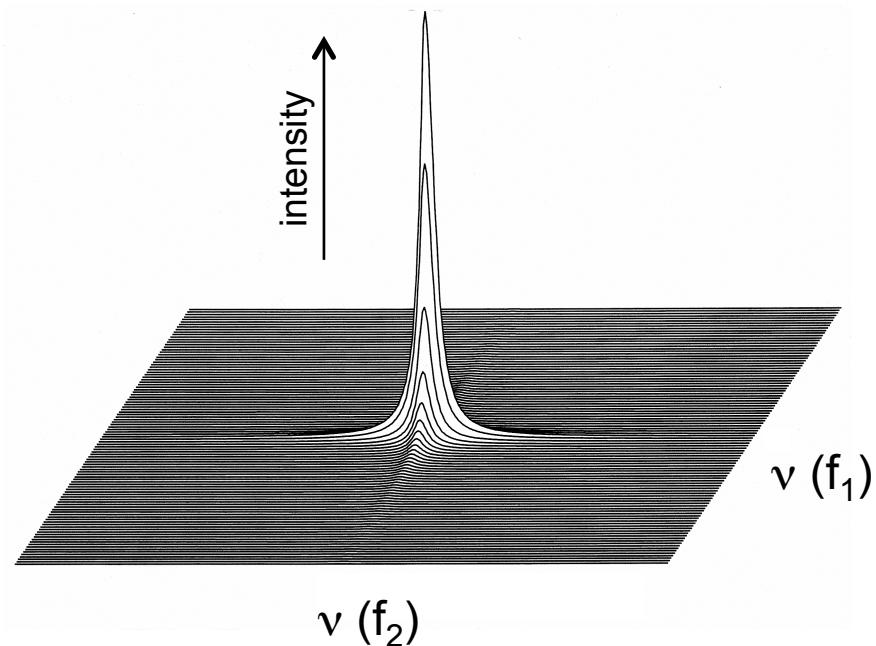
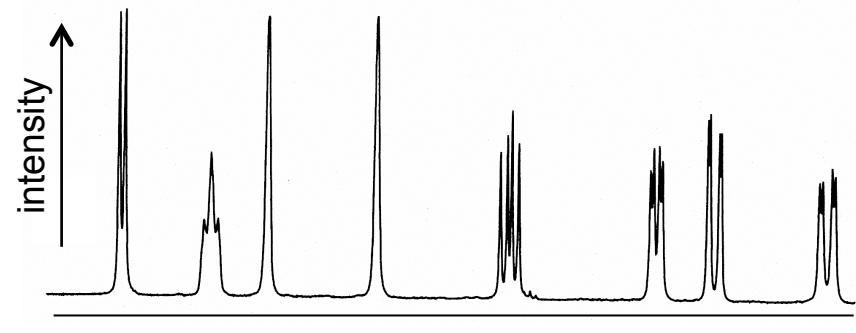
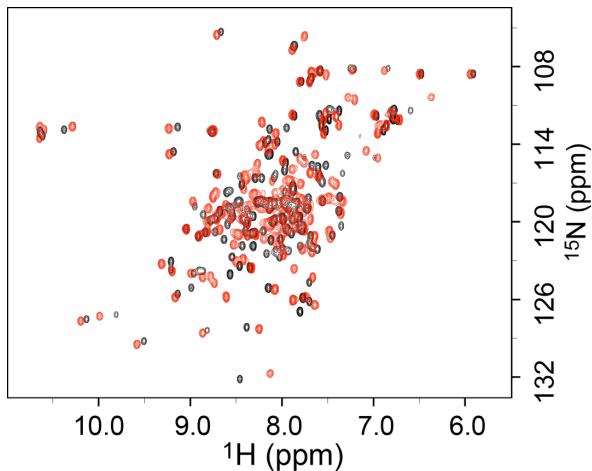


2D Correlation Experiments: HSQC, HMQC, HMBC,

BCMB/CHEM 8190

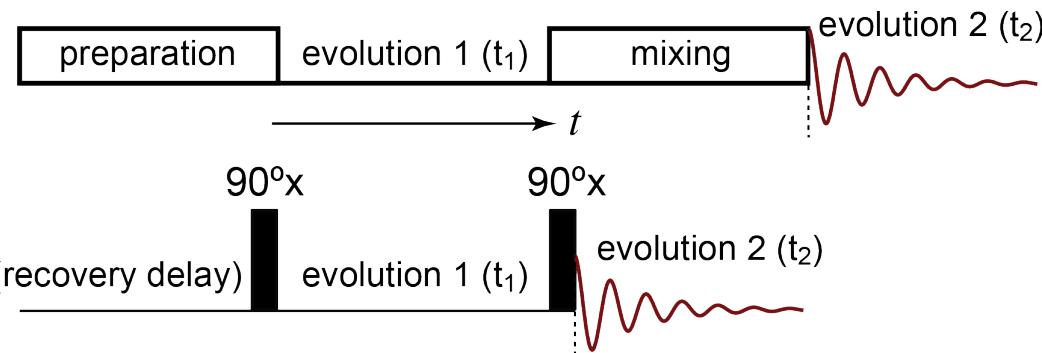
Two Dimensional NMR Spectroscopy

- 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

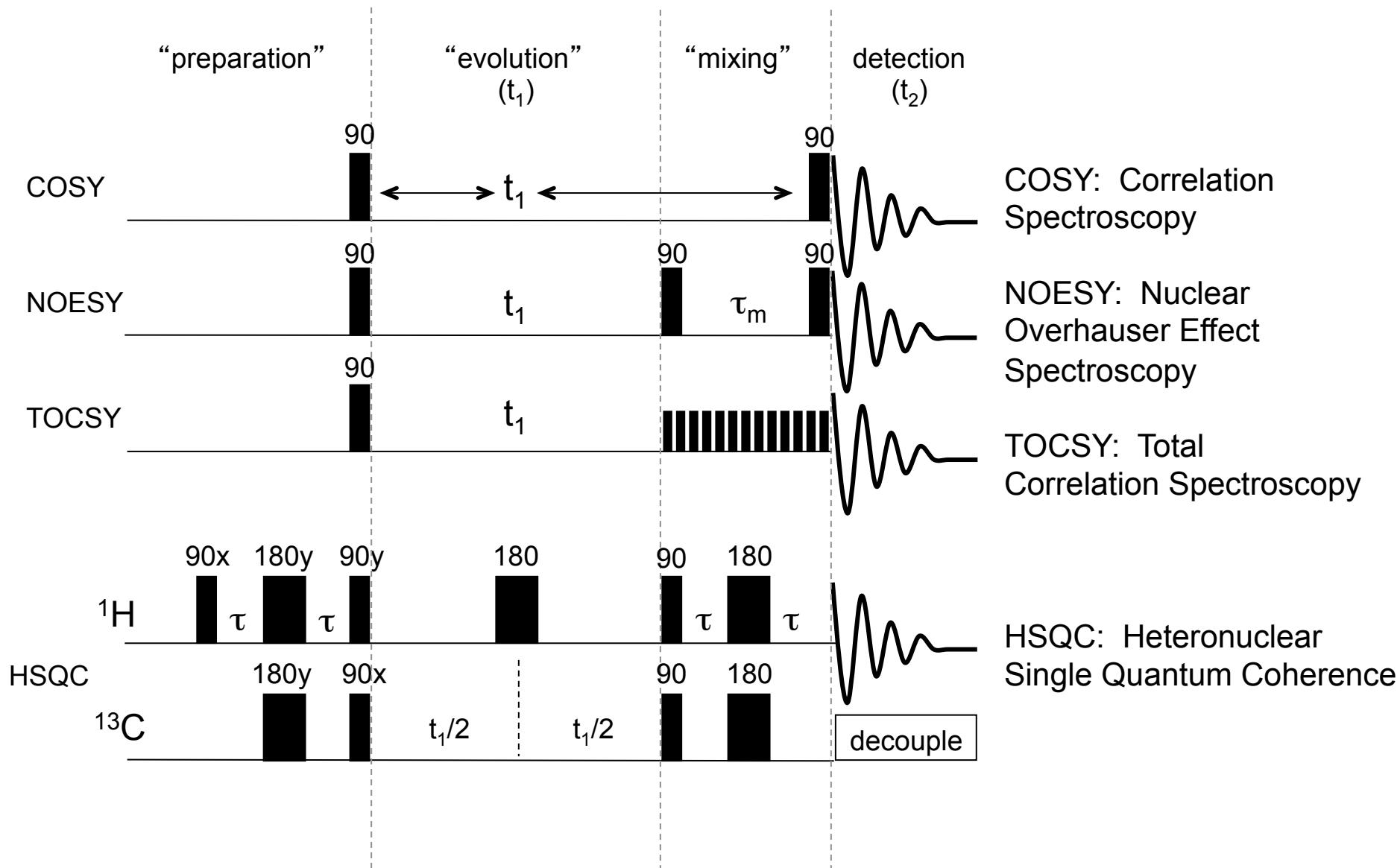
- A general scheme for 2D experiments describes these as constructed from 'preparation', 'evolution' and 'mixing' elements
 - preparation: create magnetization of interest
 - evolution 1: increment t_1 time period, evolve magnetization with information of interest (gives y -axis information, indirectly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



