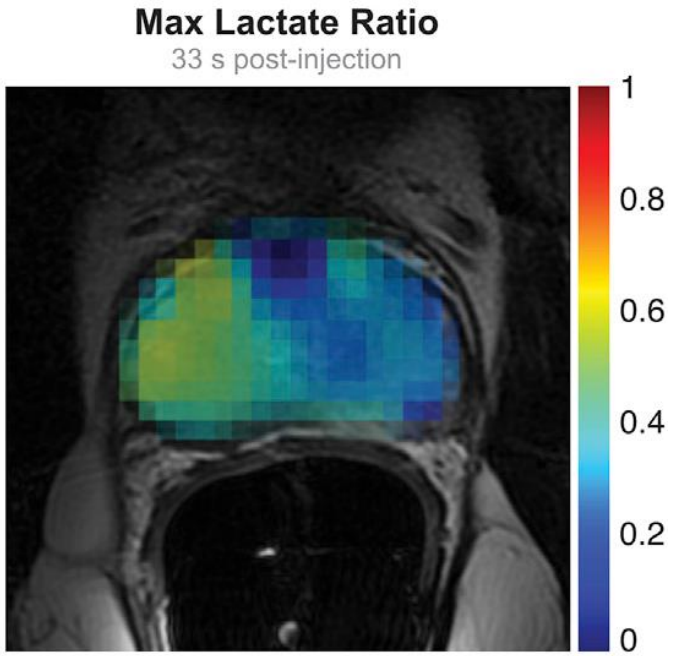
Hyperpolarized ^{13}C MRI via dissolution Dynamic Nuclear Polarization

Arnaud Comment – Aarhus Training Course Oct 2024

GE HealthCare

Why hyperpolarizing ^{13}C?

- No need to hyperpolarize ^{1}H since human body contains 40 M $^{1}H_2O$
- Other nuclei are however less concentrated and SNR is too low for MRI scans
- Carbon forms the backbone of most molecules involved in metabolic processes
- ^{13}C exhibits large chemical shift dispersion
- Real-time detection of metabolic fluxes
- High contrast-to-noise ratio images



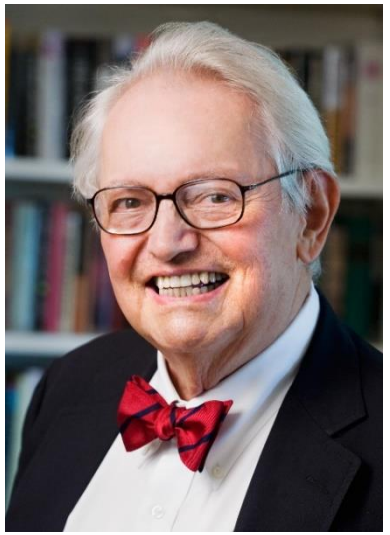
K. L. Granlund *et al.* Cell Metab. 2020

G. D. Reed *et al.* IEEE T. Med. Imaging 2014

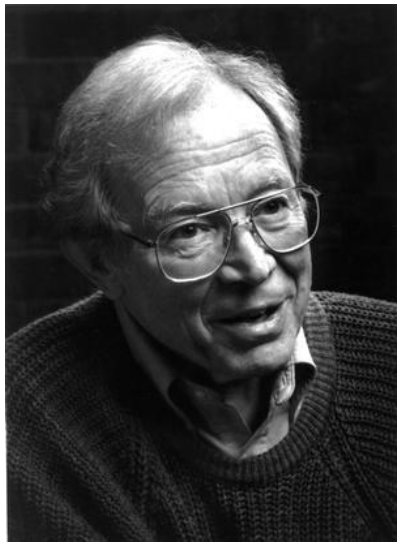
Dynamic Nuclear Polarization (DNP)

- In 1952, Overhauser predicted the possibility to transfer the large electron spin polarization to nuclear spins
- In 1953, Carver and Slichter demonstrated that nuclear spin polarization can indeed be enhanced by electrons in metals and liquids
- In 1957, Jeffries showed that nuclear spins can be polarized by electron spins using forbidden transitions in paramagnetic crystals
- In 1958, Abragam introduced the concept of “solid effect” to describe DNP in diamagnetic solids containing free radicals

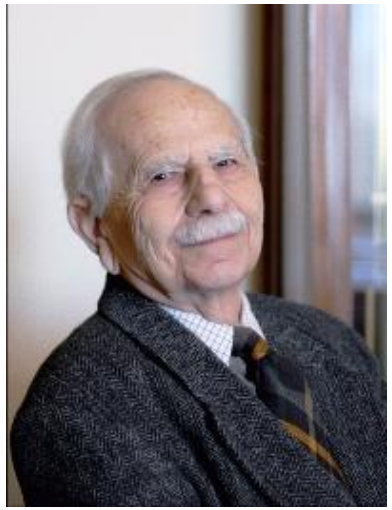
Albert W. Overhauser



Charles P. Slichter



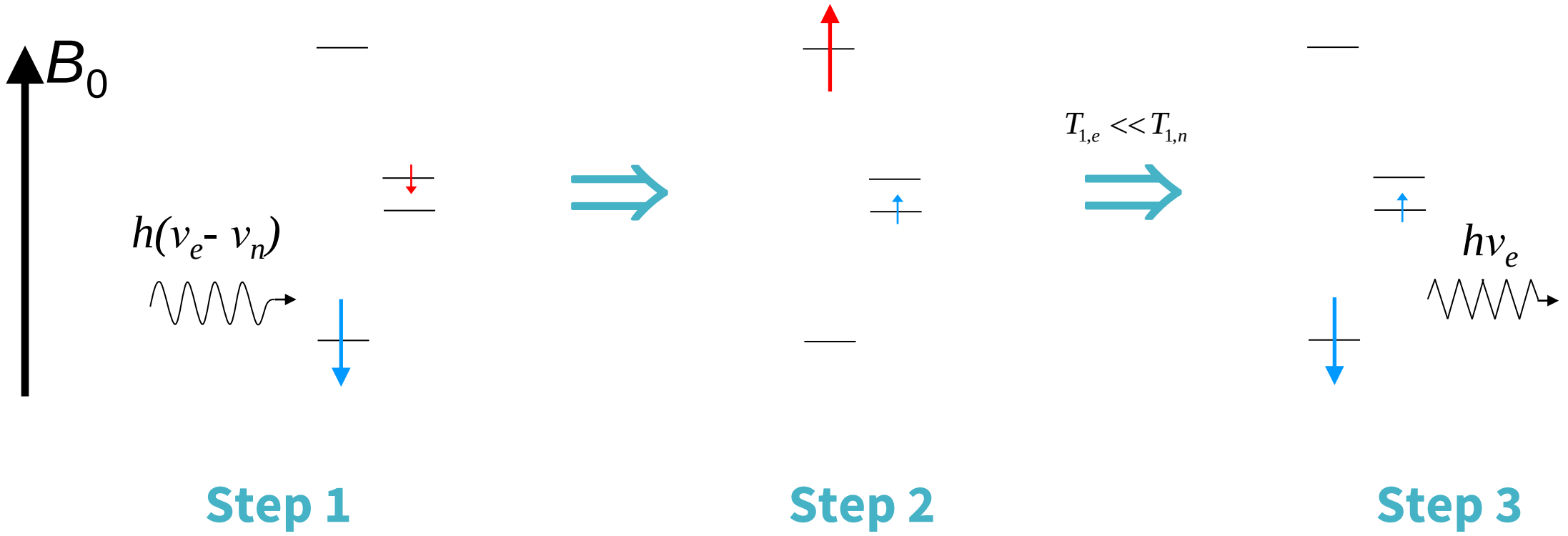
Carson D. Jeffries



Anatole Abragam

DNP by solid effect

- Polarization transfer through “forbidden” transitions



- Energy balance: $h(\nu_e - \nu_n) + h\nu_n = h\nu_e$

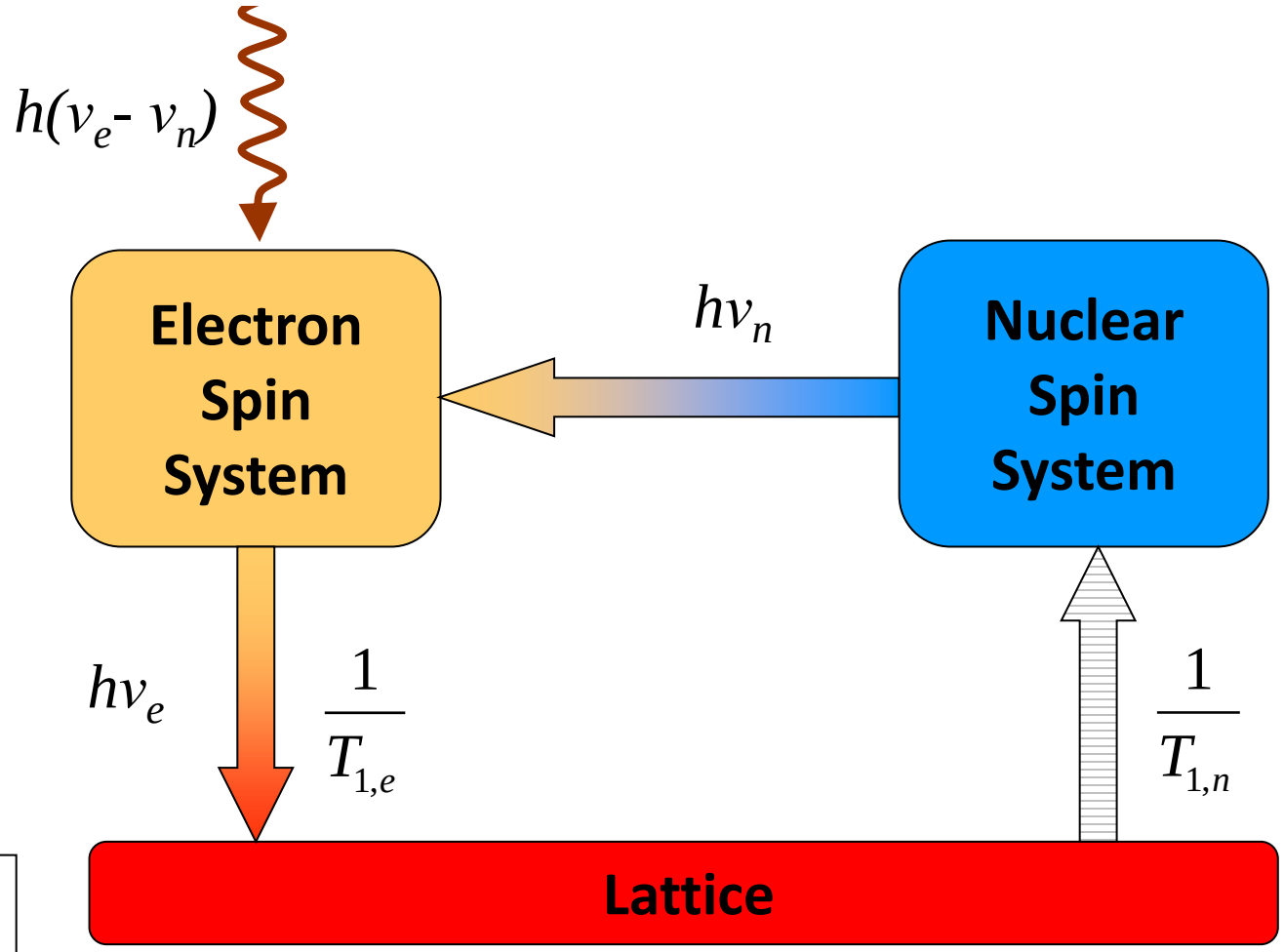
Thermodynamic description of solid effect

- Spin polarization:

$$P = \frac{\hbar \gamma B_0}{2 k_B T}$$

- Spin temperature:

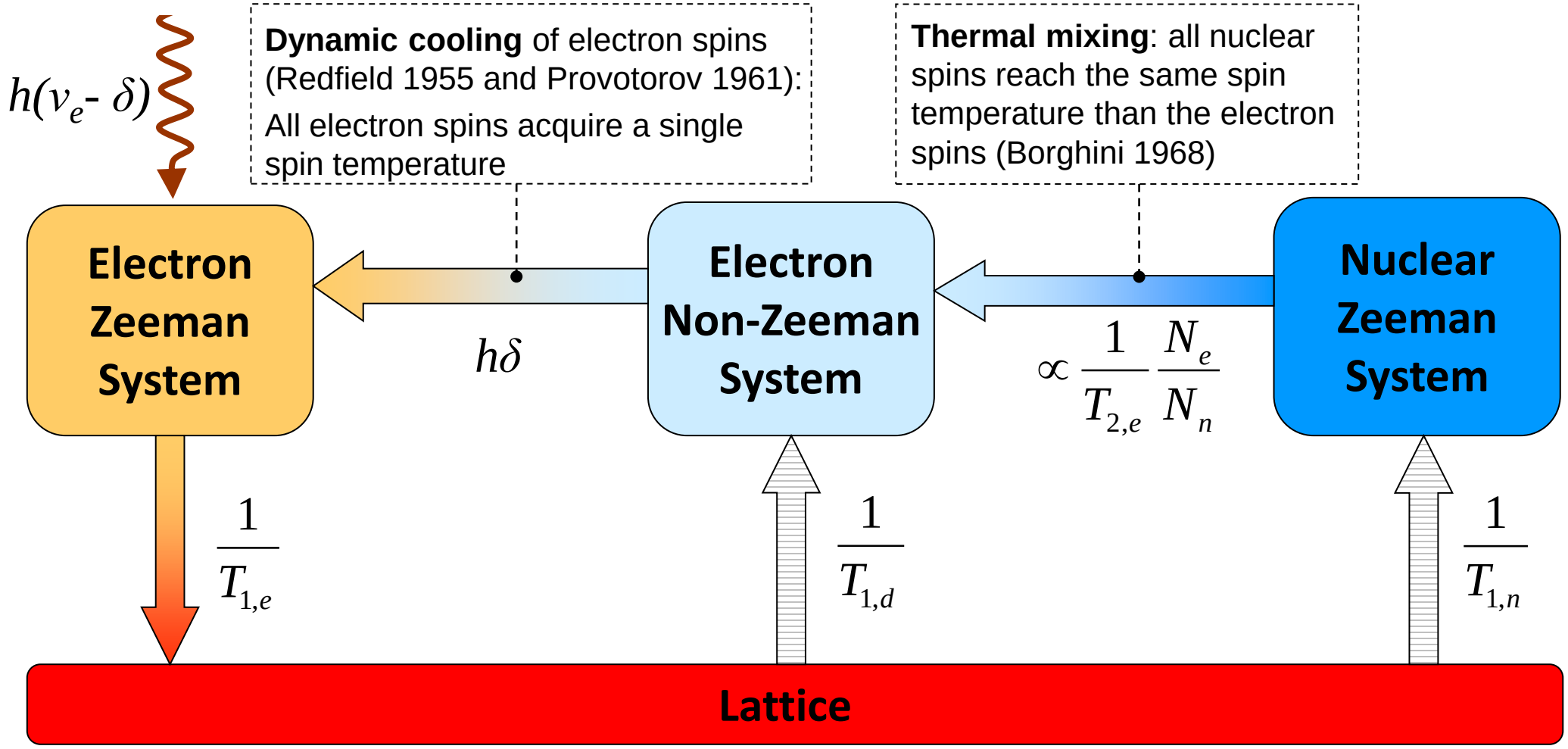
$$T_S = \frac{\hbar \gamma B_0}{2 k_B P}$$



- Coolant: polarized electron spins
- Driving power: microwaves
- Dissipation: heat into liquid He bath

