SPARKY

Graphical NMR assignment and integration program for proteins, nucleic acids, and other polymers

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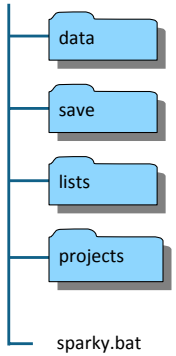
NMR data analysis with Sparky (NMR Intensiv kurs 2016)

Sparky

- Developed & maintained by Donald Kneller and Tom Goddard (University of California, San Francisco)
  - freely available for all common operating systems (Windows, Linux, Unix and Macintosh)
  - www.cgl.ucsf.edu/home/sparky/ (download & online manual)
  - tech.groups.yahoo.com/group/nmr_sparky/ (discussion group)
- Works simultaneously with any number of 2D, 3D or 4D spectra
- Can use all commonly processed NMR spectra from
  - XWinNMR, Topspin (Bruker), VNMR (Varian), NMRPipe, Felix
  - easy to convert and to edit the properties of the spectra
- NMRFAM-Sparky
  - development version with additional tools by and for NMRFAM (University of Wisconsin, Madison)

Directory setup:

- Essential folders and files for sparky datasets
  - data
    - processed NMR spectra in sparky (.ucsf) format
  - save
    - spectra (.save) files with all the display settings, peaks, assignments, labels, ...
  - lists
    - output for peak and assignment lists, spectra (postscript), ...
  - projects
    - session project (.proj) files to load multiple spectra with their relation settings
  - sparky.bat (windows)
    - not essential, but usefull executable to the start sparky project (copy from sparky/bin folder and add the variable: SPARKYHOME=.)
    - check the python version for different versions of sparky

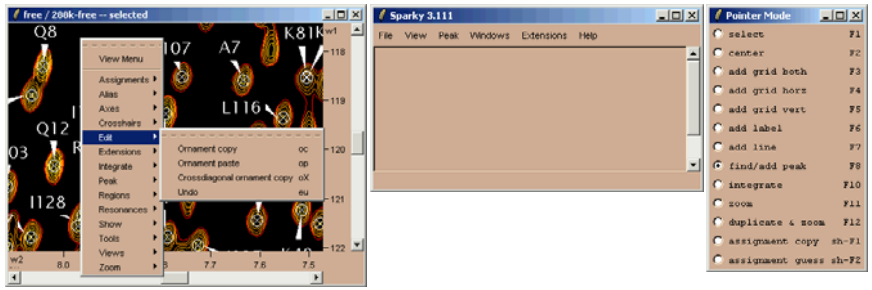


Data conversion:

- Convert spectra to .ucsf data:
  - example: **bruk2ucsf** 2rr n15hsqc.ucsf
    - will create a n15hsqc.ucsf file from the xwinnmr/topspin 2rr file
    - also: **vnmr2ucsf** for Varian spectra and **pipe2ucsf** for NMRPipe spectra
- Show spectrum information:
  - example: **ucsfdata** hnca.ucsf
    - type only 'ucsfdata' to get a full list of all the options
- Edit spectrum settings (eg. axis names, frequency, offset, ...)
  - example: ucsfdata -a2 HN n15hsqc.ucsf
    - will rename the w2-axis to 'HN')
  - example: ucsfdata -p2 -o n15noesy-2dhh.ucsf n15noesy.ucsf
    - makes a 2D $^{1}H^{1}H$-NOESY projection from the 3D $^{1}H^{1}H^{15}N$-NOESY-HSQC dataset

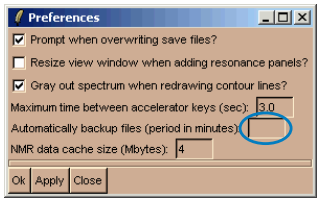
Menu & Pointer

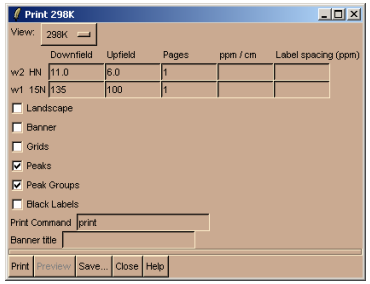
- **Commands are available via the menu and 2-letter shortcuts**
  - right-click in spectrum for direct menu access
- **The mouse pointer**
  - select function or directly change with keys F1-F12



