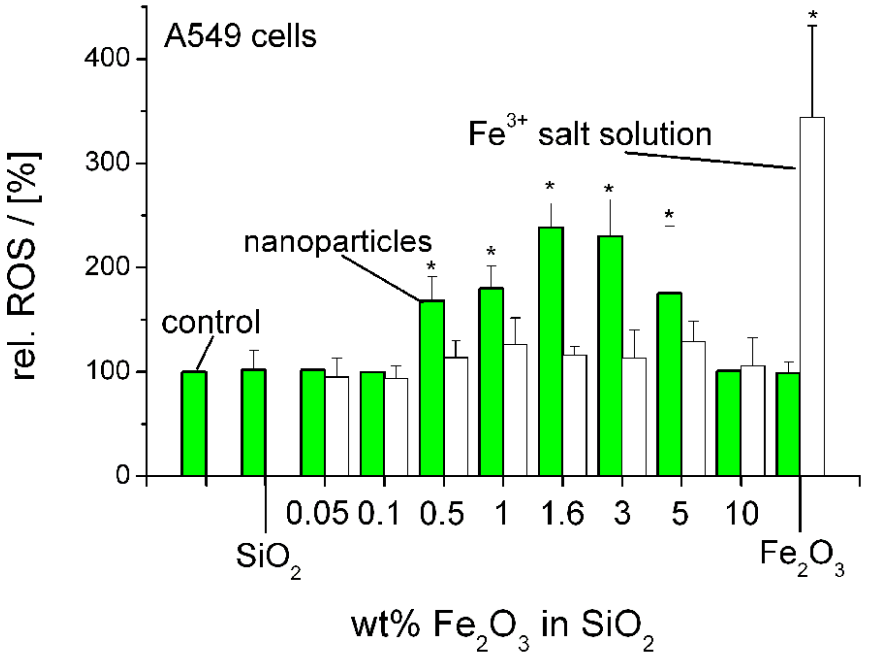
heterogeneous catalysis inside living cells





Limbach et al. ***Environ. Sci. Tech.*** 41, 4158-63, 2007

Classical toxin

- Bio-distribution
- Action
- Clearance
- Dose/Effect relations

Nanoparticles

- Agglomeration, diffusion and sedimentation change distribution kinetics
- Action; Solubility alters release
- Clearance
- Dose/effect
- Catalytic effects: Non-mass constant toxic action

Nanoparticle associated risk

acute effects

➔ apoptosis
➔ necrosis

long term effects

➔mutagenicity
➔teratogenicity

Uptake into cells, crossing cell barriers

Dissolution in cell – Trojan horse

Reactive oxygen species: ROS catalysis

Nano is not new

Global commodities:

- Silica np. (food additive)
- Titania np. (white paint, toothpaste, sunscreen)
- Carbon black np. (car tires, printing inks)

Safely used today in consumer goods

Fullerenes, carbon nanotubes (asbestos)