$T_{1,e}$ is much shorter than $T_{1,n}$

Thermodynamic description of thermal mixing

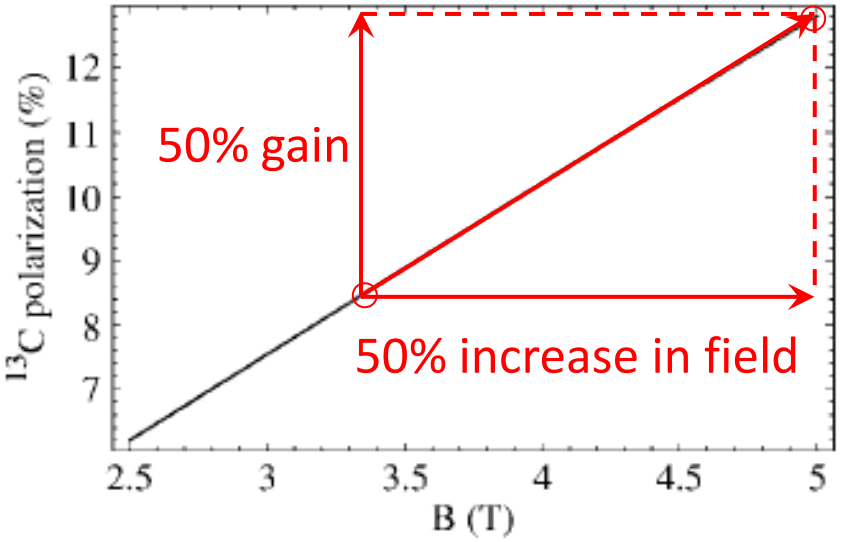


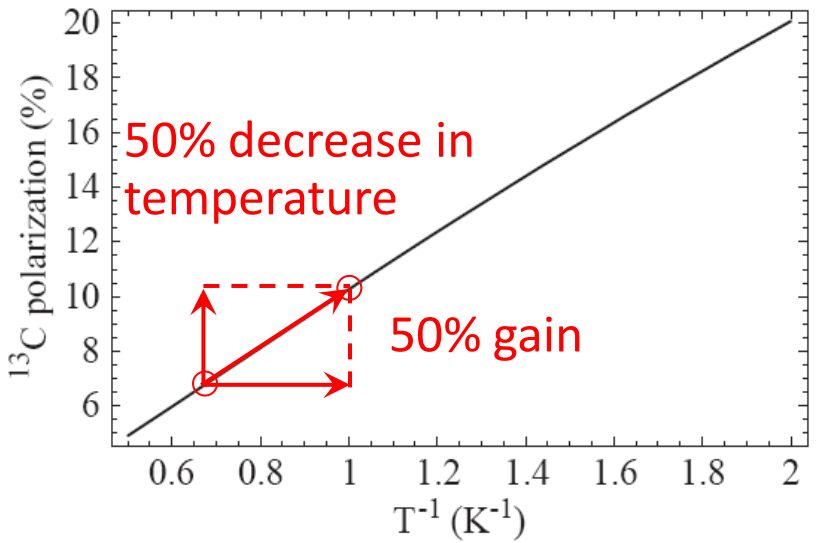
- Short $T_{2,e}$, $T_{1,e}$ and long $T_{1,n}$, $T_{1,d}$

Temperature and field dependence of DNP

- No quantitative theoretical model for DNP has been developed to date
- Qualitative trend provided by numerical solutions of Borghini model (Thermal mixing)

M. Borghini Phys. Rev. Lett 1968





A. Comment *et al.* Concepts Magn. Reson. 2007

- Experimental results match theoretical predictions

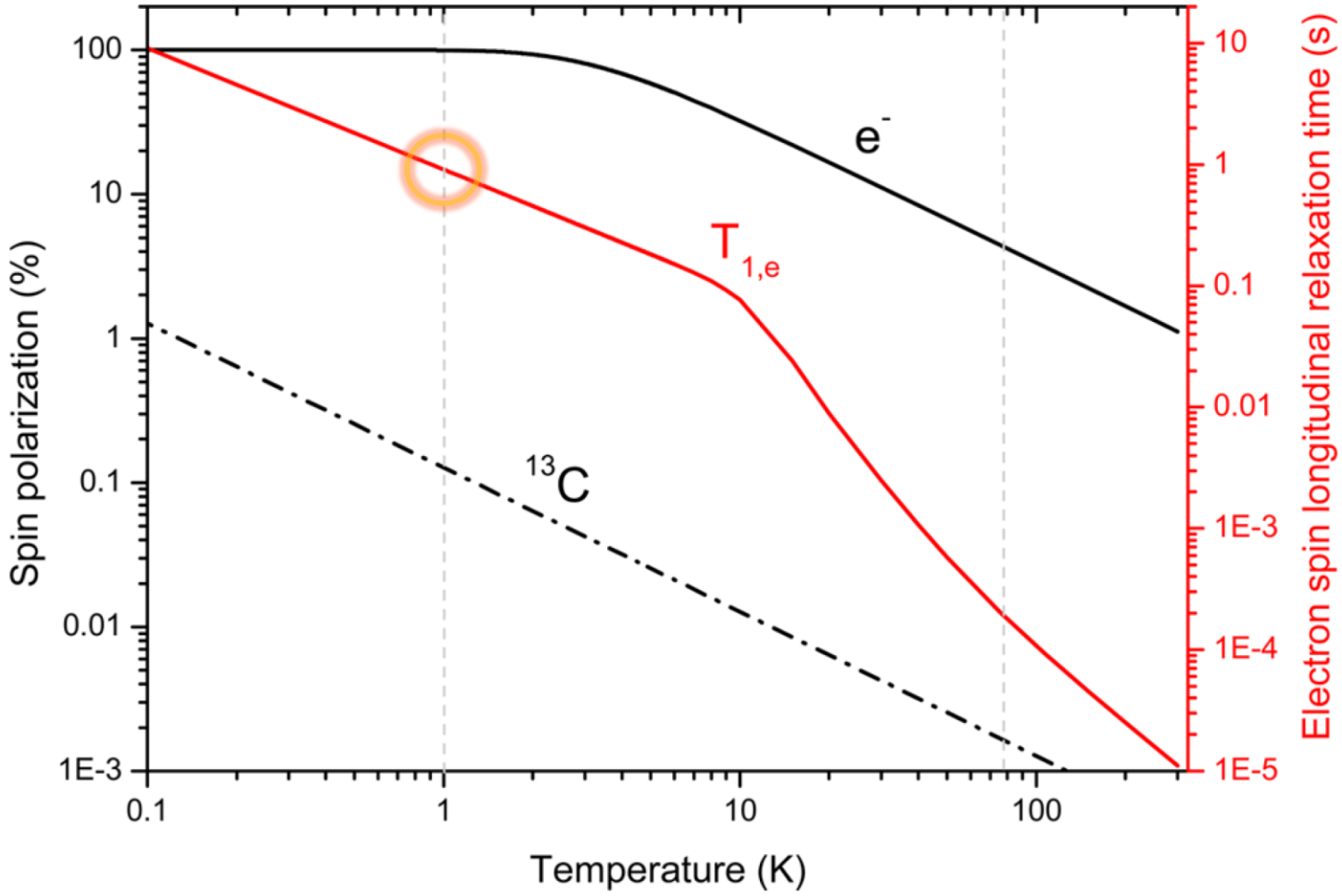
S. Jannin *et al. J. Chem. Phys.* 2008

What is the optimal temperature for DNP?

- In a field larger than 3 T, electron spins are polarized to ~100% below 2 K

$$\boldsymbol{P}_{eq} = \frac{\hbar\gamma \boldsymbol{B_0}}{2k_B \boldsymbol{T}}$$

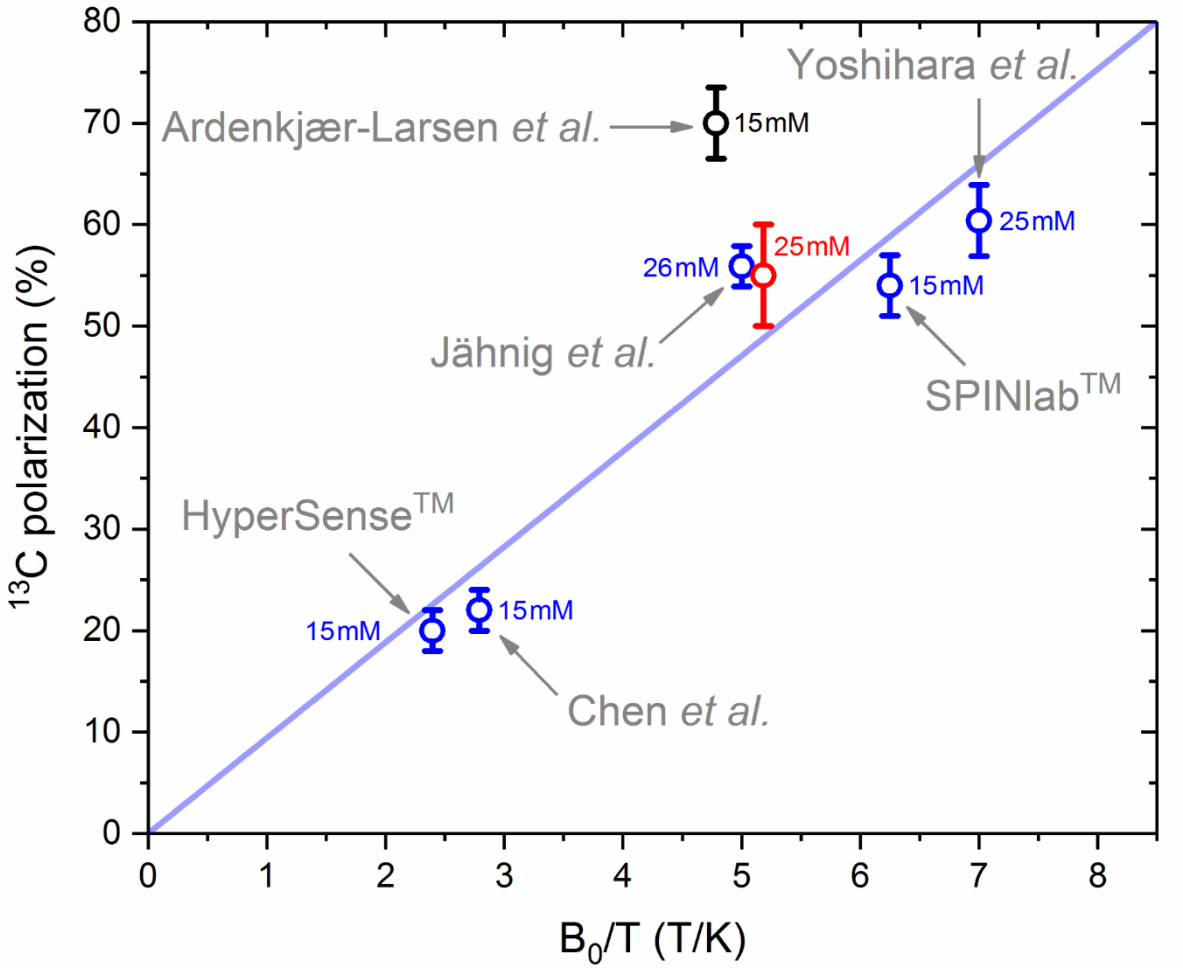
- For a given magnetic field (e.g. 5 T), the optimal temperature for DNP will mostly depend on $T_{1,e}$

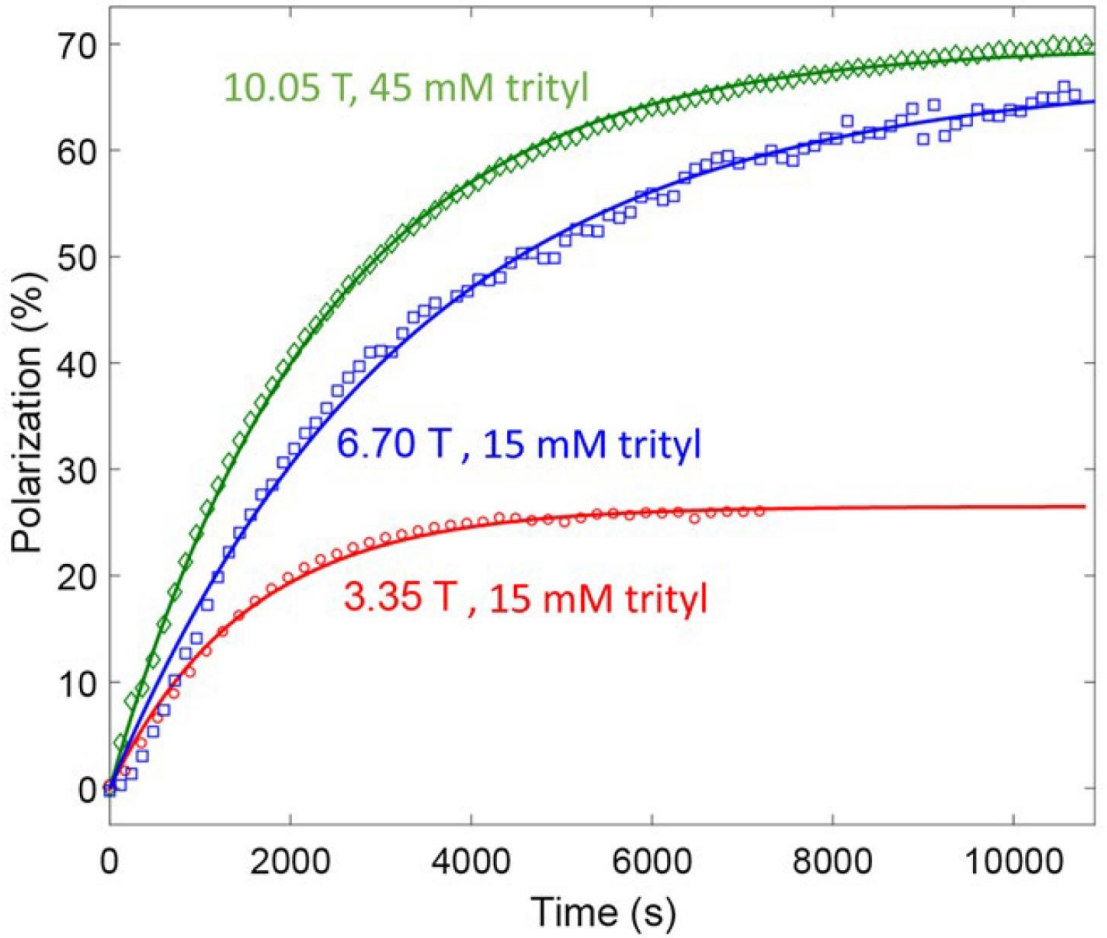


A. Comment and M. E. Merritt, Biochem. 2014

What is the ideal magnetic field for DNP?

