Lecture 12

Practical introduction to theory and implementation of multinuclear, multidimensional nuclear magnetic resonance experiment.


The main reviews from the books:
* 'Method in Enzymology.V.239, Nuclear Magnetic Resonance, Part C' by Thomas L. James and Norman J. Oppenheimer. p.3-79

Good reviews:

This book is quite difficult:
* 'Principle of Nuclear Magnetic Resonance in One and Two Dimensions' by Ernst, R Bodenhausen, G., Wokaun, A. pp 1-69 and pp.3-79
Practical introduction to theory and implementation of multinuclear, multidimensional nuclear magnetic resonance experiment.

(I) Pulse Sequences.

The easiest way to understand complex NMR experiments is to be able to identify the small number of building blocks from which all experiment are constructed. Once the input and output characteristics of the building blocks are known, a coherence flow network can be constructed to illustrate graphically the flow of coherence through an experiment. For this purposes we will introduce a small number of product operator rules. Although many of these experiments correlate four or more different spins, the majority of interactions can be understood in terms of pairwise, two-spin interaction. We will focus on the NMR experiments for through bond, sequential assignments.

Part (1): Product Operators

In the context of an 2-spin system, we distinguish one-spin and two-spin operators. One-spin operators are associated with a magnetisation. We will labelled two spins by \( I \) and \( S \). A subscript to \( I \) and \( S \) refers to the rotating frame axis along which the magnetisation is oriented. Thus \( I_x \), refer to the magnetisation of the \( I \) spin oriented along the \( x \) axis.

\[
\begin{array}{c}
\text{z} \\
\text{y} \\
\text{x}
\end{array}
\]

For the two-spin interaction there are 15 combination of the magnetization:
- \( I_z, S_z \) (longitudinal)
- \( I_x, S_x, I_y, S_y \) (transverse)
- \( 2I_zS_z \) (J-ordered spin state)
- \( 2I_xS_z, 2I_yS_z, 2S_xI_z, 2S_yI_z \) (antiphase)
- \( 2I_xS_x, 2I_yS_x, 2I_xS_y, 2I_yS_y \) (multiple quantum)

(The coefficient of 2 arises as a normalisation factor)

When only a single spin is involved, they call the inphase magnetisation.

\( x \)- and \( y \)- magnetisation are named as transverse
\( z \)-aligned along the magnetic field, is called longitudinal .

Only three operators are needed to describe most NMR experiments:
1) radio frequency (rf) pulse
2) chemical shift
3) coupling

Both the rf pulse and chemical shift operators describe simple rotations in the three-dimensional space.

The notation of product operators is:

\[
\text{operator } \rightarrow
\]

where the operator transforms \( a \) into \( b \). In our case \( a \) and \( b \) are 15 combinations presented above the operator we will discuss below. You should know that "hats" indicate an operator.
The direction of rotation from rf pulses or chemical shift better determined through "right-hand rule"

RULE:

The thumb of the right hand points along the positive axis, the direction of the rotation is the direction the fingers will close to make a fist.

(I) Radio frequency pulse

\[ I_{\beta} \xrightarrow{\phi \hat{I}_x} I_{\beta} \cos(\phi) + I_{\gamma} \sin(\phi) \]

\( \alpha, \beta, \gamma \) are x,y,z axis; \( \phi \) is the pulse flip angle

\[ I_{x} \xrightarrow{90^\circ \hat{i}_y} I_{x} \]

\[ I_{z} \xrightarrow{90^\circ \hat{i}_y} I_{x} \]

\[ I_{x} \xrightarrow{180^\circ \hat{i}_x} -I_{x} \]

\[ I_{z} \xrightarrow{180^\circ \hat{i}_x} -I_{z} \]

(II) Chemical shift

The chemical shift has the effect of an "rf" pulse along the z axis.

\[ I_{x} \xrightarrow{\Omega_{t} \hat{i}_z} I_{x} \cos(\Omega_{t} t) + I_{y} \sin(\Omega_{t} t) \]

\[ I_{y} \xrightarrow{\Omega_{t} \hat{i}_z} I_{y} \cos(\Omega_{t} t) - I_{x} \sin(\Omega_{t} t) \]

\( \Omega \) is the chemical

Except of strongly coupled state (where \( J_{IS} > \Delta \sigma \)) pulses and chemical shifts act on combination of I and S as if the other spins were absent.

