CEST & PARACEST
Theory & Practice

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ADVANCED IMAGING
AIRC
RESEARCH CENTER

ENC, April, 2007

Early history of NMR exchange techniques

THE JOURNAL OF CHEMICAL PHYSICS VOLUME 39, NUMBER 11 1 DECEMBER 1963

Study of Moderately Rapid Chemical Exchange Reactions by Means of Nuclear Magnetic Double Resonance

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AND

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(Received 1 July 1963)

A nuclear magnetic double-resonance method for the determination of chemical exchange rates has been developed. The method is applicable to systems in which a nuclear spin is reversibly transferred between two nonequivalent sites, A and B. The lifetime (τ_A) and spin–lattice relaxation time (T_{1A}) in Site A are obtained through the study of the decay to a new equilibrium value of Signal A upon the sudden saturation of Signal B. The converse experiment permits the determination of τ_B and T_{1B}. A number of data for cross checks are furthermore obtained through the study of the recovery of the signals upon the release of various combinations of saturating rf fields.

A simple theory based on the Bloch equations as modified by McConnell to incorporate the effects of chemical exchange is given. Experimental results on the hydroxyl proton exchange in the system salicylaldehyde and 2-hydroxyacetophenone are well described by this simple theory.

The present method, which can readily be extended to systems with several sites, offers a complement to the Gutowsky–Saika single-resonance method and is particularly suited to the study of exchange rates slower than those accessible by the single-resonance method.

^{31}P NMR kinetic measurements on adenylate kinase: 2ADP ⇔ ATP + AMP

Chemistry: Brown and Ogawa

Proc. Natl. Acad. Sci. USA 74 (1977) 3629

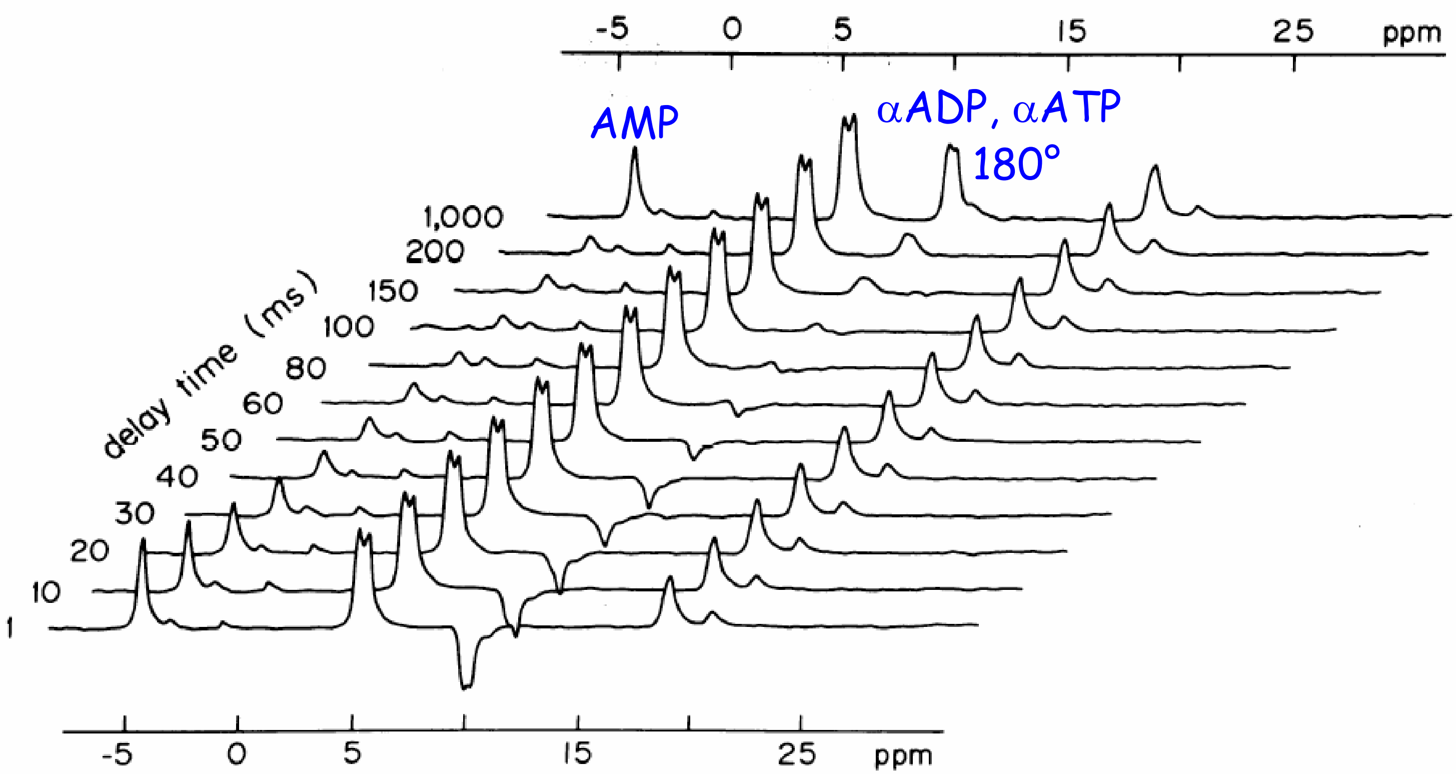
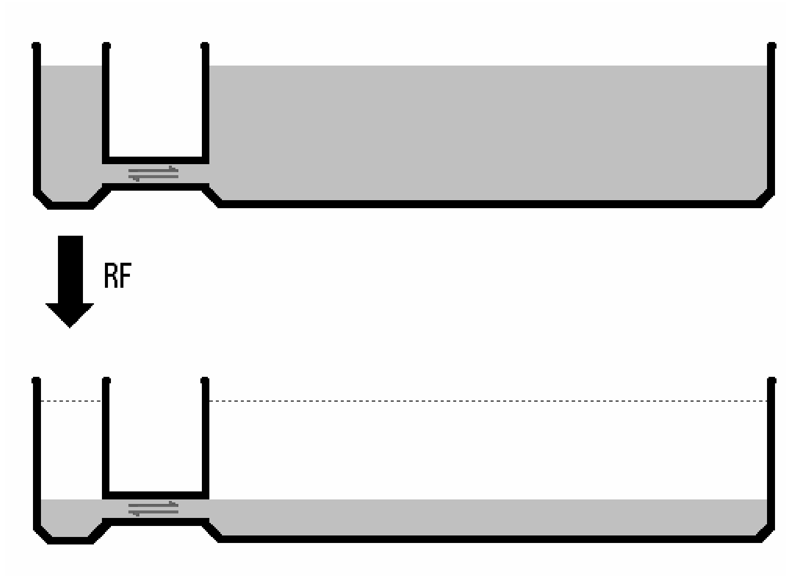
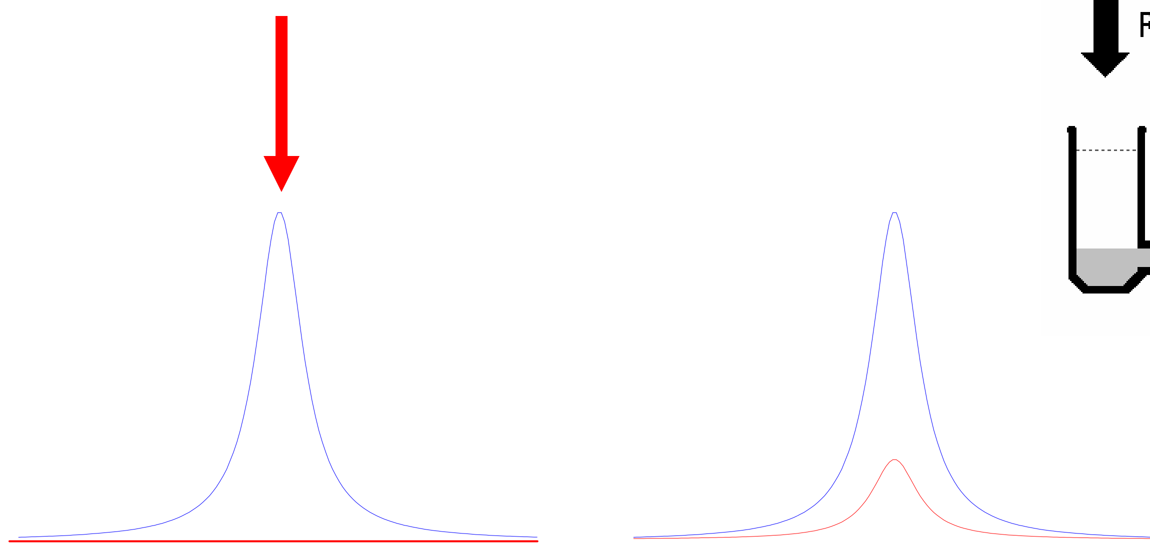


FIG. 2. A typical inversion transfer sequence. Each spectrum is 200 scans taken every 1.8 sec. The 180° pulse was applied to the α phosphates of ADP and ATP. The delay between this pulse and the 90° pulse varied from 1 ms to 1 sec and is indicated by the number to the left of each spectrum. The small peaks at −0.5 and −3.0 ppm are a marker and inorganic phosphate, respectively. The small peaks at 11 and 21 ppm are an unknown impurity which shows no sign of inversion transfer to any of the other peaks.

CEST: Chemical Exchange Saturation Transfer

$$A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B$$





$$M_{B\infty} / M_{B0} = 1 / (1 + k_2 T_{1B})$$

CEST *versus* PARACEST

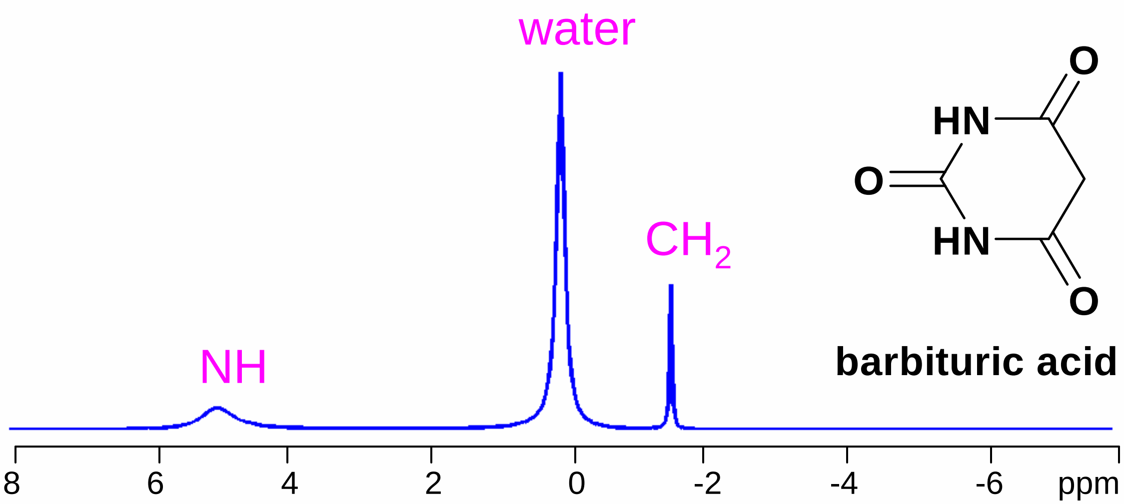
CEST agents: exogenous or endogenous compounds that exchange protons with solvent water.