- Because $T_{1,e}$ becomes too short at higher fields, higher EPA concentration is needed
- 7 T is most likely the optimal field



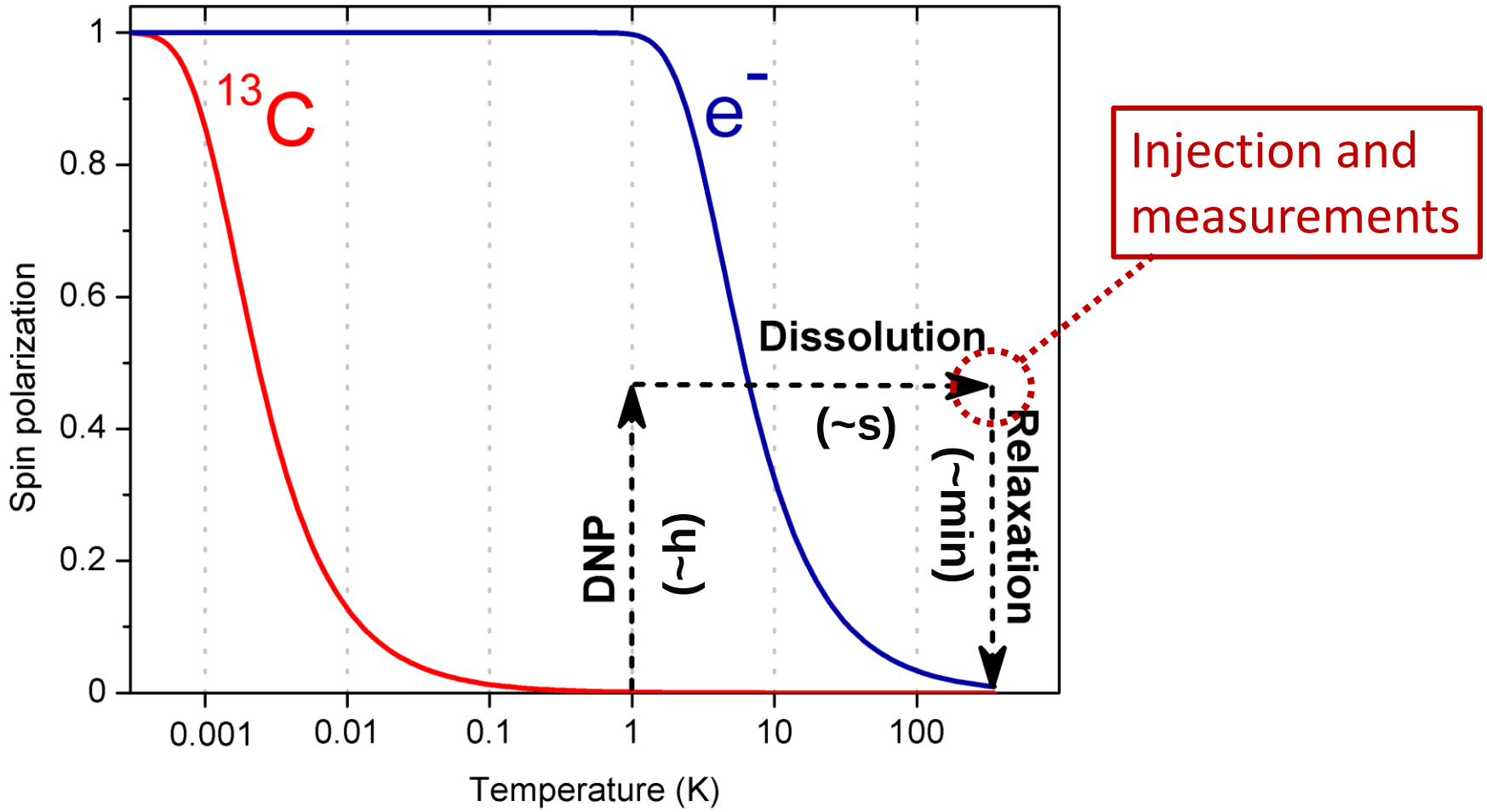


T. Cheng *et al.* NMR Biomed. 2020

J.H. Ardenkjær-Larsen *et al.*, MRM 2019

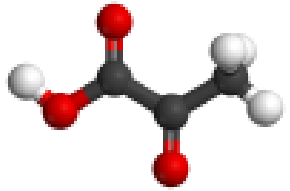
Dissolution DNP for Hyperpolarized ^{13}C MR

- Solid-to-liquid phase transition should be rapidly performed in high magnetic field environment to maintain enhanced nuclear spin polarization ⇒ ***in situ* dissolution**

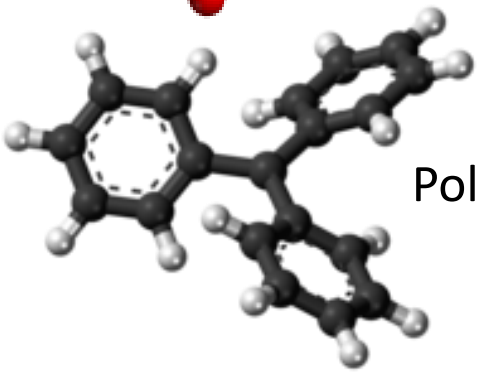


Preclinical in vivo studies

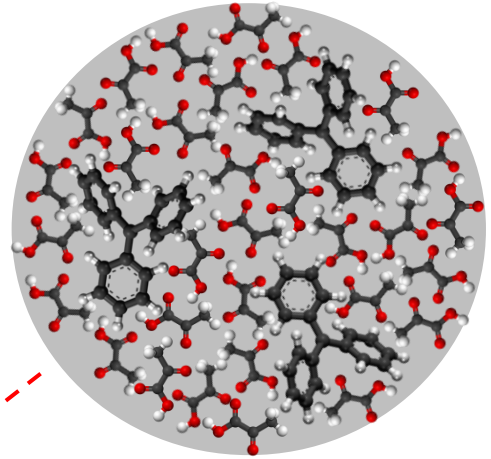
J.H. Ardenkjær-Larsen *et al.*, PNAS 2003

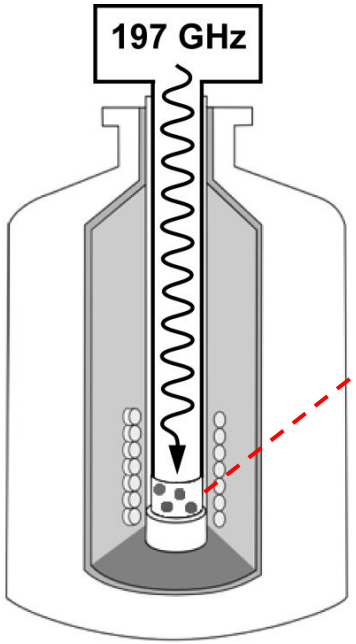


^{13}C-substrate

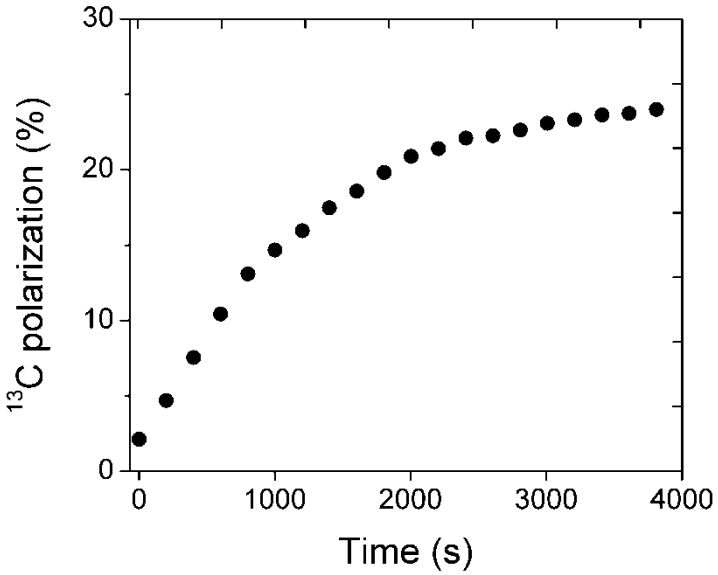


Polarizing agent (EPA)





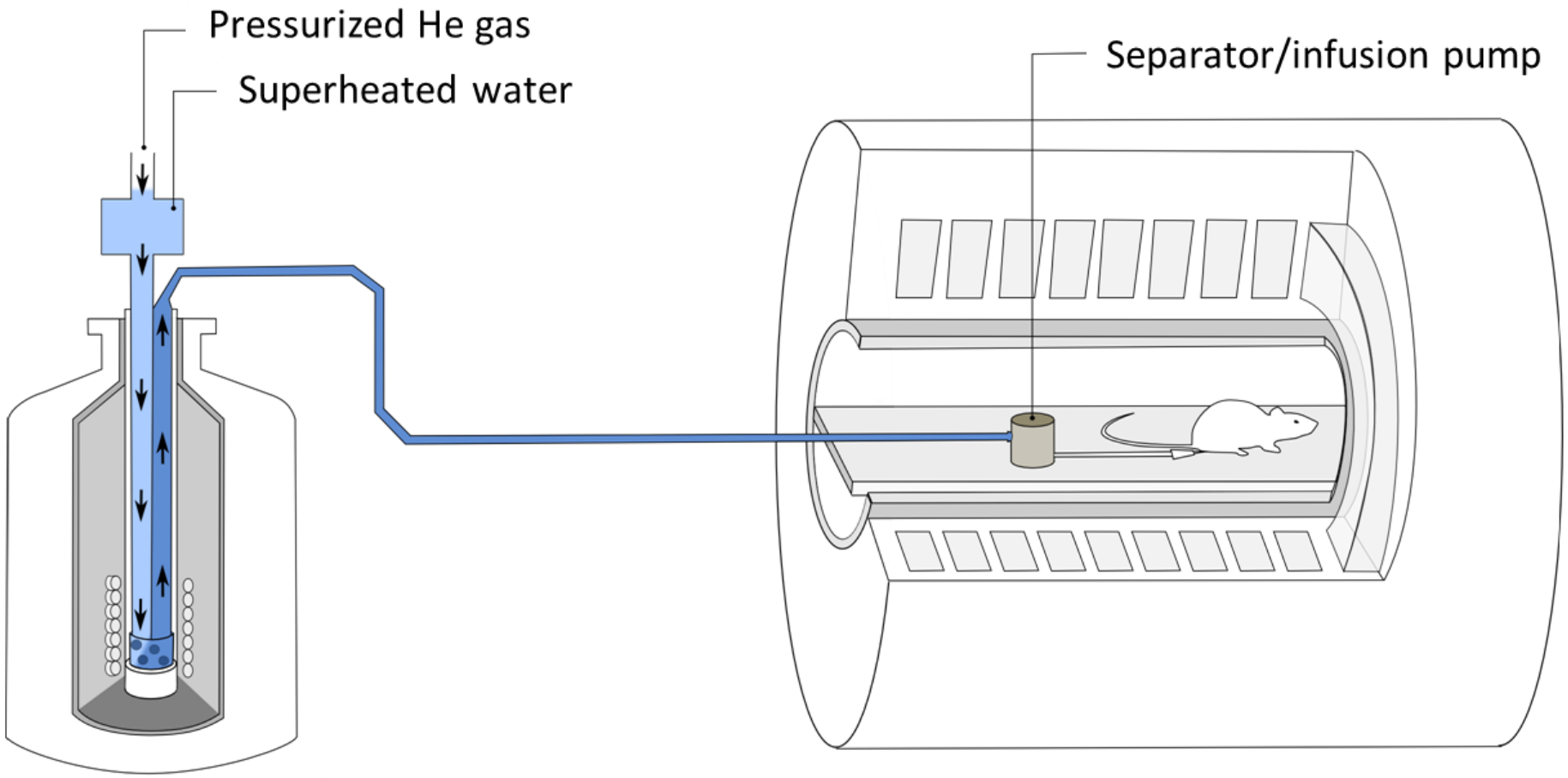
7 T / 1 K hyperpolarizer



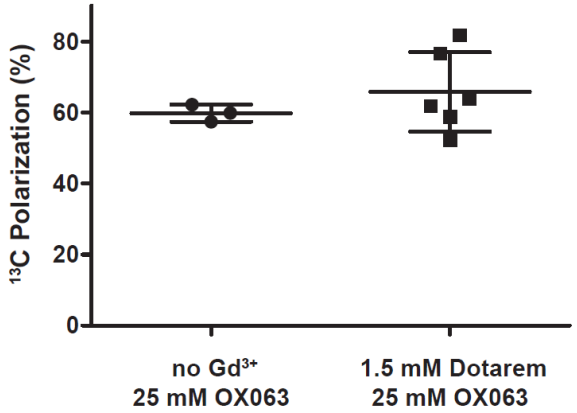
Preclinical in vivo studies

A. Comment *et al.* Concepts Magn. Reson. 2007

H.A.I. Yoshihara *et al.*, PCCP 2017



Up to 80% polarization in [1-^{13}C]pyruvate at time of injection

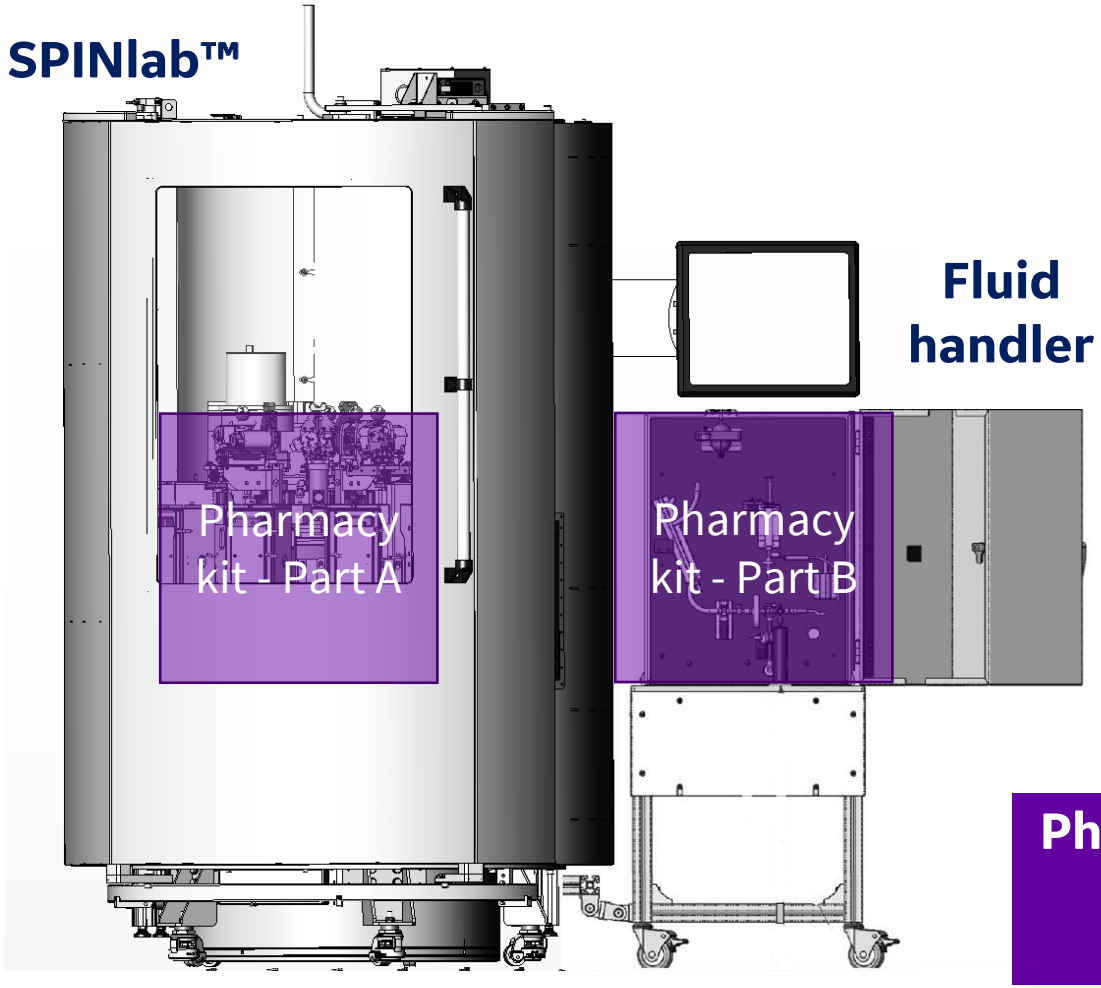


7 T / 1 K hyperpolarizer

3 s delay between dissolution and injection

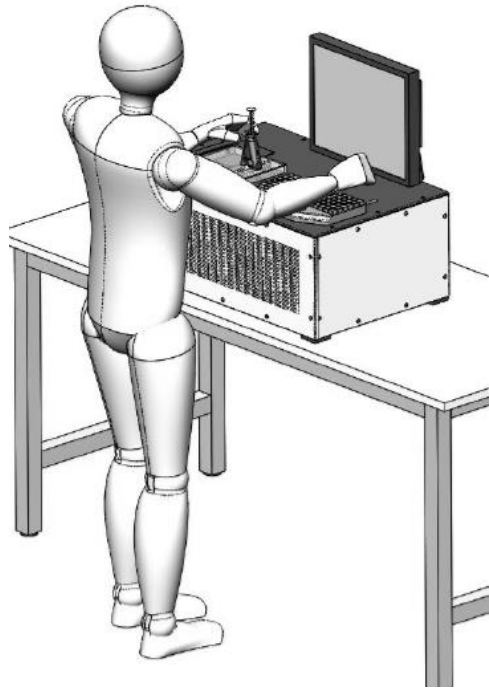
Hyperpolarized ^{13}C MRI - Hardware for clinical research