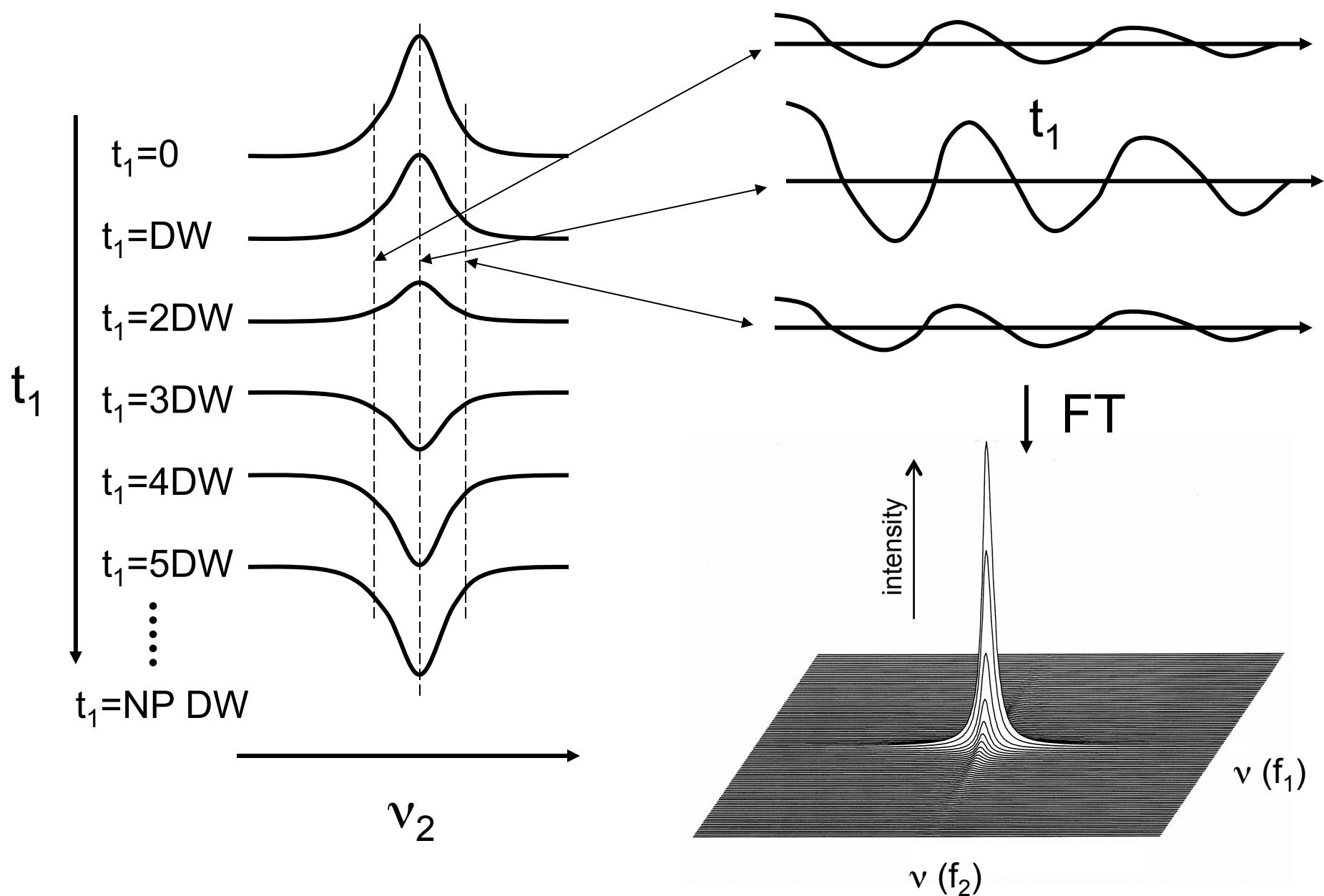
- Example: COSY
(correlation spectroscopy)

- correlates chemical shifts of coupled nuclei (usually ^1H)
- used as a stand-alone experiment for ^1H - ^1H 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

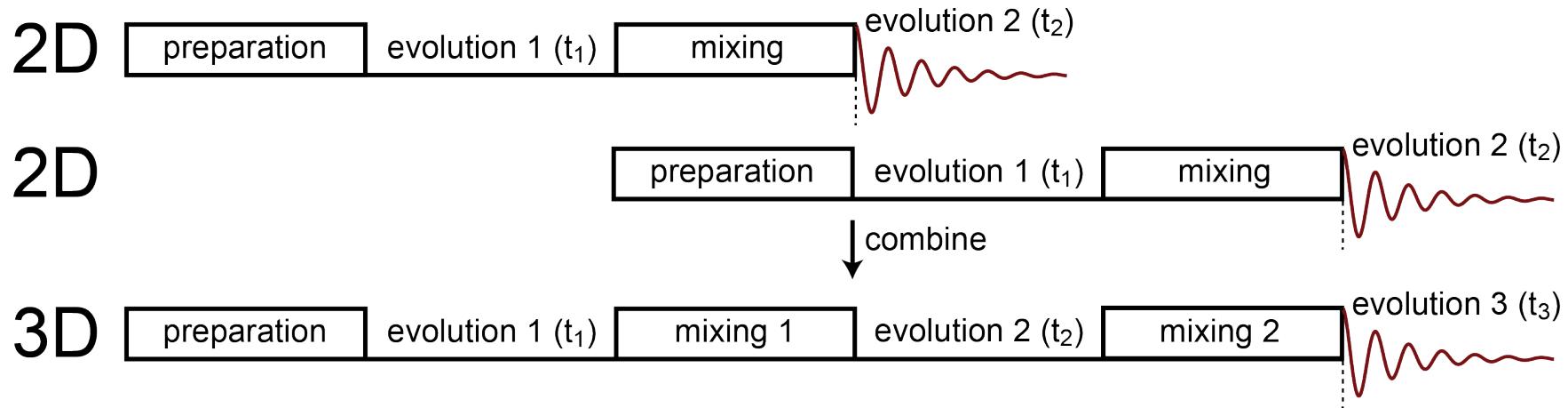


2D -NMR - FIDs are Transformed in t_2 , then in t_1



Three Dimensional NMR Spectroscopy

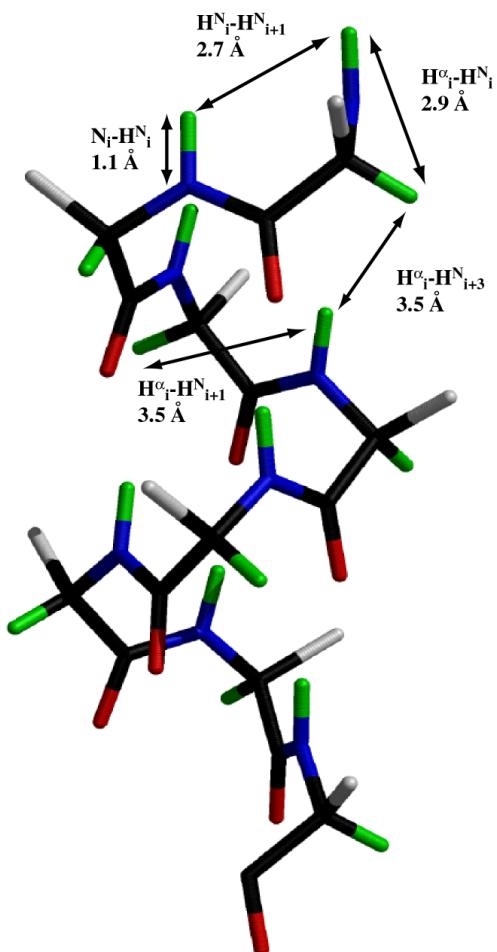
- In general, 3D experiments include the same elements as 2D experiments, just more of them
 - in a typical 3D experiment, there are three evolution periods, the first two corresponding to the two indirectly-detected dimensions (t_1 and t_2), and the third corresponding to the directly detected dimension (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

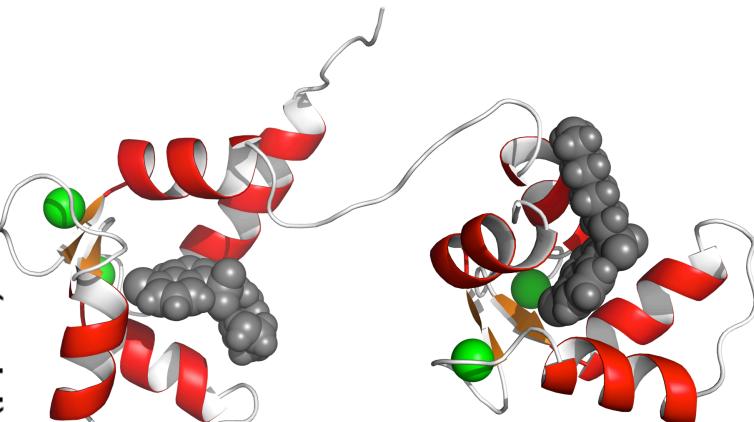
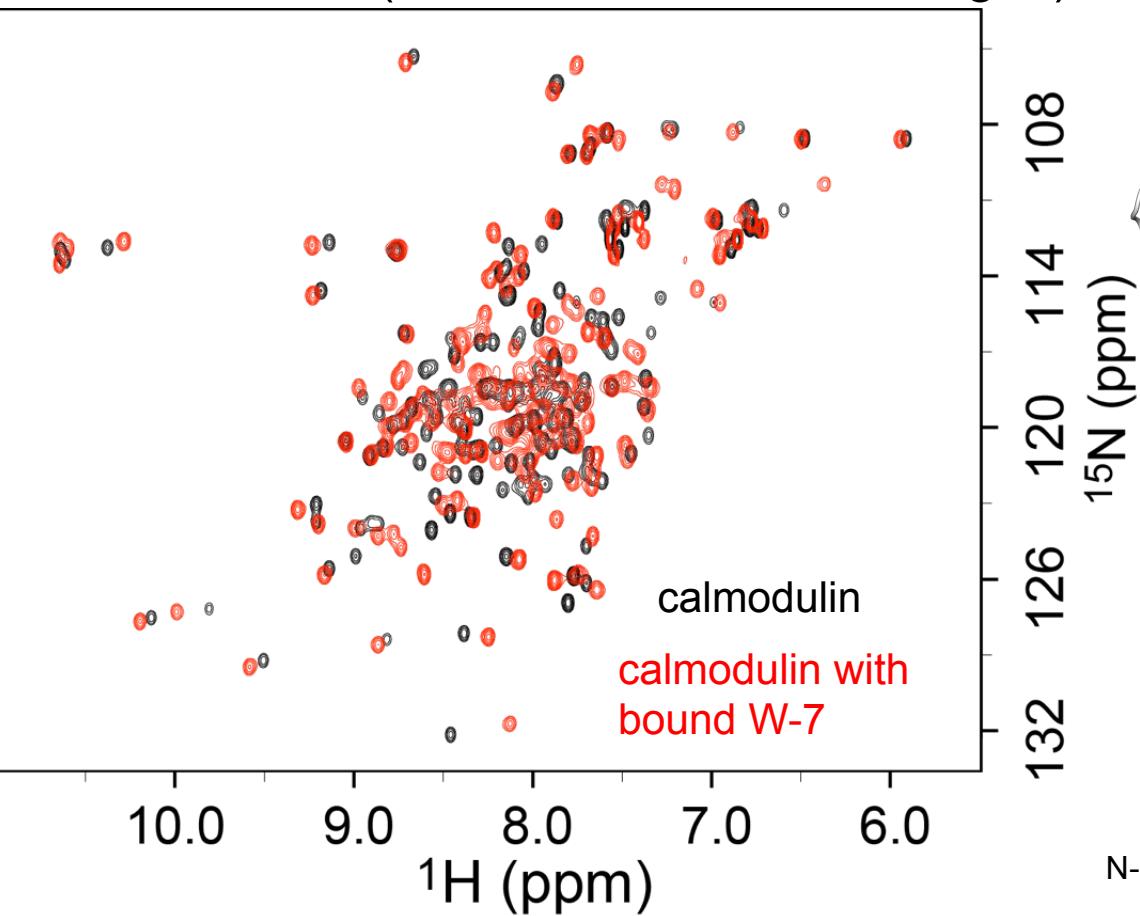
Heteronuclear Single Quantum Coherence

