Metabolic Imaging: Hyperpolarized Carbon for Measuring Kinetics in Living Systems

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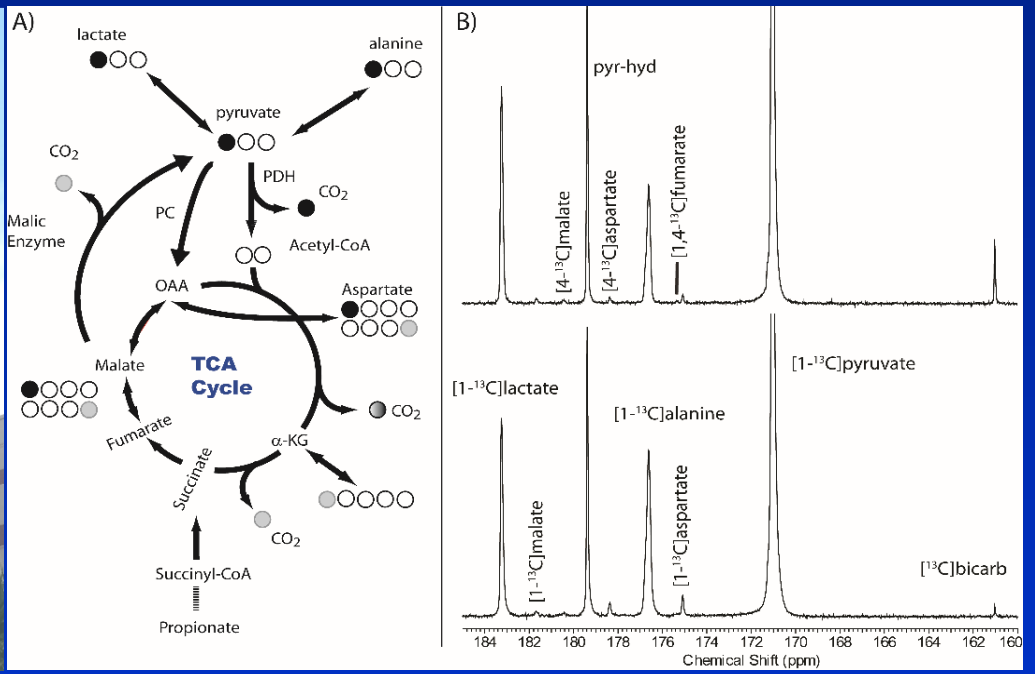

Acknowledgments

UF

Tatsiana Tsarova

Dr. Matthew Merritt

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Outline

- Understanding physical prerequisites
- Maximizing biological information
- Targets for metabolic imaging

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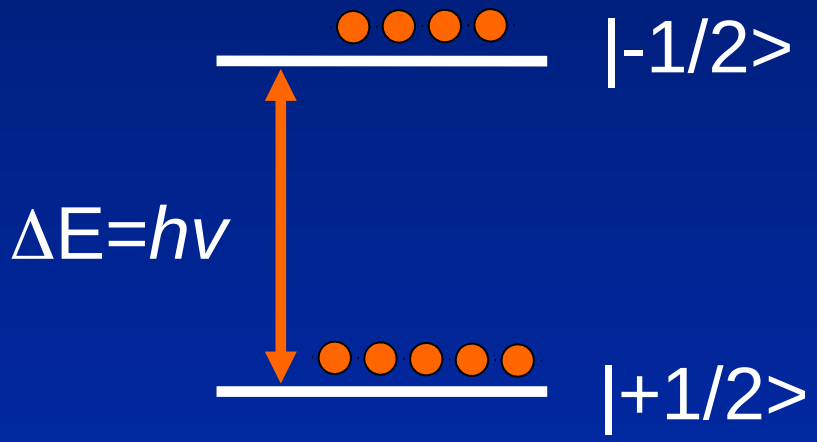
- **Understanding physical prerequisites**
- Maximizing biological information
- Targets for metabolic imaging

When will Dynamic Nuclear Polarization work?

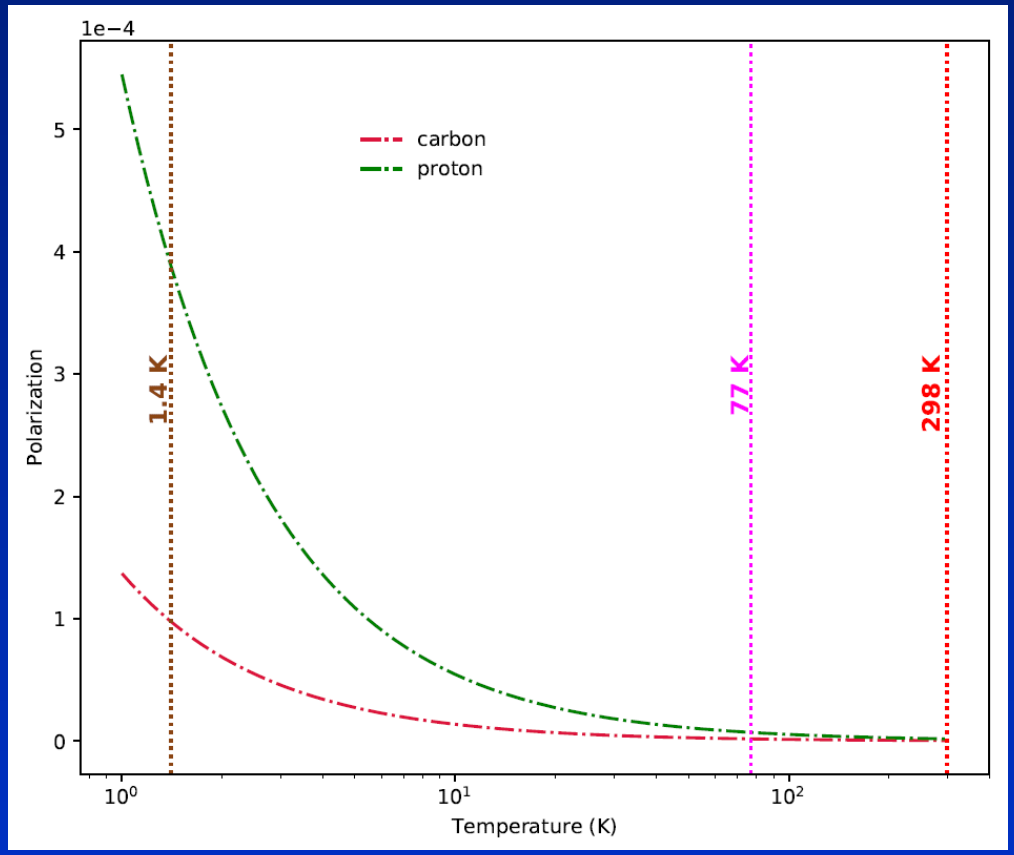
- Dope a stable free radical into a carbon-13 labeled metabolite of interest
- The sample prep must produce a *glass*

Thermal Equilibrium

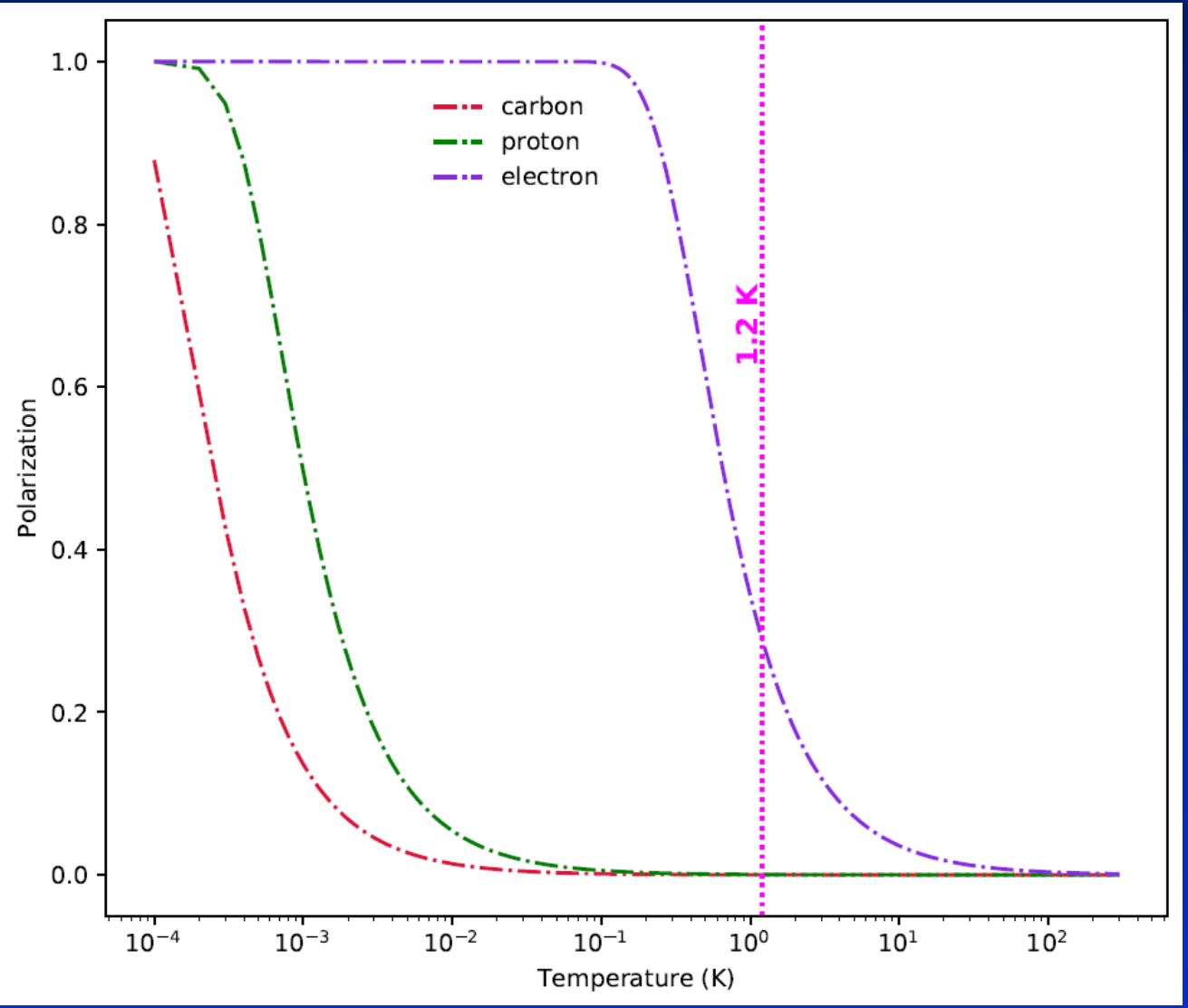
$$P = \tanh\left(\frac{\gamma \hbar B_0}{2k_B T}\right)$$



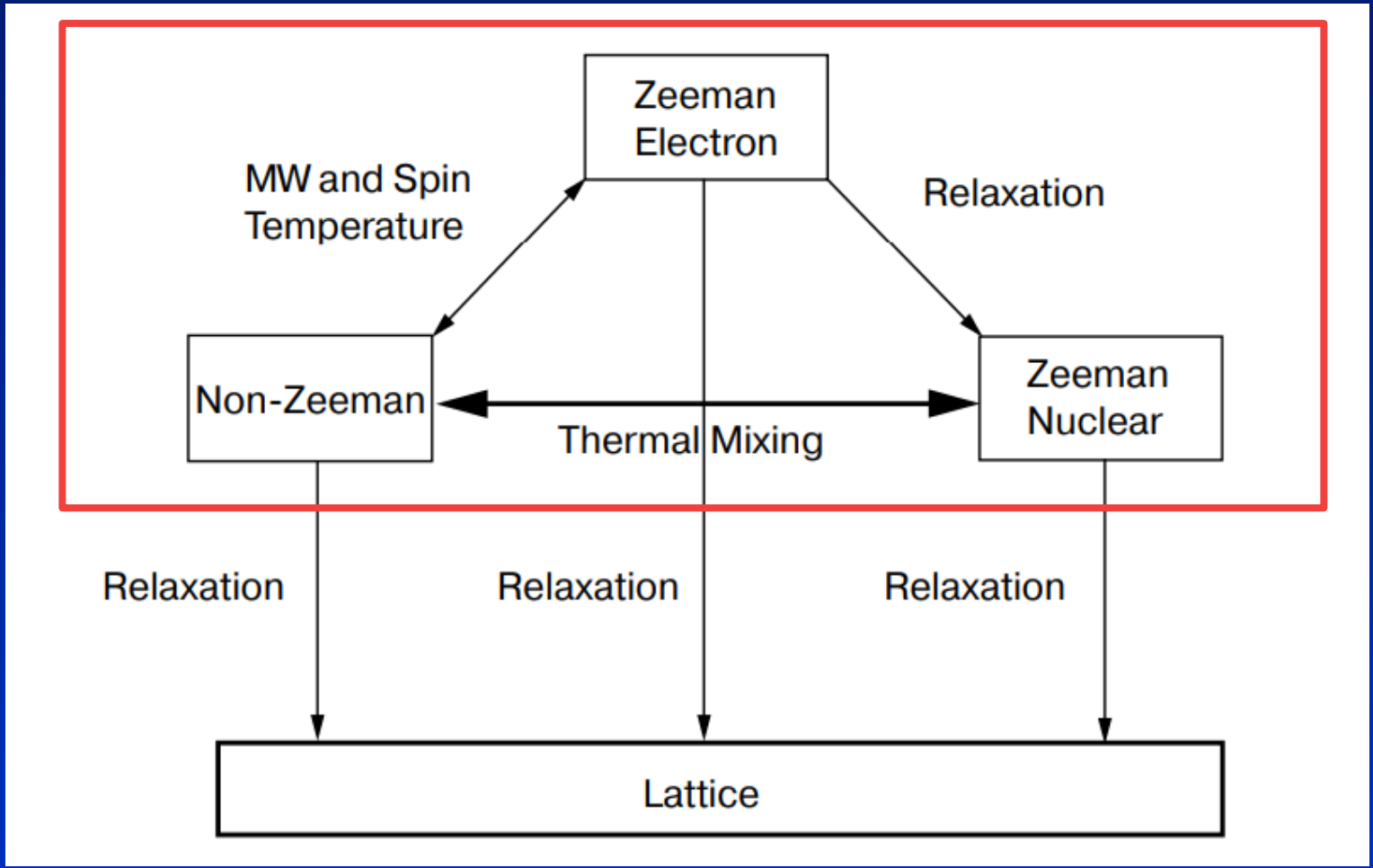
ΔE is ~ 10^{-25} J, and k is 1.38×10^{-23} J/K, so the population difference is on the order of ppm at room temperature



Dynamic Nuclear Polarization (DNP)



