

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**)

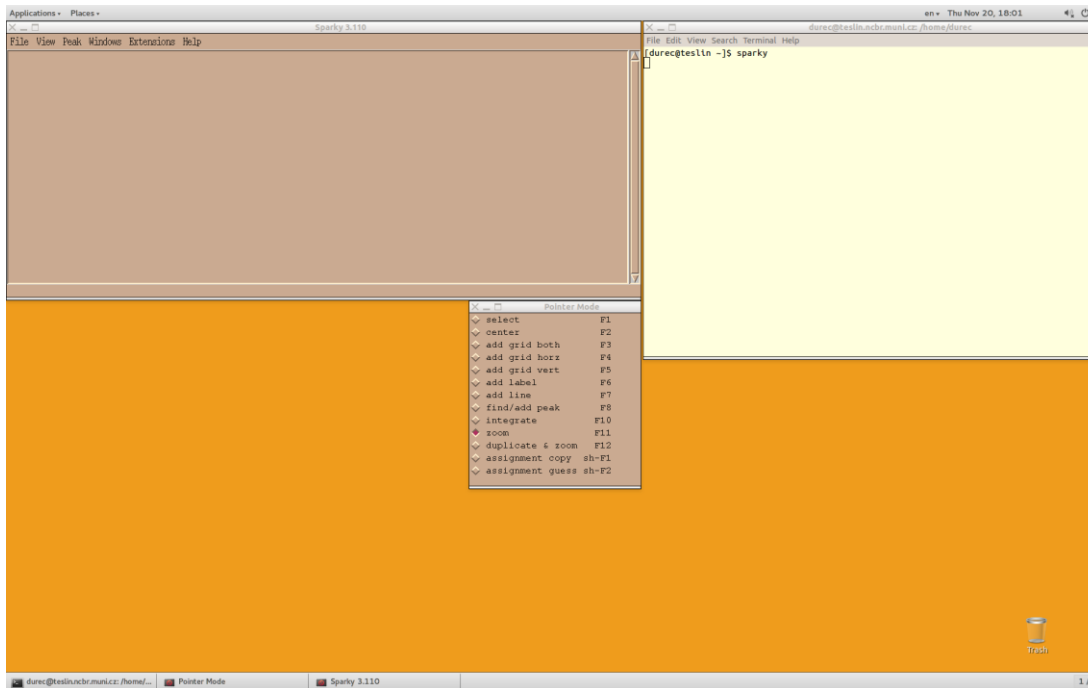
- Multiplatform (Windows and Linux OS)
- Free for download:
<https://nmrfam.wisc.edu/nmrfam-sparky-distribution/>
- Written in Python language, open source, it is not developed anymore (last version is from the 2008)
- Fast and undemanding for hardware
- **It can handle only multi-dimensional spectra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