known for toxicity

Risk of nano products

risk := damage potential * occurrence probability

linking to measurable values

Material properties

- solubility
- degradation
- fate
- activity
- behavior

…

occurrence probability

- mass, sources
- exposure concentration
- frequency
- transport
- target tissue

…

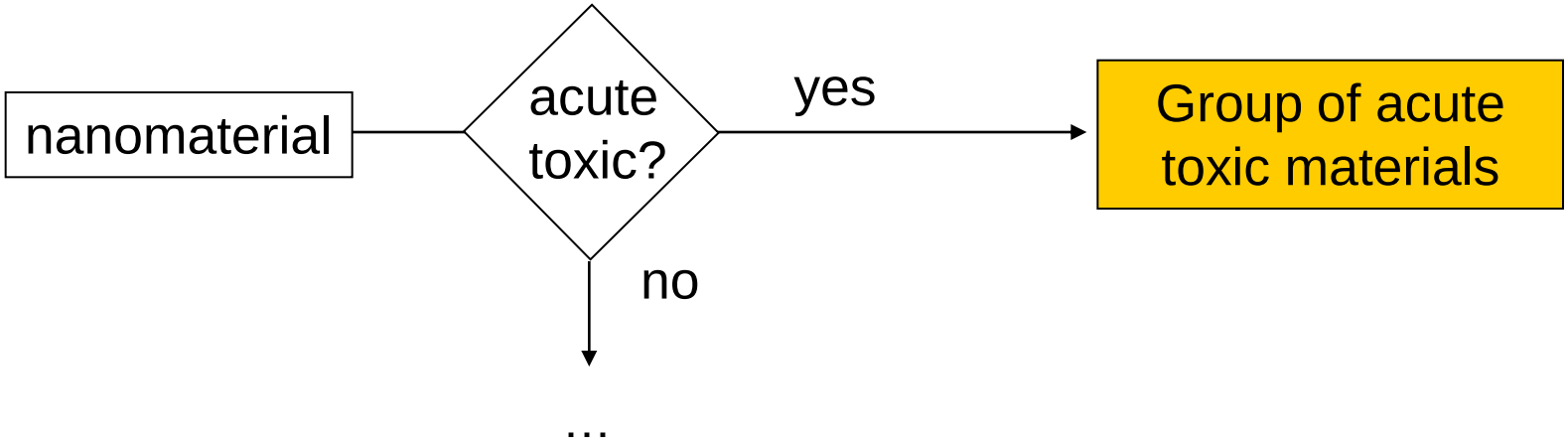
acute or long term effects

exposure and uptake

risk := damage potential * occurrence probability

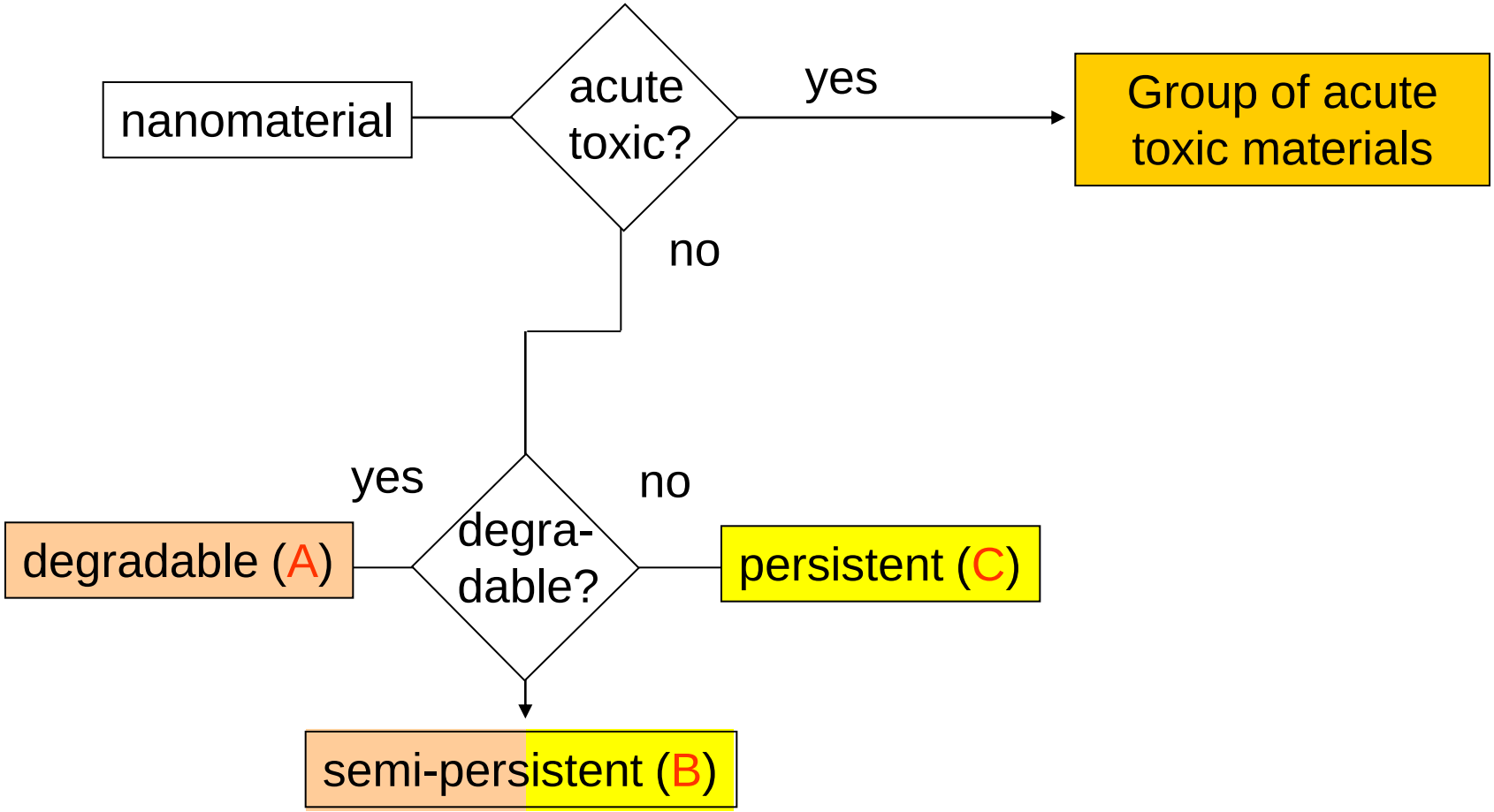
1st criterion -

is it acute toxic?