Example:

\[ 2I_{x}S_{z} \xrightarrow{\phi \hat{I}_x} 2(1_{x} \cos(\phi) - I_{y} \sin(\phi))S_{z} = 2I_{x}S_{z} \cos(\phi) - 2I_{y}S_{z} \sin(\phi) \]

\[ 2I_{x}S_{z} \xrightarrow{\Omega_{t} \hat{i}_z} 2(I_{x} \cos(\Omega_{t} t) + I_{y} \sin(\Omega_{t} t))S_{z} = 2I_{x}S_{z} \cos(\Omega_{t} t) + 2I_{y}S_{z} \sin(\Omega_{t} t) \]

Conclusion

Chemical shift and rf pulses are operations interpreted as three-dimensional rotation. They are not able to transfer magnetisation starting on spin I to spin S.
(II) Coupling
The coupling operator is the essential feature of multidimensional experiments that provide correlation through coherent transfer of magnetisation from spin I to spin S.

\[ I_\alpha \xrightarrow{\pi J_{1st}} I_\alpha \cos(\pi J_{1st}) + 2I_\beta S_z \sin(\pi J_{1st}) \]

\[ (\alpha, \beta = x, y) \]

\[ 2I_\beta S_z \xrightarrow{\pi J_{1st}} 2I_\beta S_z \cos(\pi J_{1st}) - I_\alpha \sin(\pi J_{1st}) \]

Example: if \( \Rightarrow \cos(\pi J_{1st}) = 0, \Rightarrow \pi J_{1st} = \frac{\pi}{2} \text{ then } \Rightarrow t = \frac{1}{2J_{1st}} \]

\[ I_x \xrightarrow{\pi J_{1st}} 2I_y S_z \]

\[ 2I_y S_z \xrightarrow{\pi J_{1st}} -I_x \]

**RULE:** Coupling operator can course inphase coherence to go to antiphase, or antiphase coherence to go inphase.

---

Part (2): Building Blocks and Coherence Flow Networks.
There are surprisingly few ways to transfer magnetisation (coherence) from one spin to another (for two spin system).

All of the method can be placed into two broad categories:
1) Coherent (All multinuclear, multidimensional experiments rely on one or more coherent transfer steps mediated by scalar coupling)
2) Incoherent transfer (including the dipolar interactions going rise to NOEs and ROEs as well as chemically exchanging nuclei)

For the former one, the main (coherent) building blocks are:

a) **HMOC** (heteronuclear multiple-quantum correlation spectroscopy)
b) **INEPT** (Intensive nucleus enhancement by polarisation transfer)
c) **TOCSY** (Total correlated spectroscopy)
d) **COSY** (Correlated spectroscopy)

We discuss how these building blocks work by using the product operator rules given above.
An important component of many of the "building blocks" are variations of the "spin-echo" experiment, so we will describe these first.

(I) "Spin-echo" The spin echo is simply a delay followed by $180^\circ$ pulse followed by a second delay. We will consider in present only the case with two equal delays. In part "a" we have $I_x$ (At this step it is not important how we get this magnetisation).

\[
\begin{align*}
(A),(B): & \quad \text{Dec SE} \\
& \quad I_x \\
& \quad \tau \quad \tau \\
& \quad a \quad b \quad c \quad d
\end{align*}
\]

\[
\begin{align*}
(C): & \quad J_{IS} \ SE \\
& \quad I \\
& \quad \tau \quad \tau \\
& \quad a \quad b \quad c \quad d
\end{align*}
\]

\[
\begin{align*}
(D): & \quad \text{Dec CS} \\
& \quad S \\
& \quad 180^\circ \\
& \quad a \quad b \quad c \quad d
\end{align*}
\]

Variation of the spin-echo building block. (A) and (B) Building block DEC SE which refocuses $I$ chemical shift and decouples $S$. (C) Building block $J_{IS}$ SE which refocuses $I$ chemical shift but allows coupling to occur with $S$. (D) Building block Dec CS allows chemical on $I$ to occur while decoupling $S$. 