SPINlab™



SIGNA™ Premier with MNS

Multi-Probe QC

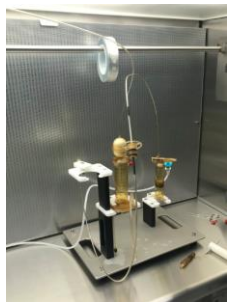


Pharmacy kit (consumable) required for preparing injectable hyperpolarized ^{13}C probes

Clinical Hyperpolarized ^{13}C MR Study - Work Flow

Pharmacy kit fill

ISO7 cleanroom or biohood



1. Admix EPA with ^{13}C-pyruvate
2. Insert mixture in sample vial
3. Fill and assemble pharmacy kit

30 min/kit (can be frozen)

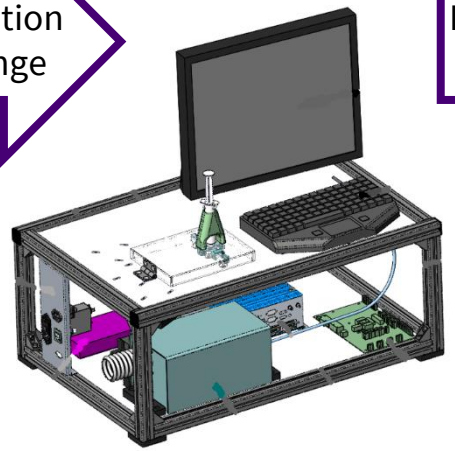
Load pharmacy kit in SPINlab

Hyperpolarization

1. Microwave irradiation
   4 kits in parallel (3h/kit)
2. Dissolve sample in hot water

Draw solution into syringe

QC measurements



1. Draw aliquot from syringe
2. Measure pH, T, pyruvate and residual EPA concentrations

Within 1 min

Place syringe on power injector

^{13}C MRI

1. Inject ^{13}C-pyruvate solution
2. Perform ^{13}C MRI scans

Less than 5 min

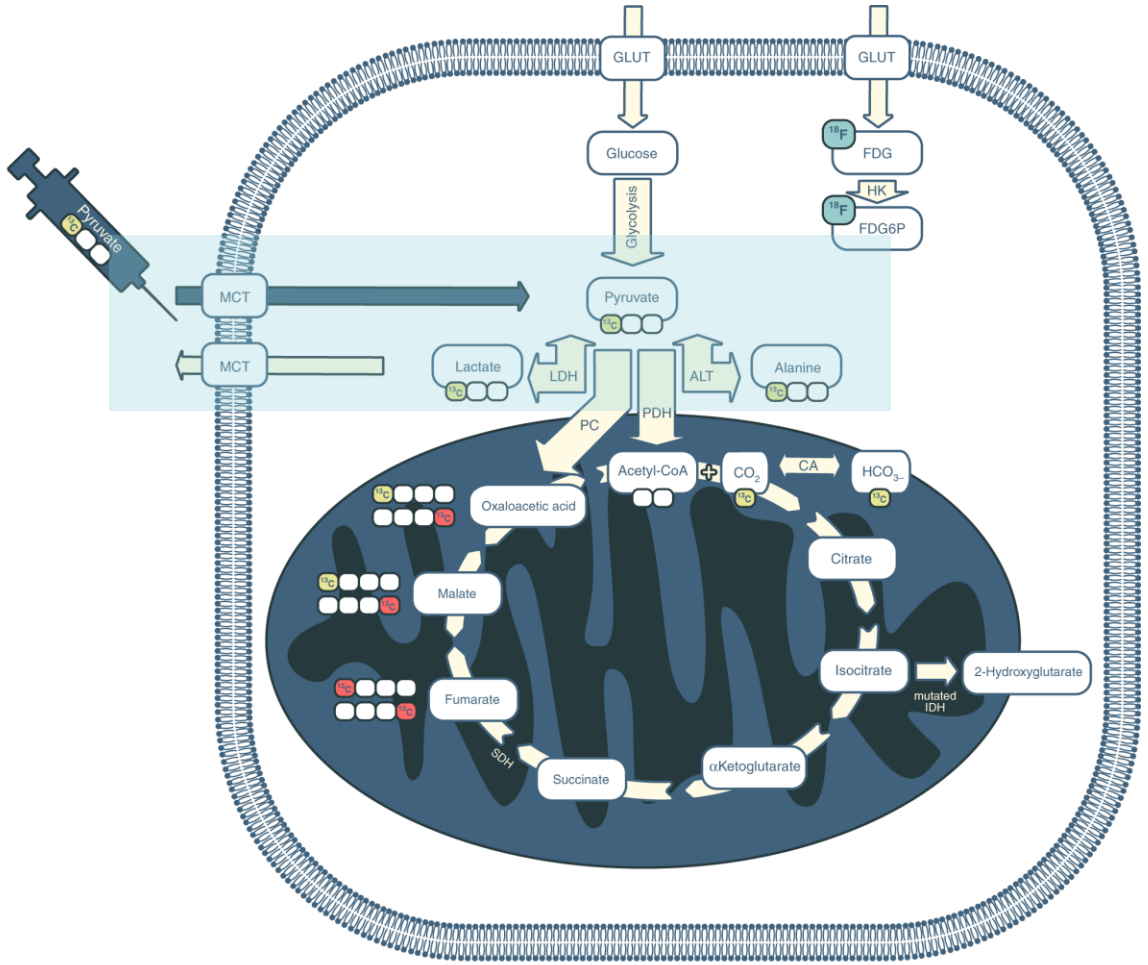
Hyperpolarized ^{13}C-pyruvate vs. ^{18}F-FDG

- While ^{18}F-FDG provides a contrast based on cellular glucose uptake, HP ^{13}C-pyruvate allows the **direct measurement of downstream metabolic products** (multiple pathways can be simultaneously probed)

  $\Rightarrow$ **potentially different metabolic contrast**

- The injected dose of HP ^{13}C-pyruvate (~0.1 mmol/kg) is much larger than ^{18}F-FDG (~0.1 nmol/kg) but ^{13}C-pyruvate is a **non-radioactive endogenous molecule (proven safety)**

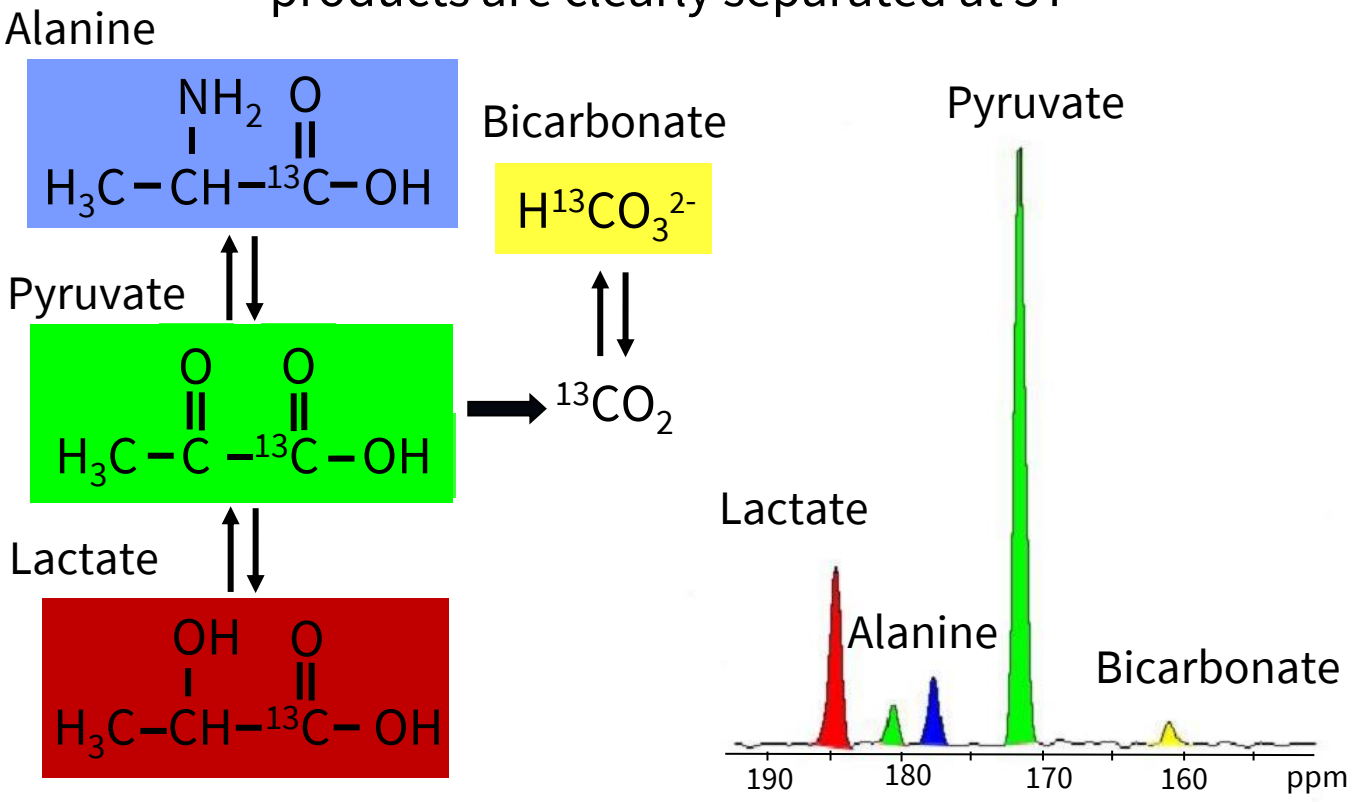
- Lifetime of the HP state of ^{13}C-pyruvate is on the order of T_1=1 min, which requires a much shorter delay between preparation and injection (typically 1 min)



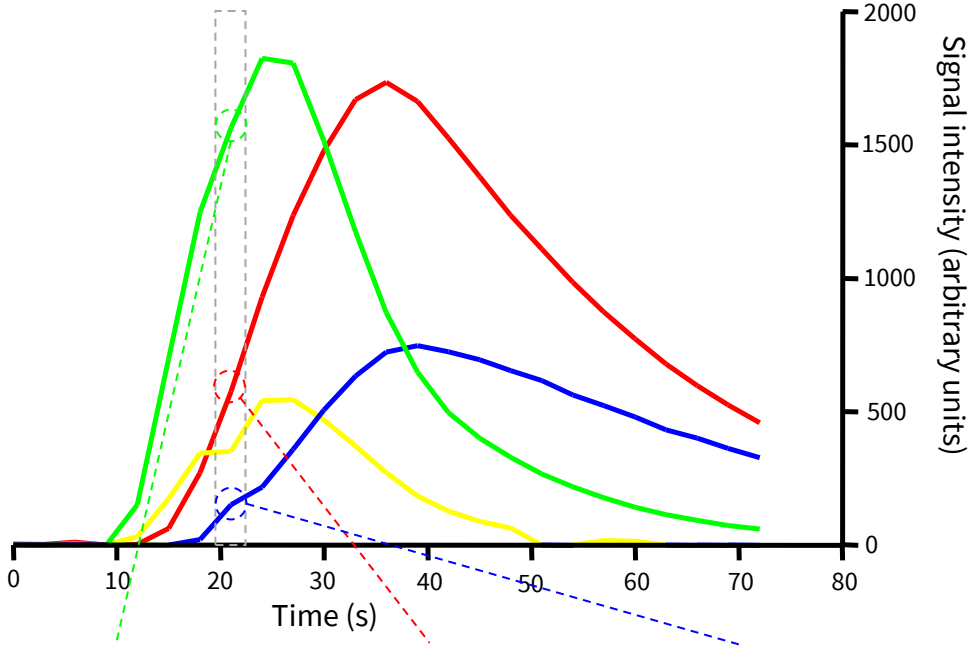
R. Woitek and F. A. Gallagher, Br. J. Canc. 124, 1187 (2021)

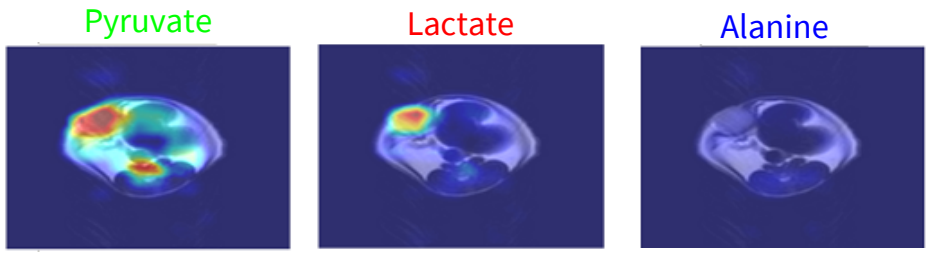
MR metabolic imaging with hyperpolarized ^{13}C-pyruvate

Resonances from [1-^{13}C]pyruvate and its metabolic products are clearly separated at 3T



