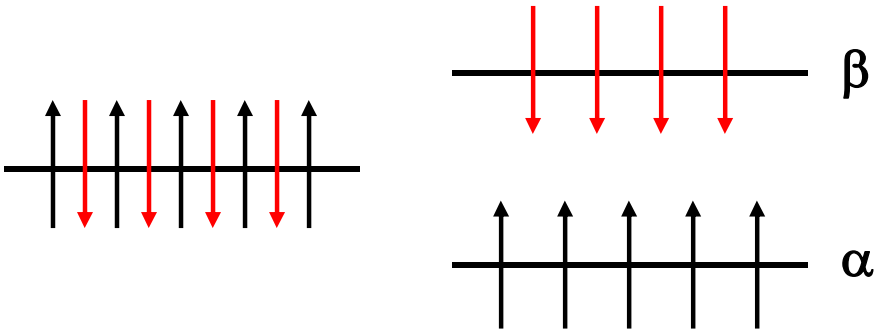
HYPERPOLARIZED CONTRAST AGENTS

What does “hyperpolarization” mean?

- **Normal Polarization:**

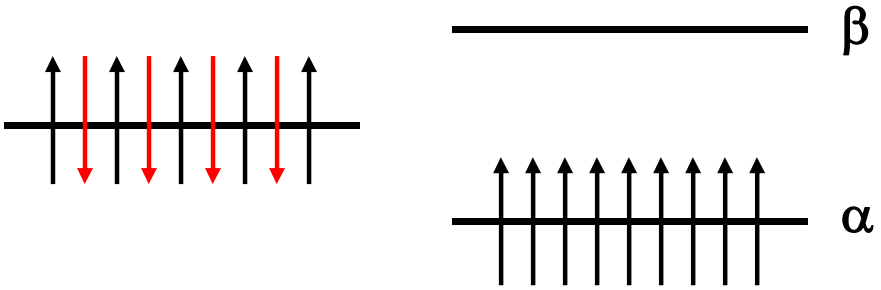


No field B_0

$$P = \frac{|N_+ - N_-|}{N_+ + N_-}$$

$\Delta N/N \approx 10^{-5}$ → Low NMR sensitivity

- **Hyperpolarization:**



No field B_0

→ 10^5 enhancement in sensitivity

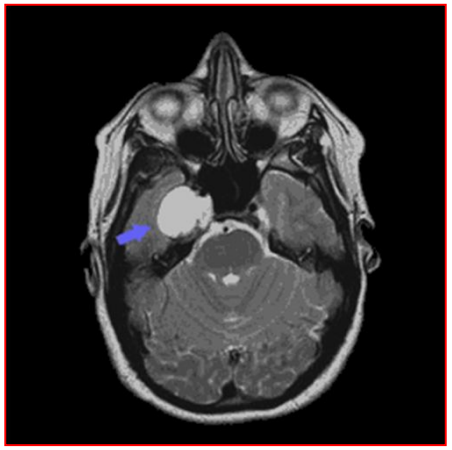
^{13}C- Magnetic Resonance Imaging with hyperpolarized contrast agents (HP-MRI)

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.



Drawback of the technique : low sensibility → Long acquisition times

The use of hyperpolarized substances as contrast agents can drastically reduce the acquisition times thanks to the strong signal enhancement

^{13}C- Magnetic Resonance Imaging with hyperpolarized contrast agents (HP-MRI)

Design of HP contrast agents

When using hyperpolarized contrast agents, images are generated by observing the CA hyperpolarized nuclei directly. The higher the concentration or the polarization, the higher the signal which will be observed.

Requisites to be satisfied by an HP molecule to be used as an MRI-CA:

- Long nuclear relaxation times (T_1)
- High number of active spins
- Biocompatibility
- Biological interest (molecular imaging)

^{13}C- Magnetic Resonance Imaging with hyperpolarized contrast agents (HP-MRI)

Design of HP contrast agents

1) LONG RELAXATION TIMES:

- T_1 is inversely proportional to the correlation time (τ_C)

→

- **Small molecules** are preferred because they are characterized by low correlation times in solution

- Dipolar interactions are proportional to the gyromagnetic ratioes (γ) of the involved nuclei and strongly depend on the distance between them

→

- Isolated low γ **heteronuclei**
- Dipolar relaxation can be reduced by **functionalization and/or perdeuteration** of the substrate

- For heteronuclei, the CSA contribution to relaxation is field dependent and is affected by the simmetry of the nucleus environment

→

- **Low field**
- **Symmetrical** configurations

^{13}C- Magnetic Resonance Imaging with hyperpolarized contrast agents (HP-MRI)

Design of HP contrast agents

2) HIGH NUMBER OF ACTIVE SPINS:

- **Isotopic labelling**

3) BIOCOMPATIBILITY:

- **Water-solubility**
- **Low toxicity** →
  - Purification after hyperpolarization or low toxicity of added substances
  - Fast dissolution in suitable saline buffers without polarization loss

4) BIOLOGICAL INTEREST:

- Hyperpolarization of **metabolites** involved in fast transformations allows the visualization of the products of metabolism few seconds after administration, exploiting the high signal intensity and absence of background noise

^{13}C- Magnetic Resonance Imaging with hyperpolarized contrast agents (HP-MRI)

Imaging considerations

- Need hardware (coils) tuned to multiple frequencies
- Gradient limitations due to lower γ

  ↳ strong gradients to get same resolution in a given time

- Non-equilibrium magnetization is not renewable
- Polarization decays to the thermal equilibrium value according to T_1

  ↓

  - Rapid trains of low flip angle pulses
  - Vary the flip angle to compensate for T_1 decay
  - Single shot imaging
  - SSFP, trueFISP to recycle transverse magnetization

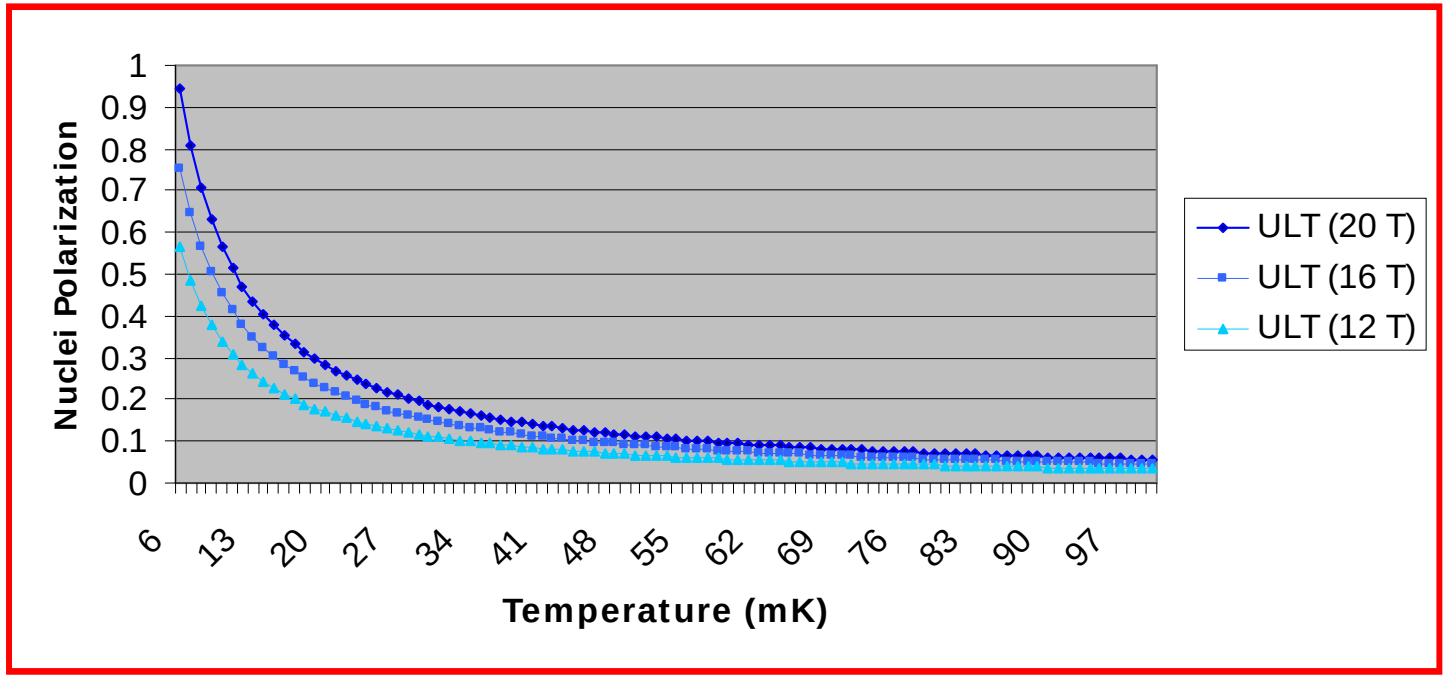
Routes to hyperpolarization

- “Brute Force”
- Optical pumping and spin exchange of noble gases
- Dynamic Nuclear Polarization (DNP)
- Para-hydrogen Induced Polarization (PHIP)

The “Brute Force” approach

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

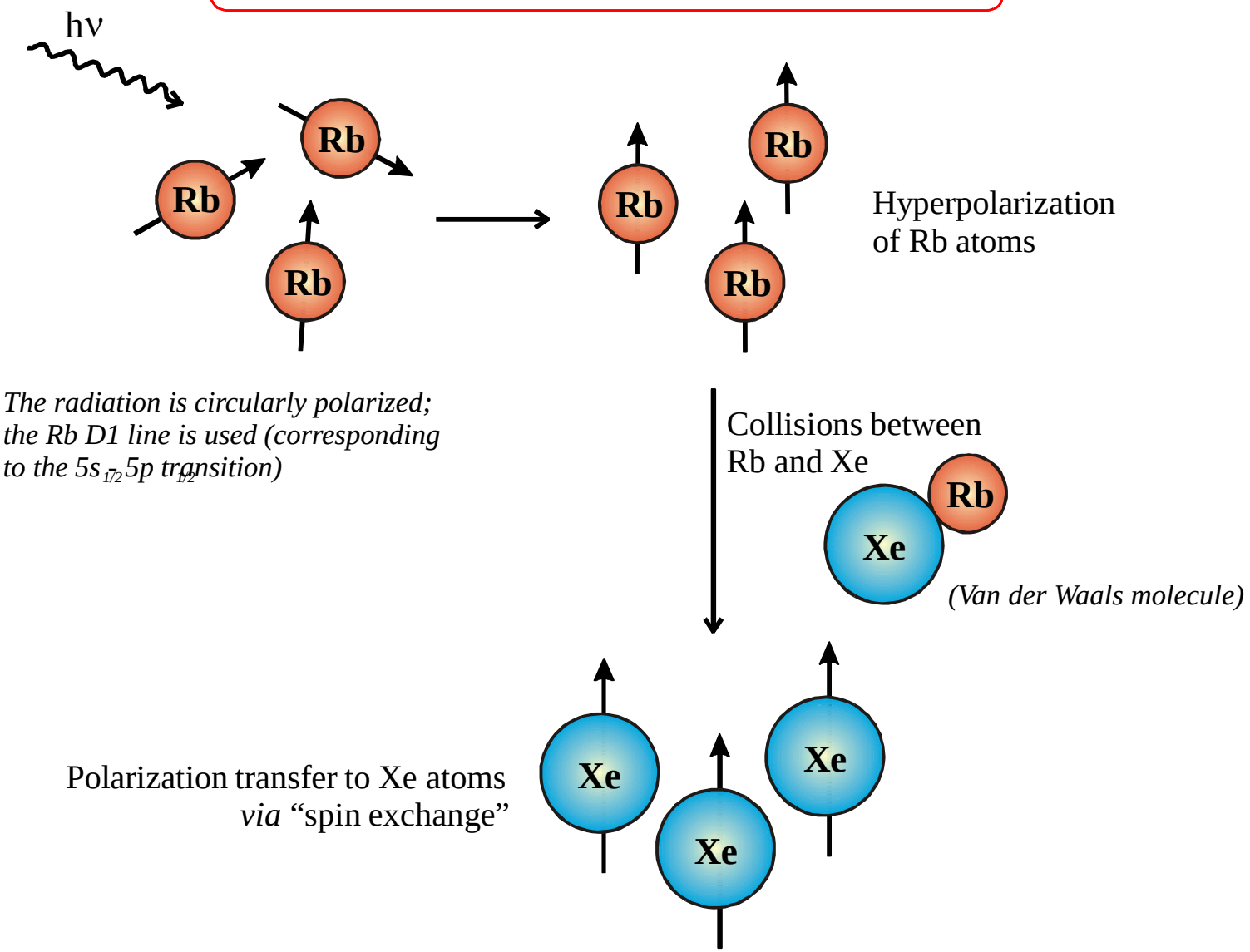
Hyperpolarization is achieved by applying High Field and Ultra-low Temperature conditions



Relaxation switches are necessary (O_2, $^3He/^4He$)

