The Vector Paradigm in Modern NMR Spectroscopy:

III. Coherence Transfer and Chemical Shift Correlation Spectroscopy

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Multidimensional NMR

Heteronuclear Single Quantum Correlation (HSQC)

Figure 1. Pulse sequence for the fast-HSQC experiment. The I and S channels are separated and represent a series of pulses and time delays. The PFG channel contains pulsed field gradients. The thin lines are $\pi/2$ pulses and the wide lines are π pulses. The filled curved pulses on the **I** channel are selective pulses, the free induction decay at the right end of the **I** channel indicates the **I** spin is detected and the striped bar on the right end of the **S** channel represents **S** spin decoupling during acquisition.
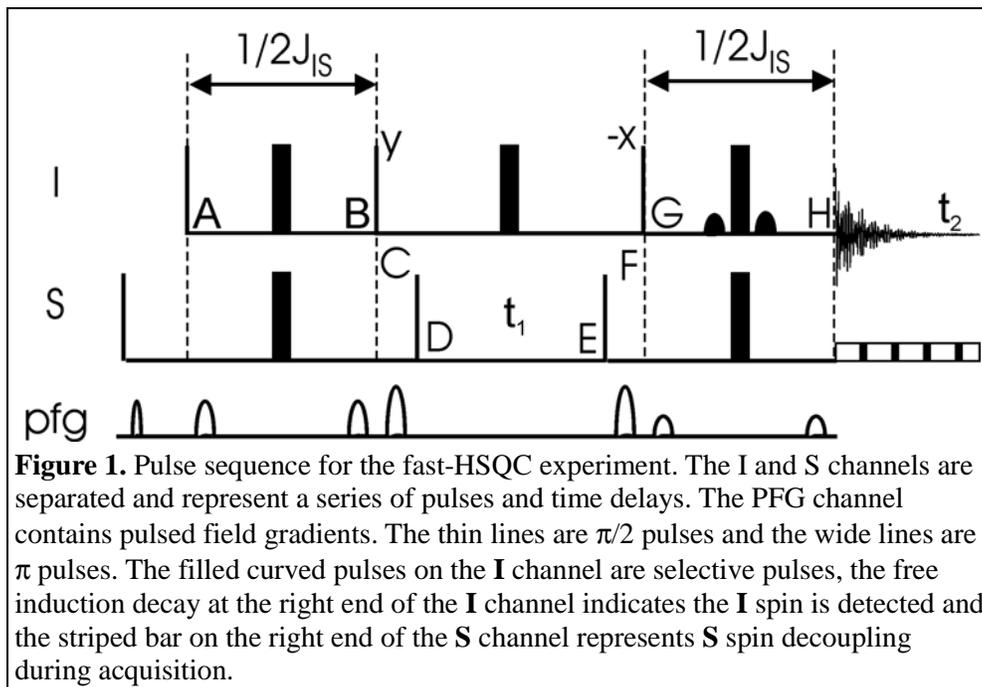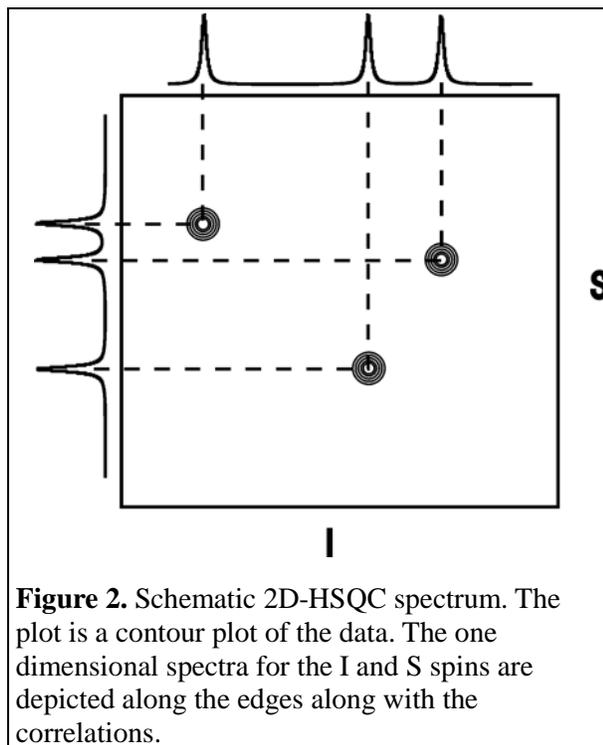
Figure 1 shows the pulse sequence for a Heteronuclear Single Quantum Correlation (HSQC) experiment for a coupled **IS** spin system. The goal of this experiment is to obtain a two-dimensional spectrum that correlates the chemical shift of an **I** spin with its coupling partner **S**. A schematic two-dimensional spectrum resulting from this experiment is shown in Figure 2. Peaks in the 2D spectrum are located at the intersection of the frequencies of the I and S coupled spins. Typically, the **I** spins are ^{1}H and the **S** spins are ^{13}C or ^{15}N.

