

# Theory and Applications of NMR Spectroscopy

**Arthur S. Edison**

**Department of Biochemistry & Molecular Biology**

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# Theory and Applications of NMR Spectroscopy

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## **Author:**

Dr. Arthur S. Edison

e-Mail: [art@ascaris.health.ufl.edu](mailto:art@ascaris.health.ufl.edu)

WWW: <http://ascaris.health.ufl.edu>

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## **About this document:**

This document is based on a course held by the author. As this is the first version there will be upcoming corrections, clarifications and enhancements of this document.

## **Prerequisites:**

Graduate student or advanced undergraduate status, physical chemistry, organic chemistry, and an understanding of basic mathematics, or by arrangement with the instructor.

## **Course Description:**

This course is an introduction to modern NMR experiments and their application to biological problems. The first half of the class will focus on an understanding of multidimensional (2D, 3D, 4D) and multinuclear ( $^1\text{H}$ ,  $^{15}\text{N}$ ,  $^{13}\text{C}$ , ...) NMR experiments. In this half, the students should gain a working knowledge of the basics of NMR spectroscopy. We will make some use of computer software for "hands on" work. The second half will cover applications to proteins and nucleic acids. Much of this will be literature-based and can be adjusted to meet the interests of the class.

Structural Biology: Theory and Applications of NMR Spectroscopy

**Week 1: Introduction to the basics: Bloch equations**

References: Most NMR books. These notes were constructed from combinations of:

"Protein NMR spectroscopy: Principles and Practice" Cavanagh, Fairbrother, Palmer, and Skelton, Academic Press, (1996).

"Biomolecular NMR Spectroscopy" Evans, Oxford University Press, (1995).

"Principles of Nuclear Magnetic Resonance in One and Two Dimensions" Ernst, Bodenhausen, and Wokaun, Oxford (1987).

**Nuclear spin and angular momentum**

Some atomic nuclei have an intrinsic property called "spin". This was first demonstrated in 1922 by the Stern-Gerlach experiment, in which a beam of silver atoms were passed through a magnet field and split into two beams. These two beams represent the two states,  $\alpha$  and  $\beta$ , of the silver (spin 1/2) nuclei.

The nuclear spin has an intrinsic angular momentum, a vector that is represented by the symbol  $\mathbf{I}$  (vectors will be in bold). Vectors have 3 orientations (x, y, and z) and a length. However, the Heisenberg Uncertainty Principle tells that we can only know one orientation and the length simultaneously. By convention in NMR, we say that we know the z-orientation of the angular momentum of a nucleus in a magnetic field. The square of the magnitude of  $\mathbf{I}$  is given by

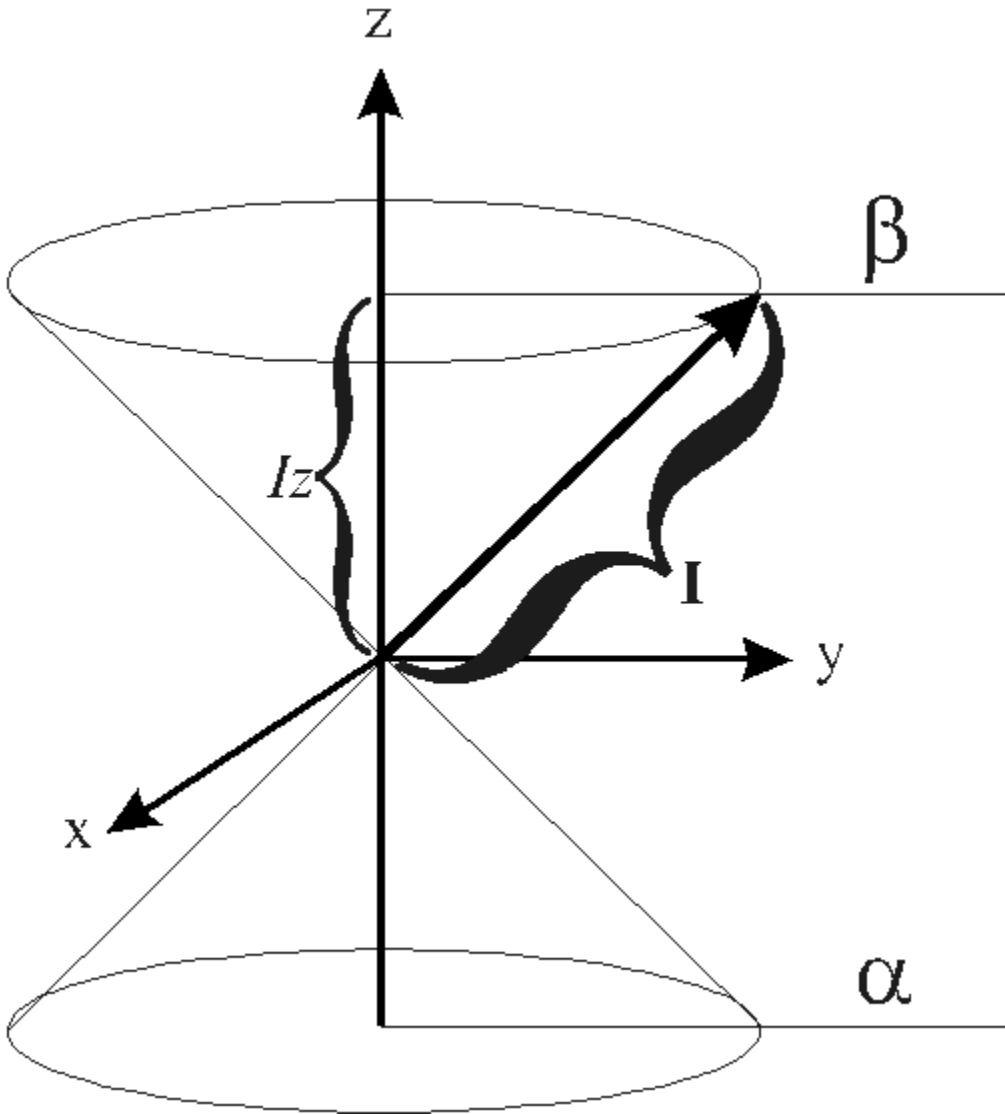
$$|\mathbf{I}^2| = \hbar^2 [I(I+1)] \quad (1-1)$$

where  $I$  is the spin quantum number (e.g. 1/2) and  $\hbar$  is Planck's constant divided by  $2\pi$ . The z-component of  $\mathbf{I}$  is given by

$$I_z = \hbar m \quad (1-2)$$

where  $m$  is the quantum number with values  $m = (-I, -I+1, \dots, I-1, I)$ . In the case of spin 1/2 nuclei,  $I = 1/2$  and  $m$  can be -1/2 and 1/2.

A diagram illustrating these principles is:



In this diagram, the coordinate system has been placed in the center of two cones. The angular momentum vector  $\mathbf{I}$  is shown on the edge of the upper cone. The projection of  $\mathbf{I}$  onto the  $z$ -axis is  $I_z$ , but  $\mathbf{I}$  is completely undetermined in the  $x,y$  plane.

Nuclei that have spin angular momentum also have a magnetic moment given by

$$\boldsymbol{\mu} = \gamma \mathbf{I} \quad (1-3)$$

where  $\gamma$  is the gyromagnetic ratio (sometimes called the magnetogyric ratio).  $\gamma$  is a physical property of each nucleus. For a given abundance, nuclei with higher values of  $\gamma$  produce higher sensitivity NMR spectra.

[Click here for values of  \$\gamma\$  and natural abundance for some important spin 1/2 biological nuclei.](#)

The z-component of the magnetic moment is thus given by

$$\mu_z = \gamma I_z = \gamma \hbar m \quad (1-4)$$

### Nuclear energies in a magnetic field

The energy of the magnetic moment of a nuclear spin in a magnetic field is given by

$$E = -\boldsymbol{\mu} \cdot \mathbf{B} \quad (1-5)$$

where  $\mathbf{B}$  is the magnetic field (in general  $\mathbf{B}$  is a vector quantity). To conduct an NMR experiment, a sample is first placed into a static magnetic field. By convention, the direction of the static magnetic field is along the z-axis, and the magnitude of the magnetic field is given by  $B_0$  (no longer a vector since it points only along the z-axis). In this case, the energy of a nuclear spin in an NMR magnet becomes

$$E = -\mu_z B_0 = -\gamma I_z B_0 \quad (1-6)$$

and the energy associated with a particular quantum number  $m$  is

$$E_m = -m \hbar \gamma B_0 \quad (1-7)$$

NMR transition energies are very small. These small energies translate into low sensitivity. In the absence of a magnetic field, the  $\alpha$  and  $\beta$  states are equally populated, leading to no net magnetization (that is why we need large static magnets to do NMR). When samples are placed into a magnetic field, a *small excess* of nuclei fall into the  $\alpha$  state. This excess of spins in the  $\alpha$  over the  $\beta$  states accounts for the entire net magnetization which is used in the NMR experiment. The ratio of the number of spins in the  $\alpha$  state to those in the  $\beta$  state is given by a Boltzman distribution

$$\frac{N_\alpha}{N_\beta} = e^{\frac{\Delta E}{k_B T}} \quad (1-8)$$

where  $\Delta E$  is the difference of energies of the  $\alpha$  and  $\beta$  states,  $k_B$  is Boltzman's constant, and T is the

absolute temperature. Typical values of magnetic field strengths produce as little as 1 in 10,000 excess  $\alpha$  spins over  $\beta$ . Higher magnetic fields produce correspondingly larger differences in spin states, leading to greater sensitivity.

Quantum mechanical selection rules dictate that NMR signals can only arise when  $m$  changes  $\pm 1$ . From Planck's law ( $E = h \nu$ ) the frequency ( $\nu_0$ ) of an NMR transition in a magnet field  $B_0$  is

$$\nu_0 = \frac{E}{h} = \frac{\gamma B_0}{2\pi} \quad (1-9)$$

The units of  $\nu_0$  are radians/second. More commonly, NMR frequencies are expressed in Hertz (Hz) from  $\nu_0 = \omega_0/2\pi$ , yielding the well-known expression

$$\omega_0 = \gamma B_0 \quad (1-10)$$

### Behavior of bulk magnetization in a magnetic field

We will now consider "bulk" magnetization  $\mathbf{M}$  that arises from all of the magnetic moments in a sample.  $\mathbf{M}$  experiences a torque when placed in a magnetic field according to

$$\frac{d\mathbf{J}(t)}{dt} = \mathbf{M}(t) \times \mathbf{B}(t) \quad (1-11)$$

where  $\mathbf{J}(t)$  is the bulk spin angular momentum. Note that all three vector quantities in Equation 11 are time dependent. The time-dependence of the magnetic field comes about when we apply radio frequency ( $rf$ ) pulses along the x- or y-axis (described below). Equation 11 is essentially identical to an equation that describes the motion of a gyroscope (see, for example, Goldstein "Classical Mechanics" Addison-Wesley, 1980):

$$\frac{d\mathbf{L}(t)}{dt} = \mathbf{r} \times m\mathbf{g} \quad (1-12)$$

where  $\mathbf{L}(t)$  is the gyroscope's angular momentum,  $\mathbf{r}$  the radius from the fixed point of rotation,  $m$  the mass, and  $\mathbf{g}$  gravity. Thus, a nuclear spin in a magnetic field will behave much like a gyroscope in a gravitational field. To make Equation 11 more useful, we use the relationship given in Equation 4 and multiply each side by  $\gamma$  to yield

$$\frac{d\mathbf{M}(t)}{dt} = \mathbf{M}(t) \times \gamma\mathbf{B}(t) \quad (1-13)$$

Equation 13 is the basis of the Bloch equations.

## Bloch equations

In 1946 Felix Bloch formulated a set of equations that describe the behavior of a nuclear spin in a magnetic field under the influence of *rf* pulses. He modified Equation 13 to account for the observation that nuclear spins "relax" to equilibrium values following the application of *rf* pulses. Bloch assumed they relax along the z-axis and in the x-y plane at different rates but following first-order kinetics. These rates are designated  $1/T_1$  and  $1/T_2$  for the z-axis and x-y plane, respectively.  $T_1$  is called spin-lattice relaxation and  $T_2$  is called spin-spin relaxation. Both of these will be described in more detail later in the class. With the addition of relaxation, Equation 13 becomes

$$\frac{d\mathbf{M}(t)}{dt} = \mathbf{M}(t) \times \gamma \mathbf{B}(t) - \mathbf{R}(\mathbf{M}(t) - M_0) \quad (1-14)$$

where  $\mathbf{R}$  is the "relaxation matrix". Equation 14 is best understood by considering each of its components:

$$\begin{aligned} \frac{dM_z(t)}{dt} &= \gamma[M_x(t)B_y(t) - M_y(t)B_x(t)] - \frac{M_z(t) - M_0}{T_1} \\ \frac{dM_x(t)}{dt} &= \gamma[M_y(t)B_z(t) - M_z(t)B_y(t)] - \frac{M(t)_x}{T_2} \\ \frac{dM_y(t)}{dt} &= \gamma[M_z(t)B_x(t) - M_x(t)B_z(t)] - \frac{M(t)_y}{T_2} \end{aligned} \quad (1-15)$$

The terms in Equation 15 that do not involve either  $T_1$  or  $T_2$  are the result of the cross product in Equation 14. Equation 15 describes the motion of magnetization in the "laboratory frame", an ordinary coordinate system that is stationary. Mathematically (and conceptually) the laboratory frame is not the simplest coordinate system, because the magnetization is moving at a frequency  $\omega_0 = \gamma B_0$  in the x-y (transverse) plane. A simpler coordinate system is the "rotating frame", in which the x-y plane rotates around the z-axis at a frequency  $\Omega = -\gamma B_0$ . In the rotating frame, magnetization "on resonance" does not precess in the transverse plane. The transformation of Equation 15 to the rotating frame is achieved by replacing each  $B_z$  (defined as  $B_0$ ) by  $\Omega/\gamma$ :

$$\begin{aligned} \frac{dM_z(t)}{dt} &= \gamma[M_x(t)B_y^r(t) - M_y(t)B_x^r(t)] - \frac{M_z(t) - M_0}{T_1} \\ \frac{dM_x(t)}{dt} &= -\Omega M_y(t) - \gamma M_z(t)B_y^r(t) - \frac{M_x(t)}{T_2} \\ \frac{dM_y(t)}{dt} &= \gamma M_z(t)B_x^r(t) + \Omega M_x(t) - \frac{M_y(t)}{T_2} \end{aligned} \quad (1-16)$$

In Equation 15, the components of  $\mathbf{B}$  have been written with r superscripts to denote that it is a rotating frame. From this point onward, the rotating frame will be assumed without the superscript.

## Physical interpretation of Bloch Equations: Single pulse experiment

We will now examine the behavior of Equation 16 under two different limiting conditions, the effect of a short *rf* pulse and free precession. The *rf* pulse will be assumed to be very short compared to either relaxation times  $T_1$  and  $T_2$  as well as the angular frequency  $\Omega$ . This assumption is valid for many typical pulsed NMR experiments in which the pulse lengths can be as short as 5  $\mu$ s. We will apply the *rf* pulse along the x-axis. These conditions allow us to neglect terms in Equation 16 that contain  $T_1$ ,  $T_2$ ,  $\Omega$ , and  $B_y$ .

$$\frac{dM_z(t)}{dt} = -M_y(t)\gamma B_x^y(t)$$

$$\frac{dM_x(t)}{dt} = 0$$

$$\frac{dM_y(t)}{dt} = M_z(t)\gamma B_x^y(t) \quad . (1-17)$$



## Homework 1

1. Calculate  $N_{\alpha}/N_{\beta}$  for the following 4 cases:

$^1\text{H}$  at 500 MHz (11.7 T)

$^1\text{H}$  at 750 MHz (17.6 T)

$^{13}\text{C}$  at 500 MHz

$^{13}\text{C}$  at 750 MHz

A 500 MHz spectrometer costs ~ \$700,000.

