Vanderbilt NMR Facilities

Instructions for Setup of 2D Homonuclear Experiments Using TOPSPIN

Index

1. 2D Acquisition Prerequisites

The common procedures involved in the setup and processing of 2D homonuclear experiments are described in this section. Specifics for each homonuclear experiment have been provided in separate section.

1.1. 1D ^{1}H Reference Spectrum

- Calibrate the 90° pulse (see manual: "Instructions for 90° Pulse Width Determination" for details)
- Acquire a 1D ^{1}H reference spectrum (**rpar AAA_proton.MV all**, lock, tune, and shim – see separate Topspin manuals).
- Transform and reference the spectrum.
- Check for lineshape and spectral quality. This spectrum may be used for the projections in the 2D homonuclear plot.
- Note the value for receiver gain, **RG**.
- Note the spectrum reference, type **SR**.
- Optional: Adjustment of parameters **SW** and **O1**
    - Using the cursors, select the region of the 1D spectrum containing all your signals. Include a minimum of 0.5 ppm of baseline on either side of the spectrum.
    - Define **SW** and **O1** by selecting the icon from the toolbar. (Fig. 1.1)

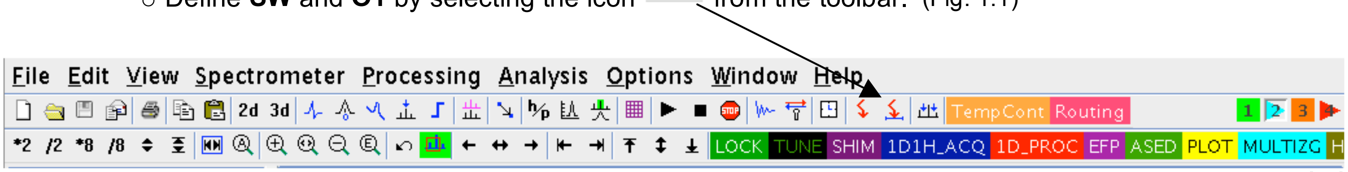


Figure 1 .1

- *Optional*: Re-acquire the 1D reference spectrum with these new values.

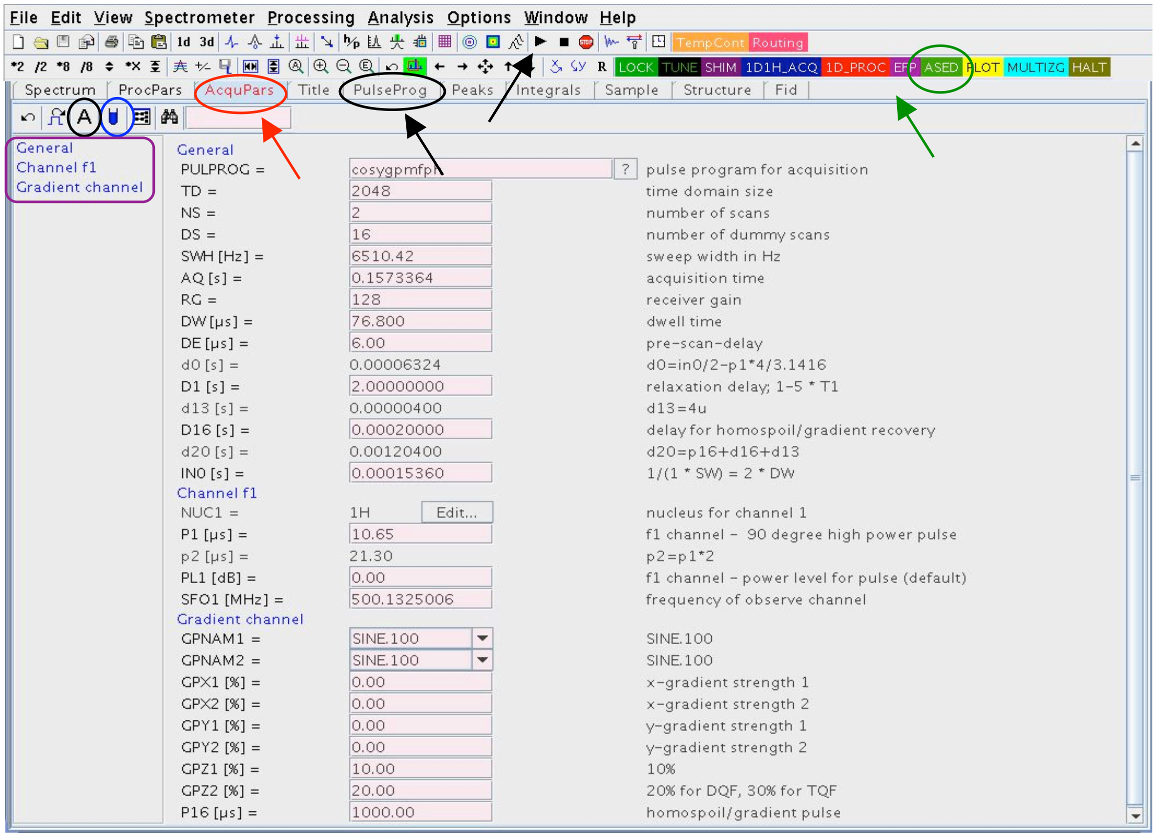
1.2. The 2D Homonuclear ^{1}H Experiment

- *Samples in organic solvents*: The default parameters starting with "**AA_**..." in their name typically produce usable spectra without modification.
- *Aqueous samples* will require customization of some experimental parameters. All experiment specific parameters (ASED) must be double checked, and set correctly, for the experiment to yield proper data.
- Check the **PulseProg** tab or parameter tables (page 18 ff) for information on specific parameter settings.

1.2.1. ASED (acquisition parameters specific to the experiment selected)

- Type **ased** or click on the button in the toolbar (Fig. 1.2).
- Click on the **getprosol** icon (Fig. 1.2). This command loads the appropriate pulses and gradient delays from a configuration table.

  - These values are typically useable for organic solvents.
  - For aqueous solutions: Type: **getprosol 1H** <P1>**us** <PL1>**db**; using the pulse width <P1> and the corresponding pulse power <PL1> as determined in section 1.1. This command recalculates all the proton pulses according to the calibrated values entered.
- Verify the correctness of **<u>all</u>** parameters in this window.



General		
PULPROG =	cosygpmfph	pulse program for acquisition
TD =	2048	time domain size
NS =	2	number of scans
DS =	16	number of dummy scans
SWH [Hz] =	6510.42	sweep width in Hz
AQ [s] =	0.1573364	acquisition time
RG =	128	receiver gain
DW [µs] =	76.800	dwell time
DE [µs] =	6.00	pre-scan-delay
d0 [s] =	0.00006324	d0=in0/2-p1*4/3.1416
D1 [s] =	2.00000000	relaxation delay; 1-5 * T1
d13 [s] =	0.00000400	d13=4u
D16 [s] =	0.00020000	delay for homospoil/gradient recovery
d20 [s] =	0.00120400	d20=p16+d16+d13
IN0 [s] =	0.00015360	1/(1 * SW) = 2 * DW
Channel f1		
NUC1 =	1H	nucleus for channel 1
P1 [µs] =	10.65	f1 channel - 90 degree high power pulse
p2 [µs] =	21.30	p2=p1*2
PL1 [dB] =	0.00	f1 channel - power level for pulse (default)
SFO1 [MHz] =	500.1325006	frequency of observe channel
Gradient channel		
GPNAM1 =	SINE.100	SINE.100
GPNAM2 =	SINE.100	SINE.100
GPX1 [%] =	0.00	x-gradient strength 1
GPX2 [%] =	0.00	x-gradient strength 2
GPY1 [%] =	0.00	y-gradient strength 1
GPY2 [%] =	0.00	y-gradient strength 2
GPZ1 [%] =	10.00	10%
GPZ2 [%] =	20.00	20% for DQF, 30% for TQF
P16 [µs] =	1000.00	homospoil/gradient pulse

