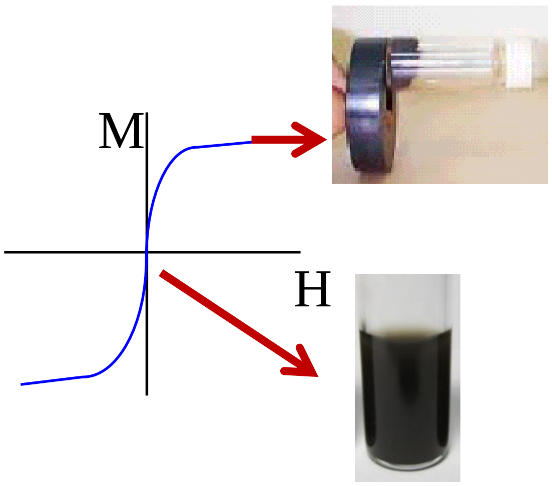
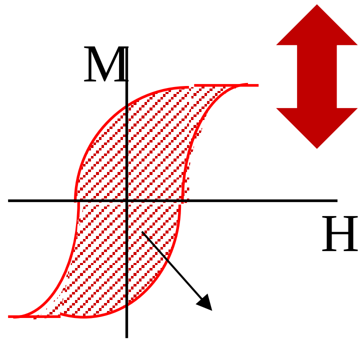
María del Puerto Morales

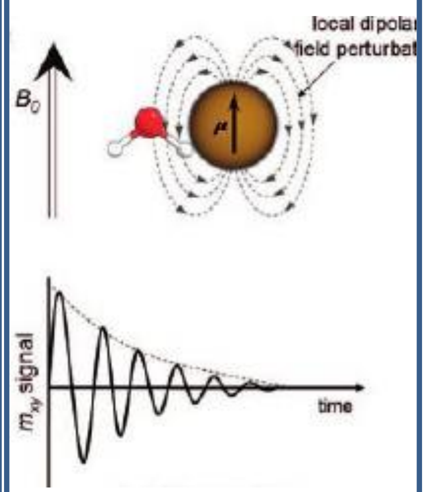
Instituto de Ciencia de Materiales de Madrid, ICMM/CSIC
Department of Biomaterials and Bioinspired Materials



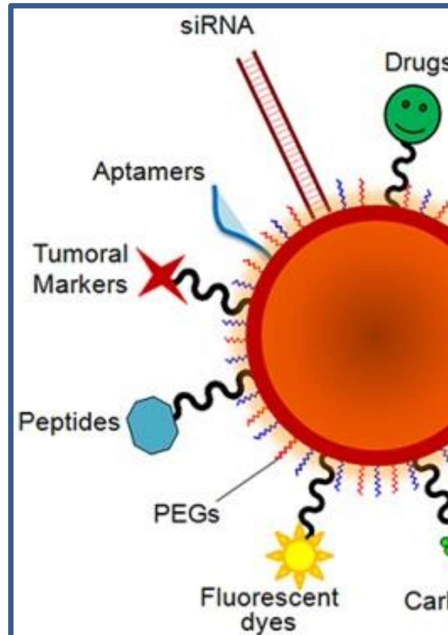
Response to a magnetic field



Thermal energy



Alter NMR signal



IEEE Summer School, *22 June 2017*
Palacio de la Magdalena, Santander, Spain

Instituto de Ciencia de Materiales de Madrid
ICMM
CSIC
Consejo Superior de Investigaciones Científicas

OUTLINE

1- Nanoparticles for medicine

2- Basic principles in magnetism

3- Biomedical applications: Separation, Diagnosis, Therapy

4- Synthesis of magnetic nanoparticles

5- Future

Nanosystems in medicine

Materials
Nano-porous
Nano-crystals
Nano-reinforced
Nano-structured surfaces

Properties
Tissue ingrowth, transport of substances
Physical, electrical, optical, mechanical properties
Mechanical properties, Biocompatibility

Applications

- Surgery
- Therapy
- Diagnostics
- Biosensors/biodetection
- Implantable materials/devices: Tissue engineering
- Textiles and wound care products
- Drug/gene delivery materials and devices

Bandages with silver nanoparticles
Curad® (www.curadusa.com)

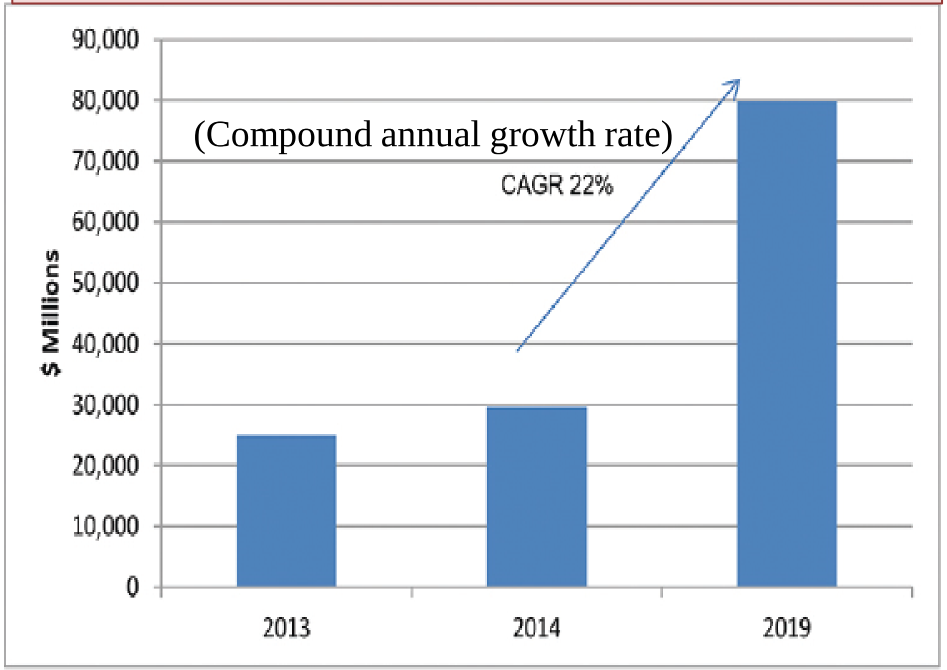
antibacterial agent.

Patrick Couvreur et al., Chem. Rev. 112, 5818, 2012
He was the first to develop nanometric capsules able to penetrate cells to deliver medicine

Nanosystems in medicine

Nanotechnology and heath care, huge potential and some risks.

Global Market for Nanoparticles



Source: BCC Research (BIO113B), August 2014

Drug delivery

- Drug release control
- Drug solubility problems
- DNA carriers
- Tissue regeneration

Areas: Cancer
Neurodegenerative
Cardiovascular
Infection

Nanoparticles in Biotechnology, Drug Development & Drug Delivery (BIO113B) by Jackson Highsmith, and **Nanotechnology in Medical Applications: The Global Market (HLC069B)** by Paul Evers. For more information, visit www.bccresearch.com. and www.drug-dev.com.

Oncology

High mortality without effective treatment

Consequences: The oncology market is the third largest pharmaceutical market, behind the cardiovascular and central nervous system therapy areas.

Treatment of cancer with traditional medicine involves surgery, ionizing radiation, and chemotherapy

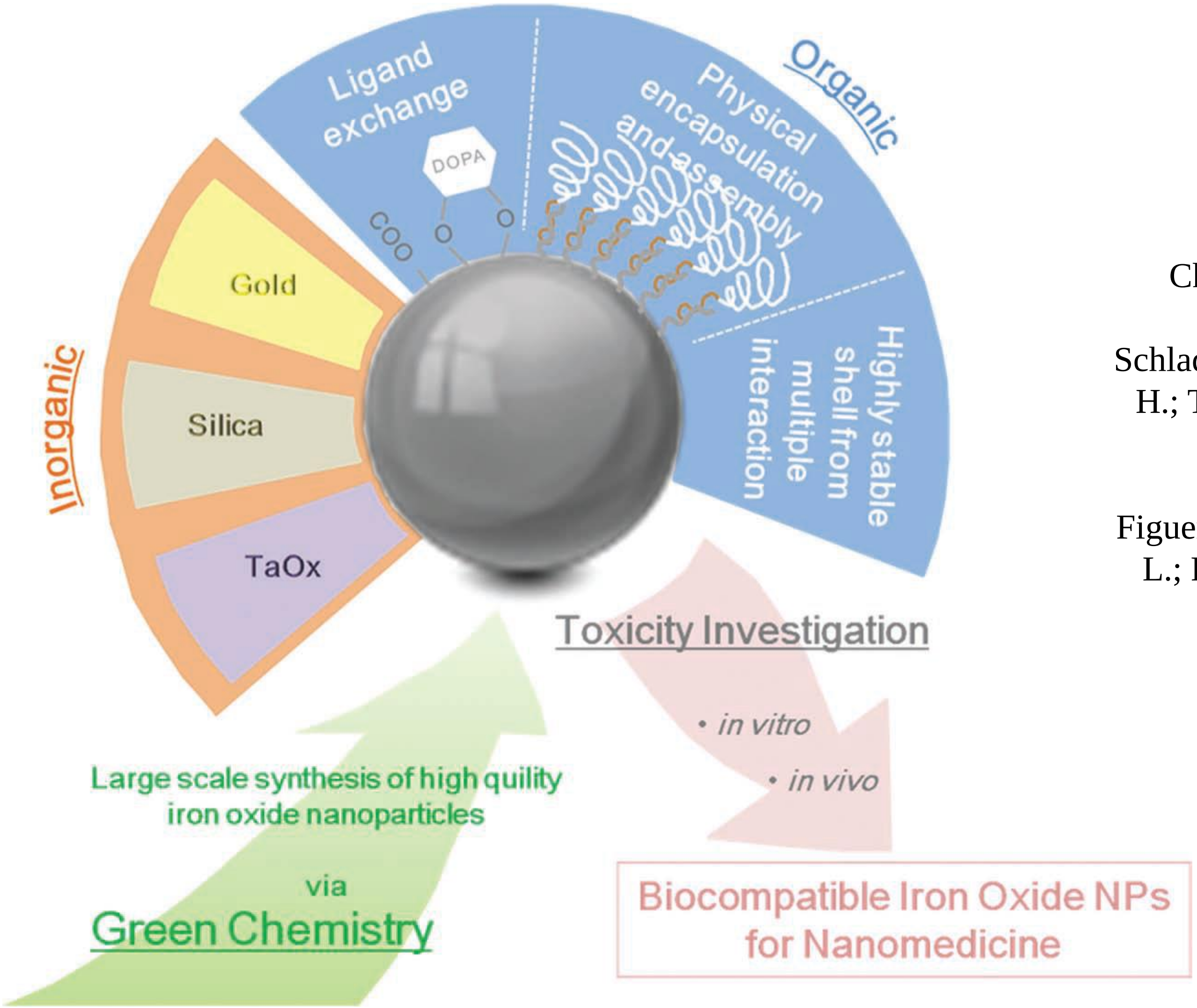
These treatments affect both tumors and healthy tissue.

Consequences: Multi-billion markets in medical and palliative expenses because systemic toxicity and undesirables side effects.

Nanoformulations approved by different regulatory agencies

Product	Nanosystem	Application	Status	Company
Doxil (Barenholz, 2012)	Doxorrubicina encapsulada en liposomas PEGilados	Cáncer de ovarios	Aprobado 11/17/1995 FDA50718	Ortho Biotech (adquirida por JNJ)
Myocet (Waterhouse et al., 2001)	Doxorrubicina encapsulada en liposomas No PEGilados	Cáncer de mama metastásico	Europa y Canadá, en combinación con ciclofosfamida	Sopherion Therapeutics, LLC EEUU y Cephalon, Inc. en Europa
DaunoXome (Forssen, 1997)	Daunorrubicina encapsulada en liposomas	Tratamiento de sarcoma de Kaposi avanzado asociado al VIH	Aprobado en E.E.U.U	Galen Ltd.
ThermoDox (Dromi et al., 2007)	Doxorrubicina encapsulada en liposomas (liberación mediada por calor)	Cáncer de mama y primeras etapas de cáncer de hígado	Aprobación esperada para el año 2013	Celsion
Abraxane (Guarneri et al., 2012)	Nanopartículas de albúmina-paclitaxel	Diferentes tipos de cáncer	Aprobado 1/7/2005 FDA21660	Celgene
Rexin-G (Gordon and Hall, 2010)	MicroRNA-122 encapsulado en liposomas	Sarcoma, osteosarcoma, cáncer de páncreas, y otros tumores sólidos	Aprobado en Filipinas, Fase II y III en E.E.U.U	Epeius Biotechnologies Corp.
Oncaspar (Avramis and Tiwari, 2006)	Asparaginasa PEGilada	Leucemia linfoblástica aguda	Aprobado 24/06/2006	Enzon Pharmaceuticals, Inc.
Resovist (Hamm et al., 1994)	Nanopartículas de óxido de hierro recubiertas de carboxidextrano	Agentes de contraste para hígado y bazo	Aprobado en Europa en 2001	Bayer Schering Pharma AG
Feridex (Weissleder et al., 1989)	Nanopartículas de óxido de hierro recubiertas de dextrano	Agentes de contraste para hígado y bazo	Aprobado por la FDA en E.E.U.U en 1996	Berlex Laboratories
Endorem (Weissleder et al., 1989)	Nanopartículas de óxido de hierro recubiertas de dextrano	Agentes de contraste para hígado y bazo	Aprobado en Europa	Guerbet

Magnetic nanoparticles



Iron Oxide

Chem. Soc. Rev., 2012, 41, 4306

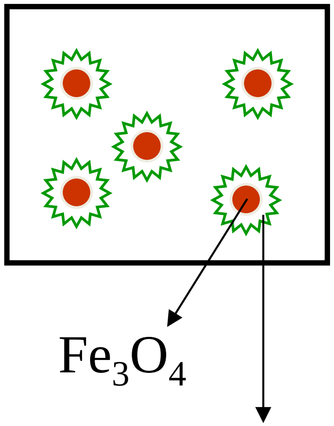
Schladt, T. D.; Schneider, K.; Schild, H.; Tremel, W. *Dalton transactions* 2011, *40*, 6315

Figuerola, A.; Corato, R. Di; Manna, L.; Pellegrino, T. *Pharmacological Research* 2010, *62*, 126

Chemical Design of Biocompatible Iron Oxide Nanoparticles for Medical Applications, *Daishun Ling a nd Taeghwan Hyeon* , **Small** 2013, *9*, No. 9–10, 1450–1466,

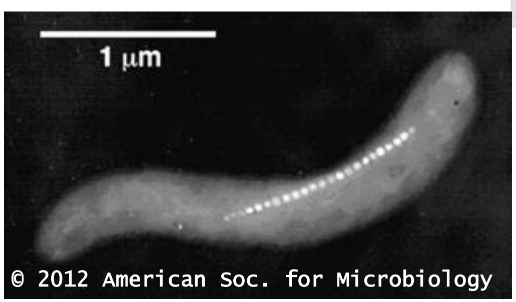
Magnetic nanoparticles

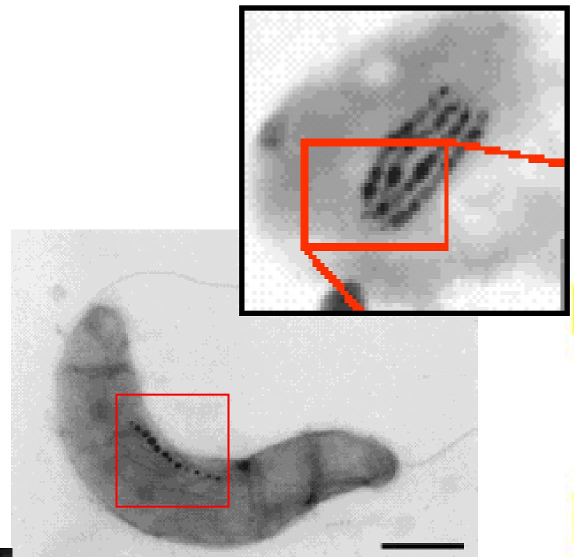
MAGNETIC NANOPARTICLES ⇔ LIVING SYSTEMS



Fe_3O_4

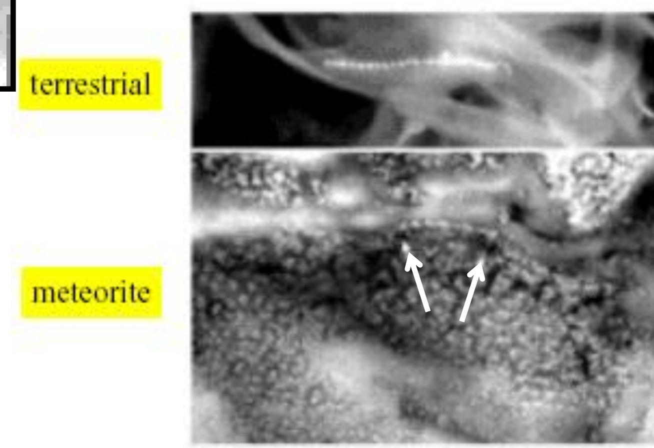
Polymer





Magnetotactic bacteria

For orientation



Life in Mars?

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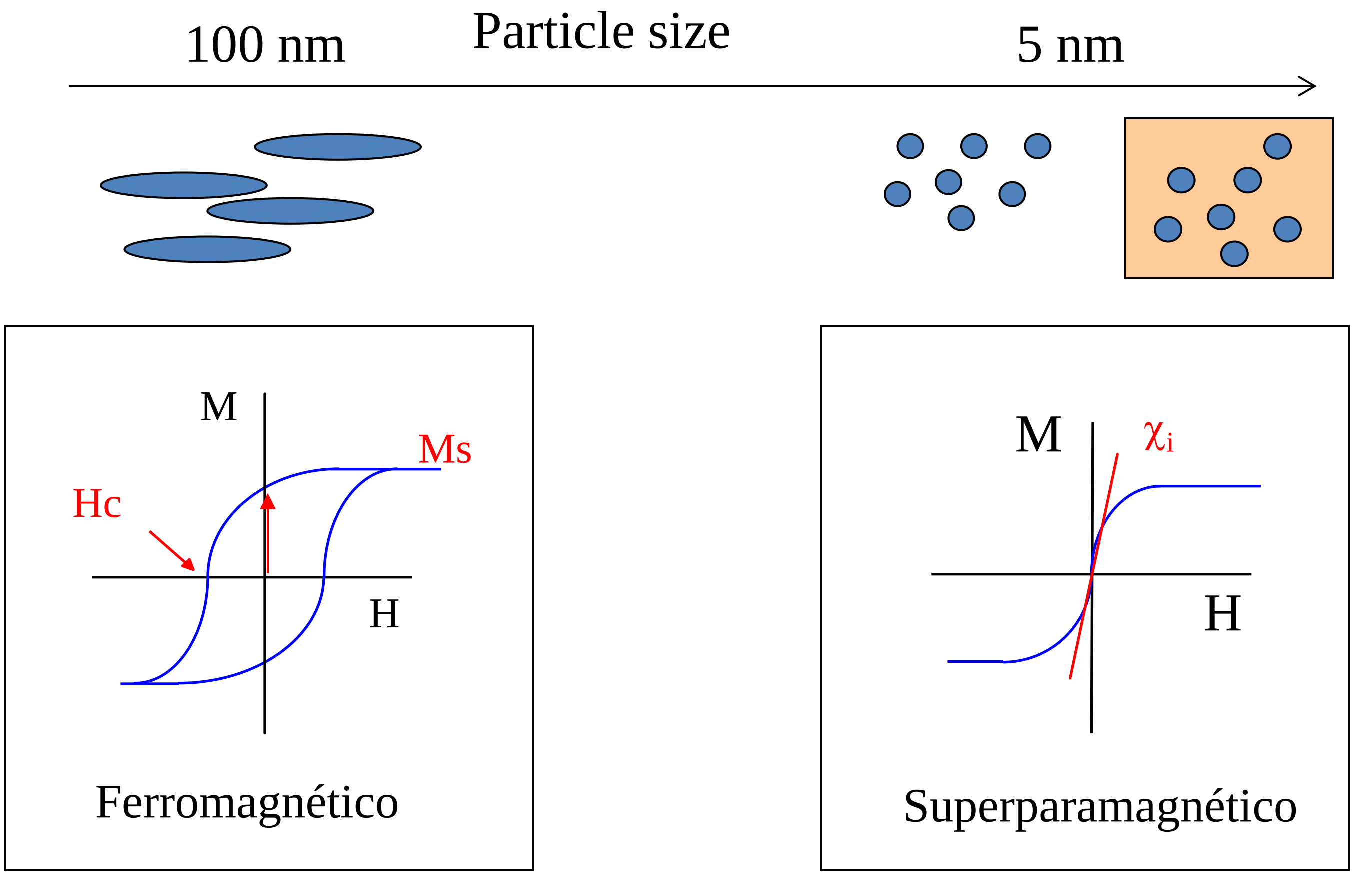
Basic principles

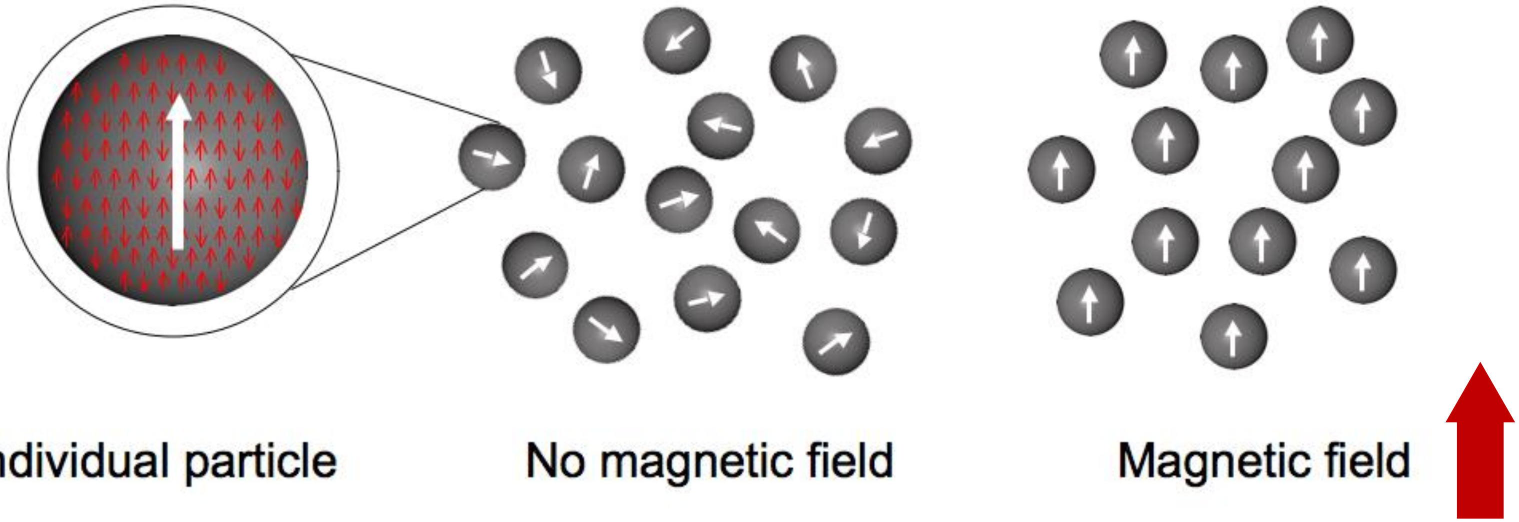


Magnetic recording

Sensors

Basic principles

Superparamagnetic particle



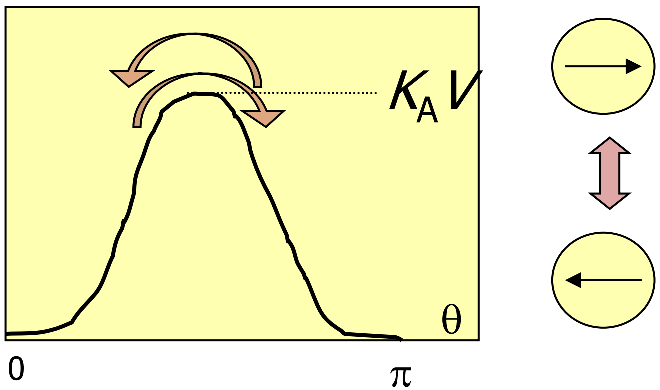
The moments within each particle are ordered (Red arrows)

The net magnetic moment of a system containing MNPs will be zero in zero field and at high enough temperatures.

In the presence of a field, there will be a net statistical alignment of magnetic moments

Basic principles

Superparamagnetism



Jump frequency

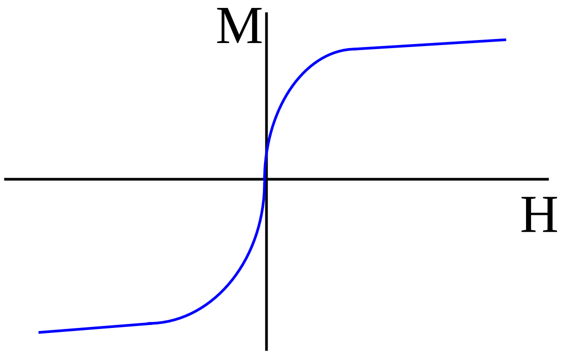
$$\upsilon = \tau_0^{-1} \exp\left(-\frac{K_a V}{k_B T}\right)$$

Relaxation time

$$\tau = \tau_0 \exp\left(\frac{K_a V}{k_B T}\right)$$

Small particle size

$$\Delta E = \mathbf{K_a V} \approx k_B \mathbf{T}$$



No particle aggregation

Reversible behaviour

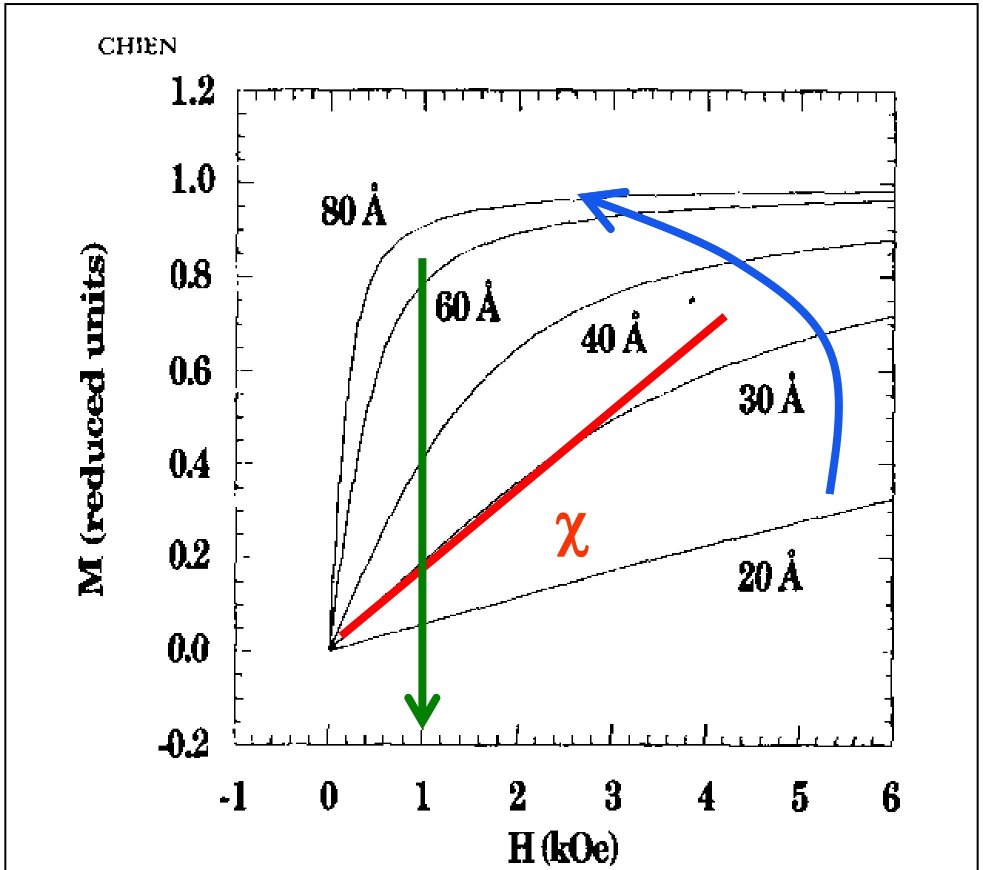
Superparamagnetism

Particle size

- Magnetic behaviour

$$\chi \approx \frac{V M_S^2}{3 k_B T}$$

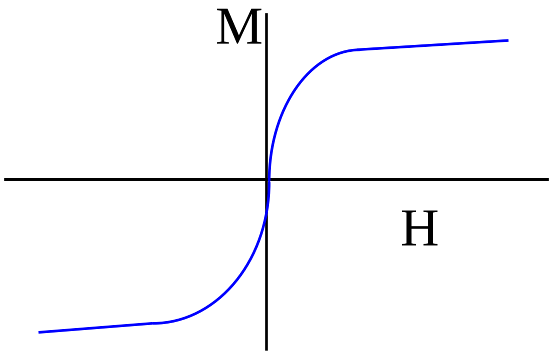
- Saturation field



...ure 7 Calculated magnetization behavior at $T = 300$ K of Fe particles of various sizes

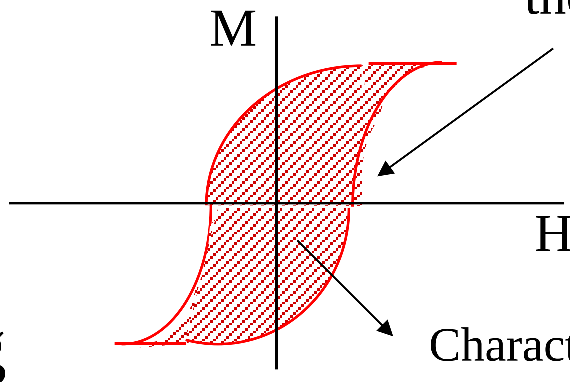
Basic principles

Ferromagnetism

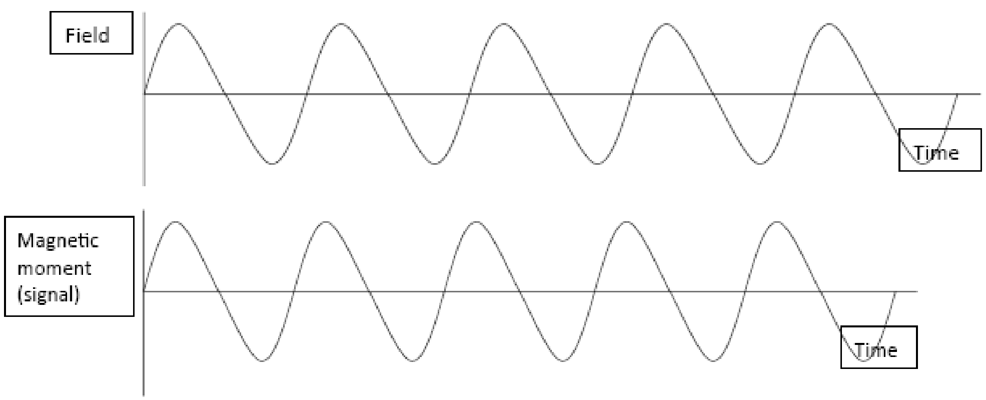


Alternating magnetic field

Delivered by the applied field



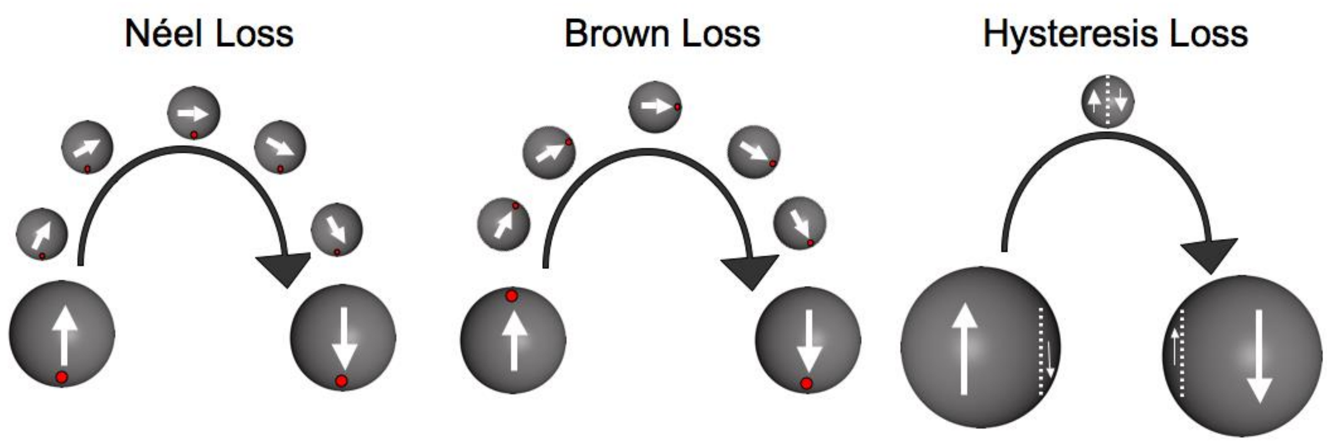
Characterised by area enclosed by the hysteresis loop



Thermal energy

Basic principles

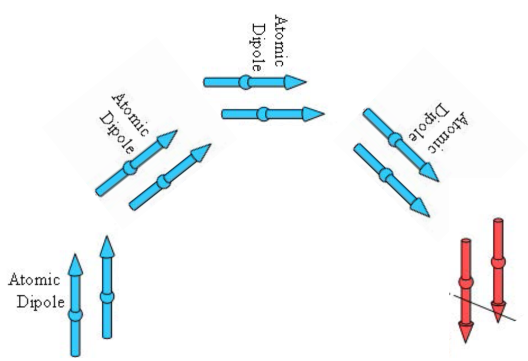
MECHANISM OF MAGNETIZATION ROTATION

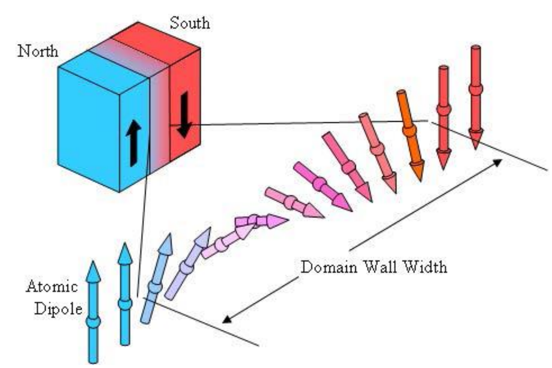


Rotation of the moment within the NP

Mechanical rotation of the NP

Movement of domain walls in multidomain





MAGNETIC MOMENT RELAXATION TIMES

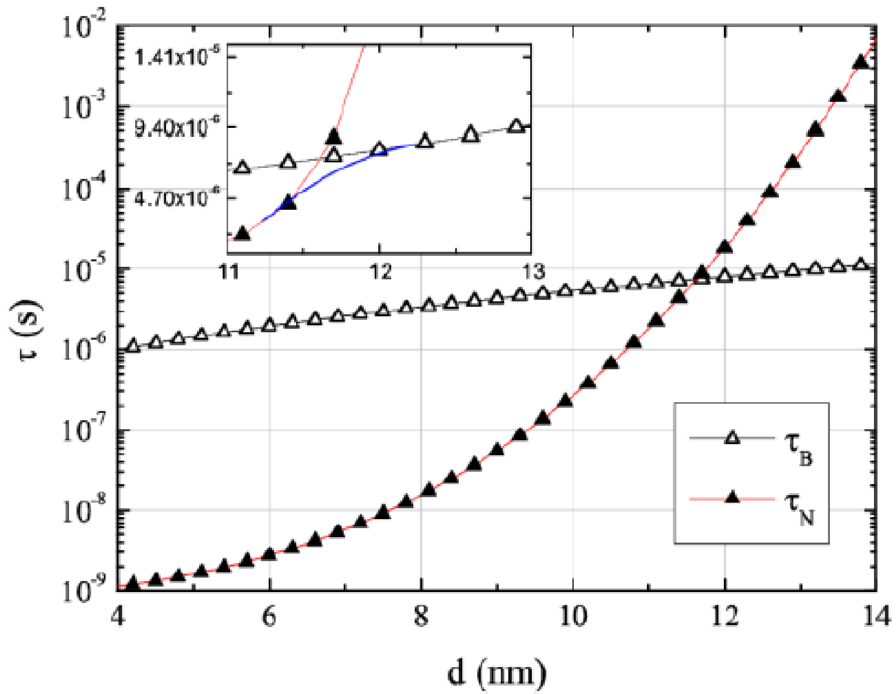
Néel relaxation = $\tau_N = \tau_0 \exp\left(\frac{K V_M}{k_B T}\right)$

Brownian relaxation= $\tau_B = \frac{3\eta V_H}{k_B T}$

Total relaxation

$$\frac{1}{\tau} = \frac{1}{\tau_B} + \frac{1}{\tau_N}$$

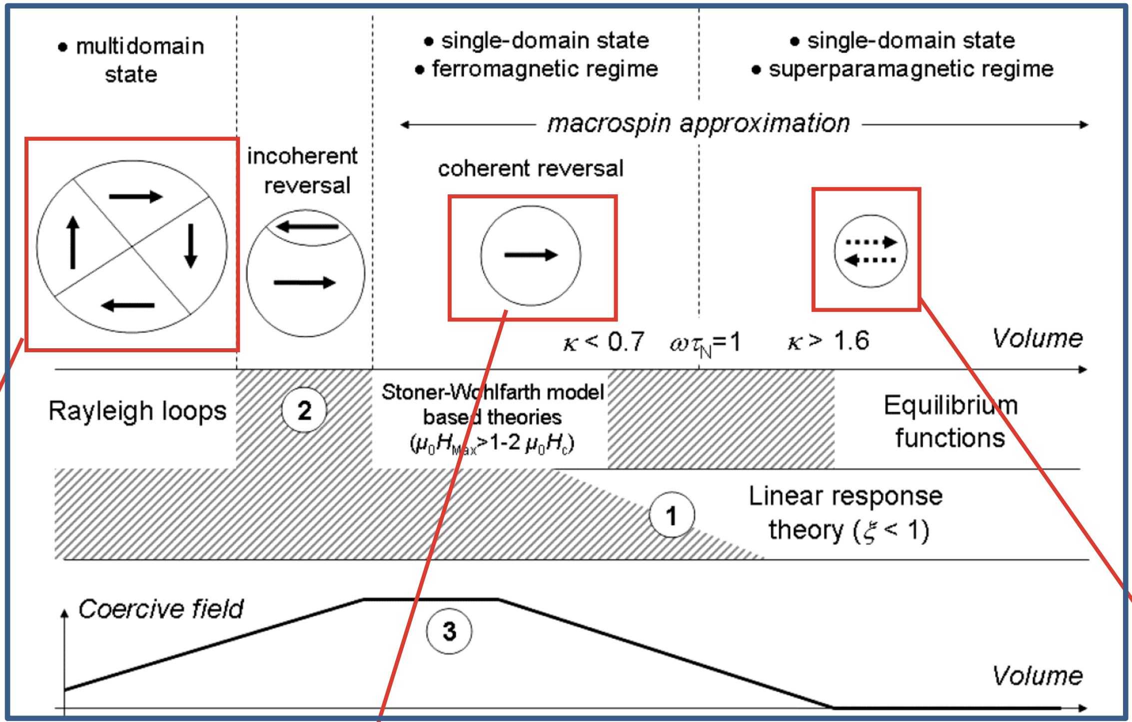
Magnetite colloid



Nanoscience, 2013, 1, 60–88 | 65

R.E. Rosensweig, **JMMM** **252** (2002) 370.

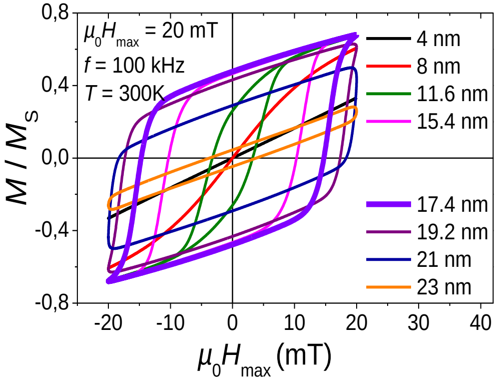
Basic principles



- Drop of coercive field→ less efficient
- No prediction possible

-No hysteresis→ no interest for heat
- Analytical calculation : Langevin…

- Open hysteresis loops→**optimized nanoparticles**
- Analytical calculations : Stoner-Wohlfarth model



J. Carrey et al. J. Appl. Phys. 110, 039902 (2011)

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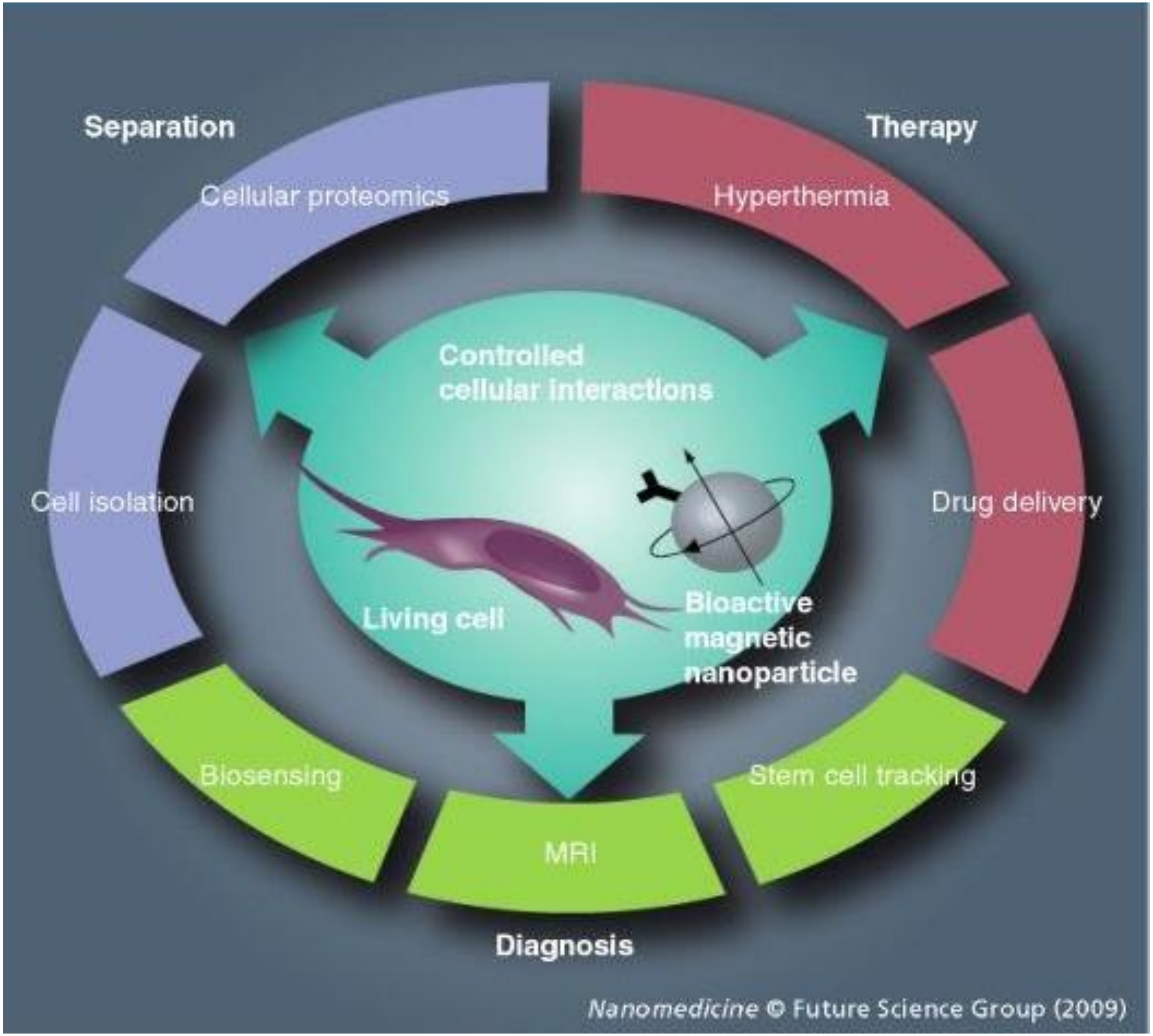
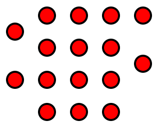
Biomedical applications



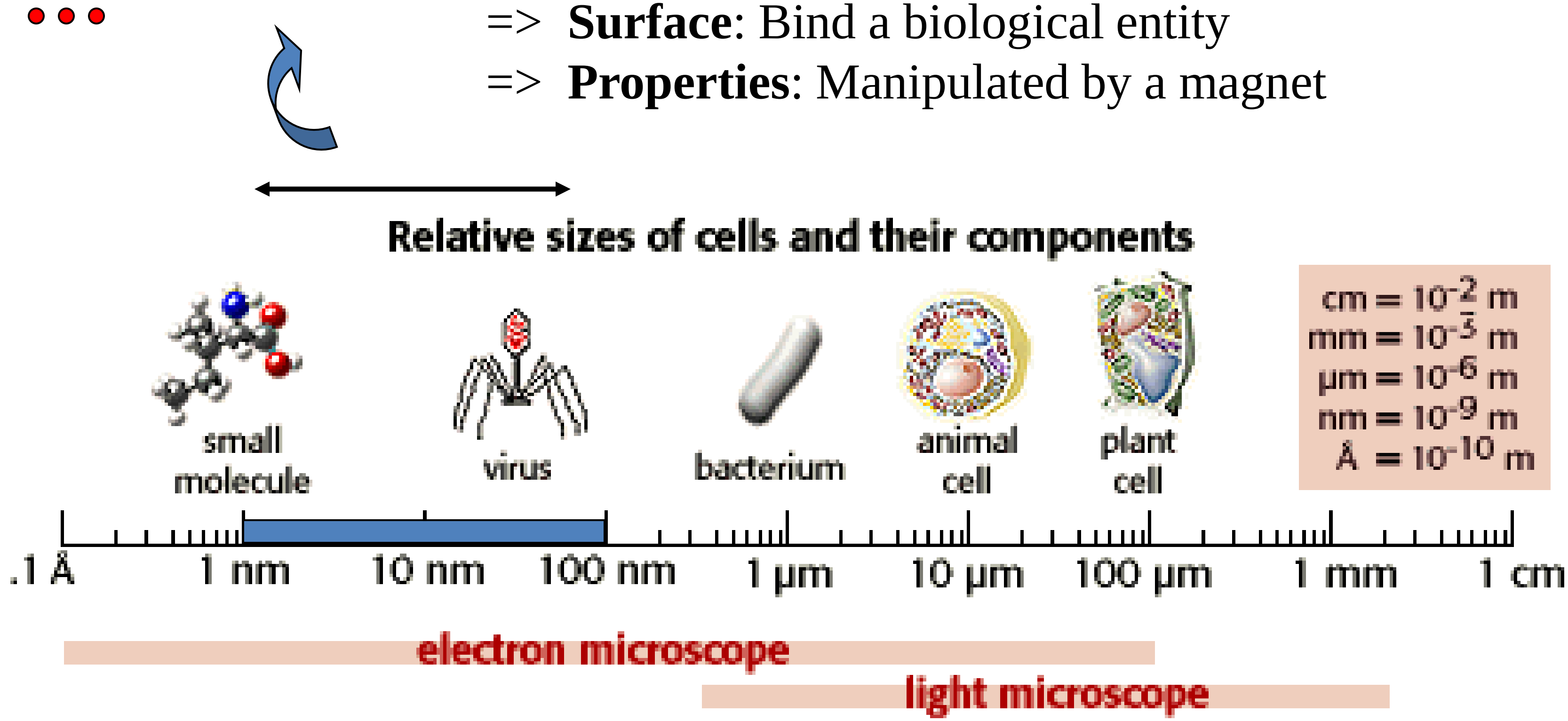
Requirements for biomedical applications



Nanometer => **Size**: Get close to a biological entity of interest

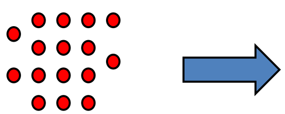
=> **Surface**: Bind a biological entity

=> **Properties**: Manipulated by a magnet



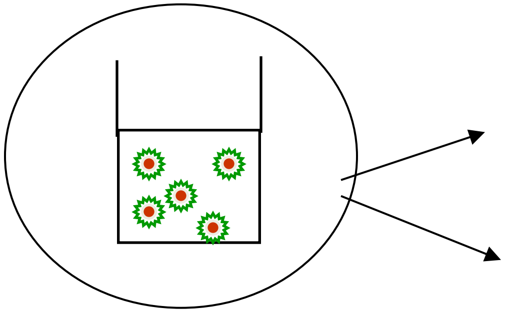
Requirements for biomedical applications

NANOPARTICLES



No toxic!!

