

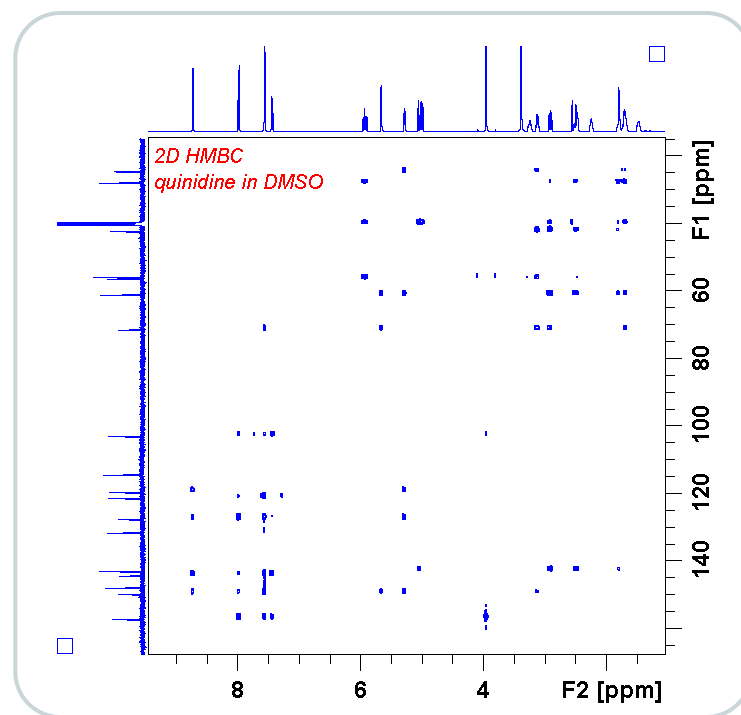
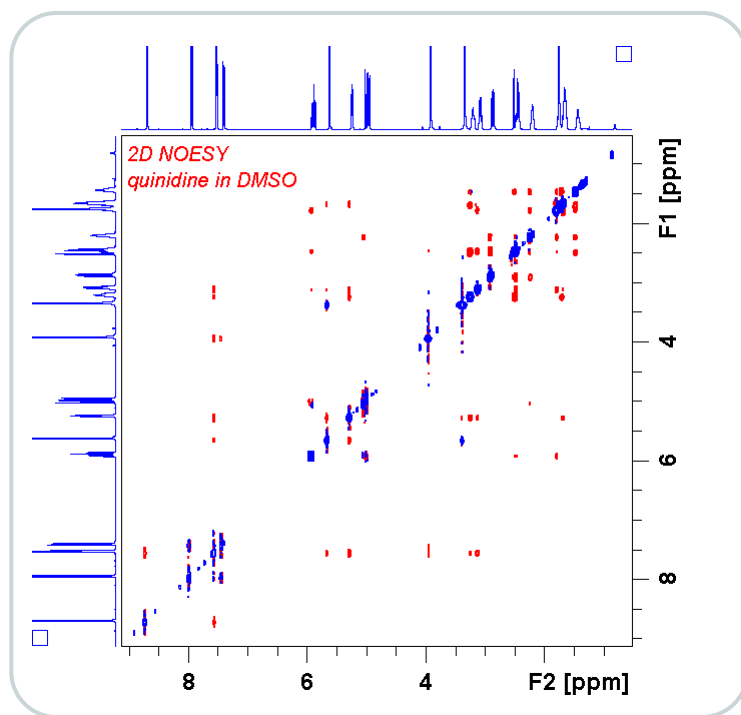
New tools for 1D and 2D selective experiments

Eric Johnson

Symposium on Frontiers in Biomolecular NMR
Vanderbilt University
May 4, 2012

Why use selective experiments?

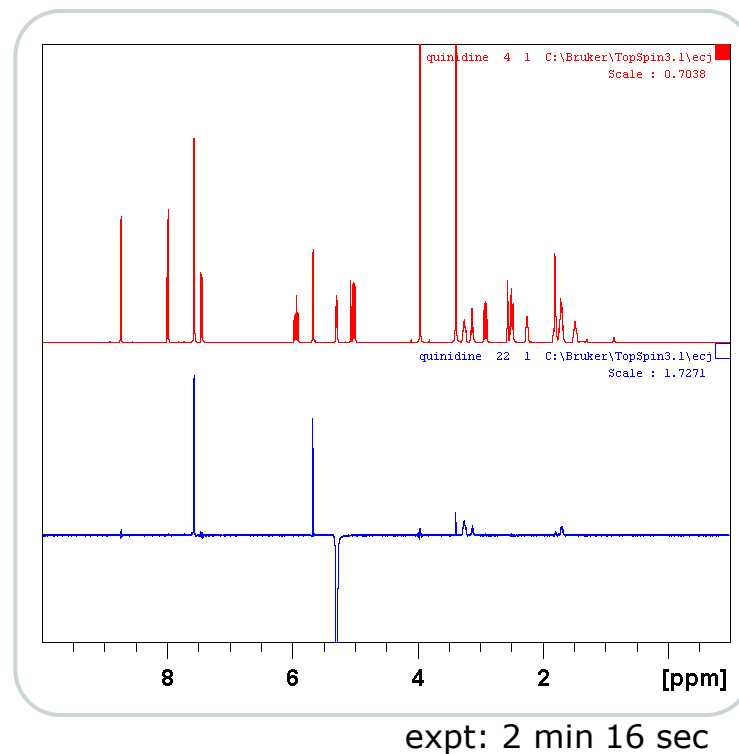
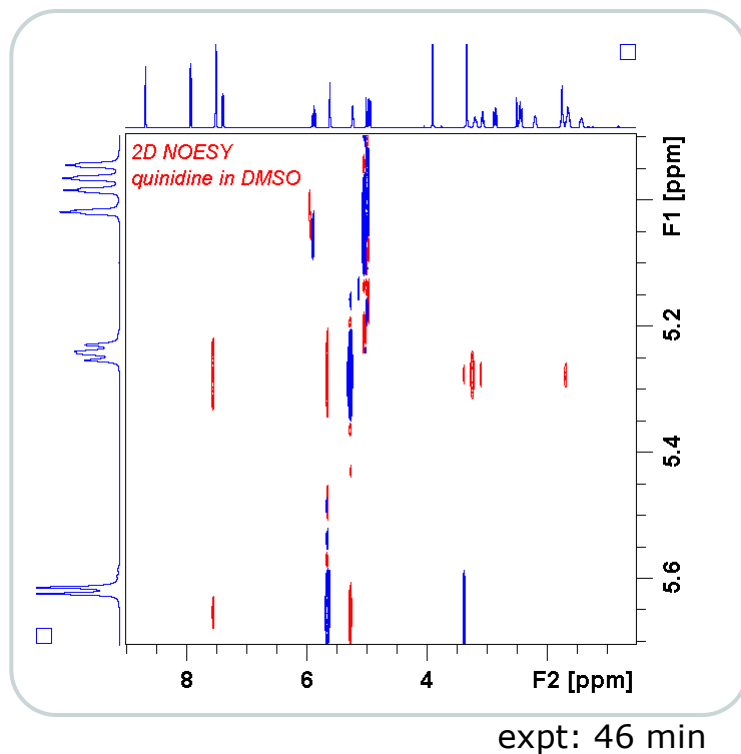
- 2D homonuclear and 2D heteronuclear experiments are easy to set up and are loaded with information



Individual experiments give correlations throughout the molecule

Why use selective experiments?

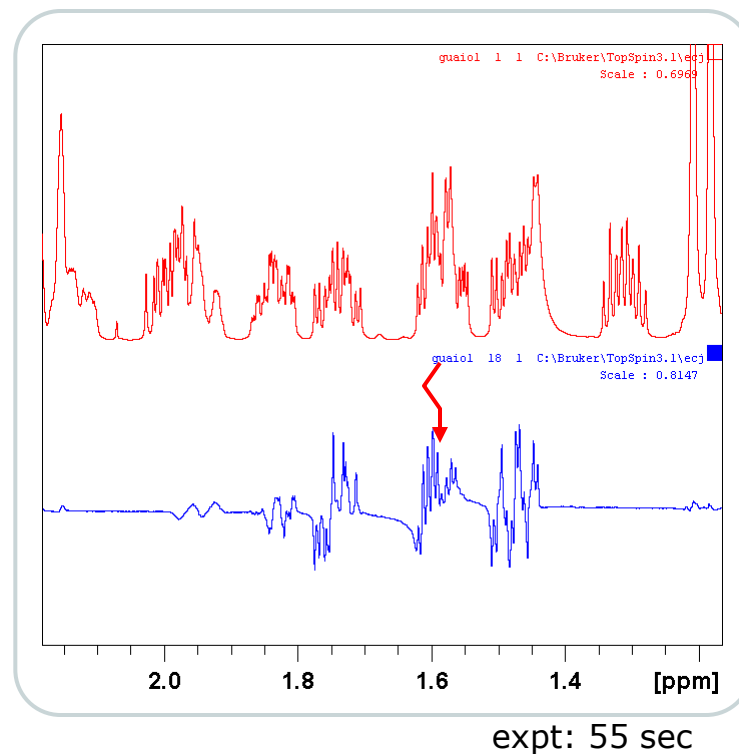
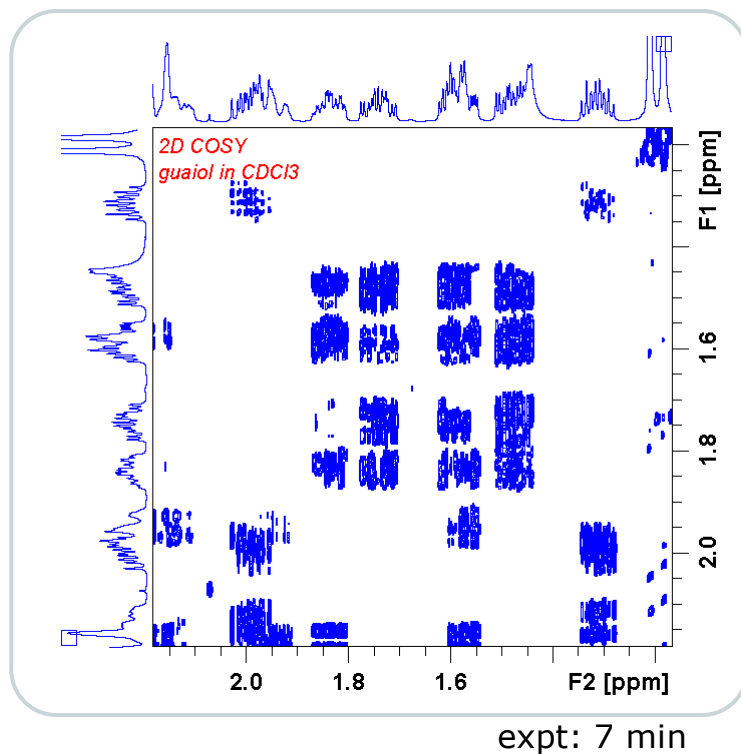
- When we're looking for a specific correlation



- Shorter acquisition time

Why use selective experiments?

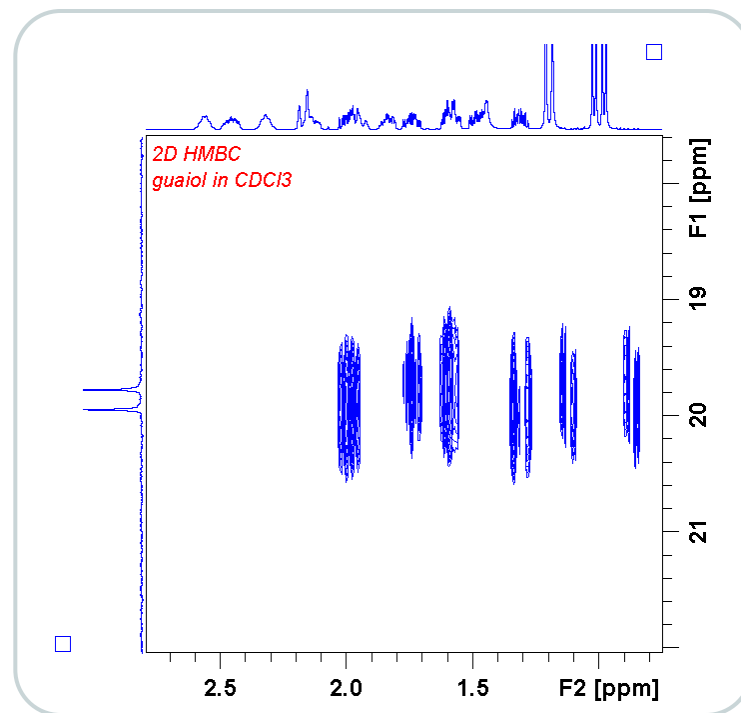
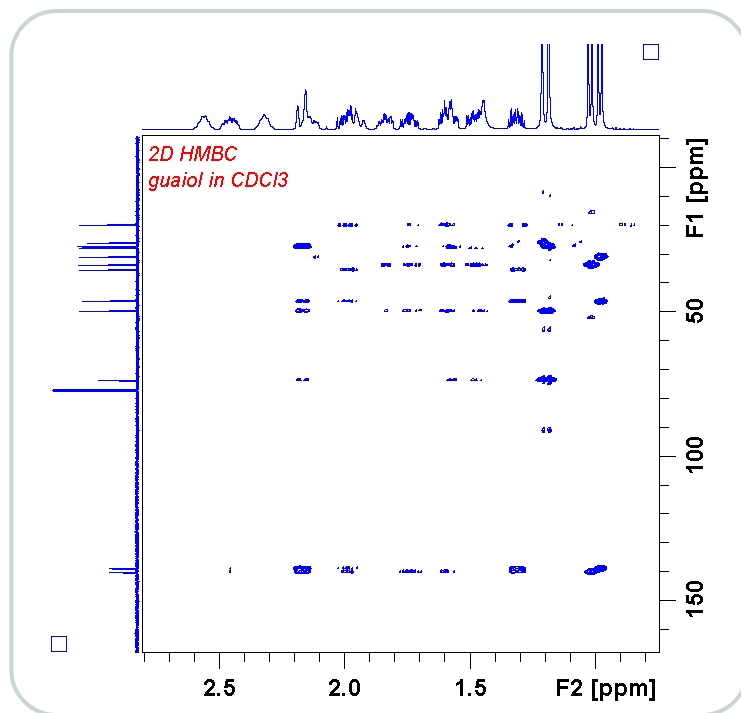
- When we're looking for a specific correlation



- Selective 1D's usually have higher resolution than 2D spectrum

Why use selective experiments?

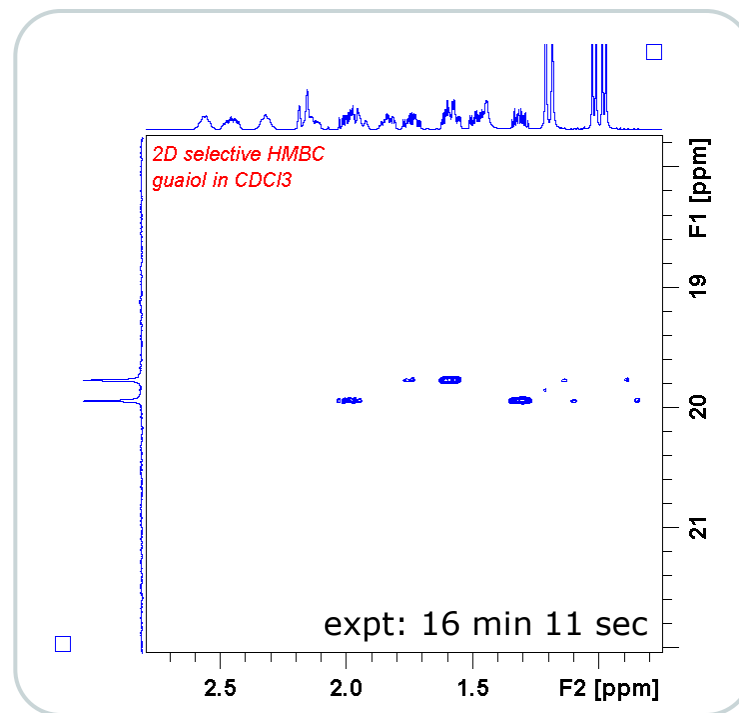
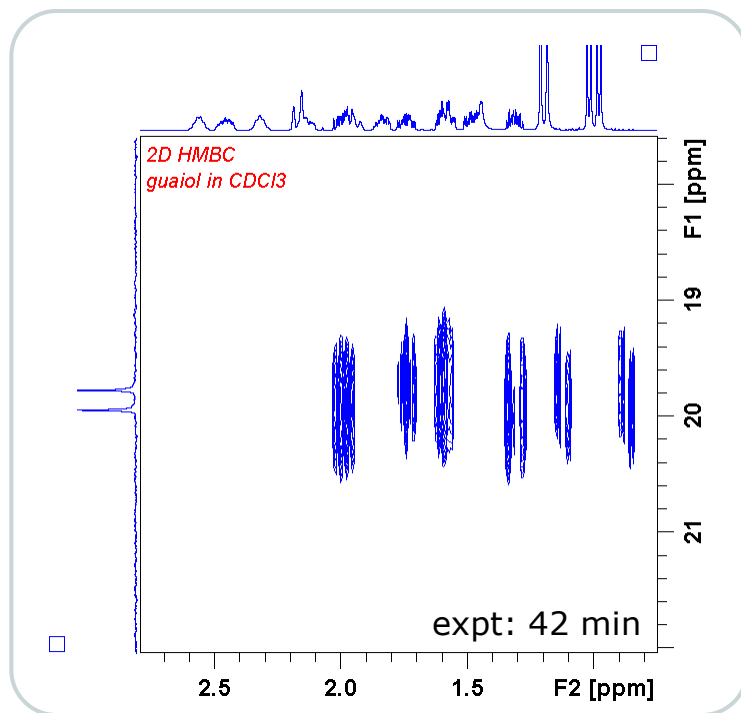
- When we need higher resolution for a specific region of the HMBC



- Increase TD(F1) → increase experiment time
- Decrease SW(F1) → aliasing/folding of peaks

Why use selective experiments?

