2D Correlation Experiments: HSQC, HMQC, HMBC,

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Two Dimensional NMR Spectroscopy

- Two-dimensional (2D) NMR spectra are presented along two orthogonal axes (rather than one for 1D NMR)
  - typically, the two axes are chemical shifts (correlation spectroscopy), but not limited to this (i.e. $J$-coupling, etc.)
  - convention is typically for directly observed dimension to be presented along the x-axis
  - correlations between x- and y-axis variables based on:
    - $J$ coupling (COSY, TOCSY, HSQC)
    - dipolar interactions (NOESY)
    - chemical exchange (EXSY)
Two Dimensional NMR Spectroscopy

- A general scheme for 2D experiments describes these as constructed from 'preparation', 'evolution' and 'mixing' elements
  - preparation: create magnetization of interest
  - evolution 1: increment $t_1$ time period, evolve magnetization with information of interest (gives $y$-axis information, indirectly observed dimension)
  - mixing: mix magnetization to get observable magnetization of interest
  - evolution 2: acquisition period (magnetization evolves, direct observation)

- Example: COSY (correlation spectroscopy)
  - correlates chemical shifts of coupled nuclei (usually $^1$H)
  - used as a stand-alone experiment for $^1$H-$^1$H correlation, and as an element in other pulse sequences for magnetization transfer/mixing
  - preparation is $d_1$ and first 90° pulse: initial $d_1$ period allows for recovery of magnetization ($T_1$ recovery), pulse creates initial transverse magnetization
  - evolution: magnetization evolves with chemical shift and scalar coupling
  - mixing period (second 90° pulse) uses scalar couplings to transfer magnetization between coupled spins to create the magnetization of interest
  - evolution 2 ($t_2$): magnetization evolves and is detected
Some Two-Dimensional NMR Experiments

- **COSY**: Correlation Spectroscopy
- **NOESY**: Nuclear Overhauser Effect Spectroscopy
- **TOCSY**: Total Correlation Spectroscopy
- **HSQC**: Heteronuclear Single Quantum Coherence

**Experiments Diagram**

- **COSY**
  - Preparation: 90°
  - Evolution: 180°, 90°
  - Mixing: 90°

- **NOESY**
  - Preparation: 90°
  - Evolution: t<sub>1</sub>
  - Mixing: τ<sub>m</sub>

- **TOCSY**
  - Preparation: 90°
  - Evolution: t<sub>1</sub>
  - Mixing: τ<sub>m</sub>

- **HSQC**
  - Preparation: 180°, 90°
  - Evolution: t<sub>1/2</sub>, t<sub>1/2</sub>

**Decoupling**

- Decoupling pulse: τ
2D-NMR - FIDs are transformed in $t_2$, then in $t_1$.
Three Dimensional NMR Spectroscopy

- In general, 3D experiments include the same elements as 2D experiments, just more of them
  - in a typical 3D experiment, there are three evolution periods, the first two corresponding to the two indirectly-detected dimensions ($t_1$ and $t_2$), and the third corresponding to the directly detected dimension ($t_3$).
  - often, two 2D experiments are combined (cut/paste) to create a 3D experiment (NOESY-HSQC, NOESY-TOCSY, etc.)

- Modern biomolecular NMR utilizes many types of 3D experiments for resonance assignment, NOE-based distance measurement, etcetera
Heteronuclear Single Quantum Coherence

- The Heteronuclear Single Quantum Coherence (HSQC) experiment is one of the most used experiments in biomolecular NMR
- the HSQC experiment is one of the fundamental building blocks of scores of multidimensional, heteronuclear and triple resonance NMR experiments
  - the HSQC experiment correlates chemical shifts of one nucleus to another (scalar coupled)
  - the \(^1\)H-\(^{15}\)N pairs in amide groups of amino acids in proteins are convenient reporters for each amino acid
  - the \(^1\)H, \(^{15}\)N-HSQC spectrum of a protein is a "fingerprint", that can be used to monitor structural changes (ligand binding, solution conditions, etc.)
  - for highest sensitivity, uniform \(^{15}\)N labeling is used, but for more concentrated samples, even natural abundance samples can be analyzed with modern, high-sensitivity instrumentation and cryogenic probes
  - not at all limited to \(^1\)H-\(^{15}\)N: \(^1\)H-\(^{13}\)C important for organic chemistry as well as biomolecular NMR
HSQC Spectrum of Amide H-N Pairs in a Protein

