CONTRIBUTION OF NANOTECHNOLOGY TO MOLECULAR IMAGING

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Imaging and nanotechnology

We shall focus our purpose on molecular imaging and will conventionally consider two separated access to the molecular level :

the direct one that uses endogenous biophysical process or specific ligands for image contrast formation ;

the indirect one that uses nanotechnology to conceptualize engineered marked probes so as to visualize cells events at the molecular level.

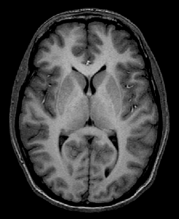
The marker is an infrared, luminescent or fluorescent probe in optical imaging, a radioactive tracer in TEP or TEMP imaging and a (super)para- or ferro- magnetic contrast agent in MRI.

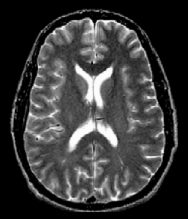
Let's considering the direct way to reach the molecular level:

image formation via endogenous biophysical processes and specific probes

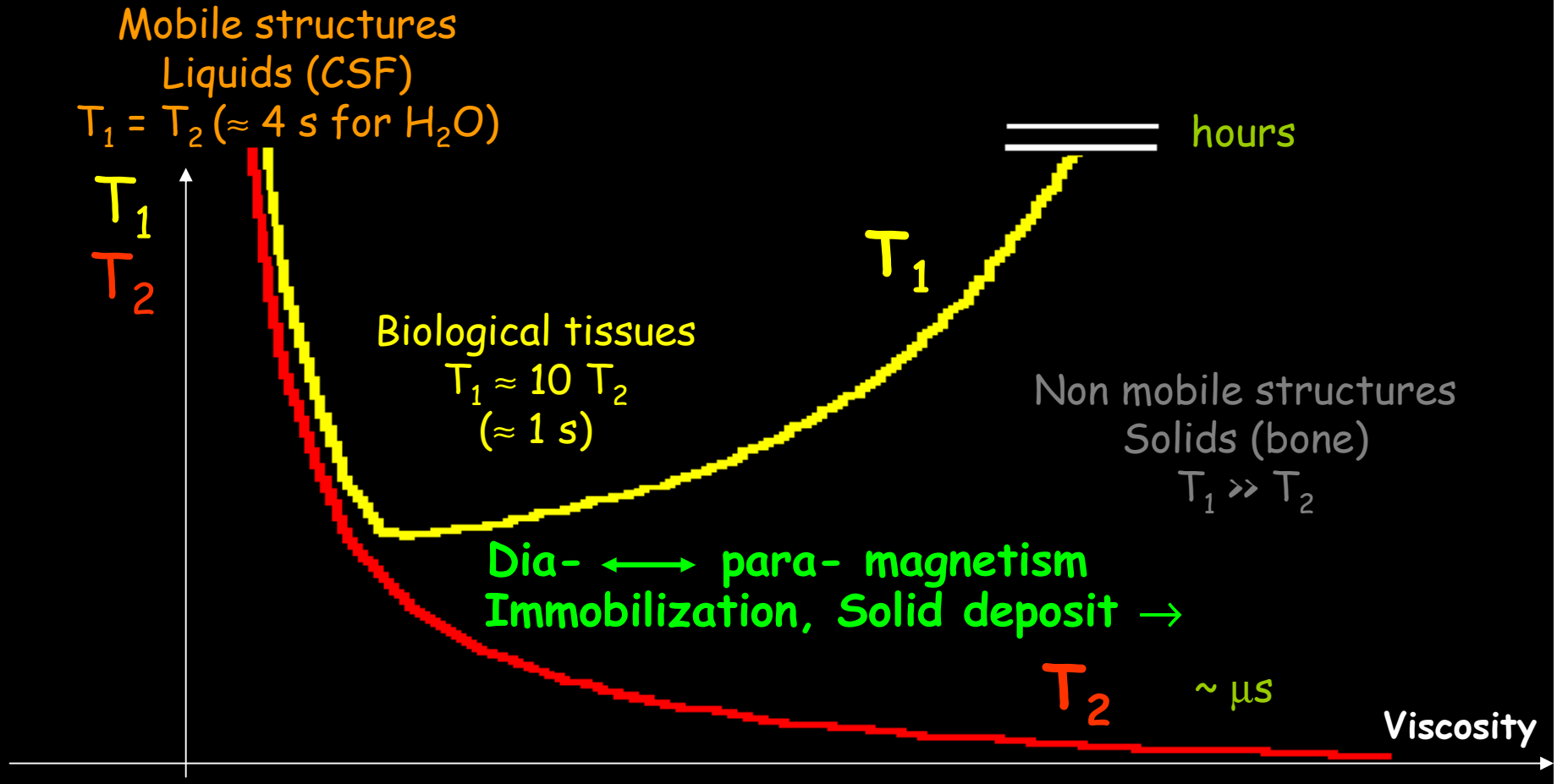
T1 & T2 modulations in MRI

... due to physiology or pathology

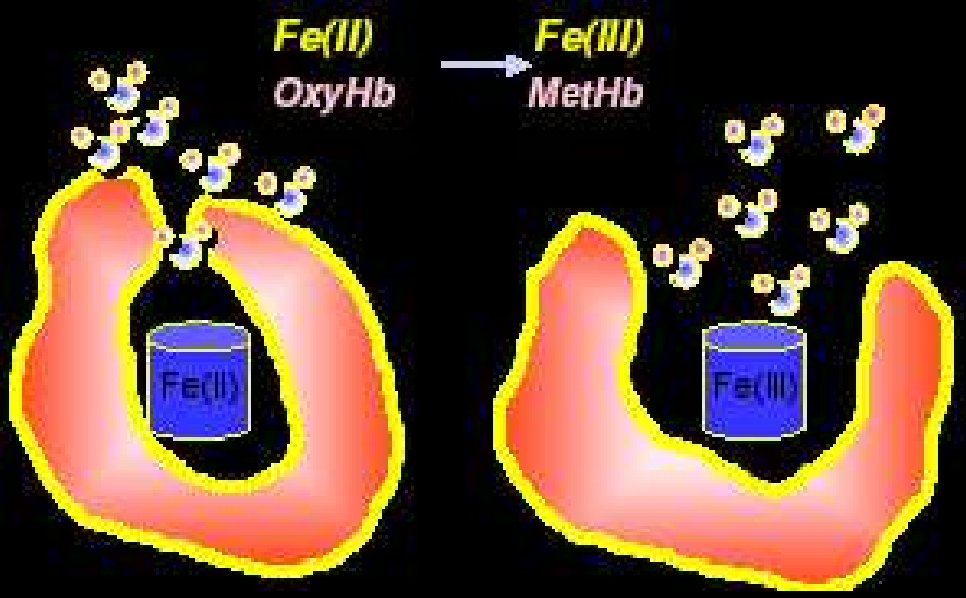


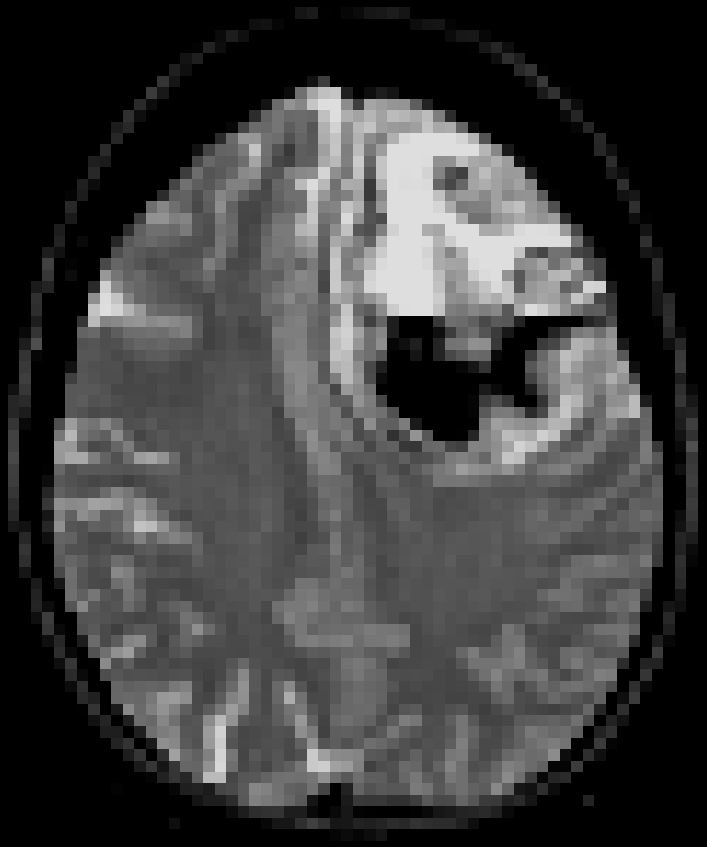


Positive contrast in $T1_w$ images, negative in $T2_w$



Spontaneous MRI T2* contrast when MetHb replaces HbO_2





HbO_2 is diamagnetic
MetHb is paramagnetic

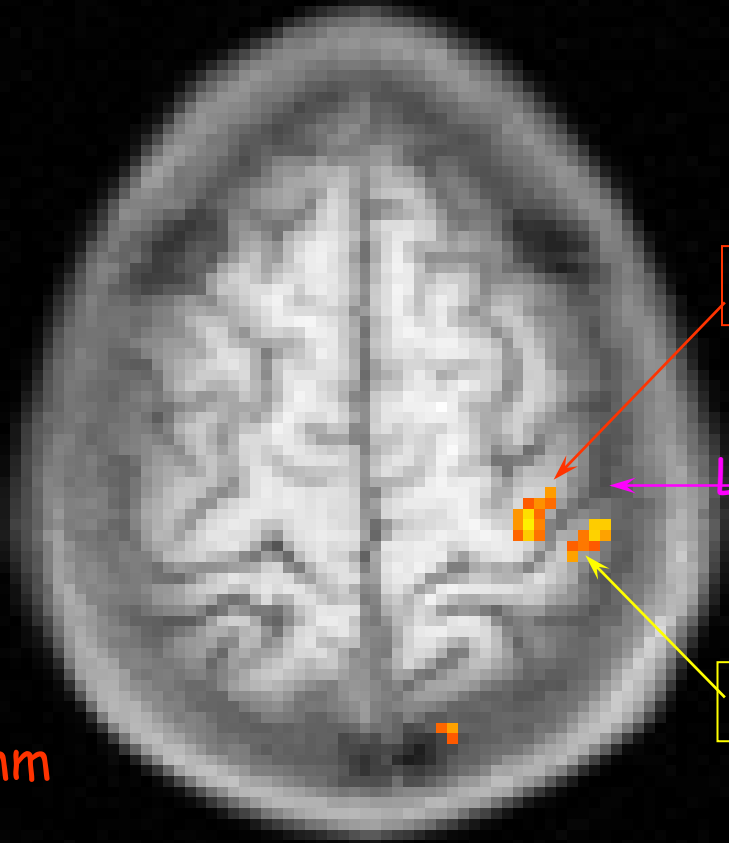
Late aspect of an hemorrhagic stroke

From M. MOSELEY, MR role in Molecular Imaging, MR4MI_2006

Spontaneous MRI contrast due to an excess of HbO_2 relatively to Hb

HbO_2 is diamagnetic
Hb is paramagnetic

Right handed subject, pre- and post- central left activations



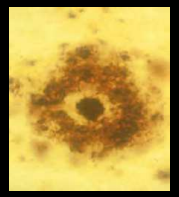
AC-PC + 70 mm

Tactile opposition, right thumb / V^{th} meta origin

M ZANCA, University Hospital & UMR/CNRS 5587, MONTPELLIER

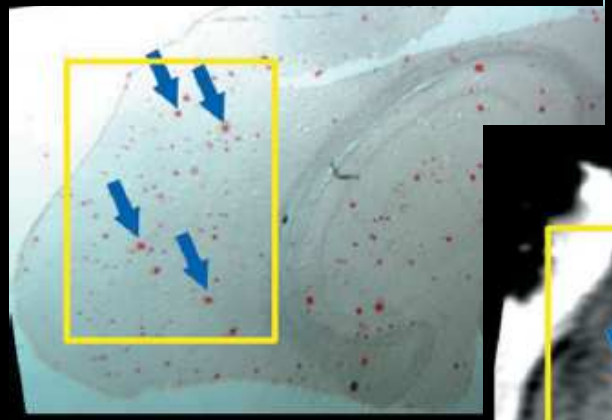
Spontaneous MRI signal loss in solid structures

Ex vivo detection of β amyloidal deposits (Alzheimer) in $T2_w$ MRI

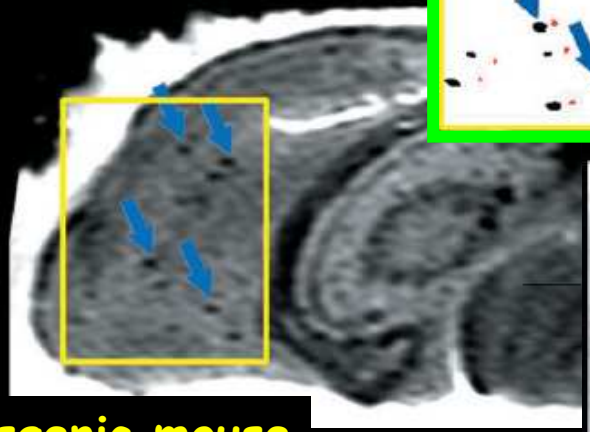


Extra cellular β amyloidal peptides deposits (20-150 μm) have very short T2*

Histology with Congo red

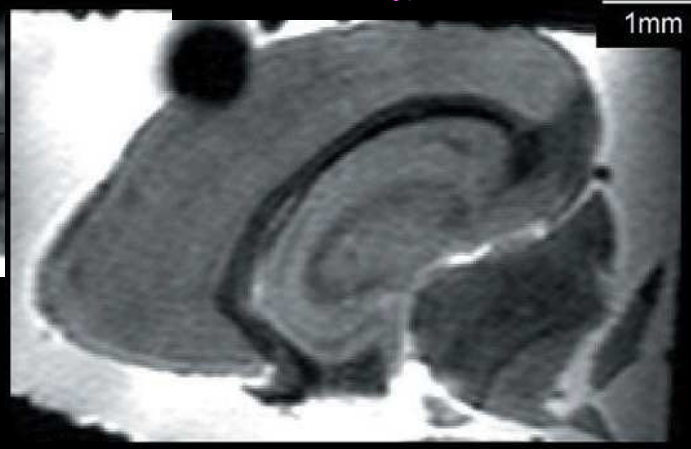


T2*-MRI



mAPP-PS1 transgenic mouse

Control mouse



mAPP : mutant human amyloid precursor protein
PS1 : mutant presenilin-1 transgenes

