Multifunctional Ferritins for Multimodal MRI-OI-SPECT Imaging

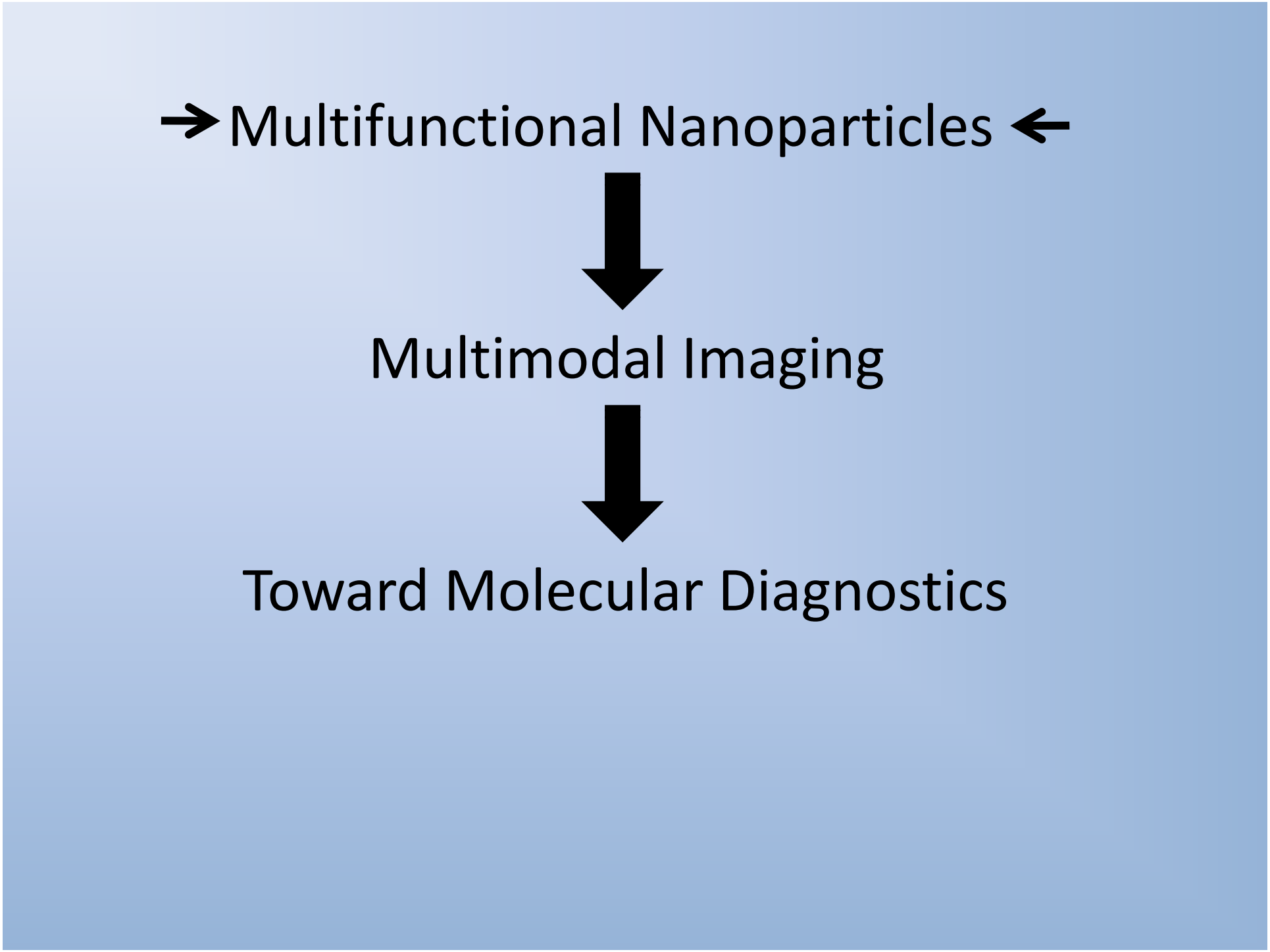
José M. Domínguez-Vera

Departamento de Química Inorgánica
Facultad de Ciencias
Universidad de Granada

Nanomedicine

Next-generation of biological diagnostic and therapeutic probes

- **Diagnosis**
  - Sensors
  - BioImaging
- **Therapy**
  - Drug delivery
  - Gene delivery
  - Hyperthermic cancer treatment
  - …

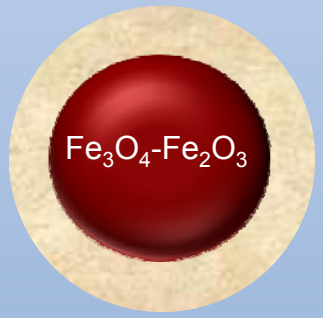

Multifunctional Nanoparticles
Multimodal Imaging
Toward Molecular Diagnostics

BIOImaging modalities

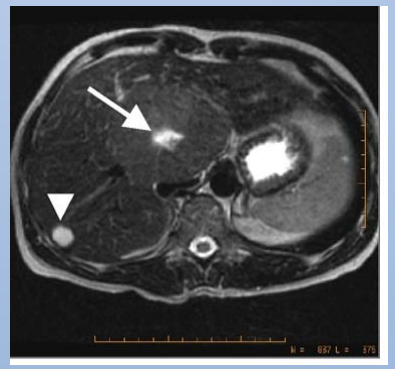
Magnetic Resonance Imaging

MRI provides images by measuring H^+ relaxation in tissues.

- Different H^+ relaxation in different biological regions due to the different chemical environments leads to different contrast, providing morphological and anatomical information.
- Sometimes, the contrast difference between tissues is subtle and then contrast agents are needed to enhance this.
- **Superparamagnetic nanoparticles**: the best candidates.



Scheme of simple SPIO



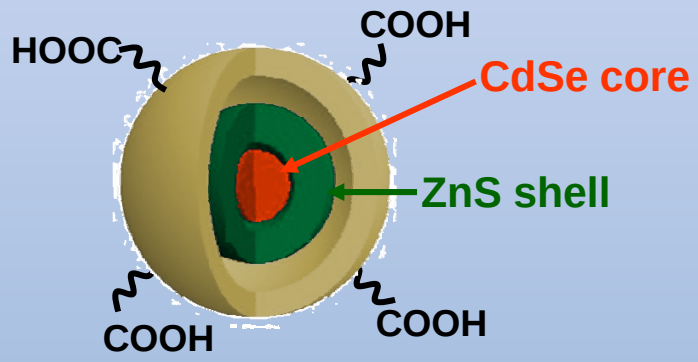
MRI of liver

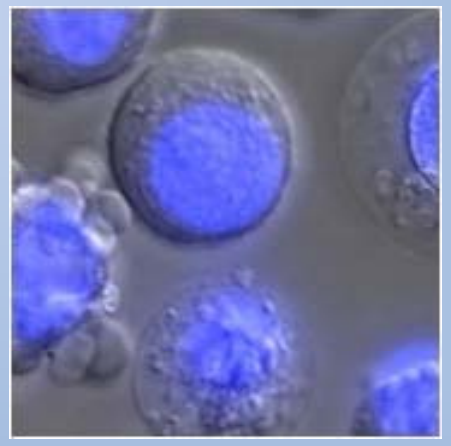
Optical Imaging

OI Fluorescent probes require excitation light to emit light at lower energy.

- Blue and green lights have poor tissue penetration.
- Ideal excitation and emission wavelengths are in the 650-900nm range.