Figure 2. Schematic 2D-HSQC spectrum. The plot is a contour plot of the data. The one dimensional spectra for the I and S spins are depicted along the edges along with the correlations.

At the beginning of the sequence, the **S** spin is saturated (S_z=0) by application of a $\widehat{\pi/2S_x}$ pulse followed by a pulsed field gradient (PFG). This is not absolutely necessary, but it removes any interference between the **S** spins that receive their polarization through coherence transfer from the **I** spin and those starting at equilibrium magnetization. At position A the $\mathbf{I_z}$ magnetization has been rotated onto the -$\mathbf{I_y}$ axis. The first pulse-interrupted free precession between points A and B serves to refocus chemical shift for the **I** spin (and **S** spin were there any transverse components) while allowing coupling to evolve for the entire period, which is set to $1/2J_{IS}$ (Eqn. 1) (Case 4 paper2##).

$$-I_y \xrightarrow{\widehat{\pi/2I_zS_z}} I_xS_z \tag{1}$$

In Eqn. 1 extra π pulses in the composite rotations (Paper2##) have been removed for simplicity. This will affect the final phase of the signals. The PFG entities during this period help to eliminate any imperfections from the π pulses on **I** and **S**. At position B an $\mathbf{I_xS_z}$ coherence is obtained. The $\widehat{\pi/2I_y}$ operator rotates the coherence into longitudinal two spin order $\mathbf{I_zS_z}$ (Eqn. 2).

$$I_xS_z \xrightarrow{\widehat{\pi/2I_y}} -I_zS_z \tag{2}$$

The application of a PFG in the state does nothing to the $\mathbf{I_zS_z}$ state while dephasing any $\mathbf{I'}$ spins that are not coupled to an **S** spin. At point B and C isolated $\mathbf{I'}$ spins, will be in the state $\mathbf{I_y}$. The PFG during C will dephase the isolated spins since they are transverse. In this experiment, we assume that the isolated spin arises from the large water signal of the solvent and that the water peak is on resonance, i.e. the frequency of the water protons is zero. One role of this experiment is to suppress the water signal (~100 M) so as to reduce the dynamic range of signal strength. A typical concentration for a solute in this experiment would be 1 mM or 10^{-5} times the water concentration. This large dynamic range causes many problems in the detection of the solute in

the absence of suppression of the water signal. Note that after every $\pi/2\mathbf{I}$ pulse in the experiment a PFG is immediately applied dephasing all signals including the large water signal. A symmetric PFG (with equal areas) at the end of each pulse-interrupted free precession period along with appropriate π pulses refocuses all coherences. This means that the water signal is not saturated (magnetization set equal to zero) during the experiment. This will be further explored below.

The $\pi/2\widehat{S_x}$ pulse rotates the longitudinal two spin order into transverse $\mathbf{S}$ coherence that is anti-phase to $\mathbf{I}$ at position D (Eqn. 3).

$$-I_zS_z \xrightarrow{\quad\pi/2\widehat{S_x}\quad} I_zS_y \tag{3}$$

The next period from D to E is a pulse-interrupted free precession in which coupling does not evolve (Case 3? Paper2##), while the transverse components of spin $\mathbf{S}$ are labeled with the $\mathbf{S}$ chemical shift frequency. The evolution is described in Eqn. 4.

$$I_zS_y \xrightarrow{\quad\omega_S t\widehat{S_z}\quad} I_zS_y\cos(\omega_S t_1) - I_zS_x\sin(\omega_S t_1) \tag{4}$$

There are two orthogonal components of the $\mathbf{S}$ spin at point E that correspond to the two components that are required for quadrature detection. Only one of these can be turned into

$$I_zS_y\cos(\omega_S t_1) - I_zS_x\sin(\omega_S t_1) \xrightarrow{\quad\pi/2\widehat{S_x}\quad}$$
$$I_zS_z\cos(\omega_S t_1) - I_zS_x\sin(\omega_S t_1) \tag{5}$$

observable magnetization in this experiment. The $\pi/2\widehat{S_x}$ turns the $\mathbf{I_zS_y}$ component back into longitudinal two-spin order, while in the other component the $\mathbf{S_x}$ spin remains transverse (Eqn 5). The PFG that is applied will dephase the transverse component and in the absence of any refocusing PFG the coherence will remain dephased and not observable. The subsequent $\pi/2\widehat{I_x}$ rotates the longitudinal two spin order component as shown in Eqn. 6.

