MRS: IN VIVO SPECTROSCOPIC IMAGING – MAIN POINTS

1. A MR spectrum can identify many metabolites other than water by:
   - Locating the peak(s) determined by a characteristic chemical shift (ppm) resulting from the shield formed by the electronic clouds surrounding nuclei in molecules
   - Characterizing several peaks (doublet, triplet) due to spin-spin coupling (or J coupling) phenomena

   A MR spectrum of water can also be very informative, as the chemical shift of the single peak arising from this "metabolite" is very sensitive to environmental factors such as temperature

2. MR Spectroscopy (MRS) exploits all these features. MRS of metabolites requires a very homogeneous magnetic field and, if not focused on water, relatively large volume voxels and a sufficient number of measurements to offset the poor signal-to-noise ratio (SNR) associated to this method. Numerous metabolites can be studied in this way by MRS; particularly if their concentration under natural or pathological conditions is in the 0.1-10 mM range. Quantification of the peak intensities, possible under certain conditions, can produce valuable quantifications of the metabolites' concentrations. This in turn can distinguish healthy from diseased tissue.
   Also, as mentioned, alternative applications of MRS involve monitoring the peak of some strong marker, like the 1Hs of water or the 31P of Pi. The former's resonance position is very sensitive to temperature and the latter to pH; enabling non-invasive *in vivo* characterizations that would otherwise be impossible.
   Fields of application of metabolic MRS include diagnosis of cancer, inflammatory and infectious pathologies, and metabolic pathologies related to brain dementias (Alzheimer, etc). Numerous developments are taking place in regard to other organs (prostate, breast, bones and joints…). Water chemical shifts are used to monitor ablation in real time, pH changes, etc.

3. *In vivo* localized spectroscopy requires special sequences. Foremost among these are two echoing sequences, PRESS and STEAM. PRESS records a conventional spin echo whereas STEAM records stimulated echoes (of weaker intensity, but which can be made cleaner from backgrounds like water or fat). Both methods use three RF pulses applied while in the presence of orthogonal gradients; one per spatial dimension, to define a single voxel. Spectroscopy then arises from a voxel of interest located at the intersection of the 3 orthogonal planes: the recorded spectrum is monitored in the absence of gradients which would otherwise ruin its resolution.

4. An alternative approach to *in vivo* spectroscopic imaging capable of scanning *multiple* voxels, is chemical shift imaging (CSI). Here multiple elements in 2D slices or 3D volumes are spatially encoded by phase gradients; each of these voxels is then characterized by its own spectrum. There are different methods of accelerating CSI data acquisition – foremost among which is EPSI, a variant of EPI. Chemical shift imaging yields multiple spectra of slices or volumes of interest. These are represented as multidimensional hybrid imaging/spectroscopy data sets, and studied on a voxel-by-voxel basis.

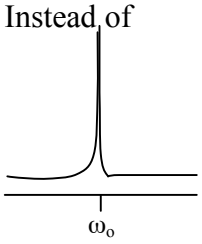
5. Any method of 1H spectroscopy, calls for suppression of the water signal and possibly of the fat & macromolecular signals: present in large quantities in the body, these have a masking effect on the metabolites close to their resonance peaks.

1. THE OBSERVABLES OF MRS: CHEMICAL SHIFT & J-COUPLINGS

Chemical shifts reflect changes in the resonance frequency of the nuclei within the molecules, due to the electrons present in the chemical bonds. The presence of an electronic cloud "shields" the nuclear sites, changing the B_0 magnetic field to which the nucleus would normally be subjected. The resulting resonance frequency difference is expressed as parts per million (ppm), a value that is independent of the amplitude of the magnetic field. The chemical shift provides information about the chemical group associated to a particular 1H resonance.

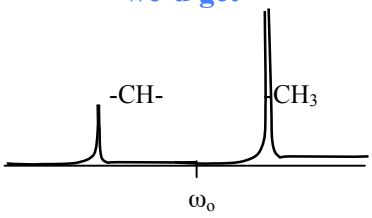
For example: FOR LACTATE: CH_3-CH(OH)-CO_2^-:

Instead of



without chemical shifts,

we'd get



with chemical shift

The integral of each peak is proportional to the number of protons in the sample that originate it:

NMR is quantitative &

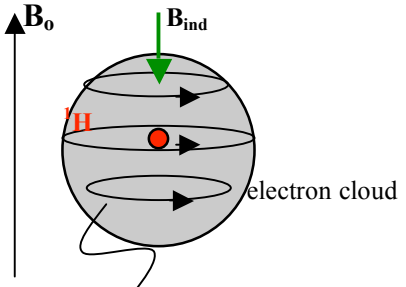
Water overwhelms everything

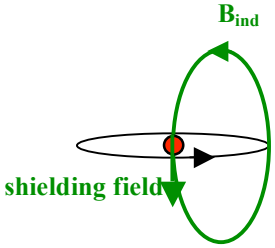
Notice that a spectrum is represented by:

- A horizontal axis denoting peak positions according to chemical shift. One metabolite may (usually will) have many inequivalent peaks. WATER IS AN EXCEPTION
- A vertical denoting the peaks' amplitudes. The area below each of these peaks will be determined by metabolite concentration. The width of the peak is inversely proportionate to T2* relaxation time.

These chemical shifts arise due to electronic currents that are induced when placing a molecule in B_0. The fields B_{ind} generated by these currents add up to the effects of B_0.

