Biomagnetism-II

(Synthetic magnetic nanoparticles, their biomedical applications and toxicity)

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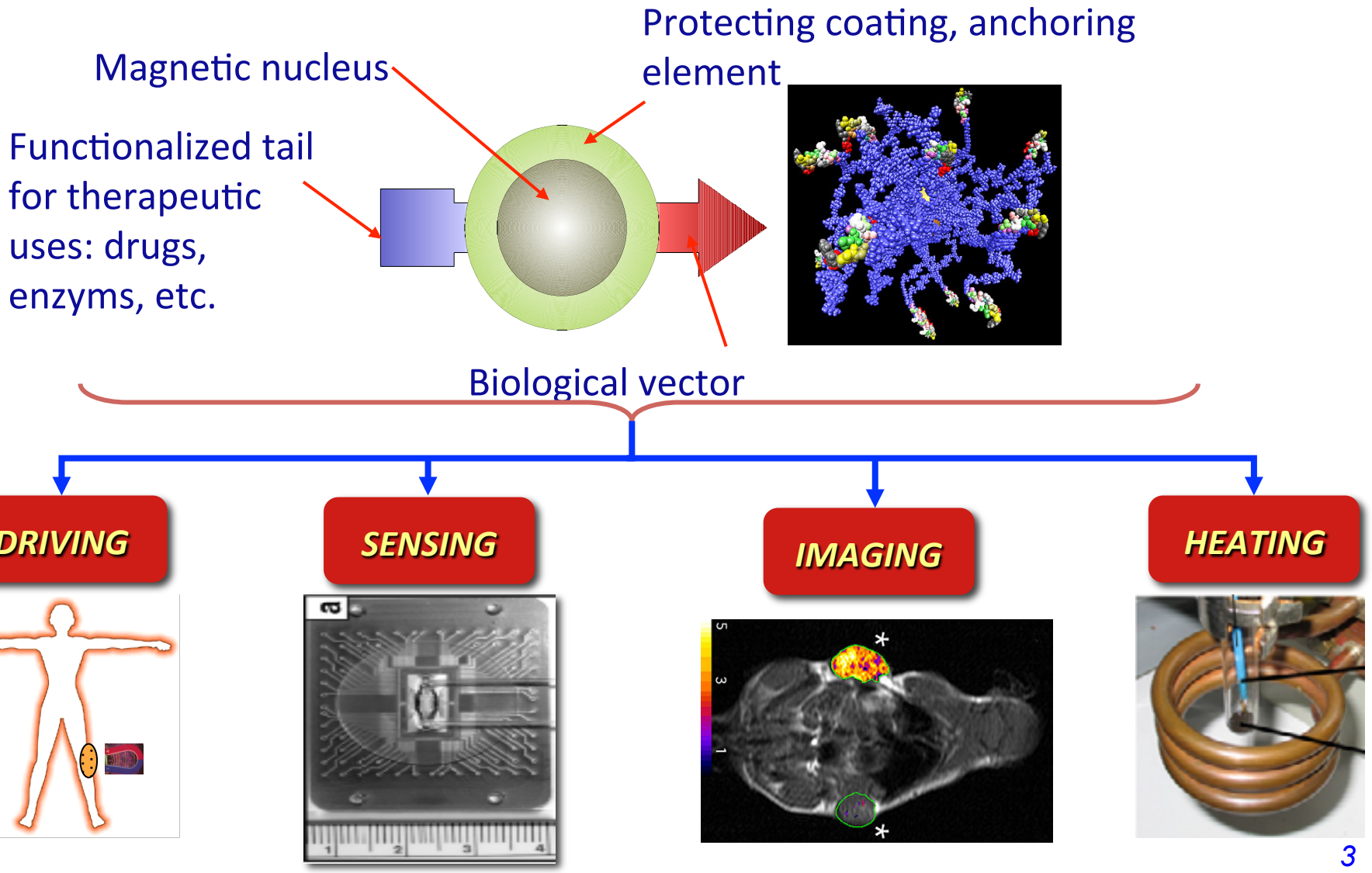
Outline P.2

In this 2nd part we will cover:

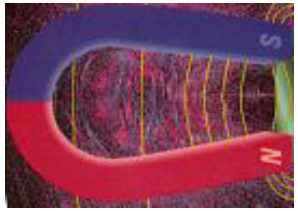
- Magnetic nanoparticles functionalities of biomedical interest:
  - moving
  - sensing
  - imaging
  - heating
- Multifunctionality
- A word on toxicological issues
- Hemocompatibility

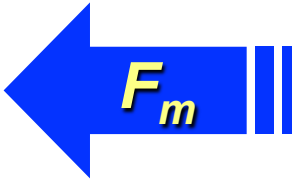
The magnetic functionality

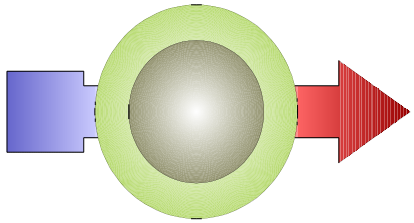


Magnetic force-driven applications

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 $B \cdot H/2$:

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

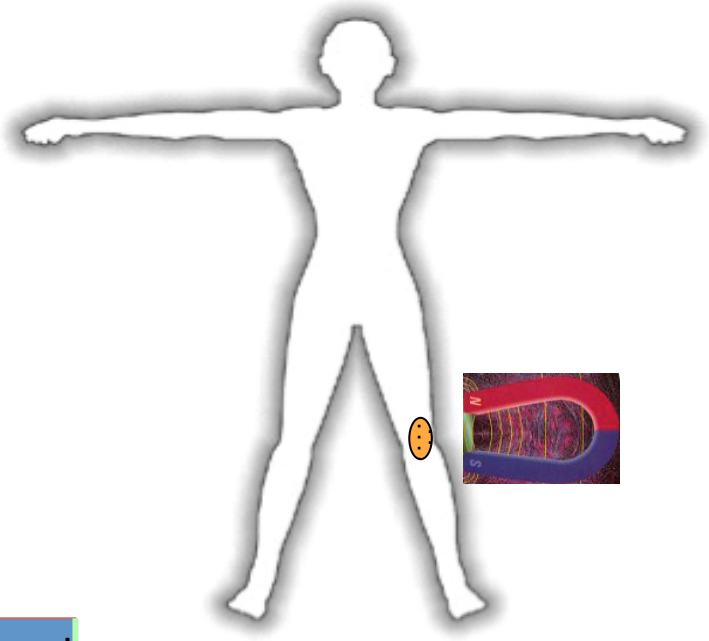
Thus if $\Delta\chi > 0$, F_m acts in the direction of the steepest ascent of the energy density scalar field.

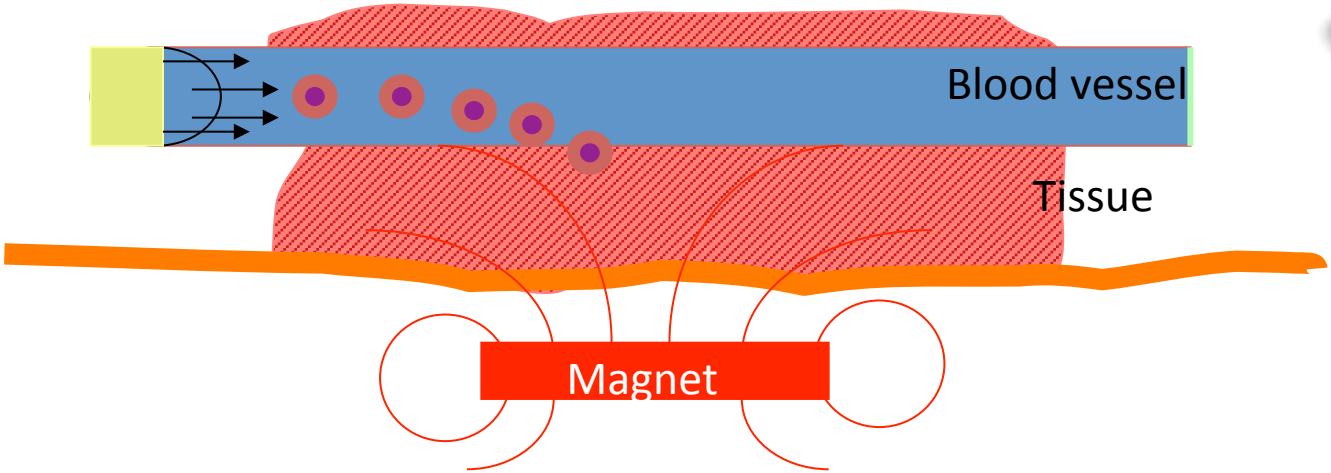
Magnetic force-driven applications

DRIVING

Targeted drug delivery

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





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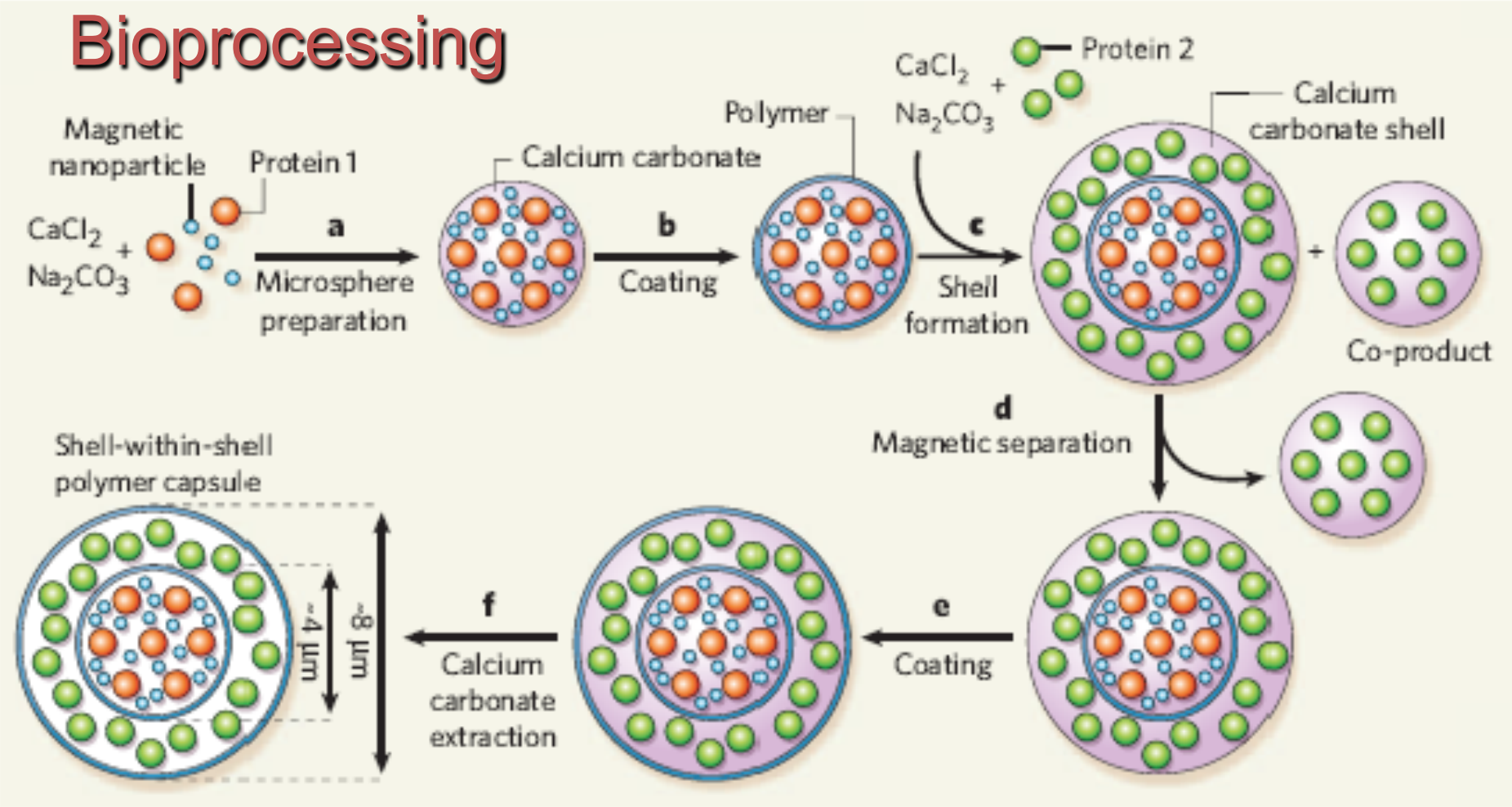
Magnetic force-driven applications

DRIVING

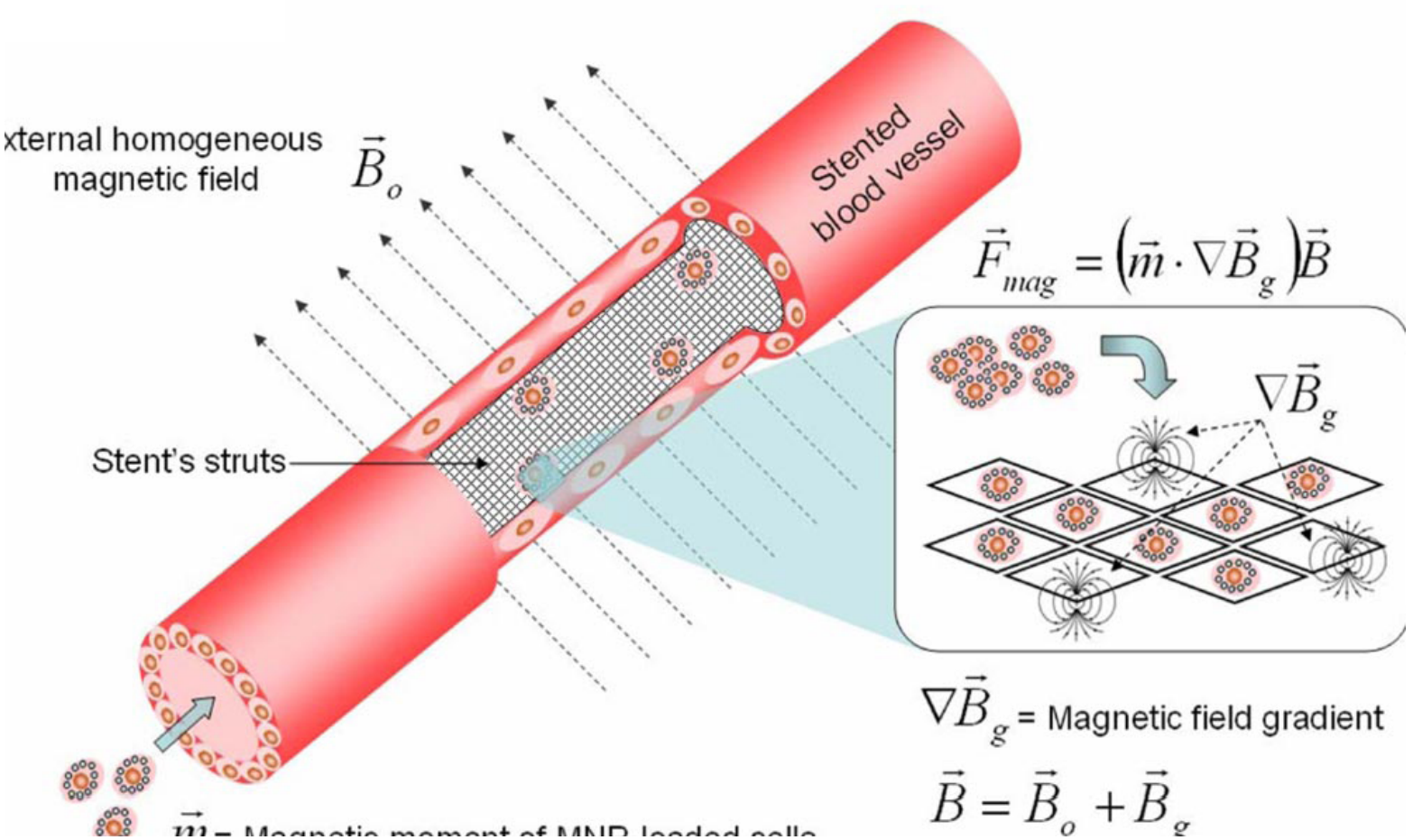
Bioprocessing



O. Kreft, Angew. Chem. Int. Edn **2007** *46* 5605

Magnetic force-driven applications

DRIVING



B. Polyak et al. PNAS **105**, 698 (2008)

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SENSING

Requirements for a good biosensor:

- Stable
- Highly specific
- Independent from external parameters (temperature, pH, others)
- Precise
- Reproducible
- Low electronic noise
- Biocompatible

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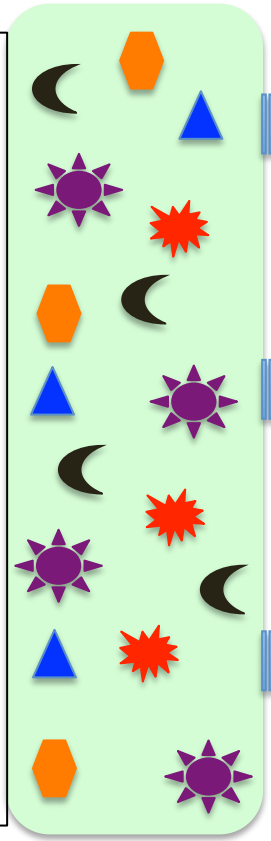
SENSING

Biofunctional membrane

- Enzimes
- Proteins
- Antibodies
- Receptors
- Microbes
- Organelles
- Animal cells
- Plant cells
- Animal tissues
- Plant tissues



Transducer

Chemical sustance	→	Electrode Semiconductor
Weigth	→	Cantileber
Light	→	Photomultiplier Photodiode
Sound	→	Sound detector
Heat	→	Thermistor
Electrical signal	→	

ELECTRICAL SIGNAL

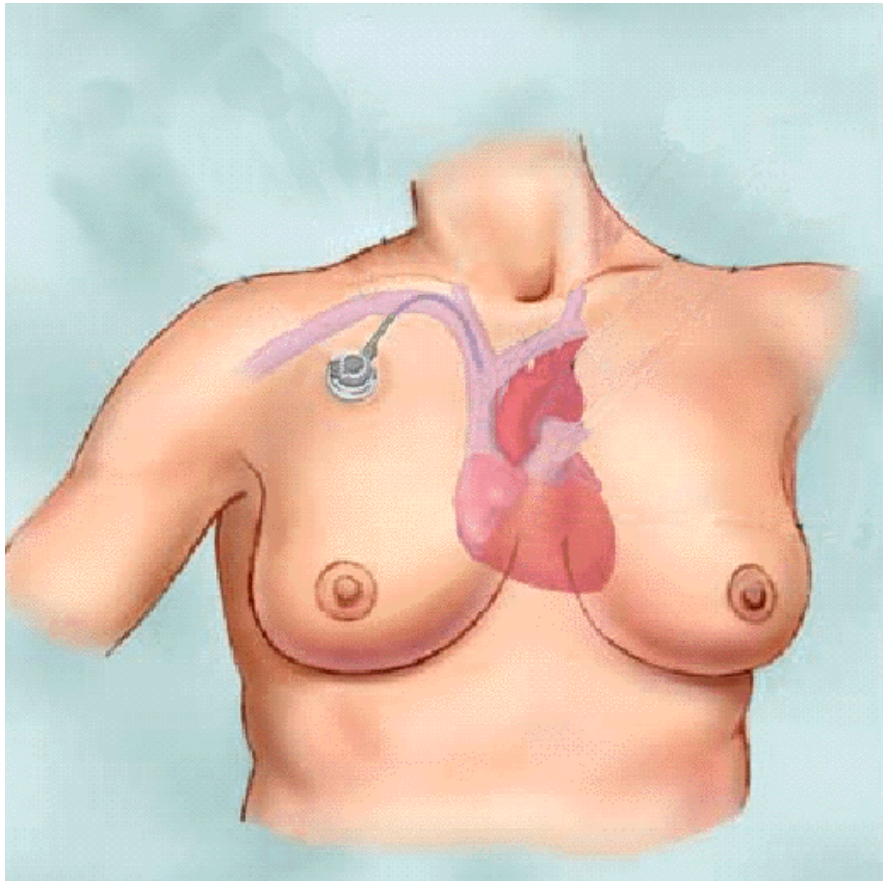
Molecular recognition system

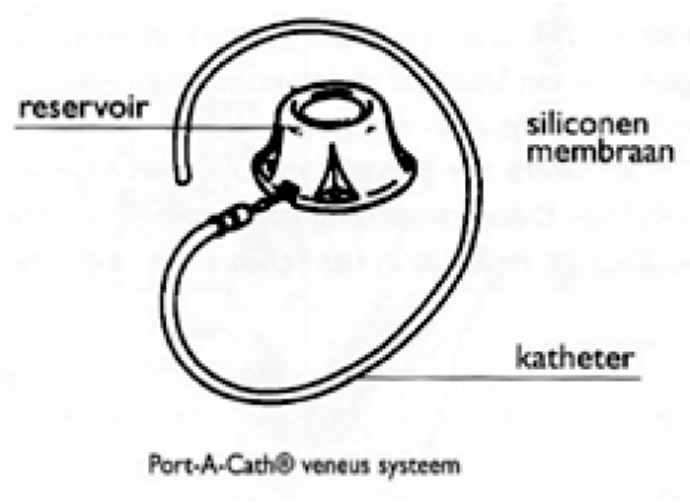
Converting molecular parameters into measurable ones