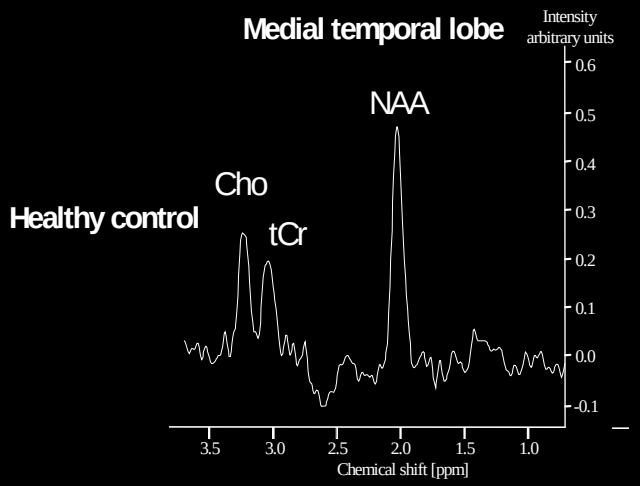
In the green surrounded frame, large MRI black spots correspond to amyloidal deposits seen by histology (red dots), moved forward for clarity and to avoid superimposition

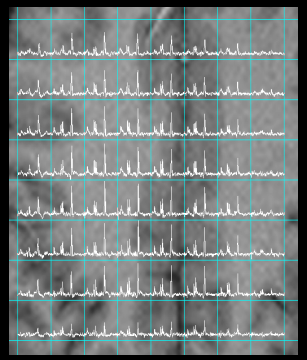
J ZHANG, et al.,, Magnetic Resonance in Medicine, 2004; 51: 452-457

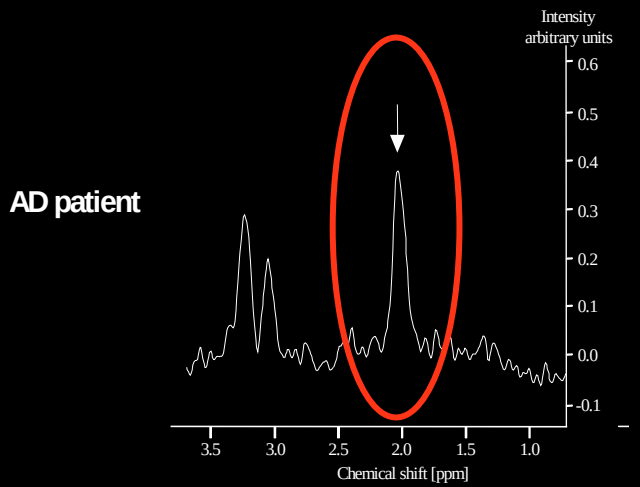
Metabolite cartography by ^{1}H-MRS & CSI

Example of Alzheimer Disease (AD)

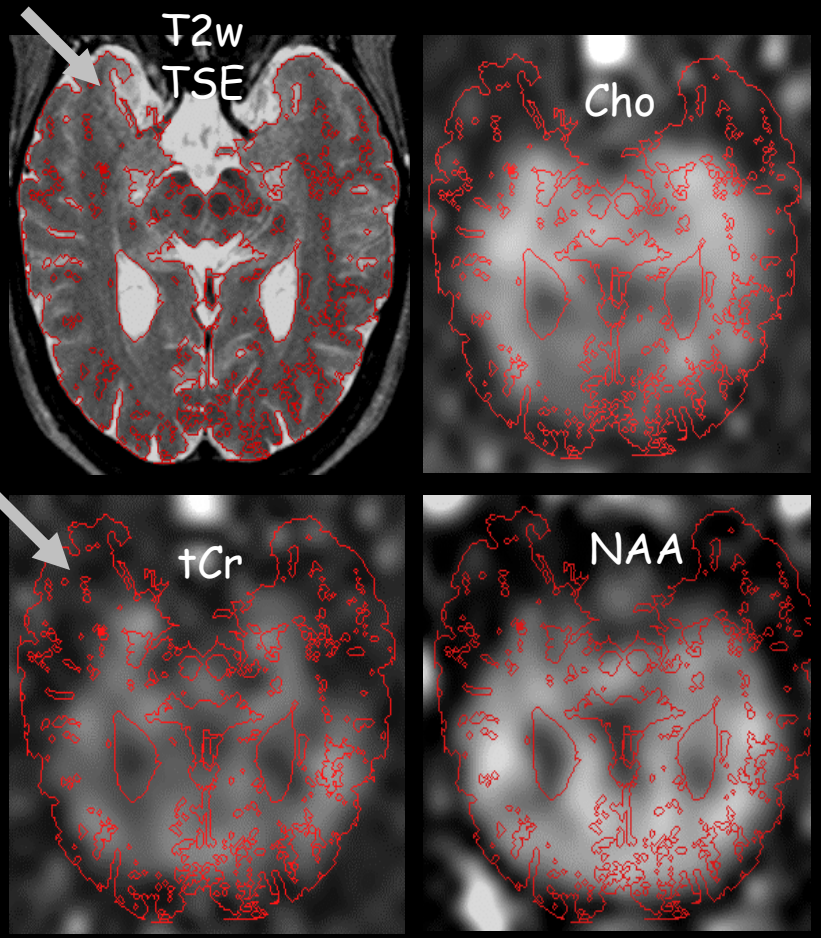
MRS





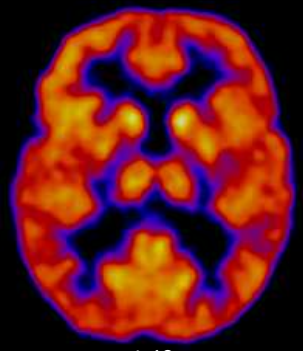


CSI



Courtesy Gwénaël HÉRIGAULT, Philips Medical Systems, 2002

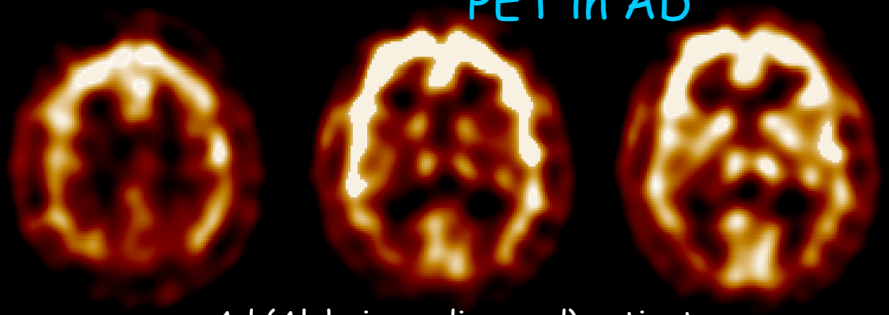
Spontaneous cerebral metabolism of ^{18}FDG



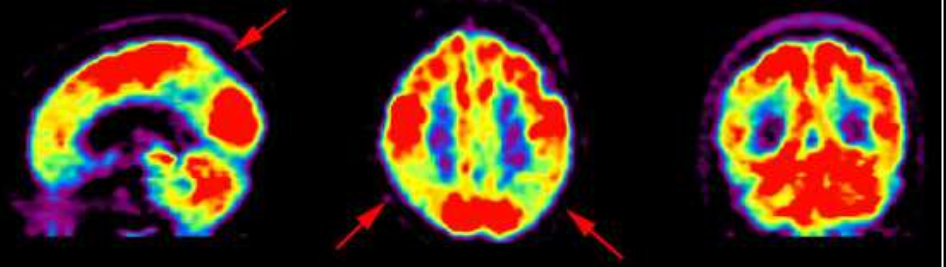
Normal ^{18}F-FDG TEP image

Glucose metabolism is equivalently distributed in the cortex for a normal (non pathological) brain, but is greatly lowered in specific regions in AD

PET in AD

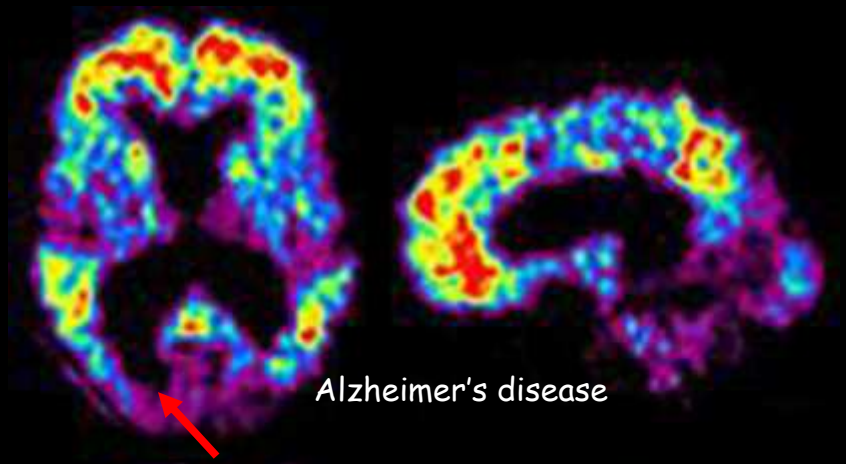


Ad (Alzheimer diseased) patient

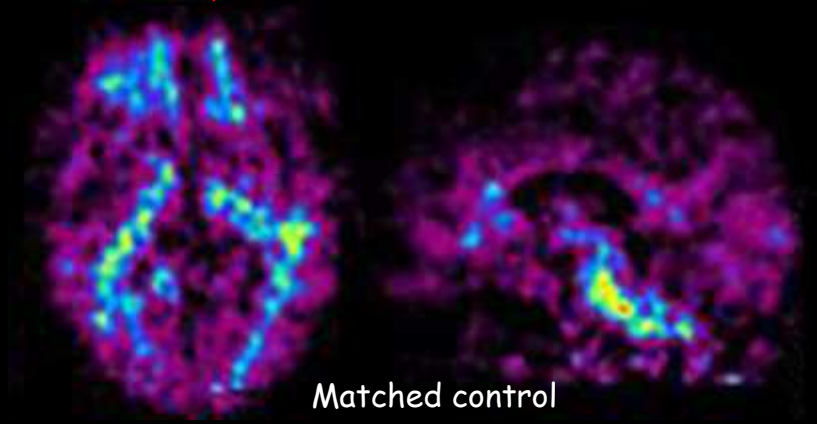


Visualizing AD β-amyloids deposits with ^{11}C-PIB PET

Pittsburgh compound B



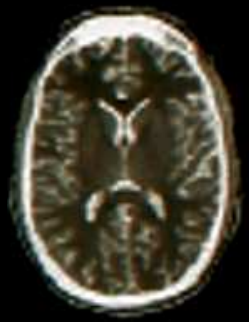
Alzheimer's disease



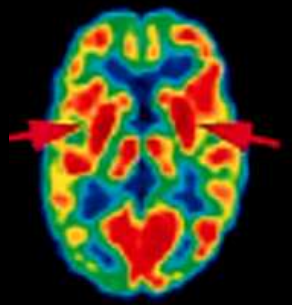
Matched control

KLUNK W, MATHIS C, et al., Pittsburgh, Associated Press posting 12 Jan 2003

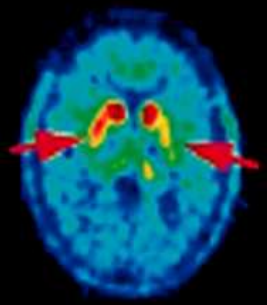
Crude and grafted Parkinson disease



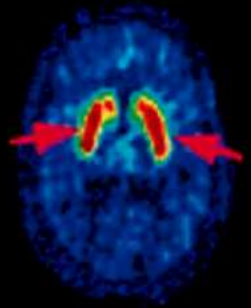
MRI
^{1}H



Metabolism
^{18}FDG



^{18}F-DOPA
Pre-synaptic
DaT



^{18}F-Ethyl
Spiperone
Post-synaptic
D_2R

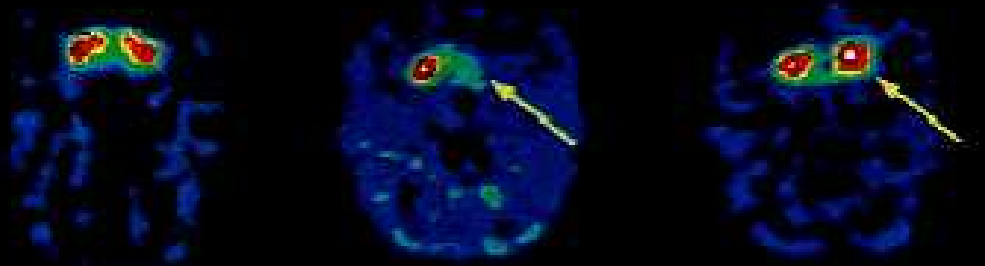
Clinically left Parkinson

Loss of DaT function in the right putamen

D_2R is normal or a little higher (up regulation)

M.E. PHELPS, JNM, 2000 ; 41 : 661-681

meta-^{18}F-tyrosine



Normal

Before Gene Therapy

After Gene Therapy

Left Parkinson generated in a monkey

A Macaques Rhesus after homolateral MPTP injection and grafted stem cells

K. BAUKIEWICZ, in M.E. PHELPS, PNAS, 2000 ; 97(16) : 9226-33

Let's now consider
the indirect way
to reach the
molecular level

using specific "engineered"
probes and ligands to
reveal cells activity

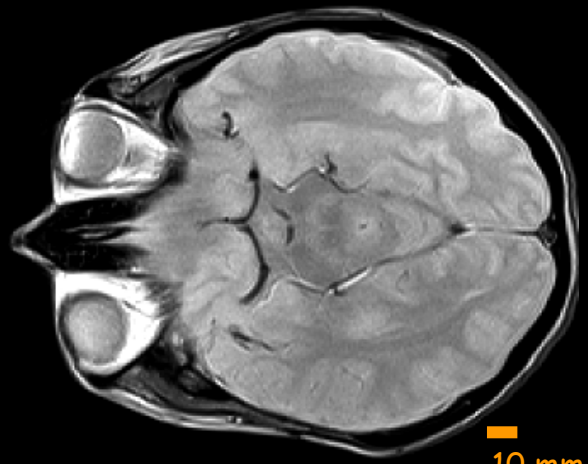
The indirect way to reach the molecular level

Apart a few examples in PET and optical imaging, we'll essentially consider MRI, due to its (very) high spatial resolution and a great variety of possible contrasts

But MRI fails by a (very) low sensitivity and we'll present some examples about how very sophisticated engineered (nano)-techniques will improve it, among which :

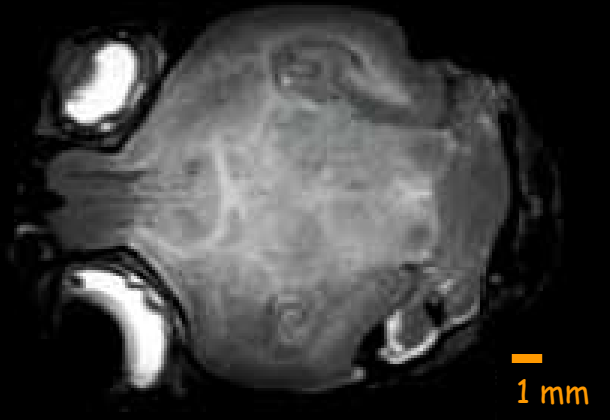
- Development of high relaxivity contrast media
- Amplification of relaxivity and targeting
- Development of smart agents whose action is limited to the targeted site and/or a chosen cellular activity

MRI gives access to the (very) high intrinsic spatial resolution



Healthy human

$R_{sp}°$ ~ 1x1x1 mm^3



Mouse tumour

$R_{sp}°$ ~ 110x110x110 μm^3

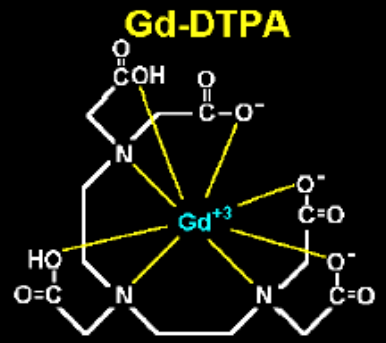
Xenopus larvae

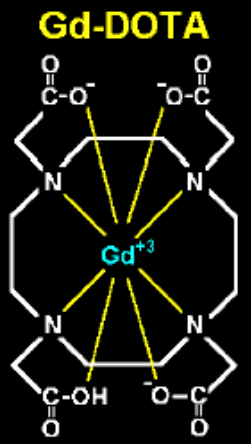
MRI spatial resolution ($R_{sp}°$) is only limited, for a given contrast, by translational movements of water molecules, of about 2 to 20 μm.

