Proton Magnetic Resonance Spectroscopy

Applications in drug research

Nivedita Agarwal

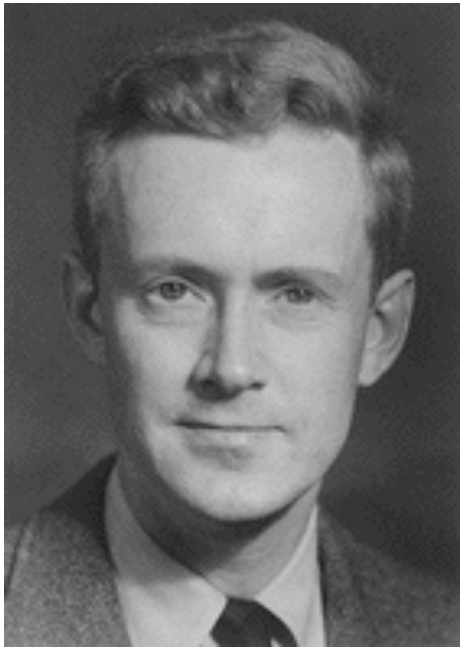
Overview

I) Proton Spectroscopy basics

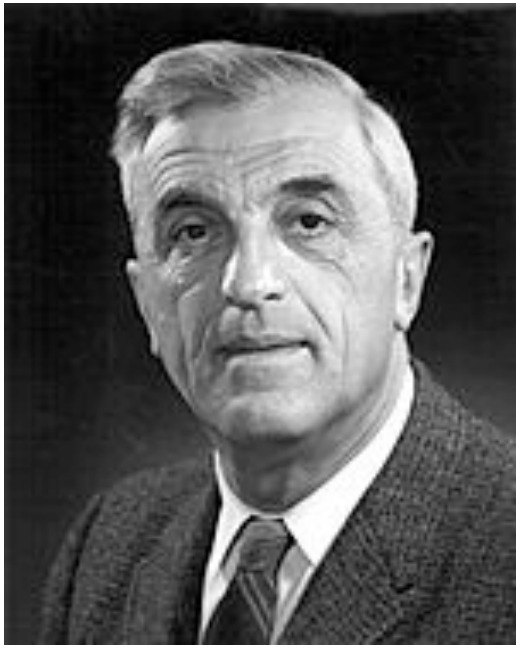
II) Major ^{1}H-MRS detectable neurotransmitters

III) Testing experimental drugs using ^{1}H-MRS – The Roche project!

Paying homage …

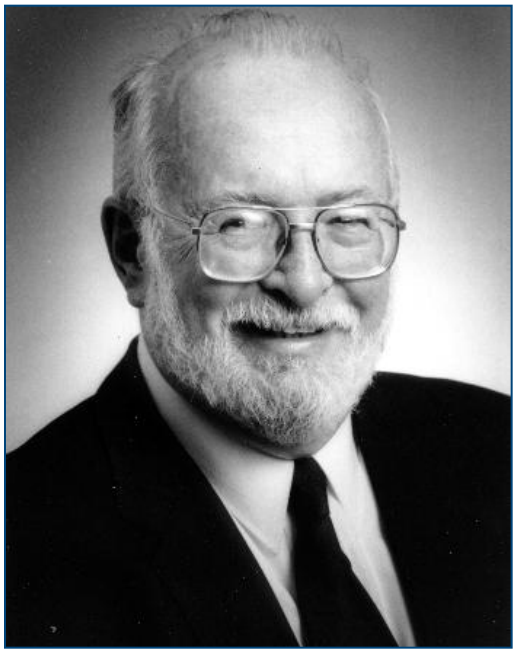


Edward Purcell
(1912-1997)
Developed ways to determine chemical structure of compounds using MR



Felix Bloch
(1905-1983)
Developed the Bloch equations – time evolution of nuclear magnetization

Paying homage …



Paul Lauterbur
(1929-2007)

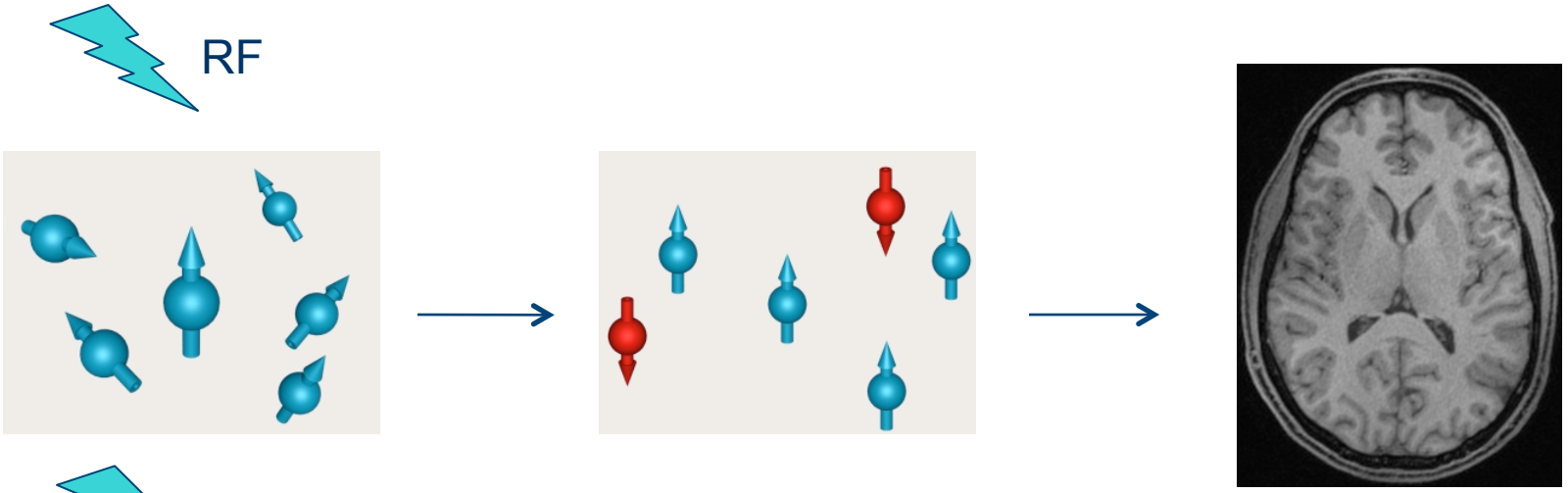
Spatial localization and creation of images

Peter Mansfield
(1933-)

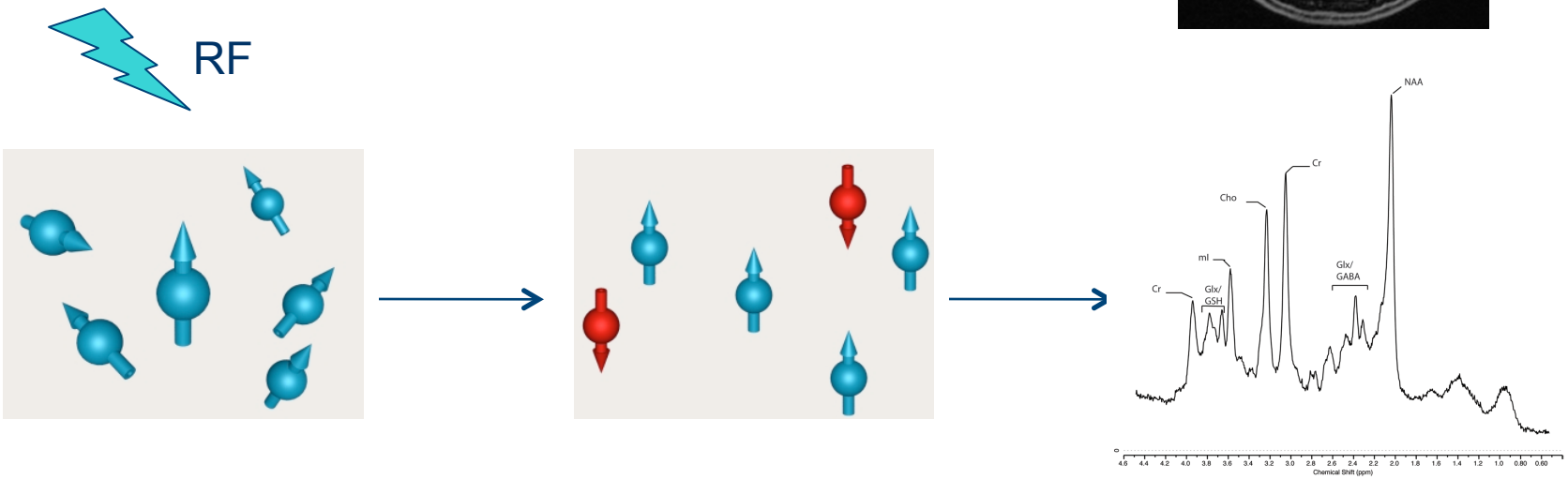
Use of RF and gradients, their interpretation and formation of signals. Also EPI made possible.

Basics - 1

MRI

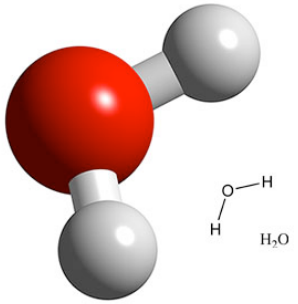


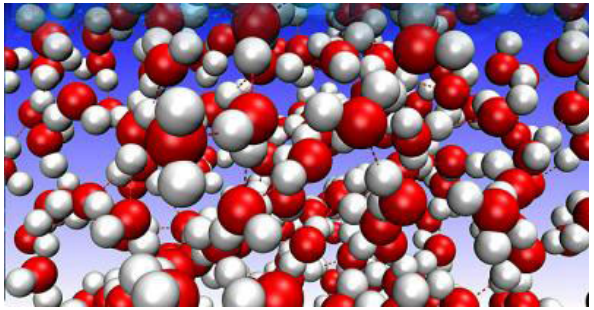
MRS

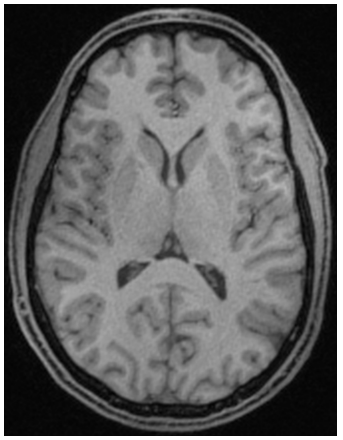


Basics - 2

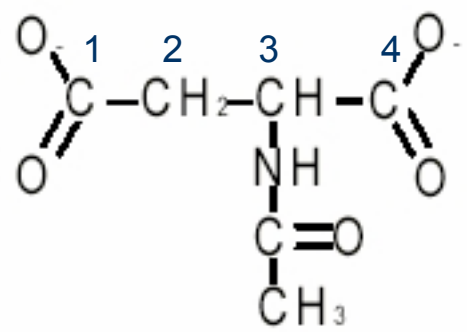
MRI

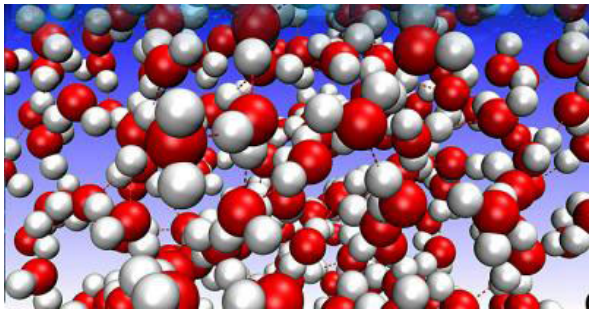




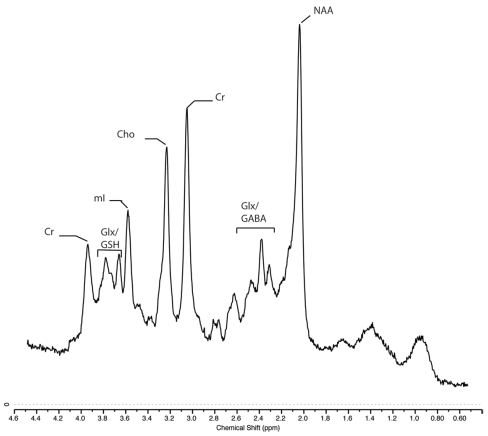


MRS

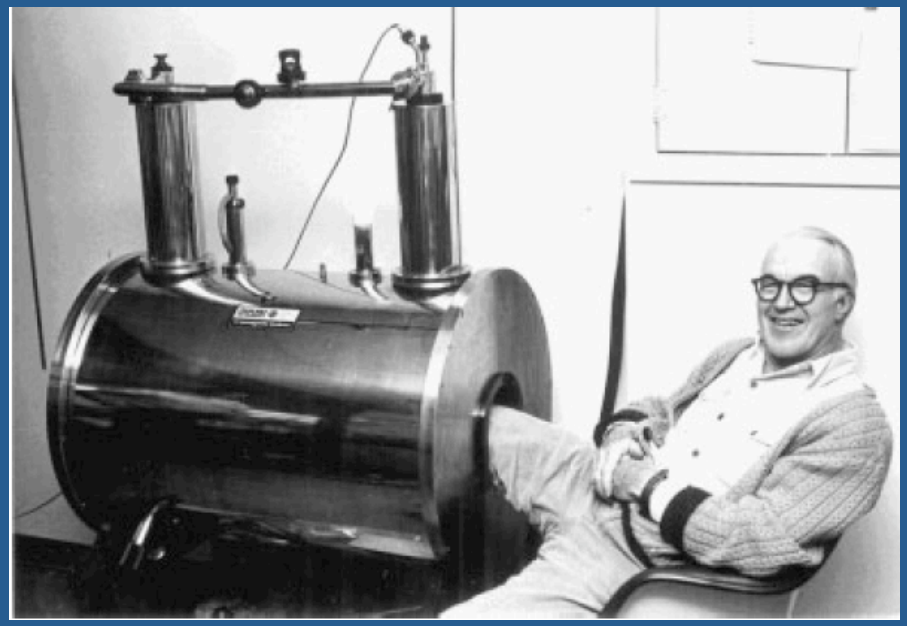




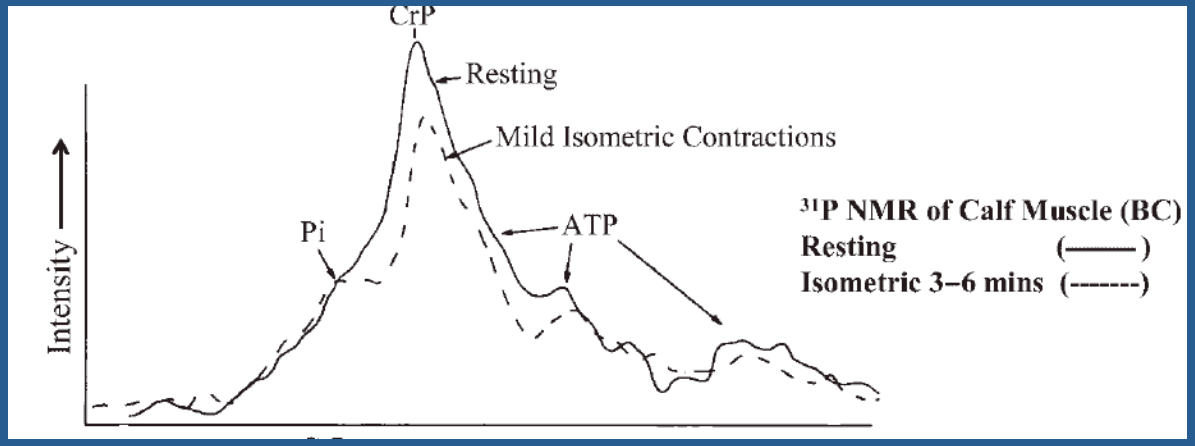
NAA



P-MRS (initial experience)

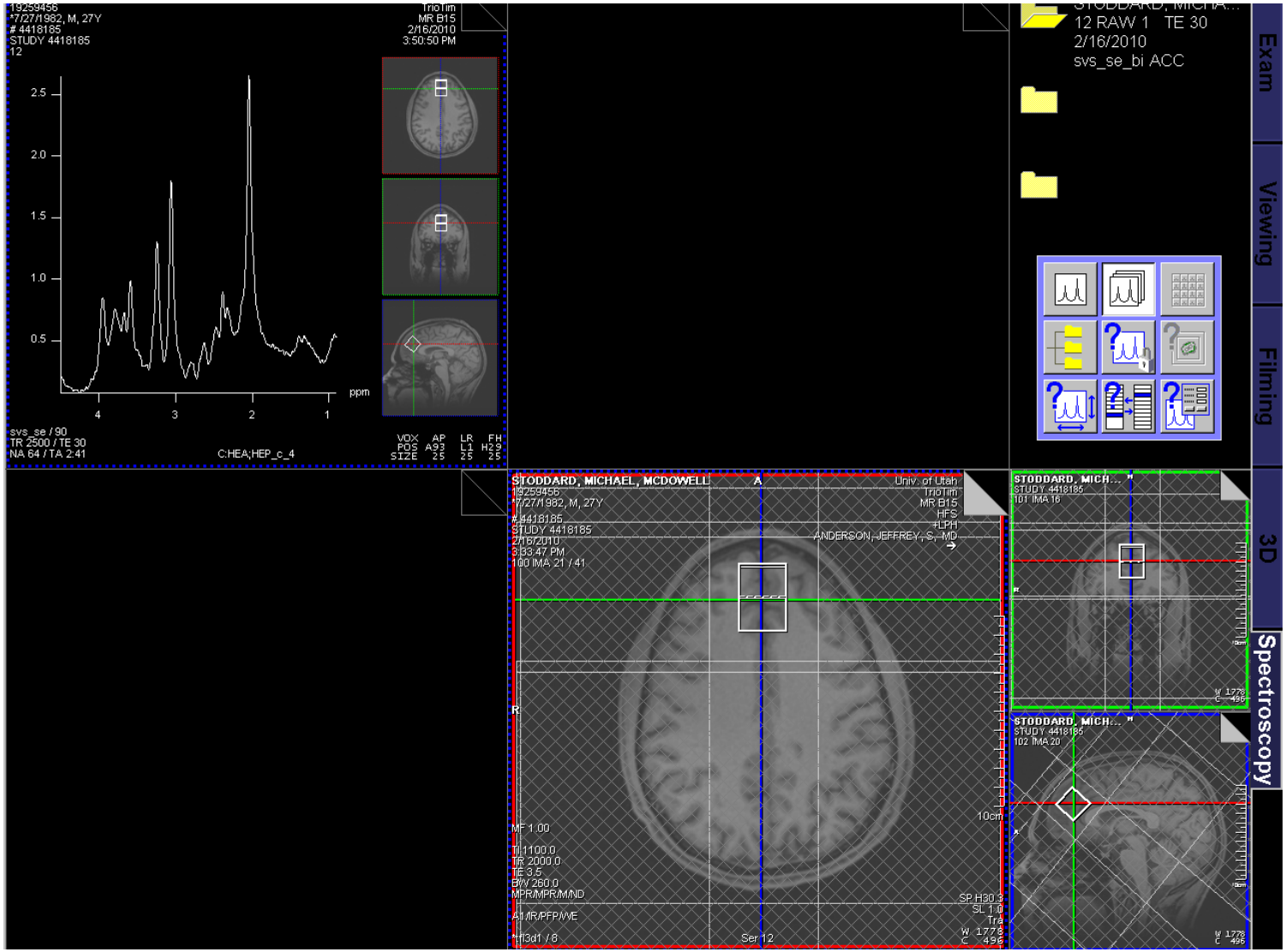


1978-79 - Radda, Chance and representatives of the Oxford Magnet Company constructed a 8” human subject magnet (sufficient to observe human extremities). The magnets were delivered to Philadelphia and Oxford simultaneously.