Transdution to electric signals

Implantable Biosensor

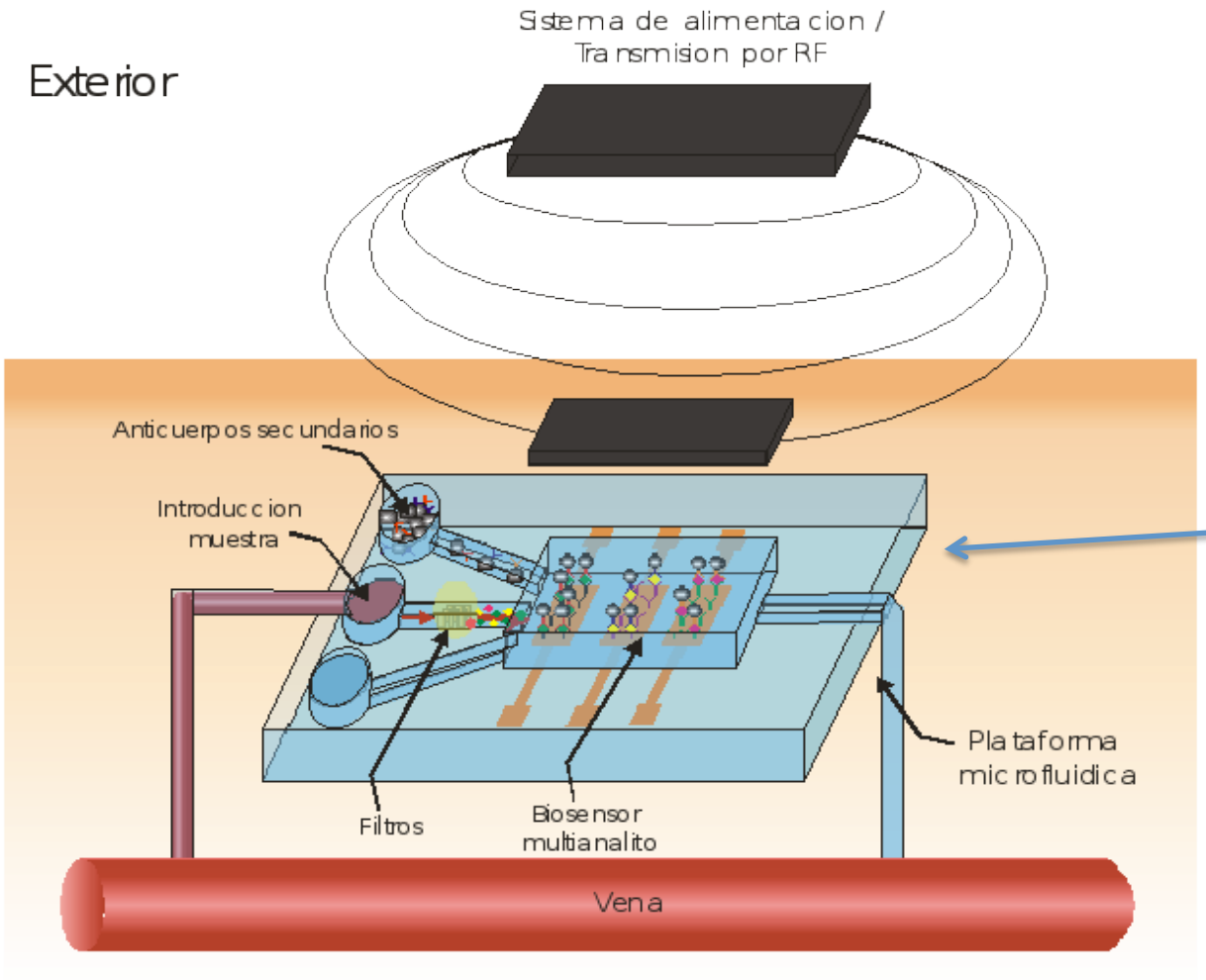
SENSING





Implantable Biosensor

SENSING



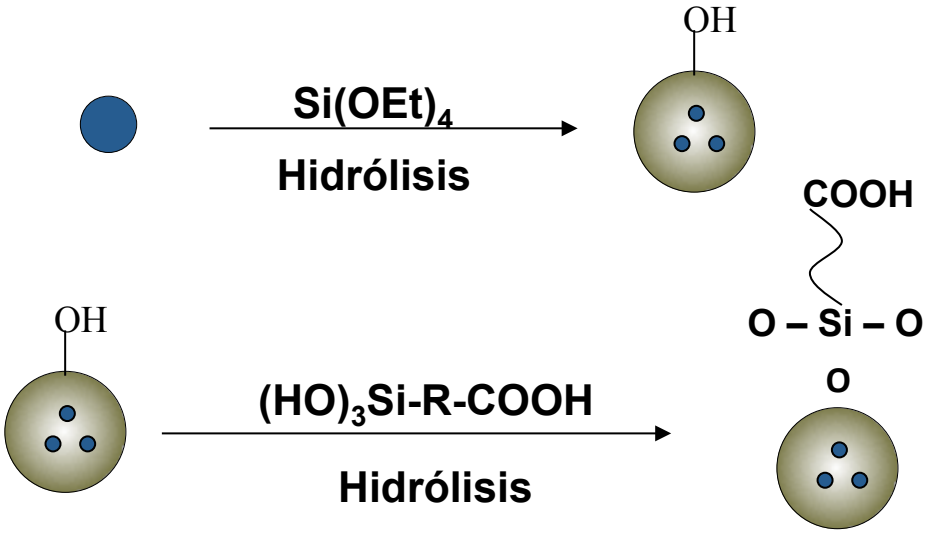
Transduction & transmision system

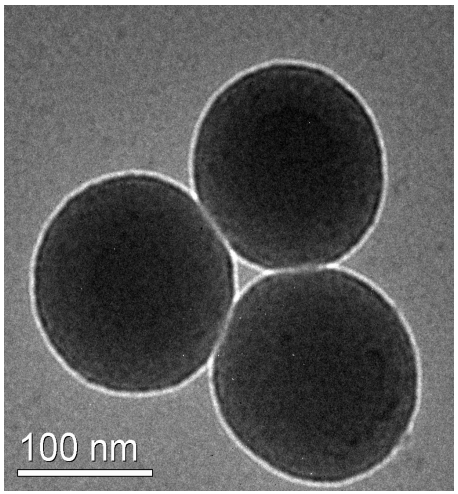
Biosensor

Microfluidic system

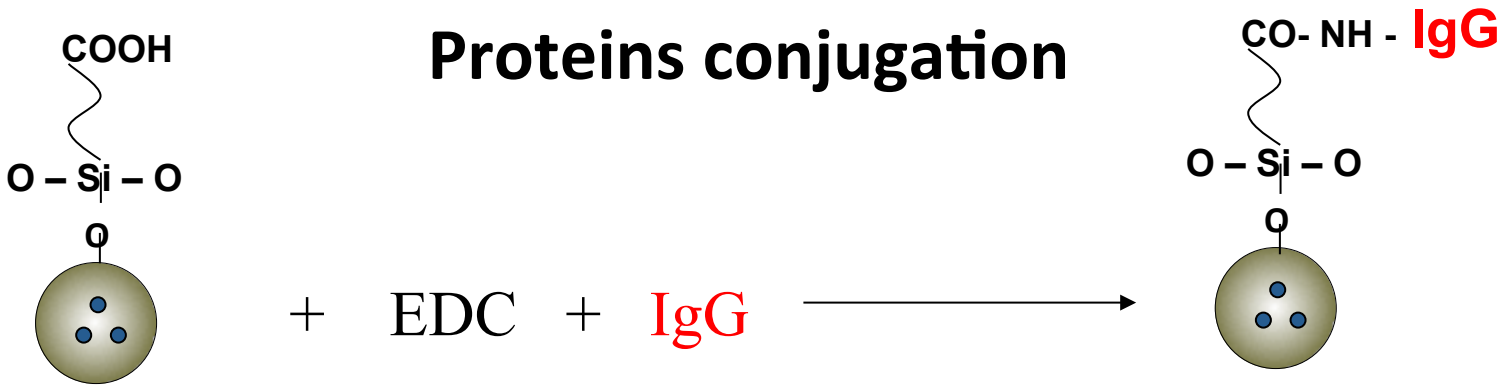
Nanobeads for a biosensor

SENSING





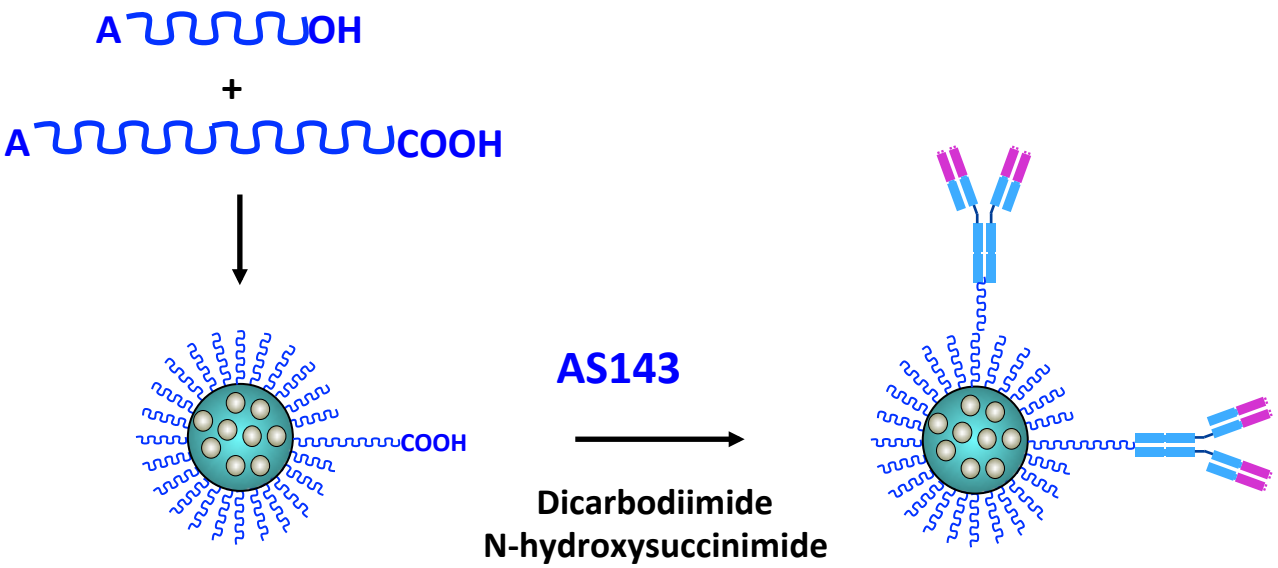
Proteins conjugation



Doping control sensor

SENSING

Antibody: AS143 (anti Methylboldenone androgenic anabolic steroid)



Doping control sensor

SENSING

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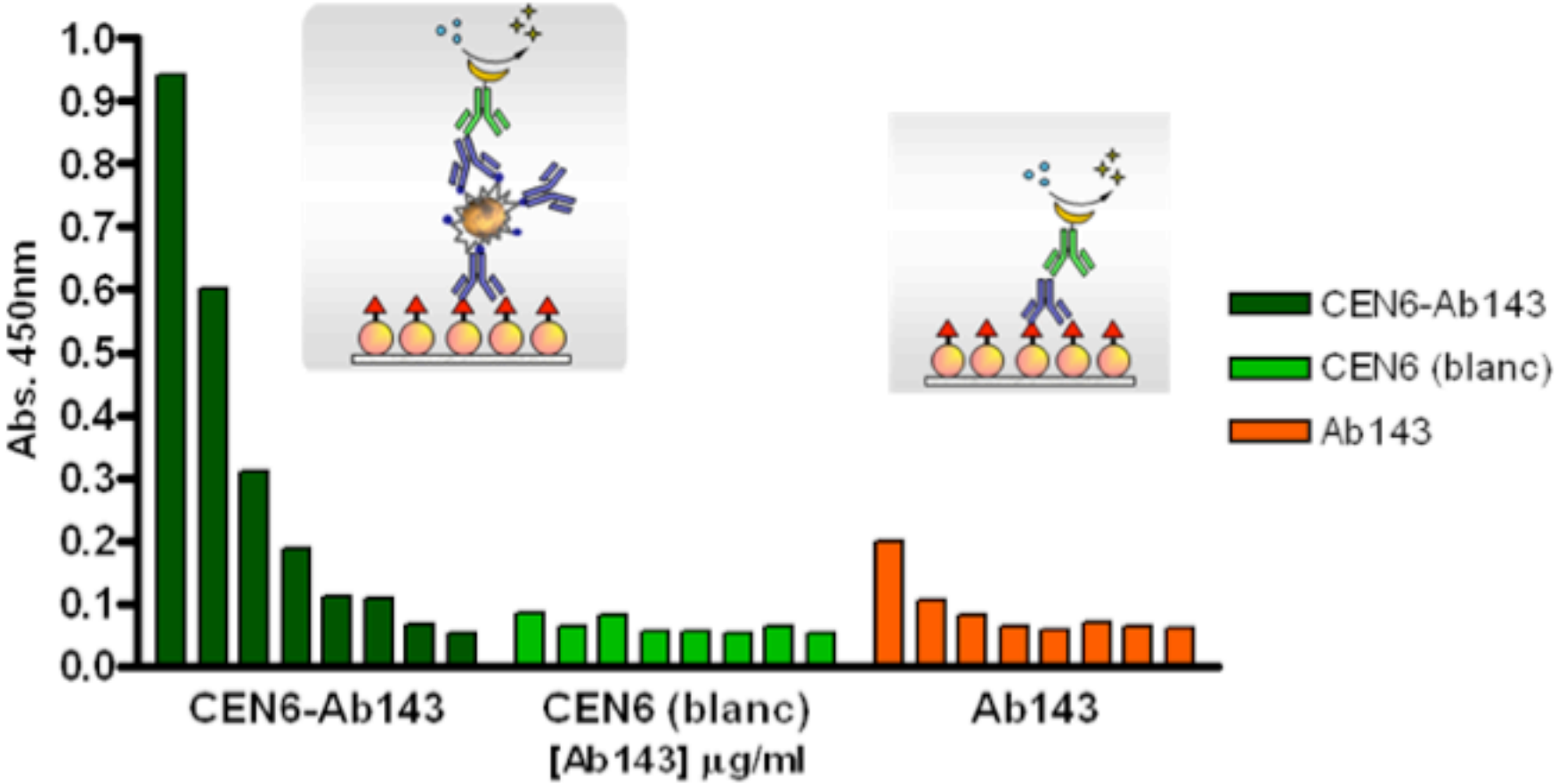

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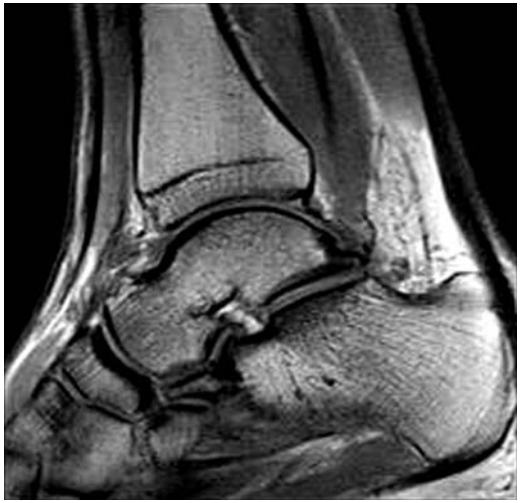
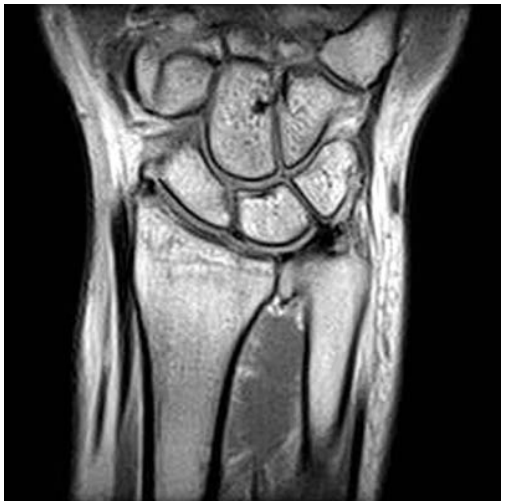
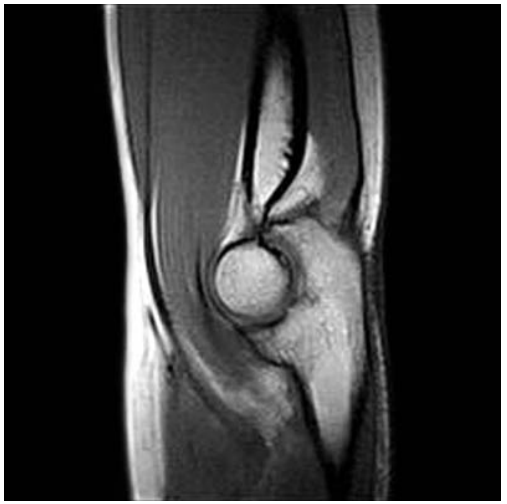
IMAGING



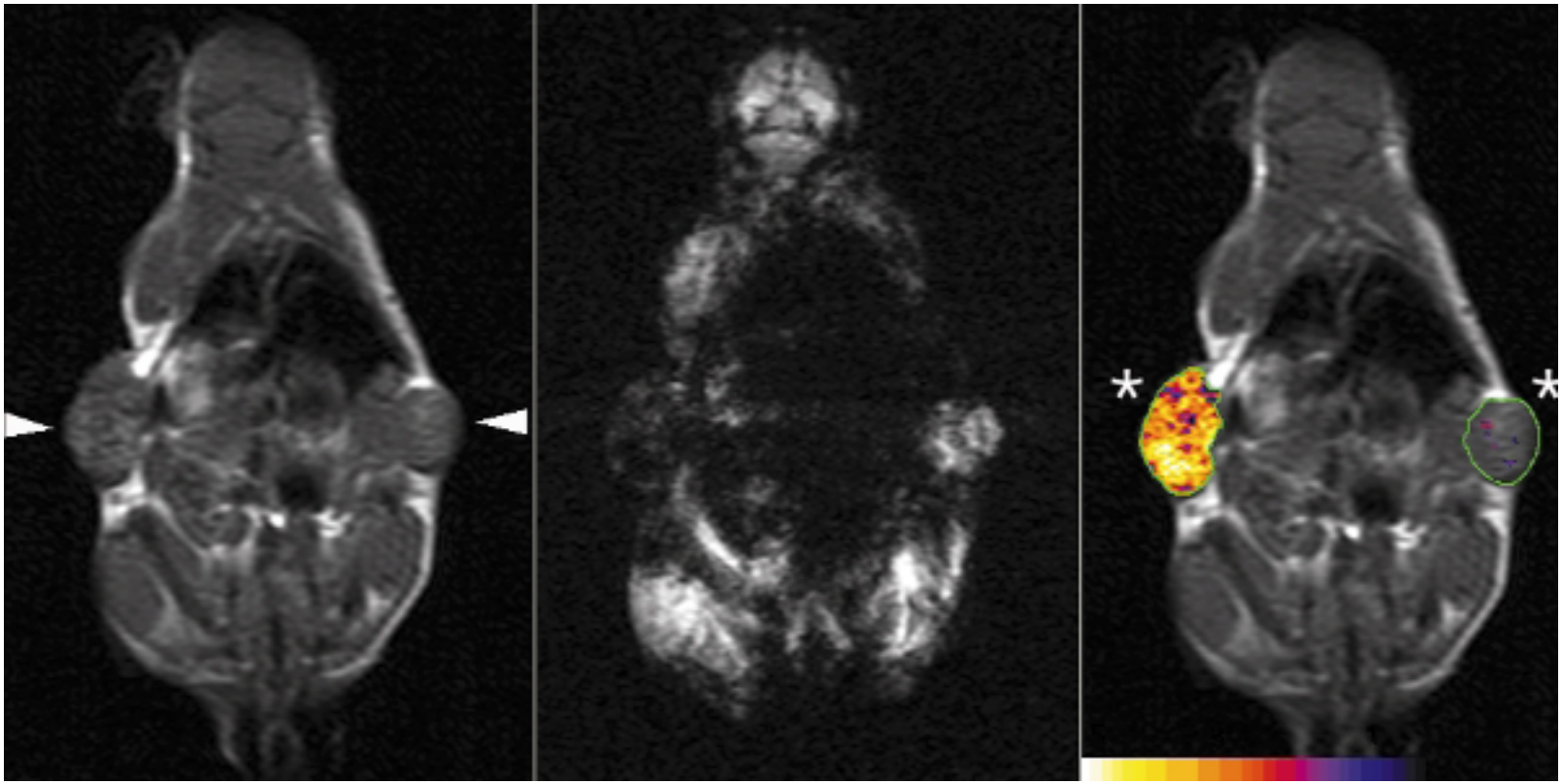




Low field 0.2 T MRI Imager by Esaote
(for ariticulations)

Magnetic nanoparticles as contrast agents for MRI

IMAGING



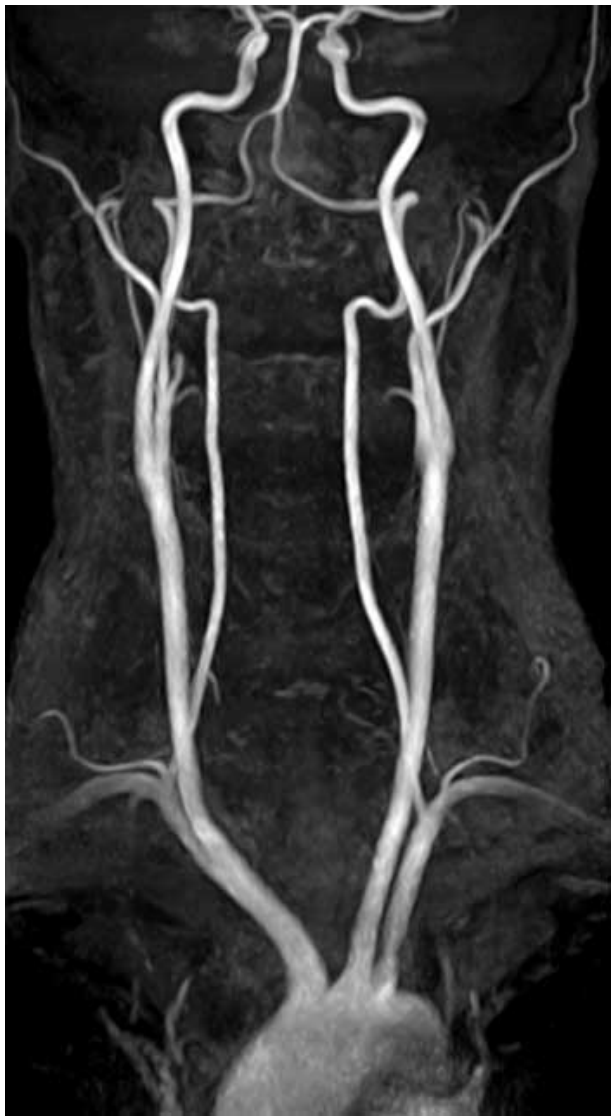
R. Weissleder et el., Nature Med. **6**(2000)351

Magnetic nanoparticles as contrast agents for MRI

IMAGING

MRI angiography by use of CA (contrast enhanced MRA)

- MRI able to evaluate the **blood vessels non-invasively**, known as **MR angiography (MRA).**
- MRA can evaluate blood vessels of the **head and neck** to detect vessel narrowing (**stenosis**), blood vessel **blockage**, cerebral **aneurysm**, arteriovenous malformation (**AVM**) and blood vessel **dissection**. This technique may be utilized for evaluation of both **arteries and veins**.
- **Contrast enhanced MRA** utilizes an intravenous injection of **MRI contrast media (Gd-DTPA).** This safe technique does not require hospital admission or arterial injection, unlike conventional angiography.



