Metabolic flux analysis for modeling dynamic MRS data

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Today's menu:

- ✓ Dynamic magnetic resonance spectroscopy (MRS)
- ✓ Metabolic tracers in MRS
- ✓ Mathematical models of metabolism
- ✓ Metabolic flux analysis (MFA) *in vivo*

^{1}H MRS for metabolite profiling *in vivo*

Tissue specificity

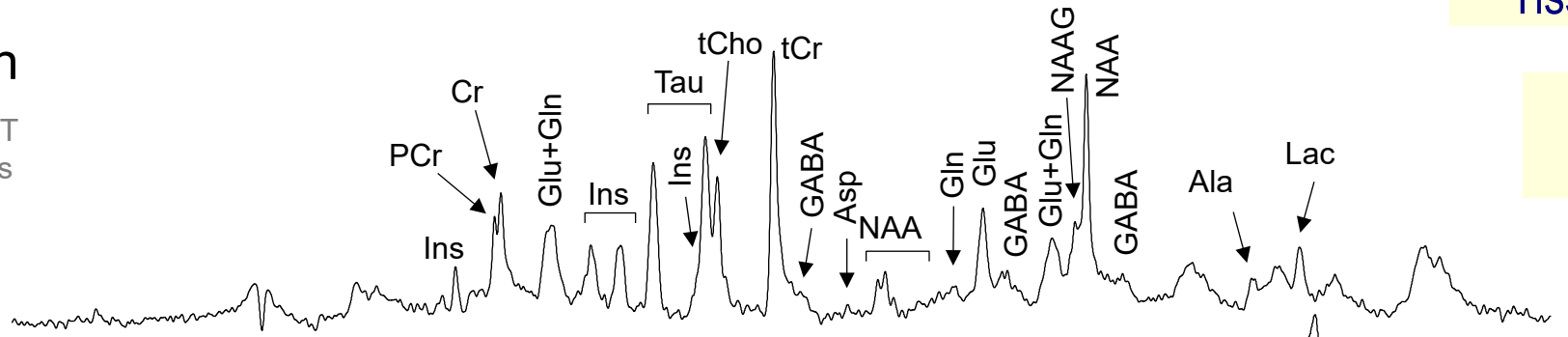
Degeneration or regeneration

Metabolic status

Tissue development and differentiation

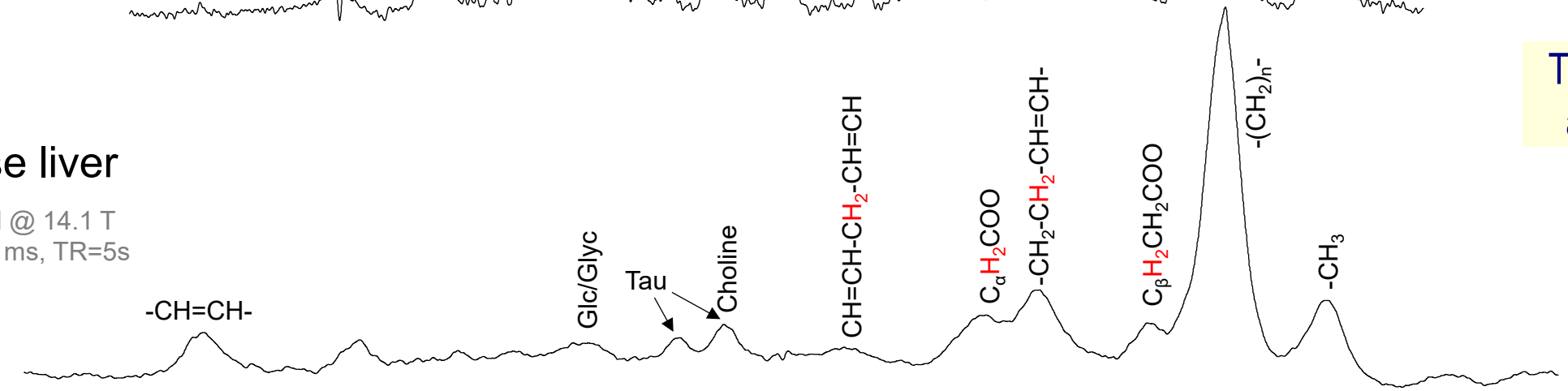
Mouse brain

SPECIAL @ 14.1 T
TE=2.8 ms, TR=4s



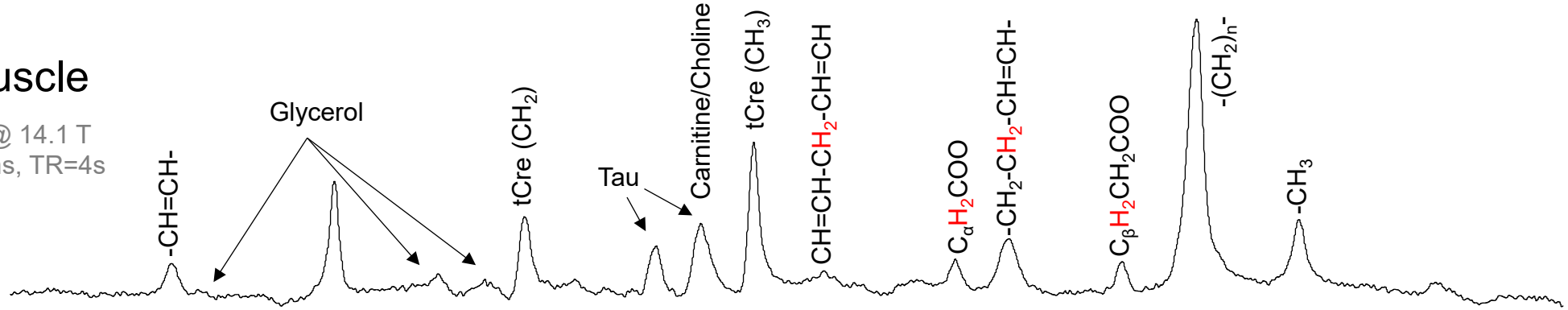
Mouse liver

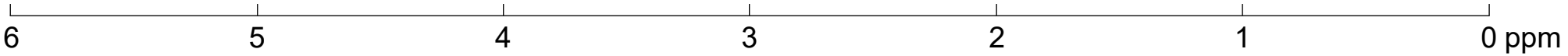
STEAM @ 14.1 T
TE=2.8 ms, TR=5s



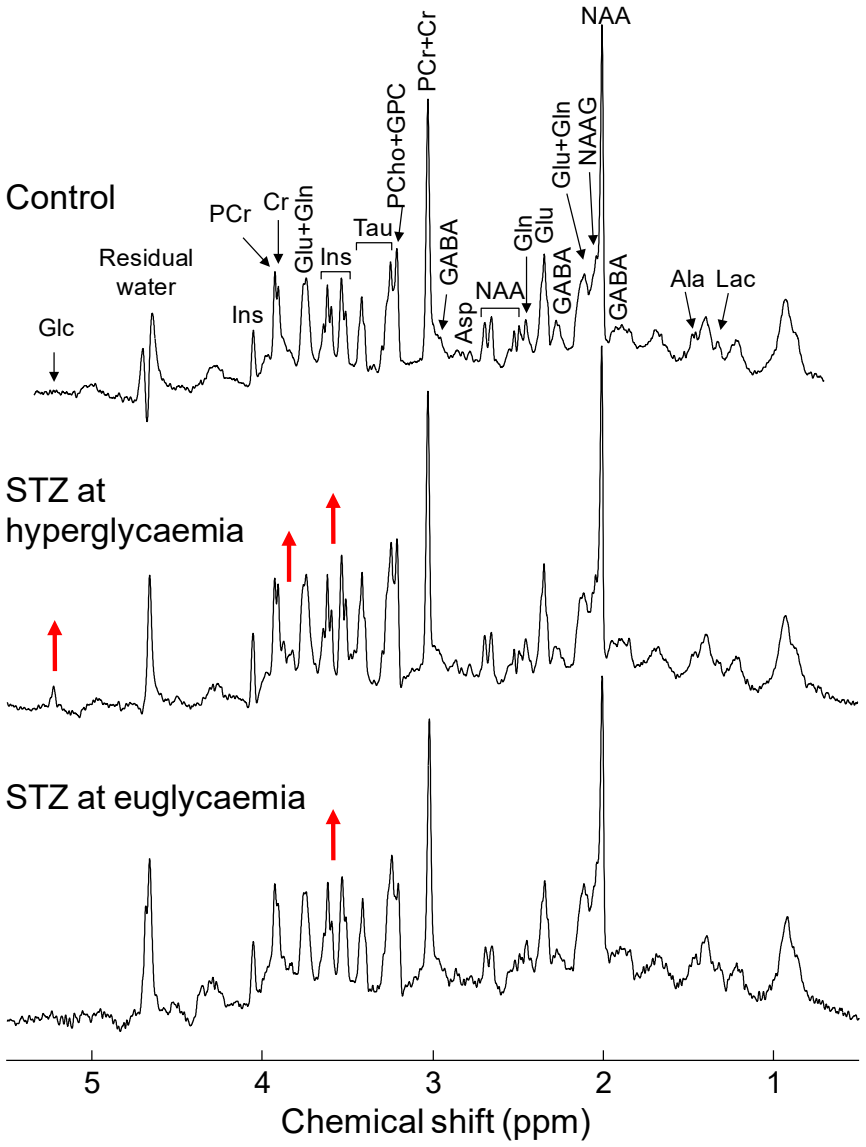
Rat muscle

STEAM @ 14.1 T
TE=2.8 ms, TR=4s





Glucose in ^{1}H MRS of the rat brain



^{1}H MRS of the rat hippocampus *in vivo* at 9.4 T

Red arrows stand out the increase in glucose and *myo*-inositol signals in the streptozotocin (STZ) model of diabetes.

Duarte *et al.*, (2009) *J Neurochem* 111:368

Recalling what you know on enzyme kinetics:
Single substrate Michaelis-Menten kinetics

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

$$v = \frac{d[P]}{dt} = k_2[ES]$$

Rapid equilibrium assumption:

Rate limiting step is ES → E + P ($k_2 << k_{-1}$)

Brown (1902) *J Chem Soc* 81:373

[S] >> [E], implying [S]o = [S]

Michaelis & Menten (1913) *Biochem Z* 49:333
based on work of Henri (1903)

Steady-state assumption: [ES] is constant

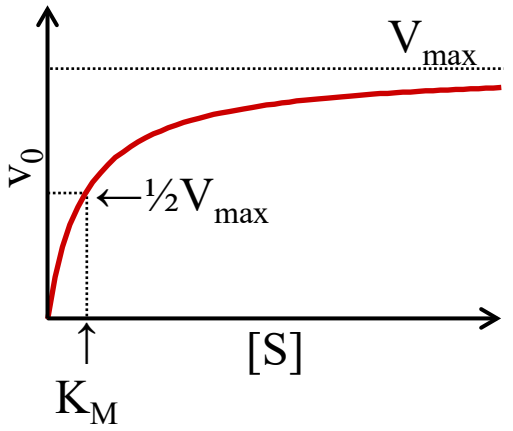
Brigs & Haldane (1925) *Biochem J* 19:338

$$\frac{d[ES]}{dt} = k_1[E][S] - (k_{-1} + k_2)[ES] = 0$$

$$\Rightarrow [ES] = \frac{[E]_t[S]}{\frac{k_{-1} + k_2}{k_1} + [S]}$$

$$\Rightarrow v_0 = \frac{k_2[E]_t[S]}{K_M + [S]}$$

$$v_0 = \frac{V_{max}[S]}{K_M + [S]}$$



Reversible Michaelis-Menten kinetics

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

$$v_0 = \frac{\frac{V^f_{max}}{K^S_M}[S] - \frac{V^r_{max}}{K^P_M}[P]}{1 + \frac{[S]}{K^S_M} + \frac{[P]}{K^P_M}}$$

$$V^f_{max} = k_2[E]_t \qquad K^S_M = \frac{k_2 + k_{-1}}{k_1}$$

$$V^r_{max} = k_{-2}[E]_t \qquad K^P_M = \frac{k_2 + k_{-1}}{k_{-2}}$$

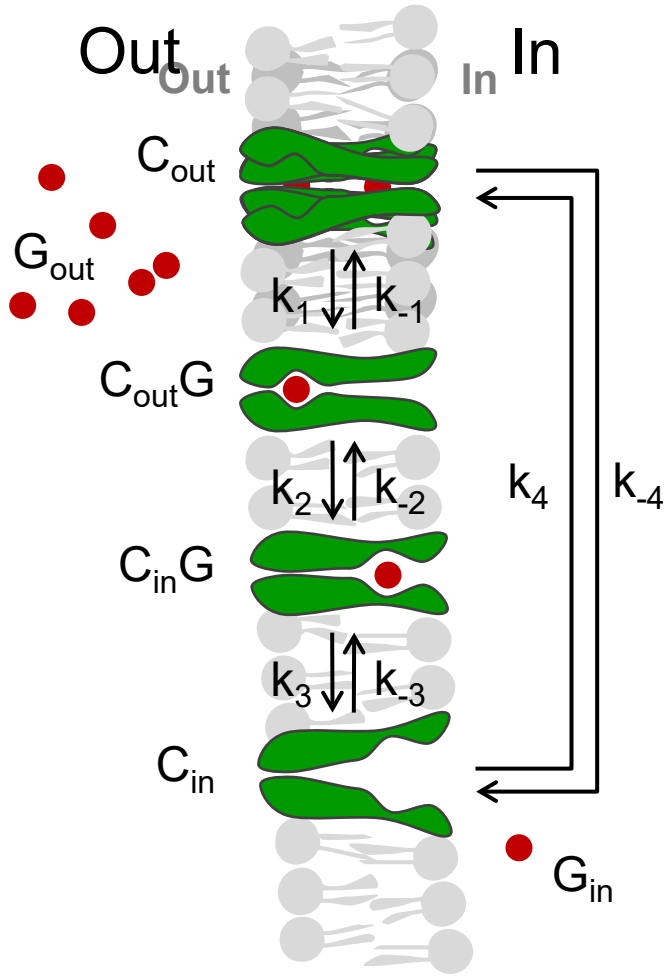
Enzyme kinetics with multiple intermediates

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EP \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E + P$$

easy with the King-Altman method

King & Altman (1956) J Phys Chem 60:1375

Glucose transport: 4-state conformational model

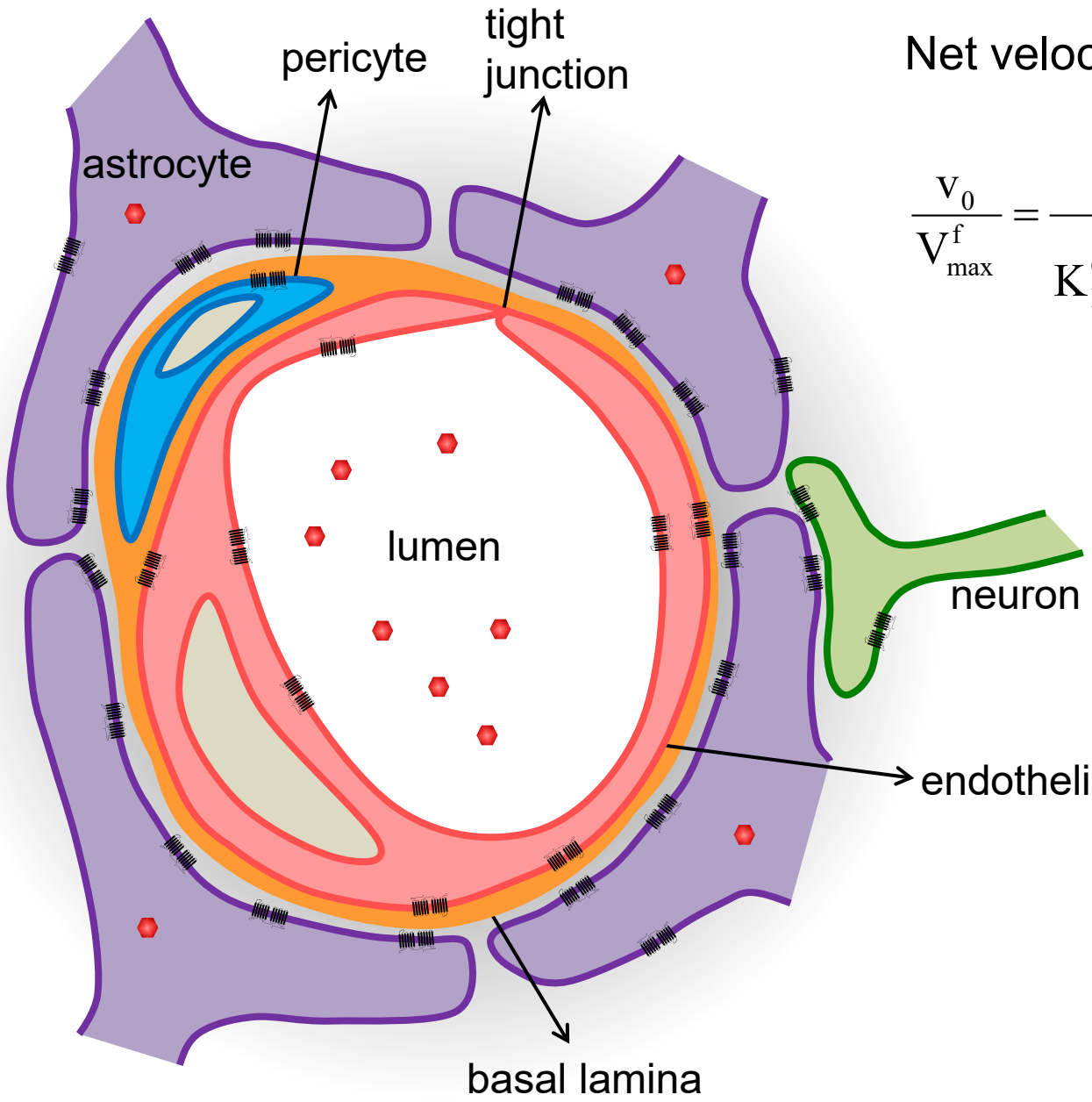


Net velocity equation:

$$\frac{v_0}{V_{max}^f} = \frac{G_{out} - \frac{G_{in}}{K_{eq}}}{K_M^{Gout}\left(1+\frac{G_{in}}{K_M^{Gin}}\right) + G_{out}\left(1+\frac{G_{in}}{K_{ii}^{Gin}}\right)}$$

Duarte *et al.* (2009) *Front Neuroenerg* 1:6

The blood-brain-barrier



Net velocity equation:

$$\frac{v_0}{V_{max}^f} = \frac{G_{out} - \frac{G_{in}}{K_{eq}}}{K_M^{Gout}\left(1+\frac{G_{in}}{K_M^{Gin}}\right) + G_{out}\left(1+\frac{G_{in}}{K_{ii}^{Gin}}\right)}$$

Model of brain glucose dynamics requires model simplification

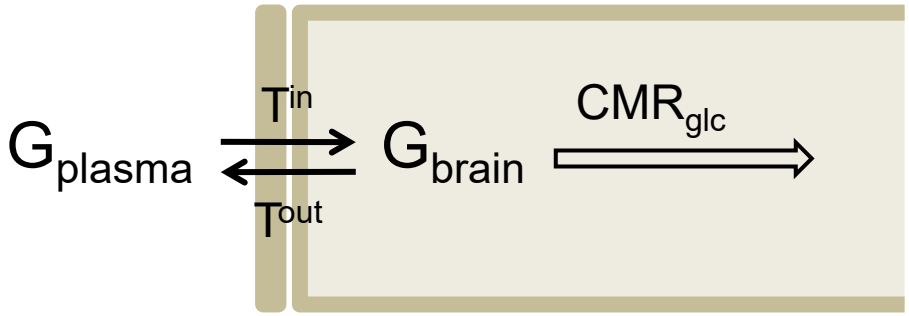
Assumptions:

BBB is a single membrane

Glucose transport is symmetric

Glucose consumption only occurs in brain parenchyma

Duarte *et al*. (2009) *Front Neuroenerg* 1:6



Net velocity equation:

$$\frac{v_0}{V_{max}^f} = \frac{G_{out} - \frac{G_{in}}{K_{eq}}}{K_M^{Gout}\left(1 + \frac{G_{in}}{K_M^{Gin}}\right) + G_{out}\left(1 + \frac{G_{in}}{K_{ii}^{Gin}}\right)}$$

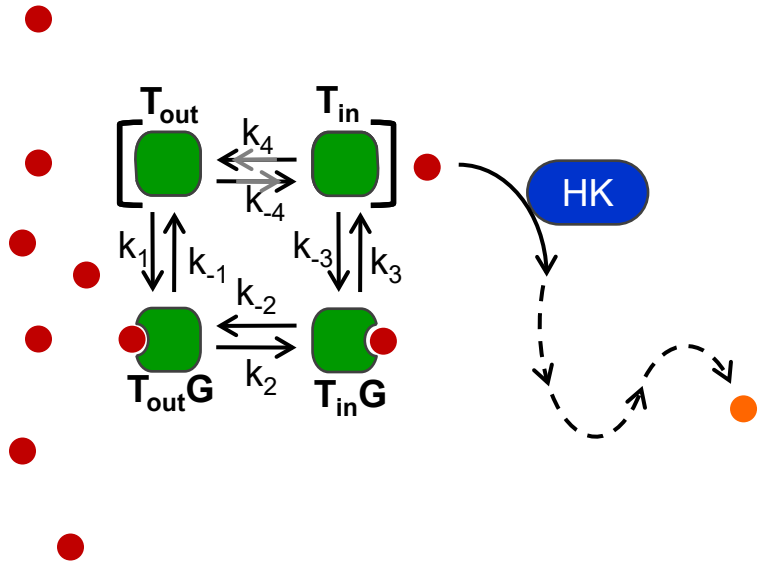
facilitated diffusion: $K_{eq} = 1$

Cuppoletti & Segel (1975) *J Theor Biol* 53;125

$$\frac{dG_{brain}}{dt} = T^{in} - T^{out} - CMR_{glc} \Rightarrow \frac{dG_{brain}}{dt} = \frac{T_{max}\left(G_{plasma} - \frac{G_{brain}}{V_d}\right)}{K_t + \frac{G_{brain}}{V_d} + G_{plasma}\left(1 + \frac{G_{brain}}{V_d K_{ii}}\right)} - CMR_{glc}$$

Further reading: **Cerebral glucose delivery, transport and metabolism: Theory and modeling using four, three, and two tissue compartments.** Seidemo et al. *J Cereb Blood Flow Metab* 2025

Fast isomerisation of unloaded carrier (Kii >> Gbrain)



$$G_{brain} = V_d \frac{\left(\frac{T_{max}}{CMR_{glc}} - 1\right) G_{plasma} - K_t}{\frac{T_{max}}{CMR_{glc}} + 1}$$

steady-state

$$0 = \frac{T_{max}\left(G_{plasma} - \frac{G_{brain}}{V_d}\right)}{K_t + \frac{G_{brain}}{V_d} + G_{plasma}} - CMR_{glc}$$

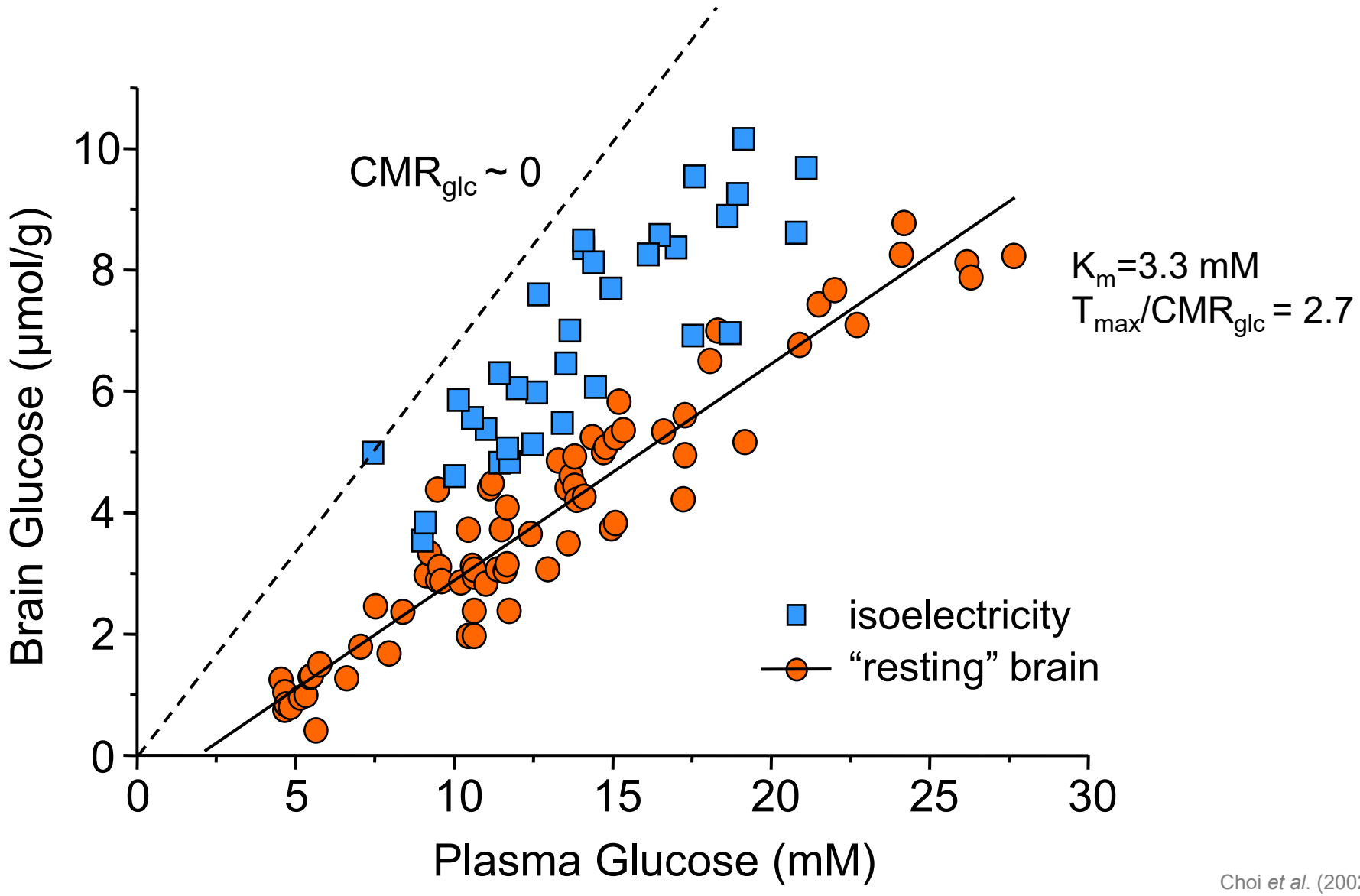
reversible Michaelis-Menten model

Analysis of steady-state brain glucose transport

	Rev. MM	4-state conf.	Rev. MM	4-state conf.	Rev. MM	4-state conf.
T_{max}/CMR_{glc}	2.3±0.2	2.7±1.2	2.7±0.1	2.9±0.3	2.3±0.1	2.4±0.4
K_t (mM)	0.6±2.0	2.1±5.2	2.9±0.5	3.5±1.0	1.4±0.7	1.5±1.1
K_{ii} (mM)		50.8±161.5		105.3±151.7		145.9±776.4

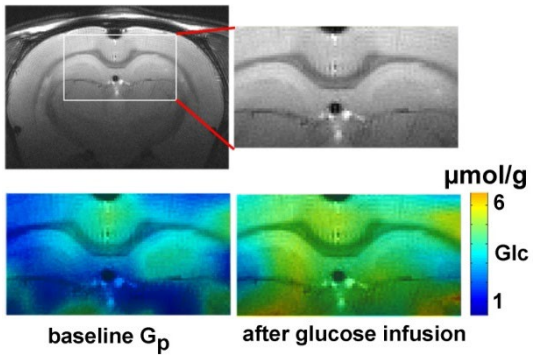
Duarte *et al*. (2009) *Front Neuroenerg* 1:6