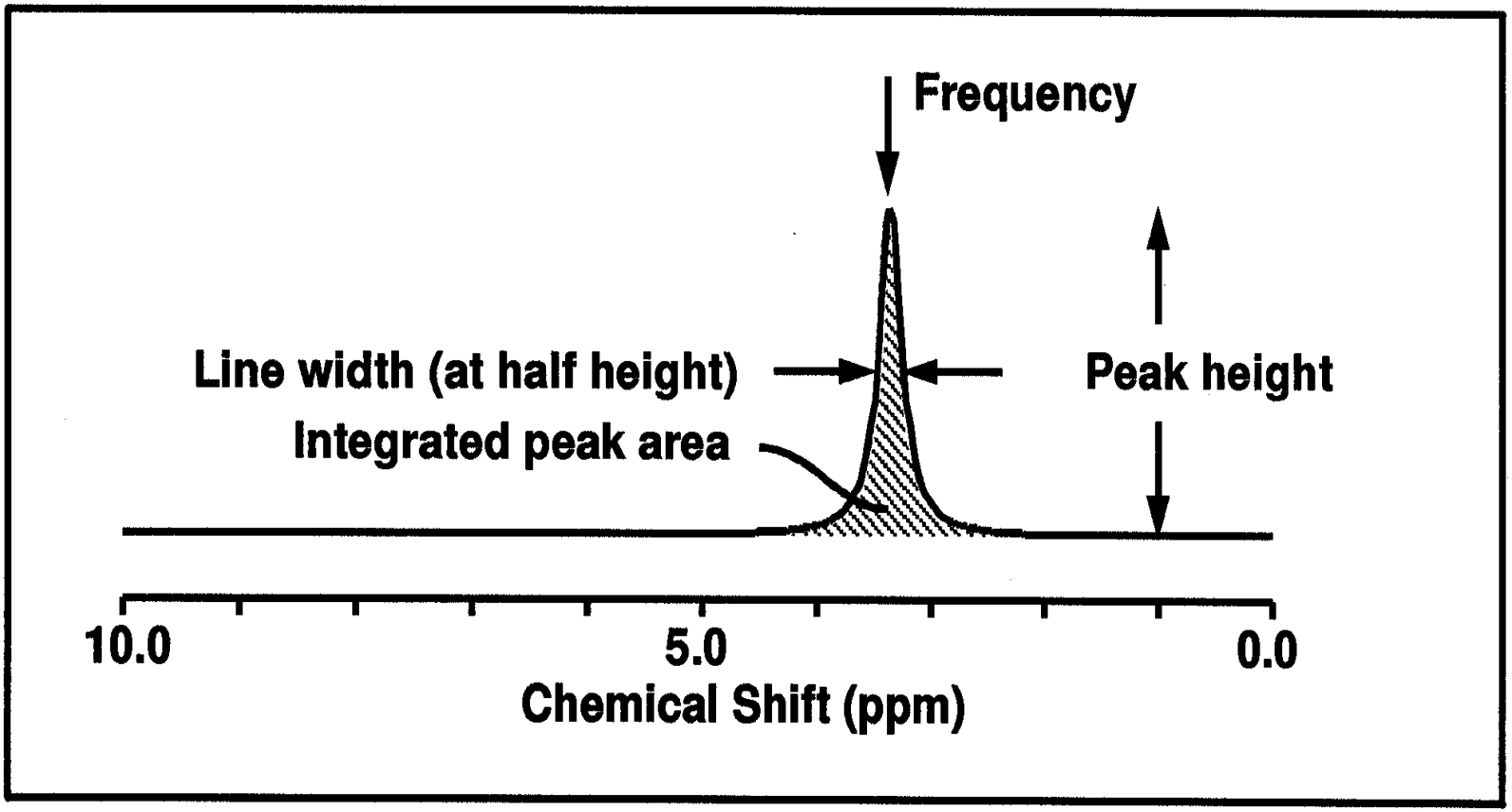


First human P-MRS spectra from a human subject’s exercising limb.

Basics - 3


Exam
Viewing
Filming
3D
Spectroscopy
12 RAW 1 TE 30
2/16/2010
svs_se_bi ACC
STODDARD, MICHAEL, MCDOWELL
ANDERSON, JEFFREY, S, MD
STODDARD, MICH...
STUDY 4418185
101 IMA 16
102 IMA 20
ppm

Basics -

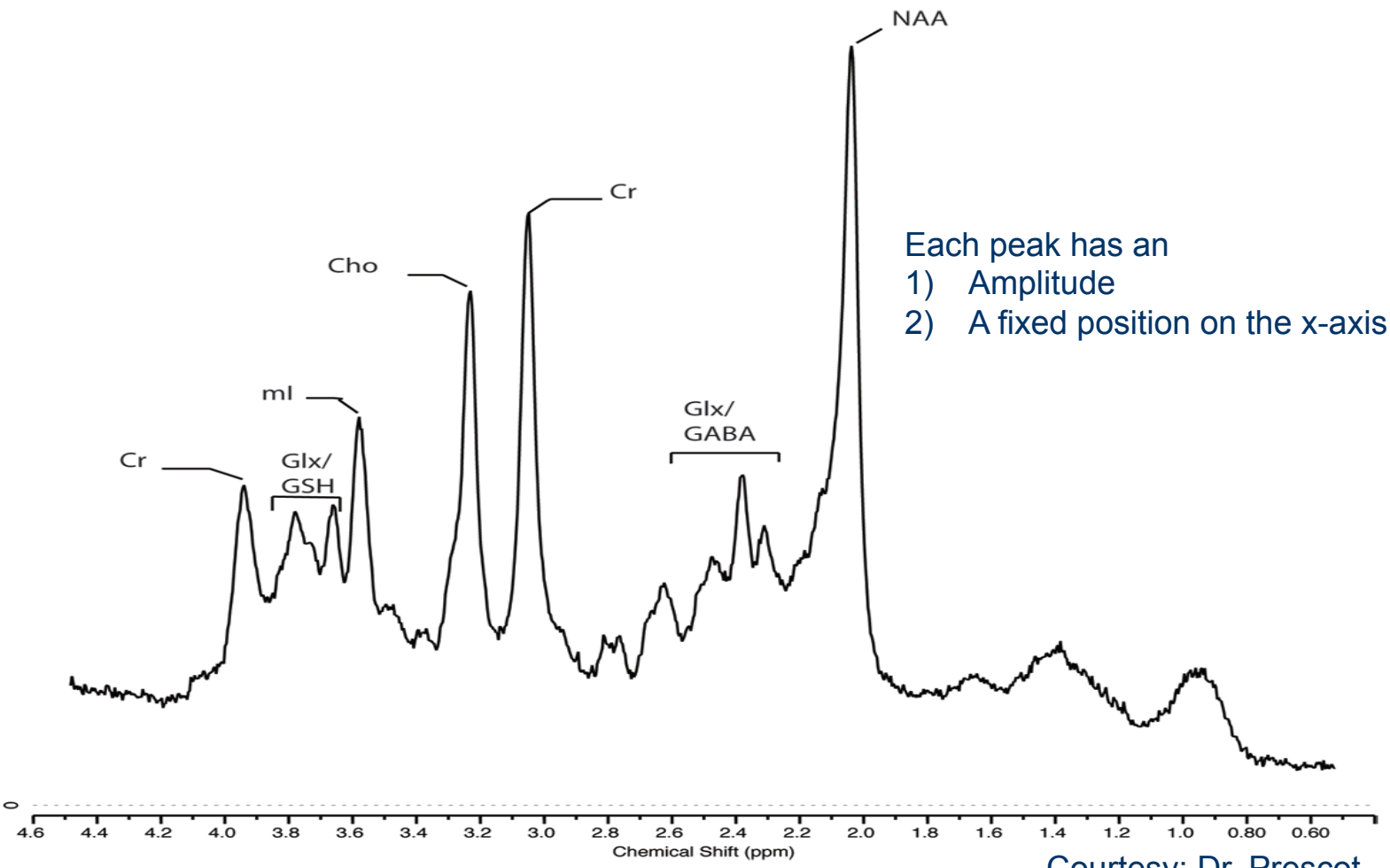

Frequency
Line width (at half height)
Integrated peak area
Peak height
10.0
5.0
0.0
Chemical Shift (ppm)

Basics -

$$\text{Chemical shift in ppm} = \frac{\text{peak position in Hz (relative to TMS)}}{\text{spectrometer frequency in MHz}}$$

Hz is directly proportional to ppm

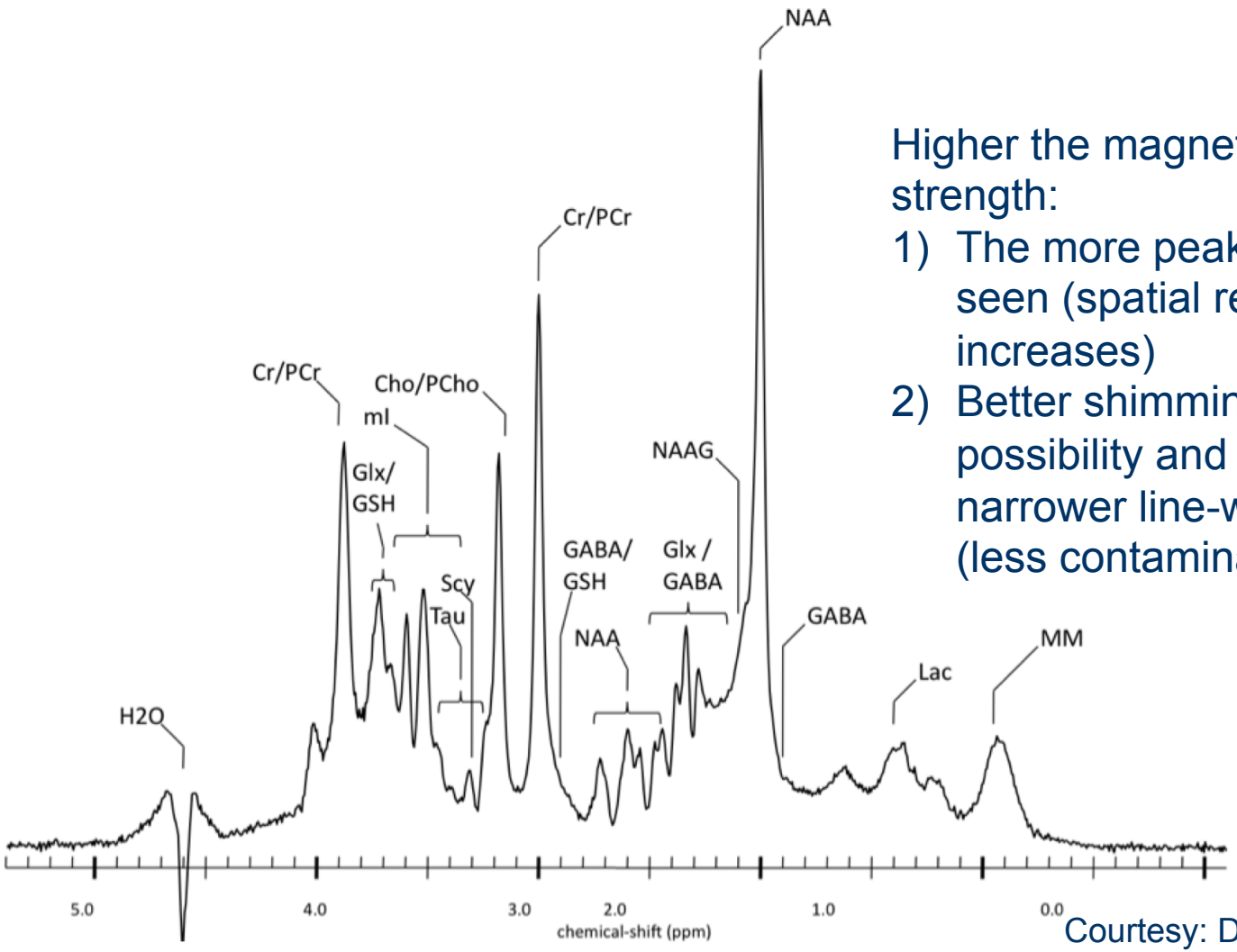
Basics – 3 Normal spectrum at 3T



Each peak has an
1) Amplitude
2) A fixed position on the x-axis

Courtesy: Dr. Prescot

Basics – 4 Normal spectrum at 4T

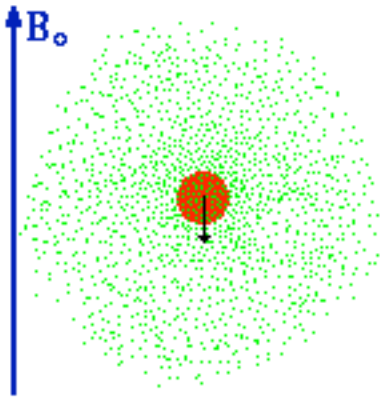


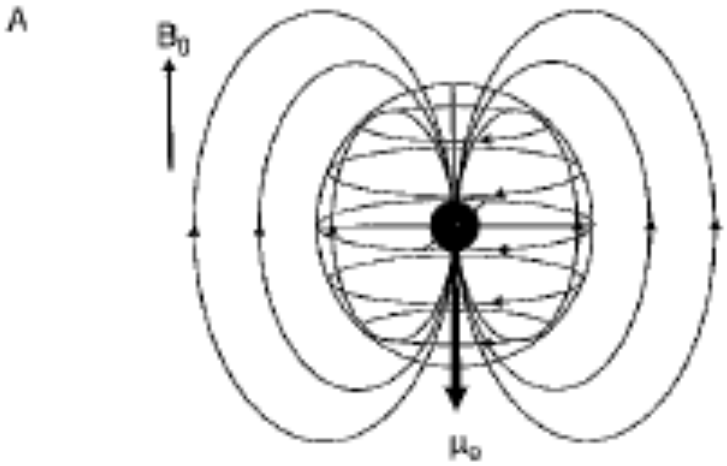
Higher the magnetic field strength:

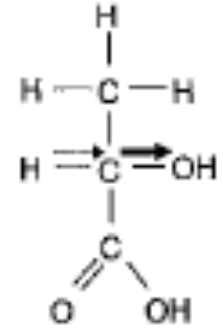
1) The more peaks are seen (spatial resolution increases)
2) Better shimming possibility and so narrower line-widths (less contamination)

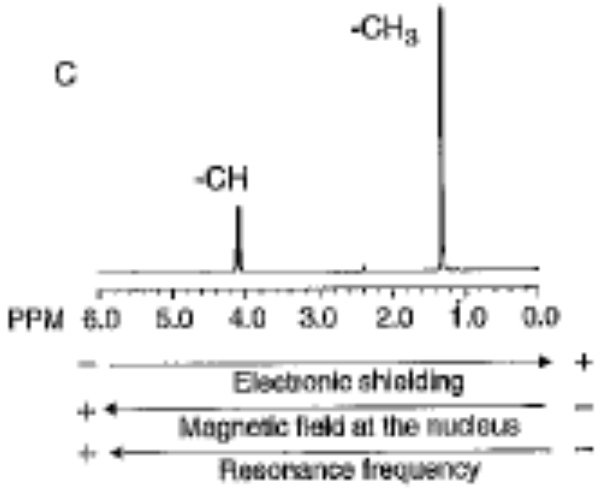
Courtesy: Dr. Jensen

Basics – 5 Chemical Shift

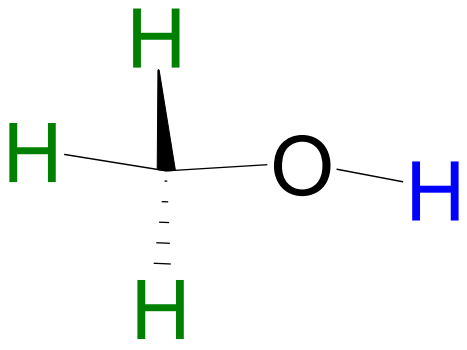






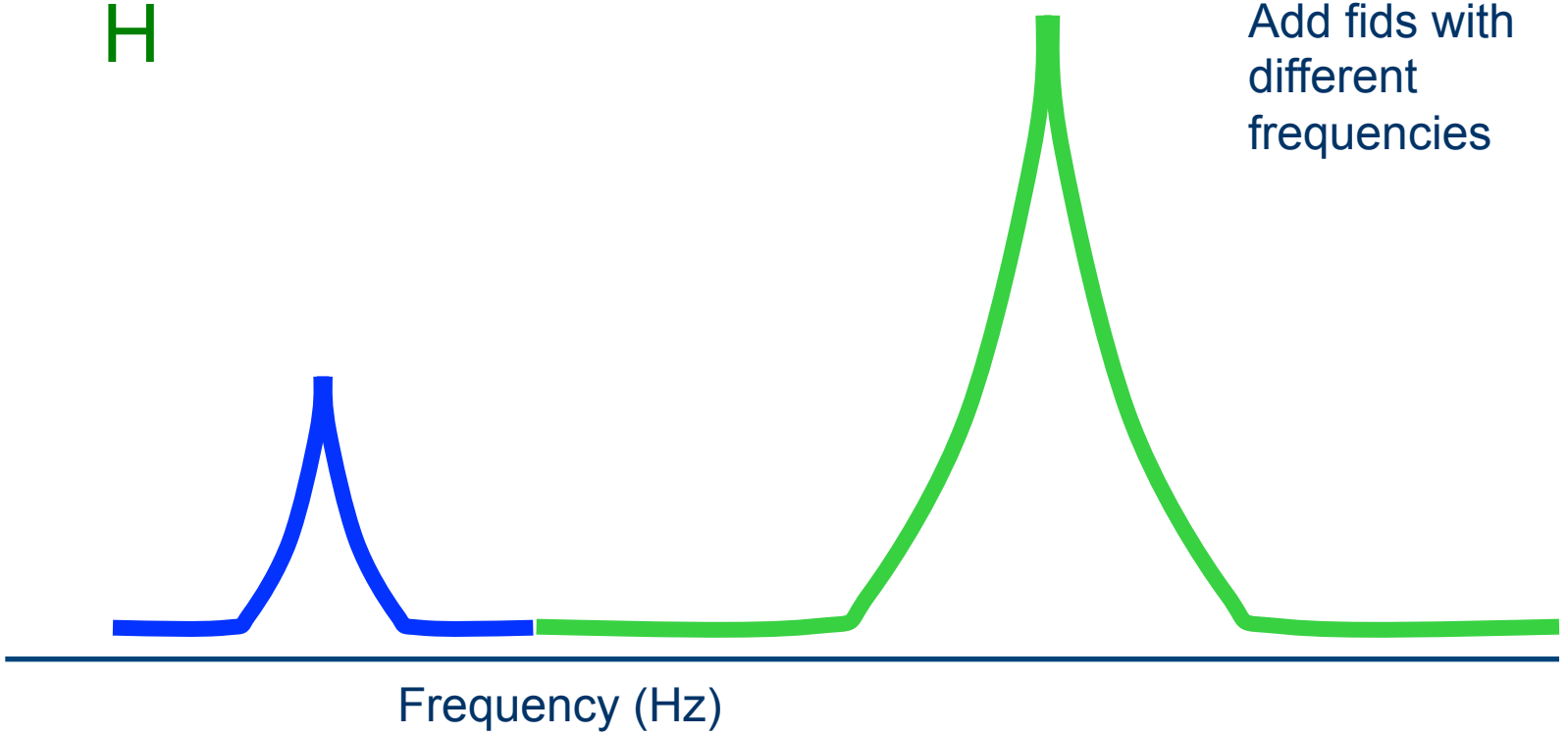


Basics – 5 Chemical Shift



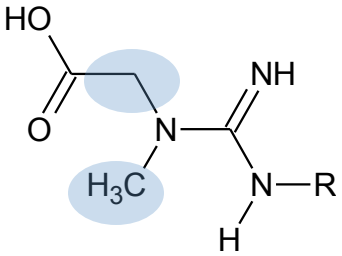
blue H is different to green H
so frequency should change

Add fids with different frequencies



Anatomy of a ^{1}H-MRS Spectrum

What gives rise to the chemical shift effect?



