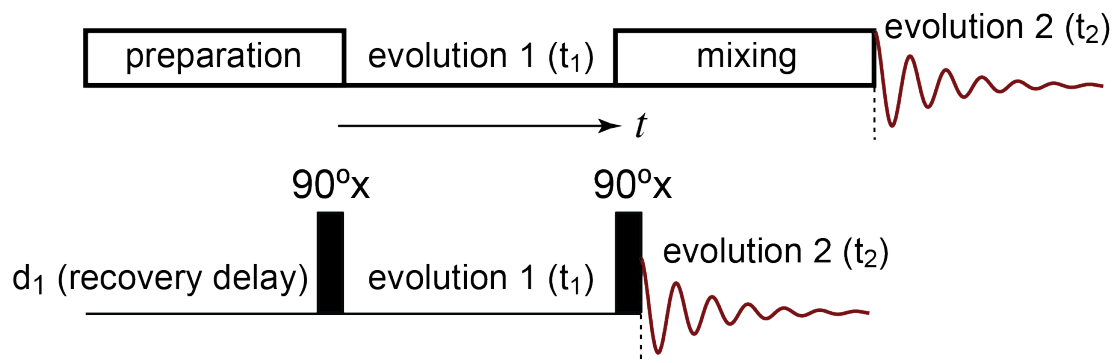


COSY and TOCSY

BCMB/CHEM 8190

COSY: Correlated Spectroscopy

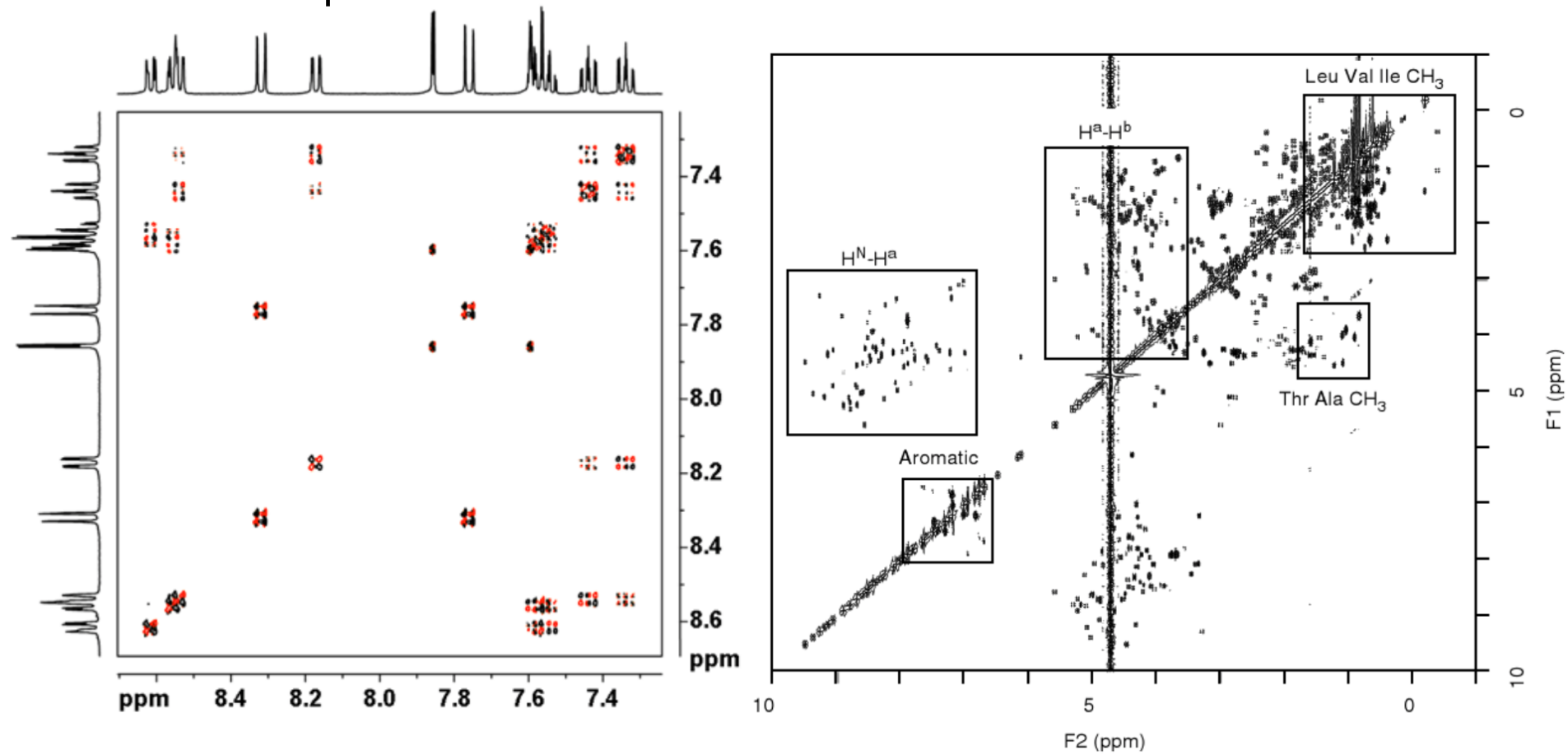
- One of the first, and most useful 2D experiments
 - one of the most common homonuclear experiments
 - workhorse experiment for small molecules to correlate coupled ^1H nuclei
 - 2- and 3-bond couplings mostly, but possible for longer range
 - good for correlating nuclei in small spin systems
- Deceptively simple looking experiment
 - preparation period: relaxation delay (d_1), then 90° x nonselective pulse to generate transverse magnetization for all ^1H nuclei
 - evolution period (t_1), all evolve according to chemical shifts and J couplings



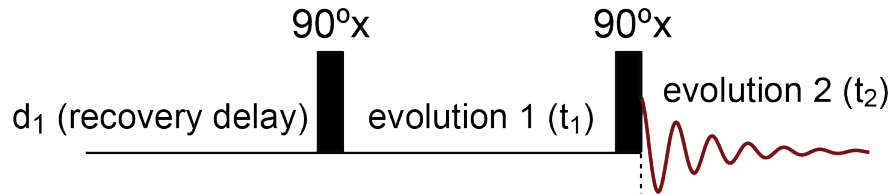
- coherence transfer resulting from the mixing period (second 90° x nonselective pulse) causes a portion of the magnetization for each spin to precess at the frequencies of coupled spins!
- crosspeaks (off-diagonal peaks) then identify frequencies of coupled spins

COSY Spectra

- Correlate chemical shifts of coupled nuclei in two dimensions
 - signals on diagonal are autocorrelated
 - signals off of the diagonal, crosspeaks, correlate chemical shifts of coupled nuclei (spectrum is symmetrical, each crosspeak appearing twice)
 - for proteins, is challenging to analyze some regions of COSY spectra for even small proteins



COSY for an AX Spin System



- Simple Hamiltonian and basis set (AX, first order system):
 - evolution due to chemical shift for spins A and X, and first order J_{AX}
$$\hat{H} = -\gamma B_0(1 - \sigma_A)\hat{I}_{Az} - \gamma B_0(1 - \sigma_X)\hat{I}_{Xz} + hJ_{AX}\hat{I}_{Az}\hat{I}_{Xz} \quad |\alpha\alpha\rangle \quad |\alpha\beta\rangle \quad |\beta\alpha\rangle \quad |\beta\beta\rangle$$
- Not so simple to analyze COSY (HSQC easier)
 - use Kanters' "POF" implementation in MAPLE to analyze COSY (limited to first-order spectra, and spin $1/2$ nuclei)
- Initial equilibrium (I_z) magnetization converted to $-I_y$ with 90°_x
 - red (input) and blue (output) from MAPLE using Kanters' "POF"
 - step1: define spin system ("spinsystem" is a defined function/object that allows the spins to be named and sets up equilibrium properties)
 - step2: 90°_x ($\pi/2_x$) pulse/rotation on step 1 for both spins, get expected y-magnetization

step1:=spinsystem([A,X]);

