NMR TRAINING

MULTI-DIMENSIONAL EXPERIMENTS

What to Cover

- Introducing a second dimension
- COSY, NOESY, TOCSY, HSQC, HMBC
- 2D Processing
- Proton T1/T2 measurement,
- Diffusion measurement

Spectrometer Preparation

For all experiments, to begin with

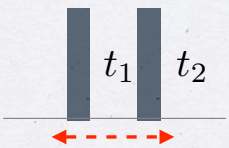
Make sure that the spectrometer is well tuned to the intended frequencies.

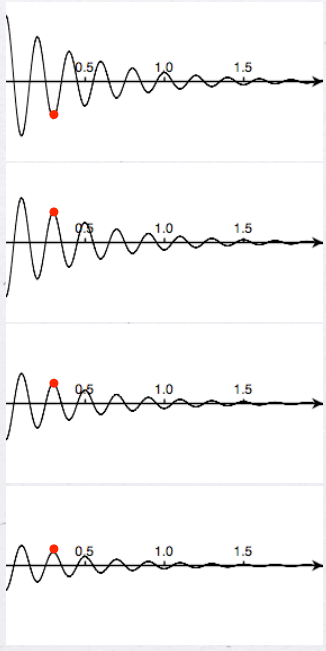
Make sure that the spectrometer is well shimmed.

Why Second Dimension

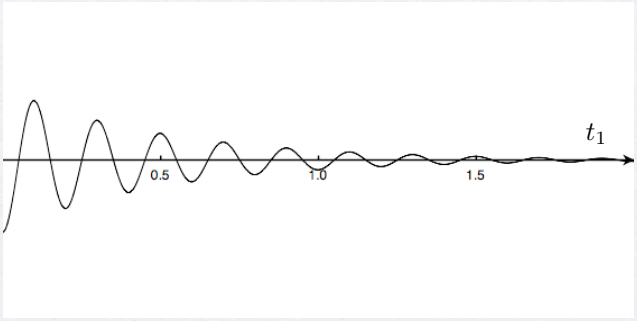
- To encode spin-spin interactions in a pulse sequence
  - Chemical shift interaction
  - J coupling
  - Dipolar coupling
- To remove spectral congestion

Introducing Second Dimension





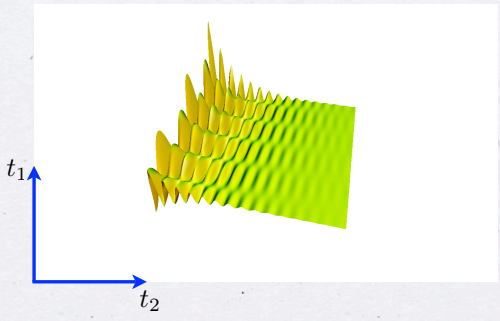
Introducing Second Dimension



FIDs in t_1 dimension

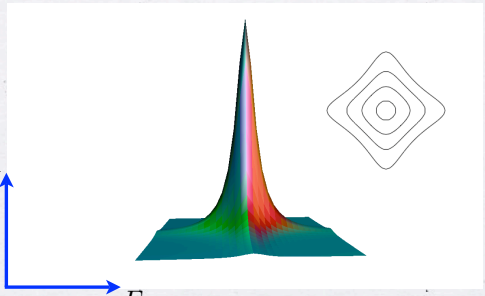
The data, $S(t_1, t_2)$, are now a function of both t_1 and t_2

Introducing Second Dimension



$S(t_1, t_2)$

Fourier Transform



$\Gamma(\Omega_1, \Omega_2)$

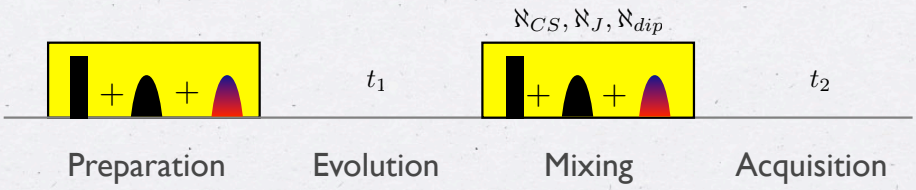
The data here were simulated for one single spin with 5Hz chemical shift and a T_2 of 0.5 second.

Introducing Second Dimension

This hypothetic experiment for one-spin system is spectacularly useless because it does not generate extra information

Introducing Second Dimension

$$I_A \longrightarrow I_A cos(\Omega_A t) \longrightarrow (\alpha I_A + \beta I_B) cos(\Omega_A t)$$

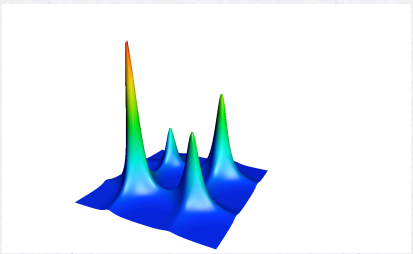


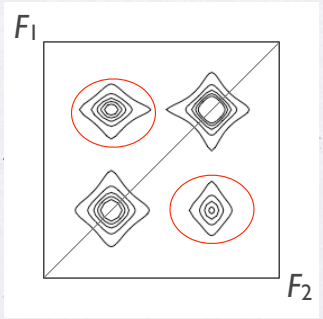
Preparing for spin state I_A

Spin state I_A gets frequency labelling during evolution

Coherence transfer from A to B during Mixing due to spin interactions

Introducing Second Dimension





Crosspeak generates information about spin interactions, which is correlated with your structural and dynamic information.