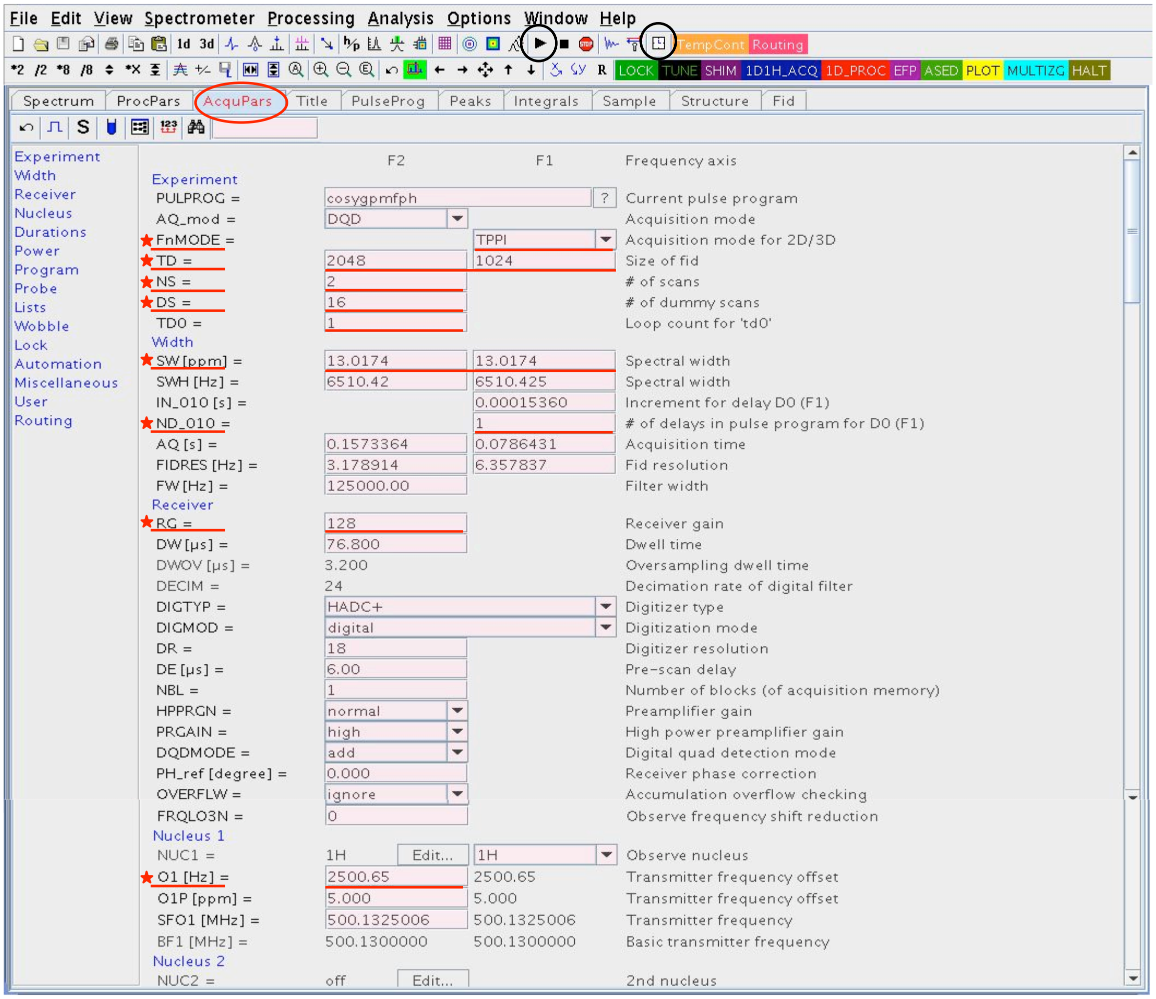
Figure 1.2

1.2.2. EDA (complete acquisition parameter list)

- Type **eda**, or click on **A** in the ASED window toolbar. (Fig. 1.2)
- Update **SW** and **O1**, as determined in section 1.1.
- **RG** equals value from the reference proton experiment as determined in section 1.1.
- Check the parameters for correctness, as outlined in the directions for the specific 2D experiment. The Selection Tabs, listed in the left column of the window, are linked to the associated parameters. (Fig. 1.3)

Time Estimate and Adjustment for 2D Experiment:

- To calculate the experiment time click on the clock in the toolbar or type **expt**.
- Note: either changing TD(F1), or NS, will affect the experiment time most.
- Changing TD(F1) directly affects the resolution in the indirect dimension, NS the signal intensity.
- Acquire the experiment with **zg** or select the ▶ button in the toolbar.
- 2D experiments can be stopped prematurely bye either command **stop** or **halt**, affecting the digital resolution in the indirect dimension.



	F2	F1	Frequency axis
Experiment			
PULPROG =	cosygpmfph		Current pulse program
AQ_mod =	DQD		Acquisition mode
★FnMODE =		TPPI	Acquisition mode for 2D/3D
★TD =	2048	1024	Size of fid
★NS =	2		# of scans
★DS =	16		# of dummy scans
TD0 =	1		Loop count for 'td0'
Width			
★SW [ppm] =	13.0174	13.0174	Spectral width
SWH [Hz] =	6510.42	6510.425	Spectral width
IN_010 [s] =		0.00015360	Increment for delay D0 (F1)
★ND_010 =		1	# of delays in pulse program for D0 (F1)
AQ [s] =	0.1573364	0.0786431	Acquisition time
FIDRES [Hz] =	3.178914	6.357837	Fid resolution
FW [Hz] =	125000.00		Filter width
Receiver			
★RG =	128		Receiver gain
DW [μs] =	76.800		Dwell time
DWOV [μs] =	3.200		Oversampling dwell time
DECIM =	24		Decimation rate of digital filter
DIGTYP =	HADC+		Digitizer type
DIGMOD =	digital		Digitization mode
DR =	18		Digitizer resolution
DE [μs] =	6.00		Pre-scan delay
NBL =	1		Number of blocks (of acquisition memory)
HPPRGN =	normal		Preamplifier gain
PRGAIN =	high		High power preamplifier gain
DQDMODE =	add		Digital quad detection mode
PH_ref [degree] =	0.000		Receiver phase correction
OVERFLW =	ignore		Accumulation overflow checking
FRQLO3N =	0		Observe frequency shift reduction
Nucleus 1			
NUC1 =	1H Edit...	1H	Observe nucleus
★O1 [Hz] =	2500.65	2500.65	Transmitter frequency offset
O1P [ppm] =	5.000	5.000	Transmitter frequency offset
SFO1 [MHz] =	500.1325006	500.1325006	Transmitter frequency
BF1 [MHz] =	500.1300000	500.1300000	Basic transmitter frequency
Nucleus 2			
NUC2 =	off Edit...		2nd nucleus

Figure 1.3

1.3. Processing the 2D Spectrum

1.3.1. RSER (command line)

This command extracts single FID's out of a nD dataset. Without further argument, the 1D FID will be stored in the ~TEMP directory.

Example: **rser 1 99** extracts the first FID of an nD and saves the FID in experiment # 99 of the current project (any argument numbers are possible)

1.3.2. EDP (processing parameter list)

- Type **edp**, or select the "**ProcPars**" tab. (Fig. 1.4)
- Check the parameters for correctness, as outlined for each specific 2D experiment (page 18 ff).

