Multifunctional magnetic nanoparticles for biomedical aplications

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Outline

- Interest of magnetic nanoparticles (MNP) in biomedical applications
- A word about size: what makes the difference?
- The magnetic functionality
- Designing magnetic nanoparticles
- Multifunctional nanoplatform: MRI, magnetic hyperthermia, fluorescence, antibodies, thermometry
- Toxicological results
- Hemocompatibility

Interest of Magnetic Nanoparticles in Biomedical Applications

- ✓ Magnetic functionality => magnetic fields penetrate human tissues
- ✓ Strong magnetic moments => can affect relaxation times of nearby protons
- ✓ They obey Coulomb's law => can be controlled at distance
- ✓ They can convert energy into heat from an alternating magnetic field
- ✓ Controllable sizes (≥ 100 nm - ≤ 10 nm) and shapes (spheres, needles, beads)

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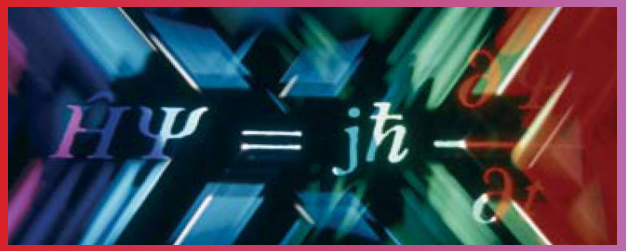
Is *nano* just a matter of size?

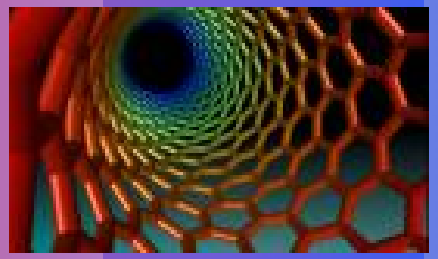
From the physics point of view Nano- is the interface separating the atomic from the macroscopic scales

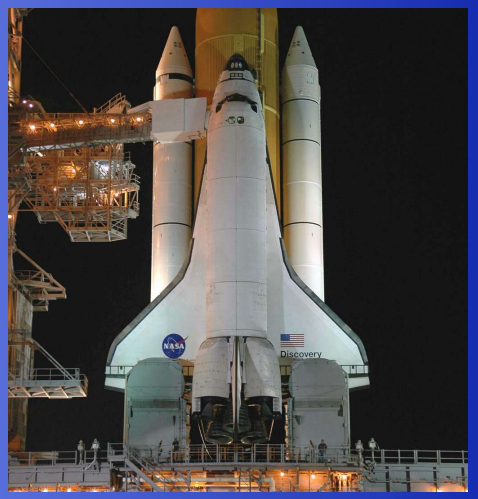
Atomic World ruled by the laws of Quantum Mechanics

NANO WORLD

Macroscopic World as we perceive it, ruled by Classical Mechanics and Electromagnetism laws







In the atomic world there are properties whose critical lengths are at the nano-scale

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Is *nano* just a matter of size?

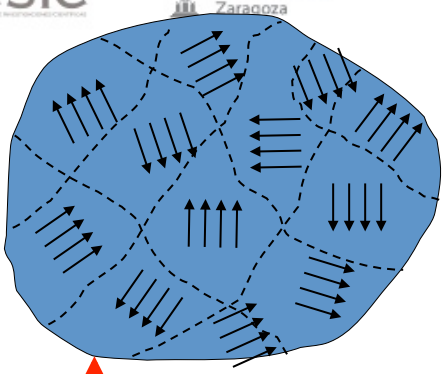
At the scale below a critical length new properties arise that can give rise to new materials and new applications

Critical lengths such as

- **one electron Fermi wavelength**
- **exciton Bohr radius**
- **single magnetic domain length**

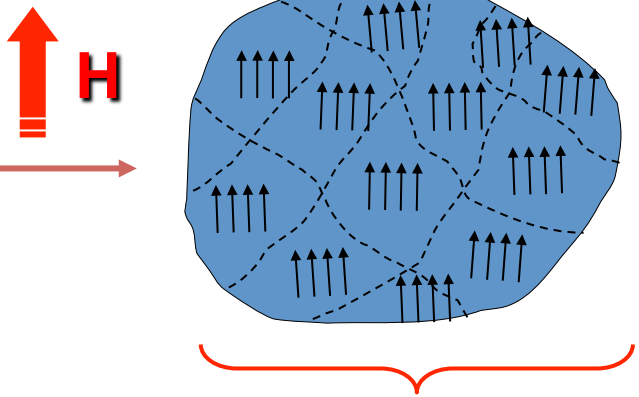
lead to Quantum Dots and Superparamagnetic particles

Magnetic Particles => Multidomain

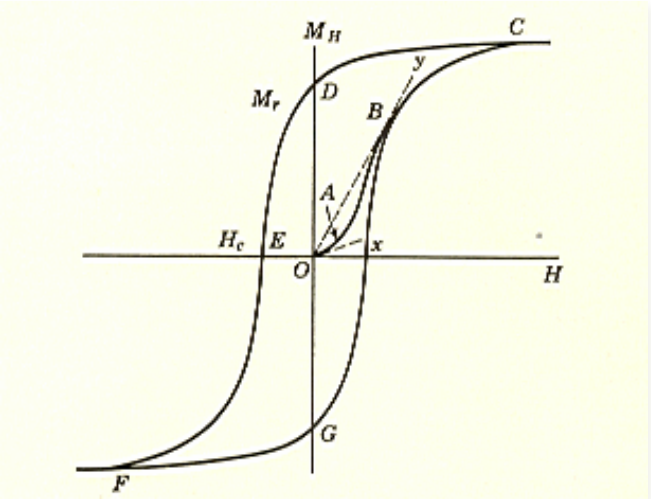


Magnetic domains

+ Magnetic field H



Magnetisation
$M = m/V_m$

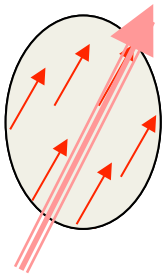


Hystheresis cycle

Single-domain particles => superparamagnets

When the size of a particle is smaller than the minimum allowing the formation of domains (≈ 20 - 30 nm), it becomes single-domain and

superparamagnetic



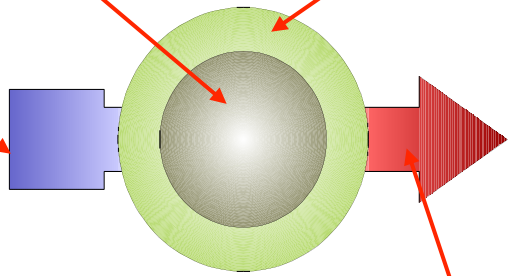
Superparamagnetic particles are hardly attracted by magnets, and the smaller the harder

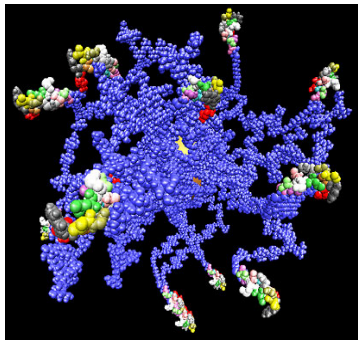
The magnetic functionality

Magnetic nucleus

Protecting coating, anchoring element

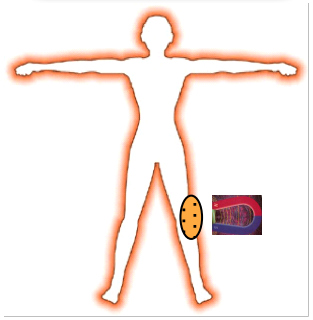
Functionalized tail for therapeutic uses: drugs, enzyms, etc.



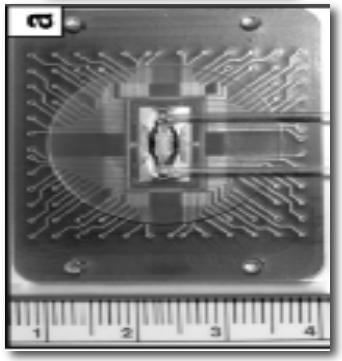


Biological vector

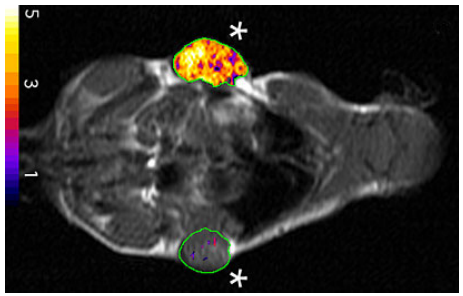
DRIVING



SENSING



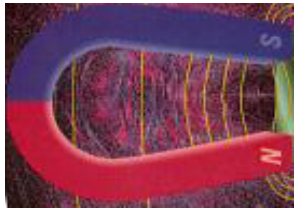
IMAGING

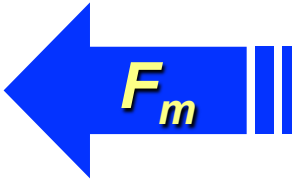


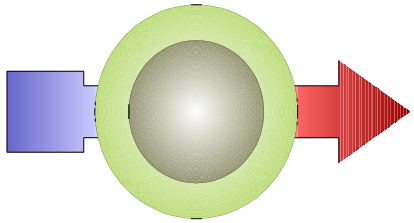
HEATING

Magnetic force-driven applications

DRIVING







Magnetic force F_m

$$F_m = (m \cdot \nabla) B$$

A force will be experienced provided there is a field gradient. More intuitively, we can relate F_m to the differential of the magnetostatic field energy density $B \cdot H/2$:

$$F_m = V_m \, \Delta\chi \, \nabla (B \cdot H/2)$$

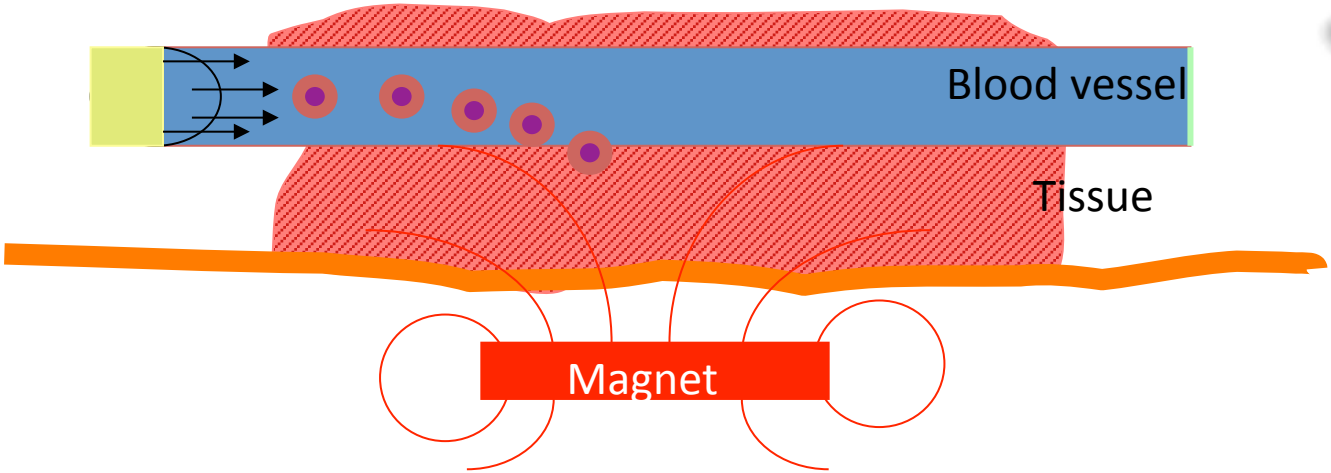
Thus if $\Delta\chi > 0$, F_m acts in the direction of the steepest ascent of the energy density scalar field.

Magnetic force-driven applications

DRIVING

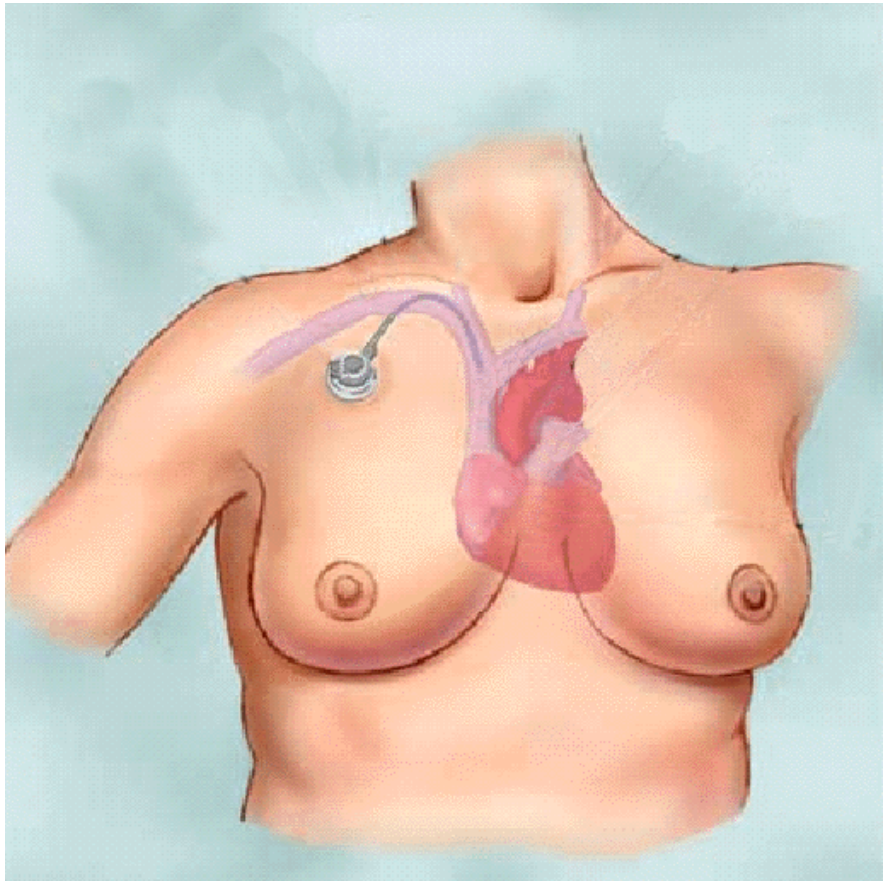
Targeted drug delivery

i.e. retaining drugs in areas of low blood irrigation or easily accessible by magnetic forces.