Time course for substrate and products relates to metabolic fluxes



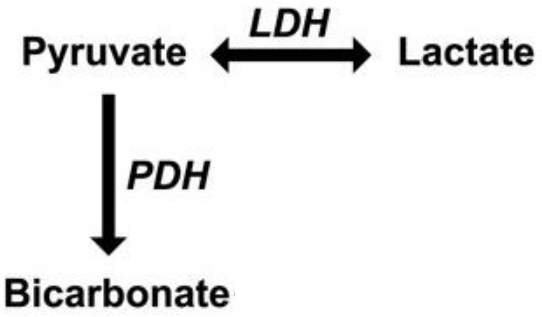


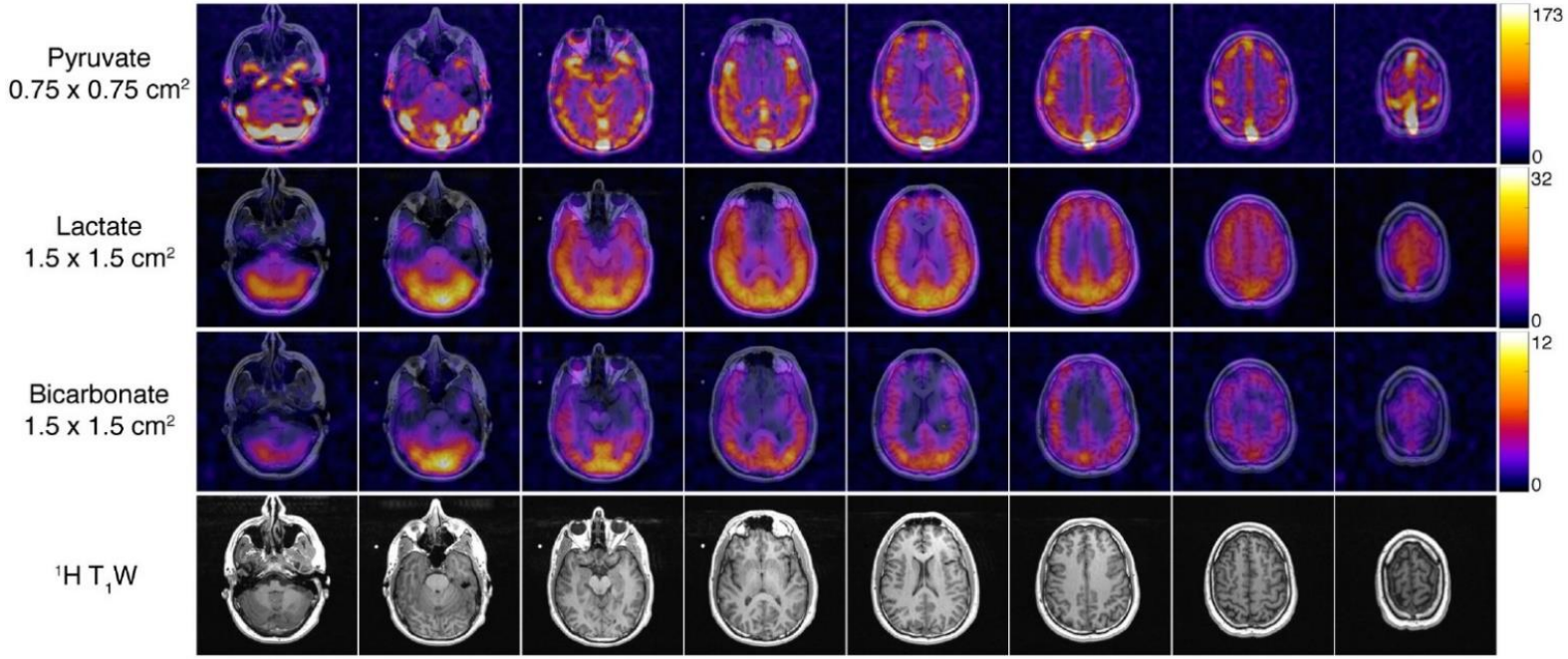
Real-time MR metabolic imaging

Enough signal to acquire image of individual metabolites at different time point

Hyperpolarized ^{13}C MRI

Unique modality to non-invasively image cellular metabolism in real time

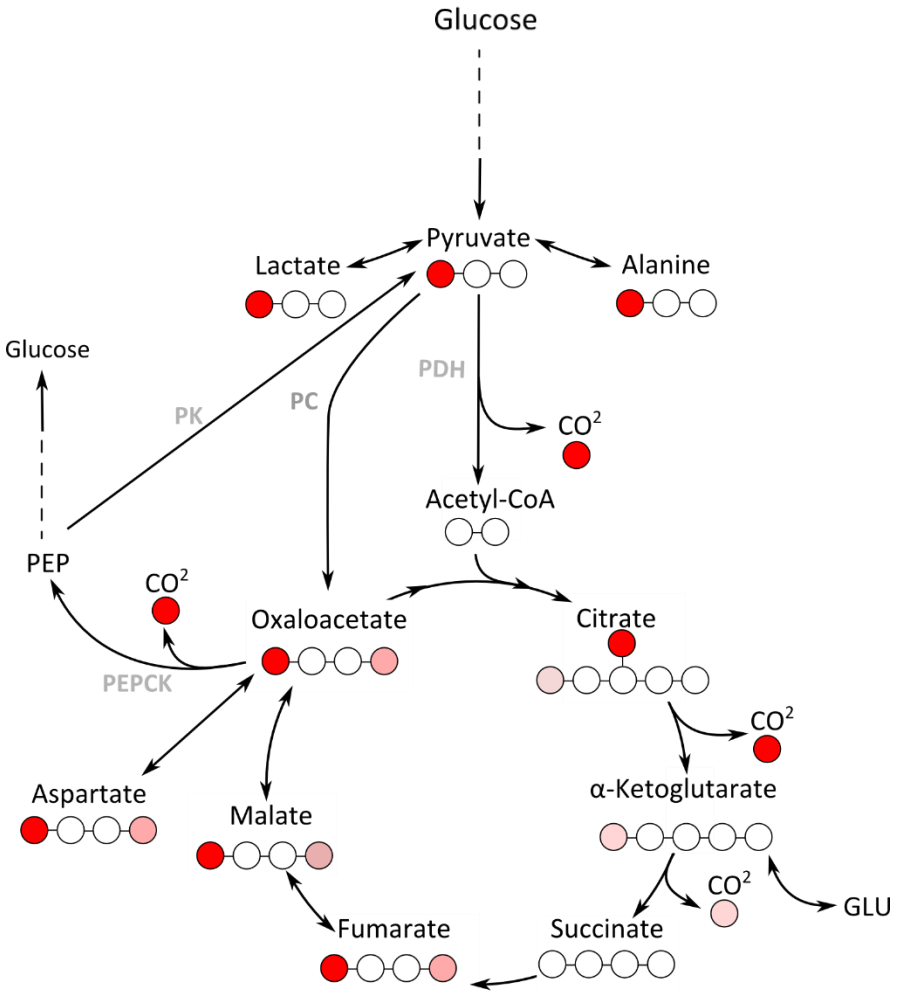




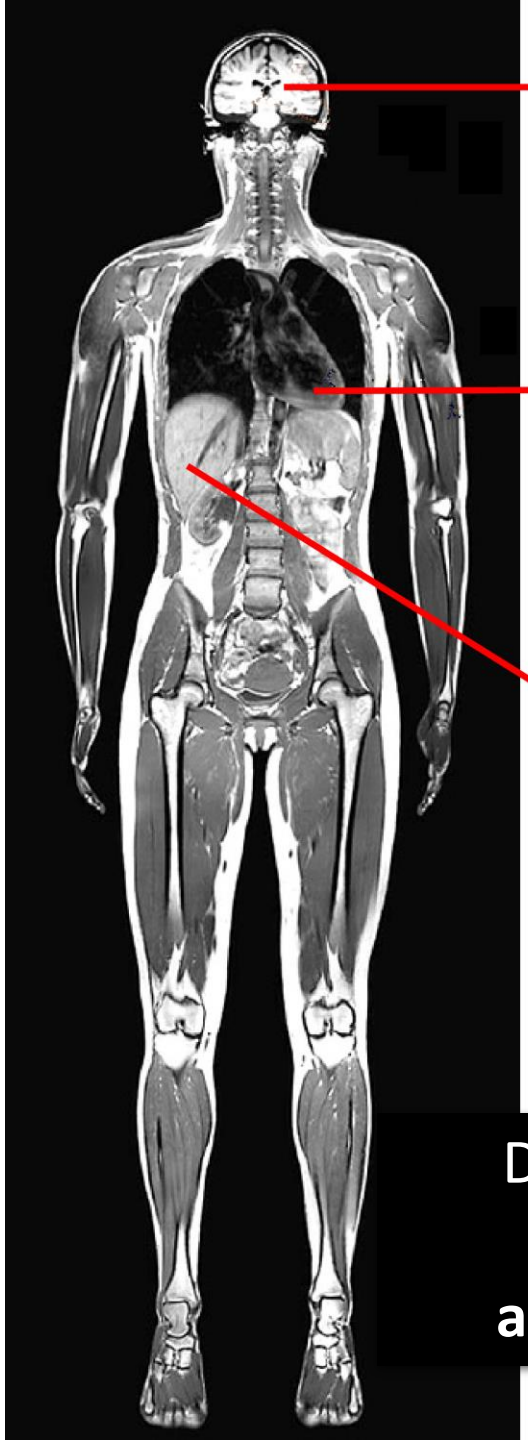
- No heavy metals
- No ionizing radiation
- Injection of ^{13}C-labelled endogenous molecules
- Neither severe nor mild adverse effects were reported following the injection of hyperpolarized ^{13}C-pyruvate (0.1mmol/kg) in nearly 1000 subjects (up to 9 injections in same subject)

Probing cell metabolism with ^{13}C

Carbon forms the backbone of most molecules involved in metabolic processes



A. Comment and M.E. Merritt, Biochem. **53** (2014)



Brain

Consumes ~50% of total blood glucose

Heart

Consumes fatty acids, ketone bodies and carbohydrates to produce mechanical work

Liver

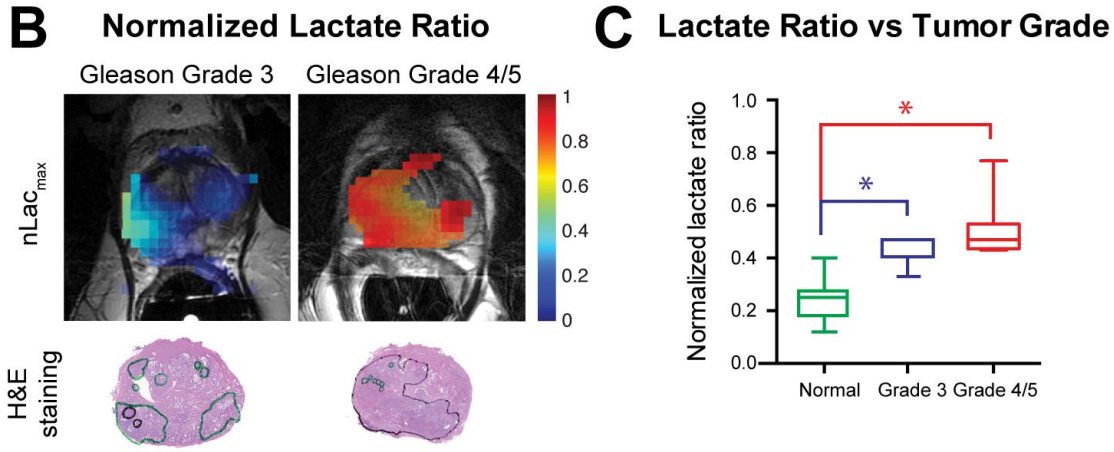
Stores and releases carbohydrates, ketone bodies and fats; regulates blood glucose level

Diseases are generally characterized by abnormal metabolism

Assessment of tumor grade in human prostate cancer

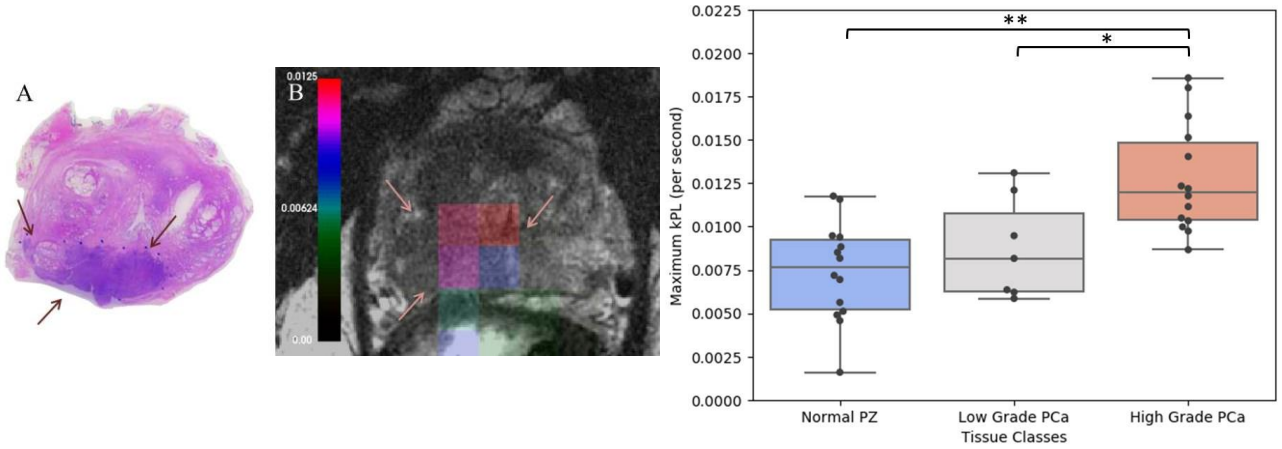
Correlate histological grade of human prostate tumor with hyperpolarized ^{13}C imaging data

MSKCC



K.L. Granlund *et al.,* Cell Metab (2020)

UCSF



EN. Korn *et al.,* ISMRM (2018)

- Conversion of HP ^{13}C pyruvate to lactate in human prostate cancer was found to increase with Gleason score.
- ^{13}C lactate can potentially be a direct imaging biomarker for tumor aggressiveness