$step1 := I_{zA} + I_{zX}$ i.e. equilibrium magnetization

step2:=xpulse(step1,{A,X},Pi/2);

$step2 := -I_{yA} - I_{yX}$ transverse y-magnetization

Evolution Step Combines All parts of Hamiltonian in Kanter's POF Approach

- Evolution step ("evolve" function/object) by default includes both chemical shift evolution and scalar coupling
 - step 3: magnetization evolves with chemical shifts of A and X, and J_{AX}
 - no surprises, straightforward
 - first type of term: 'A' (I_{yA}, I_{xA}) and antiphase 'A' ($I_{xA}I_{zA}, I_{yA}I_{zA}$) magnetization (red boxes, solid and dashed, respectively) evolving with the chemical shift of A (W_A) and J_{AX} , as a function of t_1 (first 4 terms)
 - second type of term: same as first, but for 'X' spin (second 4 terms)
 - no mixing of terms (X evolving at W_A , or A evolving with W_X) yet.....
 - if you were to observe the magnetization, would just be two signals, each a doublet

> **step3:=evolve(step2,{A,X}, t1);**

$$\begin{aligned}
 \text{step3} := & \boxed{-\cos(2\pi W_A t_1) I_{yA} \cos(\pi J_{A,X} t_1)} + 2 \cos(2\pi W_A t_1) I_{xA} I_{zX} \sin(\pi J_{A,X} t_1) \\
 & + \sin(2\pi W_A t_1) I_{xA} \cos(\pi J_{A,X} t_1) + 2 \sin(2\pi W_A t_1) I_{yA} I_{zX} \sin(\pi J_{A,X} t_1) \\
 & - \cos(2\pi W_X t_1) I_{yX} \cos(\pi J_{A,X} t_1) + 2 \cos(2\pi W_X t_1) I_{xX} I_{zA} \sin(\pi J_{A,X} t_1) \\
 & + \sin(2\pi W_X t_1) I_{xX} \cos(\pi J_{A,X} t_1) + 2 \sin(2\pi W_X t_1) I_{yX} I_{zA} \sin(\pi J_{A,X} t_1)
 \end{aligned}$$

Second Pulse: Coherence Transfer

- The second 90° pulse does many interesting things, but the most interesting is coherence transfer to create A magnetization precessing at W_X , and X magnetization precessing at W_A
 - I_{yA} and I_{yX} terms $\rightarrow I_z$ (not observable)
 - $I_{xA}I_{zX}$ and $I_{xX}I_{zA}$ terms $\rightarrow -I_{xA}I_{yX}$ and $-I_{xX}I_{yA}$ (MQ, not observable)
 - I_{xA} , modulated by $W_A t_1$ (and J_{AX}) remains the same, as does I_{xX} , modulated by $W_X t_1$ (and J_{AX})
 - during t_2 , I_A will be modulated by $W_A t_2$ as well (and J_{AX}), giving a signal in the 2D spectrum centered at W_A (f_2) and W_A (f_1) (diagonal peak/auto peak)
 - same for I_X (modulated at W_X (f_2) and W_X (f_1), diagonal peak)

> step4:= xpulse(step3, {A,X}, Pi/2);

$$\begin{aligned}
 \text{step4} := & -\cos(2\pi W_A t_1) I_{zA} \cos(\pi J_{A,X} t_1) - 2 \cos(2\pi W_A t_1) I_{xA} I_{yX} \sin(\pi J_{A,X} t_1) \\
 & + \sin(2\pi W_A t_1) I_{xA} \cos(\pi J_{A,X} t_1) - 2 \sin(2\pi W_A t_1) I_{zA} I_{yX} \sin(\pi J_{A,X} t_1) \\
 & - \cos(2\pi W_X t_1) I_{zX} \cos(\pi J_{A,X} t_1) - 2 \cos(2\pi W_X t_1) I_{xX} I_{yA} \sin(\pi J_{A,X} t_1) \\
 & + \sin(2\pi W_X t_1) I_{xX} \cos(\pi J_{A,X} t_1) - 2 \sin(2\pi W_X t_1) I_{zX} I_{yA} \sin(\pi J_{A,X} t_1)
 \end{aligned}$$

- antiphase A ($I_{yA}I_{zX}$) modulated by $W_A t_1$ (and J_{AX}) \rightarrow antiphase X ($-I_{zA}I_{yX}$) modulated by $W_A t_1$ (and J_{AX})! During t_2 this will be modulated by $W_X t_2$ (and J_{AX}) as well, giving a signal centered at W_A (f_1) and W_X (f_2)! This is a crosspeak that correlates the two chemical shifts! (same for $-I_{zX}I_{yA}$)

Observation: t_2 evolution

- During t_2 , observable terms evolve with $W_A t_2$ and $W_X t_2$
 - ignore terms from previous step that are not observable
 - also, the program essentially takes the trace of the density matrix with I_{xA} , I_{xX} , I_{yA} and I_{yX} operators to give the observables (M_x and M_y), so there are no longer any operators in the terms
 - note "I" are y (imaginary) components

> **step5:=observe(step4, {A,X}, t2,0);**

$$step5 := \frac{1}{2} \sin(2\pi \boxed{W_A t1}) \cos(\pi J_{A,X} t1) \cos(2\pi \boxed{W_A t2}) \cos(\pi J_{A,X} t2)$$

$$+ \frac{1}{2} \sin(2\pi \boxed{W_X t1}) \sin(\pi J_{A,X} t1) \cos(2\pi \boxed{W_A t2}) \sin(\pi J_{A,X} t2)$$

$$+ \frac{1}{2} \text{I} \sin(2\pi \boxed{W_X t1}) \sin(\pi J_{A,X} t1) \sin(2\pi \boxed{W_A t2}) \sin(\pi J_{A,X} t2)$$

$$+ \frac{1}{2} \sin(2\pi \boxed{W_X t1}) \cos(\pi J_{A,X} t1) \cos(2\pi \boxed{W_X t2}) \cos(\pi J_{A,X} t2)$$

$$+ \frac{1}{2} \text{I} \sin(2\pi \boxed{W_A t1}) \sin(\pi J_{A,X} t1) \sin(2\pi \boxed{W_X t2}) \sin(\pi J_{A,X} t2)$$

$$+ \frac{1}{2} \text{I} \sin(2\pi \boxed{W_X t1}) \cos(\pi J_{A,X} t1) \sin(2\pi \boxed{W_X t2}) \cos(\pi J_{A,X} t2)$$

$$+ \frac{1}{2} \sin(2\pi \boxed{W_A t1}) \sin(\pi J_{A,X} t1) \cos(2\pi \boxed{W_X t2}) \sin(\pi J_{A,X} t2)$$

$$+ \frac{1}{2} \text{I} \sin(2\pi \boxed{W_A t1}) \cos(\pi J_{A,X} t1) \sin(2\pi \boxed{W_A t2}) \cos(\pi J_{A,X} t2)$$

