Università del Piemonte Orientale "Amedeo Avogadro"
DISAV – Department of Environmental and Life Sciences

Intensive Program 2011 - "Design, Synthesis and Validation of Imaging Probes"

MRI responsive probes

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Outline

Introduction: what are MRI images and contrast agents?

1. T_1 relaxation responsive agents: the origin of responsivness (with many examples about pH responsive agents)
2. Responsive agents: the concentration issue
3. Responsive agents based on Chemical Exchange Saturation Transfer (CEST agents)
4. Responsivity to temperature, enzyme catalized reactions, metabolites, metal ions and redox potential

What are MRI images and contrast agents?

T_{1w} MRI images – Basic concepts

1. In MRI images, we ALWAYS observe the NMR signal of water

2. White pixel indicate high (maximum) intensity of the water signal; black pixel indicate low (zero) intensity of the water signal

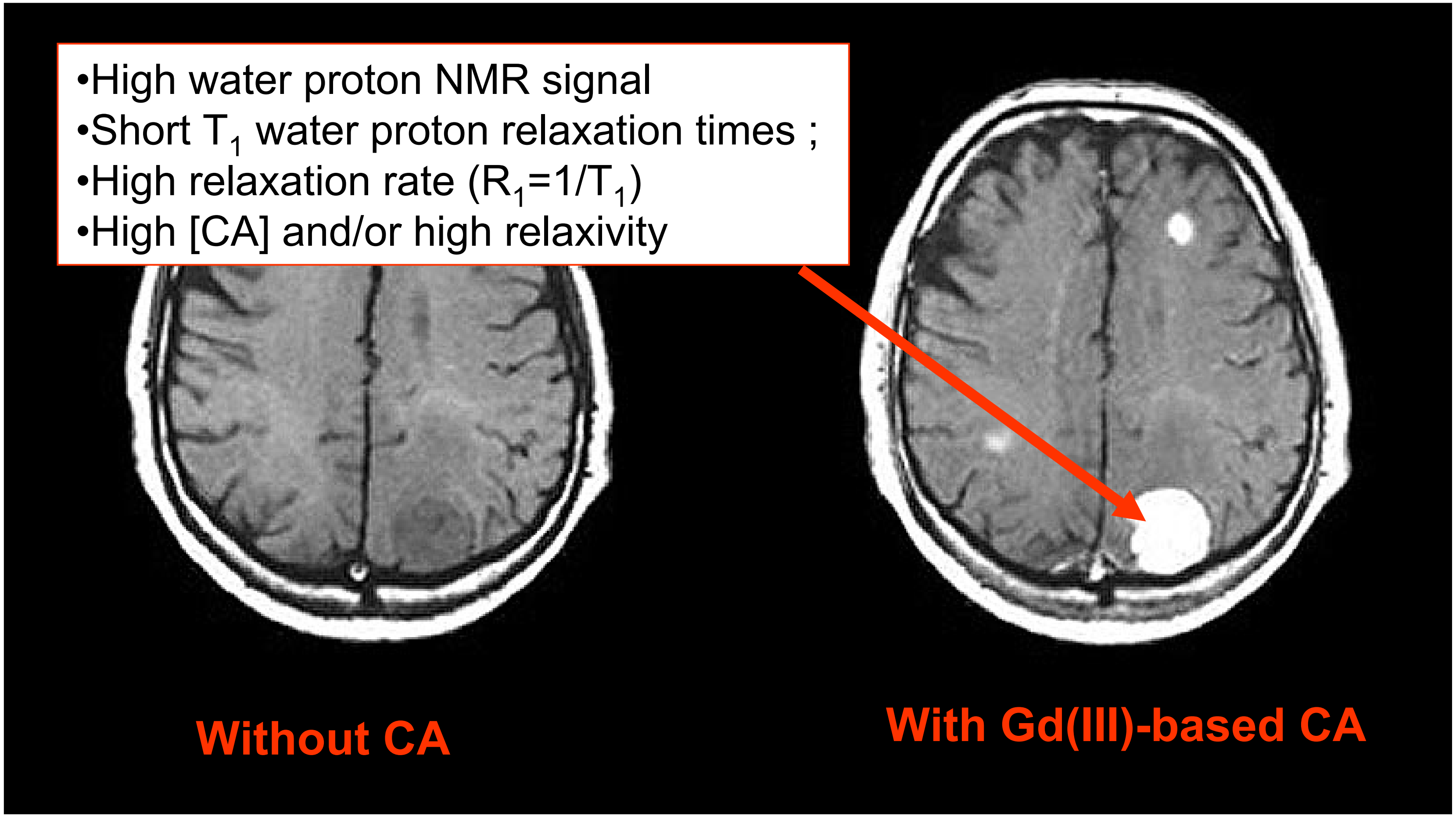
3. T_1-weighted (T_{1w}) MRI images we observe:
   -bright (**hyper-intense**) spots where water T_1 relaxation is **fast**
   -dark (**hypo-intense**) spots where water T_1 relaxation is **slow**

   Note: in this lecture I'll present almost exclusively T_{1w} images

4. Contrast agents based on Gd(III) chelates can shorten water relaxation times (*i.e.* water relaxation rates increase). The ability of a CA to shorten relaxation times can be summarized by a single parameter, called millimolar relaxivity (r_1^{mM}, $mM^{-1}s^{-1}$). The effect on T_{1w} image contrast is proportional to:

$$\text{Signal intensity} \propto R_1 = r_1^{mM} \times [CA]$$

Contrast in T_{1w} MRI images



Contrast Agents for MRI

- **Paramagnetic CAs**

Based upon paramagnetic metal ions (one or more unpaired electrons) most frequently Gd^{3+} (11 clinically approved) and Mn^{2+} (2 clinically approved).

Never used a free ions (undesiderable biodistrubution, high toxicity), rather as complexes with polyaminocarboxylic acid chelates

Typically T_1-agents (positive contrast)

- **Superparamagnetic CAs**

Based upon Iron Oxide particles (typically 5-200 nm in diameter)

Iron oxy-hydroxy aggregates made up by several thousands of magnetic ions (e.g. Fe^{3+}) have individual magnetic moments aligned, resulting in superpramagnetic properties.

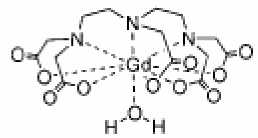
d > 50 nm → SPIO

d < 50 nm → USPIO

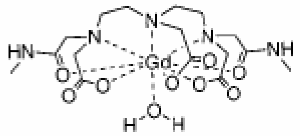
Typically T_2-agents (negative contrast), but new generation USPIOs (d< 10 nm) have T_1-enhancing properties (positive contrast)

Gd(III) complexes as T_1 agents (1)

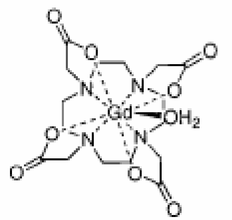
Clinical approved Gd-based agents: ExtraCellular Fluid (ECF) space



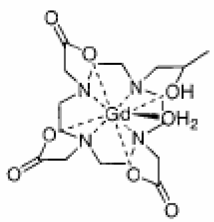
$[Gd(DTPA)(H_2O)]^{2-}$ (Magnevist™)



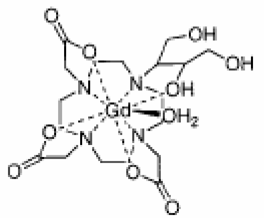
$[Gd(DTPA\text{-}BMA)(H_2O)]$ (Omniscan™)



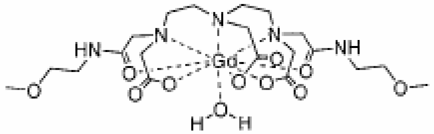
$[Gd(DOTA)(H_2O)]^-$ (Dotarem™)



$[Gd(HP\text{-}DO3A)(H_2O)]$ (ProHance™)



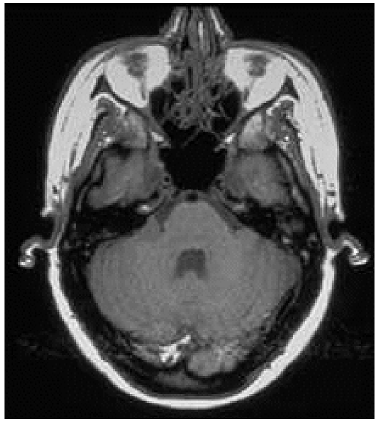
$[Gd(DO3A\text{-}butrol)(H_2O)]$ (Gadovist™)



$[Gd(DTPA\text{-}BMEA)(H_2O)]$ (OptiMARK™)

- They distribute between blood and interstitial fluid (extracellular agents)
- No specific targeting or delivery
- Fast renal excretion ($t_{1/2}$ < 1 hour)
- Clinically used in many diagnosis (mostly for detecting abnormalities blood brain barrier)
- relaxivity (0.47 T, 25 °C) of ca. 4 $s^{-1}mM^{-1}$

Clinical case: Human meningioma

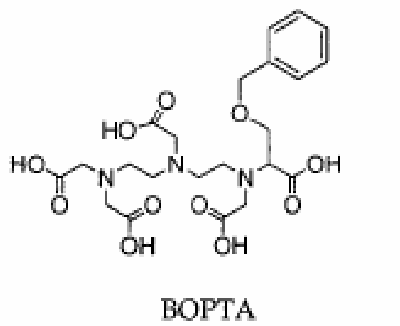


T_{1w} image without CA

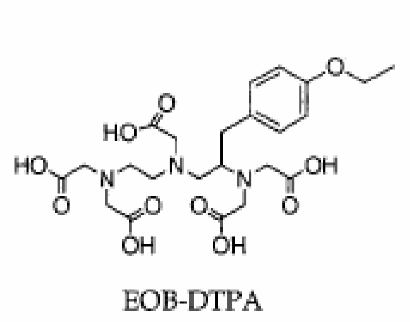
Post-admin. of a Gd-complex

Gd(III) complexes as T_1 agents (2)

Clinical approved Gd-based agents: Liver-targeted agents



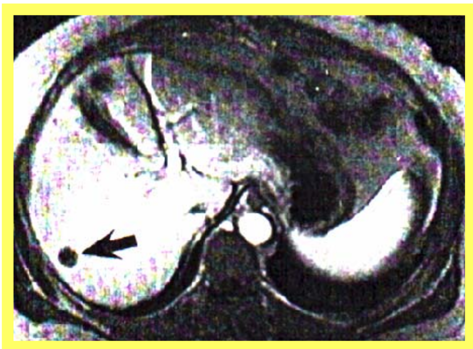
$[GdBOPTA]^{2-}$ - MultiHance™



$[GdEOB\text{-}DTPA]^{2-}$ - Primovist™ (phase III trials in USA)

The hydrophobic moieties promote the binding to serum albumin (slight increase of blood lifetime) and hepatobiliary excretion (liver imaging)

pre-contrast



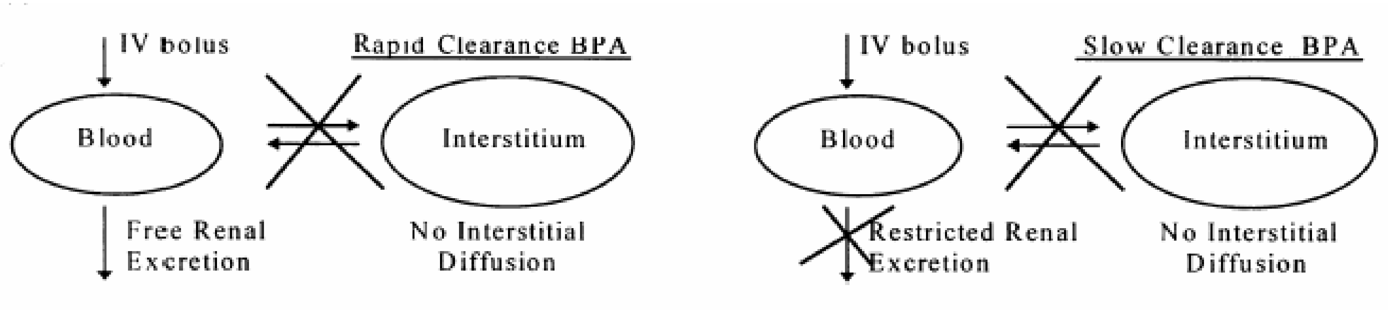
post-contrast

Clinical case:
Liver metastasis from colon cancer

Gd(III) complexes as T_1 agents (3)

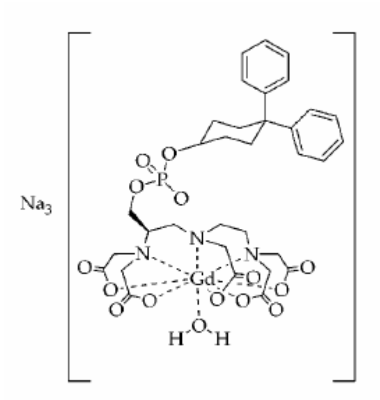
Clinically approved Gd-based agents: Blood Pool Agents (BPAs)

They have a limited (or absent) diffusion across the vascular endothelium

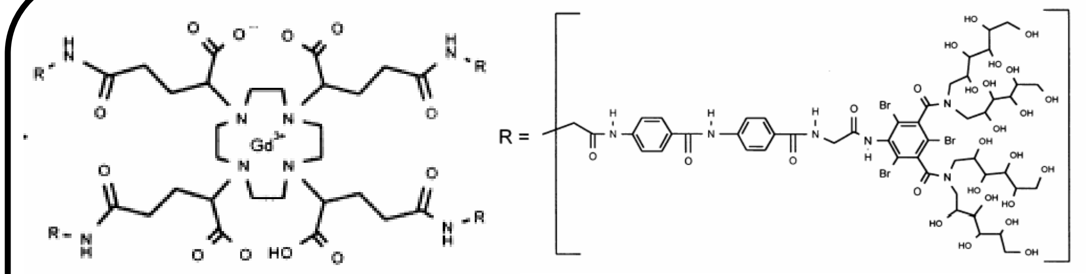


The vascular leakage is reduced by designing larger sized agents (see Vistarem™) or by promoting strong binding to serum albumin (Vasovist™)

$[Gd\text{-}MS\text{-}325]^{3-}$
Vasovist™



MS-325



P792 – Vistarem™
(currently on Clinic phase II trial)

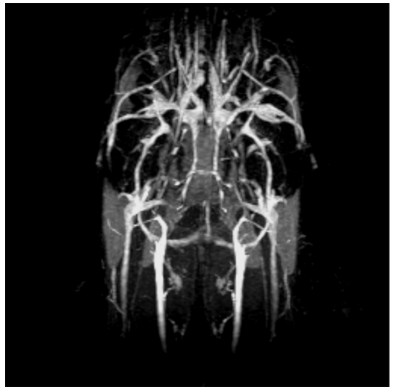
Gd(III) complexes as T_1 agents (4)

Clinical approved Gd-based agents: Blood Pool Agents (BPAs)

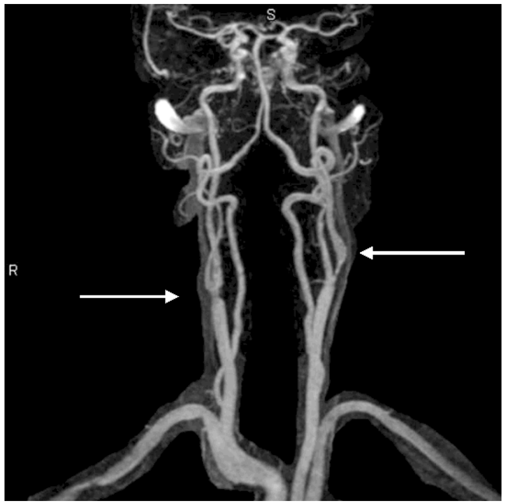
Example: MRI of rat head

pre-contrast

5 min. post
ECF agent



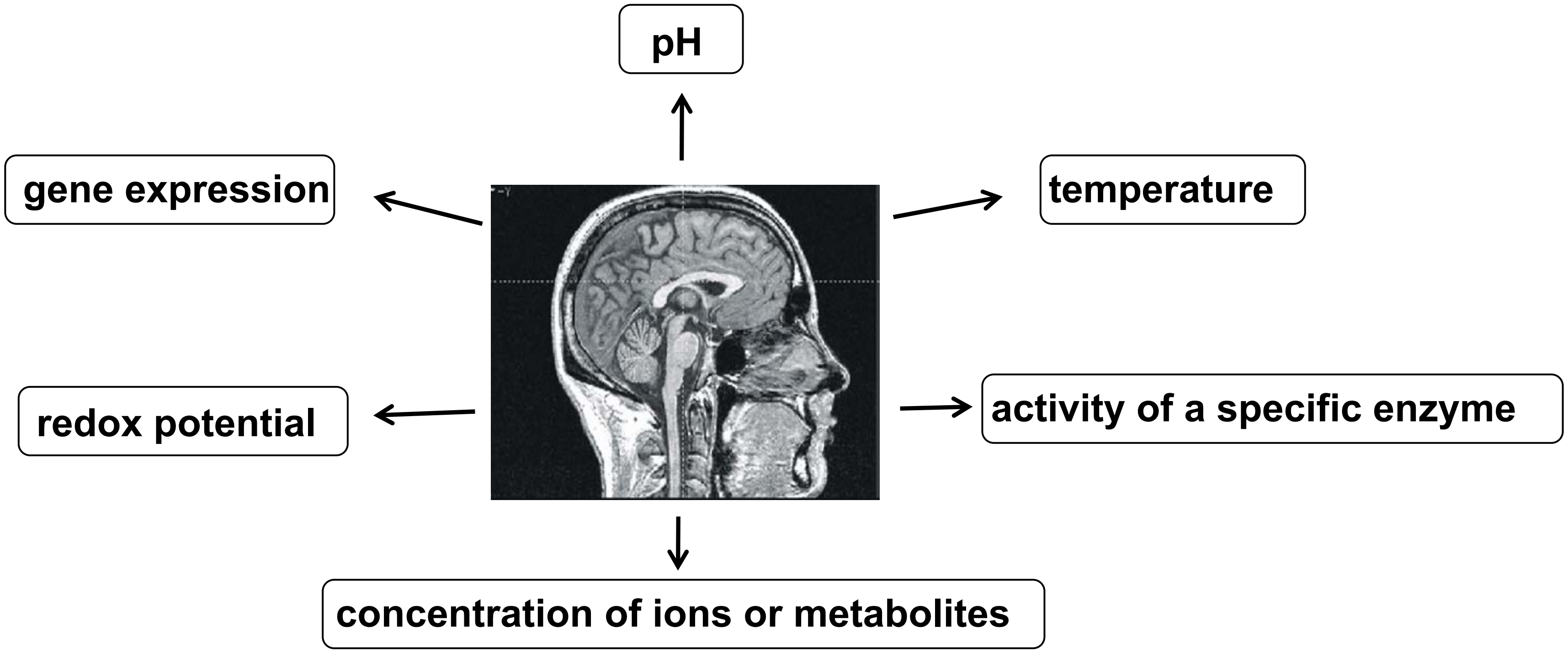
5 min. post
BPA agent



BPAs are very helpful for detecting abnormalities in blood vessels (e.g. stenosis), strokes, heart disorders,…

Responsive (also “Smart” or “Intelligent”) Agents

They are chemical probes whose image response allows the measurement of a specific physico-chemical variable characterizing their local microenvironment:

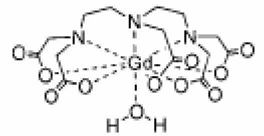


The *in vivo* assessment of such variables is very relevant for early and improved diagnosis, evaluation of therapeutic efficacy and follow-up, imaging of drug-delivery,…

„Evolution“ of MRI Contrast Agents

NOW

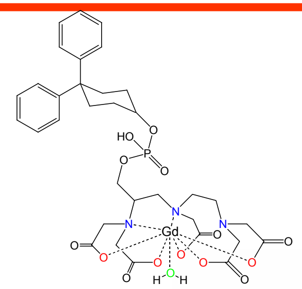
Extravasation

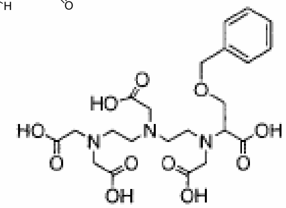


$[Gd(DTPA)(H_2O)]^{2-}$ (Magnevist™)

Blood pool

Organ specific (e.g. hepatospecific)





BOPTA

Clinically approved Gd(III) complexes

“Smart” probes

FUTURE

Responsive probes

- pH
- temperature,
- redox

-enzyme activity
-metabolites

Molecular Imaging probes

•receptors
•transporters
•other cellular processes

Cellular Imaging probes

•*Ex vivo* cell labelling

- *In vivo* cell labelling

Water proton relaxation agents: the origin of responsivness

Responsive agents

Definition: The term responsive refers to diagnostic agents whose contrasting properties are sensitive to a given physicochemical variable that characterizes the microenvironment in which the probe is distributed.

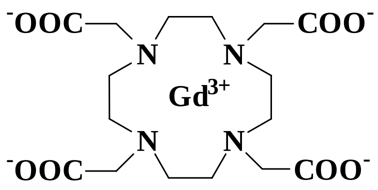
• Strictly speaking:
contrasting properties → relaxivity, CEST ;
physicochemical variable → pH, temperature, redox potential or ion concentration;

• With a wider meaning:
contrasting properties → any property, including pharmacokinetics
physicochemical variable → any variable, including binding interaction with receptor (targeted agents), trasport into cells (in vivo cell labelling agents) and biochemical transformation.

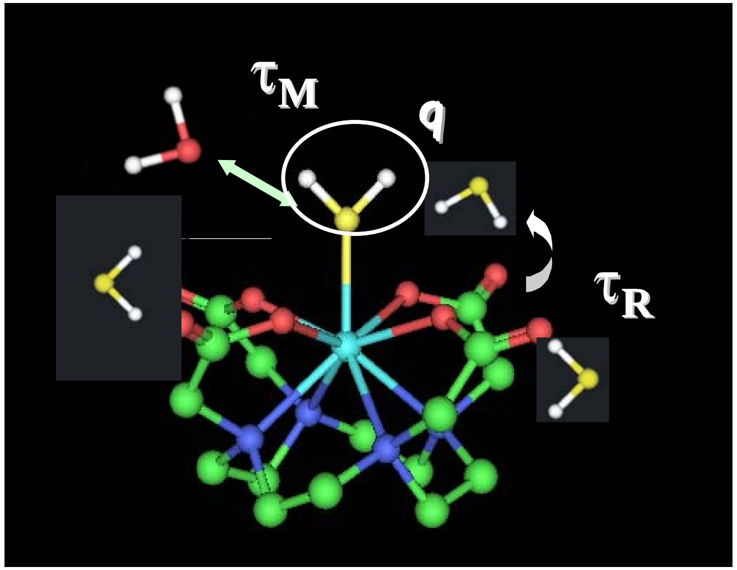
• Synonims of responsive are “smart” or “intelligent”

How can we make Gd(III) complexes responsive?

The ability of a Gd(III)-agent to make contrast (usually expressed by its relaxivity value) is dependent on several structural and dynamic parameters. The most important are:



Gd-DOTA



$$r_1^{mM} = f(\ \tau_R,\ q,\ \tau_M,\ r^{ss})$$

(…and many others…)

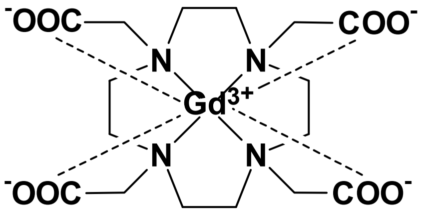
τ_R rotational mobility of the complex → slow tumbling (ns range) enhances relaxivity

q number of metal-coordinated water molecules

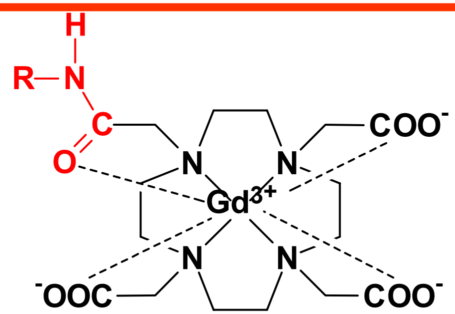
τ_M residence lifetime of the metal-coordinated water → 10-20 ns are optimal values

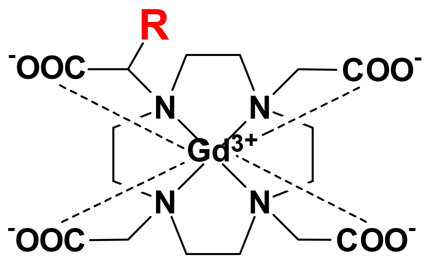
r^{ss} number of water molecules in the second hydration sphere of the metal

Most common Gd(III)-complexes macrocyclics



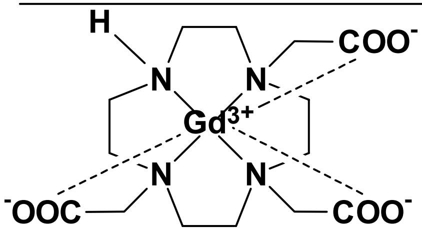
Gd-DOTA: q=1, nc=-1



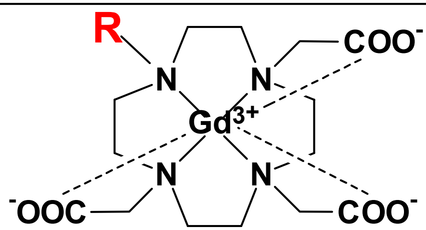


Gd-DOTAma: q=1, nc=0

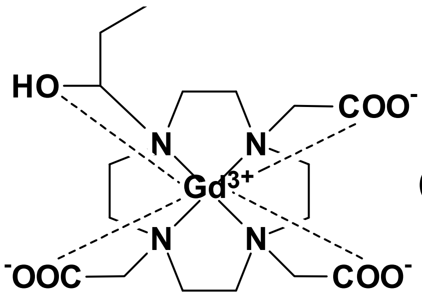
Tetramide also common; q=1, nc= +3



Gd-DO3A: q=2, nc=0

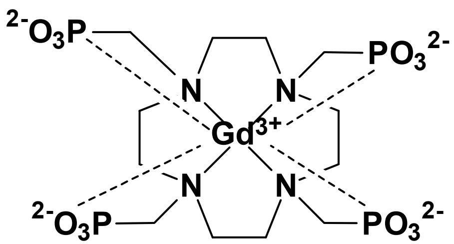


Gd-DO3A-"like": q=2, nc=0



Gd-HPDO3A

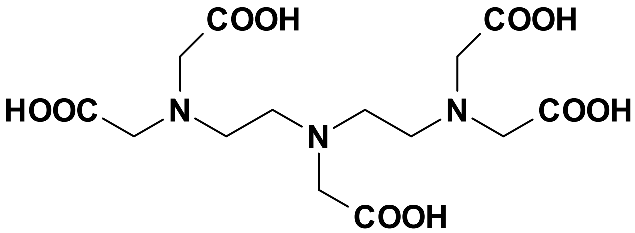
q=1, nc=0



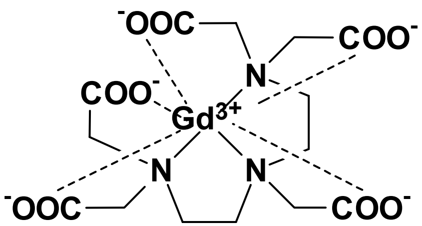
Gd-DOTP

q=0, nc=-5

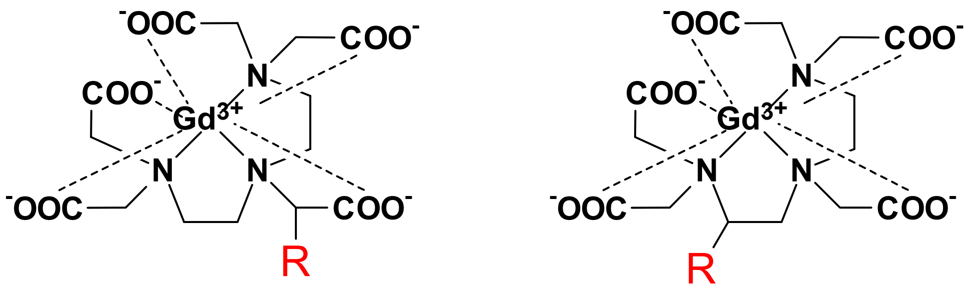
Most common Gd(III)-complexes
linear



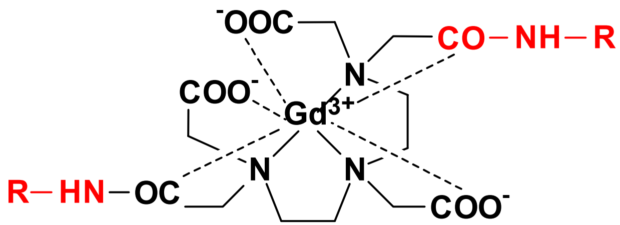
DTPA



Gd-DTPA: q=1, nc= -2



Gd-DTPA-"like", q=1, nc= -2

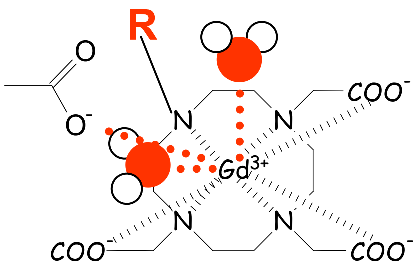


DTPA-bis amides

Gd-DTPA-BA, q=1, nc=0

A note on Gd-DO3A based complexes

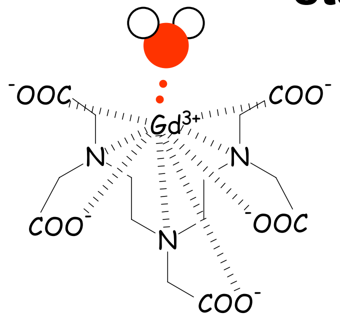
“variable” *q*



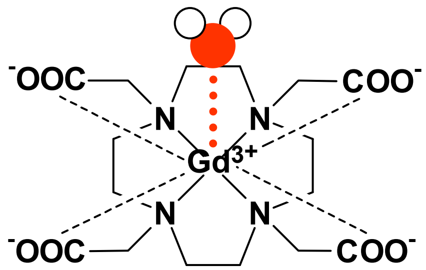
Gd-DO3A
& derivs

q may change from 2 to 1 or 0

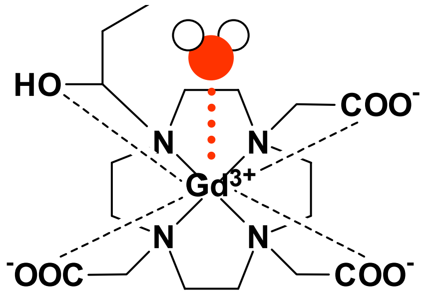
“stable” *q*



Gd-DTPA
& derivs

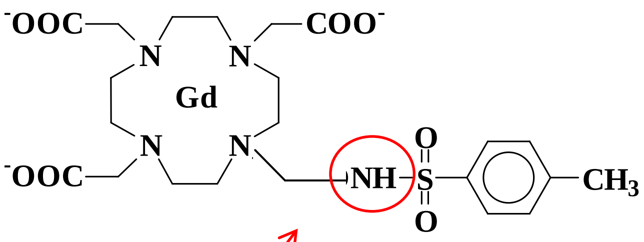


Gd-DOTA
& derivs

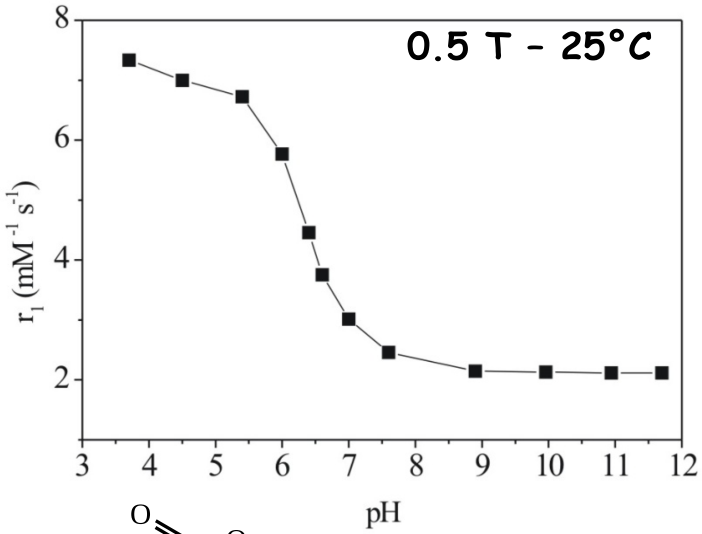


Gd-HPDO3A

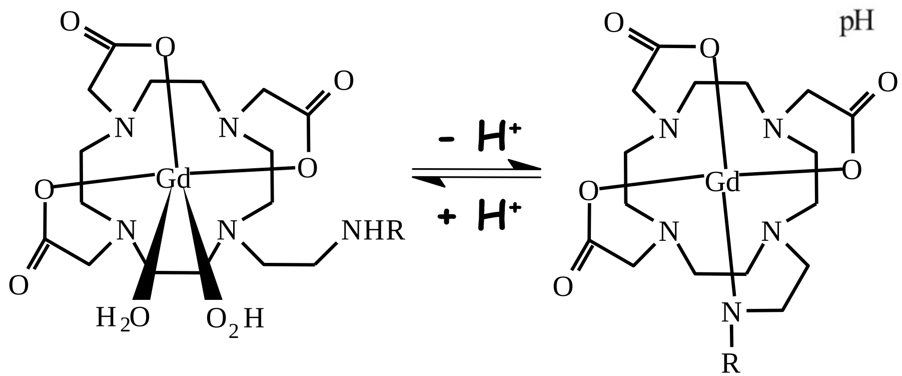
pH responsive probes: acting on *q*



pH sensitive moiety



Acid pH
$q = 2$ – High r_1

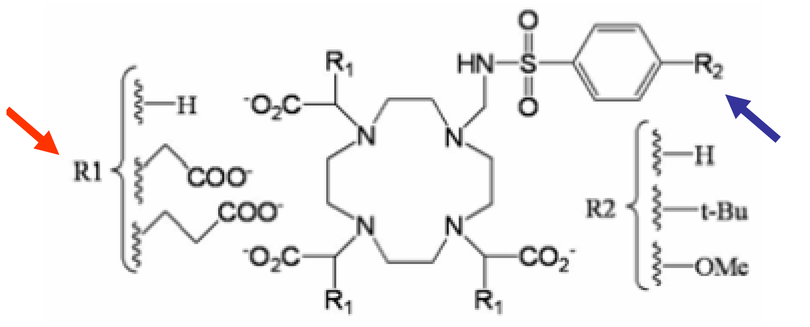


Basic pH
$q = 0$ – Low r_1

M. Lowe et al., *J. Am. Chem. Soc.*, 2001, 123, 7601.

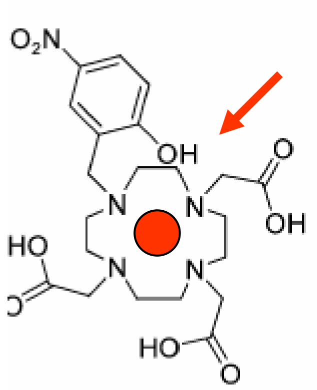
<u>Problems</u>: interferences from carbonate, lactate and the like might occur (ternary complex formation)

pH responsive probes: acting on *q*

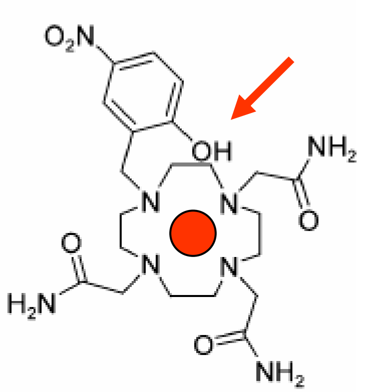


R_1: negatively charged groups to prevent interaction with endogenous organic anions
R_2: fine tuning of the protonation constant