General Practical Considerations for 2D Experiment Setup

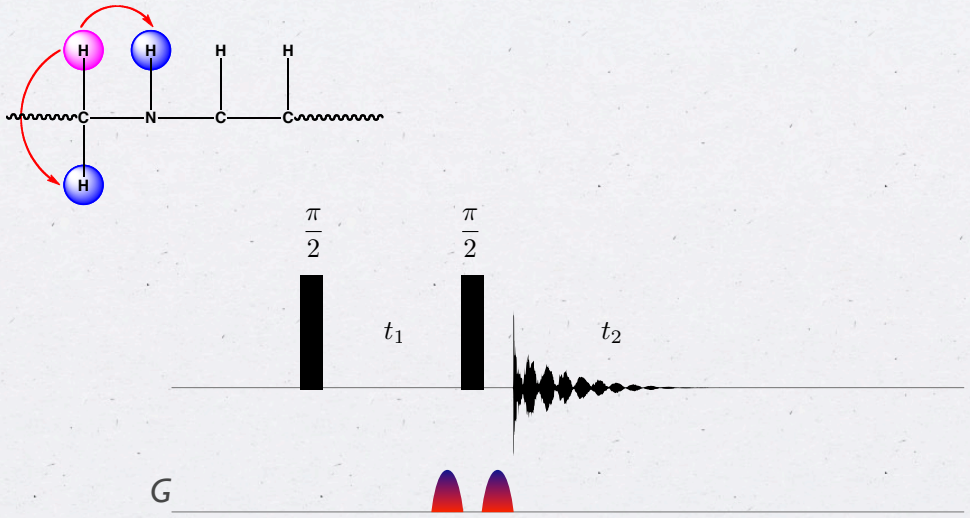
- To achieve sensitivities on F2 dimension, keep the sum of *d1* and *aq* at least 1.3 times of your T_1.
- It is rarely necessary to acquire longer *t1*s than twice of your T2s. In practice, for homonuclear experiments, keep *t1* in the range of 1/8 to 1/16 of your *t2*, and for heteronuclear experiment, set *t1* in the range of 1/128 to 1/256 of your *t2*. Linear prediction (see later) is recommended to remove data truncations in F1 dimension.
- To minimize t1 noise, 1) do not spin, 2) optimize your lock channel, 3) choose a solvent with a stronger lock if possible, 4) keep temperature constant, 5) use gradient version of a pulse sequence whenever possible.

Lock Optimization

When lock is established,

- Slowly increase the lock power until the lock signal starts to be saturated, then lower down the lock power by 3-5dBm.
- Adjust lock gain so that the lock level is around 80% of the full lock screen.
- Type loopadj to optimize loop gain and loop time. Although this is optional, sometimes it works very well to remove spectral artifacts.

[H,H]-COSY



What We Are Measuring

For diagonal signals:

$$\hat{I}_{1z} \xrightarrow{(\frac{\pi}{2})_x} -\hat{I}_{1y} \xrightarrow{\Omega_t I_z} \xrightarrow{2\pi J I_{1z} I_{2z}} \hat{I}_{1x} \sin\Omega_1 t_1 \cos\pi J t_1$$
$$\xrightarrow{(\frac{\pi}{2})_x} \hat{I}_{1x} \sin\Omega_1 t_1 \cos\pi J t_1$$

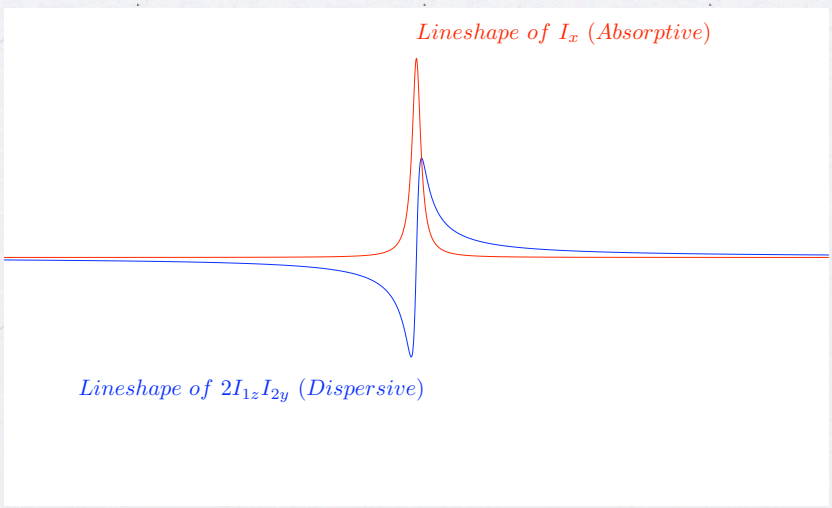
For cross peaks:

Preparation Evolution

$$\hat{I}_{1z} \xrightarrow{(\frac{\pi}{2})_x} -\hat{I}_{1y} \xrightarrow{\Omega_t I_z} \xrightarrow{2\pi J I_{1z} I_{2z}} 2\hat{I}_{1y}\hat{I}_{2z} \sin\Omega_1 t_1 \sin\pi J t_1$$
$$\text{Mixing} \xrightarrow{(\frac{\pi}{2})_x} -2\hat{I}_{1z}\hat{I}_{2y} \sin\Omega_1 t_1 \sin\pi J t_1$$

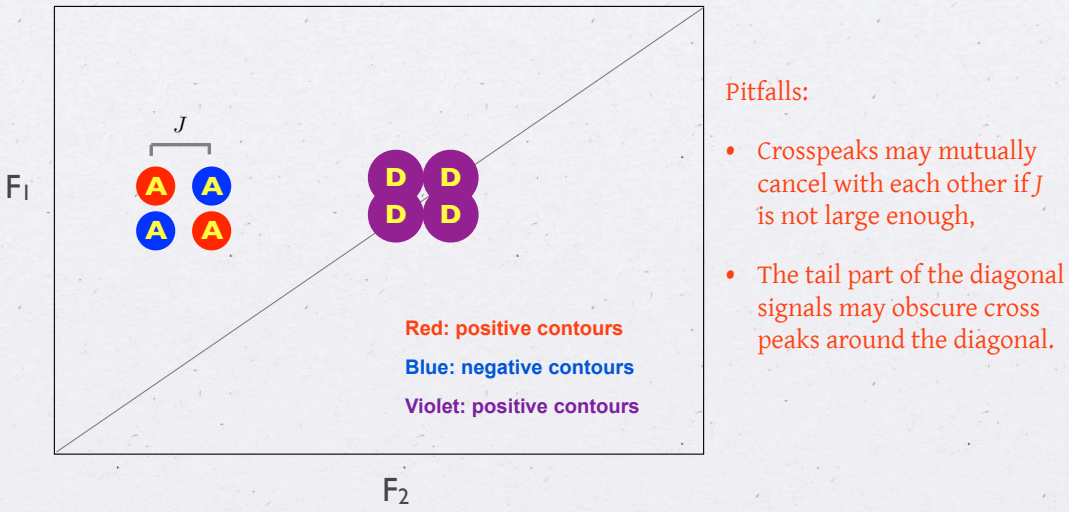
where, J is the coupling constant between I_1 and I_2, Ω is the chemical shift