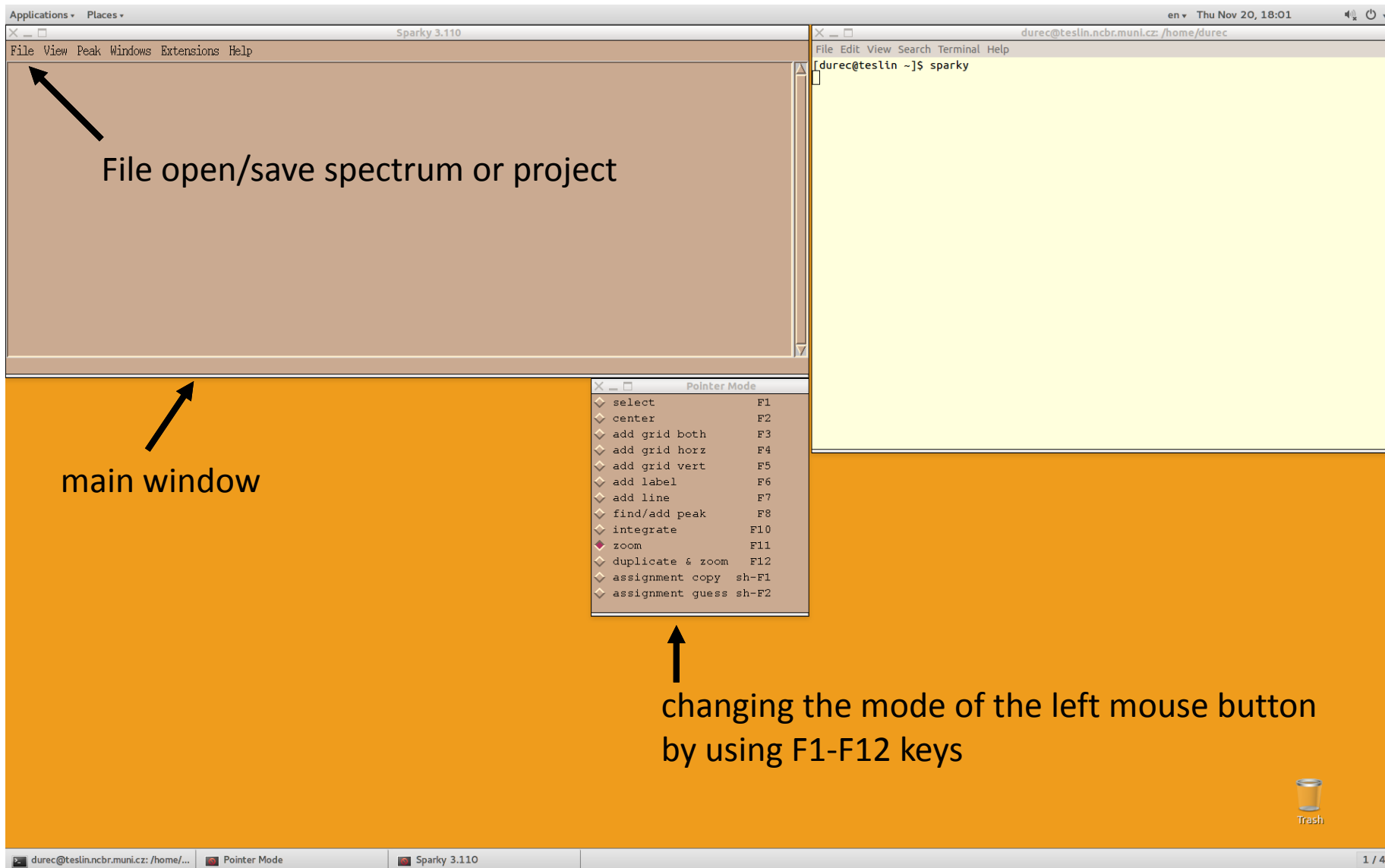
Sparky - spectra

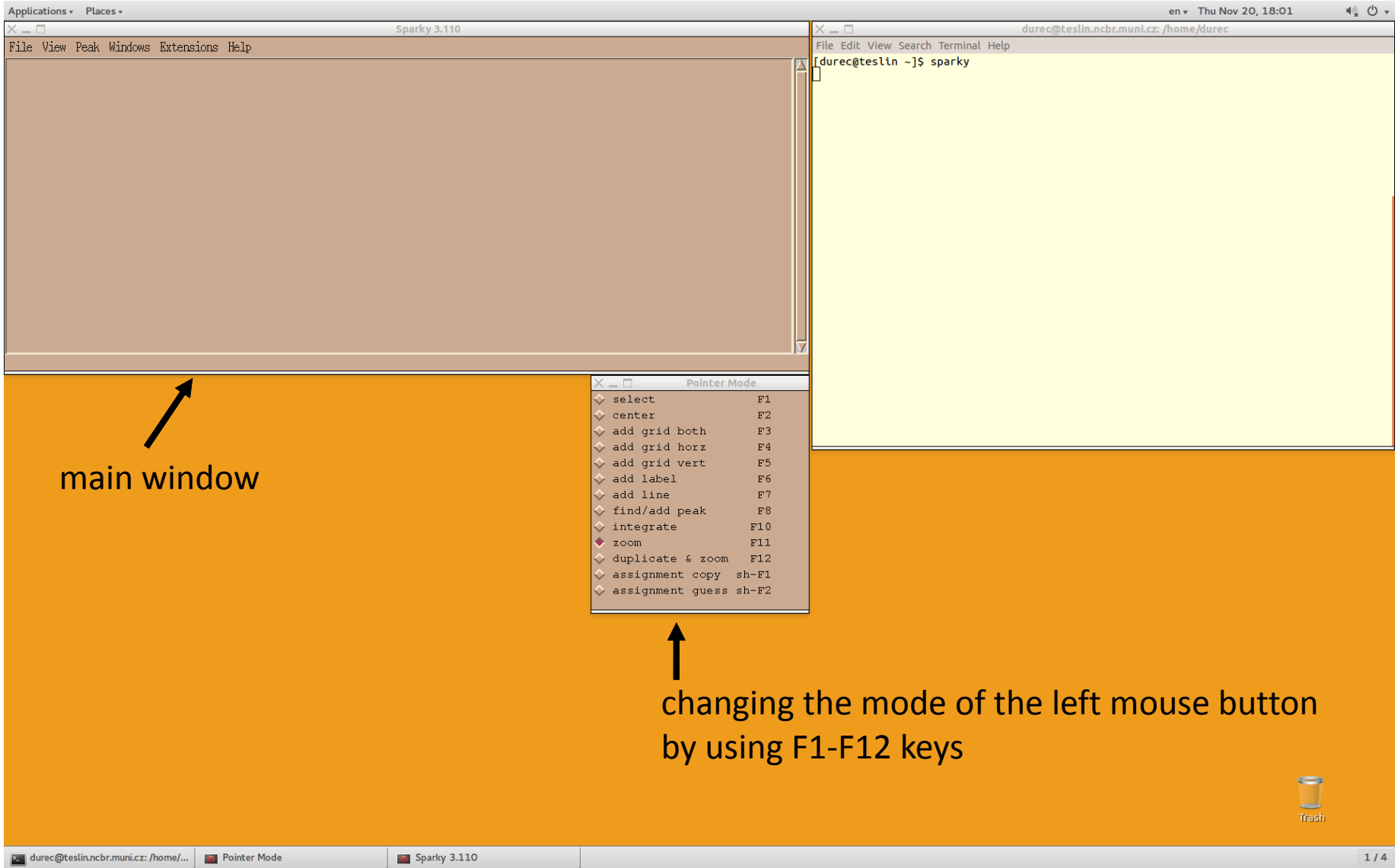
- It can work with different formats of spectra, but to work with them is hard and slow (computationally)
 - Conversion of spectra into *.ucsf format
 - Unix: `bruk2ucsf 2rr name_of_the_spectrum.ucsf`
 - Windows: on the command line `bruk2ucsf.exe 2rr name_of_the_spectrum.ucsf`
 - Spectrum does not contain 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