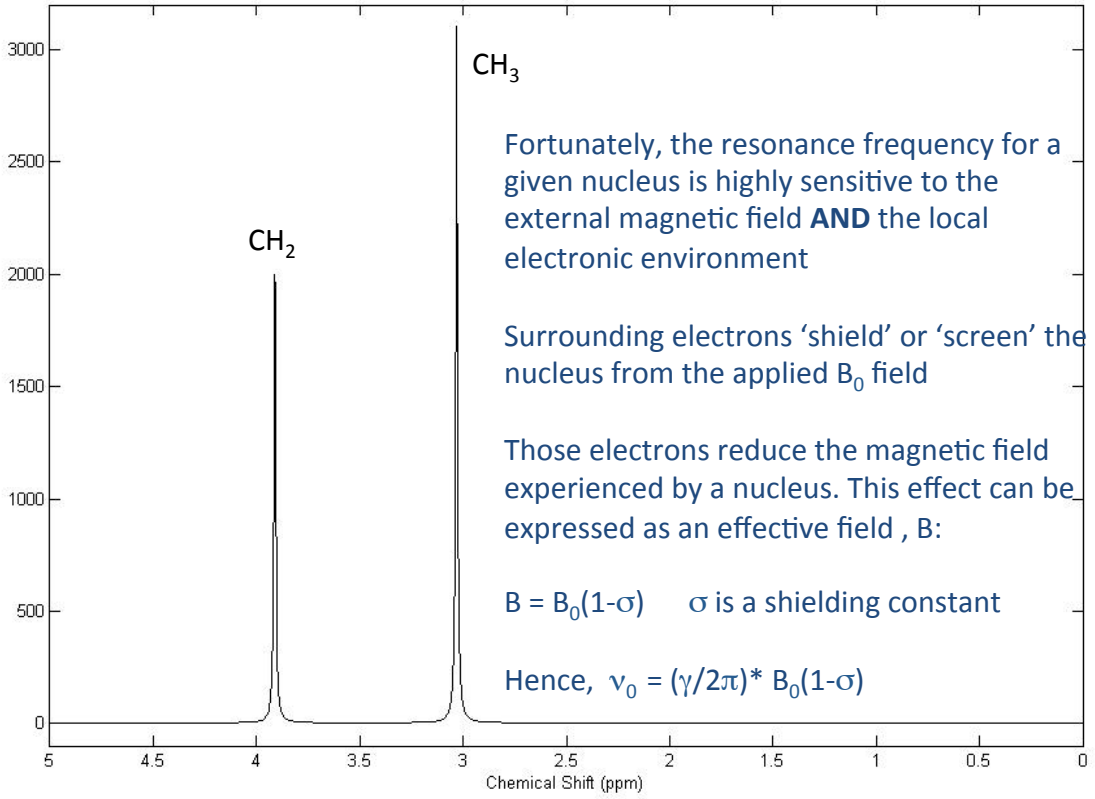
Creatine (Cre; R = H)
Phosphocreatine (PCr; R = PO_3H)

Resonance frequency (ν_0) is determined by nucleus gyromagnetic ratio (γ) and the external magnetic field (B_0)

LARMOR EQUATION: $\nu_0 = \gamma * B_0$

This infers that all nuclei would give rise to a single peak with a single resonance frequency

If this were the case then MRS would have zero application in biomedical studies!



Fortunately, the resonance frequency for a given nucleus is highly sensitive to the external magnetic field **AND** the local electronic environment

Surrounding electrons 'shield' or 'screen' the nucleus from the applied B_0 field

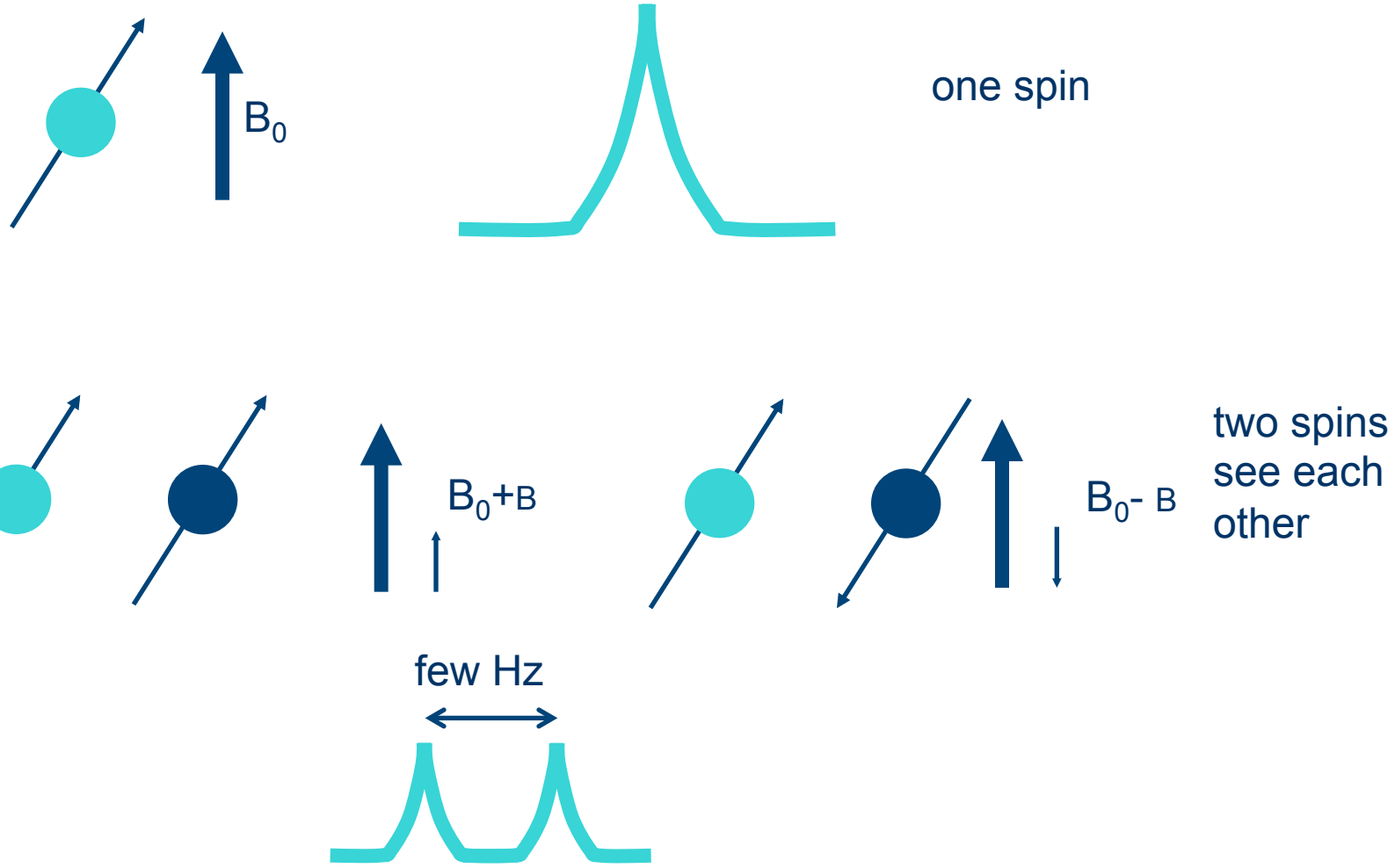
Those electrons reduce the magnetic field experienced by a nucleus. This effect can be expressed as an effective field , B:

$B = B_0(1-\sigma)$ σ is a shielding constant

Hence, $\nu_0 = (\gamma/2\pi) * B_0(1-\sigma)$

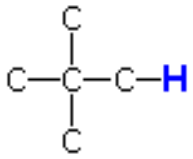
← Increasing frequency

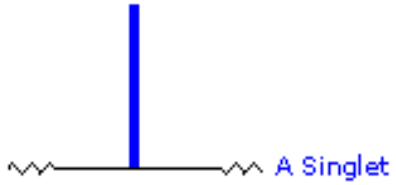
J coupling



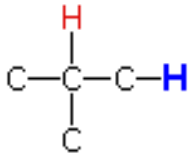
J coupling

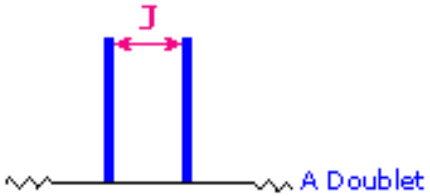
No Coupled Hydrogens



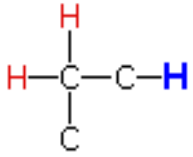


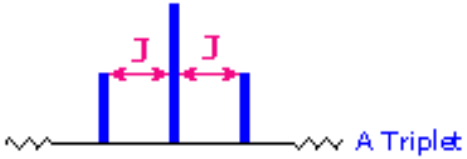
One Coupled Hydrogen



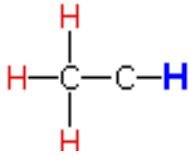


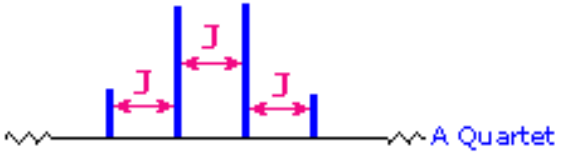
Two Coupled Hydrogens

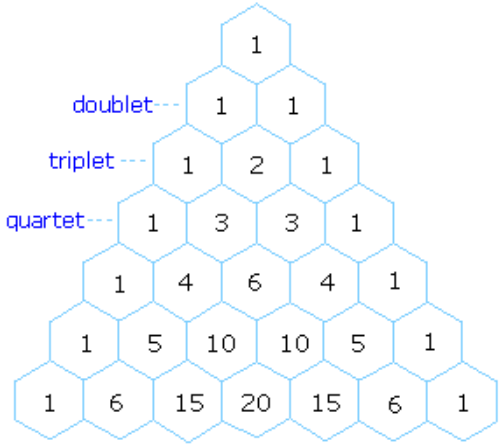




Three Coupled Hydrogens



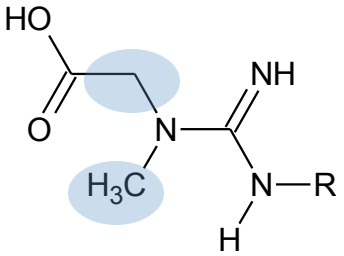




Pascal's Triangle

Anatomy of a ^{1}H-MRS Spectrum

A good starting point is to consider the Cre ^{1}H-MRS spectrum



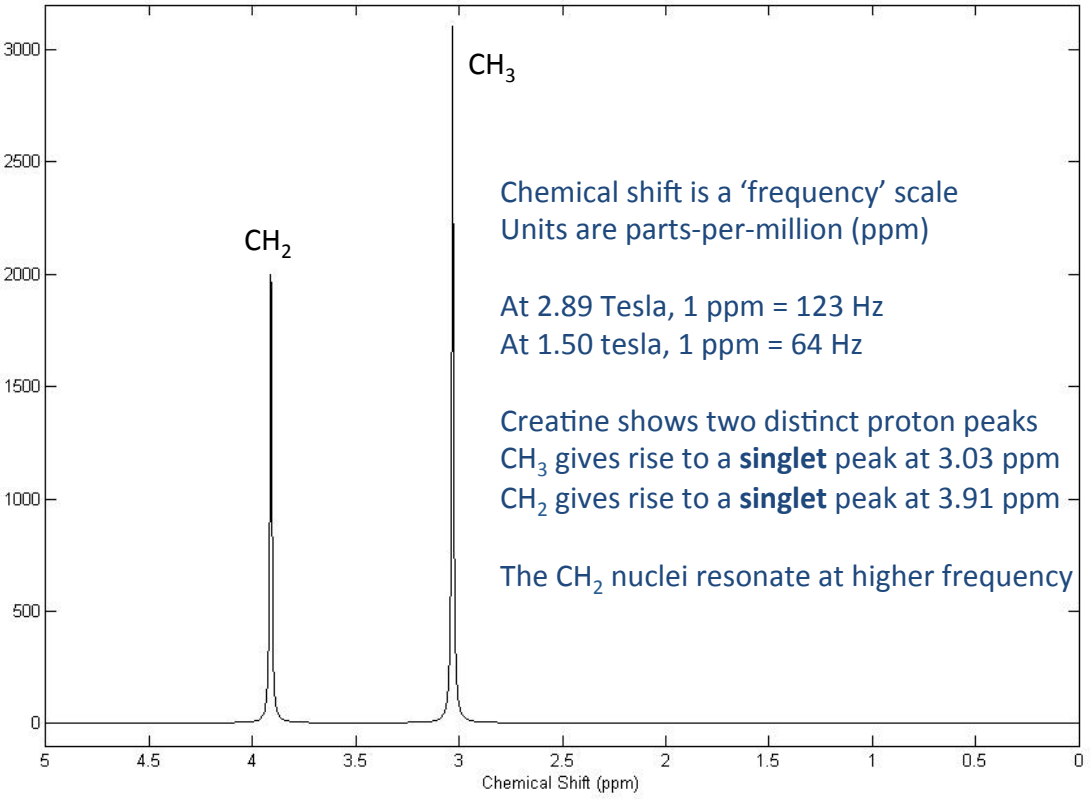
Creatine (Cre; R = H)
Phosphocreatine (PCr; R = PO_3H)

Cre has two carbon-bound proton groups that are MRS-detectable

A single methyl (CH_3) group
A single metylene (CH_2) group

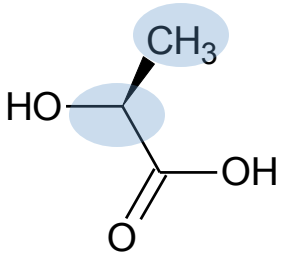
The CH_3 and CH_2 nuclei are separated by 4 covalent bonds

The nitrogen and oxygen-bound Protons are in fast exchange with Solvent water and do not give rise To observable signals



Anatomy of a ^{1}H-MRS Spectrum

Now we will consider spin-spin (*J*) coupling effects exemplified by the Lactate spectrum



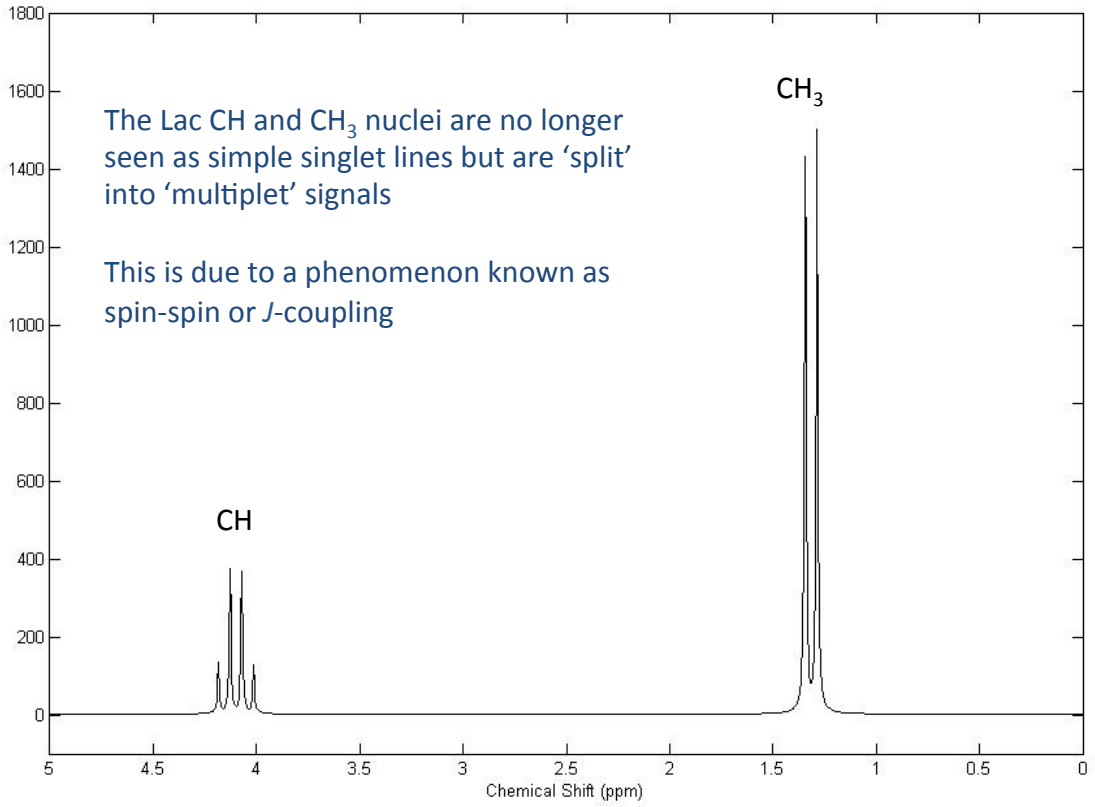
Lactic acid (Lac)

Lac has two carbon-bound proton groups that are MRS-detectable

A single methyl (CH_3) group
A single methine (CH) group

The CH group is in close proximity to two electronegative functional groups (a single OH and a carbonyl)

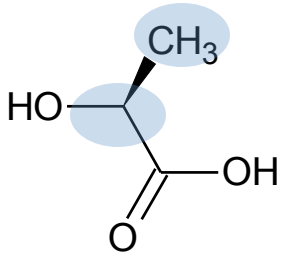
Hence, the CH resonance is chemical shifted to higher frequency (4.11 ppm) whereas the CH_3 nuclei resonate at 1.33 ppm



Conceptual approach to understanding *J*-coupling

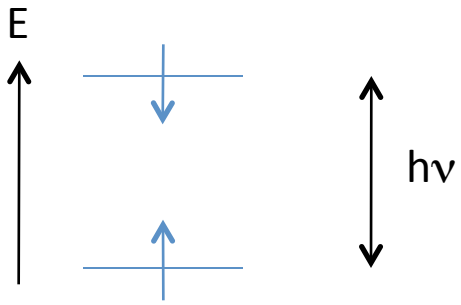
The Lac CH and CH_3 nuclei are separated by three covalent bonds – a distance which is sufficiently short enough such that the non-equivalent nuclei can 'sense' each others spin state (i.e. aligned parallel or anti-parallel to the B_0 field) and are **coupled**

This sensing of the other nuclei's spin state is propagated by bonding electrons and governed by the Pauli exclusion principle (not covered here – see De Graaf)



First consider the sensing of the CH proton nuclear spin state by the CH_3 protons

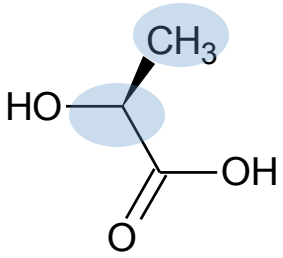
The CH proton nuclear spin could be aligned parallel or anti-parallel to main B_0 field leading to two non-degenerate energy levels sensed by the CH_3 nuclei



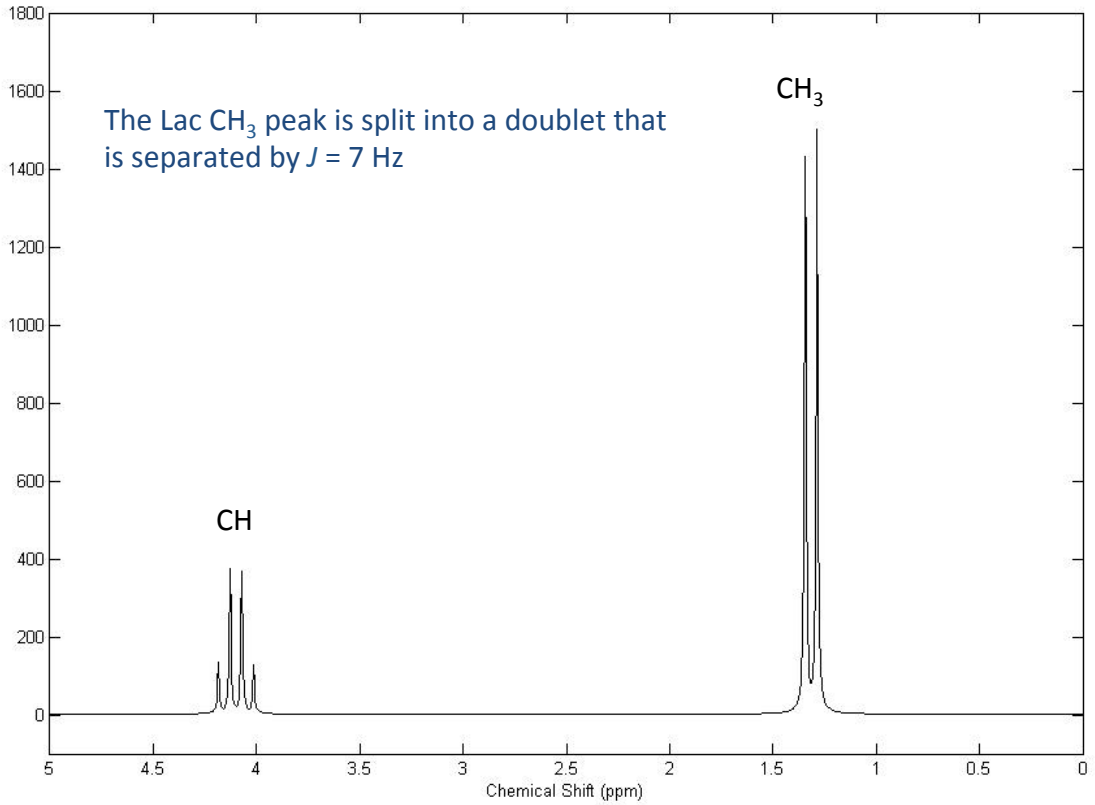
This coupling effect leads to the CH_3 resonance line being split into two lines of equal intensity (equal statistical probability of sensing the two CH spin states) separated by $\Delta E = h\nu$ or *J* Hz