Consider for instance a hydrogen atom:





$$\omega \approx \frac{e}{2m_e} B_0 \Rightarrow |\mu_{ind}| = e\frac{\omega}{2\pi}\pi r^2 = \frac{e^2}{4m_e} B_0 \cdot a_0^2 \sin^2\theta$$

a_0: Bohr radius

The spin feels a contribution B_{ind} that "shields" the nucleus and that is $\propto B_0$. Then the actual field B determining the Larmor frequency of a spin is

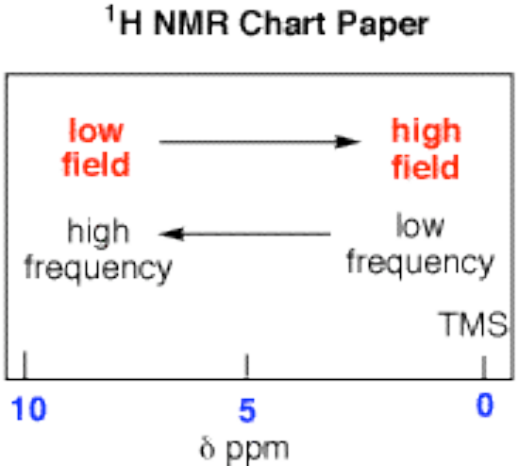
$$\mathbf{B = B_0 (1 - \sigma)}$$

σ: chemical shift or chemical shielding, a dimensionless quantity in the order of 10^{-6}

Chemically-inequivalent spins *j* in a molecule are shielded by different σ_j; these quantities are so small that they are usually expressed in parts per million (ppm's). 1 ppm = $10^6 \cdot \sigma_j$.

From a practical point of view it is convenient to express the chemical shifts with respect to a reference. In ^{1}H MRS this reference is usually chosen as tetramethylsilane (TMS): $(CH_3)_4Si$.

By measuring the difference in frequency between a peak x and the TMS resonance, the chemical shift δ can be obtained as



$$\delta(ppm) = \frac{\nu_X - \nu_{TMS}}{\nu_{TMS}} \cdot 10^6 \qquad [\nu] = \text{Hz}$$

δ in ppm is then independent of B_0: You can share *δ* values for different metabolites regardless of whether you use a 1.5, 3 or 7 T scanner.

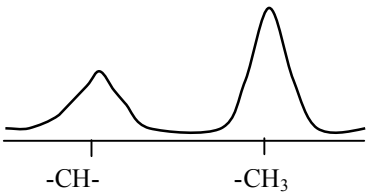
Still, the difference in frequencies compared to the reference molecule is in Hz proportional to the amplitude of the B_0 magnetic field:

Higher fields -> better separation among metabolites!!

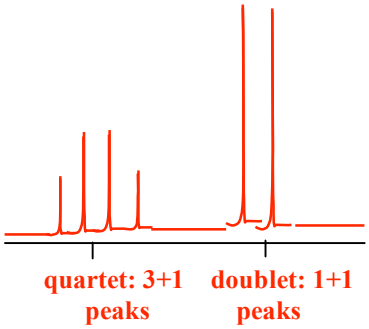
J-COUPLINGS

If the homogeneity of B_0 becomes high enough, the ^{1}H spectrum of a simple molecule such as Lactate goes

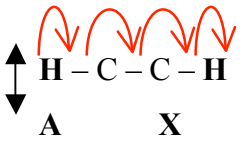
from:



to:



These splittings occur because, in addition to interacting with external magnetic fields, spins can also **create** tiny magnetic fields. Neighboring spins will therefore influence the actual precession of each other. The spin-spin fields observed in liquids do not travel through space, but mainly through the electrons in chemical bonds (hence the name indirect):



Spin X will experience an **additional field** that is

+ J/2 if spin A = ↑

- J/2 if spin A = ↓

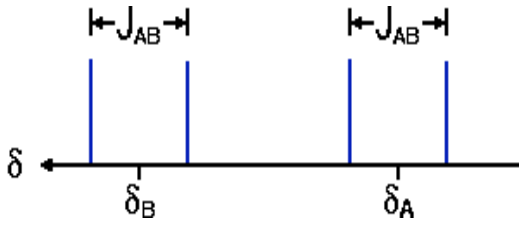
Features to notice about J-couplings

i. The coupling constant **J** is independent of B_0 ⇒ its value is expressed in Hz and not in ppm's.

ii. The coupling travels through electrons → **J** is sensitive to molecular structure

→ **J** decays as the # of bonds separating spins **A** and **X** increases

iii. **A** spins will induce on **B** spins a shift **J** that is identical to the one that **B** spins exert on **A** spins.



This may make 1H MRS look quite complex:

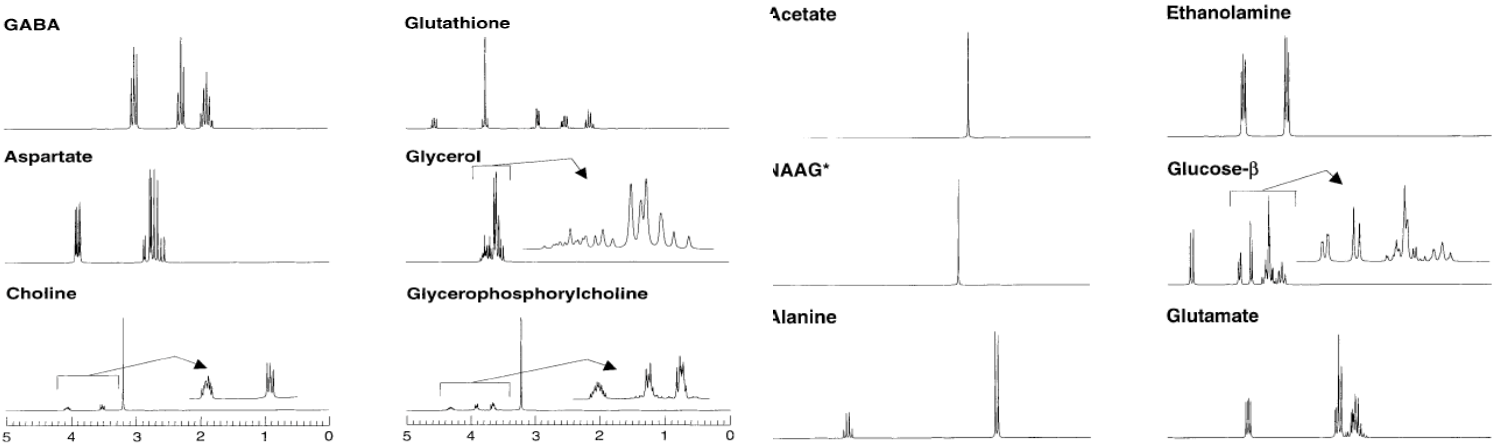


Figure 2. Spectral patterns for each metabolite at a field strength of 4.0 T with a line width of 1 Hz, simulated for FID observation using the experimentally measured NMR parameters shown in Table 1. Spectra are grouped for different ppm ranges of 0–5 ppm and 0–10 ppm. Also shown is the spectrum for DSS over the range of 0–3.5 ppm. The asterisks indicate that, for NAAG and homocarnosine, only the singlet downfield resonances were included in the simulation. The spectrum for succinate was not included as its only singlet resonance appears approximately at the pyruvate singlet resonance

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2. *In Vivo Magnetic Resonance*: MRS OF 1H & OF OTHER NUCLEI

In-vivo MRS uses scanners that are virtually identical to those used in MRI, but which also have very definite properties:

- A sufficiently strong & homogeneous magnetic field to distinguish resonance peaks: At least 3 T scanners are needed for a good resolution between the water peak, and those of metabolites. High fields are also essential to achieve sufficient sensitivity. Still, the much lower concentrations of metabolites (mM) vis-à-vis the H_2O protons (≈100 M) means that multi-scan signal averaging will be needed: MRS is very powerful, but its scans may take a long time!
- A sufficiently good field homogeneity is needed: intrinsic linewidths need to be <0.1 ppm within the VOI. This is achieved by **shimming**: an iterative addition of small external field distortions that compensate for intrinsic irregularities in the B_o.
- Sometimes nuclei other than 1Hs are sought: in particular 23Na and 31P are sufficiently sensitive isotopes (100% nat ab) and appear at sufficiently high concentrations (in kidneys for instance $[Na^+] \geq 100$ mM; in cells ATP, ADP, Pi, etc can be in 1-10 mM as well). In these cases, special transmit/receive hardware needs to be added to enable heteronuclear observations: transmitters, receivers, coils, etc.
- Specific sequences and processing methods are usually needed for MRS acquisitions. This is because one is never interested in just a spectrum; one also needs to know **where** the spectral signatures are coming from. There are therefore two main approaches to clinical MRS. One achieves spatial localization via single-voxel acquisitions, which are meant to retrieve the MRS spectrum from a single voxel only. The result is a spatially-localized 1D spectrum. The other approach is based on multidimensional separations by spectroscopic imaging (CSI: Chemical Shift Imaging); these measure the 1D MRS spectra along one axis (1D), and the positions originating it in a slice or in a volume along additional two or three additional imaging dimensions (leading in turn to 3D or 4D overall MR experiments, respectively).

Further general details of *in vivo* metabolic MRS:

Analysis of different metabolites can only be performed in the presence of sufficiently strong and homogenous magnetic fields. Weak and/or heterogeneous magnetic fields lead to resonance frequency dispersions, overlapping the peaks or even causing them to disappear into the background noise. No point in doing MRS under 1.5 T. Also, prior to any MRS acquisition the magnetic field needs to be made highly homogeneous (shimmed) –at least in the region of interest. The bigger this region the harder it may be to homogenize the magnetic field throughout it; regions that are close to bones, calcifications, air pockets, blood-rich or pulsating organs, etc., will be hard to shim. Spectroscopic quality will be poorer (or altogether unachievable) within these regions, due to perturbations in the field generated by the static and time-dependent differences in magnetic susceptibility. Some regions like the posterior part of the brain and prostate, are much easier to analyze by MRS than others. Field inhomogeneity will also be very important for adequately suppressing the water peak, which will otherwise expand and overlap/mask the much smaller metabolic peaks being sought. A sharp water peak also enables its suppression using a selective frequency pulse and dephasing gradients.

