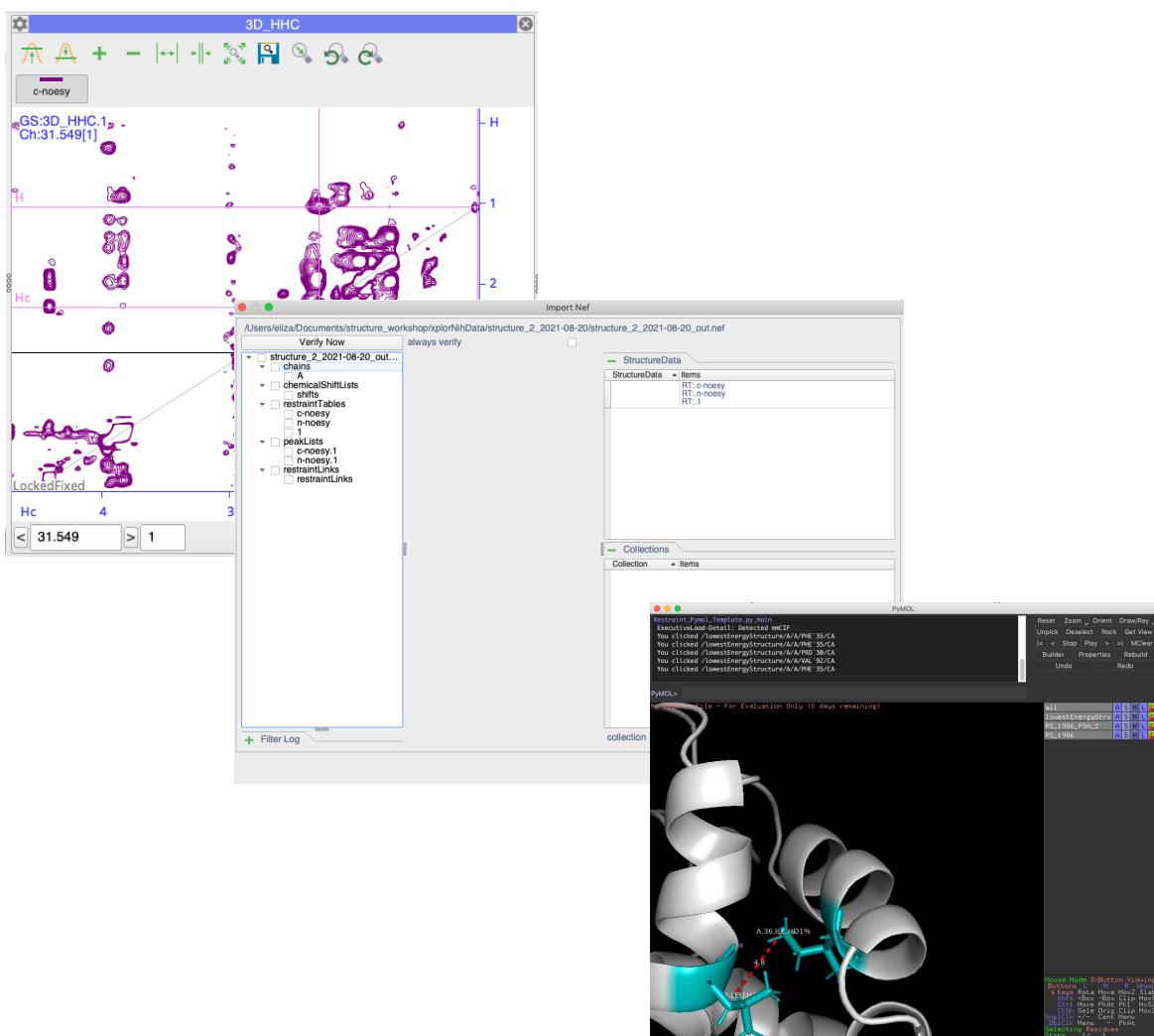


# Solving structures from NMR data



# Introduction

This tutorial will guide you through how to use CcpNmr Analysis Version 3.1.1 to prepare data for a structure calculation with XPLOR-NIH, export the data to XPLOR, re-import the results to CcpNmr Analysis and then analyse the results. It is not necessary to have XPLOR-NIH installed on your computer in order to do this tutorial, as all the results are already provided in a set of tutorial projects. We do advise that you install a version of PyMol, as this will allow you to select restraints in CcpNmr Analysis and directly show these on a structure in PyMol. This tutorial does not explain how structure calculations work, more generally – we assume that you already have an understanding of this.

We are grateful to Helen Mott for making available her Sec5 data for use in our tutorial projects. This tutorial data is available on the tutorials page of our website (<https://ccpn.ac.uk/support/tutorials/>).

The tutorial makes extensive use of the NMR Exchange Format (NEF). For more information on this see <https://github.com/NMRExchangeFormat/NEF> or <https://www.ccpn.ac.uk/manual/v3/NEF.html>.

Note that images are representative, and that there may be small differences between your setup and that shown in the tutorial.

## Contents:

1. Project Setup
2. NOESY Peak Picking
3. Structure Calculation Setup
4. Importing Results
5. Analysing Results
6. Importing wwPDB Reports

