# Hyperpolarized MRI: metabolic imaging

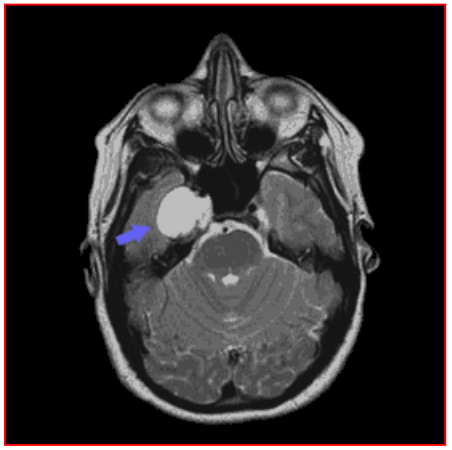
# “Classic ” MRI

Magnetic Resonance Imaging (MRI) is based on the observation of water $^{1}H$ nuclei (100% natural abundance, great quantity of water in living organisms).

Signal intensity depends on proton density and relaxation times $T_1$ and $T_2$.

**CONTRAST:** difference in signal intensity between adjacent regions of the image

**Contrast agents**: substances which enhance the contrast in the image. Usually they are paramagnetic metal complexes which alter the relaxation rates of water protons.



information about tissue morphology

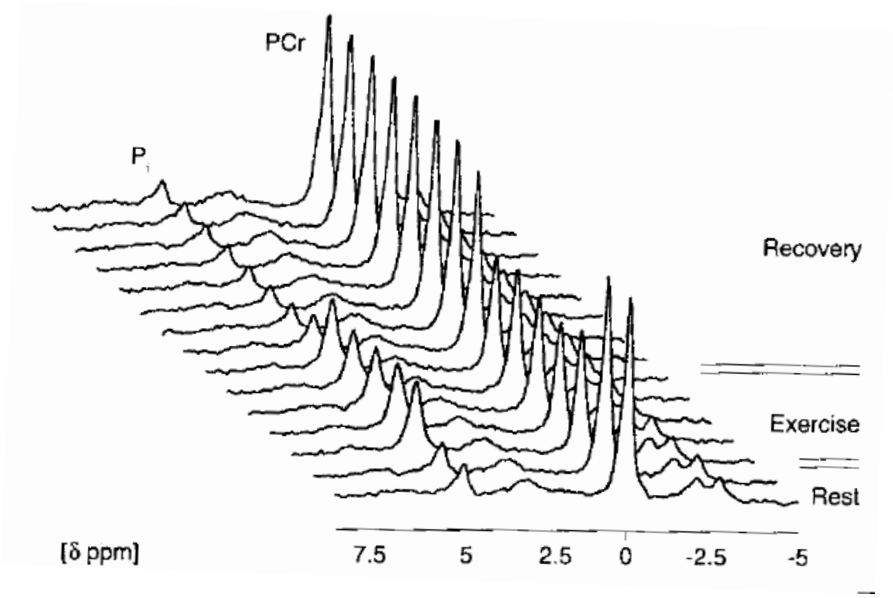
no information about molecules

Chemical shift information → metabolites can be detected in vivo

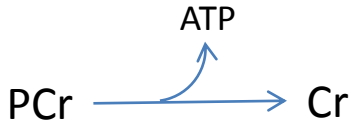
## Metabolic information from MRS

Informations about the metabolic state of a tissue can be obtained from MRS of nuclei such as $^{1}H$, $^{13}C$, $^{19}F$, $^{31}P$.

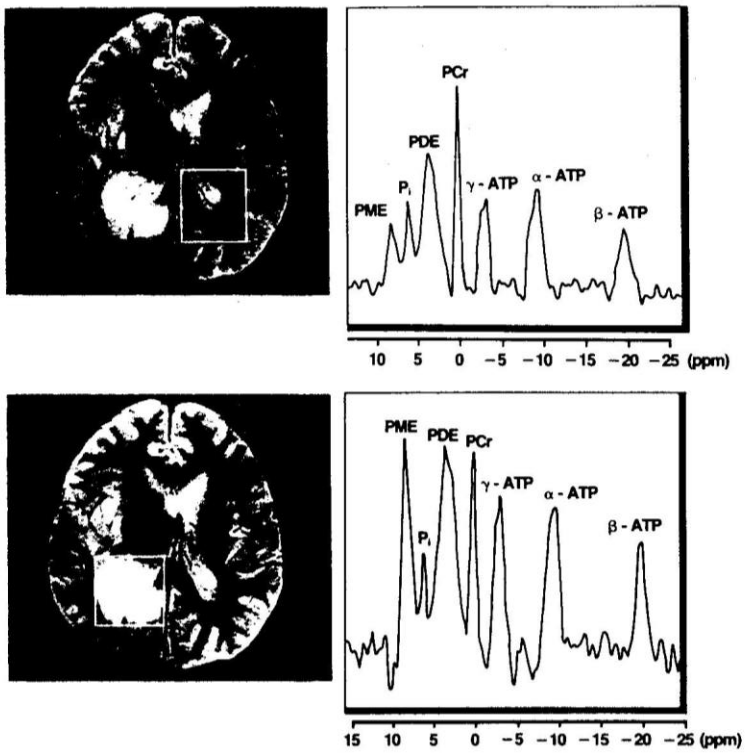
$^{31}P$ MRS of calf muscle




PCr: Phospho-creatine



# metabolic processes are altered in diseased tissues

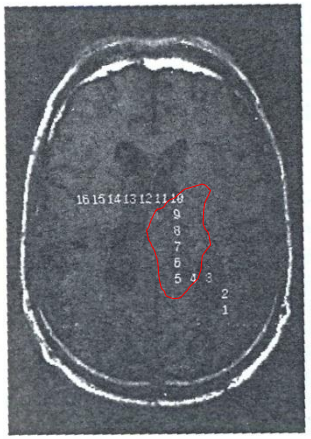



$T_1$ weighed image of brain with tumor; comparison between $^{31}P$ spectra of the healthy region (top) and brain tumor (bottom)

## metabolic processes are altered in diseased tissues

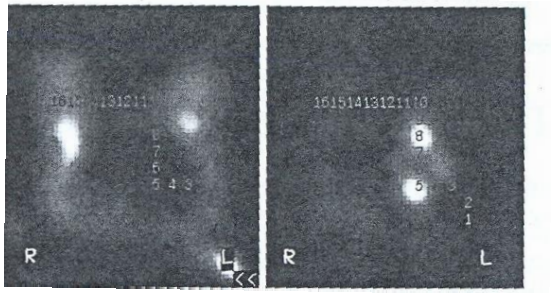
# Chemical Shift Imaging

High grade glioma

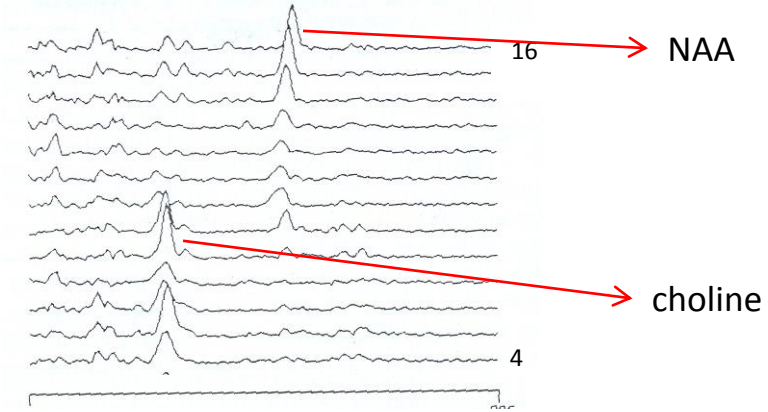


$T_1$ weighted image

. space resolution is low
. long acquisition time,
. no real time metabolic information…



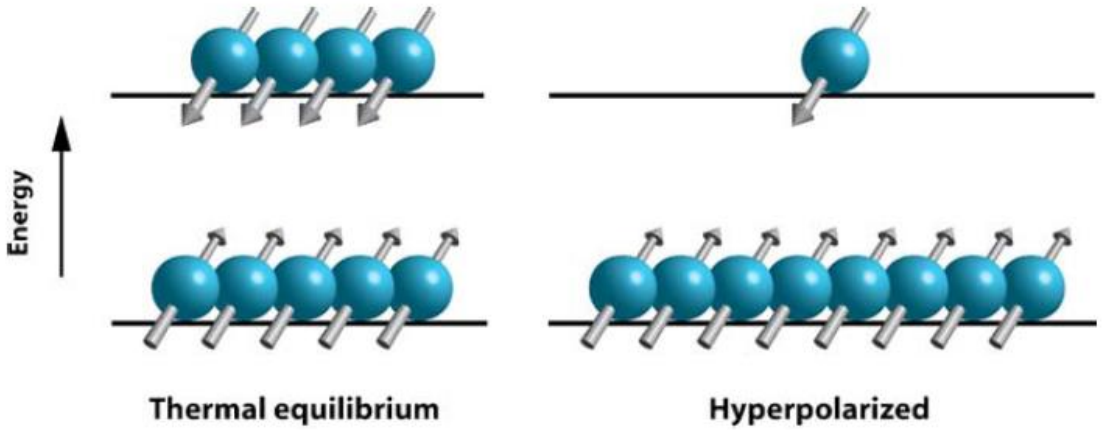

Metabolic maps ($^1$H-CSI) of NAA (left) and choline, NAA is decreased in tumor while choline is elevated




$^1$H NMR spectra from regions 4 to 16

# What does “hyperpolarization” mean?

Thermal equilibrium Hyperpolarization




$$P = \frac{N^+ - N^-}{N^+ + N^-}$$

**@ 1.5 T:**

$^{1}H$ $\Delta N/N \approx 5 * 10^{-6}$

$^{13}C$ $\Delta N/N \approx 1 * 10^{-6}$

→ Low NMR sensitivity

MR signals can be enhanced (theoretically) up to $10^5$ times

# Main features of hyperpolarized CA

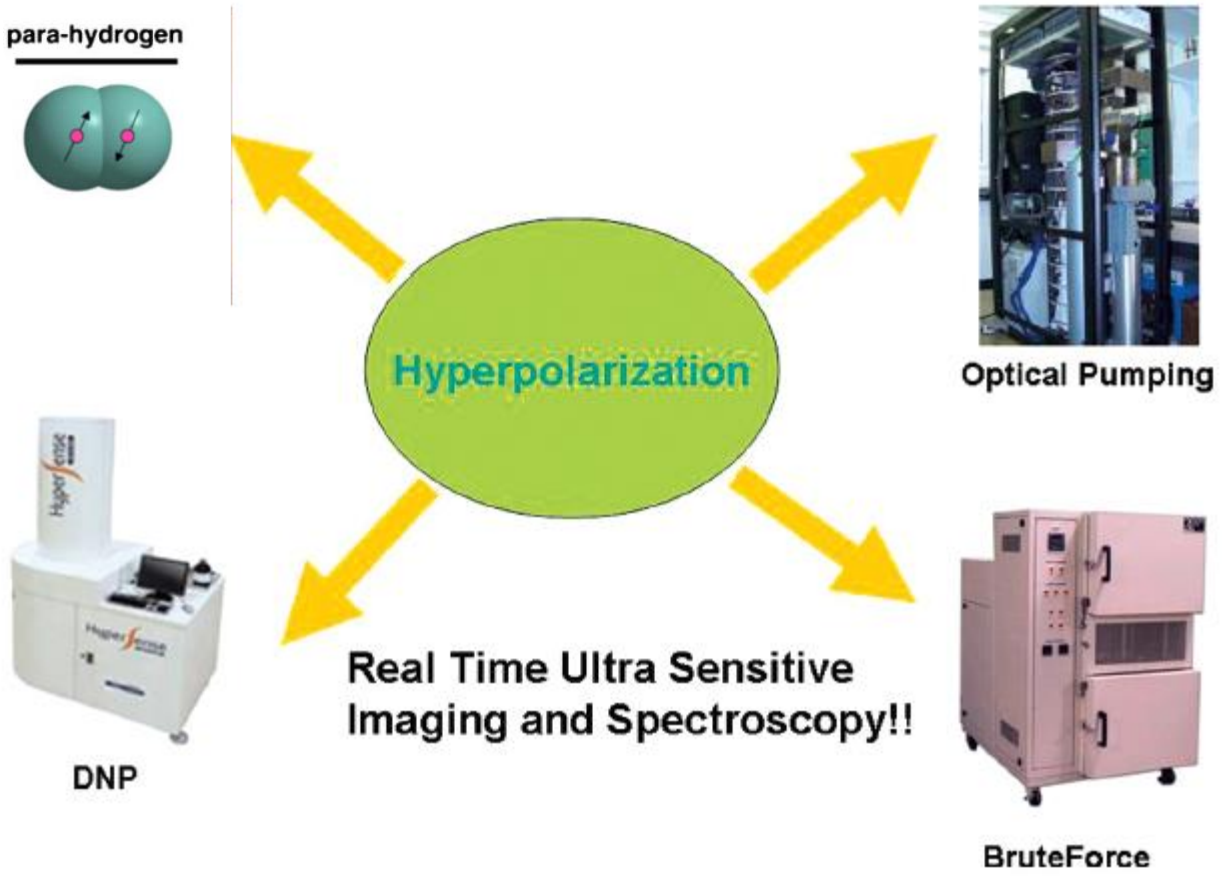
Contrast is given by the molecule itself, not by the modulation of the water signal

The endogenous signal is usually zero: optimal contrast, but $^{1}H$ images are necessary for anatomic information

Hyperpolarization tends to disappear due to relaxation processes (longitudinal relaxation) and cannot be restored

→slowly relaxing signals:
heteronuclei ($^{13}C$, $^{15}N$ ) are preferred
small molecules (small $\tau_c$, long $T_1$)
quaternary $^{13}C$ groups, carbonyl, deuterated groups...)