Reduced metabolism increases brain glucose content



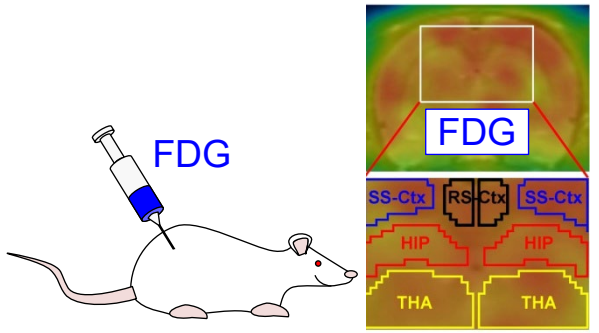
Choi *et al*. (2002) *JCBFM* 22:1343

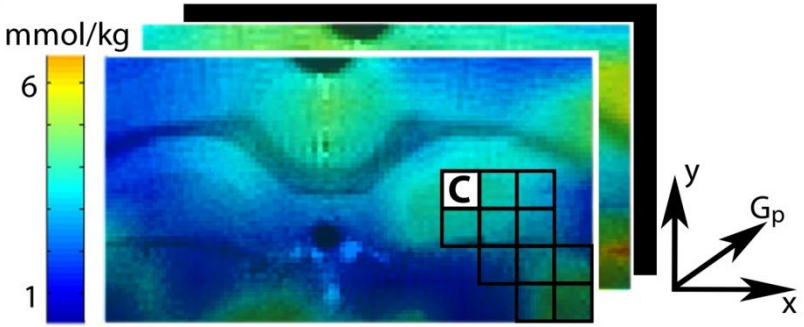
Spatial mapping of brain glucose using spectroscopic imaging methods

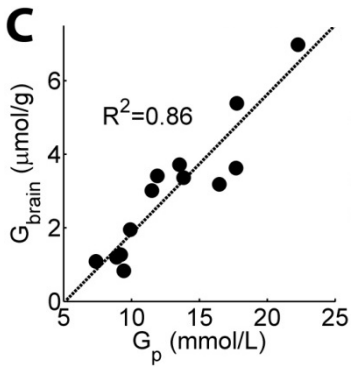


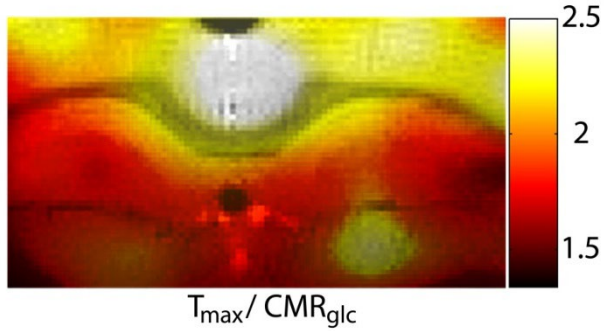
Comparable to PET

with ^{18}F-Fluorodeoxyglucose (FDG)



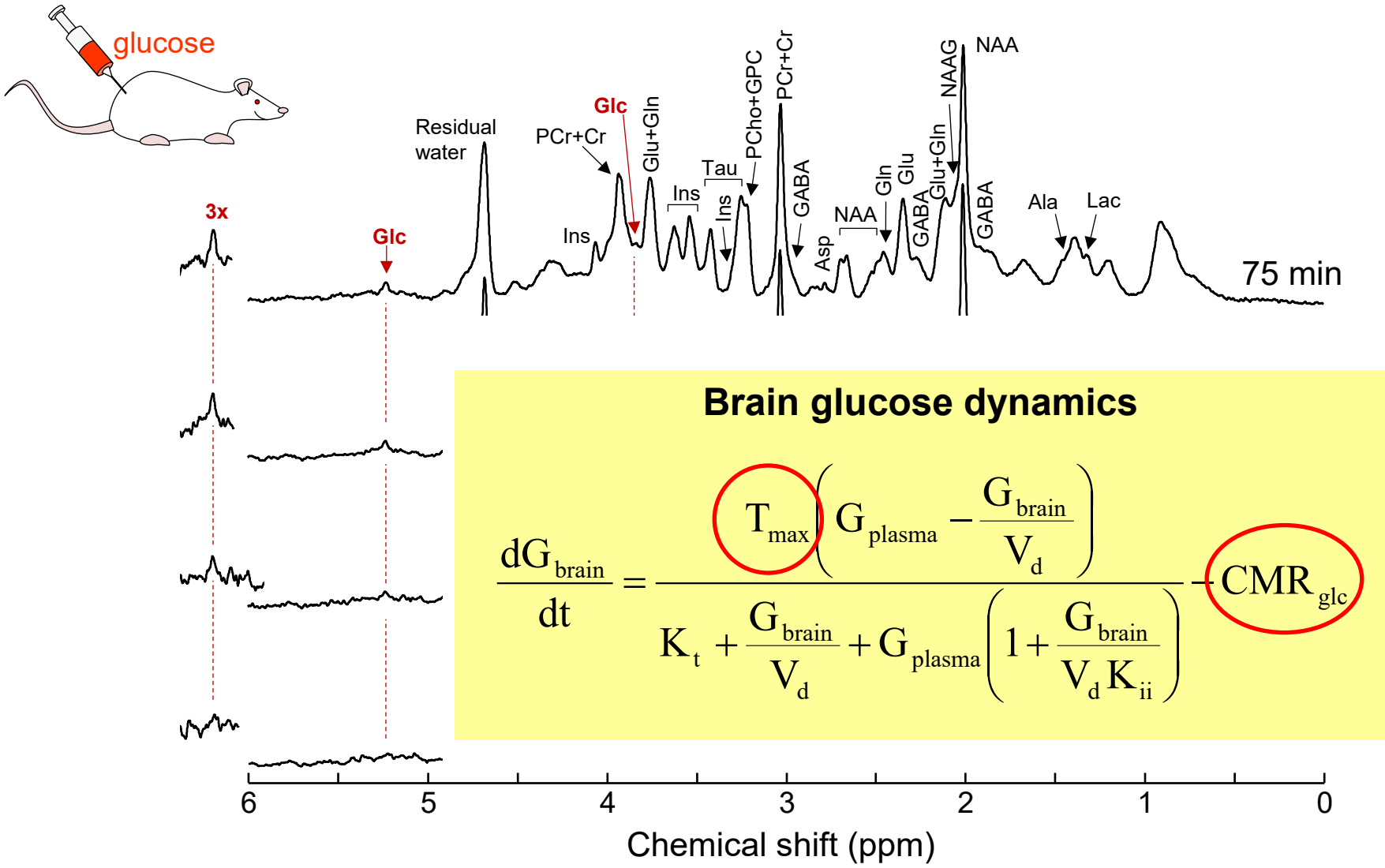






Alf *et al*. (2014) *J Neurochem* 129(4):672

Typical ^{1}H MRS at 9.4 T during the time course of glucose infusion

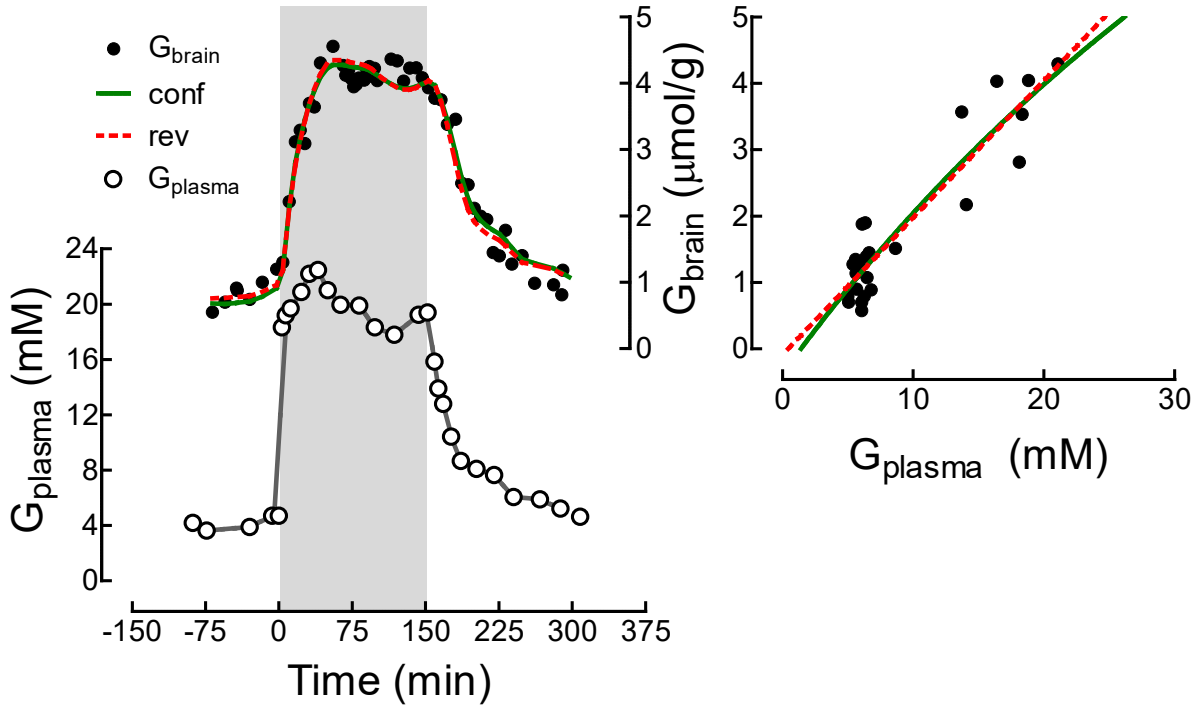


Brain glucose dynamics

$$\frac{dG_{brain}}{dt} = \frac{T_{max}\left(G_{plasma} - \frac{G_{brain}}{V_d}\right)}{K_t + \frac{G_{brain}}{V_d} + G_{plasma}\left(1 + \frac{G_{brain}}{V_d K_{ii}}\right)} - CMR_{glc}$$

Duarte & Gruetter (2012) *J Neurochem* 121(3):396

Dynamic analysis of glucose transport across the BBB



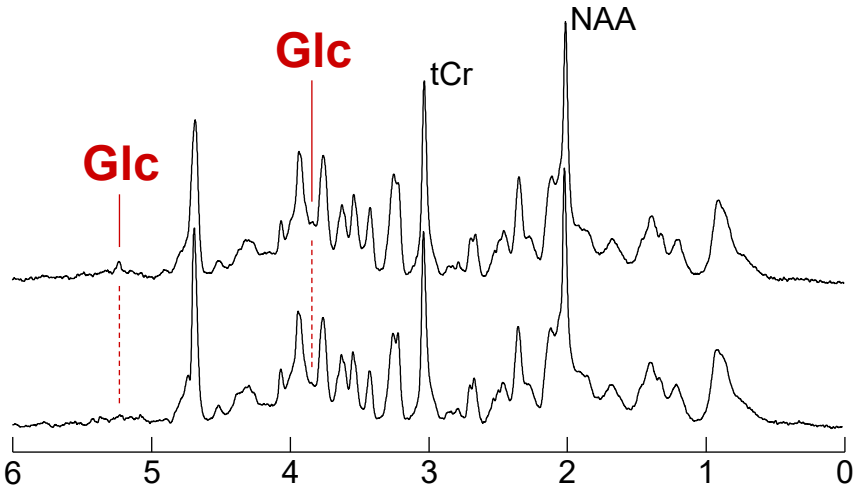
	Conformational model	Reversible MM model
T_{max}	1.03±0.18	0.99±0.18
CMR_{glc}	0.52±0.09	0.59±0.11
K_t	1.43±0.33	0.64±0.27
K_{ii}	50.3±16.0	

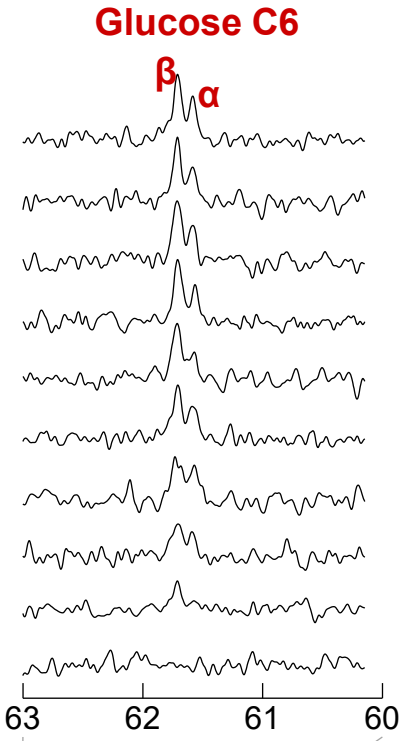
		Steady-state measurement	Dynamic measurement
Rev. MM model	T_{max}/CMR_{glc}	1.73±0.08	1.68±0.08
	K_t	0.32±0.66	0.64±0.27
Conf. model	T_{max}/CMR_{glc}	2.06±1.21	2.03±0.21
	K_t	1.49±4.32	1.43±0.33
	K_{ii}	26.4±96.8	50.3±16.0

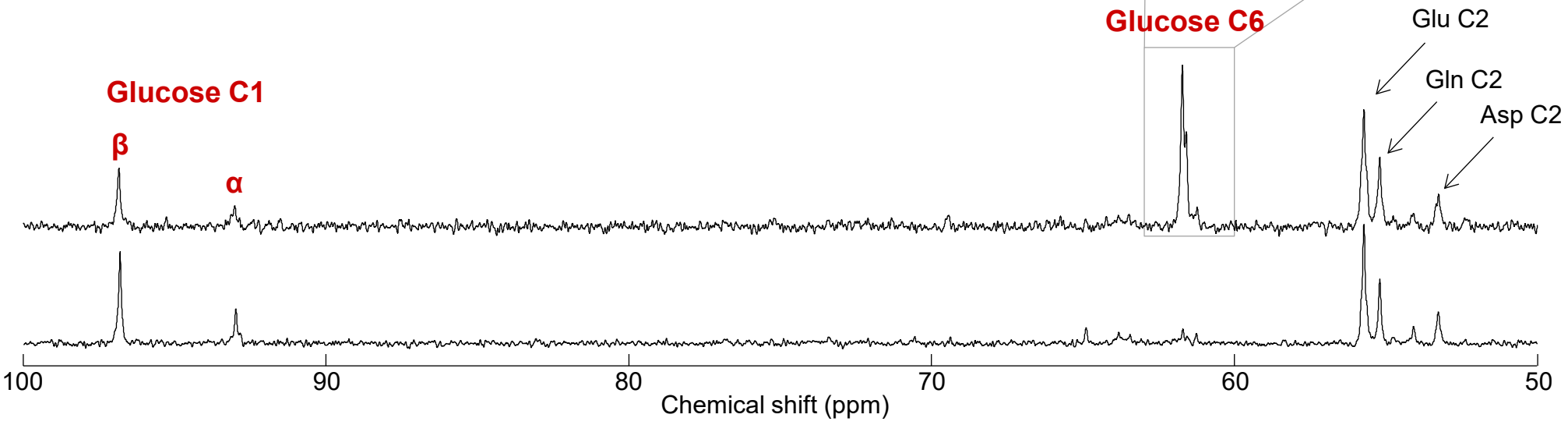
T_{max} and CMR_{glc} are given in µmol/g/min
K_t and K_{ii} are in mM (mean±SEM, n=7).

Duarte & Gruetter (2012) *J Neurochem* 121(3):396

Glucose transport by ^{13}C MRS – some advantages







Glucose transport by ^{13}C MRS – some advantages

^{12}C has zero net spin

not detectable by NMR

^{13}C tracing

(think of FDG-PET, but add chemical specificity)

^{13}C is a stable isotope with 1.1% of natural abundance (of C atoms)

gyromagnetic ratio is 1/4 that of ^{1}H

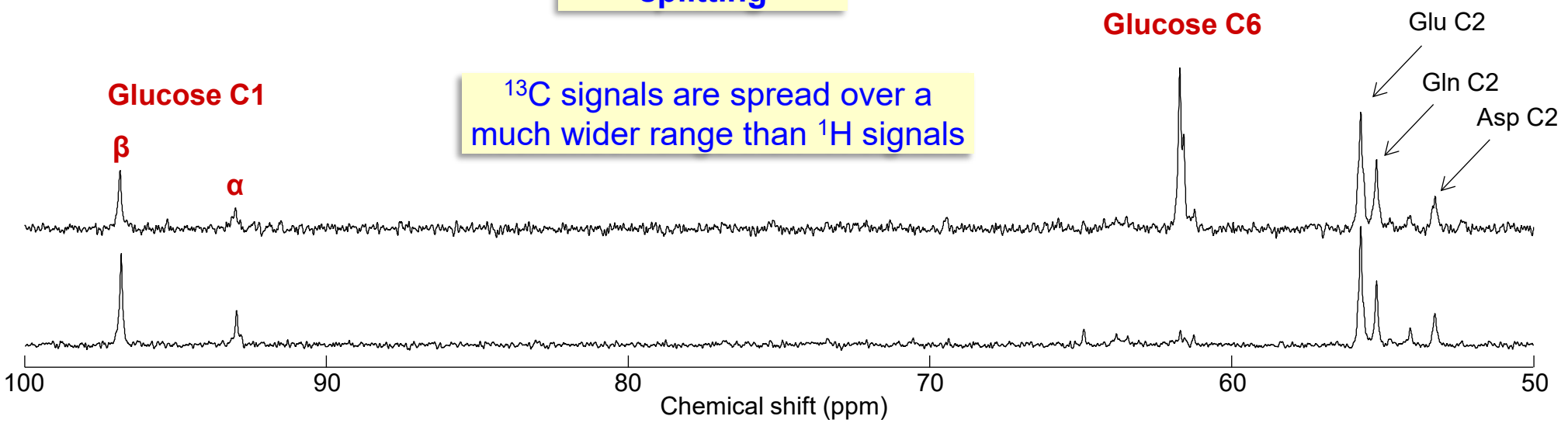
Reduced sensitivity

(~400 times less sensitive than ^{1}H)

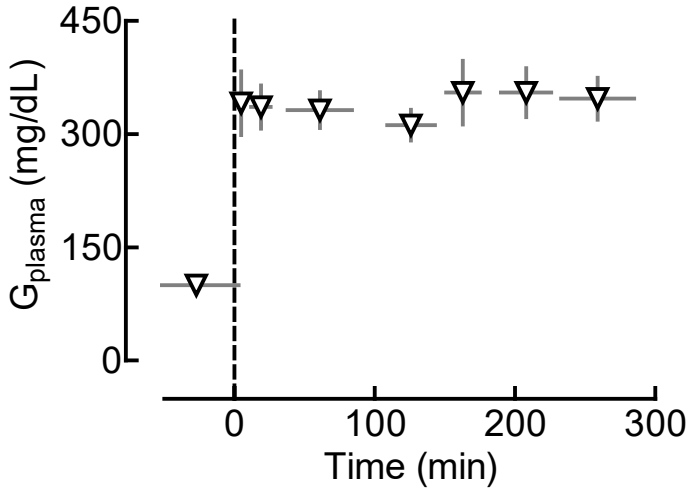
Sensitivity boost with hyperpolarized NMR

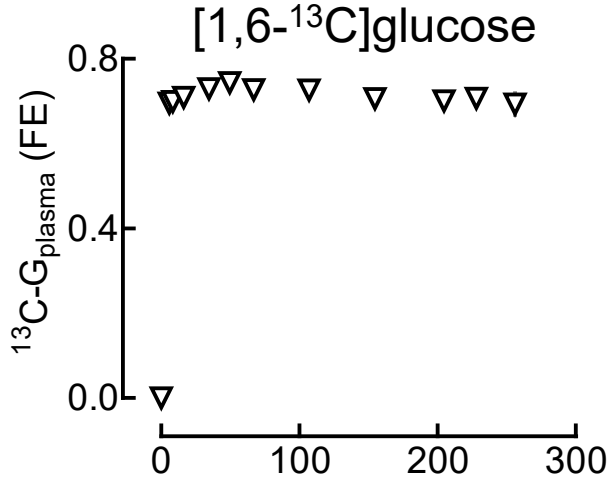
Large J-coupling constants between ^{13}C and ^{1}H

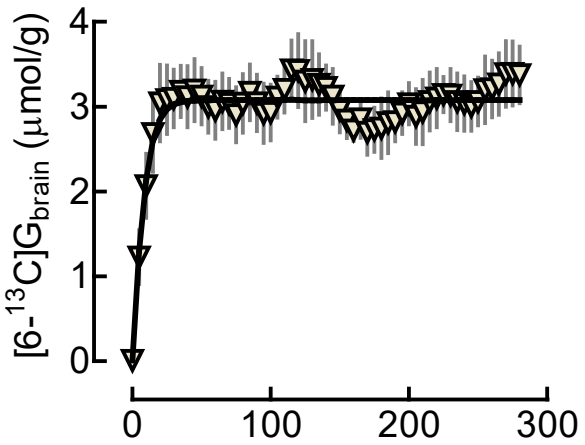
^{1}H decoupling to remove signal splitting



Glucose transport by ^{13}C MRS – similar kinetic modelling approach







$$\frac{d^{13}G_{brain}}{dt} = T_{max}\frac{^{13}G_{plasma}(t) - \frac{^{13}G_{brain}(t)}{V_d}}{K_t + \frac{G_{brain}(t)}{V_d} + G_{plasma}(t)} - CMR_{glc}\frac{^{13}G_{brain}(t)}{G_{brain}(t)}$$

$$\frac{dG_{brain}}{dt} = T_{max}\frac{G_{plasma}(t) - \frac{G_{brain}(t)}{V_d}}{K_t + \frac{G_{brain}(t)}{V_d} + G_{plasma}(t)} - CMR_{glc}$$

T_{max} = 0.91±0.03 µmol/g/min

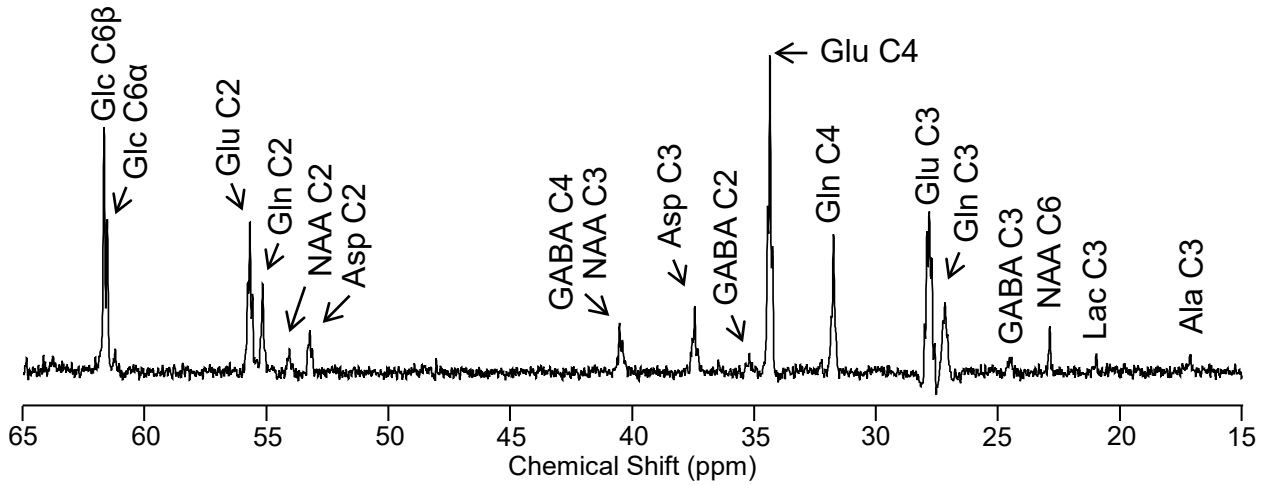
K_t = 0.32±0.10 mM

CMR_{glc} = 0.50±0.02 µmol/g/min

T_{max}/CMR_{glc} = 1.82±0.04

Duarte *et al*. (2009) *Front Neuroenergetics* 1:6

^{13}C-based metabolic flux analysis (MFA)

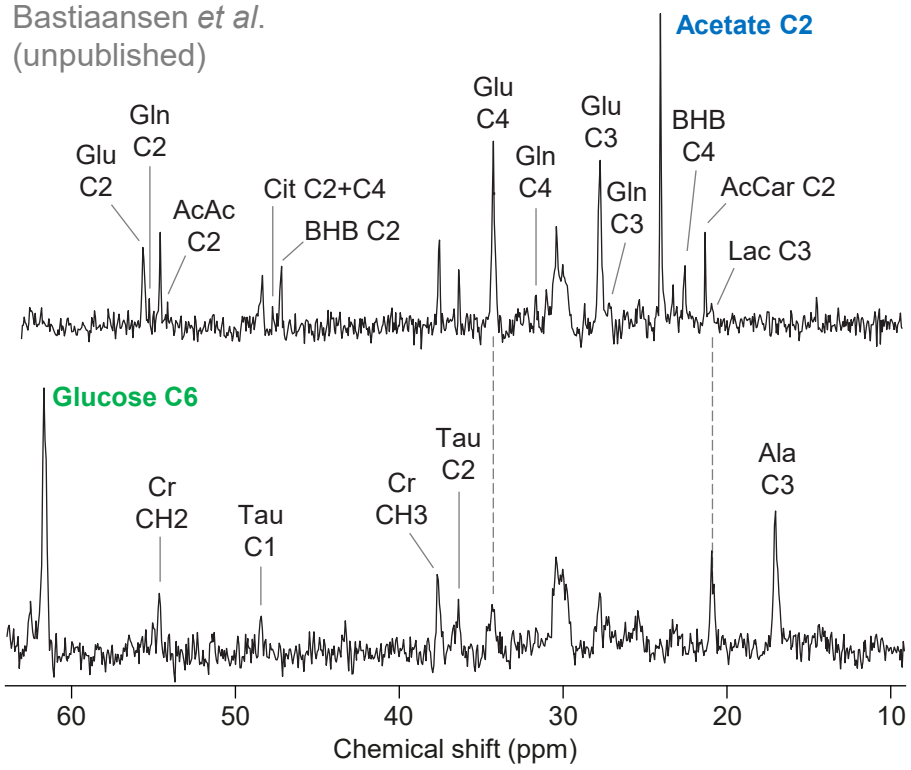


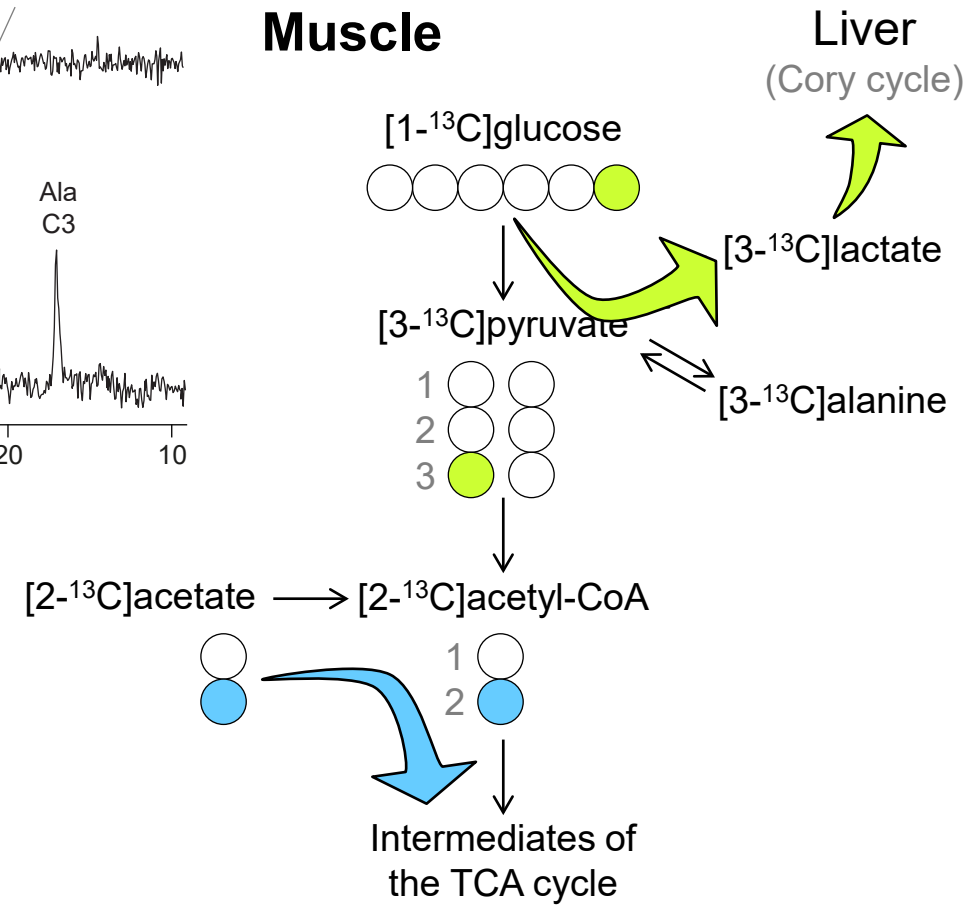
Choice of the ^{13}C tracer

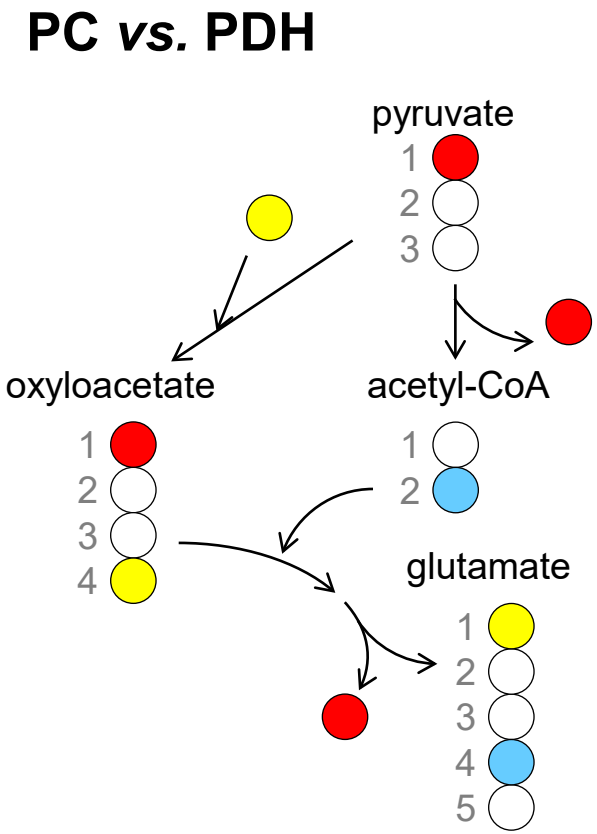
Must not affect the biochemical system

Dictates isotopomer distributions

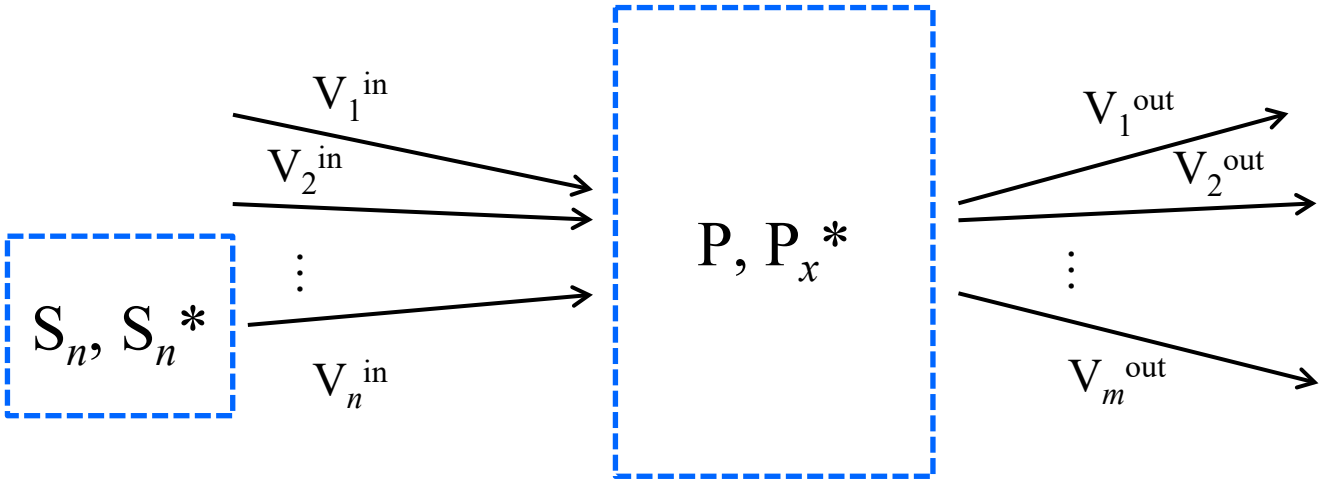
Influences flux estimation







Modeling principles



Balance equations:

$$\frac{dP(t)}{dt} = \sum_i^n V_i^{in} - \sum_j^m V_j^{out} = 0$$

$$\frac{dP_x^*(t)}{dt} = \sum_i^n V_i^{in} \frac{S_i^*(t)}{S} - \sum_j^m V_j^{out} \frac{P_x^*(t)}{P}$$

