11. TWO-DIMENSIONAL NMR

11.1 Exchange Spectroscopy (EXSY)

The basic structure of all two dimensional spectra can be divided into a sequence of four periods. The **preparation period** is used to place the spin system into the desired starting conditions. The starting condition may range from a relaxed spin system at Boltzmann equilibrium to a multiple quantum state or spin order. This period is followed by the **evolution period**, t_1, during which the spins evolve at their characteristic frequencies. This is the period that is incremented to generate the indirectly detected time domain. Since the t_1 time domain is indirectly detected, the restrictions on the "observability" of the coherences that evolve during this time are lifted. The t_1 period can witness the evolution of single quantum coherence as well as multiple quantum coherence, which is not directly observable. The next period in a two dimensional NMR experiment is the **mixing period**. The transfer of information between spins is carried out during this period. This construction of this period is very diverse. It can range from being absent to containing very complicated sequences of RF pulses and delays. The mixing period is the link between the evolution period and the **detection period**. The spectrometer's receiver is turned on during the detection period and the FID is detected and stored in the computer memory.

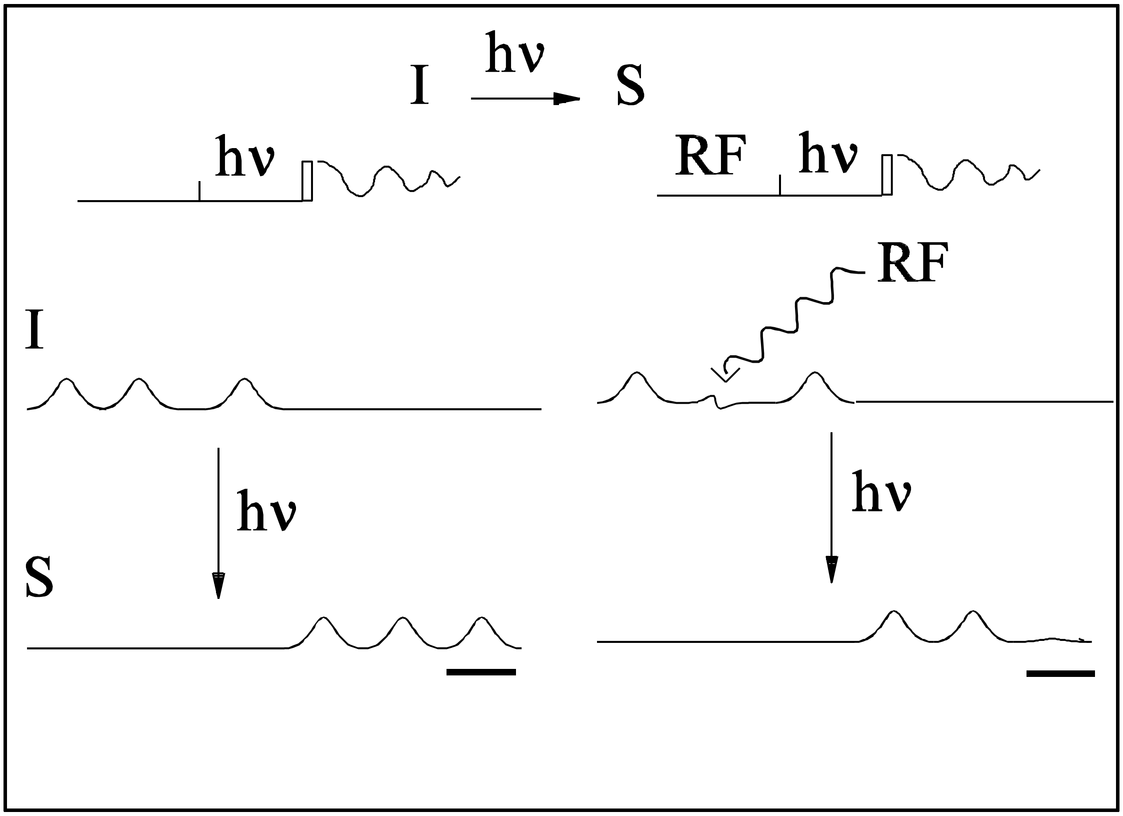


Figure 11.1.1. One-dimensional saturation transfer experiment demonstrating the transfer of "labels" from one molecule, **I,** to another, **S**.

