KJM 9250

^{1}H-^{15}N NMR spectra on the AVIIIHD-800 spectrometer.

Version 2.0

Topspin 3,5 Windows 7 AVIIIHD800

January 2020

^{15}N-^{1}H f3 (and f2) Experiments on the AVIIIHD-800 Spectrometer

1.1 Introduction

aw coded **^{15}N-^{1}H f3** (and **f2**) **HSQC, HSQC-DIPS**I2 and **HMBC** parameter sets are set up with 2048 (2K) points acquired across a **12 ppm ^{1}H** window centered at **4.7 ppm** and a **30 ppm ^{15}N window (SW)** centered at **118 ppm (O3)**. The ^{1}H and ^{15}N NMR signals of peptides and microcystins typically occur in these windows. Different **SW** and **O3** setttings may (will) be required for other nitrogen containing compounds.

1.2 Processing

HSQC and **HSQC-DIPSI2** experiments are phase sensitive experiments which should be phased **before** using the **abs1** and **abs2** commands.

HMBC experiments are absolute value experiment. Phasing is not required.

2.0 Experiments and Parameter Sets

The following aw coded **^{15}N-^{1}H f3 HSQC, HSQC-DIPS**I2 and **HMBC** parameter sets are available on the AVIIIHD-800 MHz spectrometer.

2.1 **^{1}H-^{15}N f3HSQC** spectrum

2.2 **^{1}H-^{15}N f3HSQCfp** spectrum with a flip back pulse

2.3 **^{1}H-^{15}N f3HSQC135**

2.4 **^{1}H-^{15}N f3HSQCed**

2.5 **^{1}H-15N f3TROSY**

2.6 **^{1}H-^{15}N f3HSQC-DIPSI2** spectrum

2.7 **^{1}H-^{15}N f3HSQC-NOESY**

2.8 **^{1}H-^{15}N f3HMBCET** spectrum

2.9 **^{1}H-^{15}N f3HMBCQF** spectrum

2.10 **^{1}H-^{15}N f2HSQC**

2.11 **^{1}H-^{15}N f2HSQC135**

2.12 **^{1}H-^{15}N BESTf3HSQC**

2.13 **^{1}H-^{15}N BESTf3TROSY**

2.1 ^{1}H-^{15}N HSQC spectrum

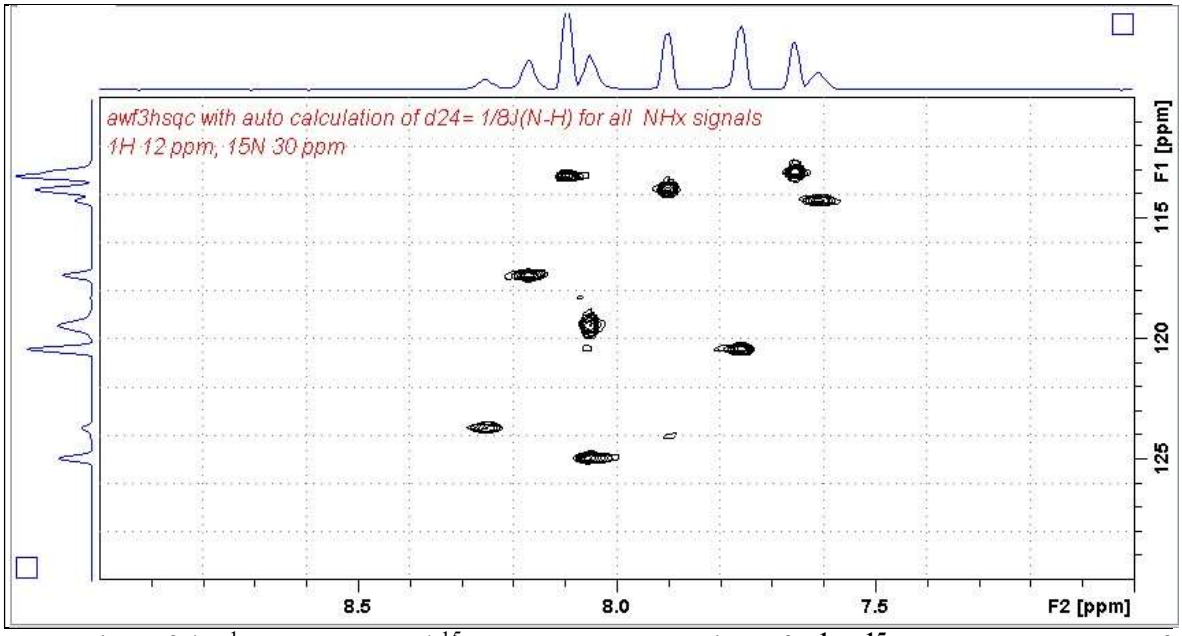
Parameter sets: **awf3hsqc (+ getprosol)**
Pulse programme: **awf3hsqc**

Type **eda** (enter) and review the following default parameters
SW ^{1}H = 12 ppm**, SW ^{15}N = 30** ppm (or other suitable values).
TD ^{1}H = 2K, **TD ^{15}N** = 128-160 (your choice).
NS = multiple of 8 or 16, **DS** = 16.

Type **ased** (enter) and review other parameters used in the job.
O1P = ^{1}H spectral window midpoint = 4.7 ppm other value of your choice.
O3P = ^{15}N spectral window midpoint = 118 ppm other value of your choice.
D1 = repetition delay = **1.0 sec** or other time of your choice.
CNST4 = ^{1}J ^{15}N-^{1}H coupling constant = **90 Hz** or other value of your choice.
D24 = **D26** = **1/4J** are auto calculated from **CNST4**
ZGOPTNS = Not used.
Check gradient settings.

Set **receiver gain** using **RGA** *(Important!).*

Process with: **SI(F2) = 2K SI(F1) = 512 or 1K**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb, abs1 and abs2



Expansion of the ^{1}H 7-9 ppm and ^{15}N 100-126 ppm region of a **^{1}H-^{15}N HSQC** spectrum of a peptide which that has 9 amino axcid units

2.2 ^{1}H-^{15}N HSQC spectrum with a flipback pulswe

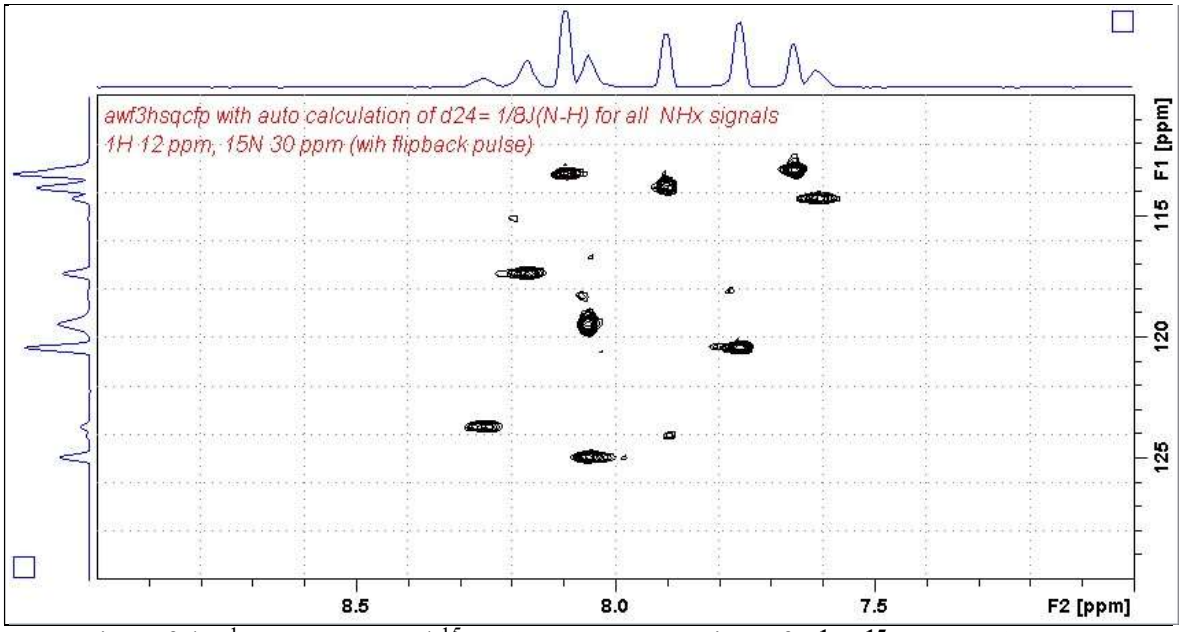
Parameter sets: **awf3hsqcfp (+ getprosol)**
Pulse programme: **awf3hsqcfp**