Other compounds, same story…



Gd(NP-DO3A)



Gd(NP-DO3AM)

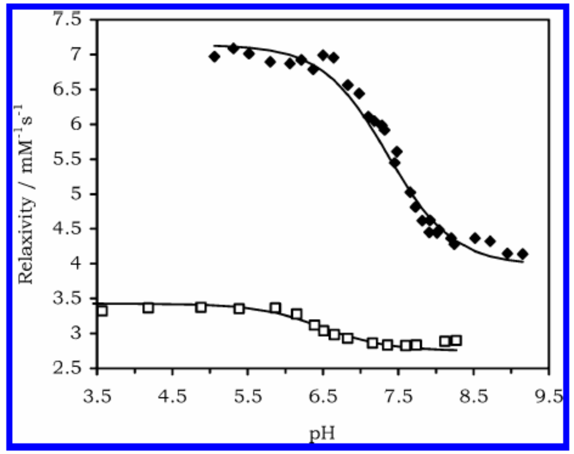
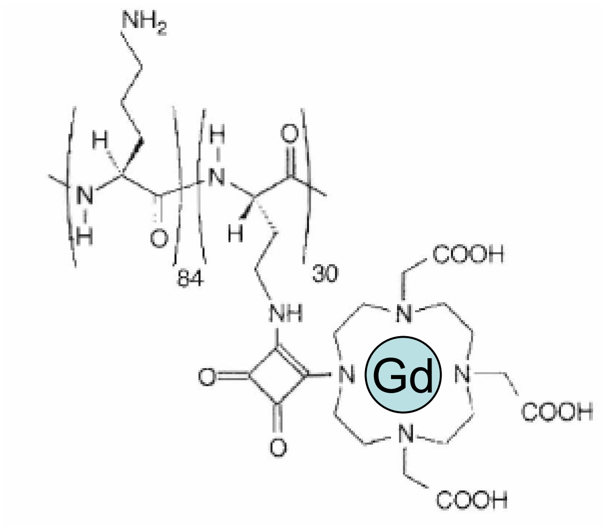
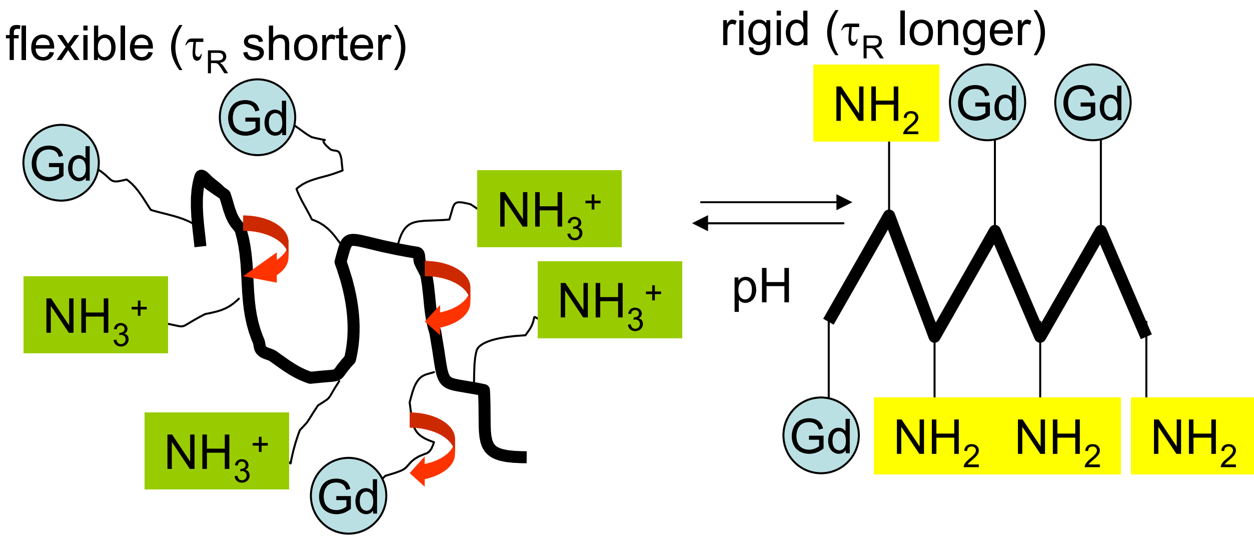


Figure 2. Relaxivity pH profiles of Gd(NP-DO3A) (filled diamonds) and Gd(NP-DO3AM) (open squares) recorded at 25 °C and 20 MHz.

Gd(NP-DO3A) - Δr about 70%
q = 1, anionic complex at high pH (r_1 = 4.1 mM^{-1} s^{-1})
q = 2, neutral complex at low pH (r_1 = 7.0 mM^{-1} s^{-1}) as the phenol becomes protonated and dissociates.
No interference from citrate, lactate, phosphate (up to 40x excess)

pH responsive probes: acting on τ_R





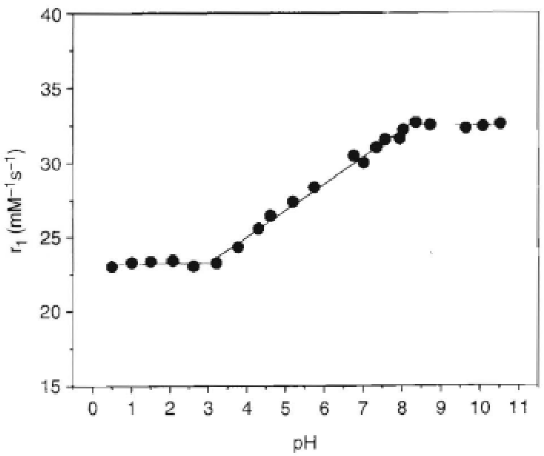


FIG. 16. pH dependence of r_1 at 20 MHz and 25°C for Gd-4 (Chart 12). The relaxivity enhancement upon increasing pH is due to an elongation of τ_R promoted by the progressive deprotonation of the NH_3^+ groups of the polymer structure.

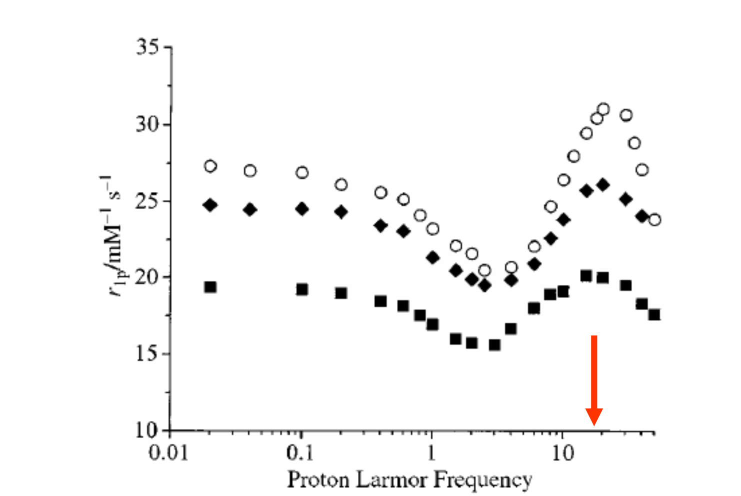
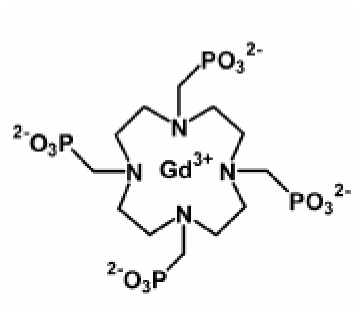


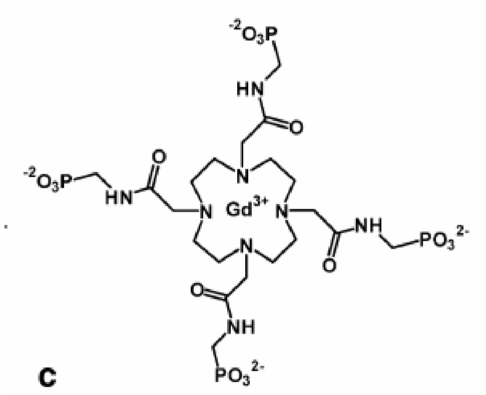
Fig. 2 $1/T_1$ NMRD profiles (298 K) of $(GdDO3ASQ)_{30}$-Orn_{114} (1 mM) at pH 4.5 (■), 7 (◆) and 8.5 (○) respectively.

Aime et al, *Chem Commun.* 1999, 1577

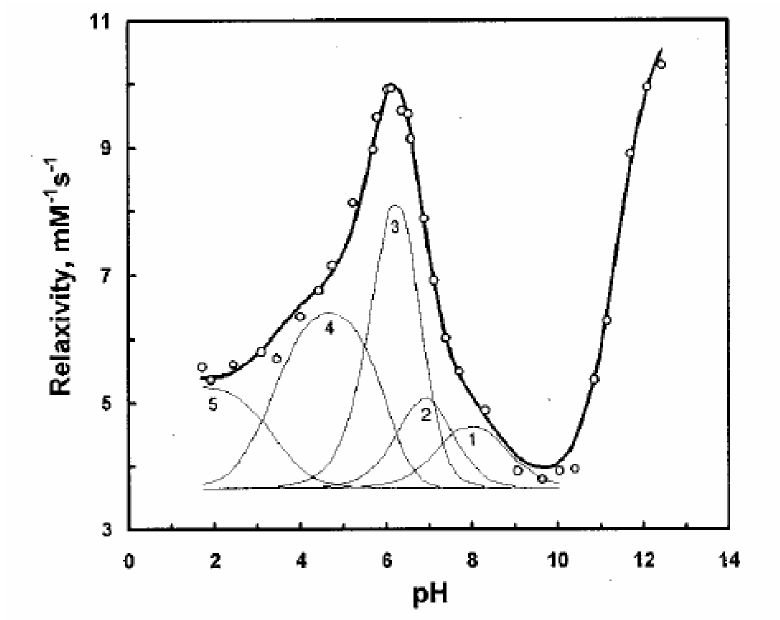
pH responsive probes: acting on second sphere water and τ_M



Gd-DOTP
Non pH sensitive
r_1= 3.0 $mM^{-1}s^{-1}$
PBS, 37°C, 4.7 T



Gd-DOTA-4amP
pH sensitive
r_1= 3.8 $mM^{-1}s^{-1}$
PBS, 37°C, 4.7 T



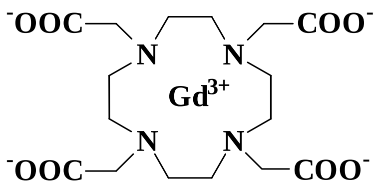
4 < pH < 6 → r_1 increases.
6 < pH < 8.5 → r_1 decreases
pH > 10 → r_1 increases

Phosphonate deprotonation, with formation/disruption of H-bond network between the phosphate groups and inner sphere water molecule;
Dynamics of 2nd sphere water molecules also affected by the ionization of posphonate groups.

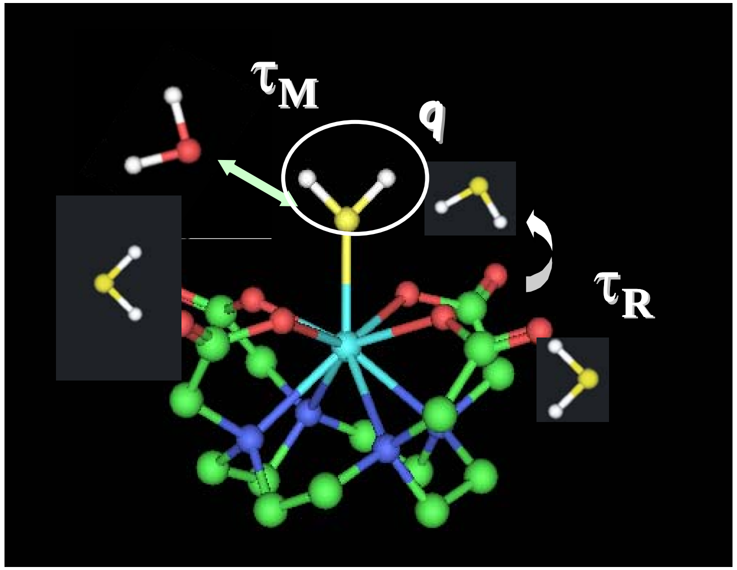
prototropic exchange of inner water molecules catalyzed by OH^-

How can we make Gd(III) complexes responsive?

The ability of a Gd(III)-agent to make contrast (usually expressed by its relaxivity value) is dependent on several structural and dynamic parameters. The most important are:



Gd-DOTA



$$r_1^{mM} = f(\, \tau_R,\ q,\ \tau_M,\ r^{ss})$$

(…and many others…)

τ_R rotational mobility of the complex → slow tumbling (ns range) enhances relaxivity

q number of metal-coordinated water molecules

τ_M residence lifetime of the metal-coordinated water → 10-20 ns are optimal values

r^{ss} number of water molecules in the second hydration sphere of the metal

Responsive agents: the concentration issue

Requisites for an "ideal" responsive agent in vivo

- high responsiveness to the parameter of interest
- high sensitivity of the imaging probe
- simplicity of the measurement protocol
- rapidity of the response
- high accuracy of the measurement

Critical issues for the accuracy *in vivo* are:

i) the dependence of the image response on the probe concentration

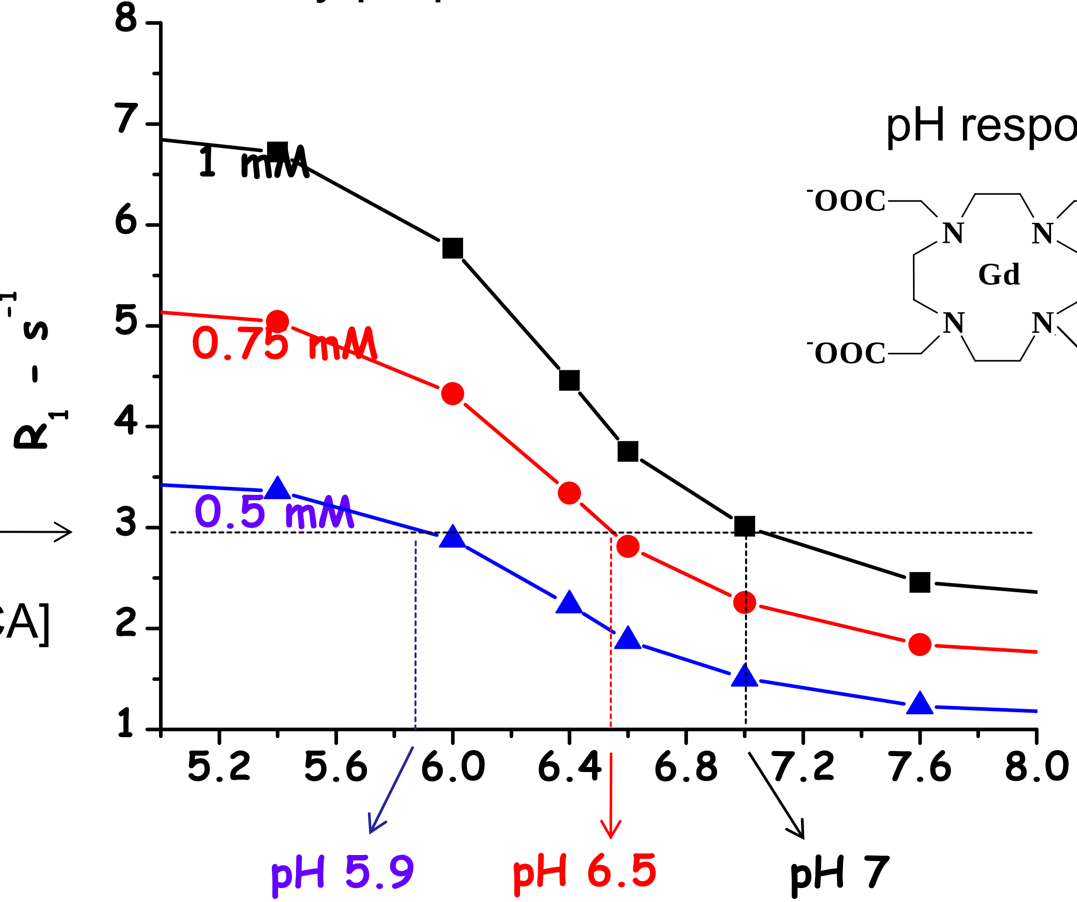
ii) the reliability of the calibration curve

Responsive probes: the concentration issue

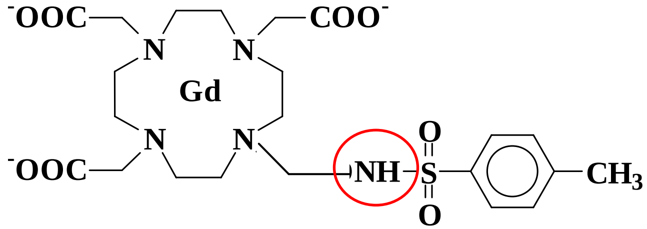
The MR response is directly proportional to the local concentration of the probe

Image Response

$\propto R_1 = r_1^{mM} \times [CA]$



pH responsive agent



The pH values are dependent on the probe concentration

How the MR response can be made independent on the probe concentration ?

i) Assessing the probe concentration *in vivo*

- •By a non responsive reference Gd-complex
- •By dual mode imaging techniques (MR-PET)

ii) Getting a concentration-independent MR response:

- to use a ratiometric approach (two experiments are needed)

- to detect a concentration-independent NMR variable (e.g, chemical shift, single experiment). We ‘ll see something after having introduced CEST probes

Assessing the probe concentration *in vivo:* *non-responsive reference*

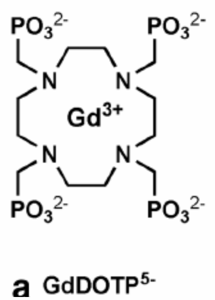
A reasonable estimation may be carried-out by using a non-responsive system whose biodistribution must be equal to that of the responsive probe (!!)

Example: mapping of extracellular pH on rat glioma

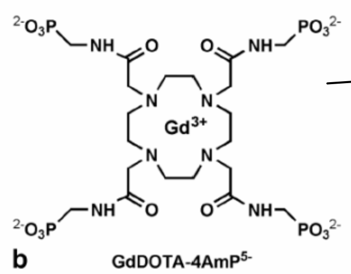
Experiment timing

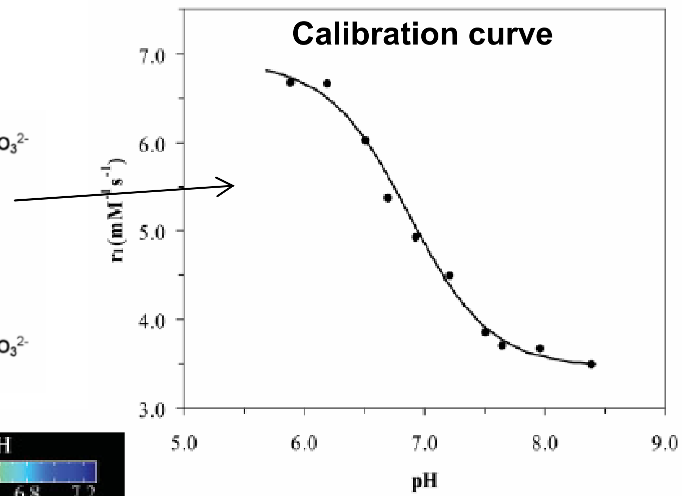
- 1st bolus (non responsive)
- DCE images to TMI1
- Washout delay
- 2nd bolus (responsive
- DCE images to TMI2
- Ratio between images

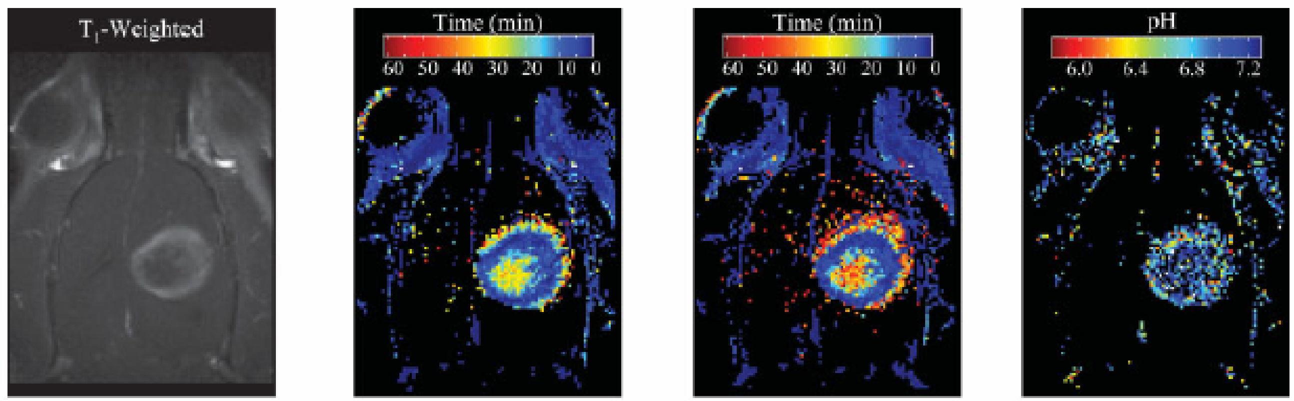
Non responsive



pH responsive







pH_e map

M. Garcia-Martin et al, *Magn. Res. Med.* , 2006, 55, 309.

Assessing the probe concentration *in vivo* with non-responsive reference: critical issues

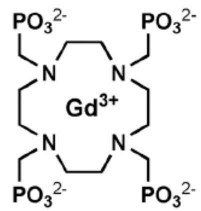
1. Reliability of the calibration curve

The relaxivity *in vivo* may be significantly influenced by:

- the interaction with biological components (e.g. proteins, cells,…) and…

Example:

Non Responsive agent

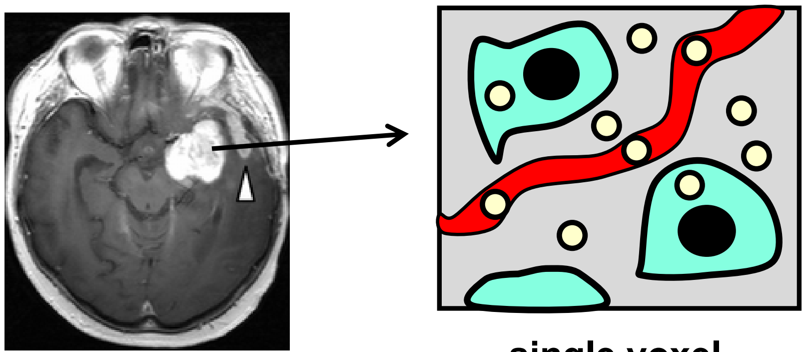


a $GdDOTP^{5-}$

r_1 in PBS buffer = 3.2 $mM^{-1}s^{-1}$

r_1 in serum = 5.7 $mM^{-1}s^{-1}$

- compartmentalization effects



single voxel

The MR contrast is strongly affected by the probe localization and distribution (intravascular, extracellular, intracellular).

Assessing the probe concentration *in vivo* with non-responsive reference: critical issues

2. Pharmacokinetics

Identical pharmacokinetics → identical structure

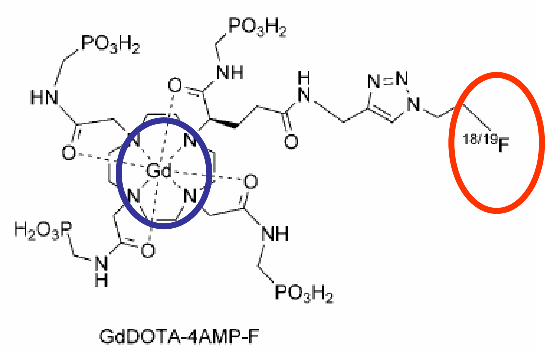
3. Sequential administration of two CAs

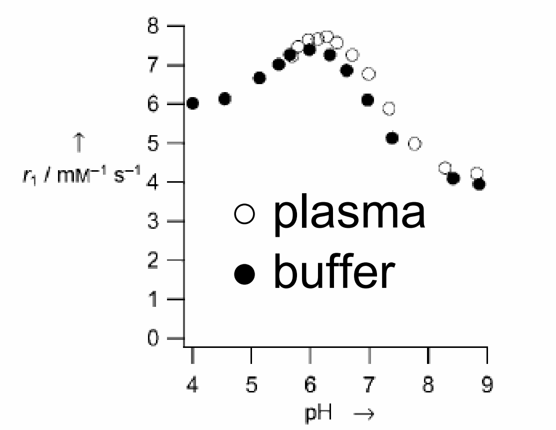
Long experiment time

- Pharmacokinetics might change because of change in the systemic blood pressure due to prolonged anesthesia

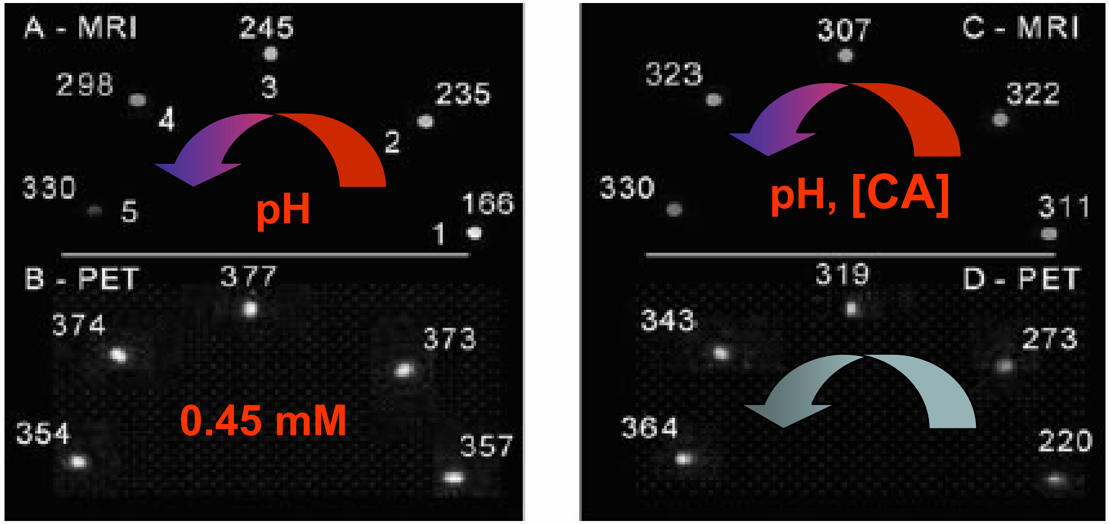
- Possible in-plane movement (image mismatch)

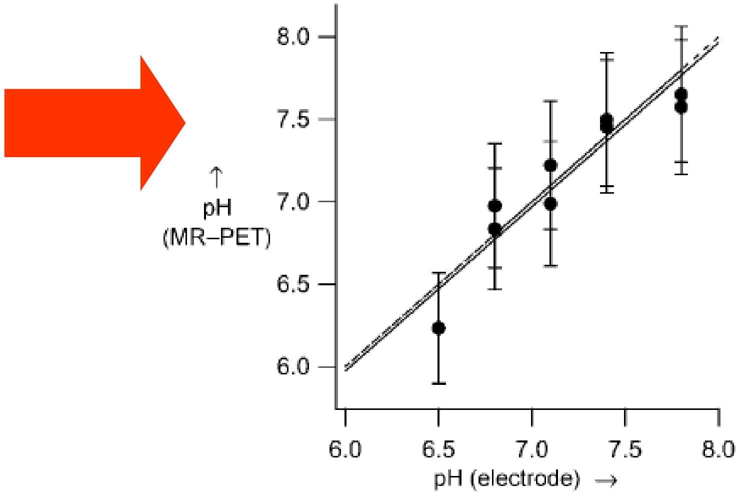
Assessing the probe concentration *in vivo:*
dual mode imaging techniques





MR-PET imaging: simultaneous acquisition of a MRI (3T) and a PET image (very specialized equipment needed!)