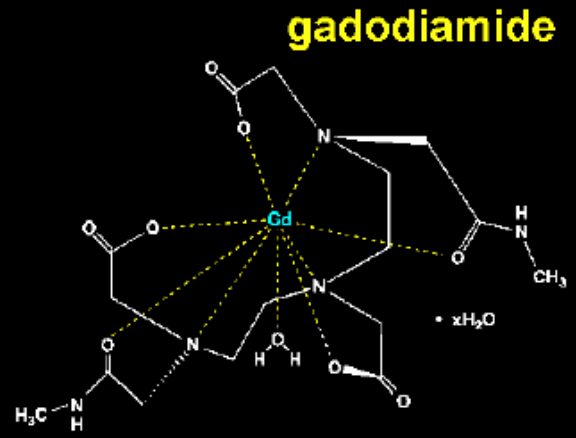
Indirect imaging:
contrast media and probes

MRI paramagnetic contrast media

Gd chelates (hypersignal in $T1_w$ MRI, toxics)







High detection threshold, added to the very low MRI sensitivity, implies high concentrations and/or smart amplification techniques

They often are coated and targeted by coupling or conjugation :
- toward receptors,
- toward cells (Gd-EOB-DTPA and hepatocytes)
- toward plaques or clots (conjugation to anti-fibrin antibodies)

WANG, Eur Radiol (2001) 11:2319-2331

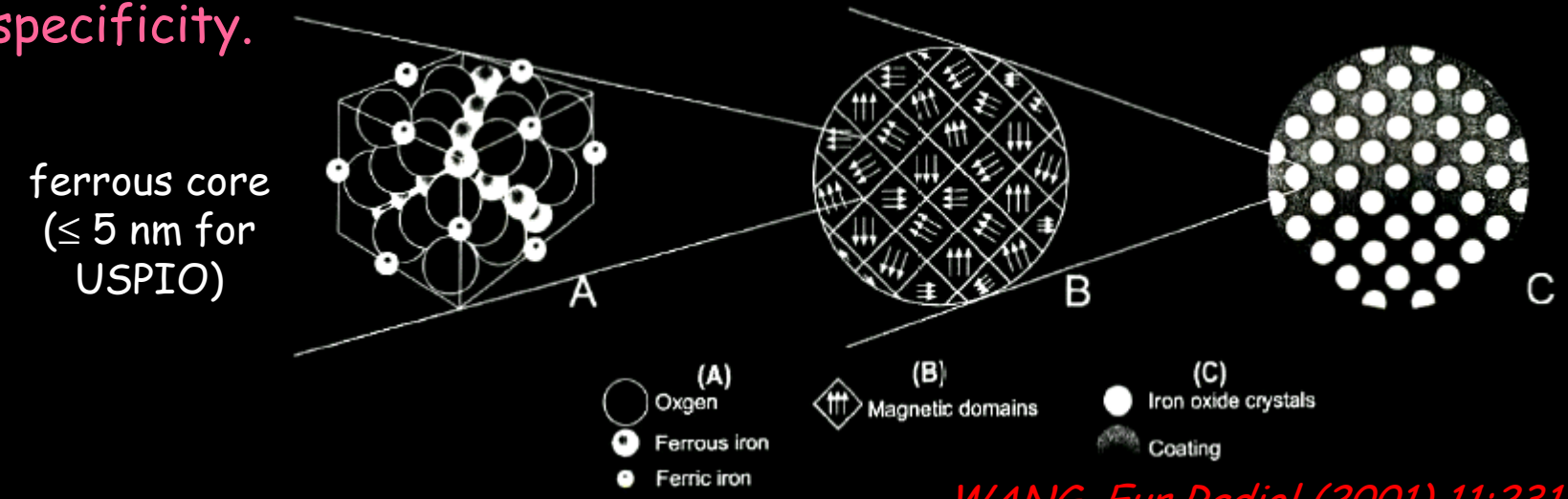
MRI superparamagnetic contrast agents

SPIO = Superparamagnetic Iron Oxyde (∅ > 50 nm), marked accumulation in RES (liver, spleen, lymph nodes).

USPIO = Ultrasmall Superparamagnetic Iron Oxyde (∅ ~ 10-50 nm), far more efficient, with a marked accumulation in monocytes and macrophages (graft reject, plaques of atherosclerosis).

Both SPIO & USPIO have a T2* main effect (hyposignal), with a detection threshold in mM for SPIO, µM or even nM for USPIO)

MIONs (Monocrystalline Iron Oxide Nanoparticles) are (U)SPIO nano particles (∅ ~ 3 mm with > 2000 Fe atoms) without any molecular specificity.

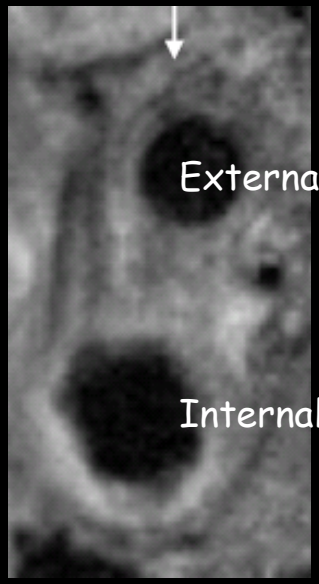


WANG, Eur Radiol (2001) 11:2319-2331

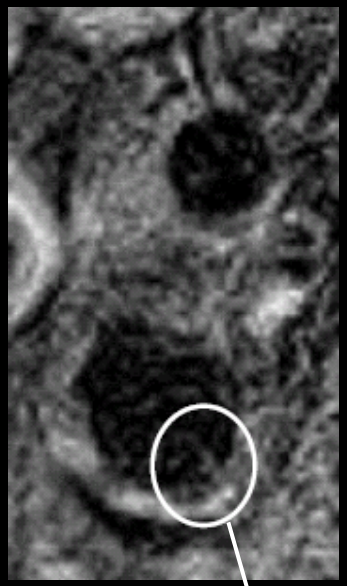
Indirect imaging via passive probe uptake and elimination

USPIO passive uptake by macrophages allows atherosclerotic plaques detection

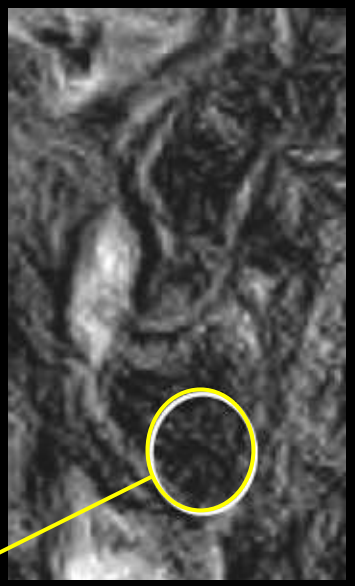
MRI



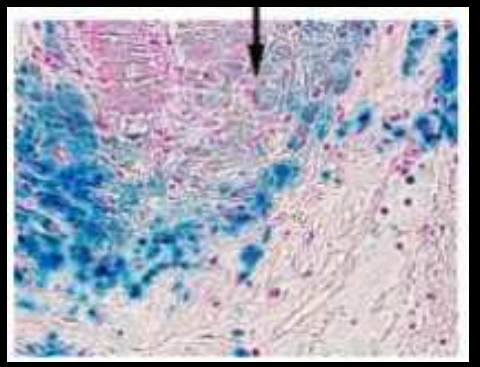
$T2_w$* MR images



USPIO
+24 h



Plaque T2* hypersignal before contrast
Plaque T2* hyposignal after USPIO



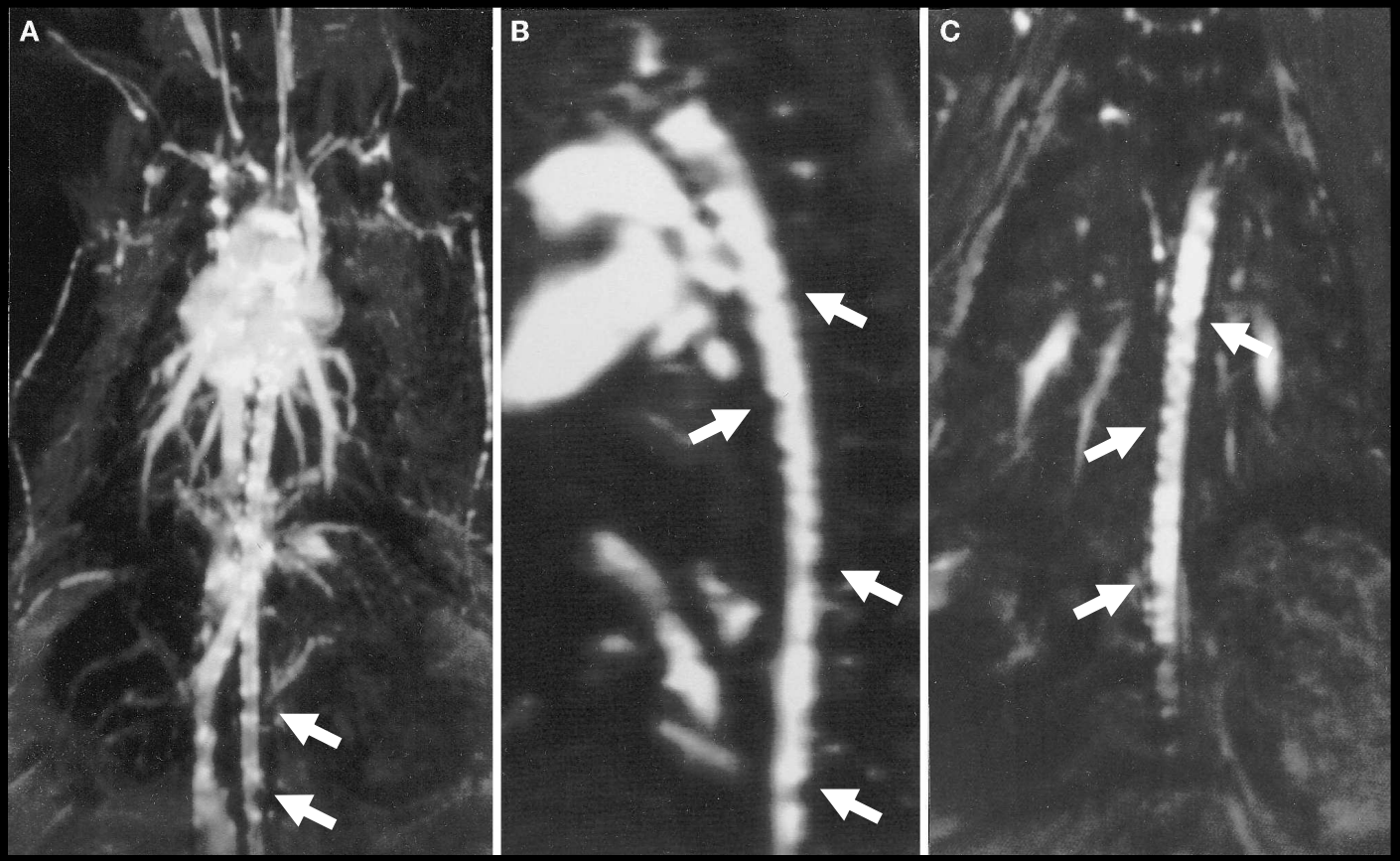
Plaques vulnerability is determined by their macrophages content

Histology :
CD68 (macrophages) – red
Perls (USPIO) - blue

ME KOOI, ISMRM Proceedings 2002

USPIO & vascular plaques detection

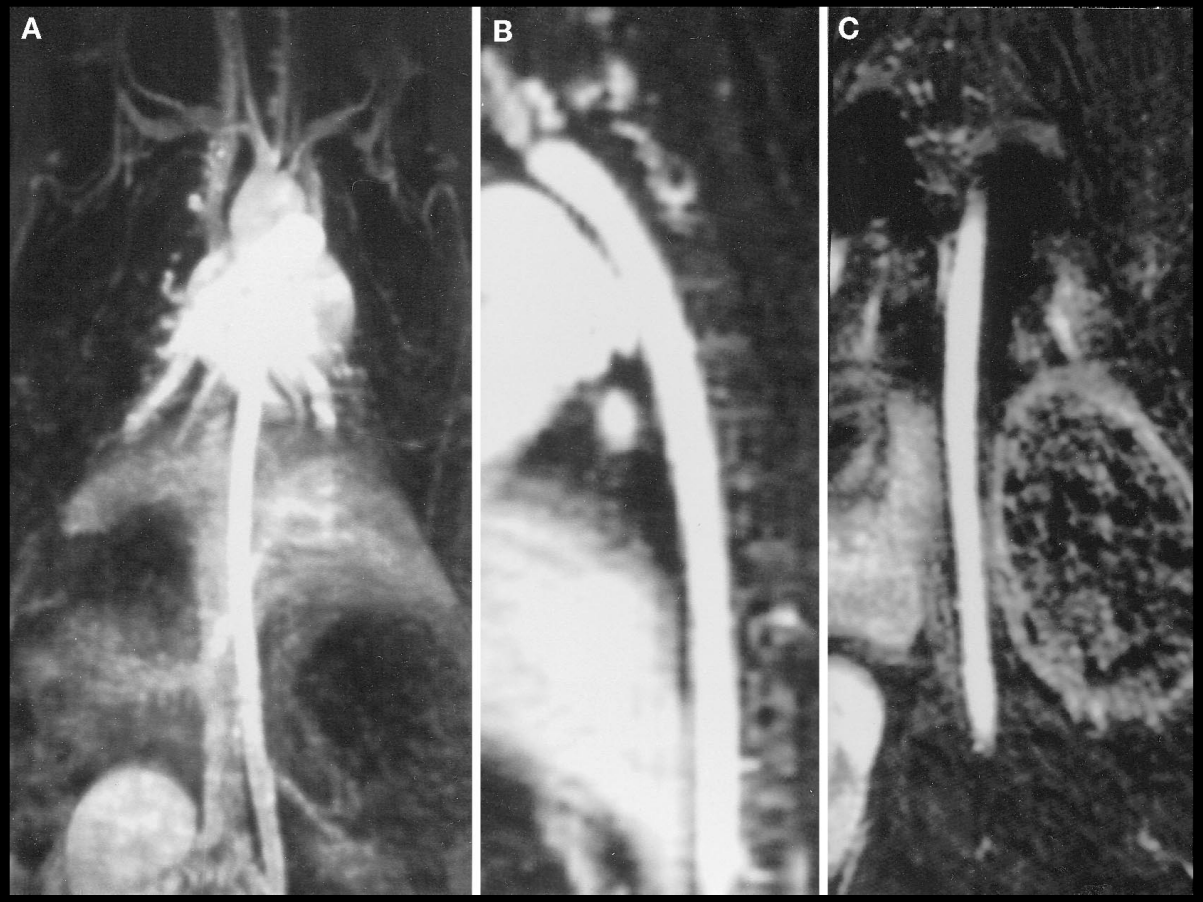
7 month old rabbit with hyperlipidemy and atherosclerosis