- diagonal peaks (autocorrelation peaks) appear on the diagonal, at the same chemical shift in both dimensions (rectangular boxes)

- crosspeaks appear off of the diagonal, at WX in one dimension and WA in the other, correlating the chemical shifts of coupled nuclei (elliptical symbols)

- note the $\sin(J_{AX} t_1)$ and $\sin(J_{AX} t_2)$ dependencies in crosspeak terms: t_1 must reach $\sim 1/(2J)$ or sin terms make crosspeaks small (so, collect lots of t_1 points)

Analysis of Terms

- Each of the terms corresponds to a signal comprised of 4 peaks
 - the following trigonometric identities can be used to express these in a familiar looking manner (may not be obvious otherwise)

$$\sin(A)\cos(B) = \frac{1}{2}[\sin(A+B) + \sin(A-B)] \quad \cos(A)\sin(B) = \frac{1}{2}[\sin(A+B) - \sin(A-B)]$$

$$\sin(A)\sin(B) = \frac{1}{2}[\cos(A-B) - \cos(A+B)] \quad \cos(A)\cos(B) = \frac{1}{2}[\cos(A+B) + \cos(A-B)]$$

- Examine one of the crosspeak terms

$$= \frac{1}{2} I \sin(2\pi W_x t_1) \sin(\pi J_{AX} t_1) \sin(2\pi W_A t_2) \sin(\pi J_{AX} t_2)$$

- this suggests magnetization precessing at W_x during t_1 and W_A during t_2 (and modulated by J_{AX})

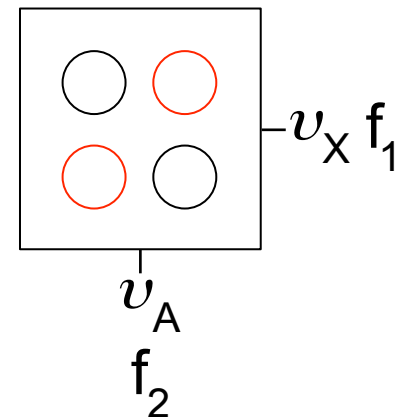
- apply the following identity: $\sin(A)\sin(B) = \frac{1}{2}[\cos(A-B) - \cos(A+B)]$

$$= \frac{1}{2} I \frac{1}{2} [\cos(2\pi W_x t_1 - \pi J_{AX} t_1) - \cos(2\pi W_x t_1 + \pi J_{AX} t_1)] \frac{1}{2} [\cos(2\pi W_A t_2 - \pi J_{AX} t_2) - \cos(2\pi W_A t_2 + \pi J_{AX} t_2)]$$

- writing this in a familiar way (frequencies $\pm J$) gives*

$$1. +(\nu_x - J_{AX})t_1 \quad 2. -(\nu_x + J_{AX})t_1 \quad 3. +(\nu_A - J_{AX})t_2 \quad 4. -(\nu_A + J_{AX})t_2$$

- this shows two antiphase doublets, the first (1 and 2) at ν_x in t_1 , and the second (3 and 4) at ν_A in t_2



Fourier Transforming these in t_1 and t_2 Gives a series of Absorptive and Dispersive peaks at $\nu \pm J/2$

- Fourier transformation changes time domains to frequencies
 - FT in t_1 and t_2 changes t_1 to ν_1 , t_2 to ν_2
 - MAPLE does the FT (first t_2 to ν_2 , then t_1 to ν_1 , output shown below)
- Example: look at first term
 - in ν_2 , frequency of X-J (absorptive), and in ν_1 , frequency of A-J (dispersive)
- Should be 16 peaks (4 signals x 4 peaks each), but are 32 terms. Why? No quadrature detection (yet).

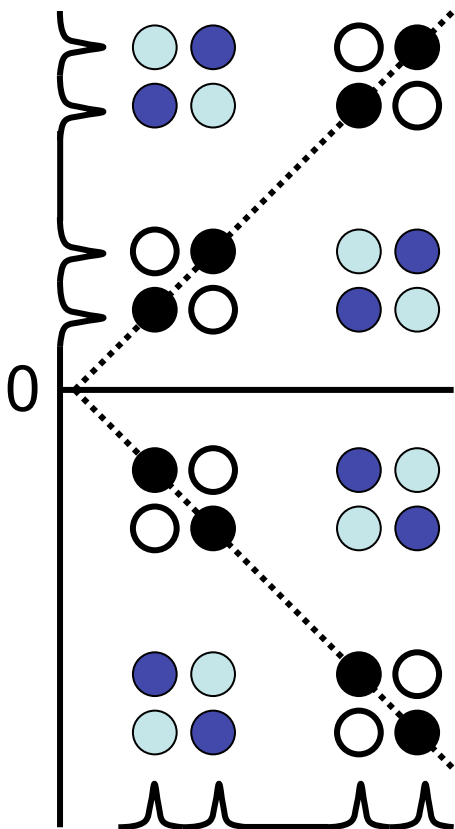
> spec1:= evalc(Re(FT(FT(step5,0,t2,v2),0,t1,v1)));

