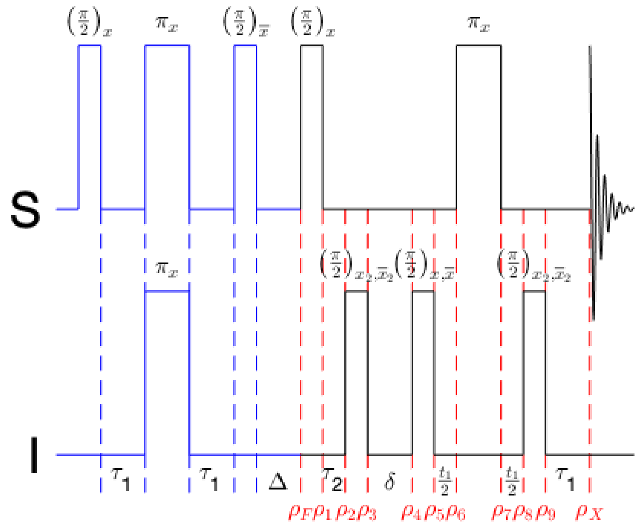
December 2022 NMR Topic of the Month: The HMBC Pulse Sequence



For what does the acronym HMBC stand?

HMBC = Heteronuclear Multi-Bond Correlation

What is the role of the HMBC sequence?

The HMBC is another cornerstone of characterization by NMR. The resulting 2D spectrum is a map of which S spins are weakly through-bond coupled to which I spins. There are many variations of this sequence, as it is easily adapted. The version shown above has a BIRD filter (see last month's topic) prepended to it (shown in blue).

How does the HMBC work?

There's no need to regurgitate the BIRD, that building block's presence conditions the initial input to the HMBC. Even still, the product-operator analysis beginning at ρ_F is lengthy and shown in the appendix. What is more important is understanding what the sequence is doing. The initial BIRD filter favors S spins that are weakly coupled (small value of J) by a choosing τ_1. This filtered magnetization is placed back in the transverse plane by the pulse at ρ_F. Next the magnetization is filtered again, this time by choosing the value of τ_2 based on a strong coupling (large value of J), but with the goal of tossing these signals out as they will be either zero or unobservable double-quantum. The value of δ is simply the difference between τ_1 and τ_2, so the S spins themselves are simply undergoing a spin echo.

There are several sequences of this kind, which is best to use?

The best one is the one that most quickly and completely answers your questions of connectivity. For example, the HMQC varies from the HMBC by the filter (first) pulse on the I spins being absent, which means the stronger couplings may obfuscate the weaker ones or it could elucidate missing intermediate couplings. There may also be concerns about transfer from spin clusters ($I_N S$) and whether an experiment's conditions are biased.

References

1. A. Bax, M.F. Summers, *J. Am. Chem. Soc.* **108**(8), 2093-2094 (1986).
2. W.F. Reynolds, R.G. Enriquez, *Magn. Reson. Chem.* **39**(9), 531-538 (2001).
3. R.R. Ernst, G. Bodenhausen, and A. Wokaun, *Principles of Nuclear Magnetic Resonance in One and Two Dimensions [Chapter 8.5]*, Oxford Science Publications, New York (1987).
4. J. Cavanagh, W.J. Fairbrother, A.G. Palmer III, N.J. Skelton, *Protein NMR Spectroscopy, Principles and Practice [Chapter 7]*, Academic Press, New York (1996).

