CHEM / BCMB   4190/6190/8189

Introductory NMR

Lecture 16
Two-Dimensional Correlated NMR Spectroscopy

1. Two-Dimensional Heteronuclear (C,H)-Correlated NMR Spectroscopy (HETCOR or C,H-COSY):

HETCOR: \textbf{HETeronuclear COR}relation

C,H-COSY: \textbf{Correlated SpectroscopY} (Observed nuclei is first)

A) Pulse sequence and vector diagram
Let's consider a two-spin AX system with $A = ^1H$ and $X = ^{13}C$ ($^{13}$CHCl3)

During $t_1$:

- $\nu(\text{MH}\{C_\alpha\}) = \nu_H - 1/2*J_{CH}$; $\nu(\text{MH}\{C_\beta\}) = \nu_H + 1/2*J_{CH}$
- $\nu_H =$ Larmor frequency in the absence of coupling
  (Here $\nu_H >$ frequency of the rotating frame)
- Ignore effect of relaxation and field inhomogeneity
- $\phi_\alpha = 2\pi(\nu_H - 1/2*J_{CH})t_1$; $\phi_\beta = 2\pi(\nu_H + 1/2*J_{CH})t_1$
  $\Theta = \phi_\alpha - \phi_\beta = 2\pi J_{CH}* t_1$

After the second $^1H$ 90°x' pulse:

- $^1H$ magnetization is transferred to the x'-z plane
- The z-magnetization components are proportional to the population differences:
  - N1 and N3 for MH{C_\alpha}
  - N2 and N4 for MH{C_\beta}
- In Figure 9-10 the populations of N1 and N3 are partially inverted and the population difference between N2 and N4 is modified:
- In general, the population differences depend on $t_1$, $\nu_H$, and $J_{CH}$.
- Population transfer from $^1H$ to $^{13}C$ as in SPI and INEPT, although here the transfer depends on $t_1$.

After the $^{13}C$ 90°x' pulse:

- Turns the two longitudinal $^{13}C$ vectors $+z$ and $-z$ into the $+y'$ and $-y'$ directions, respectively.
- Two frequencies are detected by the receiver:
  - $\nu_C - 1/2*J_{CH}$
  - $\nu_C + 1/2*J_{CH}$
B) Spectrum

- FT with respect to $t_2$ gives two $^{13}\text{C}$ signals ($\nu_{^{13}\text{C}} - \frac{1}{2}\nu_{\text{CH}}$ and $\nu_{^{13}\text{C}} + \frac{1}{2}\nu_{\text{CH}}$) along the $F_2$ axis (one positive and one negative); these signals are modulated in $t_1$ by $\nu_{^1\text{H}}$ and $J_{\text{CH}}$.
- FT with respect to $t_1$ gives two $^1\text{H}$ signals ($\nu_{^1\text{H}} - \frac{1}{2}\nu_{\text{CH}}$ and $\nu_{^1\text{H}} + \frac{1}{2}\nu_{\text{CH}}$) for each $^{13}\text{C}$ signal along the $F_1$ axis.
- 2D NMR spectrum has therefore 4 signals, 2 with negative amplitude.
- $^1\text{H}$ decoupling during acquisition would remove the signal.

*Figure 9-11.*
Schematic two-dimensional C,H-correlated NMR spectrum of a two-spin AX system (for pulse sequence see Fig. 9-10). The two signals along the $F_2$-direction correspond to the one-dimensional $^{13}\text{C}$ NMR spectrum without decoupling, except that the signals have opposite signs. Along the $F_1$-direction is seen the doublet of the $^1\text{H}$ NMR spectrum with the C,H coupling (the $^{13}\text{C}$ satellites, also with opposite signal amplitudes).
2. Modified HETCOR Pulse Sequence to Remove Splitting in F2

A) Pulse sequence

- Insertion of a delay $1/(2J_{CH})$ between the $^{13}C\ 90^\circ$ pulse and the acquisition of the FID, which allows refocusing of MC{H$\alpha$} and MC{H$\beta$}.
- BB decoupling during acquisition cause MC{H$\alpha$} and MC{H$\beta$} to precess at the same rate during that time.

![Diagram of pulse sequence]

$$\Delta_2 = 1/(2J_{CH})$$

B) Spectrum

- After FT with respect to $t_2$, only one signal is observed in F2 with a frequency $\nu_{C}$, and this signal is modulated in $t_1$ by $\nu_{H}$ and $J_{CH}$.
- FT with respect to $t_1$ gives two $^1H$ signals ($\nu_{H} - 1/2*J_{CH}$ and $\nu_{H} + 1/2*J_{CH}$) along the F1 axis.

![Diagram of spectrum]

**Figure 9-13.** Schematic two-dimensional C,H-correlated NMR spectrum of a two-spin AX system (pulse sequence as in Fig. 9-12). The 2D spectrum is reduced to two signals with opposite signs; their separation along the $F_1$ frequency axis is equal to $J (C,H)$. 