A 750 MHz spectrometer costs ~ \$2,000,000. Why spend the extra money?

2. Copy and begin to read Edison et al., *Methods in Enzymology*, V. 239, 3-79 (1994).

## **Week 2: Introduction to Product operators: Pulses and Chemical shift**

**References:** These notes were constructed from:

**Edison, A. S., Abildgaard, F., Westler, W. M., Mooberry, E. S., and Markley, J. L., "Practical introduction to the theory and implementation of multinuclear, multidimensional NMR experiments", *Methods Enzymol.*, N. J. Oppenheimer and T. L. James, eds., Vol. 239, pp. 1-79 (1994).**

**Other references include:**

**O. W. Sørensen, G. Eich, M. H. Levitt, G. Bodenhausen, and R. R. Ernst, *Prog. NMR Spectrosc.* 16, 163 (1983).**

**"Protein NMR spectroscopy: Principles and Practice" Cavanagh, Fairbrother, Palmer, and Skelton, Academic Press, (1996).**

**"Biomolecular NMR Spectroscopy" Evans, Oxford University Press, (1995).**

**"Principles of Nuclear Magnetic Resonance in One and Two Dimensions" Ernst, Bodenhausen, and Wokaun, Oxford (1987).**

**"Density matrix theory and its applications in NMR spectroscopy" Farrar and Harriman, Farragut Press, Madison, WI (1991).**

**The Bloch equations provide a good physical interpretation to an NMR experiment when there is no spin-spin (scalar or J) coupling. However, the simple picture completely breaks down in the absence of coupling. The solution to the problem is a full density matrix calculation based on the quantum mechanics of the system. Fortunately, Ernst and coworkers devised an easier way to describe NMR experiments, product operator calculations.**

### A simple case: one spin 1/2 nucleus

As we saw above in the Bloch equations, magnetization can be oriented along the x-, y-, or z-axes. Call these orientations  $I_x$ ,  $I_y$ , and  $I_z$ . As described above, we can apply rotations to these components of magnetization. In fact, almost every thing we do with product operators will be applying rotations to magnetization. The notations we will use with product operators is

$$a \xrightarrow{\text{operator}} b.$$

(2-1)

In general, we can do two things in an NMR experiment, pulse and wait. The operator for a pulse applied to an I spin is given by

$$\phi \hat{I}_\alpha$$

(2-2)

where  $\phi$  is the pulse angle and  $\alpha$  is the phase of the pulse. Operators are distinguished from the magnetization upon which they act by a "hat" (^). As we saw in the solutions to the Bloch equations, a pulse applied along the x-axis will rotate the magnetization from the z-axis to the y-axis (remember, pulses produce torque). The product operator calculation for a pulse on equilibrium magnetization applied along the x-axis is

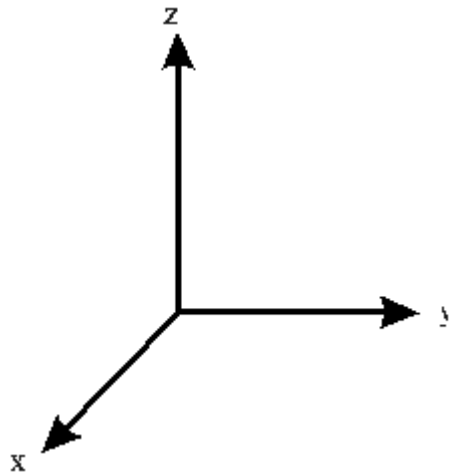
$$I_z \xrightarrow{\phi \hat{I}_x} I_z \cos(\phi) - I_y \sin(\phi)$$

(2-3)

As seen in the Bloch equations, when  $\phi = 0^\circ$ , there is no pulse (the magnetization is still  $I_z$ ) and when  $\phi = 90^\circ$ , the result is  $-I_y$ . The first "rule" to remember concerning product operator calculations is

**For every operation, the first term stays the same as it was but is multiplied by a cosine.**

The directions of rotations can be determined using the "right hand rule" with a "right handed" coordinate system:



To figure out the direction of rotation, use your right hand and point your thumb in the positive direction of the axis **around which the rotation is to occur**. The direction of rotation is the same way that you close your fingers. The same rule allows us to remember the proper orientation of the coordinate system as well; in this case, with your thumb pointing in the positive direction of a given axis, the other order of the other two axes is the proper order in the alphabet (x to y to z).

The second rule to remember is

**For every operation, the second term is the right handed rotation from the first term and multiplied by a sine.**

The third rule to remember is

## A rotation has no effect on magnetization along its axis

With these rules in mind, some other examples of *rf* pulses on **I** spins are:

$$\begin{aligned} I_z &\xrightarrow{90\hat{I}_x} -I_y \\ I_z &\xrightarrow{180\hat{I}_x} -I_z \\ I_z &\xrightarrow{90\hat{I}_y} I_x \\ I_z &\xrightarrow{-90\hat{I}_y} -I_x \\ I_x &\xrightarrow{90\hat{I}_x} I_x \end{aligned}$$

(2-4)

The chemical shift behaves exactly like a pulse but with the rotation along the z-axis. The chemical shift operator is given by

$$\Omega_I t \hat{I}_Z$$

(2-5)

where  $\Omega_I$  is the chemical shift of nucleus **I**,  $t$  is the time, and  $I_z$  is the axis around which the magnetization rotates. Following the rules outlined above we get

$$I_x \xrightarrow{\Omega_I t \hat{I}_Z} I_x \cos(\Omega_I t) + I_y \sin(\Omega_I t)$$

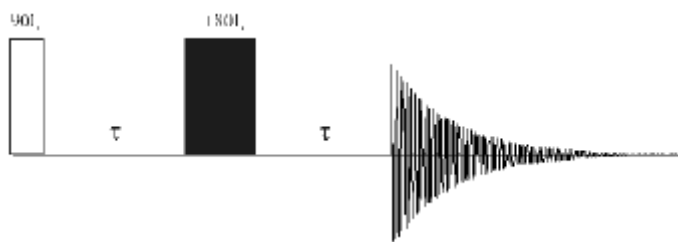
$$I_y \xrightarrow{\Omega_I t \hat{I}_Z} I_y \cos(\Omega_I t) - I_x \sin(\Omega_I t)$$

(2-6)

## Homework 2

1. Draw a right-handed coordinate system and, using vectors to represent magnetization, show the effects of a) 90 degree x-pulse and b) 90 degree y-pulse on z-magnetization. Next, with transverse magnetization (in the x-y plane) show the direction of a positive chemical shift (one that follows the right-hand rule).

2. Using both vectors and product operators, evaluate the following sequence on a single spin  $I$ :



### **Week 3: Introduction to Product operators: Scalar Coupling**

References: These notes were constructed from:

Edison, A. S., Abildgaard, F., Westler, W. M., Mooberry, E. S., and Markley, J. L., "Practical introduction to the theory and implementation of multinuclear, multidimensional NMR experiments", *Methods Enzymol.*, N. J. Oppenheimer and T. L. James, eds., Vol. 239, pp. 1-79 (1994).

Other references include:

O. W. Sørensen, G. Eich, M. H. Levitt, G. Bodenhausen, and R. R. Ernst, *Prog. NMR Spectrosc.* 16, 163 (1983).

"Protein NMR spectroscopy: Principles and Practice" Cavanagh, Fairbrother, Palmer, and Skelton, Academic Press, (1996).

"Principles of Nuclear Magnetic Resonance in One and Two Dimensions" Ernst, Bodenhausen, and Wokaun, Oxford (1987).

"Density matrix theory and its applications in NMR spectroscopy" Farrar and Harriman, Farragut Press, Madison, WI (1991).

Until now, we have worked with a single nuclear spin, I. We have also seen that the vector model represented by the Bloch equations is equivalent to the product operator description with a single spin. The power of NMR lies in its ability to correlate spins through different mechanisms (more on this later). With 2 or more coupled spins, the Bloch equations and the vector picture falls apart, in part from the number of combinations (dimension of the space for rotations) is very large. We introduce a second spin, S. Although I and S originally referred to "insensitive" and "sensitive" nuclei, they commonly are used to denote protons (I) and others (S). Two spins can exist in the following states:



$I_Z, S_Z$	Longitudinal
$I_X, S_Y, S_X, S_Y$	Transverse
$2I_Z S_Z$	J-ordered
$2I_X S_Z, 2I_Y S_Z, 2I_Z S_X, 2I_Z S_Y$	Antiphase
$2I_X S_X, 2I_Y S_X, 2I_X S_Y, 2I_Y S_Y$	Multiple Quantum

These 15 combinations of **I** and **S**, with one additional identity matrix, make up the 16-dimensional space of a pair of coupled spins. Fortunately, the rules of pulses and chemical shifts can often be applied to each of these 15 combinations in exactly the same way as they were applied to single spins. For example,

$$I_Z S_Z \xrightarrow{90\hat{I}_X} -I_Y S_Z$$

$$I_Z S_X \xrightarrow{180\hat{I}_X} -I_Z S_X$$

$$I_X S_Z \xrightarrow{\Omega_I t \hat{I}_Z} I_X S_Z \cos \Omega_I t + I_Y S_Z \sin \Omega_I t$$

### Coupling

In addition to chemical shift and *rf* pulses, there is another important operator. Scalar coupling (or J-coupling) is a purely quantum mechanical phenomenon. As such, it defies most attempts at "rational understanding". All of the equations that are shown (without proof) below "fall out" of a complete quantum mechanical treatment. For the purposes of a practical understanding of NMR experiments, we present the coupling operator as a simple set of rules:

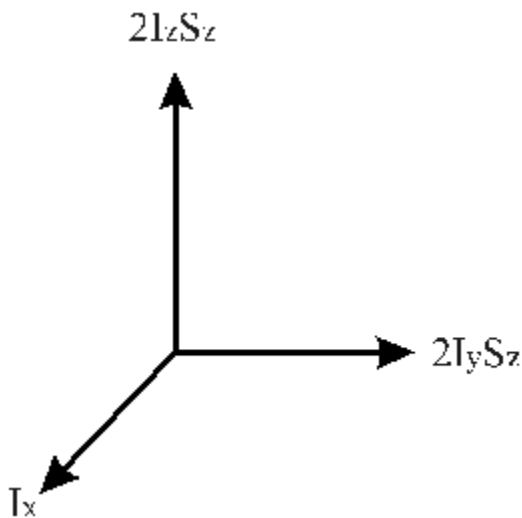
$$I_x \xrightarrow{\mathcal{J}_{IS} t 2\hat{I}_z \hat{S}_z} I_x \cos(\mathcal{J}_{IS} t) + 2I_y S_z \sin(\mathcal{J}_{IS} t)$$

$$I_y \xrightarrow{\mathcal{J}_{IS} t 2\hat{I}_z \hat{S}_z} I_y \cos(\mathcal{J}_{IS} t) - 2I_x S_z \sin(\mathcal{J}_{IS} t)$$

$$2I_y S_z \xrightarrow{\mathcal{J}_{IS} t 2\hat{I}_z \hat{S}_z} 2I_y S_z \cos(\mathcal{J}_{IS} t) - I_x \sin(\mathcal{J}_{IS} t)$$

$$2I_x S_z \xrightarrow{\mathcal{J}_{IS} t 2\hat{I}_z \hat{S}_z} 2I_x S_z \cos(\mathcal{J}_{IS} t) + I_y \sin(\mathcal{J}_{IS} t)$$

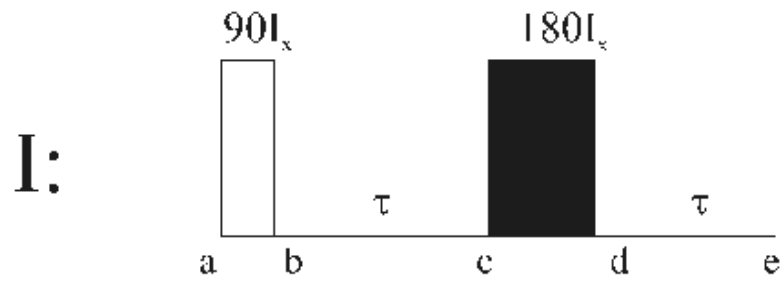
In the above equations,  $J_{IS}$  is the coupling constant (usually in Hz) between nuclei **I** and **S**. The factors of 2 multiplying the antiphase terms are normalization (and can be ignored for most practical applications). Note that the coupling operator does not act on longitudinal or J-ordered magnetization. Also note that the coupling operator is a rotation (in 16 dimensions!) and behaves exactly like all the other rotations we have discussed in that the cosine term is unchanged from the starting point. One of these rotations can be represented as:



### Homework 3

Using product operators and two coupled (e.g.  $J_{IS} \neq 0$ ) spins **I** and **S**, evaluate the following sequences:

1)

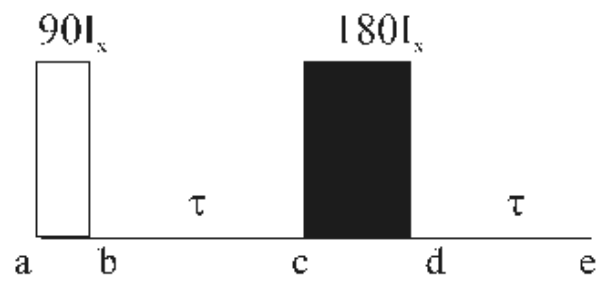


**S:**

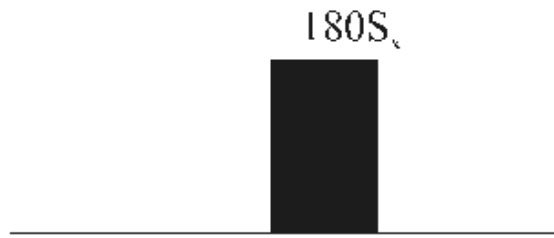
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2)

**I:**



**S:**

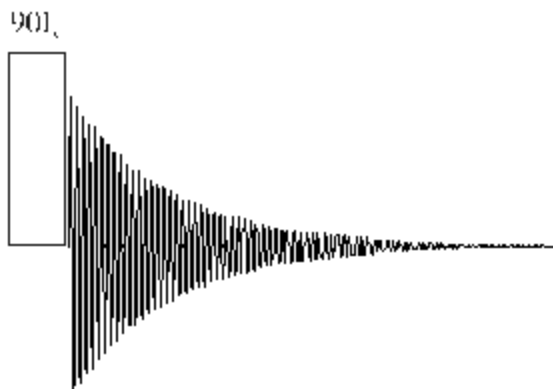


## Week 4: Introduction to two-dimensional NMR

References: These notes were constructed from scratch with help from:

"Principles of Nuclear Magnetic Resonance in One and Two Dimensions" Ernst, Bodenhausen, and Wokaun, Oxford (1987).