COLLOIDAL SUSPENSIONS



- Stable
- Biocompatible
- Reversible

APPLICATIONS

In vitro

In vivo

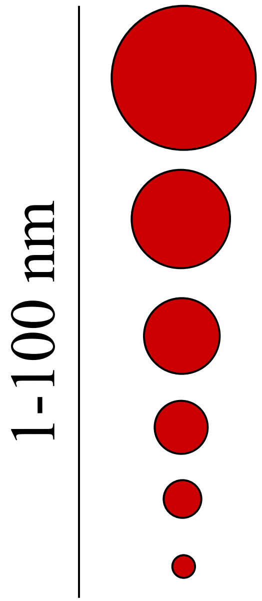
REQUIREMENTS

- **Size**
- **Surface**
- **Properties**

Requirements for biomedical applications

Size

5-50 nm = Ideal diameter for most forms of therapy



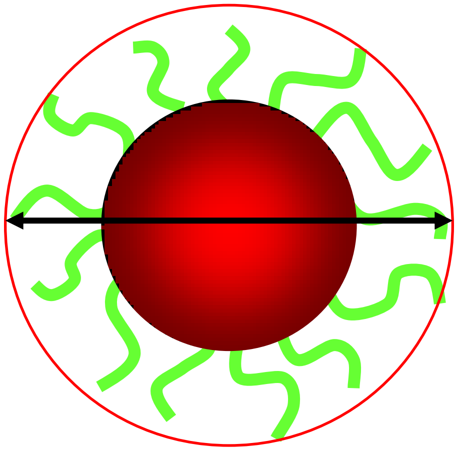
Detected by the immune system and eliminated

Remain in the body long enough to be circulated through the blood stream

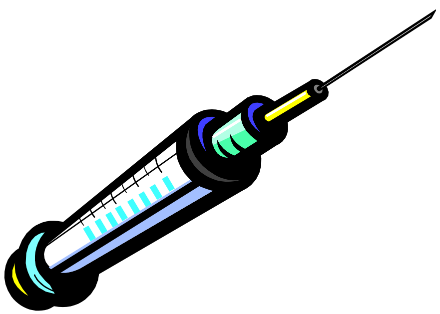
Small response to a magnetic field

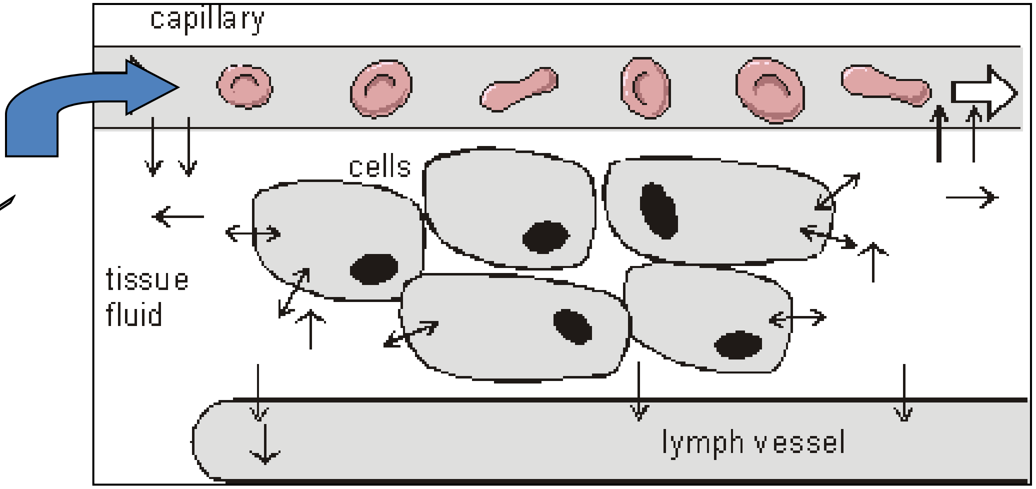
Requirements for biomedical applications

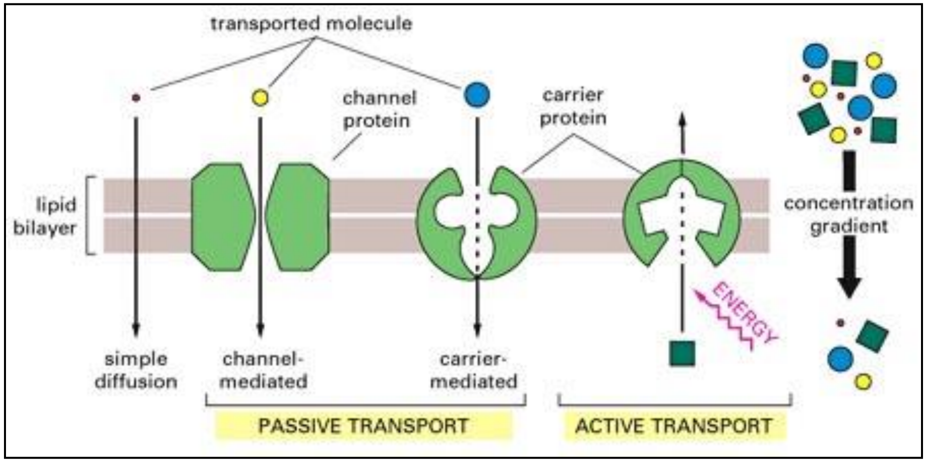
Hydrodynamic size



Core + Molecules around



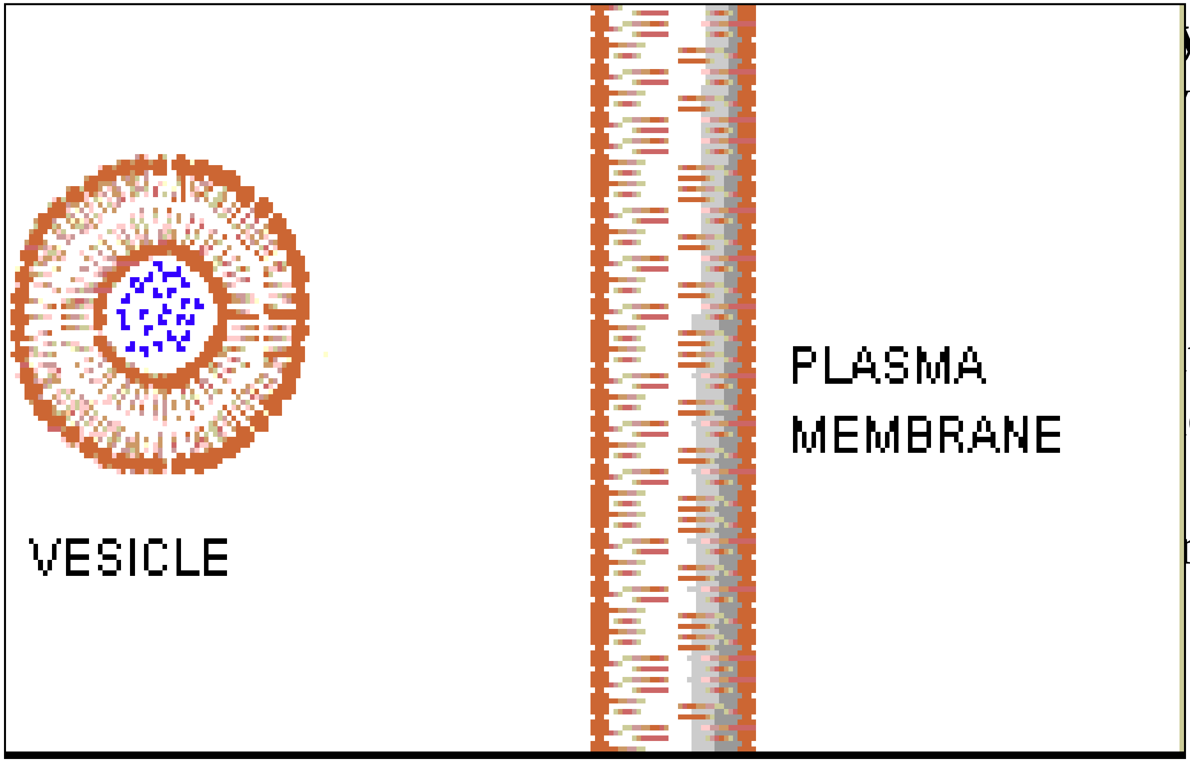




Requirements for biomedical applications

Surface

Modification of the particle´s surface to make it biocompatible and specific



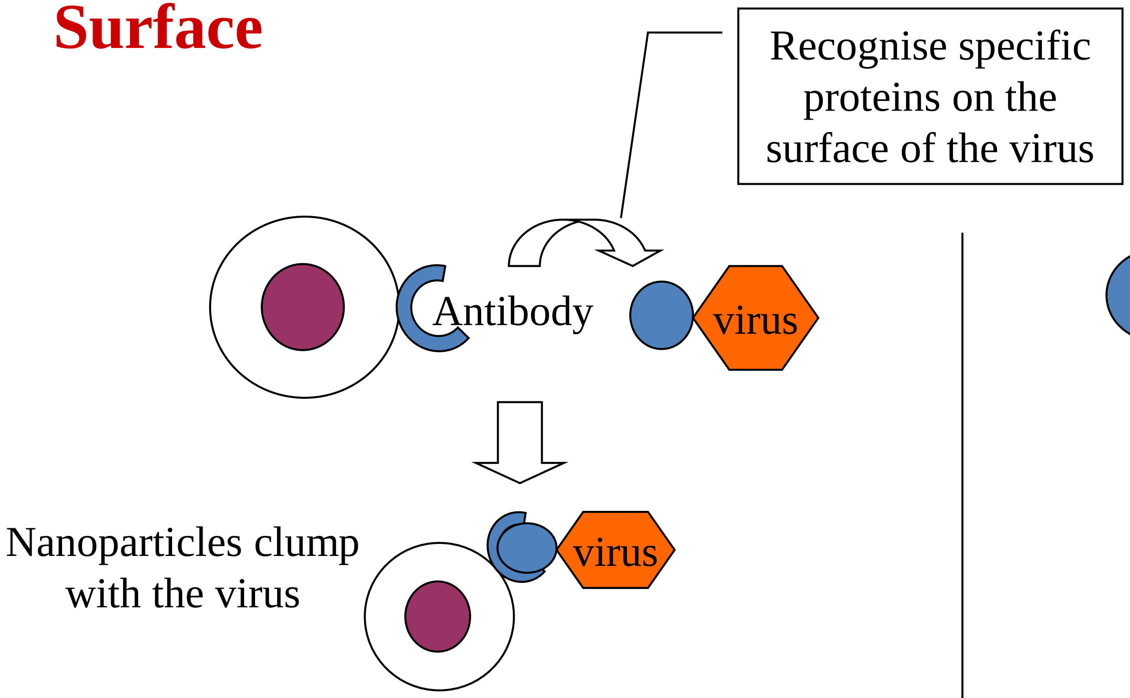
ydrophilic coating make
to the immune system

th a **biological entity** to
on in a specific manner

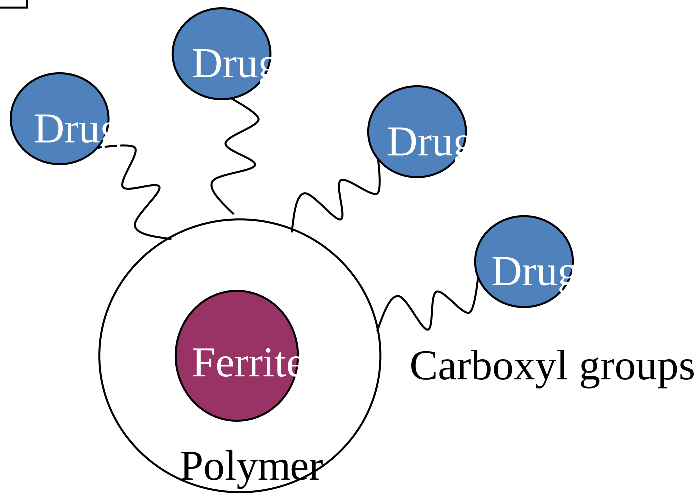
rt and delivery a biological

Requirements for biomedical applications

Surface



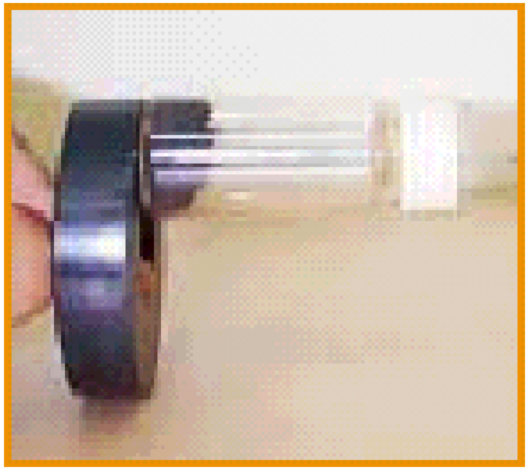
Detect virus in body fluids

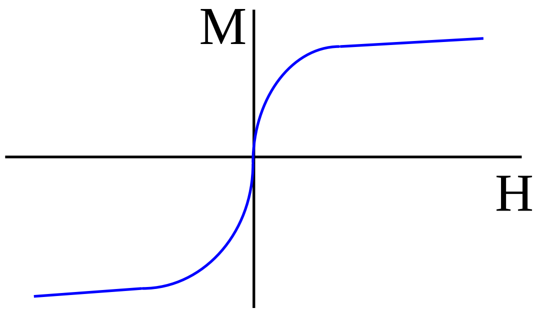


Recognise and destroy a cancer cell

Requirements for biomedical applications

Magnetic properties



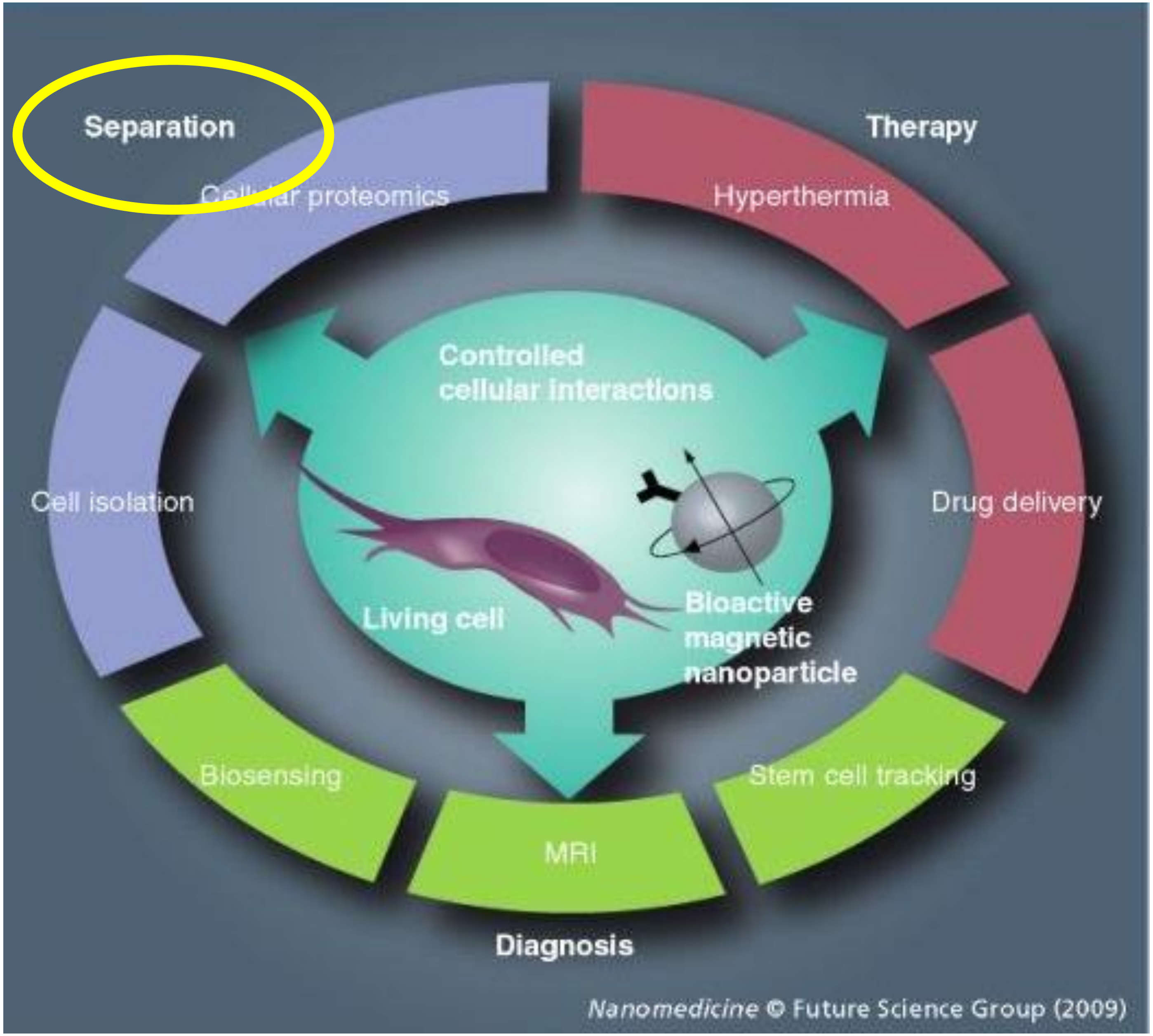


- They must **constantly and rapidly "flip" magnetic states.**
  => Mr=0

- **Saturation magnetisation** (Ms) should be **strong enough** to be manipulated by an external magnetic field

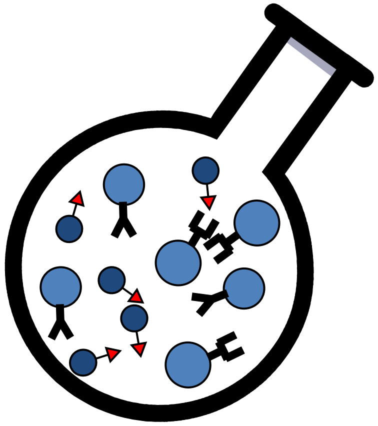
- **Resonant respond** to a time-varying magnetic field should be enough to heat up.

Biomedical applications



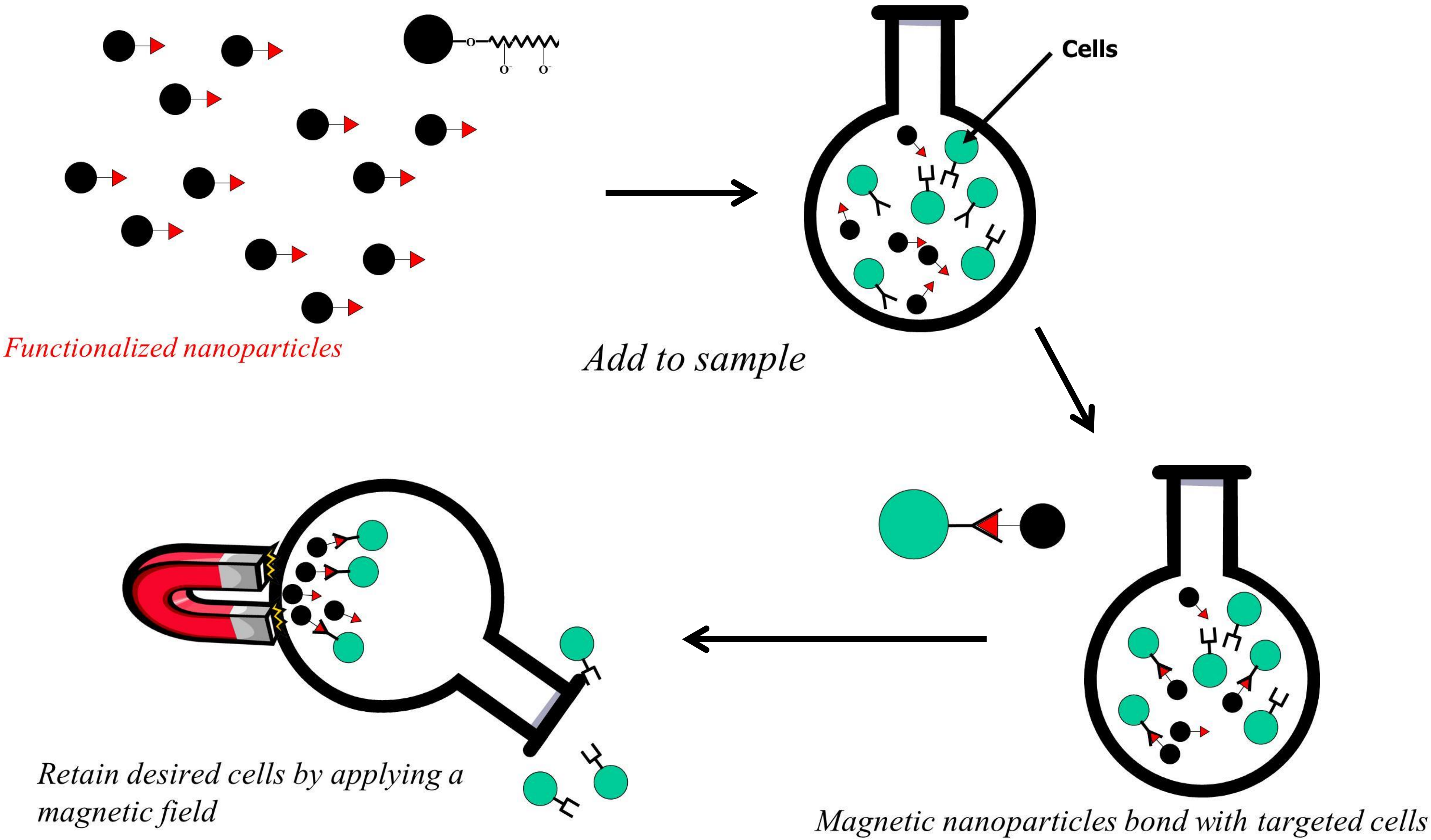
Separation/selection

- Goal: Separate/detect/isolate one type of cell from others, often when the target is present in very small quantities

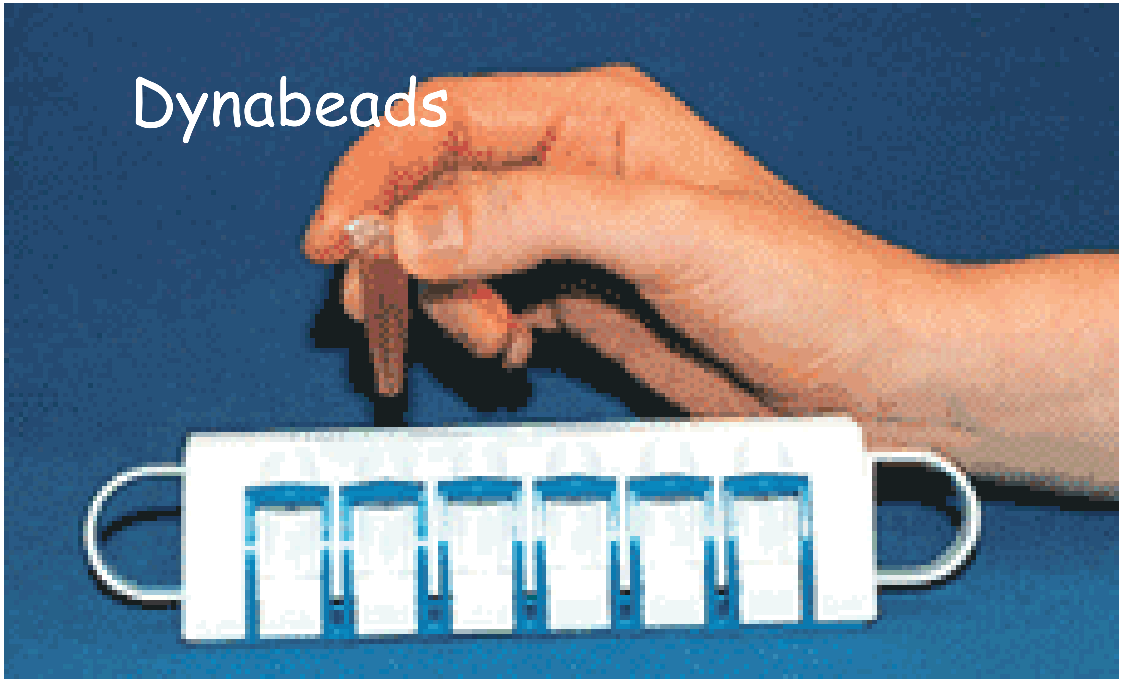


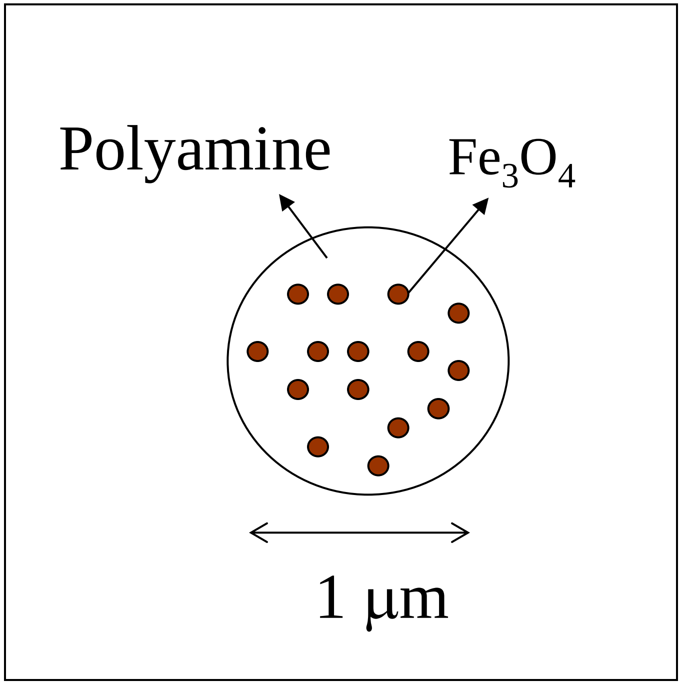
Reduce the time
Detect lower concentrations

Separation/selection



Separation/selection





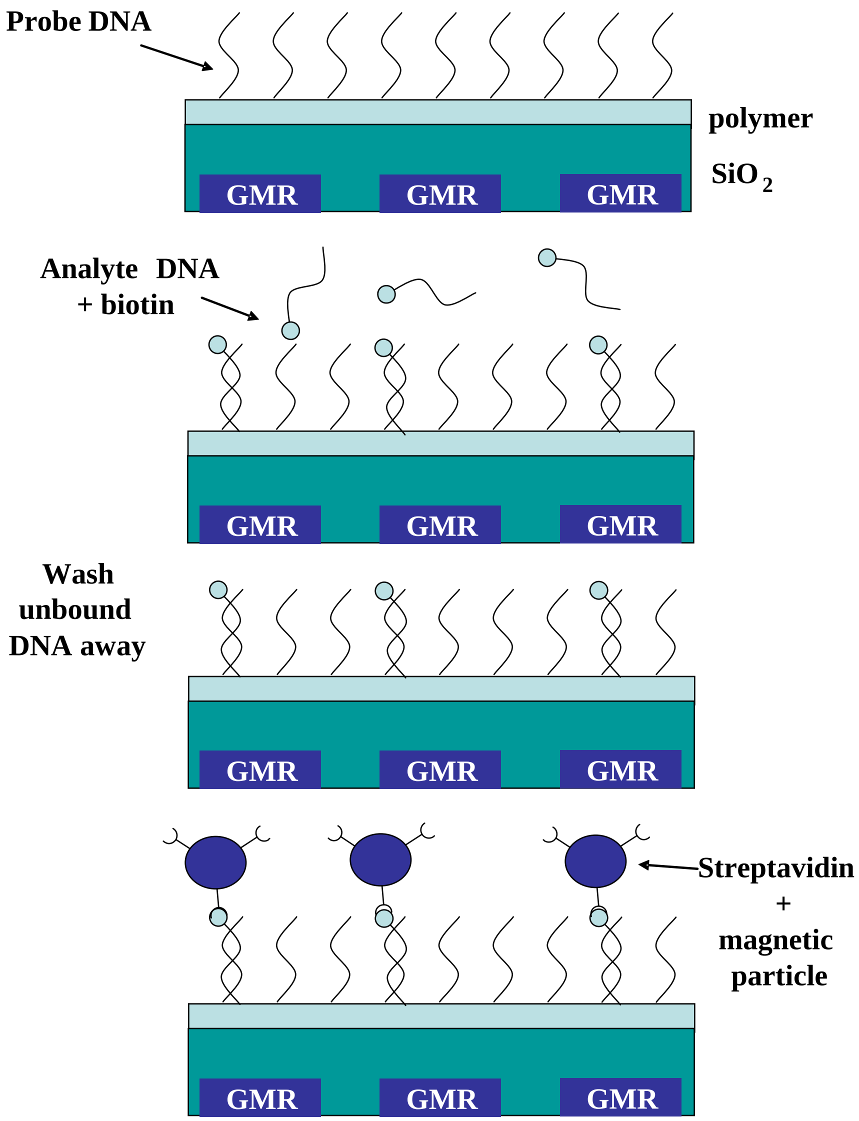
Separación y purificación

- ✓ Detection of proteins at 10^{-18} M = Prostate-specific antigen (PSA)
- ✓ Detection of DNA at 10^{-21} M

6 orders of magnitude more sensitive

J-M. Nam, C. S. Thaxton, C. A. Mirkin, Science, 301 (2003) 1884.
J-M. Nam, S. I. Stoeva, C. A. Mirkin, J. Am.Chem. Soc., 126 (2004) 5932

Separation/selection



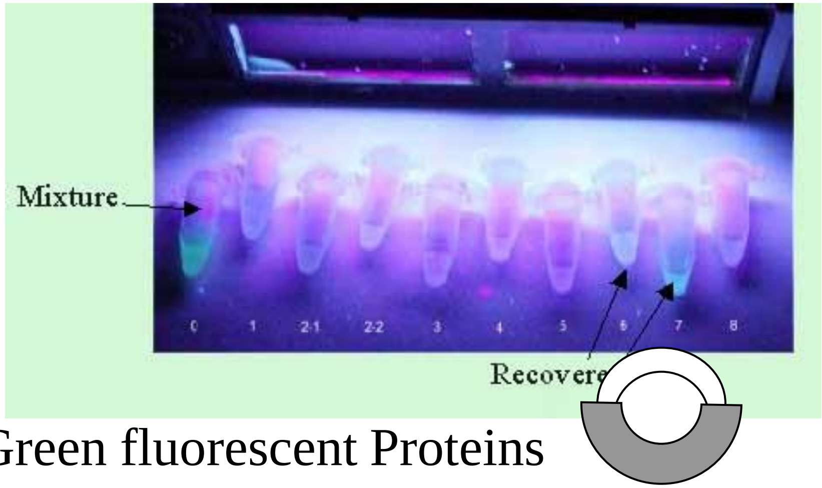
Magnetic Sorting/Detection

- **High sensitivity**
- **Multiple analytes at one time**
- **Hand-held**
- **Lightweight**
- **Fast**
- **Potential for single-bead detection**

IEEE Magn. Letter 2017, Lago Chacon et al.

Separation/selection

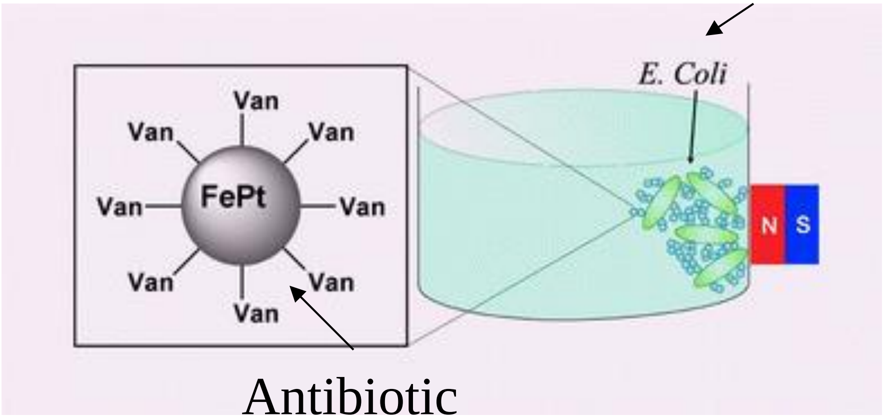
BIOMOLECULE SEPARATIONS



Green fluorescent Proteins

FOOD QUALITY CONTROL

Bacteria



Antibiotic

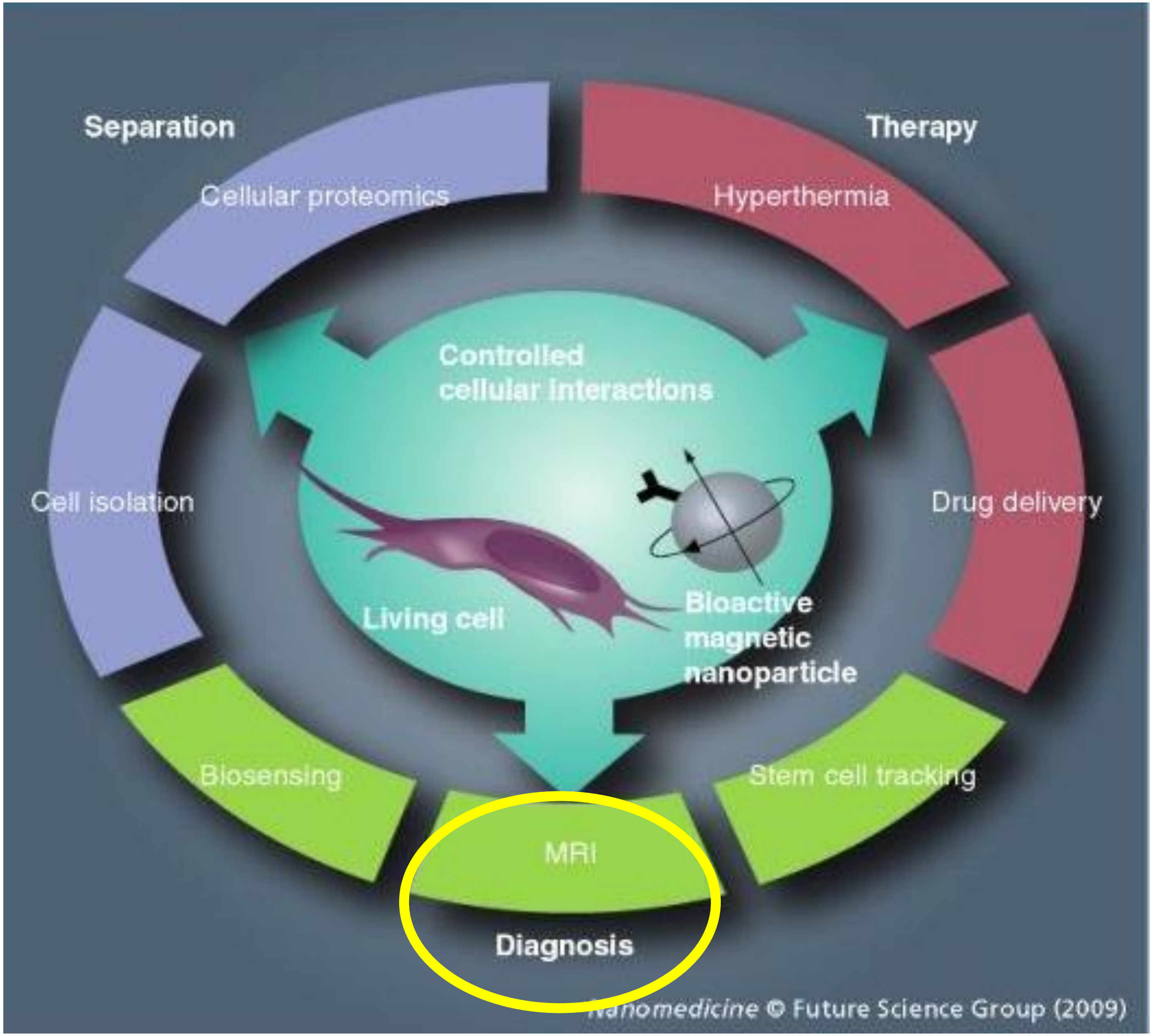
WATER PURIFICATION (Ar, Pb, Hg, Zn…)

884 millones = Personas que carecen de acceso a fuentes de abastecimiento de agua potable (una de cada ocho)

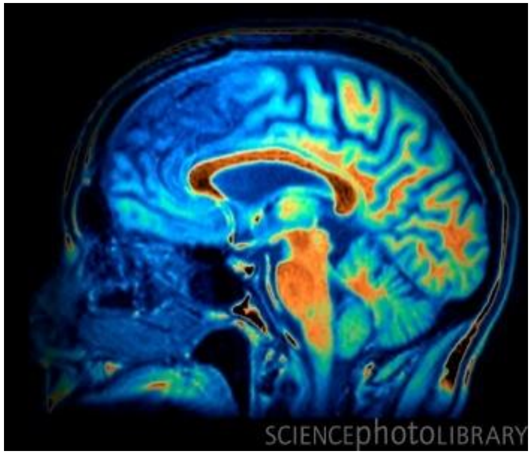
Yavuz, C. T. Prakash, A. Mayo, J. T.; Colvin, V. L. Chemical Engineering Science 2009, 64, 2510-2521;

Yavuz, C.T., Mayo, J.T., Yu, W.W. et al. Low-field magnetic separation of monodisperse Fe3O4 nanocrystals. Science 10 (2006)
Environ Geochem Health DOI 10.1007/s10653-010-9293-y
Mohan and Pittman 2007

Biomedical applications



NMR Imaging



The most powerful technique for diagnosis

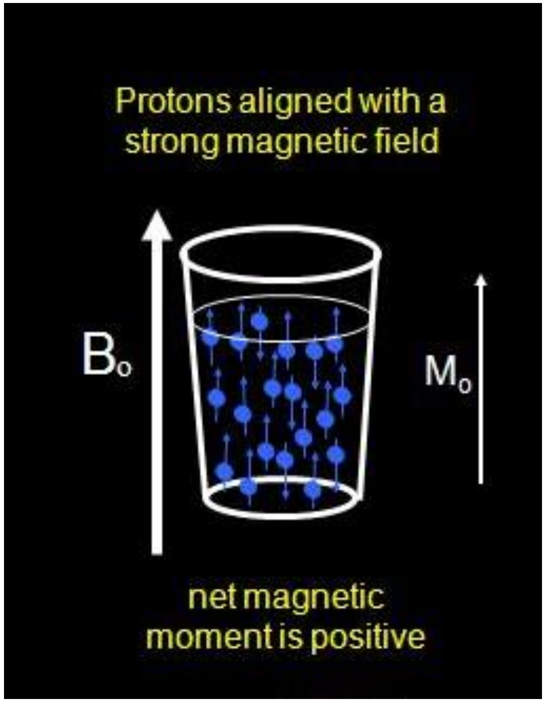
Nobel Prize 2003
Paul C. Lauterbur and Sir Peter Mansfield
"for their discoveries concerning magnetic resonance imaging"

Advantage: not use X-Rays nor any other type of "ionizing" radiation

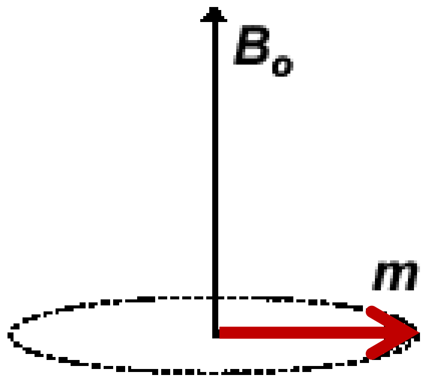
Instead: it is a technique that combines a large magnetic field and some radio frequency antennas

Measure the relaxation rate of protons in the atoms of water within the patient from their excited state to the ground state

NMR Imaging

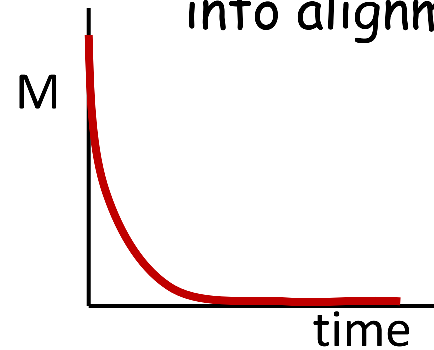


high-frequency electro-magnetic pulse



protons out of alignment

"resonance" signal as the proton goes back into alignment



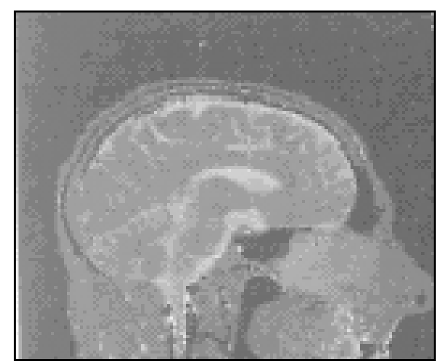
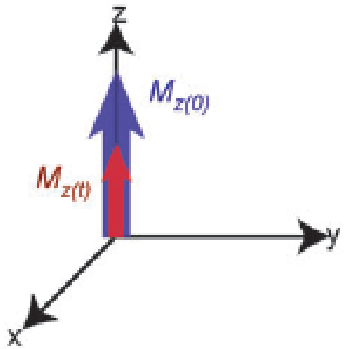
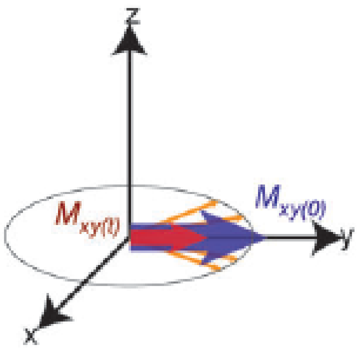


image reflects the water protons in the patient and their chemical association with proteins

T1



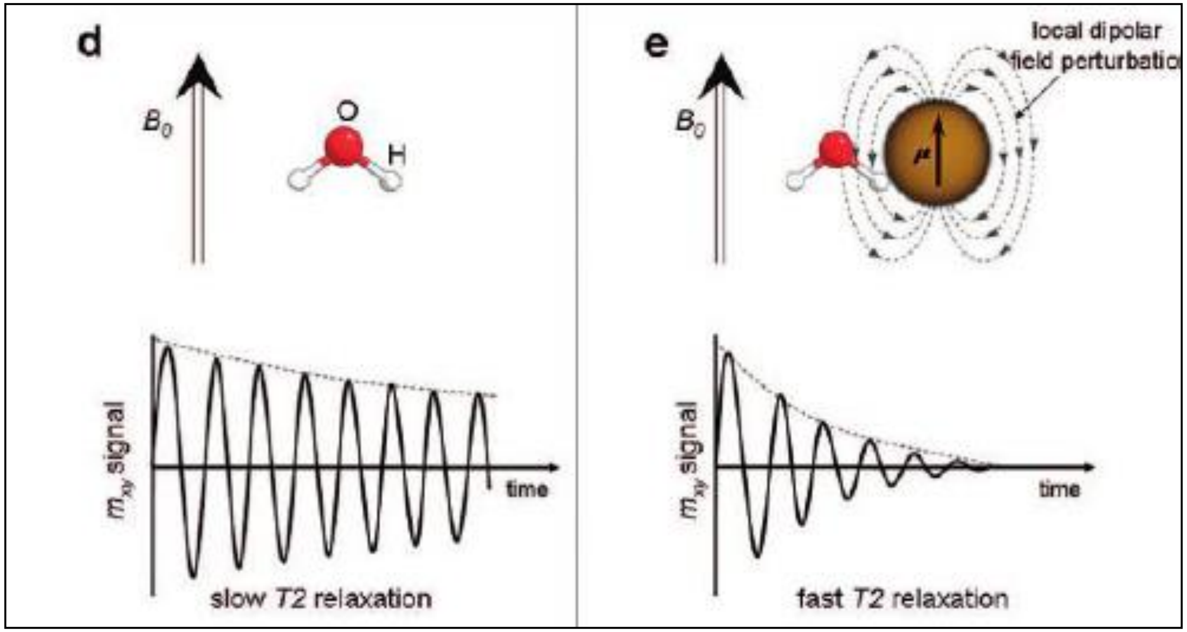


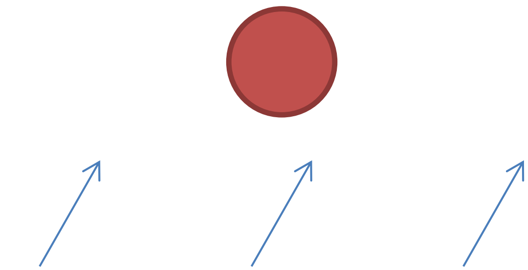
T2

Fig. 22
Coupling of a T_1- and a T_2-curve resembles a mountain with a slope. It takes longer to climb a mountain than to slide or jump down, which helps to remember that T_1 is normally longer than T_2.