- **Quantum Dots** (QD)
- Broad excitation and narrow emission peaks.
- Low energy (NIR) for excitation and emissions
- **TOXICITY**



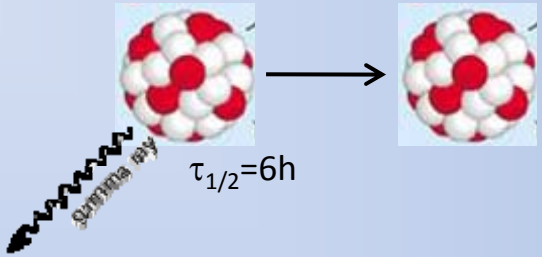


Single Photon Emission Computed Tomography

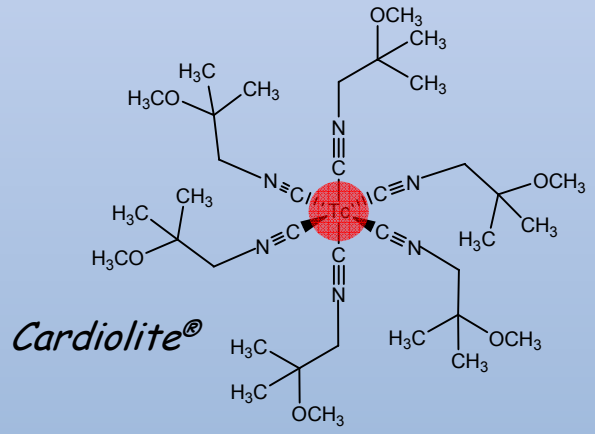
SPECT uses the γ-ray emission from radionuclides inside the body

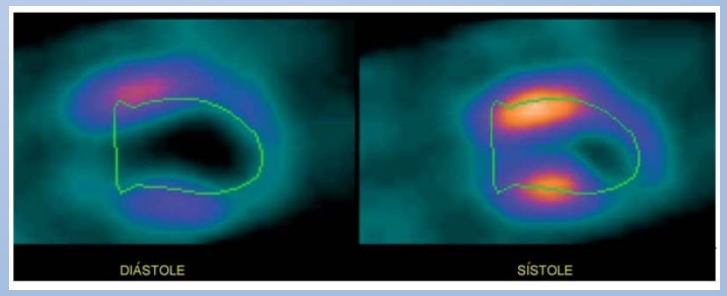
$^{99}MoO_4^{=} \rightarrow {}^{99m}TcO_4^{-} + \beta^{-}$

$^{99m}TcO_4^{-} \rightarrow {}^{99}TcO_4^{-} + \gamma$ (Single Photon)



- Only ^{99m}Tc single molecules have been commercialized.
- **One molecule - One Tc !**

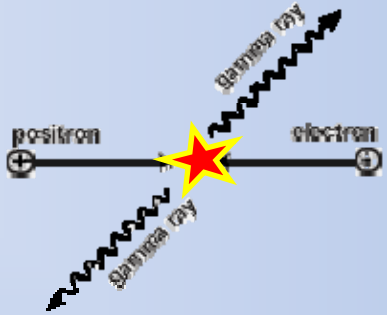




Heart SPECT

Positron Emission Tomography

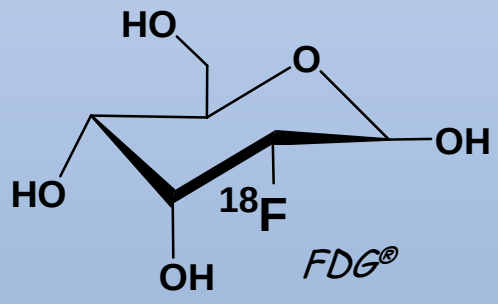
PET uses pairs of γ rays emitted by a positron-emitting radionuclide inside the body.

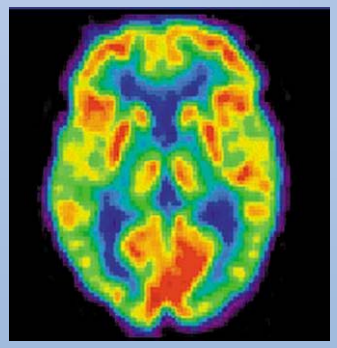


•The emitted positron e^+ (antiparticle of the e^-) is annihilated by an e^-, producing a pair of γ photons at 180º.

• **Methabolic PET**: FDG (^{18}F) for methabolic activity: brain activity.

• CANCER (accumulation of phosphorylated FDG in cancer cells)





PET of brain

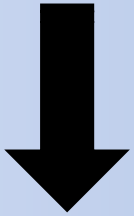
Why multimodal imaging?

Comparing in vivo Imaging Modalities

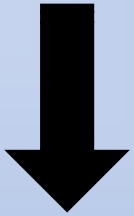
No ONE perfect modality

IMAGING TECHNIQUE	SPATIAL RESOLUTION	PENETRATIG DEPTH	SENSITIVITY	TYPES OF PROBES
MRI	25-100µm	No limit	mM Gd nM Fe	Paramagnetic ions
PET	1-2mm	No limit	pM	Positron emitters
SPECT	1-2 mm	No limit	Sub nM	Gamma emitters
CT	50200 µm	No limit	mM	Iodine, barium
OI	In vivo 2-3 mm In vitro sub-µm	mm	pM	Fluorophore QDots

→ Multifunctional Nanoparticles ←

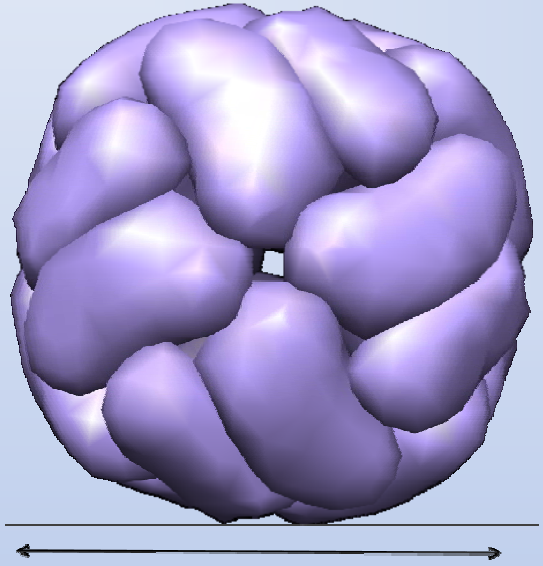


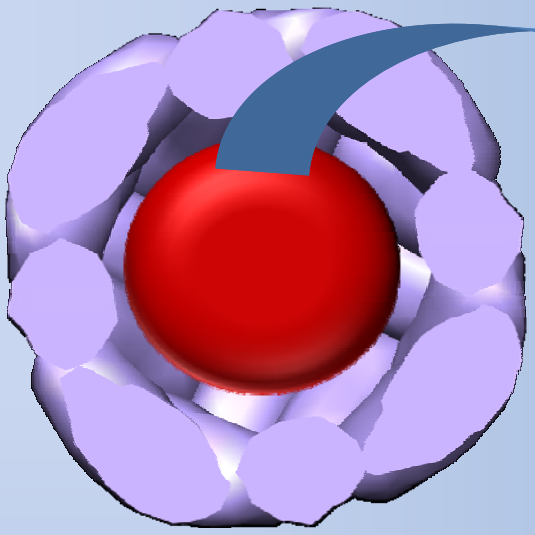
Multimodal Imaging

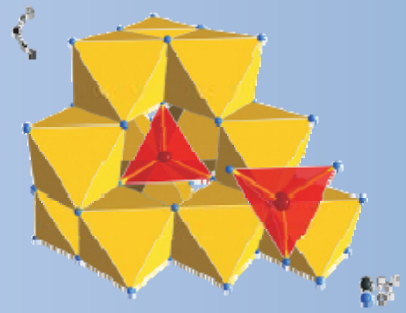


Toward Molecular Diagnostics

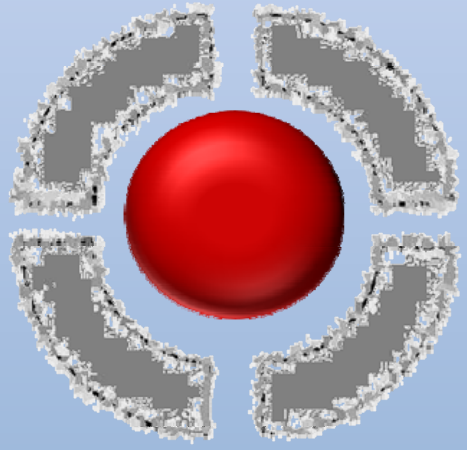
Ferritin: a paradigmatic nanocavity-containing biomolecule