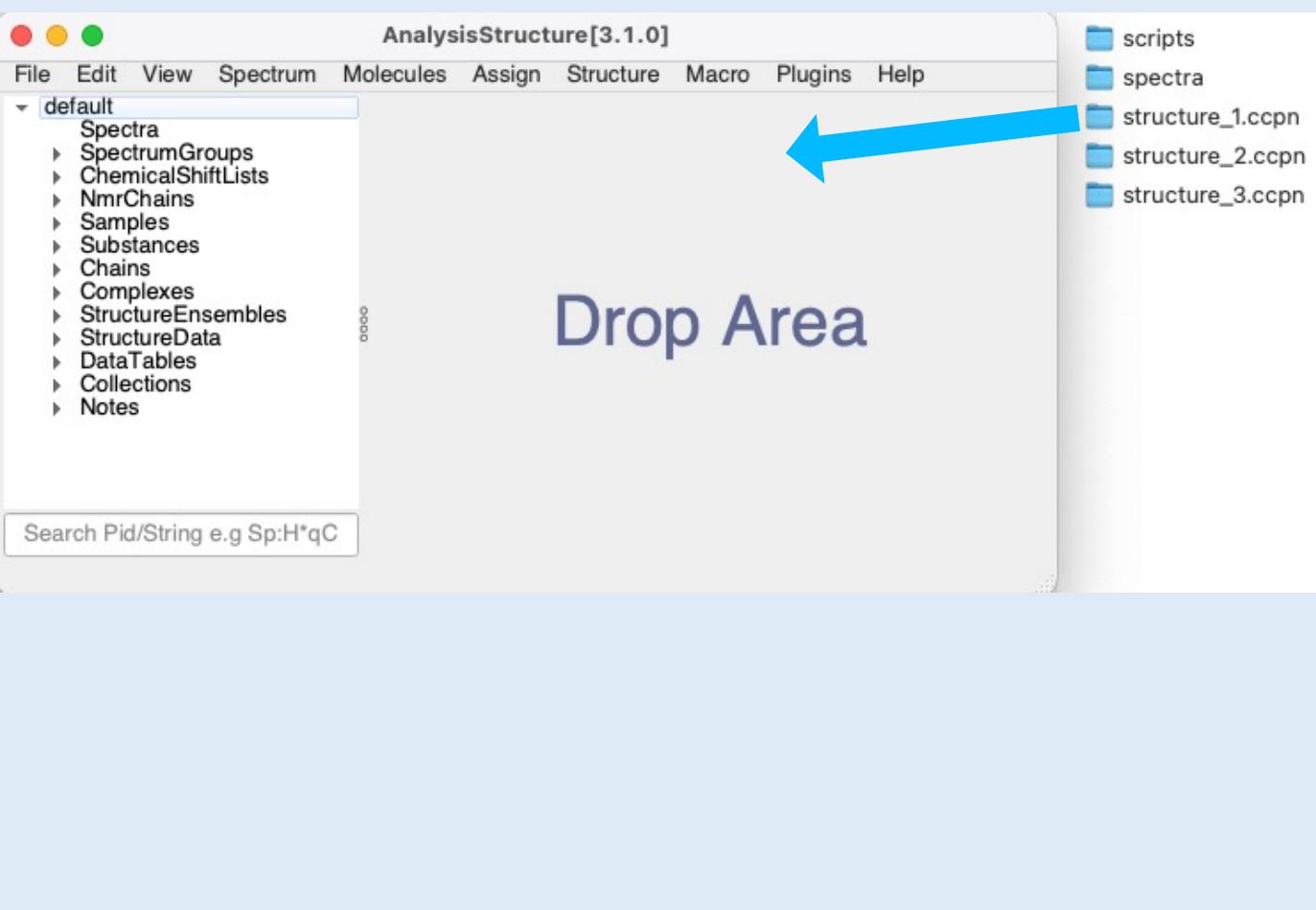
## Start CcpNmr Analysis V3

Apple users by double clicking the icon *CcpNmrAnalysis* or using the “linux” way (see below)



Linux users by using the terminal command: *bin/structure*

Windows users by double-clicking on the *structure.bat* file



## 1A Open Project structure\_1 ccpn

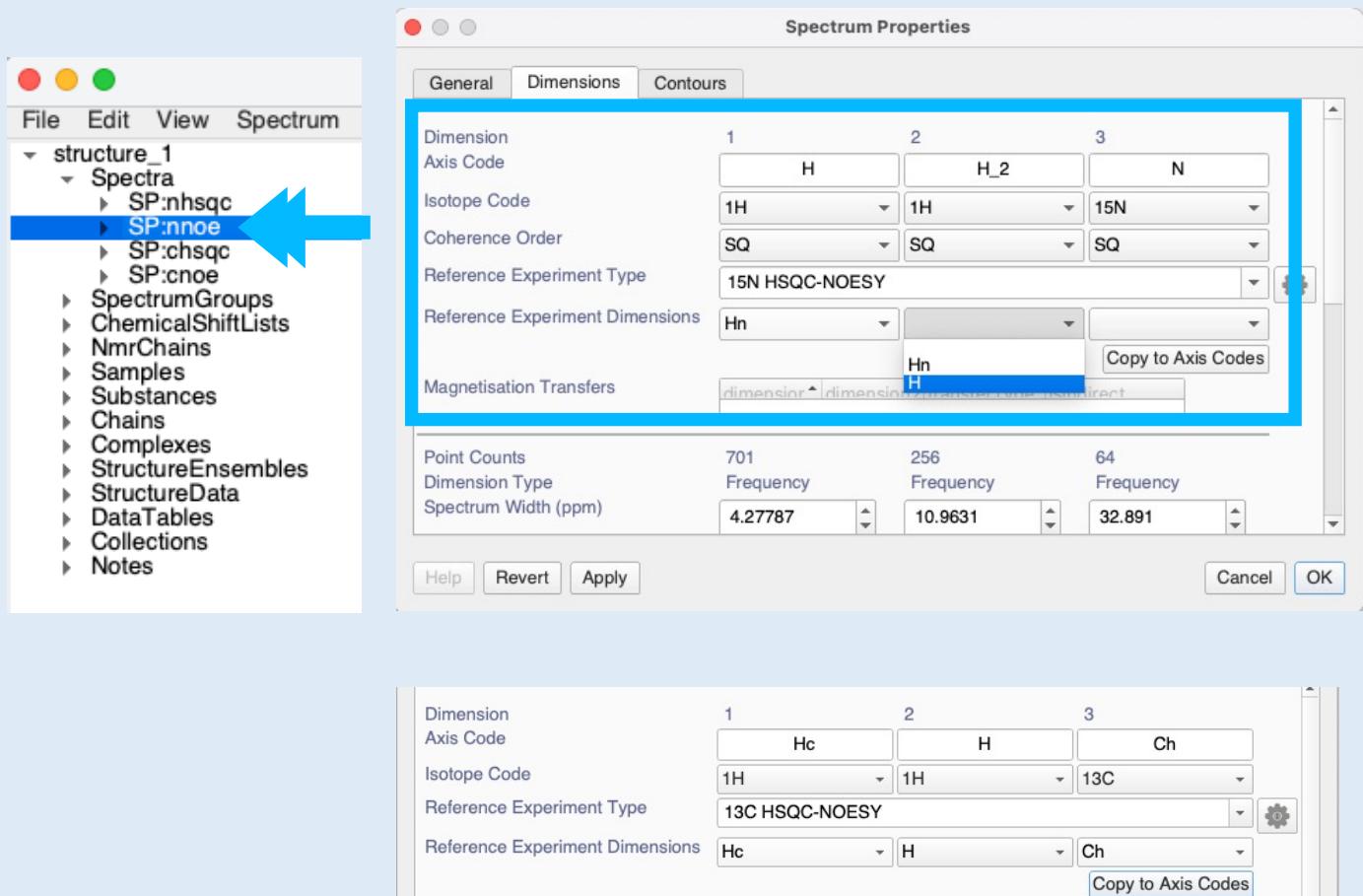
- Go to **File / Open...** and select the **structure\_1 ccpn** folder in the tutorial data directory

OR

- Drag & Drop the **structure\_1 ccpn** folder into the **Drop Area** of the program.

You may be prompted to go to **Spectrum / Validate Paths** or use shortcut **VP** to select the correct paths for to the spectrum files.

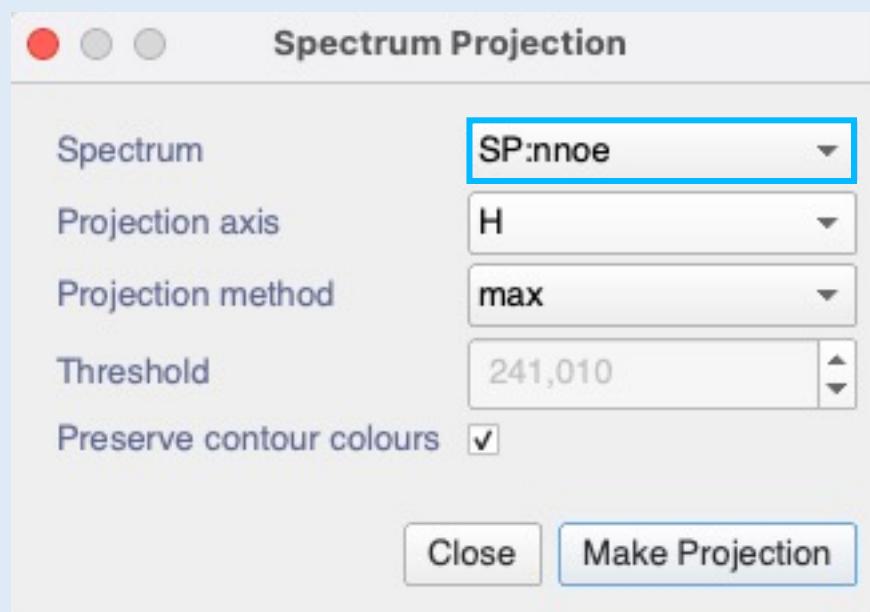
This project already contains a Chain (**MC:A**) which specifies the sequence of our protein, a Chemical Shift List (**CL:shifts**) which contains all the assigned chemical shifts and two NOESY spectra (**SP:cnoe** and **SP:nnoe**) in addition to carbon and nitrogen HSQC spectra (**SP:chsqc** and **SP:nhsqc**).