(A) In first case we assume no scalar coupling the spin I spin S.

A: Dec SE

\[ I_x \]

\[ \tau \]

\[ a \]

\[ b \]

\[ c \]

\[ d \]

The following steps show the product operator transformations from points "a" to "d":

(Let us go together through all step):

\[ a \rightarrow b: \quad I_x \xrightarrow{\Omega_1 \tau I_z} I_x \cos(\Omega_1 \tau) + I_y \sin(\Omega_1 \tau) \]

\[ b \rightarrow c: \quad I_x \xrightarrow{180^\circ \hat{I}_z} I_x \cos(\Omega_1 \tau) + [I_x \cos(180^\circ) + I_z \sin(180^\circ)] \sin(\Omega_1 \tau) \Rightarrow I_x \cos(\Omega_1 \tau) - I_y \sin(\Omega_1 \tau) \]

\[ c \rightarrow d: \quad I_x \xrightarrow{\Omega_1 \tau I_z} [I_x \cos(\Omega_1 \tau) + I_y \sin(\Omega_1 \tau)] \cos(\Omega_1 \tau) - [I_x \cos(\Omega_1 \tau) - I_y \sin(\Omega_1 \tau)] \sin(\Omega_1 \tau) = \]

\[ = I_x [\cos^2(\Omega_1 \tau) + \sin^2(\Omega_1 \tau)] + I_y [\cos(\Omega_1 \tau) \sin(\Omega_1 \tau) - \cos(\Omega_1 \tau) \sin(\Omega_1 \tau)] \Rightarrow I_x \]

If to repeat the same, but using the vector representation:

\[ \text{The net result: WE END UP WITH WHAT WE START WITH!!!} \]

**RULE:** Whenever a 180° pulse is placed symmetrically between two time intervals, no chemical shift takes place. (ECHO)

(B) In next case, consider the same spin-echo experiment in the presence of scalar coupling between I and S spin: (We neglect the chemical shift)

The following steps show the product operator transformations from points "a" to "d":

\[ a \rightarrow b: \quad I_x \xrightarrow{\pi J_{IS} \tau 2 \hat{I}_z \hat{S}_z} I_x \cos(\pi J_{IS} \tau) + 2I_z s_z \sin(\pi J_{IS} \tau) \]

\[ b \rightarrow c: \quad I_x \xrightarrow{180^\circ \hat{I}_z} I_x \cos(\pi J_{IS} \tau) + s_z [I_x \cos(180^\circ) + I_z \sin(180^\circ)] \times \sin(\pi J_{IS} \tau) \Rightarrow I_x \cos(\pi J_{IS} \tau) - 2I_z s_z \sin(\pi J_{IS} \tau) \]

\[ c \rightarrow d: \quad I_x \xrightarrow{\pi J_{IS} \tau 2 \hat{I}_z \hat{S}_z} [I_x \cos(\pi J_{IS} \tau) + 2I_z s_z \sin(\pi J_{IS} \tau)] \cos(\pi J_{IS} \tau) - [2I_z s_z \cos(\pi J_{IS} \tau) - I_x \sin(\pi J_{IS} \tau)] \sin(\pi J_{IS} \tau) \]

\[ \Rightarrow I_x [\cos^2(\Omega_1 \tau) + \sin^2(\Omega_1 \tau)] + [2I_z s_z - 2I_x - 2I_z s_z] \sin(\pi J_{IS} \tau) \cos(\pi J_{IS} \tau) \Rightarrow I_x \]

**The net result:** In addition to refocusing chemical shift, this simple experiment decouples spin I from S

This is a decoupled spin-echo:

\[ I_x \xrightarrow{\text{DecSE}} I_x \]

in term of a CFN Diagram:
(C) Let us consider two additional related experiments.

