Optimizing dissolution dynamic nuclear polarization to perform *in vivo* hyperpolarized NMR

Arnaud Comment
Studying energy metabolism *in vivo* via $^{13}$C NMR

- Thermally polarized $^{13}$C studies following $^{13}$C-labelled substrates injection
- Only the most concentrated amino acids (e.g. glutamate, glutamine and aspartate in the brain) can be detected *in vivo*

13C metabolic studies

Glycolysis

Pyruvate

Lactate

Glutamine

Glutamate

Acetyl-carnitine

CoA

Acetyl-CoA

2-oxoglutarate

Citrate

Malate

Fumarate

TCA cycle

Bicarbonate
How to get the highest possible SNR

• **#1: Hyperpolarization**
  • Obtain the highest polarization possible in the polarizer

• **#2: Optimized acquisition**
  • Use highly-sensitive coils
  • Develop optimal MR sequence

• **#2’: High-order shimming and decoupling!**

Ex-situ technique: sample spend non-negligible time outside of an MR magnet

• **#3: Nearly lossless sample transfer**
  • Fast transfer
  • Quickly remove paramagnetic impurities (radicals, Gd,...)
  • Long-lived states
DNP by solid effect

- Energy balance: $h(v_e - v_n) + h\nu_n = h\nu_e$
Principles of DNP

- Homogeneous distribution of polarizing agents
- Optimal conditions for efficient polarization transfer: \(~1\) K, 3-5T
- 1 polarizing agent per 1000 nuclei (20-50mM)
- Microwaves to force polarization transfer (electron-nucleus dipolar interaction & spin diffusion)

Trityl stable radical
Thermodynamic description of solid effect

- Short $T_{1,e}$ and long $T_{1,n}$ required
**Thermodynamic description of thermal mixing**

Dynamic cooling of electron spins (Redfield, 1955):
All electron spins acquire a single spin temperature

Thermal mixing: all nuclear spins reach the same spin temperature than the electron spins

- Short $T_{2,e}, T_{1,e}$ and long $T_{1,n}, T_{1,d}$ required
Lower temperature and increase field

- Numerical solutions of Borghini model (thermal mixing)

- Experimental results match theoretical predictions

- New challenge: increasing the field to 7T (200 GHz)


Going to higher field

- 3M [1-\textsuperscript{13}C]acetate in 2:1 D\textsubscript{2}O:d\textsubscript{6}-ethanol with 50mM TEMPOL
Going to higher field: Issues and strategies

- Microwave losses: corrugated waveguide at 1K?
  Currently testing prototype from SwissTo12

- Trityl @4.6T: 70% ($T_{\text{build}} \sim 6000$)

- Cross-polarization
- Adapt sample recipes
- Multi-sample DNP polarizer
  S. Hu et al., MRI, in press

Is the polarization time a real issue?

H. Jóhannesson, S. Macholl, and J. H. Ardenkjaer-Larsen
JMR 197, 167 (2009)
How to get the highest possible SNR

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3. **Nearly lossless sample transfer**
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RF coils

- Detection with quadrature $^{13}\text{C}$ surface coil
- Excitation with $^{13}\text{C}$ volume coil

L. Darrasse and J.-C. Ginefri
Biochimie 85, 915 (2003)
Pulse sequences

Several important developments led to the optimization of the use of the available magnetization (UCSF, Toronto, Weizmann, Freiburg, Malmö and Tübingen)

- Fast chemical shift imaging sequences
- Compressed sensing
- Frequency-selective excitation pulses
- Spatial-spectral selective excitation
- *In vivo* $^{13}$C-$^1$H polarization transfer?