- HSQC spectrum of a protein (calmodulin) in the unbound state and bound to a drug ("W-7")
- The HSQC experiment is one of the fundamental building blocks of scores of multidimensional, heteronuclear and triple resonance NMR experiments
- Chemical shift of amide $^1$H correlated to directly bonded $^{15}$N, for each amino acid (notice chemical shift ranges)

Ikura and coworkers, 1988

N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide

HSQC Spectrum of Amide H-N Pairs in a Protein (Calmodulin) in the Unbound State and Bound to a Drug ("W-7")

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Ikura and coworkers, 1988

N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide
HSQC Spectra of Other Nuclear Pairs

- HSQC (and HMQC, see later) are used to correlate many types of nuclei
- for biomolecular NMR, mostly $^1$H-$^{15}$N, $^1$H-$^{13}$C, $^1$H-$^{31}$P, etc.
- example below: $^{13}$C-$^{103}$Rh
Preparation Period for HSQC Experiment

- The INEPT sequence serves as the 'preparation' period for the HSQC experiment (and many other experiments)
  - remember, $^1$H pulses do not excite $^{15}$N, and vice versa (so, two 'channels')
  - magnetization is transferred from $^1$H to $^{15}$N in order to improve the polarization of $^{15}$N (sensitivity improved by $\gamma_{^1H}/\gamma_{^{15}N} \sim 10$) and to allow chemical shift evolution (during $t_1$) that depends on $^{15}$N chemical shift
  - initial $z$-magnetization is converted to $-y$ (90° $x$), then to antiphase $x$-magnetization (following $\tau$-180-$\tau$, no chemical shift evolution)
  - $^1$H 90° $y$ and $^{15}$N 90° $x$) convert to antiphase $^{15}$N magnetization!!

- for this pulse sequence, $\tau$ MUST be equal to $1/(4J)$
- for amide $^1$H-$^{15}$N groups in proteins, $J$ is large and very uniform (~95 Hz), so, transfer is efficient (fast, $\tau \approx 2.6$ ms), which minimizes $T_2$ magnetization losses
Preparation Period for HSQC Experiment

- Detailed product operator calculation of the preparation period (INEPT)

- Initial z magnetization on $^1H$ ($I_{1z}$ or $I_z$) is converted to -y magnetization by the first 90° $^1H$ pulse

- Just before the final 90° pulses, the magnetization is antiphase x-magnetization on $^1H$ ($I_{1x}I_{2z}$ or $I_xS_z$)

- The $I_{2z}$ term is removed by subtracting the results obtained with the final $^1H$ pulse applied along y and -y
Preparation Period for HSQC Experiment

- We described earlier the equilibrium density matrix (single spin)
- there is no net chemical shift evolution during $\tau$ - 180° - $\tau$ period (chemical shift evolution is refocused, as long as both spin 1 and spin 2 each experience a 180° pulse)

$$
\begin{align*}
\frac{\pi}{2} l_{1x} + l_{2z} & \quad \rightarrow \quad -l_{1y} + l_{2z} \\
2\pi J_{1z,2z} t & \quad \rightarrow \quad -l_{1y} \cos(\pi J_{1z,2z} t) + 2l_{1x} l_{2z} \sin(\pi J_{1z,2z} t) + l_{2z} \\
\pi l_{1y} \pi l_{2y} & \quad \rightarrow \quad -l_{1y} \cos(\pi J_{1z,2z} t) + 2l_{1x} l_{2z} \sin(\pi J_{1z,2z} t) - l_{2z} \\
2\pi J_{1z,2z} t & \quad \rightarrow \quad -l_{1y} \cos^2(\pi J_{1z,2z} t) + 2l_{1x} l_{2z} \cos(\pi J_{1z,2z} t) \sin(\pi J_{1z,2z} t) \\
& \quad \quad + 2l_{1x} l_{2z} \cos(\pi J_{1z,2z} t) \sin(\pi J_{1z,2z} t) + l_{1y} \sin^2(\pi J_{1z,2z} t) - l_{2z} \\
\text{simplify} & \quad \rightarrow \quad -l_{1y} \cos(2\pi J_{1z,2z} t) + 2l_{1x} l_{2z} \sin(2\pi J_{1z,2z} t) - l_{2z} \\
t = \tau = 1/(4J) & \quad \rightarrow \quad +2l_{1x} l_{2z} - l_{2z} \\
\frac{\pi}{2} l_{1y} \frac{\pi}{2} l_{2x} & \quad \rightarrow \quad +2l_{1z} l_{2y} + l_{2y} \\
or & \quad \quad (-2l_{1z} l_{2y} + l_{2y}) - (+2l_{1z} l_{2y} + l_{2y}) = -4l_{1z} l_{2y} \quad or \quad -2l_{1z} l_{2y}
\end{align*}
$$
**Evolution and Decoupling during $t_1$**

