



EUROPEAN UNION



Multifunctional magnetic nanoparticles for biomedical applications

Dr. Raluca Maria Fratila

Nanotechnology and Apoptosis Group

ICMA: Materiales avanzados para los retos de la sociedad

Centro de Ciencias de Benasque Pedro Pascual
February 1 – 3, 2016



Nanotechnology and Apoptosis Group

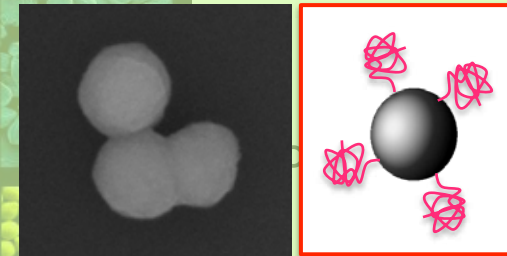
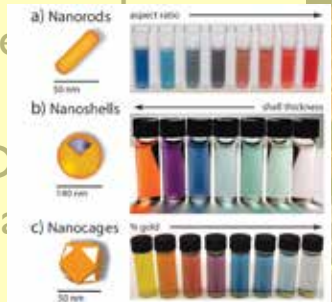
MAIN RESEARCH AREAS Scott Mitchell, Wednesday

AuNPs

Polymeric NPs

1. Development of new diagnostic tools based on nanotechnology: higher

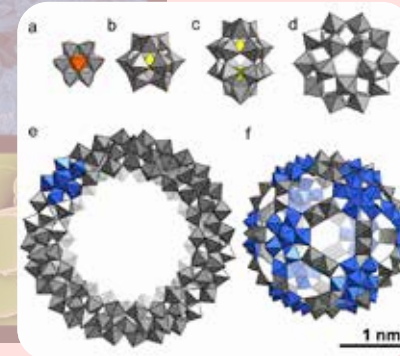
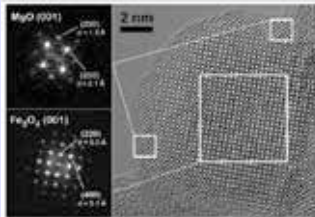
2. Drug delivery



Multidisciplinary group (Chemistry, Physics, Materials Science, Biology, Biochemistry, Biotechnology)

Magnetic NPs

Polyoxometalates

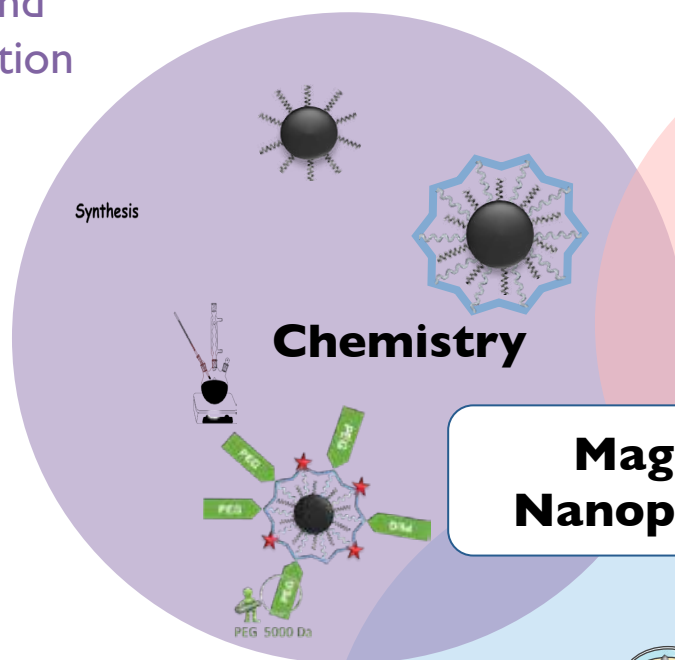


Multifunctional Magnetic Nanoparticles

Multidisciplinary work

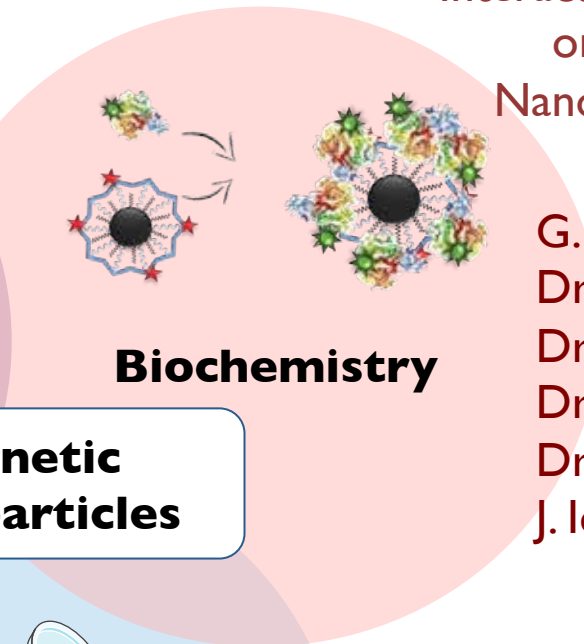
Synthesis and functionalization of MNPs

Dr. R. Fratila
V. Herrero
G. Stepien
Dr. M. Moros
M. Navascuez



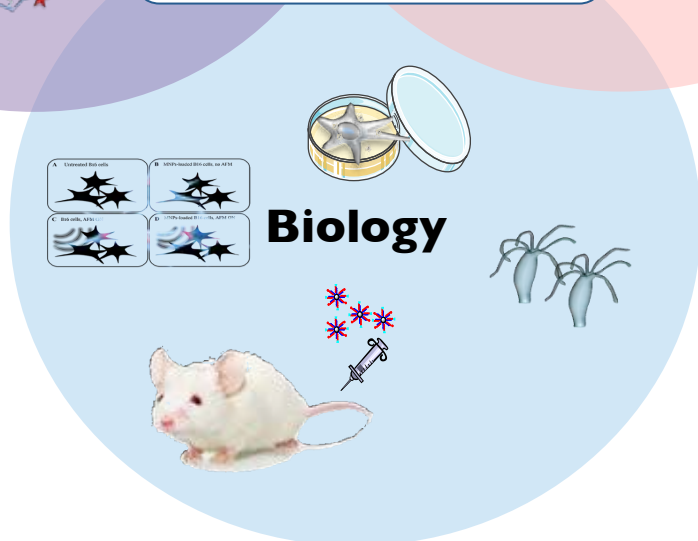
Interaction with living organisms
Nanotoxicology

G. Stepien
Dr. M. Moros
Dr. A. Ambrosone
Dr. L. Asin
Dr. R. Fratila
J. Idiago – PI²



Magnetic Nanoparticles

G. Stepien
Dr. M. Moros
Dr. L. Asin
Dr. A. Ambrosone

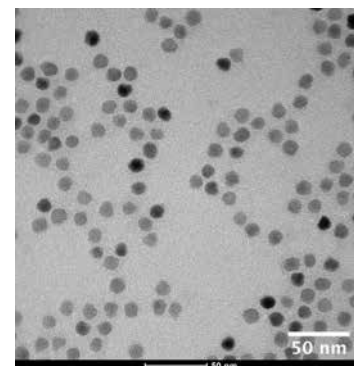
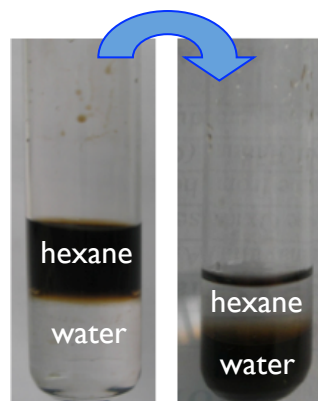
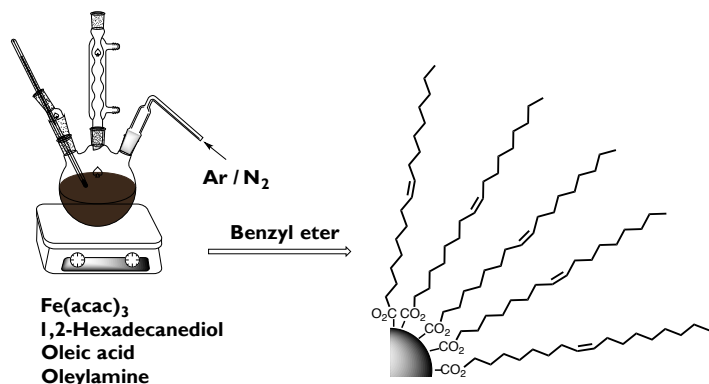


