Iron Oxide Particles

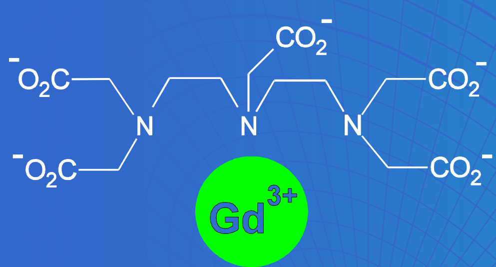
Robert N. Muller
University of Mons-Hainaut
Belgium

Reporters for MRI:

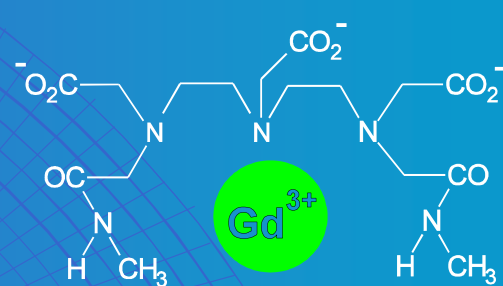
- paramagnetic
- superparamagnetic

Reporters for MRI:

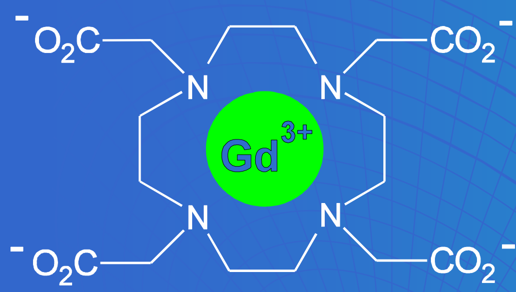
- molecules
- particles



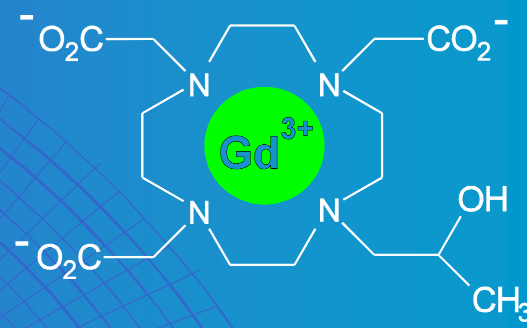
Gd-DTPA
MAGNEVIST



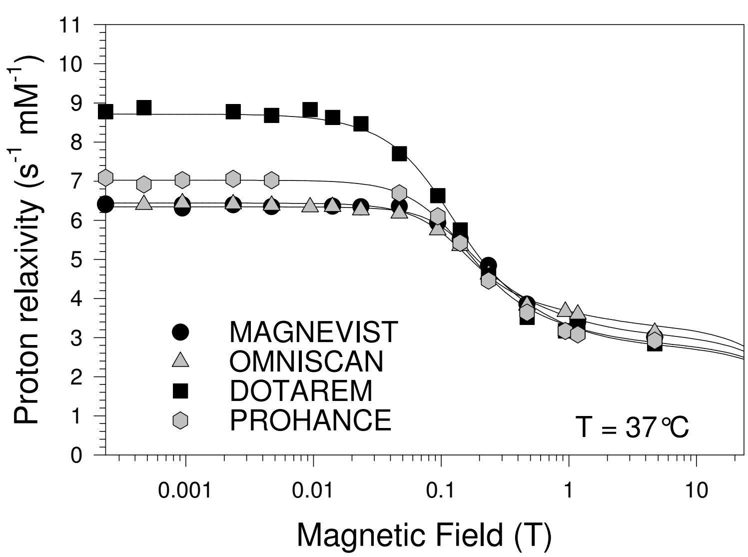
Gd-DTPA-BMA
OMNISCAN



Gd-DOTA
DOTAREM



Gd-HP-DO3A
PROHANCE



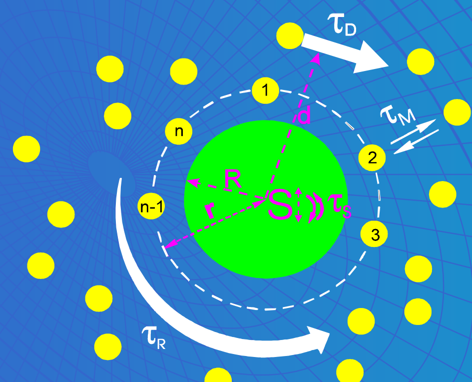
High relaxivity:

- valuable for any application
- <u>mandatory for molecular imaging</u>

Relaxivity of Paramagnetic Reporters:

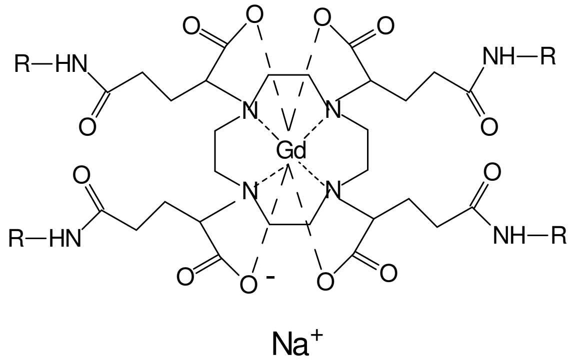
A lot has been understood

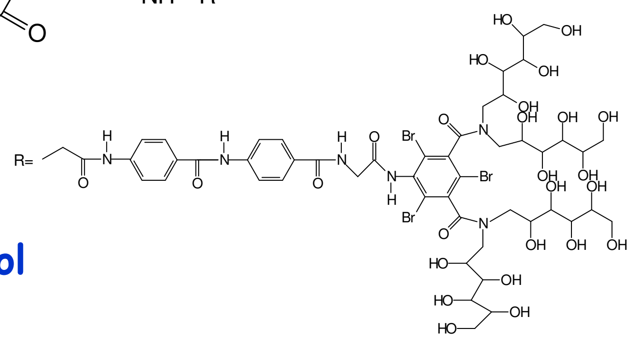
and a lot has been achieved



Chemical Structure of P792

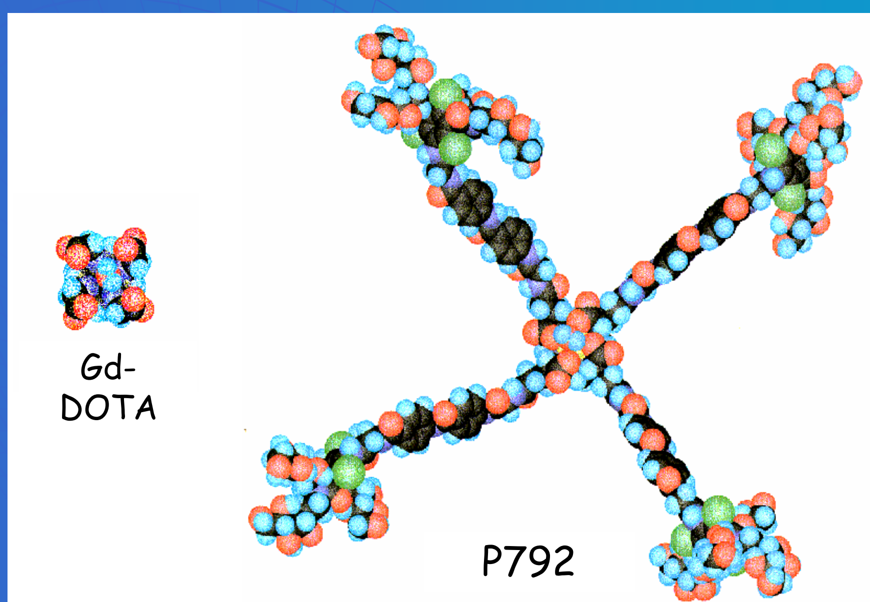
(Guerbet Laboratory, France)



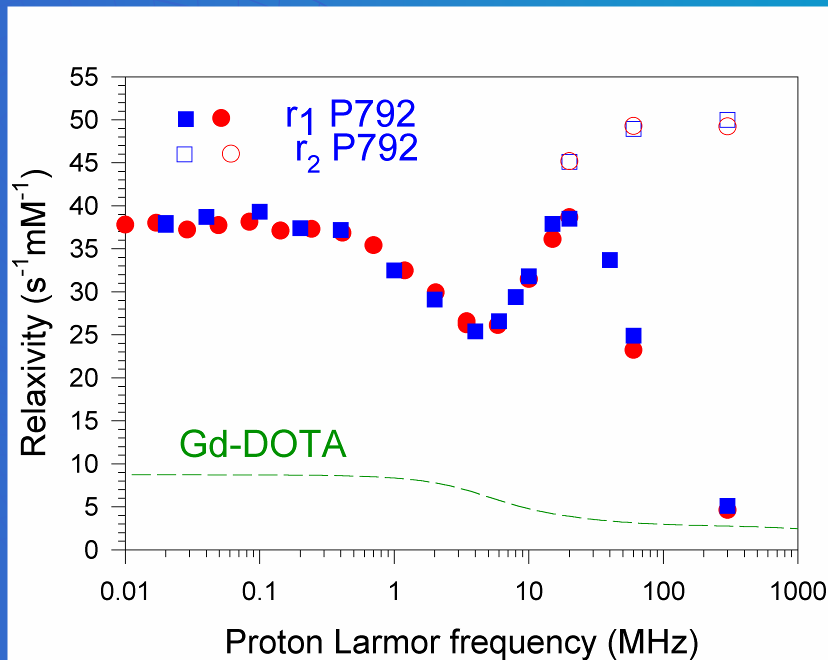


MW=6473 g/mol

Molecular modeling of P792 (SYBYL Software)



Proton NMRD Profile (37°C)

