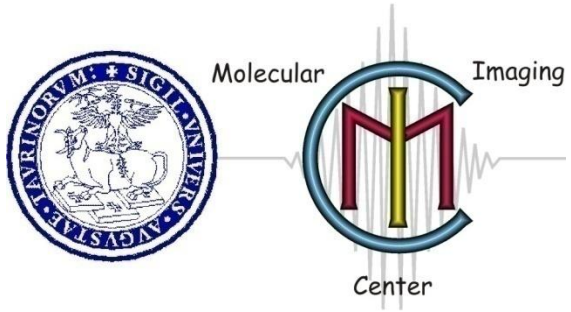


Strategies for cellular labeling

Simonetta Geninatti Crich

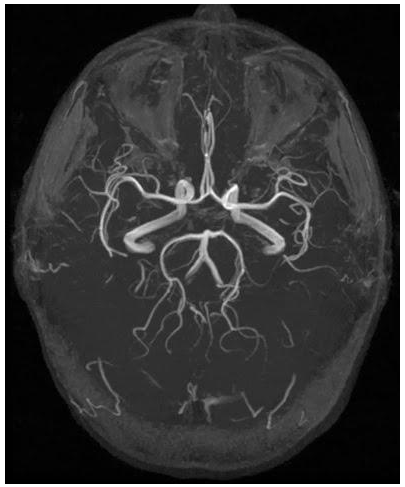
Strategies for cellular labeling

"IN VIVO"

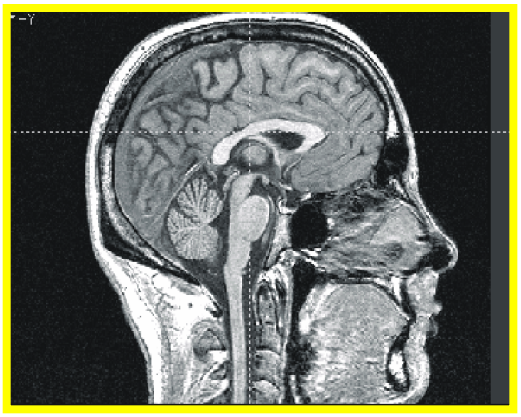
Labelling of targeted cells and visualization of cellular processes in living organisms.

"IN VITRO"

Monitoring of migration and location of transplanted cells labeled "in vitro" with contrast agents.

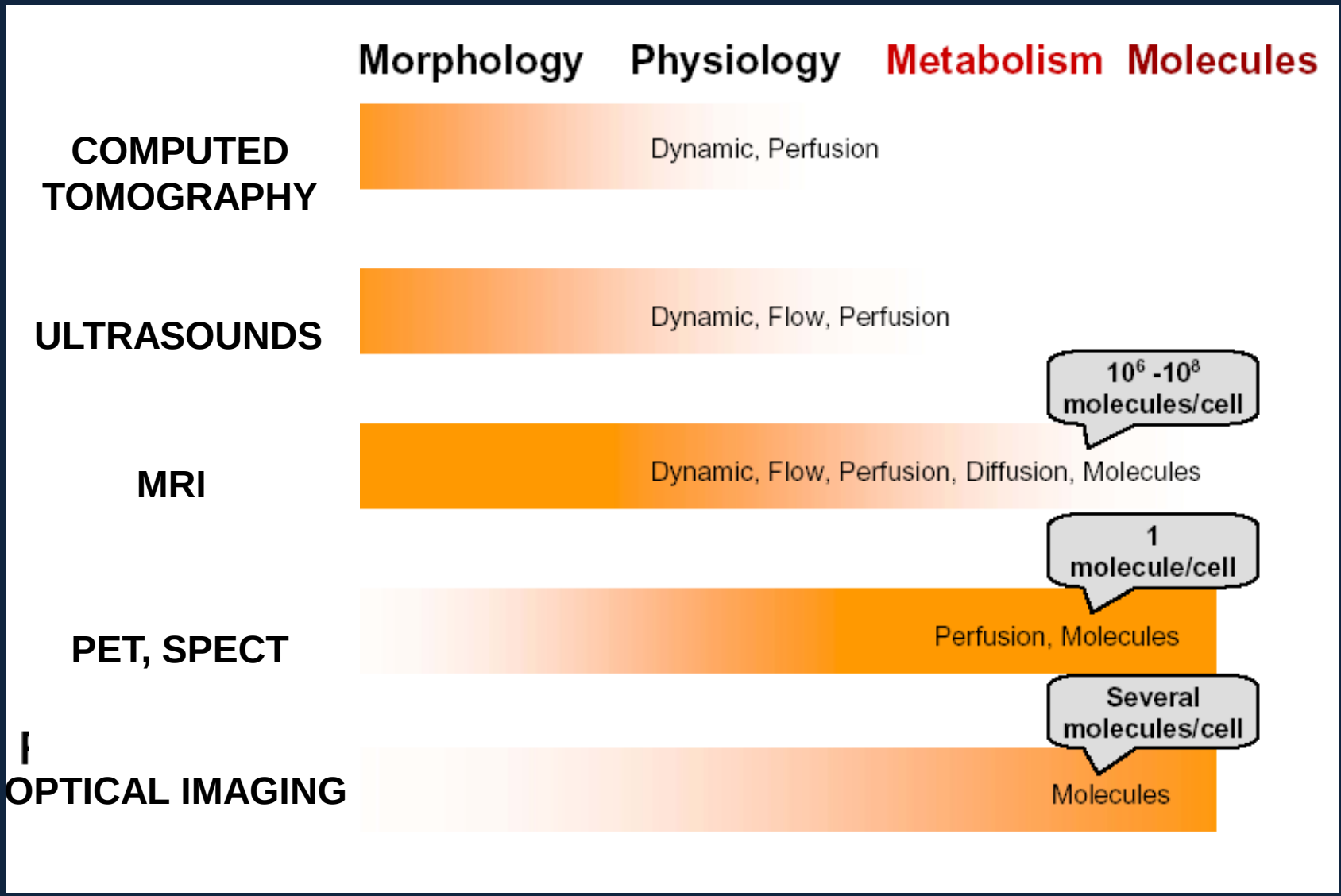


Magnetic Resonance Imaging



-Non invasive and repetitive imaging
-High resolution
-Absence of radiation
-Low sensitivity

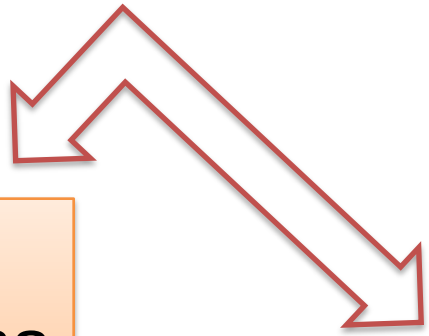
Imaging Modalities: range of detection



Courtesy of H. Siebold, Siemens Medical Solutions

MRI Contrast Agents

paramagnetic species



Complexes of paramagnetic metal ions (Gd^{3+},Mn^{2+})

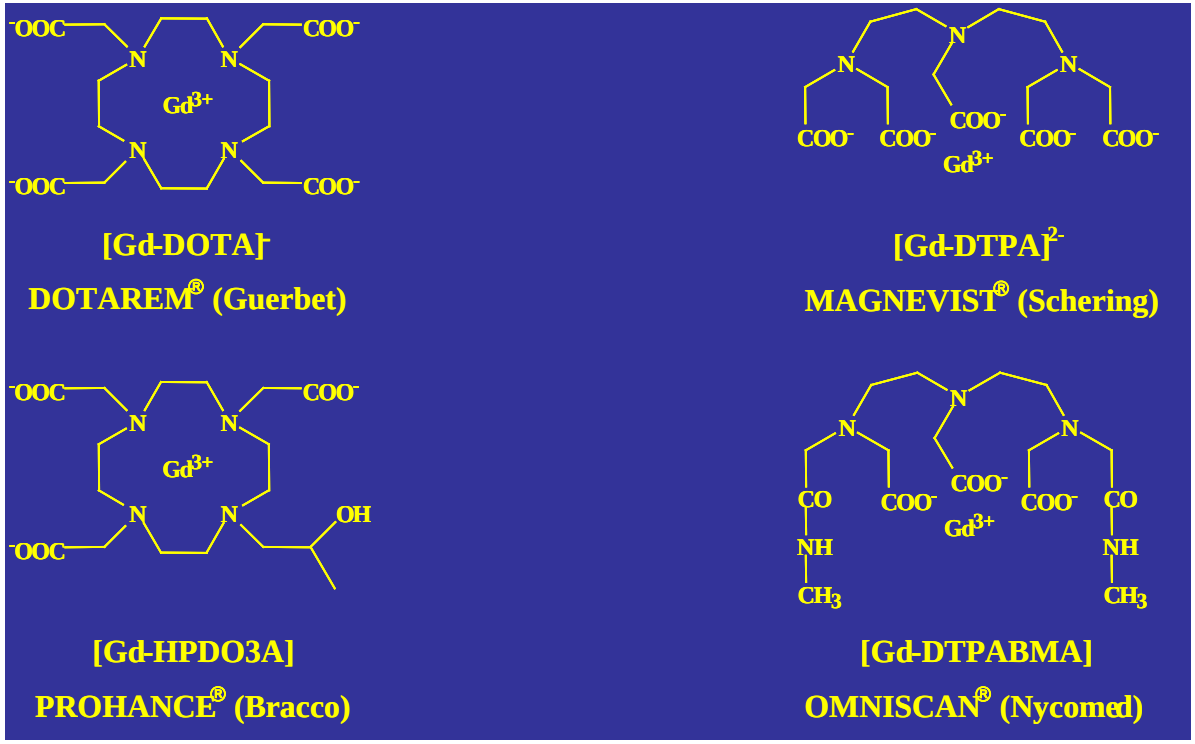
-Positive Contrast

Superparamagnetic particles (SPIOs,USPIOs)

-Negative Contrast

Clinical MRI Contrast Agents

Clinical dose 0.1 mmol/Kg

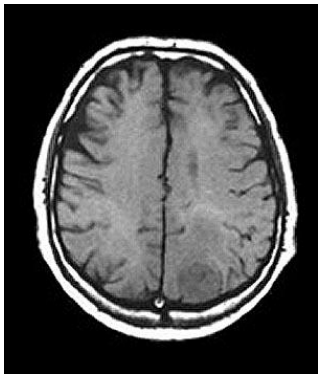


Concentration needed to obtain sufficient contrast

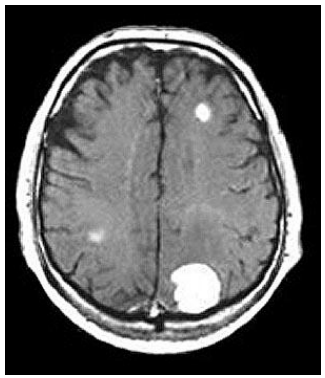
10^{-4}- 10^{-5}M

Concentration of target receptors

10^{-6}-10^{-10} M



Without CA



With Gd(III)-based CA

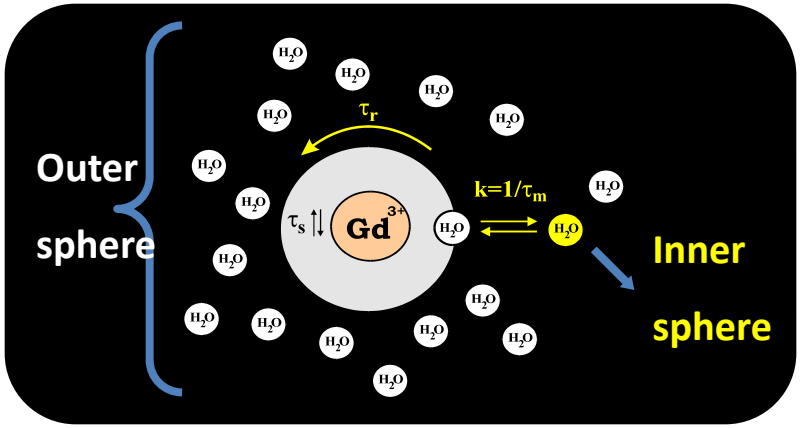
Nunn Q J Nucl Med 1997, 41, 155-162.

Can Gd^{3+} based probes be considered good candidates for MR Molecular Imaging applications?

Designing Highly Sensitive Agents ; optimization of parameters that influence relaxivity

$R1obs \propto R1(inner\ sphere)+R1(second\ sphere)$

(τ_r, τ_M, q)



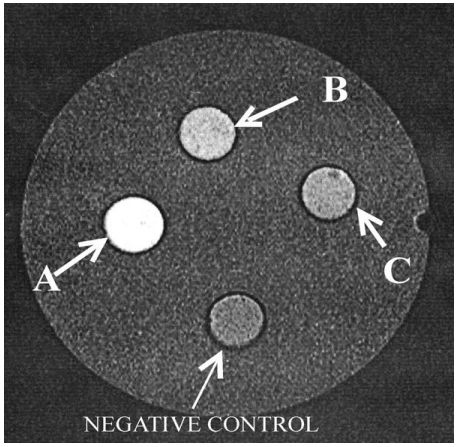
Increasing the MR-contrast agent concentration at the target site

-nanosized systems
-high capacity receptors

The minimum amount of Gd-probe necessary to visualize target Cells depends on its relaxivity

Empirical Equation:

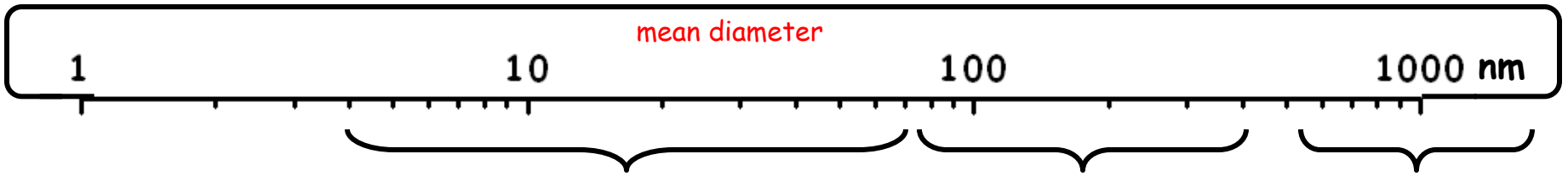
$$r_{1p} \times N° \text{ Gd-probe/cell} \geq 10^9$$

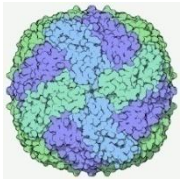


T1-weighted MR image of a phantom formed by three pellets of cells hepatocytes incubated (six hours) in media containing increasing amounts of Gd-DTPAIOPsp, and a negative control. The brightness in the image increases as the amount of internalized Gd(III) complex increases.

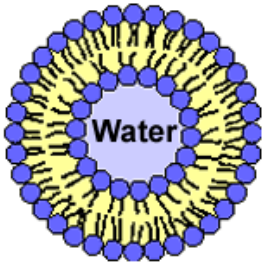
Aime S et al J Magn Reson Imaging. 2002 Oct;16(4):394-406.

Nanosized carriers for MRI probes

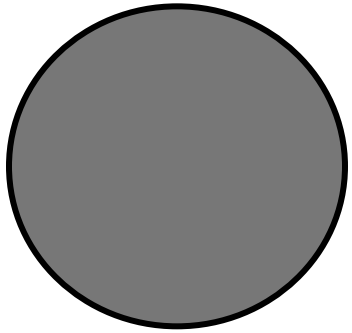




Proteins
(Apoferritin
Virus capsides,…)

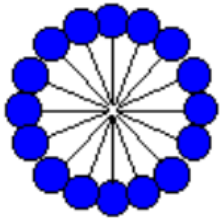


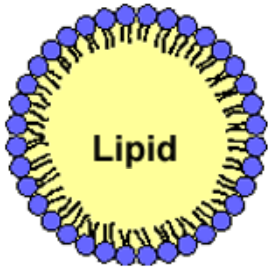
Liposomes



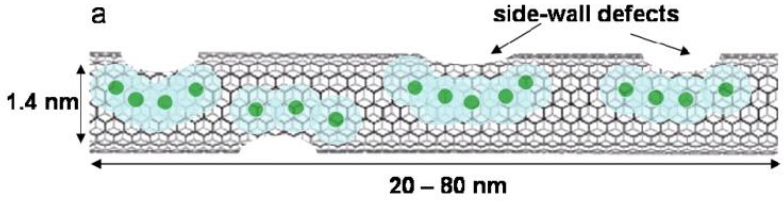
Micronized
solid particles

Micelles

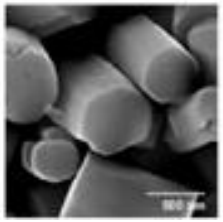




Nanoemulsions
(PFCarbon)