$\sim$90% losses because of short T$_2$ *
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A DNP protocol for *in vivo* studies

- Custom-designed polarizer
  - 5T / 1K
- 9.4T shielded MRI scanner

4.5 M $^{13}$C acetate
33 mM TEMPO $D_2O$
: $d_6EtOD$ 2:1

DNP polarization (1 to 2 hr)
DNP protocol for *in vivo* studies

- 9.4T shielded MRI scanner
- Custom-designed polarizer 5T / 1K

Rapid dissolution and transfer (3 s)

Hot D$_2$O vapor
DNP protocol for \textit{in vivo} studies

Custom-designed polarizer 5T / 1K

9.4T shielded MRI scanner

4m

Automatic infusion (9 s)

2.2 ml 0.3 M $^{13}$C acetate

A. Comment
DNP protocol for *in vivo* studies

- Custom-designed polarizer
  5T / 1K

- 9.4T shielded MRI scanner

- MR measurements (up to 1 min)
DNP protocol for in vivo studies

Custom-designed polarizer 5T / 1K

9.4T shielded MRI scanner

4m

Detection in separator (1s)

Shim set for separator

Change shim set during infusion (9s)

In vivo acquisition

A. Comment
DNP protocol for in vivo studies

**Infusion pump**

- Compatible with high magnetic field (9.4 T)
- Operated automatically
- Separate gas from hyperpolarized solution
- Optical safety device checks for the potential presence of gas at the infusion port

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Three consecutive $^{13}$C NMR measurements

- Three consecutive single-shot low flip angle $^{13}$C measurements during metabolic study without affecting the experimental timing

T. Cheng, M. Mishkovsky, O. Ouari, P. Hautle, P. Tordo, B. van den Brandt, A. Comment, *NMR Biomed* 2013
Low-field relaxation can be very short

- $^{13}\text{C}$ relaxation via scalar coupling to $^{14}\text{N}$
- $[5^{-13}\text{C}]$glutamine (E. Kubala, GE Healthcare)

- $[1^{-13}\text{C}]$urea (J. Kurhanewicz, UCSF)
Magnetic field and temperature variations

![Graph showing magnetic field and temperature variations.](image)

- **Static magnetic field** $B_0(T)$
- **Time** (s)
- **Temperature** (K)

Key events:
- **Dissolution**
- **Transfer**
Scavenging radicals in infusion pump

![Graph showing polarization loss in infusion pump](graph.png)

- Polarization loss due to transfer: ~10%
- Polarization loss avoided by scavenging TEMPO: ~9%
- $T_1 = 73\text{s}$
- $T_1 = 32\text{s}$
Scavenging radicals in infusion pump

- Large amount of Vitamin C is required to have fast scavenging
- Large amount of vitamin C leads to reduced T₁
Thermoresponsive spin-labeled hydrogel

MW irradiation

\[ \Delta T \]

1-\textsuperscript{13}C-butanol polarized by SL-hydrogel
\[ T_1 = 112 \pm 2 \text{s} \]

1-\textsuperscript{13}C-butanol polarized by TEMPO
\[ T_1 = 29 \pm 0.1 \text{s} \]

Non-degassed tert-butanol
\[ T_1 = 83.5 \pm 2.5 \text{s} \]

Degassed tert-butanol
\[ T_1 = 114.5 \pm 6 \text{s} \]
In-line filtering process

- BDPA has similar efficiency as trityls to polarize $^{13}$C

L. Lumata et al., Chemistry 17 (2011)
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Real-time *in vivo* metabolism in mouse brain

Y. Takado, T. Cheng, M. Mishkovsky, A. Comment, R. Gruetter, *ISMRM 2012*
In vivo real-time formation of 2-oxoglutarate

- Additional resonance peak at 182.05 ppm
- DNP combined with high field & high order shimming
  \[ \Rightarrow \text{increased sensitivity and high resolution in vivo spectra} \]
Homonuclear polarization transfer

**2\(^{13}\)C Acetate Detection**

\[
\begin{align*}
\text{H} & \quad \text{C}^{13} \quad \text{C}^{13} \\
\text{H} & \quad \text{CO} \\
\end{align*}
\]

**Localized homonuclear transfer sequence**

\[
\begin{align*}
\text{\(^{13}\)C} & \quad \text{OVS} \\
\text{\(^{1}H\)} & \quad \text{DECOUPLING} \\
\text{G}_r & \quad \text{OVS} \\
\end{align*}
\]

Fully adiabatic pulse sequences
Polarization transfer is carried out in a single voxel

