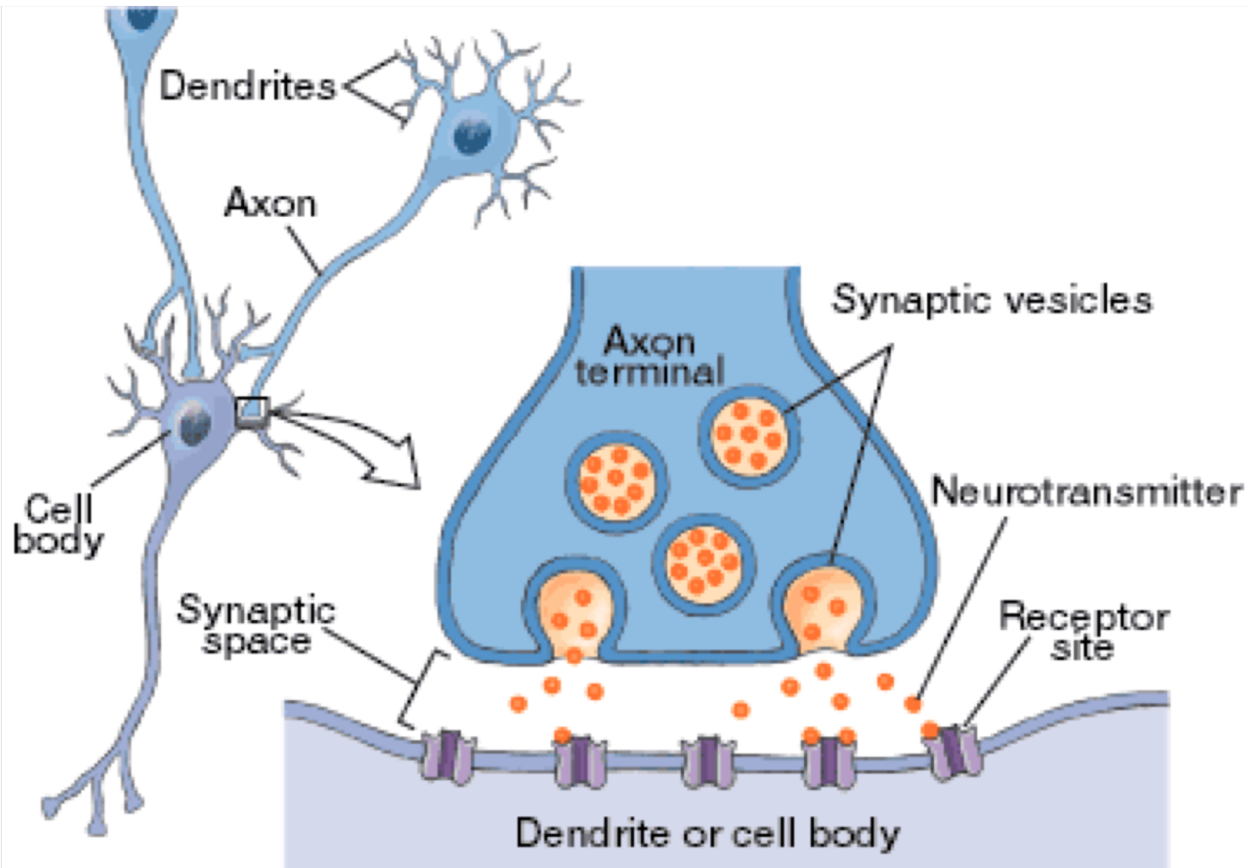
Rad226a - Lecture #17
Research topics in ^{13}C MRS

- Neurotransmission
- ^{13}C MRS
- Hyperpolarized ^{13}C MRS
- References
  - Rothman, et al., "^{13}C MRS studies of neuroenergetics and neurotransmitter cycling in humans", NMR Biomedicine, 2011 Oct;24(8):943-57.
  - Hurd et al., "Hyperpolarized ^{13}C Metabolic Imaging Using Dissolution Dynamic Nuclear Polarization", JMR 36:1314–1328 (2012) .

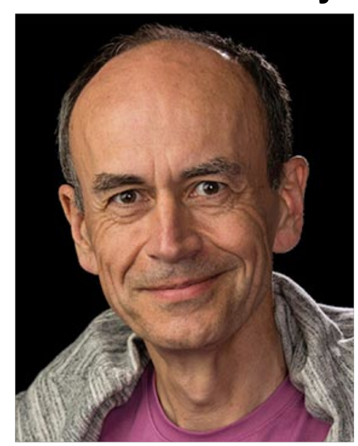
Neurons and Neurotransmission

- Neurons carry action potentials, but are not directly connected.
- Axon-dendrite connections rely on chemicals (neurotransmitters).
- Neurotransmitters can be excitatory or inhibitory.



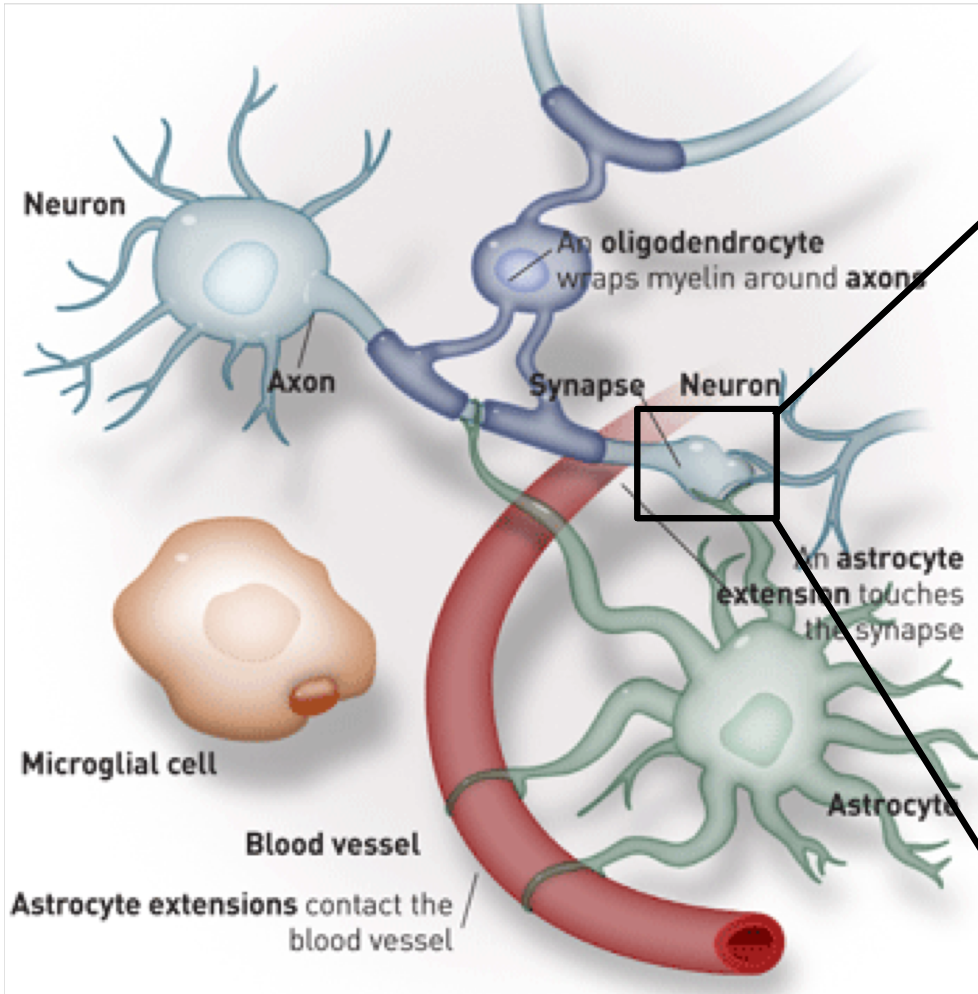
Glutamate is the major excitatory neurotransmitter in the human brain with >80% of synapses utilizing glutamate.

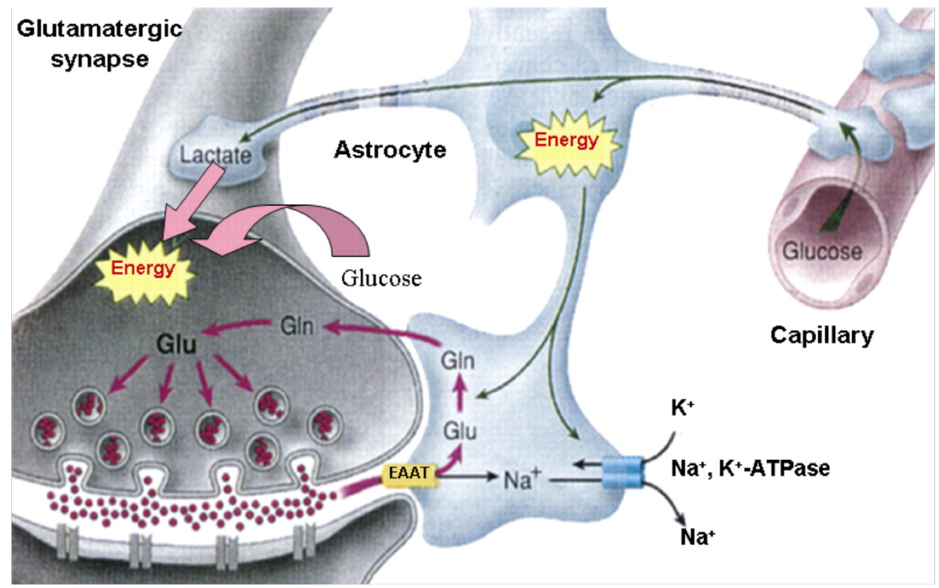
Thomas C. Südhof
Stanford University



The Nobel Prize in Physiology or Medicine 2013

The brain in more than just neurons..

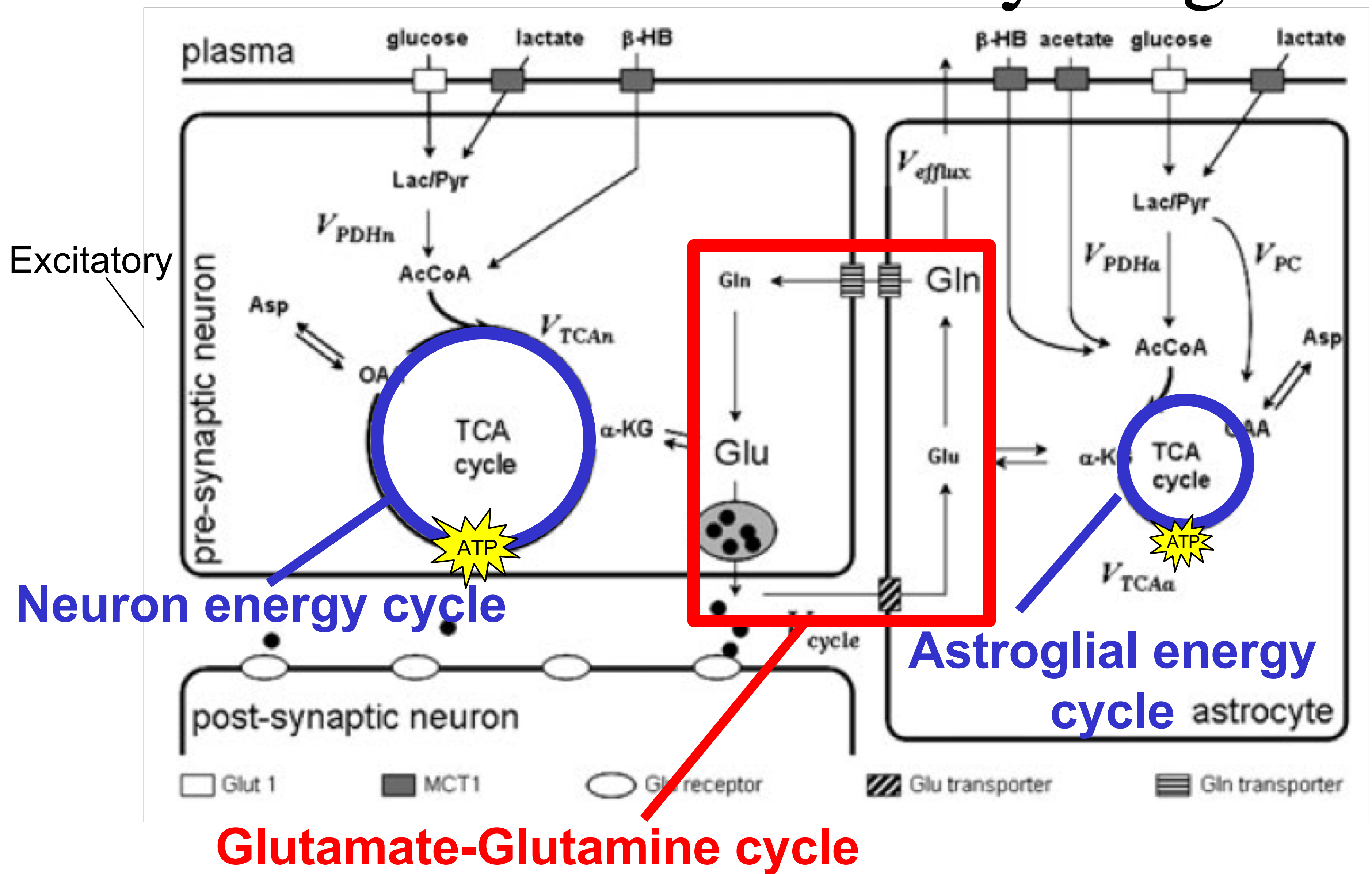




Complex metabolic interactions exist between glia and neurons.

