15. HOMONUCLEAR COSY

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In COrrelational SpectroscopY (COSY), a "fast" 2D spectrum is usually obtained first. This initial set of data takes ~15 min on ***UWChemMRF*** Bruker equipment, and has a final size after transformation of 256×256 words. The data is N-type, taken as a magnitude set. Use COSY-45 unless sensitivity is an issue. Be watchful of symmetrization traps (see Derome) with this type of data. If you decide a higher resolution COSY data set is then needed, phase sensitive DQ-COSY is recommonded (rather than HR-COSY).

I. Quick Summary for Acquiring and Plotting COSY Spectra

[section II manual setup is recommended; enough can go wrong with 2D datasets that becoming familiar with the setup in detail has considerable value]

A. Obtain a high-resolution spectrum

- use normal 1D jobfile, and set reference using **EP-G**
- write down SR
- optimize SW using **EP-<cntl>O**
- retake spectrum with new SW; set SR and CY (=15 for 8.5×11, 19 for 11×17) and **WR *filename*.001**
- write down **DW** and **O1**, then **TR** [to another job]

B. Setup 2D paramters:

- turn the spinner off and touch up non-axial shims **X Y XY XZ YZ ...**
- enter: **PJ COSYFAST.1DJ** **;**use COSYHR.*x*DJ for high-res
  RJ2D COSYFAST.2DJ
- enter aquistion parameters from 1d job:
  DW = DW(^1H 1d)
  O1 = O1(^1H 1d)
- start 2D processing screen: **ST2D**
- setup F1 SW (SW1): **I2D <ret> 1 <ret>**

C. Check 2D parameters

- make sure SI2 = SI = 2*SI1
  TD2 = TD = 4*TD1 = 4*NE
  HZ/PT2/HZ/PT1 = 1
  SW1 = 1/2 × SW2 = 1/2 × SW (setting I2D=1 will do this)
  MC2 = M
  ND0 = 1

- type **IN <ret>** , and make sure **IN** = 2*DW *10^{-6} [IN in sec, DW in μsec]
- check that **P1** = 90° ^{1}H pulse width
- make sure exp is a reasonable length using: t_{exp} = NE*(DS+NS)*(D1+AQ)
- enter: **AU COSY.AU**
  FILENAME #1: ***filename*.SER**

D. Process 2D file in DISNMR (see NUTS Cheat Sheet for NUTS processing)

1. goto step 5 if you have just taken the data; if coming back later proceed with step 2
2. goto job1, **RE *filename*.001**
   PJ *filename*.001
3. check that SR and CY are set as in step A above (if not, correct them and
   WR <ret> <ret> Y to rewrite spectrum
4. goto job2, then **PJ COSYFAST.1DJ** ;use COSYHR.*x*DJ for high-res
   RJ2D COSYFAST.2DJ
5. enter: **RE *filename*.SER <ret> ST2D <ret> XFB <ret> SYM <ret>**
   or if already XFB: **RE *filename*.SMX <ret> ST2D <ret>**
6. check that SR=SR2=SR1 and CY are set as in step A above (if not, correct them)
7. enter: **AP2D** (AC's) or **EP2D** (AM's and DataStation)
   - set contour level, and expand region if desired

   enter **<ESC>-X** to exit
8. enter: **CP2P <ret>**

```
FILENAME IN F1:  filename.001
FILENAME IN F2:  filename.001
NO. OF PENS:  1 (or 2 if have red pen)
NO. OF CONTOUR LEVELS:  4 (1-7 levels)
OUTLINE BOX:  Y
GRID:  N
```

II. Detailed Explanation of COSY Set Up

A. Take high-resolution1D profile spectrum

1. Take a regular ^{1}H NMR spectrum. While in EP, set the reference using the G command (do this here because TMS and often the solvent peaks are not included in the COSY). Write down the value of SR; set CY=15 if plotting to 8.5×11 or CY=19 for 11×17 paper; save the file with the **WR** command. This spectrum is best for profile plotting as no folded peaks are involved.

Note SR; save spectrum

2. To increase the digital resolution, set the window as small as possible leaving ~10% of the SW as baseline on either side. Ignore TMS and solvent peaks, but do not fold compound peaks unless you are certain you can avoid folding into regions of interest. Commands useful for adjusting the spectral window are: **R <move cursor> R** and then **<ctrl> O** which sets SW and O1.
3. Take another *^1H* spectrum with these settings. Note on paper DW and O1 (along with SR). Transform and phase the spectrum to insure that the new sweep width parameters are set OK. If desired, set CY=15 if plotting to 8.5×11 or CY=19 for 11×17 paper, and save the ***spectrum*** with the **WR** command.