Implications on Lineshapes



Dispersive lineshape has undesirable fat tails, therefore limits the spectral resolution

Implications on Lineshapes



Pitfalls:

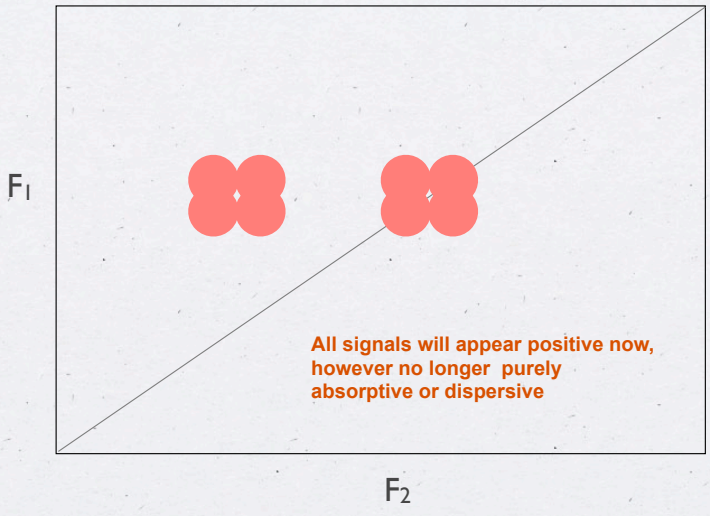
- Crosspeaks may mutually cancel with each other if J is not large enough,
- The tail part of the diagonal signals may obscure cross peaks around the diagonal.

Implications on Lineshapes

The EASIEST ways to correct the COSY lineshape problems:

- To use absolute value ($\sqrt{Re^2 + Im^2}$) to calculate the 2D spectra,
- To use a *sinebell* function to apodize your FIDs to reduce the intensity of diagonal signals relative to crosspeaks, and to improve resolution. A *sinebell* function de-emphasizes the initial parts of the FIDs, where the magnetization transfer is still minimal.

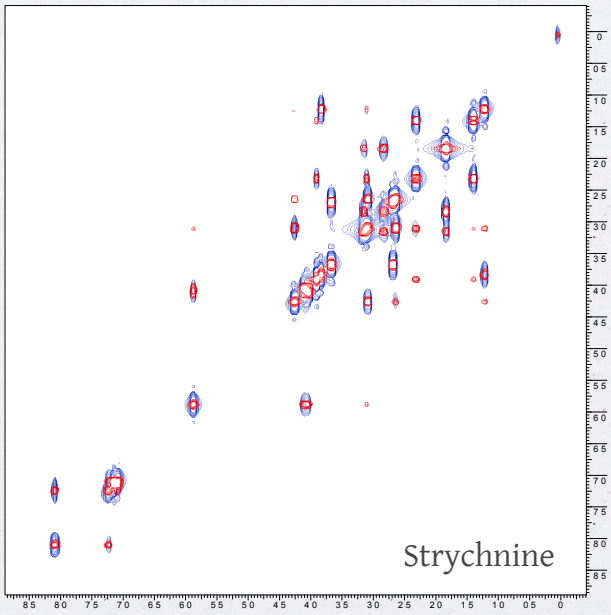
Implications on Lineshapes



Implications on Crosspeaks

- The coherence transfer is modulated by $\sin(\pi J t_1)$. For optimal transfer, t_1 should be set to $\frac{1}{4J} - \frac{1}{2J}$.
- Our default parameters are set to observe J couplings of around 10Hz.

Effects of t1 on COSY Crosspeaks



Blue: TD1=64 (t1=17ms)

Red: TD1=256 (t1=68ms)

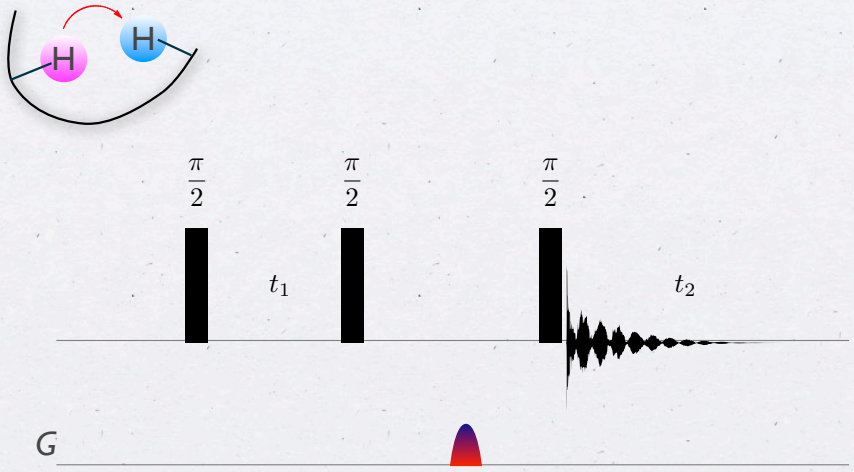
Step by Step Guide to COSY

- Run a proton spectrum, optimize SW and O1, take a note of SWH, O1 and SR,
- Create a new experiment with edc, type rpar ubc_COSY to load the standard parameter settings,
- Set your SWH, O1 and SR to the same values as in your 1D spectrum, type 1 SWH (note: there is a space between 1 and SWH) to set the frequency bandwidth in your F1 dimension exactly the same value as your SWH.
- The default TD on F1 is set to 256. If you wish to modify it, you can do so by typing 1 TD and then supply it with your desired value. Larger value than 512 is rarely necessary.
- set your number of scans (ns),

Step by Step Guide to COSY

- Type expt to calculate the total experiment time. For one scan experiment running at default parameters, COSY should take about 5 minutes,
- Type rga to automatically set the receiver gain, wait for the message: "rga: finished",
- Type zg to start the data acquisition, wait for the message: "zg: finished",
- Type xfb to do the 2D Fourier transform,
- You can automatically correct the baseline with abs2D command. You can also symmetrize your 2D map with sym command, however they will be done at your own discretion.