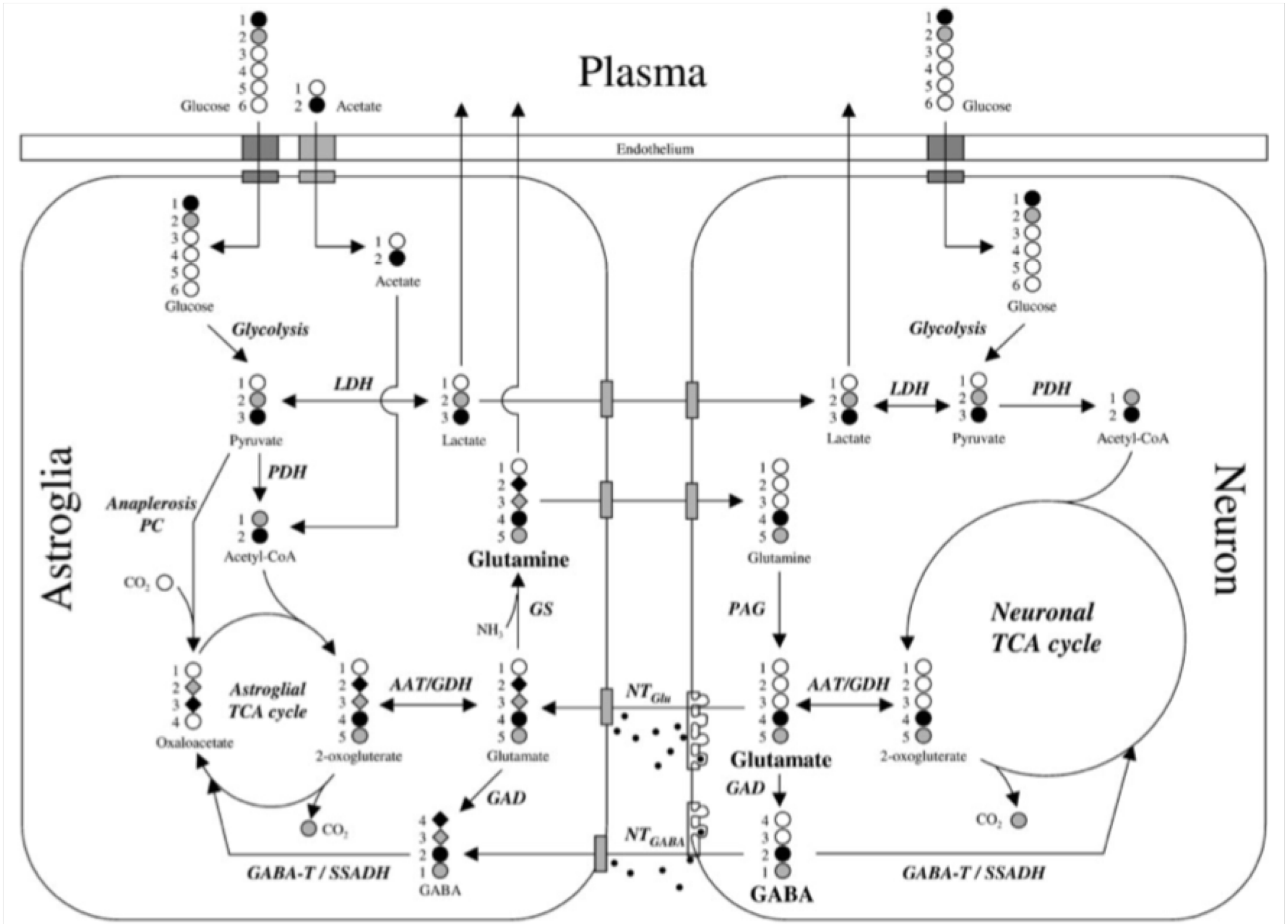
Energy metabolism is coupled with neurotransmission.

Neurotransmitter Cycling



Rothman, et al, NMR Biomedicine, 2011.

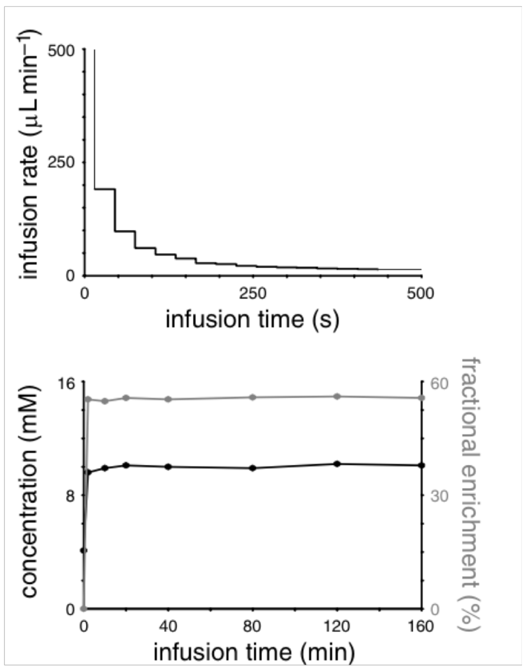
Probing Brain Function with Carbon



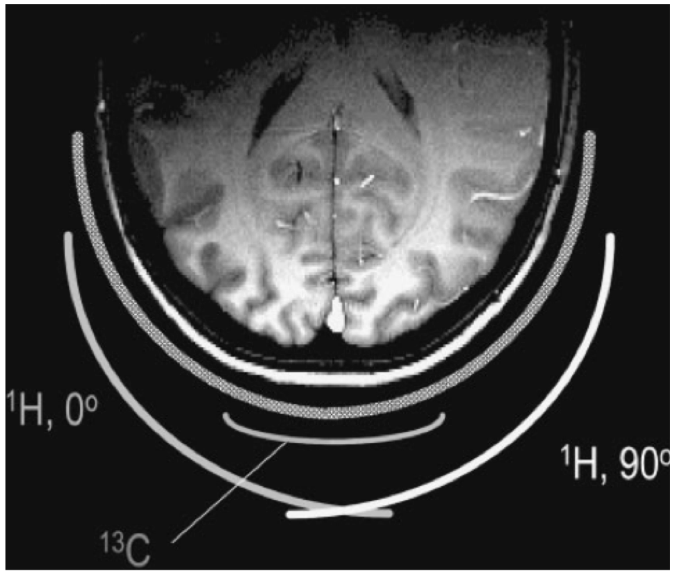
de Graaf, et al, NMR Biomedicine, 2003.

Probing Brain Function with ^{13}C MRS

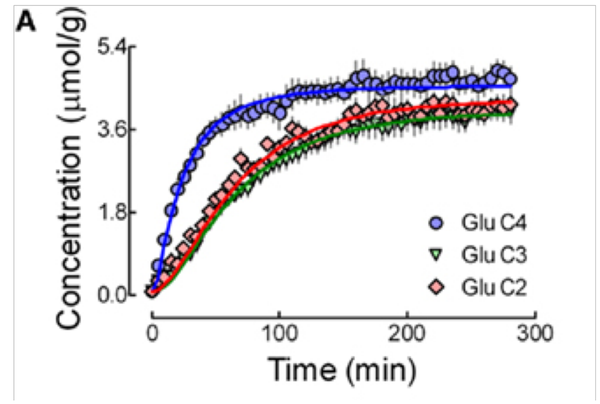
Infuse ^{13}C-glucose (or ^{13}C-acetate)

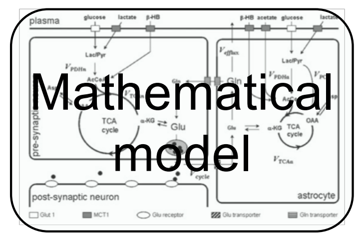


Collect spectra



Quantify spectra over time





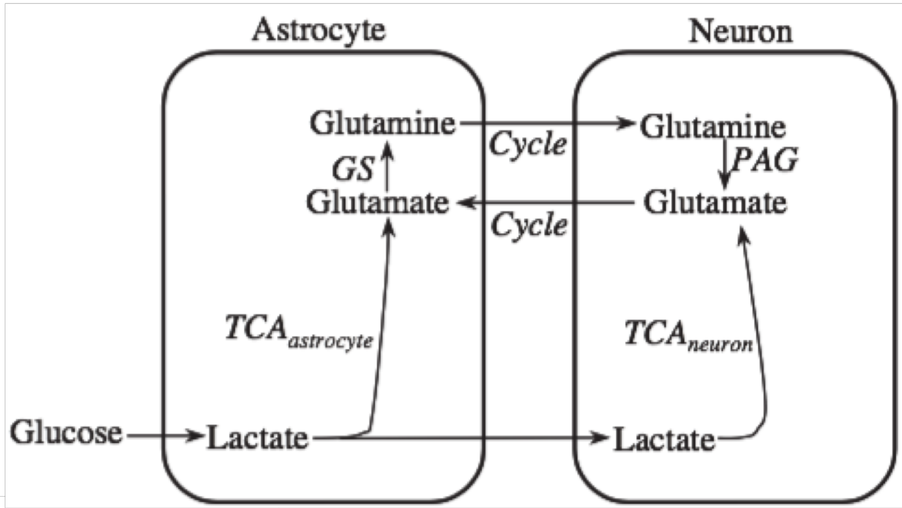
Mathematical model

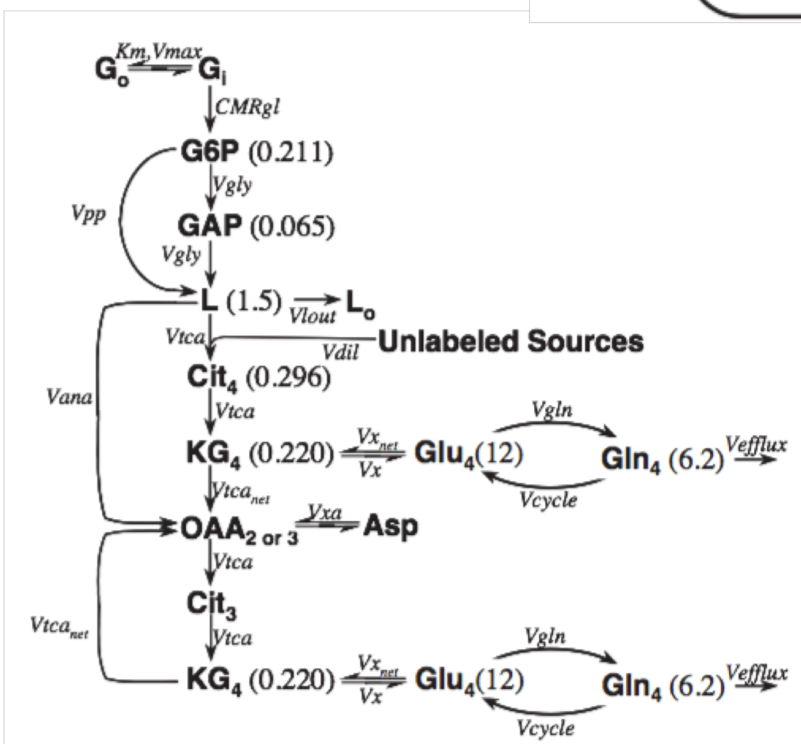
V_{TCAn} = neuron TCA flux

V_{TCAg} = glial TCA flux

V_{NT} = Glu/Gln neurotransmitter flux

Metabolic Modeling





$$\frac{d\mathrm{Glu}_4}{dt} = \frac{L_3}{L}\mathrm{Vgly} + \frac{\mathrm{Gln}_4}{\mathrm{Gln}}\mathrm{Vcycle} + (0)\mathrm{Vdil} - \frac{\mathrm{Glu}_4}{\mathrm{Gln}}(\mathrm{Vtca} + \mathrm{Vgln}),$$

$$\frac{d\mathrm{Gln}_4}{dt} = \frac{\mathrm{Glu}_4}{\mathrm{Glu}}\mathrm{Vgln} - \frac{\mathrm{Gln}_4}{\mathrm{Gln}}(\mathrm{Vcycle} + \mathrm{Vefflux}),$$

$$\frac{d\mathrm{Glu}_3}{dt} = \frac{1}{2}\frac{L_3}{L}\mathrm{Vana} + \frac{1}{2}(0)\mathrm{Vana} + \frac{1\mathrm{Glu}_4}{2\,\mathrm{Glu}}\mathrm{Vtca}_{\mathrm{net}} + \frac{1\mathrm{Glu}_3}{2\,\mathrm{Glu}}\mathrm{Vtca}_{\mathrm{net}} + \frac{\mathrm{Gln}_3}{\mathrm{Gln}}\mathrm{Vcycle} - \frac{\mathrm{Glu}_3}{\mathrm{Glu}}(\mathrm{Vtca} + \mathrm{Vgln}),$$

$$\frac{d\mathrm{Gln}_3}{dt} = \frac{\mathrm{Glu}_3}{\mathrm{Glu}}\mathrm{Vgln} - \frac{\mathrm{Gln}_3}{\mathrm{Gln}}(\mathrm{Vcycle} + \mathrm{Vefflux}),$$

$$\frac{d\mathrm{Glu}_{3,4}}{dt} = \frac{L_3}{L}\frac{\mathrm{Glu}_3}{\mathrm{Glu}}\mathrm{Vtca} + \frac{\mathrm{Gln}_{3,4}}{\mathrm{Gln}}\mathrm{Vcycle} - \frac{\mathrm{Glu}_{3,4}}{\mathrm{Glu}}(\mathrm{Vtca} + \mathrm{Vgln}),$$

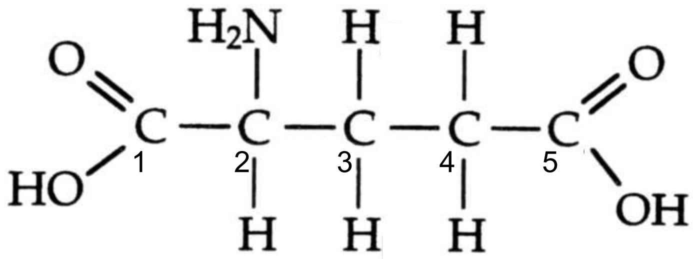
$$\frac{d\mathrm{Gln}_{3,4}}{dt} = \frac{\mathrm{Glu}_{3,4}}{\mathrm{Glu}}\mathrm{Vgln} - \frac{\mathrm{Gln}_{3,4}}{\mathrm{Gln}}(\mathrm{Vcycle} + \mathrm{Vefflux}).$$

${}^{N}V_{TCA}$, rate of neuronal TCA cycle
${}^{A}V_{TCA}$, rat of astroglial TCA cycle
V_{cycle}, rate of Glu-Gln cycle;

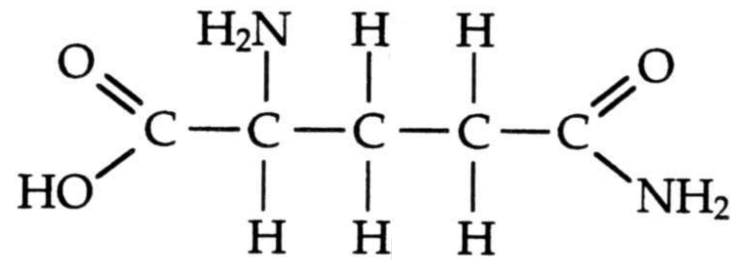
Mason, et al., Metabolic Engineering 6, 75-84, 2004