- The $t_1$ evolution period includes a 180° $^1$H pulse in the center
  - using vector diagrams, the role of this pulse can readily be visualized
  - during the first $t_1/2$ period the component vectors of the antiphase $^{15}$N magnetization ($^{15}$N with $^1$H in the $\alpha$ state – $S^{I\alpha}$, and $^{15}$N with $^1$H in the $\beta$ state – $S^{I\beta}$) rotate according to the Larmor frequency of the $^{15}$N nucleus and move apart from one another according to the scalar coupling, $J_{IS}$
  - the 180° $^1$H pulse exchanges the $^1$H $\alpha$ and $\beta$ populations (vectors are exchanged), and the second $t_1/2$ period refocuses the vectors (antiphase magnetization restored)
  - chemical shifts are NOT refocused, $^{15}$N-$^1$H couplings ARE refocused (no net evolution of coupling)
  - so, during $t_1$, signal is modulated by $^{15}$N chemical shift, NOT $J_{IS}$

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**Diagram:**

- $^1$H pulses at 0°, 180°, and 90°
- $^{15}$N pulses at 0°, 180°, and 90°
- $t_1$ evolution period
- $t_1/2$ period
- Decoupling pulse
- Vector diagrams illustrating the evolution and coupling effects.
Evolution and Decoupling during $t_1$

- During the $t_1$ evolution period the $^{15}$N antiphase $y$-magnetization evolves into $y$- and $x$-antiphase magnetization
  - the analysis here ignores scalar coupling, as we demonstrated that the $^1$H $180^\circ$ pulse centered in the $t_1$ evolution period refocused the couplings
  - below, chemical shift evolution and the $^1$H $180^\circ$ pulse are considered

$$-2l_{1z}l_{2y} \xrightarrow{\Omega_2 l_{2z} t_{1/2}} -2l_{1z}l_{2y} \cos(\Omega_2 t_1/2) + 2l_{1z}l_{2x} \sin(\Omega_2 t_1/2)$$

$$\pi l_{1x} \rightarrow 2l_{1z}l_{2y} \cos(\Omega_2 t_1/2) - 2l_{1z}l_{2x} \sin(\Omega_2 t_1/2)$$

$$\Omega_2 l_{2z} t_{1/2} \rightarrow 2l_{1z}l_{2y} \cos^2(\Omega_2 t_1/2) - 2l_{1z}l_{2x} \cos(\Omega_2 t_1/2) \sin(\Omega_2 t_1/2)$$

$$-2l_{1z}l_{2x} \cos(\Omega_2 t_1/2) \sin(\Omega_2 t_1/2) - 2l_{1z}l_{2y} \sin^2(\Omega_2 t_1/2)$$

simplify $\rightarrow 2l_{1z}l_{2y} \cos(\Omega_2 t_1) - 2l_{1z}l_{2x} \sin(\Omega_2 t_1)$

- The $^{15}$N $y$- and $x$-antiphase magnetization present following the $t_1$ evolution period is modulated by the rotating frame chemical shift of the $^{15}$N nucleus ($\Omega_2$)
  - this is how the $^{15}$N chemical shift ultimately modulates the final signal detected in $t_2$, and how the $^{15}$N chemical shift is observed in the second dimension
\[ \text{\(^{15}\text{N} \rightarrow ^{1}\text{H} \) Magnetization Transfer and Detection} \]

- Following the \( t_1 \) evolution period, the antiphase \(^{15}\text{N} \) magnetization is converted to antiphase \(^{1}\text{H} \) magnetization (and a multiple quantum term) by the \(^{15}\text{N} \) and \(^{1}\text{H} \) 90° pulses \(^{15}\text{N} \), \(^{1}\text{H} \).