MRI made easy

NMR Imaging

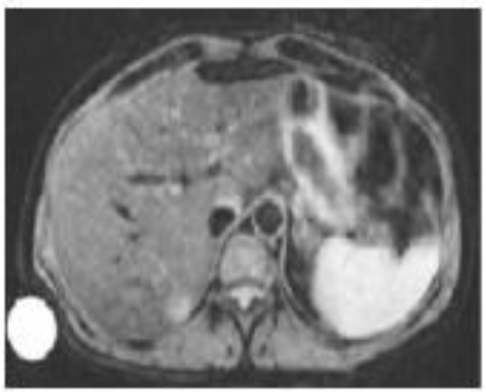


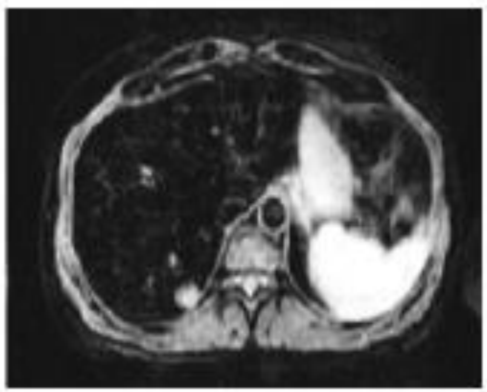


Slow White

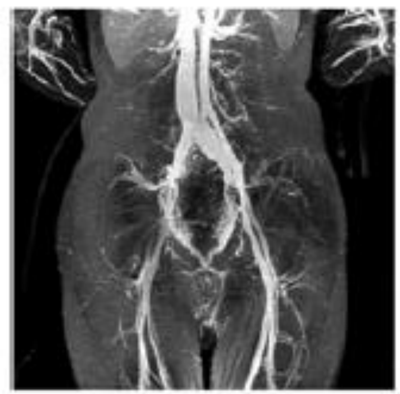
Fast Black

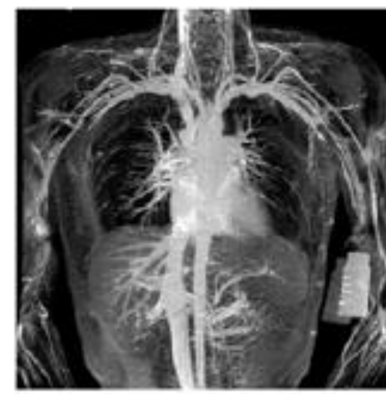
Slow white





Negative contrast from iron oxide

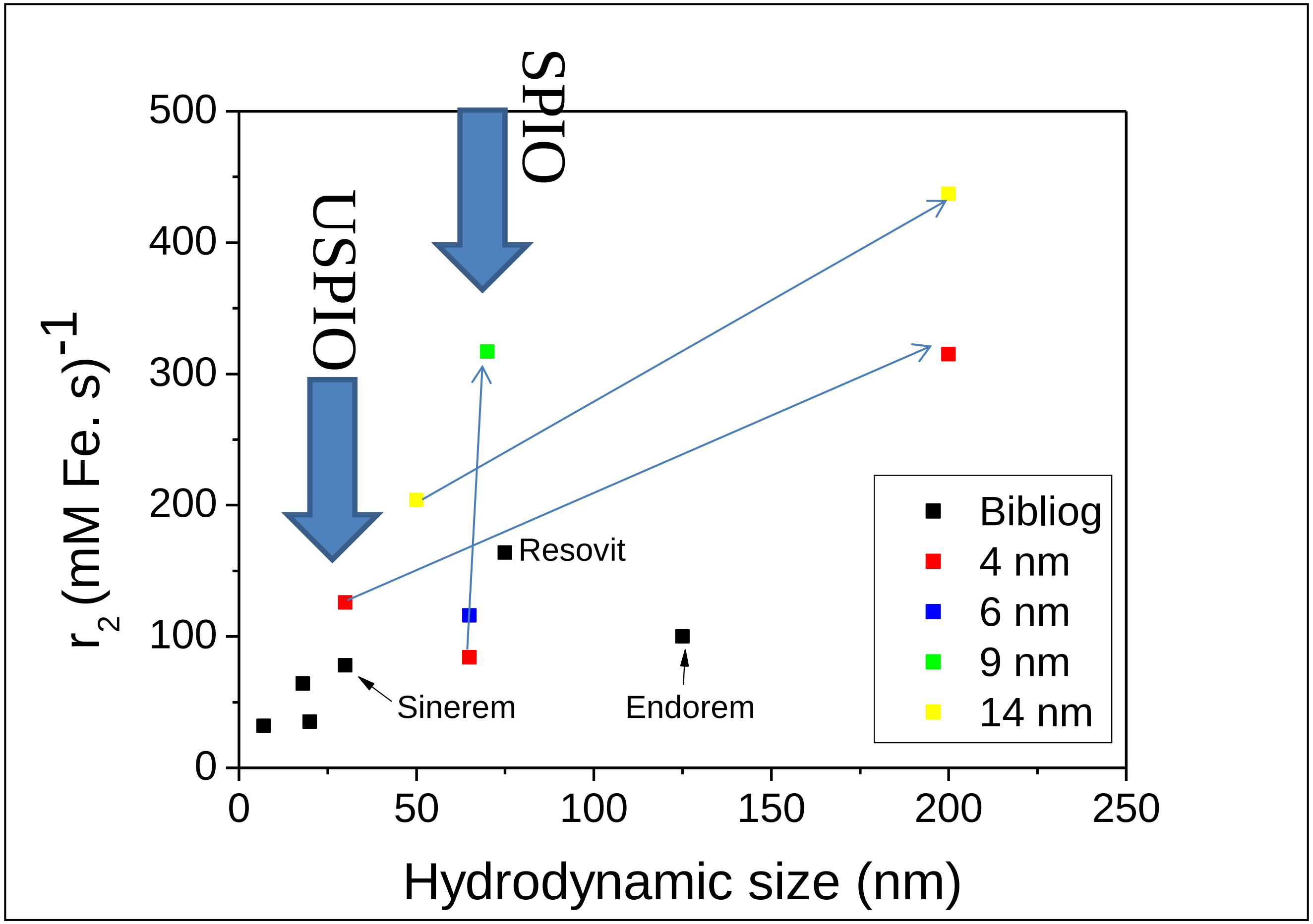




Positive contrast

NMR imaging

Commercial products = 5-10 nm



NMR Imaging

The chemistry of contrast agents in medical magnetic resonance imaging, André E. Merbach and Eva Toth, Wiley, 2001

Shorter relaxation (T_2)
=> **Darker** in the MRI

NP magnetic moment

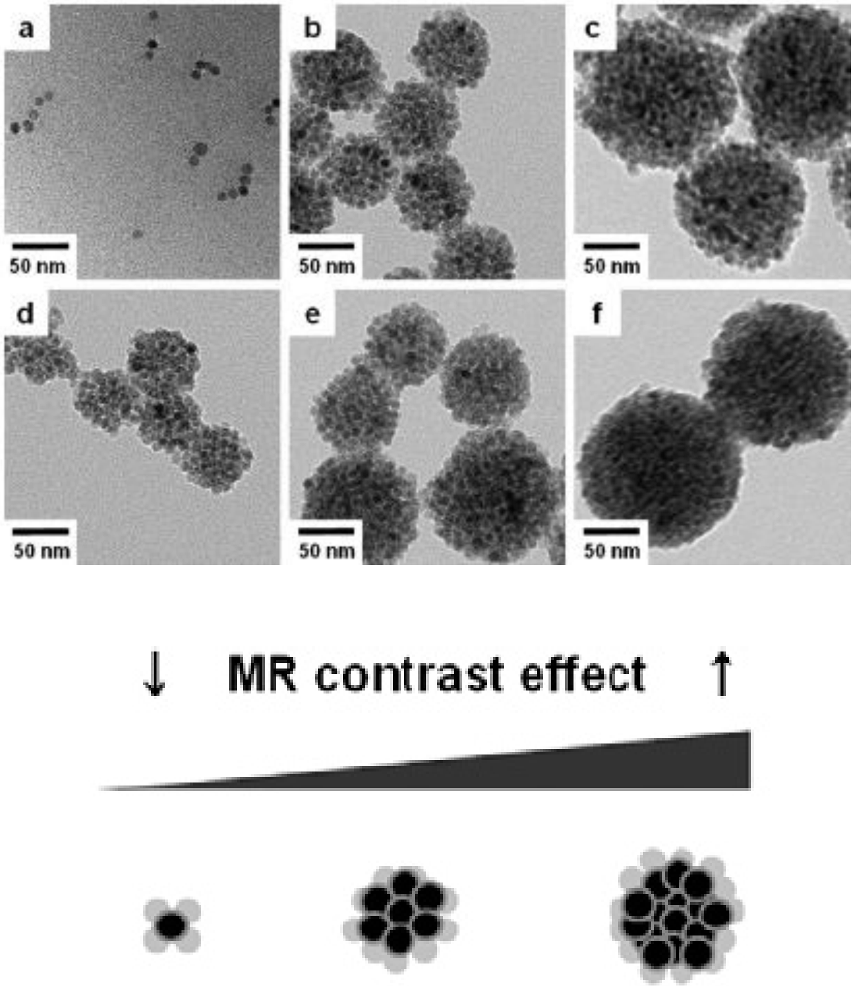
Concentration (mole.L^{-1})

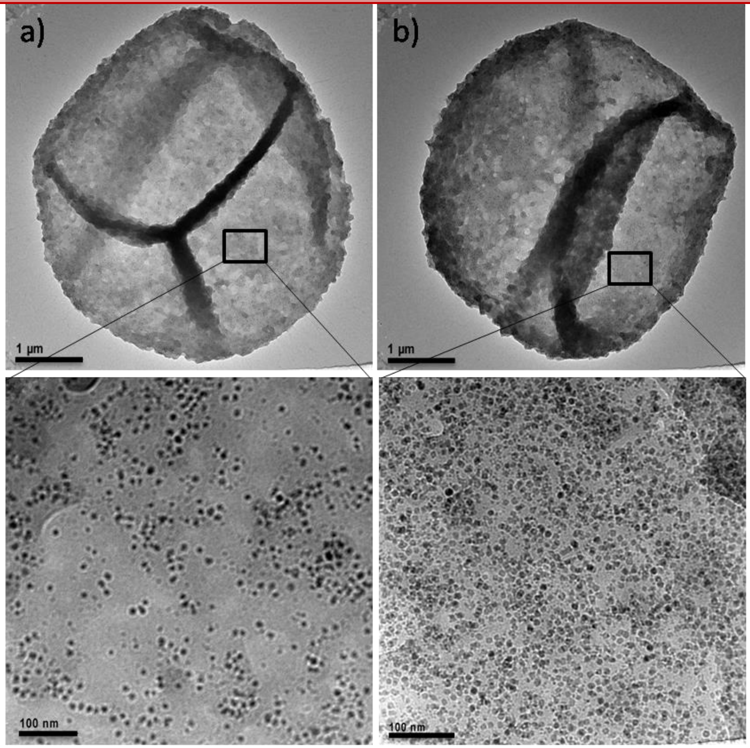
$$\frac{1}{T_2} = R_2 = \frac{\left(\frac{64\pi}{135000}\right) \gamma^2 N_A M \mu^2}{r\, D}$$

NP radio

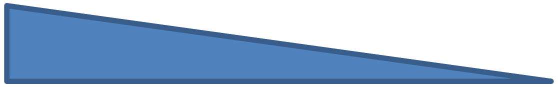
Diffusion coefficient of water molecules

NMR Imaging





↑ MR contrast effect ↓



Azhar Zahoor AbbasiJ. Phys. Chem. C 2011, 115, 6257

S.-B. Seo et al. / Journal of Colloid and Interface Science 319 (2008) 429–434

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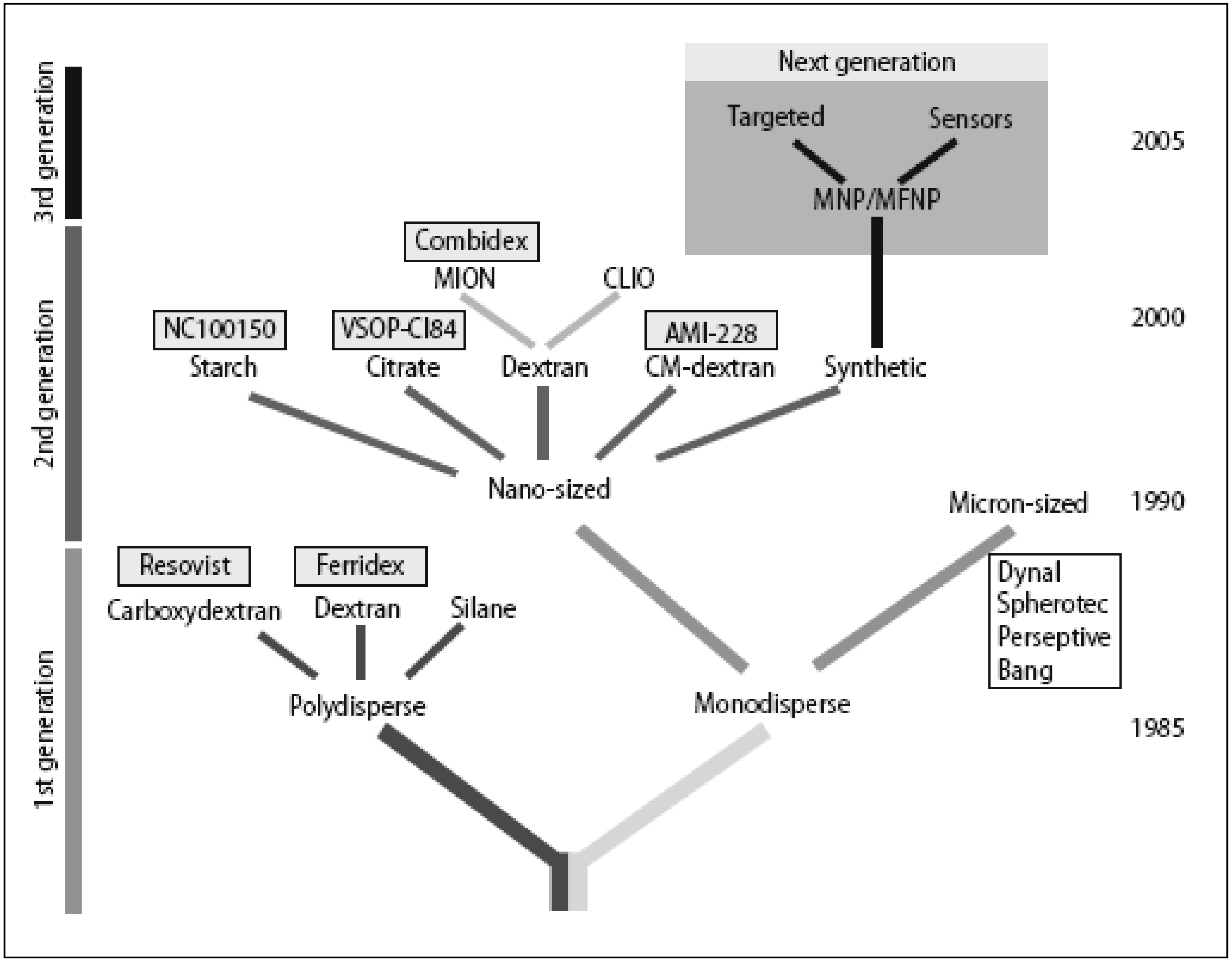
NMR Imaging



Challenges

Ralph Weissleder

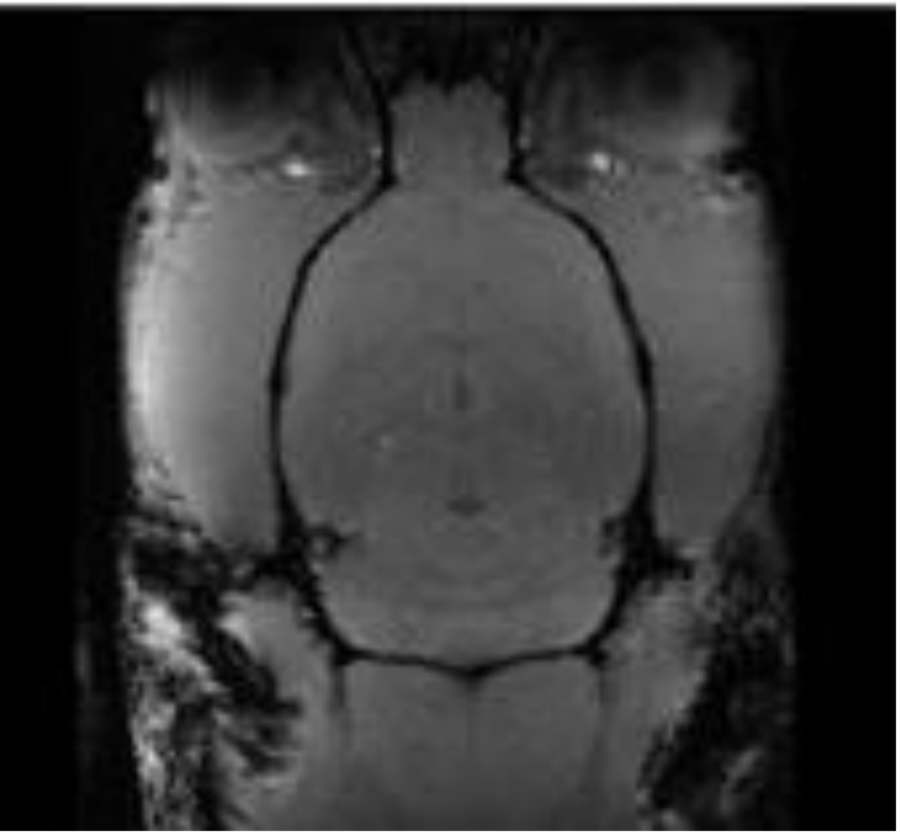
R. Weissleder et al. 2001 *Angew. Chem.*, Int. Ed. **40** 3204

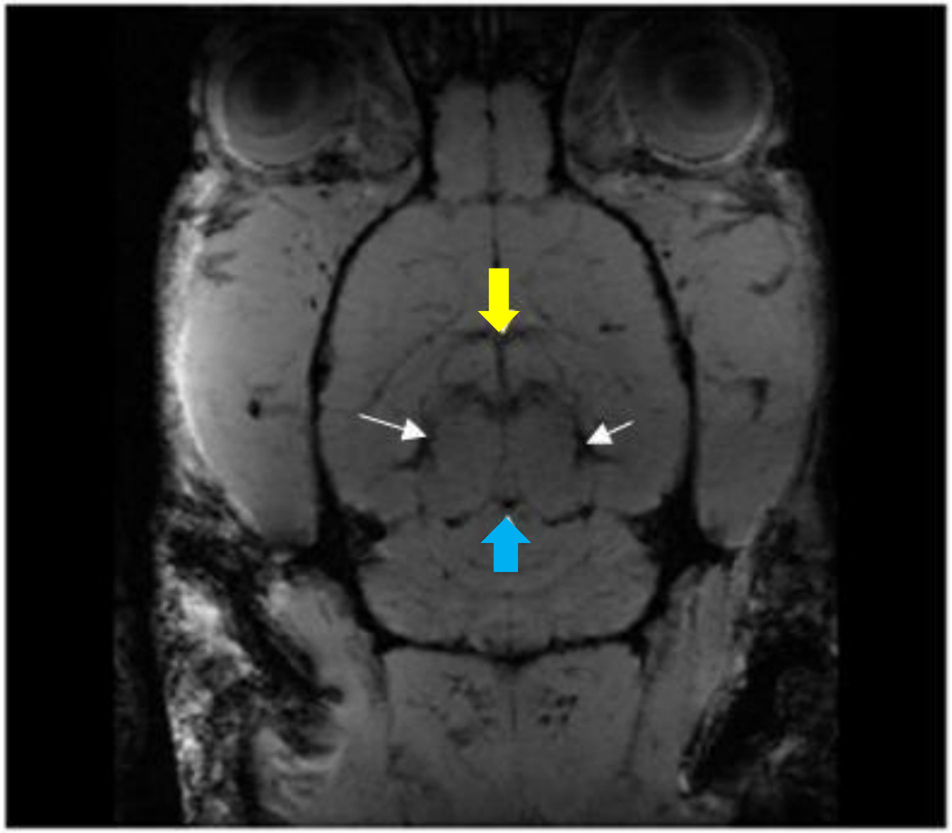
Basic Res Cardiol 103:122–130 (2008)

NMR Imaging

USPIO

NMR IMAGING OF RAT BRAIN DURING 1 HOUR





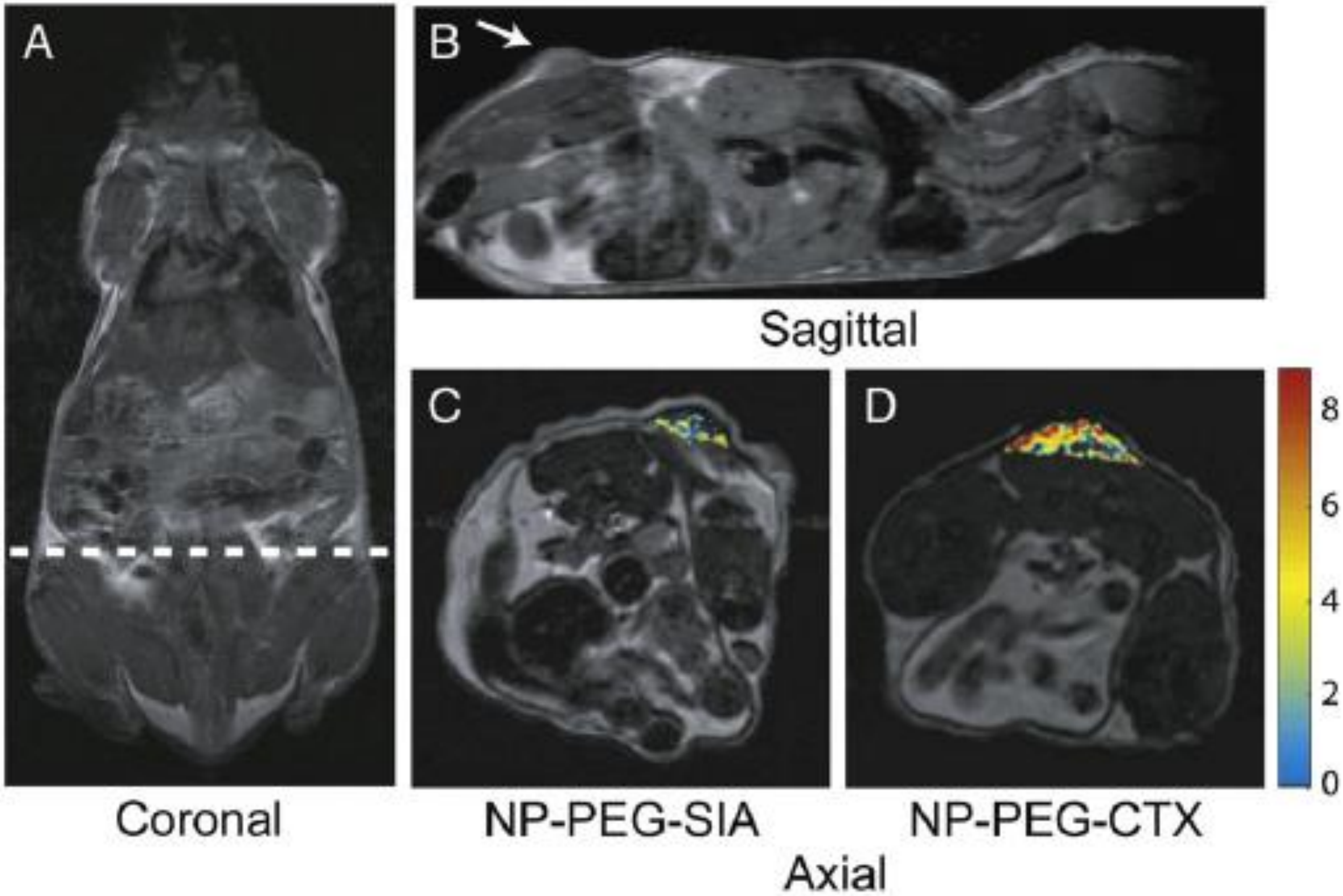
Arrowheads (*Yellow*)- Third Ventricle
Arrows (*white*)- Lateral Ventricle
Arrowheads (*blue*)- Recess *Inferior Colliculus*

R. Mejias et al., Nanomedicine 2010

NMR Imaging

Targeted imaging

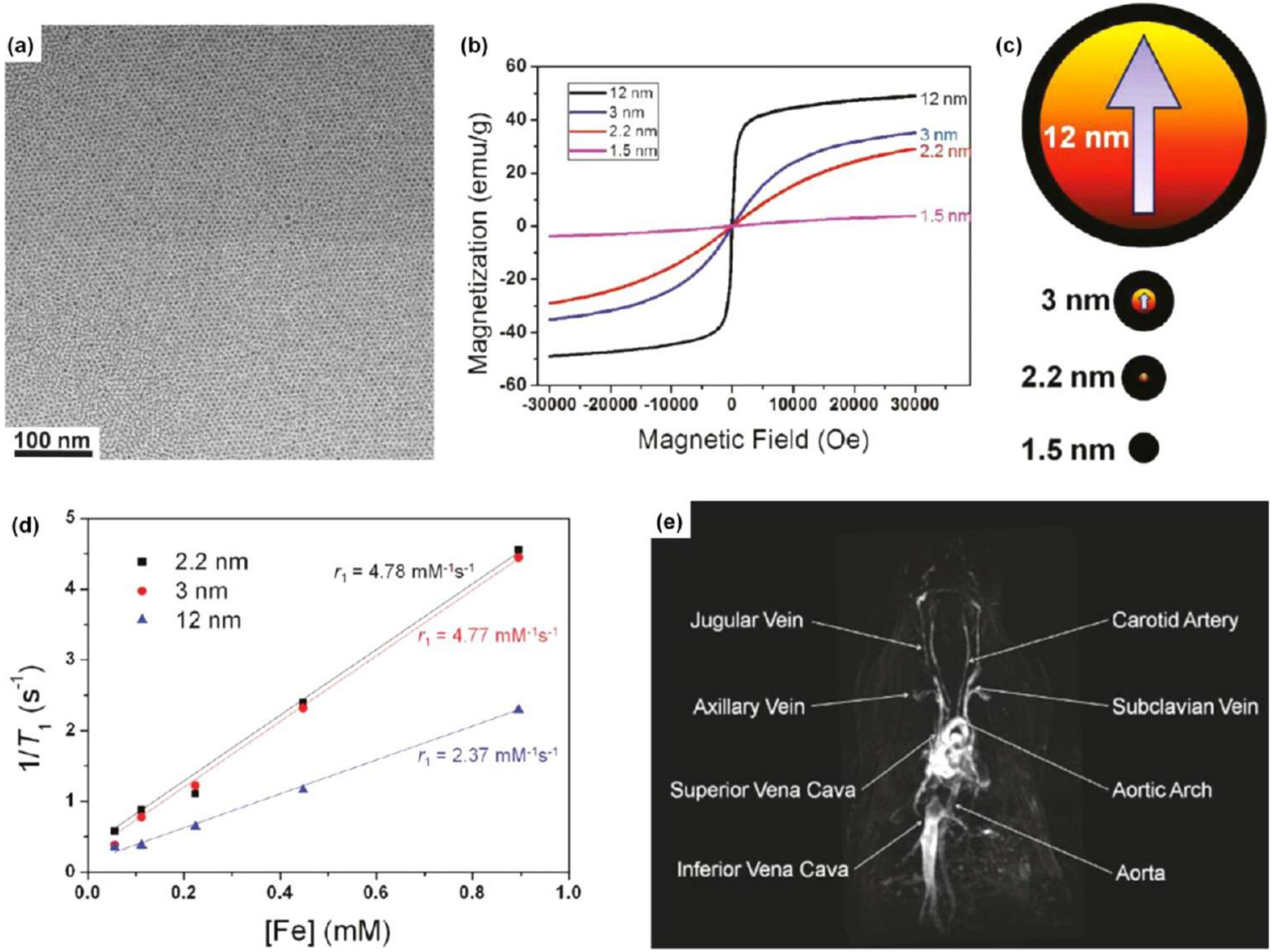
tumor



C. Sun et al. / Advanced Drug Delivery Reviews 60 (2008) 1252–1265

NMR Imaging

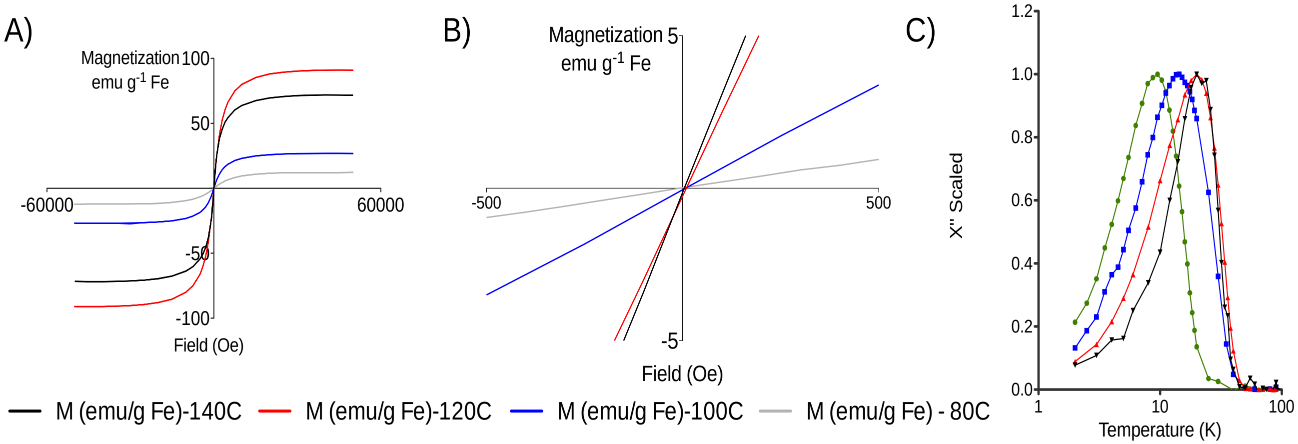
T1 contrast agents based on ultrasmall iron oxide nanoparticles

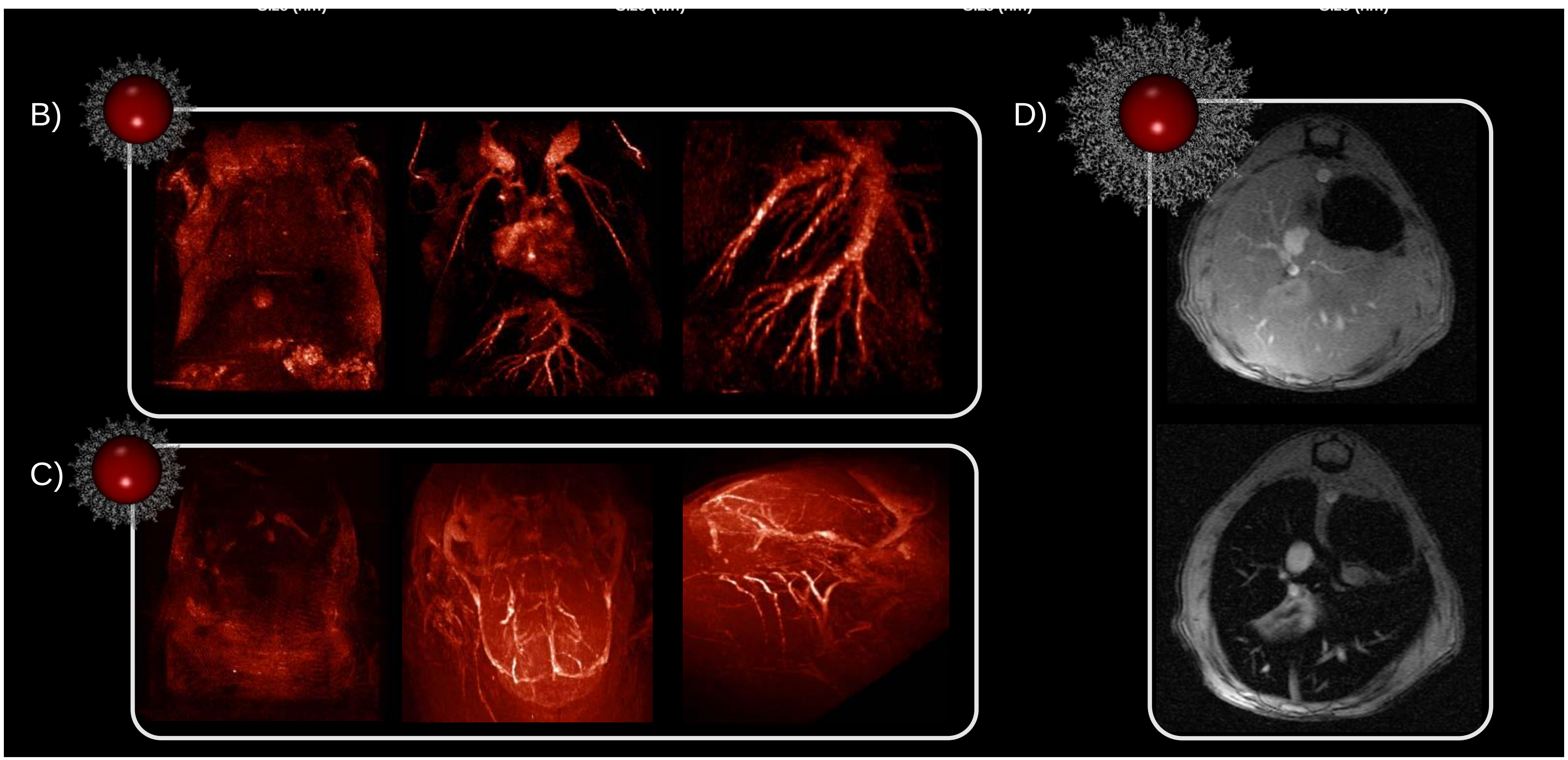


Kim, B. H.; et al., J. Am. Chem. Soc. 2011, 133, 12624.

NMR Imaging

Fernando Herranz et al

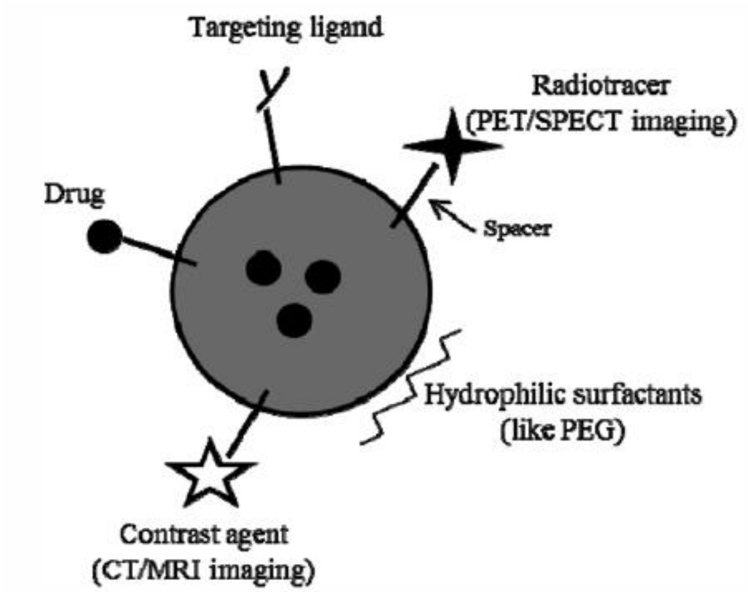




NMR Imaging

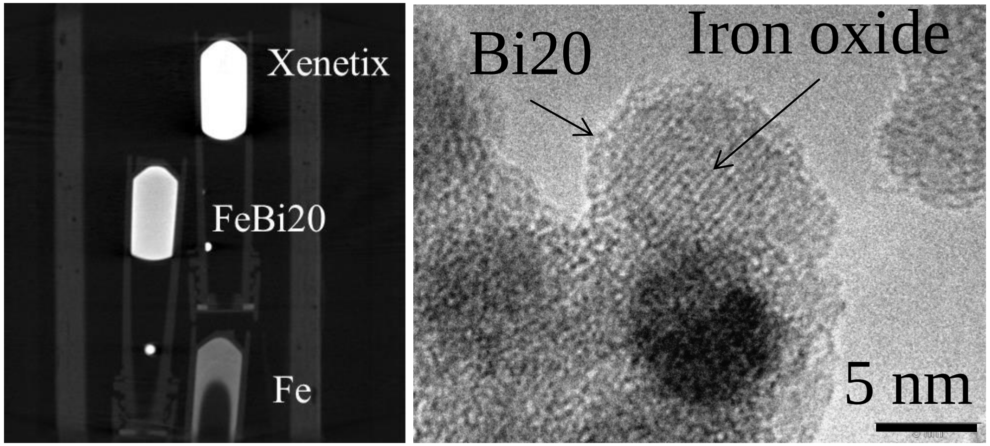
Multifunctional contrast agents

Imaging technology	Contrast agents	Spatial resolution	Toxicity	Sensitivity	Time Resolution
X-ray CT	Iodinated contrast material	sub-mm	Nephrotoxic	mM	1–2 s
MRI	Gadolinium-based	sub-mm	Nephrogenic systemic fibrosis	mM for Gd-based nM for Fe-based	1–2 s
PET/SPECT	Radioactively labelled agents	mm	Dosimetric exposure	pM	min



Core/Shell Magnetite/Bismuth Oxide Nanocrystals with Tunable Size, Colloidal, and Magnetic Properties

Evaluation as CT agent

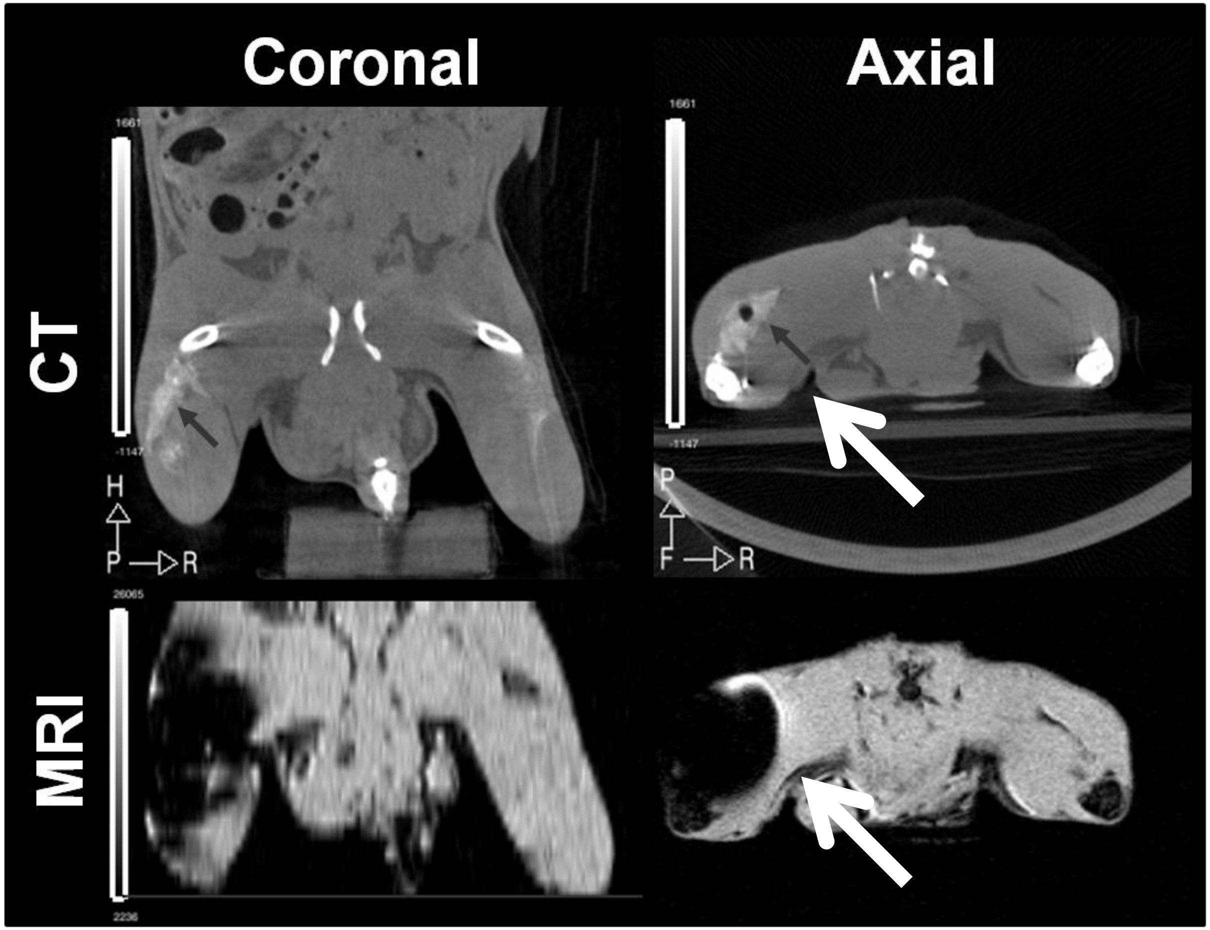


Chem. Mater. 2012, 24, 319−324

Nanotechnology 2015

Hybrids

CT+ MRI

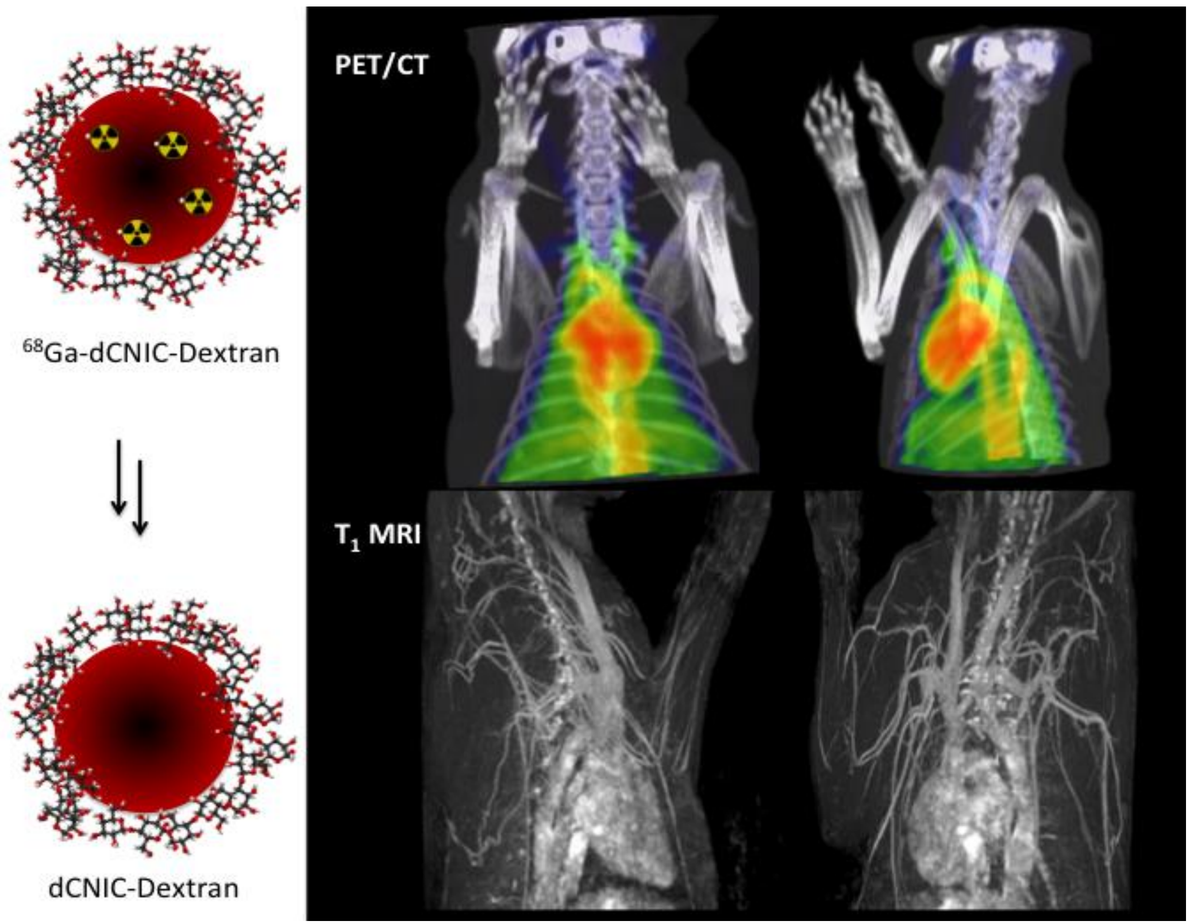


Coronal and axial images taken by CT and MRI after the subcutaneous administration of 100µL of FeBi@SiPEG (157mM Fe and 14.6mM Bi). The location of the contrast in the left leg of the mouse is marked with a arrow in the CT pictures.