P - whole content

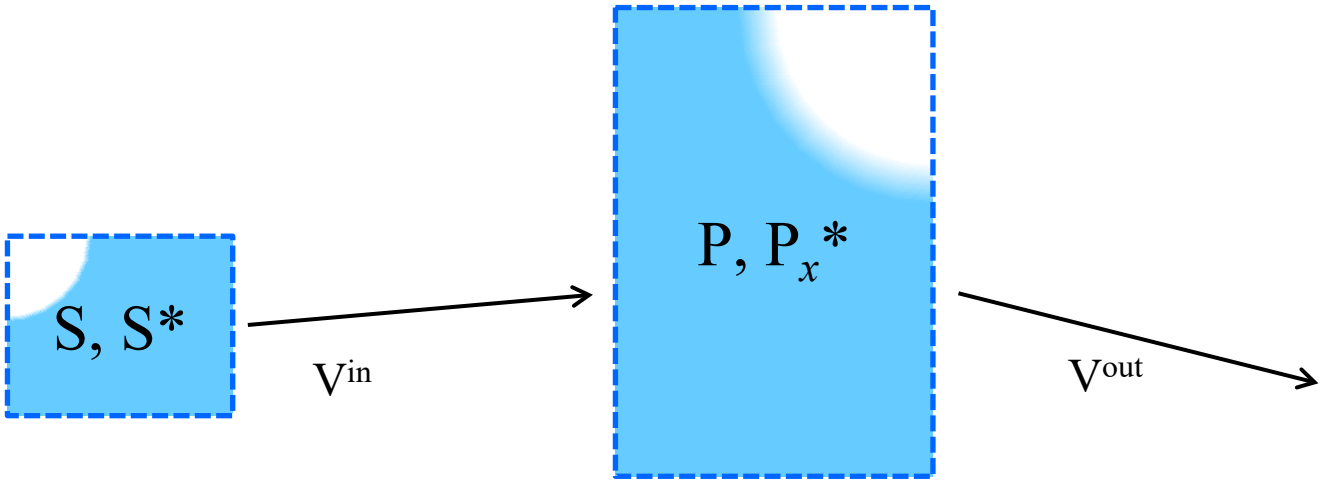
P_x^* - label fraction

Fractional Enrichment

$FE = P_x^*/P$

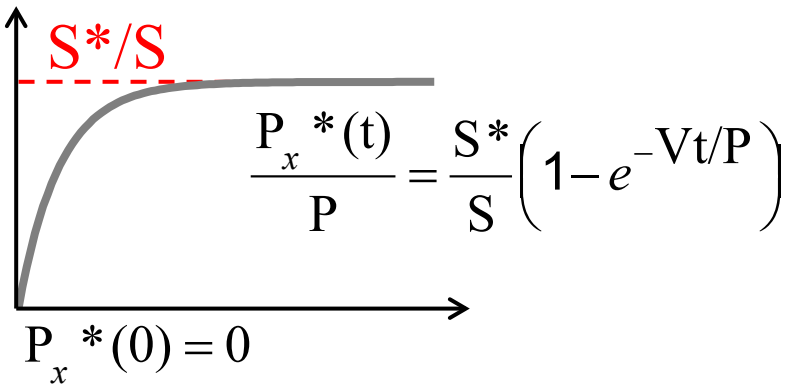
Adapted from *Front Neuroendocrinol* 4:156 (2013)

Example: single pool, substrate with constant FE



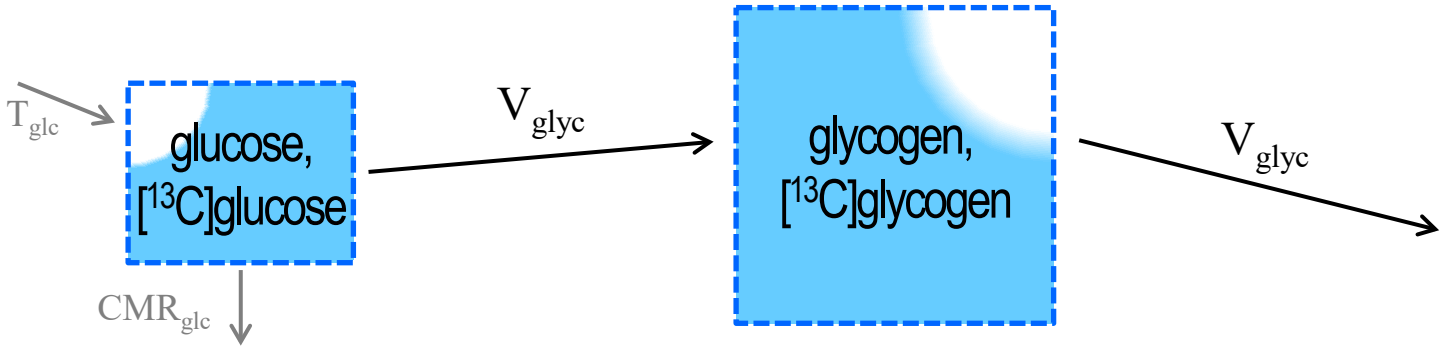
$$\frac{dP(t)}{dt} = V^{in} - V^{out} = 0$$

$$\frac{dP_x^*(t)}{dt} = V^{in}\frac{S^*(t)}{S} - V^{out}\frac{P_x^*(t)}{P}$$

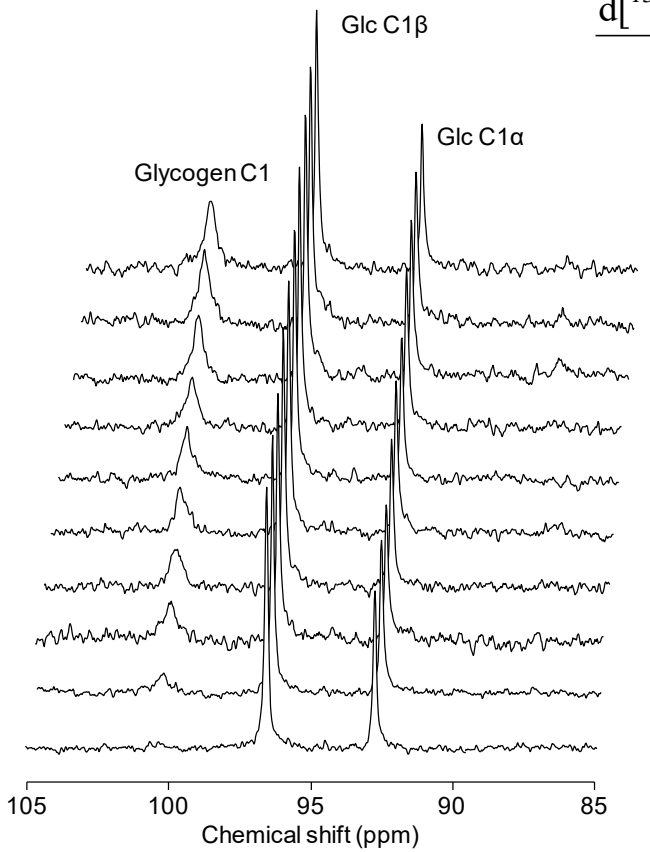


Adapted from *Front Neuroendocrinol* 4:156 (2013)

Brain glycogen metabolism in the diabetic brain



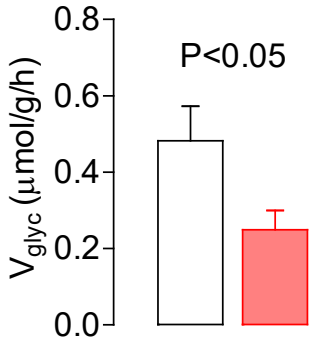
$$\frac{d[^{13}C]glycogen}{dt} = V_{glyc}\left(\frac{[^{13}C]glucose}{glucose} - \frac{[^{13}C]glycogen}{glycogen}\right)$$



important assumptions:

(1) labelling of glucose-6-phosphate resembles that of glucose because $V_{glyc} << CMR_{glc}$

(2) glycogen concentration is constant, implying that synthesis and degradation fluxes are equal (V_{glyc})



[1-^{13}C]glucose administered to GK rats under isoflurane
Soares et al., J Neurosci Res 2019

Main ingredients for designing MFA models

Abstraction level: models should be as simple as possible and as complex as necessary.

focus on pathways of interest
vs.
neglect presence or influence of all other processes

Modelling principles:
Mass and energy conservation.
Fluxes represented in terms of the system state (metabolite concentrations)

Set of balance equations describing system dynamics and/or equilibrium

Assumptions and data sources must be documented precisely.

Judge applicability conditions!

Wiechert (2002) *J Biotechnol* 94:37

Construction of a metabolic model for ^{13}C MFA

1 primary assumptions

Enzymes do not discriminate between ^{13}C and ^{12}C

not true for CO_2-fixing enzyme Rubisco

Plant Physiol 101:37, 1993

Each metabolite pool is homogeneous

There's no diffusion limitation within the same compartment

not true for channelling of metabolites in multi-enzyme complexes

Trends Biochem Sci 15:411, 1990

Labelling in a metabolite pool is well mixed before each reaction

Probability of leaving the pool is the same for all molecules

Construction of a metabolic model for ^{13}C MFA

2 <u>stoichiometric balance equations</u>

Model reduction/simplification

Omit:
- ✓repressed pathways
- ✓cofactors

Combine:
- ✓isoenzymes
- ✓linear and non-bifurcated pathways
- ✓intermediate pools that equilibrate rapidly

Keep complexity to an extent that is feasible

Reversibility of reactions

Compartmentation: Location/site of reactions; transport across compartments

Reactions with CO_2 fixation: uncertain dilution due to decarboxylation reactions

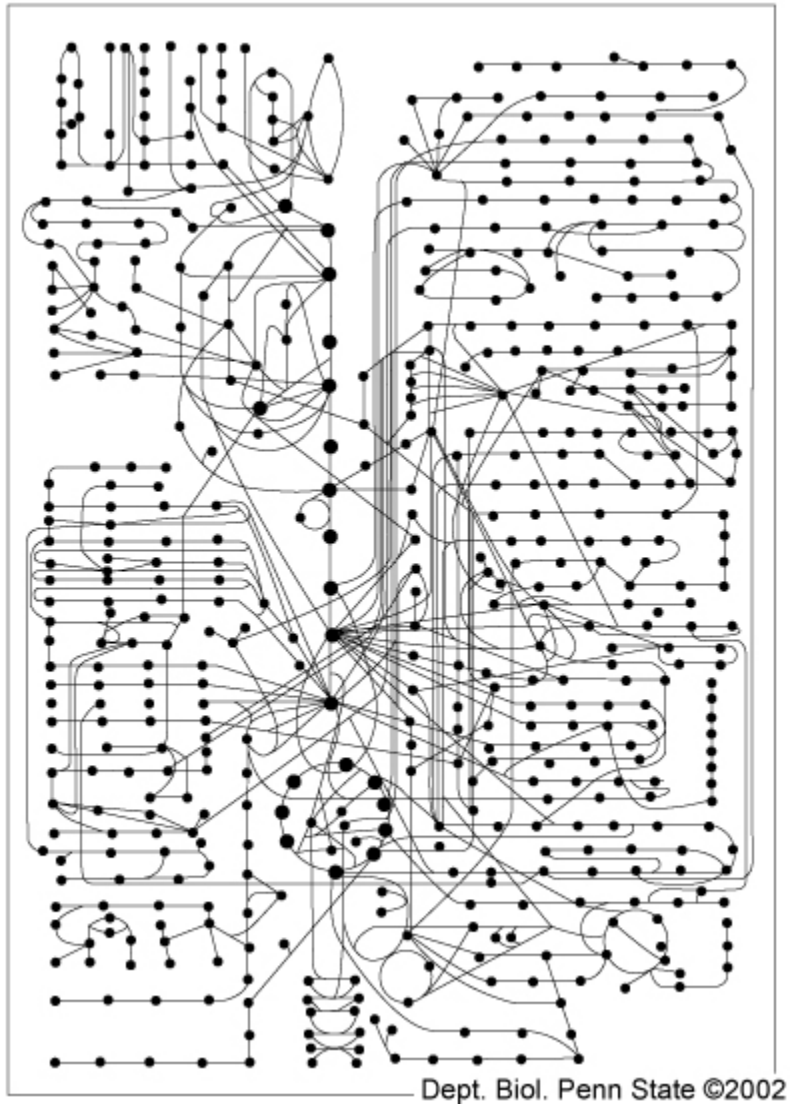


Diagram depicting only a fraction of the metabolic pathways in a cell.

Construction of a metabolic model for ^{13}C MFA

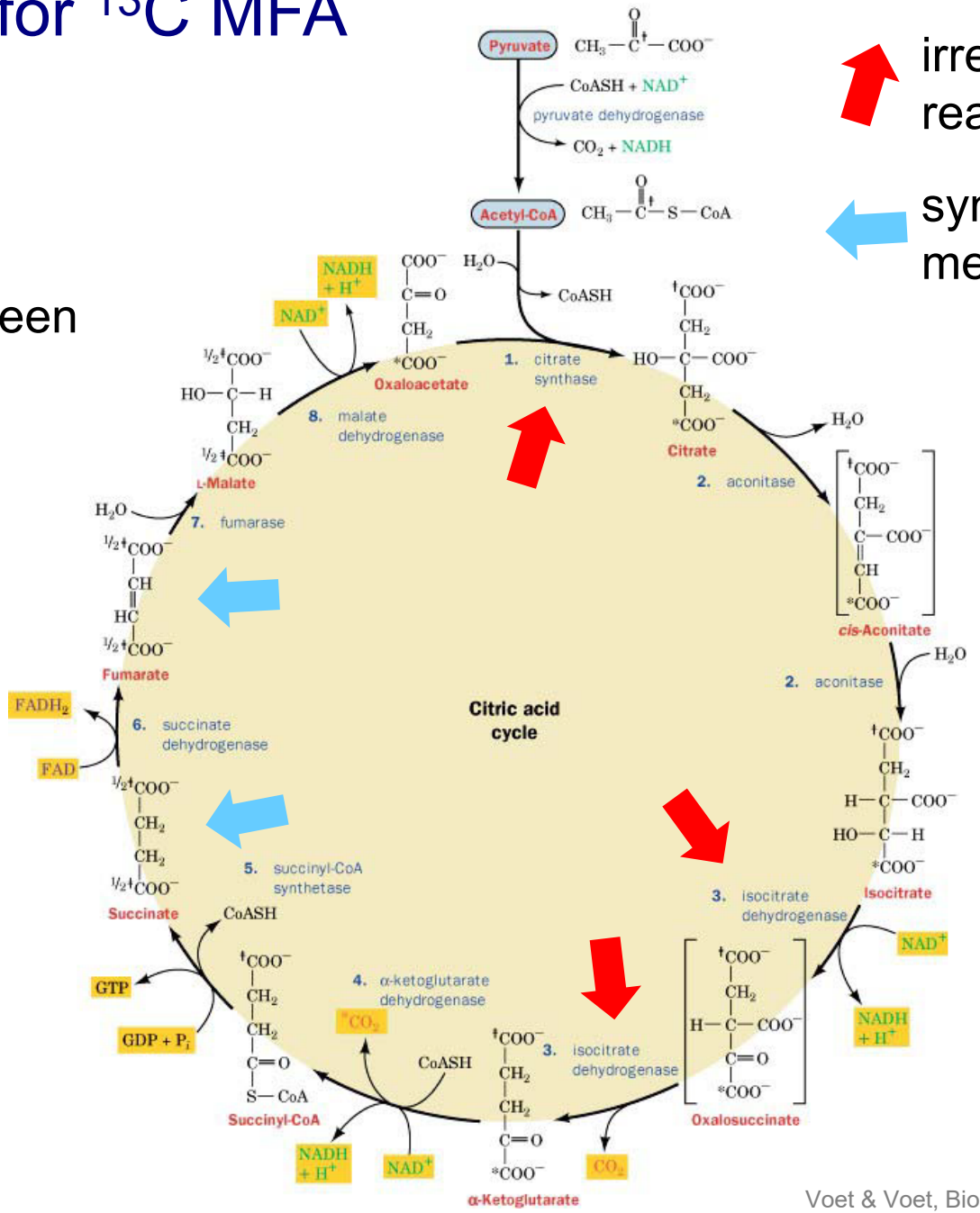
3 <u>mapping carbon atoms</u>

Describe matching of carbon atoms between each metabolite and its precursor.

- ✓Mostly conserved among species
- ✓Available from pre-existing models

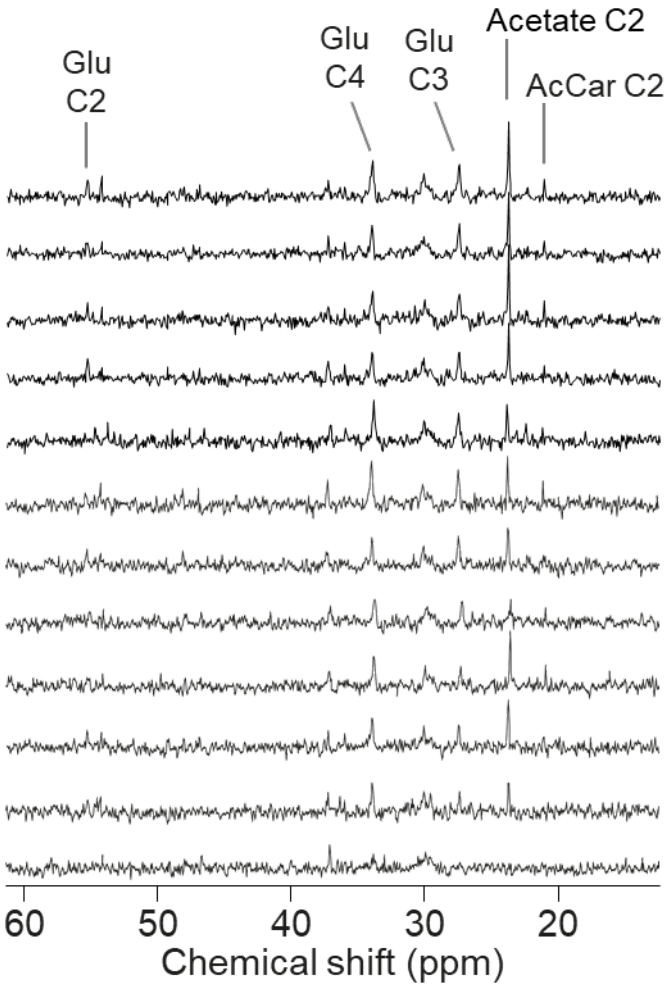
Center of symmetry in molecules causes labelling redistribution.

Origin of ^{13}C labelling, dilution reactions and labelling loss



Voet & Voet, Biochemistry, 3rd Edition

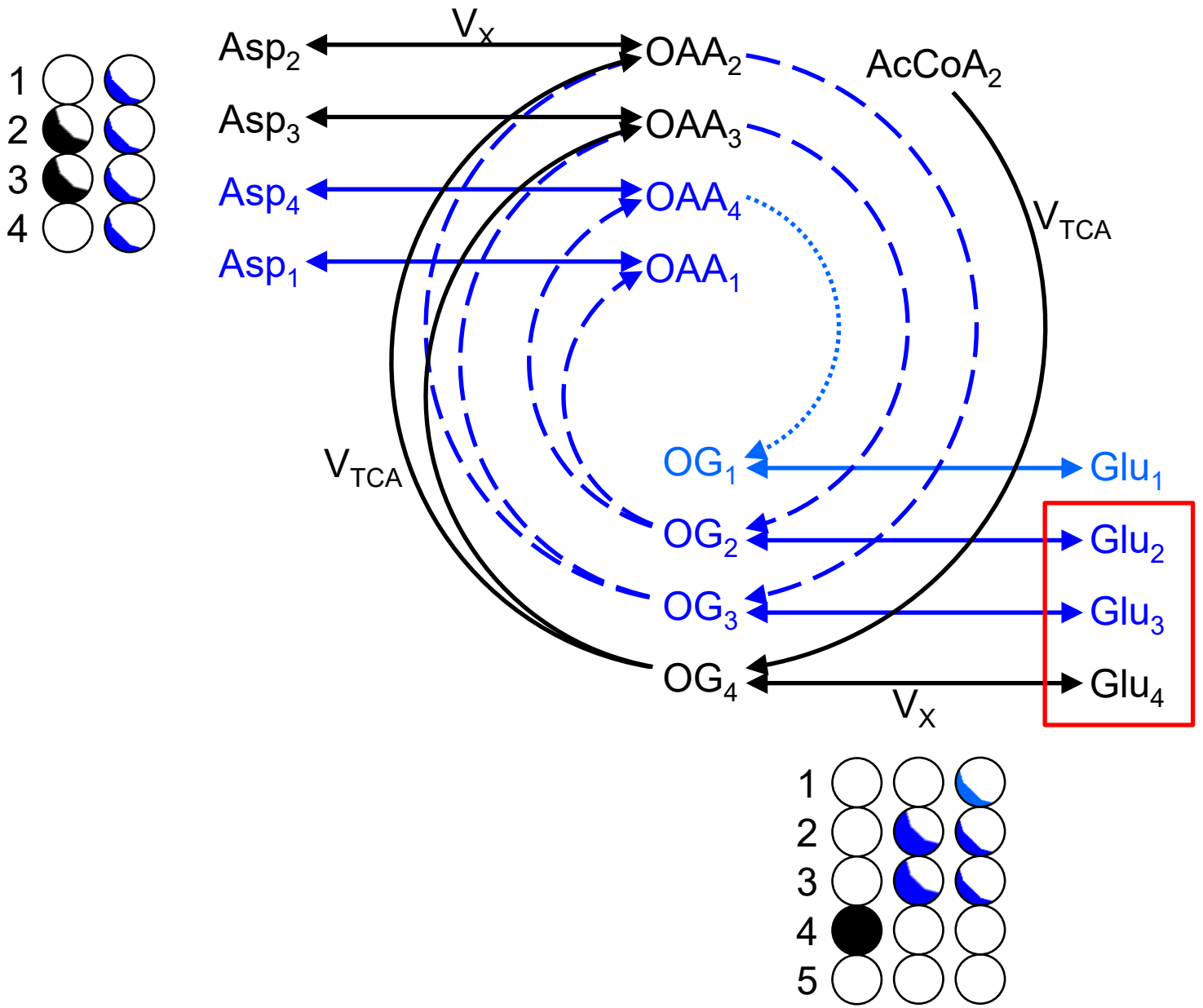
TCA cycle flux in the skeletal muscle



Bastiaansen *et al*. (unpublished)

[2-^{13}C]acetate infusion under isoflurane anesthesia; DEPT in rat leg at 14.1 T

