Classes of MR Imaging Contrast Agents and Chemical Modifications Enabling Functional Neuroimaging

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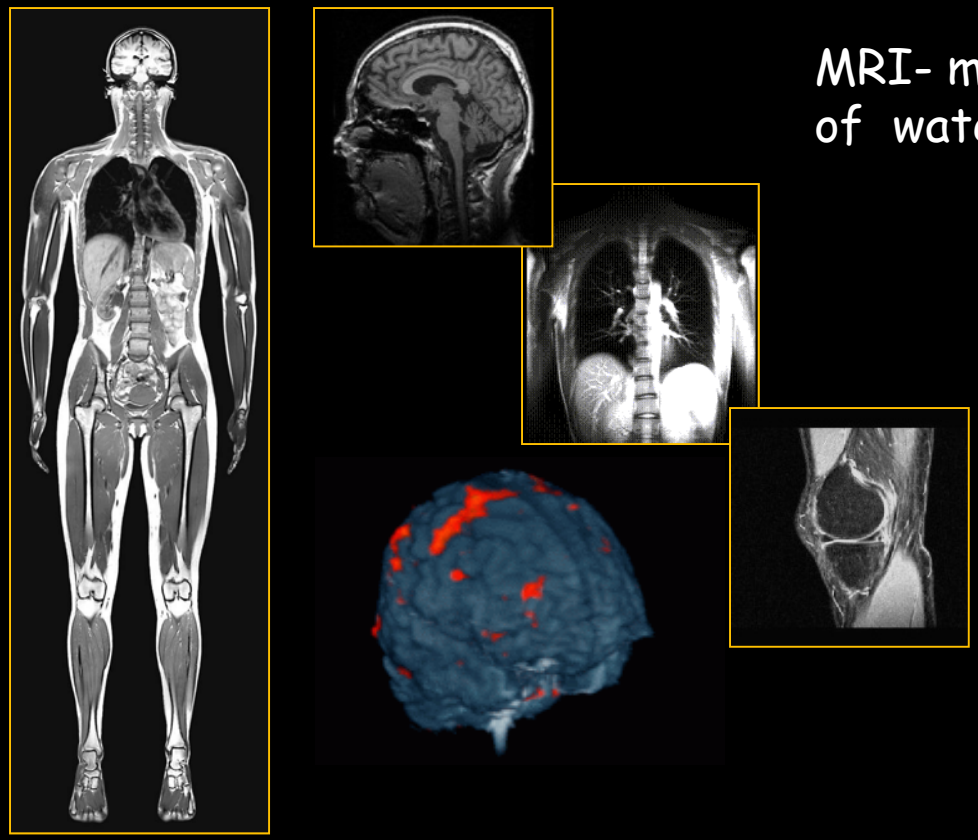
Outline

- Different types of MR imaging probes
- How to design responsive MR imaging probes for molecular neuroimaging?
- „Smart" MR imaging agents for molecular neuroimaging applications

Magnetic Resonance Imaging

Magnetic Resonance Imaging (MRI) is a noninvasive imaging technique that makes the use of the nuclear magnetic resonance to image nuclei of atoms (i.e. water protons) inside the body



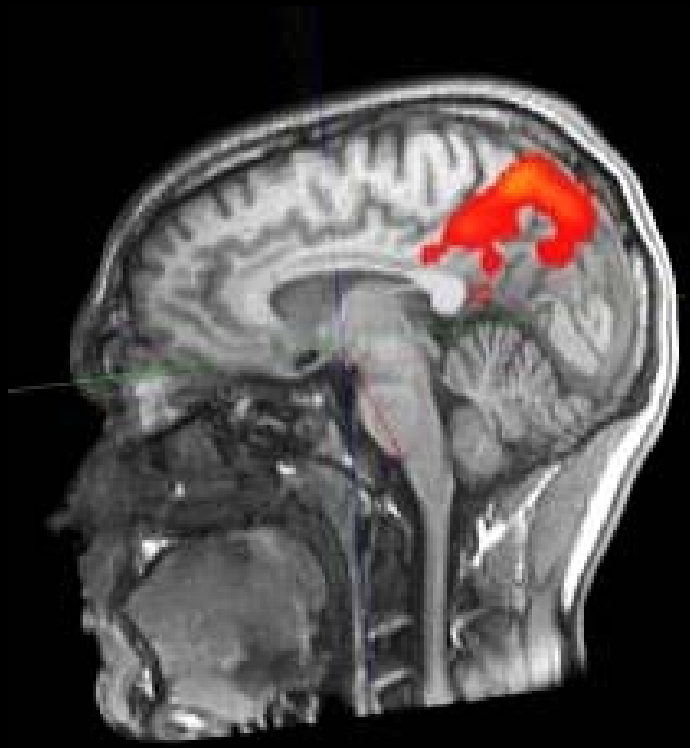
MRI- mainly looking at protons (^1H nuclei) of water molecules (70-80% of H_2O)

^1H MR image intensity :

✓different distribution of hydrogen (spin density) in various tissues

✓T_1 longitudinal and T_2 transverse relaxation times

Funtional Magnetic Resonance Imaging (fMRI)



fMRI-provides an indirect readout of neuronal activity with contrast dependent on cerebral hemodynamics

http://de.wikipedia.org/wiki/FMRI

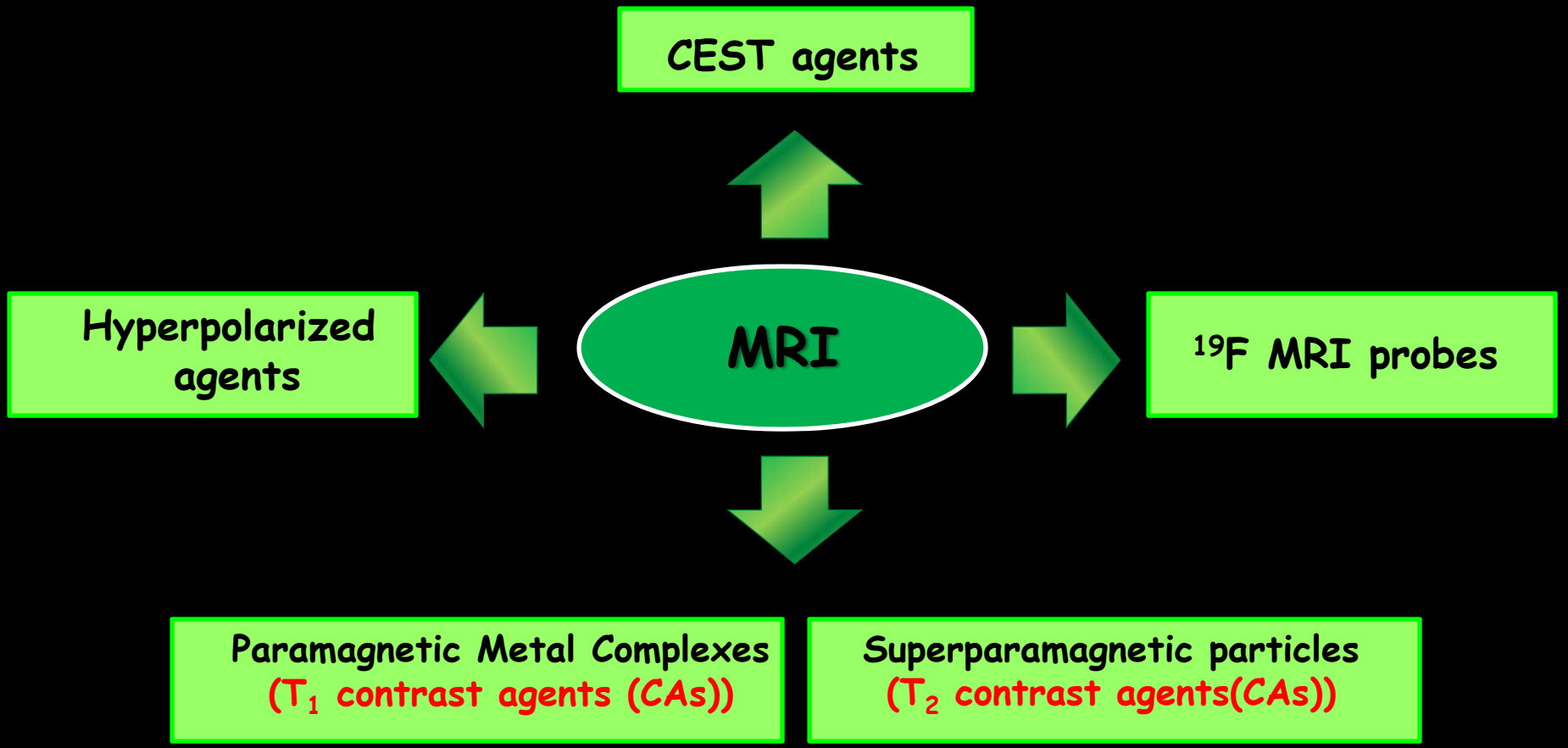
MRI Neuroimaging

How can we get a more direct readout of neuronal activity?