- The \( \tau \)-180°-\( \tau \) period results in \( x \)-magnetization modulated by the \(^{15}\text{N} \) chemical shift (and the multiple quantum term, that is not observable).

\[
\begin{align*}
2l_x l_y \cos(\Omega_2 t_1) &- 2l_x l_z \sin(\Omega_2 t_1) & 2l_x l_y \cos(\Omega_2 t_1) \cos(\pi J_{1,2} \tau) + l_x \sin(\pi J_{1,2} \tau) \quad &2l_x l_z \cos(\Omega_2 t_1) + 2l_y l_z \sin(\Omega_2 t_1) \\
\frac{2\pi J_{1,2} l_x l_z \tau}{2} &- 2l_y l_z \cos(\Omega_2 t_1) \cos(\pi J_{1,2} \tau) + l_x \cos(\Omega_2 t_1) \sin(\pi J_{1,2} \tau) - 2l_y l_z \sin(\Omega_2 t_1) \\
\frac{\pi l_y l_z \tau}{2} &- 2l_y l_z \cos(\Omega_2 t_1) \cos^2(\pi J_{1,2} \tau) - l_x \cos(\Omega_2 t_1) \cos(\pi J_{1,2} \tau) \sin(\pi J_{1,2} \tau) \\
&\quad - 2l_y l_z \cos(\Omega_2 t_1) \sin^2(\pi J_{1,2} \tau) \\
&\quad - 2l_y l_z \sin(\Omega_2 t_1) \\
&\quad \text{simplify} \\
&\quad \tau = 1/(4J) \\
&\quad \Omega \lVert t_2 \\
&\quad \lvert l_x \cos(\Omega_2 t_1) - 2l_y l_z \sin(\Omega_2 t_1) \rvert \quad \text{second term is multiple quantum, not observed} \\
&\quad \Omega \lVert t_2 \\
&\quad \lvert l_x \cos(\Omega_2 t_1) \cos(\Omega_2 t_2) - l_y \cos(\Omega_2 t_1) \sin(\Omega_2 t_2) \rvert \\
&\quad \text{The final evolution period (} t_2 \text{) results in transverse magnetization modulated by the } {^{1}\text{H}} \text{ and } {^{15}\text{N}} \text{ chemical shifts}.
\]
A Note on Decoupling

During the $t_1$ evolution period, the $180^\circ$ $x$ pulse eliminates net evolution of the $^1H$-$^{15}N$ scalar coupling (eliminates splitting of the $^{15}N$ signal by directly bonded $^1H$).

During the $t_2$ evolution period (acquisition), the 'decouple' element ($^{15}N$ channel) eliminates $^{15}N$ coupling to $^1H$ (eliminates splitting of the $^1H$ signal by directly bonded $^{15}N$).

- This is accomplished by decreasing the lifetimes of the $\alpha$ and $\beta$ states for $^{15}N$ by rapidly interconverting them with many back-to-back RF pulses.
- There are many such 'broadband' decoupling schemes (names you may encounter include 'Waltz', 'MLEV', 'GARP', 'DIPSI', etcetera).

So, rather than each signal consisting of 4 peaks, no splitting in either dimension is observed, and a single peak results.
Quadrature Detection in the Indirect Dimension

- As with the directly detected dimension (t₂), in the indirect dimension (t₁) we would like to place the carrier in the center of the chemical shift range, thus necessitating quadrature detection
  - this is accomplished by changing the phase of the first $^{15}\text{N}$ 90° pulse from 'x' to 'y'
  - the resulting term changes from $I_z S_y$ (x-pulse) to $I_z S_x$ (y-pulse), so the $^{15}\text{N}$ magnetization created with the y-pulse is orthogonal to that created with the x-pulse (i.e. 90 degrees out of phase)

- Magnetization collected during t₂ is stored separately for the x- and y-pulses, and serves as the real/imaginary components for quadrature

- This method for indirect dimension quadrature detection is called hypercomplex or 'States' (after D. J. States)...also TPPI, States-TPPI, etc.

\[
\begin{align*}
\text{x:} & \quad I_z \rightarrow -I_y \rightarrow -2I_z S_z \rightarrow -2I_z S_y \rightarrow 2I_z S_y - 2I_z S_x \rightarrow -2I_y S_z + 2I_y S_x \rightarrow -I_x \cos(\Omega_2 t_1) \\
\text{y:} & \quad I_z \rightarrow -I_y \rightarrow -2I_z S_z \rightarrow -2I_z S_x \rightarrow 2I_z S_x - 2I_z S_y \rightarrow +2I_y S_z - 2I_y S_x \rightarrow +I_x \sin(\Omega_2 t_1)
\end{align*}
\]
Important Features of $^1$H, $^{15}$N-HSQC Spectra