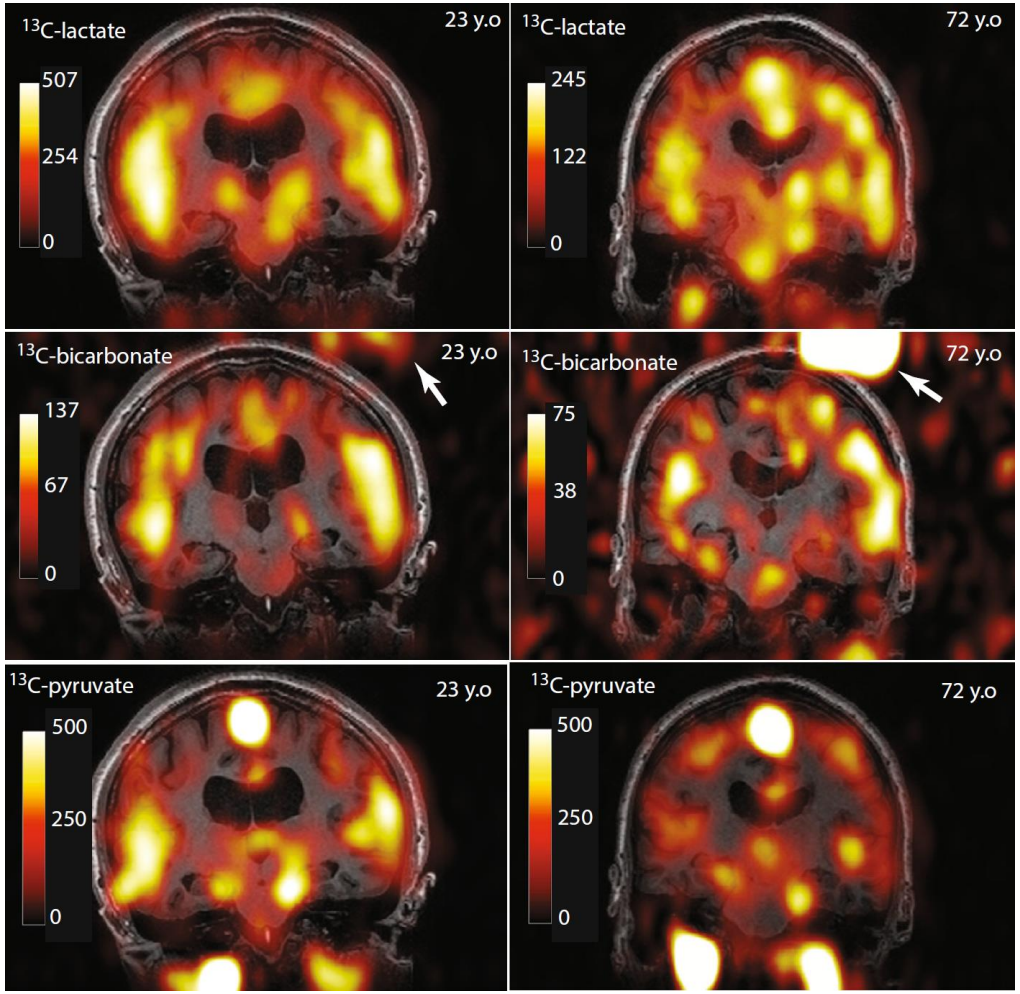
Applications in humans are SNR limited

10-15% ^{13}C polarization on [1-^{13}C]pyruvate at time of injection

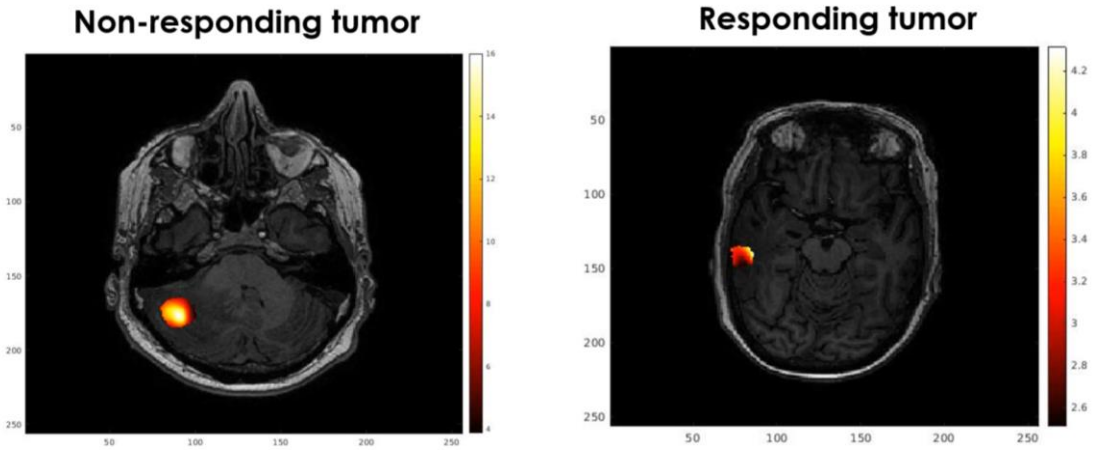
80° flip angle

80° flip angle

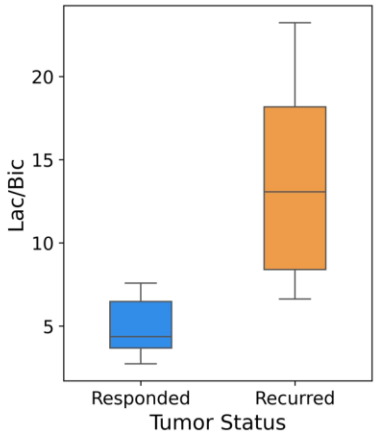
11° flip angle

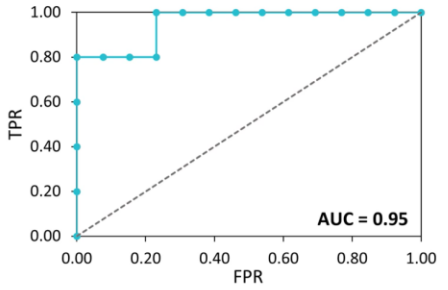


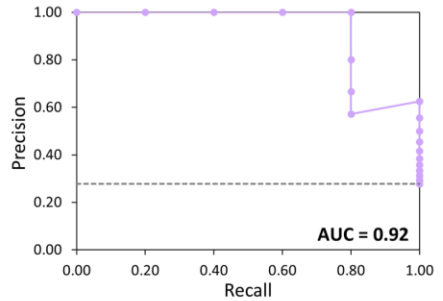
B. Uthayakumar *et al.* Hum Brain Mapp. 2023



Lactate-to-bicarbonate ratio in tumor predicts response to radiation therapy in brain metastases







C. Y. Lee *et al.* J. Neuro-Oncol. 2021

Higher SNR will allow improving the resolution and quantification of metabolites

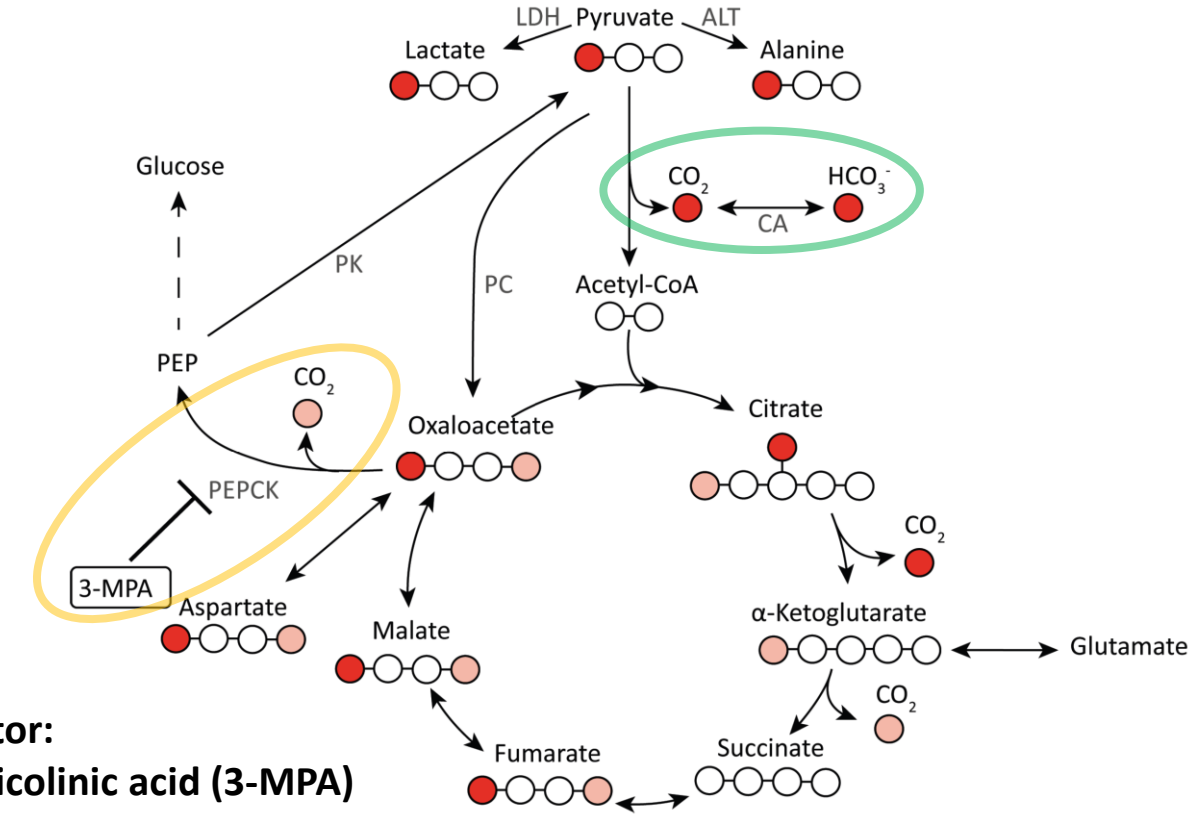
GE HealthCare

Can SNR be increased by increasing injected ^{13}C-probe dose?

- Pyruvate is typically injected at highly supraphysiological dose (about 10 times physiological dose in humans and up to 100 times in animals)
- This may alter the metabolic information obtained from the hyperpolarized ^{13}C scans

Case study: [1-^{13}C]pyruvate liver metabolism in healthy rats

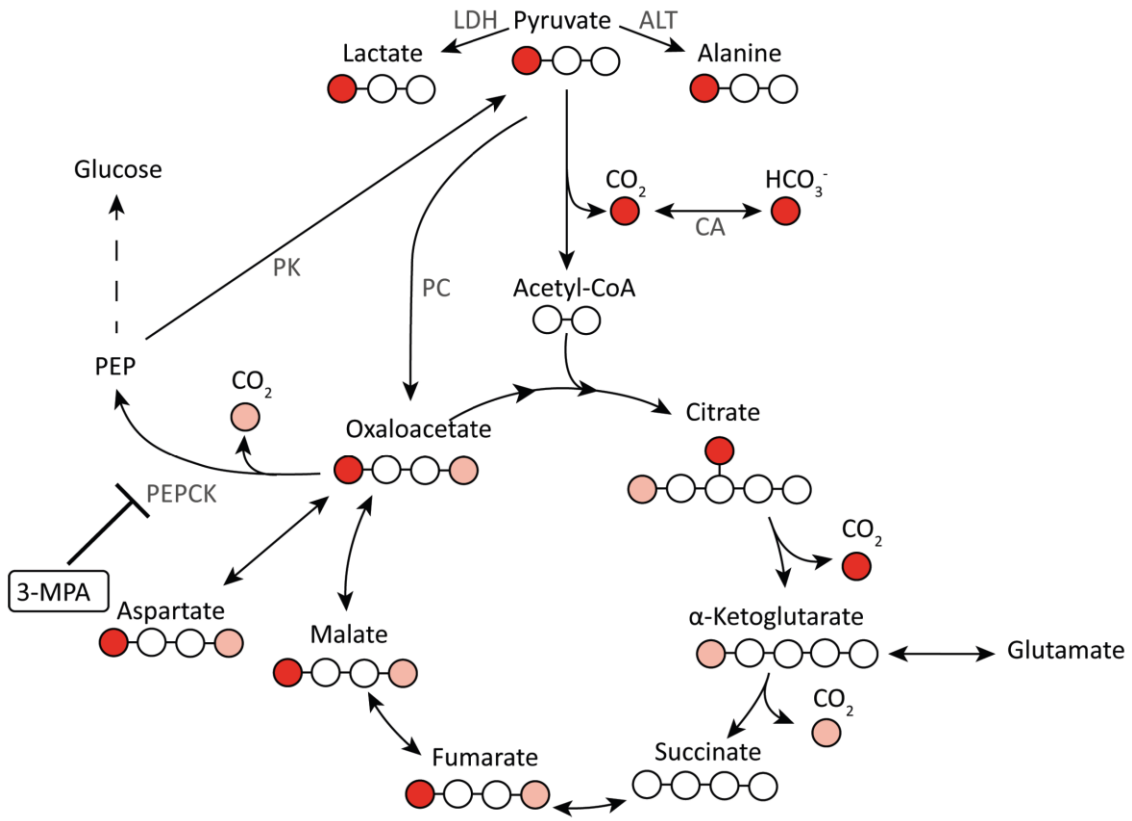
- ^{13}C-bicarbonate is from PDH activity in fed rats
- ^{13}C-bicarbonate signal is prominently from PEPCK activity in fasted rats

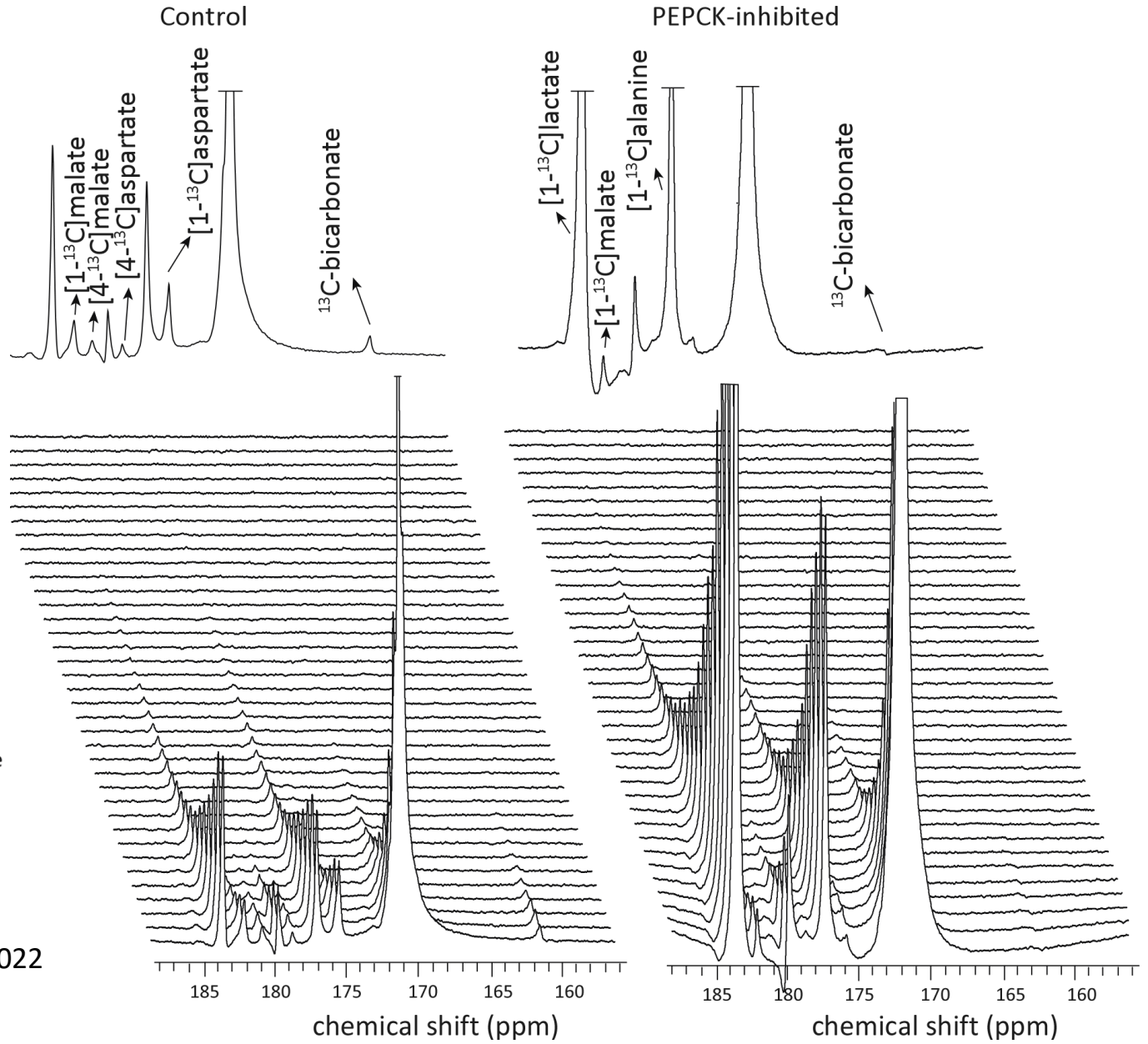


PEPCK inhibitor:
3-mercaptopicolinic acid (3-MPA)