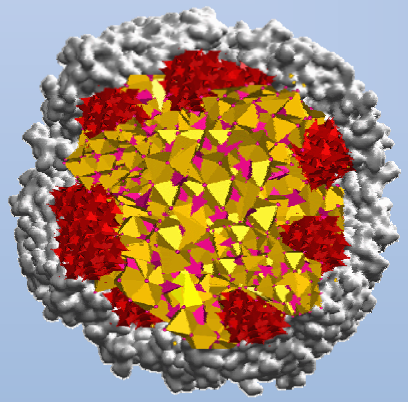


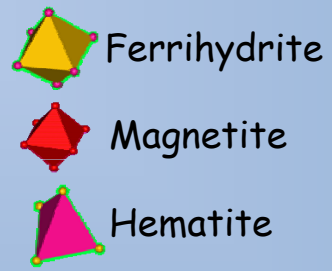


Ferrihydrite $Fe^{III}_{10}O_{14}(OH)_2$



scheme of ferritin structure

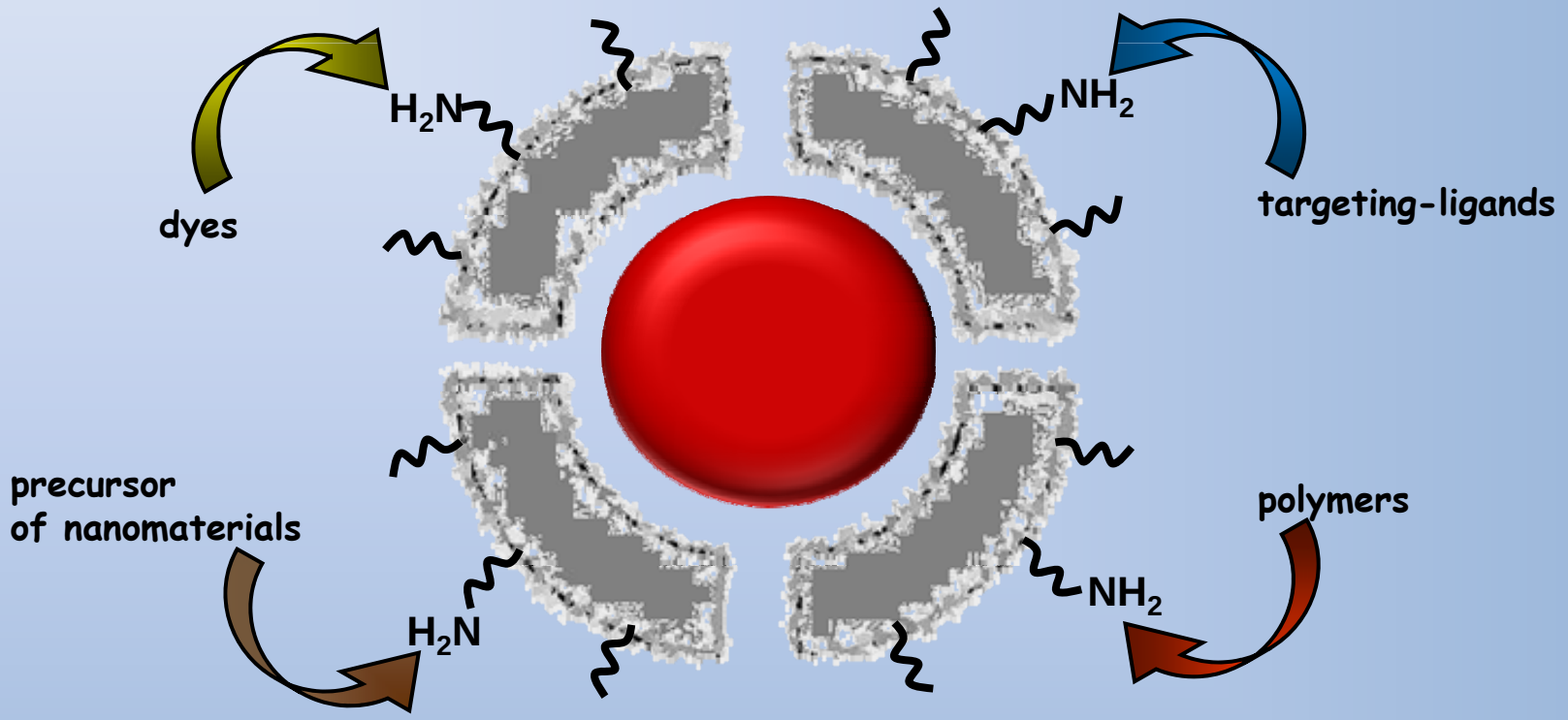




Domínguez-Vera et al.
JACS 2008, 130, 8062

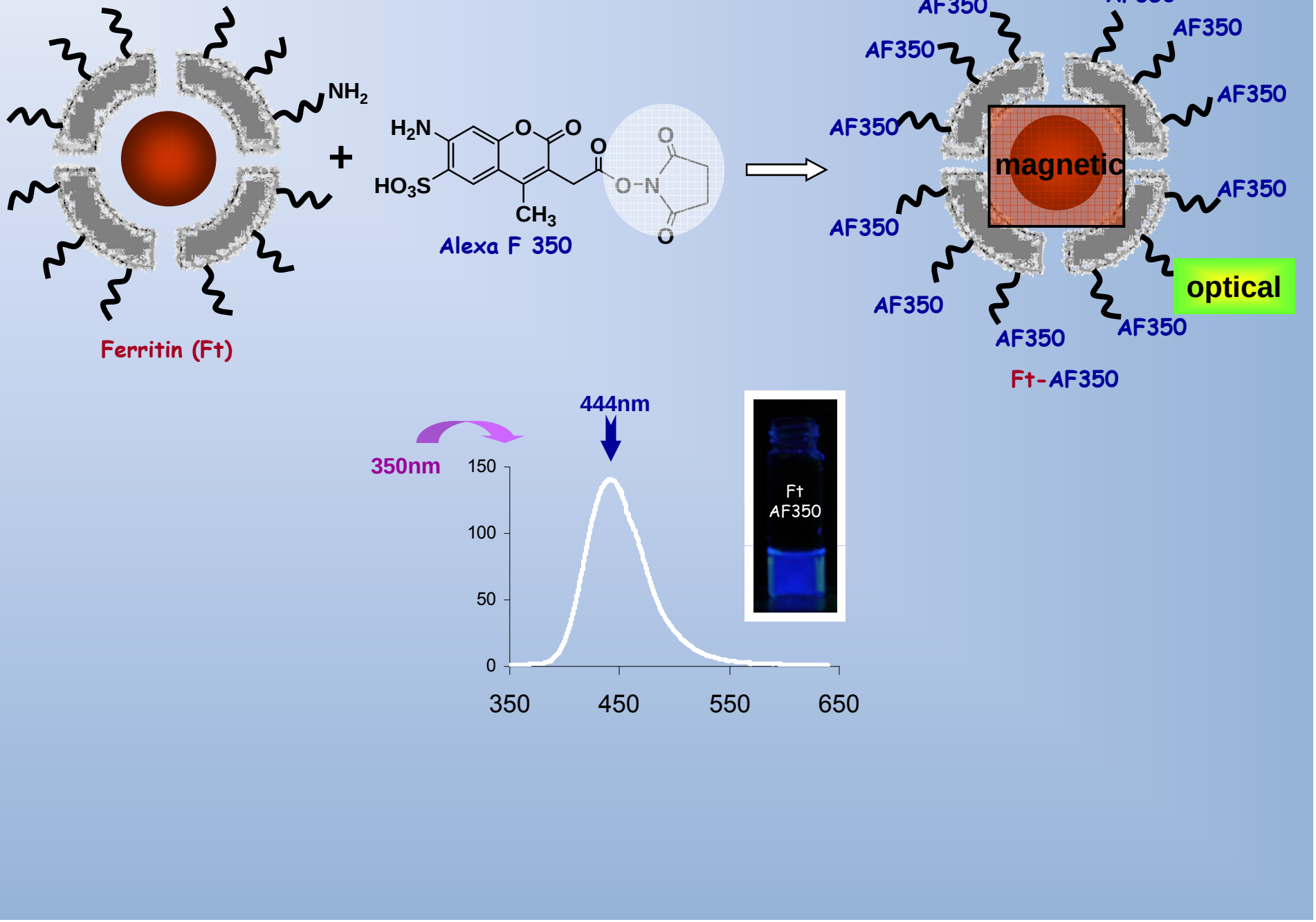
Ferritin as a multifunctional platform:

- the cavity as a nanocontainer for inorganic materials
- the external shell for “decorating” the particles



Domínguez-Vera et al.
Prussian Blue *Inorg. Chem. 2003, 42, 6983*
Co, Ni *J. Mat. Chem. 2006, 16, 2757*
Pd *J. Mat. Chem. 2007, 17, 49*
CoNi *Inorg. Chem. 2010, 49, 1705*

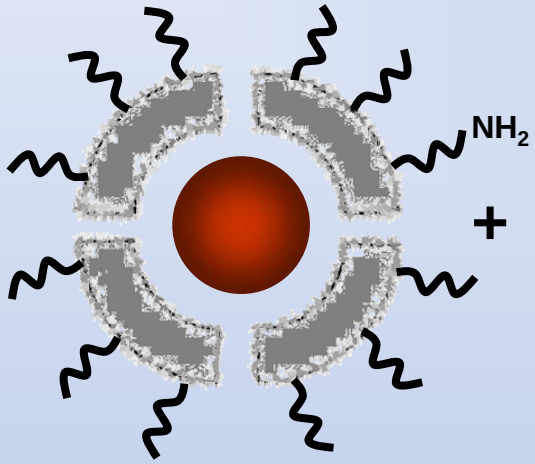
Steve Mann (University of Bristol)
Trevor Douglas (Montana University)
Ichiro Yamashita (CREST, Japan)

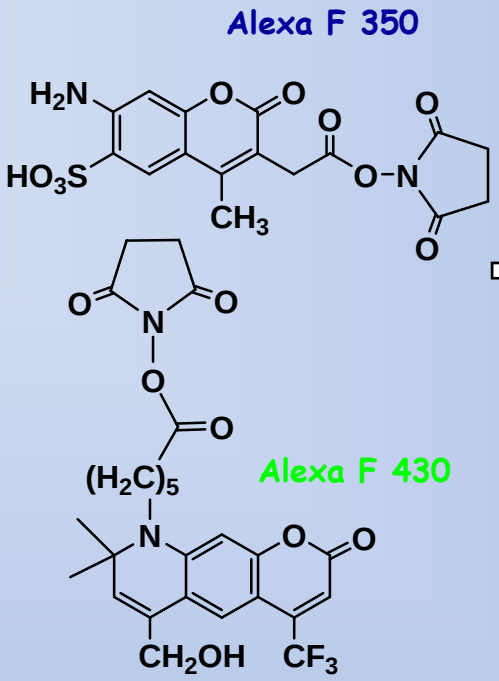

NH2
Ferritin (Ft)
H2N
HO3S
CH3
O
O-N
Alexa F 350
AF350
magnetic
optical
Ft-AF350
444nm
350nm
150
100
50
0
350
450
550
650
Ft
AF350

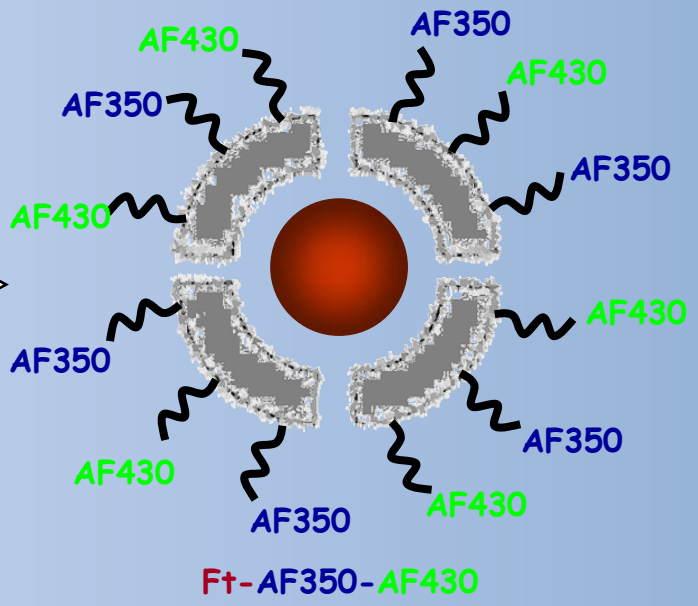
One labelled fluorophore

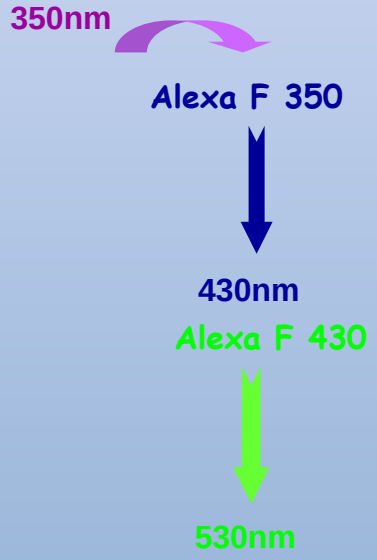
Why not two? Or three?

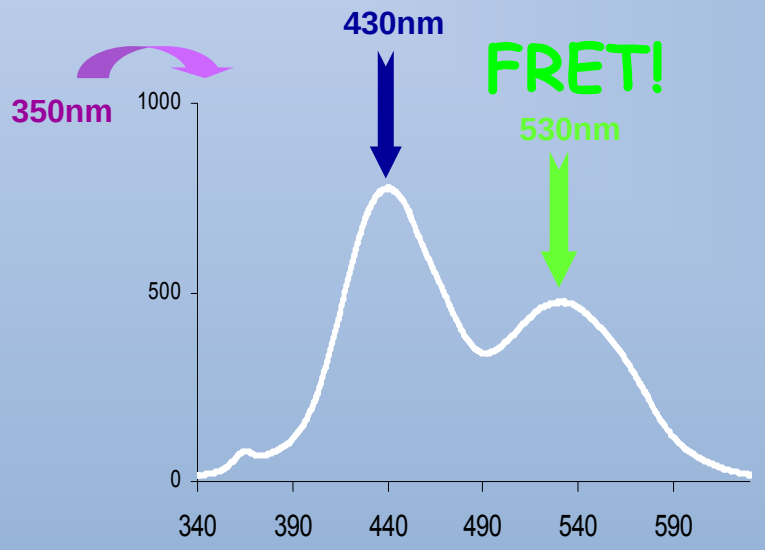
FRET (Fluorescence Resonance Energy Transfer) in a single particle



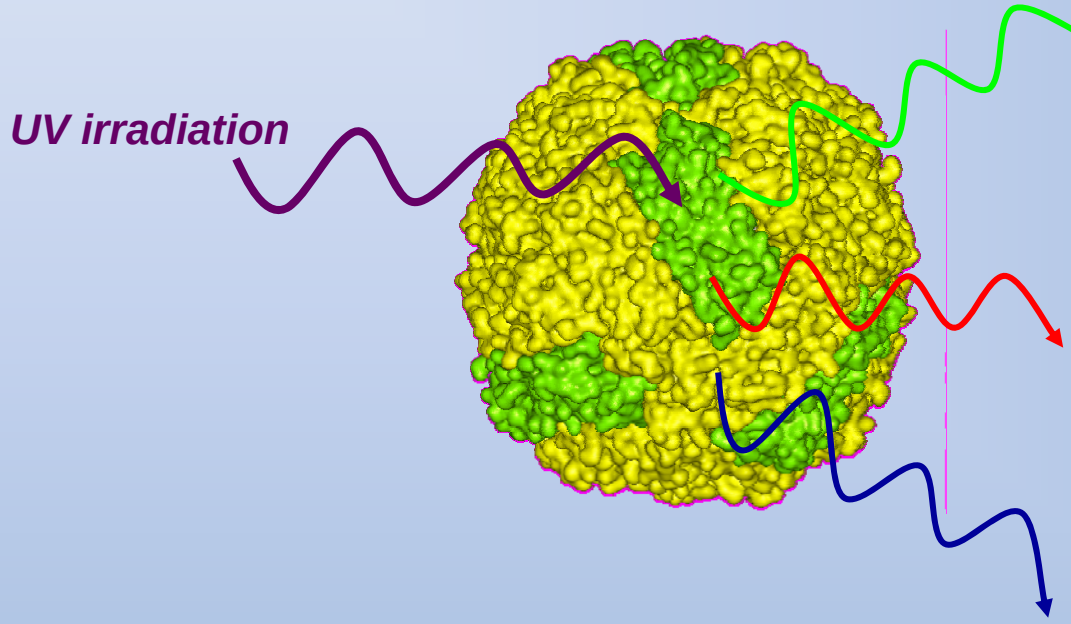








Modulated fluorescence emission by tunning the fluorophores ratio:
Toward white light emitting ferritins...