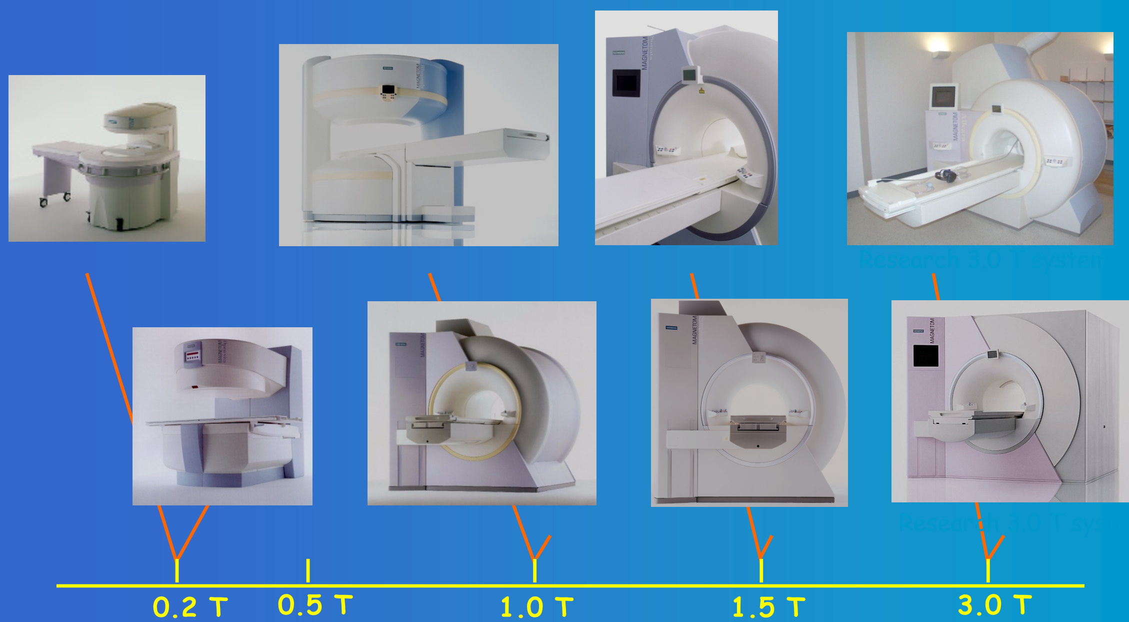


So, a lot has been understood

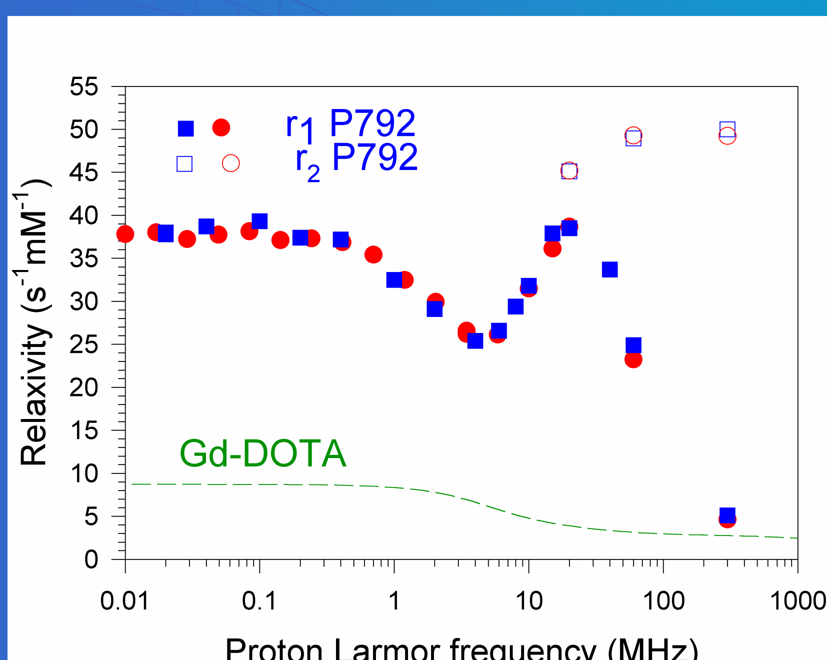
and a lot has been achieved

with paramagnetic complexes,

however ...



Proton NMRD Profile (37°C)



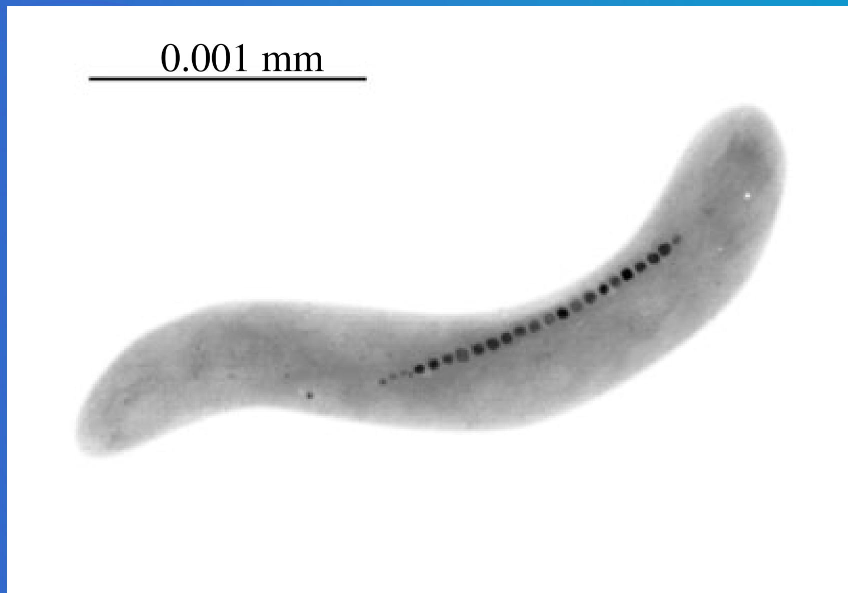
The higher the field,

the higher the r2/r1 ratio !

So, why don't we switch to other

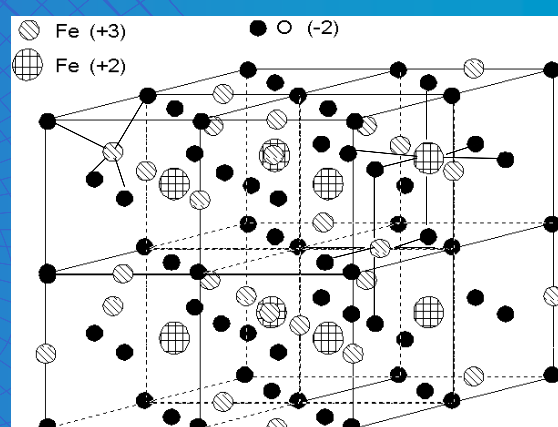
systems well-known

for their r2 effects ?



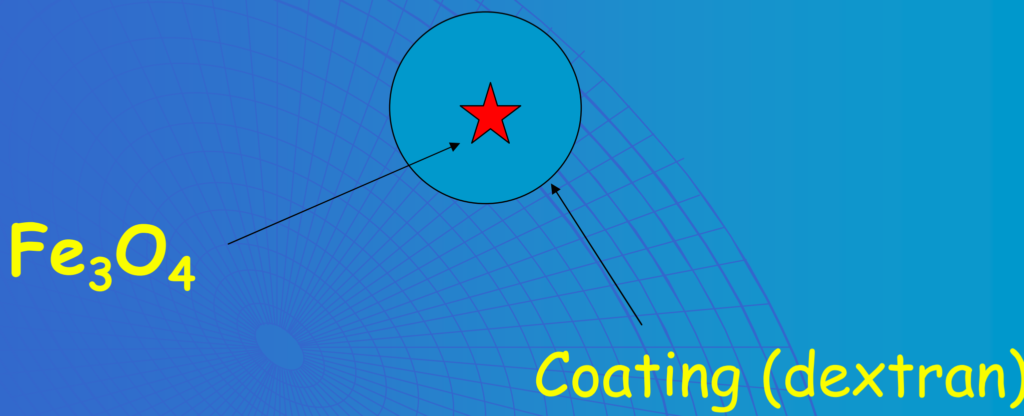
Structure of Ferrite Particles

$XO.Y_2O_3$ X : divalent cation
(Fe^{2+}, Co^{2+}, Mn^{2+}, Zn^{2+})
Y : trivalent cation
(Fe^{3+}, Al^{3+})
Magnetite :
X,Y = Fe ⇒ Fe_3O_4