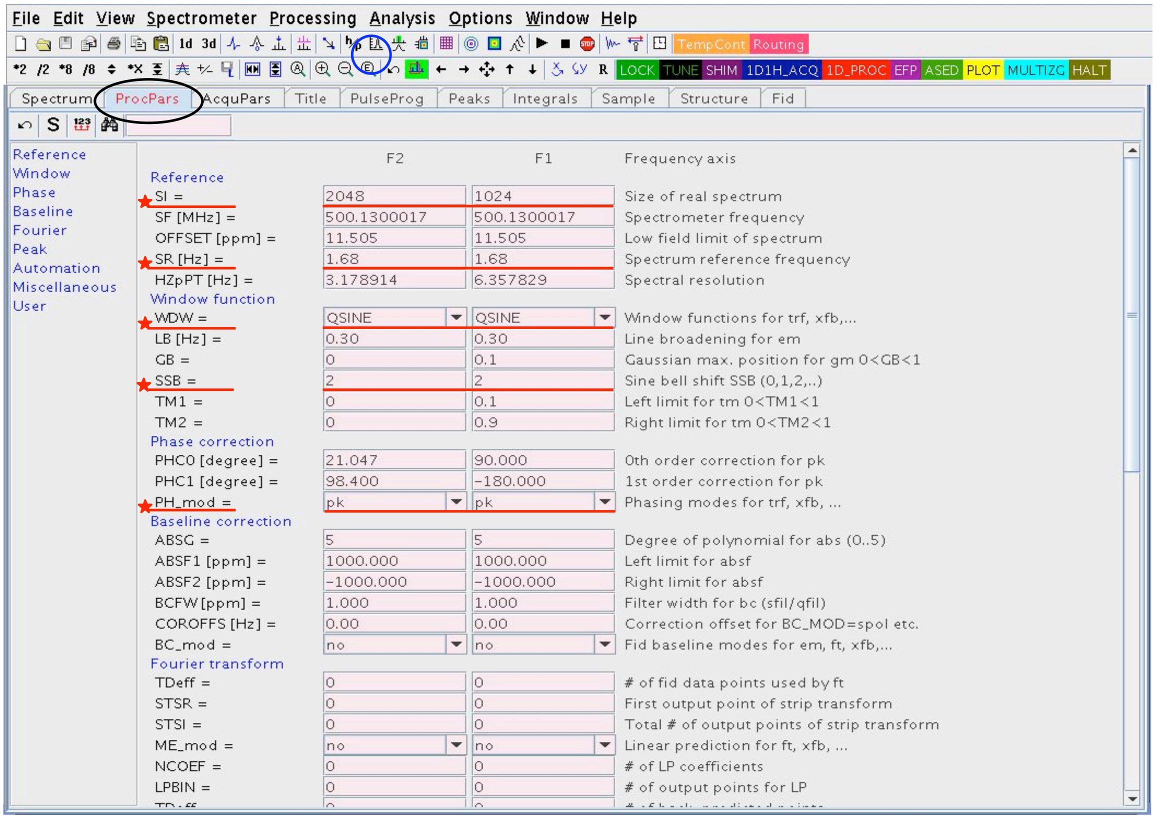
- Use "**xfb**" to transform both dimensions of the spectrum.
- Phase the spectrum according to the directions for each specific 2D experiment (chapters 2-5)
- Use **abs1** and **abs2** to correct the baseline of the spectrum.
- Using **xfb n** to transform the spectrum deletes the imaginary parts of the spectrum. The resulting data is only ¼ of the original data size, but the spectrum can't be phased without re-processing using **xfb** alone.

1.3.3. Contour Level Adjustment

Adjust the contour levels to improve visibility. Type the following command sequence:

nlev 21 (number of levels displayed)

levcalc (calculate contour level setting)



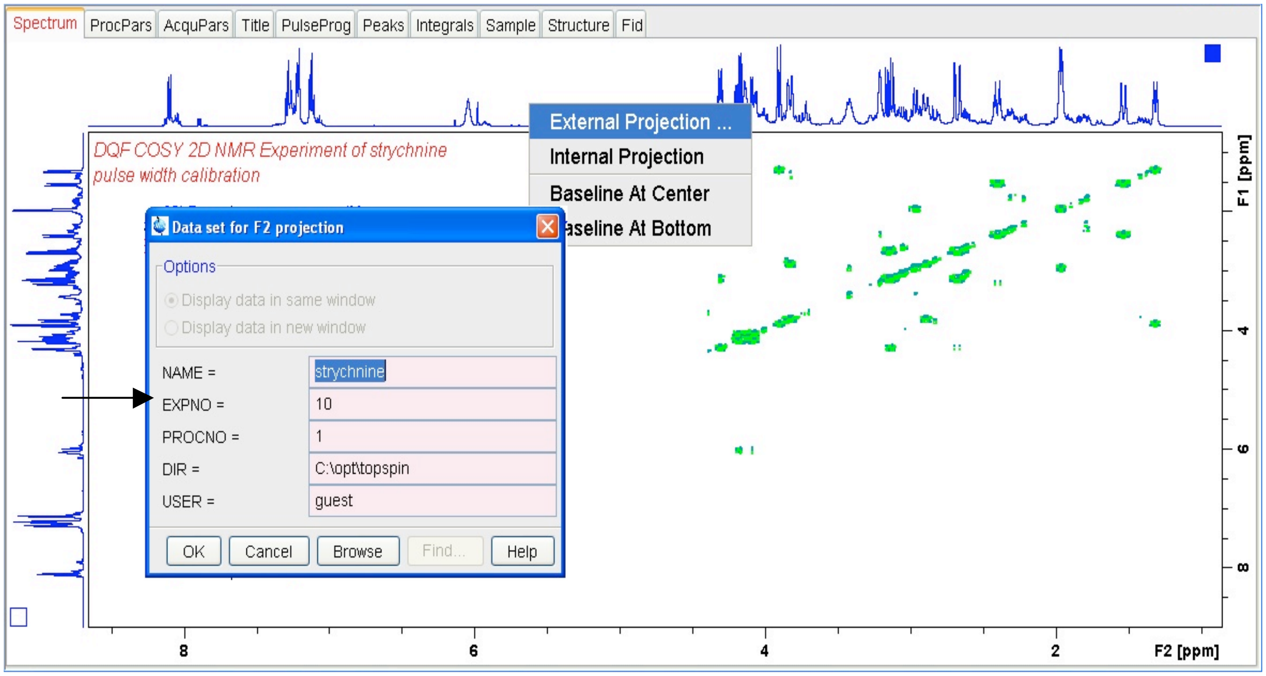
	F2	F1	Frequency axis
Reference			
SI =	2048	1024	Size of real spectrum
SF [MHz] =	500.1300017	500.1300017	Spectrometer frequency
OFFSET [ppm] =	11.505	11.505	Low field limit of spectrum
SR [Hz] =	1.68	1.68	Spectrum reference frequency
HZpPT [Hz] =	3.178914	6.357829	Spectral resolution
Window function			
WDW =	QSINE	QSINE	Window functions for trf, xfb,...
LB [Hz] =	0.30	0.30	Line broadening for em
GB =	0	0.1	Gaussian max. position for gm 0<GB<1
SSB =	2	2	Sine bell shift SSB (0,1,2,..)
TM1 =	0	0.1	Left limit for tm 0<TM1<1
TM2 =	0	0.9	Right limit for tm 0<TM2<1
Phase correction			
PHC0 [degree] =	21.047	90.000	0th order correction for pk
PHC1 [degree] =	98.400	-180.000	1st order correction for pk
PH_mod =	pk	pk	Phasing modes for trf, xfb, ...
Baseline correction			
ABSG =	5	5	Degree of polynomial for abs (0..5)
ABSF1 [ppm] =	1000.000	1000.000	Left limit for absf
ABSF2 [ppm] =	-1000.000	-1000.000	Right limit for absf
BCFW [ppm] =	1.000	1.000	Filter width for bc (sfil/qfil)
COROFFS [Hz] =	0.00	0.00	Correction offset for BC_MOD=spol etc.
BC_mod =	no	no	Fid baseline modes for em, ft, xfb,...
Fourier transform			
TDeff =	0	0	# of fid data points used by ft
STSR =	0	0	First output point of strip transform
STSI =	0	0	Total # of output points of strip transform
ME_mod =	no	no	Linear prediction for ft, xfb, ...
NCOEF =	0	0	# of LP coefficients
LPBIN =	0	0	# of output points for LP