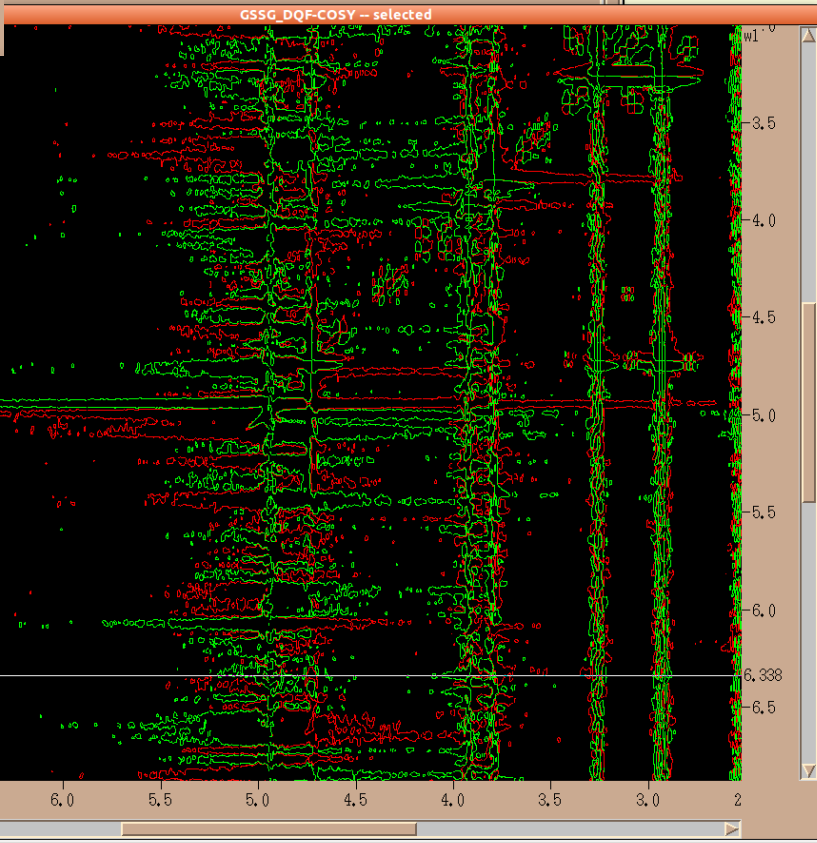


Sparky - control

- 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

The screenshot displays the Sparky 3.110 NMR software interface. The main window, titled "GSSG_DQF-COSY -- selected", shows a 2D NMR spectrum with a complex pattern of green and red peaks. The horizontal axis (w2) is labeled from 10 to -1, and the vertical axis (w1) is labeled from 1 to -9. A mouse cursor is positioned over a peak in the spectrum. The interface includes a menu bar (File, View, Peak, Windows, Extensions, Help) and a terminal window on the right showing the command "sparky". A "Pointer Mode" menu is open in the bottom right corner, listing various functions and their corresponding keyboard shortcuts.

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

System tray at the bottom shows the user "durec@teslin.ncbr.muni.cz", the application "Sparky 3.110", and the current window "GSSG_DQF-COSY -- selected". The system clock indicates "Fri 23 Jan, 14:57".



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
Positive	2.53e+04	1	1.40	red
Negative	-2.53e+04	1	1.40	green

Buttons: Ok, Apply, Close, Help

The main window shows a contour plot titled 'GSSG_DQF-COSY -- selected'. The plot displays a 2D spectrum with contour levels. The x-axis is labeled 'w2' and ranges from -1 to 10.21. The y-axis is labeled 'w1' and ranges from 0 to 9.132. 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 following icons: `durec@teslin.ncbr.muni.cz: /scratc...`, `Pointer Mode`, `Sparky 3.110`, `GSSG_DQF-COSY -- selected`, `Contour Levels GSSG_DQF-COSY`, and `1 / 4`.

adjustment of the contours:

manually overwrite the values

change by dragging the left mouse button

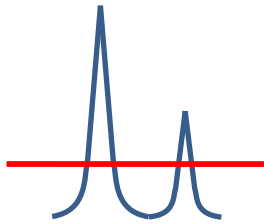
The screenshot shows the Sparky 3.110 interface with the 'Contour Levels GSSG_DQF-COSY' dialog box open. The dialog has the following fields:

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

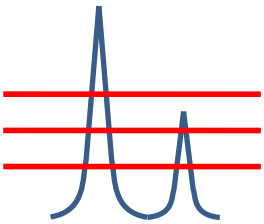
The main plot area shows a 2D contour plot with axes labeled w1 (vertical, 0 to 9) and w2 (horizontal, -1 to 10.25). A vertical slider on the right side of the plot is being adjusted, with a red oval highlighting it. The slider has a vertical scale from 0 to 9, with a current value of 9.107. A 'Pointer Mode' menu is visible in the bottom right corner, listing various actions and their corresponding function keys (F1-F12).

adjustment of the contours:

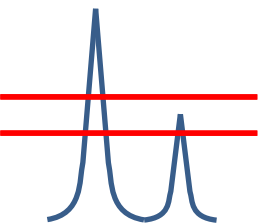
1D



Lowest positive
Levels = 1



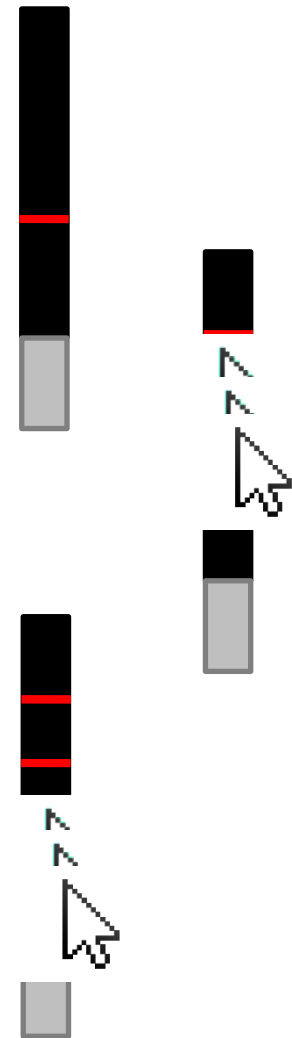
Lowest positive
Levels = 3



Lowest positive
Levels = 2



2D



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 shows a 2D NMR spectrum titled "GSSG_DQF-COSY -- selected". The x-axis is labeled "w2" and ranges from 10 to -1, with a specific value of 4.013 marked. The y-axis is labeled "w1" and ranges from 1 to 9, with a specific value of 4.087 marked. A white box highlights a region of peaks in the center of the spectrum, and a mouse cursor is positioned over it. An orange arrow points from the text "by clicking and dragging of the mouse" to the Pointer Mode menu. The Pointer Mode menu is open, showing a list of actions and their corresponding function keys. The "zoom" action is highlighted with a red circle, and its key is F11. Other actions include "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), "duplicate & zoom" (F12), "assignment copy sh" (sh-F1), and "assignment guess sh" (sh-F2). The terminal window in the background shows the command "durec@teslin ~]\$ sparky". The system tray at the bottom shows the current window title "GSSG_DQF-COSY -- selected" and the system clock "1 / 4".