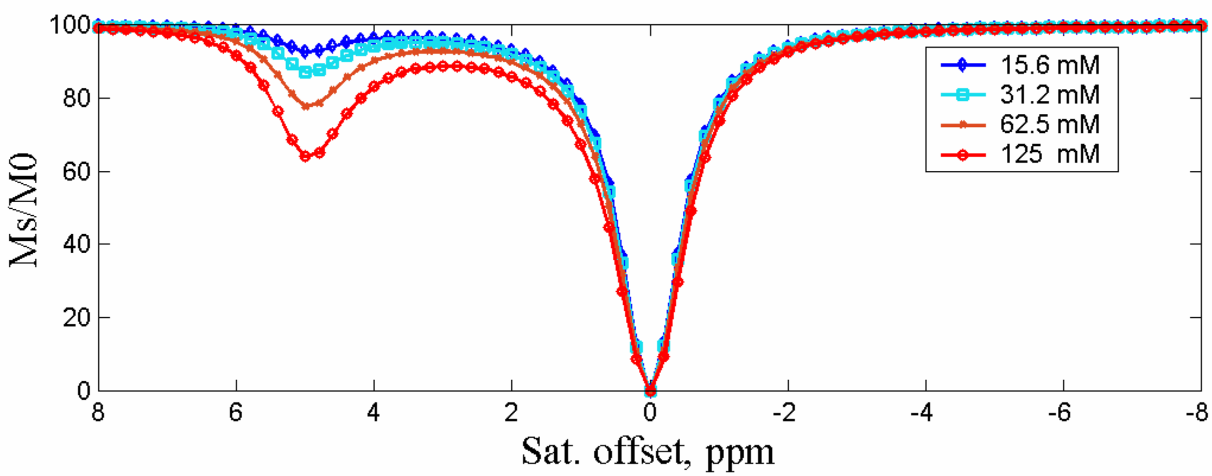
-NH, -OH, -SH with H_2O

PARACEST: exogenous paramagnetic ML complexes that exchange either protons or water molecules

EuL-NH with H_2O; EuL-OH_2 with H_2O

First report of using small molecules as Chemical Exchange Saturation Transfer (CEST) agents

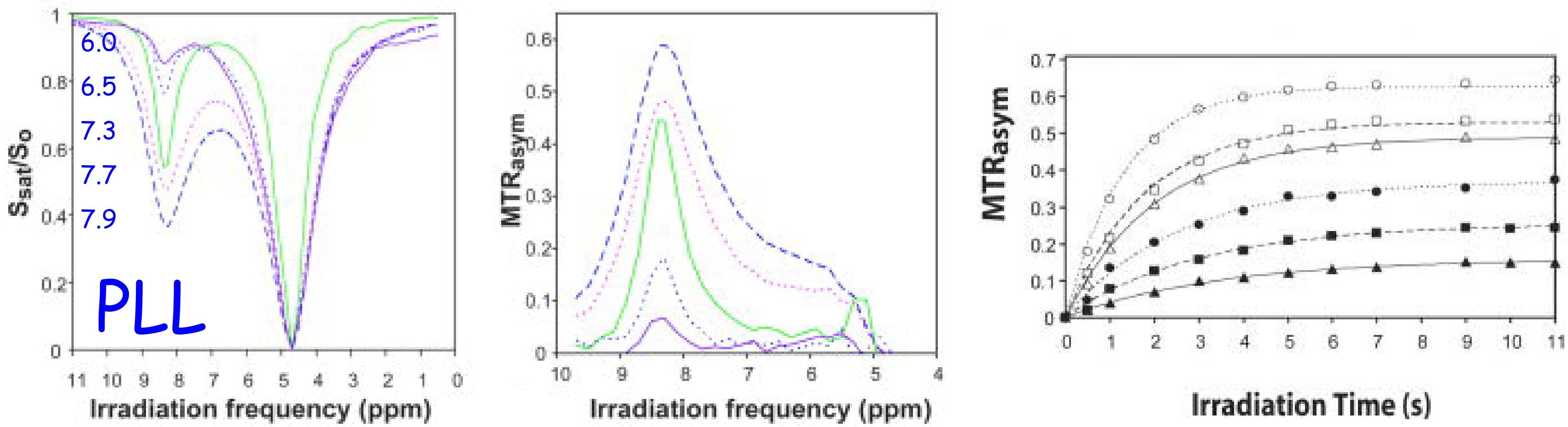




Ward, Aletras & Balaban. *J. Magn. Reson*. **143**: 79 (2000)

Quantifying exchange rates in chemical exchange saturation transfer agents,

McMahon, et al., Magn. Reson. Med., 55, 836-847 (2006)

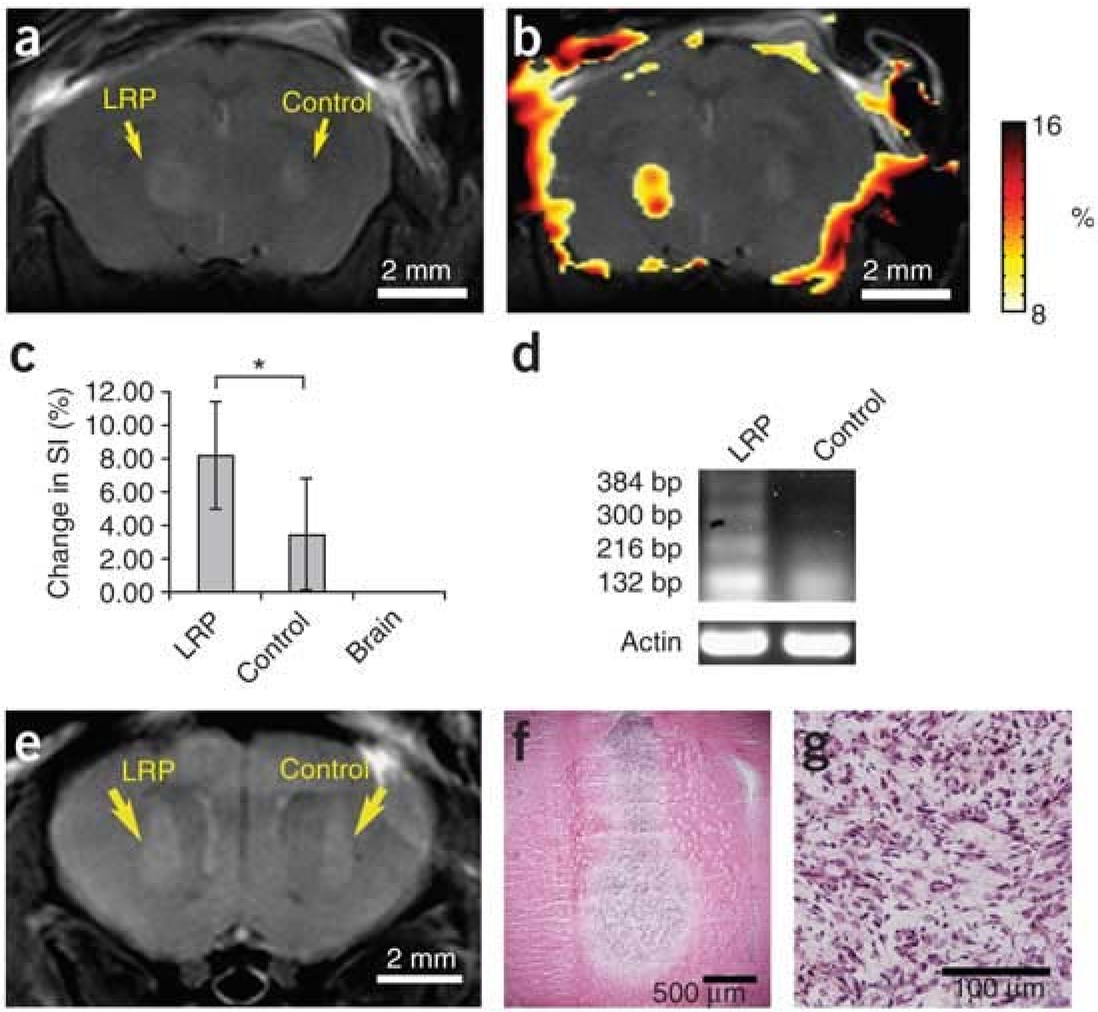


QUEST analysis: $k_{ex} = 50\ s^{-1}$ (6.0) to $1250\ s^{-1}$ (7.9)

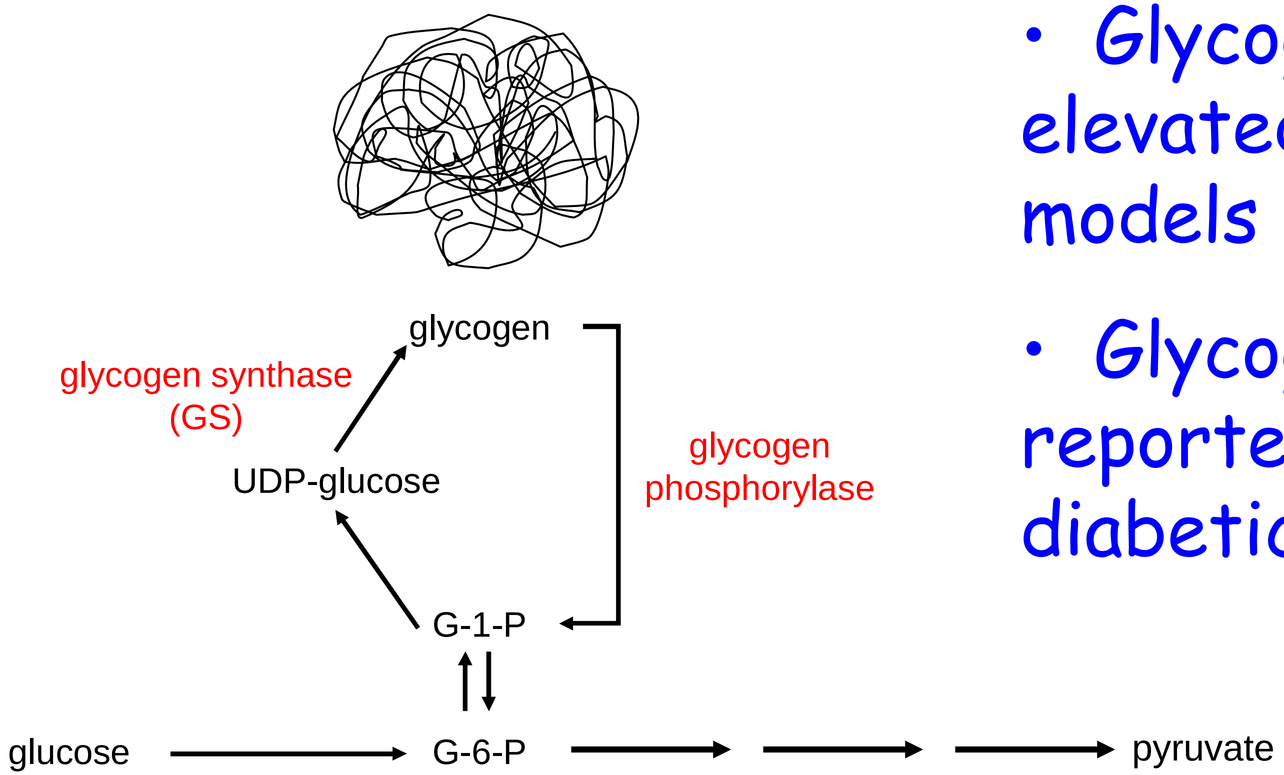
$$k_{s\rightarrow w} = 69\ s^{-1} + 1.2\ s^{-1} \times 10^{-pH} + 1.92\times10^{9}\ s^{-1} \times 10^{pH-pKw}$$

(k_0) (k_a) (k_b)

Artificial reporter gene providing MRI contrast based on proton exchange. *AA Giliad, et al.*, Nature Biotechnol. **25**, 217-219 (2007).