DNP Mechanism: Thermal Mixing



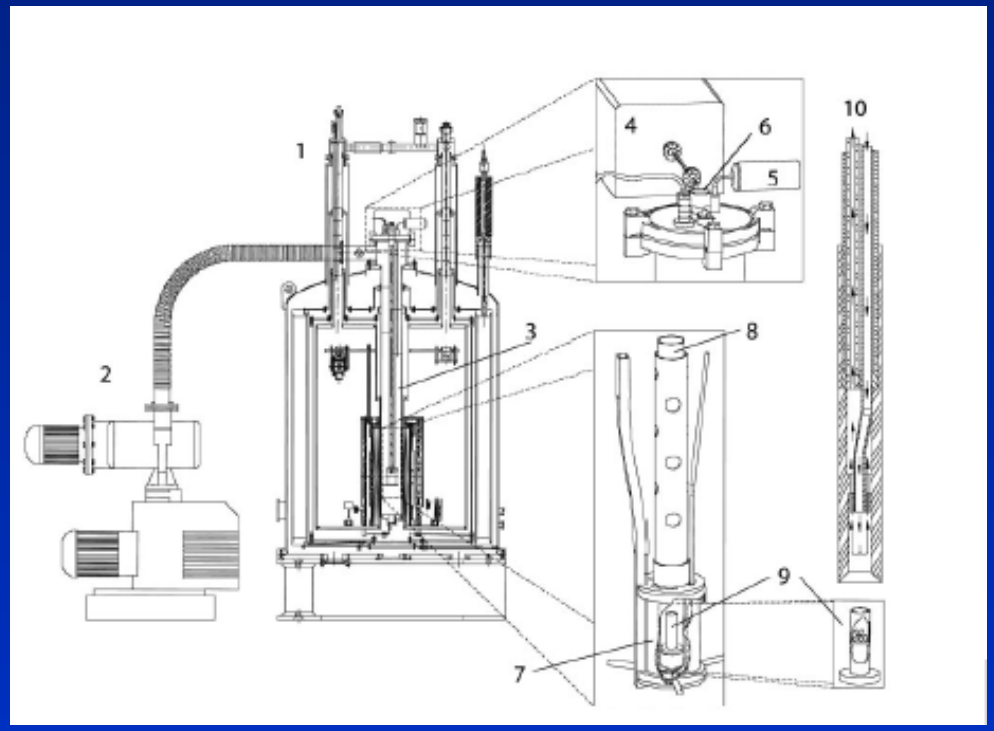
Goldman, Appl. Magn. Reson. (2008)

Increase in signal-to-noise ratio of >10,000 times in liquid-state NMR

Jan H. Ardenkjær-Larsen*, Björn Fridlund, Andreas Gram, Georg Hansson, Lennart Hansson, Mathilde H. Lerche, Rolf Servin, Mikkel Thaning, and Klaes Golman

- 3.35 T
- VTI replaces RT bore
- 94 GHz μ-wave
- 1.4 K operation

- Dissolution by boiling solvents



Melt frozen solid and inject – Dissolution DNP

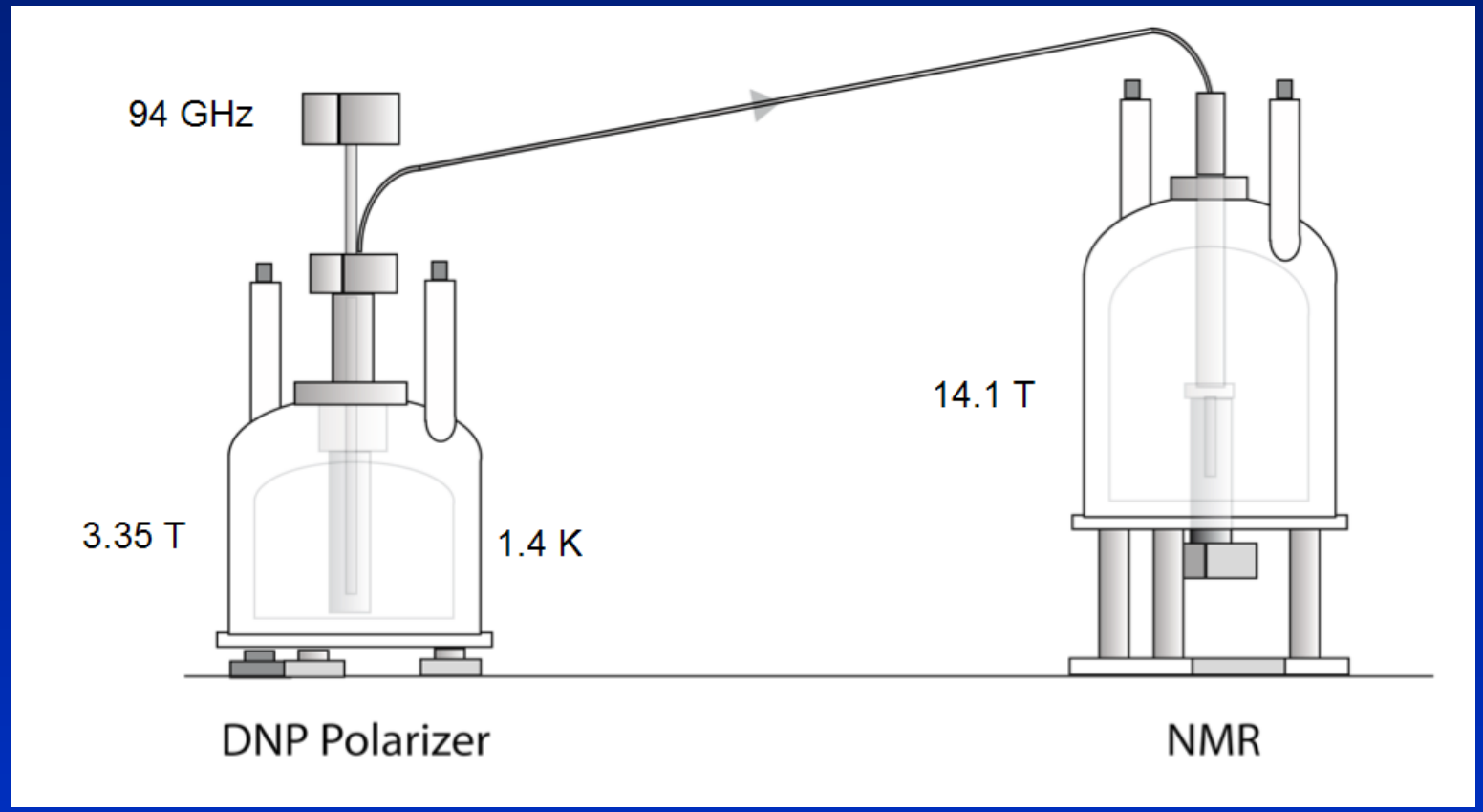


Figure Credit: CNRS, France

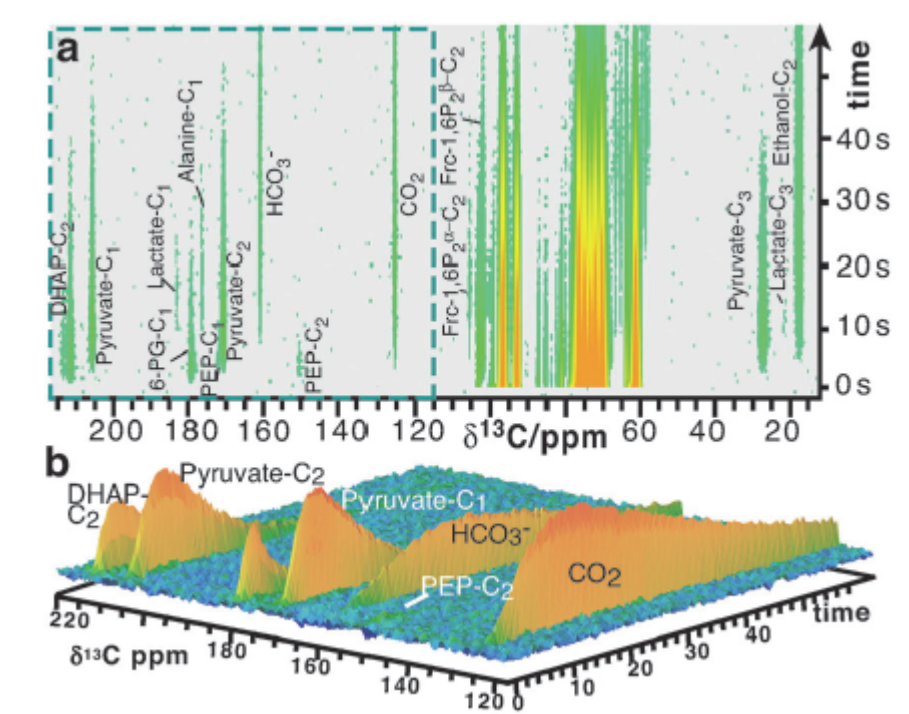
Outline

- Understanding physical prerequisites
- **Maximizing biological information**
- Targets for metabolic imaging

When will dDNP work?

- The sample prep must produce a glass
- The T_1 of the sample must be *long enough*

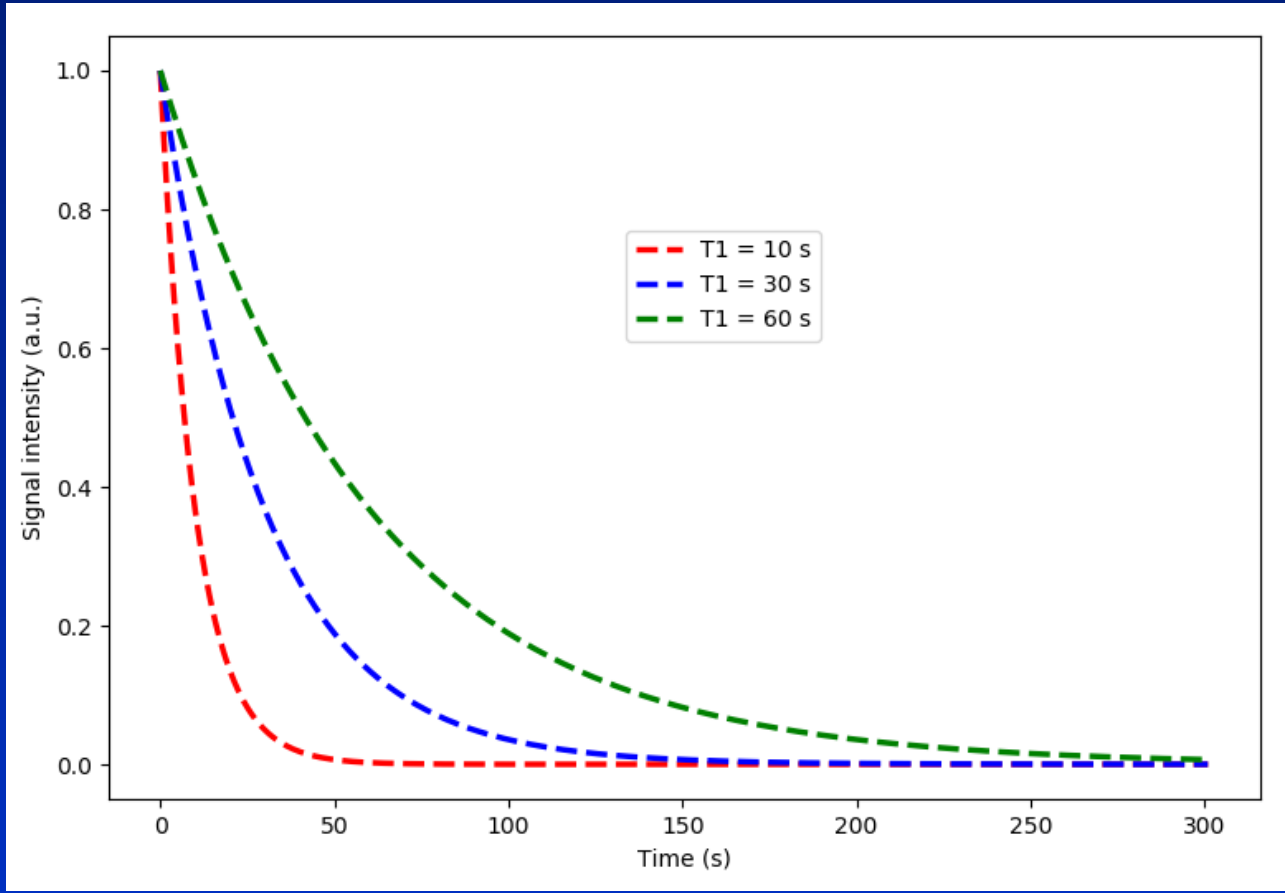
T_1: “How physical facts kill beautiful experiments”



- [U-^{2}H-$^{13}C_6$] glucose
- Glycolysis in yeast
- Most of kinetics is visible because of rapid transfer to culture

Meier, et al., Molecular Biosystems (2011)

T_1: “How physical facts kill beautiful experiments”



T_1s of heteronuclei are highly dependent on

- The number of attached hydrogens
- The chemical shift anisotropy
- The correlation time of the molecule
- The presence of paramagnetic species

Strong constraints on the types of molecules that can be imaged (Central Metabolism)

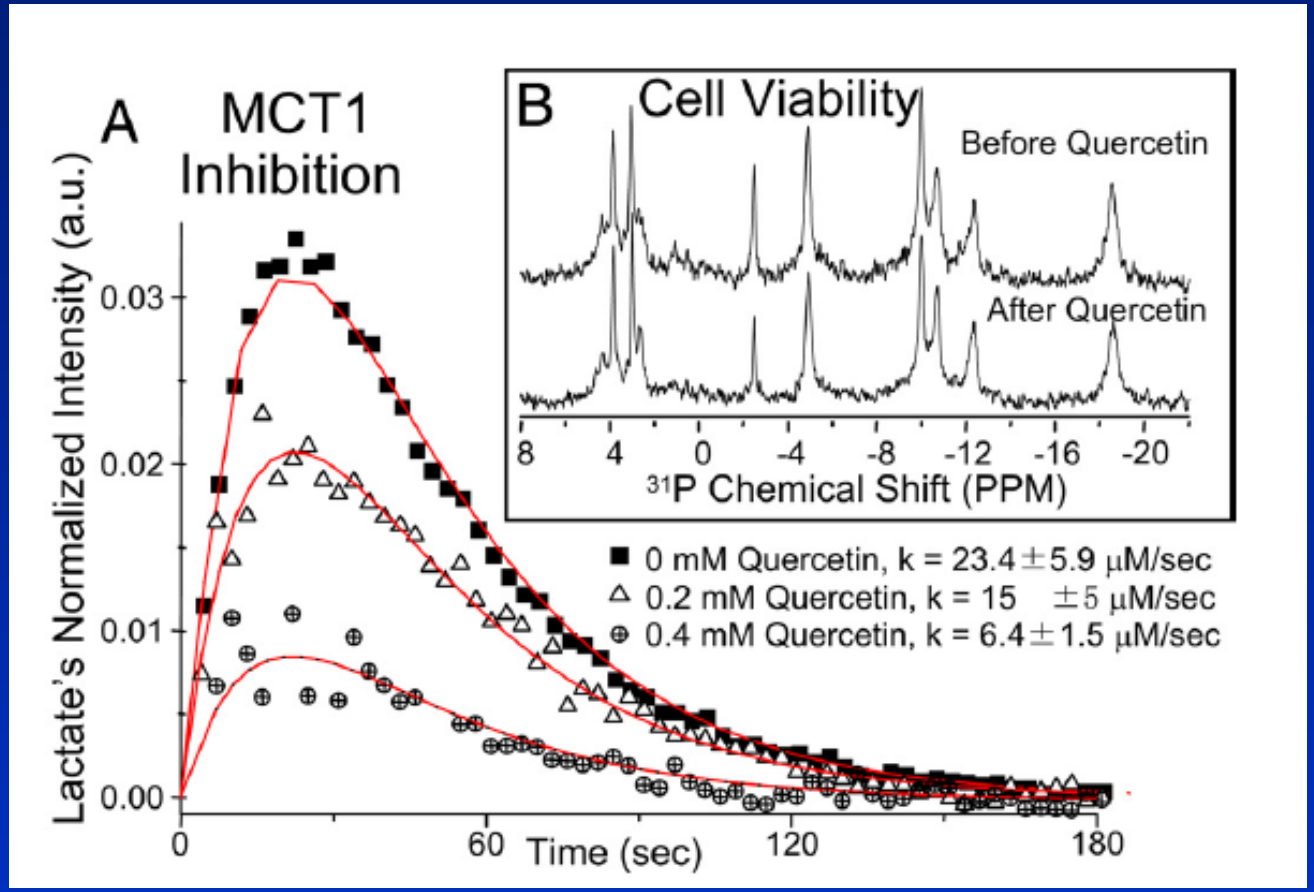
When will DNP work in living systems?