[H,H]-NOESY



What We Are Measuring

For diagonal signals:

$$\hat{I}_{1z} \xrightarrow{(\frac{\pi}{2})_x} -\hat{I}_{1y} \xrightarrow{\Omega_t I_z} -\hat{I}_{1y}\cos\Omega_1 t_1 \xrightarrow{(\frac{\pi}{2})_x} \hat{I}_{1z}\cos\Omega_1 t_1$$
$$\xrightarrow{\tau_m} a_{11}(\tau_m)\hat{I}_{1z}\cos\Omega_1 t_1 \xrightarrow{(\frac{\pi}{2})_x} -a_{11}(\tau_m)\hat{I}_{1y}\cos\Omega_1 t_1$$

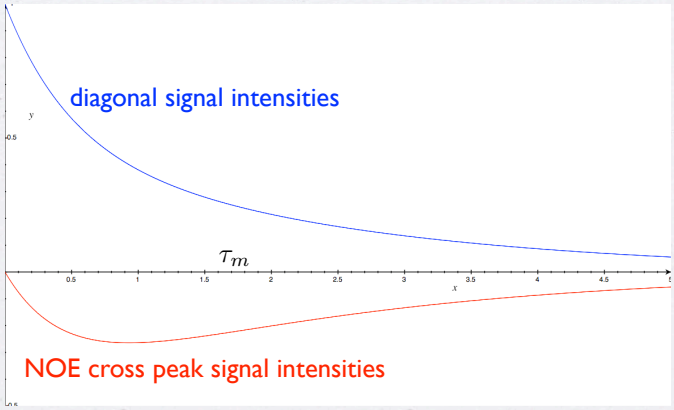
Both the diagonal and crosspeak now have the same absorptive lineshapes!

For cross peaks:

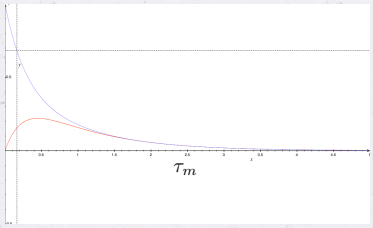
$$\hat{I}_{1z} \xrightarrow{(\frac{\pi}{2})_x} -\hat{I}_{1y} \xrightarrow{\Omega_t I_z} -\hat{I}_{1y}\cos\Omega_1 t_1 \xrightarrow{(\frac{\pi}{2})_x} \hat{I}_{1z}\cos\Omega_1 t_1$$
$$\xrightarrow{\tau_m} a_{12}(\tau_m)\hat{I}_{2z}\cos\Omega_1 t_1 \xrightarrow{(\frac{\pi}{2})_x} -a_{12}(\tau_m)\hat{I}_{2y}\cos\Omega_1 t_1$$

NOESY as a Function of τ_m

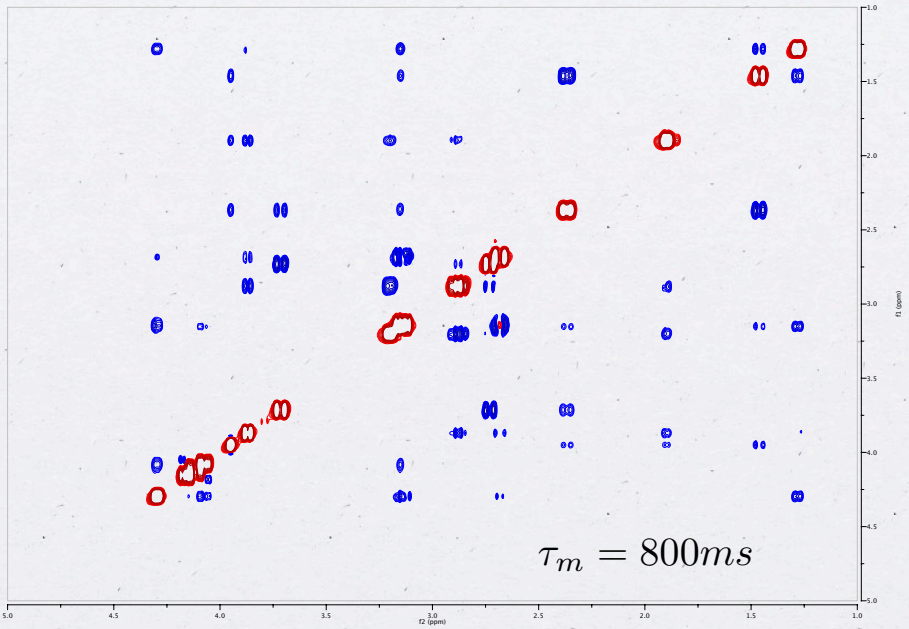
Simulation for geminal protons
of a small molecule