Anatomy of a ^{1}H-MRS Spectrum

Now we will consider spin-spin (*J*) coupling effects exemplified by the Lactate spectrum



Lactic acid (Lac)

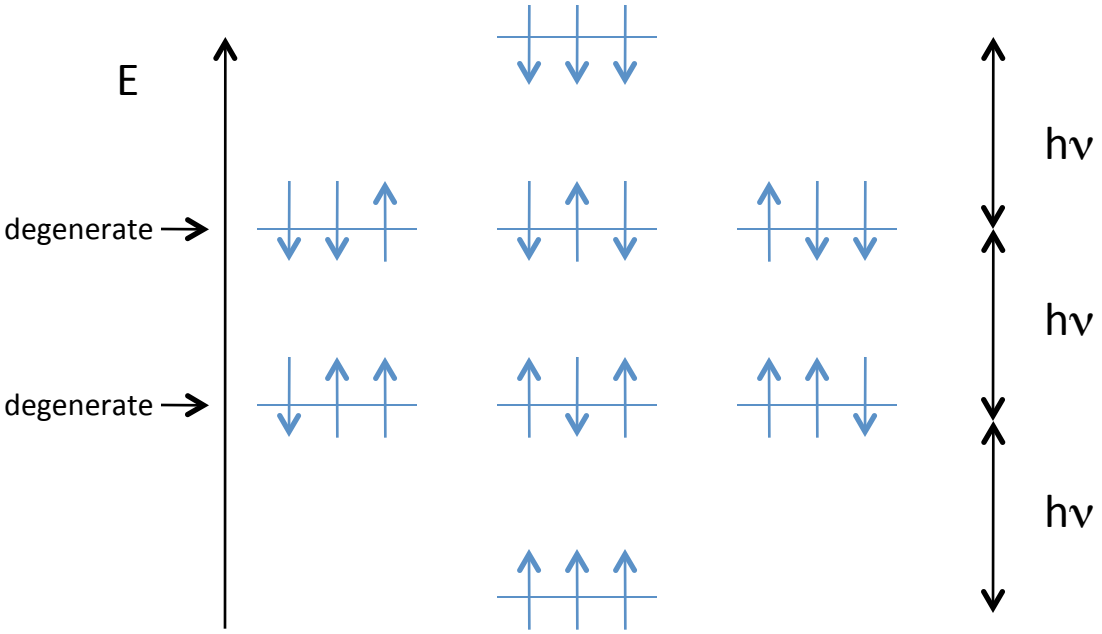


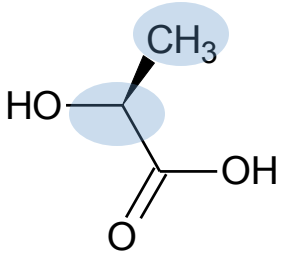
Increasing frequency

Conceptual approach to understanding *J*-coupling

Now consider the sensing of the CH_3 proton nuclear spins by the single CH proton spin

A total of 8 different spin state configurations are possible for the CH_3 spins





This coupling effect leads to the CH resonance line being split into four lines with a relative intensity of 1:3:3:1

The ratio stems from there being increased probability of two of the spin states where one nuclear spin is aligned parallel or anti-parallel to B_0 (3 degenerate states each!)

Anatomy of a ^{1}H-MRS Spectrum

So, the ^{1}H-MRS spectrum exhibits there main features:

(1) Chemical shift
(2) Signal area (concentration)
(3) J-coupling effects

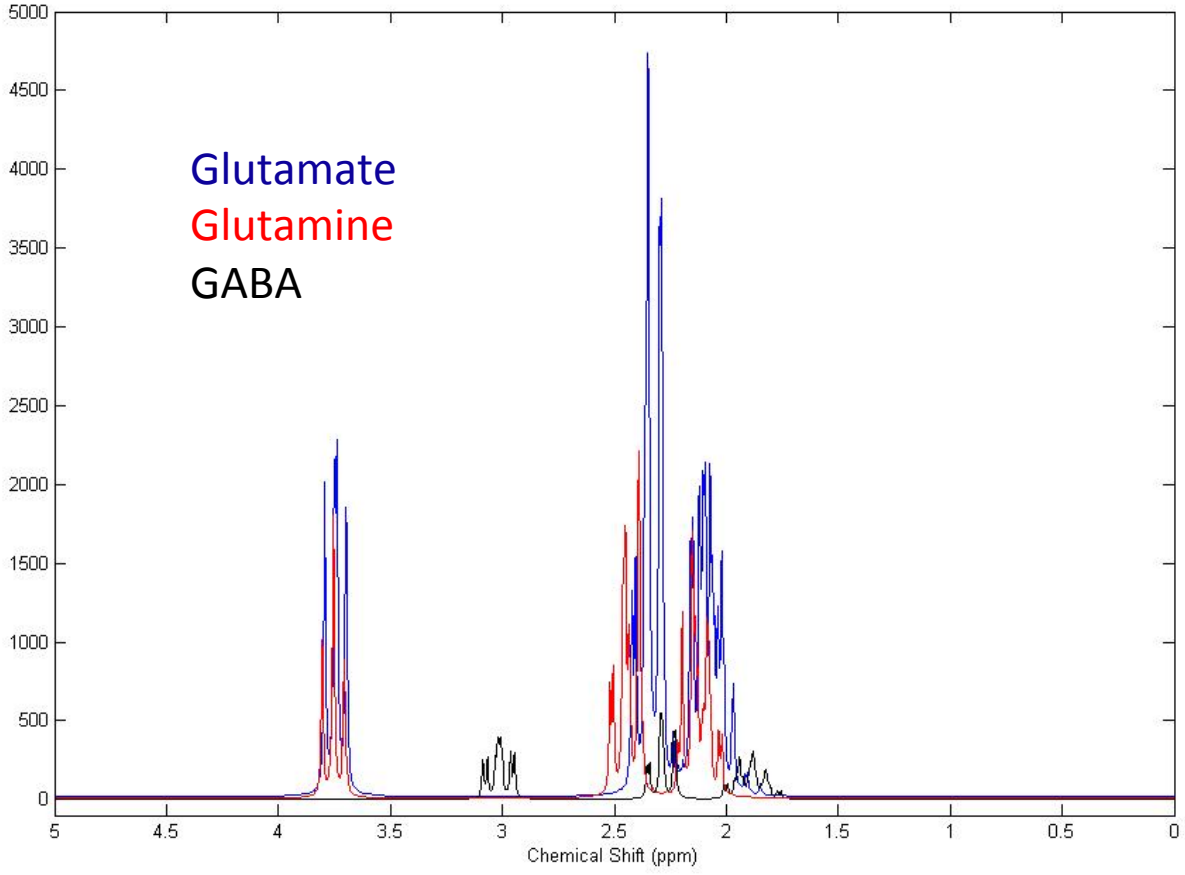
The simulated spectra on the right were generated using *in vivo* concentration ratios

A linewidth of 2 Hz was used *in vivo* linewidths would be larger

The chemical shifts for these three metabolites are VERY similar

Additional *J*-coupling effects enhances the severity of the observed spectral overlap!!!

AND THIS IS JUST THREE METABOLITES OUT OF TENS OF DETECTABLE BRAIN SPECIES!!!



METABOLITES OF CLINICAL INTEREST

METABOLITES OF INTEREST

Table. Cellular meaning and localization of the main metabolites identified by hydrogen protons spectroscopy.

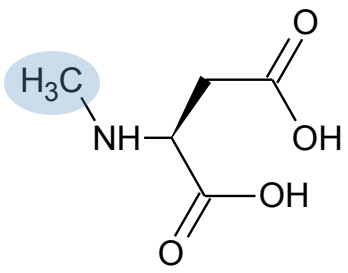
Metabolite	Cellular meanings of metabolites	PICK (ppm)	
		First	Others
NAA – N-acetyl-aspartate	Marker of number and viability of neurons	2.02	2.6
Cr – creatine	Markers of systems of energy of encephalic cells	3.03	3.9
Co – choline	Membrane markers It is related to cell membrane production and destruction High concentrations indicate hypercellularity and myelin destruction	3.2	–
Lac – lactate	Absence in normal tissue High concentrations indicate fault of cellular oxidative respiration	1.32	–
Lip – lipids	Necrosis marker (high grade tumors)	0.8	1.2–1.5
GLX – glutamine-glutamate	Neurotransmitter, neuroexcitator, detoxificator and regulator of neurotransmission activity	a – 3.65 a 3.8 b – 2.05 a 2.5	– –
mI – mio-inositol	Osmolite (osmolar regulator of cell volume) Glial marker	3.56	–

Adapted from Danielsen and Ross[39].

Proton (^{1}H) Magnetic Resonance Spectroscopy (MRS)

Many interesting neurochemical entities contain carbon-bound protons whose nuclei can potentially be detected and quantified using ^{1}H-MRS

The classic species readily identifiable using ^{1}H-MRS include:



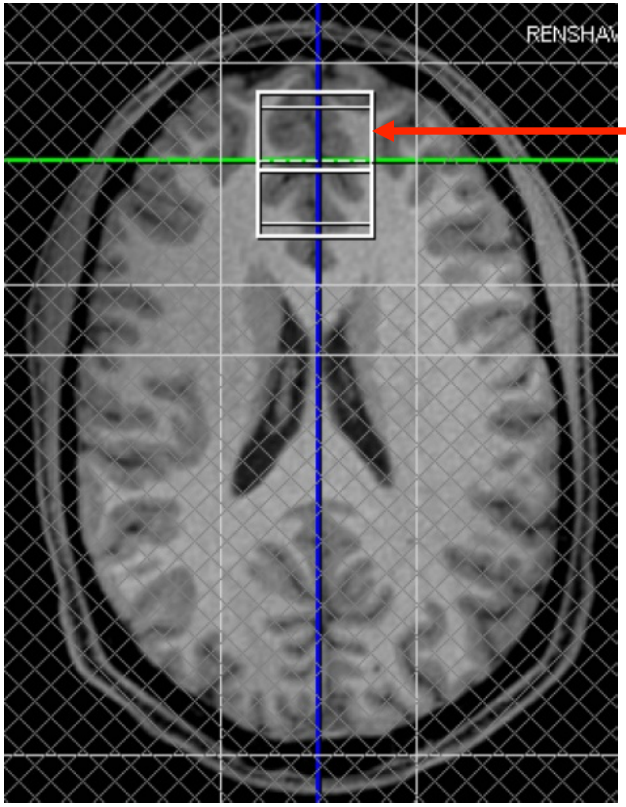
N-acetyl aspartate (NAA)

[NAA] ~ 8-17 mM
Marker for neuronal density
Osmoregulation
Breakdown product of NAAG

Raised in Canavan's disease
Decreased in MS
Reduced levels with age

In Vivo example of ^{1}H-MRS

Typical MRS voxel positioning



^{1}H MRS voxel (22.5 mL)

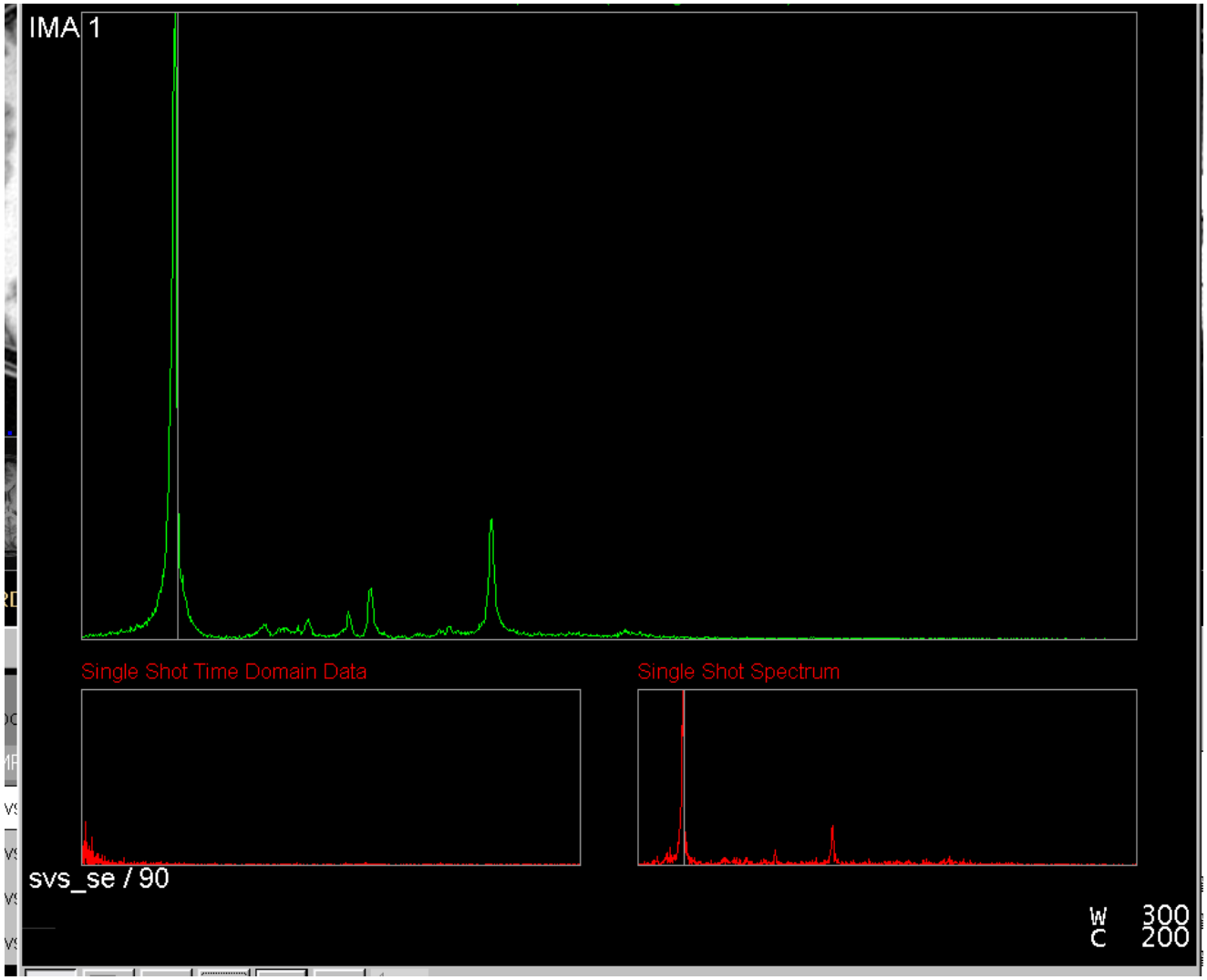
In Vivo Data Acquisition

PRESS sequence used to acquire water-suppressed ^{1}H-MRS data

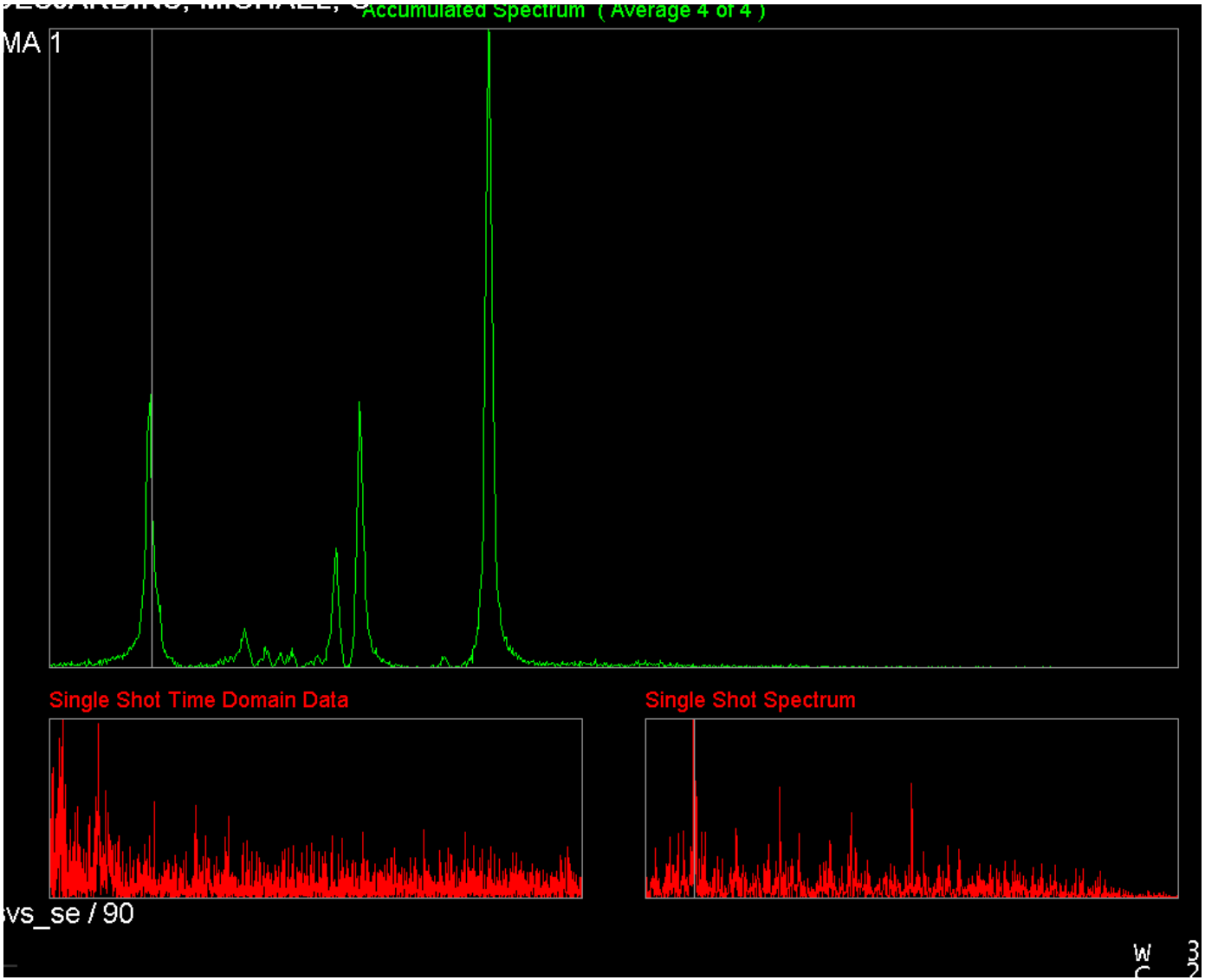
PRESS is a double spin-echo based sequence