- Experiment begins with $^1$H magnetization, then polarization is transferred to $^{15}$N: gain $\gamma^1$H/$\gamma^{15}$N factor in sensitivity (~10)
- Experiment ends with $^{15}$N magnetization transferred back to $^1$H for detection
  - theoretical sensitivity gain $(\gamma^1$H/$\gamma^{15}$N)$^3$ for detecting $^1$H vs $^{15}$N (~1000)
  - actual gain for transferring from $^{15}$N to $^1$H for detection is $\sim(\gamma^1$H/$\gamma^{15}$N)$^{3/2} \approx 30$

- For a 2D experiment, must acquire enough (complex) points in $t_1$ for the required resolution in the indirect dimension
  - can be time consuming, especially if many scans are required per fid for S/N
  - each $t_1$ time point is actually 2 separate points (real/imaginary pairs, for quadrature)
  - not uncommon to acquire 128 or 256 complex points in $t_1$ for high resolution

- For samples of higher concentration, with high sensitivity cryogenic probes, is possible to acquire spectra at natural isotopic $^{15}$N abundance (0.37%, so this is not routine)
$^{1}H$, $^{13}C$-HSQC

- Used ubiquitously in biomolecular NMR and small molecule NMR
  - for small molecules, with high concentrations, acquisition at natural isotopic abundance (~1.1%) is routine
  - example: mixture of $D$-glucose and $D$-xylose (5 mM each, 40 minute total acquisition time)

- gain $\gamma^{1}H/\gamma^{13}C$ factor in sensitivity (~4) for polarization transfer
- gain $(\gamma^{1}H)^3/(\gamma^{13}C)^3$ factor in sensitivity for detection (~64)
- Heteronuclear Multiple Quantum Coherence

- **HMQC** is an analogue of the HSQC
  - information content, spectral display are the same as HSQC
- In HMQC, multiple quantum magnetization evolves during $t_1$
  - these evolve as sums (two-quantum coherence) and differences (zero-quantum coherence) of $^1\text{H}$ and $^{15}\text{N}$ chemical shifts ($\Omega_1$ and $\Omega_S$)
  - rather than allow these to evolve during the entire $t_1$ period, the $^1\text{H}$ 180° pulse
    exchanges density matrix elements for zero- and two-quantum coherences

\[ -2I_{1x}I_{2x} \xrightarrow{\Omega_1 \tau \Omega_2 \tau} -2I_{1x}I_{2x} \cos(\Omega_1 t/2)\cos(\Omega_2 t/2) - 2I_{1y}I_{2x} \sin(\Omega_1 t/2)\cos(\Omega_2 t/2) \]

\[ -2I_{1x}I_{2y} \cos(\Omega_1 t/2)\sin(\Omega_2 t/2) - 2I_{1y}I_{2y} \sin(\Omega_1 t/2)\sin(\Omega_2 t/2) \]

\[ \xrightarrow{\pi \tau} -2I_{1x}I_{2x} \cos(\Omega_1 t/2)\cos(\Omega_2 t/2) + 2I_{1y}I_{2x} \sin(\Omega_1 t/2)\cos(\Omega_2 t/2) \]

\[ -2I_{1x}I_{2y} \cos(\Omega_1 t/2)\sin(\Omega_2 t/2) + 2I_{1y}I_{2y} \sin(\Omega_1 t/2)\sin(\Omega_2 t/2) \]

- result (see next pages) is just evolution according to $^{15}\text{N}$ chemical shift ($\Omega_S$)

- $I_z \rightarrow -I_y \rightarrow -2I_xS_z \rightarrow -2I_xS_x \rightarrow -2I_xS_x - 2I_xS_y \rightarrow -2I_xS_x - 2I_xS_z \rightarrow I_y\sin(\Omega_2 t_1)$
**Heteronuclear Multiple Quantum Coherence**

- Why use HMQC? Why use HSQC?
  - information content basically the same
  - there are differences based on the relaxation of multiple quantum magnetization as opposed to single quantum
  - there are differences based on the dipolar broadening of multiple quantum coherence as opposed to single quantum
  - there are differences based on unresolved couplings that broaden signals in the directly detected dimension
  - multiple quantum magnetization does not evolve with scalar coupling (can be an advantage)
  - these can be different for $^1$H-$^{15}$N versus $^1$H-$^{13}$C, and size of the molecule
  - these can be subtle, and depend on the application