Simulation for geminal protons
of a large molecule



NOESY of Strychnine



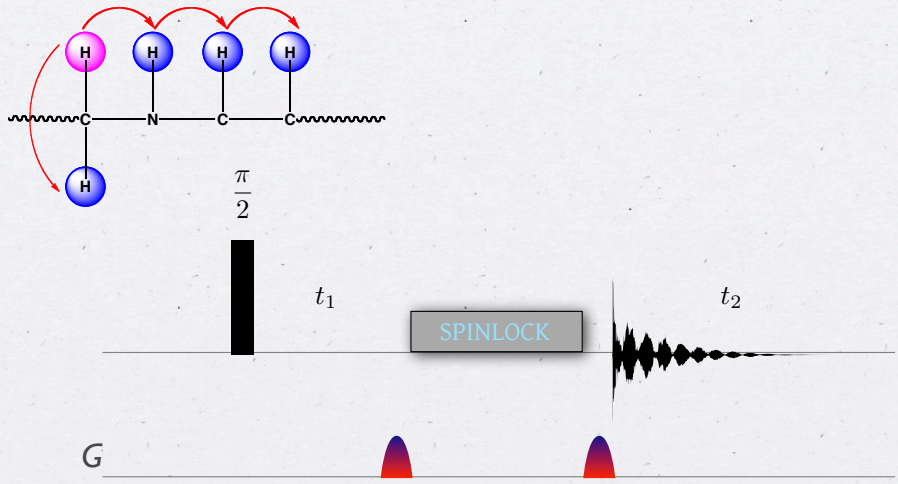
Step by Step Guide to NOESY

- Run a proton spectrum, optimize SW and O1, take a note of SWH, O1 and SR,
- Create a new experiment with edc, type rpar ubc_NOESY to load the standard parameter settings,
- Set your SWH, O1 and SR to the same values as in your 1D spectrum, type 1 SWH (note: there is a space between 1 and SWH) to set the frequency bandwidth in your F1 dimension exactly the same value as your SWH.
- The default TD on F1 is set to 400. If you wish to modify it, you can do so by typing 1 TD and then supply it with your desired value. Larger value than 512 is rarely necessary.
- Set your mixing time (d8). For small molecules, we recommend values between 600ms and 1.0s. The default is 800ms. Set your number of scans (ns=4xN), type expt to calculate the total experiment time.

Step by Step Guide to NOESY

- Type rga to automatically set the receiver gain, wait for the message: “rga: finished”,
- Type zg to start the data acquisition, wait for the message: “zg: finished”,
- Do rser 1, phase it all up, and save it as 2D.
- Type xfb to do the 2D Fourier transform. For small molecules, the diagonal signals have the opposite phase to the crosspeaks.

TOCSY



What We Are Measuring

For diagonal signals:

$$\hat{I}_{1z} \xrightarrow{(\frac{\pi}{2})_x} -\hat{I}_{1y} \xrightarrow{\Omega_t I_z} \xrightarrow{2\pi J I_{1z} I_{2z}} \hat{I}_{1x} \sin\Omega_1 t_1 \sin\pi J t_1$$

$$\xrightarrow{spin\ lock} \frac{1}{2}\hat{I}_{1x}(1+\cos(2\pi J\tau_{s-p}))\sin\Omega_1 t_1 \cos\pi J t_1$$

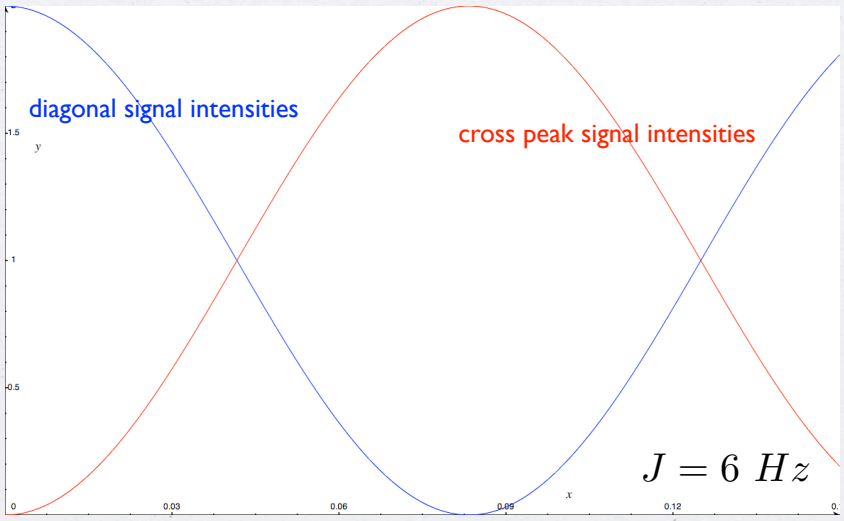
For cross peaks:

$$\hat{I}_{1z} \xrightarrow{(\frac{\pi}{2})_x} -\hat{I}_{1y} \xrightarrow{\Omega_t I_z} \xrightarrow{2\pi J I_{1z} I_{2z}} \hat{I}_{1x} \sin\Omega_1 t_1 \sin\pi J t_1$$

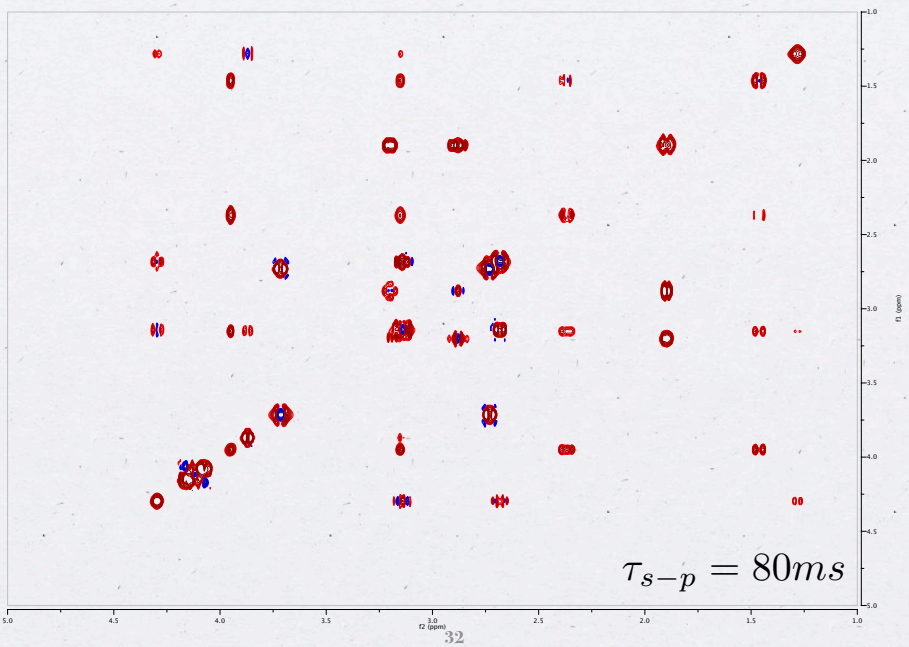
$$\xrightarrow{spin\ lock} \frac{1}{2}\hat{I}_{2x}(1-\cos(2\pi J\tau_{s-p}))\sin\Omega_1 t_1 \cos\pi J t_1$$