- 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 ^1H - ^{15}N pairs in amide groups of amino acids in proteins are convenient reporters for each amino acid
 - the ^1H , ^{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 ^1H - ^{15}N : ^1H - ^{13}C important for organic chemistry as well as biomolecular NMR

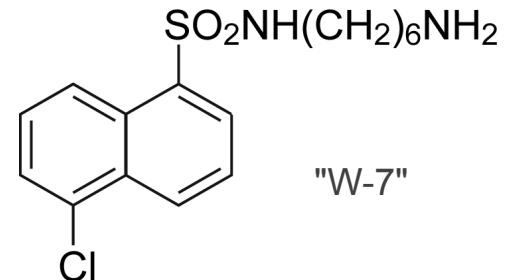


HSQC Spectrum of Amide H-N Pairs in a Protein

- 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 ^1H correlated to directly bonded ^{15}N , for each amino acid (notice chemical shift ranges)



Ikura and coworkers, 1988

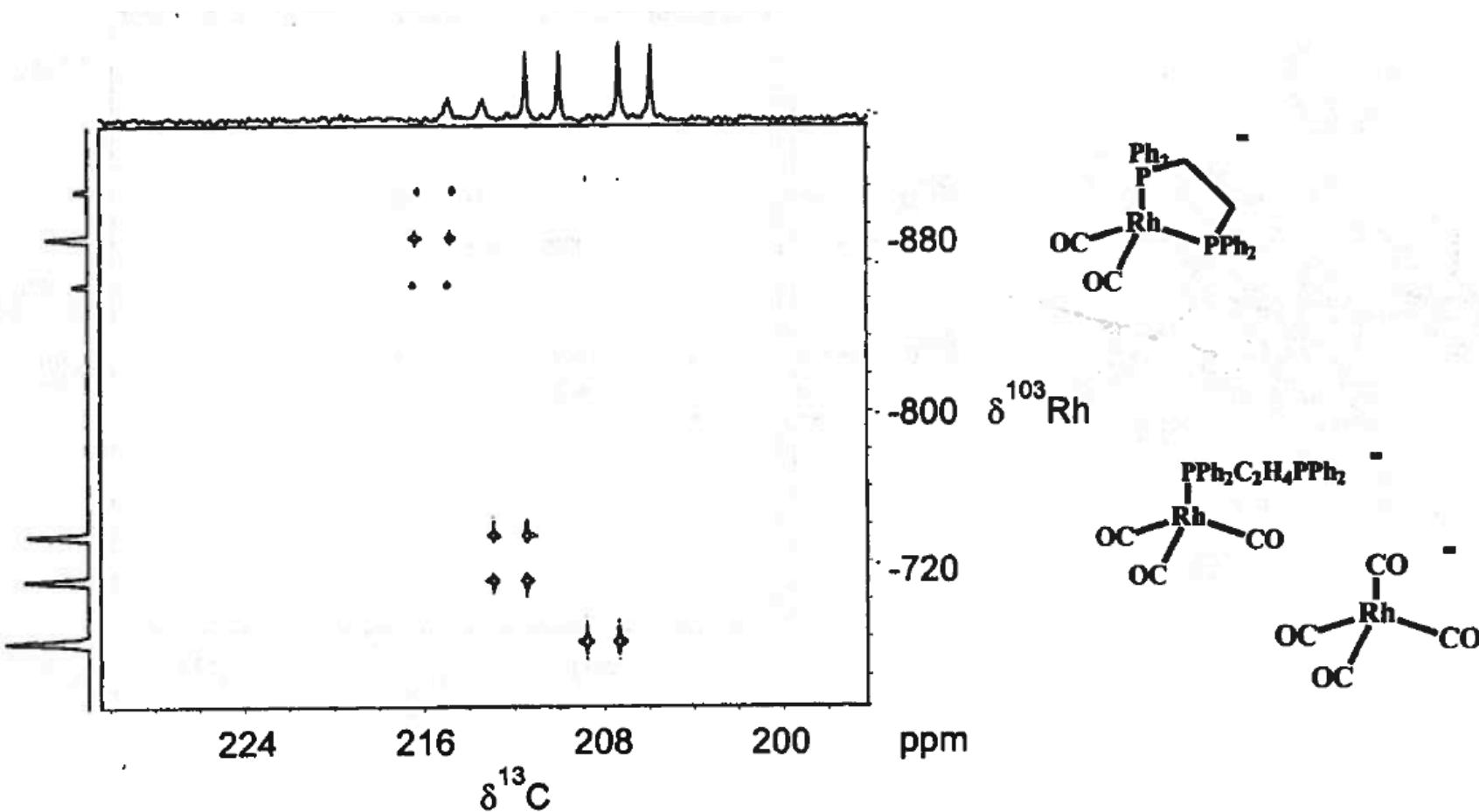


"W-7"

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

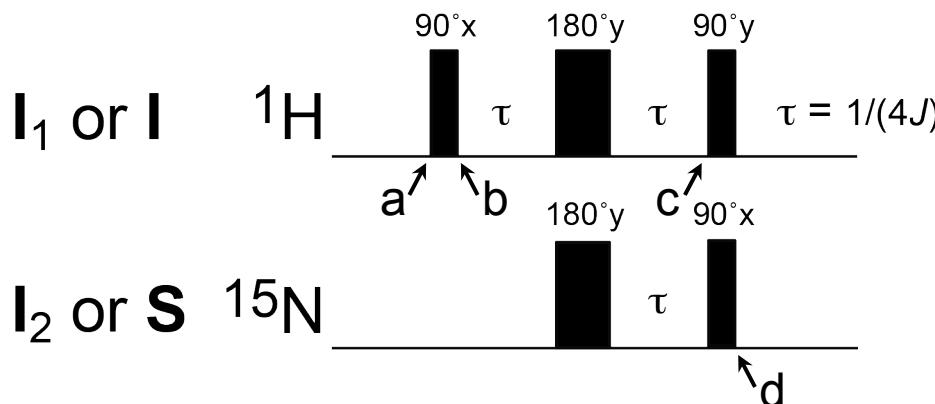
HSQC Spectra of Other Nuclear Pairs

- HSQC (and HMQC, see later) are used to correlate many types of nuclei
 - for biomolecular NMR, mostly ^1H - ^{15}N , ^1H - ^{13}C , ^1H - ^{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, ^1H pulses do not excite ^{15}N , and vice versa (so, two 'channels')
 - magnetization is transferred from ^1H to ^{15}N in order to improve the polarization of ^{15}N (sensitivity improved by $\gamma^1\text{H}/\gamma^{15}\text{N} \sim 10$) and to allow chemical shift evolution (during t_1) that depends on ^{15}N chemical shift
 - initial z-magnetization is converted to $-y$ ($90^\circ x$), then to antiphase x-magnetization (following $\tau-180^\circ-\tau$, no chemical shift evolution)
 - ^1H $90^\circ y$ and ^{15}N $90^\circ x$) convert to antiphase ^{15}N magnetization!!



- for this pulse sequence, τ MUST be equal to $1/(4J)$
- for amide $^1\text{H}-^{15}\text{N}$ 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

$$I_z \rightarrow -I_y \rightarrow -2I_x S_z \rightarrow -2I_z S_y$$

$$I_{1z} \rightarrow -I_{1y} \rightarrow -2I_{1x} I_{2z} \rightarrow -2I_{1z} I_{2y}$$