An interesting point in this experiment, is that if the PFG during period F has the same area and algebraic sign as the PFG during period C, the combination of these with the $\mathbf{I}$ spin π

pulse in the center of the D-E period will cause the transverse magnetization of water spins that were dephased during C to refocus to -$\mathbf{I_y}$ at the end of D.

$$I_z S_z \cos(\omega_S t_1) - I_z S_x \sin(\omega_S t_1) \xrightarrow{\ \pi/2\widehat{I_x}\ }$$
$$-I_y S_z \cos(\omega_S t_1) + I_y S_x \sin(\omega_S t_1)$$

(6)

The $-\pi/2\widehat{I_x}$ pulse will rotate these spins to $\mathbf{I_z}$. In the subsequent pulse-interrupted free precession period (G-H) the small rounded pulses on the $\mathbf{I}$ spin channel represent $\pi/2$ pulses that are selective for the water protons. The net result of the two $\pi/2$ and the π pulses is to rotate the water signal by 2π radians. All other $\mathbf{I}$ spins that are distant from the water frequency are not affected by the selective pulse and are rotated only by the π pulse. At position H, the water magnetization is returned to along the Z axis and is relatively close to its equilibrium value. In the case where the $\mathbf{I}$ spins are ^{1}H and the $\mathbf{S}$ spins are ^{15}N, the exchange of the water protons with those bound to nitrogen as in an amide moiety transfers any saturation of the water protons to the amide positions, decreasing the intensity of the signal. Since this experiment returns the water magnetization to the Z axis with a magnitude close to that of the equilibrium magnetization there is only a small amount of saturation that can be transferred to the amide.

For the coupled spins, during the G-H pulse-interrupted free precession period all chemical shift is refocused and coupling evolves. The term with two transverse operators, $\mathbf{I_y S_x}$, is a linear combination of non-observable zero and double quantum coherence and does not evolve under the $\pi J_{IS} t \widehat{I_z S_z}$ operator. It will not be detected.

$$-I_y S_z \cos(\omega_S t_1) + I_y S_x \sin(\omega_S t_1) \xrightarrow{\ \pi J_{IS} t \widehat{I_z S_z}\ }$$
$$I_x \cos(\omega_S t_1) + I_y S_x \sin(\omega_S t_1)$$

(7)

The observable $\mathbf{I_x}$ term, detected in quadrature during t_2, retains the modulation due to $\mathbf{S}$ chemical shift that was acquired during the t_1 period (D-E) (Eqn. 7). In Eqns. 8 and 9 the transverse relaxation terms for the coherences during the t_1 and t_2 periods are added in an ad hoc manner.

$$I_x e^{i\omega_I t_2} \cos(\omega_S t_1) e^{(-t_2/T_2^I)} e^{(-t_1/T_2^{I_z S^\pm})} \tag{8}$$

For quadrature detection in the indirect ω_1 dimension, a second experiment at the same t_1 value is collected with the phase of the first $\pi/2$ S pulse changed to y and the results are stored separately. This will lead to the detected signal shown in Eqn. 9.

$$I_x e^{i\omega_I t_2} \sin(\omega_S t_1) e^{(-t_2/T_2^I)} e^{(-t_1/T_2^{I_z S^\pm})} \tag{9}$$

A Fourier transform of these signals along t_2 yields the results in Eqn. 10. Where A_2 is the absorption signal along ω_2, D_2 is the dispersion signal along ω_2, and i is the square root of -1 making the dispersive signal 90° out of phase with respect the absorptive signal.

$$\left(A_2 + iD_2\right)\cos(\omega_S t_1) e^{(-t_1/T_2^{I_z S^\pm})} \tag{10}$$

$$\left(A_2 + iD_2\right)\sin(\omega_S t_1) e^{(-t_1/T_2^{I_z S^\pm})}$$

In order to retain pure absorption lineshapes, the imaginary part of the ω_2 signal in Eqn. 10 is set to zero before Fourier transform along t_1 (Eqn. 11). Recalling that two signals equidistant from

$$\left(A_2\right)\left(A_1^+ + iD_1^+ + A_1^- + iD_1^-\right) \tag{11}$$

$$\left(A_2\right)\left(A_1^+ + iD_1^+ - (A_1^- + iD_1^-)\right)$$

the center frequency arise from the Fourier transform of the cosine (or sine) function, the superscripts indicate the sign of the frequency for the particular signal. Quadrature detection is obtained by combining the signals for the cosine and sine modulated signals. Taking the real

portion of the sum of the two signals in Eqn. 11 gives a quadrature detected line that has a pure

absorption lineshape in both dimensions (Eqn. 12).

