INEPT, HSQC, HMQC, HMBC

BCMB/CHEM 8190
Spring, 2005
$^{1}\text{H-}^{15}\text{N}$ Pairs in Amide Bonds of Proteins Provide Convenient Reporters at Each Amino Acid Along the Backbone Just Requires $^{15}\text{N}$ Labeling
HSQC Spectrum of H-N Amides in a Protein

ACP 0.5 mM
Buffer: MES 25mM, CaCl2 5mM, DTT 5mM

gNHQC spectrum

80% apo-ACP, 20% holo-ACP
Heteronuclear Magnetization Transfer as Used in the INEPT and HSQC Experiments

\[ ^1H(\text{I}) \quad ^{15}N(\text{S}) \]

\[ \tau \quad 90-x \quad 180y \quad 90y \quad \tau \]

\[ \text{Iz} \rightarrow -\text{Iy} \rightarrow -2\text{IxSz} \rightarrow 2\text{IzSy} \quad \tau = 1/4J \]
**Evolution and Detection in HSQC**

Evolution and detection in HSQC involves a series of pulse sequences. The diagram illustrates the timing and effects of these pulses.

1. **Sz (ωS, t1)** (180 decouples I)
   - 2IzSy
   - $2IzSy \cos(\omega S t1) - 2IzSx \sin(\omega S t1)$

2. **Ix(π/2)+Sx(π/2)**
   - -2IySz \cos(\omega S t1) + 2IySx \cos(\omega St1) (unobserved 2Q)

3. **IzSz (J2τ)** (chem shift refocuses)
   - -Ix \cos(\omega S t1)

4. **Iz (ωI, t2)**
   - -Ix \cos(\omega S t1)cos(\omega I t2) -Iy \cos(\omega S t1)\sin(\omega I t2)
Obtaining Quadrature: Substitute a 90° $^{15}\text{N}$ Pulse and Save Separately (States)

$I_z \rightarrow -I_y \rightarrow -2I_xS_z \rightarrow -2I_zS_x \quad \tau = 1/4J$

Sz ($\omega S$, t1) (180° decouples I)

-2I_zS_x $\rightarrow$ -2I_zS_x cos($\omega S$ t1) + 2I_zS_y sin($\omega S$ t1)
Important Features of HSQC Spectra

• Start with $^1$H magnetization: gain $\gamma_H / \gamma_N$
  A factor of 10 in sensitivity
• Detect $^1$H magnetization: gain $(\gamma_H / \gamma_N)^2$
  Another factor of 100
• Even though you must acquire 32-64 t1 points, far more sensitive than direct detection.
$^1$H-$^{13}$C HSQC can be run at Natural Abundance
Spectral Display of HMQC Looks Just Like HSQC

\[ ^1H(I) ] \begin{array}{c}
\tau \\
90x \\
180x \\
180x \\
t_2
\end{array}
\begin{array}{c}
\tau \\
90y \\
t_1/2 \\
t_1/2 \\
\tau \\
\tau \\
\text{decouple}
\end{array}
\begin{array}{c}
15N(S) \\
90y \\
180x
\end{array}

Iz \rightarrow -Iy \rightarrow -2IxSz \rightarrow -2IxSx

-2IxSx = (-IxSx + IySy) + (-IxSx –IySy) = zero and 2Q coherence

180x changes sign of IySy parts, hence interchanges zero and 2Q

Evolves as chemical shift of 15N, just like HSQC

Differences in relaxation properties and lack of direct coupling
HMBC – coherence transfers can be tuned to eliminate one-bond coupling and emphasize long-range couplings

Choose \( \tau = \frac{1}{4}J_{IS1} \) for three-bond: \( Iz \rightarrow -Iy \rightarrow -2IxSz \rightarrow -2IxSx \)

\[-2IxSx = (-IxSx + IySy) + (-IxSx -IySy) = \text{zero and } 2Q \text{ coherence}\]

Choose \( \tau = (2n+1)\frac{1}{2}J_{IS1} \) for one-bond: \( -Iy \rightarrow Iy \rightarrow Iz = \text{no signal} \)
HMBC of Carbohydrates is Useful in Identifying Linkages
• Wouldn’t it be nice to connect resources at opposite ends of the spin system? \(\Rightarrow\) TOCSY

**TOCSY**: Total Correlation Spectroscopy

a.k.a. **HOHAHA**

(Homonuclear Hartmann-Hahn)

\[
\begin{array}{c}
90^\circ & t_1 & \Delta & t_m & t_2 \\
\end{array}
\]

• \(\Delta\): short delay (~20\,$\mu$s) to change transmitter power

• DipSI-2: isotropic mixing to transfer magnetization through several couplings during \(t_m\)

• \(t_m\): mixing time
PRODUCT OPERATOR:

\[ I_{12}^{90^\circ - \tau, -90^\circ} \rightarrow -I_{12} \cos(\omega t \tau) \cos(\tau Jt_i) - 2I_{1x} I_{2y} \cos(\omega t \tau) \sin(\tau Jt_i) + I_{1x} \sin(\omega t \tau) \cos(\tau Jt_i) - 2I_{1x} I_{2y} \sin(\omega t \tau) \sin(\tau Jt_i) \]

JUST LIKE \( \cos \gamma \)!