- The sample prep must produce a glass
- The T_1 of the sample must be *long enough*
- *A high flux transporter is highly beneficial* (e.g. MCTs)

Pyruvate Transport Can Dominate Kinetics

" … kinetics observed for the conversion of hyperpolarized ^{13}C-pyruvate to lactate in perfused T47D breast cancer cells, has a MCT1-mediated pyruvate transport as rate-limiting step."

Harris, et al., PNAS (2009)



Outline

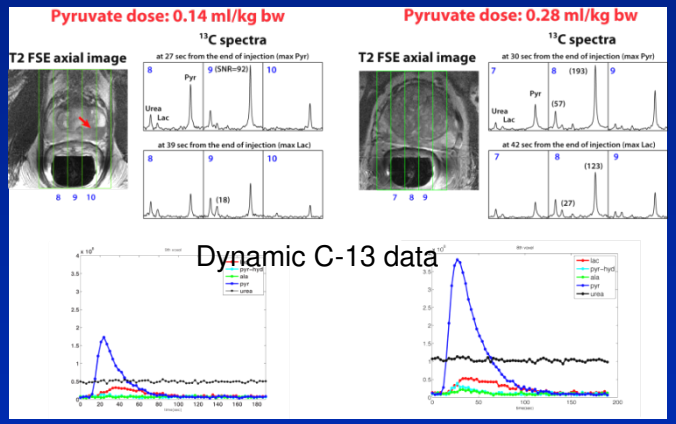
- Understanding physical prerequisites
- Maximizing biological information

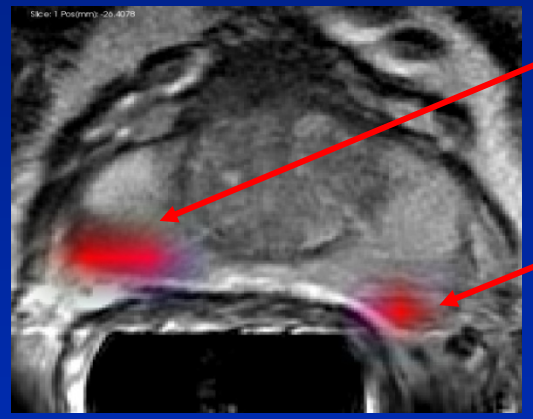
- **Targets for metabolic imaging**
  - **Cancer**

Cancer

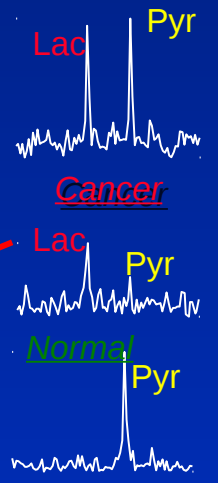
- Elevated glycolysis implies more glucose uptake (FDG-PET)
- Elevated glycolysis implies more lactate formation
- A metabolic basis for hypothesis generation

“A Phase I Ascending-dose and Exploratory Imaging Study to Assess the Safety and Tolerability and Imaging Potential of Hyperpolarized [^{13}C-1] Pyruvate Injection in Subjects With Prostate Cancer”





HP ^{13}C MRSI



UCSF University of California San Francisco

31 prostate cancer patients studied demonstrated safety and imaging feasibility in this Phase 1 dose escalation trial with no adverse events.

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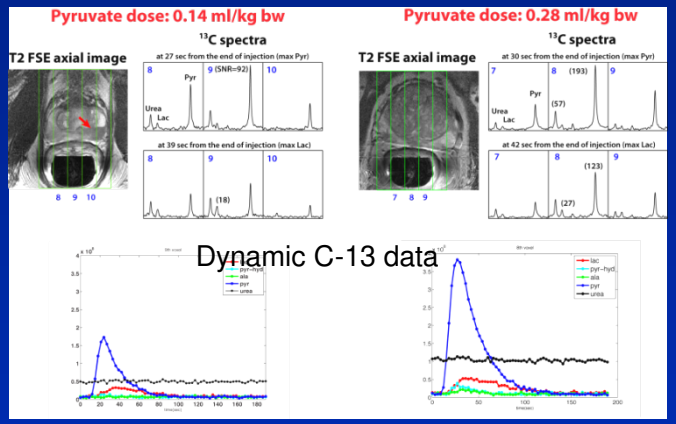

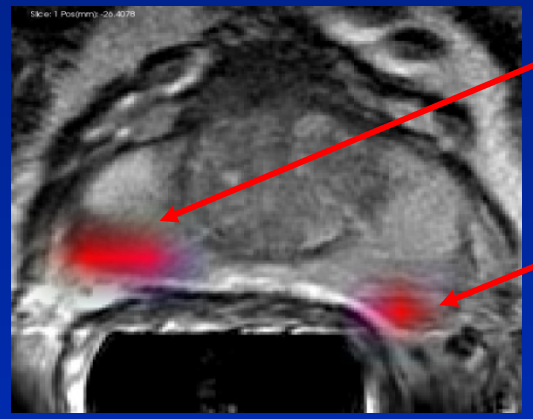

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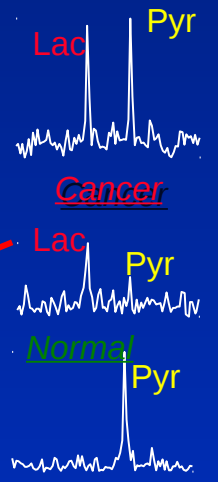

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~20 NIH funded trials currently underway

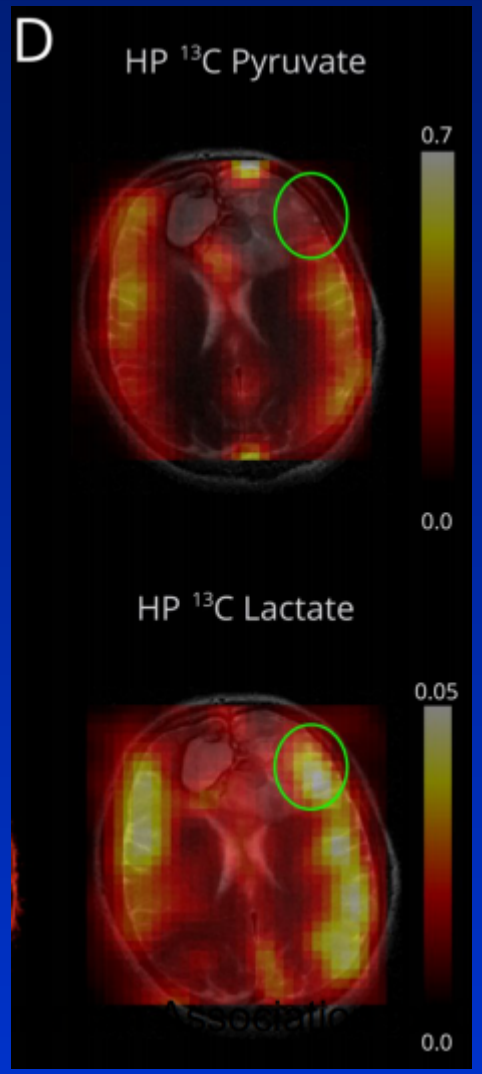
Imaging Tumor In Brain

[1 – ^{13}C] Pyruvate

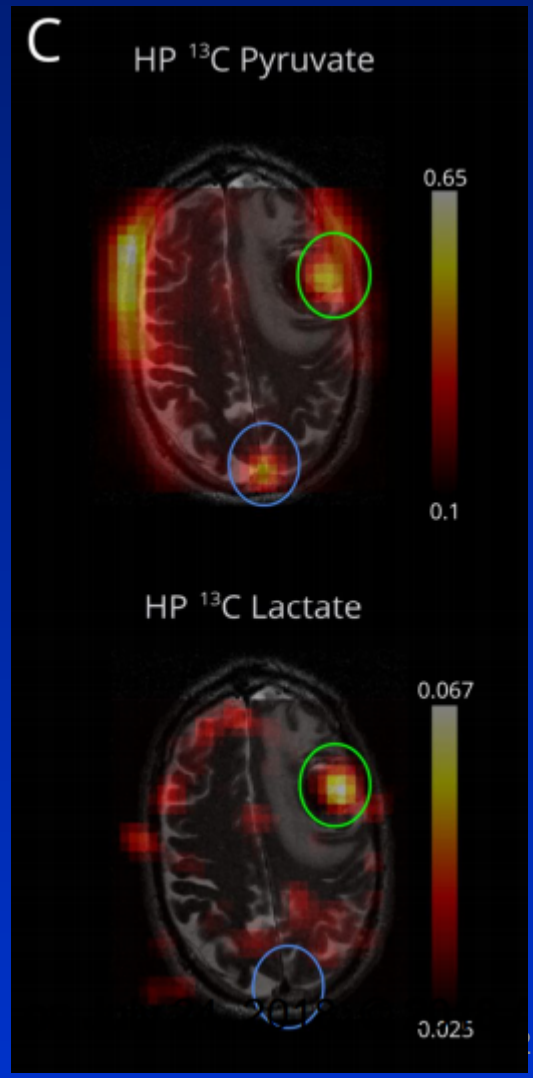
"We correlated our results with standard clinical brain MRI, MRI DCE perfusion, and in one case FDG PET/CT. Our results suggest that HP ^{13}C pyruvate-to lactate conversion may be a viable metabolic biomarker for assessing tumor response."

Miloushev, et al., Cancer Res. (2018)

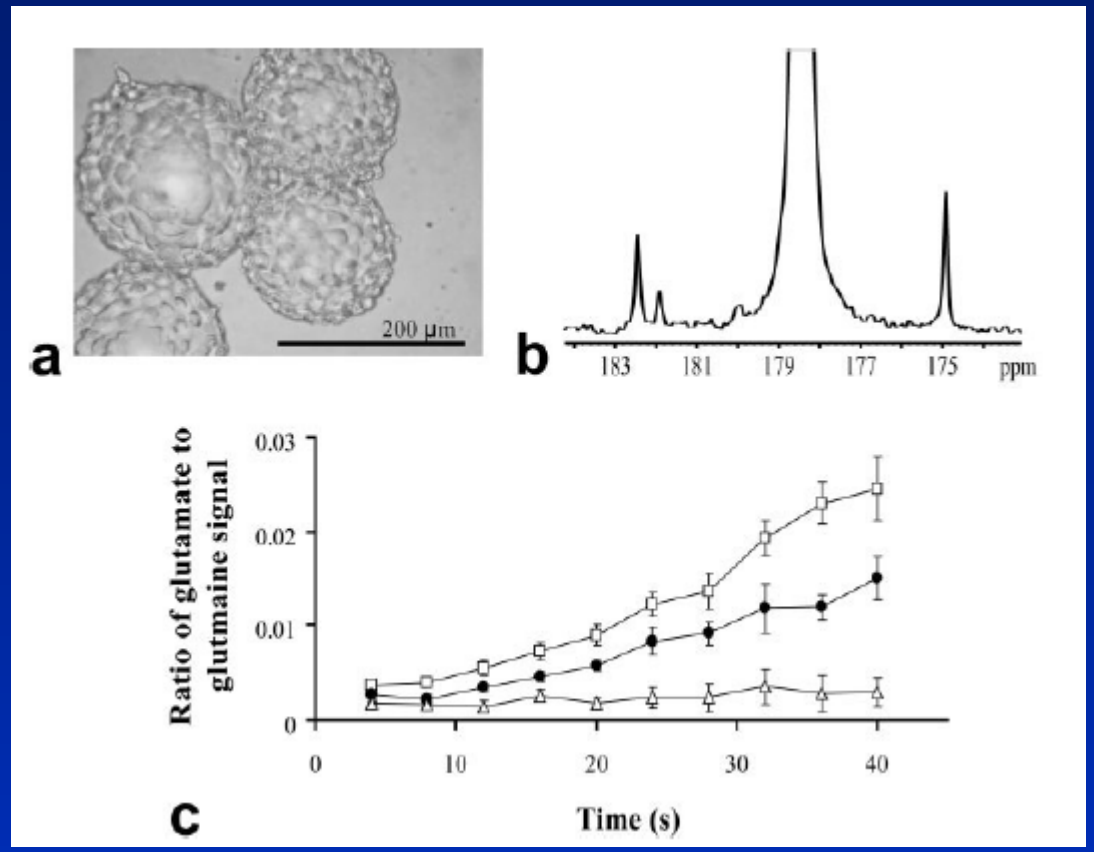
Glioblastoma



Metastatic melanoma



Tumor Cell Proliferation



Gallagher, et al., MRM (2008)

[5 – ^{13}C] Glutamine

- Anaplerosis via glutaminase to form glutamate can serve as a pathway to enhance tumor cell growth
  - hepatocellular carcinomas have a 30x increase in glutamine utilization