Technical issues due to drastic conditions and removal of relaxation switches

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

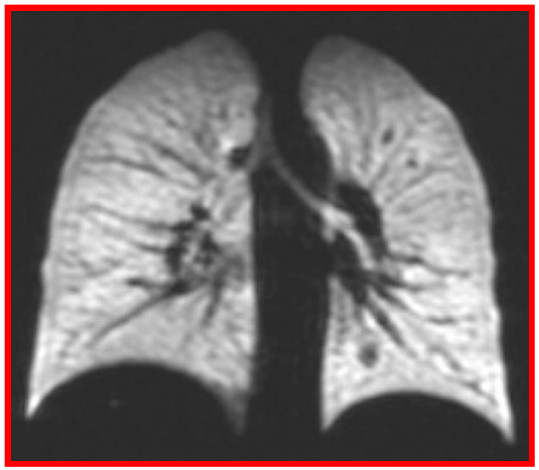


Laser-Polarized ^{3}He and ^{129}Xe

Applications

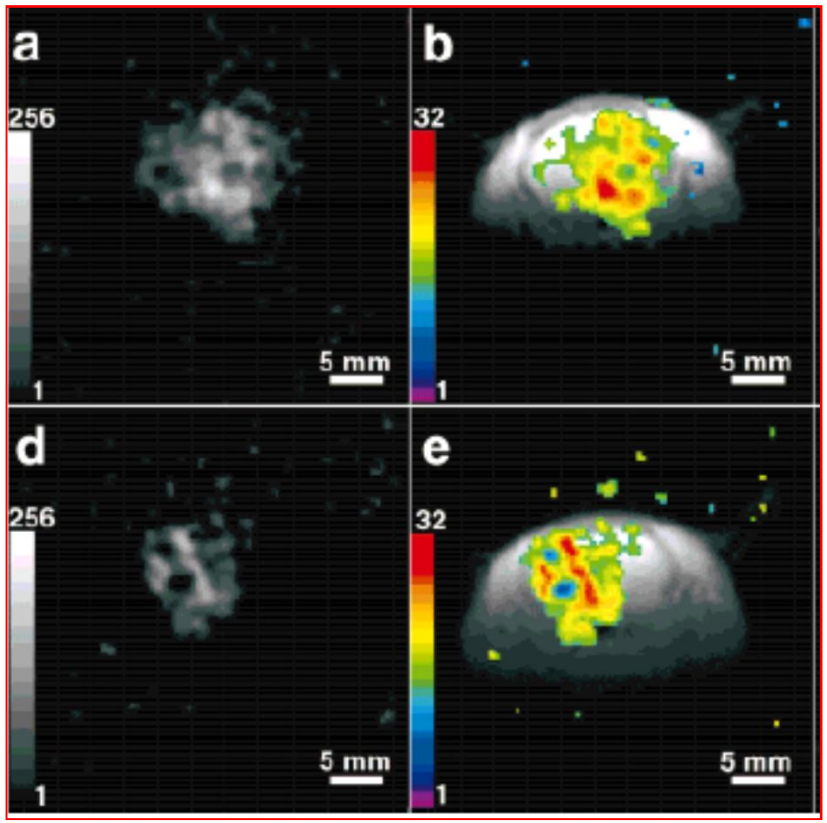
- MRI of the lungs
- perfusion studies
- ventilation studies
- diffusion studies in emphysema diagnosis
- functional MRI (which exploits the solubility and lipophilic properties of Xe)
- blood oxygenation measurements (by exploiting Xe binding to haemoglobin)

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



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

Dissolution of Xe in lipophilic biocompatible carriers → delivery through intravenous injection

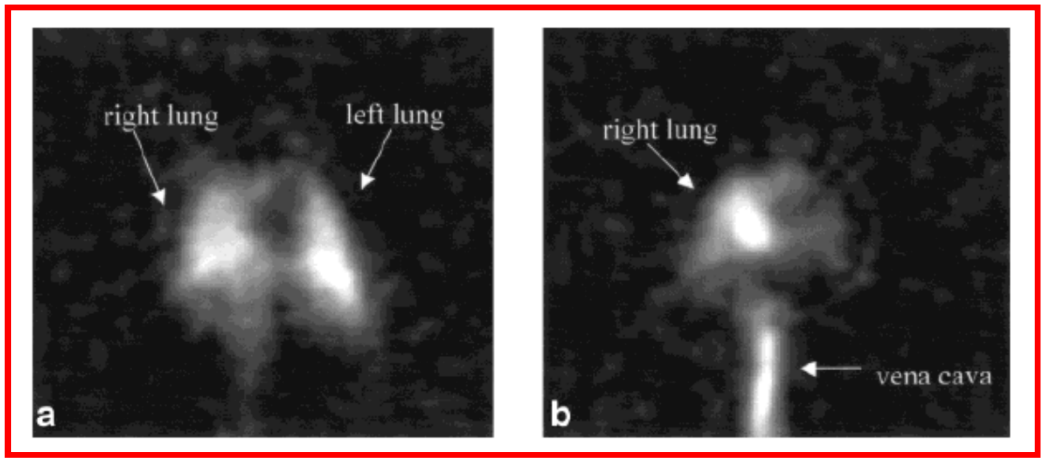


^{129}Xe transverse images of a rat brain obtained after injection of a lipid emulsion of HP ^{129}Xe either through the left (**a,b**) or through the right (**d,e**) internal carotid artery.

Magn. Reson. Med. 2001, 46, 208

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

^{3}He microbubbles → delivery through intravenous injection



Coronal projection images of the microbubble distribution in the lung vasculature (**a**) prior to pulmonary embolism and (**b**) following pulmonary embolism in the left lung

(Magn. Res. Med. 2001, 46,535)

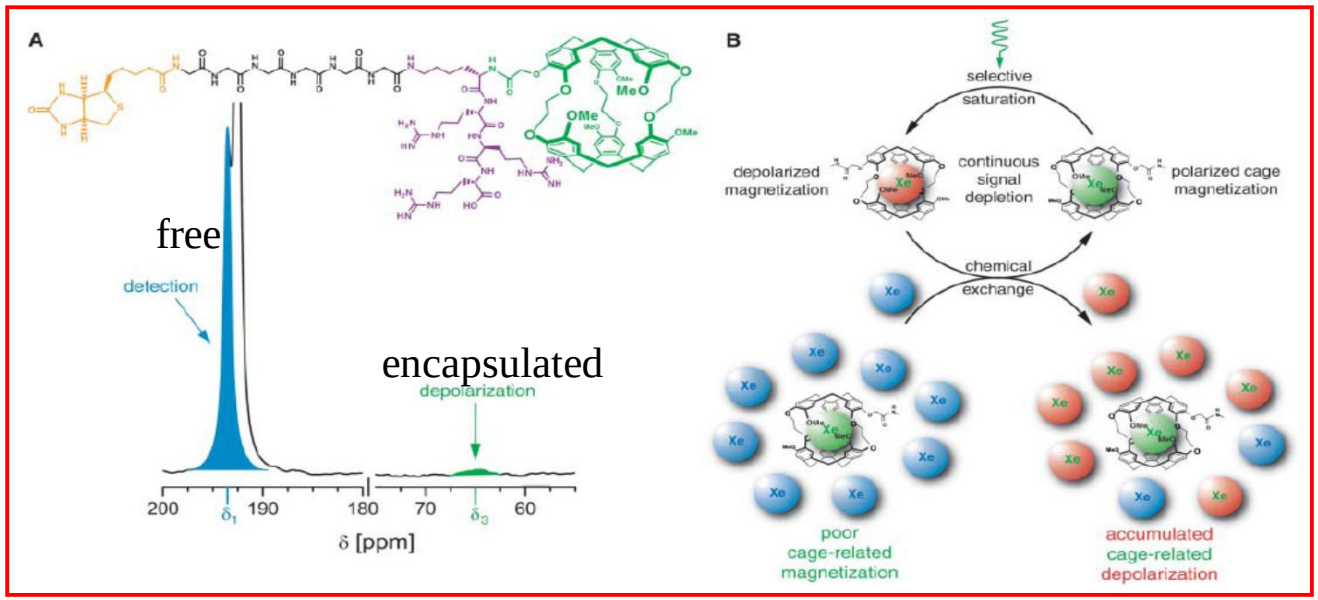
Preparation of a suspension of ^{3}He microbubbles encapsulated in a phospholipid monolayer

Polarized ^{3}He is introduced into an evacuated syringe containing a combination of lyophilized phospholipids and pharmaceutical grade polyethyleneglycol and the suspension of gas microbubbles is created by introducing saline, followed by agitation.

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

HP ^{129}Xe Biosensors

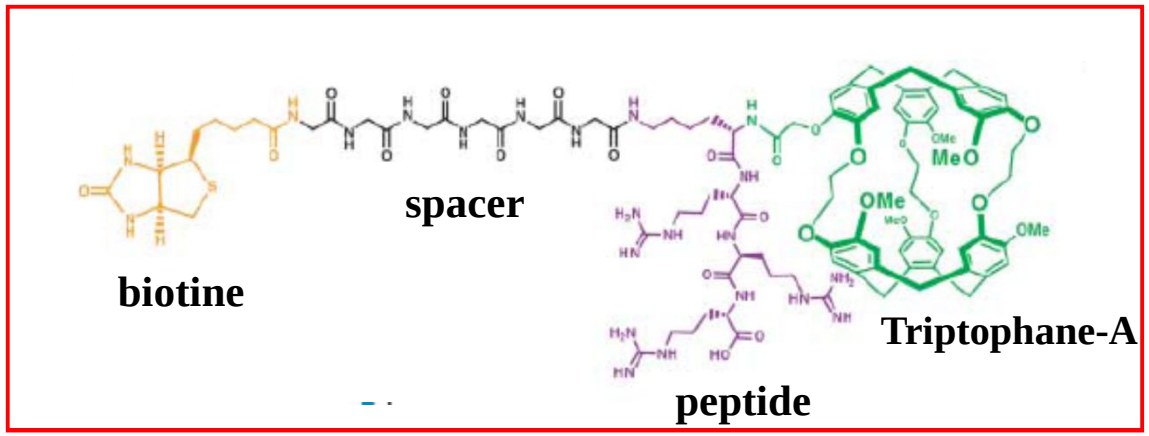
When encapsulated in molecular cages, ^{129}Xe displays a resonance that is well shifted from that of free ^{129}Xe, and the two chemically different ^{129}Xe nuclei are involved in an exchange process on the NMR time scale. By irradiating the ^{129}Xe resonance of the entrapped Xe, a saturated magnetization is transferred to the signal of the free ^{129}Xe. This procedure corresponds to continuous depolarization of cage-related magnetization that can be measured indirectly after several cycles by the difference between initial and final bulk magnetization (**HYPER-CEST**).

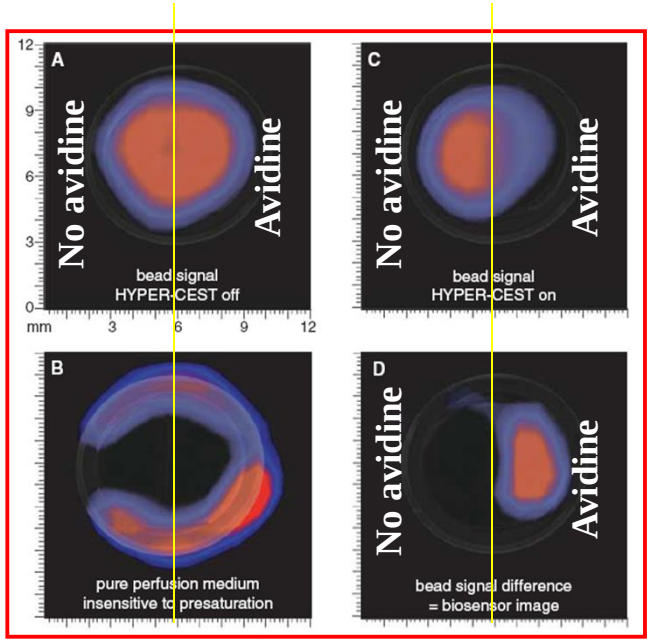


Laser-Polarized ^{3}He and ^{129}Xe

HP ^{129}Xe Biosensors

Functionalization of the cage allows molecular targeting and selective detection of different micro-enviroments.



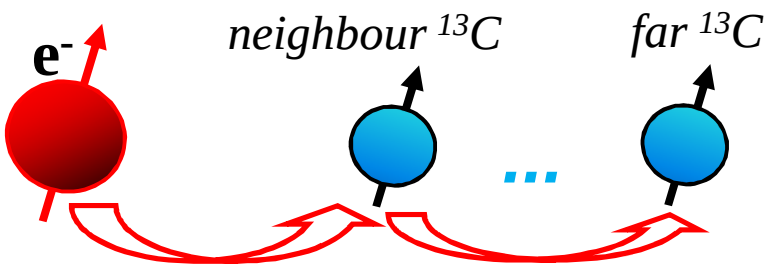


Science 2006, 314, 446

The ^{129}Xe biosensor binds avidin-agarose beads contained in one of the two compartments of a specifically designed flow system. The other compartment contained agarose beads not functionalized with avidin moieties. The application of the Hyper-CEST technique allowed to precisely localize the ^{129}Xe biosensor in the phantom, in spite of the very low sensor concentration (2 mM)

Dynamic Nuclear Polarization (DNP)

Polarization transfer from unpaired electrons coupled to nuclei through irradiation at or near the Electron Paramagnetic Resonance (EPR) frequency

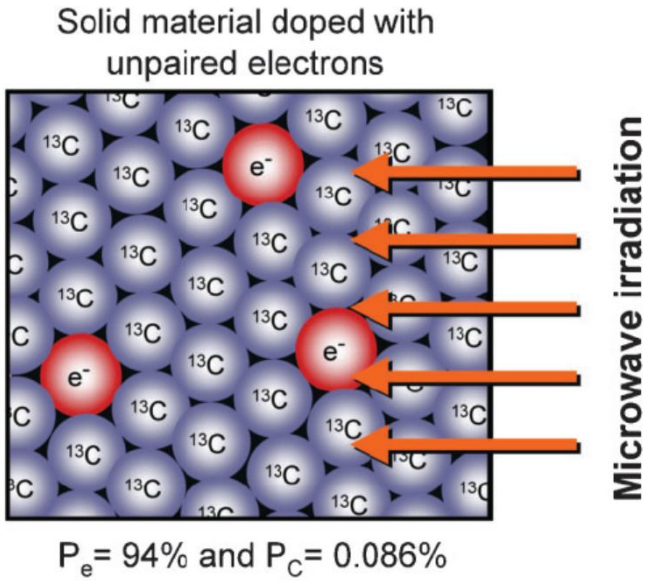


Overhauser (through MW)