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)

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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

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

- Efficacy of a contrast agent in reducing T_1 or T_2 is evaluated by measuring the **relaxivity** r_i (i=1,2), that represents the relaxation rate of hydrogen nuclei in presence of 1mM of contrast agent
- The relaxation rate is the sum of the diamagnetic contribution (absence of CA) and the praramagnetic one (presence of CA)

$$\frac{1}{T_i} = \frac{1}{T_{i,d}} + \frac{1}{T_p} \qquad \frac{1}{T_i} = R_i = \frac{1}{T_{i,d}} + r_i c$$

c = concentration of CA expressed in mM/L

Magnetic nanoparticles as contrast agents for MRI

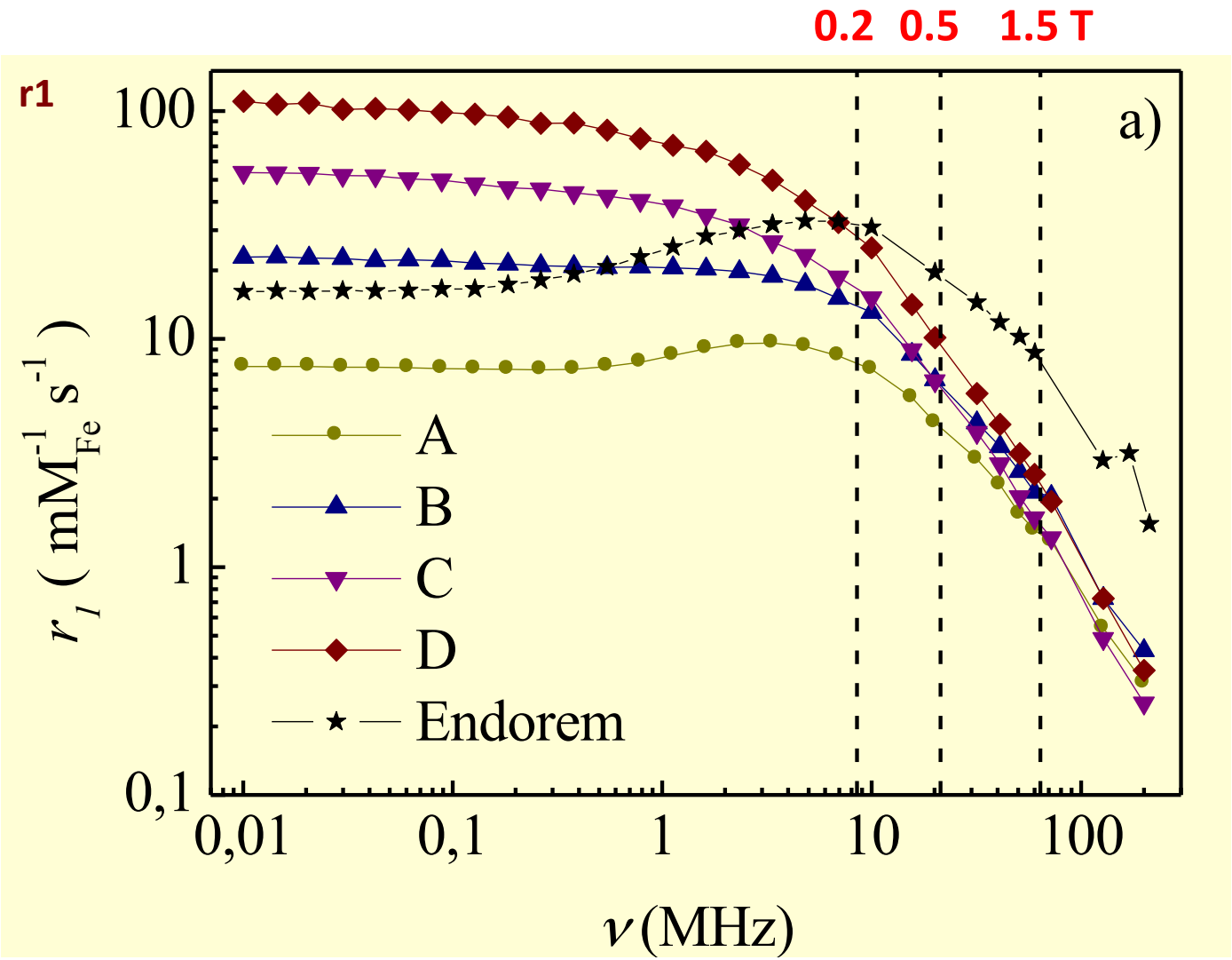
IMAGING

- 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 nanoparticles as contrast agents for MRI

IMAGING

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



Magnetic nanoparticles as contrast agents for MRI

IMAGING

A = 7.4 nm
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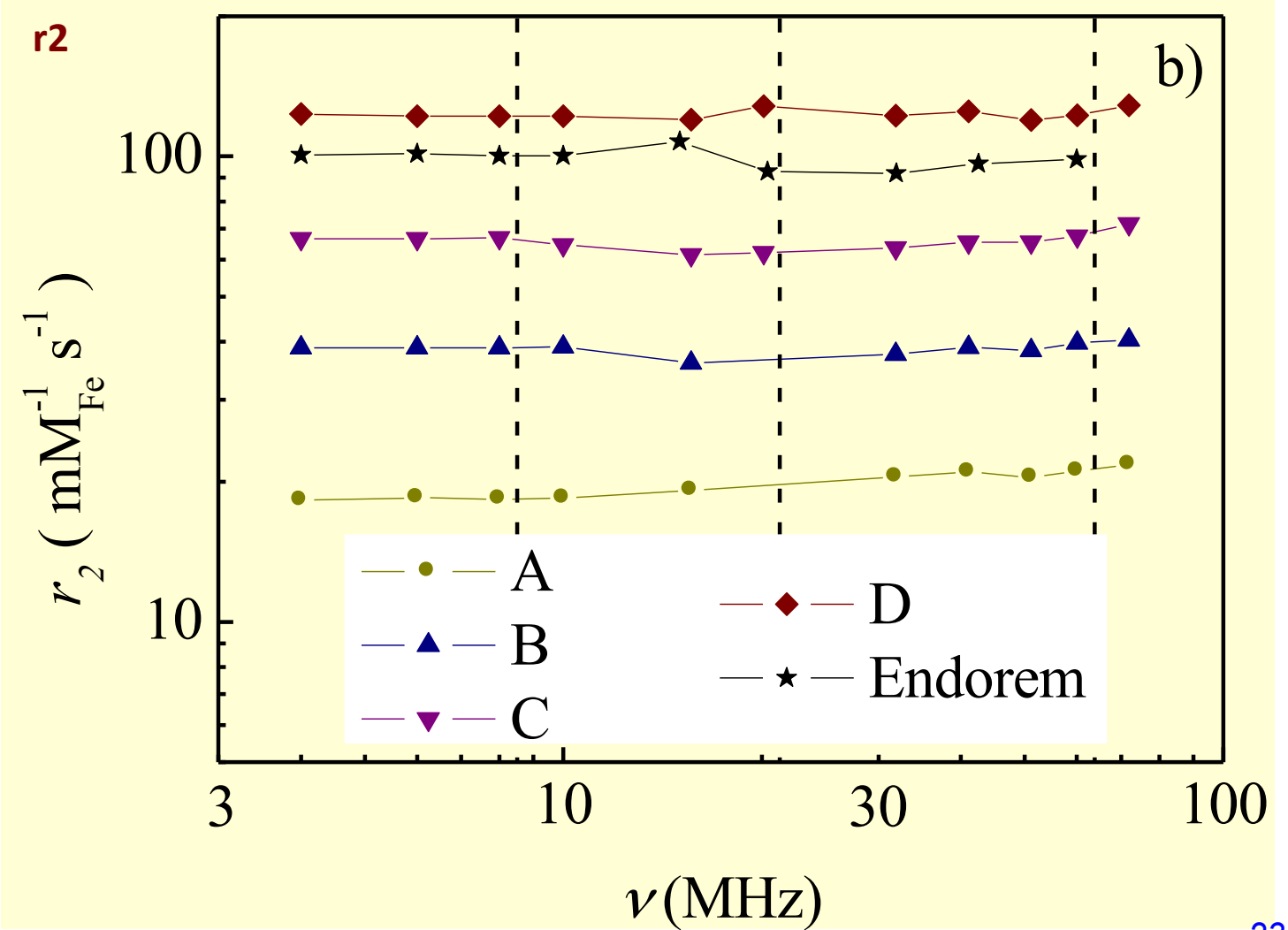
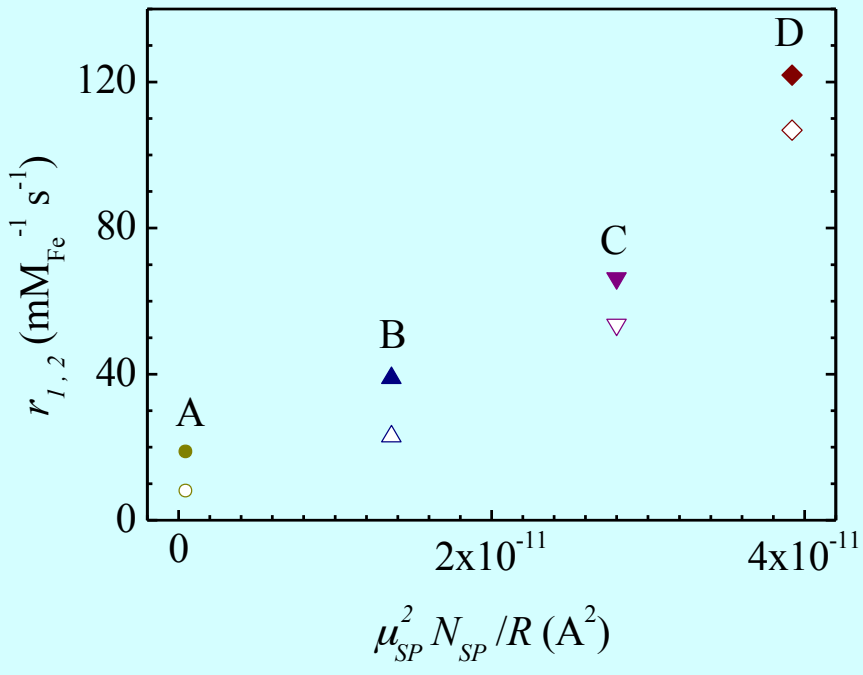


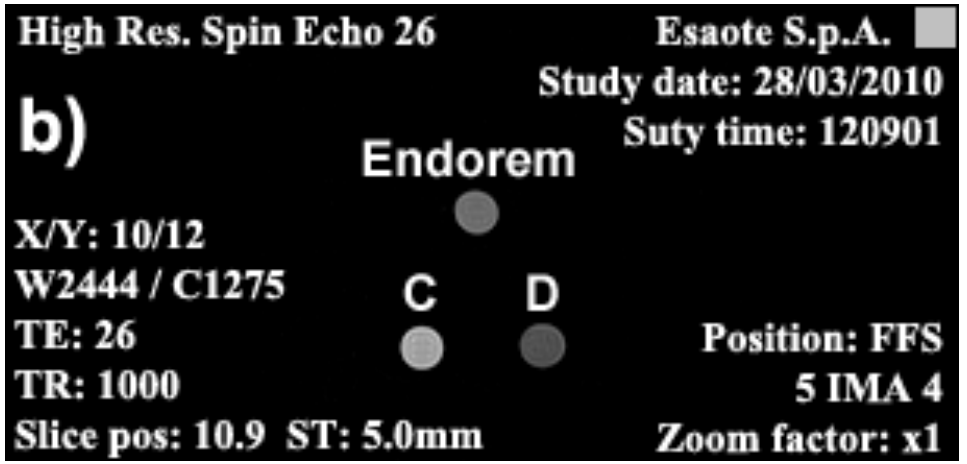
Magnetic nanoparticles as contrast agents for MRI

IMAGING

$r_{1,2}$ dependence with size



MRI imagens



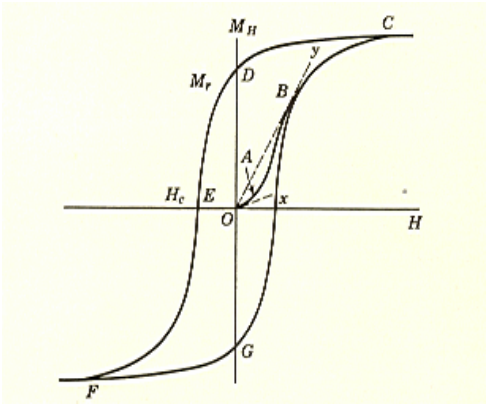
H. Amiri, et al., Magn. Res. in Medicine, **66**, 1715-1721, (2011)

Magnetic heating

HEATING

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

- inductive heating (eddy currents)
- hysteresis losses



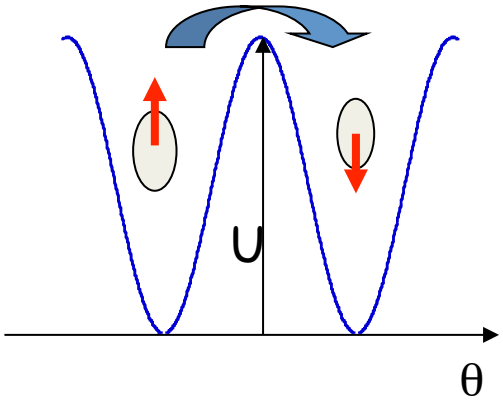
$$P_{FM} = -\mu_0 f \oint H dM$$

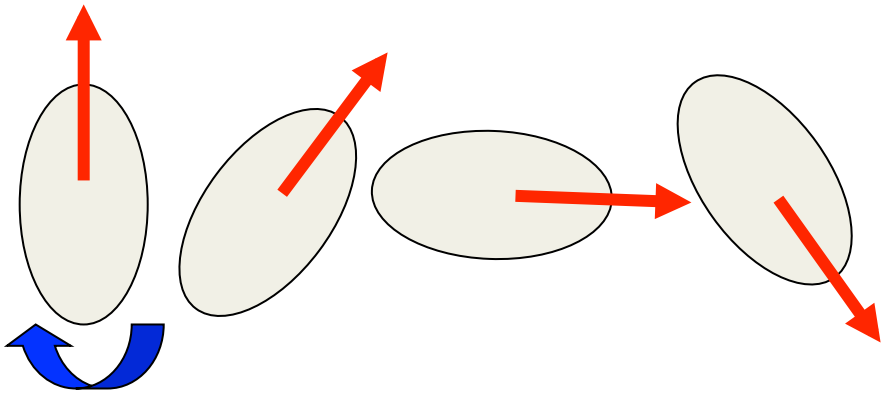
No relevant at low ac magnetic fields

Magnetic heating

HEATING

Superparamagnetic particles





Neél relaxation

Rotational Brownian motion

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

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Magnetic heating

HEATING

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

Biological limitations

50 kHz ≤ f ≤ 1200 kHz,

H < 15 kA/m

(H · f)max = 485 kHz·kA/m

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

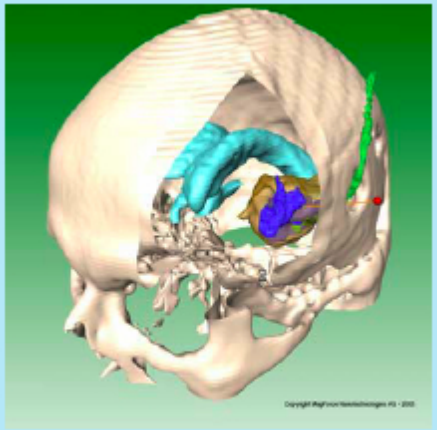
"*test person had a sensation of warmth, but was able to withstand the treatment for more than one hour without major discomfort*"

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

Magnetic heating

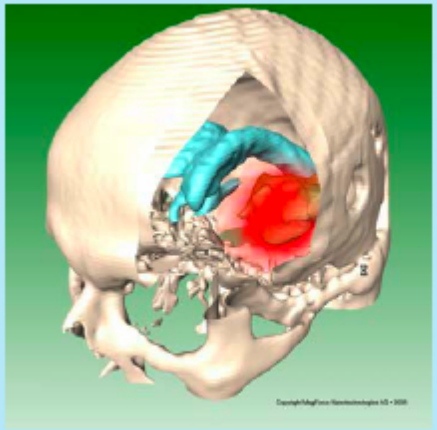
HEATING

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

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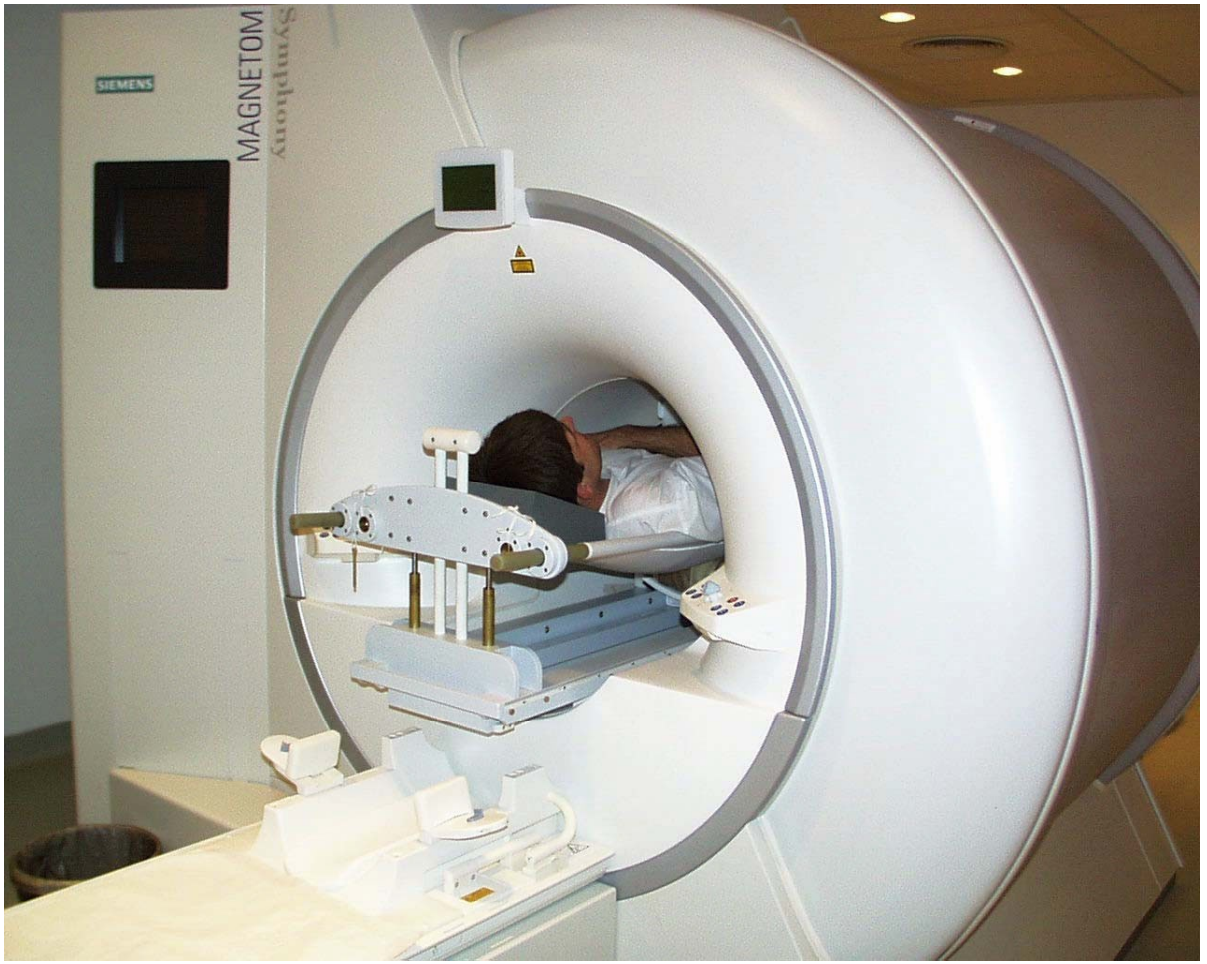
Magnetic heating

HEATING

Direct injection of MNP into the tumor: clinical phase II



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Magnetic heating

HEATING

Direct injection is

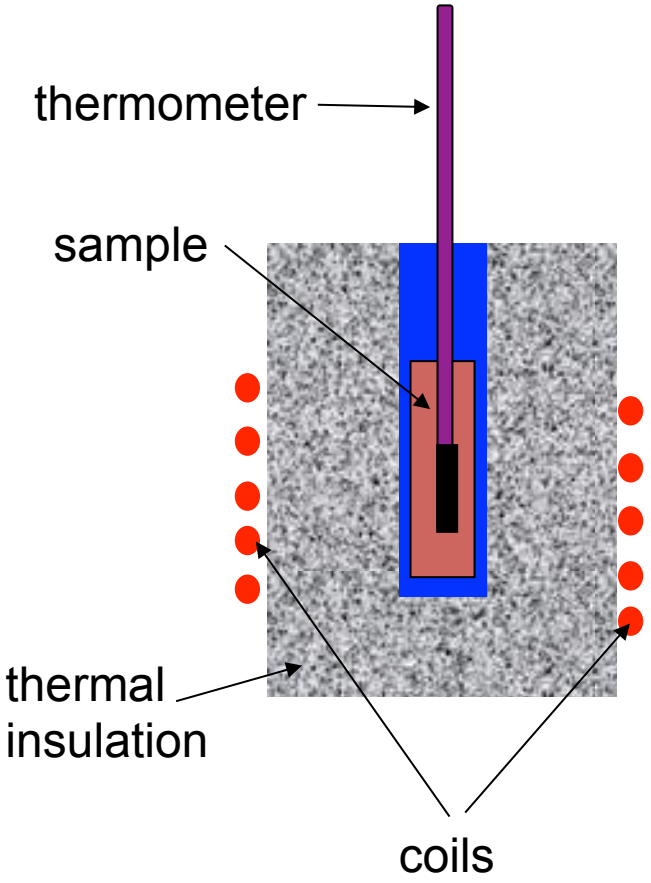
- non-selective
- contaminates healthy tissues
- only valid for large tumors
- good heating efficiency as doses can be very high