$$A_2 A_1^+ \tag{12}$$

Sensitivity enhanced HSQC
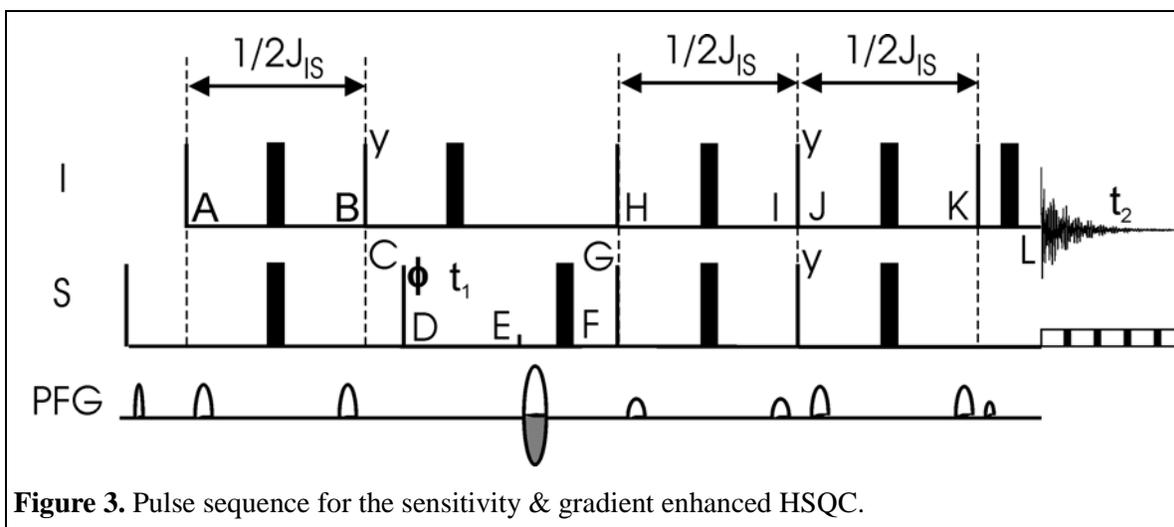
Figure 3. Pulse sequence for the sensitivity & gradient enhanced HSQC.

The pulse sequence for the sensitivity enhanced HSQC is presented in Figure 3. The experiment

is also gradient enhanced. Up to point E, the experiment is identical to the HSQC described above.

For the time being, we will skip the section between E and G and the period following the $\pi/2$

pulse at K. Ignoring E-G, at point I, the state of the coupled spin system was given in Eqn 7 and is

repeated here as Eqn. 13.

$$I_x \cos(\omega_S t_1) + I_y S_x \sin(\omega_S t_1) \tag{13}$$

A $\pm \pi/2 \widehat{S_y}$ and a $\pi/2 \mathbf{I_y}$ pulse are applied to this state yielding the state in Eqn. 14.

$$I_x \cos(\omega_S t_1) + I_y S_x \sin(\omega_S t_1)$$
$$\xrightarrow[\pm \pi/2\widehat{S_y}]{\pi/2\widehat{I_y}} -I_z \cos(\omega_S t_1) \mp I_y S_z \sin(\omega_S t_1) \tag{14}$$

The in-phase I_x magnetization is now stored along I_z. and the previously non-observable $I_y S_x$ is transformed to antiphase $I_y S_z$ coherence. The anti-phase coherence evolves into in-phase magnetization during the J-K period and the I_z commutes with the $\pi J_{IS} t \widehat{I_z S_z}$ operator (Eqn. 15).

$$-I_z \cos(\omega_S t_1) \mp I_y S_z \sin(\omega_S t_1)$$
$$\xrightarrow{\pi J_{IS} t \widehat{I_z S_z}} -I_z \cos(\omega_S t_1) \pm I_x \sin(\omega_S t_1) \tag{15}$$

The $\pi/2\widehat{I_x}$ pulse at K rotates the preserved I_z magnetization back into the transverse plane.

During detection, the signal in Eqn. 16 is obtained.

$$\left[I_y \cos(\omega_S t_1) \pm I_x \sin(\omega_S t_1) \right] e^{i\omega_I t_2} \tag{16}$$

By adding and subtracting these two signals, corresponding to the $\pm \pi/2\widehat{S_y}$ experiments that are separately stored, one obtains Eqns. 17.

$$I_y \cos(\omega_S t_1) e^{i\omega_I t_2}$$
$$I_x \sin(\omega_S t_1) e^{i\omega_I t_2} \tag{17}$$

Each of these can be Fourier transformed as in the HSQC experiment (Eqn. 11), however the results cannot be directly combined along t_1 since the I_y and the I_x components are 90° out of phase. This can be remedied by phase shifting one of the signals by 90° (Eqn. 18) this corresponds to applying a 90° zero order phase correction to every second FID along the t_1 dimension.

$$I_y \cos(\omega_S t_1) e^{i\omega_I t_2}$$
$$I_y \sin(\omega_S t_1) e^{i\omega_I t_2}$$

(18)

From this point the processing follows as that for HSQC.