Hyperfine interaction between nuclei and electrons

Spin diffusion

Dipolar interaction between nuclei



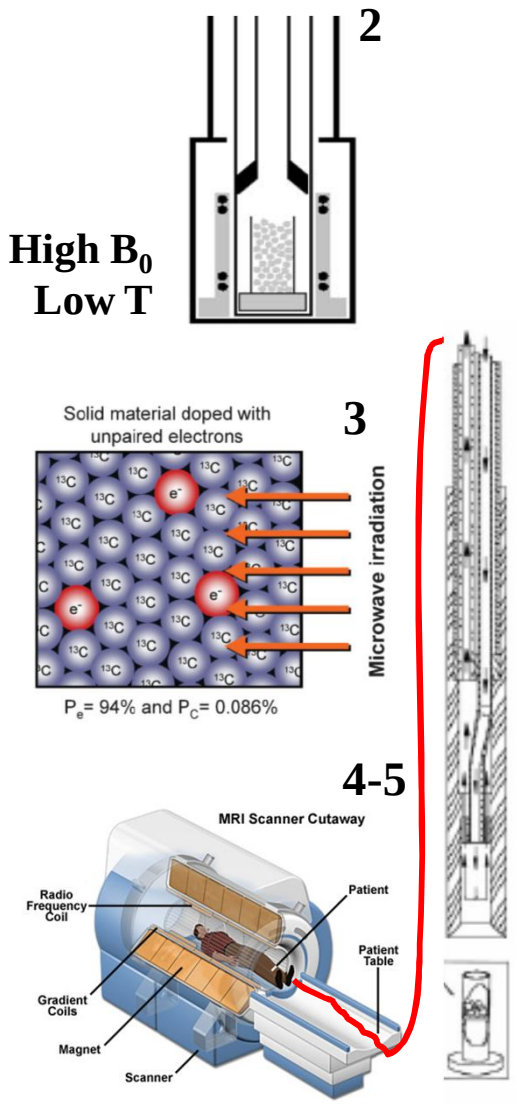
In principle every nucleus in every molecule may be polarized by DNP

Dynamic Nuclear Polarization (DNP)

DNP step by step

1. **Sample preparation:** molecules labelled with ^{13}C (or other low γ nuclei), possibly a glass-former solvent and molecules carrying unpaired electrons (e.g. organic free radical).
2. **Transfer of the sample** at low temperature and high field to obtain high electron polarization.
3. **Microwave irradiation** of the frozen sample to transfer polarization to the nuclear spins.
4. **Rapid dissolution** of the solid sample to obtain an injectable, with small polarization loss.
5. **Rapid i.v. injection** and fast image acquisition before relaxation to the thermal equilibrium.

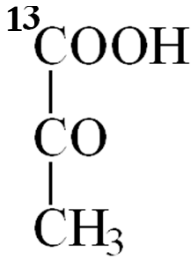
Typical time of a HP-MR procedure: 1 2 min

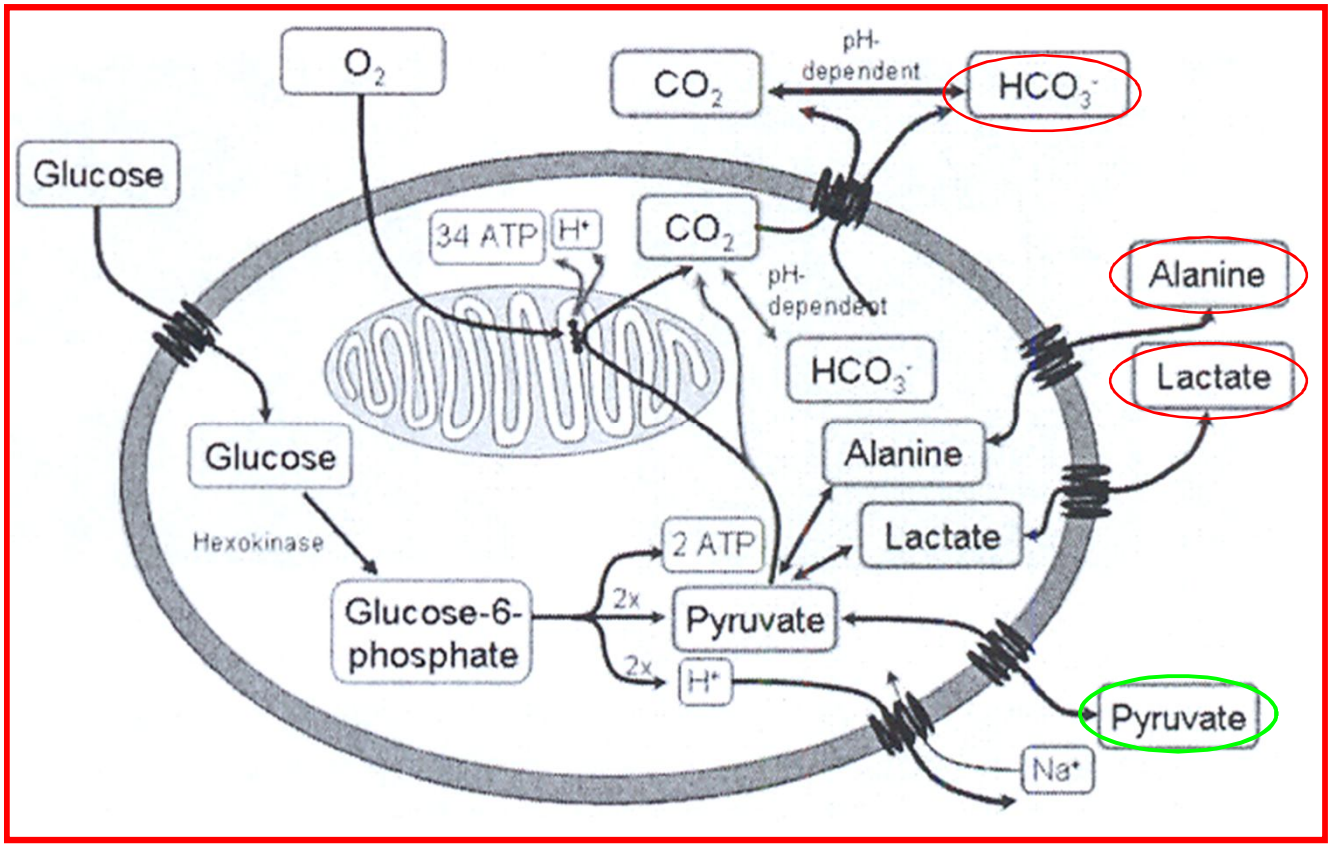


DNP hyperpolarized contrast agents – Metabolic Imaging

1-^{13}C-pyruvate

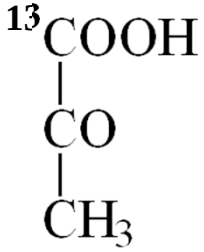
Pyruvate is a key-molecule in major metabolic and catabolic pathways in the mammalian cells, as it is **quickly** converted to alanine, lactate or carbonate to a different extent depending on the status of the cells





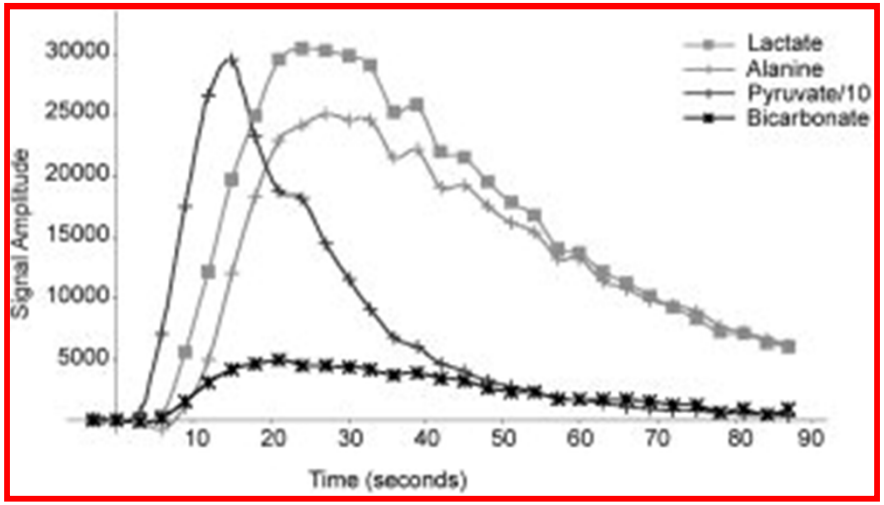
DNP HP - Metabolic Imaging

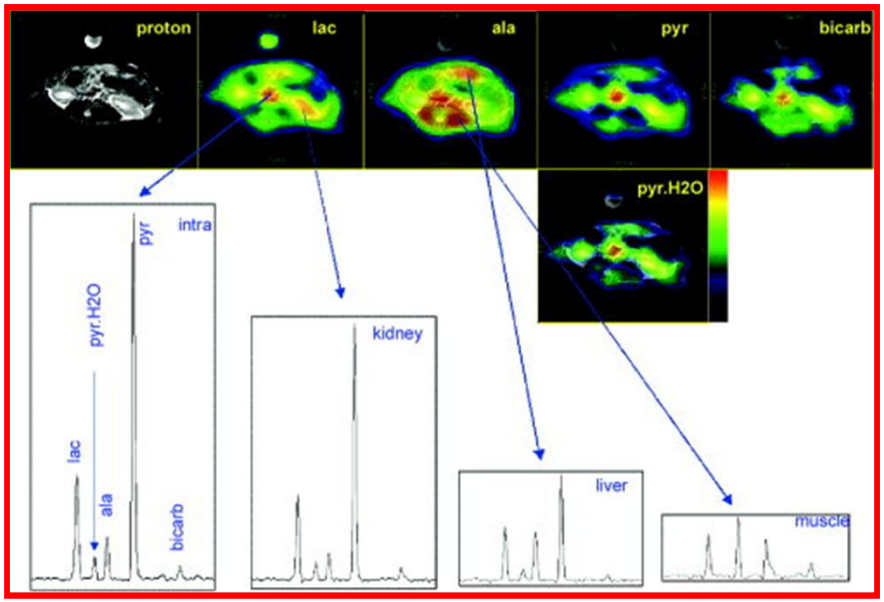
1-^{13}C- Pyruvate (P = 30%)



Conversion of pyruvate to lactate, alanine and bicarbonate occurs in few seconds.

Alanine is observed primarly in skeletal muscle and liver, while pyruvate, lactate and bicarbonate are more concentrated in skeletal muscle and kidneys.

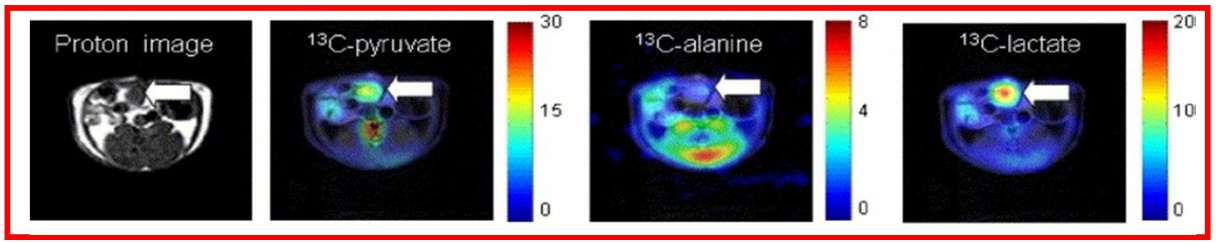




Kohler S.J. et al., Magn. Res. Med. 2007, 58, 65

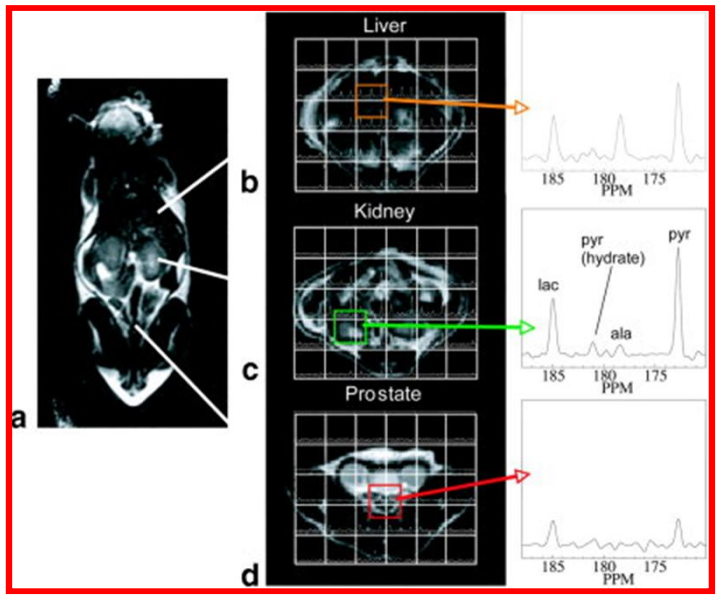
DNP HP - Metabolic Imaging

1-^{13}C- Pyruvate in tumors



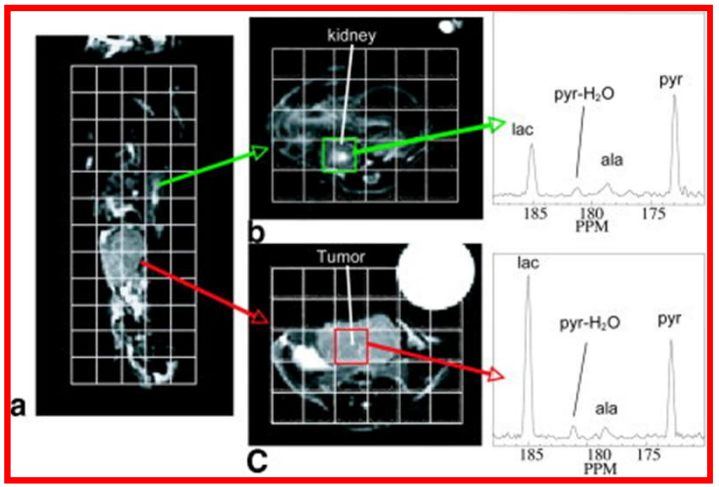
Golman K. Et al., Cancer Res. 2006, 66, 105855