Fluorescent ferritins

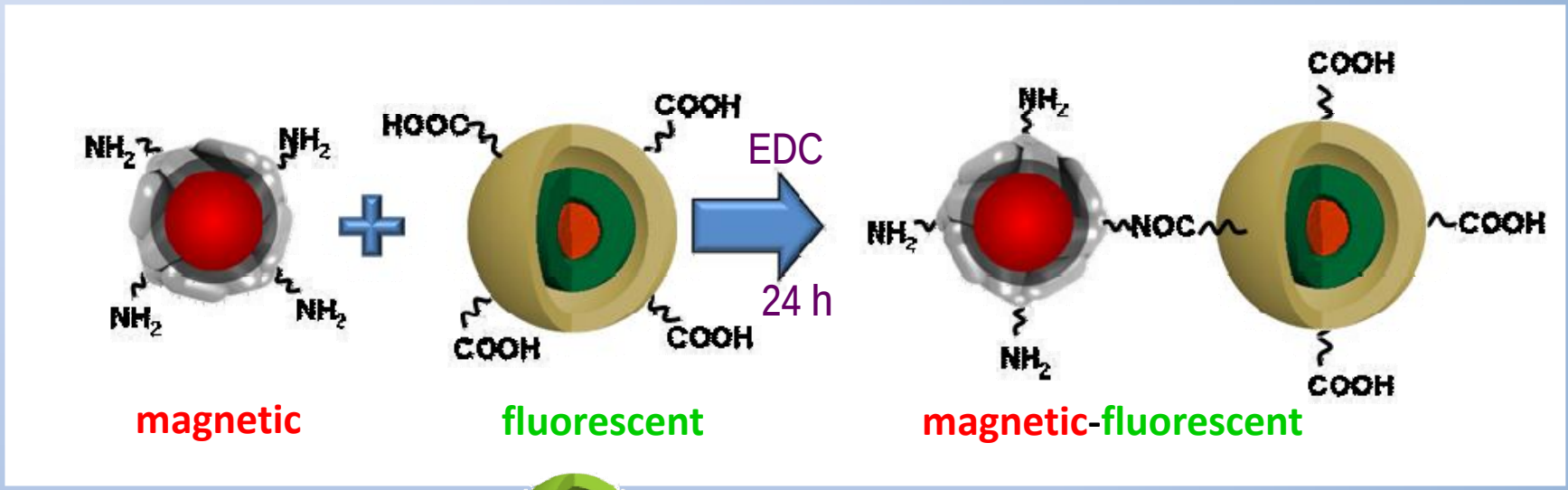
Domínguez-Vera et al.
JBIC 2008, 13, 348

labelled fluorophores

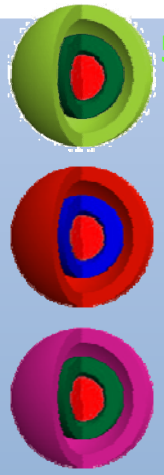
Why not quantum dots?

Magnetic-fluorescent Bioconjugates: Nanoparticles decorating Nanoparticles

Covalent coupling between the carboxylic-QD and NH_2-Ferritin



ferritins



525 nm Emission (QD525)

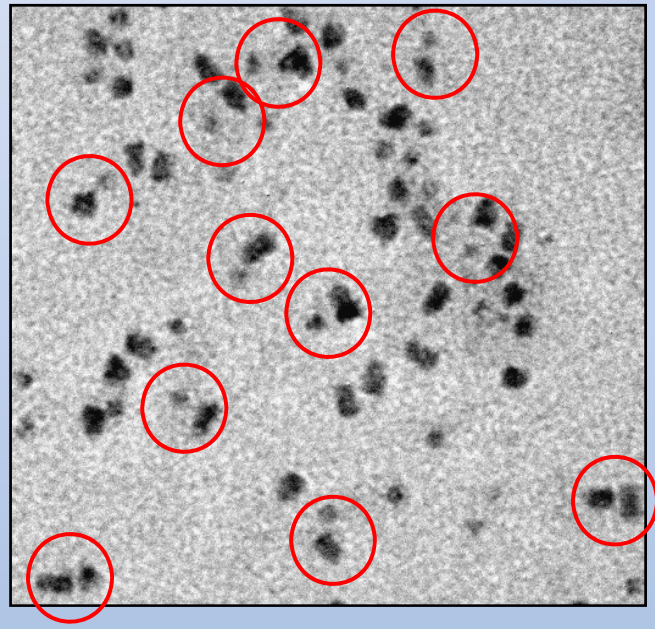
655 nm Emission (QD655)

800 nm Emission (QD800)