$$\begin{aligned} \text{spec1} := & -\frac{1}{16} \text{Ab}\left(-\frac{1}{2} J_{A,X} + W_X \nu_2\right) \text{Di}\left(-\frac{1}{2} J_{A,X} + W_A \nu_1\right) + \frac{1}{16} \text{Ab}\left(-\frac{1}{2} J_{A,X} + W_X \nu_2\right) \text{Di}\left(\frac{1}{2} J_{A,X} + W_A \nu_1\right) + \frac{1}{16} \text{Ab}\left(\frac{1}{2} J_{A,X} + W_A \nu_2\right) \text{Di}\left(-\frac{1}{2} J_{A,X} + W_X \nu_1\right) \\ & - \frac{1}{16} \text{Ab}\left(\frac{1}{2} J_{A,X} + W_A \nu_2\right) \text{Di}\left(\frac{1}{2} J_{A,X} + W_X \nu_1\right) + \frac{1}{16} \text{Ab}\left(\frac{1}{2} J_{A,X} + W_X \nu_2\right) \text{Di}\left(-\frac{1}{2} J_{A,X} + W_A \nu_1\right) - \frac{1}{16} \text{Ab}\left(\frac{1}{2} J_{A,X} + W_X \nu_2\right) \text{Di}\left(\frac{1}{2} J_{A,X} + W_A \nu_1\right) \\ & + \frac{1}{16} \text{Di}\left(-\frac{1}{2} J_{A,X} + W_A \nu_2\right) \text{Ab}\left(\frac{1}{2} J_{A,X} + W_A \nu_1\right) + \frac{1}{16} \text{Di}\left(-\frac{1}{2} J_{A,X} + W_A \nu_2\right) \text{Ab}\left(-\frac{1}{2} J_{A,X} + W_A \nu_1\right) + \frac{1}{16} \text{Di}\left(-\frac{1}{2} J_{A,X} + W_X \nu_2\right) \text{Ab}\left(\frac{1}{2} J_{A,X} + W_X \nu_1\right) \\ & + \frac{1}{16} \text{Di}\left(-\frac{1}{2} J_{A,X} + W_X \nu_2\right) \text{Ab}\left(-\frac{1}{2} J_{A,X} + W_X \nu_1\right) + \frac{1}{16} \text{Di}\left(\frac{1}{2} J_{A,X} + W_X \nu_2\right) \text{Ab}\left(\frac{1}{2} J_{A,X} + W_X \nu_1\right) + \frac{1}{16} \text{Di}\left(\frac{1}{2} J_{A,X} + W_X \nu_2\right) \text{Ab}\left(-\frac{1}{2} J_{A,X} + W_X \nu_1\right) \\ & + \frac{1}{16} \text{Di}\left(\frac{1}{2} J_{A,X} + W_A \nu_2\right) \text{Ab}\left(\frac{1}{2} J_{A,X} + W_A \nu_1\right) + \frac{1}{16} \text{Di}\left(\frac{1}{2} J_{A,X} + W_A \nu_2\right) \text{Ab}\left(-\frac{1}{2} J_{A,X} + W_A \nu_1\right) - \frac{1}{16} \text{Ab}\left(-\frac{1}{2} J_{A,X} + W_A \nu_2\right) \text{Di}\left(-\frac{1}{2} J_{A,X} + W_X \nu_1\right) \\ & + \frac{1}{16} \text{Ab}\left(-\frac{1}{2} J_{A,X} + W_A \nu_2\right) \text{Di}\left(\frac{1}{2} J_{A,X} + W_X \nu_1\right) + \frac{1}{16} \text{Ab}\left(\frac{1}{2} J_{A,X} + W_A \nu_2\right) \text{Di}\left(\frac{1}{2} J_{A,X} + W_A \nu_1\right) + \frac{1}{16} \text{Ab}\left(\frac{1}{2} J_{A,X} + W_A \nu_2\right) \text{Di}\left(-\frac{1}{2} J_{A,X} + W_A \nu_1\right) \\ & + \frac{1}{16} \text{Ab}\left(-\frac{1}{2} J_{A,X} + W_A \nu_2\right) \text{Di}\left(\frac{1}{2} J_{A,X} + W_A \nu_1\right) + \frac{1}{16} \text{Ab}\left(-\frac{1}{2} J_{A,X} + W_A \nu_2\right) \text{Di}\left(-\frac{1}{2} J_{A,X} + W_A \nu_1\right) + \frac{1}{16} \text{Di}\left(\frac{1}{2} J_{A,X} + W_A \nu_2\right) \text{Ab}\left(-\frac{1}{2} J_{A,X} + W_X \nu_1\right) \\ & - \frac{1}{16} \text{Di}\left(\frac{1}{2} J_{A,X} + W_A \nu_2\right) \text{Ab}\left(\frac{1}{2} J_{A,X} + W_X \nu_1\right) - \frac{1}{16} \text{Di}\left(-\frac{1}{2} J_{A,X} + W_A \nu_2\right) \text{Ab}\left(-\frac{1}{2} J_{A,X} + W_X \nu_1\right) + \frac{1}{16} \text{Di}\left(-\frac{1}{2} J_{A,X} + W_A \nu_2\right) \text{Ab}\left(\frac{1}{2} J_{A,X} + W_X \nu_1\right) \\ & + \frac{1}{16} \text{Ab}\left(\frac{1}{2} J_{A,X} + W_X \nu_2\right) \text{Di}\left(\frac{1}{2} J_{A,X} + W_X \nu_1\right) + \frac{1}{16} \text{Ab}\left(\frac{1}{2} J_{A,X} + W_X \nu_2\right) \text{Di}\left(-\frac{1}{2} J_{A,X} + W_X \nu_1\right) + \frac{1}{16} \text{Ab}\left(-\frac{1}{2} J_{A,X} + W_X \nu_2\right) \text{Di}\left(\frac{1}{2} J_{A,X} + W_X \nu_1\right) \\ & + \frac{1}{16} \text{Ab}\left(-\frac{1}{2} J_{A,X} + W_X \nu_2\right) \text{Di}\left(-\frac{1}{2} J_{A,X} + W_X \nu_1\right) + \frac{1}{16} \text{Di}\left(\frac{1}{2} J_{A,X} + W_X \nu_2\right) \text{Ab}\left(-\frac{1}{2} J_{A,X} + W_A \nu_1\right) - \frac{1}{16} \text{Di}\left(\frac{1}{2} J_{A,X} + W_X \nu_2\right) \text{Ab}\left(\frac{1}{2} J_{A,X} + W_A \nu_1\right) \\ & - \frac{1}{16} \text{Di}\left(-\frac{1}{2} J_{A,X} + W_X \nu_2\right) \text{Ab}\left(-\frac{1}{2} J_{A,X} + W_A \nu_1\right) + \frac{1}{16} \text{Di}\left(-\frac{1}{2} J_{A,X} + W_X \nu_2\right) \text{Ab}\left(\frac{1}{2} J_{A,X} + W_A \nu_1\right) \end{aligned}$$

Quadrature Detection in t_1

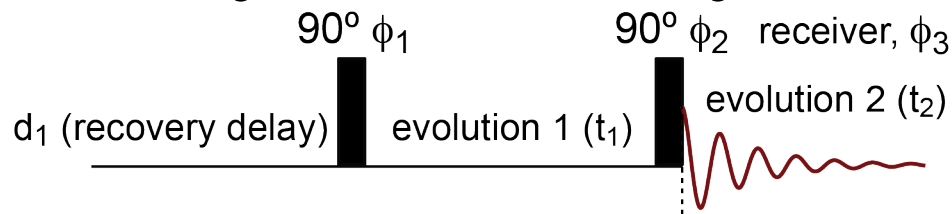
- Without quadrature detection, can't tell if signals are faster or slower than reference (so, FT gives both, so 2x actual number of peaks)
 - placing RF transmitter frequency to one side of the spectrum results in decreased S/N

no quadrature: 32 peaks
(should be 16)



- In the experiment, initial pulse created -y magnetization, so it evolved as such
 - the second pulse sampled it only along one axis
 - result of FT is two signals, one on either side of the RF transmitter (reference/carrier) frequency
- Can set up quadrature by alternating phase of second pulse (x,y)
 - so change the phase of the second pulse to get quadrature detection
- Additional cycling of pulses (and receiver) phase also assists in removing artifacts (such as "axial peaks")