Type **eda** (enter) and review the following default parameters
SW ^{1}H = 12 ppm**, SW ^{15}N = 30** ppm (or other suitable values).
TD ^{1}H = 2K, **TD ^{15}N** = 128-160 (your choice).
NS = multiple of 8 or 16, **DS** = 16.

Type **ased** (enter) and review other parameters used in the job.
O1P = ^{1}H spectral window midpoint = 4.7 ppm or other value of your choice.
O3P = ^{15}N spectral window midpoint = 118 ppm or other value of your choice.
D1 = repetition delay = **1.0 sec** or other time of your choice.
CNST4 = ^{1}J ^{15}N-^{1}H coupling constant = **90 Hz** or other value of your choice.
D24 = **1/8*J*** for all NH_x signals is auto calculated from **CNST4**
ZGOPTNS = Not used.
Check gradient settings.

Set **receiver gain** using **RGA** *(Important!).*

Process with: **SI(F2) = 2K SI(F1) = 512 or 1K**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb, abs1 and abs2



Expansion of the ^{1}H 7-9 ppm and ^{15}N 110-130 ppm region of a **^{1}H-^{15}N HSQCfp** spectrum of a peptide which that has 9 amino acid units.

2.3 ^{1}H-^{15}N HSQC spectrum

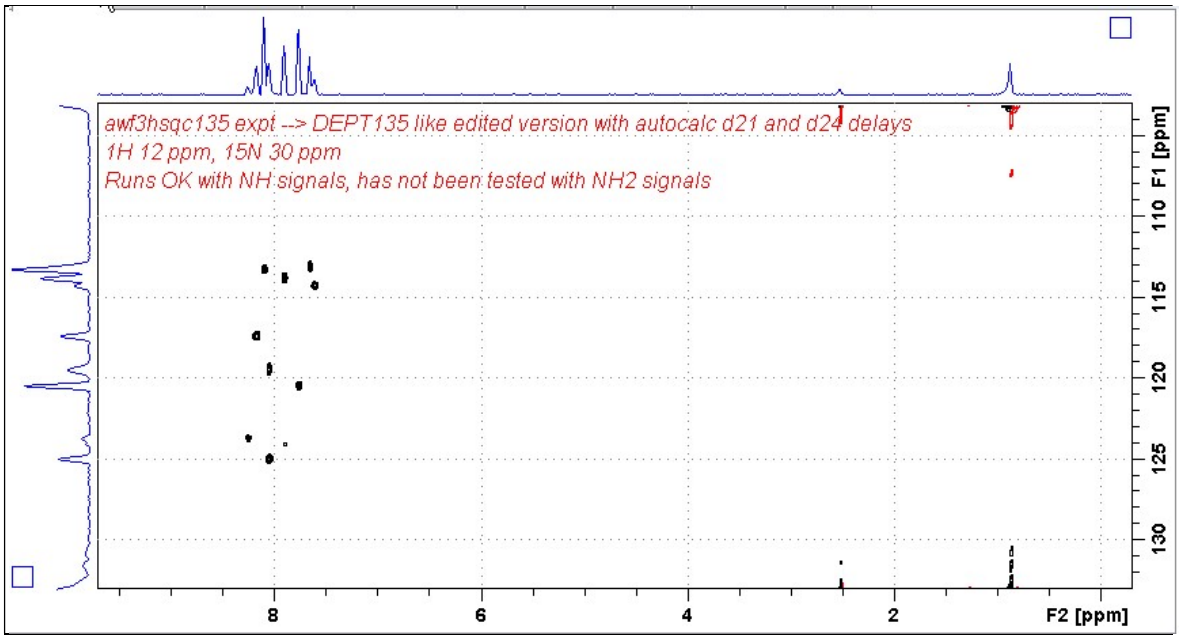
Parameter sets: **awf3hsqc135 (+ getprosol)**
Pulse programme:

Type **eda** (enter) and review the following default parameters
SW ^{1}H = 12 ppm**, SW ^{15}N = 30** ppm (or other suitable values).
TD ^{1}H = 2K, **TD ^{15}N** = 128-160 (your choice).
NS = multiple of 8 or 16, **DS** = 16.

Type **ased** (enter) and review other parameters used in the job.
O1P = ^{1}H spectral window midpoint = 4.7 ppm other value of your choice.
O3P = ^{15}N spectral window midpoint = 118 ppm other value of your choice.
D1 = repetition delay = **1.0 sec** or other time of your choice.
CNST4 = ^{1}J ^{15}N-^{1}H coupling constant = **90 Hz** or other value of your choice.
D24 = **D26** = **1/4J** are auto calculated from **CNST4**
ZGOPTNS = Not used.
Check gradient settings.

Set **receiver gain** using **RGA** *(Important!).*

Process with: **SI(F2) = 2K SI(F1) = 512 or 1K**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb, abs1 and abs2



File 8 Expansion of the ^{1}H 7-9 ppm and ^{15}N 100-126 ppm region of a **^{1}H-^{15}N HSQC** spectrum of a peptide which that has 9 amino axcid units

2.4 ^{1}H-^{15}N HSQC spectrum

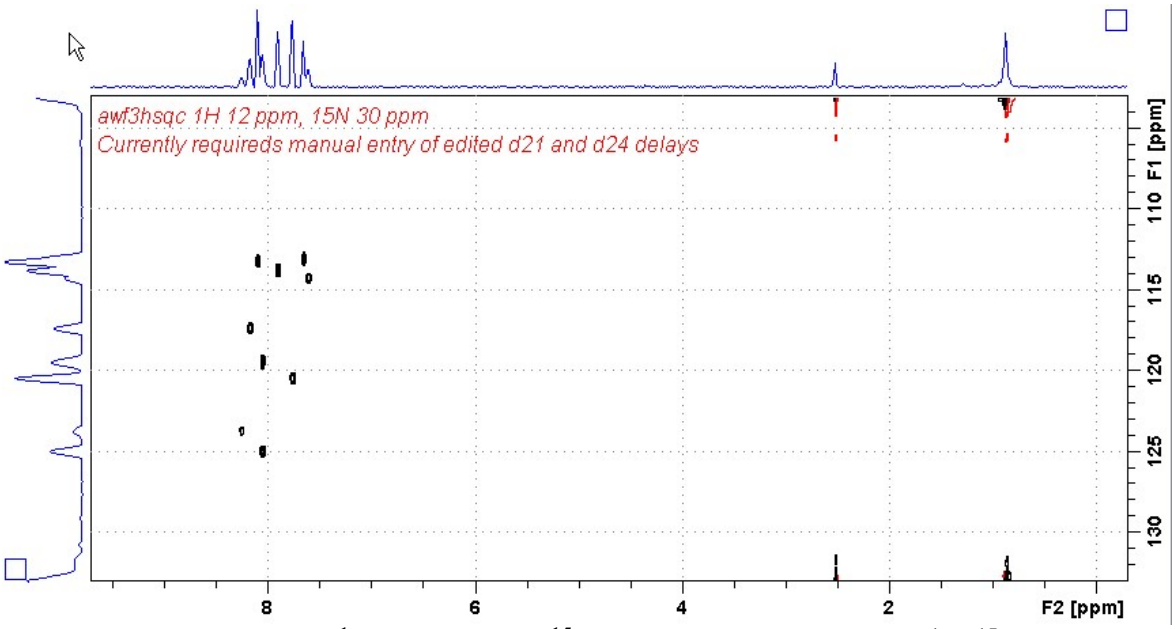
Parameter sets: **awf3hsqc (+ getprosol)**
Pulse programme: **awf3hsqc**