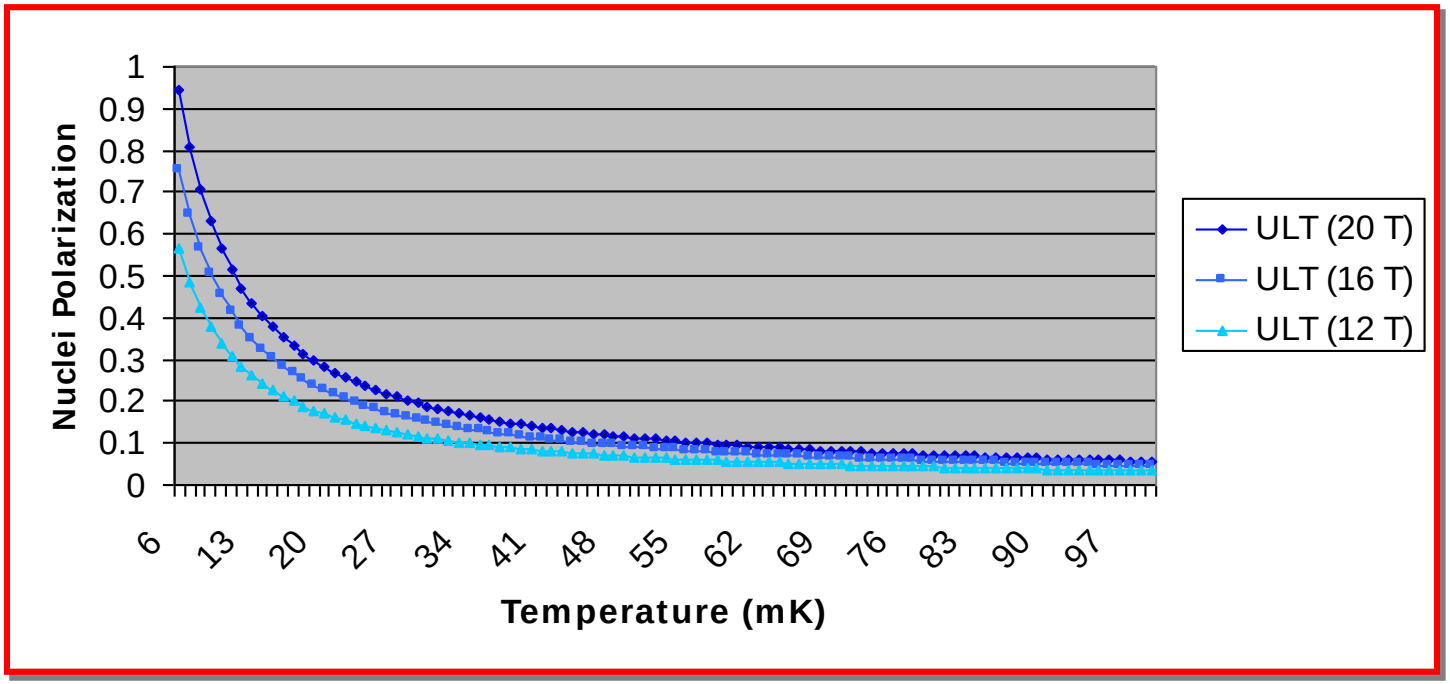
# Routes to hyperpolarziation



# The “Brute Force” approach

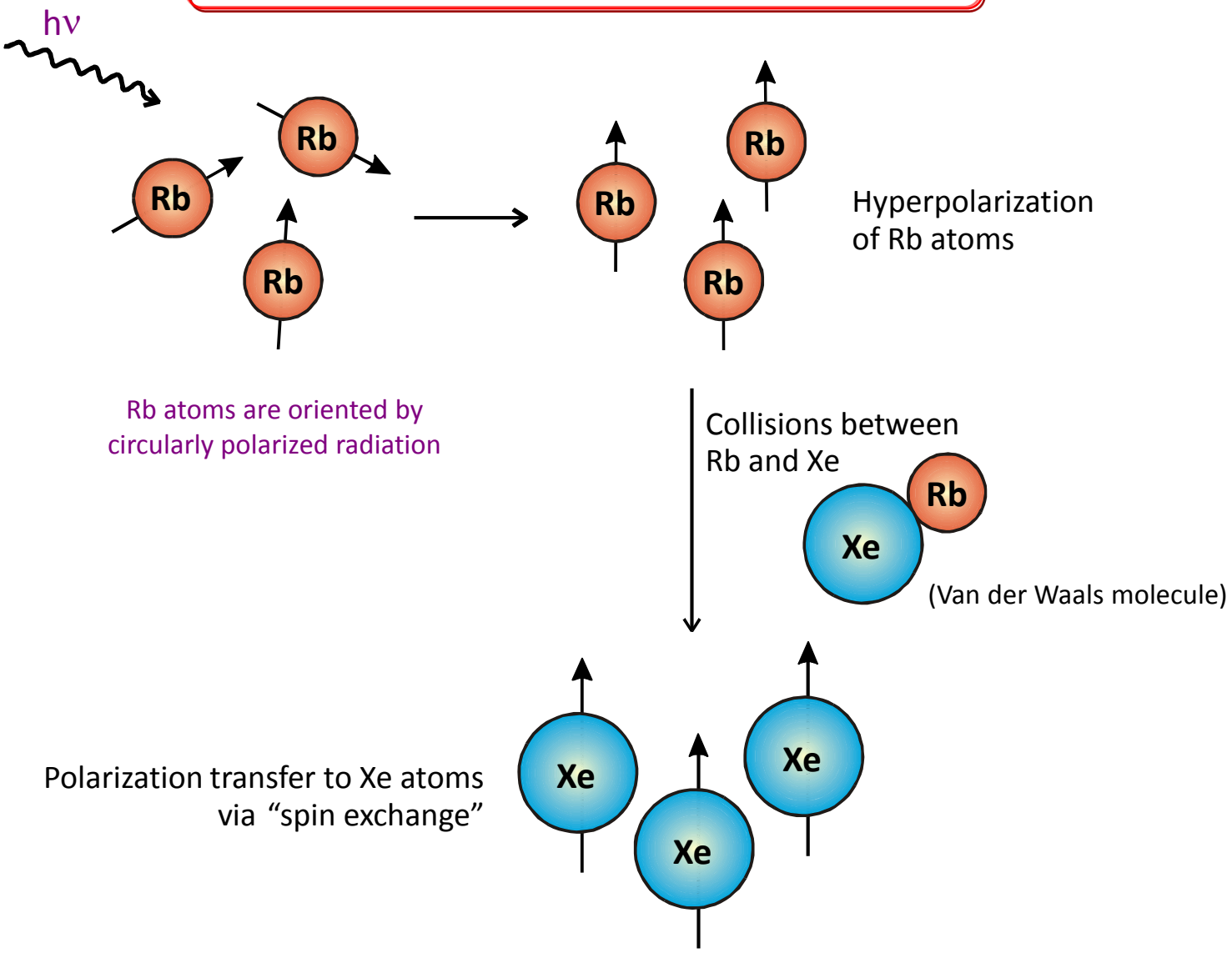
$$P = \frac{|N_+ - N_-|}{N_+ + N_-} = \tanh\left(\frac{\gamma\hbar B_0}{2k_B T}\right)$$

**Hyperpolarization is achieved by applying High Field and Ultra-low Temperature conditions**




Technical issues due to drastic conditions

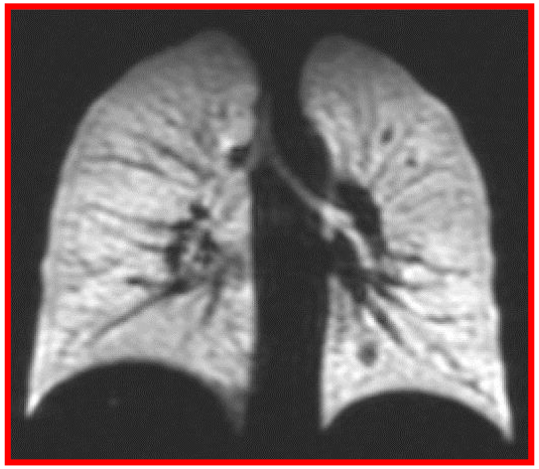
# Laser-Polarized $^{3}He$ and $^{129}Xe$



# Laser-Polarized $^{3}He$ and $^{129}Xe$

## Applications

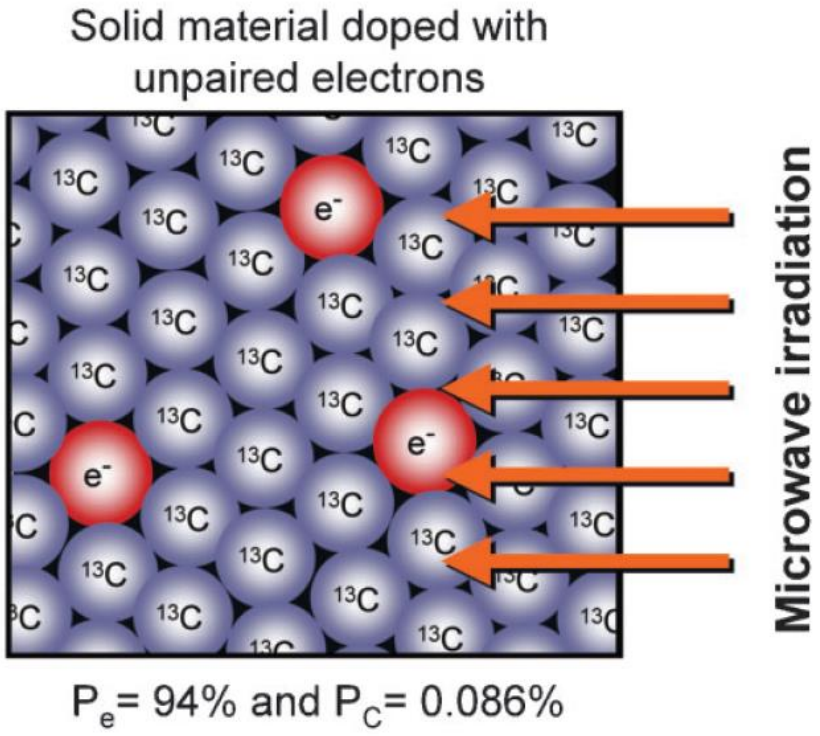
- MRI of the lungs
- perfusion studies
- ventilation studies
- diffusion studies in emphysema diagnosis
- functional MRI (which exploits the solubility and lipophilic properties of Xe)
- blood oxygenation measurements (by exploiting Xe binding to haemoglobin)



Coronal HP $^{3}He$ image of the lungs of a normal healthy human volunteer (Magn. Res. Med. 2002, 47, 1029-1051)