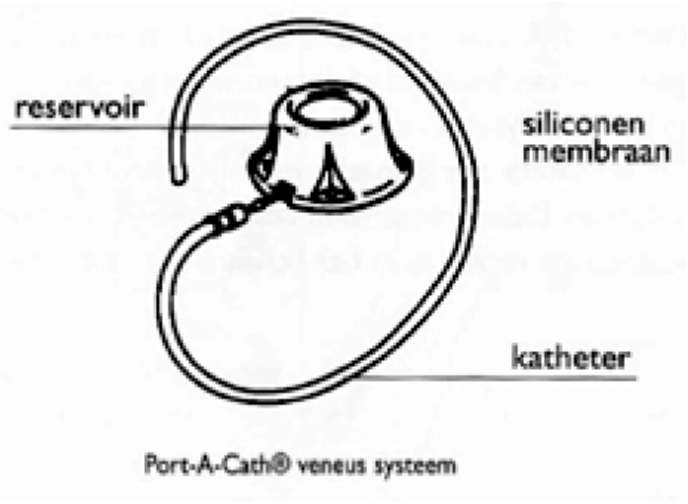


Nanobolas para el desarrollo de un biosensor

SENSING

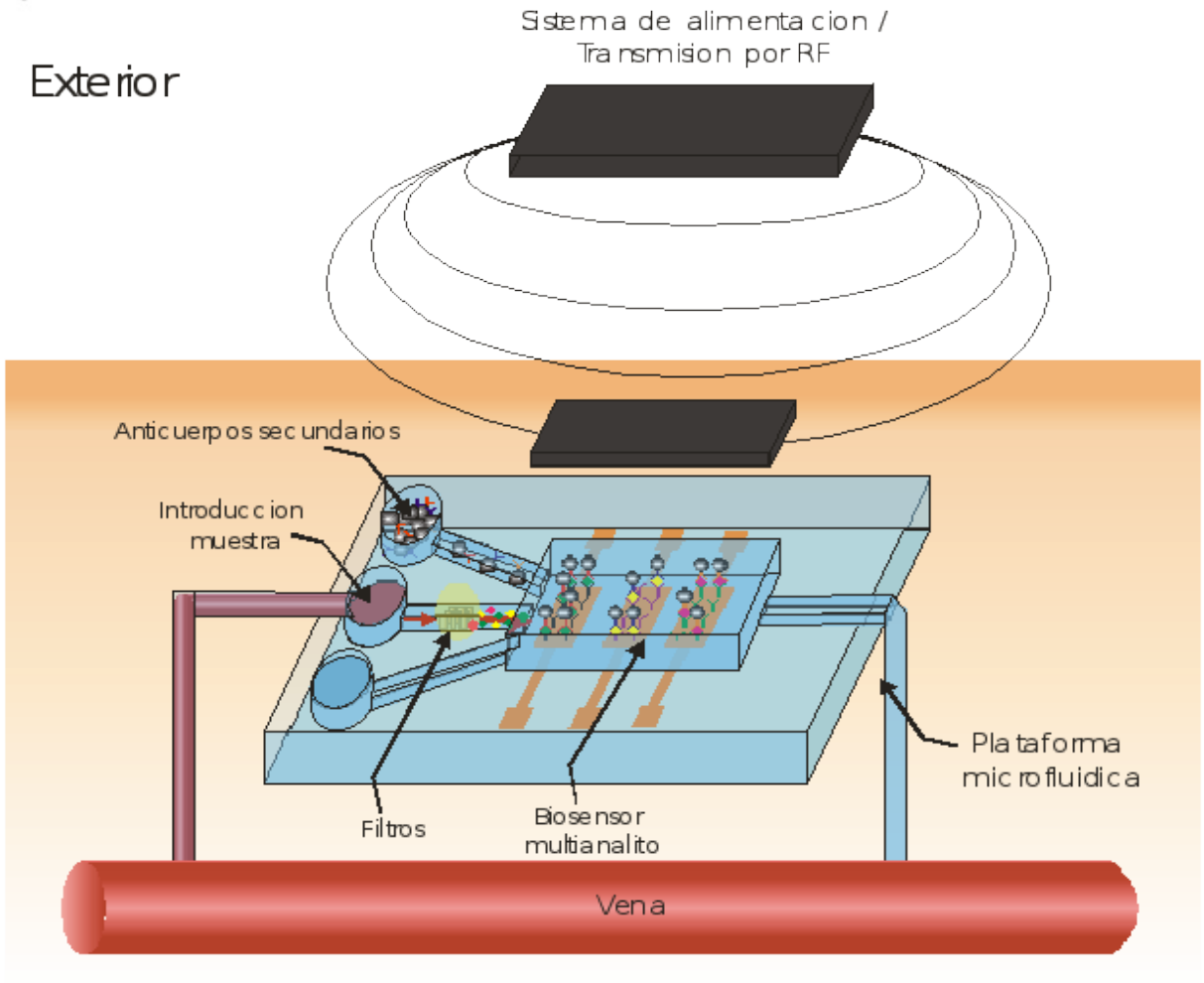


Esquema de implantación del biosensor



Nanobolas para el desarrollo de un biosensor

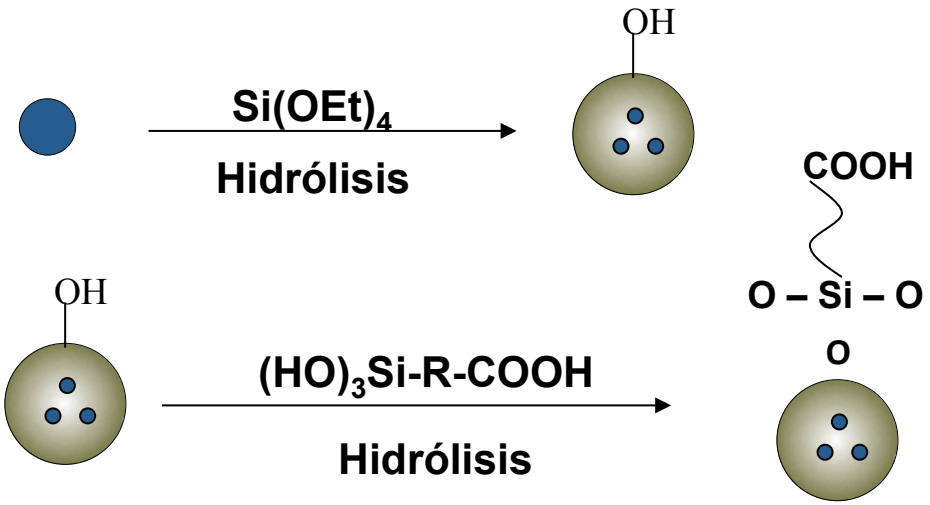
SENSING

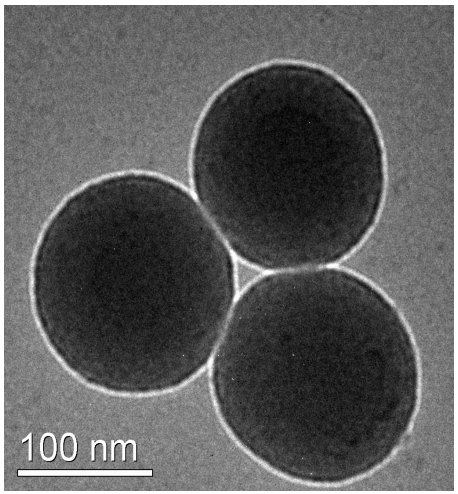


Nanobolas para el desarrollo de un biosensor

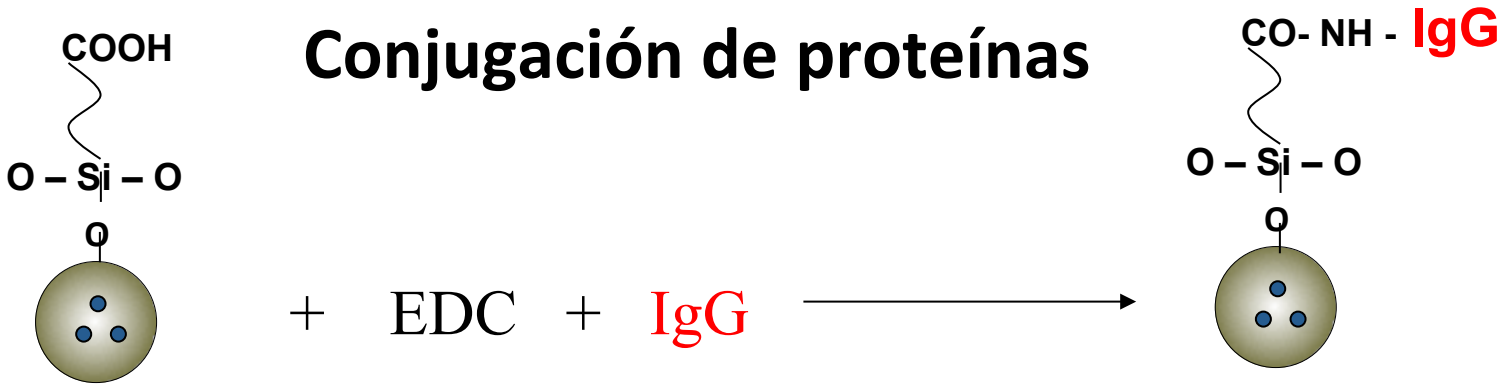
SENSING

Funcionalización de nanobolas



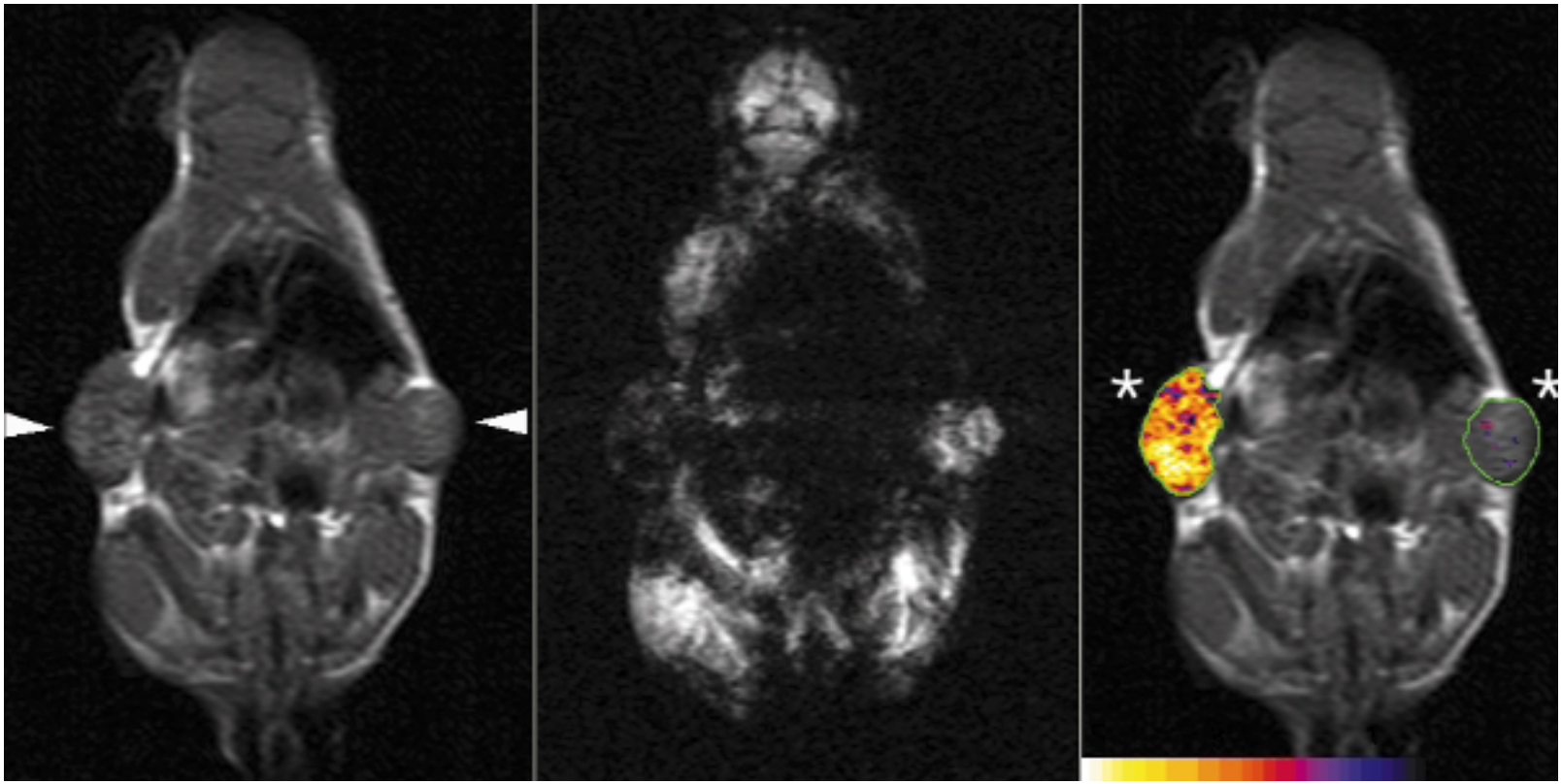


Conjugación de proteínas



Magnetic nanoparticles as contrast agents for MRI

IMAGING



R. Weissleder et el., Nature Med. **6**(2000)351

Magnetic nanoparticles as contrast agents for MRI

IMAGING

Typical clinical MRI
H = 1.5 Tesla

MRI intensity (and therefore the contrast) depends on:

Intrinsic parameters:

- local proton density N(H), (water, fat, …)
- relaxation times, T_1, T_2
- magnetic susceptibility differences

Extrinsic parameters:

- magnetic field
- timing of the pulse sequences (TE, TR)
- contrast agents (CA)

Magnetic nanoparticles as contrast agents for MRI

IMAGING

MRI signal is $s(t) = N(H)\, e^{-TE/T2^*} (1-e^{-TR/T1})$

With CA it is possible to change the nuclear relaxation times (more efficient than protons' density differences) **and so** to obtain

a better image contrast and pathology evidence

Magnetic nanoparticles as contrast agents for MRI

IMAGING

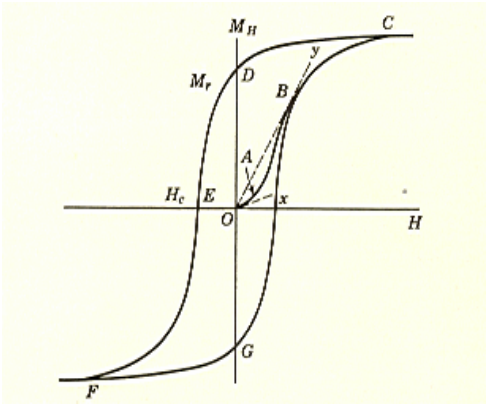
- Huge magnetic moments as compared to Gd chelates
- Proton relaxation is affected by the large magnetic field heterogeneity in the vecinity of the particles
- Can induce >10 fold increase in proton relaxivities
- Shortening of relaxation times, particularly T_2 (*negative contrast*)
- Good spacial resolution, of the order of single cell detection (10 - 50 μm^3)

Magnetic heating

HEATING

Magnetic systems can convert energy into heat under the effects of an alternating magnetic field

- **inductive heating (eddy currents)**
- **hysteresis losses**



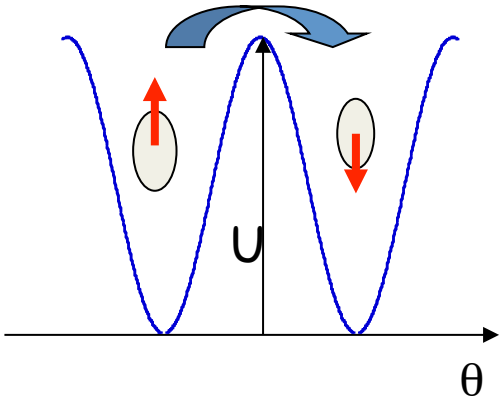
$$P_{FM} = -\mu_0 f \oint H dM$$

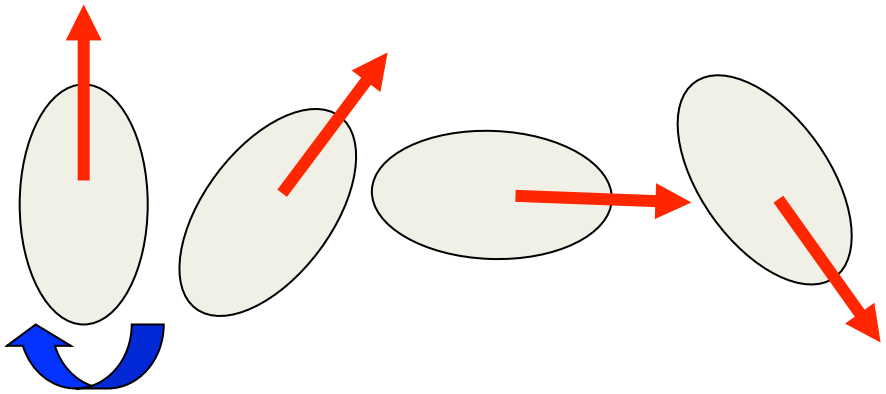
No relevant at low ac magnetic fields

Magnetic heating

HEATING

Superparamagnetic particles





Neél relaxation

Rotational Brownian motion

$$P_{SPM} = -\frac{1}{2}\mu_0 \chi'' \omega H_0^2$$

Magnetic heating

HEATING

Biological limitations

$$P_{SPM} = -\frac{1}{2}\mu_0 \chi'' \omega H_0^2$$

50 kHz ≤ f ≤ 1200 kHz,

H < 15 kA/m

(H · f)max = 485 kHz·kA/m

After Brezovich [Med. Phys. Monograph **1988** *16* 82]

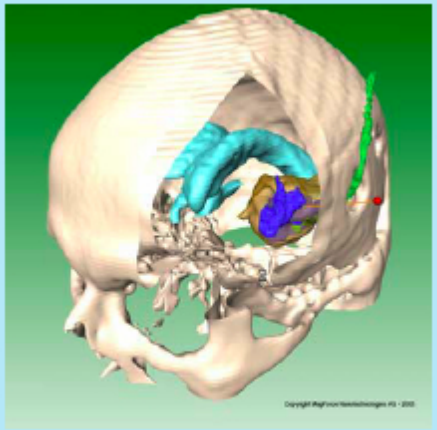
"*test person had a sensation of warmth, but was able to withstand the treatment for more than one hour without major discomfort*"

Standard values for comparative purposes: H = 4.85 kA/m, f = 100 kHz

Magnetic nanoparticles for heating

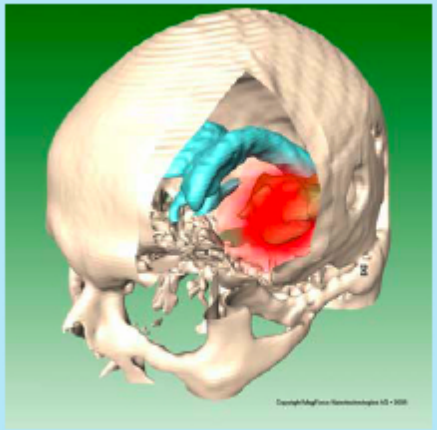
HEATING

Direct injection of MNP into the tumor: clinical phase II



Clinical Results

Phase I/II (feasibility) Study:
Nano-cancer therapy on Glioblastoma
(brain tumor trial) [3/2003-12/2004].



- All study patients (14) showed no side effects of the Nano-cancer therapy
  (2 patients fell asleep during therapy and had to be woken up after the therapy time of 60 min)
- On all study patients therapeutical temperatures of up to and more than 50°C inside the tumor were achieved.
- Indication for a local effectiveness.
- 1 patient in complete remission since 2.5 years.
- Rationale for a study of effectiveness.

Director of Study: Prof. Dr. med. Klaus Maier-Hauff,
ZE Neurosurgery, Bundeswehrkrankenhaus Berlin

Seite 9 / Dr. Dr. Dietmar Wechsler

Magnetic nanoparticles for heating

HEATING

Direct injection of MNP into the tumor: clinical phase II

Magnetic nanoparticles for heating

HEATING

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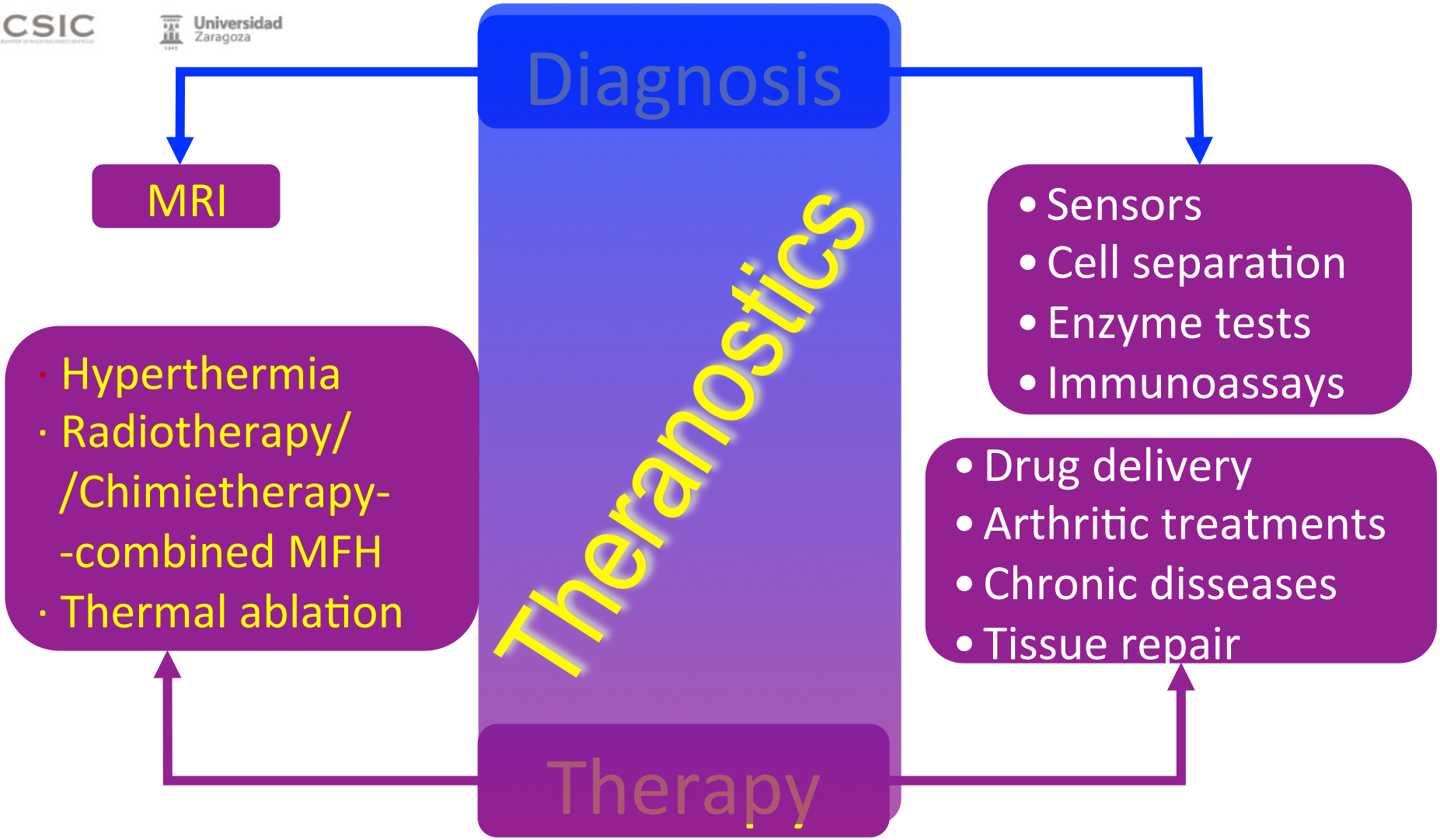
Direct injection is

- non-selective
- contaminates healthy tissues
- only valid for large tumors
- good heating efficiency as doses can be very high

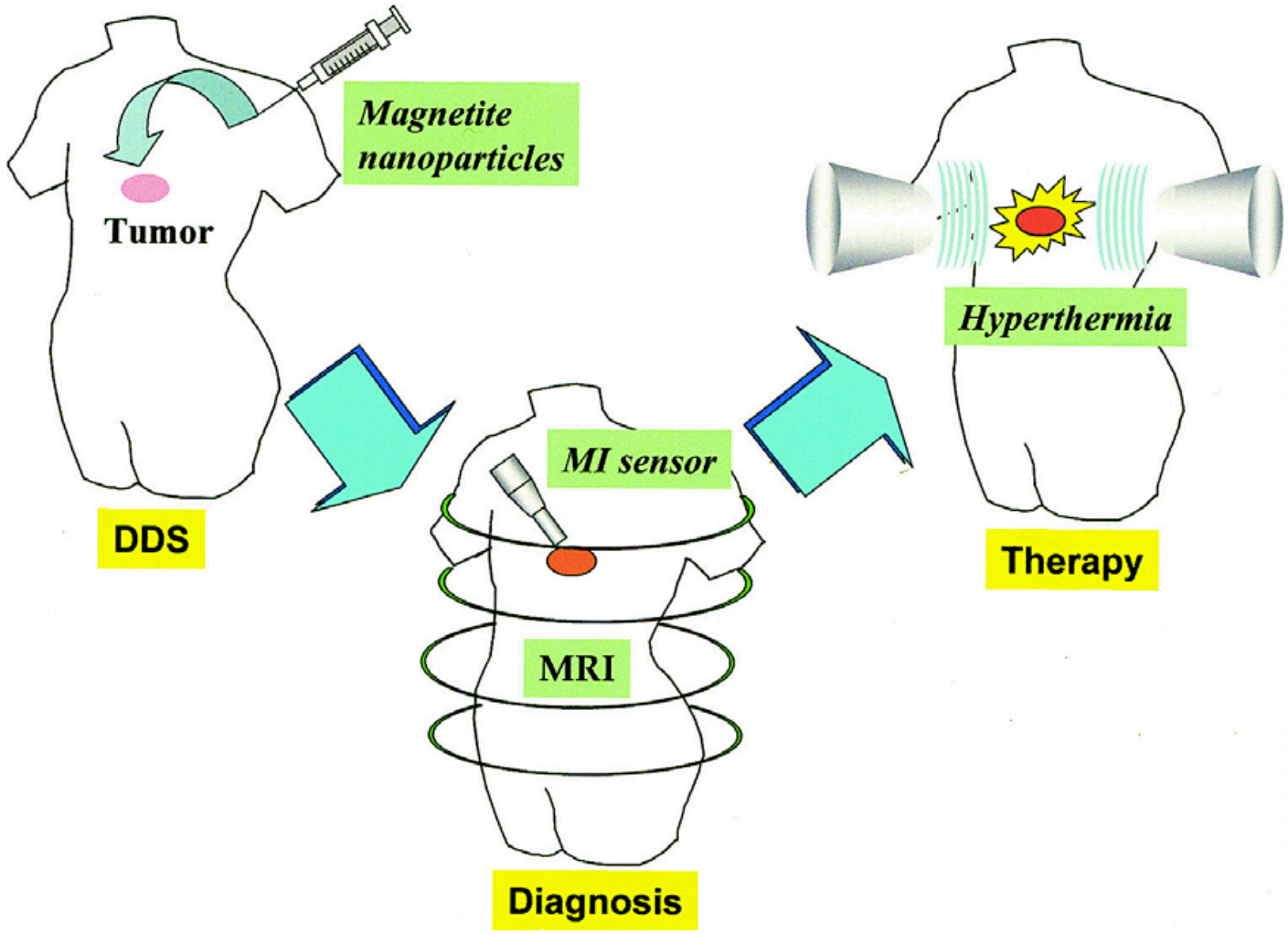
Biological vectorisation is

- highly selective (specific therapeutic target)
- heating is very localised
- valid for micro-tumors (preventive)
- heating efficiency is linked to the number of MNP recognaising the target