- 2D selective HMBC:



- Decrease $SW(F1)$
- Use ^{13}C selective pulse to prevent aliasing/folding

1D and 2D selective experiments aren't new

Pulse Programs

File Options Help Source = C:\Bruker\TOPSPIN2.1\exp\stan\nm\lists\pp

Search in names [??] Search sel*

Class = Any Dim = 1D

All

selco	selcogp	selcogp	selcorl	seldigp
selgpse	selhsqcgplrndsp	selhsqcgpsisp	selina	selineptlrrdsp
selmlgp	selmlg			
selnogpzs	selnoz			
selzgp	selzgp			

Pulse Programs

File Options Help Source = C:\Bruker\TOPSPIN2.1\exp\stan\nm\lists\pp

Search in names [??] Search shmbc*

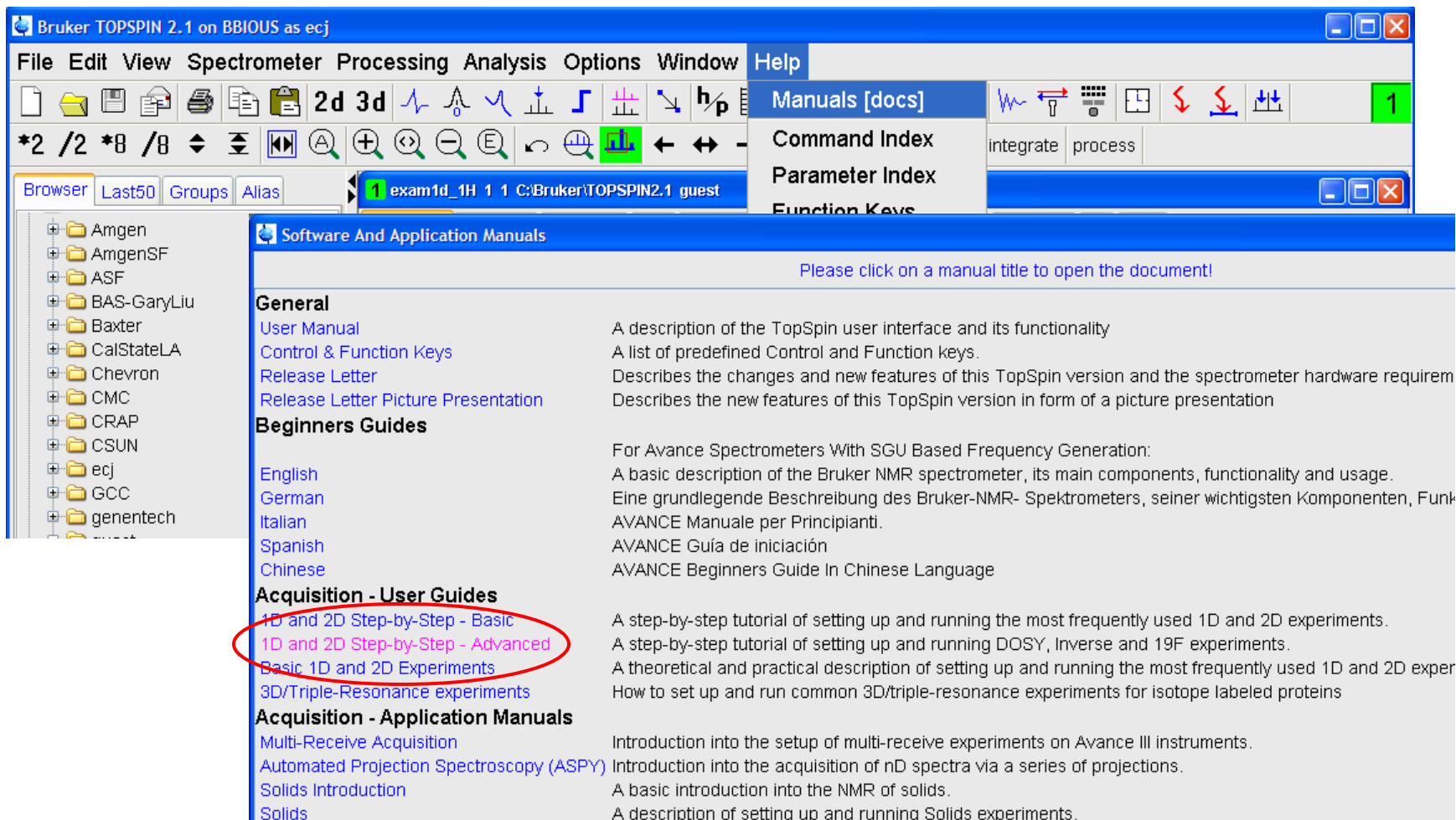
Class = Any Dim = Any

All

shmbcgpndqf				
-------------	--	--	--	--

Edit Graphical Edit Set PULPROG Close

1D and 2D selective experiments aren't new



The screenshot shows the Bruker TOPSPIN 2.1 software interface. The main window displays the 'Software And Application Manuals' section, which is open to a list of manuals. The manual titled '1D and 2D Step-by-Step - Advanced' is circled in red. The interface includes a menu bar (File, Edit, View, Spectrometer, Processing, Analysis, Options, Window, Help), a toolbar with various icons, and a file browser on the left side showing a directory structure.

Software And Application Manuals

Please click on a manual title to open the document!

General

- [User Manual](#) - A description of the TopSpin user interface and its functionality
- [Control & Function Keys](#) - A list of predefined Control and Function keys.
- [Release Letter](#) - Describes the changes and new features of this TopSpin version and the spectrometer hardware requirements
- [Release Letter Picture Presentation](#) - Describes the new features of this TopSpin version in form of a picture presentation

Beginners Guides

For Avance Spectrometers With SGU Based Frequency Generation:

- [English](#) - A basic description of the Bruker NMR spectrometer, its main components, functionality and usage.
- [German](#) - Eine grundlegende Beschreibung des Bruker-NMR- Spektrometers, seiner wichtigsten Komponenten, Funktionsweise und Anwendung.
- [Italian](#) - AVANCE Manuale per Principianti.
- [Spanish](#) - AVANCE Guía de iniciación
- [Chinese](#) - AVANCE Beginners Guide In Chinese Language

Acquisition - User Guides

- [1D and 2D Step-by-Step - Basic](#)
- [1D and 2D Step-by-Step - Advanced](#) - A step-by-step tutorial of setting up and running the most frequently used 1D and 2D experiments.
- [Basic 1D and 2D Experiments](#) - A step-by-step tutorial of setting up and running DOSY, Inverse and 19F experiments.
- [3D/Triple-Resonance experiments](#) - A theoretical and practical description of setting up and running the most frequently used 1D and 2D experiments
- [How to set up and run common 3D/triple-resonance experiments for isotope labeled proteins](#)

Acquisition - Application Manuals

- [Multi-Receive Acquisition](#) - Introduction into the setup of multi-receive experiments on Avance III instruments.
- [Automated Projection Spectroscopy \(ASPY\)](#) - Introduction into the acquisition of nD spectra via a series of projections.
- [Solids Introduction](#) - A basic introduction into the NMR of solids.
- [Solids](#) - A description of setting up and running Solids experiments.

1D and 2D selective experiments aren't new

**1D and 2D Experiments
Step-by-Step Tutorial**

**Advanced Experiments
User Guide**

6	1-D Selective NOESY	61
6.1	Introduction	61
	Reference spectrum	61
	Selective excitation region set up (example 1)	63
	On resonance	63
	Setting up the acquisition parameters	64
	Running the experiment	64
	Selective excitation region set up (example 2)	65
	Off resonance	65
	Setting up the acquisition parameters	66
	Running the experiment	67
	Selective excitation region set up (example 3)	68
	Integration region file	68
	Calculating the selective pulse width and power level	69
	Setting up the acquisition parameters	72
	Running the experiment	72

Contents

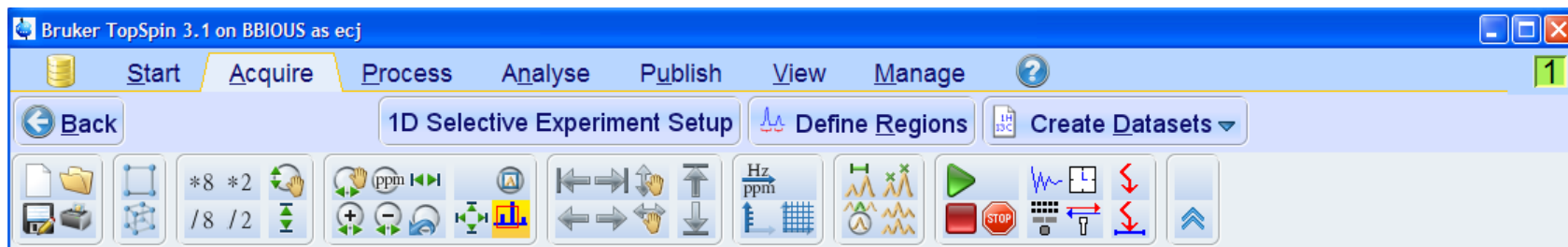
10	2-D Selective HMBC	97
10.1	Introduction	97
	Reference spectrum	97
	Limit setting	99
	Running a 2-D HMBC experiment	99
	Acquisition	100
	Processing	101
	Optimizing the parameters on the carbonyl region	102
	Set up the selective pulse	104
	Setting up the acquisition parameters	107
	Running the experiment	107

Setting up selective experiments in TopSpin2.1

- Easiest method for 1D sel. expts:
 - Selective excitation at center of spectrum (set **O1**)
 - Use shaped pulse from prosol
- Setting excitation frequency away from **O1** requires manual calculation of offset
- Adjusting selectivity requires using ShapeTool
- Setting up 2D selective HMBC requires ShapeTool to calculate shape pulse parameters