Backup & Print

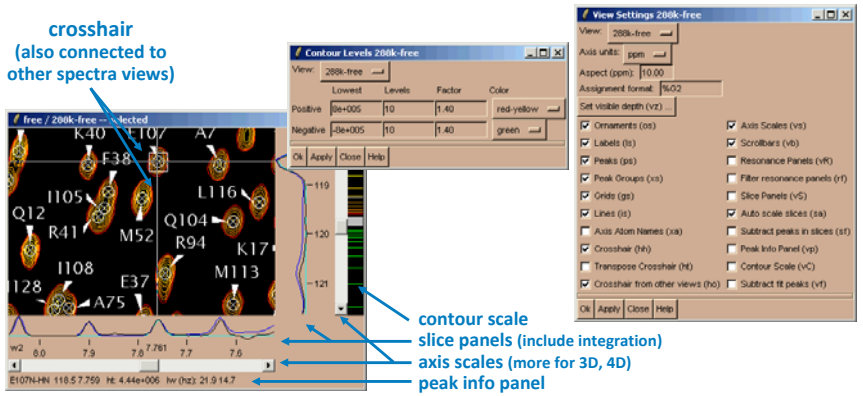
- **Automated backups**
  - set the backup time period in the preferences dialog (pf)
  - the .save file is copied to .save.BAK
  - the .proj file is copied to .proj.BAK
  - only one backup is kept
- **Undo (eu) function**
  - works only one step back
- **Print a spectrum (lt)**
  - select the spectrum and save as a postscipt file
  - print this file (use ao. ghostview)





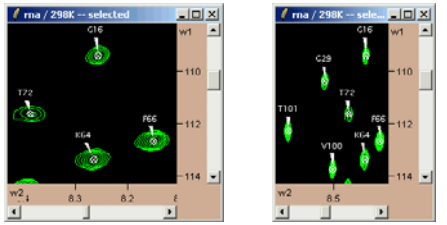
View mode

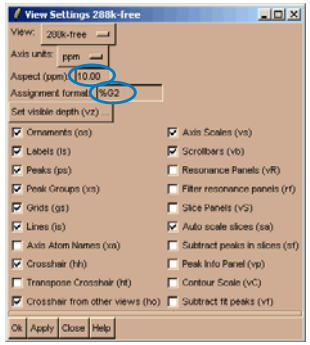
- **View options (vt) and contour levels (ct)**
  - change the look of the spectrum.
  - peaks and the label sizes can be adapted (oz)



View mode

- **Change aspect ratio**
  - widen or narrow the spectrum view





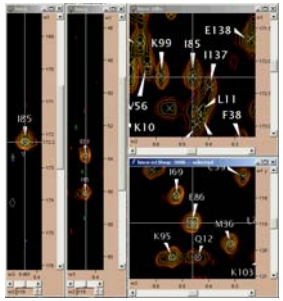
- **Adapt the asssignment format to change the peak labels**
  - %a1-%a2 full atom names and group residues G16N-HN
  - %A1-%A2 force the groups to be displayed (noesy) G16N-G16HN
  - %G1 show only residue name (of first axis) G16

Move, Zoom, Duplicate, Hide & Center

- Mouse pointer mode or shortcuts to zoom and center
  - zoom in (zi), zoom out (zo)
  - show full spectrum (zf), zoom to previous state (zp)
  - center view (vc) on peak position
- Additional views of the same spectrum
  - create a duplicate view (vd)
  - useful to assign the other side of the diagonal in a 2D NOESY (rotate with xr)
  - take care not to remove the original view instead of the duplicate!
    (a bad situation will arrise in which Sparky commands may not work)
- Manage spectrum views
  - hide a spectrum view (vh)
  - show the spectrum by selecting in the view list (pv) or in the window menu

Synchronization

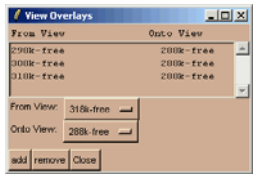
- Synchronize (yt) the axes of 2 or more selected spectra
  - the views of the connected 2D and/or 3D spectra will move simultaneously