Biomedical Applications of Magnetic Nanoparticles



Biomedical Applications of Magnetic Nanoparticles



From A. Ito et al., J Biosc. and Bioengin. **100**(2005)1

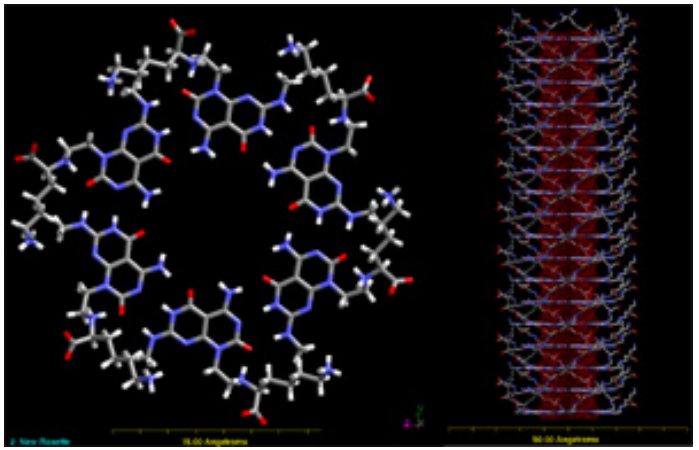
Multifunctionality

Fabrication methods

Top-down approach
It is the traditional approach: a nanosized material is obtained from a much larger source by grounding or other size-reduction actions.

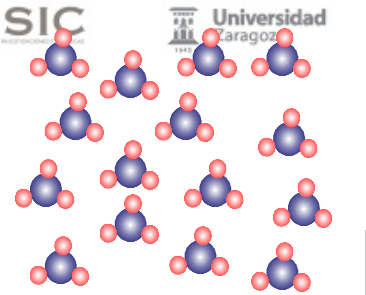
Top-down approach

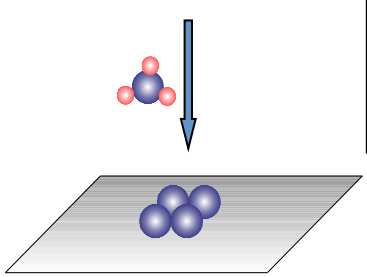
Bottom-up approach
Uses molecules or clusters and self-assemble and self-organised them like in a *Lego* toy to fabricate new materials. In many examples tends to replicate nature developing processes.

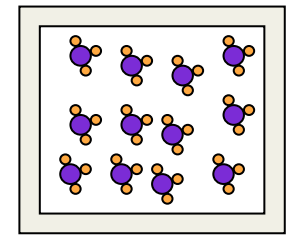


Molecular self-assembling

Bottom up preparation routes







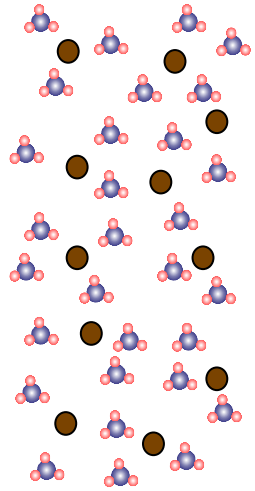
Controlled slow Transport

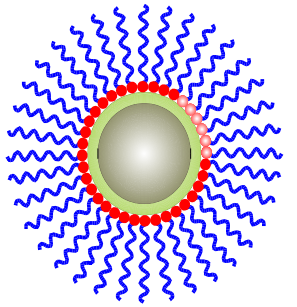
Extensive Nucleation

“Bottom-up”

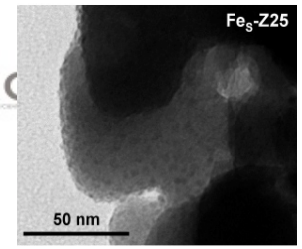
Restricted Space

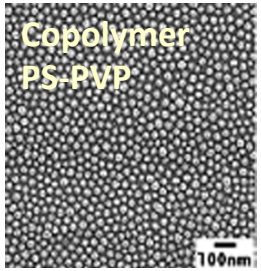
Growing Inhibition

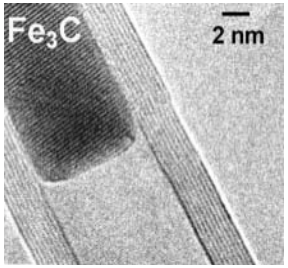


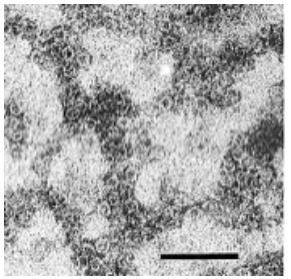


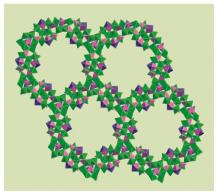
Restricted space: moulds



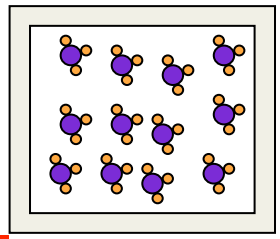




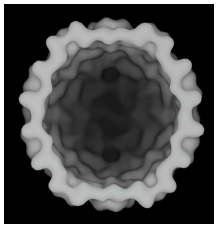


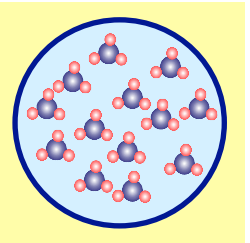


ceramic — polymer — zeolite — pores

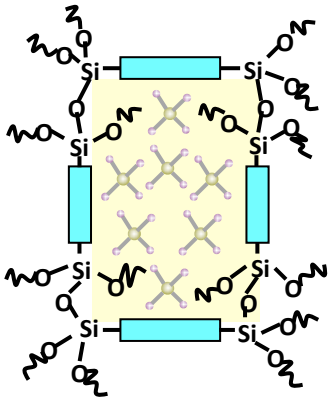


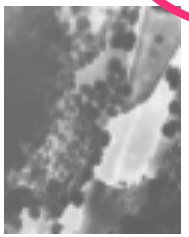
nanotube — liposome — capsules — proteine



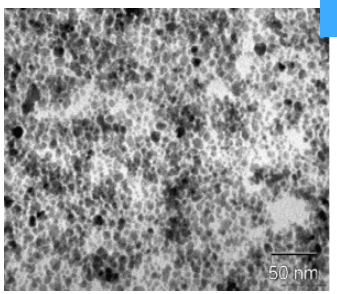


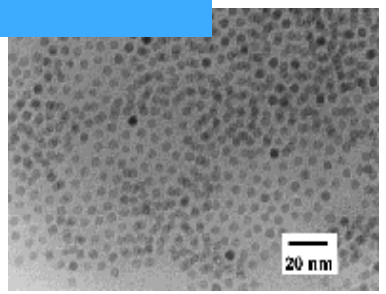
hybrid — gel — polymer



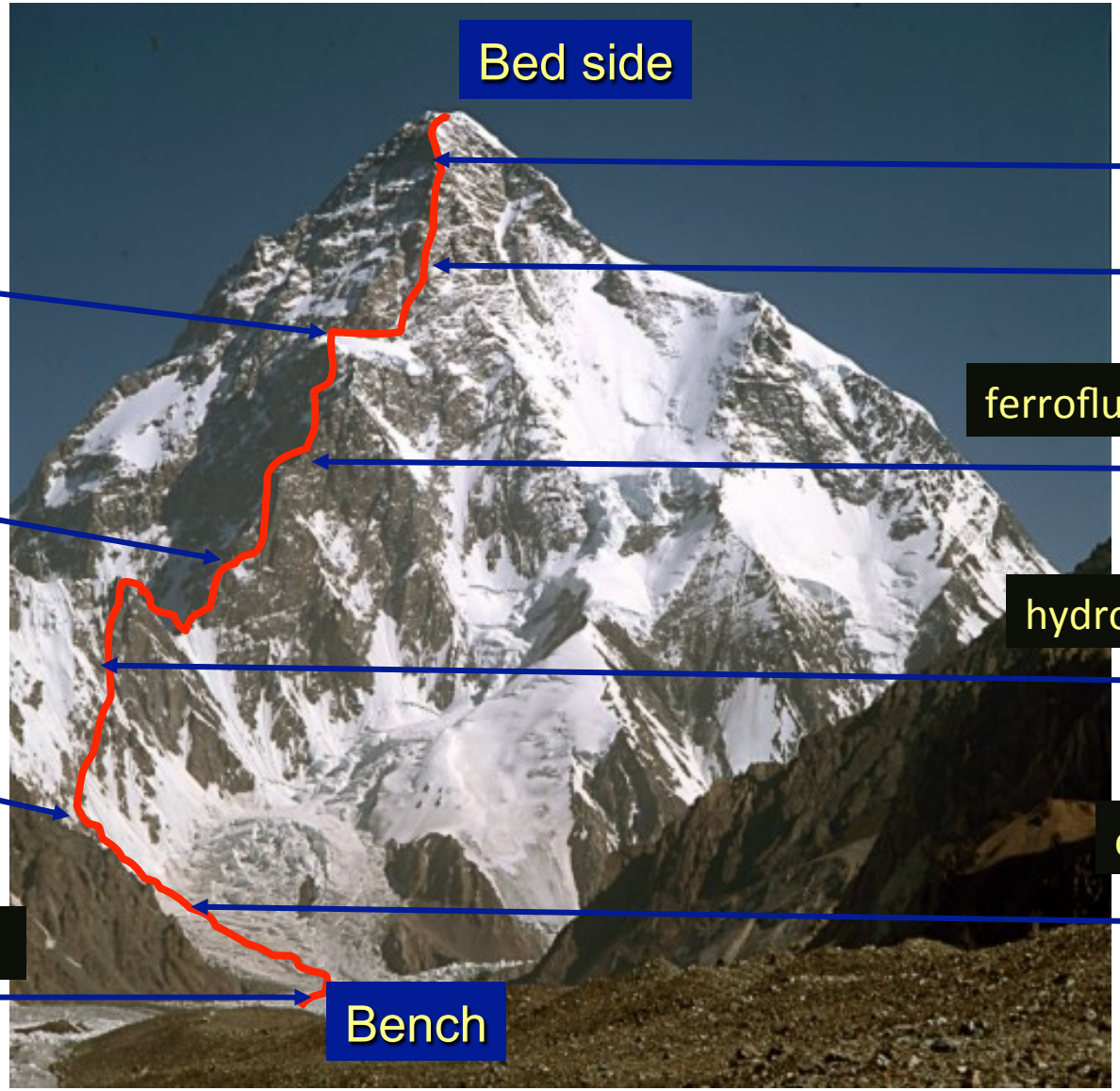


bubble — aerosol — micele — cavitation

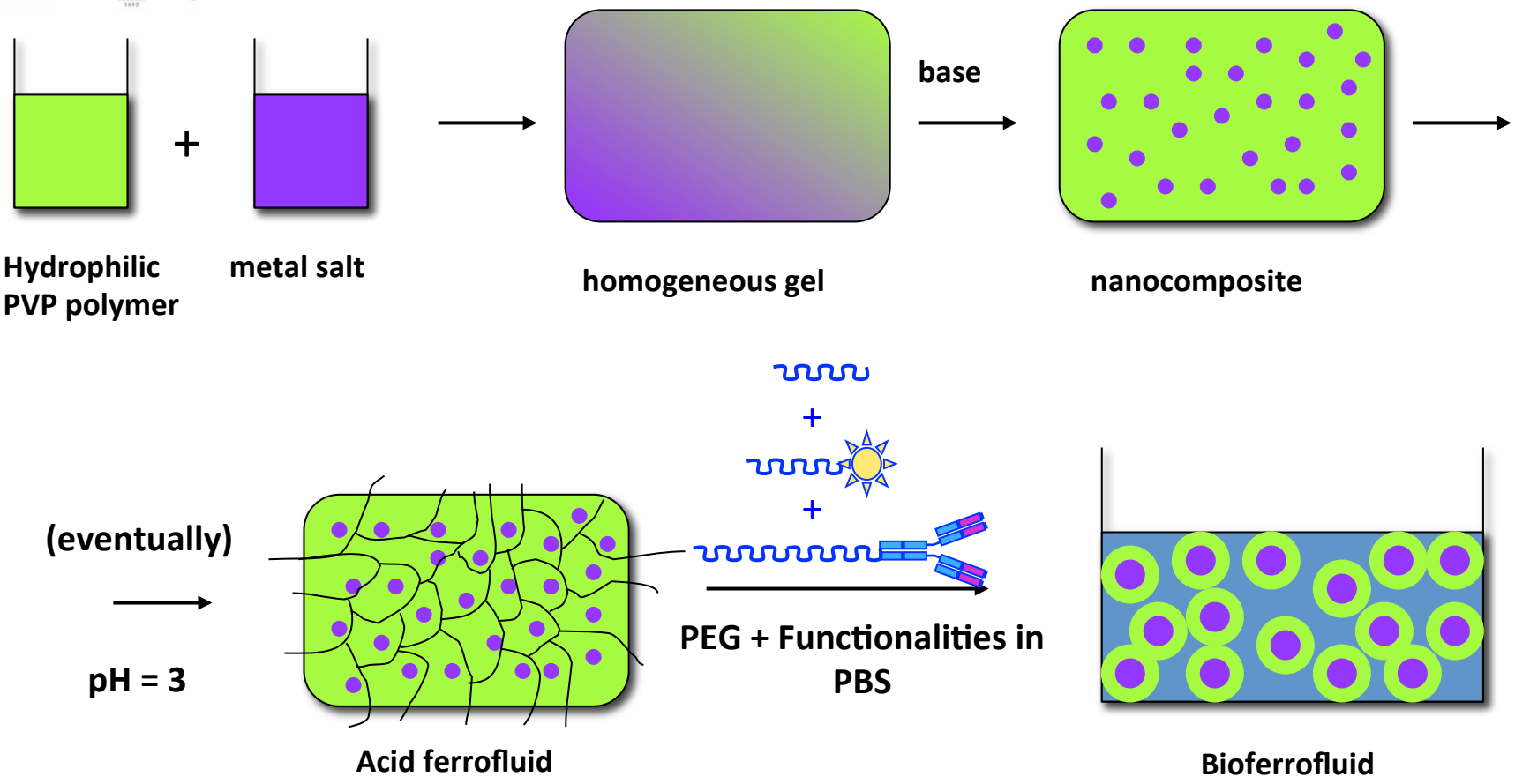




Designing MNP for Biomedical Applications



Tailoring MNP with polymers

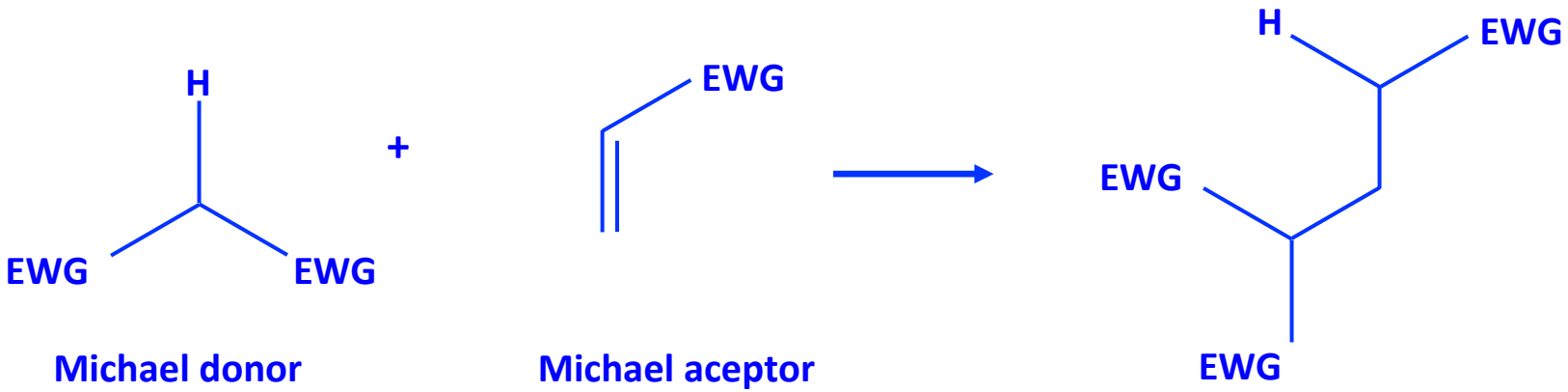


A. Millán and F. Palacio, Applied Organometallic Chemistry, **15**, 396-400 (2001)
A. Millán, F. Palacio et al., Acta Materialia **55**, 2201-9 (2007)
A. Millán, F. Palacio et al., Patent ES2308901B1

Tailoring MNP with polymers

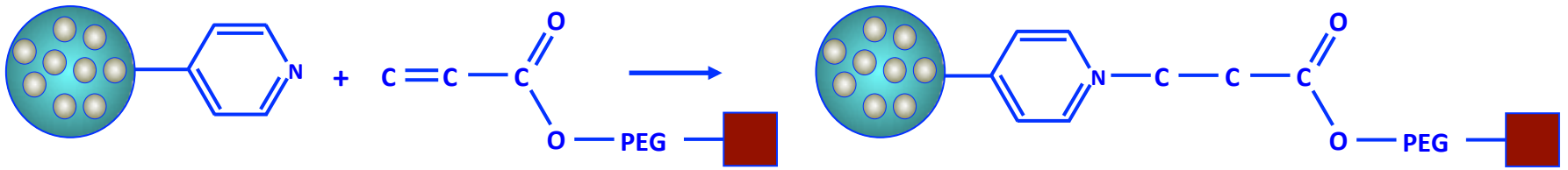
An easy route for functionalisation: Michael reaction

EWG: electron withdrawing group



Michael donor
carbanion o nucleophile

Michael aceptor
conjugated insaturated system



Physical or biological functionality

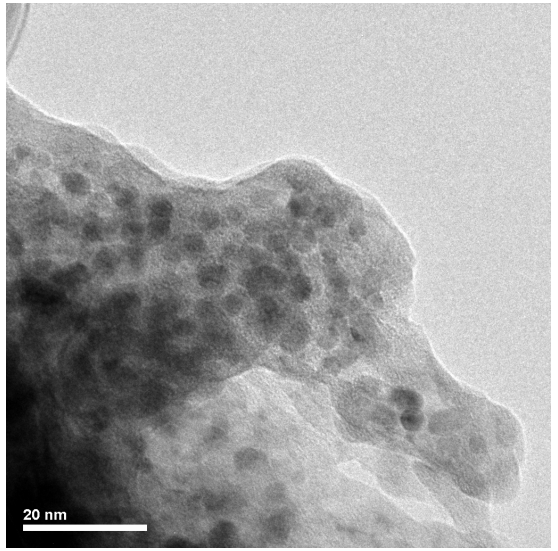
R. Piñol, A. Millán, F. Palacio et al., Patent P201031493