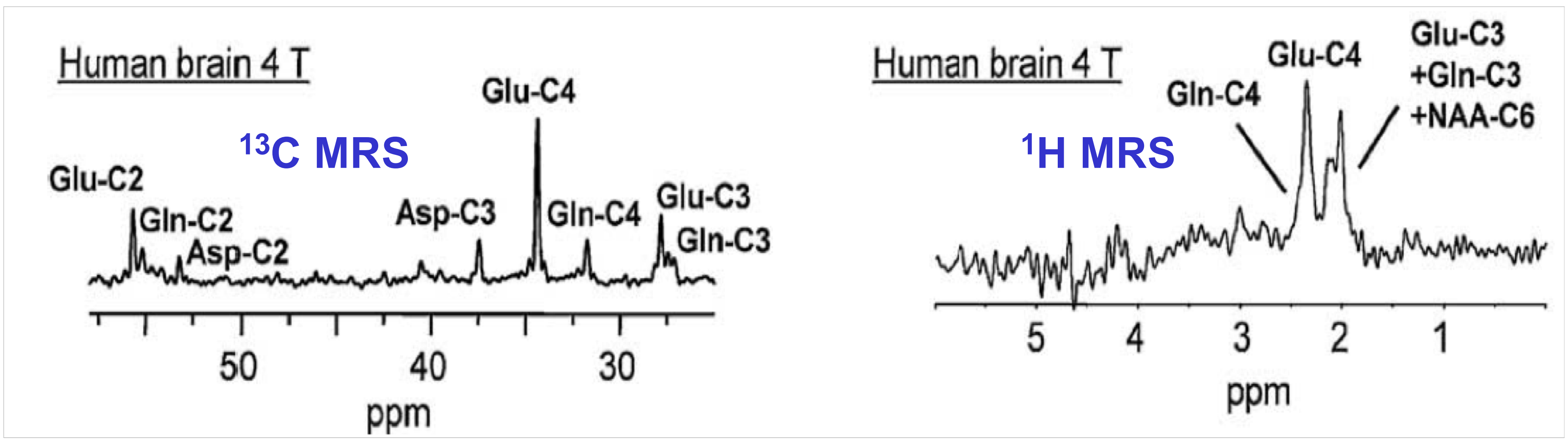
Probing Brain Function with ^{13}C MRS

Glutamate



Glutamine



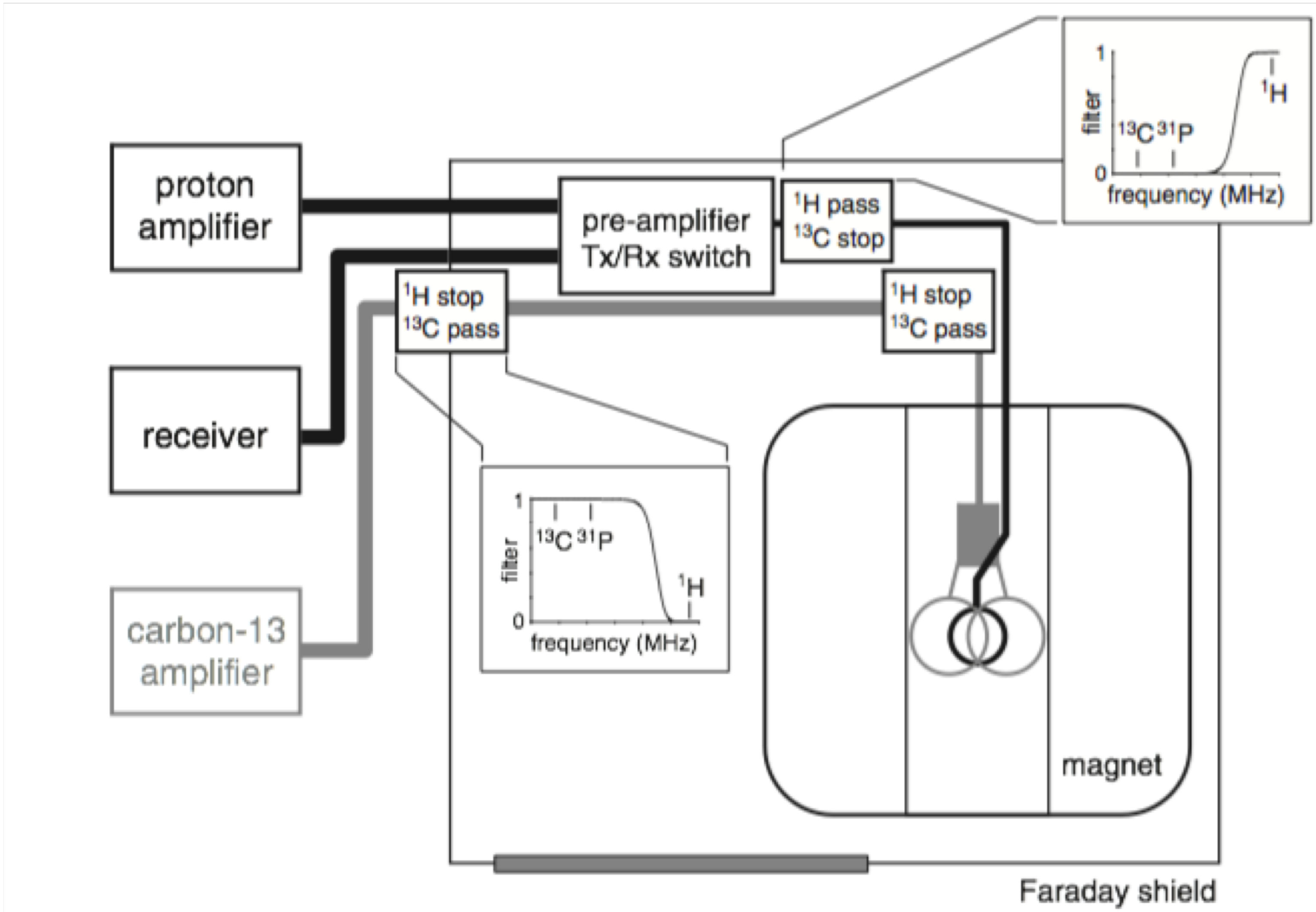


~45 cc, 10 min acq

~10 cc, 10 min acq

^{13}C and ^{1}H spectra from human visual cortex during an infusion of [1-^{13}C]glucose.

Hardware



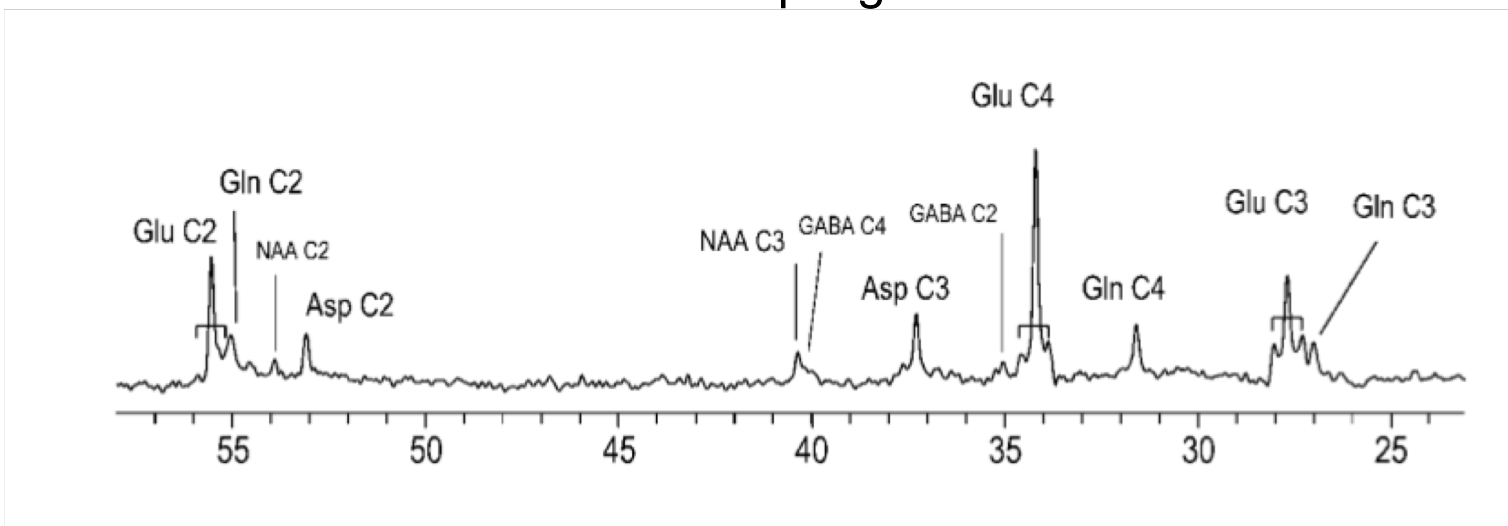
de Graaf, et al, NMR Biomed 2011

Multinuclear Pulse Sequences

Direct Detection

For in vivo ^{13}C-infusion brain studies (e.g. labeled glucose or acetate) …

- ^{13}C spectrum: low sensitivity, excellent peak discrimination.
- Simple ^{13}C excitation and detection is rarely used do to poor sensitivity
- Polarization transfer is commonly used: INEPT, DEPT
- Almost all methods use decoupling.



4T ^{13}C spectrum obtained from a 45 ml volume in the human visual cortex during an infusion of 67%-enriched [1-^{13}C]glucose. (DEPT sequence).

Gruetter, et al., NMR Biomed 2003.

Polarization Transfer

- MRI sensitivity given by

polarization; magnetic moment; ω_0; noise

$$SNR \propto \frac{\left(\frac{\gamma \hbar B_0}{2kT}\right)\left(\frac{\gamma \hbar}{2}\right)\left(\gamma B_0\right)}{\gamma B_0} = \frac{\gamma^2 \hbar^2 B_0}{4kT}$$

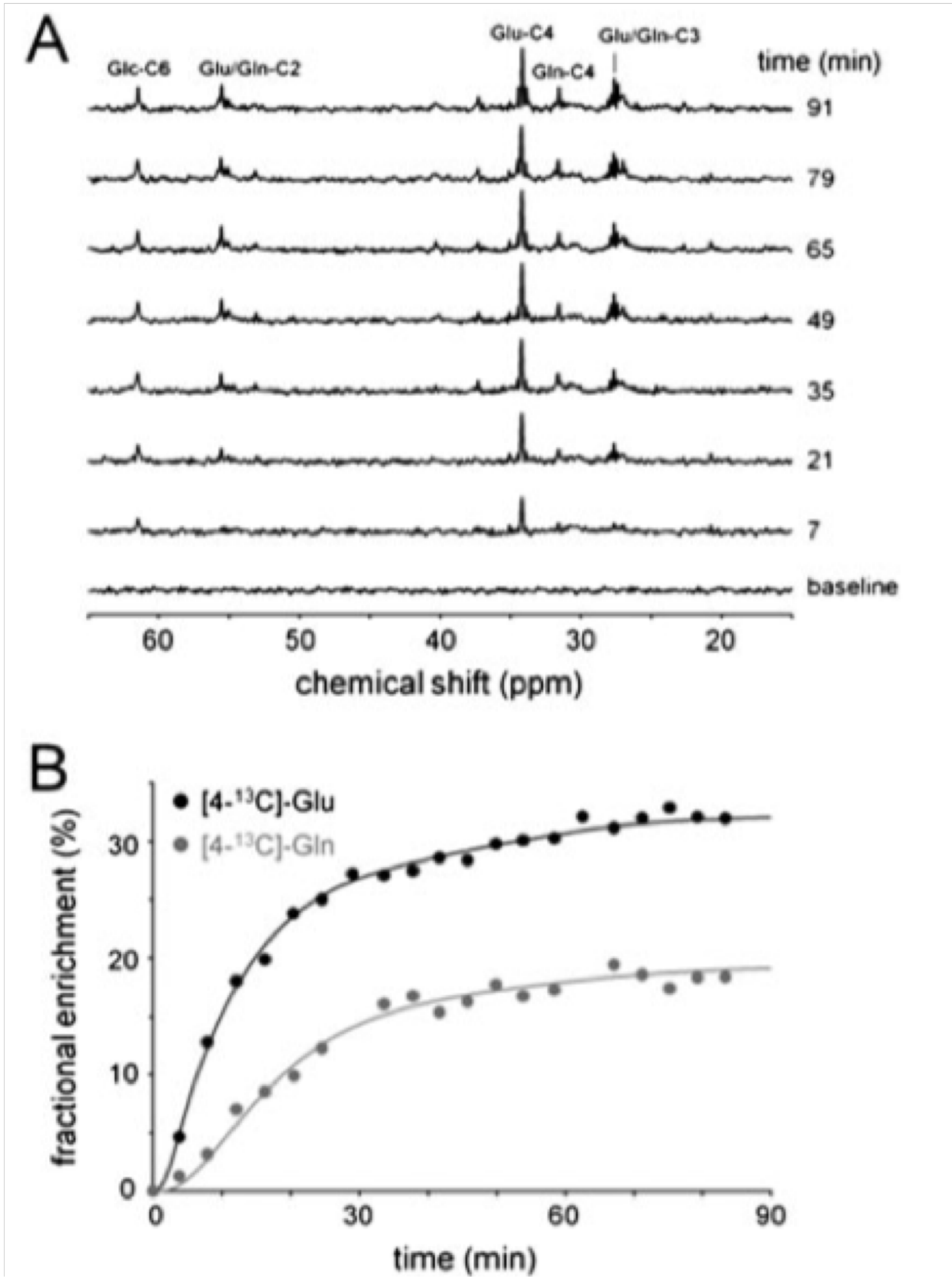
- In polarization transfer, we seek to exploit ^{1}H-^{13}C J coupling to find a pulse sequence with sensitivity given by…

"proton" polarization; "carbon" detecton

$$SNR \propto \frac{\left(\frac{\gamma_H \hbar B_0}{2kT}\right)\left(\frac{\gamma_C \hbar}{2}\right)\left(\gamma_C B_0\right)}{\gamma_C B_0} = \frac{\gamma_H \gamma_C \hbar^2 B_0}{4kT}$$

- Given $\gamma_H \approx 4\gamma_C$, this will yield a 4x sensitivity increase!