Appendix: Product-Operator

Below details the product-operator analysis of the HMBC at $t_1 = 0$, where $c_X = \frac{1}{4}\left(\gamma_X B_0 / k_B T\right)$:

$$\rho_F = -c_I I_z - c_S S_z cos\left(2\pi J \tau_1\right) \rightarrow \rho_1 = -c_I I_z + c_S S_y cos\left(2\pi J \tau_1\right) \rightarrow$$

$$\rho_2 = -c_I I_z + c_S S_y cos\left(2\pi J\tau_1\right)cos\left(\omega_S\tau_2\right)cos\left(\pi J\tau_2\right) - c_S 2I_z S_x cos\left(2\pi J\tau_1\right)cos\left(\omega_S\tau_2\right)sin\left(\pi J\tau_2\right)$$

$$- c_S S_x cos\left(2\pi J\tau_1\right)sin\left(\omega_S\tau_2\right)cos\left(\pi J\tau_2\right) - c_S 2I_z S_y cos\left(2\pi J\tau_1\right)sin\left(\omega_S\tau_2\right)sin\left(\pi J\tau_2\right) \rightarrow$$

$$\rho_3 = c_I I_y + c_S S_y cos\left(2\pi J\tau_1\right)cos\left(\omega_S\tau_2\right)cos\left(\pi J\tau_2\right) + c_S 2I_y S_x cos\left(2\pi J\tau_1\right)cos\left(\omega_S\tau_2\right)sin\left(\pi J\tau_2\right)$$

$$- c_S S_x cos\left(2\pi J\tau_1\right)sin\left(\omega_S\tau_2\right)cos\left(\pi J\tau_2\right) + c_S 2I_y S_y cos\left(2\pi J\tau_1\right)sin\left(\omega_S\tau_2\right)sin\left(\pi J\tau_2\right) \rightarrow$$

$$\rho_4 = c_I I_y cos\left(\omega_I\delta\right)cos(\pi J\delta) - c_I 2I_x S_z cos\left(\omega_I\delta\right)sin(\pi J\delta) - c_I I_x sin\left(\omega_I\delta\right)cos(\pi J\delta) - c_I 2I_y S_z sin\left(\omega_I\delta\right)sin(\pi J\delta)$$

$$+ c_S S_y cos\left(2\pi J\tau_1\right)cos\left(\omega_S\tau_2\right)cos\left(\pi J\tau_2\right)cos\left(\omega_S\delta\right)cos(\pi J\delta) - c_S 2I_z S_x cos\left(2\pi J\tau_1\right)cos\left(\omega_S\tau_2\right)cos\left(\pi J\tau_2\right)cos\left(\omega_S\delta\right)sin(\pi J\delta)$$

$$- c_S S_x cos\left(2\pi J\tau_1\right)cos\left(\omega_S\tau_2\right)cos\left(\pi J\tau_2\right)sin\left(\omega_S\delta\right)cos(\pi J\delta) - c_S 2I_z S_y cos\left(2\pi J\tau_1\right)cos\left(\omega_S\tau_2\right)cos\left(\pi J\tau_2\right)sin\left(\omega_S\delta\right)sin(\pi J\delta)$$

$$+ c_S 2I_y S_x cos\left(2\pi J\tau_1\right)cos\left(\omega_S\tau_2\right)sin\left(\pi J\tau_2\right)cos\left(\omega_S\delta\right)cos(\pi J\delta) + c_S 2I_y S_y cos\left(2\pi J\tau_1\right)cos\left(\omega_S\tau_2\right)sin\left(\pi J\tau_2\right)cos\left(\omega_S\delta\right)sin(\pi J\delta)$$

$$- c_S 2I_x S_x cos\left(2\pi J\tau_1\right)cos\left(\omega_S\tau_2\right)sin\left(\pi J\tau_2\right)sin\left(\omega_S\delta\right)cos(\pi J\delta) - c_S 2I_x S_y cos\left(2\pi J\tau_1\right)cos\left(\omega_S\tau_2\right)sin\left(\pi J\tau_2\right)sin\left(\omega_S\delta\right)sin(\pi J\delta)$$