Frullano et al *Angew. Chem. Int. Ed.* **2010**, 49, 2382

Getting a concentration-independent response: the ratiometric approach

Two independent observables (OBS1 and OBS2), both dependent on the probe concentration, have to be measured.

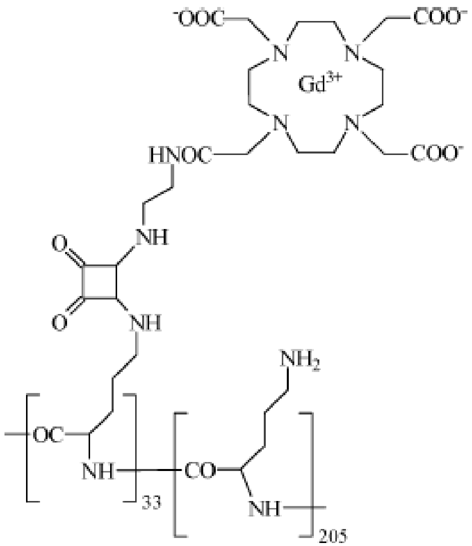
The OBS1/OBS2 ratio is the concentration-independent MR response.

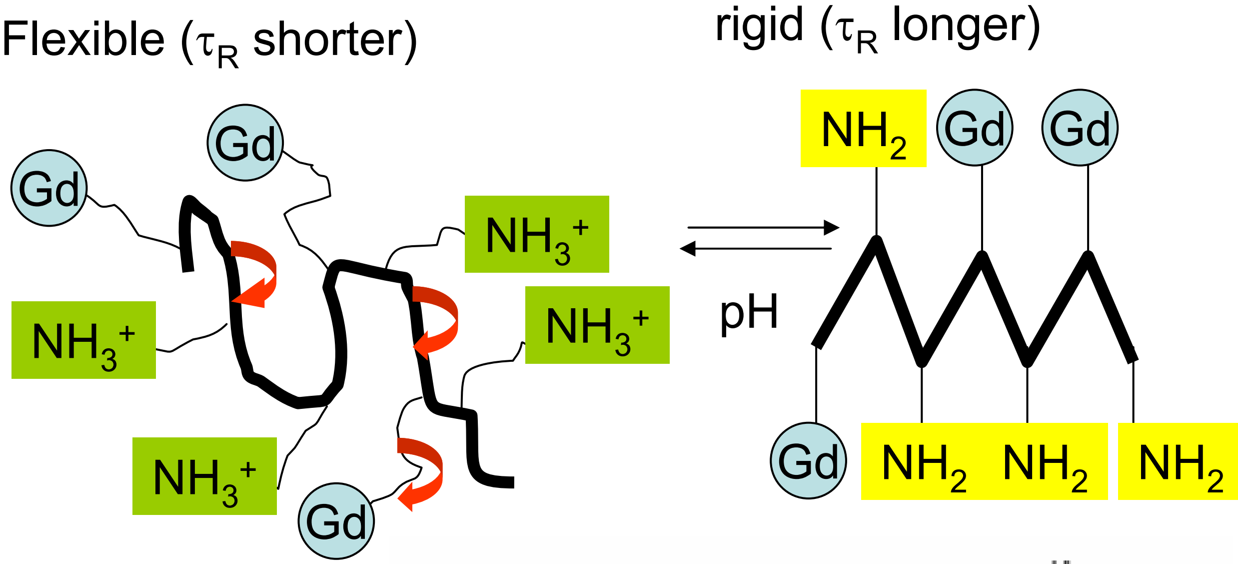
The OBS1/OBS2 ratio must be made sensitive to the parameter of interest.

High responsiveness are expected when only one of the observables is responsive or when the observables display an opposite dependence.

Ratiometric probes can be developed for both Gd(III)-based and CEST agents.

Getting a concentration-independent response: the ratiometric approach for Gd(III) complexes





The two observables are the longitudinal (R_{1p}) and the transverse (R_{2p}) relaxation rates of water protons.

$$R_{1p} \simeq \frac{P_M}{T_{1M} + \tau_M} \Longrightarrow \frac{1}{T_{1M}} = \frac{6}{15}\frac{K^{DIP}}{r_H^6}\left(\frac{\tau_C}{1 + \omega_H^2 \tau_C^2}\right)$$

$$R_{2p} \simeq \frac{P_M}{T_{2M} + \tau_M} \Longrightarrow \frac{1}{T_{2M}} = \frac{1}{15}\frac{K^{DIP}}{r_H^6}\left(4\tau_C + \frac{3\tau_C}{1 + \omega_H^2 \tau_C^2}\right)$$

τ_R contributes to τ_C

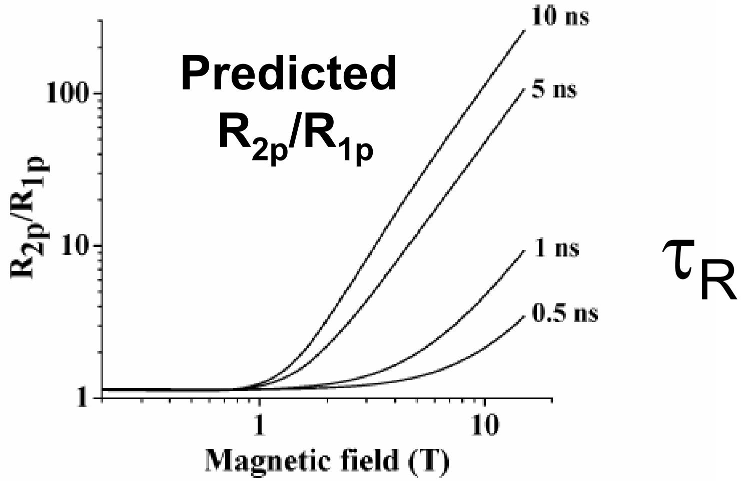
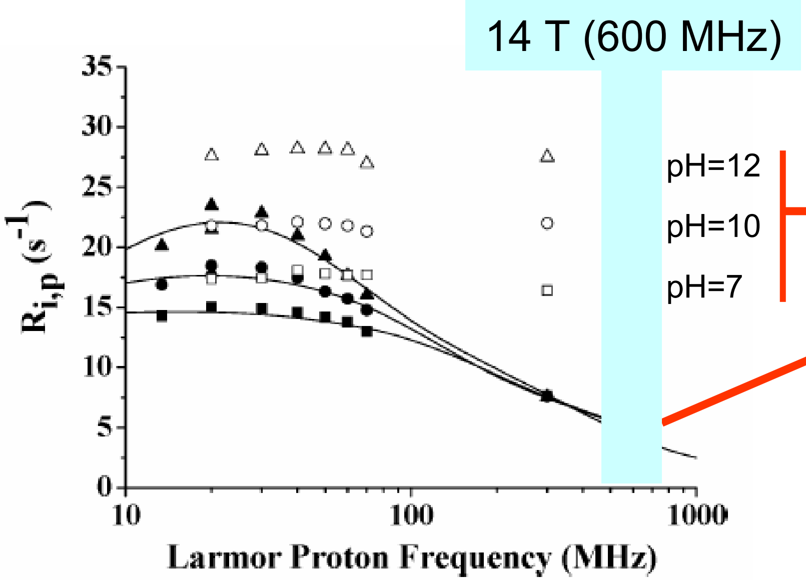


Figure 1. Calculated R_{2p}/R_{1p} values as a function of magnetic field strength for a macromolecular Gd(III) complex ($q = 1$, $r = 3$ Å, $\tau_M = 200$ ns, $\Delta^2 = 2 \cdot 10^{19}$ s^{-2}, $\tau_V = 10$ ps) endowed with τ_R values in the 0.5−10 ns range.

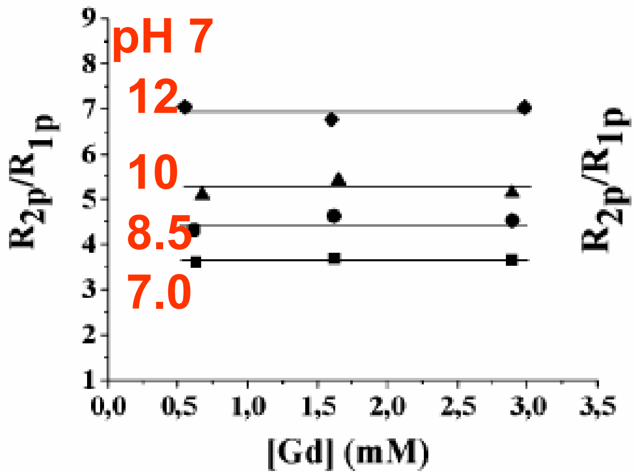
Getting a concentration-independent response: the ratiometric approach for Gd(III) complexes



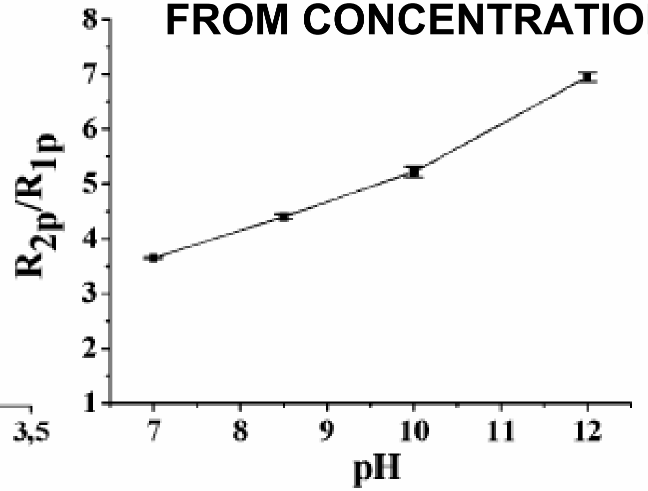
R_{2p} at high field: depends on both concn AND τ_R

R_{1p} at high field: only depends on concn, NOT on τ_R

Open symbols: R_{2p}
Solid symbols: R_{1p}



R_{2p}/R_{1p} IS INDEPENDENT FROM CONCENTRATION!!

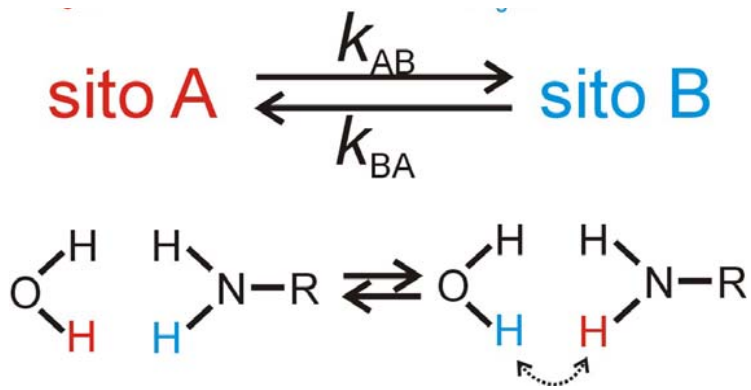


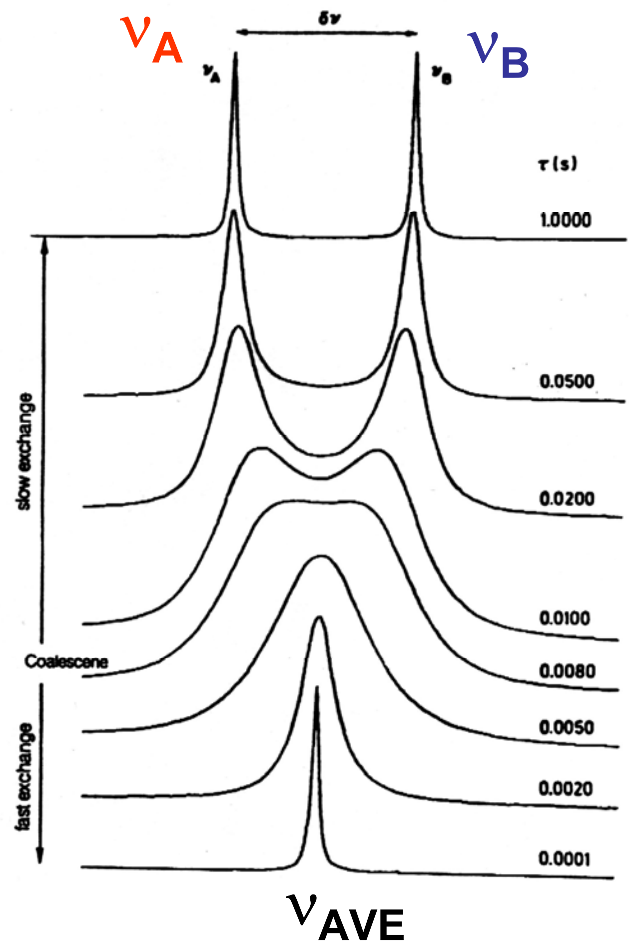
Aime et al, *J Am Chem Soc* **2006**, 128, 11326

3. Chemical Exchange Saturation Transfer contrast agents

Chemical exchange in NMR

Two protons pools, A and B, with Larmor frequency ν_A and ν_B, exchanging with a rate constant k (assuming equal populations)





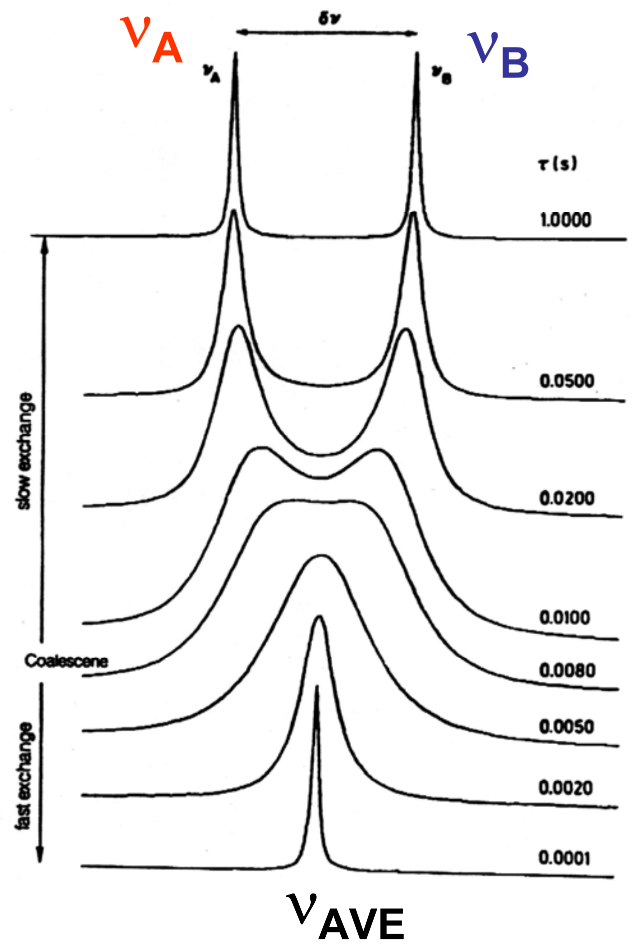
Chemical exchange in NMR

Two protons pools, A and B, with Larmor frequency ν_A and ν_B, exchanging with a rate constant k (assuming equal populations)

$k << \delta\nu$ → Slow exchange
Two separate signals at ν_A and ν_B

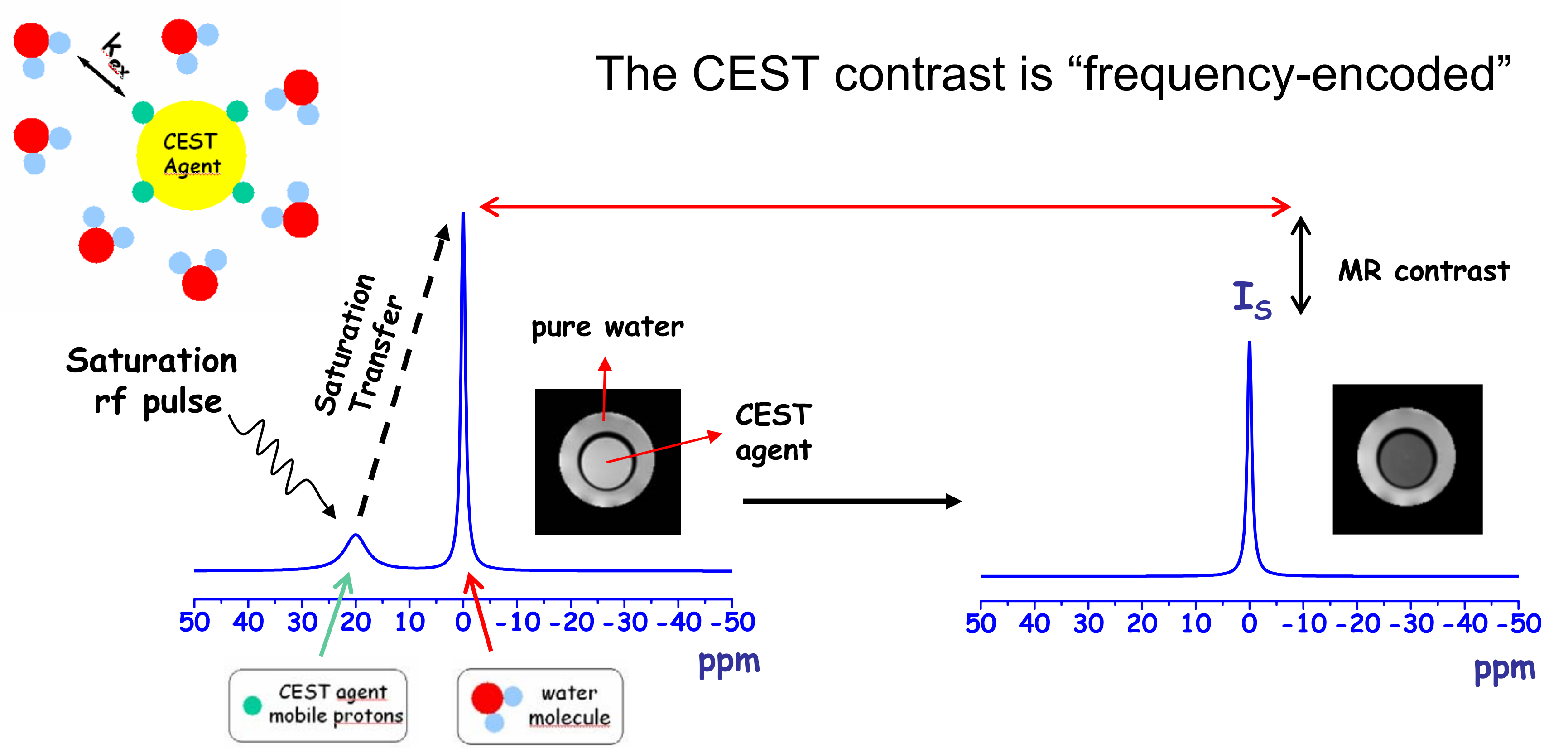
$k \approx \delta\nu$ → Intermediate exchange

$k >> \delta\nu$ → Fast exchange
One averaged NMR signal at
$\nu_{AVE} = ½ (\nu_A + \nu_B)$

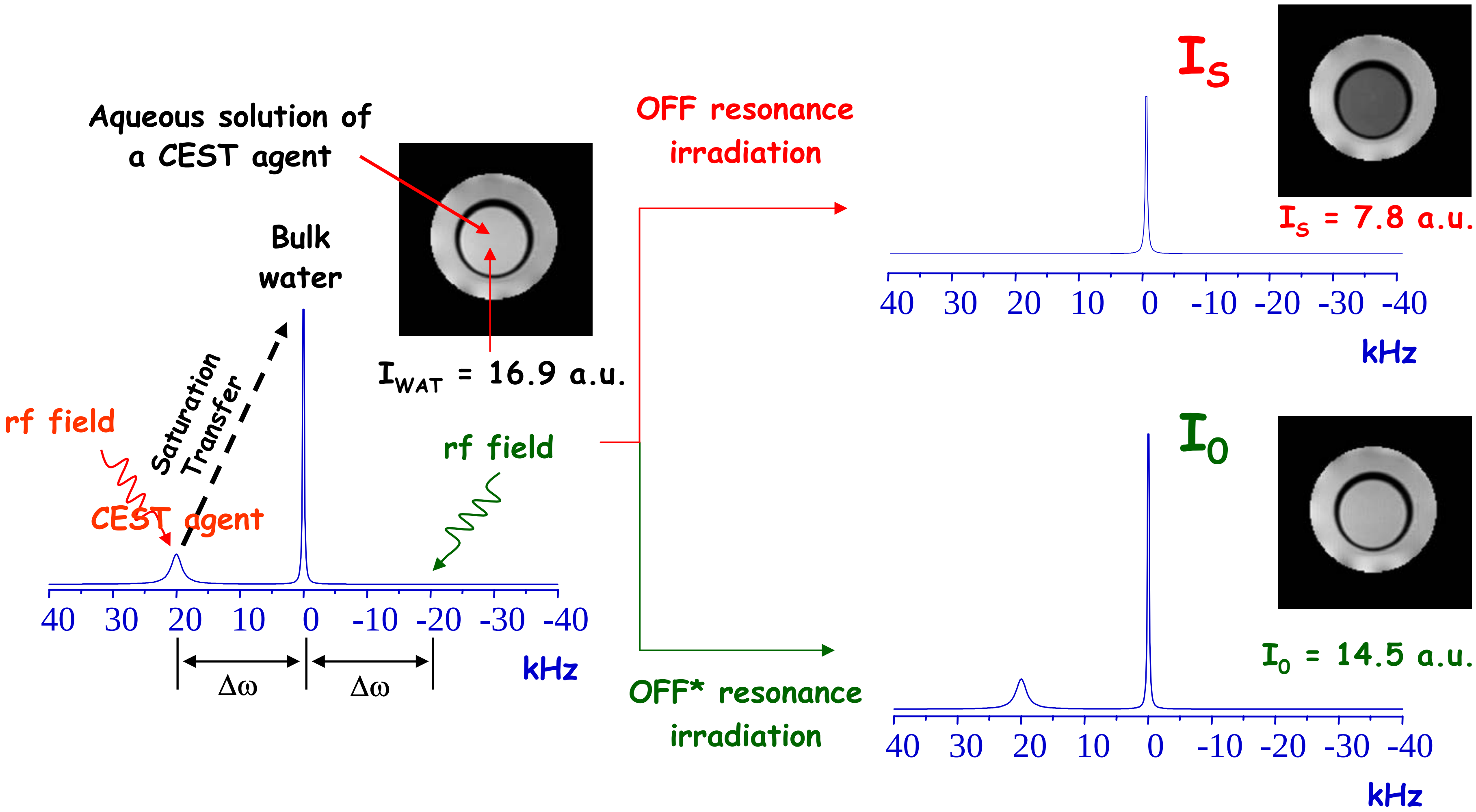


Chemical Exchange Saturation Transfer (CEST) agents

The CEST contrast is “frequency-encoded”

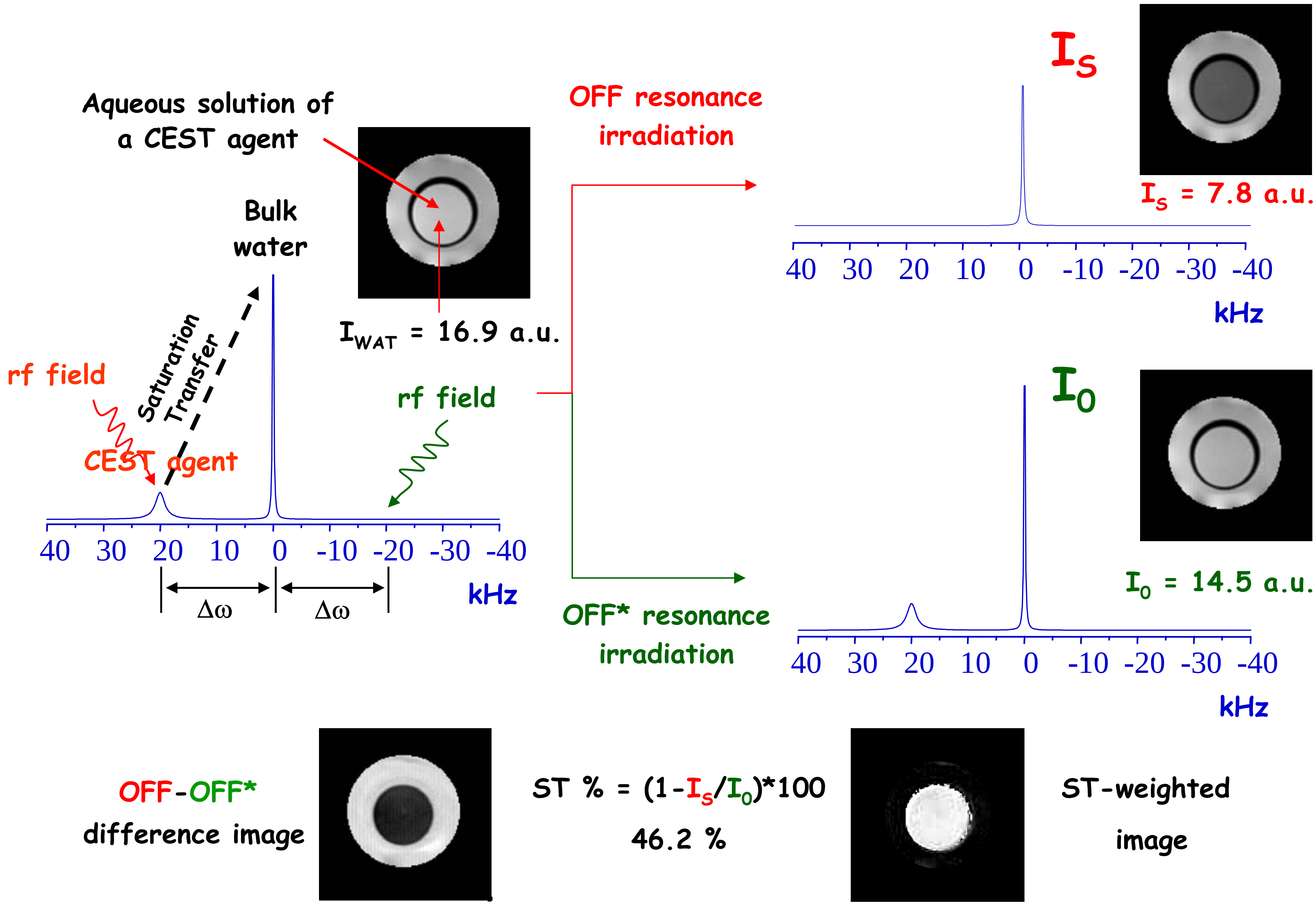


CEST contrast may be modulated by influencing the exchange rate and/or the resonance frequency of the mobile protons to be saturated.



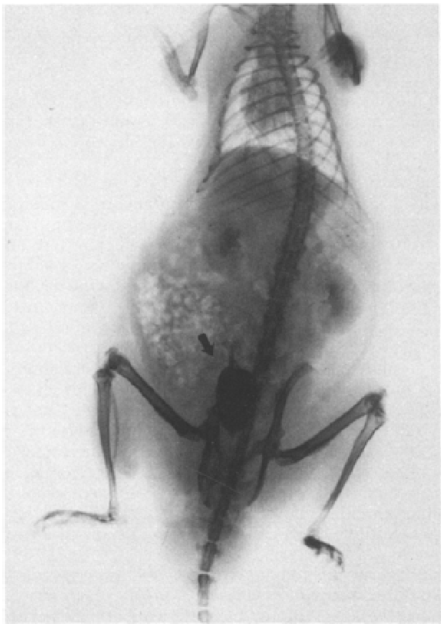
The decrease of I_{WAT} is the source of the MR contrast

The net ST % effect is calculated as $(1 - I_S/I_0) \cdot 100$

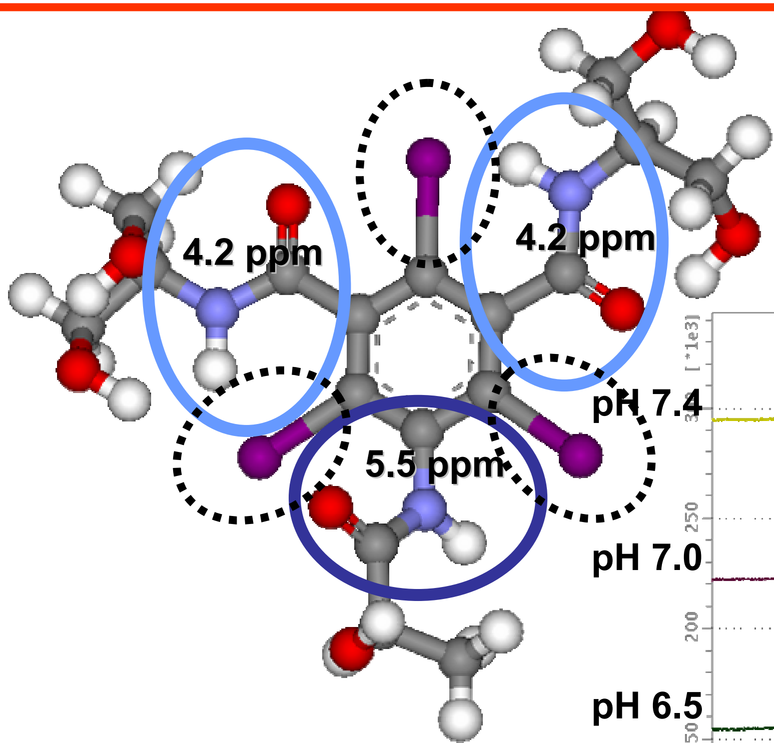

Aqueous solution of a CEST agent
Bulk water
I_{WAT} = 16.9 a.u.
rf field
Saturation Transfer
CEST agent
rf field
40 30 20 10 0 -10 -20 -30 -40
kHz
Δω
Δω
OFF resonance irradiation
I_S
I_S = 7.8 a.u.
40 30 20 10 0 -10 -20 -30 -40
kHz
I_0
I_0 = 14.5 a.u.
OFF* resonance irradiation
40 30 20 10 0 -10 -20 -30 -40
kHz
OFF-OFF* difference image
ST % = (1-I_S/I_0)*100
46.2 %
ST-weighted image

Iopamidol: a dual contrast agent for CT and CEST MRI

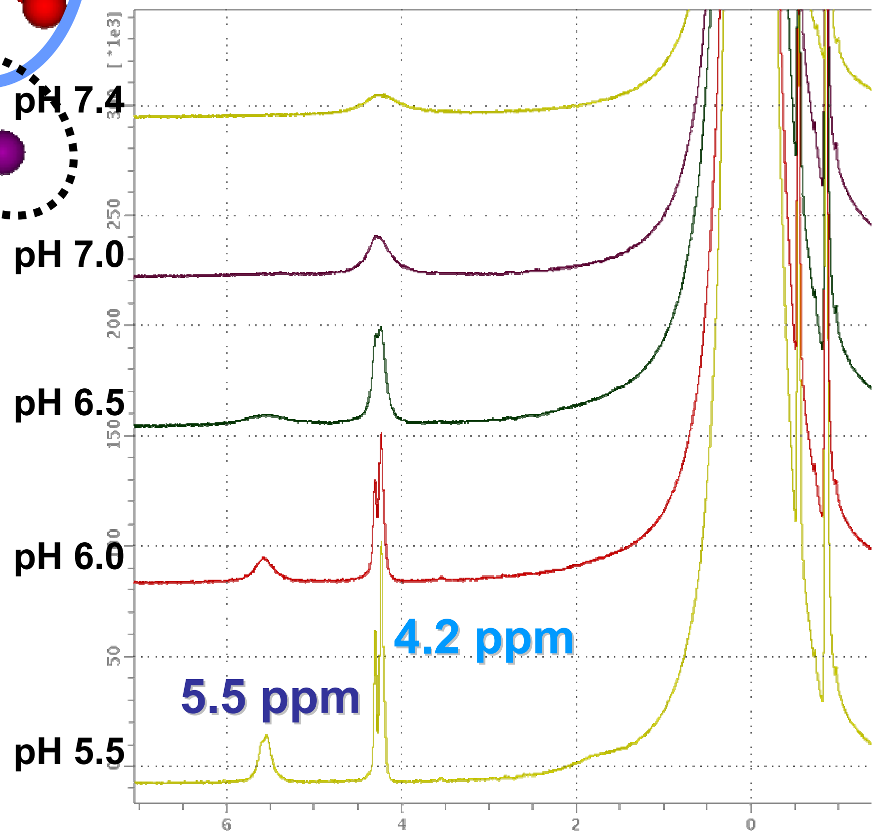
X-RAY



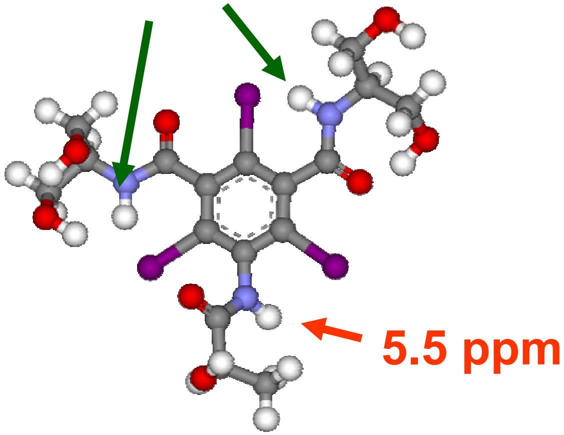
safe and effective low-osmolar non-ionic contrast medium well established with U.S. clinical experience since **1986**