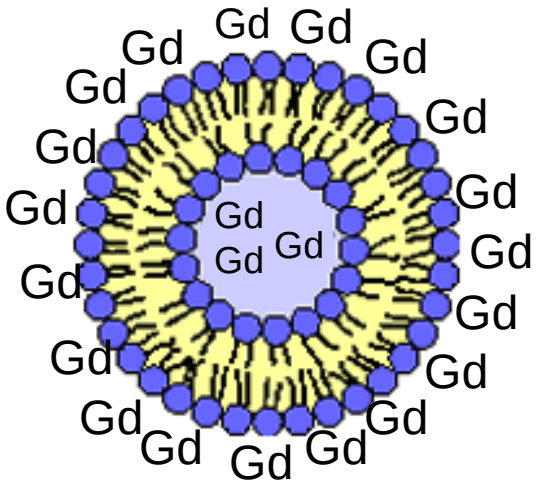


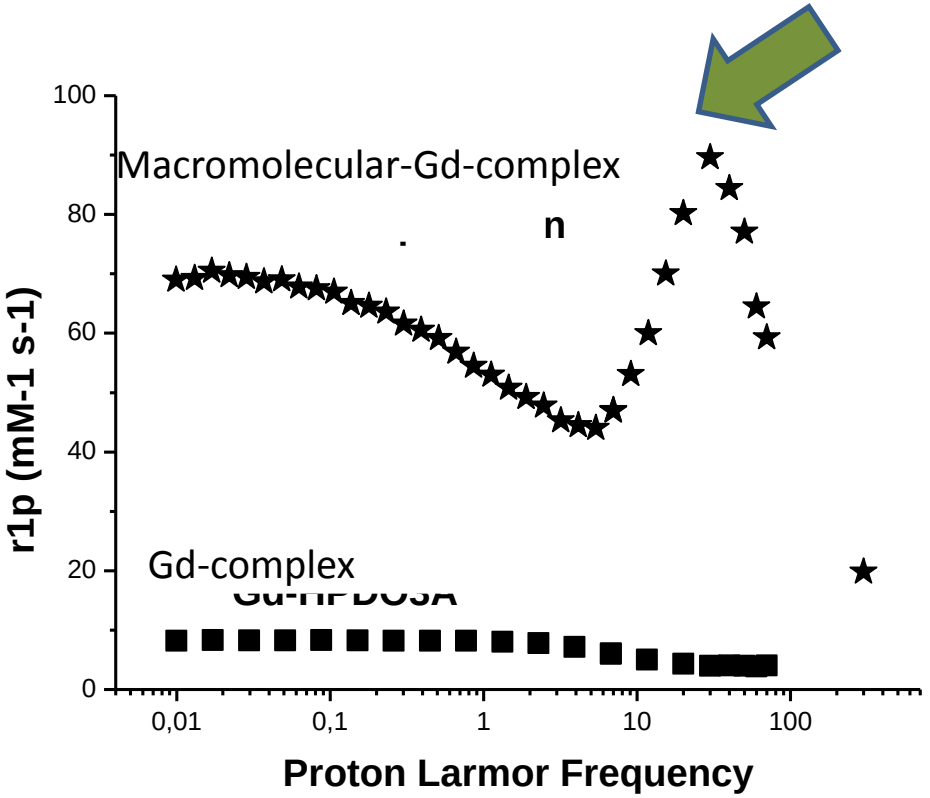
SW Carbon nanotubes



Gd-loaded Zeolites

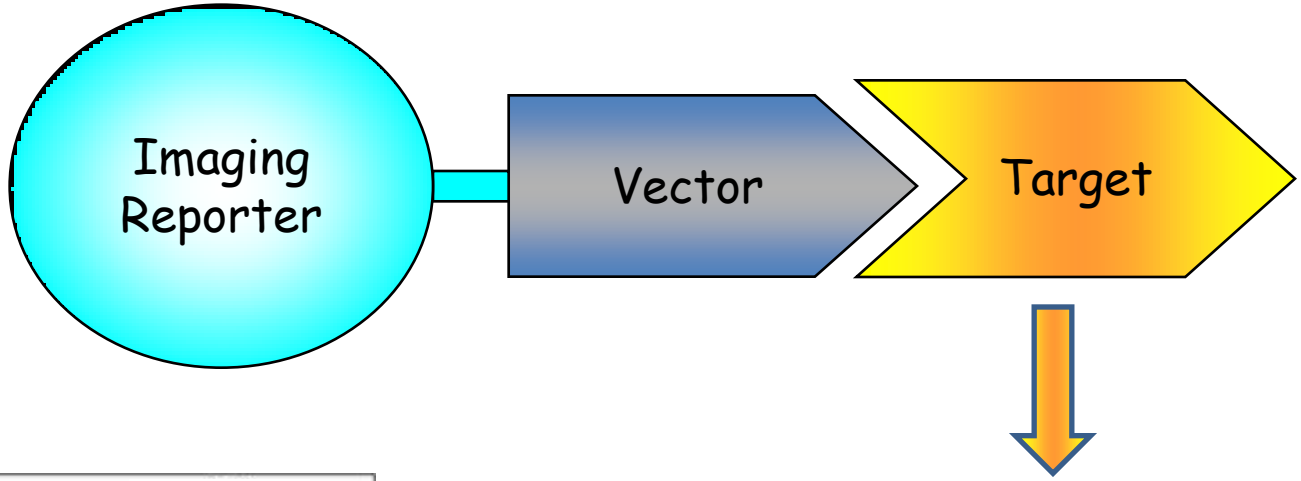
NMRD profiles of Macromolecular systems

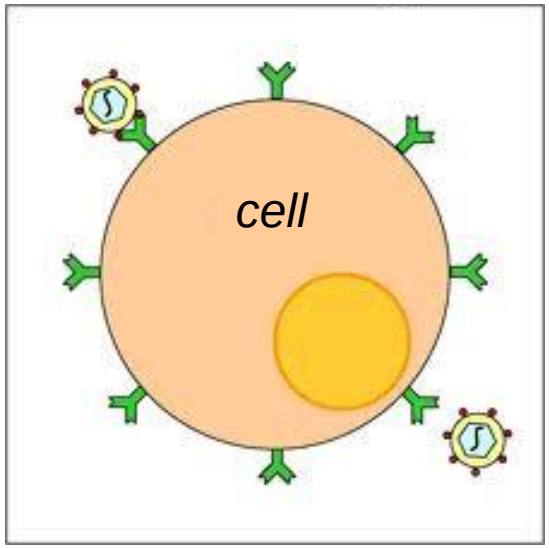




Higher relaxivity at low fields (0.5<Tesla<1.5)

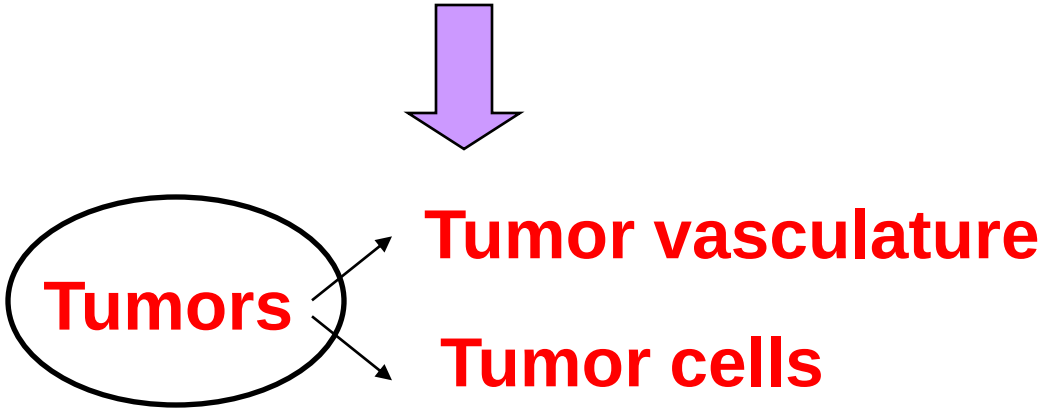
"In vivo" molecular imaging





- Overexpressed or downregulated specific cell receptors
- High capacity transporters
- Molecular processes (enzymes)

Biological Targets

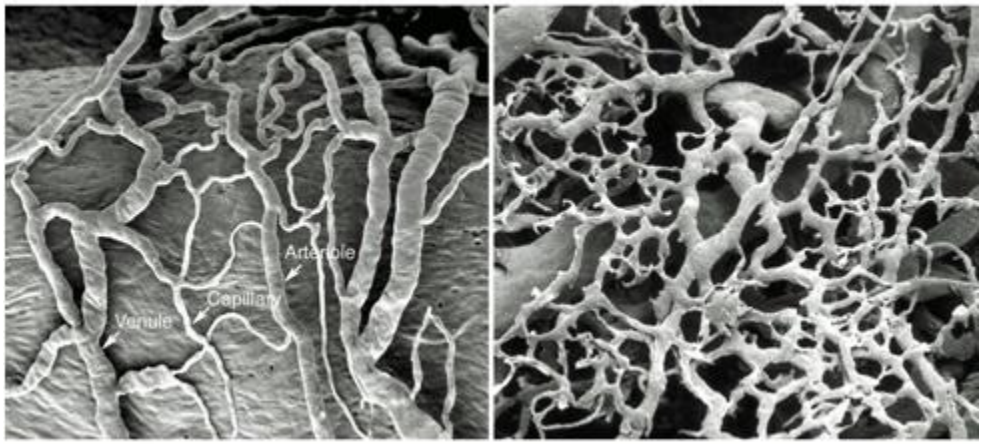


Imaging of Tumor Angiogenesis

- The identification of vessels in tumors is extremely important in oncological research.

- Imaging of angiogenesis have an additional utility in assessing the efficacy of angiogenic inhibitors used in the treatment of cancer.

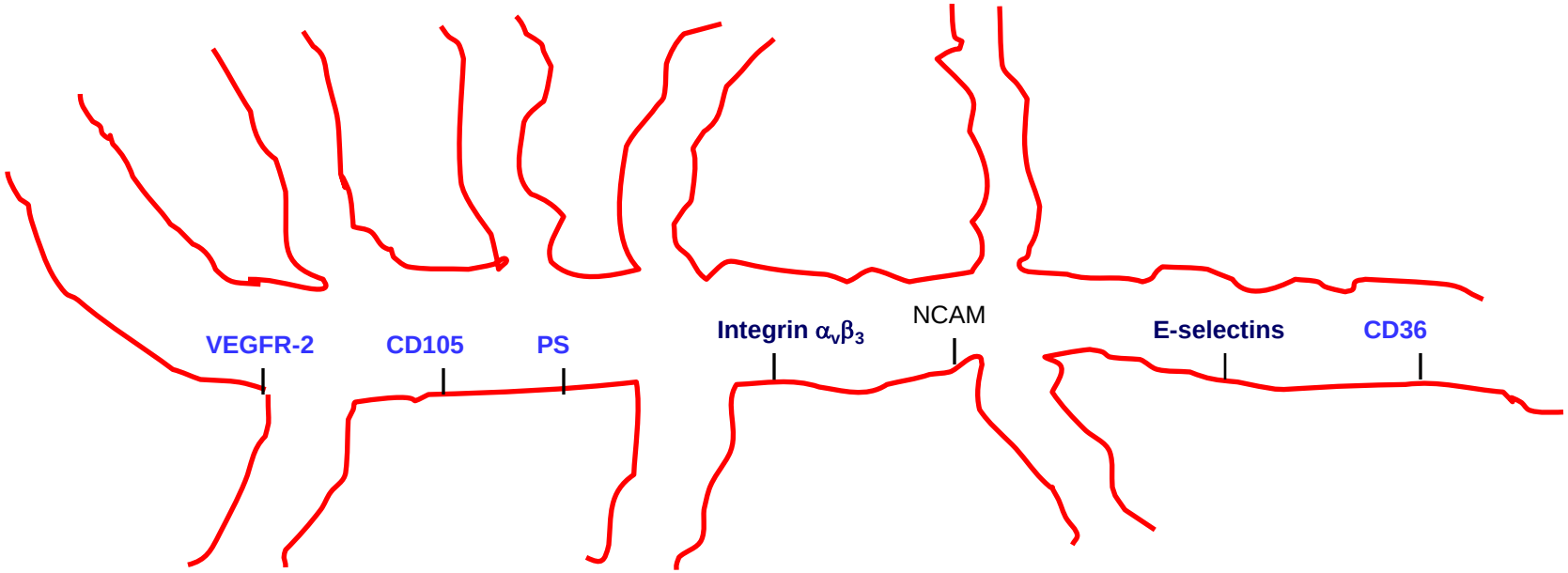
Normal Microvasculature



Tumor Microvasculature

McDonald DM, Nat Med. 2003; 9:713-25.

Molecular Biological Markers of Neoangiogenesis



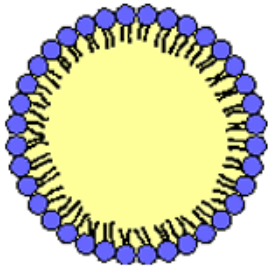
High accessibility of these markers

→

Large molecular size (nanosize) of Contrast Agents

Targeting of $\alpha_v\beta_3$ Integrins

Theragnostics for tumor and plaque angiogenesis with perfluorocarbon nanoemulsions

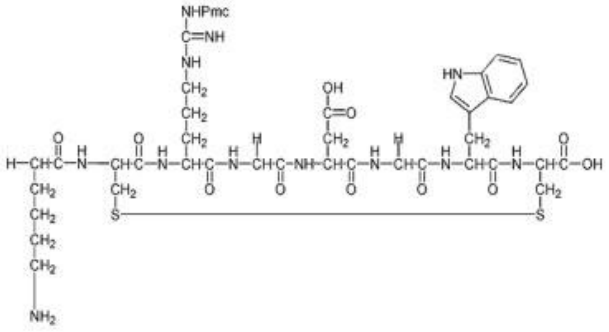


Nanoemulsions (PFCarbon)

PFC NPs are constrained within the vasculature during the targeting phase, which makes them ideal candidates for specific homing to intravascular biosignatures, such as integrins, selectins, or adhesion molecules.

Particle size (nm) 280
20% (v/v) perfluorooctylbromide
30 % Gd-DOTA-PE/particle (100000)
r1 $(s*mM)^{-1}$ [Gd] 12.5
r1 $(s*mM)^{-1}$ [Particle] 1,6 $x10^6$
Gd-perfluorocarbon nanoparticles
Covalently coupled to the 3-integrin peptidomimetic antagonist

A $\alpha_5\beta_1$**(RGD) peptide sequence (CRGDGWC)**



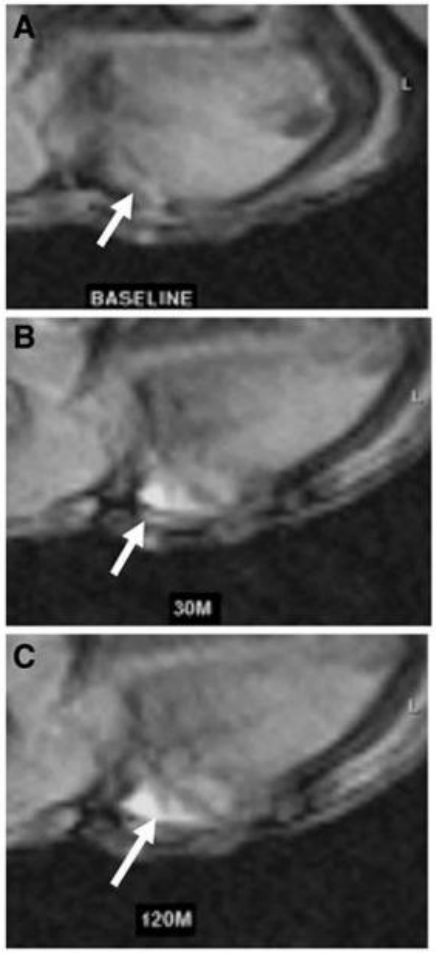
***-Lanza** et al.* Angiogenesis. 2010

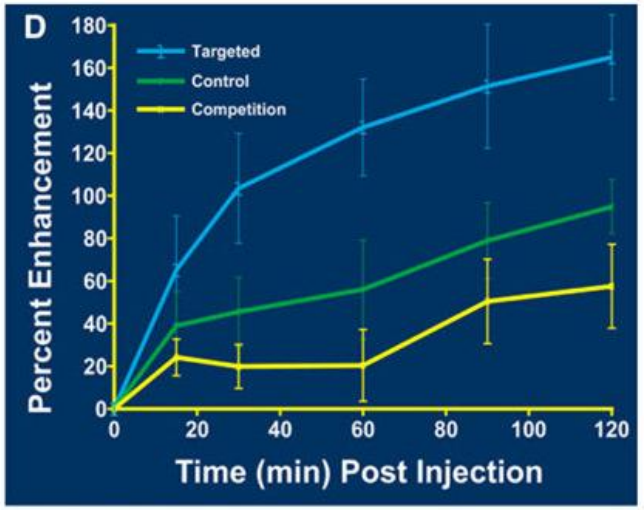
T1-weighted MR image (*axial view, 1.5 T) of a nude mouse before injection of αvβ3-targeted paramagnetic nanoparticles.*

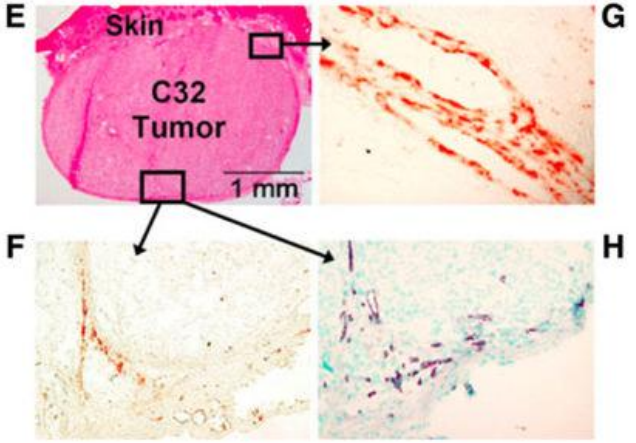
PRE

30’

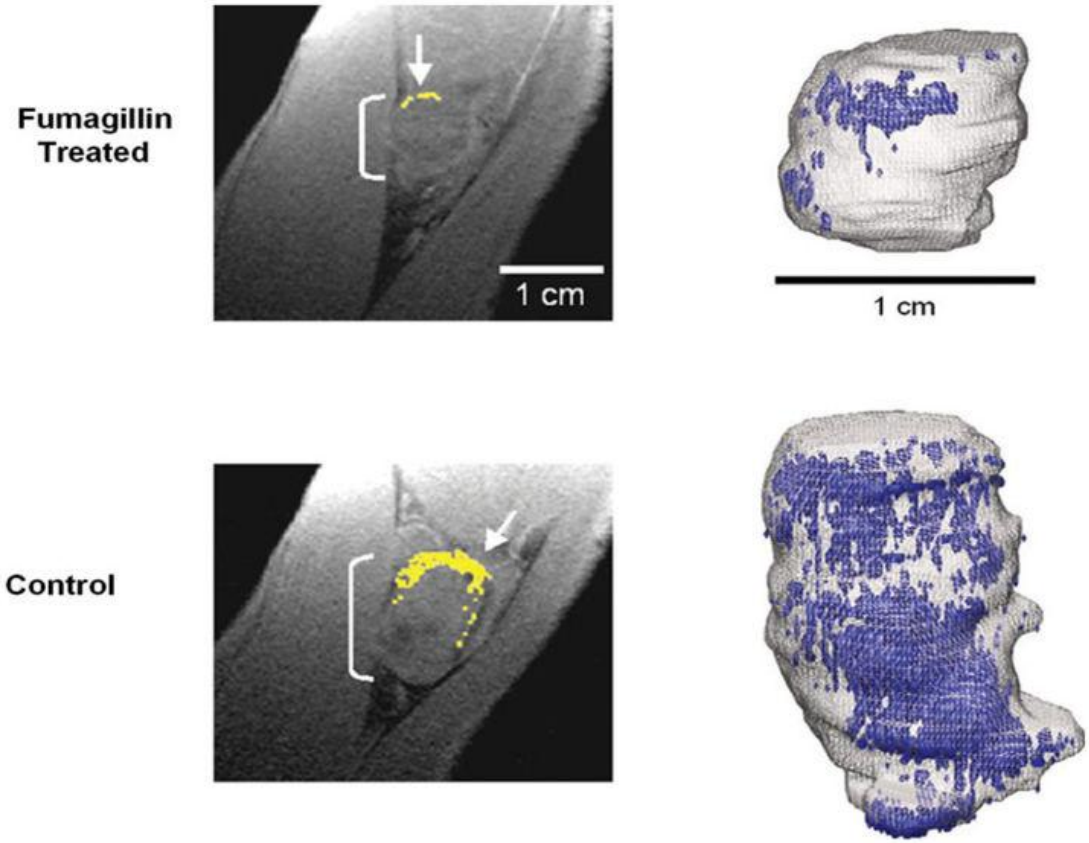
120’







Diminished *αvβ3 integrin contrast enhancement in T1-weighted, fat-suppressed, 3D gradient* echo MR single slice images in rabbits administered *αvβ3-targeted fumagillin nanoparticles (top) versus* those given *αvβ3-targeted nanoparticles without drug (bottom).*