Figure 1.4

1.3.4. Projections

To set the 1D projections first select the icon . (Fig. 1.4)

- Click the right mouse button inside the F2 projection and select "**External Projection**".
- Change the EXPNO to that of the reference spectrum collected in "**2D Acquisition Prerequisites"** (Section 1.1).
- Repeat the process for F1, if desired.



Data set for F2 projection

Options

○ Display data in same window
○ Display data in new window

NAME = strychnine
EXPNO = 10
PROCNO = 1
DIR = C:\opt\topspin
USER = guest

Figure 1.5

1.3.5. Check Data in the Indirect Dimension

Use **xf2** to transform the first dimension separately allowing for closer inspection of the acquired data in the indirect dimension (Fig. 1.6). Examine the spectrum for the presence of truncation, artifacts, or whether the experiment has adequate signal. This also helps in the selection of appropriate window functions. **xf1** will Fourier transform the spectrum in the indirect dimension (Fig. 1.7). Alternatively **xfb** can be used to process both dimensions again.

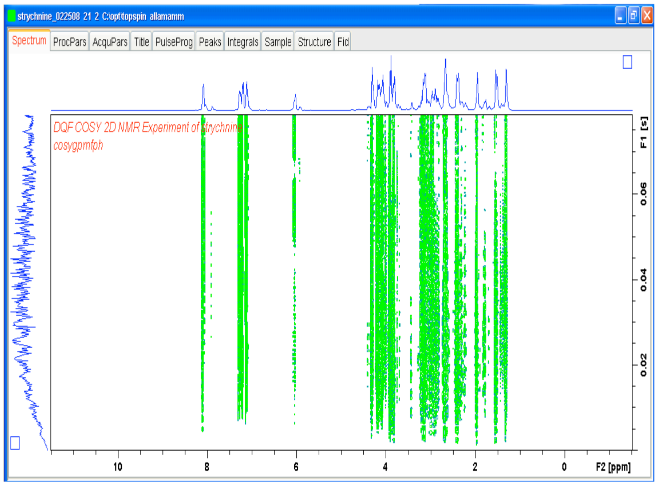


Figure 1.6

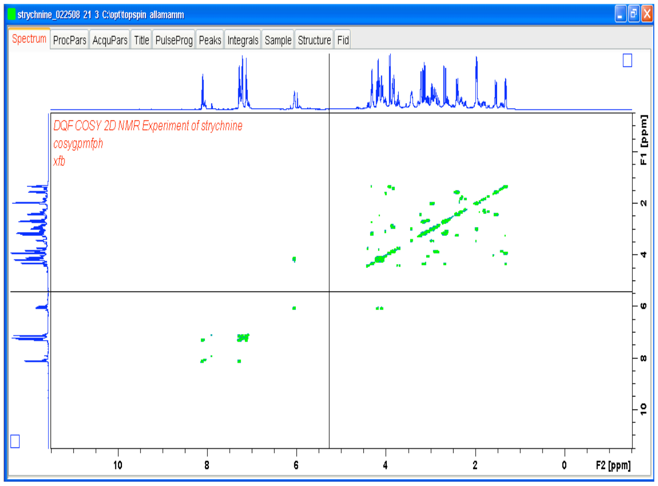


Figure 1.7

2. Magnitude COSY

2.1. Experiment

- Run a 1D proton reference experiment (see Section 1.1)
- In a new experiment, use facility default parameters (“**AA_COSY-mag.MV**”; **rpar AA_COSY-mag.MV all**)
- Users with aqueous samples will need to customize the experimental parameters. Check the **PulseProg** tab for directions indicating which parameter settings to use.

2.2. Acquisition

2.2.1. **ASED** (experiment specific acquisition parameters**)**

- Click on the **getprosol** icon
- Verify the correctness of all parameters in this window.
- For aqueous solutions: Type: **getprosol 1H** <P1>**us** <PL1>**db**; inserting the values from section 1.2.1.

2.2.2. **EDA** (complete acquisition parameter list)

Headings from the left column in the EDA window are linked to the parameters listed in **bold**. Check the following parameters in this window:

Experiment Tab:
- **AQ_mod = DQD**
- **FNMODE = QF**
- **TD = 2k** for F2, and **512** for F1
- **NS = 4** (or multiples of 4)
- **DS = 8**

Width Tab:
- Update **SW**. If using non-default values, copy and paste from the reference experiment.
- **ND010 = 1**

Receiver Tab:
- Check **RG.** Use the same value as in the reference spectrum.

Nucleus Tab:
- Update **O1**. If using a non-default value, copy and paste from the 1D reference spectrum.

Acquire the experiment with **zg** or select the ▶ button in the toolbar.

2.3. Processing

2.3.1. EDP Processing Parameters (type **edp**, or select the ProcPars tab)

Reference Tab:

- **SI = 1k** for F1 and F2
- **SR** = value copy and paste from the 1D reference spectrum.

Window Tab:

- **WDW = QSINE** for F1 and F2
- **SSB = 0** for F1 and F2

Phase Tab:

- **PH_MOD = NO** for F2, **MC** for F1

2.3.2. Process Spectrum

- **Xfb n** to transform both dimensions.
- Adjust the contour levels to improve visibility (see section 1.3.2)
- Set the projections (see section 1.3.3)
- Use **abs1** and **abs2** to correct the baseline of the spectrum.
- The spectrum may be symmetrized with the **sym** command. Use this command with caution. It will alter the dataset, real peaks may be removed or new peaks added.

3. DQF COSY

3.1. Experiment

- Run a 1D proton reference experiment (see Section 1.1)
- In a new experiment, use facility default parameters (“**AA_COSY-dqf.MV**”; **rpar AA_COSY-dqf.MV all**)
- Users with aqueous samples will need to customize the experimental parameters. Check the **PulseProg** tab for directions indicating which parameter settings to use.

3.2. Acquisition

3.2.1. ASED (experiment specific acquisition parameters)

- Click on the **getprosol** icon
- Verify the correctness of all parameters in this window.
- For aqueous solutions: Type: **getprosol 1H** <P1>**us** <PL1>**db**; inserting the values from section 1.2.1.

3.2.2. EDA (complete acquisition parameter list)

Headings from the left column in the EDA window are linked to the parameters listed in **bold**. Check the following parameters in this window:

Experiment Tab:
- **AQ_mod = DQD**
- **FNMODE = TPPI-STATES**
- **TD = 2k** for F2, and **1k** for F1
- **NS = 2** (or multiples of 2)
- **DS = 16**

Width Tab:
- Update **SW**. If using non-default values, copy and paste from the reference experiment.
- **ND010 = 1**

Receiver Tab:
- Check **RG.** Use the same value as in the reference spectrum.

Nucleus Tab:
- Update **O1**. If using a non-default value, copy and paste from the 1D reference spectrum.

