Nano Innovation 2016
Rome, 20-23 September
Conference & Exhibition

Functionalized Magnetic Nanoparticles: A Useful Tool in the Early Diagnosis and Therapy of Tumors

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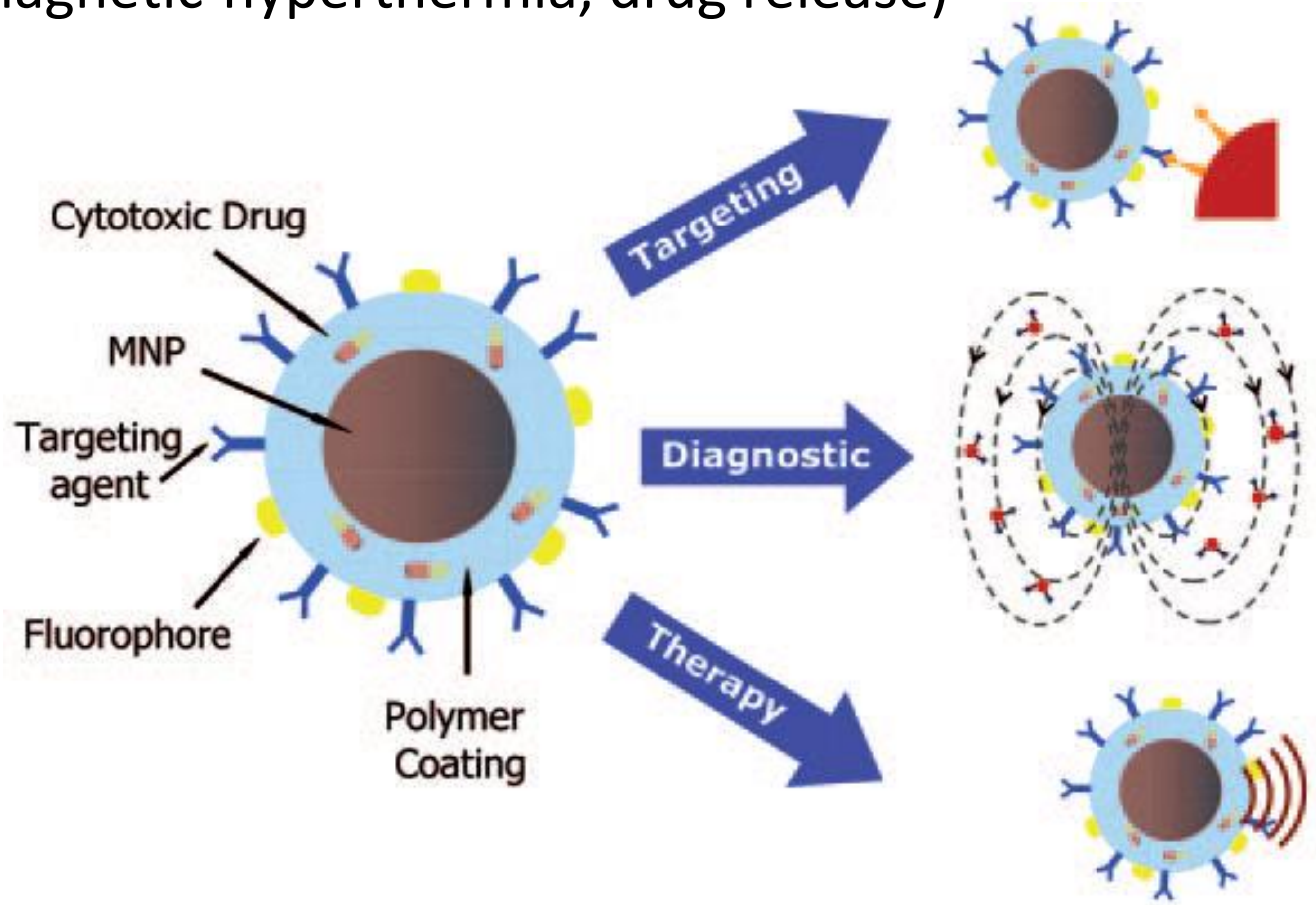
INSTM Italian National Consortium of Material Science and Technology

The ideal nanosystems

Single **theranostic** nano-objects

Diagnostics (MRI , Optical Imaging)

Therapy (magnetic-hyperthermia, drug release)



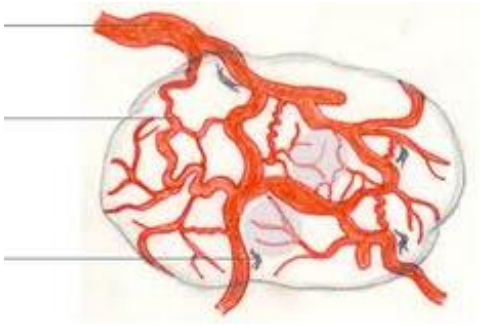
HYPERTHERMIA = extra heating of the human body or of a part of it

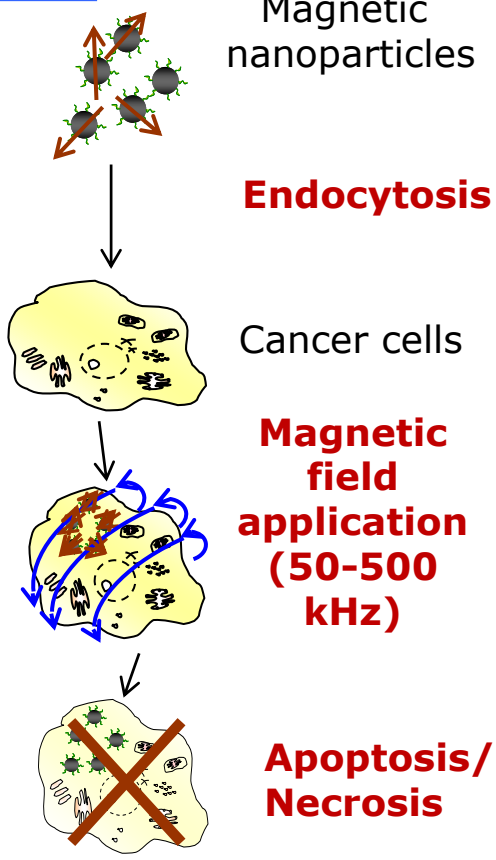
MAGNETIC FLUID HYPERTHERMIA (MFH)

hyperthermia assisted by magnetic nano-sized particles

Advantages:

- reduction of side effects because of low electric field component (eddy current)
- whole body irradiation by external application
- strong localization
- *Theranostic effects*:

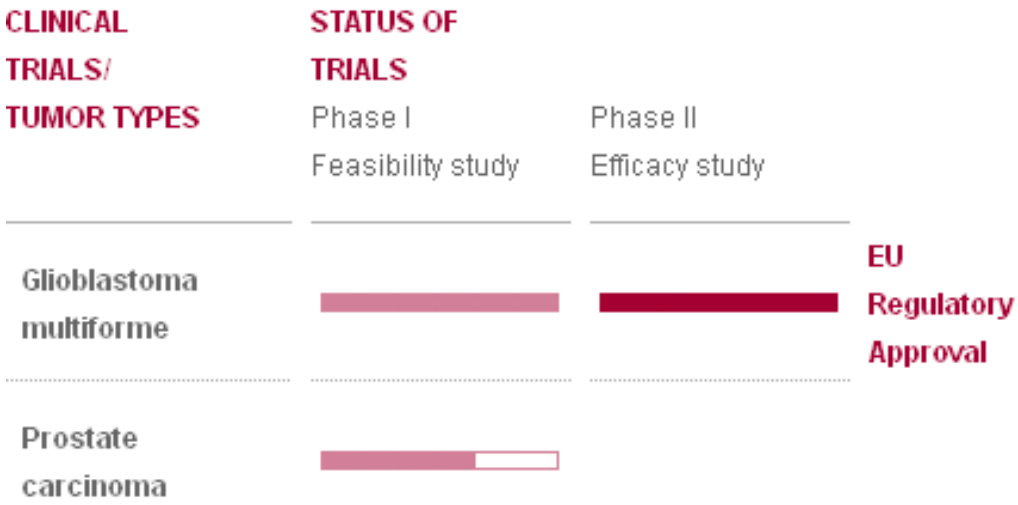




Temperature in 41-45°C range for 30min or higher for thermoablation

The feasibility of MFH has been already demonstrated in clinical tests

(MagForce Charité Hospital, Berlin, Germany and Magforce USA)



