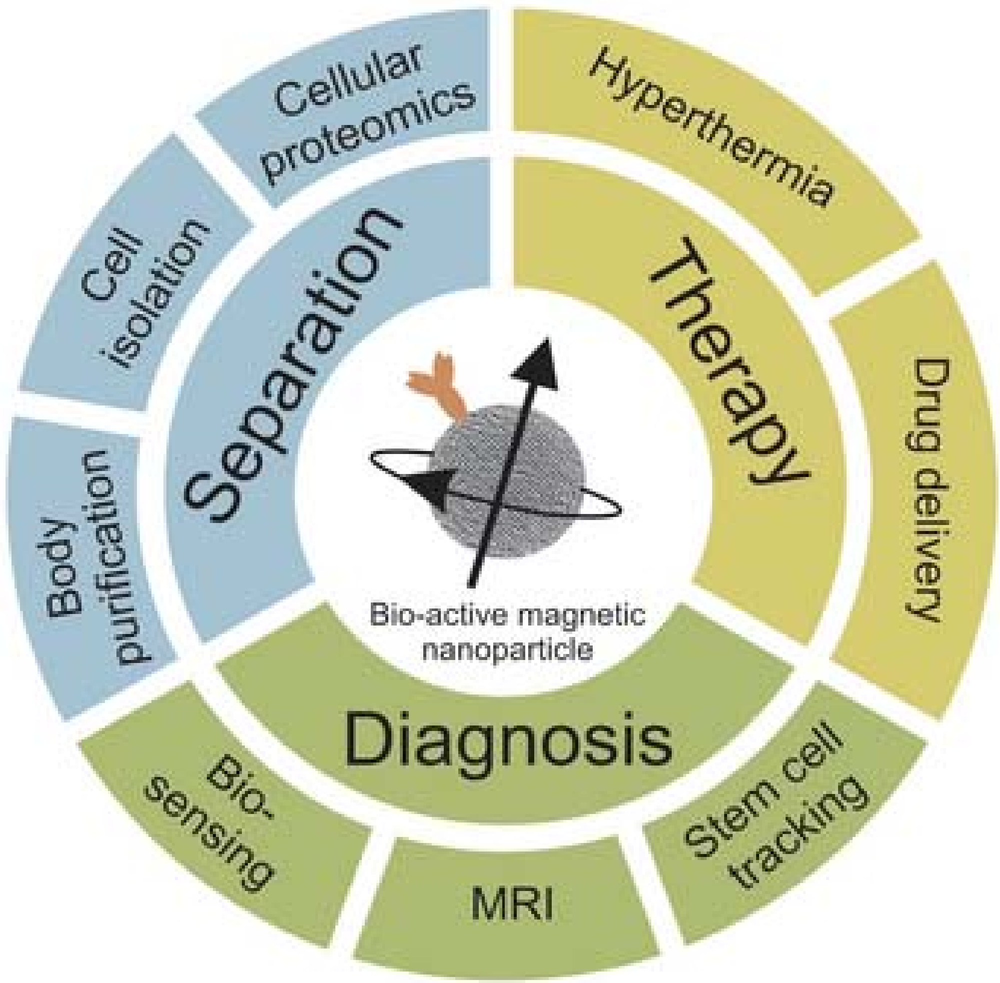
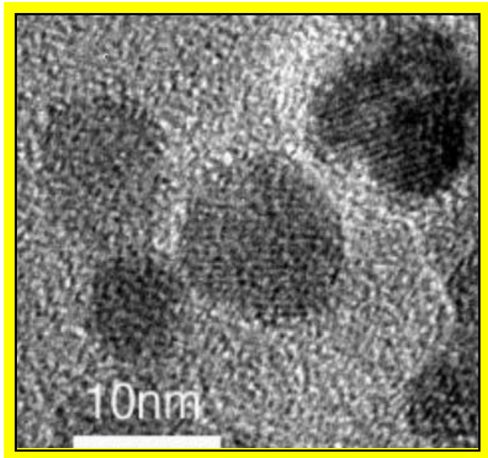
Preparation of Nanoparticle Suspension for Diagnostic and Therapeutic