2I_{1x} I_{2y} and I_{1x} : eliminated by phase cycle

2I_{1x} I_{2y} : dephased by rf inhomogeneity of isotropic mixing scheme(D3)

\[ -I_{12} \cos(\omega t \tau) \cos(\tau Jt_i) \rightarrow I_m \]

\[ \sum_{k=1}^{n} I_{12} a_{1k}(t_m) \cos(\omega t \tau) \cos(\tau Jt_i) \]

where \( a_{1k}(t_m) \) = mixing coefficient for transfer of magnetization from I1 to Ik

NOTE: 2\( R \) terms ignored.
Isotropic Mixing

- In general, Hamiltonian for a spin system contains chemical shift and scalar coupling interaction terms

\[ H = \sum_{i} -\omega_i I_{z} + \sum_{i \neq j} \pi I_{i} \cdot I_{j} \]

- Here we want to remove \(-\omega_i I_{z}\) terms such that magnetization is transferred under the strong scalar coupling Hamiltonian:

\[ H' = 2\pi \sum I_{i} \cdot I_{j} \]

where \( I_{i} \cdot I_{j} = I_{ix}I_{jx} + I_{iy}I_{jy} + I_{iz}I_{jz} \)
Isotropic mixing means:

Transfer of magnetization occurs equally well for all angular momentum components.

- Transfer is highly second order because there is no effective chemical shift difference between spins $A + X \rightarrow A_3$. If $\Delta A_1$ & $\Delta A_2$ ≠ 0 but $\Delta A_3 = 0$, still have transfer from $A \rightarrow X$.

- Product Operator in Cartesian set works only for first order systems; need new transformation rules from Liouville–von Neumann equation (Ernst).

- DiPSI-3: Very efficient mixing scheme (better than Waltz-16, WISE-17) developed by Shaka and coworkers.

\[ R = 320 \overline{410} \overline{290} \overline{285} 30 \overline{245} 375 \overline{265} 370 \]

Supercycle: $R \overline{R} \overline{R} \overline{R}$
Practical considerations:

1) In TOCSY, both crosspeaks and diagonal peaks are in phase and can both be phased in absorptive mode.

2) Crosspeaks do not indicate direct coupling necessarily. Magnetization can be transferred via $^{1}J$ & $^{3}J$ couplings.

3) The magnitude of a crosspeak depends on:
   - topology of the spin system
   - J couplings
   - efficiency of mixing sequence
   - relaxation during $T_1M$
**FIGURE 6.44** Variation of cross-peak intensity as a function of isotropic mixing time for an isoleucine spin system. (a) Cross-peak intensity for transfer from the $^1\text{H}^\alpha$ spin with the $^1\text{H}^\beta$ spin removed and (b) cross-peak intensity for magnetization transfer from the $^1\text{H}^\gamma$ spin. The curves for the destination spins are (---) $^1\text{H}^\alpha$, (-----) $^1\text{H}^\beta$, (------------) $^1\text{H}^\gamma$, (-----) $^1\text{H}^{\alpha\beta}$, and (-----) $^1\text{H}^\gamma$. The transfer functions were calculated using the following coupling constants: $J_{\text{H}^\alpha\text{H}^\beta} = 10.0$ Hz, and $J_{\text{H}^\gamma\text{H}^\delta} = 12$ Hz. Vicinal couplings to methyl groups were 6.7 Hz, all geminal couplings were $-15$ Hz, and all other vicinal couplings were 7 Hz. The effect of relaxation during the mixing was not considered.
4) Optimum $T_m$?

1) Supercycle executed an integral number of time (3.4 ms)
2) Relaxation during $T_m$
3) Goal:
   - Efficient transfer of magnetization through a single $J$ coupling:
     e.g. $^{1}H_{\text{N}} - {H}_{\text{H}} \Rightarrow T_m \sim 30 - 50$ ms
   - Maximum transfer of magnetization between resonances at extreme ends of spin system
     $\Rightarrow T_m \sim 75 - 100$ ms
Figure 6.45 Sections of H_2O TOCSY spectra acquired with mixing times of 48 (left), 83 (center), and 102 ms (right). The cross-peaks observed to the amide protons of Lys29 and Lys46 are assigned at the different mixing times. Cross-peaks to the spin-system termini are observed only at the longer mixing times.