

Sparky

**NMR Assignment and Integration
Software**

NMR software

- Paid
 - TopSpin
 - Developed and mainly used for Bruker spectrometers controlling
 - Many functions for processing, view and analysis of one- and multi-dimensional spectra
 - Basically impossible analysis of multi-dimensional
 - Mnova
 - Processing, analysis and visualization of 1D and 2D spectra
 - Support of different formats of spectra (Bruker, Jeol, Agilent...)
 - Free trial version
 - ACD/NMR processor
 - see Mnova
 - Free version for academic purposes with noncomplete functionalities

NMR software

- Freeware
 - NMRPipe
 - Multiplatform
 - Processing and analysis of any spectra
 - Based on UNIX systems → knowledge of UNIX environment is advantage
 - SpinWorks
 - For the Windows systems (emulator of Windows are needed for UNIX a Mac)
 - Processing and analysis of 1D and 2D spectra
 - User very unfriendly
 - **Sparky**

Sparky

NMR Assignment and Integration Software

Originally developed on University of California, San Francisco, currently upgraded on University of Wisconsin–Madison (as **NMRFAM-Sparky**)

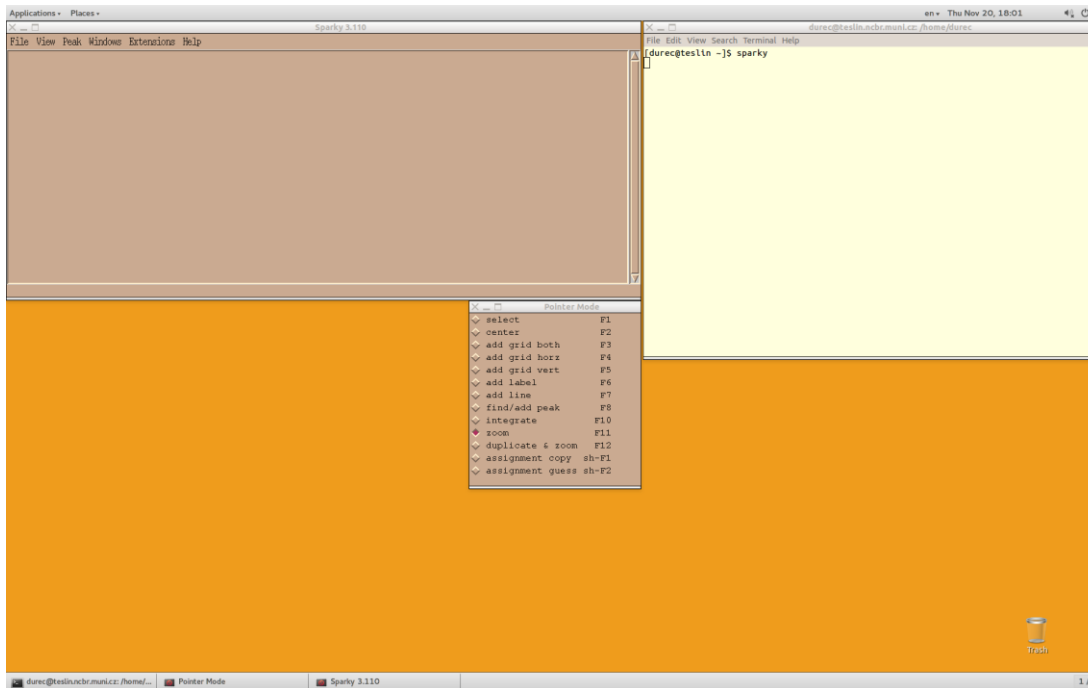
- Multiplatform (Windows and Linux OS)
- Free for download:
<https://nmrfam.wisc.edu/nmrfam-sparky-distribution/>
- Written in Python language, open source, it is not developed anymore (last version is from the 2008)
- Fast and undemanding for hardware
- **It can handle only multi-dimensional spectra (2D, 3D)**