Methods: INEPT

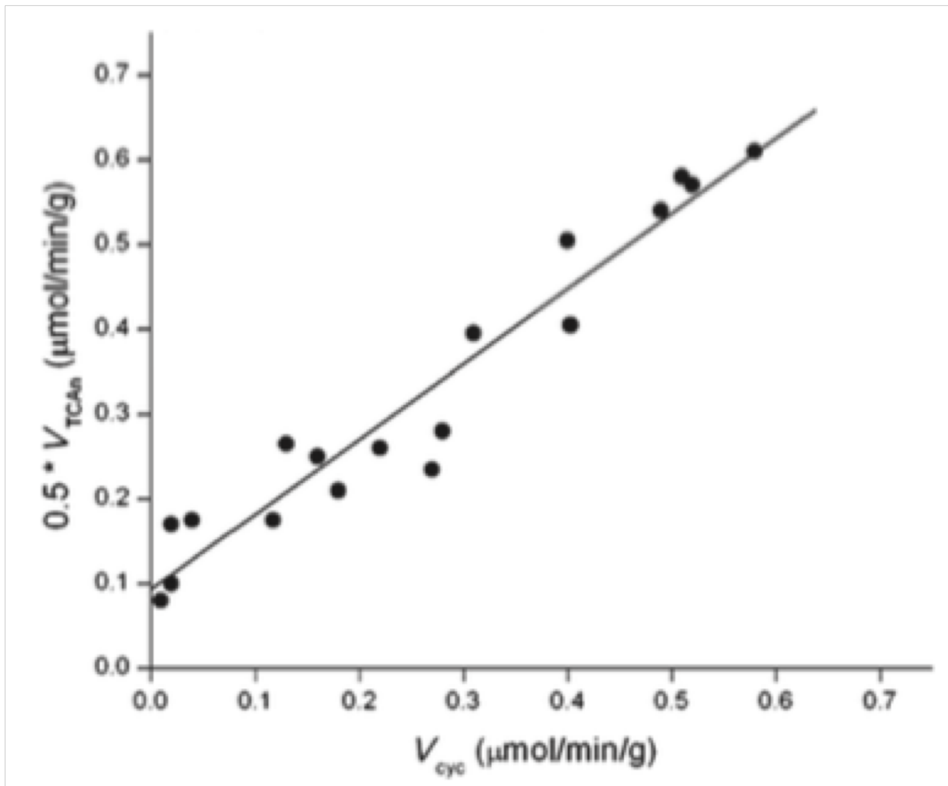


Human studies, occipital lobe, 4T

de Graaf, et al, NMR Biomed 2011

A Key Result…

- Direct linkage between neuroenergetics and neurotransmitter flux
- 1:1 relationship between neuronal TCA and Glu/Gln cycling rates



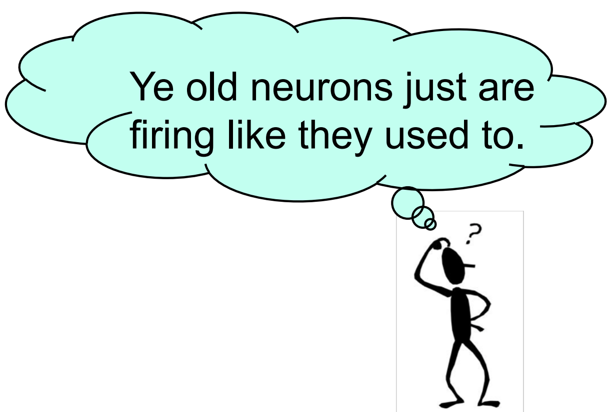
Rat cortex

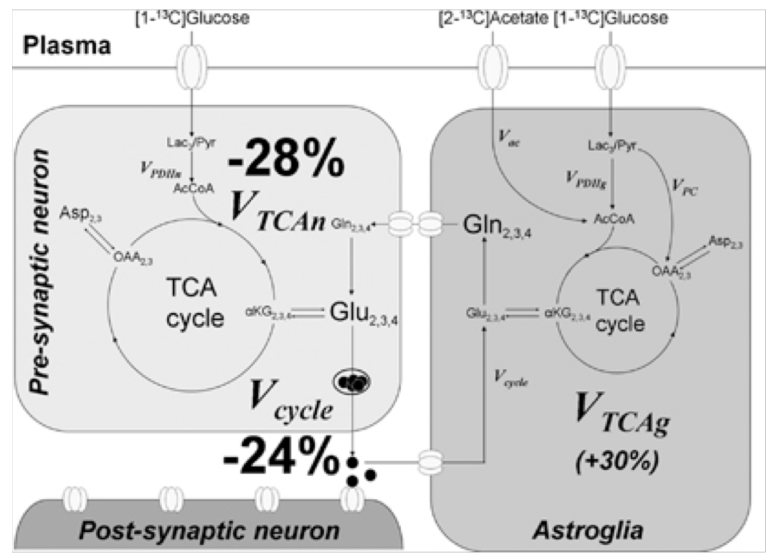
Rothman, et al, NMR Biomed 2011

Applications

- ^{13}C MRS provides the only nonivasive measurements of neurotransmitter cycling and cell-specific neuroenergetics.
- Major contributions to understanding…
  - Metabolic coupling between neurons and glia.
  - High neuronal activity of resting brain.
  - Alternations in neurological and psychiatric disease.
- Pathologies include: depression, drug addiction, epilepsy. metabolic disorders, hepatic encephalopathy, and neurodegenerative disorders.

Example: Aging





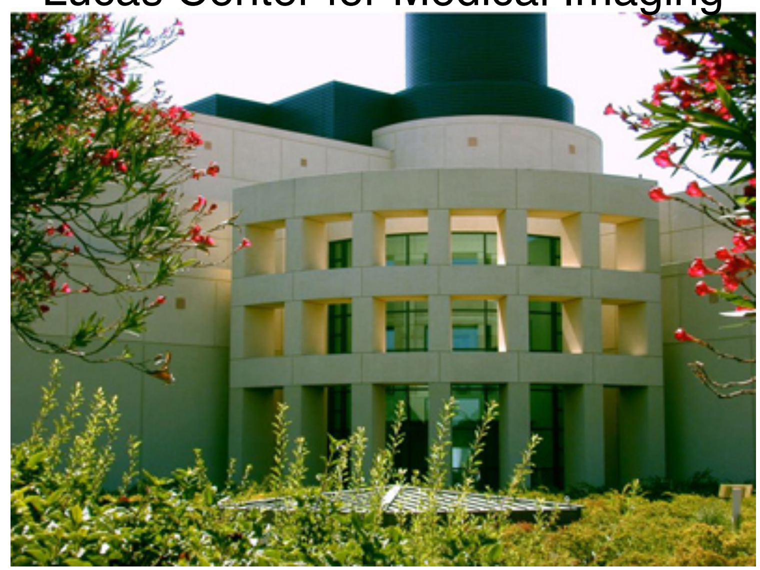
Boumezbeur, et al, Altered brain mitochondrial metabolism in healthy aging as assessed by in vivo magnetic resonance spectroscopy JCBFM, (2010) 30, 211–21

Hyperpolarized ^{13}C MR

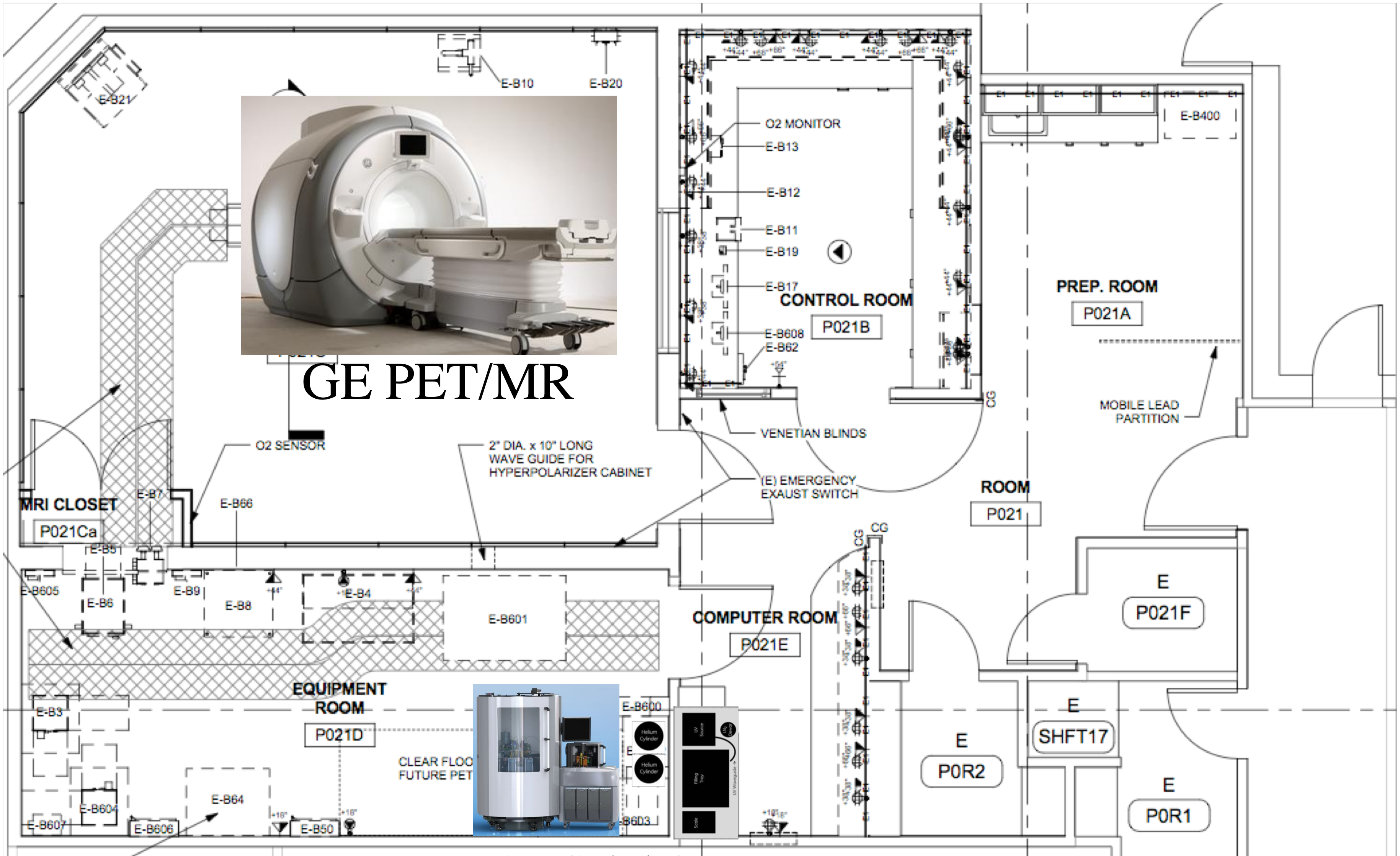
A Complementary Method to PET for Imaging In Vivo Metabolism

Daniel M. Spielman, Ph.D.
Dept. of Radiology
Stanford University
Email: spielman@stanford.edu