Phase III Clinical Trial (2014/03/25, 309 patients)
"efficacy and safety of NanoTherm® monotherapy and NanoTherm® in combination with radiotherapy versus radiotherapy alone in recurrent/progressive glioblastoma"

Indication	Patients
Glioblastoma Multiforme	80
Prostata Cancer	29
Pancreatic Cancer	7

NanoActivator® devices are installed in Berlin, Münster, Kiel, Cologne and Frankfurt

MFH & Radiotherapy Results (MagForce)

- **OS-2:** 13.4M (6.2M in previous Radio & Chemotherapy study)

  ➡ **overall increase in survival > 7.2M**

- **OS-1:** 23.2M (14.6M in previous Radio & Chemotherapy study)

  ➡ **overall increase in survival > 8.6M**

- **Few not severe side effects**

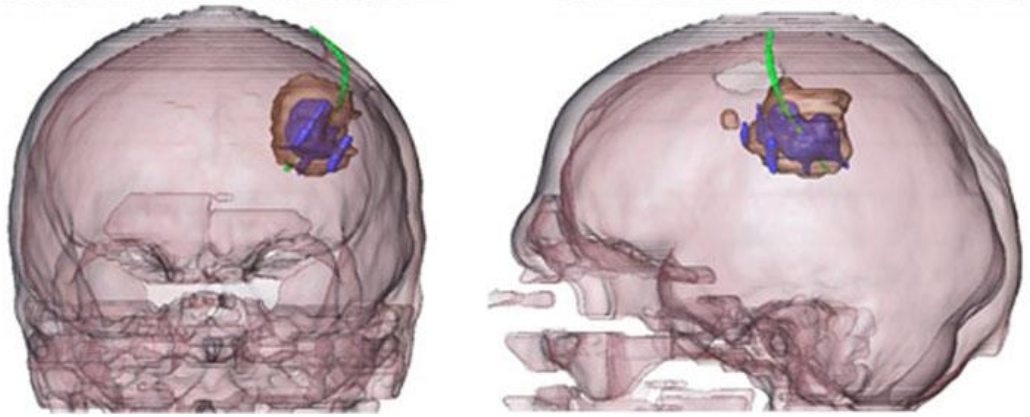
OS-2: overall survival after diagnosis of first tumor recurrence

OS-1: overall survival after primary tumor diagnosis

f=100 kHz
H=2-15 kA/m

12 nm amino-silane coated Fe_3O_4 NP

35 mg/cm^3 tumor
T_{ave}=51 °C



3-D reconstruction of fused MRI and CT showing the tumor (brown), magnetic fluid (blue) and thermometry catheter (green)

What is missing in MFH?

Too high doses of magnetic material are required

Particle surface functionalization for targeting

Increase of the power losses

- *dose reduction*
- *address small tumors*

- *lower amount of material*
- *smaller NPs (longer circulation time life)*
- *large SAR to treat smaller tumors*

Materials

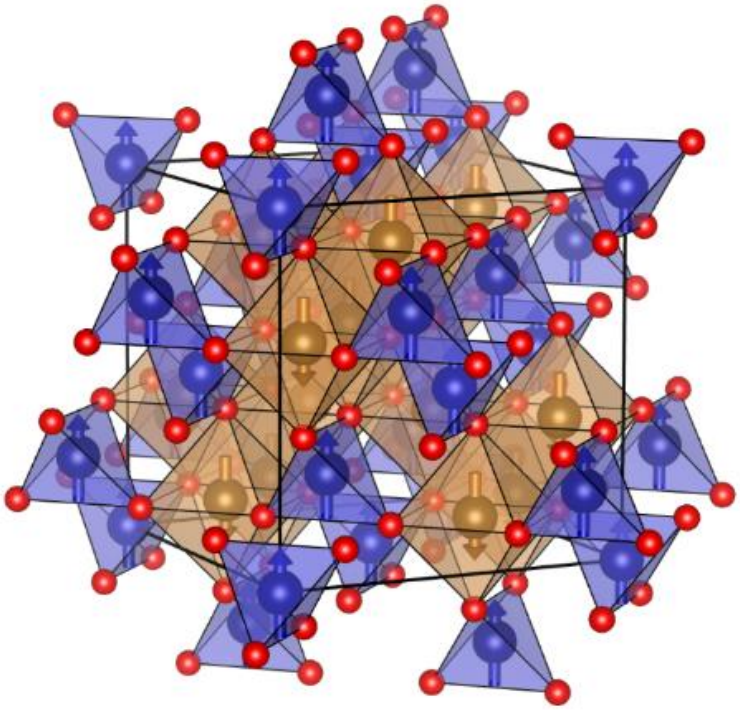
Spinel Ferrite

$(M_{1-i}Fe_i)^T[M_iFe_{2-i}]^OO_4$

i = inversion degree

i = 0 normal spinel

i = 1 inverted



Unit cell: $(AB_2O_4)_8$

cubic closed-packed array of 32 oxide ions forms 64 T_d and 32 O_h cavities

- **Ferrimagnetic** behavior due to the AF coupling of moments in T_d and O_h sites

The magnetic properties can be drastically modified by simply replacing, either completely or partially, metal ions or by modifying the inversion degree without affecting the crystal structure.

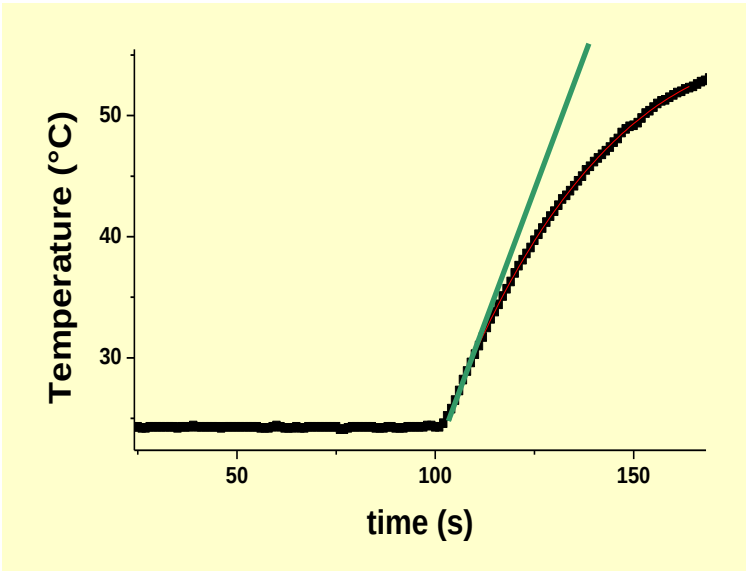
The effect of the size: Rhamnose coated Fe_3O_4 MNPs