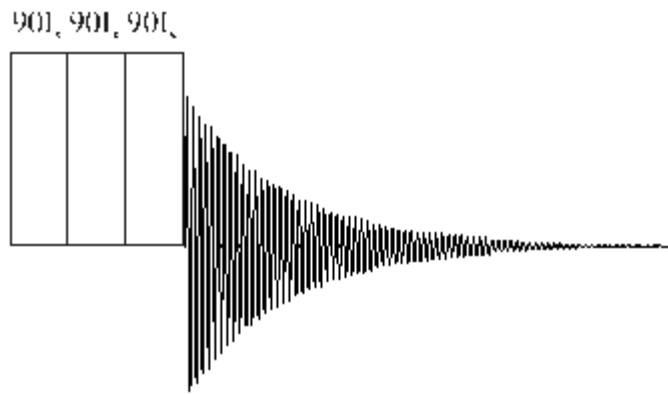
Two-dimensional NMR is not an intuitive subject, BUT it is also not difficult to understand. We will start with the simplest example, a single spin I and a single 90 degree pulse, and build from that. We have learned that a 90 degree pulse along the x-axis will rotate z-magnetization to the minus y-axis:



In product operator notation, the above picture is represented as

$$I_z \xrightarrow{90\hat{I}_x} -I_y \xrightarrow{\Omega_I t \hat{I}_z} -I_y \cos \Omega_I t + I_x \sin \Omega_I t$$

What would happen if we put an extra 180 degree x-pulse (or 2 sequential 90 degree x-pulses) after the 90 degree x-pulse? This would give the same answer but with a different sign:



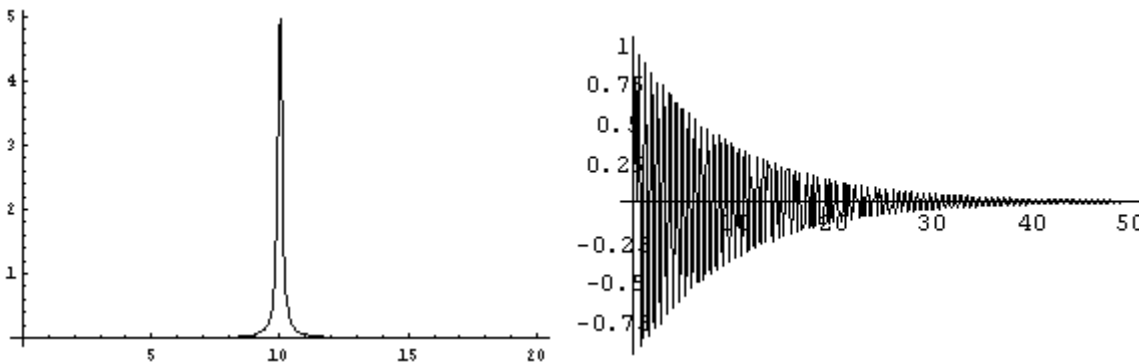
$$I_z \xrightarrow{90\hat{I}_x} \xrightarrow{90\hat{I}_x} \xrightarrow{90\hat{I}_x}$$

$$I_y \xrightarrow{\Omega_I t \hat{I}_z} I_y \cos \Omega_I t - I_x \sin \Omega_I t$$

When we include the effects of relaxation (not included in the product operator calculations), a single pulse produces the well-known NMR signal

$$I_y \cos \Omega_I t \exp-(t/T_2)$$

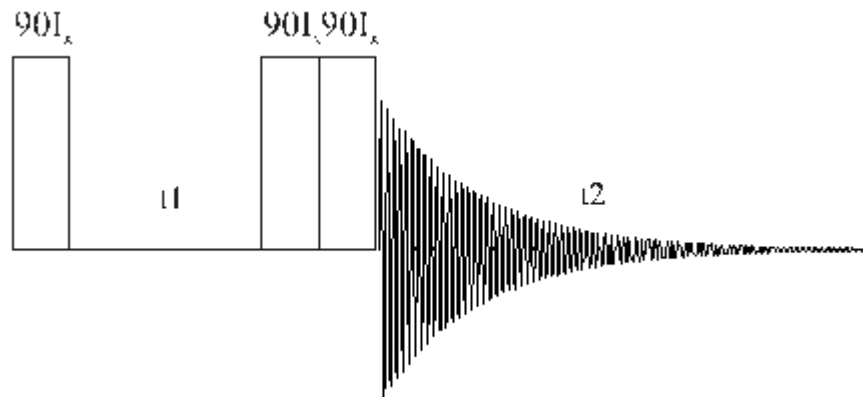
Which, with  $I_y = 1$ ,  $\Omega_I = 10$  Hz, and  $T_2 = 10$  s, looks like



Time Domain ← Fourier Transform → Frequency Domain

Now, we will add a delay, called "t1" between the first 90 degree pulse and the next two 90

degree pulses:



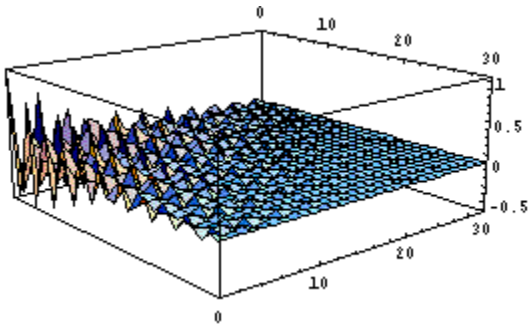
The product operator description for this sequence is:

$$\begin{aligned}
 & I_z \xrightarrow{90\hat{I}_x} -I_y \xrightarrow{\Omega_{I_1} t_1 \hat{I}_z} \\
 & -I_y \cos \Omega_{I_1} t_1 + I_x \sin \Omega_{I_1} t_1 \\
 & \xrightarrow{90\hat{I}_x} \xrightarrow{90\hat{I}_x} \\
 & I_y \cos \Omega_{I_1} t_1 + I_x \sin \Omega_{I_1} t_1 \\
 & \xrightarrow{\Omega_{I_2} t_2 \hat{I}_z} \\
 & (I_y \cos \Omega_{I_1} t_1 + I_x \sin \Omega_{I_1} t_1) \cos \Omega_{I_2} t_2 + \\
 & (-I_x \cos \Omega_{I_1} t_1 + I_y \sin \Omega_{I_1} t_1) \sin \Omega_{I_2} t_2 +
 \end{aligned}$$

This describes a signal whose frequency is modulated during two times,  $t_1$  and  $t_2$ . All components lead to an NMR signal, but the detection schemes (postponed for a later lecture) detect the cosine (called "real") and sine (called "imaginary") terms separately. The real part of the NMR signal from our simple 2D sequence is (including relaxation):

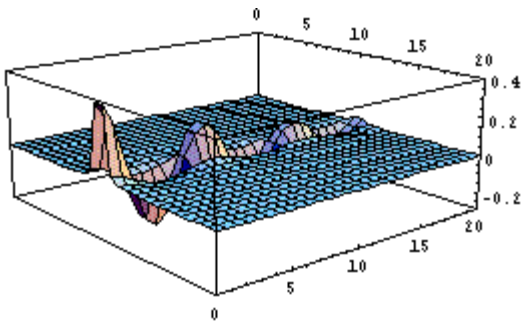
$$I_y \left( \cos \Omega_1 t_1 \exp - (t_1 / T_2) \right) \left( \cos \Omega_1 t_2 \exp - (t_2 / T_2) \right)$$

Using the same parameters as above, this signal looks like:

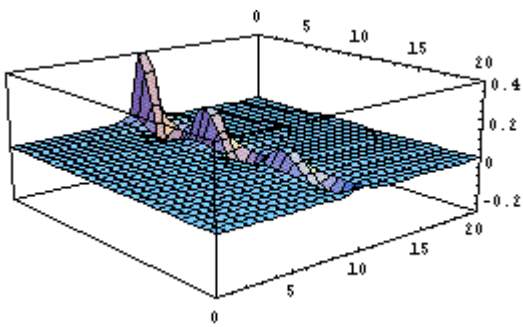


In practice, 2D NMR data is collected by recording a series of 1D spectra along t2 for different lengths of t1. Fourier Transformation along t2 gives:

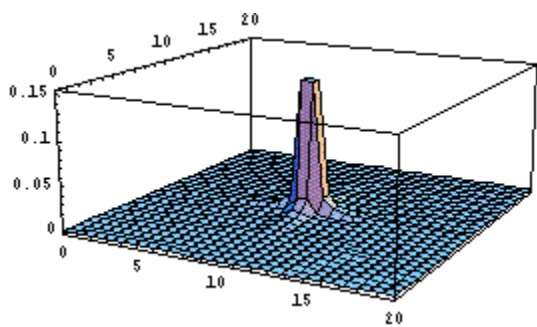




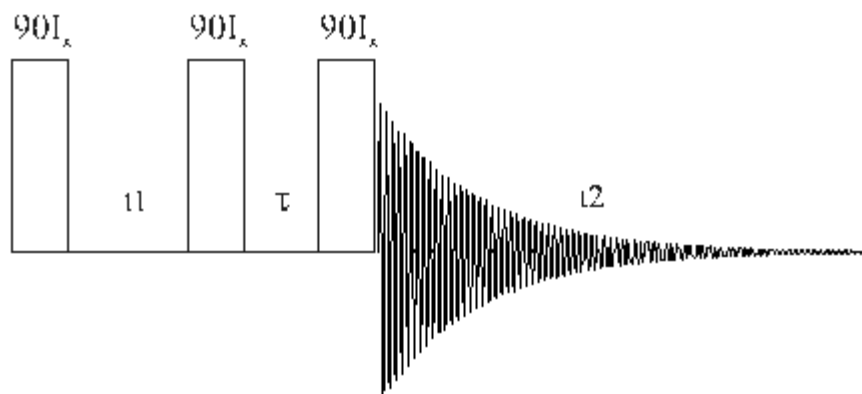
Fourier Transformation along  $t_1$  gives (same orientation in all three cases):



Finally, FT in both dimensions yields the 2D frequency domain spectrum.



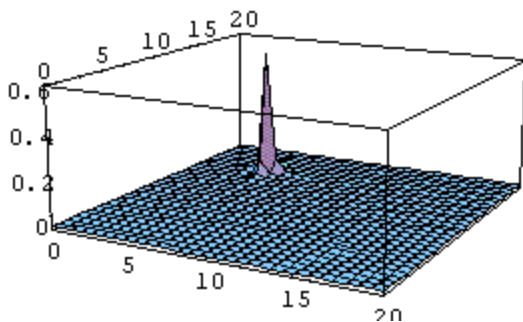
This is a simulation of a 2D NMR spectrum. You may be thinking that it is not very interesting or informative yet. However, now consider a *slightly* more complicated (and *slightly* idealized) case. Consider two chemically exchanging spins in a sample, **I** and **S**. These can interconvert by some chemical isomerization or motion (e.g. protons on an aromatic ring which is flipping back and forth). Now, we will modify the same pulse sequence used above by inserting a fixed (not variable like  $t_1$  or  $t_2$ ) delay



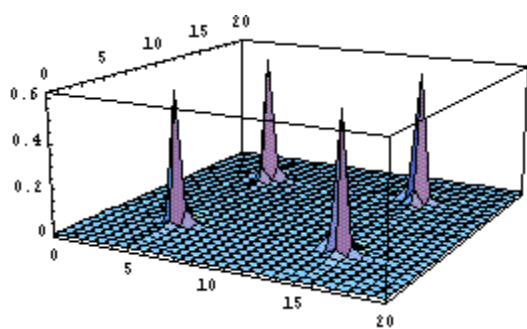
For simplicity in this example, we will assume that the magnetization all begins on spin **I** and is converted to **S** during the period  $\tau$ , which is assumed to be a long enough time for the conversion to take place. Under these assumptions, the product operator analysis of this chemical exchange sequence is:

$$\begin{aligned}
& I_z \xrightarrow{90\hat{I}_x} -I_y \xrightarrow{\Omega_{I1}t\hat{I}_z} \\
& -I_y \cos\Omega_{I1}t_1 + I_x \sin\Omega_{I1}t_1 \xrightarrow{90\hat{I}_x} \\
& -I_z \cos\Omega_{I1}t_1 + I_x \sin\Omega_{I1}t_1 \xrightarrow{\tau} \\
& -S_z \cos\Omega_{I1}t_1 + S_x \sin\Omega_{I1}t_1 \xrightarrow{90\hat{I}(\hat{S})_x} \\
& S_y \cos\Omega_{I1}t_1 + S_x \sin\Omega_{I1}t_1 \xrightarrow{\Omega_{S2}t_2\hat{S}_z} \\
& (S_y \cos\Omega_{I1}t_1 + S_x \sin\Omega_{I1}t_1) \cos\Omega_{S2}t_2 + \\
& (-S_x \cos\Omega_{I1}t_1 + S_y \sin\Omega_{I1}t_1) \sin\Omega_{S2}t_2 +
\end{aligned}$$

Note that since in this case, both **I** and **S** are the same nucleus (e.g. protons), the pulses will apply to both (thus the convenient switch in the middle). This simple chemical example is essentially the same as our previous 2D example with a single nucleus. The only difference is that the frequency (and nucleus) being detected during  $t_2$  is **S** rather than **I**. That is, we have correlated the two nuclei through a 2D spectrum. The Fourier transformed spectrum of this simple chemical exchange experiment using  $\Omega_I=5$  Hz,  $\Omega_S=15$  Hz,  $T_{2I}=20$ s, and  $T_{2S}=20$ s is:



Finally, the complete simulated spectrum of a chemical exchanging pair of nuclei with parameters as described above is



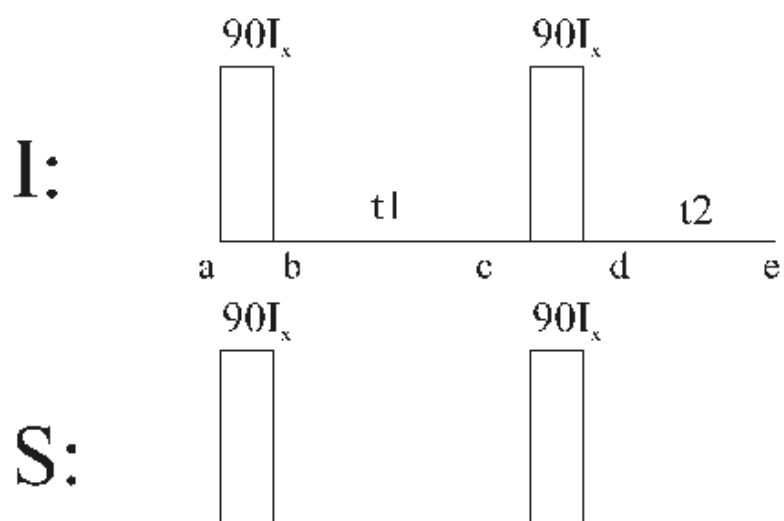
What we have seen in this simple chemical exchange simulation of 2D NMR can be generalized by realizing that all 2D experiments can be described in terms of four periods (see Ernst et al.):

<b>Preparation</b>	<b>Evolution</b>	<b>Mixing</b>	<b>Detection</b>
--------------------	------------------	---------------	------------------

The preparation period can be as simple as a delay and a pulse or a very complicated series of pulses. In the case above, it is the first 90 degree pulse. The evolution period is the variable time,  $t_1$ , in which the first chemical shifts are allowed to evolve. The mixing period is the "heart" of a 2D experiment. The type of correlation observed is a result of the type of mixing. For example, NOESY mixing allows for cross relaxation between nuclei close in space and can measure internuclear distances. TOCSY or COSY mixing periods allows for magnetization to be transferred between scalar coupled nuclei, giving rise to correlations between nuclei with couplings. In the example shown above, the mixing period allows for chemical exchange. The same sequence is used for NOESY. The detection period is the recording of the FID in  $t_2$ .