Note DW and O1

B. Setup 2D parameters

0. turn the spinner off and touch up the non-axial shims **X Y XY XZ YZ...**
1. **<ESC>** to Acquisition Parameters display.
2. Two sets of parameters are useful. Normally, acquire a fast COSY set first. If more resolution is then found to be needed, a simple modification of the fast COSY will provide a high resolution HR-COSY dataset; phase-sensitive DQ-COSY (see next Chapter) is recommended, however, rather than HR-COSY. Be aware that HR-COSY and DQ-COSY take much more time to acquire (minimum ~4 h; overnight runs are common).
3. Parameters can be entered either by accepting jobfile standard parameters, or by manual entry (***manual entry is recommended; see section 3b***):

   (a) ***Using job file parameters:***

	Fast COSY	**HR-COSY**
enter:	**RJ COSYFAST.1DJ**	**RJ COSYHR.1DJ**
	PJ COSYFAST.1DJ	**PJ COSYHR.1DJ**
	RJ2D COSYFAST.2DJ	**RJ2D COSYHR.1DJ**

   and re-enter sample-dependent acquisition parameters:

   SF = SF(*^1H* 1d)

   DW = DW(*^1H* 1d)

   O1 = O1(*^1H* 1d)

   ST2D

   I2D <ret> 1 <ret> , to make sure **HZ/PT2/HZ/PT1 = 1** (ie. SW1 = 1/2 SW2.)

   and skip table below, or

(b) ***Manual entry setup:*** follow the table below

When to enter	Parameter	Fast COSY	HR-COSY
Before ST2D	SI	512W [to 2K]	2K
Before ST2D	TD	512W [to 2K]	2K
Before ST2D	NE(=TD1)	128 [or 256]	512 to 1024
Before ST2D	ND0	1	1
Before ST2D	MC2	M	M
Enter ST2D now			
After ST2D	SW2	=SW	=SW
After ST2D	SW1 (setting I2D=1 is easier)	=SW/2	=SW/2
After ST2D	SI2(=SI)	512W [to 2K]	2K
Aer ST2D	SI1(=SI/2)	256W [to 1K]	1K
After ST2D	TD1(=NE<=SI1/2)	128W [or 256W]	512W to 1K
After ST2D	HZ/PT	4-20	0.5-4
After ST2D	WDW1=WDW2	S	S
After ST2D	SSB1=SSB2	0	0
After ST2D	SR1=SR2	SR	SR
Before ST2D	REV	N	N

Important: ***TD1 = 1/2 x SI1****. Zero-filling is reommended in F1 to increase digital resolution.*
SI1 = 1/2 x SI2*. Necessary to form square matrix for SYMmetrization.*
SW1 = 1/2 x SW2*. Full sweep width in F1 is actually +/- SW1.*
SW1/NE ≤ 24. *Increase NE if necessary, and associated parameters TD1, SI1, TD2, SI2.*

4. Make sure that:

   (a) **SI** at top of screen, says W after 512 for Fast COSY, and **TD** in both F2 and F1 dimensions equal the above values. F2 is the data acquisition dimension; F1 is formed by the second Fourier transform set of points down F2 columns.
   (b) **I2D=1**, to make sure **HZ/PT2/HZ/PT1 = 1** (ie. SW1 = 1/2 SW2.)
   (c) **SI×NE** is the size of subprogram matrix file which will be created at the beginning of the COSY acquisition. Make sure that there is enough space on disk before starting COSY; **DIR COSY.AU** will show the largest contiguous space (the .SER file has to go into this) following the listing.

5. **AS COSY.AU <ret>** [job file entry, all should be ok, but check; manual entry changes are needed]

D1 = 1S (sec; actually set to 1-3 T_1 for ^{1}H; use ~1T_1 for fast-COSY, 2T_1 for HR-COSY)
P1 = 90° *1H* pulse in μsec; observe transmitter; see pulsewidth sheet for lastest calibrations)
D0 = 3 U (initial delay)
P2 = 45° pulse in μsec = P1/2; if no close couplings, can use 90° pulse length for improved S/N
RD = PW = 0
DE = (dead time; automatically set by the spectrometer)
NS = 4 or 16 (4, 8, or 16; use 4 for fast-COSY, 16 would minimize artifacts, but 4 would still be common for HR-COSY)
DS = 2 (use 2 or 4 dummy scans)
NE = set as discussed above with NE=TD1, SW1/NE ≤ 12
IN = increment of D0; in essense DW in F1 dimension; should be set from ST2D

make sure *IN = 2*DW* (where, however, IN is given in seconds, DW in μs)

III. Acquiring COSY Spectra

A. The command **EXPT** is not accurate!! Calculate the experimental time as follows:

$$t_{exp} = NE*(DS+NS)*(D1+AQ)*1.3$$

If the experiment is too long, adjust NE (TD, SI, TD2, SI2, and SI1, accordingly), NS, or D1.