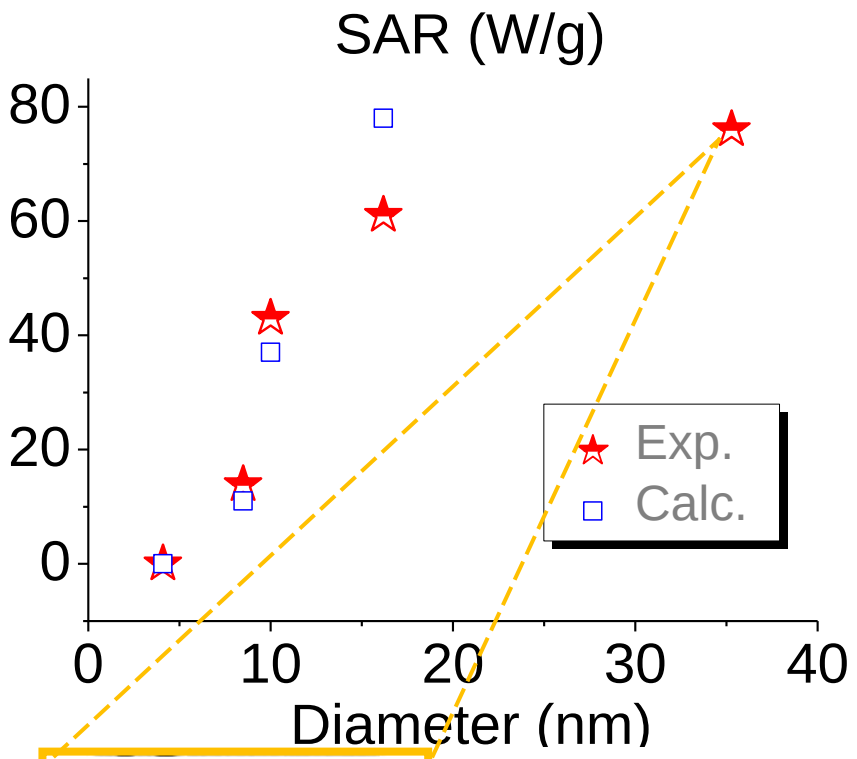
SAR (Specific Absorption Rate)

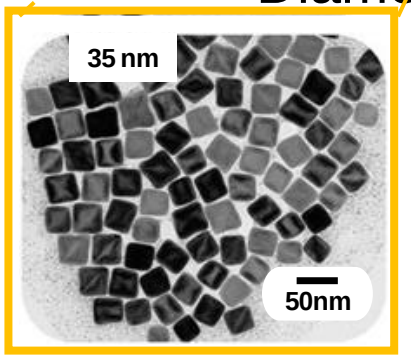
P/d = C ΔT/ Δt [W/gr]

P = absorbed power, d = density

$$P = \frac{\sum_i m_i C_{si}}{m_{Metal}} \cdot \Delta T / \Delta t$$







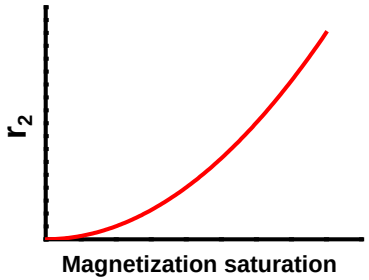
- Size in the transitional regime between SP and FM
- Interparticle interactions

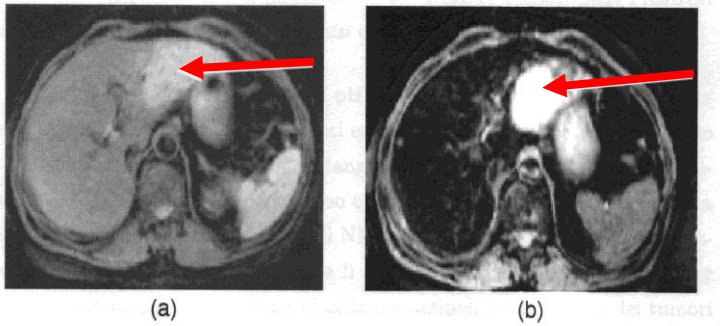
Higher M_S produces higher hyperthermal efficiency … but also

$$SAR \approx f H_0^2 M_S^2 V \frac{\omega\tau}{[1+(\omega\tau)^2]}$$

Higher MRI Relaxivity

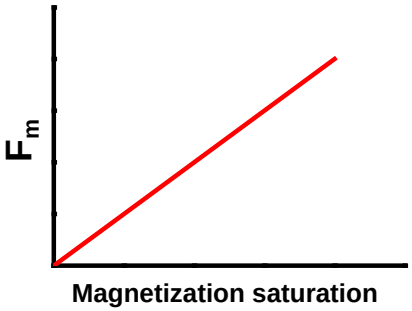
$$r_2 = 4\gamma^2\mu_0^2 M_S^2 d^2/405D$$