Preparation Period for HSQC Experiment

$$\begin{aligned}
 & \boxed{\mathbf{I}_{1z}} + \mathbf{I}_{2z} \xrightarrow{\frac{\pi}{2}\mathbf{l}_{1x}} -\mathbf{I}_{1y} + \mathbf{I}_{2z} \xrightarrow{\Omega_1\mathbf{l}_{zt} \quad \Omega_2\mathbf{l}_{zt}} -\mathbf{I}_{1y} \cos(\Omega_1 t) + \mathbf{I}_{1x} \sin(\Omega_1 t) + \mathbf{I}_{2z} \\
 & \xrightarrow{2\pi J_{1,2}\mathbf{l}_{1z}\mathbf{l}_{2z}t} -\mathbf{I}_{1y} \cos(\Omega_1 t) \cos(\pi J_{1,2} \tau) + 2\mathbf{I}_{1x}\mathbf{I}_{2z} \cos(\Omega_1 t) \sin(\pi J_{1,2} \tau) \\
 & \quad + \mathbf{I}_{1x} \sin(\Omega_1 t) \cos(\pi J_{1,2} \tau) + 2\mathbf{I}_{1y}\mathbf{I}_{2z} \sin(\Omega_1 t) \sin(\pi J_{1,2} \tau) + \mathbf{I}_{2z} \\
 & \xrightarrow{\frac{\pi}{2}\mathbf{l}_{1y}} -\mathbf{I}_{1y} \cos(\Omega_1 t) \cos(\pi J_{1,2} \tau) - 2\mathbf{I}_{1x}\mathbf{I}_{2z} \cos(\Omega_1 t) \sin(\pi J_{1,2} \tau) \\
 & \quad - \mathbf{I}_{1x} \sin(\Omega_1 t) \cos(\pi J_{1,2} \tau) + 2\mathbf{I}_{1y}\mathbf{I}_{2z} \sin(\Omega_1 t) \sin(\pi J_{1,2} \tau) + \mathbf{I}_{2z} \\
 & \xrightarrow{\frac{\pi}{2}\mathbf{l}_{2y}} -\mathbf{I}_{1y} \cos(\Omega_1 t) \cos(\pi J_{1,2} \tau) + 2\mathbf{I}_{1x}\mathbf{I}_{2z} \cos(\Omega_1 t) \sin(\pi J_{1,2} \tau) \\
 & \quad - \mathbf{I}_{1x} \sin(\Omega_1 t) \cos(\pi J_{1,2} \tau) - 2\mathbf{I}_{1y}\mathbf{I}_{2z} \sin(\Omega_1 t) \sin(\pi J_{1,2} \tau) - \mathbf{I}_{2z} \\
 & \xrightarrow{\Omega_1\mathbf{l}_{zt} \quad \Omega_2\mathbf{l}_{zt}} -\mathbf{I}_{1y} \cos^2(\Omega_1 t) \cos(\pi J_{1,2} \tau) + \mathbf{I}_{1x} \sin(\Omega_1 t) \cos(\Omega_1 t) \cos(\pi J_{1,2} \tau) \\
 & \quad + 2\mathbf{I}_{1x}\mathbf{I}_{2z} \cos^2(\Omega_1 t) \sin(\pi J_{1,2} \tau) + 2\mathbf{I}_{1y}\mathbf{I}_{2z} \sin(\Omega_1 t) \cos(\Omega_1 t) \sin(\pi J_{1,2} \tau) \\
 & \quad - \mathbf{I}_{1x} \cos(\Omega_1 t) \sin(\Omega_1 t) \cos(\pi J_{1,2} \tau) - \mathbf{I}_{1y} \sin^2(\Omega_1 t) \cos(\pi J_{1,2} \tau) \\
 & \quad - 2\mathbf{I}_{1y}\mathbf{I}_{2z} \cos(\Omega_1 t) \sin(\Omega_1 t) \sin(\pi J_{1,2} \tau) + 2\mathbf{I}_{1x}\mathbf{I}_{2z} \sin^2(\Omega_1 t) \sin(\pi J_{1,2} \tau) - \mathbf{I}_{2z} \\
 & \xrightarrow{\text{simplify}} -\mathbf{I}_{1y} \cos(\pi J_{1,2} \tau) + 2\mathbf{I}_{1x}\mathbf{I}_{2z} \sin(\pi J_{1,2} \tau) - \mathbf{I}_{2z} \\
 & \xrightarrow{2\pi J_{1,2}\mathbf{l}_{1z}\mathbf{l}_{2z}t} -\mathbf{I}_{1y} \cos^2(\pi J_{1,2} t) + 2\mathbf{I}_{1x}\mathbf{I}_{2z} \cos(\pi J_{1,2} t) \sin(\pi J_{1,2} t) \\
 & \quad + 2\mathbf{I}_{1x}\mathbf{I}_{2z} \cos(\pi J_{1,2} t) \sin(\pi J_{1,2} t) + \mathbf{I}_{1y} \sin^2(\pi J_{1,2} t) - \mathbf{I}_{2z} \\
 & \xrightarrow{\text{simplify}} -\mathbf{I}_{1y} \cos(2\pi J_{1,2} t) + 2\mathbf{I}_{1x}\mathbf{I}_{2z} \sin(2\pi J_{1,2} t) - \mathbf{I}_{2z} \\
 & \xrightarrow{t = \tau = 1/(4J)} \boxed{+2\mathbf{I}_{1x}\mathbf{I}_{2z}} - \mathbf{I}_{2z} \\
 & \xrightarrow{-\frac{\pi}{2}\mathbf{l}_{1y} \quad \frac{\pi}{2}\mathbf{l}_{2x}} \circled{+2\mathbf{I}_{1z}\mathbf{I}_{2y}} + \mathbf{I}_{2y} \\
 & \text{or} \quad (-2\mathbf{I}_{1z}\mathbf{I}_{2y} + \mathbf{I}_{2y}) - (+2\mathbf{I}_{1z}\mathbf{I}_{2y} + \mathbf{I}_{2y}) = -4\mathbf{I}_{1z}\mathbf{I}_{2y} \quad \text{or} \quad \circled{-2\mathbf{I}_{1z}\mathbf{I}_{2y}} \\
 & \xrightarrow{-\frac{\pi}{2}\mathbf{l}_{1y} \quad \frac{\pi}{2}\mathbf{l}_{2x}} \circled{-2\mathbf{I}_{1z}\mathbf{I}_{2y}} + \mathbf{I}_{2y}
 \end{aligned}$$

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

- initial z magnetization on ^1H (\mathbf{I}_{1z} , or \mathbf{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 ($\mathbf{I}_{1x}\mathbf{I}_{2z}$, or $\mathbf{I}_x\mathbf{S}_z$)

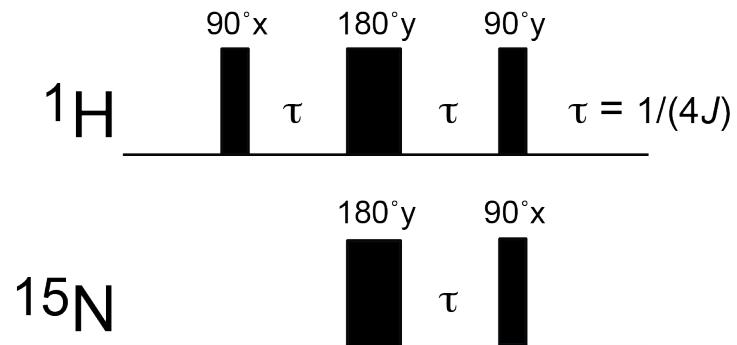
- the final pair of 90° pulses convert the antiphase x -magnetization on ^1H ($\mathbf{I}_{1x}\mathbf{I}_{2z}$, or $\mathbf{I}_x\mathbf{S}_z$) to antiphase y -magnetization on ^{15}N ($\mathbf{I}_{1z}\mathbf{I}_{2y}$ or $\mathbf{I}_z\mathbf{S}_y$), which enhances the ^{15}N magnetization by $\sim \gamma^1\text{H}/\gamma^{15}\text{N} \approx 10$

- The \mathbf{I}_{2z} term is removed by subtracting the results obtained with the final ^1H pulse applied along y and $-y$

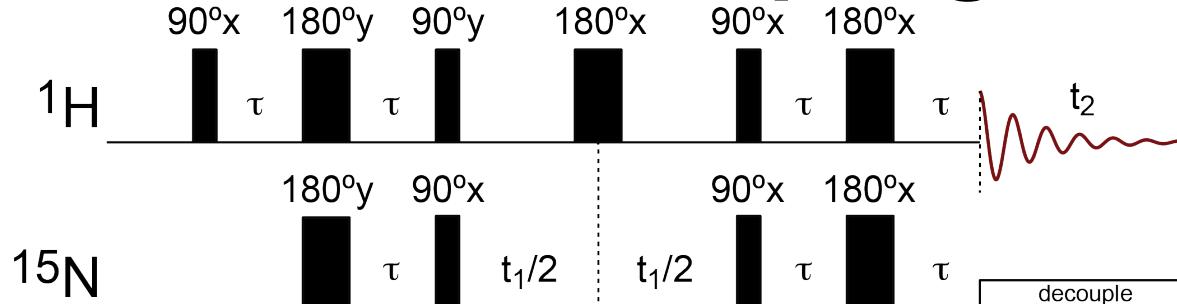
Preparation Period for HSQC Experiment

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

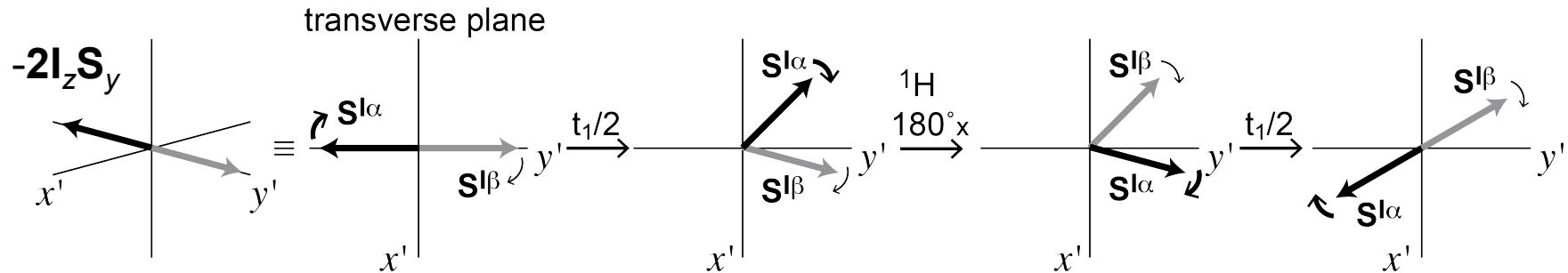
$$\begin{aligned}
 & \boxed{\mathbf{I}_{1z}} + \mathbf{I}_{2z} \xrightarrow{\frac{\pi}{2}\mathbf{I}_{1x}} -\boxed{\mathbf{I}_{1y}} + \mathbf{I}_{2z} \\
 & \xrightarrow{2\pi J_{1,2}\mathbf{I}_{1z}\mathbf{I}_{2z}t} -\mathbf{I}_{1y} \cos(\pi J_{1,2}t) + 2\mathbf{I}_{1x}\mathbf{I}_{2z} \sin(\pi J_{1,2}t) + \mathbf{I}_{2z} \\
 & \xrightarrow{\pi\mathbf{I}_{1y} \pi\mathbf{I}_{2y}} -\mathbf{I}_{1y} \cos(\pi J_{1,2}t) + 2\mathbf{I}_{1x}\mathbf{I}_{2z} \sin(\pi J_{1,2}t) - \mathbf{I}_{2z} \\
 & \xrightarrow{2\pi J_{1,2}\mathbf{I}_{1z}\mathbf{I}_{2z}t} -\mathbf{I}_{1y} \cos^2(\pi J_{1,2}t) + 2\mathbf{I}_{1x}\mathbf{I}_{2z} \cos(\pi J_{1,2}t)\sin(\pi J_{1,2}t) \\
 & \quad + 2\mathbf{I}_{1x}\mathbf{I}_{2z} \cos(\pi J_{1,2}t)\sin(\pi J_{1,2}t) + \mathbf{I}_{1y} \sin^2(\pi J_{1,2}t) - \mathbf{I}_{2z} \\
 & \xrightarrow{\text{simplify}} -\mathbf{I}_{1y} \cos(2\pi J_{1,2}t) + 2\mathbf{I}_{1x}\mathbf{I}_{2z} \sin(2\pi J_{1,2}t) - \mathbf{I}_{2z} \\
 & \xrightarrow{t = \tau = 1/(4J)} +\boxed{2\mathbf{I}_{1x}\mathbf{I}_{2z}} - \mathbf{I}_{2z} \\
 & \xrightarrow{\frac{\pi}{2}\mathbf{I}_{1y} \frac{\pi}{2}\mathbf{I}_{2x}} +\boxed{2\mathbf{I}_{1z}\mathbf{I}_{2y}} + \mathbf{I}_{2y} \\
 & \text{or } (-2\mathbf{I}_{1z}\mathbf{I}_{2y} + \mathbf{I}_{2y}) - (+2\mathbf{I}_{1z}\mathbf{I}_{2y} + \mathbf{I}_{2y}) = -4\mathbf{I}_{1z}\mathbf{I}_{2y} \text{ or } \boxed{-2\mathbf{I}_{1z}\mathbf{I}_{2y}} \\
 & \xrightarrow{-\frac{\pi}{2}\mathbf{I}_{1y} \frac{\pi}{2}\mathbf{I}_{2x}} -\boxed{2\mathbf{I}_{1z}\mathbf{I}_{2y}} + \mathbf{I}_{2y}
 \end{aligned}$$