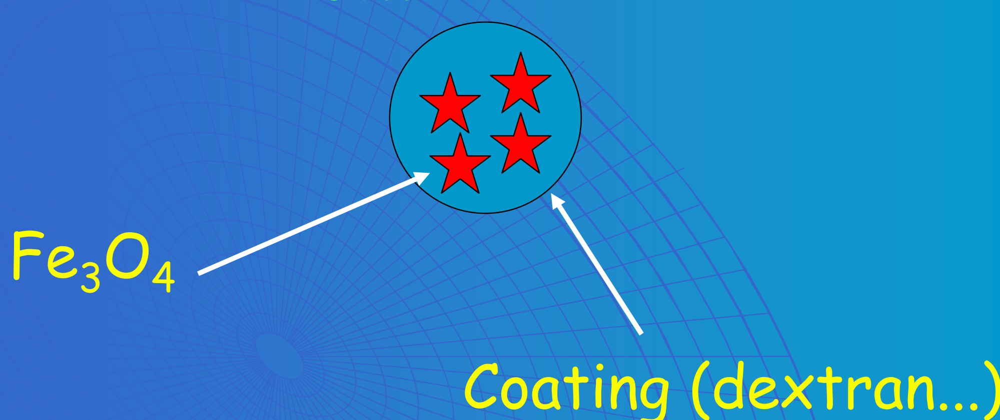
USPIO

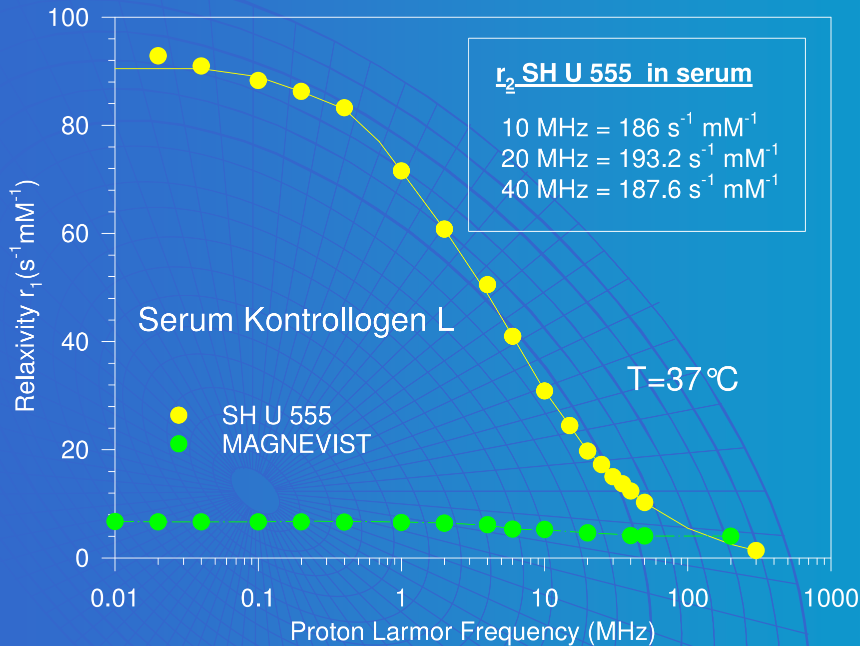
Ultrasmall Particle of Iron Oxide

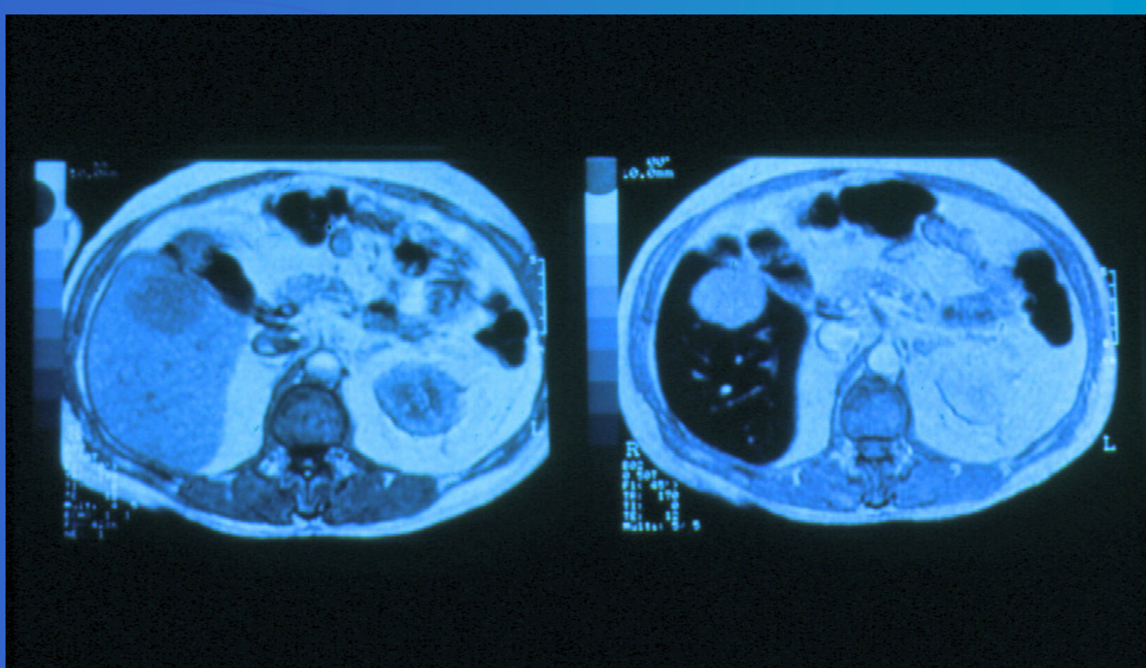


SPIO

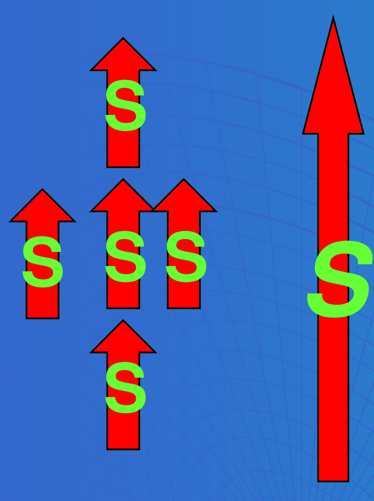
Small Particle of Iron Oxide




r2 SH U 555 in serum
10 MHz = 186 s-1 mM-1
20 MHz = 193.2 s-1 mM-1
40 MHz = 187.6 s-1 mM-1
Serum Kontrollogen L
T=37°C
SH U 555
MAGNEVIST
Relaxivity r1(s-1mM-1)
Proton Larmor Frequency (MHz)
0
20
40
60
80
100
0.01
0.1
1
10
100
1000



Why are these systems so efficient ?



$S = \Sigma s$

$r_{1,2} \longleftrightarrow \Sigma S (\Sigma S+1)$

COUPLED

Relative Relaxivities Spin= 1

NUMBER OF IONS	UNCOUPLED	COUPLED
2	4	6
10	20	110
100	200	10,100
1000	2000	1,001,000

Other good news for molecular imaging: the « Cargo » effect

One particle contains ca 5,000 Fe ions

therefore its « particulate » r2 relaxivity

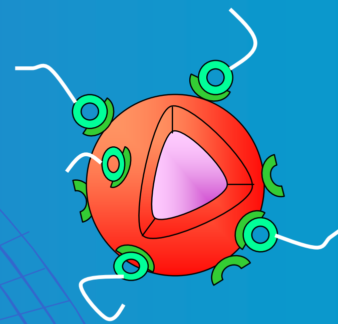
is equal to ca 1,000,000 s-1.mM (particle)

Is the sky perfectly blue ?

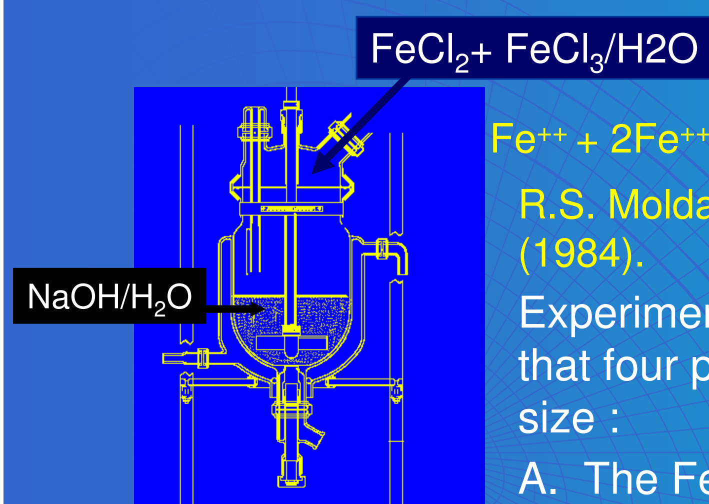
Is the sky perfectly blue ?

No !

- **Synthesis of the crystals**
  size, magnetization ?
- **Coating of the crystals**
  amount of coating molecules ? overall size ?
  charge ?
- **Grafting of the vector**
  grafting position ? number of grafted vectors ?
  charge ?



First approach : Molday synthesis.



$Fe^{++} + 2Fe^{+++} + 8OH^- \longrightarrow Fe_3O_4 + 4H_2O$

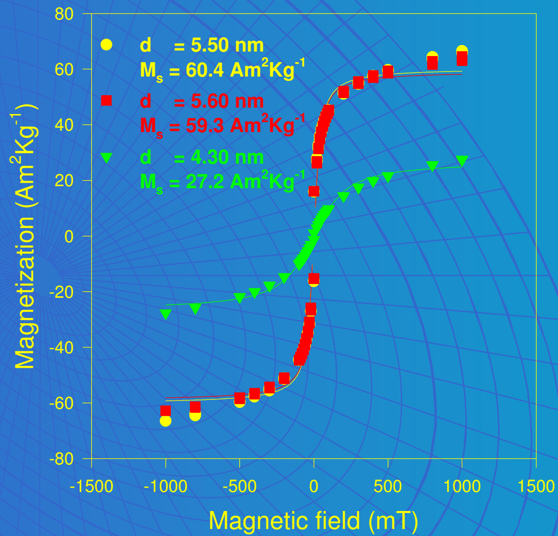
R.S. Molday, US Patent 4, 452, 773c (1984).

Experimental design analysis show that four parameters influence the size :

A. The Fe^{2+}/Fe^{3+} ratio.
B. Total iron concentration.
C. Rate of agitation.
D. Temperature.

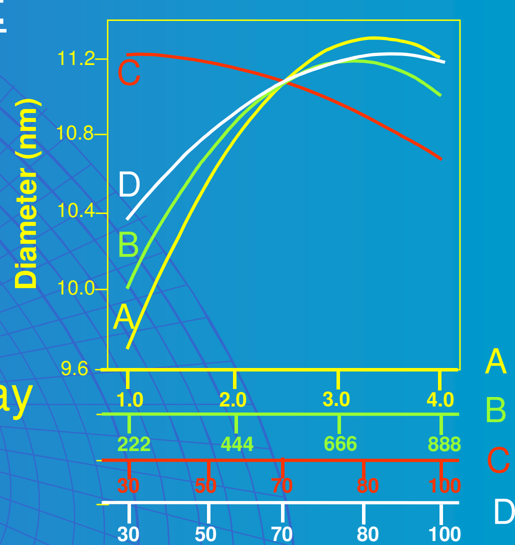
First approach : Molday synthesis.

Experimental design response used: the size obtained by fitting of the magnetization curves by the Langevin function.



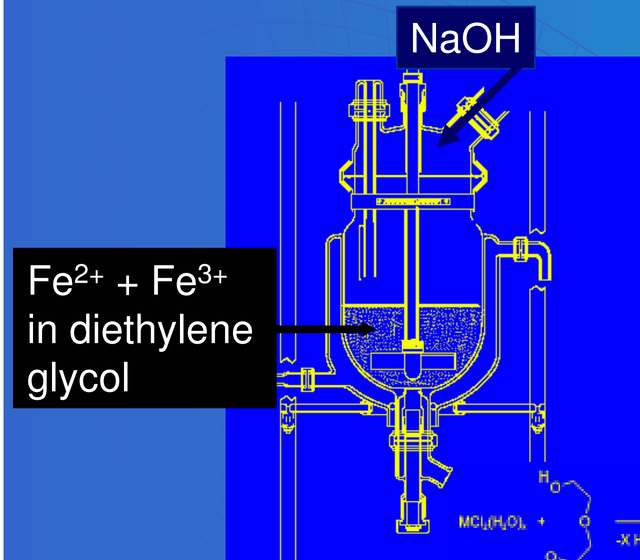
First approach : Molday synthesis.

Experimental design results :

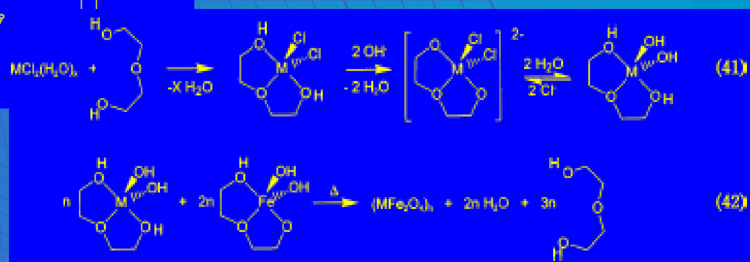


Conclusions : The maximum diameter obtained by the Molday synthesis is 11.5 nm.

Second approach : high temperature synthesis.



The formation of iron chelate and higher temperature allow a better control of the nucleation and of the crystal growing (*Caruntu, D. and all, V. Inorg. Chem.* ***2002****, 41, 6137.)*

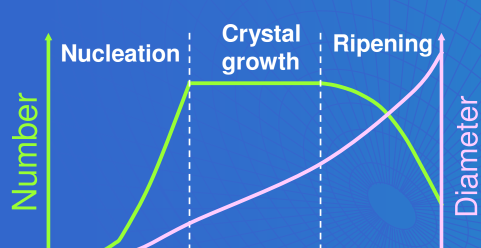


Second approach : high temperature synthesis.