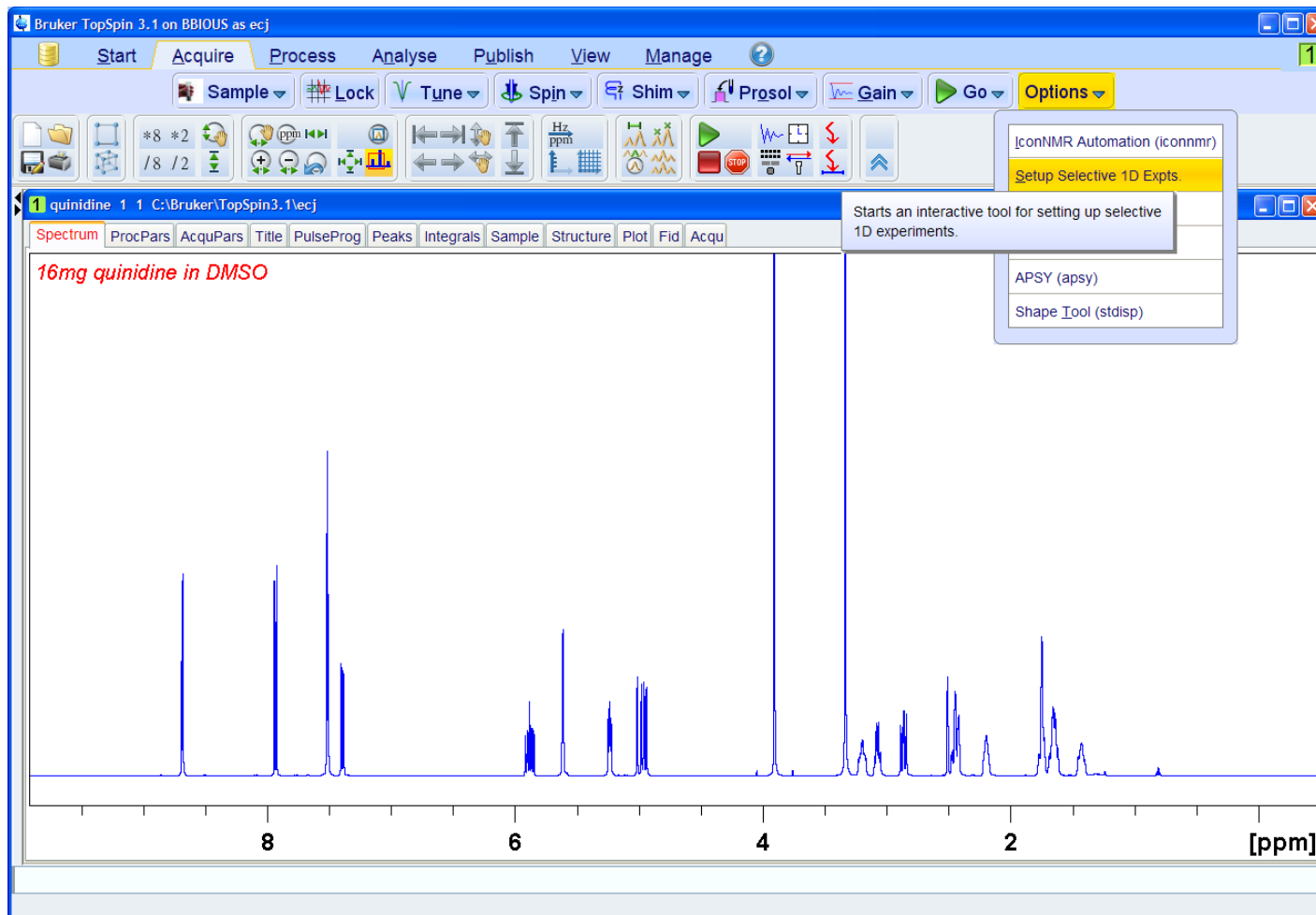
New in TopSpin3.1

- Flow interface to facilitate setting up selective experiments



- All calculations are performed automatically

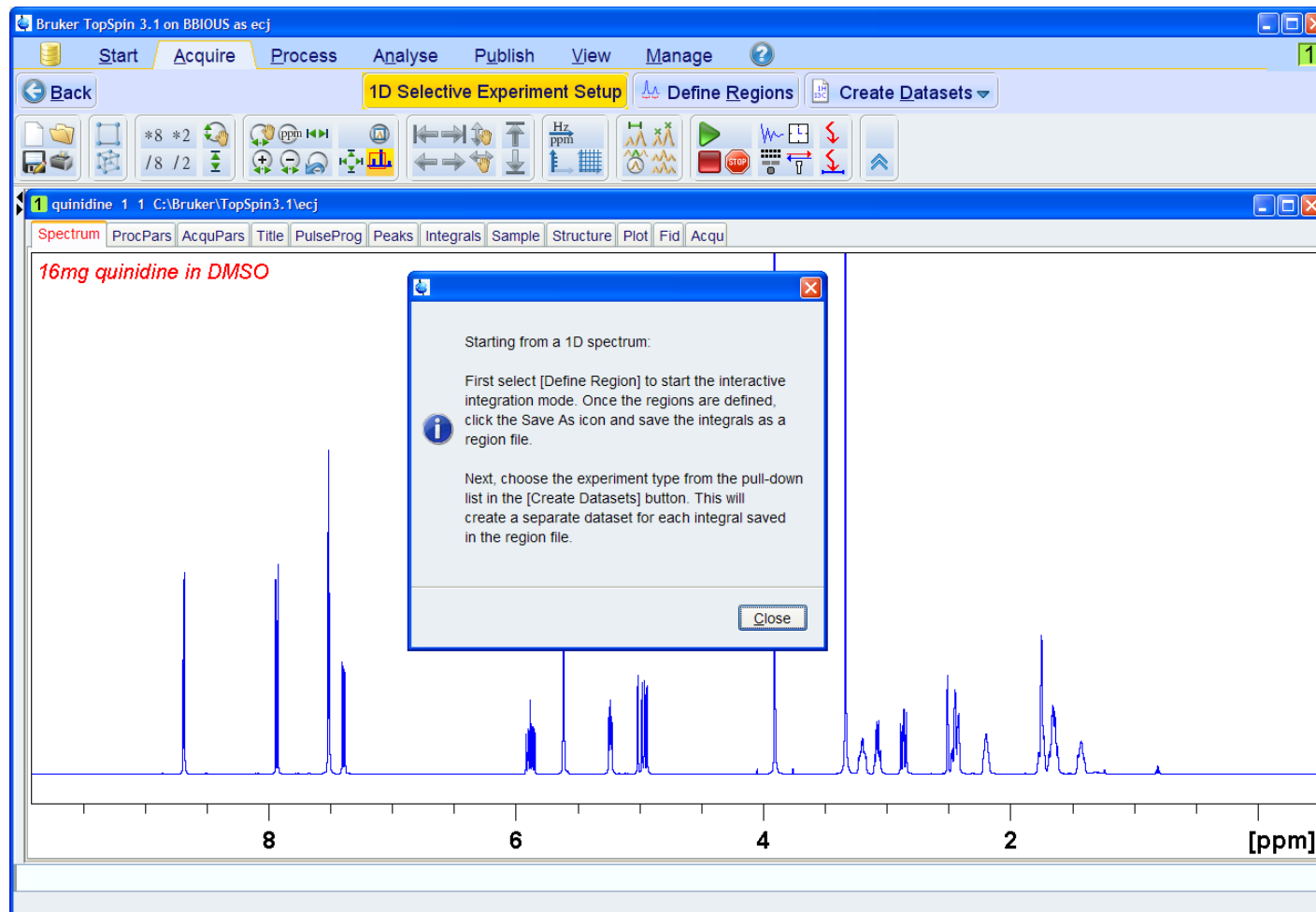
1D selective experiments



Step 1: Acquire and process a 1D PROTON

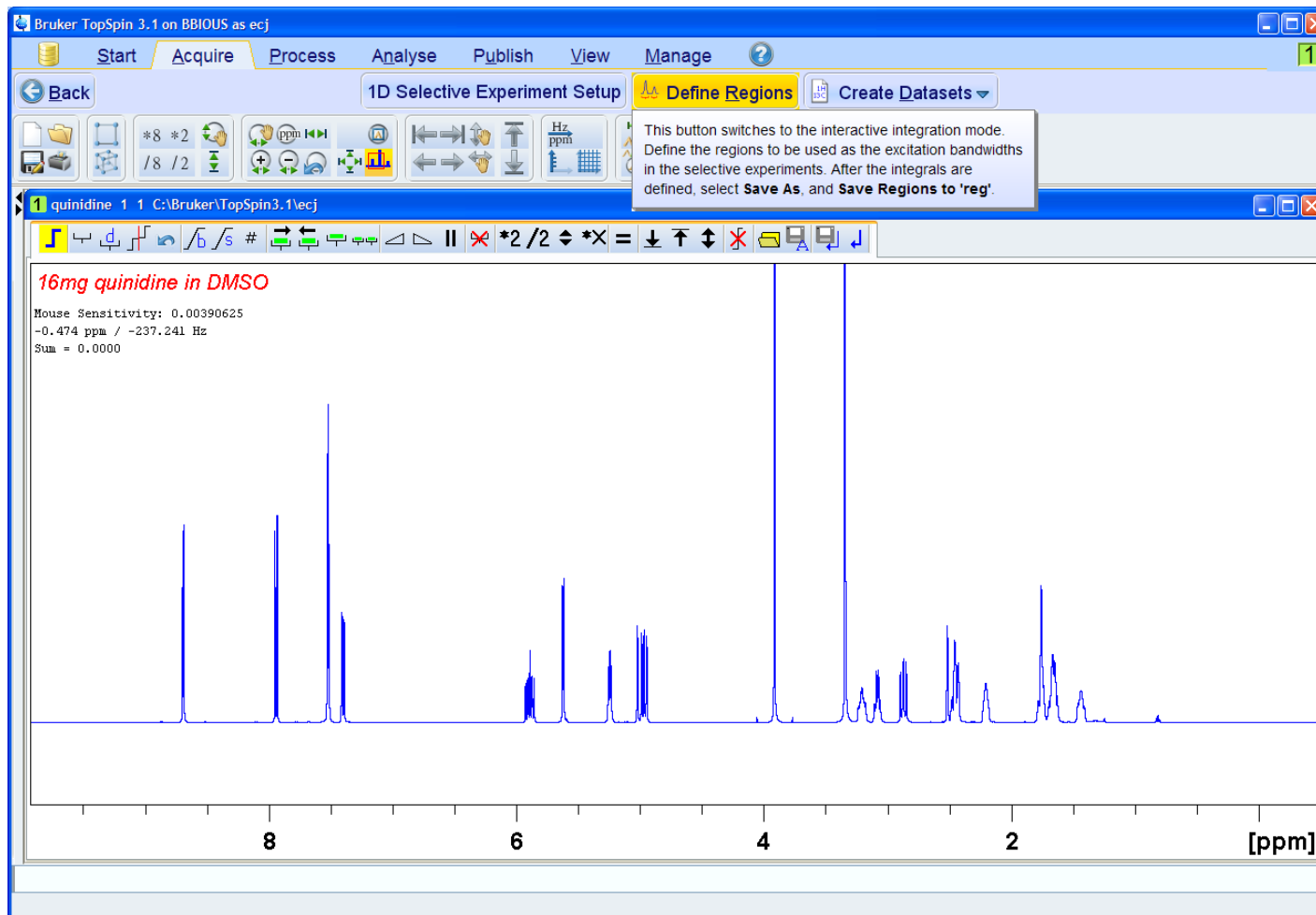
Step 2: Select **Setup Selective 1D Expts.** from **Acquire** → **Options**

1D selective experiments



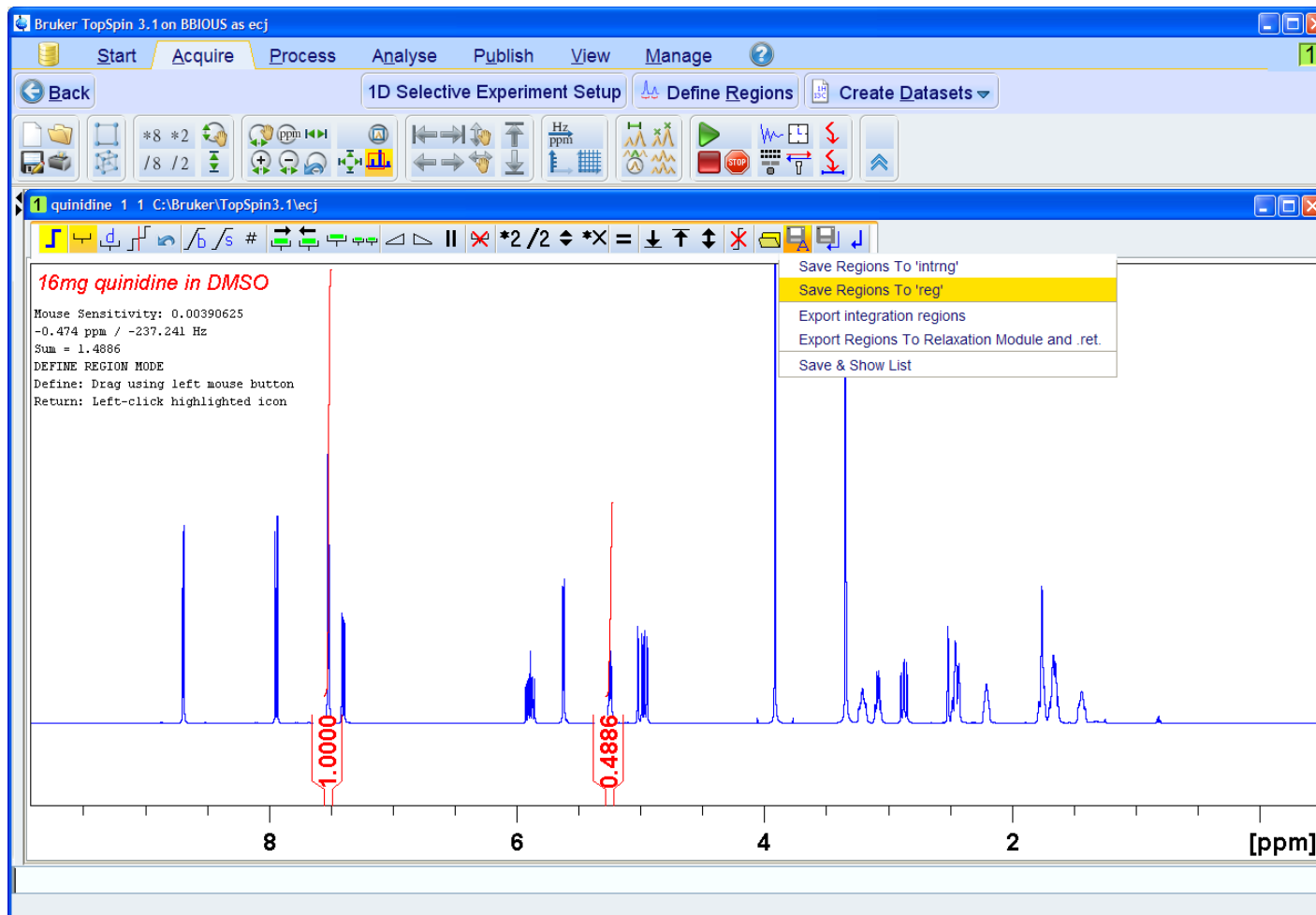
1st button gives instructions (but has no other functions)

1D selective experiments



Step 3: Click  **Define Regions** button to start the interactive integration mode

1D selective experiments



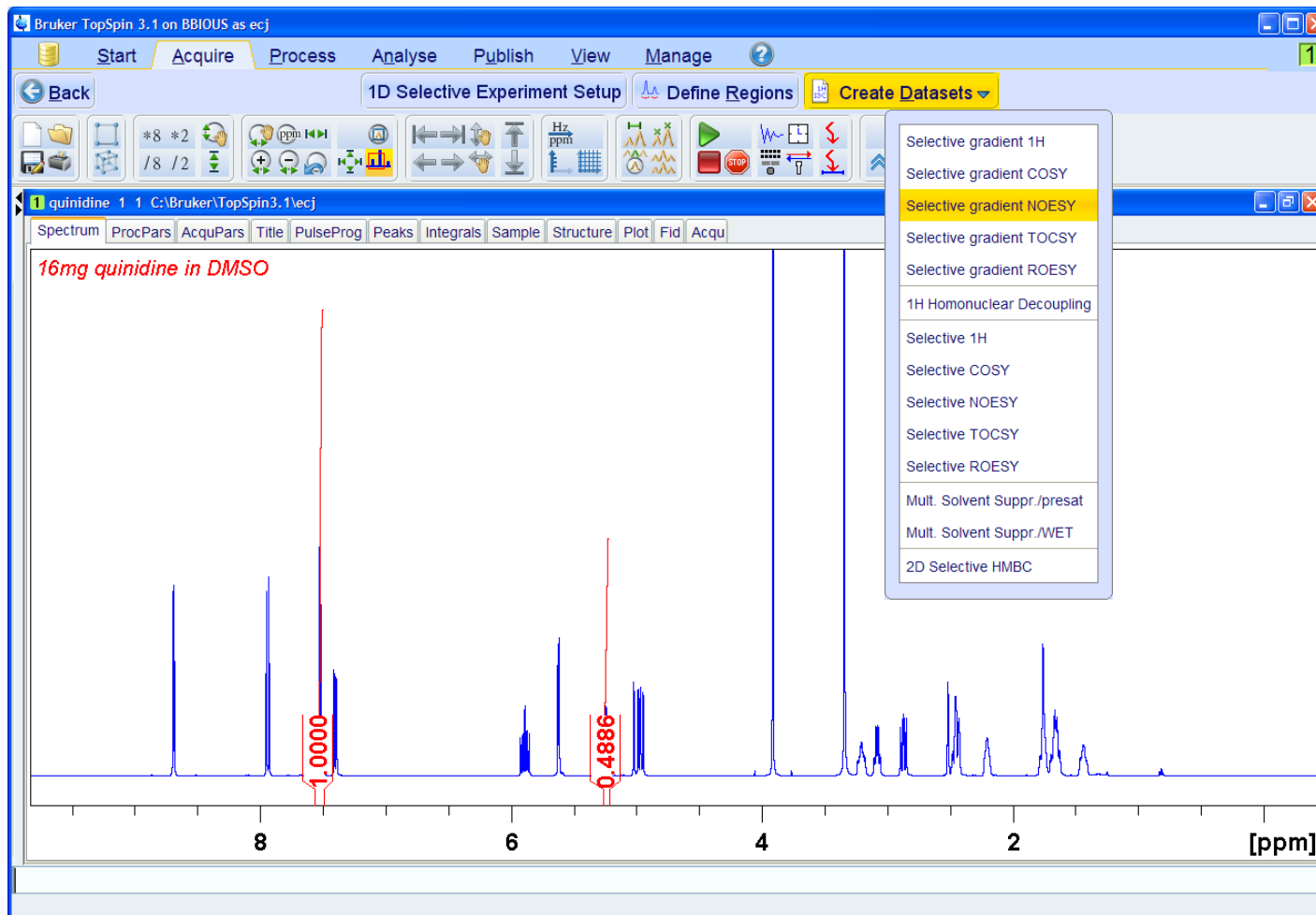
Step 4: Integrate peaks of interest and select



Save Regions To 'reg'

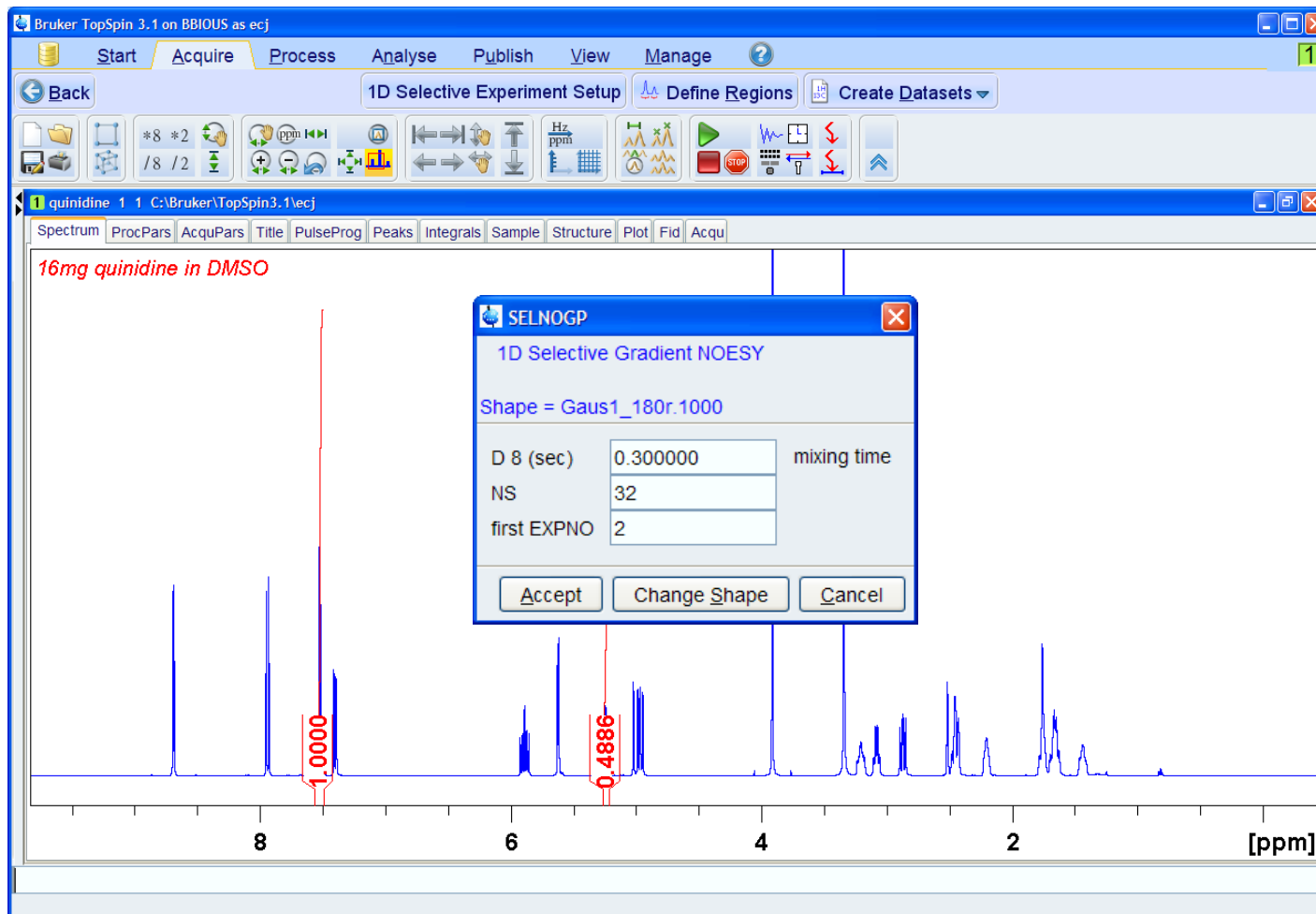
Step 5: Return from integration mode

1D selective experiments



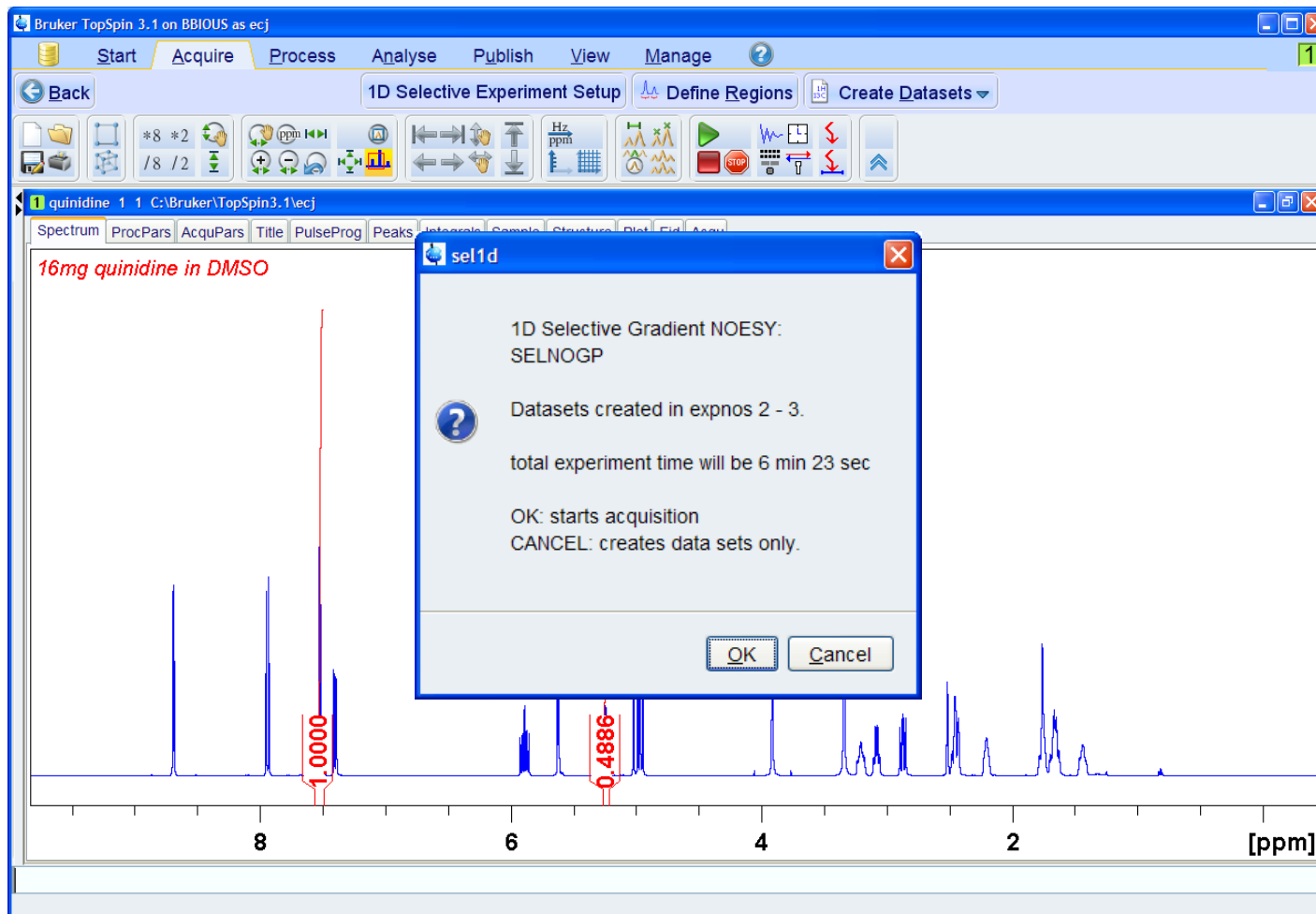
Step 6: Choose selective experiment from list under  **Create Datasets** button