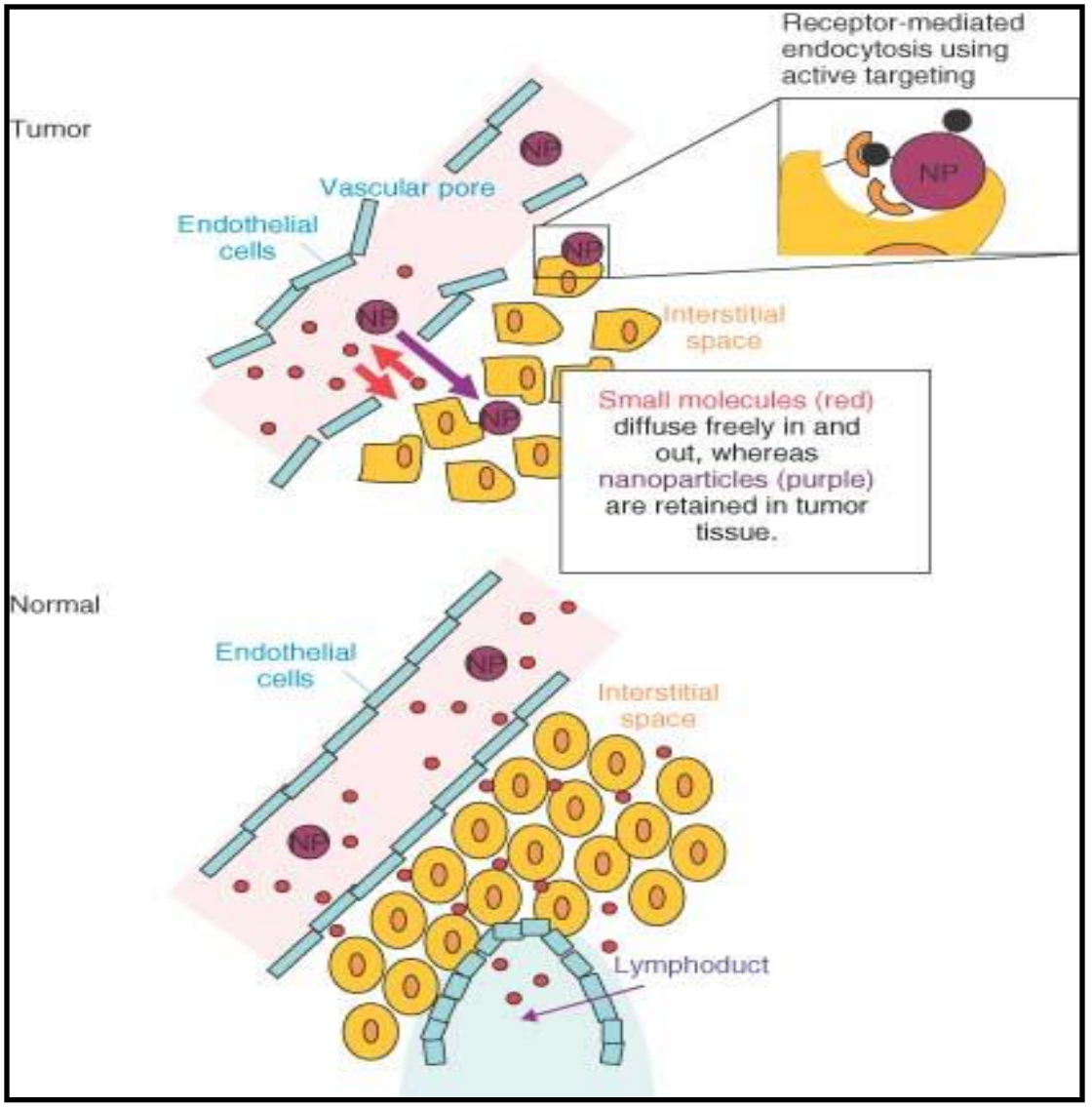


2) Imaging of Tumor Cells

-The visualization of different cell surface targets in solid tumors needs the extravasation of the MR contrast agents.

-In solid tumors the vessels formed by the process of angiogenesis show an Increase permeability due to large fenestrae (up to 400 nm)

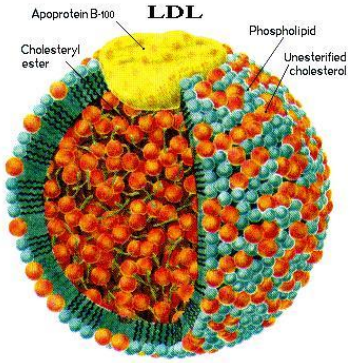
-**N**ormal vasculature endothelium consists of a continuous lining of endothelial cells tightly connected with each other.



Osamu I. et al International Journal of Pharmaceutics 190 (1999) 49–56
Pavan P. Nanomedicine and Nanotechnology, 2010

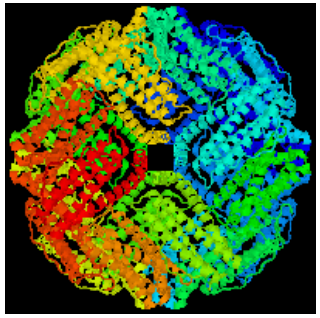
Naturally occurring biological nanocarriers loaded with paramagnetic ions

-**Lipoproteins**



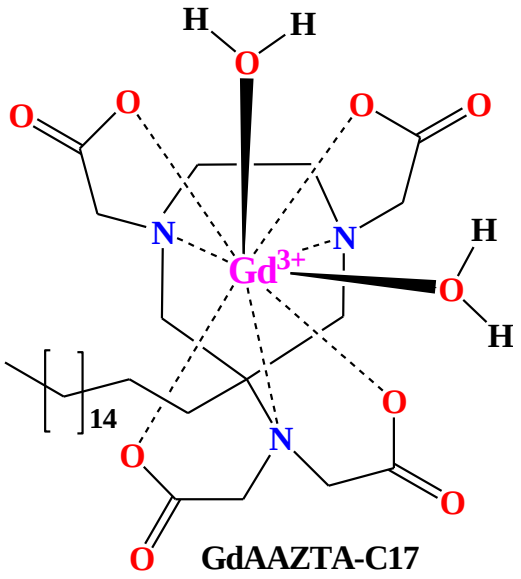
22 nm

-**Apoferritin**

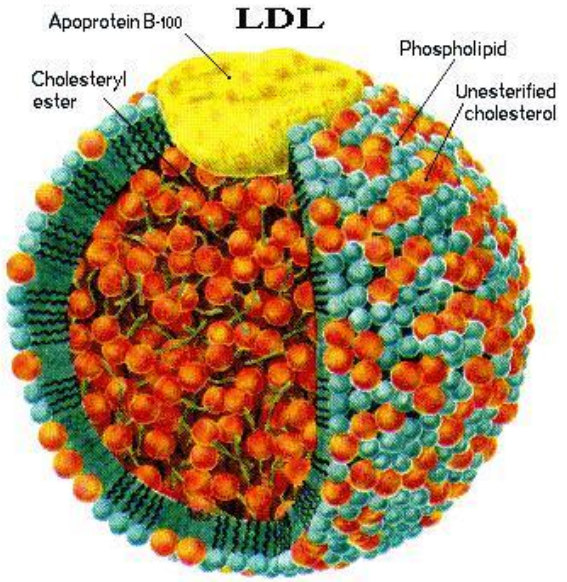


12 nm

The macromolecular imaging probe: Low Density Lipoproteins (LDL)



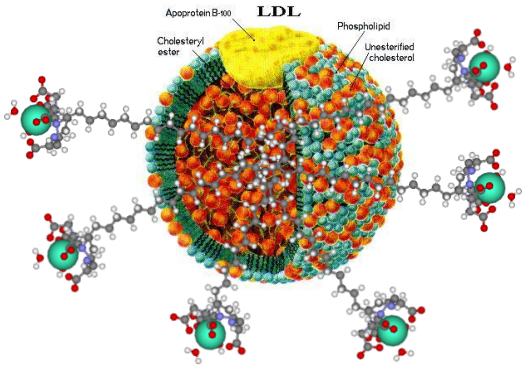
GdAAZTA-C17



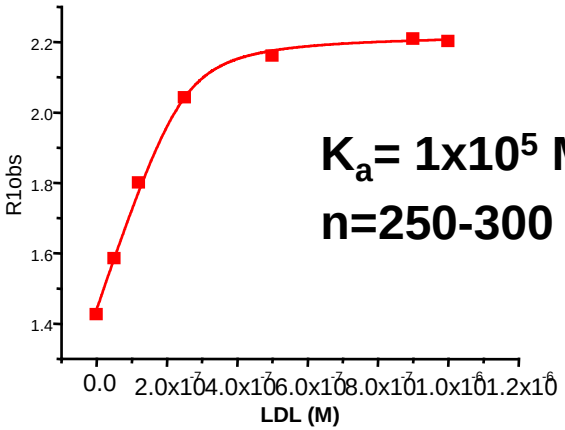
r_{1p} = 10.5 $mM^{-1}s^{-1}$ (20 MHz, 298 K)
(free complex<0.1mM)
q=2
τ_M = 70 ns
$\log\beta_{Gd\text{-}L}$ = 19.3

size = 20 nm

S. Aime, et al.; ***Inorganic Chemistry*** 2004, 43, 7588
S. Baranyai et al.; **Chemistry** 2009 15, 1696



Gd-AAZTA-C17/LDL macromolecular adduct

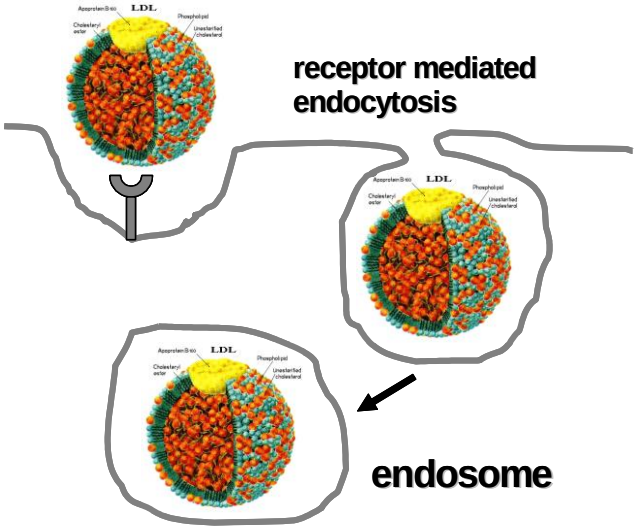


$K_a = 1x10^5\ M^{-1}$

n=250-300

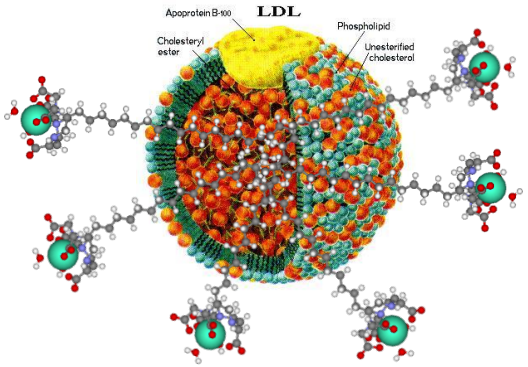
Size:
Native LDL = 21 2 nm
Gd-AAZTA-C17/LDL = 22 2 nm

altered LDL Receptors levels are found in tumor cells over-expressing LDL-R to supply the high cholesterol demand

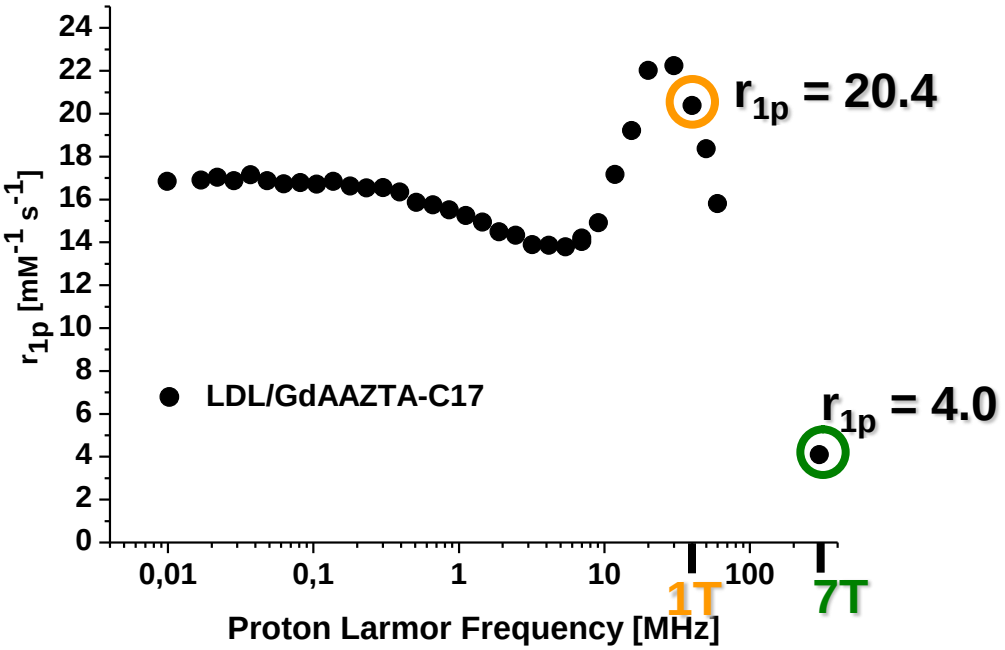


S. Geninatti Crich, et al.; ***Neoplasia*** 2007, 9, 1046

NMRD PROFILE OF Gd-AAZTA-C17/LDL



Gd-AAZTA-C17/LDL macromolecular adduct



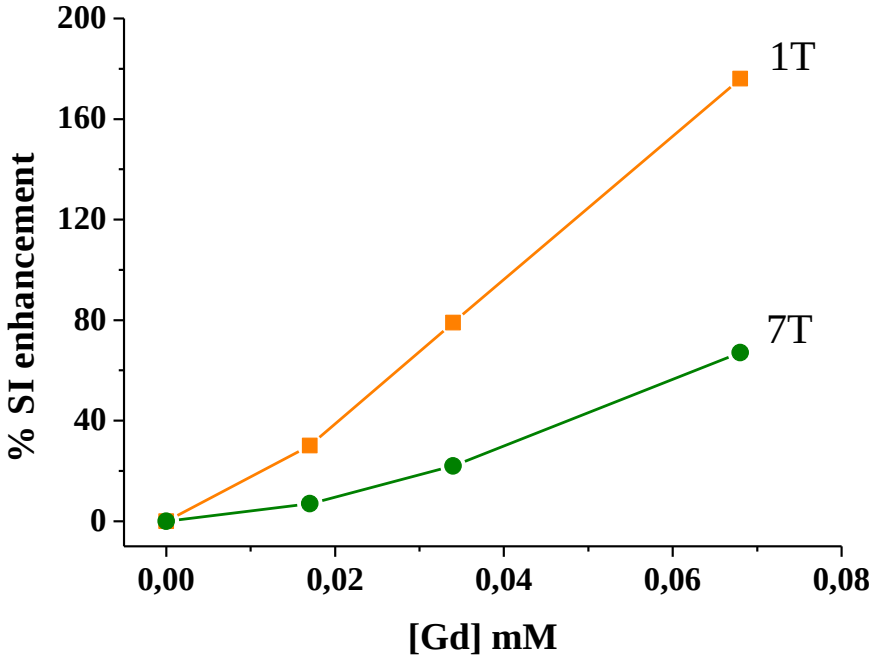
r_{1p} of the **Gd-AAZTA-C17/LDL macromolecular adduct**

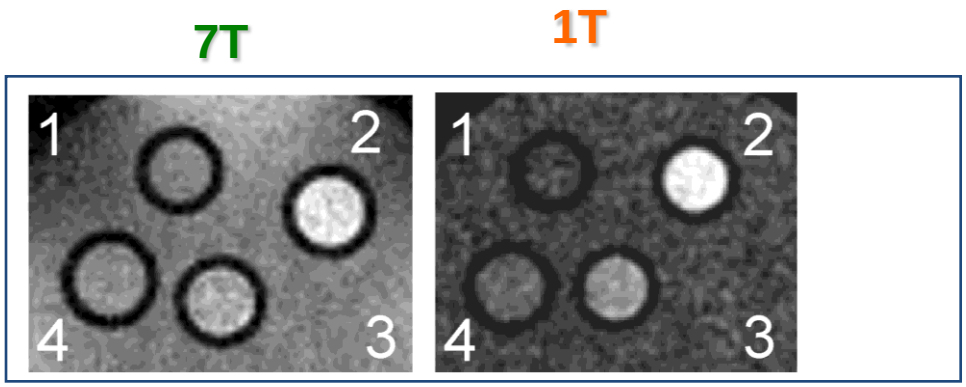
6150 mM^{-1} s^{-1} at 1T and 1200 mM^{-1} s^{-1} at 7T

IN VITRO RESULTS ON B16-F10 melanoma

cell culture – uptake

- incubation at 37 °C – 24h with 10% LPDS
- incubation with 60 μg/ml adduct for 16h
- washed 3x PBS, detached trypsin/EDTA
- transferred into glass capillaries
- Gd content determined by ICP-MS
- cell viability assessed by colorimetric assay (WST-1)

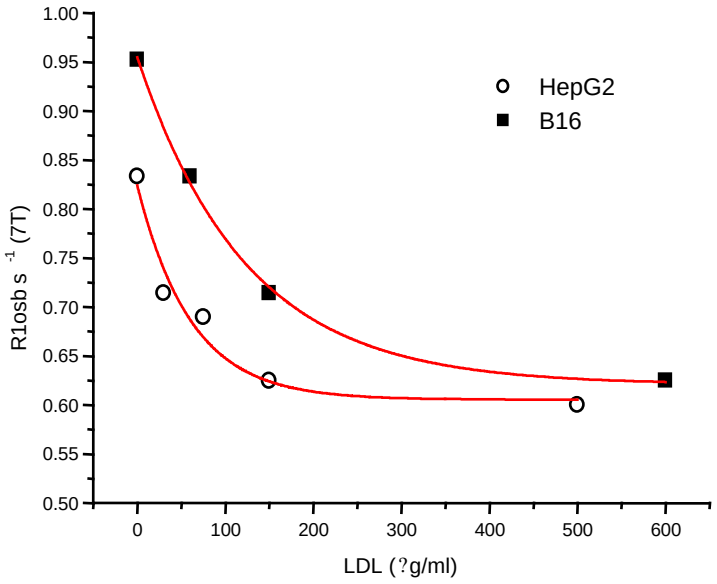




T_1-weighted spin echo image, recorded at 1 and 7T, of an agar phantom containing B16 cells labeled with Gd-AAZTAC17/LDL adduct

	cells/μl	[Gd] mM	Measured % SI (1T)	Measured % SI (7T)
2	20000	0.068	176	67
3	10000	0.034	79	22
4	5000	0.017	30	7
1	ctrl	0	0	0