Evolution and Decoupling during t_1



- The t_1 evolution period includes a 180° ^1H 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 ^1H in the α state – $\mathbf{S}^{I\alpha}$, and ^{15}N with ^1H in the β state – $\mathbf{S}^{I\beta}$) 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° ^1H pulse exchanges the ^1H α and β populations (vectors are exchanged), and the second $t_{1/2}$ period refocuses the vectors (antiphase magnetization restored)
 - chemical shifts are NOT refocused, ^{15}N - ^1H couplings ARE refocused (no net evolution of coupling)
 - so, during t_1 , signal is modulated by ^{15}N chemical shift, NOT J_{IS}



Evolution and Decoupling during t_1

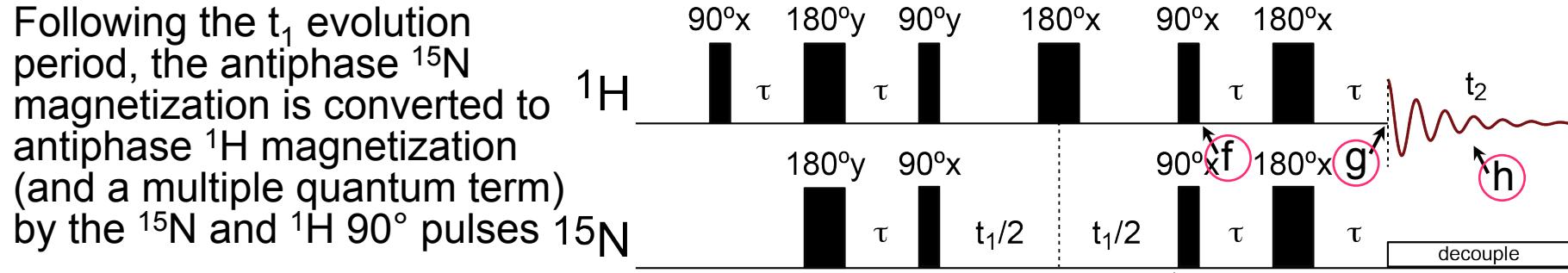
- During the t_1 evolution period the ^{15}N antiphase y -magnetization evolves into y - and x -antiphase magnetization
 - the analysis here ignores scalar coupling, as we demonstrated that the ^1H 180° pulse centered in the t_1 evolution period refocused the couplings
 - below, chemical shift evolution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

$$\begin{aligned} -2\mathbf{I}_{1z}\mathbf{I}_{2y} &\xrightarrow{\Omega_2 \mathbf{I}_z t_1/2} -2\mathbf{I}_{1z}\mathbf{I}_{2y} \cos(\Omega_2 t_1/2) + 2\mathbf{I}_{1z}\mathbf{I}_{2x} \sin(\Omega_2 t_1/2) \\ &\xrightarrow{\pi \mathbf{I}_1 x} 2\mathbf{I}_{1z}\mathbf{I}_{2y} \cos(\Omega_2 t_1/2) - 2\mathbf{I}_{1z}\mathbf{I}_{2x} \sin(\Omega_2 t_1/2) \\ &\xrightarrow{\Omega_2 \mathbf{I}_z t_1/2} 2\mathbf{I}_{1z}\mathbf{I}_{2y} \cos^2(\Omega_2 t_1/2) - 2\mathbf{I}_{1z}\mathbf{I}_{2x} \cos(\Omega_2 t_1/2)\sin(\Omega_2 t_1/2) \\ &\quad - 2\mathbf{I}_{1z}\mathbf{I}_{2x} \cos(\Omega_2 t_1/2)\sin(\Omega_2 t_1/2) - 2\mathbf{I}_{1z}\mathbf{I}_{2y} \sin^2(\Omega_2 t_1/2) \\ &\xrightarrow{\text{simplify}} 2\mathbf{I}_{1z}\mathbf{I}_{2y} \cos(\Omega_2 t_1) - 2\mathbf{I}_{1z}\mathbf{I}_{2x} \sin(\Omega_2 t_1) \end{aligned}$$

- 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 (Ω_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

$^{15}\text{N} \rightarrow ^1\text{H}$ Magnetization Transfer and Detection

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



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

e

$$2\mathbf{I}_{1z}\mathbf{I}_{2y} \cos(\Omega_2 t_1) - 2\mathbf{I}_{1z}\mathbf{I}_{2x} \sin(\Omega_2 t_1) \xrightarrow{\pi/2 \mathbf{I}_{1x} \pi/2 \mathbf{I}_{2x}} -2\mathbf{I}_{1y}\mathbf{I}_{2z} \cos(\Omega_2 t_1) + 2\mathbf{I}_{1y}\mathbf{I}_{2x} \sin(\Omega_2 t_1)$$

f

$$\xrightarrow{2\pi J_{1,2}\mathbf{I}_{1z}\mathbf{I}_{2z}\tau} -2\mathbf{I}_{1y}\mathbf{I}_{2z} \cos(\Omega_2 t_1) \cos(\pi J_{1,2}\tau) + \mathbf{I}_{1x} \cos(\Omega_2 t_1) \sin(\pi J_{1,2}\tau) + 2\mathbf{I}_{1y}\mathbf{I}_{2x} \sin(\Omega_2 t_1)$$

$$\xrightarrow{\pi \mathbf{I}_{1y} \pi \mathbf{I}_{2y}} 2\mathbf{I}_{1y}\mathbf{I}_{2z} \cos(\Omega_2 t_1) \cos(\pi J_{1,2}\tau) - \mathbf{I}_{1x} \cos(\Omega_2 t_1) \sin(\pi J_{1,2}\tau) - 2\mathbf{I}_{1y}\mathbf{I}_{2x} \sin(\Omega_2 t_1).$$

$$\xrightarrow{2\pi J_{1,2}\mathbf{I}_{1z}\mathbf{I}_{2z}\tau} 2\mathbf{I}_{1y}\mathbf{I}_{2z} \cos(\Omega_2 t_1) \cos^2(\pi J_{1,2}\tau) - \mathbf{I}_{1x} \cos(\Omega_2 t_1) \cos(\pi J_{1,2}\tau) \sin(\pi J_{1,2}\tau)$$

$$- \mathbf{I}_{1x} \cos(\Omega_2 t_1) \cos(\pi J_{1,2}\tau) \sin(\pi J_{1,2}\tau) - 2\mathbf{I}_{1y}\mathbf{I}_{2z} \cos(\Omega_2 t_1) \sin^2(\pi J_{1,2}\tau)$$

$$- 2\mathbf{I}_{1y}\mathbf{I}_{2x} \sin(\Omega_2 t_1)$$

$$\xrightarrow{\text{simplify}} 2\mathbf{I}_{1y}\mathbf{I}_{2z} \cos(\Omega_2 t_1) \cos(2\pi J_{1,2}\tau) - \mathbf{I}_{1x} \cos(\Omega_2 t_1) \sin(2\pi J_{1,2}\tau) - 2\mathbf{I}_{1y}\mathbf{I}_{2x} \sin(\Omega_2 t_1)$$

$$\xrightarrow{\tau = 1/(4J)} -\mathbf{I}_{1x} \cos(\Omega_2 t_1) - 2\mathbf{I}_{1y}\mathbf{I}_{2x} \sin(\Omega_2 t_1) \quad \text{second term is multiple quantum, not observed}$$

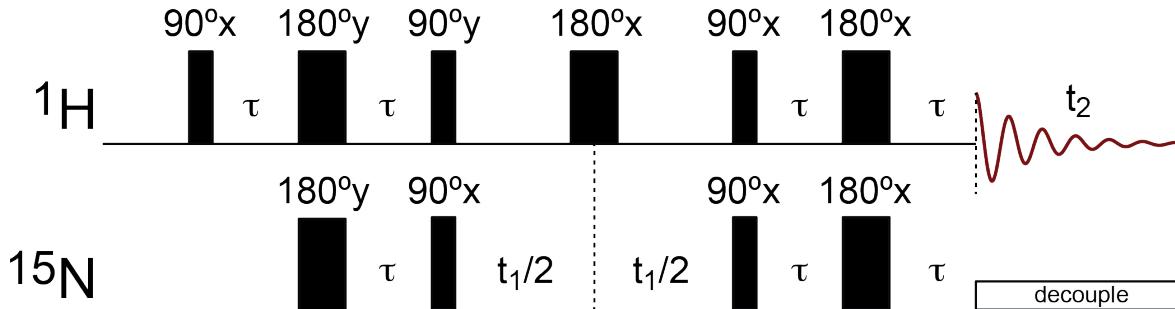
$$\xrightarrow{\Omega_1 \mathbf{I}_{2z} t_2} -\mathbf{I}_{1x} \cos(\Omega_2 t_1) \cos(\Omega_1 t_2) - \mathbf{I}_{1y} \cos(\Omega_2 t_1) \sin(\Omega_1 t_2)$$

- The final evolution period (t_2) results in transverse magnetization modulated by the ^1H and ^{15}N chemical shifts