With using molecular MRI probes sensitive to aspects of neuronal physiology…

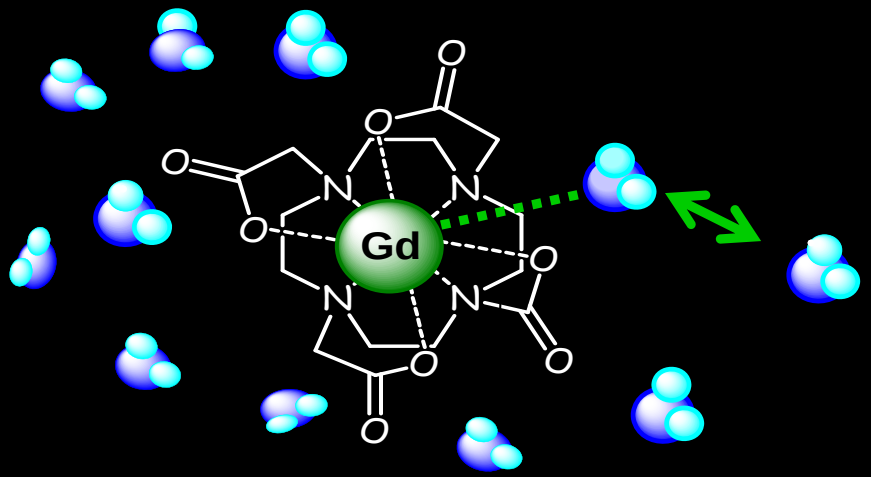
- ✓Metal ion concentration changes
- ✓pH changes
- ✓Gene expression
- ✓Neurotransmitters
- ✓Changes in membrane potential……..

MRI probes

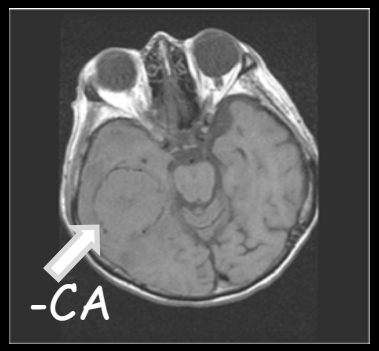

CEST agents
Hyperpolarized agents
MRI
^{19}F MRI probes
Paramagnetic Metal Complexes (T_1 contrast agents (CAs))
Superparamagnetic particles (T_2 contrast agents(CAs))

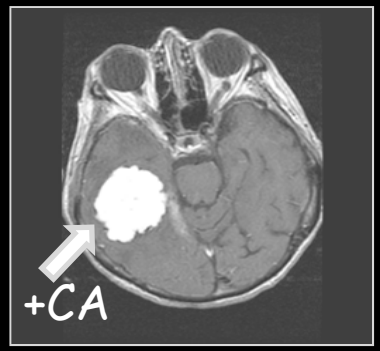
Paramagnetic contrast agents

Function by altering the relaxation times of water protons in the surrounding thereby increasing the contrast in the examined region of interest. Not visible *per se.*

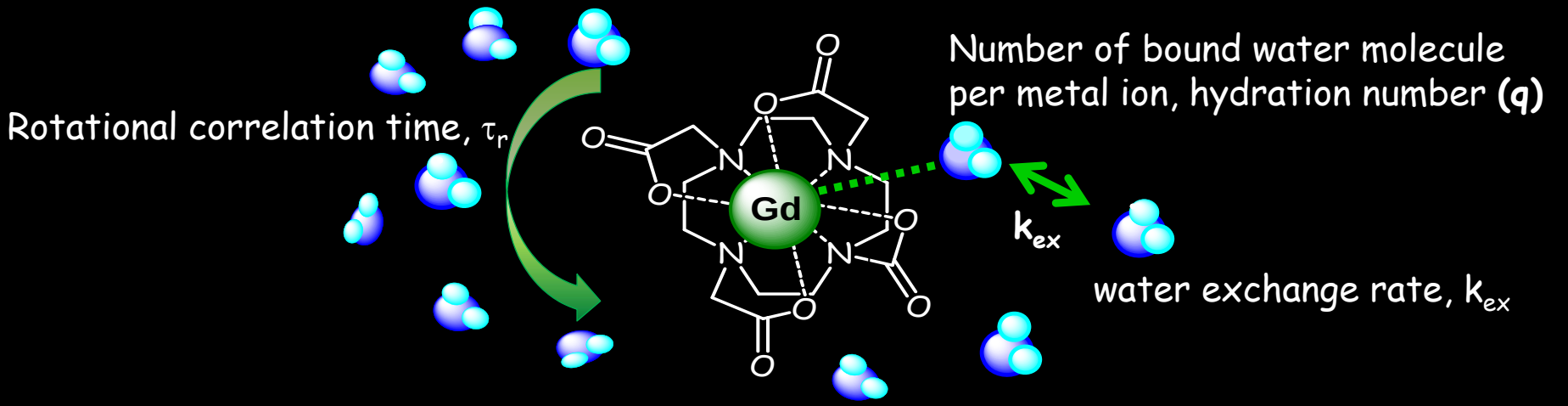


Relaxivity (r_1; r_2)- the efficiency of CA measured as the ability of its 1mM solution to increase the relaxation rate ($R_1=1/T_1$) or ($R_2=1/T_2$) of surrounding water protons





Paramagnetic contrast agents

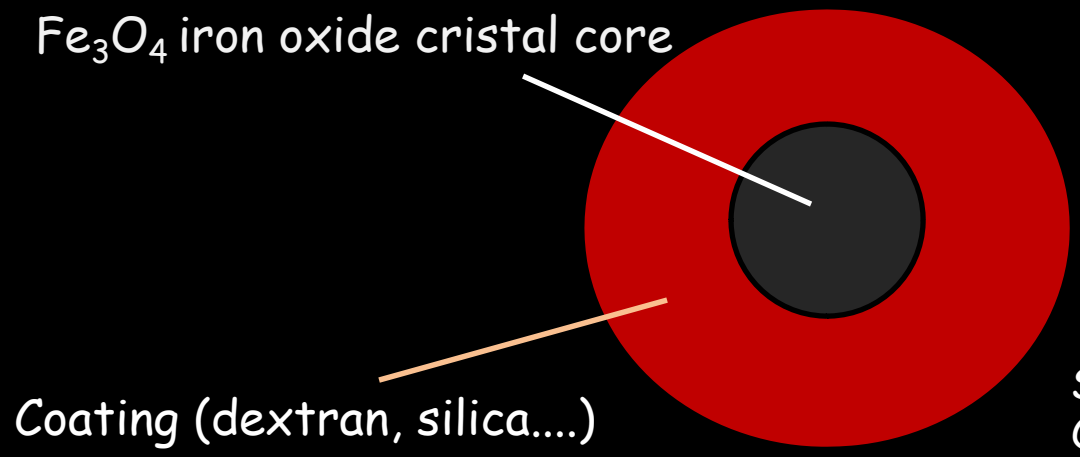


Design of responsive MRI contrast agents:

- changing q number (r_1 is increasing with increasing q number)

-changing τ_r (r_1 is increasing with lengthening of τ_r)

Nanoparticles as T_2 contrast agents



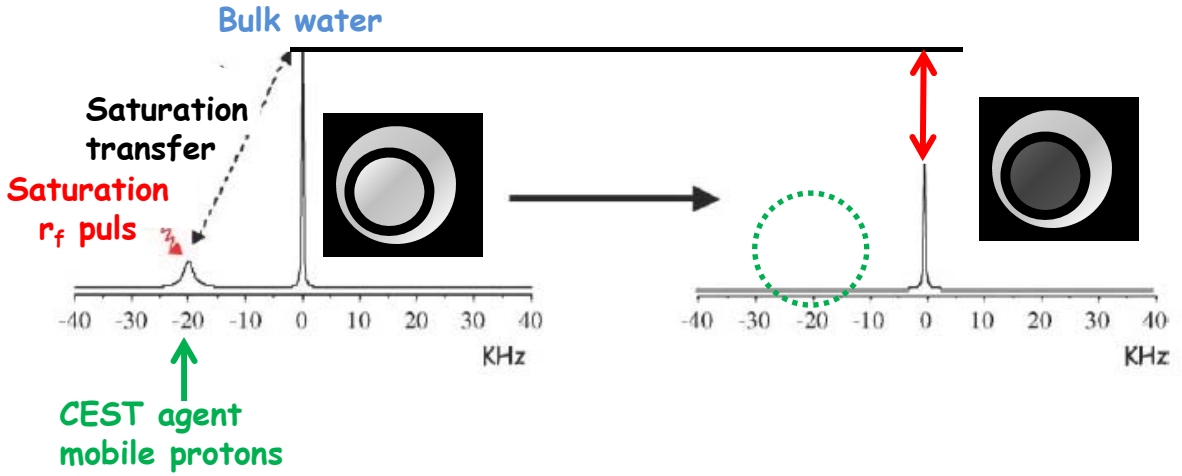
SPIO (Superparamagnetic Iron Oxides, d>50 nM)