^{13}C labeling of TCA cycle intermediates

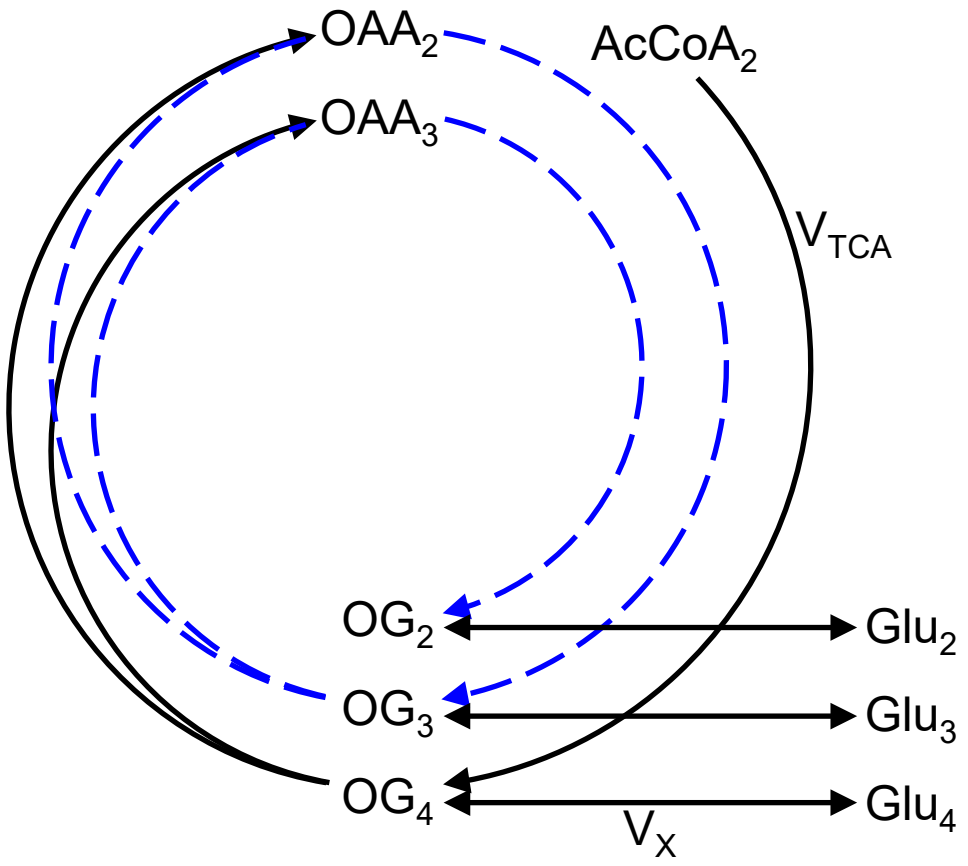


^{13}C labeling of TCA cycle intermediates

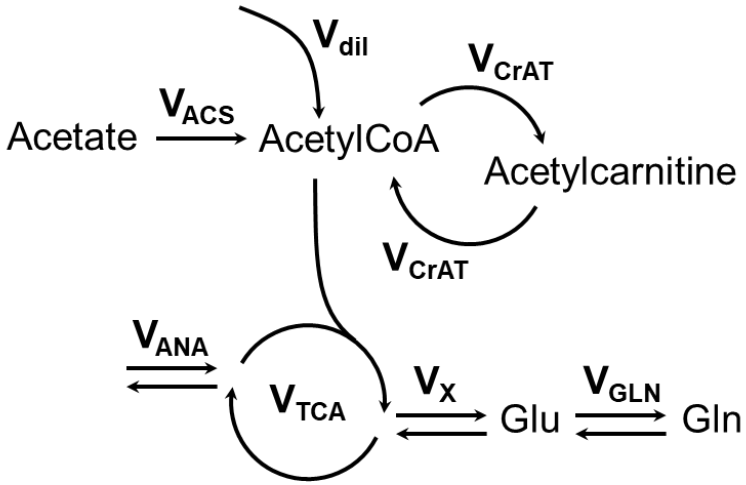
Mathematical equations defining isotopic labelling

$$\begin{cases} \dfrac{d^{13}OG_4}{dt} = V_{TCA}\dfrac{^{13}AcCoA_2}{AcCoA} + V_X\dfrac{^{13}Glu_4}{Glu} - \left(V_{TCA}+V_X\right)\dfrac{^{13}OG_4}{OG} \\ \dfrac{d^{13}OG_3}{dt} = V_{TCA}\dfrac{^{13}OAA_2}{OAA} + V_X\dfrac{^{13}Glu_3}{Glu} - \left(V_{TCA}+V_X\right)\dfrac{^{13}OG_3}{OG} \\ \dfrac{d^{13}OG_2}{dt} = V_{TCA}\dfrac{^{13}OAA_3}{OAA} + V_X\dfrac{^{13}Glu_2}{Glu} - \left(V_{TCA}+V_X\right)\dfrac{^{13}OG_2}{OG} \end{cases}$$

$$\begin{cases} \dfrac{d^{13}OAA_2}{dt} = \dfrac{1}{2}V_{TCA}\dfrac{^{13}OG_4+^{13}OG_3}{OG} + V_{ANA}0.011 - \left(V_{TCA}+V_{ANA}\right)\dfrac{^{13}OAA_2}{OAA} \\ \dfrac{d^{13}OAA_3}{dt} = \dfrac{1}{2}V_{TCA}\dfrac{^{13}OG_4+^{13}OG_3}{OG} + V_{ANA}0.011 - \left(V_{TCA}+V_{ANA}\right)\dfrac{^{13}OAA_3}{OAA} \end{cases}$$



Designing the mathematical model



Mathematical equations defining isotopic labelling

$$\frac{d^{13}AcCoA_2}{dt} = V_{AC}\frac{^{13}Ac_2(t)}{Ac(t)} + V_{dil}\,0.011 - V_{TCA}\frac{^{13}AcCoA_2}{AcCoA}$$

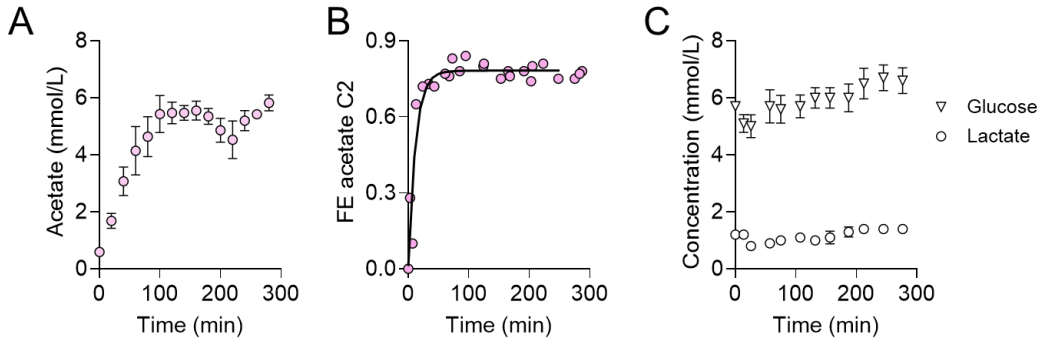
$$\frac{d^{13}Glu_i}{dt} = V_X\left(\frac{^{13}OG_2}{OG} - \frac{^{13}Glu_i}{Glu}\right)$$

Mass balance equations at steady-state

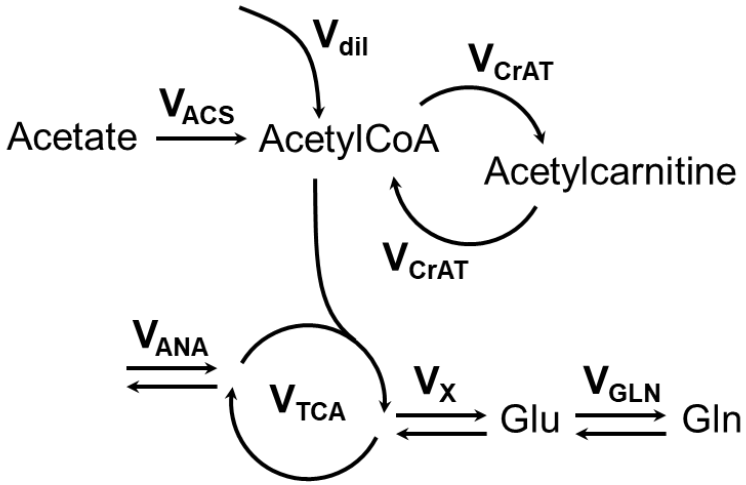
$$\frac{d\,OAA}{dt} = \frac{d\,OG}{dt} = \frac{d\,Glu}{dt} = 0$$

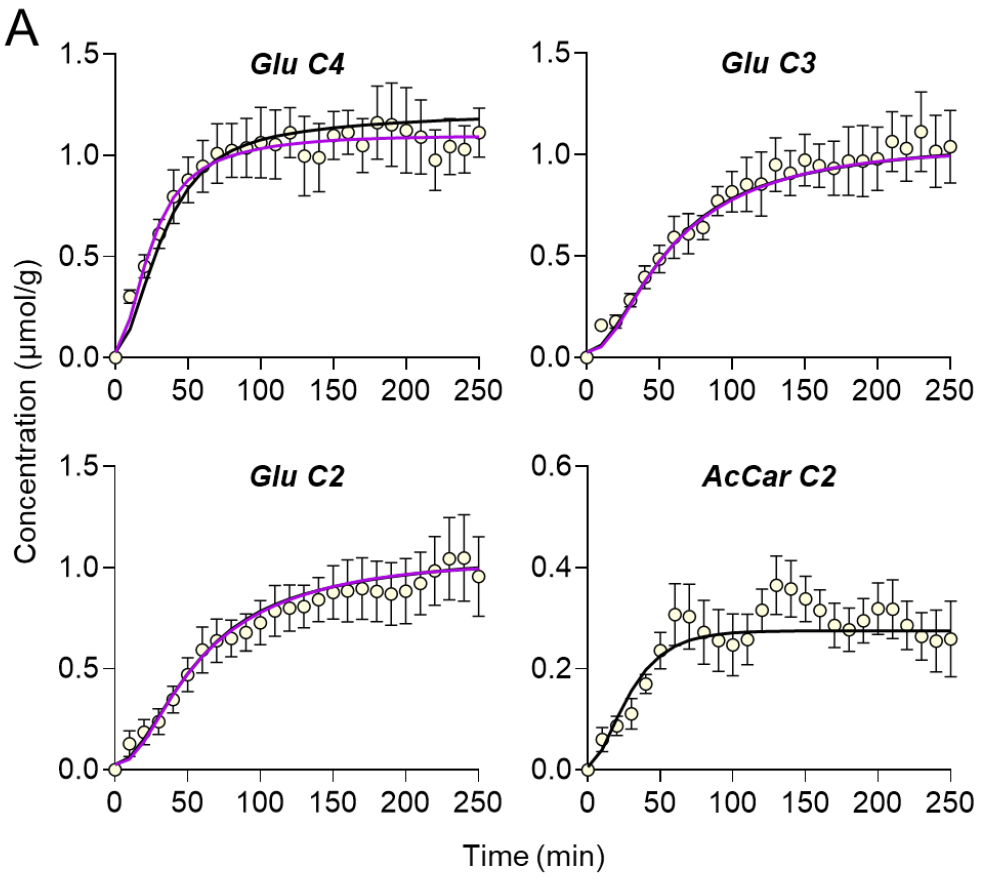
$$\frac{d\,AcCoA}{dt} = V_{AC} + V_{dil} - V_{TCA} = 0 \quad \Rightarrow \quad V_{TCA} = V_{AC} + V_{dil}$$

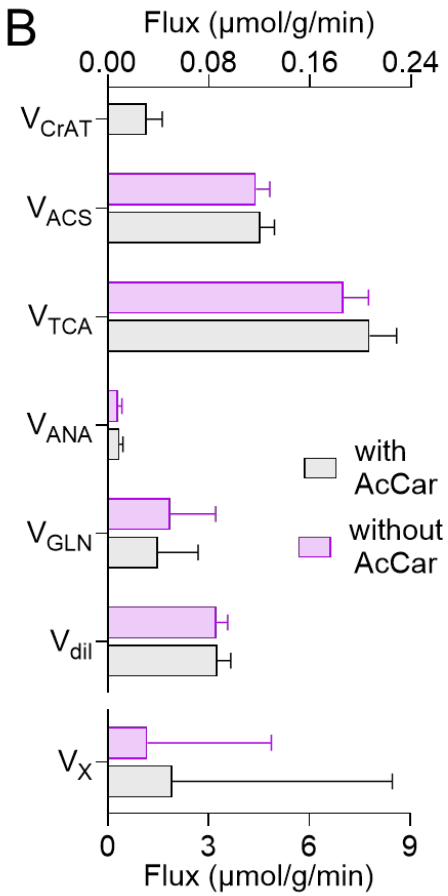
“input function” from plasma [2-^{13}C]acetate



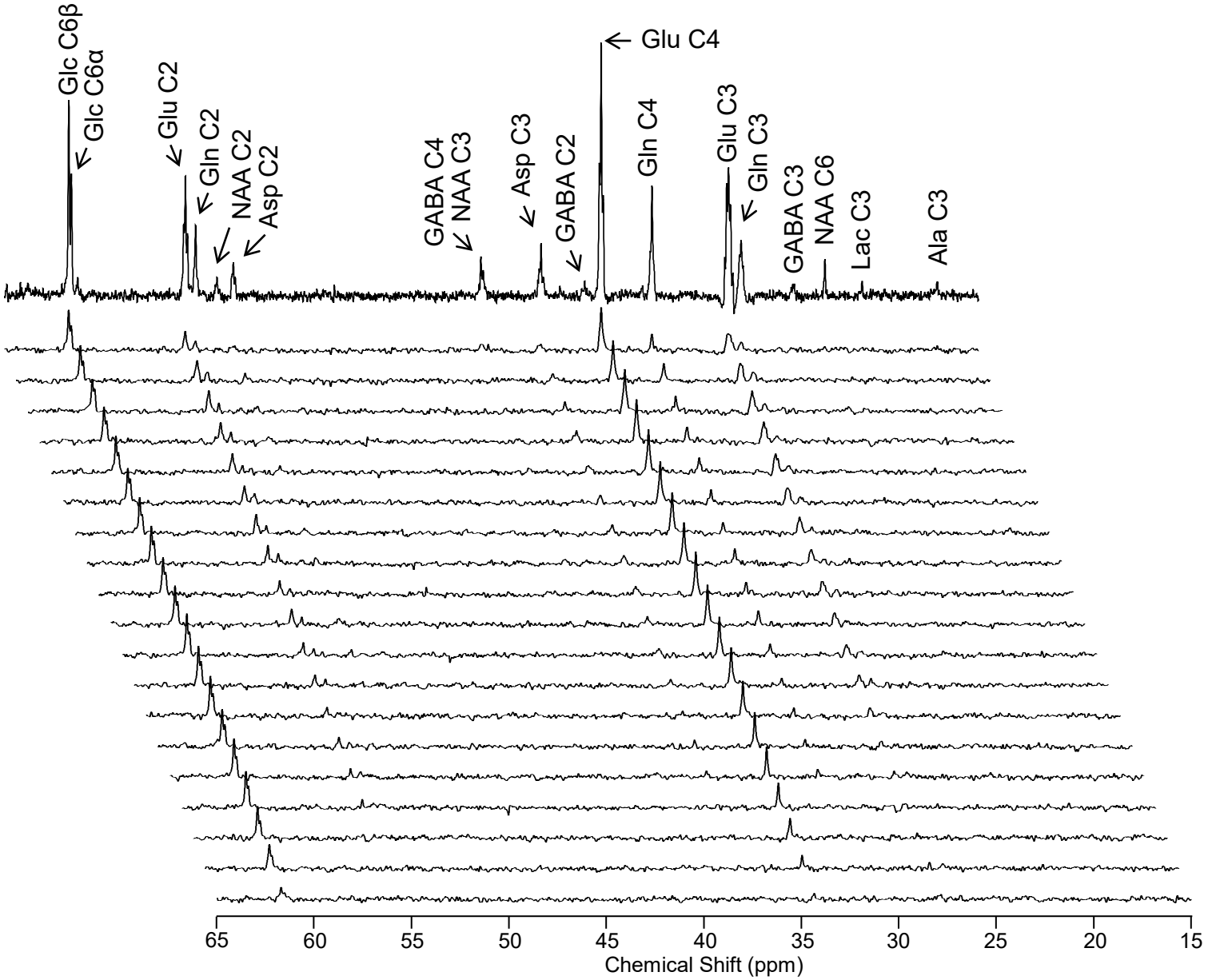
Designing the mathematical model







^{13}C MRS in the rat brain



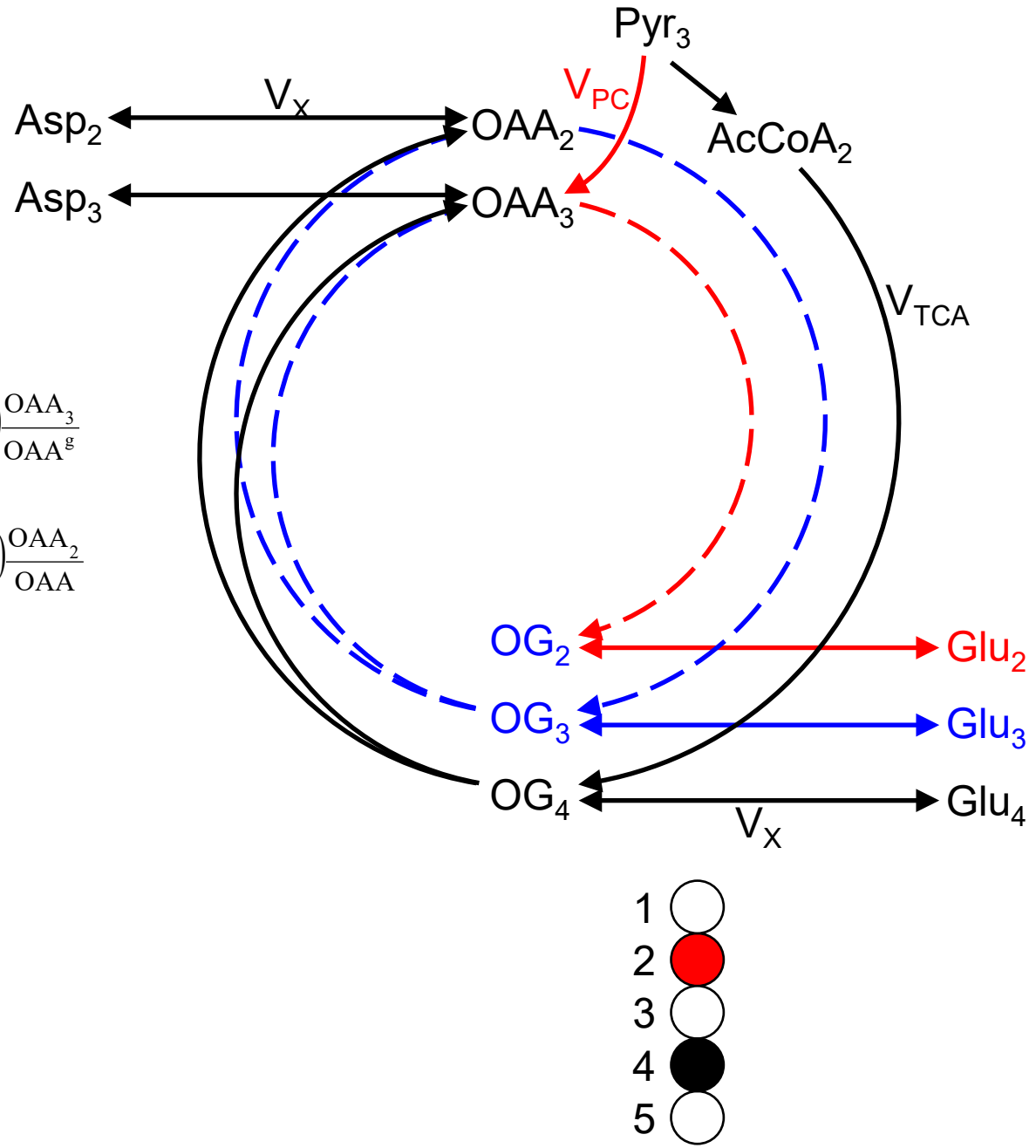
- Infusion of [1,6-^{13}C]glucose
- Direct detection by ^{13}C MRS at 14.1 T

Duarte *et al*. (2011) Front Neuroenergetics 3:3

Pyruvate carboxylation (V_{PC})

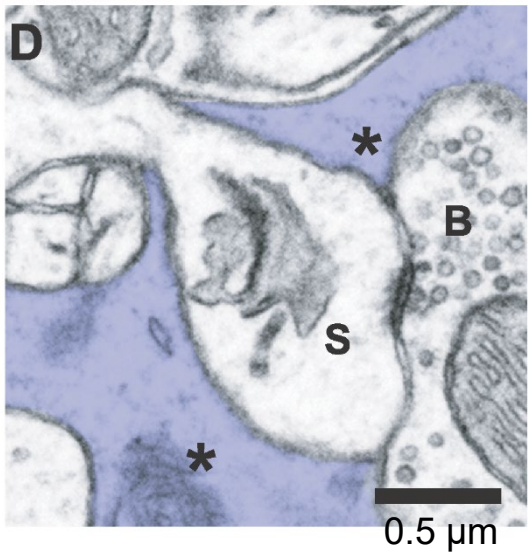
$$\frac{dOAA_3^g}{dt} = \frac{V_g}{2}\left(\frac{OG_4 + OG_3}{OG^g}\right) + V_{PC}\frac{Pyr_3}{Pyr} + V_X\frac{Asp_3}{Asp^g} - \left(V_{TCA} + V_X\right)\frac{OAA_3}{OAA^g}$$

$$\frac{dOAA_2^g}{dt} = \frac{V_g}{2}\left(\frac{OG_4 + OG_3}{OG^g}\right) + V_{PC}0.011 + V_X\frac{Asp_2}{Asp^g} - \left(V_{TCA} + V_X\right)\frac{OAA_2}{OAA}$$



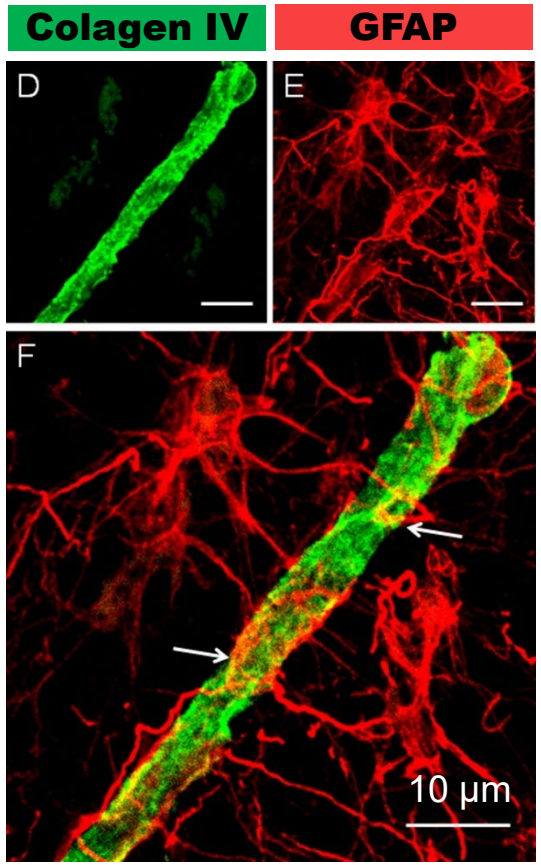
The metabolic importance of astrocytes

Astrocytic processes engulfing synapses

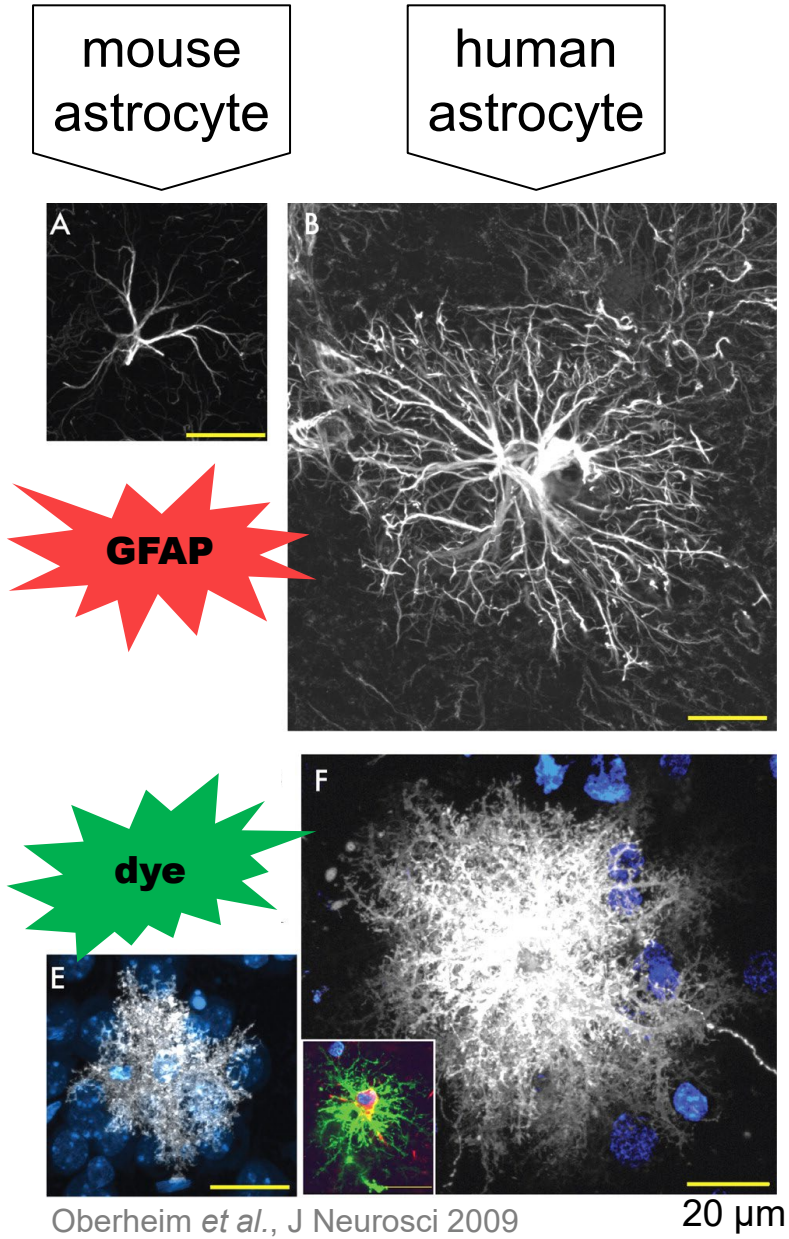


Genoud *et al.*, J Neurosci 2006

Astrocytic endfeet cover blood vessels



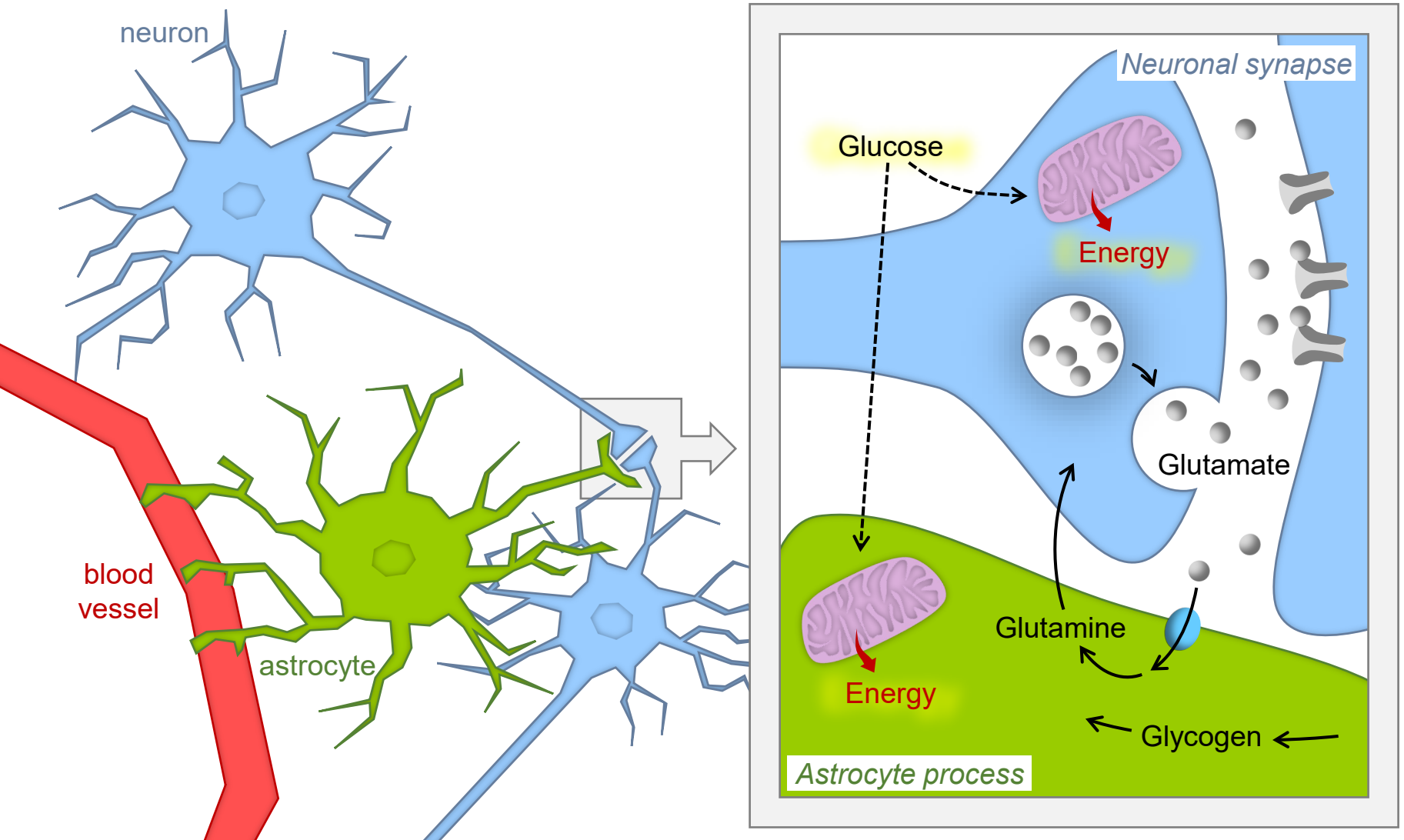
Rajkowska *et al.*, Biol Psy 2013



Oberheim *et al.*, J Neurosci 2009

Further reading: NEWS FEATURE **The 'silent' brain cells that shape our behaviour, memory and health.** Nature 648, 23-25 (2025)