2nd criterion -

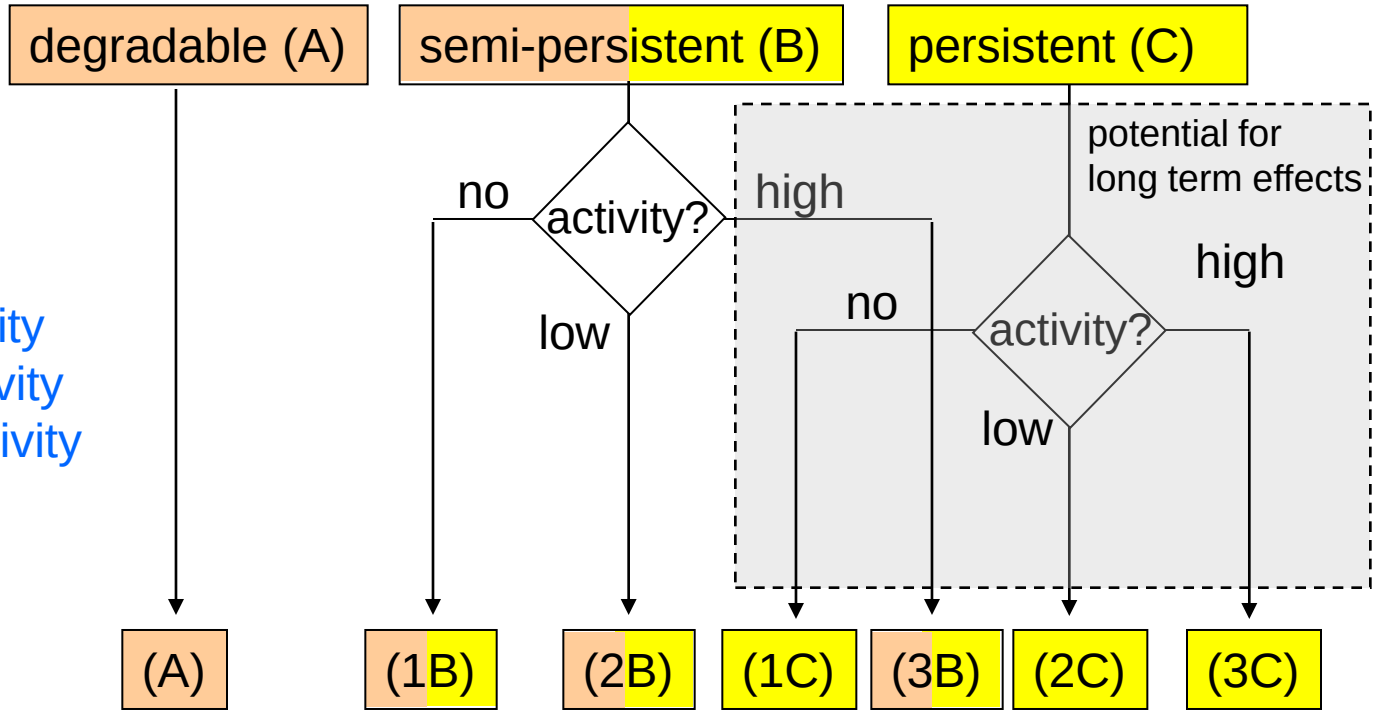
is it degradable?



3rd criterion -

chemical activity

1 - no chemical activity
2 - low chemical activity
3 - high chemical activity



Biological damage

Damage potential

ZnO	high	⇒ acute toxic
Ca_3PO_4	low	⇒ low damage potential
 CNT	high	⇒ potential long term effects

OVERALL RISK depends on the use (occurrence probability)

Occurrence probability

risk := damage potential * occurrence probability

Who is exposed?

With what ?

- an aerosol
- a liquid dispersion
- a dry powder
- a solid nanocomposite (e.g. np in polymer)

Exposure time, route of entrance

Think about

- Nanoparticles as food additive

- Nanoparticles in cosmetics

- Nanoparticles in aerosol spray can
- Nanoparticles embedded into battery material
- Nanoparticles in technical catalyst

- Nanoparticles as cancer treatment

In the laboratory routine

- Nanoparticles dispersed in liquids
  - Treat as toxic liquid chemicals
  - Wear gloves and safety glasses at all times
  - Wash up spills wearing gloves
- Nanoparticles don’t translocate through healthy skin
- Use standard chemical/biochemical laboratory practice
- Don’t wear gloves all the time
  - Doors, drawers, computers, labbook, pens only without gloves

In the laboratory routine

- Dry nanopowders (agglomerated)
  - Treat in well ventilated areas (i.e. **hoods**) **only**
    - Weighing, grinding, pooring etc.
  - Nanoparticles don't deagglomerate
    - Once stuck together as a powder, they stay together
    - Unless energy is introduced
  - Metals (even coated) are potentially pyrophoric
- Nanoparticle aerosols
  - Nanoparticle aerosols are taken up via the lung
  - In closed environments (**glove box** or similar) only!

Nanoparticle disposal

- Collect separately (including all solutions contacting nps)
- In dispersed (liquid) form
- Separated by
  - Aqueous
  - Non-aqueous
- Dispose as special waste via local authorities

Planning science with nanotoxicology

Before starting……..

- Think about product exposure
  - Manufacturing
  - Use of product/material
  - End of life

- A fast *in vitro* cell-test won't save you
  - Throwing nps on cells and measuring dead/alive is not scientific
    - Exposure scenario with correct cell lines, exposure pathway, biological endpoint, degree of aggregation and adapted concentrations **only**

Don’t be afraid…

…. be cautious!

Don’t generalize

…. differentiate

Further Reading: Stark WJ, Nanoparticles in Biological systems, *Angew. Chem. Int. Ed.* 2011**, 50**, 1242-58.