Elementary "Phase Cycle" for COSY



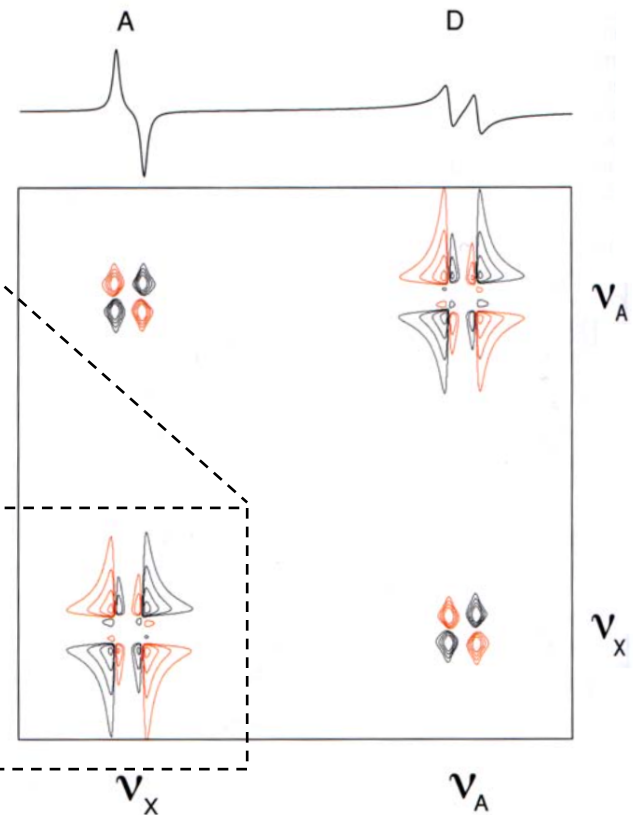
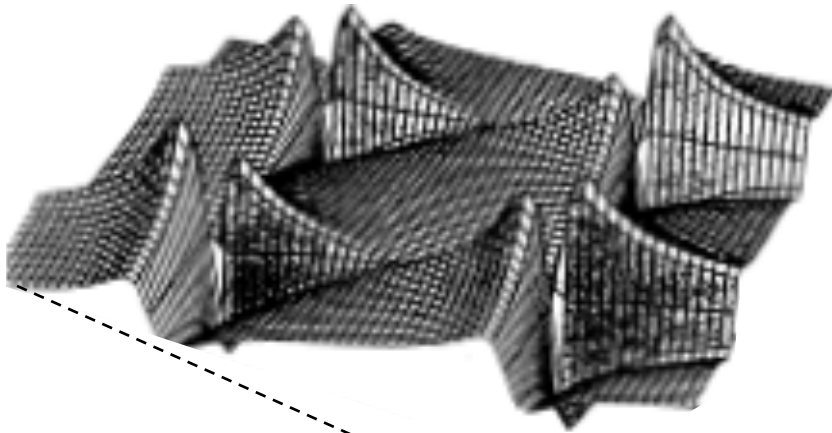
- Implementing quadrature detection in t_1
 - alternate phase of second pulse (constant receiver phase), store in memory as real and imaginary
- Removal of "axial" peaks
 - during t_1 , T_1 relaxation occurs, creating z-magnetization
 - this z-magnetization doesn't precess during t_1 , so is not modulated by chemical shift or J coupling
 - appears as peaks at zero frequency (center of spectrum with quadrature) in t_1 ("axial" peaks): annoying, no information content, obscure other peaks
 - remove by cycling first pulse and receiver (below)
- Important consideration: number of scans must be integral multiple of number of steps in the phase cycle

Phase cycle for COSY:

ϕ_1	ϕ_2	ϕ_3	memory
x	x	+	real, imag
x	y	+	imag, real
-x	x	-	real, imag
-x	y	-	imag, real

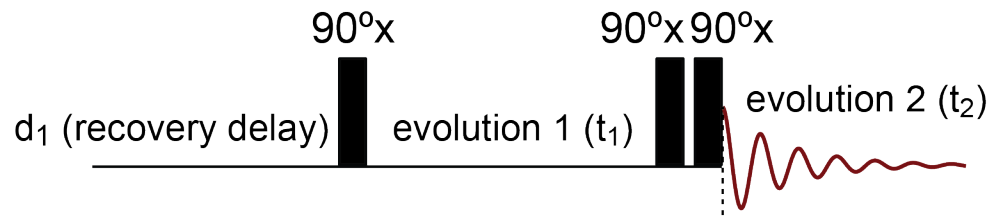
Another problem: Dispersive and Twisted Auto-Peaks (or Cross-Peaks)

- Diagonal signals are products of dispersive components
 - they give a twisted, dispersive lineshape
 - these are problematic, especially in crowded regions of spectra, as they overlap and obscure (important) crosspeaks



- crosspeaks are phased to give absorptive, antiphase signals, rendering diagonal signals with poor lineshape
- can plot as magnitude (absolute value) signals, but these are broad (poor resolution)
- still, the long "tails" of the diagonal peaks remain (crosspeaks obscured)
- are many ways to collect COSY spectra, and some alleviate this problem

Double-Quantum Filtered COSY



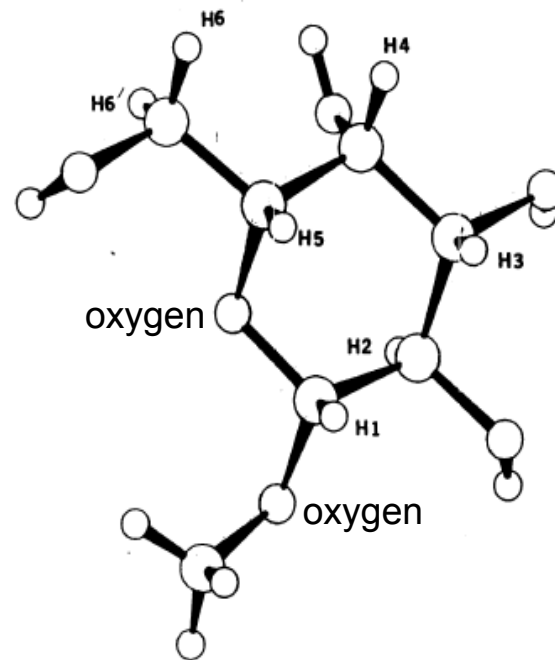
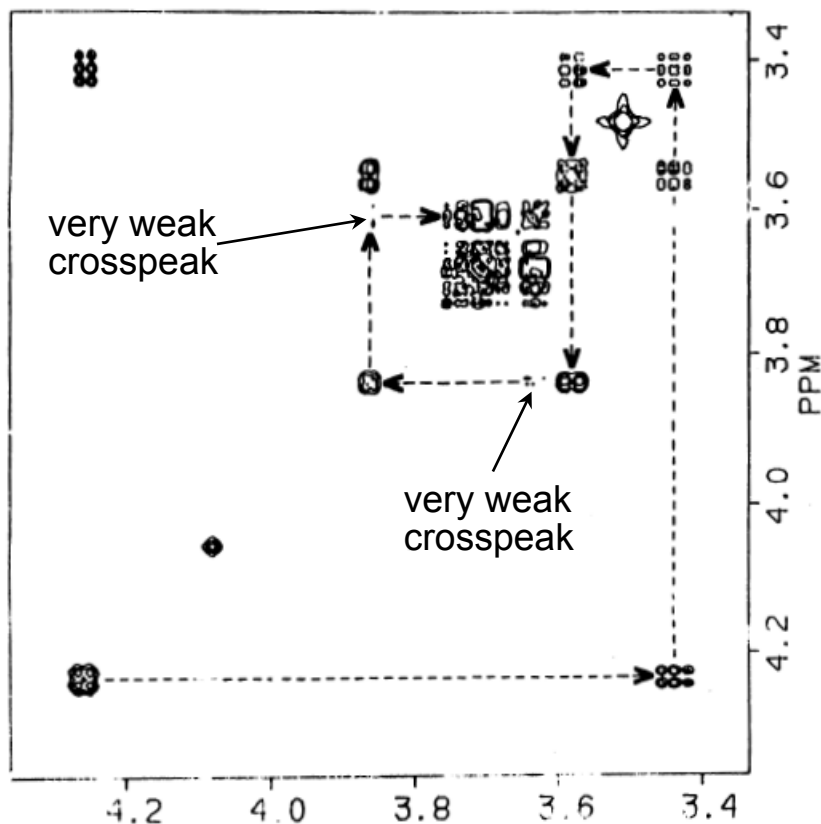
- Double-quantum filtered COSY is one variation on the COSY method that helps to alleviate the diagonal peak problem
 - consider what happens to the multiple quantum terms that follow the second 90°_x pulse when the third 90°_x pulse is applied:

$$-2I_{xA}I_{yX} \xrightarrow{\pi/2 I1_x \pi/2 I2_x} -2I_{xA}I_{zX} \quad -2I_{xX}I_{yA} \xrightarrow{\pi/2 I1_x \pi/2 I2_x} -2I_{xX}I_{zA}$$