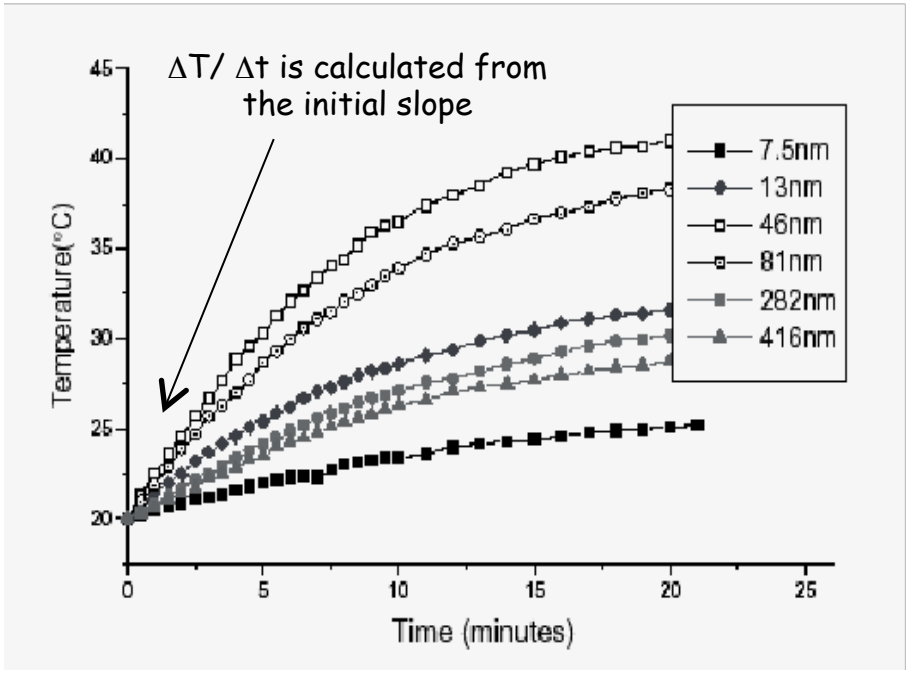
Biological vectorisation is

- 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

Magnetic heating

HEATING





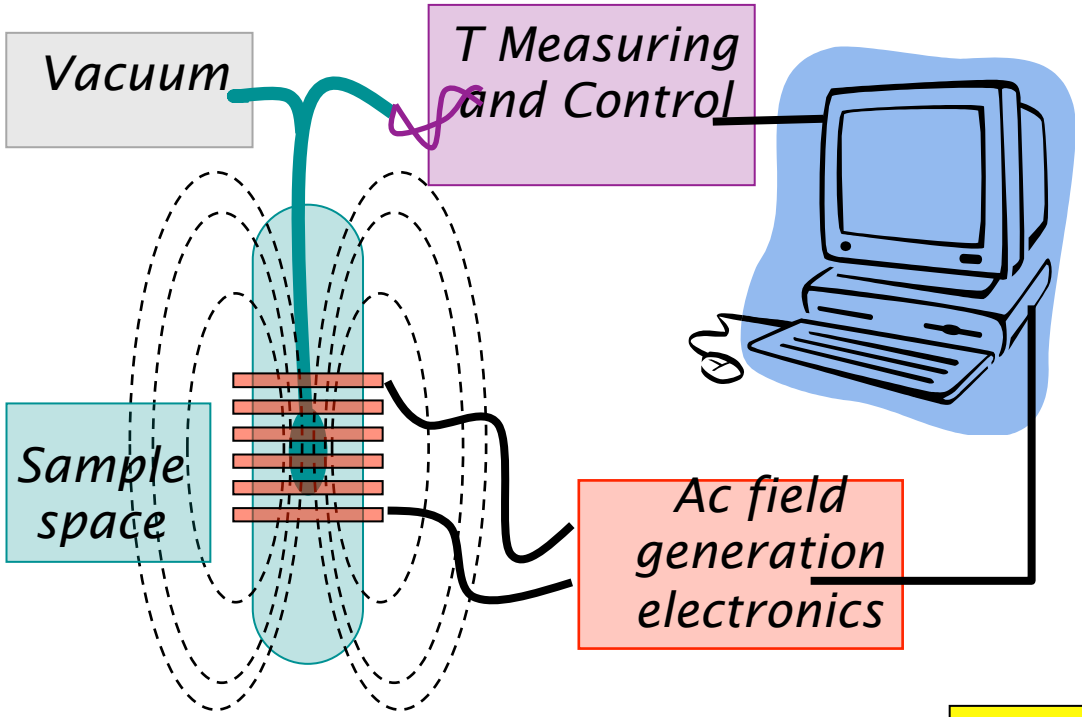
$$SAR_{(W/g)} = C\frac{\Delta T}{\Delta t}\cdot\frac{1}{m_{SPM}}$$

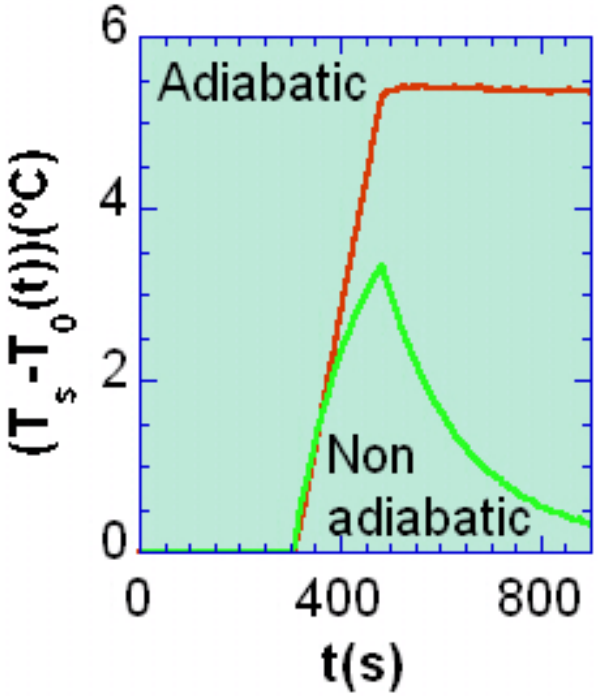
Biological applications:
$H \cdot f < 4.85 \cdot 10^8$ A/m·s

H < 15 A/m, f < 400 kHz

Magnetic heating

HEATING





$$SAR_{(W/g)} = C\frac{\Delta T}{\Delta t}\cdot\frac{1}{m_{SPM}}$$

Biological applications:

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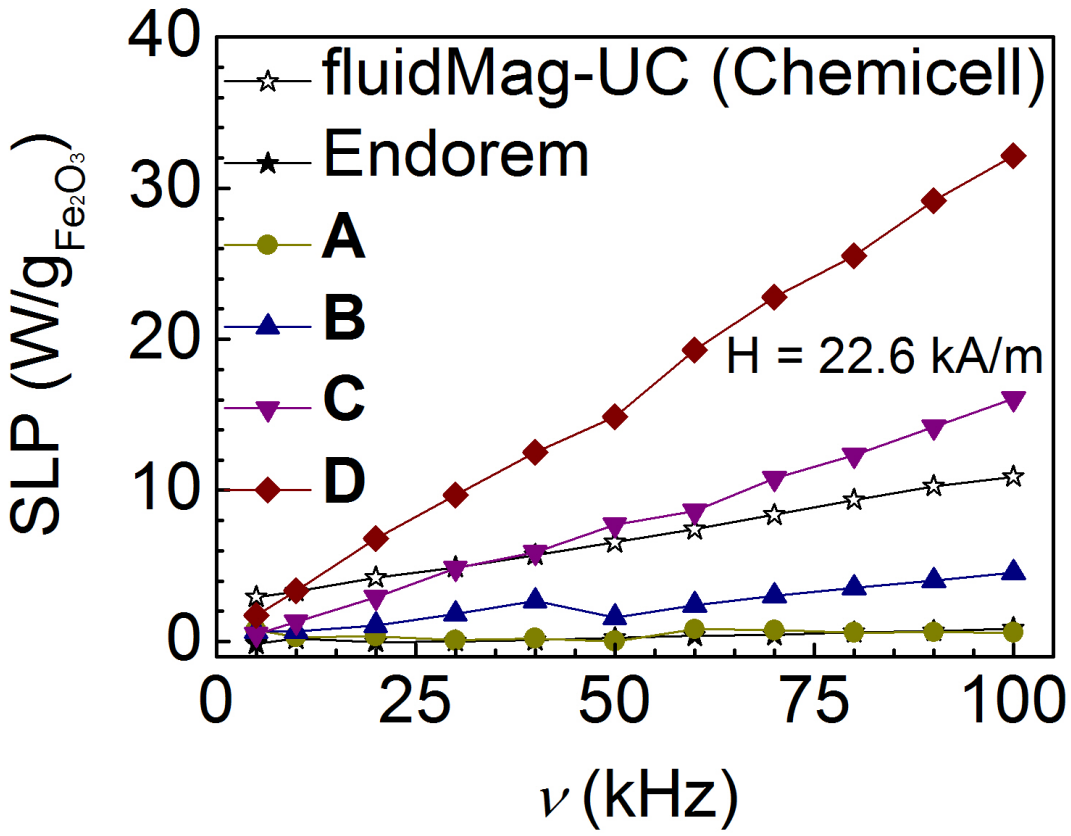
H < 15 A/m, f < 400 kHz

Magnetic heating

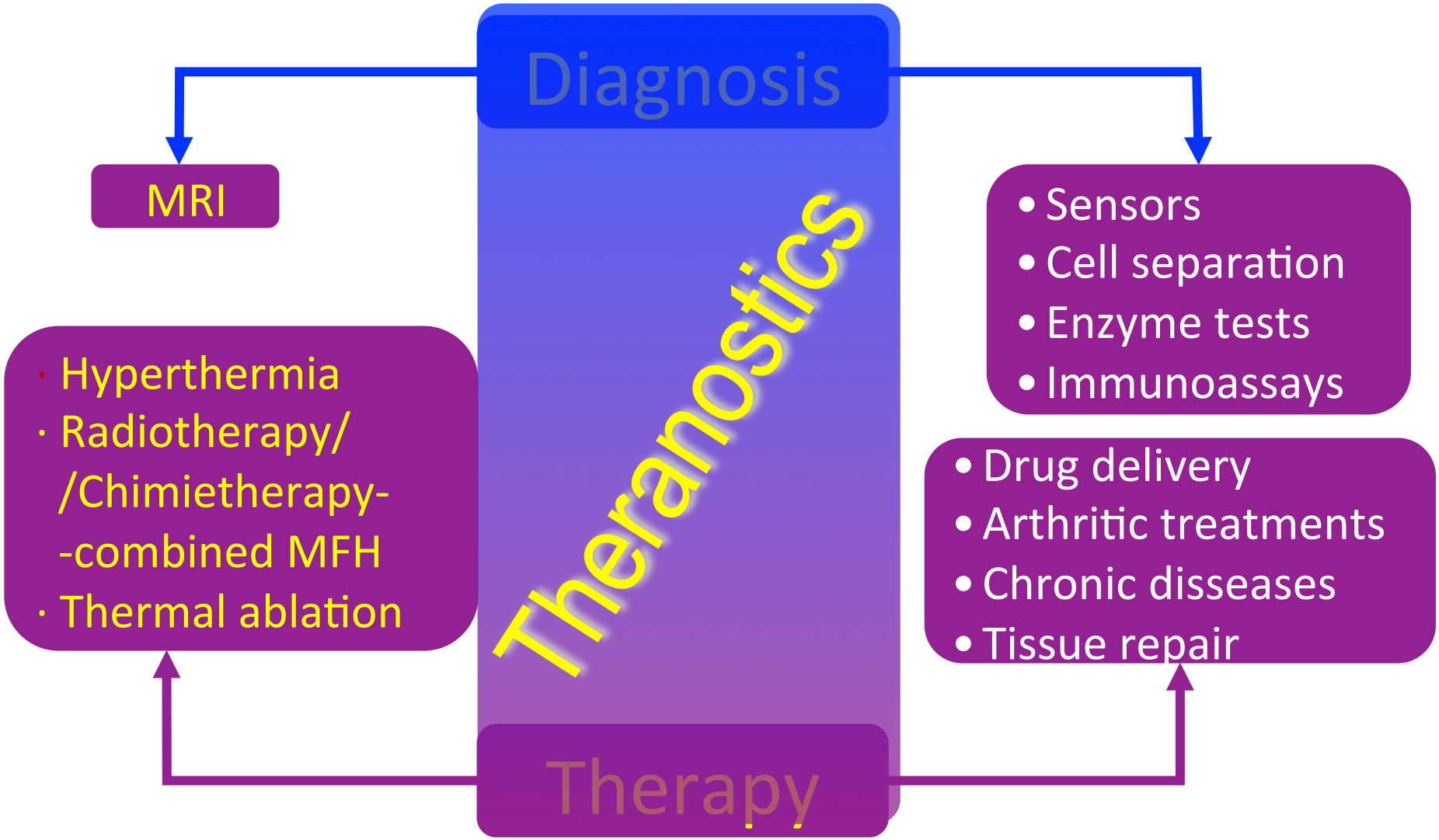
HEATING

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

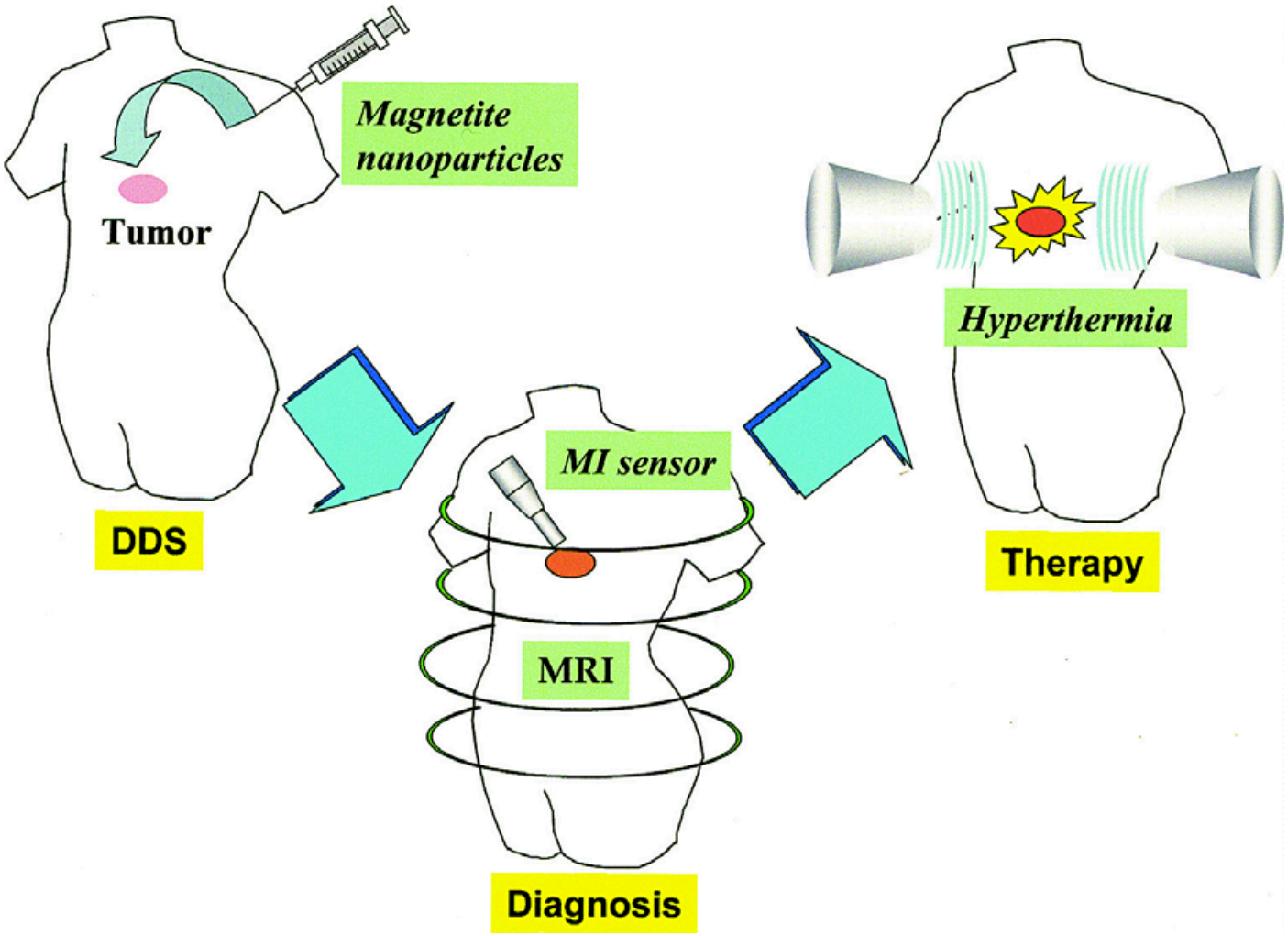


Multifunctionality

Biomedical Applications of Magnetic Nanoparticles

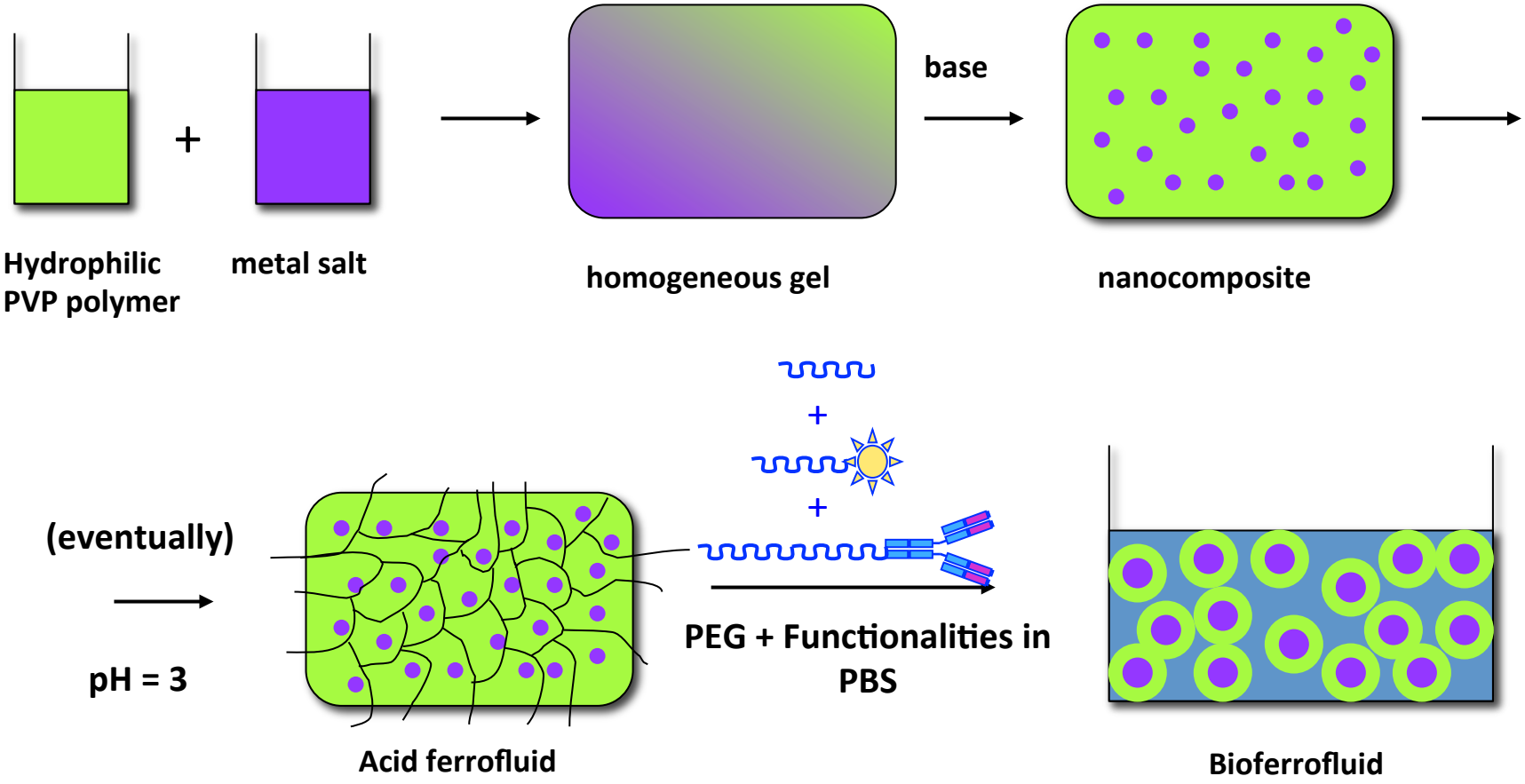


Biomedical Applications of Magnetic Nanoparticles



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

Tailoring MNP with polymers

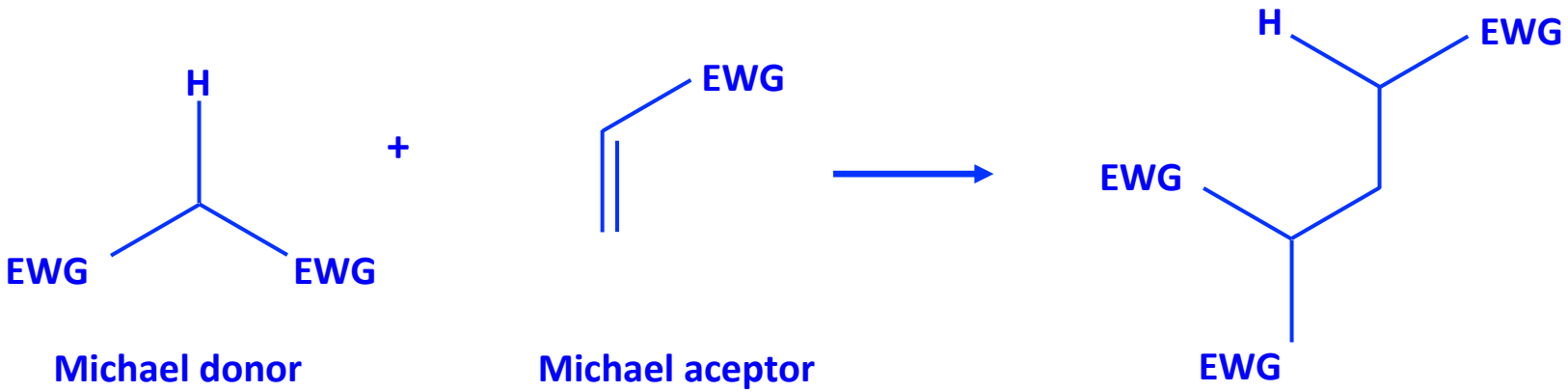


A. Millán and F. Palacio, Applied Organometallic Chemistry, **15**, 396-400 (2001)
A. Millán, F. Palacio et al., Acta Materialia **55**, 2201-9 (2007)
A. Millán, F. Palacio et al., Patent ES2308901B1