Tailoring MNP with polymers

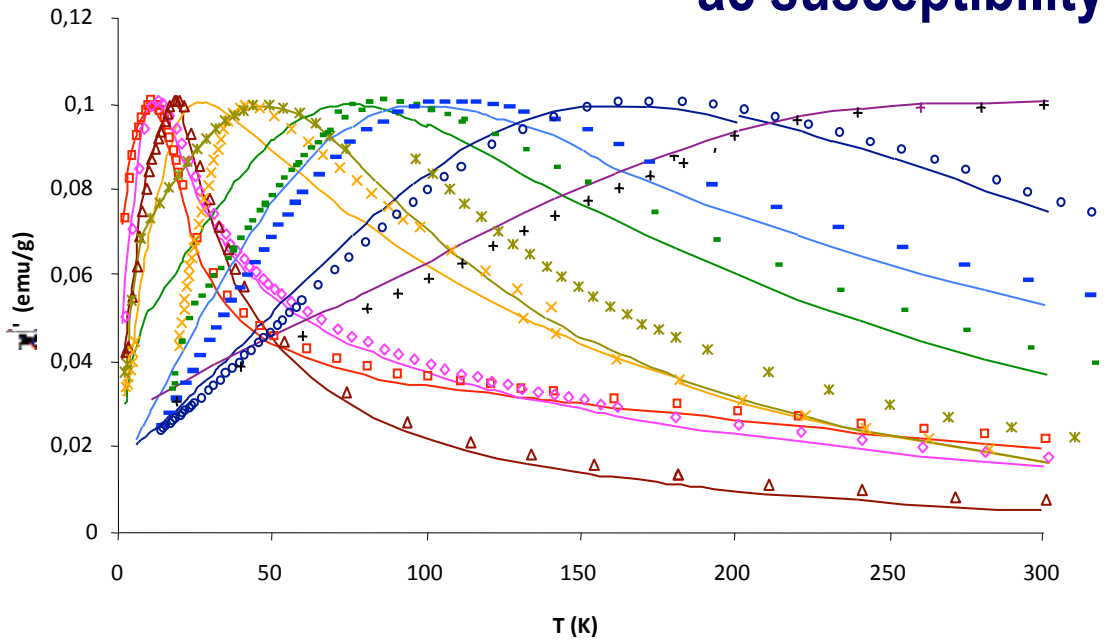
Adjustable Size

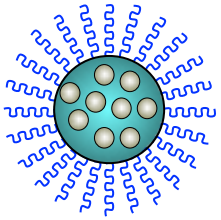
Magnetic nucleus

3 nm → 25 nm



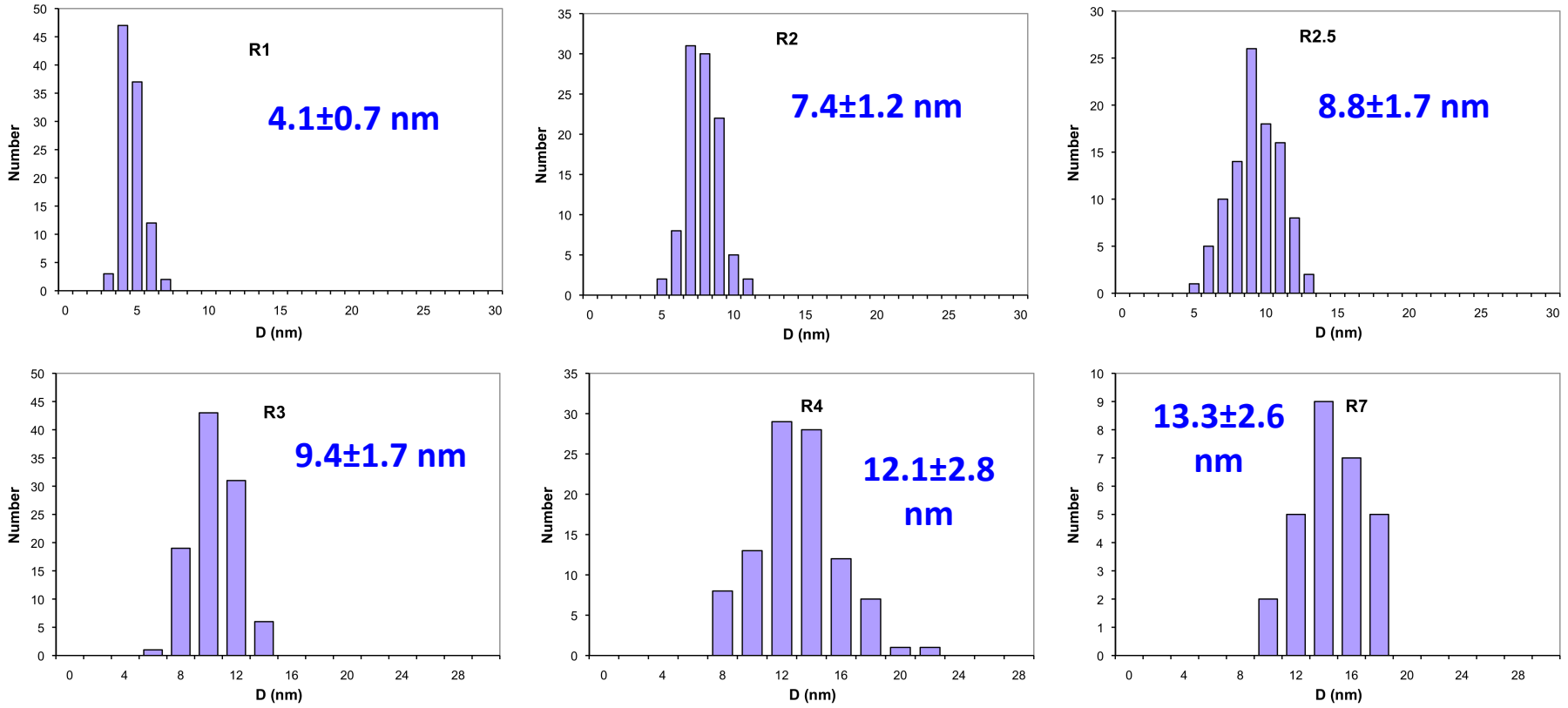
ac susceptibility





Tailoring MNP with polymers

TEM size distribution of Fe_2O_3 particles in a series of Ferrofluid samples in PBS. Same composition, but different Fe_2O_3 content

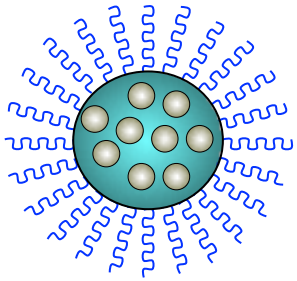


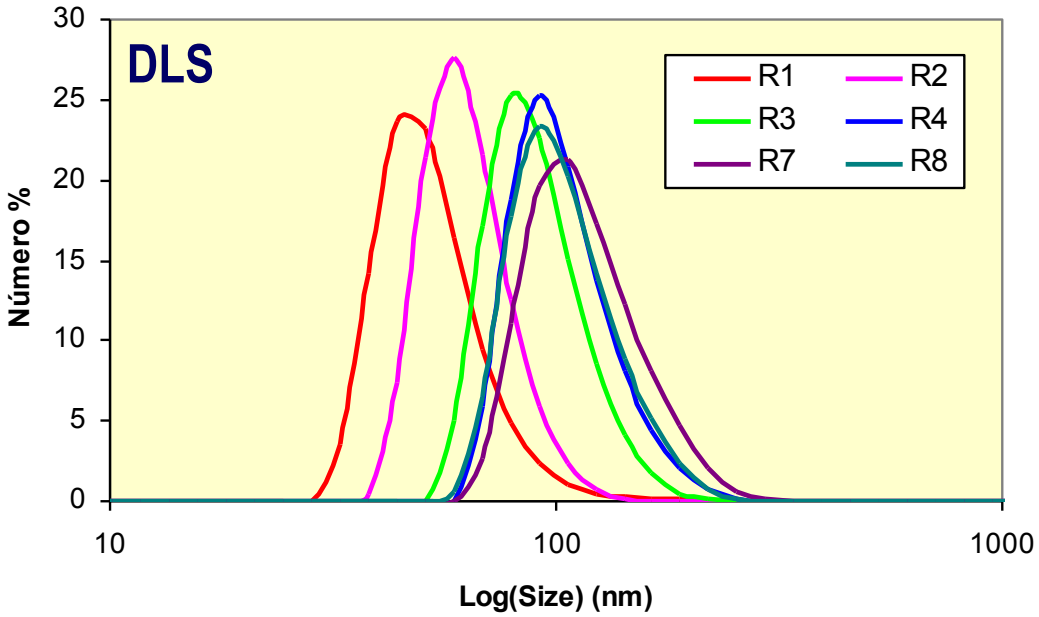
Tailoring MNP with polymers

Adjustable Size

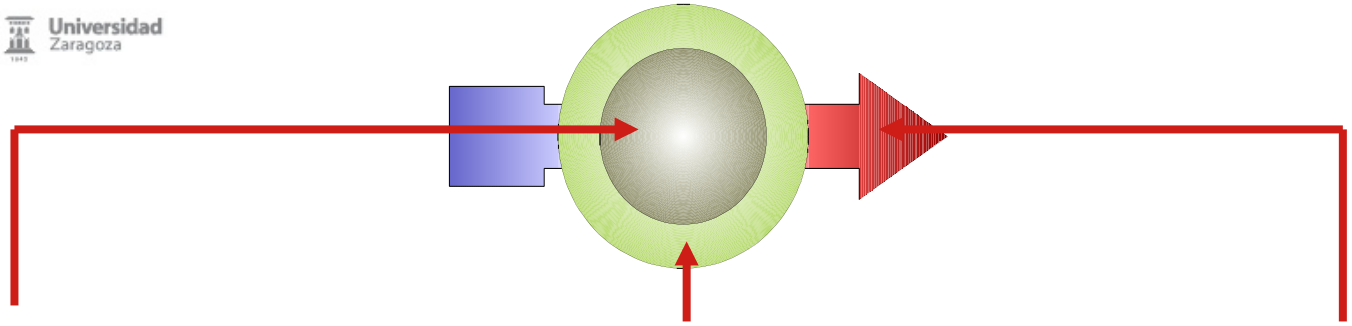
Hydrodynamic size

30 nm → 150 nm





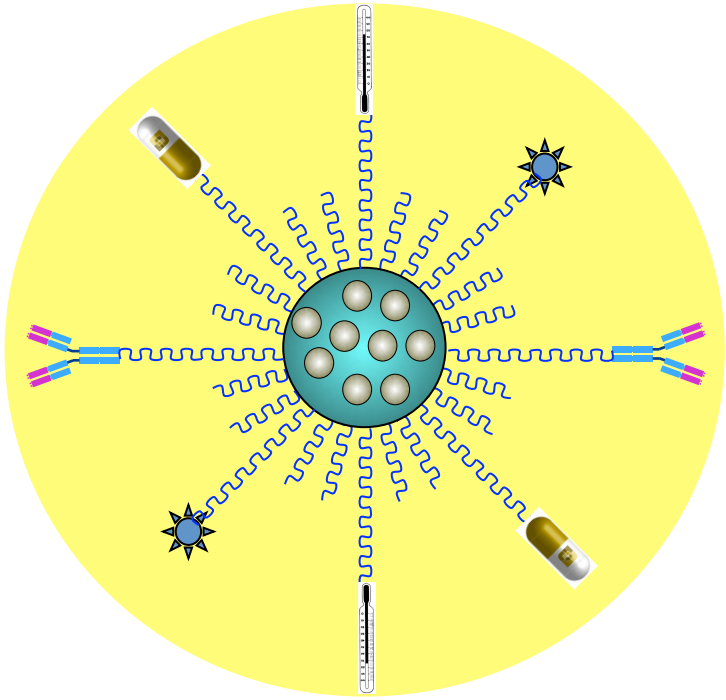
Multifunctional MNP for Biomedical Applications



Magnetic core	Protective coating	Anchoring tails
• composition (e.g., magnetic anisotropy) • doping (e.g., radio-isotopes) • size • size dispersion • biocompatibility • no core is also possible	• core protection • efficient coating • protection against macrophagous • anchoring groups and access • hydrophilic • dispersion stability at pH=7.4 • blood stability • biocompatibility	• Access to anchoring elements • multiple anchoring • selectivity (therapeutic tarjets) • blood stability • luminescent groups • antibodies • therapeutic drugs • thermometer

Tailoring MNP with polymers

Multifunctional nanoplatform



Magnetic particle(γ-Fe_2O_3)

Hydrophobic polymer

Short hydrophilic group (PEG)

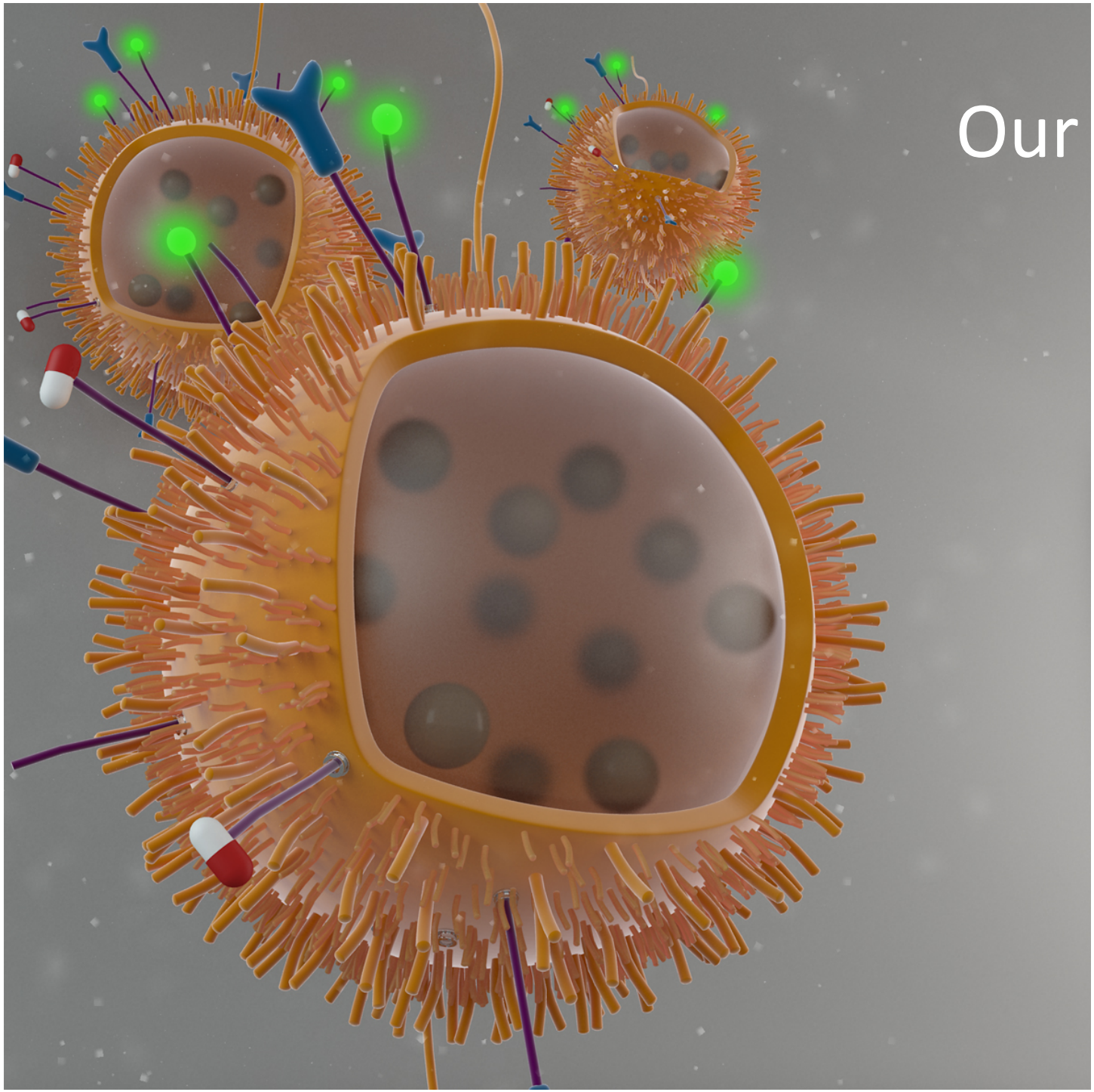
Large hydrophilic group (PEG)