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	sh-F1
assignment guess sh	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 shows a 2D NMR spectrum titled "GSSG_DQF-COSY -- selected". 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. Several peaks are visible, some highlighted in red and green. A terminal window in the top right shows the command "sparky" being executed. A "Pointer Mode" menu is open in the bottom right, listing various actions and their corresponding function keys. The "find/add peak" option is circled in red. An orange arrow points from the text above to this 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 peak at 2.813 ppm. The y-axis is labeled 'w1' and ranges from 1.0 to 4.0 ppm, with a specific peak at 2.812 ppm. The interface includes a menu bar (File, View, Peak, Windows, Extensions, Help), a terminal window showing the command 'sparky', and a 'Pointer Mode' menu with various functions like 'select', 'center', 'add grid both', etc. The bottom status bar shows the current window is 'GSSG_DQF-COSY -- selected'.

Applications ▾ Places ▾ View ▾

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

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

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 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 NMR spectra arranged in a 2x2 grid. Each spectrum shows peaks with red and green outlines and white 'X' marks. The x-axis is labeled "w2" and ranges from 3.40 to 2.75. The y-axis is labeled "w1" and ranges from 2.85 to 3.35. 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 interface. An orange arrow points from the text 'list of the peaks – *It*' to the 'Spectrum Peaks GSSG_DQF-COSY' window. This window contains a table of peak assignments with columns for 'Assignment', 'w1', and 'w2'. Below the table are search and action buttons. The main window shows a 2D NMR spectrum titled 'GSSG_DQF-COSY -- selected' with axes labeled 'w1' and 'w2'. A 'Pointer Mode' menu 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

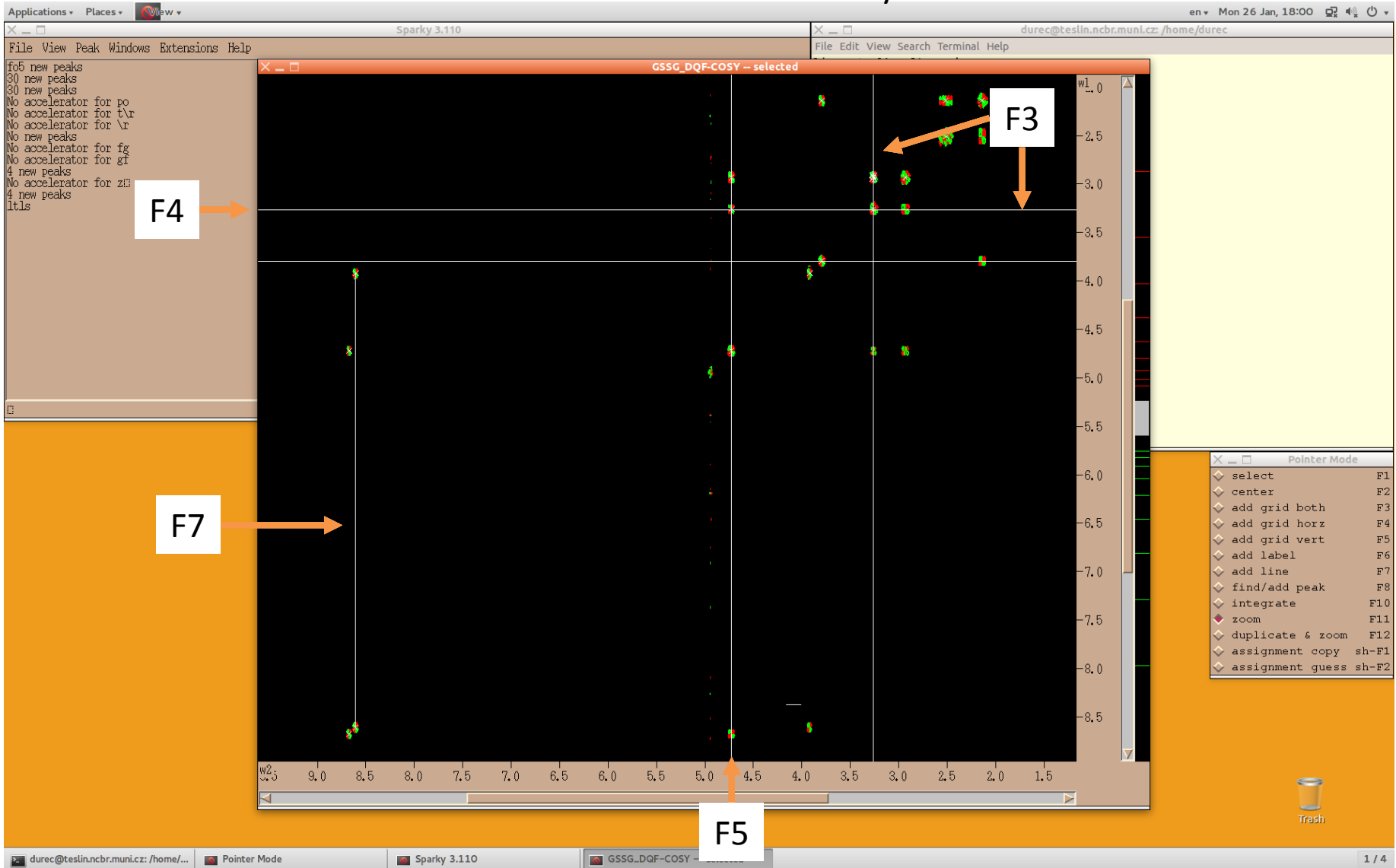
identification of the spin systems:

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:

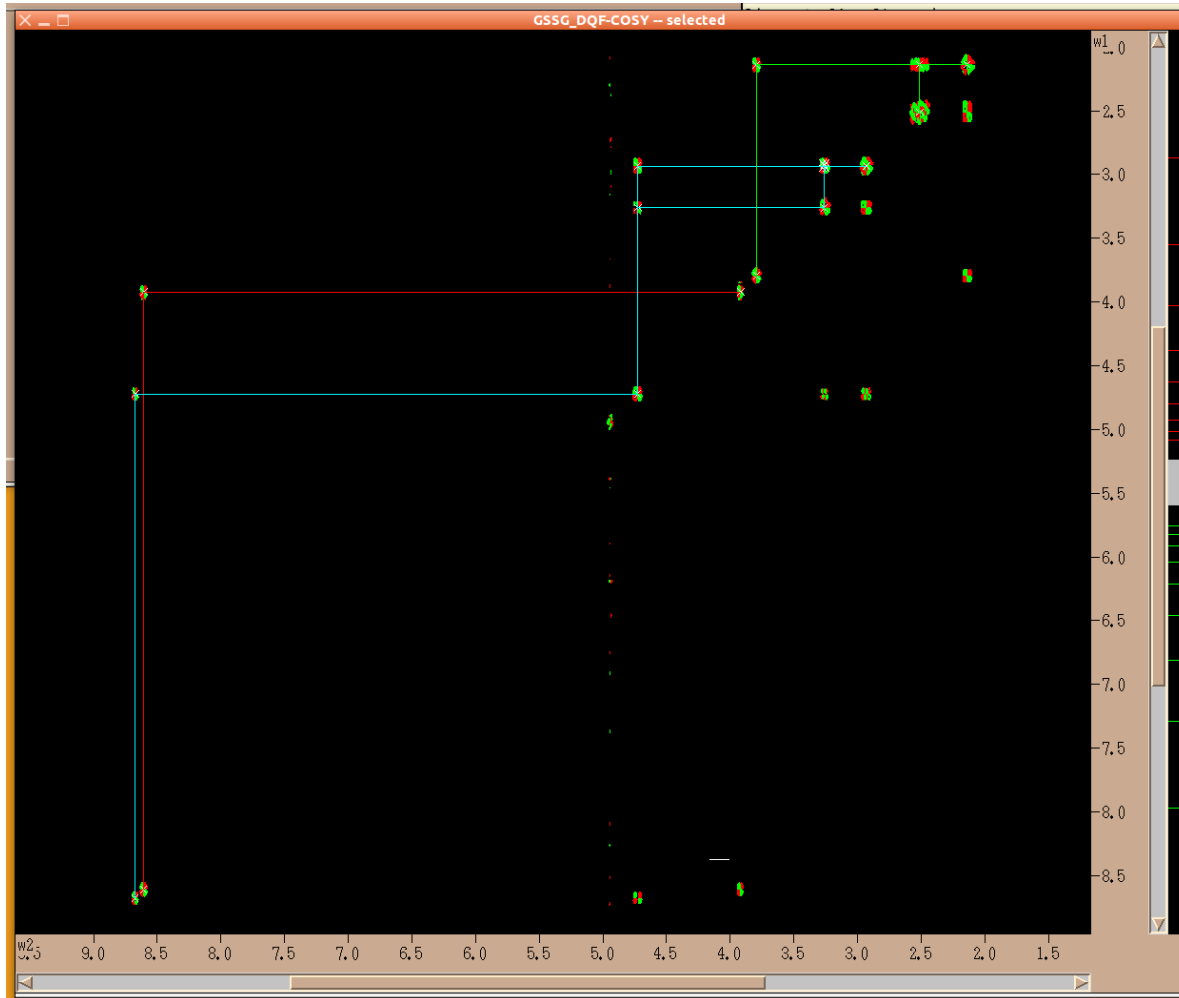
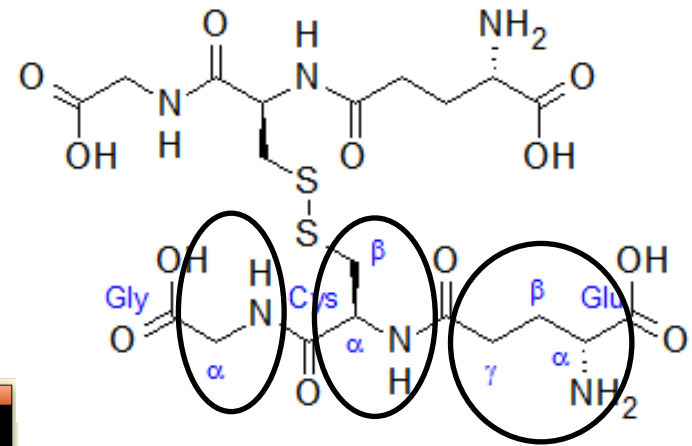
changing the color of ornaments (lines, labels...): **ot** → color →
select object/s → choose the color → apply

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 like 'select', 'center', 'add grid both', etc. The status bar at the bottom shows the current window is 'Ornament Properties' and 'Ornament Color'.

Color	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

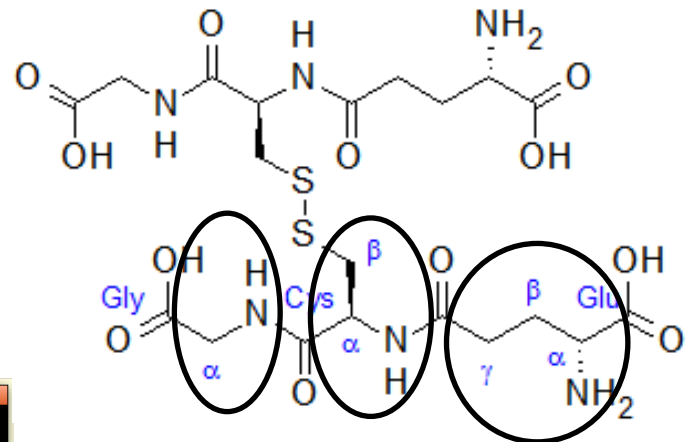
identification of the spin systems:

COSY – correlation through bonds, isolated spin systems



identification of the spin systems:

COSY – correlation through bonds, isolated spin systems



expected number of signals:

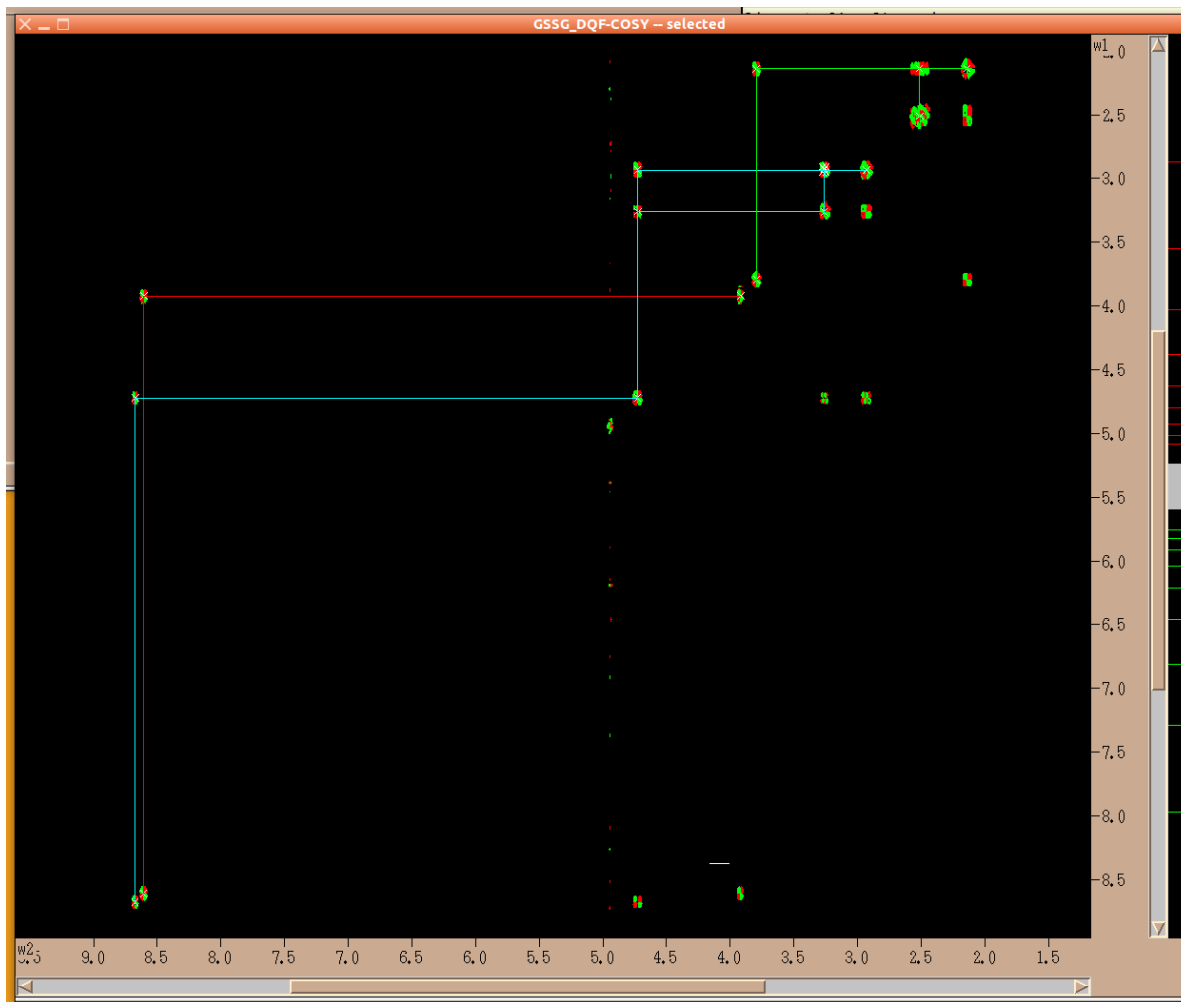
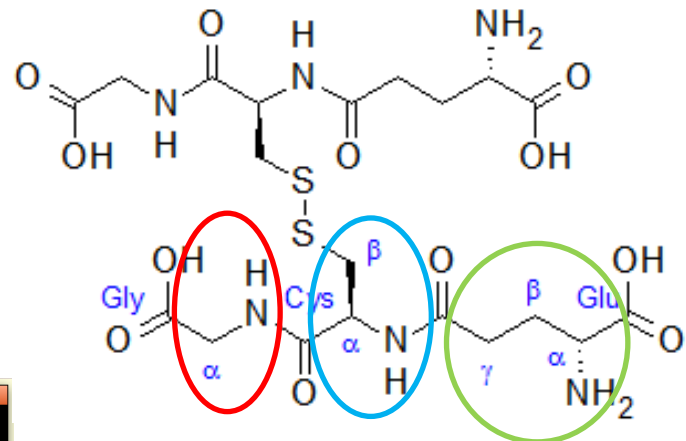
Gly – 2: H α , NH

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 NMR software interface. The main window shows a 2D COSY spectrum with two peaks highlighted in green and red. A white arrow points to the peak at approximately (8.675, 8.675). The text "CysNH-NH" is displayed in the center of the plot area. The x-axis is labeled "w2" and the y-axis is labeled "w1".

The "Assignment GSSG_DQF-COSY" dialog box is open, showing the following table:

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

Below the table, the "Resonances" section shows "Cys NH 8.675". The dialog box includes buttons for "Resonance Peaks ...", "Guessing ...", "Ok", "Apply", "Close", and "Help".

The "Pointer Mode" menu is also visible, listing various 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 system tray at the bottom shows the following windows: "durec@teslin.ncbr.muni.cz: /home/...", "Pointer Mode", "Sparky 3.110", "GSSG_DQF-COSY -- selected", and "Assignment GSSG_DQF-COSY". The page number "1 / 4" is displayed in the bottom right corner.

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 red circle highlights a peak at approximately (8.675, -4.7). The 'Assignment GSSG_DQF-COSY' dialog box is open, showing a table of assignments and a list of resonances.