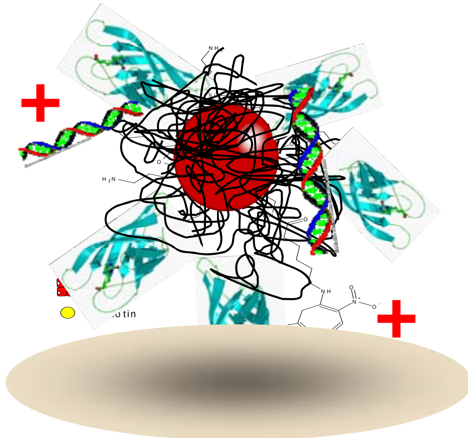
H.Hofmann, G. Coullerez, V. Bernau

Powder Technology Laboratory

Superparamagnetic Iron Oxide Nanoparticle

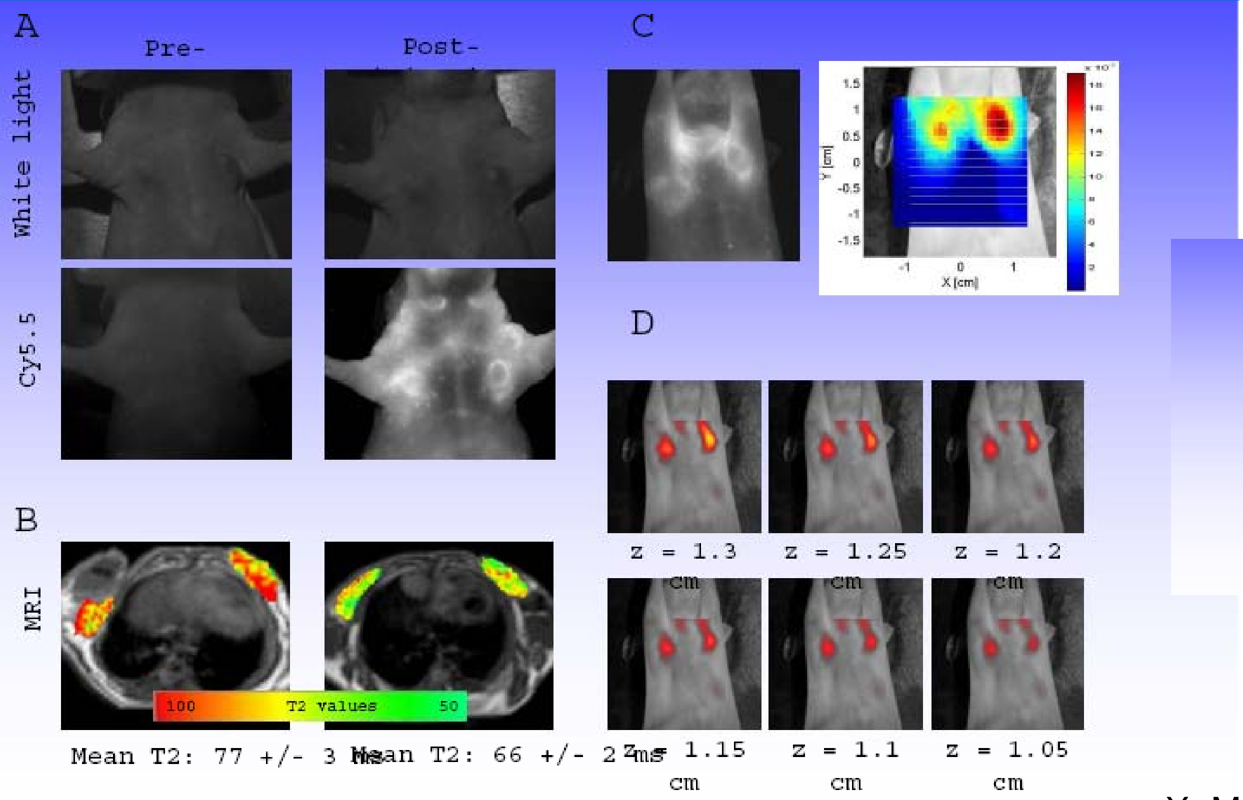


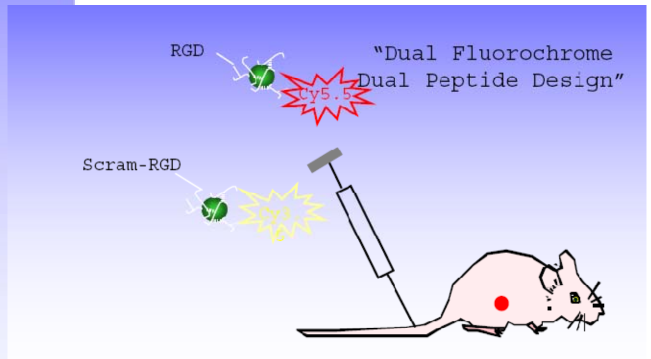




Molecular Imaging

In-vivo Imaging of RGD-aPVA-Cy5,5-SPION Uptake by BT 20

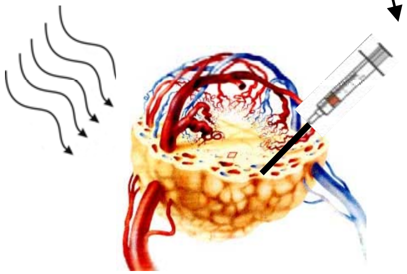




X. Montet University of Geneva,
Particles from EPFL-LTP

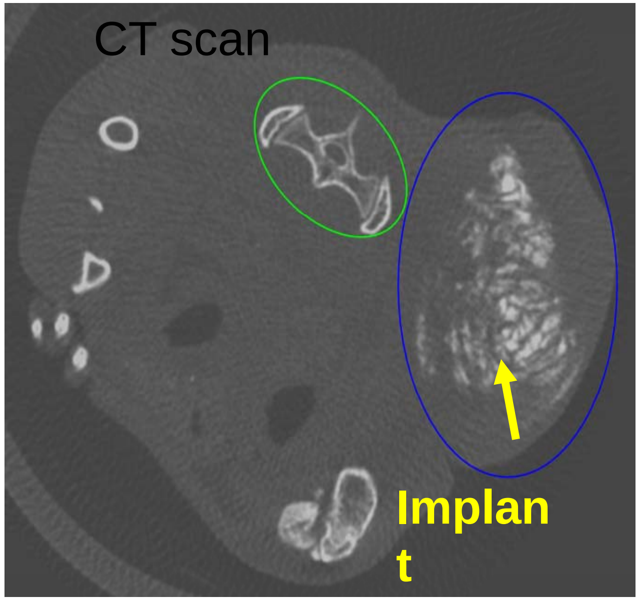
Magnetic Implant Hyperthermia

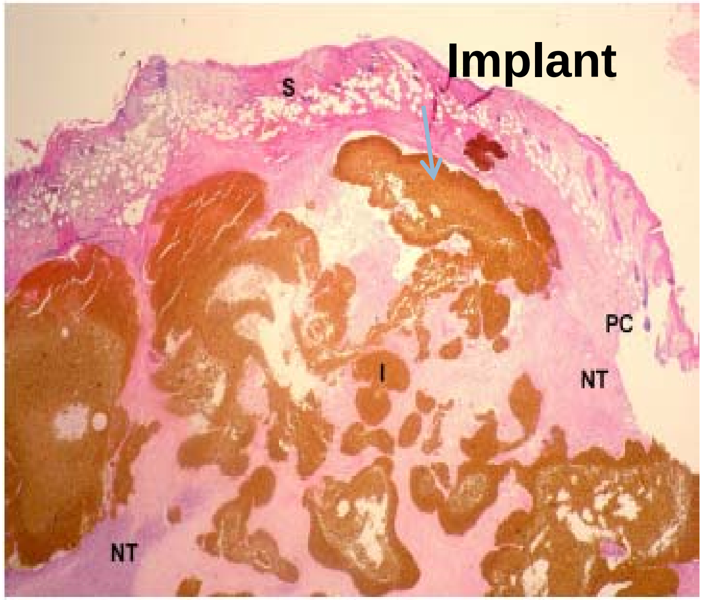
Injectable Nanoparticle/ Monomer solution

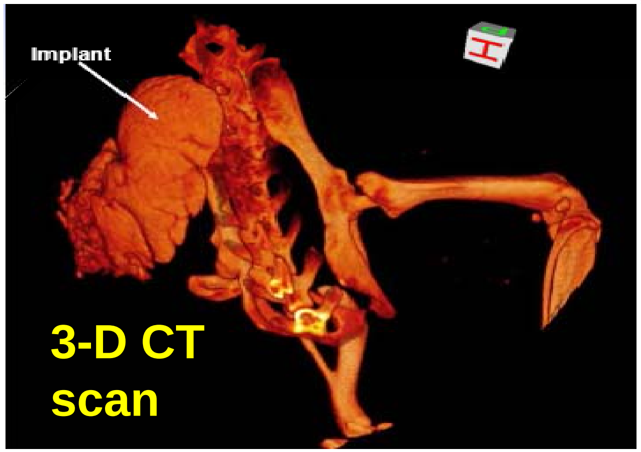


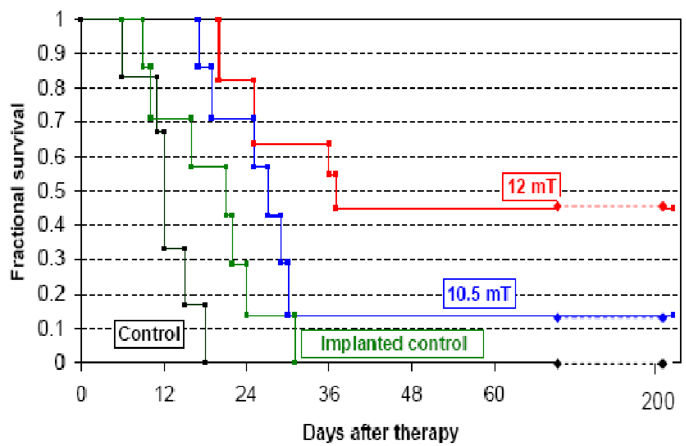
External magnetic field

144 kHz, 9554 A/m



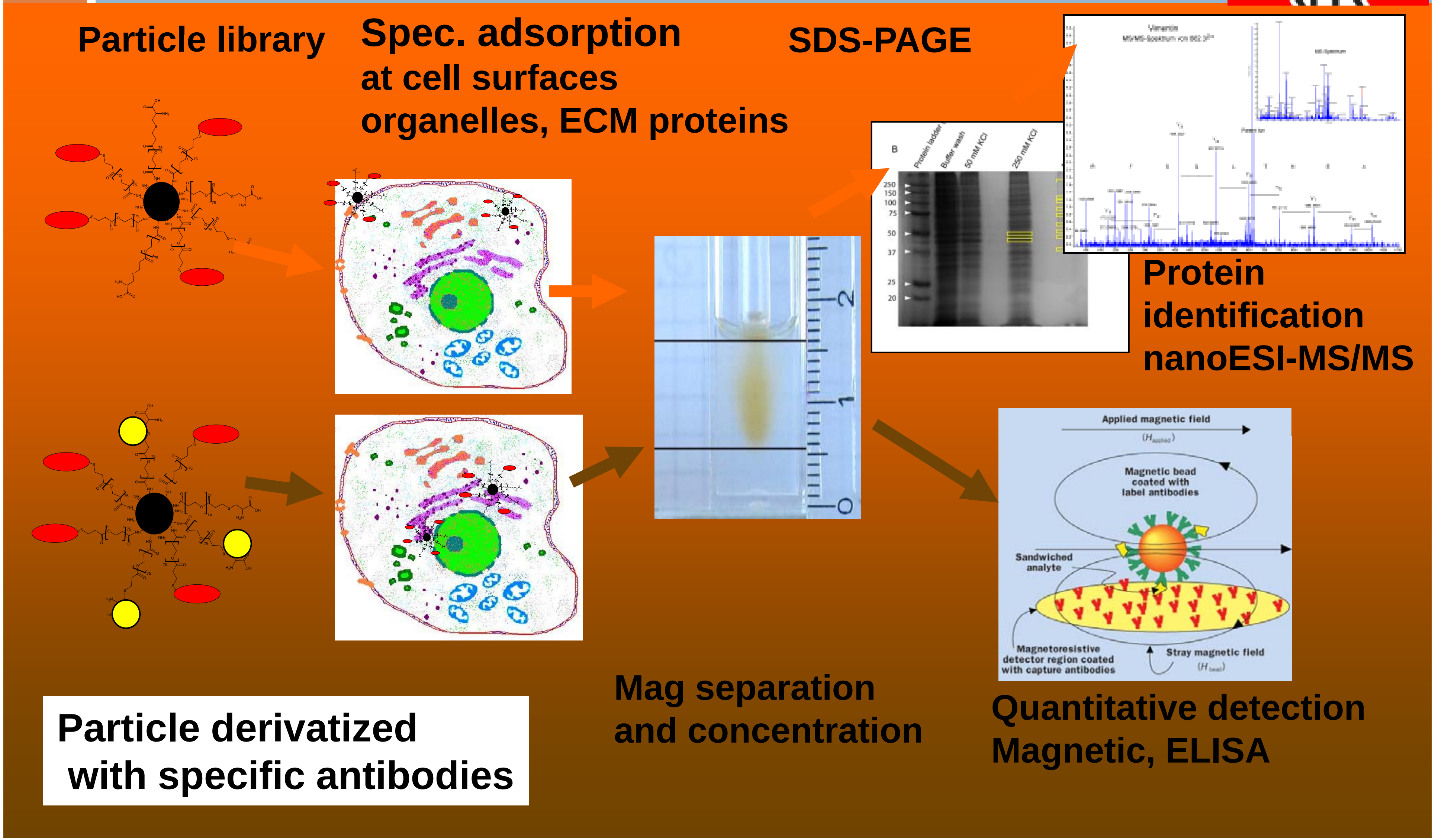






antia therapeutics

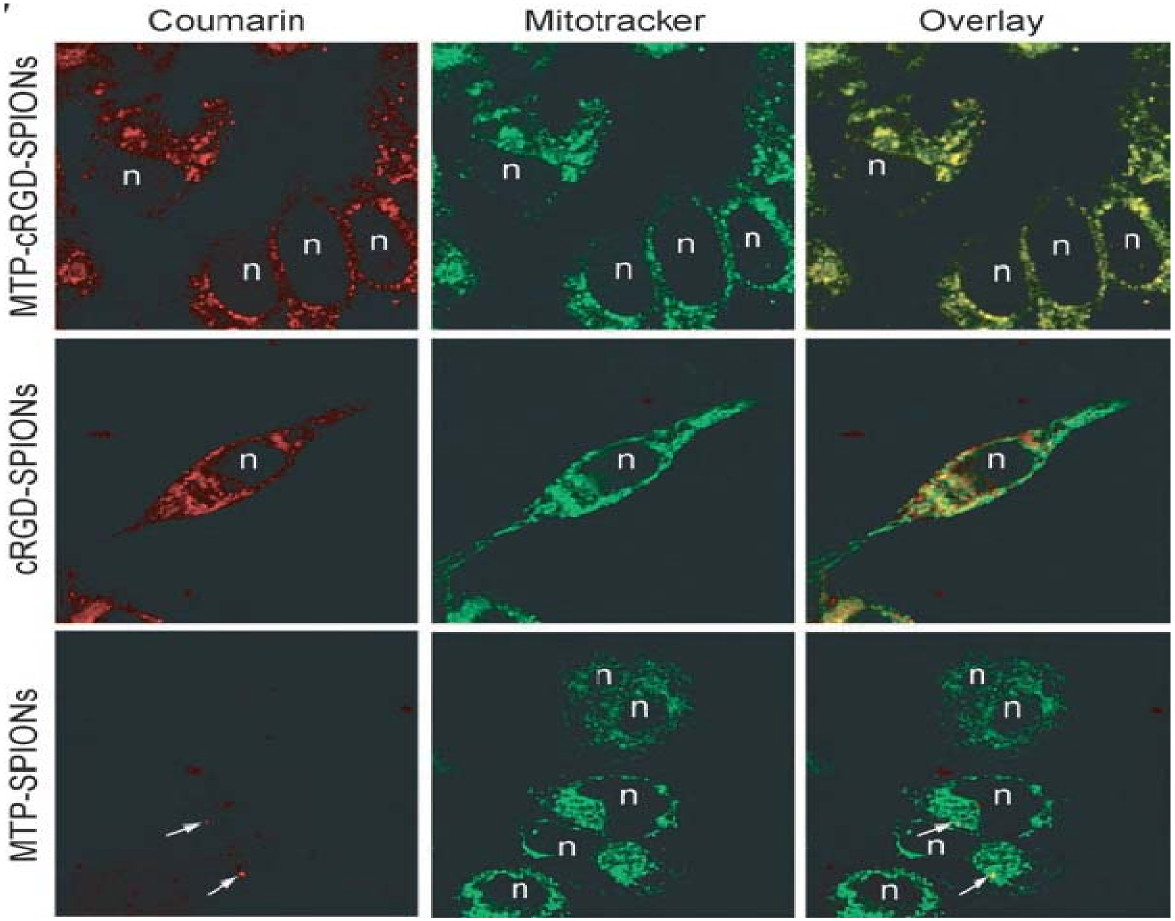
Biomarker (Protein) separation with Superparamagnetic Iron Oxide Particles