Fluorescent marker

Antibody

Molecular Thermometer

Drug

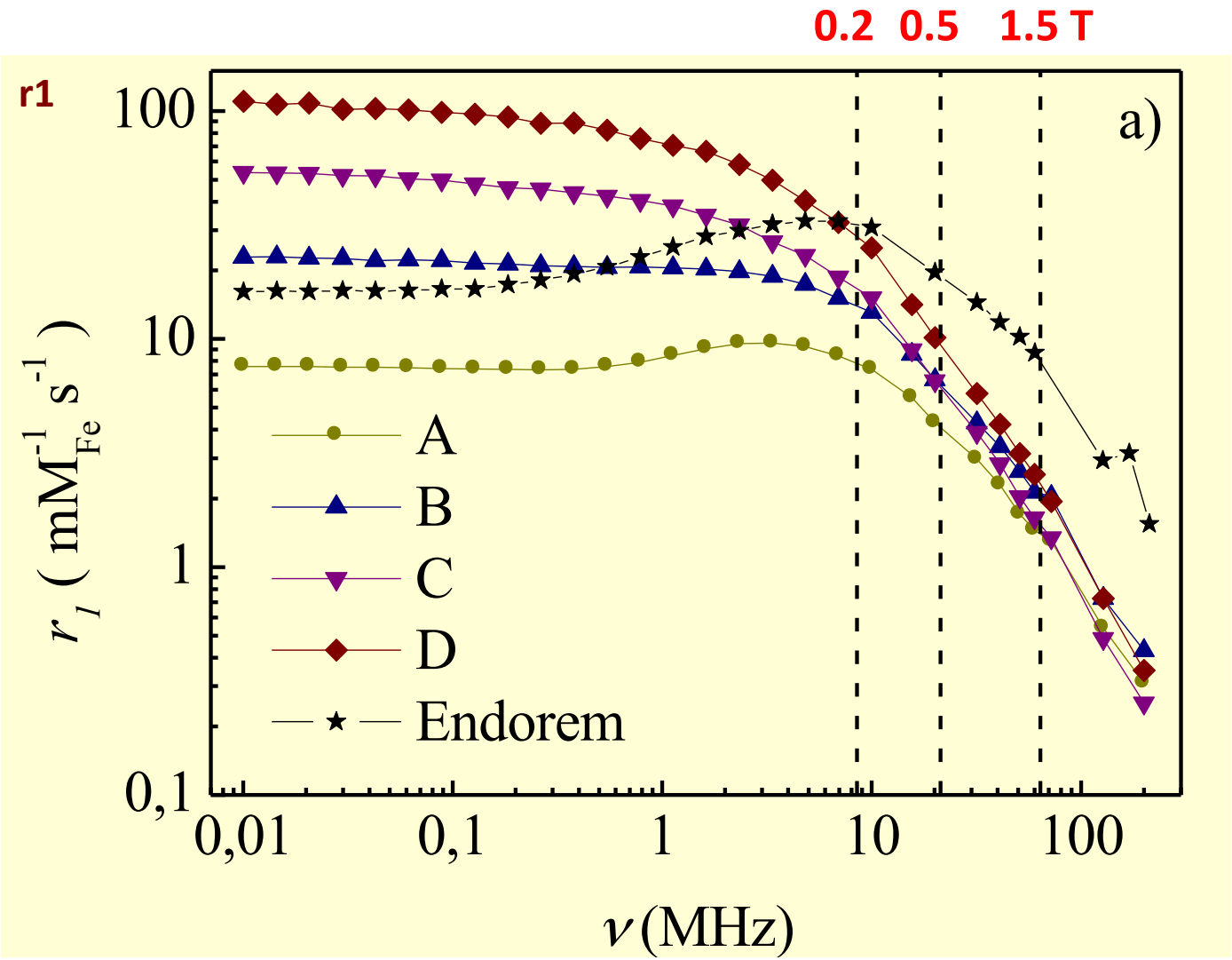


Our particles

Richard Fleischer, 1966, con Stephen Boyd, Raquel Welch, …

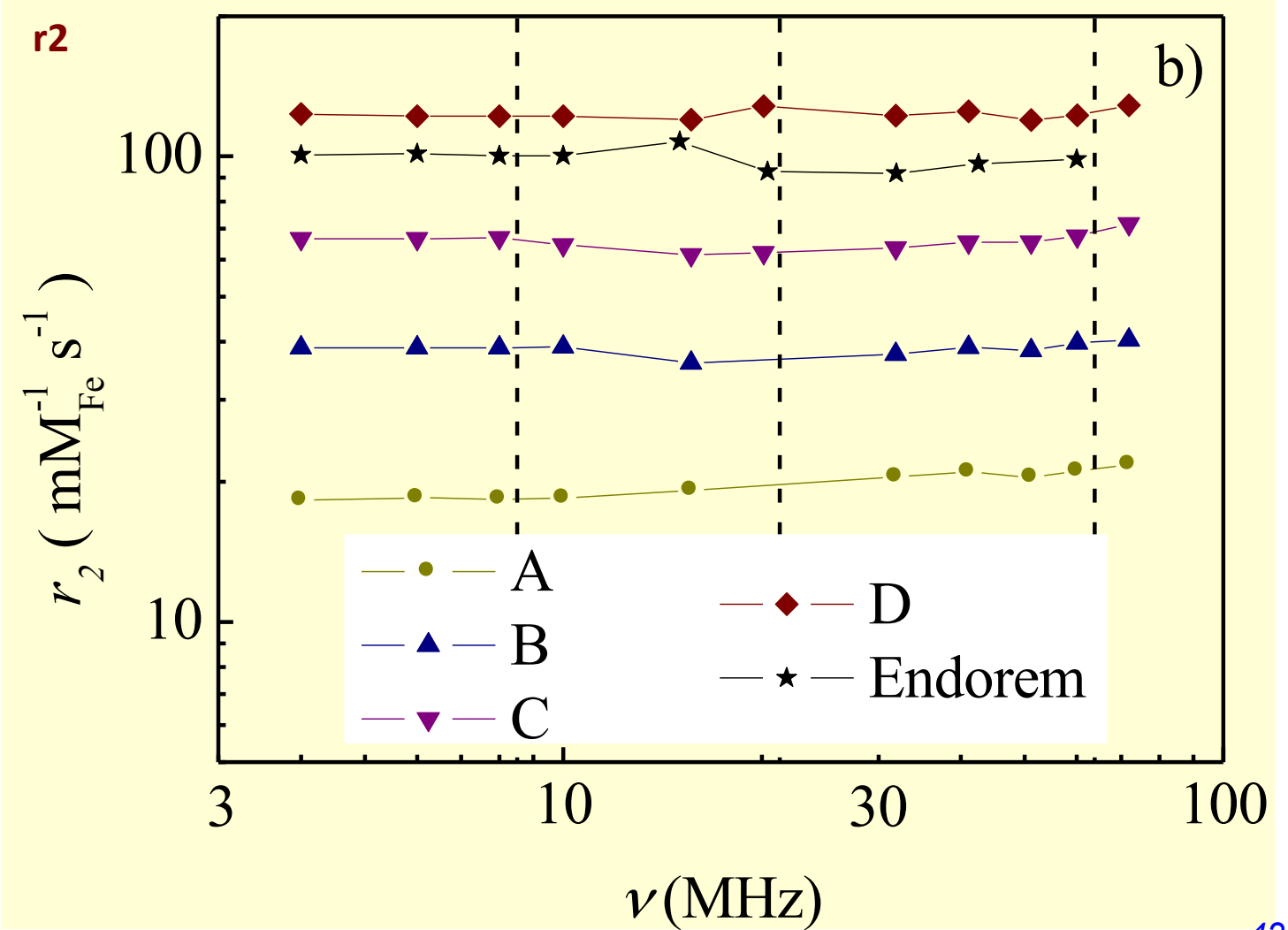
NMR relaxometry

A = 7.4 nm
B = 8.6 nm
C = 10.8 nm
D = 15 nm



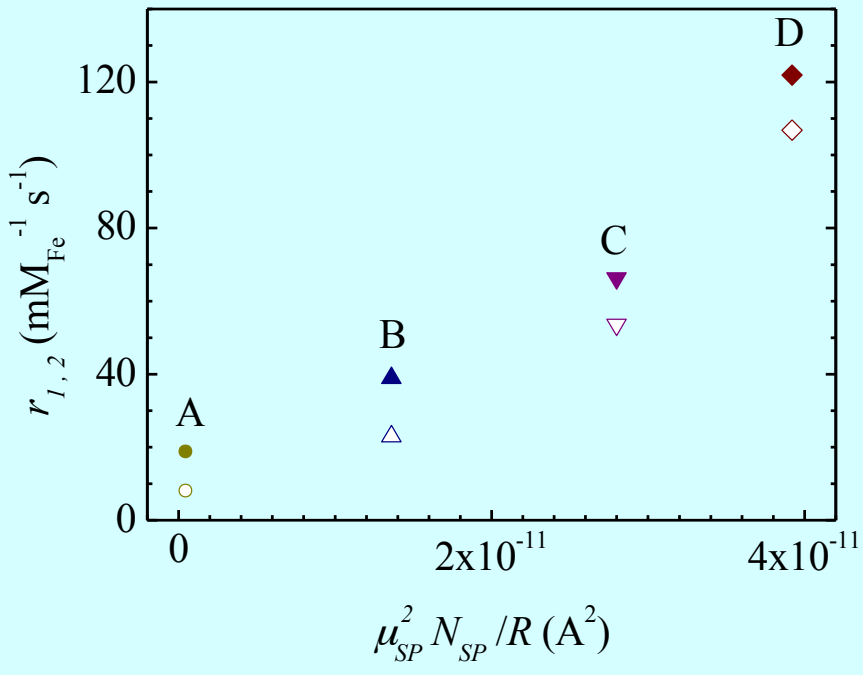
NMR relaxometry

A = 7.4 nm
B = 8.6 nm
C = 10.8 nm
D = 15 nm

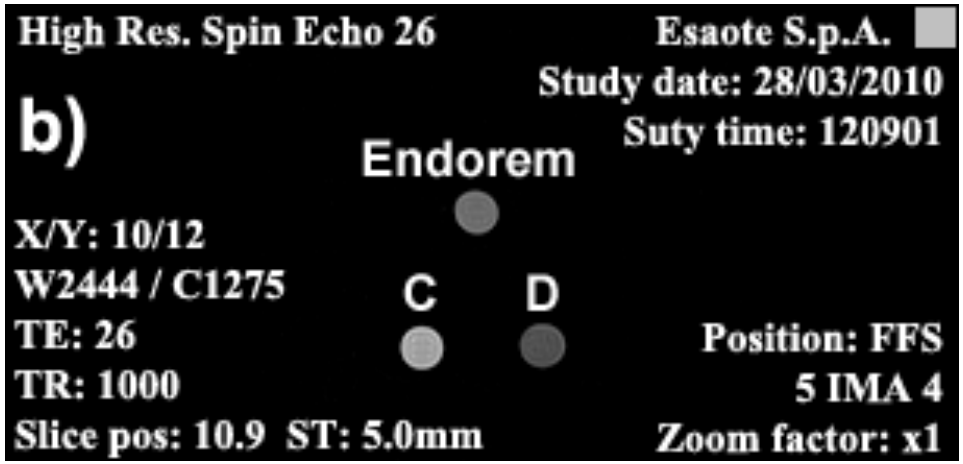


NMR relaxometry

$r_{1,2}$ dependence with size



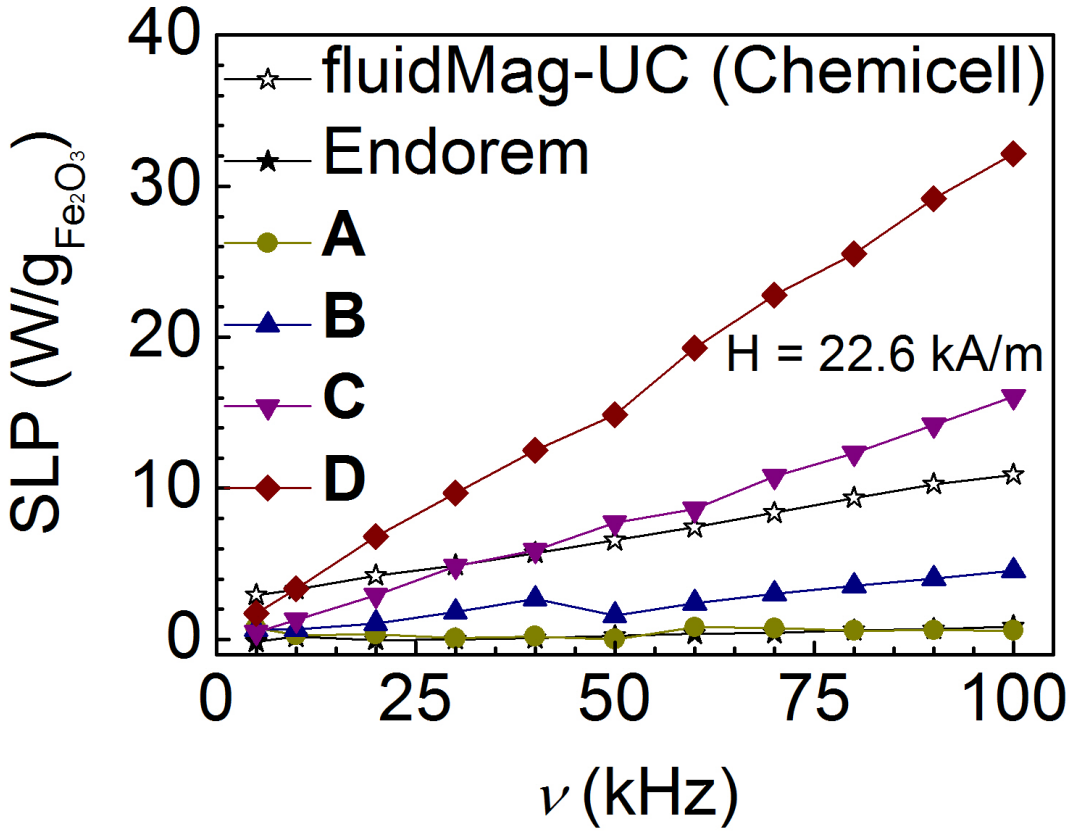
MRI imagens



H. Amiri, et al., Magn. Res. in Medicine, (2011), *DOI: 10.1002/mrm.22959*

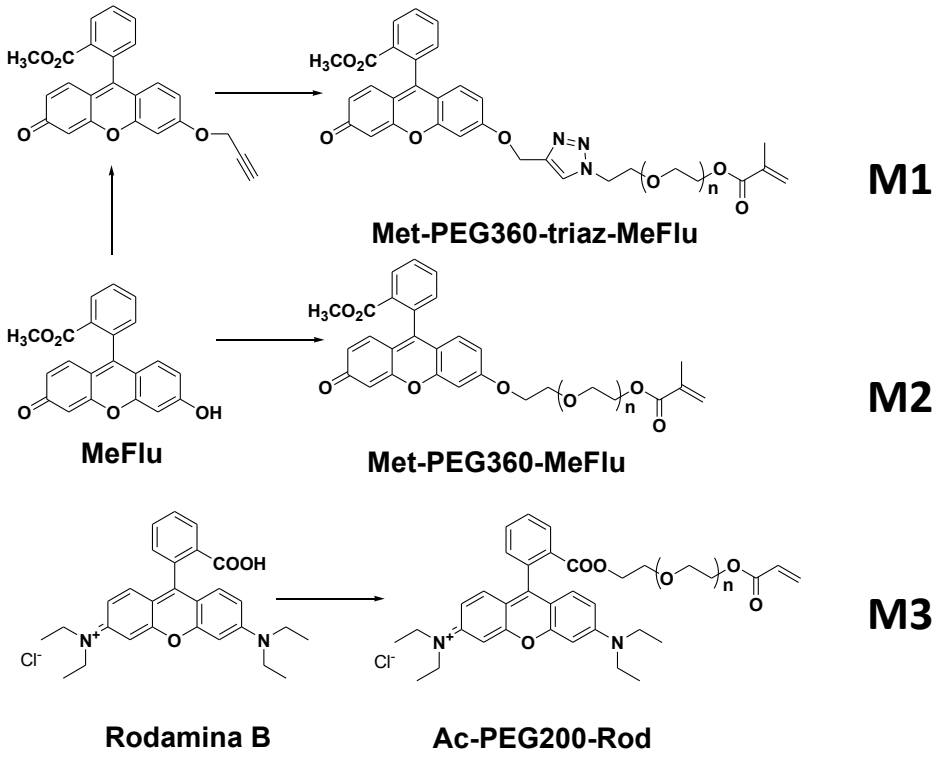
MNP-based Hyperthermia

Specific Loss Power of particles of 10.8 and 15 nm as compared with commercial products.



Multifunctional nanoplatform

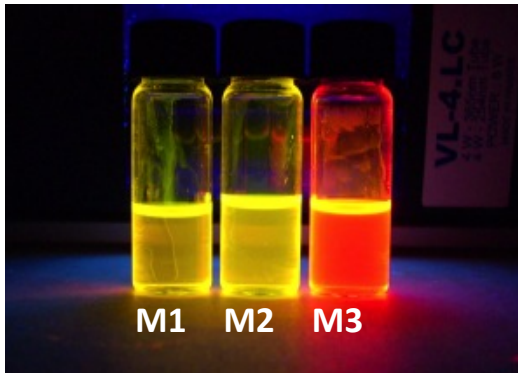
Synthesis of fluorescent groups



M1

M2

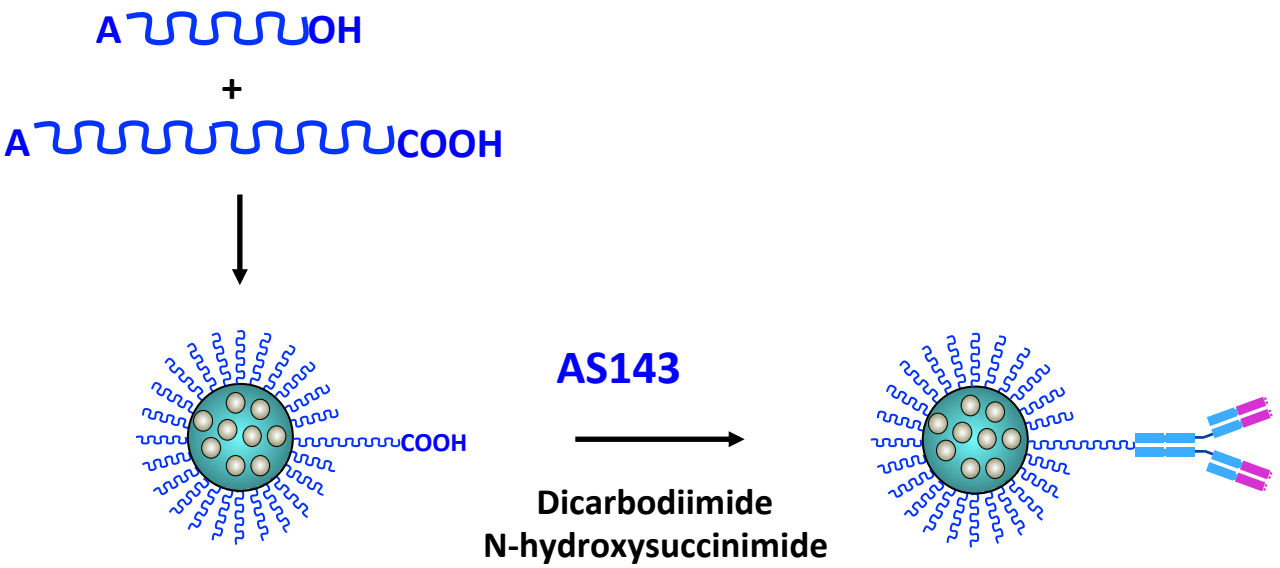
M3



c: 5.10^{-3} M (diclorometano)

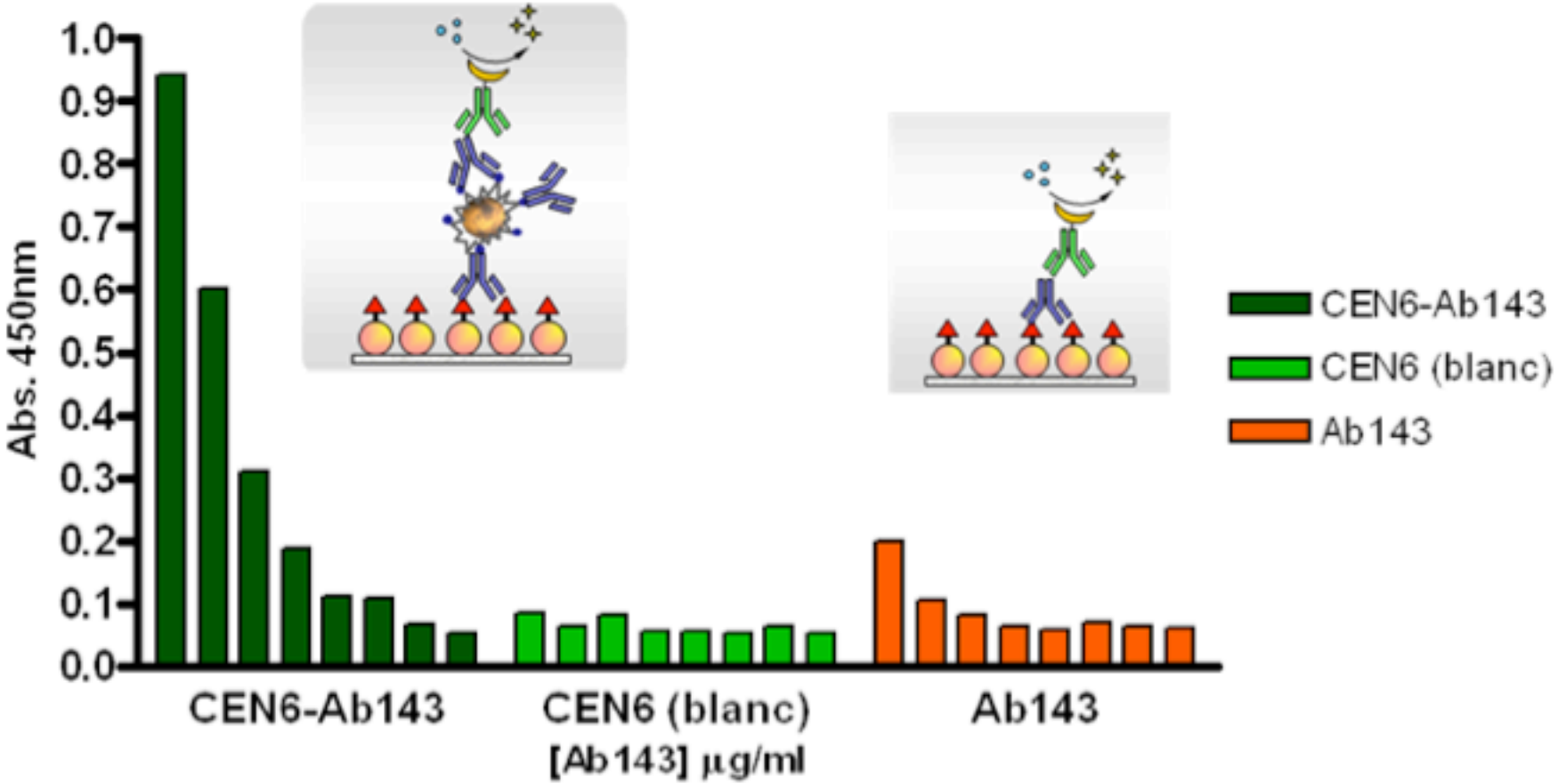
Multifunctional nanoplatform

Antibody: AS143 (anti Methylboldenone androgenic anabolic steroid)