As a paradigm of a 2D experiment, I will describe a somewhat contrived system for the study of chemical exchange. Chemical exchange is especially convenient for the explanation of the basics of two dimensional spectroscopy because the mechanism of (incoherent) magnetization transfer involves the simple physical movement of a nucleus from one chemical site to another. Since the transfer mechanism is classical in nature, the experiment can be described in the framework of classical physics. This simple experiment will be used to exemplify many general features of the 2D NMR experiment.

Consider a rapid, irreversible reaction, **I** –> **S**, which is induced by light. The protons on molecule **I** are transformed into protons on molecule **S** upon illumination of the sample with light. We would like to correlate the chemical shifts of the protons on molecule **I** with those on molecule **S**. Again for simplicity, the frequencies of the protons of **I** and of **S** are different and illumination of molecule **I** rapidly produces only molecule **S**. Assume that only **I** is present at the beginning of the experiment.

A simple approach is to "label" each individual proton of **I**, illuminate the sample, and then record the 1D spectrum of the resulting mixture. The "label" attached to the **I** proton is transferred to the corresponding proton in molecule **S**. A simple way to "label" an individual nucleus is to apply RF power at the frequency of the resonance in order to saturate the transition. A saturated resonance does not give rise to a peak in the spectrum and if an **I** proton is saturated followed by the transformation into a **S** proton by application of light, then the corresponding proton in **S** will be saturated and will not be observed in the spectrum (Figure 11.1.1). By systematically saturating each proton in a fresh sample of molecule **I**, followed by illumination, and then observation of the spectrum of the resulting mixture, each of the protons in molecule **I** could be correlated with the corresponding proton in molecule **S**. In general, this experiment consists of a preparation period (selective frequency saturation), a transfer or mixing period (illumination), followed by a detection period. A plot of the selective frequencies in **I** versus the corresponding transferred frequencies in **S** constitutes a two-dimensional map of the connectivities for the protons in **I** to the protons in **S**. If the detected spectrum is recorded as a pulsed FT spectrum then this experiment is collected as frequency (of selective saturation) versus time, $S(f_1,t_2)$, with f1 being the frequency of saturation and t_2 being the detected free induction decay (FID). A Fourier transform on each individual experiment collected with a different selective frequency then generates