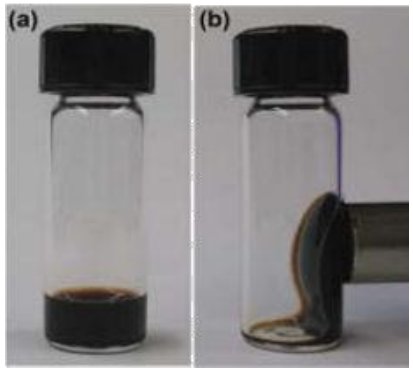




Higher Translational Attractive Force

$$F_m = V(M \cdot \nabla)B$$

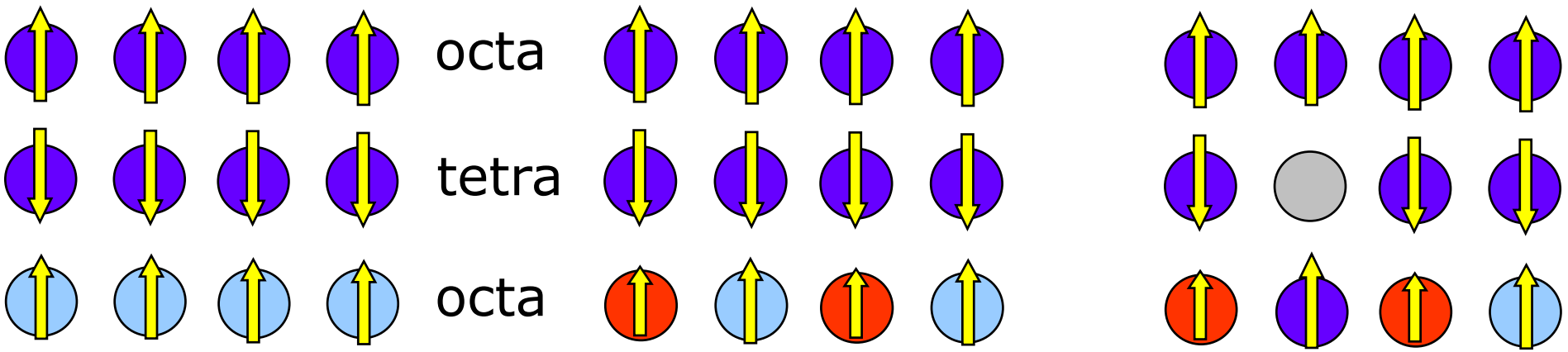




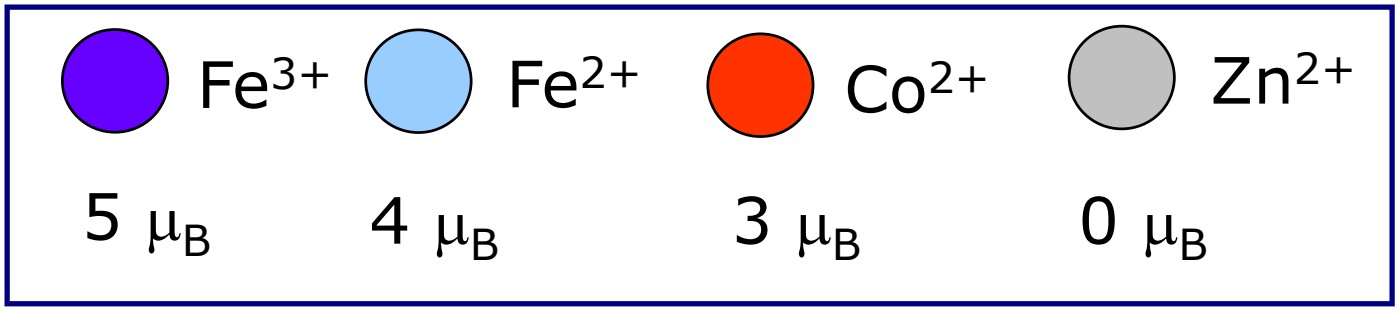
The effect of Zn doping

Magnetite $Co_xFe_{3-x}O_4$ $Co_xZn_yFe_{3-x-y}O_4$

Co doping *Zn doping*



$M = 4\ \mu_B$ $M = (4 - x)\ \mu_B$ $M = (4 - x + 6\ y)\ \mu_B$

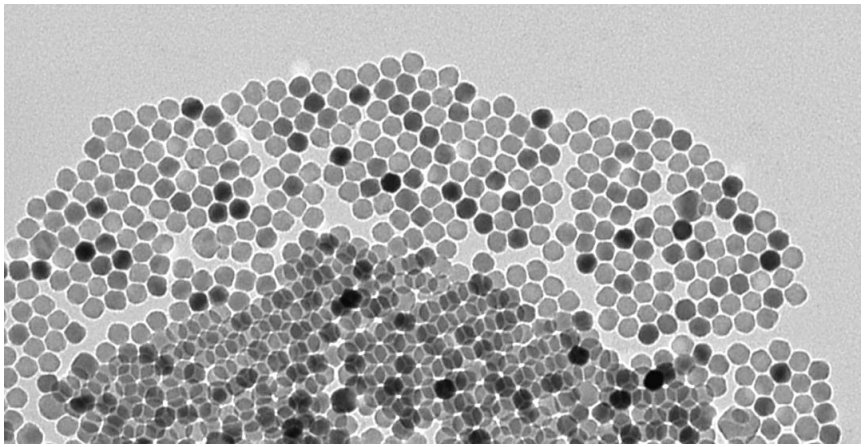


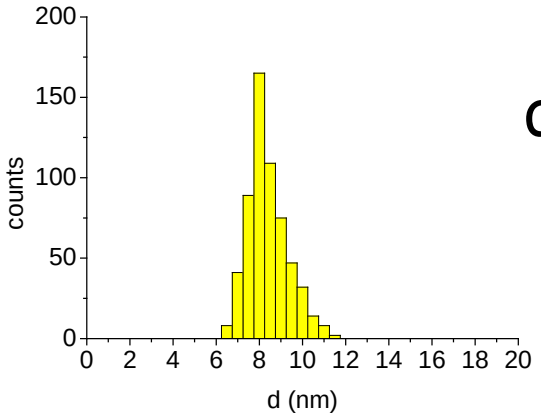
Increasing the magnetic moment

Doping with the diamagnetic Zn^{2+} ion

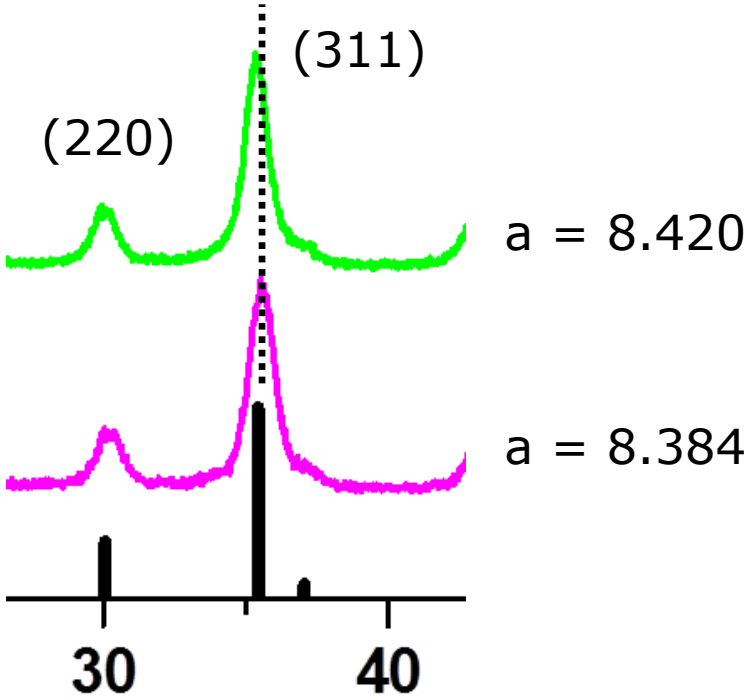
$Co_{0.57}Zn_{0.13}Fe_{2.3}O_4$

$$SAR \approx f H_0^2 M_S^2 V \frac{\omega\tau}{[1+(\omega\tau)^2]}$$

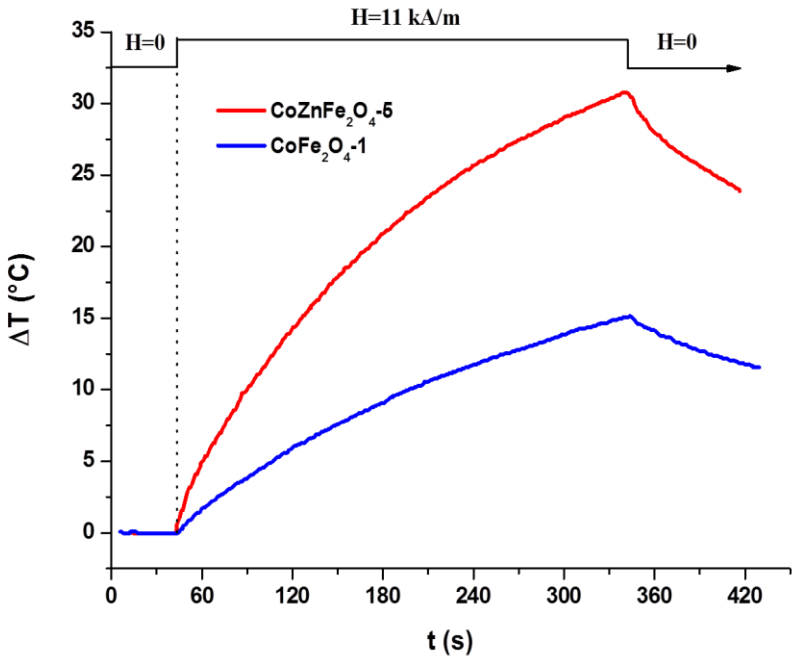


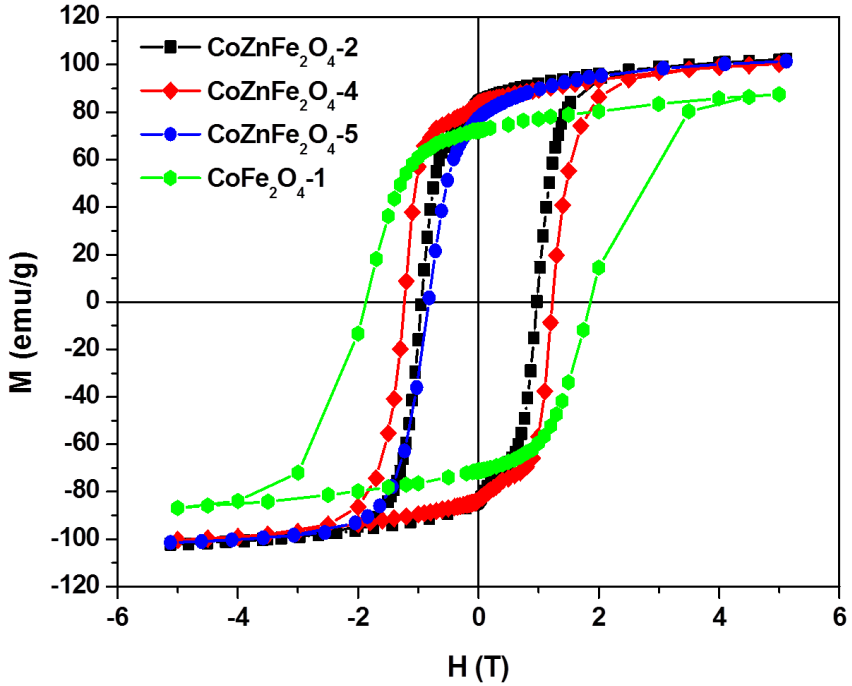


d = 8.2 nm



The effect of the Zn: $Co_{0.57}Zn_{0.13}Fe_{2.3}O_4$

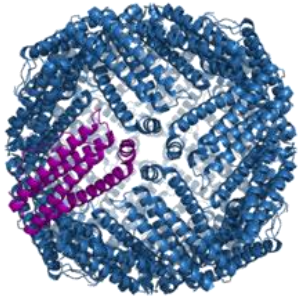




$Co_{0.61}Fe_{2.19}O_4$ **SAR 15.9 W/g** M_S=87.5 emu/g

$Co_{0.57}Zn_{0.13}Fe_{2.30}O_4$ **SAR 47.1 W/g** M_S=101.3 emu/g

