Selective internuclear coupling estimation in the solid-state NMR of multiple-spin systems

Pierre Thureau, Andrea C. Sauerwein, Maria Concistrè and Malcolm H. Levitt

Supporting Information

1 Experimental procedure

1.1 General conditions

Experiments were performed on a 9.4 T Varian Infinity plus NMR spectrometer using a 3 mm switched-angle-spinning DOTY NMR probe. The NMR probe was equipped with a Hall-effect device attached to the spinning stator. The dependence of the Hall voltage on the spinning angle was calibrated measuring the $^{13}$C spin-echo modulation curve for a fully $^{13}$C enriched glycine sample.\(^1\)

The facility to switch the spinning angle was not used for the experiments described here.

Implementation of the pulse sequence requires switching of the rf amplitude over a large dynamic range. It was found empirically that the large amplitude change between the strong rf pulses and the weak shaped pulses is accompanied by a 60° spurious phase shift on our spectrometer. We corrected for this phase shift using the pulse programming software.

1.2 Phase Cycling

The DQ and ZQ-filtered signals are obtained from separate experiments. In all experiments, a 64-step phase cycle is implemented to select the coherence transfer pathways shown in Figure S1.
Figure 1: Pulse sequence for measuring selected internuclear $^{13}$C-$^{13}$C dipolar couplings. The coherence pathways for the carbon magnetization are given in blue. The coherence pathway shown in a) selects Double Quantum (DQ) terms at the junction of the two $90^\circ$ pulses while the coherence pathway shown in b) selects Zero Quantum (ZQ) terms. Keeping all the pulse phases equal and adjusting the receiver phase allows the coherence pathways show in a) or b) to be differentiated.

The pulses phases are identical for both ZQF and DQF experiments and are given by:

- $\phi_1 = 0$
- $\phi_2 = (0, 90, 180, 270)_{16}$
- $\phi_3 = 90$
- $\phi_4 = (0, 0, 0, 0, 90, 90, 90, 90, 180, 180, 180, 180, 180, 270, 270, 270, 270)_{16}$
- $\phi_5 = 0_{16}, 90_{16}, 180_{16}, 270_{16}$

On the other hand, the receiver phase allows Double Quantum or Zero Quantum coherences to be selected and are given by:
$$\phi_{\text{rec}}(\text{DQF}) = (0, 180, 0, 180, 90, 270, 90, 270, 180, 0, 180, 0, 270, 90, 270, 90, 270, 90, 270, 90, 270, 180, 0, 180, 0, 270, 90, 270, 90, 270, 90, 270)$$

$$\phi_{\text{rec}}(\text{ZQF}) = (0, 180, 0, 180, 270, 90, 270, 90, 180, 0, 180, 0, 270, 90, 270, 180, 0, 180, 0, 270, 90, 270, 90, 270, 90, 270)$$

_N.B.: The subscript numbers indicate the number of repetitions._

### 1.3 Conditions for the individual experiments

#### 1.3.1 C2-C5 coupling measurement

For the results shown in Figure 3a, the spinning frequency was 16.5 kHz ± 0.07 kHz and the offset from the magic angle was 53.94° ± 0.05°. The recycle delay was 7s. The $^1\text{H}$ and $^{13}\text{C}$ 90° pulse lengths were 2.5 and 2.8 μs, respectively. swTPPM proton decoupling was used with a proton nutation frequency of 80 kHz, a pulse duration of 5.5 μs, and a radio-frequency phase shift $\Delta \phi$ of 18°. $^{13}\text{C}$ transverse magnetisation was created by cross polarization from $^1\text{H}$ with a $^1\text{H}$ rf field of 54 kHz and a $^{13}\text{C}$ rf field of 40 kHz. The cross-polarization contact time was 0.7 ms.

The dual-band frequency-selective pulses had a cosine-modulated Gaussian shape described by:

$$\omega_{\text{nut}}(t) = \omega_{\text{nut}}(t_0) \cos [\omega_m(t - t_0)] \exp[-\alpha(t - t_0)^2] \quad (1)$$

where $t_0$ is the middle of the selective pulse and $\alpha$ the Gaussian decay parameter. The carrier frequency of the pulse is set to the mean of the chemical shift frequencies of the selected peaks and the modulation frequency $\omega_m$ is set to one-half of the chemical shift frequency difference. To obtain the results shown in figure 3a, the relevant parameters were $\alpha = 0.968 \times 10^6 \text{ s}^{-2}$ and $\omega_m/2\pi = 930 \text{ Hz}$. The data analysis used the selective pulse time shift
of $\tau_{sh} = 1.047$ ms.