In vitro and in vivo applications

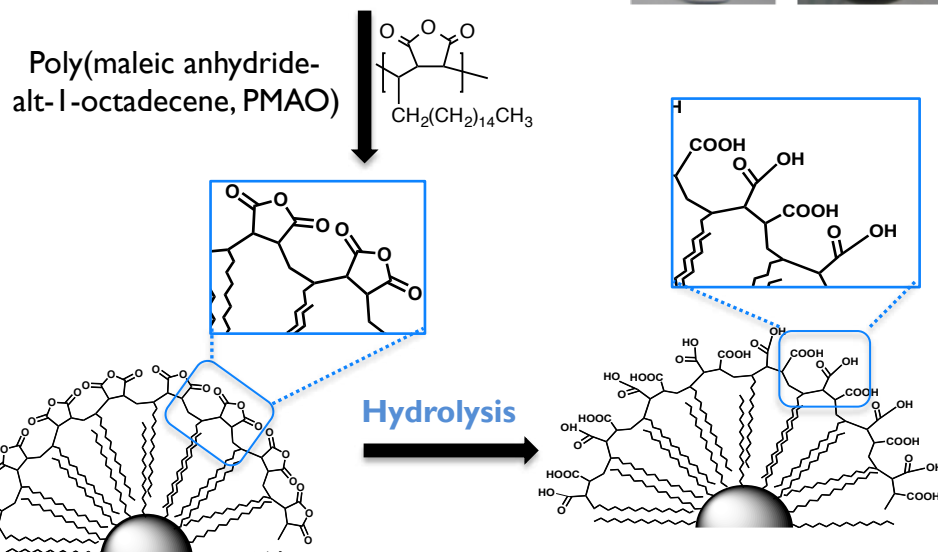
Magnetic Nanoparticles: Synthesis

Step 1: Seed-mediated growth method (thermal decomposition): **Hydrophobic MNPs**

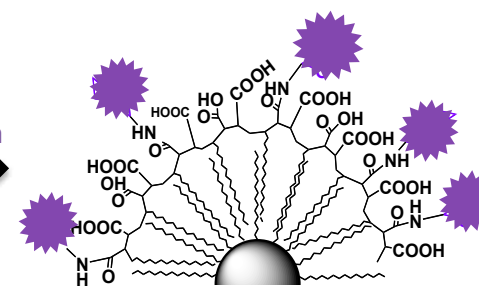
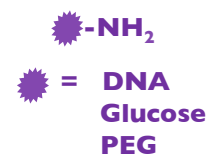
Step 2: Transfer to water with amphiphilic polymers: **Hydrophilic MNPs**



12 nm Fe₃O₄ MNPs



Functionalization



Sun et al., *J. Am. Chem. Soc.* **2002**, 124, 8204-8205
Sun et al., *J. Am. Chem. Soc.* **2004**, 126, 273-279
Moros et al., *Nanoscale* **2010**, 2, 1746-1755

Magnetic Nanoparticles: Hyperthermia applications

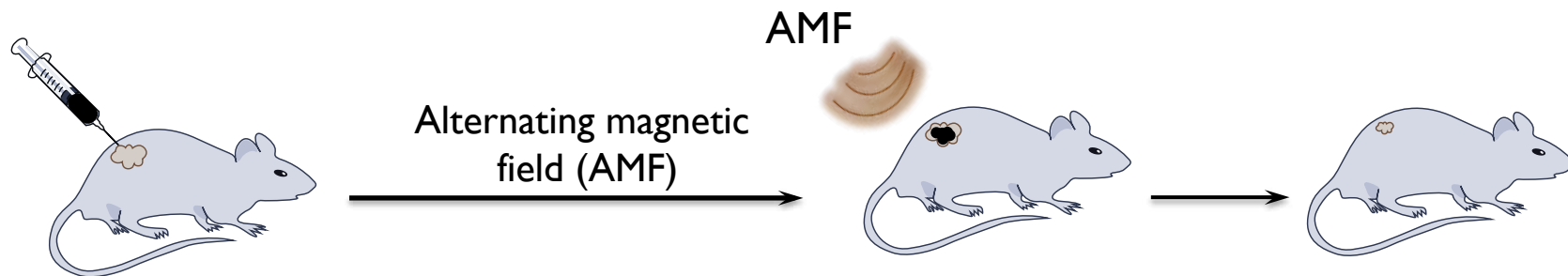
Magnetic hyperthermia

- Adjuvant therapy for cancer in combination with radio- and chemotherapy
- Heating of tissues to 41- 43 °C
- Tumour cells are more sensitive to heat than healthy ones



<http://www.magforce.de/en/produkte.html>

EU - Phase I and phase II clinical trials
(Glioblastoma, prostate and pancreas carcinoma)



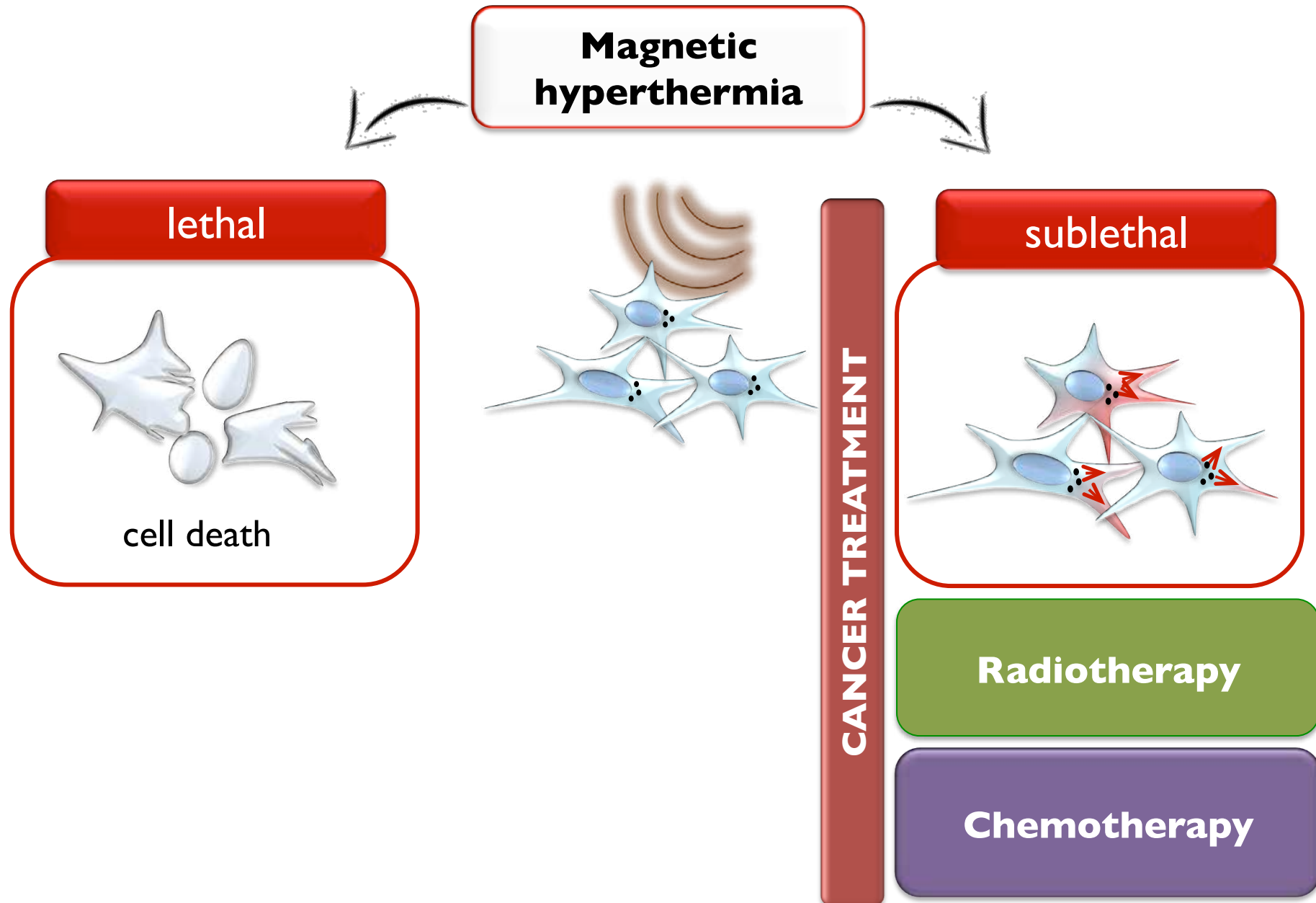
ADVANTAGES OVER CLASSICAL HYPERTHERMIA

- Magnetic field-guided delivery of MNPs
- Local heating = less damage to healthy tissues
- MNPs can be also used as drug carriers

Specific absorption rate:

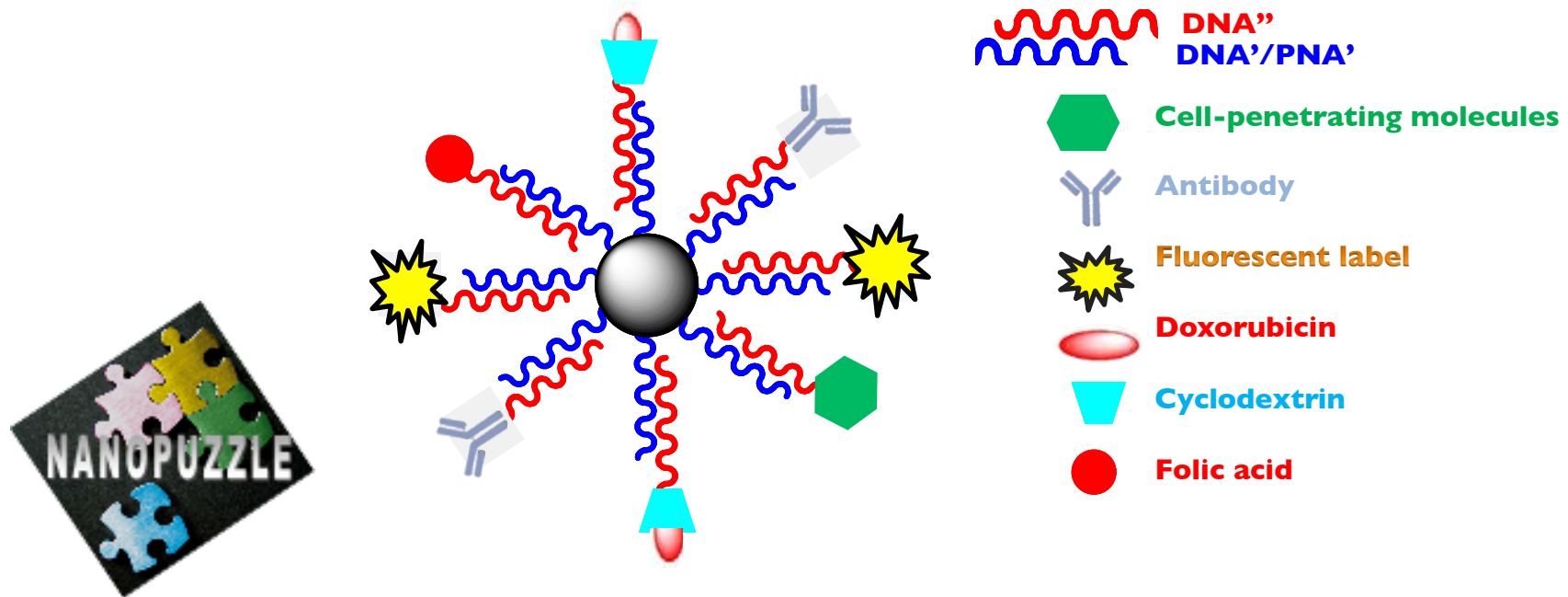
$$SAR = C/m (\Delta T/\Delta t) \quad (W \times g^{-1})$$

Magnetic Nanoparticles: Hyperthermia applications



Magnetic Nanoparticles: Hyperthermia applications

Multifunctional Magnetic Nanoparticles for Magnetic Hyperthermia Applications



ERC Starting Grant NANOPUZZLE - Multifunctional Magnetic Nanoparticles:
Towards Smart Drugs Design



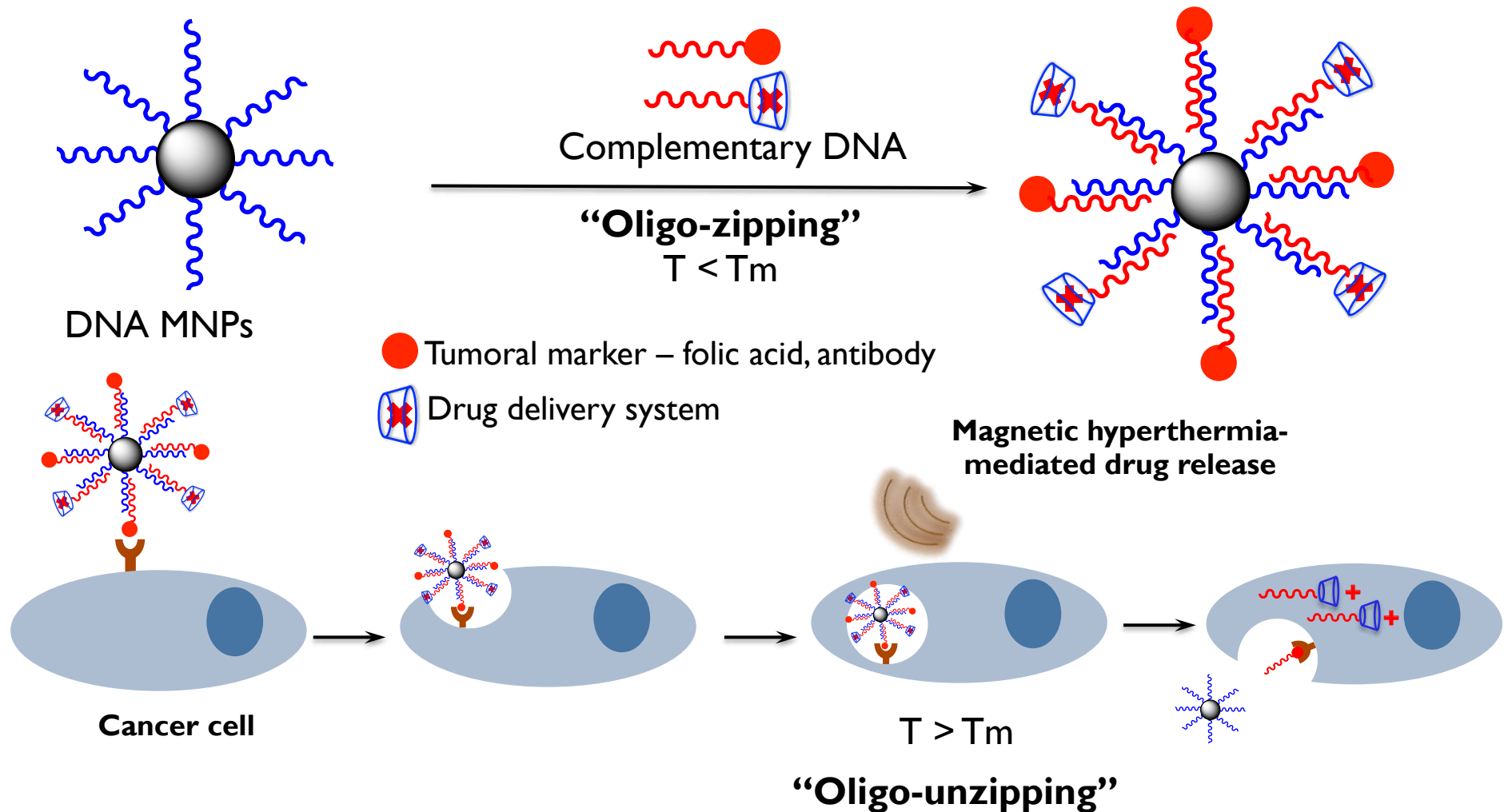
EUROPEAN UNION



European Research Council
Established by the European Commission

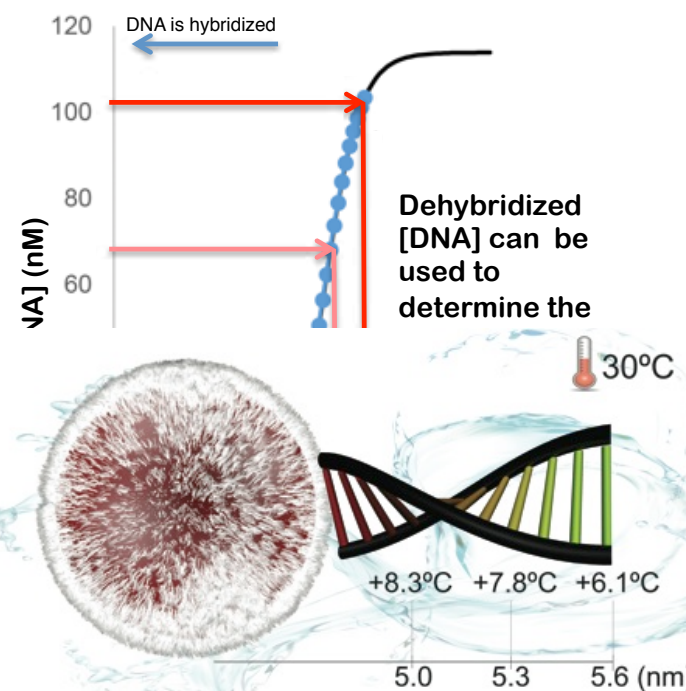
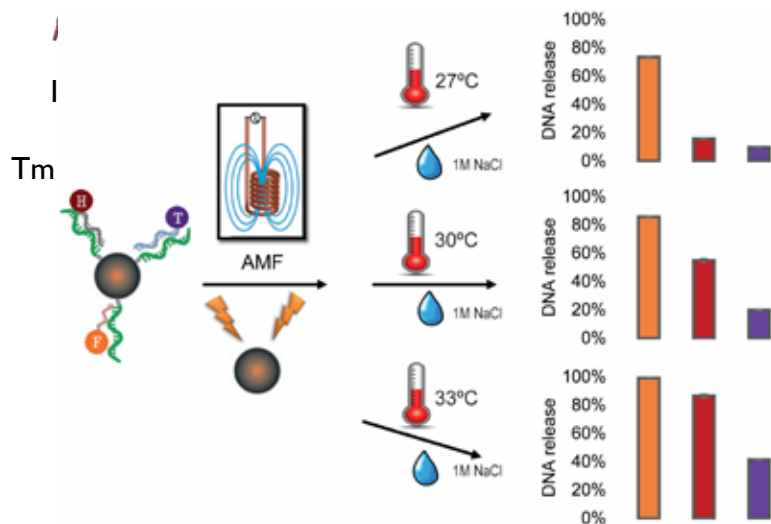
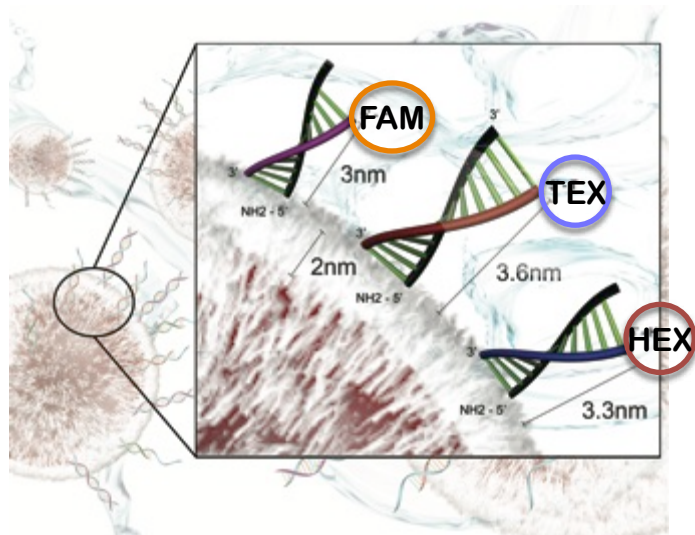
Magnetic Nanoparticles: Hyperthermia applications