Astrocytes participate in the glutamate-glutamine cycle



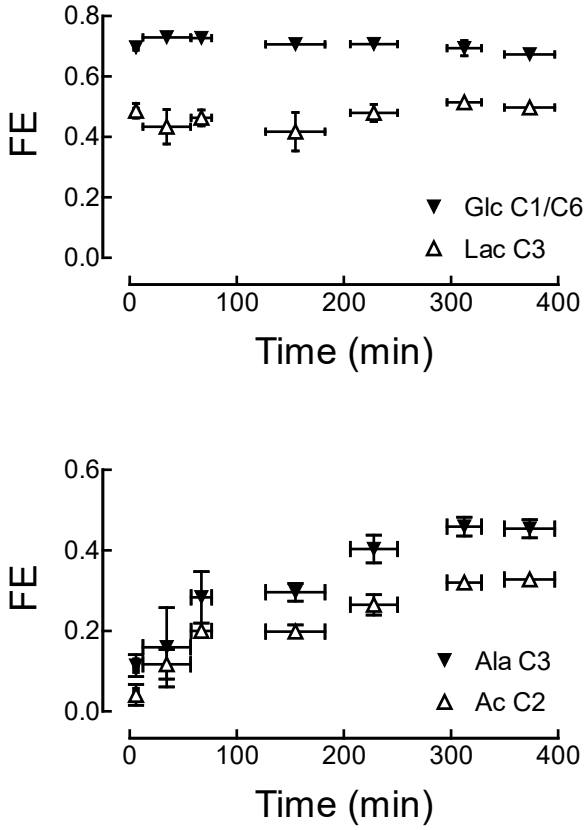
Brain glutamate

- ❑ Excitatory neurotransmitter
- ❑ Key role in plasticity and memory performance
- ❑ The most abundant free amino acid
- ❑ At the crossroad between multiple metabolic pathways
- ❑ Synthesized *de novo* from glucose

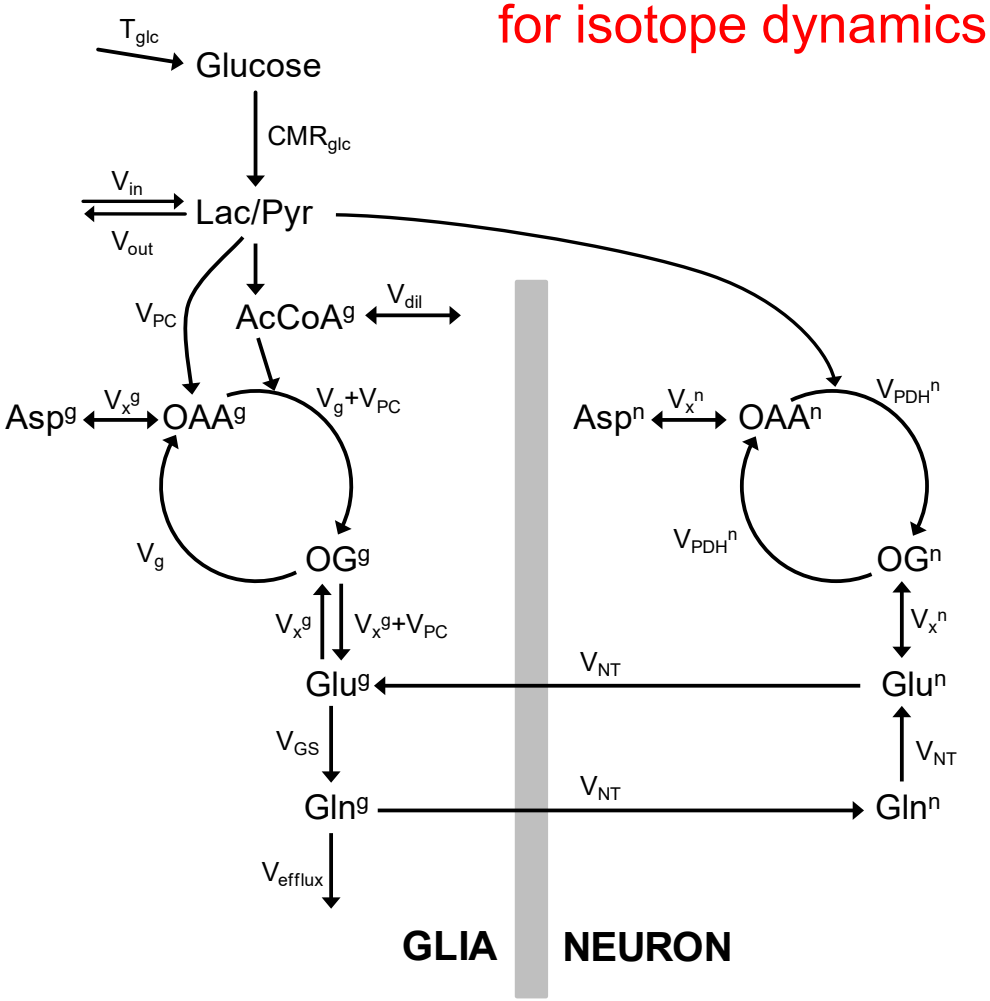
Duarte, Biochem Soc Trans 2023

Model of brain glucose metabolism

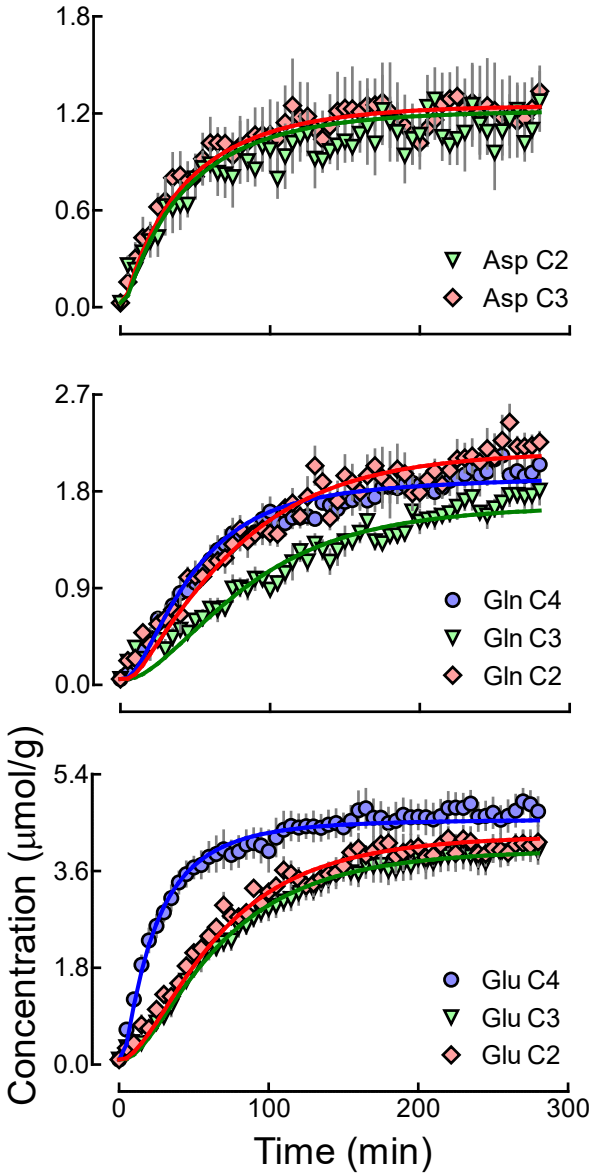
"input function" from plasma metabolites

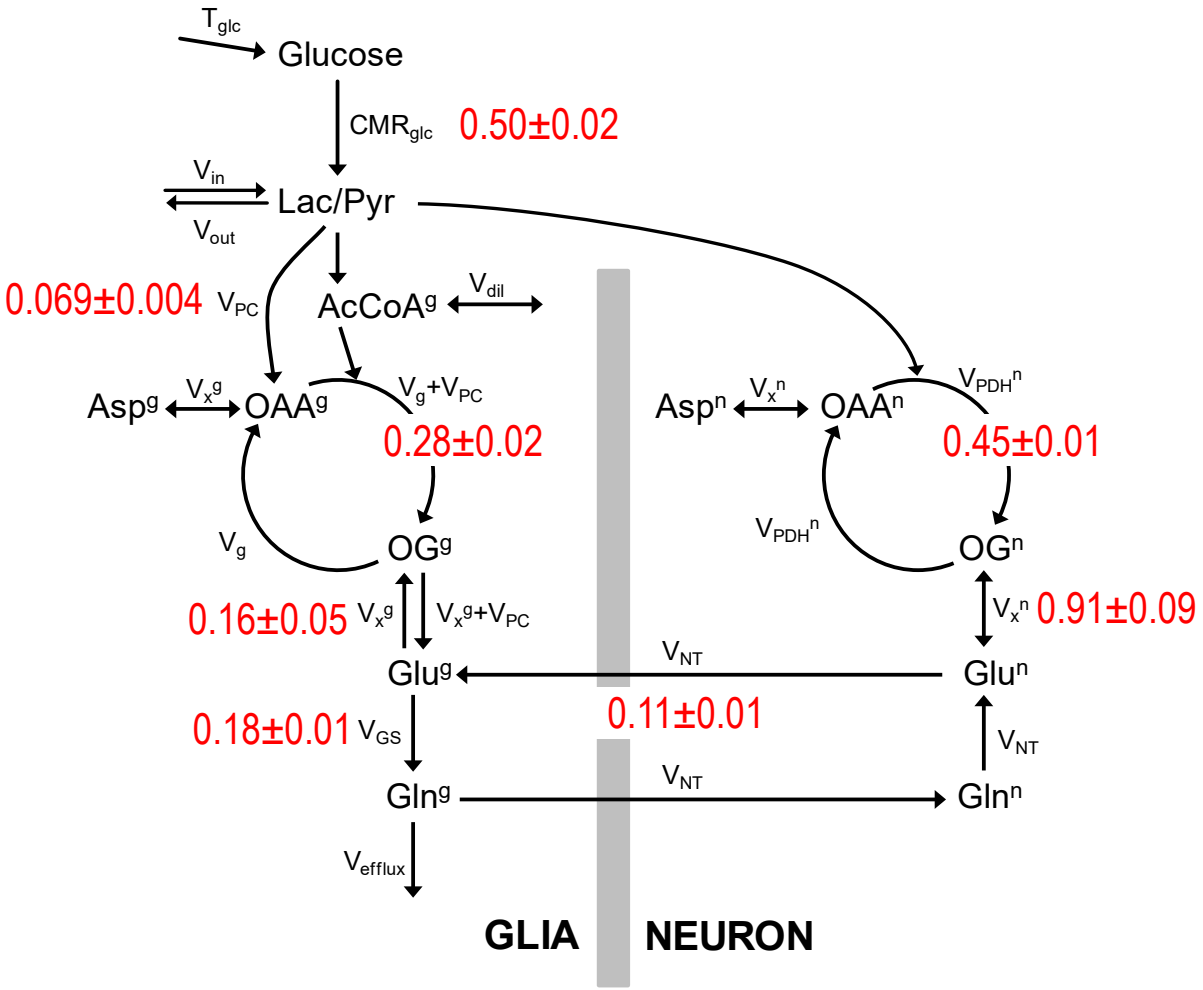


35 differential equations for isotope dynamics



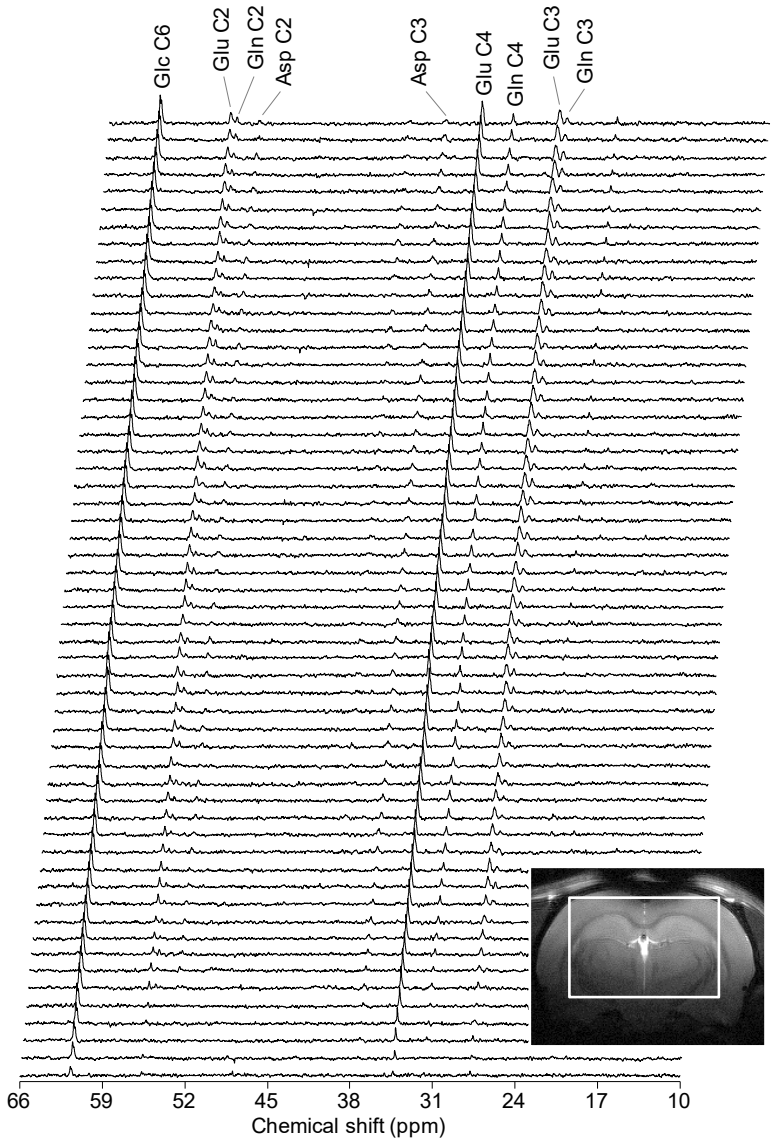
^{13}C-enriched metabolites in the brain

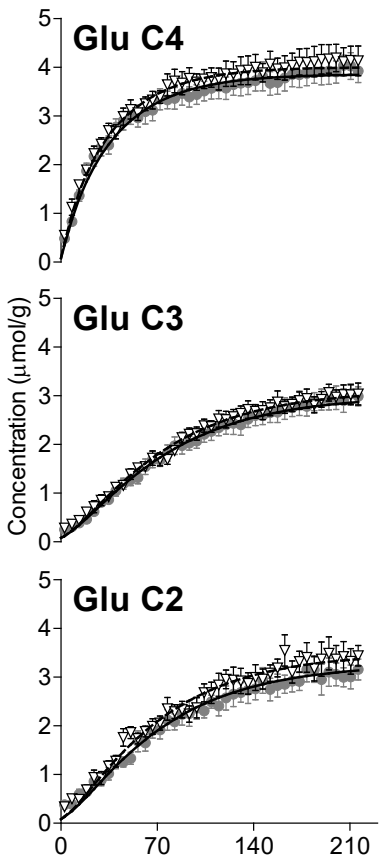


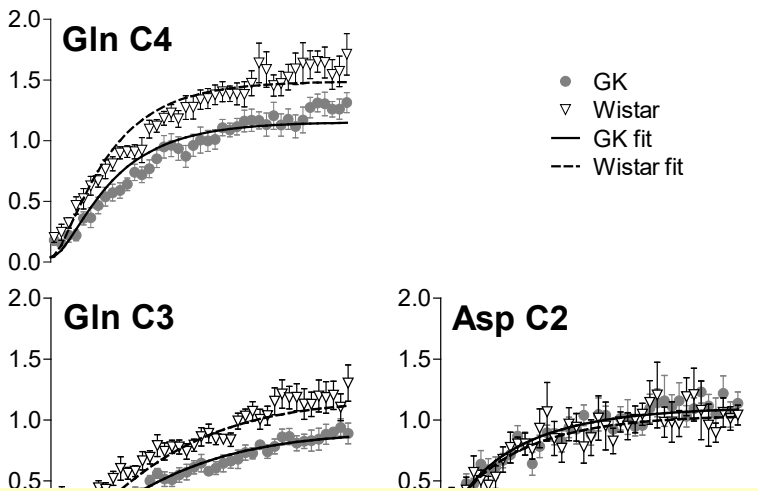


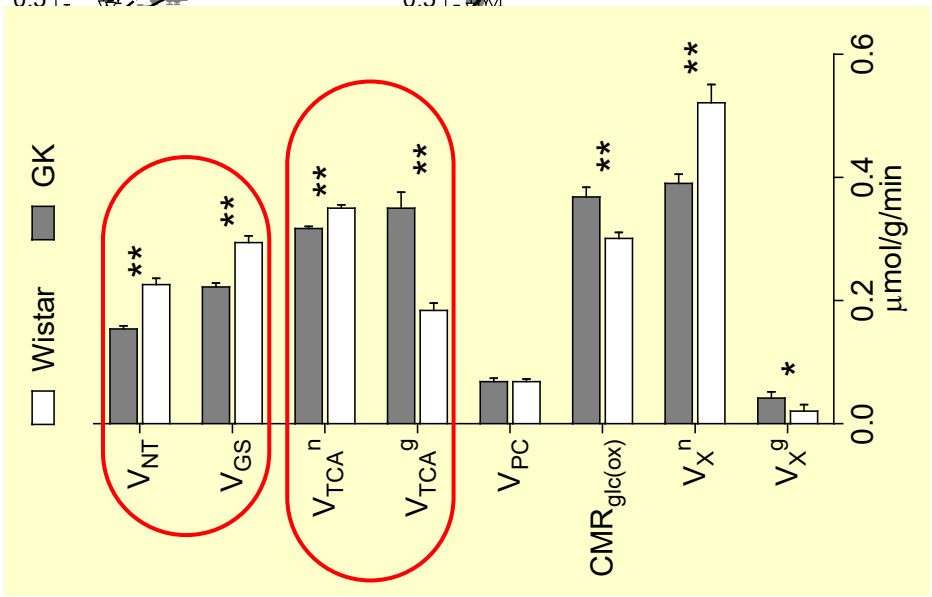
Duarte *et al.* (2011) *Front Neuroenergetics* 3:3

Brain energy metabolism in diabetic rats



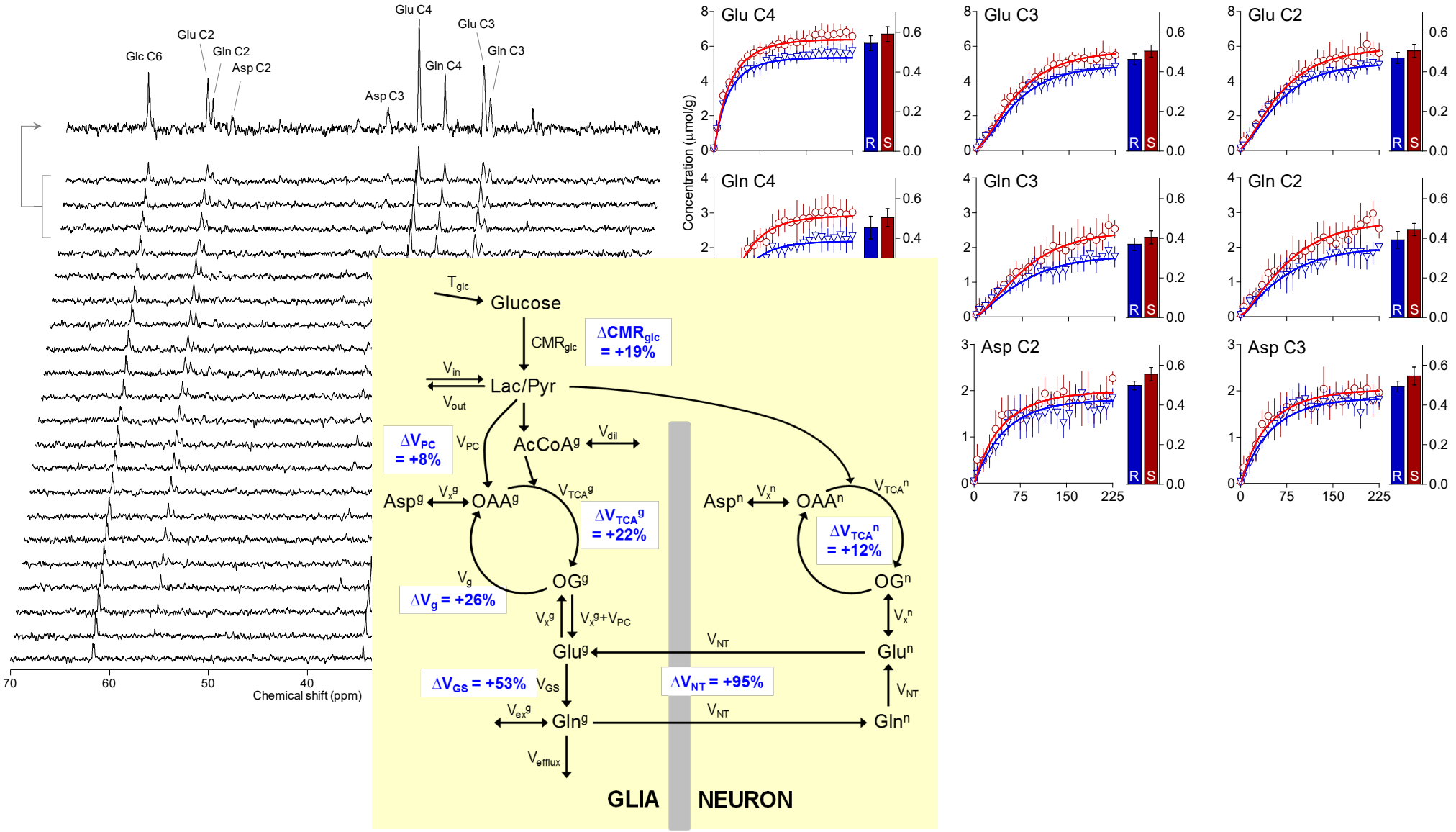




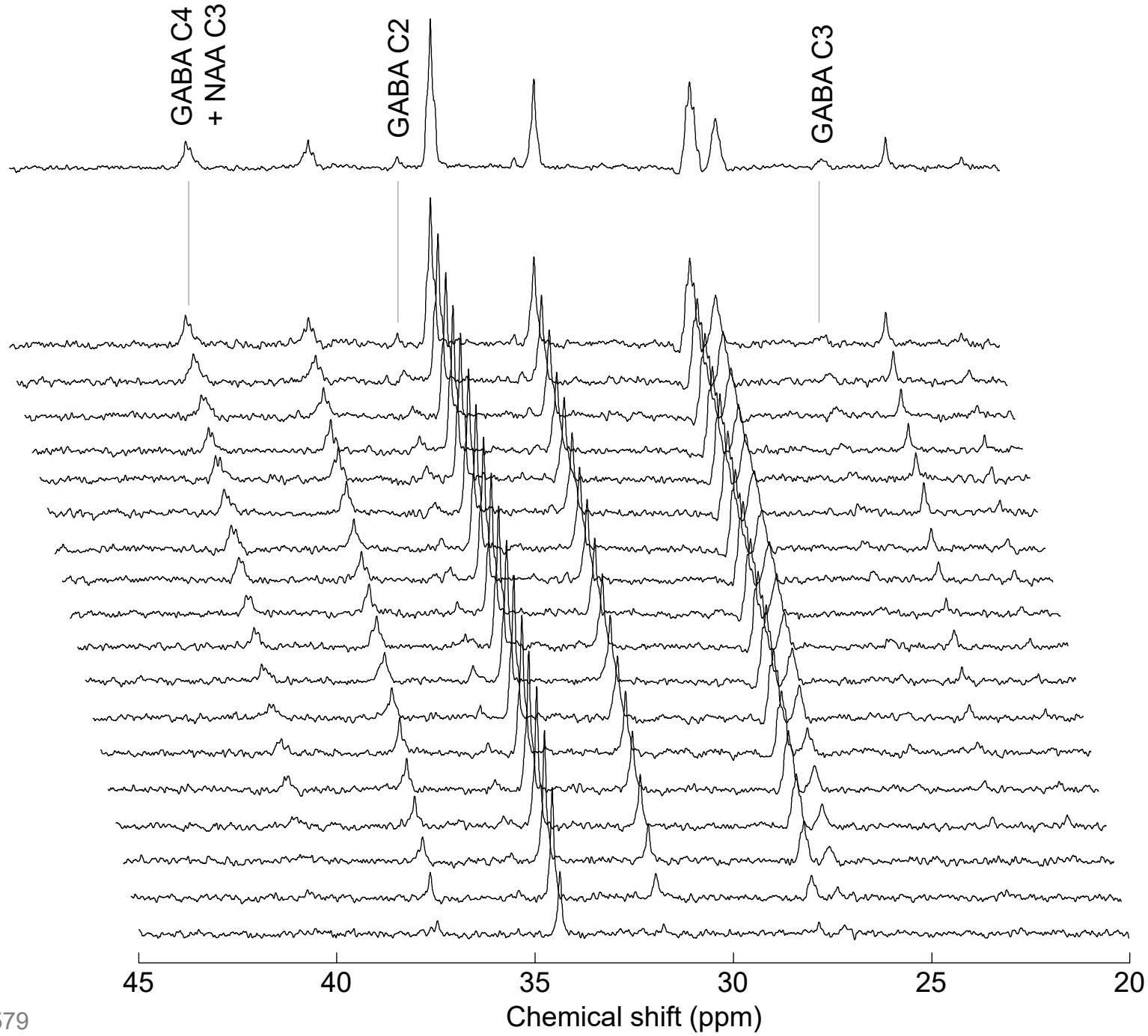


[1,6-^{13}C]glucose administered to GK rats under α-chloralose (Girault *et al*. Neurotox Res 2019)

