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 > √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** (π/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 ≥ 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, X^2-Y^2, XZ, YZ; if they change a lot, also shim XZ^2, YZ^2

a) *critical parameters*

– **ni** crucial to total experiment time and digital resolution in F1
- **time** ≈ (**at**+**d1**+**tau**) × **ni** × **nt**
- **dres1** (digital resolution in F1) = **sw1**/(2×**ni**) [without linear prediction]
- size of J-coupling you can be confident of observing ≥ dres1 / 3

– **nt** affects **time** as shown above;

for **cosy** → **nt = 4×j**,

for **gcosy/gCOSY** → **nt = 1** or **2×j**

– **d1** ~ (1 to 2) × 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

crosspeaks observed when ***J ≥ dres1 / 3 = sw1 / (6×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 ≡ wft2d foldt pcon page**
- apodization checks:
  - prun ;applies **sqsinebell** for cosy; **pi4ssbsq** for dqcosy; **gaussian** for tocsy, with t_1*(F1)* apodization matched to **celem** (not to **ni**)
  - **wft1 wti** ;should start at 0, maximize in middle, and →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→max→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.

IV. Processing and Plotting Phase-Sensitive 2D Data (any phase sensitive experiments)

- 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 the 2D experiment), then **bc2d('f2')** can work wonders here.

    → Note that care must be used in setting up the integrals. They should cover *all* areas of the spectrum that are not flat baseline. The point here is that the baseline routine will use all areas not integrated for the correction. It can also be important then to try to leave baseline regions between close multiplets, to insure the best flatness possible. This is often most important for noesy/roesy datasets where the crosspeaks are small.
  - 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.