Targeting of organelles

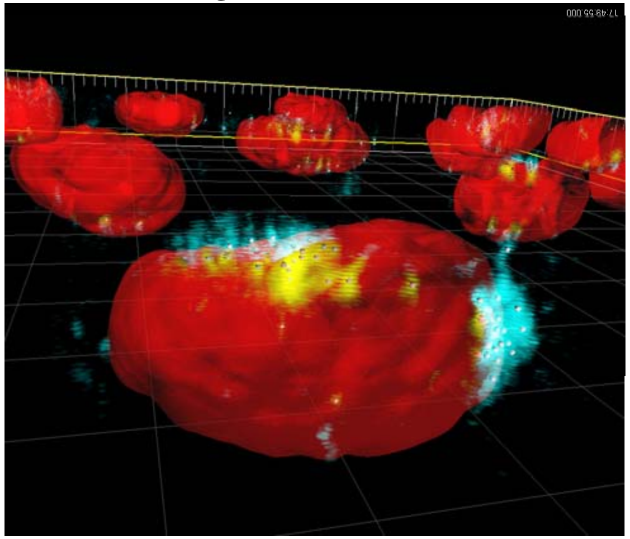
Mitochondria Targeting

SPION with Coumarin and Mitochondria targeting peptide



Nucleus Targeting

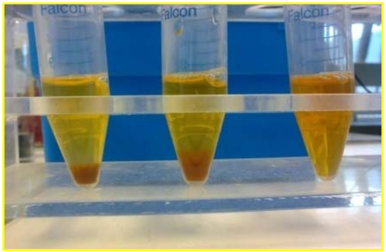
SPION with ALEXA and NTP QPSPSPTGC



Protein separation

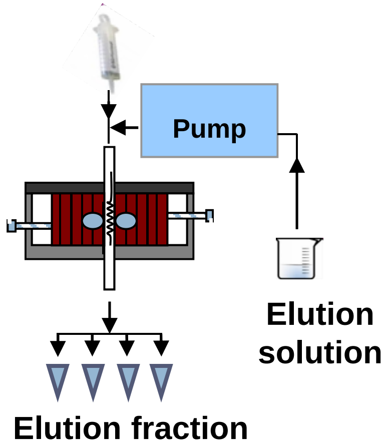
1. Particle incubation

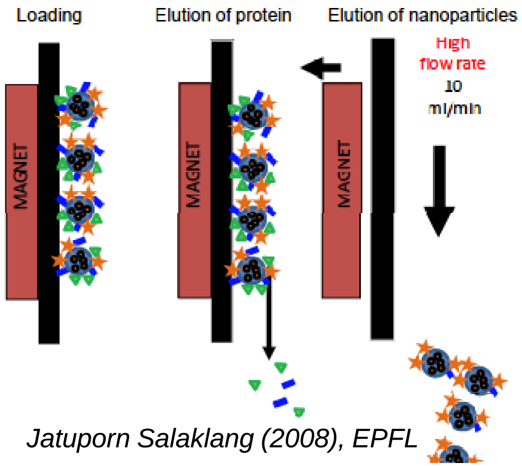
Polyvinyl alcohol (PVA) coated SPION
+ 10%serum supplemented DMEM



PVA-NH_2 PVA (neutral) PVA-COOH
\+ 0 -

2. Magnetic separation

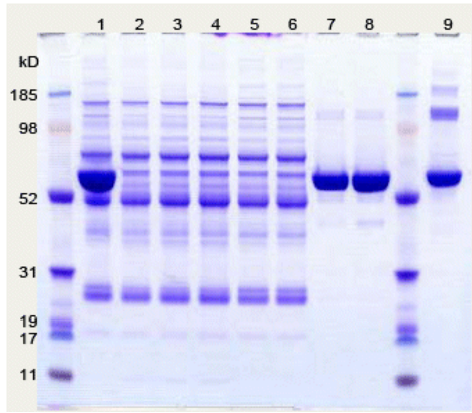




Jatuporn Salaklang (2008), EPFL

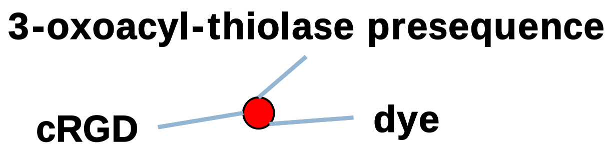
3. SDS-PAGE

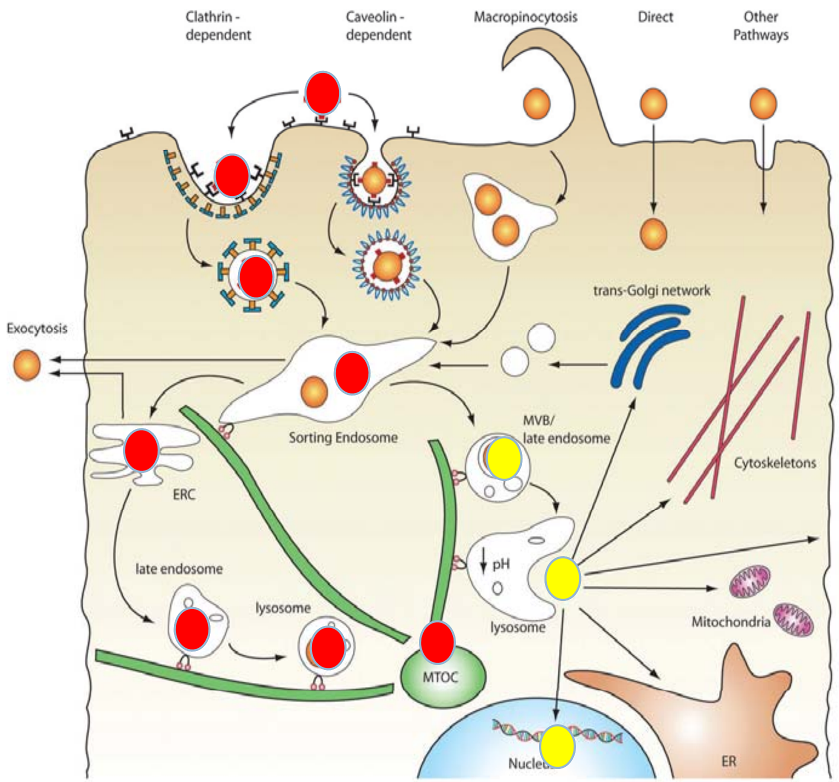
(Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis)



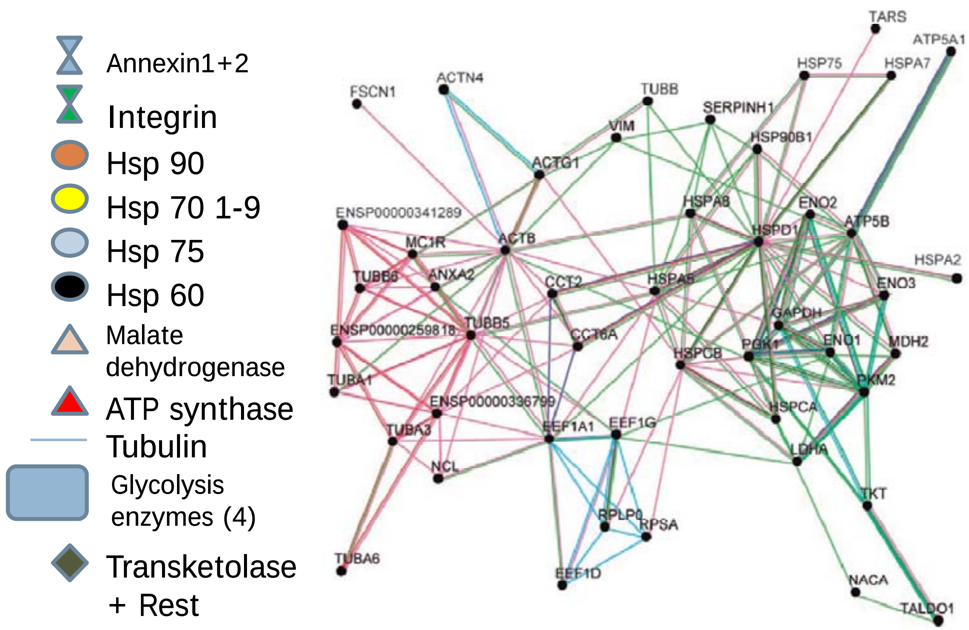
- Control volume and flow rate of elution step
- Reduce solution contamination during the process