USPIO (Ultrasmall Superparamagnetic Iron Oxides, d<50 nM)

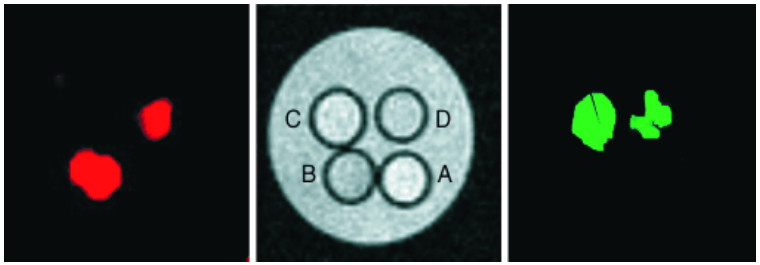
Design of responsive T_2 contrast agents:

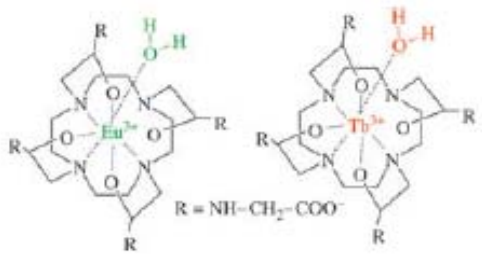
changes in relaxivity achieved by inducing the aggregation or de-aggregation of nanoparticles in the presence of target

Chemical Exchange Saturation Transfer (CEST) agents



CEST agents are able to generate a „frequency-encoded" MR contrast

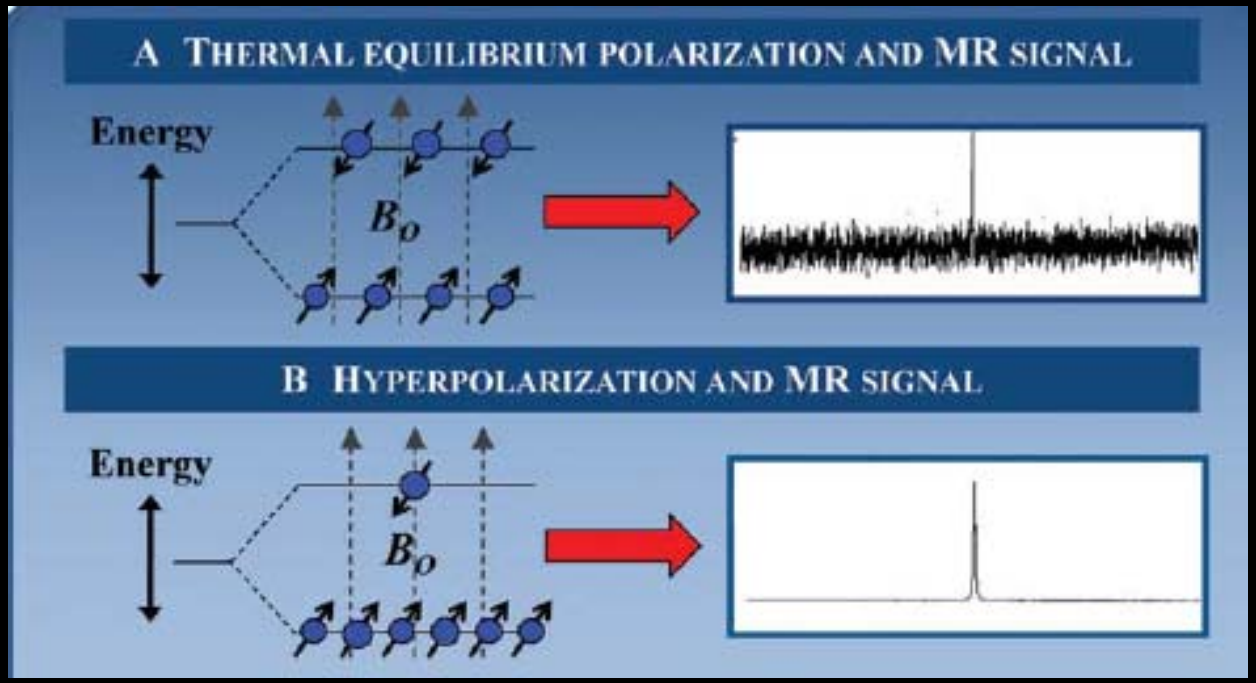


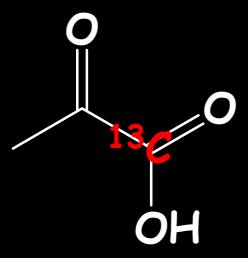


A) Unlabeled cells; B) Tb-labeled cells; C) Eu-labeled cells; D) Tb- and Eu-labeled cells

Aime et al., Angew.Chem.Int.Ed., 2005

Hyperpolarized agents



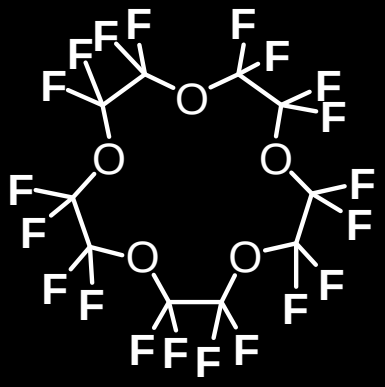


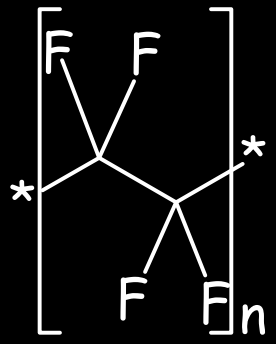
pyruvate

Polarization of 20% can obtained by forcing most spins to point in the same direction, 10000-fold increase in MR signal, reduction of acquisition time, free from background signal

Schroeder M.A., et al. Circulation, 2011)

^{19}F MRI probes





Perfluorinated compunds, direct imaging, no background

Design of responsive NMR/MRI contrast agents:

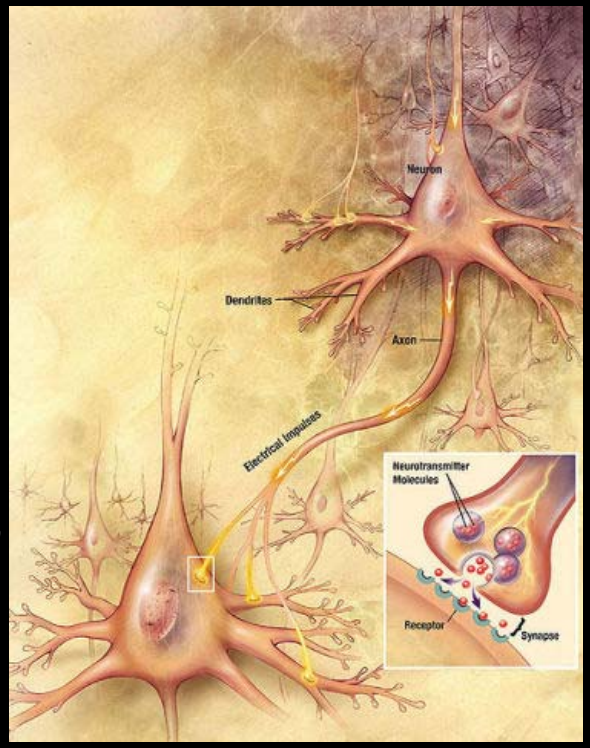
a) modulation of ^{19}F relaxation times (i.e. paramagnetic relaxation enhancement mechansim)
b) changes in ^{19}F NMR chemical shift