Multifunctional Magnetic Nanoparticles for Magnetic Hyperthermia Applications



Magnetic Nanoparticles: Hyperthermia applications

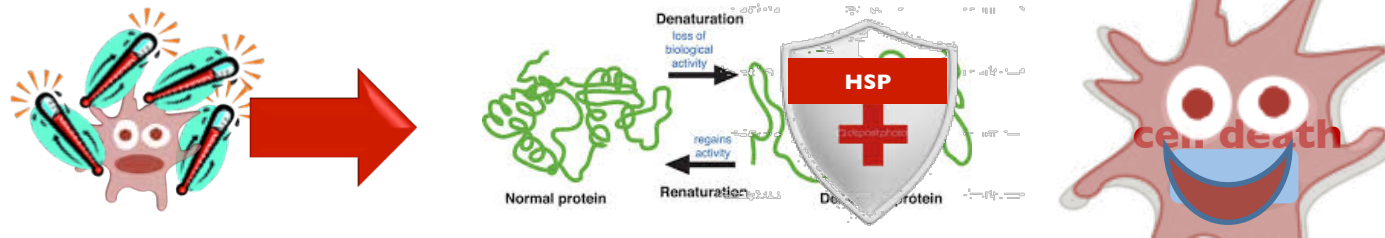
MNPs as local temperature probes: molecular thermometer



Magnetic Nanoparticles: Hyperthermia applications

Sublethal magnetic hyperthermia studies: Overexpression of Heat Shock Proteins (HSP)

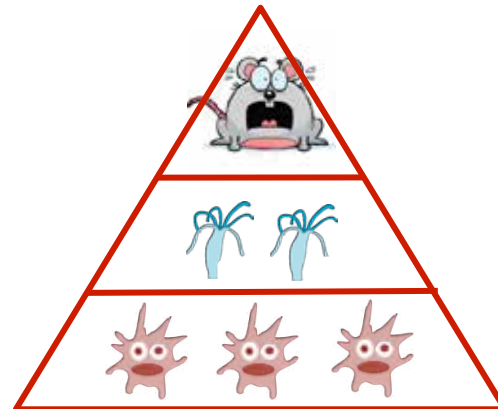
- ✓ **elevated temperature**
(type of cell stress)



Does magnetic HT change the HSP expression?



bioethics

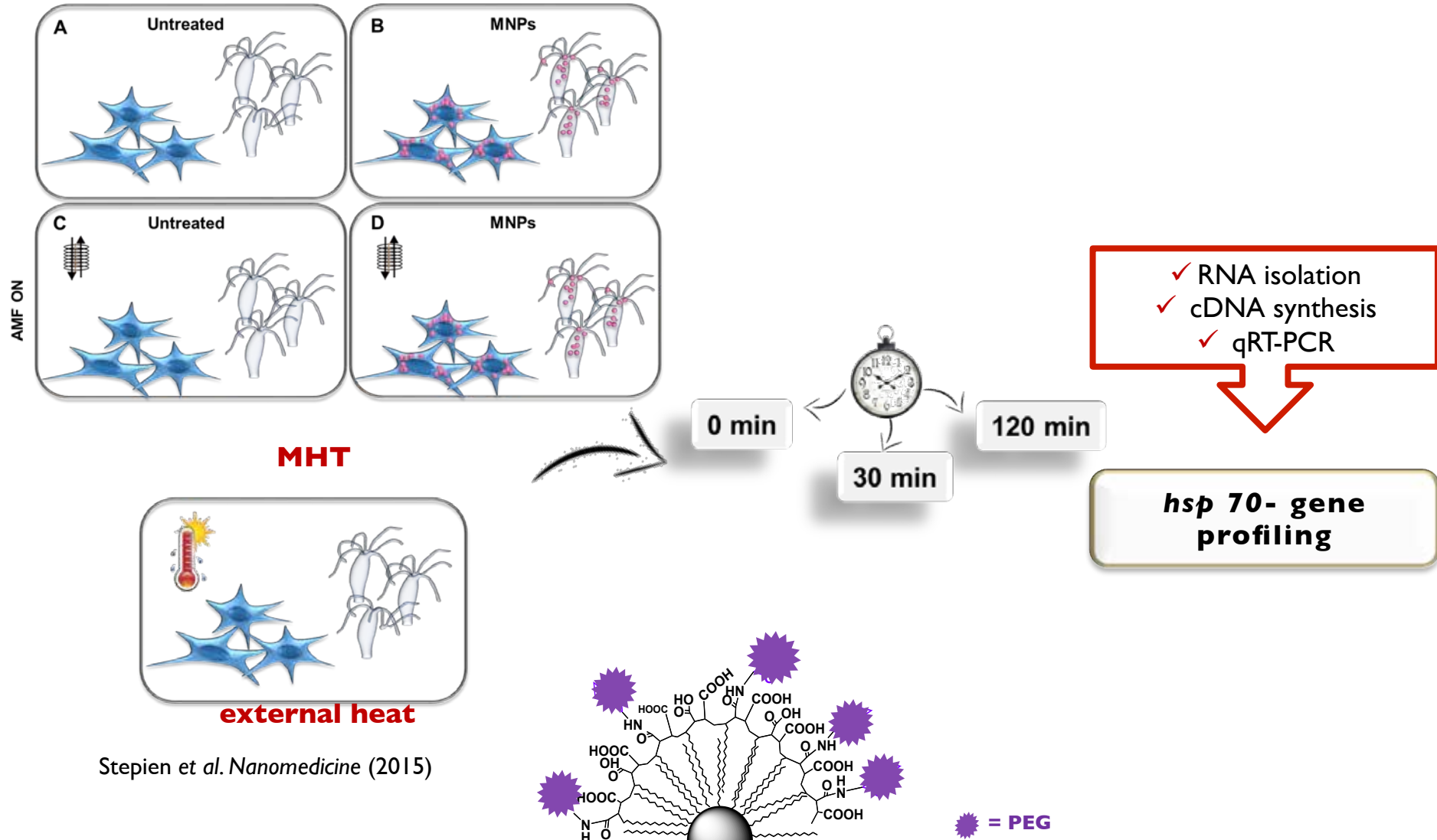


Hydra vulgaris
Dr. Alfredo Ambrosone

OBJECTIVE: a simple *in vivo* molecular model for hyperthermia treatment experiments