Protein Fishing



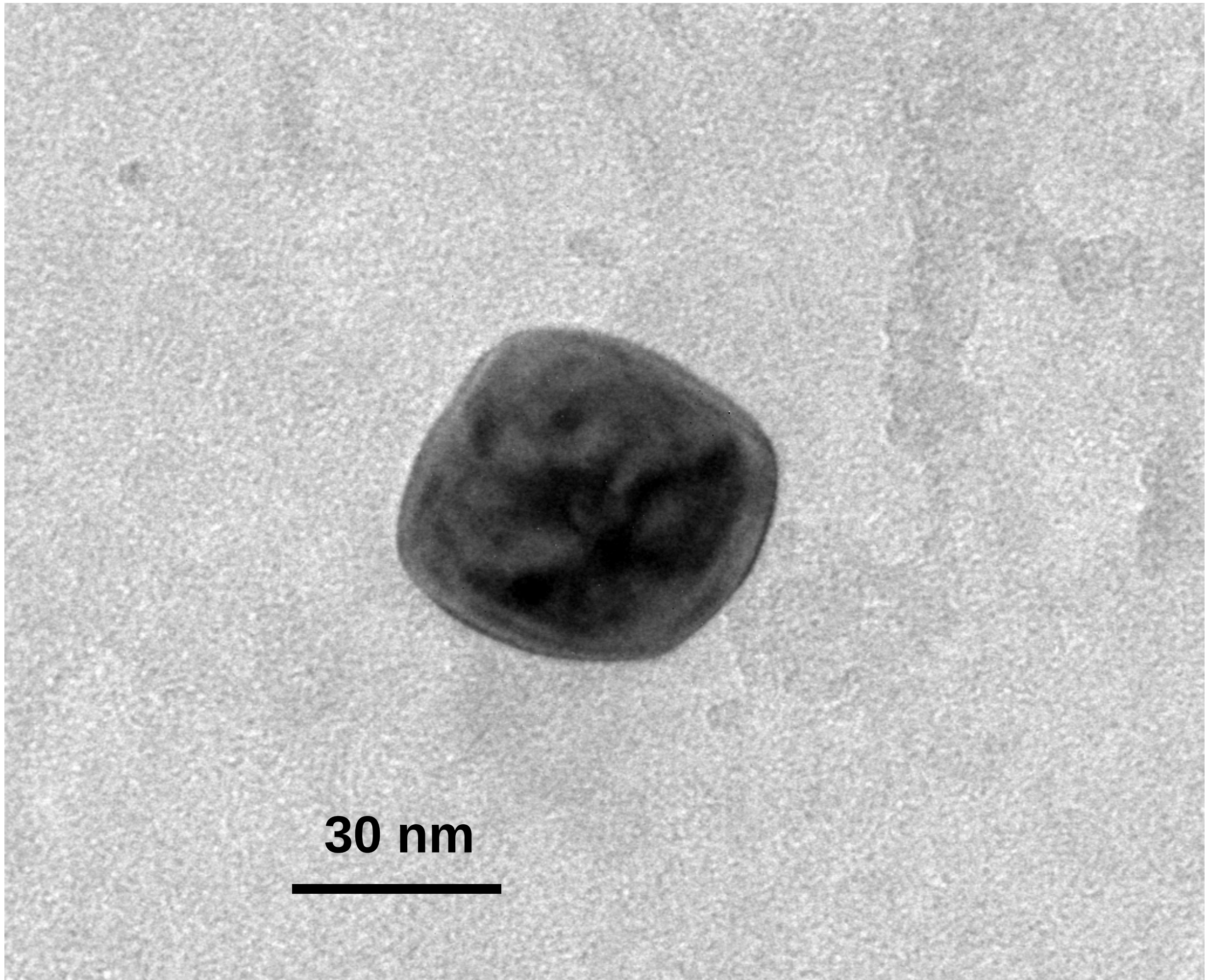


Abbreviations: ERC, endocytic recycling compartment; ER, endoplastic reticulum; MTOC, microtubule-organizing centre; MVB, multivesicular bodies;



48 out of 58 proteins could be related to: Up-take mechanism, transport to mitochondria, mitochondria membrane, including energy related processes. Evidence view of the protein interaction network in STRING

The Nanoparticle



Physical properties

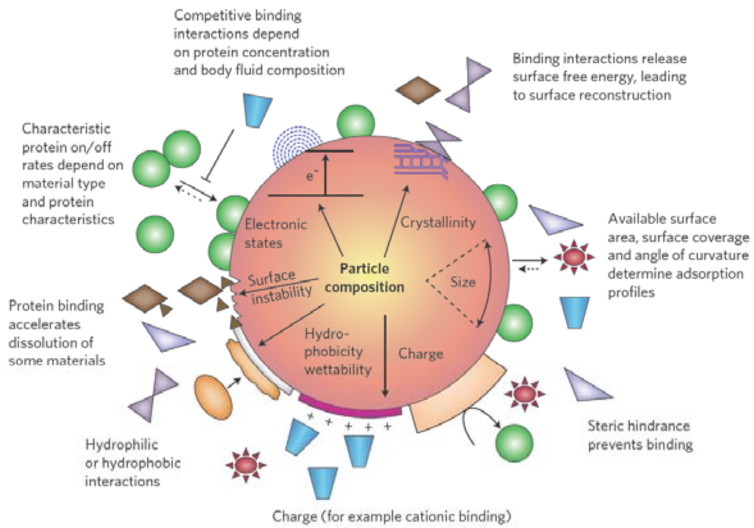
Optical
Magnetic
Electrical
chemical

Technological properties:

Colloidal stability
Biocompatibility
Reproducibility
Easy up-scaling

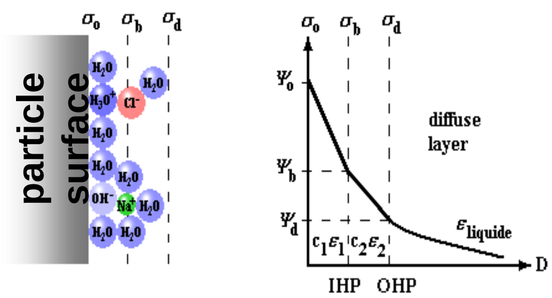
Colloidal Stability

- Van der Waals
- Electrostatic interactions
- Steric interactions
- Polyelectrolyts (Polyethylenimide PEI, etc)
- Magnetically induced interactions
- Hydrophobic/hydrophilic interactions
- Hydration forces
- Depletion forces
- Extended DLVO
- Influence of curvature (Nano)



Andre E. Nel, Nature materials **VOL 8** JULY 2009; 543

Zeta potential (ζ) potential near to the outer Helmoltz Plan



Some important parameters

Nanoparticle
Size, shape and surface area
Surface charge, energy,
Roughness and porosity
Electronic states
Functional groups
Ligands
Crystallinity and defects
Hydrophobicity and hydrophilicity

- **Solid–liquid interface**
  Surface hydration and dehydration
  Electrical double-layer formation,
  Zeta potential, isoelectric point
  Sorption of steric molecules and toxins
  Electrostat and electrosteric interactions
  Aggregation, dispersion and dissolution
  Hydrophilic and hydrophobic interactions

Suspending media
Water molecules
Acids and bases
Salts and multivalent ions
Natural organic matter
(proteins, lipids)
Surfactants
Polymers
Polyelectrolytes

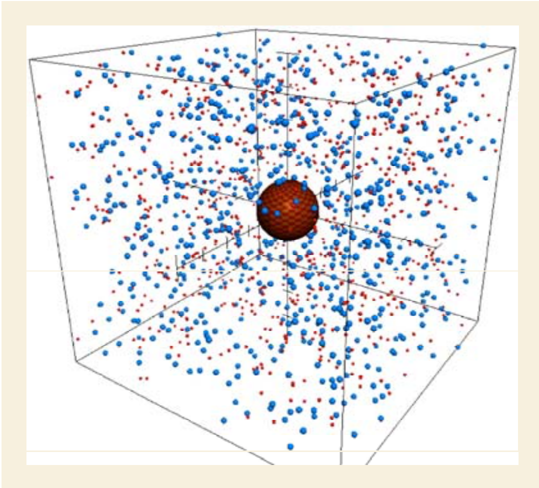
Nano–bio interface
Particle Membrane interactions
Receptor–ligand binding interactions
Membrane wrapping
Biomolecule interactions
Free energy transfer to biomolecules
Conformational change in biomolecules
Oxidant injury to biomolecules
Mitochondrial and lysosomal damage

After Andre E. Nel, Nature materials **VOL 8** JULY 2009; 543

Open questions

Most models take into assumptions particles with diameters larger than all other molecules

What happens when particles have size similar to ionic double layer or biomolecule?



Biological media complexity:

- high complex ionic strength
- complex macromolecules

Can biomacromolecules be viewed as particles?

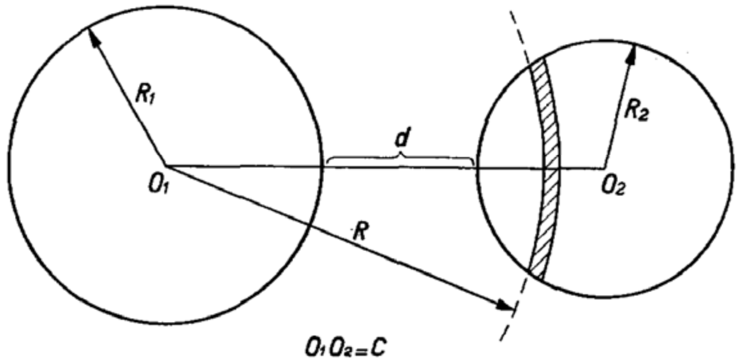
Is the DLVO theory applicable to understand the colloidal stability of nanoparticles in water or even more important in complex biofluids ?