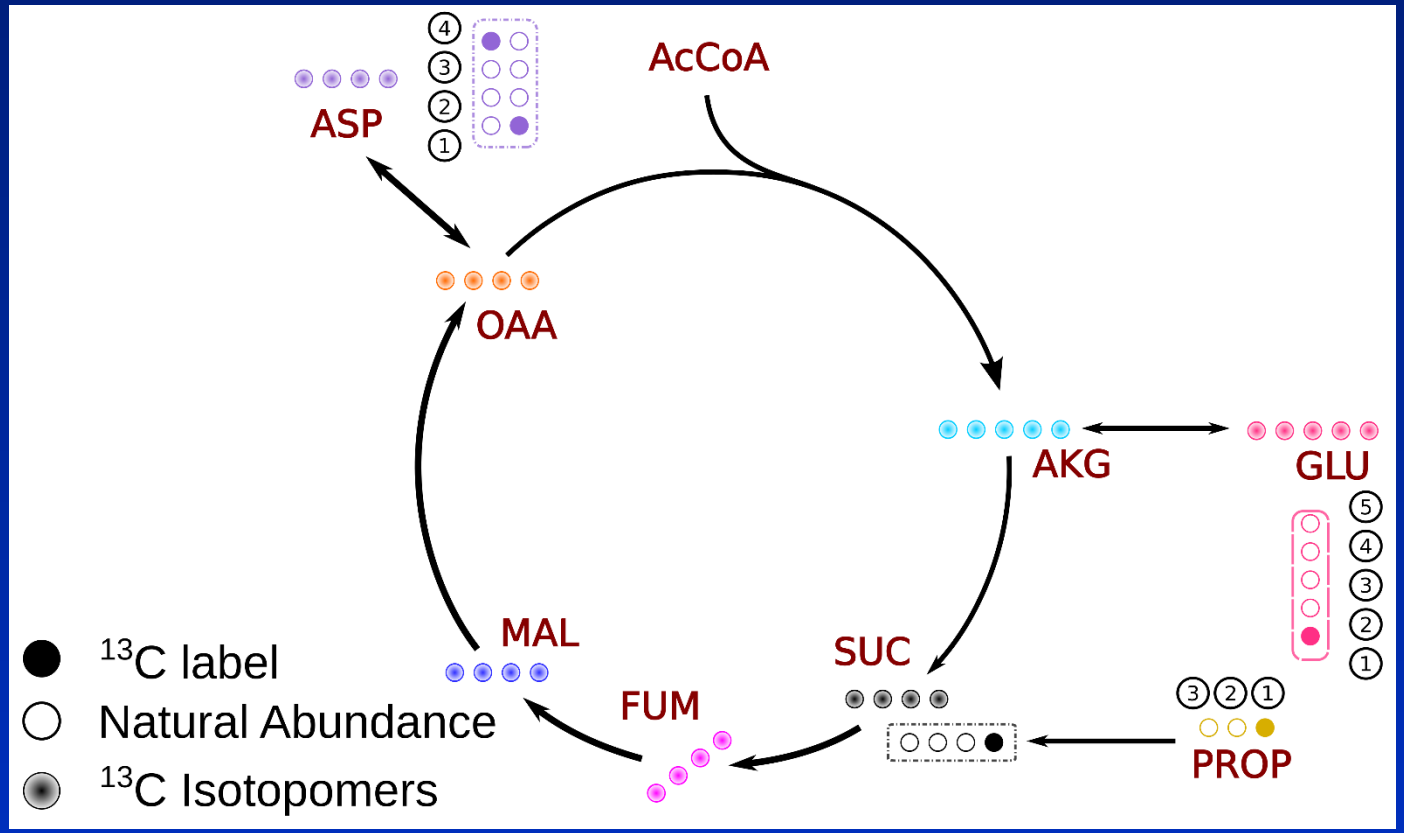
Outline

- Understanding physical prerequisites
- Maximizing biological information

- **Targets for metabolic imaging**
  - Cancer
  - **Myocardium**

Hyperpolarized ^{13}C Study of Myocardial Metabolism

- Anaplerosis replenishes TCA cycle intermediates
  - Propionate is avidly metabolized
  - High levels of PCC expressed in the heart

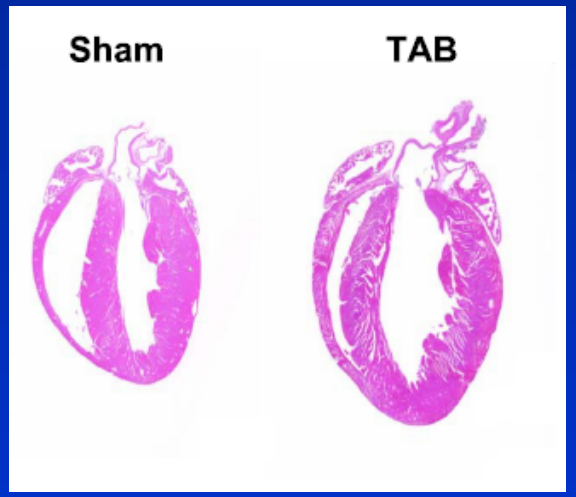


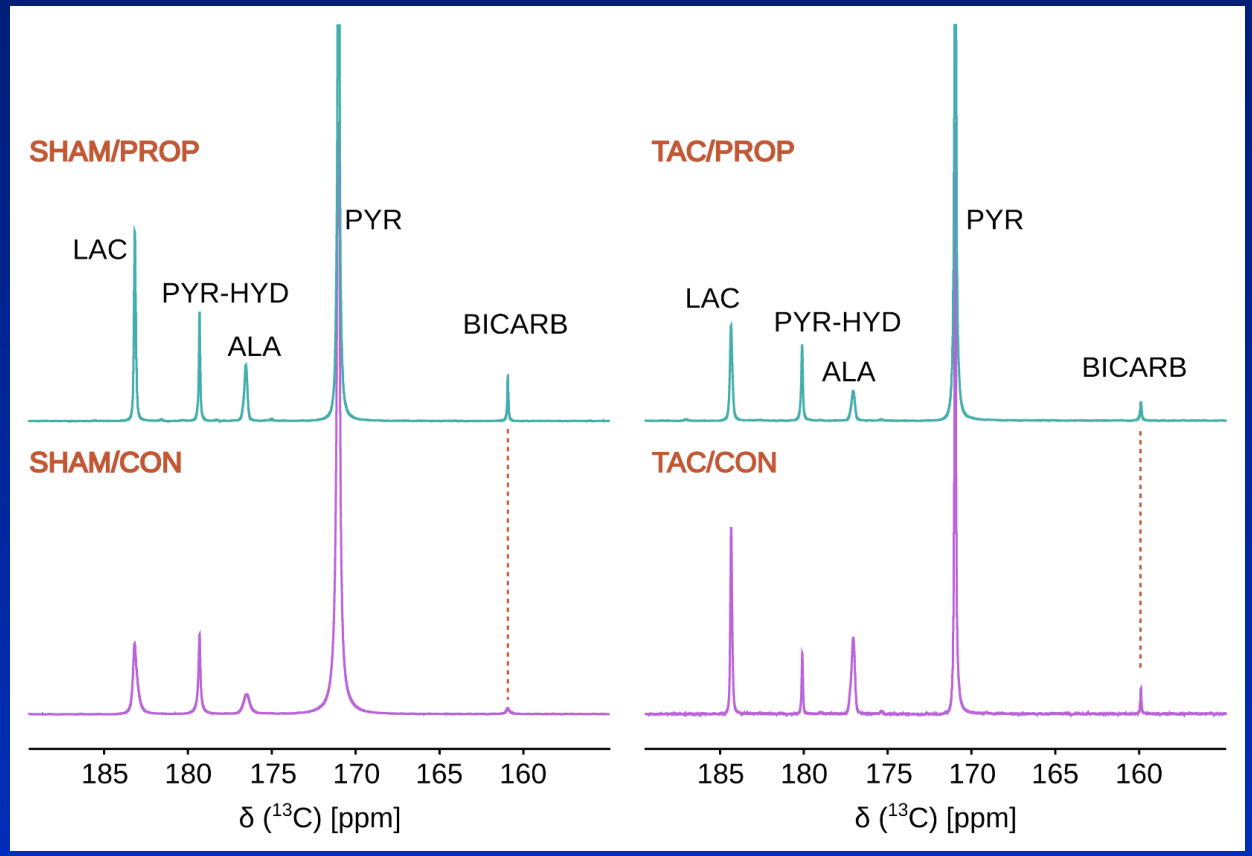
(Work in progress)

Hyperpolarized ^{13}C Study of Myocardial Metabolism

[1 – ^{13}C] Pyruvate

- Metabolic inflexibility in failing myocardium
  - Propionate Cannot Modulate PDH Flux in Hypertrophied Heart





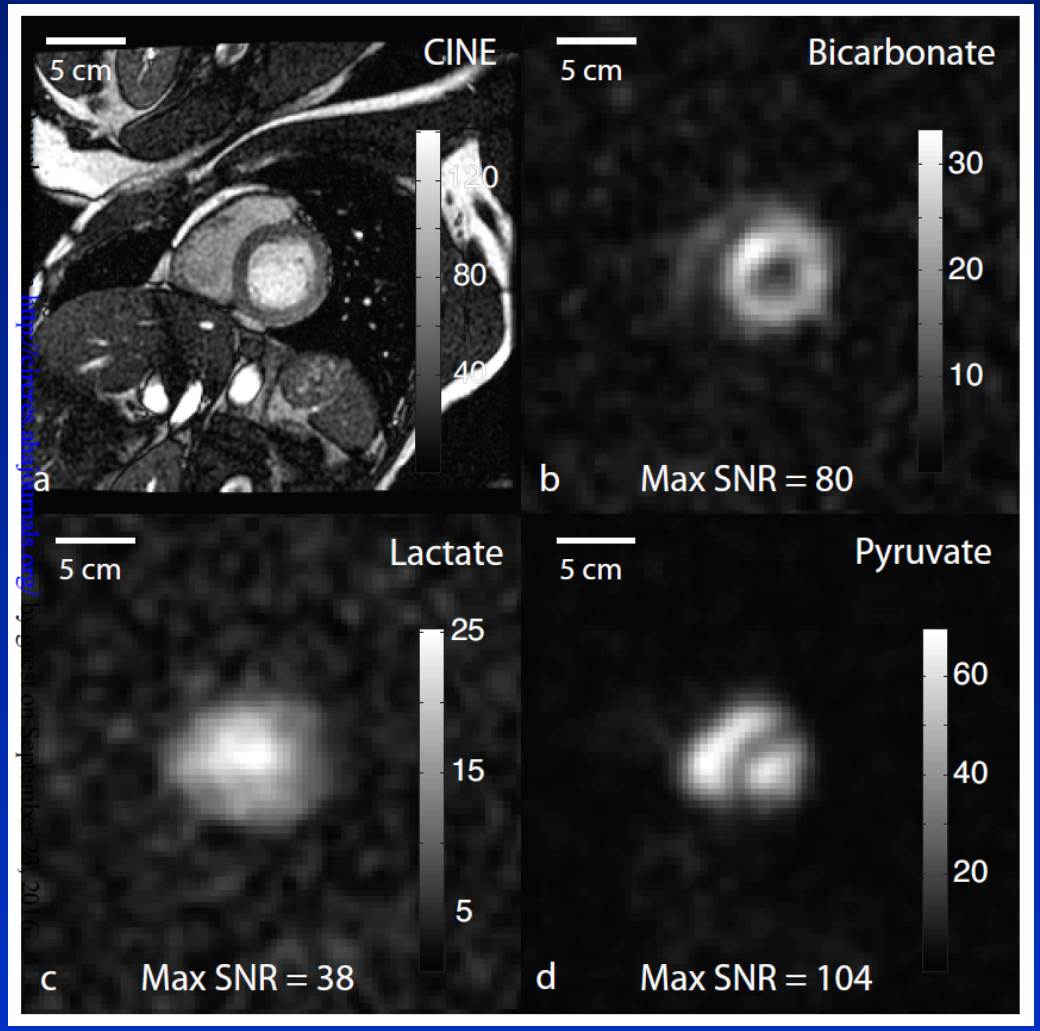
(Work in progress)

Hyperpolarized ^{13}C MRI- Human Myocardial Metabolism

LDH

PDH

Information about tissue activity and diffusion



Cunningham, et al., Circ. Res. (2016)

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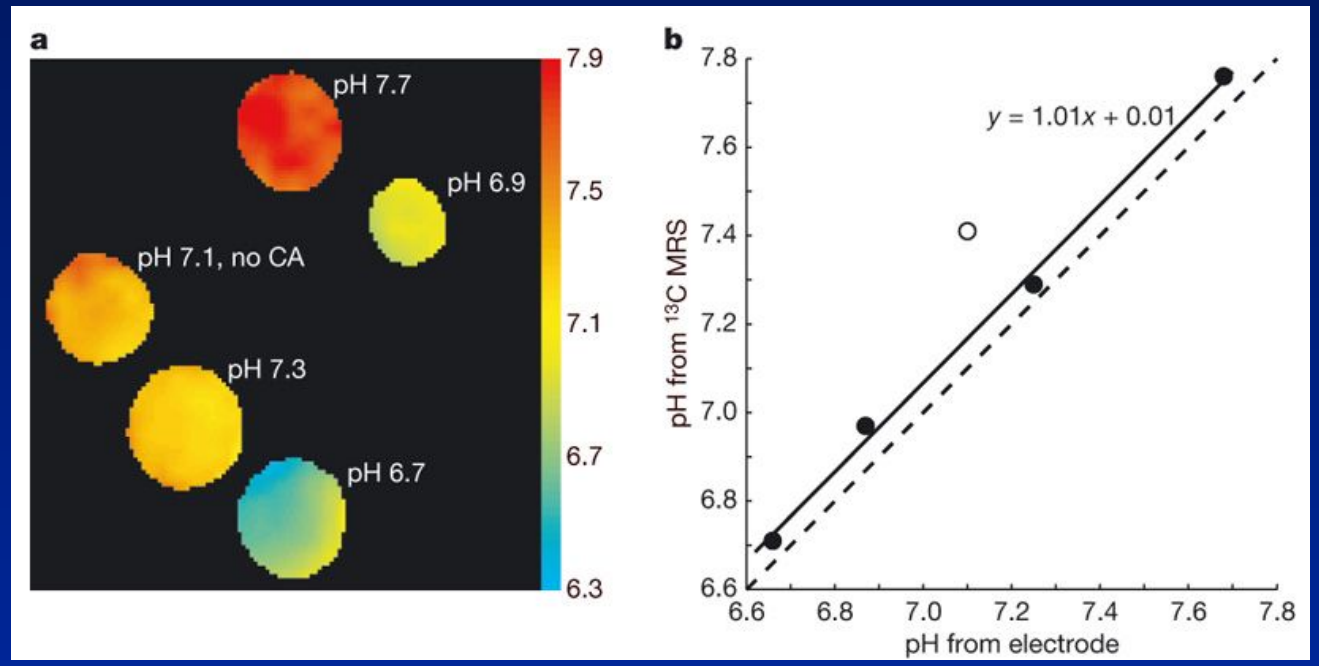
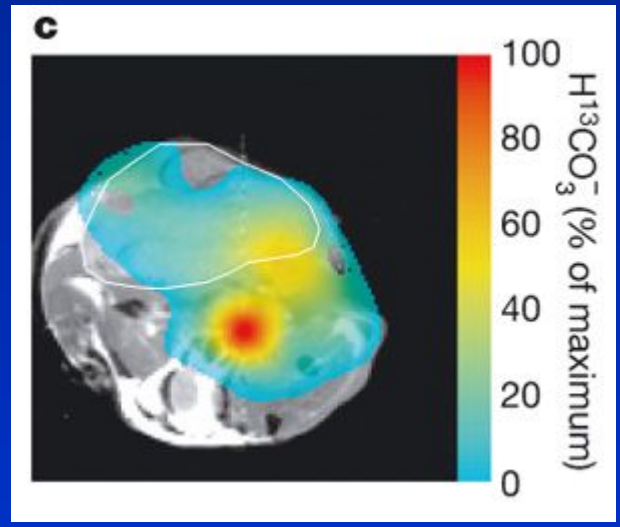
  - Cancer
  - Myocardium
  - **pH sensing**