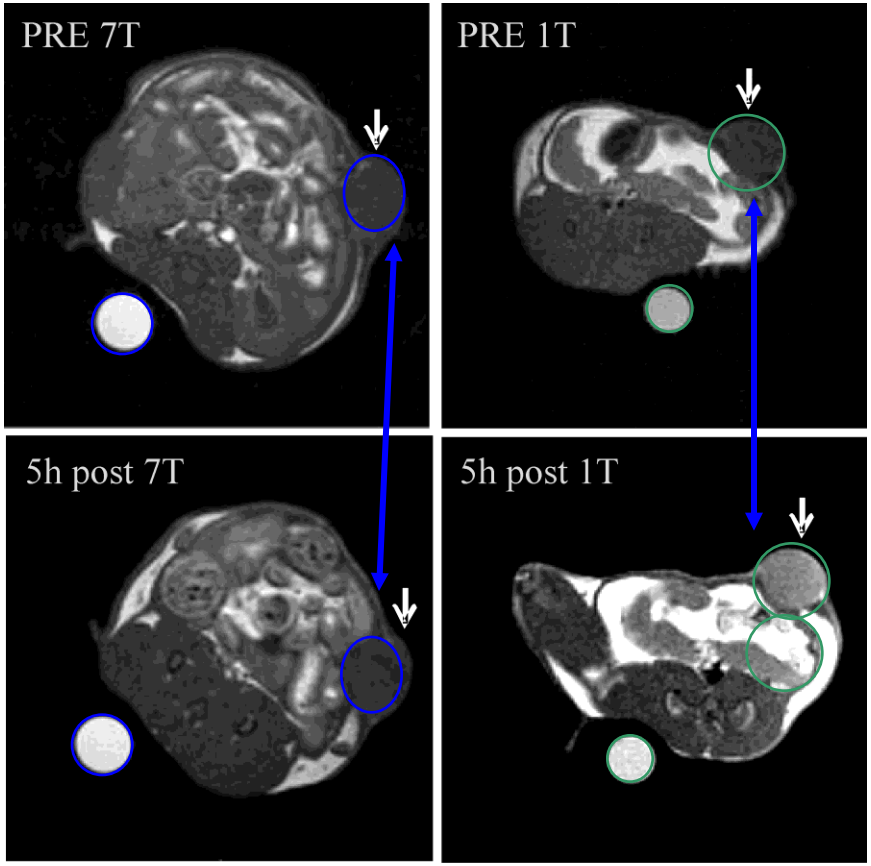
Competition study with unlabeled LDL

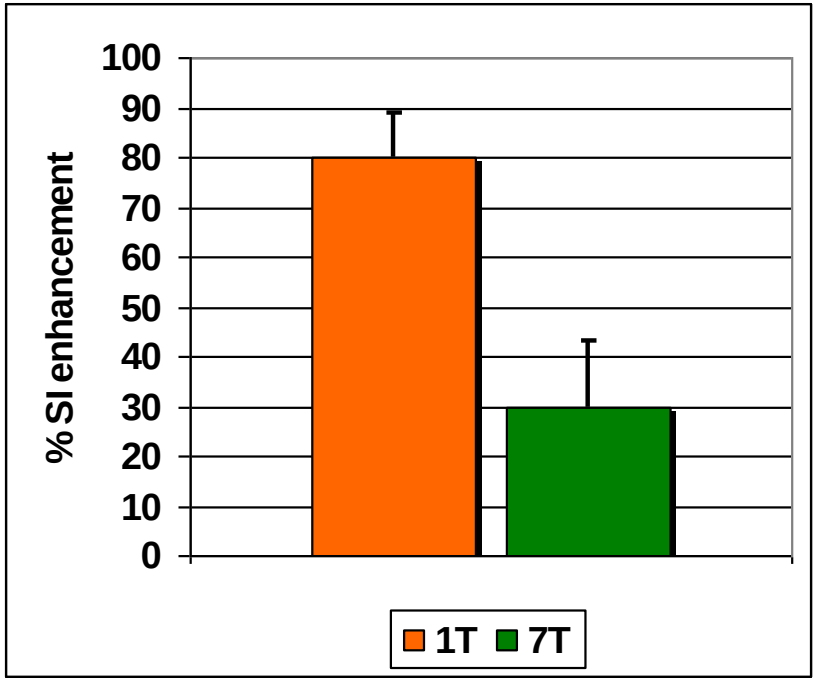


T_1-weighted multislice spin echo MR images of C57BL/6 mice grafted subcutaneously with B16 melanoma cells

PRE

POST





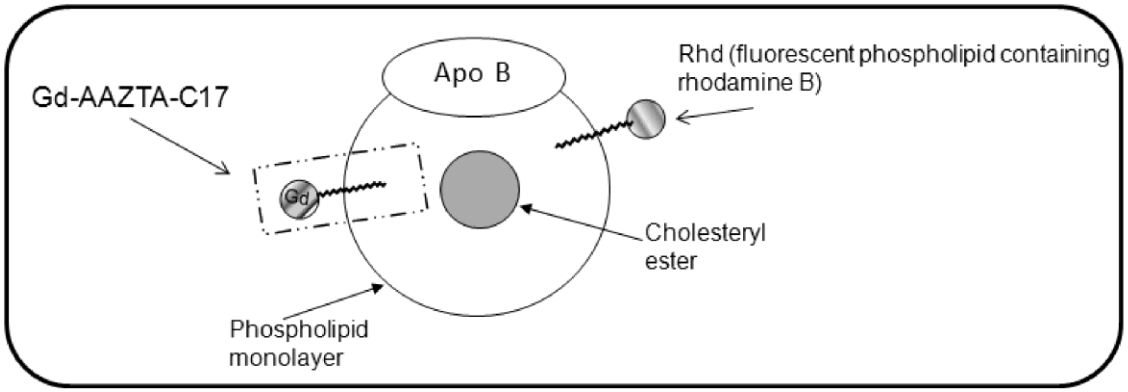
n=3

7T Bruker

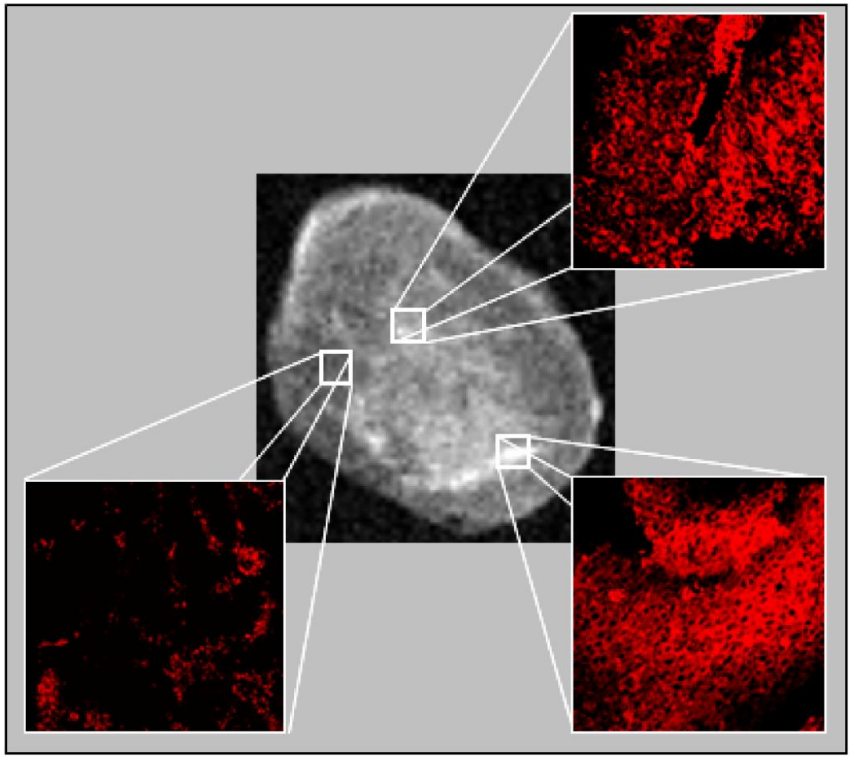
1T, Aspect

Images were obtained before (PRE) and 5 hours after (POST) the administration of Gd-AAZTAC17/LDL 0.06mmol/Kg
7T: TR/TE = 250/4 ms; in plane resolution: 78 μm
1T: TR/TE= 250/7 ms; in plane resolution: 78 μm

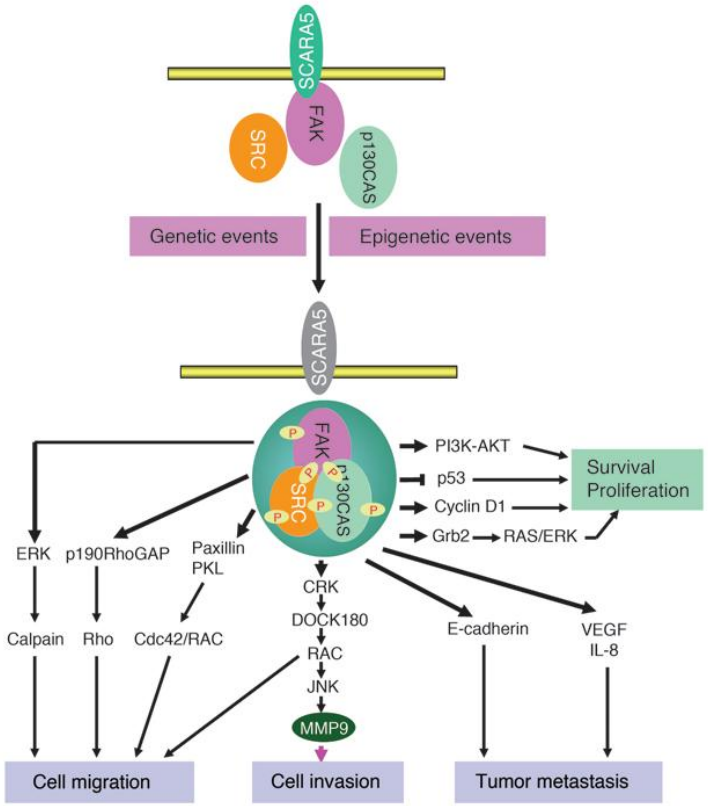
Ex vivo MRI and confocal analysis after administration of Gd-AAZTAC17/Rhd/LDL



The hyperintense areas detected in the MR images correspond to areas of greater Rhd fluorescence whereas in the other regions the Rhd signal is weak or completely absent.



Genetic and Epigenetic silencing of Scara5 may contribute to human Hepatocellular carcinoma by activating FAK signaling

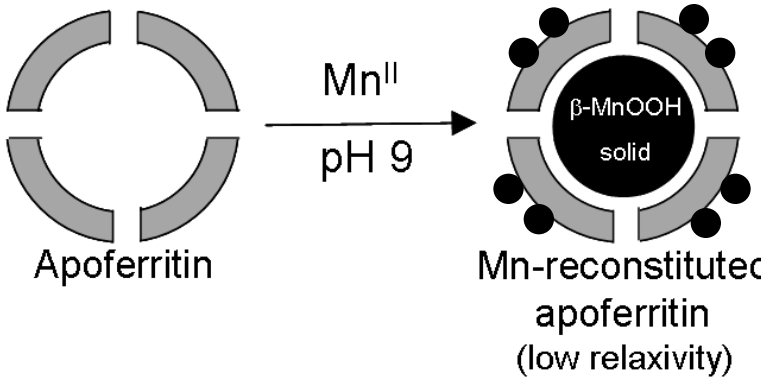


Scara5 Is a Ferritin Receptor Mediating Non-Transferrin Iron Delivery

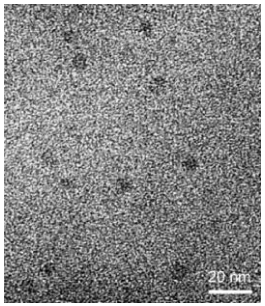
Can apoferritin loaded with paramagnetic ions act as MRI gene expression reporter ?

Jian Huang et al *The Journal of Clinical Investigation, 120 ,2010*

Reconstitution of Manganese Oxide Cores in Horse Spleen Apoferritin

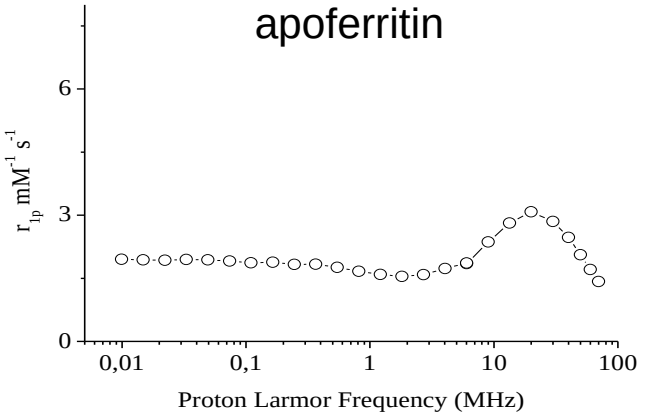


TEM image of Mn reconstituted apoferritin



- Mn(II) 5 unpaired electrons
- r_{1p}(20MHz)=8.0 mM-1s-1
- LD_{50} = 0.22 mmol/Kg
- Essential Ion

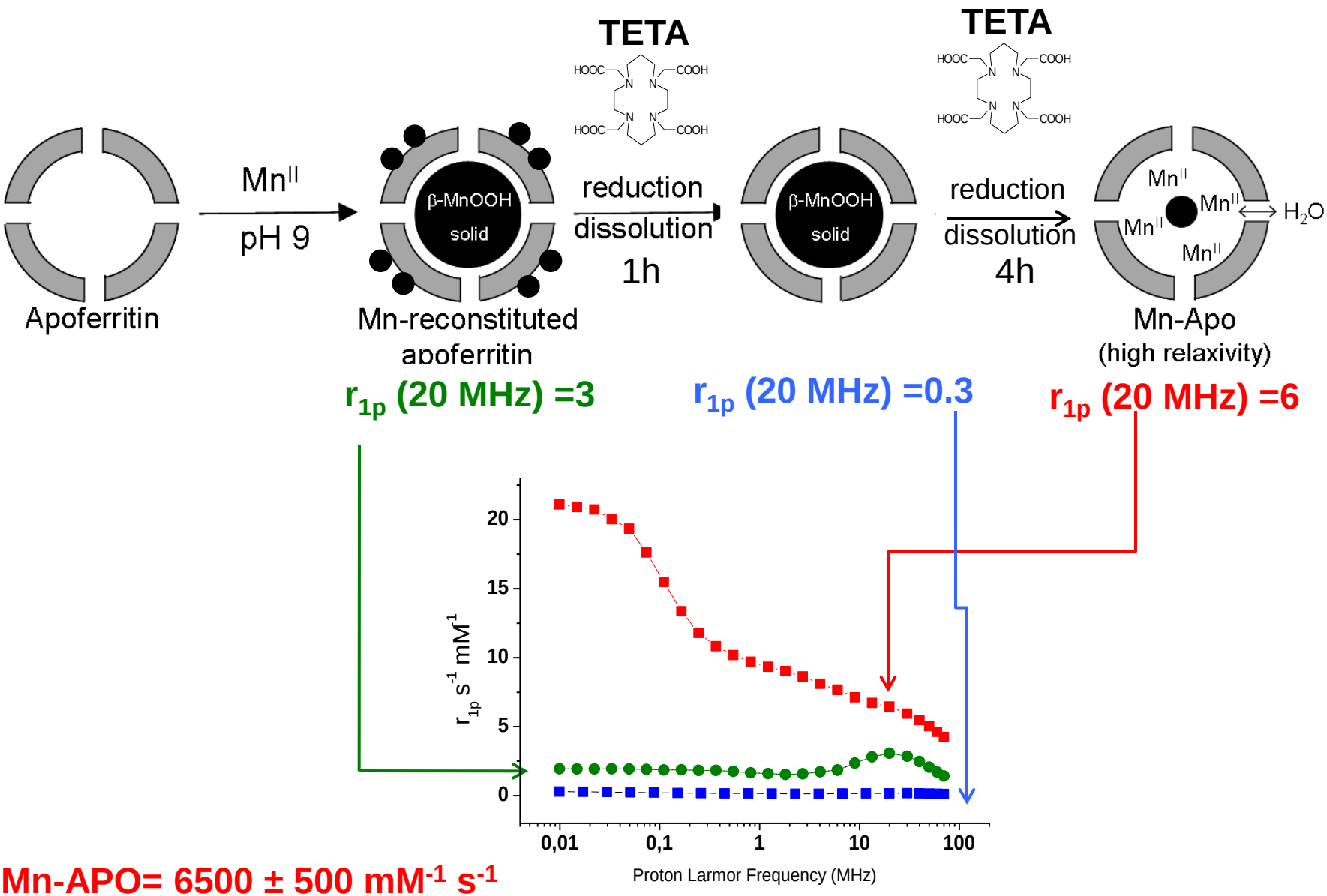
NMRD profile of Mn reconstituted apoferritin



CS Mcardell et al Environ Sci Technol 1998, 32, 2923-2930

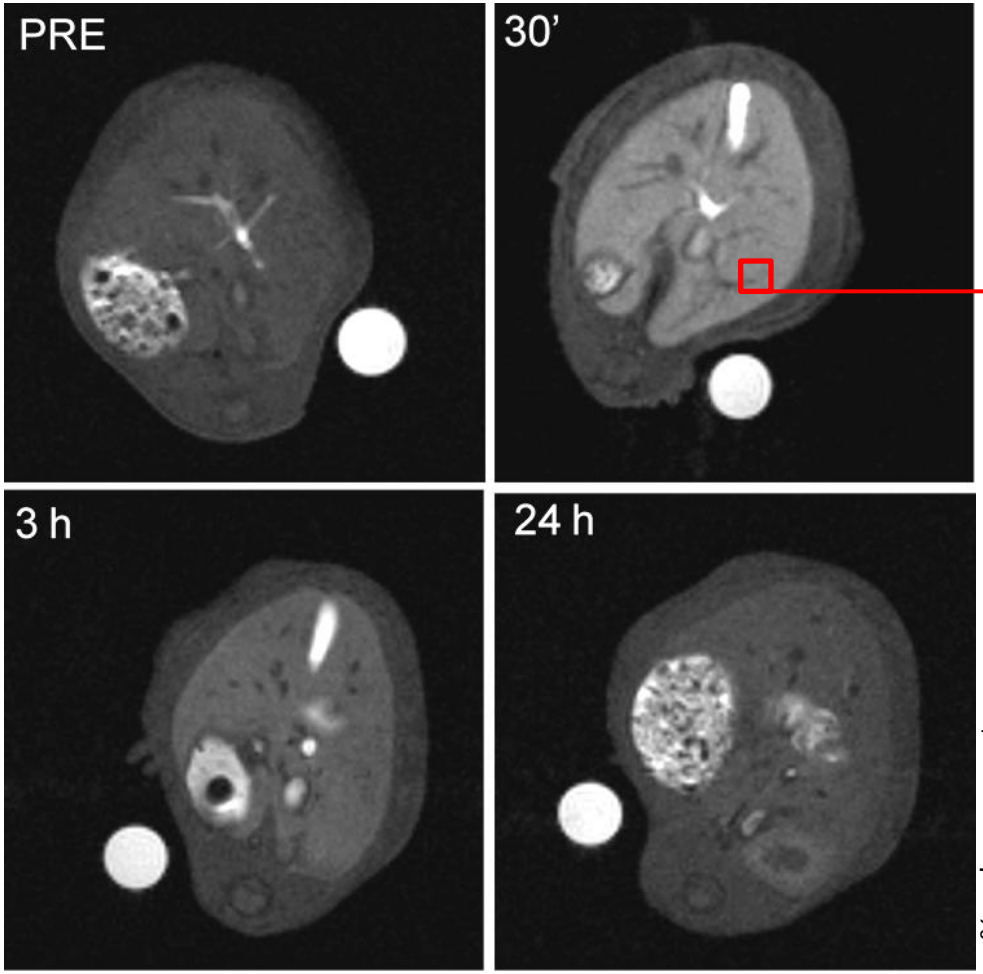
F. Kálmán, S.Geninatti-Crich and S. Aime, Angew. Chem, 2010

Mn-loaded Apoferritin as a MR Molecular Imaging probe

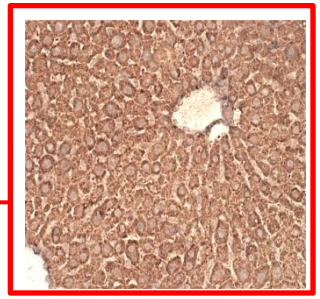


r$_{1p}$Mn-APO= 6500 ± 500 mM^{-1} s^{-1}

T_1-weighted MR images of C57BL/6 mice (liver region) acquired before and 30', 3 and 24h after the administration of Mn-Apo at a Mn dose of 0.01 mmol/kg.