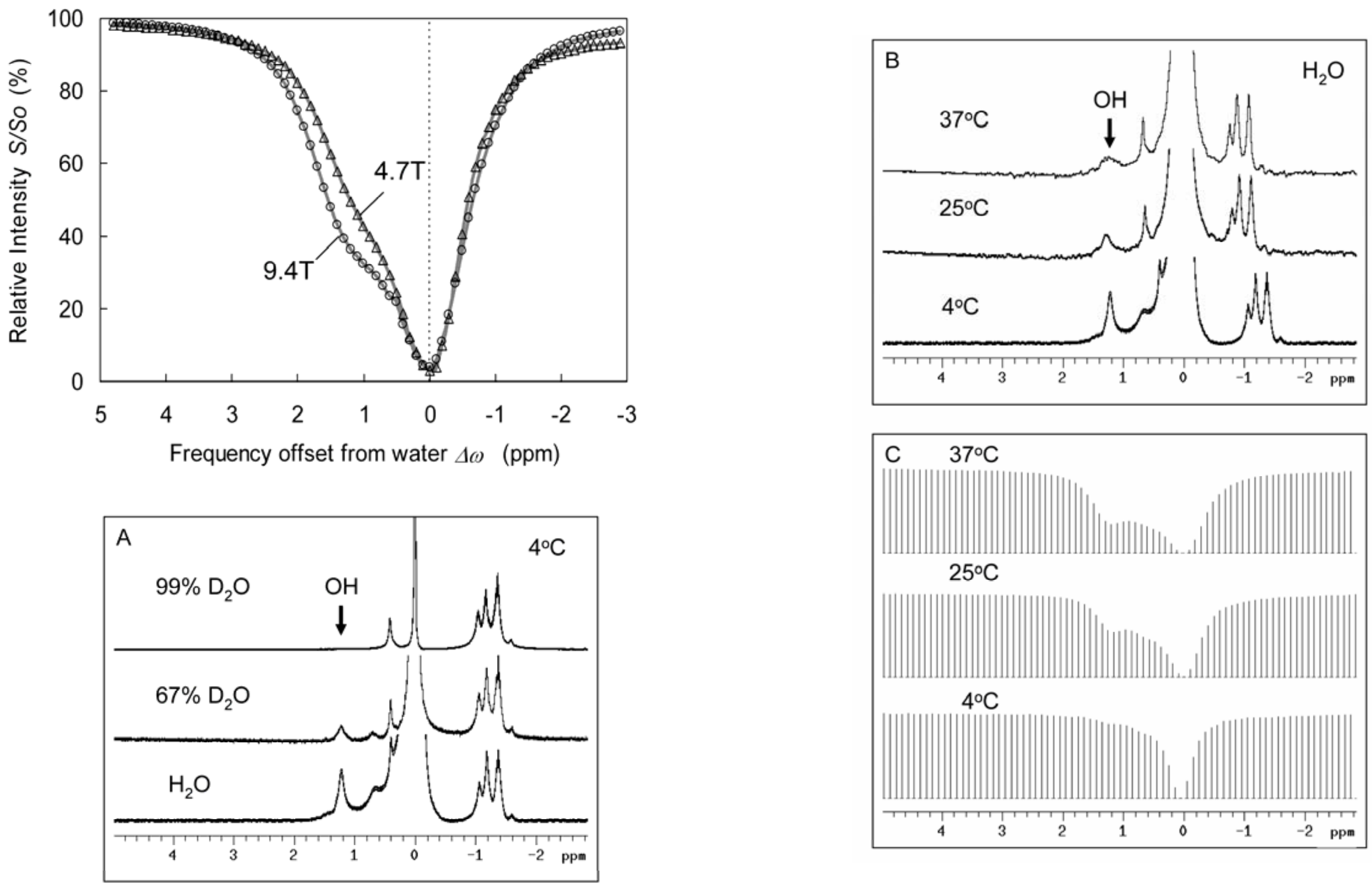


Glycogen is the main storage form of glucose in mammalian cells

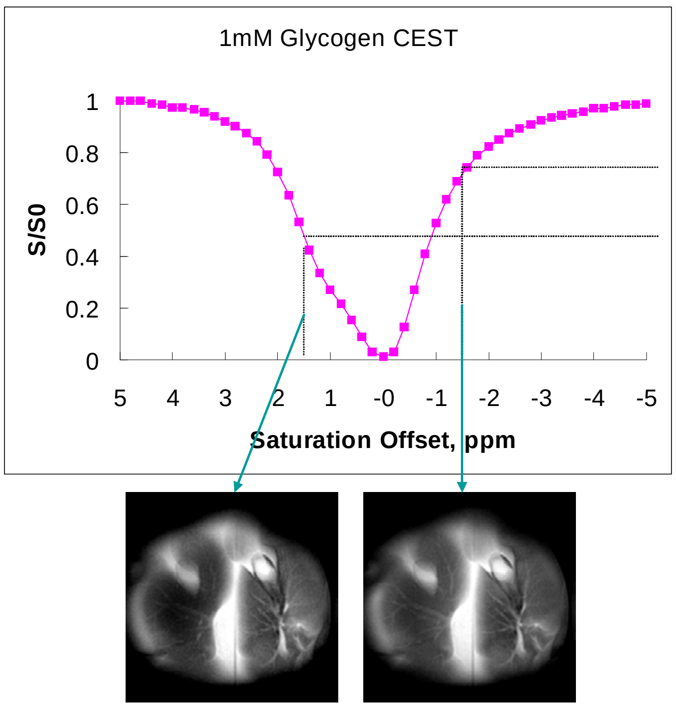


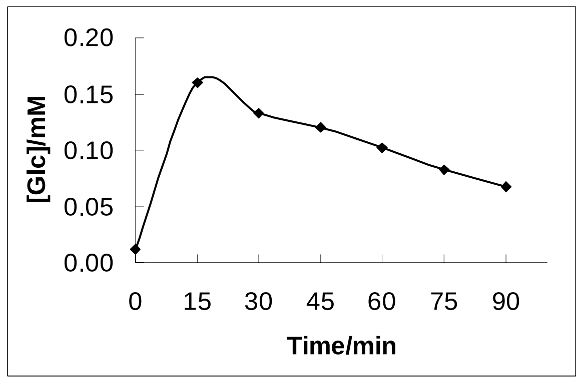
- Glycogen is elevated in animal models of diabetes
- Glycogen has been reported to be low in diabetic humans

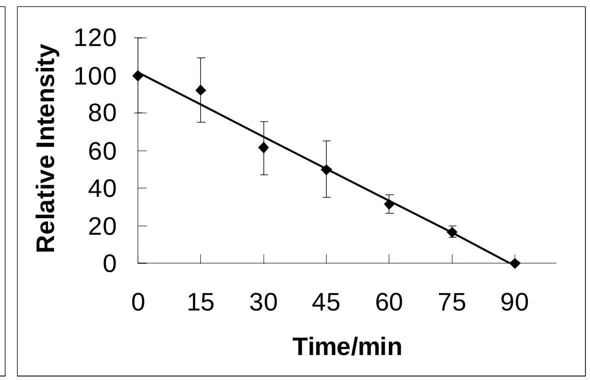
Glycogen has a large number of exchangable OH protons – Can these be detected by CEST?