1D selective experiments



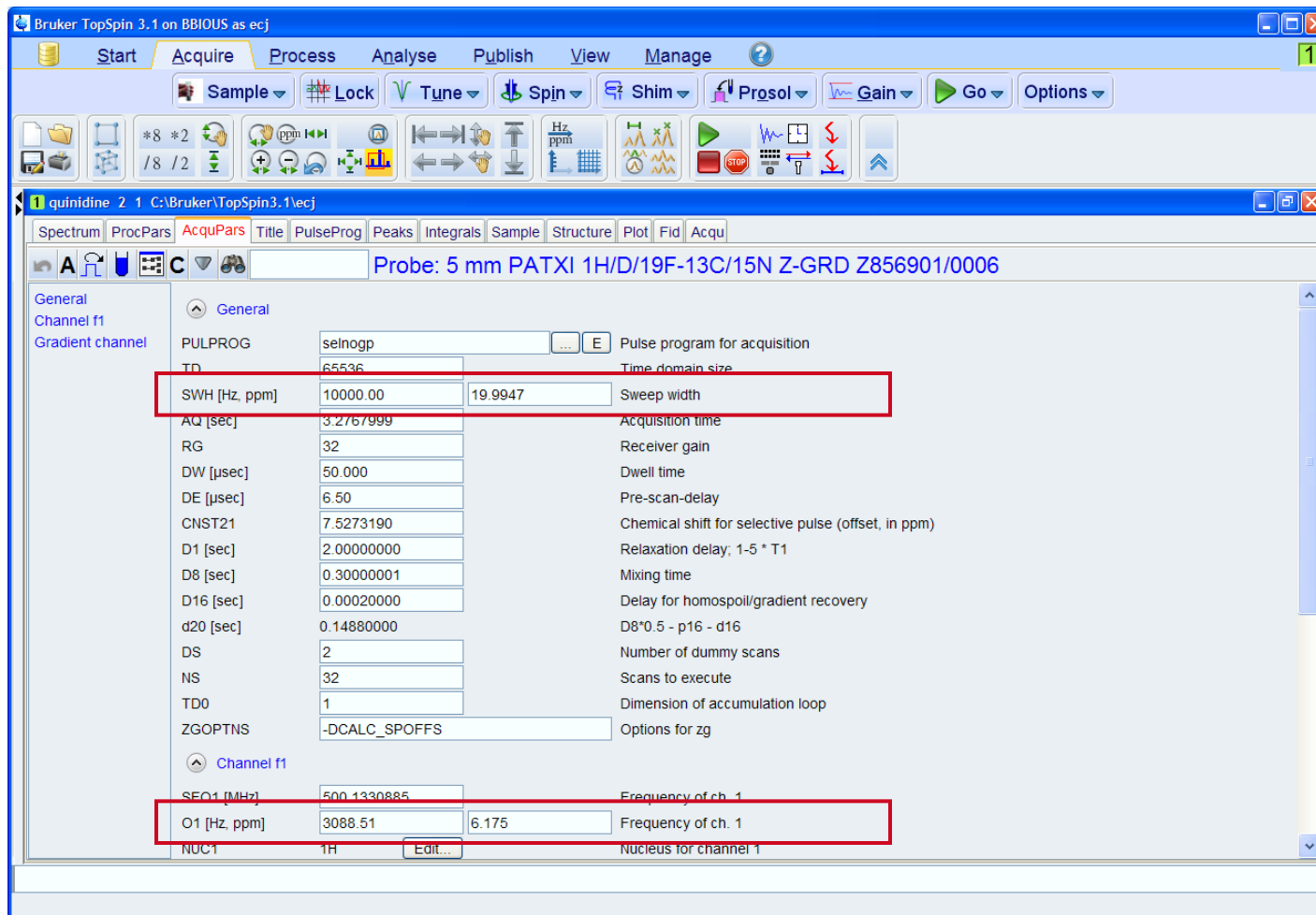
Default parameters are taken from standard parameter sets (i.e. SELNOGP)

1D selective experiments



New datasets are created and all parameters are automatically set

1D selective experiments



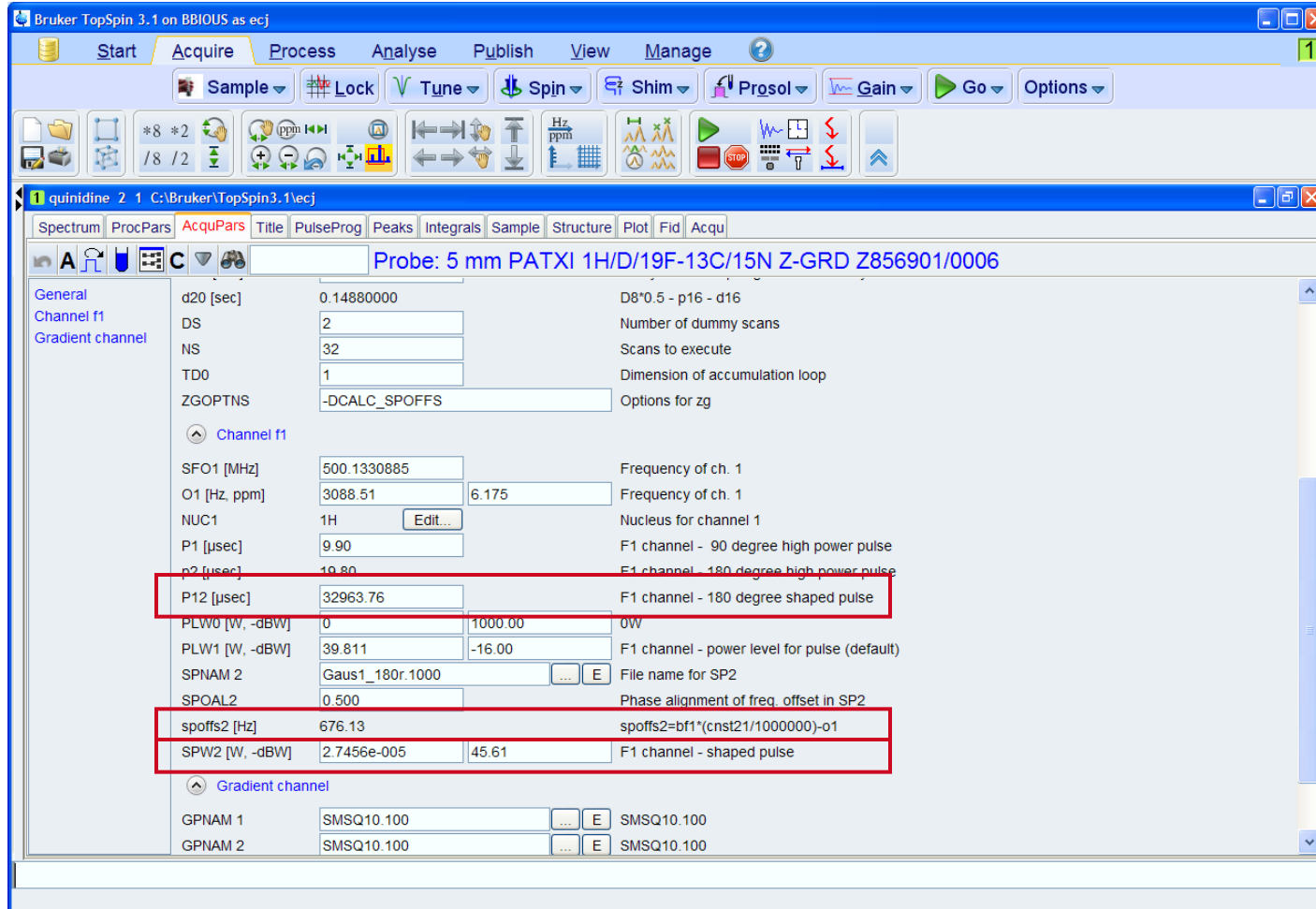
The screenshot shows the Bruker TopSpin 3.1 software interface. The main window displays the 'AcquPars' tab for a 1D selective experiment. The parameters are organized into sections: General, Channel f1, and Gradient channel. Two red boxes highlight specific parameters:

- SWH [Hz, ppm]:** 10000.00 (Frequency), 19.9947 (Sweep width)
- O1 [Hz, ppm]:** 3088.51 (Frequency), 6.175 (Offset)

Parameter	Value 1	Value 2	Description
PULPROG	selnogg		Pulse program for acquisition
TD	65536		Time domain size
SWH [Hz, ppm]	10000.00	19.9947	Sweep width
AQ [sec]	3.2767999		Acquisition time
RG	32		Receiver gain
DW [µsec]	50.000		Dwell time
DE [µsec]	6.50		Pre-scan-delay
CNST21	7.5273190		Chemical shift for selective pulse (offset, in ppm)
D1 [sec]	2.00000000		Relaxation delay; 1-5 * T1
D8 [sec]	0.30000001		Mixing time
D16 [sec]	0.00020000		Delay for homospoil/gradient recovery
d20 [sec]	0.14880000		D8*0.5 - p16 - d16
DS	2		Number of dummy scans
NS	32		Scans to execute
TD0	1		Dimension of accumulation loop
ZGOPTNS	-DCALC_SPOFFS		Options for zg
SEQ1 [MHz]	500.1330885		Frequency of ch. 1
O1 [Hz, ppm]	3088.51	6.175	Frequency of ch. 1
NUC1	1H	Edit...	Nucleus for channel 1

SW and O1 are taken from starting 1D PROTON experiment

1D selective experiments



quindine 2 1 C:\Bruker\TopSpin3.1\ecj

Probe: 5 mm PATXI 1H/D/19F-13C/15N Z-GRD Z856901/0006

Parameter	Value	Description	
d20 [sec]	0.14880000	D8*0.5 - p16 - d16	
DS	2	Number of dummy scans	
NS	32	Scans to execute	
TD0	1	Dimension of accumulation loop	
ZGOPTNS	-DCALC_SPOFFS	Options for zg	
Channel f1			
SFO1 [MHz]	500.1330885	Frequency of ch. 1	
O1 [Hz, ppm]	3088.51	6.175	Frequency of ch. 1
NUC1	1H	Nucleus for channel 1	
P1 [µsec]	9.90	F1 channel - 90 degree high power pulse	
p2 [µsec]	10.80	F1 channel - 180 degree high power pulse	
P12 [µsec]	32963.76	F1 channel - 180 degree shaped pulse	
PLW0 [W, -dBW]	0	1000.00	0W
PLW1 [W, -dBW]	39.811	-16.00	F1 channel - power level for pulse (default)
SPNAM 2	Gaus1_180r.1000		File name for SP2
SPOAL2	0.500		Phase alignment of freq_offset in SP2
spoffs2 [Hz]	676.13		spoffs2=bf1*(cnst21/1000000)-o1
SPW2 [W, -dBW]	2.7456e-005	45.61	F1 channel - shaped pulse
Gradient channel			
GPNAM 1	SMSQ10.100		SMSQ10.100
GPNAM 2	SMSQ10.100		SMSQ10.100

Power, duration and offset of shaped pulse are automatically calculated from integrals

1D selective experiments

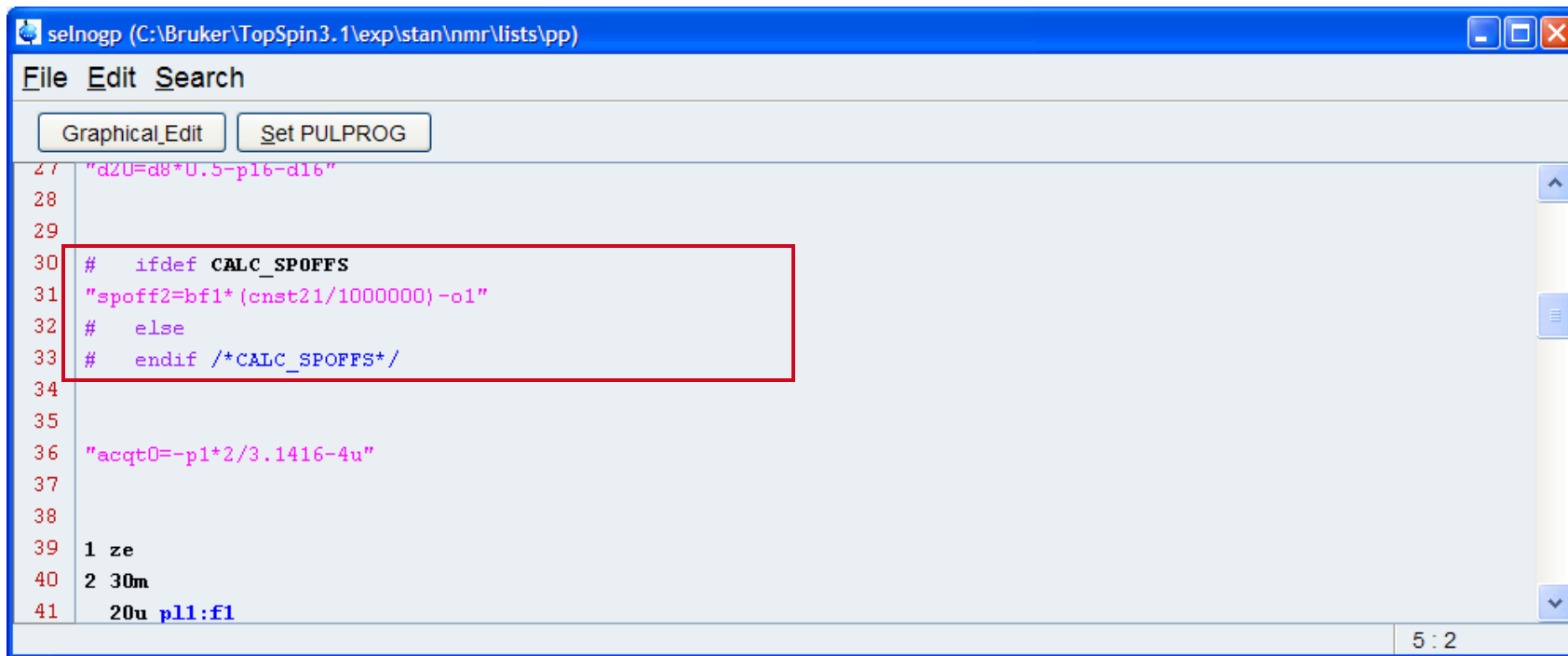
New modifications:

Compare currently installed probe with the probe used for starting EXPNO

- If same probe: `getprosol 1H <P1> <PLdB1>` From starting EXPNO
- If probes differ:



1D selective experiments

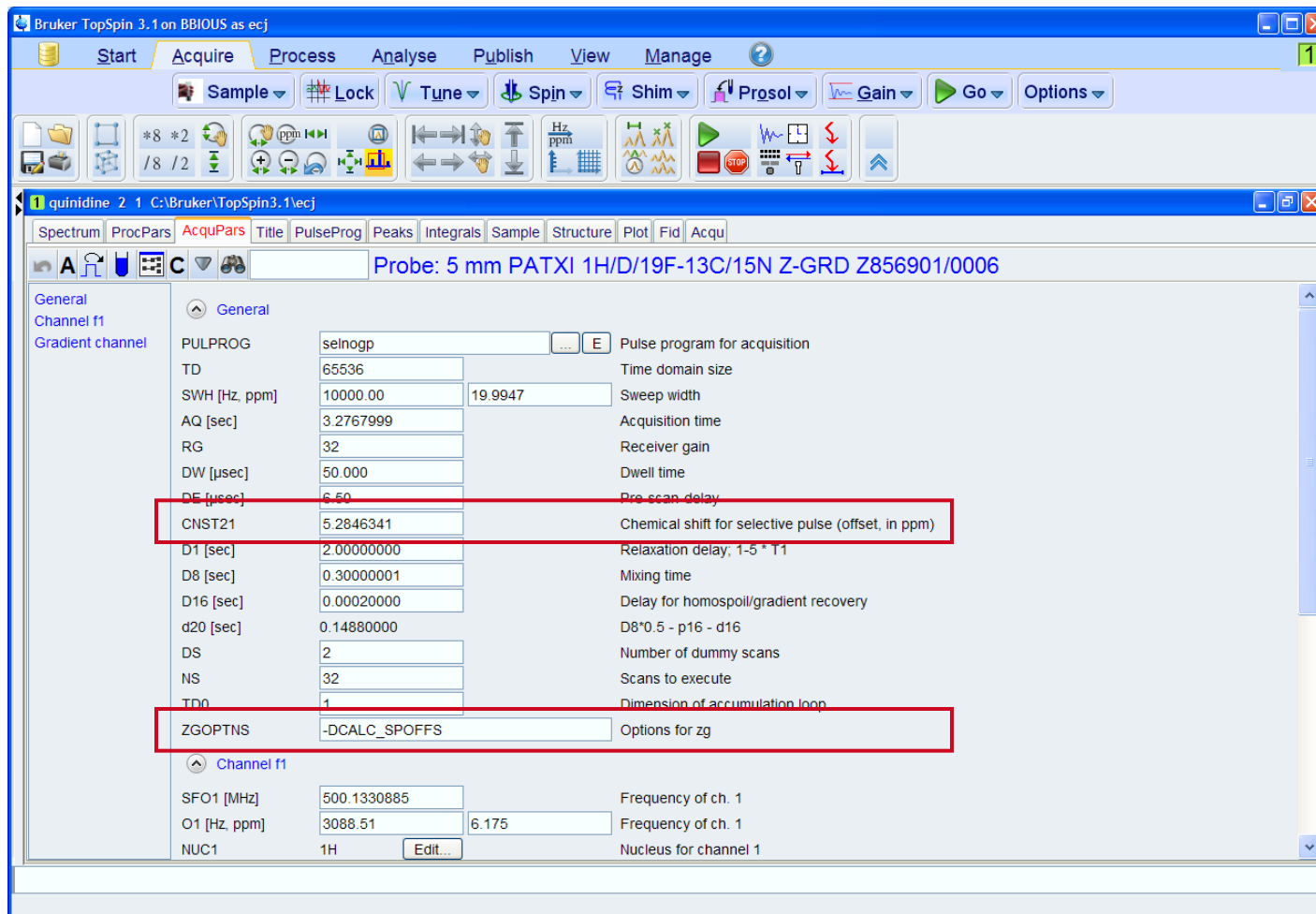


```
selnpgp (C:\Bruker\TopSpin3.1\exp\stan\nmr\lists\pp)
File Edit Search
Graphical_Edit Set PULPROG
27 "d2U=d8*U.5-p16-d16"
28
29
30 #  ifdef CALC_SPOFFS
31 "spoff2=bf1*(cnst21/1000000)-o1"
32 #  else
33 #  endif /*CALC_SPOFFS*/
34
35
36 "acqt0=-p1*2/3.1416-4u"
37
38
39 1 ze
40 2 30m
41 20u p11:f1
5 : 2
```

New modification to pulse programs calculates **SPOFFS** from **CNST21**

- Excitation frequency can be set by simply entering value into **CNST21**
- After selective experiment is set up, the spectral limits can be changed without affecting the selective excitation frequency.

1D selective experiments



quindine 2 1 C:\Bruker\TopSpin3.1\ecj

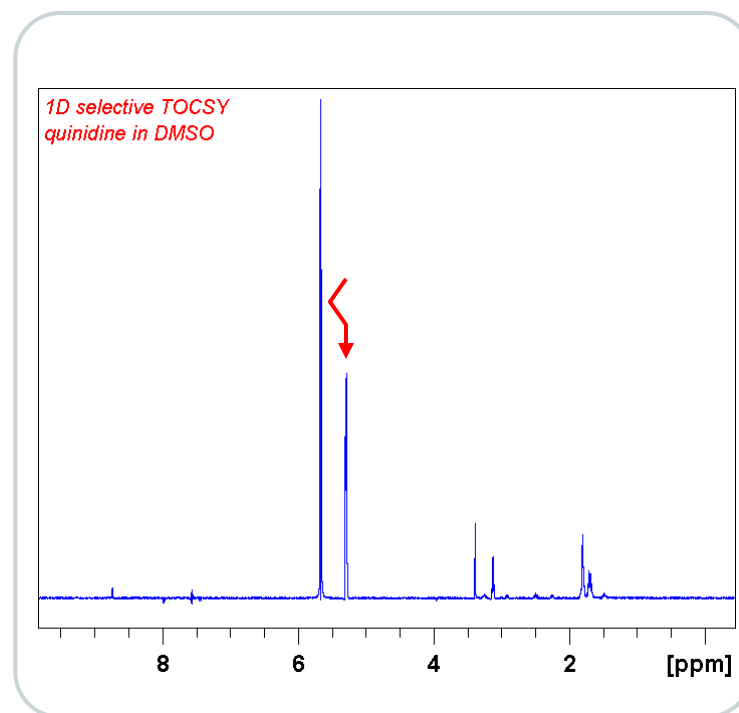
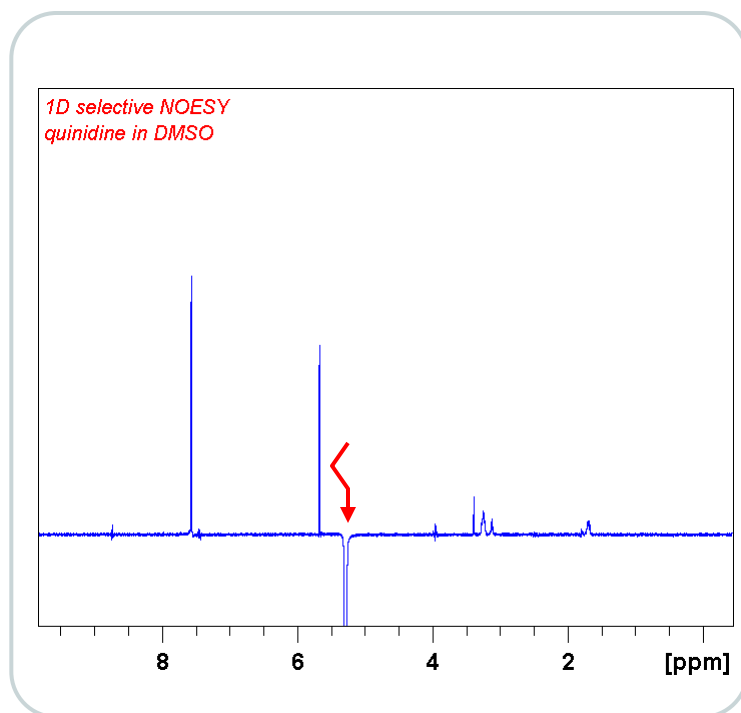
Probe: 5 mm PATXI 1H/D/19F-13C/15N Z-GRD Z856901/0006

Parameter	Value	Description	
PULPROG	selnogg	Pulse program for acquisition	
TD	65536	Time domain size	
SWH [Hz, ppm]	10000.00	19.9947	Sweep width
AQ [sec]	3.2767999	Acquisition time	
RG	32	Receiver gain	
DW [µsec]	50.000	Dwell time	
DE [µsec]	6.50	Pre-scan delay	
CNST21	5.2846341	Chemical shift for selective pulse (offset, in ppm)	
D1 [sec]	2.00000000	Relaxation delay; 1-5 * T1	
D8 [sec]	0.30000001	Mixing time	
D16 [sec]	0.00020000	Delay for homospoil/gradient recovery	
d20 [sec]	0.14880000	D8*0.5 - p16 - d16	
DS	2	Number of dummy scans	
NS	32	Scans to execute	
TD0	1	Dimension of accumulation loop	
ZGOPTNS	-DCALC_SPOFFS	Options for zg	
SFO1 [MHz]	500.1330885	Frequency of ch. 1	
O1 [Hz, ppm]	3088.51	6.175	Frequency of ch. 1
NUC1	1H	Nucleus for channel 1	

Chemical shift of excitation pulse is stored in parameter CNST21

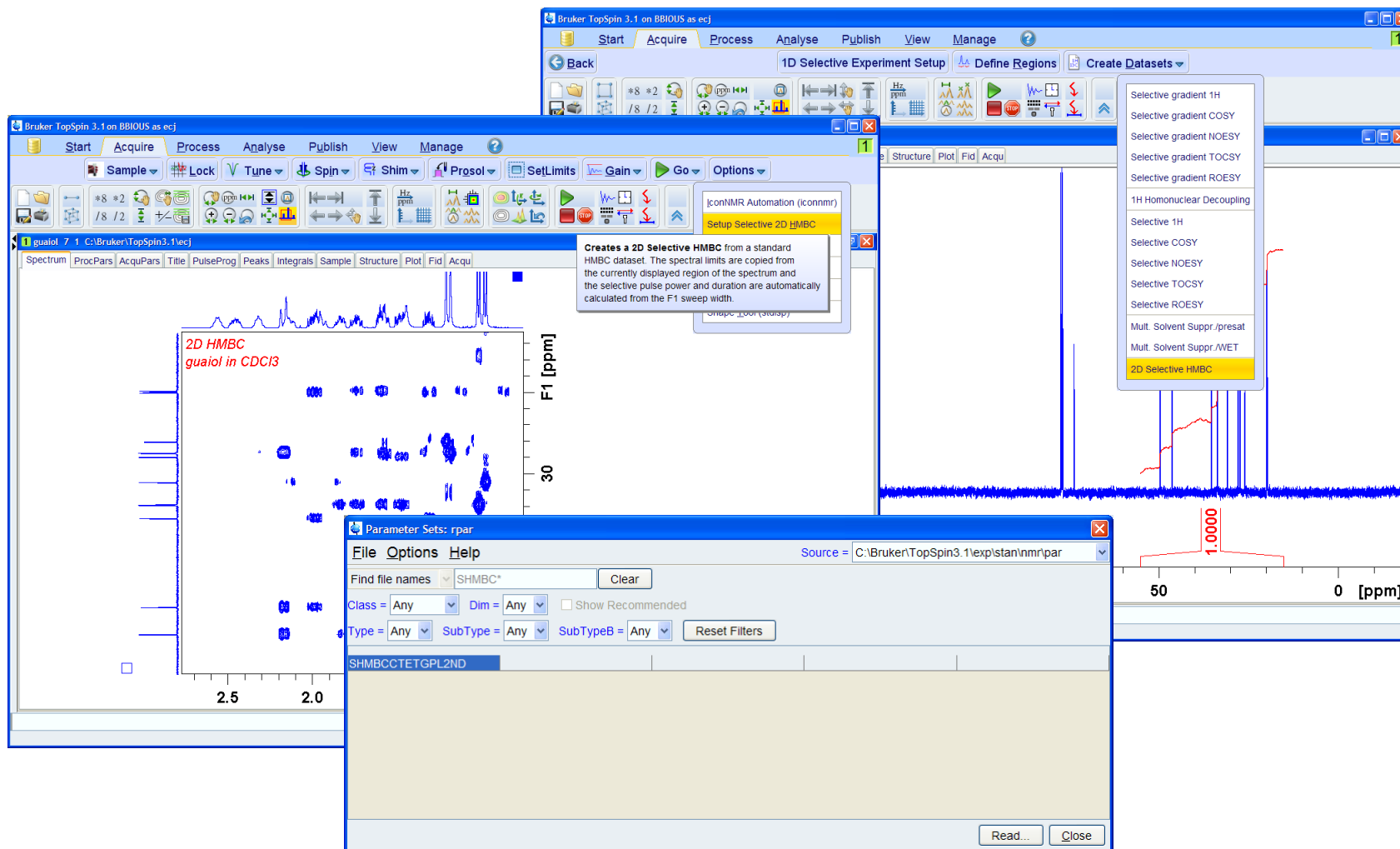
1D selective experiments

Excitation frequency is not always obvious in 1D selective experiments



CNST21 = 5.28 in both spectra

2D selective HMBC



The screenshot illustrates the Bruker TopSpin 3.1 interface for setting up a 2D selective HMBC experiment. The main window shows a 2D NMR spectrum of guaiol in CDCl₃. A '1D Selective Experiment Setup' dialog is open, displaying a list of pulse programs. The '2D Selective HMBC' option is highlighted. A 'Parameter Sets: rpar' dialog is also open, showing search results for 'SHMBC*'.

1D Selective Experiment Setup Dialog:

- Selective gradient 1H
- Selective gradient COSY
- Selective gradient NOESY
- Selective gradient TOCSY
- Selective gradient ROESY
- 1H Homonuclear Decoupling
- Selective 1H
- Selective COSY
- Selective NOESY
- Selective TOCSY
- Selective ROESY
- Mult. Solvent Suppr. presat
- Mult. Solvent Suppr. WET
- 2D Selective HMBC**

Parameter Sets: rpar Dialog:

Source = C:\Bruker\TopSpin3.1\expstan\nmr\par

Find file names: SHMBC* Clear

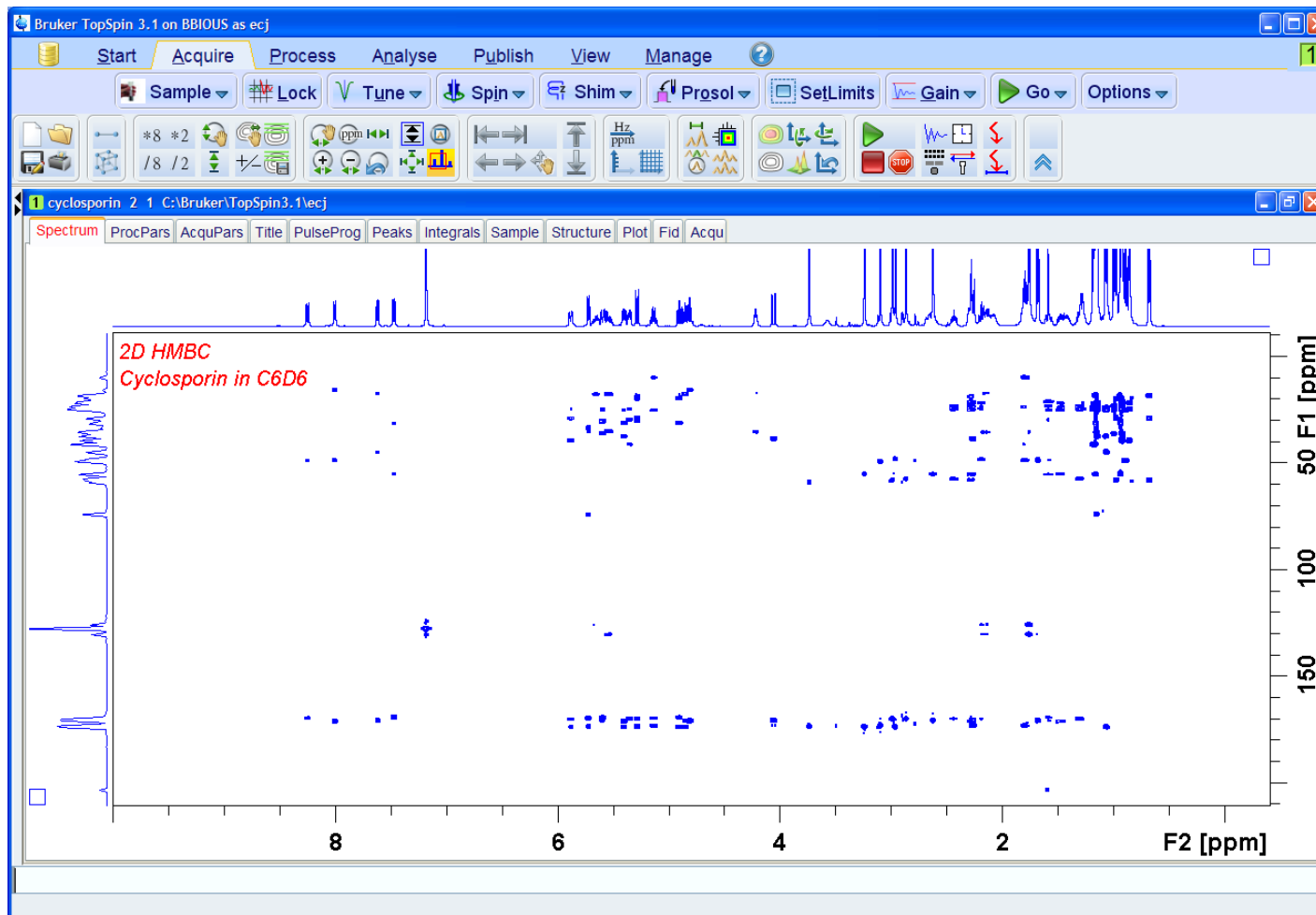
Class = Any Dim = Any Show Recommended

Type = Any SubType = Any SubTypeB = Any Reset Filters

SHMBCCTETGPL2ND

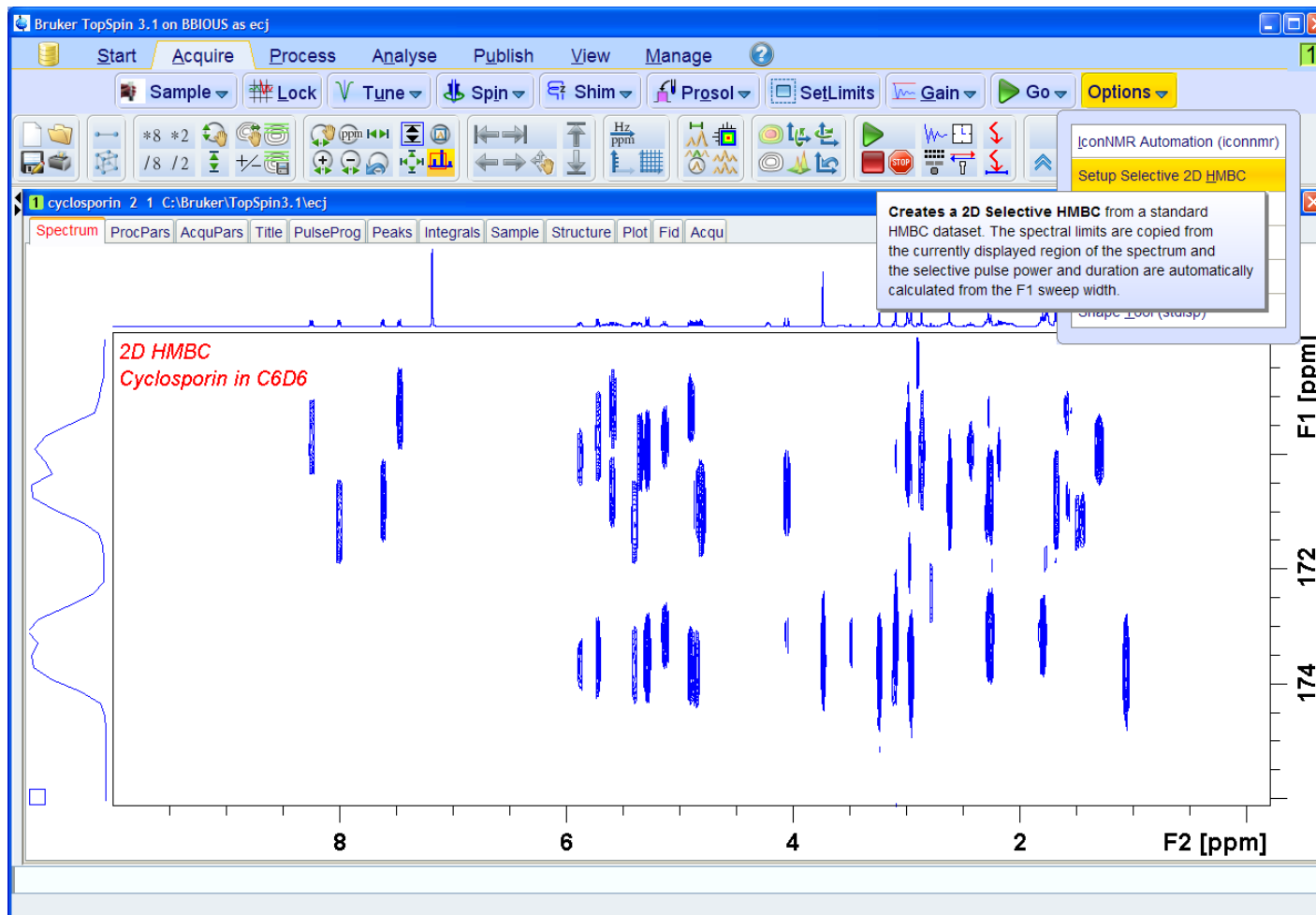
Read... Close

2D selHMBC



Method 1: Start by acquiring a standard 2D HMBC

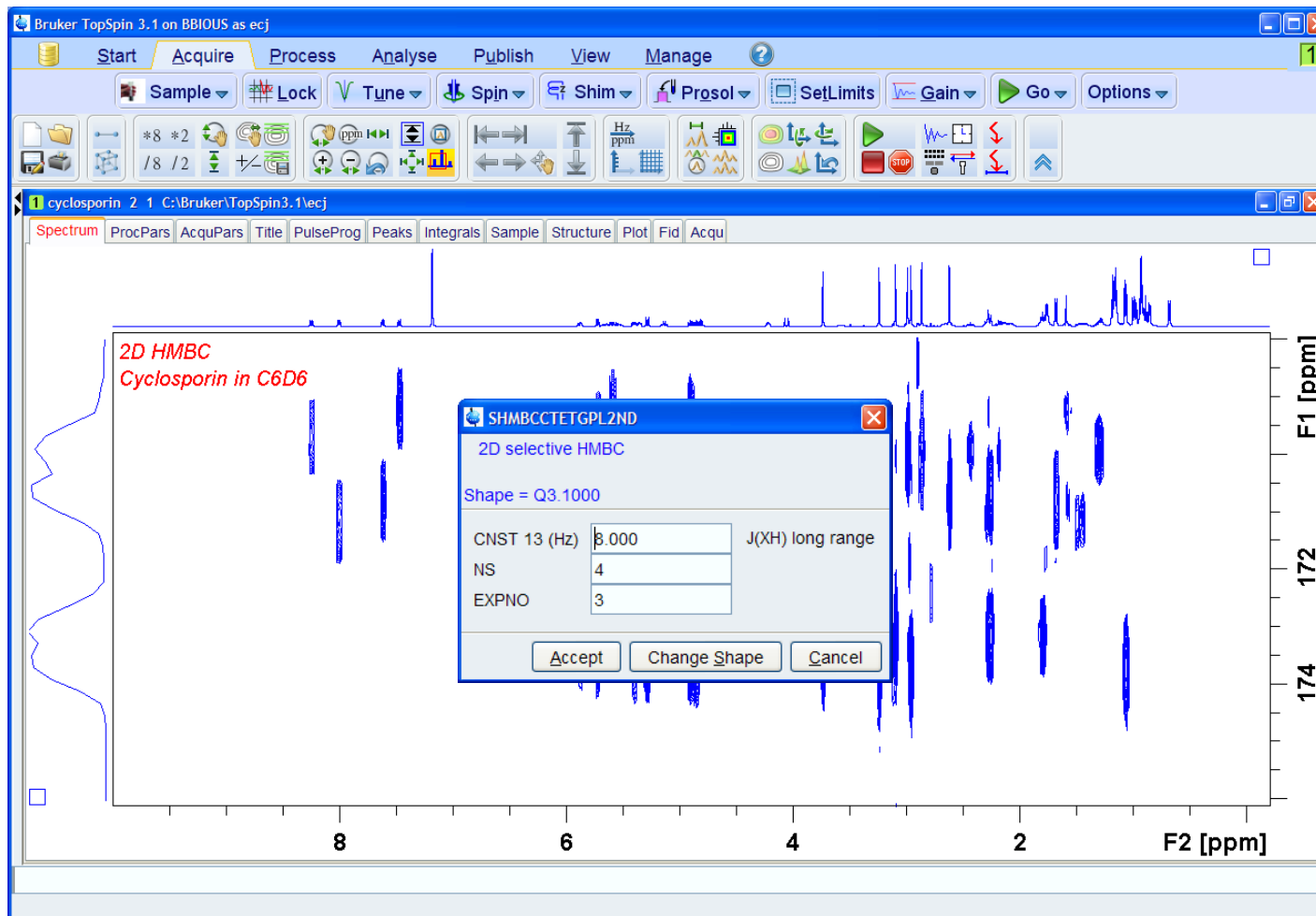
2D selHMBC



Step 2: Zoom into region of interest

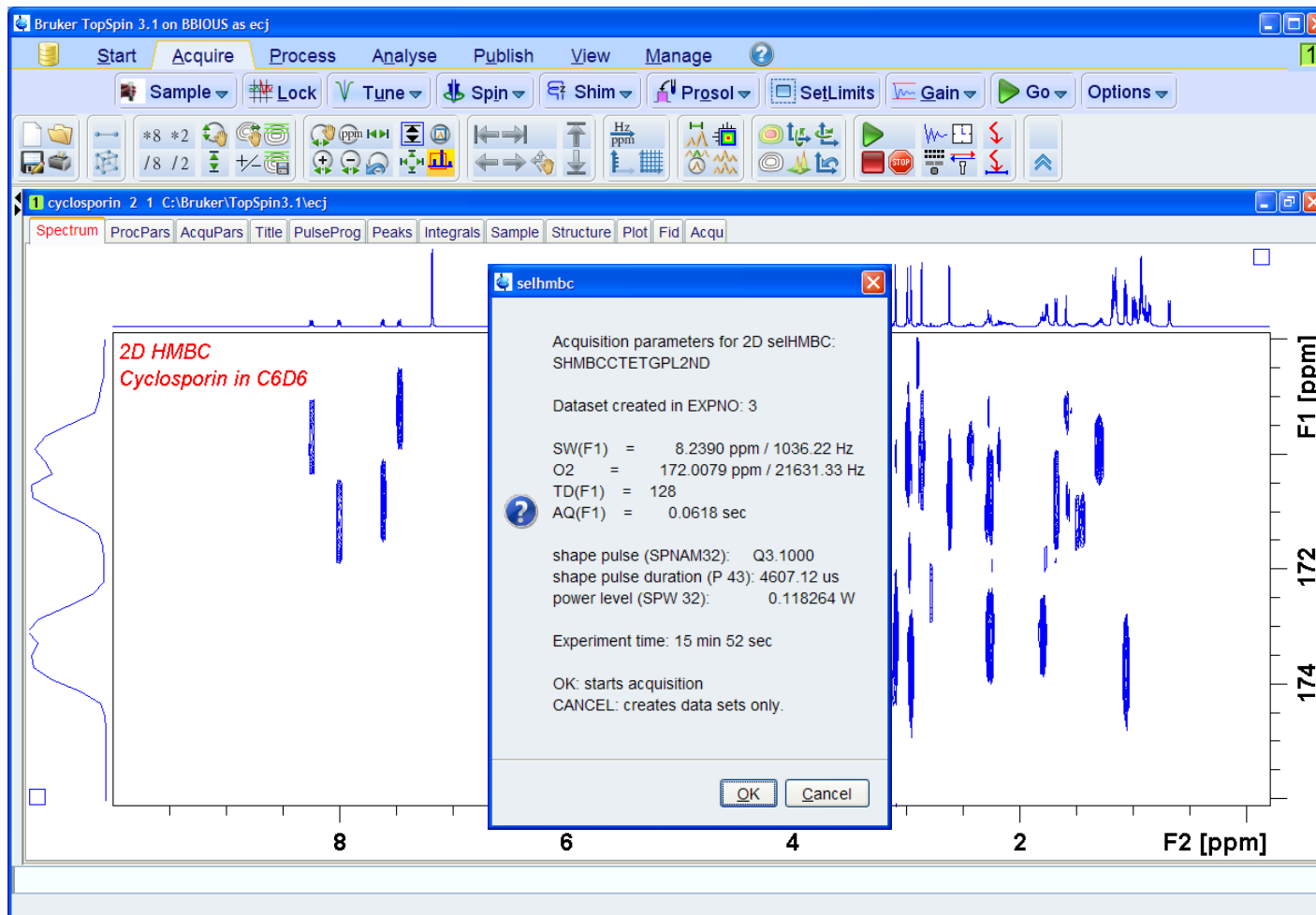
Step 3: Select **Setup Selective 2D HMBC** from **Acquire** → **Options**

2D selHMBC



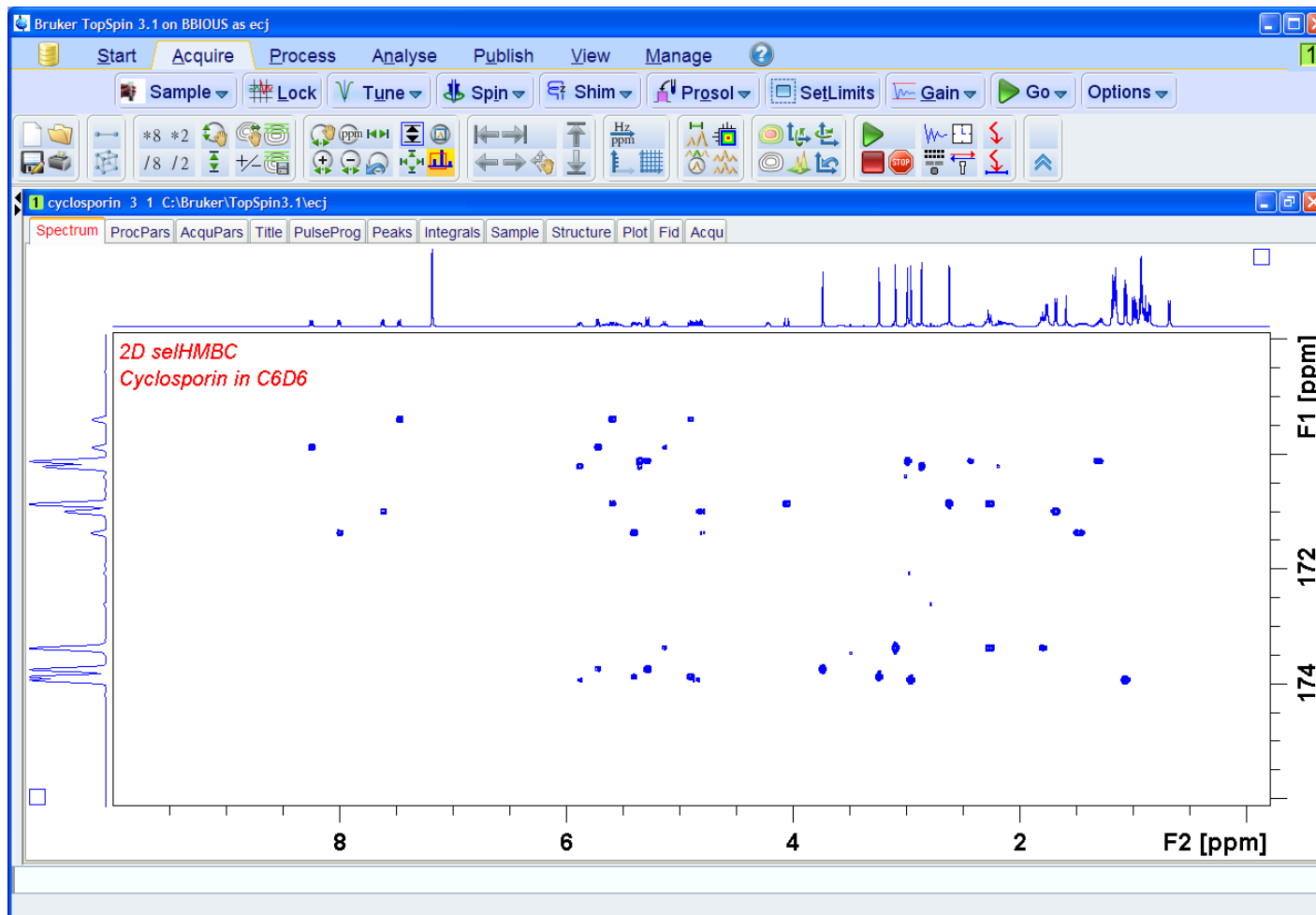
Default parameters are taken from standard parameter set (SHMBCCTETGPL2ND)