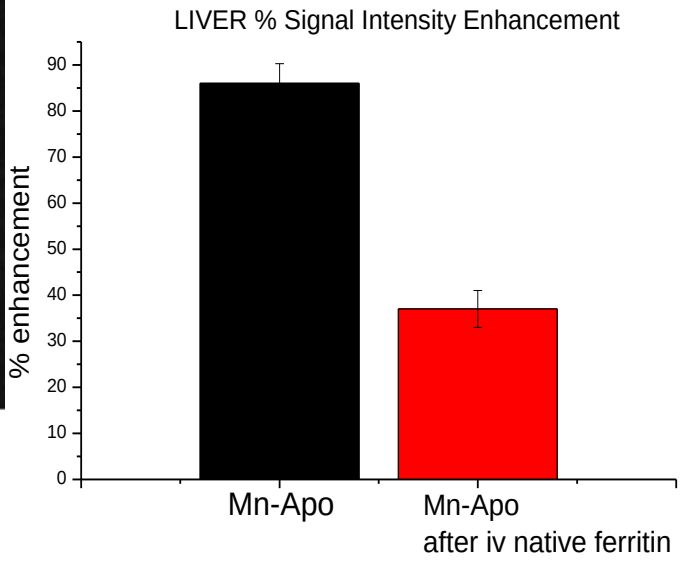


Immunohistochemistry;



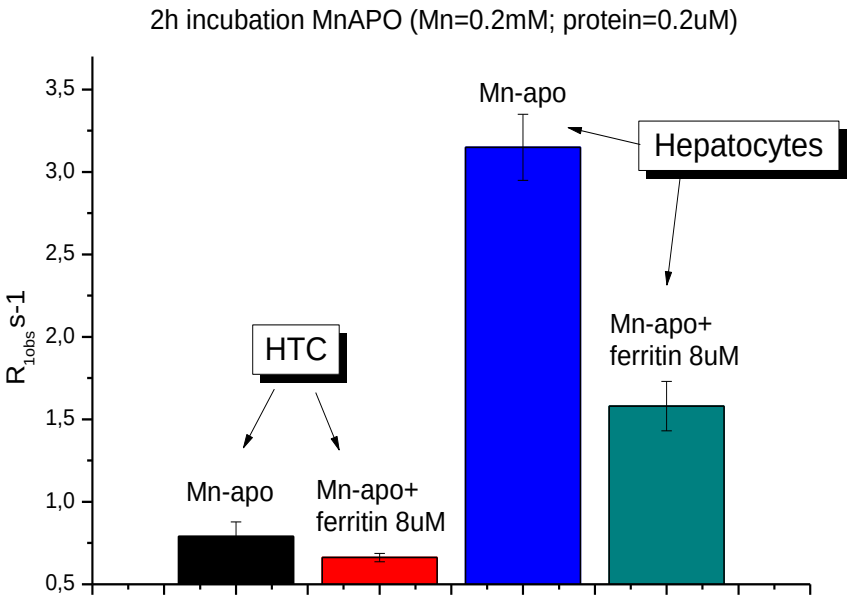
Plasma-membrane positive reaction for SCARA5 in wt mouse liver

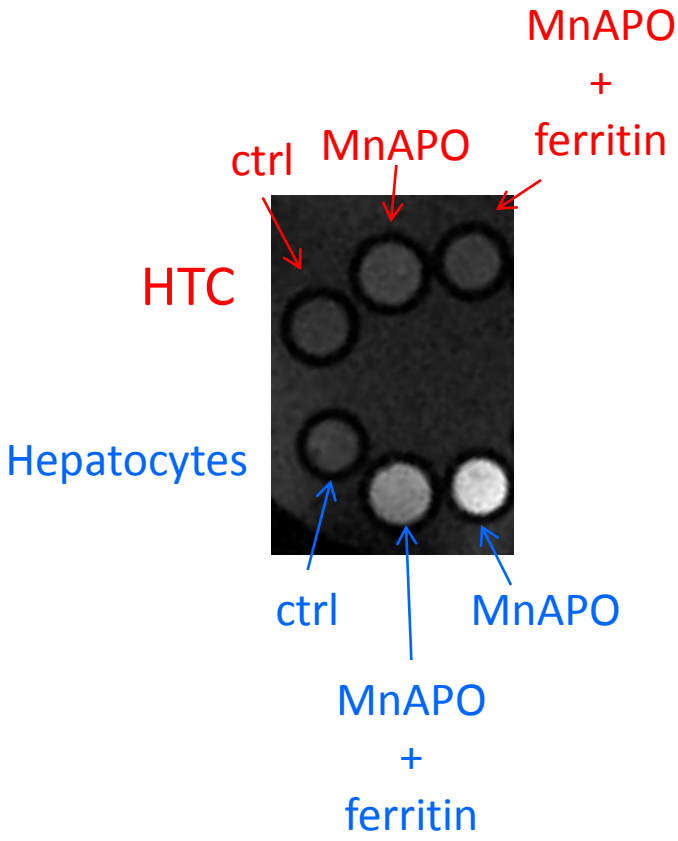
Competition with native ferritin



Uptake of Mn-APOFERRITIN by rat Hepatoma (HTC) and healthy rat hepatocytes

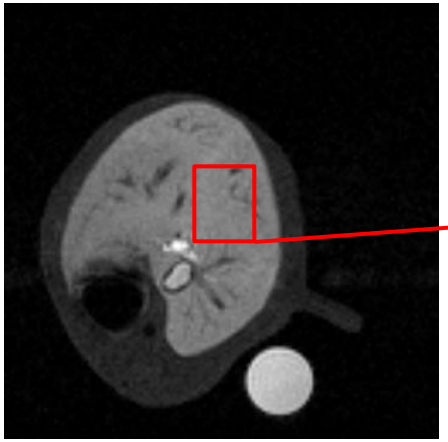
(2h incubation 0.2mM Mn, 0.2 uM protein)

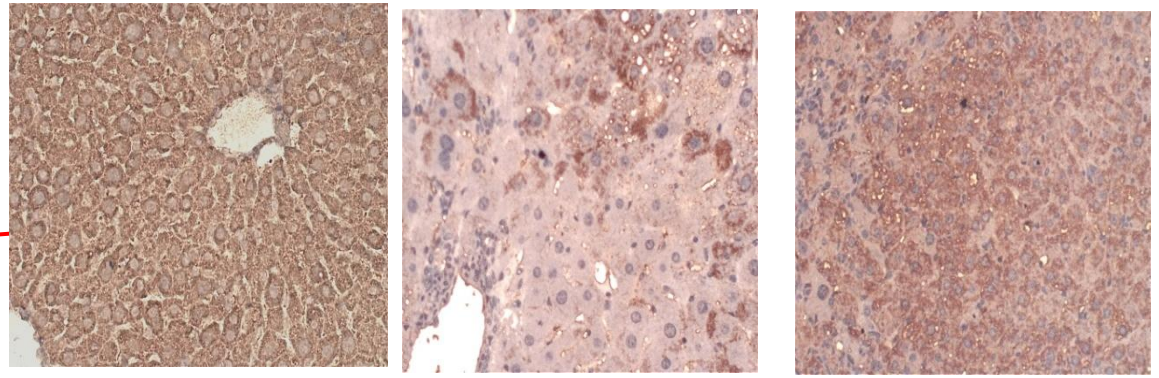




T_1-weighted MR images (liver region) of C57BL/6 w.t HBV transgenic mice at a Mn dose of 0.01 mmol/kg.

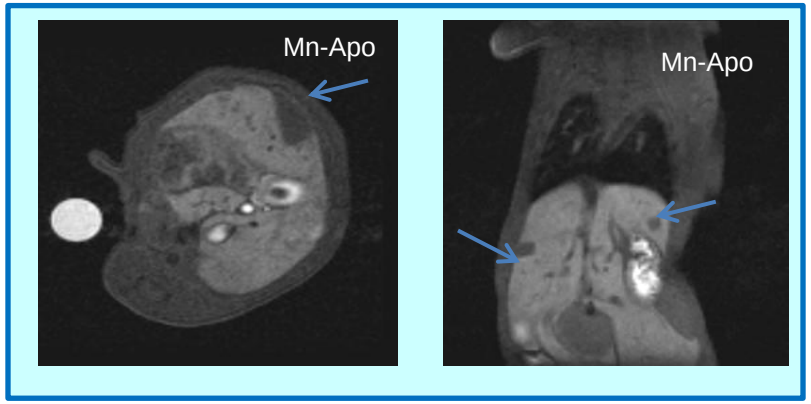
WT

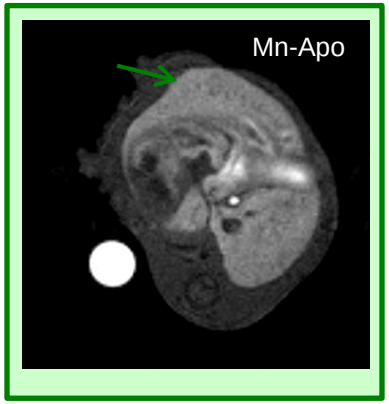




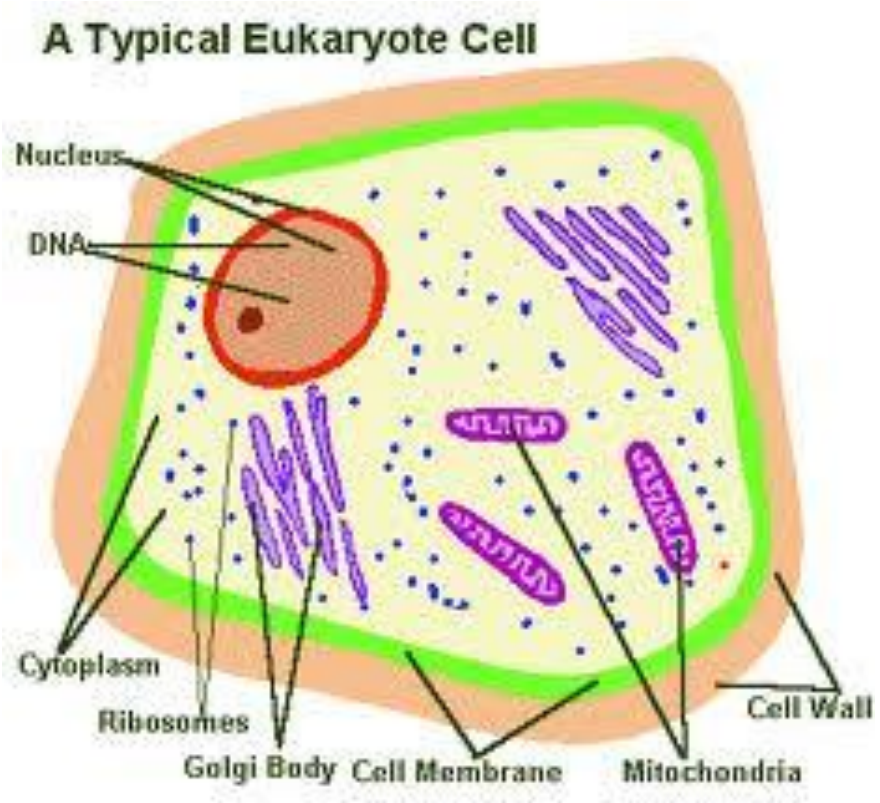
HBV-Tg-mice

13 months old





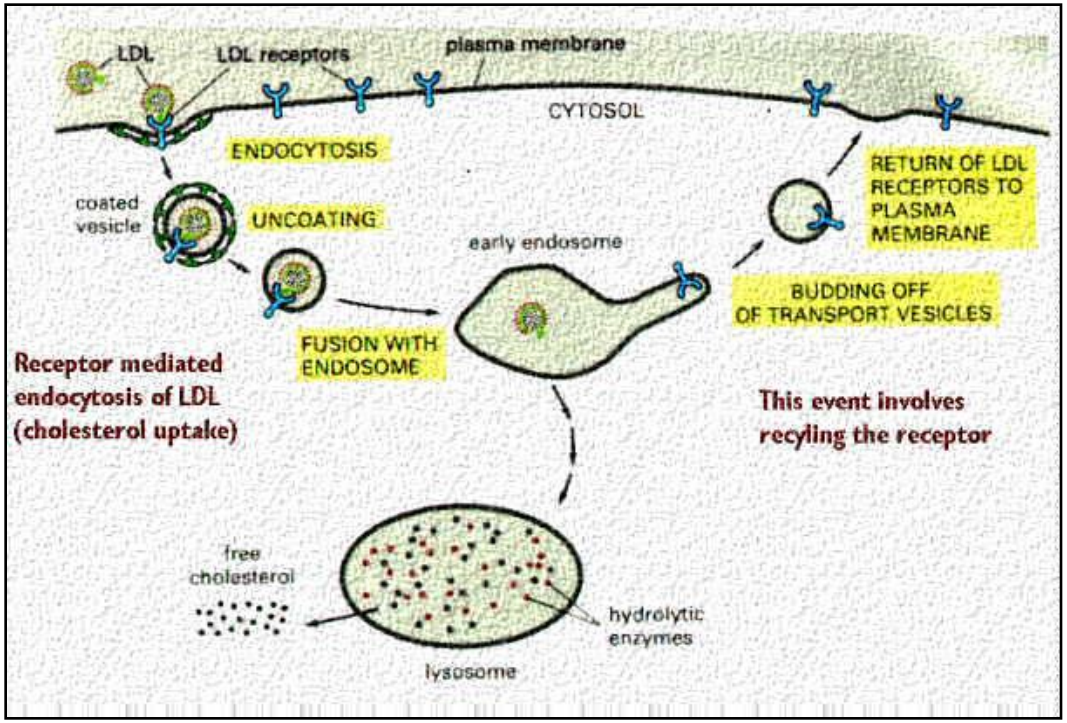
Localization of MRI Imaging Probes



Extracellular → cell membrane

Intracellular → cytoplasm or endosomes/lysosomes

Receptor Mediated Endocytoses



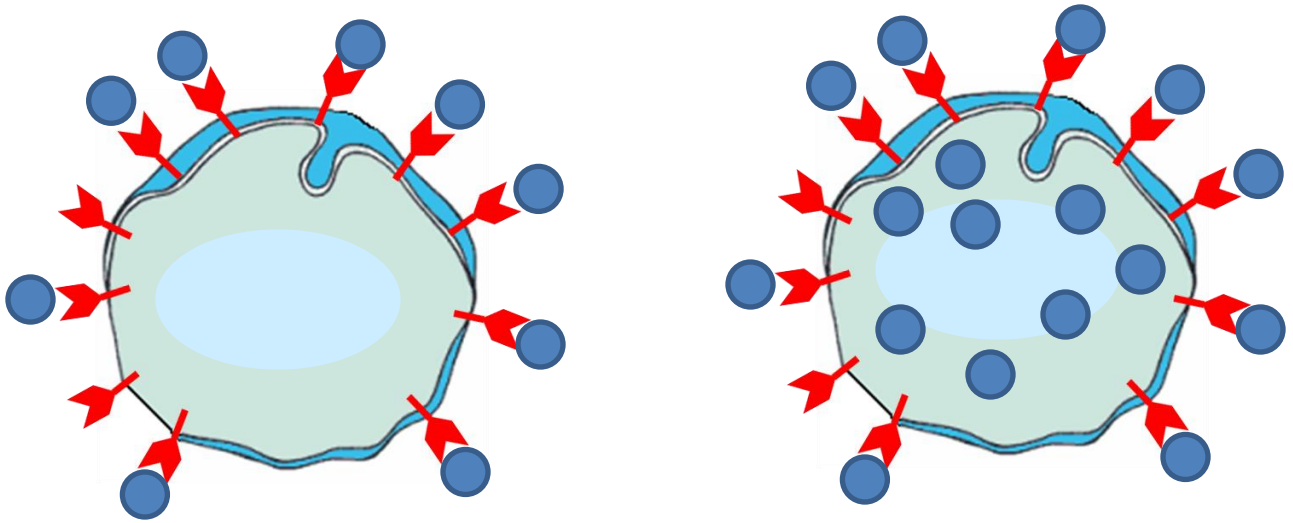
Receptor recycling time → 10’ LDL receptor
12 h Folate receptor

Number of Gd units internalized

↓

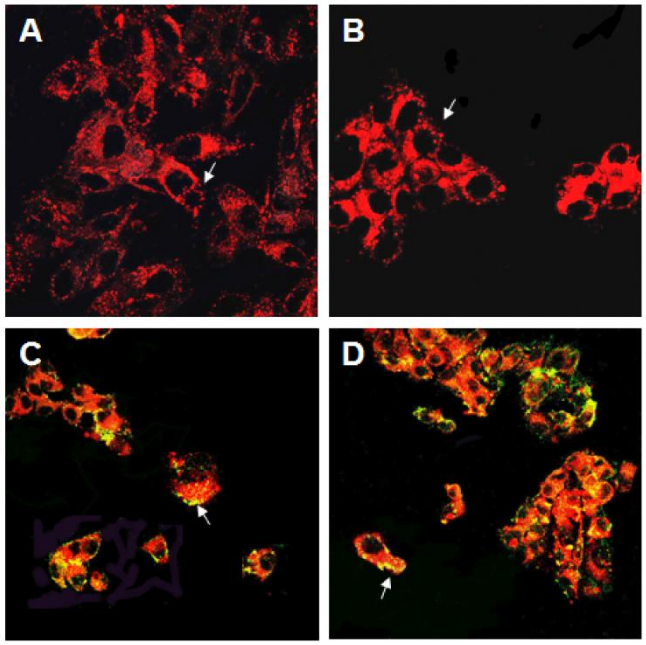
number of receptors x receptor recycling number

= Imaging probe



How can you detect Imaging Probe localization?.