Hypo signals due to passive Fe uptake in macrophages confined in atherosclerotic plaques

RUEHM, SG et al, Circulation, 2001; 103: 415

… while, with Gd-DTPA, atherosclerotic plaques are not visible

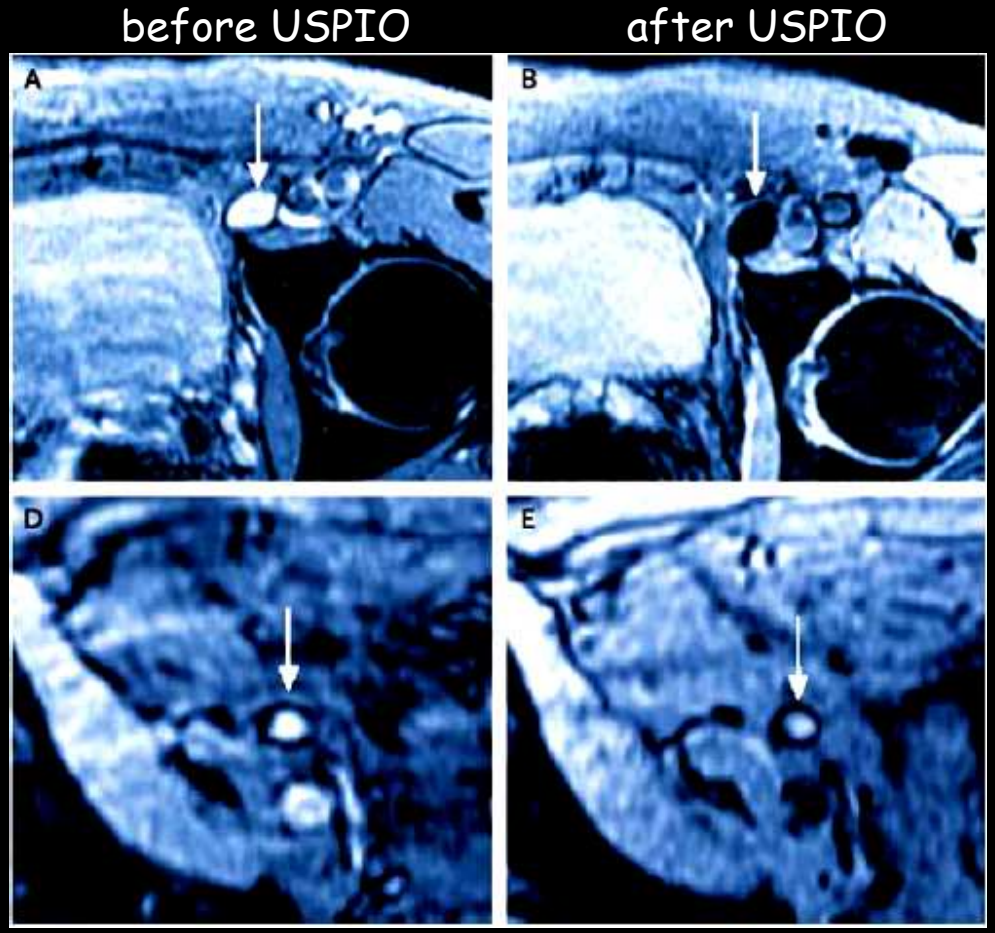


The same hyperlipidemic rabbit

RUEHM, SG et al, Circulation, 2001; 103: 415

USPIO uptake by metastatic lymph nodes

USPIO (∅ ~50 nm) improve diagnosis of metastatic axillary lymph nodes compared with precontrast MRI



Healthy lymph node captures the SPIO
T2* signal is homogeneously decreased

Tumorous lymph node does no more capture the SPIO
Persistent hypersignal

HARISINGHANI MG, et al., N. Engl. J. Med., 2003; 348: 2491-2499

Indirect imaging via targeted probe uptake and elimination

- Targeting at the tissue level
- Targeting at the cellular level
- Targeting at the molecular level

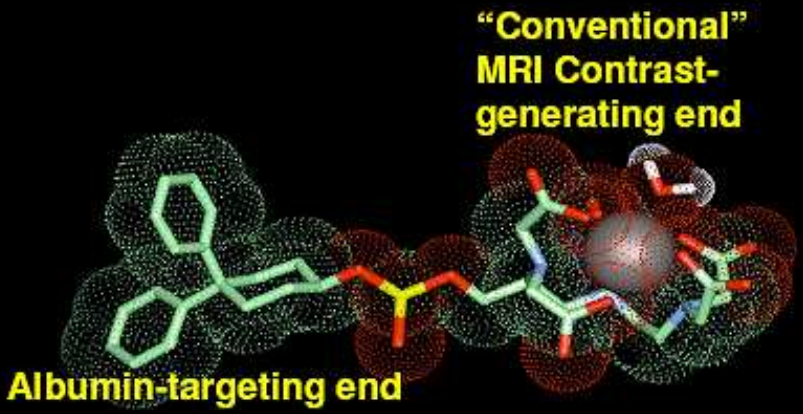
Targeting blood in vessels: Gd Complex coupled to Serum-Albumin

Albumin-binding (MS-325)

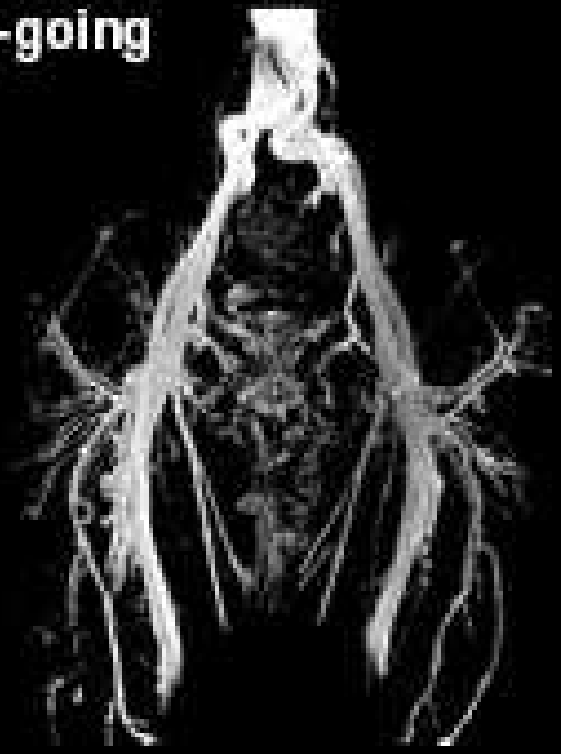
Enhanced relaxivity while in the blood

Long life = hi-res blood pool MRA

Phase III on-going



MRI

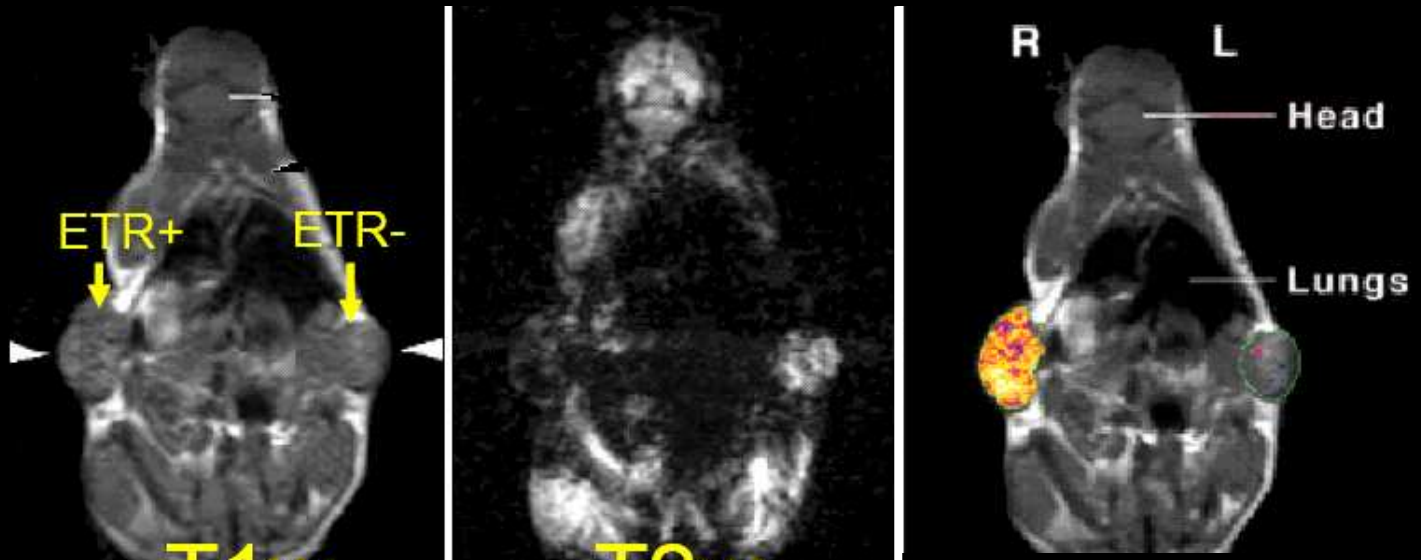


Iliac and femoral vasculature
Richard Baum, M.D.
University of Pennsylvania

From M. MOSELEY, MR role in Molecular Imaging ?, MR4MI_2006

Targeting sarcoma cells with MRI Tf-MION via transferrin receptor overexpression

Gliosarcoma cells are stably transfected with an expression plasmid containing engineered transferrin receptor (ETR+) cDNA that overexpresses high levels of the transferrin receptor protein. This will result in a marked increase in the cellular binding and uptake of MION to holotransferrin (Tf-MION), as can be seen in that living mouse with a right ETR+ flank tumour and a left ETR– flank tumour.



T1w
does not separate both tumours

T2w
drastic dropout in the ETR+ tumour

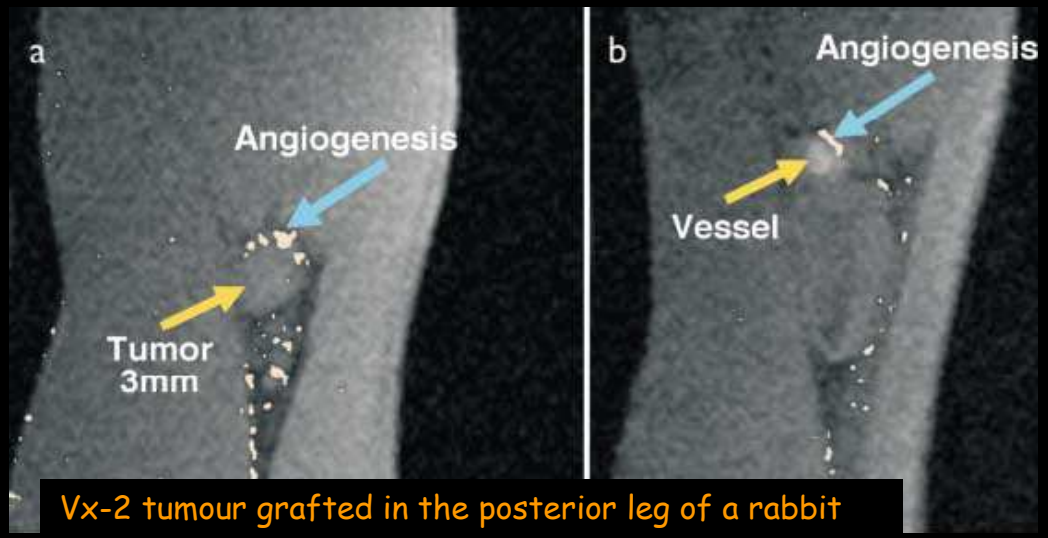
after Tf-MION,
increased uptake of iron in the right ETR+ tumour

WEISSLEDER R, Nat. Rev. Cancer, 2002 ; 2 : 11-18

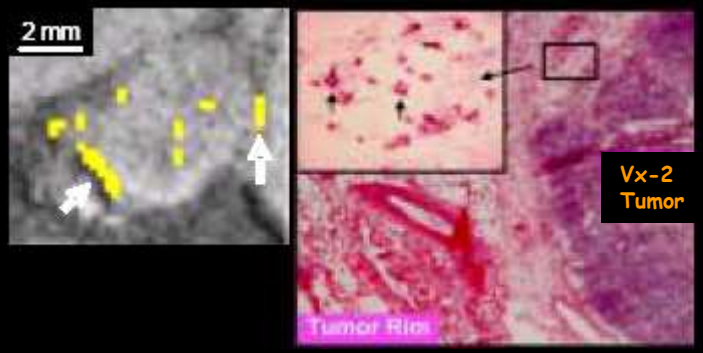
Nano particles targeted for endothelial $\alpha_v\beta_3$-integrin and angiogenesis

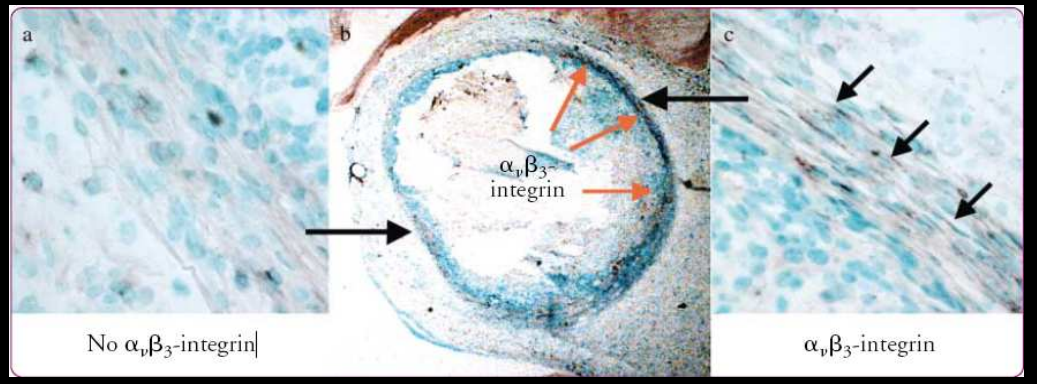
Integrin is a membrane receptor that binds peptides as fibronectin and laminin implied in cellular adhesion.