Acquire the experiment with **zg** or select the ▶ button in the toolbar. GS may be used to further optimize the parameters after the start of the acquisition.

3.3. Processing

3.3.1. EDP Processing Parameters (type **edp**, or select the ProcPars tab)

Reference Tab:
- **SI = 1k** for F1 and F2
- **SR** = value copy and paste from the 1D reference spectrum.

Window Tab:
- **WDW = QSINE** for F1 and F2
- **SSB = 2** for F1 and F2

Phase Tab:
- **PH_MOD = pk** for F2 and F1
- F2: determine phase correction (3.3.3)
- F1: **PHC0, PHC1**, **FCOR** see table 6.1 or pulse program

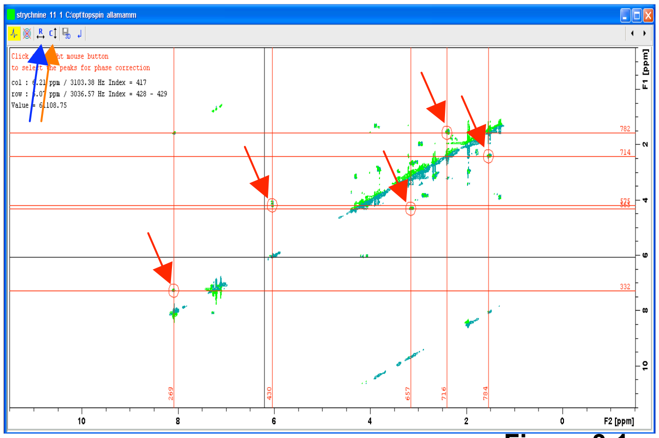
3.3.2. Processing Spectrum

- **xfb** to transform both dimensions.
- Adjust the contour levels to improve visibility (see section 1.3.2).
- Set the reference projection (see section 1.3.3).
- Use **abs1** and **abs2** to correct the baseline of the spectrum.

3.3.3. Phasing Spectrum

- Enter the 2D phase mode by selecting the icon
- In the spectral window, right click on a single peak in a set of cross peaks in the downfield region. Expansion may be necessary. Select "**Add**" (Fig. 3.1). Add 2 or more peaks in the middle to high-field range. Be sure to refer to the same peak of each multiplet (Fig. 3.2).
- Click on the row icon "**R**" to phase the F2 dimension. Select the "**0**" icon to apply zero order phase correction to the top window, and the "1" icon to apply 1st order phase correction to the lower windows. Insets in the figures below show the selected contour profiles before and after phasing (Figs. 3.3 -> 3.4).
    - The spectral peaks should be anti-phase. (Figs. 3.2 and 3.4)

- Repeat this process for the "columns", using the "**C**" icon to phase the F1 dimension (Fig. 3.1). Selection of different peaks may be necessary in order to correct the phasing in F1.



Click [illegible] ht mouse button
to sele[illegible]t [illegible]he peaks for phase correction
col : [illegible].21 ppm / 3103.38 Hz Index = 417
row : [illegible].07 ppm / 3036.57 Hz Index = 428 - 429
Value = 6[illegible]108.75

Figure 3.1

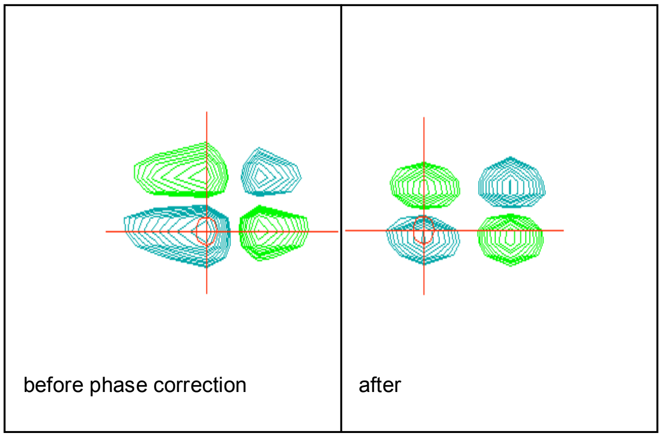
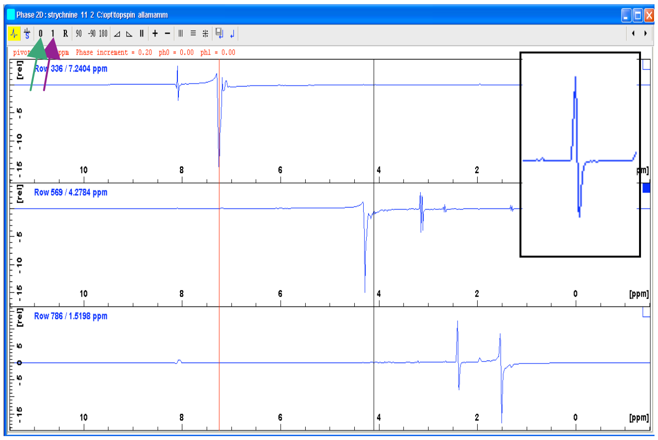
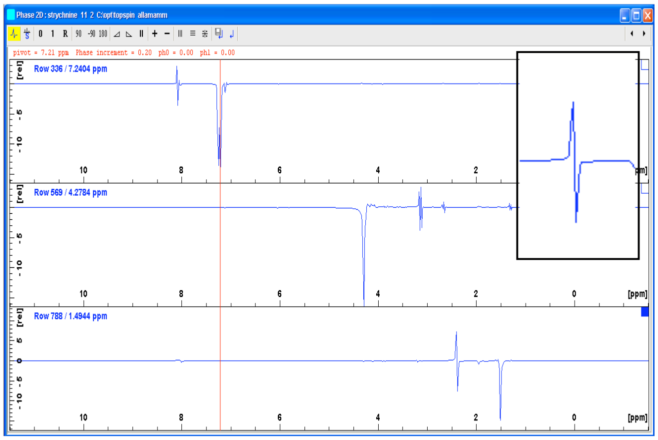


Figure 3.2



pivot [illegible] ppm Phase increment = 0.20 ph0 = 0.00 ph1 = 0.00

Figure 3.3



pivot = 7.21 ppm Phase increment = 0.20 ph0 = 0.00 ph1 = 0.00

Figure 3.4

3.3.4. General Remarks:

- The Double Quantum Filtered (DQF) COSY improves the peak resolution near the diagonal, but results in a 2-fold loss of signal intensity compared to the magnitude COSY.
- It is a good choice for coupling constant determination.
- A correctly phased DQF COSY will be purely absorptive. Both cross-peaks and diagonal multiplets have the same anti-phase absorption character. (Fig. 3.2)

4. TOCSY

4.1. Experiment

- Run a 1D proton reference experiment. (see Section 1.1)
- In a new experiment, use facility default parameters (“**AA_TOCSY-dip.MV**”; **rpar AA_TOCSY-dip.MV all**)
- Users with aqueous samples will need to customize the experimental parameters. Check the **PulseProg** tab for directions indicating which parameter settings to use.

4.2. Acquisition

4.2.1. ASED (experiment specific acquisition parameters)

- Click on the **getprosol** icon
- Verify the correctness of all parameters in this window.
- For aqueous solutions: Type: **getprosol 1H** <P1>**us** <PL1>**db**; inserting the values from section 1.2.1.
- Use the default values for the TOCSY spin-lock pulse parameters, **P6 and PL10**. These parameters are field dependent.
- The mixing time, **D9 = 80 - 120ms** (~20ms / transfer step).