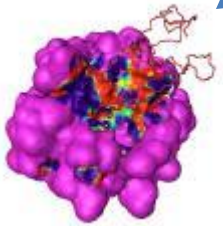
-Confocal Microscopy after labeling with a fluorescent dye



Confocal microscopy indicates that fluorescently labeled nLDL particles are taken into the cell by LDL receptors and are found together in the cell's lysosomes,

Cells detaching methods

Trypsin/EDTA



Scraper

- Intracellular Imaging Probe

Total cell associated Imaging Probe

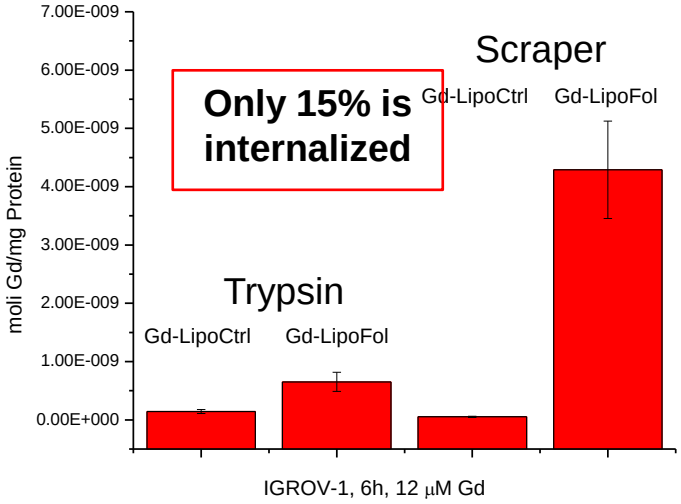
Extra- + Intra- cellular

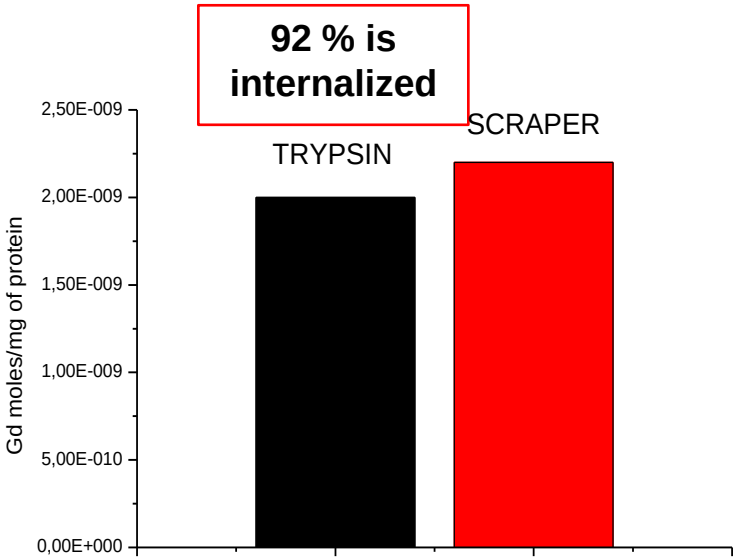
MRI + ICP-MS (Gd determination)

ICP-MS (Gd determination)

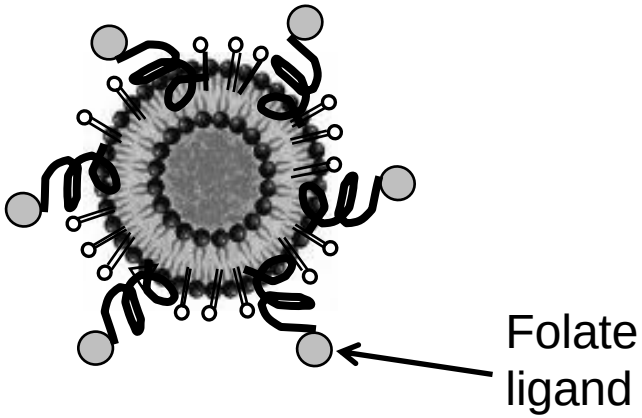
IGROV-1 cells overexpressing Folate Receptors

B16-F10 cells overexpressing LDL Receptors

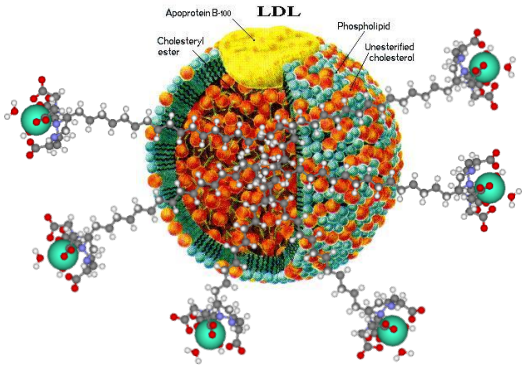




Imaging Probe:
FR targeted Gd-Loaded Liposome



Imaging Probe:
Gd-loaded/LDL



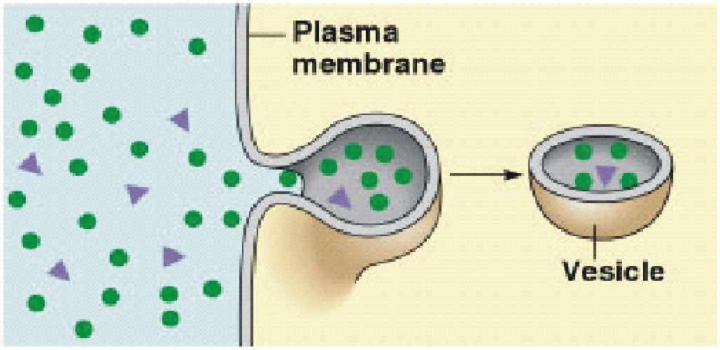
Relaxivity dependence on intracellular localization of the Gd-based imaging probe.

Pinocytoses

Electroporation

Pinocytosis

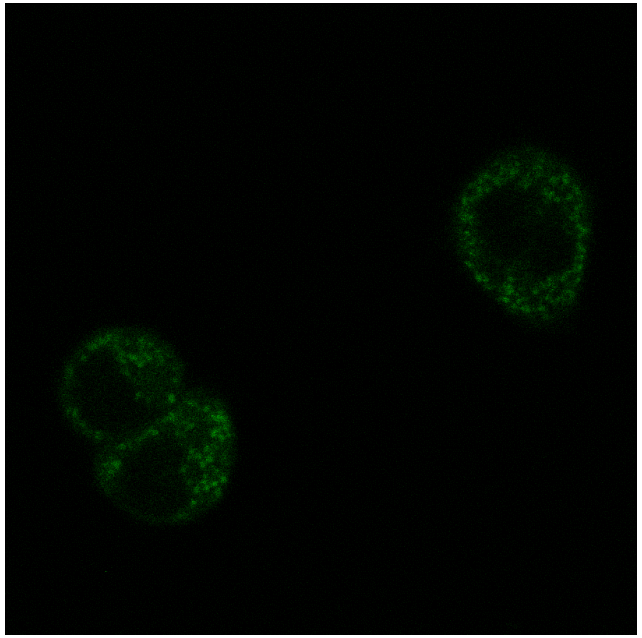
Labelling of Cells in culture
(stem cells, leucocytes, etc...)



Incubation at high concentration of well-tolerated Gd-chelates

Fluorescence Microscopy of EPCs labelled with Eu-HPDO3A

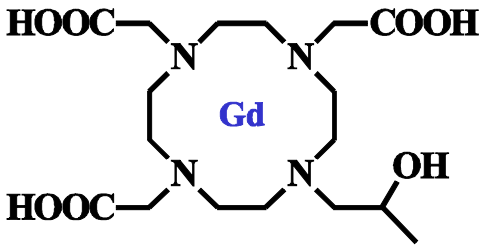
Eu-HPDO3A and Gd-HPDO3A have almost identical physico-chemical properties and therefore the same biodistribution

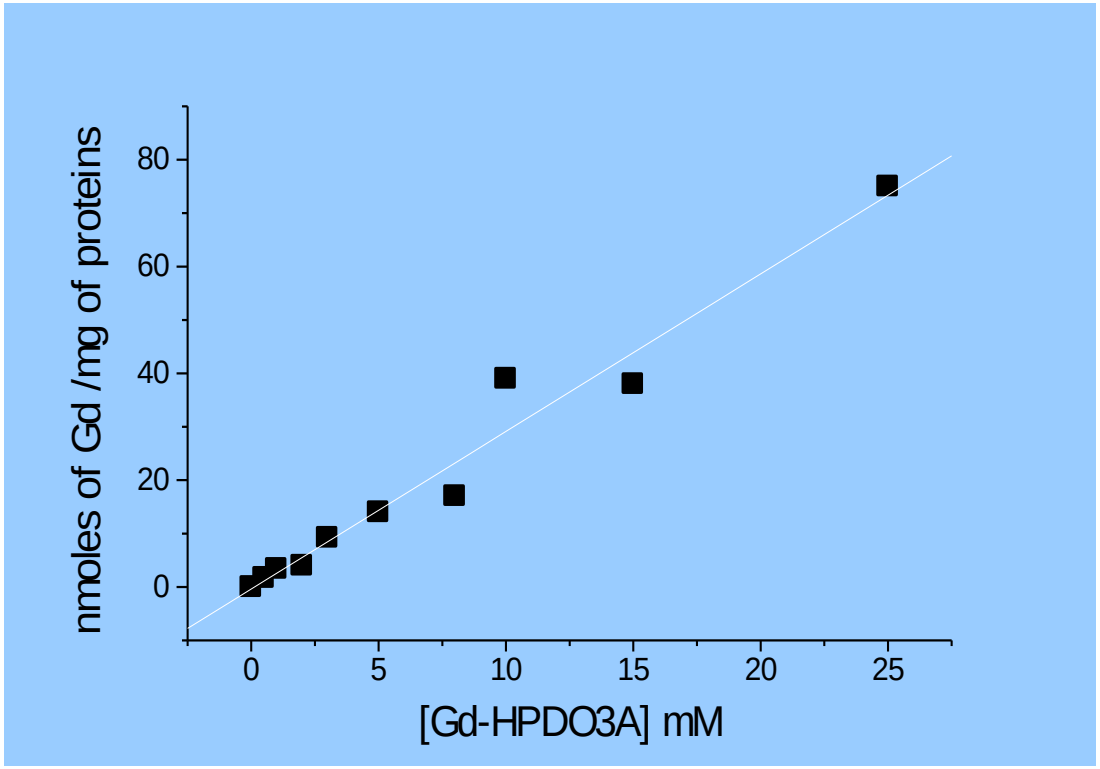


Incubation time: 16h at 37°C (in the presence of 50 mM of Eu-HPDO3A)

FLUORESCENT Eu-HPDO3A ACCUMULATES IN ENDOSOMES AROUND NUCLEOUS

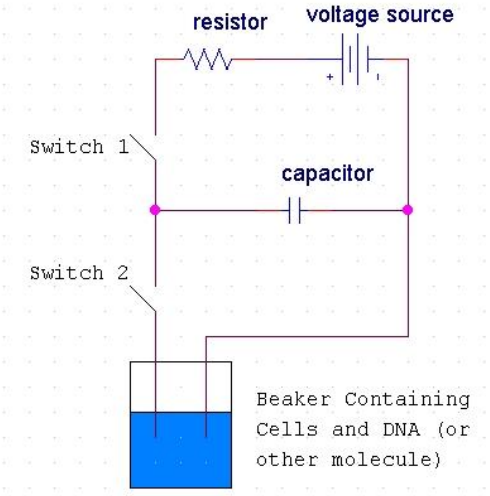
Labelling of HTC cells with Gd-HPDO3A via endosomic entrapment

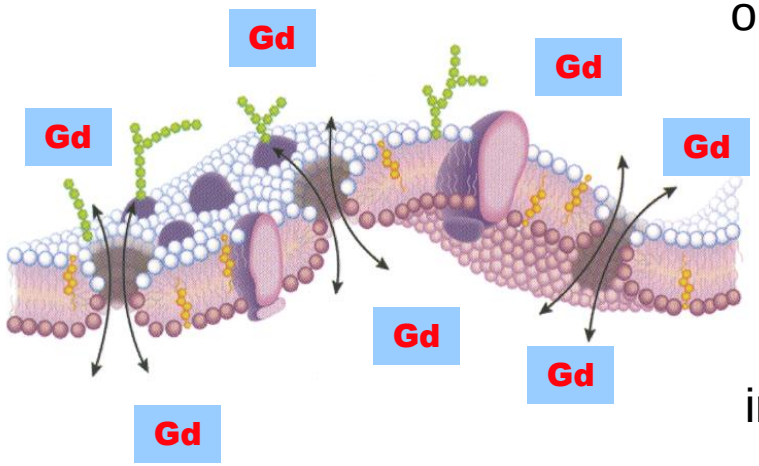


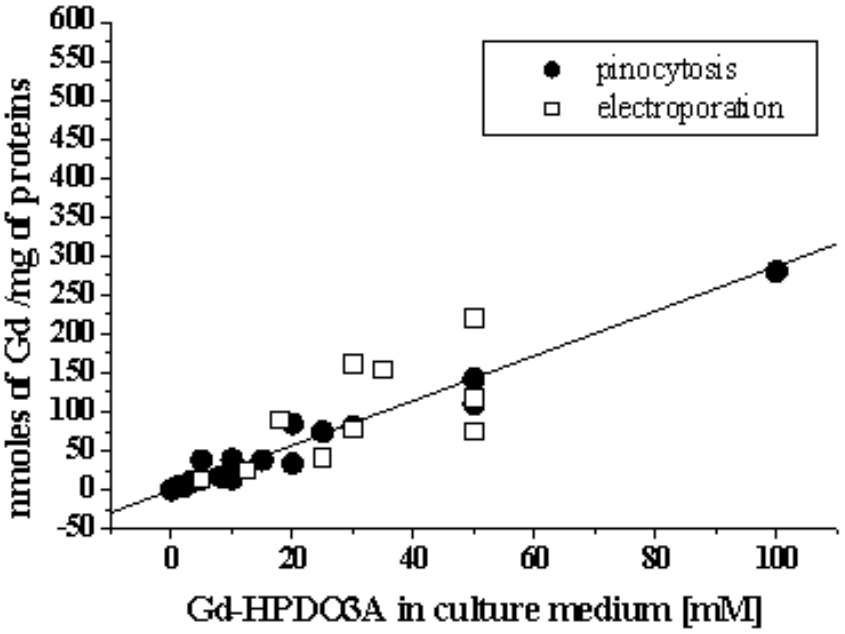


Electroporation

The imaging probe enters the cell through transient hydrophilic pores formed upon application of a suitable electric pulse



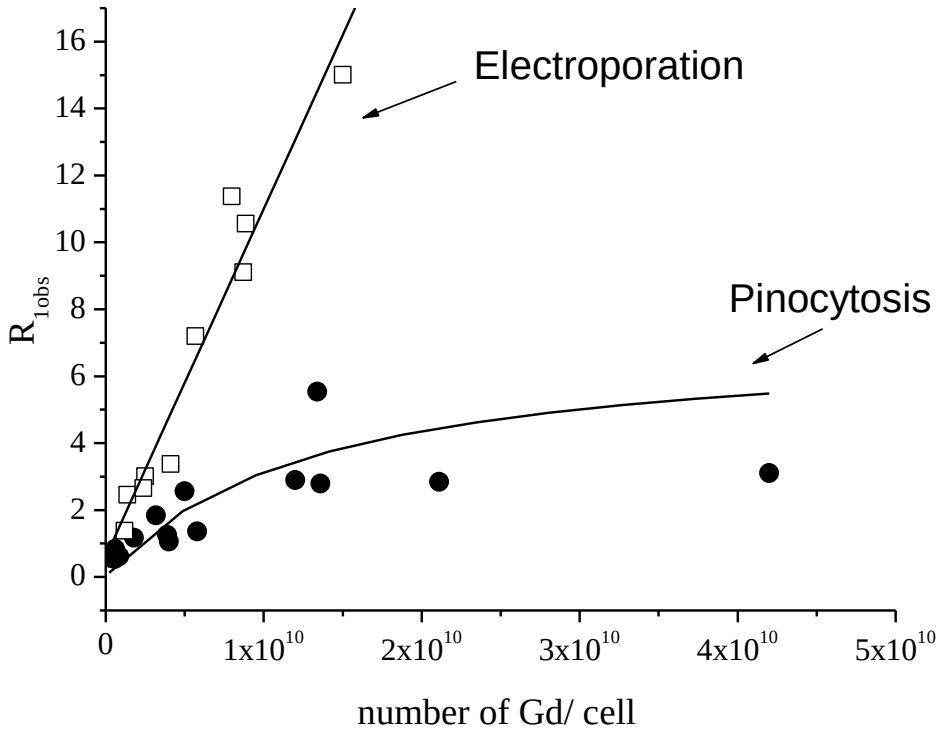




The internalization of Gd-HPDO3A into HTC cells. About 5-6 million cells were
(●) incubated at 37 C for 16 hours (pinocytosis) or (□) electroporated applyin
a pulse of 0.2 kV. Both electroporation and pinocytosis were performed in the
presence of increasing concentrations of contrast agent (Gd-HPDO3A).

How the intracellular localisation affects the attainable relaxivity

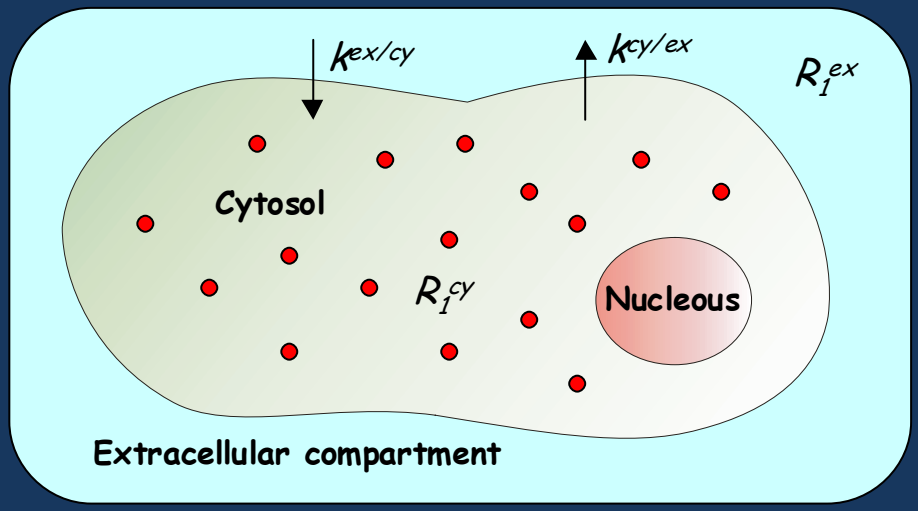
Pinocytosis	→	**Endosomes**
Electroporation	→	**Cytoplasma**



Terreno et al., Mag. Res. Med. Jan 31, 2006.

How the intracellular localisation affects the attainable relaxivity ?

● Gd(III)complex

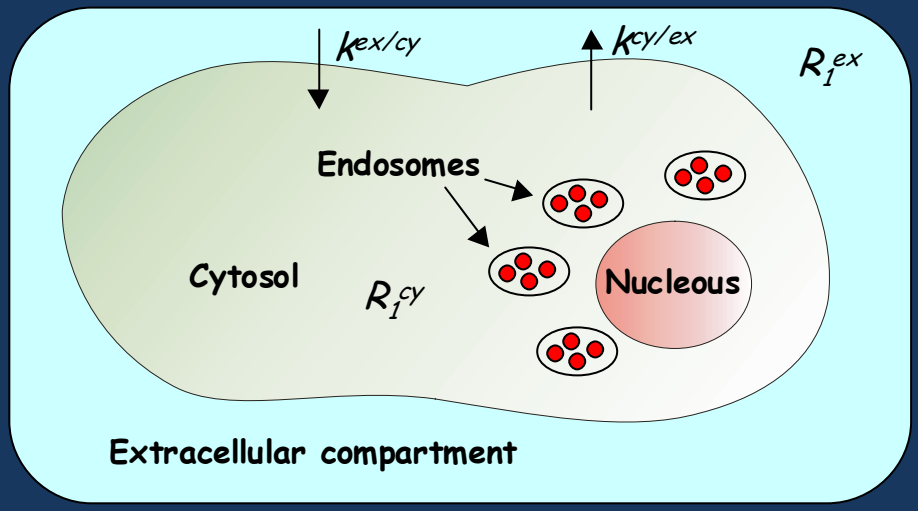


Electroporation

Two compartments (cytosol/extracellular)

$$|k^{cy/ex} + k^{ex/cy}| > |R_1^{cy} - R_1^{ex}|$$

no r_1 quenching !