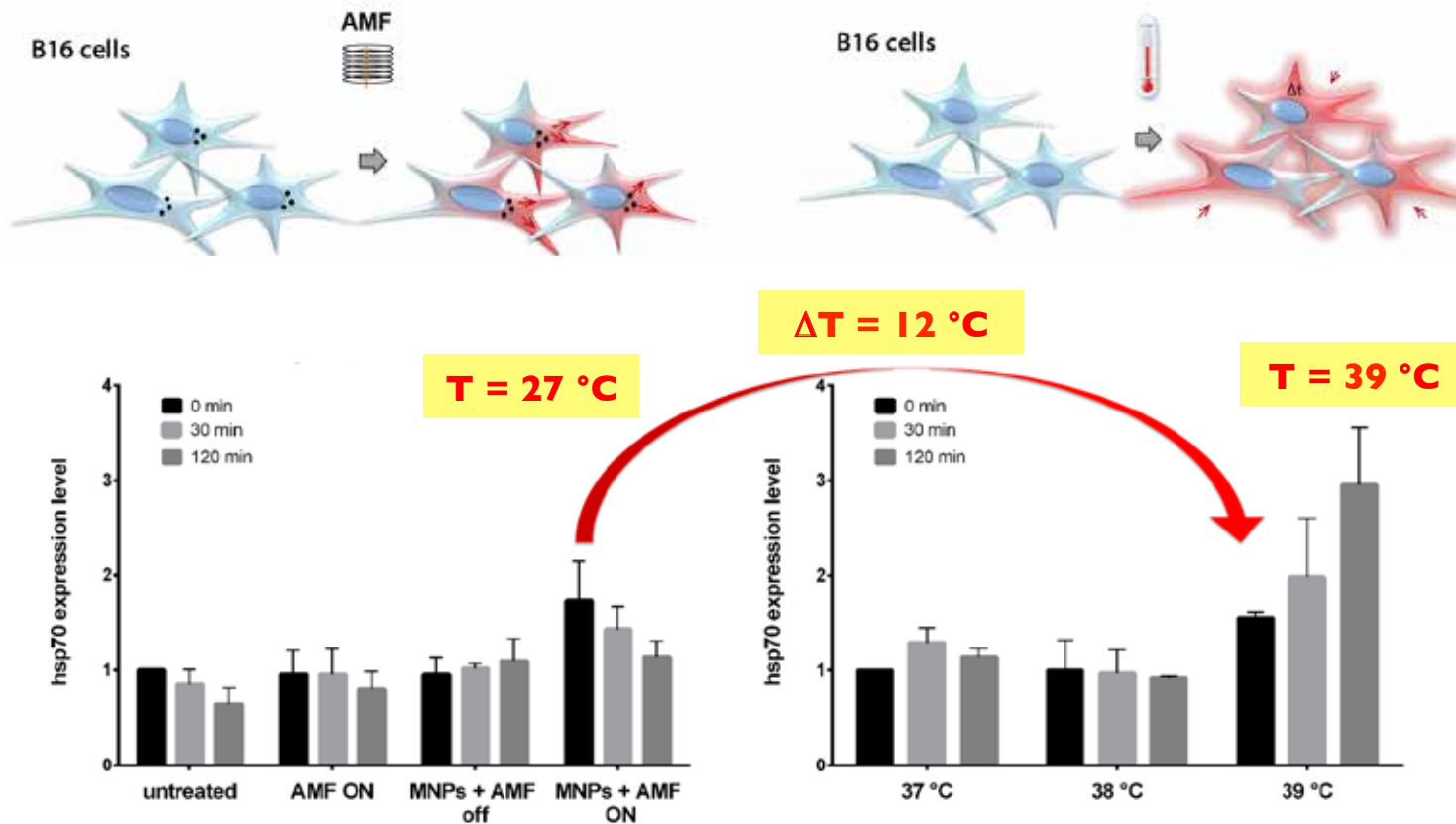
Magnetic Nanoparticles: Hyperthermia applications

Molecular analysis: Magnetic Hyperthermia (MHT) vs. external heating



Magnetic Nanoparticles: Hyperthermia applications

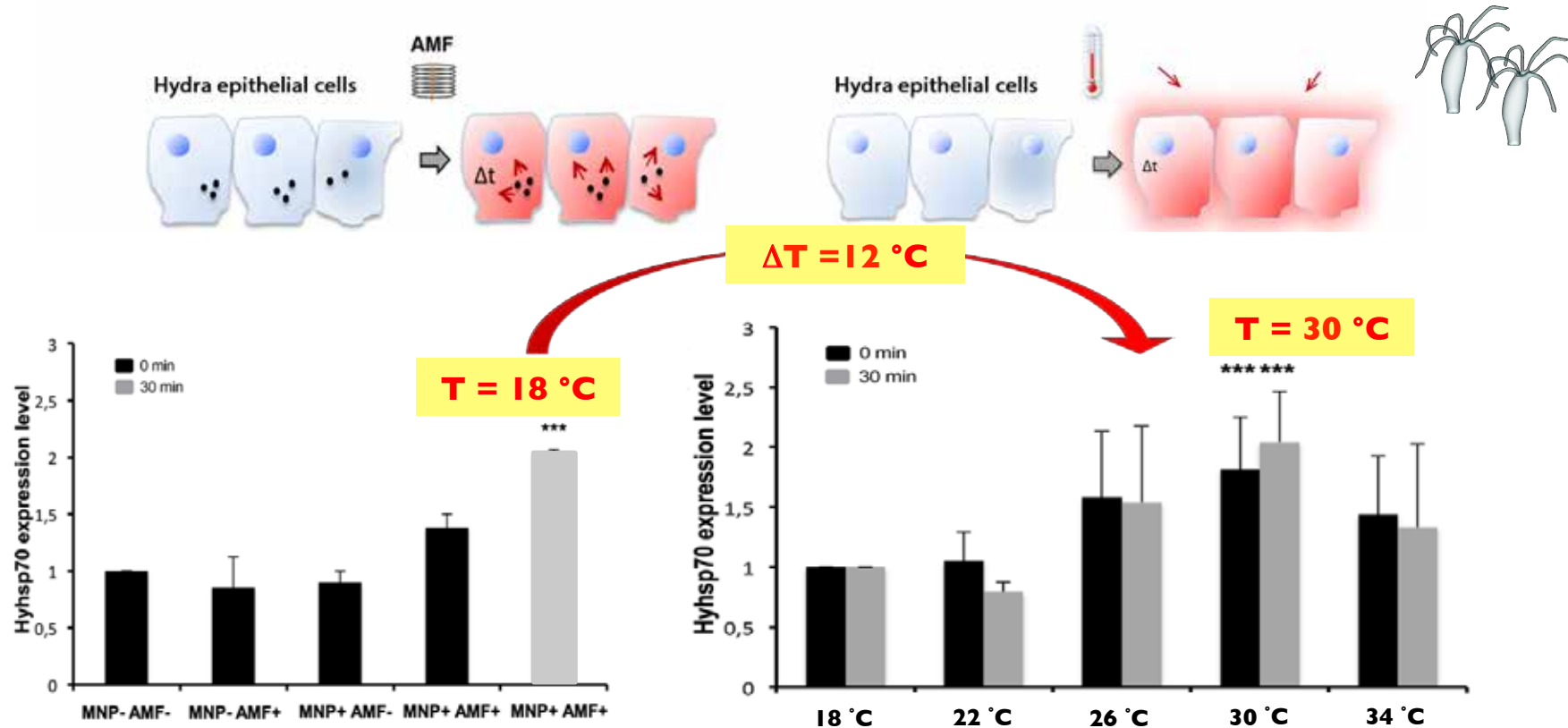
Molecular analysis: Magnetic Hyperthermia (MHT) vs. external heating



The hsp70 upregulation observed post AMF exposure mirrors the one caused by heat shocking the cells at 39°C

Magnetic Nanoparticles: Hyperthermia applications

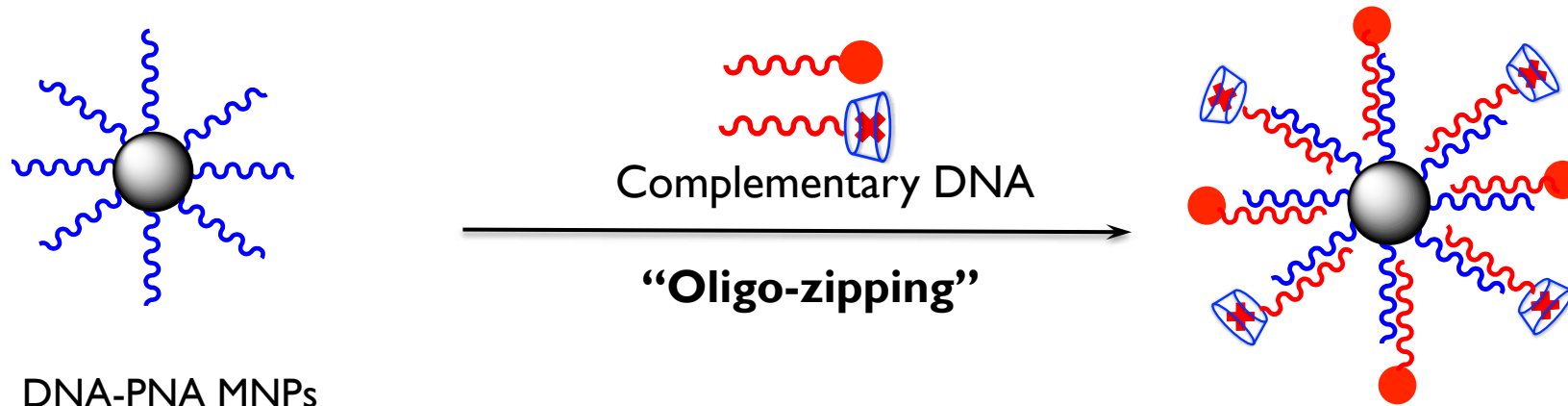
Molecular analysis: Magnetic Hyperthermia (MHT) vs. external heating