pH Imaging

$^{13}C\ HCO_3^-$

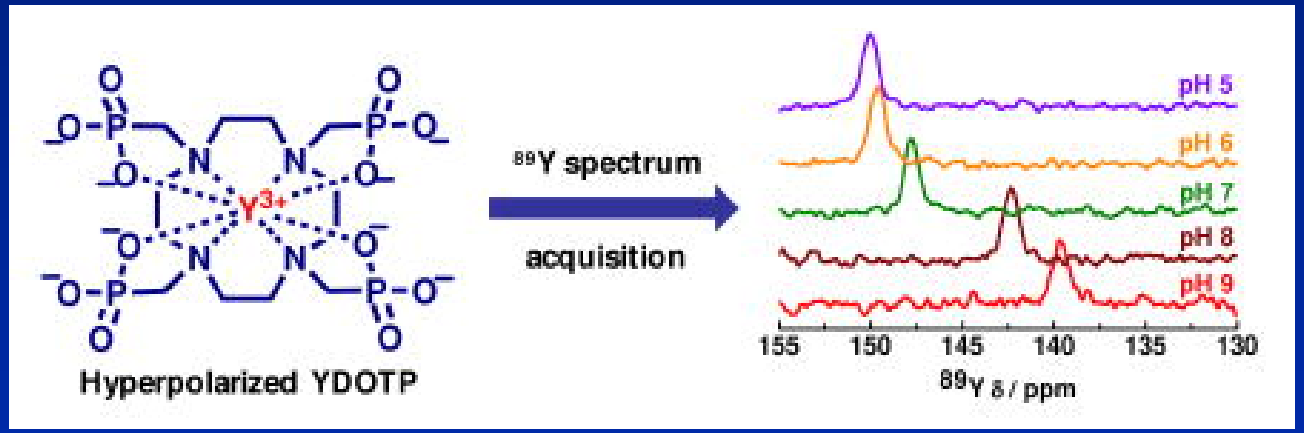
- Short T_1
- Low Concentration in Solid State

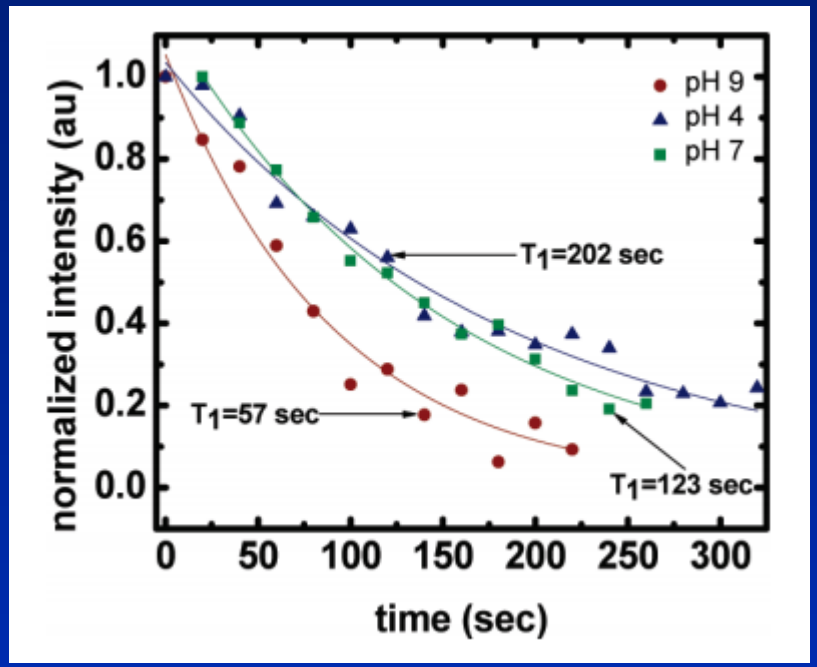




Gallagher, et al., Nature (2008)

pH Imaging





Jindal, et. al., JACS (2010)

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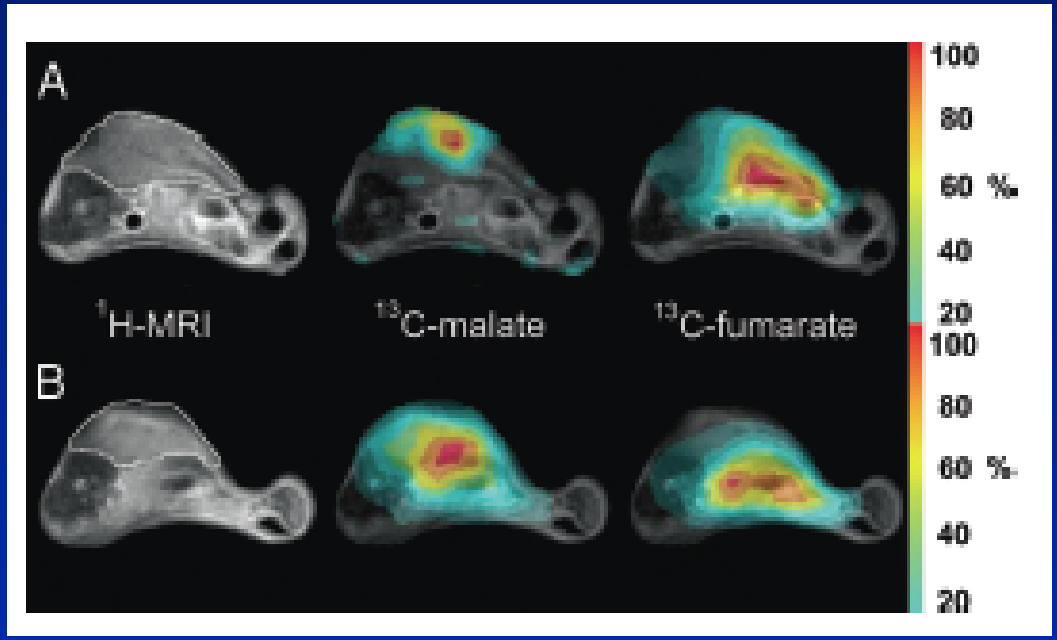
  - Cancer
  - Myocardium
  - pH sensing
  - **Necrosis**

Cell Necrosis

[1,4-^{13}C] Fumarate

- Fumarate not easily transported across cell membrane
- Appearance of malate via fumarase reaction is therefore marker of necrosis



Gallagher, et. al., PNAS 2009

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- **Targets for metabolic imaging**
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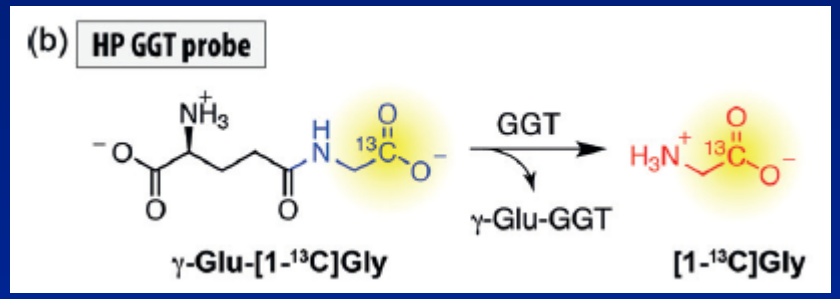
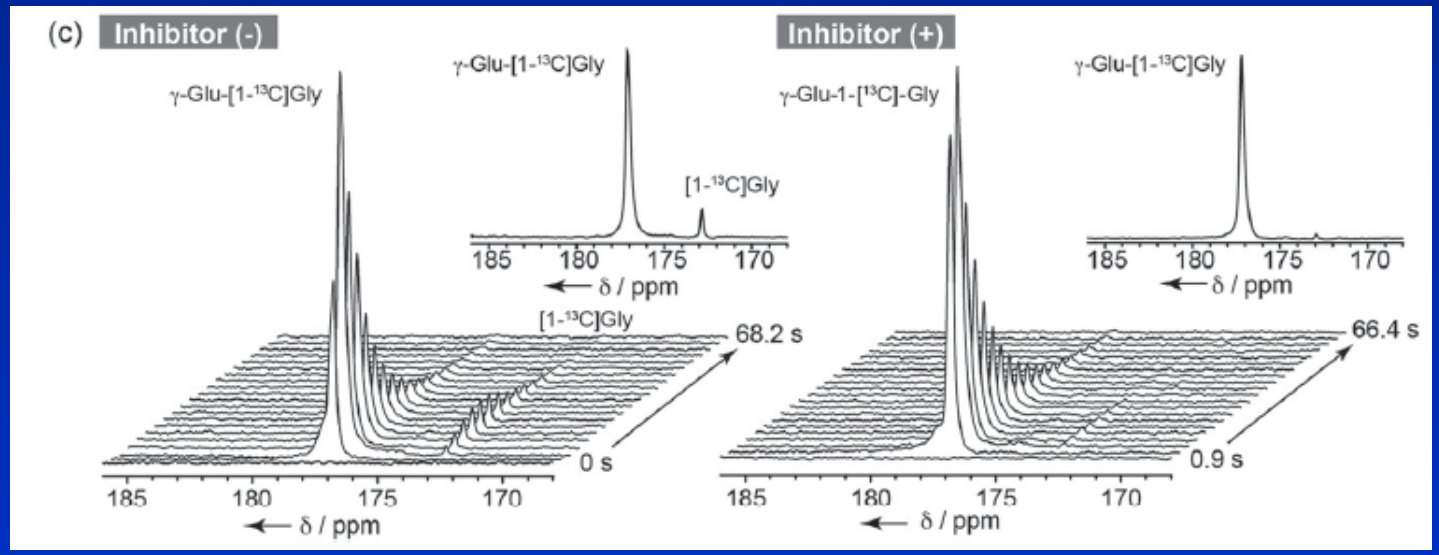
  - Myocardium
  - pH sensing
  - Necrosis
  - **Metabolic pathways**

Glutathione Homeostasis

γ – Glutamyl [1 – ^{13}C] glycine

- Probe γ-glutamyl transpeptidase activity
- T_1 > 30 s in vitro
- Rat kidney *in vivo*



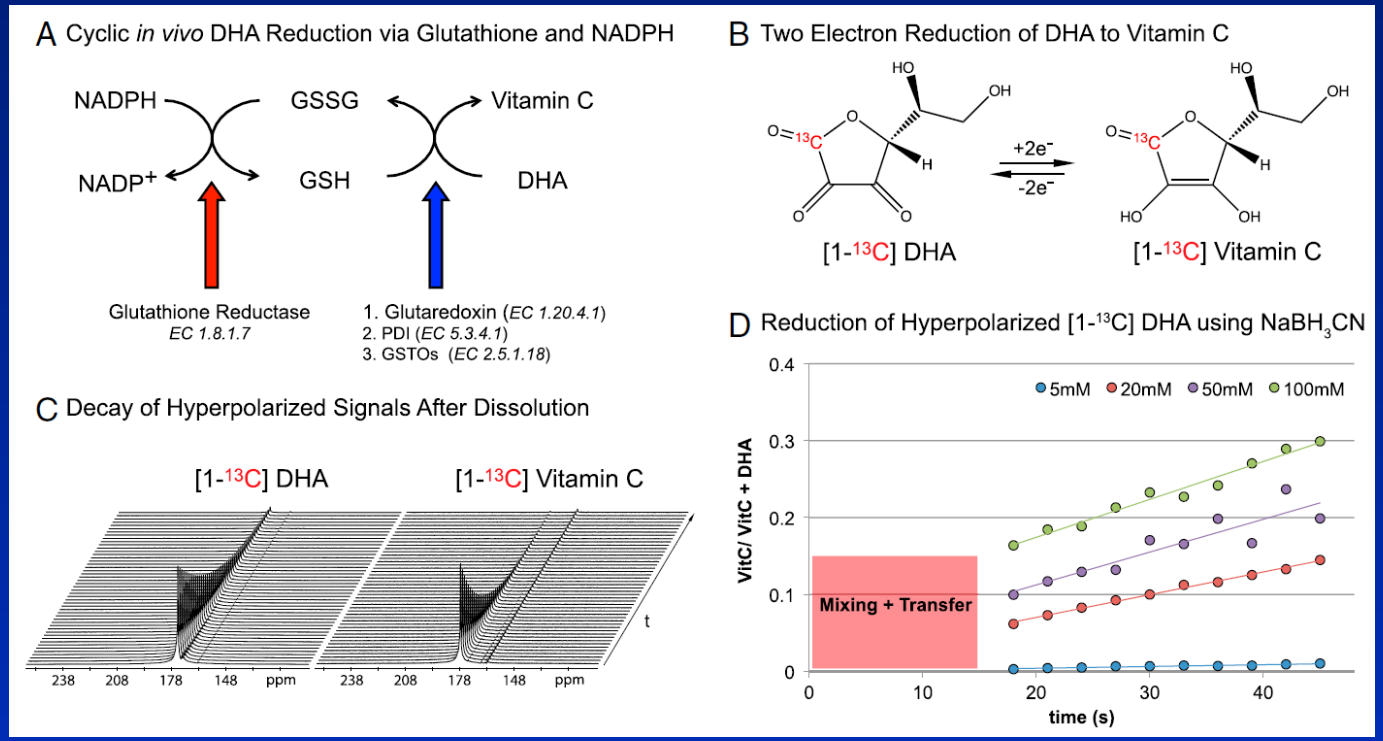


Nishihara, et al., Angew. Chemie (2016)