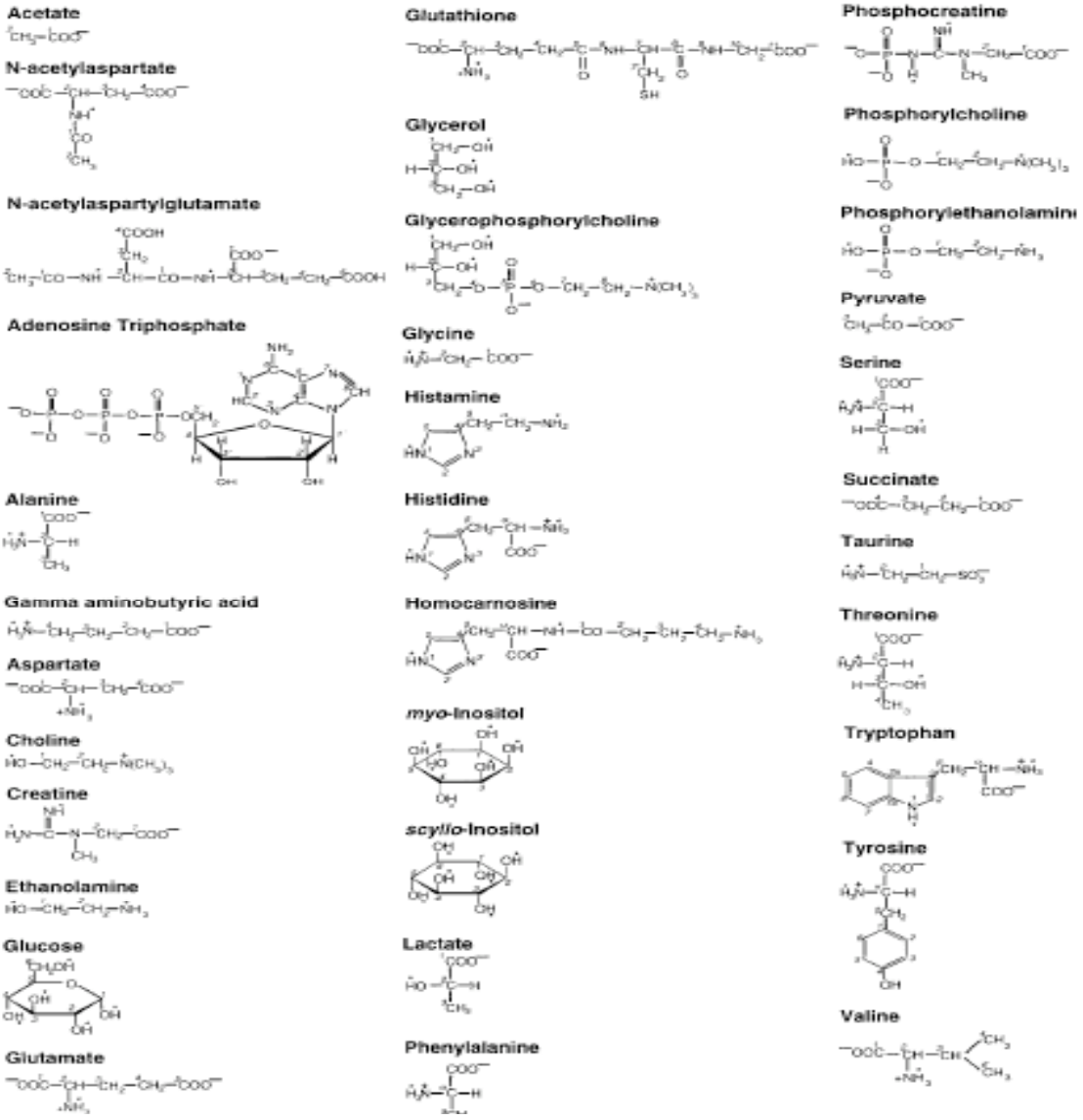
Strong fields are also crucial to deal with MRS's other main problem: its weak signal-to-noise ratio. Even with the highest fields the low concentrations being targeted entail

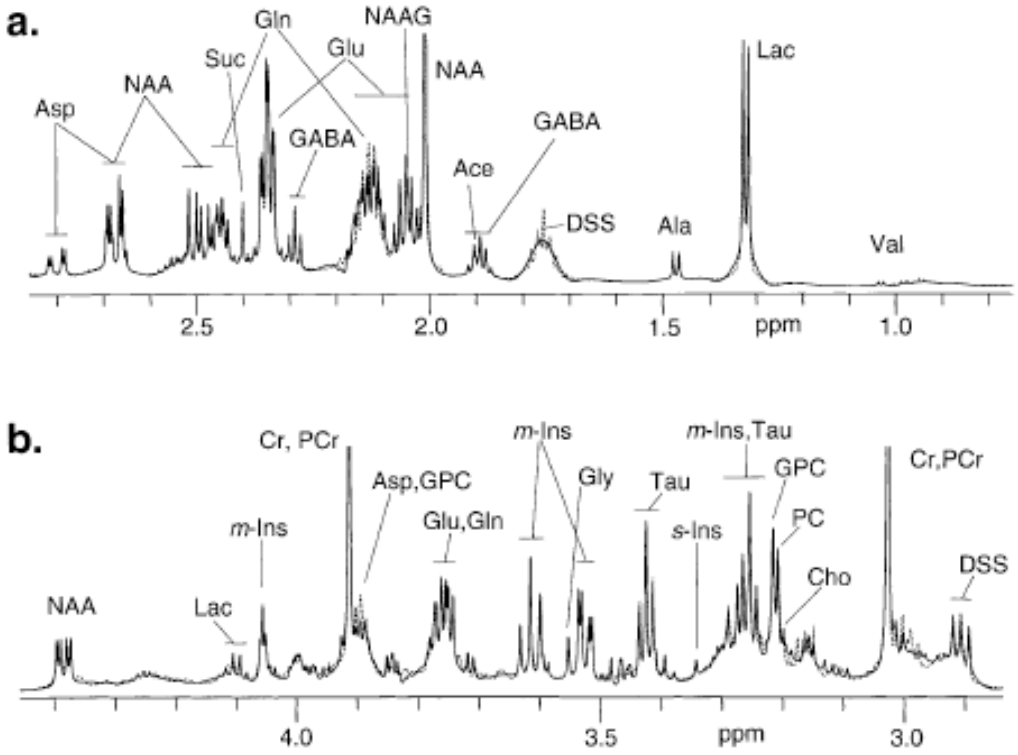
- Signal-averaging a number of measurements (# scans): SNR goes as √#scans
- Limiting the spatial resolution to a minimum voxel volume of roughly 1 cm^3
- Focusing only on the most abundant metabolites
- Using surface coils instead of volume coils (sensitivity decays with distance between coil and emitting spins)
- Acquiring relatively long time-domain signals, as spectral resolution in MRS goes as $\Delta\nu \approx 1/(\text{acquisition time})$

With these provisions, a unique number of metabolites can be observed in a non-invasive fashion by MRS – and localized by MRI:

In brain MRS, the principle molecules that can be analyzed are:

- N-acetyl-asparate (NAA) (molecule present in healthy neurones) at 2.0 ppm
- Creatine/phosphocreatine (Cr) (energy metabolism molecules) at 3.0 ppm
- Cholines(Cho) (marker in the synthesis and breakdown of cell membranes) at 3.2 ppm
- Myo-inositol (mI) (only found in glial tissue) at 3.5 ppm (figure 15.2)
- Glutamine-Glutamate-GABA complex (Glx) (neurotransmitters) between 2.1 and 2.5 ppm
- Lactate (Lac) (anaerobic metabolism): doublet at 1.35 ppm (figure 15.3)
- Free lipids (Lip): wide resonance, doublet at 1.3 and 0.9 ppm





In prostate MRS, the citrate peak is also looked for at 2.6 ppm.