- during the evolution period, these multiple quantum terms were precessing with the frequencies of A and X, and now have been converted back to observable single quantum magnetization
- With appropriate phase cycling, other terms are removed, so only those terms that precessed as multiple quantum survive
- The advantage is that all terms (those governing both diagonal and cross peaks) are pure absorptive (antiphase)
- Other types of signals are also filtered out by the double-quantum filter (contaminants, spurious peaks, solvent, any single line signals)

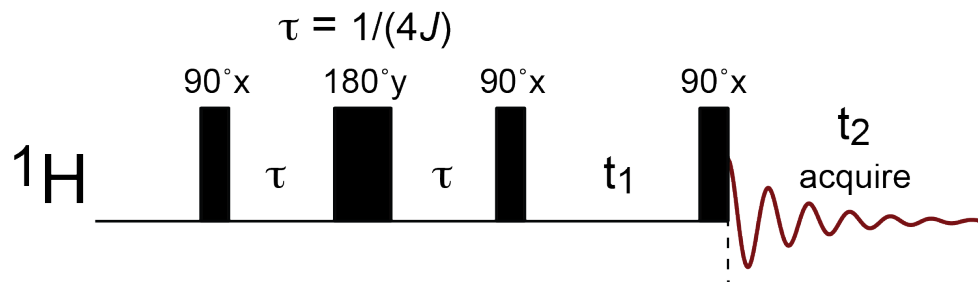
Example of 2Q-Filtered COSY

- Double quantum filtered COSY spectrum of β -methyl galactose
 - nice clean diagonal peaks and crosspeaks (pure absorptive, antiphase)
- See how many signals you can assign
 - start with anomeric hydrogen (H1): easy to pick out.....only H on a C that is bonded directly to two oxygen atoms.....
 - one crosspeak is very weak. Why? How might it be possible to make it stronger?

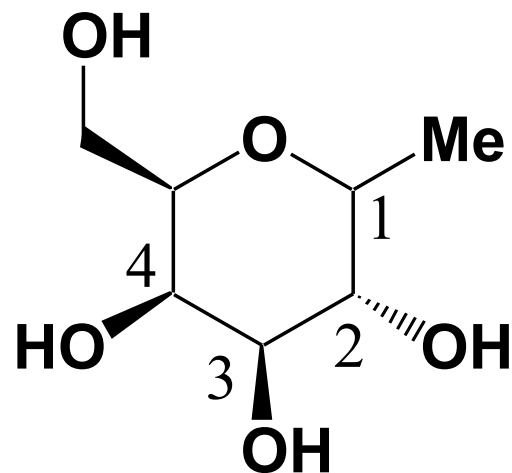
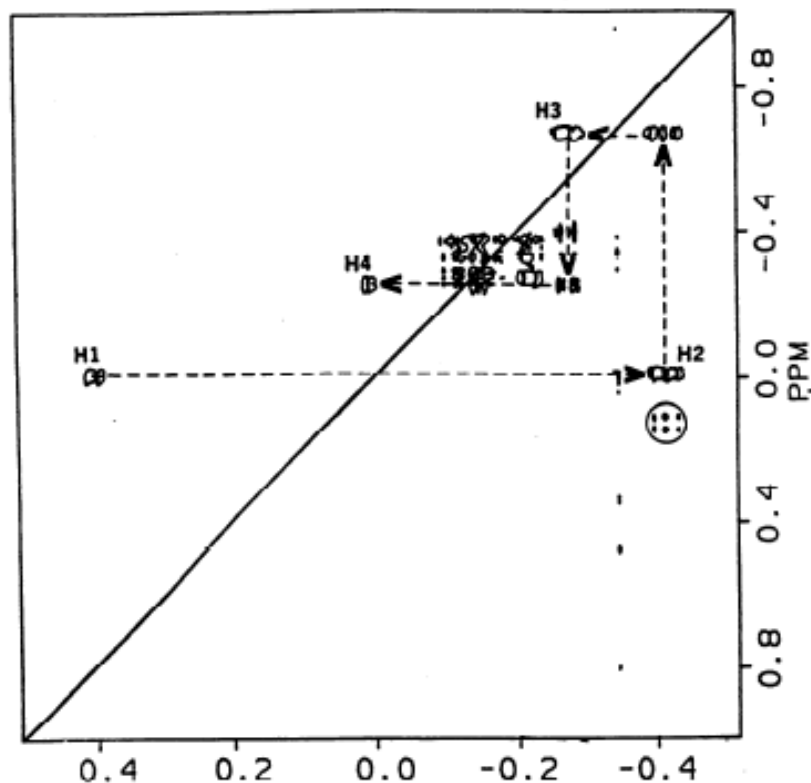


β -Me-Galactose

Recall: 2Q Spectrum of β -Me-Galactose:

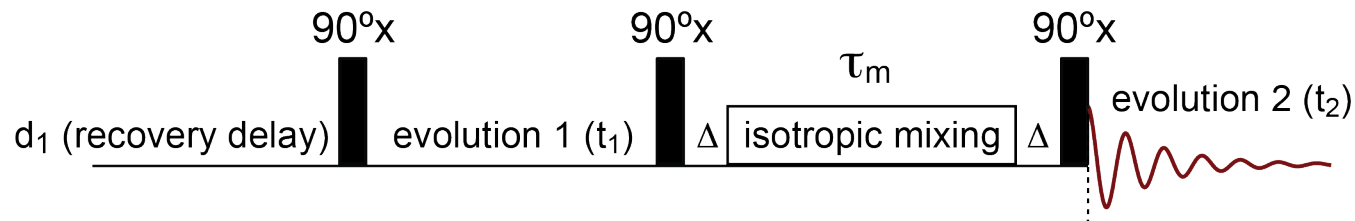


- Some advantages of the two-quantum method we saw earlier
 - no peaks on the diagonal
 - no peaks from single line signals (like solvent, same as DQF COSY)



TOCSY – Total Correlation Spectroscopy

- Method to correlate "all" spins in a given spin system
 - COSY crosspeaks indicate direct 2- or 3-bond coupling (larger J values)
 - TOCSY crosspeaks indicate that magnetization can be transferred through mutually coupled spins via 2- and 3-bond couplings
 - also useful in alleviating ambiguity in crowded regions (i.e. COSY aliphatic region) when one spin in the spin system is well resolved (anomeric proton in carbohydrates, amide proton in proteins)
 - a useful element in higher dimensional experiments (3D TOCSY-HSQC)



- Pulse sequence similar to COSY
 - mixing element is now 90° x- τ_m - 90° x rather than a single 90° x in COSY
 - Δ a short instrumental delay (transmitter power and pulse phase changes)
 - isotropic mixing sequence (sometimes called a "spin-lock") is a complex series of pulses that locks magnetization in the transverse plane
 - the behavior of the spins is similar to dozens of back-to-back spin-echo sequences: the relative frequencies now are ~identical (governed by the low rotation frequency in the transverse plane promoted by $B_{\text{eff}} \ll B_0$), but (homonuclear) couplings still active and independent of magnetic field or B_{eff} (so $\Delta\nu \sim J$, *strong coupling, highly second order behavior*)