MRI probes for Neuroimaging: practical applications

Calcium responsive CA

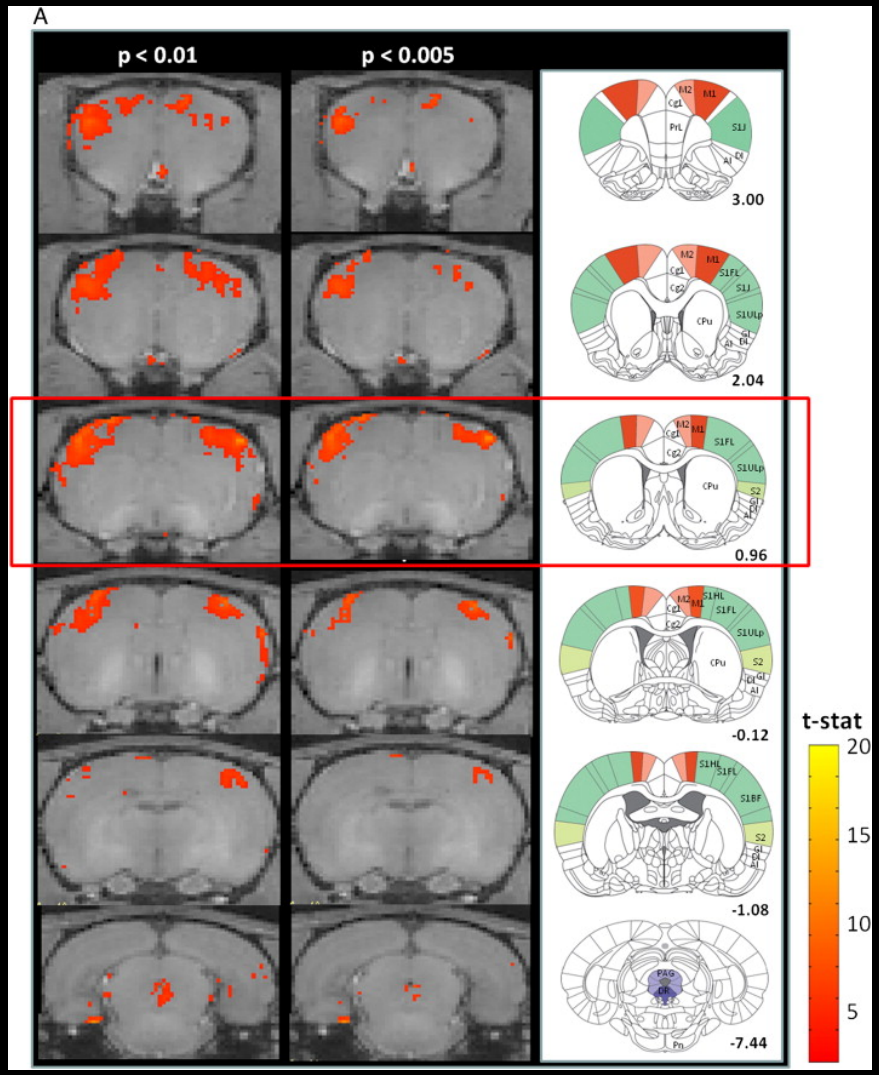
Ca^{2+} plays a critical role during neuronal activity

Changes in intracellular and extracellular concentration of Ca^{2+} during neuronal activity can be used for creating functional MRI probes



http://rienstraclinic.com/newsletter/2006/November/

Mn^{2+} enhanced MRI (MEMRI)



Regions of brain activation related to movements in the running-wheel as revealed by increased intensity of T1-weighted images due to Mn^{2+} accumulation in runners (n = 6) compared to sedentary (n = 6) rats. Right column: corresponding sections from the rat atlas with highlighted primary and secondary motor (red) and sensory (green) cortices

Mapping of functional brain activity

Characteristics :

- Mn^{2+} is a paramagnetic analog of Ca^{2+}, thus can enter voltage gated calcium ion channels

-MEMRI has specificity to image small neuronal representation that would be to small for BOLD based fMRI

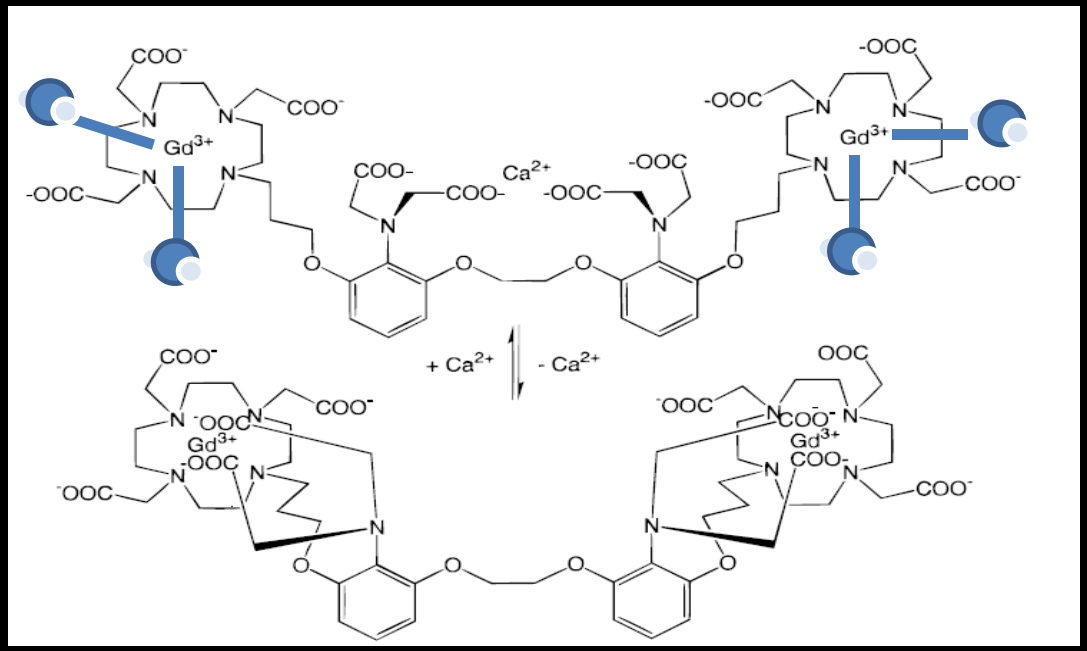
- used for brain connectivty studies

but.....

toxicity concerns as Mn^{2+} leaves the brain on the scale of weeks

Eschenko O. et al., Neuroimage, 2010

Calcium responsive Gd^{3+} based CA



r_1=5.76 $mM^{-1}s^{-1}$

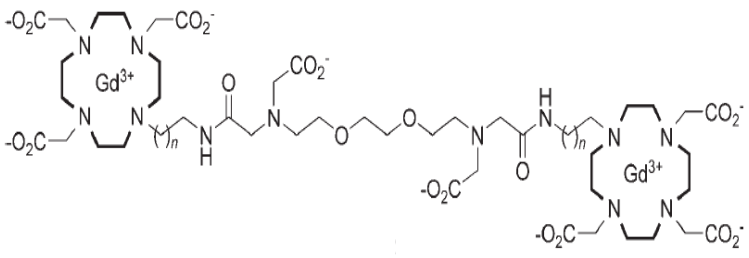
~80% change with 0.1-10 μM of $[Ca^{2+}]$

r_1=3.26 $mM^{-1}s^{-1}$

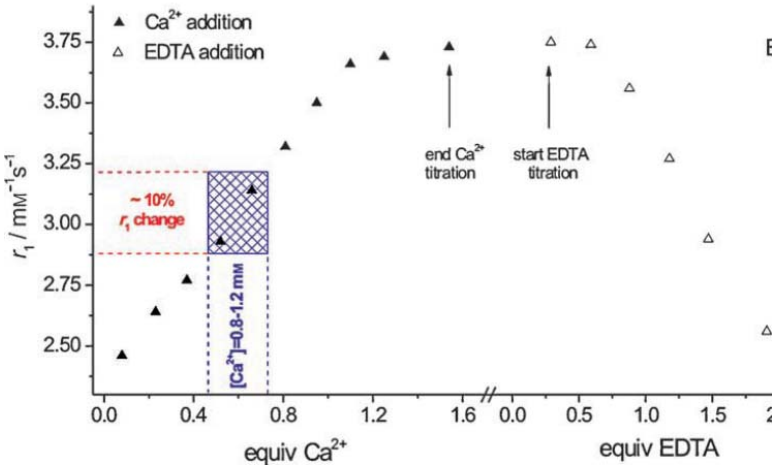
Mechanism of CA activation involving changes in „q" number upon interaction with Ca^{2+}