### 1.3.2 intermolecular CO-CO coupling measurement

For the results shown in Figure 3b, the spinning frequency was 16.5 kHz $\pm$ 0.07 kHz and the offset from the magic angle was 51.74° $\pm$ 0.05°. The recycle delay was 7s. The $^1$H and $^{13}$C 90° pulse lengths were 2.5 and 2.8 $\mu$s, respectively. swTPPM proton decoupling$^2$ was used with a proton nutation frequency of 80 kHz, a pulse duration of 5.5 $\mu$s, and a radio-frequency phase shift $\Delta\phi$ of 18°. $^{13}$C transverse magnetisation was created by cross polarization from $^1$H with a $^1$H rf field of 54 kHz and a $^{13}$C rf field of 40 kHz. The cross-polarization contact time was 2.0 ms.

The single-band frequency-selective pulses used an unmodulated Gaussian shape described by:

$$\omega_{\text{mut}}(t) = \omega_{\text{mut}}(t_0) \exp[-\alpha(t - t_0)^2]$$

where $t_0$ is the middle of the selective pulse and $\alpha$ the Gaussian decay parameter. The carrier frequency of the pulse is set to the isotropic shift frequency of the CO sites. To obtain the results shown in figure 3a, the Gaussian shape parameter was $\alpha = 11.37 \times 10^6$ s$^{-2}$. The data analysis used the selective pulse time shift of $\tau_{sh} = 0.305$ ms.

### 2 Error analysis

The confidence limits on each experimental $s_{\pm2}$ and $s_0$ points were determined by integrating several signal-free spectral regions of the same frequency bandwidth as that used for estimating the peak integrals. The standard deviation of these noise integrals were taken as the experimental confidence limits. Hence, calling $\delta_{s_{\pm2}}$ and $\delta_{s_0}$ the uncertainties for respectively the $s_{\pm2}$ and $s_0$ data points, the uncertainty on the $s_{\pm2}/s_0$ data points has been estimated according to:

$$\delta_{s_{\pm2}/s_0} = \left( \frac{|\delta_{s_{\pm2}}|}{s_{\pm2}} + \frac{|\delta_{s_0}|}{s_0} \right) \times \frac{|s_{\pm2}|}{|s_0|}$$
Then, the confidence limits on the determined RDCs were determined by weighted non-linear fitting to the analytical formula for the ratio $s_{\pm 2}(\tau)/s_0(\tau)$. Calling $\delta_{RDC}$ the uncertainty on the determined residual coupling and $\delta_{\beta}$ the uncertainty on the spinning angle, the uncertainty on the overall estimated RDC $\delta_b$ has been estimated according to

$$\delta_b = |\delta_{RDC}| + \left|\frac{\delta_{\beta}}{\beta}\right| \times RDC$$

3 Analytical Formula for the Powder-Average Multiple-Quantum-Filtered Signal

Considering the case of two weakly coupled spins, $i$ and $j$, we can describe the NMR signal with the help of the product operator theory.\(^3\)

The spin Hamiltonian $H_{ij}$ for the two spins $i$ and $j$ can be written as follows:

$$\hat{H}_{ij} = \omega_i \hat{I}_{iz} + \omega_j \hat{I}_{jz} + \omega_A 2\hat{I}_{iz}\hat{I}_{jz}$$

where the two coupled terms depend on the direct and indirect dipole-dipole couplings as follows:

$$\omega_A = \pi J + d$$

where $J$ is the scalar coupling and $d$ is the secular component of the through-space dipole-dipole coupling.

In a rotating sample, the chemical shift frequencies $\omega_i$ and $\omega_j$, and the secular dipole-dipole coupling, are time dependent. The time dependent terms are expressed as a Fourier series as follows:

$$\omega_Q(t) = \sum_{m=-2}^{2} \omega_Q^{(m)} \exp(i\omega_r t)$$

where $\omega_r$ is the spinning frequency and $Q$ refers to any of $A$, $i$ or $j$.

In the case of off-magic-angle spinning, the most important term is the zero-order Fourier component of $\omega_A$

$$\omega_A^{(0)} = \pi J + b \times d_{00}^2(\beta_{PR})d_{00}^2(\beta_{RL})$$
where $b$ is the dipole-dipole coupling constant, $d_{00}^2(\beta)$ is an element of the second-rank reduced Wigner matrix, $\beta_{PR}$ is the angle between the internuclear vector and the rotor axis, and $\beta_{RL}$ is the angle between the rotor axis and the static magnetic field.