Isotropic Mixing

- Product operators we've developed can't describe isotropic mixing
 - these assume first-order, isotropic mixing based on second order processes
- First order Hamiltonian: $\mathbf{H} = \sum_i -\omega_i \mathbf{I}_{iz} + \sum_{i \neq j} \pi 2 J_{ij} \mathbf{I}_{iz} \mathbf{I}_{jz}$
- Hamiltonian in small B_{eff} : $\mathbf{H} = \sum_i -\omega_i \mathbf{I}_{iz} + \sum_{i \neq j} \pi 2 J_{ij} \mathbf{I}_i \cdot \mathbf{I}_j$
- $\mathbf{I}_i \cdot \mathbf{I}_j = \mathbf{I}_{ix} \mathbf{I}_{jx} + \mathbf{I}_{iy} \mathbf{I}_{jy} + \mathbf{I}_{iz} \mathbf{I}_{jz}$
- The additional operators mix all coupled spin states
- Cartesian product operators no longer have nice one-to-one inter-conversion rules (only apply to first order)
- Could be done with simple B_1 field but complex pulse sequences work better and suppress relaxation effects:
- "DIPSI-2" (Shaka) uses super cycles $R \bar{R} \bar{R} R$
- $R=320^\circ \quad \overline{410^\circ} \quad 290^\circ \quad \overline{285^\circ} \quad 30^\circ \quad \overline{245^\circ} \quad 375^\circ \quad \overline{265^\circ} \quad 370^\circ$

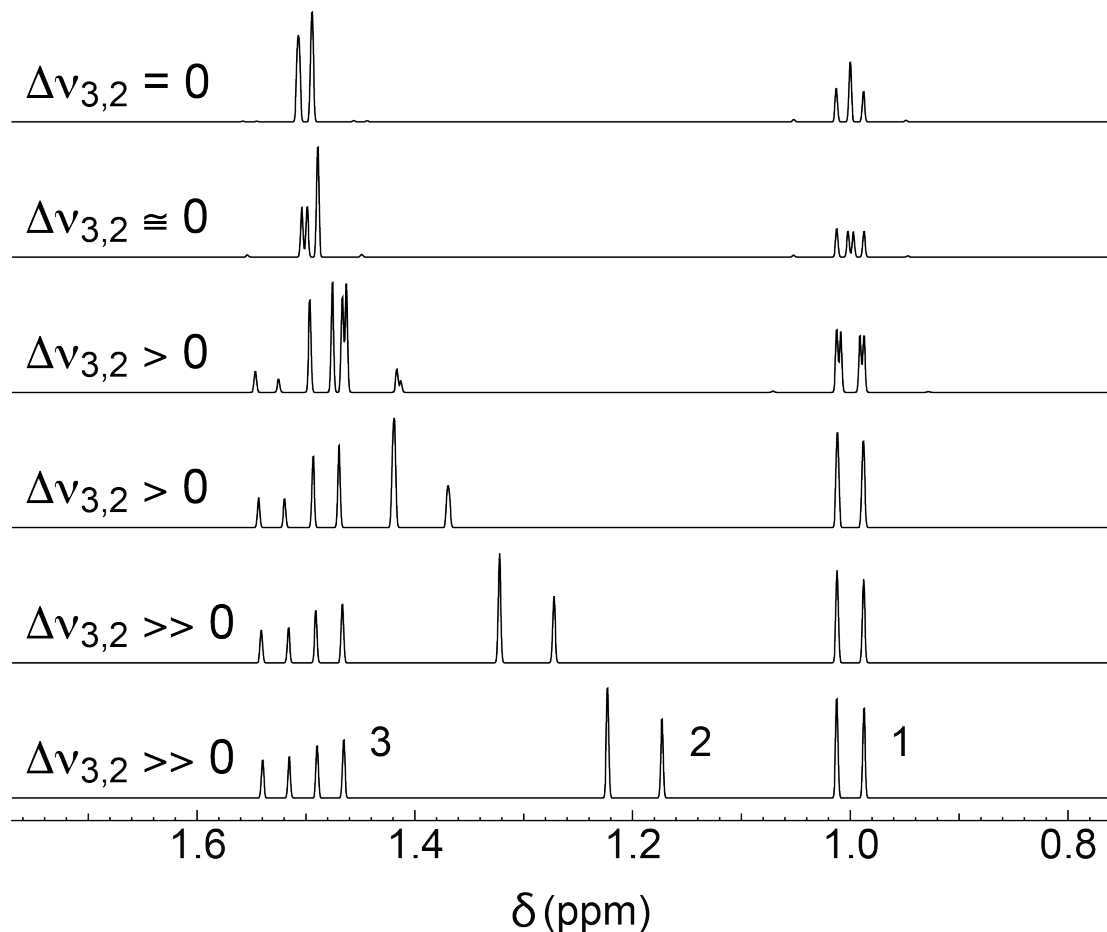
Practical Considerations for TOCSY

- Advantage: both cross-peaks and auto-peaks are in-phase and can be phased absorptive (unlike COSY)
 - good for large molecules where often get lots of peak overlap, so overlapping peaks with + and - intensities don't cancel one another
- Cross-peaks do not necessarily indicate direct coupling – but show virtual coupling as in second order spectra; $J_{13} \neq 0$, $J_{23} \neq 0$, $J_{12} = 0$; still see 1-2 splitting and cross-peak.
- The magnitude of cross-peaks depends on:
 - the topology of the spin system
 - magnitudes of all couplings involved
 - efficiency of mixing sequence
 - relaxation during τ_m .
- Chose τ_m 75-100 ms for long transfer, 30-50 ms for one to two couplings.

Virtual Coupling Examples

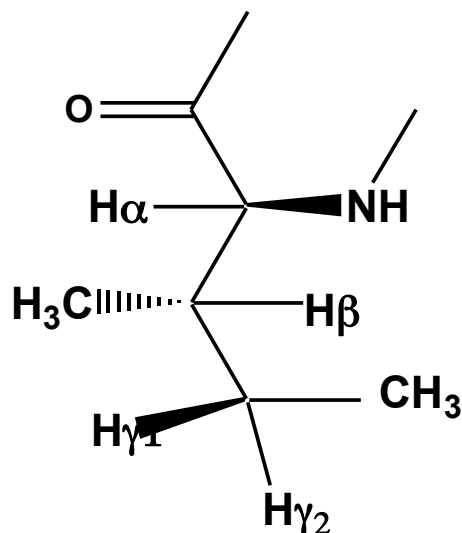
- Three spins (1, 2, and 3), $J_{1,3}=5$ Hz, $J_{2,3}=10$ Hz, $J_{1,2}=0$ Hz
 - no coupling between 1 and 2 ($J_{1,2}=0$ Hz)
 - however, as system becomes second order ($\Delta\nu_{3,2} \rightarrow 0$), virtual coupling to 1 from 2 (via 3) occurs

$$J_{1,3} = 5 \text{ Hz} \quad J_{2,3} = 10 \text{ Hz} \quad J_{1,2} = 0 \text{ Hz}$$



TOCSY Transfers in Isoleucine

- Transfer functions indicate complex magnetization exchange
 - in general, shorter mixing times favor short-range transfers
 - longer mixing times favor better transfer to all spins in the system