Preparation Period for HMQC Experiment

- For this version of the HMQC experiment, the preparation period is identical to the HSQC experiment, except for the final 90° pulse (for HMQC, only a $^{15}$N 90° pulse, no $^1$H 90° pulse)

\[
\begin{align*}
I_z + I_{2z} \xrightarrow{\pi/2 I_x} & -I_y + I_{2z} \\
2\pi J_{1,2} I_z I_{2z} t \xrightarrow{\pi I_y \pi I_{2y}} & -I_y \cos(\pi J_{1,2} t) + 2I_{1x} I_{2z} \sin(\pi J_{1,2} t) + I_{2z} \\
2\pi J_{1,2} I_z I_{2z} t \xrightarrow{\pi I_y \pi I_{2y}} & -I_y \cos(\pi J_{1,2} t) + 2I_{1x} I_{2z} \sin(\pi J_{1,2} t) - I_{2z} \\
\text{simplify} & -I_y \cos(2\pi J_{1,2} t) + 2I_{1x} I_{2z} \sin(2\pi J_{1,2} t) - I_{2z} \\
& t = \tau = 1/(4J) + 2I_{1x} I_{2z} - I_{2z} \\
& \pi/2 I_{2y} \xrightarrow{\pi/2 I_{1y}} + 2I_{1x} I_{2z} - I_{2z} \\
\text{or} & (-2I_{1x} I_{2z} + I_{2x}) - (+2I_{1x} I_{2z} + I_{2x}) = -4I_{1x} I_{2z} \text{ or } -2I_{1x} I_{2z} \\
- \frac{\pi}{2} I_x \text{ (first 90° pulse)} \xrightarrow{-} & -2I_{1z} I_{2y} + I_{2x}
\end{align*}
\]

- initial z magnetization on $^1$H ($I_{1z}$, or $I_z$) is converted to -y magnetization by the first 90° $^1$H pulse

- just before the final 90° pulses, the magnetization is antiphase x-magnetization on $^1$H ($I_{1x} I_{2z}$, or $I_x S_z$)

- the 90° $^{15}$N pulse convert the antiphase x-magnetization on $^1$H ($I_{1x} I_{2z}$, or $I_x S_z$) to multiple quantum x-magnetization on $^{15}$N ($I_{1x} I_{2x}$ or $I_x S_x$) ($^{15}$N magnetization enhanced by $\sim \gamma^{1H}/\gamma^{15N} \approx 10$)

- The $I_{2z}$ term is removed by subtracting the results obtained with the initial $^1$H pulse applied along x and -x
Evolution and Decoupling During $t_1$

- During the $t_1$ evolution period the multiple quantum magnetization evolves with the chemical shift of both $^1H$ ($\Omega_1$) and $^{15}N$ ($\Omega_2$)
- however, the $180^\circ$ $^1H$ pulse changes the sign of the $I_y$ terms, which exchanges two-quantum and zero-quantum terms
- this results in final terms (end of $t_1$) that include only $^{15}N$ chemical shift ($\Omega_2$)
- in other words, $180^\circ$ $^1H$ pulse in effect refocuses $^1H$ chemical shift evolution
- $J$ coupling operator doesn't change multiple quantum terms (so, don't consider $J$ coupling)

$$-2I_{1x} I_{2x} \rightarrow -2I_{1x} I_{2x} \cos(\Omega_1 t/2)\cos(\Omega_2 t/2) - 2I_{1y} I_{2x} \sin(\Omega_1 t/2)\cos(\Omega_2 t/2)$$

$$-2I_{1x} I_{2y} \cos(\Omega_1 t/2)\sin(\Omega_2 t/2) - 2I_{1y} I_{2y} \sin(\Omega_1 t/2)\sin(\Omega_2 t/2)$$

- **simplify**
  $$-2I_{1x} I_{2x} \cos(\Omega_2 t) - 2I_{1x} I_{2y} \sin(\Omega_2 t)$$
Multiple Quantum $\rightarrow ^1\text{H}$ Magnetization Transfer and Detection