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

Resonances	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 such as 'select', 'center', 'add grid both', etc., with 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

lt

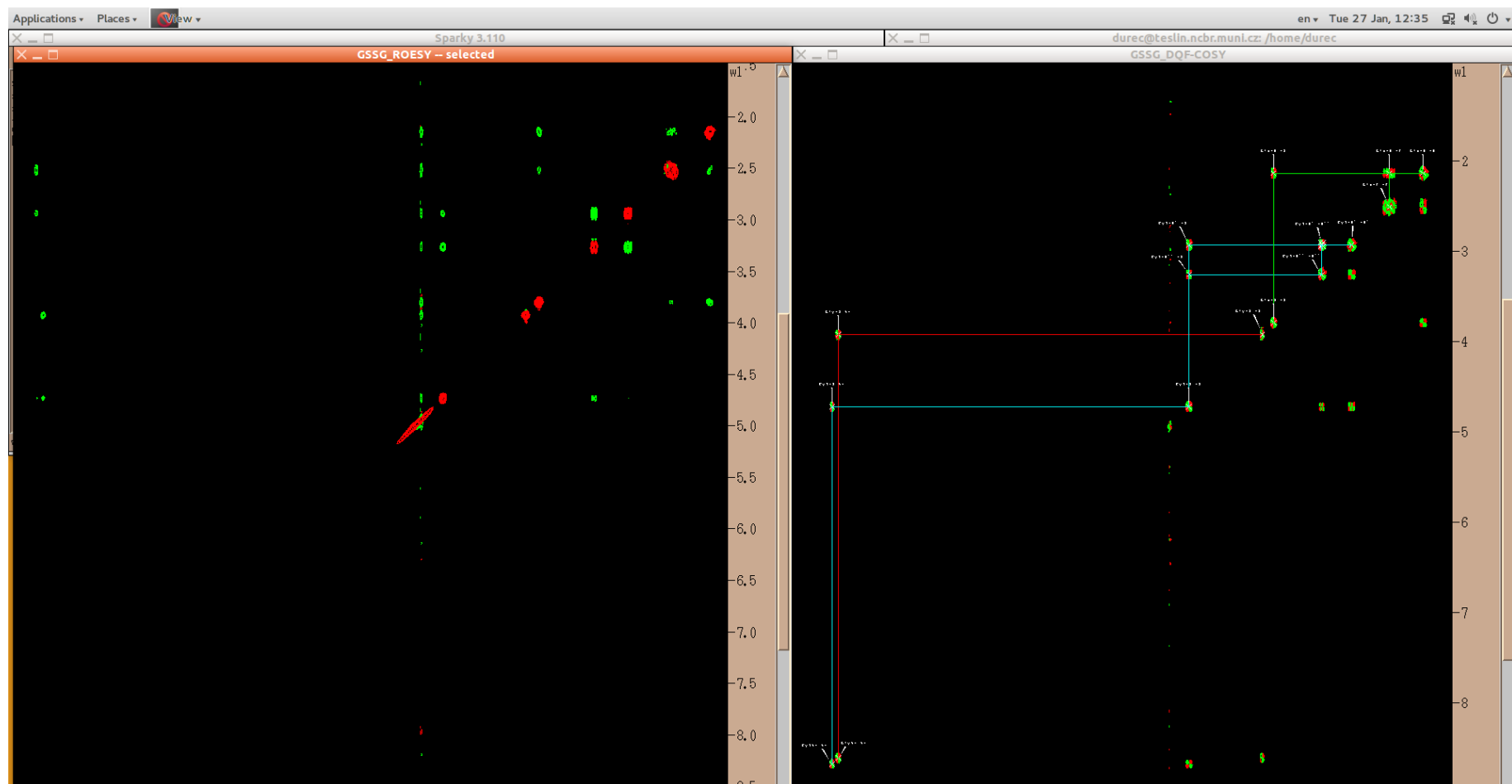
Assignment	w1	w2
CysHa-Ha	4.720	4.727
CysHa-NH	4.722	8.673
CysHb'-Ha	2.935	4.727
CysHb'-Hb'	2.932	2.930
CysHb''-Hb''	2.930	3.261
CysHb''-Ha	3.258	4.725
CysHb''-Hb''	3.263	3.261
CysNH-NH	8.676	8.675
GluHa-Ha	3.795	3.793
GluHb-Ha	2.138	3.795
GluHb-Hb	2.138	2.139
GluHb-Hc	2.137	2.513
GluHc-Hc	2.512	2.509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