## Homework 4

Using product operators and two coupled (e.g.  $J_{IS} \neq 0$ ) spins **I** and **S**, evaluate the following sequence:



## **Week 5: Heteronuclear correlations and introduction to Phase cycling**

**References:** These notes were constructed from:

**Edison, A. S., Abildgaard, F., Westler, W. M., Mooberry, E. S., and Markley, J. L., "Practical introduction to the theory and implementation of multinuclear, multidimensional NMR experiments", *Methods Enzymol.*, N. J. Oppenheimer and T. L. James, eds., Vol. 239, pp. 1-79 (1994).**

**Other references include:**

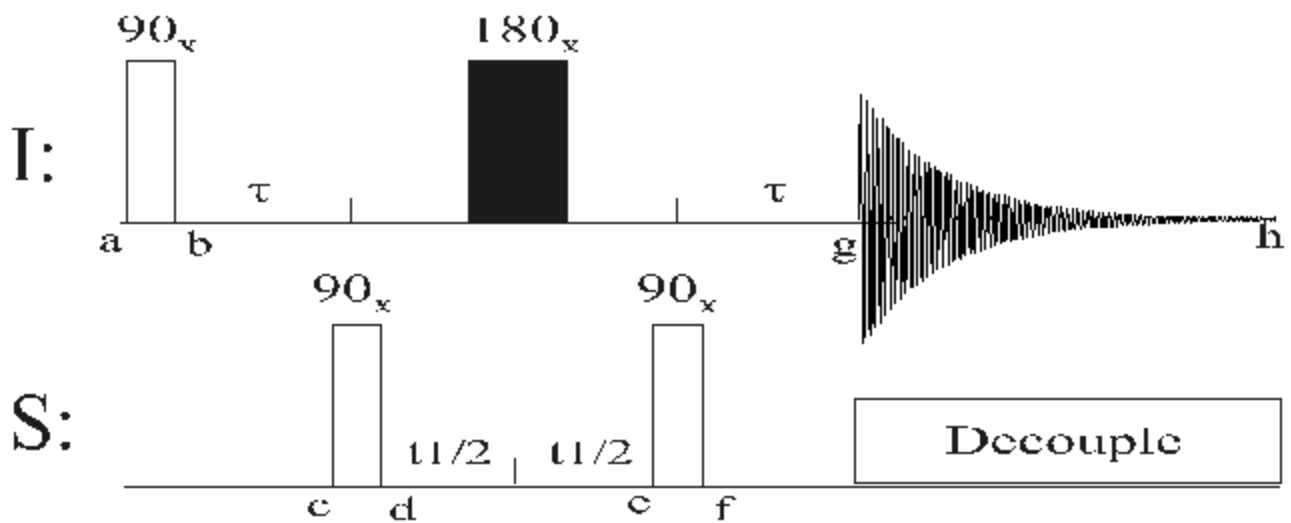
**"Protein NMR spectroscopy: Principles and Practice" Cavanagh, Fairbrother, Palmer, and Skelton, Academic Press, (1996).**

**"Principles of Nuclear Magnetic Resonance in One and Two Dimensions" Ernst, Bodenhausen, and Wokaun, Oxford (1987).**

### **Heteronuclear correlations**

**Much of protein NMR spectroscopy relies on spectral editing techniques using  $^{13}\text{C}$  or  $^{15}\text{N}$  nuclei (heteronuclei). Spectral editing allows a subset of an entire spectrum to be observed. Normally, we observe a subset of  $^1\text{H}$  spectra that has been selected based upon which nucleus the protons are attached to. The same techniques involved in spectral editing allow the measurement of heteronuclear correlations (which, for example, allows you to know which  $^1\text{H}$  are attached to which  $^{13}\text{C}$ ). The most basic sequence to achieve this is the Heteronuclear Multiple Quantum Correlation (HMQC) experiment:**





Before starting the product operator analysis of the HMQC sequence, we can take note of an already common feature that will simplify the problem: The 180 degree pulse on the I spin is placed symmetrically in the sequence. From our previous work on spin echo sequences, we found that such a pulse will refocus (eliminate) chemical shift. Therefore, we won't worry about chemical shift on I until the acquisition time (g to h). In addition, similar examples using spin echo sequences showed that a single 180 degree pulse will decouple I from S during the  $t1$  period (students are encouraged to verify this). The simplified product operator analysis is:

$$a \rightarrow b: I_z \xrightarrow{90\hat{I}_x} -I_y$$

$$b \rightarrow c: -I_y \xrightarrow{\mathbb{1}_{IS} \mathbb{1}_{2\hat{I}_z\hat{S}_z}} 2I_x S_z \quad (\tau = 1/2J)$$

$$c \rightarrow d: 2I_x S_z \xrightarrow{90\hat{S}_x} -2I_x S_y$$

$$d \rightarrow e: -2I_x S_y \xrightarrow{\Omega_S t_1 \hat{S}_z} -2I_x S_y \cos \Omega_S t_1 + 2I_x S_x \sin \Omega_S t_1$$

$$e \rightarrow f: -2I_x S_y \cos \Omega_S t_1 + 2I_x S_x \sin \Omega_S t_1 \xrightarrow{90\hat{S}_x} \\ -2I_x S_z \cos \Omega_S t_1 + 2I_x S_x \sin \Omega_S t_1$$

$$f \rightarrow g: -2I_x S_z \cos \Omega_S t_1 + 2I_x S_x \sin \Omega_S t_1 \xrightarrow{\mathbb{1}_{IS} \mathbb{1}_{2\hat{I}_z\hat{S}_z}} \\ -I_y \cos \Omega_S t_1 + 2I_x S_x \sin \Omega_S t_1 \quad (\tau = 1/2J)$$

$$g \rightarrow h: -I_y \cos \Omega_S t_1 + 2I_x S_x \sin \Omega_S t_1 \xrightarrow{\Omega_I t_2 \hat{I}_z}$$

$$(-I_y \cos \Omega_S t_1 + 2I_x S_x \sin \Omega_S t_1) \cos \Omega_I t_2$$

$$+(I_x \cos \Omega_S t_1 + 2I_y S_x \sin \Omega_S t_1) \sin \Omega_I t_2$$

are several points of the above product operator analysis worth noting. First, by knowing that the I 180 degree pulse refocuses chemical shift and decouples I from S during t1, the number of terms to worry about is greatly reduced. Second, the multiple quantum part of HMQC is from the

**multiple quantum term during the  $t_1$  period. In the pulse sequence shown, there are multiple quantum terms that do not get converted back to antiphase (e.g.  $I_x S_z$ ) magnetization. These terms are carried through to the end and are not observable (remember that only  $I_x$ ,  $I_y$ ,  $S_x$ , and  $S_y$  are observable). Finally, many of the "extra" terms can be eliminated by gradients and/or phase cycling. This topic will be covered in next week's notes.**

## Week 6: Heteronuclear correlations and introduction to Phase cycling

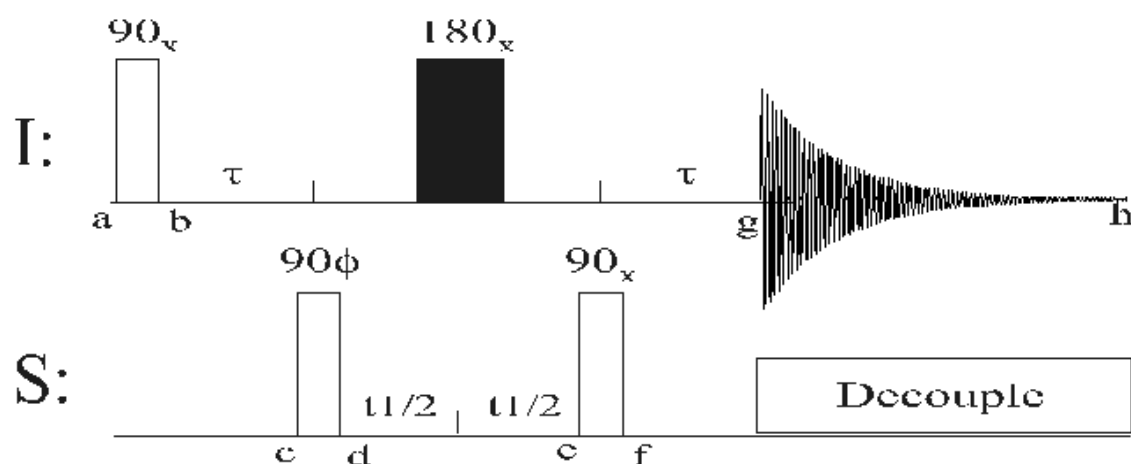
References: These notes were constructed from:

Edison, A. S., Abildgaard, F., Westler, W. M., Mooberry, E. S., and Markley, J. L., "Practical introduction to the theory and implementation of multinuclear, multidimensional NMR experiments", *Methods Enzymol.*, N. J. Oppenheimer and T. L. James, eds., Vol. 239, pp. 1-79 (1994).

### Phase Cycling basics

#### *Spectral Editing*

One of the simplest examples of a phase cycle is the following modification of the HMQC sequence:



The first 90 degree pulse on the S channel is now  $90^\phi$  rather than  $90_x$ . This is a phase cycle, in which the phase of the pulse will be systematically varied. For this example,  $\phi = x, -x$ . This means that at least two FIDs need to be collected for each  $t1$  time point. The first will be  $\phi = x$ , the second will be  $\phi = -x$ . If there are more FIDs collected,  $\phi$  will continue in this pattern. Therefore, we will need an even number of FIDs collected per  $t1$  point. In the notes from the previous week, we analyzed the result when  $\phi = x$ . The product operator analysis for  $\phi = -x$  is shown next:

$$a \rightarrow b: I_z \xrightarrow{90\hat{I}_x} -I_y$$

$$b \rightarrow c: -I_y \xrightarrow{\mathbb{J}_{IS} \mathbb{J} 2\hat{I}_z \hat{S}_z} 2I_x S_z \quad (\tau = 1/2J)$$

$$c \rightarrow d: 2I_x S_z \xrightarrow{90\hat{S}_{-x}} 2I_x S_y$$

$$d \rightarrow e: 2I_x S_y \xrightarrow{\Omega_S t_1 \hat{S}_z} 2I_x S_y \cos \Omega_S t_1 - 2I_x S_x \sin \Omega_S t_1$$

$$e \rightarrow f: 2I_x S_y \cos \Omega_S t_1 - 2I_x S_x \sin \Omega_S t_1 \xrightarrow{90\hat{I}_x} \\ 2I_x S_z \cos \Omega_S t_1 - 2I_x S_x \sin \Omega_S t_1$$

$$f \rightarrow g: 2I_x S_z \cos \Omega_S t_1 - 2I_x S_x \sin \Omega_S t_1 \xrightarrow{\mathbb{J}_{IS} \mathbb{J} 2\hat{I}_z \hat{S}_z} \\ I_y \cos \Omega_S t_1 - 2I_x S_x \sin \Omega_S t_1 \quad (\tau = 1/2J)$$

$$g \rightarrow h: I_y \cos \Omega_S t_1 - 2I_x S_x \sin \Omega_S t_1 \xrightarrow{\Omega_I t_2 \hat{I}_z}$$

$$(I_y \cos \Omega_S t_1 - 2I_x S_x \sin \Omega_S t_1) \cos \Omega_I t_2$$

$$+ (-I_x \cos \Omega_S t_1 - 2I_y S_x \sin \Omega_S t_1) \sin \Omega_I t_2$$

The effect of the change from  $90_x$  to  $90_{(-x)}$  is to change the sign of the magnetization that went through the antiphase (e.g.  $I_x S_z$ ) state. If we subtract the  $\phi_{=-x}$  signal from the  $\phi_{=x}$

signal, we will add the observable magnetization. This is done by also cycling the phase of the receiver from  $x$  to  $-x$  (with the net result of subtracting signals). To see the point of this phase cycle, we must consider an  $I$  spin that is not coupled to an  $S$  ( $J_{IS}=0$ ). Using the same assumptions about the effect of the 180 degree  $I$  pulse on  $I$  chemical shift, we get (for  $\phi=x$ ):

$$a \rightarrow b: I_z \xrightarrow{90\hat{I}_x} -I_y$$

$$b \rightarrow c: -I_y \xrightarrow{\pi_{IS} \hat{I}_z \hat{S}_z} -I_y$$

$$c \rightarrow d: -I_y \xrightarrow{90\hat{S}_x} -I_y$$

$$d \rightarrow e: -I_y \xrightarrow{\Omega_S t_1 \hat{S}_z} -I_y$$

$$e \rightarrow f: -I_y \xrightarrow{90\hat{S}_x} -I_y$$

$$f \rightarrow g: -I_y \xrightarrow{\pi_{IS} \hat{I}_z \hat{S}_z} -I_y$$

$$g \rightarrow h: -I_y \xrightarrow{\Omega_I t_2 \hat{I}_z} -I_y \cos \Omega_I t_2 + I_x \sin \Omega_I t_2$$

It should be fairly clear that the phase of the  $S$  pulses will have no effect on the results with just an  $I$  spin. Therefore, the sign of the signal will not change with  $\phi$ . The net result is that the  $I$  spins not coupled to  $S$  are eliminated with the phase cycle described above.

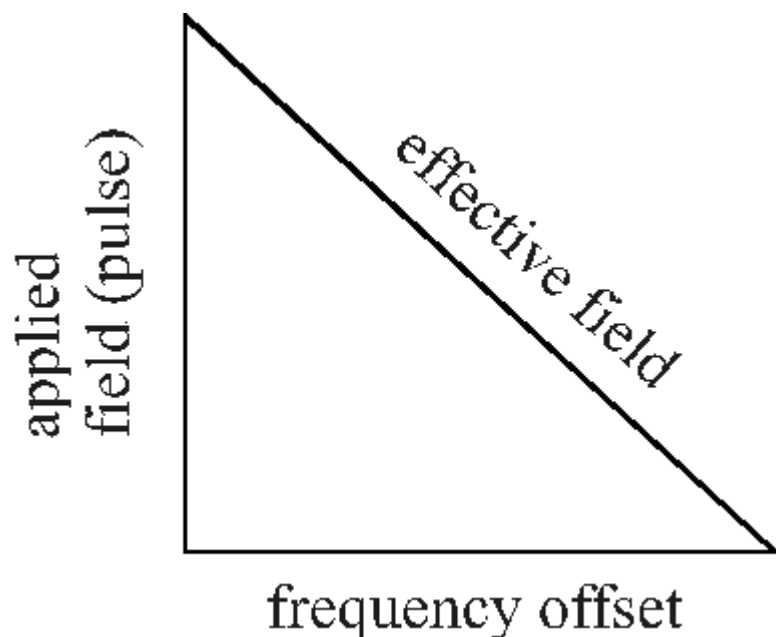
### Exorcycle

Another frequently used phase cycle in NMR is called exorcycle, which is designed to

remove pulse imperfections. The pulse imperfections arise from at least two main problems. First, they might be poorly calibrated. Second, and more important, a 90 (or 180) degree pulse delivered "on resonance" (that is, sitting at the frequency of a particular nucleus) is not going to be a 90 (or 180) degree pulse far off-resonance. This is a result of the power of the pulse (stronger pulses will cover a greater distance) and the effective field generated by the pulse.