# Dynamic Nuclear Polarization (DNP)

Principle of the method

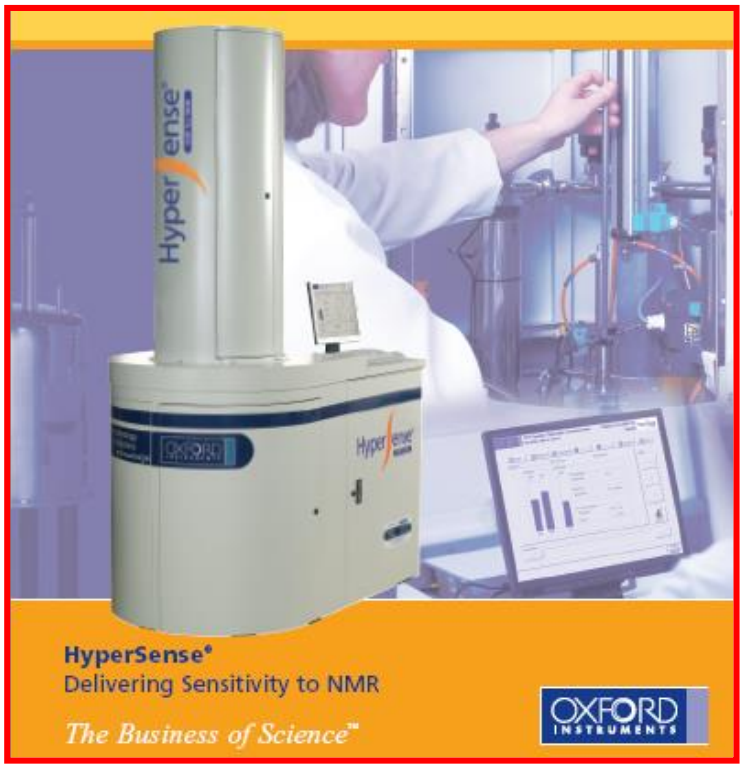



-glassy solution of the polarizing material and a paramagnetic molecule (unpaired electron)
-Low temperature (1K)
-High magnetic field (3T)
-Microwave irradiation

Microwaves allow polarization transfer from electrons (Pe 95%) to nuclei (Pc 0.08 %) through nOe

# Dynamic Nuclear Polarization (DNP)

## Steps to polarization




- A solution of the substrate and a stable radical (usually a nitroxide- or triaryl-based radical) is made (it must form a glass when freezed)
- The solution is placed into a strong magnetic field (3T).
- The solution is frozen (tipically at 1.5 K)
- microwave irradiation is applied (about 1-2 hours)
- polarization transfer takes place
-Switch off rf
-the sample is raised upon the liquid Helium level
- Dissolution in hot water (inside the magnet)
-Quick transfer for observation in the NMR scanner.

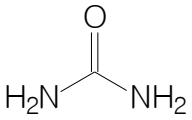
# Dynamic Nuclear Polarization (DNP)

## pros

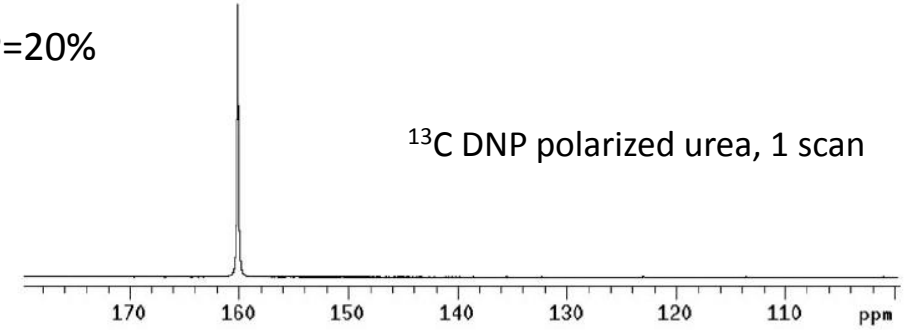
Potentially **any nucleus** in **any molecule** may be polarized by DNP

## cons

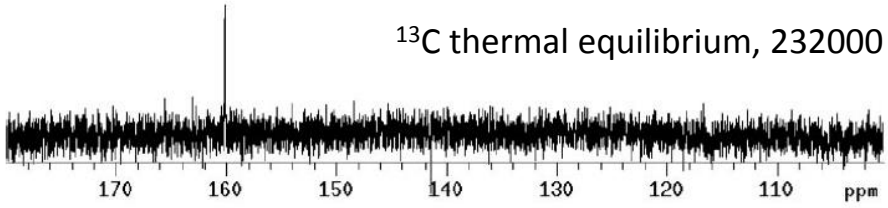
Quite expensive equipment
Cryogenic fluids are consumed
Long polarization time is necessary




P=20%




$^{13}$C DNP polarized urea, 1 scan



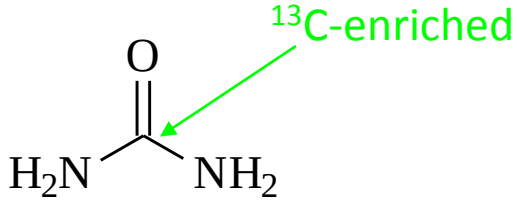

$^{13}$C thermal equilibrium, 232000 scans

Ardenkjaer-Larsen et al., PNAS 2003

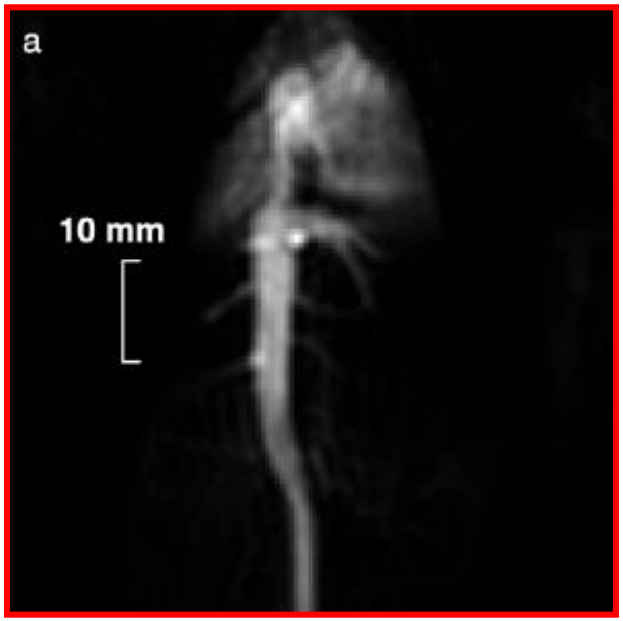
# DNP hyperpolarized contrast agents

## Early examples.

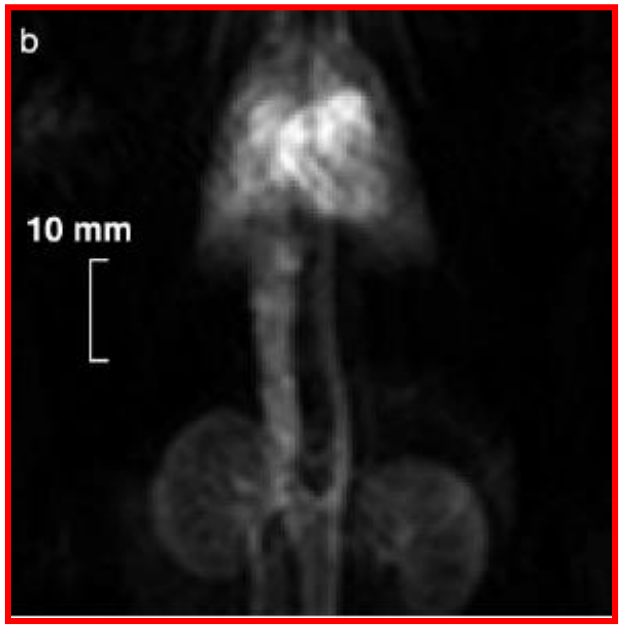
$^{13}$C coronal projection images of a rat obtained after injection of DNP HP $^{13}$C-urea (angiography)




Immediately after completing the injection of the contrast agent:




2 s later:

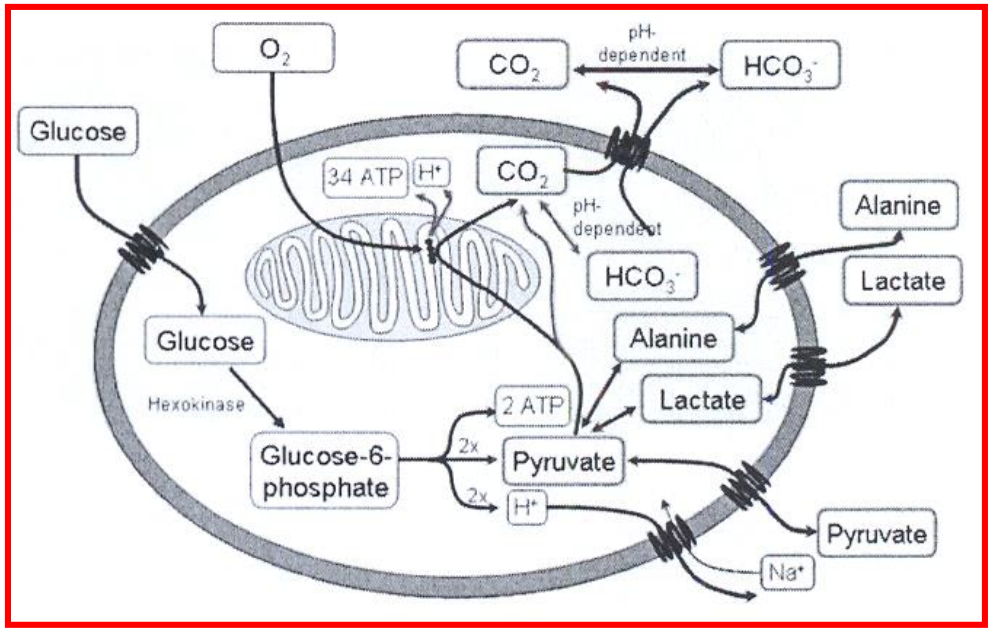


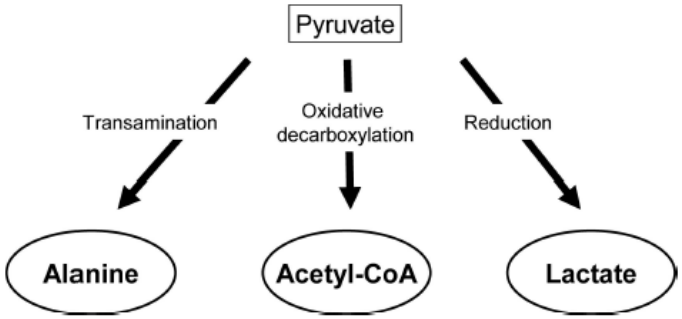

Golman K.et al., Proc. Natl. Acad. Sci. USA 2003, 100, 10435

# DNP hyperpolarized contrast agents – Metabolic Imaging

## 1-$^{13}$C-pyruvate

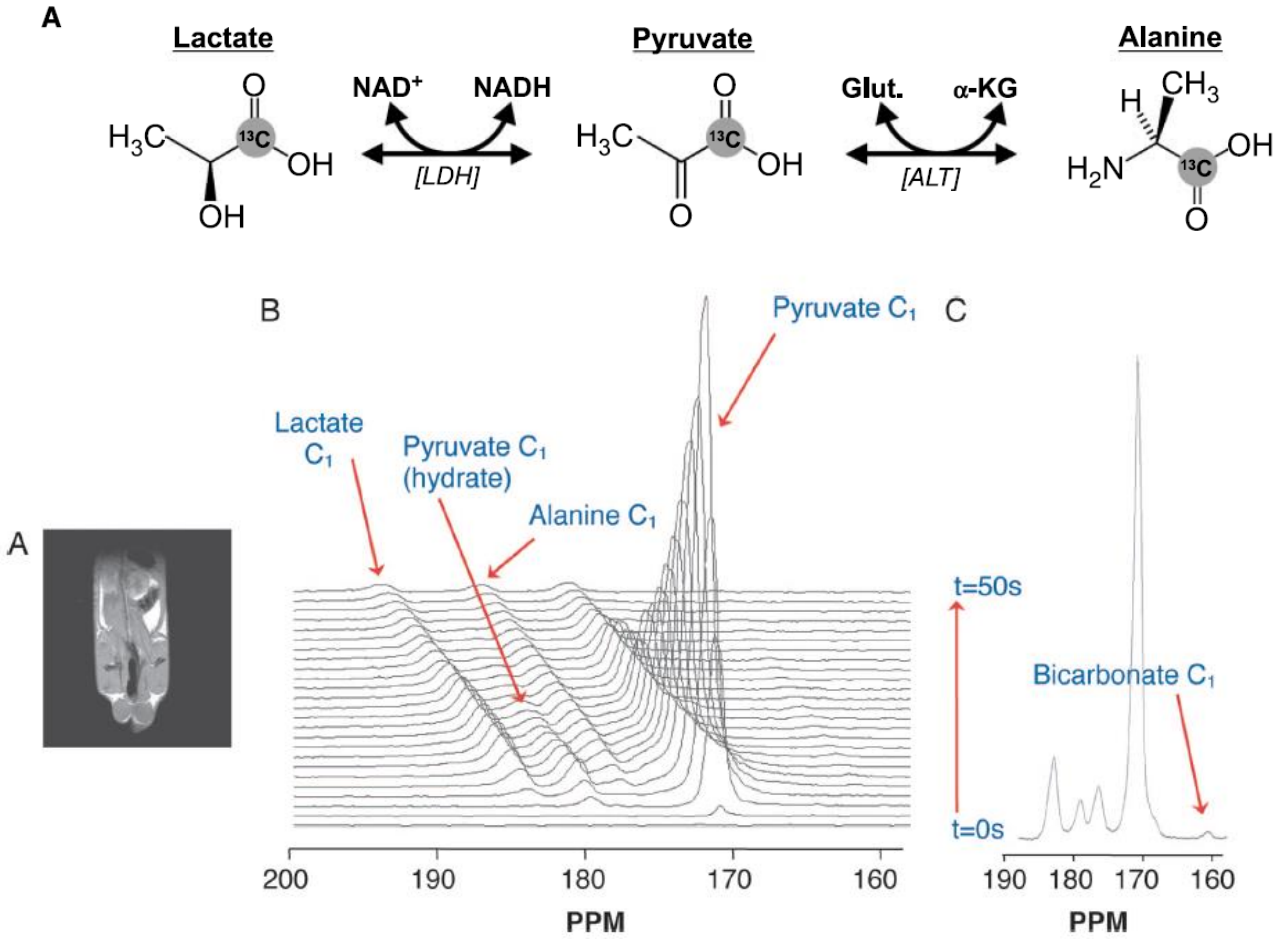
Pyruvate is a key-molecule in major metabolic and catabolic pathways in the mammalian cells, as it is converted to alanine, lactate or carbonate to a different extent depending on the status of the cells