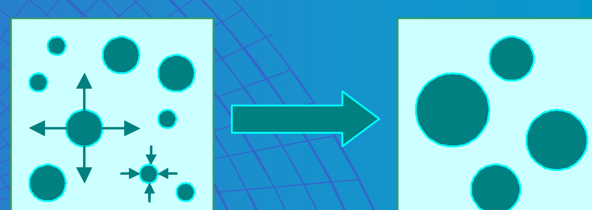
Important parameters of this synthesis :

1 The ripening time.

2 The ripening temperature.



Ripening process



Interest of the ripening:

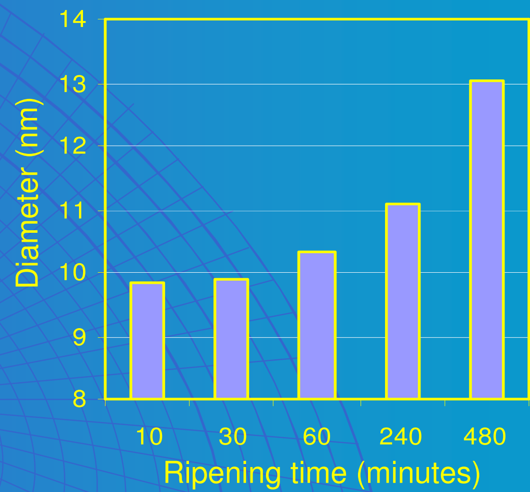
1 Allows a size control.

2 Should decrease the width of the size distribution.

Second approach : high temperature synthesis.

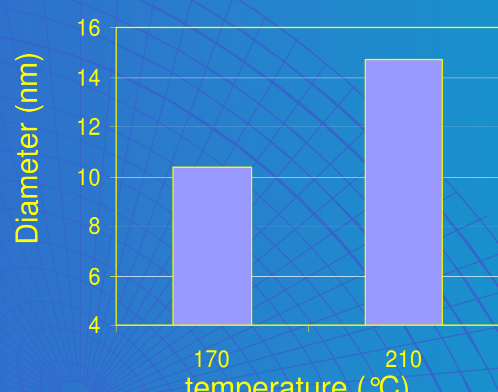
Observed effect of the ripening time :

⇒ An increase of the diameter (from 9.9 to 13nm)



Temperature = 170℃

Second approach : high temperature synthesis.



Ripening time = 60 minutes

Observed effect of the ripening temperature :

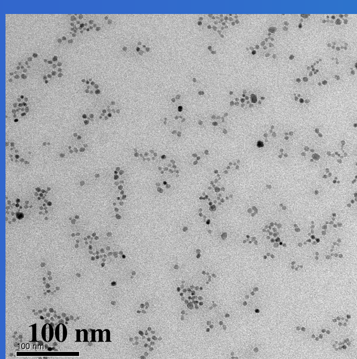
⇒ An increase of the diameter with temperature (from 10 nm to above 14 nm)

- High temperature process permit to obtain optimum crystal diameter for biomedical use (about 14 nm).
- Ripening occuring at high temperature allows a better control of the diameter.
- Low and high temperature route of magnetic synthesis seems to be complementary :
  - ⇒ Low temperature produces diameters between 9.0 and 11.5 nm.
  - ⇒ High temperature produces at least diameters between 10 nm and 14.5 nm.

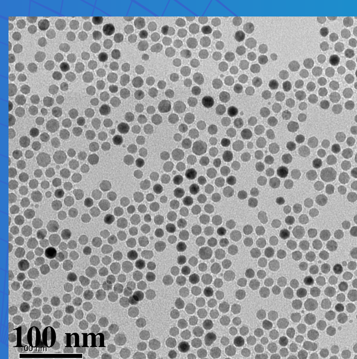
CRYSTAL SIZE

- Electron Microscopy: average over five hundred measurements on a picture.
- Magnetometry: fitting of the magnetization curve by one Langevin function.
- Relaxometry: fitting of the NMRD curve

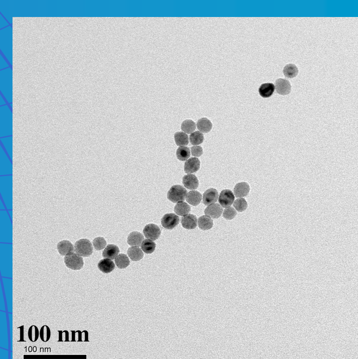
Transmission electron microscopy



6nm

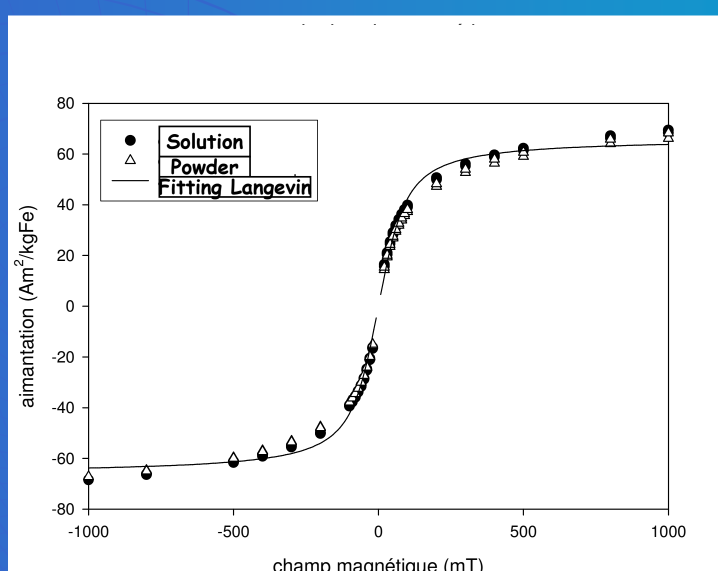


17nm



25nm

Magnetometry



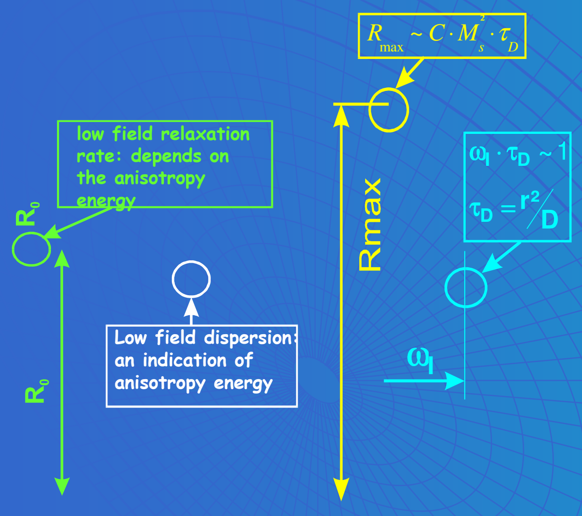
Relaxometry

The complete relaxometer

The air coil magnet

The cooling system

Parameters determined from the NMRD profile

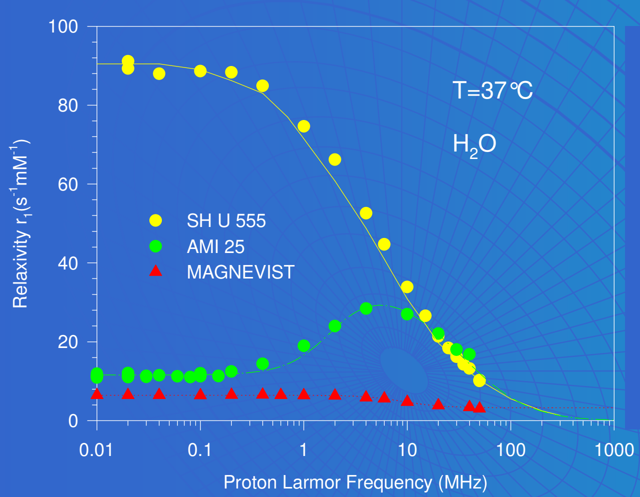


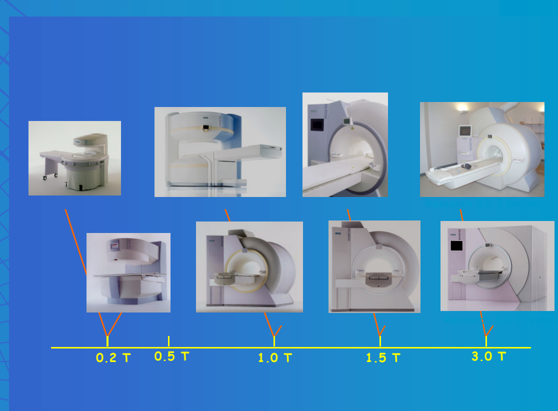
Crystal size
High field dispersion

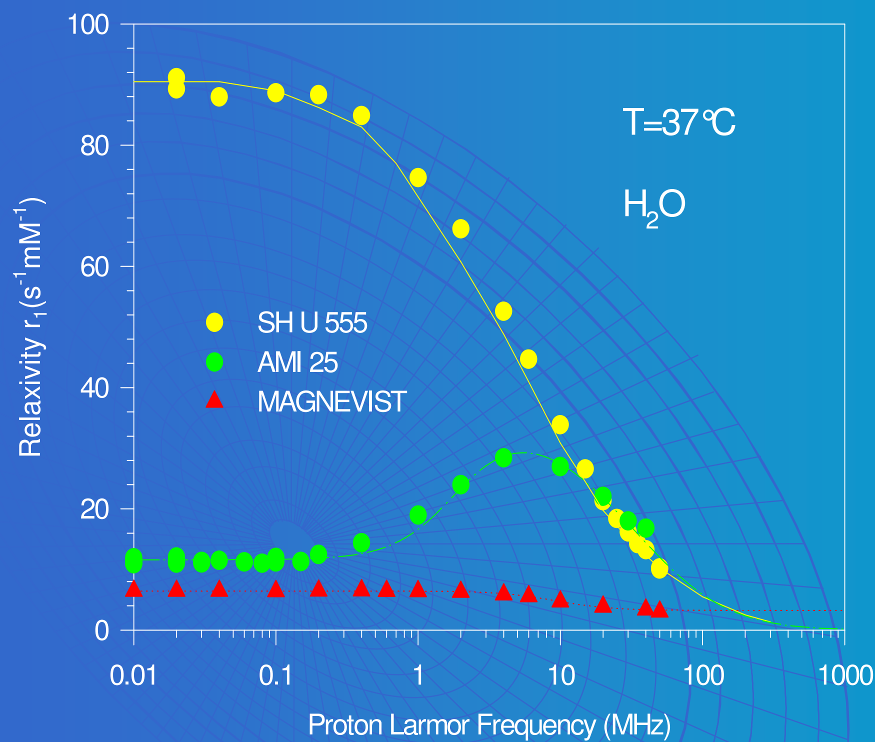
Magnetization
Maximum of relaxivity value