B. You may want to save the 2D parameters in a job file using **WJ2D** (I often use .2DJ extension). All parameters except NE (TD1 is saved, though, and NE=TD1) will be saved. Many of the 2D parameters will *not* be saved with the .SER file.

C. **ZE**; **AU <ret>**

FILENAME #1: **<filename>.SER <ret>** ...Provide a filename with the extension .SER
(do *not* deviate from .SER; it is required!!)

D. After first scan, check FID and if necessary, adjust receiver gain. **<ctrl> E**; **ZE**; **AU** to restart COSY.

III. Processing 2D Data in DISNMR (see NUTS Cheat Sheet for NUTS processing)

(follow the next steps *exactly* to get correct projection plots)

A. Setup 1D projection file

1. Go to job1 and **PJ COSYSMAL.1DJ** [for 8.5×11; use COSYLARG.1DJ for 11×17]
   then read spectrum file using
   RE <filename>.001 (or unique *number* [no characters allowed!] extension)
2. Either set reference in EP, or type in SR from step I.A.1. above.
3. Check CY=15 (8.5×11) or 19 (11×17), and then enter **WR <ret> <ret> Y** to re-write spectrum

B. Setup and plot .SER 2D files

[see next section if you have already processed .SER and have .SMX to plot]

1. Enter **PJ COSYFAST.1DJ <ret>**
   RJ2D COSYFAST.2DJ <ret>
   RE <filename>.SER <ret>
   [change COSYFAST.1DJ to COSYFT17.1DJ if you are plotting to 11×17 paper,
   and change to your own .J2D file if you saved one]
2. Check all parameters, especially SI, TD, and window functions for both F2 and F1.
3. Check that **NE** = TD1
4. **<esc>** to spectrum/FID display.
5. **XFB** (window multiplication and Fourier transform in both F2 and F1 dimensions)

   This transformation takes a while on AM's (~2-10 min fast-COSY; 20-40 min HR-COSY), but is much faster on AC's (~ factor of 10). Progress is indicated by a number in the top portion of the display, decrementing from SI to 1. Final FID is displayed at completion.
6. **SYM** (symmetrizing function to reduce noise)

   SYM is not applicable to heteronuclear COSY!

Progress is indicated by a number in the top portion of the display, incrementing from 1 to 7, 2 to 7, so on, until it stops at completion.

7. set references: **SR <ret>** **[**value from I.A.1. or III.A.2.**]**

   SR1 = SR

   SR2 = SR

8. **AP2D** on AC's, **EP2D** on AM's and Datastation (2D contour plot and projection display)

   + or **-** to move the starting contour level up or down; adjust it to exclude artifacts/baseline noise.
   C- and **D**-knobs to move the cursor through contour display.
   C to go to a particular column.
   R to go to a particular row.
   I to increment the column or row number.
   D to decrement the column or row number.
   O toggles the data display on and off.
   L,...,L to do contour expansion; **<ctrl> R** to return to contour display.
   <ctrl> Q to update and to restart plotting updated display.
   <esc> X to exit AP2D/EP2D to Command Interpreter.

9. **CP2P <ret>**

```
FILENAME IN F1:  <filename>.001
FILENAME IN F2:  <filename>.001
NO. OF PENS:  1 (use 2 if have red pen and want all but lowest contour red)
NO. OF CONTOUR LEVELS:  4 (1-7 levels).                    (1 is lowest level; 7 highest)
OUTLINE BOX:  Y
```

Note: Set DSPL=1 to check plot on display; set back to DSPL=0 to plot on plotter.