Healty mouse



Chen A.P. et al., Magn. Res. Med. 2007, 58, 1099

TRAMP mouse



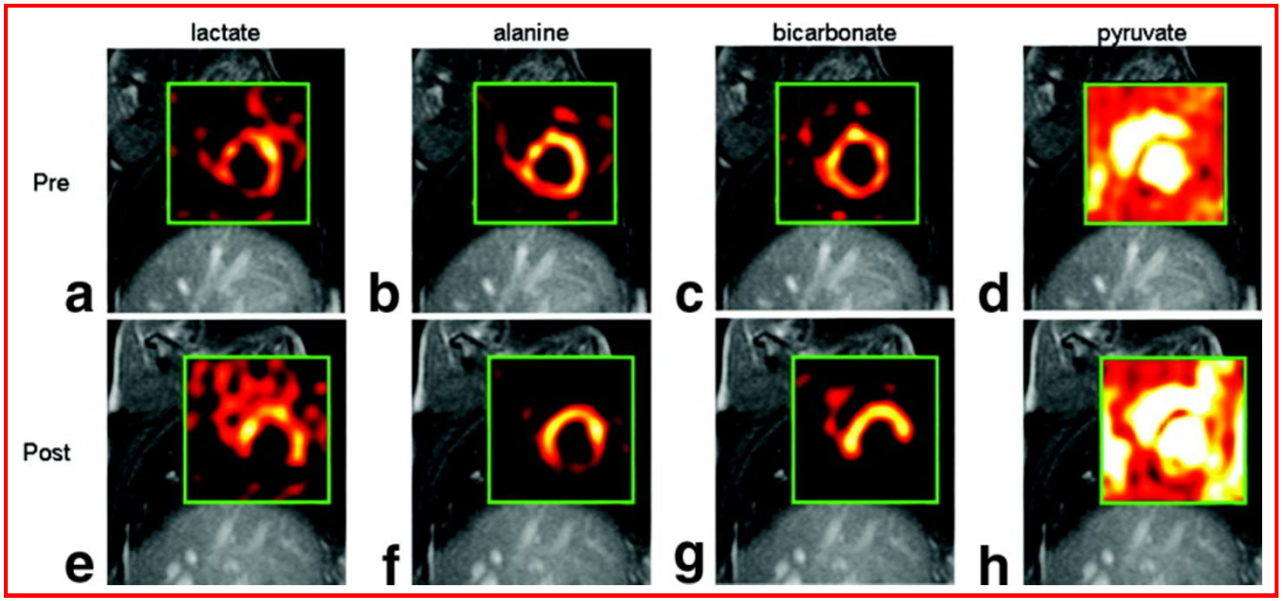
High lactate concentration in tumor

- Stadiation
- Monitoring of the response to treatment

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

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

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

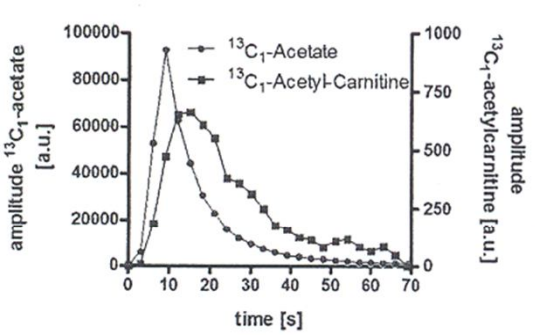


Figure 1. Dynamics of 1-^{13}C-Acetyl-Carnitine in mouse heart after injection of 1-^{13}C-Acetate.

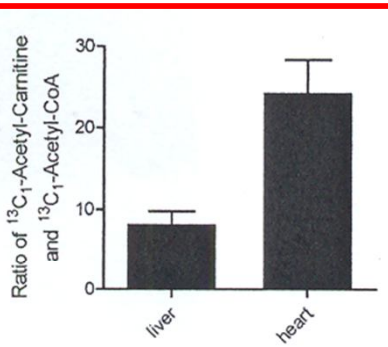
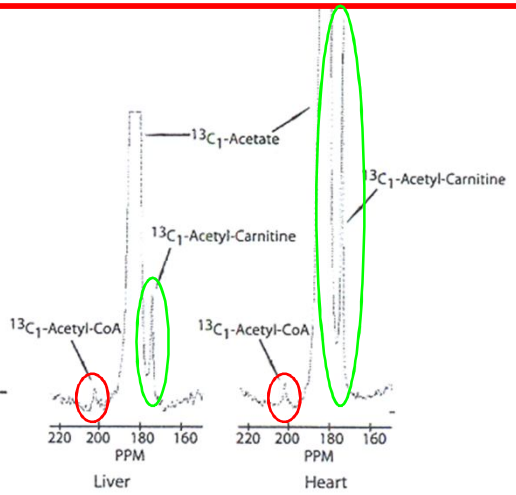


Figure 2. Distribution of the ratio of 1-^{13}C-acetyl-Carnitine and 1-^{13}C-acetyl-CoA in mouse heart and liver (n=6).



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

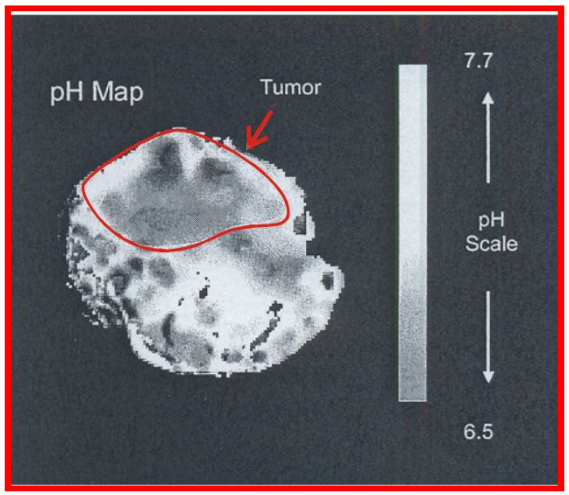
J. Biol. Chem. 2009, 284, 36077

DNP HP - Metabolic Imaging

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

$$HCO_3^- \xrightarrow{\text{Carbonic anhydrase}} CO_2$$

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



In vivo pH map calculated from $H^{13}CO_3^-$ and $^{13}CO_2$ peak intensities following injection of HP $H^{13}CO_3^-$

- Method validated *via* ^{31}P-MRS
- Bicarbonate is not toxic
- Measurement independent from concentration

Gallagher FA et al., Nature 2008;453:940–943.

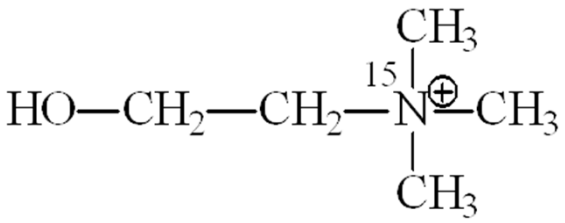
Molecules That Have Been Polarized Using DNP as Well as the Published Polarization Levels at 3.35 T, Method for Polarization, and the Biological Process(es) That Can Be Probed with Each Substrate

Molecule	Reported polarization	Method for polarization	What it can probe	References
[1-^{13}C]Pyruvate	Up to 40% at 3.35 T (up to 64% at 4.64 T)	Neat acid	LDH NADH/NAD$^+$ Glycolysis Treatment response PDH Intracellular pH ALT	18–22,26–33
[1-^{13}C]Ethyl pyruvate	28–35%	Ethanol	LDH Brain metabolism	34
[1-^{13}C]Lactate	7%	DMSO	LDH	35
^{13}C-Bicarbonate	15%	$CsH^{13}CO_3$ or $NaH^{13}CO_3$	pH Carbonic anhydrase activity	36,37
[1,4-$^{13}C_2$]Fumarate	26–35%	DMSO	Fumarase Necrosis	38–41
[2-^{13}C]Fructose	12%	Aqueous solution	GLUT5 Hexokinase Glycolysis PPP	42
[1-^{13}C]Ketoisocaproate	32% (~2-fold higher at 4.64 T)	Neat acid	BCAT Glutamate	43
[5-^{13}C]Glutamine	5%	Glycerol	Glutaminase	44
[1-^{13}C]Glutamate	28%	Tris base	ALT α-Ketoglutarate	45
[1-^{13}C]Urea	37%	Glycerol	Perfusion	10,11,37,46

K. Brindle et al., Magn. Res. Med. 66:505–519 (2011)

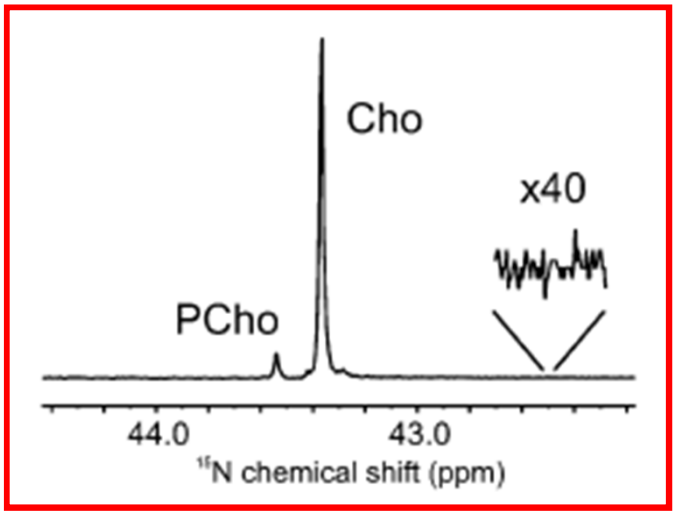
DNP HP - Metabolic Imaging

^{15}N-Choline (P = 5%)

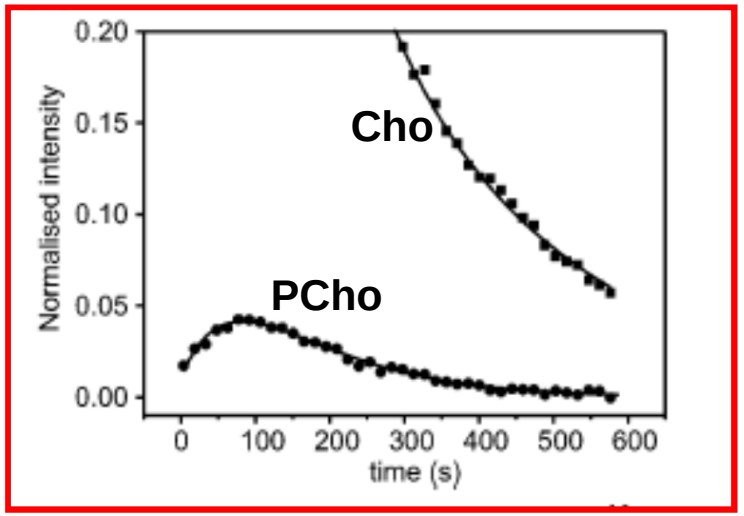


T_1 = 285 s (11.7 T, D_2O/H_2O)
T_1 = 120 s in human blood at 37°C

Choline + ATP —(Choline kinase)→ **PhosphoCholine + ADP**



^{15}N spectrum recorded after 114 s conversion (maximum PCho build-up)



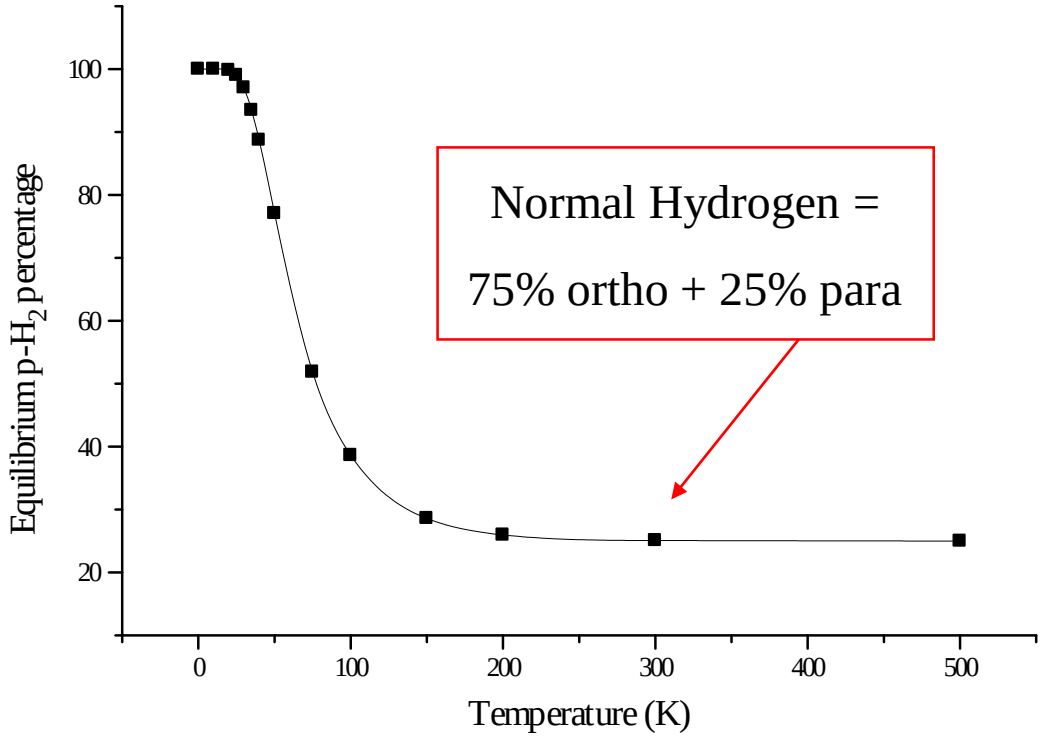
Gabellieri C. et al, JACS 2008, 130, 4598