Anisotropy
determines low field $r_{1,2}$

Fitted parameters:
r = 4 nm,
M_S = 2.7 10^5 A.m^{-1}

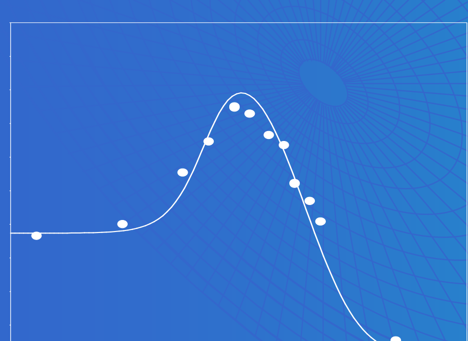






Magnetic filtration :

NMRD profiles of Endorem [(R)] and of the successive magnetic fractions

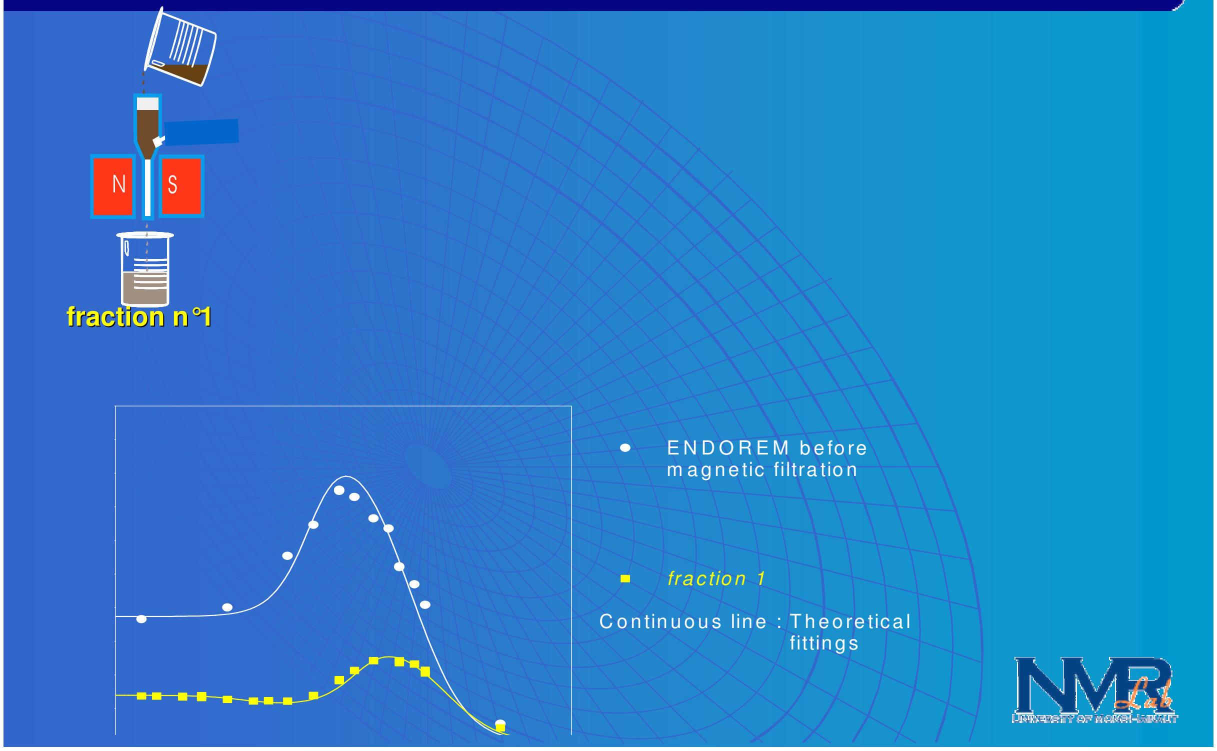


ENDOREM before magnetic filtration

Continuous line : Theoretical fittings

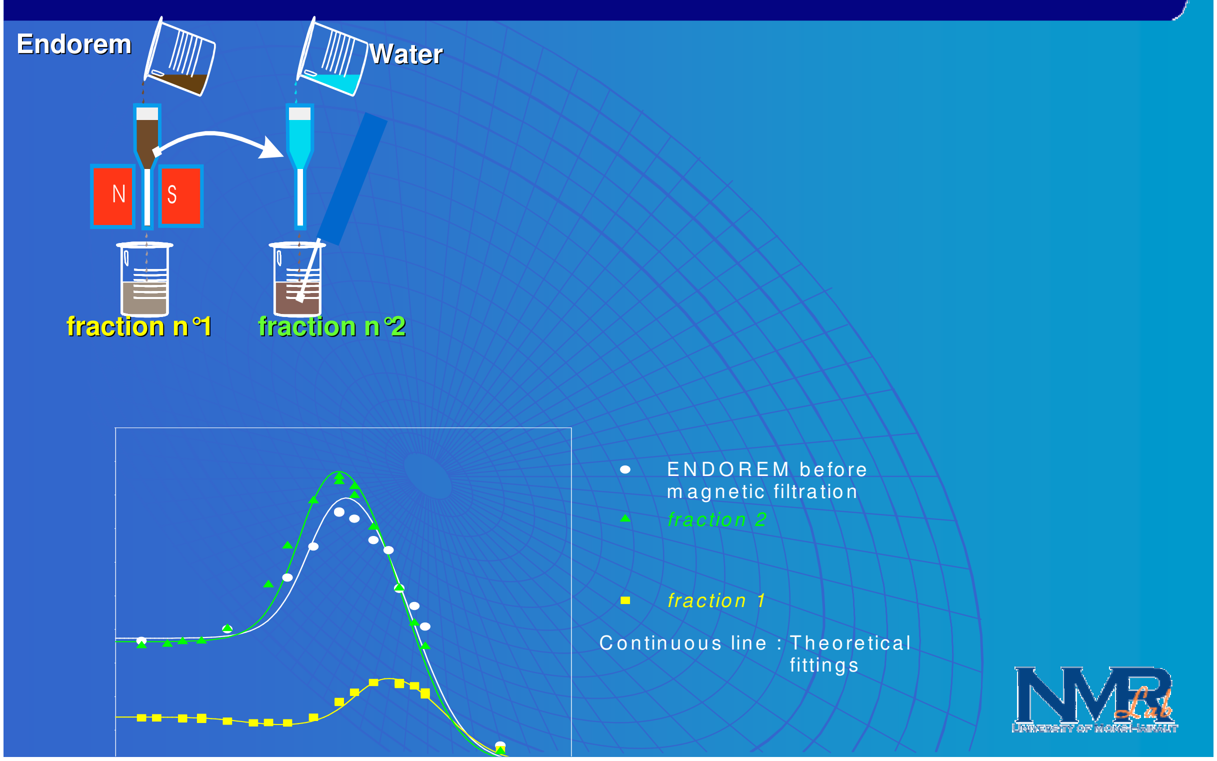
Magnetic filtration :

NMRD profiles of Endorem [(R)] and successive magnetic fractions



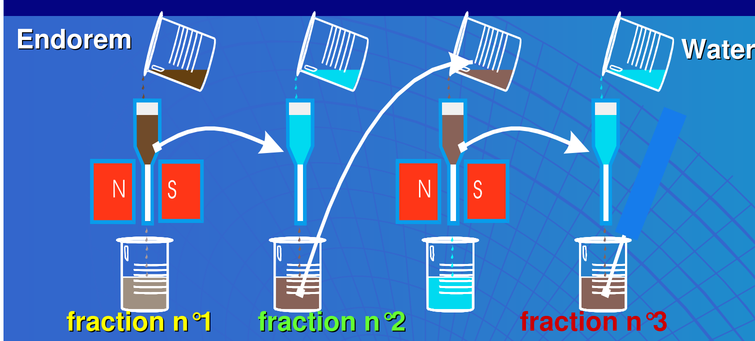
Magnetic filtration :

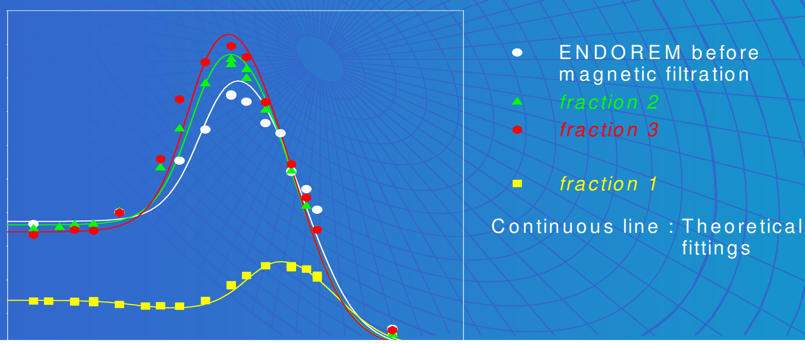
NMRD profiles of Endorem [(R)] and of the successive magnetic fractions



Magnetic filtration :

NMRD profiles of Endorem (R) and of the successive magnetic fractions

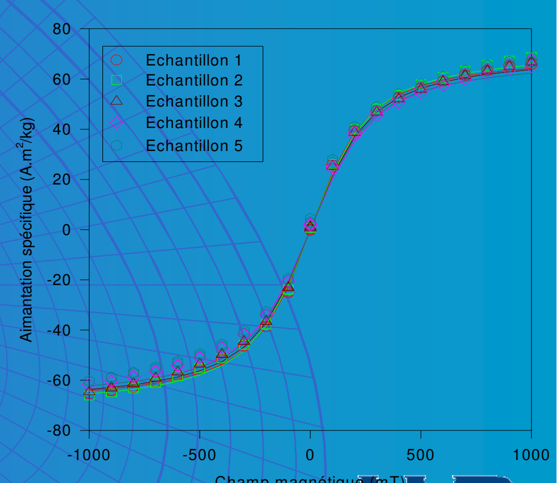




Reproducibility evaluated by magnetometry

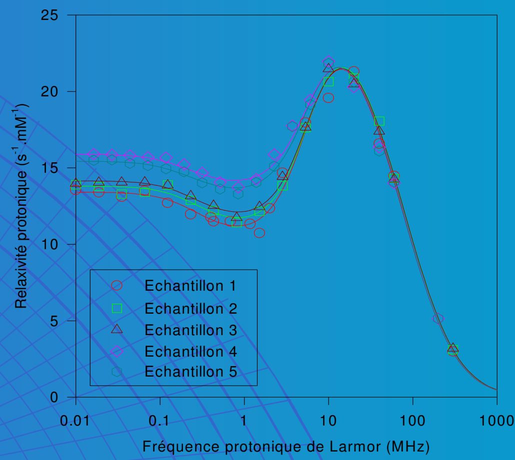
- constant $[Fe^{2+}]/[Fe^{3+}]$ ratio (0.67)
- constant [Fe]/[dextran] ratio (54)

