

SBS for COSY-, DQCOSY- and TOCSY-Type Experiments

Usage guide: **gcosy** is recommended for general use
cosy-90 and **DQCOSY** are the most sensitive forms of cosy-spectroscopy
gDQCOSY provides the best resolution, e.g., for measurement of J-couplings
TOCSY/TOCSY1D are good alternatives for peptides & oligosaccharides

- gcosy**
- **gcosy-90** variant is the default
 - is less sensitive than **cosy** by a factor of 2
 - faster than **cosy**, so **gcosy** is the preferred sequence unless concentration is low (in which case, **DQCOSY** is recommended)
 - minimum phase cycle $nt=1$ ($nt=2$ is $> \sqrt{2}$ better)
 - **gcosy-45** (set with **p1=pw90 pw=pw90/2**) minimizes width of the diagonal, so is useful if important crosspeaks involve protons having small differences in δ ;
 - in samples having strong singlets (including solvent peaks) interfering with in the spectrum, **gDQCOSY** is better
 - **gcosy-45** can provide the sign of J-couplings: vicinal having positive J values, versus geminal often having negative J values
 - **tau**≠0 sets up a long-range cosy; **tau**=0.1 is a typical value; **tau**~ $1/(2J_{lr})$ is the theoretical optimum value (for $-CH<$), but **tau** > 0.2 is unusual due to relaxation losses that would occur
 - **wft2d**, **sinebell** or **sqsinebell** processing, no linear prediction (**prun** is an alternative)
- cosy**
- **cosy-45** is the default
 - $nt=4$ is minimum phase cycle (thus **gcosy** is usually better)
 - **cosy-90** is setup by typing **p1=pw90 pw=pw90**;
 - **cosy-90** is better than **gcosy** when amount of sample is very limited (but **DQCOSY** is then the recommended variant for such samples)
 - **cosy-45** and long-range cosy options and processing are same as with **gcosy**
- gCOSY**
- Varian's ChemPack sequence (Varian's current standard cosy)
 - identical to **gcosy**, except **cosy90** only and no long-range option
 - **wft2d**, **sqsinebell** (sinebell-squared) processing [**prun** does by default], turns *on* 2× linear-prediction by default (which usually is OK)
- gDQCOSY**
- a very good double-quantum cosy (ChemPack) sequence;
 - removes all singlets, including large uncoupled methyl and solvent peaks
 - **wft2da**, **pi4ssbsq** ($\pi/4$ -sinebell-squared) [**prun** does by default]; turns *off* 2× linear-prediction by default
- DQCOSY** – better sensitivity than **gDQCOSY**, but more artifacts; otherwise same as above
- TOCSY**
- Varian's ChemPack sequence
 - sets up a good spin lock pulse with **mix** = 80 msec; recommend acquiring an additional tocsy with **mix** = 30 ms (acquiring a 3rd experiment/mix is not uncommon)
 - **wft2da**, **gaussian** processing [**prun** does by default], turns *off* 2× linear-prediction by default; can turn back on using **setLP1(2*ni) gaussian.↓**

Step-by-Step

I. For all experiments, start by acquiring a normal 1D proton

- **nt=8 ss=2 ga** [acquire good 1H 1d, and svf]
- **movesw nt=1 ss=0 ga** [baseline on each end of spectrum should be $\geq 10\%$ of **sw**; **ga** should be performed after the **movesw**]
- check **pw90** [not required prior to a **cosy**, **gcosy**, or **gCOSY**—presuming the probe is properly tuned!—but is recommended prior to **gDQCOSY** or **TOCSY**]
- check **gain** at **pw=pw90** [gain might be too high if set with the standard 30-40° pw]
- **mf(1,2) jexp2 dsx** [assumes 1H 1d in exp1; useful to keep 1H 1d around]

II. SetUp of 2D COSY-type Experiments

MAIN MENU → SETUP → SEQUENCES → **cosy** or **gcosy** or **gCOSY** or **gDQCOSY** or **DQCOSY** or **TOCSY**

Can just type the sequence name in. Sequence names are *case sensitive*.

- **Make certain the spinner is OFF!** Shim on X, Y, XY, X²-Y², XZ, YZ; if they change a lot, also shim XZ², YZ²

a) critical parameters

- **ni** crucial to total experiment time and digital resolution in F1
 - **time** $\approx (\text{at} + \text{d1} + \text{tau}) \times \text{ni} \times \text{nt}$
 - **dres1** (digital resolution in F1) = $\text{sw1} / (2 \times \text{ni})$ [without linear prediction]
 - size of J-coupling you can be confident of observing $\geq \text{dres1} / 3$
- **nt** affects **time** as shown above;
 - for **cosy** → **nt = 4 × j**,
 - for **gcosy/gCOSY** → **nt = 1** or **2 × j**
- **d1** $\sim (1 \text{ to } 2) \times T_1$ for protons of interest

a) other parameters

- **sw** need 10% baseline for each edge; **dres2=sw/np** (no zerofill, so **fn=np**)
- **sw1 = sw** and **fn1 = fn**; required for symmetrization; **dres1** set as above

$$\boxed{\text{crosspeaks observed when } J \geq \text{dres1} / 3 = \text{sw1} / (6 \times \text{ni}) .}$$