Both the diagonal and crosspeak now have the same absorptive lineshapes! Note: some COSY lineshapes may be observed due to the nature of spin-locking

TOCSY as a Function of Spin-Lock



TOCSY of Strychnine



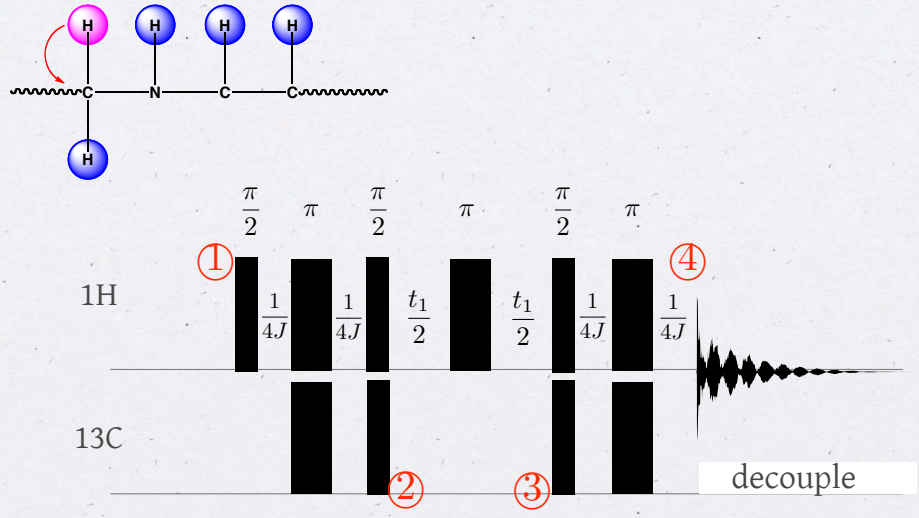
Step by Step Guide to TOCSY

- Run a proton spectrum, optimize SW and O1, take a note of SWH, O1 and SR,
- Create a new experiment, type rpar ubc_TOCSY to load the standard parameter settings,
- Set your SWH, O1 and SR to the same values as in your 1D spectrum, type 1 SWH (note: there is a space between 1 and SWH) to set the frequency bandwidth in your F1 dimension exactly the same value as your SWH.
- The default TD on F1 is set to 384. If you wish to modify it, you can do so by typing 1 TD and then supply it with your desired value. Larger value than 512 is rarely necessary.
- Set your Spin-Lock time (d9). The default is 80ms. Set your number of scans (ns=4xN), Type expt to calculate the total experiment time.

Step by Step Guide to TOCSY

- Type rga to automatically set the receiver gain, wait for the message: “rga: finished”,
- Type zg to start the data acquisition, wait for the message: “zg: finished”,
- Type xfb to do the 2D Fourier transform.
- Phase correct on both dimensions (see instructions later).