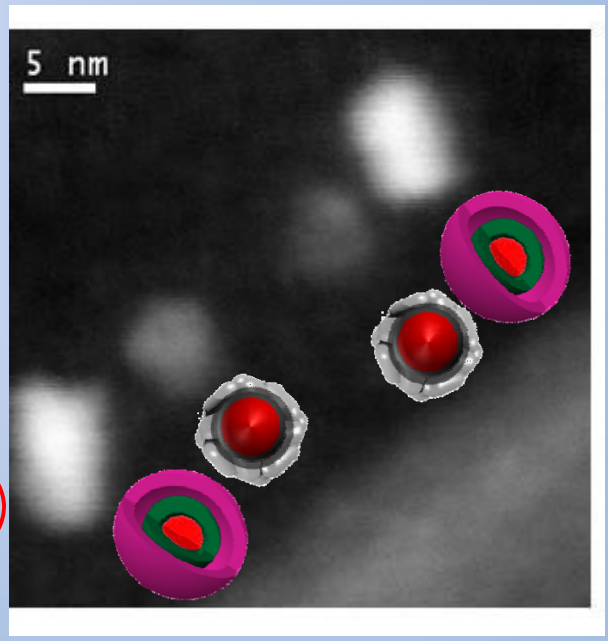
CdSe

Magnetic-fluorescent Bioconjugates: TEM and STEM-HAADF

TEM

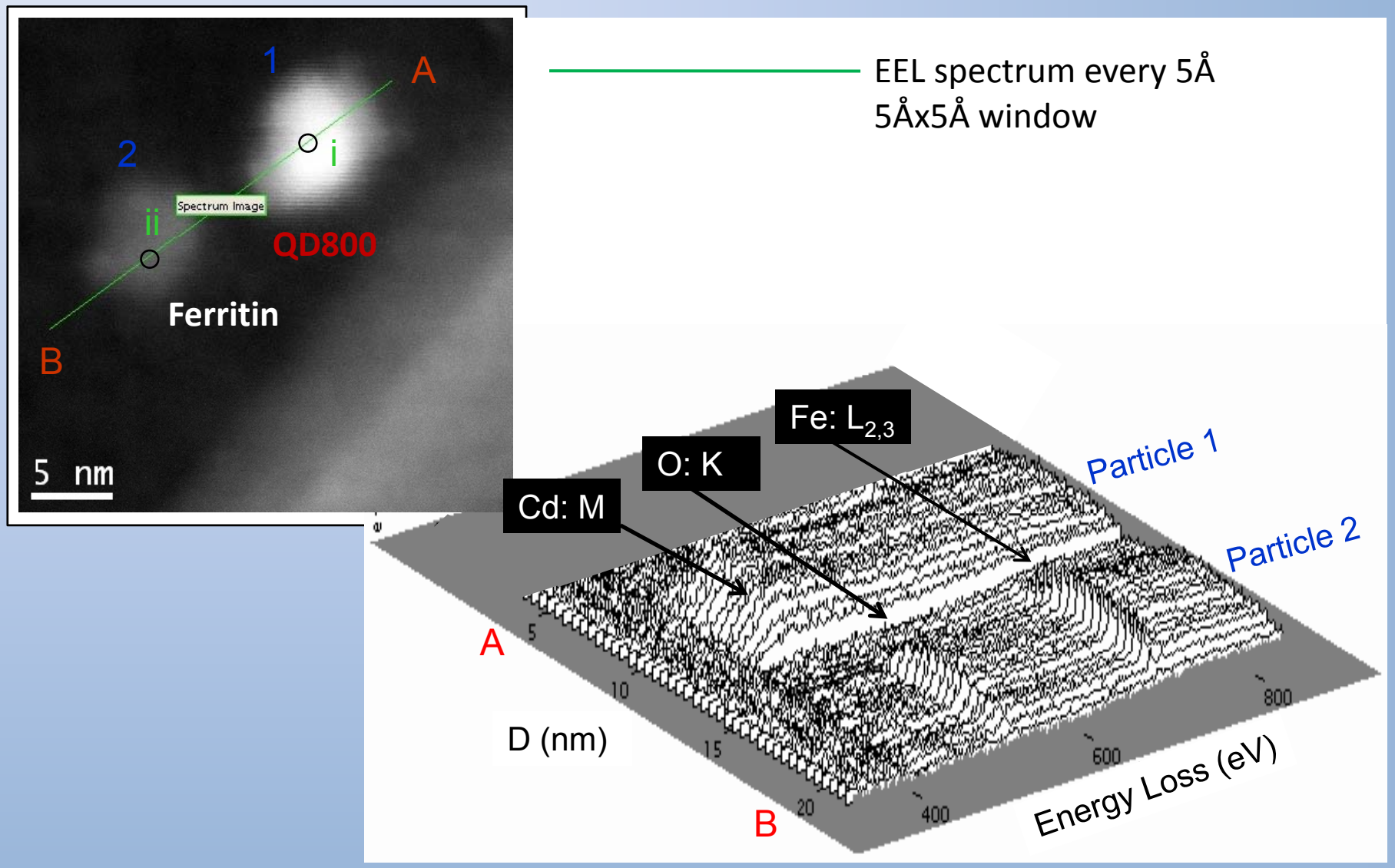


STEM-HAADF

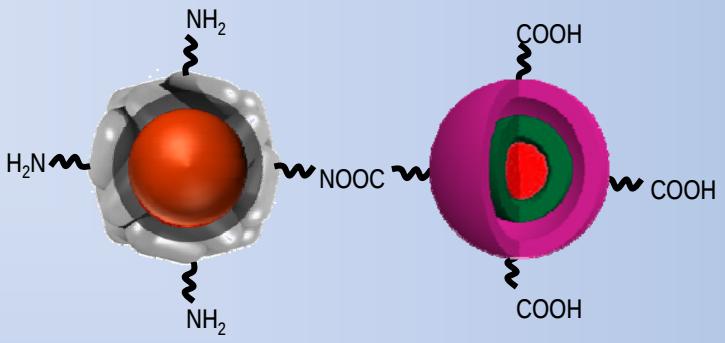


Domínguez-Vera et al.
Adv. Funct. Mater. 2008, 18, 3931

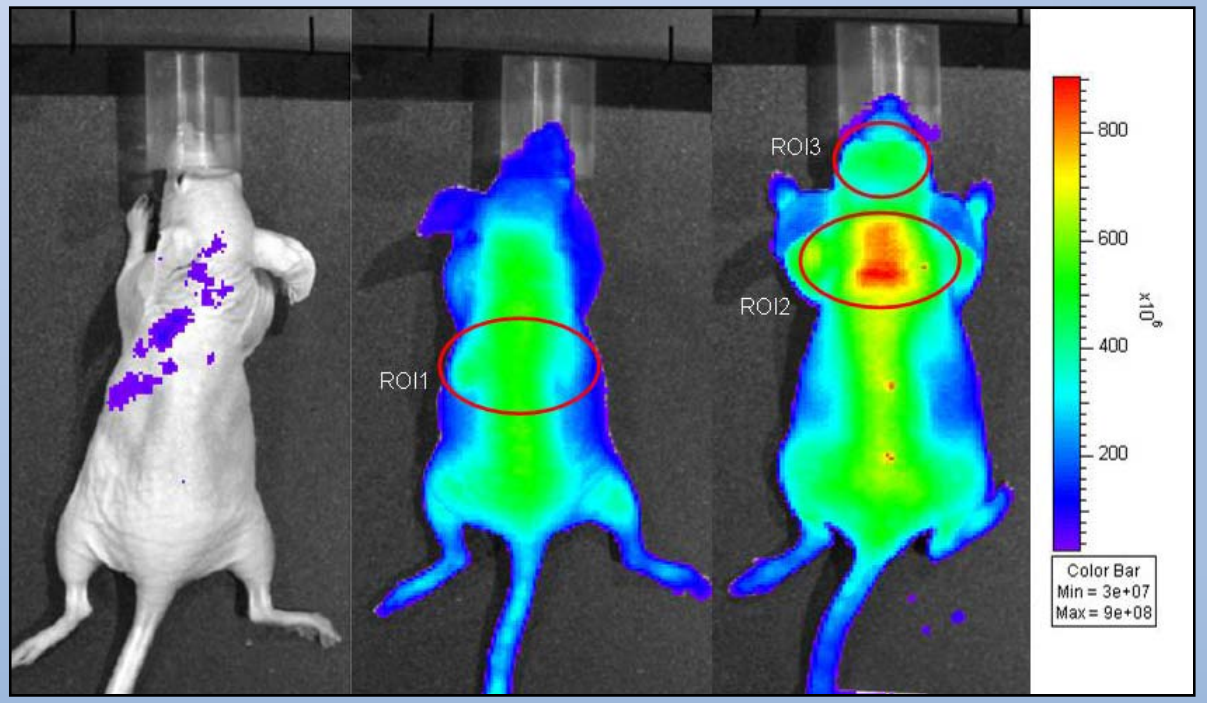
Magnetic-fluorescent Bioconjugates: EELS line



Magnetic-fluorescent Bioconjugates: Optical Imaging

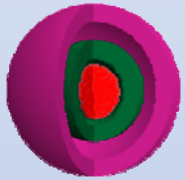


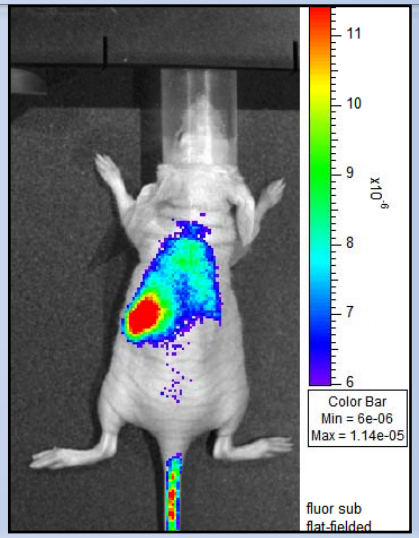
In vivo OI



t=0 t=1h t=3h

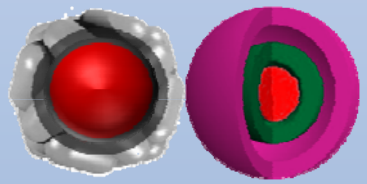
Magnetic-fluorescent Bioconjugates: Optical Imaging

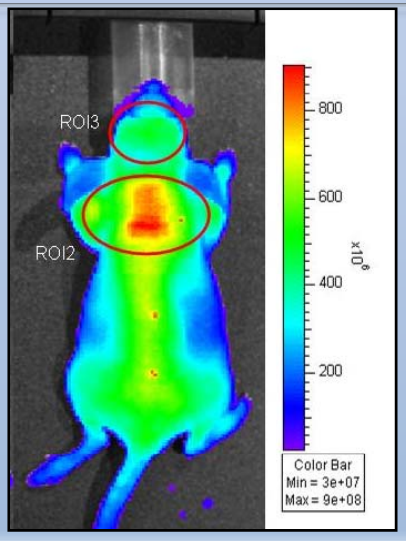




Free QD800 is detected early by Mononuclear Phagocite System (MPS):