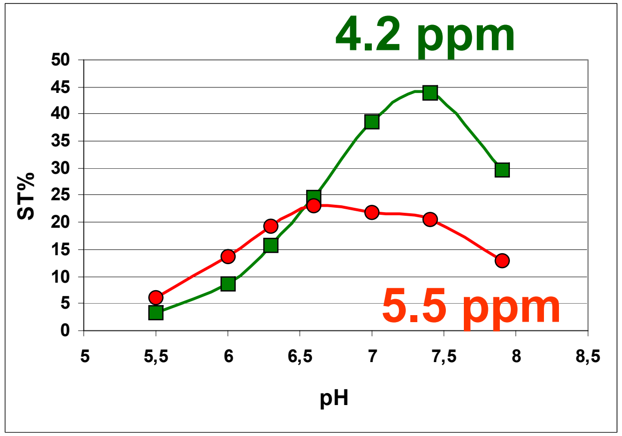


CEST-NMR

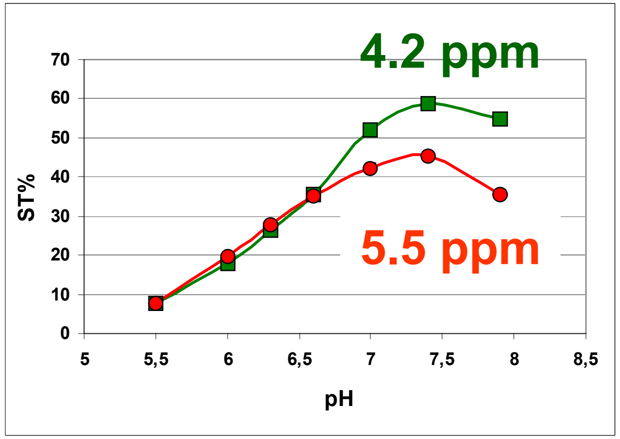


Iopamidol as a pH responsive CEST MRI CA

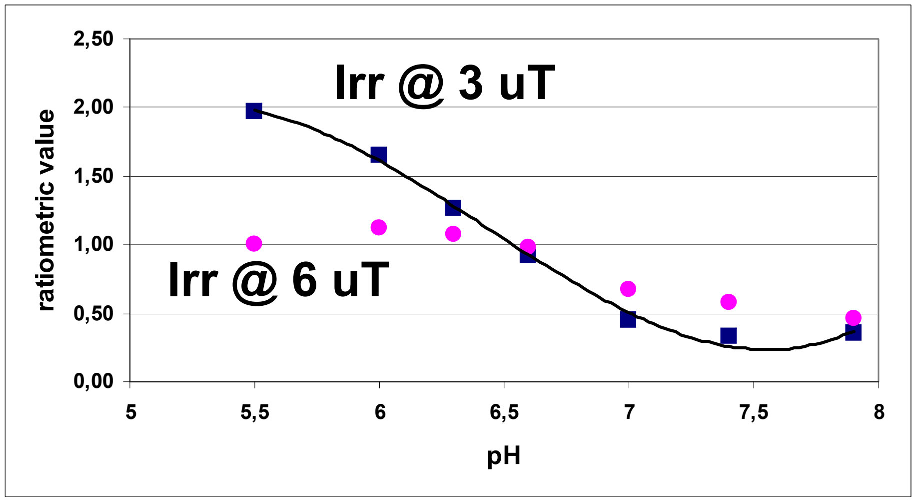




Irr @ 3 uT

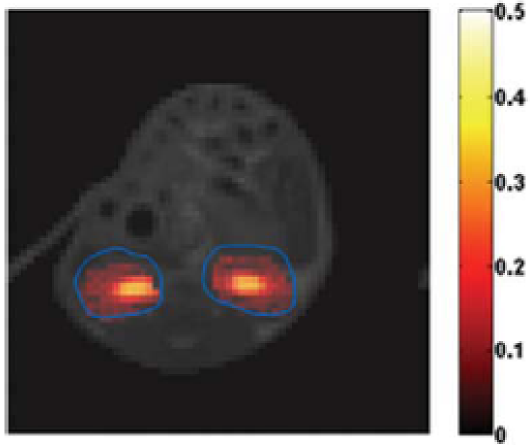


Irr @ 6 uT

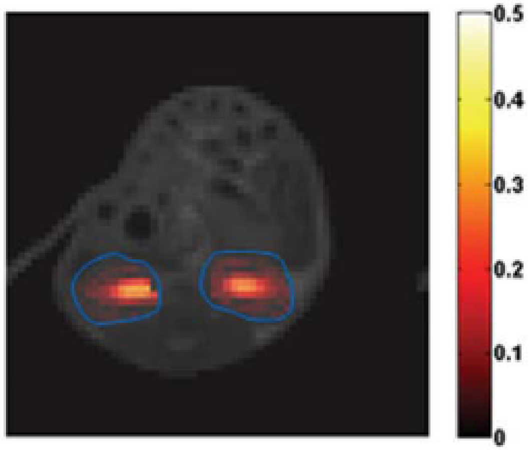


pH dependence of the ratio between the CEST effect of the two amide protons

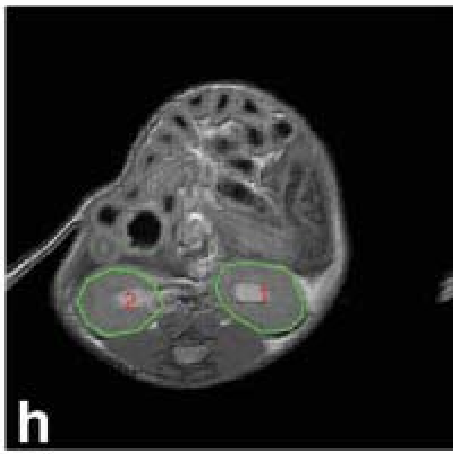
Iopamidol: pH maps of the kidneys



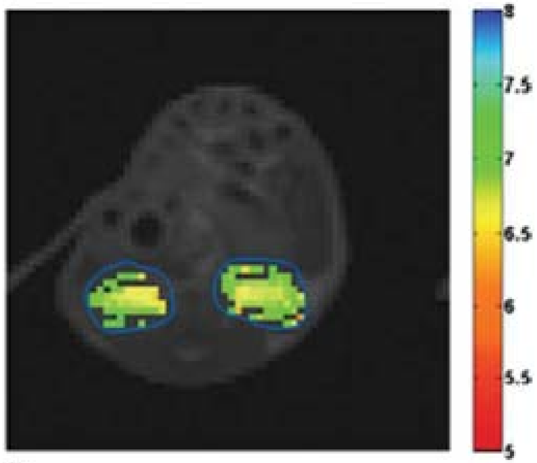
ST% 4.2 ppm



ST% 5.5 ppm



T2w

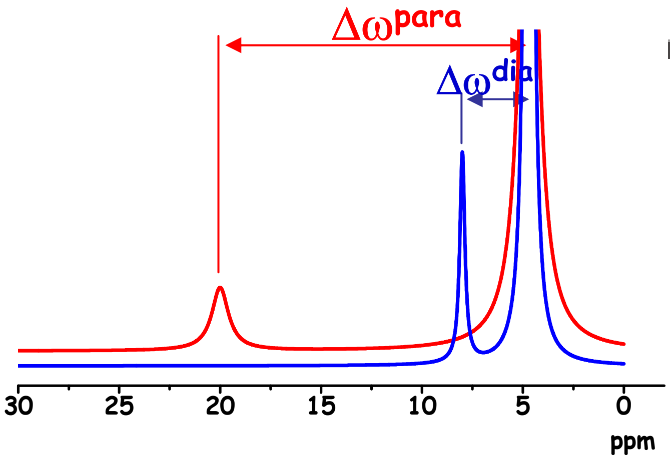


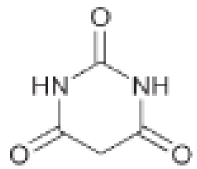
pH map

Longo et al. *Magn Res Med* **2011**, *65*, 202

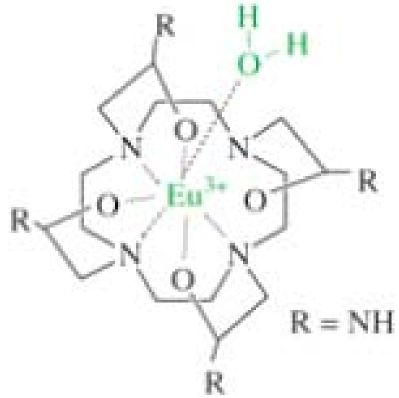
PARACEST agents

	DIACEST	**PARACEST**
Size:	small diamagnetic molecules	paramagnetic lanthanide complexes
Mobile protons:	< 10	< 10
Sensitivity:	mM	mM





Barbituric acid

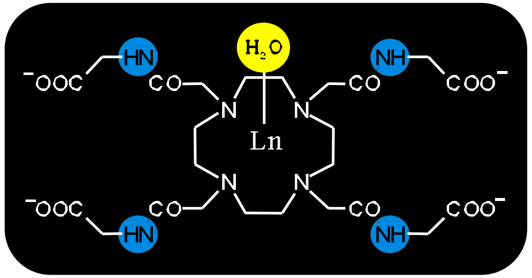


Terreno et al *Angew. Chemie Int. Ed.* **2005**, 44, 5513

pH responsive PARACEST probe: ratiometric

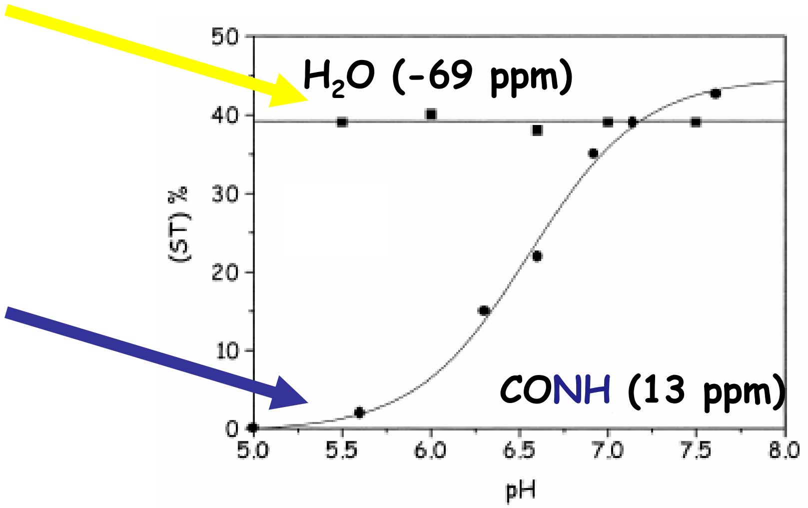
Usually, the two observables are the ST effects measured upon irradiating two magnetically different pools of mobile protons in the same CEST system.

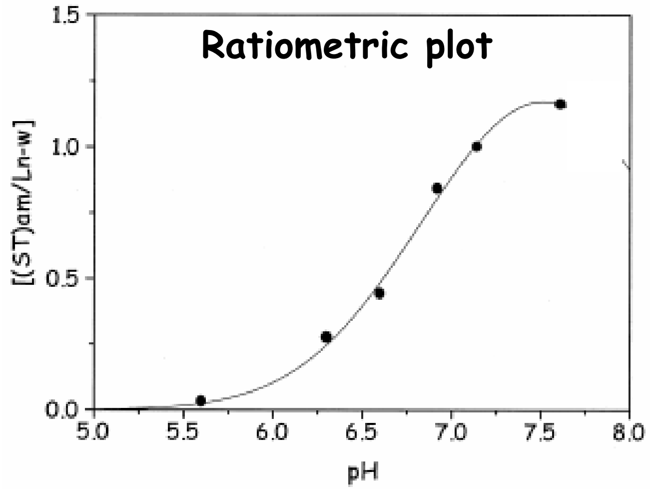
Example: [Ln-DOTAMGly]⁻ complexes as pH responsive probes



The resonance frequency of the two pool is very different and dependent on the Ln(III) ion

Pr(III) complex

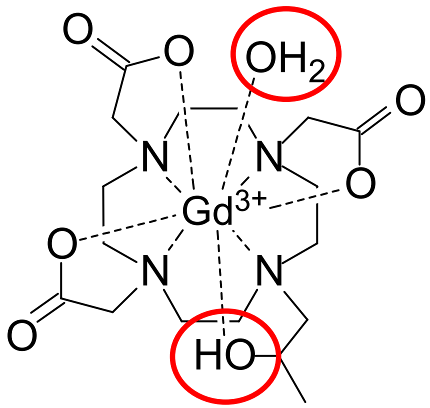




E. Terreno et al *Invest. Radiol.* **2004**, 39, 235.

Yb-HPDO3A: PARACEST pH responsive agent

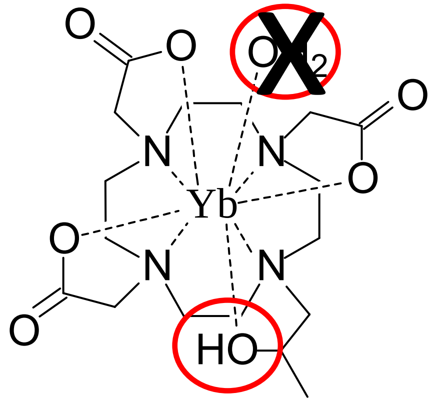
Gd(III)HPDO3A



ProHance

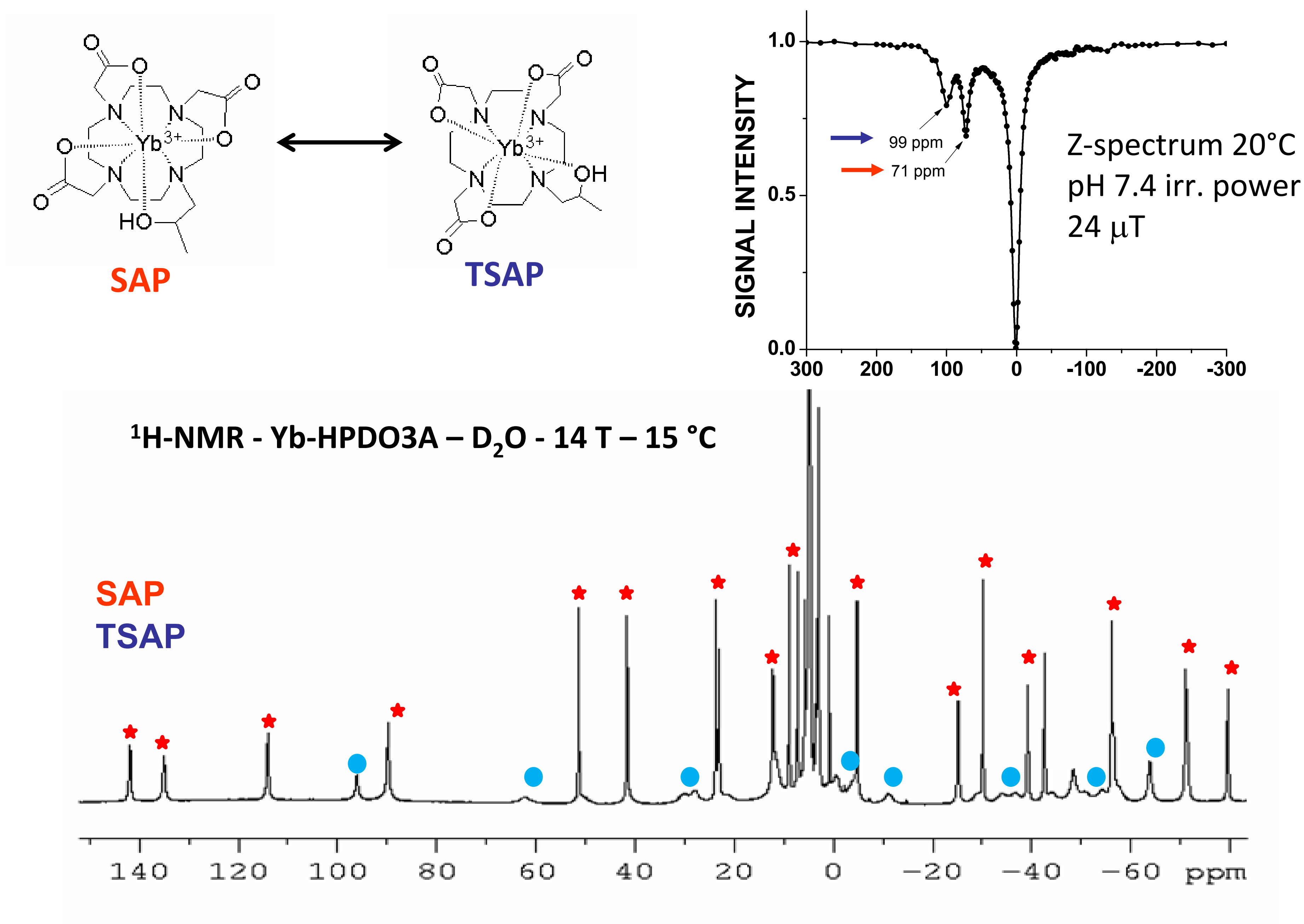
Ln≠Gd

Yb(III)HPDO3A

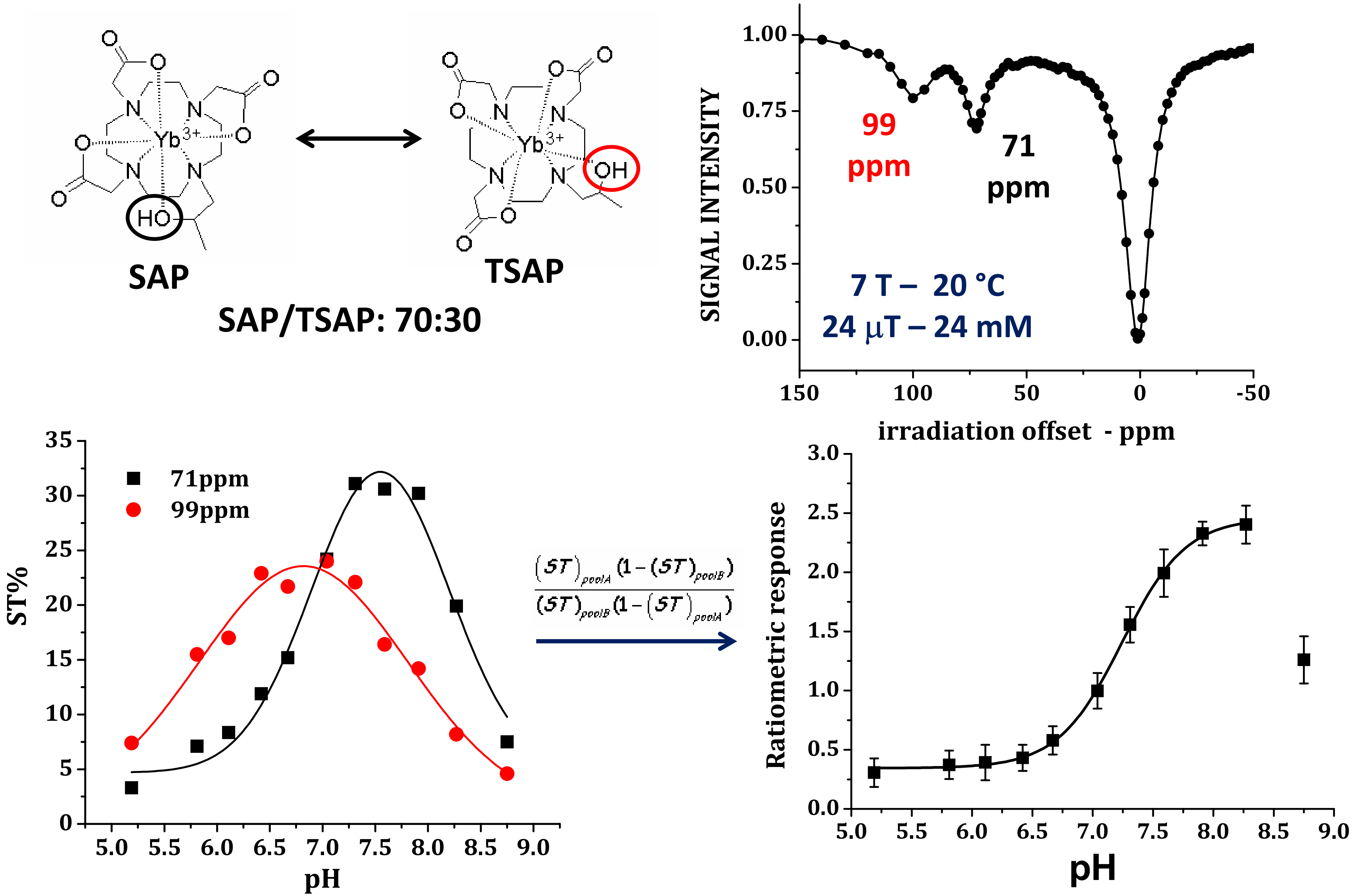


$k_{ex} > \Delta\omega$

	58 Ce	59 Pr	60 Nd	61 Pm	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb
C^D	- 6.3	- 11	- 4.2		- 0.7	+ 4.0	0	- 86	- 100	- 39	+ 33	+ 53	+ 22

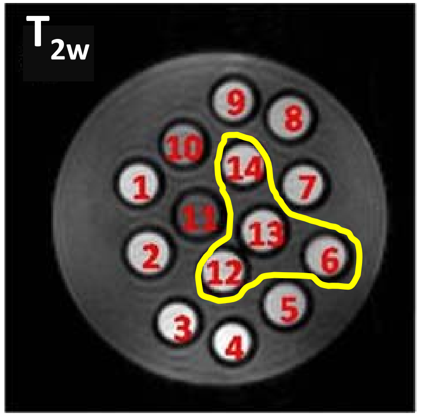


SAP
TSAP
Yb3+
SIGNAL INTENSITY
1.0
0.5
0.0
300 200 100 0 -100 -200 -300
99 ppm
71 ppm
Z-spectrum 20°C
pH 7.4 irr. power
24 μT
1H-NMR - Yb-HPDO3A – D2O - 14 T – 15 °C
SAP
TSAP
140 120 100 80 60 40 20 0 −20 −40 −60 ppm


SAP
TSAP
SAP/TSAP: 70:30
SIGNAL INTENSITY
99 ppm
71 ppm
7 T – 20 °C
24 µT – 24 mM
irradiation offset - ppm
ST%
71ppm
99ppm
pH
(ST)poolA(1-(ST)poolB) / (ST)poolB(1-(ST)poolA)
Ratiometric response
pH

Yb-HPDO3A as concentration-independent pH sensor

7 T - 20°C - Irr time 2 s - Irr. power 24 μT

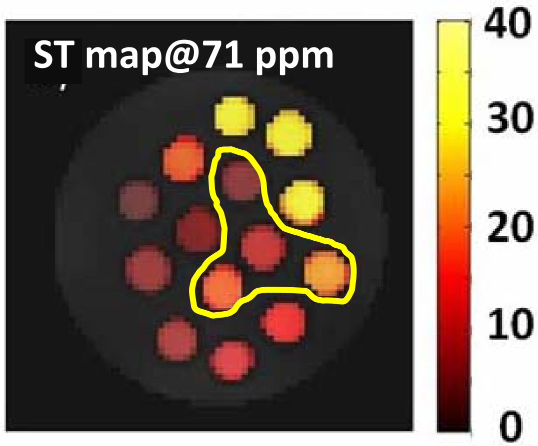


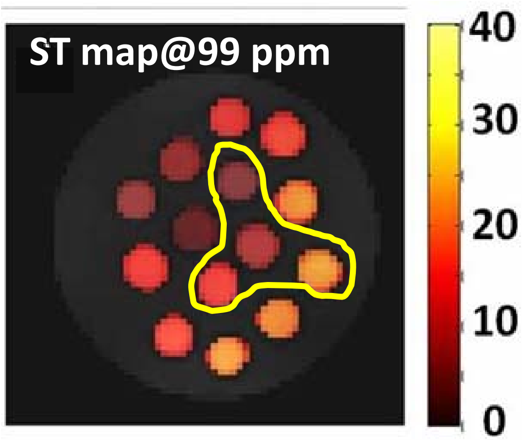
[YbHPDO3A] 24 mM

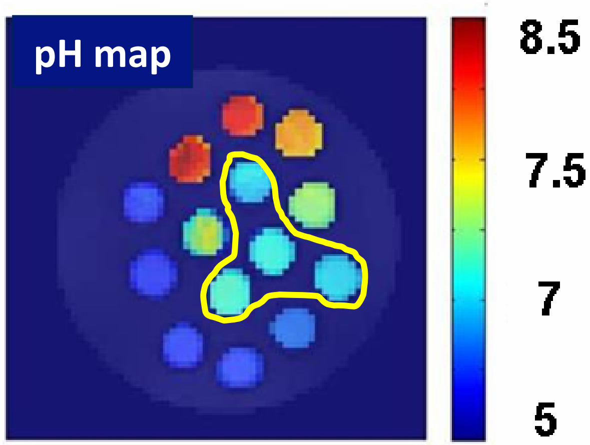
1. pH = 5.2
2. pH = 5.8
3. pH = 6.1
4. pH = 6.4
5. pH = 6.7
6. pH = 7.0
7. pH = 7.3
8. pH = 7.6
9. pH = 7.9
10. pH = 8.3
11. pH = 8.7

pH 7

6. 24 mM
12. 12 mM
13. 6 mM
14. 3 mM







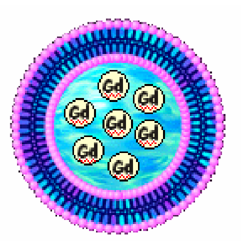
4. Bored by pH measurements?

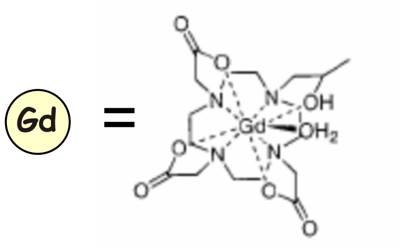
More biochemical parameters to play around with…

Temperature responsive probes

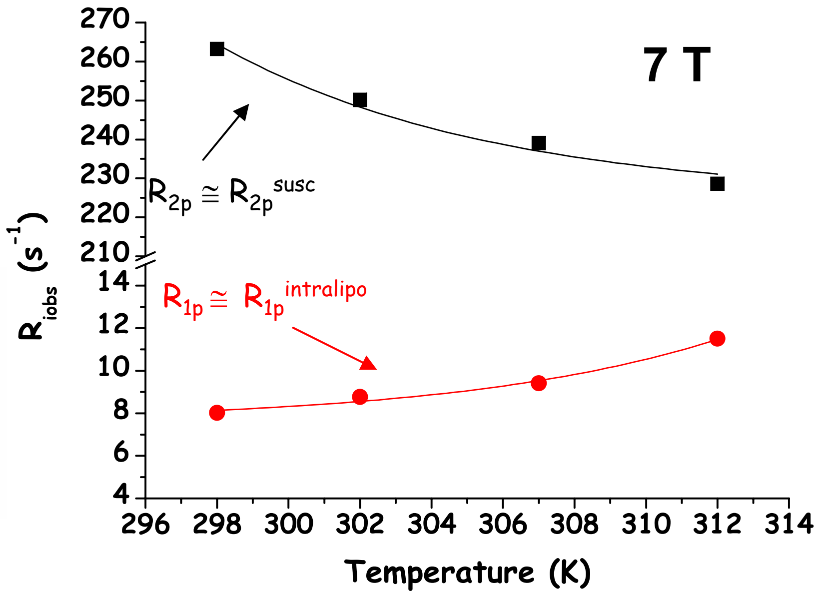
Gd(III)-doped liposomes as ratiometric probes for monitoring Temperature

In the presence of specific T_2-relaxation processes (e.g. magnetic susceptibility), R_{2p}/R_{1p} ratio becomes >> 1 and it can be made responsive to specific parameters.