Tailoring MNP with polymers

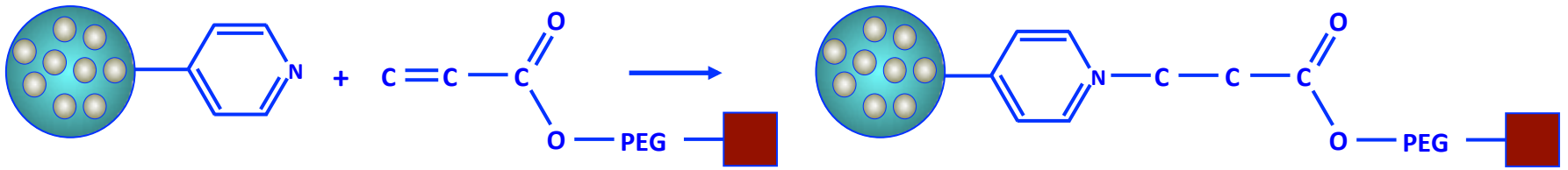
An easy route for functionalisation: Michael reaction

EWG: electron withdrawing group



Michael donor
carbanion o nucleophile

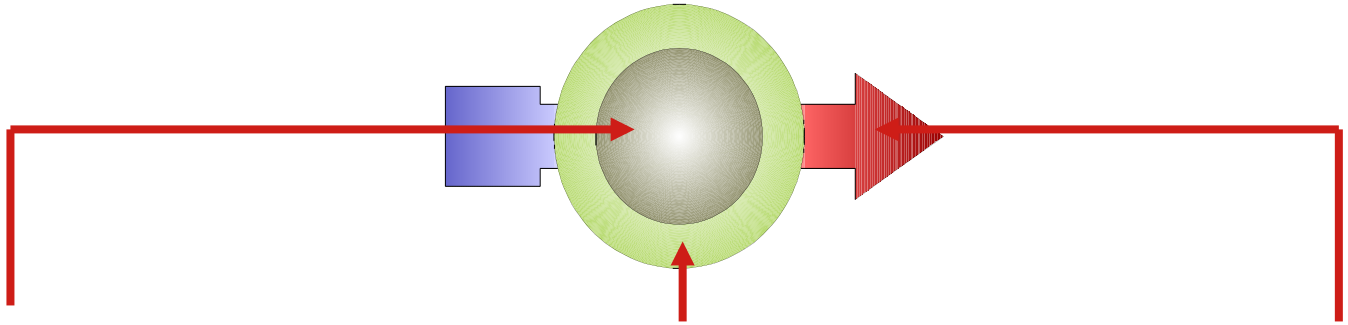
Michael aceptor
conjugated insaturated system



Physical or biological functionality

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

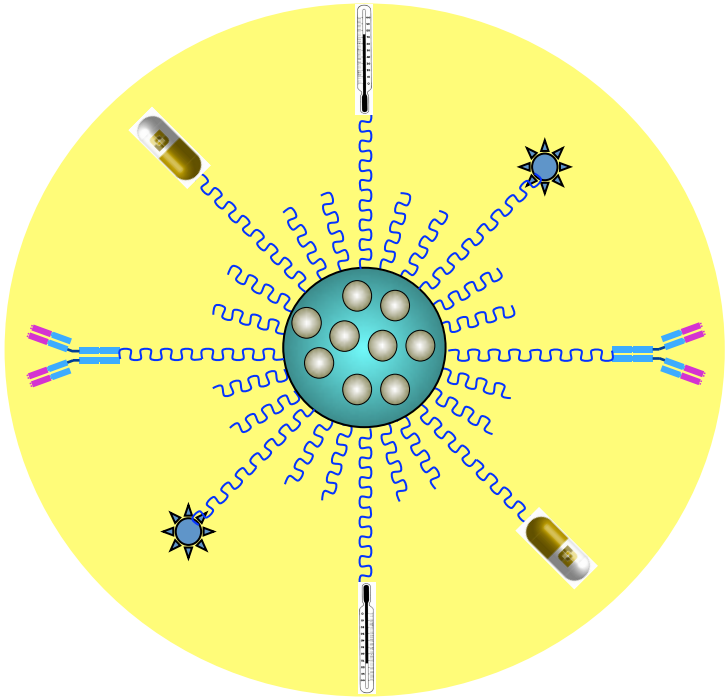
Multifunctional MNP for Biomedical Applications



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

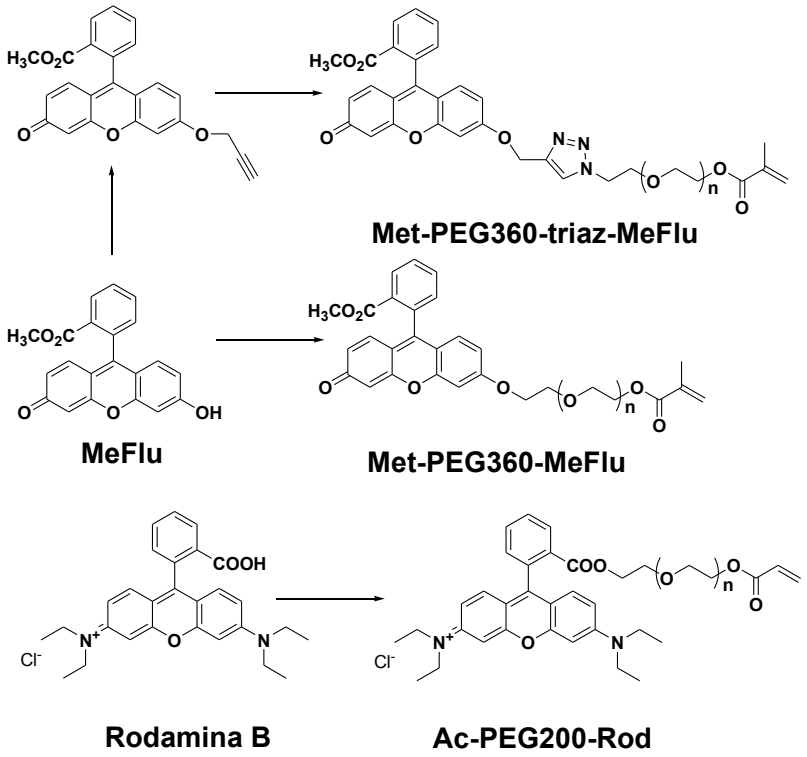
Antibody

Molecular Thermometer

Drug

Multifunctional nanoplatform

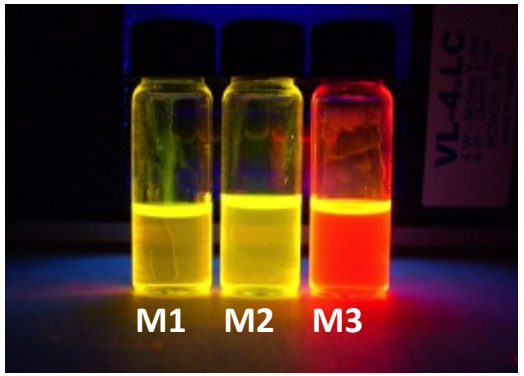
Synthesis of fluorescent groups



M1

M2

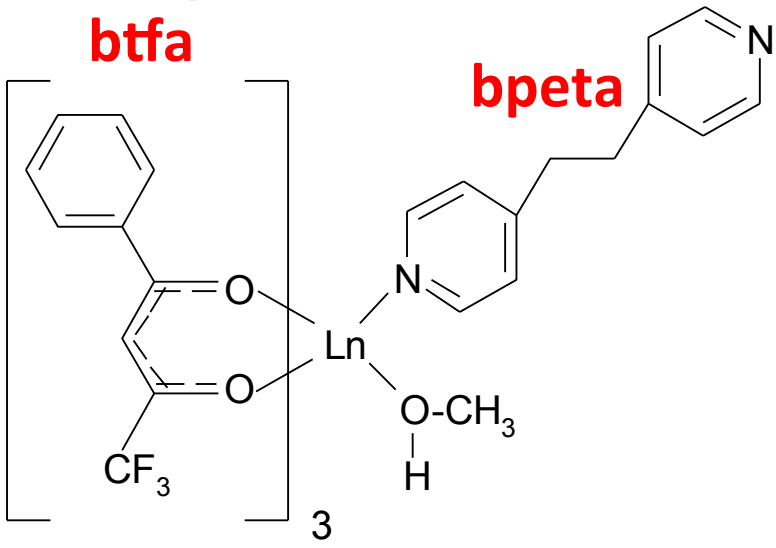
M3



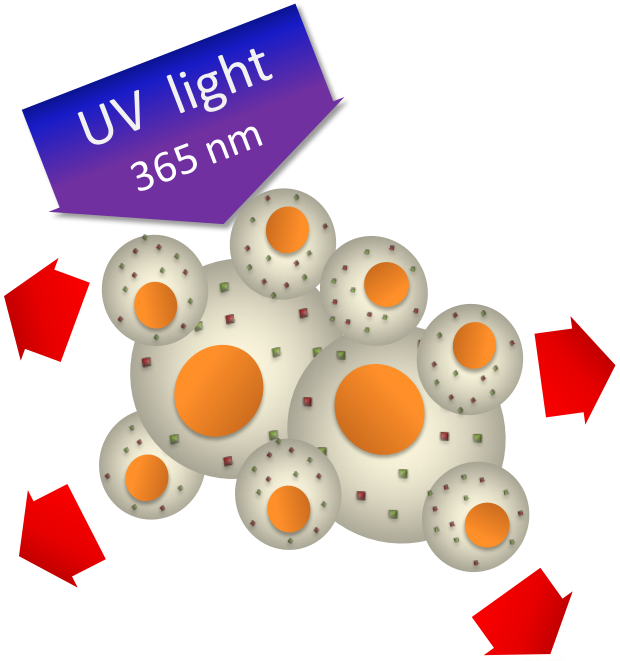
c: 5.10^{-3} M (diclorometano)

Thermometer design

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



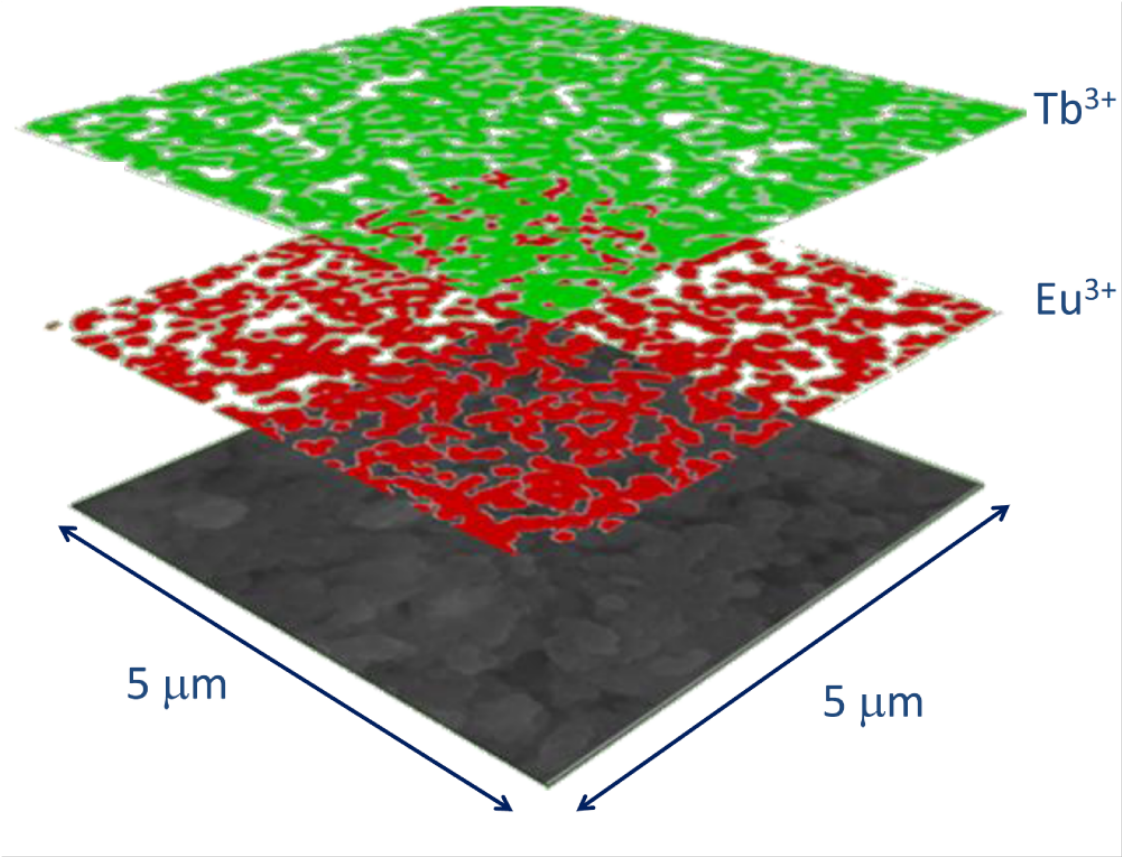
- Organic-inorganic hybrid NPs formed by a maghemite (γ-Fe_2O_3) magnetic core coated with a tetraethyl orthosilicate/ aminopropyltriethoxysilane ***(TEOS/ APTES)*** organosilica shell (modified Stöber method)



- 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)**

EDS mappings

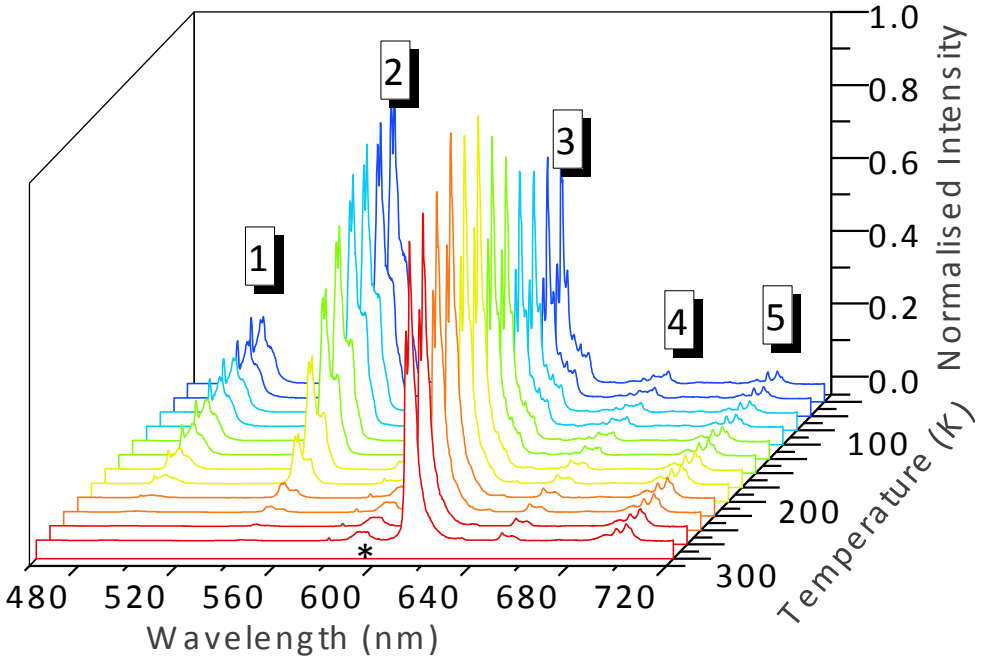
EDS mappings show Eu^{3+} and Tb^{3+} distributions with contours and shapes similar to those of the NPs



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

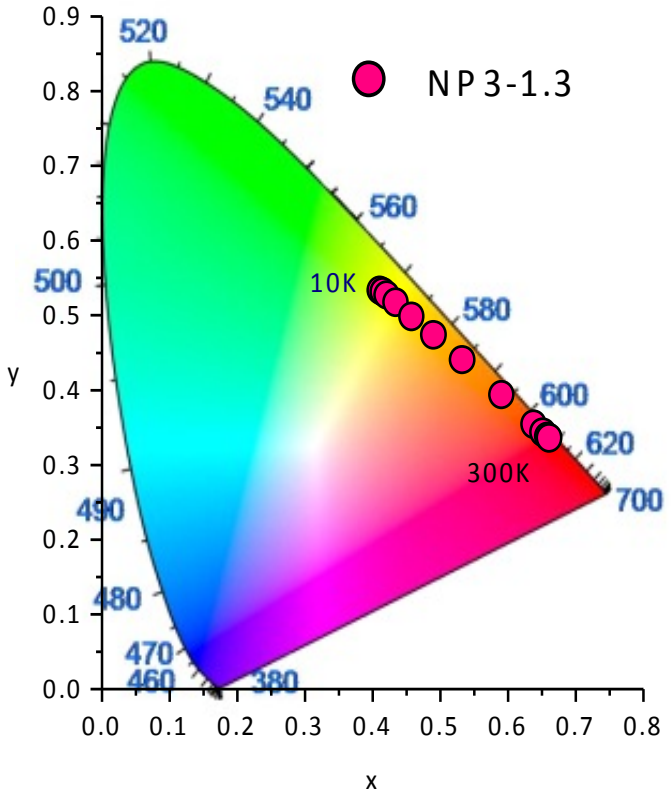
Photoluminescence

γ-Fe_2O_3@TEOS/APTES **NP3-1.3**

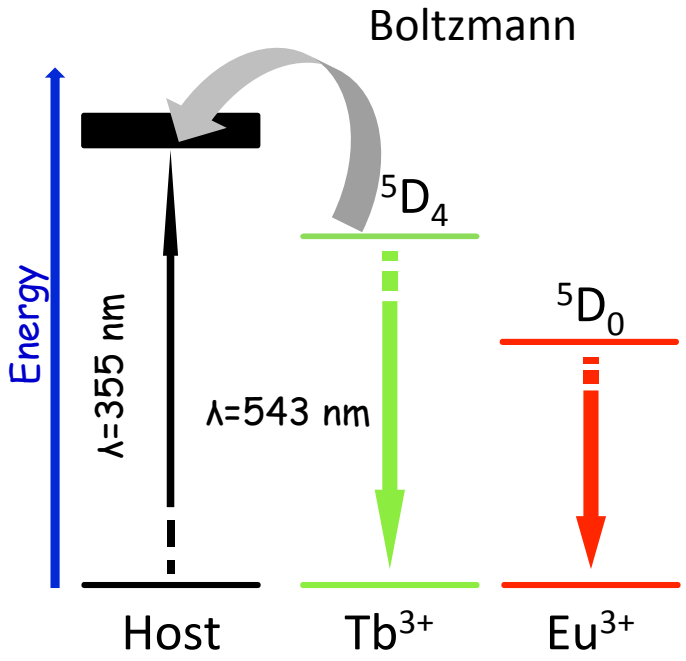


- **1** & **2**: $^5D_4 \Rightarrow {}^7F_{6,5}$ (Tb^{3+})
- **3**, **4** & **5**: $^5D_0 \Rightarrow {}^7F_{2-4}$ (Eu^{3+})
- Area marked with an asterisk: Eu^{3+}/Tb^{3+} ($^5D_0 \Rightarrow {}^7F_{0,1}$)/($^5D_4 \Rightarrow {}^7F_4$) overlapping