# DNP HP - Metabolic Imaging

**1-$^{13}$C- Pyruvate (P = 30%)**

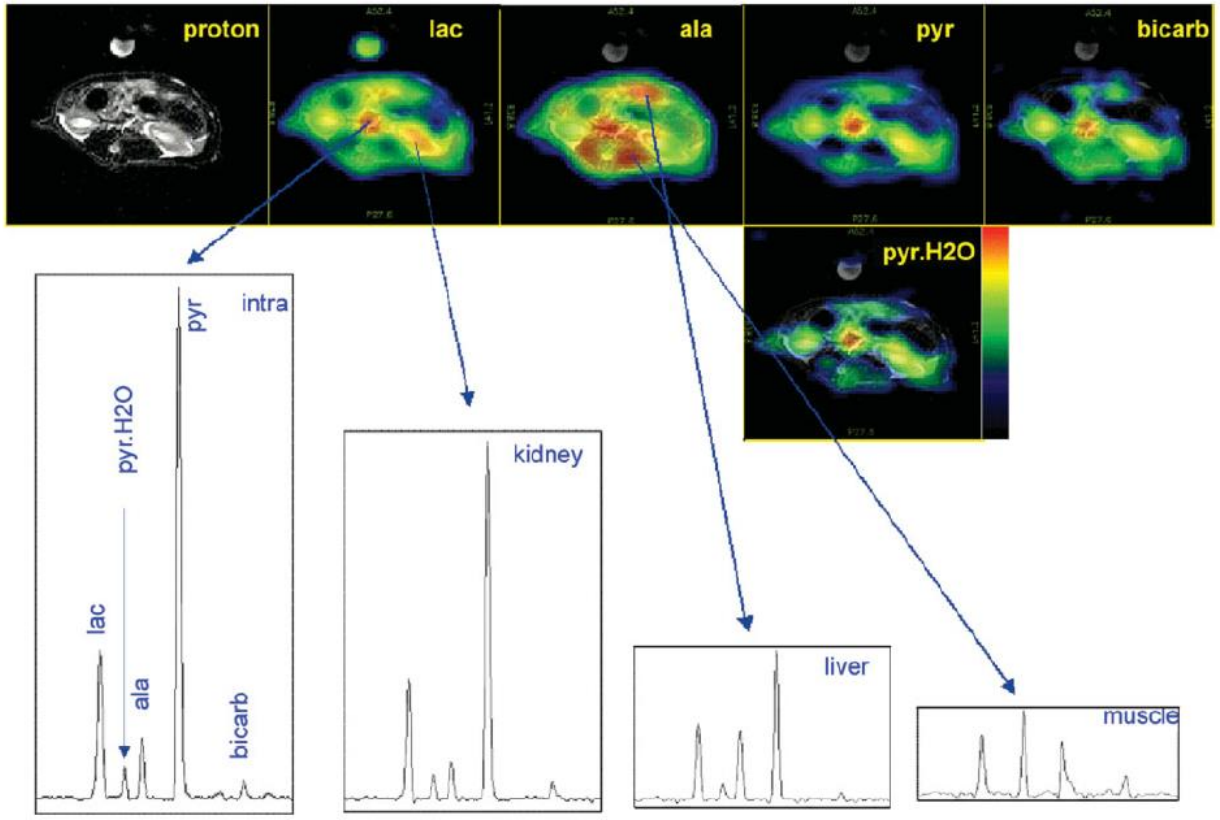



Metabolic production of Lactate and Alanine after the injection of $^{13}$C-polarized pyruvate

K.Golman et al., PNAS 2006

# DNP HP - Metabolic Imaging (Pyruvate)

Different tissues have different metabolism




CSI acquisitions show metabolite maps and idividual spectra at each tissue tipe

Kohler S.J. et al., Magn. Res. Med. 2007, 58, 65

# DNP HP - Metabolic Imaging (Pyruvate)

Metabolic processes are altered in tumors

$^{13}$C MRS from prostate tumor slice of a TRAMP mice

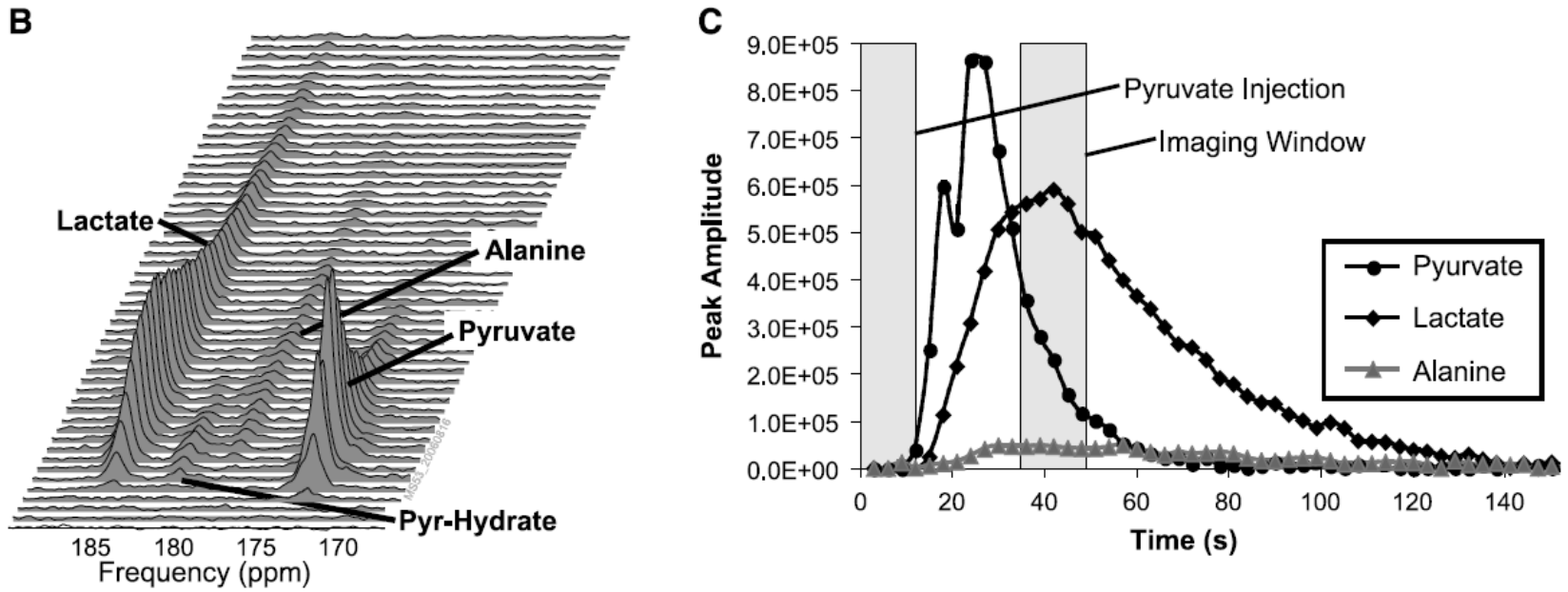



**Figure 1.** *A*, diagram of the [1-$^{13}$C] pyruvate and the metabolic pathways relevant to this study. The hyperpolarized $^{13}$C spectra (*B*) and peak height plots (*C*) show the time course for the hyperpolarized [1-$^{13}$C] pyruvate and its metabolic products following the injection of 350 μL of hyperpolarized pyruvate. The pyruvate was injected at a constant rate from 0 to 12 s. The MR spectra were acquired every 3 s from a 28-wk-old TRAMP mouse with a high-grade primary tumor using a 5° flip angle and a 10-mm-thick slice. The peak height plot was corrected for the amount of magnetization used to record the previous n spectra by dividing each peak height by $\cos^n(5°)$. The hyperpolarized pyruvate quickly reached a maximum at 24 s before being converted to lactate and alanine. Based on this time course, the subsequent MRSI data were recorded between 35 and 49 s, a time when the hyperpolarized lactate signal was roughly constant. *Glut.*, glutamate; *α-KG*, α-ketoglutarate; *ALT*, alanine transaminase.

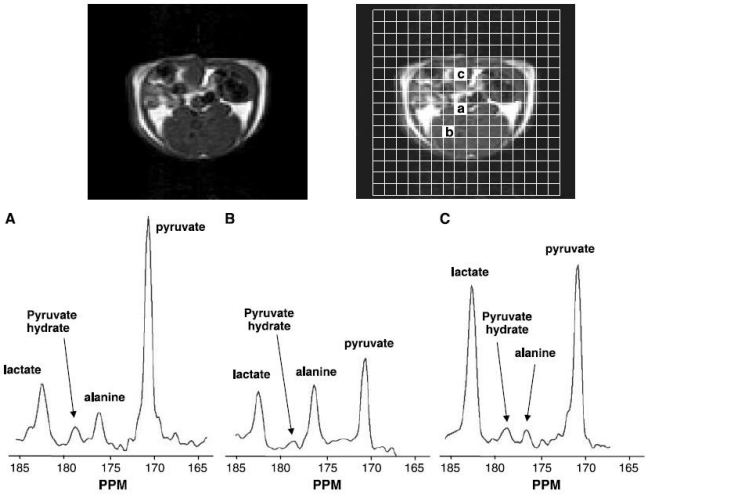
$^{13}$C spectra acquired with 5° pulses, 10mm slice centered on the primary tumor (prostrate cancer)

M.J Albers, *Cancer res.* 2008

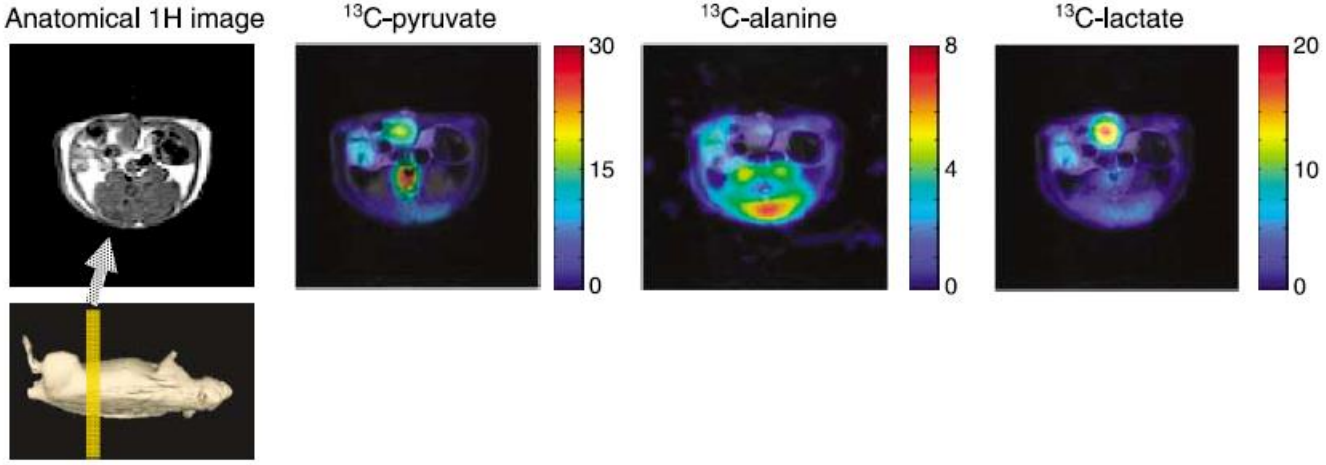
# DNP HP - Metabolic Imaging (Pyruvate)