Gd-HPDO3A



R_2 ↓

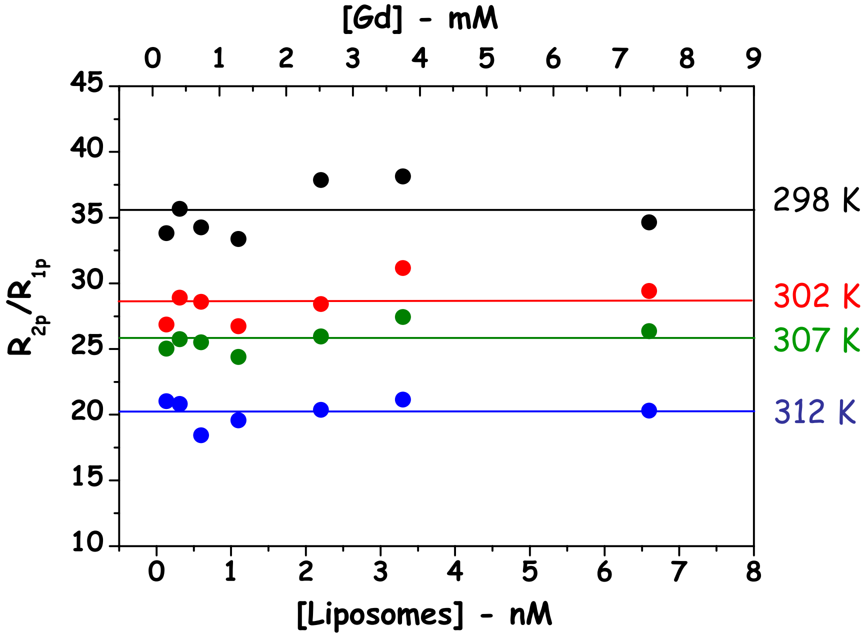
The susceptibility contribution decreases upon increasing temperature

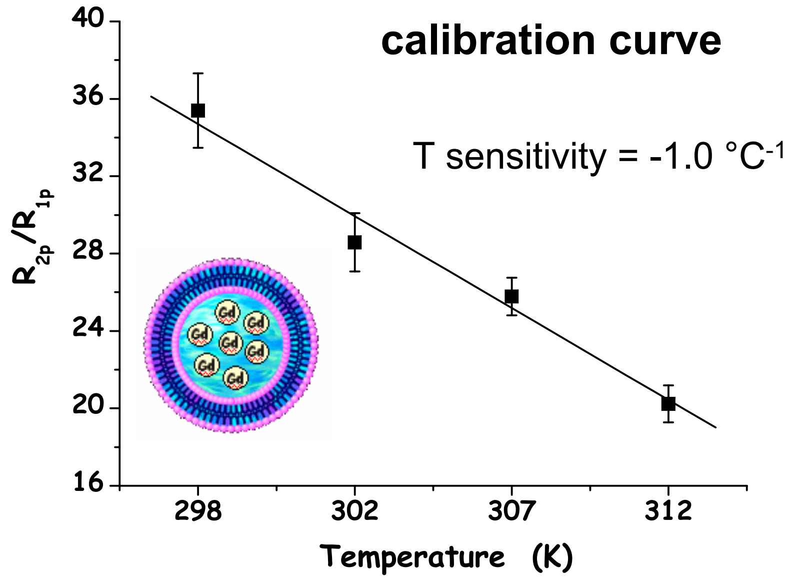
R_1 ↑

The water permeability increases upon increasing temperature

Temperature responsive probes

Ratiometric approach

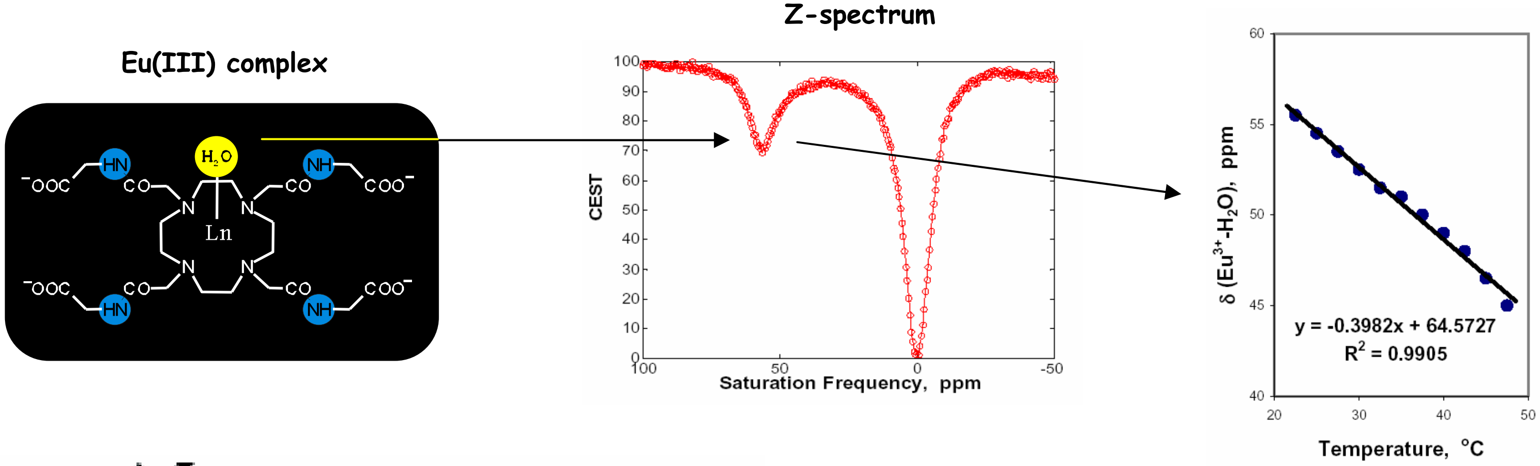


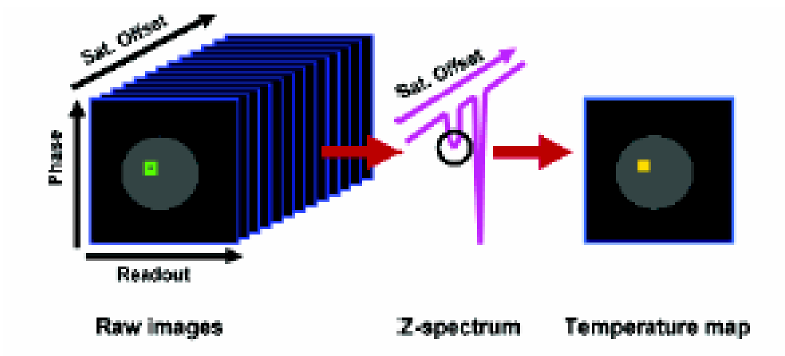


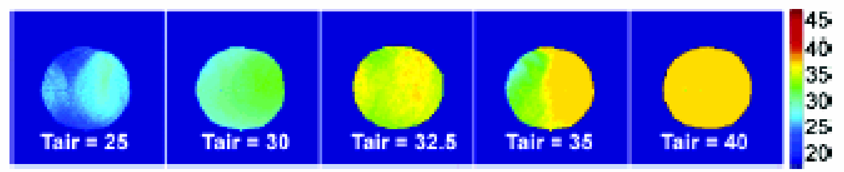
Temperature maps

Temperature responsive PARACEST probes: monitoring a concentration independent observable

Typically, the resonance frequency of an NMR signal is concentration-independent. The “frequency-encoding” of CEST contrast allows the design of concentration-independent probes that do not require the ratiometric analysis.

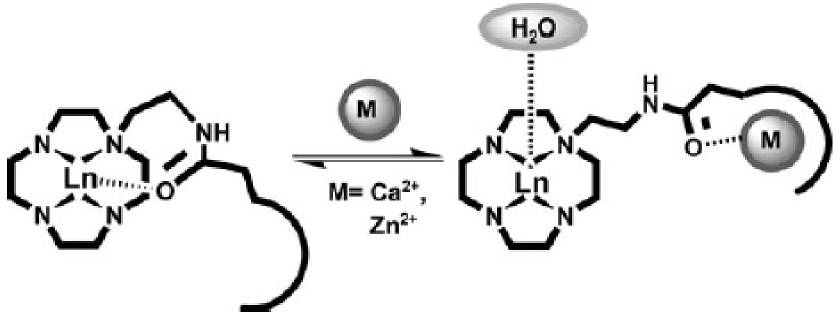


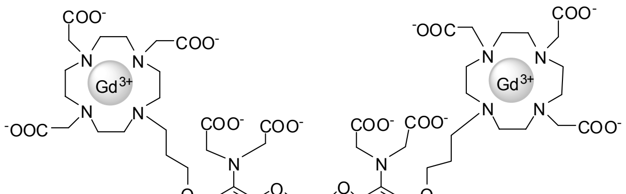




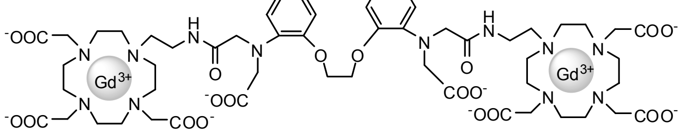
S. Zhang et al., *J. Am. Chem. Soc.* **2005**, 127, 17572.

Metal ion responsive probes: calcium





GdDOPTA **1**



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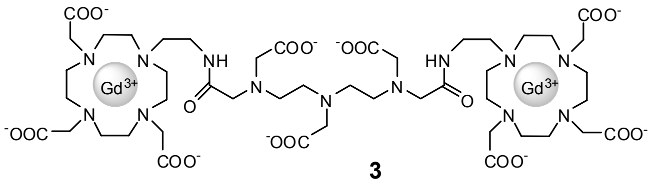

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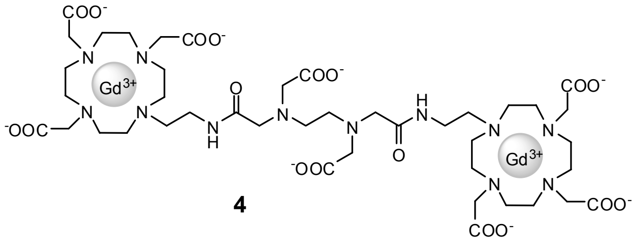

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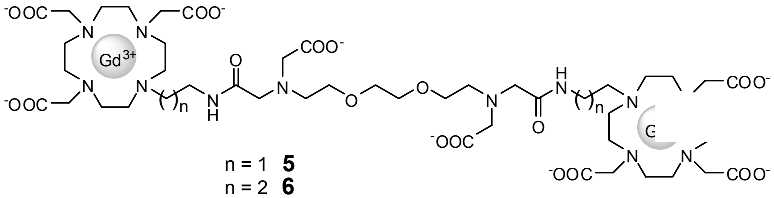
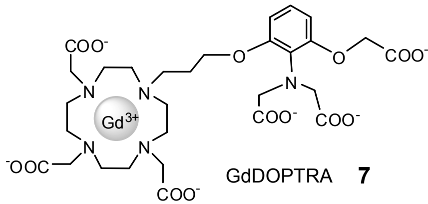
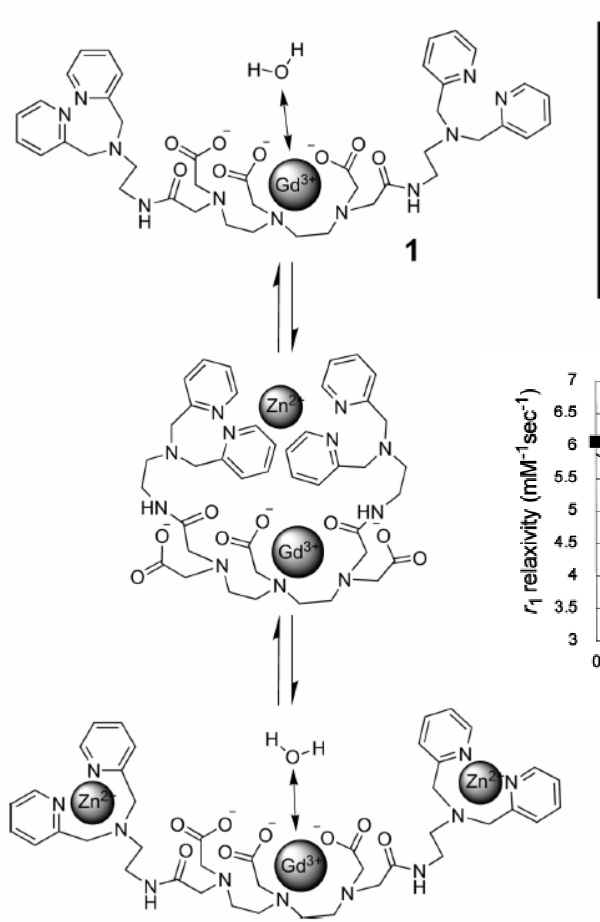


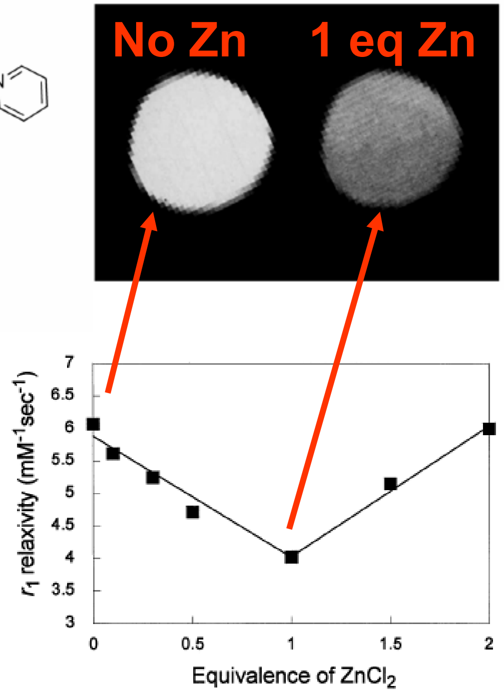
n = 1 **5**
n = 2 **6**

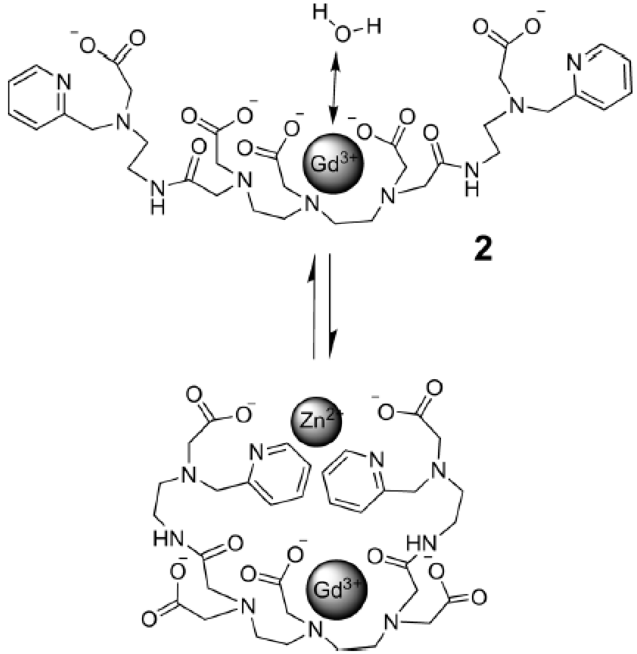


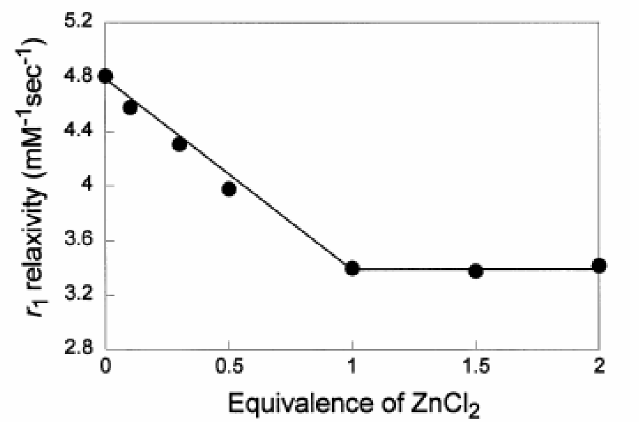
GdDOPTRA **7**

Metal ion responsive probes: zinc





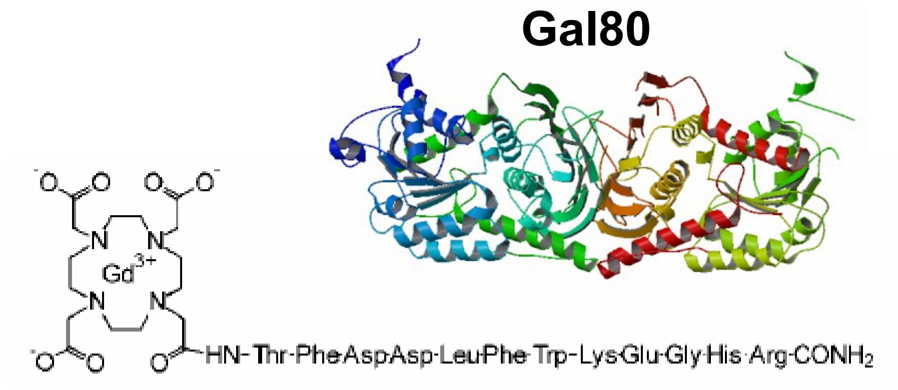




Hanaoka, Chem. Pharm. Bull. **2010**, 58(10) 1283

Protein responsive probes: Gal80

Gd-G80BP



yeast transcription repressor Protein Gal80 (20 MHz, 25 °C, PBS pH 7.4)

- Free form: low relaxivity

$r_1^{mM} = 8.3\ mM^{-1}s^{-1}$

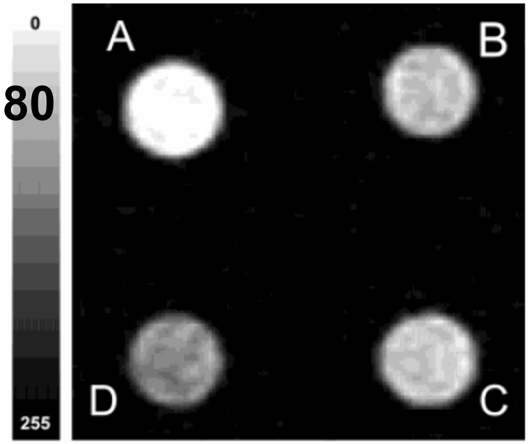
- Bound to Gal80: higher relaxivity

$r_1^{mM} = 44.8\ mM^{-1}s^{-1}$

14 μM Gd-G80BP +

11 μM Gal80

11 μM BSA



Buffer only

No protein

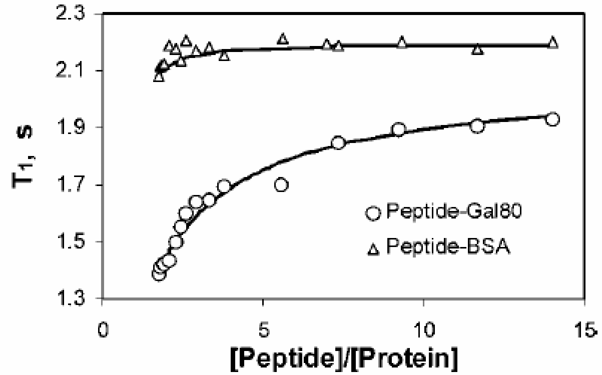


Figure 1. Water proton spin−lattice relaxation times for a 14 μM solution of Gd^{3+}-G80BP upon addition of either BSA (△) or Gal80 (○). In each case, the protein concentration was varied from 1 to 8 μM.

$$\text{Gd-L + Prot} \rightleftharpoons \text{GdL-Prot}$$

$K_a = 5 \times 10^5\ M^{-1}$

Sherry et al. *J Am Chem Soc* **2002**, 124, 3514

Enzyme responsive probes: β-Gal (acting on q/τ_M)

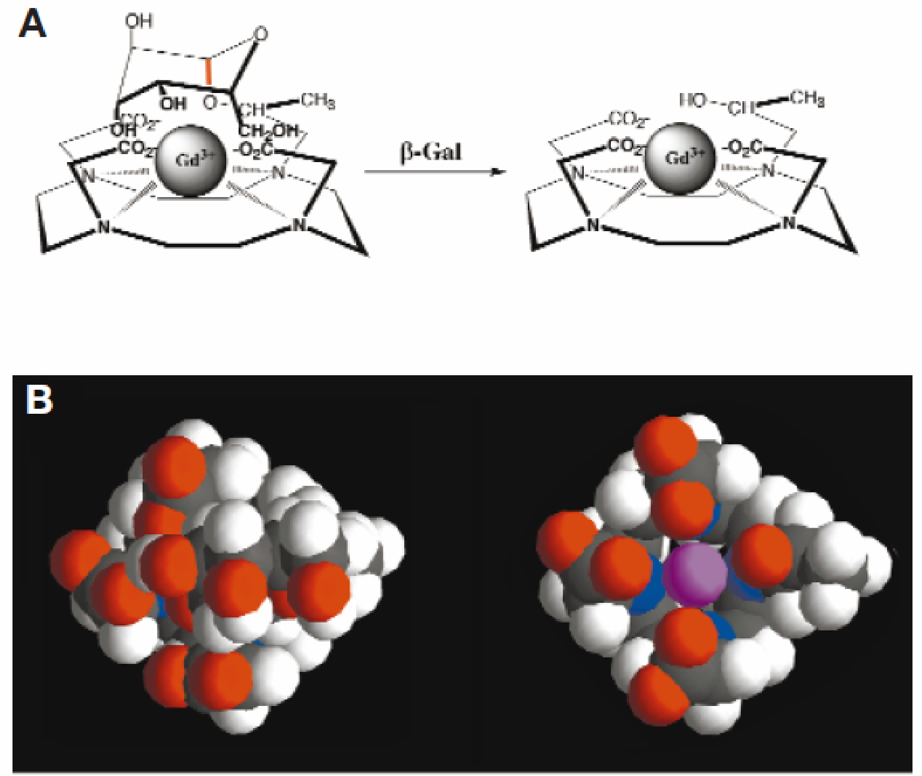


Figure 1. Schematic of the transition of EgadMe from a weak to a strong relaxivity state. (A) Schematic diagram representing the site-specific placement of the galactopyranosyl ring on the tetraazamacrocycle (side view). Upon cleavage of the sugar residue by β-galactosidase (at red bond), an inner sphere coordination site of the Gd^{3+} ion becomes more accessible to water. (B) Space-filling molecular model (top view, from above the sugar residue) of the complex before (left) and after cleavage by the β-gal (right), illustrating the increased accessibility of the Gd^{3+} ion (magenta) upon cleavage: white, H; red, O; blue, N; gray, C.

EgadMe: cleavable by β-Gal

Uncleaved form: low relaxivity
r_1^{mM} = 0.9, q=0

Cleaved form: higher relaxivity
r_1^{mM} = 2.72, q=1

T.J. Meade et al. *Angew. Chemie Int. Ed.*, **1997**, 37, 726

From responsive to molecular probes

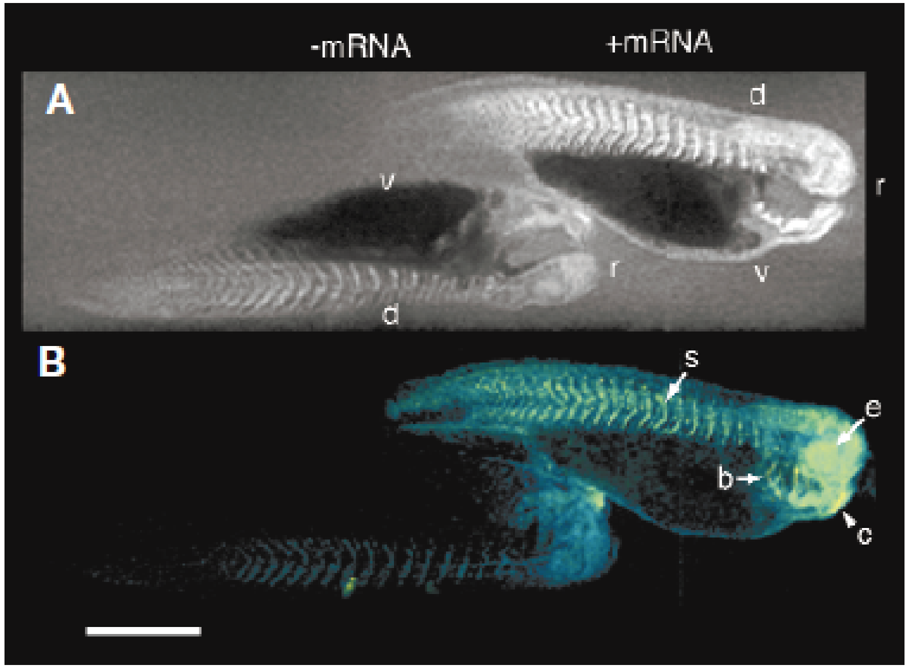


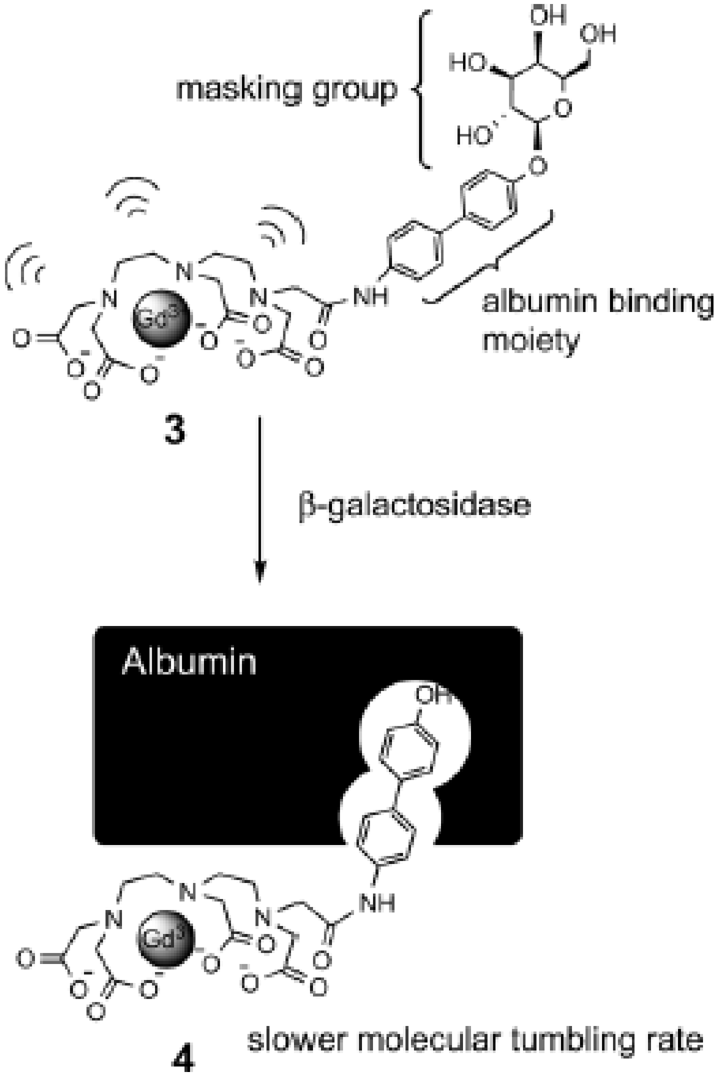
Figure 2. MRI detection of β-galactosidase mRNA expression in living *X. laevis* embryos. MR images of two embryos injected with EgadMe at the two-cell stage. (A) Unenhanced MR image. The embryo on the right was also injected with β-gal mRNA, resulting in the higher intensity regions. The signal strength is 45–65% greater in the embryo on the right containing β-gal (contrast-to-noise ratio ranges from 3.5 to 6). The cement gland has intrinsically short T_1, thus is visible as a bright structure on both embryos. (B) Pseudocolor rendering of same image in (A) with water made transparent. The image correction makes it possible to recognize the eye, and brachial arches in the injected embryo: d, dorsal; v, ventral; r, rostral; e, eye; c, cement gland; s, somite; b, brachial arches. Scale bar = 1 mm.

EgadMe injected with/without mRNA expressing β-Gal (commonly used to detect gene expression)

…promise of in vivo mapping of gene expression in transgenic animals and validate a general approach for constructing a family of MRI contrast agents that respond to biological activity.

Louie et al *Nat. Biotec* **2000**, 18, 321-325

Enzyme responsive probes: β-Gal (acting on τ_R)

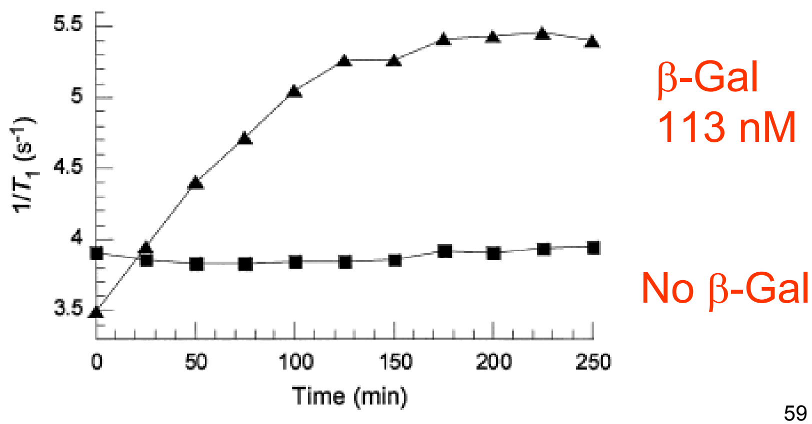


Compound 3: cleavable by β-Gal
r_1^{mM} = 7.0 $mM^{-1}s^{-1}$
@ 20 MHz, PBS pH 7.4, 37 °C

Compound 4: cleaved form of compound 3
r_1^{mM} similar to parent compound

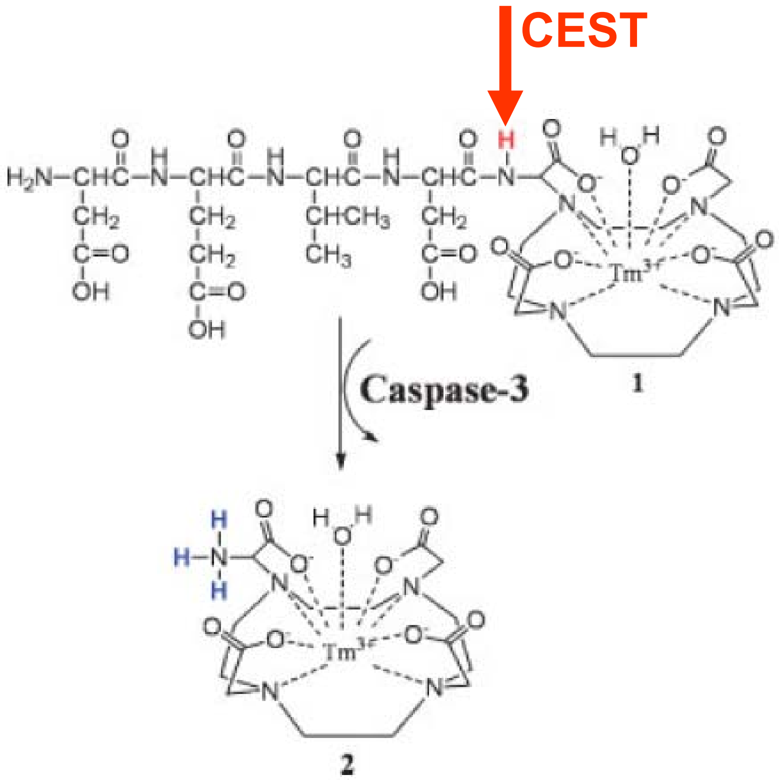
Compound 4, albumin bound:
r_1^{mM} = 11.0 $mM^{-1}s^{-1}$

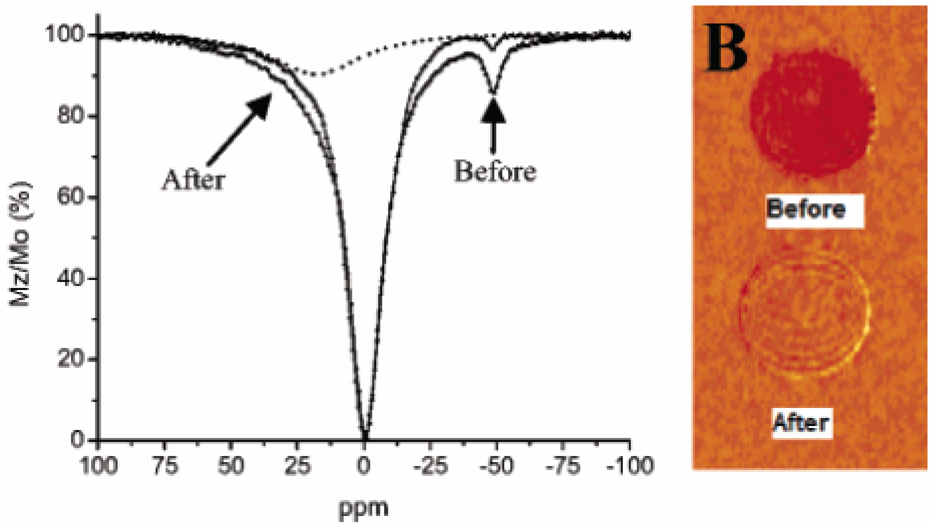
Remark: albumin and β-Gal must be in the same biological compartment…