Type **eda** (enter) and review the following default parameters
SW ^{1}H = 12 ppm**, SW ^{15}N = 30** ppm (or other suitable values).
TD ^{1}H = 2K, **TD ^{15}N** = 128-160 (your choice).
NS = multiple of 8 or 16, **DS** = 16.

Type **ased** (enter) and review other parameters used in the job.
O1P = ^{1}H spectral window midpoint = 4.7 ppm other value of your choice.
O3P = ^{15}N spectral window midpoint = 118 ppm other value of your choice.
D1 = repetition delay = **1.0 sec** or other time of your choice.
CNST4 = ^{1}J ^{15}N-^{1}H coupling constant = **90 Hz** or other value of your choice.
D24 = **D26** = **1/4J** are manaully set
ZGOPTNS = Not used.
Check gradient settings.

Set **receiver gain** using **RGA** *(Important!).*

Process with: **SI(F2) = 2K SI(F1) = 512 or 1K**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb, abs1 and abs2



File 9 Expansion of the ^{1}H 7-9 ppm and ^{15}N 100-126 ppm region of a **^{1}H-^{15}N HSQC** spectrum of a peptide which that has 9 amino axcid units

2.5 ^{1}H-^{15}N TROSY spectrum

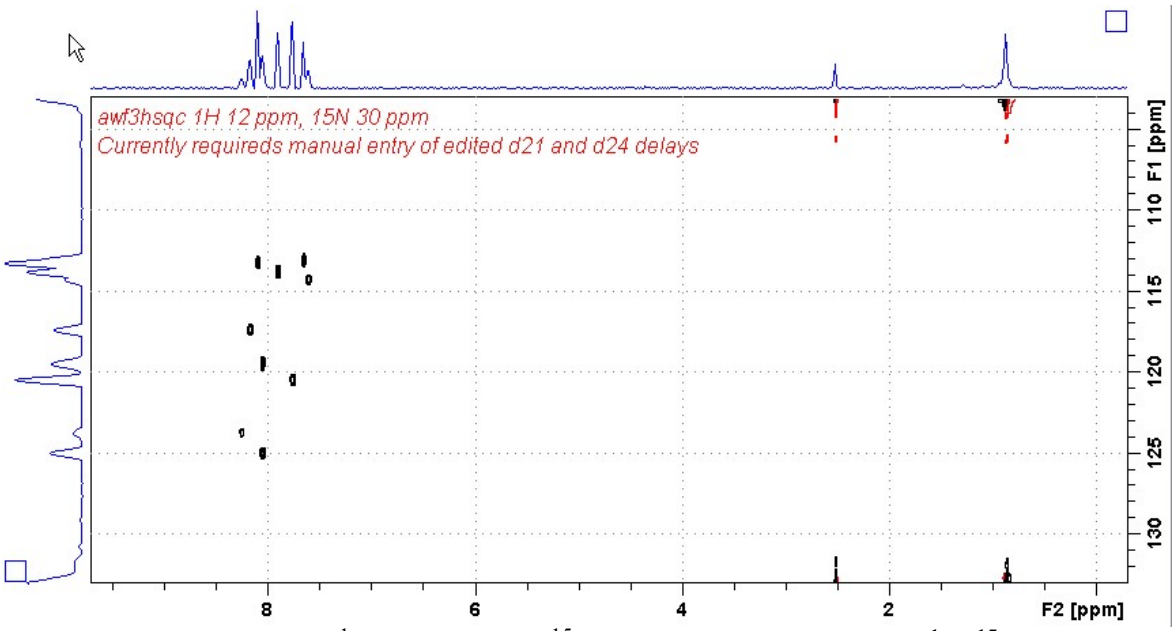
Parameter sets: **trosyeff3gpsi (+ getprosol)**
Pulse programme: **awf3trosy**

Type **eda** (enter) and review the following default parameters
SW ^{1}H = 12 ppm**, SW ^{15}N = 30** ppm (or other suitable values).
TD ^{1}H = 2K, **TD ^{15}N** = 128-160 (your choice).
NS = multiple of 8 or 16, **DS** = 16.

Type **ased** (enter) and review other parameters used in the job.
O1P = ^{1}H spectral window midpoint = 4.7 ppm other value of your choice.
O3P = ^{15}N spectral window midpoint = 118 ppm other value of your choice.
D1 = repetition delay = **1.0 sec** or other time of your choice.
CNST4 = ^{1}J ^{15}N-^{1}H coupling constant = **90 Hz** or other value of your choice.
ZGOPTNS = Not used.
Check gradient settings.

Set **receiver gain** using **RGA** *(Important!).*

Process with: **SI(F2) = 2K SI(F1) = 512 or 1K**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb, abs1 and abs2



File 5 Expansion of the ^{1}H 7-9 ppm and ^{15}N 100-126 ppm region of a **^{1}H-^{15}N HSQC** spectrum of a peptide which that has 9 amino acid units

2.6 ^{1}H-^{15}N HSQC-DIPSI spectrum

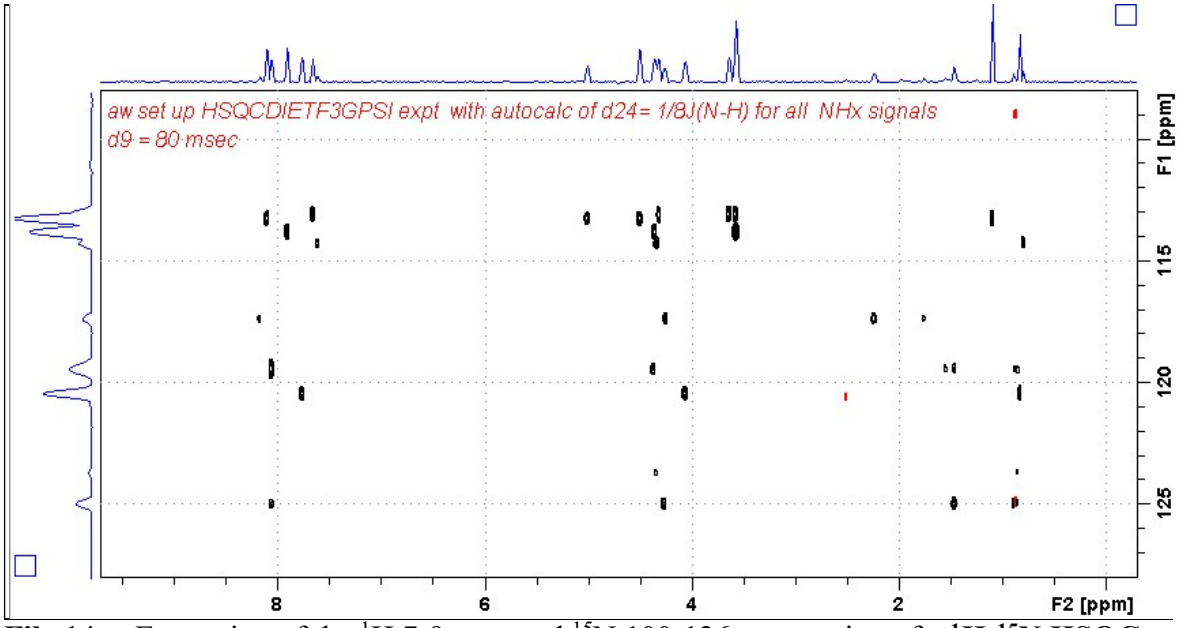
Parameter sets: **awf3hsqdietf3gpsic (+ getprosol)**
Pulse programme: **awf3hsqc-dipsi**

Type **eda** (enter) and review the following default parameters
SW ^{1}H = 12 ppm**, SW ^{15}N = 30** ppm (or other suitable values).
TD ^{1}H = 2K, **TD ^{15}N** = 128-160 (your choice).
NS = multiple of 8 or 16, **DS** = 16.