$$- c_S S_x cos\left(2\pi J\tau_1\right)sin\left(\omega_S\tau_2\right)cos\left(\pi J\tau_2\right)cos\left(\omega_S\delta\right)cos(\pi J\delta) - c_S 2I_z S_y cos\left(2\pi J\tau_1\right)sin\left(\omega_S\tau_2\right)cos\left(\pi J\tau_2\right)cos\left(\omega_S\delta\right)sin(\pi J\delta)$$

$$- c_S S_y cos\left(2\pi J\tau_1\right)sin\left(\omega_S\tau_2\right)cos\left(\pi J\tau_2\right)sin\left(\omega_S\delta\right)cos(\pi J\delta) + c_S 2I_z S_x cos\left(2\pi J\tau_1\right)sin\left(\omega_S\tau_2\right)cos\left(\pi J\tau_2\right)sin\left(\omega_S\delta\right)sin(\pi J\delta)$$

$$+ c_S 2I_y S_y cos\left(2\pi J\tau_1\right)sin\left(\omega_S\tau_2\right)sin\left(\pi J\tau_2\right)cos\left(\omega_S\delta\right)cos(\pi J\delta) - c_S 2I_y S_x cos\left(2\pi J\tau_1\right)sin\left(\omega_S\tau_2\right)sin\left(\pi J\tau_2\right)cos\left(\omega_S\delta\right)sin(\pi J\delta)$$

$$- c_S 2I_x S_y cos\left(2\pi J\tau_1\right)sin\left(\omega_S\tau_2\right)sin\left(\pi J\tau_2\right)sin\left(\omega_S\delta\right)cos(\pi J\delta) + c_S 2I_x S_x cos\left(2\pi J\tau_1\right)sin\left(\omega_S\tau_2\right)sin\left(\pi J\tau_2\right)sin\left(\omega_S\delta\right)sin(\pi J\delta) =$$

$$c_I I_y cos\left(\omega_I\delta\right)cos(\pi J\delta) - c_I 2I_x S_z cos\left(\omega_I\delta\right)sin(\pi J\delta) - c_I I_x sin\left(\omega_I\delta\right)cos(\pi J\delta) - c_I 2I_y S_z sin\left(\omega_I\delta\right)sin(\pi J\delta)$$

$$+ c_S S_y cos\left(2\pi J\tau_1\right)cos\left(\omega_S\tau_1\right)cos\left(\pi J\tau_2\right)cos(\pi J\delta) - c_S 2I_z S_x cos\left(2\pi J\tau_1\right)cos\left(\omega_S\tau_1\right)cos\left(\pi J\tau_2\right)sin(\pi J\delta)$$

$$- c_S S_x cos\left(2\pi J\tau_1\right)sin\left(\omega_S\tau_1\right)cos\left(\pi J\tau_2\right)cos(\pi J\delta) - c_S 2I_z S_y cos\left(2\pi J\tau_1\right)sin\left(\omega_S\tau_1\right)cos\left(\pi J\tau_2\right)sin(\pi J\delta)$$

$$+ c_S 2I_y S_x cos\left(2\pi J\tau_1\right)cos\left(\omega_S\tau_2 + \pi J\delta\right)sin\left(\pi J\tau_2\right)cos\left(\omega_S\delta\right) + c_S 2I_y S_y cos\left(2\pi J\tau_1\right)sin\left(\omega_S\tau_2 + \pi J\delta\right)sin\left(\pi J\tau_2\right)cos\left(\omega_S\delta\right)$$

$$- c_S 2I_x S_x cos\left(2\pi J\tau_1\right)cos\left(\omega_S\tau_2 + \pi J\delta\right)sin\left(\pi J\tau_2\right)sin\left(\omega_S\delta\right) - c_S 2I_x S_y cos\left(2\pi J\tau_1\right)sin\left(\omega_S\tau_2 + \pi J\delta\right)sin\left(\pi J\tau_2\right)sin\left(\omega_S\delta\right) \rightarrow$$

