

## TOCSY

TOCSY spectra are 2-D proton homonuclear experiments used to find through multiple through bond couplings. This experiment can identify isolated spin systems, such as single amino acids in a peptide chain. A TOCSY pulse sequence utilizes a spin lock that must be calibrated. Solvent presaturation with the transmitter is optional.

### *Basic TOCSY (upper case)*

1. Collect a 1-D proton spectrum. Find the 90-degree pulse width and set **pw90=pw**. Adjust the sweep width, **tof**, and **gain** of the spectrum. Make sure **probe='HCN'**. Call the macro **>TOCSY**.
2. Set **sw=sw1**, **nt**, **ni**, **gain**, **d1**, and **phase=1,2**. Set up presaturation parameters if desired.
3. This pulse sequence also has a parameter for **spinlock** that will be set automatically if left as **spinlock=0**. If desired, set **spinlock=2\*sw**.
4. Set the **mix** time. The mix time depends on how far the correlations to be observed. Mix times vary from 0.05 to 0.07 seconds. Suggested value, **mix=0.05**.
5. Check the **>time** and adjust the delays, transients, or increments to fit the time allowed. To start the experiment, **>go**.
6. Processing:
  - >setLP1**
  - >gaussian**
  - >wft2da**

If this is a long experiment, set **proc1='ft'** to turn off the linear prediction and omit the **setLP1**, otherwise the processing will take a very long time.

Alternative processing, (similar to NOESY):

**[ProcMenu]**

**[Phase F2]**

The first increment will appear. Phase the baseline as a 1-D spectrum.

**[Adjust Weighting]**

Use any weighting function desired, but a common one for a TOCSY is a gaussian (**gf**).

**[Return] [Transform F2]**

A spectrum will appear. Place the cursor on a FID (not solvent) that has a reasonable signal and **[Adjust Weighting]**. Again use a gaussian function or other function.

**[Return] [Transform F1]**

The spectrum should appear on the screen. If an error about being outside of range occurs, use **>f full dcon1**. To further correct phase on the spectrum and/or do a drift correct. For drift correct:

**>dc('f1')** or **dc('f2')**

or **>abc**

Alternatively, process the spectrum as follows:

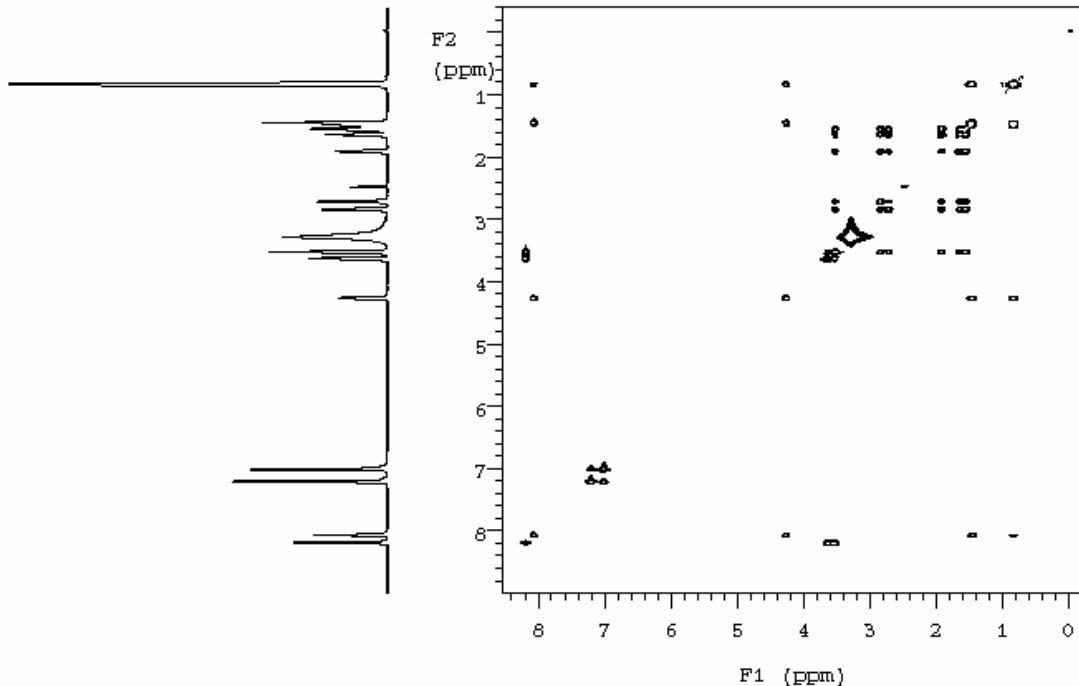
**>wft(1)** Phase the spectrum. **>wti**