Li W.-H. et al., J. Am. Chem. Soc., 1999

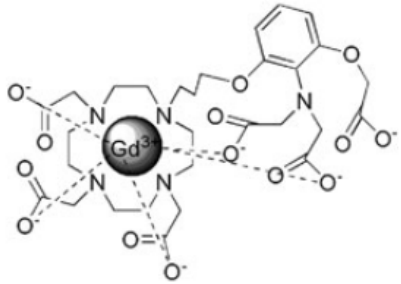
Calcium responsive Gd^{3+} based CA



r_1=3.44 $mM^{-1}s^{-1}$ —Ca^{2+}— 6.29 $mM^{-1}s^{-1}$
83% change (buffer), 7T



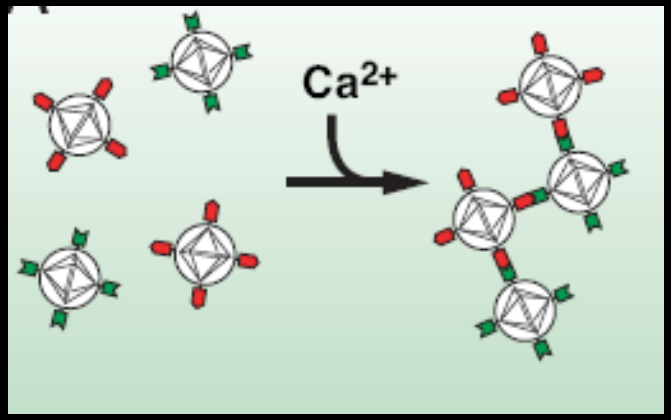
Angelovski G. et al., ChemBioChem, 2008



r_1=3.5 $mM^{-1}s^{-1}$ —Ca^{2+}— 6.9$mM^{-1}s^{-1}$
~100% change (buffer),
~25% change (artificial extracellular matrix) (7T)

Dhingra K. et al., Chem Comm, 2008

Calcium responsive T_2 CA



Reversible aggregation of functionalized SPIO particles in the presence of Ca^{2+}:

Red-------Ca^{2+} binding protein calmodulin (CaM)
Green-----short basic peptides:M13 or RS20 binding to CaM in the presence of Ca^{2+}

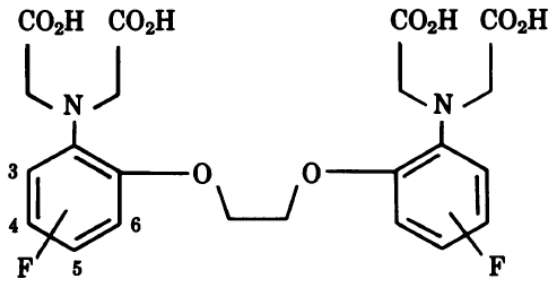
5-fold relaxivity change in the presence of Ca^{2+}:

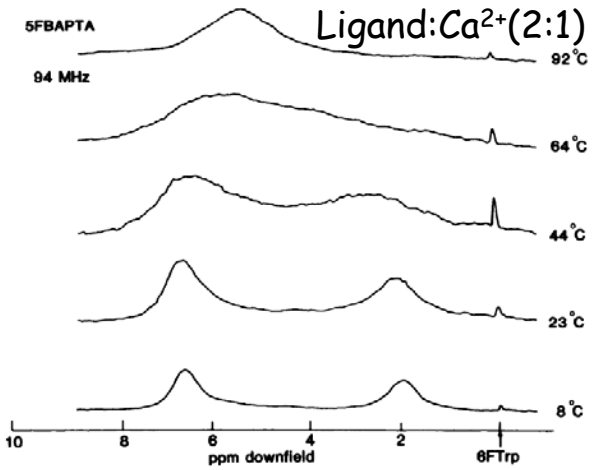
r_2= 220 $mM^{-1}s^{-1}$ (without Ca^{2+}) & 45 $mM^{-1}s^{-1}$ (after addition of Ca^{2+})
r_2= 200 $mM^{-1}s^{-1}$ (without Ca^{2+}) & 34 $mM^{-1}s^{-1}$ (after addition of Ca^{2+})

Atanasijevic T., Proc. Natl. Acad. Sci. USA , 2006

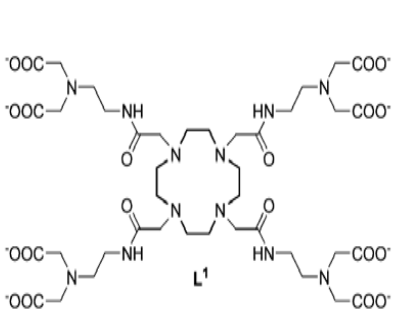
Calcium responsive MRI agents

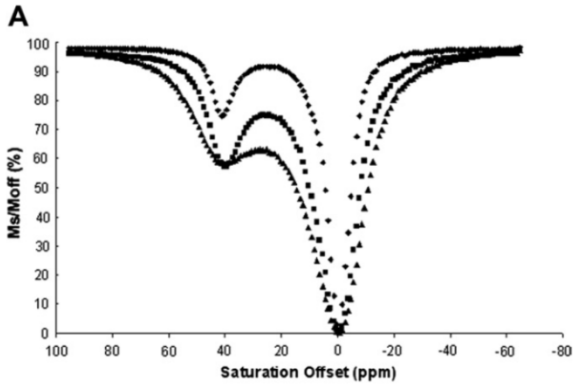
^{19}F chemical shift agent

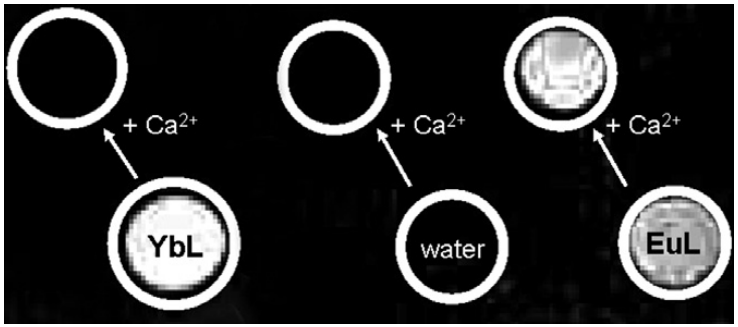




PARACEST agent





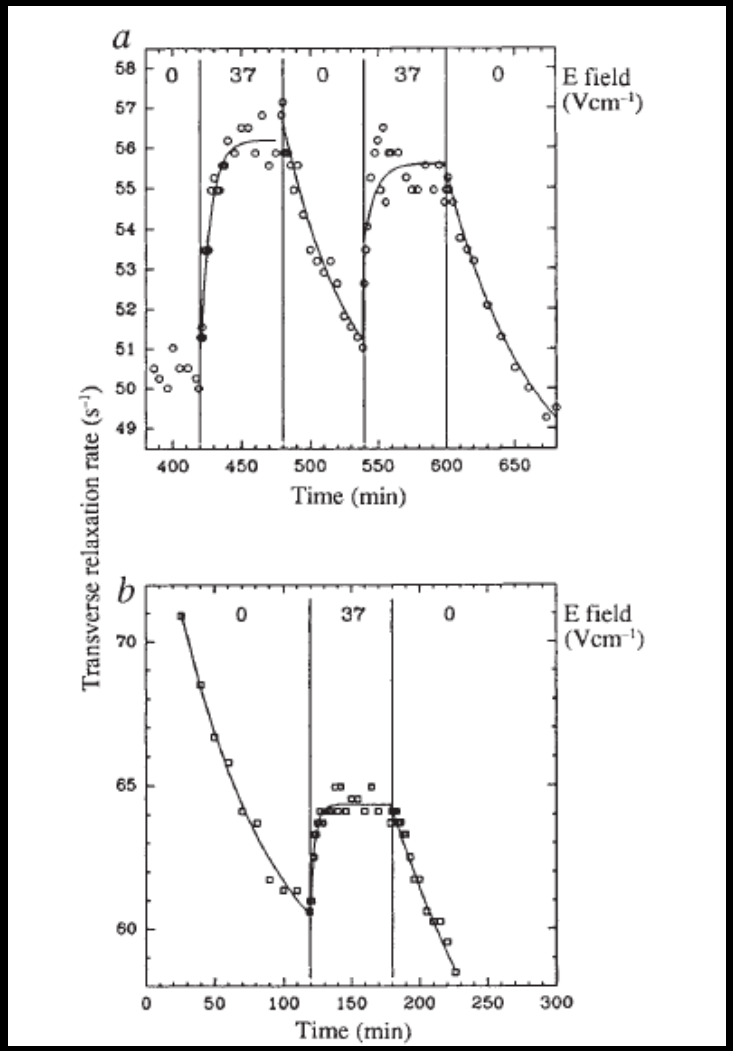


The CEST images represent the intensity difference between the spin-echo images for saturation at δ = 11 ppm and δ = +11 ppm (20 mM YbL1) or at δ = +41 ppm and δ = +26 ppm (20 mM EuL1), 23 C, 9.4 T