$$\rho_5 = c_I I_z cos\left(\omega_I\delta\right)cos(\pi J\delta) - c_I 2I_x S_z cos\left(\omega_I\delta\right)sin(\pi J\delta) - c_I I_x sin\left(\omega_I\delta\right)cos(\pi J\delta) - c_I 2I_z S_z sin\left(\omega_I\delta\right)sin(\pi J\delta)$$

$$+ c_S S_y cos\left(2\pi J\tau_1\right)cos\left(\omega_S\tau_1\right)cos\left(\pi J\tau_2\right)cos(\pi J\delta) + c_S 2I_y S_x cos\left(2\pi J\tau_1\right)cos\left(\omega_S\tau_1\right)cos\left(\pi J\tau_2\right)sin(\pi J\delta)$$

$$- c_S S_x cos\left(2\pi J\tau_1\right)sin\left(\omega_S\tau_1\right)cos\left(\pi J\tau_2\right)cos(\pi J\delta) + c_S 2I_y S_y cos\left(2\pi J\tau_1\right)sin\left(\omega_S\tau_1\right)cos\left(\pi J\tau_2\right)sin(\pi J\delta)$$

$$+ c_S 2I_z S_x cos\left(2\pi J\tau_1\right)cos\left(\omega_S\tau_2 + \pi J\delta\right)sin\left(\pi J\tau_2\right)cos\left(\omega_S\delta\right) + c_S 2I_z S_y cos\left(2\pi J\tau_1\right)sin\left(\omega_S\tau_2 + \pi J\delta\right)sin\left(\pi J\tau_2\right)cos\left(\omega_S\delta\right)$$

$$- c_S 2I_x S_x cos\left(2\pi J\tau_1\right)cos\left(\omega_S\tau_2 + \pi J\delta\right)sin\left(\pi J\tau_2\right)sin\left(\omega_S\delta\right) - c_S 2I_x S_y cos\left(2\pi J\tau_1\right)sin\left(\omega_S\tau_2 + \pi J\delta\right)sin\left(\pi J\tau_2\right)sin\left(\omega_S\delta\right) \rightarrow$$

Appendix: Product-Operator (Continued)

$$\rho_7 = c_I I_z cos(\omega_I \delta) cos(\pi J \delta) + c_I 2I_x S_z cos(\omega_I \delta) sin(\pi J \delta) - c_I I_x sin(\omega_I \delta) cos(\pi J \delta) + c_I 2I_z S_z sin(\omega_I \delta) sin(\pi J \delta)$$

$$- c_S S_y cos(2\pi J \tau_1) cos(\omega_S \tau_1) cos(\pi J \tau_2) cos(\pi J \delta) + c_S 2I_y S_x cos(2\pi J \tau_1) cos(\omega_S \tau_1) cos(\pi J \tau_2) sin(\pi J \delta)$$

$$- c_S S_x cos(2\pi J \tau_1) sin(\omega_S \tau_1) cos(\pi J \tau_2) cos(\pi J \delta) - c_S 2I_y S_y cos(2\pi J \tau_1) sin(\omega_S \tau_1) cos(\pi J \tau_2) sin(\pi J \delta)$$

$$+ c_S 2I_z S_x cos(2\pi J \tau_1) cos(\omega_S \tau_2 + \pi J \delta) sin(\pi J \tau_2) cos(\omega_S \delta) - c_S 2I_z S_y cos(2\pi J \tau_1) sin(\omega_S \tau_2 + \pi J \delta) sin(\pi J \tau_2) cos(\omega_S \delta)$$

$$- c_S 2I_x S_x cos(2\pi J \tau_1) cos(\omega_S \tau_2 + \pi J \delta) sin(\pi J \tau_2) sin(\omega_S \delta) + c_S 2I_x S_y cos(2\pi J \tau_1) sin(\omega_S \tau_2 + \pi J \delta) sin(\pi J \tau_2) sin(\omega_S \delta) \rightarrow$$