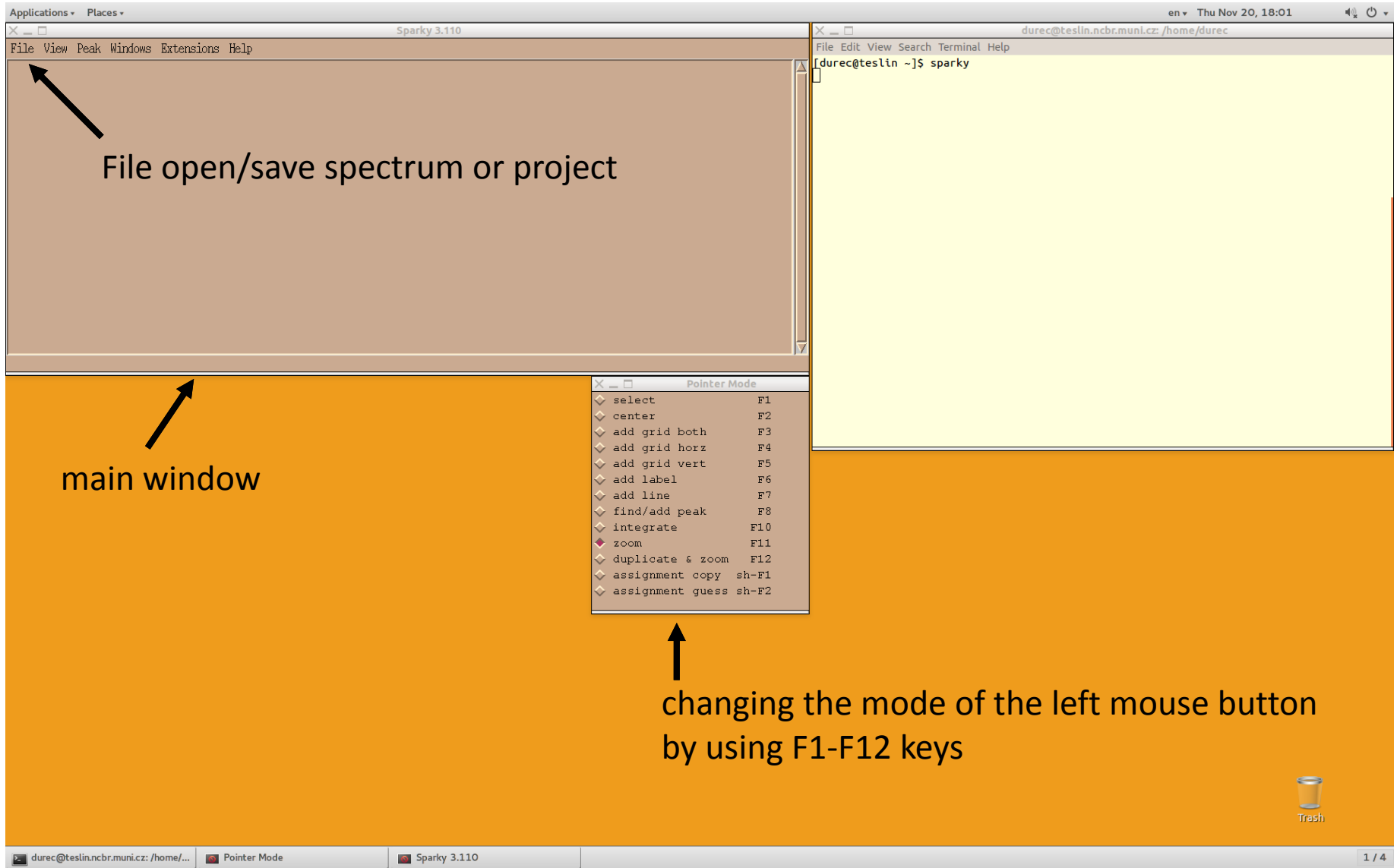
Sparky - spectra

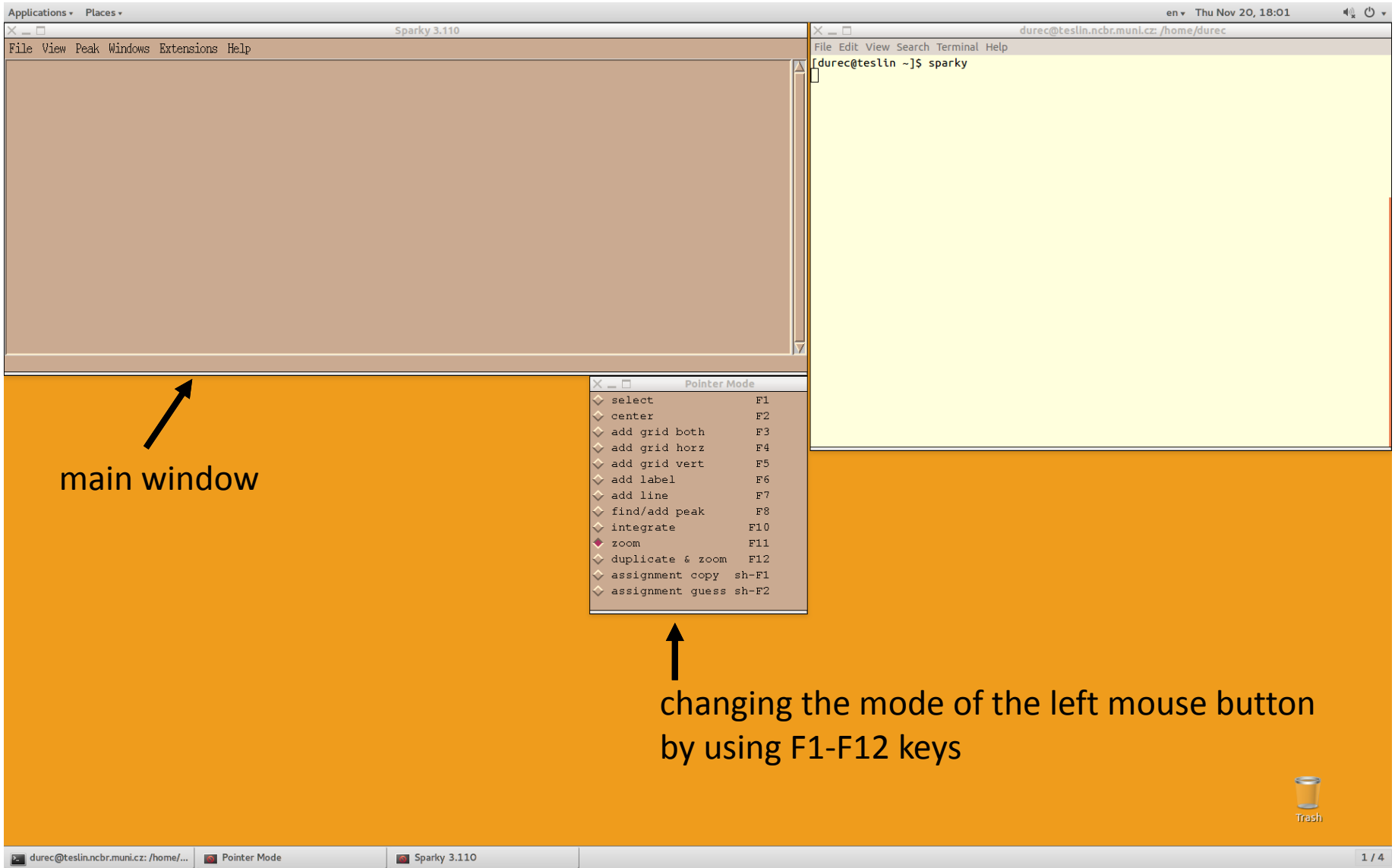
- It can work with different formats of spectra, but to work with them is hard and slow (computationally)
 - Conversion of spectra into *.ucsf format
 - Unix: `bruk2ucsf 2rr name_of_the_spectrum.ucsf`
 - Windows: on the command line `bruk2ucsf.exe 2rr name_of_the_spectrum.ucsf`
 - Spectrum does not contain any information about acquisition, temperature and so on → it is necessary to give the *.ucsf file proper name (`colchicine_noesy_800_D2O_25C.ucsf`)
- It has its own structure of folders
 - Lists, Projects, Save
- Sparky start
 - Windows: `/sparky/bin/sparky.bat`

Sparky - start

- Windows: batch file – sparky.bat





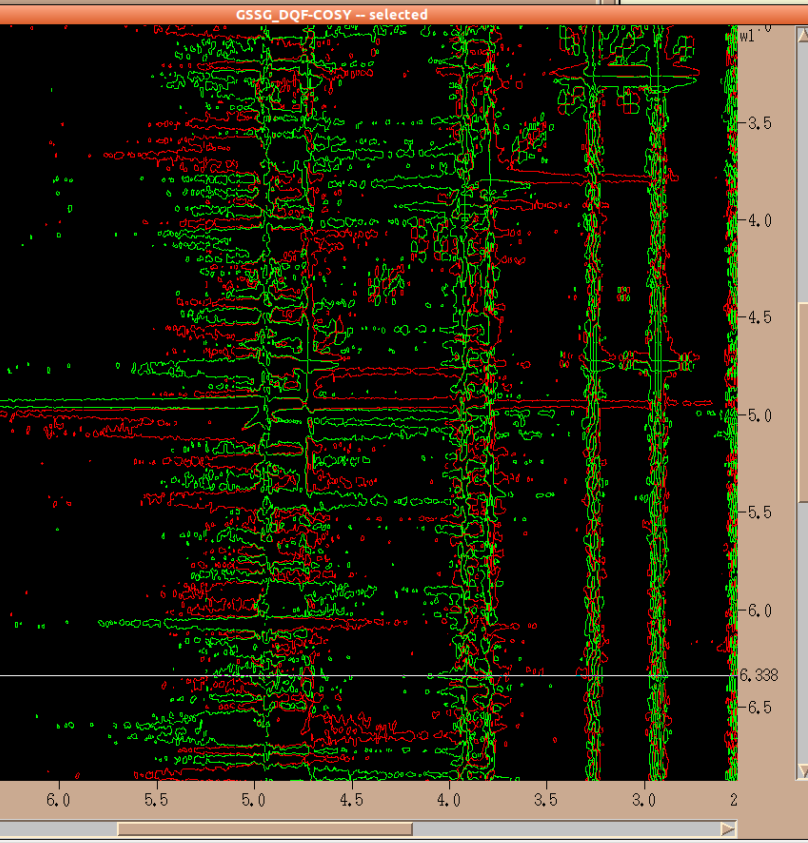


Sparky - control

- Control by using
 - Two letter shortcuts (case-sensitive)
 - Menu in the main window
- *.ucsf file - spectral data from a NMR exp
- *.save file – Sparky format (plain text) contains all settings, assignment
- *.proj file – Sparky project file contains link to multiple *save files
- Open spectrum/project: *fo/jo*
- Save spectrum/project as: *fa/ja*
- Save spectrum/project: *fs/js*
- Backup files *.bak are created automatically during the saving of the spectrum

fo ←

```
[dures@teslin FRVS_spektra]$ sparky
```



fo

- Pointer Mode
- select F1
 - center F2
 - add grid both F3
 - add grid horz F4
 - add grid vert F5
 - add label F6
 - add line F7
 - find/add peak F8
 - integrate F10
 - zoom F11
 - duplicate & zoom F12
 - assignment copy sh-F1
 - assignment guess sh-F2



zf – full view

Applications ▾ Places ▾ View ▾

Sparky 3.110

File View Peak Windows Extensions Help

fo

durec@teslin.ncbr.muni.cz /scratch/durec/FRVS_spektra

File Edit View Search Terminal Help

[durec@teslin FRVS_spektra]\$ sparky

GSSG_DQF-COSY -- selected

w1

1

2

3

4

5

6

7

8

9,868

w2

10 9,623 9 8 7 6 5 4 3 2 1 0 -1

Pointer Mode

- ◇ select F1
- ◇ center F2
- ◇ add grid both F3
- ◇ add grid horz F4
- ◇ add grid vert F5
- ◇ add label F6
- ◇ add line F7
- ◇ find/add peak F8
- ◇ integrate F10
- ◇ zoom F11
- ◇ duplicate & zoom F12
- ◇ assignment copy sh-F1
- ◇ assignment guess sh-F2

durec@teslin.ncbr.muni.cz /scratch... Pointer Mode Sparky 3.110 GSSG_DQF-COSY -- selected