Adjust the weighting function for the FID and use **>wft1da**.

Select a trace and use **>wti**. Apply the second weighting function and use **>wft2da**.

*Adding presaturation to basic TOCSY*

Set **d1=0**, **satmode='y'**, **satfrq=tof**, **satdly=1.5**, **satpwr=2**. The **satfrq** is not required to be equal to the **tof**. Values of **satpwr** and **satdly** vary with the sample. Saturation powers (**satpwr**) of 2dB are usually sufficient for residual solvent peaks. **Do not use satpwr>10dB**.



**Figure 28:** The TOCSY spectrum of the same three-residue peptide in Figure 18 shows longer range coupling than the COSY.

### Versions of TOCSY

**tocsy** – (lower case) older version, no gradients. Beware! The spin lock power for this pulse sequence is **tpwr** (lower power!) and the spin lock pulse width is **pw** (longer pulse). The hard 90-degree pulse and power are **p1** and **p1lvl**. Do not swap these or a high power pulse for the spin lock will be used and may damage the probe. Minimum **nt=4**.

**ntocsy** – lower case, presat TOCSY, (BioPack version), supports multiple solvent suppression

**[Setup] [Proteins][Standard Exps][Homonuclear/Auto1Ds/Auto2D][HH2Ds][TOCSYs][Presat (mlev)]**

The spin lock should be set up by the computer based on the proton pw90 in the probe file. Minimum **nt=2** (multiple of 8 recommended). Value of **window~pw**. Spinlock **strength~0.6 \* sw** (in Hz, the computer will likely set this).

### **Watergate TOCSY (wgtocsy)**

This TOCSY is the watergate version found in BioPack. Make sure **probe='HCN'**.

1. **[Setup] [Water] [AutoCalibration] [Watergates][Tocsy]** -or- **[3-9-19 TOCSY]**.
2. Answer the questions as prompted. Enter a delay time (**d1**) and a **mix** time (in seconds).
3. For the spin-lock strength, use a value equal to at least 60% of **sw** or greater than the spectral window (i.e. **sw=8000**, use 8000.) The spinlock will be set up based on the proton pw90 found in the probe file.
4. For the shape pulse, use a value between 1 and 2 msec.
5. After the optimization finishes, change **phase=1,2** and check **nt, ni, sw, sw1** before starting the experiment.

### **DIPSI TOCSY (called dipsih2o\_cl\_wg or sometimes gtocsywg)**

This a pulse sequence adapted from Lewis Kay's lab and is occasionally found in the main vnmr in the Structural Biology NMR Lab but is generally copied from user and is not part of standard VNMR. (Warning: not tested since upgrade to VNMRj 1.1D!)

Collect an optimized autowatergate 1D (gwatergate.c) and print out the parameters (**printon dg printoff**). Load the tocsy parameters. The hard 90 is **pw** is at **tpwr**. Set **tof** and **phincr1** from the watergate. Set **pw\_sl** equal to the **p1** from the 1-D watergate and set **tpwrs1** to the value for **p1lv1** from the watergate. Set **p180** equal to that from the watergate.

The **pw\_d** is the low power pulse (should be in upper 20s) at the power level of **ttrimpwr** (spin lock power) (~47-48 dB). Do this 90-deg. calibration in a separate experiment. **ncyc** sets the mixing time. To see the mixing time, type **dps**. **ncyc** must stay below 20 and must be an even number.