Lucas Center for Medical Imaging

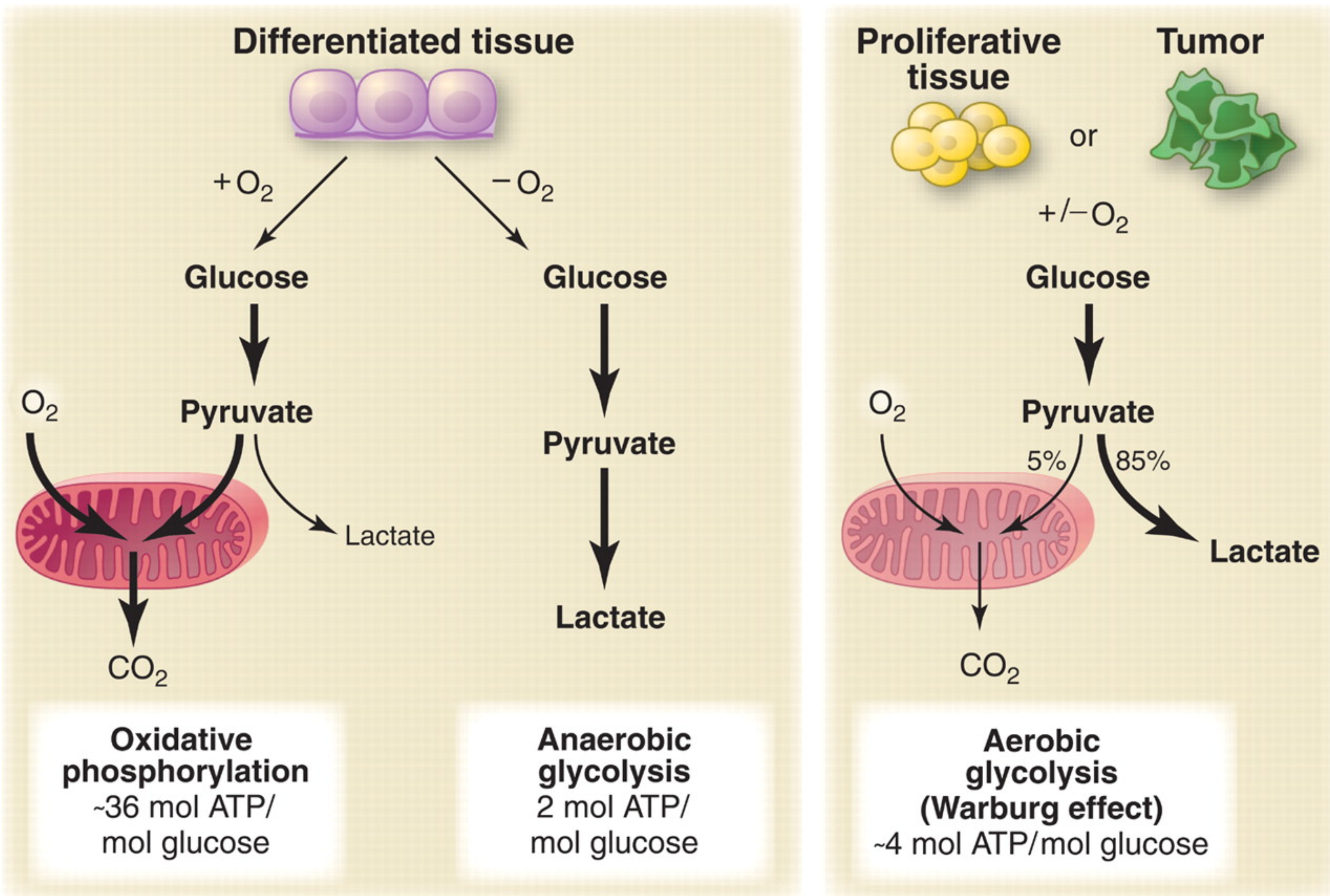


PET/MR/^{13}C Center for Metabolic Imaging



GE Spinlab

Cancer and the Warburg Effect



Energy production while preserving biomass

Metabolic Therapy Challenges

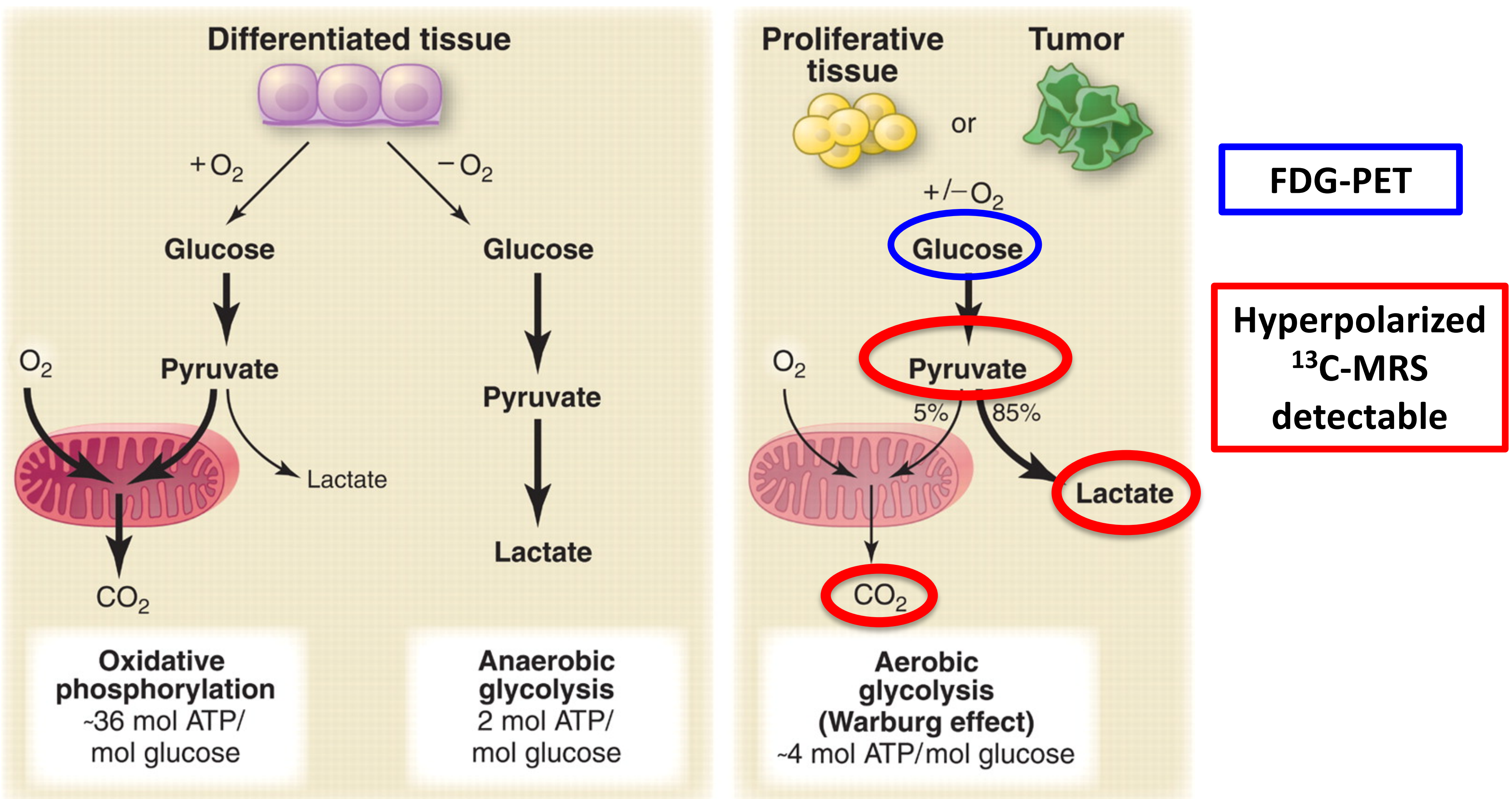
- Metabolic reprogramming represents a shifted balance towards glycolysis (GLY) from oxidative phosphorylation (OXPHOS)
- Malignant glioma are ideal candidates
  - Highly resistant to conventional treatments
  - Robustly manifest metabolic reprogramming
- Critical clinical obstacle: robust measurement of response

Proposed metric: $$\text{Metabolic therapy index} = \frac{[\text{Glycolysis}]}{[\text{OXPHOS}]}$$

How can we measure GLY/OXPHOS in vivo?

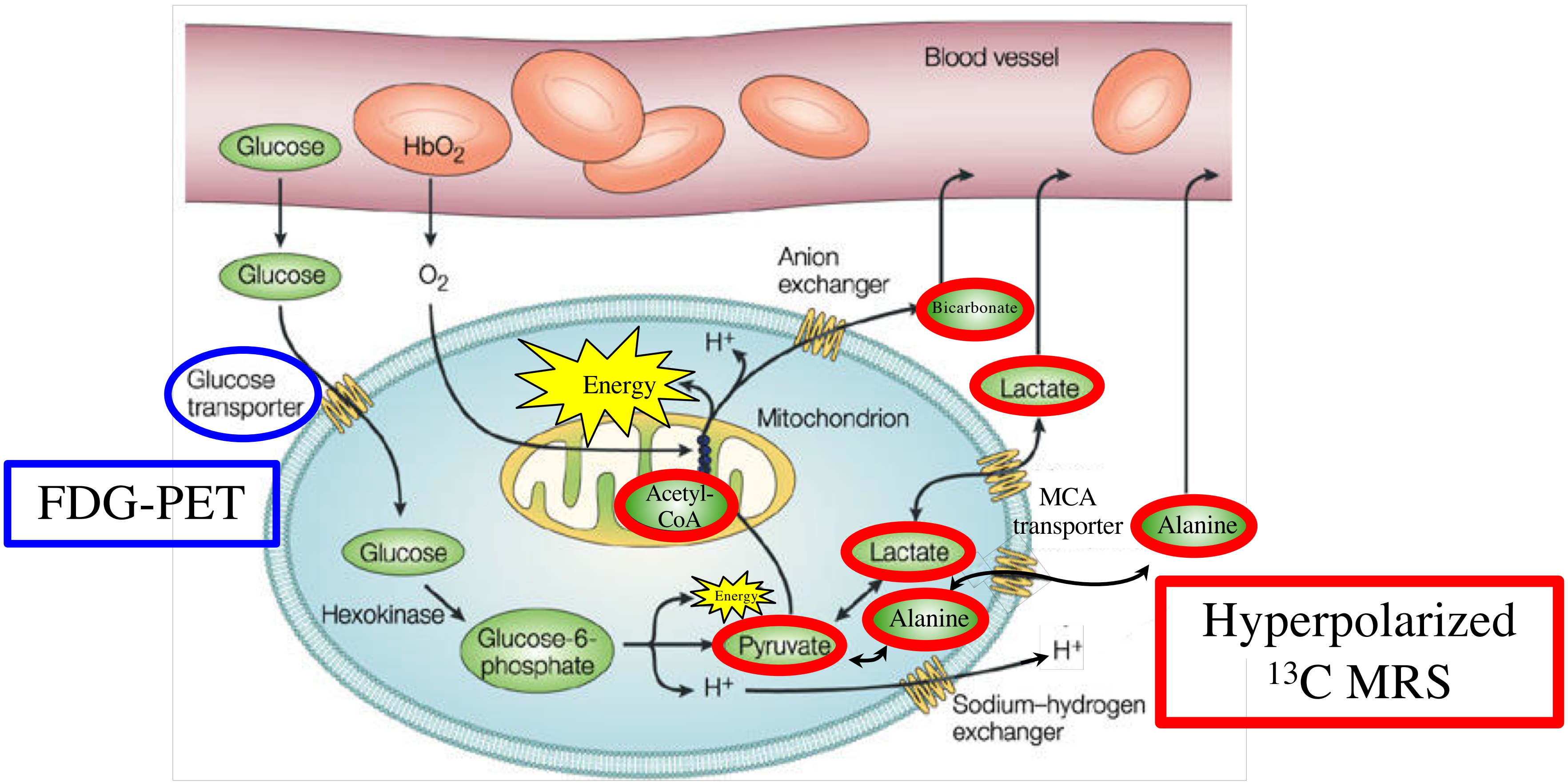
Hyperpolarized ^{13}C MRS

Hypothesis: Imaging of hyperpolarized [1-^{13}C]pyruvate can provide one such metabolic therapy index.



PET vs Hyperpolarized ^{13}C MRS

- Key idea: inject a “magnetically” enhanced biological substrate and image both the substrate and its downstream metabolic products.



Key technology: A polarizer that magnetically prepares the substrate to boost its MR visibility by >10,000 fold.

MR Sensitivity

- Thermal equilibrium magnetization:

$$M_0 = \rho \frac{\gamma^2 \hbar^2 B_0}{4kT} = \underbrace{\rho}_{\text{Spins/unit volume}} \underbrace{\left(\frac{\gamma\hbar}{2}\right)}_{\text{Magnetic moment}} \underbrace{\left(\frac{\gamma\hbar B_0}{2kT}\right)}_{\text{Polarization}}$$

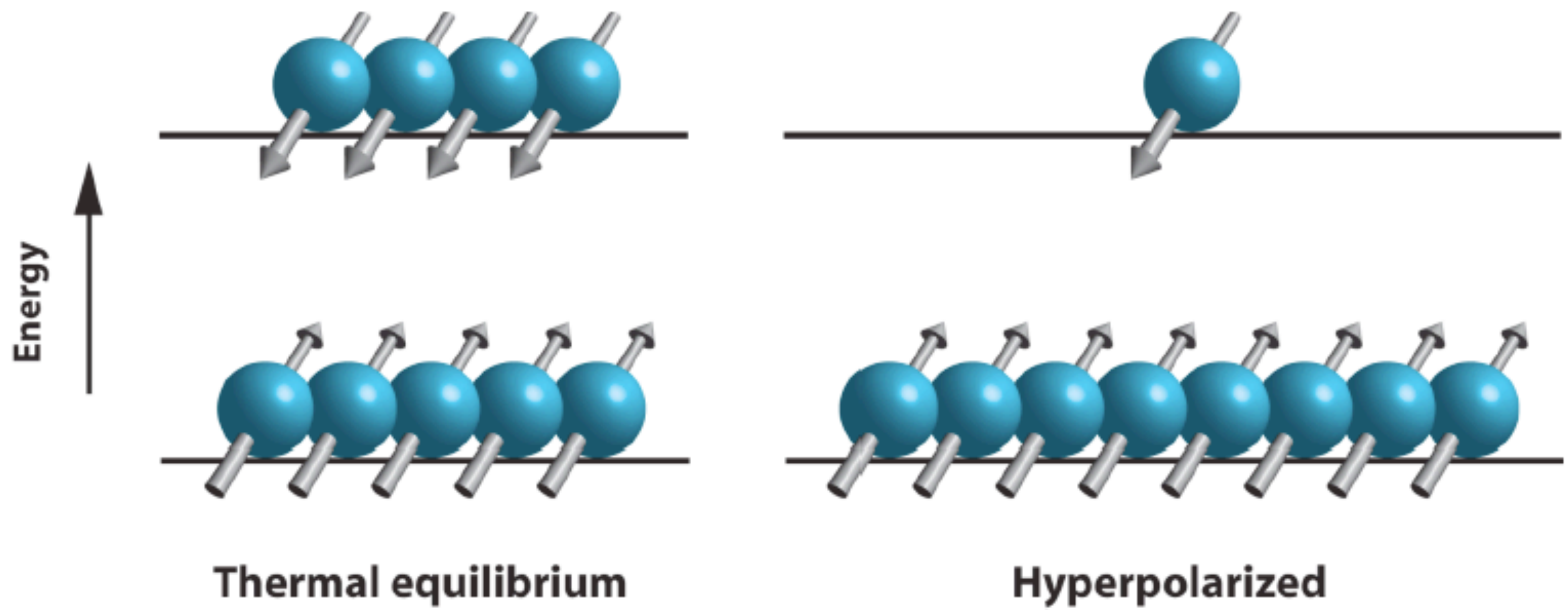
Polarization $P = \frac{N^+ - N^-}{N^+ + N^-}$

- Increasing sensitivity
  - Increase ρ (*e.g.* isotopically enriched ^{13}C substrates vs natural abundance)
  - Increase polarization:
    - → Higher B_0
    - → Lower T
    - → Change "effective" γ?!?

In vivo hyperpolarized ^{13}C via DNP exploits all of these effects!

Hyperpolarization

- Hyperpolarization: creating nuclear spin polarization much greater than that achieved at normal thermal equilibrium.



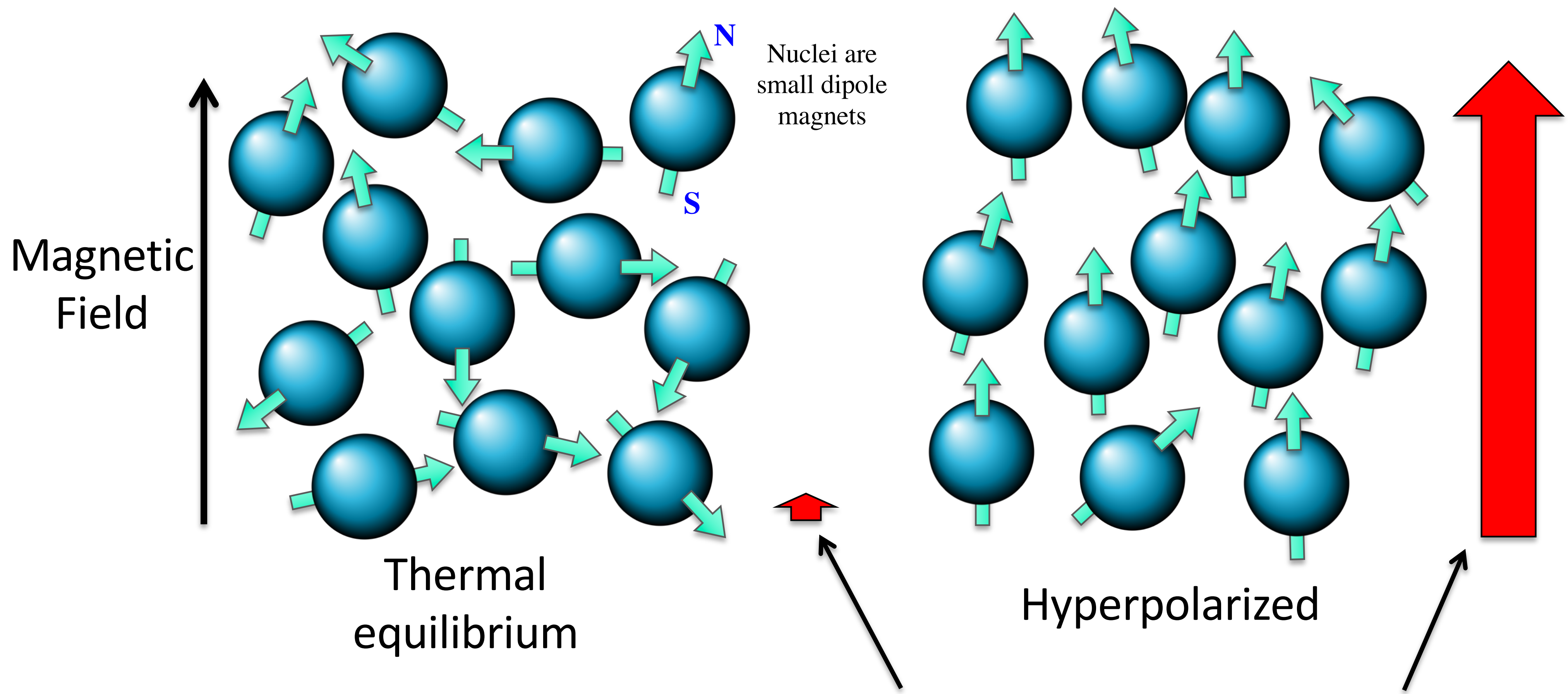
$\approx 10^{-5}$ %

≈ 25-50%
(or higher)

MR signal proportional to population difference!