Type **ased** (enter) and review other parameters used in the job.
O1P = ^{1}H spectral window midpoint = 4.7 ppm other value of your choice.
O3P = ^{15}N spectral window midpoint = 118 ppm other value of your choice.
D1 = repetition delay = **1.0 sec** or other time of your choice.
CNST4 = ^{1}J ^{15}N-^{1}H coupling constant = **90 Hz** or other value of your choice.
D24 = **D26** = **1/4J** are auto calculated from **CNST4**
D9 = 80 msec
ZGOPTNS = Not used.
Check gradient settings.

Set **receiver gain** using **RGA** *(Important!).*

Process with: **SI(F2) = 2K SI(F1) = 512 or 1K**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb, abs1 and abs2



File 14 Expansion of the ^{1}H 7-9 ppm and ^{15}N 100-126 ppm region of a **^{1}H-^{15}N HSQC** - DIPSI2 spectrum of a peptide which that has 9 amino acid units

2.7 ^{1}H-^{15}N HSQC-NOESY spectrum

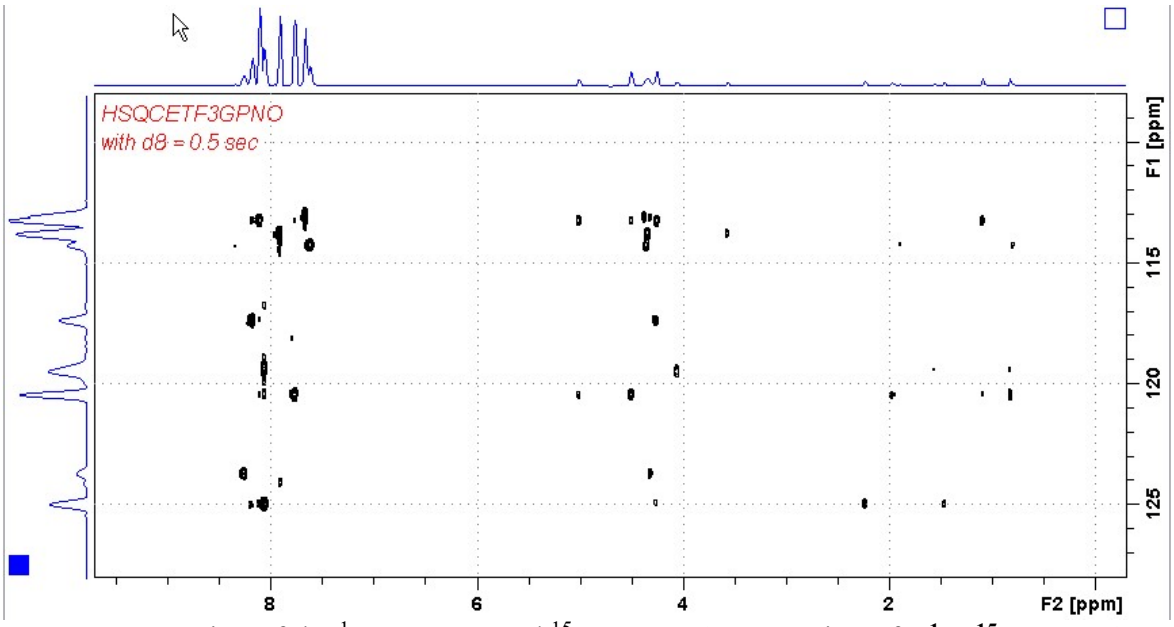
Parameter sets: **awf3hsq-noesy + getprosol)**
Pulse programme: **hsqcetf3gpno**

Type **eda** (enter) and review the following default parameters
SW ^{1}H = 12 ppm**, SW ^{15}N = 30** ppm (or other suitable values).
TD ^{1}H = 2K, **TD ^{15}N** = 128-160 (your choice).
NS = multiple of 8 or 16, **DS** = 16.

Type **ased** (enter) and review other parameters used in the job.
O1P = ^{1}H spectral window midpoint = 4.7 ppm other value of your choice.
O3P = ^{15}N spectral window midpoint = 118 ppm other value of your choice.
D1 = repetition delay = **1.0 sec** or other time of your choice.
CNST4 = ^{1}J ^{15}N-^{1}H coupling constant = **90 Hz** or other value of your choice.
D24 = **D26** = **1/4J** are auto calculated from **CNST4**
D9 = 80 msec
ZGOPTNS = Not used.
Check gradient settings.

Set **receiver gain** using **RGA** *(Important!).*

Process with: **SI(F2) = 2K SI(F1) = 512 or 1K**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb, abs1 and abs2



File 7 Expansion of the ^{1}H 7-9 ppm and ^{15}N 100-126 ppm region of a **^{1}H-^{15}N HSQC - NOESY** spectrum of a peptide which that has 9 amino acid units

2.8 ^{1}H-^{15}N HMBCET spectrum

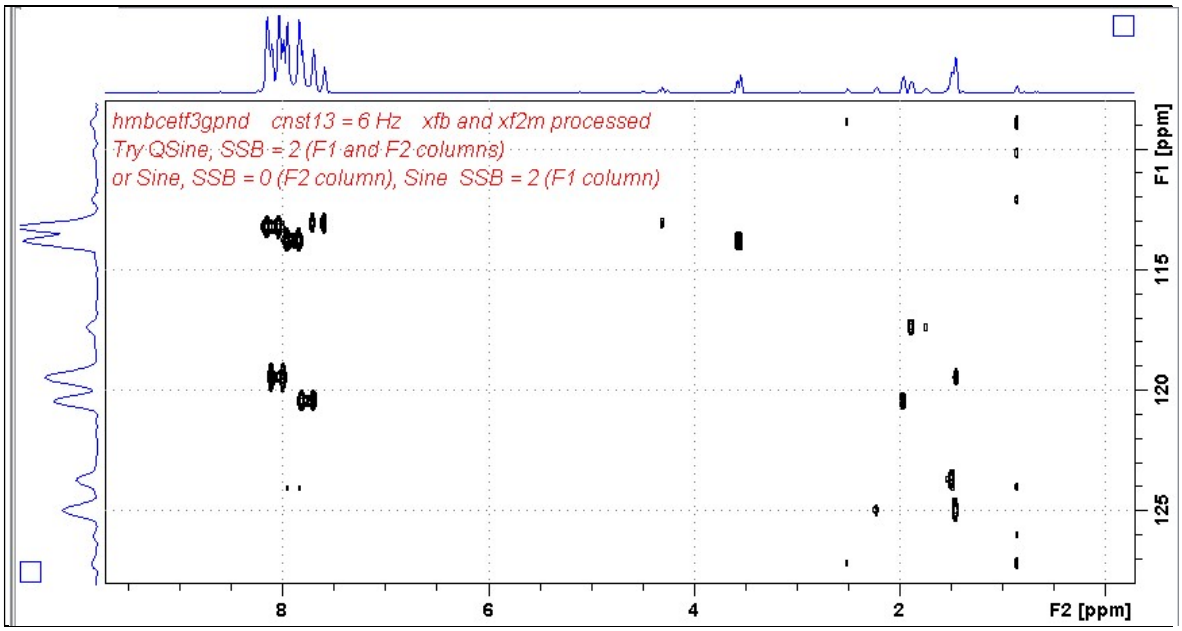
Parameter sets: **awf3hmbcet+ getprosol)**
Pulse programme: **hmbcetf3gpnd**

Type **eda** (enter) and review the following default parameters
SW ^{1}H = 12 ppm**, SW ^{15}N = 30** ppm (or other suitable values).
TD ^{1}H = 2K, **TD ^{15}N** = 128-160 (your choice).
NS = multiple of 8 or 16, **DS** = 16.