Para-Hydrogen Induced Polarization (PHIP)

Para-Hydrogen

$$c(\alpha\beta - \beta\alpha)\ \begin{matrix}\beta\beta \\ \\ \alpha\alpha\end{matrix}$$ **Triplet (ortho)**

$$c(\alpha\beta + \beta\alpha)$$ **Singlet (para)**

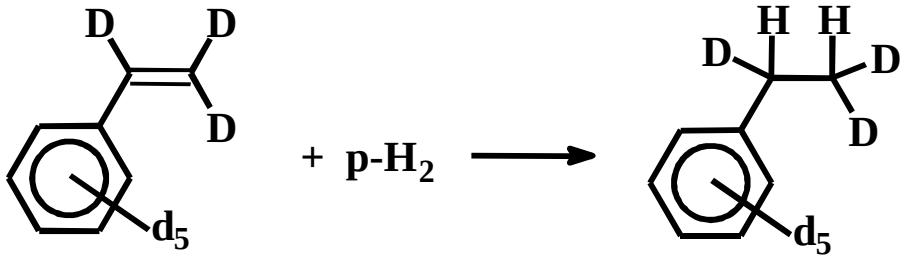


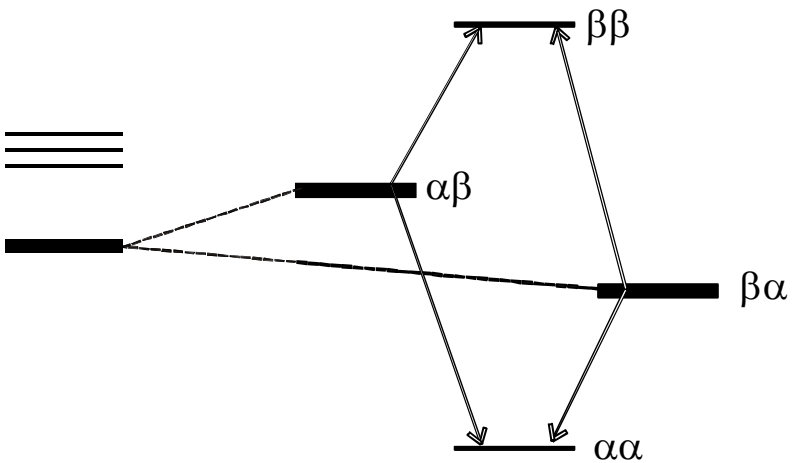
Hydrogen can be enriched in the para form at low temperature in the presence of a paramagnetic catalyst:

- about 50% para at liq. N_2
- 100% para at liq. He

Para-Hydrogen Induced Polarization (PHIP)

Following the addition of para-H_2 to an unsaturated substrate, the perturbation of the spin populations is maintained in the product, and typical NMR spectra characterized by strongly enhanced signals and adsorption-emission patterns are observed:





Para-H_2 **AX spin system**

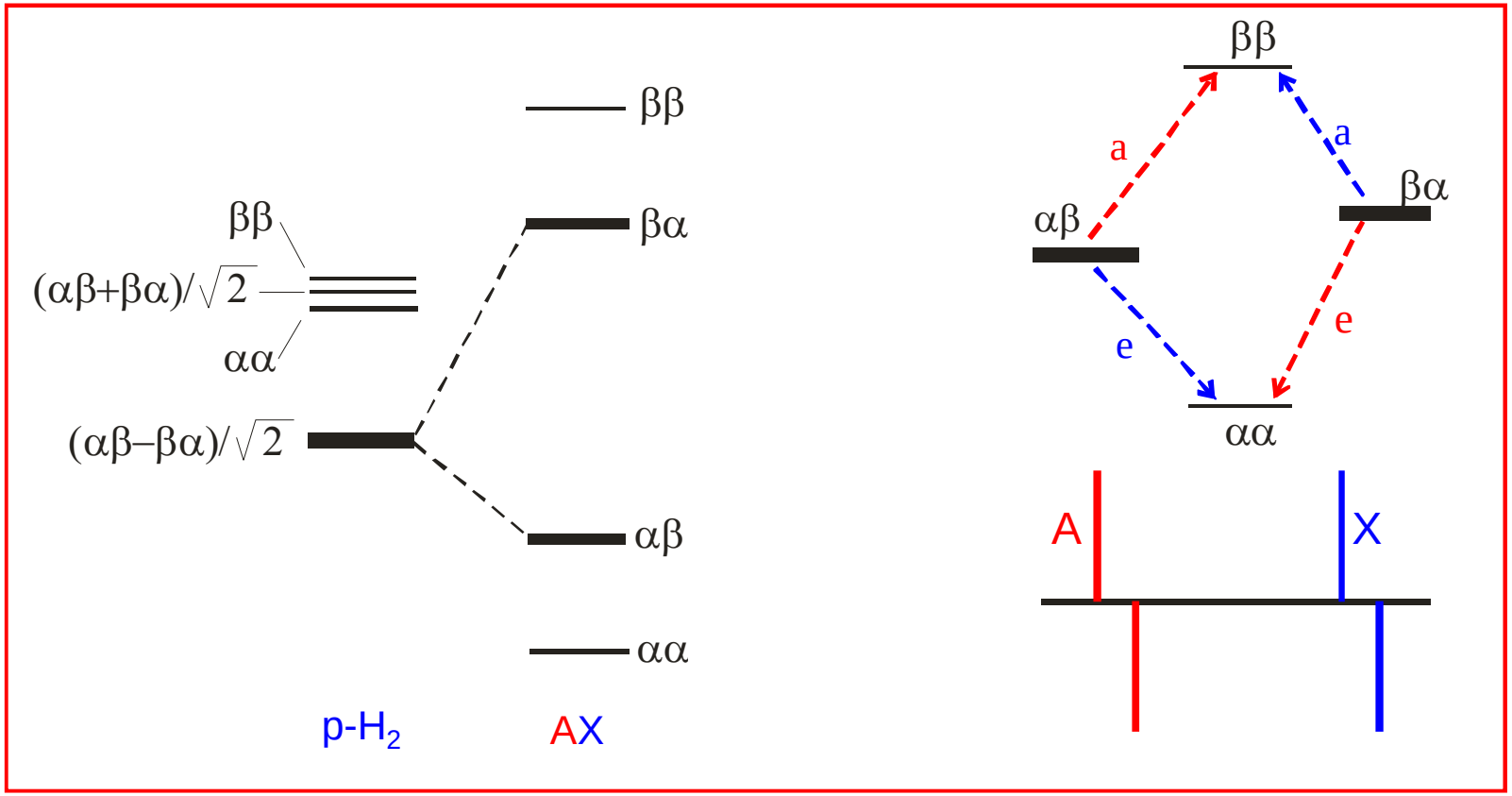
Resulting ^{1}H NMR spectrum:

Para-Hydrogen Induced Polarization (PHIP)

Parahydrogen And Synthesis Allow Dramatically Enhanced Nuclear Alignment

PASADENA

The reaction is carried out at high magnetic field (inside the NMR spectrometer)

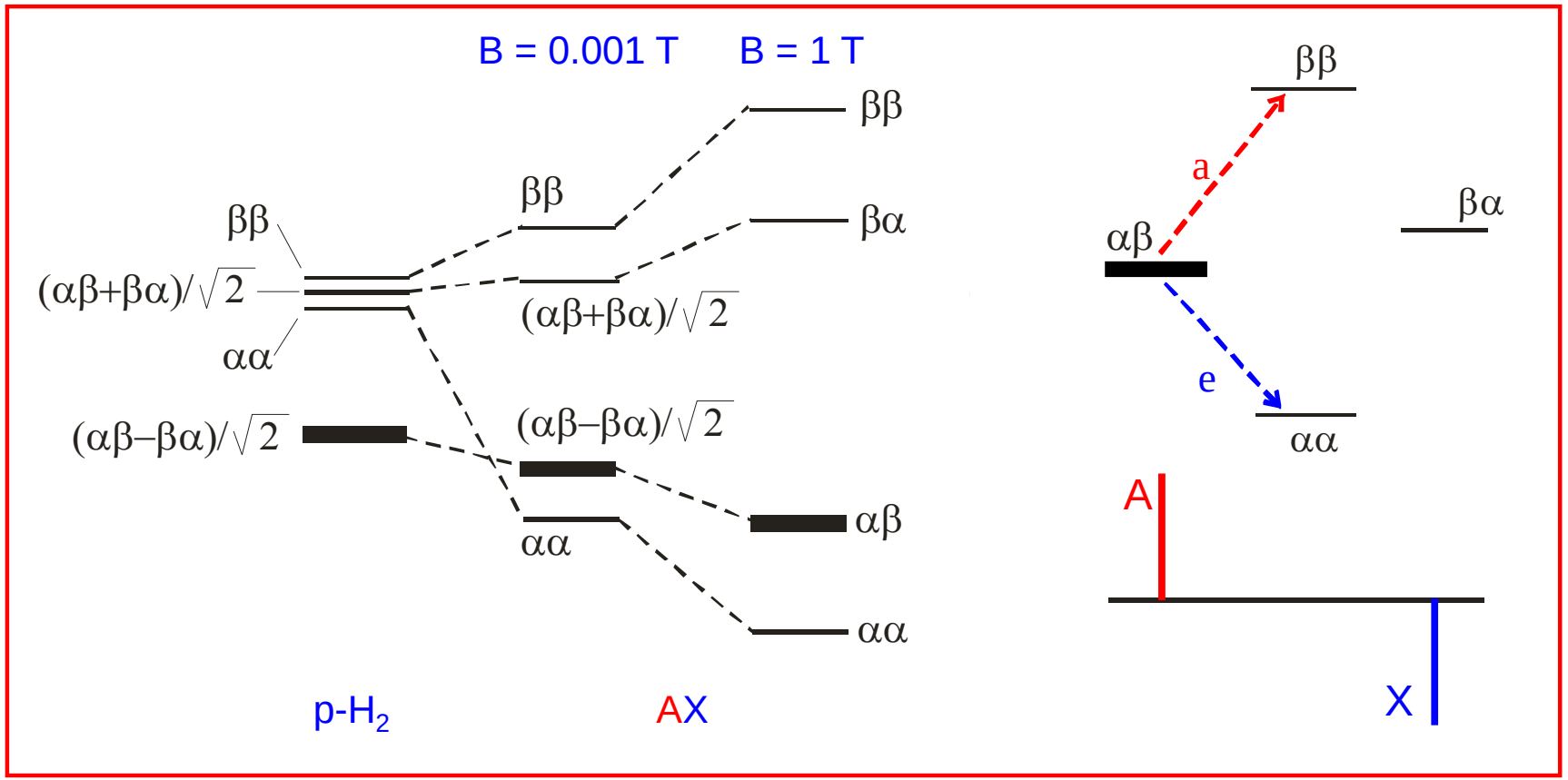


Para-Hydrogen Induced Polarization (PHIP)

Adiabatic Longitudinal Transport After Dissociation Engenders Net Alignment

ALTADENA

The reaction is carried out at low magnetic field
(the sample is introduced in the spectrometer after the reaction has taken place)



Para-Hydrogen Induced Polarization (PHIP)

Requisites to be satisfied in order to observe PHIP

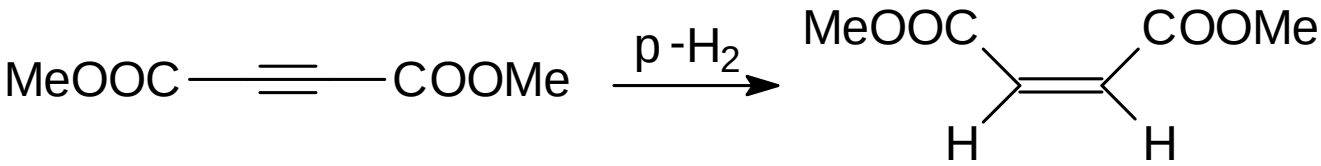
- Para-Hydrogen atoms must be transferred PAIRWISE to the same substrate molecule
- Para-Hydrogen atoms must occupy non equivalent positions in the product
- If para-hydrogen atoms occupy equivalent positions in the product molecule, symmetry can be broken by the presence of scalarly coupled ^{13}C atoms
- PHIP can be observed even in symmetrical molecules if asymmetrical intermediates are formed along the reaction pathway (this leads to unbalanced populations in the final product)
- The overall process must be faster than thermal spin relaxation

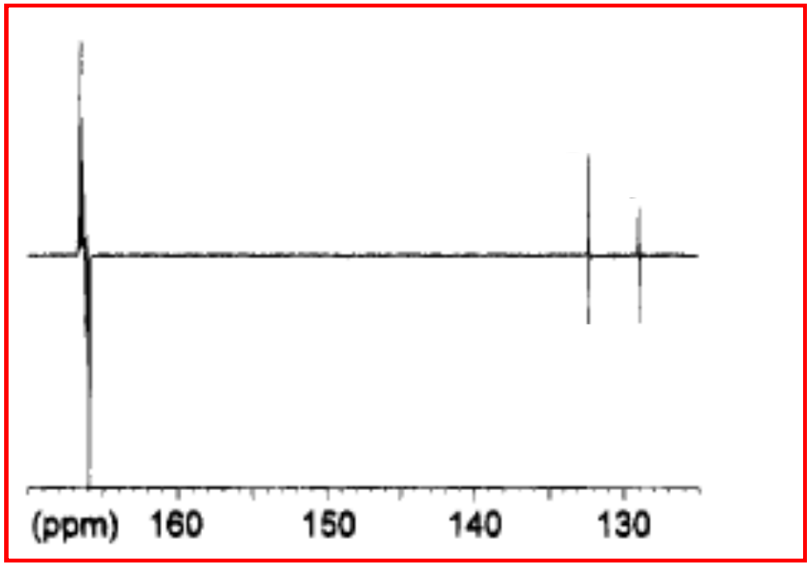
  - High reaction rates
  - Long nuclear relaxation times (T_1) in the products
  - Long relaxation times in the intermediates
  - Short lifetime of fast-relaxing intermediate species