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

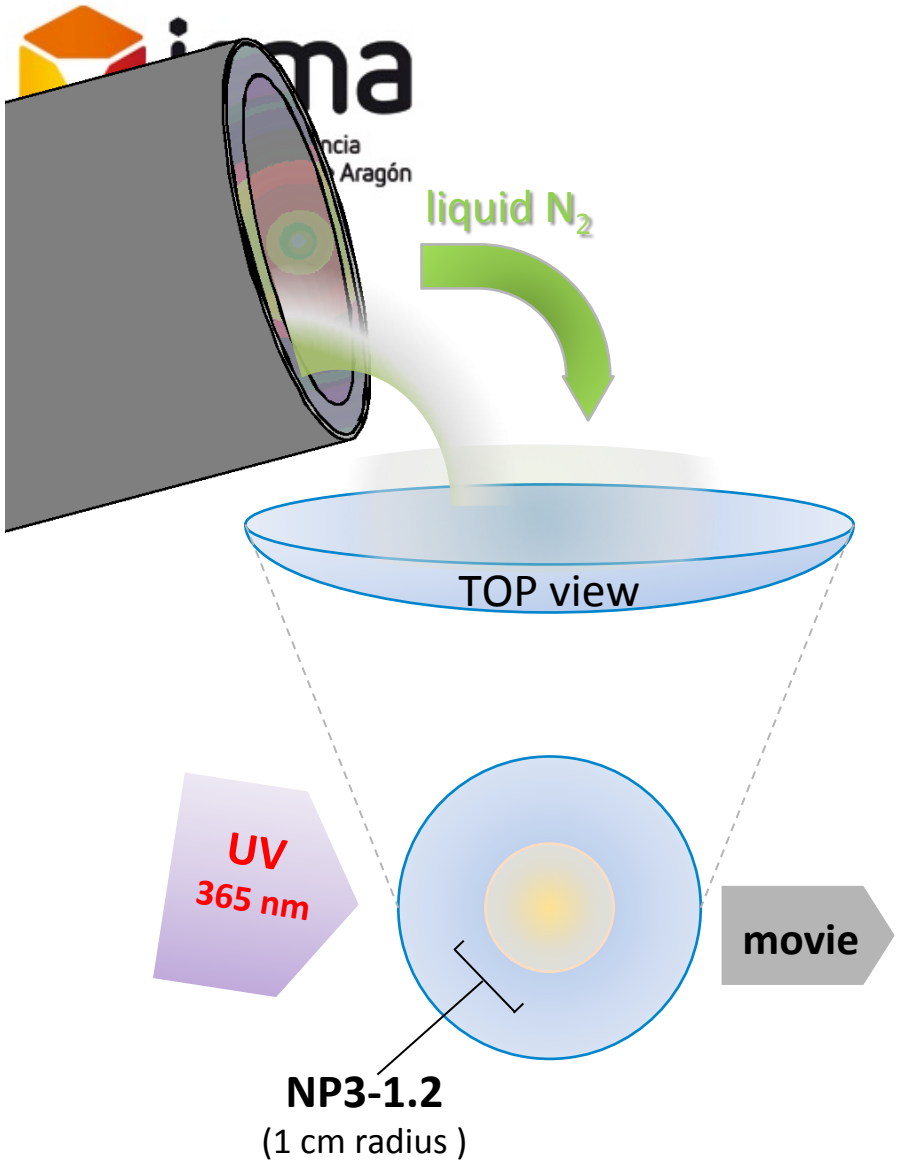


Eu/Tb luminescent nanothermometer

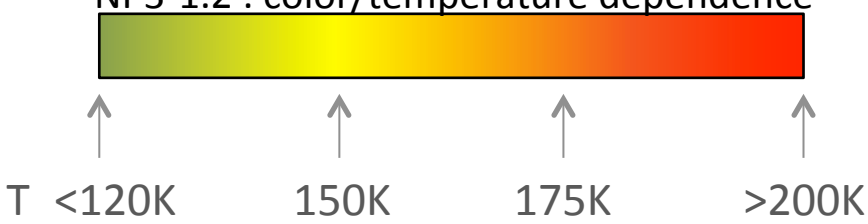


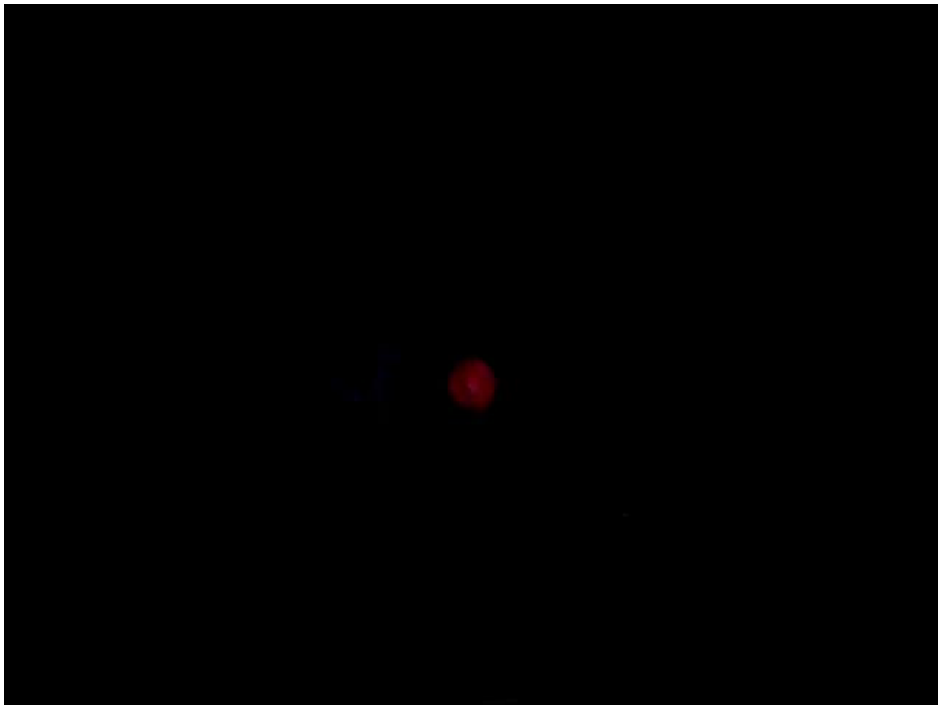
- ***Host rational design***; an excited triplet with energy above that of the Tb^{3+} $^5D_4$ state, thus warranting the occurrence of thermally-driven $^5D_4$⇨host energy transfer
- ΔE between that triplet state and the Eu^{3+} $^5D_0$ emitting level is too large to permit thermally-driven depopulation
- 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)

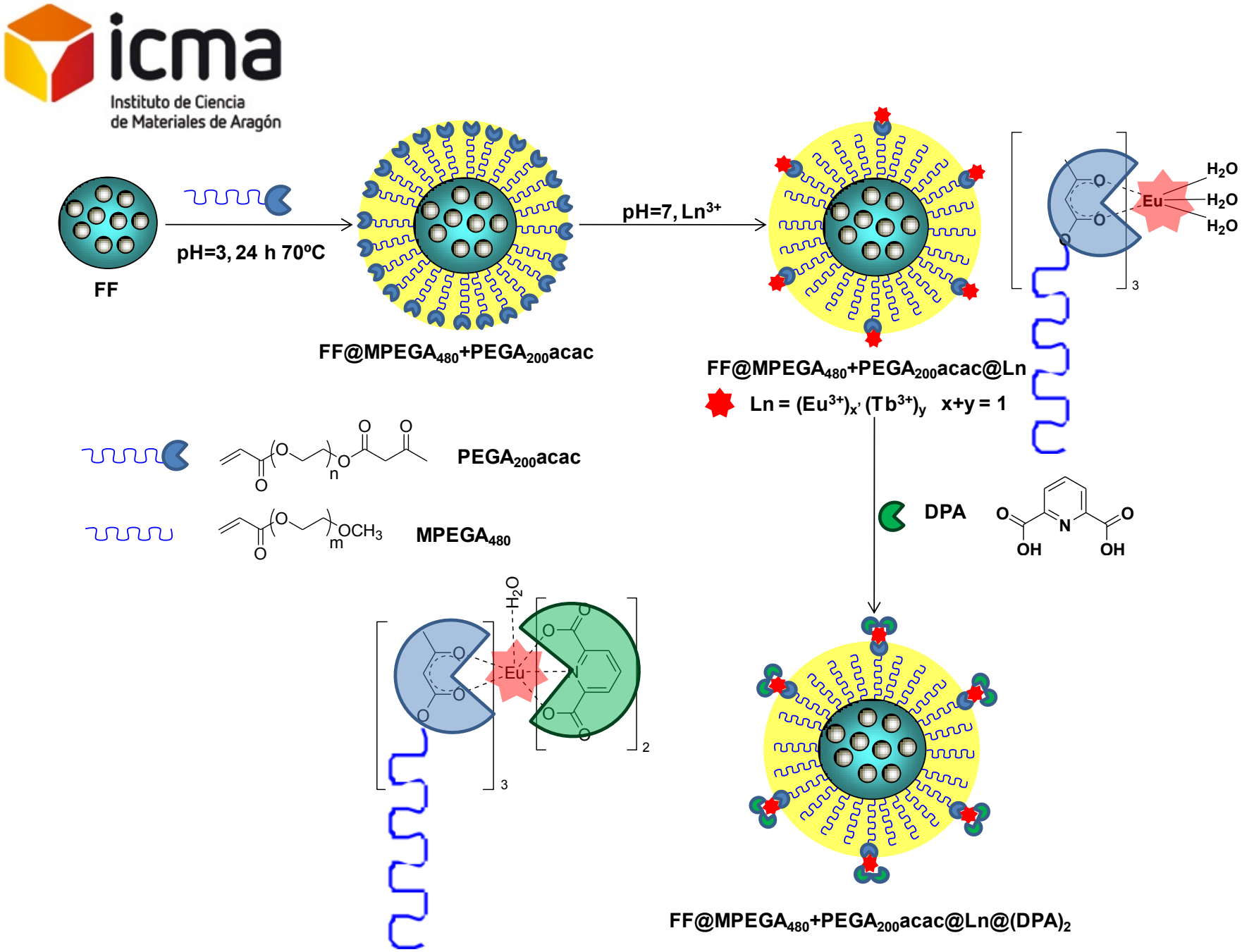
Adv. Mater., 2010, 22, 4499; **New J. Chem.**, 2011, 35, 1177
Spain Patent P200930367, 2009; PCT/ES2010/070430



NP3-1.2 : color/temperature dependence



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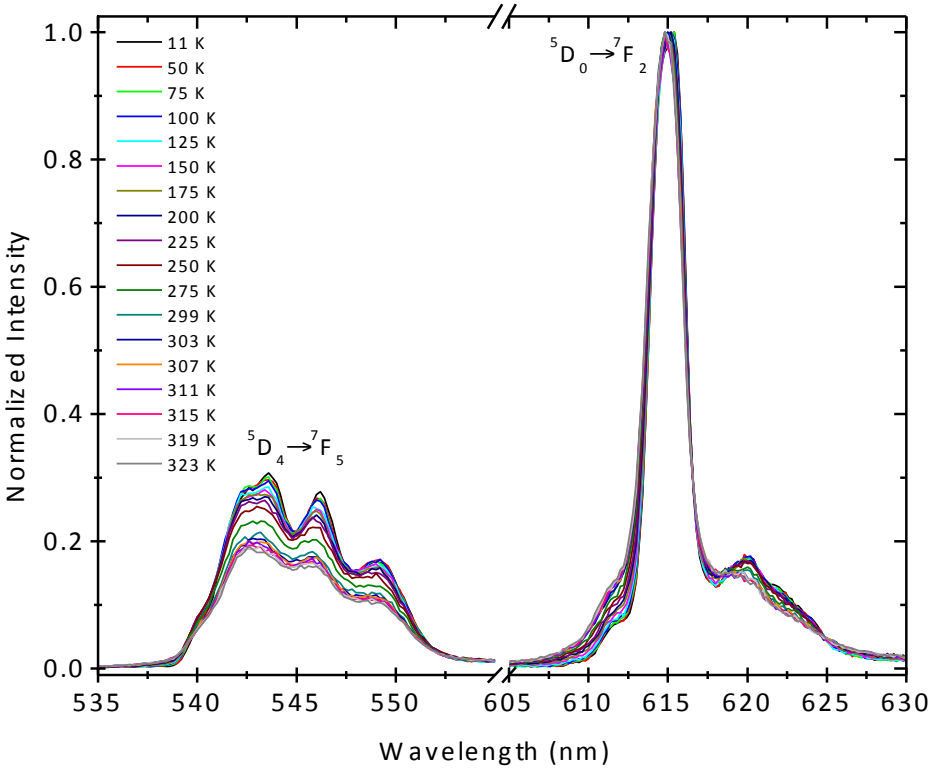
FF
pH=3, 24 h 70ºC
FF@MPEGA480+PEGA200acac
pH=7, Ln3+
FF@MPEGA480+PEGA200acac@Ln
Ln = (Eu3+)x, (Tb3+)y x+y = 1
PEGA200acac
MPEGA480
DPA
FF@MPEGA480+PEGA200acac@Ln@(DPA)2

Multifunctional nanoplatform

Emission spectra

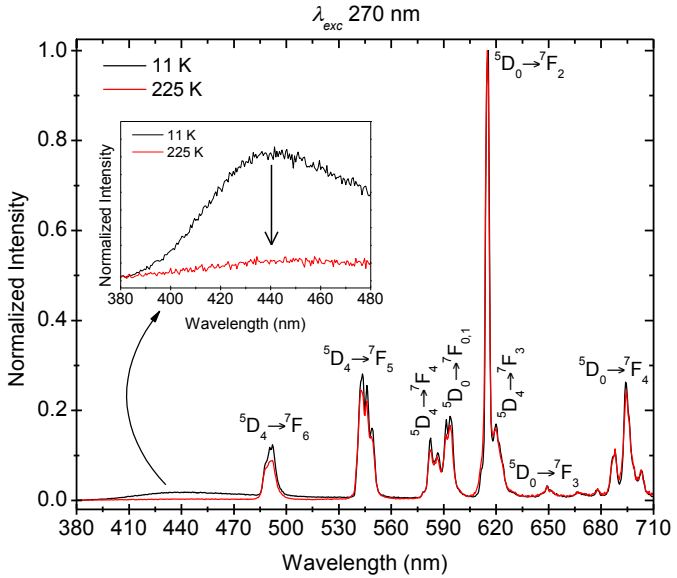


M3 = FF6@MPEGA+PEGAacac@
$Eu_{0.25}Tb_{0.75}$@DPA

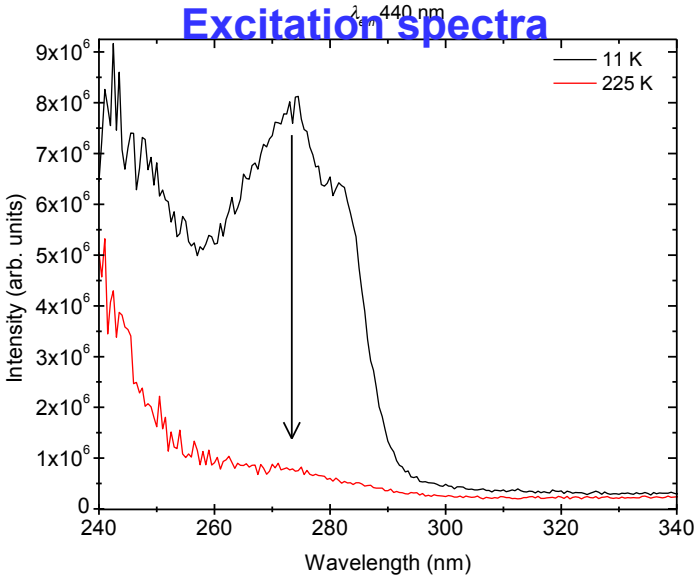
Solid

Dialyzed Sample

Emission spectra



Excitation spectra

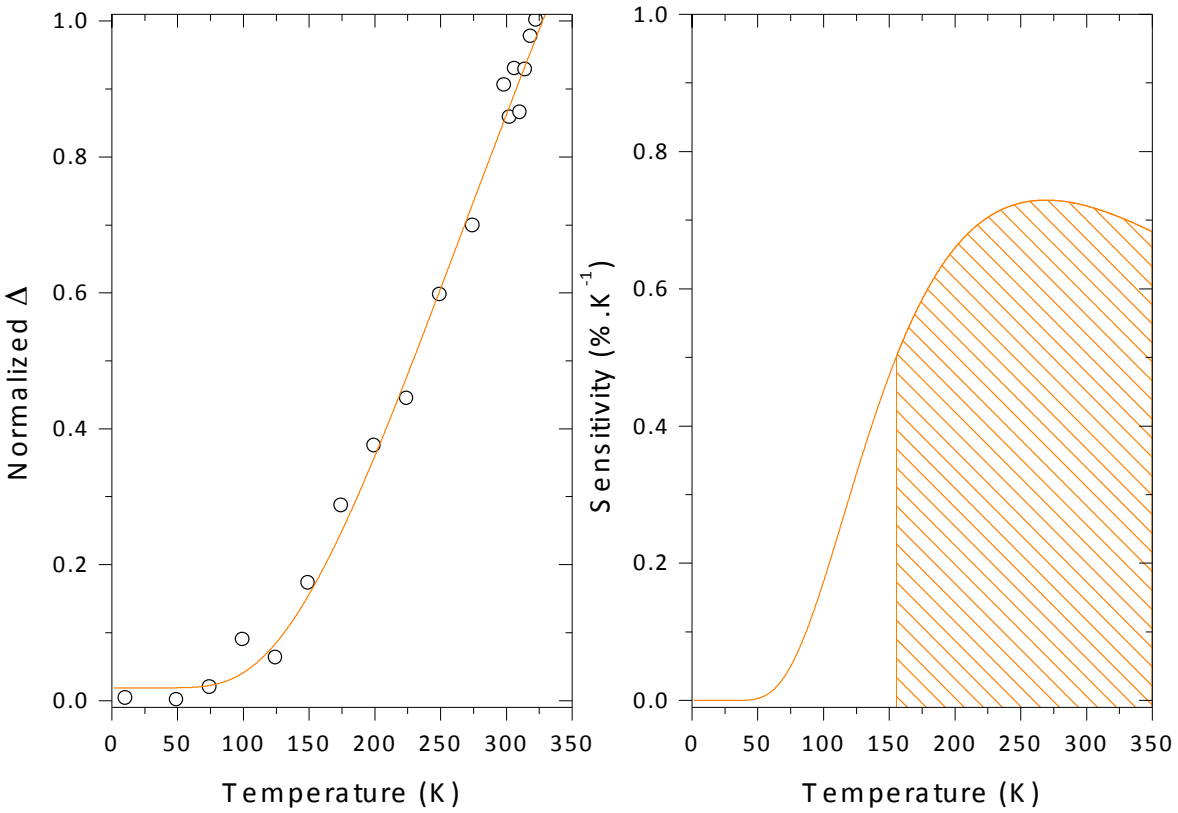


Multifunctional nanoplatform

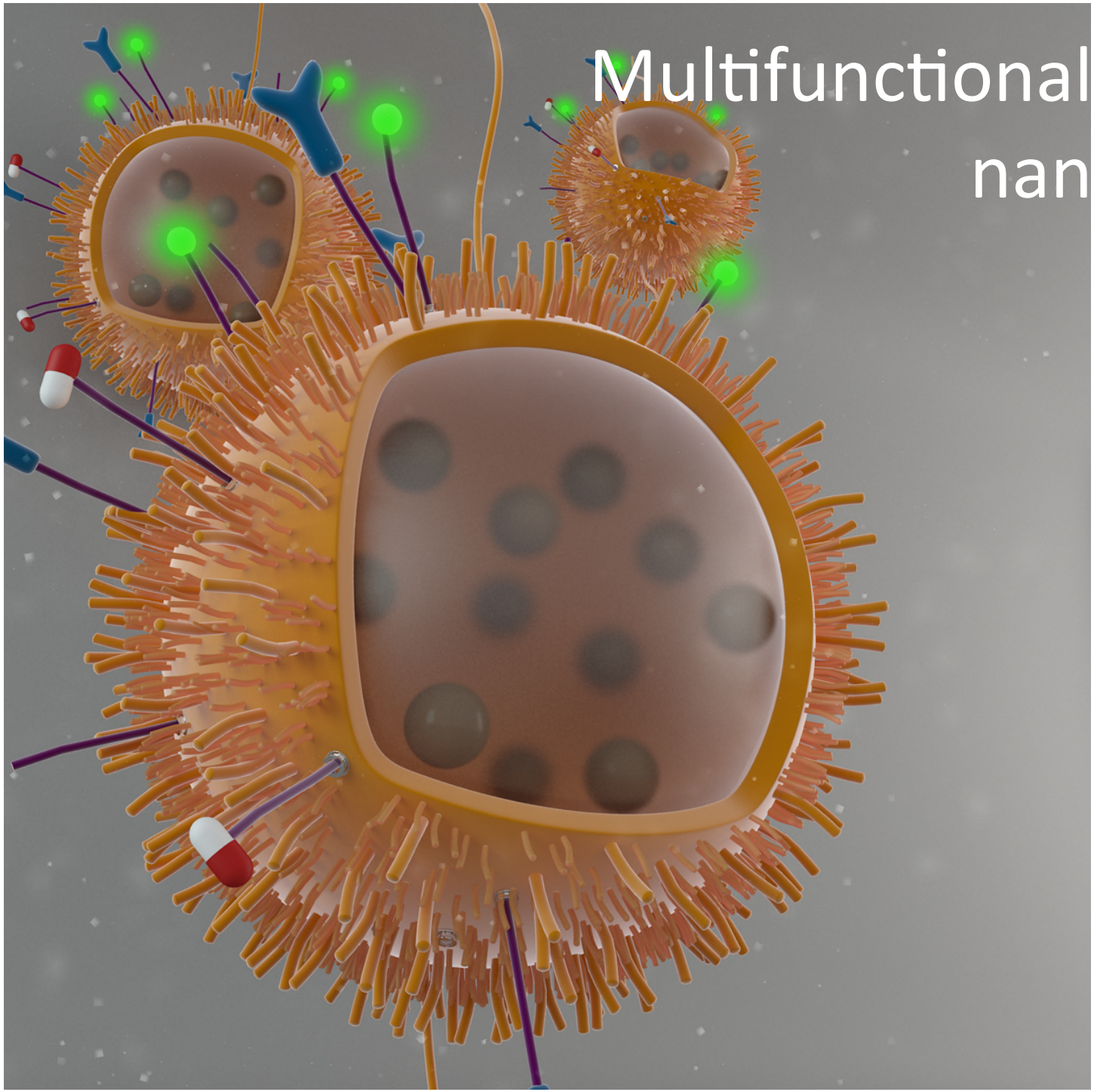
M3 = FF6@MPEGA+PEGAacac@$Eu_{0.25}Tb_{0.75}$@DPA

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*



Multifunctional magnetic nanoparticles

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

Based on a novel from Otto Klement and Jerome Bixby, screenplay written by Isaac Asimov