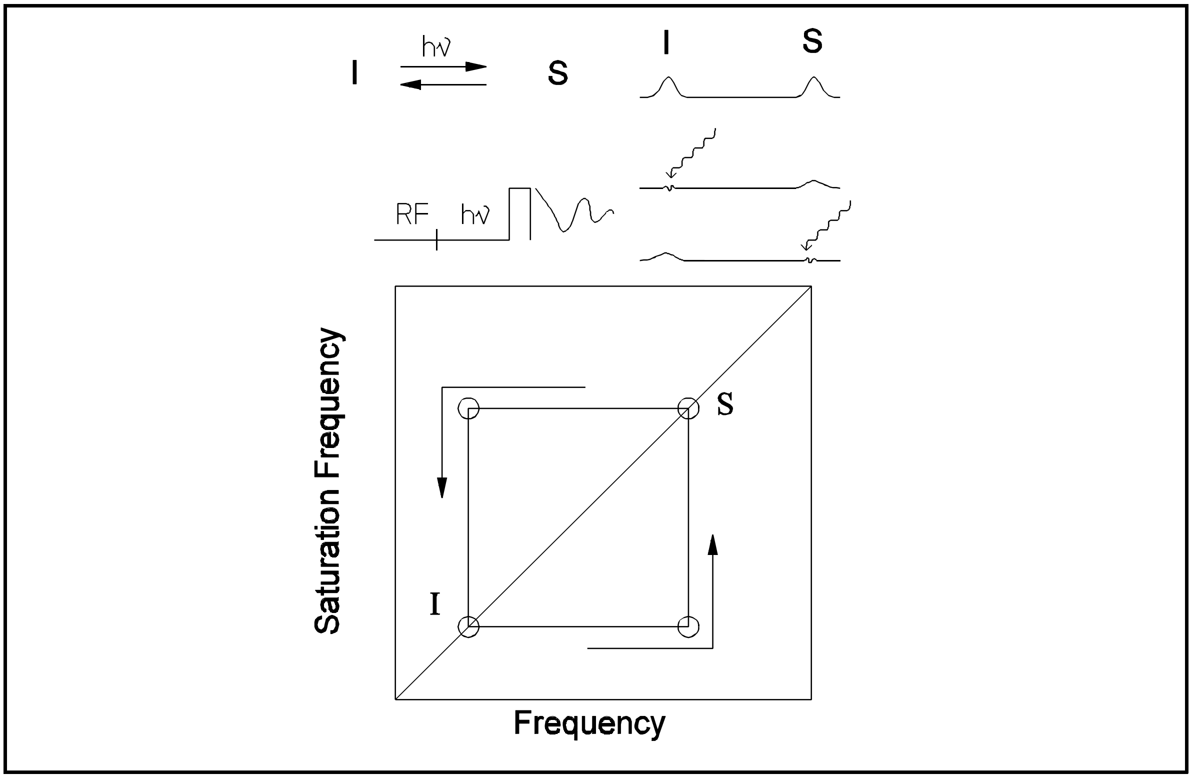


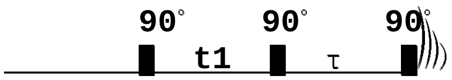
Figure 11.1.2. Two dimensional representation of a saturation transfer experiment.

the final frequency versus frequency, $S(f_1,f_2)$ spectrum (Figure 11.1.2).

This 2D spectrum could be collected in a mindless fashion by systematically moving the saturation frequency by a small, fixed amount across the width of the spectrum. At frequencies not resonant with any **I** protons, no effect would be seen on the spectrum, but at positions where the saturating frequency is resonant with an **I** spin then both the **I** spin and corresponding **S** spin will be saturated and be absent from the spectrum. Ideally this experiment would suffice for obtaining the correlations between **I** and **S** protons; in the real world, the saturating frequency source has a finite bandwidth and a range of frequencies are excited when it is applied. For peaks that are close together in frequency, the saturating field will excite both spins and make the correlation to the **S** spins ambiguous.

11.2 Time-Time Two-Dimensional Spectroscopy

The Fourier transform defines a relationship between time and frequency. This relationship suggests that an experiment that is collected as a function of frequency can be substituted with an equivalent experiment collected as a function of time. An experiment equivalent to the saturation transfer experiment described can be collected as a time versus time, $S(t_1,t_2)$, two dimensional spectrum. The details of the transfer are not exactly the same for the frequency-time and the time-time experiments but the results are essentially the same. In the frequency-time experiment, saturated magnetization is transferred, whereas in a time-time experiment the selective frequency information is passed as a time dependent difference magnetization. The pulse sequence for the two dimensional EXSY experiment is shown in Eqn. 11.1.1



(11.1.1)

The preparation period for this experiment consists of allowing the spin system to come to thermal equilibrium in the static magnetic field followed by a 90°, which generates transverse magnetization. The preparation period is followed by the evolution delay t_1, then two 90° pulses separated by the mixing time, τ_m, and a final acquisition time, t_2.

The first 90° pulse tips the protons of **I** onto the -Y axis of the transverse plane, starting the evolution period (Figure 11.1.4). In terms of product operators, this is described by

$$\mathbf{I}_z \; =\pi/2\hat{\mathbf{I}}_x => -\mathbf{I}_y. \qquad (11.1.2)$$

Here **I** represents a proton spin in molecule **I**. If the receiver were turned on at this time the spectrum of **I** would be recorded; however, instead of detecting the signal during t_1, a series of spectra is recorded with t_1 values that differ by a dwell time increment. The dwell time (DW) is related to the frequency range or sweep width (SW) that is to be covered by DW= 1/(2*SW). During each of these t_1 periods, the protons in **I** precess at their characteristic frequency, ω_I. During a given t_1 period, a particular proton will rotate in the transverse plane through an angle, $\omega_I t_1$ (Eqn. 11.1.3).