Para-Hydrogen Induced Polarization (PHIP)

Polarization can be transferred from protons to heteronuclei by:

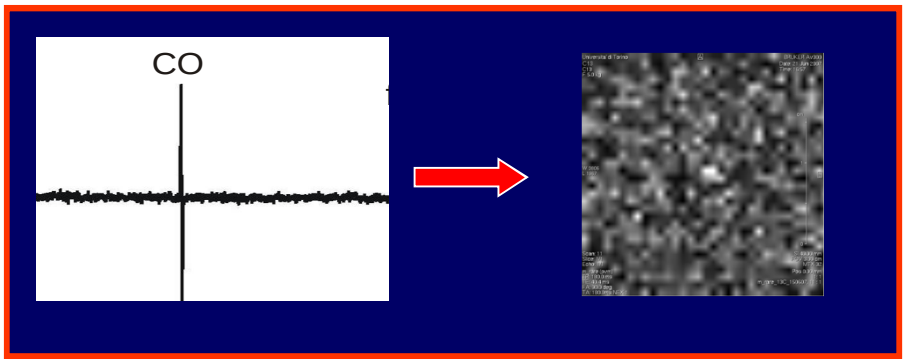
- nuclear Overhauser effect (nOe)
- Scalar Coupling

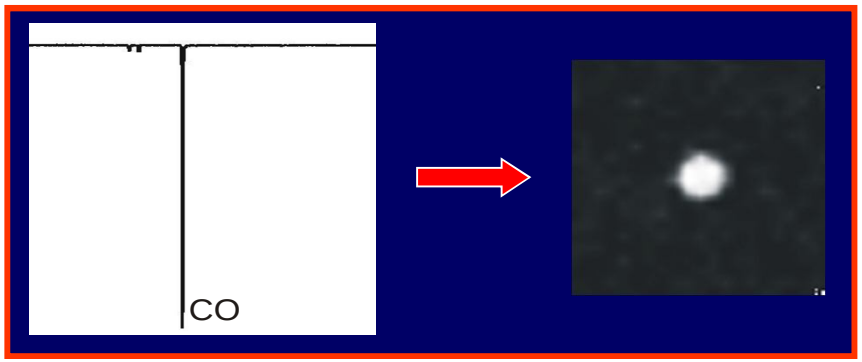




Para-H_2 containing molecules as hyperpolarized contrast agents

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

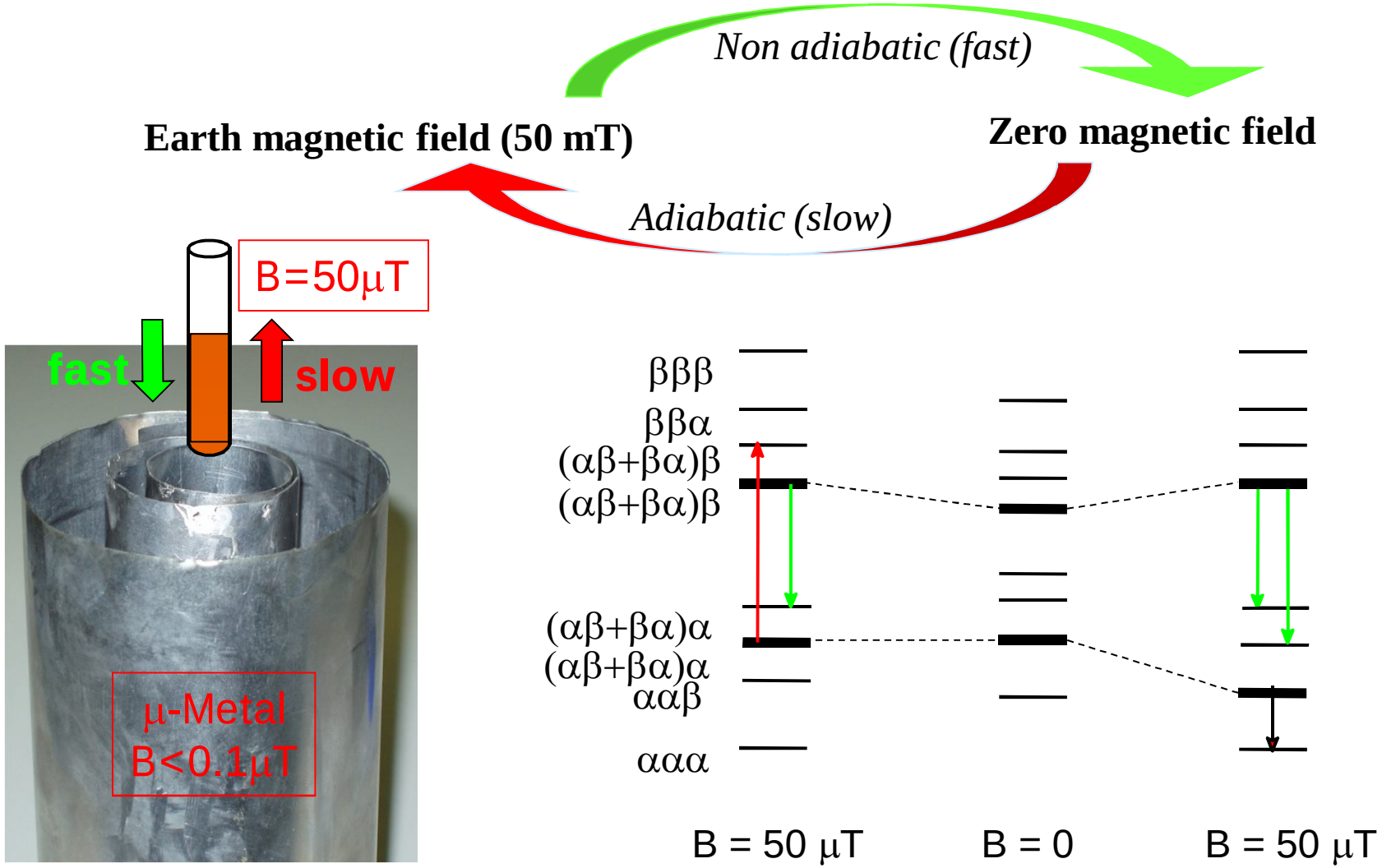




- Magnetic field cycling
- Pulse sequences

Para-H_2 containing molecules as hyperpolarized contrast agents

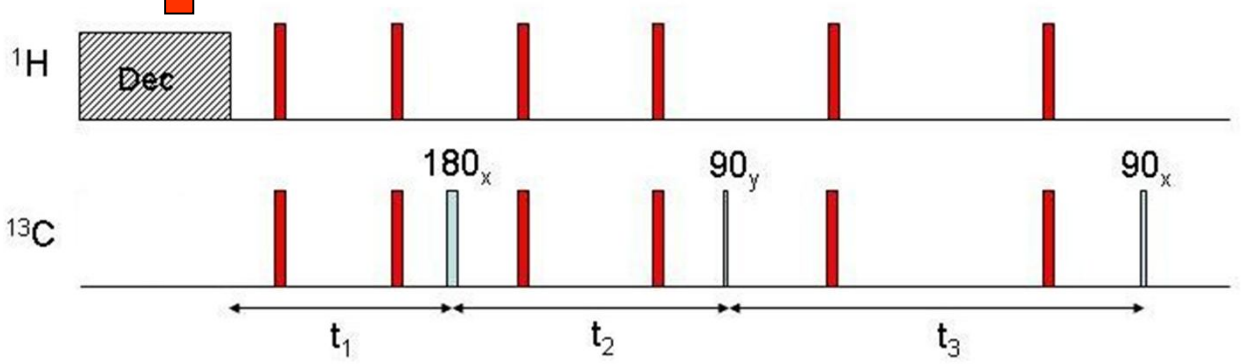
MAGNETIC FIELD CYCLING



Para-H_2 containing molecules as hyperpolarized contrast agents

✦ PULSE SEQUENCE

Loss of longitudinal spin order during the hydrogenation reaction is minimized by continuous decoupling.



Goldman M., Johannesson H., C.R.Phisique 2005, 6, 575

Many echoes are introduced to correct defects due to field inhomogeneity

Conversion of the spin order into net ^{13}C polarization

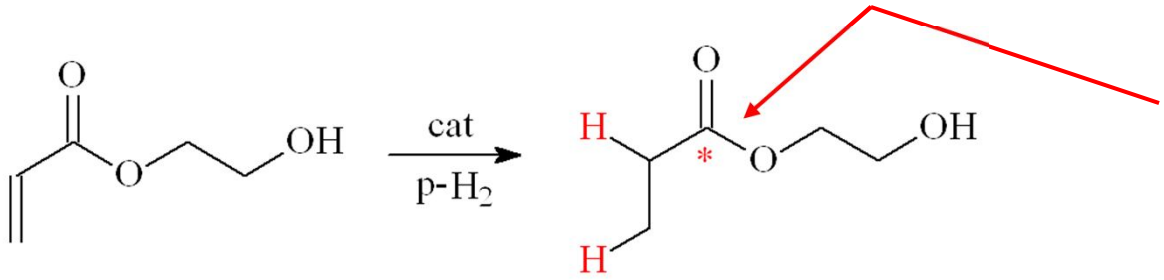
The time intervals between pulses are calculated on the basis of the J_{HX} coupling constants (between the heteronucleus and the added p-H_2 protons)

Para-H_2 containing molecules as hyperpolarized contrast agents

PHIP step by step

1. **Sample preparation:** the homogeneus hydrogenation catalyst (usually Rh(I) cationic complexes) in deuterated solvent is mixed with the substrate (labelled with ^{13}C or other low γ nuclei), and p-H_2 is added. The mixture is heaten if necessary.
2. **Hydrogenation**: the tube is vigorously shaken at low magnetic field (ALTADENA), or p-H_2 is bubbled in the tube inside the NMR spectrometer (PASADENA) for few seconds.
3. **Field cycling or pulse sequence application** to obtain net heteronuclear magnetization.
4. **FAST Purification** to eliminate the catalyst and organic solvent (if any).
5. **Rapid i.v. injection** and fast spectrum/image acquisition before relaxation to the thermal equilibrium.

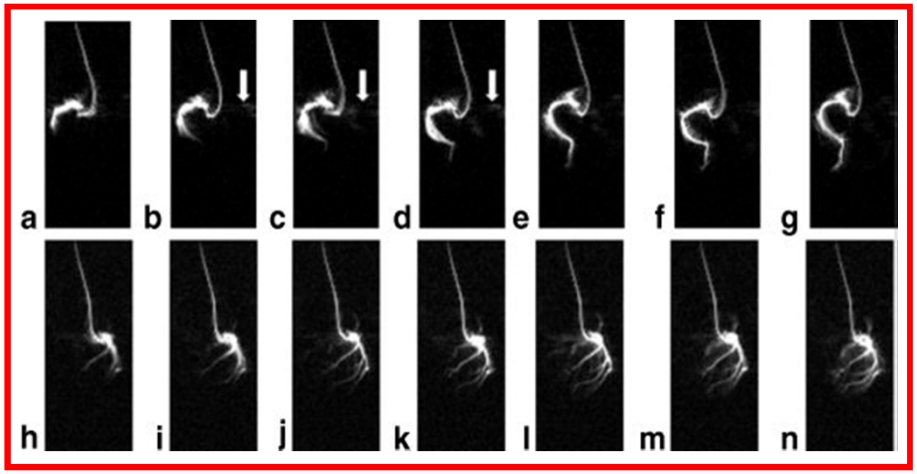
Para-H_2 containing molecules as hyperpolarized contrast agents



2-hydroxyethylacrylate *2-hydroxyethylpropionate*

HP ^{13}C Angiograms of a pig heart

SSFP, images acquired in a continuous series

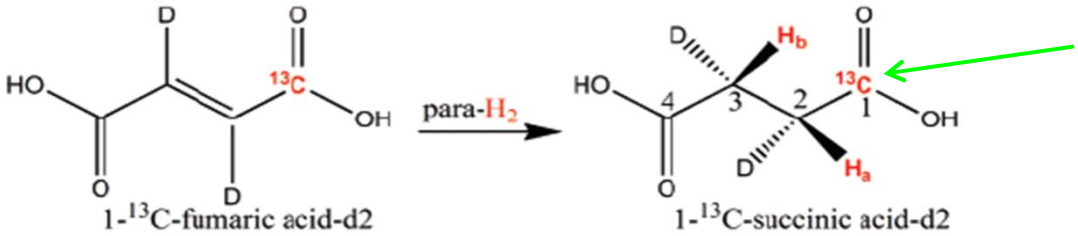


Olsson S. et al.,Magn. Res. Med. 2006, 55, 731

- **Rh(I) cationic water-soluble catalyst**
- **Para-hydrogenation in water**
- **Automatic PHIP polarizer by GE**
  - ***10 bar p-H_2***
  - **Pulse sequence for magnetization transfer to ^{13}C**
- **Catalyst must be removed before in-vivo injection**