Nanoparticles mineralized in ferritin

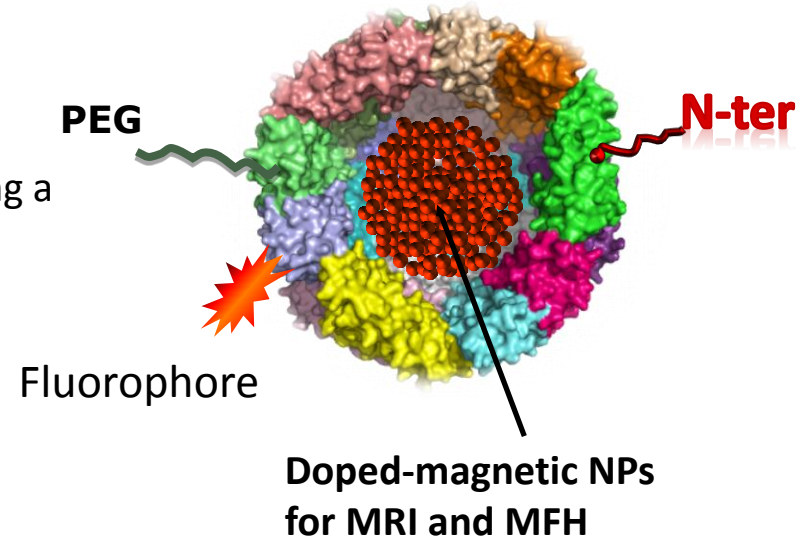


Ferritin (Ft) is an ubiquitary protein
✓24 subunits assembled in a **cage-like architecture**
✓**internal cavity of 8 nm** diameter
✓external diameter of **12 nm**
→ **Involved in iron homeostasis (iron sequestration and storage)**

→**natural system** that can be **finely tailored** for the realization of theranostic applications
- ✓ possibility of **mineralizing different inorganic materials in the Ft cavity**
- ✓ genetical and chemical **modifications on the protein surface**

Several advantages for biomedical applications:

- Naturally tailored for iron sequestration and NPs storage
- Adequate size to freely circulate in the body avoiding a rapid clearence by kidney
- High thermal and pH stability
- Naturally monodispersed
- Stability against aggregation

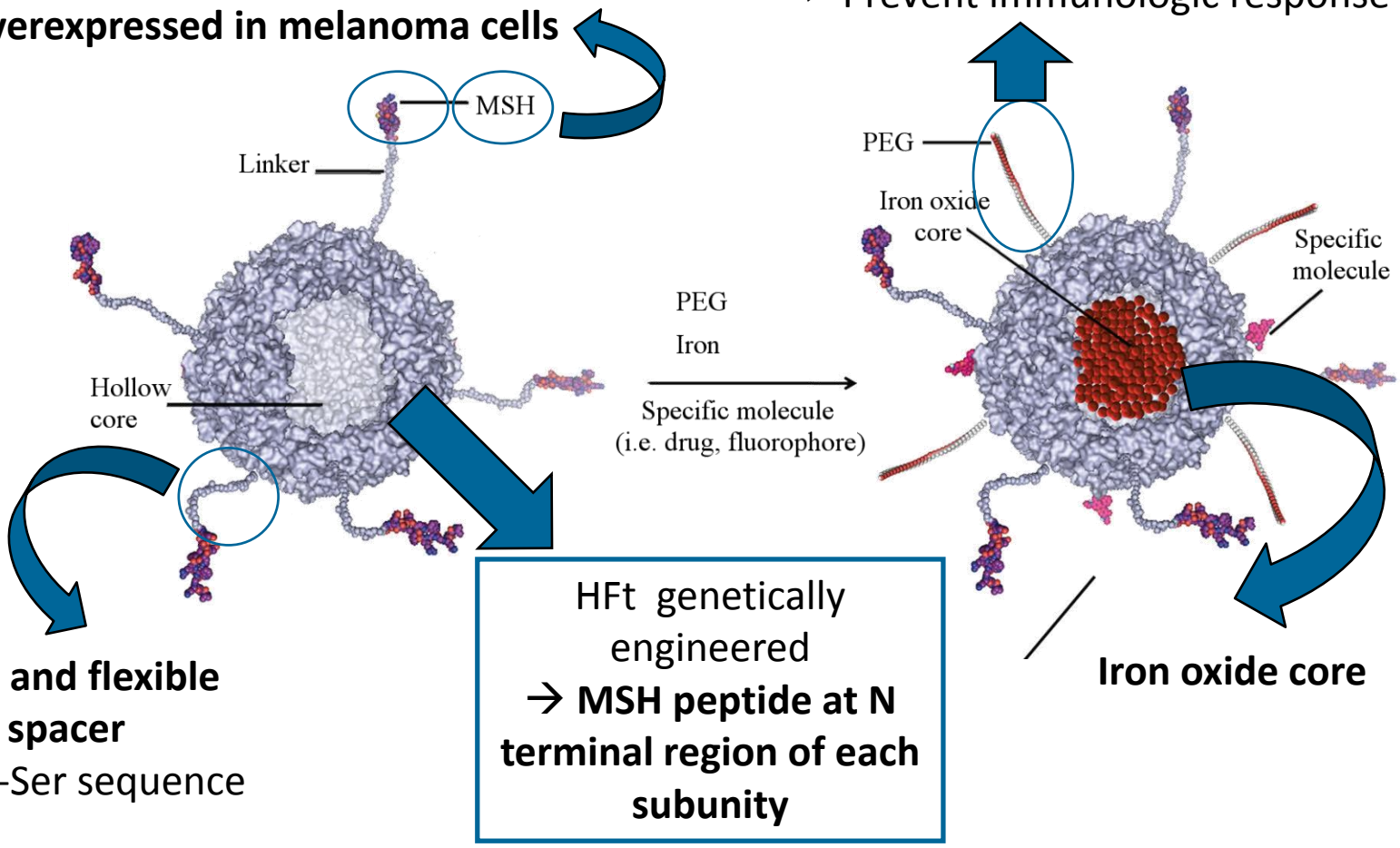


Human H chain Ft (HFt) →Potential low or null immunogenicity!