	Size PCS (nm)	r_2/r_1	magneto. radius (nm)	magneto M_s. ($A.m^2/kg$)
Sample 1	26	1.93	3.0	71.1
Sample 2	30	1.96	2.9	73.0
Sample 3	30	1.83	2.9	71.1
Sample 4	26	1.83	2.9	70.2
Sample 5	28	1.85	3.0	69.6



Reproducibility evaluated by relaxometry

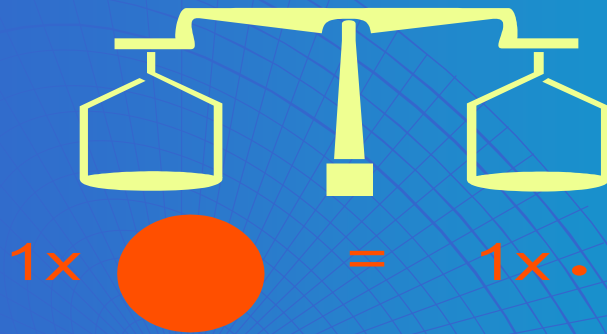
	Radius relaxo. (nm)	M_s relaxo. ($A.m^2/kg$)	τ_N (10^{-9}s)
Sample 1	4.0	53.0	1.64
Sample 2	3.9	54.1	1.67
Sample 3	3.9	54.1	1.77
Sample 4	3.9	52.8	2.47
Sample 5	4.0	52.8	2.29



Quality control: NMRD more sensitive than magnetometry (importance of the low field part !)

Amazingly, different techniques rarely give the same value for the crystal diameter

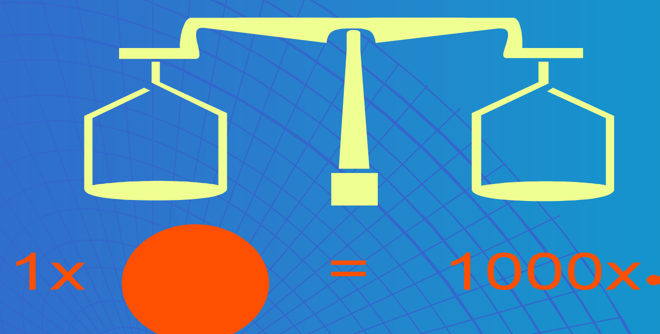
ELECTRON MICROSCOPY



Mixture: 1particle of 10nm + 1000 particles of 1nm

d(Elec. Mic.) =1nm

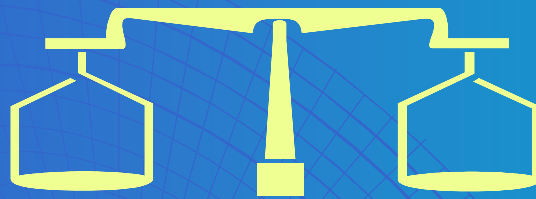
MAGNETOMETRY



Mixture: 1particle of 10nm + 1000 particles of 1nm

d(Magn.) =ca 5nm

RELAXOMETRY



1x ● = 100 000x •

Mixture: 1particle of 10nm + 1000 particles of 1nm

d(relax.) =7.1nm

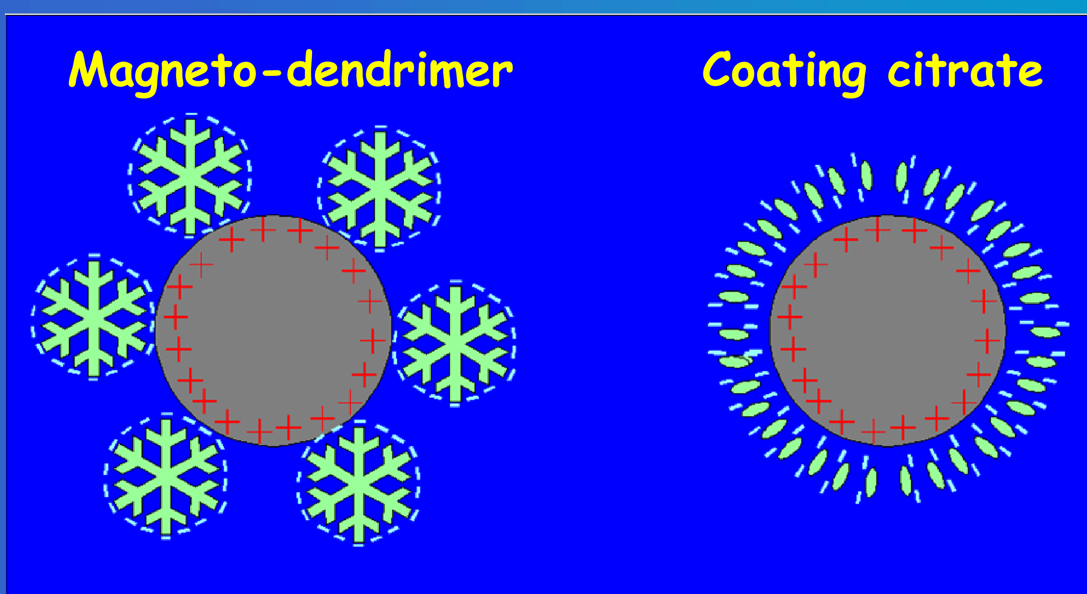
NMR Lab

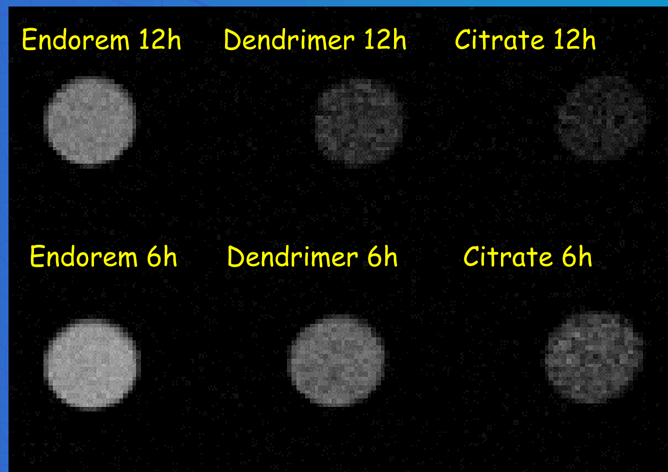
Applications

- cellular imaging
- molecular imaging

NMR Lab

cellular imaging

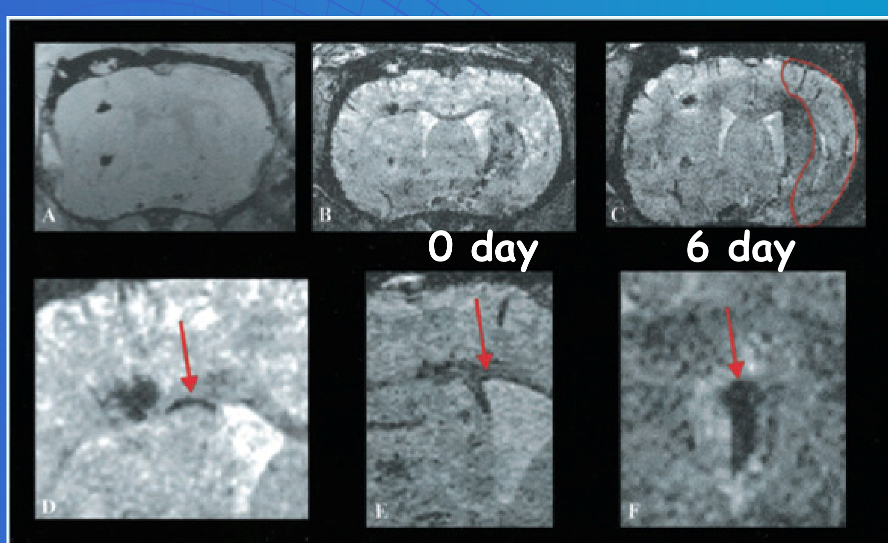




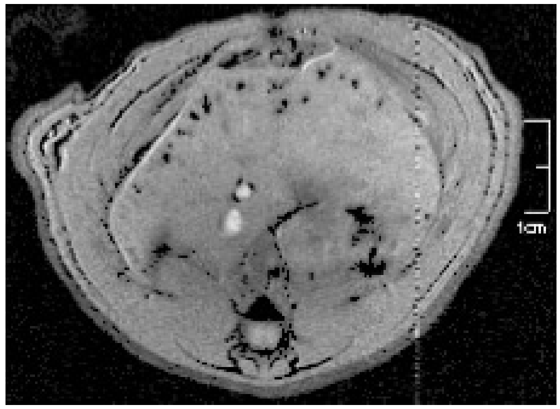
T_2 MRI Image (TR/TE : 2000/20 ms, 16 echoes, FOV : 4 cm, matrix 256x256): samples with cells incubated with Endorem®, dendrimer coated particles or citrate.

Labeled stem cells in cerebral stroke:

Hoehn et al. (2002)



Labeled and transplanted PI Clusters

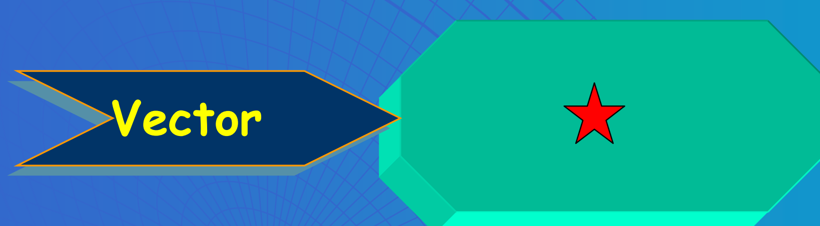


Jirak D, Strzelecki M et al: ISMRM 2008.
Evgenov N et al; Diabetes 55:2419-2428, 2006.