Para-H_2 containing molecules as hyperpolarized contrast agents

PASADENA hyperpolarization of a biologycal molecule: succinic acid

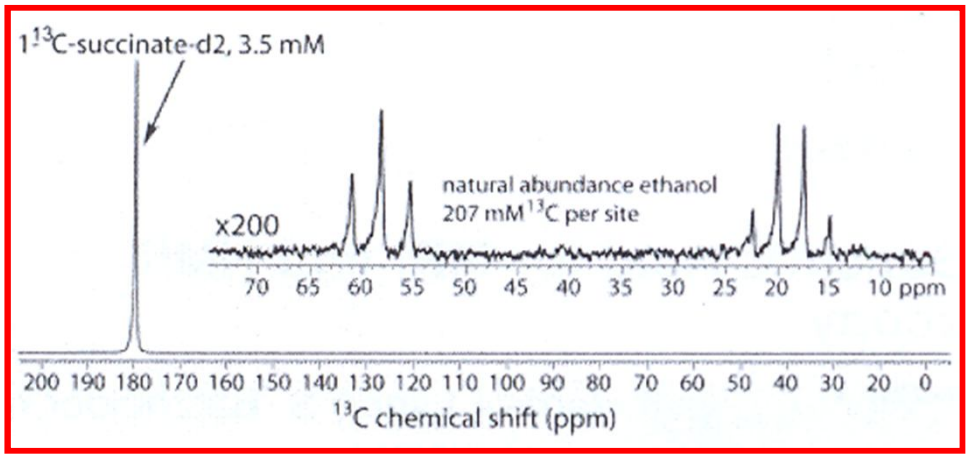


1-^{13}C-fumaric acid-d2 → 1-^{13}C-succinic acid-d2

^{13}C-enriched

T_1 = 39 s in D_2O (pH 3)

Typical ^{13}C spectrum of 3.5 mM 1-^{13}C-succinate-d_2 hyperpolarized at <u>pH = 2.9</u>



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

- The coupling constants values depend on pH and are not determined at phisiological values due to chemical exchange: the pulse sequence to obtain net ^{13}C magnetization can only be applied under acidic conditions
- A purification step is required after para-hydrogenation

Para-H_2 containing molecules as hyperpolarized contrast agents

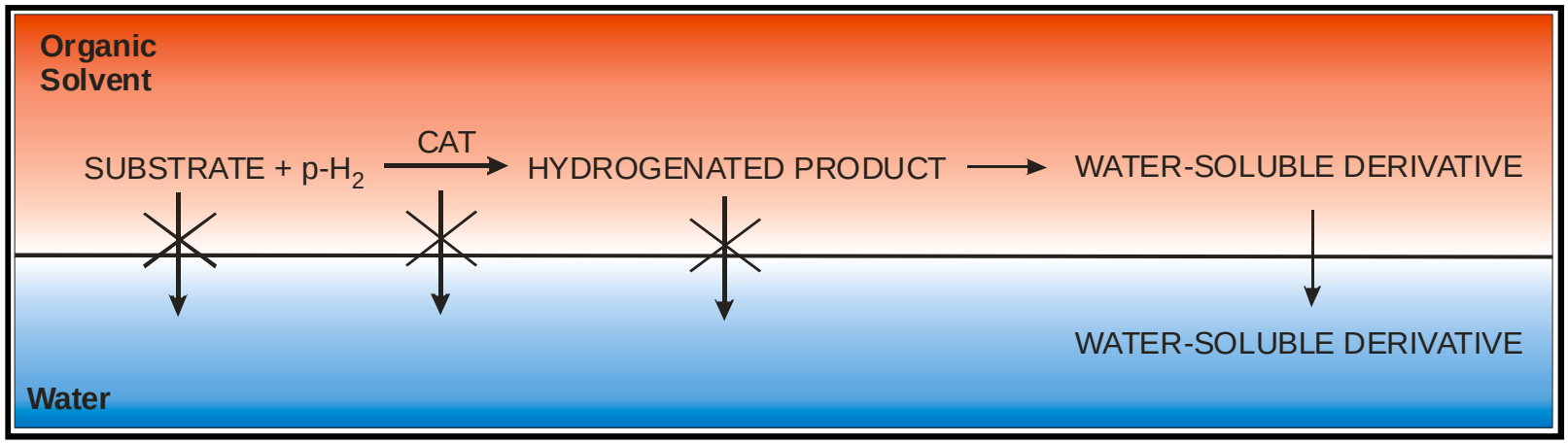
Organic solvent and catalyst removal after para-hydrogenation

Necessary for *in-vivo* measurements

- Cationic catalyst removal by ion exchange and solvent removal by spray distillation → Polarization loss
- Para-hydrogenation in water and catalyst removal by ion exchange → Water soluble Rh catalysts; High pressure required for H_2 dissolution in water
- Preparation of water solutions by phase extraction

Para-H_2 containing molecules as hyperpolarized contrast agents

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

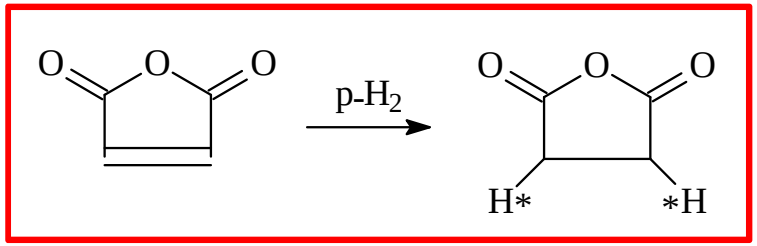


A lipophylic precursor of the compound of interest is para-hydrogenated in an organic solvent not miscible with water, then it is quickly converted to the hydrophilic derivative by addition of an aqueous medium and it is extracted in the water phase as a pure compound.

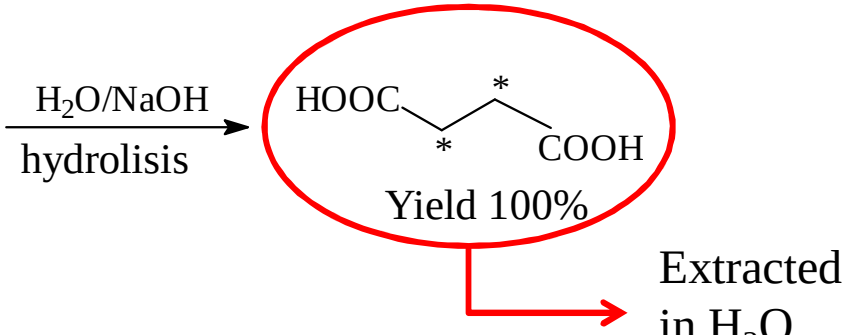
The procedure allows to obtain pure water solution of the compounds of interest in one step only by a simple phase extraction process, avoiding the use of high para-H_2 pressures and expensive experimental set-ups.

Para-H_2 containing molecules as hyperpolarized contrast agents

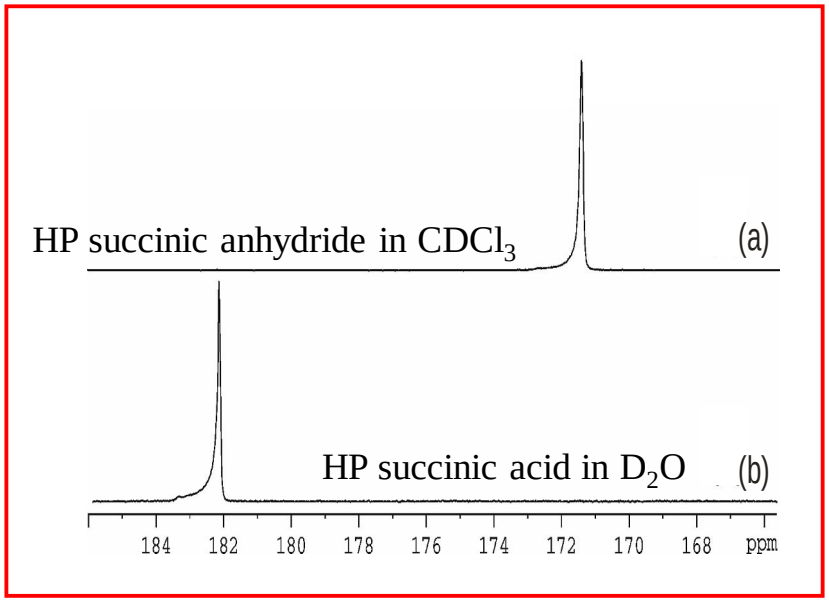
Example: para-hydrogenation of maleic anhydride and subsequent hydrolisis afford succinic acid



Reaction and pulse sequence in chloroform



- The coupling constants values of succinic anhydride in chloroform are well determined and the time intervals for the pulse sequence can be accurately calculated
- A pure aqueous solution of succinic acid is obtained in one step only by addition of basic water and phase extraction



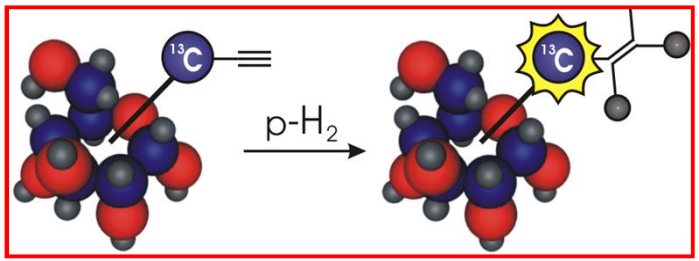
Para-H_2 containing molecules as hyperpolarized contrast agents

Design of novel para-hydrogenated contrast agents

- Non toxic
- Water- soluble
- Hydrogenatable

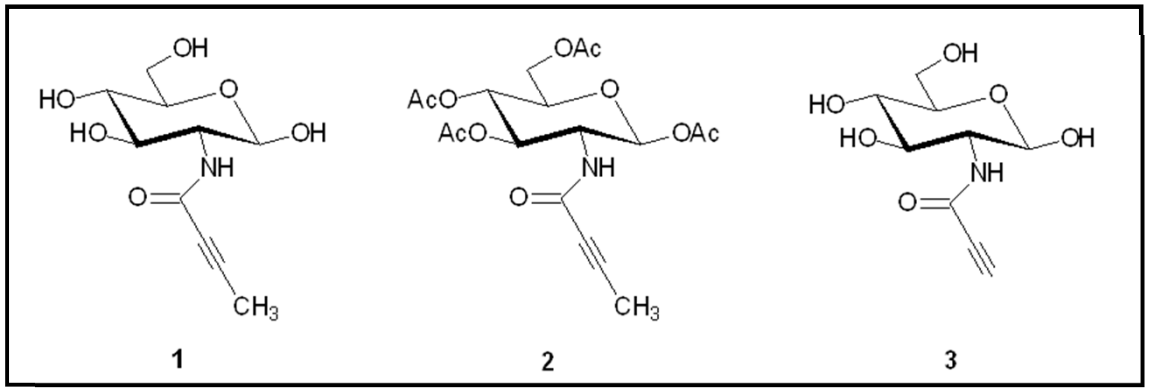
Binding of an hydrogenatable synthone to a biological substrate

Example: Conjugation of butynoic acid to Glucose

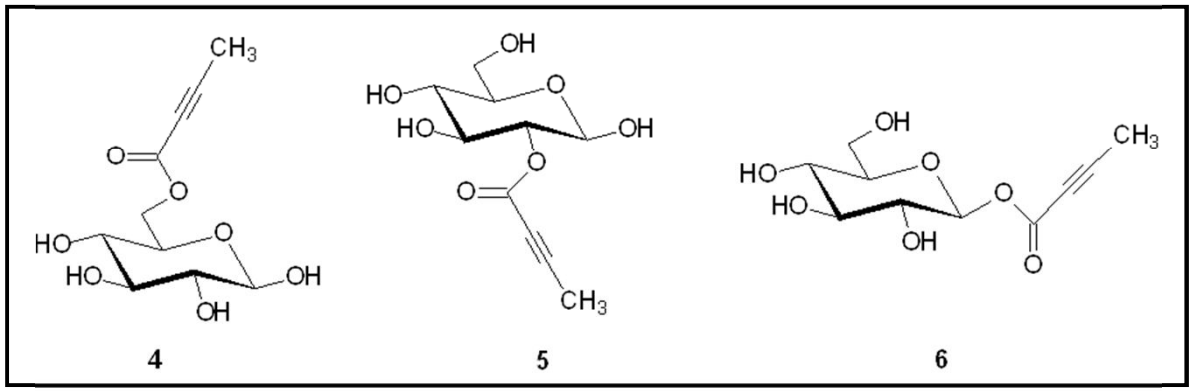


Toxicity tests: OK
Cellular Uptake experiments: OK

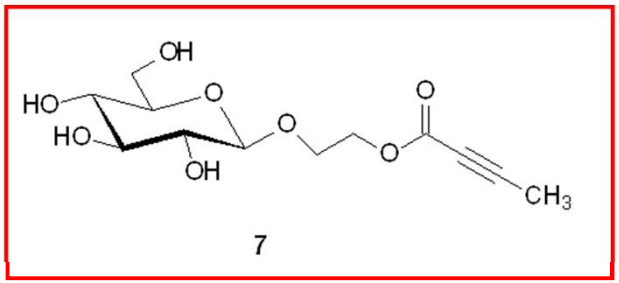
Para-H_2 containing molecules as hyperpolarized contrast agents



Amidic derivatives are hydrogenated in low yield and do not allow ^{13}C-PHIP detection.