Integrin increases in cancer angiogenesis, at capillaries epithelial cells membranes level



Vx-2 tumour grafted in the posterior leg of a rabbit





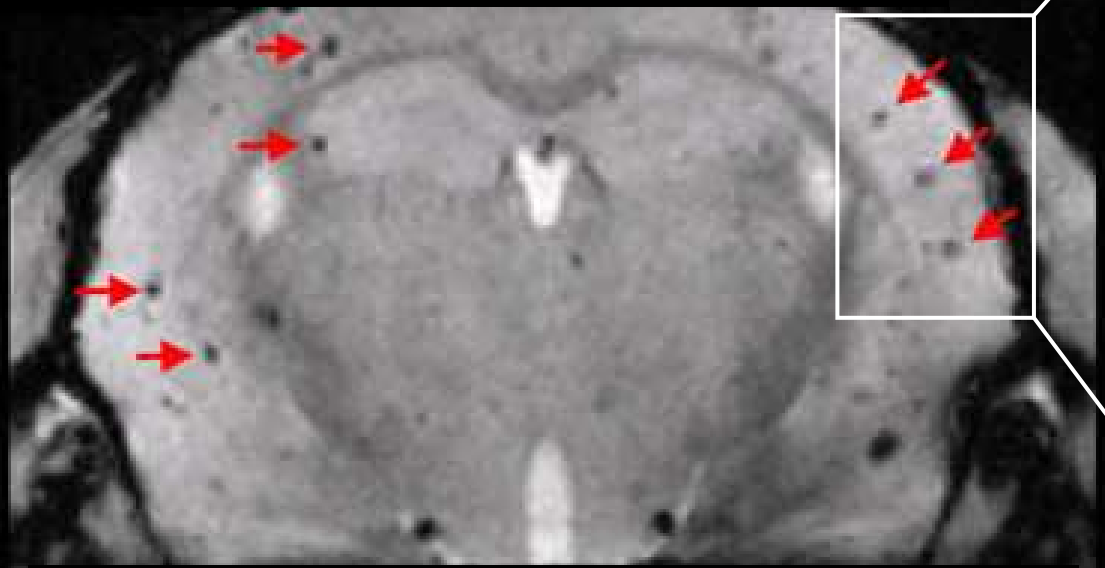
Exact correspondence between histology and MRI

DA SIPKINS et al., Nature Medicine, 1998 ; 4(5) : 623 – 626

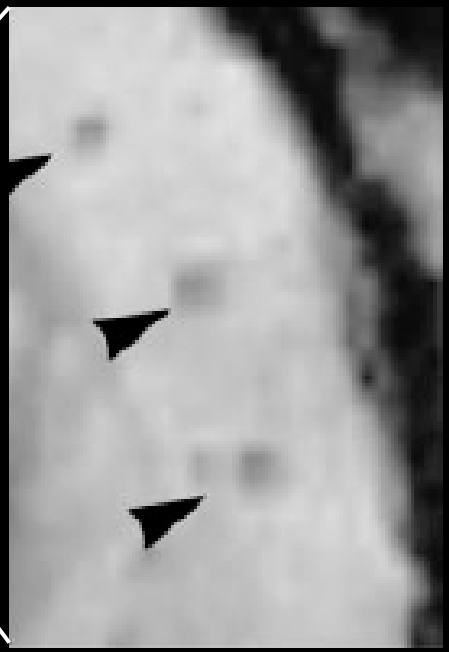
SPIO-peptides targeted for Amyloid ß plaques

Magnetically labeled peptides enable in vivo detection of amyloid-ß (arrows) in the brains of transgenic mice used to model Alzheimer's Disease

Plaques in *in vivo* T2* MRI
78x78x250 mm, acquisition 59 min



6-month-old APP/PS1-transgenic mouse brain

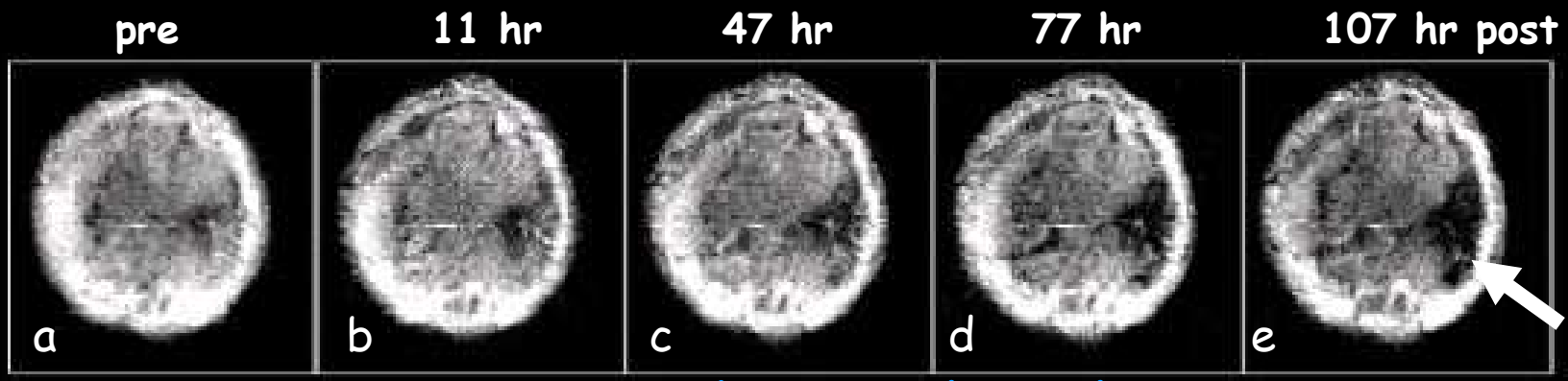


Arrows emphasize the neat agreement with plaques shown by histologic coloration

http://www.med.nyu.edu/cgi-bin/bk/showresimg.py?pid=37233

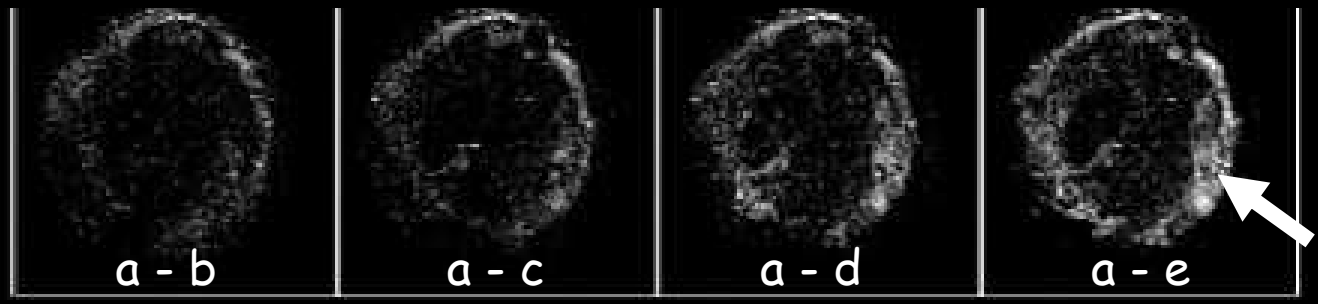
C2-Synaptotagmin SPIO and apoptosis

Synaptotagmin I binds to anionic phospholipids (phosphatidylserin, PS).
During apoptosis, PS translocates from inner to outer layer of cellular plasma membranes.
Thus, when cells are dying by apoptosis, SPIO conjugated to C2 domain of synaptotagmin I will bind to PS and reveal the apoptotic area (arrow).



Murine lymphoma following treatment with cyclophosphamide and etoposide

Cells in apoptosis



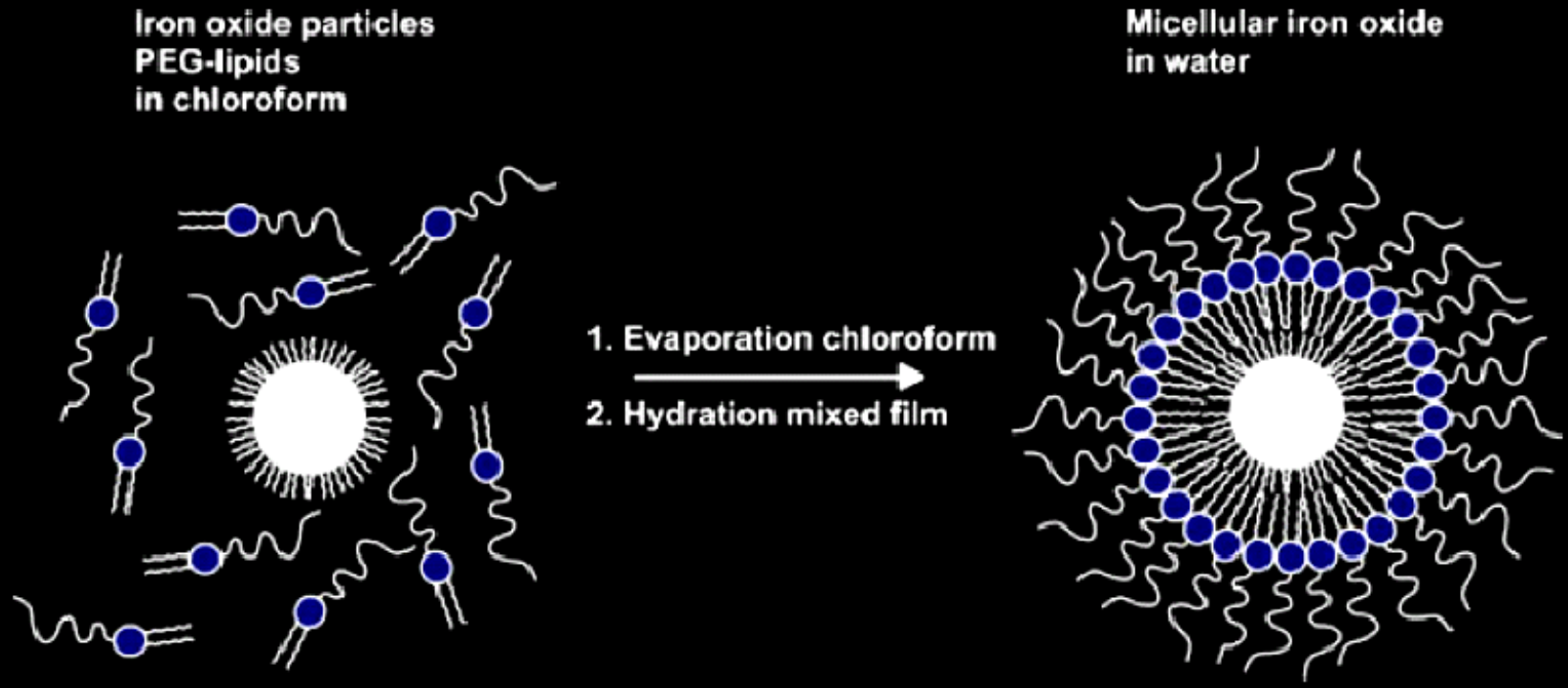
M ZHAO et al., Nature Medicine, 2001 ; 7 : 1241-44

Indirect imaging via contrast over concentration (amplification)

- using (targeted) micelles
- using (targeted) dendrimers
- using carbon nanotubes
- using cellular enrichment in iron affine structures

Nanoparticle-containing micelles

Schematic representation of the encapsulating procedure of hydrophobic nanoparticles in micelles.



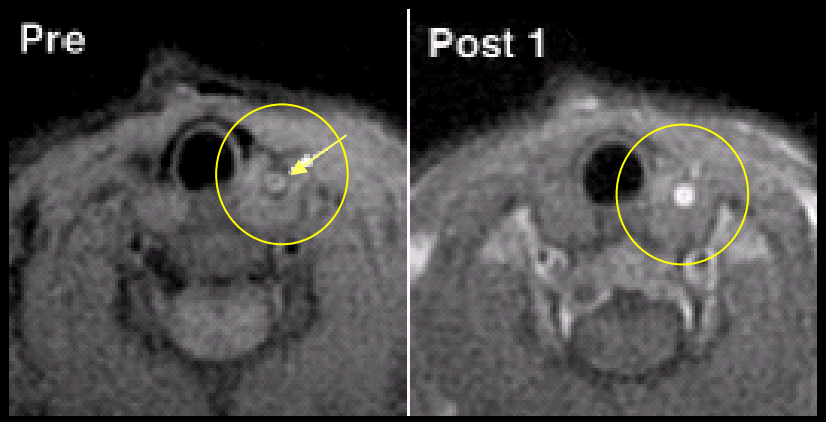
Lipids are mixed with the nanoparticles in an apolar solvent. The mixed film obtained is hydrated. Thereafter, the nanoparticle-containing micelles and empty micelles are separated by centrifugation

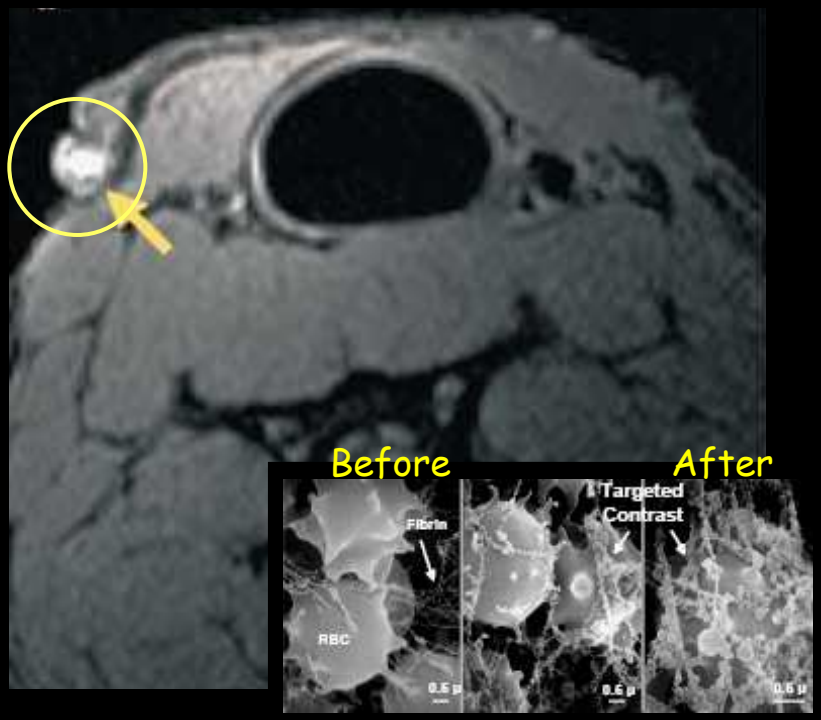
WJM MULDER, GJ STRIJKERS et al., NMR Biomed. 2006;19:142-164

The targeting with fibrin-specific micelles containing paramagnetic nanoparticles visualizes thrombus in the external jugular vein

Fibrin plays the role of anti thrombic antigen

There exists a neat $T1_w$ contrast increase where paramagnetic nano particules conjugated to anti-fibrin antibody fragments accumulate (yellow arrows and circles).