Nanotechnology 26 (2015) 135101

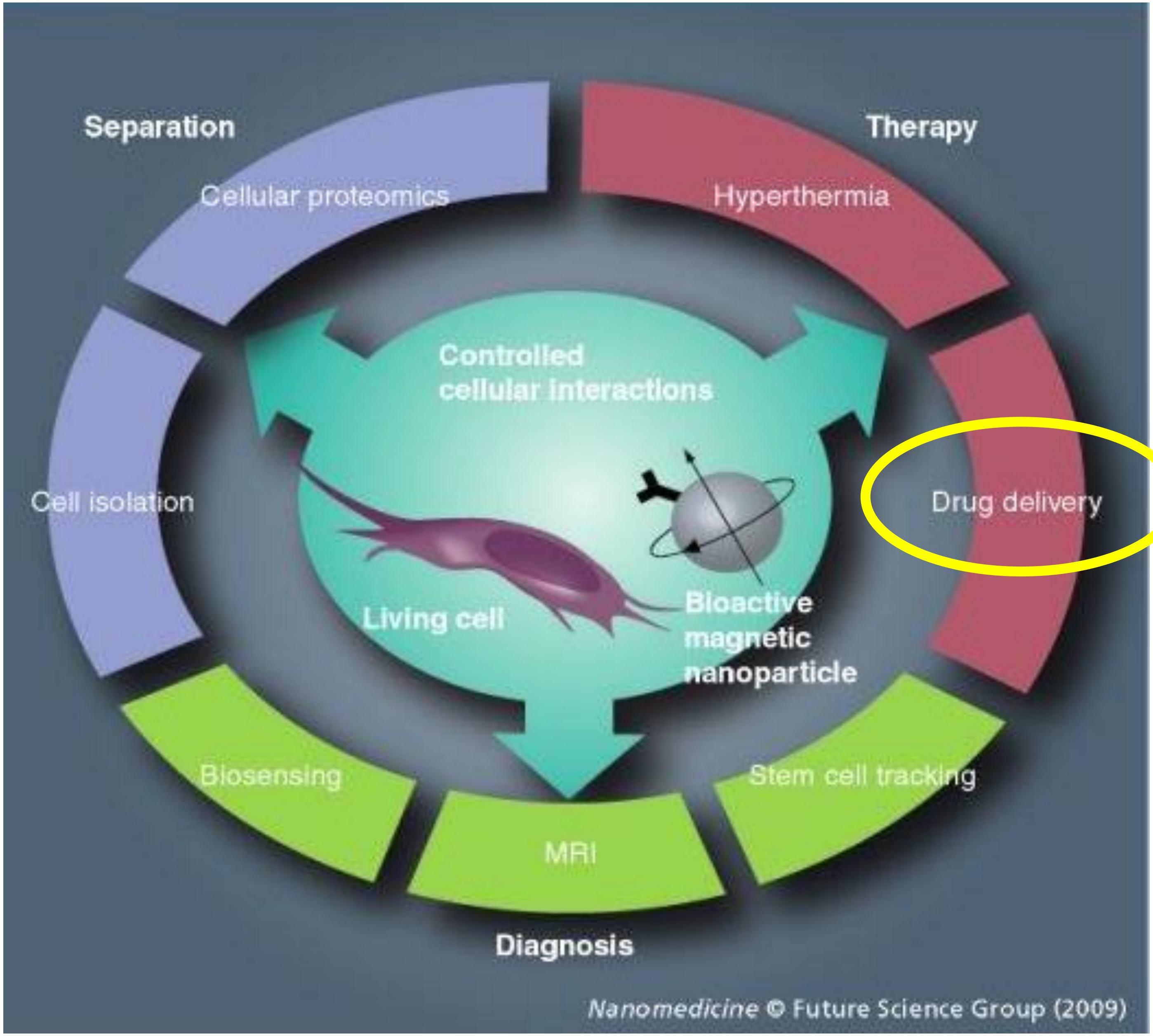
Hybrids

PET + MRI



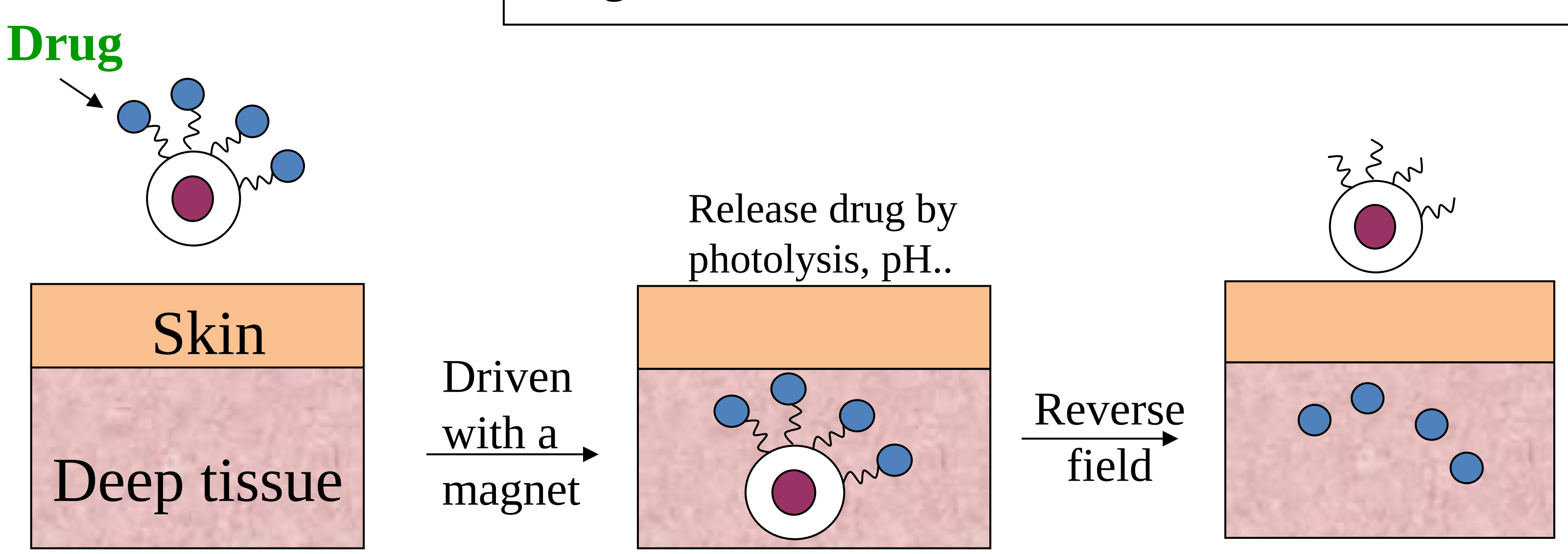
Pellico et al, Contrast Media Mol Imaging (2016)

Biomedical applications



DRUG DELIVERY

Targeting of a drug immobilised on magnetic nanoparticles under the action of an external magnetic field.



IONP ionically or covalently binding a drug

- **Specific** -> Reducing side effects
- **High local concentration** -> Reducing the dosage
- **Problem** -> Field strength

→ Human preliminary test

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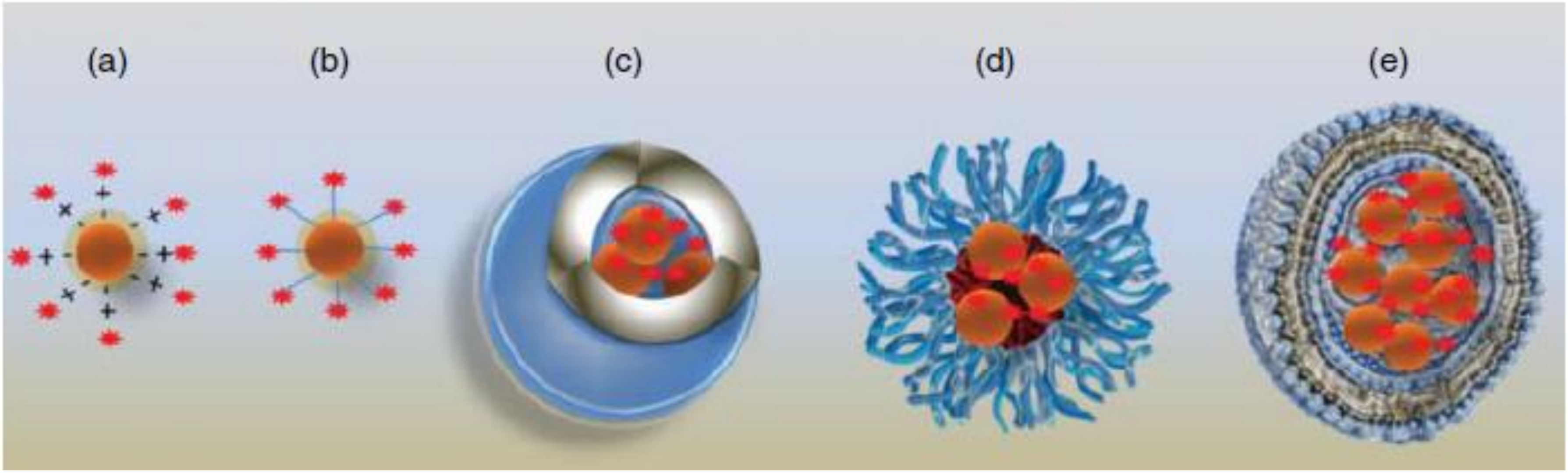
Drug carrier systems



IONP ionically or covalently binding a drug

Polymer coated nanosystems loaded with drugs and IONPs

Drug loaded magnetic micelles

Lipid vesicles loaded with drugs and IONPs

Drug delivery

Cytokine IFN-γ

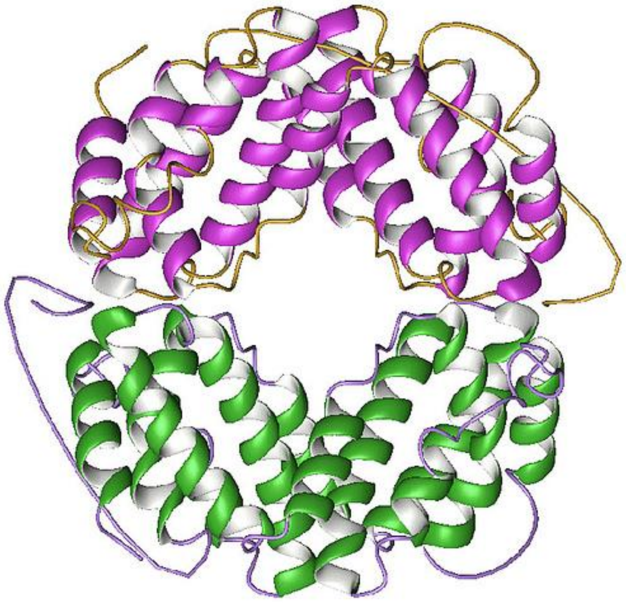
CNB

Cancer inmunotherapy : Activating immune response to removal primary tumor and prevent metastases.

Cytokine: small protein produced by macrophages and T lymphocytes

Activity:

- Activate macrophages production
- Induce cancer cell apoptosis



IFN-γ the most effective cytokine in tumor elimination

Magnetic nanoparticles: Controlled local release of cytokines

R. Mejías, Journal of Controlled Release 130,168, 2008

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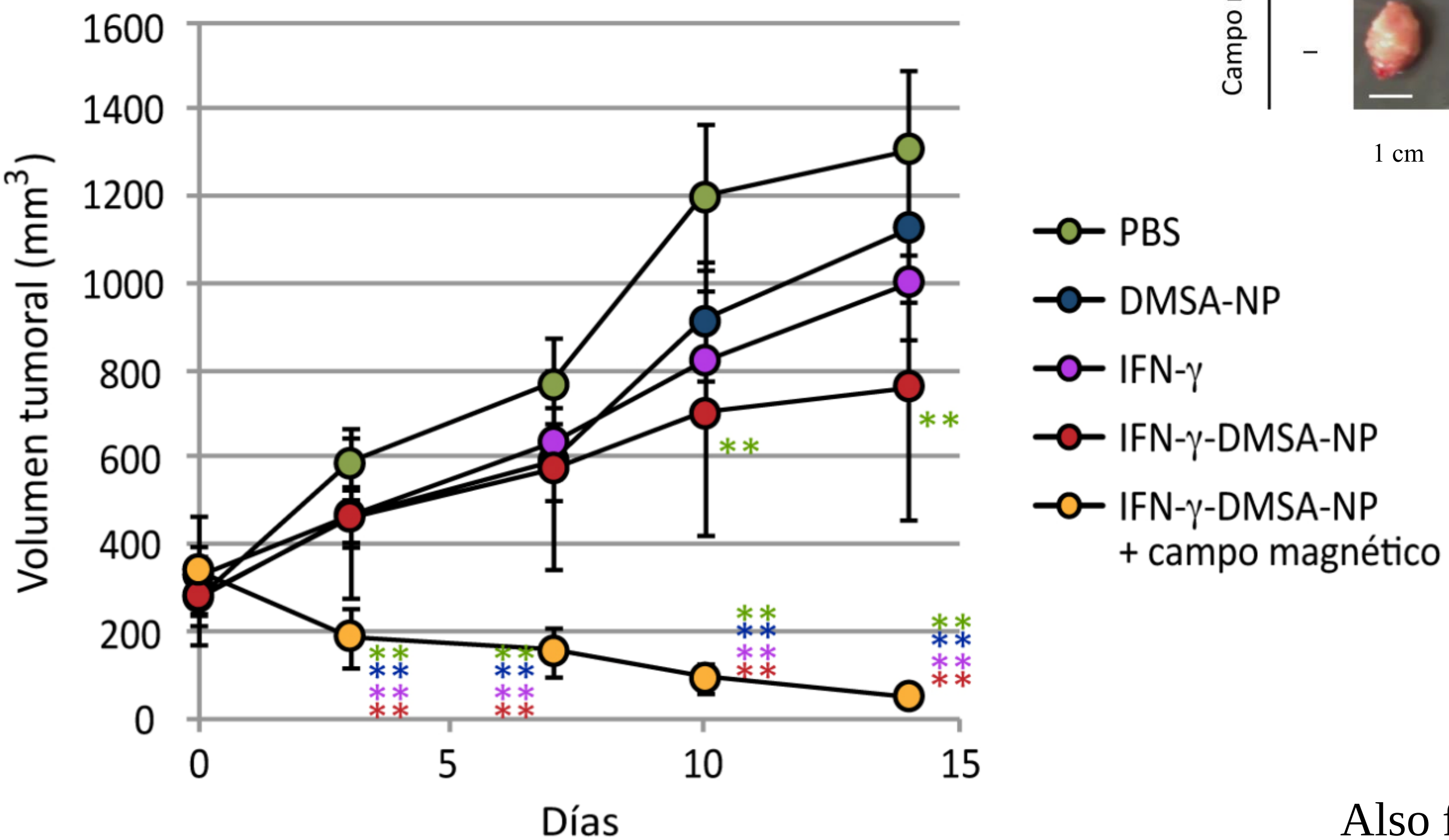
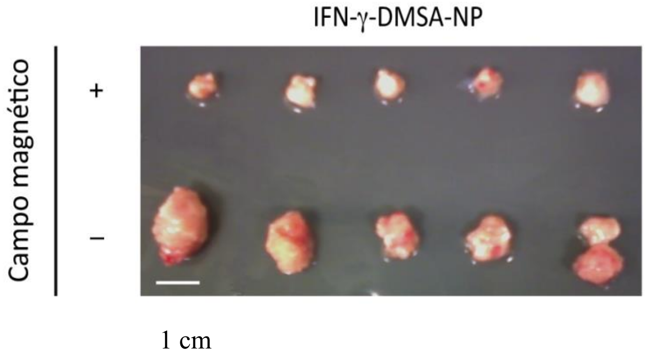
Drug delivery

Tumor size



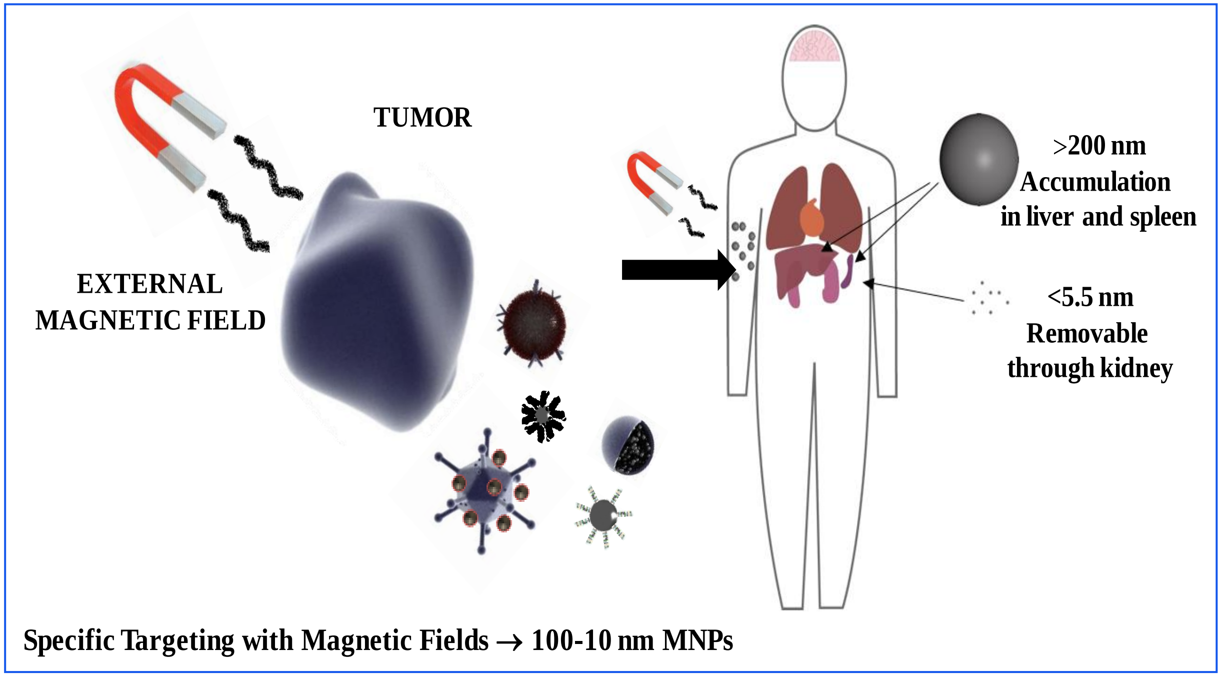


Also for induced tumours
with 3-methylcholanthrene (MCA)

Biomaterials 32, 2938, 2011.

Drug delivery

Main limitation



- Large MNPs (> 200 nm) will be easily detected by the immune system and removed from the blood and delivered to the liver and the spleen.
- Very small MNPs (< 5.5 nm) can be excreted through the kidneys.
- Different magnetic biocomposites can be transported to reach the tumor area inside the body thanks to the applied magnetic field.

Drug delivery

Challenges

Nanoparticle-based chemotherapy

Proof-of-principle, in vivo studies

Nanoparticle-based radiotherapy

In vitro and in vivo studies

Nanoparticle-based phototherapy

Proof-of-principle

Drug delivery across blood-brain barrier

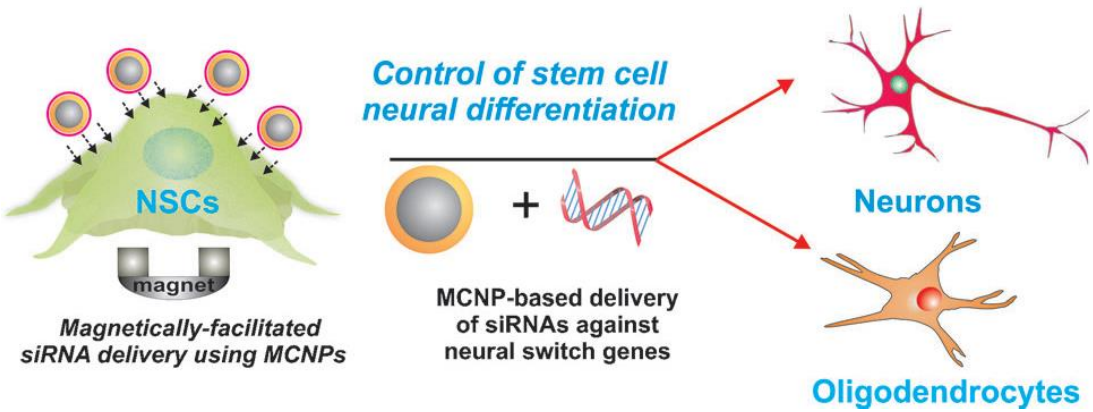
- Treatment of brain tumours, Alzheimer's, and Parkinson's – *development phase*

  NanoDel Technologies GmbH, Germany

Nanovectors for gene therapy

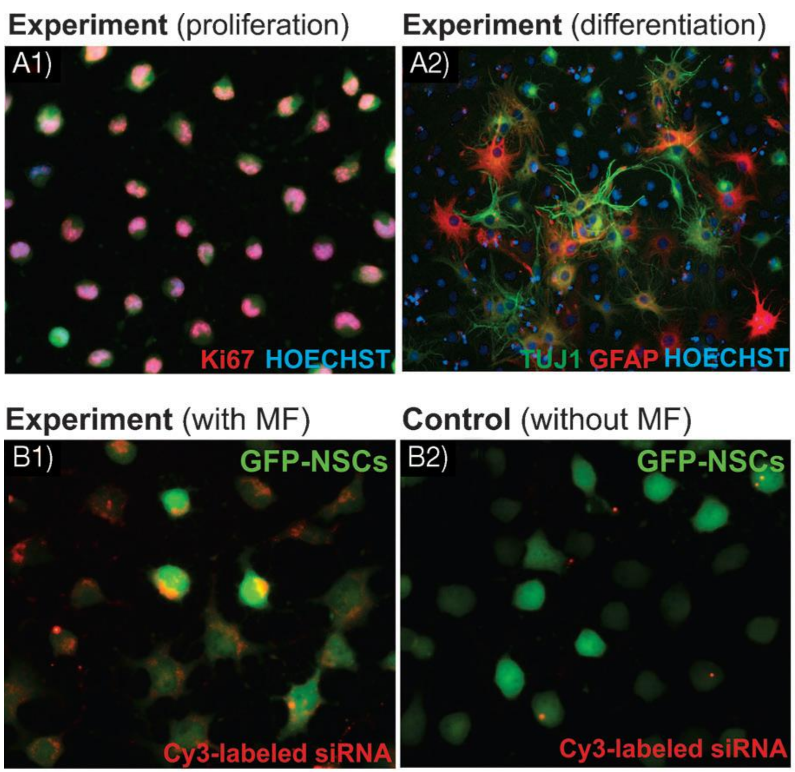
- Non-viral gene delivery systems – *in vitro studies*

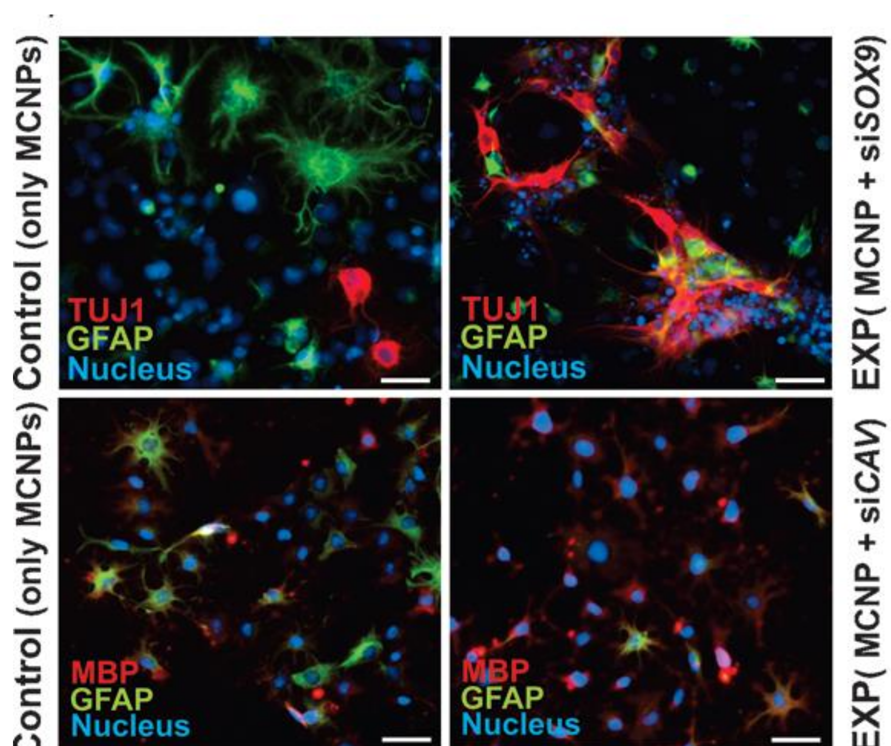
Delivery of genetic materials (siRNA and pDNA) into stem cells



Delivery of siRNA against SOX9 (siSOX9) and CAVEOLIN-1 (siCAV) gens for inducing neural differentiation of NSCs

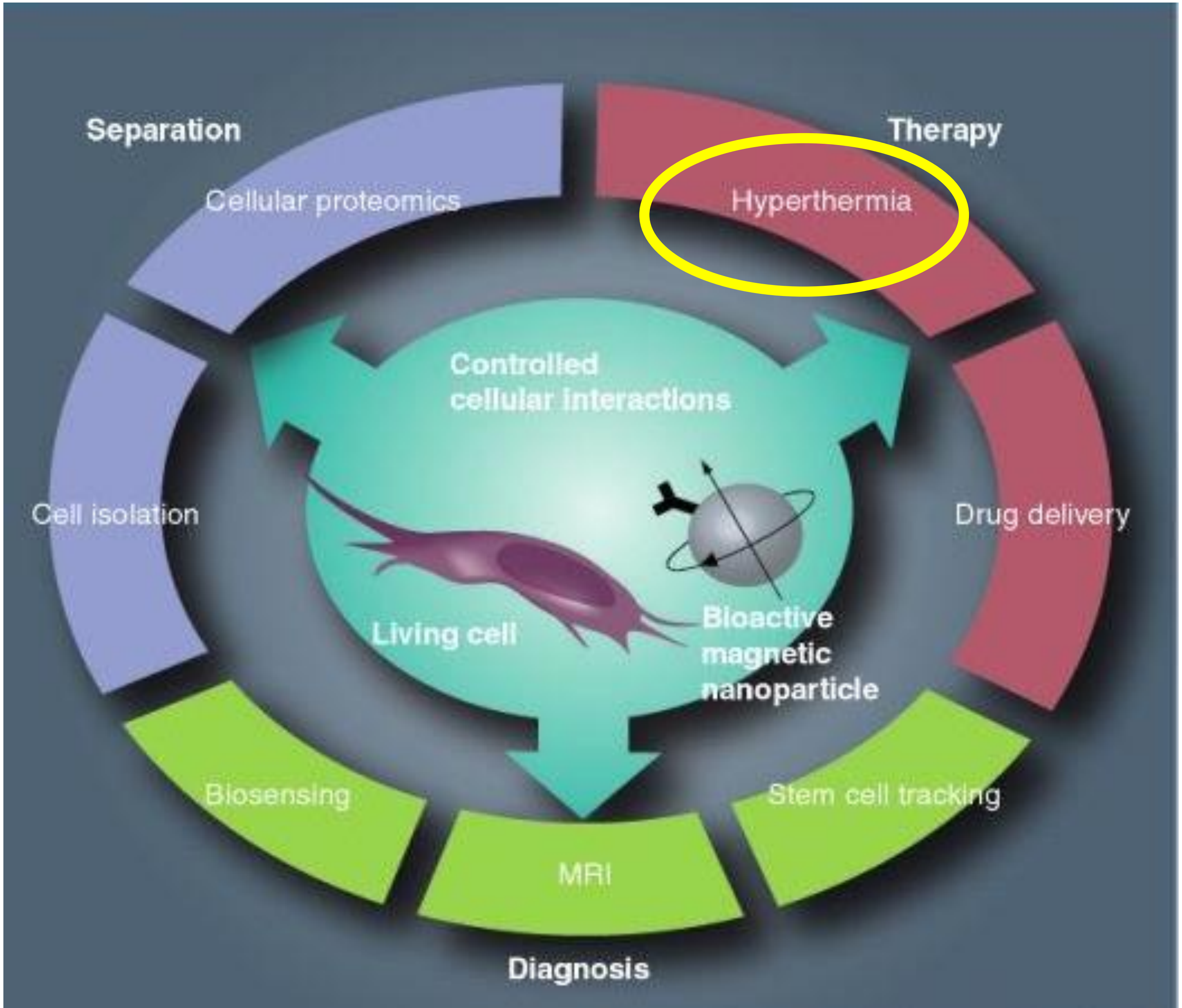
Angew. Chem. Int. Ed. 2013, 52, 6190 –6195





TUJI= Neurons, MBP= Oligodendroncytes

Biomedical applications

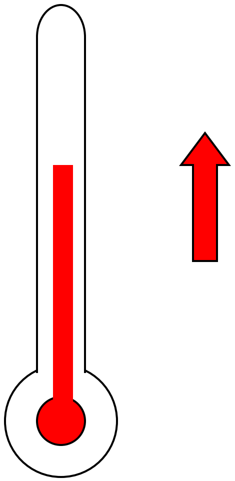


Nanomedicine © Future Science Group (2009)

Hyperthermia

HYPERTHERMIA

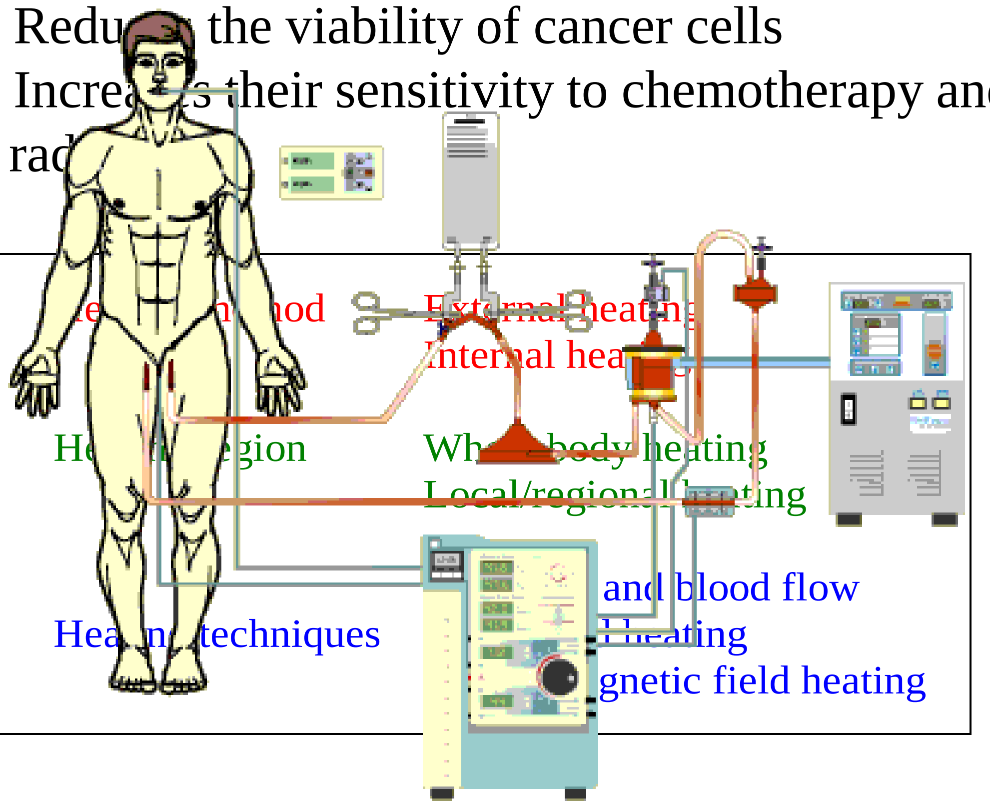
Heating of a target tissue to the temperatures between 42-43 ºC



42-43ºC

- Reduces the viability of cancer cells
- Increases their sensitivity to chemotherapy and radiotherapy

Conventional Hyperthermia

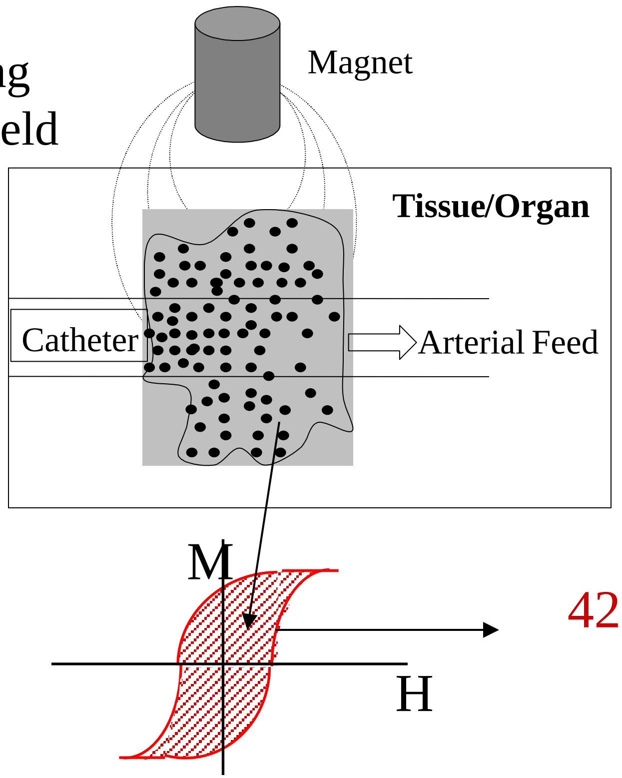


Heating method	External heating Internal heating
Heating region	Whole body heating Local/regional heating
Heating techniques	and blood flow heating gnetic field heating

Hyperthermia

Magnetic Hyerthermia

Alternating
magnetic field



Advantages of using magnetic nanoparticles

- Avoid heating healthy tissues

- Combining other therapies
  Targeting radionuclides

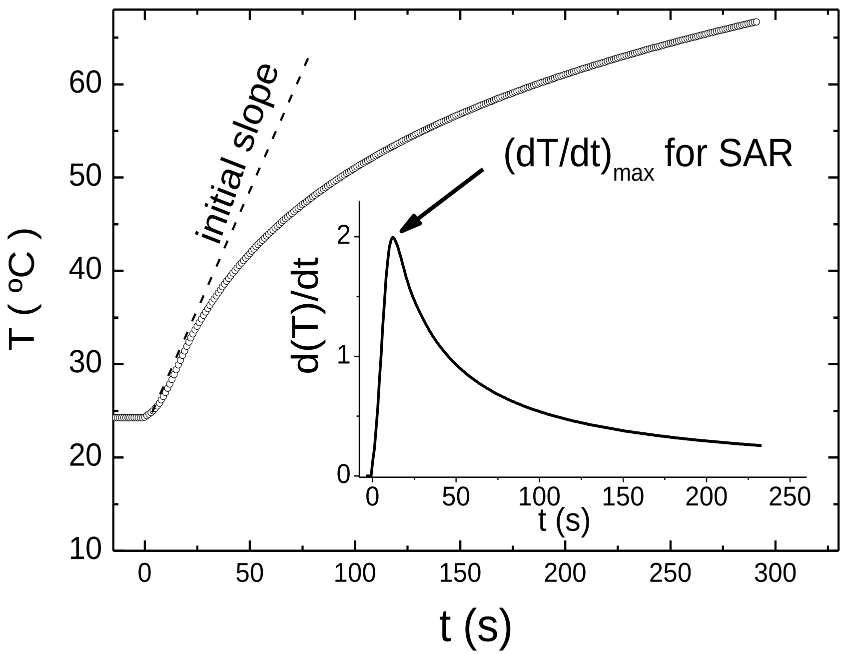
42ºC / 30 min → Cancer is destroyed

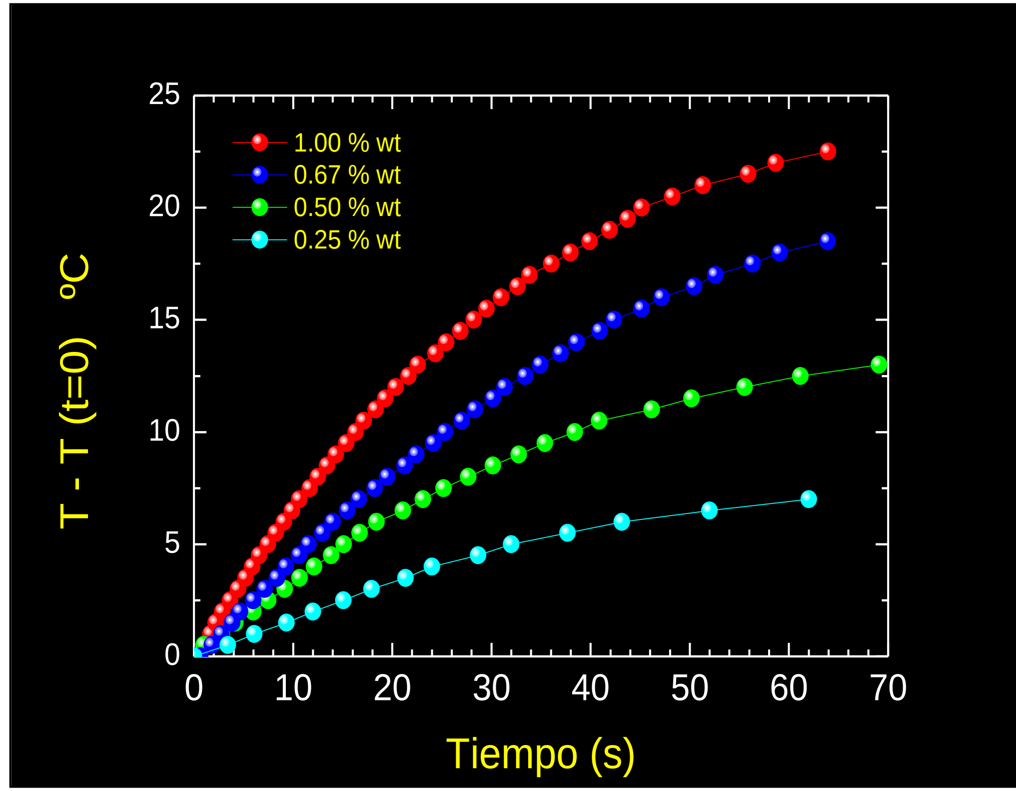
Goya et al, Current Nanoscience 2008, 4, 1-16

Nearly complete regression of tumors via collective behavior of magnetic nanoparticles in hyperthermia, C L Dennis et al., Nanotechnology 20 (2009)

Hyperthermia

What we actually measure…





$$SAR= C_m \phi(\Delta T/\Delta t)$$

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Important parameters

Specific Absorption Rate = Specific Loss Power = Experimental

$$SAR = C_m\, \phi(\Delta T/\Delta t)$$

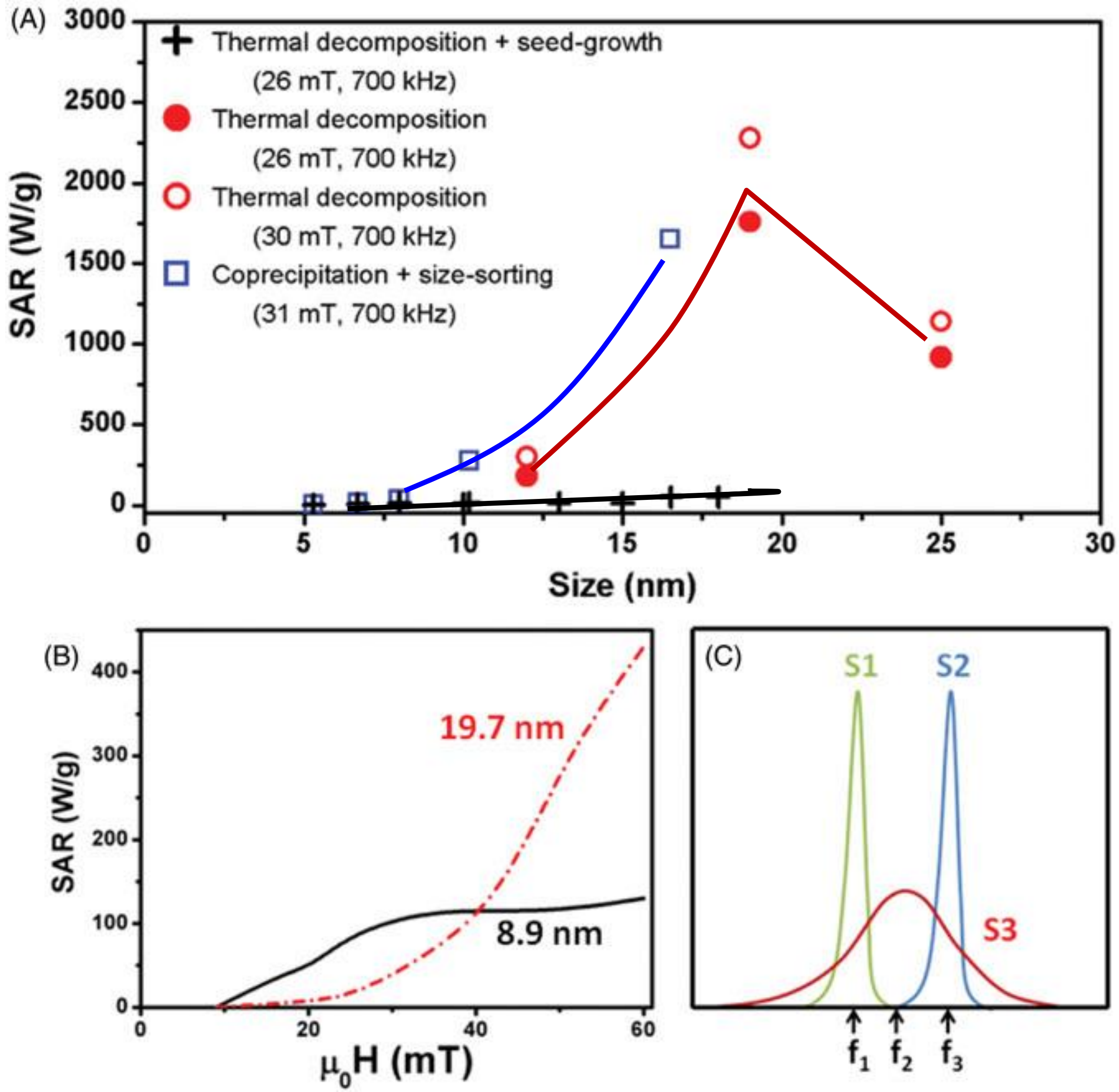
$$SLP = \mu 0 \cdot \pi \cdot f \cdot H^{2} \cdot \chi''(f)$$

where Cm is the specific heat capacity of the sample

Specific hysteresis loss= SHL = Theoretical area

Intrinsic Loss Parameter = $ILP = SAR/ H^2.f$

Hyperthermia



~~SAR max~~

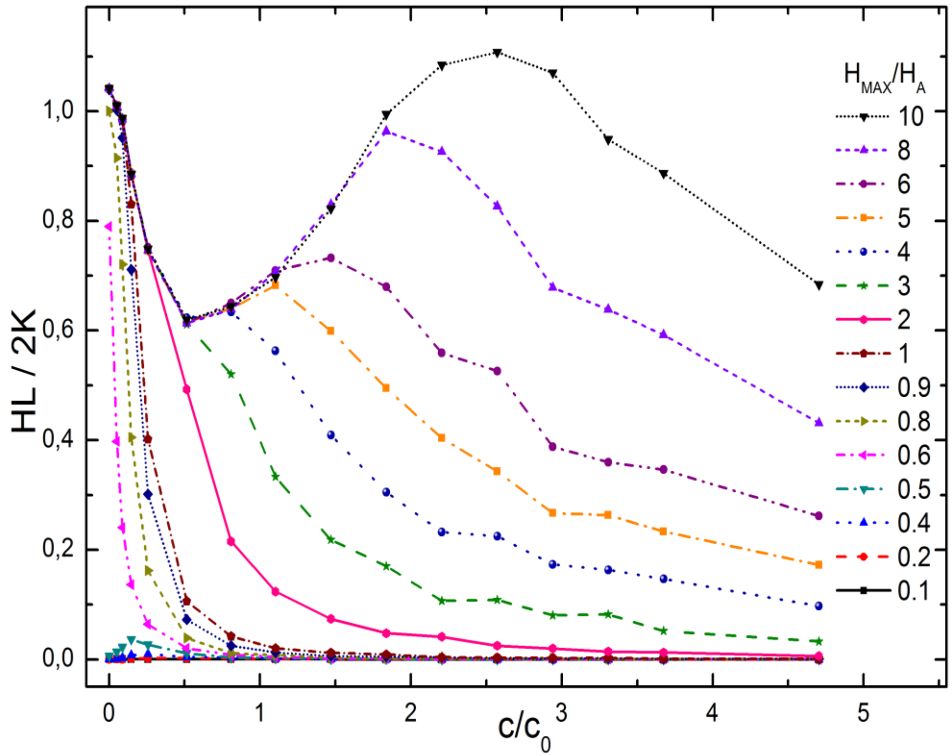
SAR max

G. Salas, et al., International Journal of Hyperthermia, 29, 8, 768-776, 2013

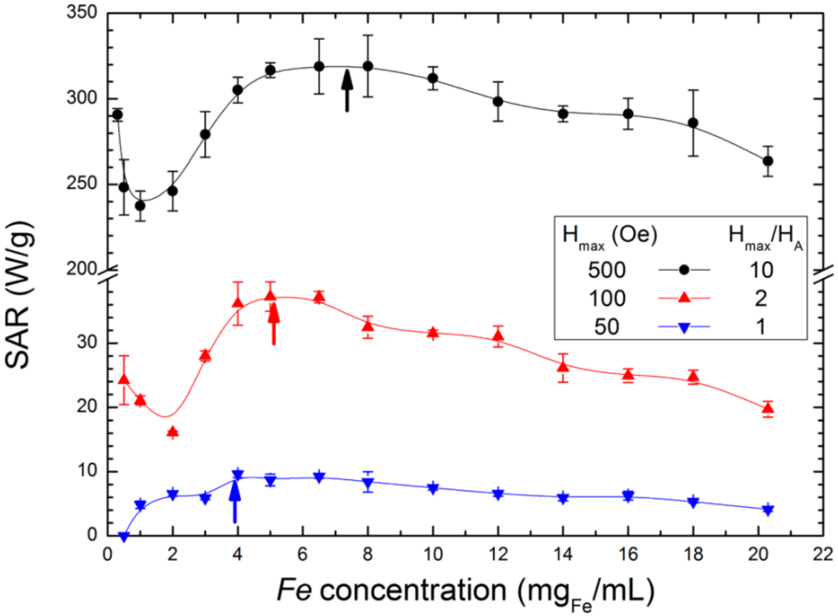
EFFECT OF NP CONCENTRATION

multifun

IMDEA Nanoscience (Madrid)