Pinocytosis

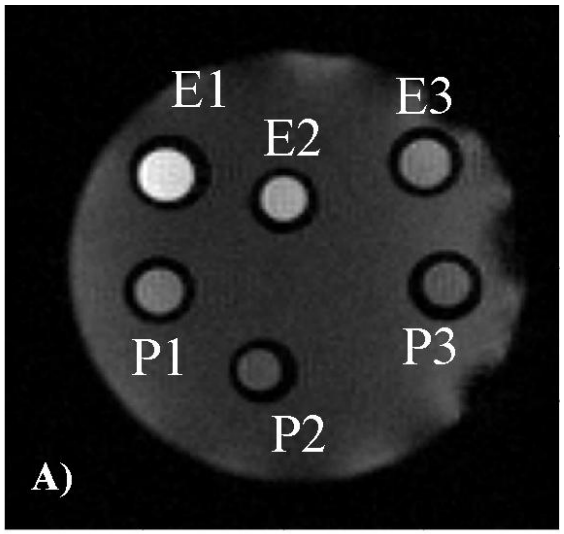
Three compartments

(endosomes/cytosol/extracellular)

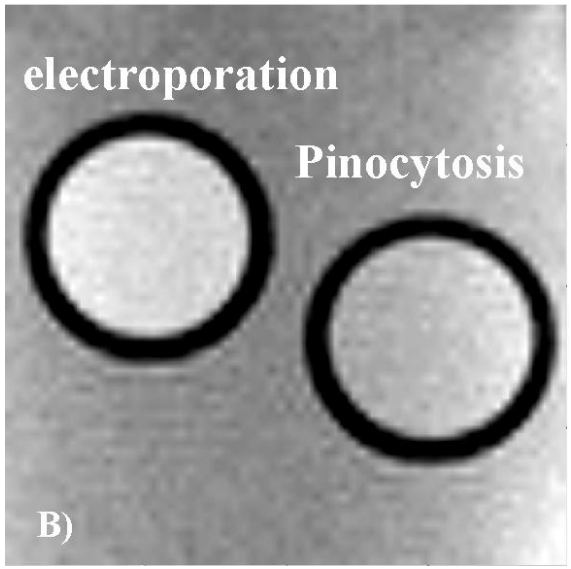
$$|k^{cy/end} + k^{end/cy}| < |R_1^{end} - R_1^{cy}|$$

$$|k^{cy/ex} + k^{ex/cy}| > |R_1^{cy} - R_1^{ex}|$$

r_1 quenching !



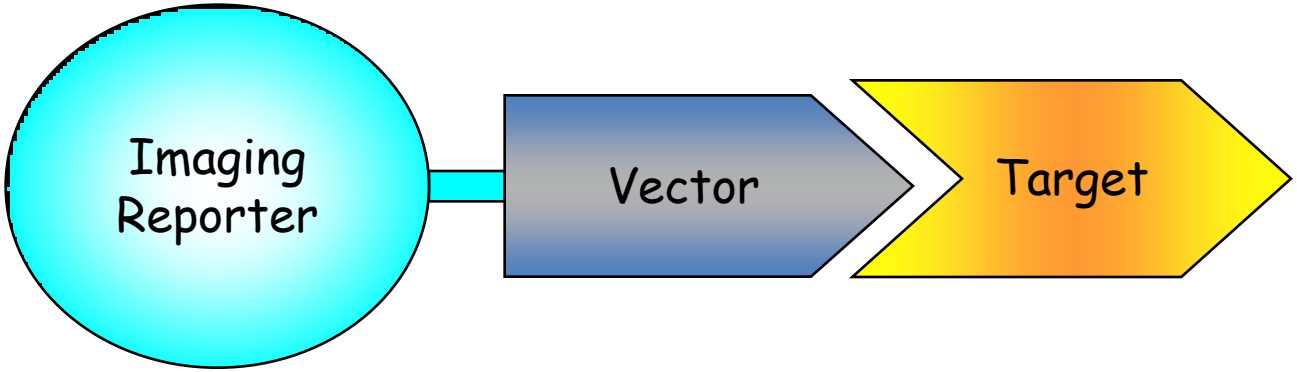
A) T_1 weighted spin echo image of an agar phantom containing HTC cells labeled with GdHPDO3A internalized by pinocytosis (P) or electroporation (E). Cells are dispersed in agar at different densities 1)$1x10^4$ cells/μl, 2)$5x10^3$cells/μl, 3)$1x10^3$cells/μl.

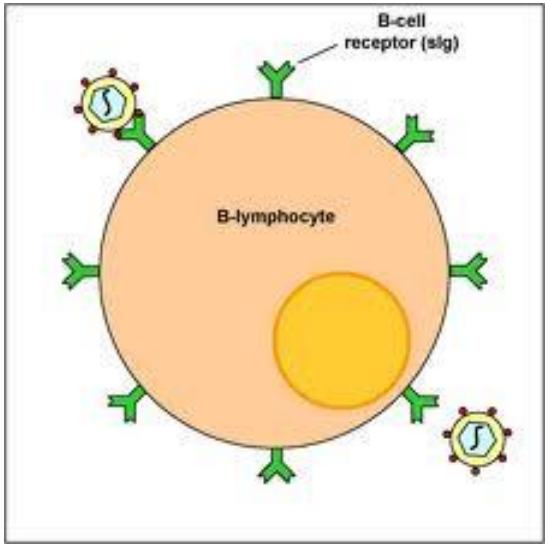


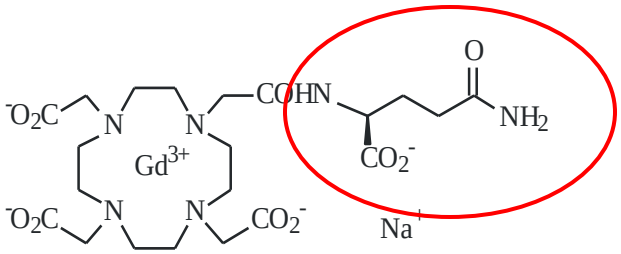
B) T_1 weighted spin echo image of an agar phanto containing HTC cells ($5x10^2$cells/μl) labeled wit GdHPDO3A internalized by pinocytosis or electroporation.

Minimum number of cells detectable x μl = 5000 Pinocytosis
500 Electroporation

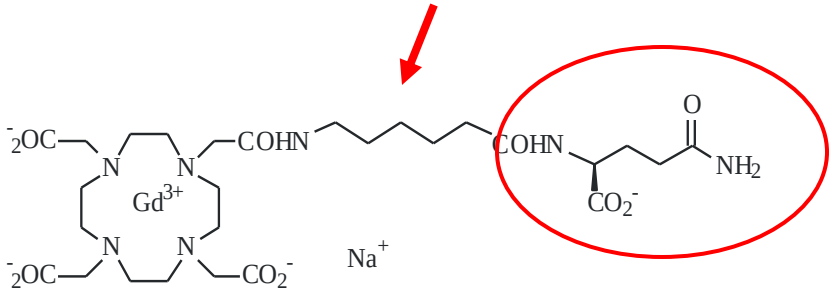
Affinity of the Imaging Probe for the target receptor





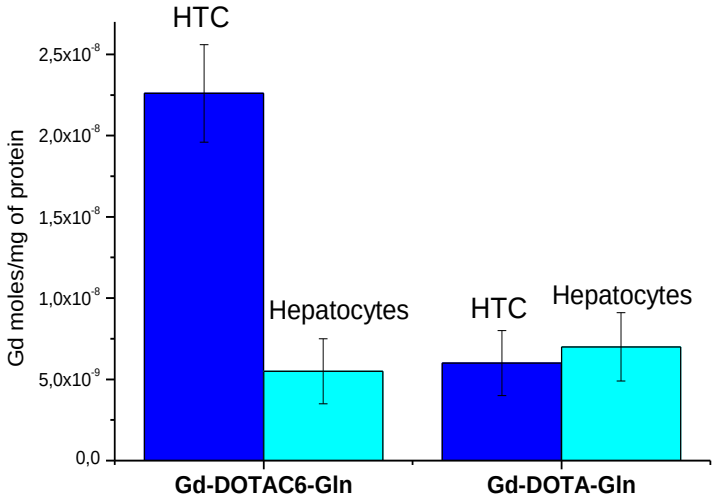


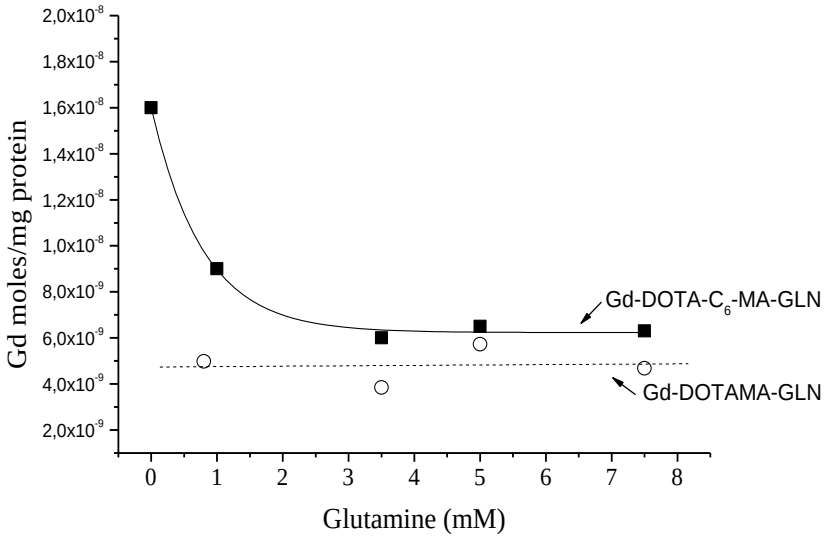
Na[Gd-DOTAMA-Gln]



Na[Gd-DOTAMA-C_6-Gln]

Competition with glutamine in HTC cells





S. Geninatti Crich, C. Cabella, A.Barge, S Belfiore, C Ghirelli, L Lattuada, S Lanzardo, A Mortillaro, L Tei, M Visiga G Forni, S Aime ***J Med Chem. 2006;49:4926-36****.*

Strategies for cellular labeling

"IN VIVO"

Labelling of targeted cells and visualization of cellular processes in living organisms.

"IN VITRO"

Monitoring of migration and location of transplanted cells labeled "in vitro" with contrast agents.

“In vivo” Cell Tracking

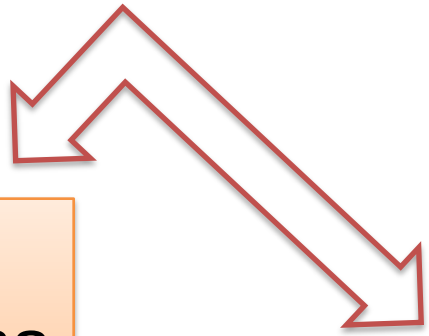
- The use of stem and progenitor cells in human clinical Studies will require a technique that can monitor their tissue biodistribution noninvasively

-Whole body imaging will be useful to study the biodistribution of injected cells and help reveal undesired seeding in nontarget organs.

Characteristics of the Ideal Imaging Agent
Real-time visualization of injected cells
Cell quantification
Long term, serial traceability
Single cell sensitivity in any location
Less false-positive imaging
Minimal or no transfer of contrast agent to other cells

MRI Contrast Agents

paramagnetic species



Complexes of paramagnetic metal ions (Gd^{3+},Mn^{2+})

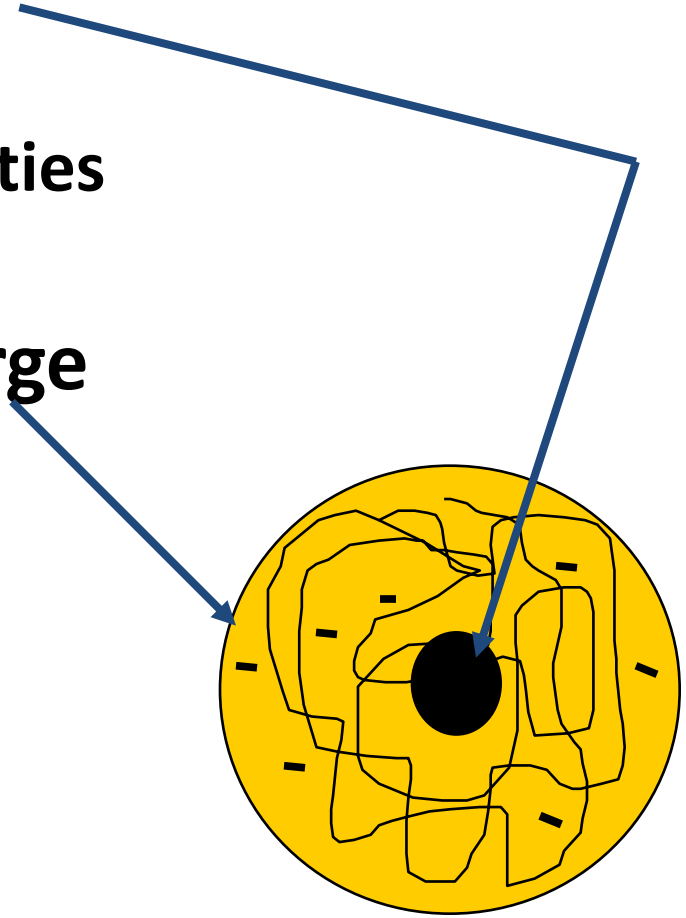
-Positive Contrast

Superparamagnetic particles (SPIOs,USPIOs)

-Negative Contrast

Iron Oxide Nanoparticles
Schematic view

- **Iron Oxide cristal core**
  - **Superparamagnetic properties**
- **Hydrophilic Coating + charge**
  - **Biological behavior**



Monitoring of implanted stem cell migration in vivo: A highly resolved in vivo magnetic resonance imaging investigation of experimental stroke in rat

Mathias Hoehn et Al. *PNAS* ***December 10, 2002,*** *99 no. 25* ***16267–16272***

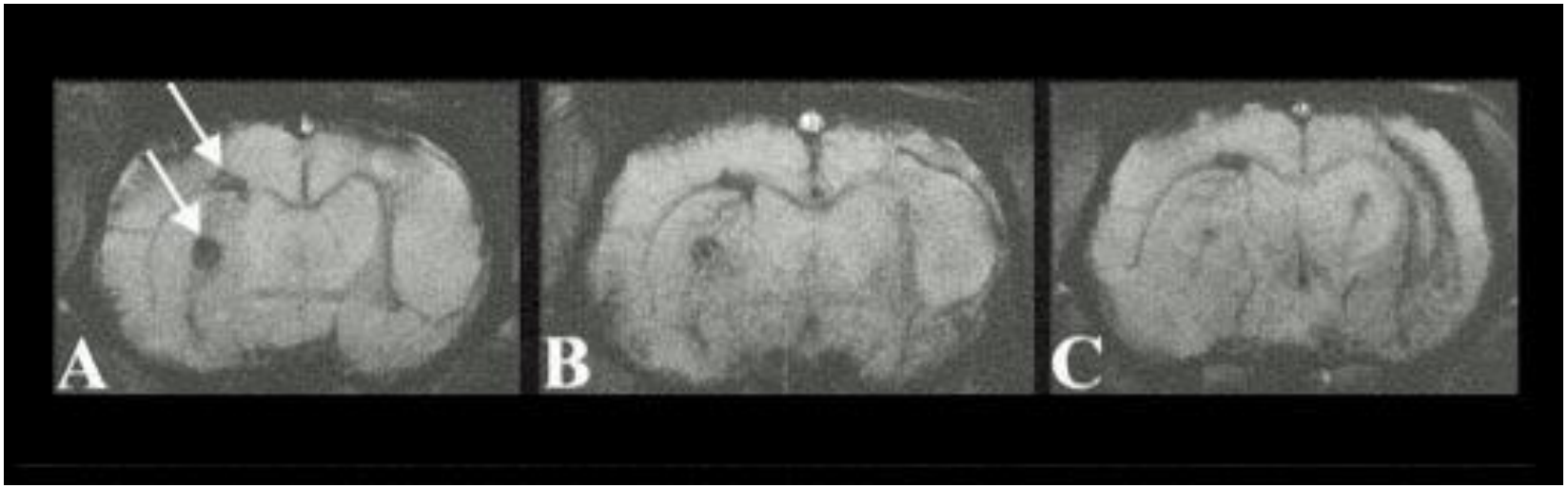
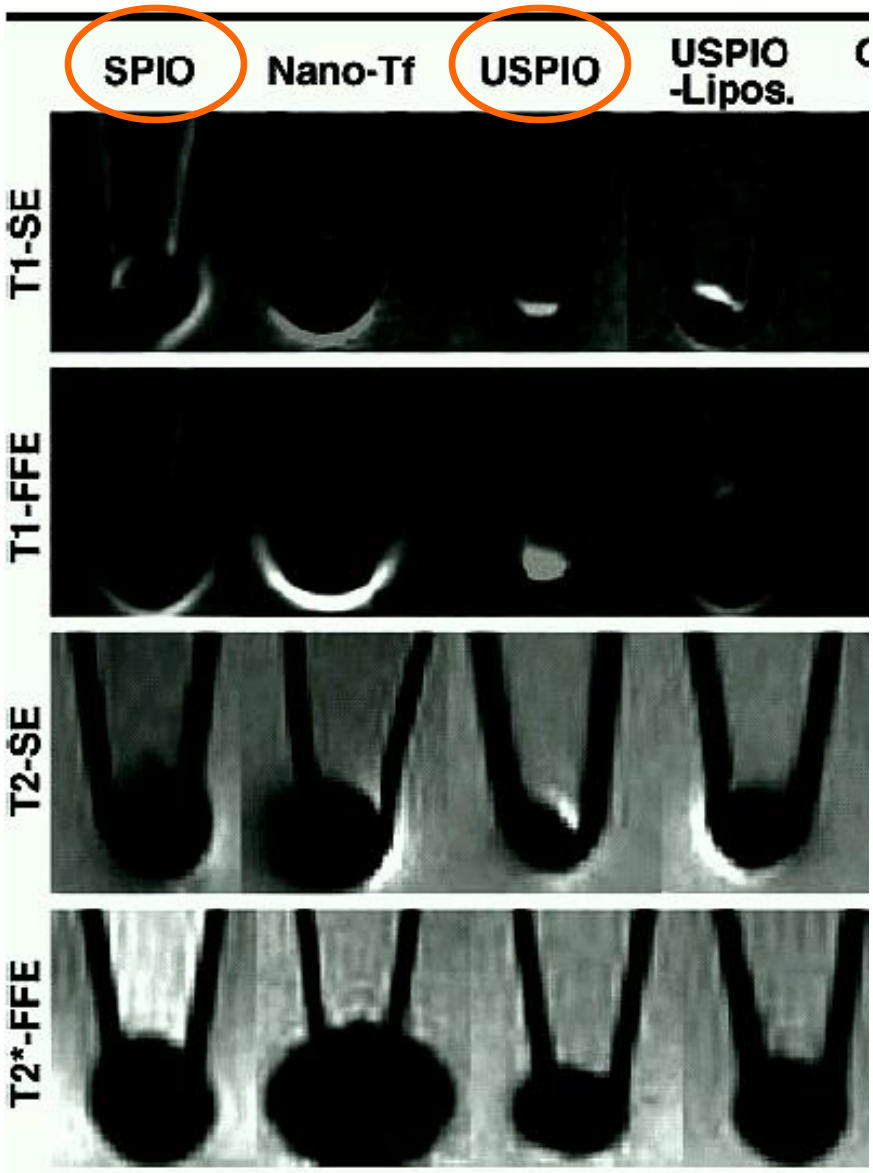


Fig. 2. Coronal sections from a 2D multislice MRI experiment through a rat brain at the day of stem cell implantation (*A*) and 8 days (*B*) and 16 days (*C*)
after implantation. Transient focal cerebral ischemia of the right hemisphere (60 min) had been induced 14 days before implantation.