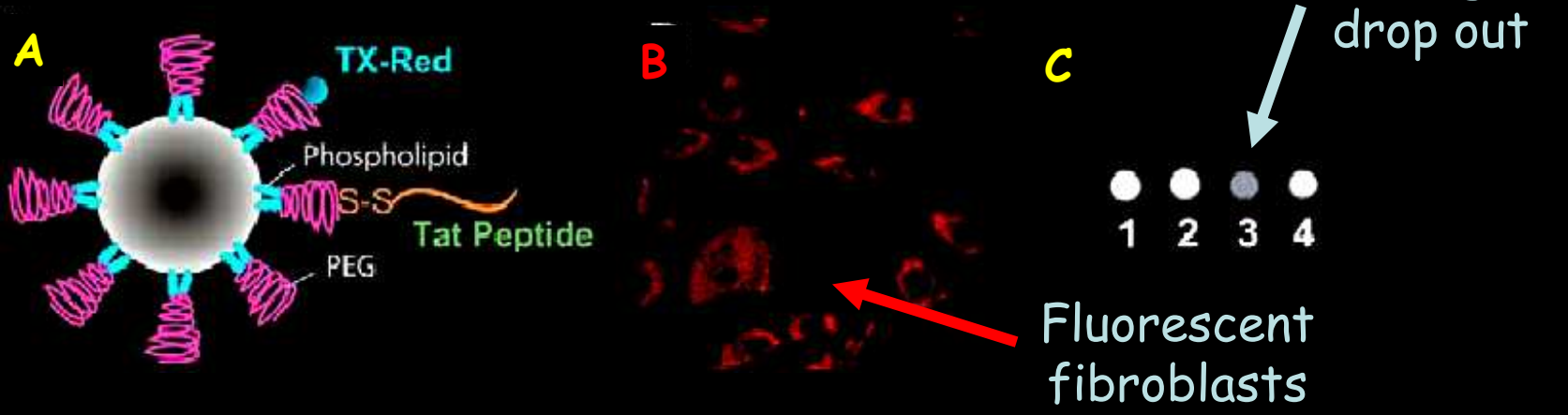




Microscopic MRI

FLACKE S, et al., Circulation, 2001 ; 104(11) : 1280-1285.

Micelle-encapsulated SPIO conjugated with Tat peptide and the fluorescent label Texas Red



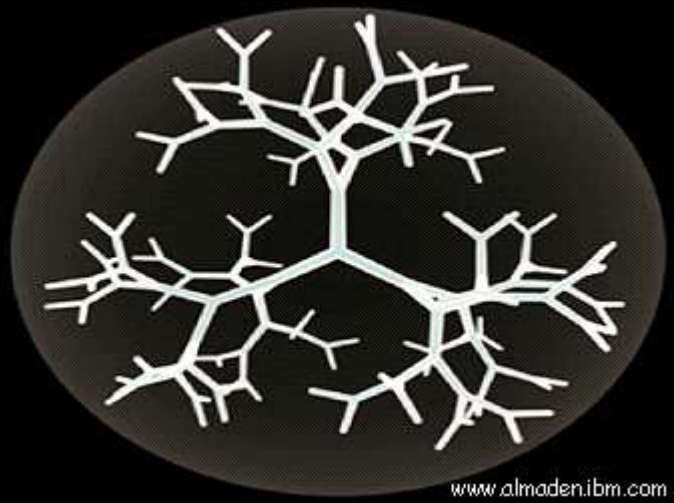
(A) Schematic of a micelle-encapsulated SPIO conjugated with Tat peptide and the fluorescent label Texas Red (TX-Red) for cellular delivery and combined optical imaging and MRI.

(B) Fluorescent images of Tat-linked, TX-Red-labeled SPIOs in human dermal fibroblast (HDF) cells. Images were obtained using a Zeiss confocal microscope with excitation at 543 nm and emission detection at 560 nm.

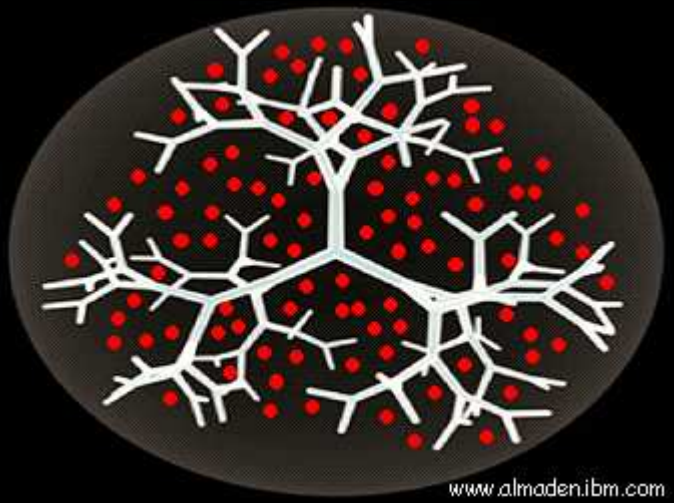
(C) MRI images of four different samples: (1) culture media only, (2) cells without SPIOs, (3) cells with SPIOs, (4) culture media only. Images were obtained using a 3 T Siemens TRIO MRI machine.

L LACONTE, N NITIN, and G BAO, Nano Today, Mai 2005
http://www.bme.gatech.edu/groups/bao/mion.html

Over concentration of magnetic nano particles in dendrimers



"Virgin" dendrimer



Gd^{3+} or MION Magnetodendrimer

A dendrimer component is a polymeric, 3D tree-like structure

It contains a great number of 3D voids acting as pockets carrying numerous particles of contrast agent (Gd^{3+} or super-paramagnetic nanoparticles as MION).

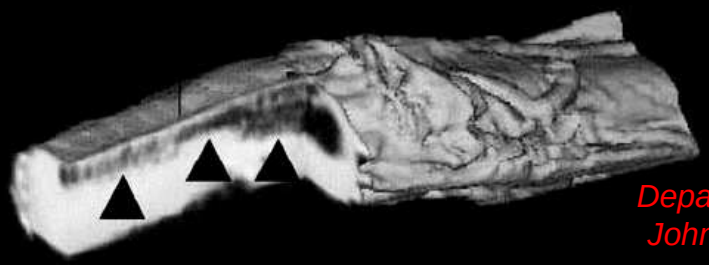
Magnetodendrimers allow efficient labeling of mammalian cells, including human neural stem cells and mesenchymal stem cells.

Their use in MRI allows growth tracking of new neural pathways from the stem cell transplant.

Nanotechnology and Medicine, Nanopedia, the web course of nanotechnology
http://nanopedia.case.edu/NWPrint.php?page=nw.ppm2.med3

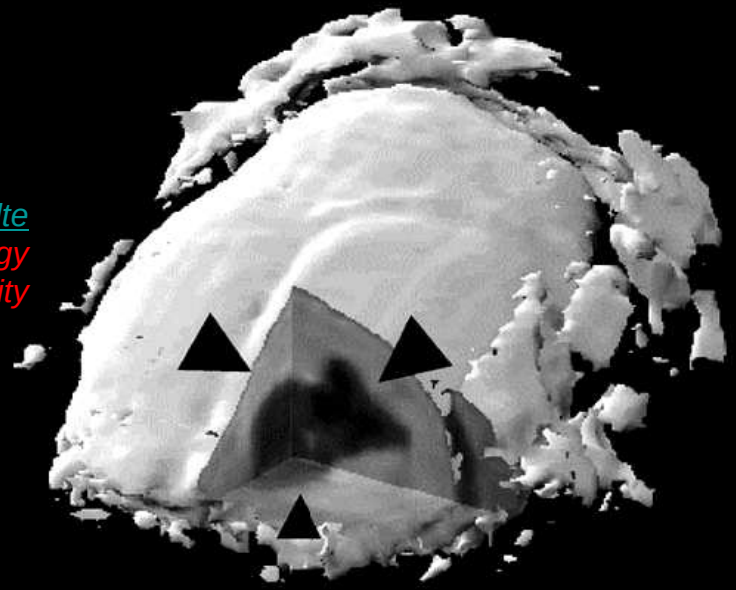
Application of magnetodendrimers as cellular markers after transplantation

Stem cells containing magnetic nano particles in dendrimers were transplanted in dysmyelinated rat spinal cord



Jeff Bulte
Department of Radiology
John Hopkins University

At 10 days following transplantation, 3D *ex vivo* MR image shows the migration of labeled cells along the dorsal column away from the injection site.



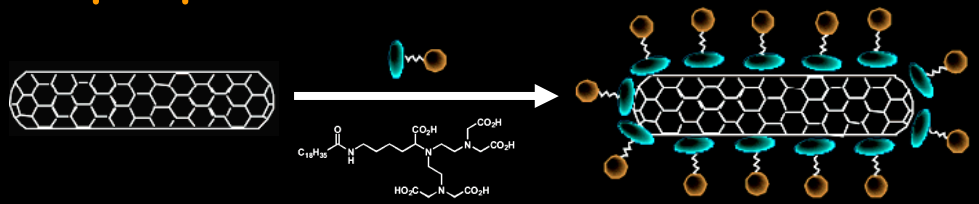
At 6 weeks following transplantation, 3D *in vivo* MR image shows the migration of labeled cells into the parenchyma away from the ventricle

There exists an excellent concordance between MRI and immunohistochemical coloration of new formed neural myelin

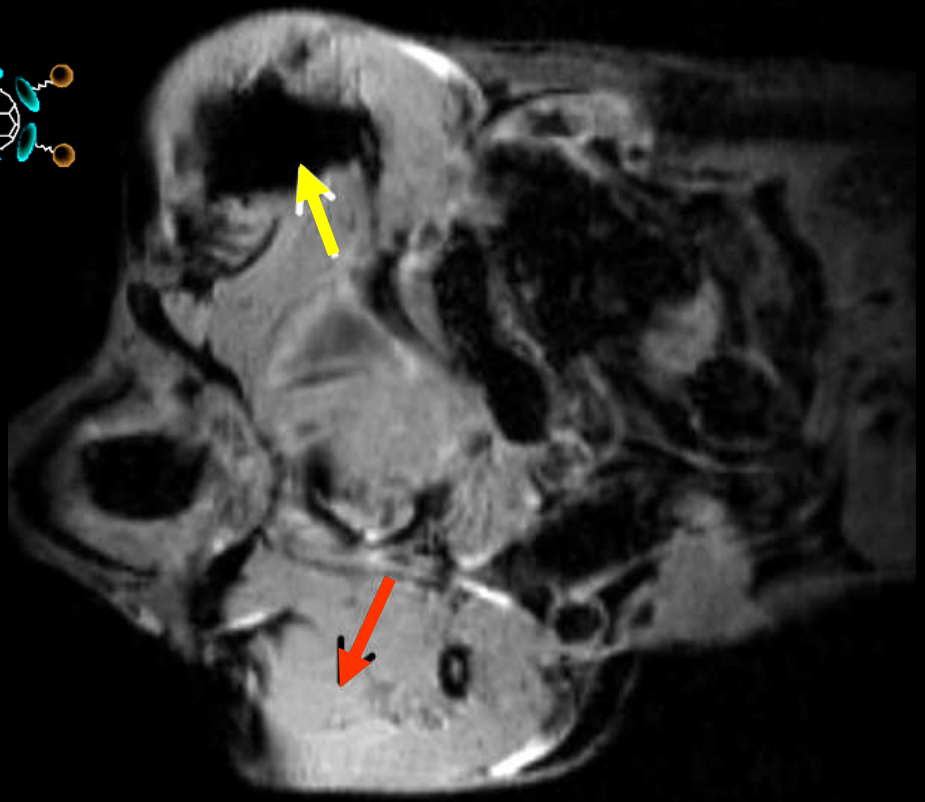
JWM BULTE, Nature Biotech, 2001
JWM BULTE, Journal of Magnetism and Magnetic Materials, 289 (2005) 423–427

Amplification and targeting with carbon nanotubes loaded with hydrated Gd^{3+} ions

Carbon nanotubes can be noncovalently functionalized by amphiphilic Gd^{3+} chelates.

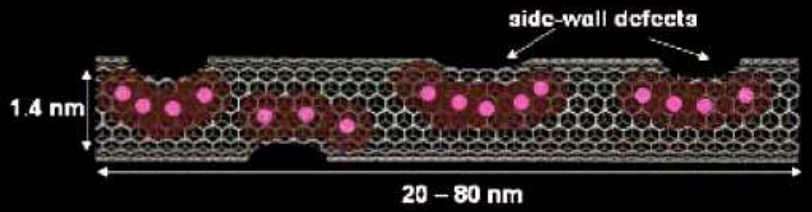


Coronal in vivo MR image of the muscle of a mouse legs … after Gd^{3+} multiwalled carbon nanotubes injection … and lipid injection



C RICHARD,ET al., Nano Lett. 2008, Vol. 8, No. 1: 232-236

Here is another way to load carbon nanotubes with Gd^{3+}



B. SITHARAMAN, et al., Chem. Commun., 2005, 3915–3917

Modifying images by activable contrasts

- smart or reporter agents
- Magnetic relaxation switch of CLIO
- Magnetic relaxation amplification
- Reporter gene imaging

Smart contrast agents

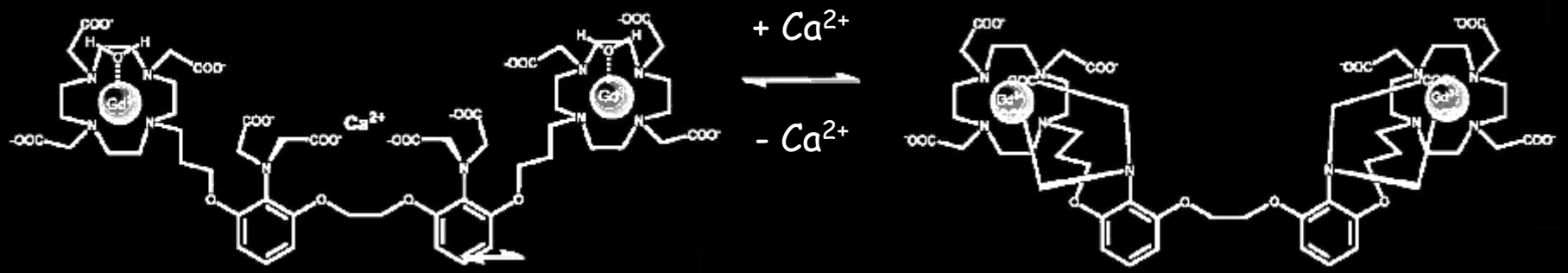
Smart contrast agents are activable agents that undergo a large change in relaxivity upon activation: one state is *off* and corresponds to low contrast enhancement, while the other state, the *on* state, corresponds to high contrast enhancement.

