Pulse sequences and spectral editing for NMR based metabolomics

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Overview

- A workshop presentation
  - Please feel free to interrupt and contribute!
- One dimensional applications for metabolomics
  - The basics
  - $T_2$ and the spin echo delay
  - $T_1$, quantification and BIRD filter
  - DOSY and diffusion
- Questions and contributions from the floor
NMR based metabolic fingerprinting

- A relatively insensitive approach compared with mass spectrometry
- **BUT**
  - Robust
  - Cheap per sample
  - Capable of monitoring intact or in vivo tissues
- A combined MRI, MRS, HRMAS and high res NMR approach to following PCD in tumours
- During PCD lipids increase in intensity for both saturated and unsaturated resonances
  - $\delta$ 5.3, 2.8 $\uparrow$ 3-fold
  - $\delta$ 1.3 $\uparrow$ 2-fold
  - Lipid changes associated both with PCD and cell debris region
A Brief aside on sample preparation for NMR based metabolomics I

- Depending on the sample we will have to prepare our samples differently
- BUT within a study a SOP is very important
- Urine
  - phosphate buffer to maintain pH
  - But peaks will still ‘move’ because of cation chelation etc
  - CSF can be treated in a similar manner
- Tissue extracts
  - Extraction procedure is vital
    - Perchloric acid
    - Acetonitrile/water
    - Chloroform/methanol
- Blood plasma/serum
  - Maintain osmolarity to keep lipoprotein structure
  - Need to avoid red blood cell lysis
  - TSP – good or bad?
NMR Samples, particularly in solution, are dynamic systems.

Molecules tumble and change location within the active area.

They therefore experience magnetic influences from:
- field inhomogeneities
- the motion of other nearby nuclear magnets

These dynamic processes contribute to the relaxation process as:
- information is lost
- energy is dissipated

To understand these processes better, we need to know a bit about PHASE.
We talk about phase when we process NMR spectra and we talk about phase when we look at the dynamics of nuclear magnets.

It’s very easy to get confused so let’s clear up phase-correcting spectra first.

The pulse is a radio wave and thus has frequency and phase.

The detected signal is a composite radio wave containing various frequencies, each of which has a phase relative to the pulse.

There is normally a difference between the pulse phase and the detected signal phases.

Phase-correction must therefore be carried out to compensate for these differences and bring the whole spectrum into ‘Absorptive Mode’.
Some time after being pulsed the nuclei will have returned to their equilibrium state.

This is called RELAXATION (a bit like hot objects cooling down).

The relaxation process entails loss of information ($T_2$ relaxation) and loss of energy to the surroundings ($T_1$ relaxation).

Larger molecules tend to relax quicker than smaller ones.

A time delay between the pulse and starting to collect the FID would remove contributions from larger molecules (often broad and unhelpful).

Unfortunately this would lead to massive phase errors.

By inserting a ‘Spin-Echo’ sequence between the pulse and the FID, this phase error problem can be overcome.
Useful for looking at small molecules in tissues (HRMAS), blood plasma and other biofluids with protein in it

- Typically 1ms – 500 us interpulse delay (d20)
- Typical T2 filter
  - $2 \times \tau \times L_4 = 20$ ms to 200 ms
  - But beware of heating effects
  - Funny peak shapes – have you got the 180 right? Is d20 too long?
Spectra Decomposition according to the $T_2$ time
$T_1$ measurements

- Spin-lattice relaxation along the z axis
- Slower than $T_2$ (typically 1-2 s; maybe longer)
- Pulse sequence requires $d_2$ to be varied between say 50 ms up to 3-4 s to get......
- T1 is important in quantification – a long T1 usually means a small signal
  - Need to consider saturation factors (and d1; relaxation delay)
  - Often don’t ‘see’ carbonyls
  - But sometimes long T1s are very useful - hyperpolarisation

- Can also edit spectra using a T1 filter – send a problem peak through the null!

Golman et al., 2006. Metabolic imaging of 13C labelled cmpds

Gallagher et al., 2008
Consider, e.g. all the acetate protons in the sample. In a 14.1 Tesla field the net magnetisation of these protons is rotating around the z-axis at about 600001200 revs per second.

This angular velocity is proportional to the experienced external magnetic field.

Gradient field coils can be fitted to the probe which superimpose a small non-uniform magnetic field onto the main field.

This additional field is designed to be proportional to position along the z-axis (i.e. main field direction).
ANOTHER THOUGHT EXPERIMENT!

Pulse the sample in the usual way

Switch on the gradient field. Now the acetate proton magnetisation at the top of the sample will be rotating round the z-axis faster than the acetate proton magnetisation at the bottom of the sample (or vice versa)

After a short period switch off the gradient then, after a suitable delay, switch it on again the other way round for the same duration as before

Build in some spin-echo type sequence to make sure the spectrum can be phase-corrected and collect the FID
FIELD GRADIENTS

WHAT DOES THIS MEAN?

Proton magnetisation will experience a change in angular velocity with the first gradient being switched on.

With the gradient switched off the original angular velocity will be restored but there will have been some phase shift because of the temporary acceleration/deceleration.

Molecules remaining at the same position along the z-axis will have this phase shift reversed with the second, reversed, gradient field.

All molecules with no net phase shift will give a normal FID.

Molecules diffusing along the z-axis between gradient applications represent a net dephasing of magnetisation and will contribute little or nothing to the FID.

Slow-moving molecules will thus suffer less peak attenuation than faster ones.
With relaxation editing we can remove the contributions of large molecules from NMR spectra.

With gradient coils we can apply diffusion editing to remove the contributions of small molecules from NMR spectra.

Equations exist by which diffusion coefficients can be calculated if the gradient field strengths and time delays are known.

Running a sequence of these gradient experiments with varying parameters can give a ‘pseudo-2D’ plot of chemical shift against diffusion coefficient. This is called Diffusion-Ordered Spectroscopy or ‘DOSY’.

Gradients can also be used for solvent suppression (‘WET’, ‘WATERGATE’).

And last – and far from least – gradients are what make Magnetic Resonance Imaging possible.
Diffusion ordered spectroscopy

- DOSY
- Can process using exp fitting/Laplace transform
  - Provides an extra dimension for separation
  - Screen shots from GIFA
- Can even ignore this and used statistical correlated (work of John Lindon et al.)
  - Great for separating complex mixtures
We can use diffusion to probe the molecular environment of metabolites in situ.

- Top: Spectra at diffusion gradient strengths for rat testes demonstrating the high diffusion coefficient of creatine.

- Bottom – water in endomitrial cells:
  - Probing intracellular environments?
  - Mitochondria (S. Cerdan, M. Bollard)
Conclusions

- Relaxation and diffusion are both processes that can be easily used to provide contrast.
- As well as spectral editing, one can probe intracellular environment.
Thanks to....