Design of a theranostic platform: HFt-MSH NPs

MSH = α-melanocyte-stimulating hormone peptide
→**binds to melanocortine receptors** that are **overexpressed in melanoma cells**

Role of PEG:
✓ Shield to non specific uptake
✓ Grant free circulation in the bloodstream
✓ Prevent immunologic response



Inert and flexible spacer
→ Gly-Ser sequence

HFt genetically engineered
→ **MSH peptide at N terminal region of each subunity**

Iron oxide core

Dr. P. Ceci *C.N.R. Inst. of Molecular Biology and Pathology, Dept. of Biochemical Sciences', La Sapienza, Univ.of Rome*

Optimization for magnetic fluid hyperthermia

Iron oxide NPs@HFt-MSH

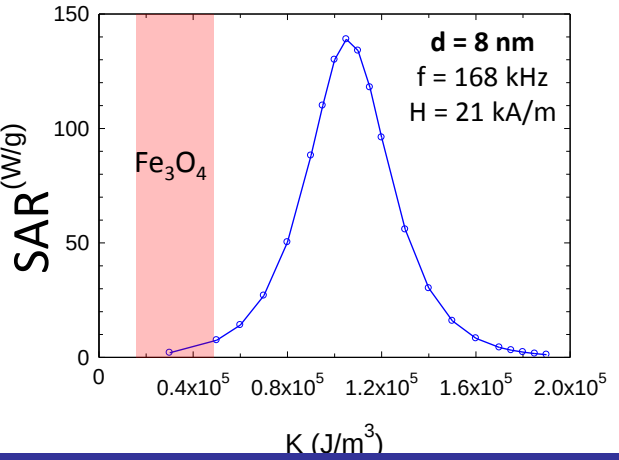
✓Promising candidate as MRI–CA and drug delivery
✓Excellent targeting properties
✓ High biocompatibility

× Constrains on size: **maximum 8 nm**, too low in order to observe hyperthermic efficiency for magnetite

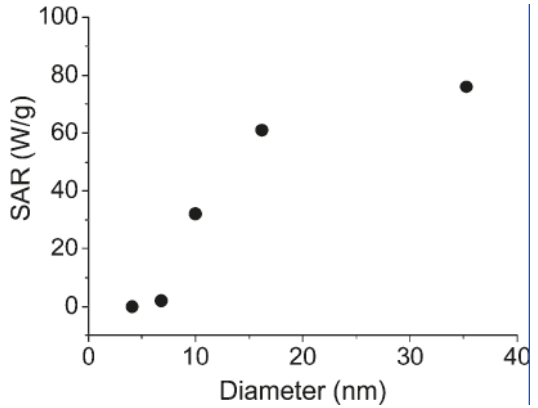
How to enhance the hyperthermic efficiency?

$$SAR \approx f \cdot H_0^2 \cdot M_S^2 \cdot V \cdot \frac{\omega\tau}{1+(\omega\tau)^2} \qquad \tau = \tau_0 \exp(KV/k_BT)$$

Increasing magnetic anisotropy

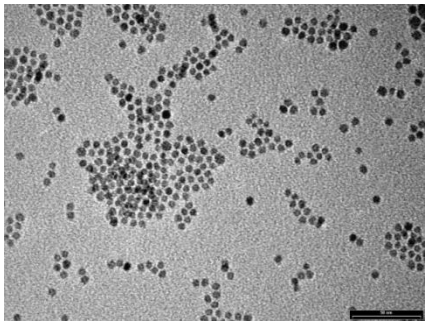


Increasing mean NPs size



L. Lartigue; C, Sangregorio *et al. J. Am. Chem. Soc.* **2011**, *133*, 10459

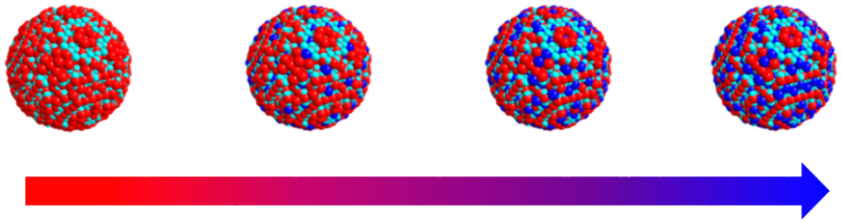
Increasing the magnetic anisotropy: Co doping



Replacing of divalent iron with Co^{2+}

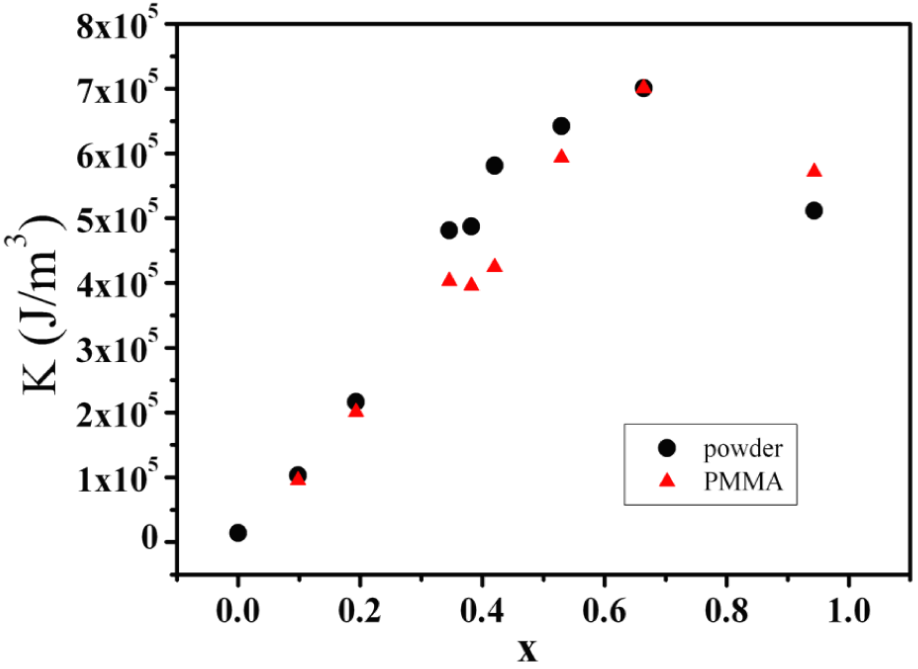
K_{bulk} of cobalt ferrite ca. 20 times larger than magnetite

$Co_xFe_{3-x}O_4$ NPs



Co content

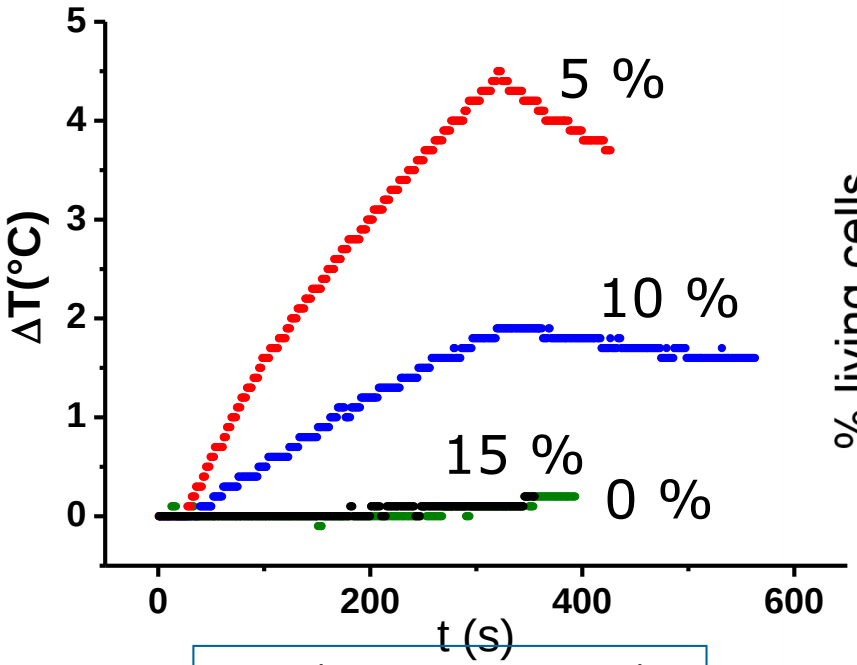
Strong increase of the magnetic anisotropy on Co substitution
→even for small Co %