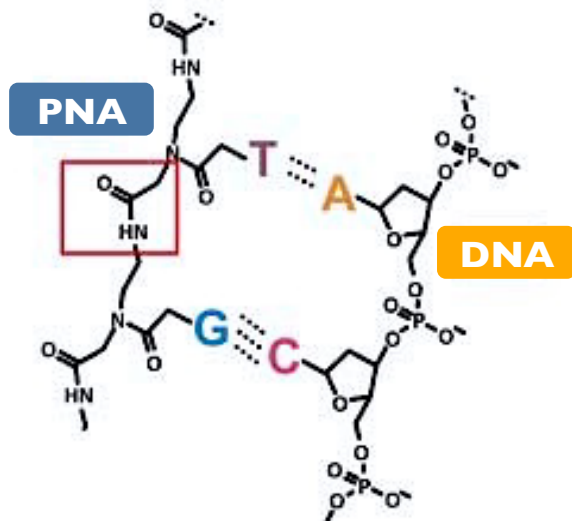
The hsp70 upregulation observed post AMF exposure mirrors the one caused by heat shocking the animals at 30 °C

Magnetic Nanoparticles: Hyperthermia applications

Multifunctional Magnetic Nanoparticles for combined targeting, MHT and drug delivery - ongoing work



PNA = Peptide Nucleic acids



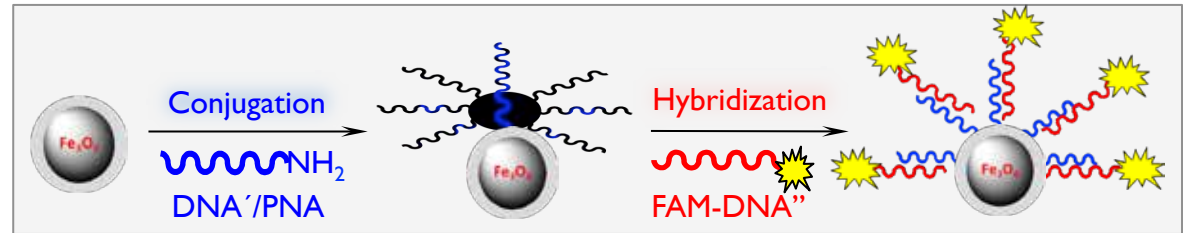
- pH stable
- No negatively charged backbone
- Resistance to enzymatic degradation
- Maintain hybridization specificity with DNA

Magnetic Nanoparticles: Hyperthermia applications

Multifunctional Magnetic Nanoparticles for combined targeting, MHT and drug delivery - ongoing work

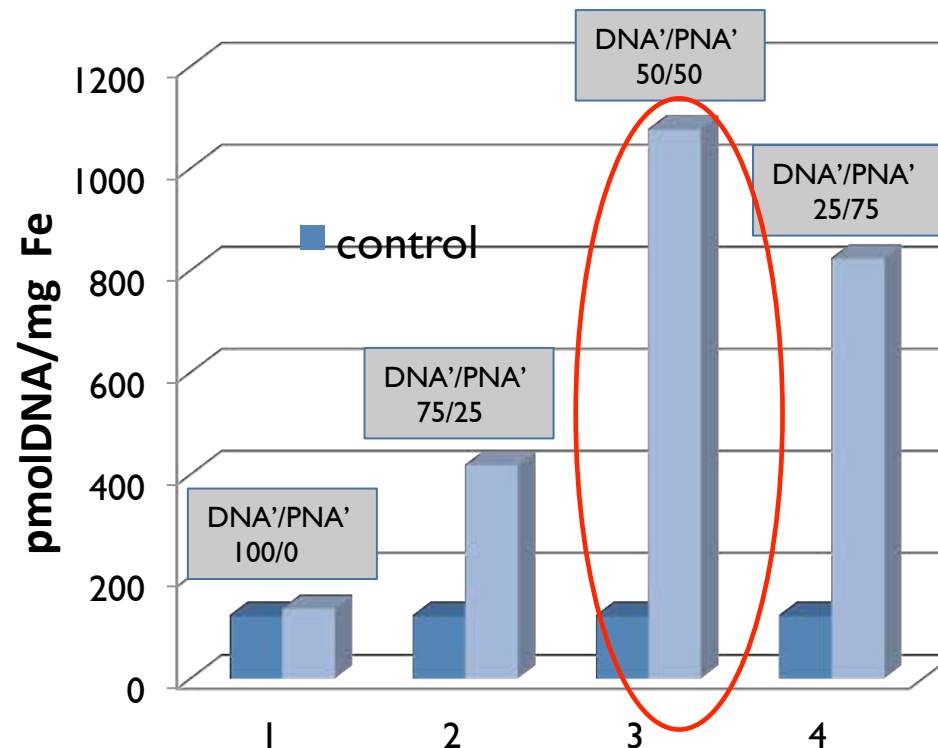
DNA/PNA ratio optimization

MNPs@PMAO-DNA'/PNA'-DNA''



DNA %	PNA %
100	0
75	25
50	50
25	75

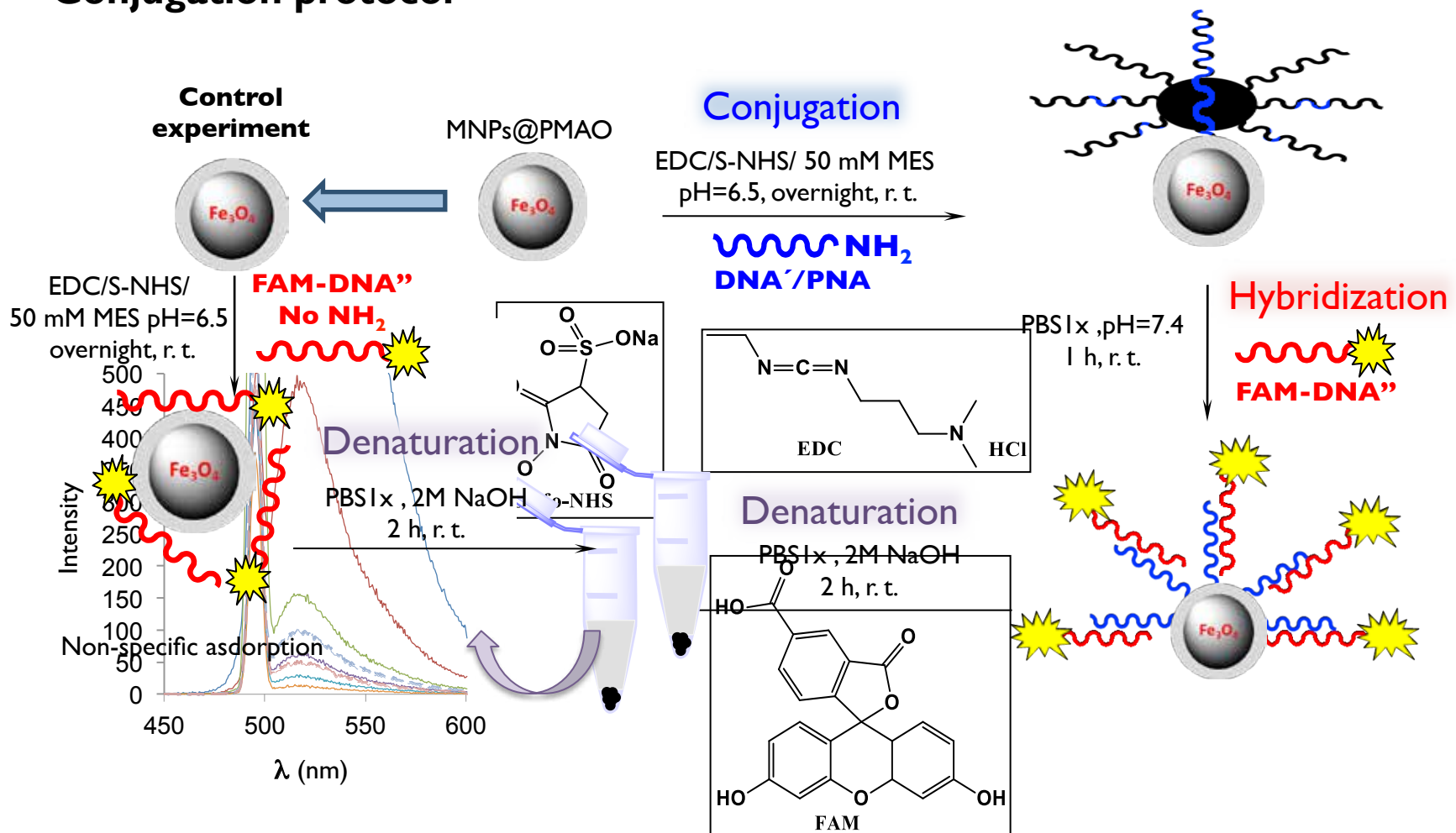
Fratila et al., in preparation



Magnetic Nanoparticles: Hyperthermia applications

Multifunctional Magnetic Nanoparticles for combined targeting, MHT and drug delivery - ongoing work