## 1B Setting experiment types

To access the full functionality of Analysis V3 you should specify the **Experiment Types** and **Experiment Dimensions** of your NOESY spectra. This will ensure that magnetization transfers are correctly defined for each dimension.

- Double-click on the **SP:nnoe** in the sidebar to bring up the **Spectrum Properties** dialog box.
- Click on the **Dimensions** tab.
- Make sure **15N HSQC-NOESY** is selected from the **Reference Experiment Type** drop-down menu.
- In the **Reference Experiment Dimensions** select **Hn** for the first dimension, **H** for the second and **Nh** for third.
- (*Optional but recommended*) Click on **Copy to Axis Codes** button to propagate the selected **Reference Experiment Dimensions** names to the **Axis Codes**.
- Repeat this for the **SP:cnoe** spectrum using the **Reference Experiment Dimensions** names shown in the figure above.



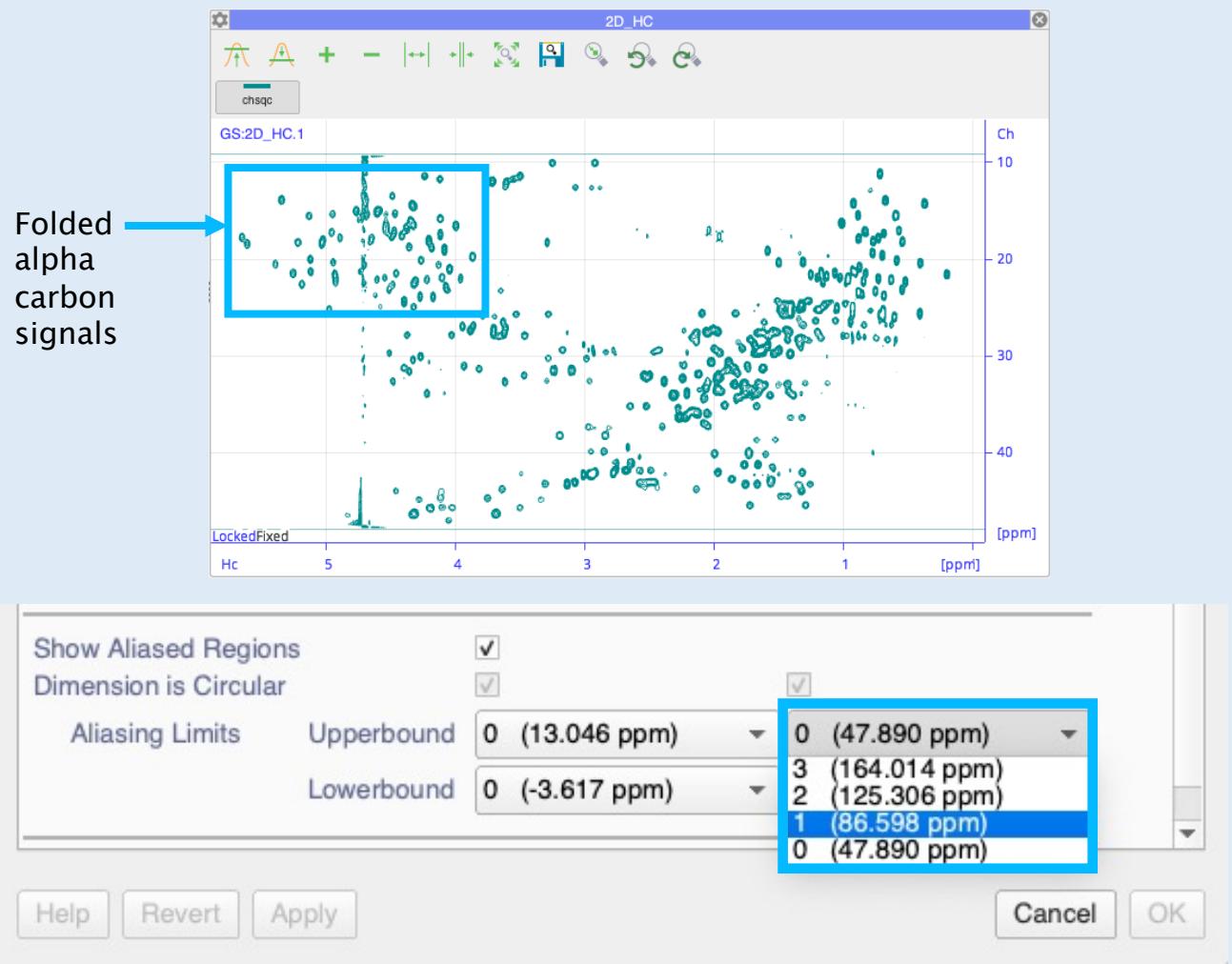
## 1C Making 2D projections of 3D NOESY spectra

Creating a 2D projection of your 3D NOESY spectra is an easy way to check if your 3D spectrum is correctly aligned with your 2D HSQC spectrum or to investigate the sweep width of your spectrum.

- Go to **Main Menu > Spectrum > Make Projection...** (shortcut **PJ**)
- Change **Spectrum** to **SP:n-noesy** and **Projection axis** to **H**, so that the projection is made along the NOESY dimension. Leave the other settings as they are. The projection routine will save new spectrum in hdf5 format alongside your original 3D spectrum (unless the folder is Read-Only, in whichy case it will be saved in the spectra folder inside your project).
- Click **Make Projection**.

You will see that an additional spectrum has been added to the sidebar.

You can now drag the **SP:nnoe\_projection** spectrum into the Drop Area, adjust the contours and then drag the **SP:hsqc** spectrum on top. You will see that they overlap well, so the spectra do not need to be re-referenced.

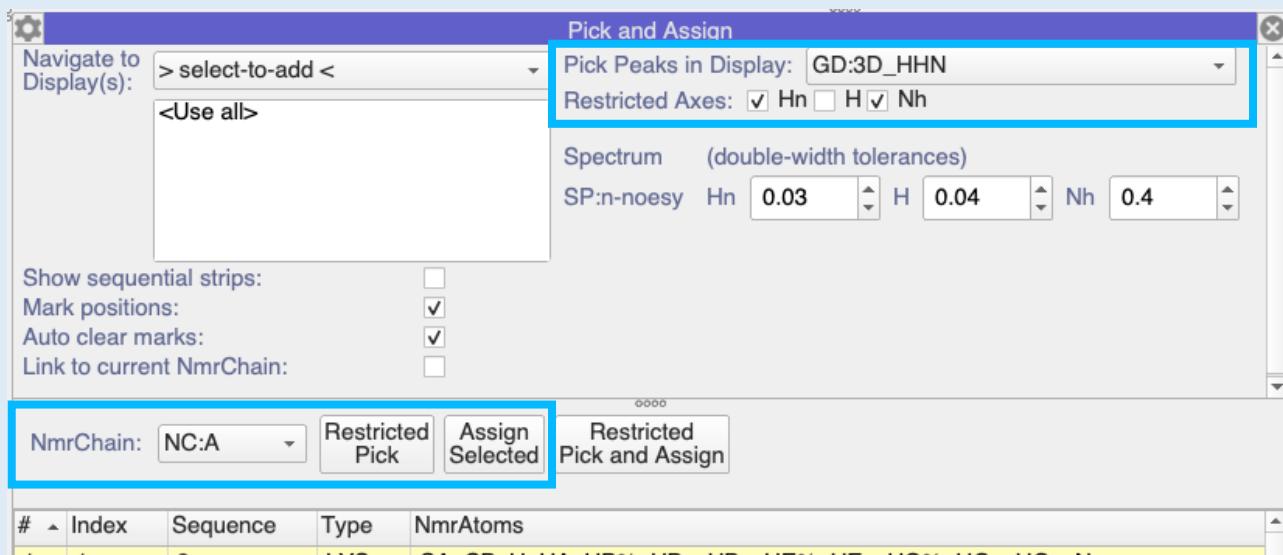


## 1C Unaliasing (unfolding) spectra

The  $^{13}\text{C}$ -NOESY dataset used in this tutorial has a folded carbon dimension.