DLVO

$$V_{p1-p2} = V_{disp} + V_{elec}$$

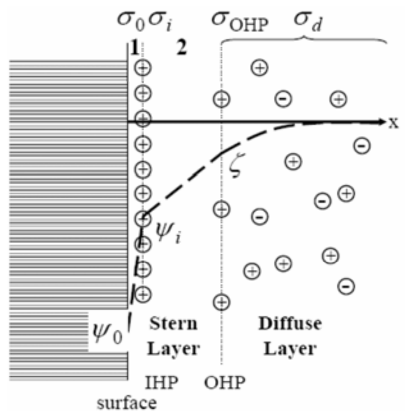
Hamaker[3]

$$V_{disp} = -\frac{A_H}{6}\left[\frac{2R_1R_2}{C^2-(R_1+R_2)^2} + \frac{2R_1R_2}{C^2-(R_1-R_2)^2} + \ln\frac{C^2-(R_1+R_2)^2}{C^2-(R_1-R_2)^2}\right]$$



Hogg-Healy-Furstenau[4]

$$V_{elec} = \pi\varepsilon\varepsilon_0 \frac{R_1R_2}{R_1+R_2}\left[(\psi_1+\psi_2)^2 \ln\left(1+e^{-\kappa d}\right) + (\psi_1-\psi_2)^2 \ln\left(1-e^{-\kappa d}\right)\right]$$

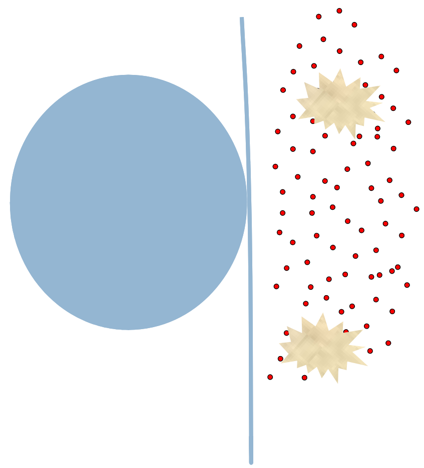


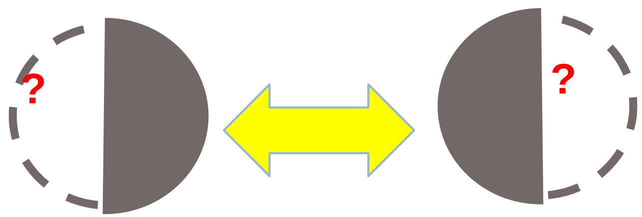
Mean field theory, point charges

[3] Hamaker, Physika 4 (1937) 1058
[4] Hogg, Trans. Faraday Soc. 62 (1966) 1638

Effect of size & size distribution

- Electrostatic interaction / double layer
  - HHF[4] only "looks" at the charges on one side of the particle, assumes that other side is to far away for influence





[4] Hogg, Trans. Faraday Soc. 62 (1966)

Superparamagnetic properties and colloidal particles

- (Superpara-) Magnetic properties
  - With small sizes & ferromagnetic material, superparamagnetic behavior appears
  - Rotating magnetic moment still present, with characteristic flipping time τ_N:
    - For iron oxide, $d=15$ nm, $T = 300$ K, $\boldsymbol{\tau_N \approx 460}$ **µs**
    - For iron oxide, $d= 5$ nm, $T = 300$ K, $\boldsymbol{\tau_N \approx 1.6}$ **ns**
    - The 5nm particle switches side ~ $3*10^5$ times while the 15nm particle switches once!

$$\tau_N = \tau_0 e^{KV/k_B T}$$

Effect of size & size distribution

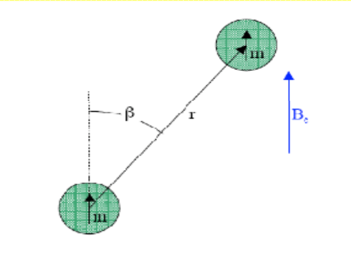
- According to Dobson & Gray[7], residual magnetic force without ext. magn. field
  - Similar to vdW force
  - Effect expected to be enhanced by particle size distribution
    - Small particles “see” bigger ones as quasi-permanent magnets

Alignment due to dipole-dipole interaction

$E_{d\text{-}d} = \mu_0/4\pi\, m^2/r\,(3\cos^2\beta - 1)$

B < 54.7° : attraction

B > 54.7° : repulsion

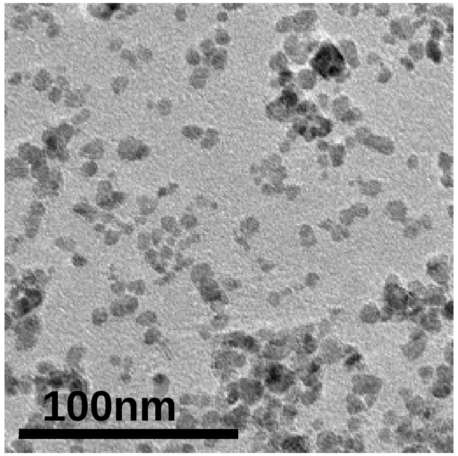


- According to Müller[8], SPION with d_{TEM} <7.5 nm should not really be able to aggregate in aligned manner as magn. forces too small compared with V_{elec} & V_{vdW} & thermal energy

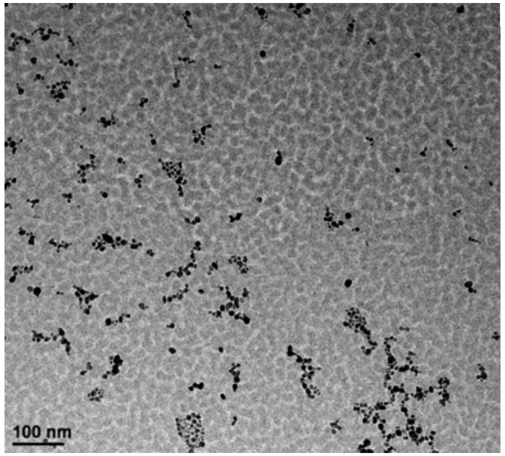
[7] Dobson, Arxiv (2009)
[8] Müller, Master Thesis LTP (2010)

Superparamagnetic particles

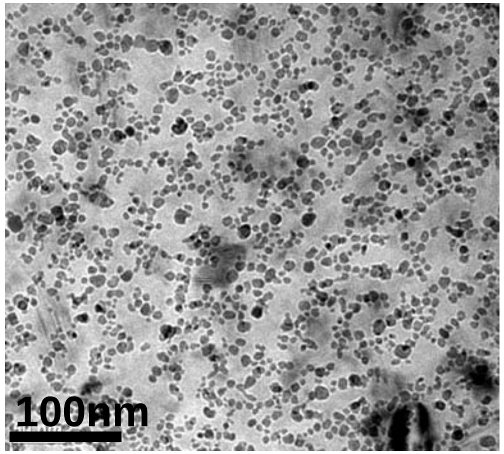
TEM on amorph. carbon grid	cryo-TEM on holey-carbon grid	cryo-TEM on holey-carbon grid



$H_{ext} = 0$



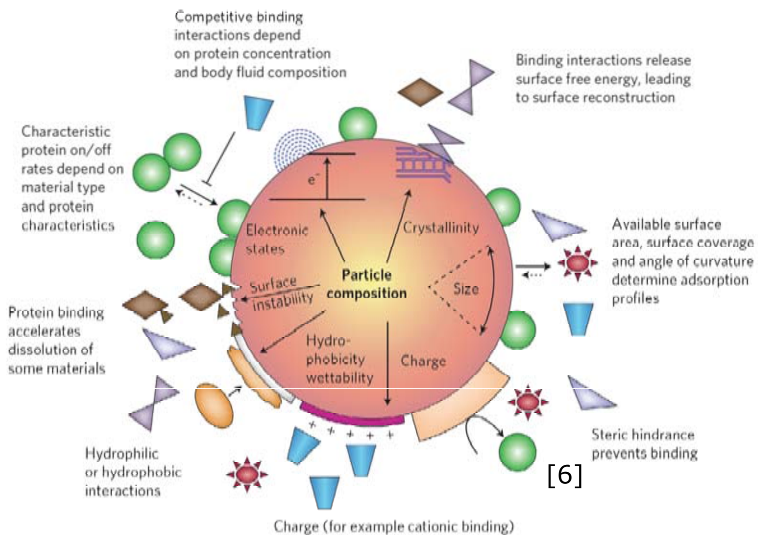
$H_{ext} = 0$



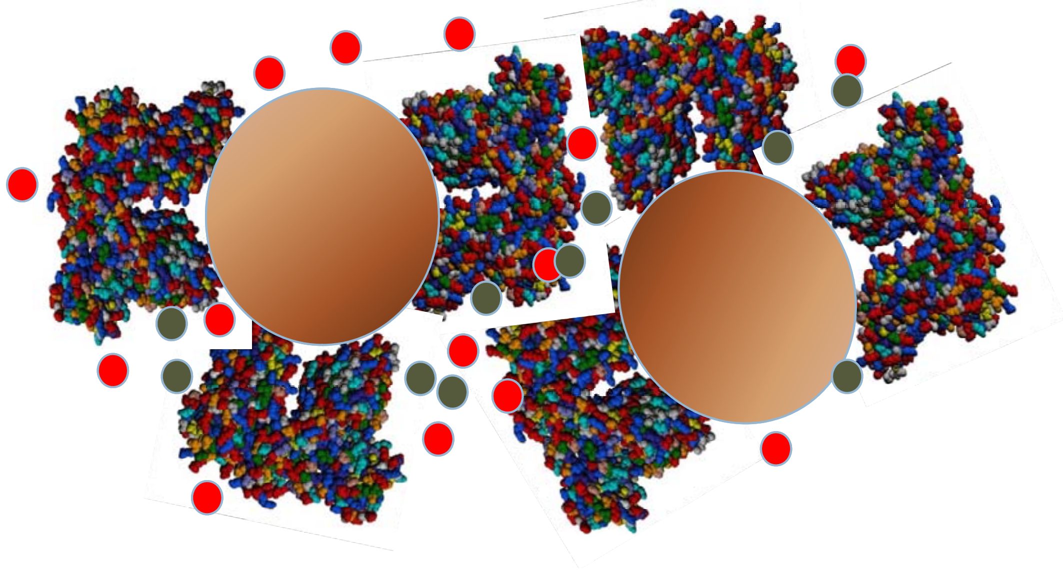
$H_{ext} > 0$

- Small aggregates and single particles
- Chain-like structures of cryo-TEM observed small aggregates
- Longer chains & orientation in magn. field