- 90° $^{15}\text{N}$ pulse converts multiple quantum back to antiphase $x$-magnetization ($I_xS_z$)
- remaining double quantum term is not observable
- the $\tau$ -180° - $\tau$ period results in $x$-magnetization modulated by the $^{15}\text{N}$ chemical shift (and the multiple quantum term, that is not observable)

\[
-2I_{1x}I_{2x}\cos(\Omega_2t) - 2I_{1y}I_{2y}\sin(\Omega_2t) \xrightarrow{\frac{\pi}{2}I_{2x}} -2I_{1x}I_{2x}\cos(\Omega_2t) - 2I_{1x}I_{2z}\sin(\Omega_2t)
\]

\[
2\pi J_{1,2}I_{1z}I_{2z} \tau
\]

\[
-2I_{1x}I_{2x}\cos(\Omega_2t) - 2I_{1y}I_{2z}\sin(\Omega_2t)\cos(\pi J_{1,2}t) - I_{1y}\sin(\Omega_2t)\sin(\pi J_{1,2}t)
\]

\[
\pi I_{1x} \pi I_{2x}
\]

\[
-2I_{1x}I_{2x}\cos(\Omega_2t) + 2I_{1x}I_{2z}\sin(\Omega_2t)\cos(\pi J_{1,2}t) + I_{1y}\sin(\Omega_2t)\sin(\pi J_{1,2}t)
\]

\[
2\pi J_{1,2}I_{1z}I_{2z} \tau
\]

\[
-2I_{1x}I_{2x}\cos(\Omega_2t) + 2I_{1x}I_{2z}\sin(\Omega_2t)\cos^2(\pi J_{1,2}t) + I_{1y}\sin(\Omega_2t)\cos(\pi J_{1,2}t)\sin(\pi J_{1,2}t)
\]

\[
+ I_{1y}\sin(\Omega_2t)\cos(\pi J_{1,2}t)\sin(\pi J_{1,2}t) - 2I_{1x}I_{2z}\sin(\Omega_2t)\sin^2(\pi J_{1,2}t)
\]

simplify \[
-2I_{1x}I_{2x}\cos(\Omega_2t) - 2I_{1x}I_{2z}\sin(\Omega_2t)\cos(2\pi J_{1,2}t) + I_{1y}\sin(\Omega_2t)\sin(2\pi J_{1,2}t)
\]

simplify ($\tau = 1/(4J)$) \[
-2I_{1x}I_{2x}\cos(\Omega_2t) + I_{1y}\sin(\Omega_2t)\text{ multiple quantum term evolves to multiple quantum, not observable}
\]

\[
\Omega_{1z}t_2 \rightarrow I_{1y}\cos(\Omega_1t_2)\sin(\Omega_2t_2) - I_{1y}\sin(\Omega_1t_2)\sin(\Omega_2t_2)
\]

- final transverse magnetization modulated by $\Omega_1$ and $\Omega_2$
Heteronuclear Multiple Bond Correlation (HMBC)

- Experiment permits correlations between $^1$H and $^{15}$N (or $^{13}$C) via 2-, 3- or more bond couplings (as opposed to 1 bond couplings for HSQC, HMQC)
  - can be performed in either a single quantum or multiple quantum mode (here is diagrammed the multiple quantum type)
- Delays ($\tau$) tuned to the scalar coupling of interest: still $1/(4J_{IS})$, but for coupling constants much smaller than 1-bond $^1$H-$^{15}$N or $^1$H-$^{13}$C
  - for 1-bond experiments, $^1J_{H,N} = 95$ Hz (for amide bond in protein), so $1/(4J) = 2.6$ ms, and $^1J_{H,C} = 125$ Hz (typical for H-C in proteins), $1/(4J) = 2.0$ ms
  - for HMBC experiments, 2- or 3-bond couplings are much smaller, say 4 Hz, so $^3J = 4$Hz, so $1/(4J) = 62.5$ ms
- Delay ($\tau$) is also tuned to correspond to an odd integer multiple of $1/(2^1J_{IS})$ ($\tau = (2n+1)/(2^1J_{IS})$), which removes signal from 1-bond couplings
Example: Identifying Carbohydrate Linkages

- How can linkages be established? By correlating $^1$H nuclei on one monomer with $^{13}$C nuclei on another
  - here, the $^1$H chemical shift of one hydrogen nucleus on monomer '1' is correlated to 3 separate $^{13}$C atoms (each 3 bonds away), one of these on a different monomer
  - thus, the linkage between monomer '1' and monomer '3' is established
  - works even across glycosydic bonds