Redox Sensor For *in vivo* Metabolic Imaging

[1 – ^{13}C] Dehydroascorbate

- Measuring *in vivo* redox state with dehydroascorbate (DHA)
- DHA reduction by NADPH via glutathione
- Glutathione mediates response to ROS



Keshari, et al., PNAS (2011)

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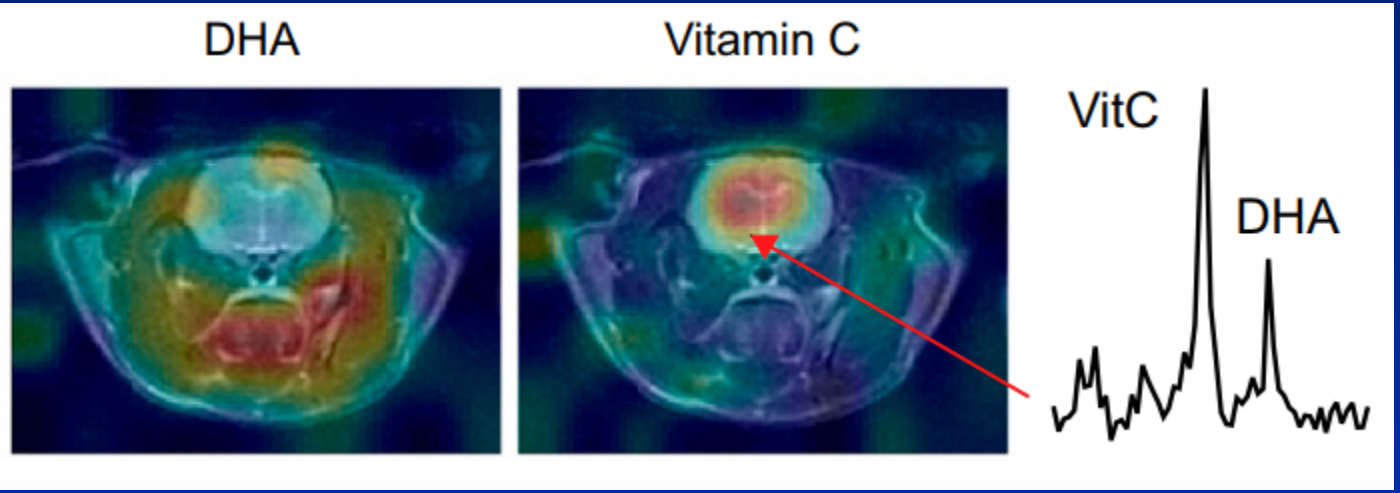


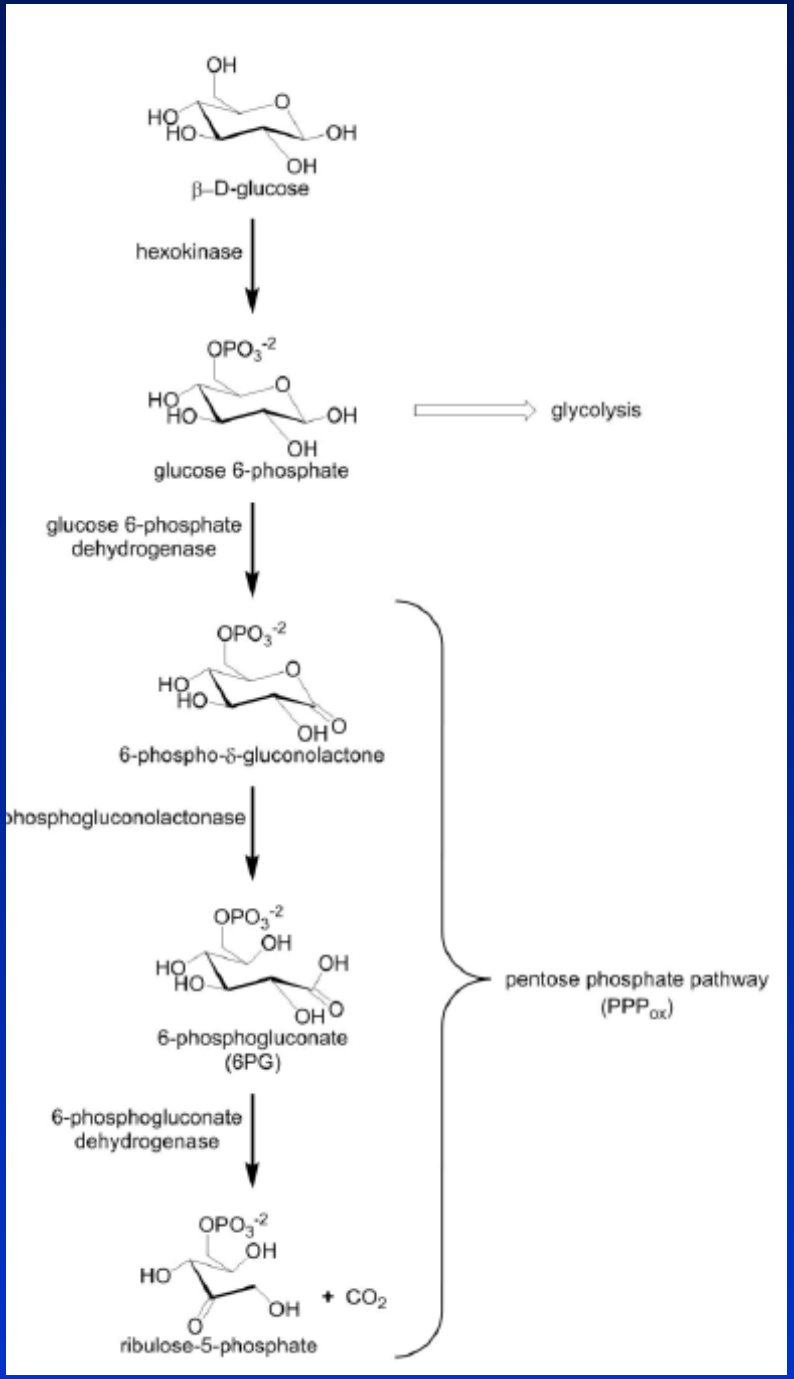
Keshari, et al., PNAS (2011)

Pentose Phosphate Pathway

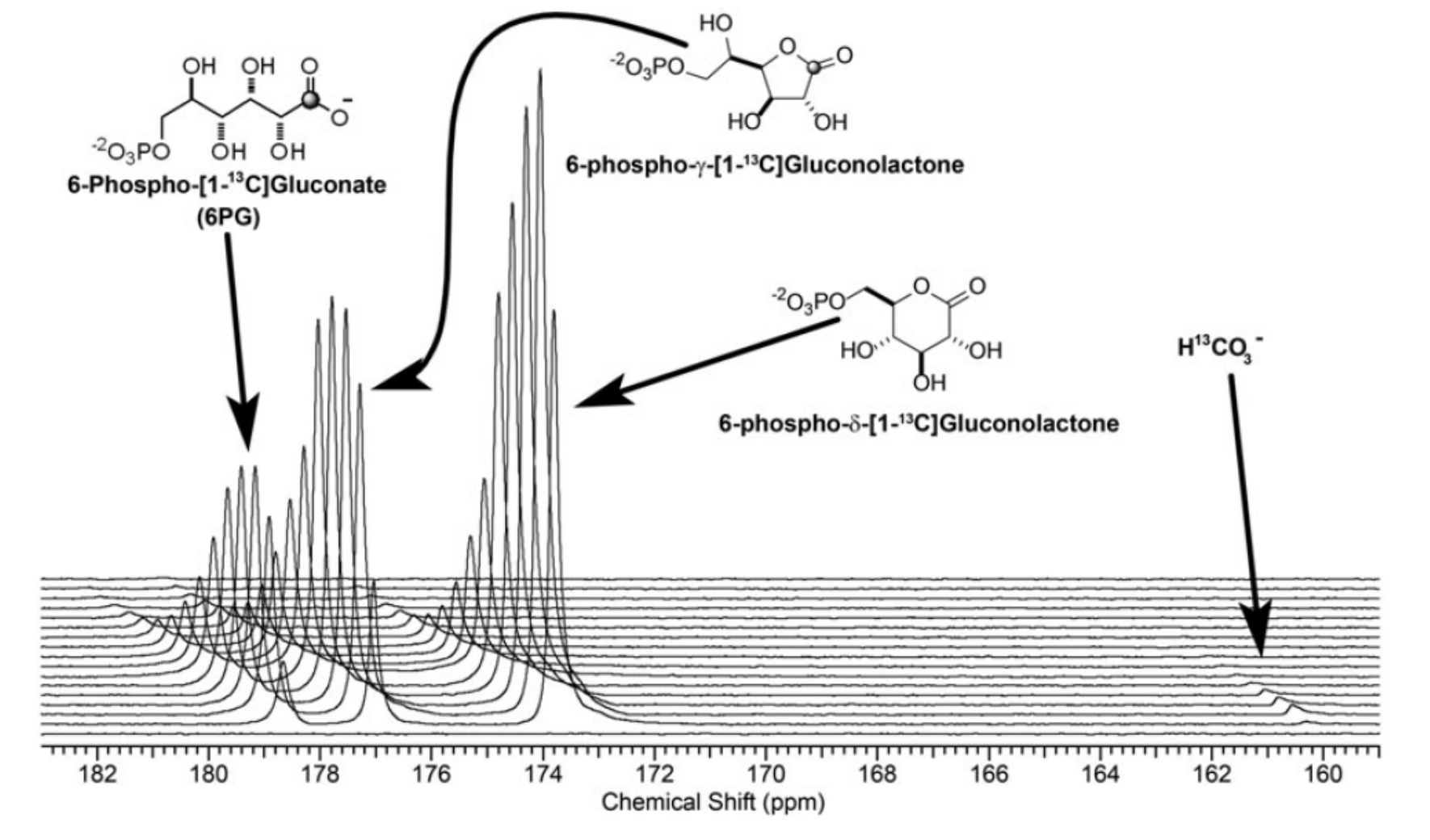
δ-[1 – ^{13}C] Gluconolactone

- Image carbon metabolism related to PPP flux
- Gluconolactone labeled at 1 position can only produce $^{13}CO_2$ via PPP

Moreno, et al., NMR Biomed (2017)



HP bicarb production in perfused liver



Moreno, et al., NMR Biomed (2017)

Arginine mediated T-cell proliferation

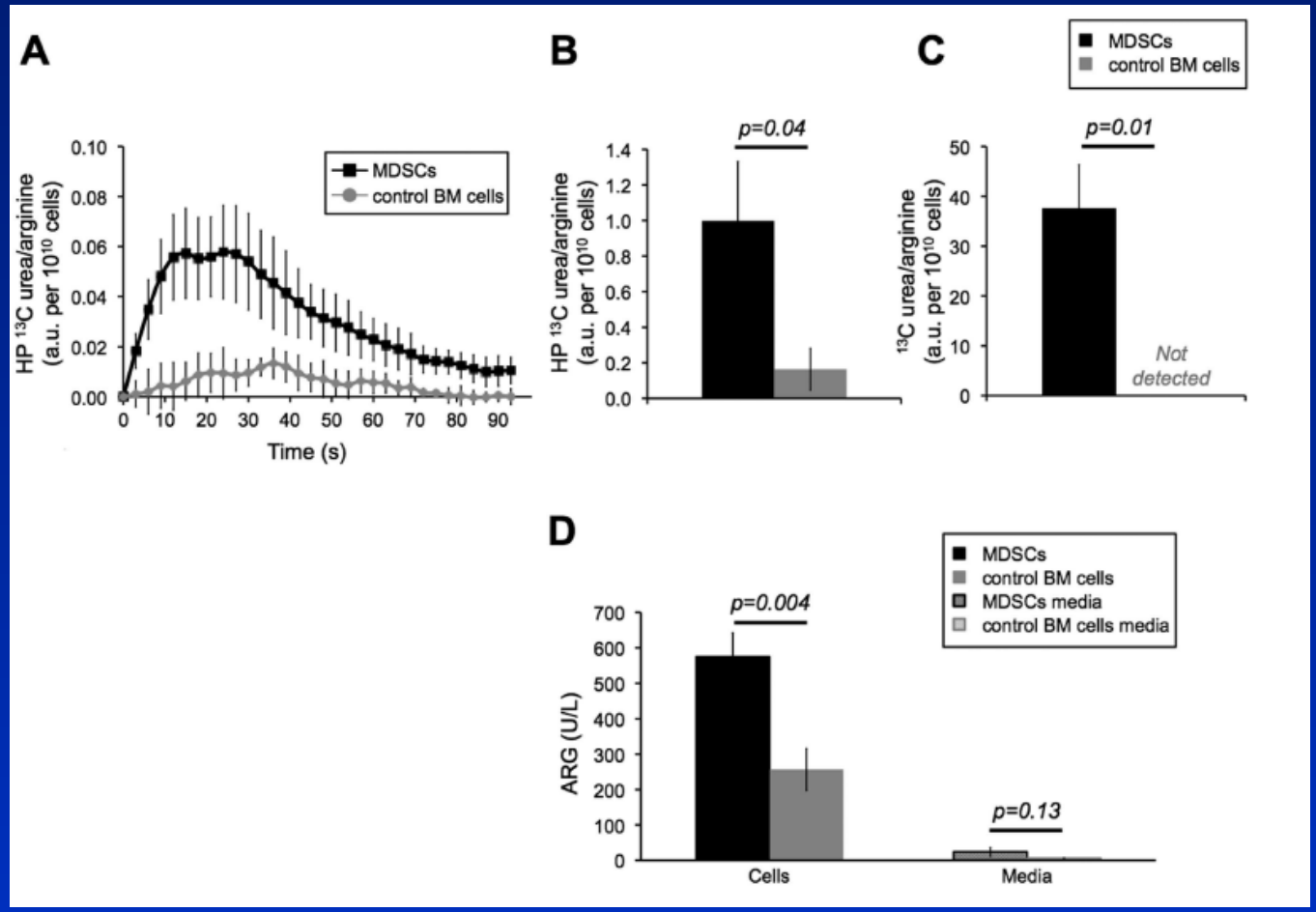
- Inflammatory myeloid-derived suppressor cells (MDSCs) promote tumor development

- T-cells proliferate in presence of arginine, which can be metabolized to ornithine and urea by arginase
  - Arginine disposal prevents T-cell inhibition of cancer growth