C. Plot .SMX 2D files

1. Go through section III.A. if you haven't already, *Setup 1D projection file*
2. Enter **PJ COSYFAST.1DJ <ret>**

   RJ2D COSYFAST.2DJ <ret>

   RE <filename>.SMX <ret>

   [change COSYFAST.1DJ to COSYFT17.1DJ if you are plotting to 11×17 paper,
   and change to your own .J2D file if you saved one]

3. **<esc>** to spectrum/FID display.
4. set references: **SR <ret>** **[**value from I.A.1. or III.A.2.**]**

   SR1 = SR

   SR2 = SR

5. **AP2D** on AC's, **EP2D** on AM's and Datastation (2D contour plot and projection display)

   + or **-** to move the starting contour level up or down; adjust it to exclude artifacts/baseline noise.
   C- and **D**-knobs to move the cursor through contour display.
   C to go to a particular column.
   R to go to a particular row.
   I to increment the column or row number.
   D to decrement the column or row number.

O toggles the data display on and off.
L,...,L to do contour expansion; **<ctrl> R** to return to contour display.
<ctrl> Q to update and to restart plotting updated display.
<esc> X to exit AP2D/EP2D to Command Interpreter.

6. **CP2P <ret>**

```
FILENAME IN F1:  <filename>.001
FILENAME IN F2:  <filename>.001
NO. OF PENS:  1 (use 2 if have red pen and want all but lowest contour red)
NO. OF CONTOUR LEVELS:  4 (1-7 levels).                    (1 is lowest level; 7 highest)
OUTLINE BOX:  Y
```

Note: Set DSPL=1 to check plot on display; set back to DSPL=0 to plot on plotter.

IV. Listing of Automation Routine

```
;  COSY.AU
;  HOMONUCLEAR SHIFT-CORRELATED 2-D NMR (JEENER)
;  W. P. AUE, E. BARTHOLDI, R. R. ERNST, J. CHEM. PHYS. 64, 2229 (1976)
;  K. NAGAYAMA ET AL, J. MAGN. RES. 40, 321 (1980)

;  D1 - 90 - D0 - 90 OR 45 - FID

;  SYMMETRIC MATRIX WITH SHIFTS AND COUPLINGS IN F1, F2
;  OFF-DIAGONAL PEAKS CORRELATE SPINS WHICH SHARE A
;  SCALAR COUPLING J.

   1  ZE
   2  D1 ; RELAXATION
   3  P1 PH1  ; 90 DEG EXCITATION PULSE
   4  D0 ; EVOLUTION OF SHIFTS AND COUPLINGS
   5  P2 PH2  ; MIXING PULSE, 90 OR 45 DEG
   6  GO = 2  ; ACQUIRE FID
   7  WR #1   ; STORE FID
   8  IF #1   ; INCREMENT FILE NUMBER
   9  IN = 1  ; INCREMENT D0 AND LOOP FOR NEXT EXPER.
   10 EXIT

    PH1 =  A0 A0 A0 A0 A1 A1 A1 A1     ; PHASE PROGRAMS CANCEL AXIAL
           A2 A2 A2 A2 A3 A3 A3 A3     ; PEAKS (SCANS 1-2), SELECT N-TYPE
                                       ; PEAKS (SCANS 3-4), SUPPRESS F2
    PH2 =  A0 A2 A1 A3 A1 A3 A2 A0     ; QUAD IMAGES (SCANS 5-8), AND CANCEL
           A1 A3 A2 A0 A2 A0 A3 A1     ; ARTEFACTS FROM P1 (SCANS 9-16).

;  PROGRAM REQUESTS FILENAME WITH .SER EXTENSION
;  NE DEFINES NUMBER OF FIDS = TD1
;  USE QP, NS = 4, 8, OR 16 (COMPLETE PHASE CYCLE)
;  DS = 2 OR 4
;  RD = PW = 0
;  D1 = 1-5 X T1
;  P1 = 90 DEG
;  P2 = 90 DEG FOR MAX. SENSITIVITY
;          = 45 DEG FOR MINIMAL DIAGONAL (GOOD FOR TIGHT AB SYSTEMS)
;                   AND 'TILTED' CORREL. PEAKS (SIGNS OF COUPLINGS).
;  D0 = 3E-6 INITIAL DELAY
;  IN = 0.5 / SW1 = 2 X DW
;  ND0 = 1
;  I2D = 1, SW1 = SW / 2

;  CHOOSE SW AND SI SO THAT HZ/PT = CA. 2-6 HZ
;  TYPICALLY USE TD = SI, NO ZERO-FILLING IN F2
;                                   NE = SI / 4, ZERO-FILL IN F1
;  MATRIX CAN BE SYMMETRIZED ABOUT DIAGONAL.
```