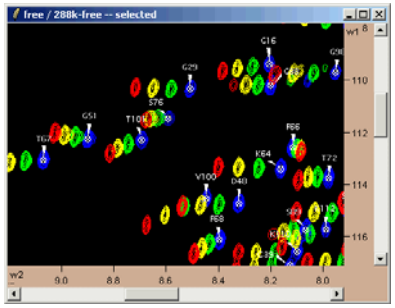


- Align spectra (al) with each other by matching peak positions
  - reference one spectrum to another
  - the names of the axis have to be the same
  - check the shift offset values in spectrum settings (st)

Overlay Spectra

- Overlay (ol) the contours of one spectrum onto another spectrum view
  - spectrum type has to be the same (dimension & axis names)
  - the contour levels of each spectrum are seperately adjusted
  - overlay as many spectra onto one view
  - only the peaks and labels of the original view are shown





Peak Picking

- Peaks can be picked using the 'find/add peak' pointer mode
  - manual or automatic peak picking by dragging a box in a region of your spectrum
    - the peak pick parameter settings (kt) can be adjusted
  - folded peaks can be aliased to the correct frequency
- Select, adjust, integrate and copy peaks
  - select all peaks in the spectrum (pa)
  - center the peak positions (pc)
  - Integrate (pi) the peak volume(s)
    - settings can be adjusted (it) and overlapping peaks can be fitted simultaneously
    - unintegrate (pU) a peak, lock (pk) or unlock (pu) peak positions
  - copy and paste the peaks and their assignment labels (ornaments) from one spectrum to another (oc,op)

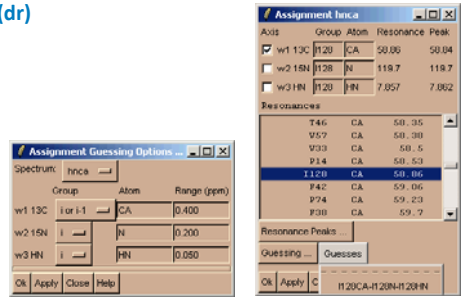
Peak Assignment

❖ Assign the selected peak using the assignment dialog (at)

- resonances known in the current project and closest in frequency to the peak position for the selected axis will be highlighted and can be selected
- For new resonances the residue group and atom name have to be entered manually
- rename, merge or swap resonances (rr)
- remove unused project resonances (dr)

❖ Assignment guessing

- assist peak assignment based on known resonances (select ppm ranges and the optional group and atoms)
- the values are also used by the assignment guessing pointer mode (sh-F2)



Lists of peaks and table of assignments

❖ Every spectrum in sparky has a peak list (lt) associated

- double clicking on a peak in this list will show and center this peak in the spectrum (or select the peak and type vc)
- output is customisable and exportable to other applications

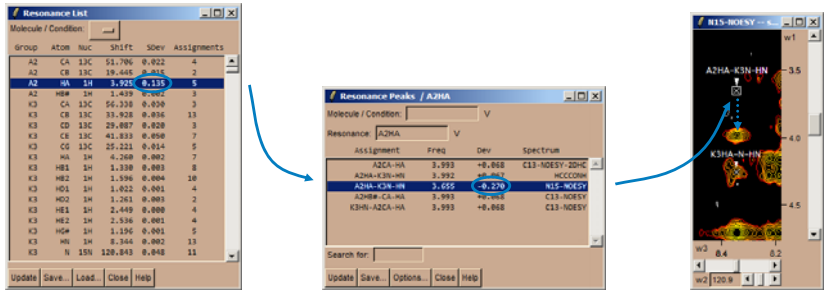
❖ Assignment table (tb)

- multiple tables possible by defining different molecules or conditions in the spectrum settings (st)

Lists of resonances

❖ Resonance list (rl)

- convenient list of the combined resonance assignments
- inspect the standard deviation of each resonance to find assignment mistakes
- select the resonance to open the resonance assignments (ra) which lists all the peaks that have been assigned for this resonance - trace the deviating peak assignments and jump to the corresponding peak in the spectrum



More features in Sparky

❖ 3D, 4D, ..., spectra

- restricted peak picking
- strip plots

❖ Connection to structure

- view NOE crosspeak - Atom-Distance
- assignment assistance
- completeness check with spin graphs

❖ Relaxation Data

- fit the exponential decay from a series of 2D spectra