1 / 4

ct – contour levels

vc – contour scale

The screenshot displays the Sparky 3.110 interface. A red circle highlights the 'Contour Levels GSSG_DQF-COSY' dialog box, which is used to set contour levels. The dialog box contains the following information:

View:	Lowest	Levels	Factor	Color
GSSG_DQF-COSY	2.53e+04	1	1.40	red
Positive				
Negative	-2.53e+04	1	1.40	green

Buttons: Ok, Apply, Close, Help

The main window shows a contour plot of the GSSG_DQF-COSY spectrum. The x-axis is labeled 'w2' and ranges from -1 to 10.21. The y-axis is labeled 'w1' and ranges from -9.132 to 1. The plot shows a complex pattern of peaks, with red and green contours overlaid. A red circle highlights the vertical axis (w1) and the plot area, indicating the contour scale.

A terminal window in the background shows the command `[durec@teslin FRVS_spektra]$ sparky`.

A 'Pointer Mode' menu is visible in the bottom right corner, listing various actions and their corresponding function keys (F1-F12).

The system tray at the bottom shows the date and time: Fri 23 Jan, 15:00. The taskbar includes the following items: `durec@teslin.ncbr.muni.cz: /scratc...`, `Pointer Mode`, `Sparky 3.110`, `GSSG_DQF-COSY -- selected`, and `Contour Levels GSSG_DQF-COSY`. The page number `1 / 4` is also visible.

adjustment of the contours:

manually overwrite the values

change by dragging the left mouse button

The screenshot shows the Sparky 3.110 interface. The 'Contour Levels GSSG_DQF-COSY' dialog box is open, showing the following settings:

View:	Lowest	Levels	Factor	Color
Positive	3.94e+06	9	1.40	red
Negative	-3.94e+06	9	1.40	green

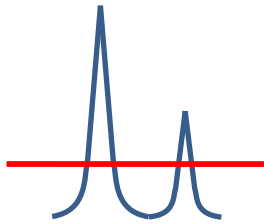
The 'Pointer Mode' menu is also visible, listing various actions and their corresponding function keys:

- select F1
- center F2
- add grid both F3
- add grid horz F4
- add grid vert F5
- add label F6
- add line F7
- find/add peak F8
- integrate F10
- zoom F11
- duplicate & zoom F12
- assignment copy sh-F1
- assignment guess sh-F2

The plot area shows a 2D contour plot with a vertical axis labeled 'w1' ranging from 0 to 9.107 and a horizontal axis labeled 'w2' ranging from -1 to 10.25. The plot displays several peaks, with some highlighted in red and green. A red circle highlights the vertical axis, and another red circle highlights the 'Contour Levels GSSG_DQF-COSY' dialog box.

adjustment of the contours:

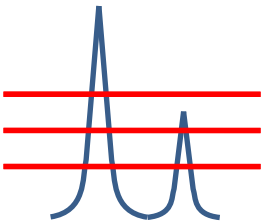
1D



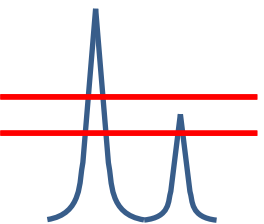
Lowest positive
Levels = 1



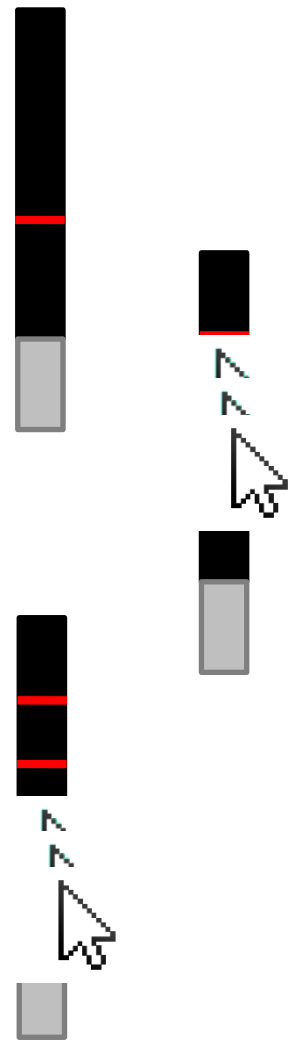
2D



Lowest positive
Levels = 3



Lowest positive
Levels = 2



zooming the spectrum:

keyboard shortcut: **zi** (zoom in)

preset zoom in

alternatively use of middle mouse button

pointer mode– zoom: key F11

selected region is zoomed

by clicking and dragging of the mouse

The screenshot displays the Sparky 3.110 interface. The main window, titled "GSSG_DQF-COSY -- selected", shows a 2D NMR spectrum with a white rectangular region of interest highlighted. A mouse cursor is positioned over this region. An orange arrow points from the text "selected region is zoomed by clicking and dragging of the mouse" to the highlighted area. In the bottom right corner, a "Pointer Mode" menu is open, listing various functions and their corresponding keyboard shortcuts. The "zoom" function, associated with F11, is circled in red. The terminal window in the background shows the command "sparky" being executed. The system tray at the bottom includes a "Trash" icon and the system clock showing "1 / 4".

Function	Shortcut
select	F1
center	F2
add grid both	F3
add grid horz	F4
add grid vert	F5
add label	F6
add line	F7
find/add peak	F8
integrate	F10
zoom	F11
duplicate & zoom	F12
assignment copy sh	F1
assignment guess sh	F2

PEAK PICKING:

pointer mode – **find/add peak: key F8**

one peak is added by one left click,

peaks in every local maximum/minimum are added by selecting the rectangle region

The screenshot displays the Sparky 3.110 interface. The main window, titled "GSSG_DQF-COSY -- selected", shows a 2D NMR spectrum with several peaks highlighted in red and green. The x-axis is labeled "w2" and ranges from 4.2 to 1.8. The y-axis is labeled "w1.0" and ranges from 2.2 to 4.0. A terminal window in the background shows the command "sparky" being executed. A "Pointer Mode" menu is open in the bottom right corner, listing various actions and their corresponding function keys. The "find/add peak" option, associated with F8, is circled in red. An orange arrow points from the top right of the terminal window down to the "find/add peak" option in the menu.

Action	Key
select	F1
center	F2
add grid both	F3
add grid horz	F4
add grid vert	F5
add label	F6
add line	F7
find/add peak	F8
integrate	F10
zoom	F11
duplicate & zoom	F12
assignment copy	sh-F1
assignment guess	sh-F2

peak picking: changing the size of ornaments (peaks, labels...): *ot* → sizes → change the size as desirable (proper value 0.05) → apply

The screenshot displays the Sparky 3.110 NMR software interface. The main window shows a 2D GSSG_DQF-COSY spectrum with several peaks highlighted in red and green. The x-axis is labeled 'w2' and ranges from 4.2 to 1.8 ppm, with a specific value of 2.813 ppm marked. The y-axis is labeled 'w1' and ranges from 1.0 to 4.0 ppm, with a specific value of 2.812 ppm marked. A terminal window in the top right shows the command 'durec@teslin ~]\$ sparky'. A 'Pointer Mode' menu is open in the bottom right, listing various actions and their corresponding function keys (F1-F12).