[1-^{13}C]pyruvate liver metabolism in fasted rats

Significant decrease in bicarbonate levels only in FASTED rats

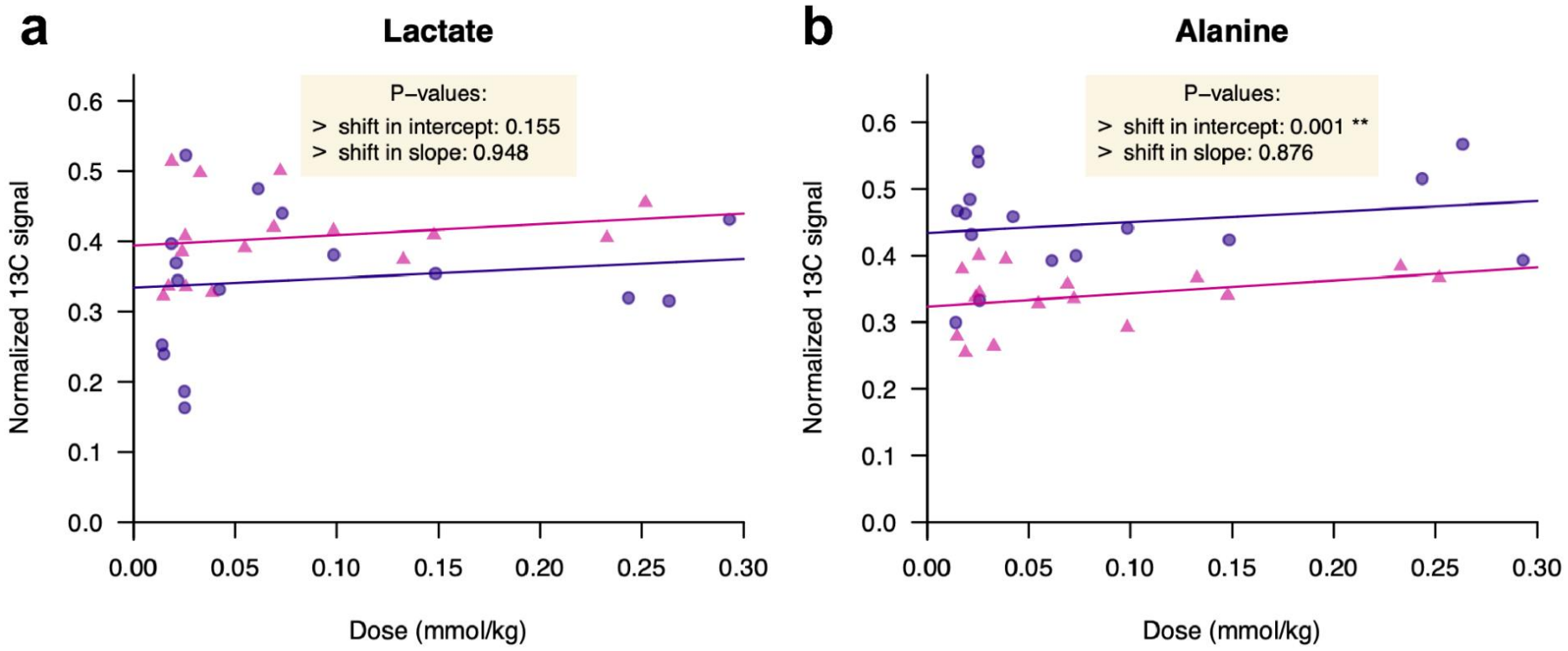




E. Can *et al.* Comm. Biol. 2022

Pyruvate dose study in rat liver

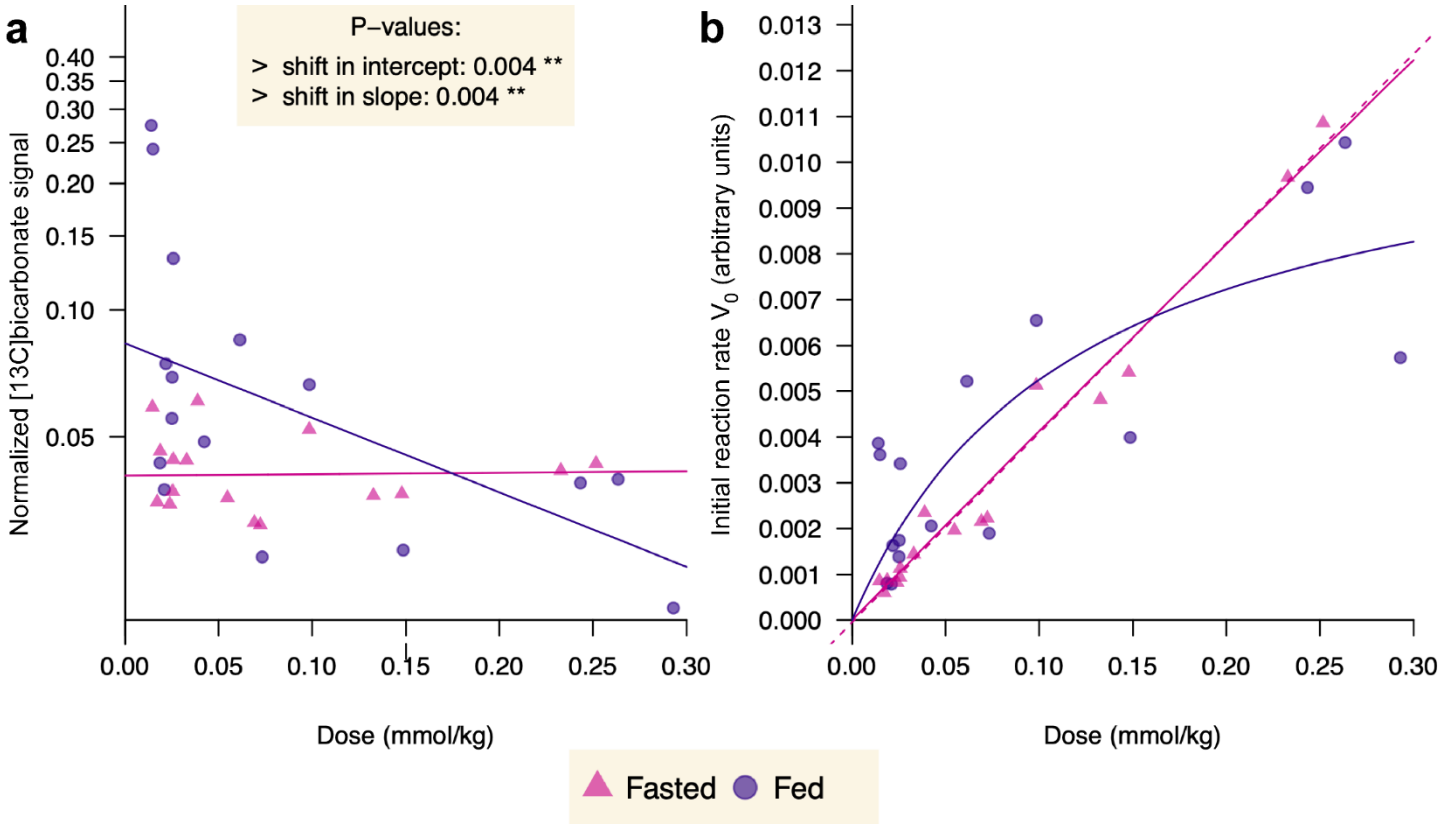
- Doses as low as 0.023mmol/kg were injected, leading to about 2 to 3-fold the normal basal physiological level
- Alanine decreases in fasted state for all doses (consistent with Hu *et al.*, Mol Imag Biol 2009)



E. Can *et al.* Comm. Biol. 2022

Pyruvate dose study in rat liver

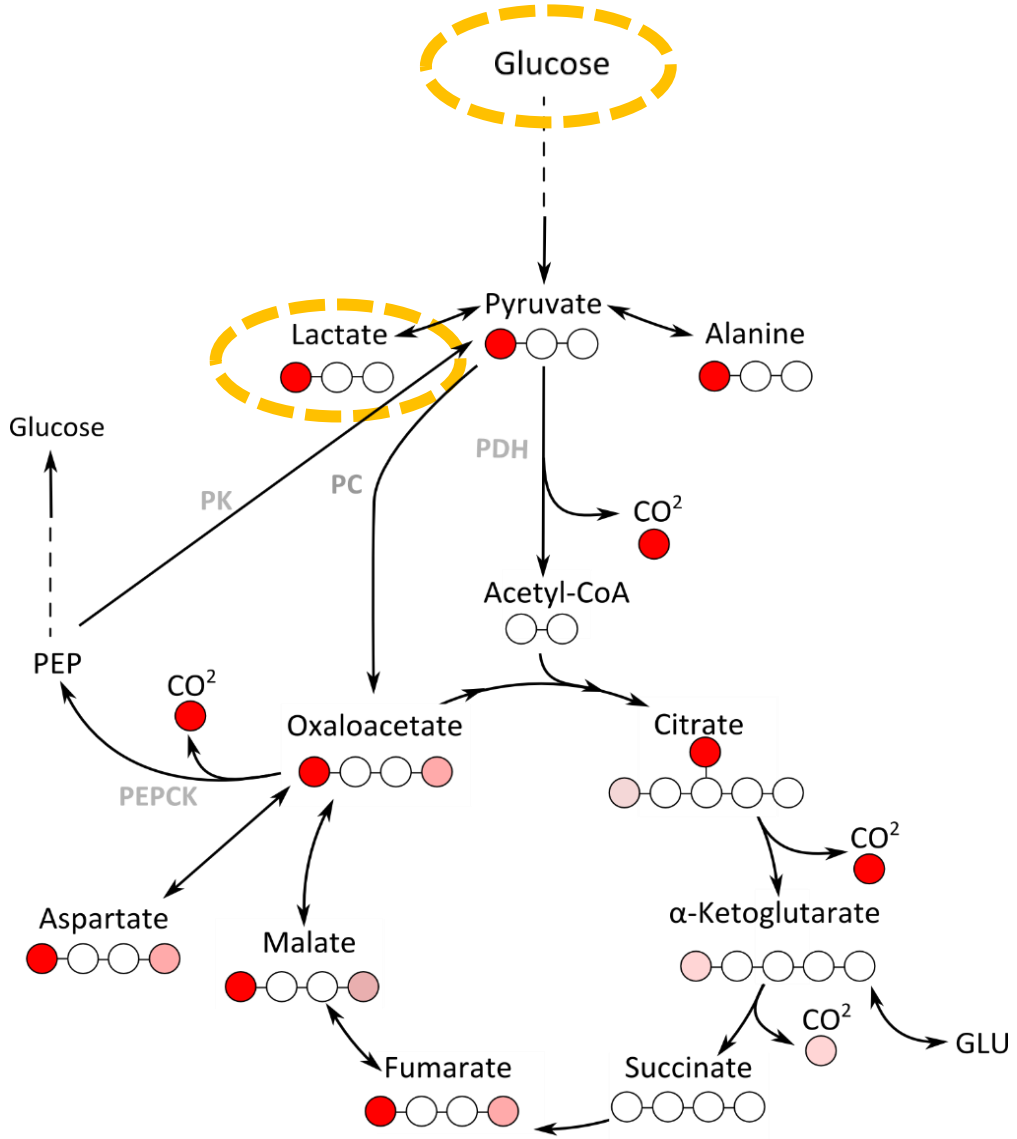
- Bicarbonate signal significantly decreases in fasted state but only apparent at lower doses
- PDH is most likely saturated at higher doses in fed state
- In fasted state, liver bicarbonate signal should provide a direct relation to flux through PEPCK
- **Results will depend on the dose in fed state**



E. Can *et al.* Comm. Biol. 2022

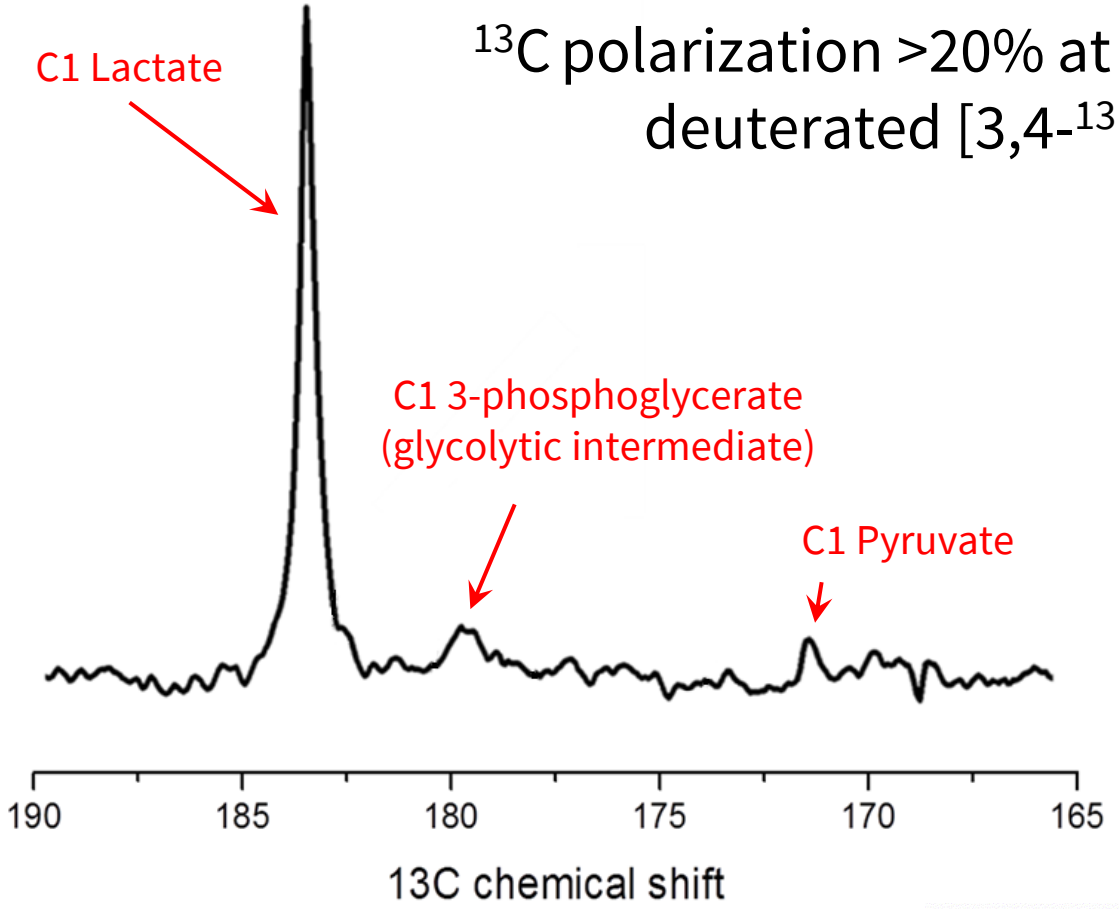
^{13}C-probes that can be injected at physiological doses

- Glucose and lactate blood concentration are on the order of mM
- Hyperpolarized ^{13}C-glucose and ^{13}C-lactate can be injected at physiological dose

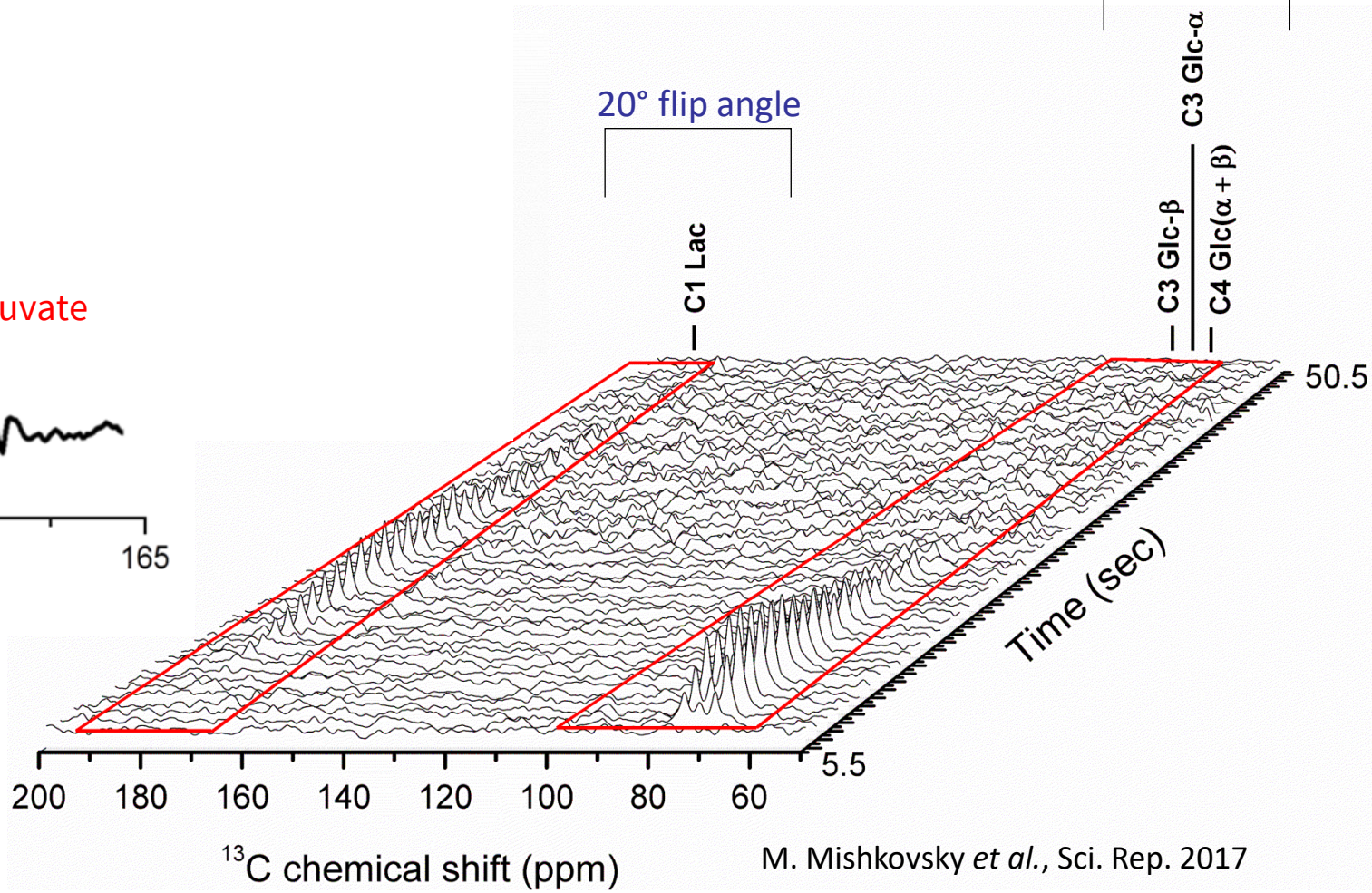


Real-time cerebral glucose metabolism

^{13}C polarization >20% at time of injection of hyperpolarized deuterated [3,4-$^{13}C_2$]glucose in healthy mouse