Nano-Particles and Proteins



[6]

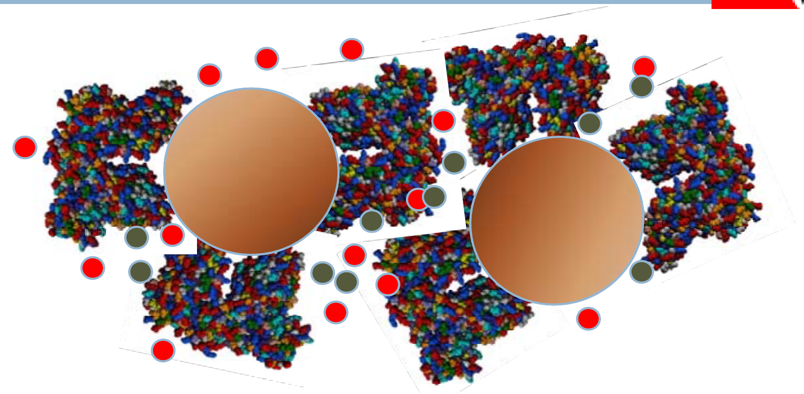


Velegol[5] proposes a new, *extended and extendable* potential, still under the assumption that contributions are additive:

$$\Phi = \Phi_{vdw} + \Phi_{es} + \Phi_{dep} + \Phi_{bio} + \Phi_{solvophobic} + \Phi_{solv} + \Phi_{steric} + \Phi_{\mathrm{magn}}$$

[5] Velegol, J. Nanophoton. 1 (2007) 012502
[6] Nel, Nature Mater. 8 (2009) 543

Van der Waals Attraction

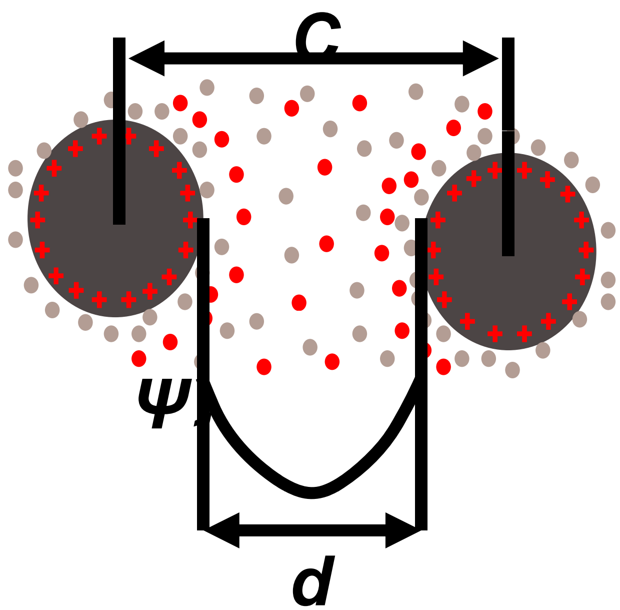


Hamaker constants are not trivial to determine anymore, could be that at some point quantum mechanical effects appear. It can be expected that retardation effects will be important at smaller interparticle distances for smaller particles, as the dipole fluctuation inside smaller particles is believed to be faster than for larger ones.

It is expected that van der Waals type interactions appear not only between particles, but also between particles and biological species . Also, the Hamaker constant will become less and less trivial to determine, as local distribution of macromolecules will change very heterogeneously the polarizability of the fluid in between two particles.

Electrostatic interaction

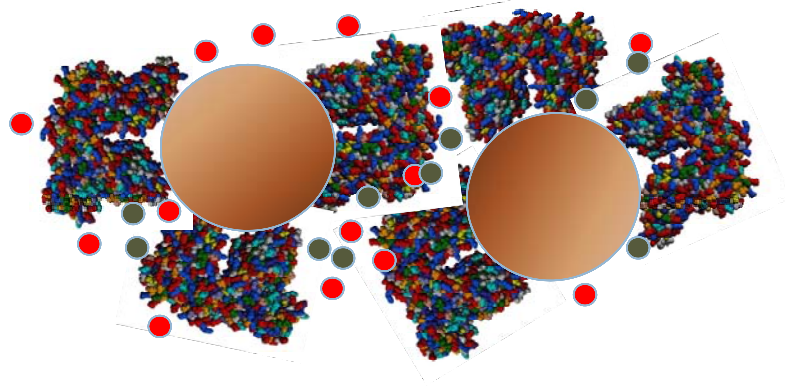
For small sizes (or bigger sizes and low ionic strength), κa can be (very much) smaller than 10. In other terms, Hogg-Healy-Fürstenau (HHF) theory is not believed to be correct in the nano-size range



Screening/shielding of the electrostatic field making typically the electrostatic interaction less important in complex (biological) media, comes more and more important at high ionic strength.

Steric stabilisation

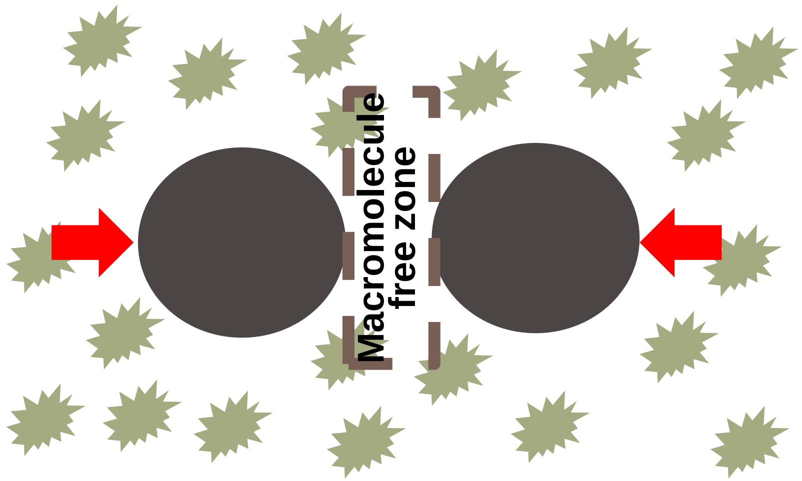
As particles become smaller and smaller, the size ratio between particles and macromolecules starts to matter. Especially, when the particles are about the same size than the molecules, the question of mechanism of adsorption and steric hindrance have to be rethought.



In complex media, steric interaction will probably be the leading repulsive force between particles (as electrostatic repulsion is probably very strongly reduced due to squeezing of the ionic double layer).

Depletion

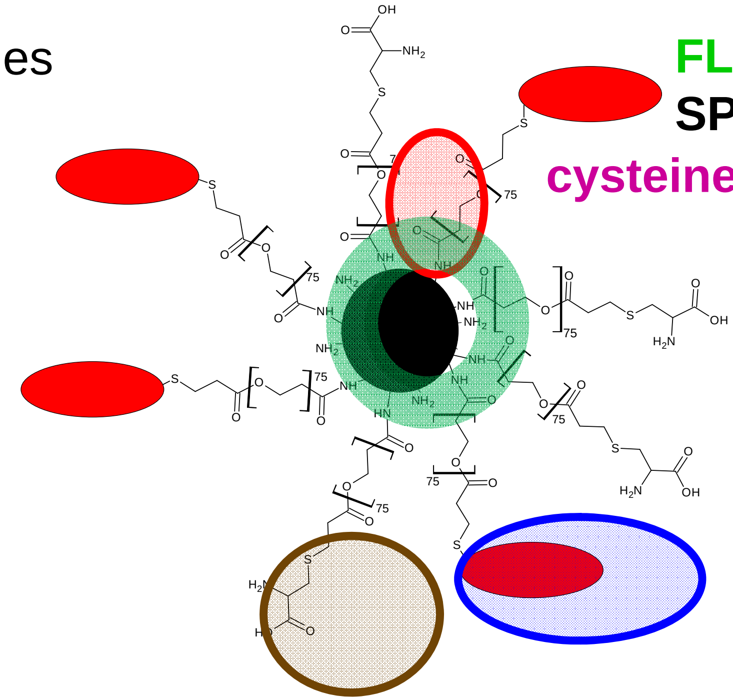
Depletion becomes important in media with (high) content of solvated macromolecules. When macromolecules are in the same size range than the particles, it will be difficult to determine where the effect is still a "depletion", or when we have steric hindrance because of adsorption of the molecules, or even when these molecules will have to be treated as an other type of particles in suspension.



Multifunctional Core-shell Nanoparticles

Highly complex compounds:

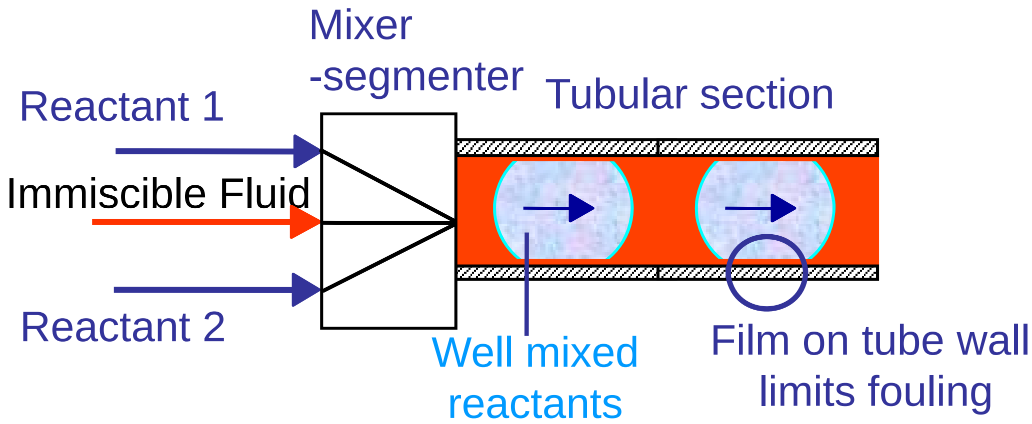
- Core: magnetic and/or fluorescent materials
- Shell I: inorganic materials (SiO_2….)
- Shell II: functionalizable layer (COOH, SH, NH_2..)
- Therapeutic and/or imaging payload
- „Stabilizer“
- Targeting moieties