Evaluation of Co doping effect on hyperthermic properties of HFt-MSH NPs

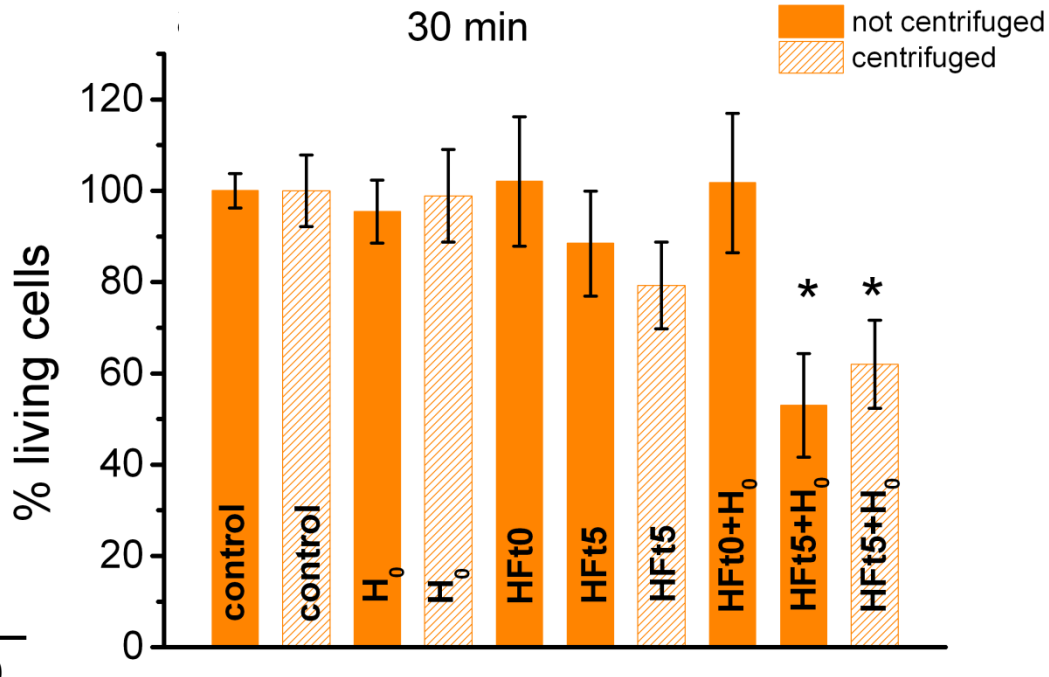
E. Fantechi *et al. J. Phys. Chem. C* **2012**, 116, 8261–8270
E. Fantechi *et al. J. Magn. Magn. Mat*. **2014** doi:10.1016/j.jmmm.2014.10.082

Synthesis and Characterization of Co-doped HFt-MSH NPs



1.2 w/w, 183 kHz, 12 kA/m

- ✓ Co doping strongly increases SAR **up to 5%**
- ✓ Above 5% an unxepected **decrease** is observed



183 kHz, 17.0 kA/m, 30 min

- ✓ No effect of magnetic field alone
- ✓ No effect observed for HFt_0% Co
- ✓ **Significant effect for HFt_5 % Co**

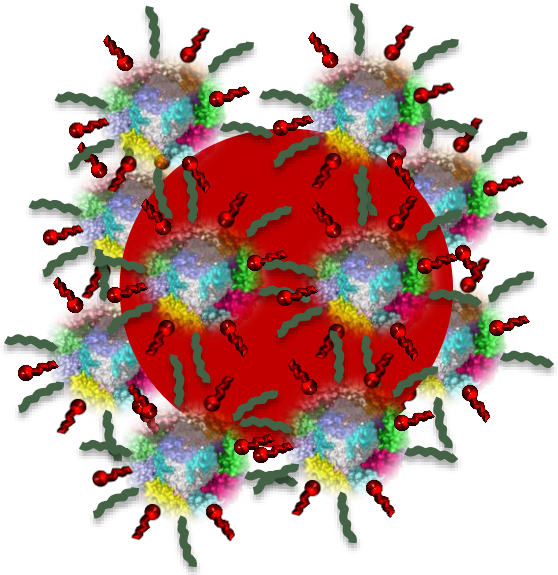
- ➢ Good hyperthermic cytotoxicity even with very low SAR
- ➢ High degree of cellular internalization

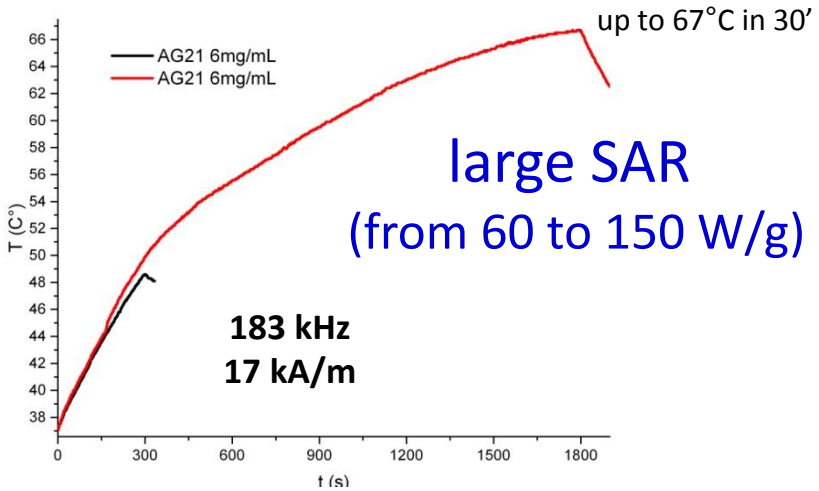
E. Fantechi *et al.* ACS Nano **2014**, *8*, 4705.

M. Zanardelli, Dr. L. Di Cesare Mannelli, Prof. C. Ghelardini
Dip. NEUROFARBA - Sez. Farmacologia e Tossicologia, Univ. di Firenze

Magnetite Nanoparticles functionalized with *apo*-HFt

- System with higher SAR with respect to Co-doped magnetite NPs mineralized in HFt
- Excellent properties of HFt retained
- Avoiding the use of Co^{2+}, potential issue of toxicity



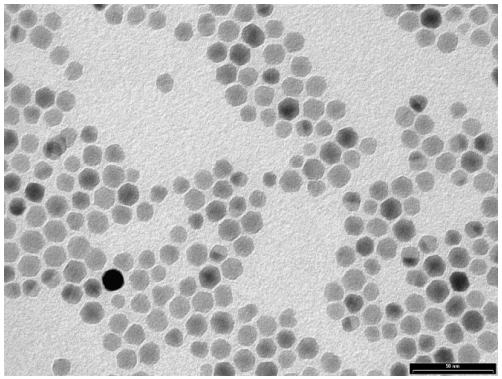


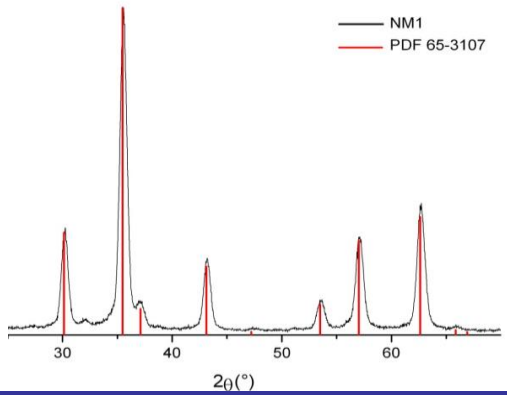
up to 67°C in 30'

large SAR
(from 60 to 150 W/g)