4.2.2. EDA (complete acquisition parameter list)

Headings from the left column in the EDA window are linked to the parameters listed in **bold**. Check the following parameters in this window:

Experiment Tab:
- **AQ_mod = DQD**
- **FNMODE = Echo-Antiecho**
- **TD = 2k** for F2, and **512** for F1
- **NS = 8** (or multiples of 8)
- **DS = 16**

Width Tab:
- Update **SW**. If using non-default values, copy and paste from the reference experiment.
- **ND010 = 1**

Receiver Tab:
- Check **RG**

Nucleus Tab:
- Update **O1**. If using a non-default value, copy and paste from the 1D reference spectrum.

Acquire the experiment with **zg** or select the ▶ button in the toolbar. GS may be used to further optimize the parameters after the start of the acquisition.

4.3. Processing

4.3.1. **EDP** Processing Parameters (type **edp**, or select the **ProcPars** tab)

Reference Tab:
- **SI = 1k** for F1 and F2
- **SR** = value copy and pasted from the 1D reference spectrum.

Window Tab:
- **WDW = QSINE** for F1 and F2
- **SSB = 2** for both, F1 and F2

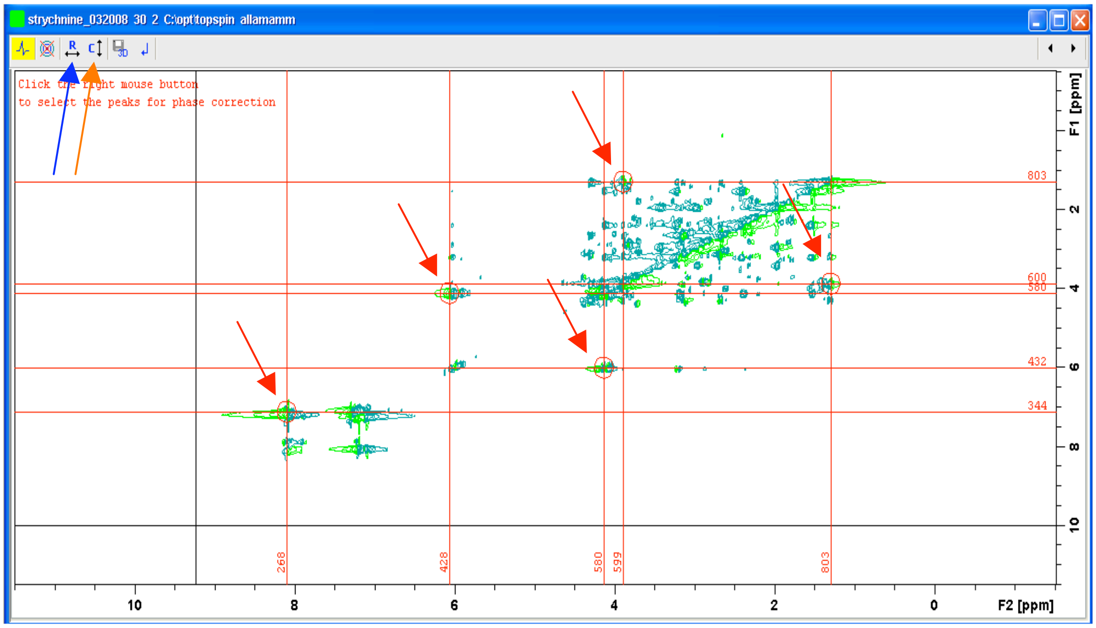
Phase Tab:
- **PH_MOD = pk** for both, F2 and F1
- F2: determine phase correction (4.3.3)
- F1: **PHC0, PHC1**, **FCOR** see table 6.2 or pulse program

4.3.2. Processing Spectrum

- **xfb** to transform both dimensions.
- Adjust the contour levels to improve visibility (see section 1.3.2)
- Set the projections (see section 1.3.3)
- Use **abs1** and **abs2** to correct the baseline of the spectrum.

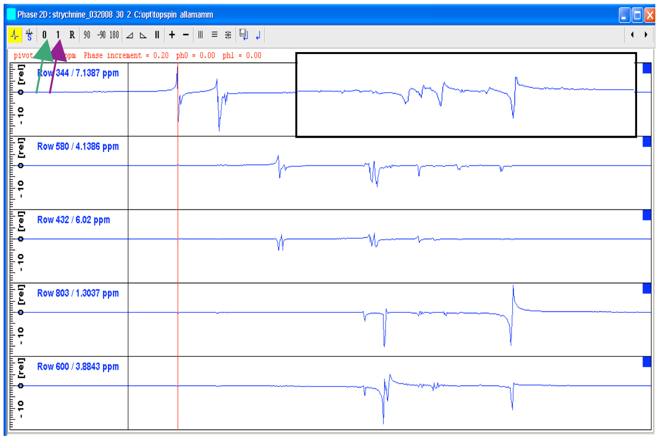
4.3.3. Phasing Spectrum

- Enter the 2D phase mode by selecting the icon
- In the spectrum window, right click on a single, downfield cross-peak and select "**Add**" (Fig. 4.1). Add 2 or more other peaks moving towards the upfield side of the spectrum.
- Click on the row icon "**R**" to phase the F2 dimension. Select the "**0**" icon to apply zero order phase correction to the top window, and the "**1**" icon to apply 1st order phase correction to the lower windows. Insets in the figures below show the selected contour profiles before and after being phased positive (Fig. 4.2 → 4.3)
- Repeat this process for the "columns", using the "**C**" icon to phase the F1 dimension if necessary. (Fig. 4.1)



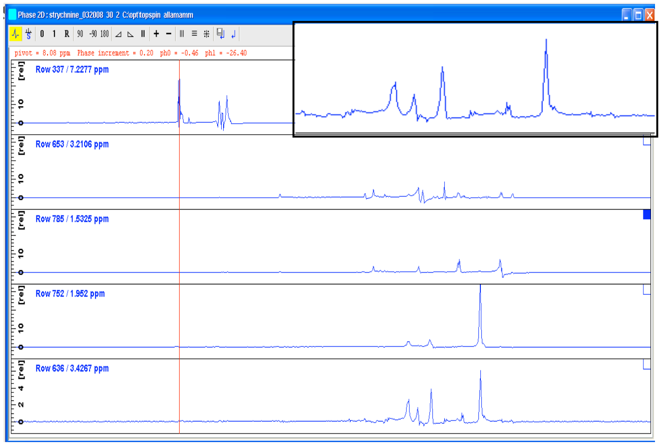
Click the right mouse button
to select the peaks for phase correction

Figure 4.1



```
pivot = ... ppm  Phase increment = 0.20  ph0 = 0.00  ph1 = 0.00
```

Figure 4.2



```
pivot = 8.08 ppm  Phase increment = 0.20  ph0 = -0.46  ph1 = -26.40
```

Figure 4.3

4.3.4. General Remarks:

Rule of Thumb: Mixing Time, T_m = 12 to 20 ms per transfer step. Short spin-lock durations of 20 ms will produce a spectrum similar to the COSY. Longer spin-lock times will yield the desired TOCSY spectrum.

The advantages of the TOCSY spectrum are:

- Connect whole spin systems
- Sensitive correlation experiment
- All peaks are in-phase
- Narrow diagonal peaks

5. NOESY

5.1. Experiment

- Run a 1D proton reference experiment (see Section 1.1).
- In a new experiment, use facility default parameters (“**AA_NOESY-ph.MV**”; **rpar AA_NOESY-ph.MV all**)
- Users with aqueous samples will need to customize the experimental parameters. Check the **PulseProg** tab for directions indicating which parameter settings to use.