MR signal with SPIO-USPIO



Cells pellet in test tubes
Incubation with SPIO-USPIO

T1, T2 and T2* images

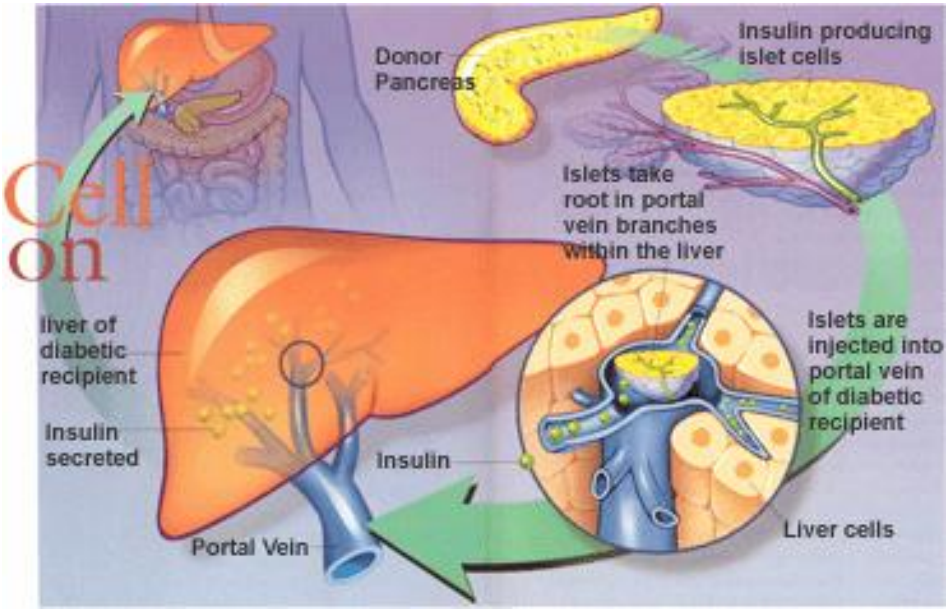
T2*
Good for sensitiviy,
Macrophages uptake
Bad for spatial resolution
High field is needed

Susceptibility artifacts

HE Daldrup et al. Radiology, 2003

Bulte JWM Krathchman NMR in BIOMEDICINE 2004

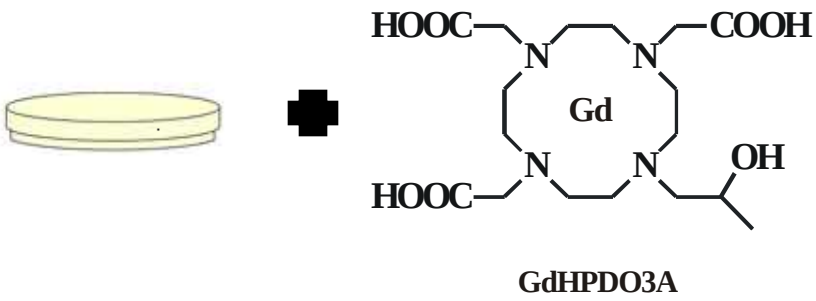
- **Islet transplantation** is now considered as a viable routine option for t
treatment of insulin-dependent diabetes mellitus but……..



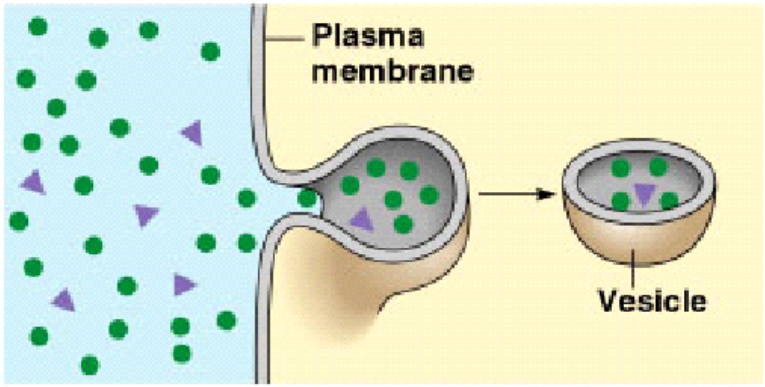
……the first phases of an islet transplant are critical and the possibility
of initial monitoring of the engraftment only possible throughout indirect
functional data, representing a severe limitation for research and clinica
transplantation.

LABELLING PROCEDURE

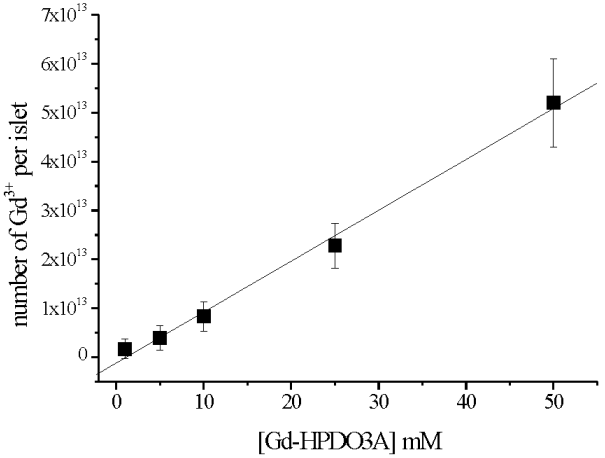
About 1000 islets were incubated for 16 hours with increasing concentrations of the complex (1-50mM)



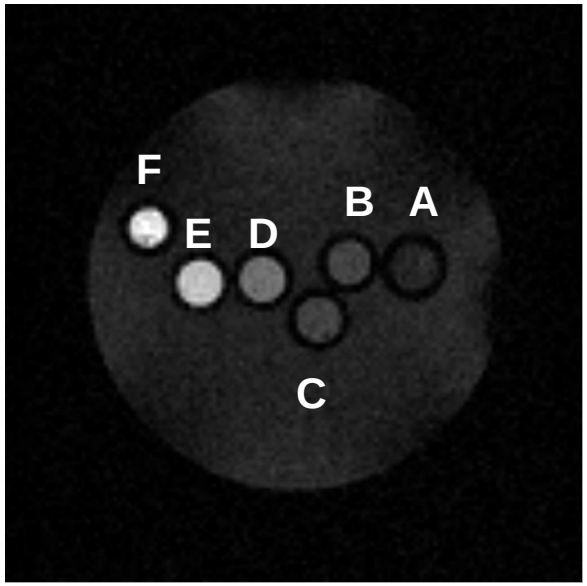
GdHPDO3A



(b) Pinocytosis

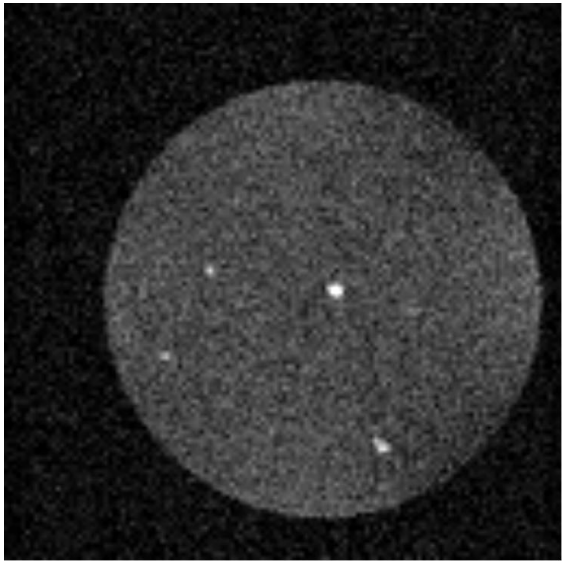


T_1 weighted spin echo image of glass capillaries placed in a agar phantom containing:

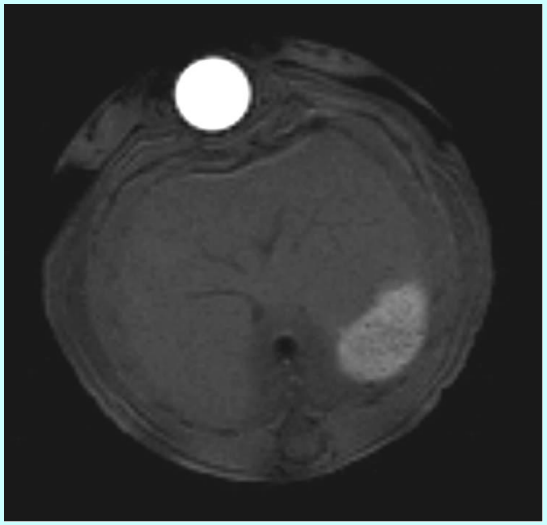


A) human islets incubated with GdHPDO3A at increasing concentrations. Each pellet (ca. 1000 islets) was incubated with Gd-HPDO3A: 0 (A), 1 (B), 5 (C), 10 (D), 25 (E) 50 (F) mM respectively.

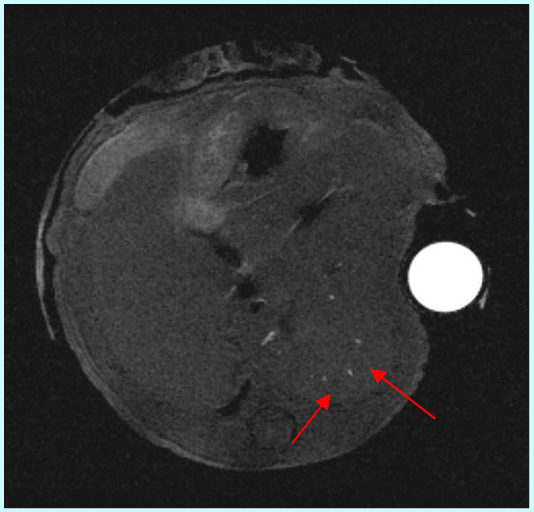
200 IEQ of human islets dispersed in about 200 ul of AGAR (each hyperintense spot corresponds to the signal deriving from one islet).



T1 weighted spin echo images of SCID mice transplanted into the liver via portal vein with human islets and analyzed one day after transplantation.



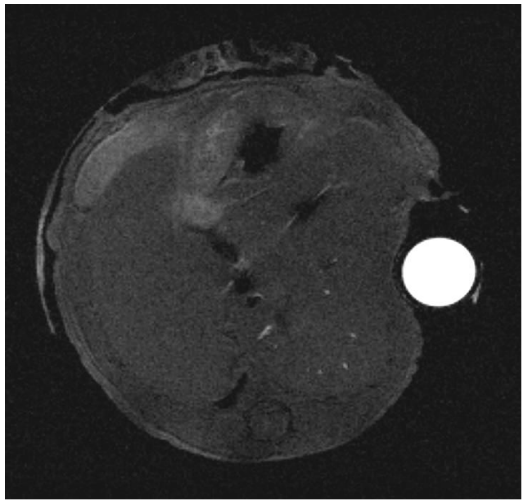
Mouse liver with unlabeled islets

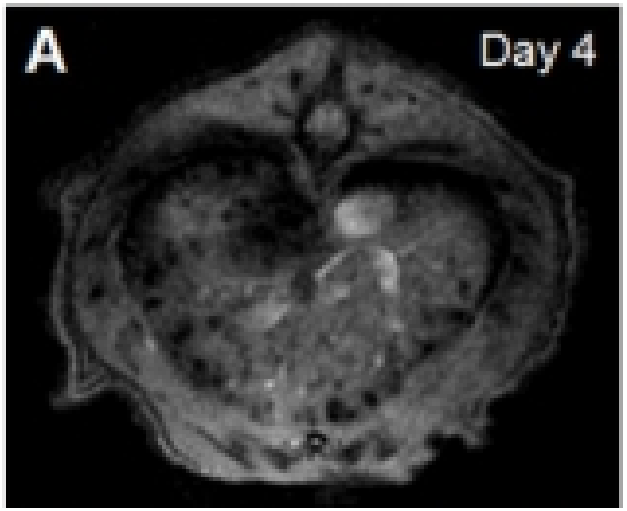


Mouse liver with isletslabeled with 50 mM Gd-HPDO3A for 16 hrs in culture prior to infusion

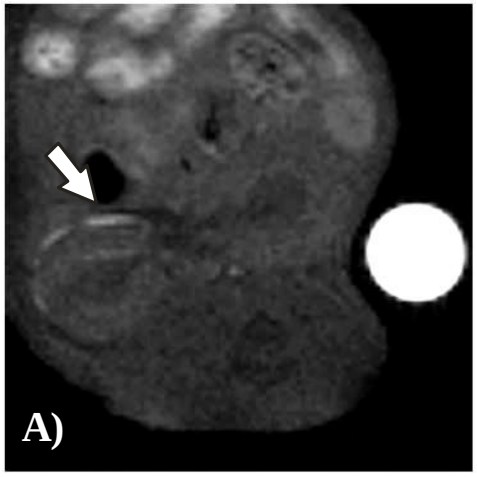
Spin-echo T1-weighted images: TR/TE/NEX 260/4.4/64

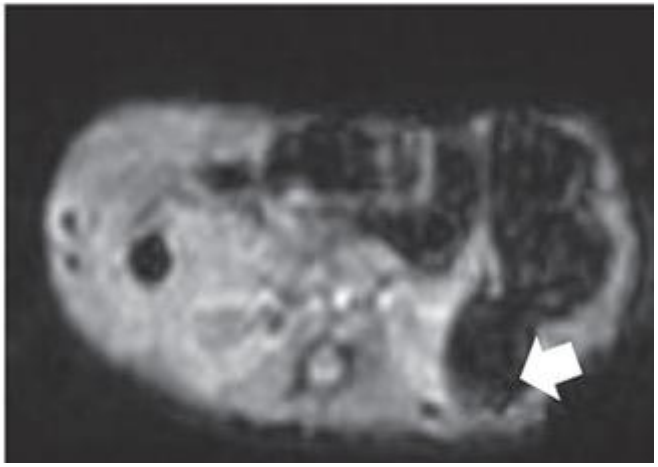
Comparison between Gd and iron-oxide labeled islets





LIVER





KIDNEYS

Gd

Fe

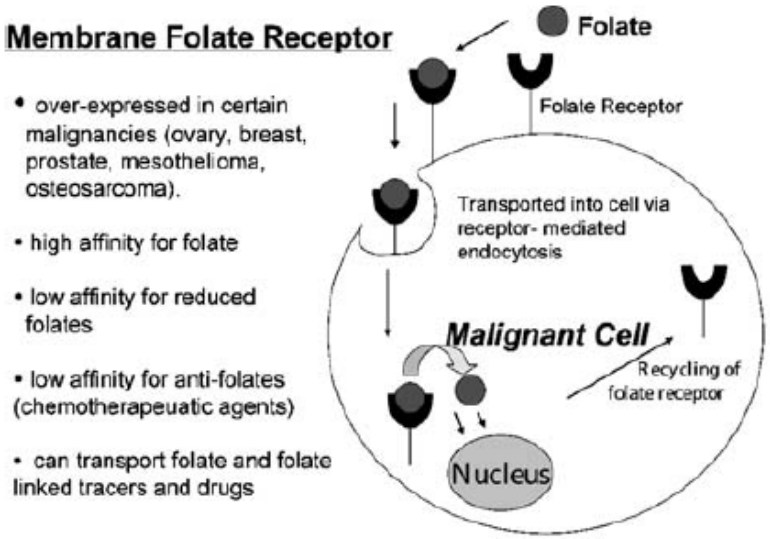
Biancone et al NMR Biomed. 2007

Jung MJ et al Biomaterials. 2011
Lee et al Proc Natl Acad Sci U S A. 2011

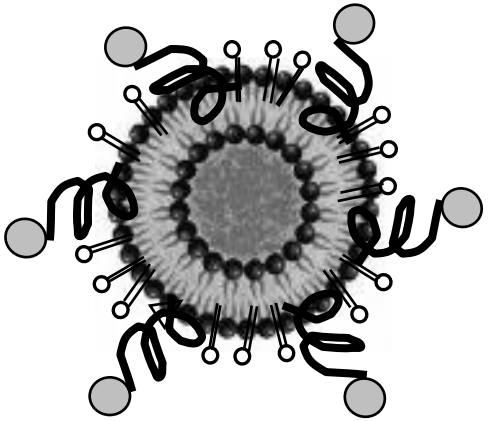
Introduction to the practical session.

Cellular Labelling by receptor mediated Endocytosis

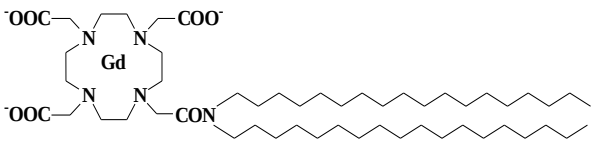
- Target: Cells over expressing Folate receptors (IGROV Human ovarian cancer Cells)
- Vector: Folic acid
- MRI Probe Gd-loaded Liposomes



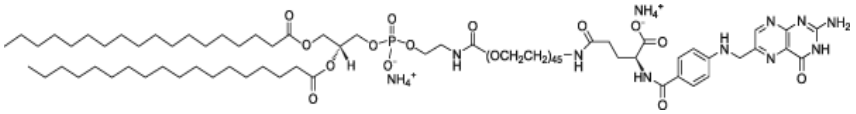
Folic acid Targeted Liposomes



= Amphiphilic Gd-complex



= Folate Fospholipid



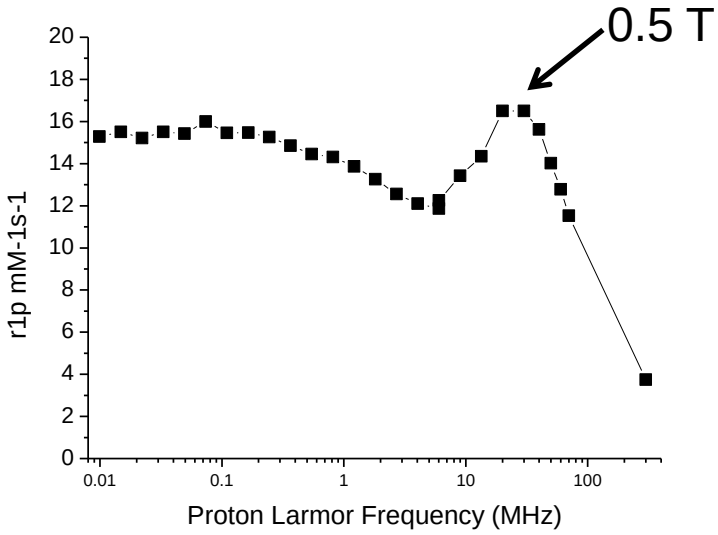
Liposome Formulation

POPC	59%
CHOLESTEROL	23%
Gd-COMPLEX	15%
DSPE-PEG2000	2%
DSPE-PEG2000-FOLATE	1%

r_{1p} (mM^{-1} sec^{-1}): 16.5

Size (nm): ≈ 150

NMRD Profile



Uptake experiment:

- Cells were incubated in the presence of FA- targeted and non-targeted liposomes 24h before at 37°C, 5% CO2 at the same concentration.
-Washing with PBS
-Dethaching with trypsin/EDTA
-Re-suspension of cell pellets within 100 µl of PBS and transferring in glass capillaries for MRI measurements.