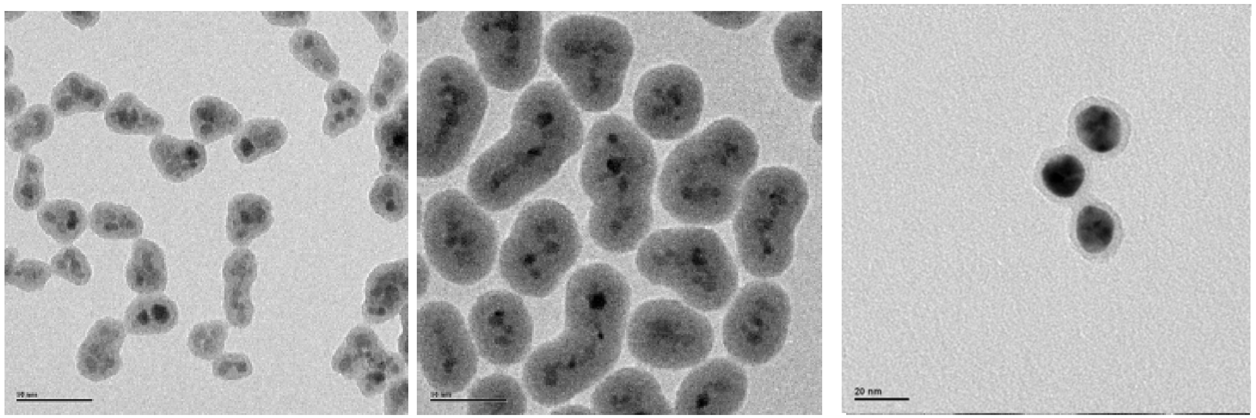


APTES-SPIONs
(~25-35 nm; **+ 33 mV**)
↓
PEG-APTES-SPIONs
1 hr recirculation
(~70 nm & **-20 mV**)
↓
FL-protein-PEG-APTES-SPIONs
↓
cysteine-protein-FL-PEG-APTES-SPIONs
(~80 nm & **-13 mV**)

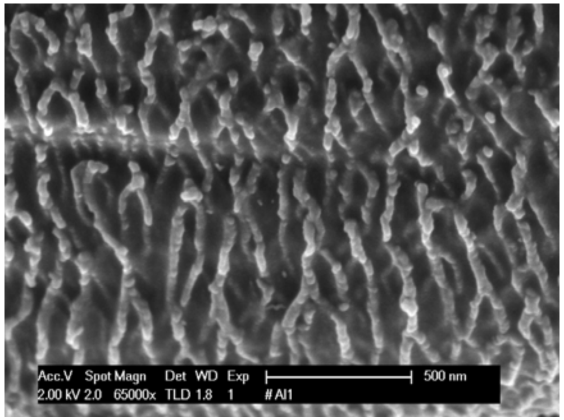
APTES: aminopropyltriethoxysilane

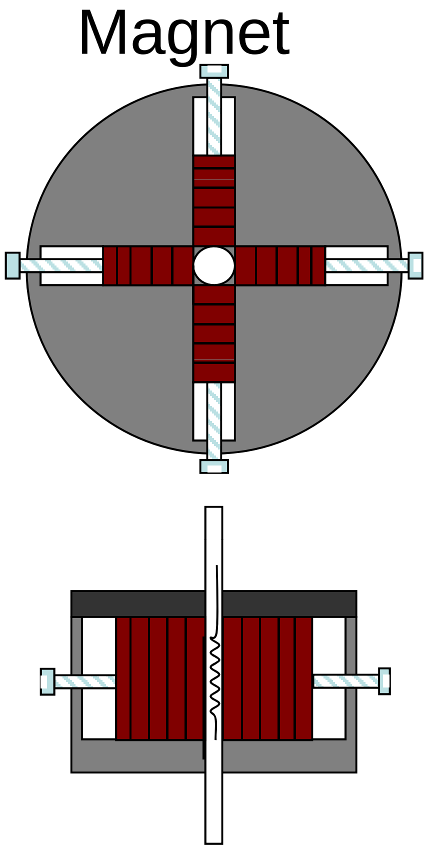
Segmented Tubular Flow Reactor for inorganic coating (Fe_2O_3/SiO_2)





Magnetic fixed bed reactor (Lab scale) for organic surface modification

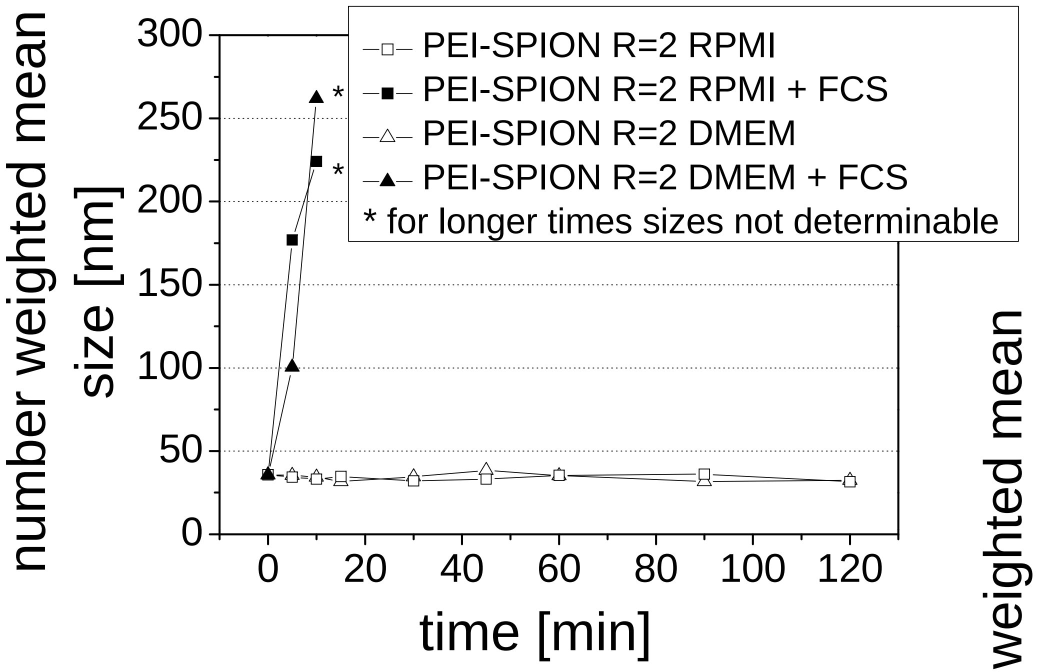




SPION alignement : mean distance 100 nm

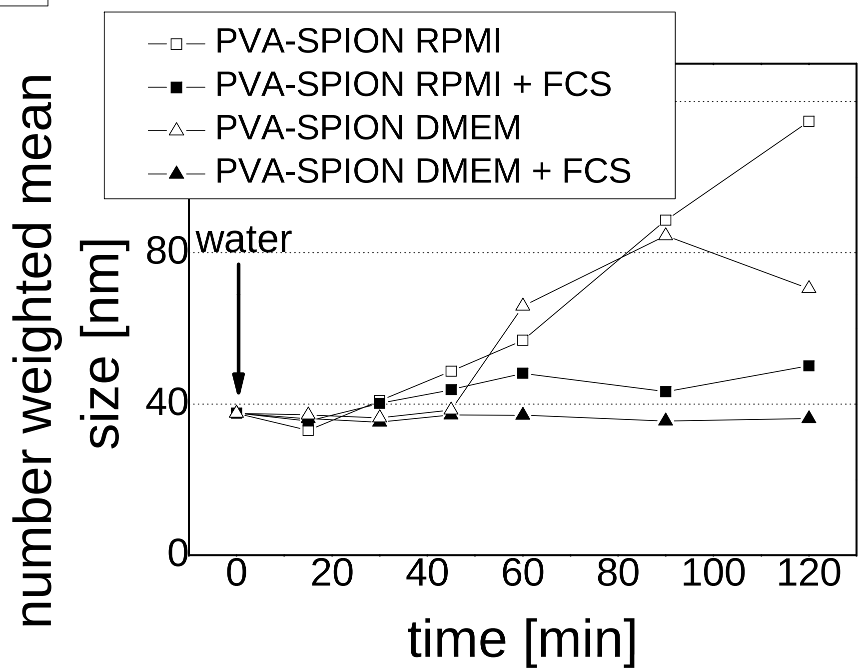
Particles in biological media:

Colloidal stability = f(coating and of protein adsorption)