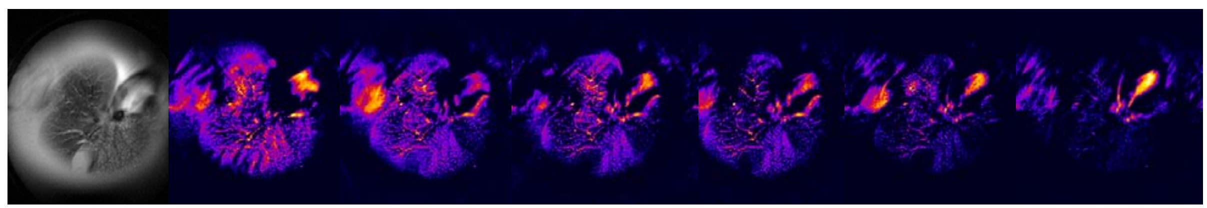


Endogenous glycoCEST imaging of glycogen in liver





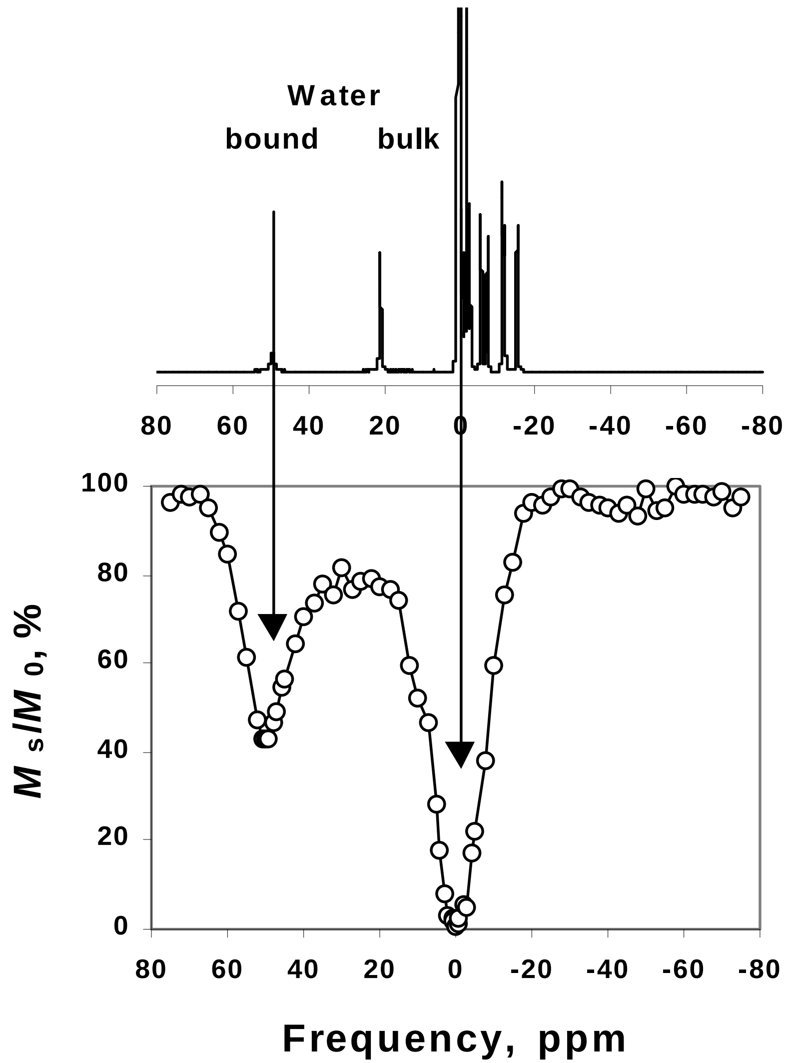


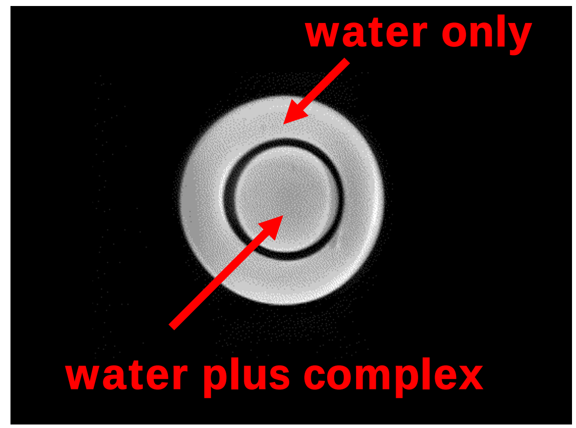


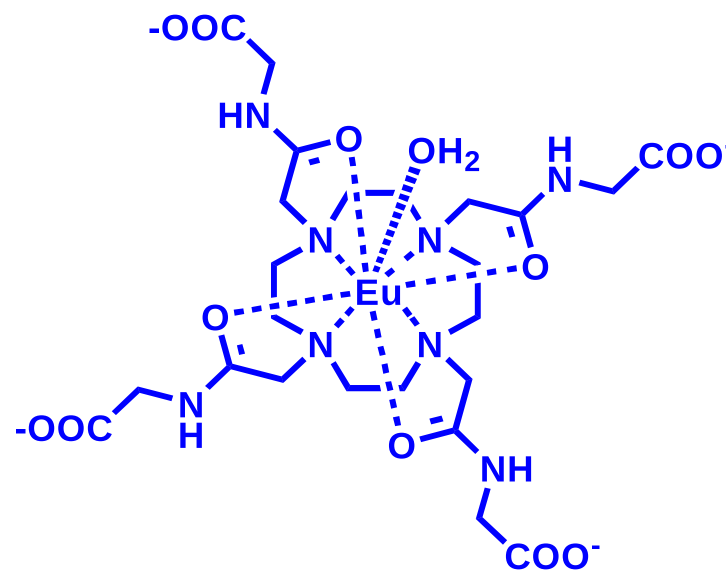
CEST images showing depletion of glycogen in liver after exposure to glucagon

van Zijl, et al., PNAS, 104, 4359-4364 (2007)

EuDOTA-4AmC⁻ as a PARACEST agent







Zhang, et al., J. Amer. Chem. Soc. **123**, 1517-1518 (2001).

Some simple predictions from exchange theory

$$EuL\text{-}H_2O \underset{k_2}{\overset{k_1}{\rightleftharpoons}} H_2O_{solvent}$$

Saturation of the Eu-bound water molecule for an extended period yields a new SS intensity in bulk water.

$$Z = M_z^{H2O}/M_0^{H2O} = \tau_{H2O}/(T_{1H2O} + \tau_{H2O})$$

From mass balance, $\tau_{H2O} = \tau_{EuL}\,(C_{H2O}/C_{EuL})$

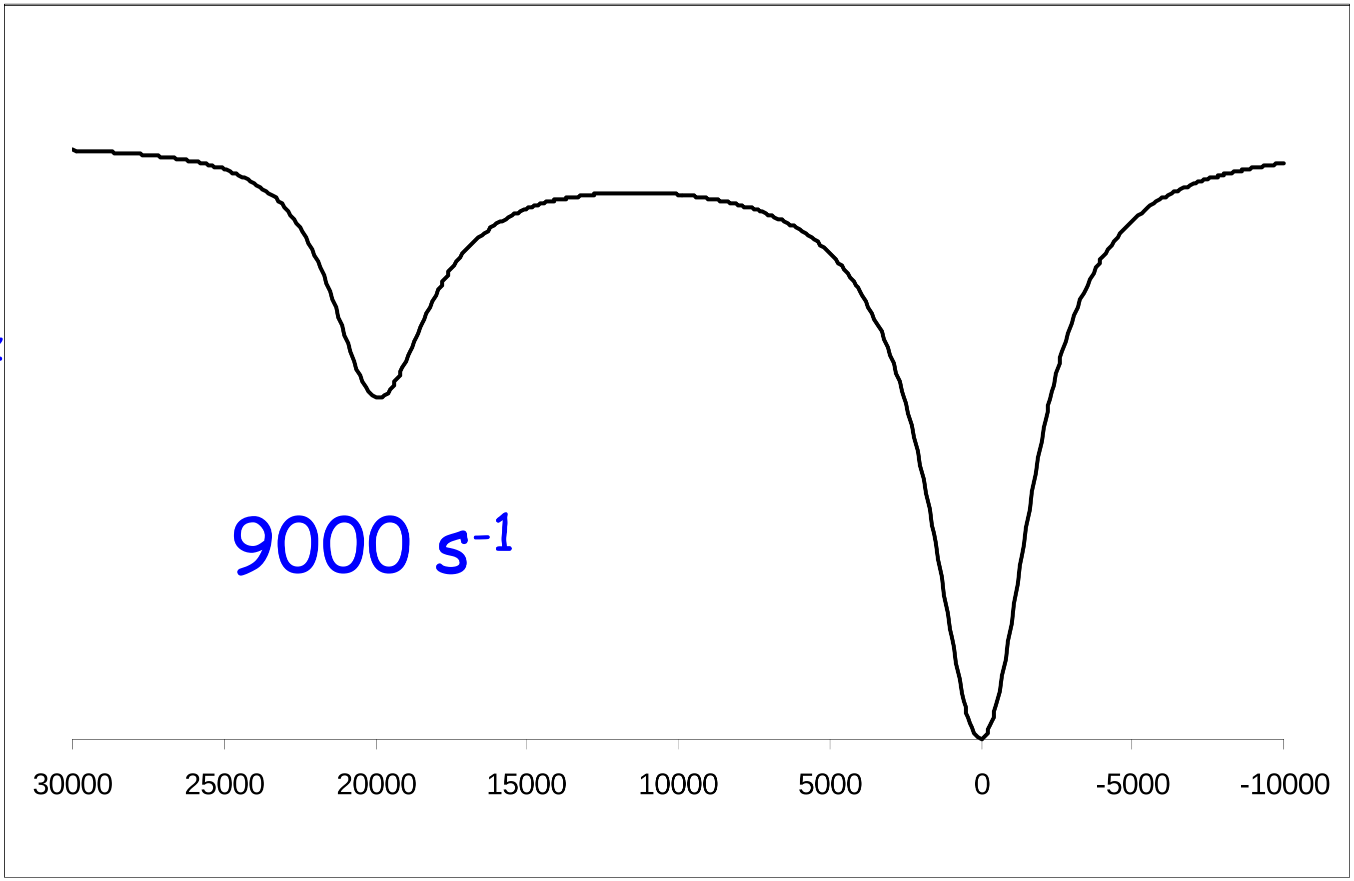
$$Z = M_z^{H2O}/M_0^{H2O} = \tau_{EuL}/(T_{1H2O}(C_{EuL}/C_{H2O}) + \tau_{EuL})$$

shorter τ_{EuL}, long T_{1H2O}, high C_{EuL} $\Rightarrow$ bigger CEST effect

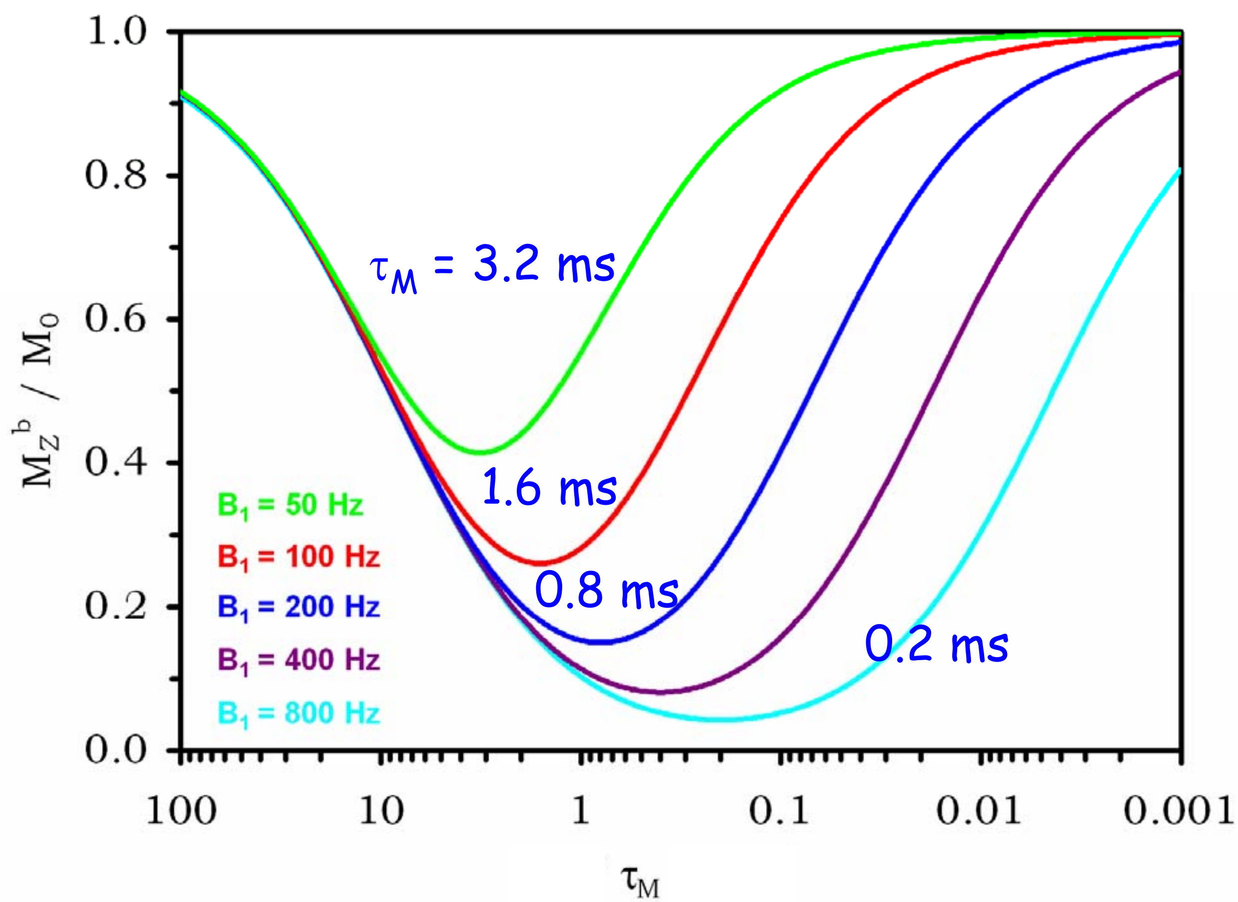
A PARACEST agent with two exchanging pools