## 1-$^{13}$C- Pyruvate in tumors

Higher lactate production in tumor than in other tissues




Vena cava Muscle tissue P22 tumor tissue

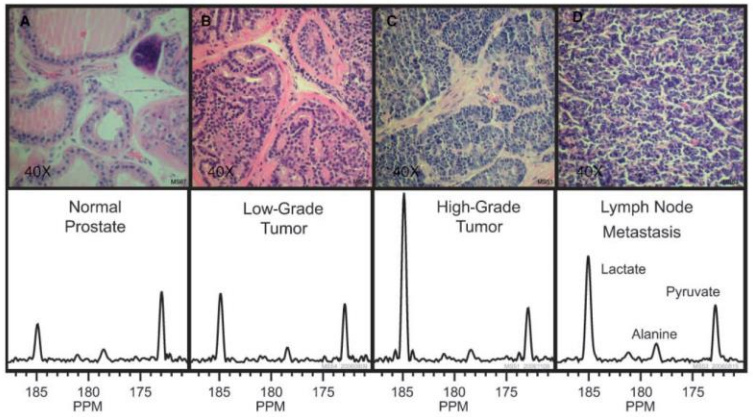


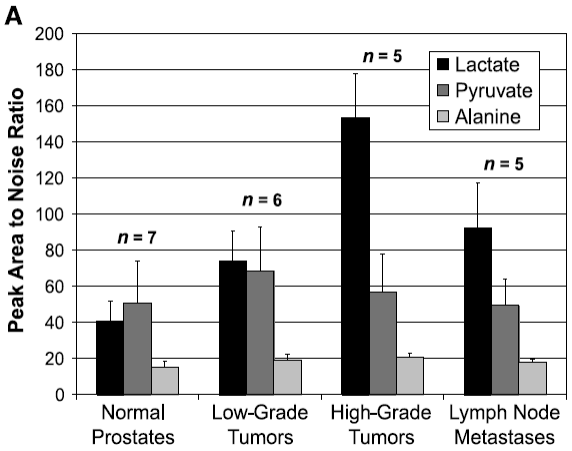

K. Golman, *Cancer res.* 2006

# DNP HP - Metabolic Imaging (Pyruvate)

## 1-$^{13}$C- Pyruvate in tumors

HP pyruvate allow to monitor the tumor progression (spectra taken from CSI voxels)



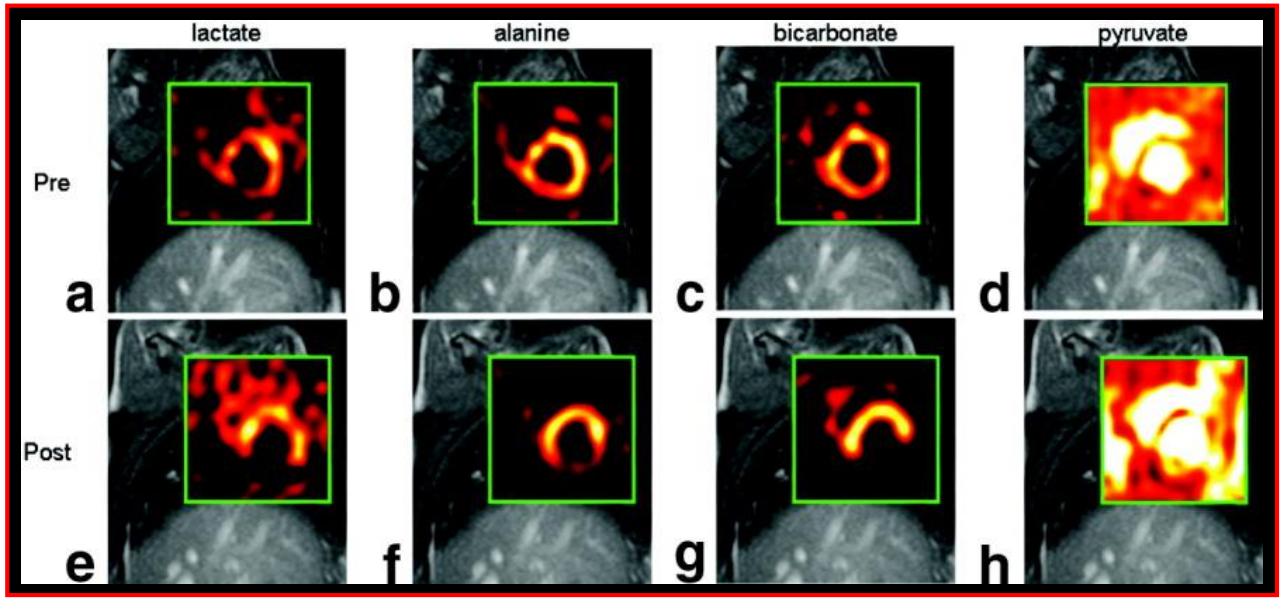




The amount of lactate is grade dependent, low grade and high grade tumors can be distinguished.

# DNP HP - Metabolic Imaging

## 1-$^{13}$C- Pyruvate for the assessment of cardiac metabolism

The bicarbonate level in the myocardium is indicative of ischemic or post-ischemic tissue, being lower or absent in the areas where ischemia was present due to a decreased activity of pyruvate deyhdrogenase (PDH)



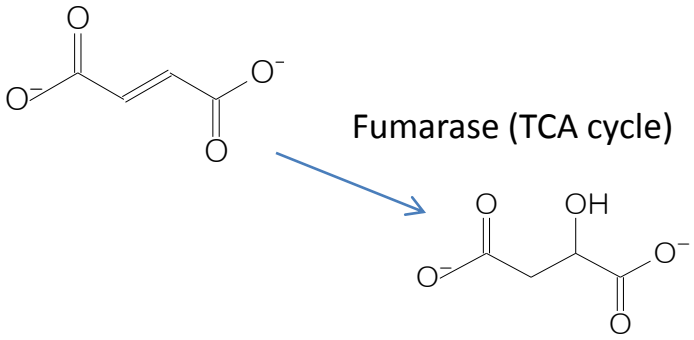

$^{13}$C-CSI maps of lactate, alanine, bicarbonate and pyruvate from a pig heart obtained pre- and post-45-min occlusion

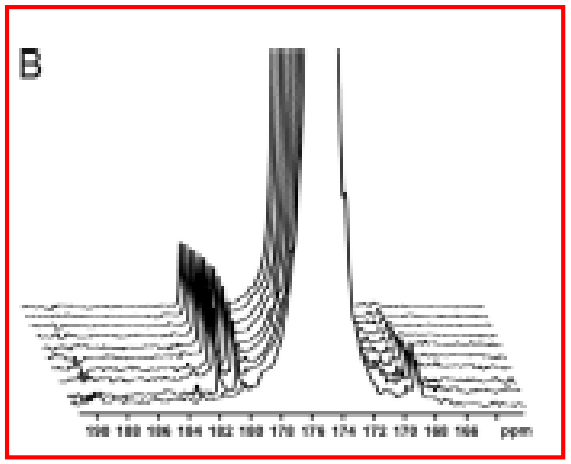
Magn. Res. Med. 2008, 59:1005

# DNP HP - Metabolic Imaging

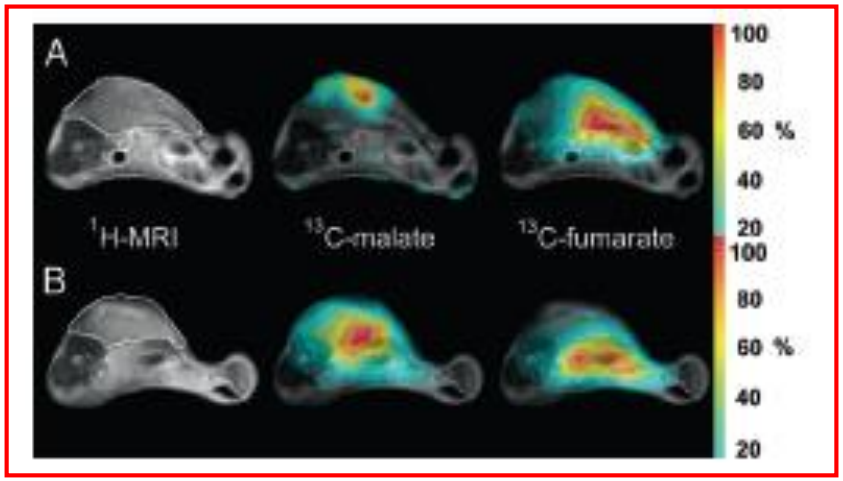
Production of HP [1,4-$^{13}C_2$]-**malate** from [1,4-$^{13}C_2$]-**fumarate**
as a marker of cell necrosis and tumor response to treatment

Tumor response to treatment is currently addressed by changes of tumor size or by means of FDG-PET




Images from untreated (A) and etoposide-treated (B) mice with implanted lymphoma tumors




The increased malate production in necrotic or treated cells is explained by increased access of fumarate to fumarase

PNAS 2009, 106, 19801

# DNP HP - Metabolic Imaging

## Production of HP [1,4-$^{13}C_2$]-malate from [1,4-$^{13}C_2$]-fumarate as a marker of cell necrosis and tumor response to treatment

Tumor response to treatment is currently addressed by changes of tumor size or by means of FDG-PET

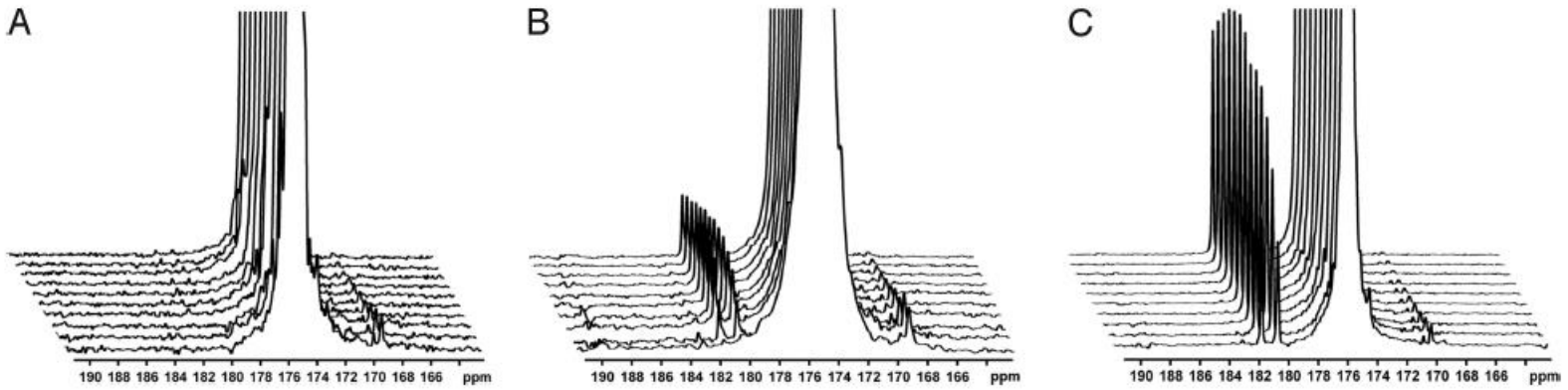



**Fig. 1.** $^{13}C$ spectra acquired over a period of 1 min after injection of hyperpolarized [1,4-$^{13}C_2$]fumarate into suspensions of intact murine lymphoma cells (≈5 × $10^7$ cells) or lysed cells; these are representative spectra from the data shown in Fig. 2. For clarity, only every third spectrum is shown and each series has been scaled to the maximum fumarate signal. (*A*) Untreated cells. (*B*) Cells 16 h after etoposide treatment. (*C*) Lysed cells. The truncated signal from the hyperpolarized [1,4-$^{13}C_2$]fumarate is at 175.4 ppm, the signal from [1-$^{13}C$]malate at ≈181.8 ppm, and the signal from [4-$^{13}C$]malate at ≈180.6 ppm.