We can use the  $^{13}\text{C}$  HSQC ('SP:chsqc') spectrum to assess how much we need to unfold the 3D c-noesy.

- Drag your 'SP:chsqc' spectrum into to the Drop Area to open it in a spectrum display.
- You will notice that there are a number of peaks for which the carbon chemical shifts are not correct (see above).
- Double-click on SP:chsqc to open the Spectrum Properties pop-up.
- In the Dimensions tab scroll down to the bottom where you can see Aliasing Limits and change the Upperbound to 1 and click OK.
- You can see that a copy of the spectrum has appeared above the original. Note that peaks can only be picked across one aliased region of the spectrum at a time. Folded peaks are shown with a faded cross symbol.
- Open the Spectrum Display Settings panel to change how aliased peaks are displayed.
- Set the aliasing regions for the sp:cnoe spectrum (Upperbound to 1).



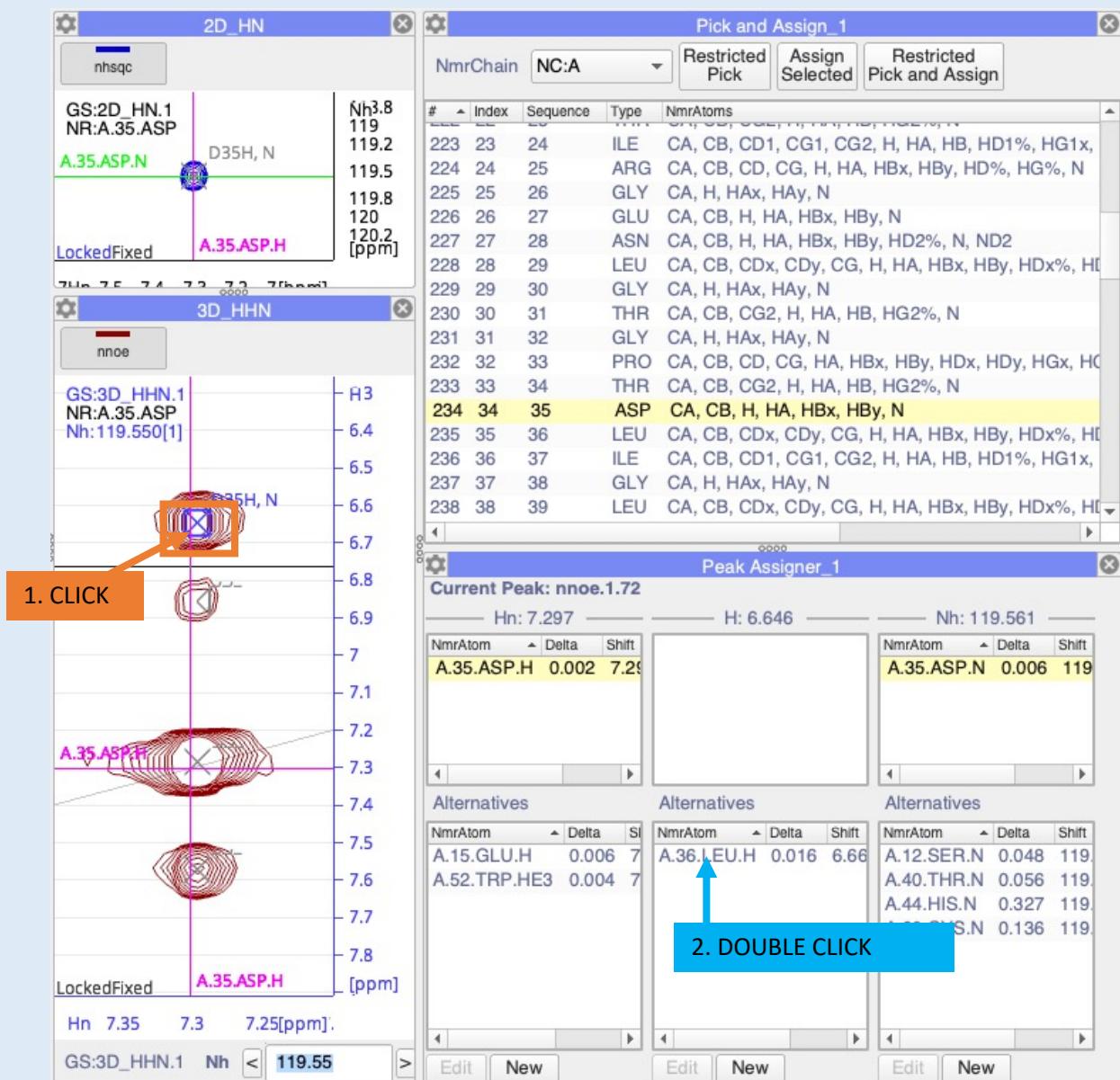
## 2A Manual picking of $^{15}\text{N}$ -NOESY peaks

As well as peak picking your NOESY spectra automatically (see sections 2C and 2E), you can also do this manually. This may enable you to have a "cleaner" peak list with less noise, or you may like to use the opportunity to assign a few peaks at the same time, e.g. those characteristic of secondary structure elements.

- Open Spectrum Displays of only the **SP:nnoe** and **SP:nhsqc** spectra.
- Go to **Main Menu > Assign > Pick and Assign** or use shortcut **PA**.
- Open the gear box setting of the Pick and Assign module and select **GD:3D\_HHN** from the **Pick Peaks in Display** drop-down menu and untick **H** in the **Restricted Axes** row.
- Close the Settings and select **NC:A** from the **NmrChain** drop-down menu.
- **Double-click** on the **35 ASP** row.  
Both spectra will navigate to the 35 ASP HN position.
- Click on **Resticted Pick** to pick all peaks in 35 ASP strip of the 3D spectrum.
- If desired, click on **Assign Selected** (while the peaks are still selected) to assign the peaks to 35 ASP in the **Nh** and **Hn** dimensions.

# NOESY peak picking

structure\_1



## 2B Manual assigning of N-NOESY peaks

- Go to Main Menu > Assign > Peak Assigner or use shortcut AP
- Click on the peak just above the diagonal of the 35 ASP strip. You can see that one of the options for assigning the H dimension in the Peak Assigner is 36 LEU. Double-click on this to assign A.36.LEU.H to this peak dimension.
- Add another strip to the spectrum display (green plus sign on the toolbar).
- Double click on the previous row with 34 THR in the Pick and Assign module. This will navigate the spectrum in the next active strip to the NH position for 34 THR. Pick and assign the peaks as for 34 THR.



## 2C Pick 3D peaks from 2D roots

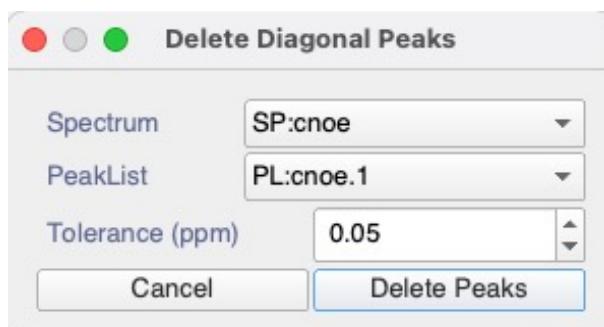
An alternative to manual picking is to pick a 3D based on a set of 2D peaks all in one go, rather than one residue/peak at a time.