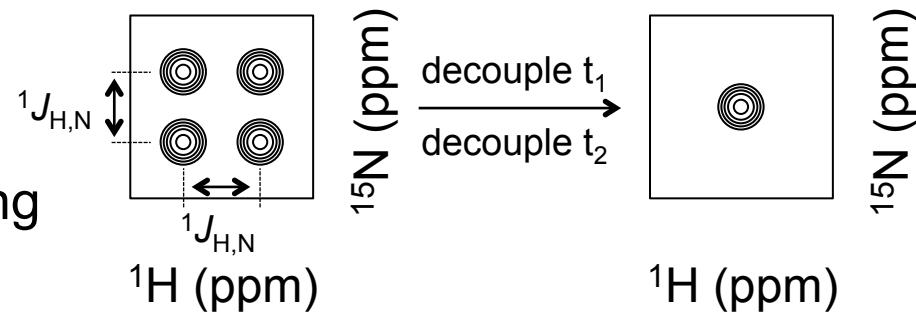
g

h

A Note on Decoupling

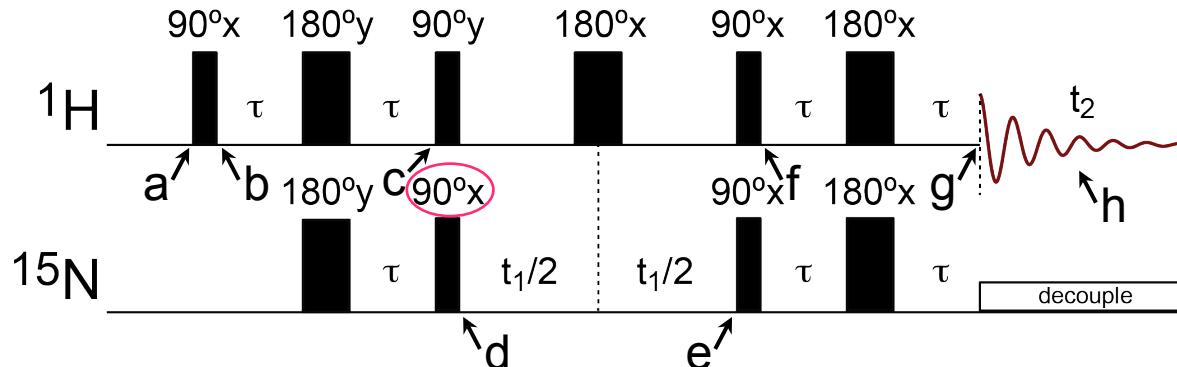


- During the t_1 evolution period, the $180^\circ x$ pulse eliminates net evolution of the $^1\text{H}-^{15}\text{N}$ scalar coupling (eliminates splitting of the ^{15}N signal by directly bonded ^1H)
- During the t_2 evolution period (acquisition), the 'decouple' element (^{15}N channel) eliminates ^{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 α and β 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 (t_2), in the indirect dimension (t_1) 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}N 90° pulse from 'x' to 'y'
 - the resulting term changes from $\mathbf{I}_z \mathbf{S}_y$ (x-pulse) to $\mathbf{I}_z \mathbf{S}_x$ (y-pulse), so the ^{15}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 t_2 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.

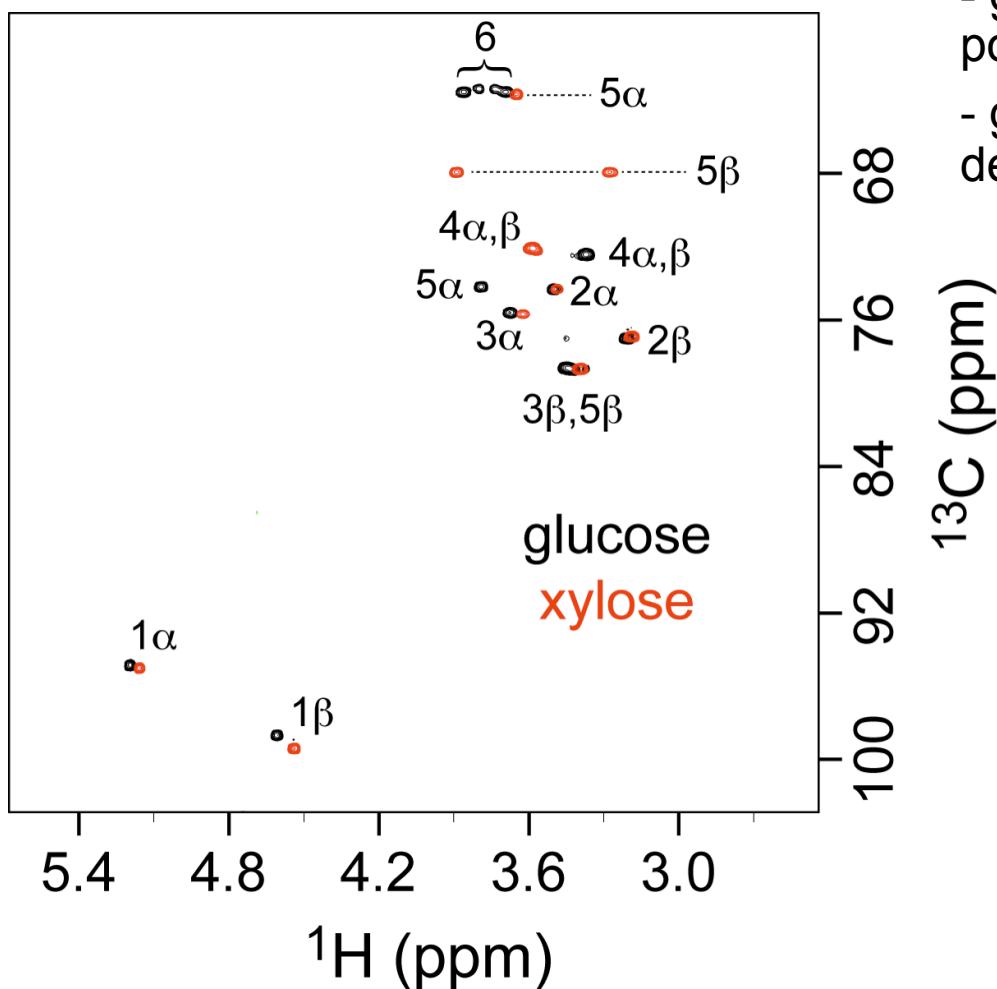
	a	b	c	d	e	f	g
x	$\mathbf{I}_z \rightarrow -\mathbf{I}_y \rightarrow -2\mathbf{I}_x \mathbf{S}_z \rightarrow -2\mathbf{I}_z \mathbf{S}_y$				$-2\mathbf{I}_z \mathbf{S}_y \rightarrow 2\mathbf{I}_z \mathbf{S}_y - 2\mathbf{I}_z \mathbf{S}_x \rightarrow -2\mathbf{I}_y \mathbf{S}_z + 2\mathbf{I}_y \mathbf{S}_x \rightarrow -\mathbf{I}_x \cos(\Omega_2 t_1)$		
y	$\mathbf{I}_z \rightarrow -\mathbf{I}_y \rightarrow -2\mathbf{I}_x \mathbf{S}_z \rightarrow -2\mathbf{I}_z \mathbf{S}_x$				$\rightarrow 2\mathbf{I}_z \mathbf{S}_x - 2\mathbf{I}_z \mathbf{S}_y \rightarrow +2\mathbf{I}_y \mathbf{S}_z - 2\mathbf{I}_y \mathbf{S}_x \rightarrow +\mathbf{I}_x \sin(\Omega_2 t_1)$		

Important Features of ^1H , ^{15}N -HSQC Spectra

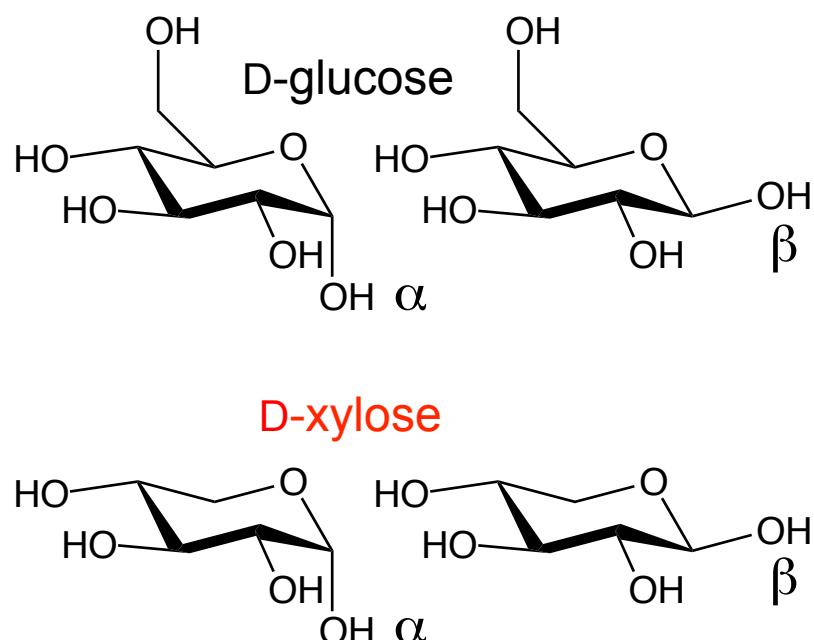
- Experiment begins with ^1H magnetization, then polarization is transferred to ^{15}N : gain $\gamma^1\text{H}/\gamma^{15}\text{N}$ factor in sensitivity (~ 10)
- Experiment ends with ^{15}N magnetization transferred back to ^1H for detection
 - theoretical sensitivity gain $(\gamma^1\text{H}/\gamma^{15}\text{N})^3$ for detecting ^1H vs ^{15}N (~ 1000)
$$emf = dB'/dt \propto dM/dt \propto \gamma B_0 M_0 = N \gamma^3 B_0^2 \hbar^2 I(I+1)/(3k_B T)$$
 - actual gain for transferring from ^{15}N to ^1H for detection is $\sim (\gamma^1\text{H}/\gamma^{15}\text{N})^{3/2} \approx 30$
- 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)