# DNP HP - Metabolic Imaging

## $^{13}$C-acetate (P = 25%) – fatty acid metabolism

**Acetate** —CoA→ **AcCoA** —Carnitine→ **AcetylCarnitine + CoA**

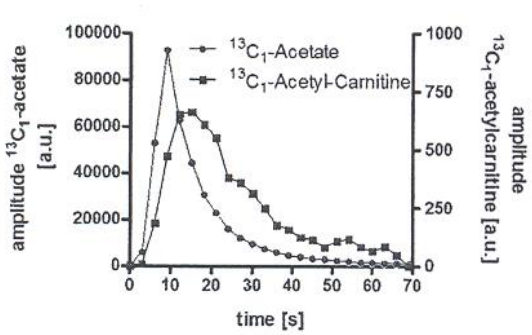



**Figure 1.** Dynamics of 1-$^{13}$C-Acetyl-Carnitine in mouse heart after injection of 1-$^{13}$C-Acetate.

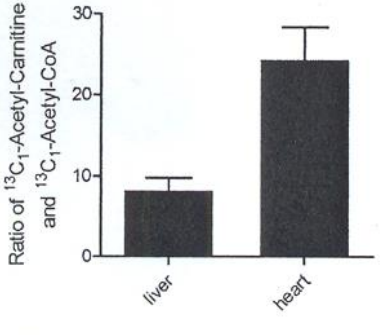
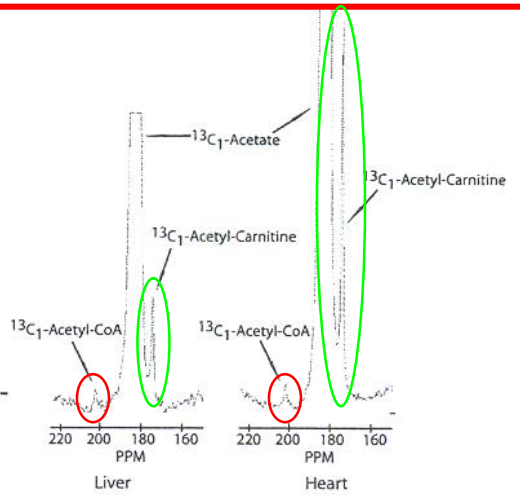



**Figure 2.** Distribution of the ratio of 1-$^{13}$C-acetyl-Carnitine and 1-$^{13}$C-acetyl-CoA in mouse heart and liver (n=6).




After injection of HP $^{13}$C-acetate, its metabolic conversion to $^{13}$C-acetyl-CoA and $^{13}$C-acetylcarnitine is observed

Reduced acetate metabolism is observed after ischemia

J. Biol. Chem. 2009, 284, 36077

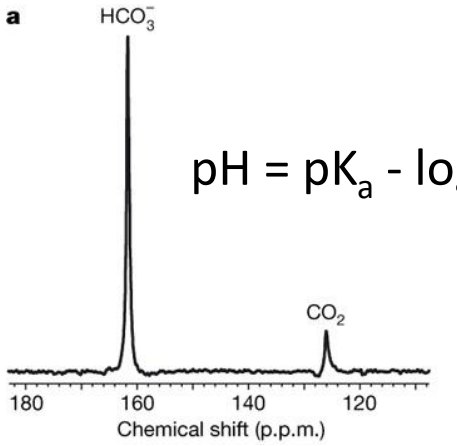
# $^{13}$C-bicarbonate (P = 20%) – pH evaluation

*In-vivo* pH measurement

$^{13}$C-bicarbonate-$CO_2$ pKa = 6.17

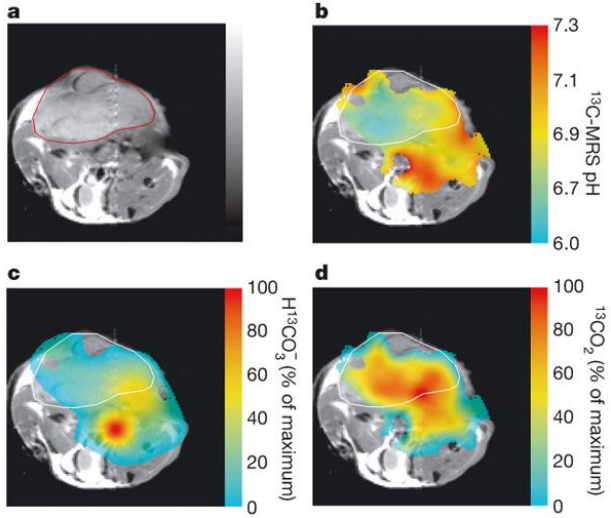
$$H^{13}CO_3^- + H_3O^+ \xleftrightarrow{\text{Carbonic anhydrase}} {}^{13}CO_2 + H_2O$$

**Slow exchange**: two separate signals $HCO_3^-$ and $CO_2$




$$pH = pK_a - \log \frac{[HCO_3^-]}{[CO_2]}$$

$^{13}$C spectrum of murine lymphoma *in vivo* (pH ≈ 6) , after the intravenous injection of hyperpolarized $H^{13}CO_3^-$,

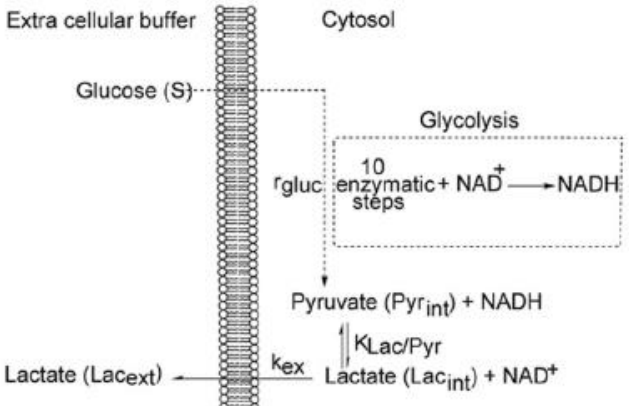


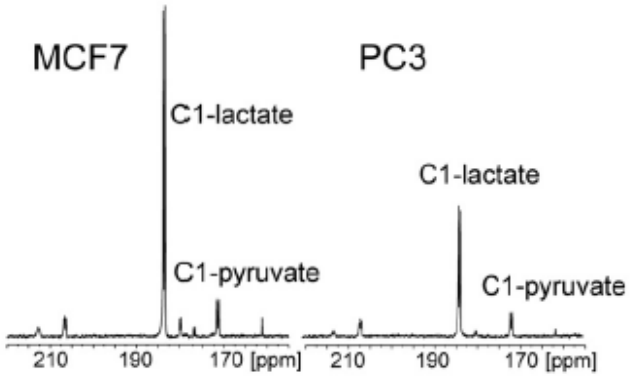

**a**, $^1$H image of a mouse with a subcutaneously implanted EL4 tumour (red). **b**, pH map calculated from the ratio of the $H^{13}CO_3^-$ (**c**) and $^{13}CO_2$ (**d**) voxel intensities in $^{13}$C chemical shift images after intravenous injection of 100 mM hyperpolarized $H^{13}CO_3^-$.

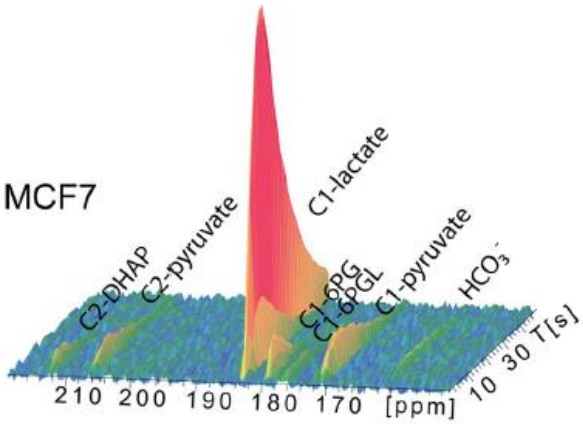
Gallagher, F. et al. Nat. Lett. 2008 (vol. 453)

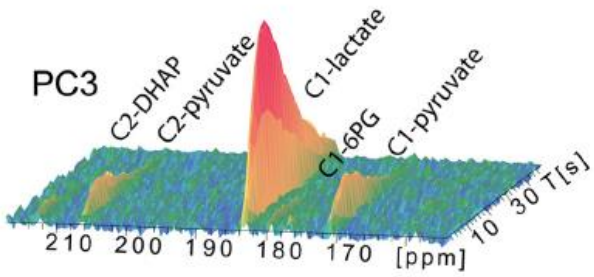
# HP-$^{13}$C labelled [U-$^{2}$H, U-$^{13}$C] **glucose** (P= 15%)

The whole glycolitic pathway is monitored in cell cultures



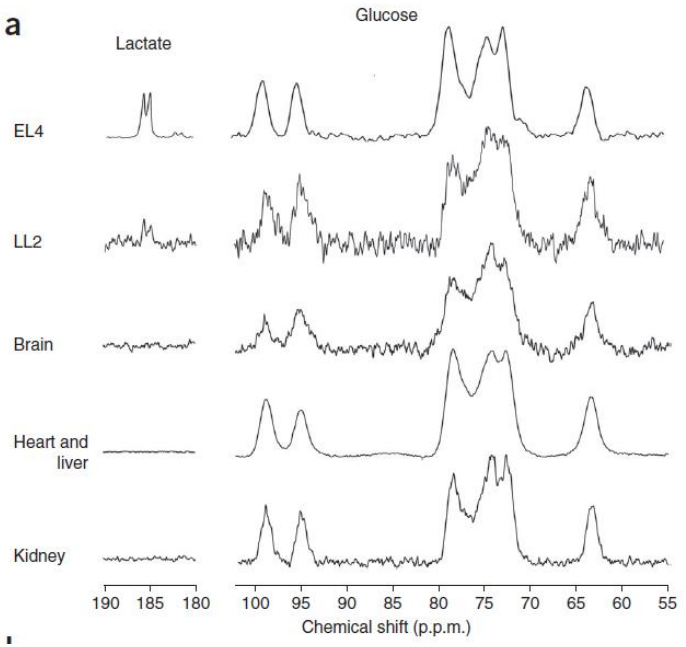

6PG: 6-phosphogluconate; DHAP: dihydroxy acetone phosphate

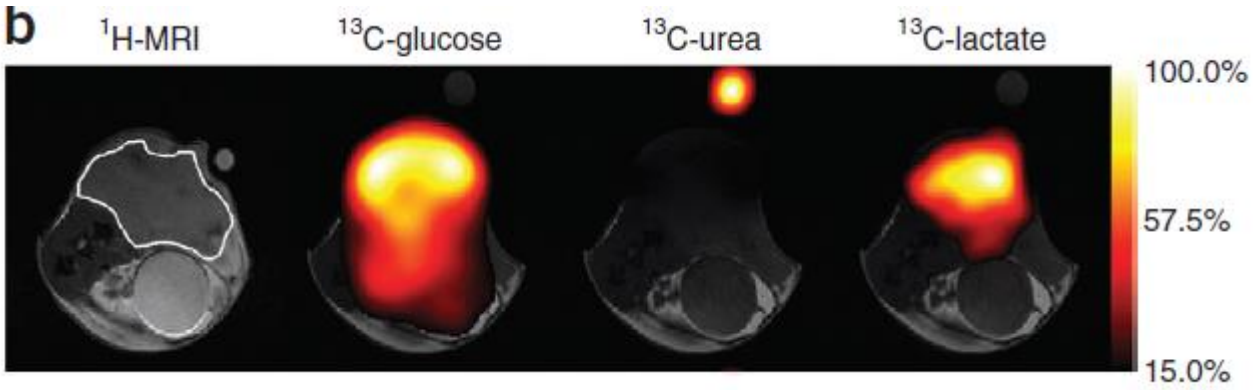
C.E.Christensen et al. J Biol Chem. 2014