Arginine mediated T-cell proliferation

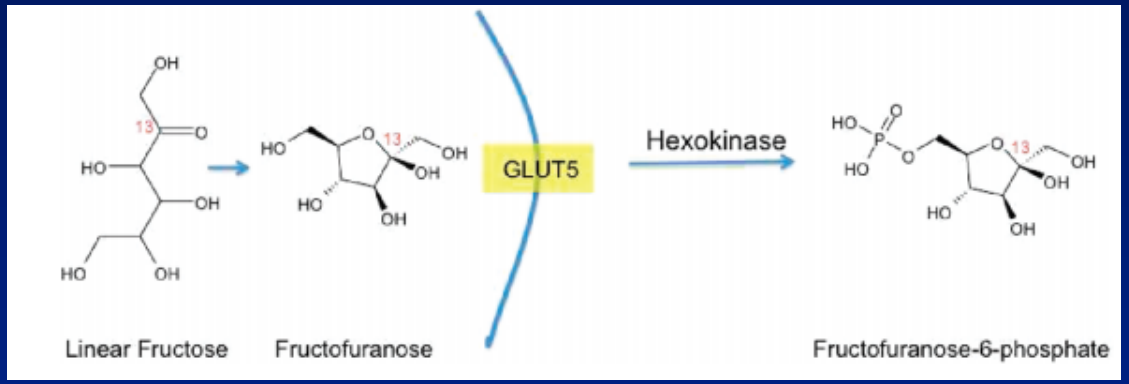
[6 – ^{13}C] Arginine

- Urea production is correlated with arginase concentration *in vivo*



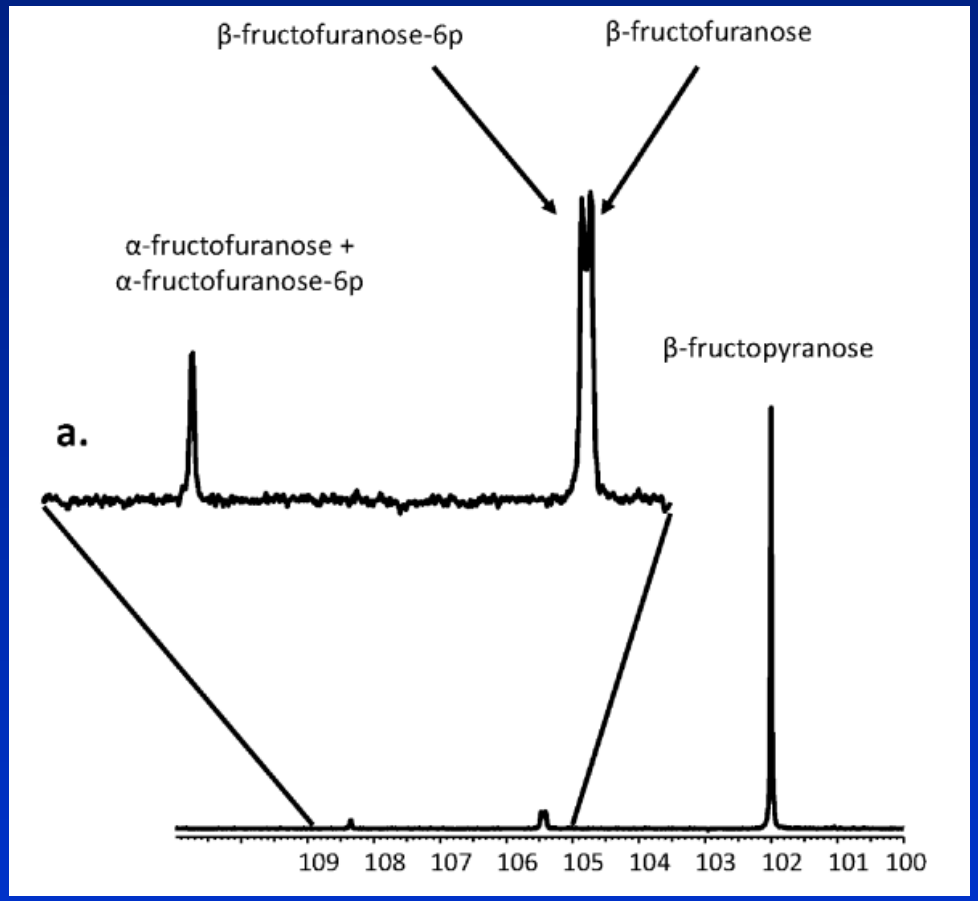
Najac, et al., Scientific Reports (2016)

Hexokinase Flux



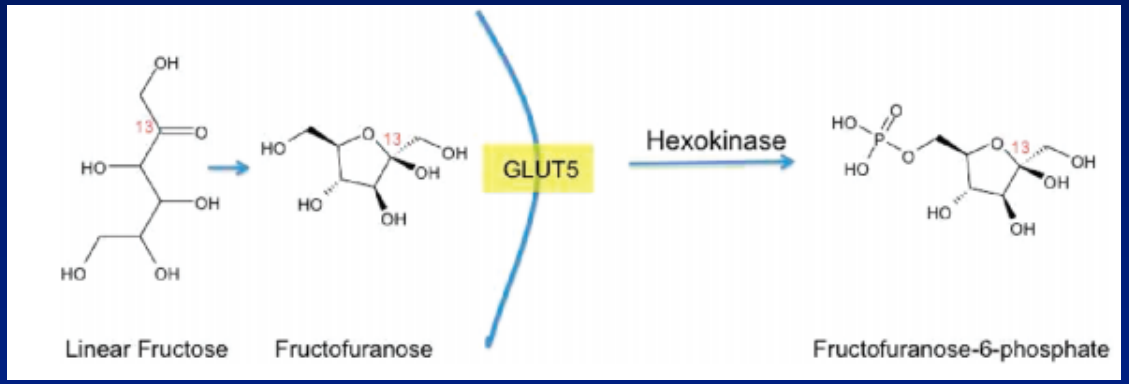
[2-^{13}C]Fructose

- Longer T_1 than glucose gives better prospects for *in vivo* use in mammals



Keshari, et al., JACS (2009)

Hexokinase Flux



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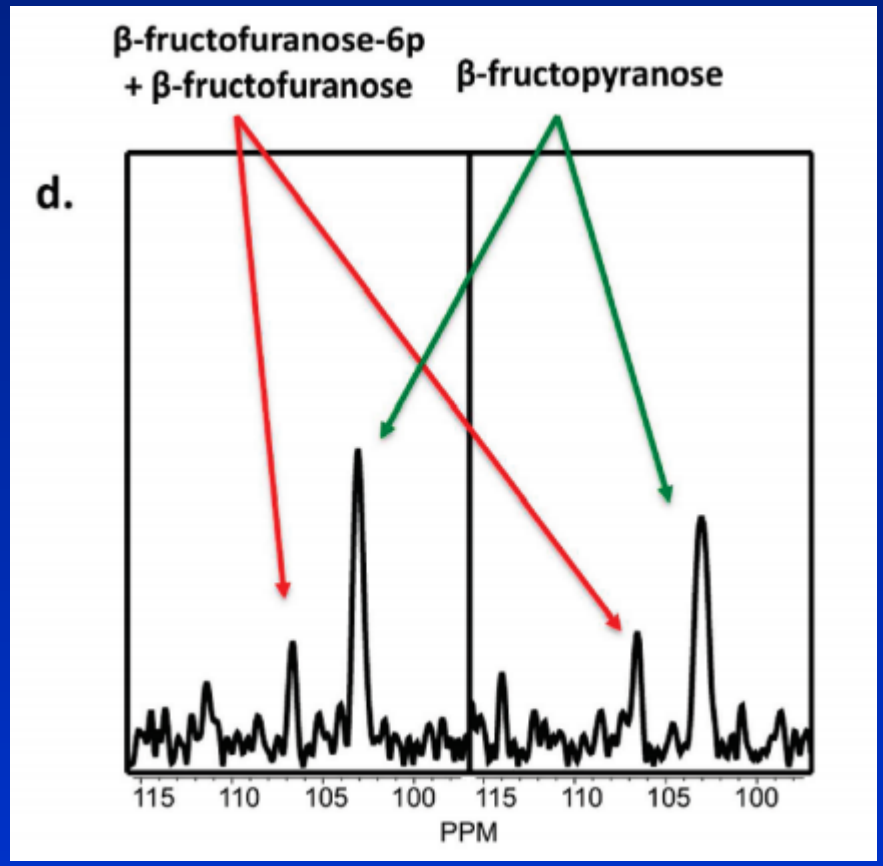
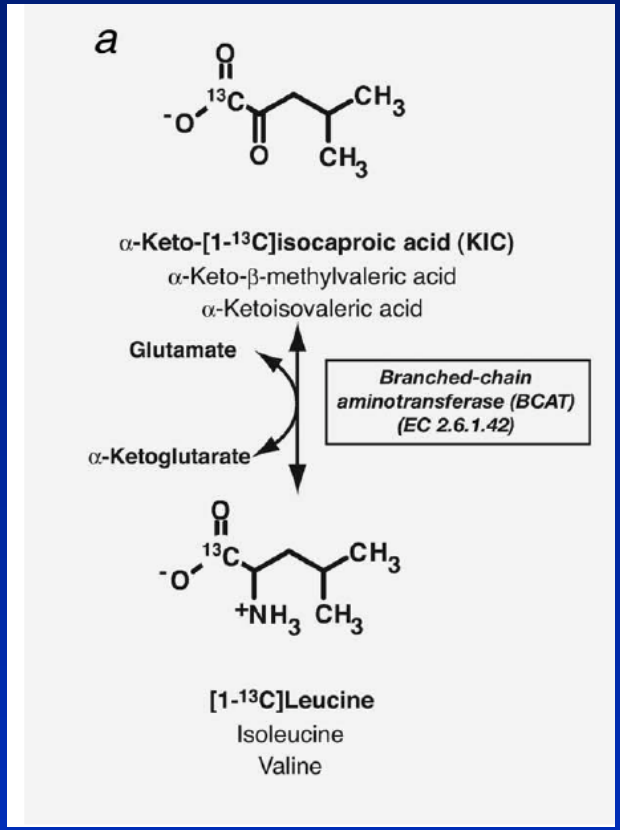


Keshari, et al., JACS (2009)

BCAA Metabolism

[1-^{13}C] Ketoisocaproate

- Branched-chain aminotransferase (BCAT) i.e. leucine metabolism
- BCAT expression found to be up-regulated in some cancers



Karlsson, et al., IJC (2009)

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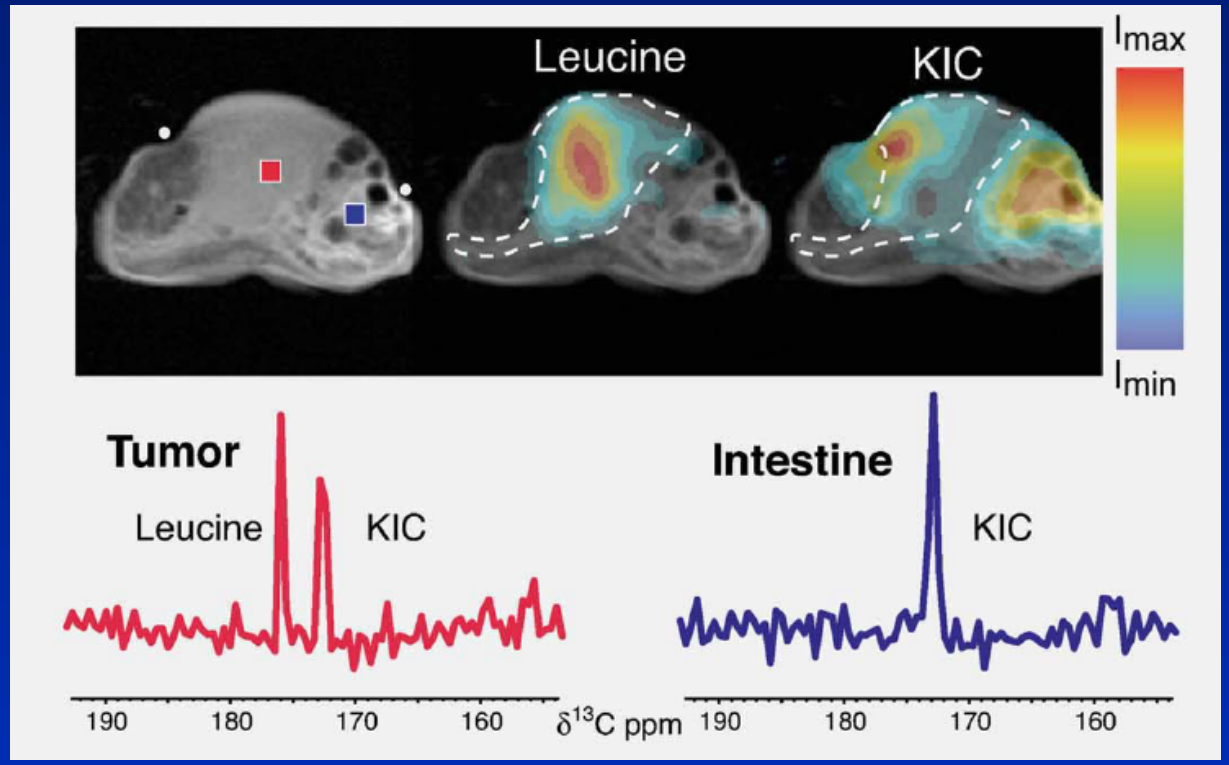
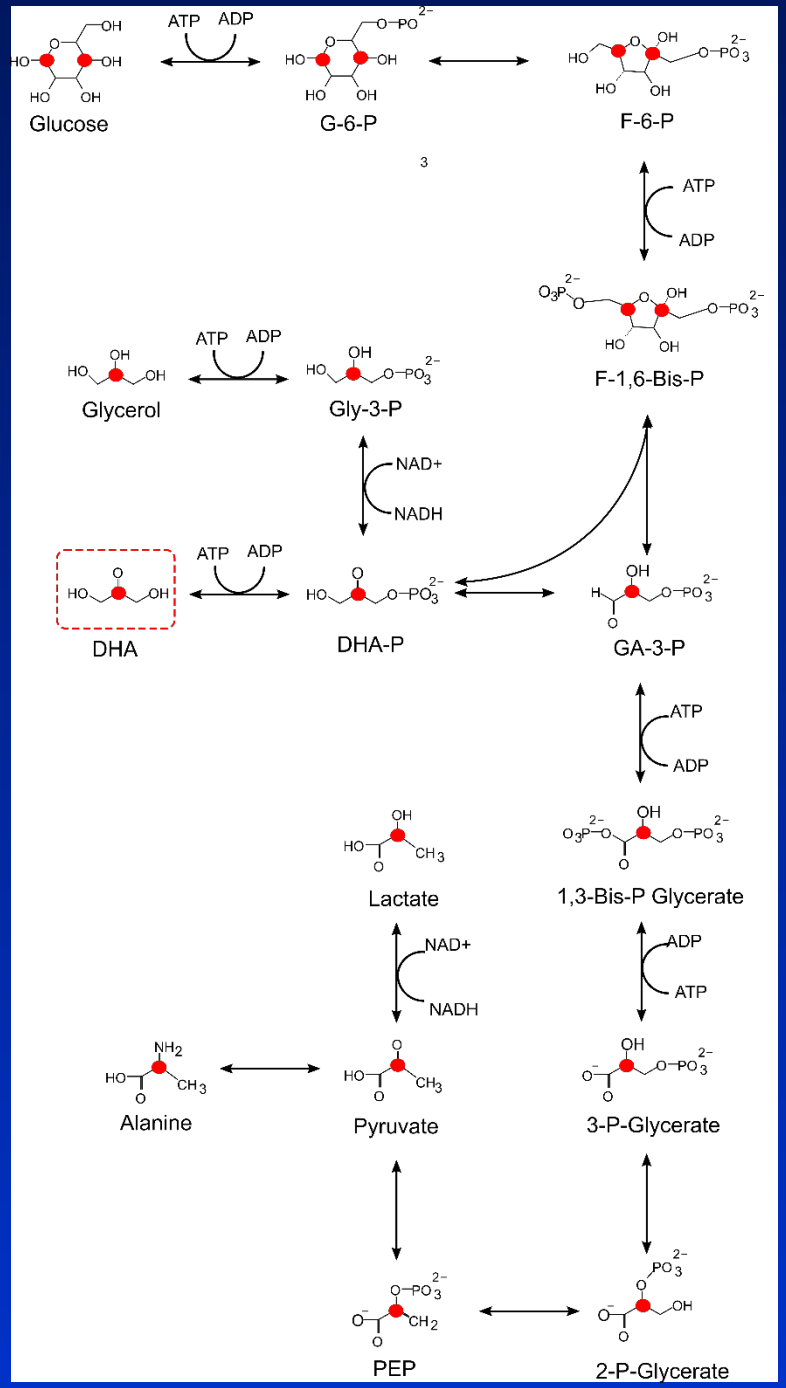