### Short Digression into Effective Fields

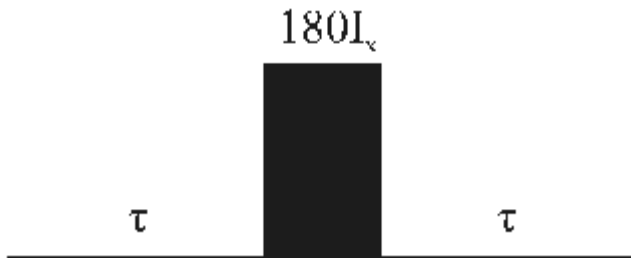
Until now, we have just considered a pulse along the x-axis as just that. Well, like most things, it is more complicated. A spin that is offset from the center pulse frequency will not experience the same field as a spin that is at the pulse frequency. Instead it will experience an effective field, given by



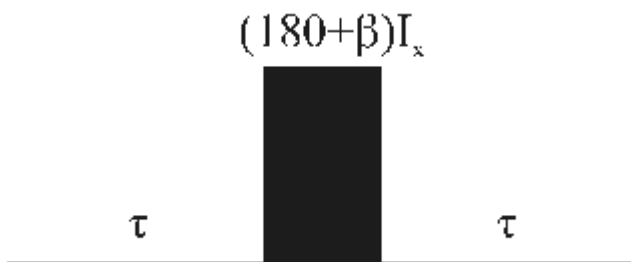
The important point here is that the pulse that a nucleus feels is a complicated function that depends on the distance from the center of the pulse and the strength of the pulse. A much more complete treatment of effective fields can be found in most NMR books.

### Back to the Main Story

Both poor calibrations and field effects lead to greater problems with 180 degree pulses than with 90 degree pulses. To appreciate the impact of pulse imperfections, consider a simple spin echo refocusing sequence on an uncoupled spin **I** in the transverse plane ( $I_x$ ).



This sequence should be familiar by now, and students should be able to verify that if  $I_x$  goes in,  $I_x$  goes out. Now consider a more realistic modification to that sequence:



The pulse imperfection factor  $\beta$  is a function of many variables. Here we will consider its effect on refocusing  $I_x$  magnetization (no coupling):



$$\begin{aligned}
& I_x \xrightarrow{\Omega_I \tau \hat{I}_Z} I_x \cos \Omega_I \tau + I_y \sin \Omega_I \tau \\
& \xrightarrow{180 + \beta \hat{I}_X} I_x \cos \Omega_I \tau + I_y \sin(\Omega_I \tau) \cos(180 + \beta) \\
& + I_z \sin(\Omega_I \tau) \sin(180 + \beta) \\
& \xrightarrow{\Omega_I \tau \hat{I}_Z} (I_x \cos \Omega_I \tau + I_y \sin(\Omega_I \tau) \cos(180 + \beta)) \cos \Omega_I \tau \\
& + (I_y \cos \Omega_I \tau - I_x \sin(\Omega_I \tau) \cos(180 + \beta)) \sin \Omega_I \tau \\
& + I_z \sin(\Omega_I \tau) \sin(180 + \beta)
\end{aligned}$$

Clearly, the factor  $\beta$  could cause many problems and artifacts. One fix to the problem is the exorcise sequence, which is the following:



The phase  $\psi$  is  $(n_x, n_y, n(-x), n(-y))$  where  $n$  is an integer number. Using the same input  $I_x$  as above (and assuming a perfect 180 degree pulse) we get for phases  $x$  and  $y$ :

$$\begin{aligned}
I_x \xrightarrow{\frac{\Omega_I \hat{\tau}}{I} Z} & I_x \cos \Omega_I \tau + I_y \sin \Omega_I \tau \\
\xrightarrow{180 \hat{I}_x} & I_x \cos \Omega_I \tau - I_y \sin(\Omega_I \tau) \\
\xrightarrow{\frac{\Omega_I \hat{\tau}}{I} Z} & (I_x \cos \Omega_I \tau - I_y \sin(\Omega_I \tau)) \cos \Omega_I \tau \\
& + (I_y \cos \Omega_I \tau + I_x \sin(\Omega_I \tau)) \sin \Omega_I \tau \\
& = I_x
\end{aligned}$$

$$\begin{aligned}
I_x \xrightarrow{\frac{\Omega_I \hat{\tau}}{I} Z} & I_x \cos \Omega_I \tau + I_y \sin \Omega_I \tau \\
\xrightarrow{180 \hat{I}_y} & -I_x \cos \Omega_I \tau + I_y \sin(\Omega_I \tau) \\
\xrightarrow{\frac{\Omega_I \hat{\tau}}{I} Z} & (-I_x \cos \Omega_I \tau + I_y \sin(\Omega_I \tau)) \cos \Omega_I \tau \\
& + (-I_y \cos \Omega_I \tau - I_x \sin(\Omega_I \tau)) \sin \Omega_I \tau \\
& = -I_x
\end{aligned}$$

Notice that the sign changes going from x to y. The student can verify that the sign will change each time going from x to y to -x to -y. Thus the receiver phase will need to be (n(x), n(-x), n(x), n(-x)) for the exorcycle. This phase cycle will eliminate many of the artifacts generated by imperfect pulses.

## Week 9: Assignments in proteins and peptides using TOCSY or COSY and NOESY or ROESY

References: These notes were constructed from:

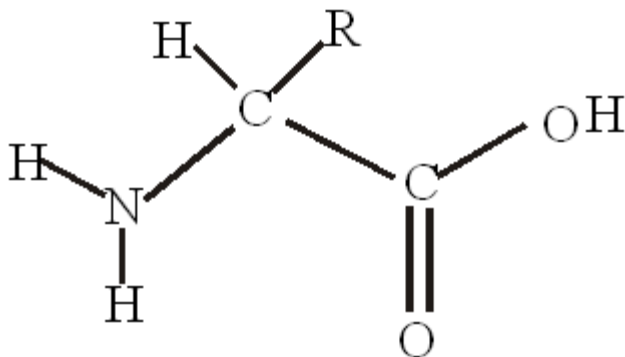
"NMR of Proteins and Nucleic Acids" by Kurt Wüthrich, Wiley Interscience (1986).

### Amino Acids and the peptide bond

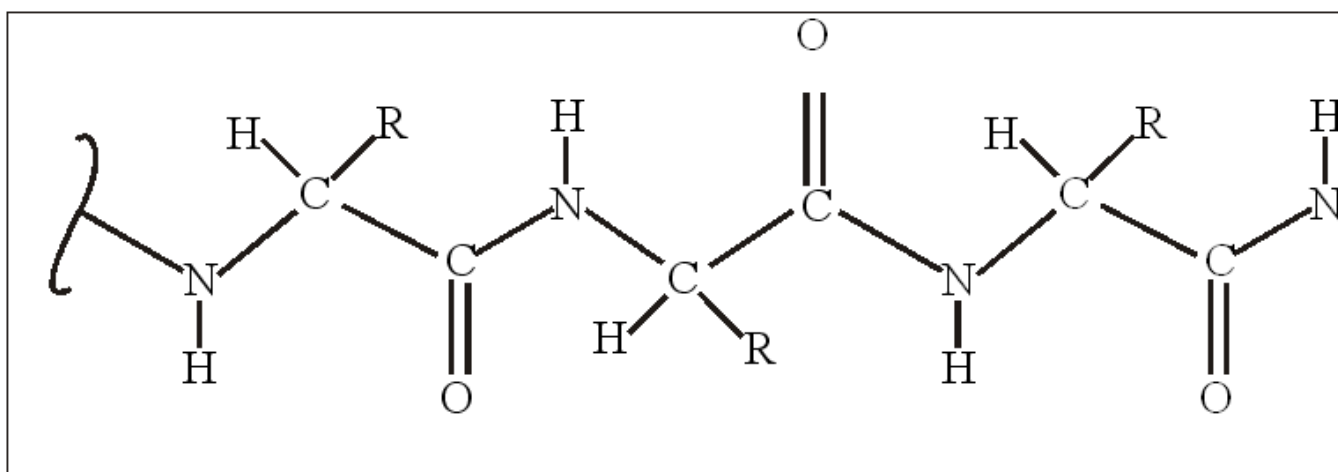
**This is just a short summary of some facts. Students who are unfamiliar with amino acids, peptides, and proteins should consult any introductory biochemistry text for more complete information.**

**There are 20 naturally occurring amino acids that are combined as linear polymers to make peptides and proteins. The distinction between peptides and proteins is not clear and depends on who you talk to. In general, peptides are short (let's say 2-30 amino acids) and proteins are longer (greater than 30 amino acids). Some people will disagree about the cutoff point. The major practical difference (and maybe a good defining property) is that peptides tend to be quite flexible in solution while proteins are much more structured.**

**Amino acids have the general structure:**



**Where R is one of the 20 amino acid side chains. Amino acids are combined through peptide bonds in a linear chain:**



**For a list of the different amino acids please refer to a textbook.**

### **$^1\text{H}$ NMR properties of peptides and amino acids**

**Kurt Wüthrich was the major person who developed the initial strategies of NMR analysis and structure determination of peptides and proteins. Any serious student should read his book. Here I will just outline a few of the main principles.**

**In a so-called "random coil" each amino acid has characteristic (not always unique!) patterns of NMR peaks. The term random coil is meant to mean a completely random (not ordered) state. Recent studies on unfolded proteins or partially folded proteins have shown that often times there is no true random coil state. However, for the purposes of NMR analysis and assignments, the random coil properties are useful and a good starting point for identification of amino acids.**

Random Coil H Chemical Shifts for the 20 common Amino Acid Residues

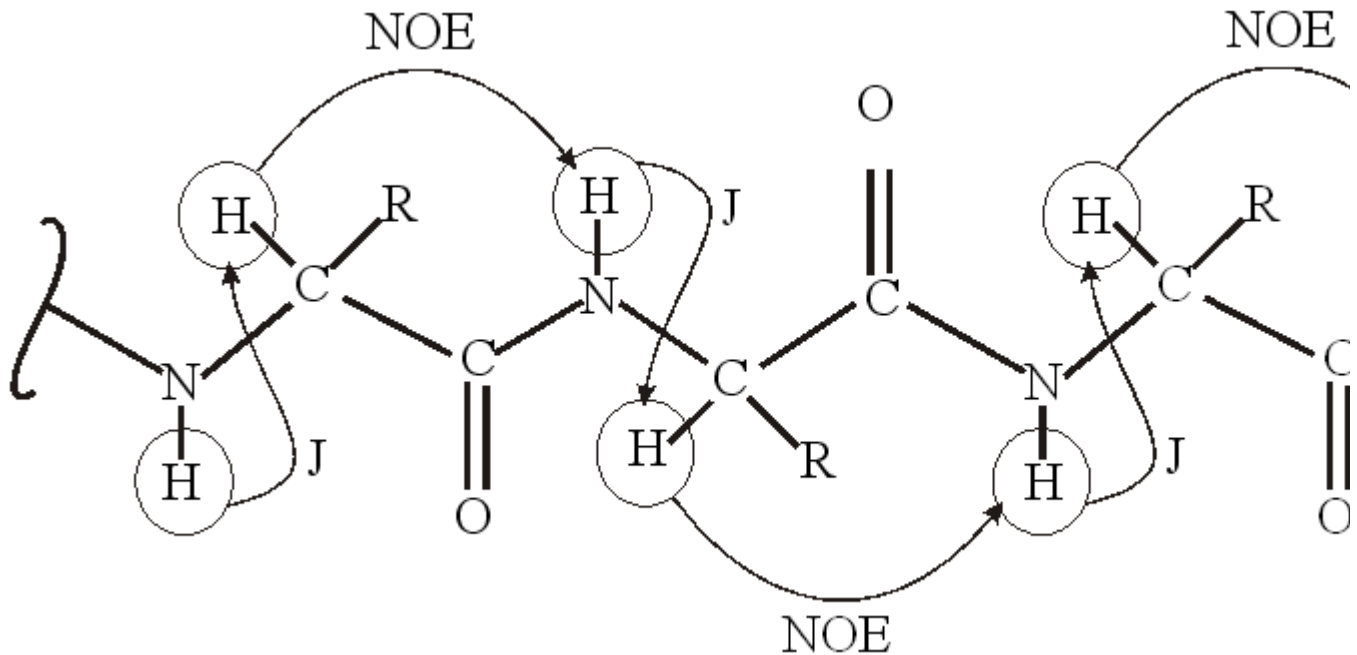
Residue	NH	alpha-H	$\beta\epsilon\alpha$ -H	Others ( $\gamma$ = gamma) ( $\delta$ = delta) ( $\epsilon$ = epsilon)
Gly	8.39	3.97		
Ala	8.25	4.35	1.39	

Val	8.44	4.18	2.13	$\gamma\text{CH}_3$ 0.97, 0.94
Ile	8.19	4.23	1.90	$\gamma\text{CH}_2$ 1.48, 1.19 $\gamma\text{CH}_3$ 0.95 $\delta\text{CH}_3$ 0.89
Leu	8.42	4.38	1.65, 1.65	$\gamma\text{H}$ 1.64 $\delta\text{CH}_3$ 0.94, 0.90
Pro		4.44	2.28, 2.02	$\gamma\text{CH}_2$ 2.03, 2.03 $\delta\text{CH}_2$ 3.68, 3.65
Ser	8.38	4.50	3.88, 3.88	
Thr	8.24	4.35	4.22	$\gamma\text{CH}_3$ 1.23
Asp	8.41	4.76	2.84, 2.75	
Glu	8.37	4.29	2.09, 1.97	$\gamma\text{CH}_2$ 2.31, 2.28
Lys	8.41	4.36	1.85, 1.76	$\gamma\text{CH}_2$ 1.45, 1.45 $\delta\text{CH}_2$ 1.70, 1.70 $\epsilon\text{CH}_2$ 3.02, 3.02 $\epsilon\text{NH}_3^+$ 7.52
Arg	8.27	4.38	1.89, 1.79	$\gamma\text{CH}_2$ 1.70, 1.70 $\delta\text{CH}_2$ 3.32, 3.32 NH 7.17, 7.17
Asn	8.75	4.75	2.83, 2.75	$\gamma\text{NH}_2$ 7.59, 6.91
Gln	8.41	4.37	2.13, 2.01	$\gamma\text{CH}_2$ 2.38, 2.38 $\delta\text{NH}_2$ 6.87, 7.59
Met	8.42	4.52	2.15, 2.01	$\gamma\text{CH}_2$ 2.64, 2.64 $\epsilon\text{CH}_3$ 2.13
Cys	8.31	4.69	3.28, 2.96	
Trp	8.09	4.70	3.32, 3.19	2H 7.24 4H 7.65 5H 7.17 6H 7.24 7H 7.50 NH 10.22
Phe	8.23	4.66	3.22, 3.19	2,6H 7.30 3,5H 7.39 4H 7.34
Tyr	8.18	4.60	3.13, 2.92	2,6H 7.15 3,5H 6.86





The schematic of the TOCSY and NOESY experiments suggests a way in which peptides and proteins can be sequentially assigned using these two experiments. Much more detail is provided by Wüthrich, but the general idea is shown below.



In the above figure, connections indicated by "J" are present in TOCSY or COSY (and often also NOESY or ROESY), but those indicated by "NOE" are only present in NOESY or ROESY).