Type **ased** (enter) and review other parameters used in the job.
O1P = ^{1}H spectral window midpoint = 4.7 ppm other value of your choice.
O3P = ^{15}N spectral window midpoint = 118 ppm other value of your choice.
D1 = repetition delay = **1.0 sec** or other time of your choice.
CNST4 = ^{1}J ^{15}N-^{1}H coupling constant = **90 Hz** or other value of your choice.
CNST13 = 6 Hz
ZGOPTNS = Not used.
Check gradient settings.

Set **receiver gain** using **RGA** *(Important!).*

Process with: **SI(F2) = 2K SI(F1) = 512 or 1K**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb *and* **xf2m + abs1 and abs2**



File 16 Expansion of the ^{1}H 7-9 ppm and ^{15}N 100-126 ppm region of a **^{1}H-^{15}N HMBCET** spectrum of a peptide which that has 9 amino acid units

2.9 ^{1}H-^{15}N HMBCQF spectrum

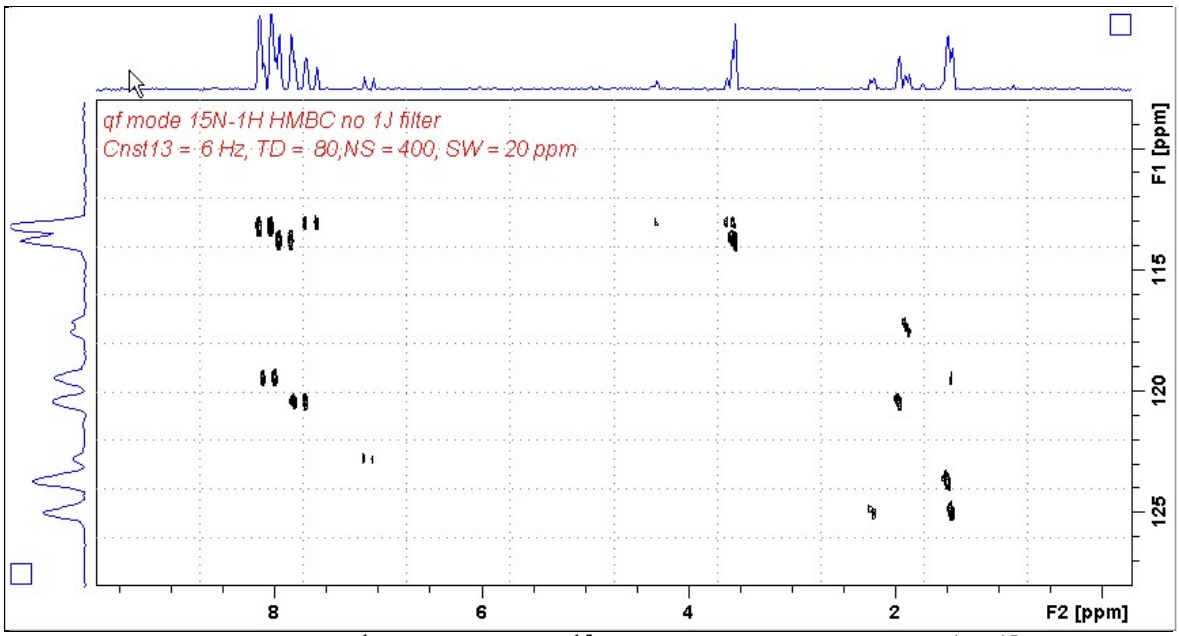
Parameter sets: **awf3hmbcqf + getprosol)**
Pulse programme: **hmbcf3gpndqf**

Type **eda** (enter) and review the following default parameters
SW ^{1}H = 12 ppm**, SW ^{15}N = 30** ppm (or other suitable values).
TD ^{1}H = 2K, **TD ^{15}N** = 128-160 (your choice).
NS = multiple of 8 or 16, **DS** = 16.

Type **ased** (enter) and review other parameters used in the job.
O1P = ^{1}H spectral window midpoint = 4.7 ppm other value of your choice.
O3P = ^{15}N spectral window midpoint = 118 ppm other value of your choice.
D1 = repetition delay = **1.0 sec** or other time of your choice.
CNST4 = ^{1}J ^{15}N-^{1}H coupling constant = **90 Hz** or other value of your choice.
CNST13 = 6 Hz
ZGOPTNS = Not used.
Check gradient settings.

Set **receiver gain** using **RGA** *(Important!).*

Process with: **SI(F2) = 2K SI(F1) = 512 or 1K**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb *and* **xf2m + abs1 and abs2**



File 17 Expansion of the ^{1}H 7-9 ppm and ^{15}N 100-126 ppm region of a **^{1}H-^{15}N HMBCQF** spectrum of a peptide which that has 9 amino acid units

2.10 ^{1}H-^{15}N f2HSQC spectrum

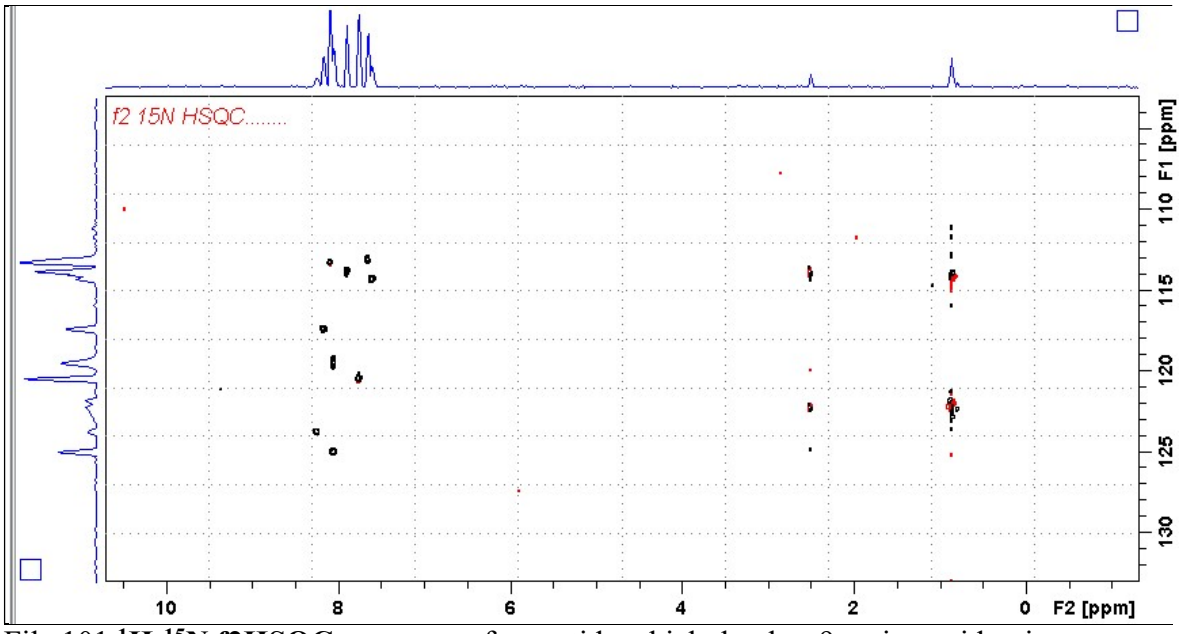
Parameter sets: **awhsqcetgp (+ getprosol)**
Pulse programme: **awf2hsqc**

Type **eda** (enter) and review the following default parameters
SW ^{1}H = 12 ppm**, SW ^{15}N = 30** ppm (or other suitable values).
TD ^{1}H = 2K, **TD ^{15}N** = 128-160 (your choice).
NS = multiple of 8 or 16, **DS** = 16.