2D selHMBC



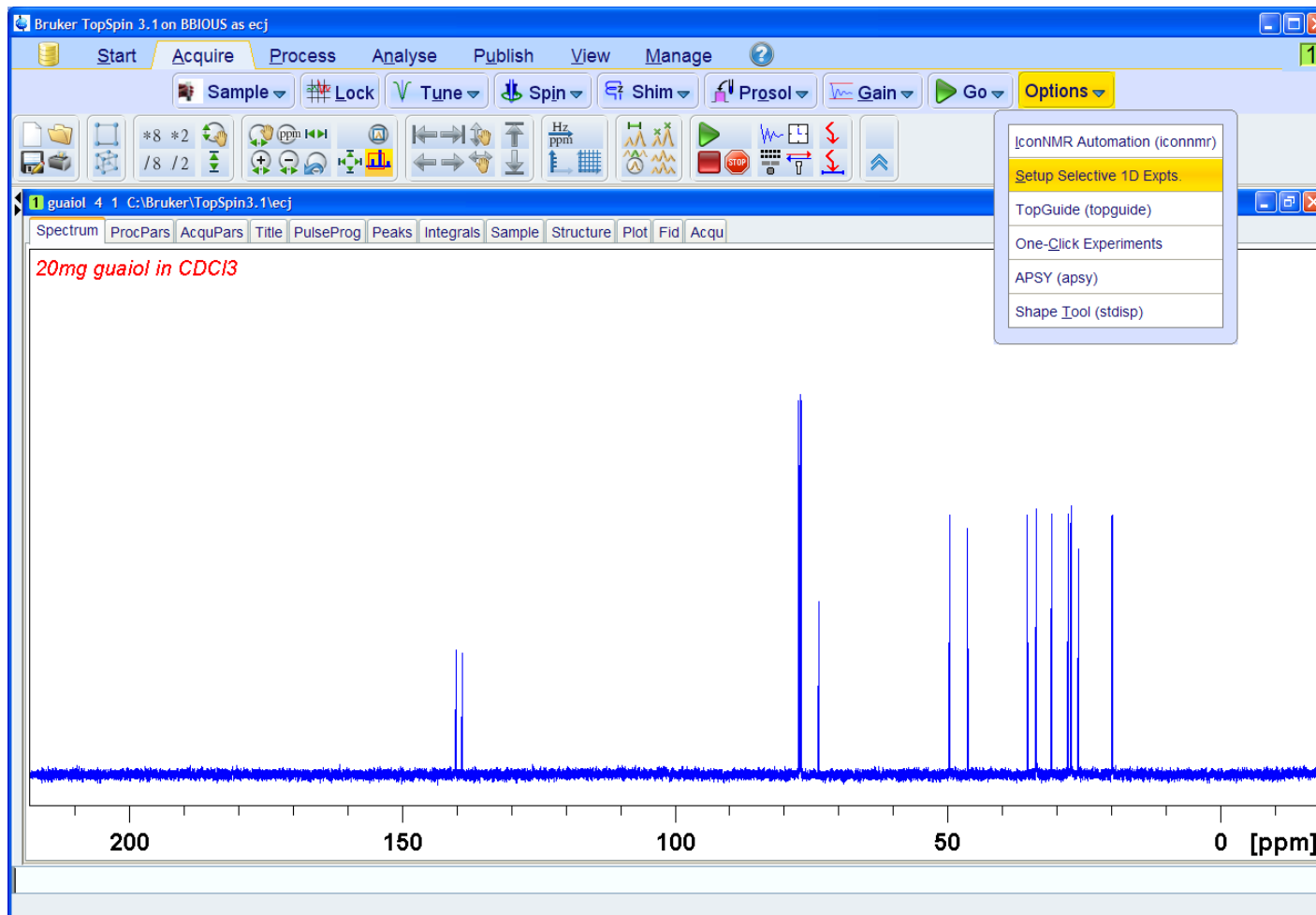
All parameters are automatically calculated and stored in new dataset

2D selHMBC



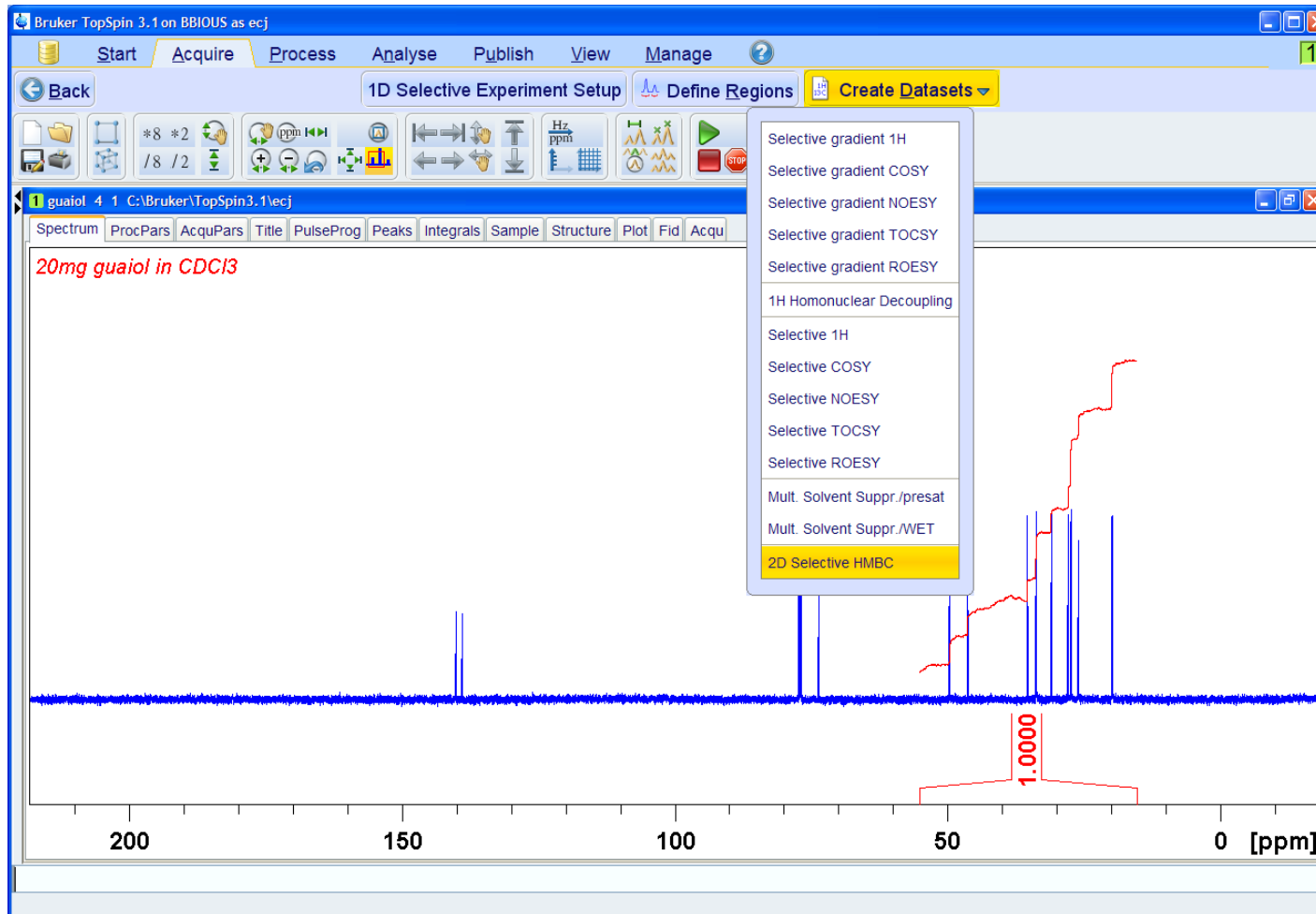
Significantly higher ^{13}C resolution compared to standard HMBC

2D selHMBC



Alternate method: start from a 1D ^{13}C spectrum

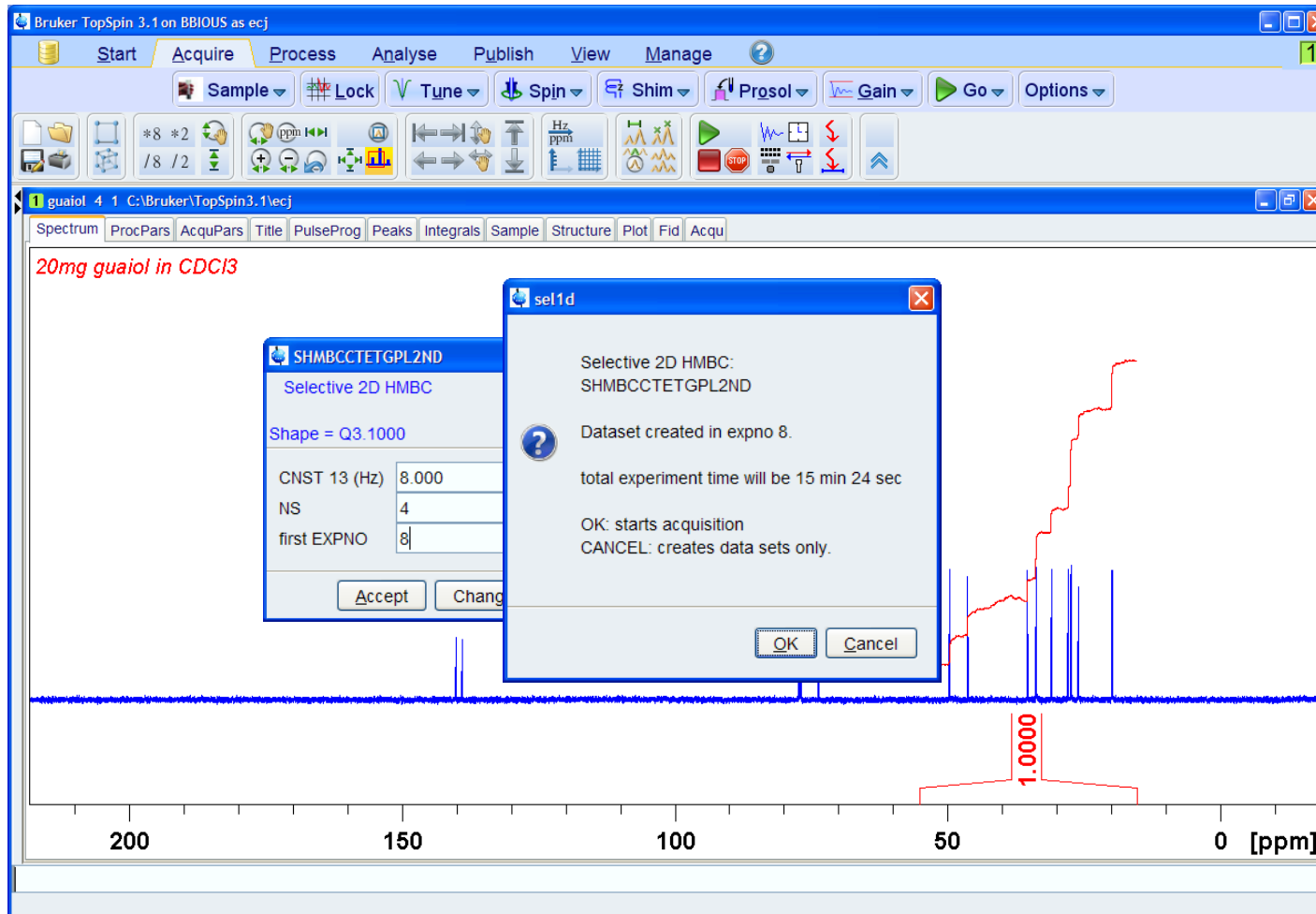
2D selHMBC



Follow same flow as 1D selective experiments:

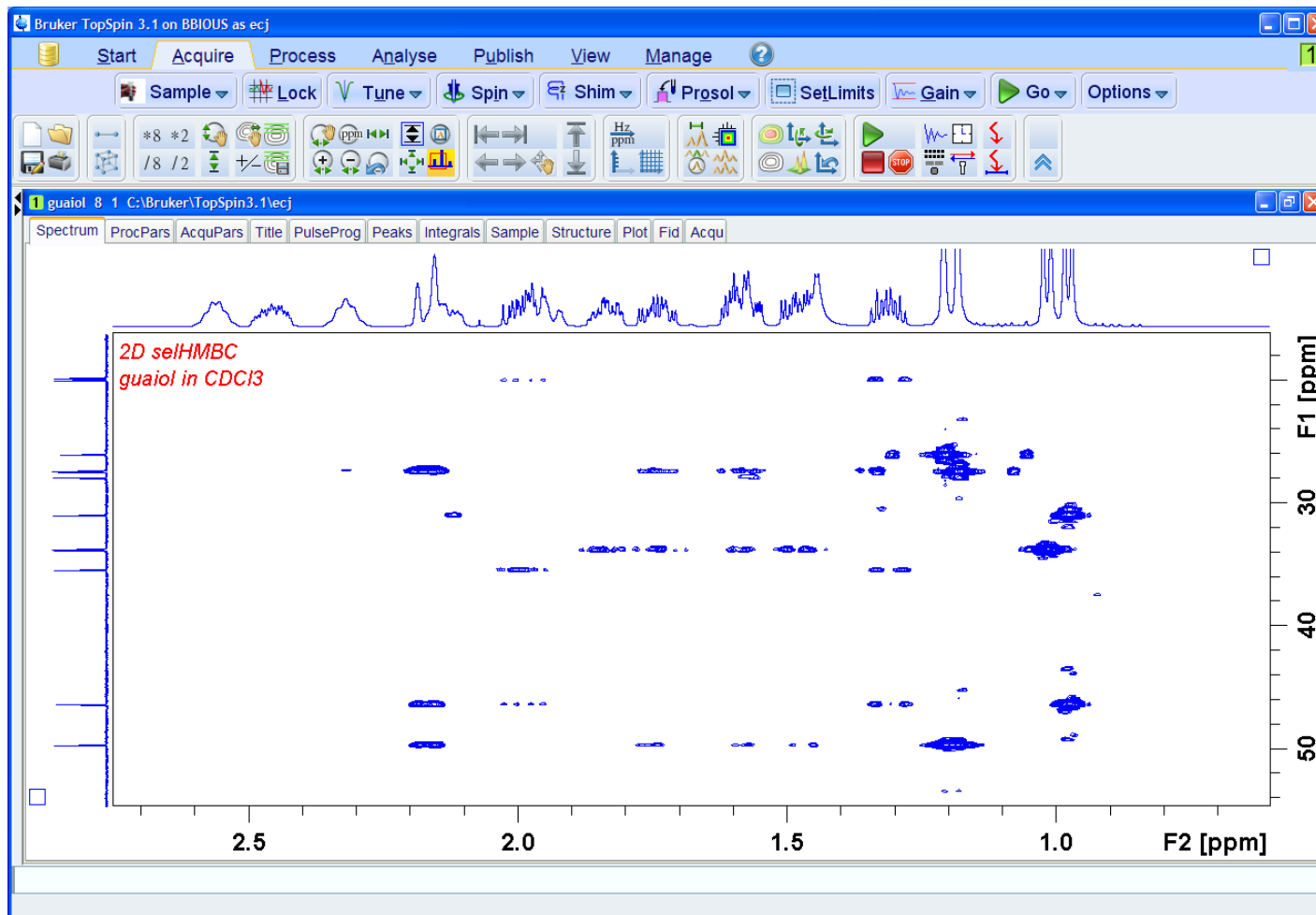
- Save integral to region file...

2D selHMBC

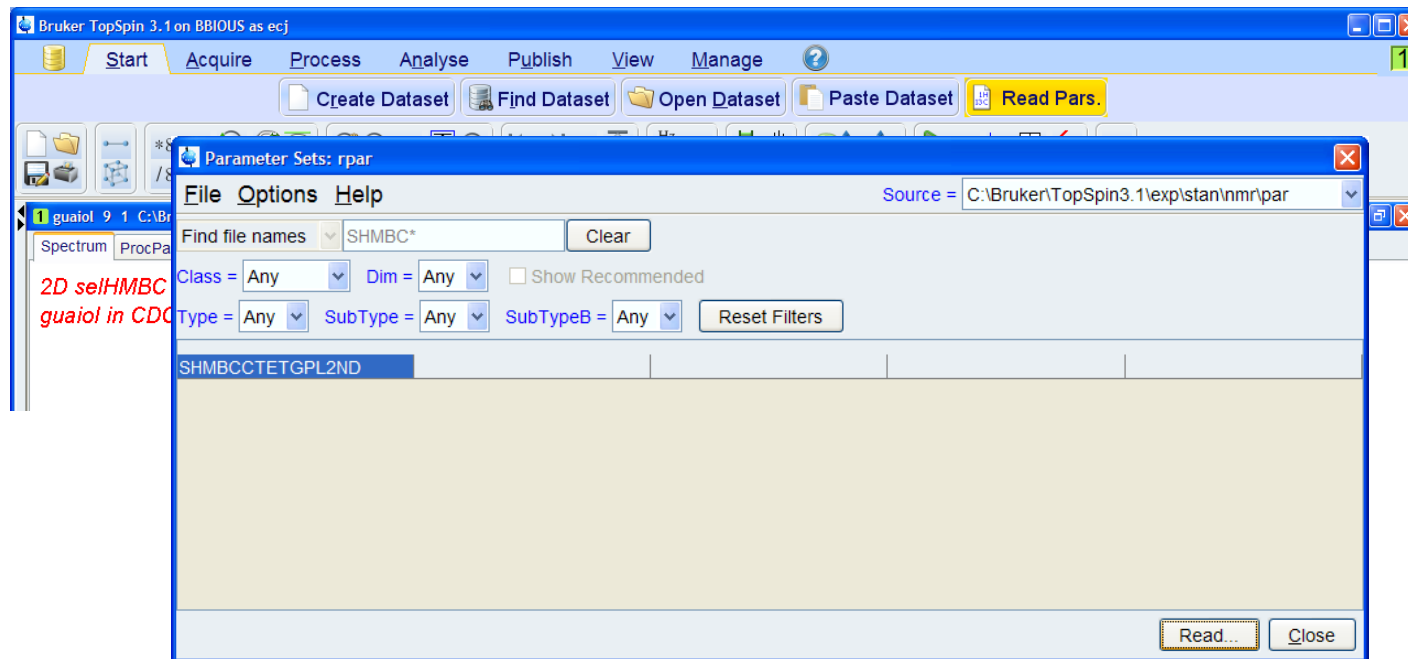


Datasets are automatically created

2D selHMBC



2D selHMBC



Next alternate method: start from standard parameter set: SHMBCCTETGPL2ND

- Default SW(F1) is 10.6 ppm



2D selHMBC

edprosol

File Edit View Help

Saved Observe and Saved Decouple Prosol Parameter Set for:

Probe: 5 mm PATXI 1H/D/19F-13C/15N Z-GRD Z856901/0006 [36] Solvent: generic

Observe: 1H, generic, generic
Decouple: 13C, generic, generic

Observe Comment: Default 1H obs 500 Decouple Comment: Default 13C dec

90 deg. Pulses | Square Pulses | Shape Pulses | Others

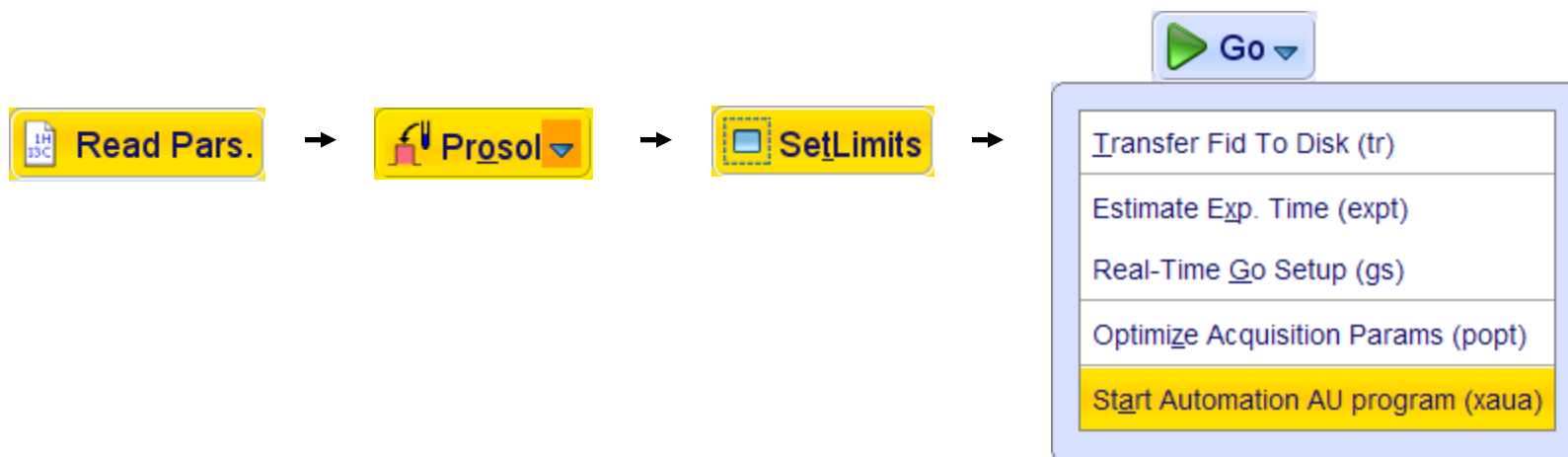
Observe								Decouple							
Filename	α [°]	RFF[Hz]	Ali	PuW[μ s]	A[-dBW]	#		Filename	α [°]	RFF[Hz]	Ali	PuW[μ s]	A[-dBW]		
selective excitation	Gaus1_270.1000	270.00	22.78	1.0	80000.00	44.81	0	selective excitation	Q5.1000	90.00	114.66	1.0	40000.00	23.25	
select. inversion/refocussing	Gaus1_180r.1000	180.00	15.19	0.5	80000.00	48.33	1	select. inversion/refocussing	Q3.1000	180.00	82.52	0.5	40000.00	26.11	
bandsel. excitation	Q5.1000	90.00	458.63	1.0	10000.00	18.73	2	bandsel. excitation	Q5.1000	90.00	1273.98	1.0	3600.00	2.33	
bandsel. inv./refoc.	Q3.1000	180.00	330.08	0.5	10000.00	21.59	3	bandsel. inv./refoc.	Q3.1000	180.00	916.88	0.5	3600.00	5.19	
off-resonance presat. (powe	Squa100.1000	90.00	2.50	0.5	100000.00	64.00	4	adiabatic inversion	Crp60,0.5,20.1	180.00	9772.05	0.5	500.00	-15.36	
90° flip back (H2O)	Squa100.1000	90.00	250.00	0.5	1000.00	24.00	5	adiabatic refocussing	Crp60comp.4	180.00	9772.05	0.5	2000.00	-15.36	

Last Save | Print | Copy to Solvent | Copy to Probe | Save

SHMBCCTETGPL2ND uses Q3.1000 pulse in prosol

2D selHMBC

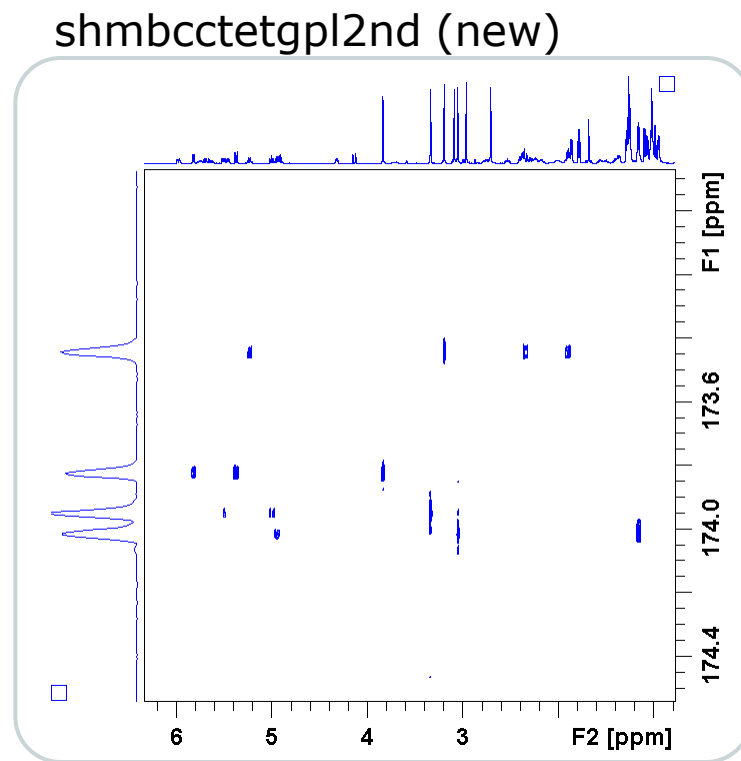
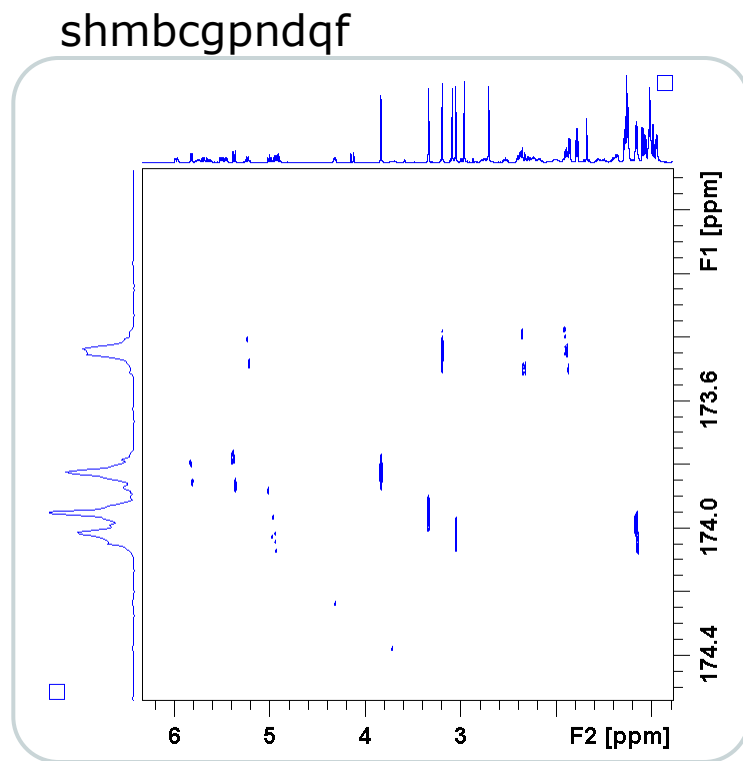
Starting from parameter set SHMBCCTETGPL2ND, but manually setting SW(F1)...



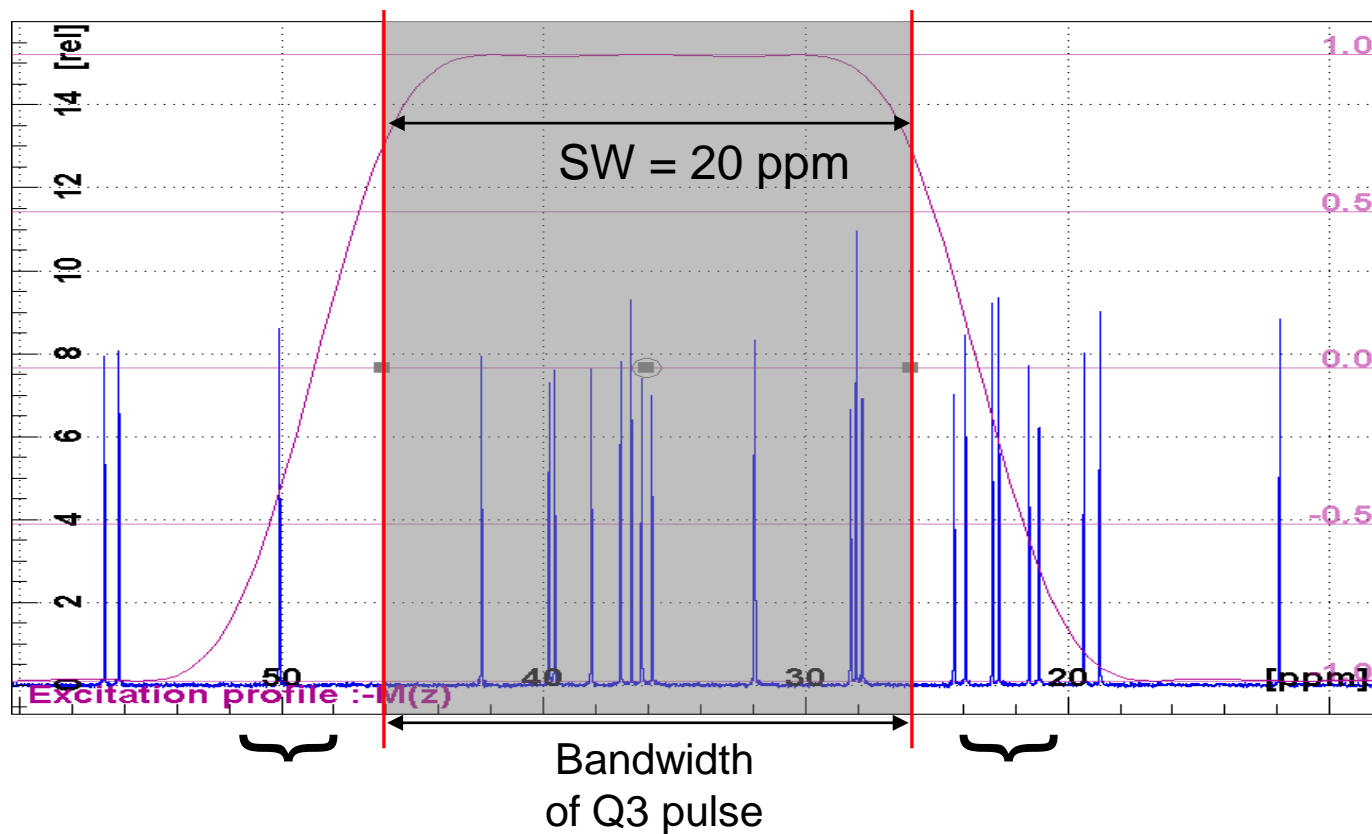
Automation AU program ***au_selhmbc*** will calculate shape pulse parameters based on SW(F1) and starts acquisition

2D selHMBC: pulse sequence

Use constant time version to remove J_{HH} couplings



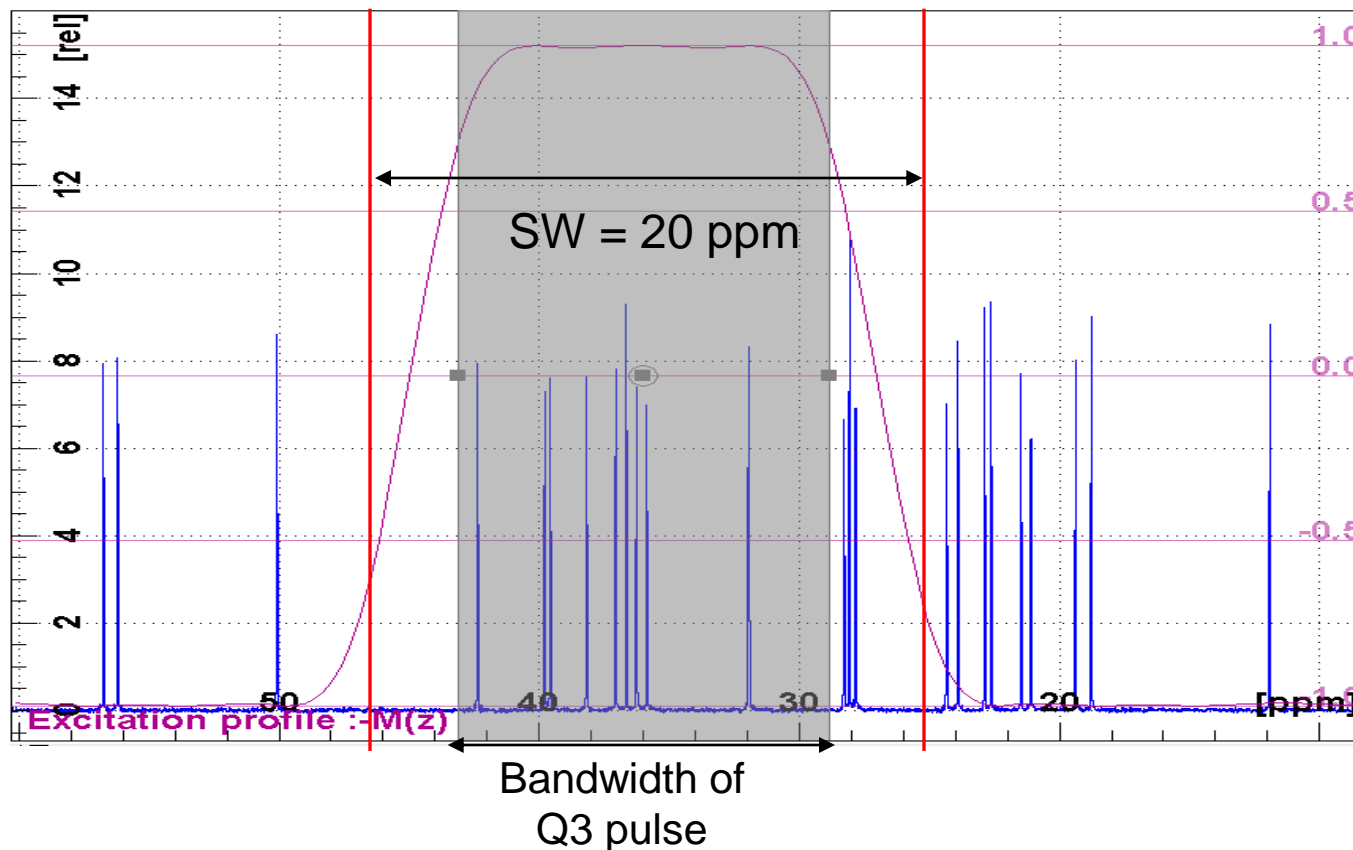
2D selHMBC: excitation profile



If SW matches the bandwidth of excitation of selective pulse:

- Peaks outside SW can alias into spectrum

2D selHMBC: excitation profile

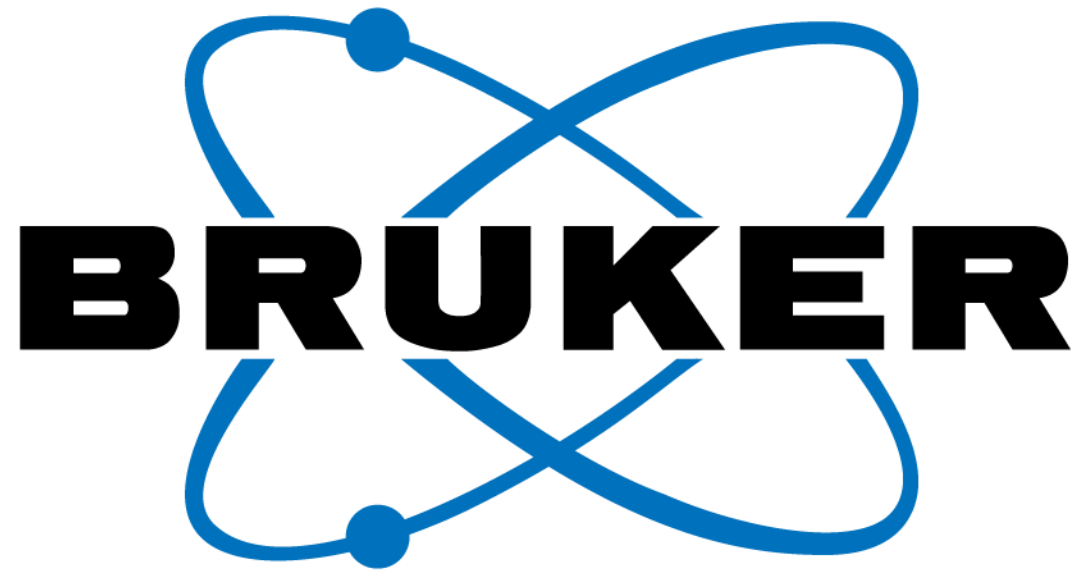


Compromise: Reduce bandwidth of selective pulse by a factor of 1.4

- Less aliasing of peaks outside SW.
- Some attenuation of peaks near edges of spectrum.

Availability

- Everything is included in Topspin 3.1
- Topspin 3.0: I can provide the AU programs and the modifications to the Flowbar



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