Cortical energy metabolism upon somatosensory stimulation



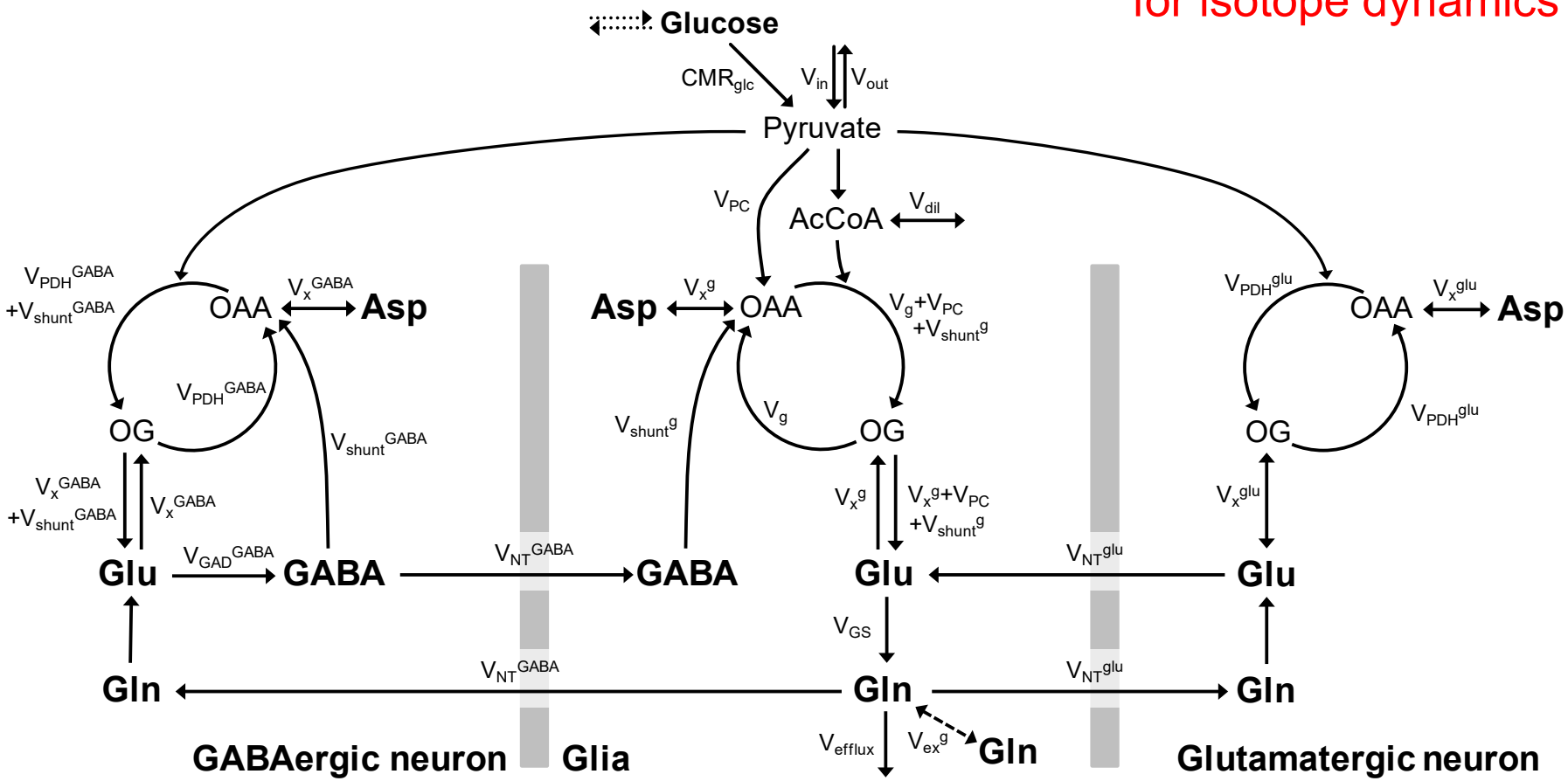
Sonnay *et al.*, JCBFM 2016



Duarte & Gruetter (2013) *J Neurochem* 126(5):579

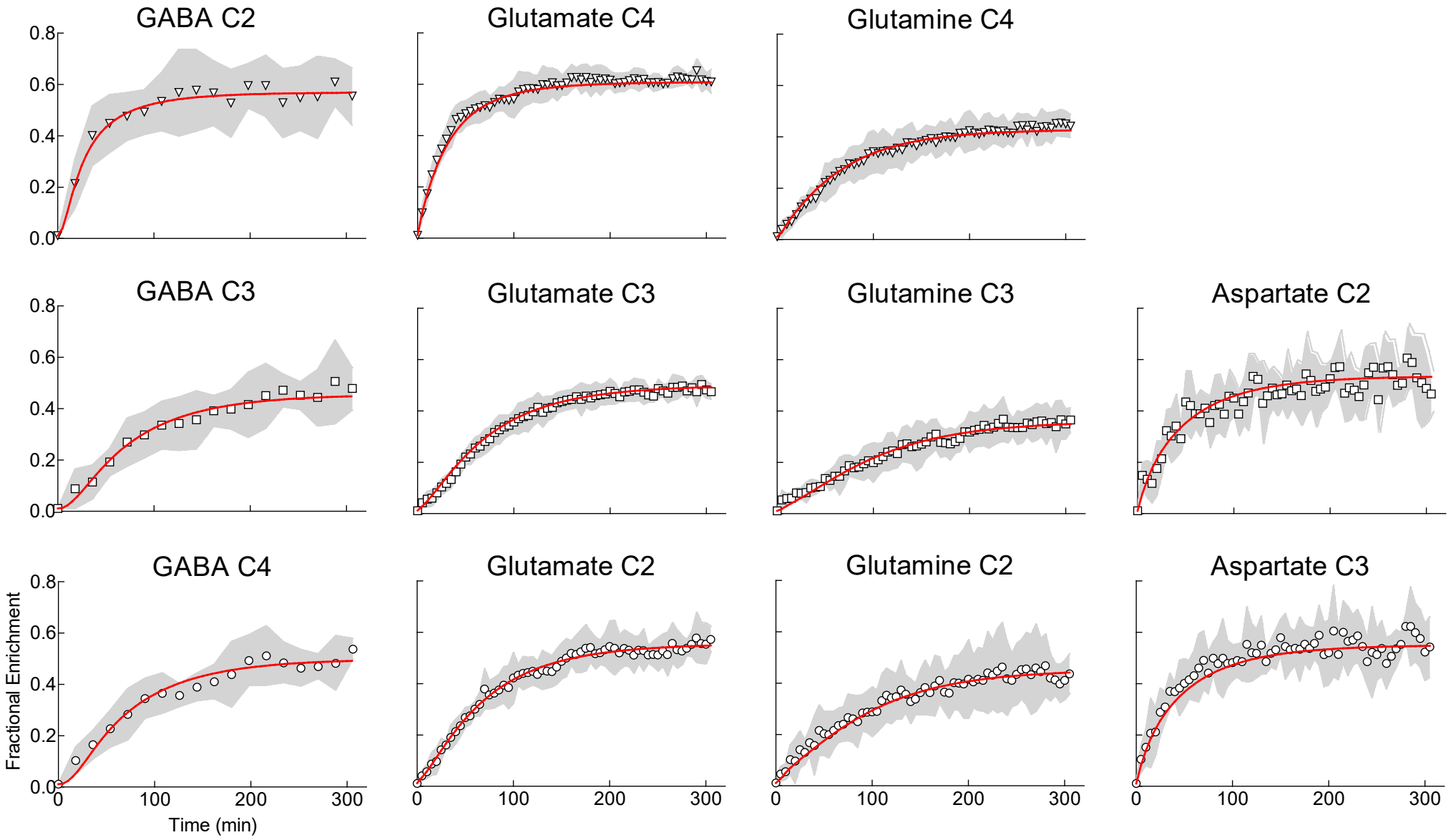
3-compartment model of brain glucose metabolism

52 differential equations
for isotope dynamics



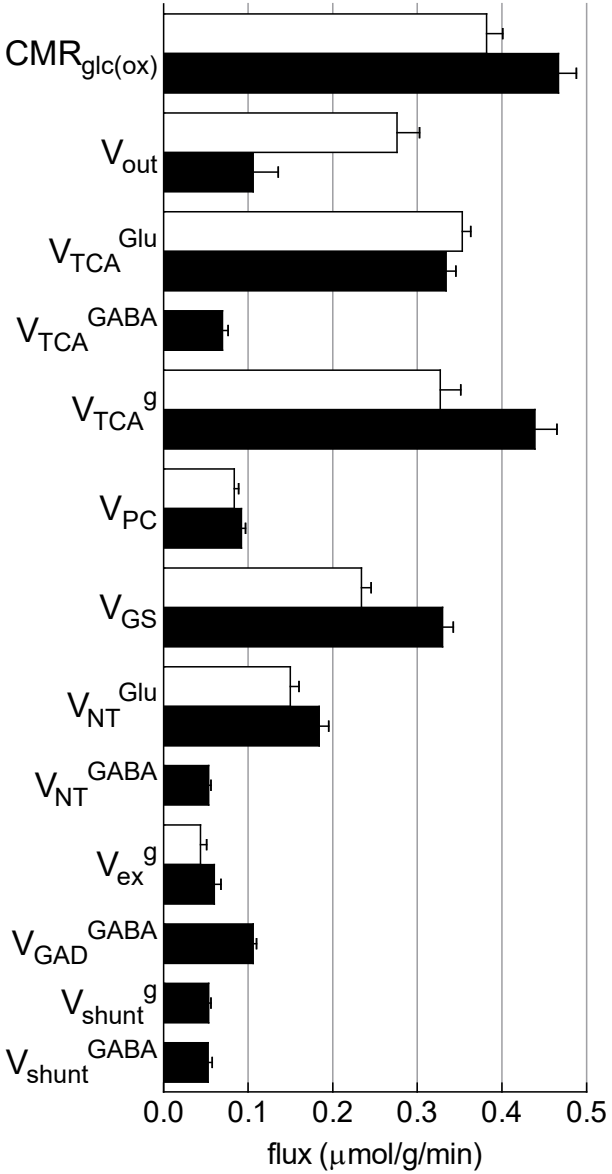
Duarte & Gruetter (2013) *J Neurochem* 126(5):579

^{13}C-enriched metabolites in the brain



Duarte & Gruetter (2013) *J Neurochem* 126(5):579

Metabolic fluxes from the 3 compartment model



Compartments	**1**	**2**	**3**
Experimental ^{13}C curves	3	8	11
Parameters to fit	4	8	12
Mean SD of fluxes	28% 7% V_{TCA} 69% V_X	10% 3% V_{TCA}^{n} 31% V_X^{g}	19% 3% V_{TCA}^{n} 103% V_X^{g}

Duarte & Gruetter (2013) *J Neurochem* 126(5):579

Isotopomer analysis

Isotopologue

chemical specie that differ only in the isotopic composition

Hydrogen-related isotopologues of water:

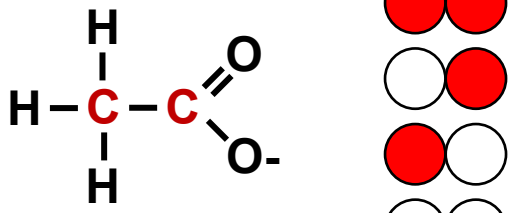
$^{1}H_2O$	light water
$^{1}H^{2}HO$	semi-heavy water
$^{2}H_2O$	heavy water
$^{3}H_2O$	tritiated water (super-heavy)

Isotopomer

Malloy *et al.* (1988) *J Biol Chem* 263:6964

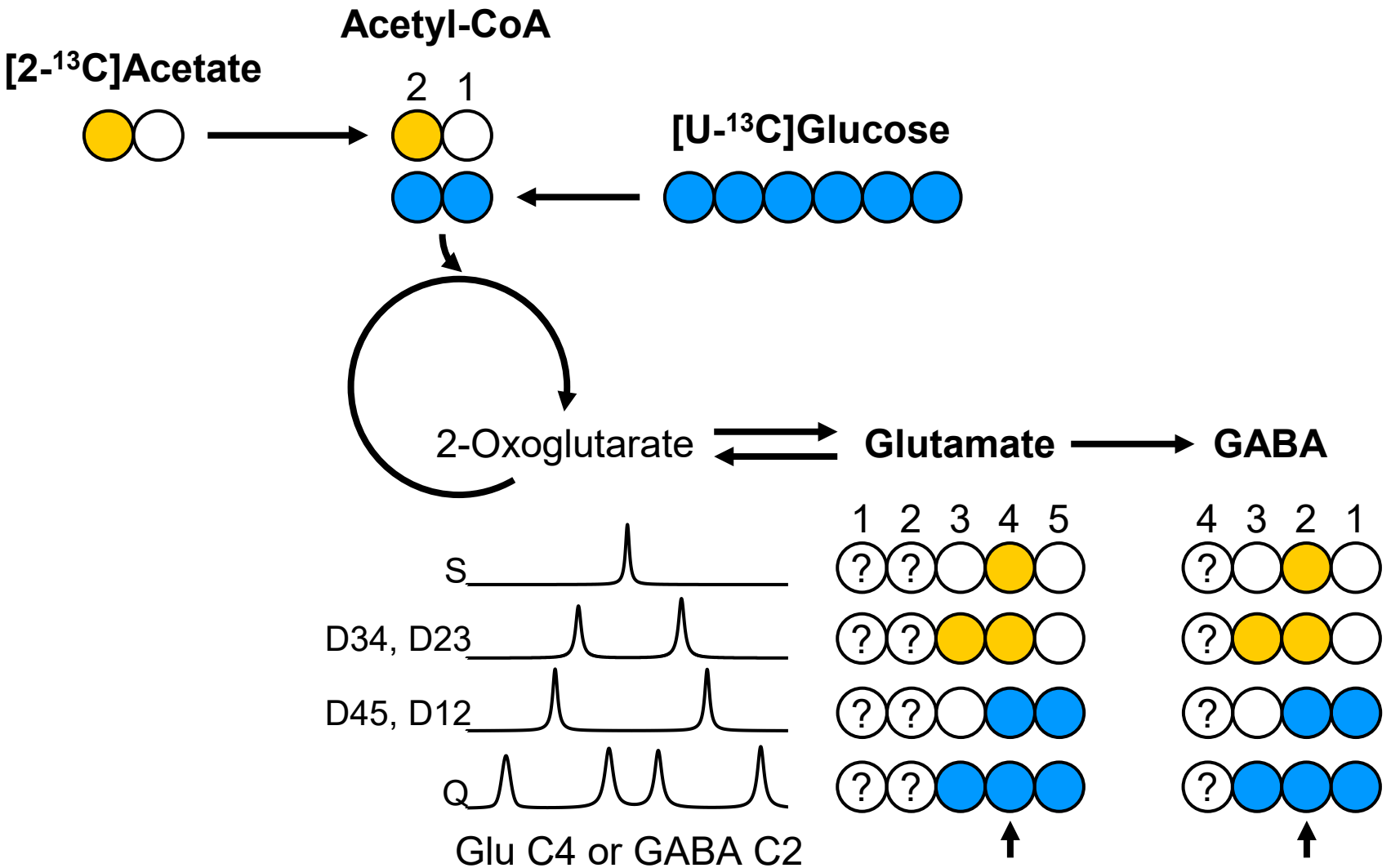
Isomers with same isotopic atom but different position

^{13}C in acetate



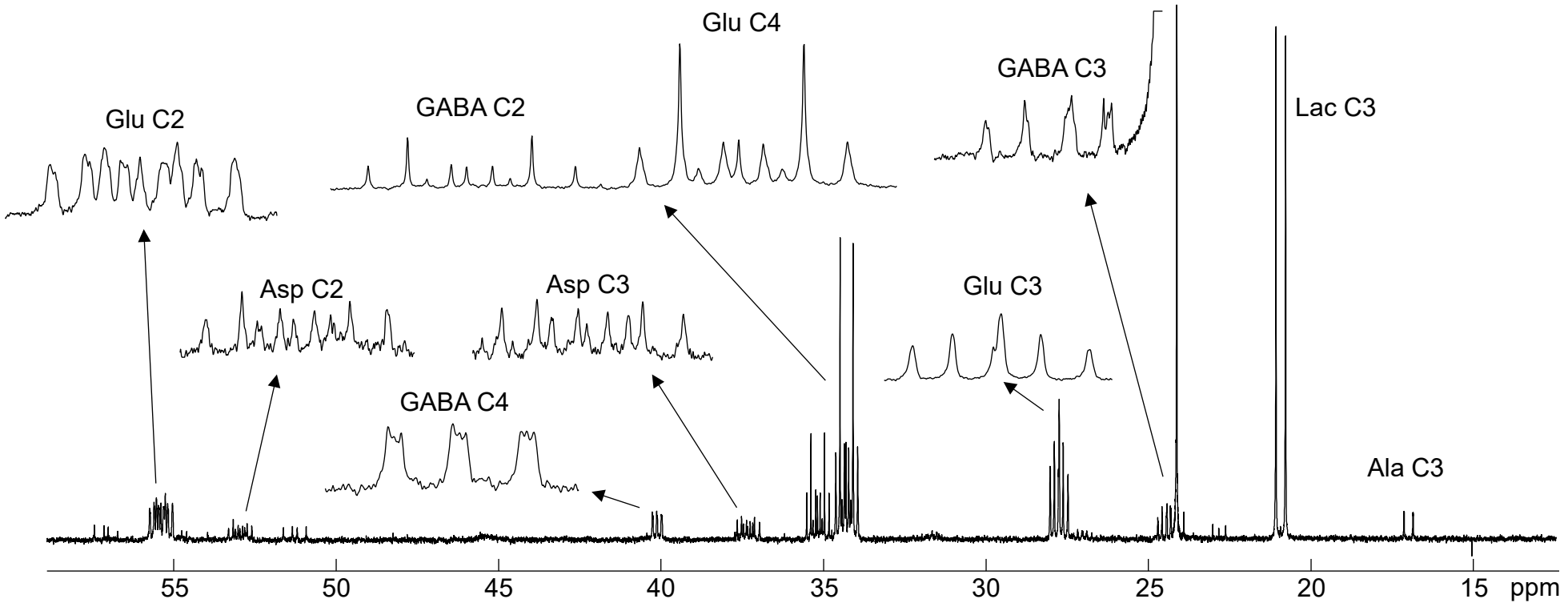
The positional enrichment of a carbon within a metabolite is the sum of all its isotopomer fractions where that carbon atom is labeled.

TCA cycle: oxidation of [2-13C]acetate & [U-13C]glucose



Duarte *et al*. (2007) *Neuroscience* 144:1305

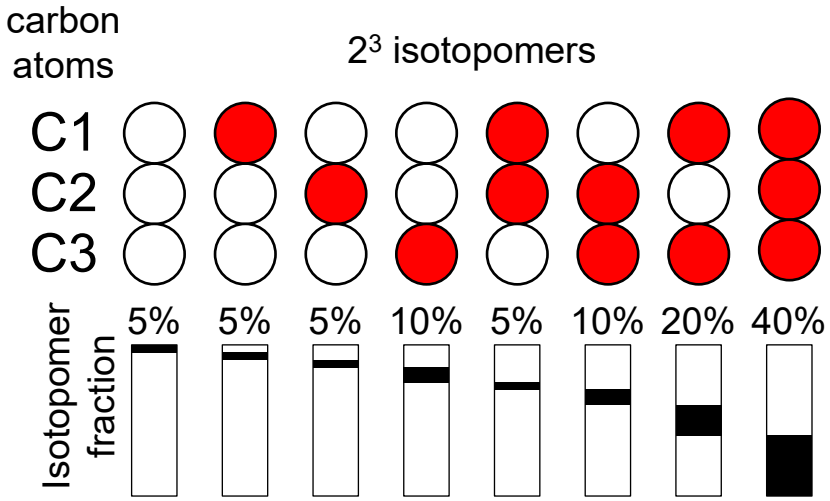
Hippocampal slices with [2-^{13}C]acetate & [U-^{13}C]glucose



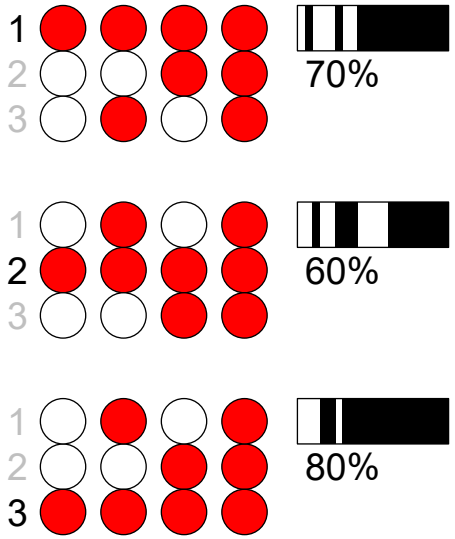
Duarte *et al*. (2007) *Neuroscience* 144:1305

Duarte *et al*. (2012) *Neurochem Int* 60:1

Cumulated isotopomer: “cumomer”

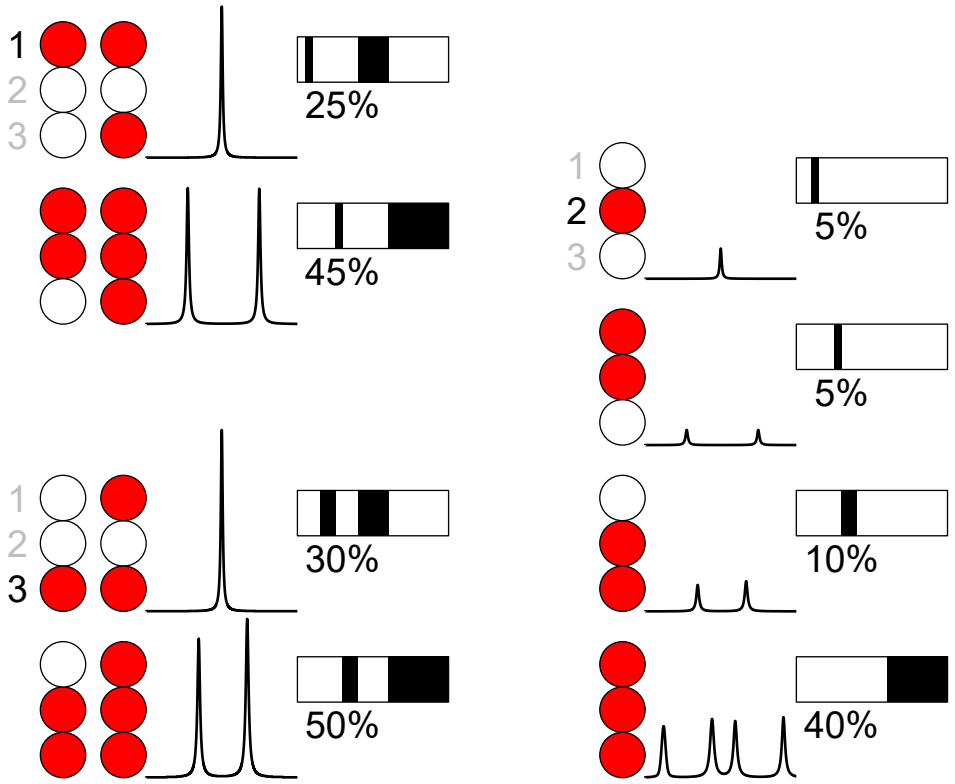


Positional enrichments



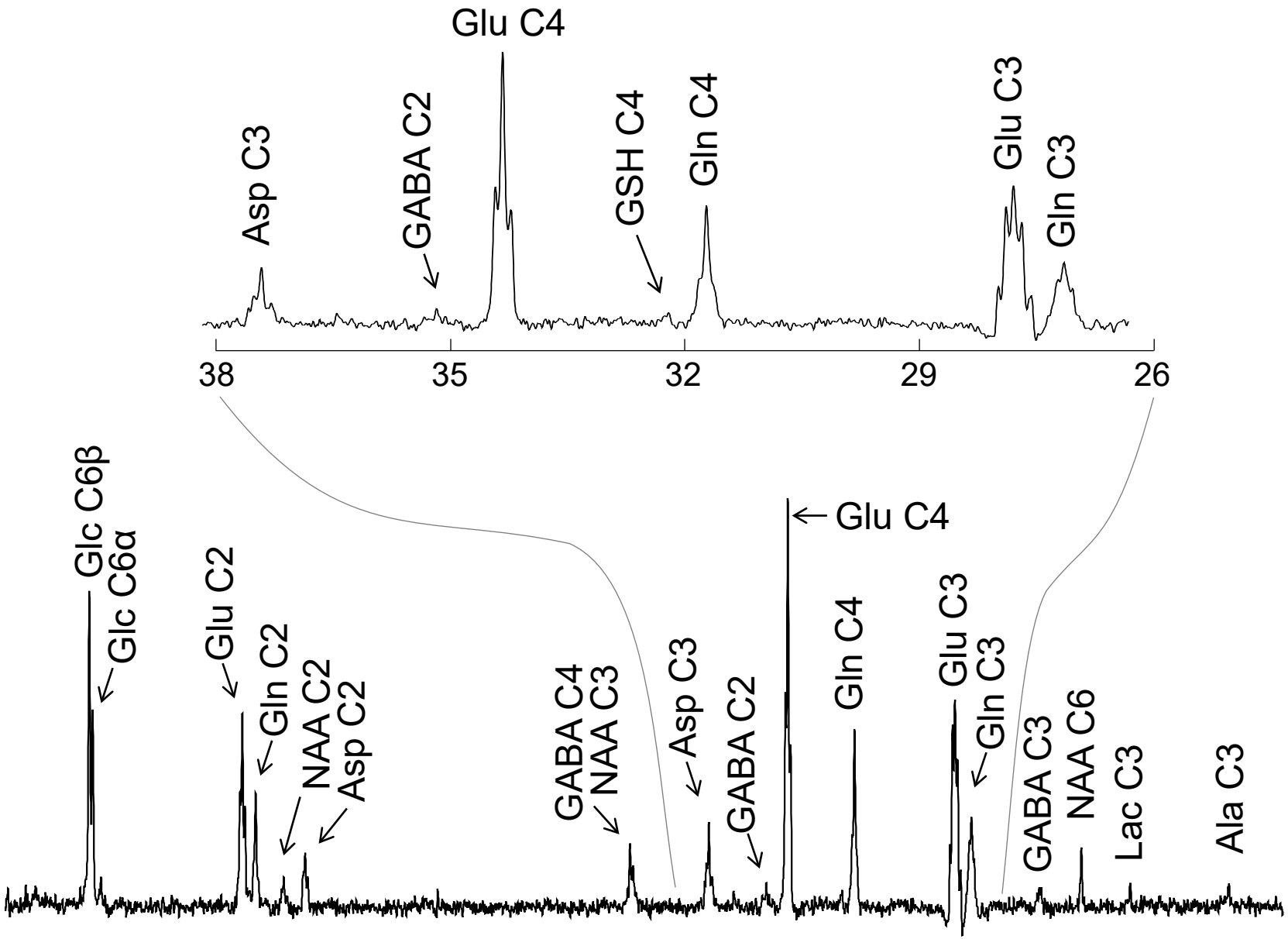
Multiplets in ^{13}C MRS

(cumomer fractions)

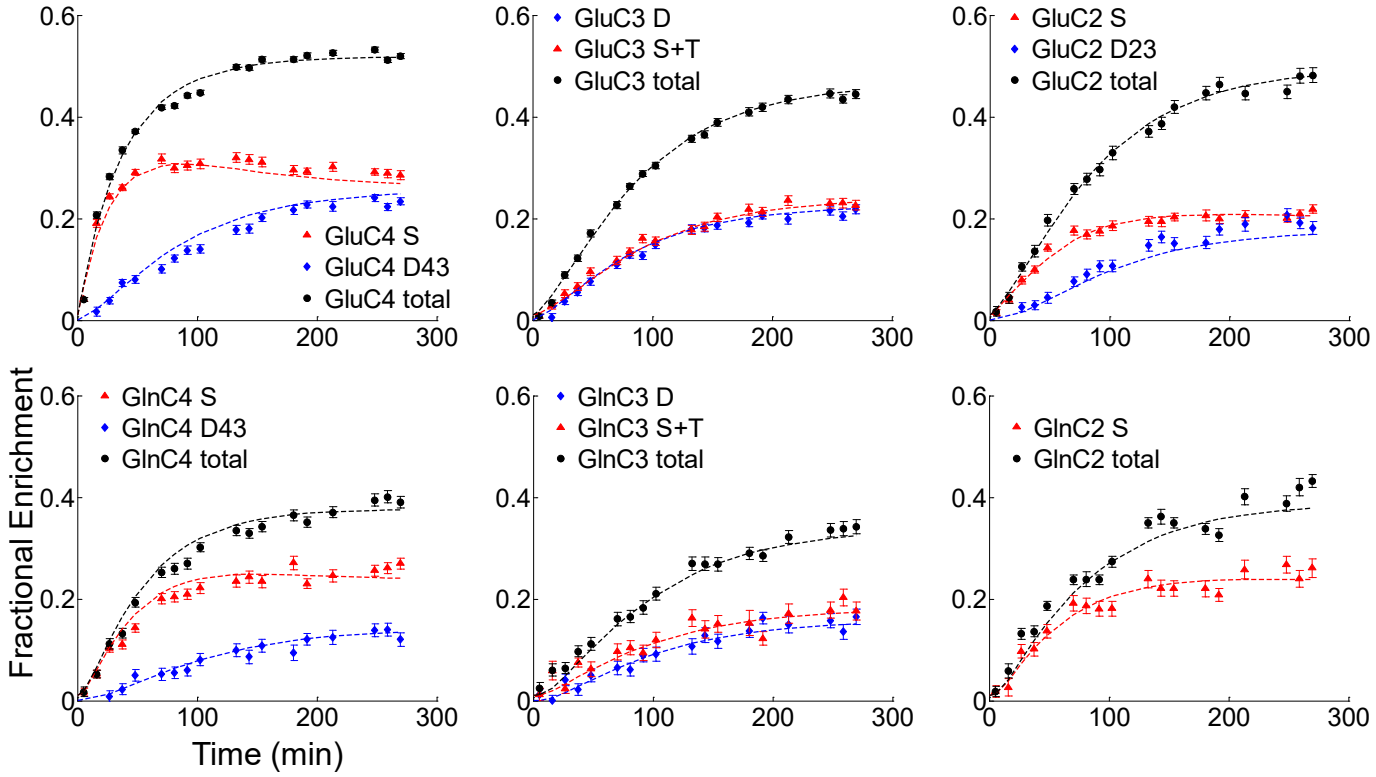


Wiechert *et al*. (1999) *Biotechnol Bioeng* 66:69

Cumomers in addition to positional enrichments



Cumomers in addition to positional enrichments



model	positional	positional + cumomer
Experimental ^{13}C curves	6	17
Parameters to fit	7	7
Mean SD of fluxes	9% 3% V_{TCA}^{n} 18% V_{NT}	5% 3% V_{TCA}^{n} 10% V_{dil}