First, we include a $180^\circ$ pulse on $S$ spin simultaneous to the $180^\circ$ pulse on $I$ spin

\[ \text{JIS SE} \]

\[ \text{(presence of scalar coupling between } I \text{ and } S \text{ spin)} \]

**Note:** That since $I_x$ and $S_x$ do not interfere with unlike spins ($S_y \rightarrow 180^\circ I_x \rightarrow S_y$) a $180^\circ$ $S$ and $I$ pulses can be applied together or in tandem, in either order.

Once again, we start with $I_x$ magnetisation and neglect chemical shift which is refocused by a $180^\circ I$ pulse.

\[ a \rightarrow b: \quad I_x, \pi J_{IS} 2 I \hat{z} \frac{\hat{S}_I}{2} \rightarrow I_x \cos(\pi J_{IS} \tau) + 2 I_s \sin(\pi J_{IS} \tau) \]

\[ b \rightarrow c: \quad 180^\circ I \frac{\hat{I}_z}{2} \rightarrow I_x \cos(\pi J_{IS} \tau) + S_z \left[ I_x \cos(180^\circ) + I_x \sin(180^\circ) \right] \times \sin(\pi J_{IS} \tau) \Rightarrow I_x \cos(\pi J_{IS} \tau) - 2 I_s \sin(\pi J_{IS} \tau) \]

\[ 180^\circ S \frac{\hat{S}_I}{2} \rightarrow I_x \cos(\pi J_{IS} \tau) - 2 I_s \sin(\pi J_{IS} \tau) \frac{\hat{S}_S}{2} \cos(180^\circ) - S_z \sin(180^\circ) \Rightarrow I_x \cos(\pi J_{IS} \tau) + 2 I_s \sin(\pi J_{IS} \tau) \]

\[ c \rightarrow d: \quad \pi J_{IS} 2 I \hat{I} \frac{\hat{S}_I}{2} \rightarrow I_x \cos(\pi J_{IS} \tau) + 2 I_s \sin(\pi J_{IS} \tau) \]

\[ \Rightarrow I_x \left[ \cos^2(\pi J_{IS} \tau) - \sin^2(\pi J_{IS} \tau) \right] = \cos(2 \pi J_{IS} \tau) \]

\[ 2 \sin(\pi J_{IS} \tau) \cos(\pi J_{IS} \tau) = \sin(2 \pi J_{IS} \tau) \]

\[ \Rightarrow \frac{1}{2} \cos(2 \pi J_{IS} \tau) + 2 I_s \sin(\pi J_{IS} \tau) \]

**The net result:** The result of this building block is the same as the single operation, $\pi J_{IS} 2 \tau 2 I \hat{z} S_z$

Unlike the previous example, simultaneous by a $180^\circ$ pulses on both $S$ and $I$ in spin echo experiment leave the two spin **coupled**. Turn back to the final result:

\[ I_x \cos(\pi J_{IS} 2 \tau) + 2 I_s S_z \sin(\pi J_{IS} 2 \tau) \]

\[ \pi J_{IS} 2 \tau = \frac{\pi}{2} \Rightarrow \tau = \frac{1}{4 J_{IS}} \]

Then \[ I_x, \pi J_{IS} 2 I \hat{z} \frac{\hat{S}_I}{2} \rightarrow 2 I_s, S_z \leftrightarrow I_x, \text{JIS SE} \rightarrow 2 I_s, S_z \]

or \[ 2 I_s S_z, \pi J_{IS} 2 I \hat{z} \frac{\hat{S}_S}{2} \rightarrow -I_x \leftrightarrow 2 I_s, S_z, \text{JIS SE} \rightarrow -I_x \]

In term of a CFN.