20 mM EuL

B_1 = 512 Hz



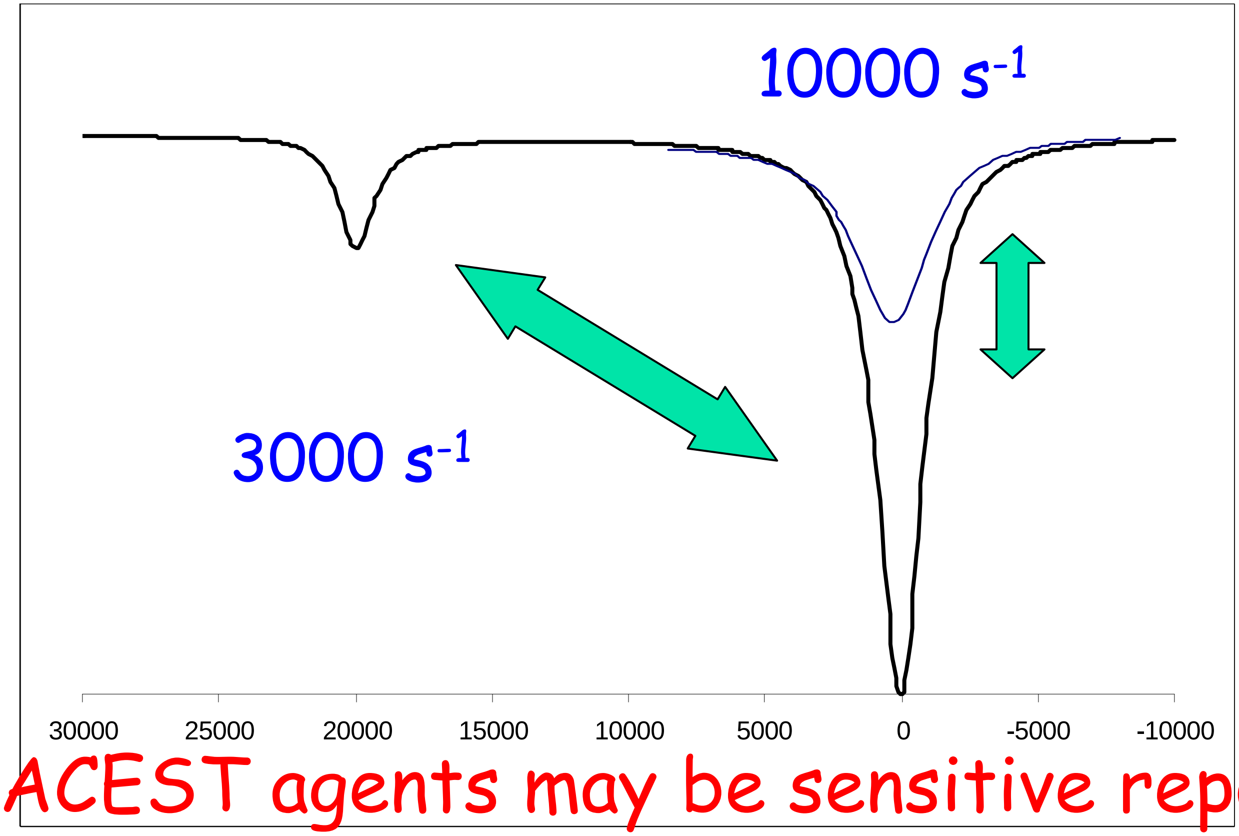
- There is an optimal exchange rate for CEST
- CEST increases with applied B_1
- Optimal exchange rate = $2\pi B_1$



Does the presence of endogenous proton pools in exchange with water alter CEST efficiency?

20 mM
EuL

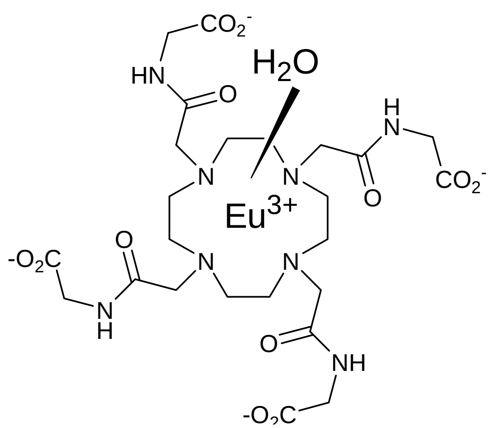
B_1 = 512 Hz

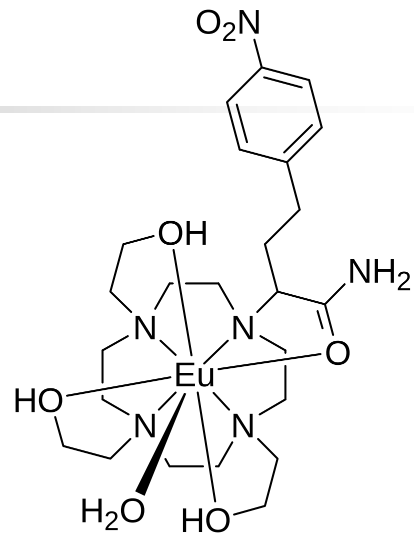


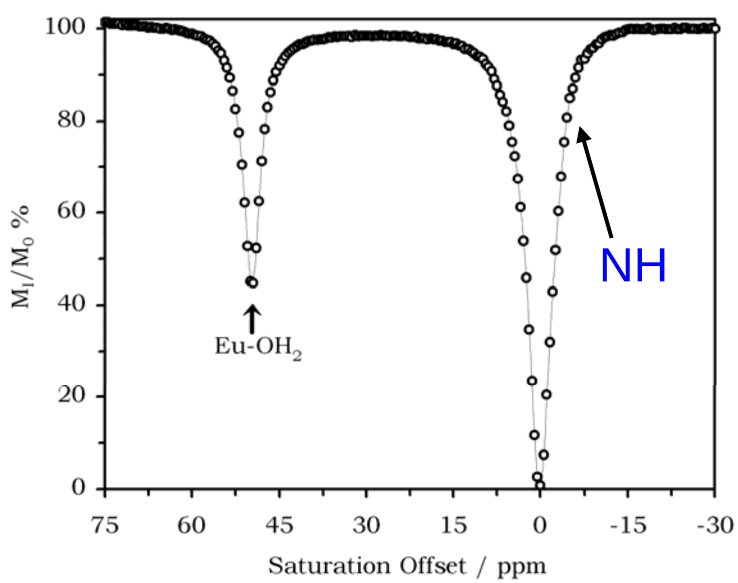
40 mM
endogenous
protons

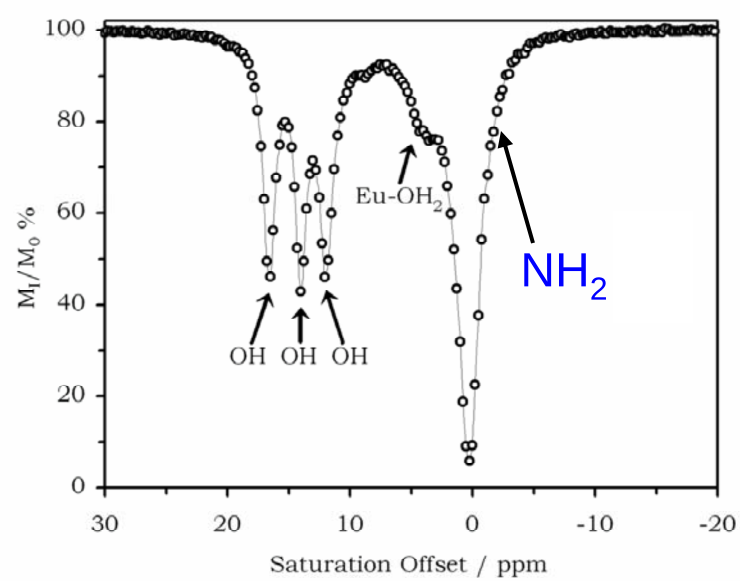
PARACEST agents may be sensitive reporters of other exchange reactions that occur in tissues.

Fitting Z-spectra to the Bloch equations modified for exchange





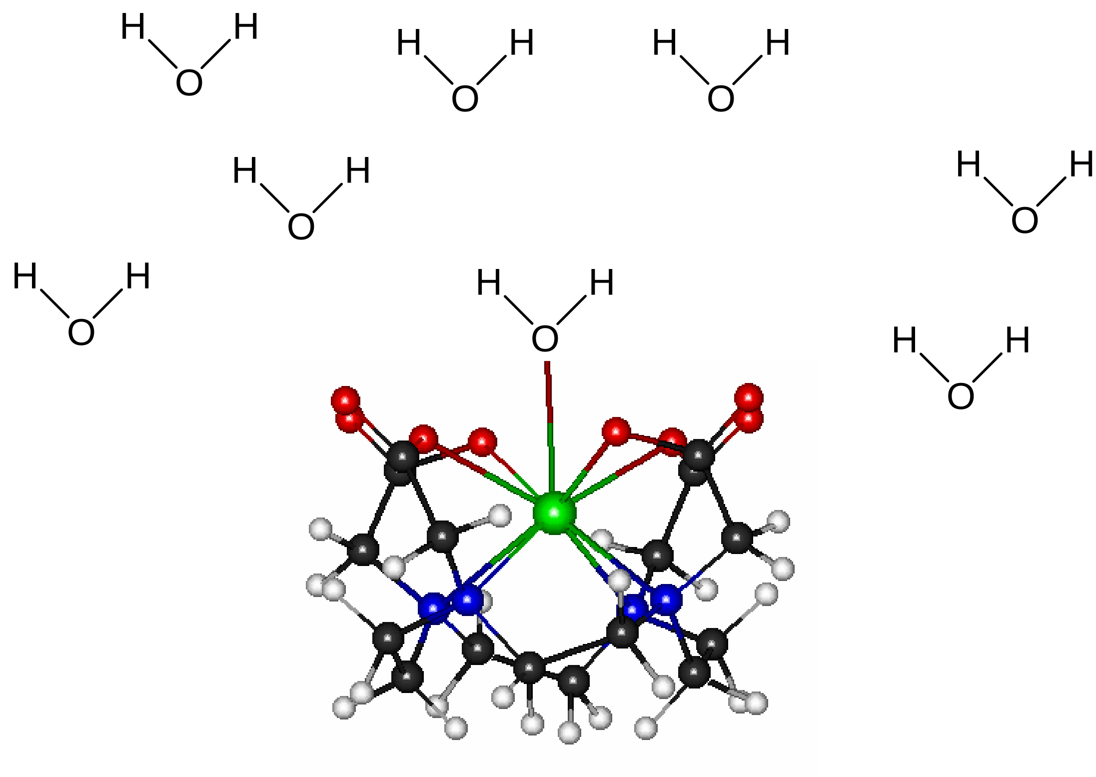




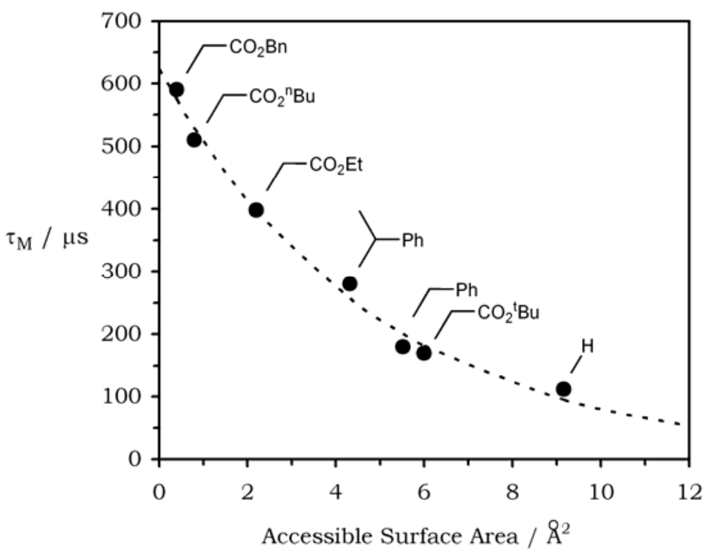
Some references to methods & models used for fitting Z-spectra

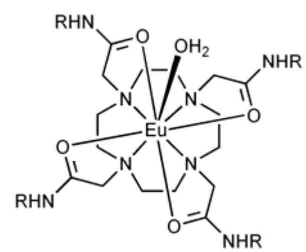
1. "A numerical solution of the Bloch equations provides insights into the optimal design of PARACEST agents for MRI", DE Woessner, et al., Magn. Reson. Med., **53**, 790-799 (2005).
2. "Quantifying exchange rates in chemical exchange saturation transfer agents using the saturation time and saturation power dependencies of the magnetization transfer effects on the magnetic resonance imaging signal (QUEST and QUESP): pH calibration for poly-L-lysine and a starburst dendrimer", MT McMahon, et al., Magn. Reson. Med., **55**, 836-847 (2006).
3. "Chemical exchange saturation transfer imaging and spectroscopy", J Zhou & P van Zijl, Prog. NMR Spectros., **48**, 109-136 (2006).