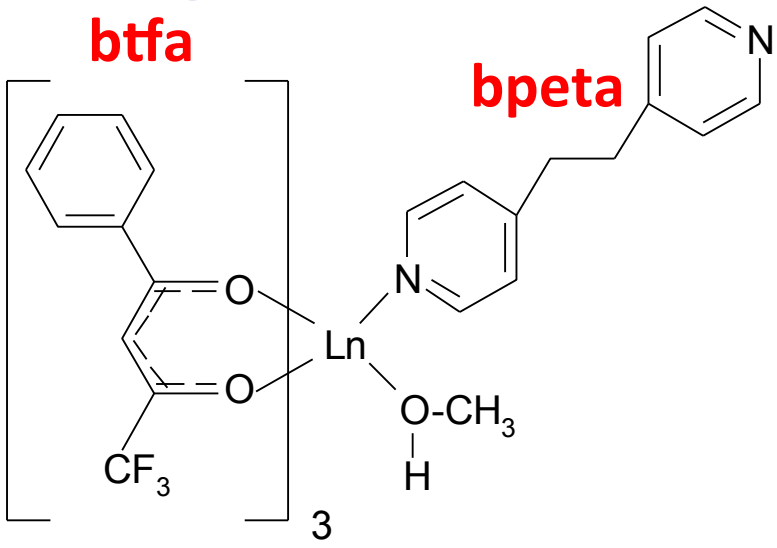
Multifunctional nanoplatform

Antibody: AS143 (anti Methylboldenone androgenic anabolic steroid)

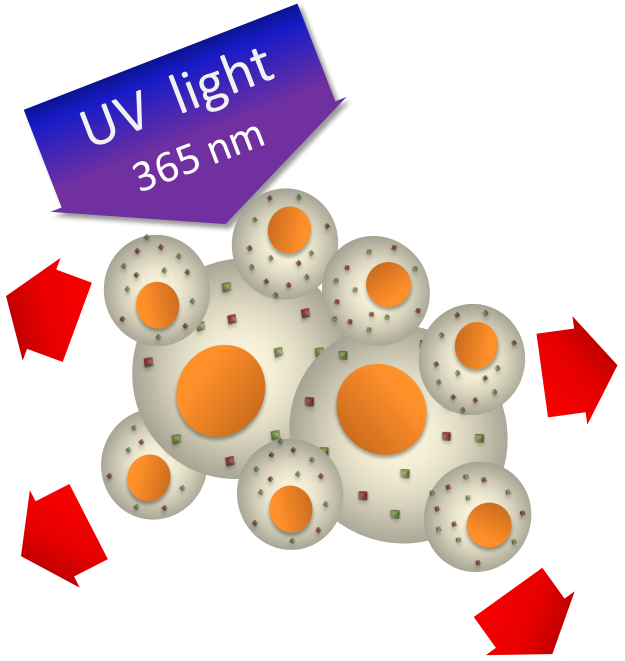


Thermometer design

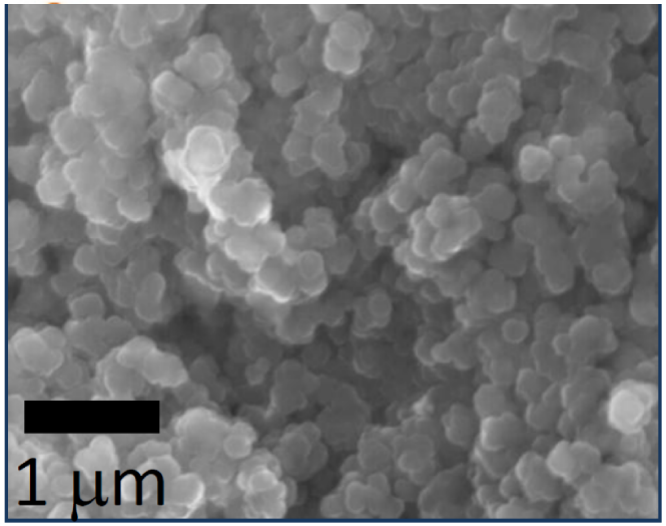
- $[Ln(btfa)_3(MeOH)(bpeta)]$ (Ln=Eu & Tb) β-diketonates

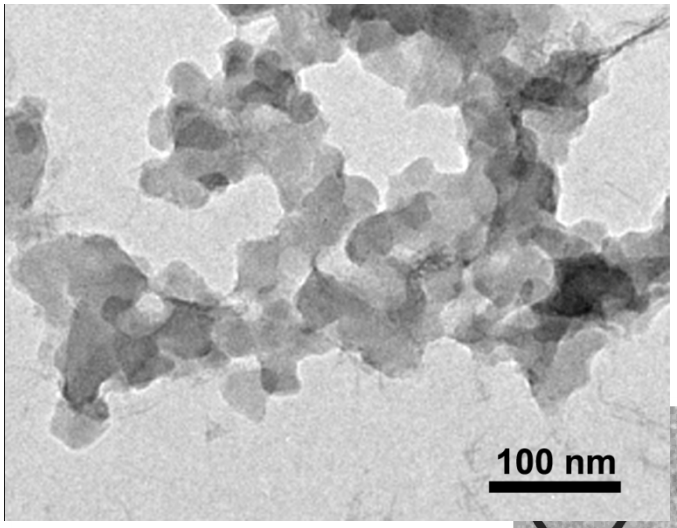


- Organic-inorganic hybrid NPs formed by a maghemite (γ-Fe_2O_3) magnetic core coated with a tetraethyl orthosilicate/ aminopropyltriethoxysilane ***(TEOS/ APTES)*** organosilica shell (modified Stöber method)



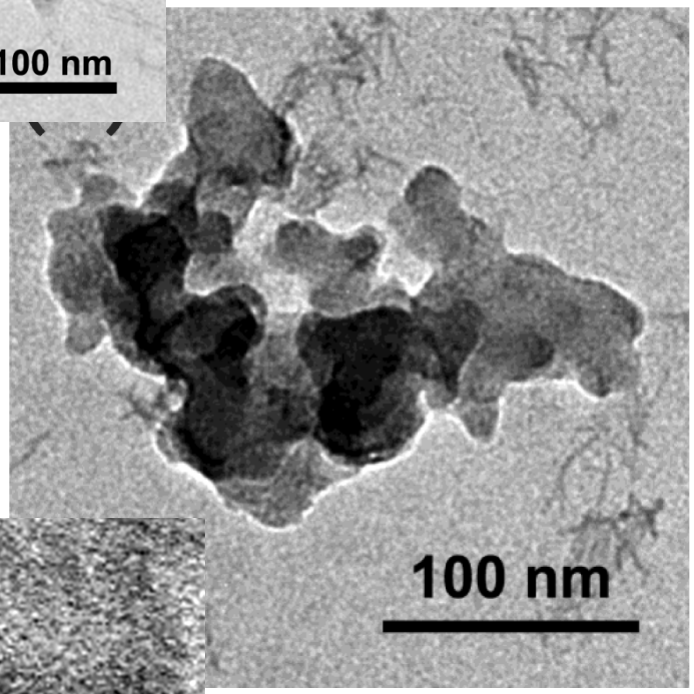
- Eu/Tb co-doped NPs with Eu:Tb ratios of 2:1 **(NP3-2.1)**, 1:1 **(NP3-1.1)**, 1:2 **(NP3-1.2)**, 1:3 **(NP3-1.3)** and 1:10 **(NP3-1.10)**



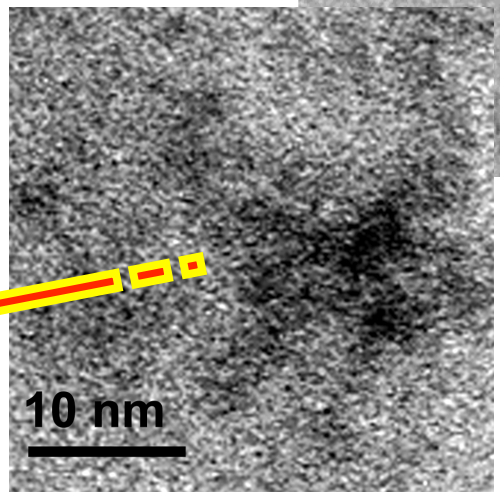


SEM & TEM

- High contrast: Eu^{3+}/Tb^{3+}
- Low contrast: APTES/TEOS



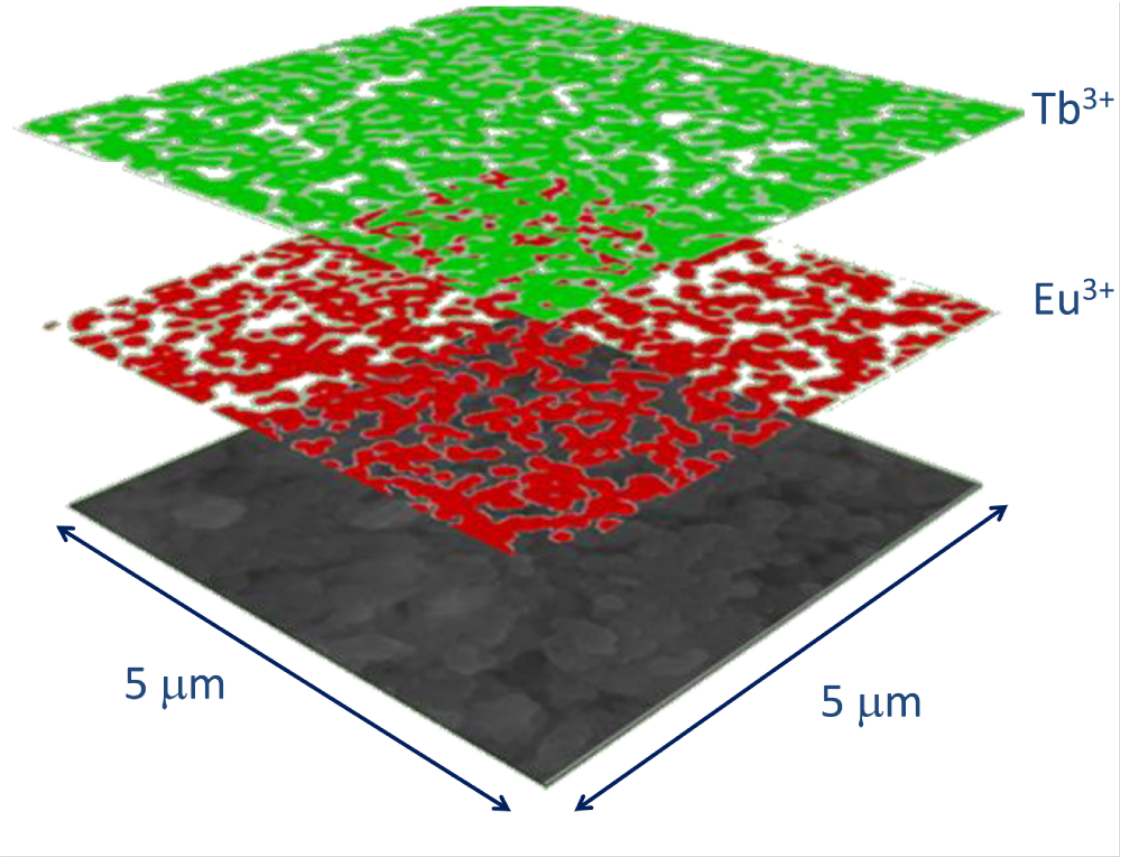
- NPs aggregation occurs during evaporation of the dispersions



- γ-Fe_2O_3

EDS mappings

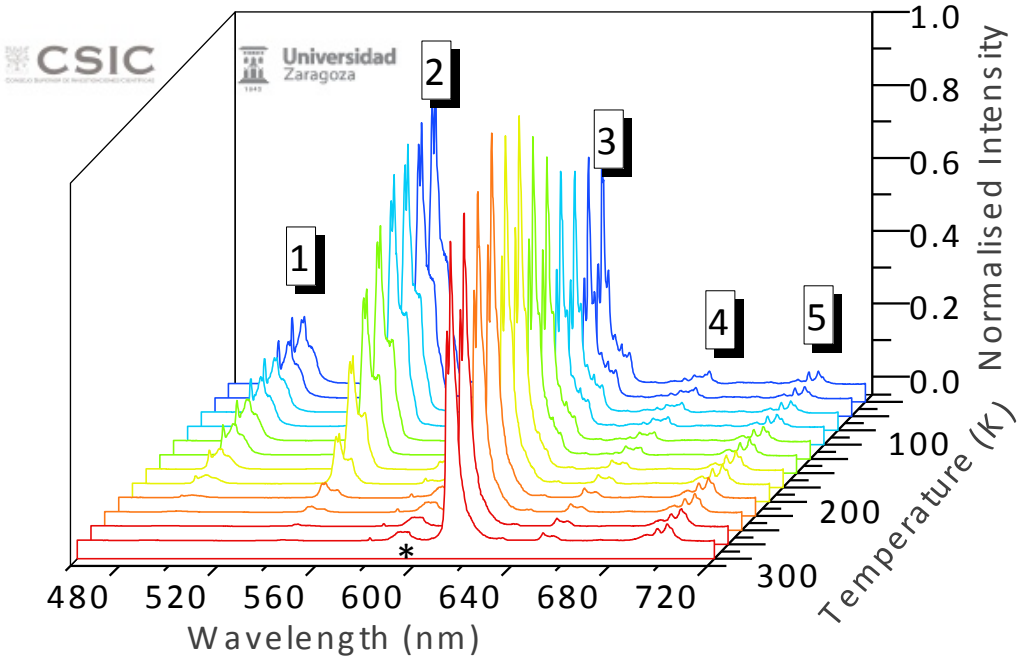
EDS mappings show Eu^{3+} and Tb^{3+} distributions with contours and shapes similar to those of the NPs



The NPs contain both Eu^{3+} and Tb^{3+}

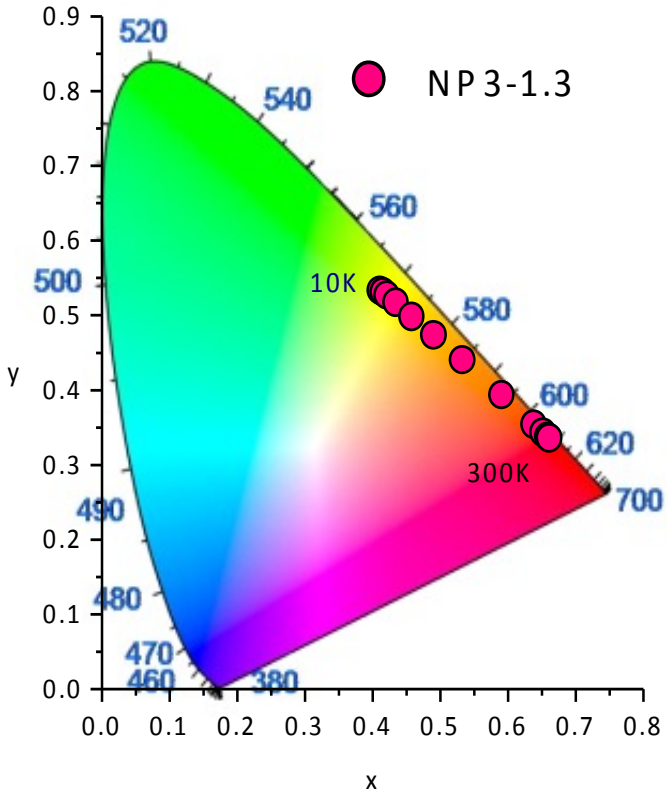
Photoluminescence

γ-Fe_2O_3@TEOS/APTES **NP3-1.3**

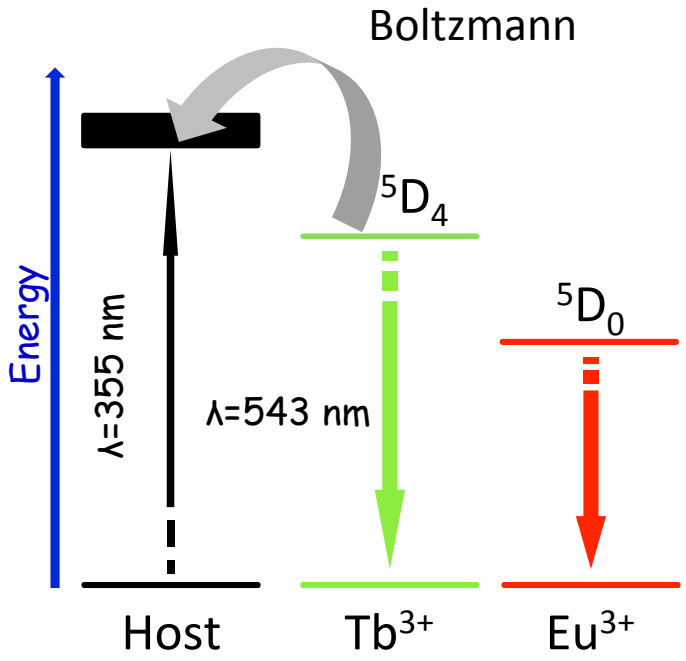


- **1** & **2**: $^5D_4 \Rightarrow {}^7F_{6,5}$ (Tb^{3+})
- **3**, **4** & **5**: $^5D_0 \Rightarrow {}^7F_{2\text{-}4}$ (Eu^{3+})
- Area marked with an asterisk: Eu^{3+}/Tb^{3+} ($^5D_0 \Rightarrow {}^7F_{0,1}$)/($^5D_4 \Rightarrow {}^7F_4$) overlapping

Commission Internacionale d'Éclairage (CIE) (x,y) color coordinates illustrates the dependence on T:

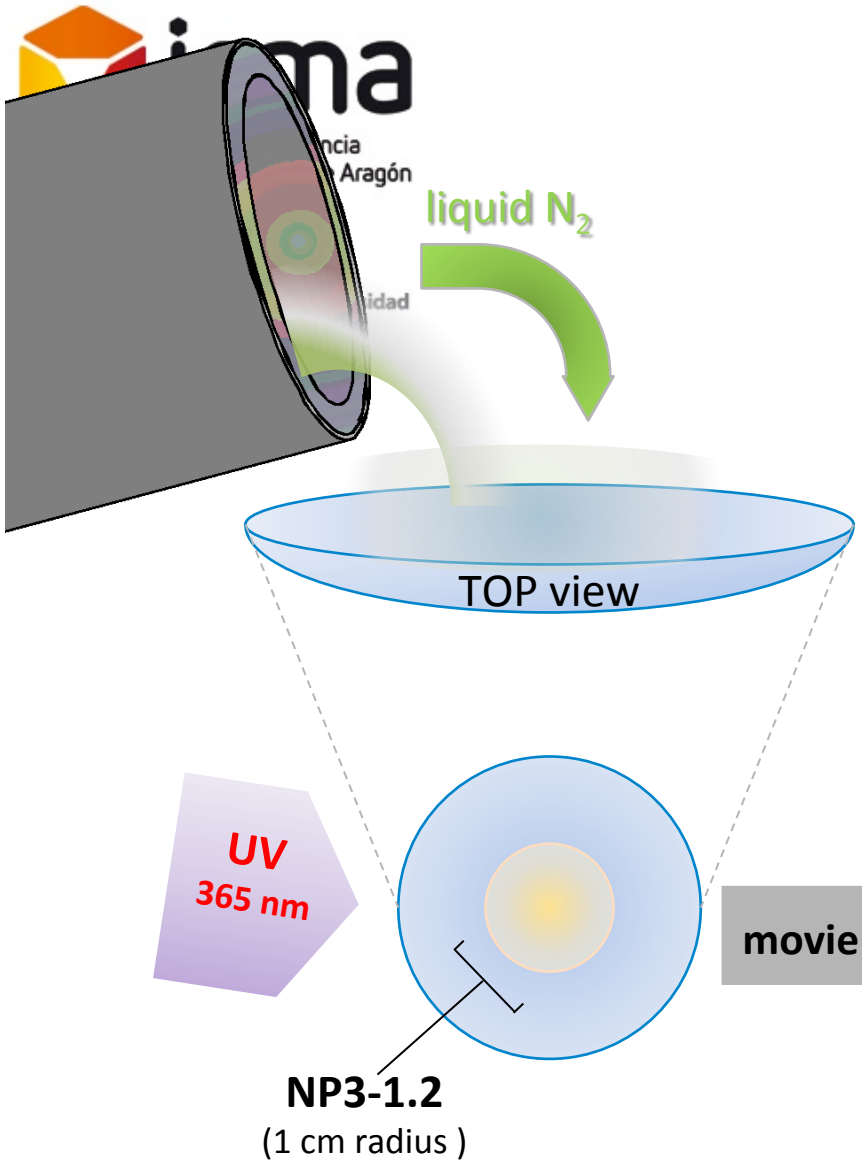


Eu/Tb luminescent nanothermometer

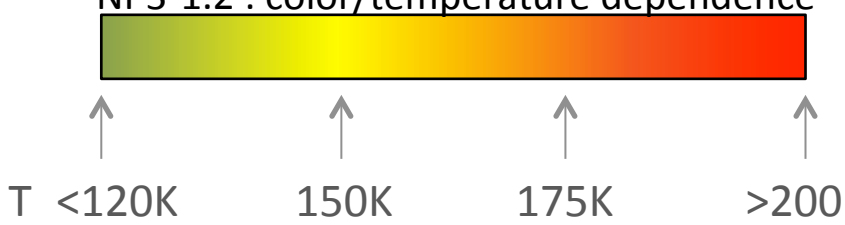


- ***Host rational design***; an excited triplet with energy above that of the Tb^{3+} $^5D_4$ state, thus warranting the occurrence of thermally-driven $^5D_4$⇨host energy transfer
- ΔE between that triplet state and the Eu^{3+} $^5D_0$ emitting level is too large to permit thermally-driven depopulation
- The Tb/Eu relative intensity guarantees **absolute measurement of temperature**
- **The self-calibration** (relative intensities) overcomes the well-known drawbacks of intensity-based measurements (*e.g.* sensor concentration and drifts of the lamp and detectors)