**CFN represents changes in state both from in-phase to antiphase and antiphase to in-phase.**
Finally, consider the experiment shown in Fig. 137: Dec CS

\[ I \]

\[ S \]

As above, we start the analysis with \( I_x \) magnetization at point "a", but this time we cannot neglect chemical shift!!!

\[ a \rightarrow b: I_x \rightarrow I_x \cos(\Omega_1 \tau) + I_y \sin(\Omega_1 \tau) \]

\[ \pi/2 \hat{S}_z \rightarrow [I_x \cos(\Omega_1 \tau) + I_y \sin(\Omega_1 \tau)] \cos(\pi/2 \tau) + [2I_z \cos(\Omega_1 \tau) - 2I_z \sin(\Omega_1 \tau)] \sin(\pi/2 \tau) \]

\[ b \rightarrow c: -180^\circ \hat{S}_x \rightarrow [I_x \cos(\Omega_1 \tau) + I_y \sin(\Omega_1 \tau)] \cos(\pi/2 \tau) + [2I_z \cos(\Omega_1 \tau) - 2I_z \sin(\Omega_1 \tau)] \sin(\pi/2 \tau) \]

\[ S_z \cos(180^\circ) - S_z \sin(180^\circ) \Rightarrow [I_x \cos(\Omega_1 \tau) + I_y \sin(\Omega_1 \tau)] \cos(\pi/2 \tau) - [2I_z \cos(\Omega_1 \tau) - 2I_z \sin(\Omega_1 \tau)] \sin(\pi/2 \tau) \]

\[ c \rightarrow d: \pi/2 \hat{S}_z \rightarrow [I_x \cos(\Omega_1 \tau) + I_y \sin(\Omega_1 \tau)] \cos^2(\pi/2 \tau) + [2I_z \cos(\Omega_1 \tau) - 2I_z \sin(\Omega_1 \tau)] \sin(\pi/2 \tau) \cos(\pi/2 \tau) - \]

\[ -[I_x \cos(\Omega_1 \tau) + I_y \sin(\Omega_1 \tau)] \sin^2(\pi/2 \tau) = \Rightarrow I_x \cos(\Omega_1 \tau) + I_y \sin(\Omega_1 \tau) \]

\[ \Omega_1 \hat{t} \rightarrow I_x \cos^2(\Omega_1 \tau) + I_y \cos(\Omega_1 \tau) \sin(\Omega_1 \tau) + I_z \cos(\Omega_1 \tau) \sin(\Omega_1 \tau) - I_2 \sin^2(\Omega_1 \tau) = \]

\[ \Rightarrow I_2 \cos^2(\Omega_1 \tau) - \sin^2(\Omega_1 \tau) + 2I_1 \sin(\Omega_1 \tau) \cos(\Omega_1 \tau) = \Rightarrow I_x \cos(\Omega_2 \tau) + I_y \sin(\Omega_2 \tau) \]

The net result: The experiment decouples \( I \) from \( S \) while letting \( I \) evolve under chemical shift for the period \( 2\tau \).

This experiment is referred to as a decoupled chemical shift (DecCS) and can be represented as:

\[ I_x \xrightarrow{\text{DecCS}} I_x \cos(\Omega_2 \tau) + I_y \sin(\Omega_2 \tau) \]

In term of a CFN

**RULES**

1. Only products having a single transverse component give rise to detected NMR signal (e.g., \( I_x, S_y, 2I_xS_z, \) and \( 2I_yS_y \)).
2. Products having more than one transverse component correspond to multiple quantum coherence, and are not detected (e.g., \( 2I_xS_y, 2I_yS_x, \) or for a three-spin system, \( 4I_xS_xS_y, 4I_xS_zS_x \)).
3. Products having one transverse term and one or several \( z \) terms represent antiphase signal. In other words, the \( I \) doublet appears in the spectrum with one line up and the other line down, having an overall integral of zero.