Conjugation protocol

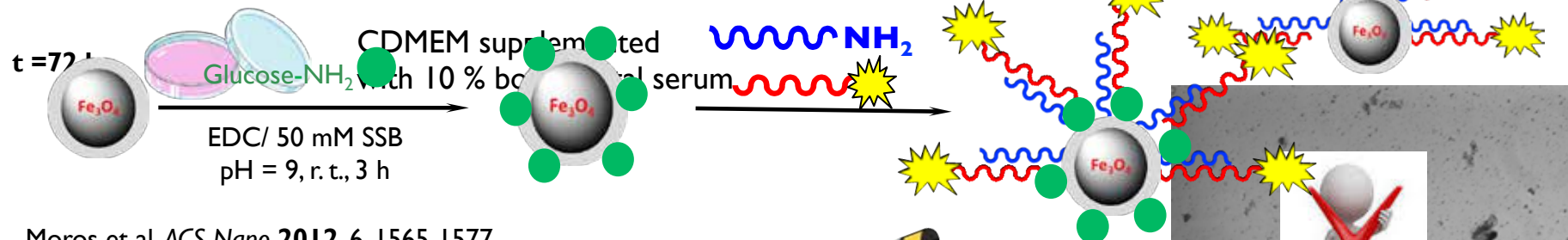


Magnetic Nanoparticles: Hyperthermia applications

Multifunctional Magnetic Nanoparticles for combined targeting, MHT and drug delivery - ongoing project

Cell culture medium stability assay

MNPs@PMAO-GNADPNAPNDNA



Moros et al. *ACS Nano* **2012**, 6, 1565-1577

$t = 72 \text{ h}$

- MNP functionalization with antibodies and cyclodextrin
- Hyperthermia studies: MHT vs. external heating

Magnetic Nanoparticles: Hyperthermia applications

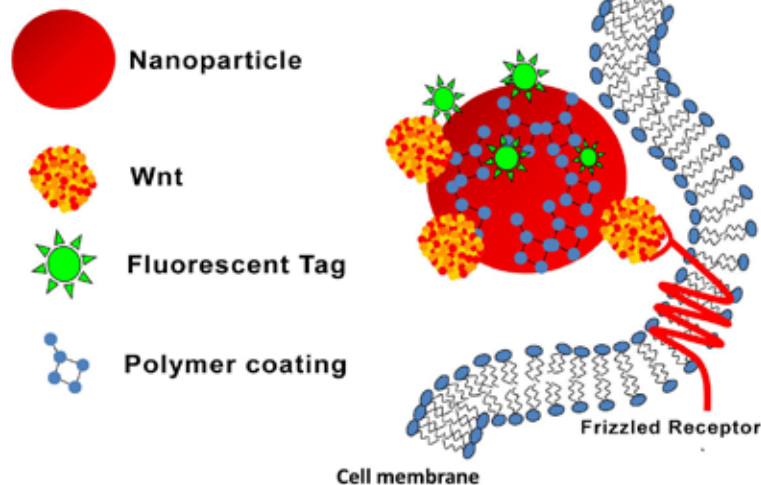
CONFINES: Controlling Cell Fate through Smart Nanoheaters – ongoing project



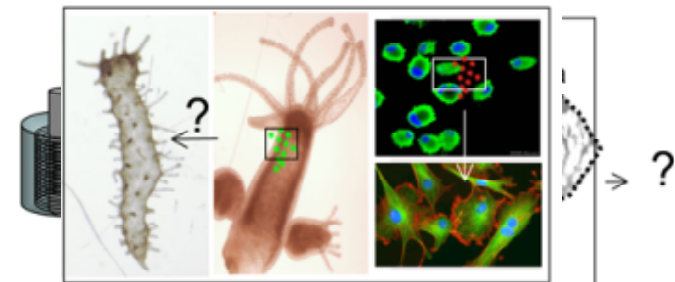
MSCA - IEF
Dr. Alfredo Ambrosone
September 2015 – August 2017

- Targeting of a specific cell signalling – Wnt signalling
- Wnt signalling plays crucial roles in the animal life by controlling the genetic programs of embryonic development and adult homeostasis and stem cell maintenance in all metazoans
- Canonical Wnt pathway is aberrantly regulated in many cancers

NanoWnt



To stimulate Wnt signalling where it is inactive (Developmental disorders)



Wnt ON

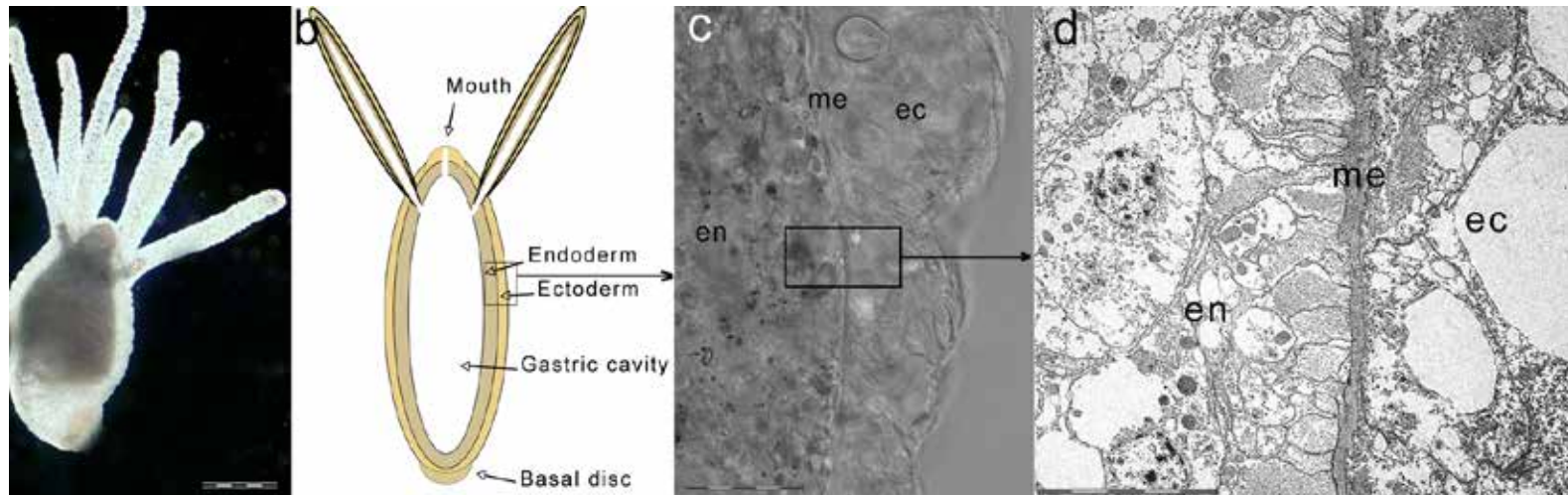
To target and kill the cells where the pathway is aberrantly activated (Cancer)

Magnetic Nanoparticles: Hyperthermia applications

CONFINES: Controlling Cell Fate through Smart Nanoheaters – ongoing project

Why Hydra?

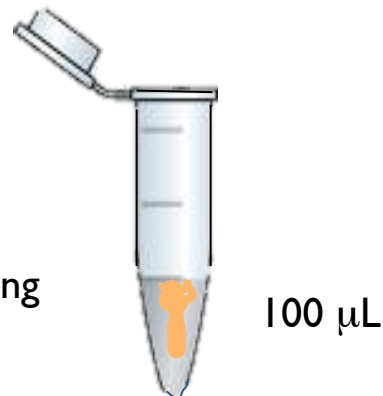
Hydra is a simple invertebrate model at the base of animal evolution



Key points:

Development
Stemness/Ageing

Easy and cost-effective culturing
Rapid growth
No bioethical issues



Magnetic Nanoparticles: Hyperthermia applications

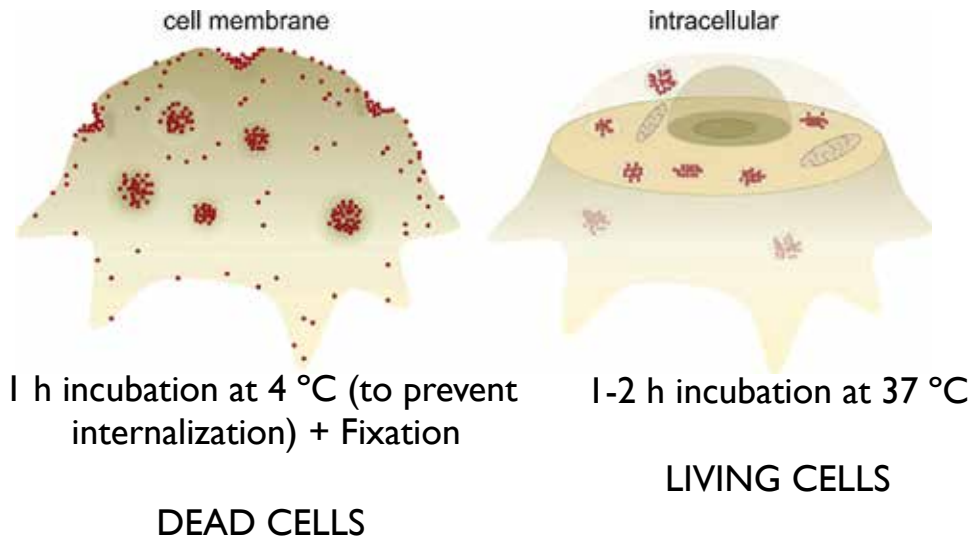
OUTstanding: Cell-surface immobilized vs. internalized magnetic nanoparticles for magnetic hyperthermia studies – ongoing project



MSCA - IEF
Dr. Raluca M. Fratila
September 2015 – August 2017

Purpose: Immobilize magnetic nanoparticles on the surface of **living cells** for magnetic hyperthermia studies

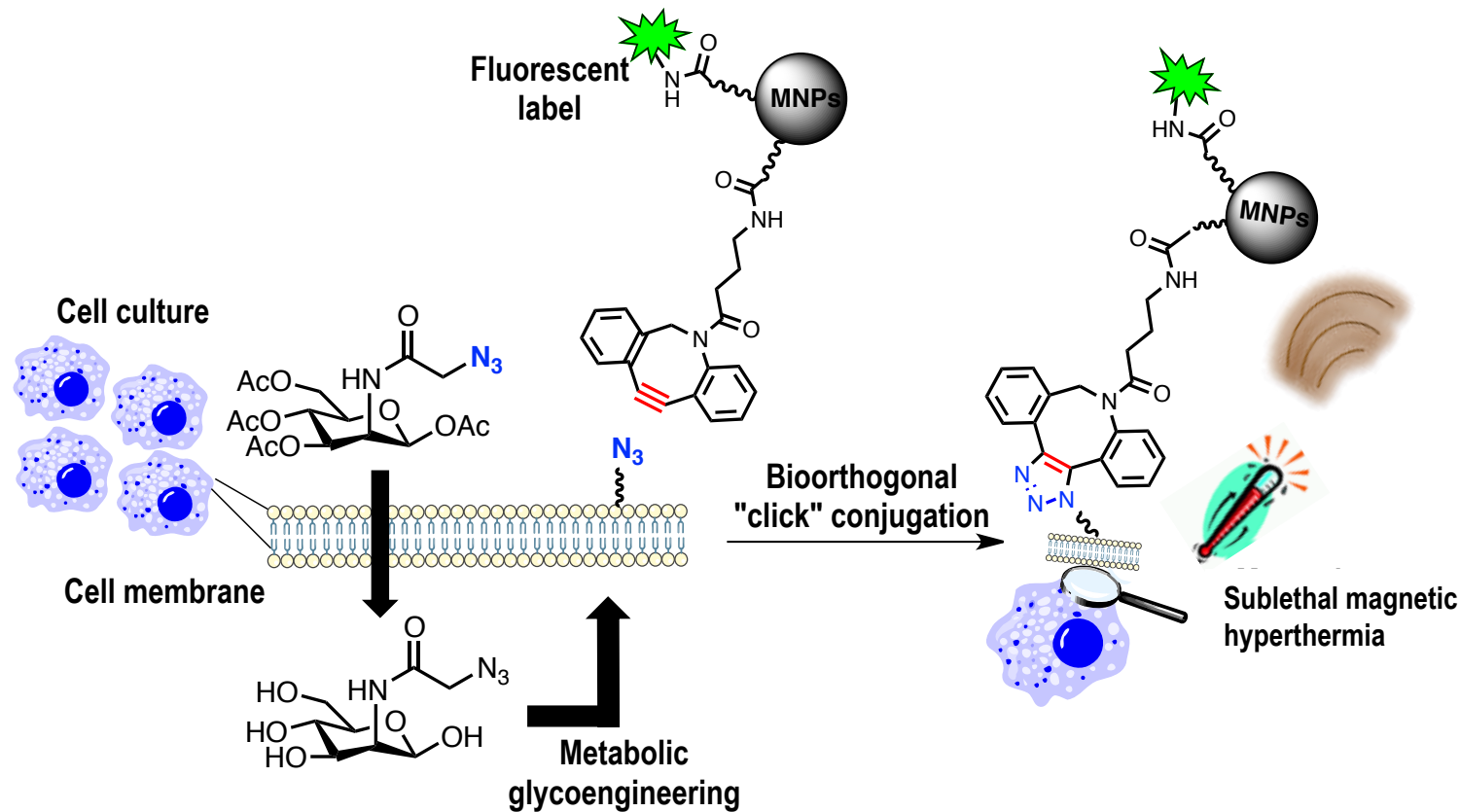
What happens when the nanoparticles are placed in the cellular environment?



Similar SAR values for both cellular environments, but **always lower** than in solution

Magnetic Nanoparticles: Hyperthermia applications

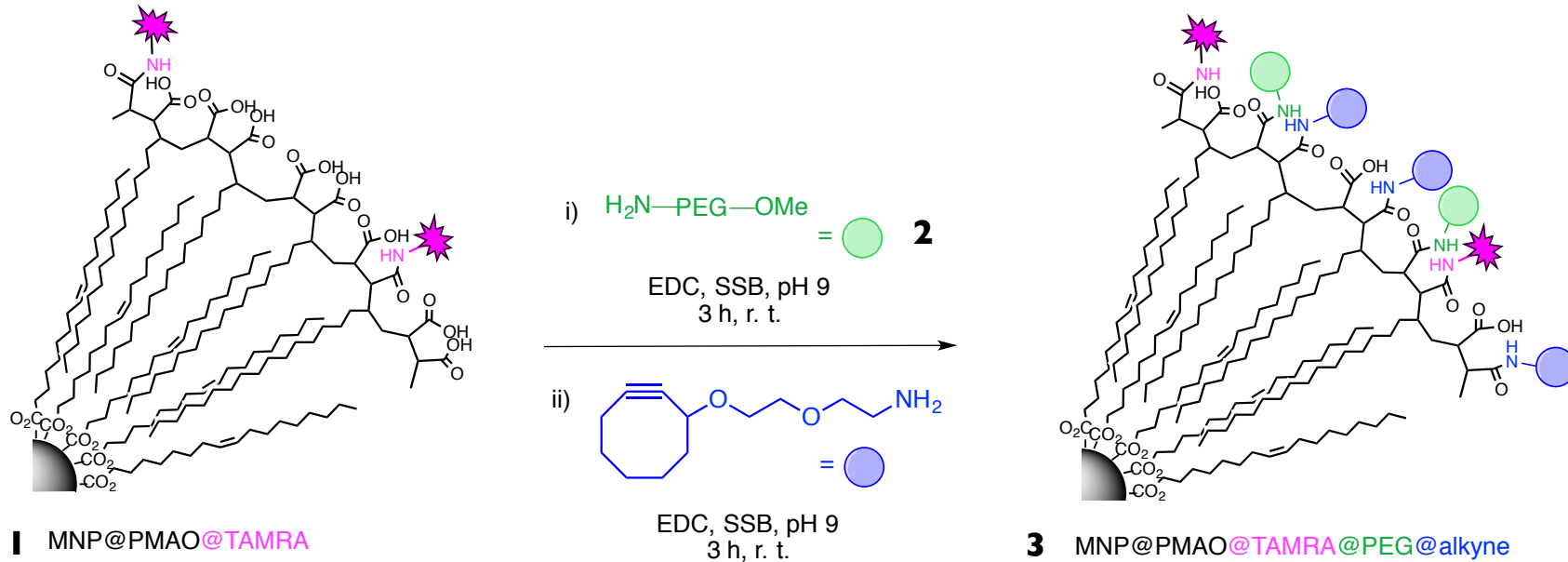
OUTstanding: Cell-surface immobilized vs. internalized magnetic nanoparticles for magnetic hyperthermia studies – ongoing project



- 1) Assess how the cell attachment of MNPs affects their heating efficiency when compared to internalized MNPs
- 2) How MNPs immobilization and sub-lethal hyperthermia treatment affect biophysics of cell membranes (permeability, fluidity)

Magnetic Nanoparticles: Hyperthermia applications

OUTstanding: Cell-surface immobilized vs. internalized magnetic nanoparticles for magnetic hyperthermia studies – ongoing project



- Good stability in cell culture medium (complete DMEM supplemented with 10% FBS at 5 $\mu\text{g Fe}/50 \mu\text{L CDMEM}$, 24 h)



- Cell/hydra toxicity studies
- Incubation with azide-labelled Vero cells and hydra

THANK YOU FOR YOUR ATTENTION!