^1H , ^{13}C -HSQC

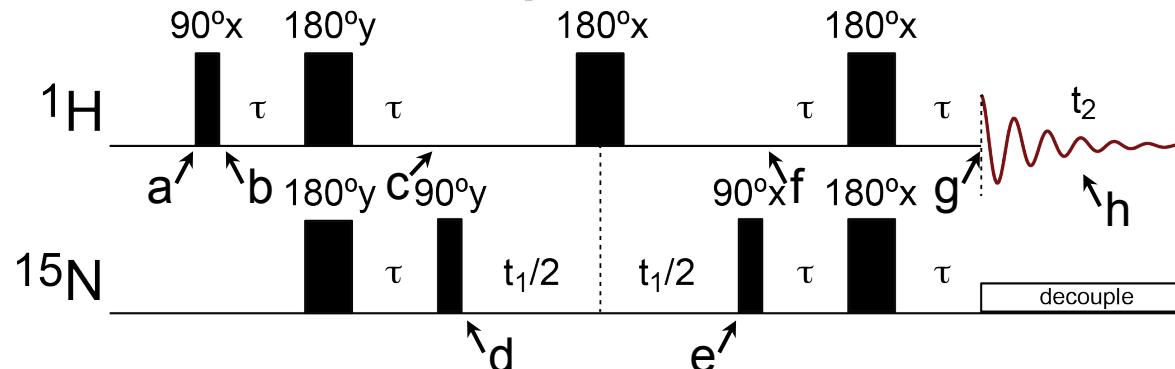
- Used ubiquitously in biomolecular NMR and small molecule NMR
 - for small molecules, with high concentrations, acquisition at natural isotopic abundance (~1.1%) is routine
 - example: mixture of D-glucose and D-xylose (5 mM each, 40 minute total acquisition time)



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



Heteronuclear Multiple Quantum Coherence



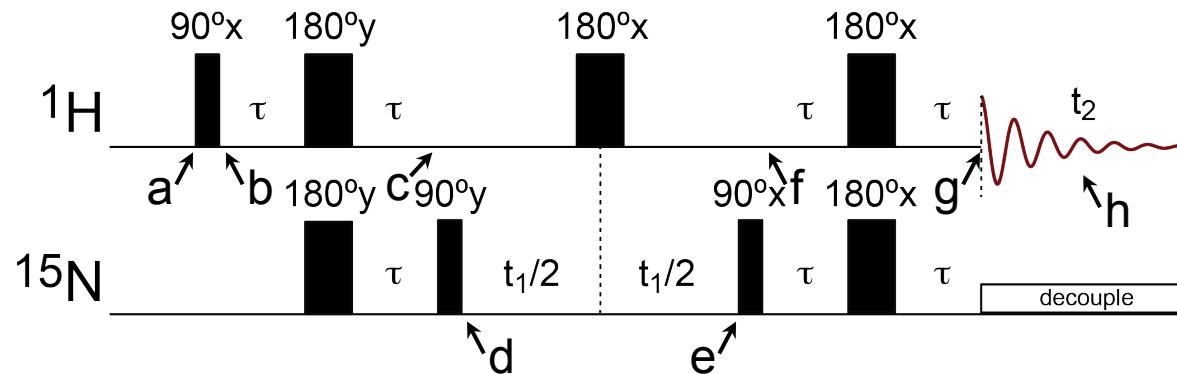
- HMQC is an analogue of the HSQC
 - information content, spectral display are the same as HSQC
- In HMQC, multiple quantum magnetization evolves during t_1
 - these evolve as sums (two-quantum coherence) and differences (zero-quantum coherence) of ^1H and ^{15}N chemical shifts (Ω_1 and Ω_S)
 - rather than allow these to evolve during the entire t_1 period, the ^1H 180° pulse exchanges density matrix elements for zero- and two-quantum coherences

$$\begin{aligned}
 -2\mathbf{I}_{1x}\mathbf{I}_{2x} &\xrightarrow{\Omega_1\mathbf{I}_{2x}\Omega_2\mathbf{I}_{2x}} -2\mathbf{I}_{1x}\mathbf{I}_{2x} \cos(\Omega_1 t/2)\cos(\Omega_2 t/2) - 2\mathbf{I}_{1y}\mathbf{I}_{2x} \sin(\Omega_1 t/2)\cos(\Omega_2 t/2) \\
 &\quad - 2\mathbf{I}_{1x}\mathbf{I}_{2y} \cos(\Omega_1 t/2)\sin(\Omega_2 t/2) - 2\mathbf{I}_{1y}\mathbf{I}_{2y} \sin(\Omega_1 t/2)\sin(\Omega_2 t/2) \\
 &\xrightarrow{\pi\mathbf{I}_{1x}} -2\mathbf{I}_{1x}\mathbf{I}_{2x} \cos(\Omega_1 t/2)\cos(\Omega_2 t/2) + 2\mathbf{I}_{1y}\mathbf{I}_{2x} \sin(\Omega_1 t/2)\cos(\Omega_2 t/2) \\
 &\quad - 2\mathbf{I}_{1x}\mathbf{I}_{2y} \cos(\Omega_1 t/2)\sin(\Omega_2 t/2) + 2\mathbf{I}_{1y}\mathbf{I}_{2y} \sin(\Omega_1 t/2)\sin(\Omega_2 t/2)
 \end{aligned}$$

- result (see next pages) is just evolution according to ^{15}N chemical shift (Ω_S)

a	b	c	d	e	f	g
$\mathbf{I}_z \rightarrow -\mathbf{I}_y \rightarrow -2\mathbf{I}_x\mathbf{S}_z \rightarrow -2\mathbf{I}_x\mathbf{S}_x \rightarrow -2\mathbf{I}_x\mathbf{S}_x - 2\mathbf{I}_x\mathbf{S}_y \rightarrow -2\mathbf{I}_x\mathbf{S}_x - 2\mathbf{I}_x\mathbf{S}_z \rightarrow \mathbf{I}_y \sin(\Omega_2 t_1)$						

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 $^1\text{H}-^{15}\text{N}$ versus $^1\text{H}-^{13}\text{C}$, and size of the molecule
- these can be subtle, and depend on the application

HSQC vs HMQC analysis for proteins: Bax and coworkers, *J. Magn. Reson.* **86**, 304-318 (1990)

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 ^1H 90°pulse)

$$\mathbf{I}_{1z} + \mathbf{I}_{2z} \xrightarrow{\pi/2 \mathbf{I}1x} -\mathbf{I}_{1y} + \mathbf{I}_{2z}$$

- initial z magnetization on ^1H (\mathbf{I}_{1z} , or \mathbf{I}_z) is converted to -y magnetization by the first 90° ^1H pulse

$$\xrightarrow{2\pi J_{1,2} \mathbf{I}_{1z} \mathbf{I}_{2z} t} -\mathbf{I}_{1y} \cos(\pi J_{1,2} t) + 2\mathbf{I}_{1x} \mathbf{I}_{2z} \sin(\pi J_{1,2} t) + \mathbf{I}_{2z}$$

$$\xrightarrow{\pi \mathbf{I}1y \pi \mathbf{I}2y} -\mathbf{I}_{1y} \cos(\pi J_{1,2} t) + 2\mathbf{I}_{1x} \mathbf{I}_{2z} \sin(\pi J_{1,2} t) - \mathbf{I}_{2z}$$

$$\begin{aligned} \xrightarrow{2\pi J_{1,2} \mathbf{I}_{1z} \mathbf{I}_{2z} t} & -\mathbf{I}_{1y} \cos^2(\pi J_{1,2} t) + 2\mathbf{I}_{1x} \mathbf{I}_{2z} \cos(\pi J_{1,2} t) \sin(\pi J_{1,2} t) \\ & + 2\mathbf{I}_{1x} \mathbf{I}_{2z} \cos(\pi J_{1,2} t) \sin(\pi J_{1,2} t) + \mathbf{I}_{1y} \sin^2(\pi J_{1,2} t) - \mathbf{I}_{2z} \end{aligned}$$

$$\xrightarrow{\text{simplify}} -\mathbf{I}_{1y} \cos(2\pi J_{1,2} t) + 2\mathbf{I}_{1x} \mathbf{I}_{2z} \sin(2\pi J_{1,2} t) - \mathbf{I}_{2z}$$

- just before the final 90° pulses, the magnetization is antiphase x-magnetization on ^1H ($\mathbf{I}_{1x} \mathbf{I}_{2z}$, or $\mathbf{I}_x \mathbf{S}_z$)

$$\xrightarrow{t = \tau = 1/(4J)} +2\mathbf{I}_{1x} \mathbf{I}_{2z} - \mathbf{I}_{2z}$$

- the 90° ^{15}N pulse convert the antiphase x-magnetization on ^1H ($\mathbf{I}_{1x} \mathbf{I}_{2z}$, or $\mathbf{I}_x \mathbf{S}_z$) to multiple quantum x-magnetization on ^{15}N ($\mathbf{I}_{1x} \mathbf{I}_{2x}$ or $\mathbf{I}_x \mathbf{S}_x$) (^{15}N magnetization enhanced by $\sim \gamma^1\text{H}/\gamma^{15}\text{N} \approx 10$)

$$\xrightarrow{\pi/2 \mathbf{I}2y} +2\mathbf{I}_{1x} \mathbf{I}_{2x} - \mathbf{I}_{2x}$$

or

$$(-2\mathbf{I}_{1x} \mathbf{I}_{2x} + \mathbf{I}_{2x}) - (+2\mathbf{I}_{1x} \mathbf{I}_{2x} + \mathbf{I}_{2x}) = -4\mathbf{I}_{1x} \mathbf{I}_{2x} \text{ or } \boxed{-2\mathbf{I}_{1x} \mathbf{I}_{2x}}$$

$$\xrightarrow{-\pi/2 \mathbf{I}1x \text{ (first 90° pulse)}} -2\mathbf{I}_{1z} \mathbf{I}_{2y} + \mathbf{I}_{2x}$$