TR = 2000 ms, TE = 30 ms, NEX = 128

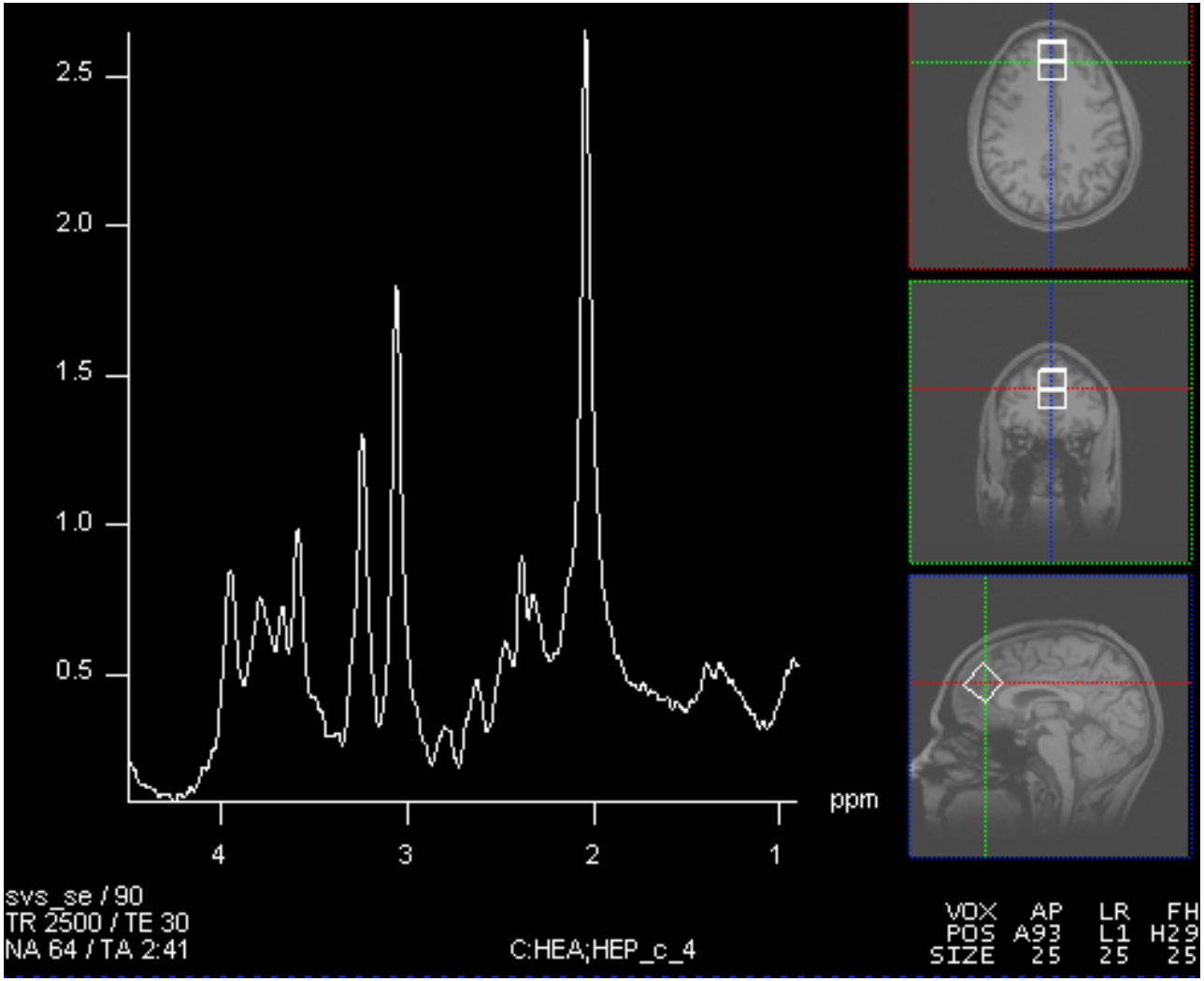
Water peak


IMA 1
Single Shot Time Domain Data
Single Shot Spectrum
svs_se / 90
W 300
C 200

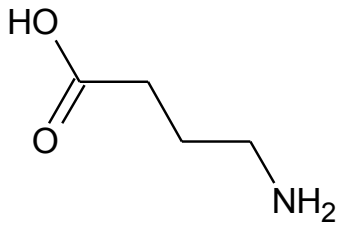
Water peak suppression online



Final spectrum



Species pertinent to psychiatric and neurologic illness and ^{1}H-MRS detectable include:



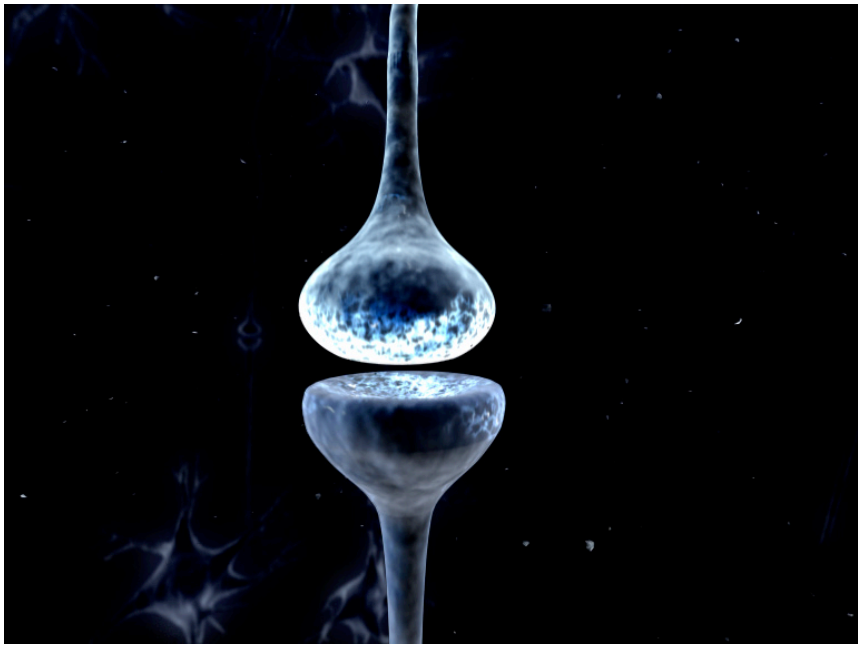
γ-amino butyric acid (GABA)

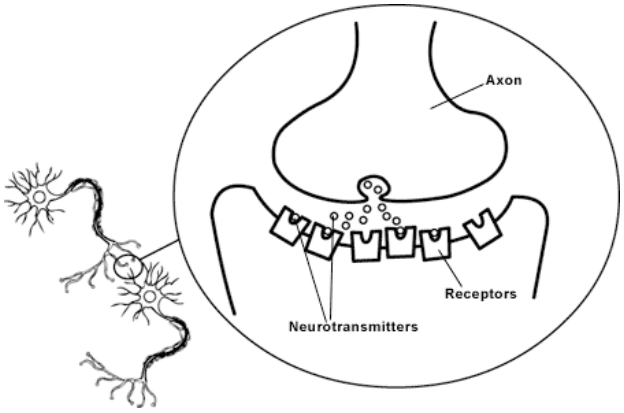
[GABA] ~ 1 mM
Major **inhibitory** amino acid
neurotransmitter in human CNS

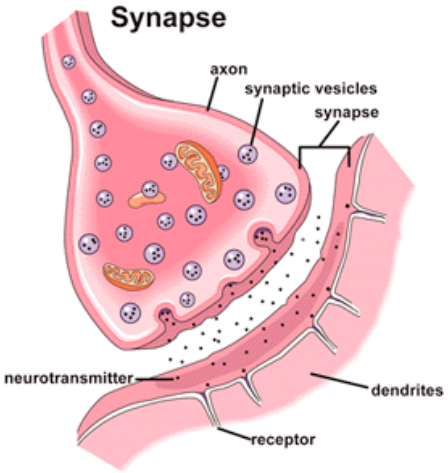
Reduced in epilepsy. Increasing
GABA levels with e.g. vigabatrin
is one line of therapy with ^{1}H-MRS
utilized as a monitoring approach
Reduced in major depression

See Govindaraju et al NMR in Biomedicine (2000) 13:129-153 for excellent source of MRS-detectable brain metabolite information

Synapse

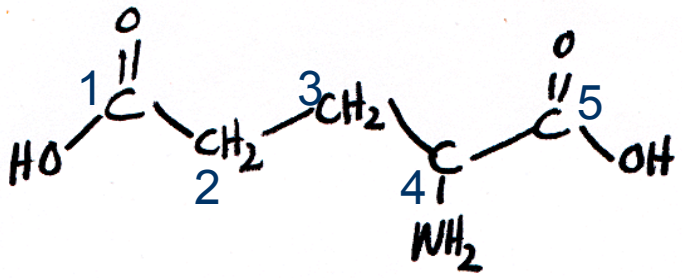






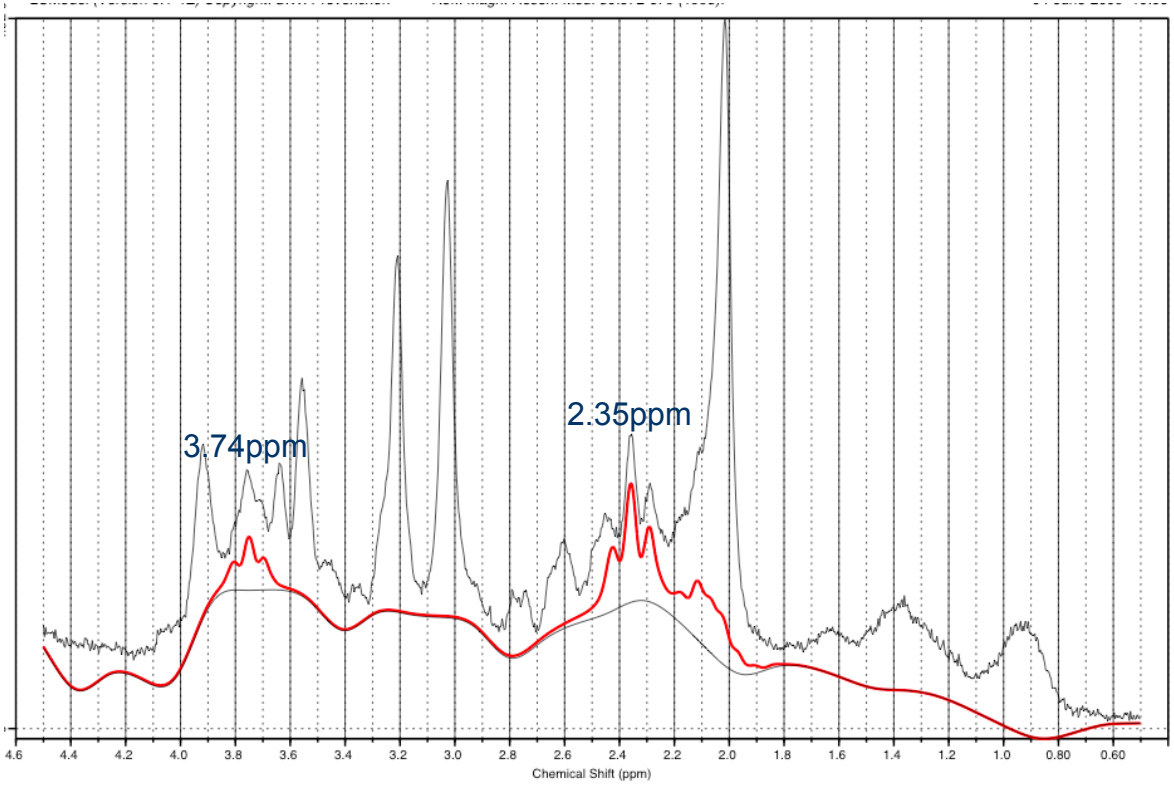
presynaptic membrane
post synaptic membrane
synapse
vesicles

Glutamate

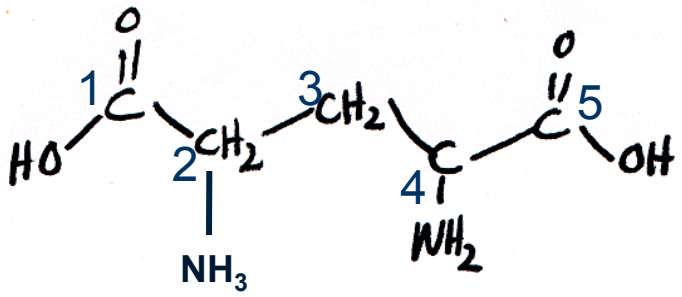


$Glu_{[Gray\ matter]}$ = 10-15mmol/L
$Glu_{[white\ matter]}$ = 4-6mmol/L

Group	ppm
^{2}CH	3.74
$^{3}CH_2$	2.03, 2.12
$^{4}CH_2$	2.33, 2.35



Glutamine



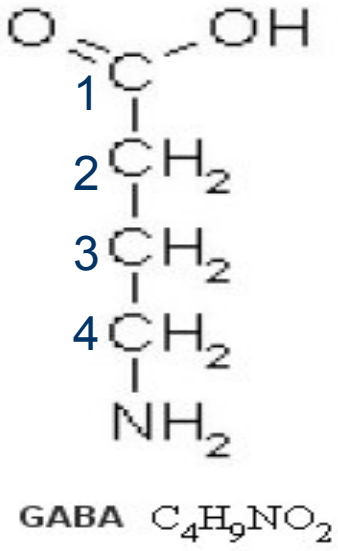
Important precursor for glutamate.
Important for the detoxification of free ammonia.
Exclusively synthesized in glial cells via glutamine synthetase.

Gln= 2-4mmol/L

Group	ppm
^{2}CH	3.75
$^{3}CH_2$	2.11, 2.13
$^{4}CH_2$	2.43, 2.45

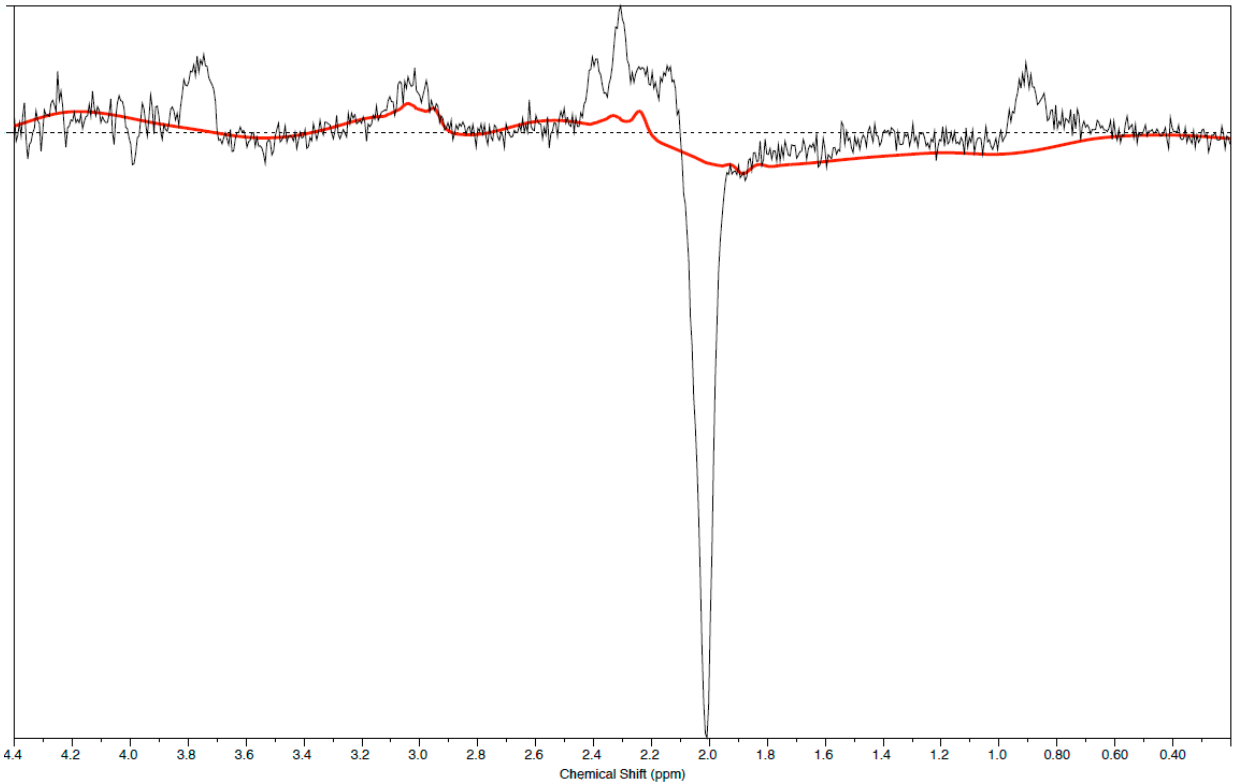
Group	ppm
^{2}CH	3.74
$^{3}CH_2$	2.03, 2.12
$^{4}CH_2$	2.33, 2.35

GABA=γ-aminobutyric acid

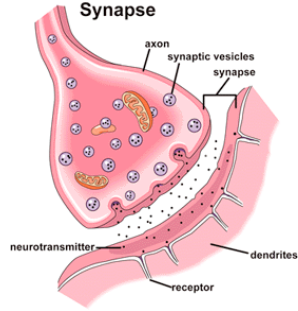


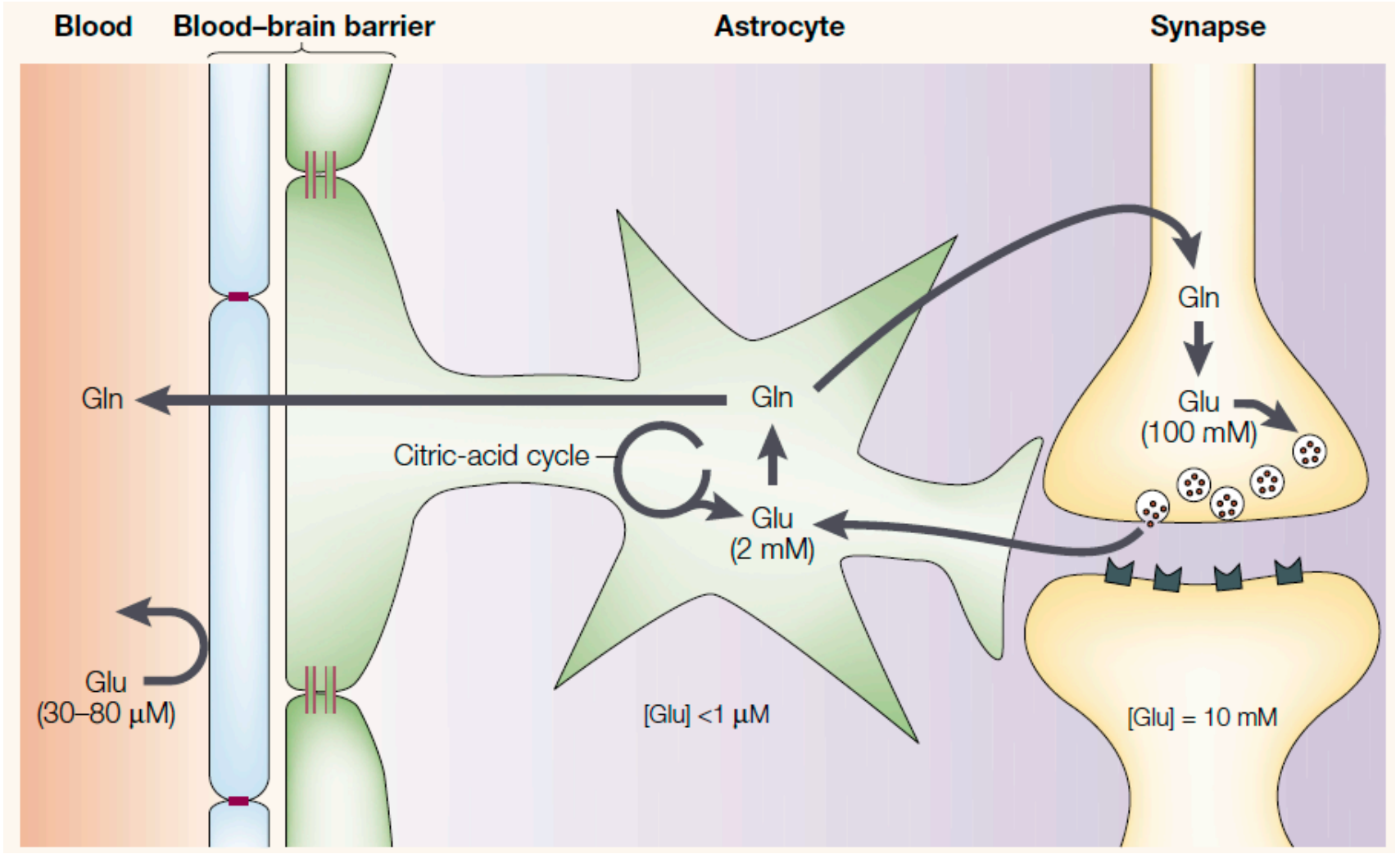
Major inhibitory neurotransmitter
GABA: 1-2 mmol/L
Glucose is the main precursor of GABA