Adv. Mater., 2010, 22, 4499; **New J. Chem.**, 2011, 35, 1177
Spain Patent P200930367, 2009; PCT/ES2010/070430

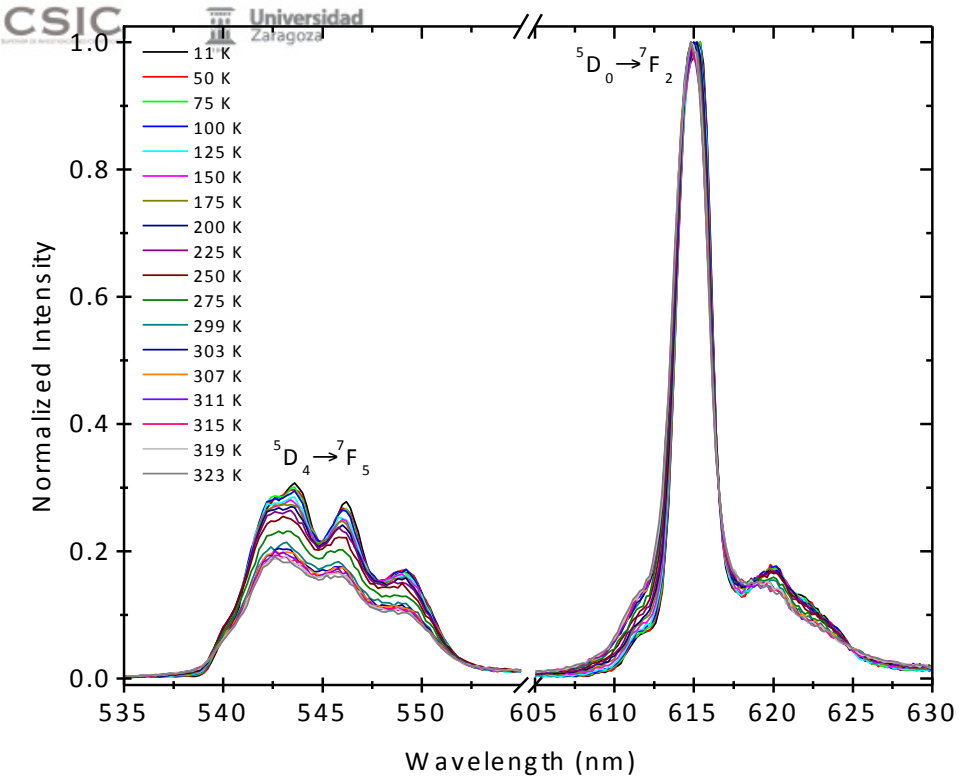


NP3-1.2 : color/temperature dependence

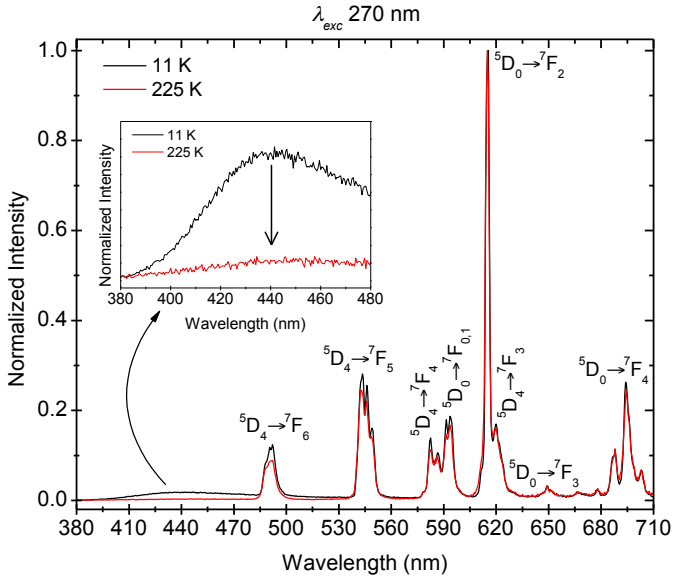


Multifunctional nanoplatform

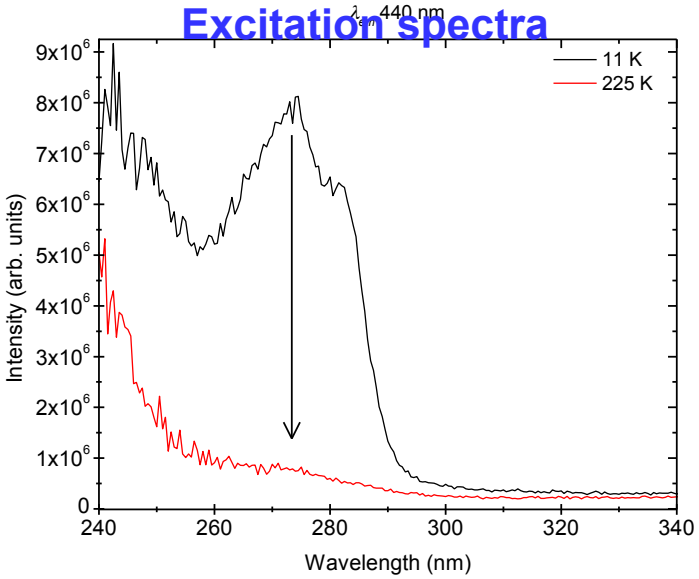
Emission spectra



Emission spectra



Excitation spectra



M3 = FF6@MPEGA+PEGAacac@

$Eu_{0.25}Tb_{0.75}$@DPA

Solid

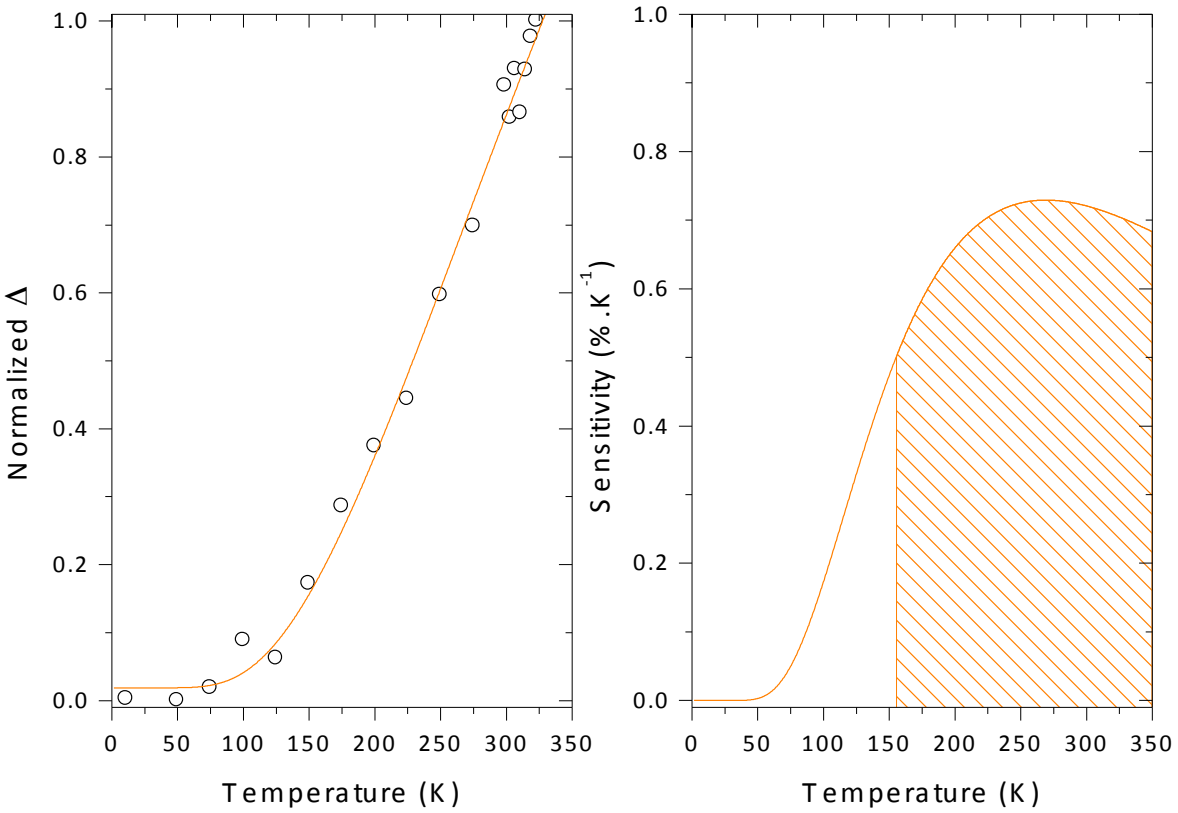
Dialyzed Sample

Multifunctional nanoplatform

M3 = FF6@MPEGA+PEGAacac@$Eu_{0.25}Tb_{0.75}$@DPA

Solid

Relative Sensitivity and Temperature Range of Operation



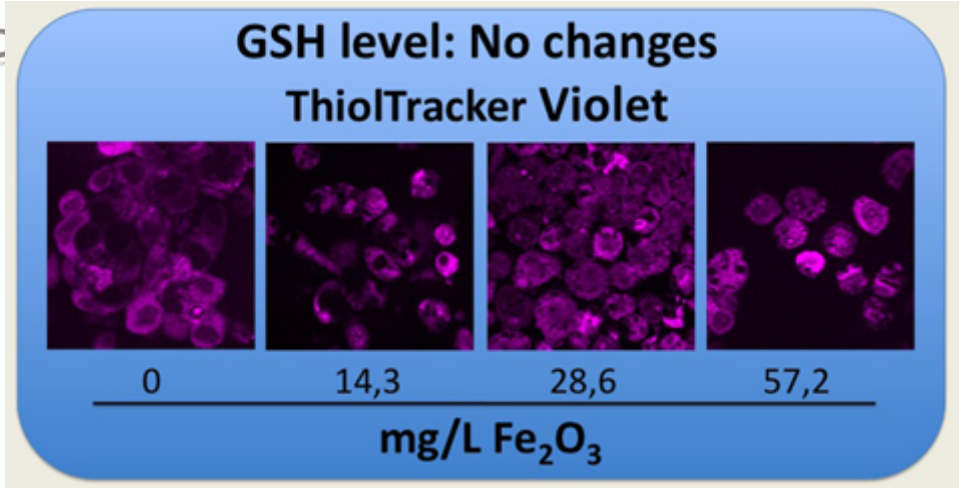
- The Δ parameter presents a temperature dependence almost linear in the temperature range 150-350K.
- The maximum sensitivy is 0.73 $\%.K^{-1}$ at 270 K and the sensitivity is above 0.5 $\%.K^{-1}$ for temperatures T>150K*s*

Cytotoxicity

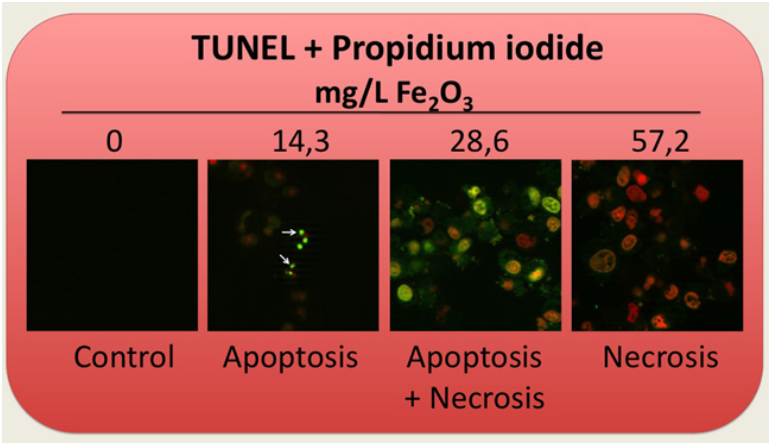
Lamiaa M.A. Ali
Prof. Victor Sorribas
Dept. of Toxicology

Cytotoxicity

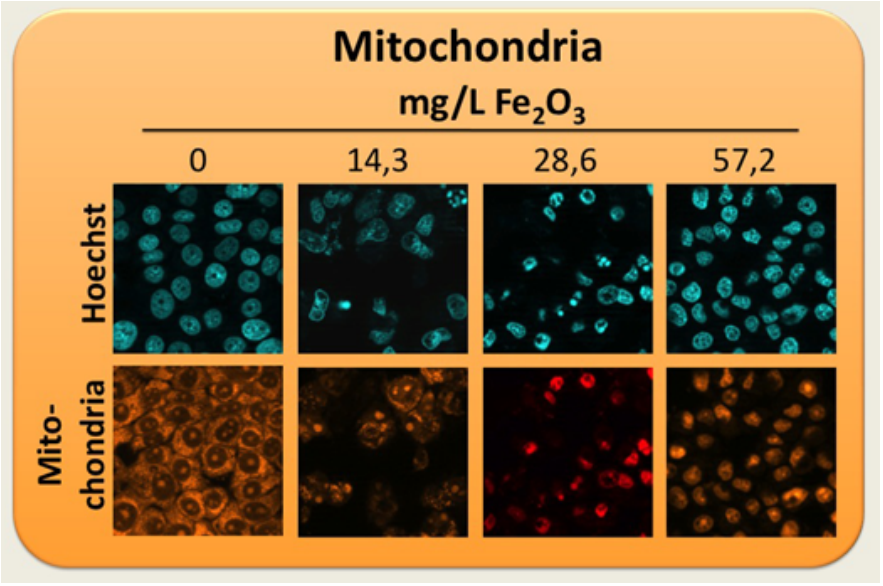
No oxidative stress



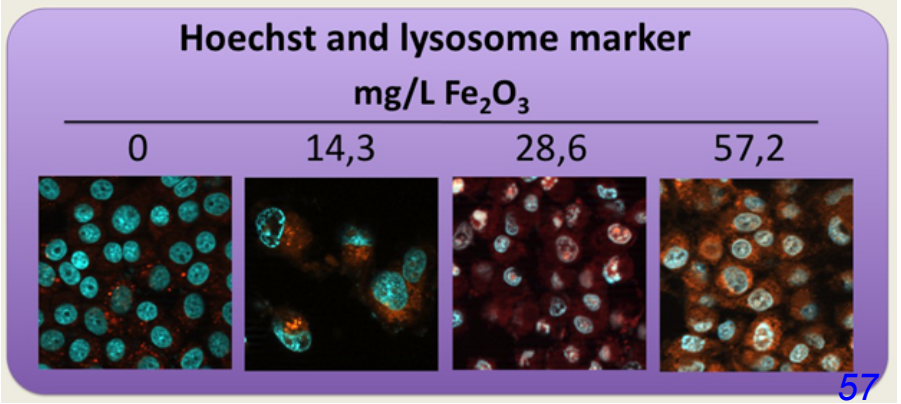
Apoptosis and necrosis at higher concentration



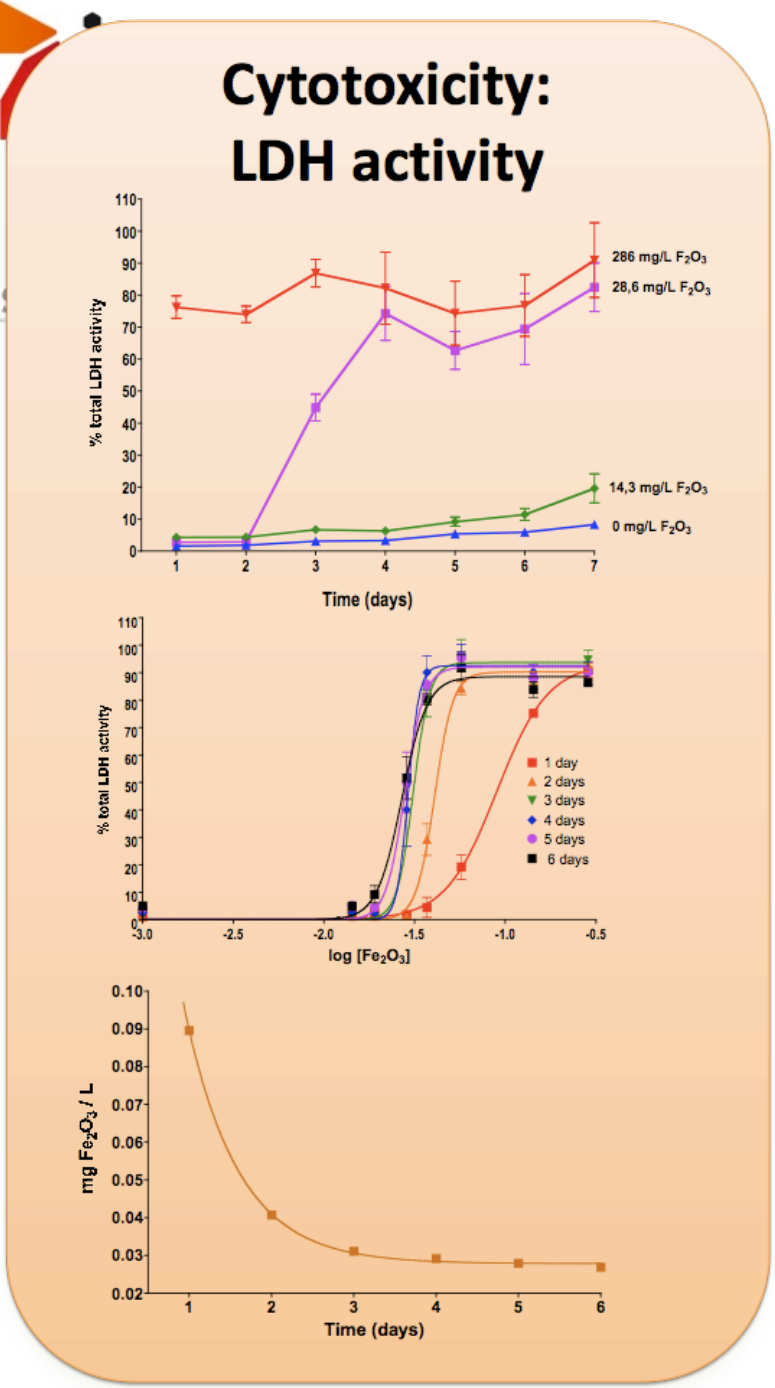
Mitocondria are not affected



Lysosomes are enlarged or proliferate



Cytotoxicity



Activity of cytosolic **lactate dehydrogenase** in culture medium of OK cells incubated with nanoparticles at different concentrations and times.

This activity indicates that cell membrane has broken and intracellular content has leaked.