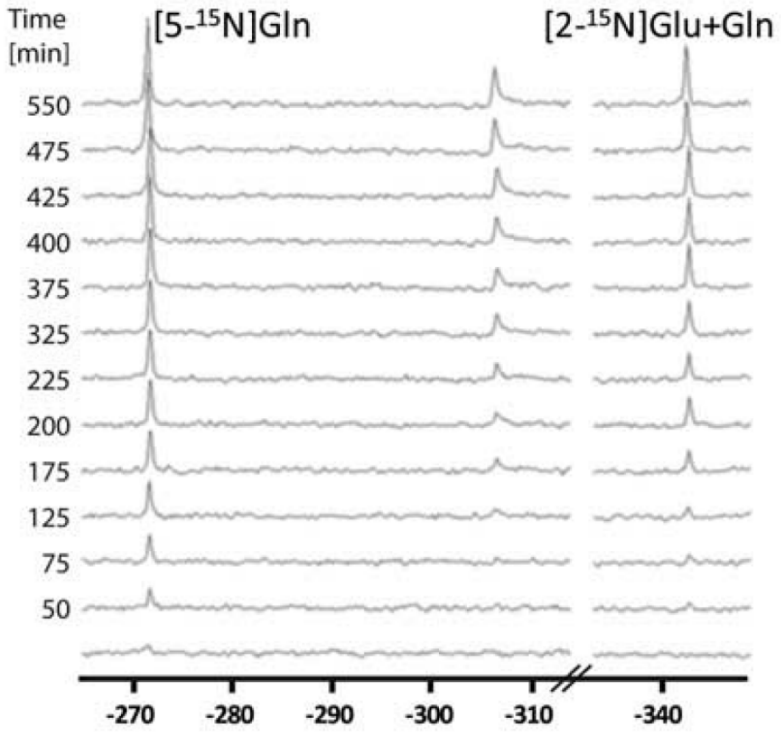
Dehghani *et al*. ASN Neuro 2016

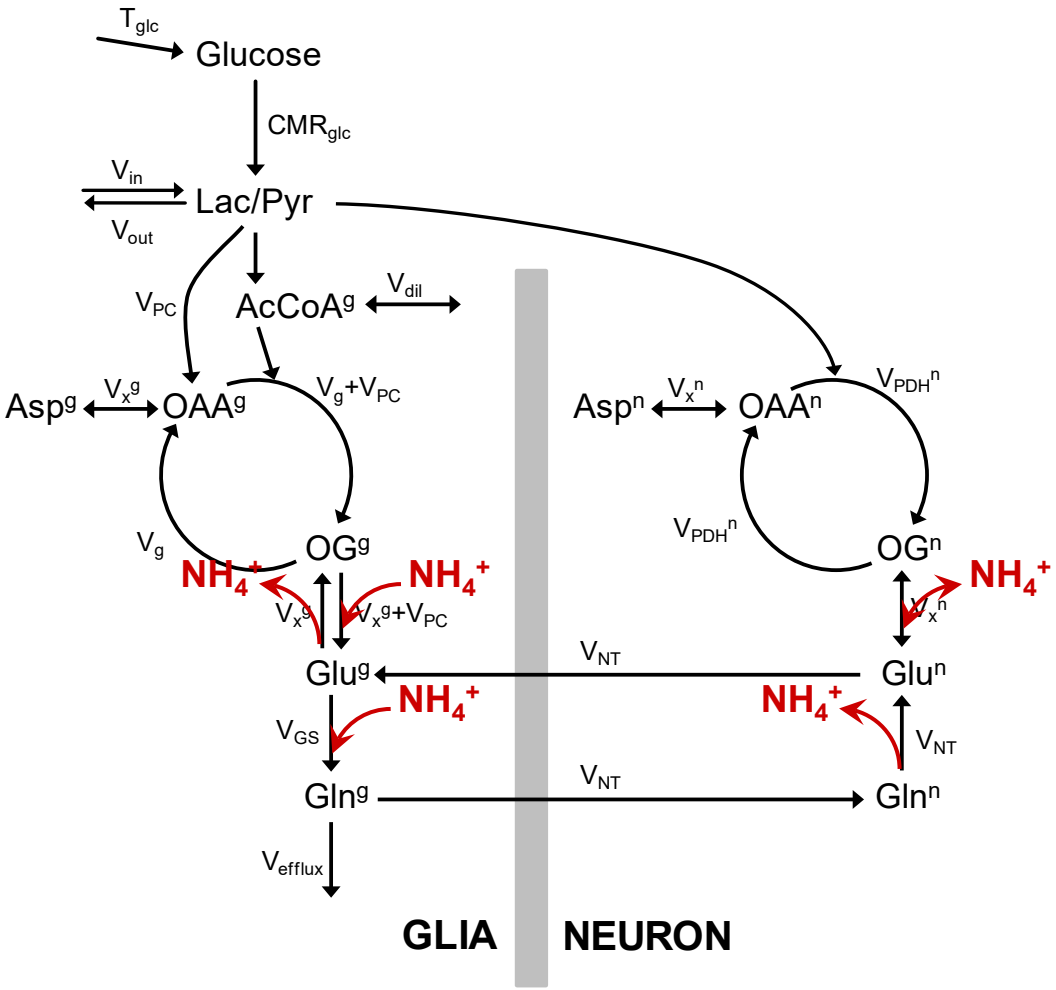
Error bars ±CRLB

Non-stationary MFA: example with ^{15}N MRS

$^{15}NH_4Cl$ infusion

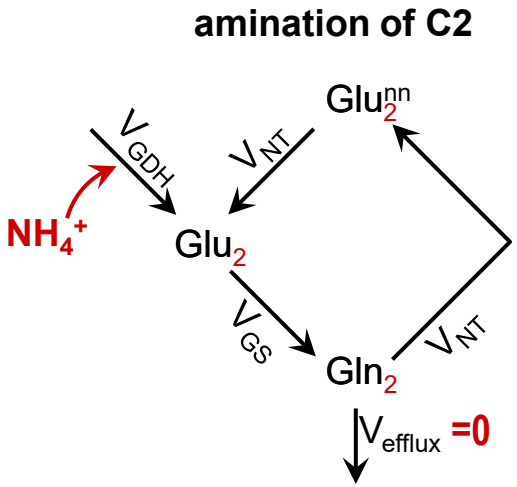
^{15}N MRS in the rat brain

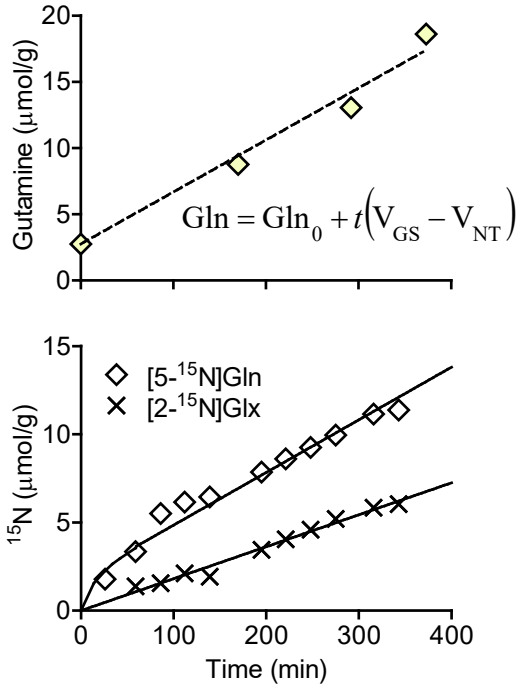


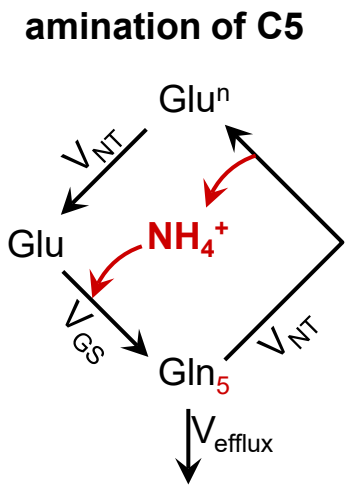


Cudalbu *et al.*, *JCBFM* 32:696 (2012)

Model for 15N incorporation into glutamate & glutamine







Mass balance equations

$$\frac{d\,NH_4^+}{dt} = V_{in}^{net} + V_{NT} - V_{GS} - V_{GDH} = 0$$

$$\frac{d\,Glu}{dt} = \frac{d\,Glu^n + d\,Glu^g}{dt} = V_{GDH} + V_{NT} - V_{GS} = 0$$

$$\frac{d\,Gln}{dt} = V_{GS} - V_{NT} - V_{efflux} \neq 0$$

Isotope balance equations

$$\frac{d\,{}^{15}NH_4^+}{dt} = V_{in}^{net} FE_{pl} + V_{NT}\frac{Gln_5}{Gln} - (V_{GS} + V_{GDH})\frac{{}^{15}NH_4^+}{NH_4^+}$$

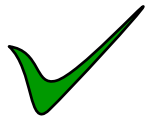
$$\frac{d\,Gln_2}{dt} = V_{GS}\frac{Glu_2^g}{Glu^g} - V_{NT}\frac{Gln_2}{Gln}$$

$$\frac{d\,Glu_2^n}{dt} = V_{NT}\left(\frac{Gln_2}{Gln} - \frac{Glu_2^n}{Glu^n}\right)$$

$$\frac{d\,Glu_2^g}{dt} = V_{GDH}\frac{{}^{15}NH_4^+}{NH_4^+} + V_{NT}\frac{Glu_2^n}{Glu^n} - V_{GS}\frac{Glu_2^g}{Glu^g}$$

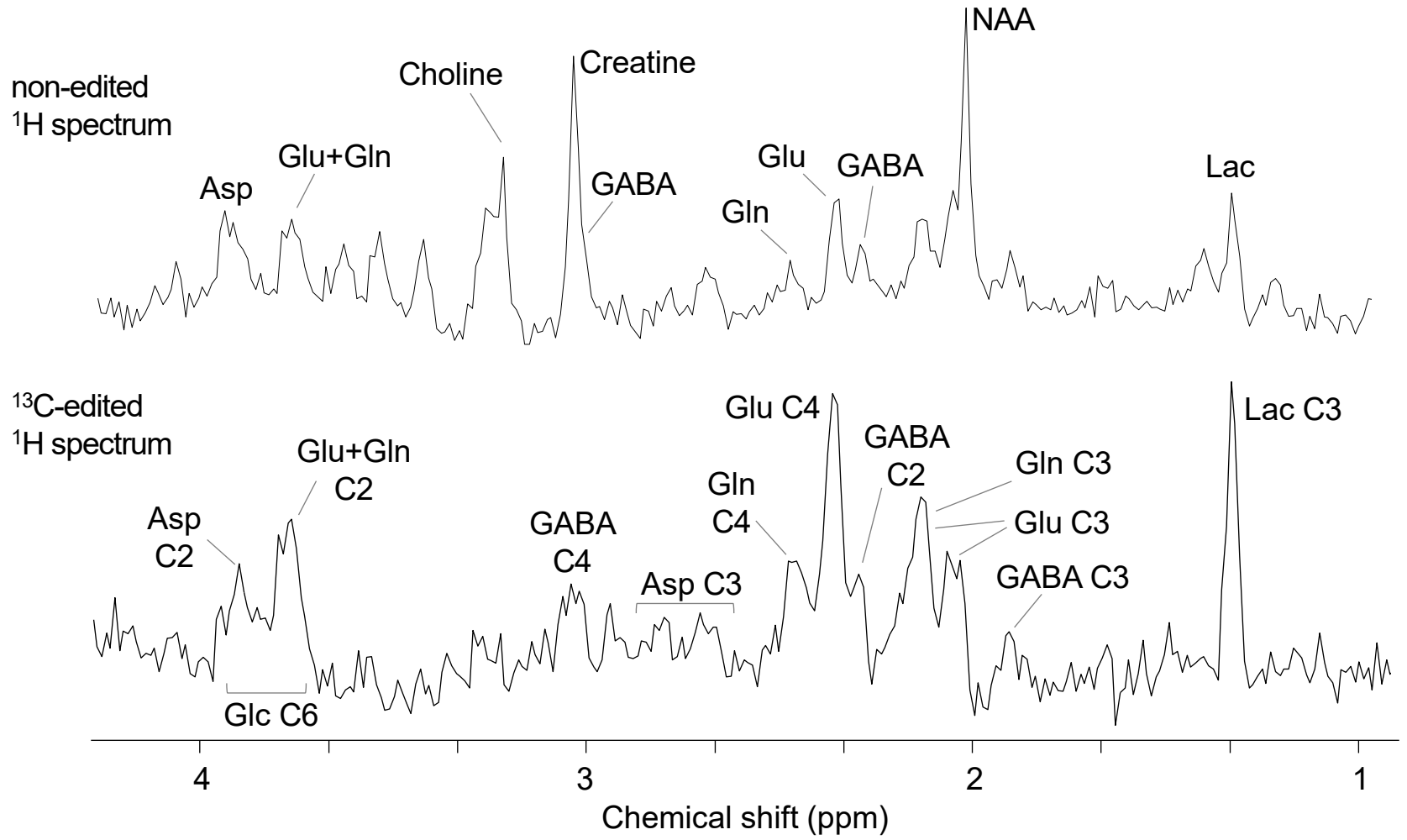
$$\frac{d\,Gln_5}{dt} = V_{GS}\frac{{}^{15}NH_4^+}{NH_4^+} - (V_{NT} + V_{efflux})\frac{Gln_5}{Gln}$$

^{13}C MFA *in vivo*: take home message(s)

- Models as simple as possible.
- Collect as much data as possible from the system to be modelled.
- Document model assumptions.
- Model comparison and reliability/validity testing.
- Matching carbons between each molecule and its precursor.
- MFA models contain no information about regulatory mechanisms
- Little predictive power!
- **Metabolic Control Analysis (MCA)** 

SNR is an important limitation

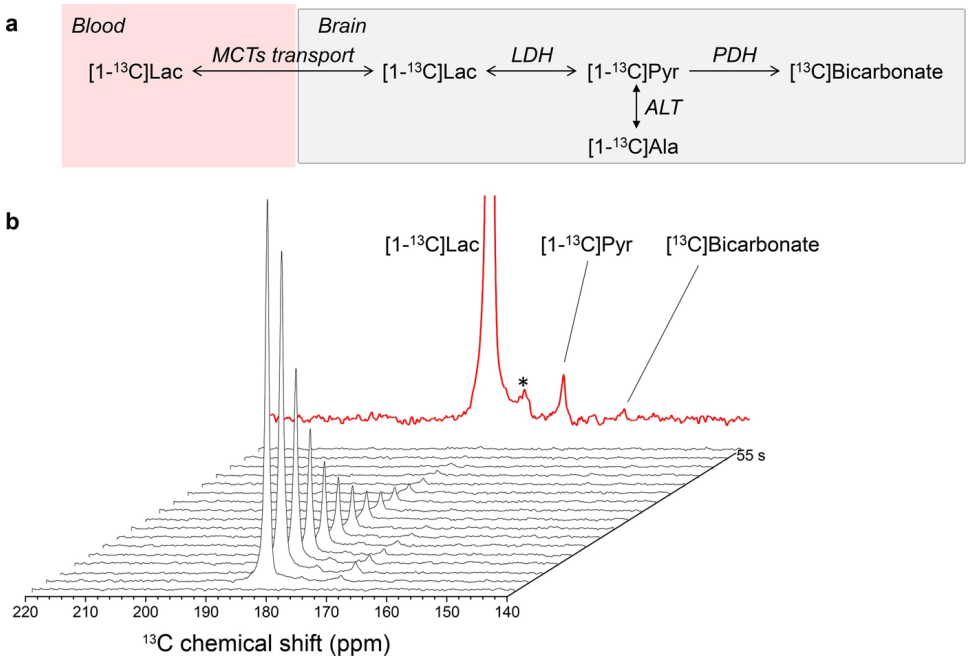
Indirect detection of ^{13}C



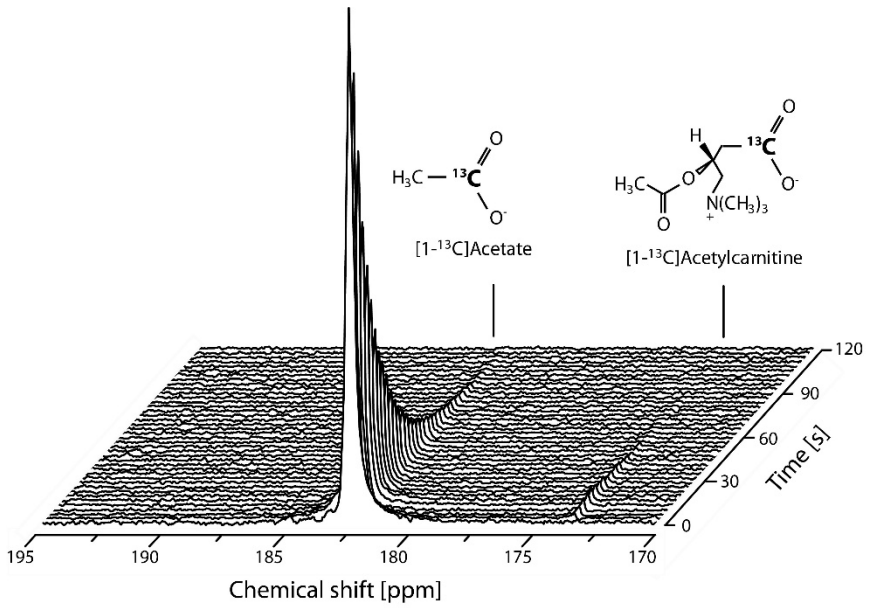
Lizarbe *et al.*, Int J Obes 2019 (see discussion in Duarte, Neurochem Res 2025)

SNR is an important limitation

Hyperpolarized MRS



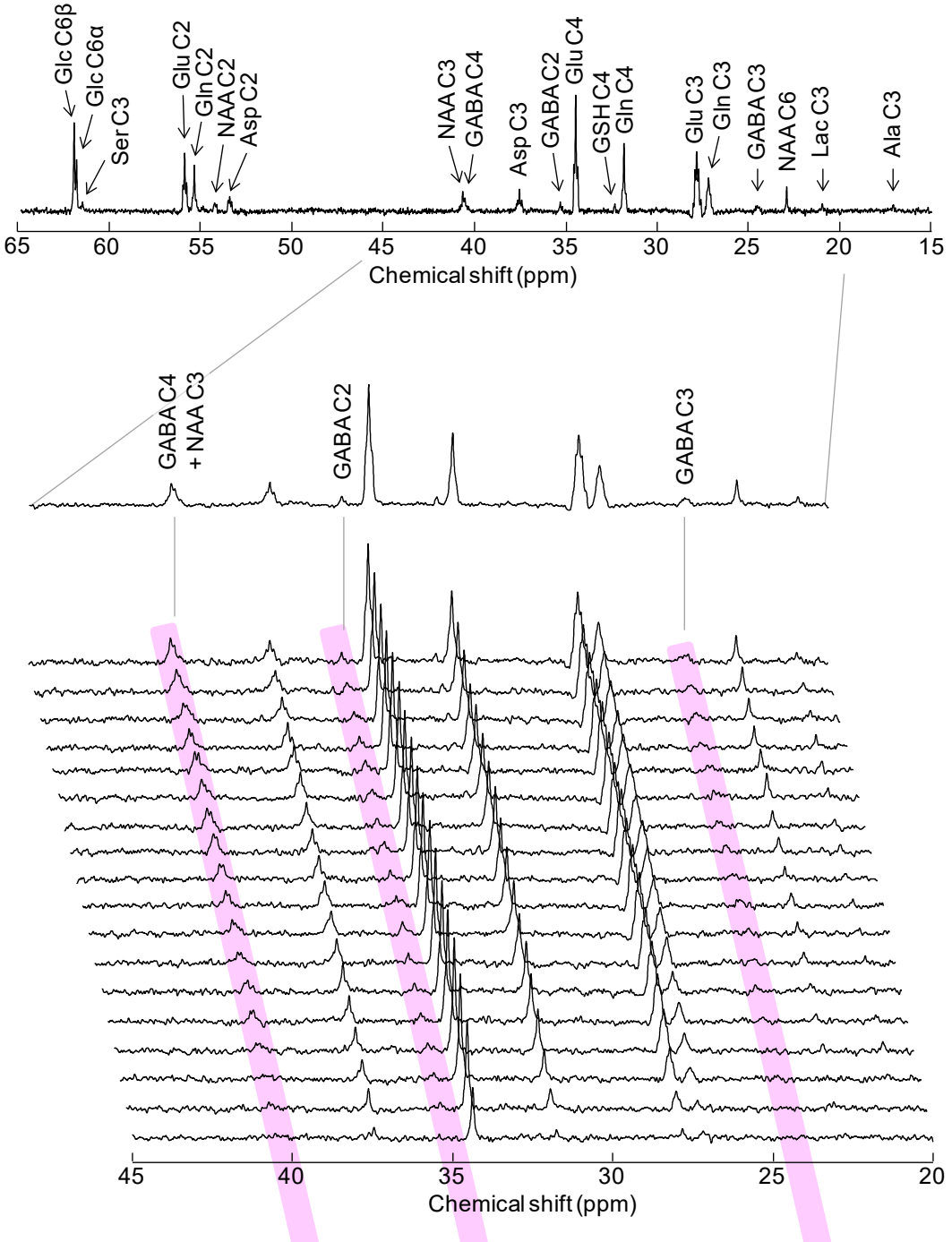
Hyacinthe *et al*. Sci Rep 2020



Bastiaansen *et al*. BBA 2013

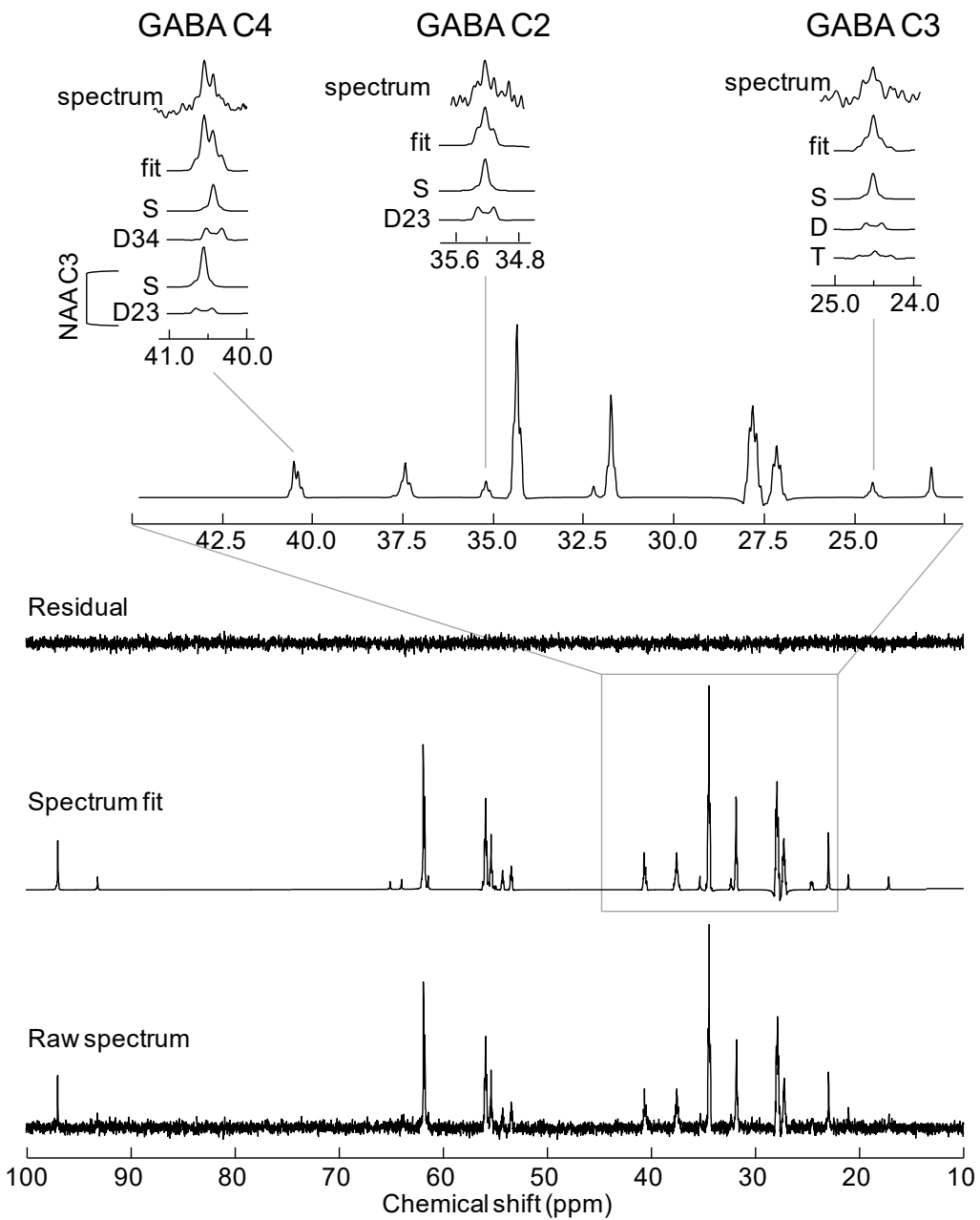
^{13}C NMR spectra from the rat brain *in vivo*

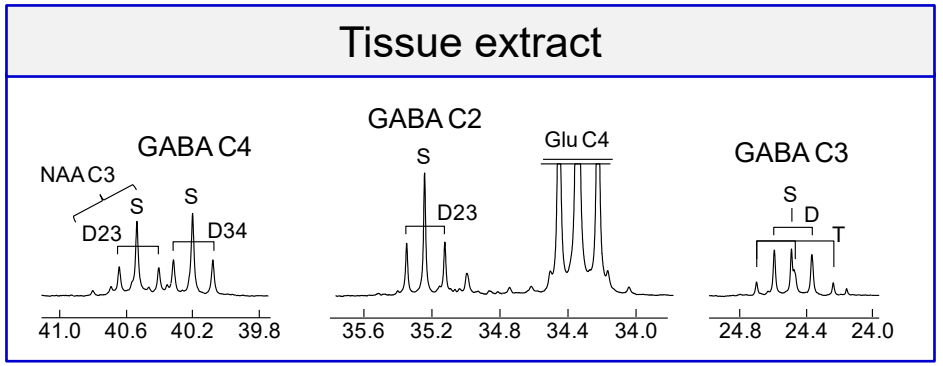
GABA is 4-fold smaller than glutamate

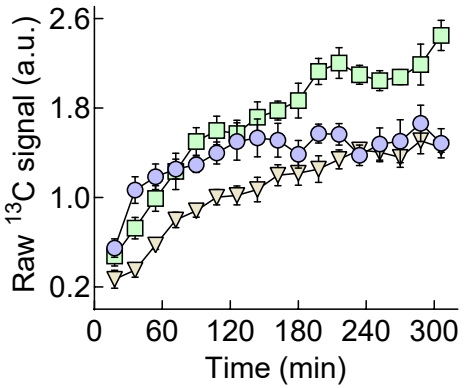


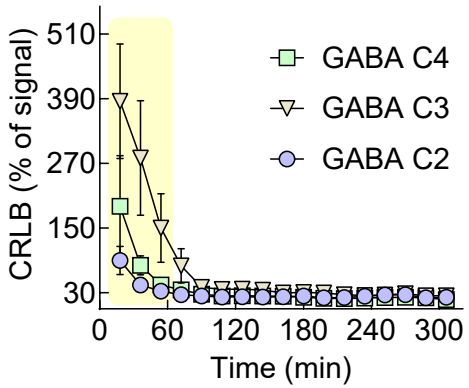
Bottom:
4x128 scans with TR of 2.5 s (21.3 min)
Lorentzian–Gaussian apodization (lb=5, gf=0.08, sbs=0.02)
Top:
Sum of 3 last spectra (~1-h)
Shifted Gaussian (gf=0.08, gfs=0.02)

Quantification of ^{13}C NMR spectra using LCModel





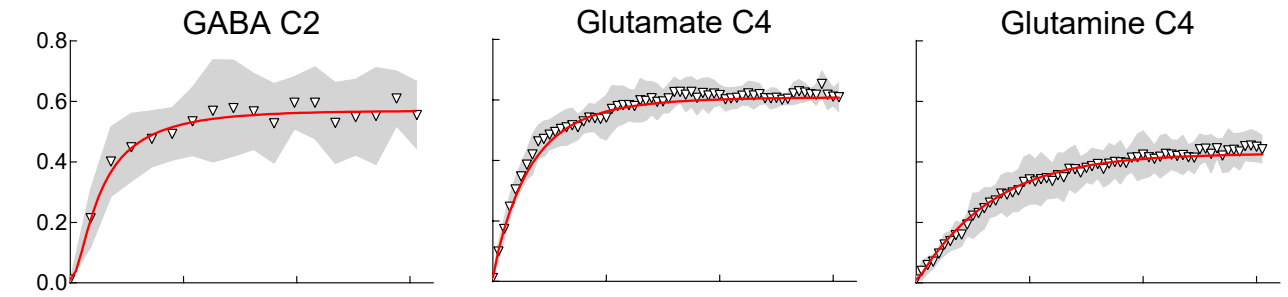


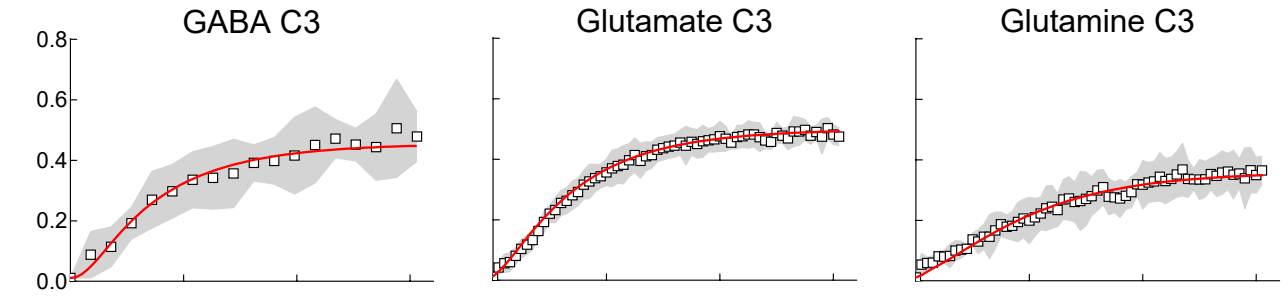


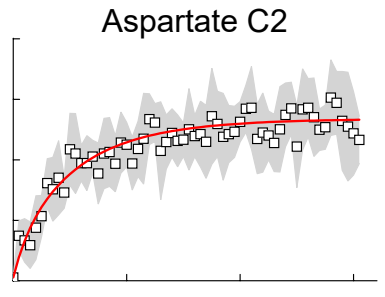
mean±SEM, n=8 rats

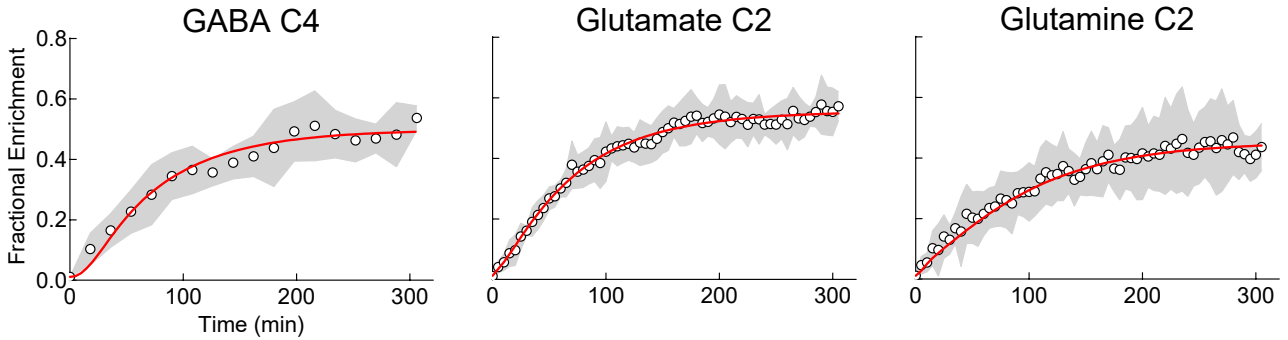
Inaccurate estimation of GABA during the 1st hour !