$$-\mathbf{I}_y \ =\omega_I t_1 \hat{\mathbf{I}}_z=> -\mathbf{I}_y \cos(\omega_I t_1) + \mathbf{I}_x \sin(\omega_I t_1) \qquad (11.1.3)$$

The second 90° pulse along the X axis begins the mixing period by turning all of the Y components of the spin vectors into the Z axis (Eqn 11.1.4).

$$-\mathbf{I}_y \cos(\omega_I t_1) + \mathbf{I}_x \sin(\omega_I t_1) =\pi/2\hat{\mathbf{I}}_x=> -\mathbf{I}_z \cos(\omega_I t_1) +\mathbf{I}_x \sin(\omega_I t_1) \qquad (11.1.4)$$

The amount of Y component to be turned into the Z axis is directly related to the precession frequency (chemical shift). A proton that is on resonance with the carrier frequency does not precess in the rotating frame ($\omega = 0$). Irrespective of the value of t_1, the vector will remain along the -Y axis. The second 90° pulse along the X axis will turn the vector into the -Z axis. For a proton that is not on resonance, the vector precesses at a frequency, $\omega_I t_1$, in the rotating frame. At a value of $t_1 = 0$, the vector will lie along the -Y axis and will be turned along the -Z axis by the second 90° x pulse. As the value of t_1 is incremented by the dwell time, the vector will precess in the XY plane by an

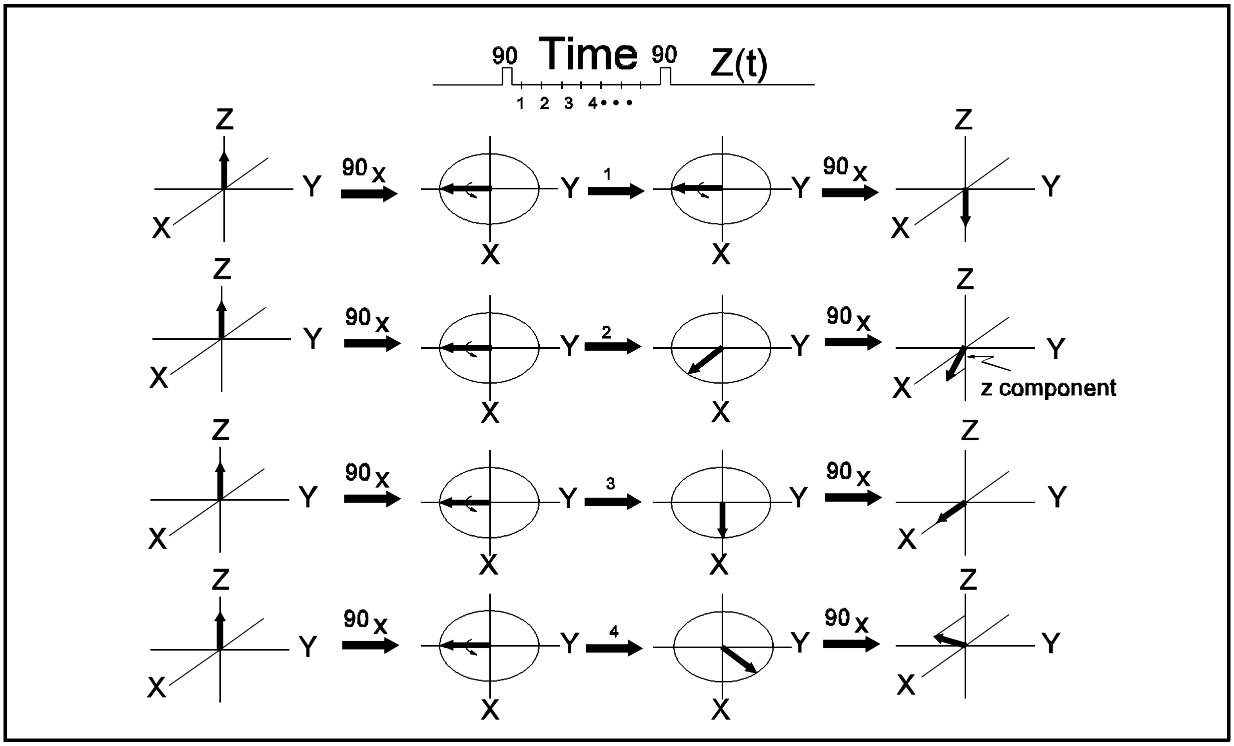


Figure 11.1.4. Schematic representation of frequency labeling of Z magnetization by chemical shift.