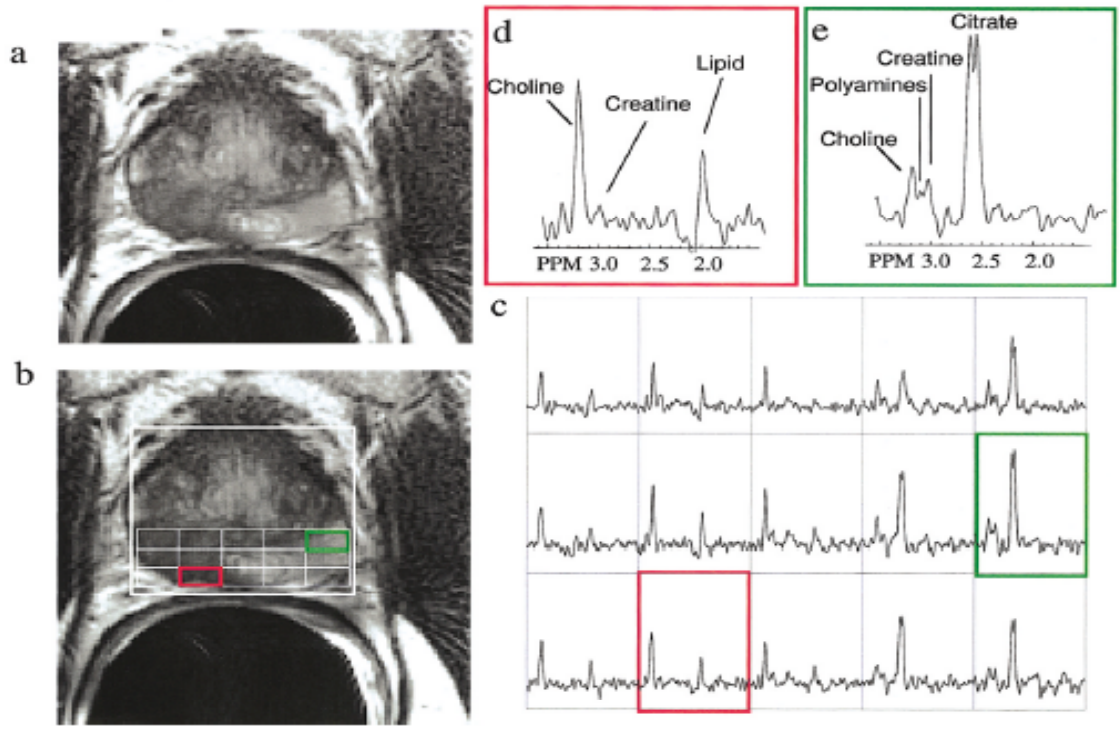


Figure 2. a: A representative reception-profile corrected T2-weighted FSE axial image taken from a volume data set demonstrating a large tumor in the right midgland to base (same patient as in Fig. 1). The selected volume for spectroscopy (bold white box) and a portion of the $16 \times 8 \times 8$ spectral phase-encode grid from one of eight axial spectroscopic slices is shown overlaid (fine white line) on (b) the T2-weighted image with (c) the corresponding 0.3 cm^3 proton spectral array. Spectra in (d, red box) regions of cancer demonstrate dramatically elevated choline, and a reduction or absence of citrate and polyamines relative to (e, green box) regions of healthy peripheral zone tissue. In this fashion, metabolic abnormalities can be correlated with anatomic abnormalities from throughout the prostate. The strength of the combined MRI/MRSI exam is demonstrated when changes in all three metabolic markers (choline, polyamines, and citrate) and imaging findings are concordant for cancer. The focus of future studies will be to increase the number of metabolic markers and to better understand the cause of these changes through correlation with earlier protein and genetic changes.

The number of discernable metabolites will vary according to the TE of the spectroscopy sequence: longer TEs (135 or 270 ms) select metabolites with longer T2. In most of these cases, suppression of the water signal (and sometimes also of fat) is essential.

31P MRS

31P spectroscopy provides information regarding the energetic status of the tissue
31P MRS does not suffer from water-suppression complications, but is challenged the need of specialized hardware, lower γ values (ie lower sensitivity and weaker gradi effects for a given G), etc.