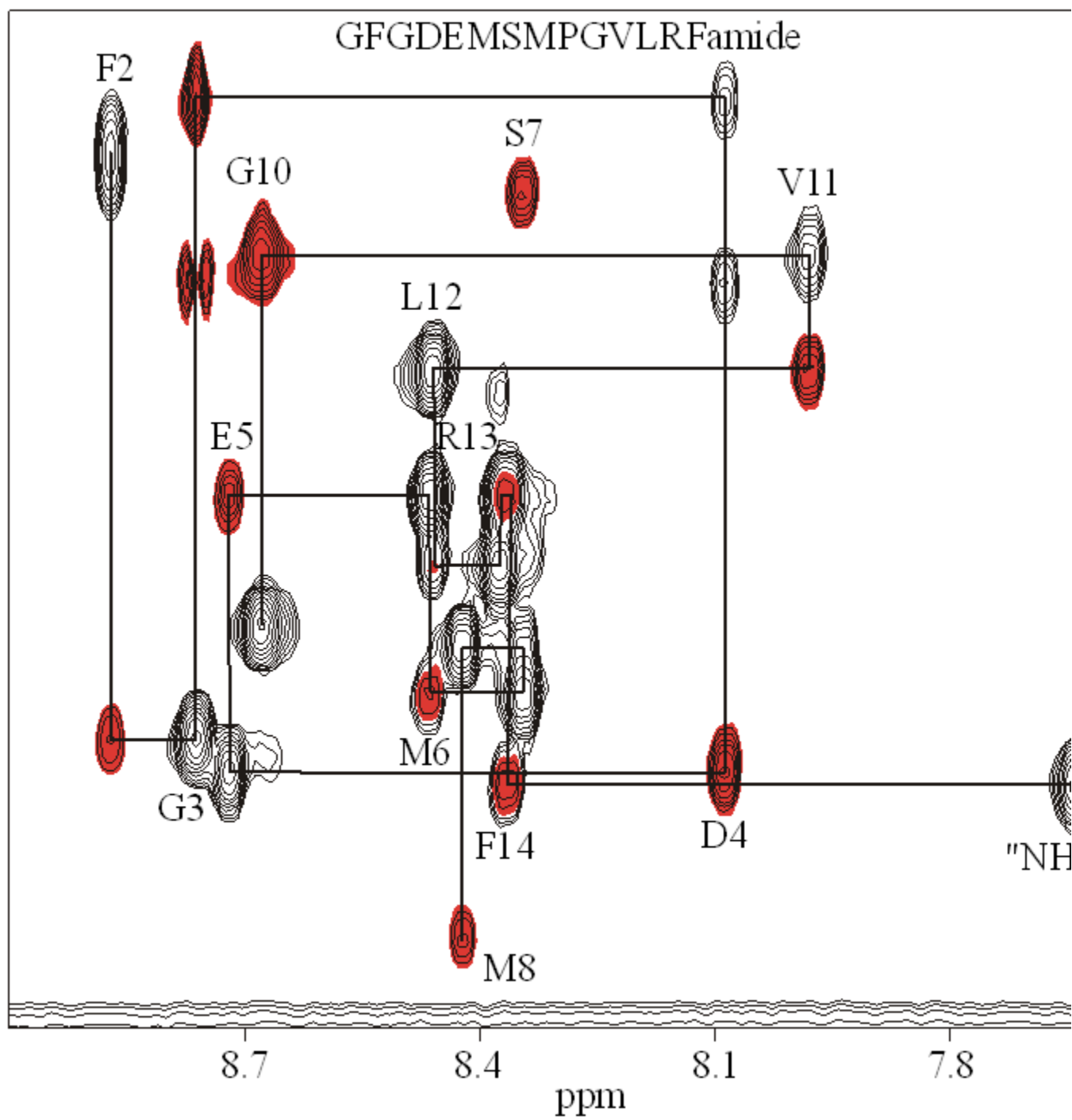
A real example of this is shown below for a 14 amino acid neuropeptide with the amino acid sequence

**Gly-Phe-Gly-Asp-Glu-Met-Ser-Met-Pro-Gly-Val-Leu-Arg-Phe-NI**

The spectrum is a ROESY, but peaks that are colored black are also in the TOCSY. Note that often times the NH peak of first amino acid in a peptide is missing due to rapid exchange with solvent. Also note that the chain that is traced out connecting the amino acids is broken by a proline. Why?



GFGDEMSPGVLRamide



## **Week 10: Relaxation and the nuclear Overhauser effect**

References: These notes were constructed from:

"Protein NMR spectroscopy: Principles and Practice" Cavanagh, Fairbrother, Palmer, and Skelton, Academic Press, (1996).

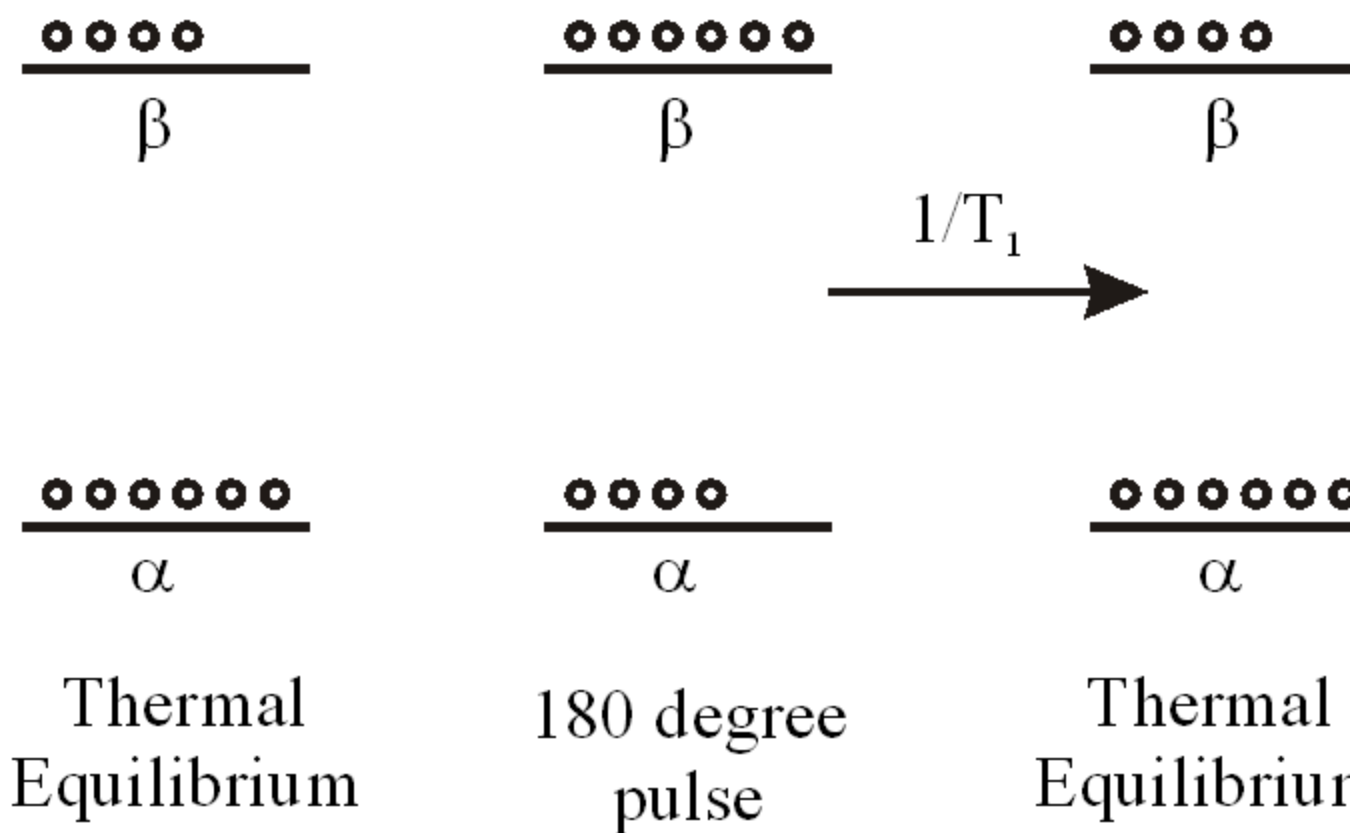
"The Nuclear Overhauser Effect in Structural and Conformational Analysis" Neuhaus and Williamson, VCH (1989).

"Biomolecular NMR Spectroscopy" Evans, Oxford University Press, (1995).

"Principles of Nuclear Magnetic Resonance in One and Two Dimensions" Ernst, Bodenhausen, and Wokaun, Oxford (1987).

### **Physical interpretation of $T_1$ and $T_2$**

Recall from the first week the relaxation terms  $T_1$  (longitudinal or spin-lattice) and  $T_2$  (transverse or spin-spin).  $T_1$  is the rate that magnetization from a single spin relaxes along the z-axis.  $T_2$  is the rate that magnetization relaxes in the x-y plane. In the next lecture we will describe in more detail the mathematical properties that influence  $T_1$  and  $T_2$ . For the present discussion,  $T_1$  is the rate of the following process:



The circles in the diagram above represent the Boltzmann population distribution of nuclei in a magnetic field. (Recall from the first homework that the true distribution is much smaller than the 6:4 shown above.)

The transition between the two states is caused by fluctuating local magnetic fields created by neighboring chemical environments. These fluctuations are a result of molecular motion. In liquids with spin  $1/2$  nuclei, the major contributors to relaxation are dipolar effects and chemical shift anisotropy (CSA). The dipolar effects, as the name implies, are results of interactions between two nuclei with magnetic dipoles. CSA is somewhat more complicated and is due to the fact that the chemical shift of a given nucleus is dependent on the orientation of that nucleus with the external field (e.g. chemical shift is a tensor). Motions of the molecule will thus cause fluctuations in the chemical shifts; certain nuclei, especially atoms in the peptide

bond of proteins, have large CSAs and thus produce larger effects.

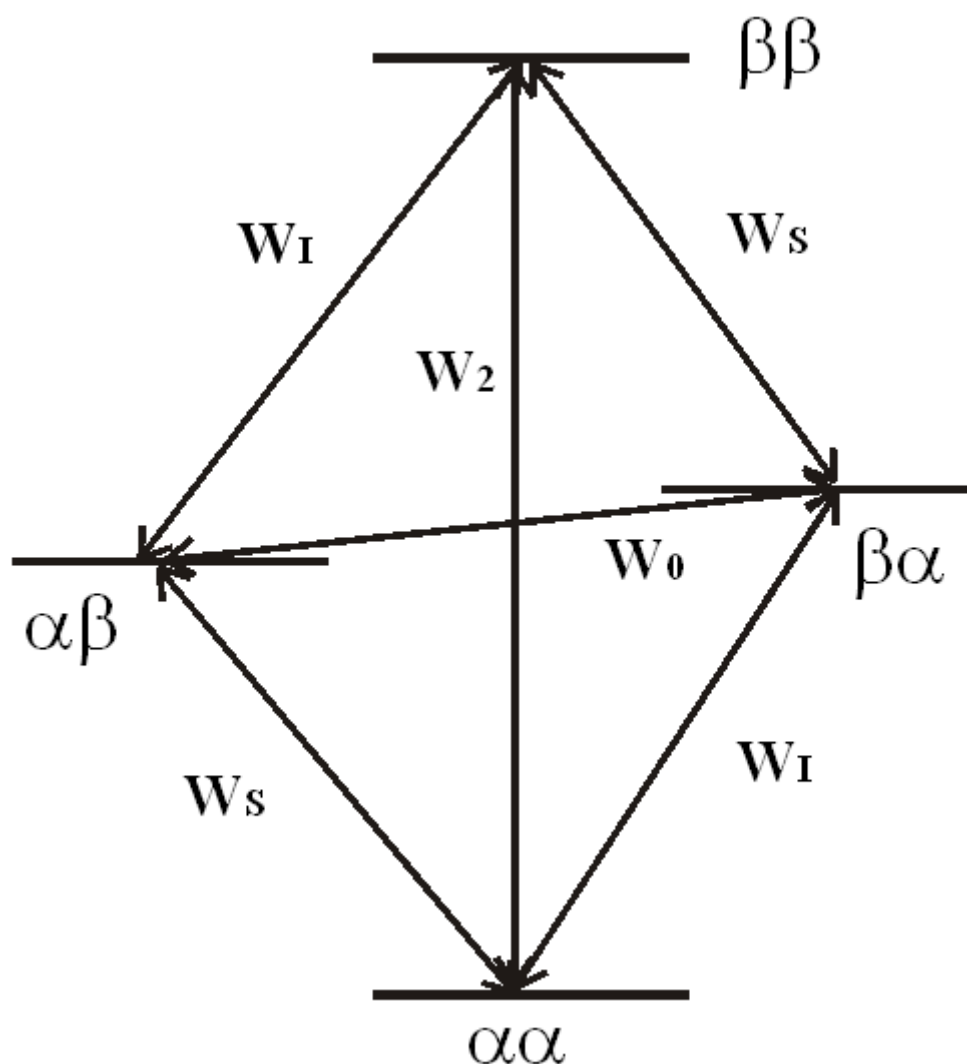
$T_2$  relaxation is a "fanning out" of transverse magnetization in the x-y plane. A good analogy for  $T_2$  is to imagine a room full of people with old-fashioned spring operated watches. At 12 noon on Monday all watches are synchronized (e.g. there is a *coherent* rf pulse that brings all the magnetization into the x-y plane). The next day the times on the watches are compared (e.g. the linewidth of a resonance is measured). Not surprisingly, it would be found that some of the watches are slow and some are fast. The distribution of the watch speeds would depend on the quality and homogeneity of the watches. With peaks in an NMR spectrum, it is found that some are broad (a big mix of times) and some are narrow (almost the same time). The major factor that influences the fanning out is size of the molecule: the larger the molecule (really the slower it moves in solution), the broader the lines.

### A Note on Coherent vs. Incoherent processes

Most of what we have described in class so far is "coherent". This includes the effects of rf pulses and scalar coupling. Relaxation, on the other hand, is incoherent. Neuhaus and Williamson describe the difference between coherent and incoherent very well: A fluctuating dipole will influence the relaxation of a neighboring nucleus and thus can be thought of as a "local pulse" delivered in a small region of a molecule. In contrast to a regular rf pulse, which would pass the magnetization through a *coherent* transverse state (e.g.  $I_z \rightarrow I_y \rightarrow -I_z$ ), a "local pulse" will only lead to a transition along the z-axis (e.g.  $-I_z \rightarrow I_z$ ). The local fluctuations are thus *incoherent*.