Type **ased** (enter) and review other parameters used in the job.
O1P = ^{1}H spectral window midpoint = 4.7 ppm or other value of your choice.
O2P = ^{15}N spectral window midpoint = 118 ppm or other value of your choice.
D1 = repetition delay = **1.0 sec** or other time of your choice.
CNST4 = ^{1}J ^{15}N-^{1}H coupling constant = **90 Hz** or other value of your choice.
ZGOPTNS = Not used.
Check gradient settings.

Set **receiver gain** using **RGA** *(Important!).*

Process with: **SI(F2) = 2K SI(F1) = 512 or 1K**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb, abs1 and abs2



File 101 **^{1}H-^{15}N f2HSQC** spectrum of a peptide which that has 9 amino acid units.

2.11 ^{1}H-^{15}N f2HSQC spectrum

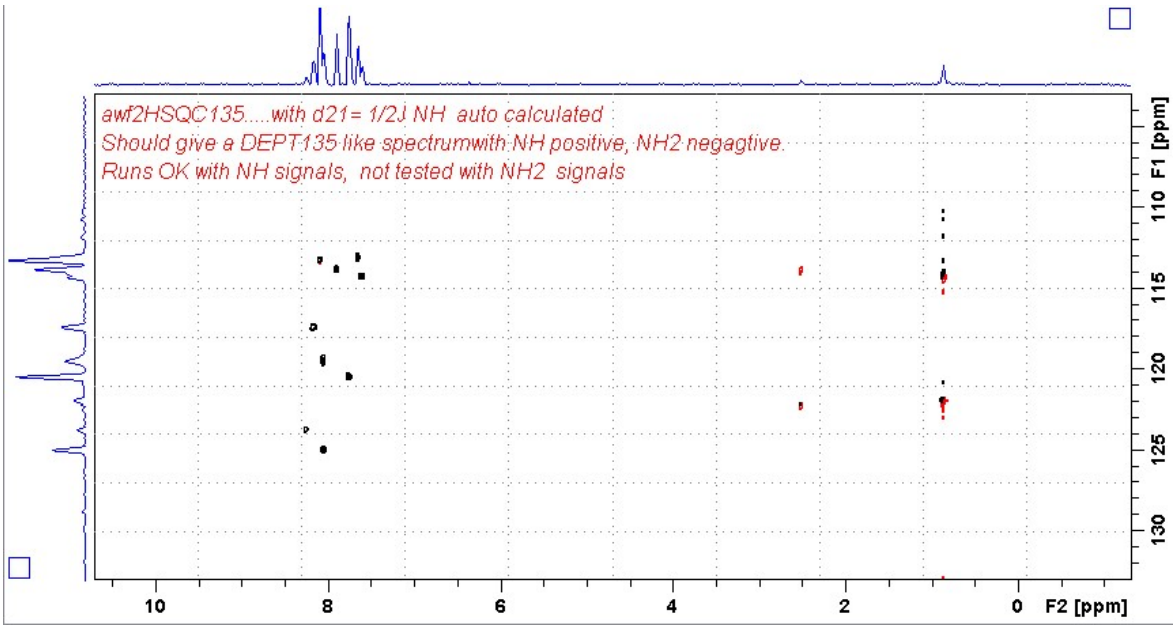
Parameter sets: **awf2hsqc135 (+ getprosol)**
Pulse programme: **awhsqcedetdp-135**

Type **eda** (enter) and review the following default parameters
SW ^{1}H = 12 ppm**, SW ^{15}N = 30** ppm (or other suitable values).
TD ^{1}H = 2K, **TD ^{15}N** = 128-160 (your choice).
NS = multiple of 8 or 16, **DS** = 16.

Type **ased** (enter) and review other parameters used in the job.
O1P = ^{1}H spectral window midpoint = 4.7 ppm other value of your choice.
O2P = ^{15}N spectral window midpoint = 118 ppm other value of your choice.
D1 = repetition delay = **1.0 sec** or other time of your choice.
CNST4 = ^{1}J ^{15}N-^{1}H coupling constant = **90 Hz** or other value of your choice.
D24 = **D26** = **1/4J** are auto calculated from **CNST4**
ZGOPTNS = Not used.
Check gradient settings.

Set **receiver gain** using **RGA** *(Important!).*

Process with: **SI(F2) = 2K SI(F1) = 512 or 1K**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb, abs1 and abs2



File 104 ^{1}H-^{15}N **f2HSQC** spectrum of a peptide which that has 9 amino acid units

2.12 ^{1}H-^{15}N BESTF3HSQC spectrum

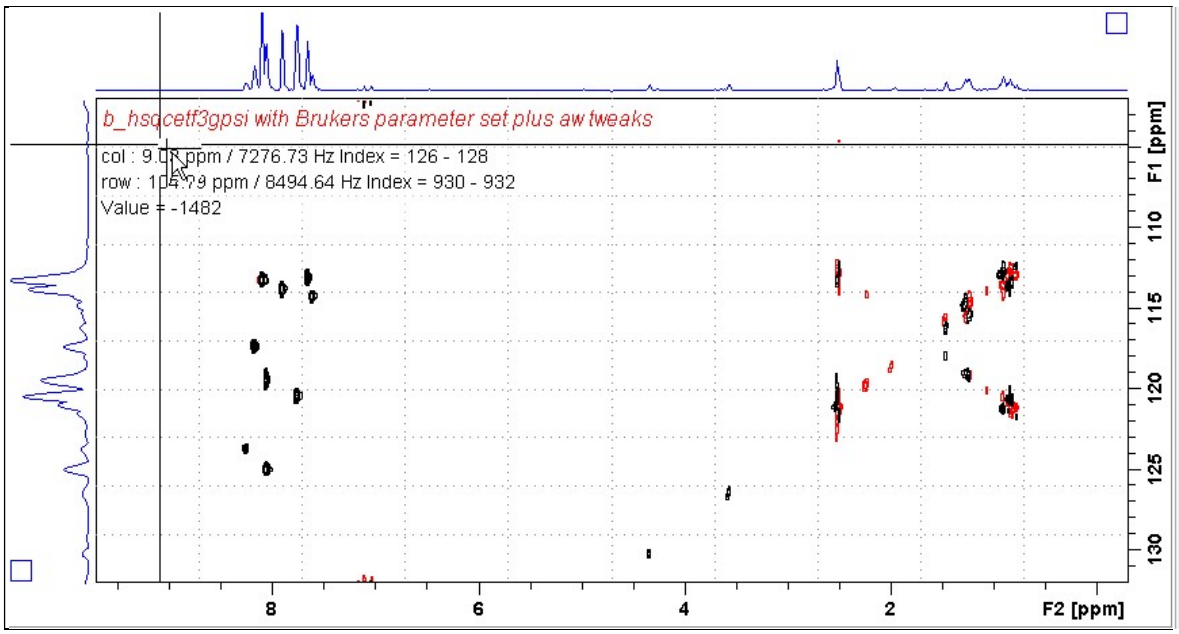
Parameter sets: **awbestf3hsqc (+ getprosol)**
Pulse programme: **b_hsqcetf3gpsi**

Type **eda** (enter) and review the following default parameters
SW ^{1}H = 12 ppm**, SW ^{15}N = 30** ppm (or other suitable values).
TD ^{1}H = 2K, **TD ^{15}N** = 128-160 (your choice).
NS = multiple of 8 or 16, **DS** = 16.