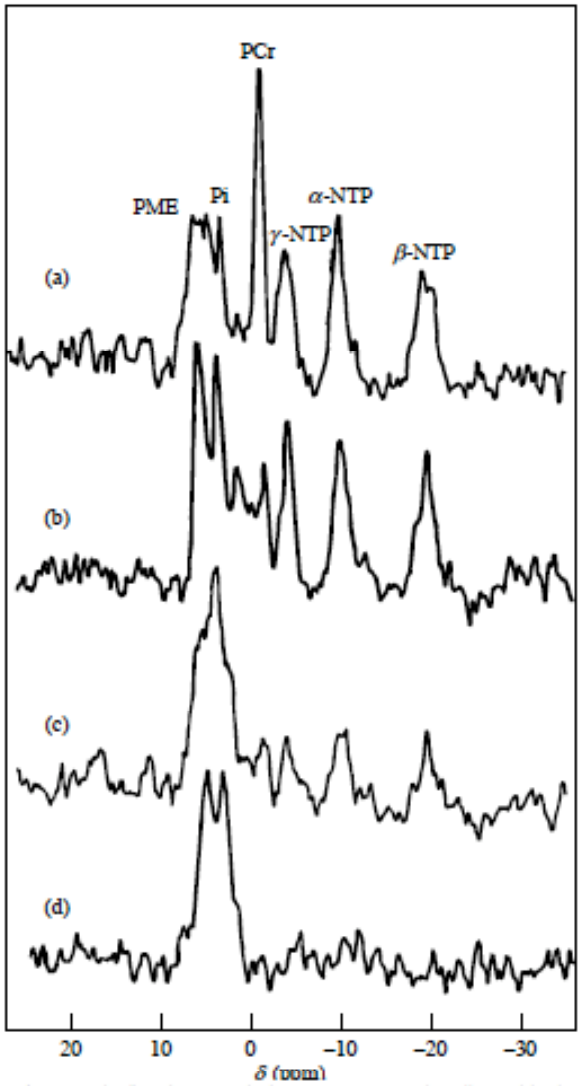
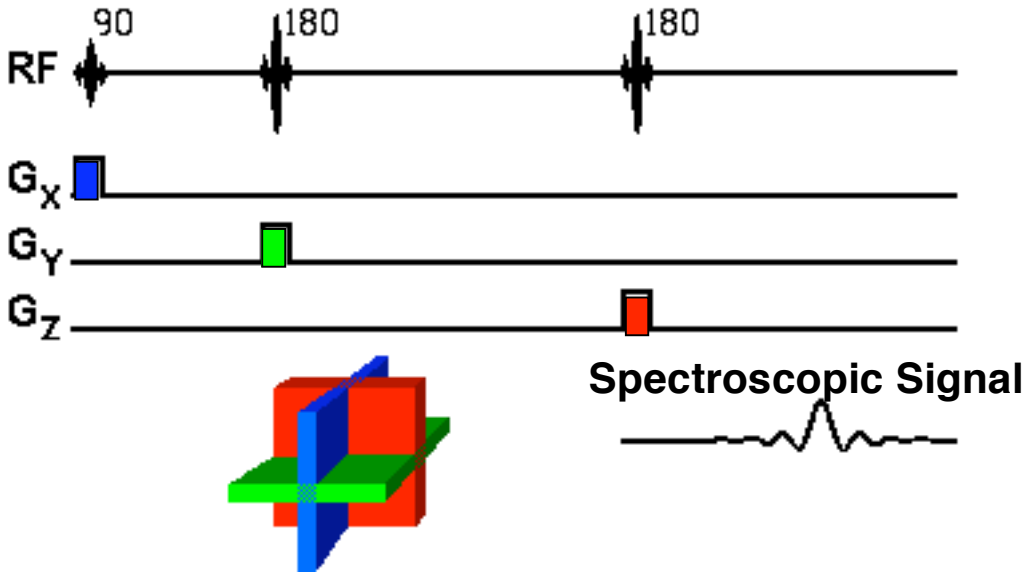


Figure 1 Metabolic changes during tumor growth reflected in in vivo ^{31}P NMR spectra, showing progressive decline of high-energy phosphate signals (NTP, nucleoside 5′-triphosphate, mainly ATP, adenosine 5′-triphosphate; PCr, phosphocreatine) and an increase of inorganic phosphate (Pi) signals. The concentration of phosphomonoesters (PME), which are attributed to precursors of major membrane phospholipids, is elevated. (a) Base line spectrum and subsequent spectra recorded at (b) day 3, (c) day 7, and (d) day 11. The pH value of the tumor tissue dropped from 7.1 for (a) to 6.6 for (c). Experimental tumor: subcutaneously implanted MOPC 104E myeloma (modified from Evanochko et al.[2])

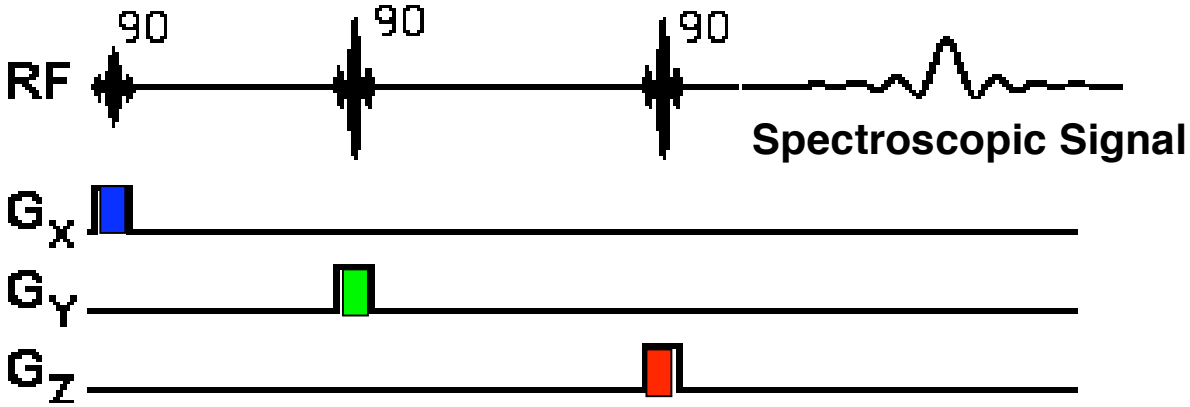
3. SPATIALLY-LOCALIZED SPECTROSCOPIC SEQUENCES: PRESS AND STEAM

Single-voxel approaches to MRS rely on retrieving signals from a limited volume. Selecting a given volume of interest (VOI) can usually be achieved by a succession of three selective radiofrequency pulses, applied in the presence of associated gradients along the three directions of space. These pulses determine three orthogonal planes whose intersection corresponds to the volume studied. As only a MRS signal from this voxel will arise, spatial localization has been achieved. The two main approaches to achieve this are PRESS and STEAM

In the (Point RESolved Spectroscopy) PRESS method, the RF pulses have flip angles of 90° - 180° - 180° respectively. The signal eventually acquired is a spin echo, emitted solely by the voxel of interest. In many ways, this sequence involves a conventional spin echo:

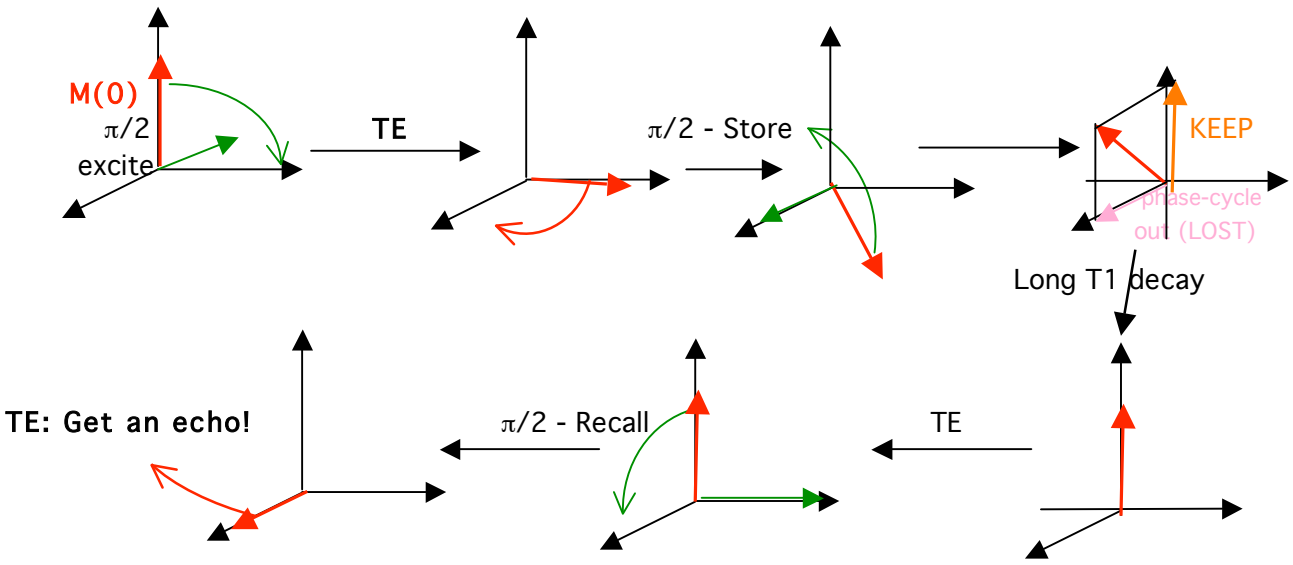


In the STEAM (Stimulated Echo Acquisition Mode) pulse sequence, the three selection RF pulses have flip angles of 90°. The stimulated echo is recorded from the cumulated effect of the three pulses, thus corresponding to the signal from the only voxel of interest



This is a more "complicated" echo – also called a stimulated echo – which involves both T2 and T1 decay.

The principles of such pulse sequence can be understood by following a single-spin magnetization model:



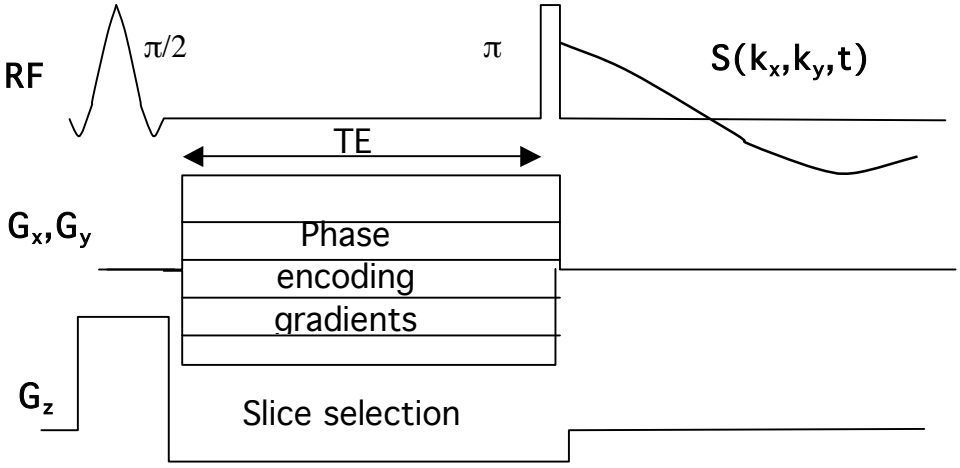
This technique is particularly adapted to spectral acquisitions requiring short TEs because of fast transverse relaxation. But the amplitude of these spin echos is only up to ½ as strong as that of the PRESS echo.

Regardless of which of these spatial localization sequences is used, the spectroscopic signal is not collected in the presence of a readout gradient, which would otherwise completely distort the very fine spectral information. ***All that is done is to Fourier transform the resulting time-domain signal – the position of the voxel originating the resulting spectrum has been defined by the offset of the slice-selective RF pulses***

4. PURE SPECTROSCOPIC IMAGING METHODS

Another approach to this problem is given by so-called Chemical Shift Imaging (CSI) methods. These consist of recording the spectroscopic data for a group of voxels, in a single high-dimensional data set. If it is done so as to provide resolved spectra from voxels in a selected slice(s) it is a spectral-spatial-spatial 3D acquisition. If it seeks the full volume information it becomes a spectral-spatial-spatial-spatial 4D experiment. Needless to say these are long experiments; but they can be speeded up on the basis of EPI-like arguments.

The basis of CSI methods is a repetition of spin-echo sequences (of the 90/180-type as in PRESS, or of the stimulated echo 90/90/90 type as in STEAM), where signals are detected in the absence of gradients in order to achieve maximal spectral resolution. To these sequences one then adds gradient-based elements, like phase-encoding gradients acting during TE, or slice-selective pulses in unison with the excitation. For instance a spatial-spatial-spectral version of these experiments, could look like