## NOE and T1

A single spin  $1/2$  nucleus has 2 energy levels,  $\alpha$  and  $\beta$ . A two spin (I and S) energy system can be described with four energy levels,  $\alpha\alpha$ ,  $\alpha\beta$ ,  $\beta\alpha$ , and  $\beta\beta$ . In this nomenclature, the first term refers to the I spin and the second to the S spin. The energy level diagram is often shown as:

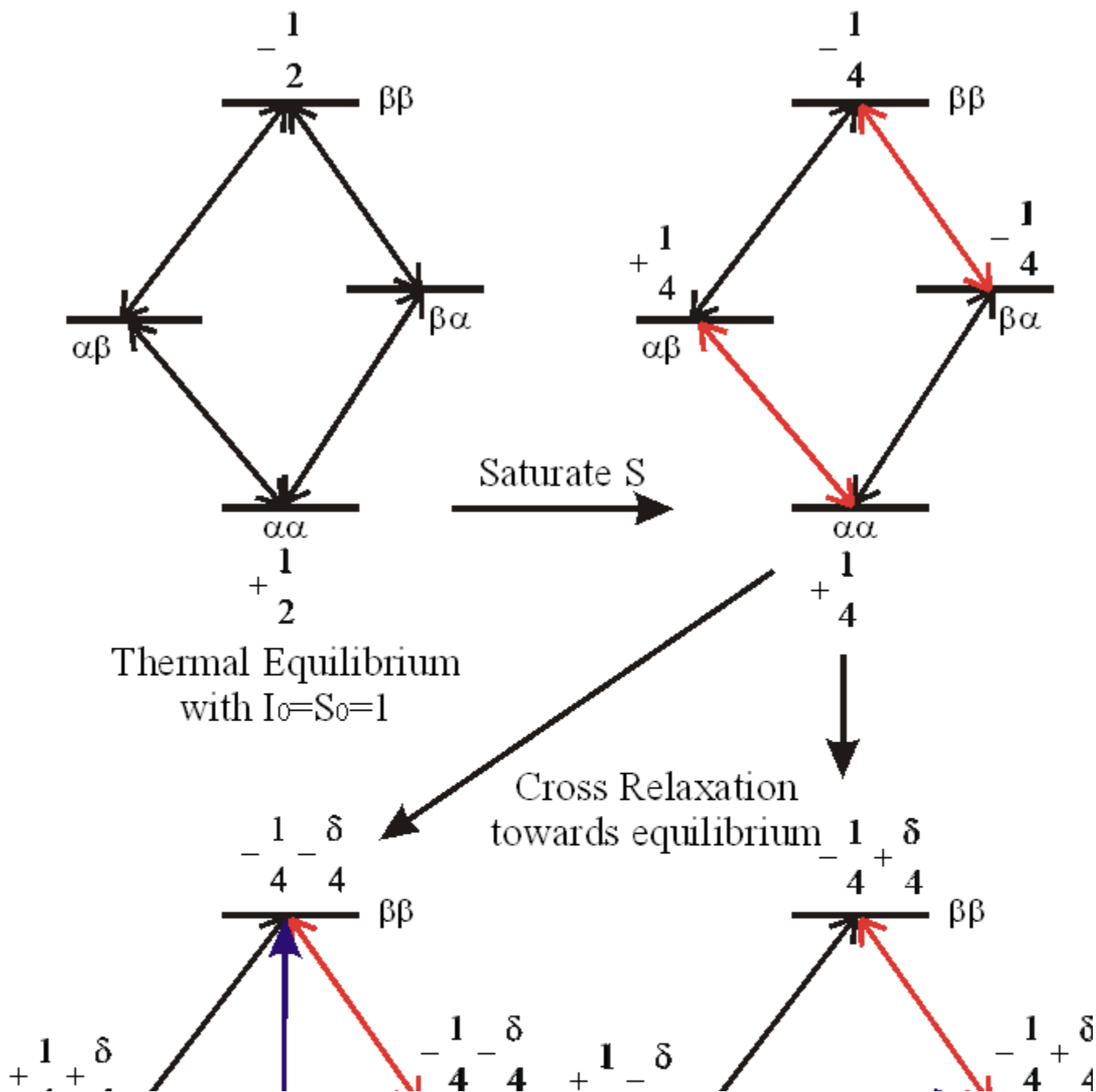


The levels each refer to the state of the system (e.g.  $\alpha\alpha$  is I in  $\alpha$  and S in  $\alpha$ ). The  $W$ 's are the transition frequencies between the levels. If there is no *cross-relaxation*, characterized by  $W_0$  and  $W_2$ , then the transition frequencies along the edge ( $W_I$  and  $W_S$ )

correspond directly to the  $T_1$  relaxation rates of I and S.

However, when  $W_0$  and  $W_2$  are not zero there the interpretation of  $T_1$  is complicated, and there can be an NOE between the two spins.

Neuhaus and Williamson give a good pictorial, description of the NOE based on the 2 spin energy level diagram:



The numbers given for each energy level diagram are populations of each state. The intensity of the I and S spins are given by:

$$I_z = P_{\alpha\alpha} + P_{\alpha\beta} - P_{\beta\alpha} - P_{\beta\beta}$$

$$S_z = P_{\alpha\alpha} + P_{\beta\alpha} - P_{\alpha\beta} - P_{\beta\beta}$$

The terms like  $P_{\alpha\alpha}$  are the populations of spins in the corresponding (e.g.  $\alpha\alpha$ ) energy level. Thus, at equilibrium in the example above,  $I^0 = S^0 = 1$  (The 0 refers to the value of the magnetization at equilibrium). When we saturate or apply a 90 degree pulse to S (indicated by the red transition), there is no net S magnetization, but I is still 1. The system is no longer at equilibrium. What will it do? Like any dynamic system, it will come to equilibrium. The route that it takes to get there depends on many factors.

When conditions favor (to be described shortly) the W2 pathway, some amount ( $\delta/4$  here) of magnetization is transferred from  $\beta\beta$  to  $\alpha\alpha$ . Since S is still saturated, the  $\alpha\beta$  and  $\beta\alpha$  levels will also adjust to keep the net magnetization on S = 0. However, I now has

$1 + \delta$  magnetization. This is a positive NOE.

When conditions favor the  $W_0$  pathway,  $\delta/4$  of magnetization is transferred across from  $\alpha\beta$  to  $\beta\alpha$  until equilibrium is reached.

Since S is still saturated,  $\beta\beta$  and  $\alpha\alpha$  adjust to make  $S = 0$ . Now the intensity of I is  $1 - \delta$ . This is a negative NOE.

Whether the NOE is positive or negative depends on the pathway. For any transition there needs to be motion that is producing a local field in the vicinity. The motion must be matched to the transition frequency.  $W_0$  is close to 0 Hz for homonuclear spins.  $W_2$ , on the other hand, is equal to the sum of  $\omega_I$  and  $\omega_S$  transitions. This frequency is dependent on the magnetic field: at 11.74 T (500 MHz), the frequency gap for two protons is 1 GHz ( $10^9$ /second). Small molecules that tumble rapidly will be able to excite these frequencies and thus show positive NOEs. Large macromolecules, on the other hand, tumble slowly and are matched more closely with  $W_0$  transitions. At intermediate molecular weights the two pathways cancel and lead to no NOE signal.



## Week 11: Spectral Density and Dynamics

References: These notes were constructed from:

Peng and Wagner, "Investigation of Protein Motions via Relaxation Measurements" *Methods in Enzymology* 239, pp 563-596 (1994).

Peng and Wagner, "Mapping of the Spectral Densities of N-H Bond Motions in Eglin c using Heteronuclear Relaxation Experiments" *Biochemistry* **31**, 8571-8586 (1992).

"Protein NMR spectroscopy: Principles and Practice" Cavanagh, Fairbrother, Palmer, and Skelton, Academic Press, (1996).

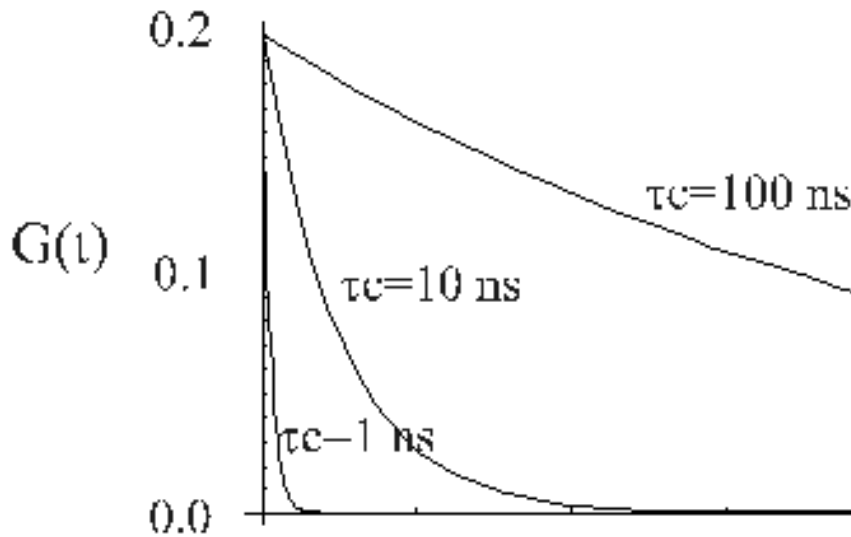
### **Autocorrelation**

Correlation is an important concept in NMR. Consider a particular covalent bond in a molecule (for example the NH bond along the backbone of proteins). The autocorrelation function describes the decay of the orientation of that vector (more properly the ensemble of those vectors in a sample) with time. The two extremes are most instructive: when there is absolutely no tumbling or internal motion over a period of time, there is no change in autocorrelation, so it is constant over that period of time. On the other hand, if the motion is so rapid that the vector no longer has a component along its starting axis, then there is no correlation over that period of time. For simple isotropic Brownian motion the autocorrelation function is simply

$$G(t) = \frac{1}{5} \exp\left(-\frac{t}{\tau_c}\right)$$

where  $\tau_c$  is the correlation time. Correlation times range from less

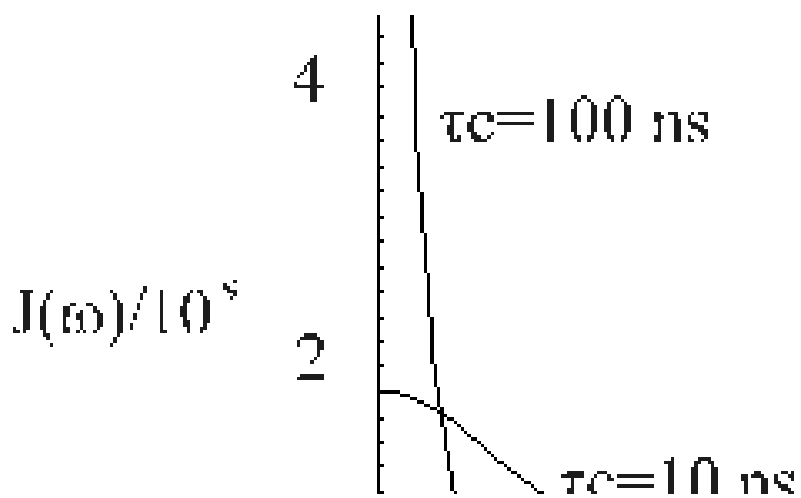
than 1 ns for small molecules to several hundred nanoseconds for large proteins. Typical small proteins (e.g. 100 amino acids) have correlation times in the tens of nanoseconds. The correlation time is strongly dependent on molecular weight but also is a function of the shape of the molecule.



The spectral density function is the Fourier transform of the autocorrelation function. For the simple case of isotropic Brownian motion shown above, the spectral density function becomes

$$J(\omega) = \frac{2}{5} \left( \frac{\tau_c}{1 + \omega^2 \tau_c^2} \right)$$

Using the same values of correlation time as the plots above, the spectral density functions are



The spectral density is the distribution of frequencies giving rise to a particular autocorrelation function. What this means in practice is that a different range of frequencies are present for different correlation times.

Recall that relaxation in NMR is dependent on perturbations at particular frequencies. Thus, by measuring relaxation rates of different spin states which are sensitive to different frequencies, the spectral density can be "mapped". Applications of spectral density mapping can be found in some of the references listed at the top.

## Answers for Homework 1

1. Calculate  $N_\alpha/N_\beta$  for the following 4 cases:

$^1\text{H}$  at 500 MHz (11.7 T)

$^1\text{H}$  at 750 MHz (17.6 T)

$^{13}\text{C}$  at 500 MHz

$^{13}\text{C}$  at 750 MHz

A 500 MHz spectrometer costs ~ \$700,000.

A 750 MHz spectrometer costs ~ \$2,000,000. Why spend the extra money?

ANSWER:

$$\frac{N_\alpha}{N_\beta} = e^{\frac{\Delta E}{k_B T}}$$

$$\Delta E_m = 1/2 \hbar \gamma B_0 - (-1/2 \hbar \gamma B_0) = \hbar \gamma B_0$$

$$\gamma (^1\text{H}) = 26.7510 \times 10^7 \text{ T}^{-1} \text{ s}^{-1}$$

$$\gamma (^{13}\text{C}) = 6.7263 \times 10^7 \text{ T}^{-1} \text{ s}^{-1}$$

$$\hbar = 1.05459 \times 10^{-34} \text{ J s}$$

$$k_B = 1.38066 \times 10^{-23} \text{ J K}^{-1}$$

$$T = 298 \text{ K}$$

Plugging it in gives:

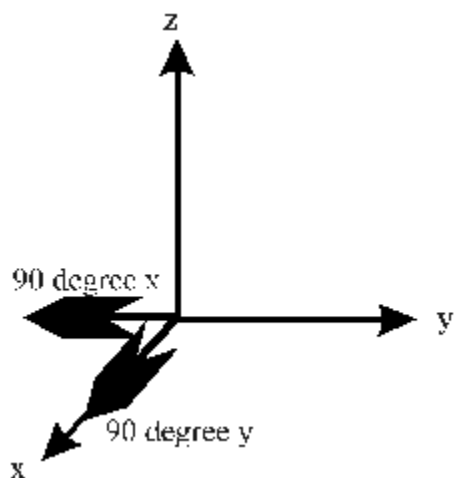
	500 MHz (11.7 T)	750 MHz (17.6 T)
$^1\text{H}$	1.0000802	1.0001206867
$^{13}\text{C}$	1.00002017	1.000030344

Because of a higher gyromagnetic ratio,  $^1\text{H}$  is more sensitive than  $^{13}\text{C}$ . This is a major reason that most protein NMR experiments start on  $^1\text{H}$  and end on  $^1\text{H}$  and visit less sensitive nuclei in the middle.

The higher magnetic field strength leads to higher polarization (more spins in the ground energy state). This translates into higher signal to noise.

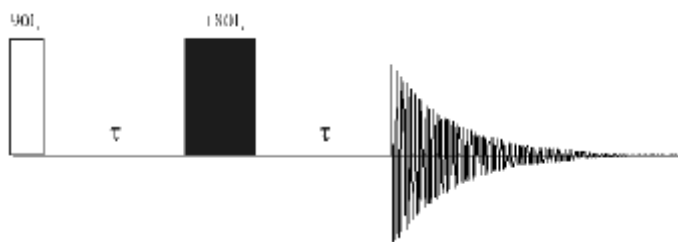
## Answers for Homework 2

1. Draw a right-handed coordinate system and, using vectors to represent magnetization, show the effects of a) 90 degree x-pulse and b) 90 degree y-pulse on z-magnetization. Next, with transverse magnetization (in the x-y plane) show the direction of a positive chemical shift (one that follows the right-hand rule).