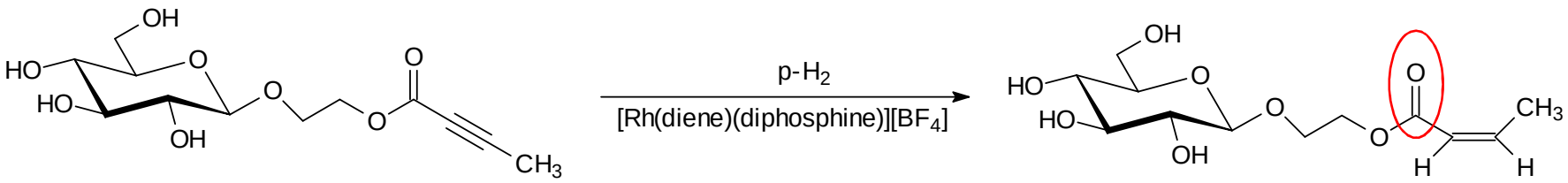


Ester derivatives are hydrogenated in high yield and afford highly enhanced ^{13}C signals, but the ^{13}C T_1 of the CO group is too short.

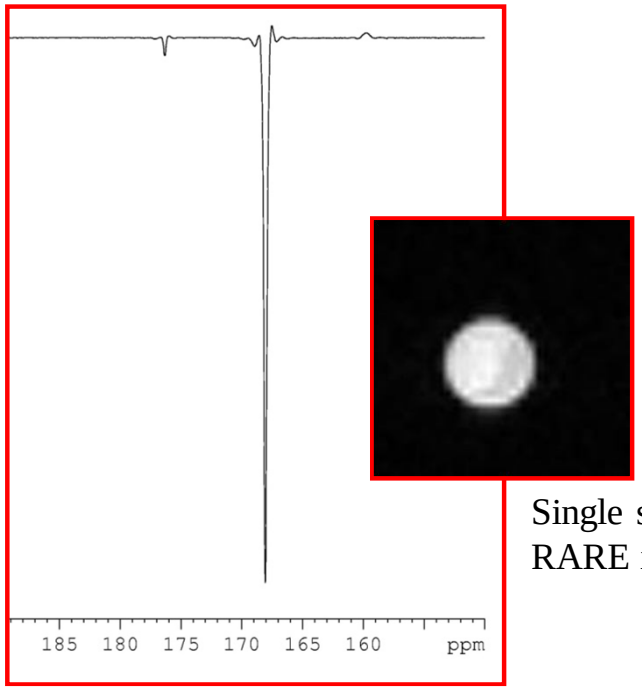


The insertion of a spacer between the glucose ring and the unsaturated synthone is efficient in maintaining the ^{13}C T_1 of the carbonyl group long enough for further manipulations and NMR/MRI experiments.

Para-H_2 containing molecules as hyperpolarized contrast agents



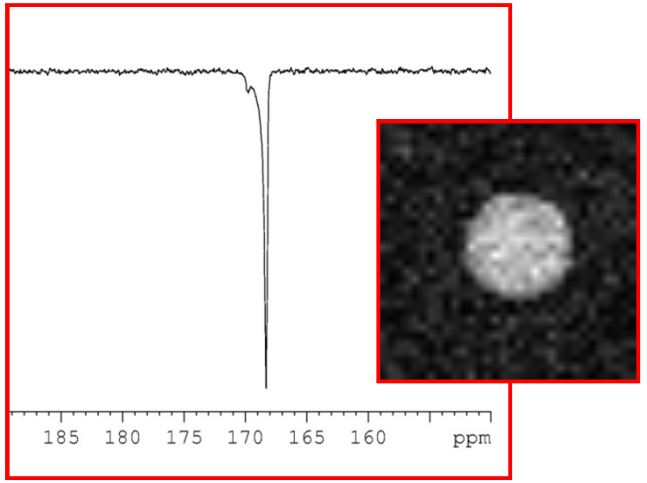
Para-hydrogenation in D_2O (^{13}C enriched substrate, field cycling)



Single scan ^{13}C spectrum

Single shot ^{13}C RARE image

Catalyst removal by ion exchange



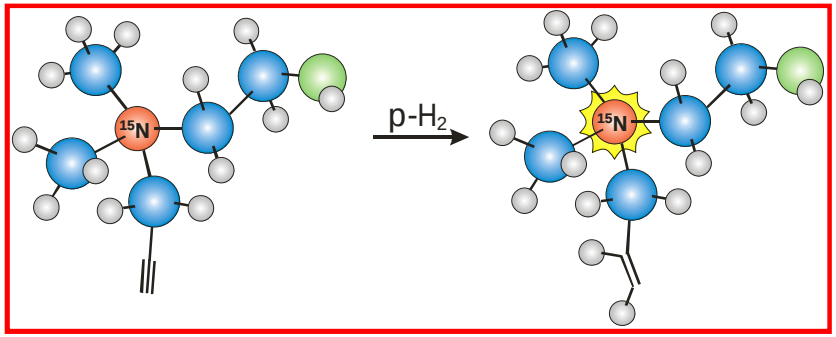
Para-H_2 containing molecules as hyperpolarized contrast agents

Design of novel para-hydrogenated contrast agents

- Non toxic
- Water- soluble
- Hydrogenatable

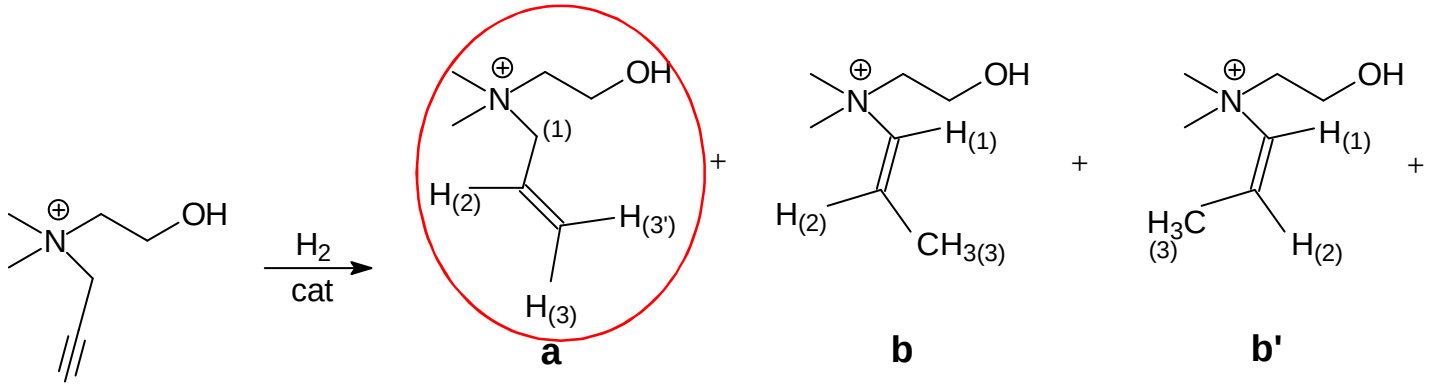
Binding of an hydrogenatable synthone to a biological substrate

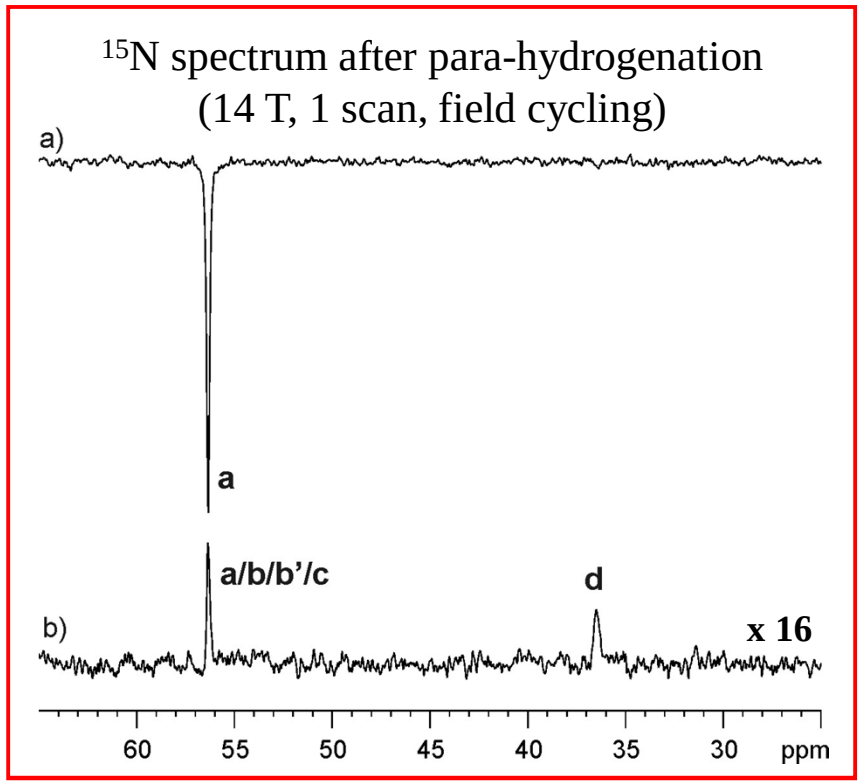
Example: Conjugation of propargyl to ^{15}N-choline

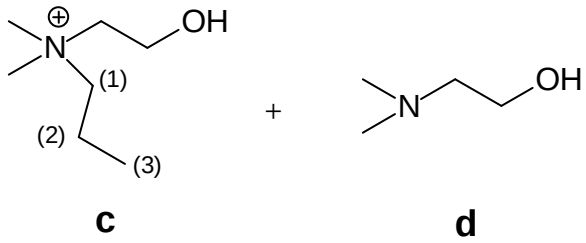


Toxicity tests: OK
Cellular Uptake experiments: OK

Para-H_2 containing molecules as hyperpolarized contrast agents



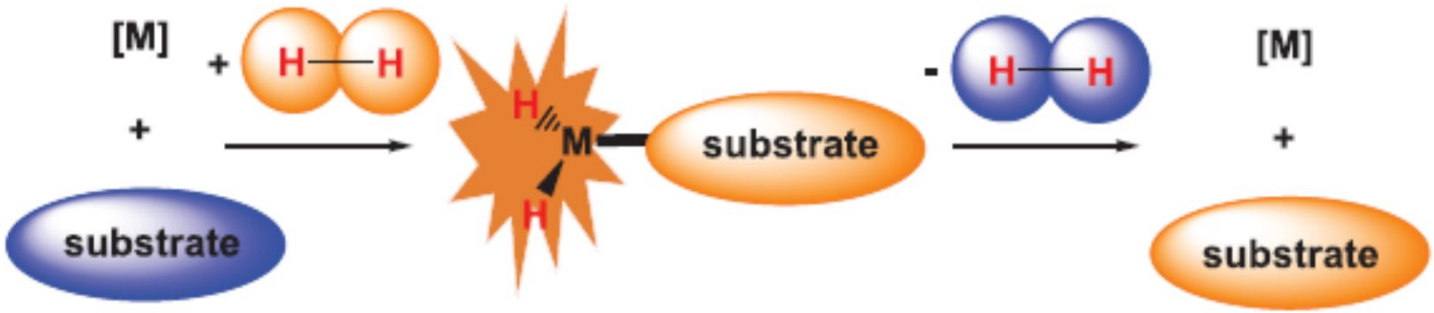




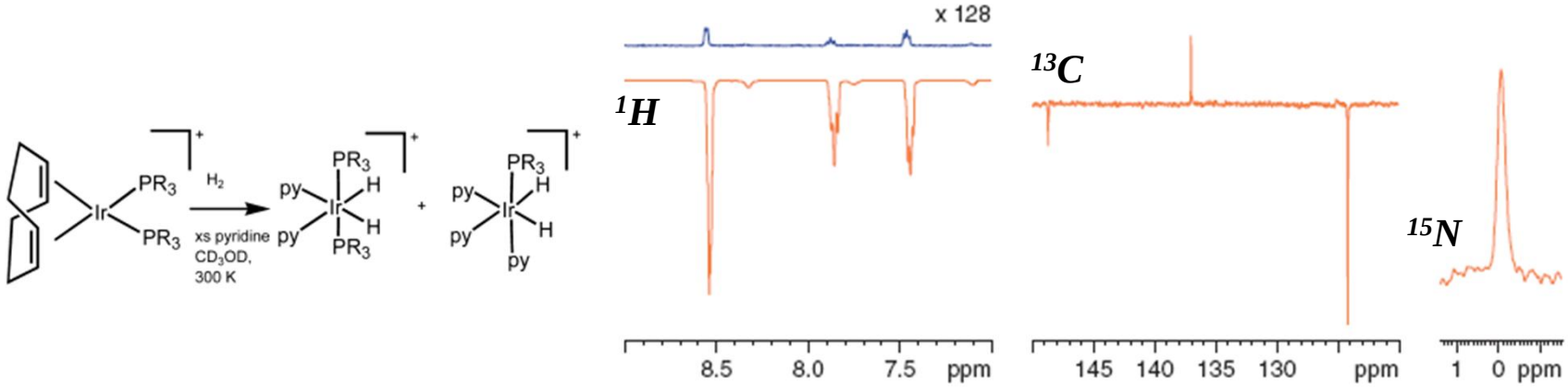
Field cycling experiments, theoretical studies, experiments with deuterium/ deuterated propargylcholine and inverse INEPT tests have shown that **a** is the only product in which polarization transfer to ^{15}N takes place, **via the small $J^3_{H,N}$**

Signal Amplification By Reversible Exchange (SABRE)

Tramsfer of para-H_2 derived spin order to other molecules via reversible interactions, **without hydrogenation**



Example: polarization of ^{15}N in pyridine and other N-containing molecules via reversible interaction with an Ir complex



Science ***2009****, 323, 1708; Inorg. Chem.* ***2009****, 48, 663*

Conclusions

What to keep in mind when designing an HP probe

- Long relaxation times
- Isotopic labelling
- Biocompatibility / biological interest
- Chemical requirements for PHIP

Laser polarized noble gases

- Limited applications

DNP

- Wide variety of candidate molecules
- Expensive
- Low quantities

PHIP

- Cheap and easy technique
- Continuous flow is possible
- Limited number of candidate molecules
- Purification issues