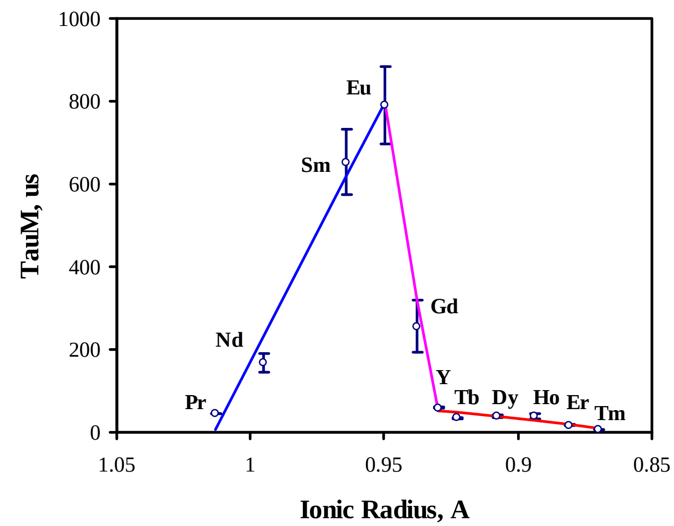
How might one alter τ_M ?



Chemical factors that influence water exchange rates in Ln^{3+}-DOTA-tetraamides

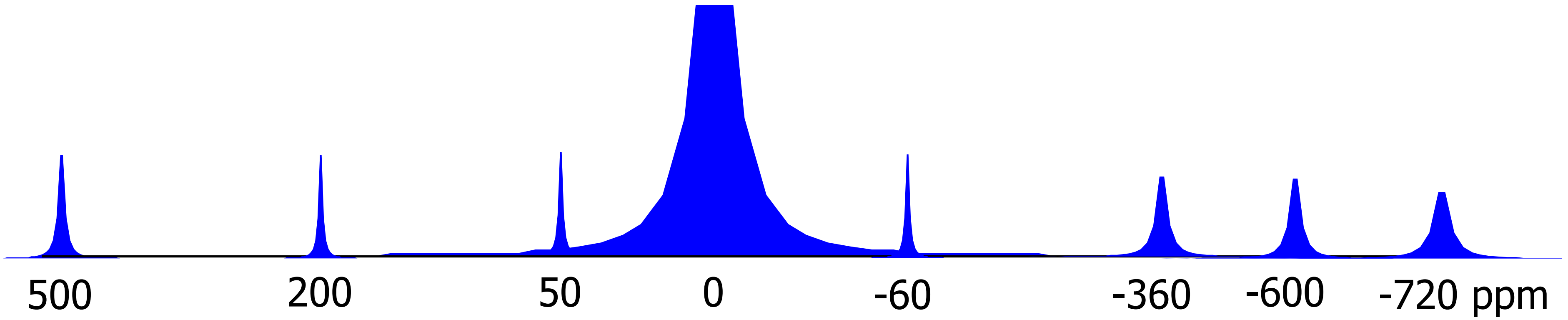






1. Coordination geometry: SAP vs. TSAP
2. Electronics of appended aromatic groups

Which lanthanides are most useful for PARACEST imaging?

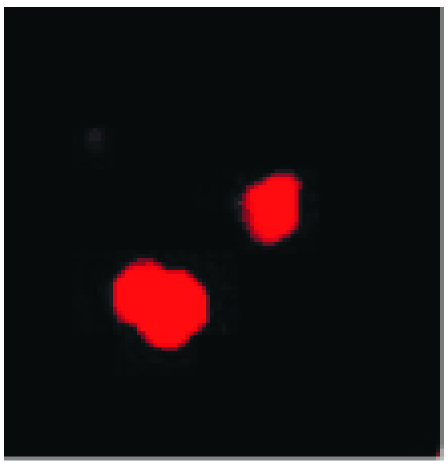


57	58	59	60	61	62	63	**64**	65	66	67	68	69	70	71
La	**Ce**	**Pr**	**Nd**	**Pm**	**Sm**	**Eu**	**Gd**	**Tb**	**Dy**	**Ho**	**Er**	**Tm**	**Yb**	**Lu**

diamagnetic | unstable | isotropic | diamagnetic

Tunable Imaging of Cells Labeled with MRI-PARACEST Agents

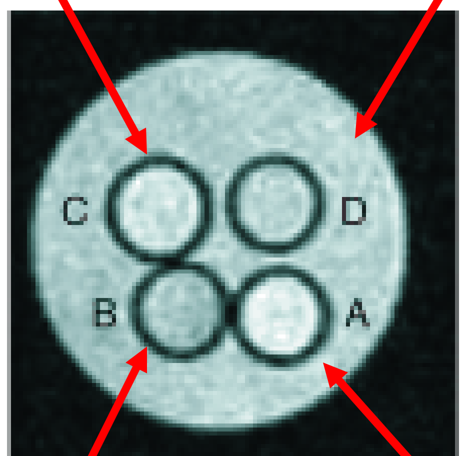
S Aime, et al., Angew Chem Int Ed, 44:1813 (2005)



CEST images after irradiation @ 50 ppm

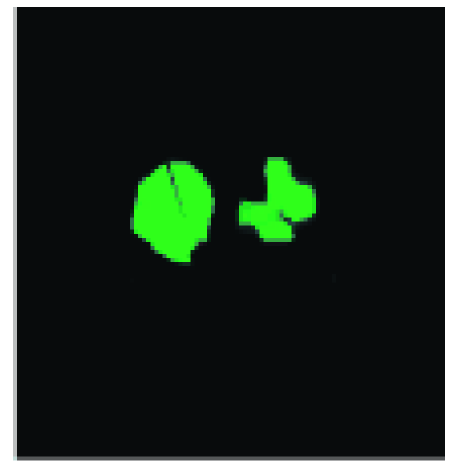
TbL loaded HTC cells

Mixture of cells loaded EuL or TbL



EuL loaded HTC cells

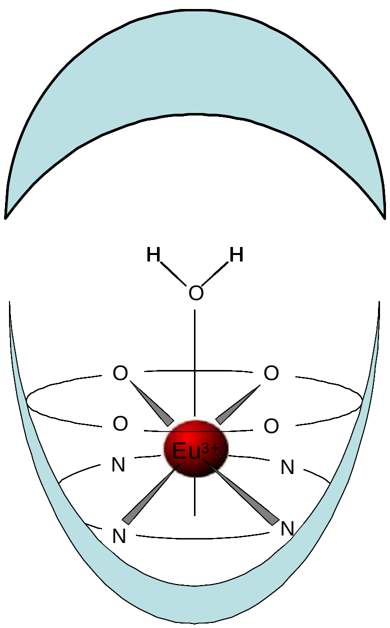
HTC cells



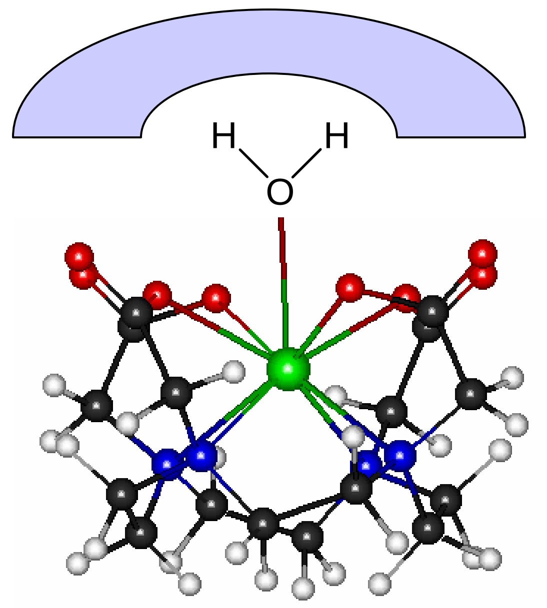
CEST images after irradiation @ -600 ppm