Smith G.A. et al., Proc. Natl. Acad. Sci. USA , 1983

Angelovski G. et al., Bioorg. & Med. Chem, 2011

Voltage-sensitive CA



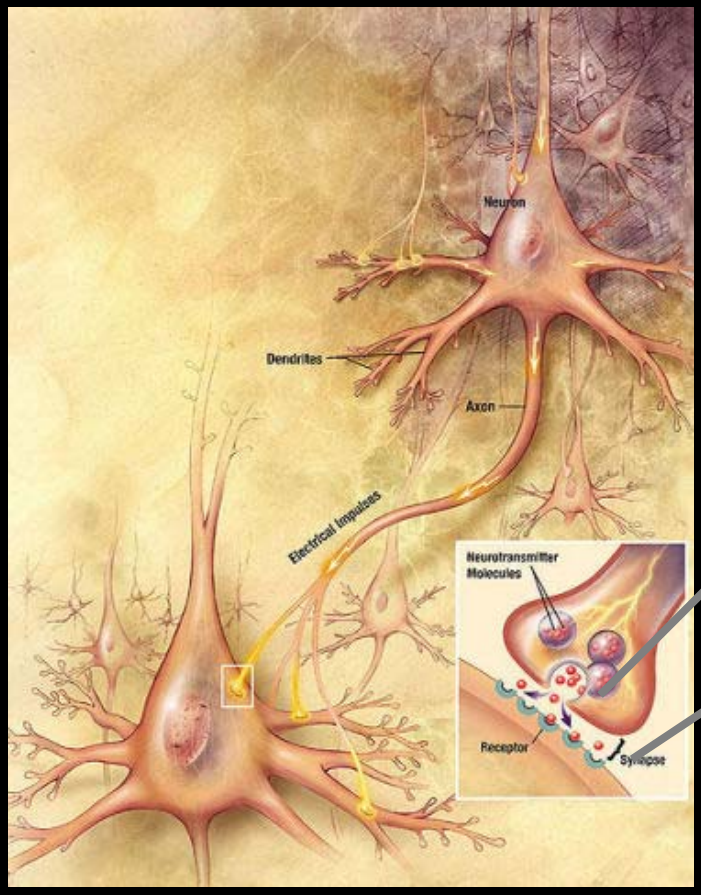
Suspension of magnetic polyelectrolyte gel microparticles (SPIO) with two different sizes of particles. Time course of R_2 with and without an applied field.

Frank & Lauterbur, Letters to Nature, 1993

Neurotransmitter responsive CA

Neurotransmitters: endogenous brain chemicals that allow transmission of signal from neuron to target cell across synapses

Dopamine (DA)
Noradrenaline (NA)
Serotonine (SE)
Glutamate
γ-aminobutyric acid (GABA)
Histamine (5-HT)
Acetocholine (Ach).....

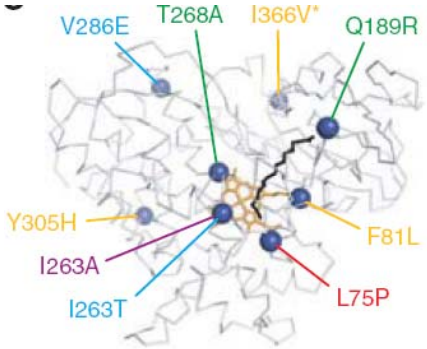


Neurotransmitter imaging

Receptor imaging

http://rienstraclinic.com/newsletter/2006/November/

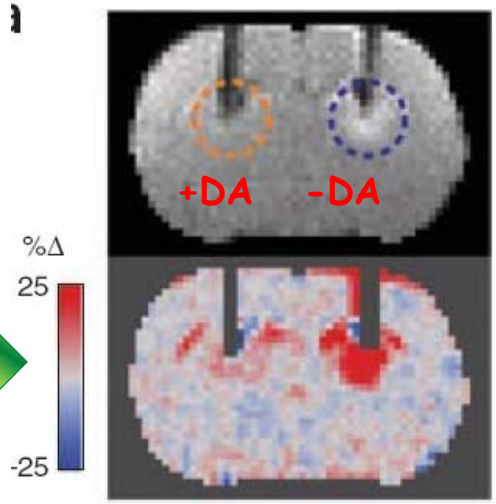
Neurotransmitter responsive CA



Dopamine (DA) sensors was derived from heme domain of the bacterial cytochrom P450-BM3 (BM3h)

Dopamine binding to a site near BM3h's paramagnetic heme iron led to a drop in MRI signal enhancement

BM3h-8C8 sensor can report on dopamine in vivo



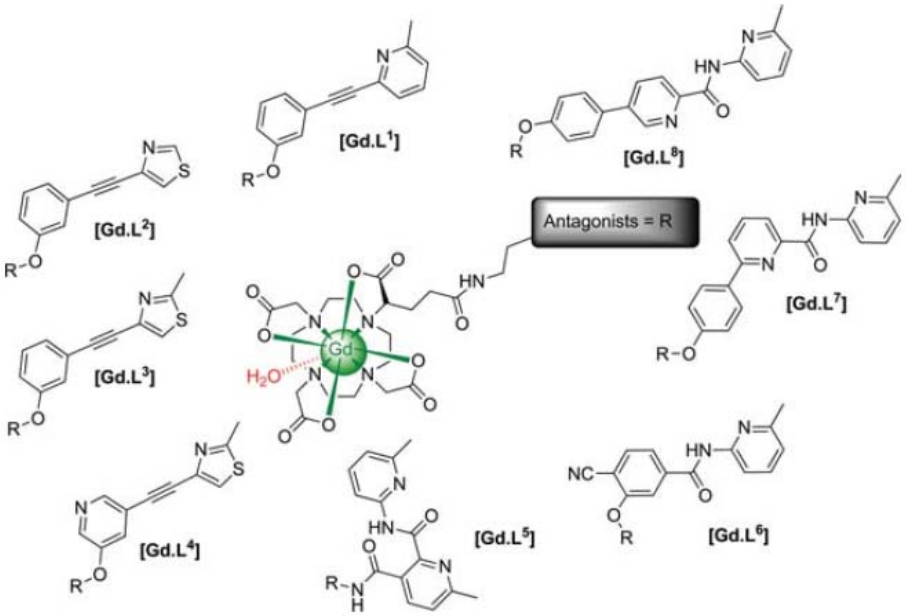
Coronal MRI image of rat injected with 500 μM BM3h-8C8 in the presence or absence of dopamine (500 μM)

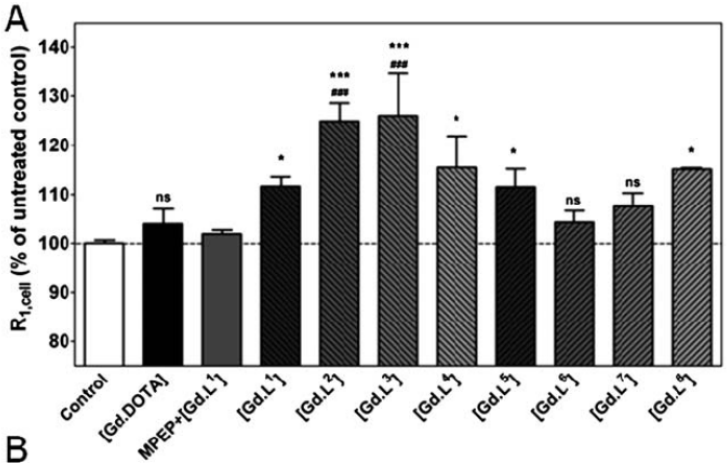
Map of percent signal change (%Δ) computed by comparing pre- and post-injection MRI signal

Shapiro M.G. et al., Nature Biotech. 2010

Neuroreceptor targeting

Glutamate receptors ($mGluR_5$)