Hyperpolarization

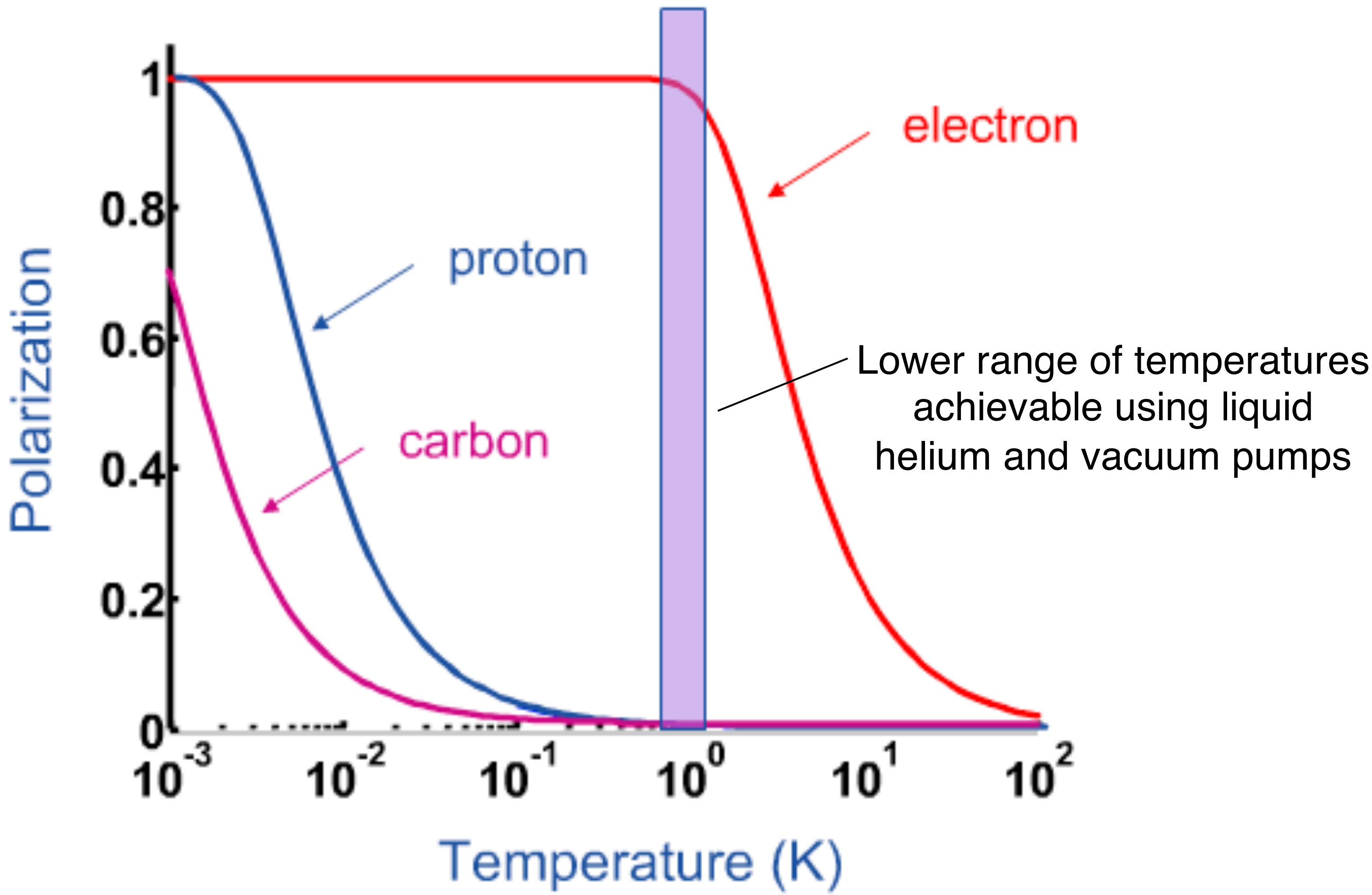
- Increases net nuclear magnetization
- Does **NOT** change chemical properties.



MR signal proportional to the net magnetization!

Brute Force

Boltzmann distribution at 3T



Dissolution DNP

Step 1: Polarization (~2 hrs)

- ^{13}C-labeled substrate + free radical
- 5T magnet, 1K temperature, 20 mM microwave

Step 2: Dissolution (~30 s)

- Water is heated and pressurized
- Melts the sample and fills the syringe
- Quality control checks before syringe is released

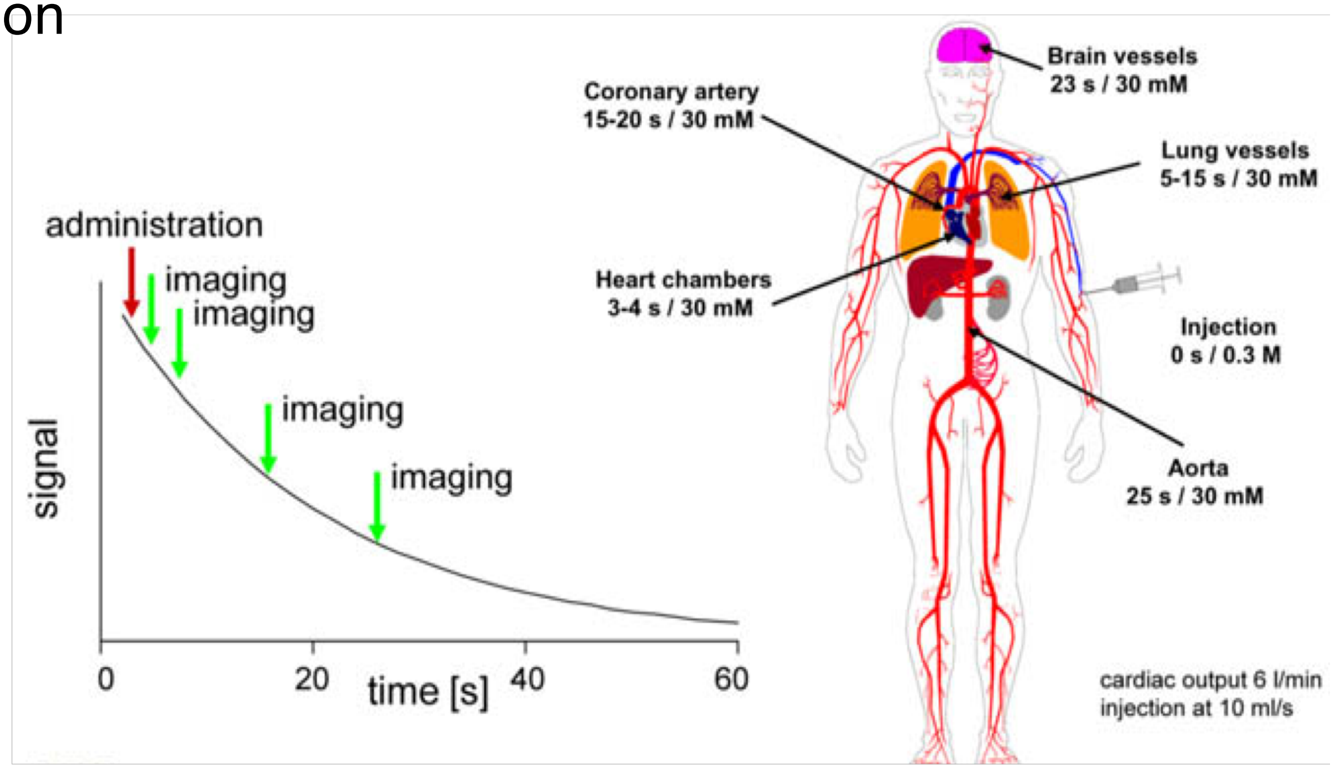
Inject and image before signal disappears (~ 3 min)!

40% polarization ~ 50,000 fold signal gain!
In vivo T_1 = 40 s

In Vivo Imaging Requirements

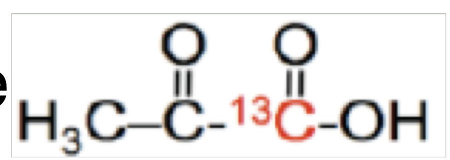
- Low toxicity (mM conc.)
- Long NMR relaxation times
- Chemical shift separation
- Rapid cellular uptake
- Rapid metabolism

Signal decays by relaxation and dilution



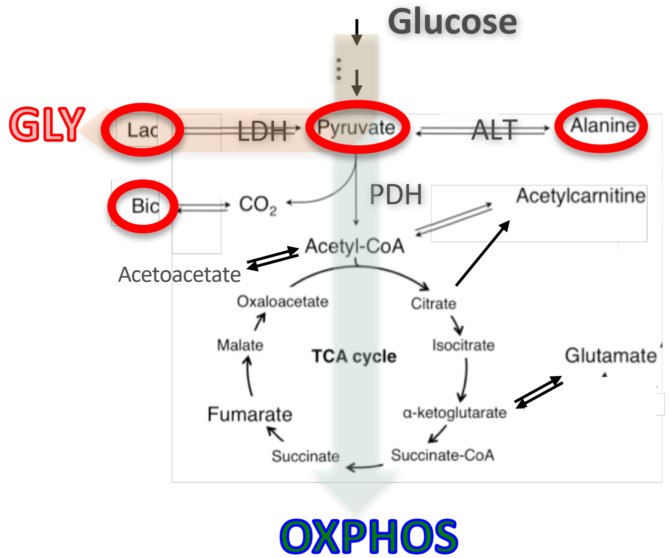
➡ Focus on low molecular weight endogenous compounds.

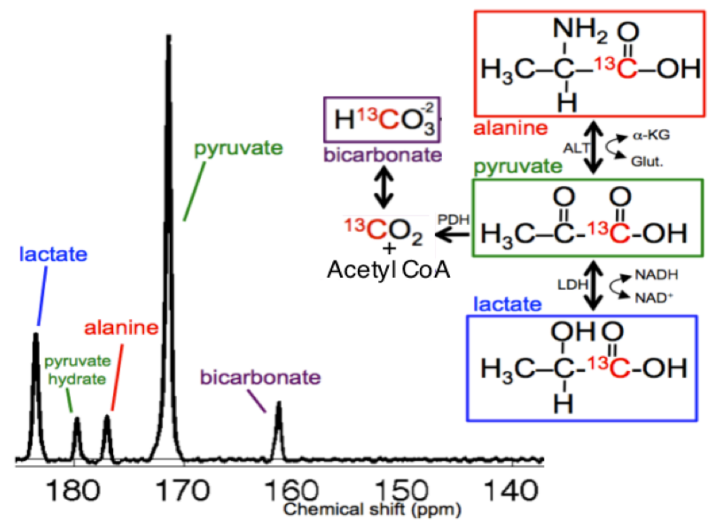
Example: [1-^{13}C]pyruvate

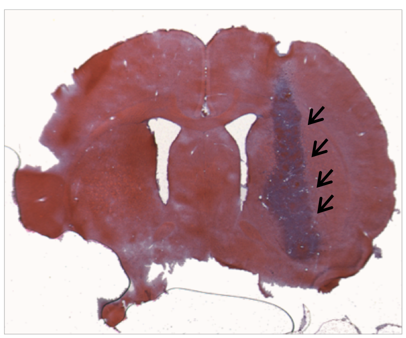


25% polarization ~ 30,000 fold signal gain!
In vivo T_1 = 30 s

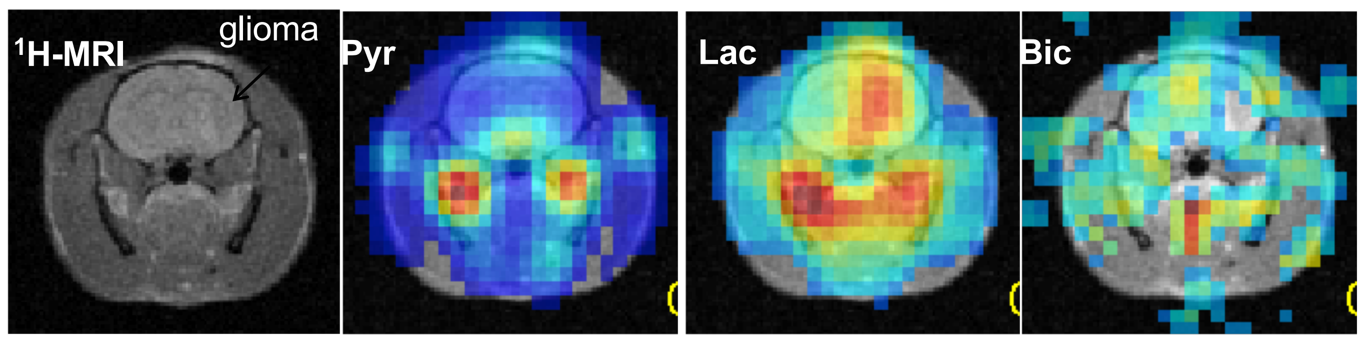
Hyperpolarized [1-^{13}C]Pyruvate







C6 rat glioma model



Hyperpolarized [1-^{13}C]Pyr imaging

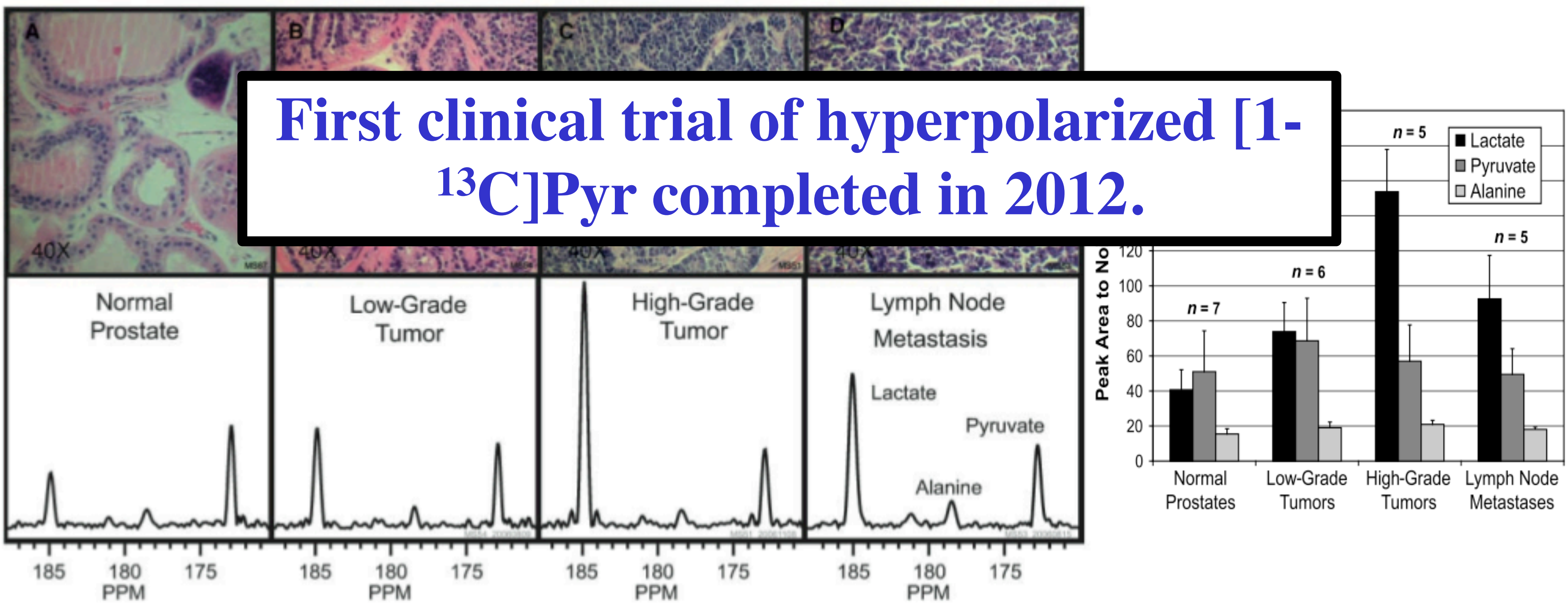
Proposed metric: Metabolic therapy index $= \dfrac{[^{13}\text{C-Lactate}]}{[^{13}\text{C-Bic}]}$

Cancer Res 2008; 68: (20). October 15, 2008 Research Article

Hyperpolarized ^{13}C Lactate, Pyruvate, and Alanine: Noninvasive Biomarkers for Prostate Cancer Detection and Grading

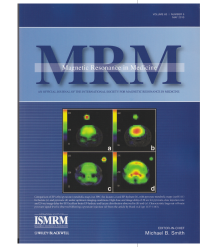
Mark J. Albers,[1,2] Robert Bok,[2] Albert P. Chen,[2] Charles H. Cunningham,[3] Matt L. Zierhut,[1,2] Vickie Yi Zhang,[2] Susan J. Kohler,[4] James Tropp,[5] Ralph E. Hurd,[5] Yi-Fen Yen,[5] Sarah J. Nelson,[1,2] Daniel B. Vigneron,[1,2] and John Kurhanewicz[1,2]

[1]Department of Bioengineering, University of California San Francisco and University of California Berkeley; [2]Department of Radiology and Biomedical Imaging, University of California San Francisco, San Francisco, California; [3]Sunny Brook Health Sciences Centre, Toronto, Ontario, Canada; [4]Union College, Schenectady, New York; and [5]GE Healthcare, Menlo Park, California



First clinical trial of hyperpolarized [1-^{13}C]Pyr completed in 2012.