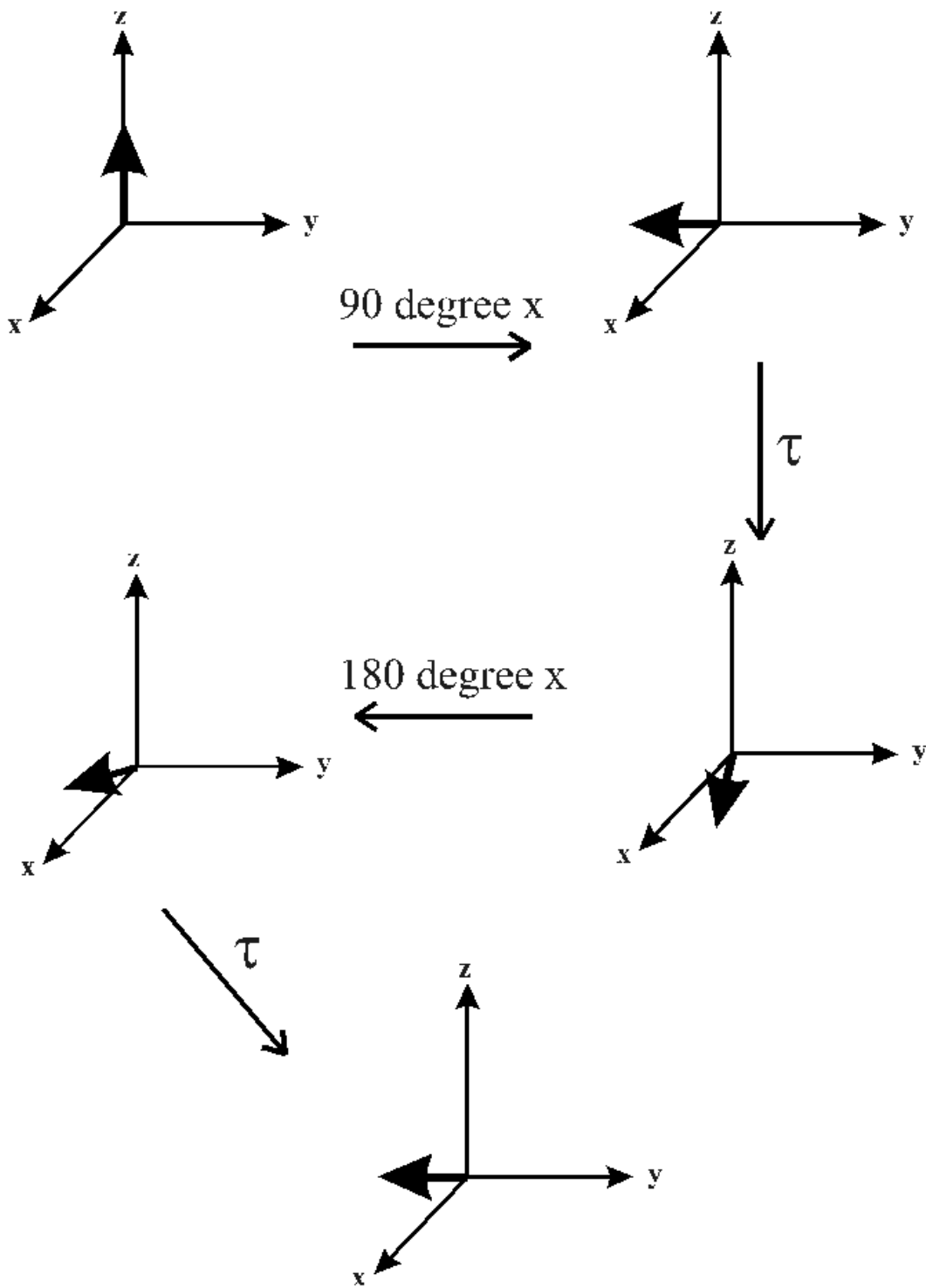


*Positive chemical shift rotation is from x to y to -x to -y in the (x,y) plane.*

2. Using both vectors and product operators, evaluate the following sequence on a single spin  $I$ :



*Vectors:*



*Product operators:*

$$\begin{aligned}
& I_z \xrightarrow{90\hat{I}_x} -I_y \xrightarrow{\Omega_I \tau \hat{I}_z} \\
& -I_y \cos \Omega_I \tau + I_x \sin \Omega_I \tau \xrightarrow{180\hat{I}_x} \\
& I_y \cos \Omega_I \tau + I_x \sin \Omega_I \tau \xrightarrow{\Omega_I \tau \hat{I}_z} \\
& (I_y \cos \Omega_I \tau + I_x \sin \Omega_I \tau) \cos \Omega_I \tau + \\
& (-I_x \cos \Omega_I \tau + I_y \sin \Omega_I \tau) \sin \Omega_I \tau \\
& = I_y \cos^2 \Omega_I \tau + I_y \sin^2 \Omega_I \tau \\
& = I_y
\end{aligned}$$

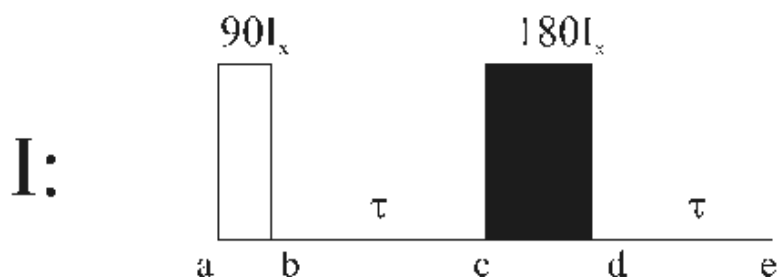
NOTE THE DIFFERENCE IN SIGN BETWEEN THE PRODUCT OPERATOR AND VECTOR PICTURE. THIS IS A RESULT OF AN EARLY DECISION TO CHANGE THE SIGN OF  $\omega_0 = \gamma B_0$ .



### Answers for Homework 3

Using product operators and two coupled (e.g.  $J_{IS} \neq 0$ ) spins **I** and **S**, evaluate the following sequences:

1)



**S:**

---

Noting the results from homework 2 in which the chemical shift of I was refocused with a symmetric 180 degree pulse, we will neglect chemical shift:

$$a \rightarrow b: I_z \xrightarrow{90\hat{I}_x} -I_y$$

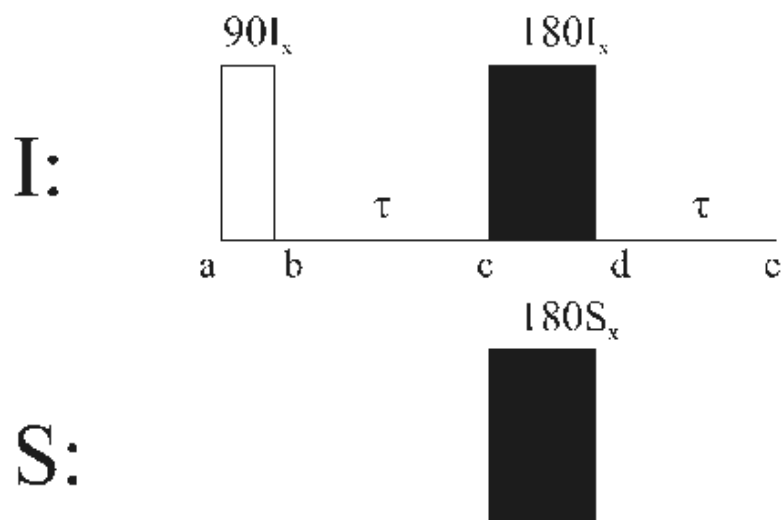
$$b \rightarrow c: -I_y \xrightarrow{\pi J_{IS} \tau 2\hat{I}_z \hat{S}_z} -I_y \cos(\pi J_{IS} \tau) + 2I_x S_z \sin(\pi J_{IS} \tau)$$

$$c \rightarrow d: -I_y \cos(\pi J_{IS} \tau) + 2I_x S_z \sin(\pi J_{IS} \tau) \xrightarrow{180\hat{I}_x} \\ I_y \cos(\pi J_{IS} \tau) + 2I_x S_z \sin(\pi J_{IS} \tau)$$

$$d \rightarrow e: [I_y \cos(\pi J_{IS} \tau) + 2I_x S_z \sin(\pi J_{IS} \tau)] \cos(\pi J_{IS} \tau) \\ + [-2I_x S_z \cos(\pi J_{IS} \tau) + I_y \sin(\pi J_{IS} \tau)] \sin(\pi J_{IS} \tau) \\ = I_y$$

Thus, the results of 1) is to decouple I from S and refocus the chemical shift of I.

2)



$$a \rightarrow b: I_z \xrightarrow{90\hat{I}_x} -I_y$$

$$b \rightarrow c: -I_y \xrightarrow{\pi J_{IS} \tau 2\hat{I}_z \hat{S}_z} -I_y \cos(\pi J_{IS} \tau) + 2I_x S_z \sin(\pi J_{IS} \tau)$$

$$c \rightarrow d: -I_y \cos(\pi J_{IS} \tau) + 2I_x S_z \sin(\pi J_{IS} \tau) \xrightarrow{180\hat{I}_x + 180\hat{S}_x} I_y \cos(\pi J_{IS} \tau) - 2I_x S_z \sin(\pi J_{IS} \tau)$$

$$d \rightarrow e: [I_y \cos(\pi J_{IS} \tau) - 2I_x S_z \sin(\pi J_{IS} \tau)] \cos(\pi J_{IS} \tau) \\ + [-2I_x S_z \cos(\pi J_{IS} \tau) - I_y \sin(\pi J_{IS} \tau)] \sin(\pi J_{IS} \tau)$$

$$= I_y [\cos^2(\pi J_{IS} \tau) - \sin^2(\pi J_{IS} \tau)] - 4I_x S_z \sin(\pi J_{IS} \tau) \cos(\pi J_{IS} \tau)$$

$$= I_y \cos(2\pi J_{IS} \tau) - 2I_x S_z \sin(2\pi J_{IS} \tau)$$

The last result is from the following two trig relations:

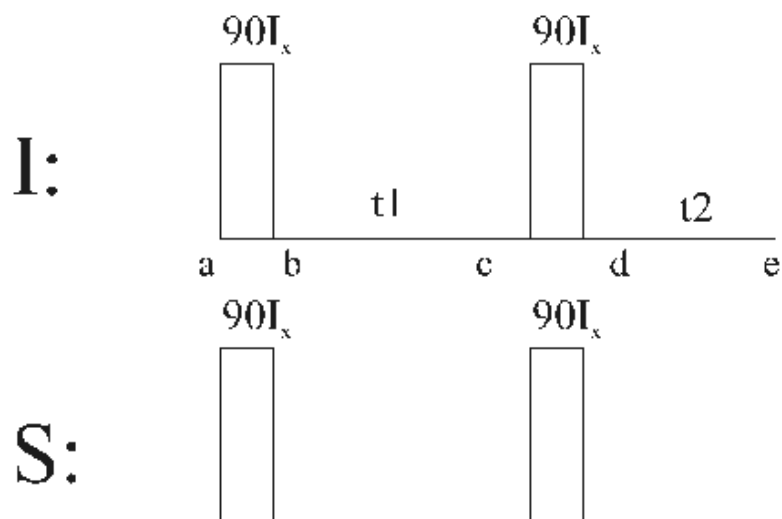
22  
S

1  
S

**Also note that when  $\tau=1/4J$ , the result of 2) is to create an antiphase term  $I_xS_z$ .**

## Answers for Homework 4

Using product operators and two coupled (e.g.  $J_{IS} \neq 0$ ) spins **I** and **S**, evaluate the following sequence:



Inspection of the pulse sequence suggests that we should get a symmetric spectrum with respect to I and S. To simplify the number of terms, we will first just start with the I term alone:

$$\begin{aligned}
a \rightarrow b: & I_z \xrightarrow{90\hat{I}_x} -I_y \\
\rightarrow c: & \xrightarrow{\Omega_I t_1 \hat{I}_z} -I_y \cos \Omega_I t_1 + I_x \sin \Omega_I t_1 \\
& \xrightarrow{\pi J t_1 2 \hat{I}_z \hat{S}_z} [-I_y \cos \Omega_I t_1 + I_x \sin \Omega_I t_1] \cos(\pi J t_1) \\
& + [2I_x S_z \cos \Omega_I t_1 + 2I_y S_z \sin \Omega_I t_1] \sin(\pi J t_1) \\
\rightarrow d: & \xrightarrow{90\hat{I}_x} \xrightarrow{90\hat{S}_x} [-I_z \cos \Omega_I t_1 + I_x \sin \Omega_I t_1] \cos(\pi J t_1) \\
& + [-2I_x S_y \cos \Omega_I t_1 - 2I_z S_y \sin \Omega_I t_1] \sin(\pi J t_1)
\end{aligned}$$

We can now make another simplification by dropping longitudinal (e.g.  $I_z$ ) and multiple quantum (e.g.  $I_x S_y$ ) terms that will never become observable without another pulse (which we don't have):

observables at d:

$$\begin{aligned}
& I_x \sin(\Omega_I t_1) \cos(\pi J t_1) - 2I_z S_y \sin(\Omega_I t_1) \sin(\pi J t_1) \\
\rightarrow e: & \xrightarrow{\pi J t_2 2 \hat{I}_z \hat{S}_z} \\
& [I_x \sin(\Omega_I t_1) \cos(\pi J t_1) - 2I_z S_y \sin(\Omega_I t_1) \sin(\pi J t_1)] \cos(\pi J t_2) \\
& + [2I_y S_z \sin(\Omega_I t_1) \cos(\pi J t_1) + S_x \sin(\Omega_I t_1) \sin(\pi J t_1)] \sin(\pi J t_2)
\end{aligned}$$

We can now make YET another simplification by dropping all antiphase (e.g.  $I_z S_y$ ) terms that will never become observable without another precession period (which we don't have):

observables at e:

$$I_x \sin(\Omega_I t_1) \cos(\pi J t_1) \cos(\pi J t_2) + S_x \sin(\Omega_I t_1) \sin(\pi J t_1) \sin(\pi J t_2)$$

$$\xrightarrow{\Omega_I t_2 \hat{I}_z} \xrightarrow{\Omega_S t_2 \hat{S}_z}$$

$$I_x \sin(\Omega_I t_1) \cos(\pi J t_1) \cos(\pi J t_2) \cos(\Omega_I t_2)$$

$$+ I_y \sin(\Omega_I t_1) \cos(\pi J t_1) \cos(\pi J t_2) \sin(\Omega_I t_2)$$

$$+ S_x \sin(\Omega_I t_1) \sin(\pi J t_1) \sin(\pi J t_2) \cos(\Omega_S t_2)$$

$$+ S_y \sin(\Omega_I t_1) \sin(\pi J t_1) \sin(\pi J t_2) \sin(\Omega_S t_2)$$

Now, we need to think about this answer for just a minute (and remember that it is only 1/2 of a symmetric pair). Notice for the top two terms with  $I_x$  and  $I_y$  that they are modulated by the chemical shift of  $I$  in both  $t_1$  and  $t_2$ . This is a diagonal peak. On the other hand, the terms involving  $S_x$  and  $S_y$  are modulated by the chemical shift of  $I$  in  $t_1$  and  $S$  in  $t_2$ . These are cross peaks. Finally, notice the coupling terms multiplying the diagonal and cross peaks: for the diagonals it is  $\cos(\pi J t)$  and for the cross peaks it is  $\sin(\pi J t)$ . The Fourier transform of a cosine of a coupling gives two peaks pointing in the same direction; these are called in-phase. The FT of a sine of a coupling gives two peaks that are opposite in sign and are called anti-phase. This is seen more clearly from the following relationship:

$$\sin(\Omega_I t) \cos(\pi J t) = \frac{1}{2} [\sin(\Omega_I t - \pi J t) + \sin(\Omega_I t + \pi J t)]$$

$$\sin(\Omega_I t) \sin(\pi J t) = \frac{1}{2} [\cos(\Omega_I t - \pi J t) - \cos(\Omega_I t + \pi J t)]$$

Note that both of these relations have peaks at the center frequency



(I) + or – the coupling. The 2nd term with  $\sin(\pi Jt)$  has two peaks with different signs.

The other symmetric part of the spectrum is easily obtained by replacing S for I (along with the appropriate chemical shift changes).