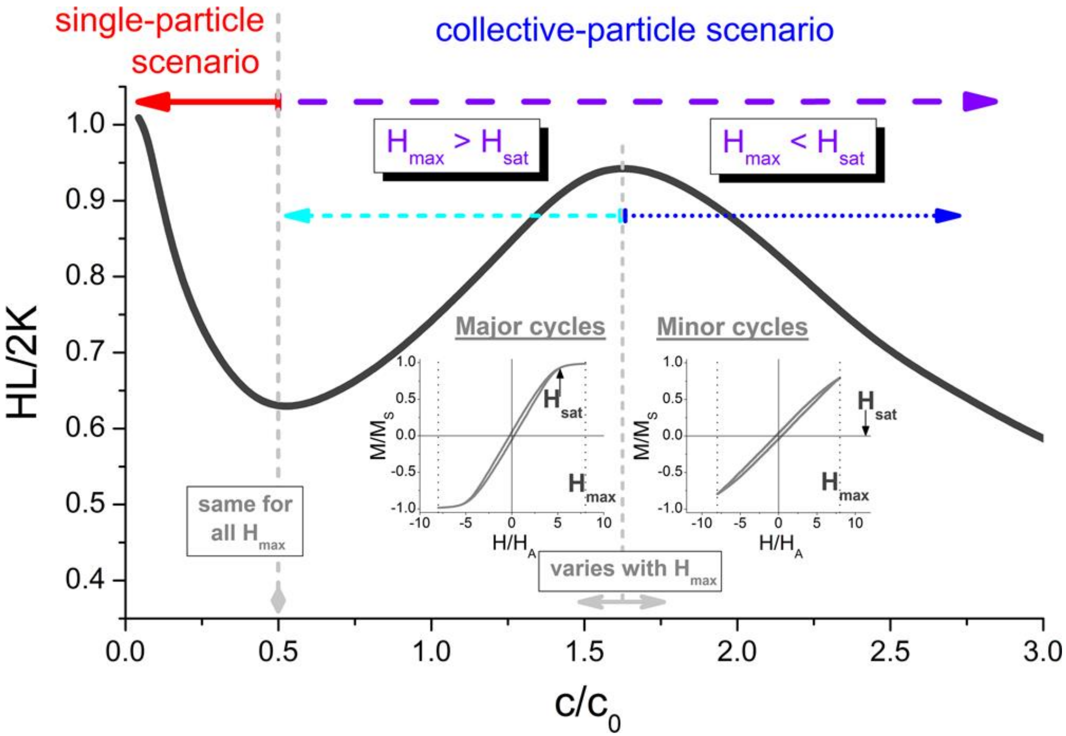
22 nm



Dependence of the SAR values for magnetite colloids, at different HMAX for a given frequency (107 kHz). Arrows depict the concentration at which the SAR value is maximum for each HMAX

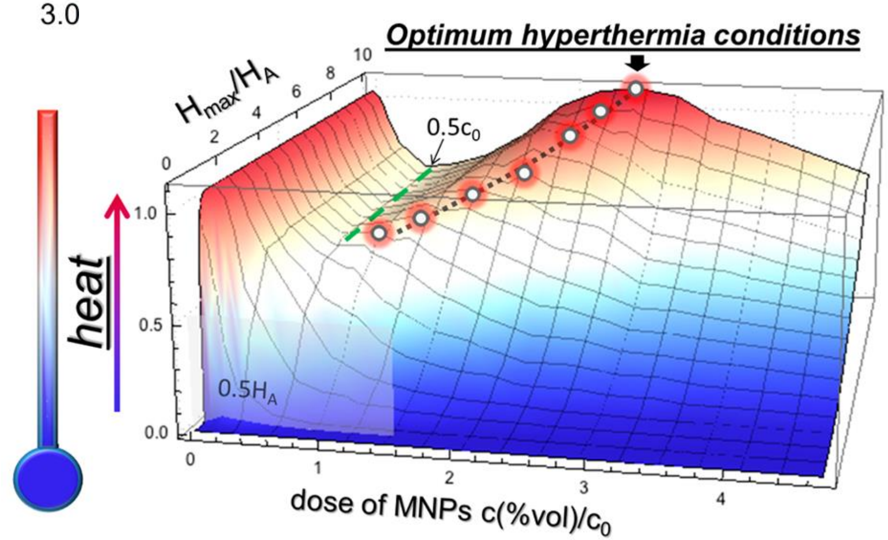
Salas et al, J. Phys. Chem. C 2014, 118, 19985–19994

A Single Picture Explains Diversity of Hyperthermia Response of Magnetic Nanoparticles



Non-monotonic concentration dependence of heating efficiency

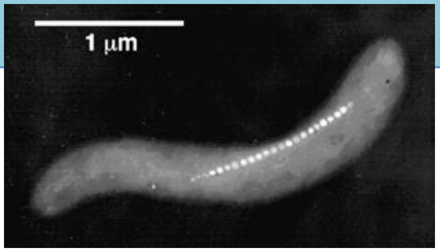
- Intrinsic features
- Experimental conditions



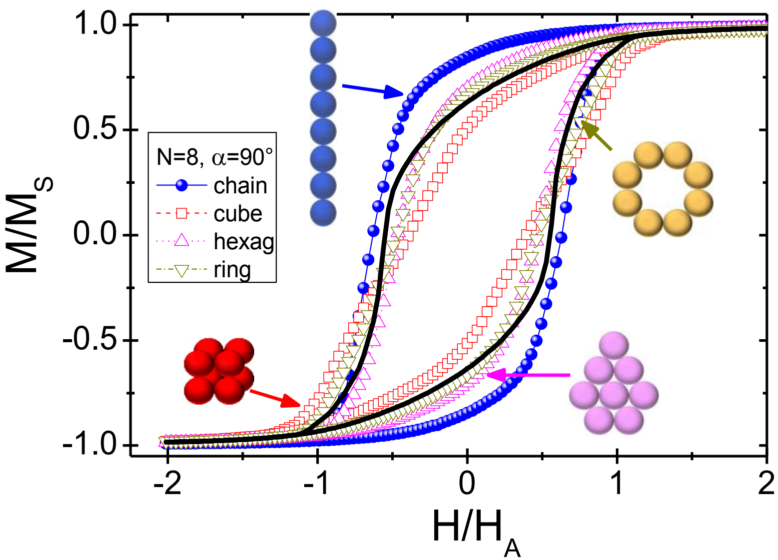
J. Phys. Chem. C, **2014**, *118* (11), pp 5927

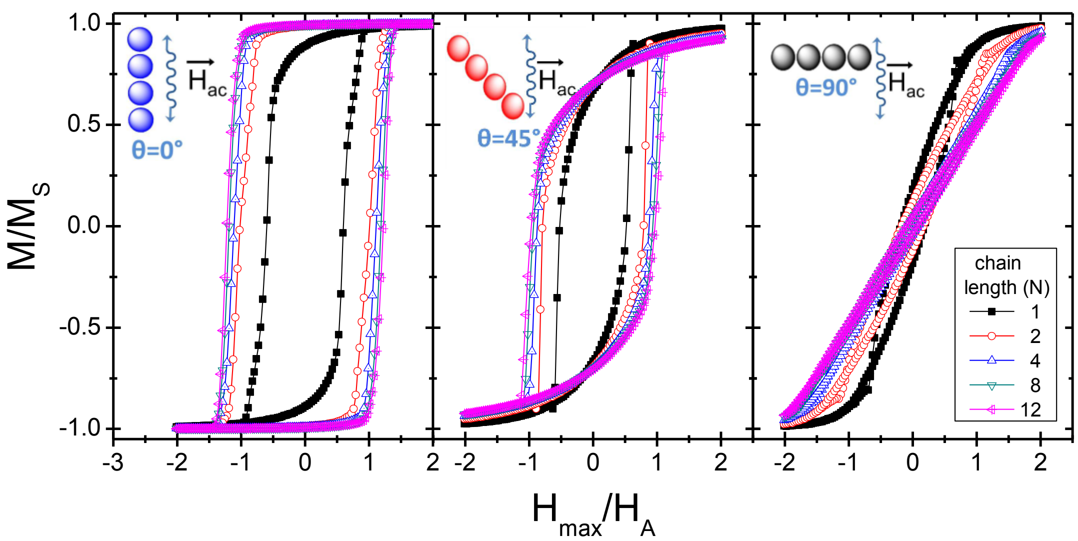
Langmuir, **2016**, *32* (5), pp 1201–1213

NANOPARTICLE ASSEMBLING



David Serantes, I. Conde-Leborán, D. Baldomir, K. Simeonidis, M. Angelakeris, Ò. Iglesias, O. Chubykalo-Fesenkoa and C. Martínez-Boubeta





- The highest area M(H) curve is attained for chain-like shape
- Dependence of area M(H) curve on *chain length and orientation*

Chain-like arrangements produce higher hyperthermia output (up to 5 times!)

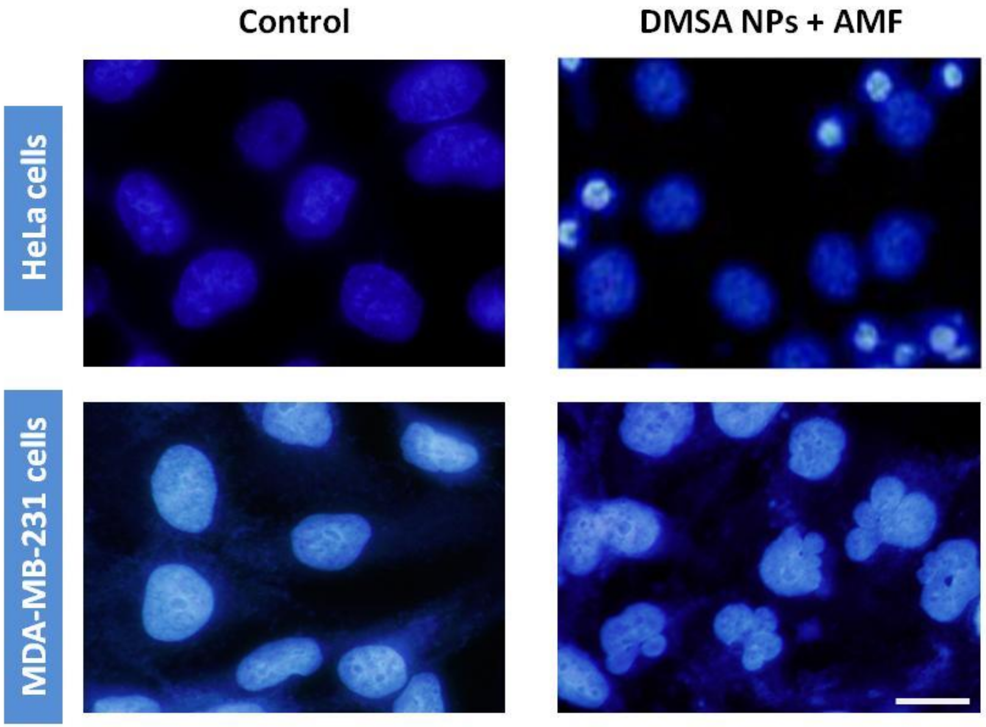
Hyperthermia

UAM-IMDEA

HeLa and MDA-MB-231 cells (stained with Hoechst 33258) were incubated with DMSA coated NPs, and exposed to an AC magnetic field (AMF).

24 h after the treatment apoptotic cells (HeLa) and giant and multinucleated cells (MDA-MB-231) were observed.

DMSA (anionic)



AMF conditions: 161 kHz, 210 G, 15 min exposure (HeLa cells), 225 kHz , 150 G , 45 minutes exposure (MDA-MB-231 cells).

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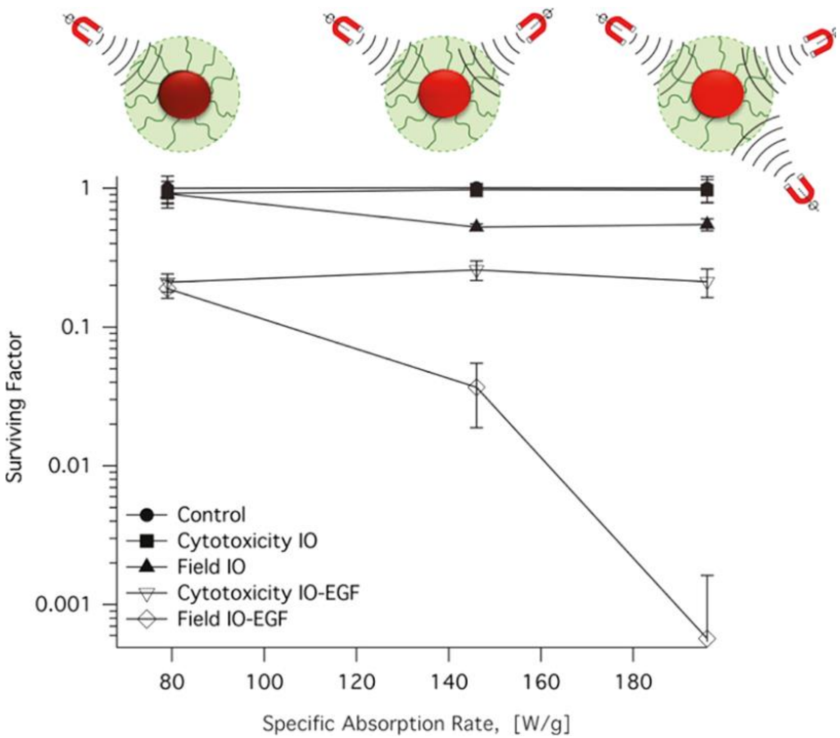
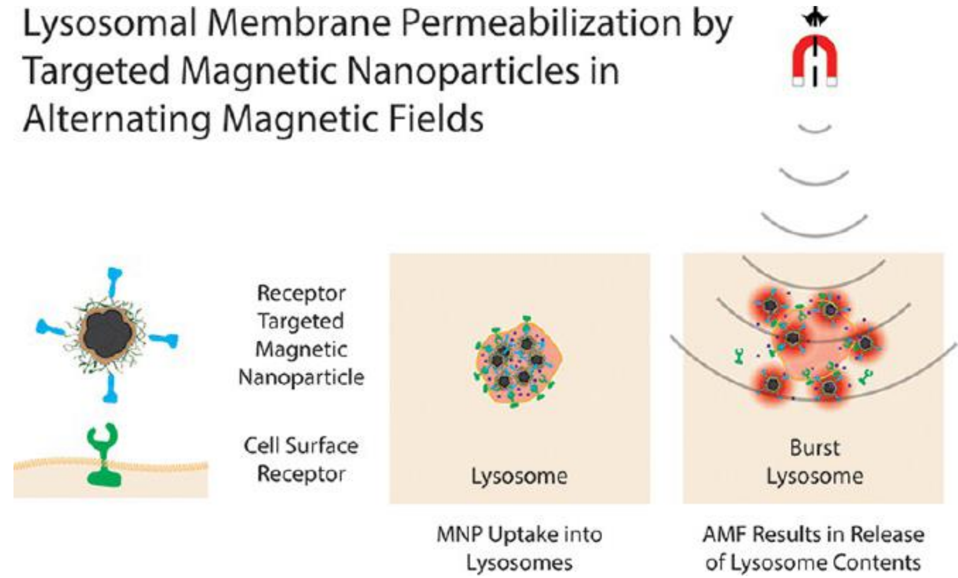
Thermal damage
Non-thermal damage

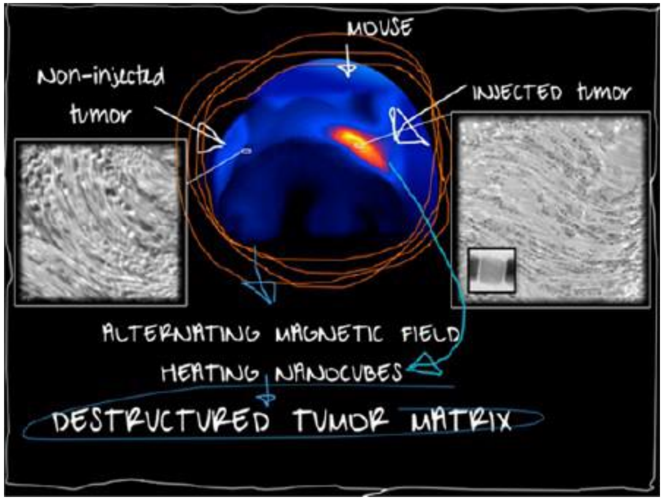


Intracellular hyperthermia at different specific absorption rates.

VOL. 5 ’ NO. 9 ’ 7124–7129 ’ 2011 acsnano



ACS Nano 2013, 10.1021/nn405356r



Hyperthermia could be used to locally modify tumor stroma and thus improve drug penetration

No perceptible change in temperature

Hyperthermia

Table 3. Summary of nanoparticle features favoring MRI and/or magnetic hyperthermia applications.

MRI (contrast)	Nanoparticle feature	Magnetic hyperthermia (heating)
+	High magnetization (size and surface coating)	+
+	SPIO	-
+	USPIO	-
+/-	Large size (core diameter >10 nm)	+
+	Sequestration by MPS	-
-	Long plasma half-life (targeting)	+
+	Short plasma half-life (targeting)	-

+: Favoring feature/parameter; -: Disfavoring feature/parameter; MPS: Monocyte phagocyte system; SPIO: Superparamagnetic iron oxide; USPIO: Ultrasmall superparamagnetic iron oxide.

Ingrid Hilger et al., Nanomedicine 2012

http://www.magforce.de/en
http://www.youtube.com/watch?v=BZLmD3SOR_Y
http://www.clinicaltrials.gov/ct2/show/study/NCT00003052
http://www.mhaus.org/

Hyperthermia

COMPANY PRODUCTS CLINICAL TRIALS RESEARCH PRESS & INVESTORS CONTACT

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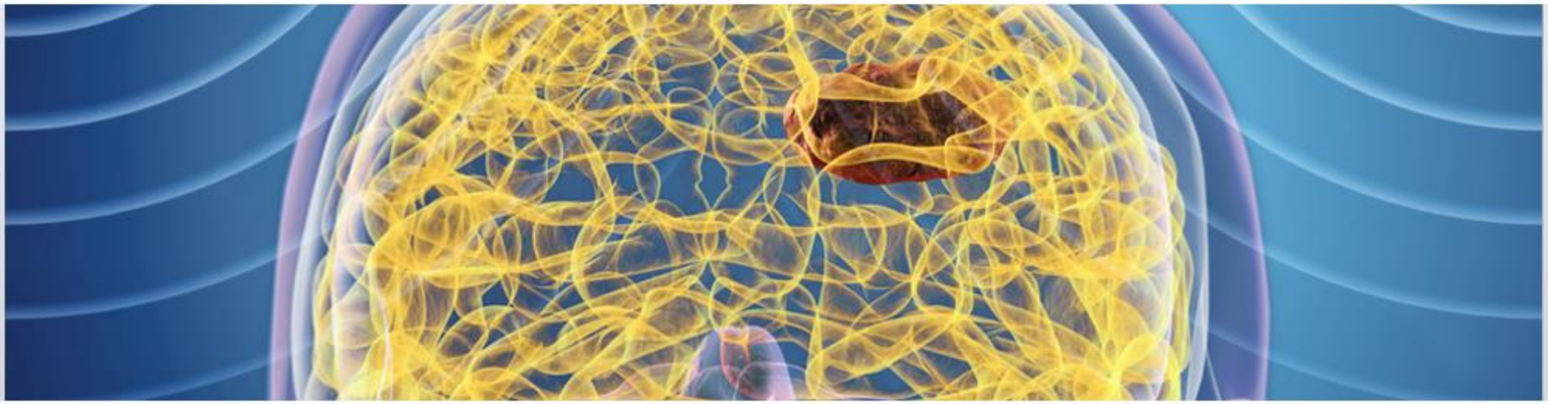


- NanoTherm™ therapy
- NanoTherm™
- NanoPlan®
- NanoActivator®

Fighting cancer more effectively and with fewer side effects

HOW DOES NANOTHERM™ THERAPY WORK?

NanoTherm™ therapy is a new approach to the local treatment of solid tumors. The method is based on the principle of introducing magnetic nanoparticles directly into a tumor and then heating them in an alternating magnetic field. At approximately 15 nanometers in diameter, the nanoparticles, which are suspended in water, are extremely small (a nanometer is one millionth of a millimeter), and comprise an iron oxide core with an aminosilane coating. The particles are activated by a magnetic field that changes its polarity up to 100,000 times per second, generating heat.

MED CERT

Compliant with European Standard

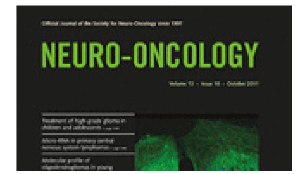
NanoTherm™

Hyperthermia

- Overview
- New glioblastoma study
- Patient Information
- Physicians' Information
- Publications
- Contact

Growing nanomedicine into a cancer therapy of the future

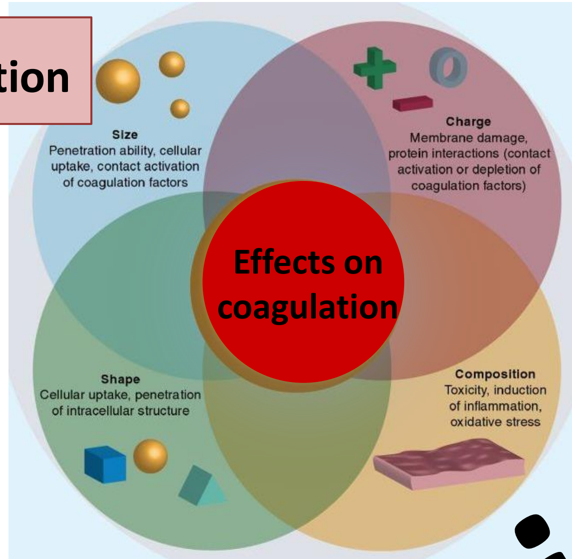
CLINICAL TRIALS/ TUMOR TYPES	STATUS OF TRIALS Phase I Feasibility study	Phase II Efficacy study	EU Regulatory Approval
Glioblastoma multiforme			
Prostate carcinoma			
Pancreatic carcinoma			



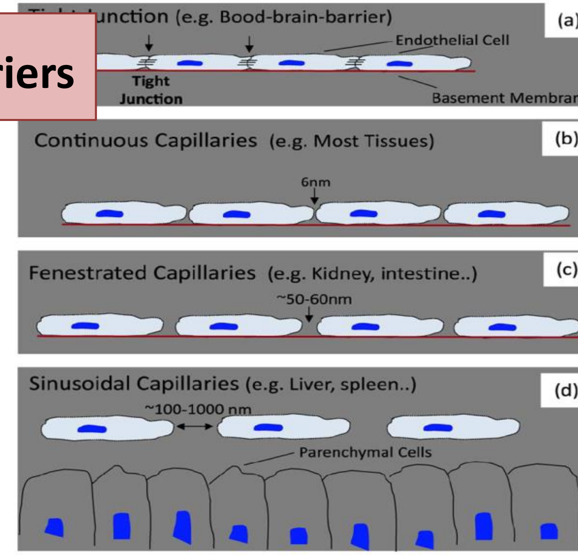
Study results

LIMITATIONS

Blood interaction



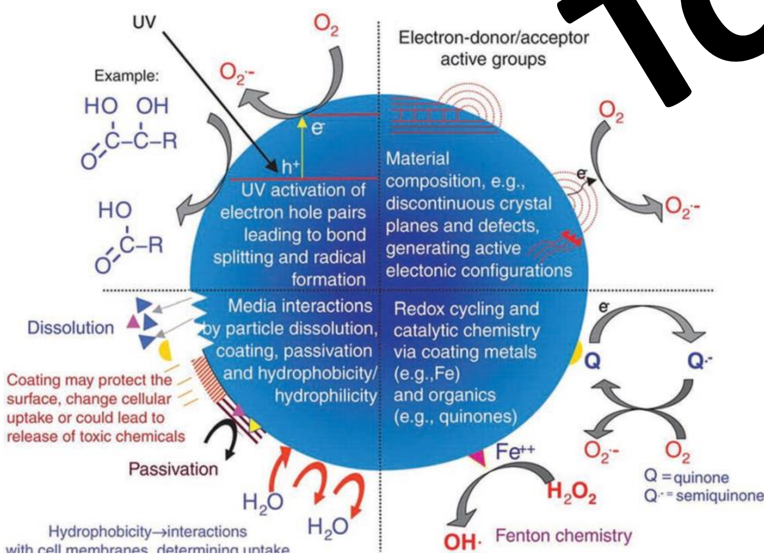
Biological barriers



Y. Okuhata, *Adv. Drug Del. Rev., 1999.*

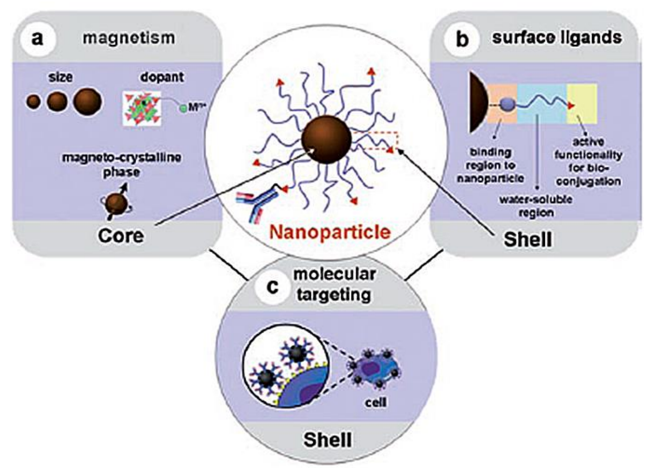
Toxicity

Nanoparticle-cell interactions



A. Nel , T. Xia , L. Mädler , N. Li, *Science* **2006** , *311* , 622 .

Design a nanoparticle for each application

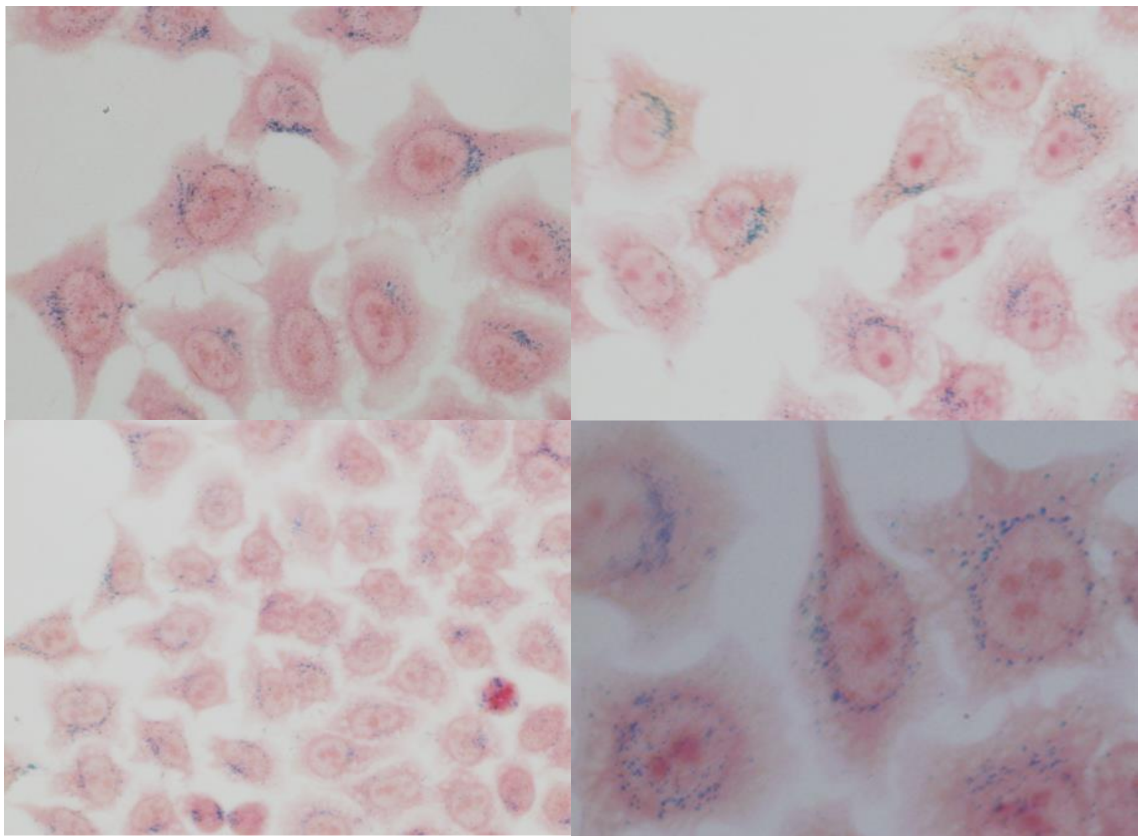


Cheon et al, Accounts Chem. Research 2008

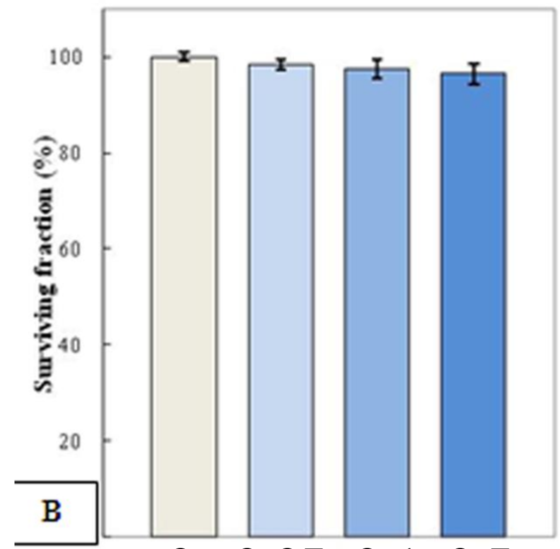
Interaction with cells

HeLa cells-DMSA

0,5 mg Fe/mL- 24 horas



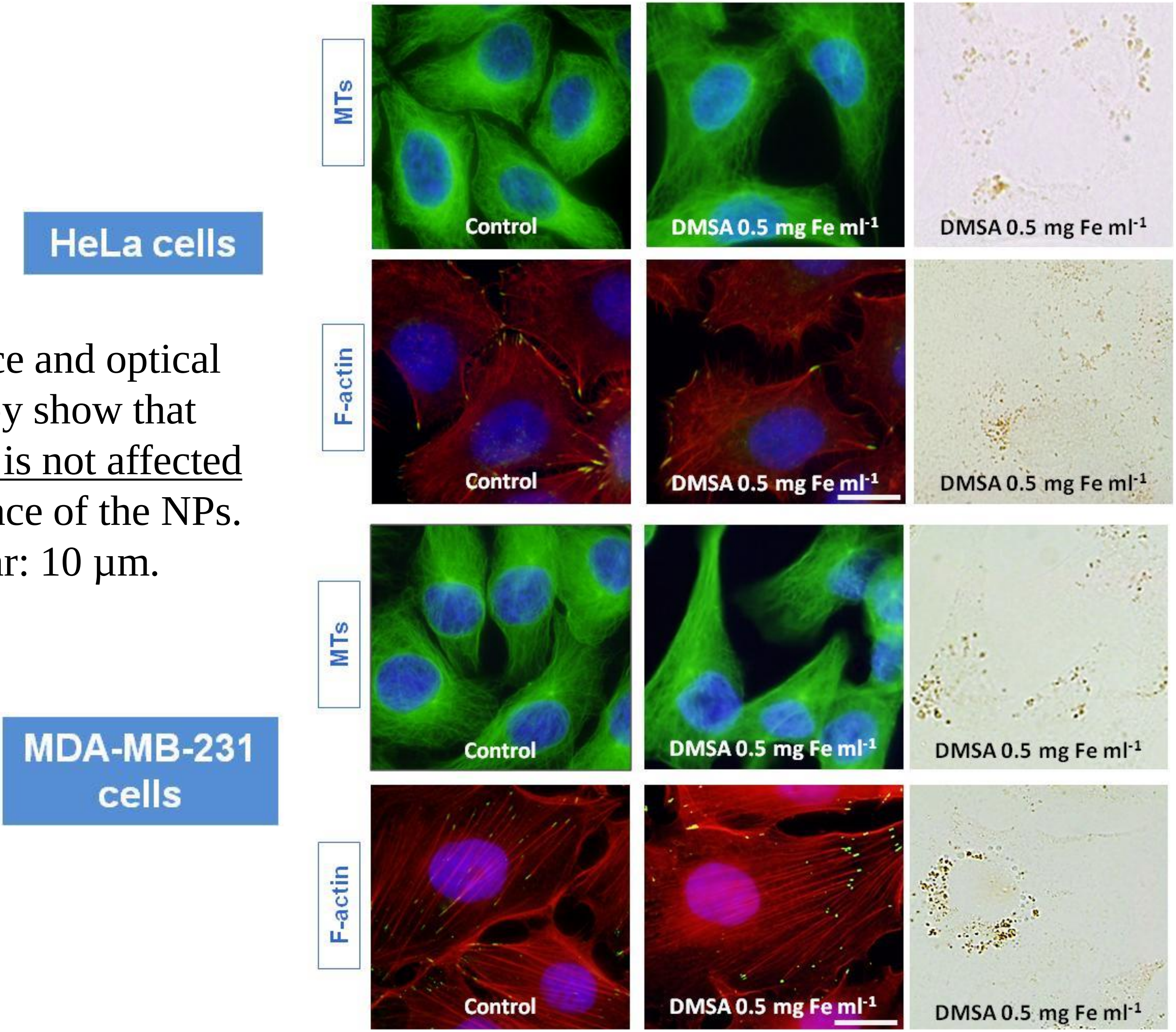
MTT TEST



0 0,05 0,1 0,5
mg/ml

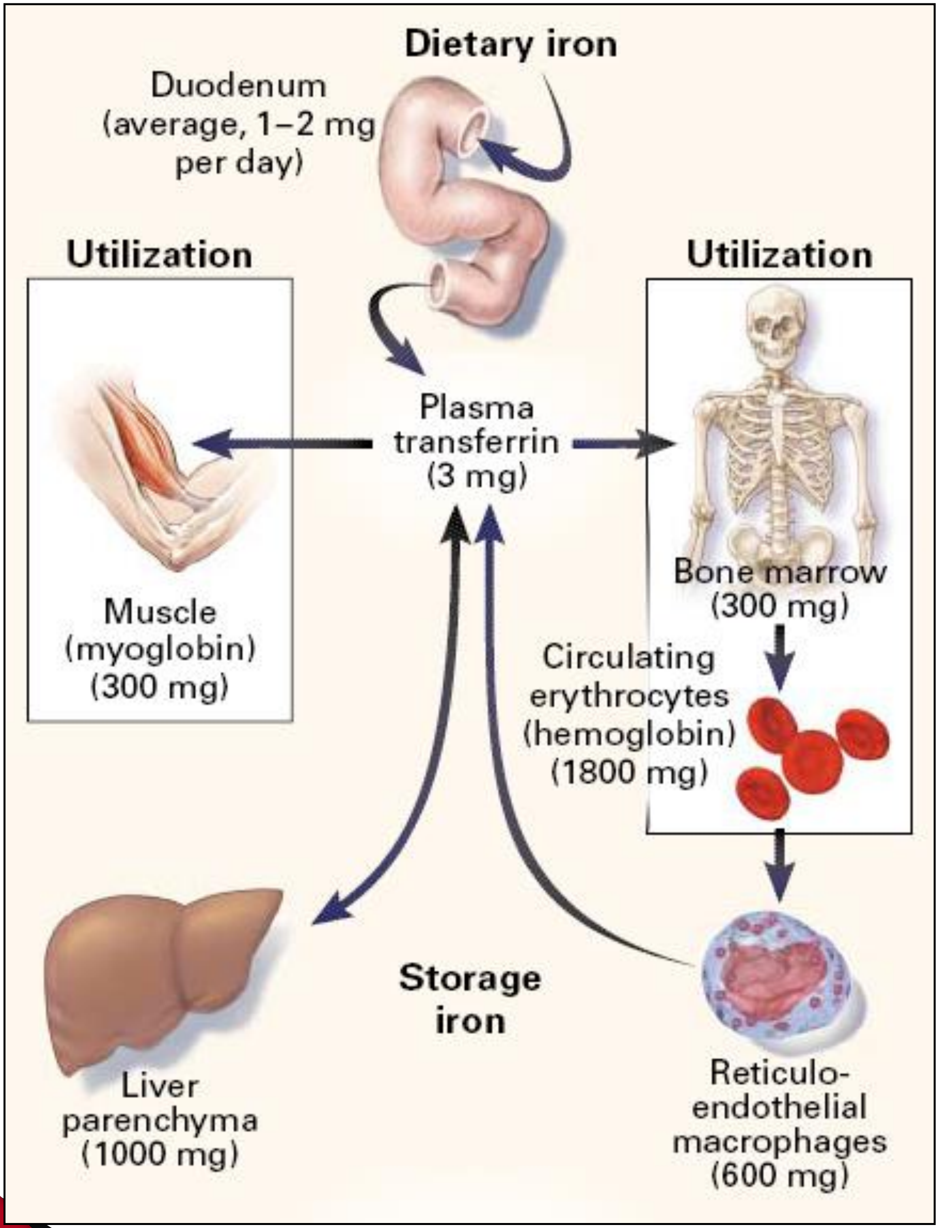
NPs concentration

Interaction with cells



Fluorescence and optical microscopy show that cytoskeleton is not affected by the presence of the NPs. Scale bar: 10 µm.