Type **ased** (enter) and review other parameters used in the job.
O1P = ^{1}H spectral window midpoint = 4.7 ppm other value of your choice.
O3P = ^{15}N spectral window midpoint = 118 ppm other value of your choice.
D1 = repetition delay **= 0.xx sec** or other time of your choice.
CNST4 = ^{1}J ^{15}N-^{1}H coupling constant = **90 Hz** or other value of your choice.
ADD best set up info
ZGOPTNS = Not used.
Check gradient settings.

Set **receiver gain** using **RGA** *(Important!).*

Process with: **SI(F2) = 2K SI(F1) = 512 or 1K**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb, abs1 and abs2



File 18 Best f3hsqc expt

2.13 ^{1}H-^{15}N BESTf3TROSY spectrum

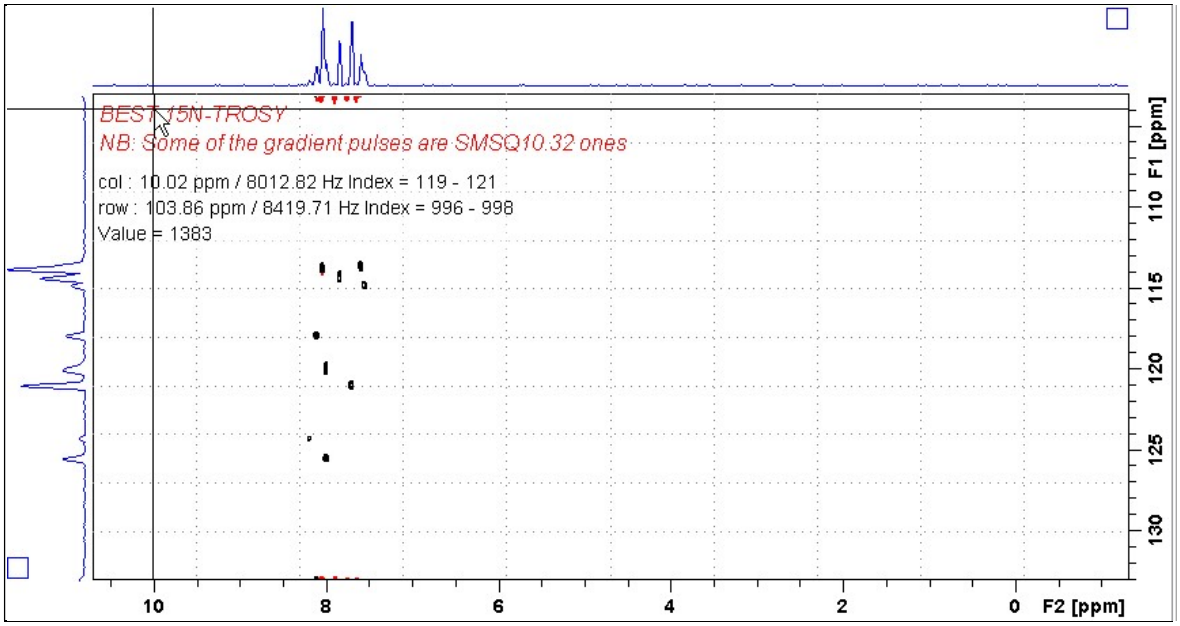
Parameter sets: **awbestf3trosy (+ getprosol)**
Pulse programme: **b_rosyetf3gpsi**

Type **eda** (enter) and review the following default parameters
SW ^{1}H = 12 ppm**, SW ^{15}N = 30** ppm (or other suitable values).
TD ^{1}H = 2K, **TD ^{15}N** = 128-160 (your choice).
NS = multiple of 8 or 16, **DS** = 16.

Type **ased** (enter) and review other parameters used in the job.
O1P = ^{1}H spectral window midpoint = 4.7 ppm other value of your choice.
O3P = ^{15}N spectral window midpoint = 118 ppm other value of your choice.
D1 = repetition delay **= 0.xx sec** or other time of your choice.
CNST4 = ^{1}J ^{15}N-^{1}H coupling constant = **90 Hz** or other value of your choice.
ADD besttrosy set up info
ZGOPTNS = Not used.
Check gradient settings.

Set **receiver gain** using **RGA** *(Important!).*

Process with: **SI(F2) = 2K SI(F1) = 512 or 1K**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb, abs1 and abs2