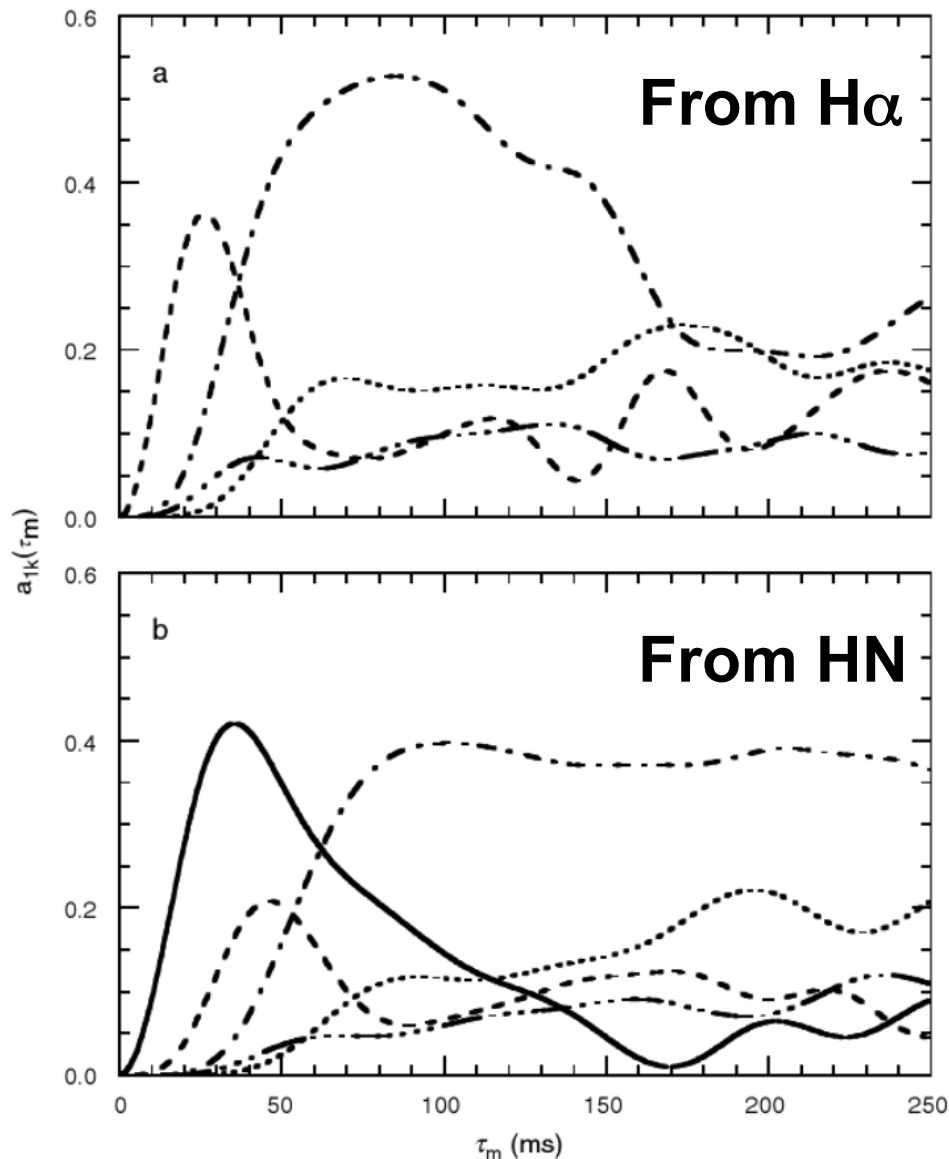
$H\alpha$ —, $H\beta$ ----, $H\gamma1$ -...-, $H\gamma2$ -.-, $H\delta$

$^3J_{HNH\alpha} = 10 \text{ Hz},$

$^3J_{H\alpha H\beta} = 12 \text{ Hz}, \quad ^3J_{HCH3} = 7 \text{ Hz},$

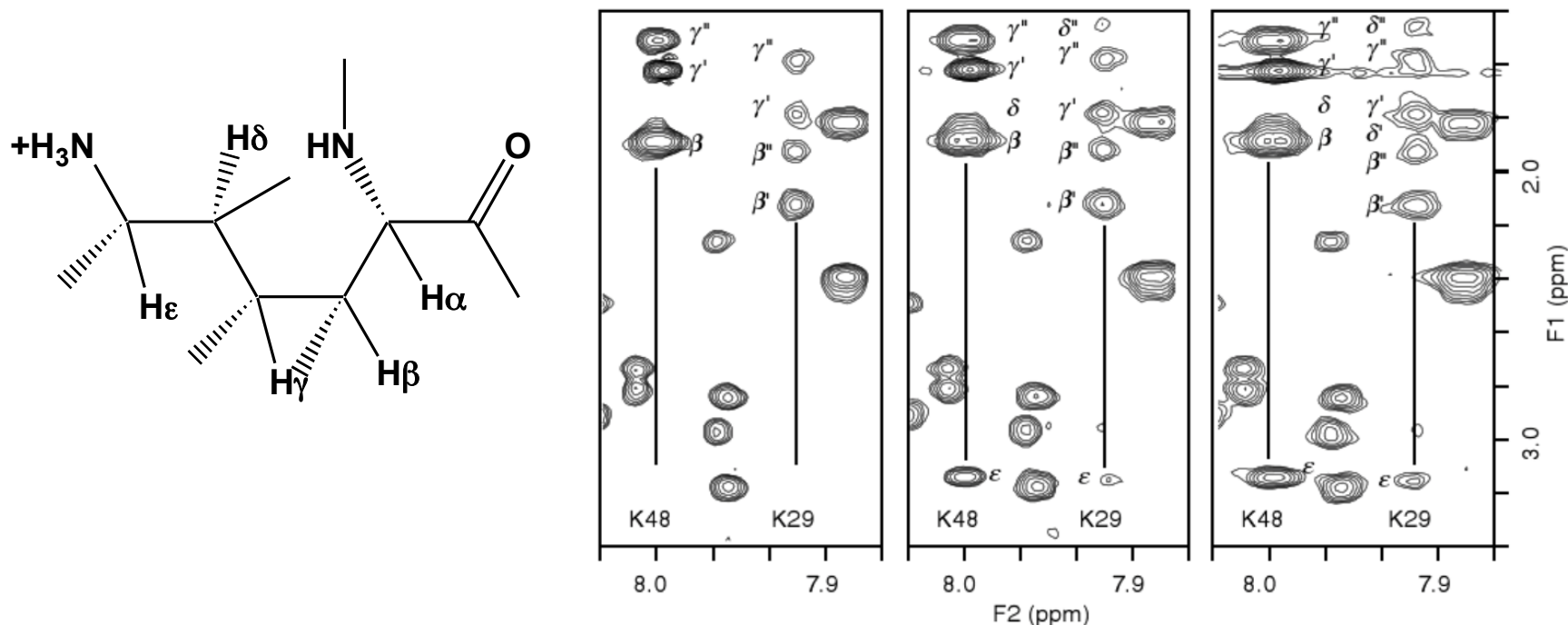
$^3J_{\text{geminal}} = -15 \text{ Hz},$

From Cavanagh .. and Palmer



Example TOCSY for Lysine

- Results depend on mixing time
 - compromise between mixing time length and magnetization losses due to relaxation
 - often a good idea to collect a TOCSY at 2 or 3 mixing times to get most complete set of data
 - TOCSY data also can assist in amino acid type identification



- Mixing times of 48, 83, and 102 ms
- From Cavanagh, Fairbrother, Palmer and Skelton