Problem: Biodistribution



100 mg Fe => Endorem
(1-5 mg/Kg)

Distribution of Iron in Adults

NANCY C. ANDREWS

The New England Journal of Medicine
Volume 341 Number 26, 1986, 1999

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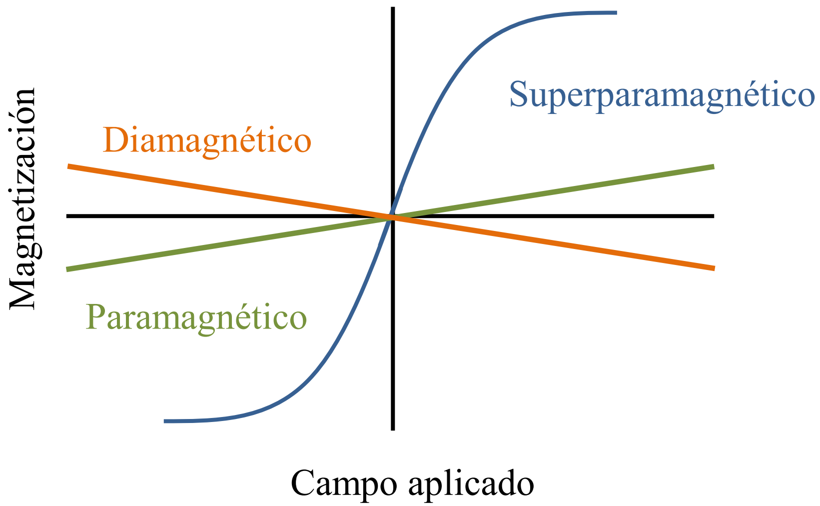
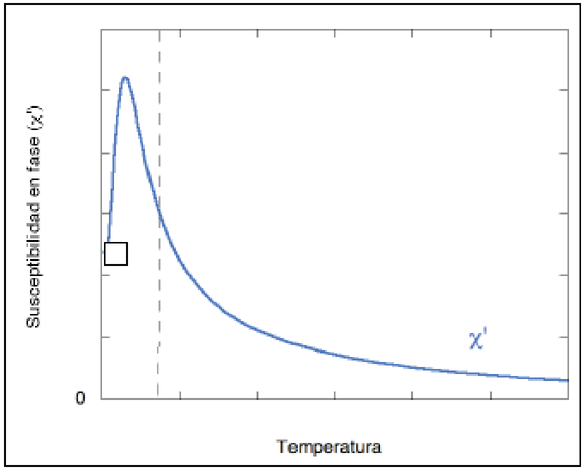
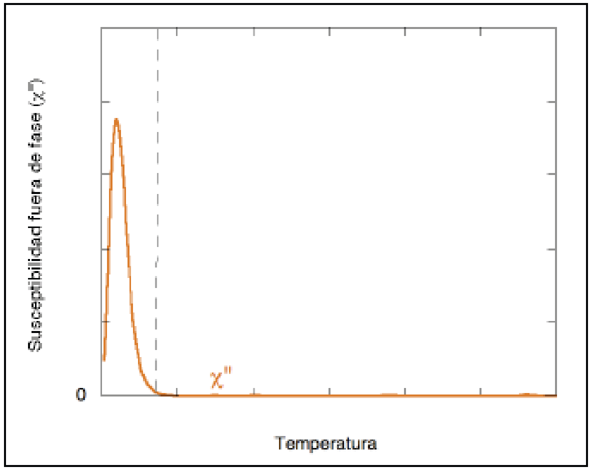
Magnetisation curves



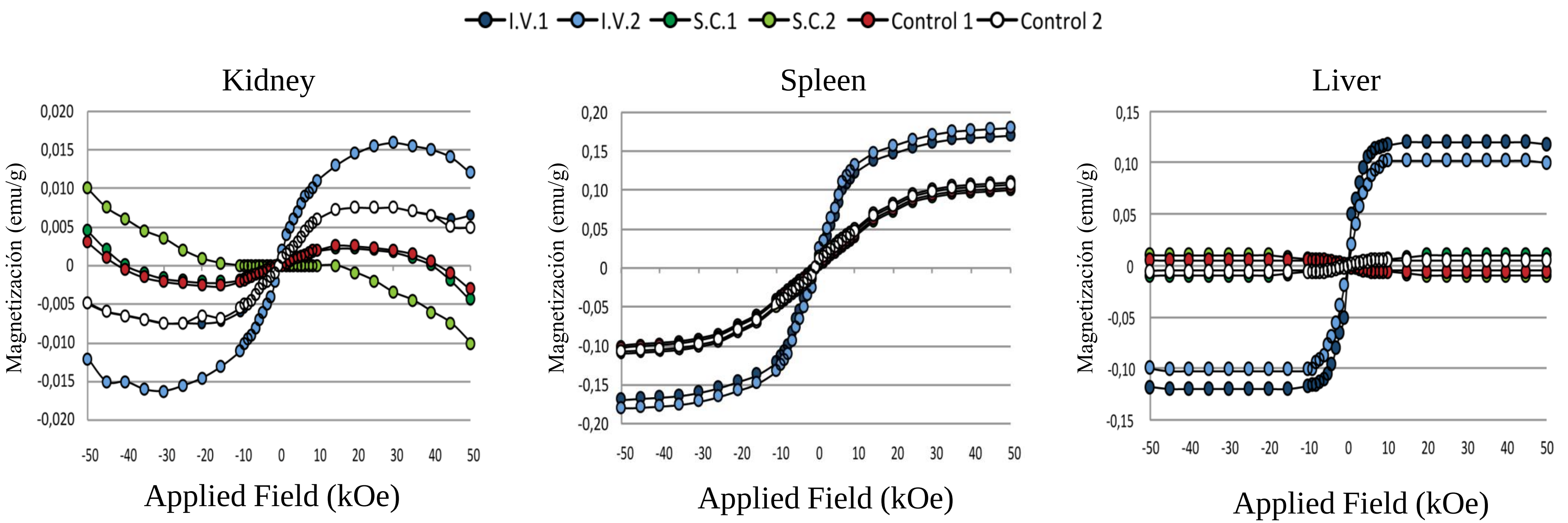
All materials are magnetic to some extent with their magnetic response depending on their atomic structure and temperature

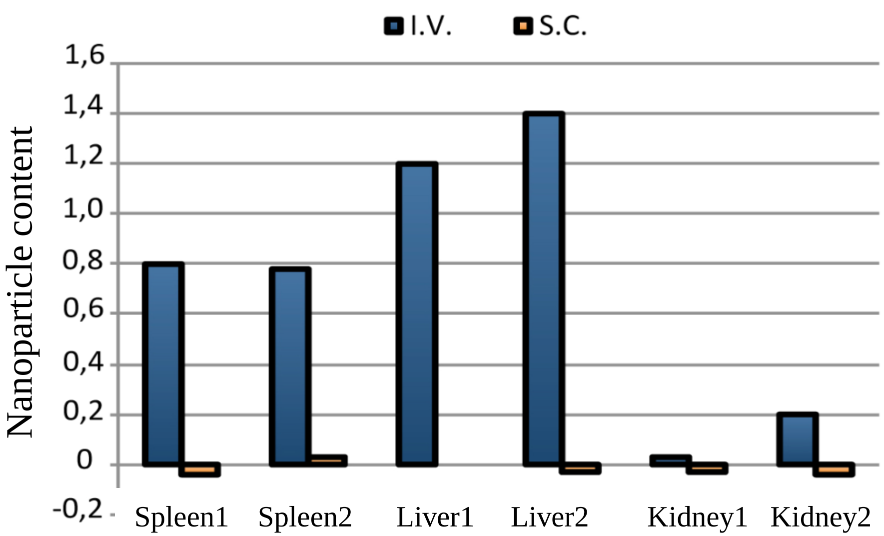
Suscepbilidad AC





Biodistribution in vivo: Magnetic methods

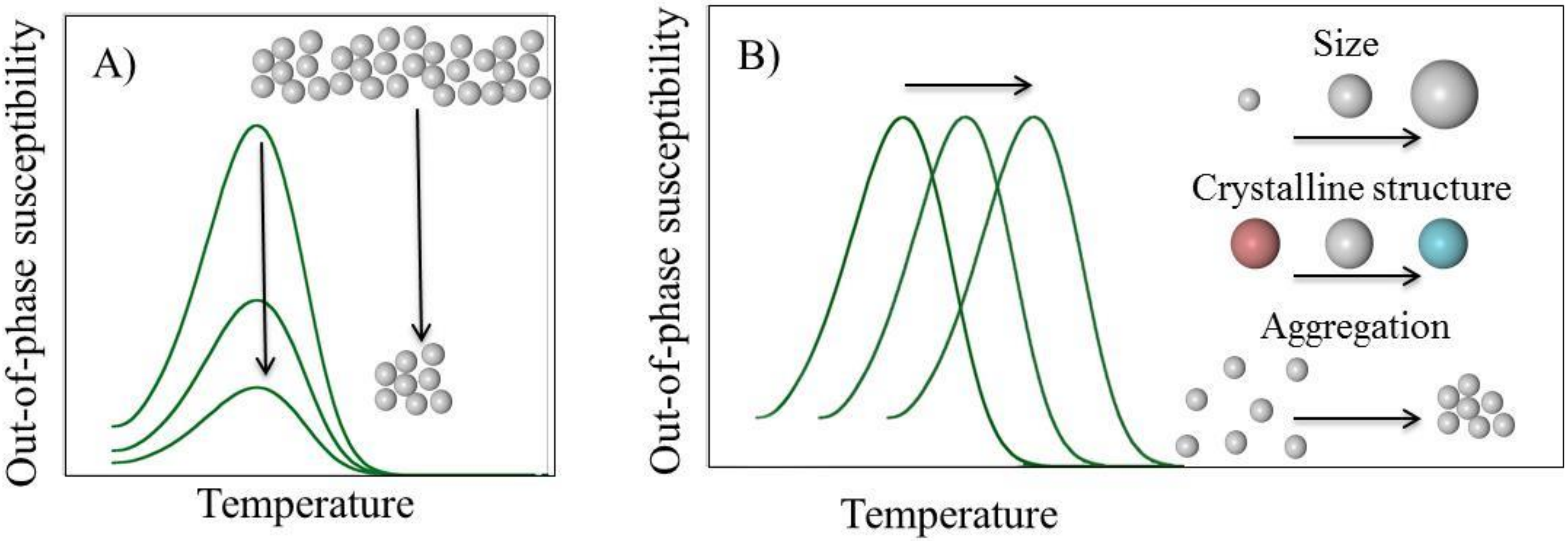




Liver and brain imaging through dimercaptosuccinic acid-coated iron oxide nanoparticles
Nanomedicine 5(3), 397- 408, 2010

Biodistribution: Characterization

AC MAGNETIC SUSCEPTIBILITY



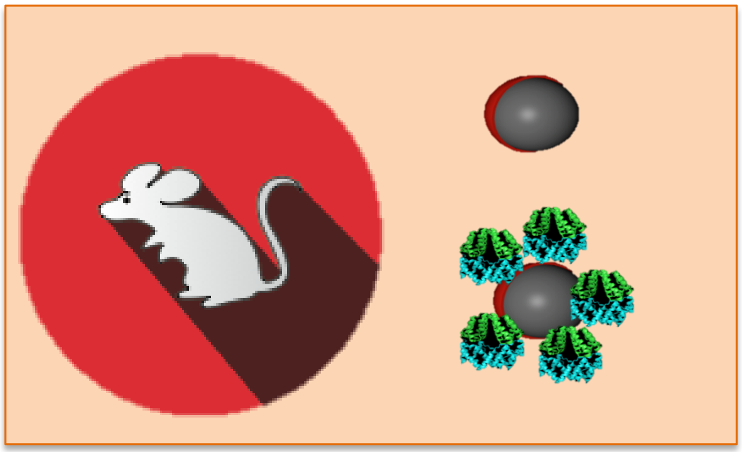
With the appropriated standards it is possible to calculate the amount of the total iron that is in the form of the magnetic nanoparticles.

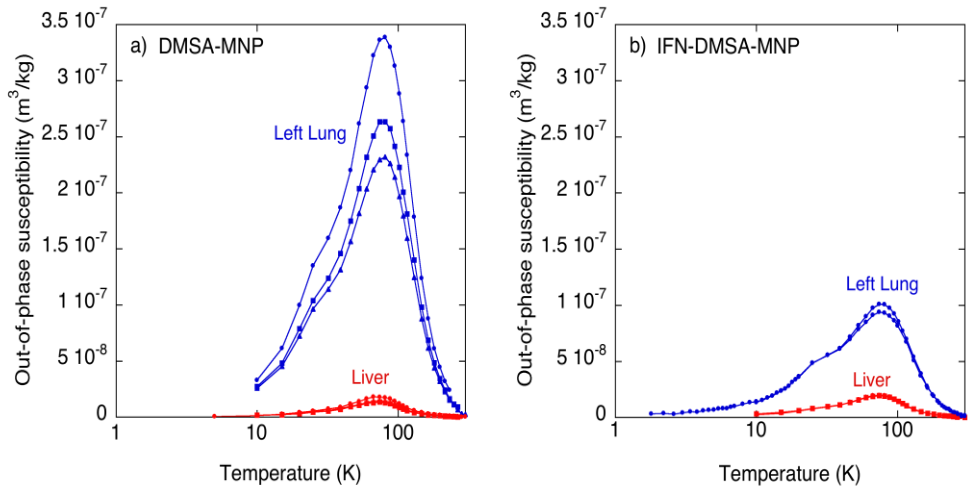
Gutiérrez et al, Phys.Chem.Chem.Phys.,2014, 16, 4456.

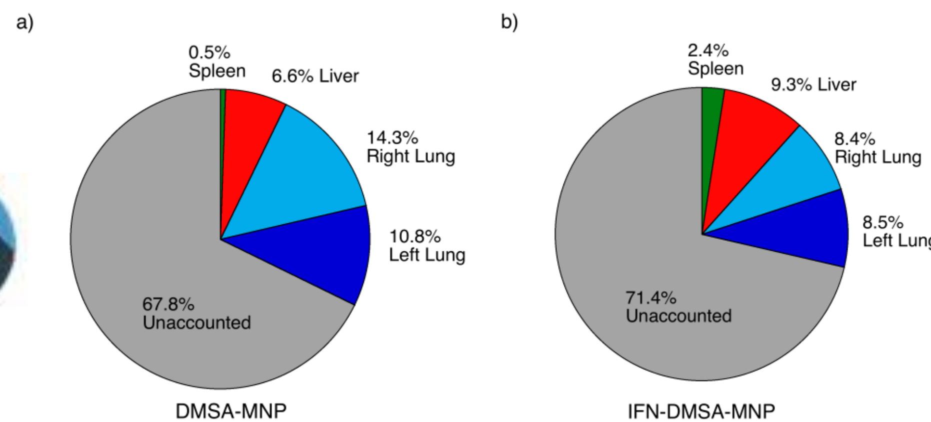
Biodistribution

In vivo

Coating







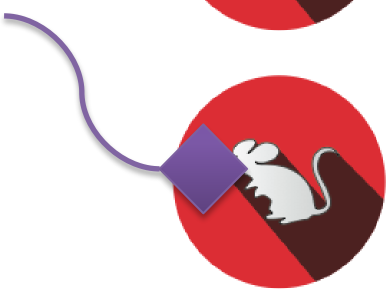
L.Gutiérrez et al, J. Phys. D: Appl. Phys. 44 (2011)

Biodistribution

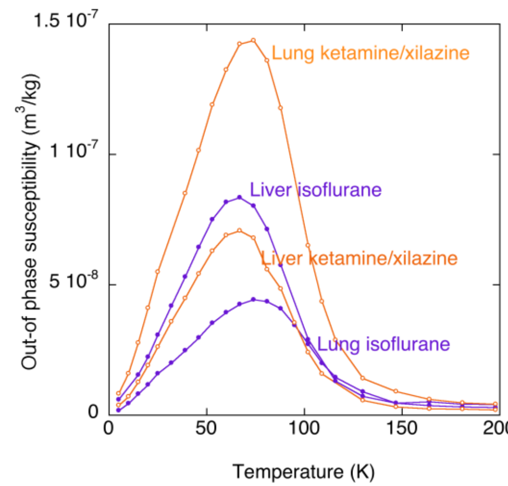
In vivo

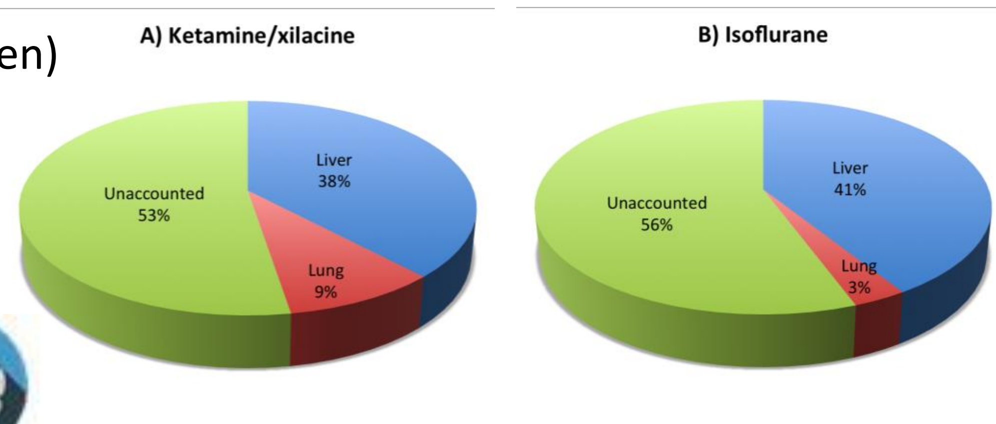
Anestesia

Ketamine and xylazine
Intraperitoneal



Isoflurane
Inhaled (0.5% in oxygen)



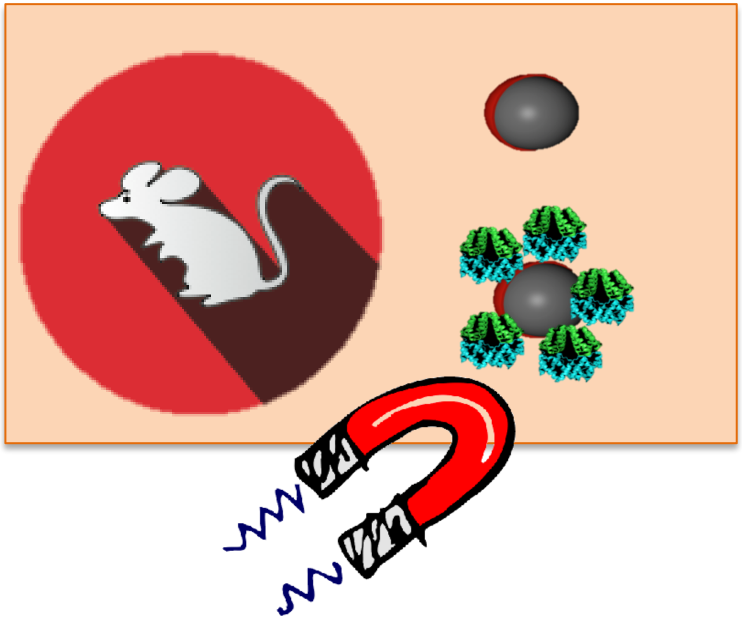


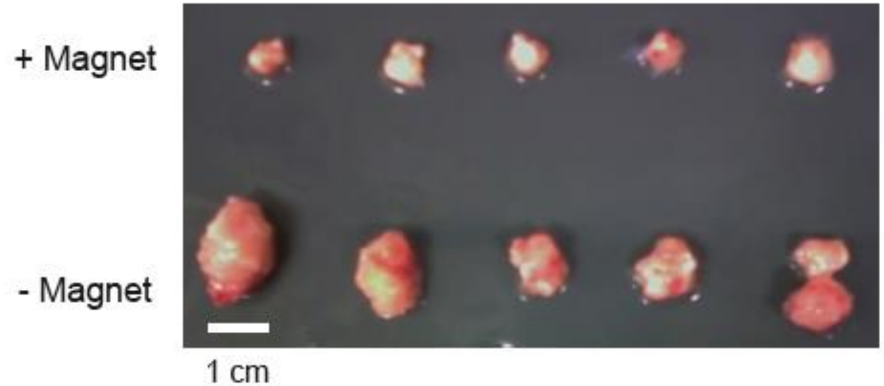
L.Gutiérrez et al, IEEE Trans. Magn., 49, (2013)

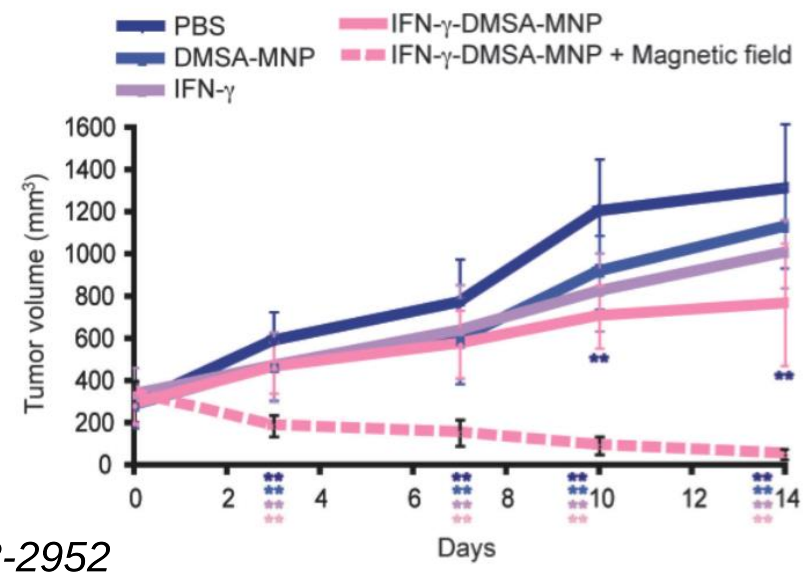
Biodistribution

In vivo

External magnetic field



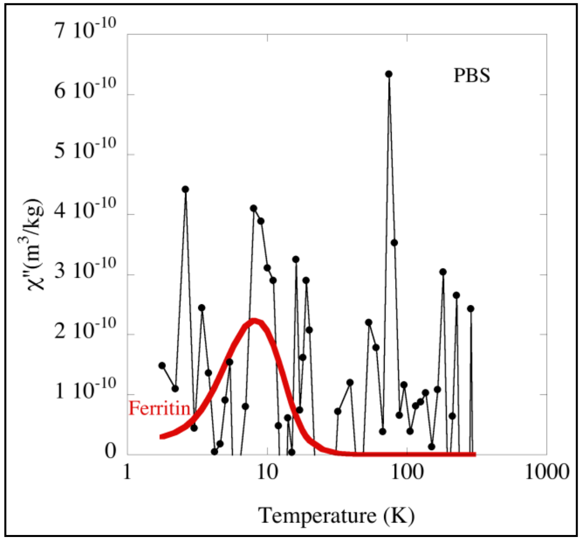




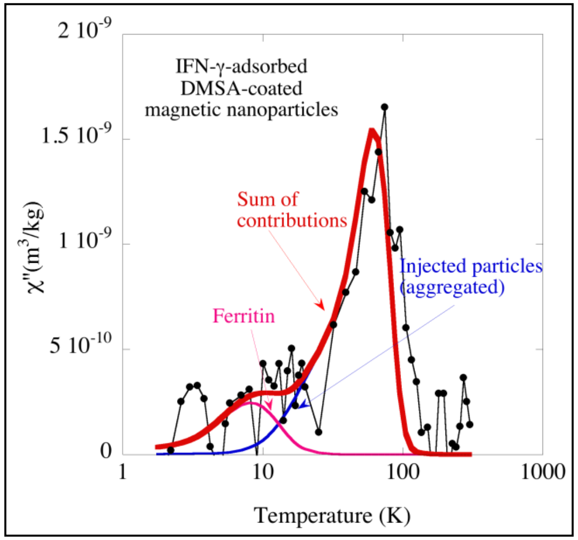
R. Mejías et al, Biomaterials 32 (2011) 2938-2952

Quantification of NPs in tumor

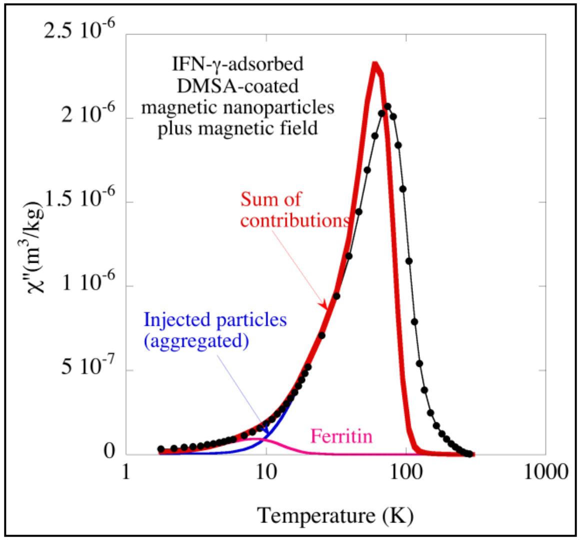
Susceptibilidad magnética



NP-γ-IFN



NP-γ-IFN- Magnet



Nanomedicine 5(3), 397- 408, 2010

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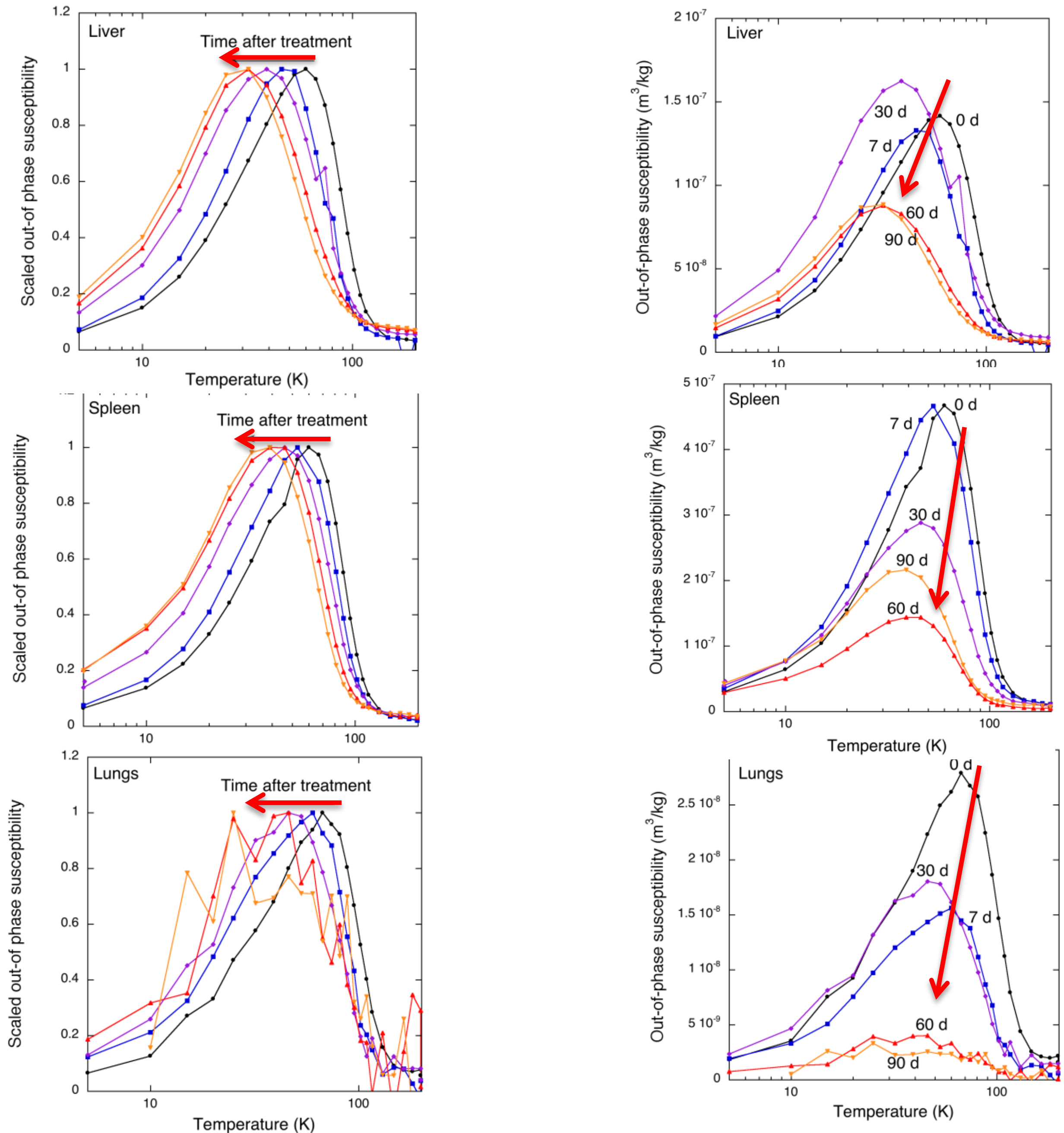
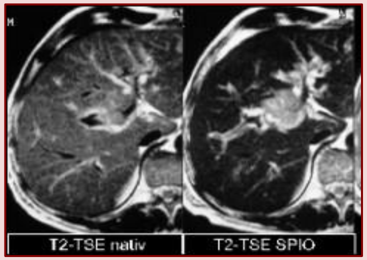
Long term particle transformations



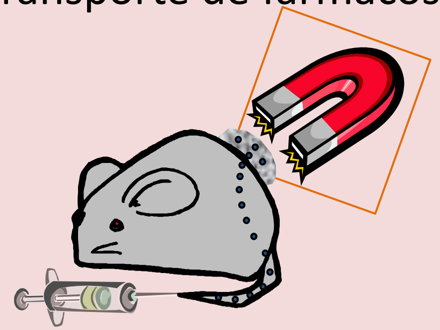
Future Research

Contrast agents



- More efficient agents
- Targeting
- Multimodal imaging agents
  - MRI/TC
  - MRI/PET

Transporte de fármacos



- NP-Chemotherapy
- NP-Radiotherapy
- NP-Phototherapy
- Across the blood-Brain barrier
- Gene therapy

Hipertermia

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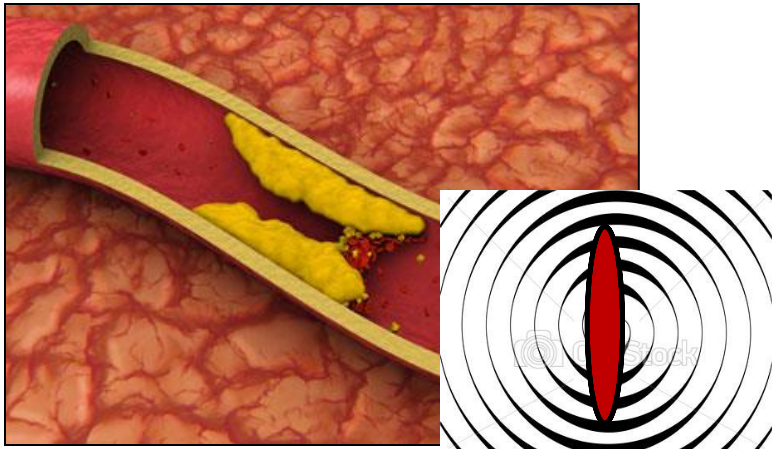
- Optimization of agents and heat generator
- Mechanism of cell death
- i.v. injection

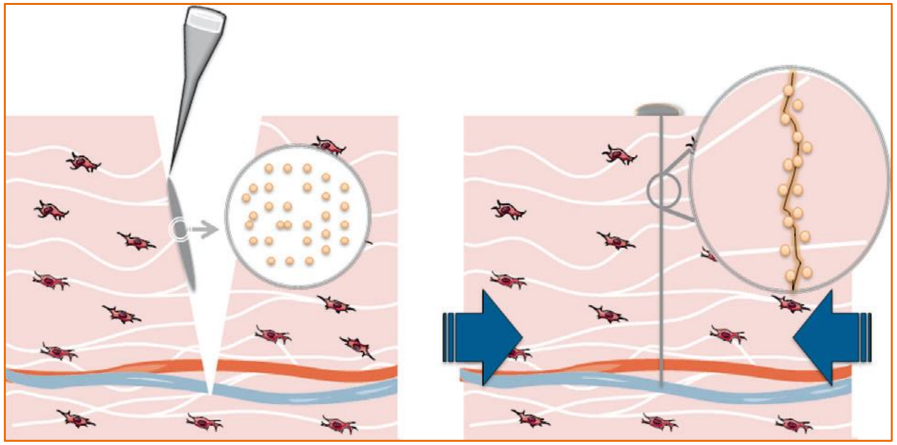
New applications

Pulse Therapeutics

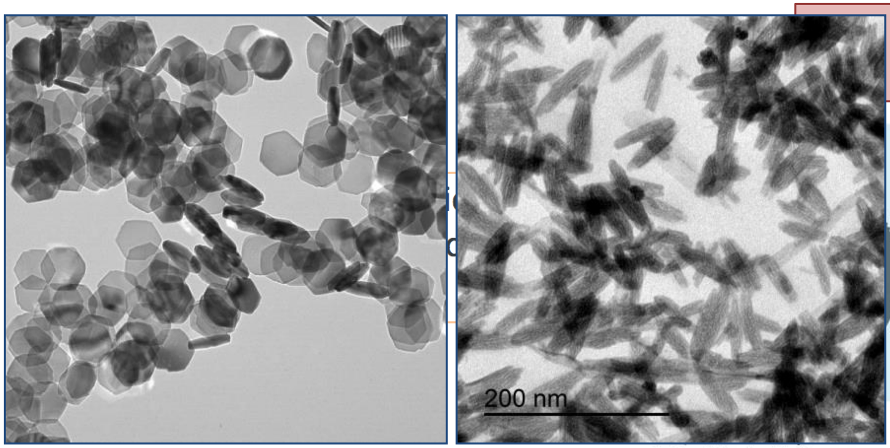


http://scitation.aip.org/content/aip/magazine/physicstoday/news/10.1063/PT.5.5029

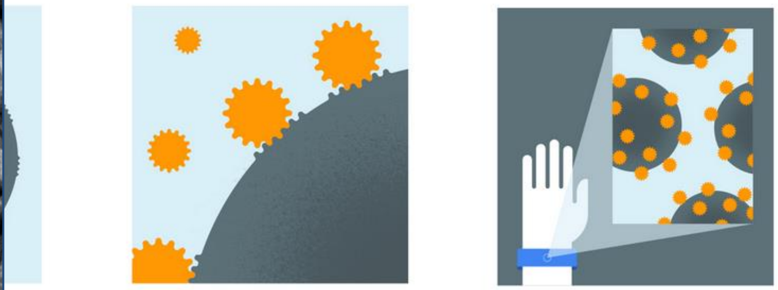
Regeneration



Angewandte Chemie International Edition
53, 6369-6373, 16 APR 2014 DOI: 10.1002/anie.201401043



Detecting disease early



single red blood cell is the same width as about 2000 nanoparticles.

Nanoparticles circulate in the blood and can be built to attach to particular types of cells, such as circulating cancer cells.

A device worn on the outside of the body can detect the nanoparticles and provide useful information to physicians.

http://www.zmescience.com/medicine/google-nanoparticle-pill-5344/

OUTLINE

1- Nanoparticles for medicine

2- Basic principles in magnetism

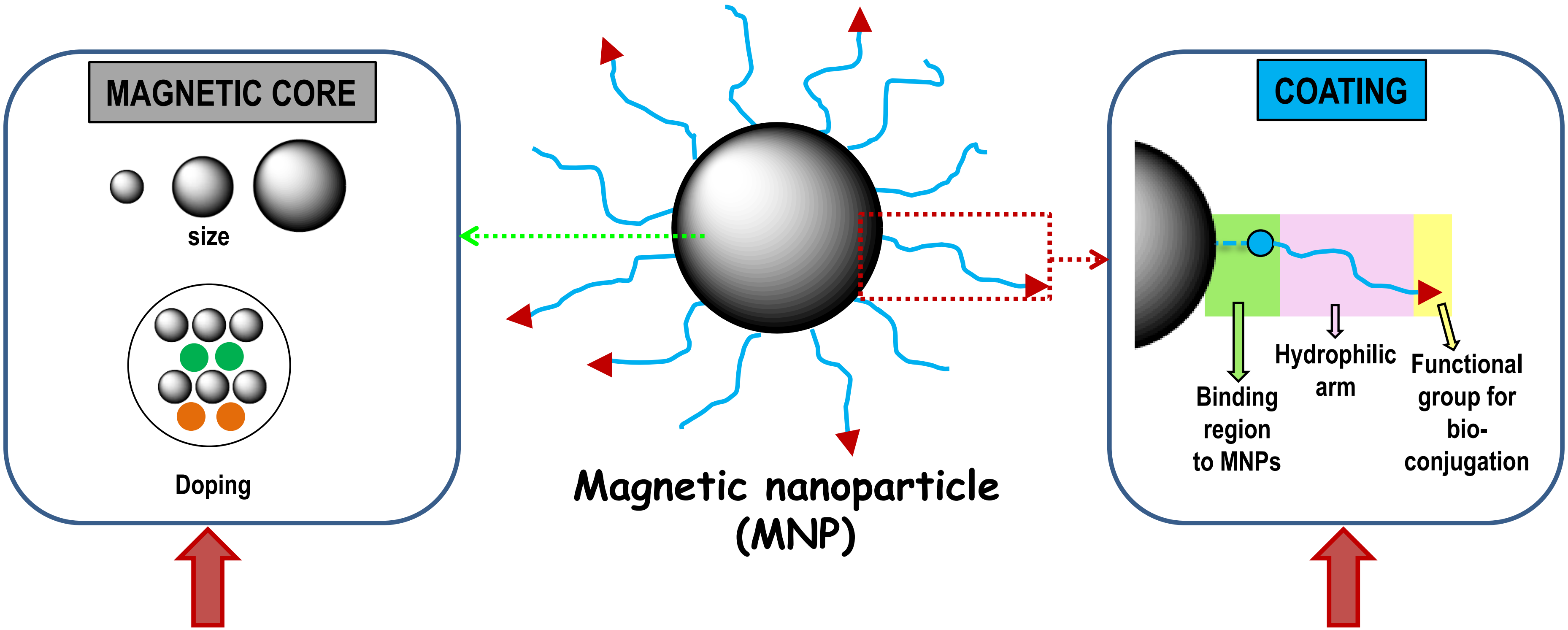
3- Biomedical applications: Separation, Diagnosis, Therapy

4- Synthesis of magnetic nanoparticles

5- Future

Design a Nanoparticle for each application

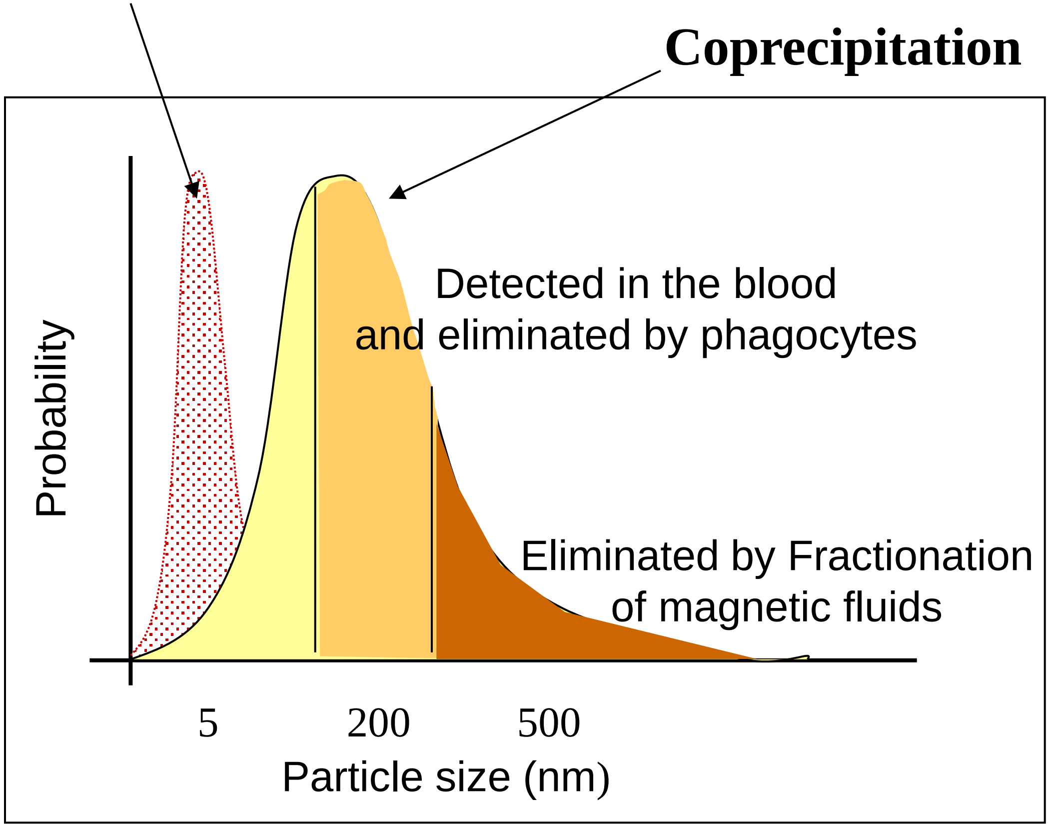
IMPORTANT PARAMETERS



- Different core size
- Different core composition

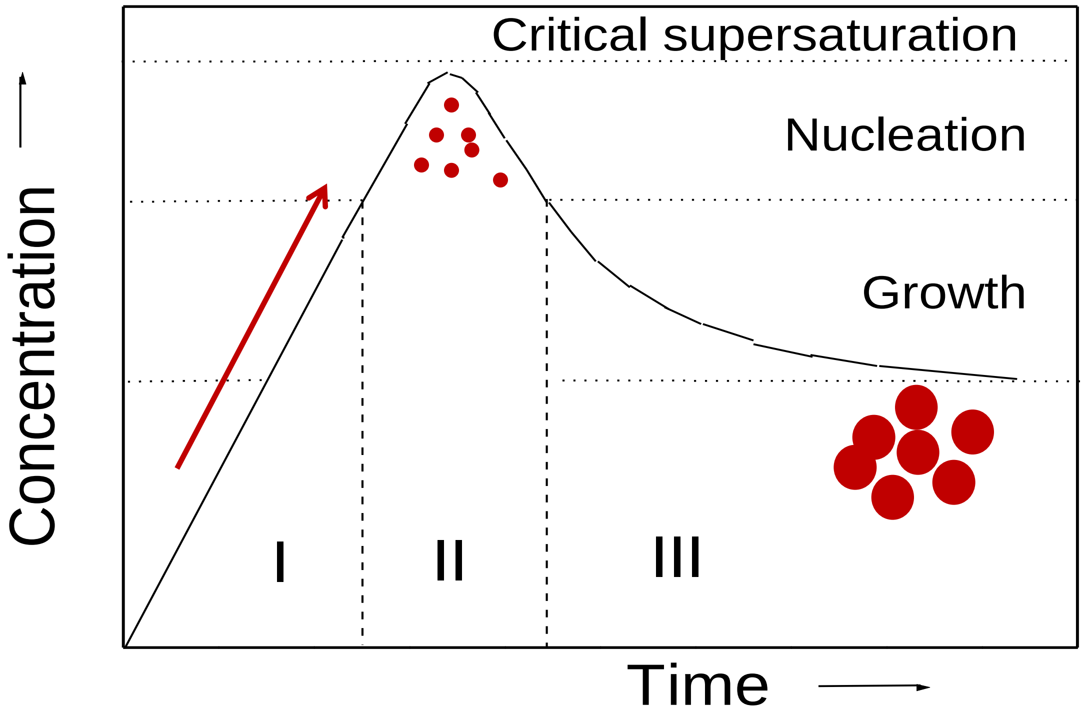
- Biocompatible polymers
- Colloidal stability
- Strong anchoring
- > MNPs blood life-time
- Core protection

UNIFORM NANOPARTICLES



Upon application, only a small number of particles contribute to the desired magnetic effect.

Nanoparticle synthesis routes

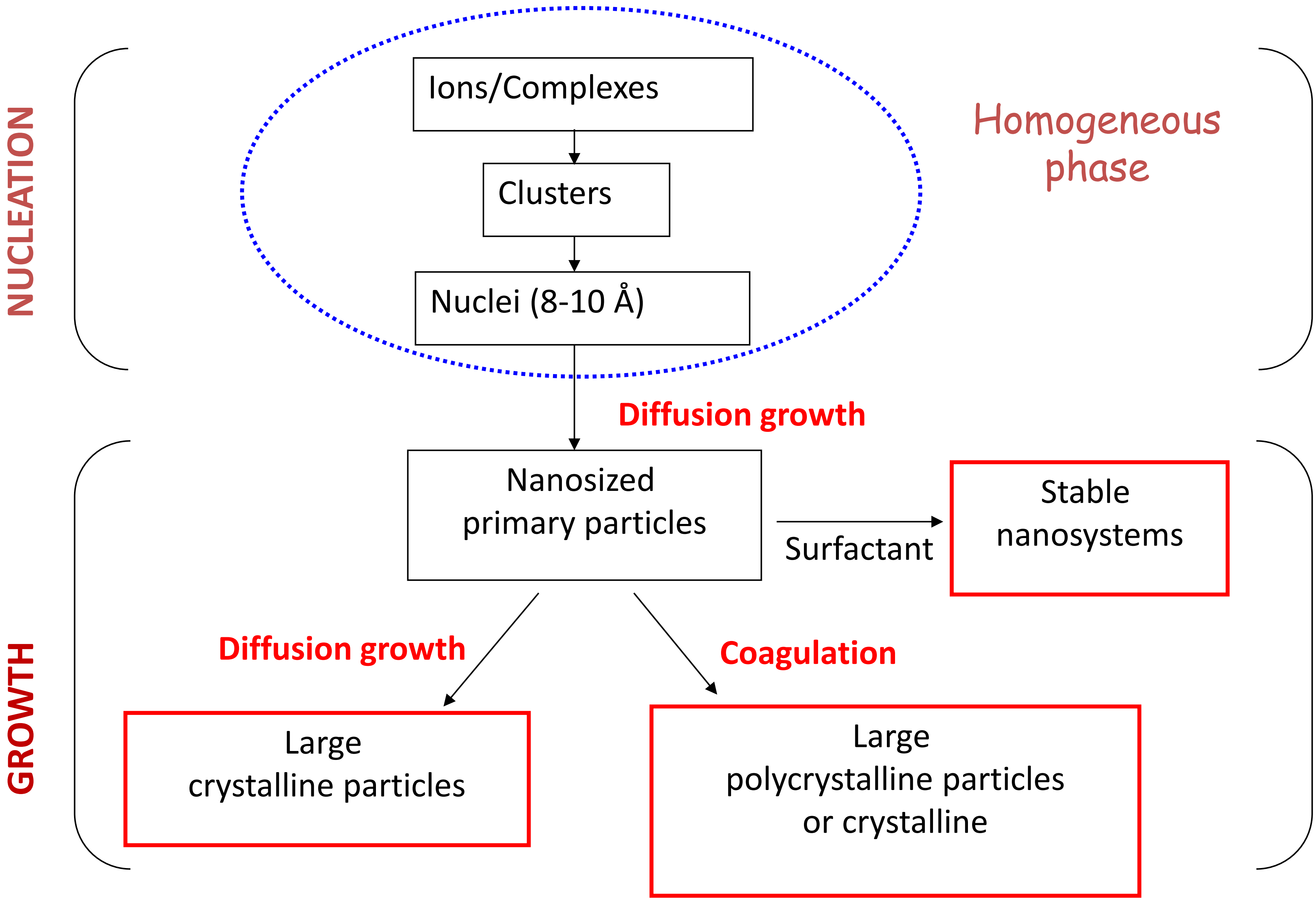


Modelo Clásico

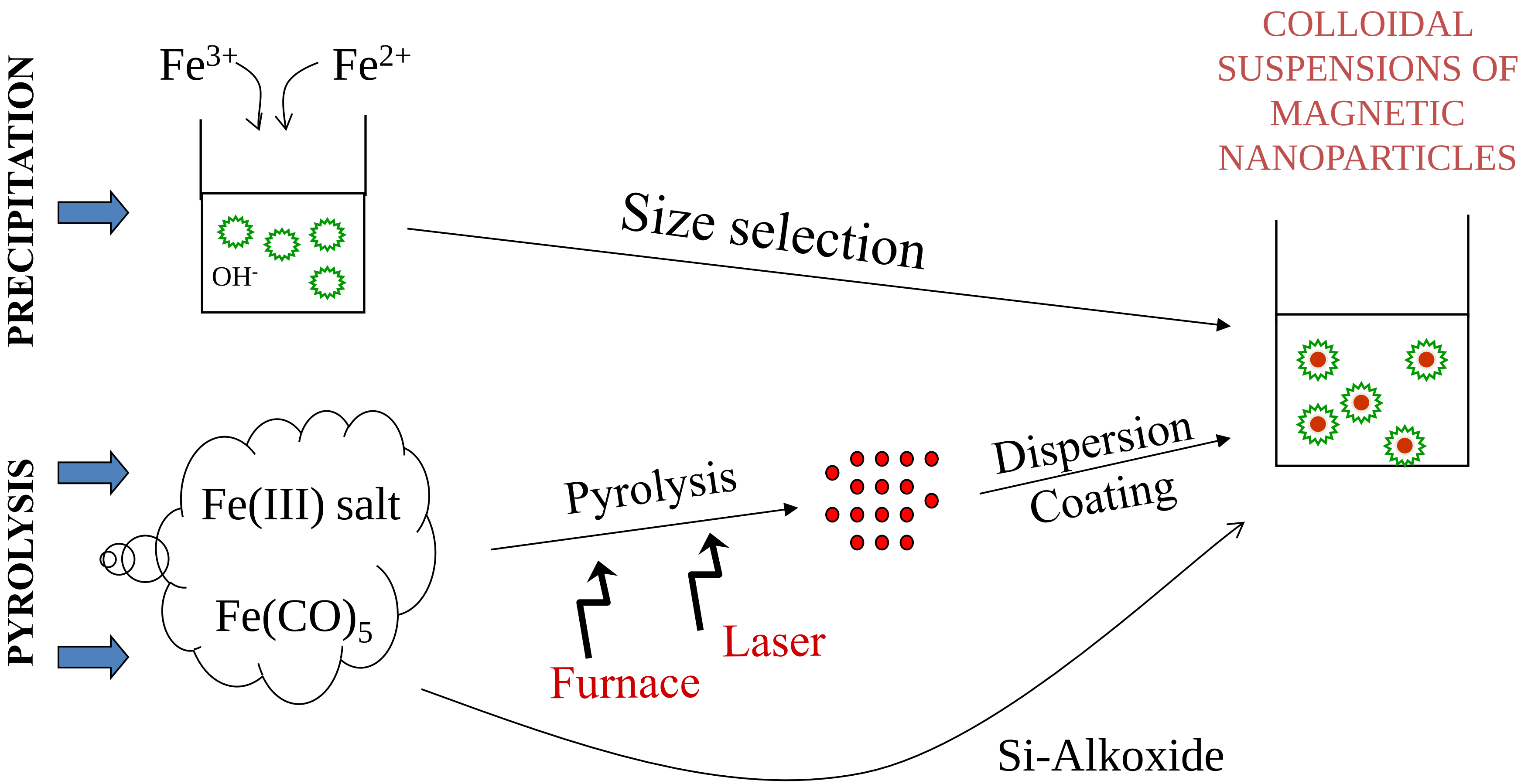
LaMer and Dinegar

Synthesis and Characterization of Nanoparticles: Synthesis of Inorganic Nanoparticles,
Gorka Salas, Rocio Costo and María del Puerto Morales
Part I, Vol. 4 Nanobiotechnology, Inorganic Nanoparticles vs Organic Nanoparticles
edited by J.M. de la Fuente and V. Grazu, 2012 Elsevier Ltd, FRONTIERS OF NANOSCIENCE, Series, Editor: R. E. Palmer, UK.