Group	ppm
$^{2}CH2$	2.28
$^{3}CH_2$	1.88
$^{4}CH_2$	3.01

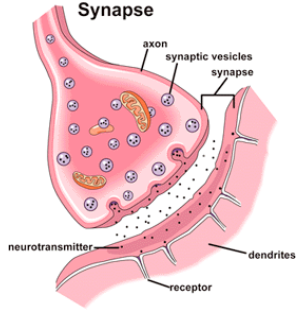


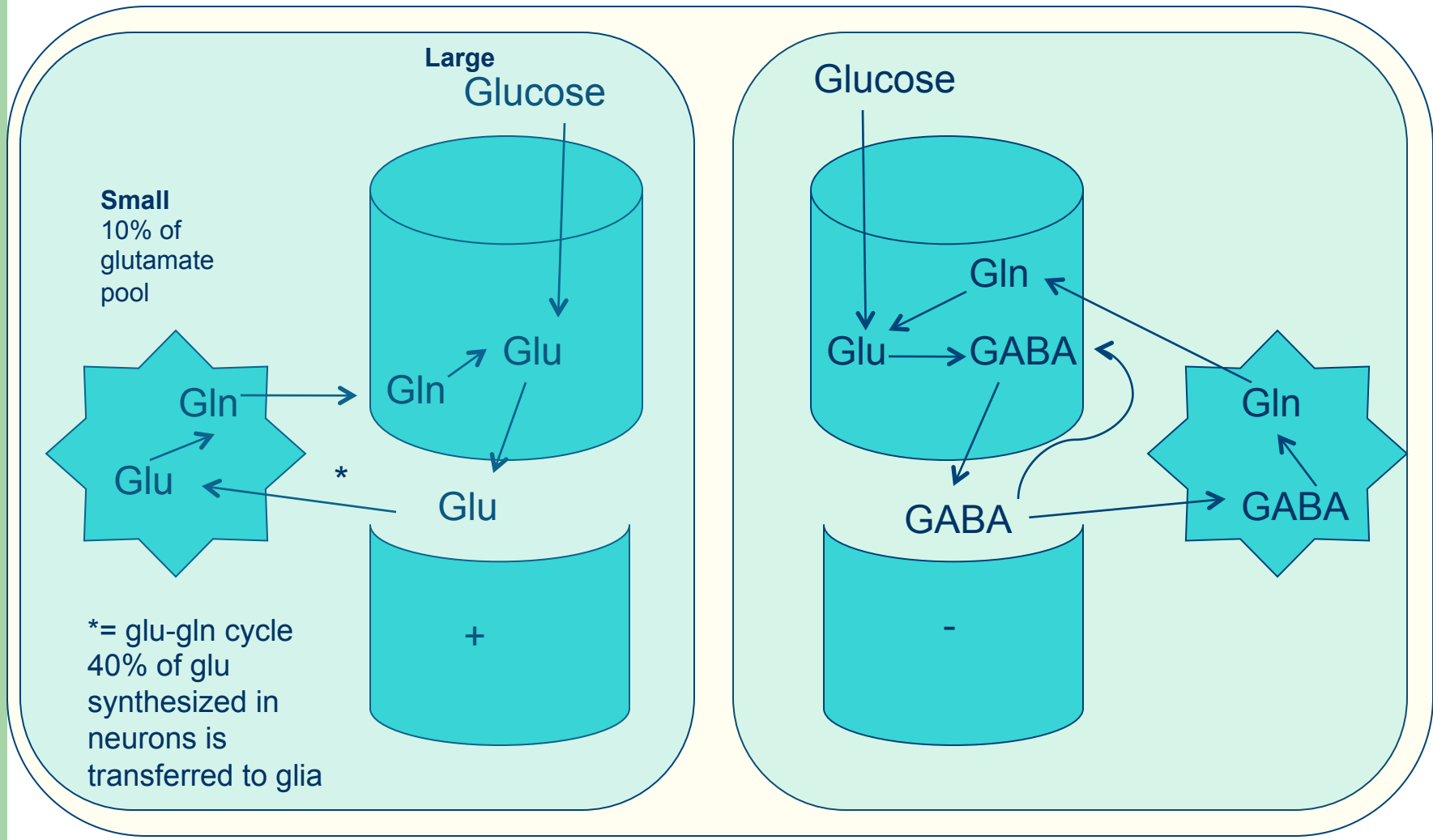
Two metabolic compartments





Two metabolic compartments





glutaminase = in neurons; Glutamine synthetase = in glia; GAD=GABAergic neurons

Pros and cons of ^{1}H-MRS

Advantages	Disadvantages
Makes use of typical MRI hardware e.g. amplifiers, coils and simple to implement	Low chemical shift dispersion of ~ 3.5 ppm (430 Hz at 3.0 T) places demands on good B_0 homogeneity (shimming)
High sensitivity due to high γ and high natural abundance	Severe peak overlap makes quantification of several metabolites problematic
Many biologically interesting species can potentially be detected	Peak overlap further exacerbated by J-coupling effects

In Vivo example of ^{1}H-MRS

Data Processing

The commercially-available Linear-Combination (LC)-Model[1] spectral fitting package was used to fit all ^{1}H MRS spectra using a simulated basis set containing:

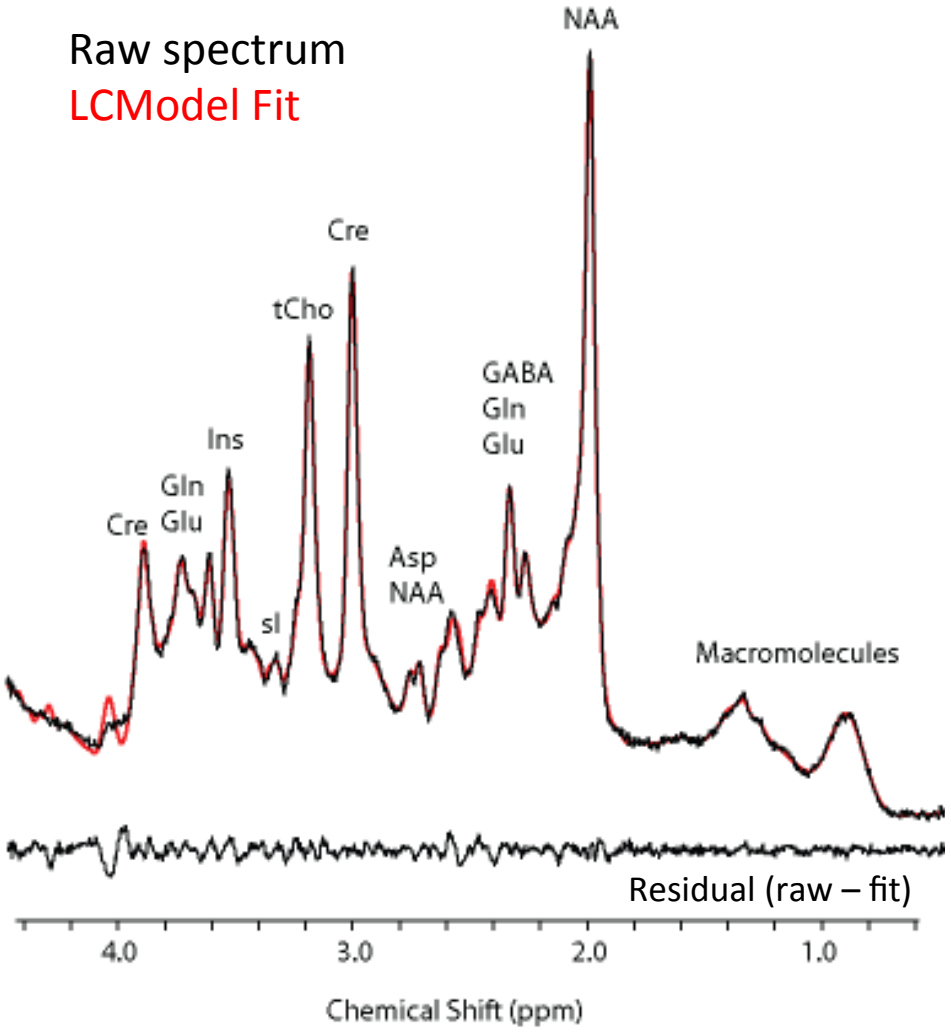
alanine (Ala)
creatine (Cr)
glutamine (Gln)
glycerophosphocholine (GPC)
lactate (Lac)
N-acetyl aspartyl glutamate (NAAG)
scyllo-inositol (sI)
aspartate (Asp)
γ-amino butyric acid (GABA)
glutamate (Glu)
myo-inositol (Ins)
N-acetyl aspartate (NAA)
phosphorylcholine (PCh)
taurine (Tau)

[1] Provencher S. J Magn Reson Med. 1993;30(6):672-9.

In Vivo example of ^{1}H-MRS

Example ^{1}H MRS Spectra

Raw spectrum
LCModel Fit



LC-Model provides an index of metabolite fit reliability in the form of a Cramer Rao lower bound (CRLB)
Estimation of standard deviation for linear model parameters

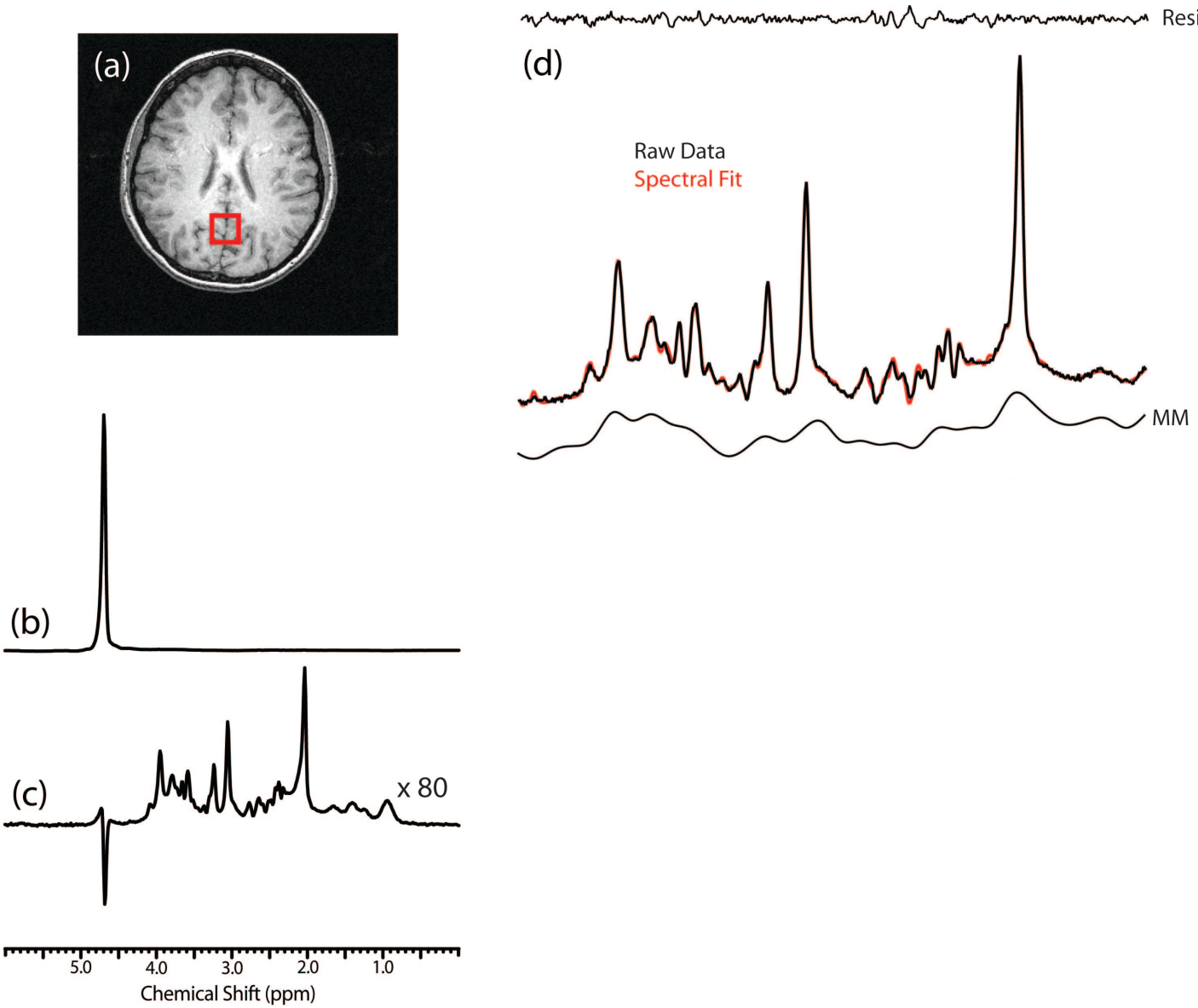
Metabolite	CRLB (%)
Ala	146
Asp	12
tCho	2
Cre	2
GABA	53
Gln	28
Glu	6
Ins	3
Lac	999
NAA	2
NAAG	11
Scyllo	999
Tau	29

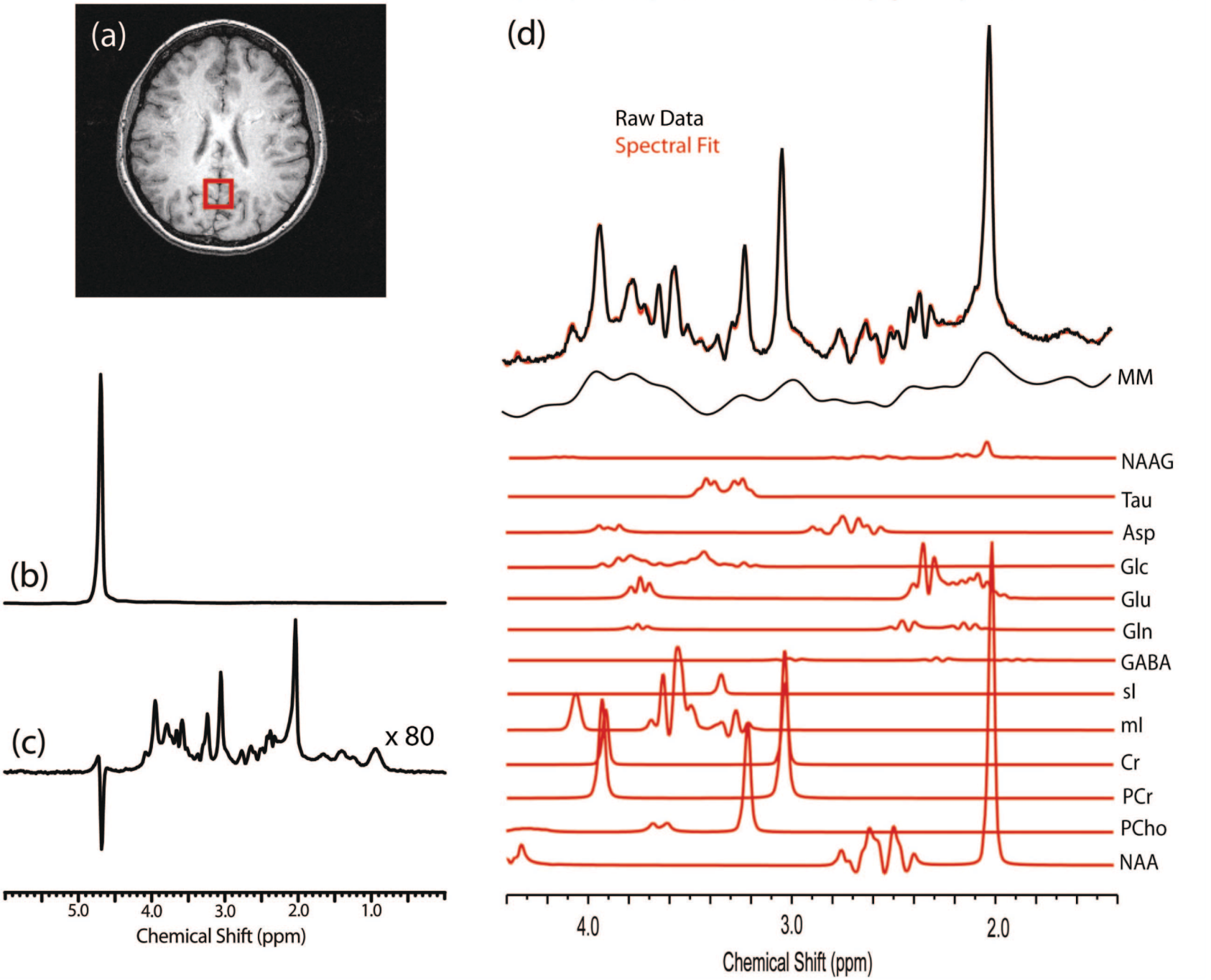
Good fit

No fit

Acceptable fit

In Vivo example of ^{1}H-MRS: individual fits




(a)
(b)
(c)
x 80
5.0
4.0
3.0
2.0
1.0
Chemical Shift (ppm)
Residual
(d)
Raw Data
Spectral Fit
MM
NAAG
Tau
Asp
Glc
Glu
Gln
GABA
sI
mI
Cr
PCr
PCho
NAA
4.0
3.0
2.0
Chemical Shift (ppm)

In Vivo example of ^{1}H-MRS

LC-Model can provide good reliable measures for Asp, NAA, tCho, Cre, Ins, Glu when used to quantify conventional short TE ^{1}H-MRS spectra

The reliability of LC-Model fits for 'interesting' metabolites-of-interest particularly GABA, Gly, Gln, NAAG, GSH etc is sub-optimal and has led to the development of optimized ^{1}H-MRS data acquisition and post-processing techniques for isolating these species with increased precision and reliability

Several so-called metabolite-editing methods have emerged over the years for resolving and measuring single species such as GABA, GSH, Lac and Gly.

We ourselves have been using a 'MEGAPRESS' sequence for GABA detection at 3.0 Tesla

The problem with many of these methods is a rather lengthy measurement time with the ultimate resolution of only a single species

Two-dimensional (2D) ^{1}H-MRS

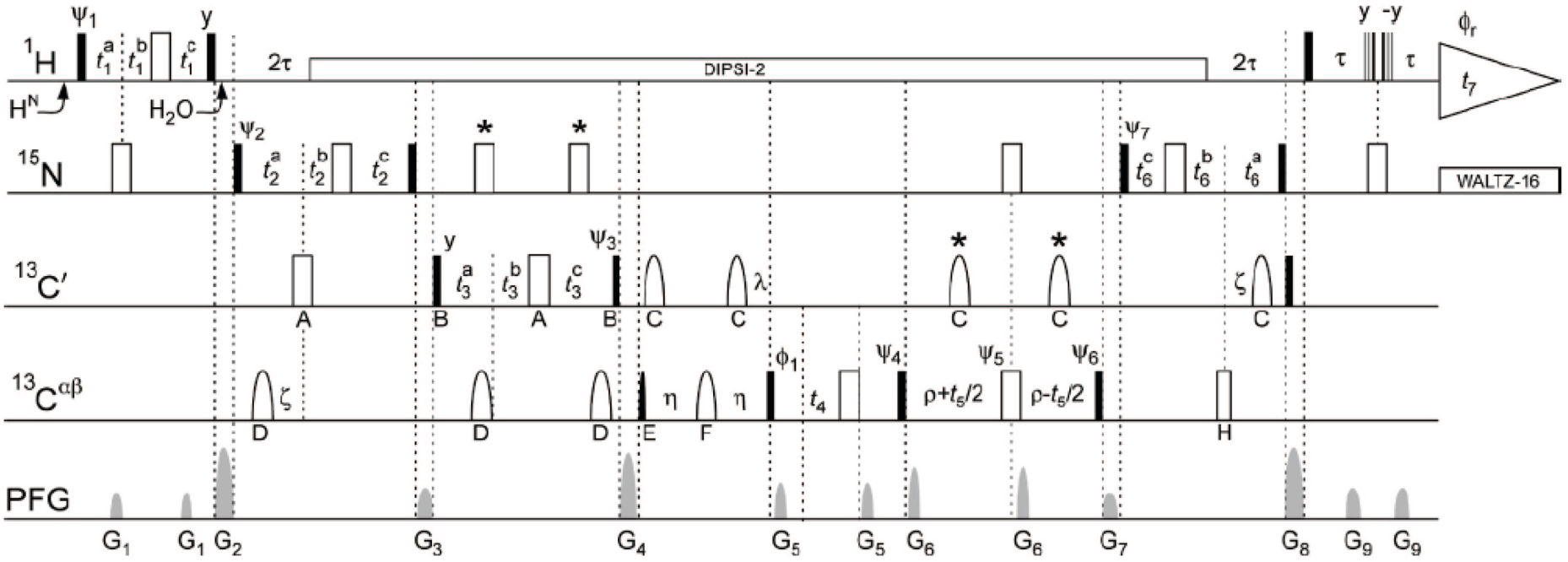
Since its proposition by the Jean Jeener in 1971, multi-dimensional (ND) ^{1}H-MRS (or NMR!) has become a crucial tool used by chemists and molecular biologists

The goal of high-resolution ND ^{1}H-MRS is structure elucidation of small, medium-sized and macromolecular entities often achieved by probing through-bond connectivity

In addition, sophisticated ND ^{1}H-MRS methods probe through-space nuclear spin interactions thus providing unique information on 3D molecular conformation

Higher-dimensional (7D) ^{1}H-MRS methods now exist for protein structure elucidation

Seven-dimensional (7D) ^{1}H-MRS



!!!!