[1H, 13C]-HSQC



What We Are Measuring

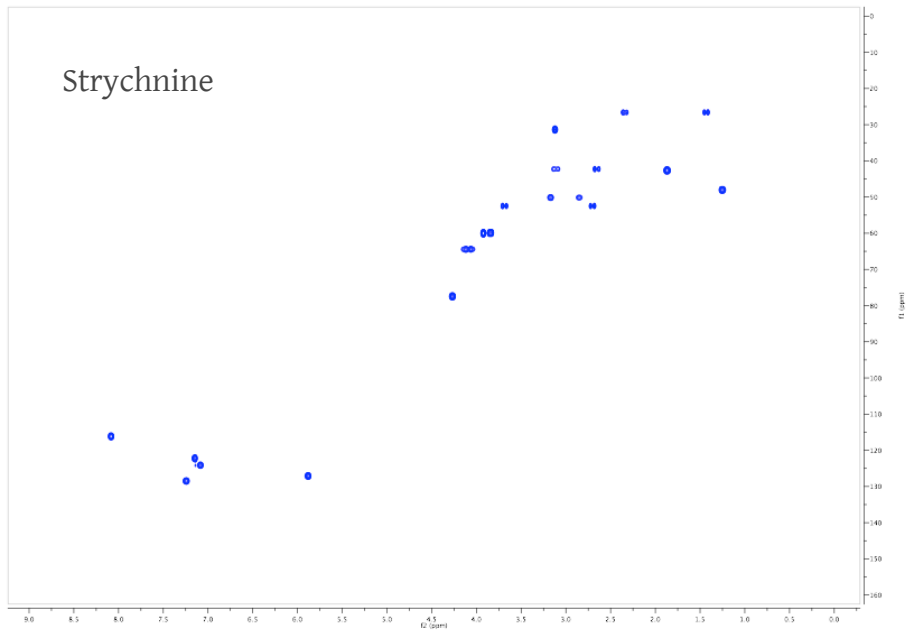
① $\hat{I}_z$

② $-2\hat{I}_z\hat{S}_y$

③ $2\hat{I}_z\hat{S}_y\cos(\Omega_S t_1)$

④ $\hat{I}_x\cos(\Omega_S t_1)$

[1H, 13C]-HSQC



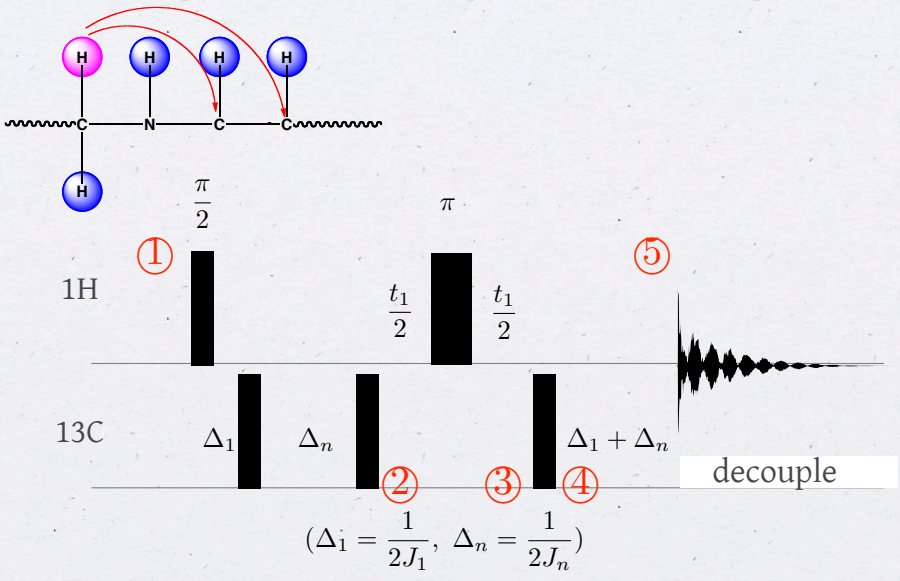
Step by Step Guide to HSQC

- Run a proton spectrum, optimize SW and O1, take a note of SWH, O1 and SR for your proton spectrum,
- Run a carbon spectrum, find out the optimum bandwith for all protonated Carbon-13 signals,
- Create a new experiment with edc, type rpar ubc_HSQC to load the standard parameter settings,
- Set your SWH, O1 and SR to the same values as in your 1D proton spectrum,
- You can use the default parameters for your bandwidth (0 - 165ppm) in C13-dimension. Alternatively, to customize your spectrum window in your C13 (F1) dimension, type 1 SW (note: there is a space between 1 and SW) to set the spectrum window of your F1 dimension; type O2p to set the desired frequency offset in F1; type 1 SR to set the reference frequency to your SR of your carbon spectrum,

Step by Step Guide to HSQC

- The default TD on F1 (carbon dimension) is set to 256. If you wish to modify it, you can do so by typing 1 TD and then supply it with your desired value,
- Set your number of scans (ns) of each increment,
- Type expt to calculate the total experiment time,
- Type rga to automatically set the receiver gain, wait for the message: "rga: finished",
- Type zg to start the data acquisition, wait for the message: "zg: finished",
- Type xfb to do the 2D Fourier transform,
- Phase correct the 2D spectra on both dimensions (see instructions later).

[1H, 13C]-HMBC



What We Measure

① $\hat{I}_z$

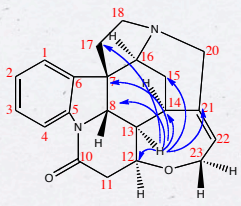
② $-2\hat{I}_x\hat{S}_y\cos\Omega_I\tau - 2\hat{I}_y\hat{S}_y\sin\Omega_I\tau$

③ $-2\hat{I}_x\hat{S}_y\cos(\Omega_I\tau)\cos(\Omega_S t_1) + 2\hat{I}_x\hat{S}_x\cos(\Omega_I\tau)\sin(\Omega_S t_1)$
$+2\hat{I}_x\hat{S}_y\sin(\Omega_I\tau)\cos(\Omega_S t_1) - 2\hat{I}_x\hat{S}_y\sin(\Omega_I\tau)\sin(\Omega_S t_1)$

④ $-2\hat{I}_x\hat{S}_z\cos(\Omega_I\tau)\cos(\Omega_S t_1) + 2\hat{I}_x\hat{S}_x\cos(\Omega_I\tau)\sin(\Omega_S t_1)$
$+2\hat{I}_y\hat{S}_z\sin(\Omega_I\tau)\cos(\Omega_S t_1) - 2\hat{I}_x\hat{S}_y\sin(\Omega_I\tau)\sin(\Omega_S t_1)$

⑤ $-\hat{I}_y\cos(\Omega_S t_1)$

CIGAR-HMBC of Strychnine



Step by Step Guide to HMBC

- Run a proton spectrum, optimize SW and O1, take a note of SWH, O1 and SR,
- Run a carbon spectrum, optimize SW and O1, take a note of SWH, O1 and SR for your carbon spectrum,
- Create a new experiment with edc, type rpar ubc_HMBC to load the standard parameter settings,
- Set your SWH, O1 and SR to the same values as in your 1D spectrum, type 1 SWH (note: there is a space between 1 and SWH) to set the frequency bandwidth in your F1 dimension exactly the same value as your SWH.
- You can use the default parameters for your bandwidth (0 - 250ppm) in C13-dimension. Alternatively, to customize your spectrum window in your C13 (F1) dimension, type 1 SWH (note: there is a space between 1 and SWH) to set the frequency bandwidth in your F1 dimension exactly the same value as your SWH of your carbon spectrum; type O2 and supply it with the O1 value of your carbon spectrum; type 1 SR to set the reference frequency to your SR of your carbon spectrum,

Step by Step Guide to HMBC

- The default TD1 is set to 400. If you wish to modify it, you can do so by typing 1 TD and then supply it with your desired value. Larger value than 512 is rarely necessary.
- set your number of scans (ns),
- Type expt to calculate the total experiment time,
- Type rga to automatically set the receiver gain, wait for the message: “rga: finished”,
- Type zg to start the data acquisition, wait for the message: “zg: finished”,
- Type xfb to do the 2D Fourier transform.

2D Data Processing

Type edp for the processing menu:

- SI: controls the size of the real part of the transformed NMR spectrum in each dimension. In F2 dimension, set SI=TD/2, in F1 dimension, set SI = 256 or 512. An excessively large SI in F1 can be very expensive in terms of computing speed and disk space and completely unnecessary.
- MC2 (F1): depends on your pulse program for data acquisition. This parameter is set according to how frequency discrimination on F1 is achieved. Inputs are States, States-TPPI, TPPI or Echo-AntiEcho)
- WDW and SSB: controls how you apodize your 2D FIDs. The fids in 2D experiments often appear as echos rather than exponential decays, the window functions are therefore different than the 1D case. The most common settings for WDW in each dimension are SINE (sine multiplication) or QSINE (squared sine multiplication), and SSB controls the phase of the sine function (or squared sine function) used in the fid apodization. When SSB=0, a pure sine wave is multiplied, and when SSB=2, a pure cosine wave.