5.2. Acquisition

5.2.1. ASED (experiment specific acquisition parameters)

- Click on the **getprosol** icon
- Verify the correctness of all parameters in this window.
- For aqueous solutions: Type: **getprosol 1H** <P1>**us** <PL1>**db**; inserting the values from section 1.2.1.
- The mixing time, **D8 = 40 – 1000 ms** (SMF: 600 ms, CSB: 80-150 ms).

5.2.2. EDA (complete acquisition parameter list)

Headings from the left column in the EDA window are linked to the parameters listed in **bold**. Check the following parameters in this window:

Experiment Tab:
- **AQ_mod = DQD**
- **FNMODE = TPPI-States**
- **TD = 2k** for F2, and **512** for F1
- **NS = 2** (or multiples of 2)
- **DS = 16**

Width Tab:
- Update **SW**. If using non-default values, copy and paste from the reference experiment.
- **ND010 = 1**

Receiver Tab:
- Check **RG**

Nucleus Tab:
- Update **O1**. If using a non-default value, copy and paste from the 1D reference spectrum.

Acquire the experiment with **zg** or select the ▶ button in the toolbar. GS may be used to further optimize the parameters after the start of the acquisition.

5.3. Processing

5.3.1. EDP Processing Parameters (type **edp**, or select the **ProcPars** tab)

Type **edp**, or select the **ProcPars** tab.

Reference Tab:
- **SI = 1k** for F1 and F2
- **SR** = value copy and pasted from the 1D reference spectrum.

Window Tab:
- **WDW = QSINE** for F1 and F2
- **SSB = 2** for F1 and F2

Phase Tab:
- **PH_MOD = pk** for F2 and F1
- F2: determine phase correction (see 5.3.3)
- F1: **PHC0 = 90, PHC1 = -180**; **FCOR(F1) = 1**

5.3.2. Process Spectrum

- **xfb** to transform both dimensions.
- Adjust the contour levels to improve visibility (see section 1.3.2)
- Set the reference projections (see section 1.3.3)
- Use **abs1** and **abs2** to correct the baseline of the spectrum.

5.3.3. Phase Spectrum Using the First Increment

- Start with the directly detected dimension, F2. Type “rser 1 99”; where 99 represents a location for processing the file (procno).
- Phase the increment displayed, selecting the icon (Fig. 5.1)
    - Small molecules: phase 1st increment negative (down)
    - Large molecules: phase 1st increment positive (up, Fig. 5.1)
- Once phased, select “save as 2D” and return

5.3.4. Phase 2D Spectrum interactively

- Enter the 2D phase mode by selecting the icon
- In the spectrum window, right click on a single, downfield cross-peak and select “**Add**” (Fig. 5.2). Add 2 or more other peaks moving towards the upfield side of the spectrum
- Click on the row icon, “**R**”, to phase the horizontal dimension. Select the “**0**” icon to apply zero order phase correction, and the “**1**” icon to apply 1st order phasing. (Fig. 4.2). For small molecules, the phase of the NOE peaks typically is opposite of the diagonal peaks, while molecules > 1-3 kDa produce all positive peaks.
- Insets in the figures 5.3 and 5.4, show the selected contour profiles of a small molecule before and after phasing.

- Repeat this process for the “columns”, using the “**C**” icon to phase the vertical dimension.

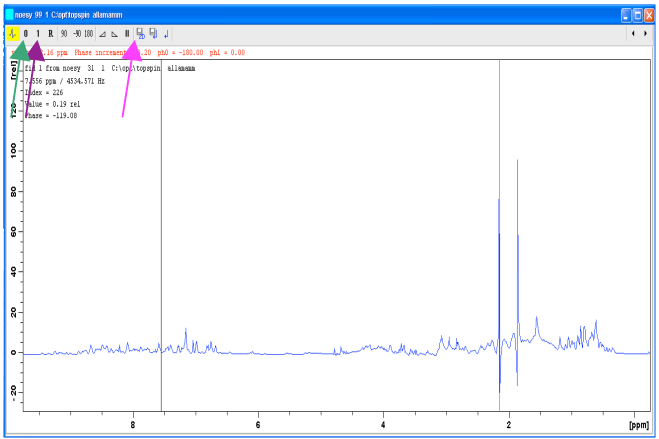


Figure 5.1

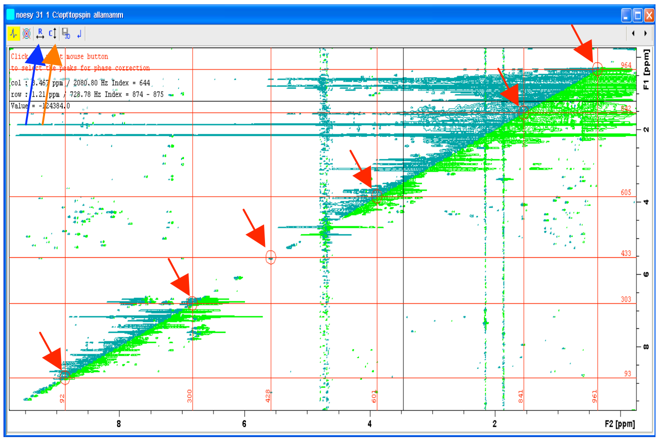


Figure 5.2

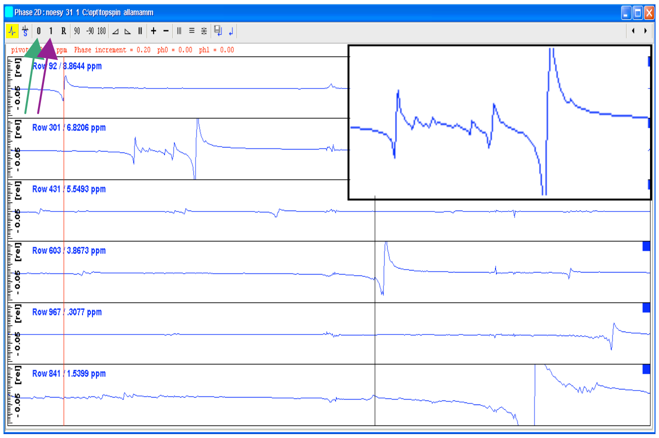


Figure 5.3

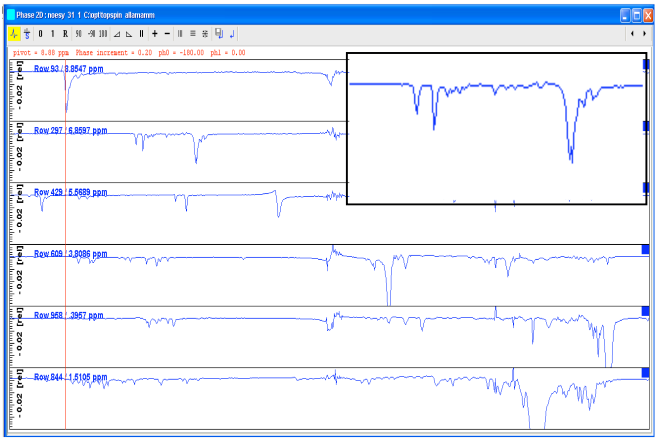


Figure 5.4

5.3.5. General Remarks:

For MW’s between 1k and 3 kDa, transient and steady state NOE’s approach zero. In this case, the appropriate experiment would be a ROESY.