The activatable agent can be *switched* from one state to the other by the occurrence of a metabolic or physiological event.

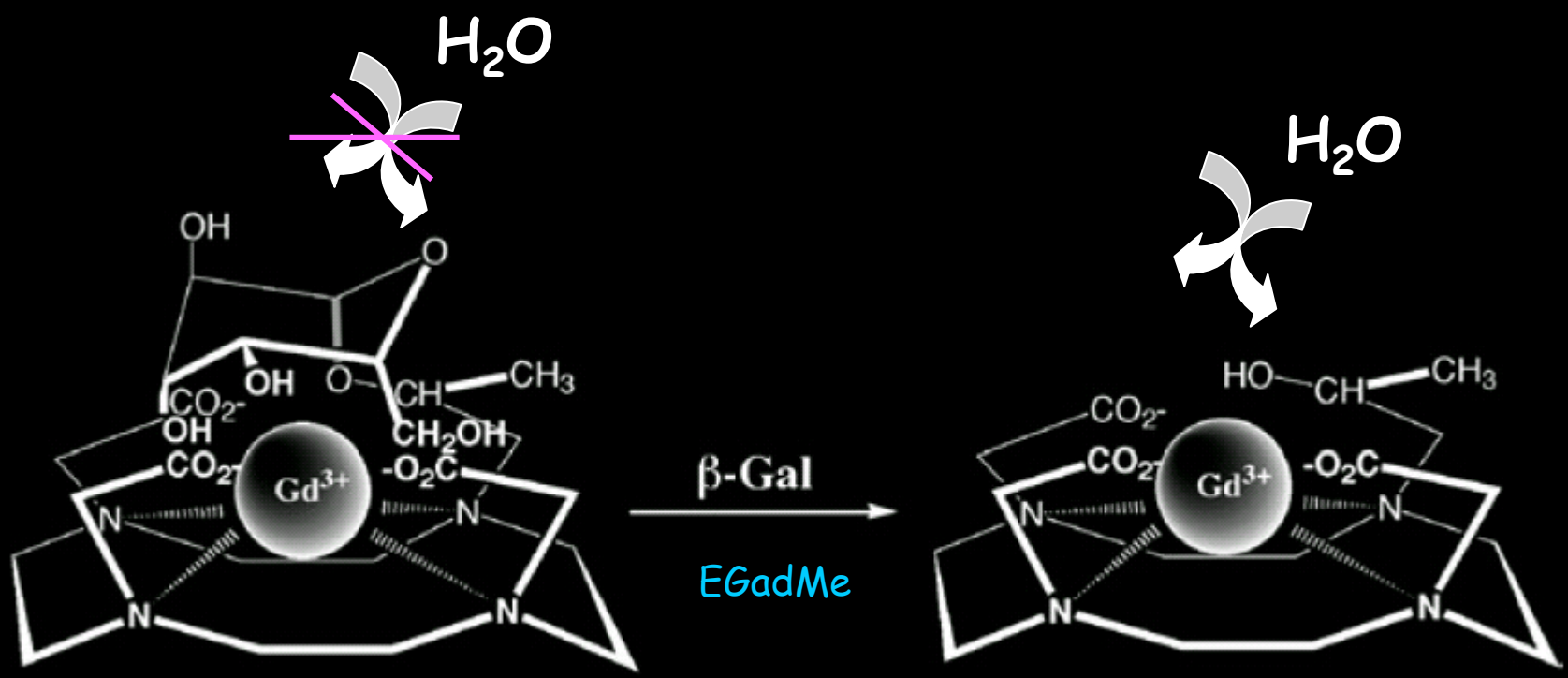
With Gd^{3+} agents, contrast enhancement is generally linked to a decrease in T1 but may follow a chemical exchange saturation transfer (CEST) event as the *switch*.

For iron oxide agents, contrast enhancement is due to an enhanced anisotropy that leads to a dramatic decrease in *T2*.

Examples of a "smart" MRI probes



DZIK-JURASZ, The British Journal of Radiology, 76 (2003), S98-S109



AY LOUIE et al., Nature biotechnology, March 2000, Volume 18 No 3 : 321-25

EGadMe - MRI detection of β-galactosidase mRNA expression in living *X. laevis* embryos

EGadMe has been used as a MR functional reporter agent for displaying *in vivo* β-galactosidase activity

Brighter images of Xenopus embryos injected with β-gal mRNA and EgadMe (top) compared with EgadMe alone (bottom)

AY. LOUIE et al., Nature biotechnology, March 2000, Volume 18 No 3 : 321-25

Fluorochromes-labeled smart probe, activated by cathepsin-B

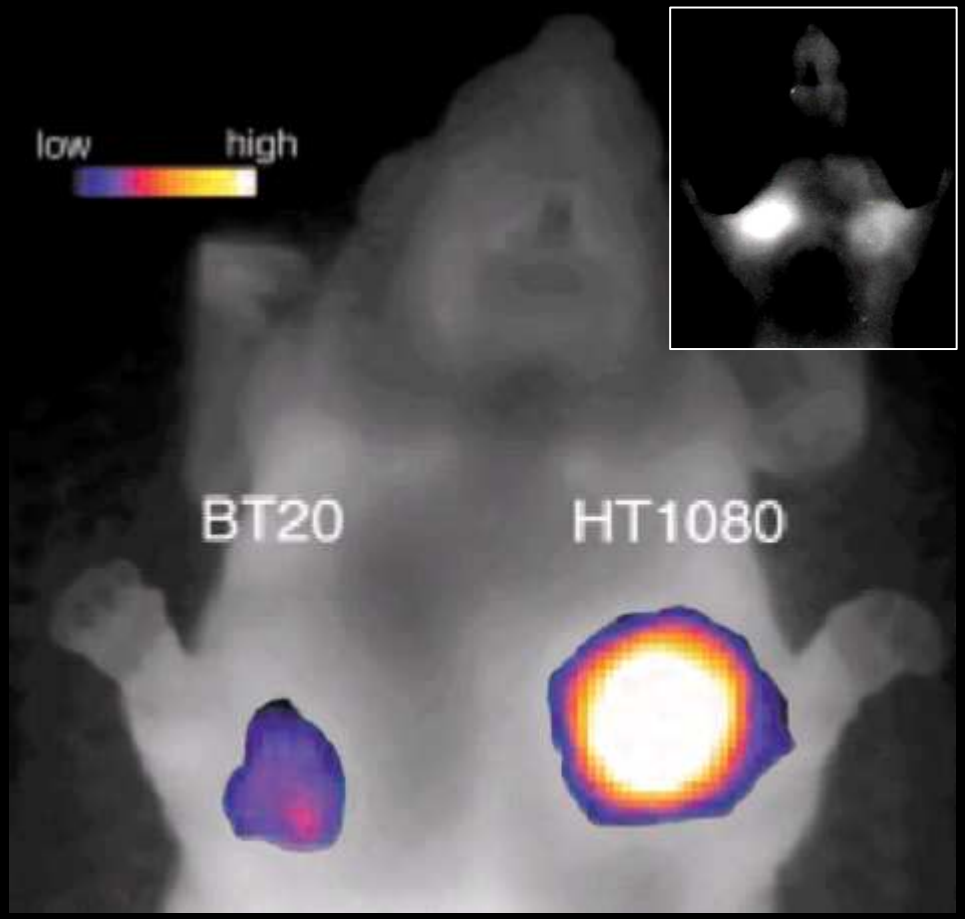


Image courtesy of Ralph Weissleder, CMIR

Color-encoded near-infrared fluorescence image of a mouse implanted with two different human breast tumors differing in tissue invasiveness.

The mouse was injected with a fluorochromes-labeled smart probe, activated by cathepsin-B.

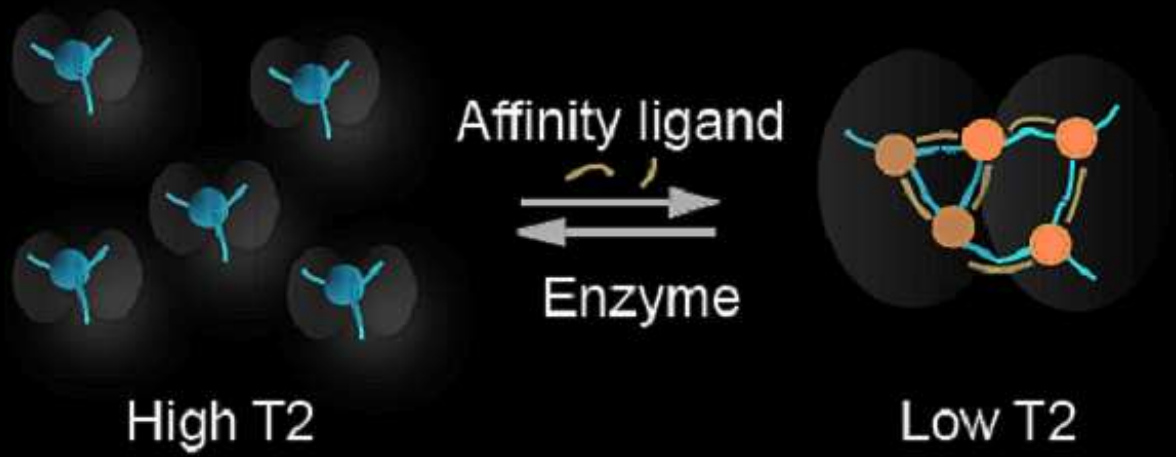
The agent is more activated in the more invasive right tumor (where is more cathepsin-B).

SR CHERRY, Phys. Med. Biol. 49 (2004) R13-48

Magnetic relaxation switch of covalently coupled SPIO to biomolecules

When (U)SPIO are covalently coupled to oligo-nucleotids, nucleic acids, small molecules, peptides, receptors ligands, proteins, antibodies, …, they form CLIO (Cross Linked Iron Oxides), interacting with molecular targets (DNA, small molecules, proteins, enzymes, …).

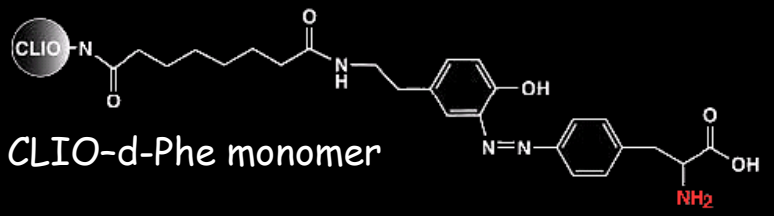
The cooperative auto aggregation of CLIO at the target level greatly increases their relaxing power by a local over concentration and a partial immobilization, allowing to visualize the aggregation site by the T2* hyposignal it implies



JM PEREZ et al., Nature Biotech., 2002; 20: 816-20

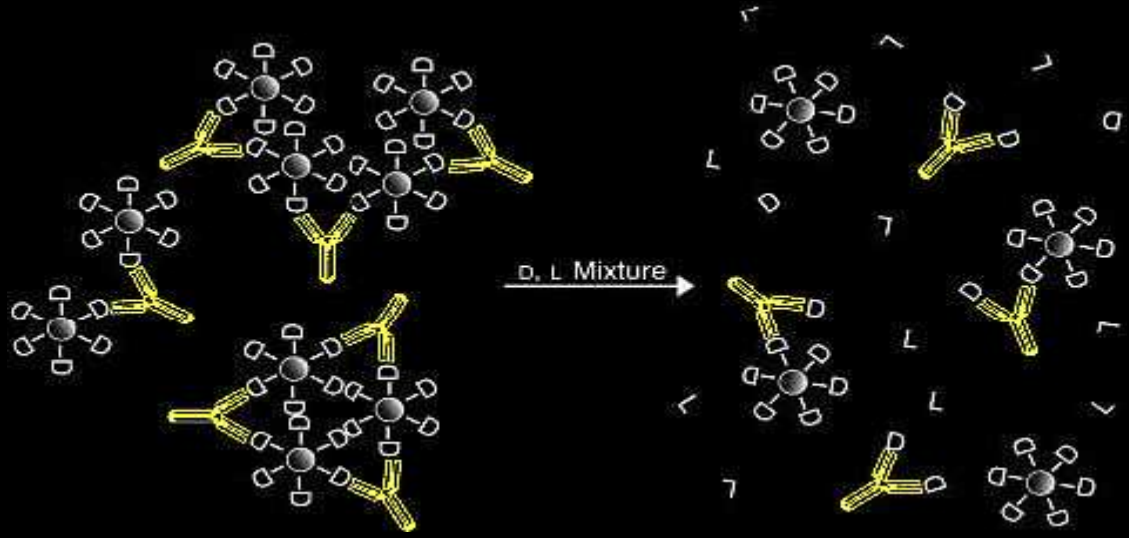
MR switch detection of L-Phenylalanine

In this example, a **CLIO**, made of an SPIO core coated with an aminated dextran, is covalently coupled to a derivative of d-phenylalanine (d-Phe)



Structure of a CLIO-d-Phe monomer

Addition of anti d-Amino Acids Antibody leads to monomers aggregation. The T2* relaxivity is thus high because of concentration & immobilization