Two-dimensional (2D) ^{1}H-MRS

The goal of ND ^{1}H-MRS for *in vivo* application is quite different in the sense that we know the structure of our relatively simple brain metabolites

The goal of *in vivo* 2D ^{1}H-MRS is to spread metabolite resonances over a two-dimensional surface to increase the effective spectral resolution

J-Resolved 2D ^{1}H-MRS

The conventional PRESS sequence is comprised of one slice-selective excitation RF pulse and two slice-selective refocusing RF pulses for single-voxel localization:

WATER SUPPRESSION – 90^{o} (SS) – TE1/2 – 180^{o} (SS) – TE1/2 – TE2/2 - 180^{o} (SS) – TE2/2 – acquire

Total TE = TE1 + TE2

Consider the effect of the PRESS sequence on Cre, GABA and Lac spectra at various TE's

The MATLAB script will display Cre, GABA and Lac spectra for TE = 2 → 500 ms

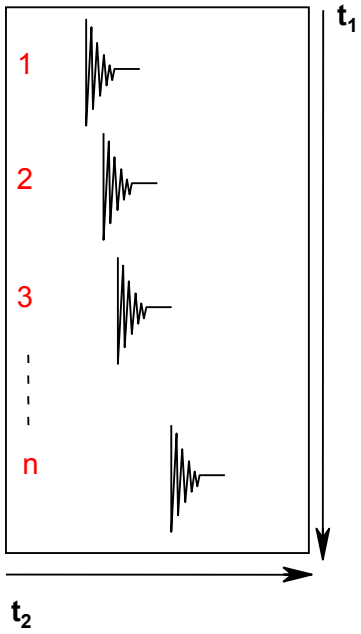
This demonstrates that although chemical shift is refocused by a spin echo sequence **homonuclear *J*-coupling effects are NOT**

Can we encode this phase-modulation such that we can spread J-coupled resonances onto a second frequency axis?

J-Resolved 2D ^{1}H-MRS

This is actually very straightforward and the method is illustrated below:

$S(t_1, t_2)$



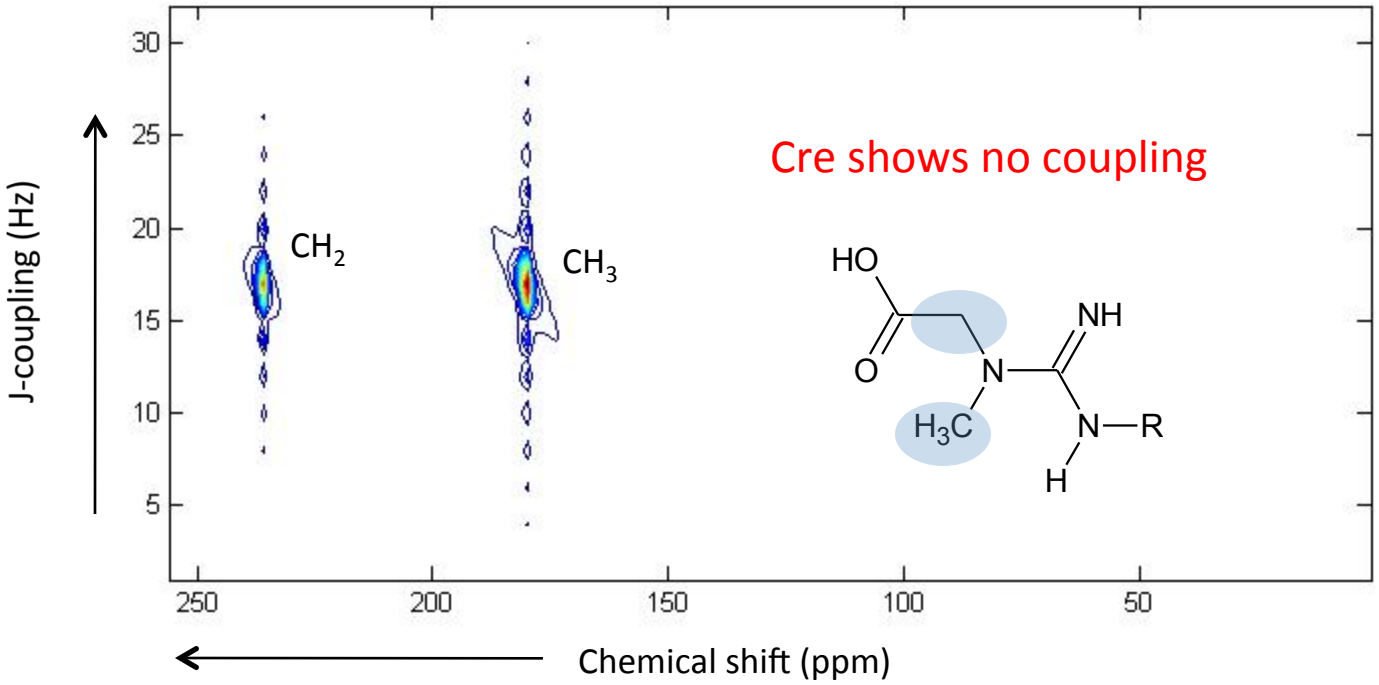
Collect N echoes with
incremental TE period
e.g. 100 TE steps, ΔTE = 2 ms

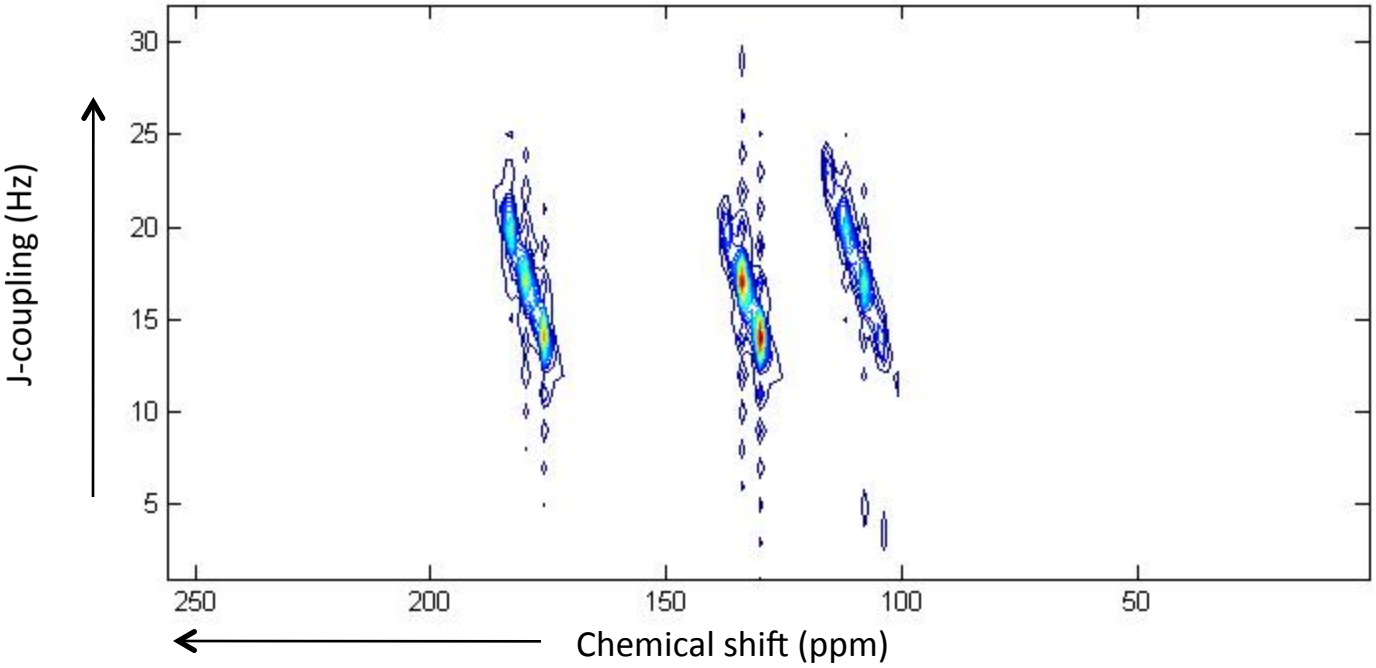
Spatially Localized *J*-Resolved 2D ^{1}H-MRS

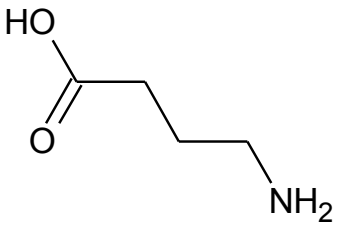
The conventional PRESS sequence can be readily made into a 2D analogue by inserting an incremental time period (t_1) into the TE2 period

WATER SUPPRESSION – 90° (SS) – TE1/2 – 180° (SS) – TE1/2 – TE2/2 – $t_1/2$ – 180° (SS) – $t_1/2$ – TE2/2 – acquire

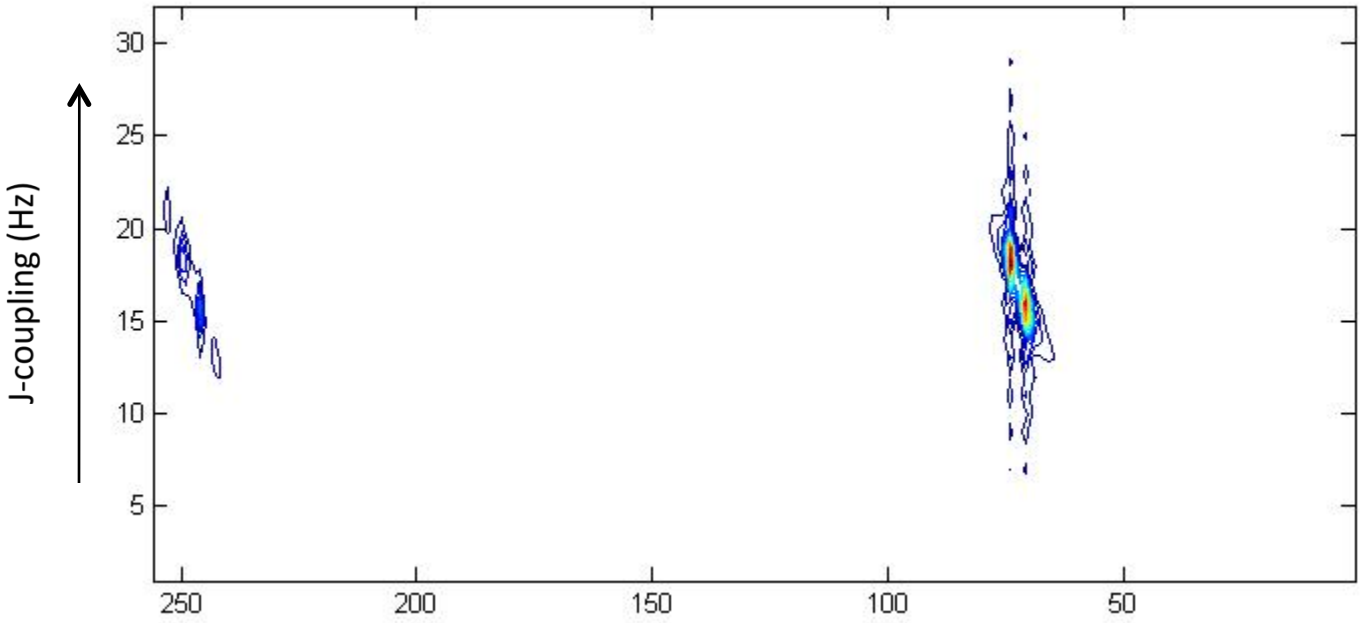
Total TE = TE1 + TE2 + t1

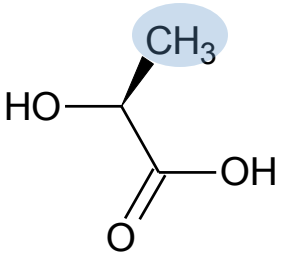






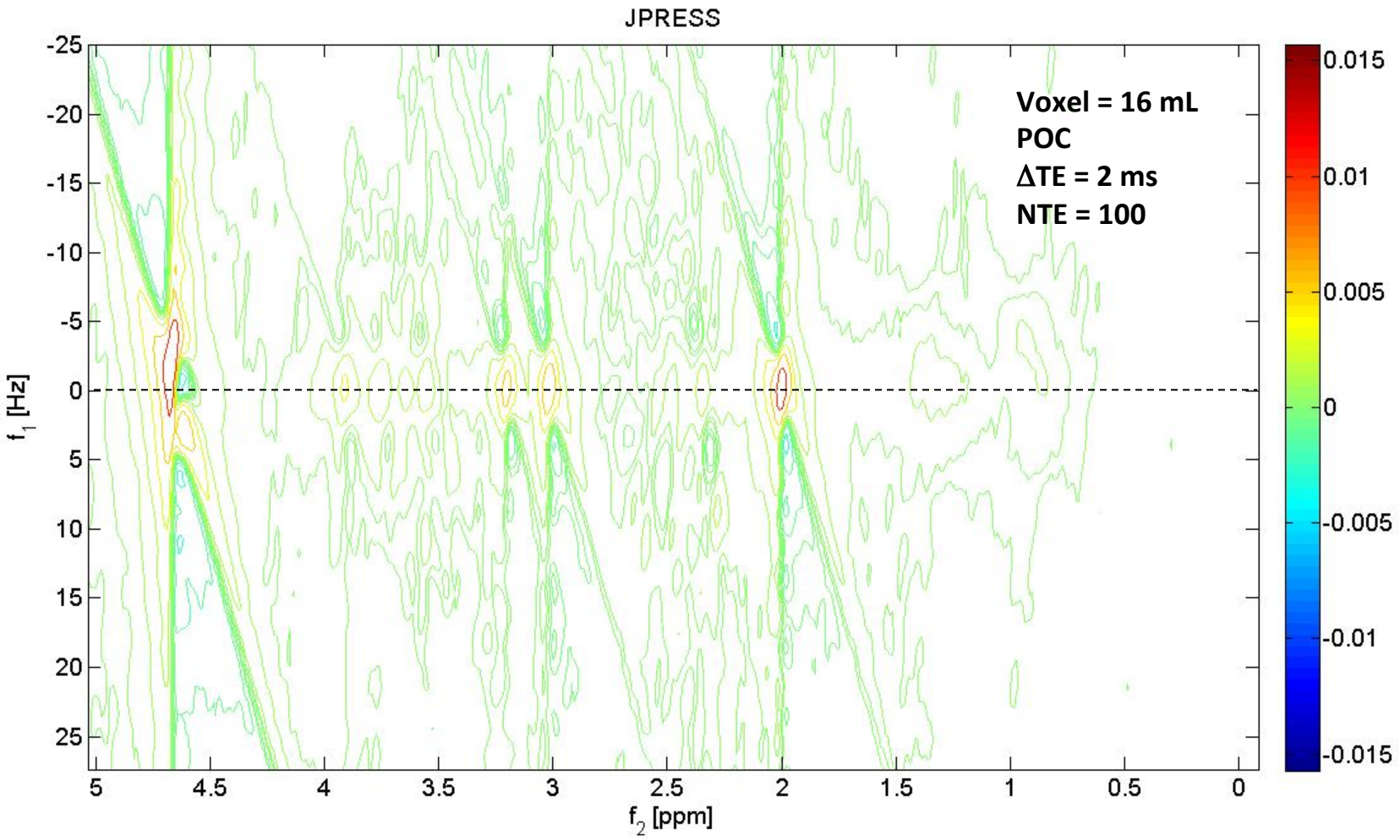
J-coupled structure of GABA peaks spread over second dimension





J-coupled structure of Lac peaks spread over second dimension

In Vivo J-Resolved 2D ^{1}H-MRS



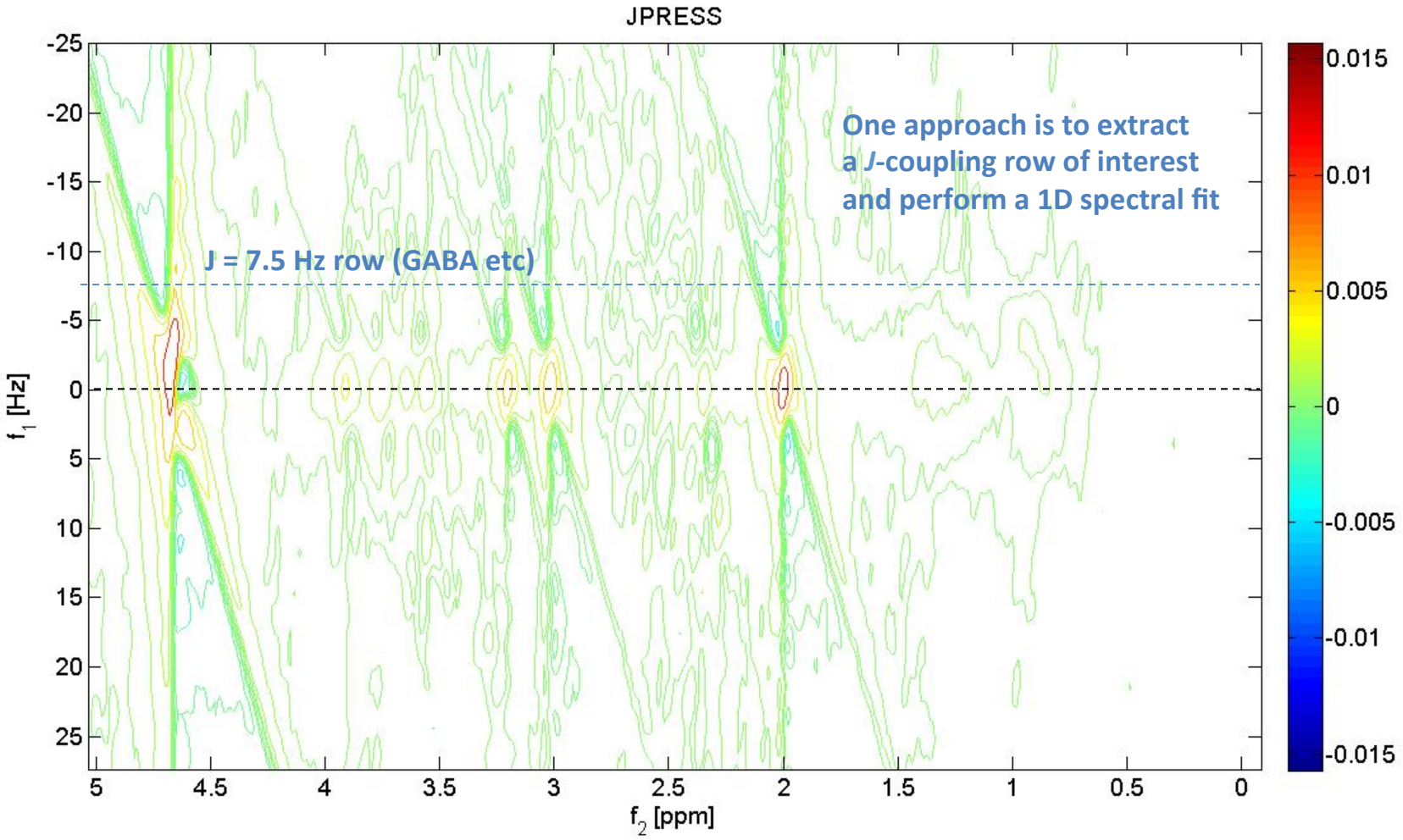
J-Resolved 2D ^{1}H-MRS: Precedent Literature