- **tau** used for long-range cosy (this parameter allows **ni** to stay reasonably small when observing/confirming crosspeaks involving small J-couplings); typical tau = 0.1s, ranging 50ms to 500ms
- **p1** only for **cosy** and **gcosy**; adjusts flip-angle of last pulse;
 - = **pw/2** for cosy-45-type (minimize diagonal; obtain sign of coupling)
 - = **pw** for cosy-90-type (maximizes sensitivity)

- **gDQCOSY, DQCOSY, and TOCSY**
 - recommend defining integral regions on 1D prior to entering 2D setup; be certain the integral regions cover *all* areas of the spectrum that are not noise, as what is not in a region will be used by the baseline (**bc**) fitting routines [vnmr processing only]
 - after running setup (by typing name of sequence), acquire 1st increment and **wft1**
 - [esp. important for TOCSY] phase as best you can, then enter **calfa** and re-acquire the 1st increment; should be no (or very small) 1st-order phase error (**lp~0**)
- **TOCSY mix** set depending on information wanted:
 - = 0.015 to 0.030 will be cosy-like, showing 2- and 3-bond couplings only
 - = 0.055 common intermediate value
 - = 0.080 common longer value, showing full spin network
 - = 0.200 longest value that should be tried (ask cgf if longer is wanted)

III. Processing 2D cosy-type (cosy, gcosy, gCOSY; magnitude-mode) Experiments

- standard processing commands:
 - **wft2d** or **wft2da** to transform ; try the new macro **pcon**
 - **dconi** or **dpconi** or **dqcon** for contour plot display
 - **symm** or **foldt** to symmetrize
 - **pcon pap page** to plot [a good plotting alternative is **plot2dhr**]
 - **do2d** is run if use **au** ; utilizing **wexp='do2d'**; **do2d** \equiv **wft2d foldt pcon page**
- apodization checks:
 - **prun** ;applies **sq sinebell** for cosy; **pi4ssbsq** for dqcosy; **gaussian** for tocsy, with $t_1(FI)$ apodization matched to **celem** (not to **ni**)
 - **wft1 wti** ;should start at 0, maximize in middle, and $\rightarrow 0$ at end of data
 - **wft1da ff dconi** ;ff will push full screen and full sw modes
 - set trace, **ds** ;note Index # at top of vnmr prior to **ds**, is row being displayed
 - **wti** ;shows apodization to t_1 row, $0 \rightarrow \max \rightarrow 0$ matching data/fid
 - **wft2da** ;final display prior to symmetrization
 - **symm** ;symmetrize (smallest point method)
- To set intensities for 2d displays (**dconi** followed at end by **dqcon**):
 - click middle button on the right-hand bar to show all colors (click next to the 0)
 - click middle button on the plot somewhere in the baseline (not on a peak)
 - click middle button again if necessary; it will toggle the intensity between higher and lower intensities; you want the lower intensity
 - click middle button on right-hand bar to remove one or two of the colors
- Plot cosy spectra using the **plot2dhr** macro. More details are given in the next section.

This page is a copy of section E of the DQCOSY write-up in VUG, for phase-sensitive processing of 2D datasets

IV. Processing and Plotting Phase-Sensitive 2D Data (dqcosy & tocsy)

- Often should not need to phase DQCOSY data at all.
- *phase sensitive 2d data* data should be processed something like the following:
 - Set **pmode='full'** ; allows phasing along F2 in 2d spectrum
 - **wft(1)** ; transform just first spectrum
 - **wtia** ; interactive phasing; middle button scales, left sets **lb**
 - **wft1da** ; perform first transform (on t_2 dimension)
 - If integrals have been setup (best done on high-res 1d done prior to setting up dqcosy), then **bc('f2')** can work wonders here.
 - Click on **TRACE** and select strong intensity trace.
 - ; trace='f1' changes columns→rows, trace='f2' goes back
 - **wtia** ; interactive phasing on t_1 trace, left button sets **lb/gf**
 - **wft2da** ; performs second (or both) transform(s)
 - Pick off two (or 3) traces that have crosspeaks
 - ; downfield trace save number as **r1**
 - ; upfield trace save number as **r2**
 - **ds(r1)** do 0-order phase only
 - **ds(r2)** do 1st-order phase only (click left mouse button on downfield position sets toggle pt)
 - Iterate between **ds(r1)** and **ds(r2)** (and often useful, a 3rd trace) to get good phase.
 - **dconi** ; should now have good phasing (**dqcon** give nicer display)
 - **trace='f2'** **dconi** allows phasing along F2 (similar to above) if needed
 - If integrals have been setup (as above), and only if **fn1=fn**, then **bc('f1')** can sometimes work wonders here.
 - **rl(..p)** references the F2 axis, **rl1(..p)** references the F1 axis.
 - To plot, **plot2dhr** is a macro that works quite well; if you want 1d projections, load the high-resolution 1d spectra into separate experiments before issuing the macro command. Otherwise, the parameters: **wc=130 wc2=wc sc=0** work well; this leaves room for a vertical projection or to print parameters on the page (use **disp2d** to set these).
 - **plot2dps** is exactly the same as **plot2dhr**, but it does not issue a **page** at the end; give a **page('filename')** to plot postscript or hpgl (preferred), depending on plotter selected.
 - **pconpos** or **pconneg** can have additional utility for plotting phase sensitive spectra (or just see **pcon** description in the Varian documentation).
 - Maximum printable parameters on 8.5×11 paper are **wc=230 wc2=150** ; square plots then use **wc=wc2=150**. **sc** will shift the plot from full right (**sc=0**) to the left by **sc** mm.