2D Data Processing

- PH_mod: controls the phase mode implemented after the 2D FT. When set to "pk", phase correction is performed using the values of PHC0 and PHC1, when set to "mc", it calculates the magnitude spectrum. For 2D magnitude experiments such as COSY or HMBC, PH_mod(F2) is set to "no" and in F1 set to "mc". For phase sensitive experiments, PH_mod should be set to "pk" according to appropriate PHC0 and PHC1.
- ME_mod: controls linear prediction calculations on fids. To enable linear prediction, ME_mod can be set to LPfr (forward predict, real data), LPfc (forward predict, complex data), LPbr (backward predict, real data), or LPbc (backward predict, complex data). Set NCOEF to be approximately the same as expected signals. Usually linear prediction is only needed in indirect dimensions.
- Once all processing parameters are correctly prepared, type xfb to start 2D FT transformation. Note, if you load our parameter files (ubc*) to run your 2D experiments, these parameters should be correctly set already.

Phasing
"Phase Sensitive" 2D Experiments

- Generally, a 2D spectrum is first phase corrected in the F2 dimension (rows), and then in the F1 dimension (columns). To phase correct the spectrum in F2, three rows each with a cross peak should be selected. The cross peak of one row should be to the far left of the spectrum, the cross peak of the second row should be close to the middle, and the one of the third row should be to the far right of the spectrum.
- Enter the phase correction menu by clicking on the phase button.
- Select one row by clicking on row with the left mouse button to tie the cursor to the 2D spectrum appearing in the upper left corner of the display. Move the mouse until the horizontal cross hair is aligned with a row that has a cross peak. Select the row by clicking the middle mouse button. If the selected row does not intersect the most intense portion of the cross peak, click + or - with the left mouse button until it does. Once the desired row is selected, click on mov 1 with the left mouse button to move the row to window 1 appearing in the upper right hand corner of the display.
- Repeat the selection of rows described above for a row with a cross peak in the middle and another row with a cross peak at the right edge of the spectrum and move them to window 2 and 3, respectively.

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- Now that three rows have been selected, the 0th- and 1st-order phase corrections in F2 are determined by hand exactly as described for the 1D spectrum:
- Click on the big 1 or the cur 1 button to tie the cursor to the biggest peak of the row in window 1. Phase Correct this row using the 0th-order phase correction button ph0. Correct the 1st-order phase correction for the other two rows using the ph1 button and observe the rows in window 2 and 3, respectively.
- Save the phase correction by returning to the main window (select Save & return at the prompt).
- To phase correct the spectrum in F1, repeat the above procedure by selecting three columns rather than rows.

Step-by-Step to T1/T2 Measurement

- Setup your temperature with edte, and wait for the equilibrium,
- Create a new experiment with edc, and run a proton spectrum, optimize SW and O1, write them down,
- Create the next experiment with i or wrpa #n, load T1 or T2 parameter files with rpar ubc_T1 or ubc_T2, type in your SW and O1,
- Check if the default d1 and delay lists (t1_tau for T1 and t2_tau for T2) are appropriate for you. If not, you can create your own delay list files with different names. set your ns to 8xN,
- Type rga and zg to start acquisition, wait for message: "zg: finished".

Step-by-Step to T1/T2 Measurement

- Type rser 1, Fourier transform the fid, phase and baseline correct the spectrum and store it to 2D. Enter basl, from here click on def-pts . Use the middle mouse button to select the signals for which T1 or T2 will be calculated. Take care to select the point of maximum intensity for each peak. When finished, click the left-hand mouse button to release the cursor from the spectrum. Type wmisc , select baslpnts from the list, type in your file name to store the selected peak info. Click on return and select save and return to exit. click on 2D to return to the 2D data set.
- Type xf2 to process all the fids to spectra, and abs2 to baseline correct all the spectra,
- Type edt1, set NUMPNTS to 16 or your own number of delays, set LISTTYPE to vdlist (for T1) or vclist (for T2), set FCTYPE to invrec (for T1) or expdec (for T2). From the Analysis pulldown menu, select Relaxation (T1/T2), load the peak info file you just saved with rmisc, type pd0 to find the peak intensities for all spectra, click seen to ignore the error message, type simfit all calculate T1 or T2 values. **NOTE: The calculated T2s are in ms.**

Step-by-Step Guide to Measure Diffusion Coefficient

- Set up temperature with edte, create a new experiment, rpar ubc_DOSY1d, the default parameter file sets Δ (d20) = 50ms, and δ (p30=0.5ms), the gradient strength gpz6 is set to 2% of the maximum gradient strength of each spectrometer;
- Type rga and zg to acquire the data, optimize the SW and O1 if needed, re-acquire the data and store the data by wrp 2 (store it in PROCNO 2). This spectrum will be used as a reference for the parameter optimization process;
- Increase gpz6 to 95%, acquire the data, and compare this spectrum with the reference spectrum, the intensity will now go down. Adjust d30 so that the intensity will go down approximately to 5% of the reference spectrum. note: the smallest signals have to be above the noise, if not, increase number of scans;
- Type rpar ubc_DOSY2d to load 2D DOSY parameters, type in your optimized d20 and p30, as well as your SW and O1.
- Use dosy macro to set up diffusion measurement. dosy 2 95 16 l y y will set the starting gradient strength to 2%, the final gradient strength to 95% in 16 steps linearly, and the acquisition will start after rga.

Step-by-Step Guide to Measure Diffusion Coefficient

- Type rser 1, Fourier transform the fid, phase and baseline correct the spectrum and store it to 2D. Enter basl, from here click on def-pts . Use the middle mouse button to select the signals for which diffusion coefficients will be calculated. Take care to select the point of maximum intensity for each peak. When finished, click the left-hand mouse button to release the cursor from the spectrum. Type wmisc , select baslpnts from the list, type in your file name to store the selected peak info. Click on return and select save and return to exit. click on 2D to return to the 2D data set.
- Type xf2 to process all the fids to spectra, and abs2 to baseline correct all the spectra,
- Type setdiffparm, this will transfer all your experimental parameters, i.e. Δ and δ, and automatically set all your parameters under edt1 menu; From the Analysis pulldown menu, select Relaxation (T1/T2), load the peak info file you just saved with rmisc, type pd0 to find the peak intensities for all spectra, click seen to ignore the error message, type simfit all to calculate the diffusion coefficients.