- Short plasma half-life
- No biodistribution

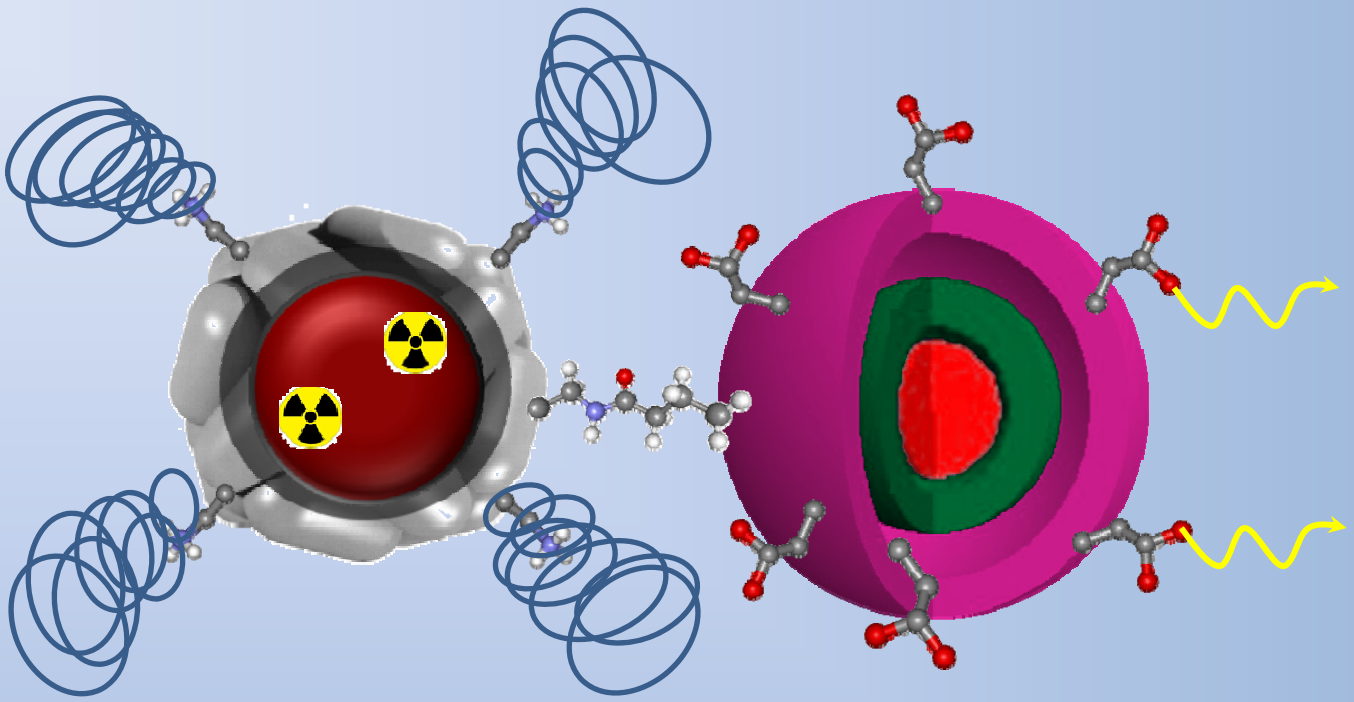




QD800+Ferritin:

- High plasma half-life
- Biodistribution

Toward multi(more than two)modal nanoparticles



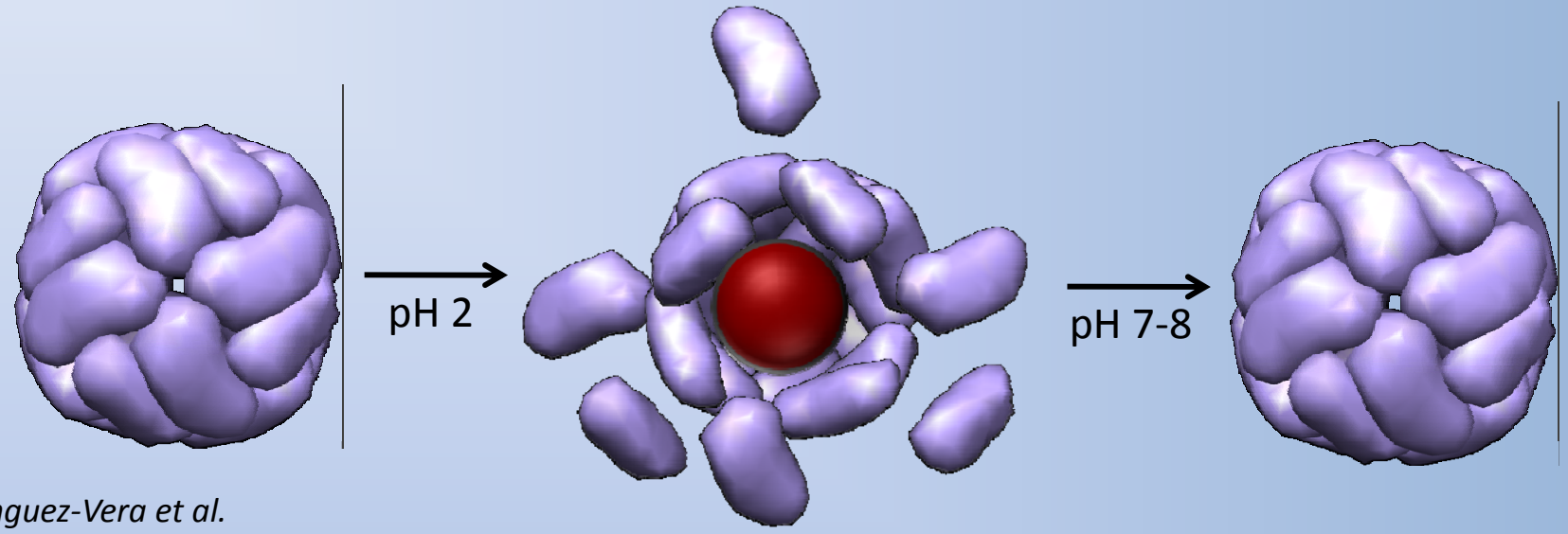
magnetite (↑M/g, ↑ r_2)

Hydrophobic polymers

Radionuclide *(Patent ES1634.24A)*

Targeting ligand *(Patent ES1634.24C)*

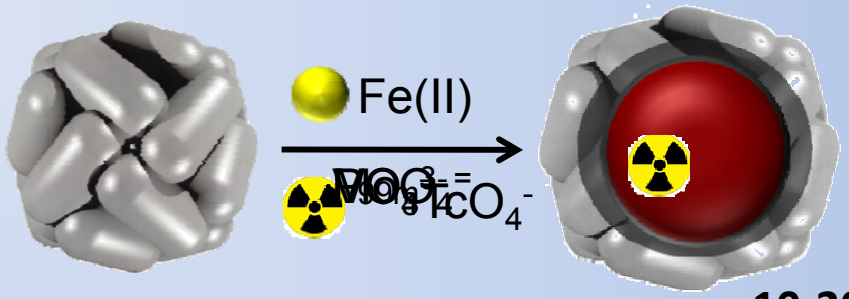
Some flash results: improving r_2



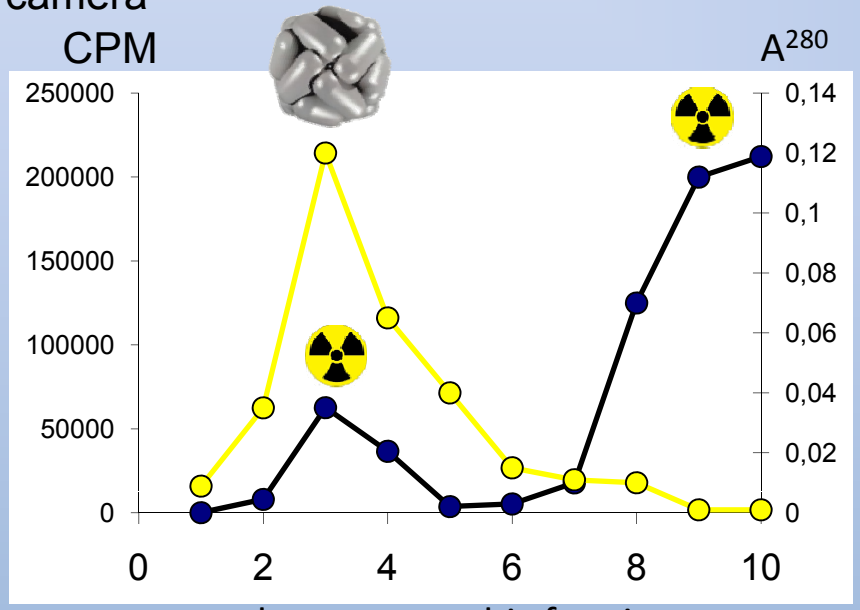
Domínguez-Vera et al.
Inorg. Chem. 2003, 42, 6983
J. Inorg. Biochem. 2004, 98, 469