Pointer Mode		
select		F1
center		F2
add grid both		F3
add grid horz		F4
add grid vert		F5
add label		F6
add line		F7
find/add peak		F8
integrate		F10
zoom		F11
duplicate & zoom		F12
assignment copy	sh-	F1
assignment guess	sh-	F2

Applications ▾ Places ▾ View ▾
Sparky 3.110
File View Peak Windows Extensions Help
fo5 new peaks
30 new peaks
30 new peaks
durec@teslin.ncbr.muni.cz: /home/durec
File Edit View Search Terminal Help
[durec@teslin ~]\$ sparky
GSSG_DQF-COSY -- selected
w1.0
2.2
2.4
2.6
2.812
3.0
3.2
3.4
3.6
3.8
4.0
w2 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.813 2.6 2.4 2.2 2.0 1.8
durec@teslin.ncbr.muni.cz: /home/... Pointer Mode
Sparky 3.110 GSSG_DQF-COSY -- selected
Trash
1 / 4

PEAK SELECTION

selection: pointer mode – **select: key F1**

one object is selected by clicking on it,

more object are selected by dragging the mouse (or Shift+click)

The screenshot shows the Sparky 3.110 interface. The main window, titled 'GSSG_DQF-COSY -- selected', displays four 2D NMR spectra. The top-left and bottom-right spectra have peaks highlighted with green and red outlines. The top-right and bottom-left spectra also show peaks but are not highlighted. A 'Pointer Mode' menu is open in the bottom right corner, listing various actions and their corresponding function keys. An orange arrow points from the top right towards the menu.

Action	Key
select	F1
center	F2
add grid both	F3
add grid horz	F4
add grid vert	F5
add label	F6
add line	F7
find/add peak	F8
integrate	F10
zoom	F11
duplicate & zoom	F12
assignment copy	sh-F1
assignment guess	sh-F2

peak picking: adding of central peak from the selected group of peaks – *pg*
(useful for multiplets in COSY spectra)

Applications ▾ Places ▾ View ▾

en ▾ Mon 26 Jan, 17:40

Sparky 3.110

durec@teslin.ncbr.muni.cz: /home/durec

File View Peak Windows Extensions Help

```
fo5 new peaks
30 new peaks
30 new peaks
No accelerator for po
No accelerator for t\r
No accelerator for \r
No new peaks
No accelerator for fg
No accelerator for gf
```

File Edit View Search Terminal Help

```
[durec@teslin ~]$ sparky
```

GSSG_DQF-COSY -- selected

w1

2.85

2.90

2.95

3.00

3.05

3.078

3.10

3.15

3.20

3.25

3.30

3.35

w2

3.40 3.35 3.30 3.25 3.20 3.15 3.10 3.05 3.00 2.95 2.90 2.85 2.80 2.75

Pointer Mode

- ◆ select F1
- ◆ center F2
- ◆ add grid both F3
- ◆ add grid horz F4
- ◆ add grid vert F5
- ◆ add label F6
- ◆ add line F7
- ◆ find/add peak F8
- ◆ integrate F10
- ◆ zoom F11
- ◆ duplicate & zoom F12
- ◆ assignment copy sh-F1
- ◆ assignment guess sh-F2

durec@teslin.ncbr.muni.cz: /home/... Pointer Mode Sparky 3.110 GSSG_DQF-COSY -- selected

1 / 4

peak picking: list of the peaks – *It*

The screenshot displays the Sparky 3.110 software interface. The main window shows a 2D NMR spectrum titled "GSSG_DQF-COSY -- selected". The x-axis is labeled "w2" and ranges from 9 to 1. The y-axis is labeled "w1" and ranges from 1 to 8. The spectrum shows several cross-peaks, with some highlighted in green and red. A "Spectrum Peaks GSSG_DQF-COSY" window is open on the left, displaying a list of peaks with their assignments and coordinates. A terminal window on the right shows the command "sparky" being executed. A "Pointer Mode" window is open in the bottom right corner, listing various actions and their corresponding function keys.

Assignment	w1	w2
?-?	3.795	3.793
?-?	2.932	2.930
?-?	2.930	3.261
?-?	2.138	2.139
?-?	2.138	3.795
?-?	8.676	8.675
?-?	8.607	8.608
?-?	3.921	8.606
?-?	4.722	8.673
?-?	4.720	4.727
?-?	3.923	3.918
?-?	2.935	4.727
?-?	3.258	4.725
?-?	2.137	2.513
?-?	2.512	2.509
?-?	3.263	3.261

Pointer Mode

- select F1
- center F2
- add grid both F3
- add grid horz F4
- add grid vert F5
- add label F6
- add line F7
- find/add peak F8
- integrate F10
- zoom F11
- duplicate & zoom F12
- assignment copy sh-F1
- assignment guess sh-F2

1 / 4

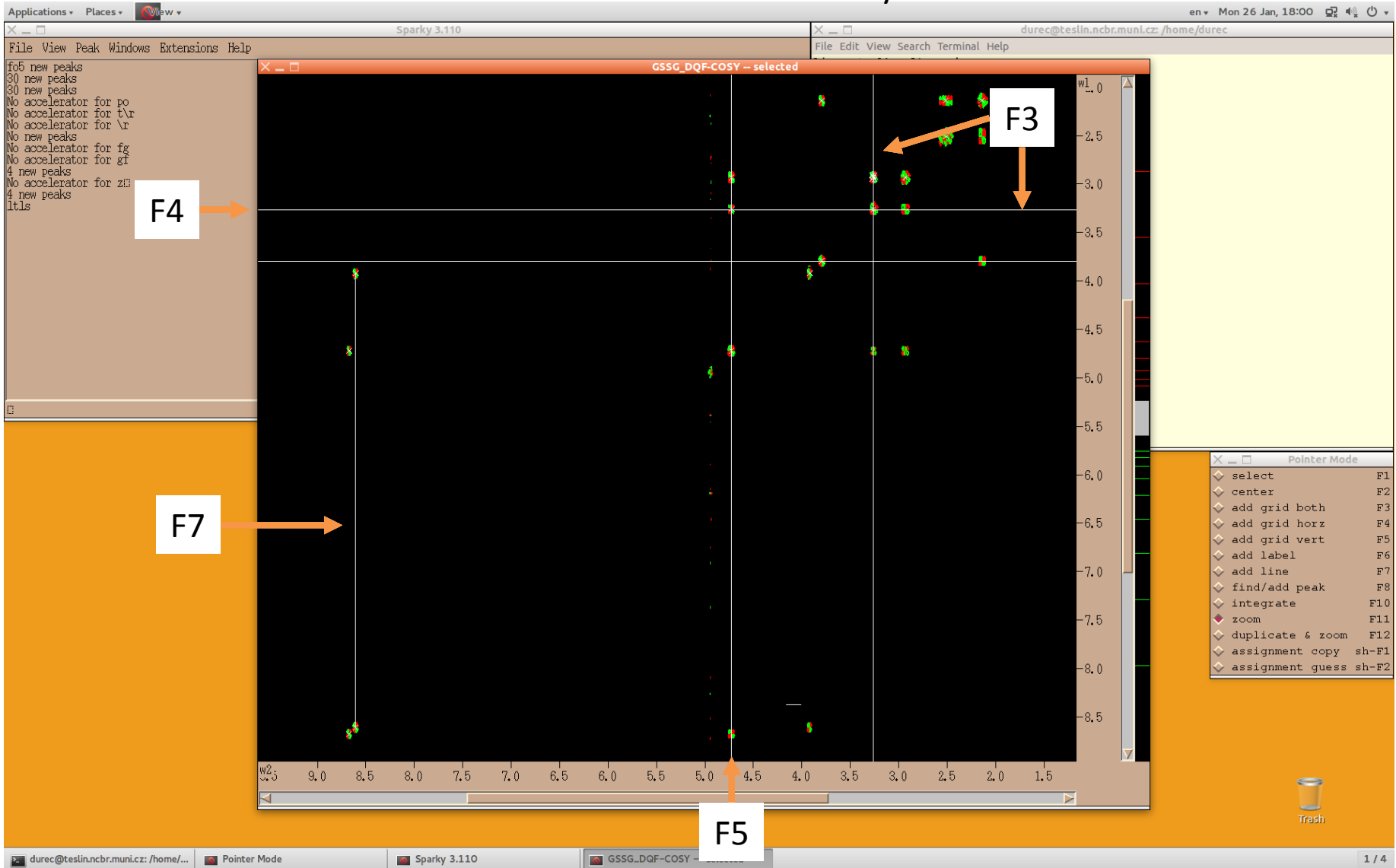
identification of the spin systems:

add vertical and horizontal line: key F3

add horizontal line: key F4

add vertical line: key F5

add a vector: key F7



identification of the spin systems:

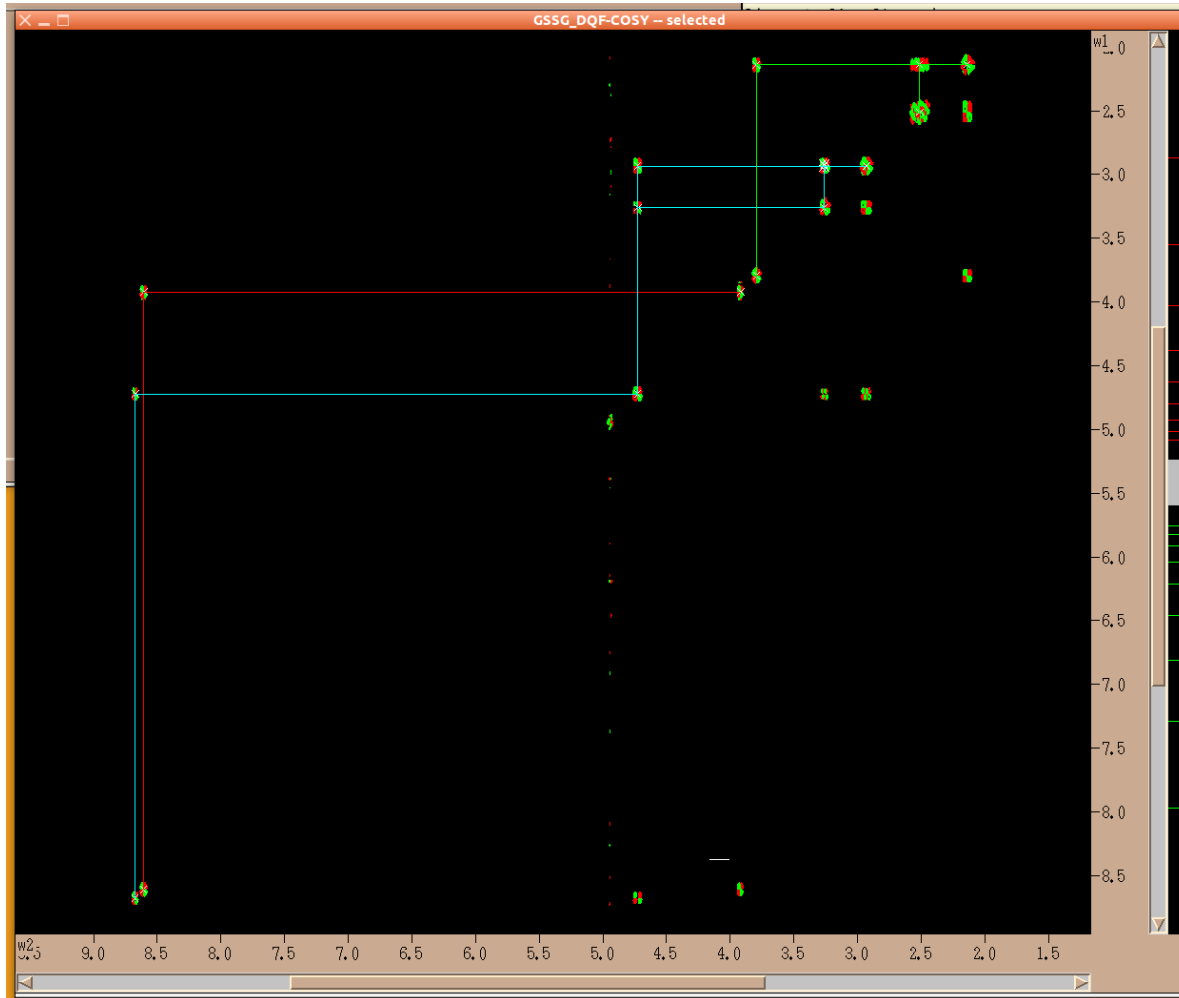
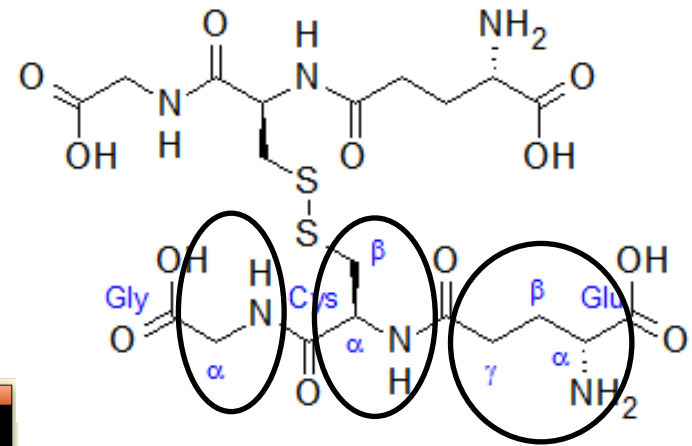
changing the color of ornaments (lines, labels...): **ot** → color →
select object/s → choose the color → apply

The screenshot shows the Sparky 3.110 interface with the 'Ornament Properties' dialog box open. The dialog box has a red circle around the 'Color ...' button. Below the dialog is a color palette with various color options. The main window displays an NMR spectrum with several peaks and lines. A 'Pointer Mode' menu is visible in the bottom right corner, listing various actions and their corresponding function keys.

Action	Function Key
select	F1
center	F2
add grid both	F3
add grid horz	F4
add grid vert	F5
add label	F6
add line	F7
find/add peak	F8
integrate	F10
zoom	F11
duplicate & zoom	F12
assignment copy	sh-F1
assignment guess	sh-F2

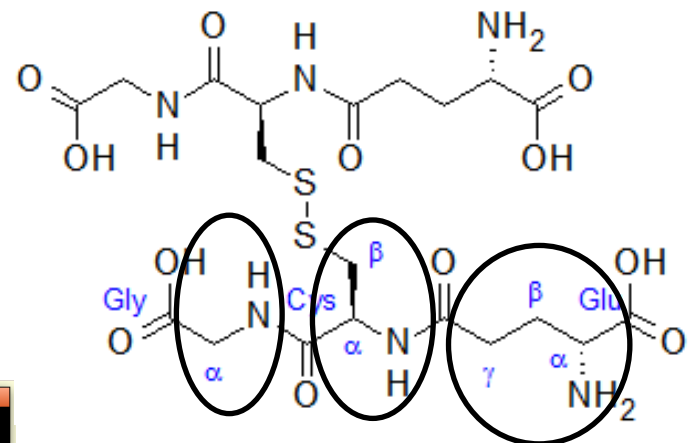
identification of the spin systems:

COSY – correlation through bonds, isolated spin systems



identification of the spin systems:

COSY – correlation through bonds, isolated spin systems



expected number of signals:

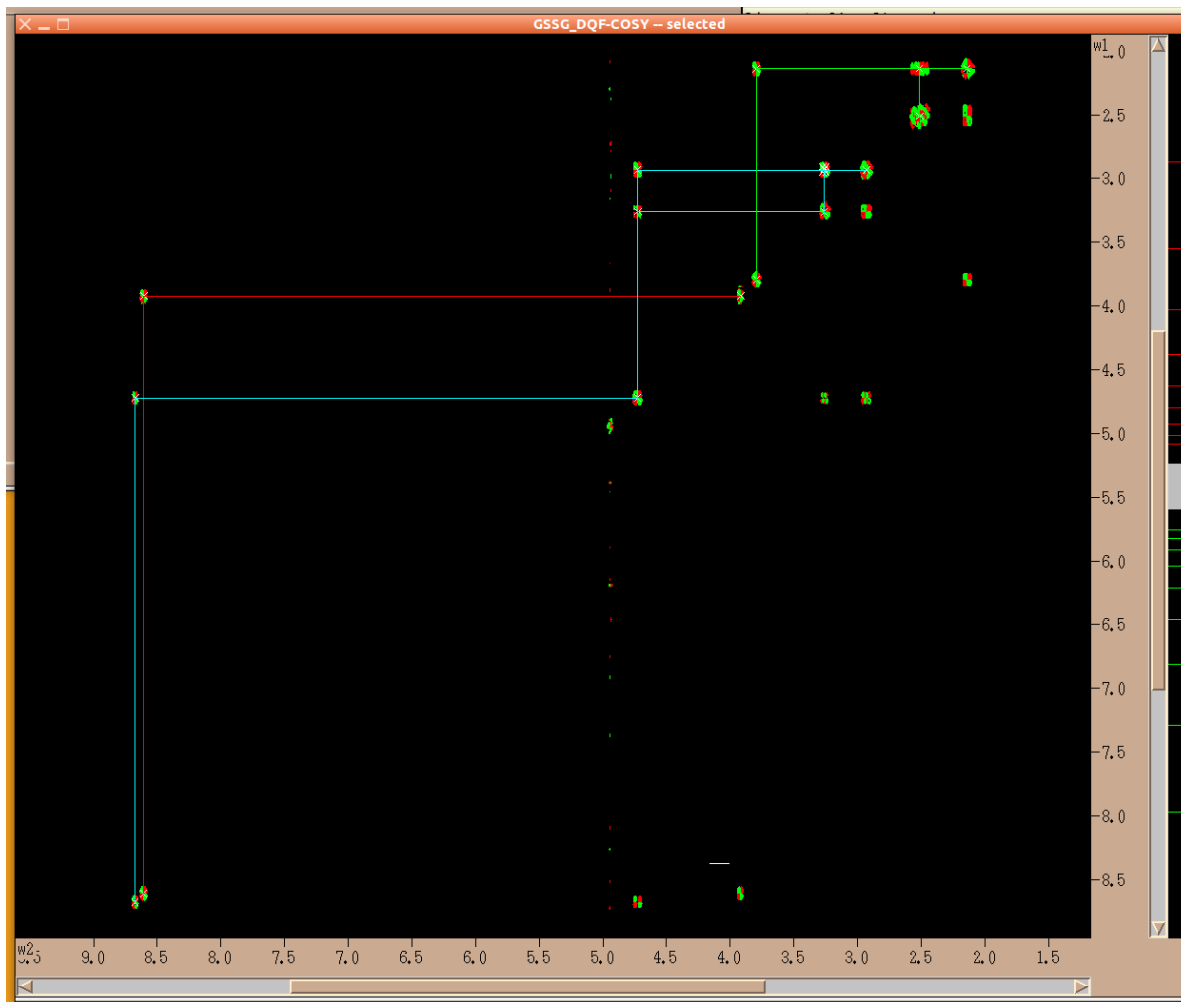
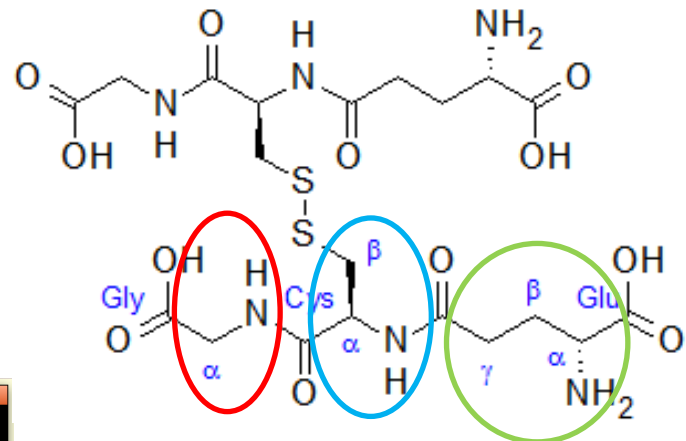
Gly – 2: H α , NH

Cys – 4: H α , NH, H β' , H β''

Glu – 3: H α , H β , H γ

identification of the spin systems:

COSY – correlation through bonds, isolated spin systems



expected number of signals:

Gly – 2: H_α, NH

Cys – 4: H_α, NH, H_β', H_β''

Glu – 3: H_α, H_β, H_γ

Signal assignment:

select the peak: pointer mode – select: key F1 → shortcut **at** – assignment → fill in the name of the group (e.g. abbreviation of residuum/molecule) and the name of the atom (eg. NH) according to the resonance in w1 and w2 axis → type apply

The screenshot displays the Sparky 3.110 software interface. The main window shows a 2D NMR spectrum with two peaks highlighted in green and red. A white arrow points to the peak at approximately (8.675, 8.675) in the w1-w2 plane. The text "CysNH-NH" is displayed in the center of the plot area. The w1 axis ranges from 8.30 to 8.35, and the w2 axis ranges from 8.00 to 8.05. The assignment window is open, showing the following table:

Axis	Group	Atom	Resonance	Peak
w1	1H	Cys	NH	8.675
w2	1H	Cys	NH	8.675

The assignment window also shows the text "Resonances" and "Cys NH 8.675". The "Pointer Mode" window is open, showing a list of shortcuts:

- select F1
- center F2
- add grid both F3
- add grid horz F4
- add grid vert F5
- add label F6
- add line F7
- find/add peak F8
- integrate F10
- zoom F11
- duplicate & zoom F12
- assignment copy sh-F1
- assignment guess sh-F2

The status bar at the bottom shows the current window is "GSSG_DQF-COSY -- selected" and the assignment window is "Assignment GSSG_DQF-COSY".

Signal assignment:

the assignment with the closest resonance to the current selected resonance is highlighted when clicking on the name of the axis. (not necessarily correct one!!), double click on the highlighted line adds the name of the **group** and **atom label** to currently selected peak. Use unique naming, otherwise resonances will be averaged. Display position of resonance by **vR** command (view Resonances).