angle $\omega_I t_1$. The component of the vector along the -Y axis is given by $\cos(\omega_I t_1)$. At $t_1 = 1/(4*\omega_I/2\pi)$, a vector precesses to lie along the X axis and the second 90°x pulse does not effect it. A plot of the Z magnetization after the second 90°x pulse versus t_1 be the function $\cos(\omega_I t_1)$ (Figure 11.1.5). A Fourier transform of this time dependent function gives the frequencies, ω_I, of the protons in molecule **I**.

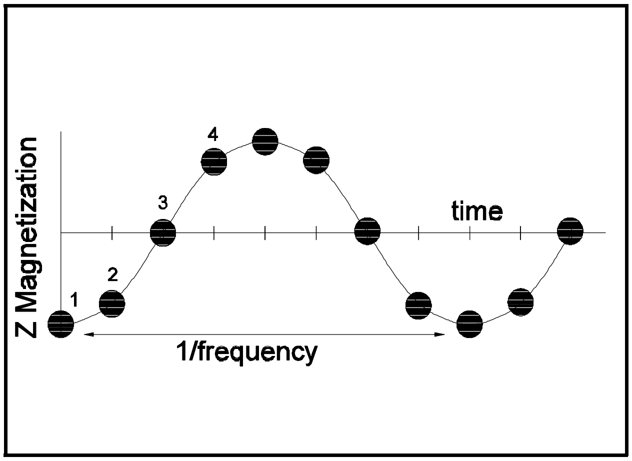


Figure 11.1.5. Plot of the chemical shift modulated Z magnetization of Figure 11.1.4.

During the delay τ_m, the light is turned on to allow the reaction to proceed. An amount λ of **I** is converted to **S** (Eqn. 11.1.5).

$$-\mathbf{I}_z \cos(\omega_I t_1) + \mathbf{I}_x \sin(\omega_I t_1) \;\; {=}h\nu{\Rightarrow} \;\; (1-\lambda)*[-\mathbf{I}_z \cos(\omega_I t_1) + \mathbf{I}_x \sin(\omega_I t_1)] + \lambda[-\mathbf{S}_z \cos(\omega_I t_1) + \mathbf{S}_x \sin(\omega_I t_1)) \qquad (11.1.5)$$

The values of the Z magnetization for the protons of **I** that were established during the 90° - t_1 - 90° period do not change during the application of light and thus the Z information is transferred from protons of **I** molecules to those of **S** molecules. The protons on **S** now have components along the Z axis that are a function of the frequency of the protons on **I**. Also during τ_m, the X components of **I** protons that were not turned into the Z axis by the second 90° pulse precess and are converted into **S** proton transverse magnetization. These components are not desired and can be eliminated by phase cycling. We will defer the details of phase cycling to a later section. The elimination of the transverse components, in effect, leaves only Z magnetization at the end of τ_m (Eqn. 11.1.6).

$$(1-\lambda)*[-\mathbf{I}_z \cos(\omega_I t_1)] + \lambda[-\mathbf{S}_z \cos(\omega_I t_1)] \qquad (11.1.6)$$

The final 90° pulse applied to both **I** and **S** spins, turns the Z magnetization along the Y axis and the FID is collected during the detection period (Eqn. 11.1.7).

$$(1-\lambda)*[-\mathbf{I}_z \cos(\omega_I t_1)] + \lambda[-\mathbf{S}_z \cos(\omega_I t_1)] \;\; {=}\pi/2(\hat{\mathbf{I}}_x+\hat{\mathbf{S}}_x){\Rightarrow} \;\; (1-\lambda)*[\mathbf{I}_y \cos(\omega_I t_1)] + \lambda[\mathbf{S}_y \cos(\omega_I t_1)] \qquad (11.1.7)$$