# HP-$^{13}$C labelled [U-$^{2}$H, U-$^{13}$C] **glucose** (P= 15%)

Glycolisys is monitored in vivo in mice bearing lymphoma and lung tumors

Lactate is observed after 10 enzimatic steps



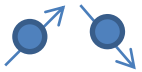




T.B. Rodrigues et al. Nat.Med. 2014

# ParaHydrogen Induced Polarization

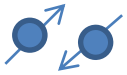
parahydrogen

$\alpha\beta - \beta\alpha = S$

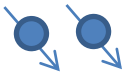


orthohydrogen

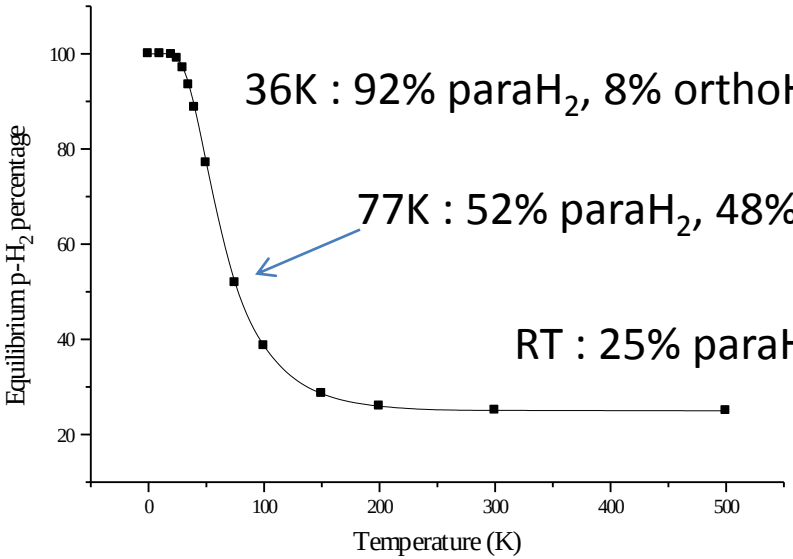
$\beta\beta = T_-$



$\alpha\beta + \beta\alpha = T_0$



$\alpha\alpha = T_+$

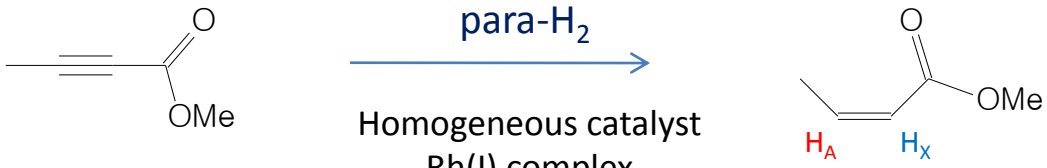



36K : 92% para$H_2$, 8% ortho$H_2$

77K : 52% para$H_2$, 48% ortho$H_2$

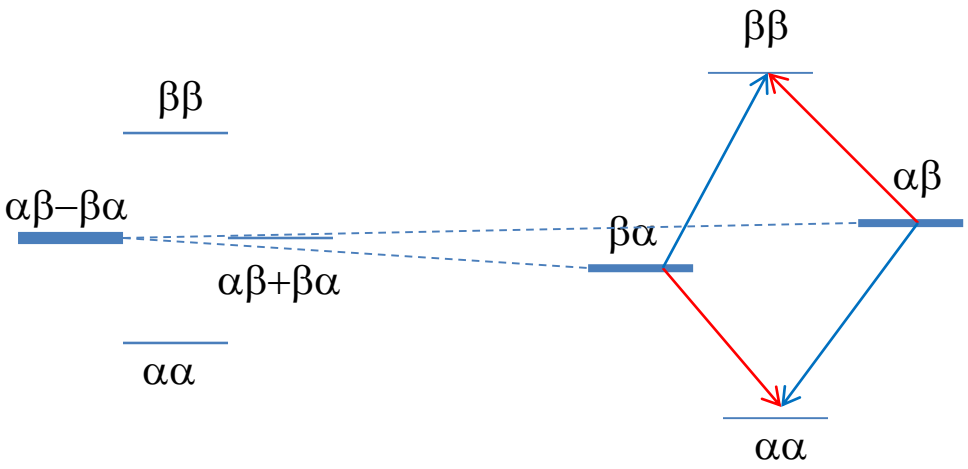
RT : 25% para$H_2$, 75% ortho$H_2$

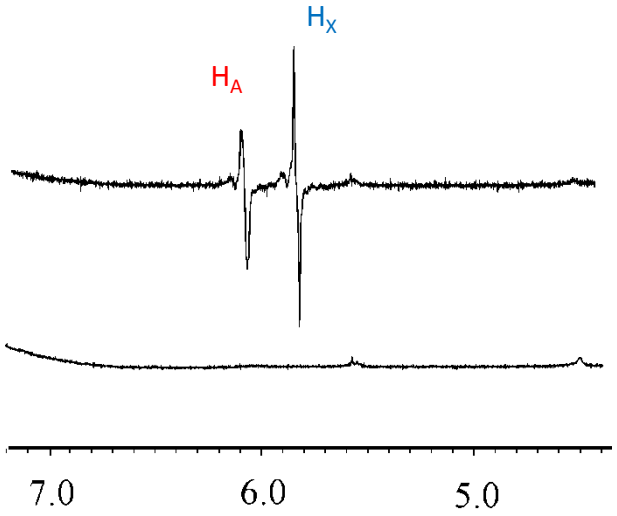
# ParaHydrogen Induced Polarization




Para-$H_2$

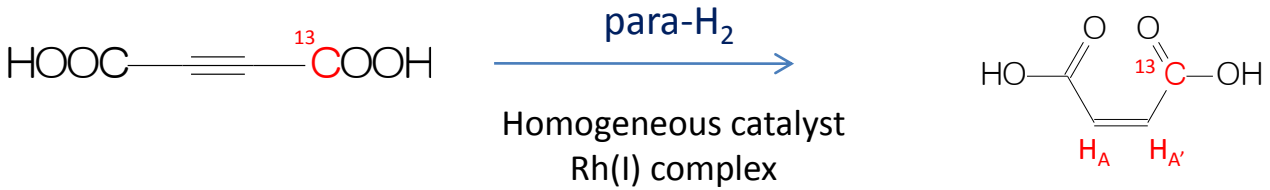
AX-spin system



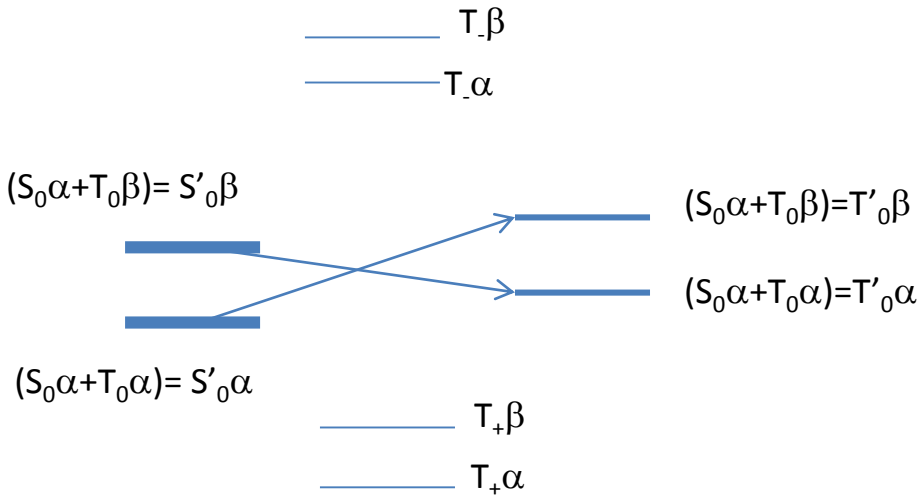

Hyperpolarization is obtained from non-equilibrium spin states population

# $^{13}$C- ParaHydrogen Induced Polarization

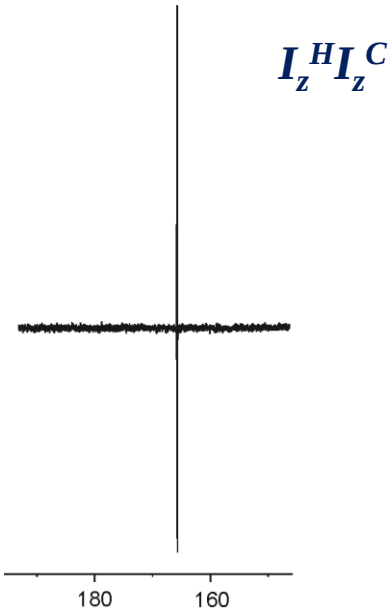



Polarization is transferred to heteronuclei through J couplings

## AA'X spin system

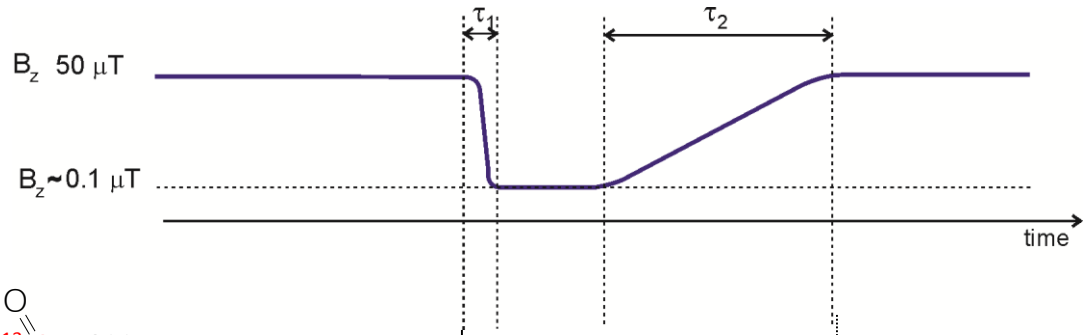


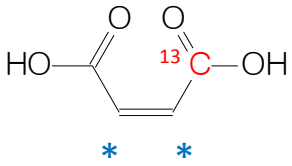

## Hyperpolarized $^{1}$H-$^{13}$C spin order



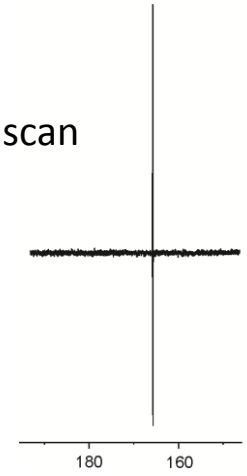
# $^{13}$C- ParaHydrogen Induced Polarization

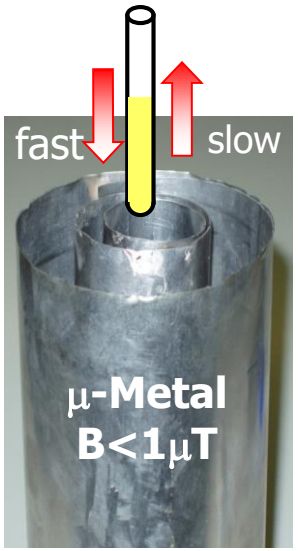
The antiphase signal obtained after polarization transfer to $^{13}$C must be converted into an in-phase signal in order to be suitable for image acquisition. This can be achieved by a magnetic field cycling procedure or by applying an opportune pulse sequence to the sample



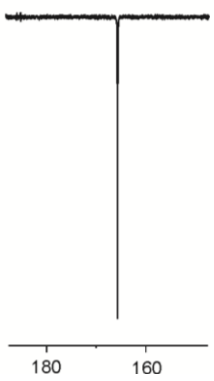




$^{13}$C-NMR, 1 scan



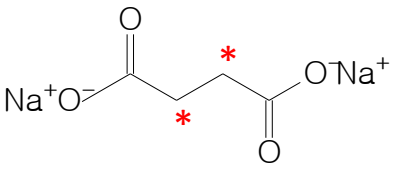

$^{13}$C-NMR, 1 scan



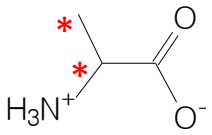
# PHIP

## on $^{13}C$ of biologically relevant molecules

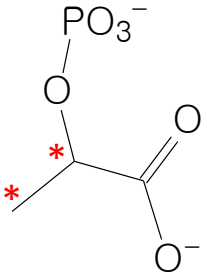
### Unsaturated precursor available



Succinate (maleic acid)



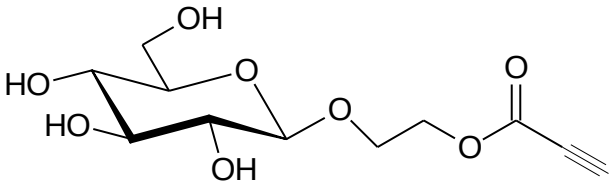
alanine (dehydro-AA)