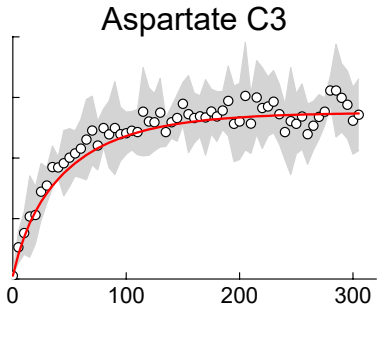
Model fit to ^{13}C curves



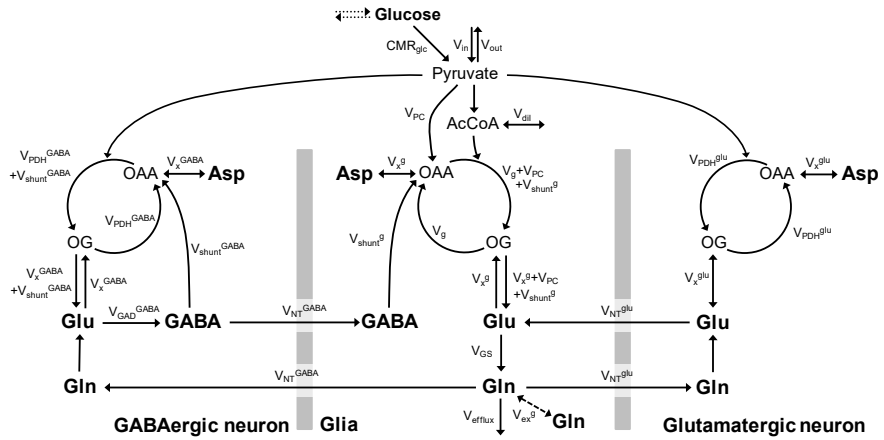








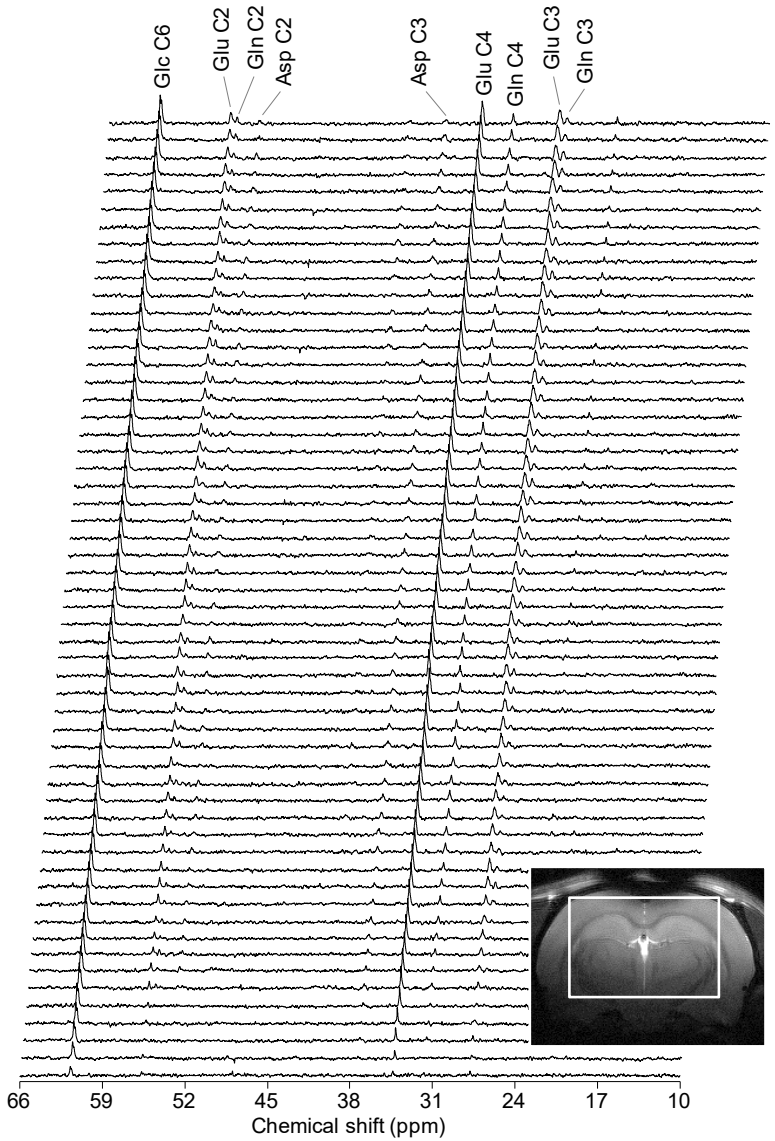
Model of brain energy metabolism

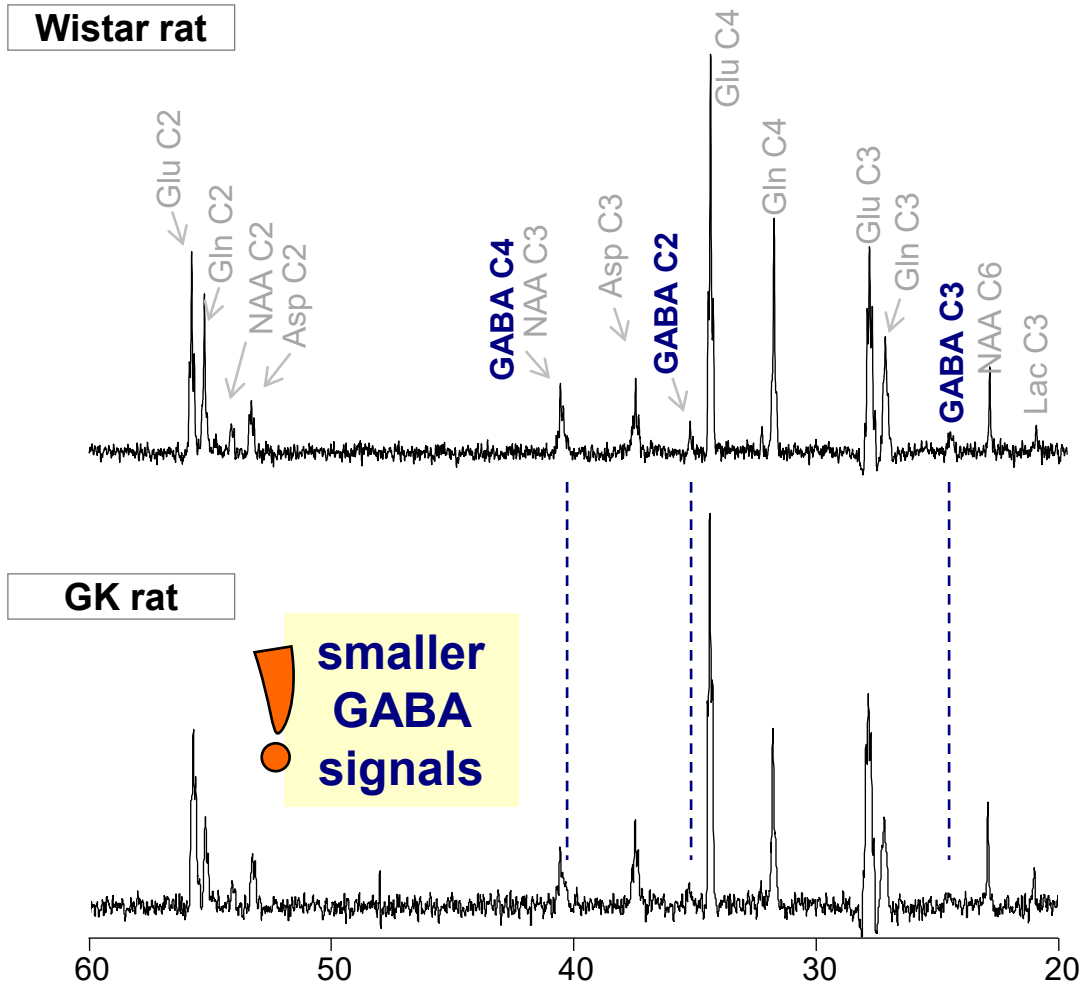


low time resolution for GABA

Duarte & Gruetter, *J Neurochem* 2013

Brain metabolism in diabetic rats



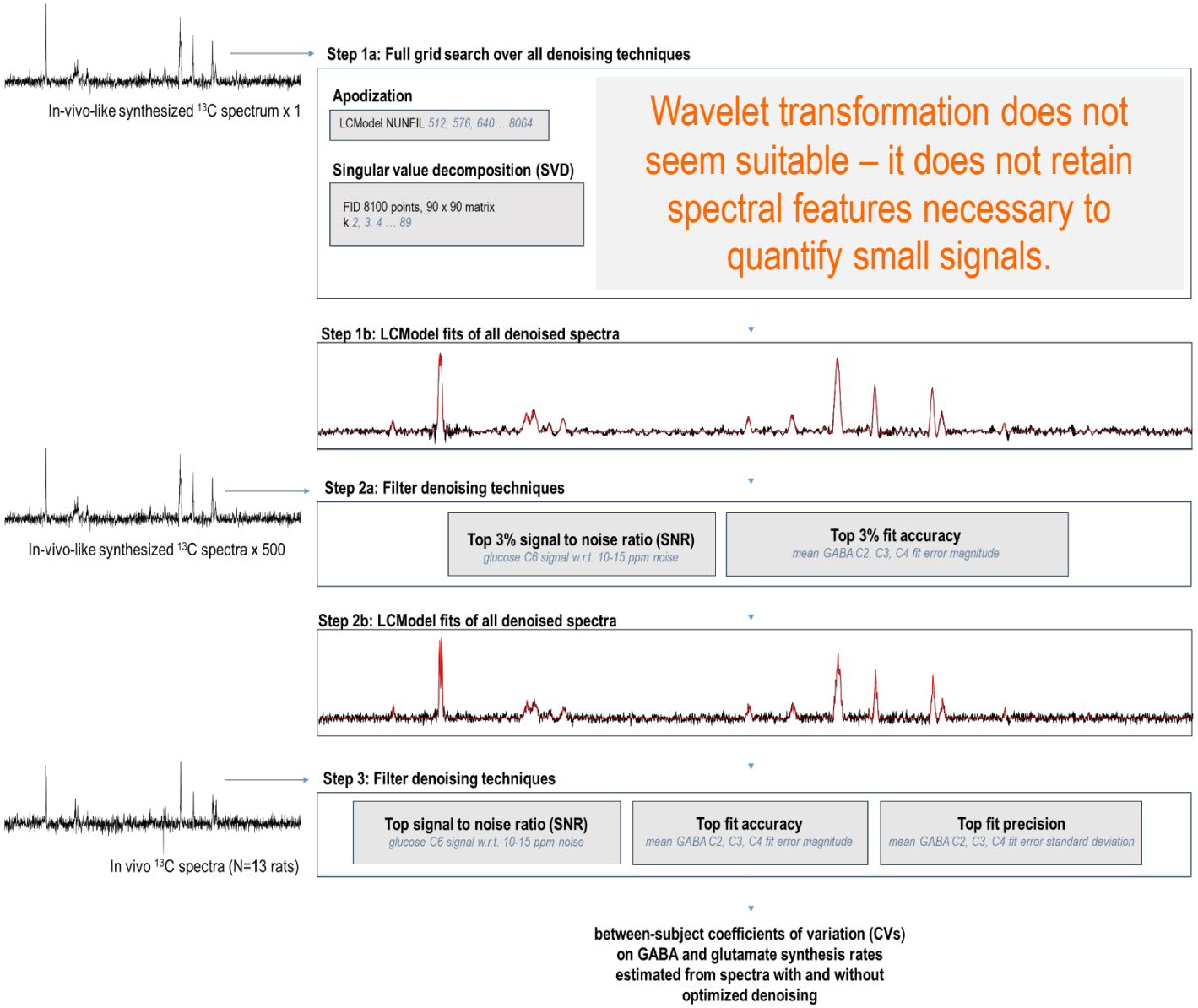


Left:
128 scans with TR of 2.5 s (5.3 min) for about 5 h
Lorentzian–Gaussian apodization (lb=7, gf=0.08, sbs=0.02)

Right:
Sum of 3 last spectra (~1-h)
Shifted Gaussian (gf=0.08, gfs=0.02)

Girault *et al.*, *Neurotox Res* 2019

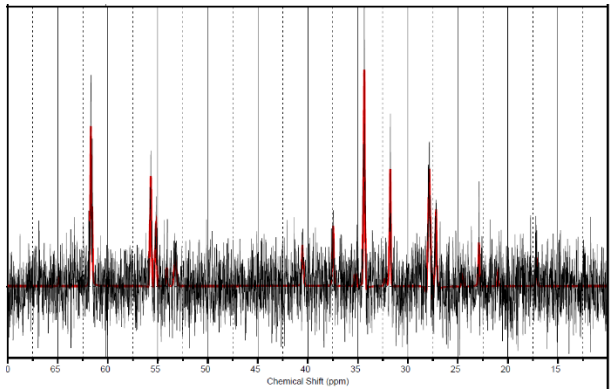
Can spectral denoising techniques improve the reliability of GABA estimates by ^{13}C NMR spectroscopy?



Kelley Swanberg
@ Lundgaard lab

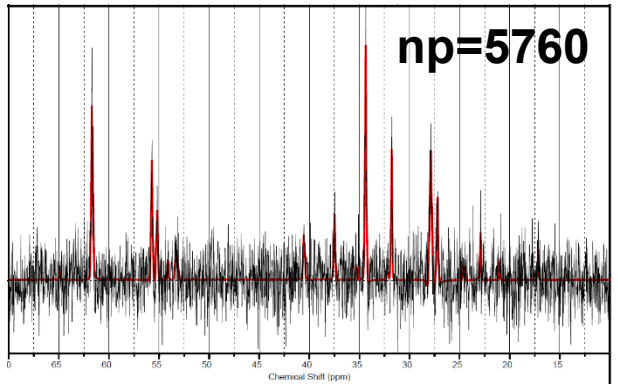
- Apodization/FID truncation impacts analysis of multiplets.
- SVD seems promising for increasing precision but needs to be further tested *in vivo*.

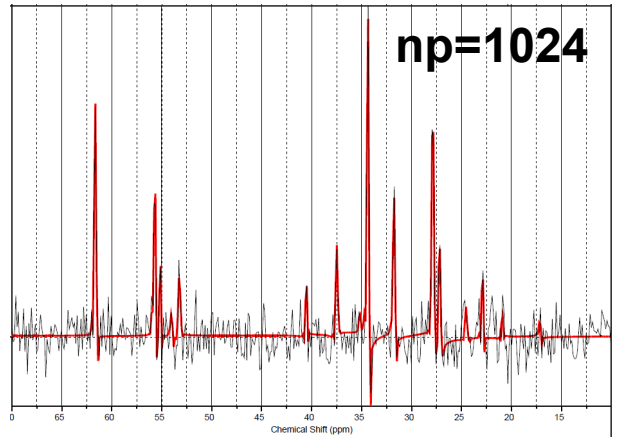
Results Analysis 3: *in vivo* application – fit of 128 scans



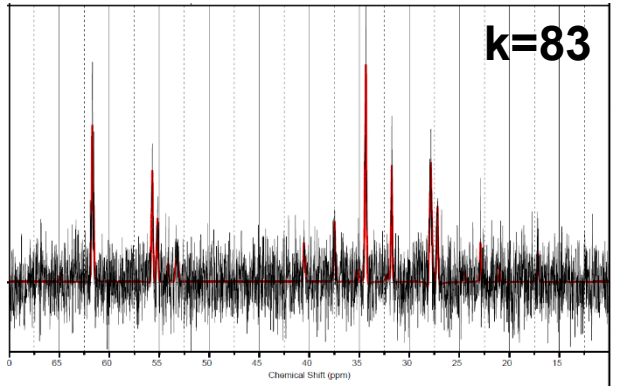
Original spectrum
(np=8065)

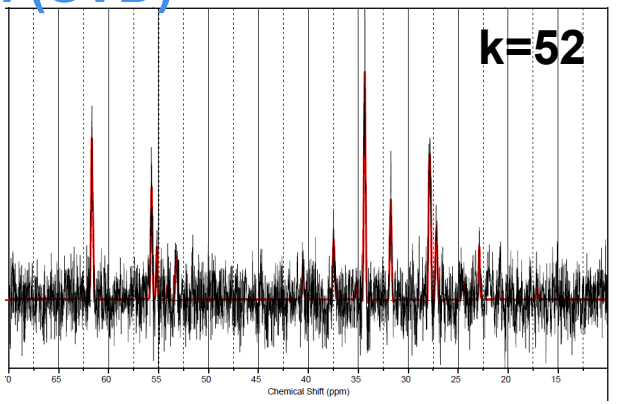
FID truncation



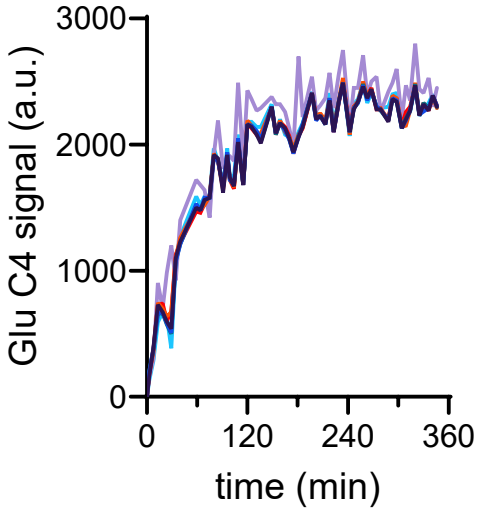


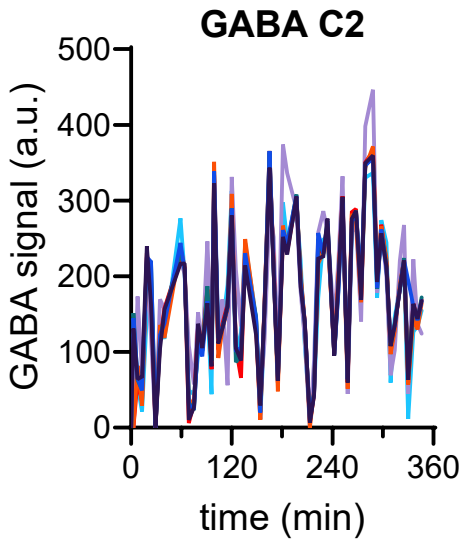
Singular value decomposition (SVD)

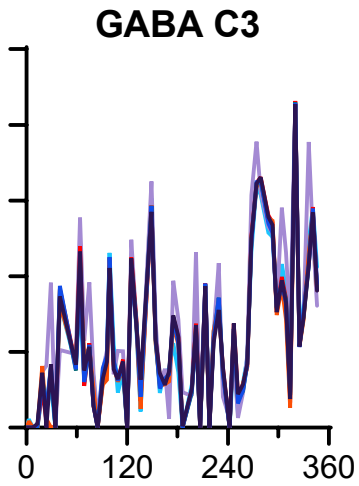


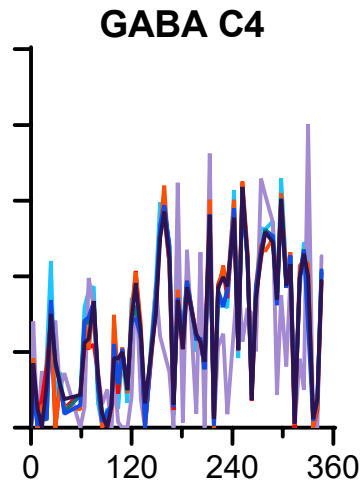


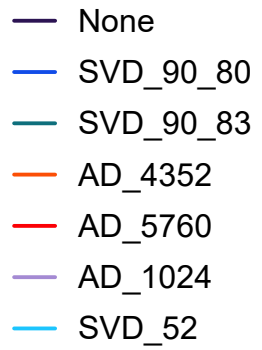
Results Analysis 3: *in vivo* application – fit of 128 scans





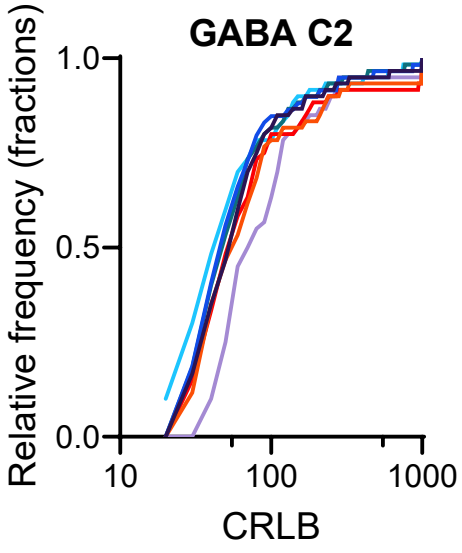


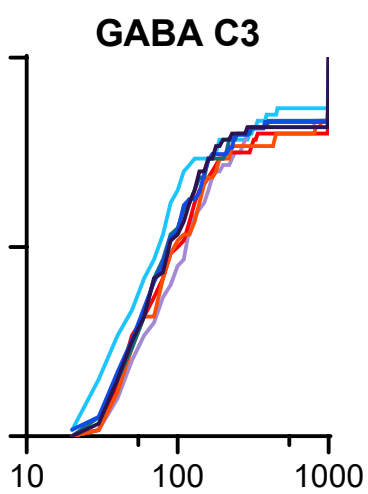


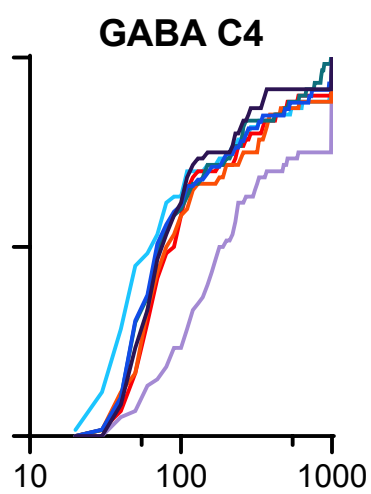


There's no noticeable improvement in signal fit result

General decrease of CRLBs with SVD_52





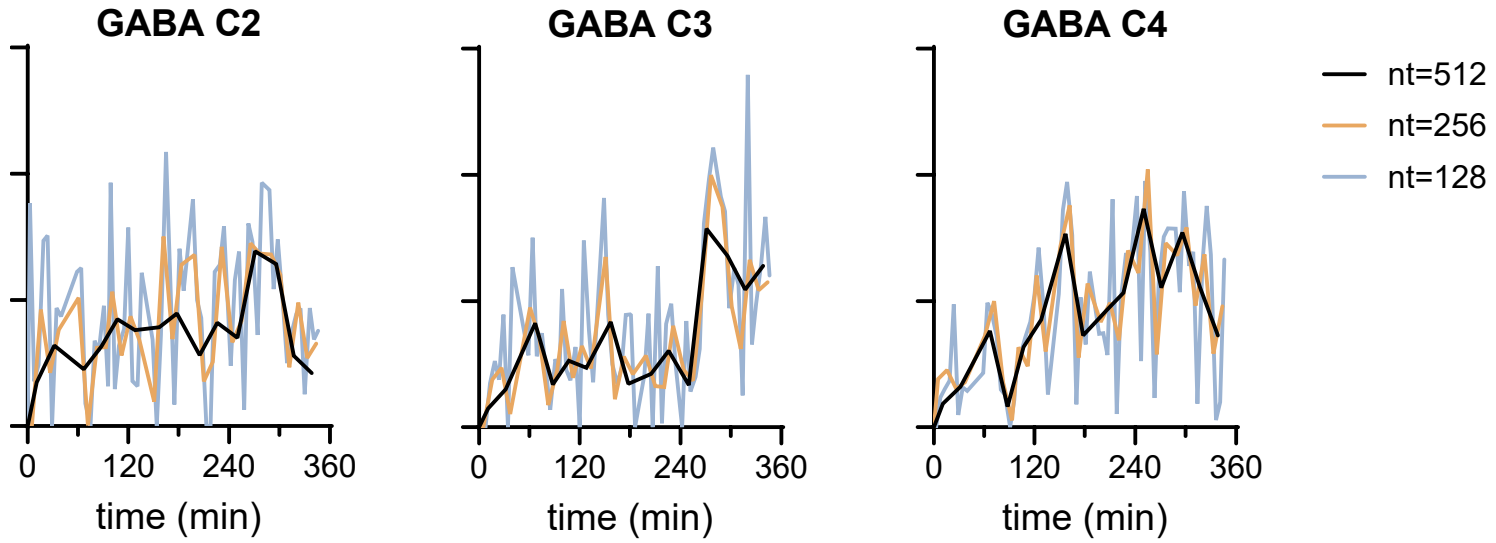


One typical experiment

Conclusion

- Noise reduction is essential, but not straightforward
- Testing SNR enhancing methods that take in account that *spectral features are consistent over time* might be interesting.
  (1) PCA of redundant data (Veraart et al. NeuroImage 2016)
  (2) Stationary Wavelet Denoising for Multiple Similar scans (Song et al., JMR 2024)
- Denoising using neuronal networks

The best approach remains
the sum of more spectra
= improve SNR at acquisition



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LUND UNIVERSITY

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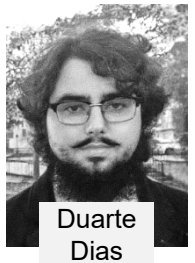
Diabetes & Brain Function unit


Annelise Francisco


Nadiia Kravchenko


Duarte Dias


João Duarte


Valentina Cesaroni

Postdoc on metabolism NMR+MS coming soon

@DiaBrainFun

main funding: Wallenberg Center for Molecular Medicine, Albert Påhlsson, Swedish Research Council, Diabetesfonden, European Commission

Funded by the European Union

exodiab
Excellence of Diabetes Research in Sweden

Knut and Alice Wallenberg Foundation