Magnetic Resonance in Medicine 59:1005–1013 (2008)

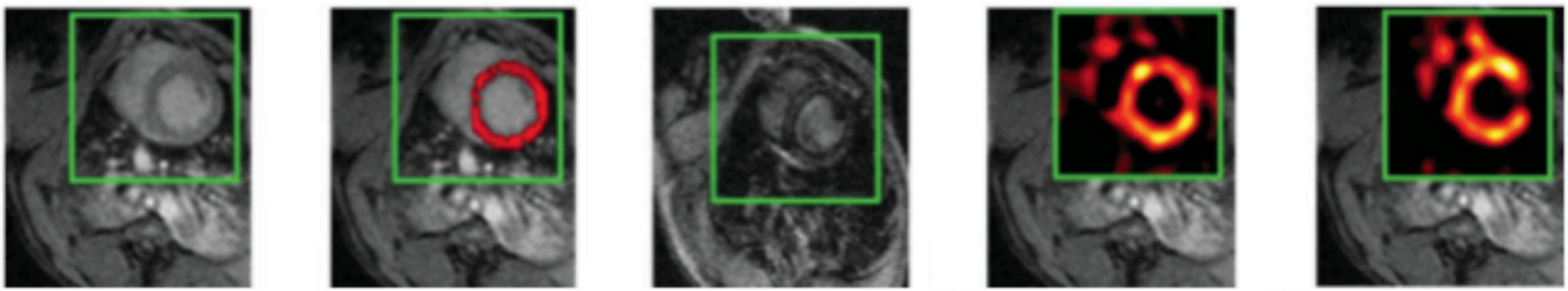


Cardiac Metabolism Measured Noninvasively by Hyperpolarized ^{13}C MRI

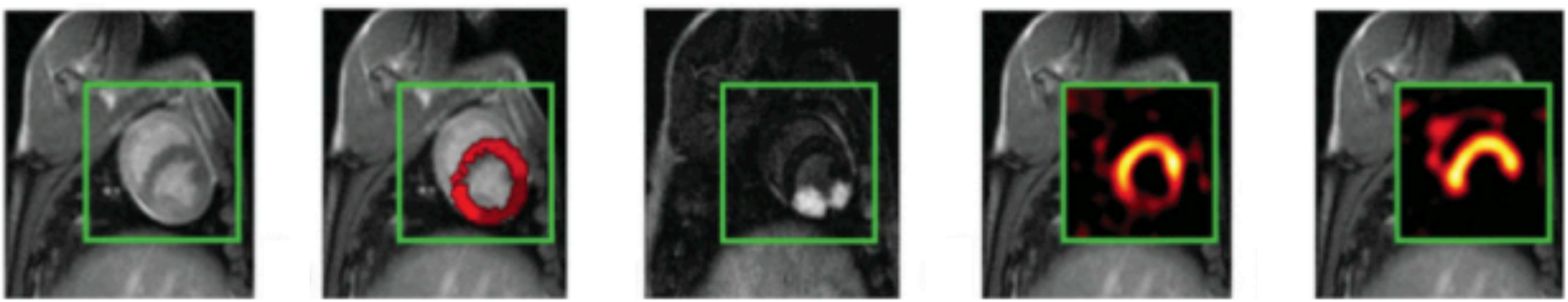
Klaes Golman,[1] J. Stefan Petersson,[1*] Peter Magnusson,[1] Edvin Johansson,[1] Per Åkeson,[2] Chun-Ming Chai,[3] Georg Hansson,[1] and Sven Månsson[3]

Pig Coronary Occlusion Model

Proton Perfusion Delayed enhancement Alanine Bicarb



15 min occlusion (stunned) + 2 hrs reperfusion

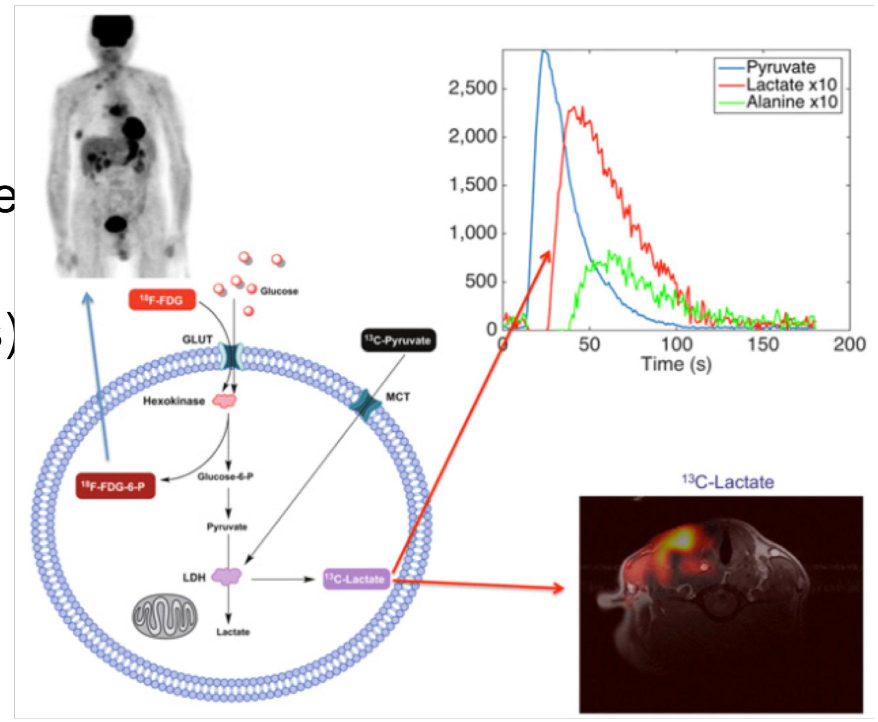


45 min occlusion (infarcted) + 2 hrs reperfusion

PET + Hyperpolarized ^{13}C

- Opportunity: currently only two Spinlab systems installed adjacent to PET/MR scanners.

- ^{18}F-FDG and ^{13}C-pyruvate
  - Measures glucose metabolic pathway at multiple points: uptake vs. glycolysis/oxidative phosphorylation balance
  - Tumors hot on FDG: initial studies (animals) show elevated ^{13}C Lac labeling
  - Tumors cold on FDG: hyp ^{13}C-pyr ?
  - Inflammation: FDG vs. hyp ^{13}C-pyr ?