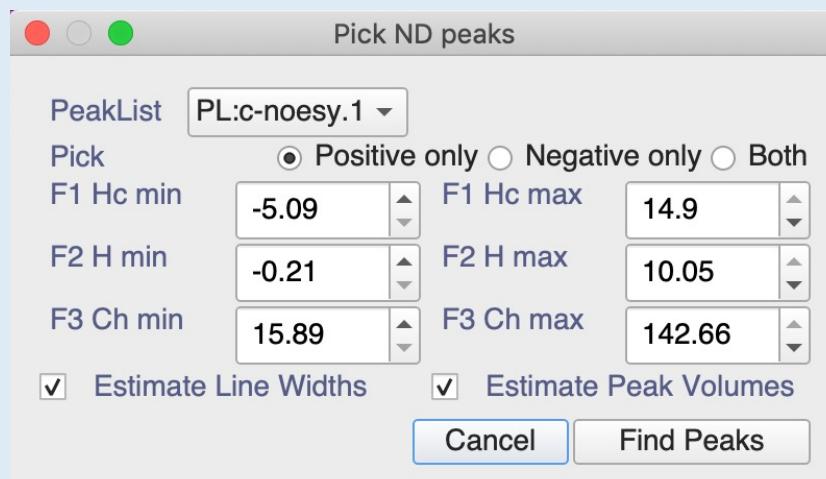
- Close all your modules except for the 2D **SP:nhsqc** Spectrum Display and the 3D **SP:nnoe** Spectrum Display.
- Select some or all of the peaks in your 2D HSQC.
- Go **Main Menu > Structure > Restricted Peak Pick** and select the **PL:nnoe.1** peak list as your target peak list.
- Click on **Pick Restricted Peaks**.

## 2D Remove diagonal peaks

Diagonal peaks are currently not excluded when picking peaks. If you would like to remove these, then:

- Go to **Main Menu > Structure > Remove Diagonal Peaks** and select the spectrum, peak list and tolerances of your choice, e.g.
- Click on **Delete Peaks** to remove the diagonal peaks.





## 2E Automatic peak picking

This may be particularly suited to structure calculation programs/algorithms which do not use prior assignments and deal well with noisy data.

- Close the **SP:nnoe** spectrum for now and open the **SP:cnoe** spectrum instead.
- Move to the methyl region by typing **11.0** in the Z-navigation toolbar.
- Inspect the **SP:cnoe** spectrum by going through the Z-planes. Either use the arrows on the navigation toolbar or use **Ctrl(Cmd on Mac)+mouse wheel**.

Remember that peak picking is done on the contours currently visible in your spectrum, so you will need to ensure your spectrum is at a suitable contour level and that negative contours are on/off as required:

- **Double-click** on the **SP:cnoe** in the sidebar, raise the contour levels to 650,000 and turn off the negative contours. Close the pop-up again.

Pick peaks in the methyl regions:

- Go to **Spectrum > Pick Peaks > Pick ND Peaks...** or use shortcut **PP**.
- Pick the following two regions using the **PL:cnoe** peak list and **Positive only**:

Region 1:

**Hc:** 0.0 – 1.5  
**H:** 0.0 – 10.05  
**Ch:** 10.0 – 20.0

Region 2:

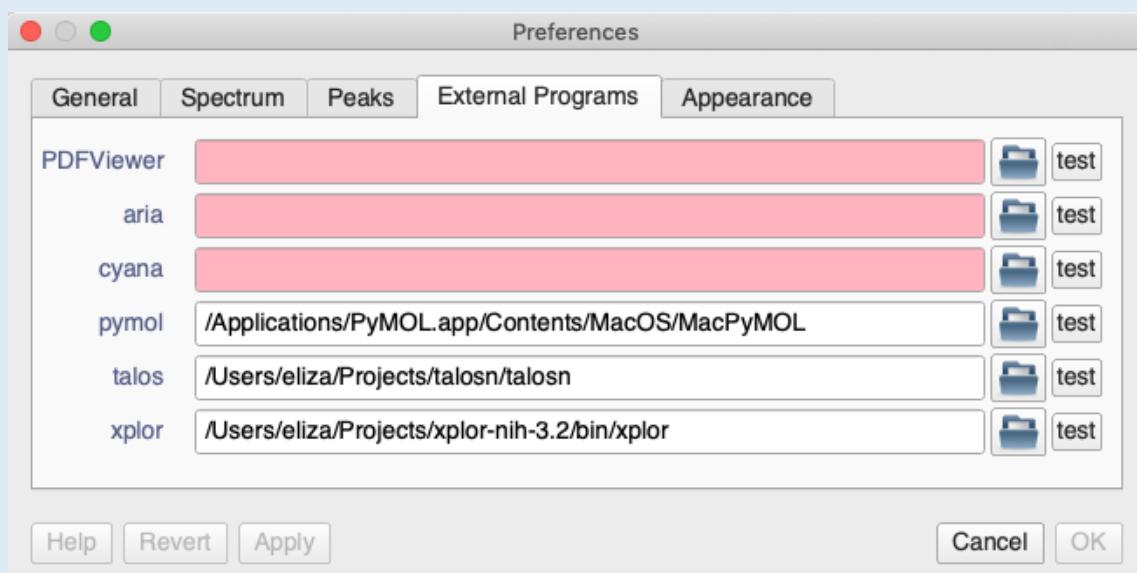
**Hc:** 0.0 – 2.6  
**H:** 0.0–10.05  
**Ch:** 22.0 – 26.0

You can now remove diagonal peaks using the process outlined in Section 2D.

# 3 Structure Calculation Setup

structure\_2

## Open Structure\_2.ccpn



## 3A Setting external program paths

Once both n-noesy and c-noesy PeakLists are ready, we can set up a structure calculation. We will create a directory with a NEF file containing all the information needed to perform the structure calculation, as well as all the XPLOR-NIH scripts needed to drive the calculation. You can follow the tutorial steps if you have installed XPLOR-NIH and TalosN on your computer, otherwise you can use folders that were already prepared in the workshop materials.

- Open the **structure\_2.ccpn** project from the tutorial data.  
This project now contains fully peak picked NOESY spectra.
- Go to **File > Preferences > External Programs** and enter the paths to the PyMol, talosN and xplor\_nih executables if you have them.
- Click **OK** to close the window.

The paths should look roughly like this:

/Users/user1/programs/talosn/talosn

/Users/user1/programs/xplor-nih-3.5/bin/xplor

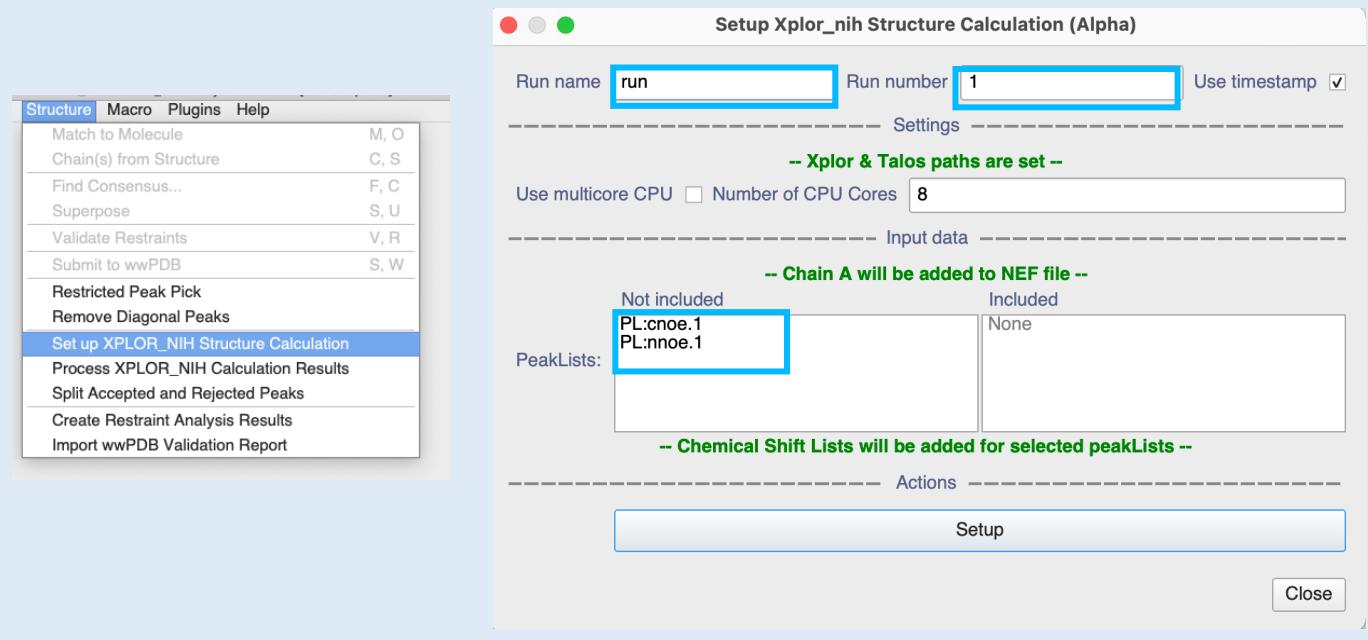
/Applications/MacPyMOL.app/Contents/MacOS/MacPyMOL (Mac)

C:\Program Files\PyMOL\ (Windows)

/usr/lib/python2.7/dist-packages/pymol (Linux)

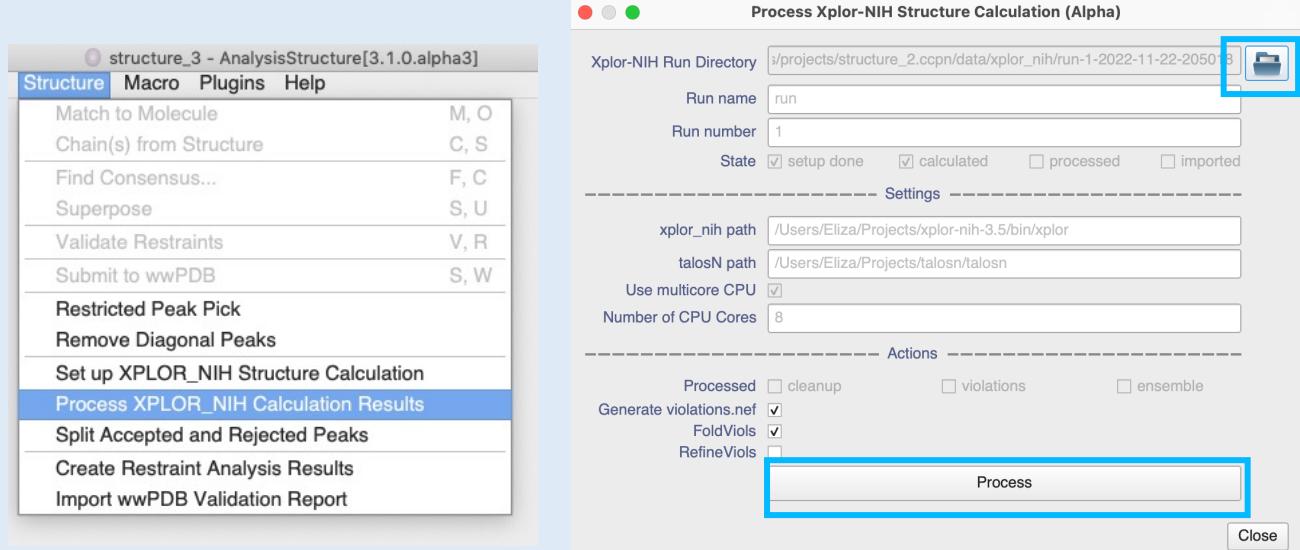
# 3 Structure Calculation Setup

structure\_2



## 3B Setting up and running Xplor-NIH

- Go to **Main Menu > Structure > Setup XPLOR\_NIH Structure Calculation**. You will see a pop-up window.
  - Define your **Run name** (e.g. ‘myRun’) and ‘Run number’ (default is ‘1’); leave the **Use timestamp** checked.
  - As the project has only one chain, you do not need to define it.
  - Drag the **PL:nnoe.1** and **PL:cnoe.1** peakLists from **Not included** to **Included**.
- Note:* Only peak lists belonging to spectra with **through-space magnetization transfer** will be listed in the pop-up.
- Chemical shift list will be added automatically for selected peaks lists.
  - (*Optional*) If you wish to run it on a multi core processor you can tick the **Use multicore CPU** tick box and type the number of cores you wish to use in **Number of CPU Cores** box.
  - Click **Setup**. In the structure\_2 project (at ‘structure\_2 ccpn / data / xplor\_nih /’) a new directory ‘myRun-1-date-timestamp’ (e.g. ‘myRun-1-2022-02-28-223619’) will have been created. This directory should contain files copied from the xplor\_nih installation, scripts for controlling each part of the calculations, as well as:
    - **input.nef** – a NEF file containing chain, chemical shift list, nnoe and cnoe peak lists.
    - **script.sh** – a shell script for running the calculation.
  - To start your calculation, you need to execute the shell script (by typing: `./script.sh`) in a terminal window in the setup directory.
  - These calculations are computationally very intensive; it is therefore advisable to run them on a multiprocessor system, if possible.



/structure\_2 ccpn /data/xplor\_nih/run-1-2022-11-22-205018

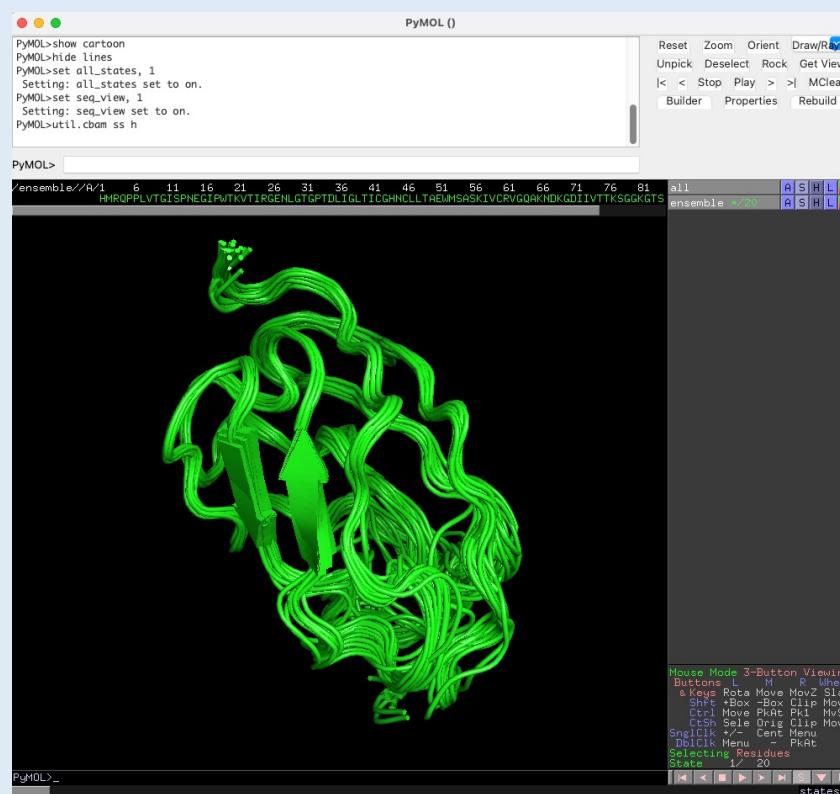
## 4A Processing Xplor-NIH structure calculation results

The XPLOR-NIH calculation yields a vast amount of data, that we first rearrange and pre-process.