6. Parameter Tables

6.1. Summary of COSY parameters

NOTE: Changing one parameter might impact others, hence the integrity of all acquisition parameters must be verified. This can be done using the resources found in the pulse sequence description in the **PulseProg** tab, or by requesting help from a facility staff member.

ased parameters

Experiment	Magnitude COSY	Phase Sensitive DQF COSY	Echo/AntiEcho DQF COSY	Water suppression DQF COSY
Parameter set	AA_COSY-mag.MV	AA_COSY-dqf.MV	AA_COSY-dqfea.MV	AA_COSY-dqfes.MV
Bruker PP name	cosygpqf	cosygpmfph	cosydfetgp.1	cosydfesgpph
Description	No phasing, sensitive, use for connectivity	Phase sensitive lower S/N than magnitude COSY, measurable coupling constants	re more scans than the phase sensitive DQF COSY	Phase sensitive, water suppression via excitation sculpting
Pulses	P0 = 20° to 90° of P1 (1)	N/A	N/A	SP1 = shaped pulse PL9 = presat pwr lvl
P1 @ PL1	P1 (90°)	P1 (90°)	P1 (90°)	P1 (90°)
GPNAM1 (2)	SMSQ10.100	SMSQ10.100	SMSQ10.100	SMSQ10.100
GPNAM2 (2)	SMSQ10.100	SMSQ10.100	SMSQ10.100	SMSQ10.100
GPZ1	10%	30%	30%	31%
GPZ2	10%	30%	30%	11%

(1) For optimal sensitivity, a P0 of 60° is recommended, while of 45 ° improves the intensity ratios and diagonal streamlining effect with only a moderate loss in signal-to-noise.

(2) **SINE.100** may be used as well in these experiments, but is considered less efficient

eda parameters

Bruker PP name	cosygpqf	cosygpmfph	cosydfetgp.1	cosydfesgpph
FnMODE	QF	States, **States-TPPI**, TPPI	Echo/Anti-Echo	States, **States-TPPI**, TPPI
TD F2	1024, **2048**, 4096	1024, **2048**, 4096	1024, **2048**, 4096	1024, **2048**, 4096
TD F1	128, 256, **512**, 1024	128, 256, **512**, 1024	128, 256, **512**, 1024	128, 256, **512**, 1024
NS (minimum)(3)	1	2	8	16
SW F2	= SW F1	= SW F1	= SW F1	= SW F1
ND_010	1	1	1	1

(3) **NS** may be increased by multiples of n (whole numbers)

Underlined values represent common choices

edp parameters

Bruker PP name	cosygpqf	cosygpmfph	cosydfetgp.1	cosydfesgpph
SR (F1=F2)	Value from 1H ref spectrum	Value from 1H ref spectrum	Value from 1H ref spectrum	Value from 1H ref spectrum
WDW (F1=F2)	QSINE	QSINE	QSINE	QSINE
SSB (F1=F2)	0	2 - 3	2 - 3	2 - 3
PH_mod F2	no	pk	pk	pk
PH_mod F1	mc	**pk**, no	**pk**, no	**pk**, no
F2: PHC0; PHC1	N/A	determine	determine	determine
F1: PHC0; PHC1	N/A	90; -180	determine	90; -180
F1: FCOR	N/A	1	1	1

6.2. Summary of TOCSY parameters

NOTE: Changing one parameter might impact others, hence the integrity of all acquisition parameters must be verified. This can be done using the resources found in the pulse sequence description in the **PulseProg** tab, or by requesting help from a facility staff member.

ased parameters

Experiment	Echo/AntiEcho TOCSY	Echo/Antiecho TOCSY with Presaturation	Phase Sensitive TOCSY with Water Suppression
Parameter set	AA_TOCSY-dipea.MV	AA_TOCSY-dipet.MV	AA_TOCSY-mleves.MV
Bruker PP name	dipsi2etgp	dipsi2etgppr.mv	mlevesgpph
Description	For samples in organic solvents, Phase sensitive	For samples in D2O, Uses presaturation, Phase sensitive	For aqueous samples, Water suppression via excitation sculpting, Phase sensitive
D9 (mixing)	80-120 ms	80-120 ms	80-120 ms
D20 (1st z-filter)	20 us	10 us	N/A
D21 (2nd z-filter)	20 us	10 us	N/A
P1 @ PL1	P1(90˚)	P1(90˚)	P1(90˚)
P6 (spin-lock)	P6(90˚) (25-30 us, field dependent) @ PL10	P6(90˚) (25-30us, field dependent) @ PL10	P6(90˚) (25-30us, field dependent) @ PL10
PL10	power level for TOCSY spin-lock	power level for TOCSY spin-lock	power level for TOCSY spin-lock
GPNAM1 (1)	SMSQ10.100	SMSQ10.100	SMSQ10.100
GPNAM2 (1)	SMSQ10.100	SMSQ10.100	SMSQ10.100
GPZ1	10%	30%	31%
GPZ2	10%	30%	11%

(1) **SINE.100** may be used as well in these experiments, but is considered less efficient

eda parameters

Bruker PP name	dipsi2etgp	dipsi2etgppr.mv	mlevesgpph
FnMODE	Echo/Anti-Echo	Echo/Antiecho	States, **States-TPPI**, TPPI
TD F2	1024, **2048**, 4096	1024, **2048**, 4096	1024, **2048**, 4096
TD F1	128, 256, **512**	128, 256, **512**	128, 256, **512**
NS (minimum) (2)	8	8	2
SW F2	SW F1	SW F1	SW F1
ND_010	1	1	1

(2) **NS** may be increased by multiples of n (whole numbers)

edp parameters

Bruker PP name	dipsi2etgp	dipsi2etgppr.mv	mlevesgpph
SR (F1=F2)	1H spectral ref.	1H spectral ref.	1H spectral ref.
WDW (F1=F2)	QSINE	QSINE	QSINE
SSB (F1=F2)	2	2	2
PH_mod (F1=F2)	pk	pk	pk
F2: PHC0; PHC1	determine	determine	determine
F1: PHC0; PHC1	determine	determine	180; -180
F1: FCOR	1	1	1

6.3. Summary of NOESY parameters

NOTE: Changing one parameter might impact others, hence the integrity of all acquisition parameters must be verified. This can be done using the resources found in the pulse sequence description in the **PulseProg** tab, or by requesting help from a facility staff member.

ased parameters

Experiment	NOESY	NOESY with Water Suppression
Parameter set	AA_NOESY-ph.MV	AA_NOESY-phes.MV
Bruker PP name	noesygpph	noesyesfpgpphrs
Description	Phase sensitive, With gradient pulses	Water suppression using excitation sculpting and filpback pulse, optimized for radiation damping suppression
D8 (mixing)	40-1000 ms (SMF: 600ms, CSB: 100-250ms)	40-1000 ms (SMF: 600ms, CSB: 100-250ms)
P1 @ PL1	P1(90°)	P1(90°)
GPNAM1,2 (1)	SMSQ10.100	SMSQ10.100
GPZ0	N/A	2
GPZ1	N/A	50
GPZ1	40.00	31
GPZ2	-40.00	11

(1) **SINE.100** may be used as well in the gradient experiments, but is considered less efficient

eda parameters

Bruker PP name	noesygpph	noesygpphpr.mv
FnMODE	TPPI, States, States-TPPI	TPPI, States, States-TPPI, Echo/Anti-Echo
TD F2	1024, **2048**, 4096	1024, **2048**, 4096
TD F1	128, 256, **512**, 1024	128, 256, **512**, 1024
NS (minimum) (2)	2	8
SW (F2 = F1)	SW	SW
ND_010	1	1

(2) **NS** may be increased by multiples of n (whole numbers)

edp parameters

Bruker PP name	noesygpph	noesygpphpr.mv
SR (F1=F2)	1H spectral ref.	1H spectral ref.
WDW (F1=F2)	QSINE	QSINE
SSB (F1=F2)	2	2
F2: PHC0; PHC1	determine	determine
F1: PHC0; PHC1	determine	90; -180
F1: FCOR	1	1