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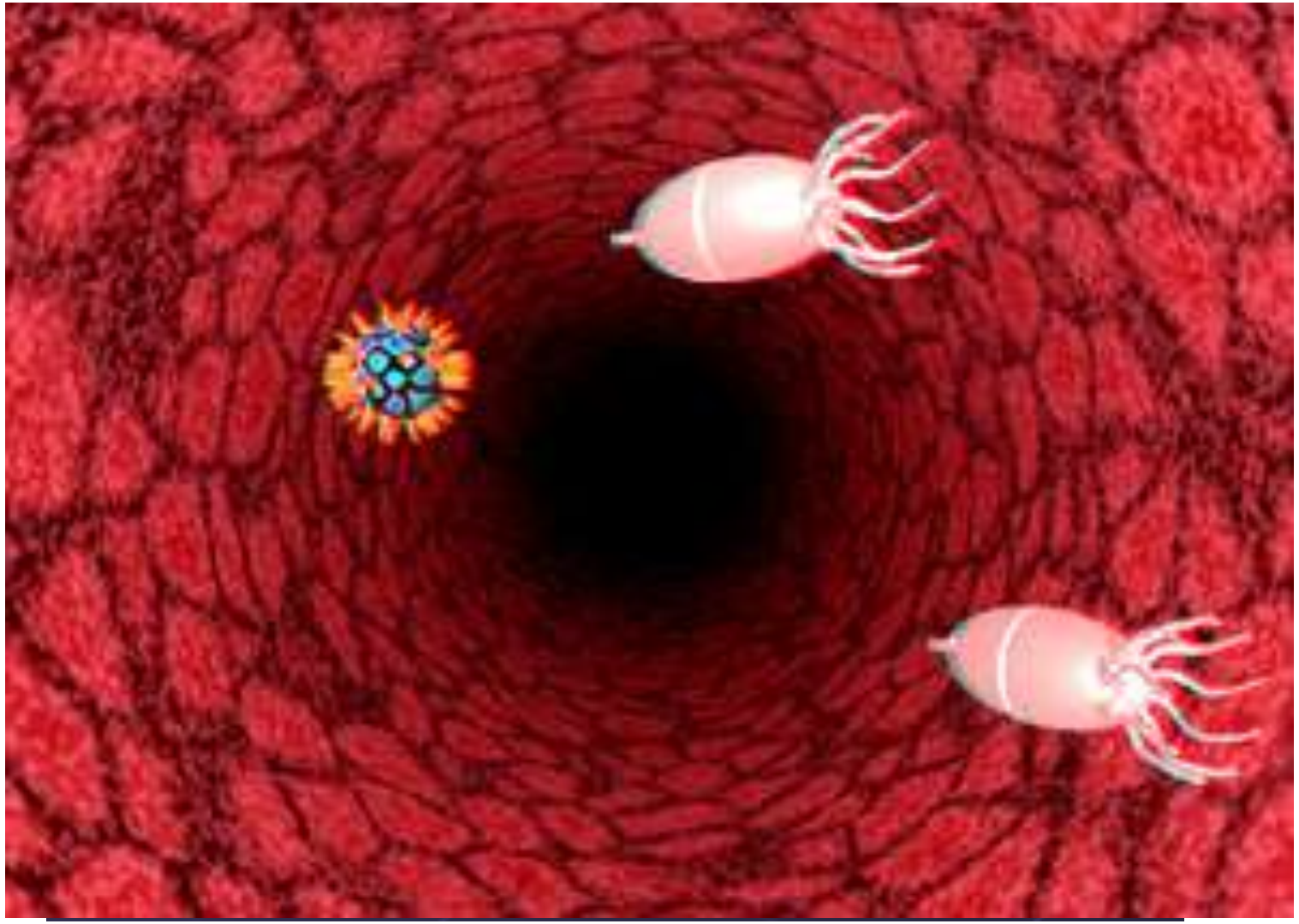
“Biosubmarins” for the s. XXIst



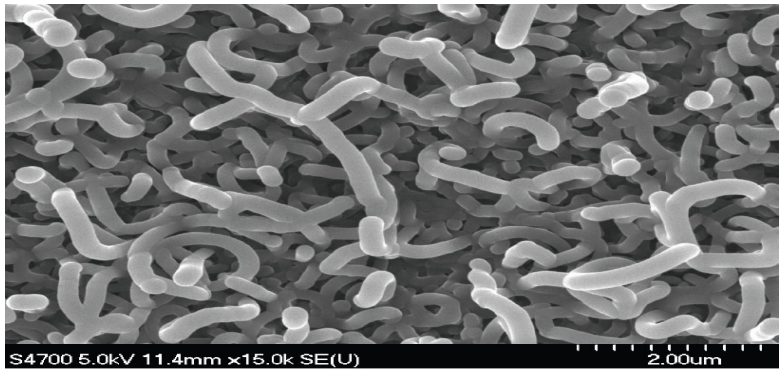
Toxicology issues

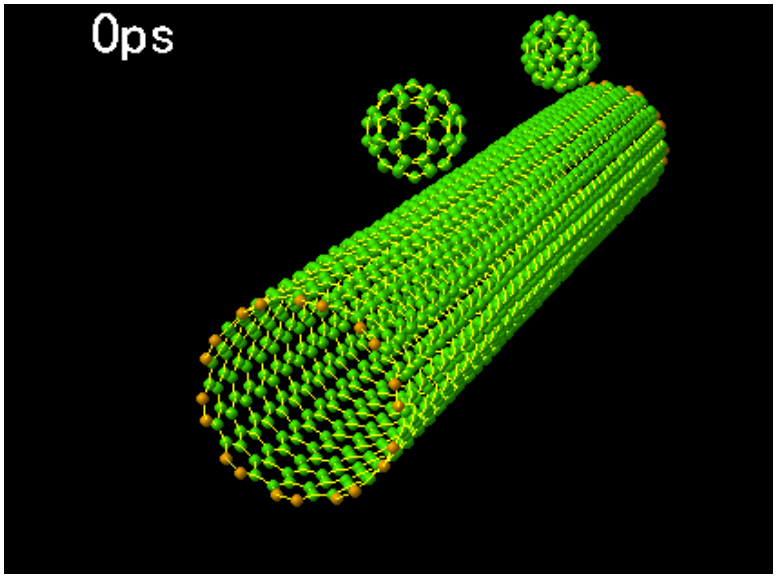
Toxicology issues

Nanopartícles, nanowires, carbon nanotubes, etc. can bring new risks and toxicology problems due to their size.

Attention should be paid to

- fabrication processes
- use
- disposal





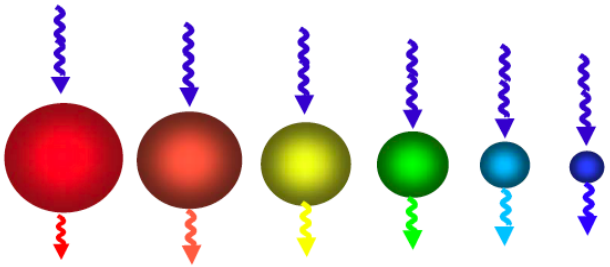
Nanopartícles and other nanomaterials can be

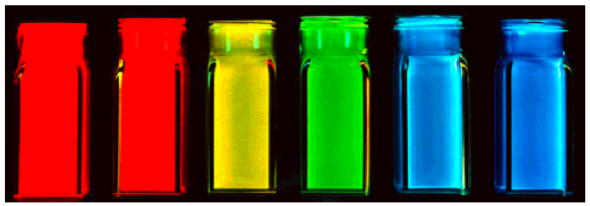
- naturals, e.g.: dust
- incidentals, e.g.: polution
- manufactured, e.g.: MNP, CNT

Toxicology issues

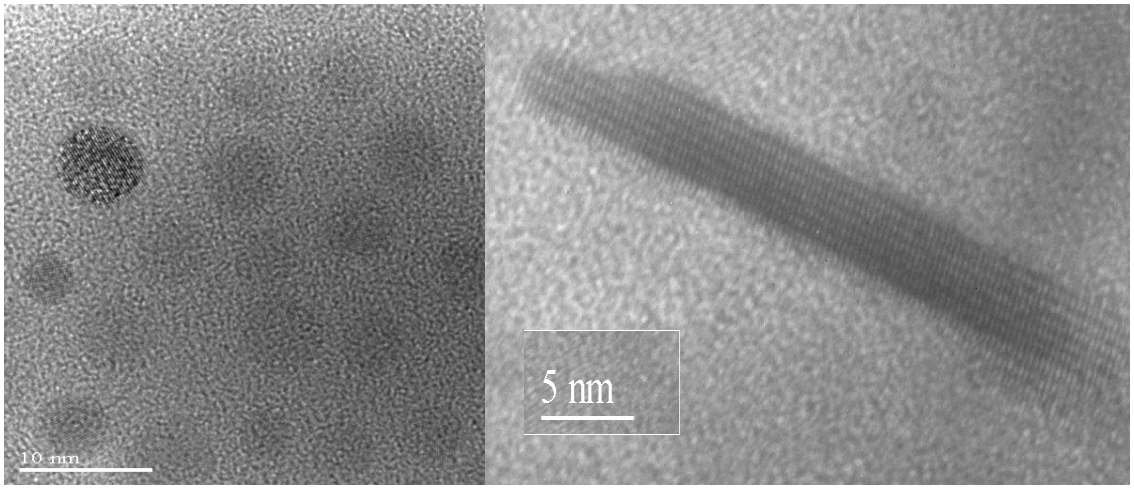
Nanosized materials can be:

- metalic oxides
- nanoclays and nanocomposites
- nanotubes and fullerenes
- quantum dots





Felice Frankel

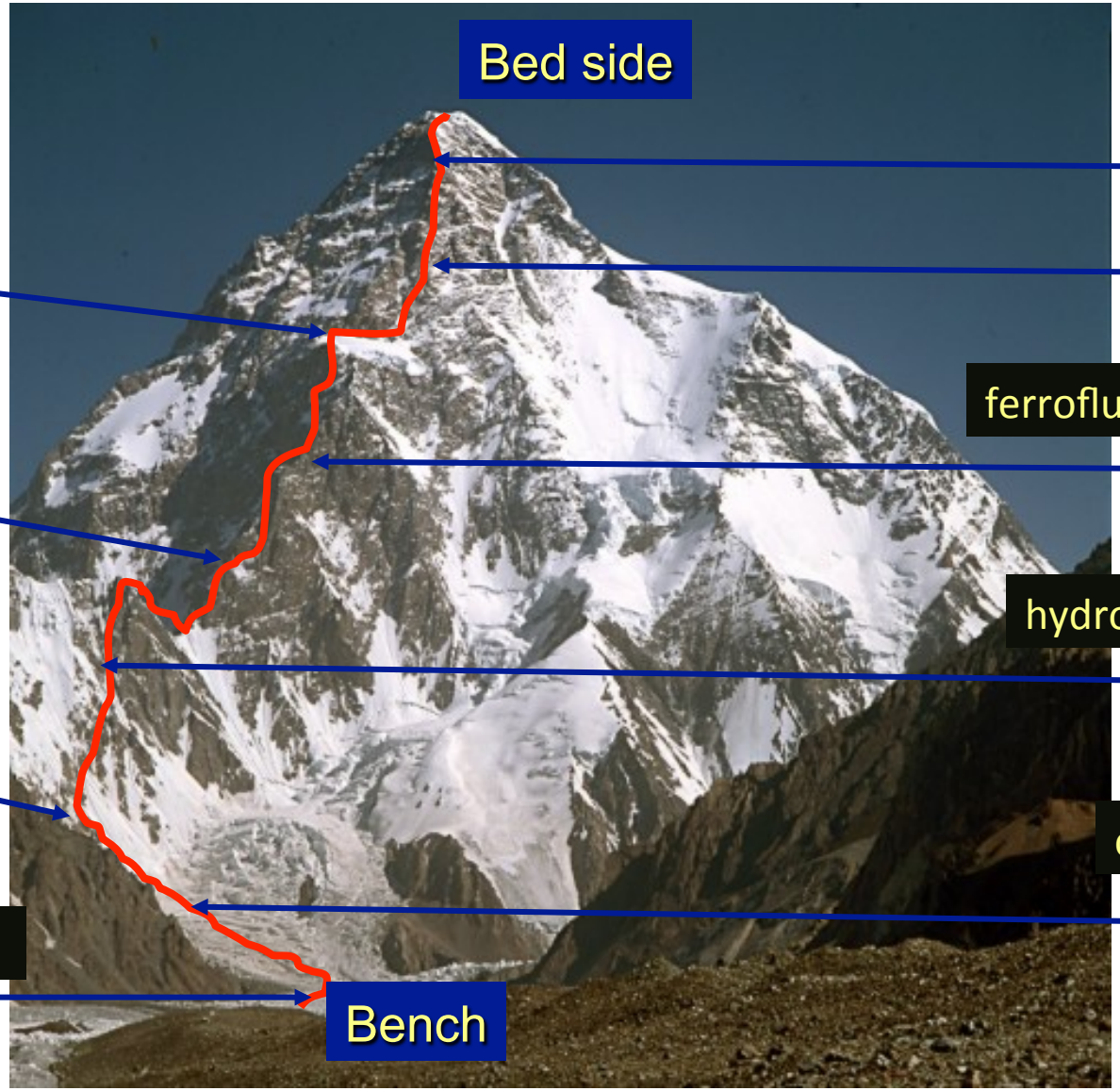


In nanotoxicology,

- form
- size
- purity

Are about as important as quantity

Designing MNP for Biomedical Applications



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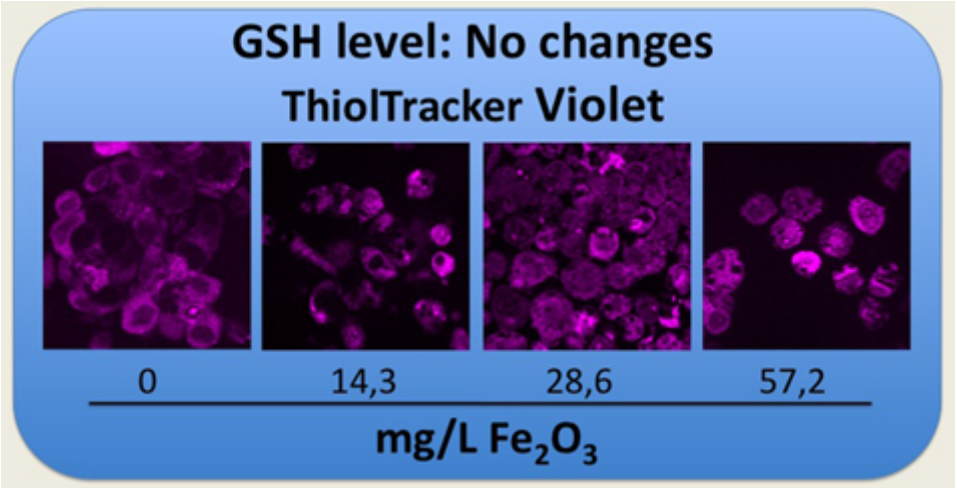
Toxicology issues

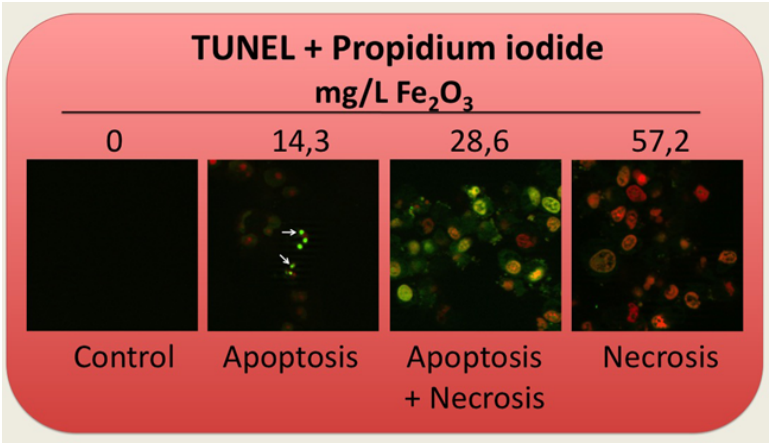
- Short-term toxicity
  - ✓ *in vitro cell toxicity*
  - ✓ Pathology
    - o Hart, Liver, lung, kidney...
    - o Examination of macrophages
      - – Spleen, liver, lung, bone marrow
- Long-term toxicity
  - ✓ Blood and urine analysis

Cytotoxicity

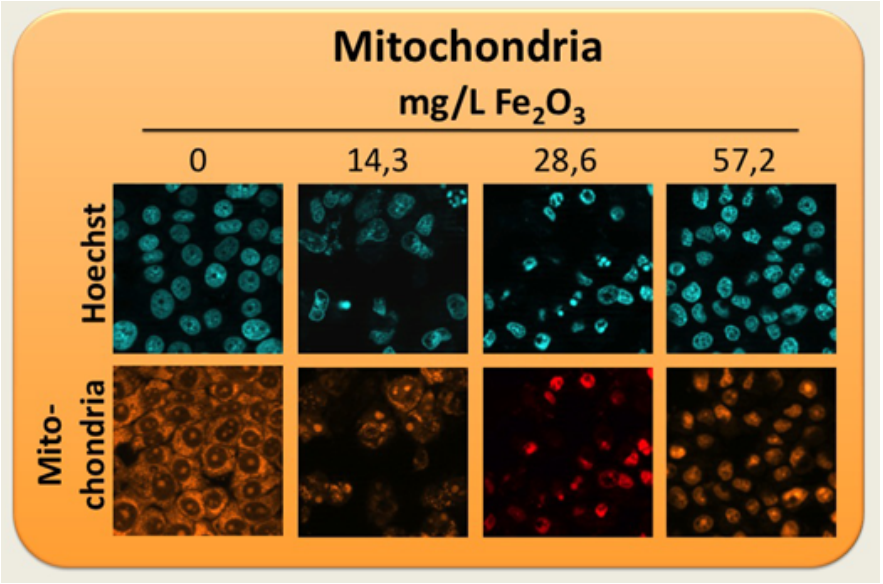
No oxidative stress



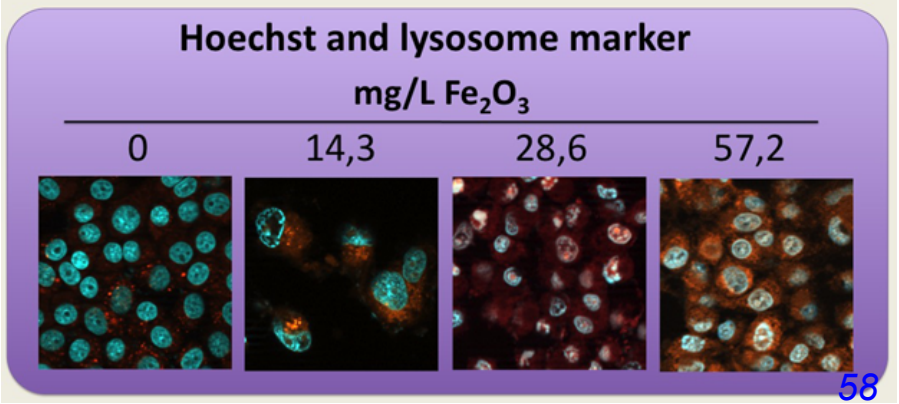
Apoptosis and necrosis at higher concentration



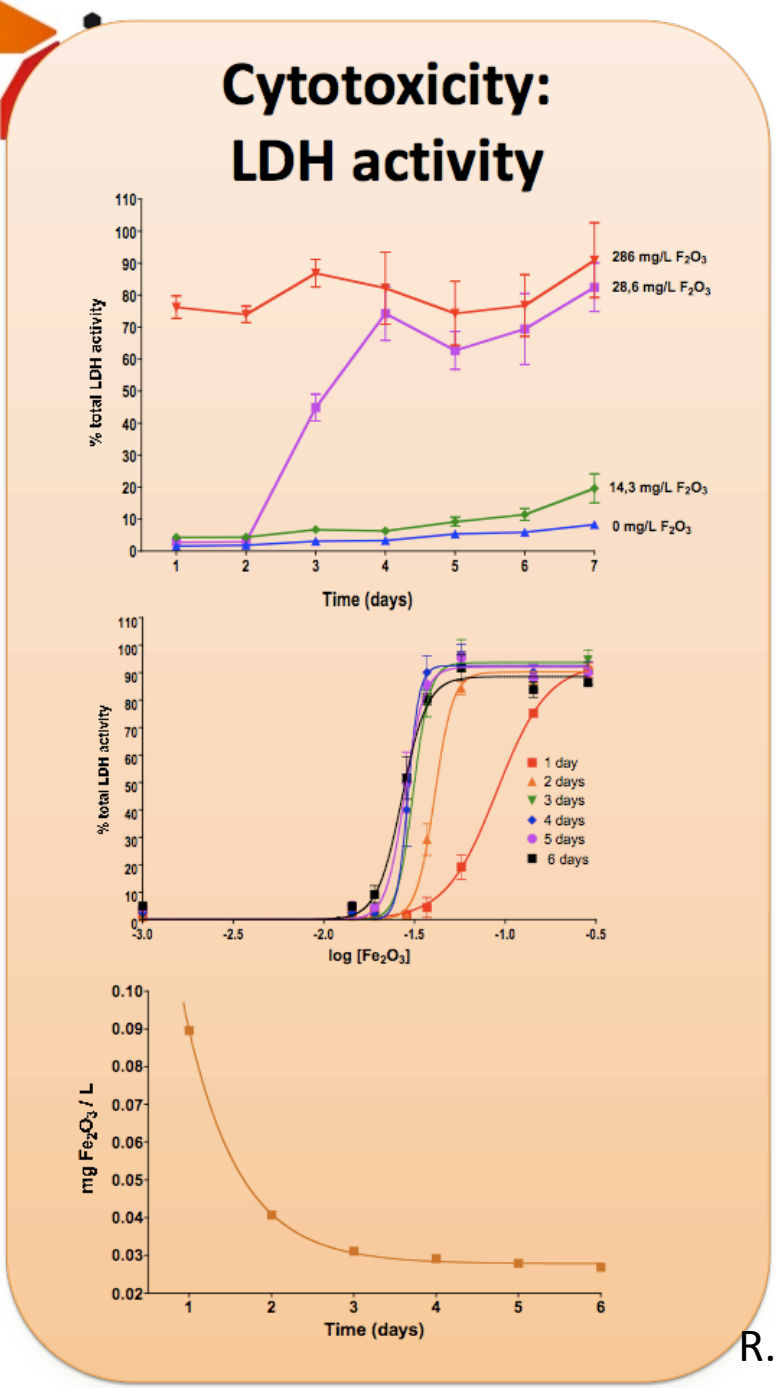
Mitocondria are not affected



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 g/L is not toxic, and 28.6 g/L only after 3 days**.