Hanaoka, Chem. Pharm. Bull. **2010**, 58(10) 1283

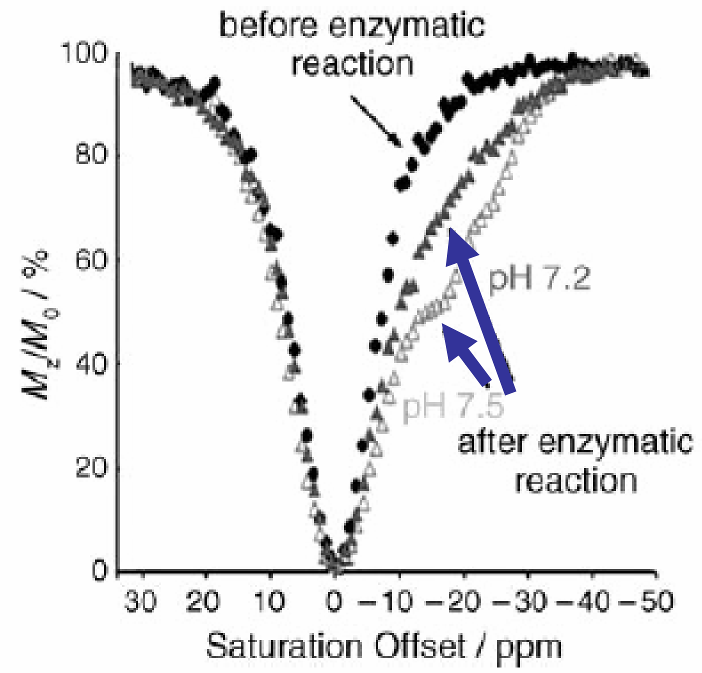
Responsive CEST-MRI agent for caspase-3

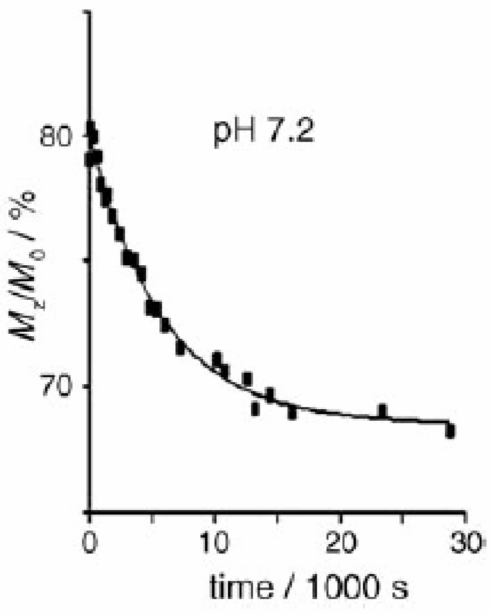


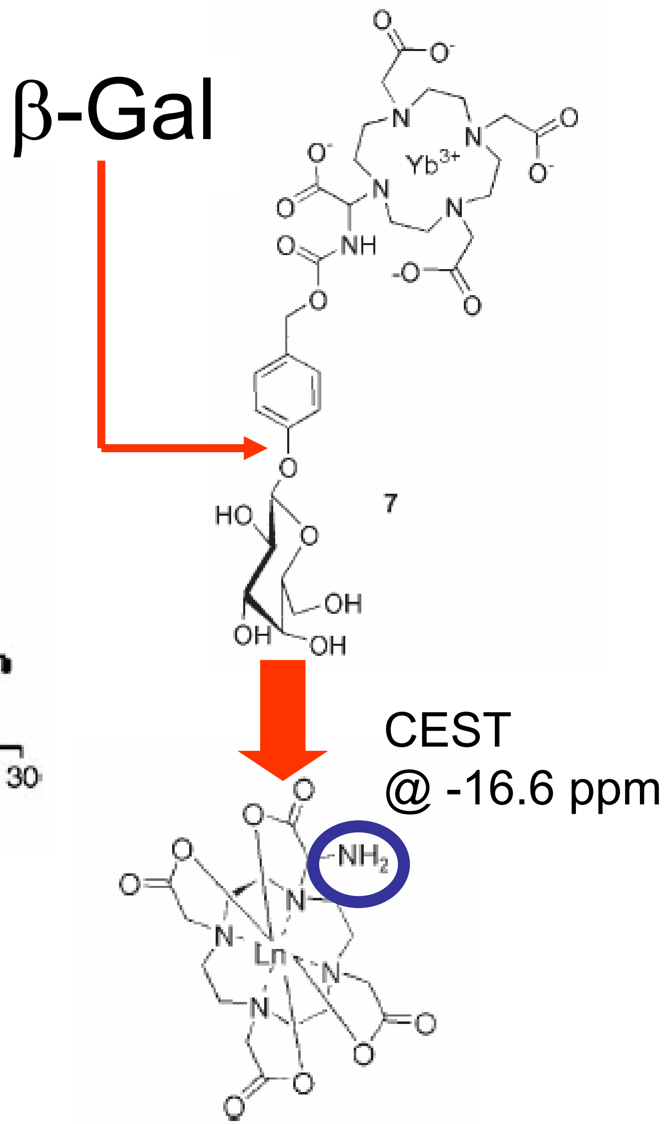


Yoo & Pagel *J. Am. Chem. Soc.* **2006**, 128, 14032

„Self-immolative“ CEST-MRI agents



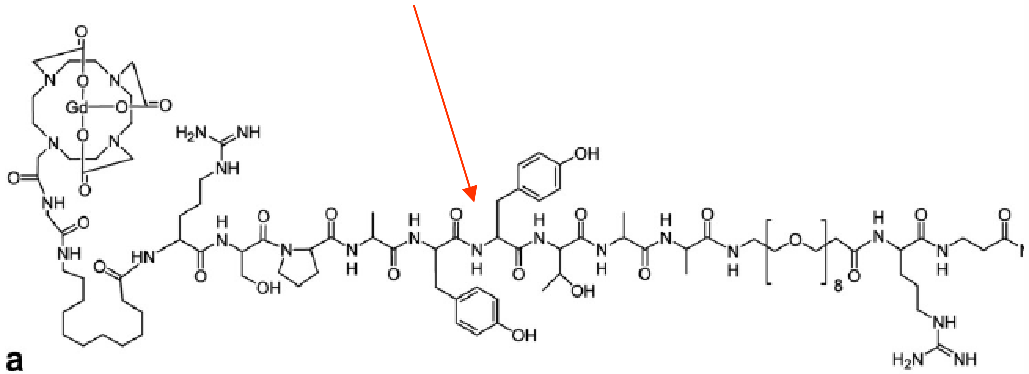




Toth et al. *Angew. Chem. Int. Ed.* **2008**, 47, 4370

Solubility switchable MRI agent for MMP-2

-The probe is a substrate specific for Matrix Metalloproteinase 2 (MMP-2))
MMP-2 is extracellular !
-After cleavage, an “aggregating” moiety is produced
-Pharmacokinetics of the cleaved/uncleaved probe are different



a

WT: MMP-2^+
KD: MMP-2^-

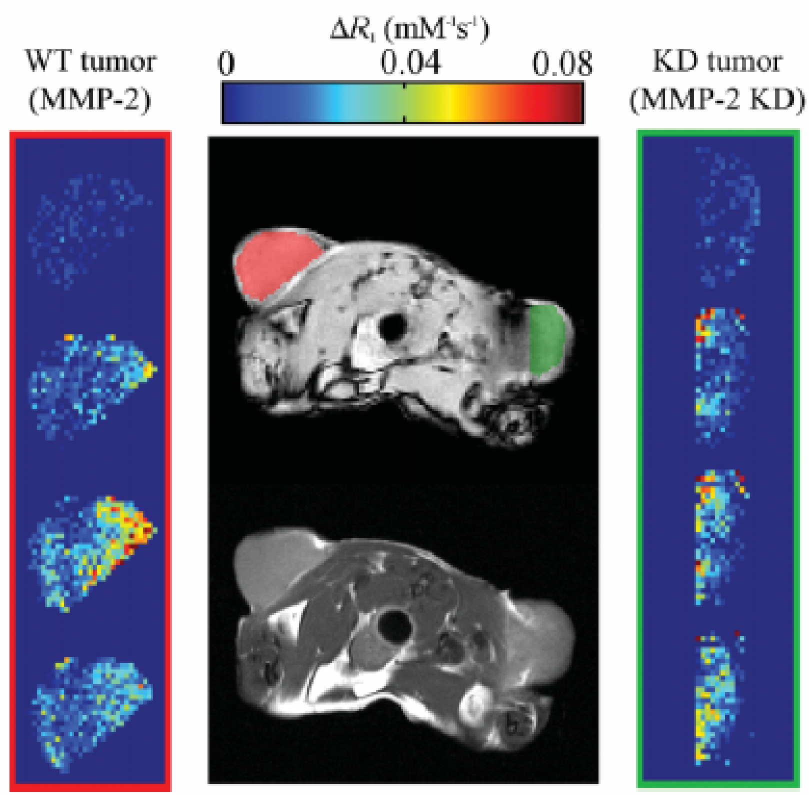


FIG. 4. T_1-weighted image (middle top) after injection of PCA2-switch and the corresponding preinjection T_2-weighted (middle bottom) axial image for one slice of one animal. ROIs were drawn on the WT tumor (left, red overlay) and the KD tumor (right, green overlay) of each animal, carefully avoiding regions where partial volume artifacts were observed on either T_1-weighted or T_2-weighted images. The left and right columns are composed of relative ΔR_1 maps for the WT and the KD tumors, respectively, at four different time points (0, 16, 42, and 76 min). For both tumors, the relative ΔR_1 increases and is eventually washed out.

Lebel et al *Magn Reson Med* **2008**, 60, 1056

Solubility switchable MRI agent for MMP-2

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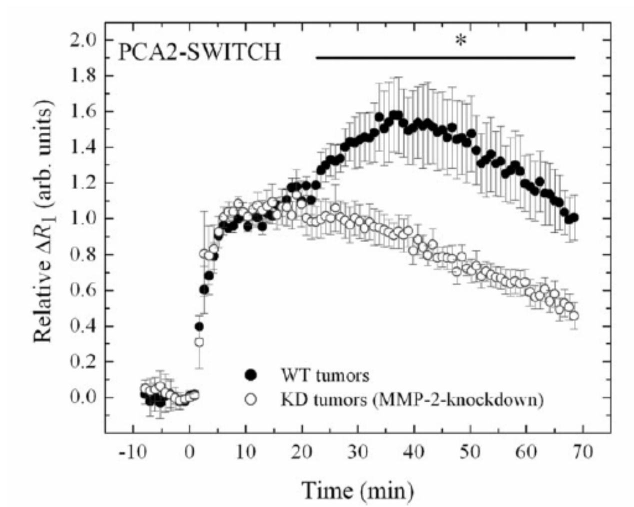
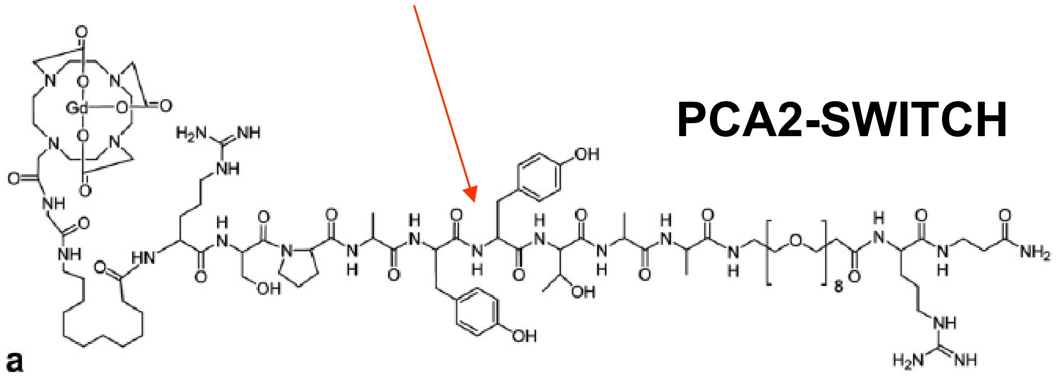
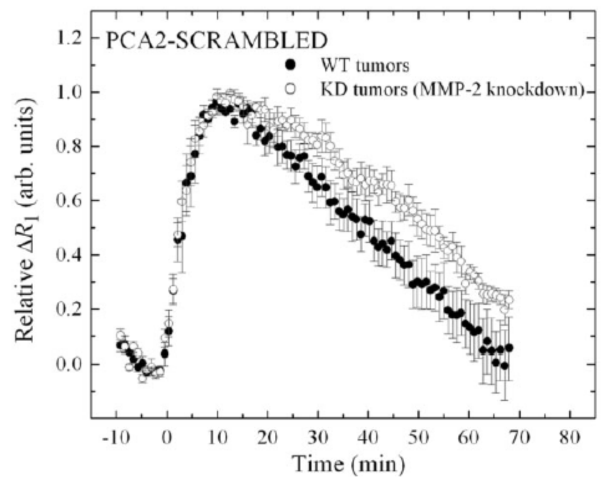




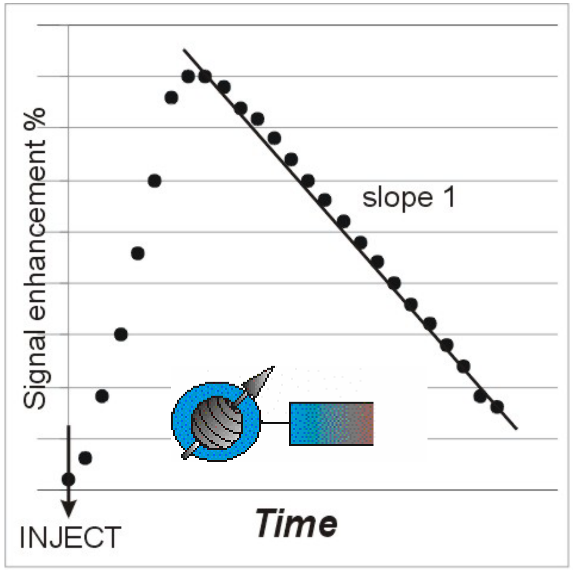


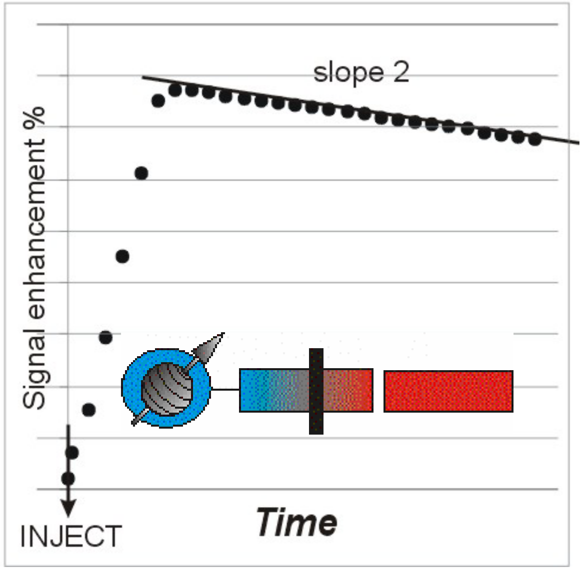
WT: MMP-2^{+}
KD: MMP-2^{-}

Lebel et al *Magn Reson Med* **2008**, 60, 1056

MMP responsive MRI agent for MMPs (another version)

MMP-sensitive probe: amphiphilic molecule (Gd-peptide) turned into a hydrophylic fragment upon MMP dependent cleavage, with change in wash-out kinetics from tissue





HIGH MMP ACTIVITY

- The probe is cleaved into a more hydorphilic fragment
- Weak interactions with the ECM components
- **FAST** wash-out expected

LOW MMP ACTIVITY (INHIBITED)

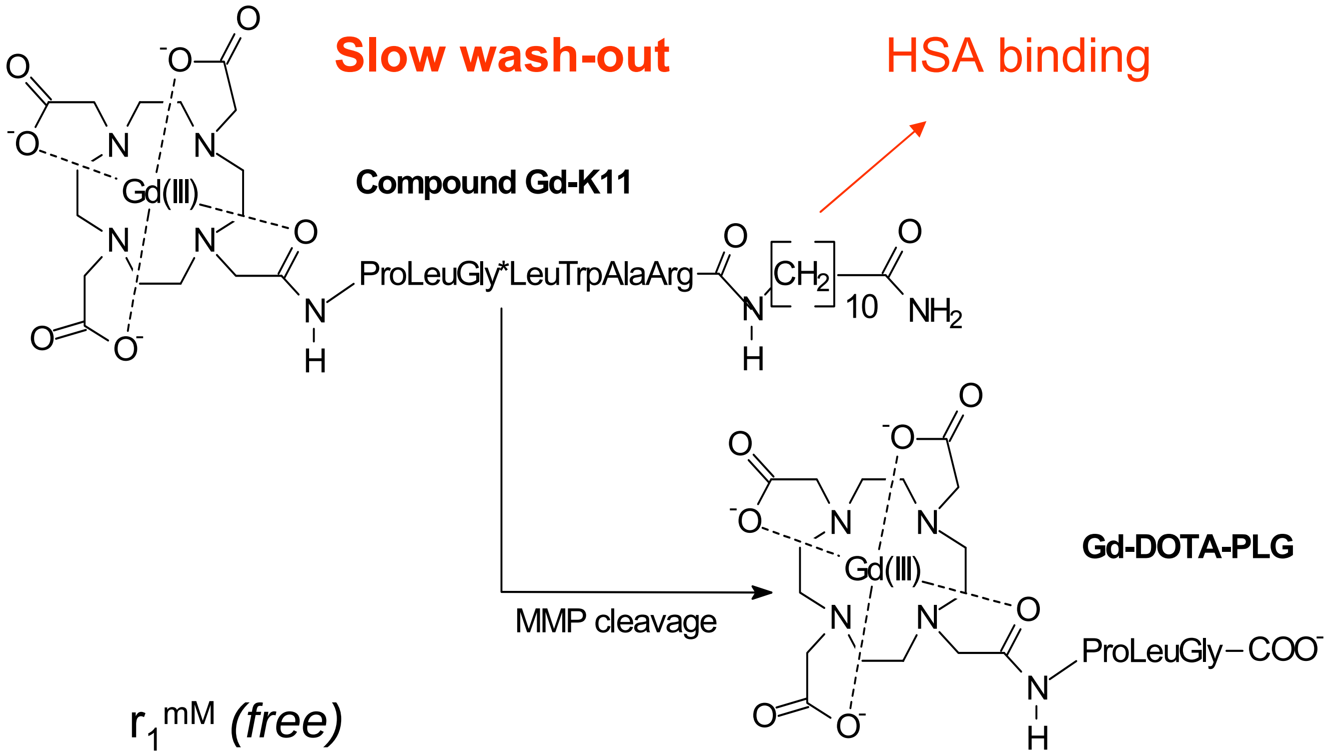
- The probe is not cleaved
- Strong interactions with the ECM components
- **SLOW** wash-out expected

MMP responsive MRI agent for MMPs (another version)

r_1^{mM} *(free)*
= *8.5* $mM^{-1}s^{-1}$

r_1^{mM} *(albumin bound)*
= *16.3* $mM^{-1}s^{-1}$

HSA binding:
K_D=350±30 μM



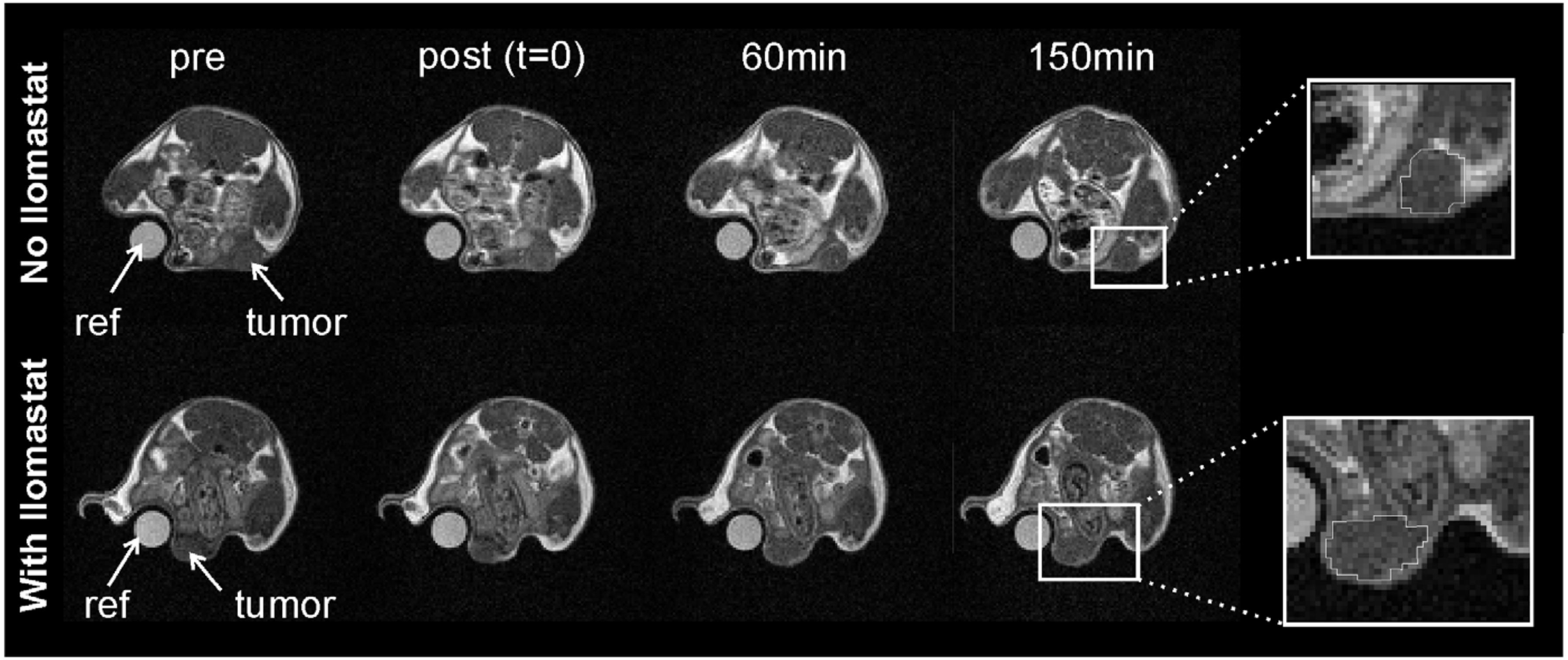
r_1^{mM} *(free)*
= *6.1* $mM^{-1}s^{-1}$

Fast wash-out

Decrease of contrast in T_1w MR images expected as a result of faster wash-out and lower relaxivity of the cleaved probe

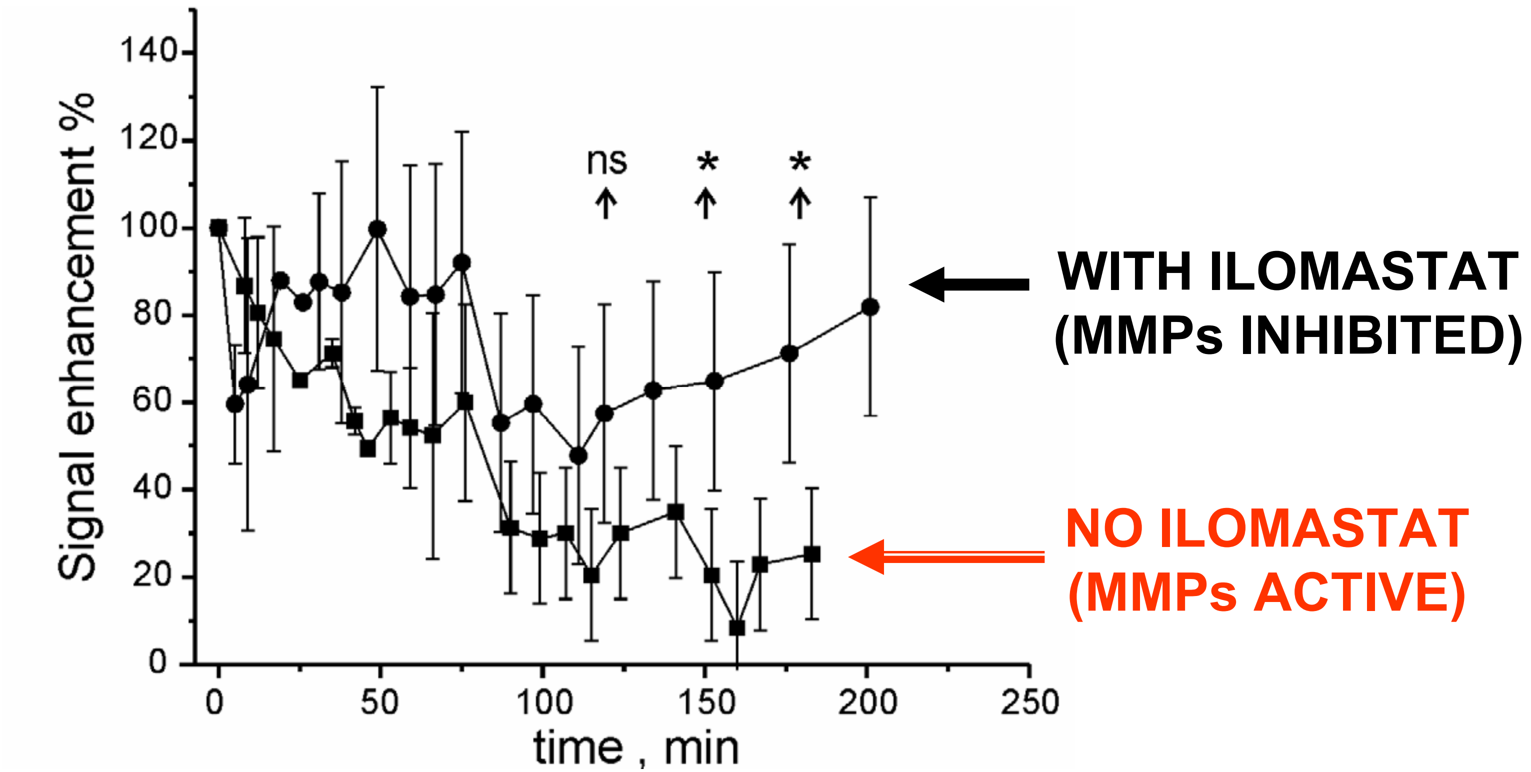
MMP responsive MRI agent for MMPs (another version)

Pre-contrast and post-contrast (Gd-K11 at 0.03 mg/kg) T_{1w}-SE axial images at 1 T (M2 ASPECT) of mice subcutaneously grafted with a B16 melanoma.



MMP responsive MRI agent for MMPs (another version)

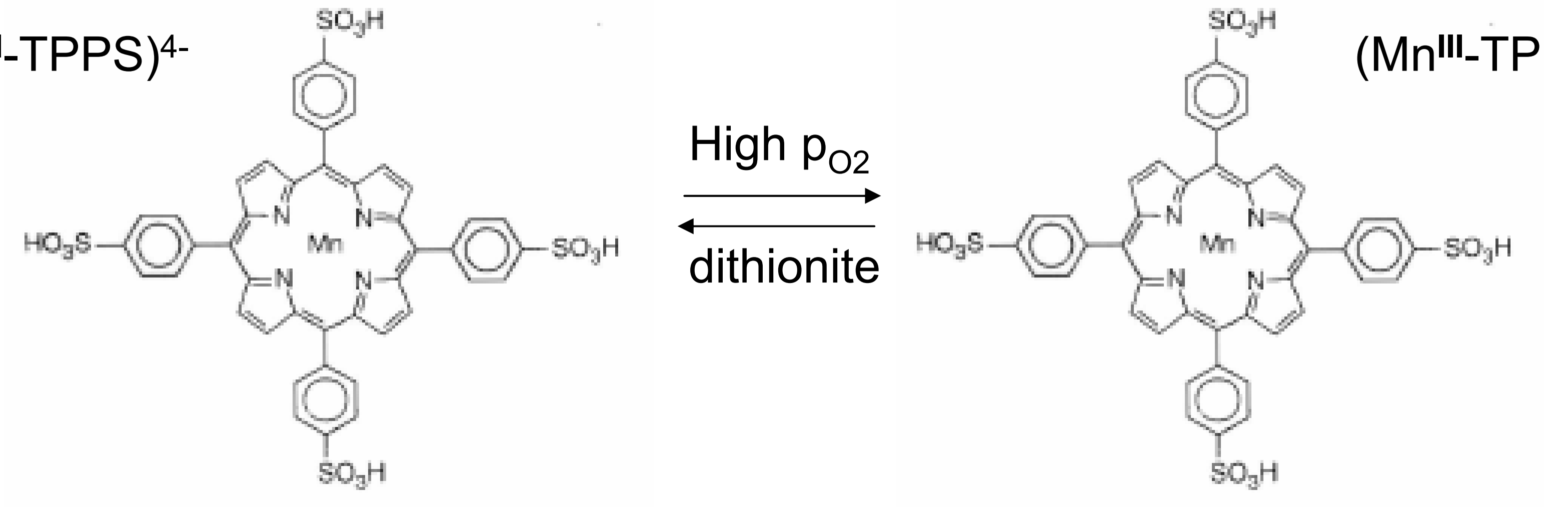
Time course of signal enhancement in tumor after iv administration of the MMP-responsive Gd-complex (at 0.03 mg/kg) with/without treatment of the tumor with the MMP inhibitor Ilomastat



Gringeri et al. *Contrast Media & Mol Imaging* **2011**, 10.1002/cmmi.478

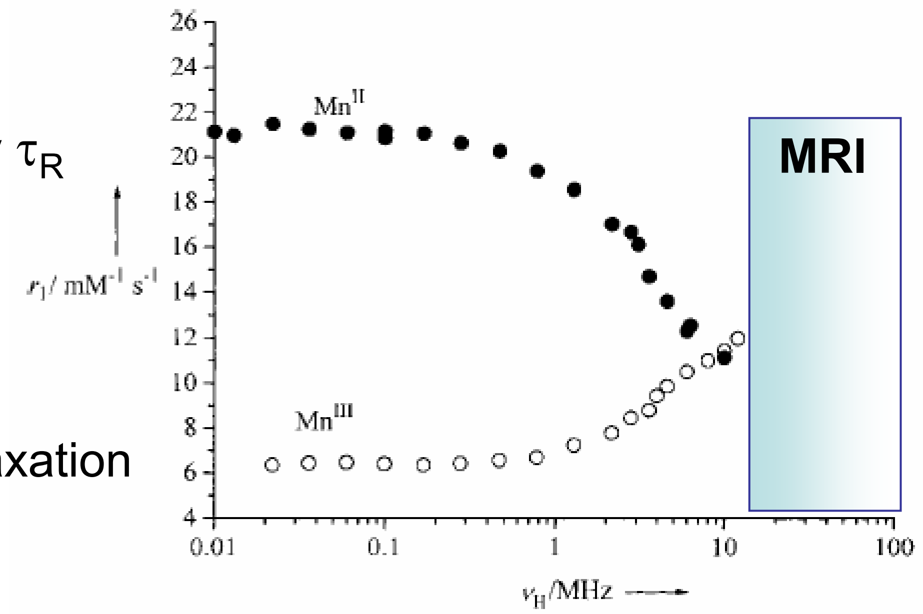
Redox responsive probes:Mn(II)/Mn(III)

$(Mn^{\mathbf{II}}\text{-TPPS})^{4-}$ $(Mn^{\mathbf{III}}\text{-TPPS})^{3-}$



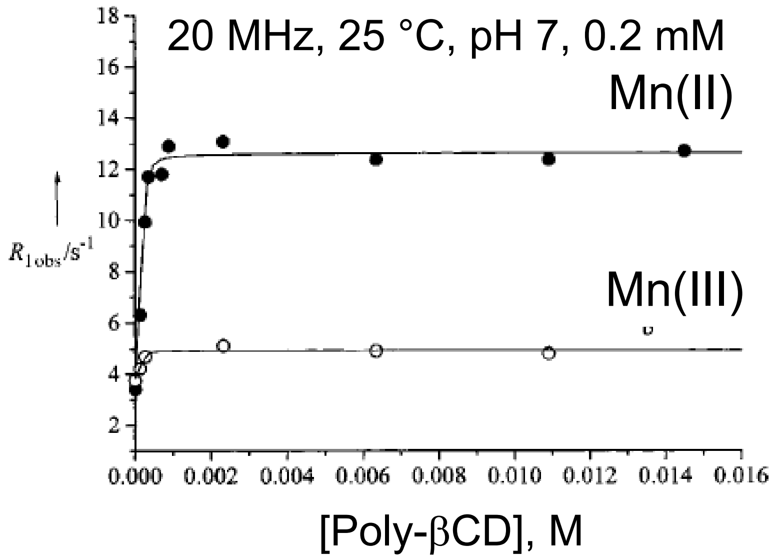
Relaxivity determined by τ_R

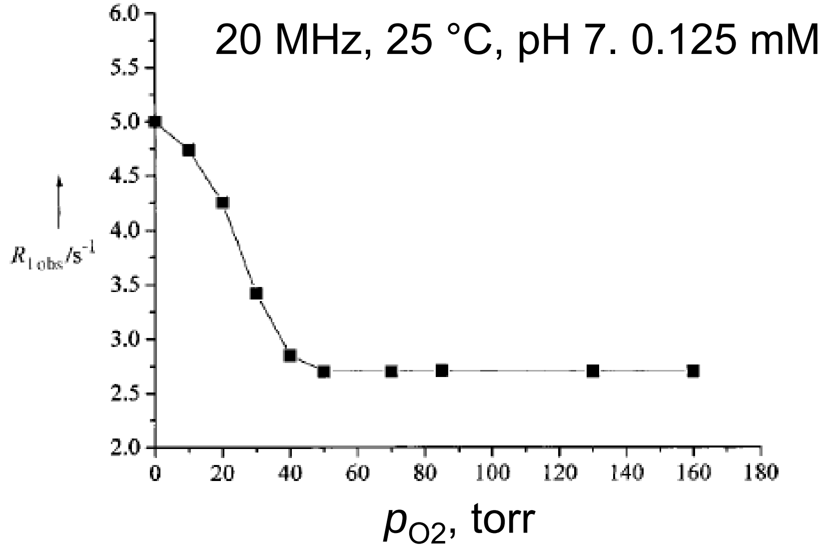
Relaxivity determined by electronic relaxation

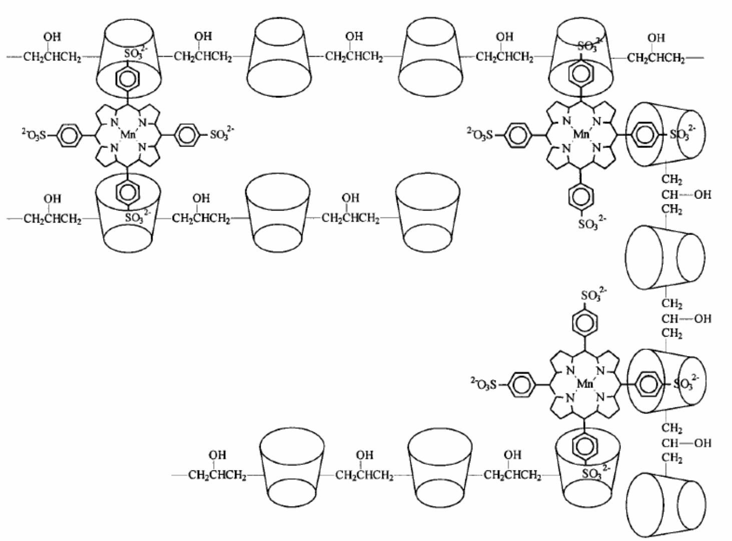


Small difference of relaxivity at clinically relevant fields !