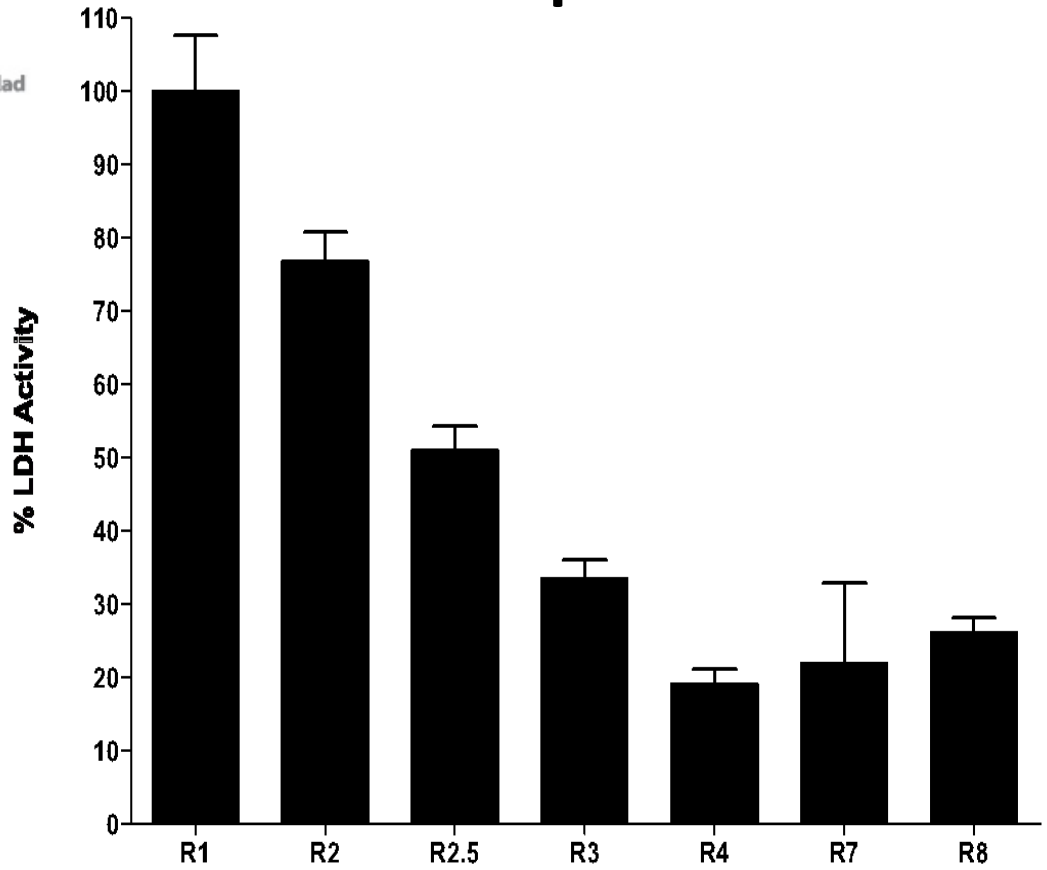
Top: Four time courses with different concentrations of Fe_2O_3. **14.3 mg/ml is not toxic, and 28.6 mg/ml only after 3 days**.

Center: Dose-responses at different days. Lethal mean concentrations (LC50) are calculated for each curve.

Bottom: Representation of LC50 evolution with the time, showing that the LC50 diminishes with time, ie. **the effect is accumulative**.

Cytotoxicity

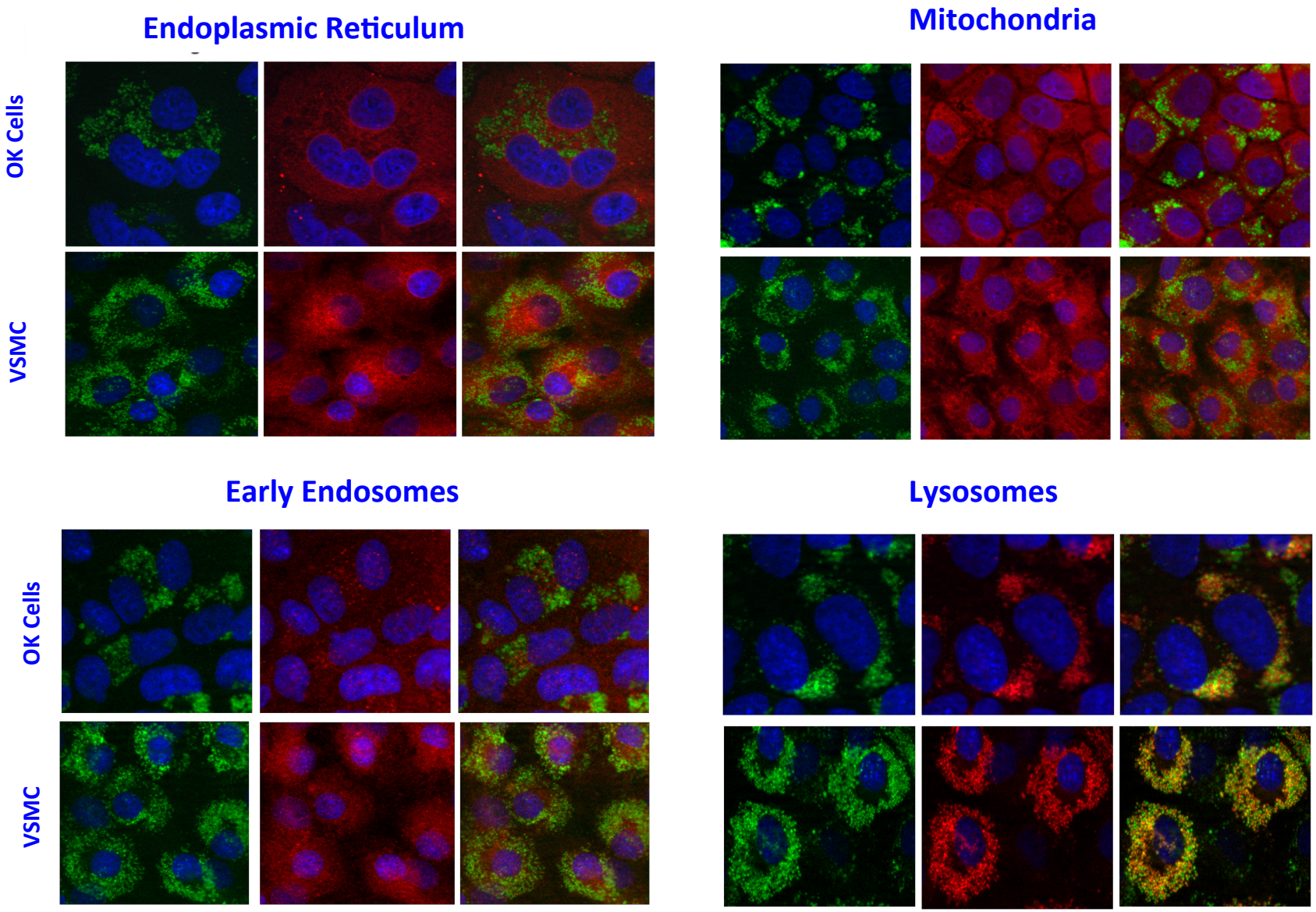
Size dependence



Effect of the nanoparticle diameter on the OK cell death after 7 days of incubation with 0.01 g/l Fe_2O_3

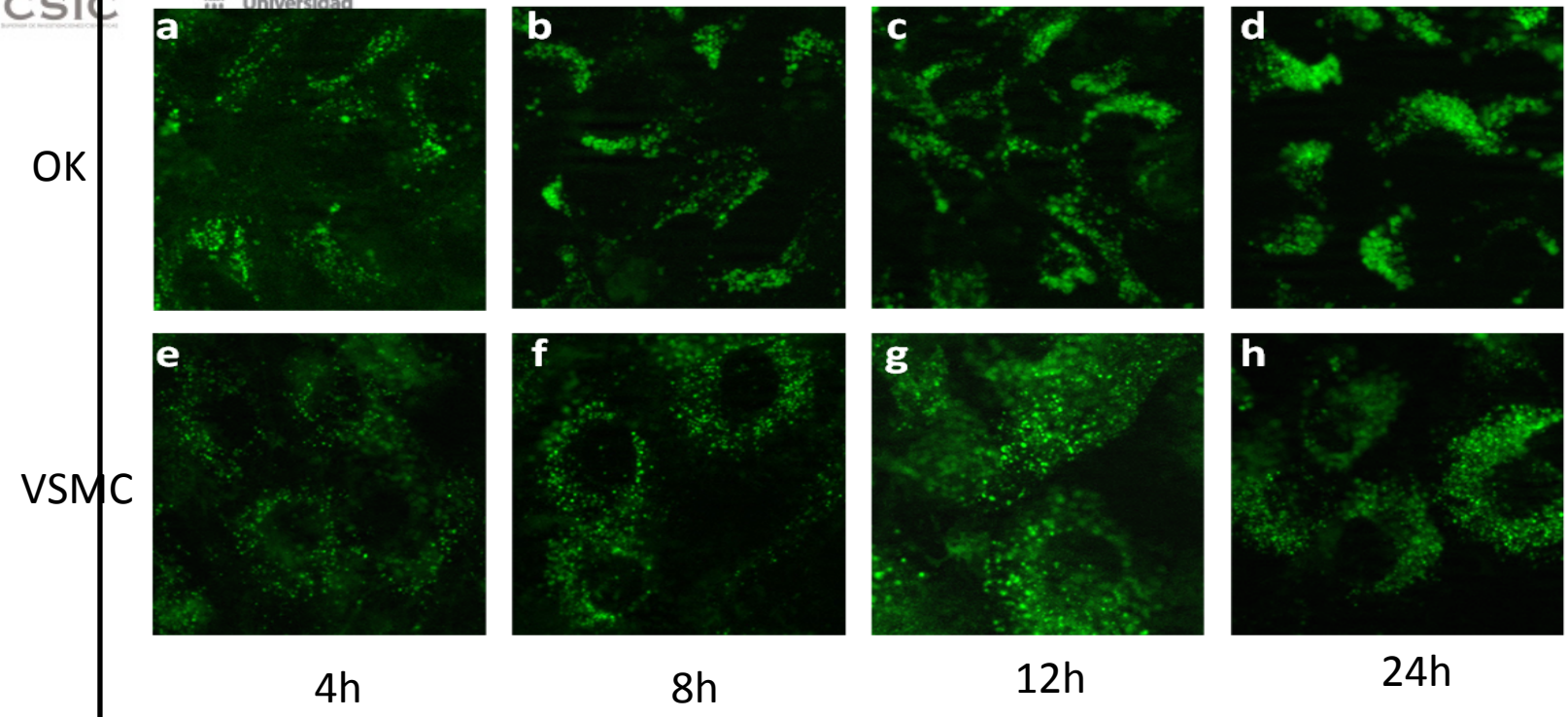
Toxicity is inversely proportional to diameter of the MNPs

Subcellular localization of fluorescent nanoparticles: LYSOSOMES.



The only location of SPIONS in cells are the lysosomes.

Uptake kinetics



Internalization of fluorescently labeled R8 (112.5nm) in OK and VSMC at different time points of incubation. Upper panel is OK cells after 4h (a), 8h (b), 12h(c), and 24h (d) of incubation, lower panel is VSMC after 4h (e), 8h (f), 12h (g), and 24h (h) of incubation.

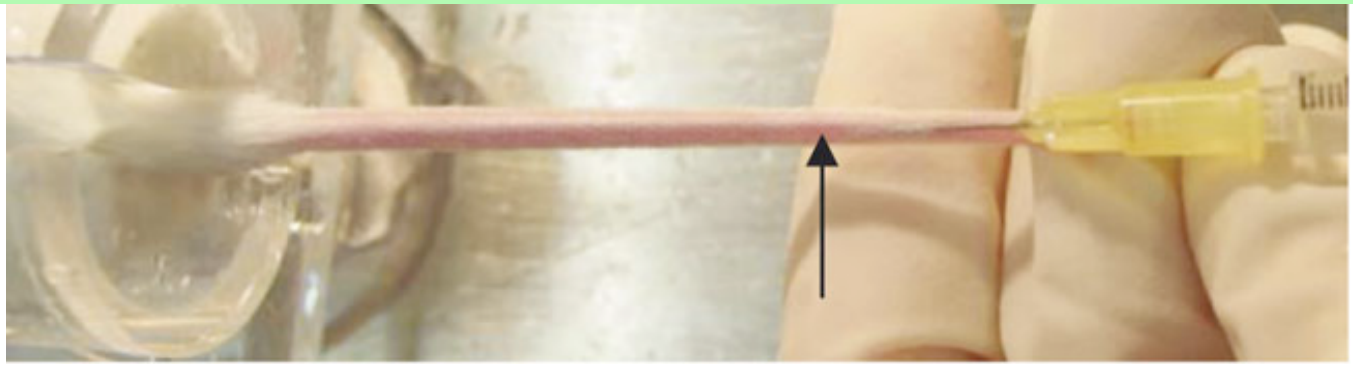
In vivo assays

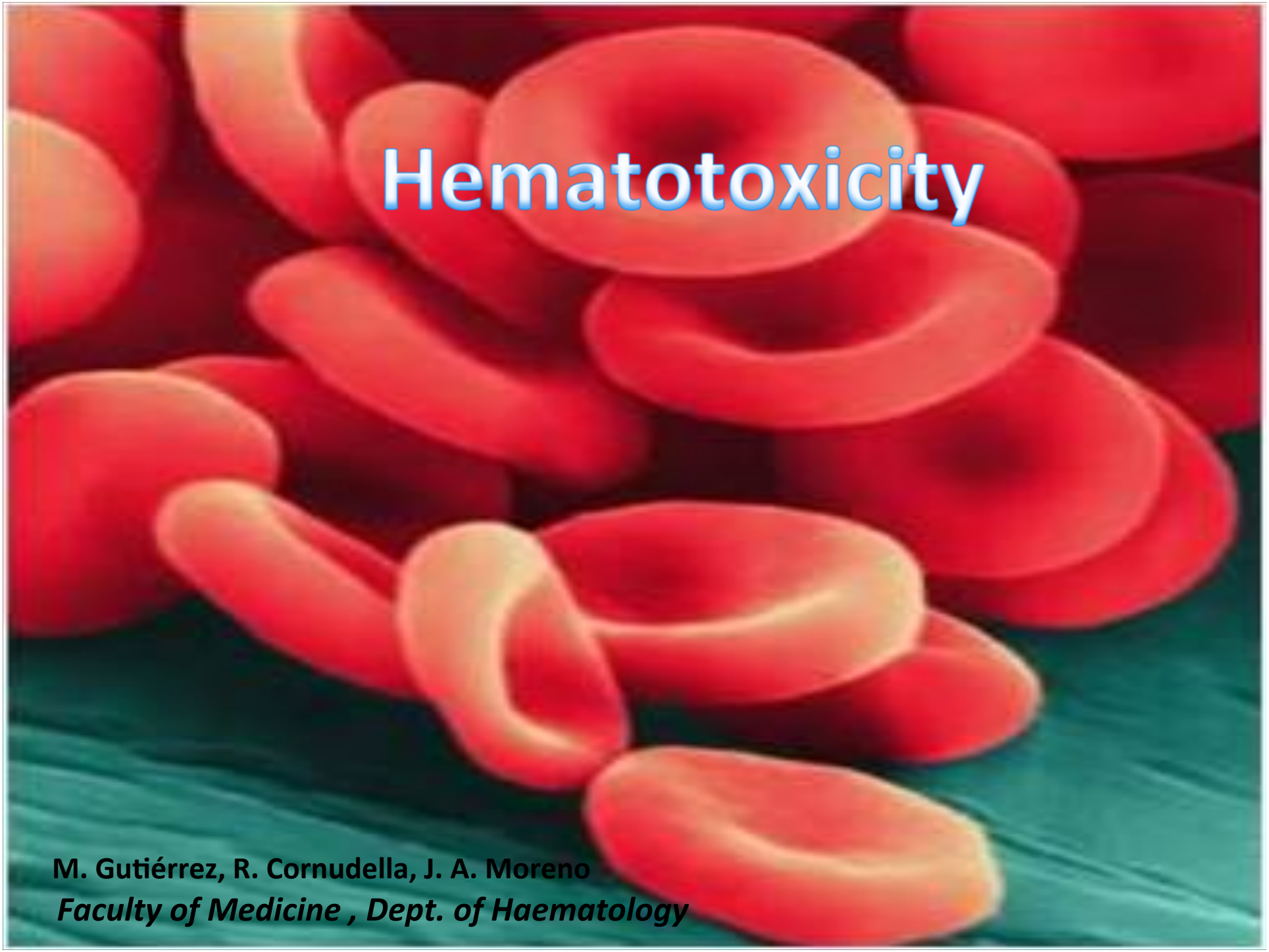
(Current work)

Intraperitoneal injection shows
No significant effects after 1 month.
No damage in organs

Intravenous injection of 5XEndorem shows after 10 days no excess of iron in a variety of tissues neither anomalies in the pathologic anathomy inspection.

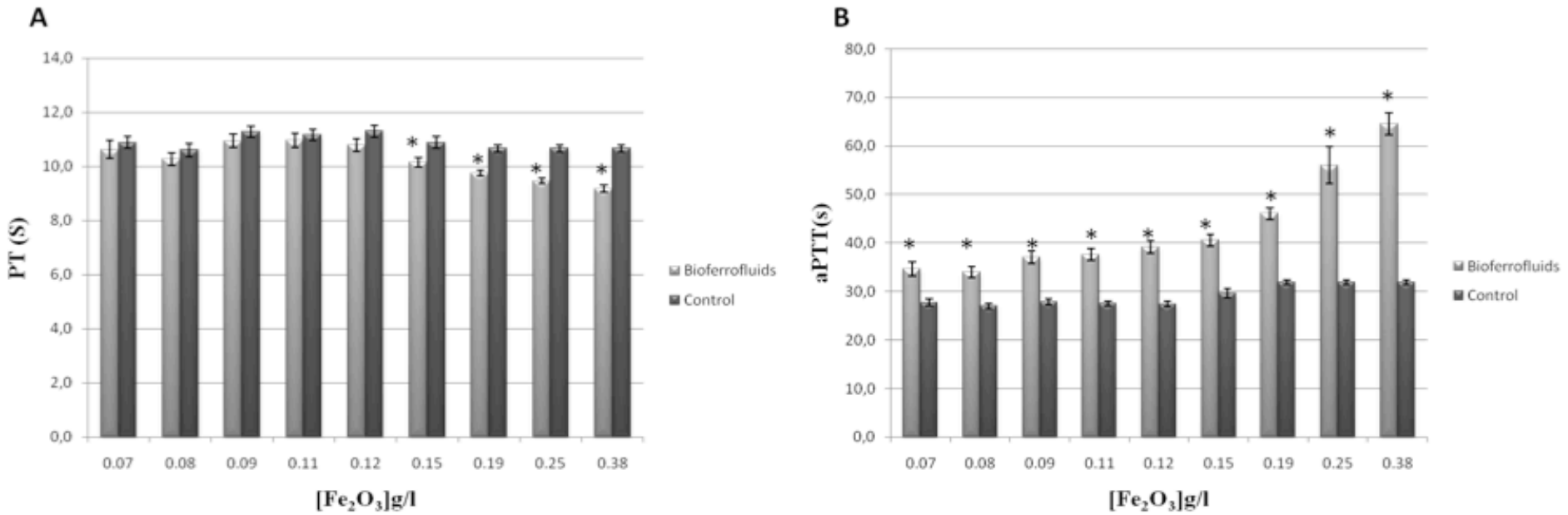
Zn-doped modified particles and same doses lead to the same results





Hematotoxicity

M. Gutiérrez, R. Cornudella, J. A. Moreno
Faculty of Medicine , Dept. of Haematology



The effect of bioferrofluids on: (A) the prothrombin time (PT) in seconds, (B) the activated partial thromboplastin time (aPTT) in seconds

Haematotoxicity

- No changes in Prothrombine Time (PT) observed.
- Activated partial Thromboplastine Time (aPTT) increases with the concentration of MNPs.
- Combine PT and aPTT results indicate that the bioferrofluids act as non- specific inhibitor / anticoagulant circulating agent for coagulation process.
- While PEG component does not seem to have any effect on the coagulation process, the coating copolymer P4VP-g-PEG shows strong anticoagulant behaviour indicating that P4VP is at the origin of the effect.
- These effect may not appear in vivo

Haemotoxicity

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Complete blood picture (CBC)

- There is no significant changes in CBC between test and control in :
- Erythrocyte count
- Leukocyte count
- Hemoglobin
- Platelets

Plasmatic viscosity

Our nanoparticles does not show any change the plasmatic viscosity.

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M4 Group

(Multifunctional Magnetic Molecular Materials)

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- **CONSOLIDER** - MAT2007-61621
- Integrated Spanish-Portuguese
- Action PT2009-0131
- MAT2011-25991

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