^{1}H images of HTC cells embedded in agar

Molecular recognition complexes



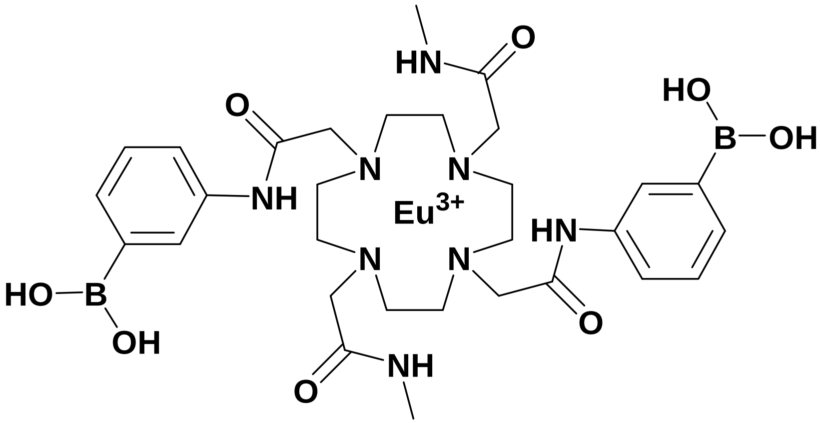
Type II

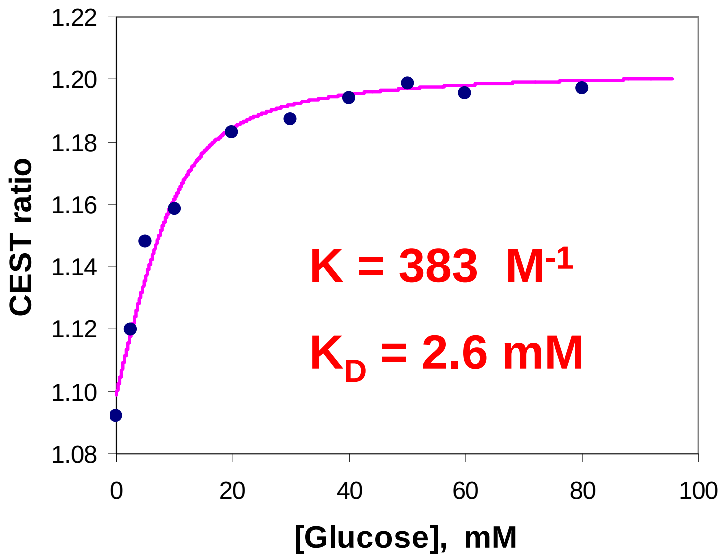


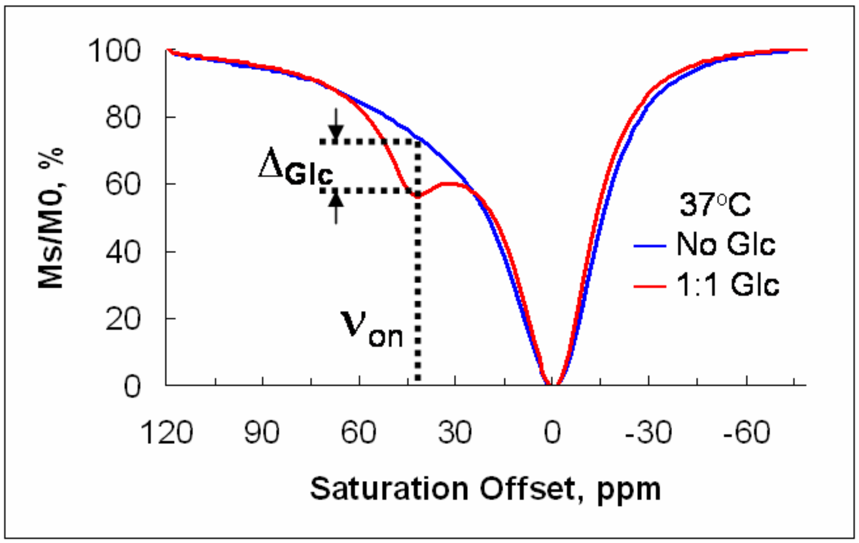
Begin with a fast water exchange system

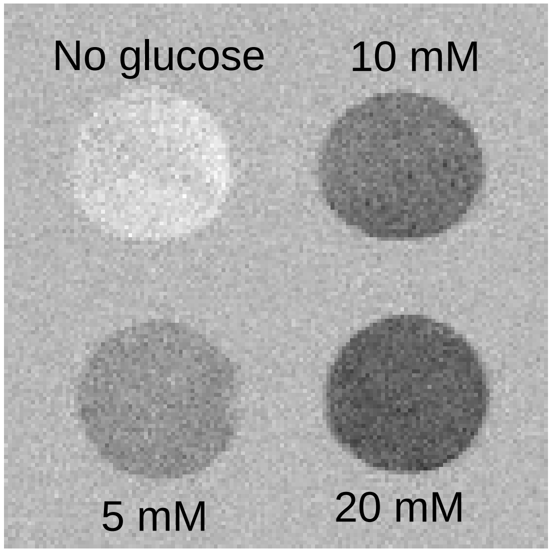
➔ Slow water exchange by molecular recognition $\Rightarrow$ turn "on" CEST

A glucose-sensitive PARACEST agent



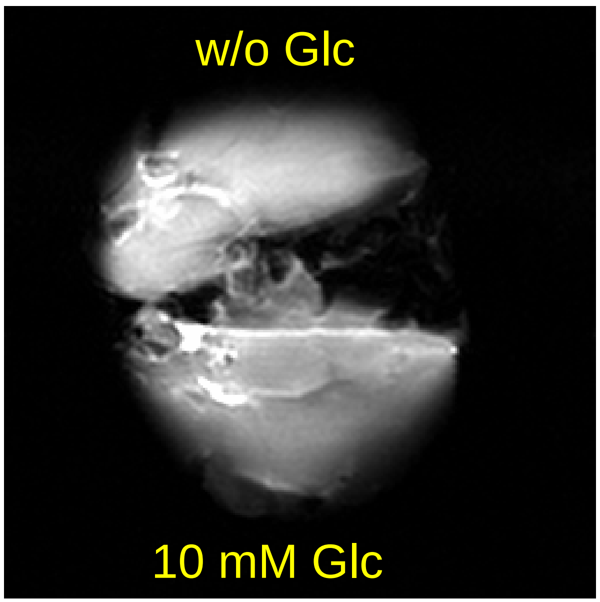




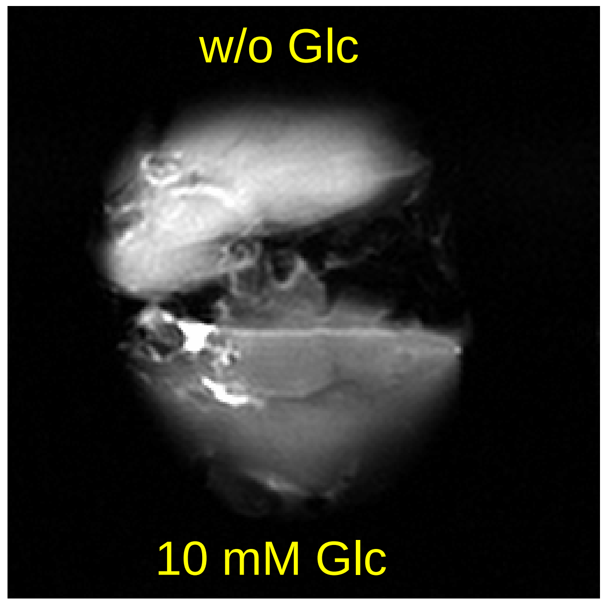


EuDOTMA-2PB: a glucose sensor

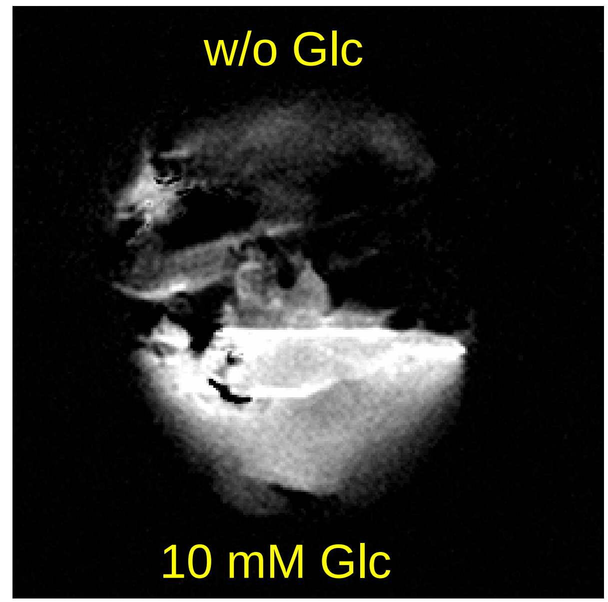
Spin Echo Images | **CEST Images**



Presat. freq. off resonance
(A)



Presat. on Eu^{3+}-bound water @ 42 ppm
(B)



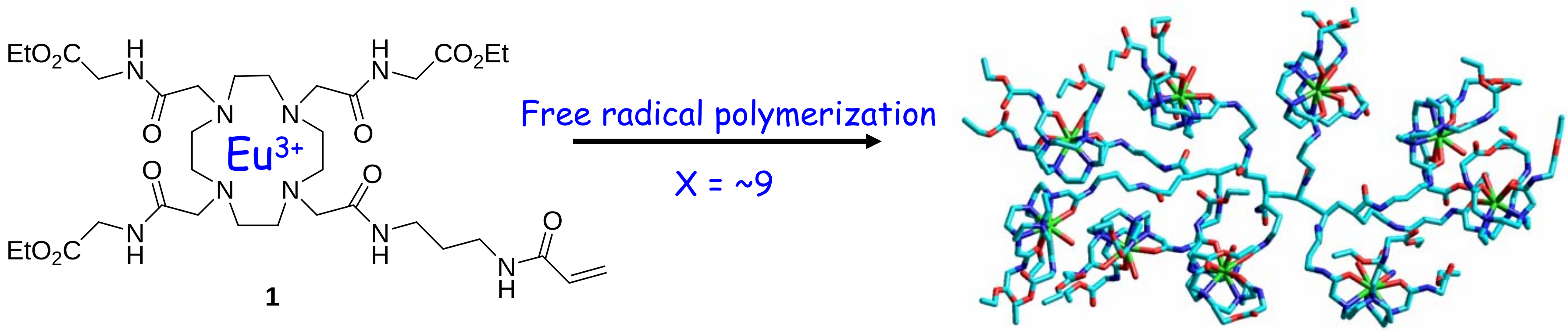
(A) – (B)

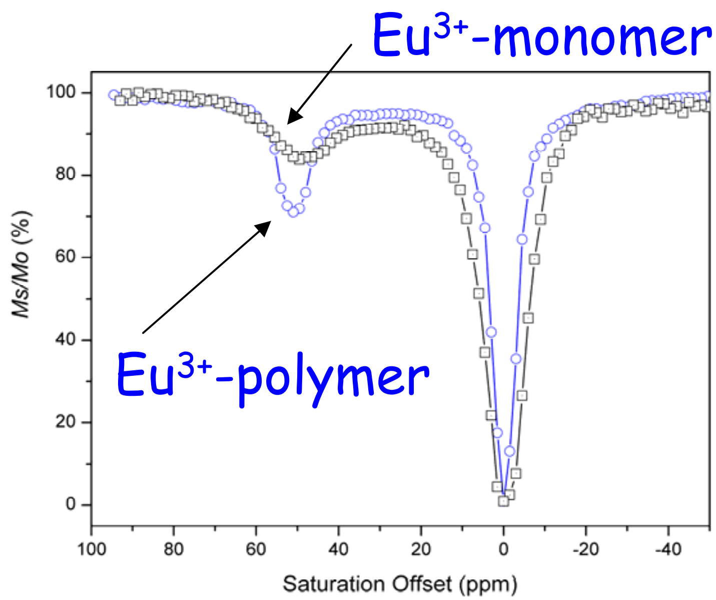
Strategies to enhance PARACEST sensitivity

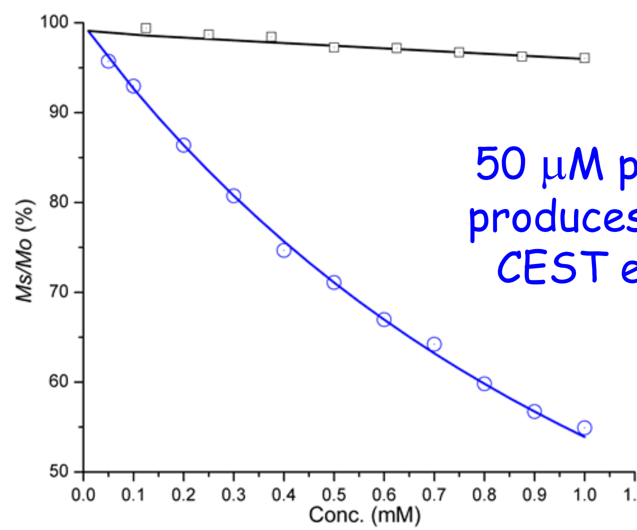
- Amplify the number of exchangable sites
  - Polymeric systems
  - Nanoparticles
  - LIPOCEST