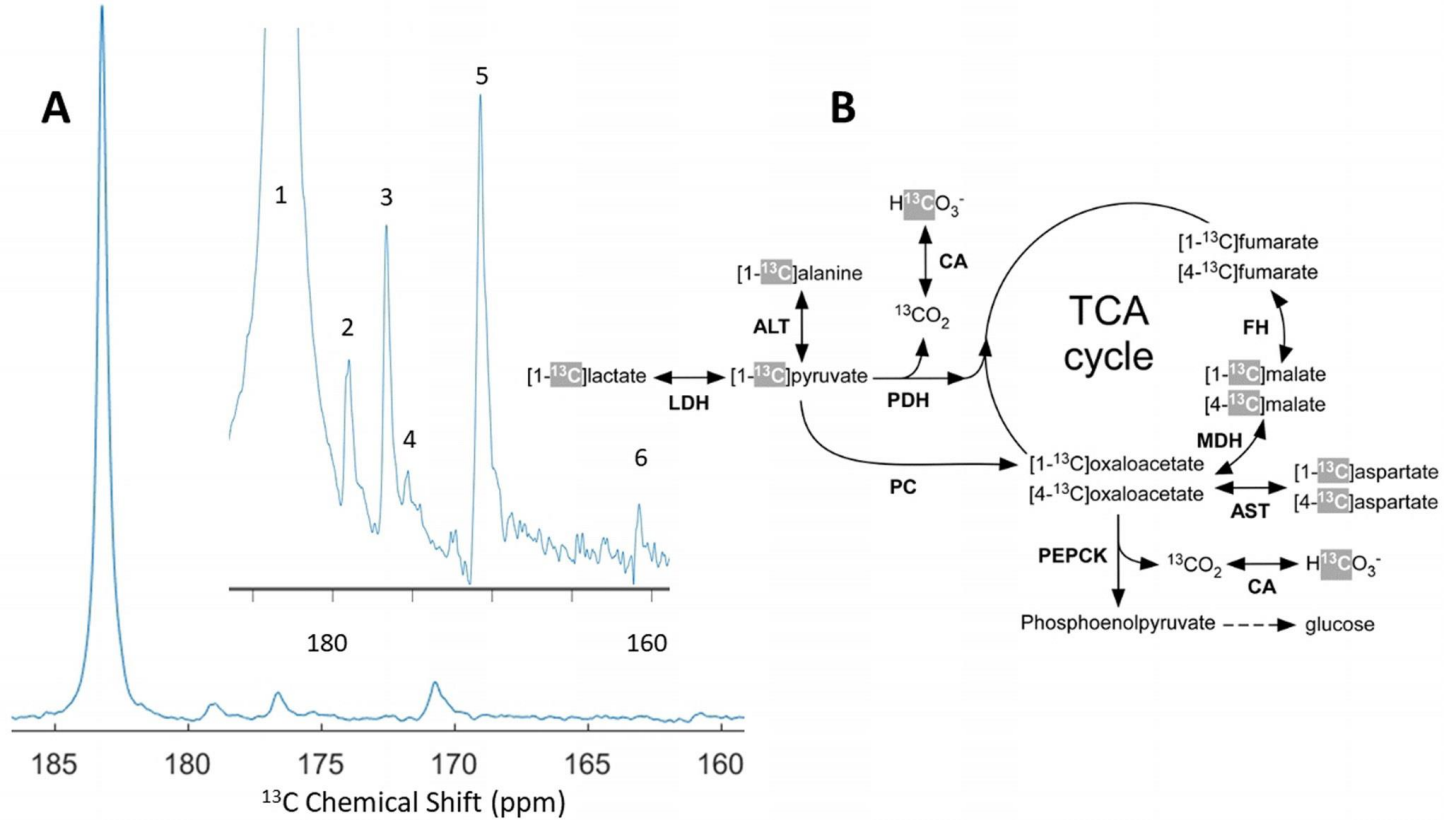
Lactate-to-pyruvate ratio
17.6 ± 2 (n = 5)



M. Mishkovsky *et al.*, Sci. Rep. 2017

Real-time liver lactate metabolism

- Higher ^{13}C polarization allows injecting lower doses
- Injection of 1mL of a 40mM lactate solution in a 350g rat
- Plasma lactate concentration is on the order of 1.5mM, which is the physiological level in rats
- Lactate-to-pyruvate ratio is about 15, which is what one can expect in the rat liver.

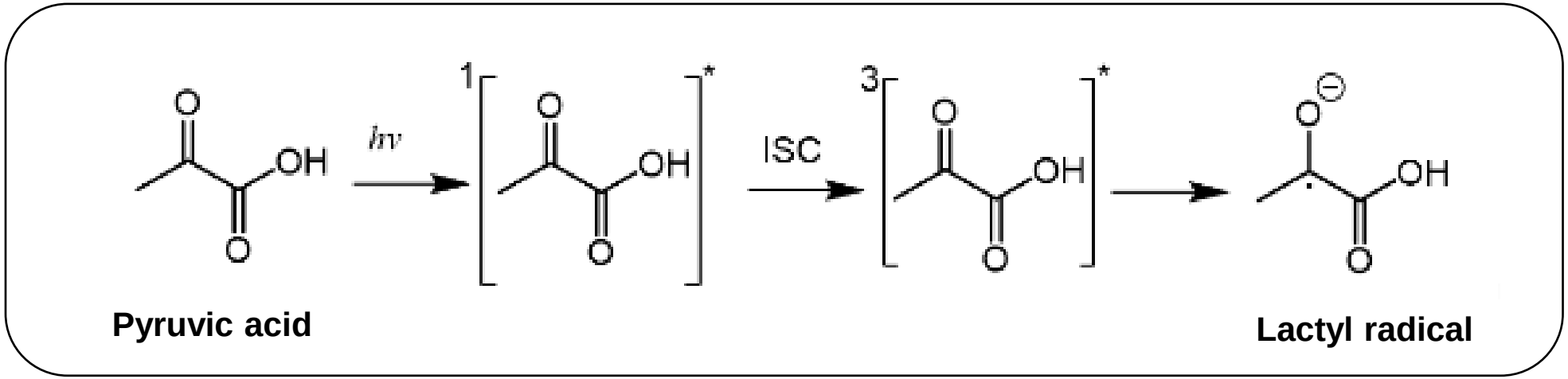


A. Gaunt *et al.*, Angew. Chem. 2021

Minimizing delay between dissolution and injection into humans

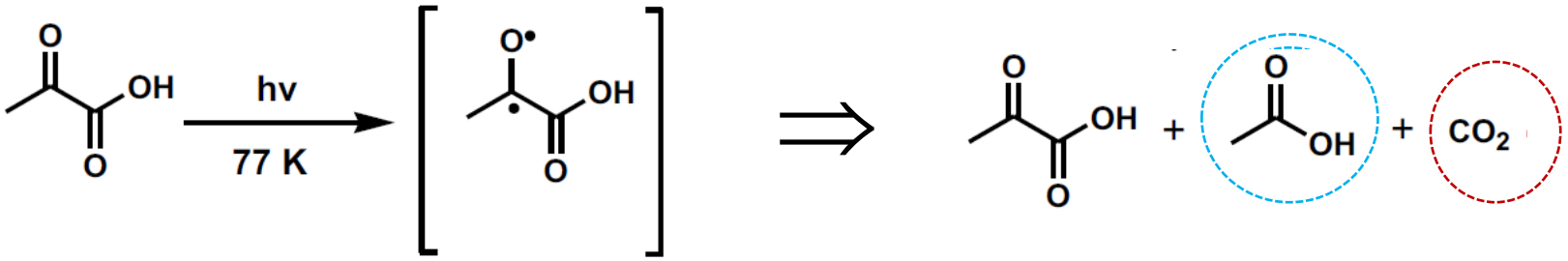
- Rapid dissolution of large sample (1-2mL) + Neutralization (10s)
- Need to filter polarizing agents (radicals) out
- Transfer into QC syringe
- Fast Quality Control (T, pH, ^{13}C-probe concentration, residual radical concentration)

- Can photo-induced polarizing agents help?

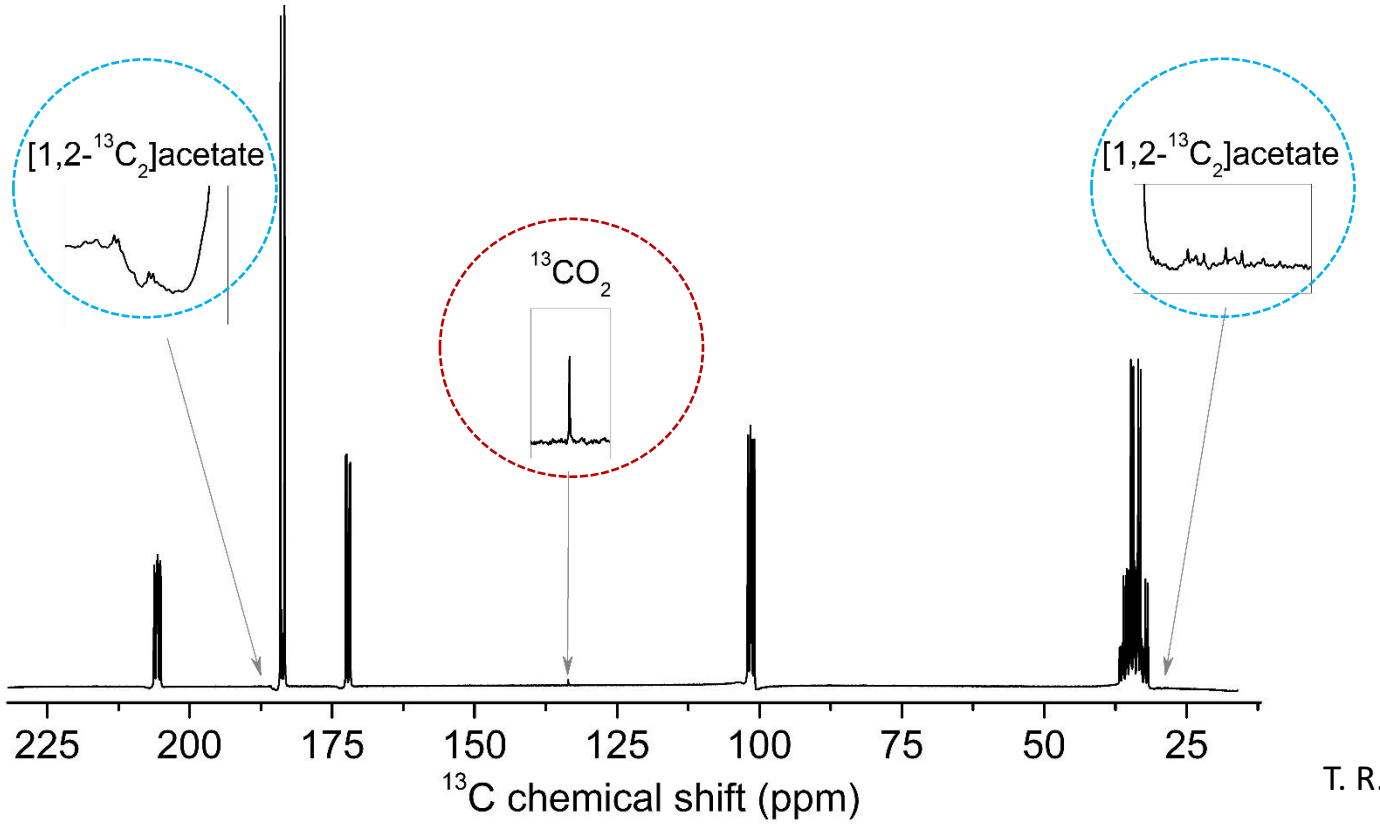


T. R. Eichhorn, *et al.*, PNAS 2013

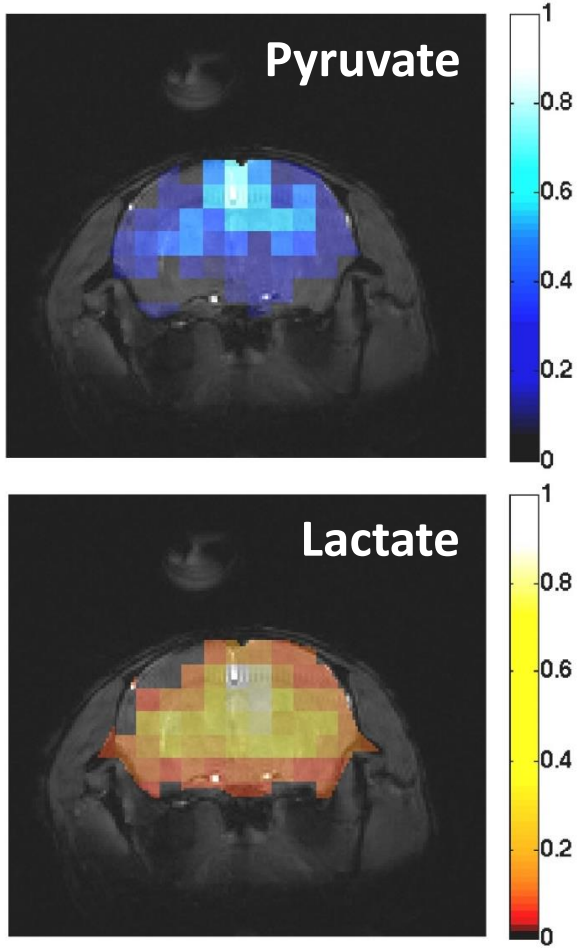
Spontaneous radical quenching upon dissolution



[U-^{13}C]pyruvate and [U-^{13}C]pyruvate hydrate



Hyperpolarized [1- ^{13}C]pyruvate in mouse head



T. R. Eichhorn, *et al.*, PNAS 2013

Conclusions

- dDNP provides **high level of ^{13}C polarization** enabling metabolic imaging in humans and it is **safe and versatile**
- Depending on the metabolic pathways probed by HP ^{13}C-pyruvate, the supraphysiological dose from the bolus injection may not reflect the normal physiology: **HP ^{13}C-pyruvate is not a tracer and ^{13}C needs to be polarized to the highest level possible for ^{13}C MRI since low polarization cannot be compensated by increasing concentration (unlike in PET imaging)**
- Because of their higher normal plasma levels, HP ^{13}C-lactate and HP ^{13}C-glucose experiments can be performed while maintaining a physiological level but **low metabolic product pool sizes or shorter T_1 means that a large ^{13}C polarization is still required**
- Higher ^{13}C polarization can be obtained by improving solid-state DNP but also by **decreasing the delay between dissolution and injection**
- Photo-induced radicals would **remove the need for radical filtration** and enable producing purely endogenous imaging agents

Acknowledgments

Adam Gaunt
Jennifer Lewis
Irene Marco-Rius
Tian Cheng
Andrea Capozzi
Emine Can
Tim Eichhorn
Jessica Bastiaansen
Yuhei Takado
Mor Mishkovsky
Najat Salameh
Hikari Yoshihara
Jean-Noël Hyacinthe
Jacques van der Klink

Kevin Brindle
Friederike Hesse
Alan Wright
Felix Kreis
Dominique-Laurent Couturier

Albert Chen

Rolf Gruetter
Christophe Roussel

Mathilde Lerche
Magnus Karlsson

Matthew Merritt

European Research Council

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SWISS NATIONAL SCIENCE FOUNDATION


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