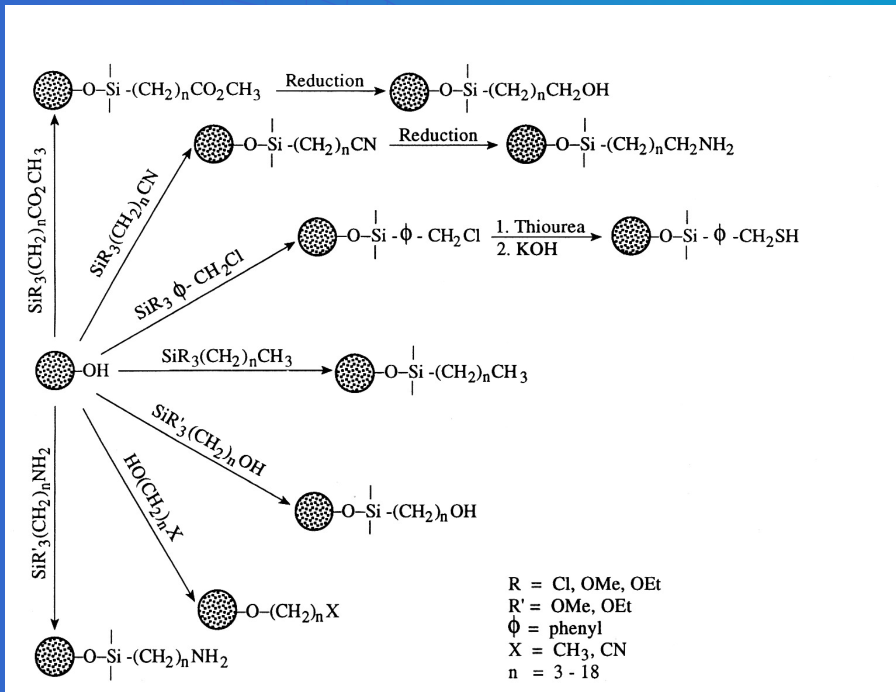
molecular imaging

Molecular Imaging = Vectorized Reporters

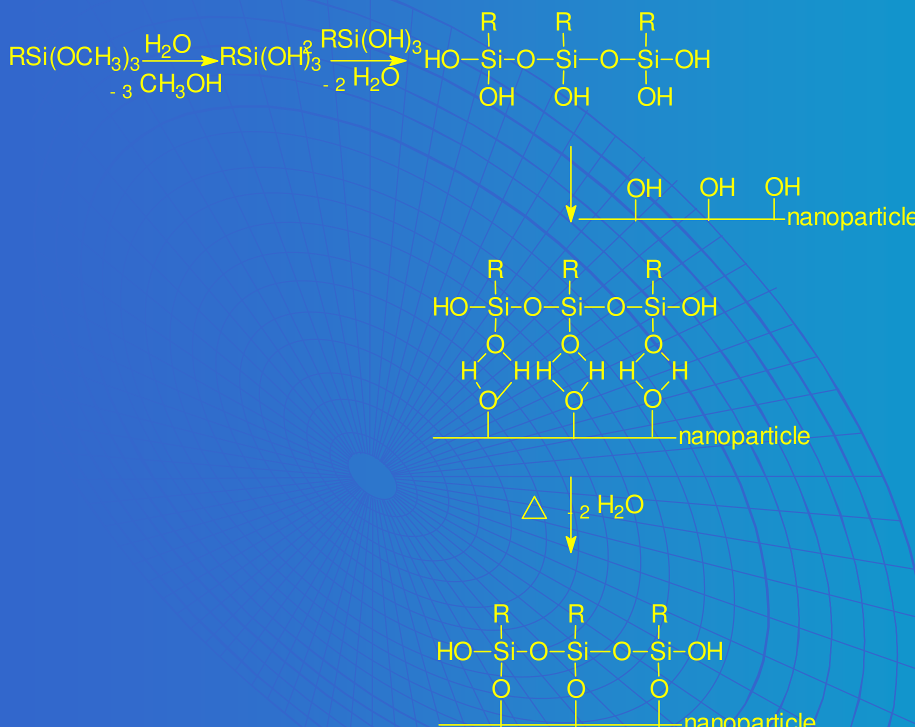
Molecular Imaging = Vectorized Reporters