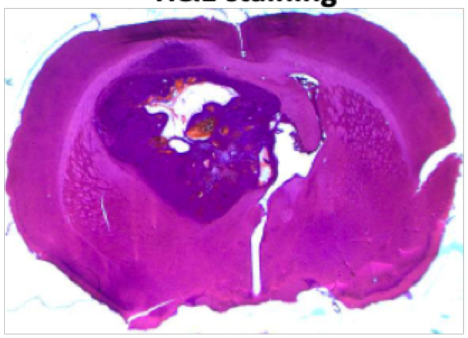


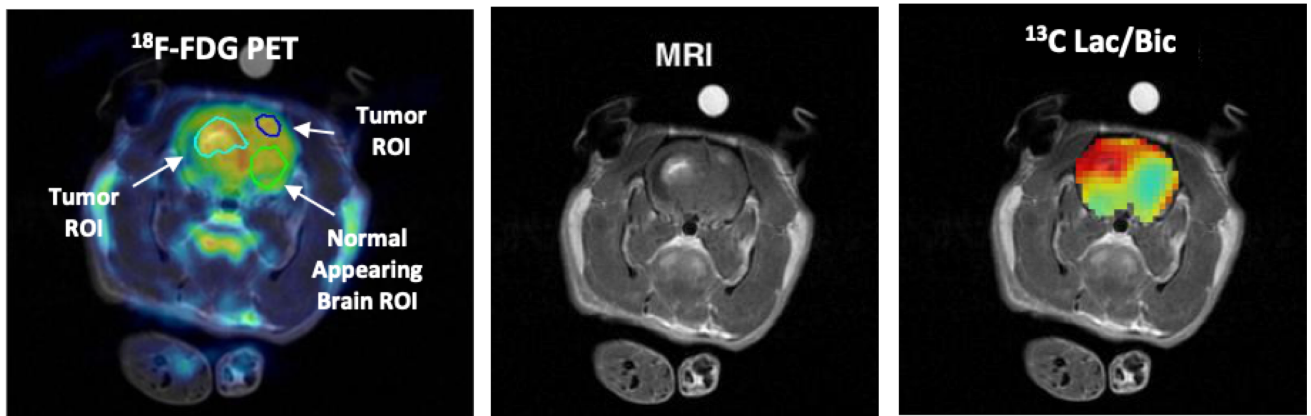
- Other tracers/substrates

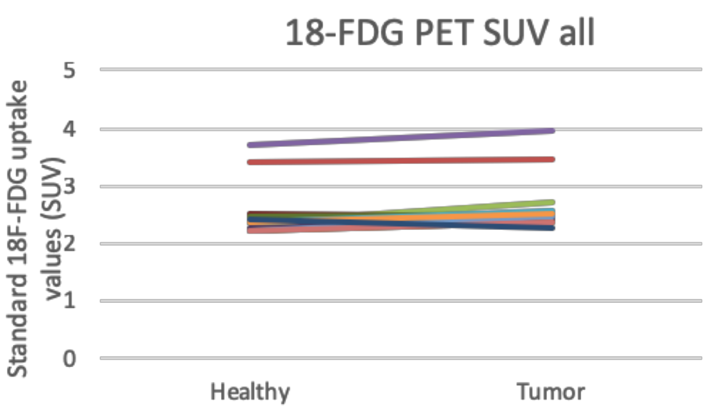
PET + Hyperpolarized ^{13}C

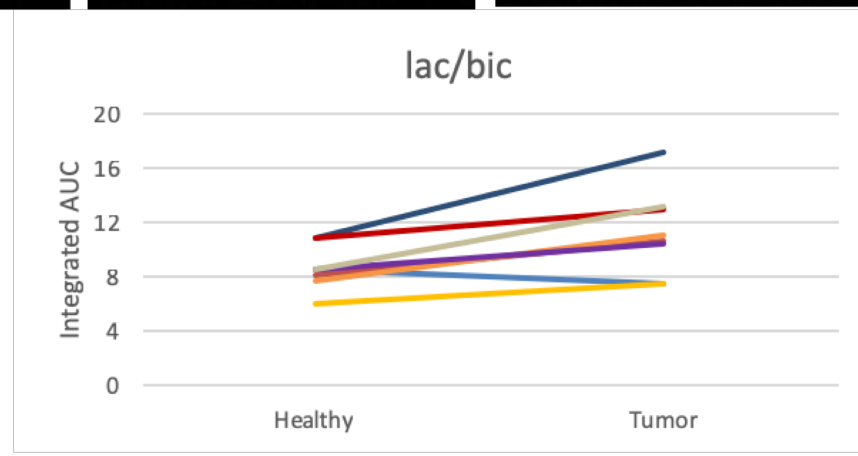
Rat ENU tumor model

H&E staining



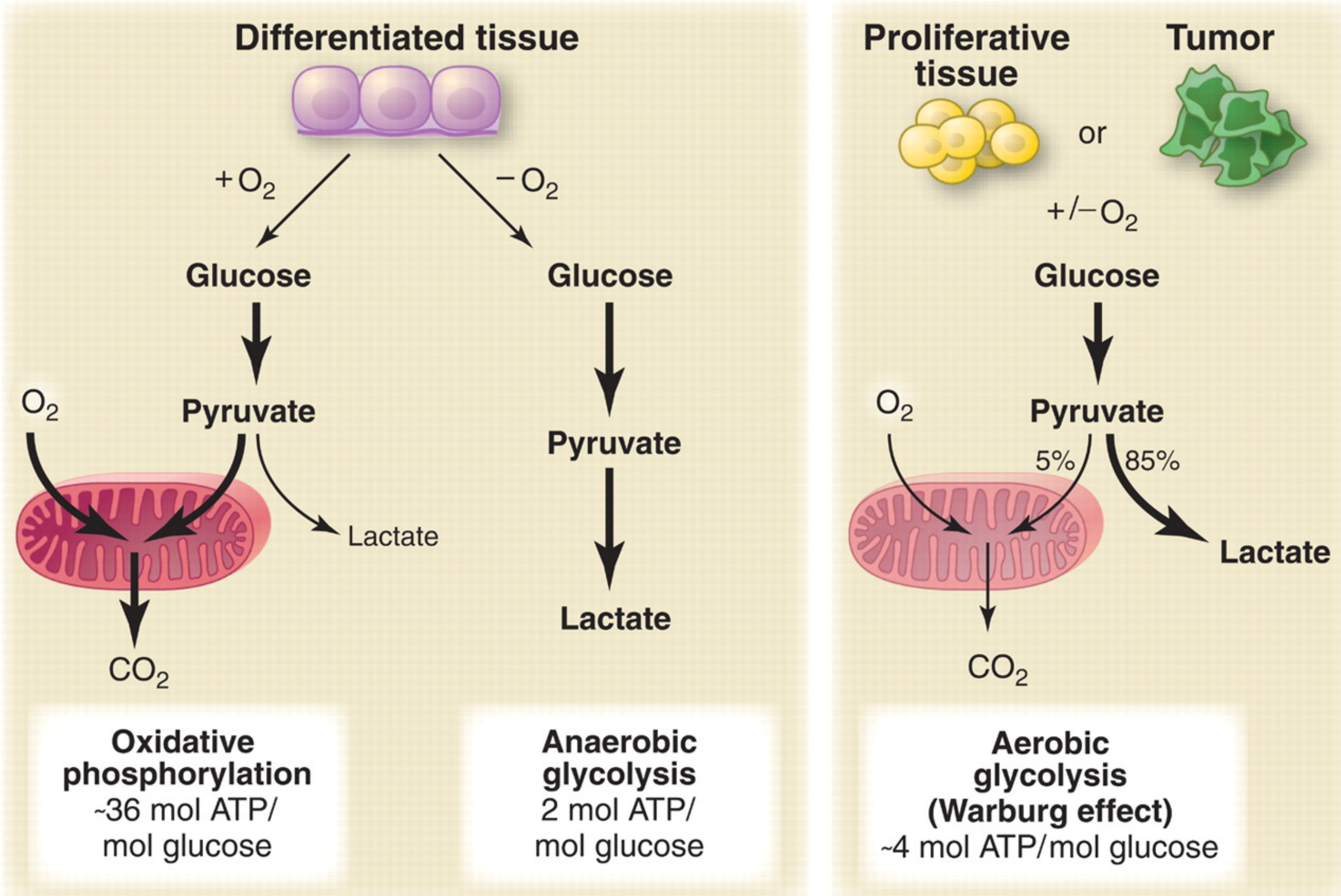






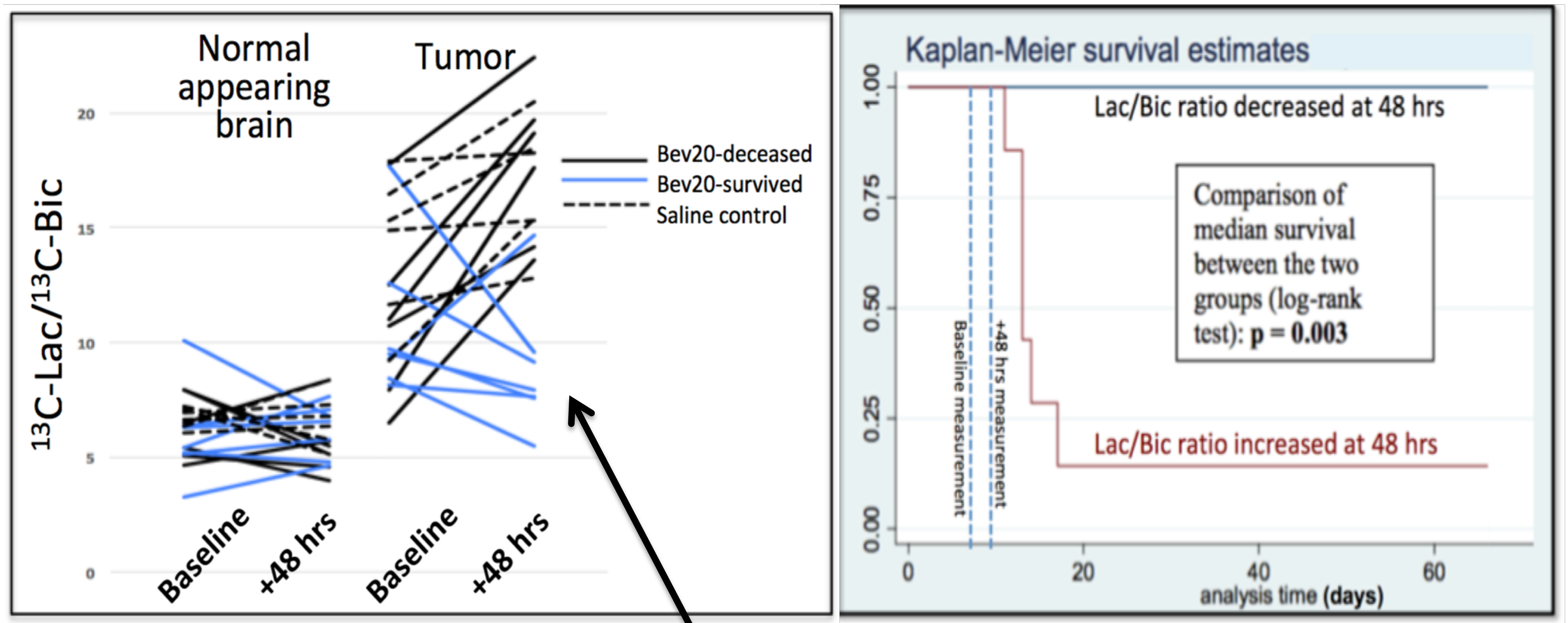
Are there synergies to exploit with simultaneous PET/HP^{13}C?

Cancer and the Warburg Effect



Will reversing the Warburg Effect slow tumor growth?

Rat C6 Glioma Bevacizumab Study



Decreased Lac/Bic ratio predicts survival

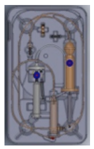
Note: no correlations found with respect to tumor size.

Clinical Hyperpolarized ^{13}C Studies

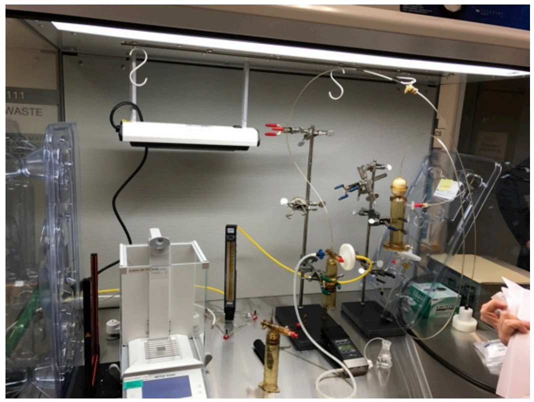
- FDA IND ##135767–"Hyperpolarized [1-13C]Pyruvate Injection",
- Stanford IRB, protocol #39845–"A Pilot Study to Assess Lactate and Bicarbonate Detection within Malignant Brain Tumors Using [1-13C]-pyruvate DNP Magnetic Resonance Spectroscopy (MRS)".

Step 1:
Pharmacy kit preparation 24 hrs prior to injection

Sterile fluid path



^{13}C-pyruvate + reagents (GMP)

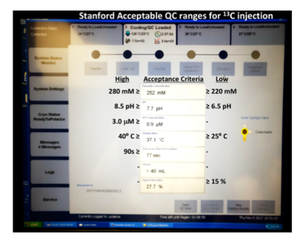


Lucas Center Radiochemistry Cleanroom

Step 2:
Polarization 2.5-3 hrs prior to injection

Polarizer
~ 3 hrs

Step 3:
Dissolution and quality control checks
~90s prior to injection



Fast filter test

Step 4:
Pyr injection and MRI scan 2-3 min

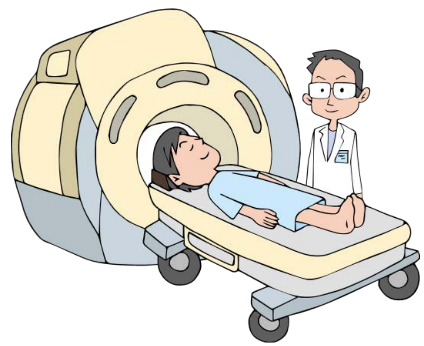
MR scanner
~ 2-3 min scan

MEDRAD power injector

Recruitment - volunteers

STANFORD
CANCER INSTITUTE

Volunteers wanted for a pyruvate tracer Research Study



Looking for healthy volunteers

We are conducting a research study to assess the safety of infusing 13C-labeled hyperpolarized pyruvate (HP-pyruvate) prior to performing magnetic resonance imaging (MRI)

The study requires 3 visits:

Visit 1: Review and sign consent form, medical history review, vital signs, ECG, and blood draw (Approximately 2 hrs)

Visit 2: MRI using HP-pyruvate and observation (Approximately 2 hrs)

3-5 hours later: Vital signs and ECG (Approximately 30 mins)

Visit 3: Follow up visit or telephone call (Approximately 30 mins)

Participants will receive financial compensation for their time spent in this research study. There will be no direct benefits to you from participating in this study, but the knowledge gained from this study may help future cancer patients.

Key eligibility requirements:

- Must be 18 years old or older
- Must not be undergoing active treatment for malignancy
- No history of allergic reactions to MRI contrast (gadolinium)
- Must be able to undergo an MRI
- Volunteers cannot be pregnant or nursing.
- Please note that this is an abbreviated list of study requirements.

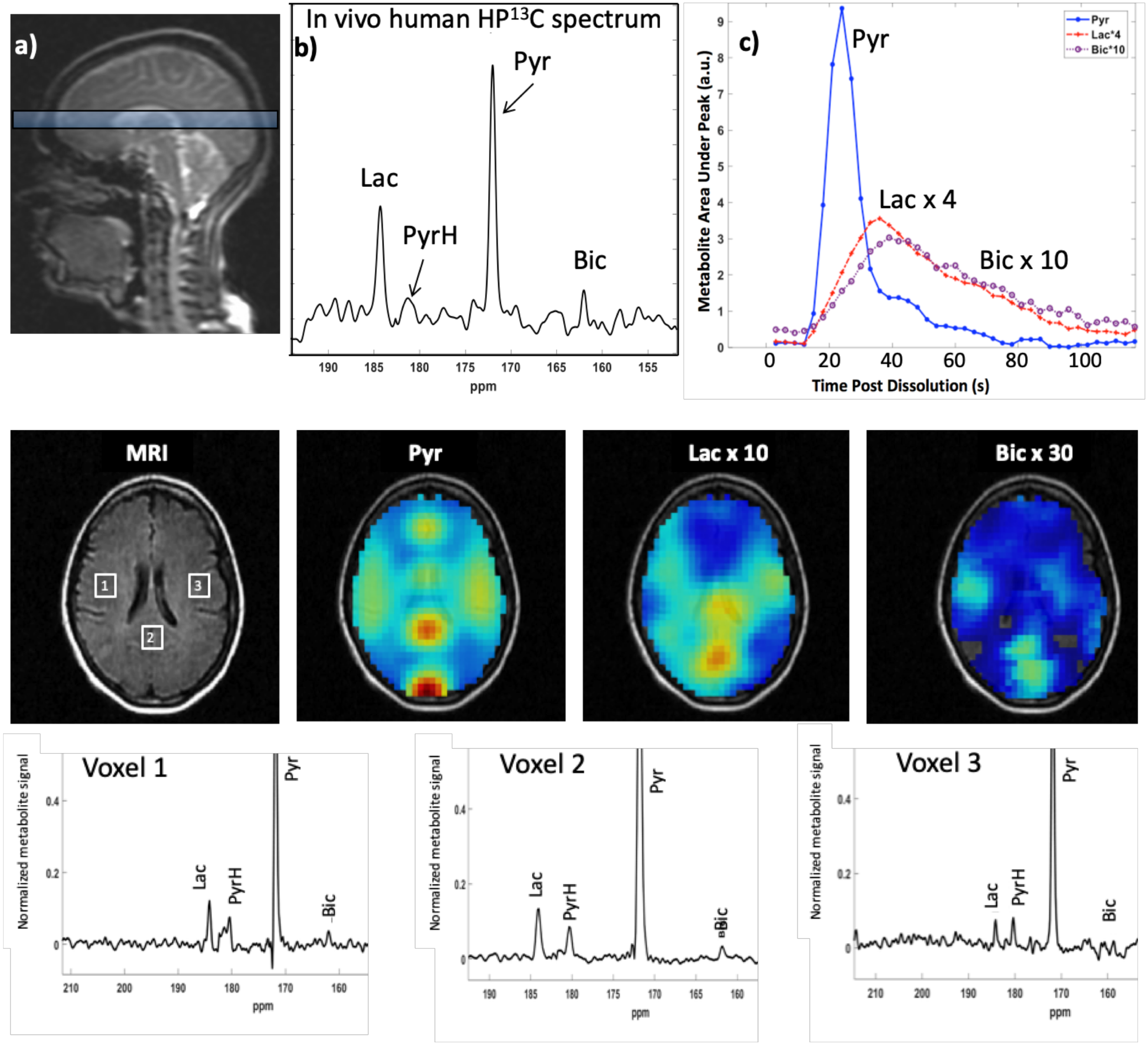
If you are interested, please contact the research nurse below for more information

Stephanie Lewis, RN, MSN
Email: lewisste@stanford.edu
Tel: (650) 723-0381

First Stanford Human Hyperpolarized ^{13}C Results

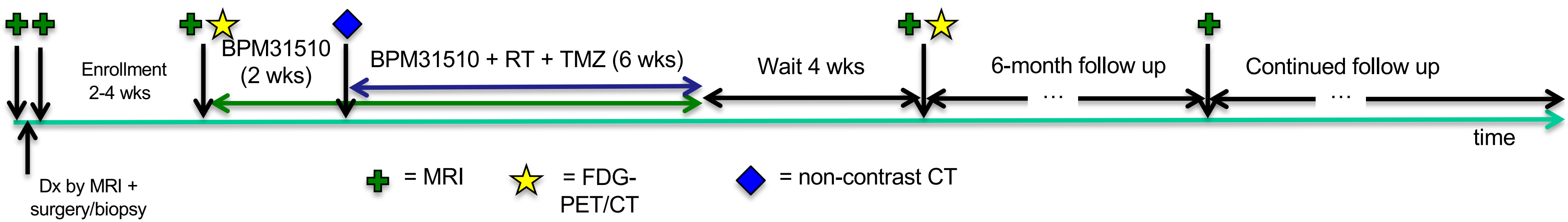
Subject 1

Subject 2

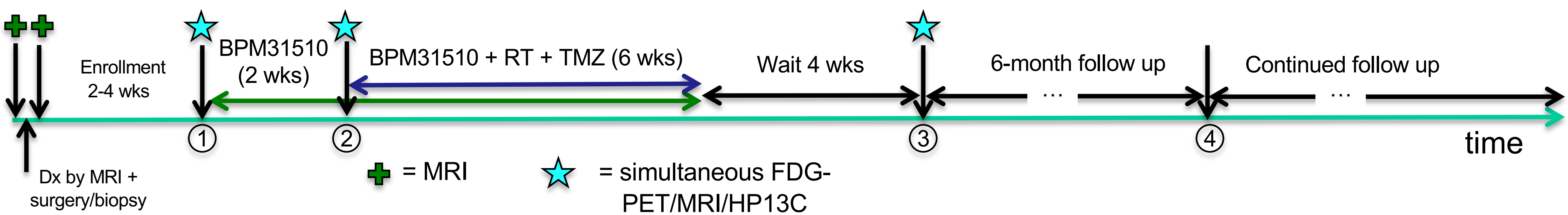


BPM31510 Phase II GBM Trial

Trial as planned



Proposed modification with HP ^{13}C-pyruvate



Next lecture: Brain metabolic changes during heart/lung bypass surgery