The screenshot displays the Sparky 3.110 software interface. The main window shows a 2D NMR spectrum plot with a vertical axis labeled 'w1' and a horizontal axis labeled 'w2'. The vertical axis ranges from -3.9 to -4.7, and the horizontal axis ranges from 9.1 to 8.1. A red crosshair is positioned at approximately (8.675, -3.9). A green crosshair is positioned at approximately (8.675, -4.7). A red circle highlights the 'w2' label on the horizontal axis. Another red circle highlights the 'w1' label on the vertical axis. A third red circle highlights the 'w2' label on the horizontal axis. A fourth red circle highlights the 'w1' label on the vertical axis. The Assignment dialog box is open, showing the following table:

Axis	Group	Atom	Resonance	Peak
w1	1H			4.722
w2	1H	Cys	NH	8.675

Below the table, the Resonances list is shown:

Group	Atom	Resonance
Gly	NH	8.607
Cys	NH	8.675

The dialog box also includes buttons for 'Resonance Peaks ...', 'Guessing ...', 'Guesses', 'Ok', 'Apply', 'Close', and 'Help'. A Pointer Mode menu is visible on the right side of the plot, listing various actions and their corresponding function keys (F1-F12).

signal assignment:

shortcut **tb** – assignment table
residues vs. atoms, statistical
data can be shown as well

both can be exported
as a plain text file
(→ Save)

shortcut **lt** – list of the peaks
already with assignments, or
with other information
(→ Options) ! Always check
SDev column for consistency of
the assignment

Assignment Table

	Ha	Hb	Hb'	Hb''	Hc	NH
Cys	4.724	-	2.932	3.261	-	8.675
Glu	3.794	2.138	-	-	2.511	-
Gly	3.921	-	-	-	-	8.607

Spectrum Peaks GSSG_DQF-COSY

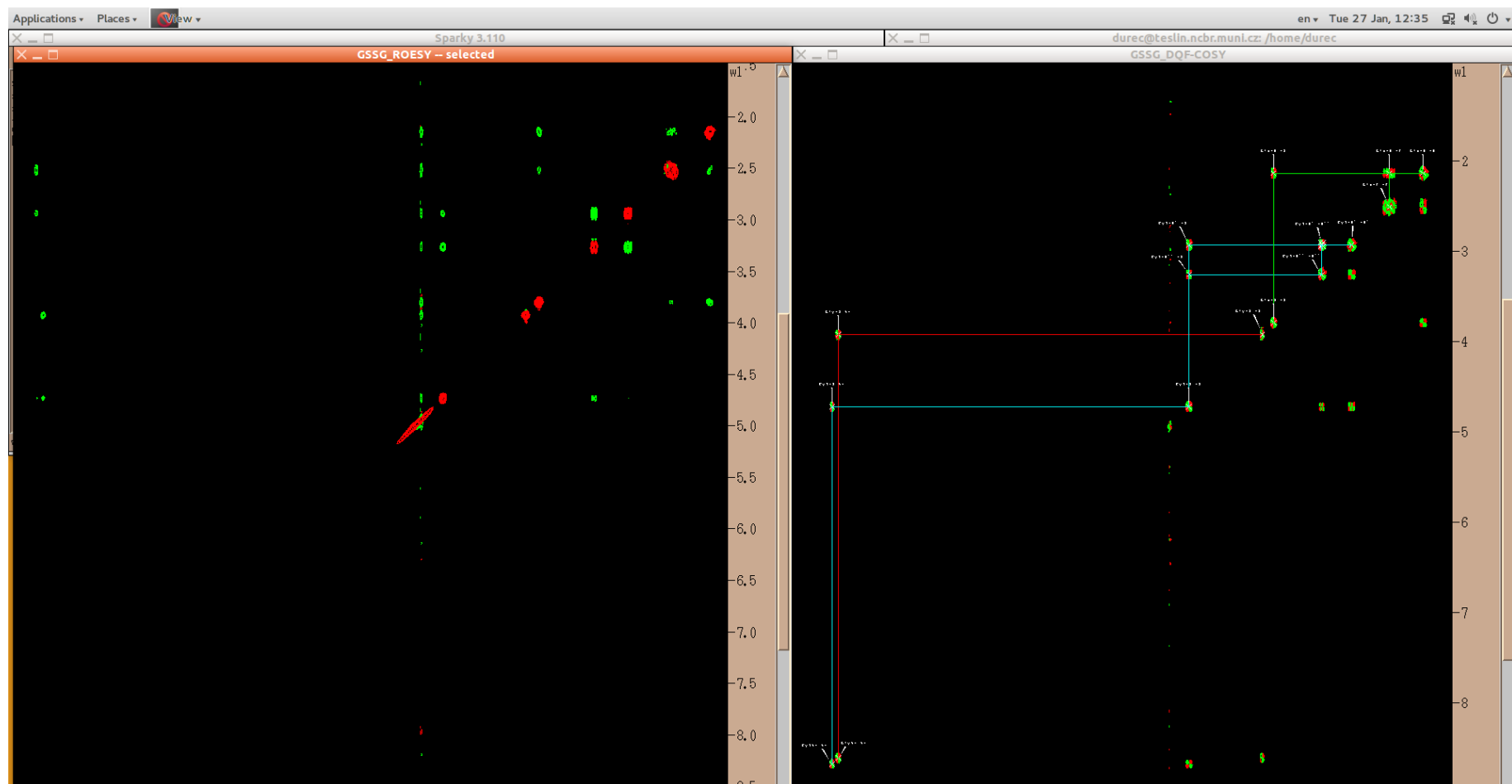
Assignment	w1	w2
CysHa-Ha	4.720	4.727
CysHa-NH	4.722	8.673
CysHb'-Ha	2.935	4.727
CysHb'-Hb'	2.932	2.930
CysHb''-Hb''	2.930	3.261
CysHb''-Ha	3.258	4.725
CysHb''-Hb''	3.263	3.261
CysNH-NH	8.676	8.675
GluHa-Ha	3.795	3.793
GluHb-Ha	2.138	3.795
GluHb-Hb	2.138	2.139
GluHb-Hc	2.137	2.513
GluHc-Hc	2.512	2.509
GlyHa-Ha	3.923	3.918
GlyHa-NH	3.921	8.606
GlyNH-NH	8.607	8.608

Pointer Mode

- select F1
- center F2
- add grid both F3
- add grid horz F4
- add grid vert F5
- add label F6
- add line F7
- find/add peak F8
- integrate F10
- zoom F11
- duplicate & zoom F12
- assignment copy sh-F1
- assignment guess sh-F2

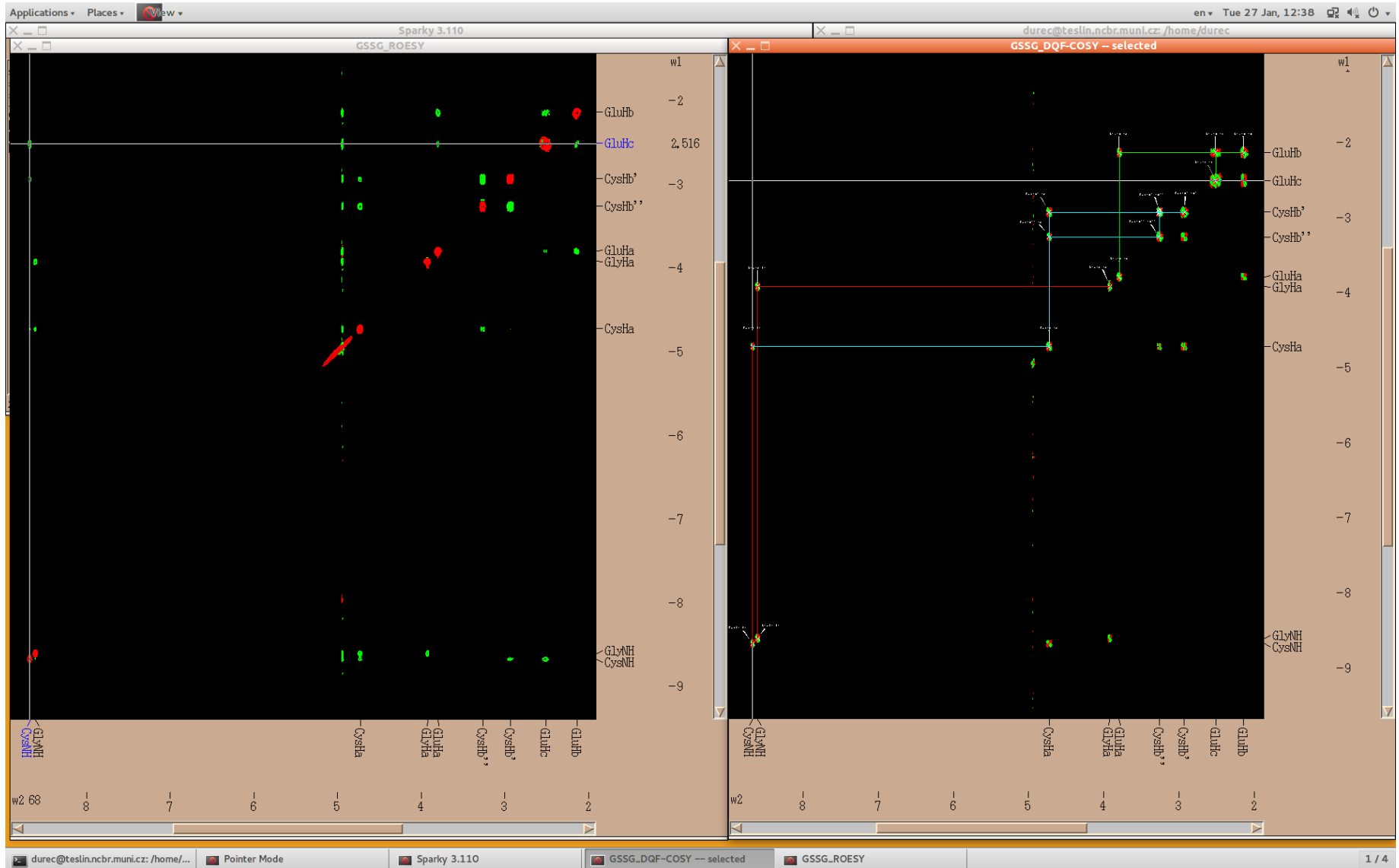
Working with more spectra:

Assignment made in one spectrum is available/shared also for other spectra, peak assignment can be completed using different experiments on condition that experiments were recorded at the same temperature, identical sample etc. During work you may save separately the spectra (file save – experiments of active window) and subsequently save the entire project



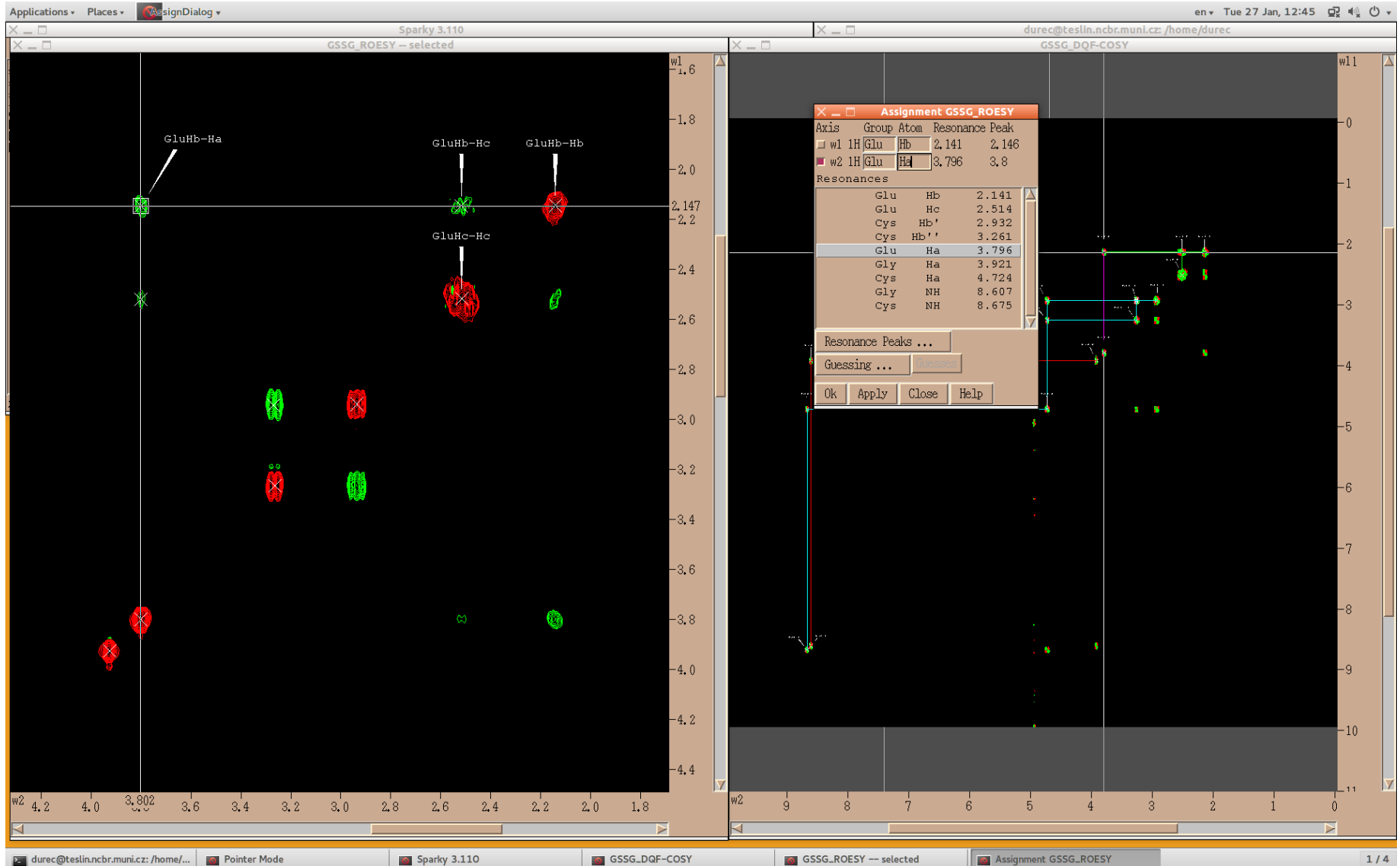
working with more spectra:

view assigned (averaged) resonances on the edge of the spectra - **vR**



working with more spectra:

assign ROESY spectrum by using **at**:



working with more spectra:

synchronize views of spectra: shortcut **yt** – it allows simultaneously move synchronized axes of spectra when moving one of them,

click on the axes in spectra we want synchronize → Synchronize

The image shows a software interface for NMR data analysis. On the left, a dialog box titled "Synchronize views" is open. It contains a table with two columns, w1 and w2, and two rows of spectra names. The first row is "GSSG_DQF-COSY" with "1H a" in both columns. The second row is "GSSG_ROESY" with "1H a" in both columns. Below the table are four buttons: "Synchronize", "Unsynchronize", "Close", and "Help". A mouse cursor is pointing at the "Synchronize" button. The main area of the software displays two 2D NMR spectra side-by-side. The left spectrum is a GSSG_DQF-COSY spectrum, and the right is a GSSG_ROESY spectrum. Both spectra have a vertical axis (w1) ranging from 1.6 to 4.4 and a horizontal axis (w2) ranging from 4.2 to 1.8. The spectra show cross-peaks between various protons, with labels such as "GluHc-Ha", "GluHc-Hc", "CysHb'-Hb''", "CysHb'-Hb'", "GluHa-Ha", "GlyHa-Ha", and "a-Ha". The two spectra are synchronized, meaning their axes are linked together. At the bottom of the software window, there is a taskbar with several icons and labels: "durec@teslin.ncbr.muni.cz: /home/...", "Pointer Mode", "Sparky 3.110", "GSSG_DQF-COSY", "GSSG_ROESY -- selected", "Synchronize views", and a page indicator "1 / 4".

	w1	w2
GSSG_DQF-COSY	1H a	1H
GSSG_ROESY	1H a	1H