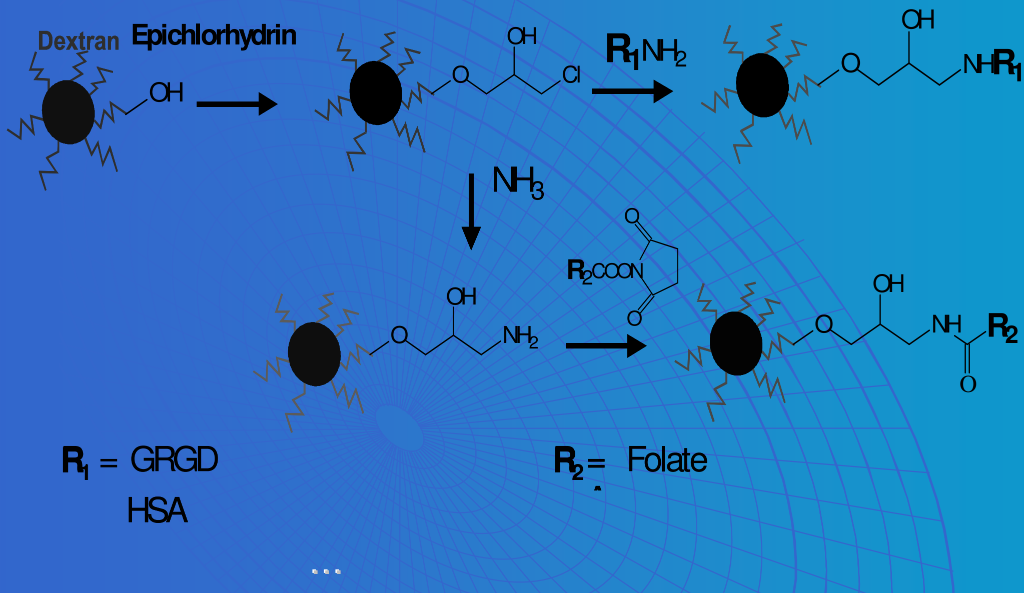
Chemistry of silane compounds



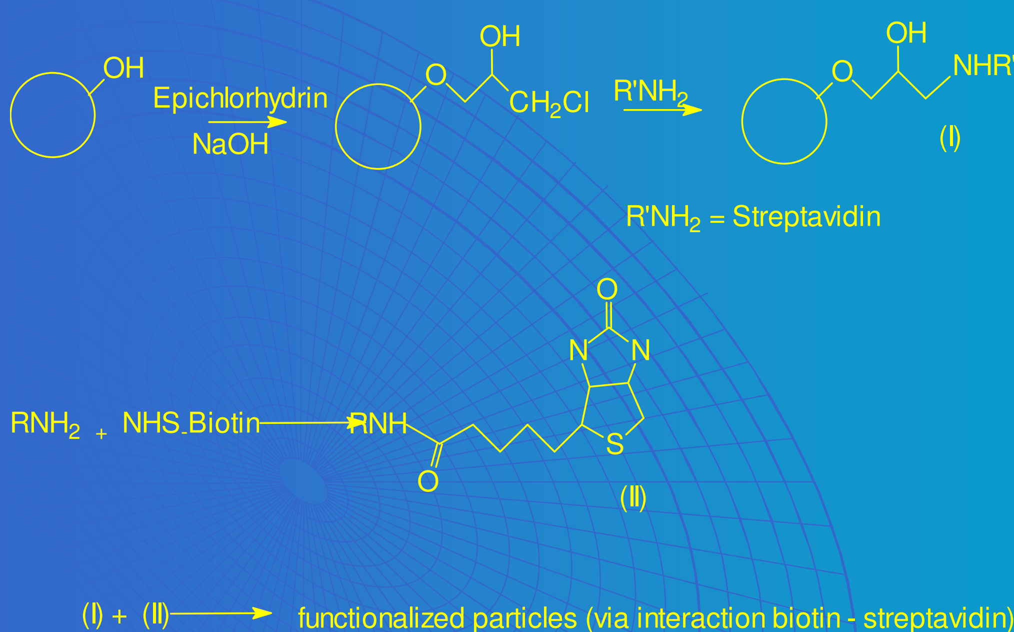
Coating with trimethylsilane



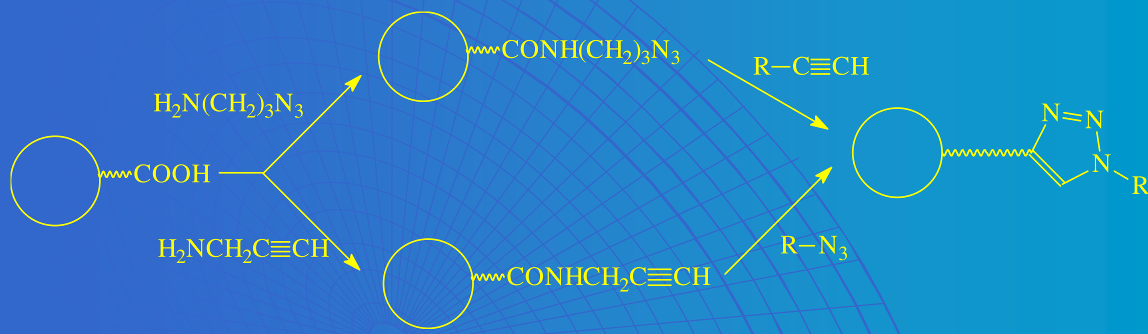
Grafting with epichlorhydrin



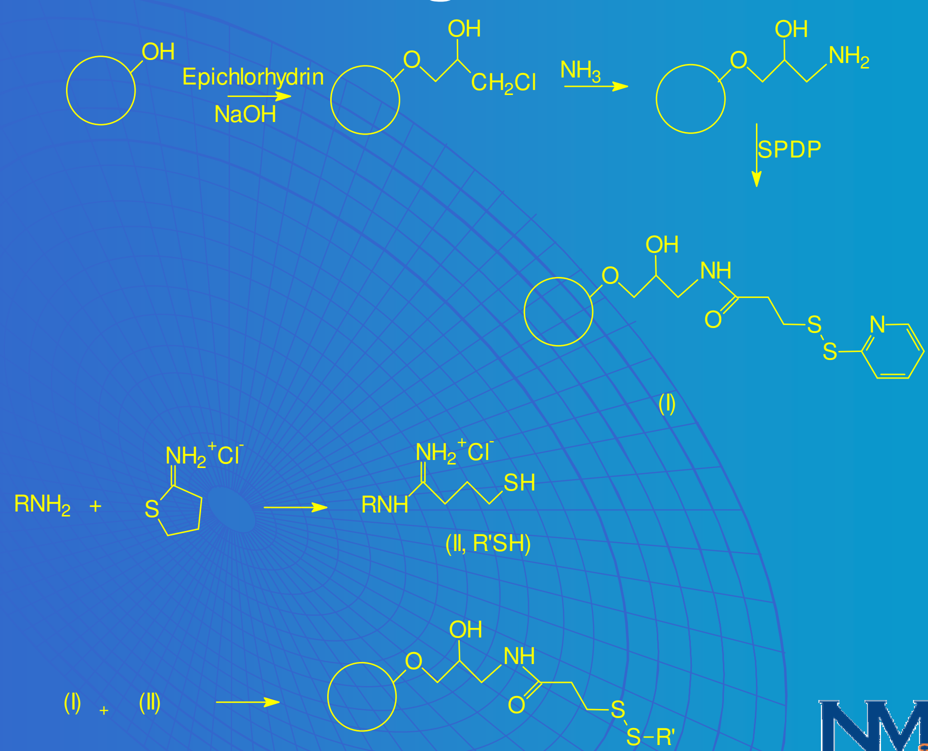
Biotin-streptavidin system



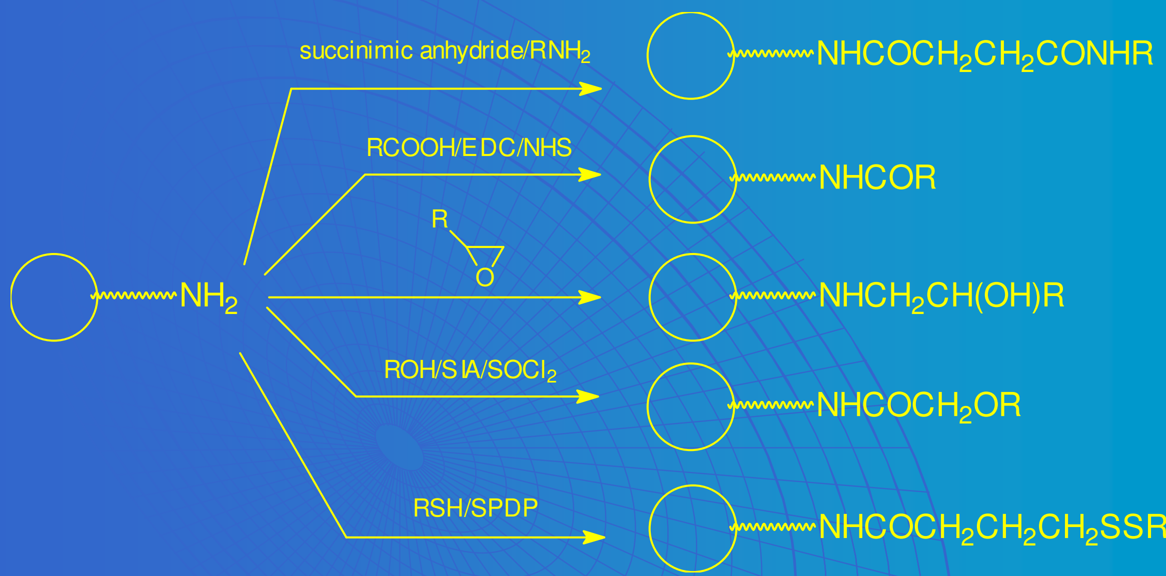
Click chemistry



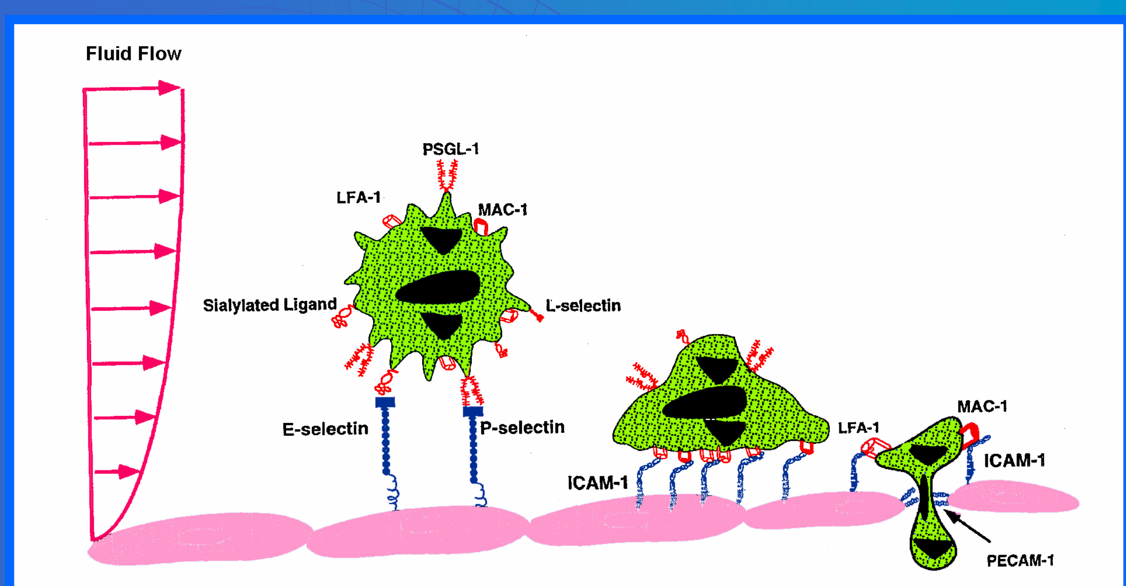
Disulfur bridges

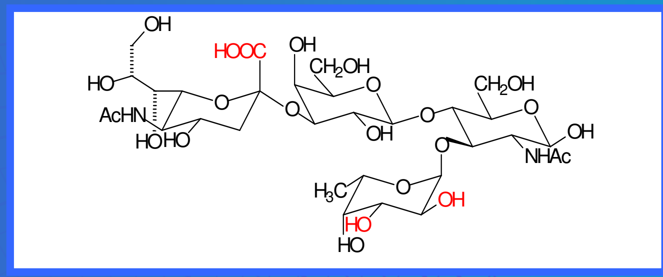


Conjugation strategies

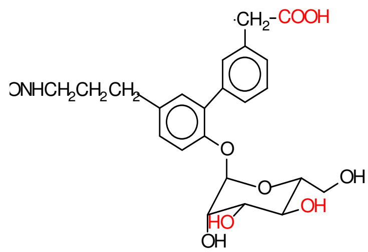


THE ADHESION MOLECULES AND INFLAMMATION



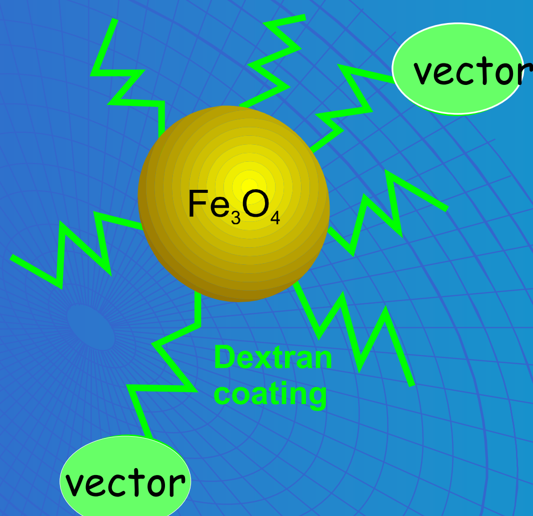


Sialyl-Lewis^x,
ligand of E-selectin



Muller et al. Eur. J. Org. Chem. 2002

Vectorization of Superparamagnetic Particles



Imaging of E-Selectin expression on TNF-α stimulated HUVECs

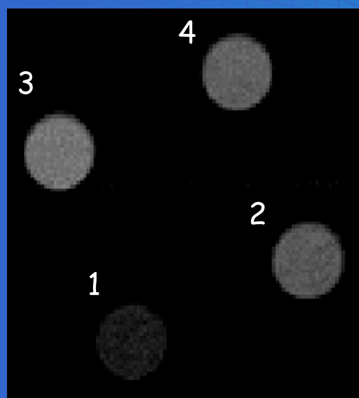
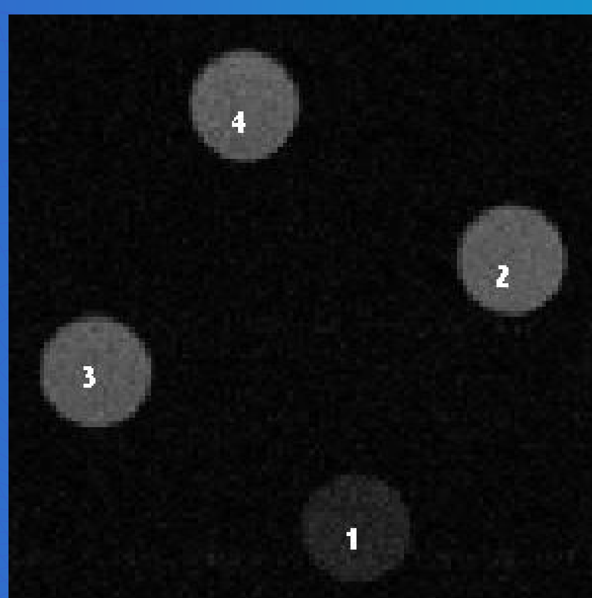


Image at 150 ms

TR/TE: 3000/15 ms, 24 echoes

1. TNF-α stimulated HUVECs + USPIO-g-sLeX
2. Unstimulated HUVECs + USPIO-g-sLeX
3. TNF-α stimulated HUVECs + USPIO
4. Unstimulated HUVECs + USPIO

In Vitro Targeting of Apoptosis



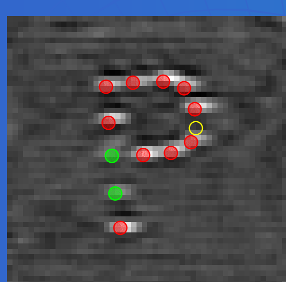
Apoptotic (1) and healthy (2) cells incubated with USPIO-peptide,
Healthy (3) and apoptotic cells (4) incubated with USPIO.

C. Laumonier et al, University of Mons-Hainaut, Belgium

Something new …

Tomographic imaging using the nonlinear response of magnetic particles
B. Gleich1 & J. Weizenecker Nature, Vol 435, 30 June 2005

Magnetic Particle Imaging



- 10mT RF amplitude
- 3.4 T/m gradient
- 13 holes (0.5 x 1.0 mm), filled with Resovist® from Schering AG (*approved* MR-Contrast Agent)
- 9.4 mm x 9.4 mm Image Size

Tomographic imaging using the nonlinear response of magnetic particles
B. Gleich1 & J. Weizenecker Nature, Vol 435, 30 June 2005

Courtesy of Philips Medical Systems

CONCLUSIONS

Magnetic Nanoparticles are :

•relatively difficult to synthesize in a perfectly reproducible way

•relatively easy to vectorize (nevertheless, exact number of grafted vectors as well as their grafted structure are still uncertain)

•excellent (although negative) reporters for MRI in the context of molecular and cellular imaging

CONCLUSIONS

•Magnetic Nanoparticles could be used in the context of localized hyperthermia (and drug delivery)

•MPI ...?

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