We consider the $(\pm 1)$-quantum coherences at the start of free-evolution interval $\tau/2$, thus the initial density operator $\hat{\rho}_0$ is:

$$\hat{\rho}_0 = \hat{I}_{ix} + \hat{I}_{jx}$$

During the first interval $\tau/2$, the $(\pm 1)$-quantum coherences evolve under the influence of the weak coupling $\omega_A$. As a result, at the end of the first free-evolution interval $\tau/2$, we have:

$$\hat{\rho}_0 \xrightarrow{2\omega_A(\tau/2)\hat{I}_{ix}\hat{I}_{ix}} \cos \left[ \frac{\omega_A T}{2} \right] (\hat{I}_{ix} + \hat{I}_{jx}) + 2 \sin \left[ \frac{\omega_A T}{2} \right] (\hat{I}_{iy}\hat{I}_{jz} + \hat{I}_{iz}\hat{I}_{jy})$$

Afterwards, the first central $\pi/2$ pulse generates ZQ and DQ terms, such as:

$$-\cos \left[ \frac{\omega_A T}{2} \right] (\hat{I}_{iz} + \hat{I}_{jz}) + 2 \sin \left[ \frac{\omega_A T}{2} \right] (\hat{I}_{iy}\hat{I}_{jx} + \hat{I}_{ix}\hat{I}_{jy})$$

where, the cosine term is a pure ZQ coherence while the sine term is a pure DQ coherence. Adjusting the phase cycle of the second central $\pi/2$ pulse we can select the ZQ or the DQ term.

After reconversion into observable magnetization during the second $\tau/2$ time interval, we obtain:

$$s_0(\tau) = \cos^2 \left[ \frac{\omega_A T}{2} \right]$$

$$s_{\pm 2}(\tau) = \sin^2 \left[ \frac{\omega_A T}{2} \right]$$

As a result, for a single crystal orientation the ZQ signal is given by:

$$s_0(\tau) = \cos \left[ \frac{\tau}{2} \left( \pi J + bd_{00}^2(\beta_{PR})d_{00}^2(\beta_{RL}) \right) \right]^2$$
and the DQ signal is given by:

\[ s_{\pm 2}(\tau) = \sin \left[ \frac{\tau}{2} (\pi J + bd_{00}^2(\beta_{PR})d_{00}^2(\beta_{RL})) \right]^2 \]

After powder averaging the ZQ and DQ signals become:\(^4\)

\[ s_0(\tau) = \frac{1}{2} \int_{0}^{\pi} \cos \left[ \frac{\tau}{2} (\pi J + b \times d_{00}^2(\beta_{PR})d_{00}^2(\beta_{RL})) \right]^2 \sin(\beta_{PR})d\beta_{PR} \quad (3) \]

\[ = \frac{1}{2} + \frac{1}{2x} (Fc(x) \cos \varphi + Fs(x) \sin \varphi) \quad (4) \]

\[ s_{\pm 2}(\tau) = \frac{1}{2} \int_{0}^{\pi} \sin \left[ \frac{\tau}{2} (\pi J + b \times d_{00}^2(\beta_{PR})d_{00}^2(\beta_{RL})) \right]^2 \sin(\beta_{PR})d\beta_{PR} \quad (5) \]

\[ = \frac{1}{2} - \frac{1}{2x} (Fc(x) \cos \varphi + Fs(x) \sin \varphi) \quad (6) \]

thus, the ratio \( s_{\pm 2}(\tau)/s_0(\tau) \) is given by:

\[ \frac{s_{\pm 2}(\tau)}{s_0(\tau)} = \frac{x - Fc(x) \cos \varphi - Fs(x) \sin \varphi}{x + Fc(x) \cos \varphi + Fs(x) \sin \varphi} \]

It should be noted that for the case of exact magic angle spinning (MAS) \( (\beta_{RL} = \arctan \sqrt{2}) \), Equ. 3 and 5 reduce to

\[ s_0(\tau) = \cos \left[ \frac{\pi J \tau}{2} \right]^2 \]

\[ s_{\pm 2}(\tau) = \sin \left[ \frac{\pi J \tau}{2} \right]^2 \]

Hence, in the case where the pulse sequence presented here is performed at exact MAS conditions, one can measure precisely the isotropic scalar \( J \)-coupling. This experiment can be used in the case of protein/peptide structure determination where spins share dipolar and scalar coupling.
References