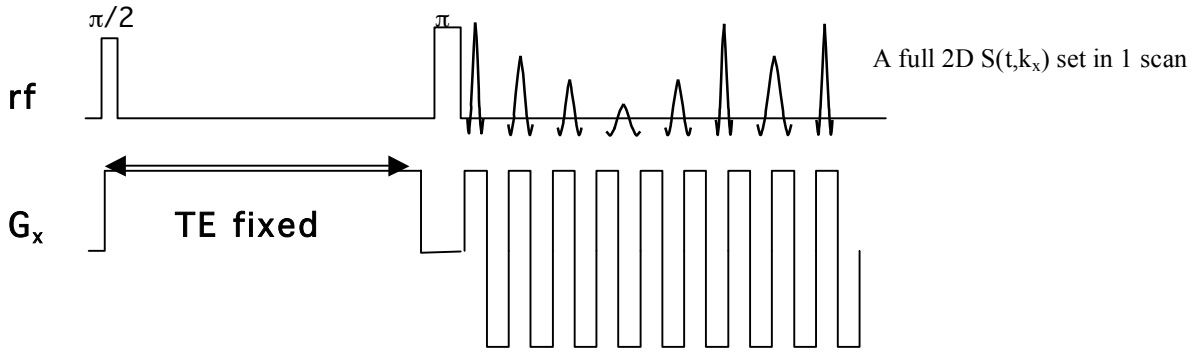
The number and direction of the phase encodings G's define the FOV & resolution along the corresponding axes. The t-span does the same for the spectral dimension

The duration of a sequence like the one here shown will equal ≈ TR (including Tacq+Te+recycledelay) · NϕX · NϕY · NssZ · #scans (perϕ). It could end up being a fairly long acquisition, particularly if spatial resolution is to be high (meaning small voxels, meaning low SNR).

To reduce acquisition times, variants of the basic sequence have been proposed:

* Multiple Slice CSI: can accelerate the acquisition: monitor several different slices while waiting for spins in the previous slices to relax

* Single-scan CSI: provides an important speed gain compared to the above-mentioned sequence, by performing both spatial and spectral detection during the course of the signal acquisition by means of oscillating gradients. This is similar to what happens in an echo planar sequence: **Echo-Planar Spectroscopic Imaging**. This technique is less sensitive than the classic CSI sequence, but the speed gain is particularly useful in 1D spectroscopic + 3D spatial imaging, or when dealing with motion artifacts:



Signal processing MRS data calls on Fourier transforms (1 for each phase-encoded dimension phase + 1 for the spectral analysis). It also requires the full set of data processing tools normal in MR spectroscopy: phase-cycling, correction of the baseline, phasing, weighting, zero-filling, etc.

One of the most important requirements needed to implement *in vivo* 1H MRS of metabolites is good **WATER SUPPRESSION**

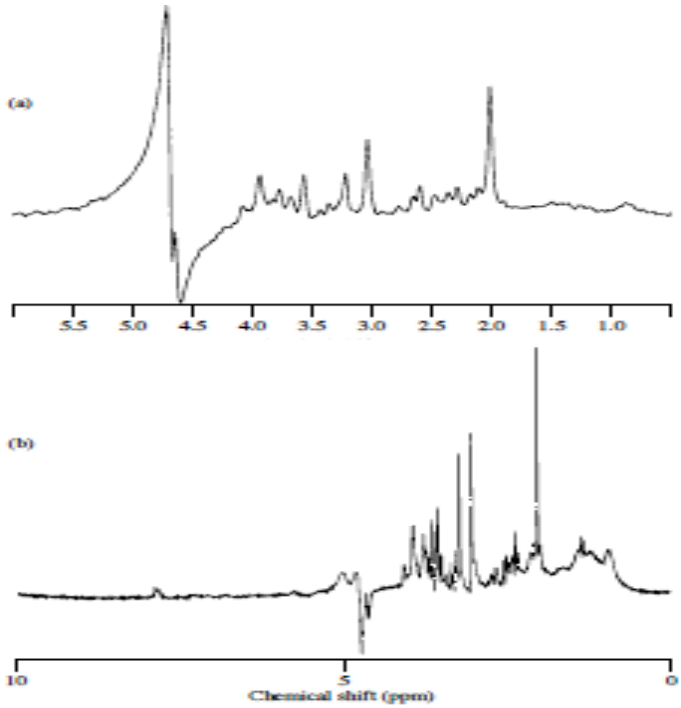


Figure 1 Examples of high quality water suppression in in vivo proton spectroscopy of (a) human and (b) animal brain. (a) In vivo proton NMR spectrum of an 18 ml volume obtained from normal gray matter of a healthy volunteer. The spectrum is obtained with STEAM localization (*TR/TE/TM* = 6000/20/30 ms), and three preceding CHESS water suppression procedures. The spectrum was obtained in approx. 6 min. A 2.0 T instrument was used, with a conventional quadrature head coil. Only exponential multiplication was used prior to FT, and no further data manipulation was applied. Courtesy of Dr. Jens Frahm, Biomedizinische NMR Forschungs GmbH, Max Planck Institut für Biophysikalische Chemie, Göttingen, Germany. (b) In vivo proton NMR spectrum from a 0.7 mL volume located in the superior part of the cat brain using STEAM localization (*TR/TE/TM* = 1000/15/80 ms). A 4.7 T instrument was used, equipped with 20 mT m^{-1} shielded gradients. A surface coil (outer diameter 48 mm) was used for transmission and reception. Three preceding CHESS water suppression sequences, and three CHESS water suppression sequences in the *TM* period were used. Lorentzian-to-Gaussian transformation was used prior to FT. For more details, see Moonen and van Zijl.[12]

The most commonly used method to suppress the water peak **is CHESS (CHEmical Shift Selective)** suppression. CHESS consists in applying three pairs of 90° RF pulses + dephasing gradients in each spatial direction. The bandwidth of these RF pulses is narrow and centered on the resonance frequency of the water peak in order to saturate the water signal and preserve the signal from the other metabolites:

Sel π/2 Pulse on H_2O – G_x - Sel π/2 Pulse on H_2O – G_y - Sel π/2 Pulse on H_2O – G_z –

Start your regular spectroscopic imaging sequence

Another possibility: **PRESAT**. A long, frequency selective pulse is sent on resonance of the water frequency until it destroys the water macroscopic magnetization.

Another group of techniques achieves water/fat suppression by applying a 180° inversion pulse, and then suitable inversion-recovery TI time to eliminate the undesired signal (e.g., the fat signal in breast or liver spectroscopy):