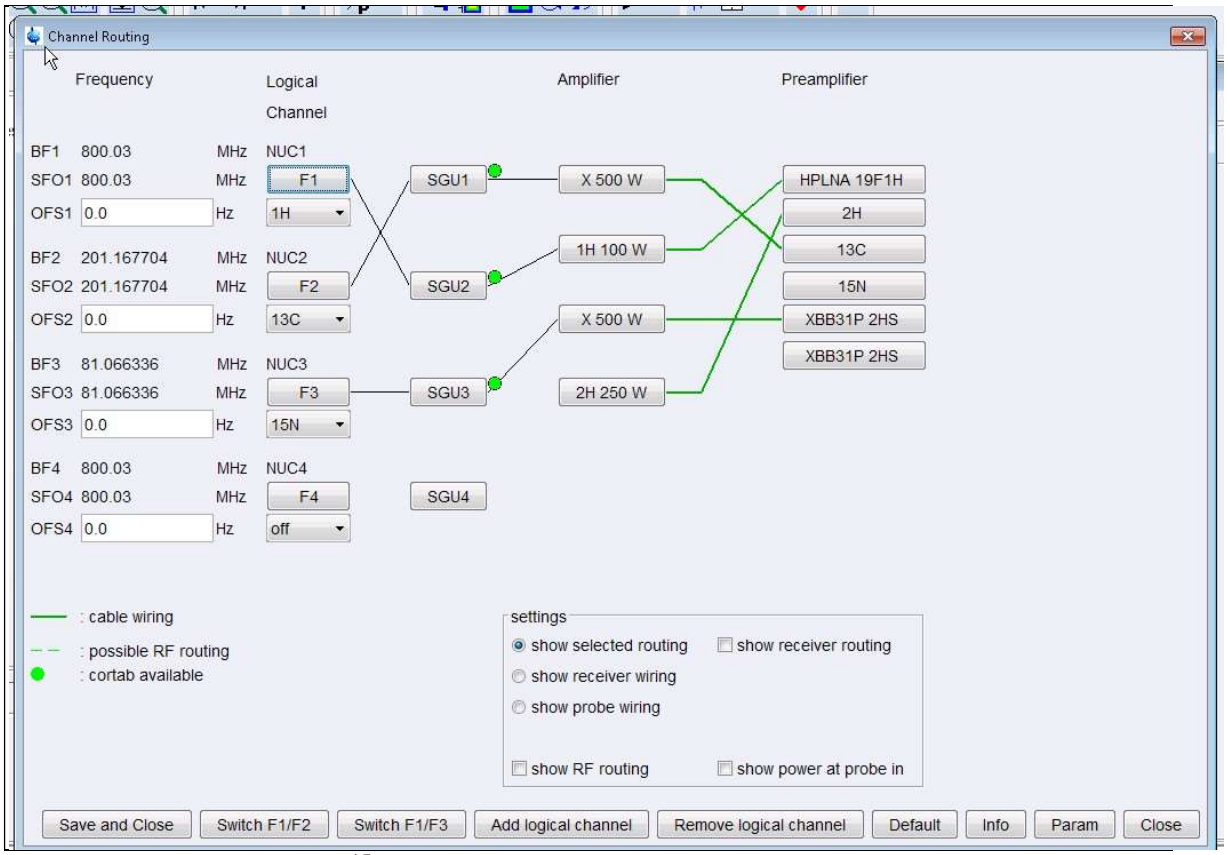


File 6 Best f3trosy expt

Appendix ....or standalone f2/f3 1H-15N set up info file

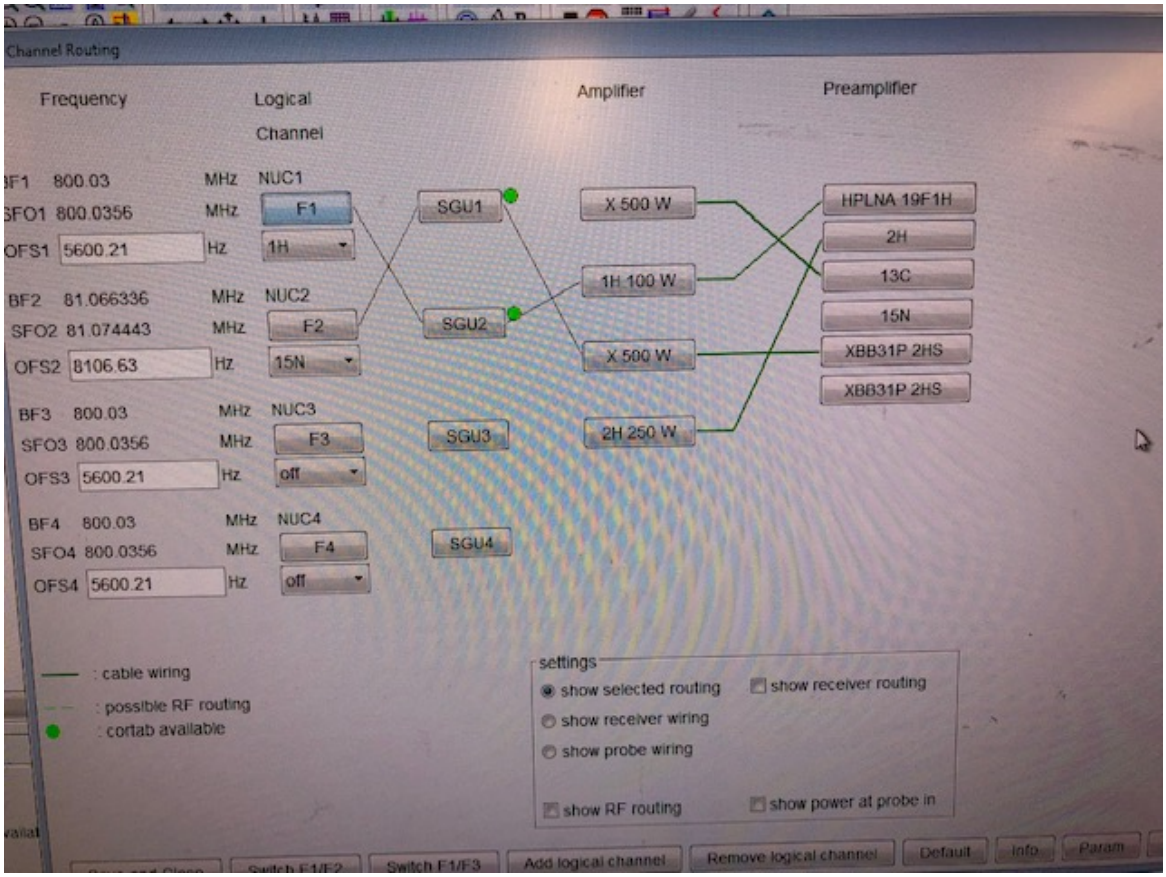
Spectrometer routings

The required spectrometer routing for a **^{15}N f3** channel experiment is shown in Figure 1. The routing can be checked by typing: **edsp (return).** If a red error line appears between the second (lower) **X 500 W Ampifler** and the **^{15}N Preampifer** can be removed by selecting the **Default** and **Save and Close** buttons, after which **O1**, O2 if used and **O3** must be re-entered since they will have been reset to **0 Hz**. The correct routing for an **f2** channel **^{15}N** experiment is shown on the next page.



Channel routings for an **f3 ^{15}N** experiment.

The **f2** channel can optionally be set to **OFF** if **^{13}C** pulses are not used.

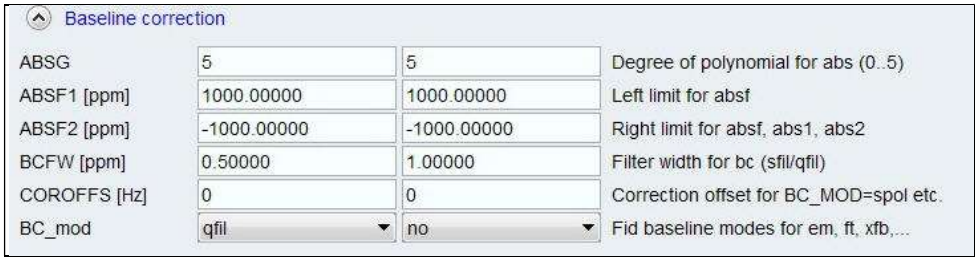


Channel routings for a **f2** ^{15}**N** experiment.

Processing options

A large residual **H_2O/HOD** line when spectra are run in **9:1 H_2O:D_2O** or other solvents can by suppressed by setting up the experimen with its **O1** value in **Hz**, or **O1P ppm** value (typically ~ 4.7 ppm), to that of the **HOD** line and pProcessing the spectrum using the following **ProcPars (edp)** settings:

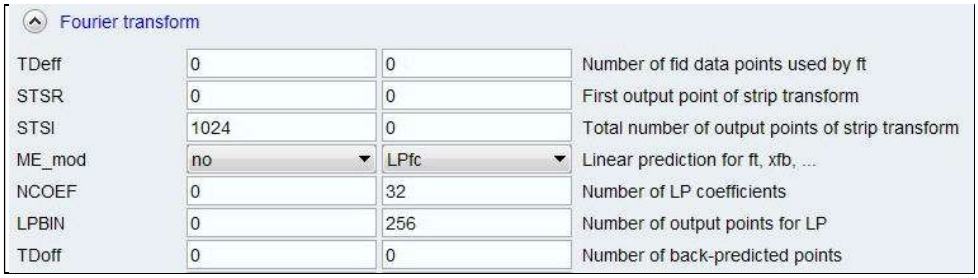
BC_MOD = qfil
BCFW = 0.5 ppm or other suppresion band width value of your choice
STSI = 0 (not used) **or 1024** points when a data data set acquired and processed with **2048** ^{1}H points to *ONLY* display spectral data to the left hand side (higher ppm side) of the noisy residual H_2O/HOD line.



Qfil setttings.

The defaul values are: **BC_MOD = no, BCFW = 0 or 1.00000**

Provided the s/n ratio of Fourier transformed **^{15}N** axis data points is reasonable, **linear prediction** can be applied to improve the apparent resolution of that axis.



STSI and linear prediction setings

The defaul values for these parameters when they are not used are:
ME_MOD = no, NCOEF = 0, LPBIN = 0