M. Mishkovsky et al., MRM 68, 349 (2012)
In vivo spectrum after polarization transfer

- Detection of TCA cycle intermediate in the brain
- Double constraint for metabolite assignment: C(1) and C(2)
In vivo Brain Metabolism – TCA cycle

- First observation of in vivo TCA cycle intermediate in brain
- Observation of 2OG and lack of Glu signal implies that transport across the inner mitochondria membrane is rate limiting in the brain

M. Mishkovsky, A. Comment, R.Gruetter
Acetate heart metabolism

- Metabolism in the heart is fast
- Acquisition synchronized with respiration and blood pressure

- Formation of acetyl-carnitine is significantly reduced in ischemic muscle and in particular in the myocardium
1-$^{13}$C pyruvate cardiac metabolism

- Possible to monitor the pH using the Bicarbonate to CO2 ratio
- Transformation of pyruvate into acetyl-CoA via PDH flux is strongly reduced in fasted animals (switch to fatty acid metabolism)
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Acetate skeletal muscle metabolism

- Monitoring the real-time conversion of acetate to acetyl carnitine *in vivo* in muscle

Model to deduce kinetics

\[
\frac{dM_A}{dt} = -R_{1,A} [M_A - M_{A,eq}] - kM_A
\]

\[
\frac{dM_C}{dt} = kM_A - R_{1,C} [M_C - M_{C,eq}]
\]

- Only 2 free parameters: \( R_{1,A} \) and \( k \)

\[
M_A(t) = A_0 (\cos \theta)^t / TR e^{(-k-R_{1,A})t}
\]

\[
M_C(t) = A_0 (\cos \theta)^t / TR \left( b e^{(-k-R_{1,A})t} - be^{-R_{1,C}t} \right)
\]

\[
b = \frac{k}{-k - R_{1,A} + R_{1,C}}
\]
Molecular imaging by MRI

- Many brain pathologies are associated with disruption of the blood brain barrier

\[ \text{In vivo detection of sub-\text{\(\mu\)M}} \]
concentration of Gd-DOTP in a rat head using hyperpolarized \(^6\text{Li}\)

\[
\frac{1}{\tau_{\text{CA}}} = \frac{1}{\tau_{\text{pure}}} + r_1 [\text{CA}] \quad \text{with } r_1 = 11 \text{ mM}^{-1}\text{s}^{-1}
\]

\[ \Rightarrow [\text{CA}] = 820 \pm 300 \text{ nM} \]

- **Strategy**: combining \(T_1\)-relaxing contrast agents and hyperpolarized substances
**In vivo hyperpolarized $^{89}$Y studies**

**Pros:**
- 100% natural abundance
- *in vitro* $T_1$ up to about 500 s
- large chemical-shift range
- FDA-approved contrast agents such as Gd(DOTA)$^-$

**Cons:**
- gyromagnetic ratio very small
  ($\sim$20 times smaller than $^1$H)

In vivo pH imaging using hyperpolarized $^{89}$Y

- Ligands leading to pH-sensitive chemical shift

- Dissolution with ascorbate to scavenge nitroxy radical

Hyperpolarized $^{129}\text{Xe}$ via DNP

- Hyperpolarized $^{129}\text{Xe}$: lung imaging, vascular and perfusion imaging, and potential use with biosensors
- Liquid xenon mixed with radical doped solvent
  $\Rightarrow$ DNP $\Rightarrow$ Sublimation $\Rightarrow$ NMR

- Advantage of DNP over optical pumping method: large volumes can be produced in small amount of time

Encapsulated hyperpolarized $^{129}$Xe

Strategies for microbubble targeting

Conclusions

• SNR is everything! There is never too much

• Each 10% you gain can make a difference *in vivo*.

• $^{13}$C MR will never be as sensitive as $^1$H MR but:

  a) Contrast-to-noise is HUGE
  
  b) Hyperpolarized $^{13}$C MR gives access to a new time scale in terms of 
  biochemistry

• A tentative number for the ultimate detection level for 13C metabolites 
  is a few nmol (Boltzmann *in vivo* $^{13}$C NMR: 10mM in 1cm$^3$)
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http://sdnpi.epfl.ch