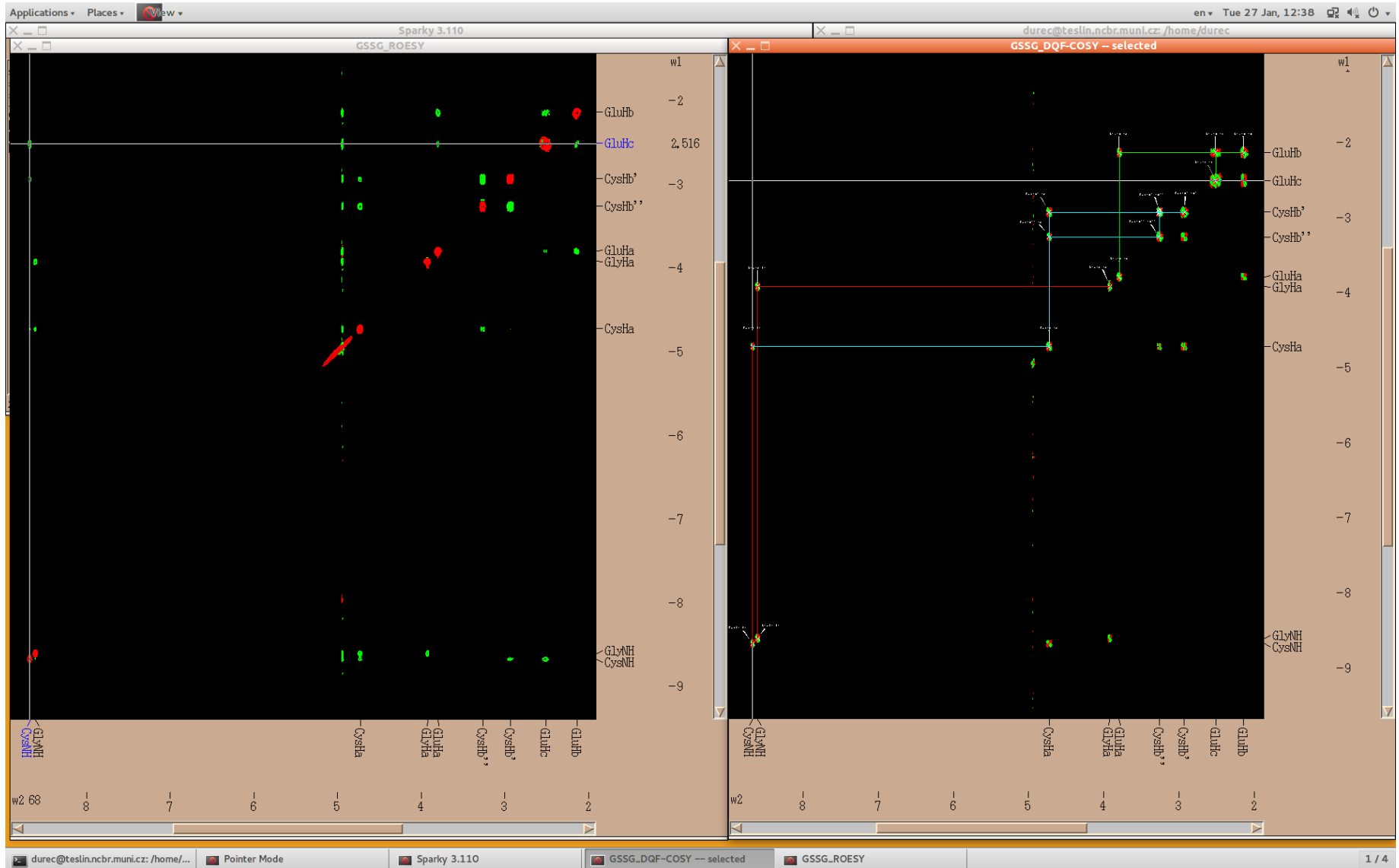
Working with more spectra:

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



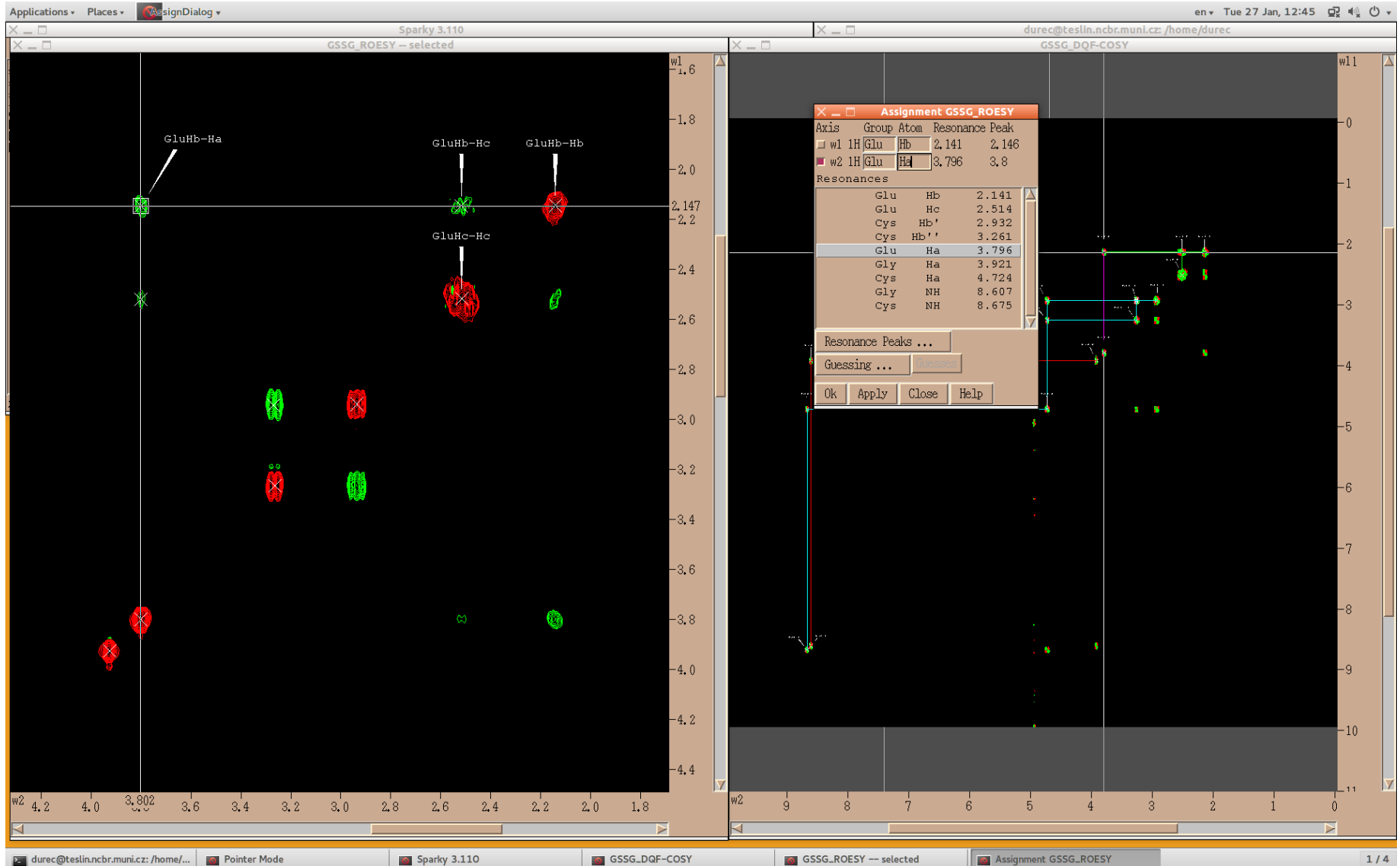
working with more spectra:

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