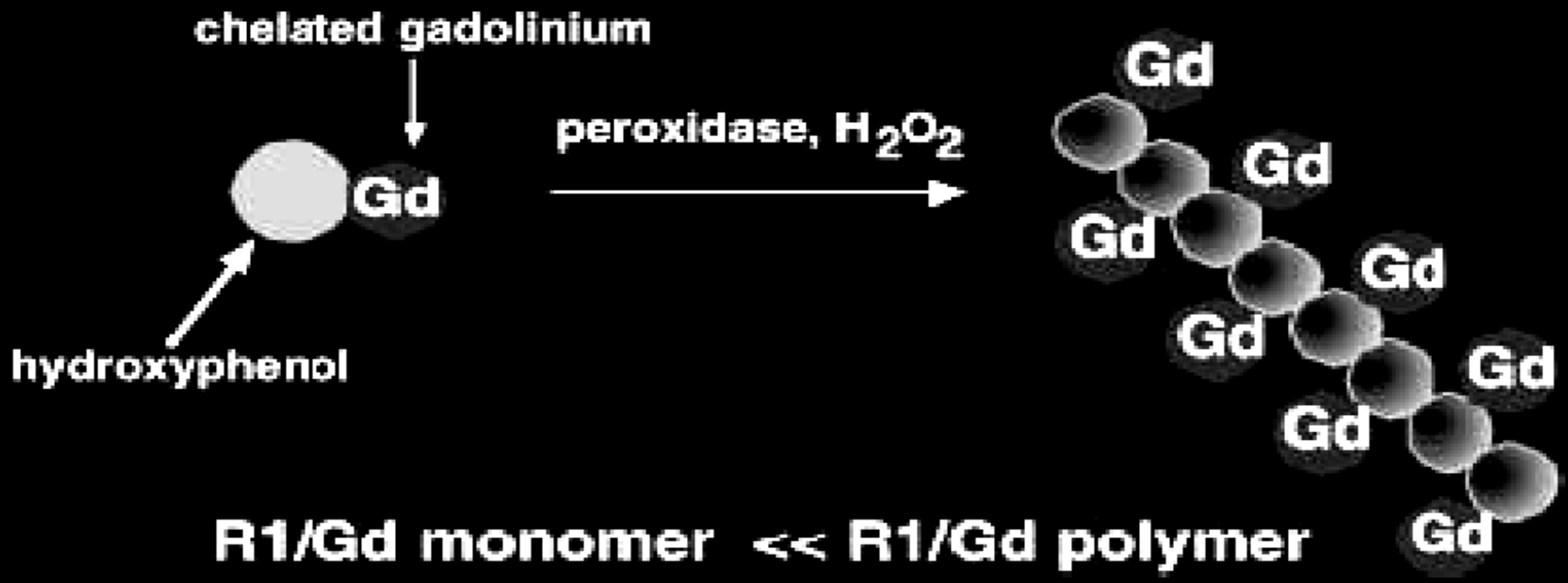


When l-Phe is present, CLIO-d-Phe/anti d-AA aggregates disrupt, inducing a T2* relaxivity decrease leading to a signal increase by MR switch

A TSOURKAS et al., Angew. Chem. 2004, 116, 2449 -2453

Magnetic relaxation amplification: Enzymatic activity by MRamp

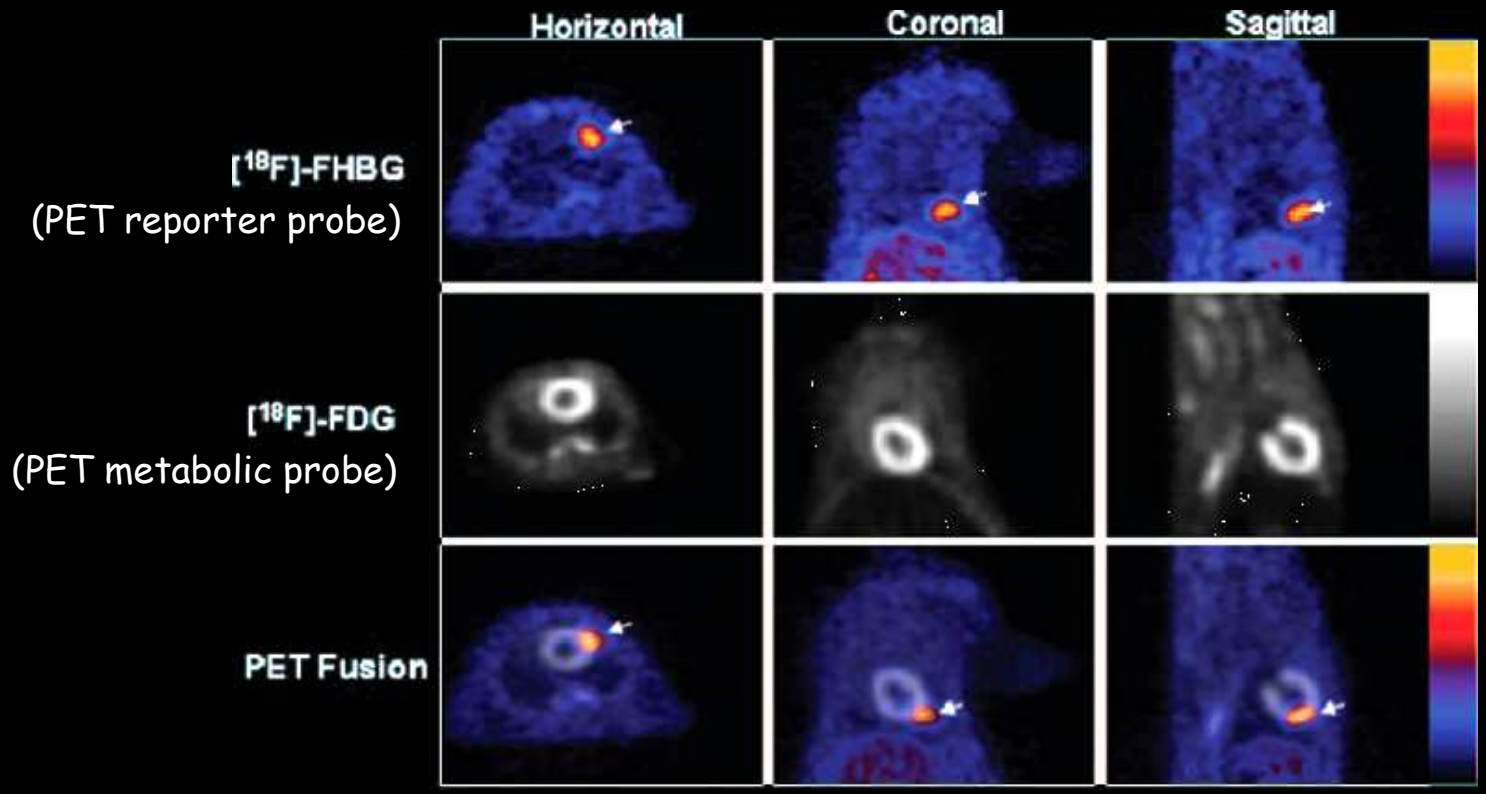
Activation of hydroxyphenol by peroxidase in the presence of H_2O_2 results in spontaneous condensation and polymerization of chelated gadolinium and an hypersignal in $T1_w$ MRI.



A TSOURKAS et al., Angew. Chem. 2004, 116, 2449 -2453

Reporter probe imaging

To define the location of transplanted Embryonic Stem cells in the myocardium, cells were stably transduced with a lentiviral vector carrying a novel triple-fusion (TF) reporter gene that consists of firefly luciferase, monomeric red fluorescence protein, and truncated thymidine kinase (fluc-mrfp-ttk).

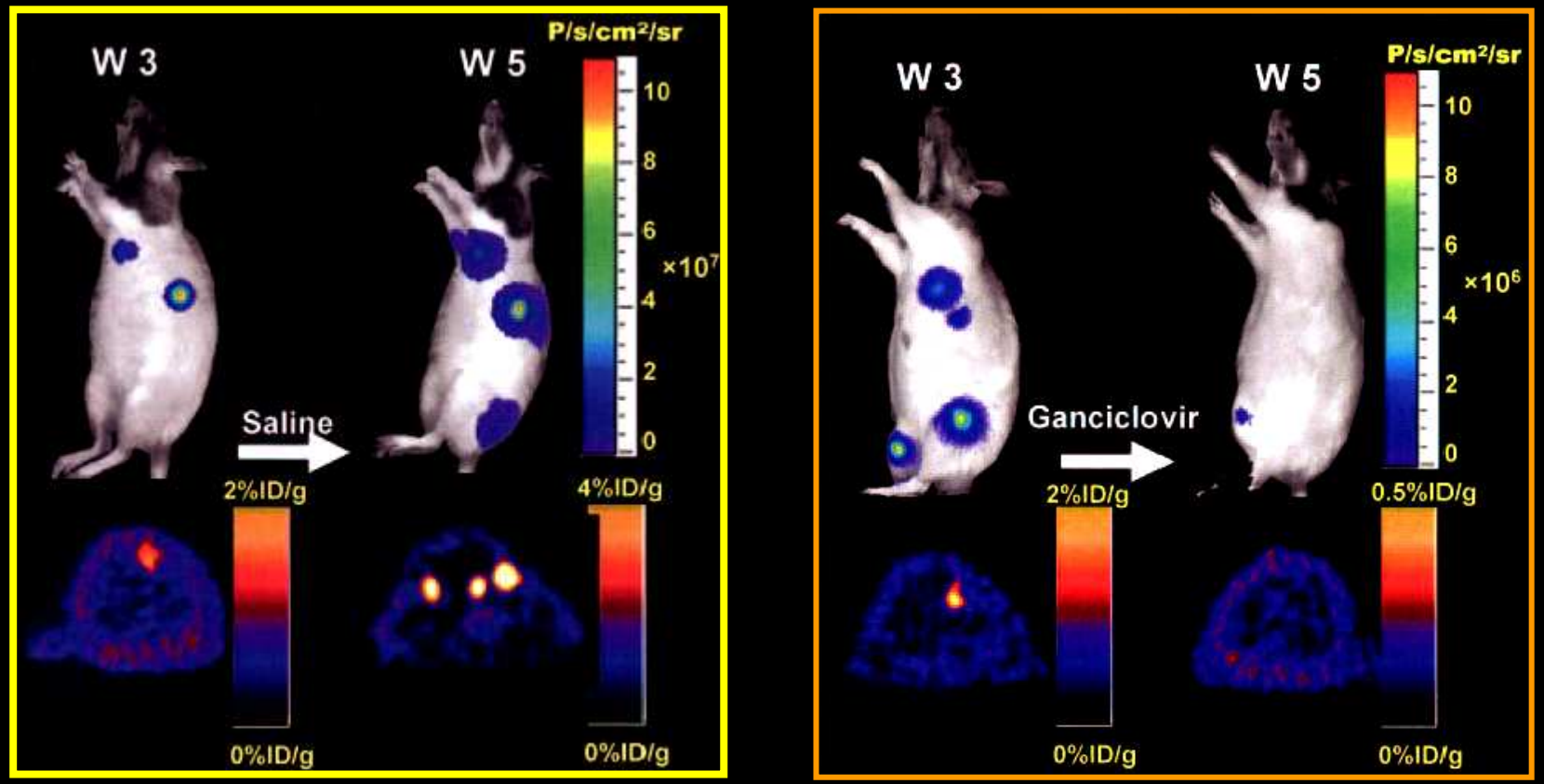


Two weeks after cell transplantation, animals underwent [18F]- FHBG reporter probe imaging (top row) followed by [18F]-FDG myocardial viability imaging (middle row).

F. CAO, S. LIN, et al., Circulation 2006;113;1005-1014

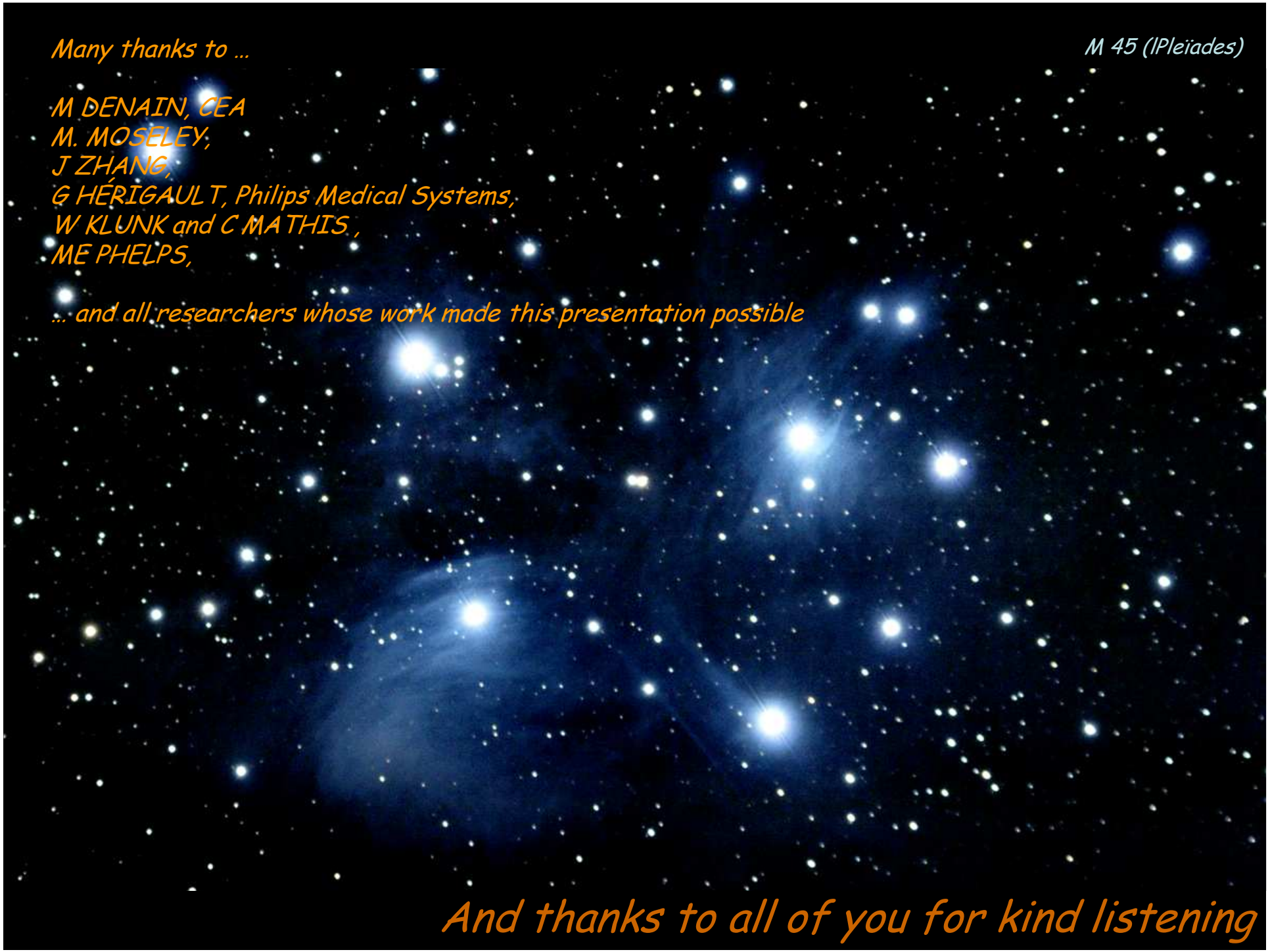
Embryonic Stem Cell–Derived Teratoma Formation Can Be Selectively Ablated by Ganciclovir Therapy

Ablation of teratoma formation with the PET reporter gene ttk (truncated thymidine kinase) as both a reporter and a suicide gene



Treatment of control animals with saline resulted in multiple teratoma formation by week 5. In contrast, study animals treated with ganciclovir for 2 weeks showed abrogation of both bioluminescence and PET imaging signals

F. CAO, S. LIN, et al., Circulation 2006;113;1005-1014


Many thanks to …
M DENAIN, CEA
M. MOSELEY,
J ZHANG,
G HÉRIGAULT, Philips Medical Systems,
W KLUNK and C MATHIS ,
ME PHELPS,
… and all researchers whose work made this presentation possible
M 45 (lPleïades)
And thanks to all of you for kind listening