The Y magnetization in the FID arises from two sources: 1). the protons on **I** molecules that did not react during τ_m and 2). the protons on **S** that were created during τ_m. Both types of protons retain the information about **I** proton frequencies that was stored as an amplitude modulation of the Z magnetization, $\cos(\omega_I t_1)$. During t_2, **I** and **S** protons precess at their characteristic frequencies, ω_I and ω_S (Eqn. 11.1.8).

$$\begin{aligned}
&(1-\lambda)\,[\mathbf{I}_y \cos(\omega_I t_1)] \\
&\qquad + \lambda[\mathbf{S}_y \cos(\omega_I t_1)] \;\; =\omega_I t_2 \hat{\mathbf{I}}_z + \omega_S t_2 \hat{\mathbf{S}}_z => (1-\lambda)\cos(\omega_I t_1) \\
&\qquad\qquad\qquad\qquad\qquad\qquad * [\mathbf{I}_y \cos(\omega_I t_2) - \mathbf{I}_x \sin(\omega_I t_2)] \\
&\qquad\qquad\qquad\qquad\qquad\qquad\quad + \lambda \cos(\omega_I t_1) \\
&\qquad\qquad\qquad\qquad\qquad\qquad\quad * [\mathbf{S}_y \cos(\omega_S t_2) - \mathbf{S}_x \sin(\omega_S t_2)] \qquad (11.1.8)
\end{aligned}$$

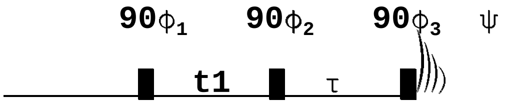
By collecting a series of experiments with an incrementation of t_1 by the dwell time, a two dimensional time-time spectrum, $S(t_1,t_2)$, is built up. A two-dimensional Fourier transform extracts the frequencies from the time dependent signal to generate a final spectrum $S(f_1,f_2)$.

In 2D spectra, all peaks have two frequency coordinates corresponding to the frequency f_1 that the spin experiences during the first time period t_1 and the frequency f_2, which it has during the detection period t_2. In our example, an **I** spin in t_1 that is transformed into a **S** spin during t_2 will have the frequency coordinates, (ω_I, ω_S). This type of peak is known as a **cross peak**. An **I** spin that remains a **I** spin during the experiment will have the same frequency coordinates in both dimensions (ω_I, ω_I). This type of peak is known as a **diagonal peak**. In the simple experiment described here, the peaks that are observed are the diagonal peaks of **I** representing material that does not react during illumination and a cross peak at (ω_I,ω_S) that represents molecules of **I** that were transformed into **S** upon illumination. In a situation where the exchange proceeds in both directions simultaneously, **I**-->**S** and **S**-->**I**, a cross peak would also be observed at the symmetric coordinates (ω_S,ω_I) and a diagonal peak would appear at (ω_S,ω_S).

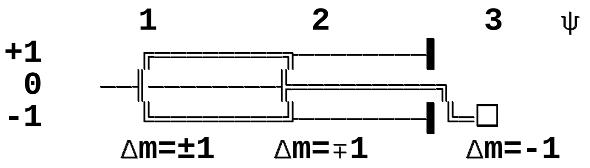
Another type of peak observed in 2D nmr spectra arises from magnetization that relaxes during the evolution period. **Axial peaks** are found at zero frequency along f_1, $(0,\omega_I)$ and $(0,\omega_S)$. The relaxation of the magnetization during evolution produces Z magnetization without any frequency labeling. These components have not precessed during the t_1 period and therefore appear to have zero frequency. These peaks can be removed by phase cycling.

11.3 EXchange SpectroscopY (EXSY) Phase Cycling:

The EXSY experiment consists of three 90° pulses; the first two pulses are separated by the t_1 frequency labeling period and the second and third pulses are separated by the mixing time during which magnetization is exchanged among the spins.



From knowledge of the coherence order at each step of the pulse sequence a coherence transfer pathway can be constructed.