$$\rho_9 = - c_I I_y cos(\omega_I \delta) cos(\pi J \delta) + c_I 2I_x S_z cos(\omega_I \delta) sin(\pi J \delta) - c_I I_x sin(\omega_I \delta) cos(\pi J \delta) - c_I 2I_y S_z sin(\omega_I \delta) sin(\pi J \delta)$$

$$- c_S S_y cos(2\pi J \tau_1) cos(\omega_S \tau_1) cos(\pi J \tau_2) cos(\pi J \delta) + c_S 2I_z S_x cos(2\pi J \tau_1) cos(\omega_S \tau_1) cos(\pi J \tau_2) sin(\pi J \delta)$$

$$- c_S S_x cos(2\pi J \tau_1) sin(\omega_S \tau_1) cos(\pi J \tau_2) cos(\pi J \delta) - c_S 2I_z S_y cos(2\pi J \tau_1) sin(\omega_S \tau_1) cos(\pi J \tau_2) sin(\pi J \delta)$$

$$- c_S 2I_y S_x cos(2\pi J \tau_1) cos(\omega_S \tau_2 + \pi J \delta) sin(\pi J \tau_2) cos(\omega_S \delta) + c_S 2I_y S_y cos(2\pi J \tau_1) sin(\omega_S \tau_2 + \pi J \delta) sin(\pi J \tau_2) cos(\omega_S \delta)$$

$$- c_S 2I_x S_x cos(2\pi J \tau_1) cos(\omega_S \tau_2 + \pi J \delta) sin(\pi J \tau_2) sin(\omega_S \delta) + c_S 2I_x S_y cos(2\pi J \tau_1) sin(\omega_S \tau_2 + \pi J \delta) sin(\pi J \tau_2) sin(\omega_S \delta)$$

The next step is free evolution for τ_1, which will not transform I spin magnetization or zero/double quantum terms into observable S spin magnetization. This means the only terms that need to be kept from ρ_9 are:

$$\rho_9' = - c_S S_y cos(2\pi J \tau_1) cos(\omega_S \tau_1) cos(\pi J \tau_2) cos(\pi J \delta) + c_S 2I_z S_x cos(2\pi J \tau_1) cos(\omega_S \tau_1) cos(\pi J \tau_2) sin(\pi J \delta)$$

$$- c_S S_x cos(2\pi J \tau_1) sin(\omega_S \tau_1) cos(\pi J \tau_2) cos(\pi J \delta) - c_S 2I_z S_y cos(2\pi J \tau_1) sin(\omega_S \tau_1) cos(\pi J \tau_2) sin(\pi J \delta) \rightarrow$$

$$\rho_X = - c_S S_y cos(2\pi J \tau_1) cos(\pi J \tau_2) cos[\pi J(\tau_1 + \delta)] + c_S 2I_z S_x cos(2\pi J \tau_1) cos(\pi J \tau_2) sin[\pi J(\tau_1 + \delta)]$$

To help with interpretation, remember that $\tau_2 \ll \delta$ and as $\tau_2 \rightarrow 0$ then $\delta \rightarrow \tau_1$. Under this limit:

$$\rho_X = - c_S S_y cos(2\pi J \tau_1) cos(2\pi J \tau_1) + c_S 2I_z S_x cos(2\pi J \tau_1) sin(2\pi J \tau_1)$$

Which, sure enough, looks just like a Hahn echo on the S spin. The additional pulses in the HMBC are there to filter out the ^1J couplings and leave you with a 2D mapping of S spins that are next nearest neighbors (and beyond) to I spins. But even doubly filtered sometimes ^1J couplings bleed through, so the HMBC is generally not I spin decoupled during t_2 (like the HSQC is) to facilitate identifying these signals. Another oddity that often occurs is that on phenyl rings (and similar) the next nearest coupling is too strong and is filtered out, so the HMBC “skips around the ring”. Experimental conditions may be adjusted to ameliorate these flaws.