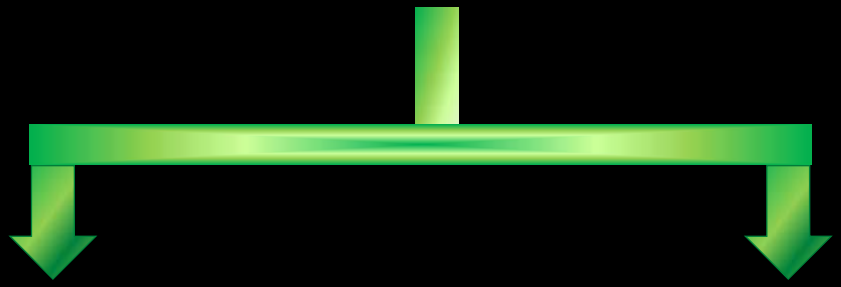


Cortical primary astrocytes from newborn Wistar-rats image after incubation with 100 μM of CA, 45 min incubation time (3T, ~25 C)

Mishra A. et al., Chem Sci, 2012

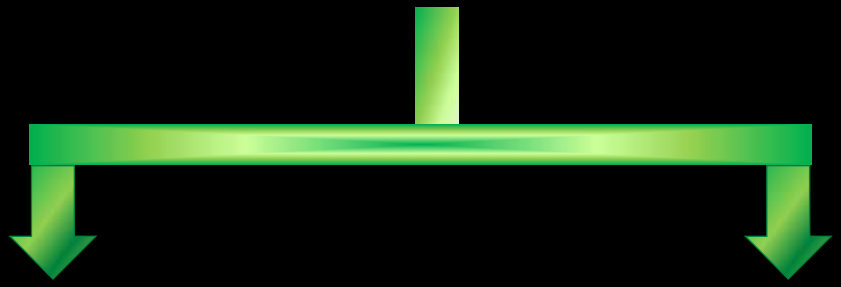
Genetically controlled CAs

Expression of genetically encoded protein



Under control of promoter known to be regulated under neuronal activity

Neuronal stem cells tracking without using pre-labelling approach

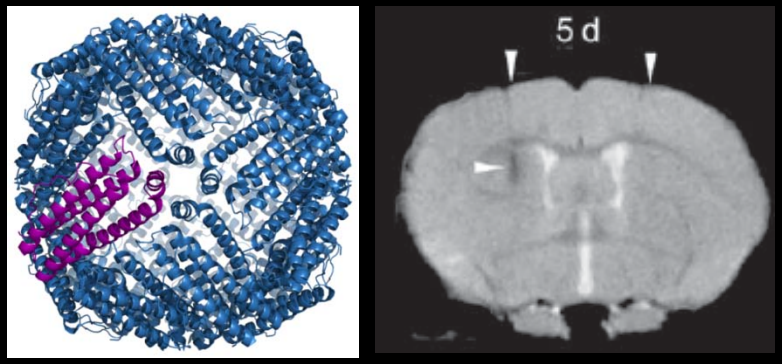


Genetically encodable proteins directly detectable by MRI

"Semi-genetic" approach using reporter genes and exogenous MRI agents

Genetically encoded proteins for MRI

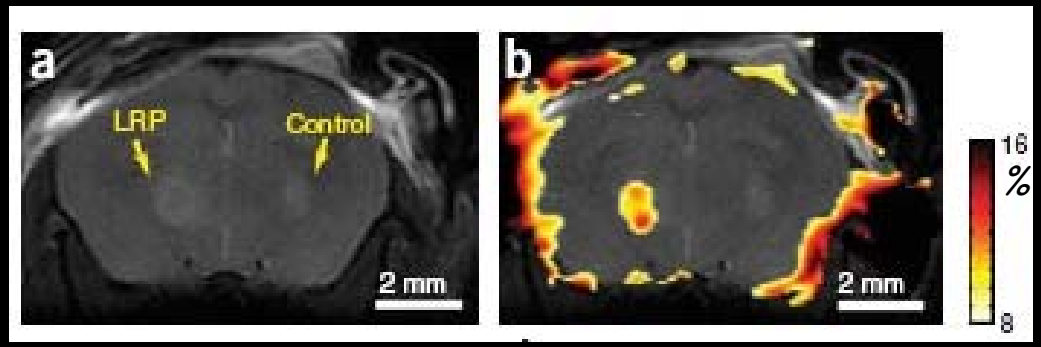
Ferritin, iron storage protein as T_2 contrast agent



In vivo longitudinal results of MRI reporter expression in the mouse brain. Adenovirus containing the MRI reporter was inoculated into the striatum. **(a) T2-weighted image 5 d after injection** showing the inoculated sites (left arrows, MRI reporter; right arrow, AdV-lacZ control).

Genove et al., Nat. Med., 2005

Artificial lysine rich protein (LRP) as CEST agent

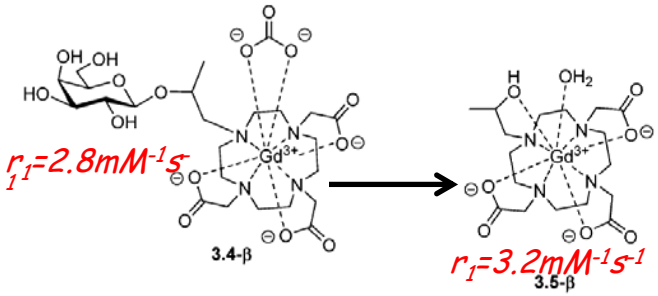


In vivo imaging of LRP. (a) Anatomical image. (b) CEST signal intensity-difference map overlaid on a distinguishes LRP-expressing and control xenografts;

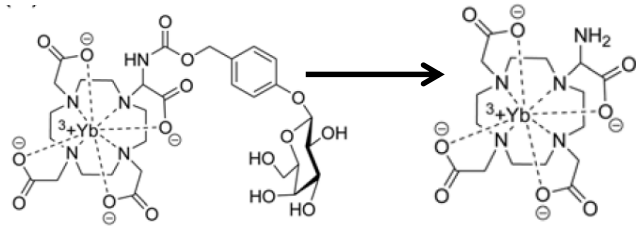
Gilad et al., Nat. Biotech., 2005

(+) genetically targeted to specific cell types
(-) very strong promoter have been used or transfected cells to get enough protein for MRI imaging

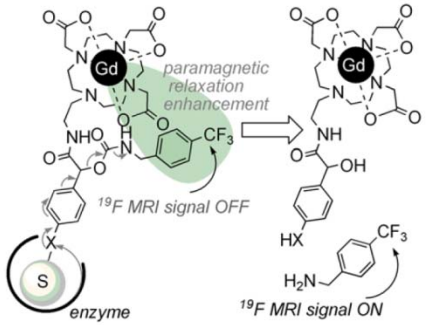
Examples of „Semi-genetic approaches" with reporter gene