3. Modified HETCOR Pulse Sequence to Remove Splitting in F1 and F2

A) Pulse sequence

B) Vector Diagram

- Insertion of a $^{13}$C 180° pulse in the middle of $t_1$, which allows refocusing of $MH(C\alpha)$ and $MH(C\beta)$.
- Insertion of a delay $1/(2J_{CH})$ after $t_1$ and before the second $^1$H 90° pulse. This constant delay is needed for optimal population transfer. After a delay of $1/(2J_{CH})$ $MH(C\alpha)$ and $MH(C\beta)$ have a 180° phase difference.
- The magnitude of the polarization transfer depends only on $\varphi$, which is independent of $J_{CH}$. $\varphi\alpha = \varphi\beta = 2\pi (\nu_H) t_1$

Figure 9-14. A: Pulse sequence for a two-dimensional C,H-correlated NMR experiment which reduces the 2D spectrum of a two-spin AX system to only one peak. B: The vector diagrams a to f show the positions of the $^1$H magnetization vectors $M_{H1}^{t=0}$ and $M_{H1}^{t=0}$ or their $z$-components (f) at the instants indicated in A; in diagrams a to d only the $x',y'$-plane is shown.
C) Spectra

- FT with respect to $t_2$ gives one $^{13}$C signal along the $F_2$ axis with a frequency $\nu_C$, and this signal is modulated in $t_1$ by $\nu_H$ only.
- FT with respect to $t_1$ gives one $^1$H signal along the $F_1$ axis with a frequency $\nu_H$.

For $^{13}$CHCl$_3$:

![Diagram showing $^{13}$C and $^1$H signals]

For more complex molecules:

![Diagram showing correlation peaks]

NOTE: 1) Easy to assign $^{13}$C signals if $^1$H signals are assigned, or vice versa. 2) Little overlap of the correlation peak.
4. Two-Dimensional Homonuclear (H,H)-Correlated NMR Spectroscopy. H,H-COSY:

A) Pulse sequence

Let's consider the case where $\Theta x' = 90^\circ x'$ and let's consider a homonuclear two-spin AX system.

First 90°x' pulse:

- Tilt both vectors $M_A$ and $M_X$ along $y'$
- Due to $J_{AX}$, $M_A$ has two components $M_A(X_\alpha)$ and $M_A(X_\beta)$, and $M_X$ has two components $M_X(A_\alpha)$ and $M_X(A_\beta)$.

During $t_1$:

- $\nu_A (X_\alpha) = \nu_A - 1/2*J_{AX}$; $\nu_A (X_\beta) = \nu_A + 1/2*J_{AX}$
- $\nu_X (A_\alpha) = \nu_X - 1/2*J_{AX}$; $\nu_X (A_\beta) = \nu_X + 1/2*J_{AX}$

- $\varphi(M_A(X_\alpha))=2\pi (\nu_A - 1/2*J_{AX}) t_1$; $\varphi(M_A(X_\beta))=2\pi (\nu_A + 1/2*J_{AX}) t_1$
- $\varphi(M_X(A_\alpha))=2\pi (\nu_X - 1/2*J_{AX}) t_1$; $\varphi(M_X(A_\beta))=2\pi (\nu_X + 1/2*J_{AX}) t_1$

- At the end of $t_1$, the vectors have components along $x'$ and $y'$
After the second 90° x' pulse:

- The y and -y vector components are tilted along z and -z, which results in polarization transfer. The transfer depends on \( t_1, \nu \), and \( J_{AX} \).
- Four frequencies are detected by the receiver:
  \[
  A_2 = \nu A (X_\alpha) \\
  A_1 = \nu A (X_\beta) \\
  X_2 = \nu X (A_\alpha) \\
  X_1 = \nu X (A_\beta)
  \]

B) Magnitude Spectrum

- FT with respect to \( t_2 \) yields four signals at \( A_1, A_2, X_1, \) and \( X_2 \). These signals are modulated in \( t_1 \) with these same four frequencies.
- FT with respect to \( t_1 \) gives a 2D NMR spectrum with four groups of signals, each containing four signals.
  - Groups centered at \( (\nu A, \nu A) \) and \( (\nu X, \nu X) \) are diagonal peaks.
  - Groups centered at \( (\nu A, \nu X) \) and \( (\nu X, \nu A) \) are cross peaks.
  - Within each group, separation in \( F_1 \) and \( F_2 \) is \( J_{AX} \).

**Figure 9-18.**
Schematic representation of a COSY experiment on a two-spin AX system in which A and X are protons. The signal amplitudes are shown here as absolute values. In an actual spectrum the peaks on the diagonal are dispersion signals, while the correlation peaks (cross peaks) are absorption signals with alternating signs. The diagonal peaks of a pair of mutually coupled nuclei and their cross peaks form the corners of a square.
C) Phase-Sensitive Spectrum

Figure 5.19. The phase-sensitive COSY for a coupled two-spin AX system. Diagonal peaks have broad, in-phase double-dispersion lineshapes (D), whereas crosspeaks have narrow, antiphase double- absorption lineshapes (A), as further illustrated in the row extracted from the spectrum.
5. Two-Dimensional Homonuclear (H,H)-Correlated NMR Spectroscopy. **COSY-45:**

Same as COSY-90, but second $^1$H pulse is $45^\circ x'$ instead of $90^\circ x'$. Reduces the intensity of the signal, but simplifies the spectrum.
6. Two-Dimensional Homonuclear (H,H)-Correlated NMR Spectroscopy. Long-range COSY:

A fixed delay $\Delta$ (0.1 to 0.4 s) is added before and after the second $^1$H 90°x' pulse:

$$90^\circ_x - t_1 - \Delta - 90^\circ_x - \Delta - \text{FID}$$

Allows development of correlation effects for very weakly coupled $^1$H.