Ryner L., et al. 3D Localized 2D NMR Spectroscopy on an MRI Scanner. J Magn Reson. **1995** 107:126-137

Several localized 2D MRS sequences tested including J-Res, COSY etc

J-Resolved 2D ^{1}H-MRS: Quantification

Quantification of 2D ^{1}H-MRS datasets has been a major drawback for these methods and standardization of quantification methods between different sites must be achieved



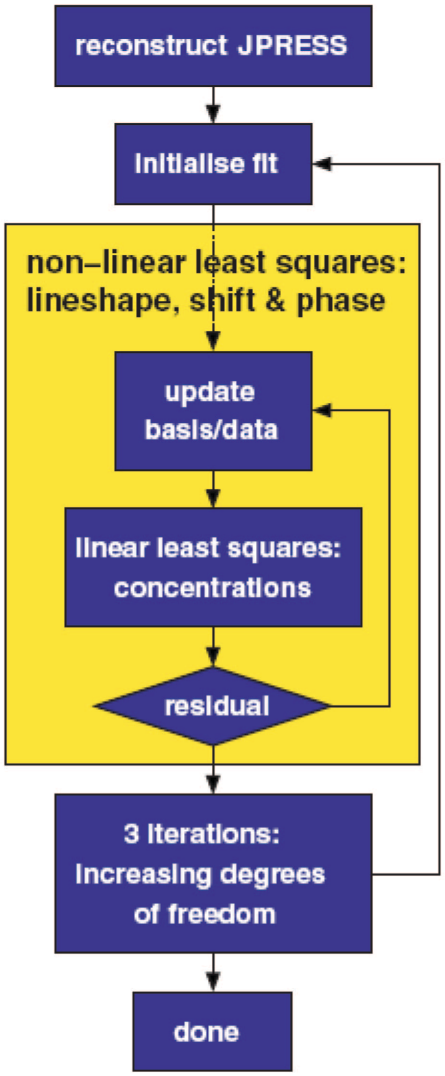
J-Resolved 2D ^{1}H-MRS: Quantification

Jensen et al recently reported the use of GAMMA-simulated basis sets in conjunction with LC-Model for fitting all rows of a 2D *J*-resolved ^{1}H-MRS dataset

Jensen E., et al. Quantification of *J*-resolved proton spectra in two-dimensions with LCModel using GAMMA-simulated basis sets at 4 Tesla. **2009** NMR in Biomed. 22(7):762-769

A major problem with this frequency-domain approach has been extracting reliability indices for each metabolite fit

The ProFit Algorithm (from Schulte et al)



Implementation of ProFit at the Uof U

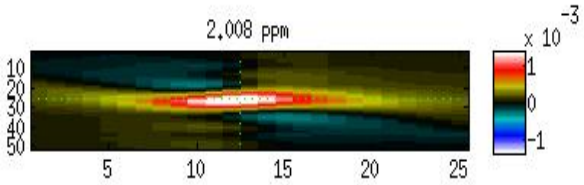
Data Acquisition:

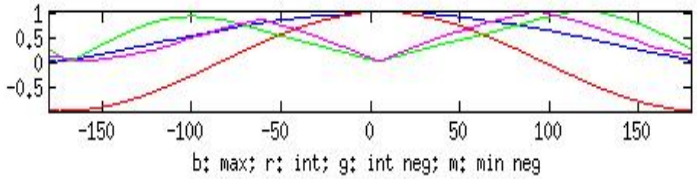
Shulte et al reported an optimized *J*-resolved ^{1}H-MRS acquisition scheme immediately after publishing the ProFit algorithm

Schulte R., et al. Improved two-dimensional *J*-resolved spectroscopy. **2006** NMR in Biomed. 19:264-270

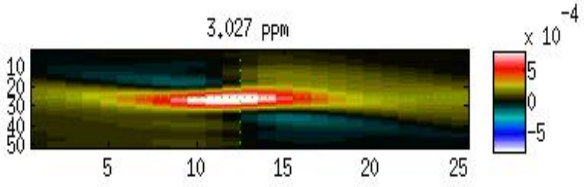
Step 1: phase-correction and referencing

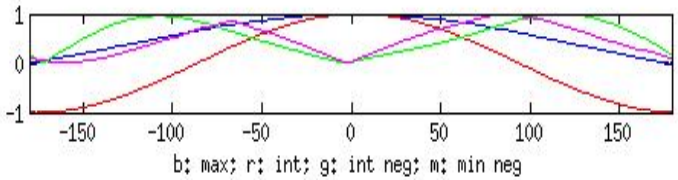
NAA





Cre





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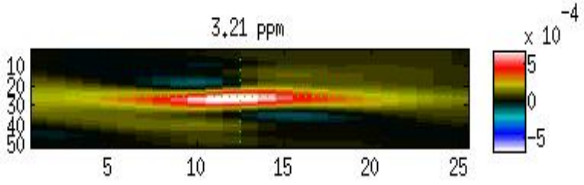
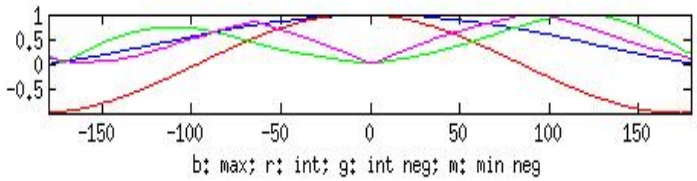
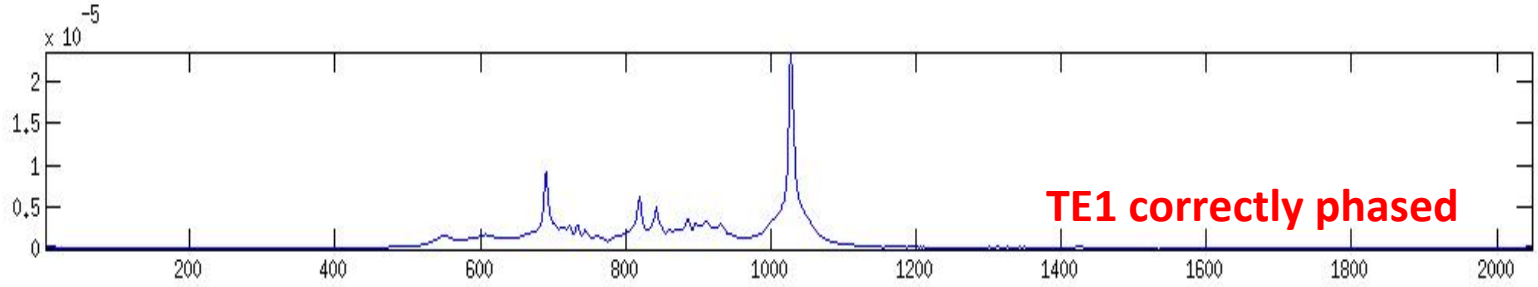
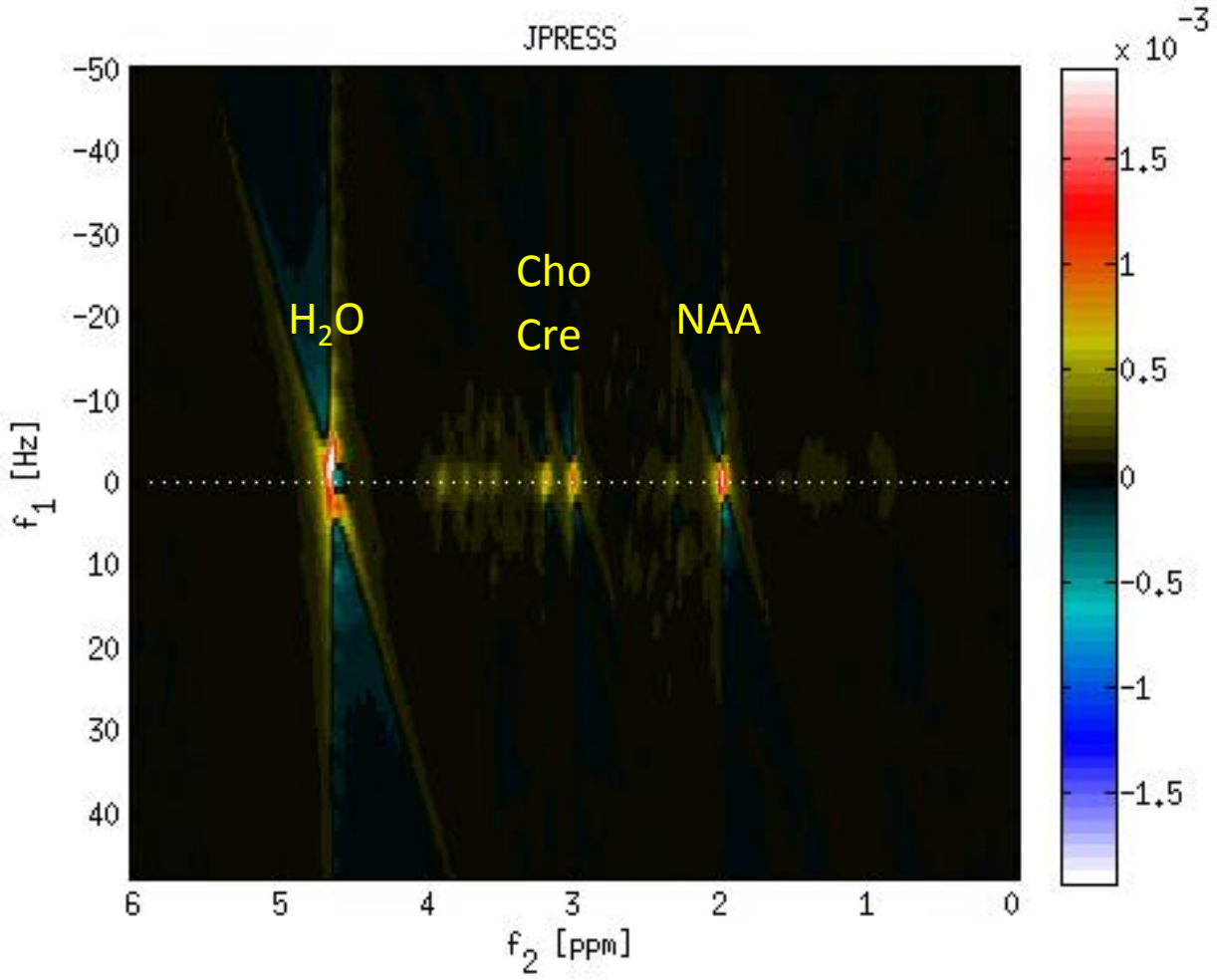


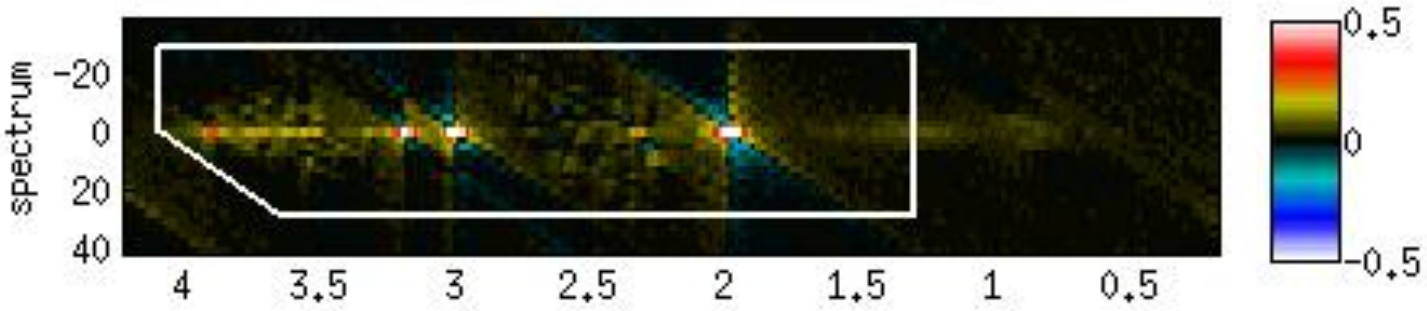




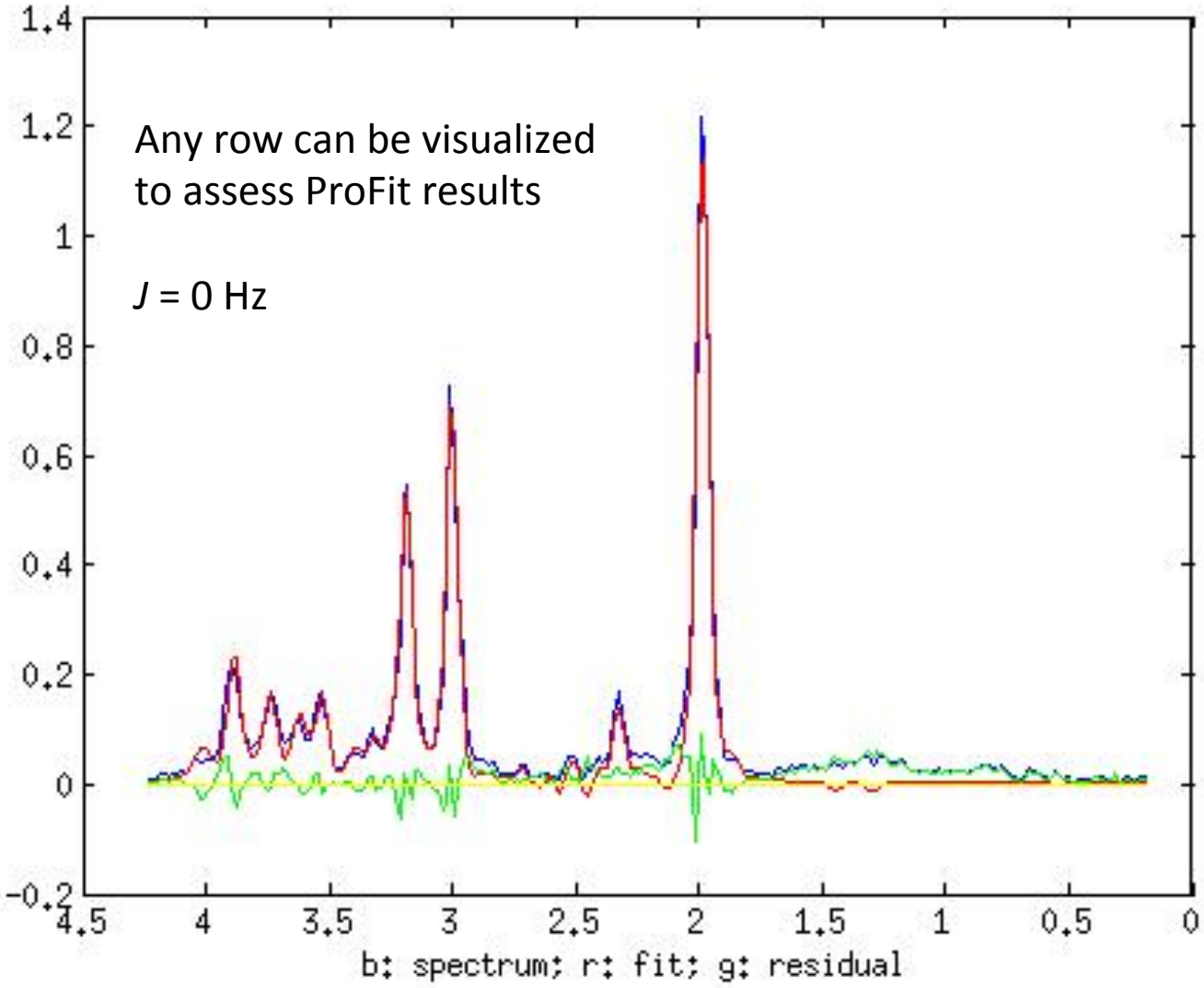
Step 2: correct referencing visualized in 2D



Step 3: 2D Visualization of 2D Fit Results

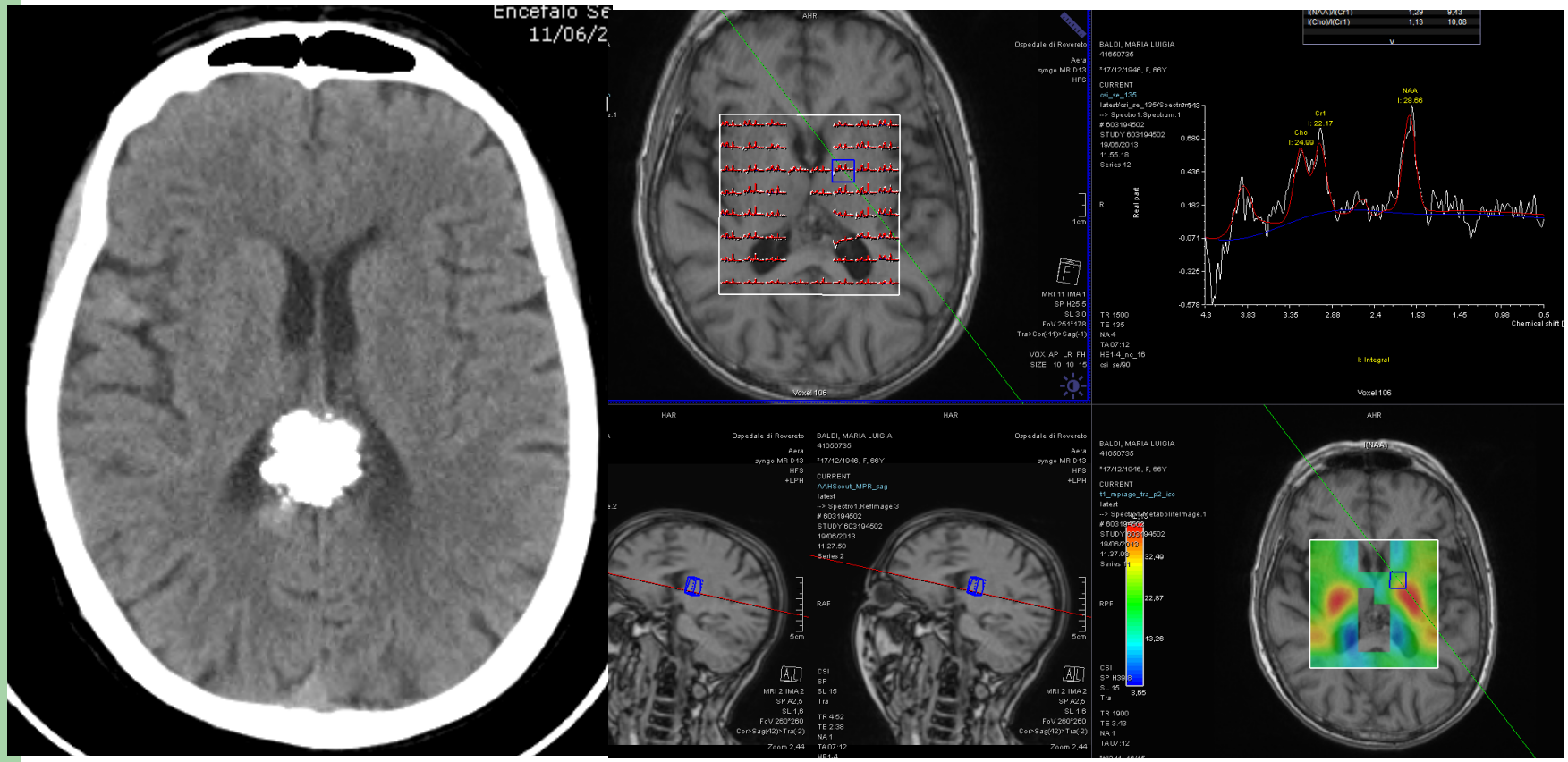


Step 4: 1D Visualization of 2D Fit Results



Esempi

Calcium - artefact



Scalp lipids- artefact