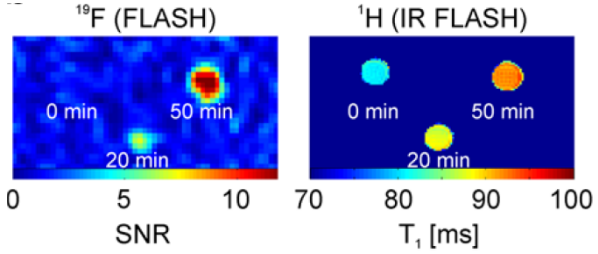


Louie et al, Nat Biotech., 2001

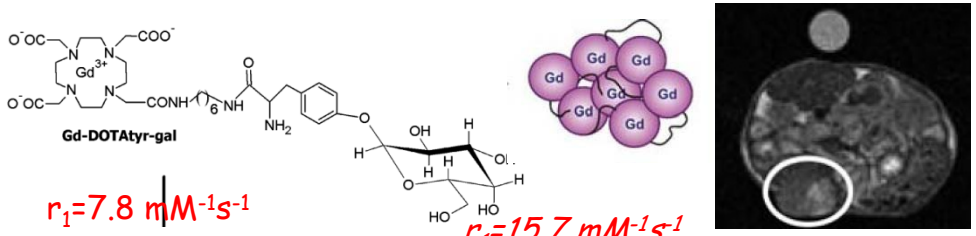


Chauvin et al., Angew. Chem. Int. Ed., 2010

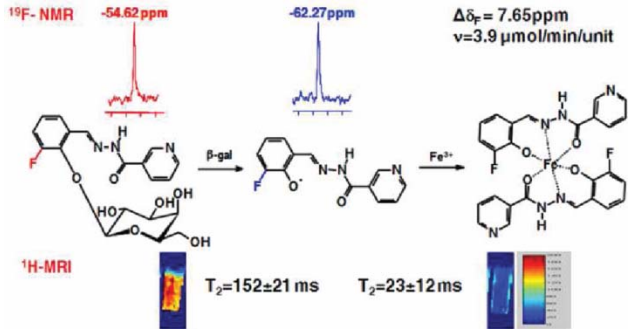




Keliris A. et al., CMMI,2012

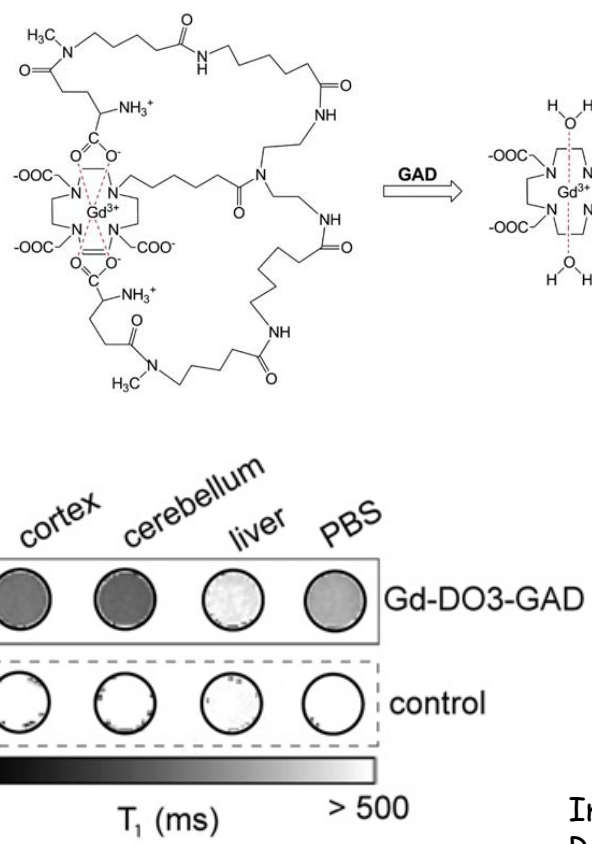


Arena et al., Bioconjug. Chem., 2011

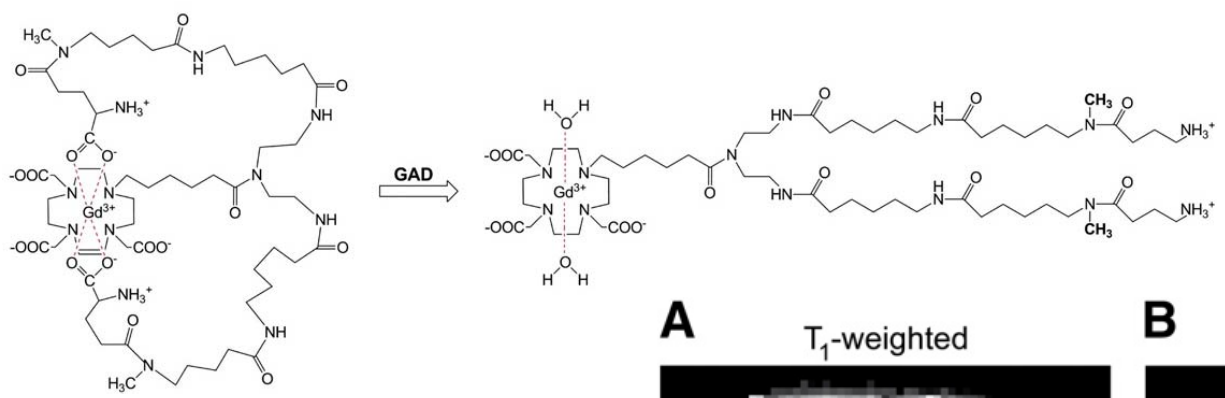


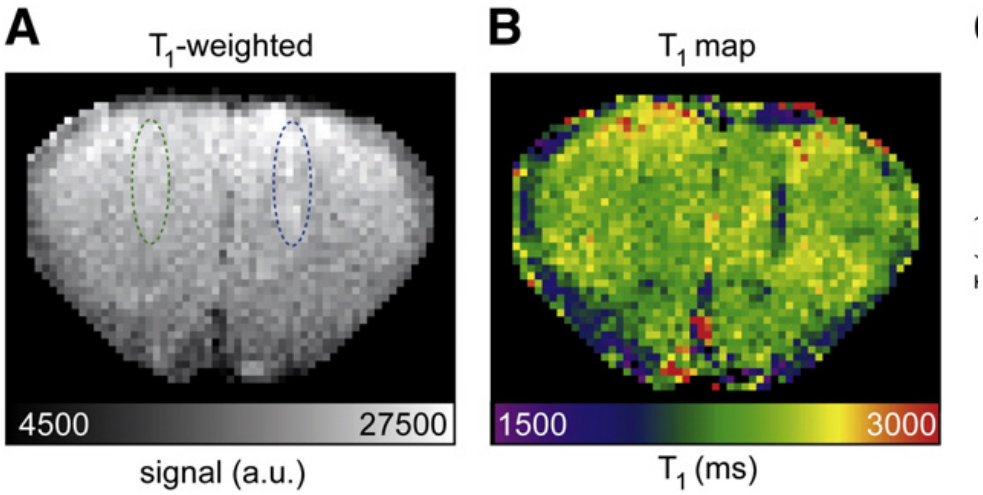
Yu et al., Bioconjug. Chem., 2012

Imaging of GABArgenic neurons



Tissue cell lysates containing GAD



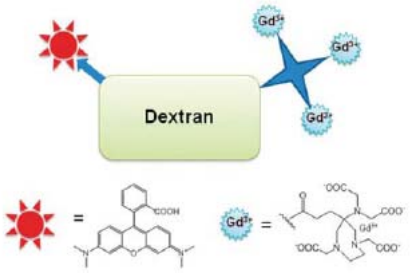


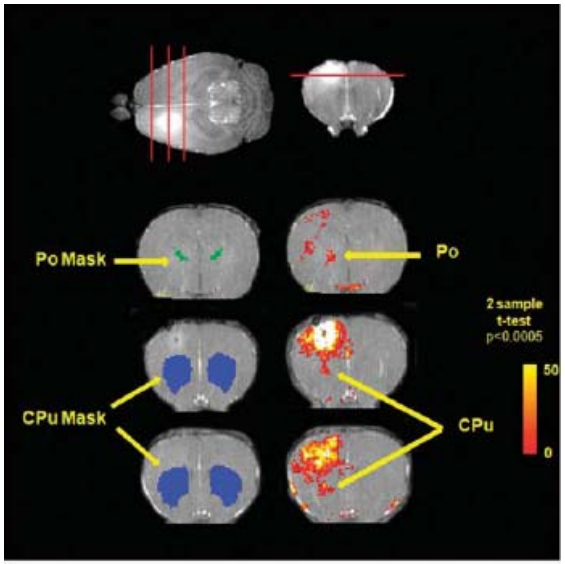
Implanted native and differentiated cells in the mouse brain. A) Representative T_1 weighted image at 0.5 h after implantation of labeled differentiated cells (right striatum) and labeled undifferentiated cells (left striatum). B) Corresponding T_1 map revealing distinct lower relaxation rate for the differentiated cell graft.

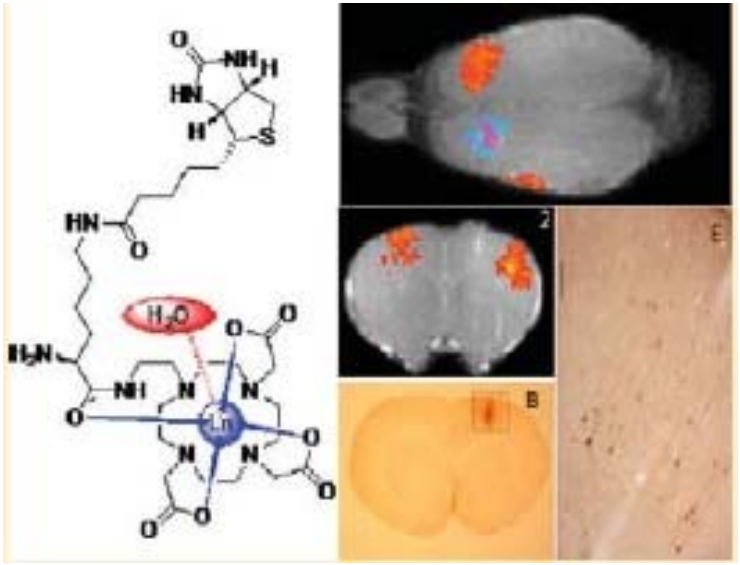
Aswendt et al., Neuroimage, 2012

Neuroanatomical MRI tracers

Tract-tracing techniques help to reveal valuable information about connectivity of neuronal networks between various regions in the brain







Mamedov et al, Chem. Comm. 2011

Mishra et al, ASC Chem. Neuroscien. 2011