- Go to **Main Menu > Structure > Process XPLOR\_NIH Calculation.**  
You will see a pop-up window.
- Click the ‘file selection’ button and select ‘**run-1-2022-11-22-205018**’ in the **structure\_2 ccpn /data/xplor\_nih** folder which contains the data from a completed structure calculation
- Leave the ‘Generate violations.net’ and ‘FoldViols’ boxes ticked.
- Click **Process**.

This will create directories like ‘pass2’, ‘pass3’ and ‘fold’ in the ‘run-1-2022-11-22-205018’ directory. The **fold** directory contains the folded protein ensemble file, to be used in step **4B**. Scripts, logs and talosN files can be found in the appropriately named directories.

We also have provided the results of this step in the **xplor\_nih.done/run-1-2022-11-22-205018.done** directory; this also contains wwPDB validation reports.



## 4B Assessing structures in a molecular viewer

The first step when analyzing results should be to inspect the structure models in a molecular viewer, ensuring all models are inspected.

- Open PyMol
- In your file browser, go to: structure\_2 ccpn / data / xplor\_nih.done / run - 1 - 2022-11-22-205018.done / fold
- Drop **ensemble.pdb** onto your opened Pymol window.
- To have a better view of the structure you may wish to execute following commands in the command window:

```
dss
show cartoon
hide lines
set all_states, 1
set seq_view, 1
util.cbam ss h
```

The screenshot shows two windows side-by-side. The left window is titled 'Import Nef' and displays the contents of an imported NEF file. It includes a sidebar with project files like 'out.nef', 'chains', 'chemicalShiftLists', 'restraintTables', 'peakLists', and 'restraintLinks'. A central tree view shows 'NEF MOLECULAR SYSTEM' and 'nef\_sequence'. A green plus sign icon with an orange border is highlighted with a red box and an arrow pointing to it, labeled 'click to view'. Below this, a callout box says 'Field to view individual parts (saveframes) of the NEF file'. The right window is titled 'NEF Importer' and shows a 'StructureData' section with items 'RT:cnoe', 'RT:nnoe', and 'RT:1'. A green plus sign icon is also present here. A callout box on the right says 'Fields to organise data in the project'. At the bottom of both windows are 'Cancel' and 'Import' buttons.

Contents of imported NEF file

Fields to organise data in the project

Ticked for import

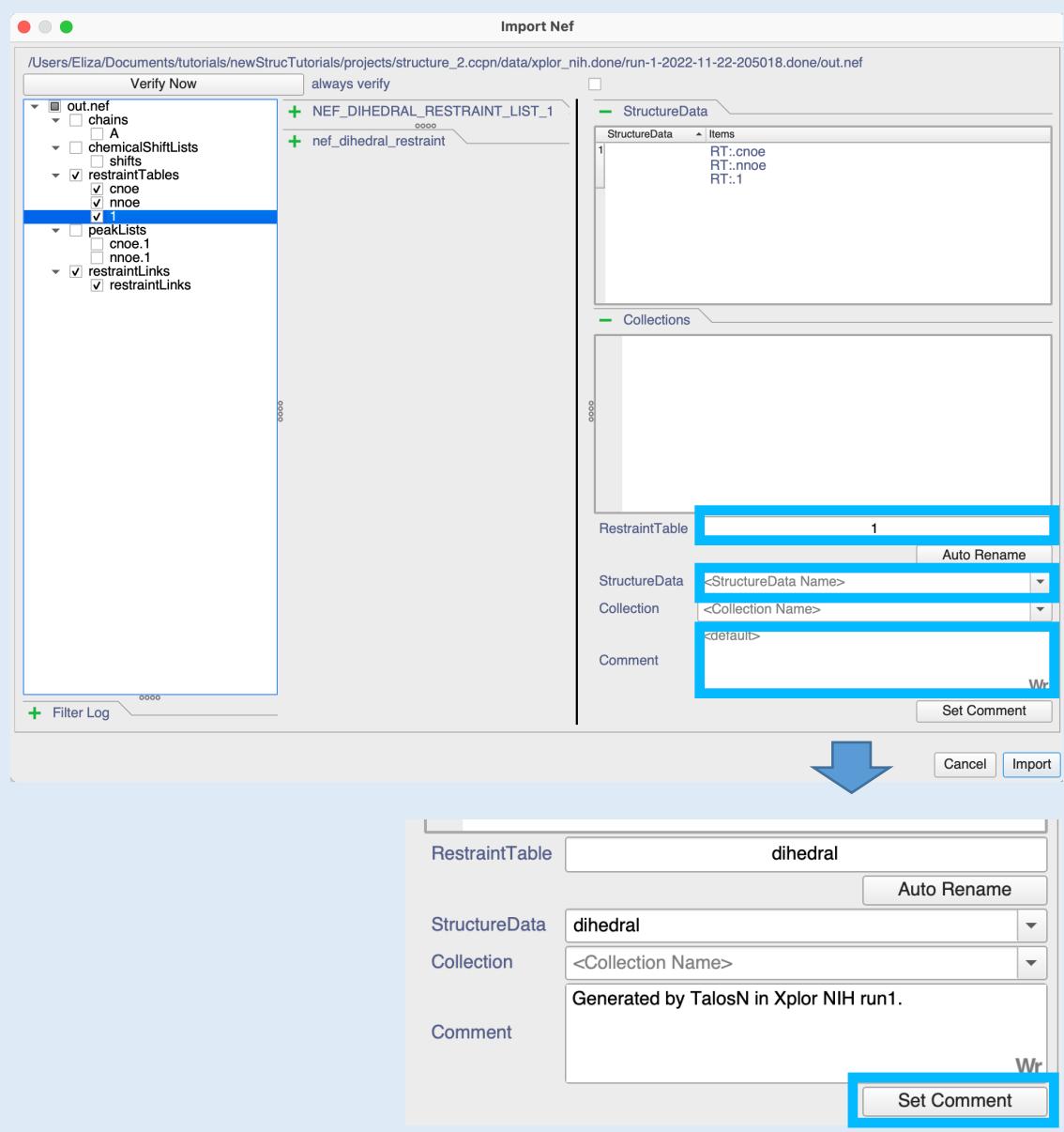
Selected for edit

#### 4C Loading generated restraints from NEF

- With the **structure\_2 ccpn** project open go to **File > Import > Nef File**
- In the resulting window select:  
**structure\_2 ccpn / data / xplor\_nih.done / run-1-2022-11-22-205018.done / out.nef** (or drag this file onto the sidebar).
- Click **Import** at the prompt.
- After a moment you will see the NEF Importer dialog window.
- Items chosen for import must be ticked
- You can select item(s) for editing by clicking on the item (the background will be highlighted in blue). If you start to edit an item, it will be ticked by default.
- You can view/inspect the contents of items by clicking on the green plus sign in the middle of the pop up window.