Redox responsive probes:Mn(II)/Mn(III)



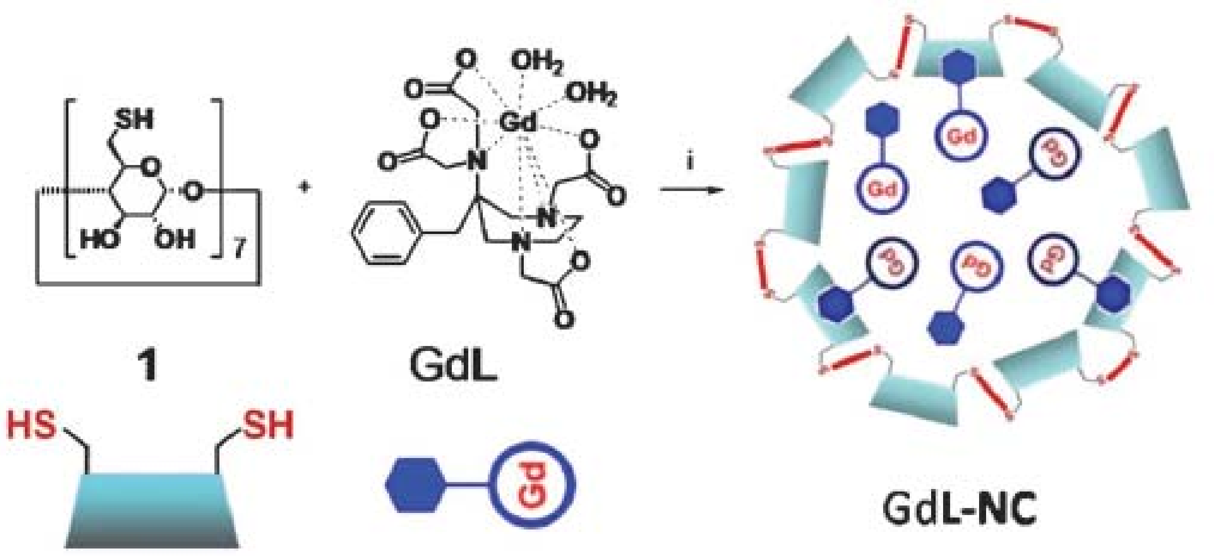




A larger difference of relaxivity (at clinically relevant fields) between the +3 and +2 oxidation states can be reached by exploiting the macromolecule effect, affecting Mn(II)-TPPS but not Mn(III)-TPPS.

Aime et al *Angew. C hem.* **2000**, 112.

Redox responsive probes: thiol/disulphide



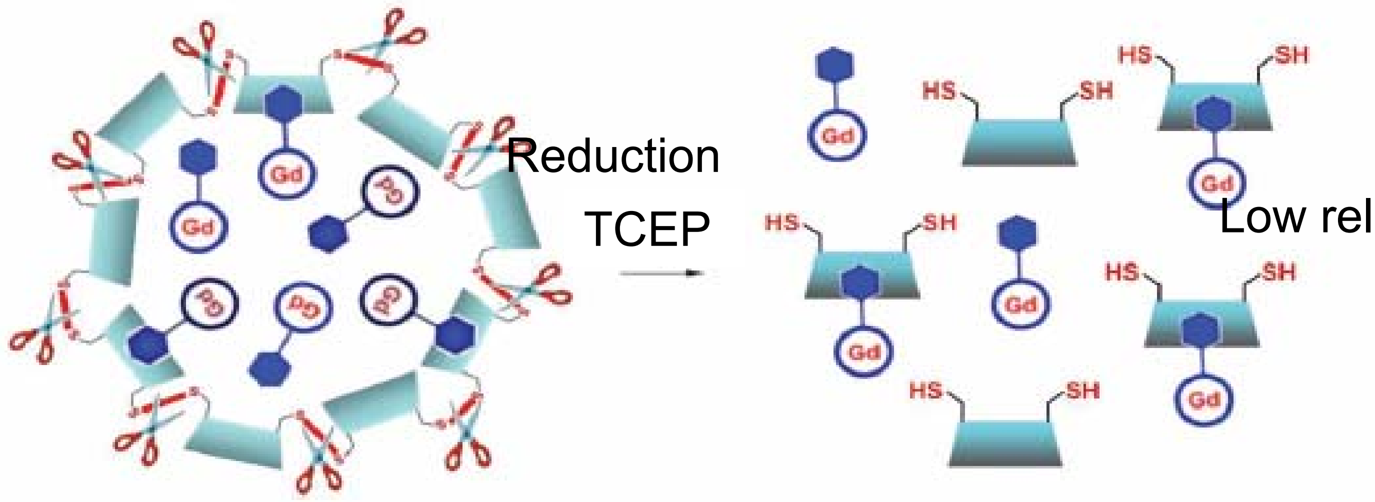
Gd-loaded nanocapsule

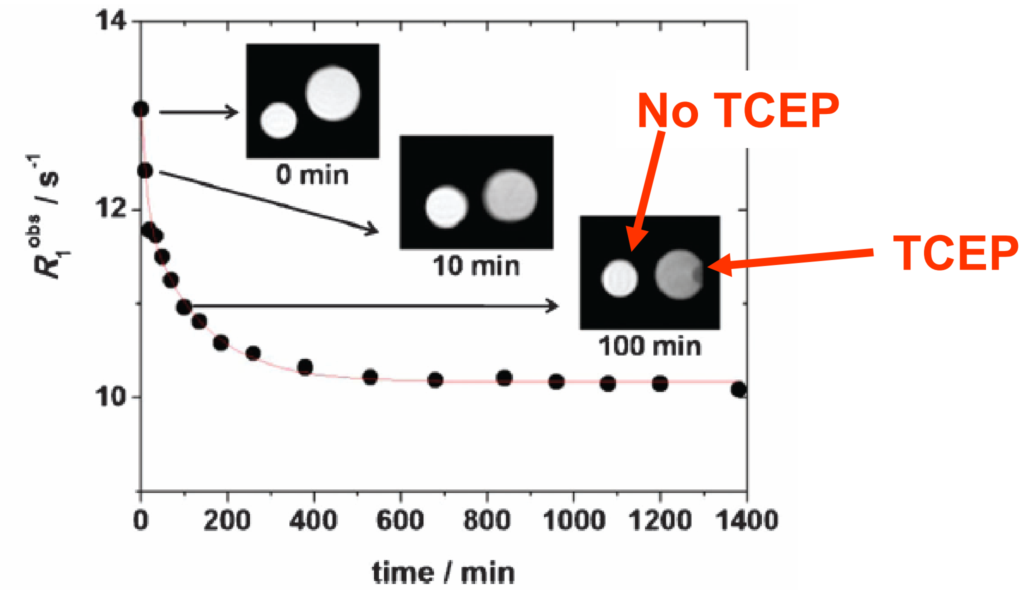
	Size[a]/nm	[Gd][b]/mmol g^{-1}	r_{1p}[c]/mM^{-1} s^{-1}	
			25 °C	37 °C
NC	233 ± 2	0	N/A[d]	N/A[d]
GdL-NC	238 ± 6	0.14	20.7	19.3
GdL	—	—	9.0	7.3

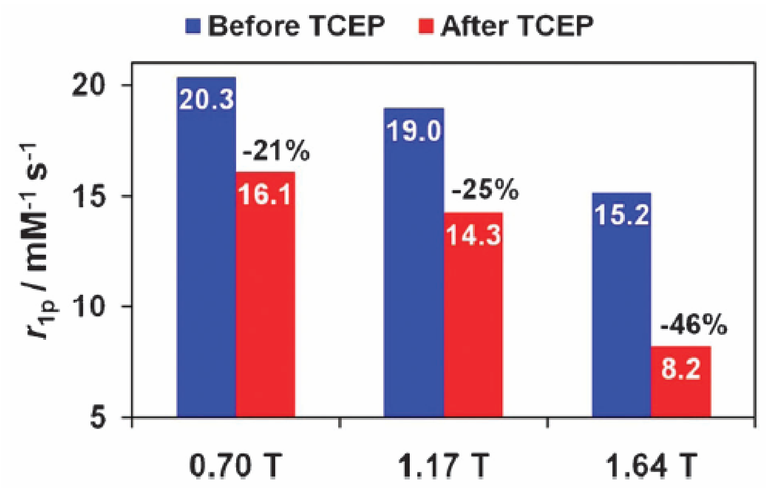
[a] By DLS analysis. [b] By ICP-MS analysis. [c] At 0.47 T. [d] R_1 of a solution of empty capsules (4.6 mg mL^{-1}) is 0.38 s^{-1} at 25 °C and 0.28 s^{-1} at 37 °C.

Redox responsive probes: thiol/disulphide

High relaxivity

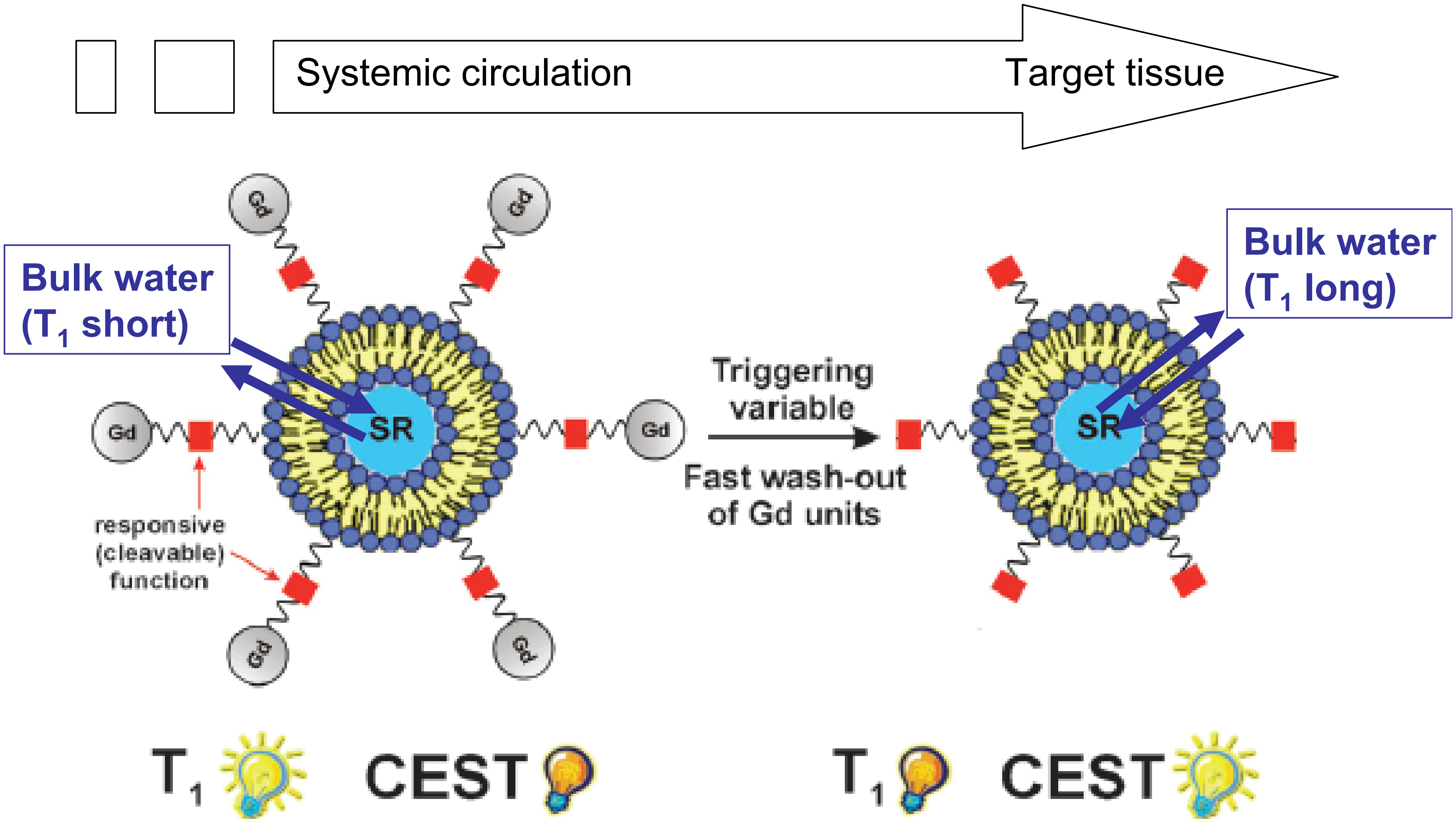






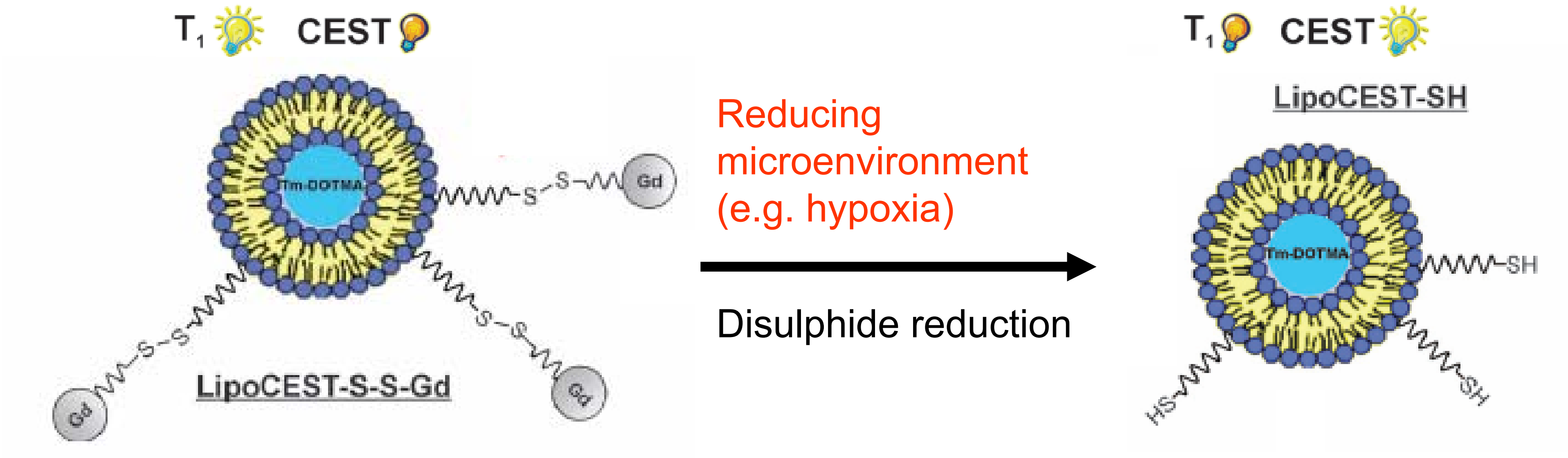
Martinelli et al *Chem. Commun.* **2011**, 47, 3144

Redox responsive probes: T_1 / CEST dual agent

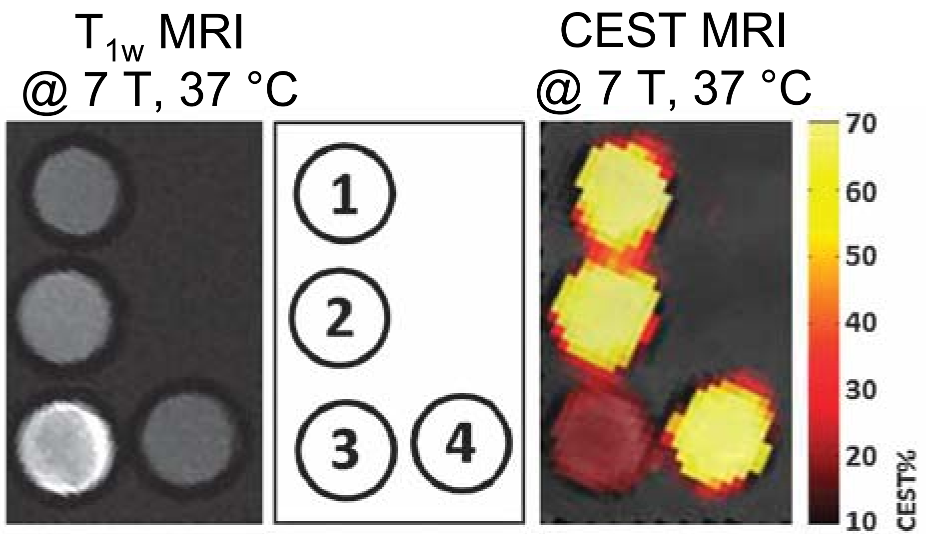


Terreno et al *Chem. Commun.* **2011**, 47, 4667

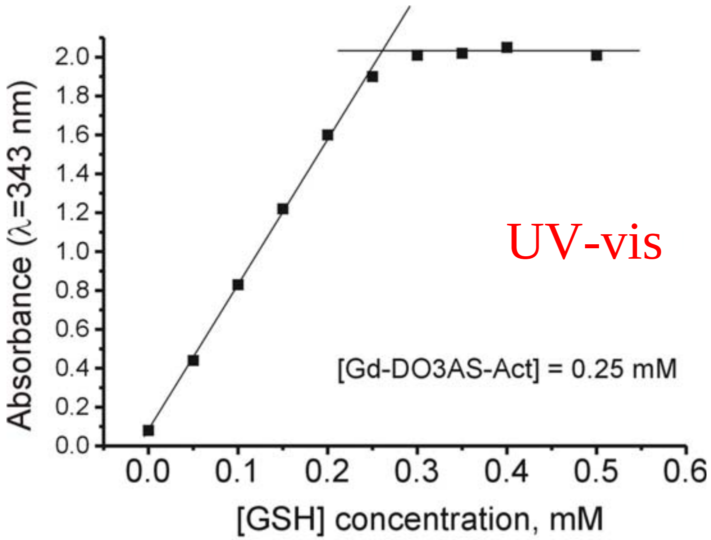
Redox responsive probes: T_1 / CEST dual agent
Thiol/disulfide redox

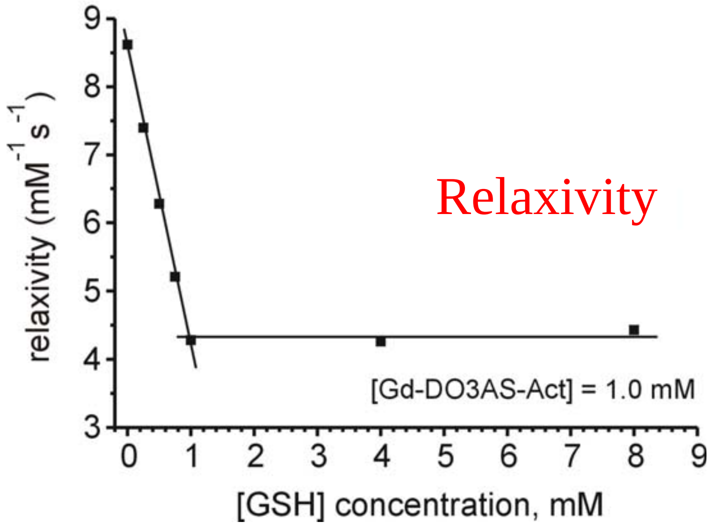


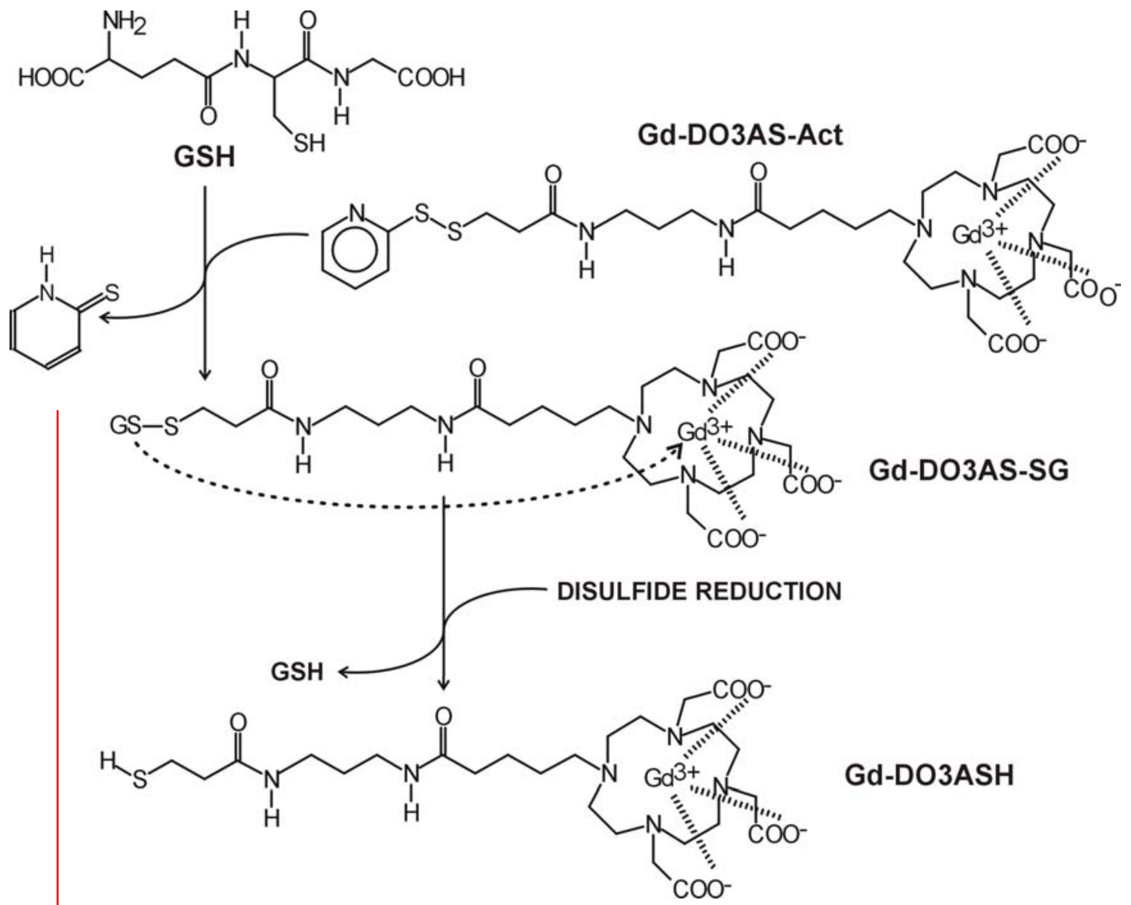
1. Not relevant…
2. LipoCEST-SH
3. LipoCEST-SS-Gd
4. LipoCEST-SS-Gd after reduction of the disulfide and wash-out of LMW Gd complex



Thiol-responsive agents: reduced glutathione



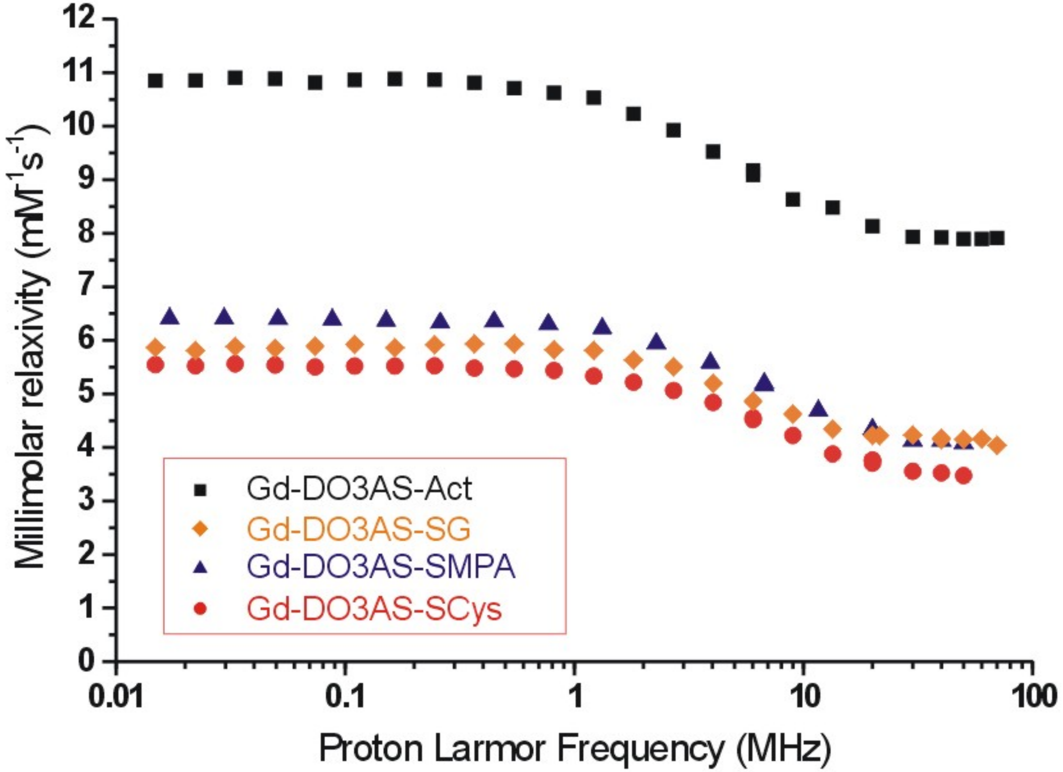


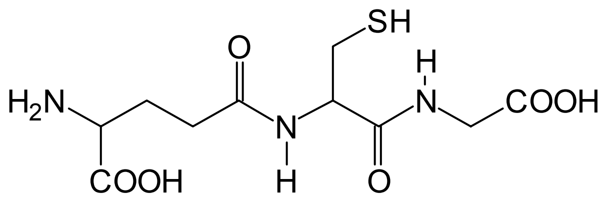


ε_{343nm}=8,081 $M^{-1}cm^{-1}$

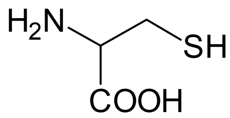
Carrera et al. *Dalton Trans.* **2007**, 4980

Thiol-responsive agents: reduced glutathione

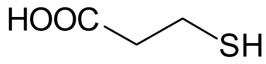




glutathione (GSH)

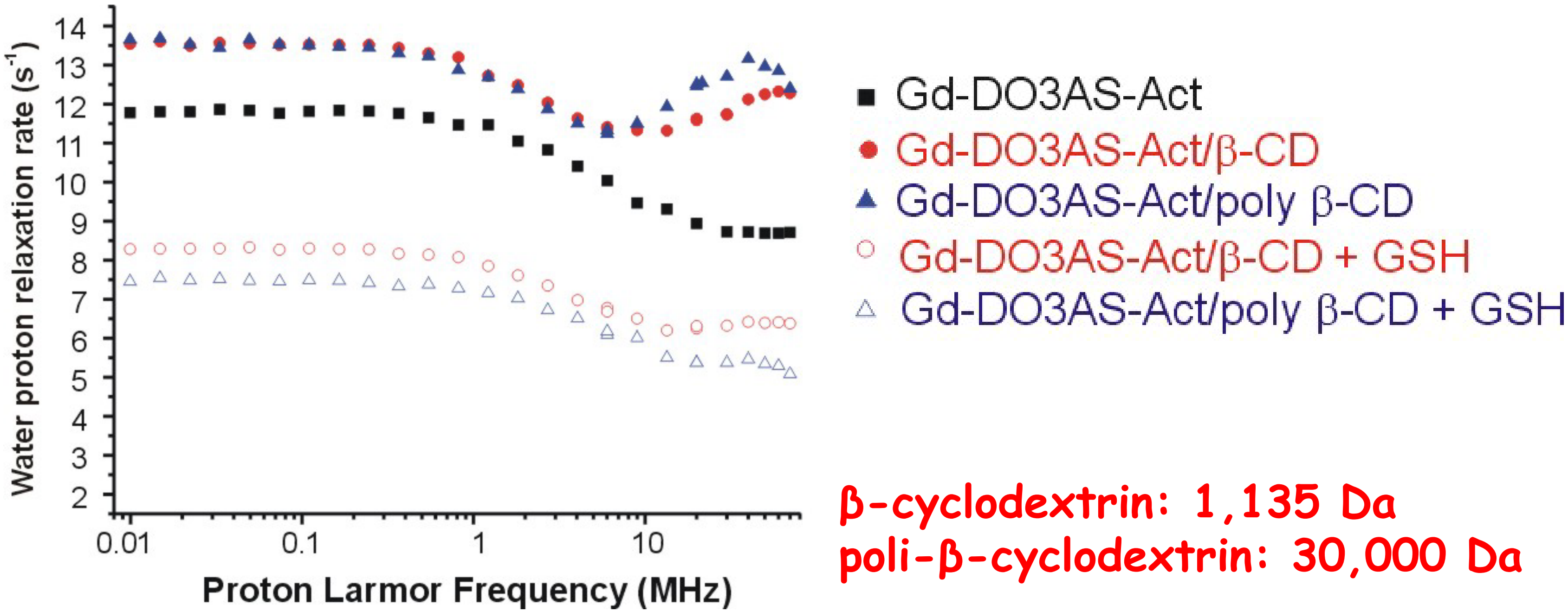


L-cysteine (Cys)