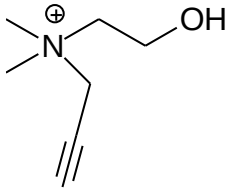


Plac

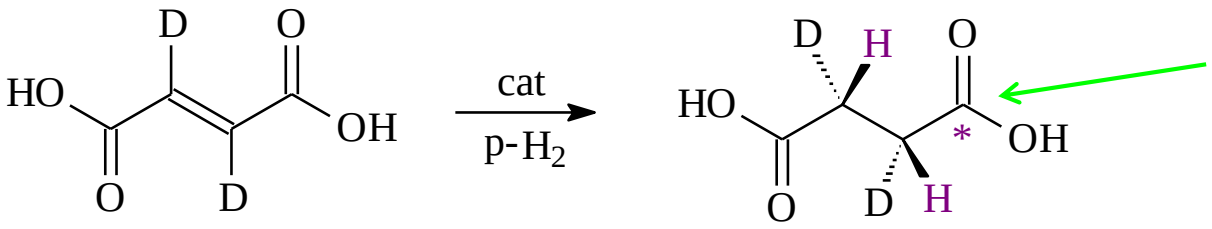
Phospho-Lac

### Binding of a parahydrogenable group to a biological substrate



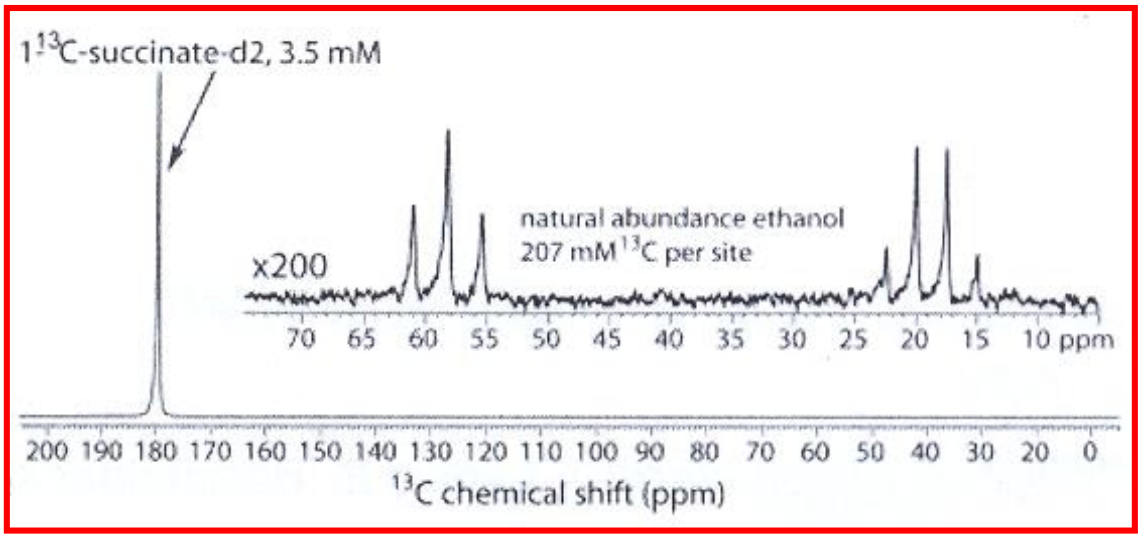


# $^{13}$C-PHIP polarized succinate

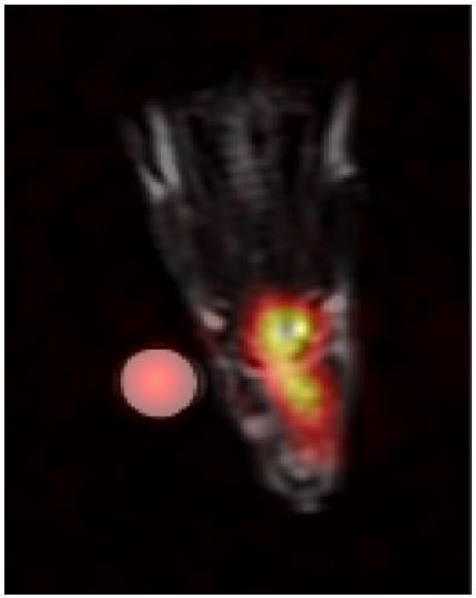



$^{13}$C-enriched

$T_1$ = 39 s in $D_2O$ (pH 3)

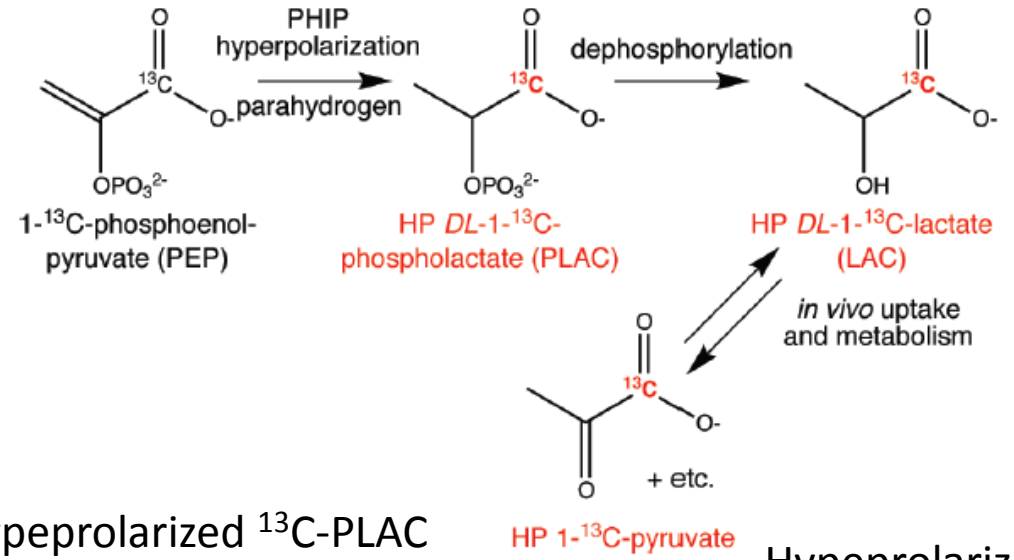



Chekmenev E.Y. et al.,JACS 2008, 4212

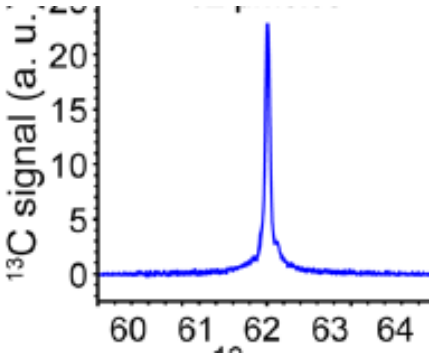


13C image of rat brain
using 13C-PHIP succinate

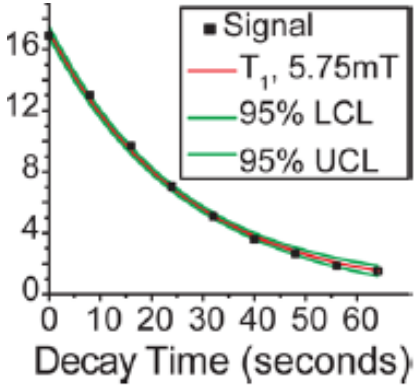
# $^{13}$C-PHIP Phospho Lactate




Hypeprolarized $^{13}$C-PLAC
P= 15.6%

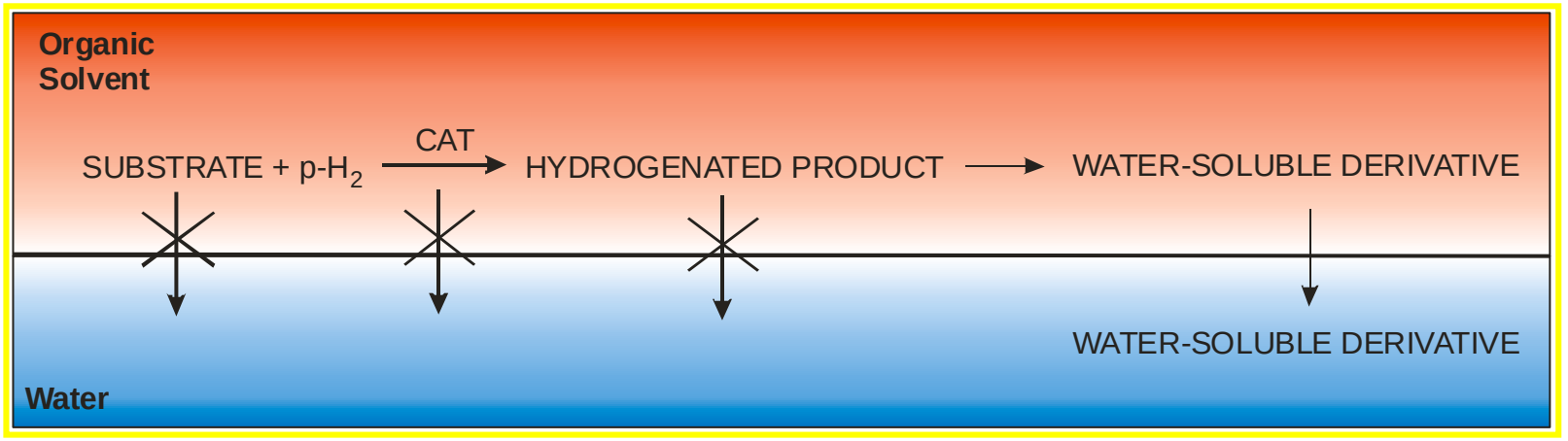



Hypeprolarization decay
$T_1$ =51 s



# Para-$H_2$ containing molecules as hyperpolarized contrast agents

## Preparation of pure hyperpolarized water-soluble compounds by para-hydrogenation and phase extraction

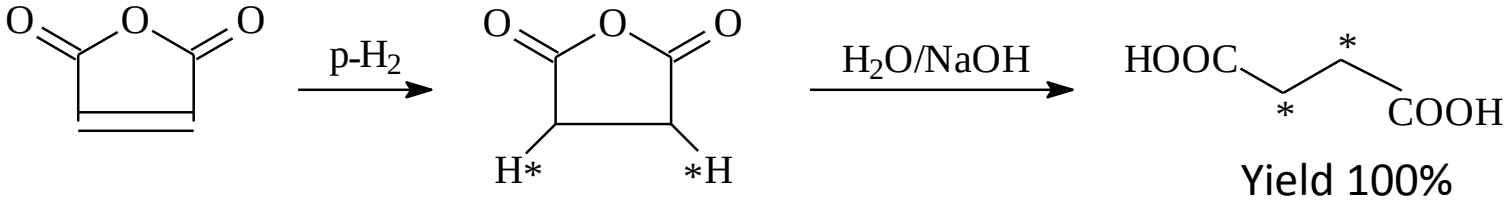



A lipophylic precursor of the compound of interest is para-hydrogenated in an organic solvent not miscible with water, then it is quickly converted to the hydrophilic derivative by addition of an aqueous medium and it is extracted in the water phase as a pure compound.

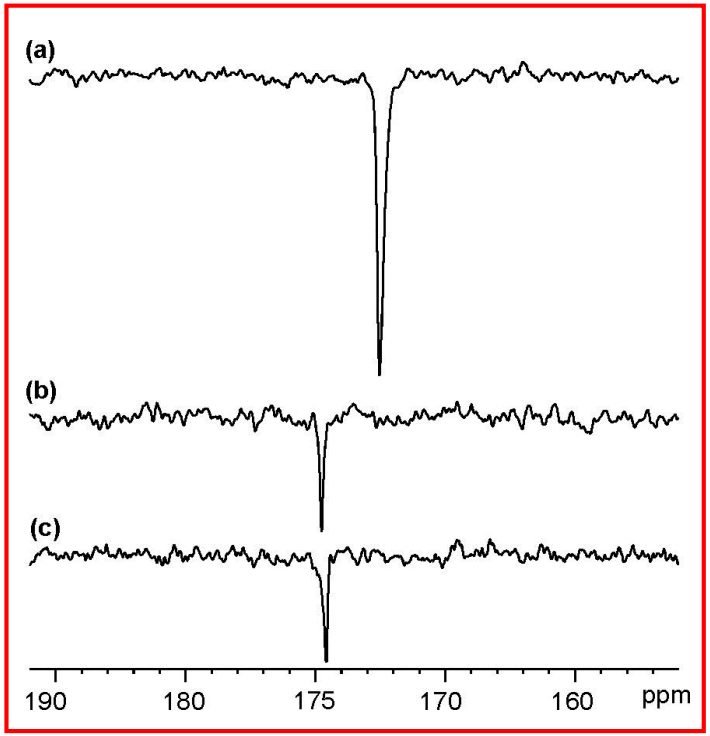
The procedure allows to obtain pure water solution of the compounds of interest in one step only by a simple phase extraction process, avoiding the use of high para-$H_2$ pressures and expensive experimental set-ups.

# Para-$H_2$ containing molecules as hyperpolarized contrast agents

## Example: para-hydrogenation of maleic anhydride and subsequent hydrolisis afford succinic acid




Yield 100%




(a) Succinic anhydride obtained by para-hydrogenation of maleic anhydride in acetone-$d^6$

(b) hyperpolarized succinic acid obtained by para-hydrogenation of maleic anhydride in acetone-$d^6$ and successive hydrolysis

(c) hyperpolarized succinic acid obtained by para-hydrogenation of maleic anhydride in $CDCl_3$ and successive hydrolysis and extraction in basic $D_2O$

# Conclusion

Metabolic imaging is currently carried out using DNP polarization

High polarization level, any molecule can be polarized

High costs, technically challenging, few polarizers

ParaHydrogen Induced Polarization

Easily accessible technique, but few molecules can be polarized:
research is ongoing…