A polymeric PARACEST agent

Y Zhou, M Woods, G Kiefer & AD Sherry, unpublished results



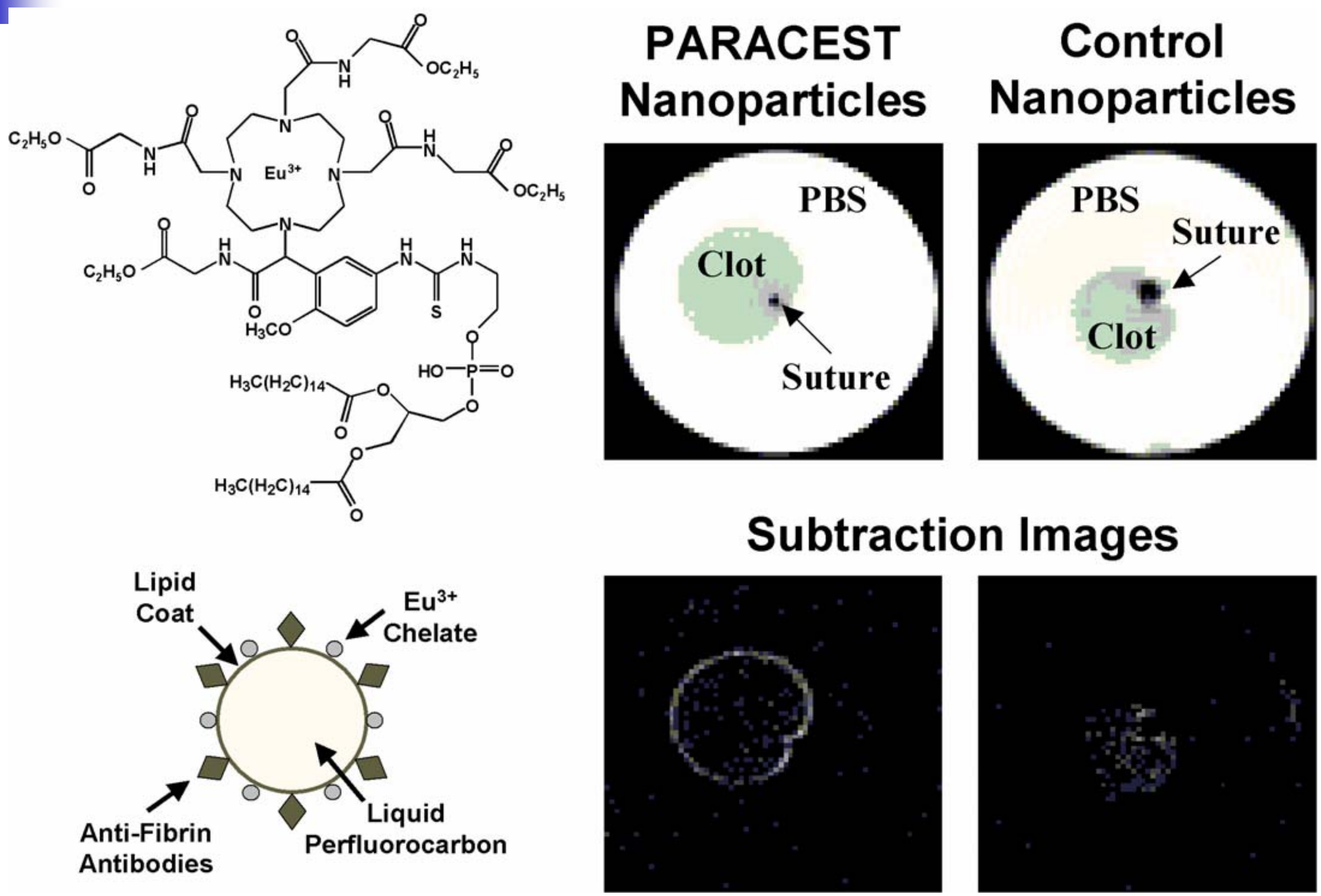




50 μM polymer produces a ~5% CEST effect

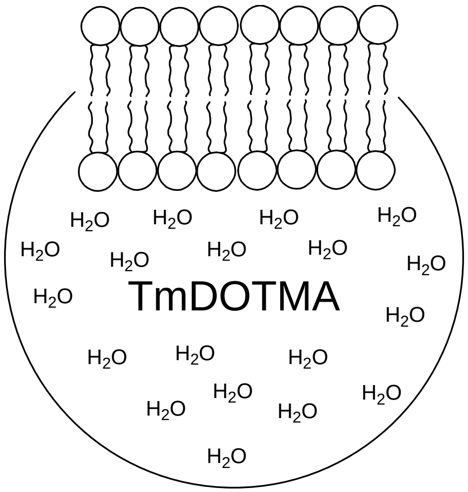
Targeted PARACEST nanoparticles for the detection of fibrin

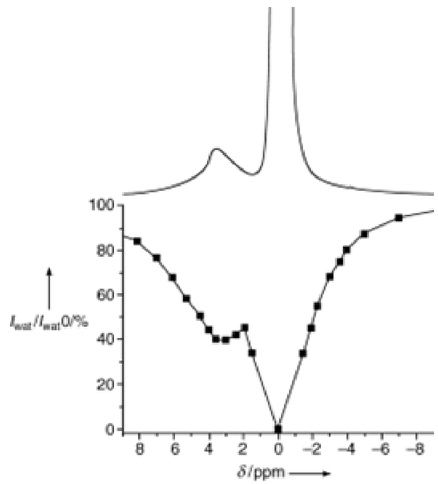
PM Winter, Magn. Reson. Med., 56, 1384-1388 (2006)

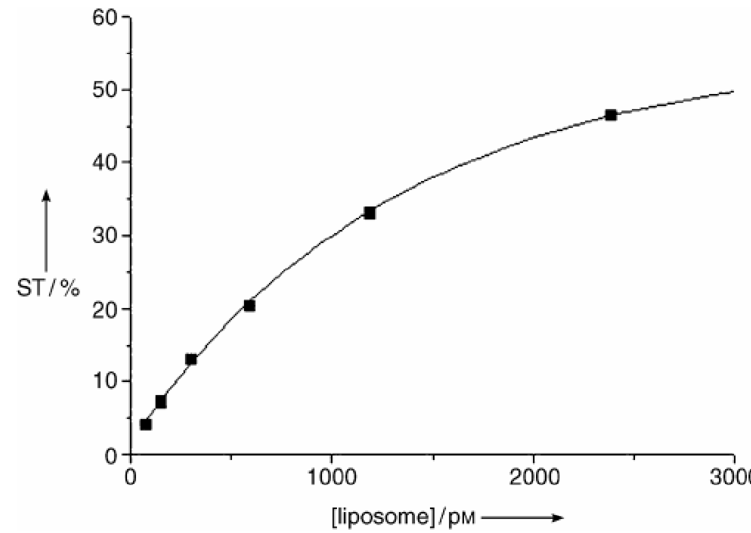


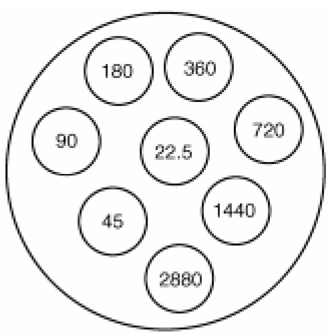
Highly Sensitive MRI Chemical Exchange Saturation Transfer Agents Using Liposomes

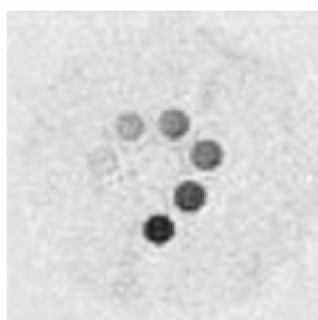
Aime, et al., Angew Chem Int Ed., 44, 5513 (2005)







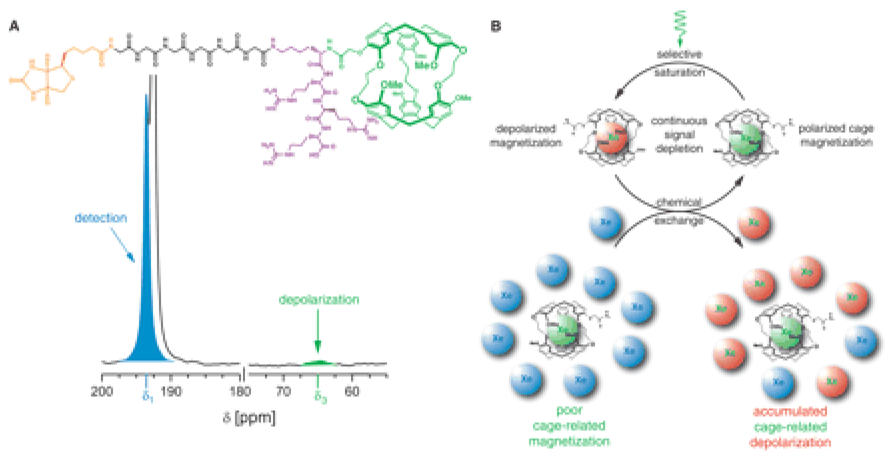


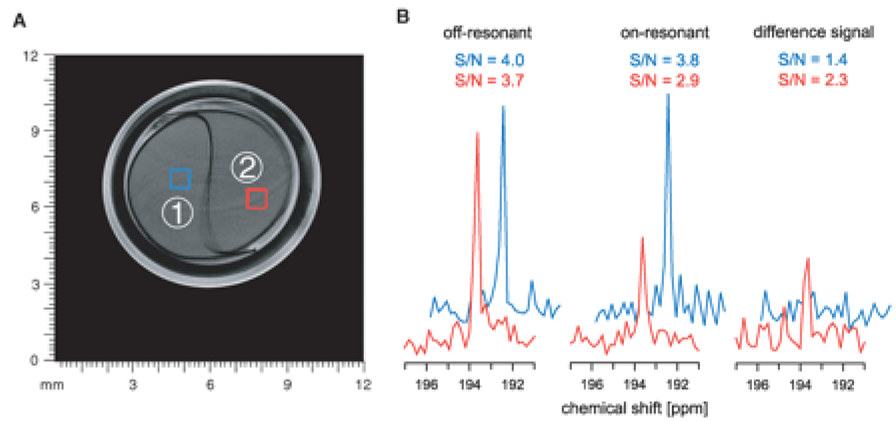


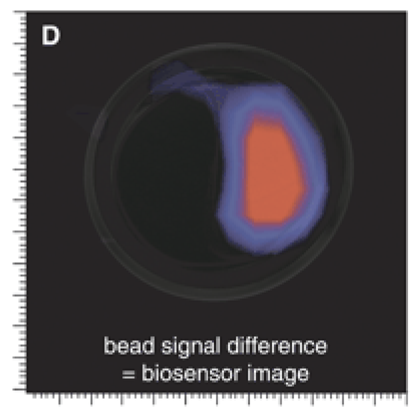
Detection limit
~ 90 pM

Molecular Imaging Using a Targeted Magnetic Resonance Hyperpolarized Biosensor

L Schröder, Science, 314, 446-449 (2006)







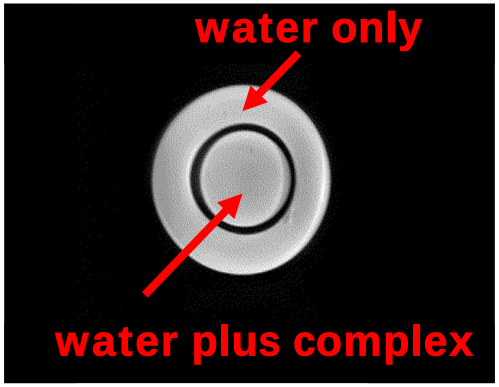
Hyper CEST imaging

Conclusions

- Chemical exchange saturation transfer (CEST) offers new opportunities for imaging metabolism using both endogenous biomolecules and exogenous agents.

- Given the requirement, $\Delta\omega\ \tau_M \geq 1$, the larger $\Delta\omega$ offered by PARACEST agents allows for faster water exchange systems (shorter τ_M), hence greater sensitivity.

- Multiple types of PARACEST agents may be administered simultaneously and activated individually & selectively.



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JHU: Peter van Zijl, Craig Jones
BIDMC: Bob Lenkinski, Elena Vinogradov
GE: Tom Dixon, Ileana Hancu

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