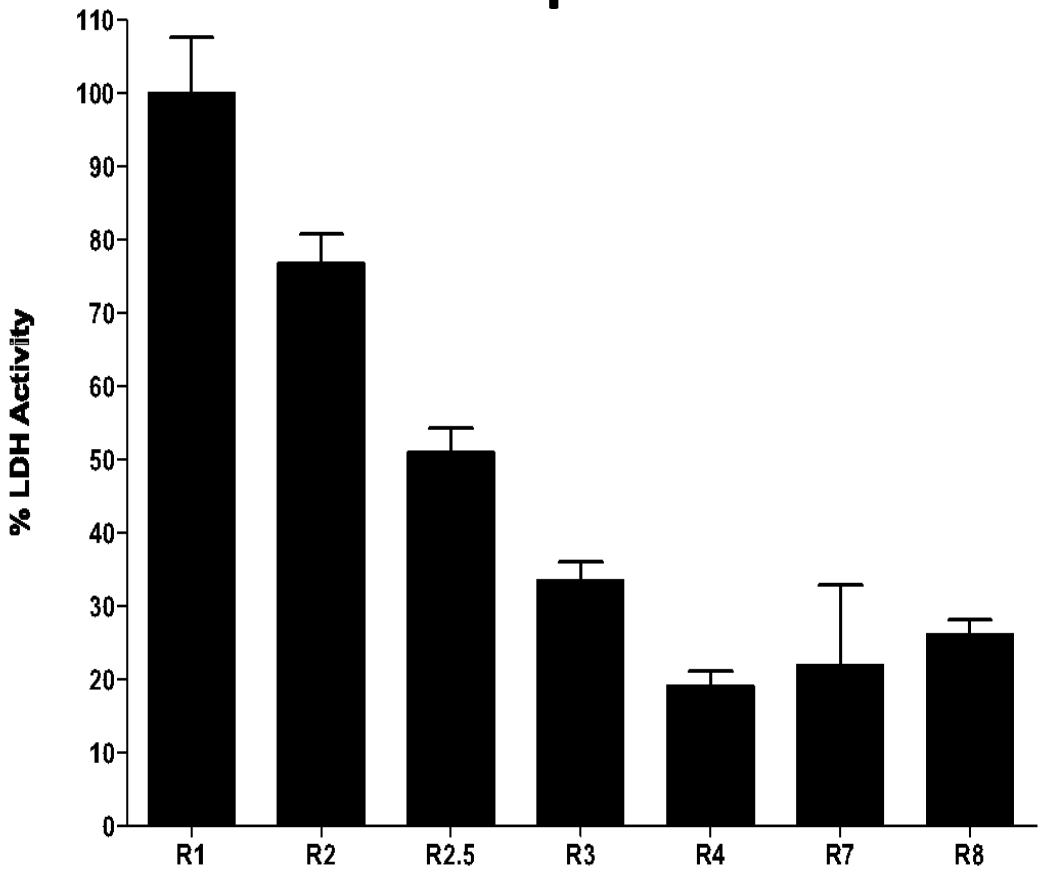
Center: Dose-responses at different days. Lethal mean concentrations (LC50) are calculated for each curve.

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

R. Villa-Bellosta *et al.*, Toxicol. Lett., **180**, S221, (2008)

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

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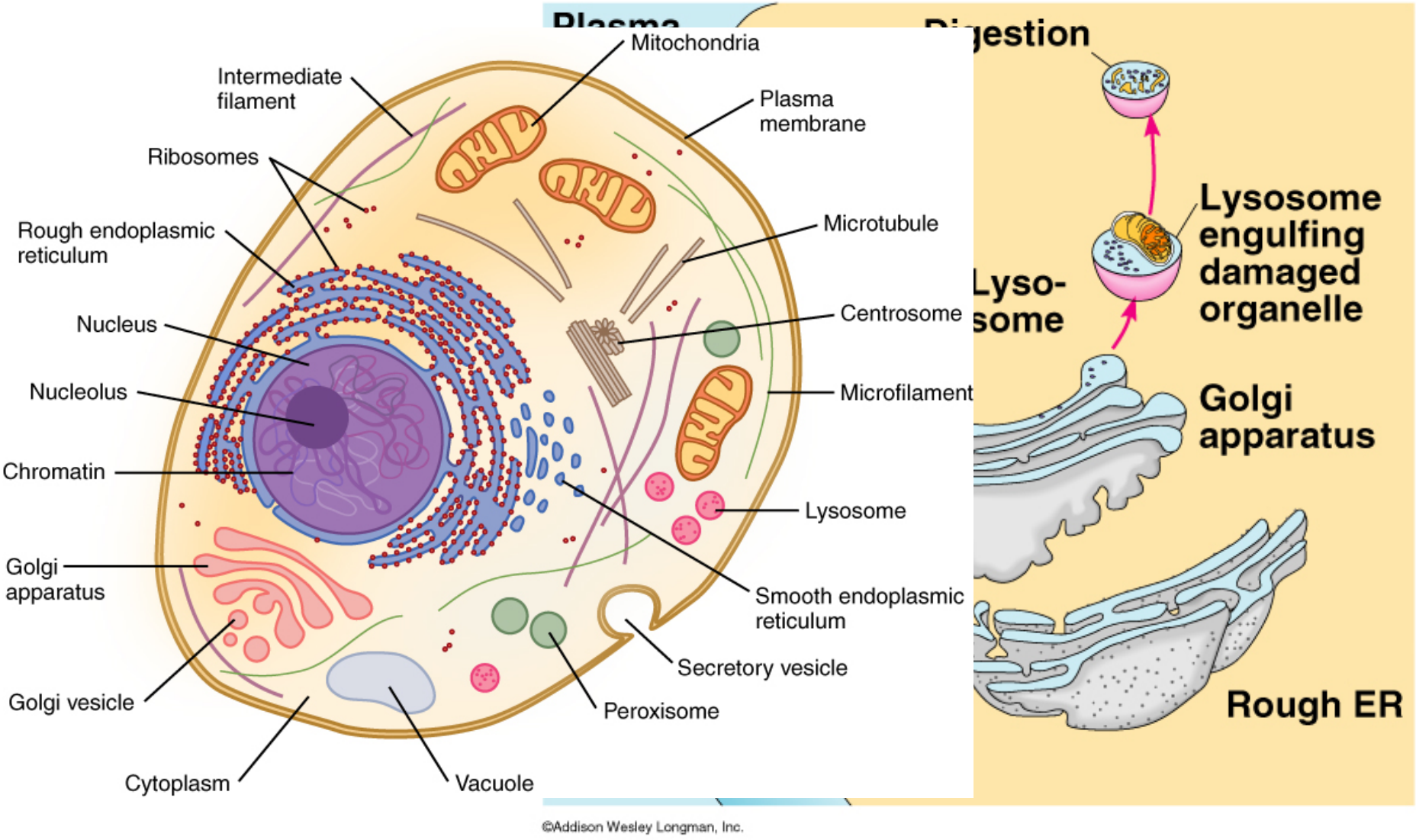
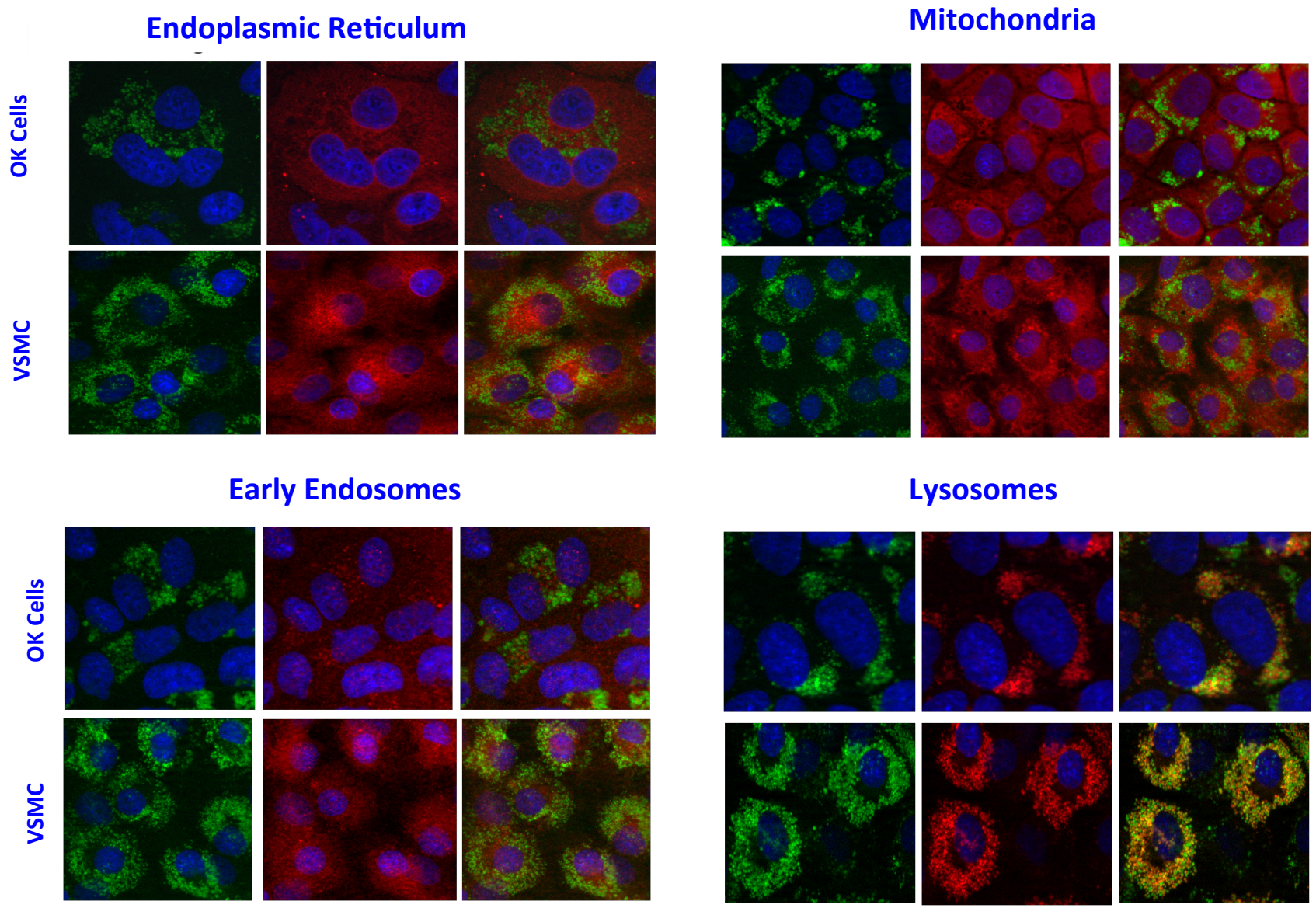
Where particles go into the cell?



Subcellular localization of fluorescent nanoparticles: LYSOSOMES.



The only location of SPIONS in cells are the lysosomes.

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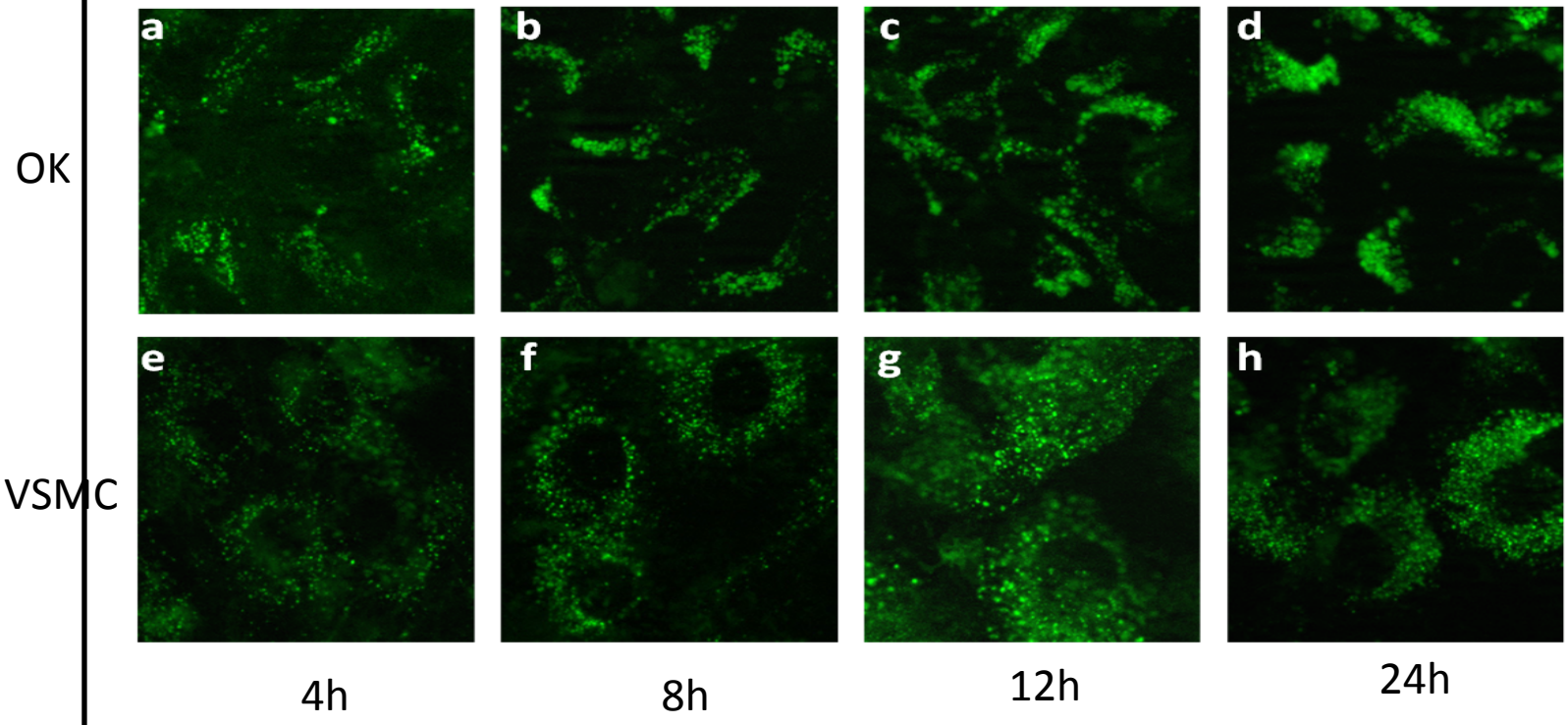
Uptake kinetics



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.

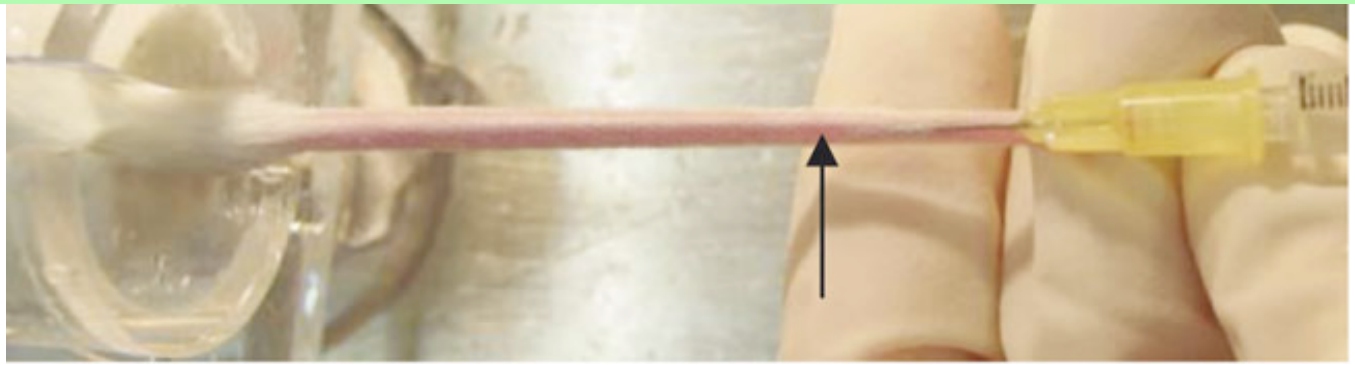
In vivo assays

(Current work)

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

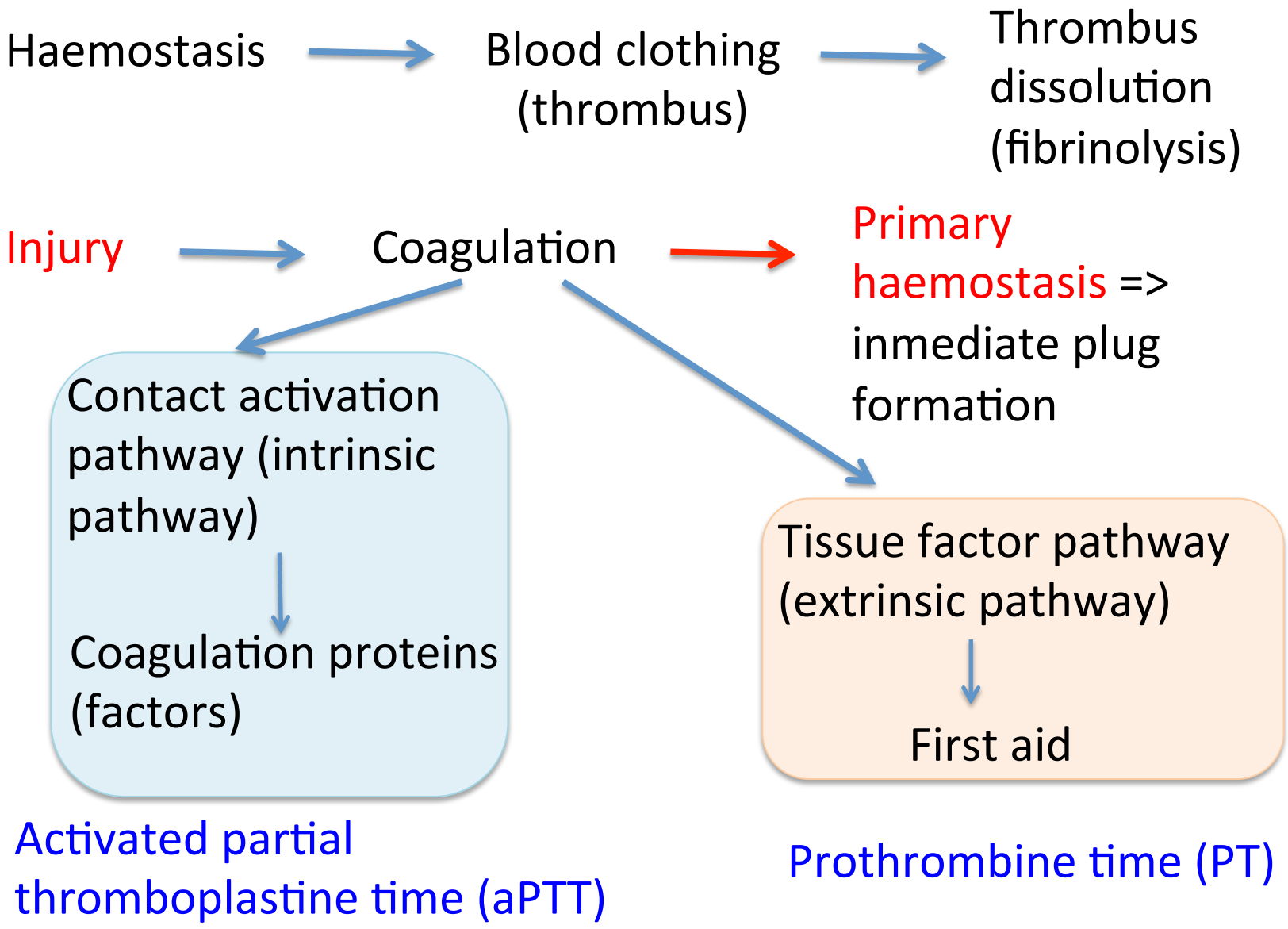
Intravenous injection of 5XEndorem shows after 10 days no excess of iron in a variety of tissues neither anomalies in the pathologic anathomy inspection.

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

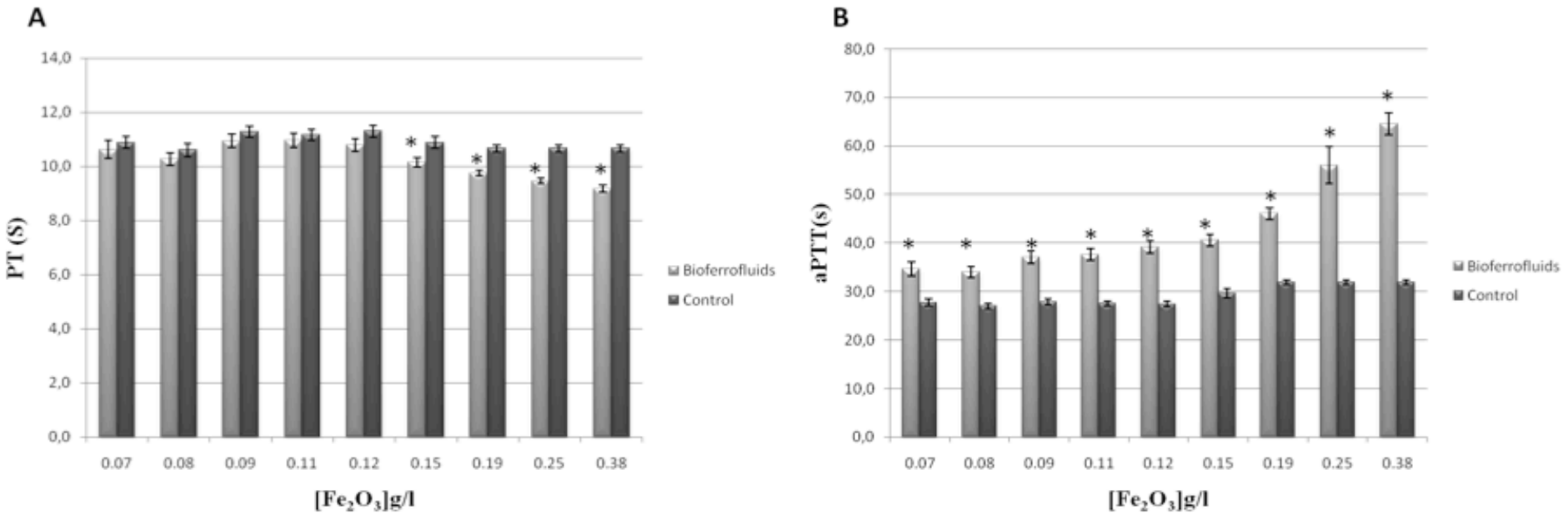



Hematotoxicity

Hematotoxicity



Hematotoxicity



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

Lamiaa M.A. Ali, *et al.*, J. Biomed. Nanotechn. (in press) (2013)

Hematotoxicity

Other parameters to control compatibility in blood

Complete blood picture (CBC)

Plasmatic viscosity

- Erythrocyte count
- Leukocyte count
- Hemoglobin
- Platelets

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Conclusión

IMAGINATION IS MORE IMPORTANT THAN KNOWLEDGE.

KNOWLEDGE IS LIMITED.

IMAGINATION ENCIRCLES THE WORLD.

A. Einstein

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M4 Group

(Multifunctional Magnetic Molecular Materials)

Acknowledgements

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