Nanoparticle synthesis routes



Nanoparticle synthesis routes



A:G. Roca et al. J. Phys. D:Apply Phys., 42, 224002, 2009

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Nanoparticle synthesis routes

TABLE 2.1 Summary Comparison of the Synthetic Methods.

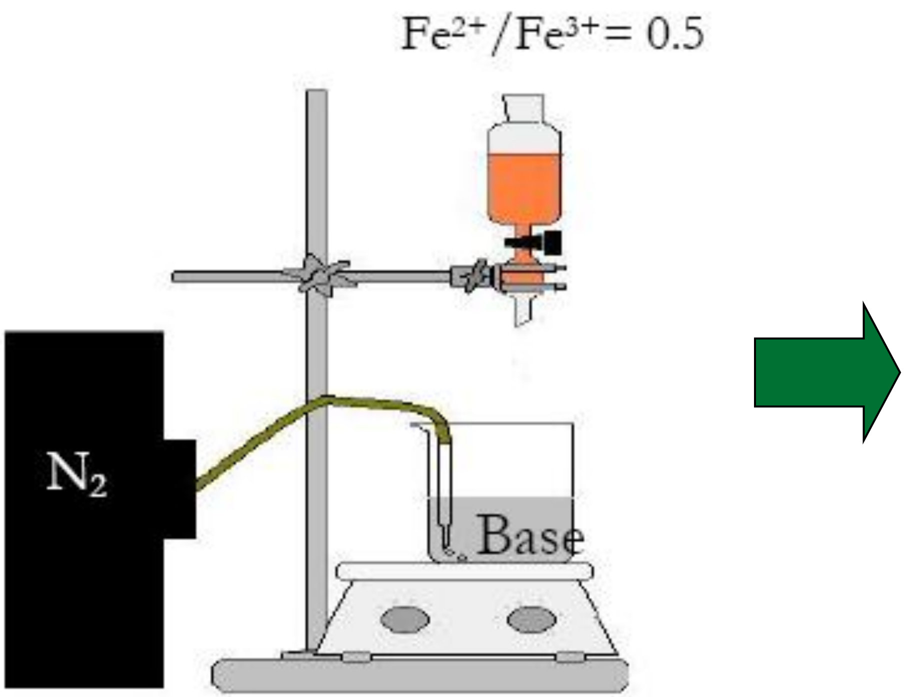
Synthesis Method	Reaction Time	Solvent	Surface-Capping Agent	Sizes	Size Distribution	Shape Control	Yield
Coprecipitation	Minutes	Water	No	2–15	Broad	Not good	Medium
Thermal decomposition	Hours–days	Organic compound	Yes	4–30	Very narrow	Very good	Medium
Polyol process	Hours	Polyglycol	Yes	5–150	Narrow–broad	Good	Medium
Microemulsion	Hours	Organic compound	Yes	5–50	Narrow	Good	Low
Spray pyrolysis	Seconds	Water and volatile solvents	No	2–10	Broad	Not good	High
Laser pyrolysis	Milliseconds	Gases	No	2–10	Very narrow	Good	High

Synthesis and Characterization of Nanoparticles: Synthesis of Inorganic Nanoparticles,
Gorka Salas, Rocio Costo and María del Puerto Morales
Part I, Vol. 4 Nanobiotechnology, Inorganic Nanoparticles vs Organic Nanoparticles edited by J.M. de la Fuente and V. Grazu, 2012 Elsevier Ltd, FRONTIERS OF NANOSCIENCE, Series, Editor: R. E. Palmer, UK.

Synthesis by precipitation in water

Coprecipitation

Sal de Fe(II) y Fe(III)



- Concentration
- Temperature
- Atmosphere
- Stirring

Fe_3O_4

(Magnetite)

R. Massart, IEEE Trans. Magn. Magn., 17, (1981) 131 and J.Chem.Phys. 84, 967 (1987)

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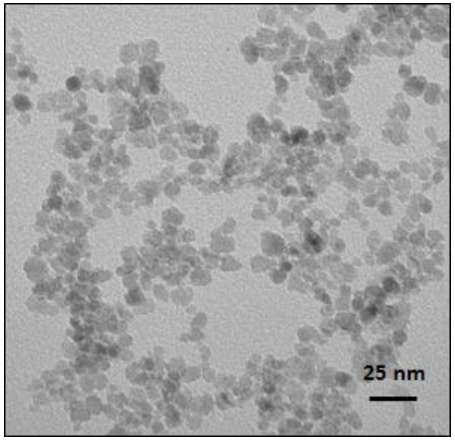
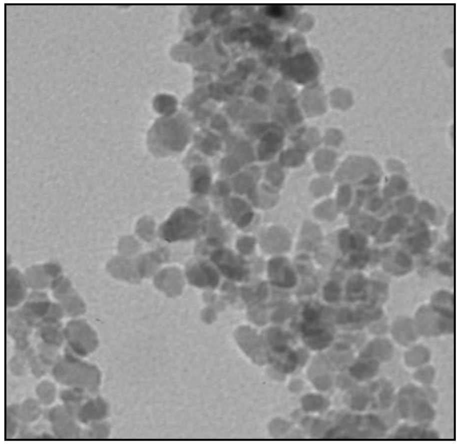
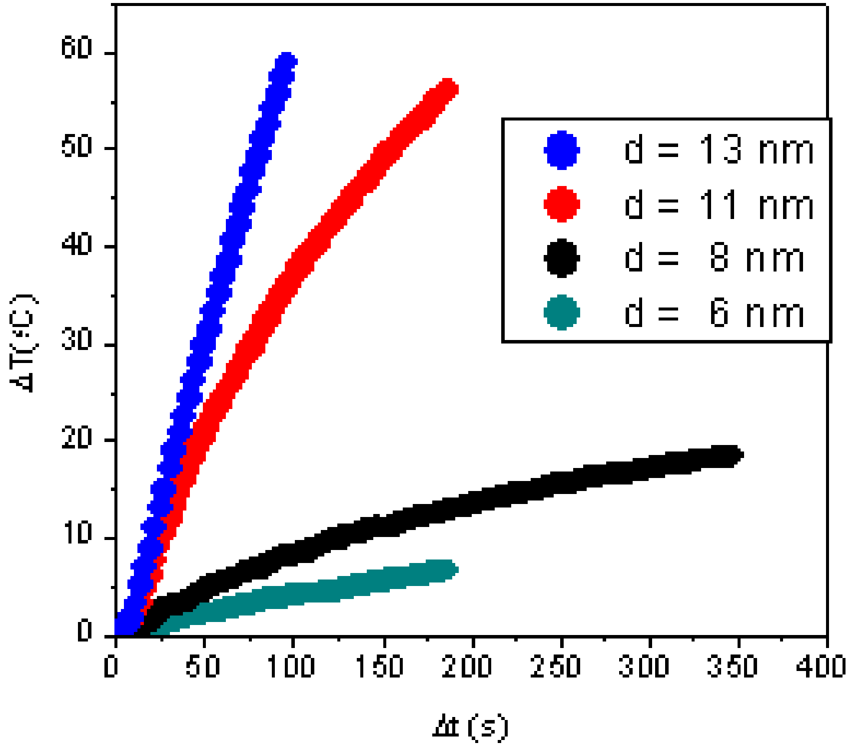
Synthesis by precipitation in water

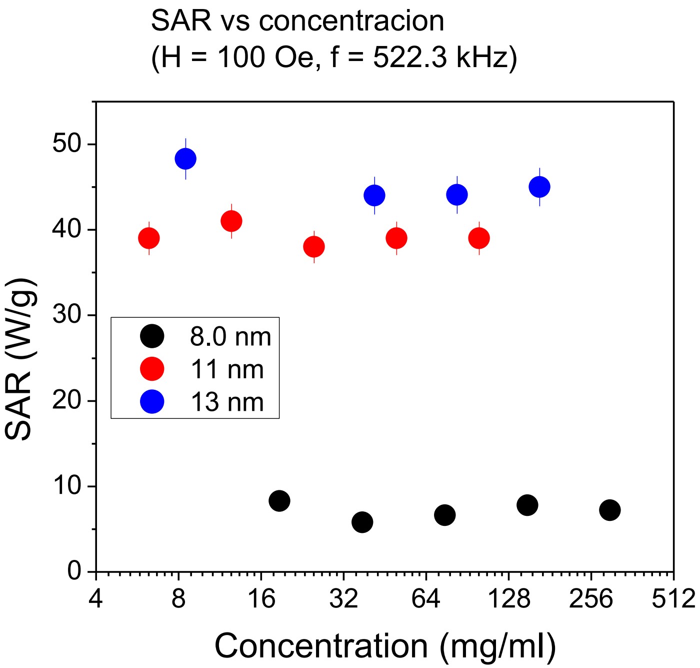
Instituto de Magnetismo Aplicado
Laboratorio "Salvador Velayos"
UCM-ADIF-CSIC

H = 100 Oe
f = 522.7 Hz
c = 50 mg/ml

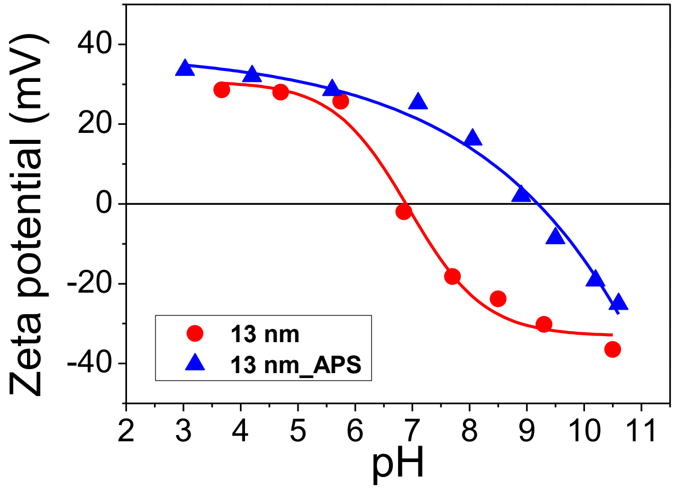




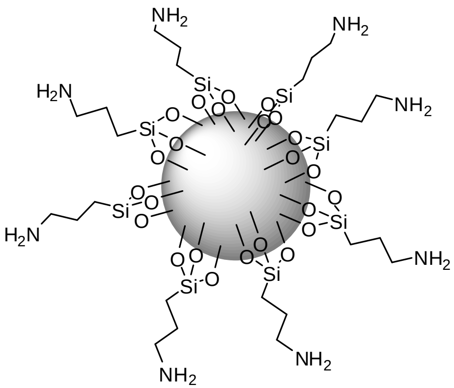


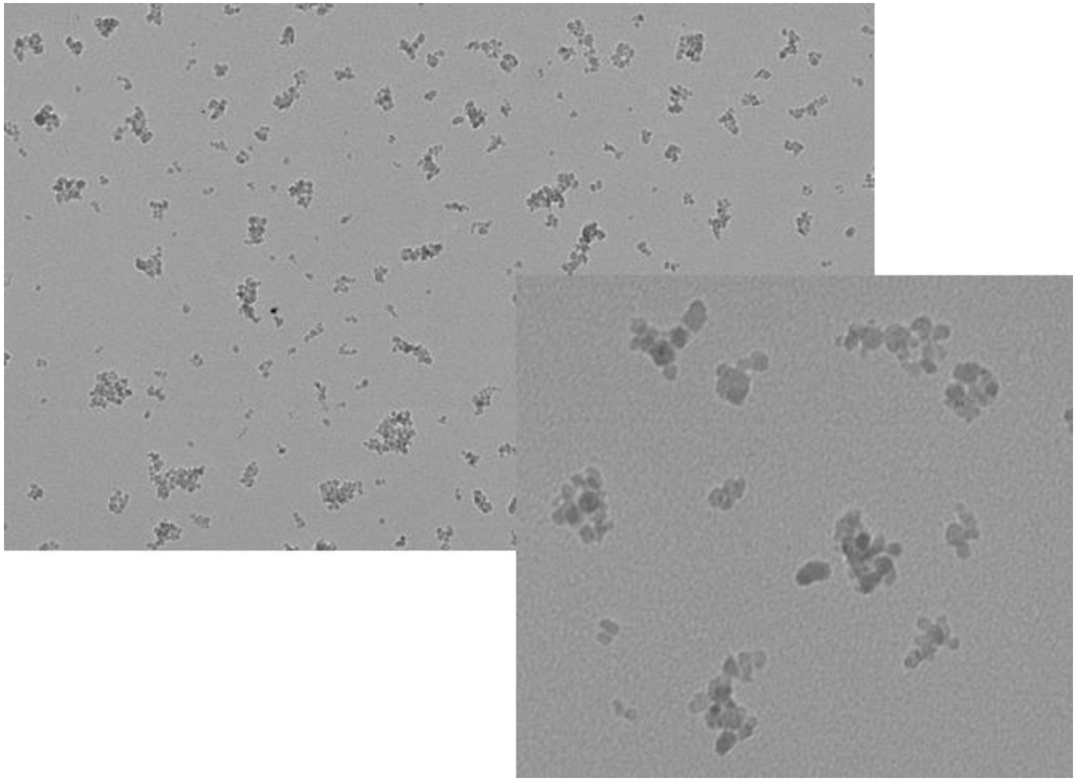


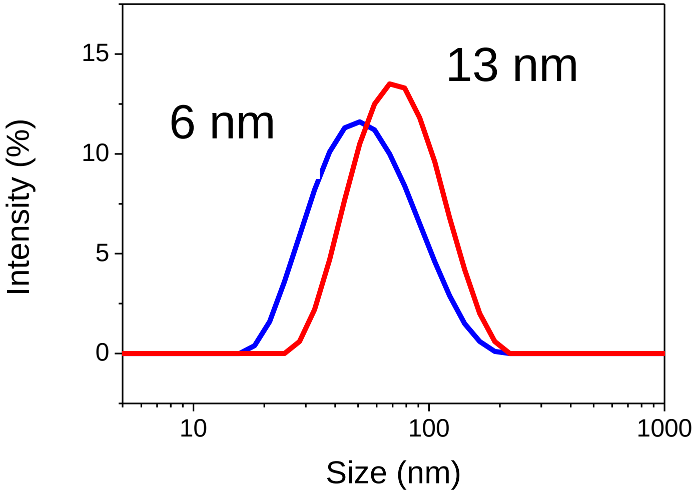
Synthesis by precipitation in water



APS coating





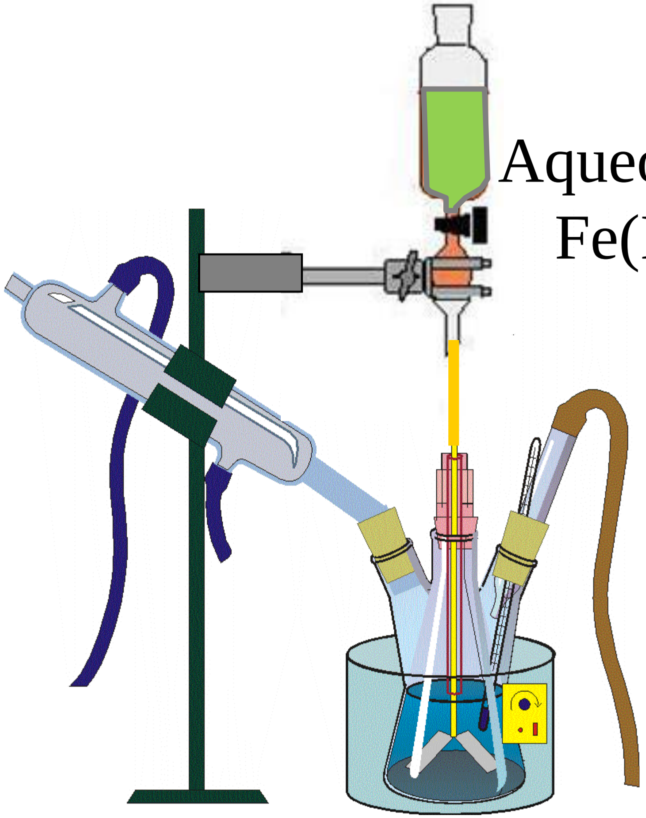


D core= 6.3 nm (PDI=0.2)
D hyd= 47.81 nm (PDI=0.23)

D core= 13.7 nm (PDI=0.2)
D hyd=106.3 nm (PDI= 0.17)

Synthesis by precipitation in water

Sal de Fe(II)



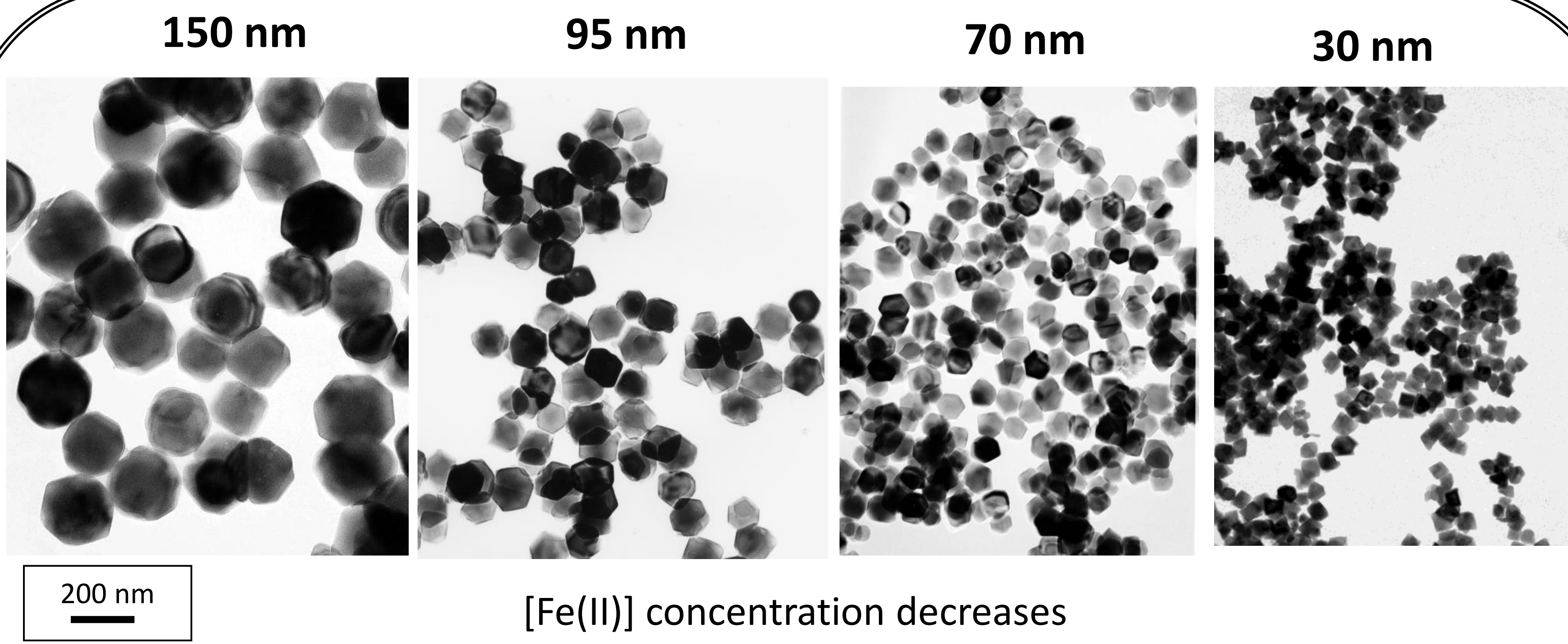
Aqueous solution
$Na(OH) + KNO_3$

90ºC ± 0.1
24 hours

Undisturbed system

Synthesis by precipitation in water

Control de la oxidación



[Fe(II)] concentration decreases

$[OH]_{exc}$ increases from 0.0002 M to 0.02 M

Particle size decreases from 300 nm to 30 nm

M. Andres et al. J. Phys. D:Appl. Phys., 2008

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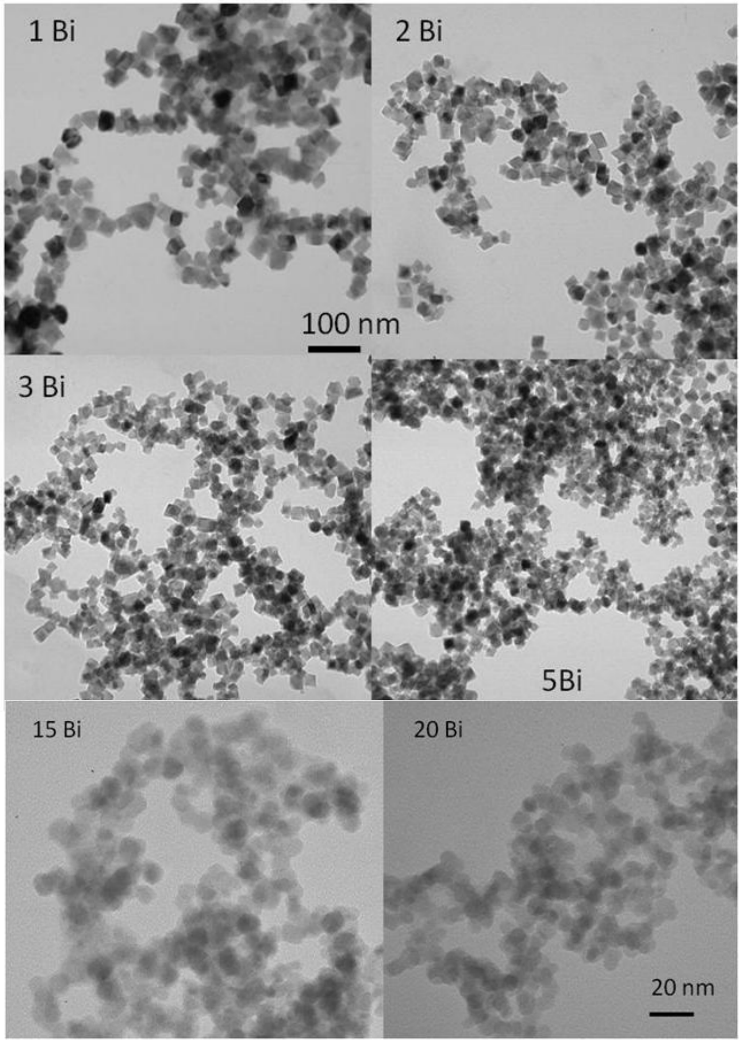
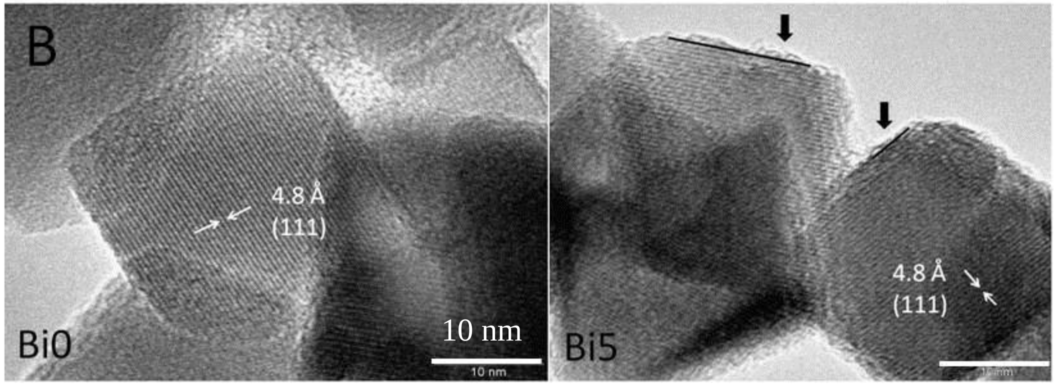
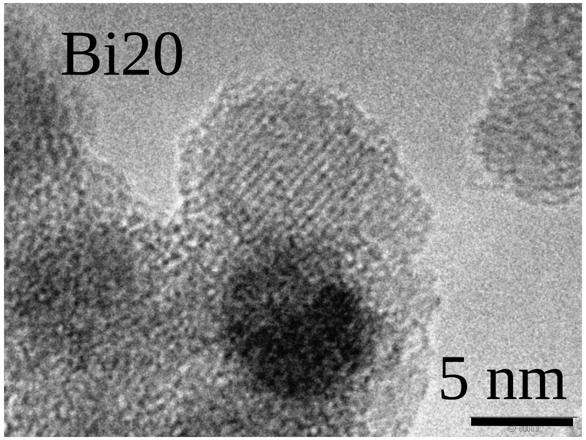
Hyperthermia + Dual imaging agent (NMR + CT)

Core/Shell Magnetite/Bismuth Oxide Nanocrystals with Tunable Size, Colloidal, and Magnetic Properties







Chem. Mater. 2012, 24, 319−324
Nanotechnology 2015

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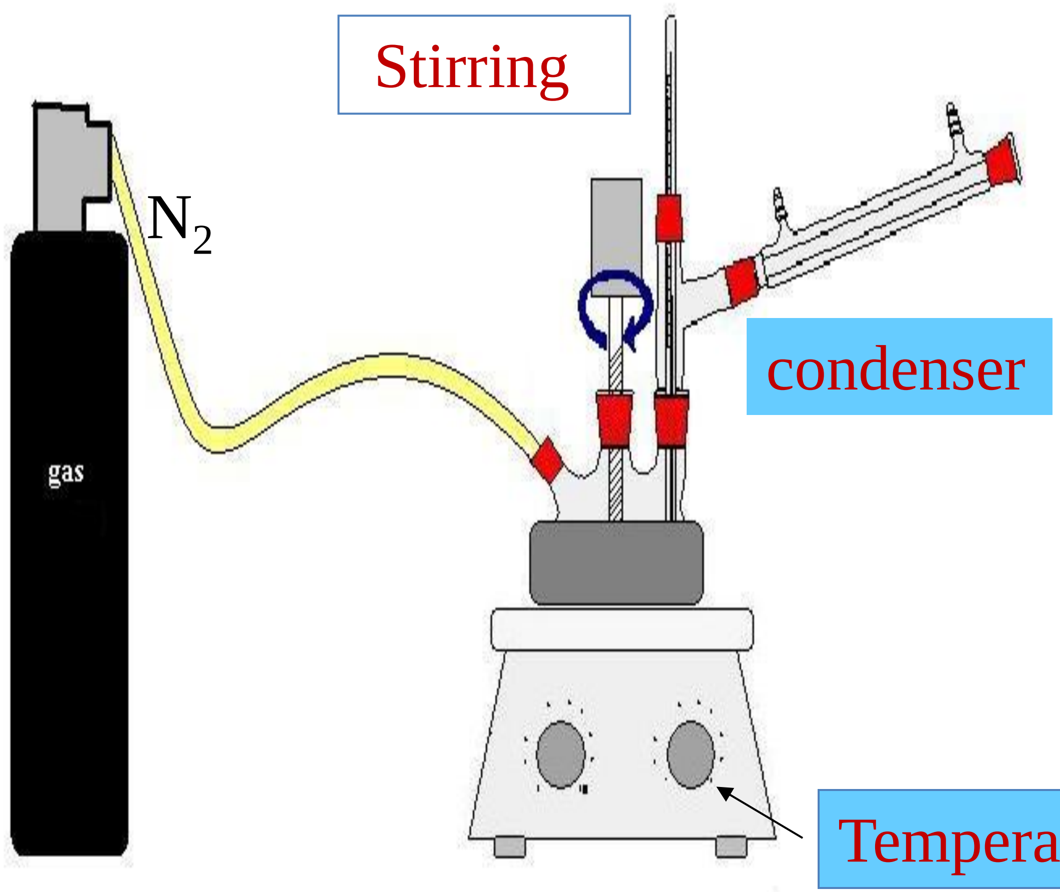
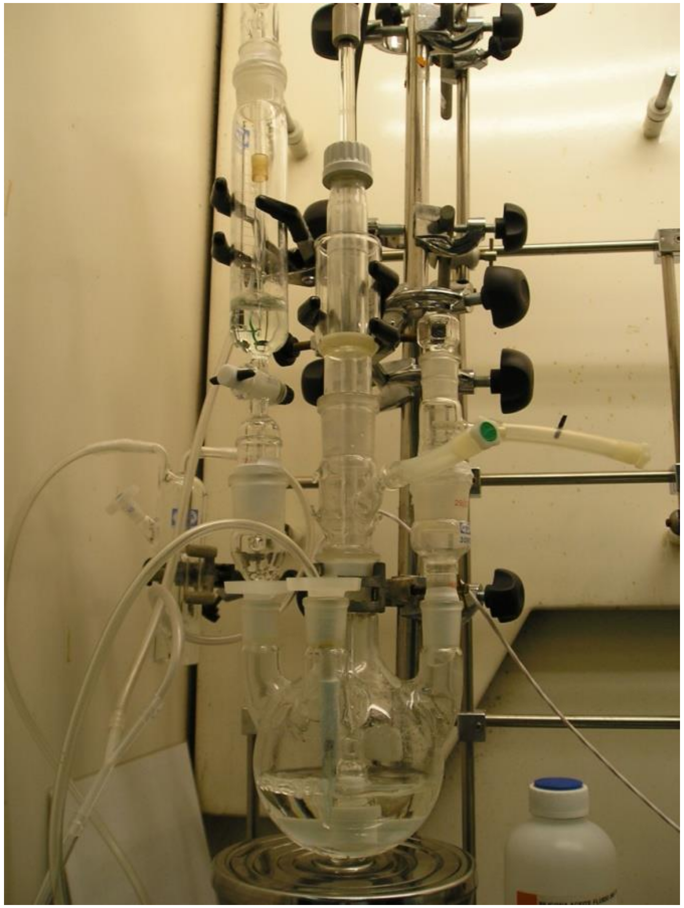
High temperature decomposition of organic precursors





Atmosphere control

High temperature decomposition of organic precursors

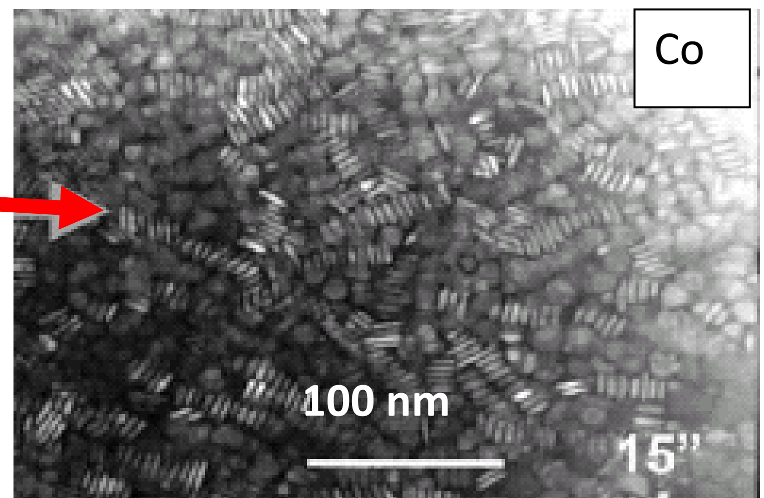
Surfactante

- El surfactante tiene la habilidad de controlar el crecimiento de la partícula

- Combinando diferentes surfactantes es posible controlar tamaño y forma de partícula.

$Co_2(CO)_8$

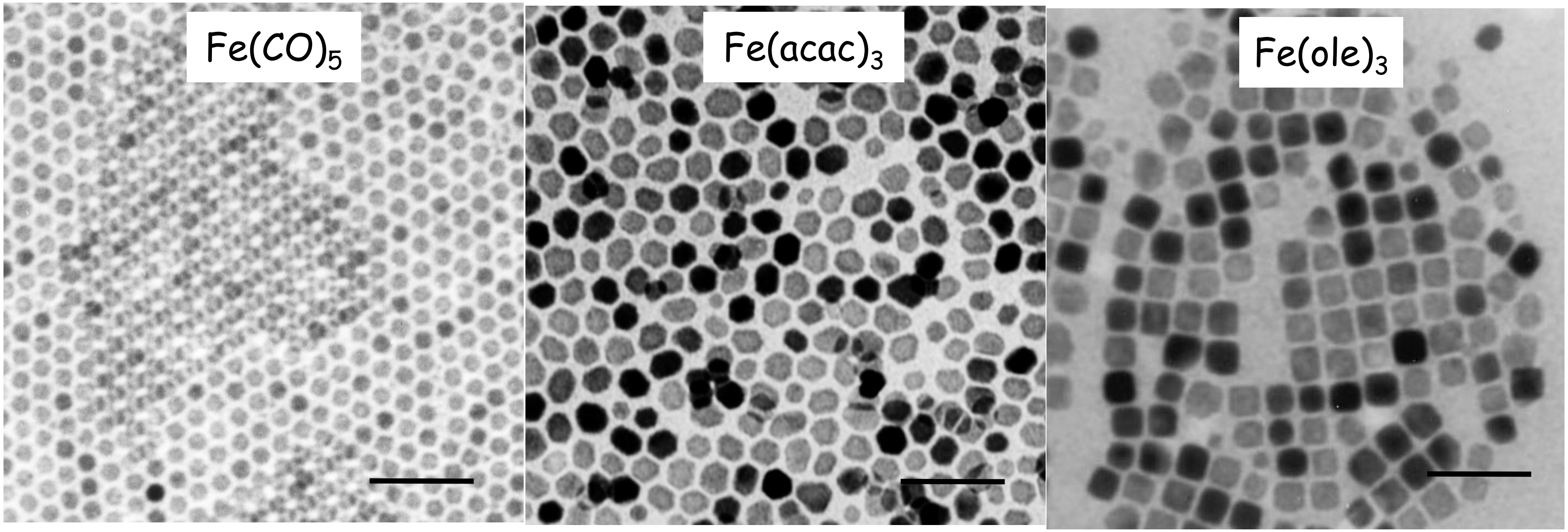
Oleic acid
+
TOPO

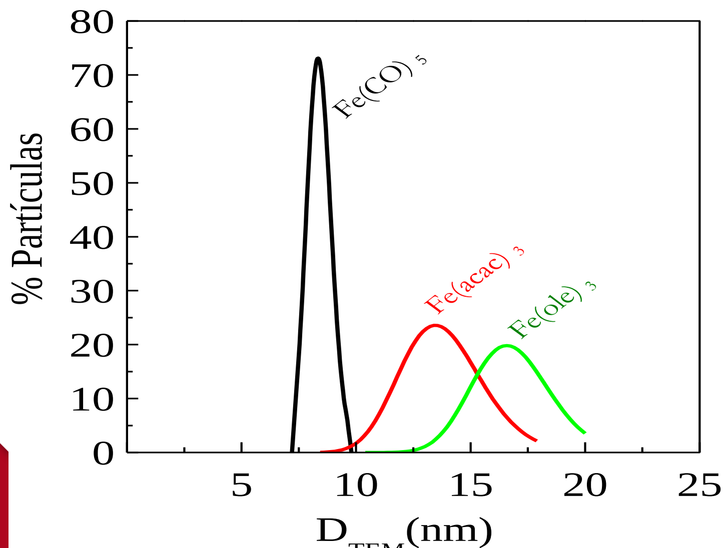


V.F. Puntes et al., Science 291 (2002) 2115

High temperature decomposition of organic precursors

Precursor





$Fe(acac)_3$+ a.oleico ⇄ $(Fe\text{-}oleico)_x$+ subproducts

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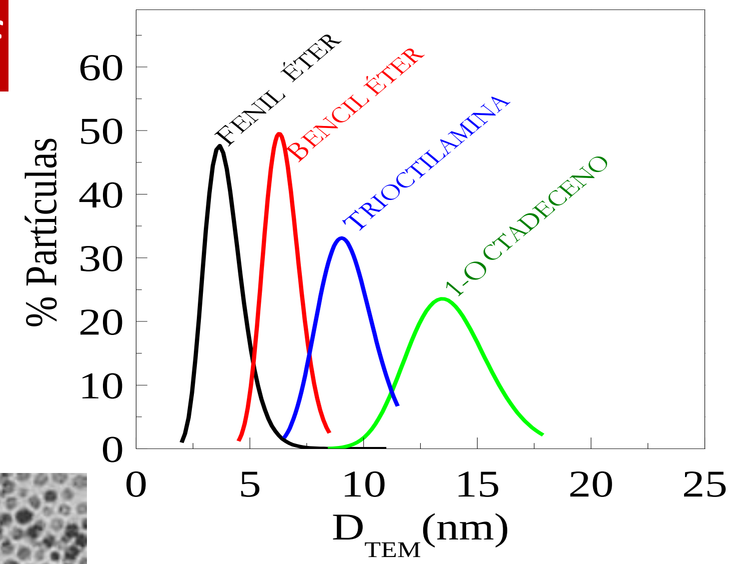
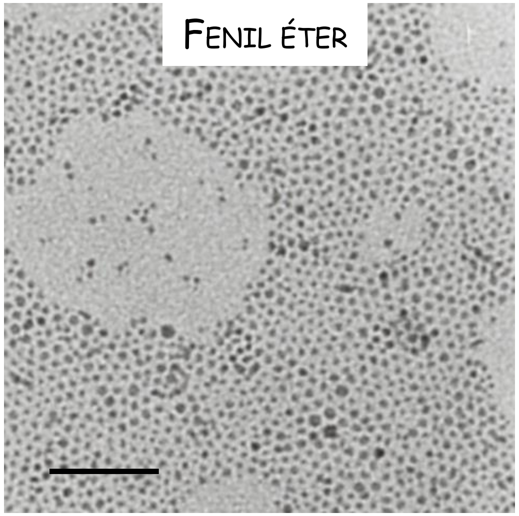
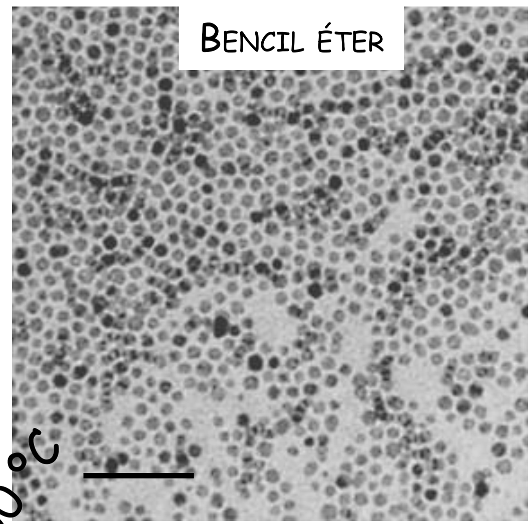
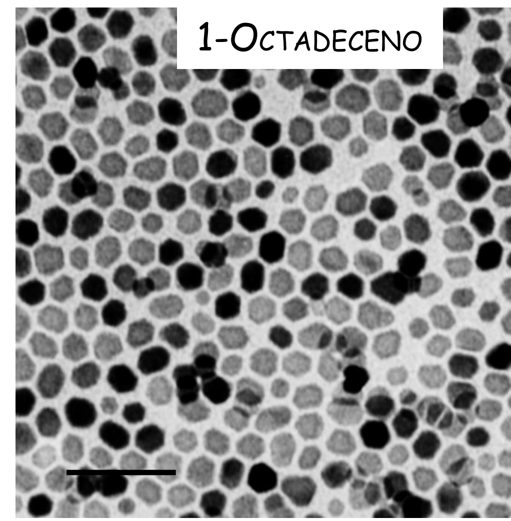
High temperature decomposition of