The first 90° pulse excites equilibrium magnetization to coherence order ±1. From thermal equilibrium this is the only possibility with a single pulse. Of course some magnetization may remain along the Z axis due to pulse misset or imperfections. The change in coherence order is the parameter that is important in designing a phase cycling routine, for pulse 1: Δm = ±1.

Following the procedure of Section 10.6, for pulse 1 we list and select the desired coherence order changes.

ϕ_1**:** (**-1** 0) +1.

The -1 value was chosen as the desired value (+1 could have been chosen with the same results). The number of members is:

N=2.

Computing the phases for pulse 1 from Eqn. 10.6.1:

$\phi_1 = 0,\pi$

The role of the second pulse is to generate Z magnetization from the precessing transverse magnetization. Any remaining transverse magnetization after this pulse is undesirable. The phase cycle is designed to only pass zero order coherence, such as Z, during the mixing period. The coherence transfer pathway is drawn such that the ±1 coherences are transferred to the 0 level. Note that since the magnetization is not at thermal equilibrium, the ±1 selection rules are dropped. This is not important in the isolated spin system described here, but in a spin system with **n** coupled spins the coherence levels **+n .. -n** can become populated. These higher order coherences must be considered in the overall phase cycle.

Following the same procedure as for pulse 1, the list of Δm values with the proper marking is

ϕ_2**:** -1(0 **+1**).

Note that to go from order -1 to order 0, $\Delta m = +1$. Again N=2 and the phases are calculated as before

$\phi_2 = 0,\pi$

For the third pulse, the coherence is transferred to order -1 that is then detected by the receiver. Quadrature images arise from magnetization that transfers to the +1 level. As above, from the list of Δm values select the subset of coherences:

ϕ_3: (+2 +1 0 $\underline{-1}$) -2

Since there is not another value above +2 that we want, we move to the farthest point on the list; we see that N=4. Unlike in the CYCLOPS phase cycle, Section 10.9, where we needed to add a +2 coherence order level to obtain 90° phase shifts, the ±2 coherence order changes are not fictitious in this step in the phase cycle.

The phases are:

$\phi_3 = 0,\pi/2,\pi,3\pi/2$

Using the formula Eqn. 10.6.2, the receiver phase can be calculated. Each pulse is systematically and independently cycled through the proper phases. The receiver is phase cycled to cause constructive interference for signals that have taken the "right" pathway and to cause destructive interference for those that have not.

Let us calculate the phase for one step in the phase cycle. Let: $\phi_1=\pi$; $\phi_2=\pi$; and $\phi_3=3\pi/2$. The receiver phase, Ψ, is given by

$\psi = -[(-1)(\pi) + (+1)(\pi) + (-1)(3\pi/2)] = 3\pi/2.$

Notice that the path that was followed was $\Delta m_1=-1:\Delta m_2=+1:\Delta m_3=-1$. The same phase cycle is obtained by following the path: $\Delta m_1=+1:\Delta m_2=-1:\Delta m_3=-1$. Finally, the phase table for the EXSY experiment follows.

ϕ_1: 8 $(0,\pi)$
ϕ_2: 4 $(0,0,\pi,\pi)$
ϕ_3: 4 (0), 4 $(\pi/2)$, 4(π), 4$(3\pi/2)$
ψ: $(0,\pi,\pi,0)$ $(\pi/2,3\pi/2,3\pi/2,\pi/2)$ $(\pi,0,0,\pi)$ $(3\pi/2,\pi/2,\pi/2,3\pi/2)$

In this notation, 4 (0) represents four steps of 0 phase, 8 $(0,\pi)$ represents eight pairs of 0 and π phases, etc. This experiment is carried out by collecting 16 individual acquisitions and combining them in the computer memory.

Both + and - pathways must be taken during the t_1 evolution period to obtain spectra with pure phase. By allowing both pathways, the frequency is transferred by amplitude modulation that provides for pure phase spectra. If only one pathway is selected the frequency is transferred by phase modulation and the resulting line shapes are an inseparable mixture of absorptive and dispersive peaks. This is not a good situation for obtaining high resolution spectra. As well, the choice of a single pathway, as in "magnitude COSY," throws away ~30% of the signal amplitude, leading to poor signal-to-noise.