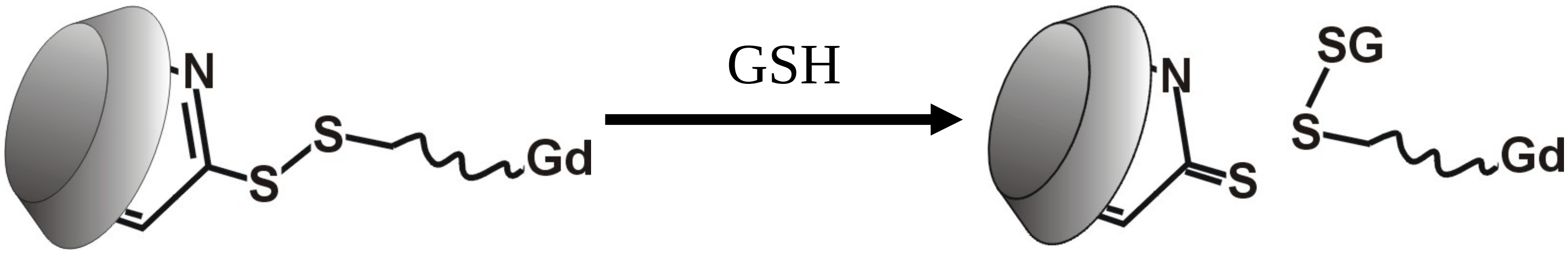


β-mercaptopropionic acid (MPA)

Thiol-responsive agents: reduced glutathione



β-cyclodextrin: 1,135 Da
poli-β-cyclodextrin: 30,000 Da

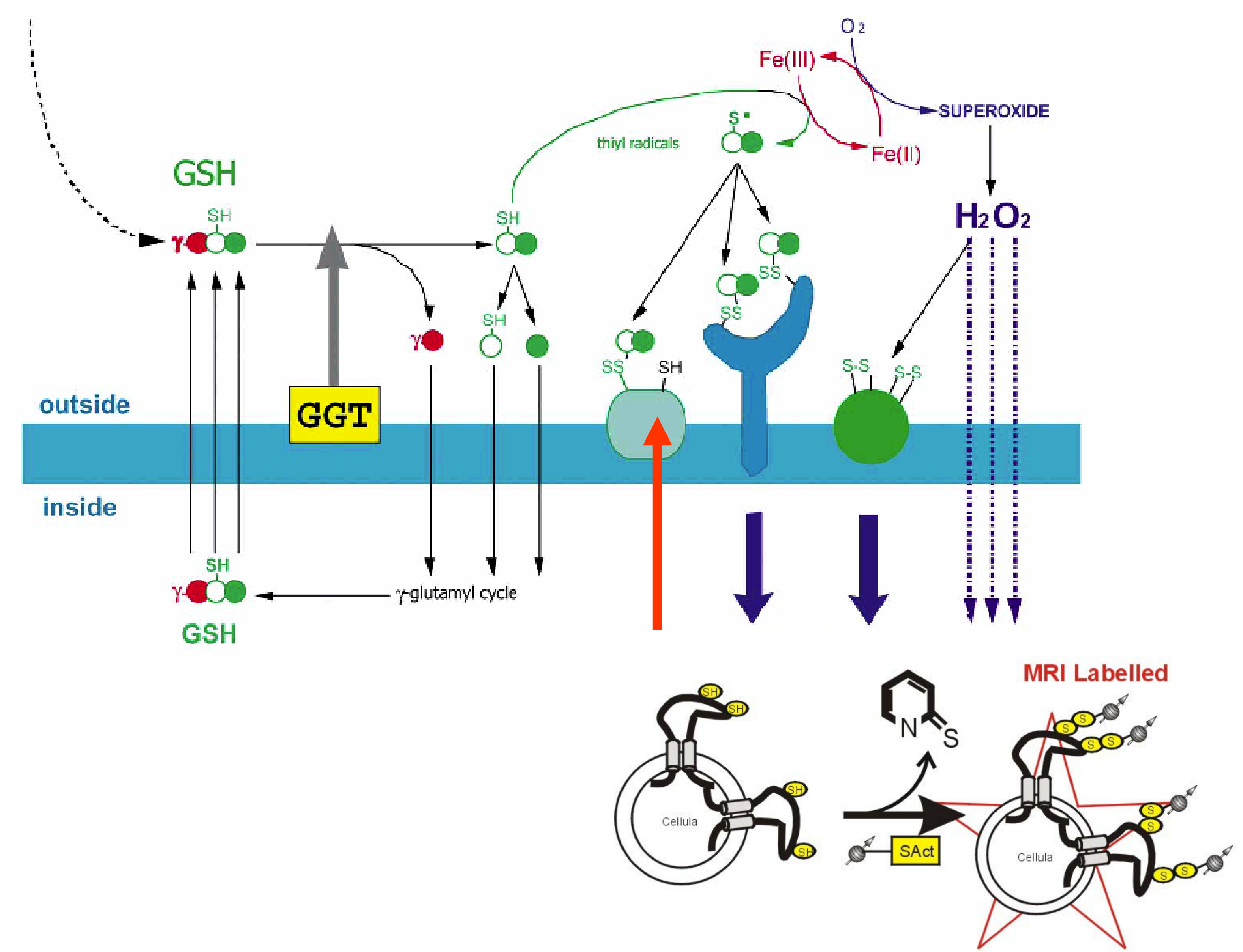


τ_R effect

No τ_R effect

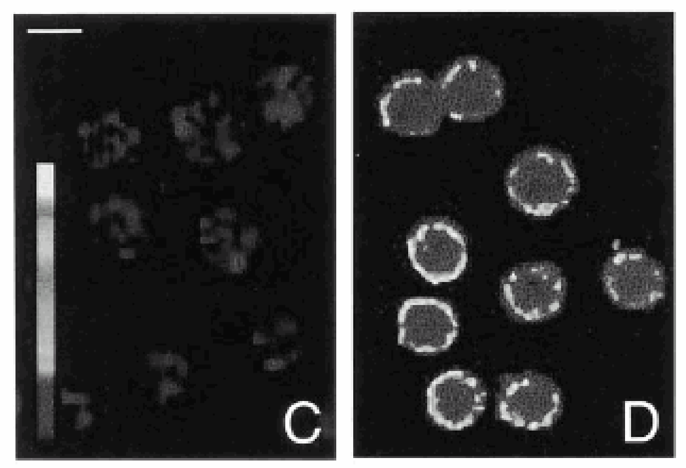
Extracellular redox through thiol-responsive agents: exofacial protein thiols (EPTs)

From Paolicchi et al., *Biochem Pharmacol* 2002, 1027



Exofacial Protein Thiols (EPTs)

Visualization of exofacial protein thiols in U937 cells by confocal microscopy



From Dominici et al. *Free Rad Biol Med*, 1999, 623

Cell concn of EPTs:

- 5-37 10^9 n-SH/cell (CHO-Chinese Hamster Ovary)
- 3-9 10^9 n-SH/cell (PBMC-peripheral blood mononuclear cells)
- 9-18 10^9 n-SH/cell (HT1080-human fibrosarcoma cells)
- 5-7 10^9 n-SH/cell (K562-human myeloid leukemia)

For MRI visualization: $r_1 \times N > 10^9$

N → number of Gd(III) ions/cell

r_1 → relaxivity ($mM^{-1}s^{-1}$) in the cellular environment

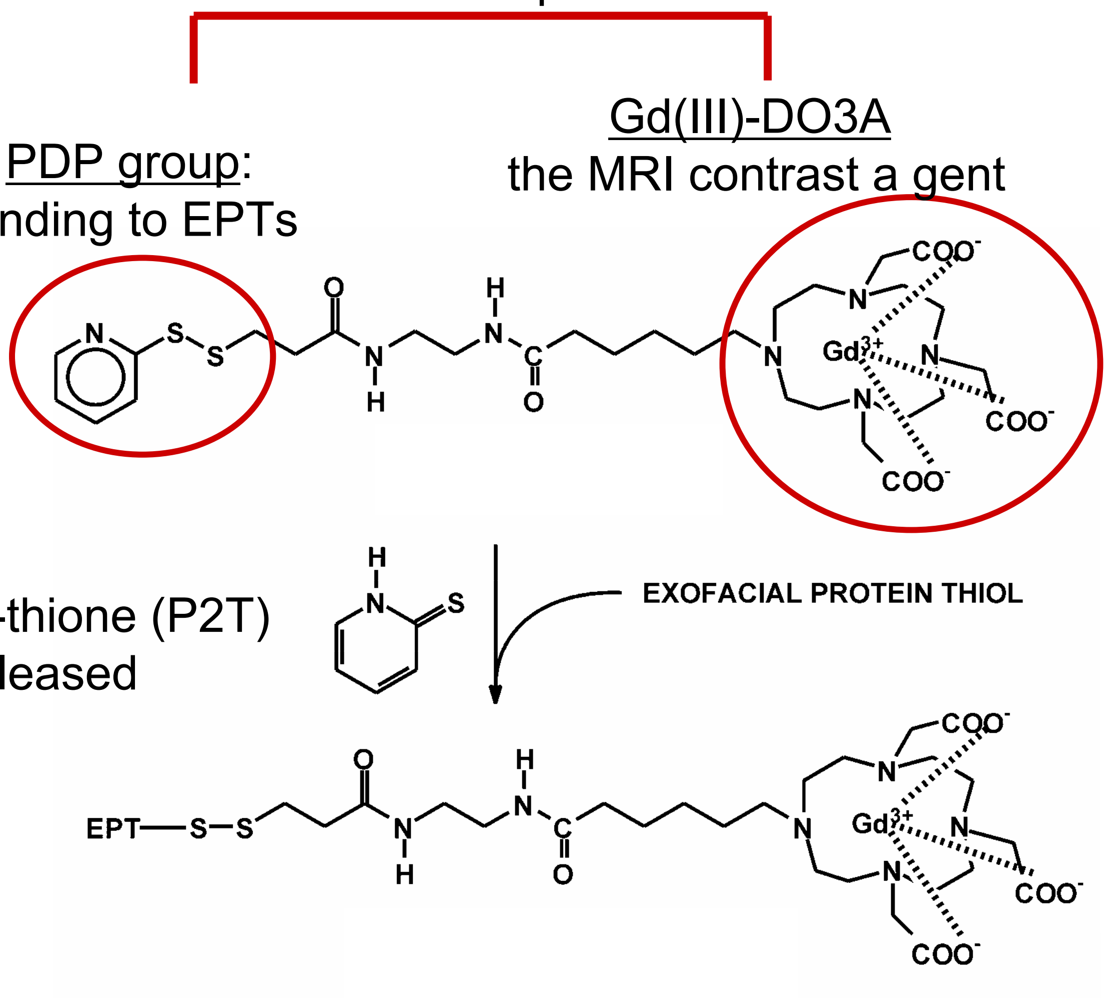
Aime et al. *J Magn Reson Imaging*, 2002, 394.

Design of probes responsive to EPTs

Flexible spacer

<u>Gd(III)-DO3A</u>
the MRI contrast a gent

<u>PDP group</u>:
binding to EPTs



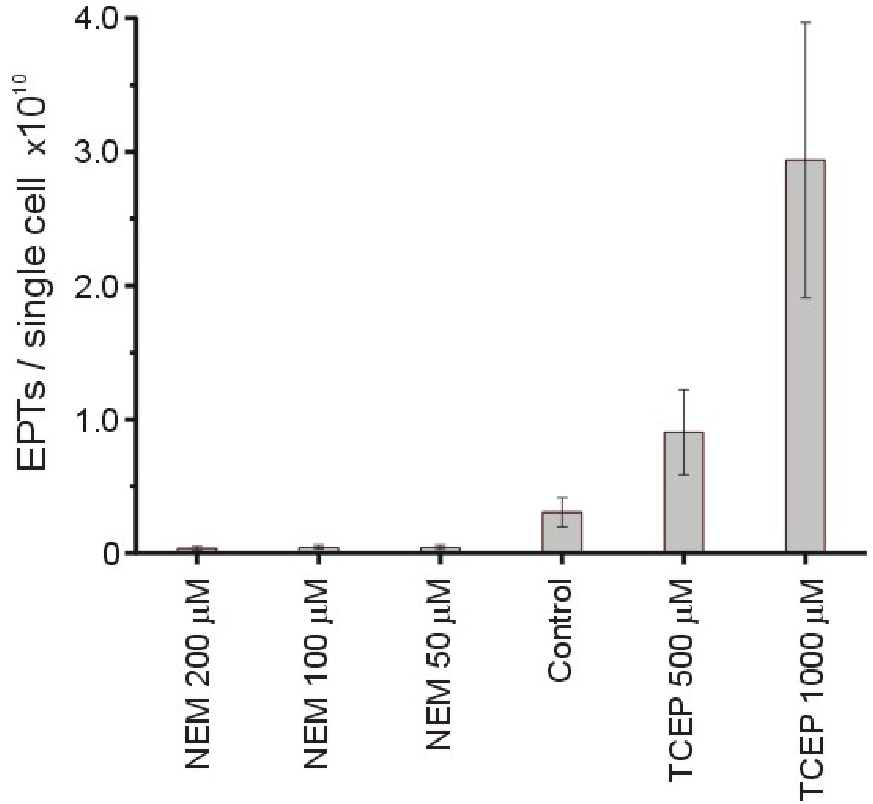
Free probe:
Gd-DO3A-PDP

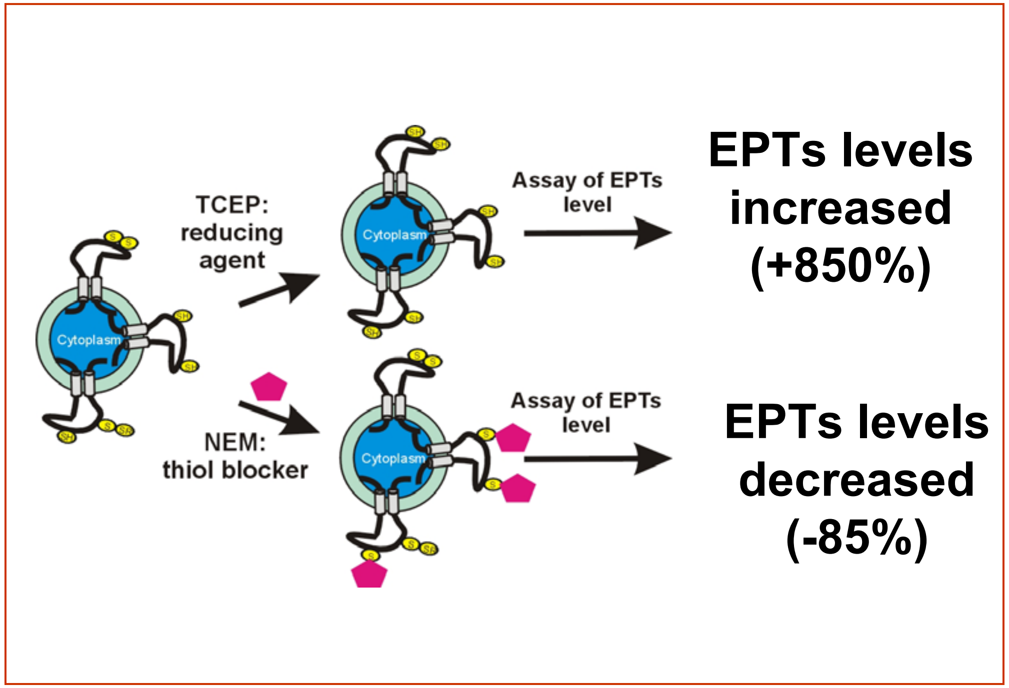
Pyridine-2-thione (P2T)
is released

Probe disulfide bridged to membrane proteins:
Gd-DO3A-EPT

Digilio et al., *Chem. Commun. 2009, 893.*

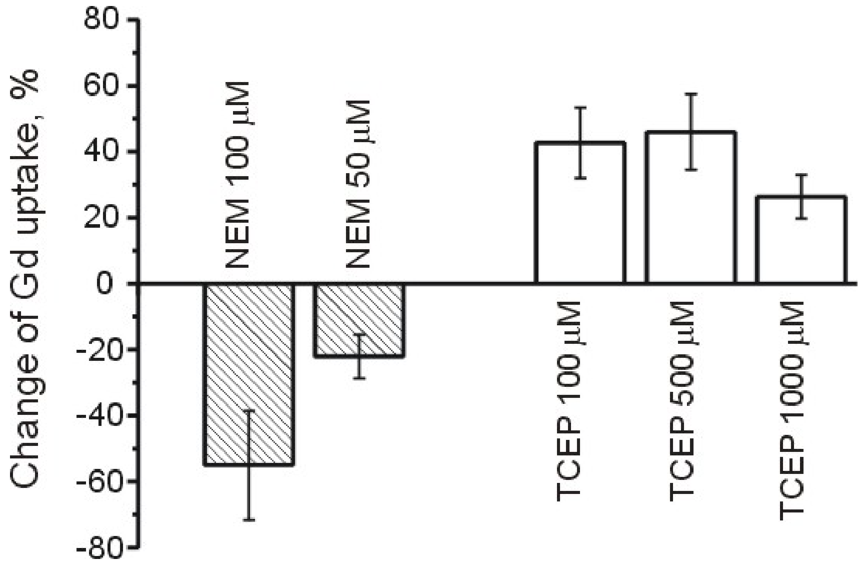
EPTs levels in mouse melanoma B16 cells

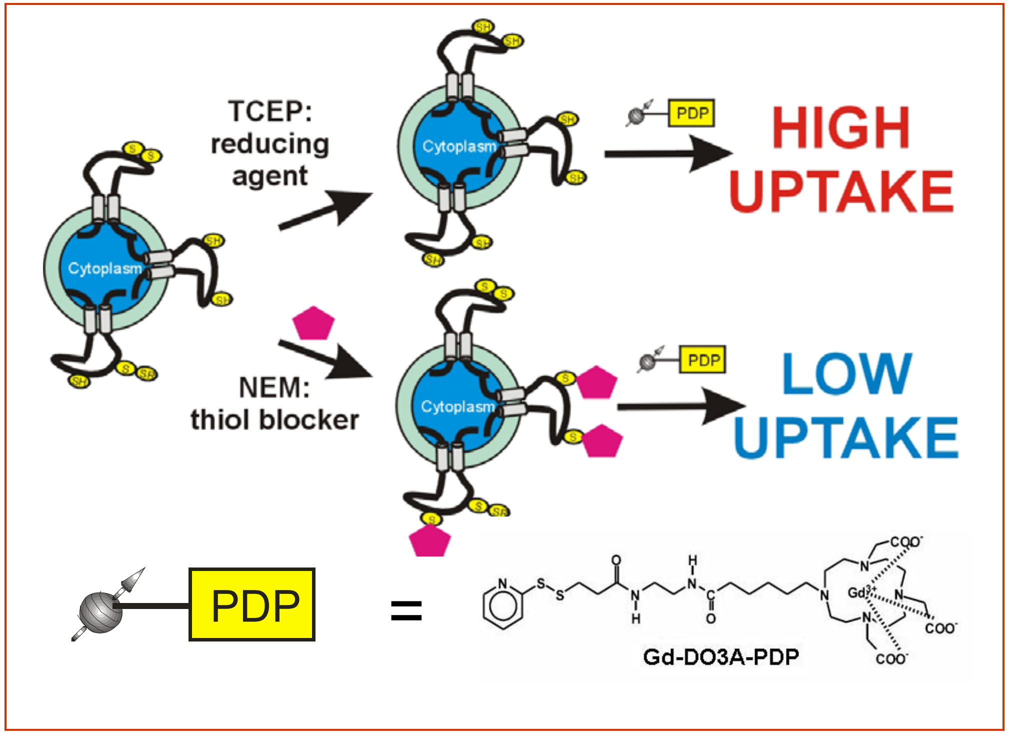




The levels of EPTs in cultured B16 cells can be altered by incubating cells with chemical reductants (TCEP) or thiol blocker (NEM)

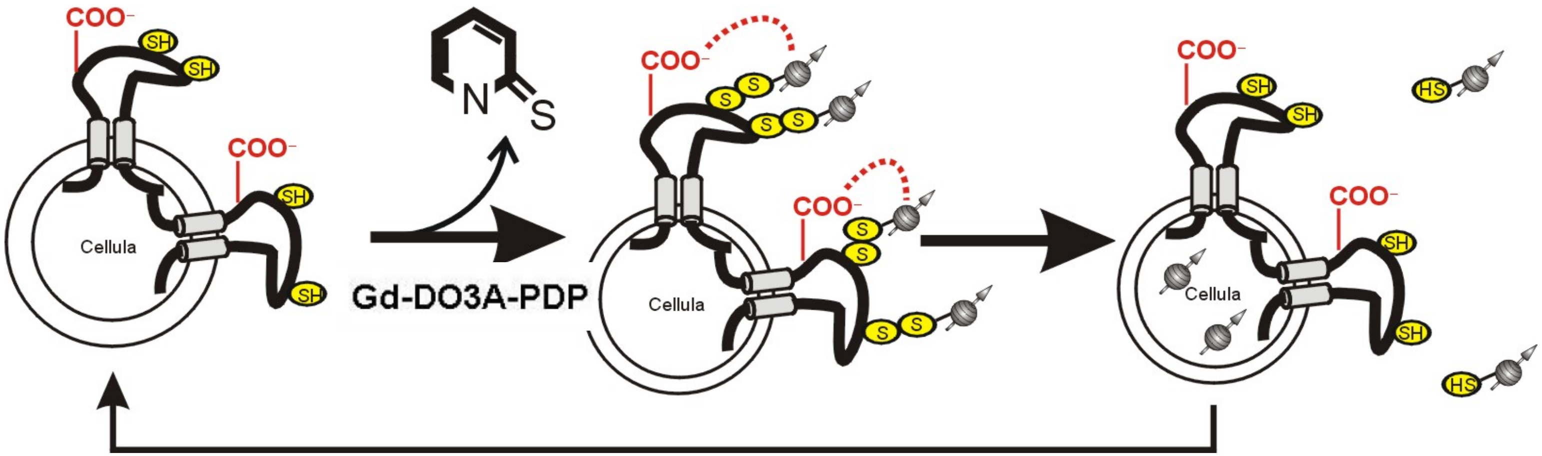
Responsivity of Gd-DO3A-PDP to EPTs

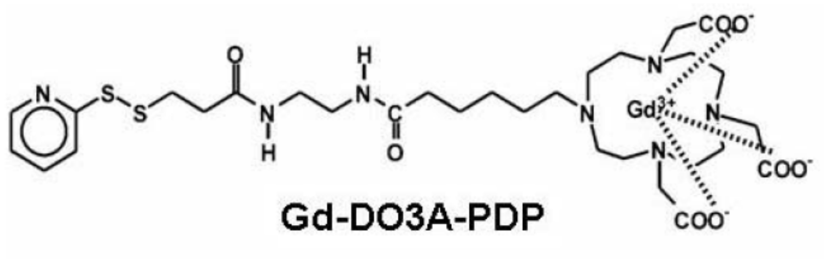




- <u>High EPT level</u> → High Gd uptake (up to 1.5×10^{10} Gd/cell)
- <u>Low EPT level</u> → Low Gd uptake
- Uptake (*ex vivo*) of Gd → proportional to EPT → responsive to extracellular redox.
- What about *in vivo* ?

Responsivity of Gd-DO3A-PDP to EPTs

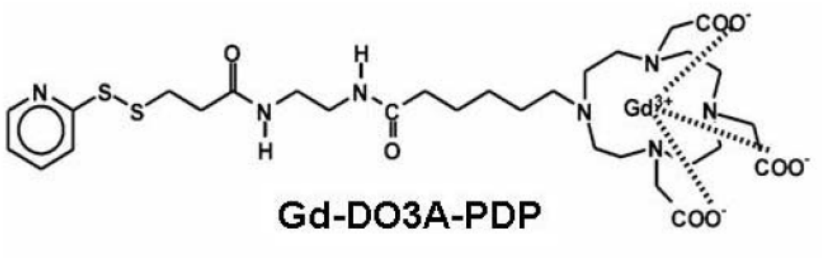




<u>What was observed</u>: after binding of the thiol-sensitive Gd-DO3A-PDP probe to the EPTs, the complex is accumulated (internalized) within the cytoplasm and EPTs are restored.

Digilio et al., *J. Med. Chem. 2010, 53, 4877–4890.*

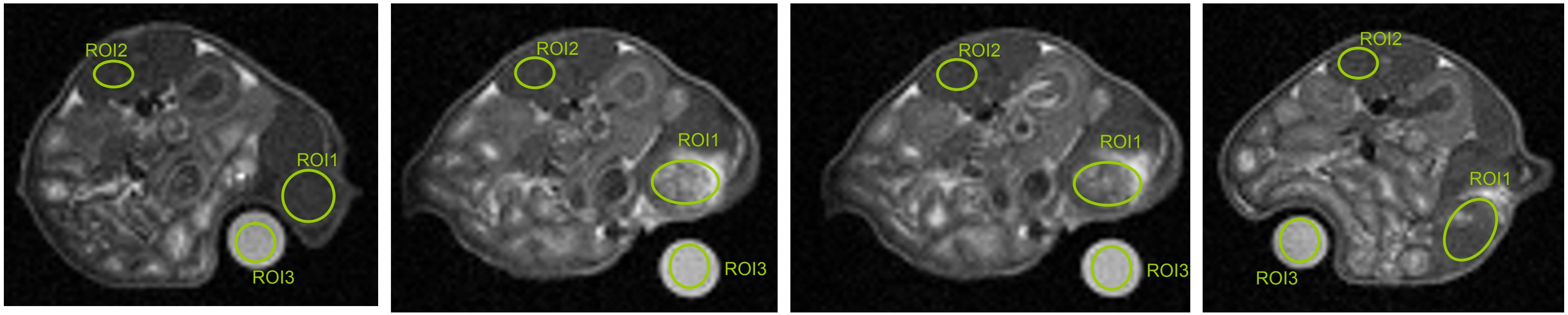
Responsivity of Gd-DO3A-PDP to EPTs: *in vivo*



Intratumor injection

T_1-weighted SE images @ 1T

ROI_1 = Tumor; ROI_2 = Muscle; ROI_3 = Reference



Pre injection

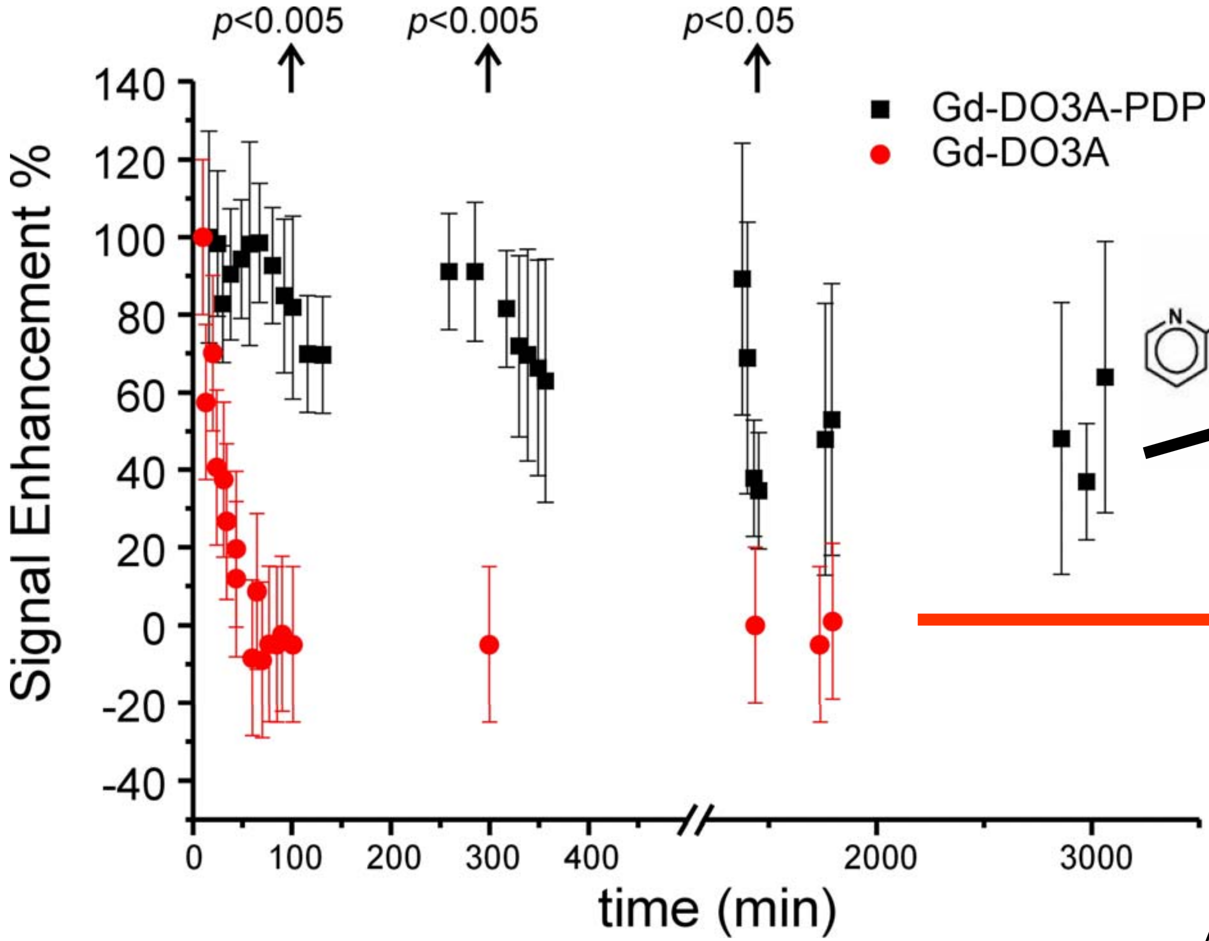
5min **2 hours** **24 hours**

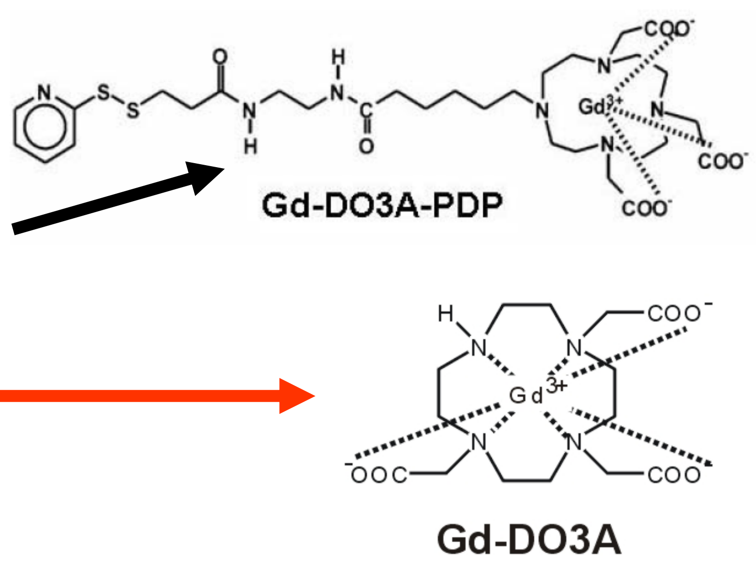
Time post-injection

$$SE\,\% = \frac{100 \times (SI_{tumor}/SI_{reference})_{post} - (SI_{tumor}/SI_{reference})_{pre}}{(SI_{tumor}/SI_{reference})_{pre}}$$

Responsivity of Gd-DO3A-PDP to EPTs: *in vivo*

Time course of the signal enhancement afer intratumor injection of PDP (control: Gd-DO3A)





Aime et al. *Mol. Pharmaceutics* **2011**, in press

Suggested readings

S. Aime et al. “Gd(III)-based contrast agents for MRI” Adv. Inorg. Chem. 2005, 57, 173-175

B. Yoo & M.D. Pagel “An overview of responsive MRI contrast agents for molecular imaging” Frontiers in Bioscience 2008, 13, 1733-1752.

M. Woods et al. “Paramagnetic lanthanide complexes as PARACEST agents for medical imaging” Chem. Soc. Rev. 2006, 35, 500-511.

A.E. Merbach & E. Toth (eds) “The chemistry of contrast agents in medical magnetic resonance imaging” 2001, John Wiley & Sons, Chichester, UK. ISBN 0-471-60778-9

L.M. De Leon-Rodriguez et al. “Responsive MRI agents for sensing metabolism in vivo” Accounts of Chemical Research, 2009, 42(7), 948-957.

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Questions ?