254 °C

300 °C

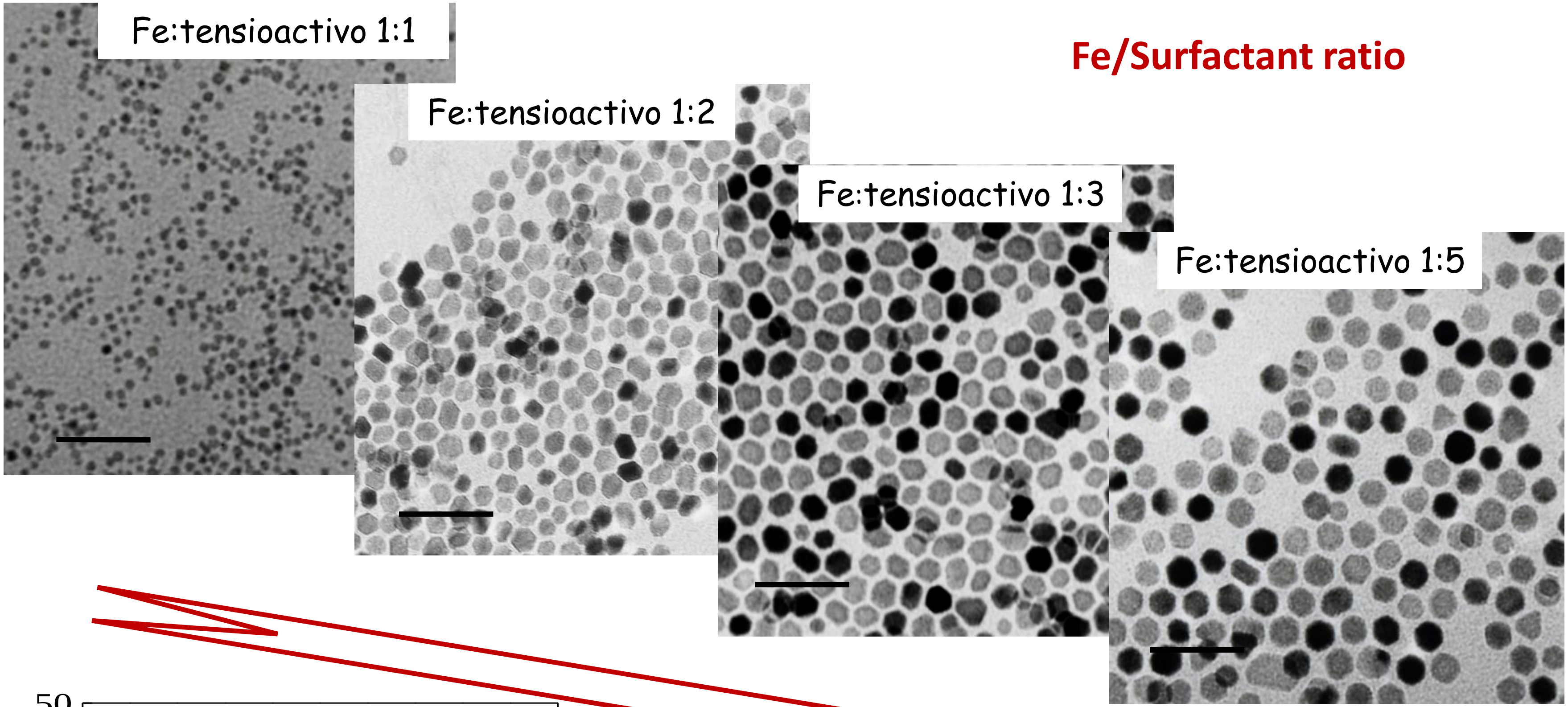
320 °C

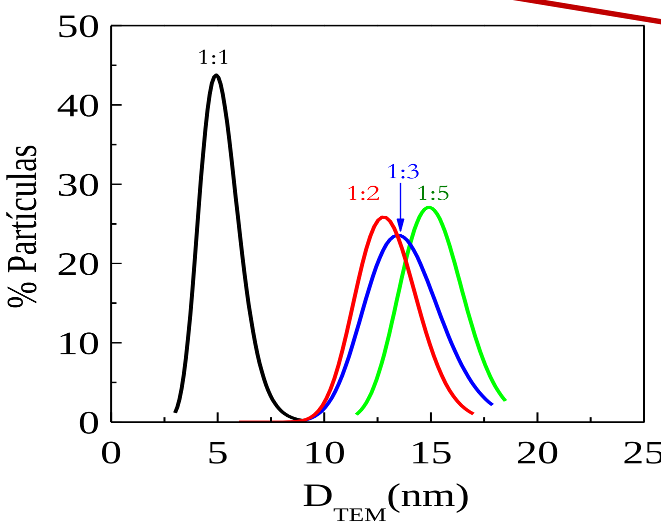
Boiling point

Solvent

High temperature decomposition of organic precursors

Fe/Surfactant ratio





Fe(acac)$_3$+ a.oleico ⇄ (Fe-oleico)$_x$ + subproductos

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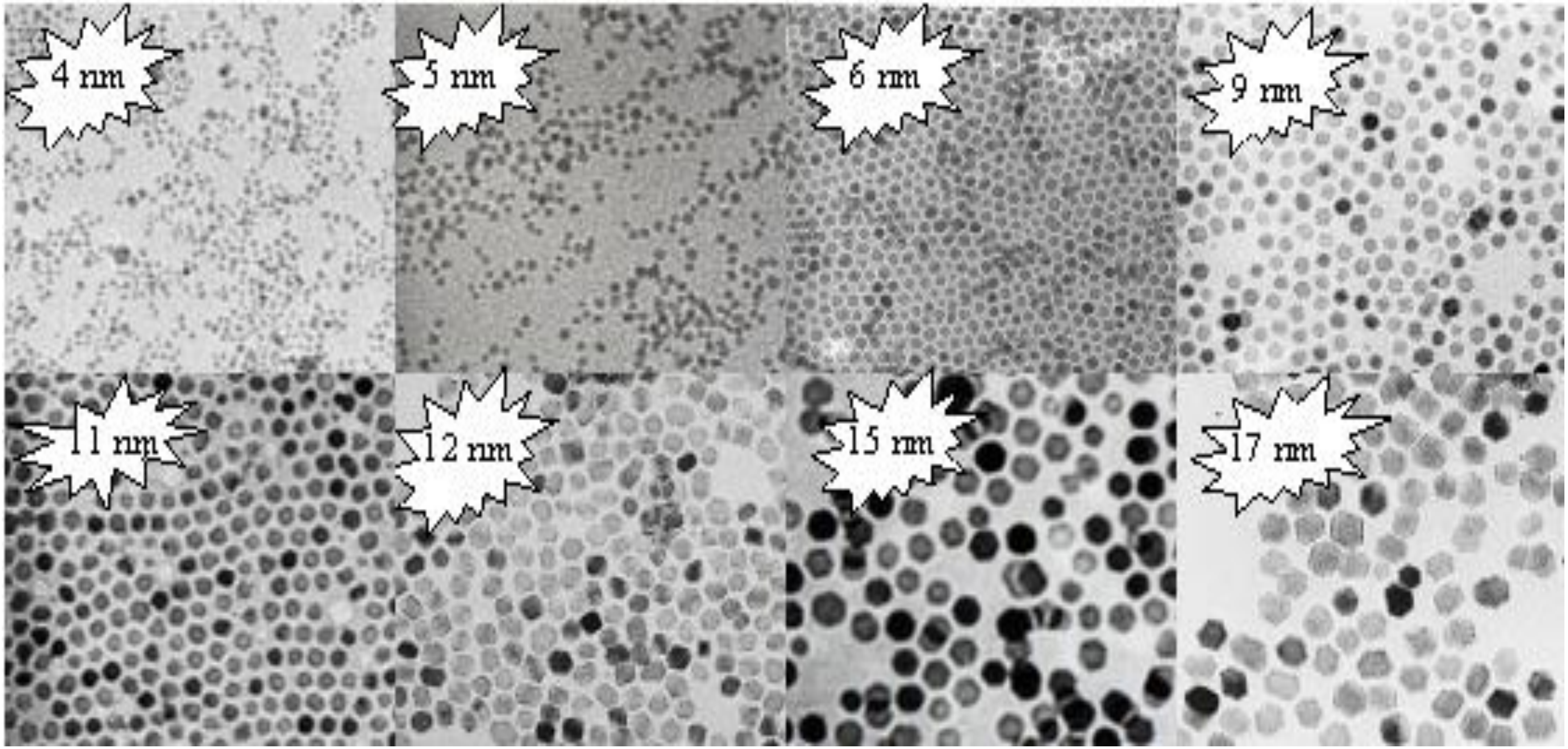
High temperature decomposition of organic precursors

Size control

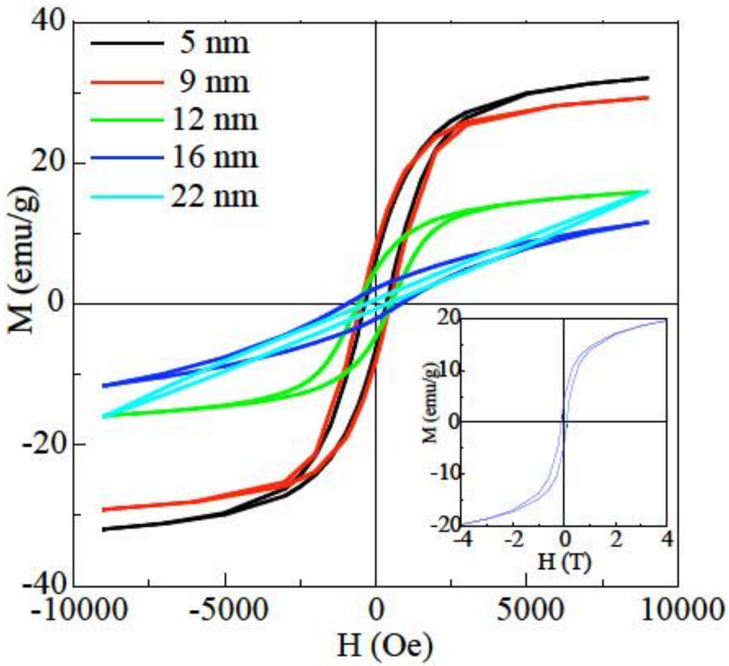


Iron oxide nanoparticles showing one nanometer increments in diameter

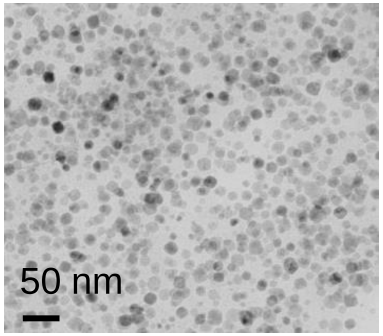
Roca, A. G. et al *Nanotechnology* **2006**, *17*, 2783-2788.

IEEE TRANSACTIONS ON MAGNETICS, 42, 3025 (2006)

Problems: low Ms at larger sizes

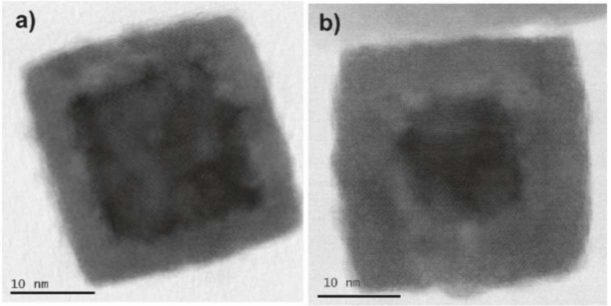


Park, J. *et al. Nat. Mater.* **2004**, *3,* 891-895



Broad size-distribution

Other phases

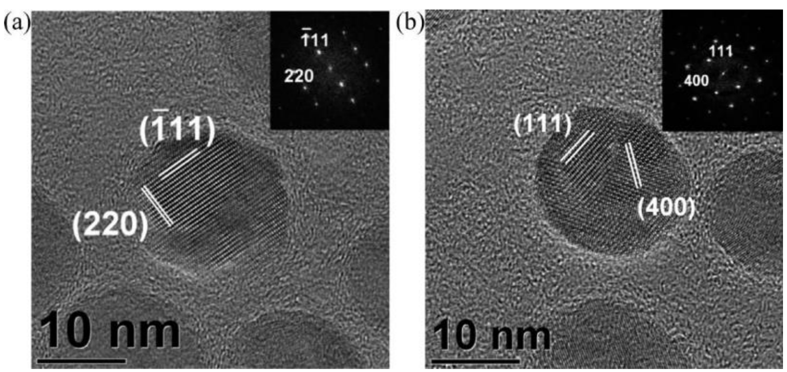


wüstite-spinel core-shell structure

FeO γ-Fe_2O_3/Fe_3O_4

Pichon et al *Chem. Mater.* **2011**, *23*, 2886-2900

Structural imperfections



|Chem. Mater. 2011, 23, 4170–4180

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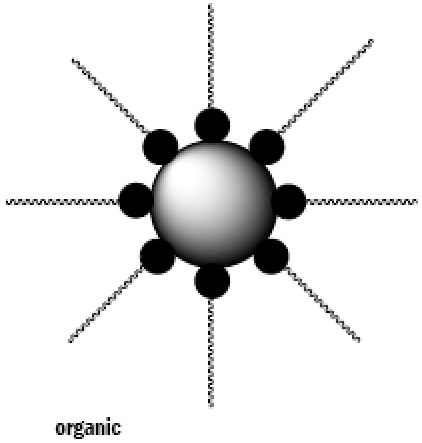
Hydrophilic Nanoparticles

The major challenge in the development of nanoparticles for biomedical applications is to make them hydrophilic, stable at physiological conditions and without significant aggregation.

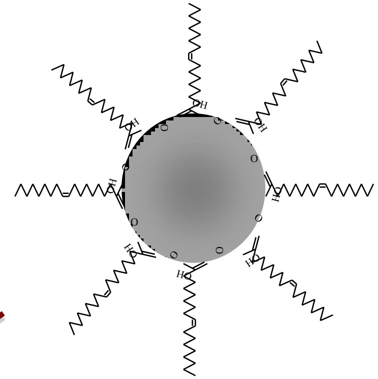
Easy and reproducible experimental set up.

Functional groups for water stability.

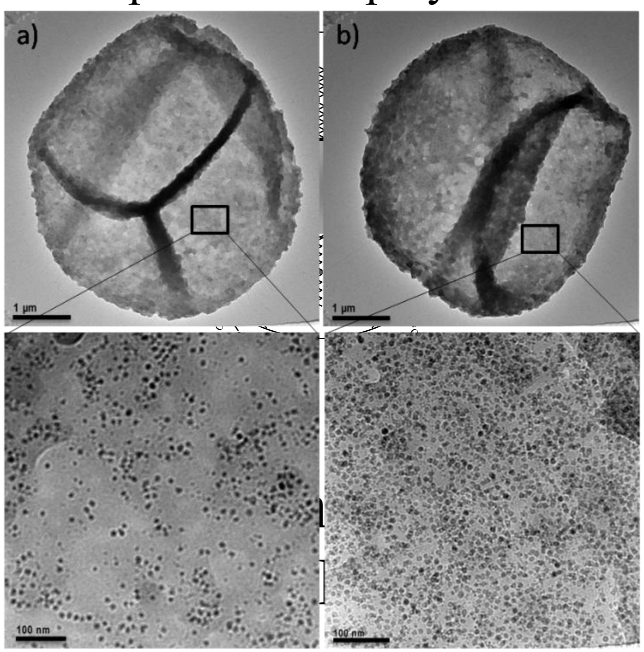
Ready for further functionalization.



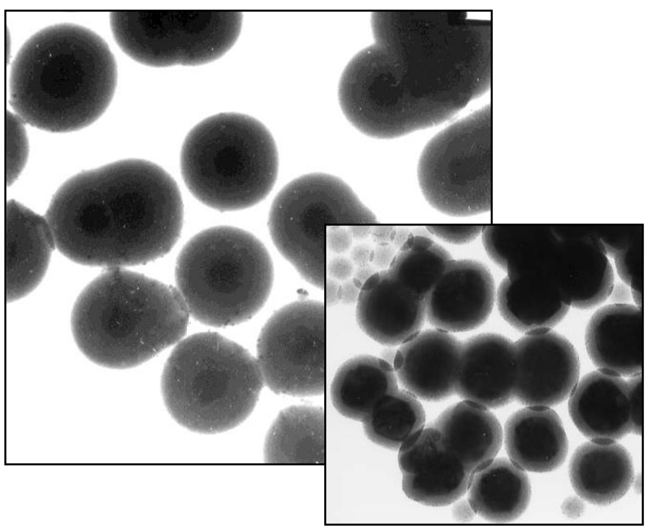
Hydrophilic Nanoparticles



Encapsulation in polymers

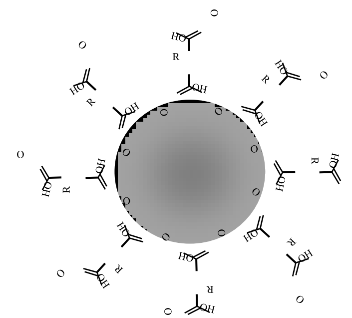


Morales et al., J. Mater. Chem., 2009, 19, 6381; J. Phys. Chem C 2011



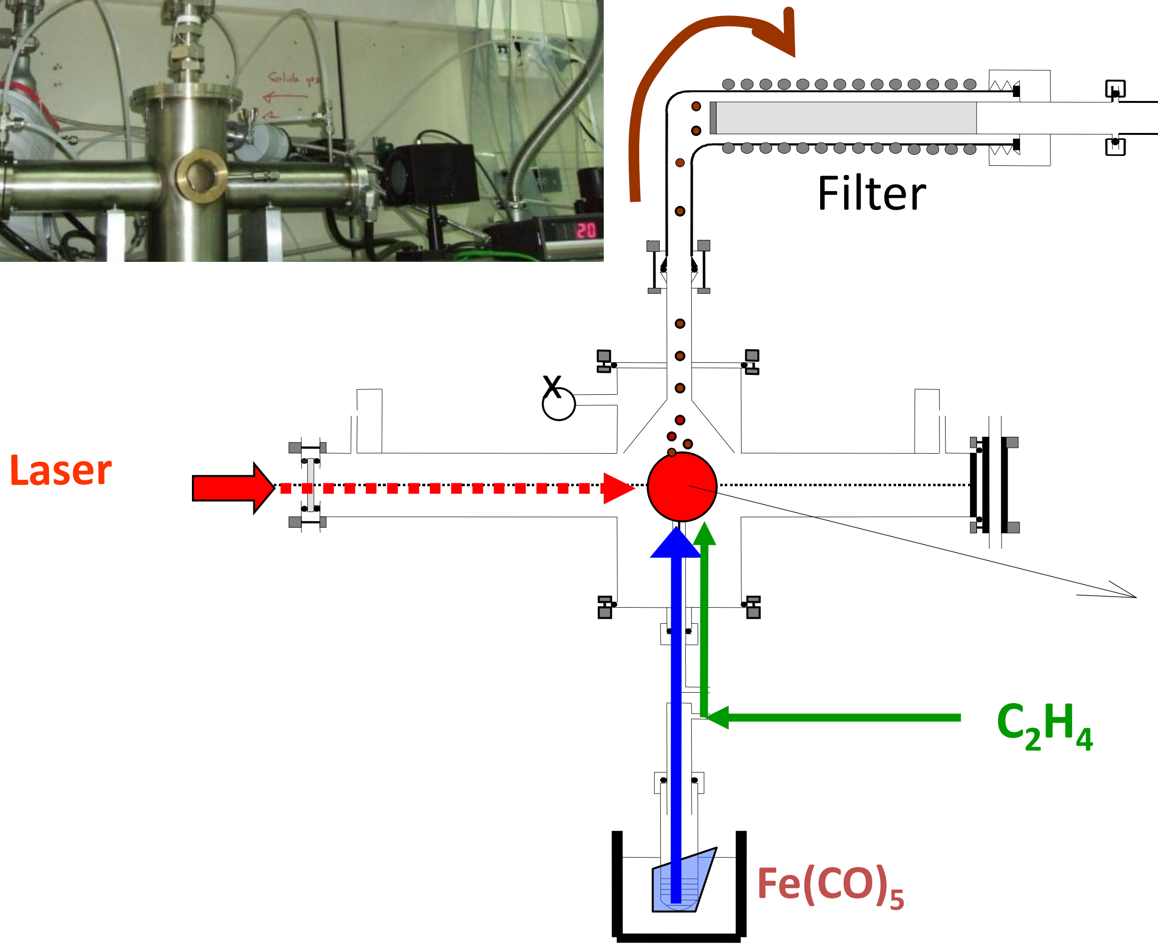
Pita et al, Journal of Colloid and Interface Science 321 (2008) 484–492

Ligand exchange o modification



F. Herranz et al., Chemistry - A European Journal, 14, p 9126 2008 Contrast Media Mol. Imaging 2008, 3 215–222

Laser Pyrolysis



S. Veintemillas-Verdaguer, O. Bomati-Miguel and M.P. Morales. **Scripta Mater.**, 47, 589-593 (2002)

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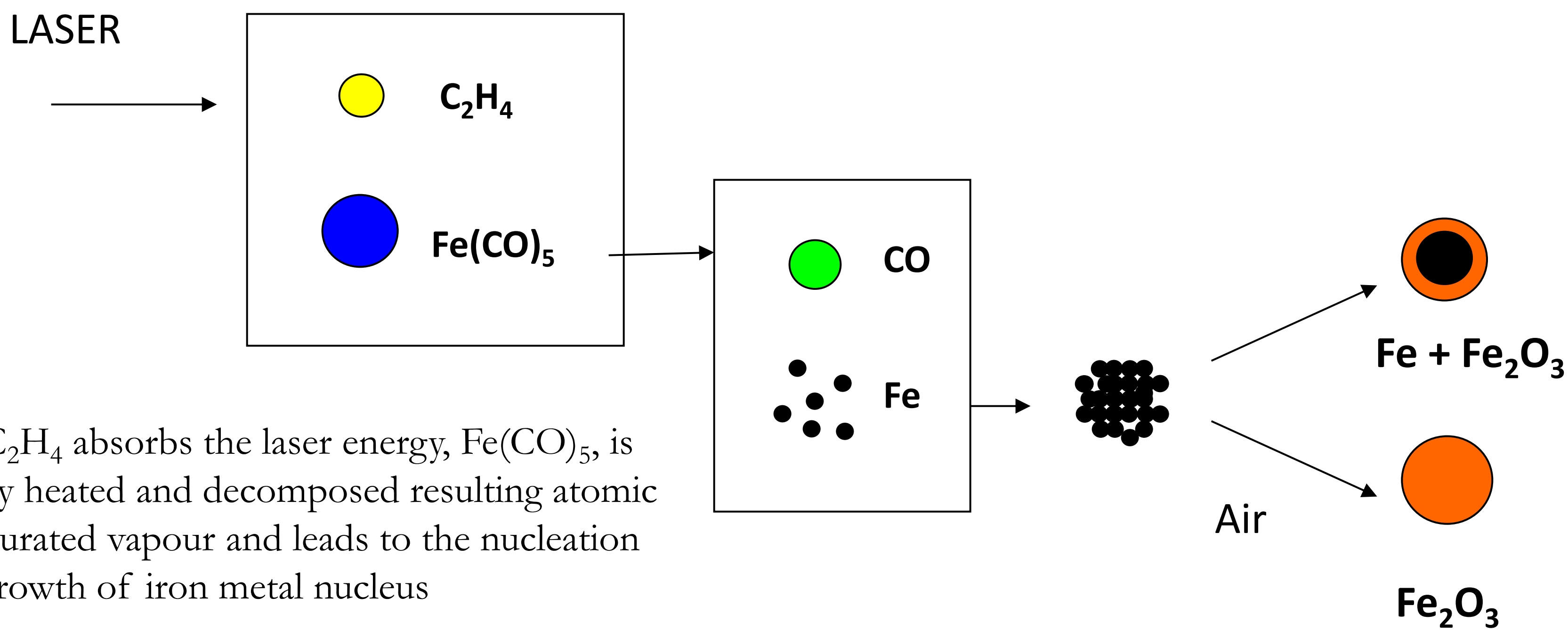
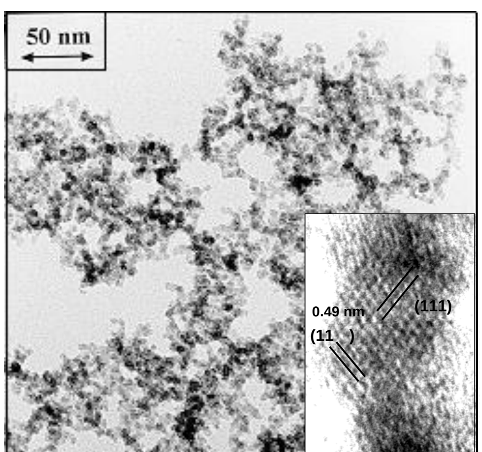
MECHANISM OF NANOPARTICLE FORMATION



The C_2H_4 absorbs the laser energy, $Fe(CO)_5$, is rapidly heated and decomposed resulting atomic Fe saturated vapour and leads to the nucleation and growth of iron metal nucleus



To stabilise the powders, a mixture of air and ethylene can be introduced together with the iron pentacarbonyl (hard oxidation) or after the laser pyrolysis (soft oxidation).

Laser Pyrolysis

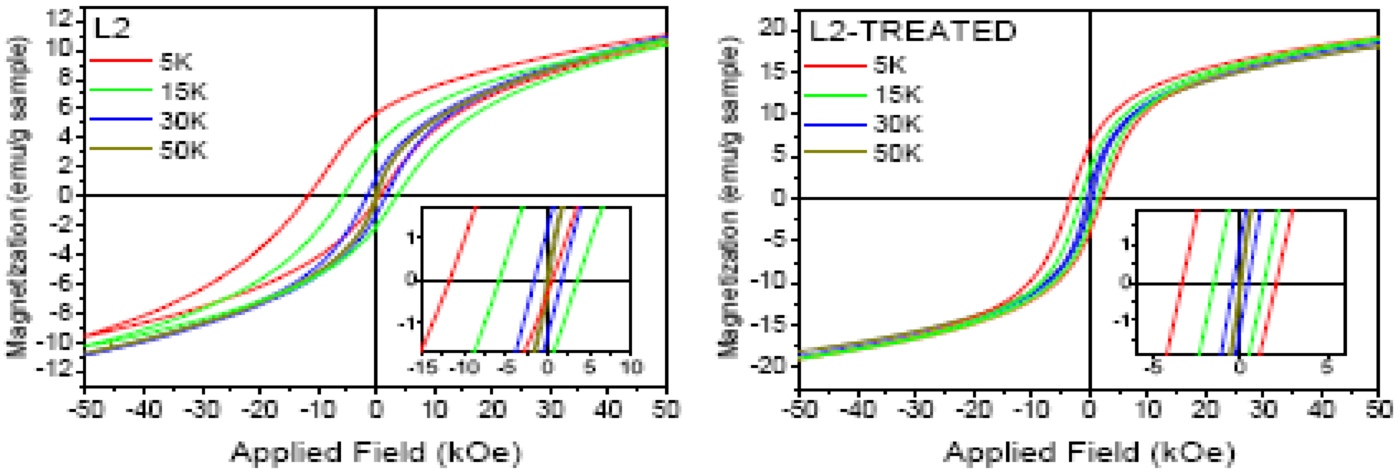
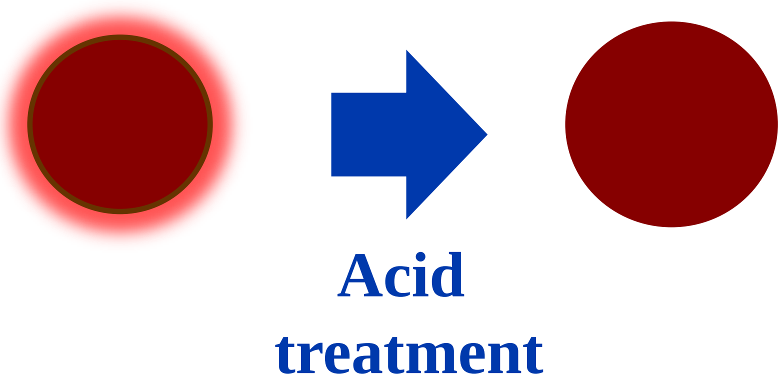


Figure 4.35: Field Cooling hysteresis loops of the samples at 5, 15, 30 and 50K. The insets show the low field area where the loop shift and the coercivity increase can be clearly observed.



Costo et al. Langmuir 2012, 28, 178–185

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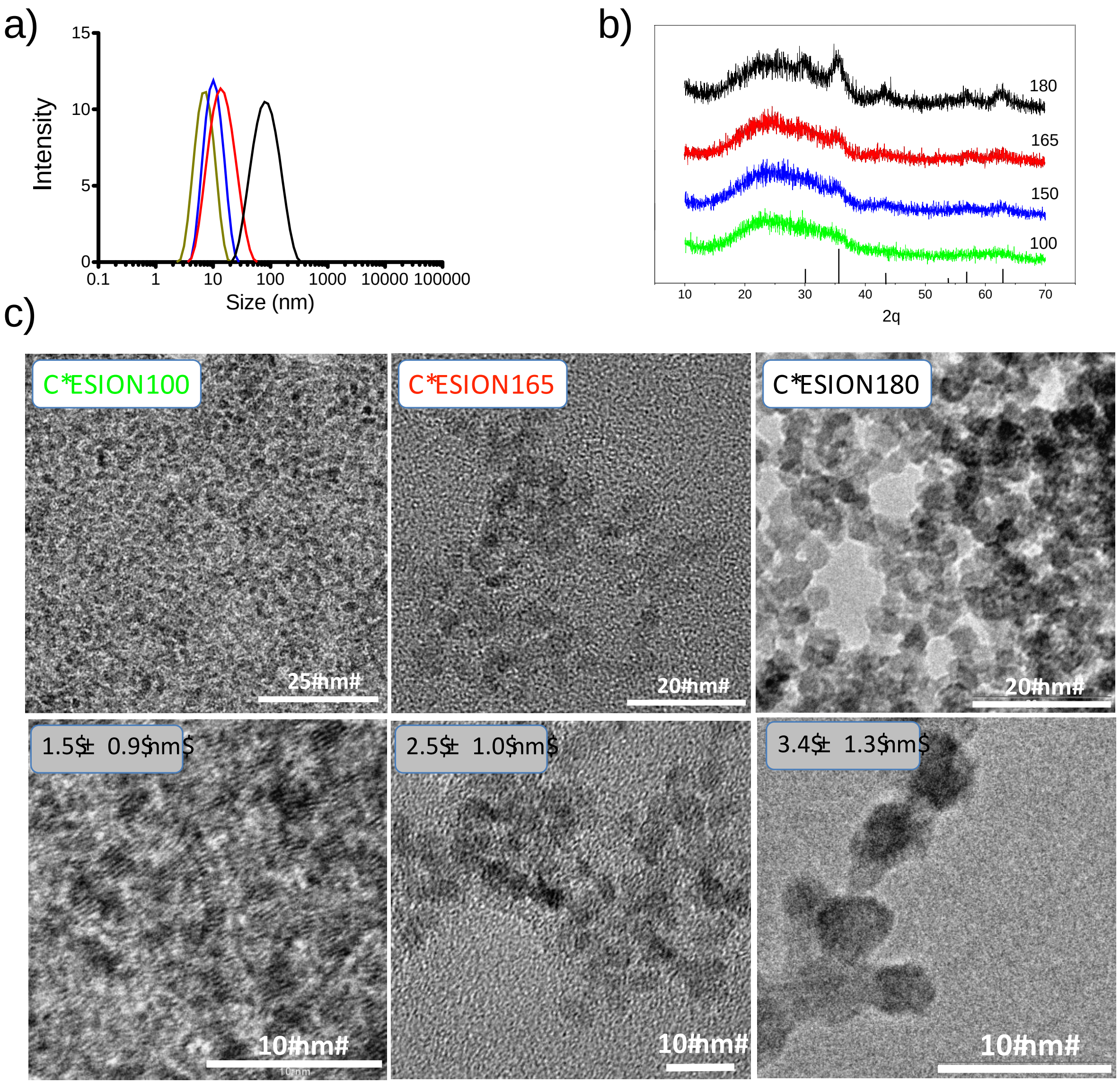
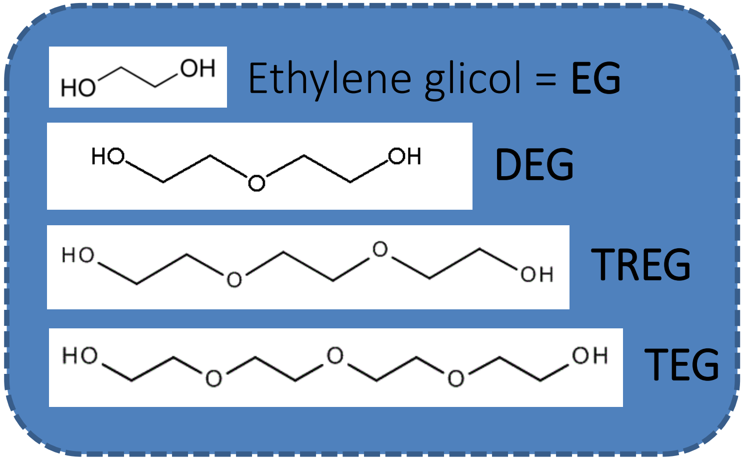
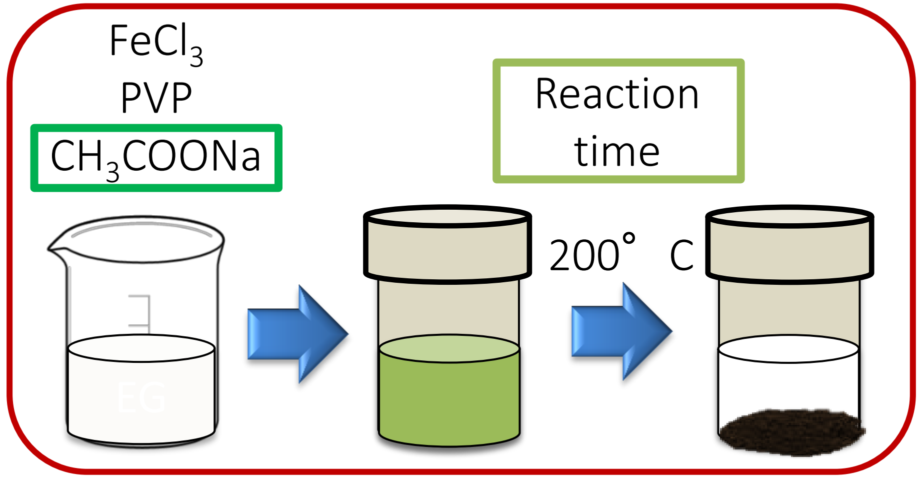
Microwave



a)
15
10
5
0
Intensity
0.1
1
10
100
1000
10000
100000
Size (nm)
b)
180
165
150
100
10
20
30
40
50
60
70
2q
c)
C*ESION100
C*ESION165
C*ESION180
25#nm#
20#nm#
20#nm#
1.5$± 0.9$nm$
2.5$± 1.0$nm$
3.4$± 1.3$nm$
10#nm#
10#nm#
10#nm#

Polyol Mediated Process





- ✓ $\uparrow \varepsilon_r, \uparrow T_B$
- ✓ Hydrophilic coating
- ✓ Easily dispersed in aqueous media and other polar solvents

- ✓ $\uparrow T$ favors higher crystallinity and $\uparrow M_s$
- ✓ Narrow Size distribution

Other reagents present:
Iron precursor, precipitator, surfactant/stabilizer

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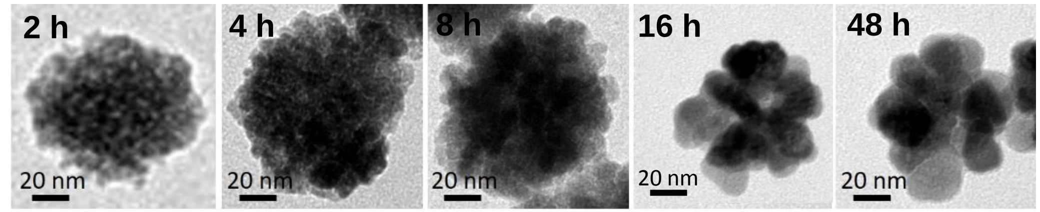
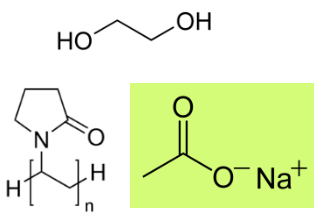
Polyol Mediated Process

VARYING THE CORE SIZE OF THE NANOFLOWER

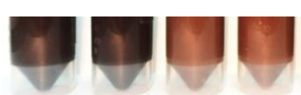


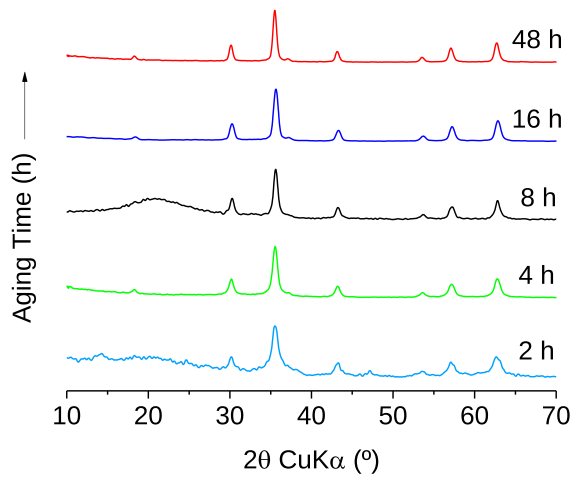
$FeCl_3$

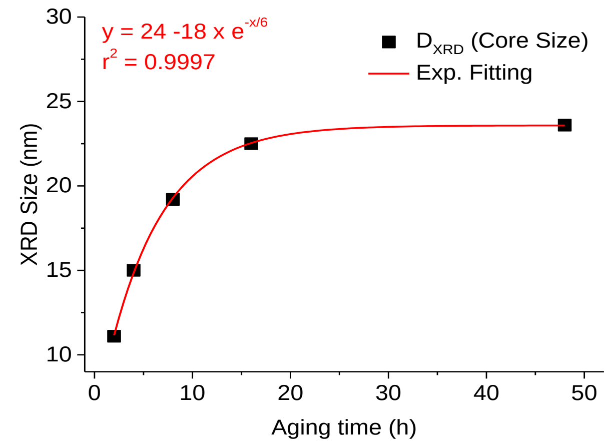
+

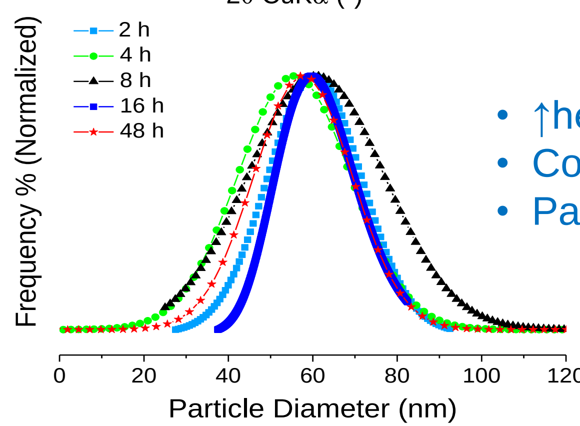


heating time (h)









- ↑heating time, ↑d_{XRD}
- Core grows exponentially over time
- Particle size remains unchanged

H. Gavilán et al. (2017) "Formation mechanism of γ-Fe_2O_3 nanoflowers synthesized by polyol mediated process" (Submitted)

icmm

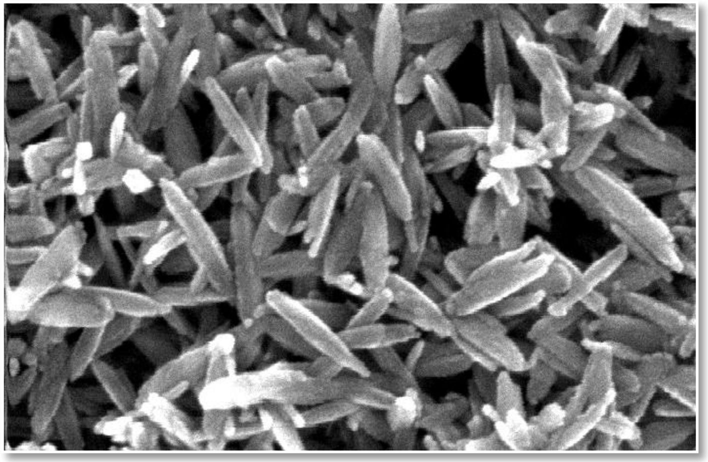
Shape control

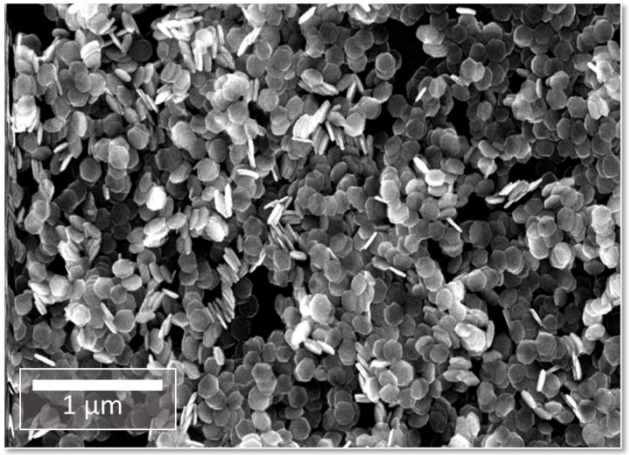
Induced anisotropy growth

Anisotropic growth along a particular direction => choosing appropriate capping reagents (e.g. surfactant and additives)

The density of Fe atoms on the surface of {100} and {110} is higher than that on the {001} facet. Phosphate and amine bond preferentially onto {100} and {110} facets through covalent bonding rather than on the {001} facet.

Phosphate and amine	Elongated nanoparticles
Silicate anions and citric acid (CA)	Discs or prismic



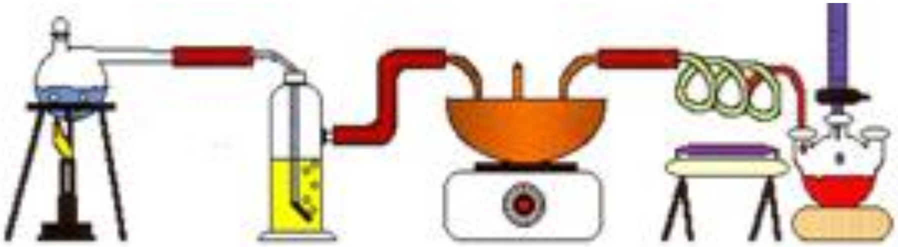


- There is an urgent for development of adequate **testing protocols and metrology standards** to assess the quality and hazard of, and exposure to nanomaterials.

- ***NanoMag* project** addresses this task in the field of magnetic nanoparticles for medical applications.

- We have to **defined and standardized ways of analysing these nanostructures**.

  ✓ Define the **relevant measurands**
  ✓ Describe the available techniques : their **limits, uncertainty**
  ✓ Summarize existing standards and develop **new standards**
  ✓ Provide **reference materials**



Wrapping up

- Thermal decomposition require :
  - Long reaction times and high temperatures
  - Control of the oxygen free atmosphere
  - Toxic reagents and byproducts
  - Particles are hydrophobic= need extra steps

- Hydrothermal/Solvothermal methods produce high quality nanoparticles with relatively lower reaction temperatures (<200ºC), relatively simple equipment and process. Polyol process allow using higher temperatures (~250ºC) and render hydrophilic nanoparticles.

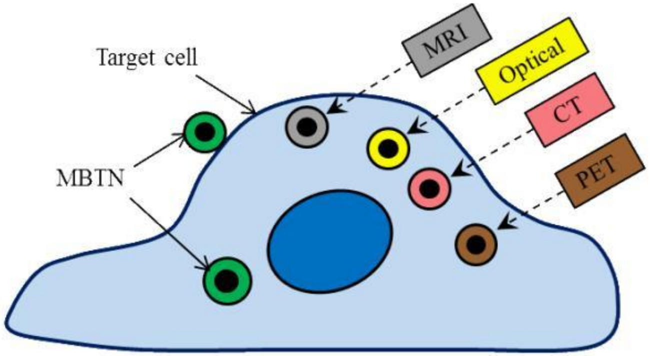
Toward the standardization of the synthesis of magnetic nanoparticles

- Homogeneous temperature distribution for large scale production
- Short reaction times

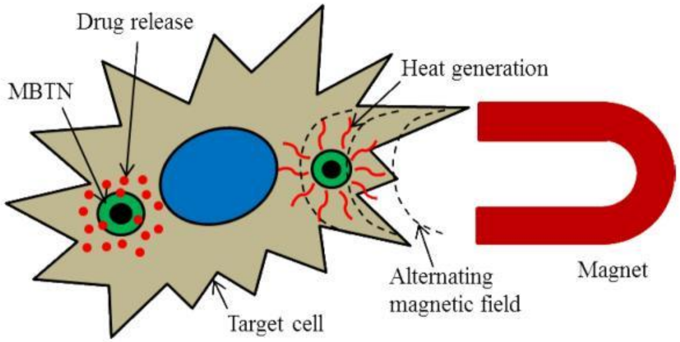
Summarizing

Magnetic nanoparticles could help to improve clinical practice in the treatment of cancer, most probably in synergy with other conventional treatments.

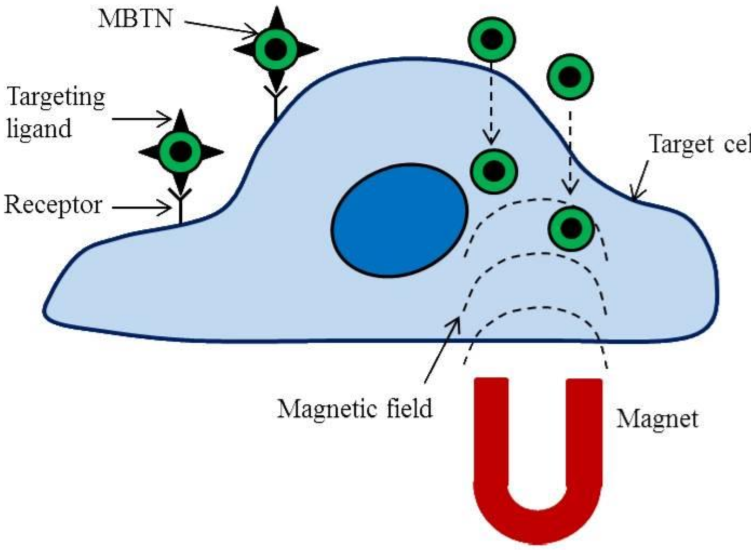
Imaging agents



Therapeutic agents



Magnetic nanoparticles advantages



Magnet assisted

Design and Application of Magnetic-based Theranostic Nanoparticle Systems, Aniket S. Wadajkar, et al. Recent Pat Biomed Eng. Apr 1, 2013; 6(1): 47–57

AKNOWLEDGEMENTS

Thank you!!!

CHARACTERISATION

Magnetic properties
Jana Vejpravova (A. of Science)
Daniel Niznansky (Charles U.)
Patricia de la Presa (IMA)

Heating efficiency
Dr. Gorka Salas (IMDEA Nanoscience)
Dr. Francisco Terán (IMDEA Nanoscience)
Prof. Marc Respaud (INSA, Toulouse)
Prof. Ingrid Hilger (UHJ, Jena)

NMR imaging
Dr. Fernando Herranz (CNIC)
Prof. Jesús Ruiz Cabello (CNIC)

BIOMEDICAL
Cell therapy
Dr. Angeles Villanueva (UAM)
Dr. Domingo Barber (CNB)

MODELLING
Dr. Oksana Chubykalo-Fesenco (ICMM)

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- Scientific and industrial challenges of developing nanoparticle-based theranostics and multiplemodality contrast agents for clinical application, Yì Xiáng J. Wáng, Jean-Marc Idée and Claire Corot, Nanoscale, 2015, 7, 16146.

- Chemical Synthesis and Assembly of Uniformly Sized Iron Oxide Nanoparticles for Medical Applications, Daishun Ling, Nohyun Lee, and Taeghwan Hyeon, Acc. Chem. Res. 2015, 48, 1276−1285.

- Magnetite nanoparticles for cancer diagnosis, treatment, and treatment monitoring: recent advances, Materials Today Volume 19, Number 3 April 2016

- Free course on magnetic nanoparticles for biomedical applications, **http://www.npl.co.uk/commercial-services/products-and-services/training/e-learning/magnetic-nanoparticles-standardisation-and-biomedical-applications/**

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