Karlsson, et al., IJC (2009)



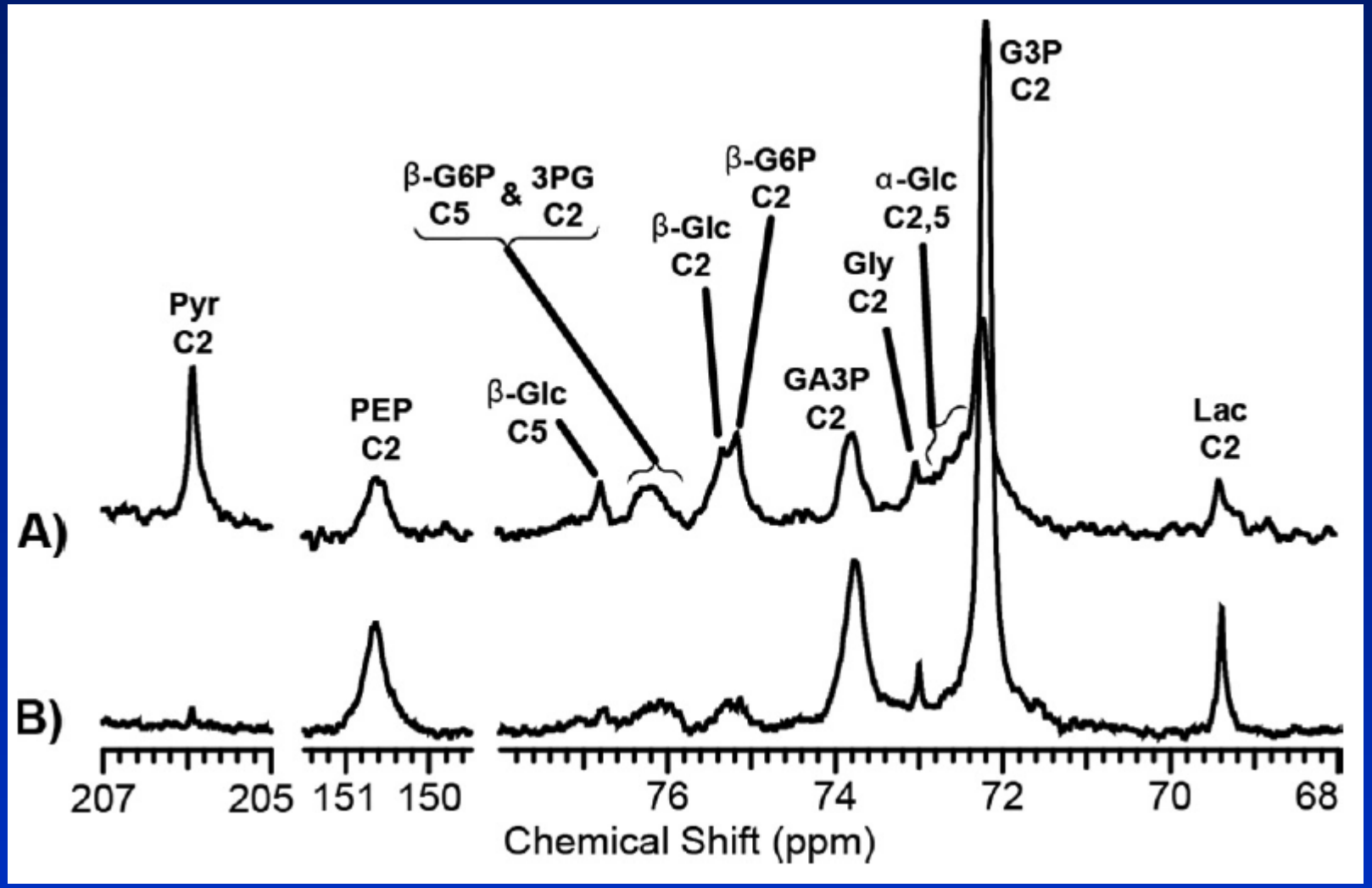
Hepatic Gluconeogenesis (GNG)

[2-^{13}C] Dihydroxyacetone

- Long T_1 (~40 s)
- Avidly consumed by liver
- Multiple metabolic fates

- First step of metabolism requires ATP for production of DHAP
- Glycerol production is redox dependent

[2-^{13}C]Dihydroxyacetone

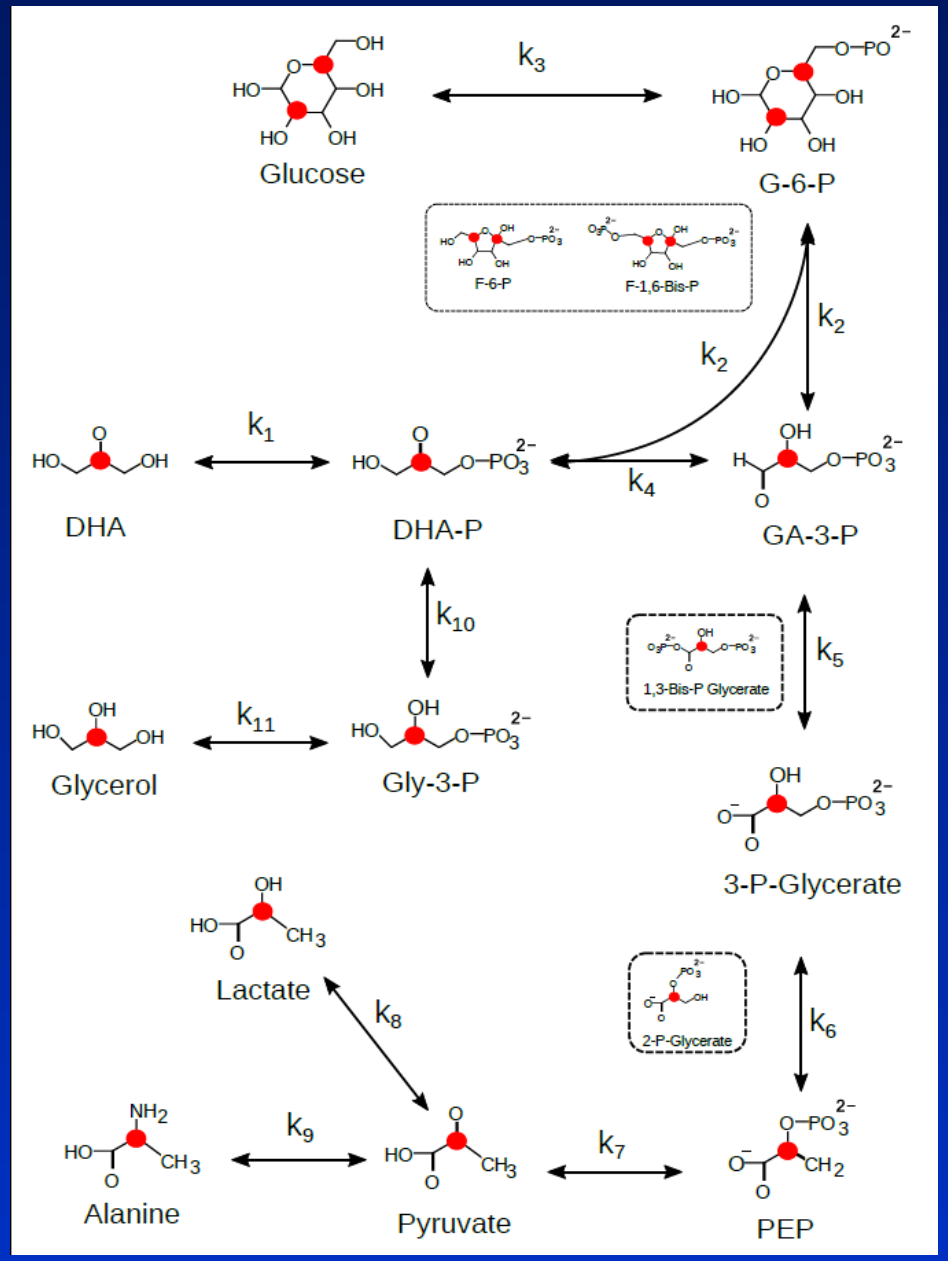


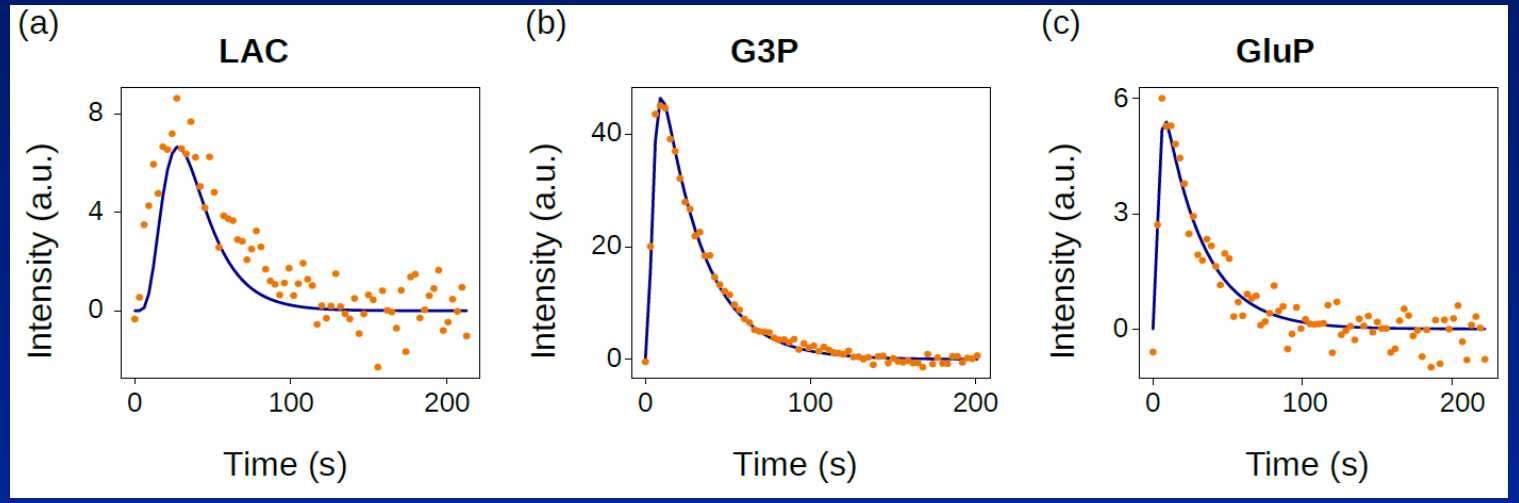
Gluconeogenic

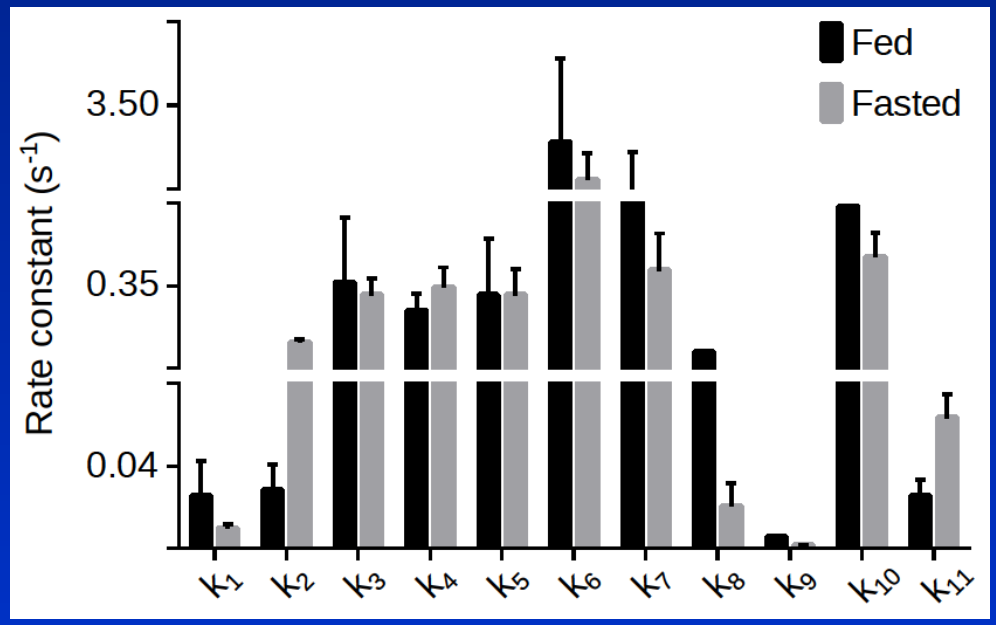
Glycogenolytic

Moreno, et al., JBC (2014)

Kinetics







(Manuscript under preparation)

Summary

- A new MR technique opens research
  - In basic biological sciences
  - *In vivo* diagnostic imaging
- Metabolic imaging with hyperpolarized nuclei is rapidly growing
- Clinical applications are on the immediate horizon
  - Several clinical trials are underway

Thank You