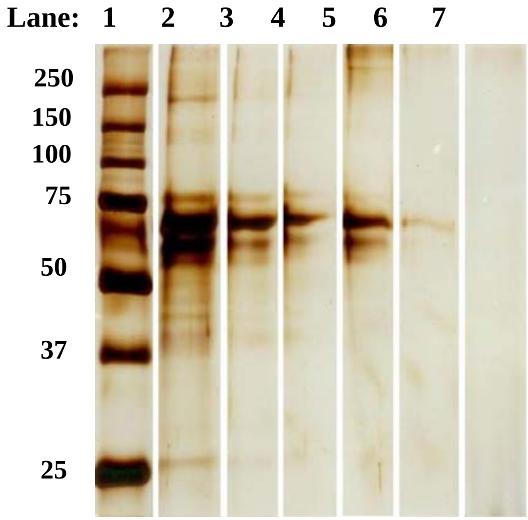
PEI-SPIONs

PVA-SPIONs

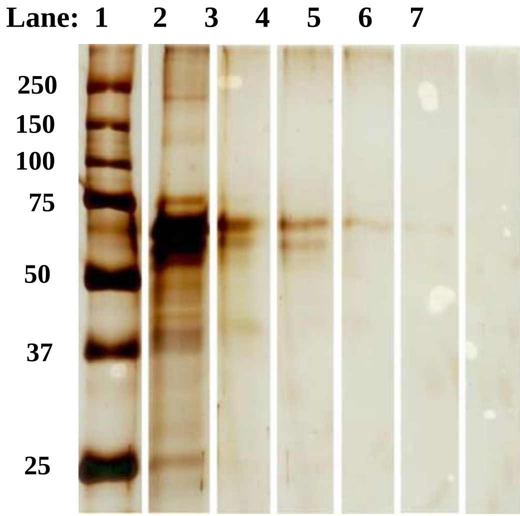


Protein adsorption (PVA-coated SPION)

positively charged SPION

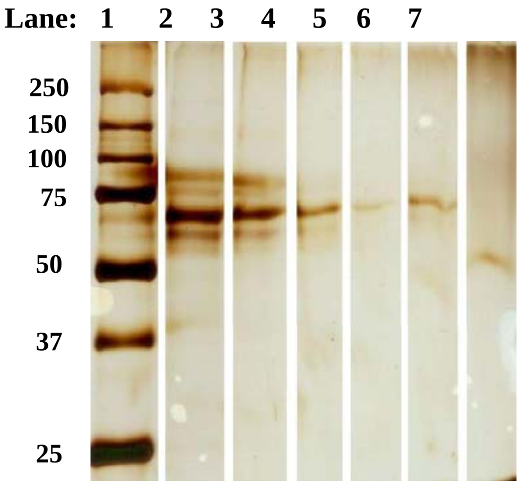


b. Neutral SPION



Lane1: Marker (kDa)
Lane 2: Washing
Lane 3: 0.2 M KCl
Lane 4: 0.5 M KCl
Lane 5: 1.0 M KCl
Lane 6: 2.0 M KCl
Lane 7: Tightly bound protein on nanoparticles

c.



negatively charged SPION

Incubation of particles with serum. The serum to particle surface ratio was fixed at 2.8 ml per m^2 of particle.

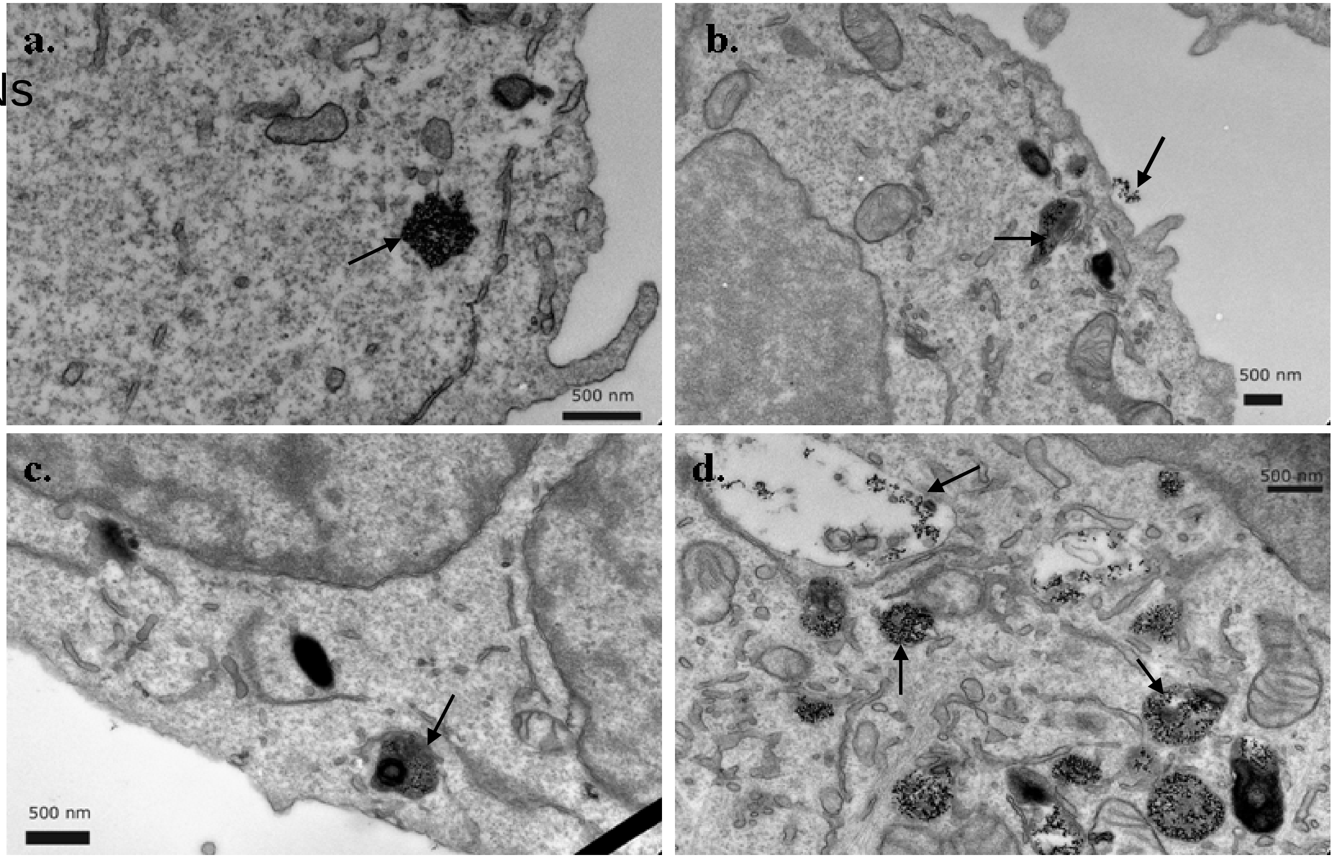
HeLa cells after incubation with SPION

naked SPIONs

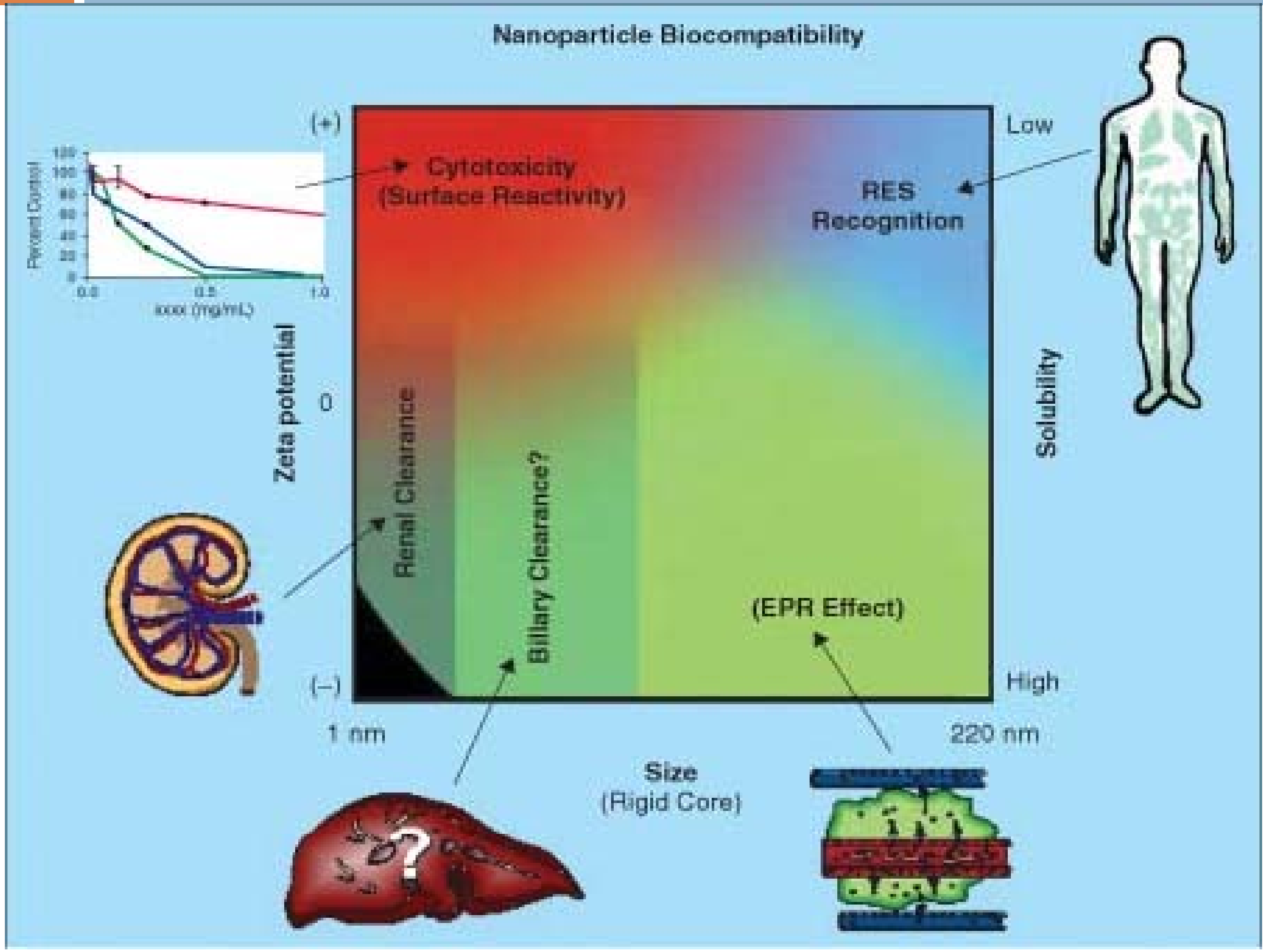
Negatively charged

Neutral charged

Positively charged



Biocompatibility



retikulo-endotheliales System or
The **mononuclear phagocyte system**

Enhanced Permeability and Retention (**EPR**) **effect**

A. Neel, Nature materials

Outlook

- Development of a **simulation tool** (Monte Carlo) for predicting the structure of ionic double layer / resulting repulsive forces between the particles
- Addition of vdW forces and determination of validity of classical DLVO for nanosized systems in simple media
- Development of a simple experiment set that enables to characterize best colloidal stability
- Investigation in hetero-particle systems as model for biomolecules

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