183 kHz
17 kA/m

Synthesis by surfactant-assisted thermal decomposition of organo-metallic precursors





Viability test

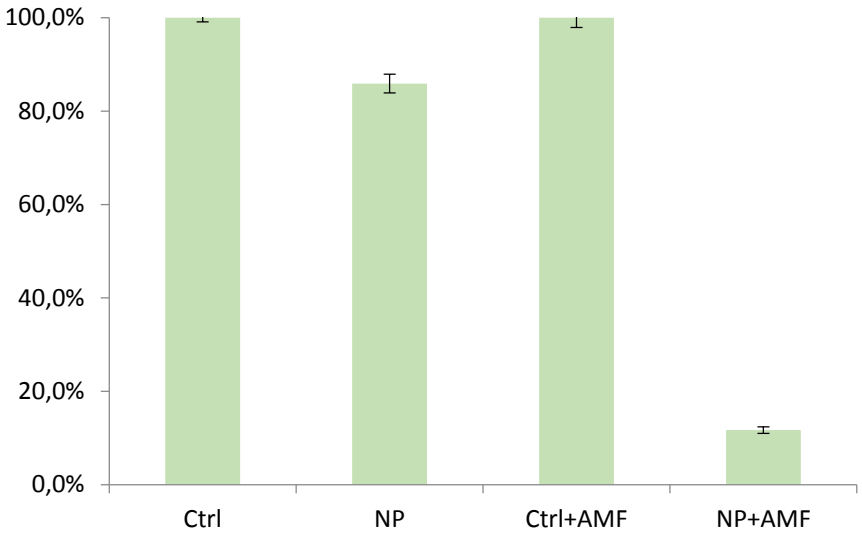
$T_{ext}^{o} = 42°C$

0.3% w/w

$f = 183kHz$

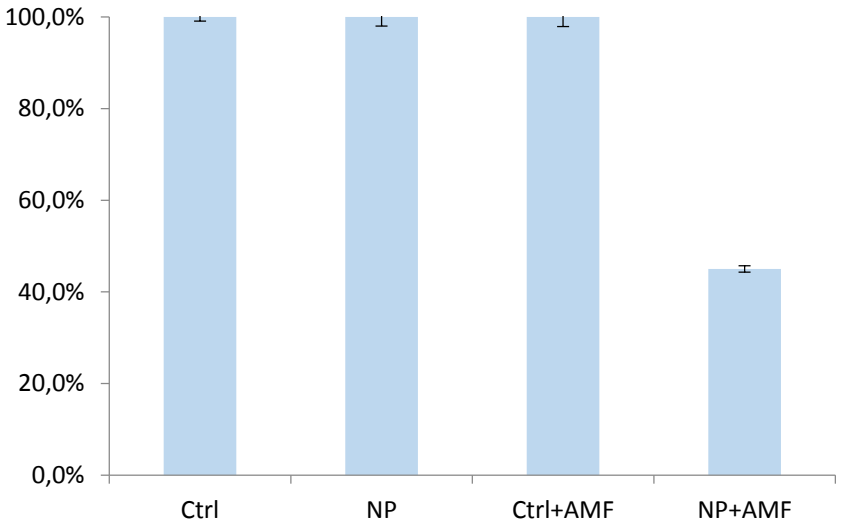
$H = 17KAm^{-1}$

$t = 60'$

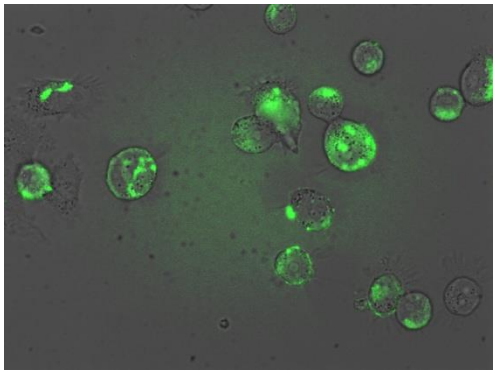


$T_{ext}^{o} = 39°C$

0.1% w/w

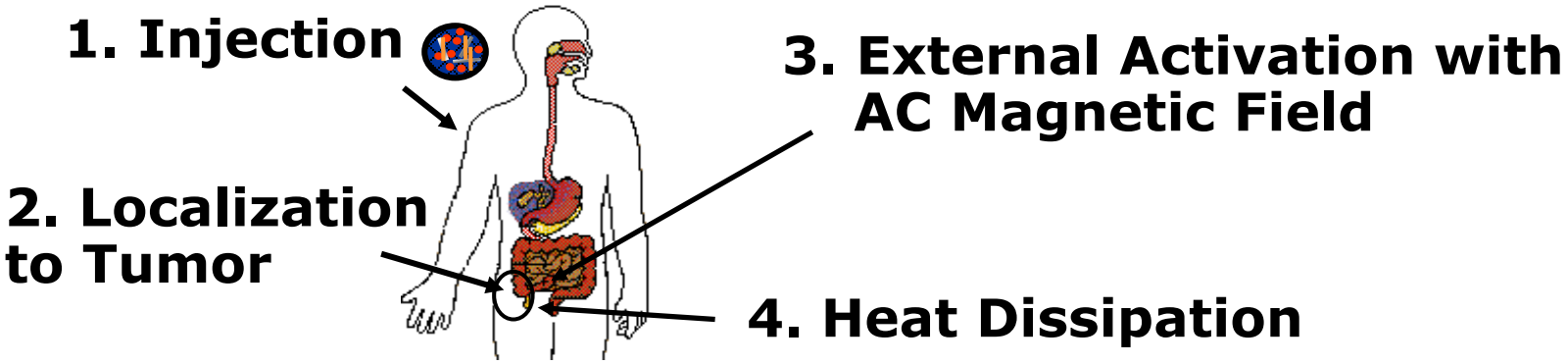


PC3 human prostate cancer lines



The ideal clinical application of MFH

- Create a versatile nanoplatform with multiple functionalities to target, image and treat cancerous cells



- **Toxicity**
- **Maximize SAR and minimize dose** (too high concentration of MNPs)
- **Nanoparticle delivery** to the tumour
- Intratumoral MFH **need of controlled homogenous distribution of MNPs in the tumor mass**
- Local **uniform heating diffusion** to tumour tissues
- **Study of heat flow** into surrounding tissue and through it
- **Elimination of necrotic material**
- **Combination therapies**
- Understanding **MNP – cell interactions**
- **Macrophages uptake/protein corona** (too big MNPs hydrodynamic diameter?)
- **MNPs fate**

Acknowledgements

INSTM-La.M.M.
Univ. di Firenze

C. Innocenti
E. Fantechi
M. Albino
A. Guerrini

Collaboration

A. Lascialfari (*Univ. Milano*)
P. Ceci (*CNR-IBPM*)
E. Falvo (*CNR-IBPM*)
M. Zanardelli (*Dip. Neurofarba – Univ. Firenze*)
L. Di Cesare Mannelli (*Dip. Neurofarba – Univ. Firenze*)
C. Ghelardini (*Dip. Neurofarba – Univ. Firenze*)
A Ponti *(CNR-ISTM)*
A. Ferretti *(CN-ISTM)*

Thank you for your attention

Financial support:

INSTM – Reg. Lombardia MAGNANO

MIUR
Project FIRB Rinaame

AIRC Projects No.
MFAG10545 & IG 11993