Maghemite-ferritin r_2>100mM^{-1}s^{-1}
ferritin r_2<10mM^{-1}s^{-1}

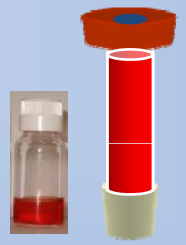
Some flash results: adding a new modality, SPECT



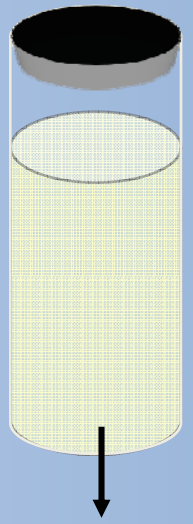
^{99m}Tc detected by γ camera



10-20 ^{99m}Tc / nanoparticle!
Cardiolite: 1 ^{99m}Tc / molecule

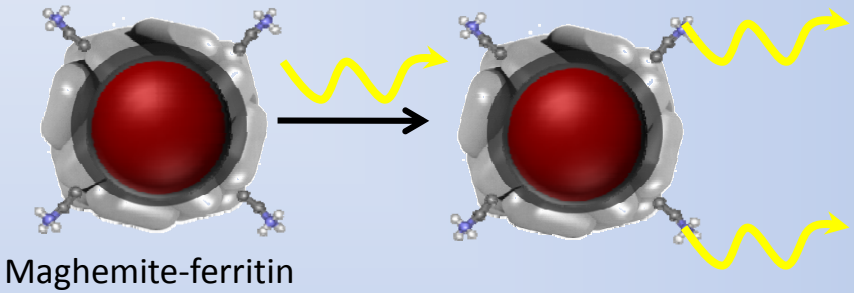


^{99m}Tc-magnetite (fraction 3)

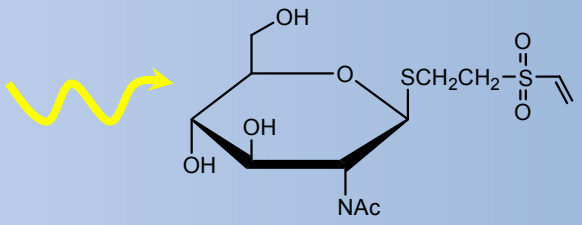


No free ^{99m}Tc after 3h

Some flash results: searching for specificity



Maghemite-ferritin



N-acetyl glucosamine

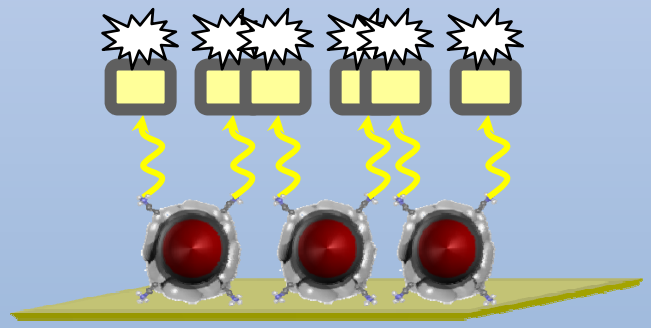
NGA

Test carbohydrate-lectin binding

NGA

WGA (hepatocytes)

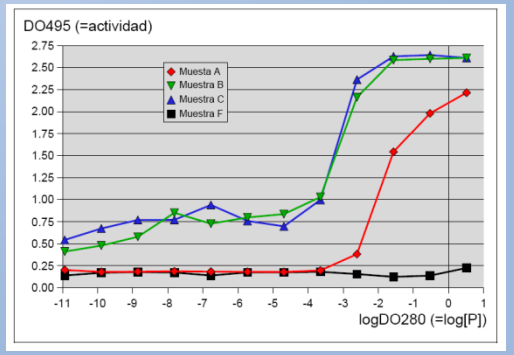
WGA-peroxidase

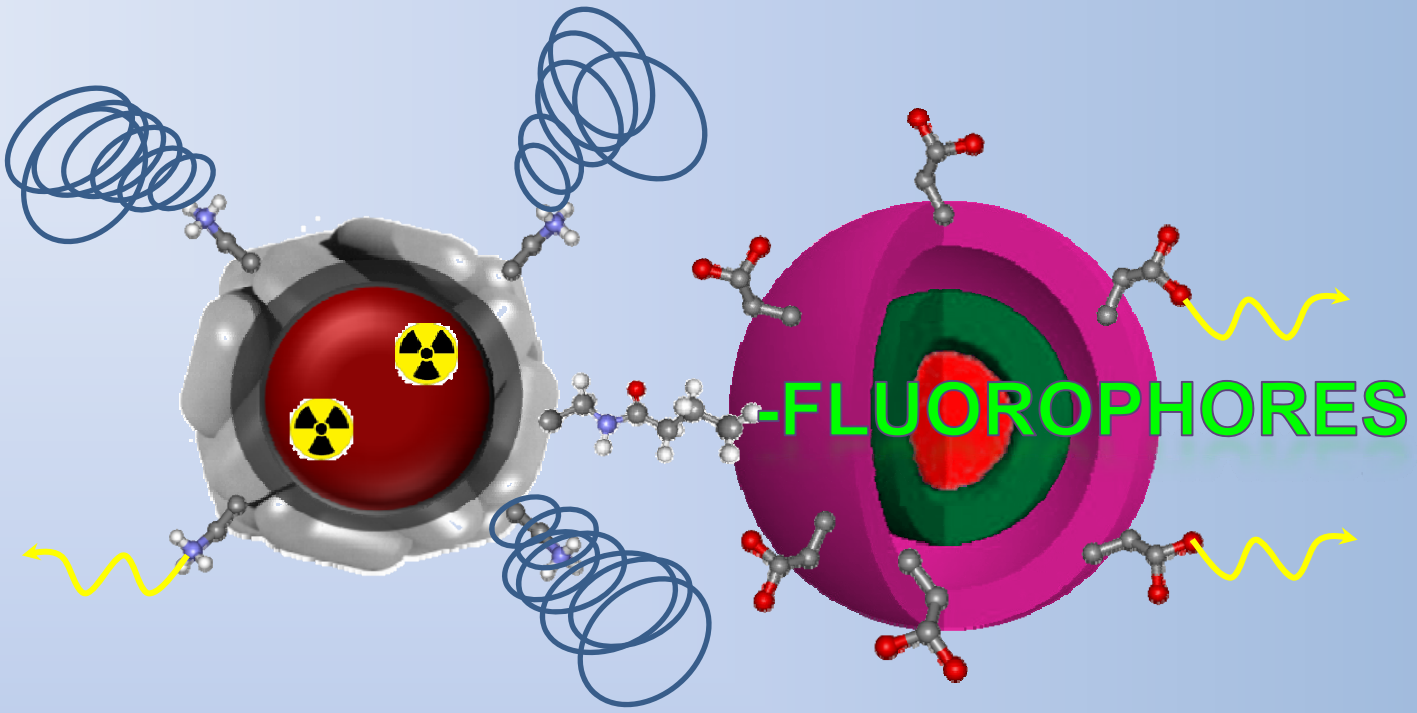


NGA/maghemite

[particles]

blank





NaNo-IKEA

for Multimodal Imaging

Thanks to:

Pasquina Marzola and Laura Calderan (MRI and Optical Imaging)
University of Verona, Italy

J. J. Calvino Group (Electron Microscopy)
Universidad de Cádiz, Spain

Roger Alberto Group (Radiochemistry)
University of Zurich, Switzerland

Paco Santoyo Group (carbohydrate targeting ligands)
Universidad de Granada, Spain