- The \mathbf{I}_{2z} term is removed by subtracting the results obtained with the initial ^1H 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 (Ω_1) and ^{15}N (Ω_2)
 - however, the 180° ^1H pulse changes the sign of the \mathbf{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 (Ω_2)
 - in other words, 180° ^1H pulse in effect refocuses ^1H chemical shift evolution
 - J coupling operator doesn't change multiple quantum terms (so, don't consider J coupling)

$$\begin{aligned}
 -2\mathbf{I}_{1x}\mathbf{I}_{2x} &\xrightarrow{\Omega_1\mathbf{I}_z t \Omega_2\mathbf{I}_z t} -2\mathbf{I}_{1x}\mathbf{I}_{2x} \cos(\Omega_1 t/2)\cos(\Omega_2 t/2) - 2\mathbf{I}_{1y}\mathbf{I}_{2x} \sin(\Omega_1 t/2)\cos(\Omega_2 t/2) \\
 &\quad - 2\mathbf{I}_{1x}\mathbf{I}_{2y} \cos(\Omega_1 t/2)\sin(\Omega_2 t/2) - 2\mathbf{I}_{1y}\mathbf{I}_{2y} \sin(\Omega_1 t/2)\sin(\Omega_2 t/2) \\
 &\xrightarrow{\pi\mathbf{I}_{1x}} -2\mathbf{I}_{1x}\mathbf{I}_{2x} \cos(\Omega_1 t/2)\cos(\Omega_2 t/2) + 2\mathbf{I}_{1y}\mathbf{I}_{2x} \sin(\Omega_1 t/2)\cos(\Omega_2 t/2) \\
 &\quad - 2\mathbf{I}_{1x}\mathbf{I}_{2y} \cos(\Omega_1 t/2)\sin(\Omega_2 t/2) + 2\mathbf{I}_{1y}\mathbf{I}_{2y} \sin(\Omega_1 t/2)\sin(\Omega_2 t/2) \\
 &\xrightarrow{\Omega_1\mathbf{I}_z t \Omega_2\mathbf{I}_z t} -2\mathbf{I}_{1x}\mathbf{I}_{2x} \cos^2(\Omega_1 t/2)\cos^2(\Omega_2 t/2) - 2\mathbf{I}_{1y}\mathbf{I}_{2x} \cos(\Omega_1 t/2)\cos^2(\Omega_2 t/2)\sin(\Omega_1 t/2) \\
 &\quad - 2\mathbf{I}_{1x}\mathbf{I}_{2y} \cos^2(\Omega_1 t/2)\cos(\Omega_2 t/2)\sin(\Omega_2 t/2) - 2\mathbf{I}_{1y}\mathbf{I}_{2y} \cos(\Omega_1 t/2)\cos(\Omega_2 t/2)\sin(\Omega_1 t/2)\sin(\Omega_2 t/2) \\
 &\quad + 2\mathbf{I}_{1y}\mathbf{I}_{2x} \sin(\Omega_1 t/2)\cos^2(\Omega_2 t/2)\cos(\Omega_1 t/2) - 2\mathbf{I}_{1x}\mathbf{I}_{2x} \sin^2(\Omega_1 t/2)\cos^2(\Omega_2 t/2) \\
 &\quad + 2\mathbf{I}_{1y}\mathbf{I}_{2y} \sin(\Omega_1 t/2)\cos(\Omega_2 t/2)\cos(\Omega_1 t/2)\sin(\Omega_2 t/2) - 2\mathbf{I}_{1x}\mathbf{I}_{2y} \sin^2(\Omega_1 t/2)\cos(\Omega_2 t/2)\sin(\Omega_2 t/2) \\
 &\quad - 2\mathbf{I}_{1x}\mathbf{I}_{2y} \cos^2(\Omega_1 t/2)\sin(\Omega_2 t/2)\cos(\Omega_2 t/2) - 2\mathbf{I}_{1y}\mathbf{I}_{2y} \cos(\Omega_1 t/2)\sin(\Omega_2 t/2)\sin(\Omega_1 t/2)\cos(\Omega_2 t/2) \\
 &\quad + 2\mathbf{I}_{1x}\mathbf{I}_{2x} \cos^2(\Omega_1 t/2)\sin^2(\Omega_2 t/2) + 2\mathbf{I}_{1y}\mathbf{I}_{2x} \cos(\Omega_1 t/2)\sin^2(\Omega_2 t/2)\sin(\Omega_1 t/2) \\
 &\quad + 2\mathbf{I}_{1y}\mathbf{I}_{2y} \sin(\Omega_1 t/2)\sin(\Omega_2 t/2)\cos(\Omega_1 t/2)\cos(\Omega_2 t/2) - 2\mathbf{I}_{1x}\mathbf{I}_{2y} \sin^2(\Omega_1 t/2)\sin(\Omega_2 t/2)\cos(\Omega_2 t/2) \\
 &\quad - 2\mathbf{I}_{1y}\mathbf{I}_{2x} \sin(\Omega_1 t/2)\sin^2(\Omega_2 t/2)\cos(\Omega_1 t/2) + 2\mathbf{I}_{1x}\mathbf{I}_{2x} \sin^2(\Omega_1 t/2)\sin^2(\Omega_2 t/2)
 \end{aligned}$$

simplify

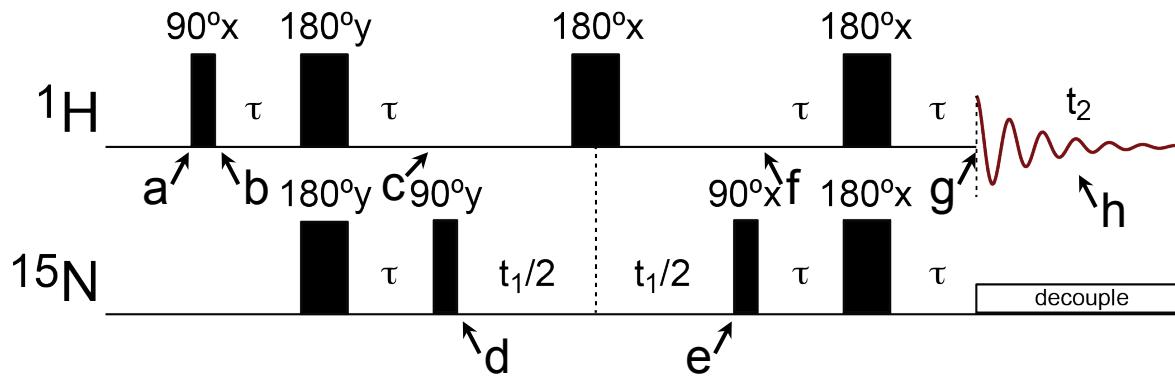
$$\xrightarrow{\quad} -2\mathbf{I}_{1x}\mathbf{I}_{2x} \cos(\Omega_2 t) - 2\mathbf{I}_{1x}\mathbf{I}_{2y} \sin(\Omega_2 t)$$

Multiple Quantum $\rightarrow ^1\text{H}$ Magnetization Transfer and Detection

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

$$\begin{aligned}
 & -2\mathbf{I}_{1x}\mathbf{I}_{2x} \cos(\Omega_2 t) - 2\mathbf{I}_{1x}\mathbf{I}_{2y} \sin(\Omega_2 t) \xrightarrow{\pi/2 \text{ I2x}} -2\mathbf{I}_{1x}\mathbf{I}_{2x} \cos(\Omega_2 t) - 2\mathbf{I}_{1x}\mathbf{I}_{2z} \sin(\Omega_2 t) \\
 \xrightarrow{2\pi J_{1,2} \mathbf{I}_{1z}\mathbf{I}_{2z} \tau} & -2\mathbf{I}_{1x}\mathbf{I}_{2x} \cos(\Omega_2 t) - 2\mathbf{I}_{1x}\mathbf{I}_{2z} \sin(\Omega_2 t) \cos(\pi J_{1,2} \tau) - \mathbf{I}_{1y} \sin(\Omega_2 t) \sin(\pi J_{1,2} \tau) \\
 \xrightarrow{\pi \mathbf{I} \mathbf{l}_x \pi \mathbf{I} \mathbf{l} 2x} & -2\mathbf{I}_{1x}\mathbf{I}_{2x} \cos(\Omega_2 t) + 2\mathbf{I}_{1x}\mathbf{I}_{2z} \sin(\Omega_2 t) \cos(\pi J_{1,2} \tau) + \mathbf{I}_{1y} \sin(\Omega_2 t) \sin(\pi J_{1,2} \tau) \\
 \xrightarrow{2\pi J_{1,2} \mathbf{I}_{1z}\mathbf{I}_{2z} \tau} & -2\mathbf{I}_{1x}\mathbf{I}_{2x} \cos(\Omega_2 t) + 2\mathbf{I}_{1x}\mathbf{I}_{2z} \sin(\Omega_2 t) \cos^2(\pi J_{1,2} \tau) + \mathbf{I}_{1y} \sin(\Omega_2 t) \cos(\pi J_{1,2} \tau) \sin(\pi J_{1,2} \tau) \\
 & + \mathbf{I}_{1y} \sin(\Omega_2 t) \cos(\pi J_{1,2} \tau) \sin(\pi J_{1,2} \tau) - 2\mathbf{I}_{1x}\mathbf{I}_{2z} \sin(\Omega_2 t) \sin^2(\pi J_{1,2} \tau) \\
 \xrightarrow{\text{simplify}} & -2\mathbf{I}_{1x}\mathbf{I}_{2x} \cos(\Omega_2 t) - 2\mathbf{I}_{1x}\mathbf{I}_{2z} \sin(\Omega_2 t) \cos(2\pi J_{1,2} \tau) + \mathbf{I}_{1y} \sin(\Omega_2 t) \sin(2\pi J_{1,2} \tau) \\
 \xrightarrow{\text{simplify } (\tau = 1/(4J))} & -2\mathbf{I}_{1x}\mathbf{I}_{2x} \cos(\Omega_2 t) + \mathbf{I}_{1y} \sin(\Omega_2 t) \quad \text{multiple quantum term evolves to multiple quantum, not observable} \\
 \xrightarrow{\Omega_1 \mathbf{I} z t_2} & \mathbf{I}_{1y} \cos(\Omega_1 t_1) \sin(\Omega_2 t_2) - \mathbf{I}_{1x} \sin(\Omega_1 t_1) \sin(\Omega_2 t_2) \quad \text{-final transverse magnetization} \\
 & \quad \text{modulated by } \Omega_1 \text{ and } \Omega_2
 \end{aligned}$$

Heteronuclear Multiple Bond Correlation (HMBC)



- Experiment permits correlations between ^1H 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 (τ) tuned to the scalar coupling of interest: still $1/(4J_{\text{IS}})$, but for coupling constants much smaller than 1-bond $^1\text{H}-^{15}\text{N}$ or $^1\text{H}-^{13}\text{C}$
 - for 1-bond experiments, $^1J_{\text{H},\text{N}} = 95$ Hz (for amide bond in protein), so $1/(4J) = 2.6$ ms, and $^1J_{\text{H},\text{C}} = 125$ 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 (τ) is also tuned to correspond to an odd integer multiple of $1/(2^1J_{\text{IS}})$ ($\tau = (2n+1)/(2^1J_{\text{IS}})$), which removes signal from 1-bond couplings

Example: Identifying Carbohydrate Linkages

- How can linkages be established? By correlating ^1H nuclei on one monomer with ^{13}C nuclei on another
 - here, the ^1H chemical shift of one hydrogen nucleus '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 glycosidic bonds