# Importing Results

structure\_2

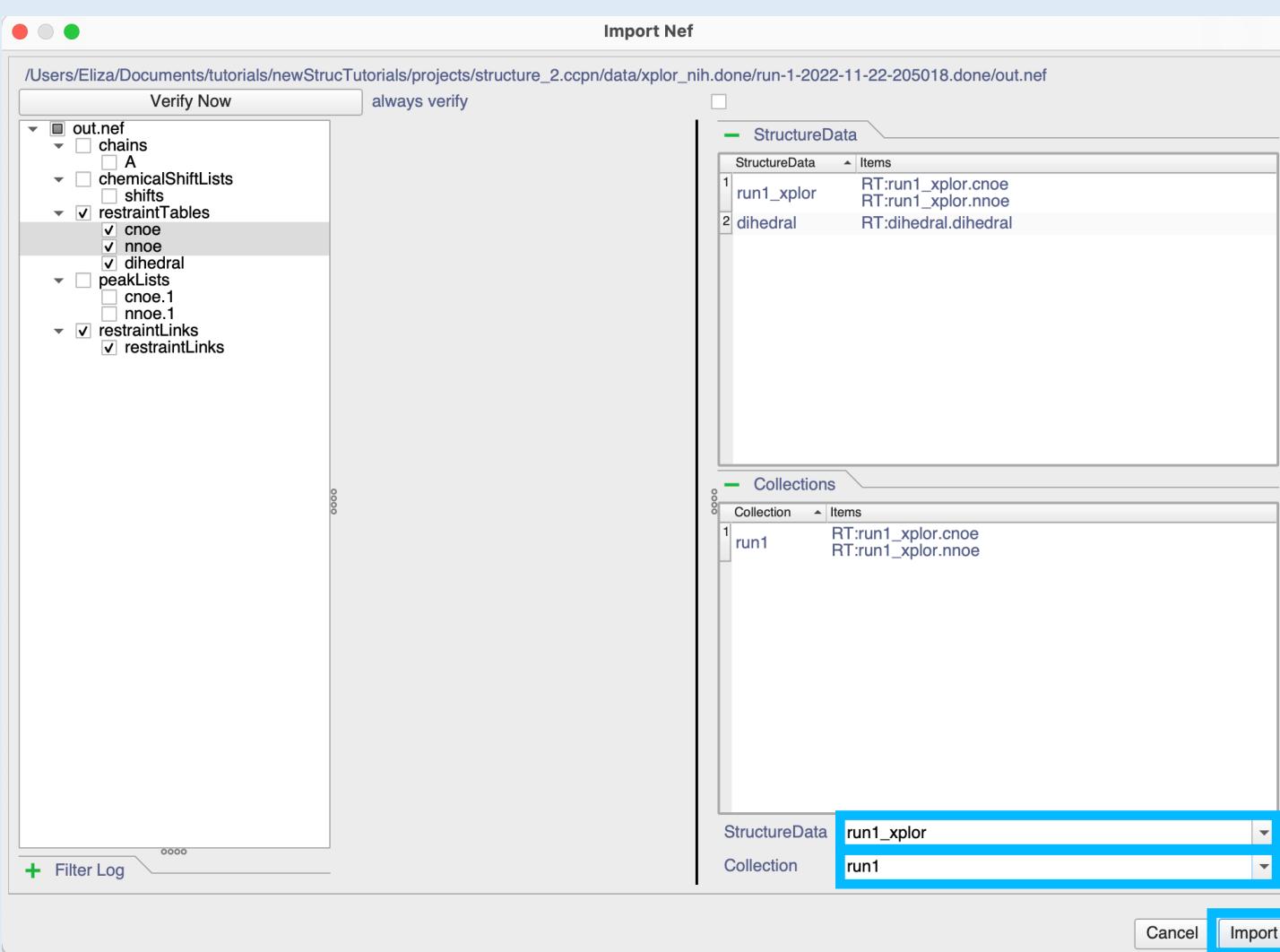


## 4C Loading generated restraints from NEF (continued)

- Tick **restraintTables**, this will highlight the whole group: **cnoe**, **nnoe** and **1**.
- Select (click) on **1** in the list.
- On the right hand side in the **RestraintTable** field change the name to **dihedral** (or another name of your choice). **Press 'Return' to confirm**.
- In the **StructureData** field type **dihedral**, which will become the name for the data group created in your project. **Press 'Return' to confirm**. You will see that in the **StructureData** tab above, that a **dihedral** group was created containing the **RT:dihedral.dihedral** restraint list:

| StructureData |                            |
|---------------|----------------------------|
| StructureData | Items                      |
| 1             | RT:cnoe<br>RT:nnoe<br>RT:1 |
| 2 dihedral    | RT:dihedral.dihedral       |

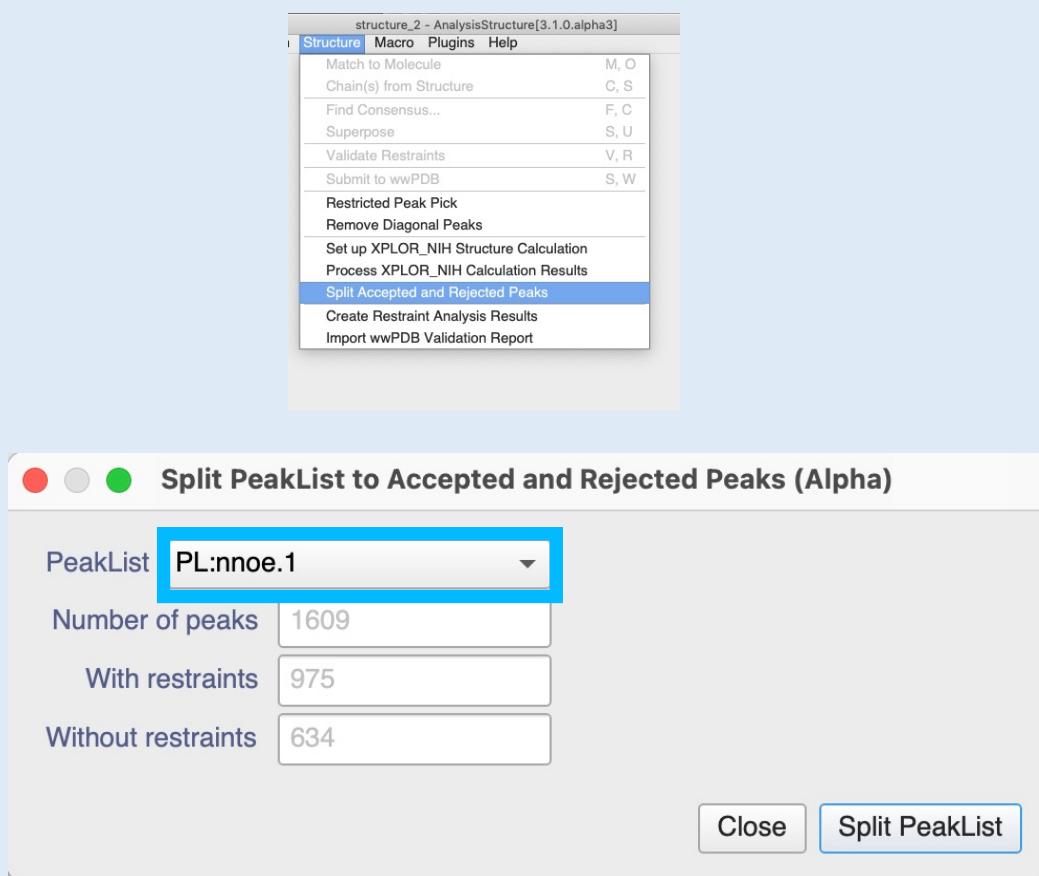
- You may add a comment (e.g. "Generated by TalosN in Xplor NIH run1"). Click **Set Comment** to confirm.



## 4C Loading generated restraints from NEF (*continued*)

- Select **c noe** and **n noe**.
- In the **StructureData** field type **run1\_xplor**. Press 'Return' to confirm.  
You will see that in the StructureData tab above the **run1\_xplor** group was created with the **RT: run1\_xplor.c noe** and **RT: run1\_xplor.n noe** restraint lists.
- In the Collection field type **run1**. Press 'Return' to confirm.
- Ensure that **restraintLinks** are ticked (they should be ticked by default when you tick the **restraintTables**) and click **Import** to complete.

In this step we have created a new **Collection**. A Collection is a data item in the CCPN project that contains other data items, we can think of it as a box for grouping data. It can accommodate a mix of **any** kind of data, including Collections themselves.



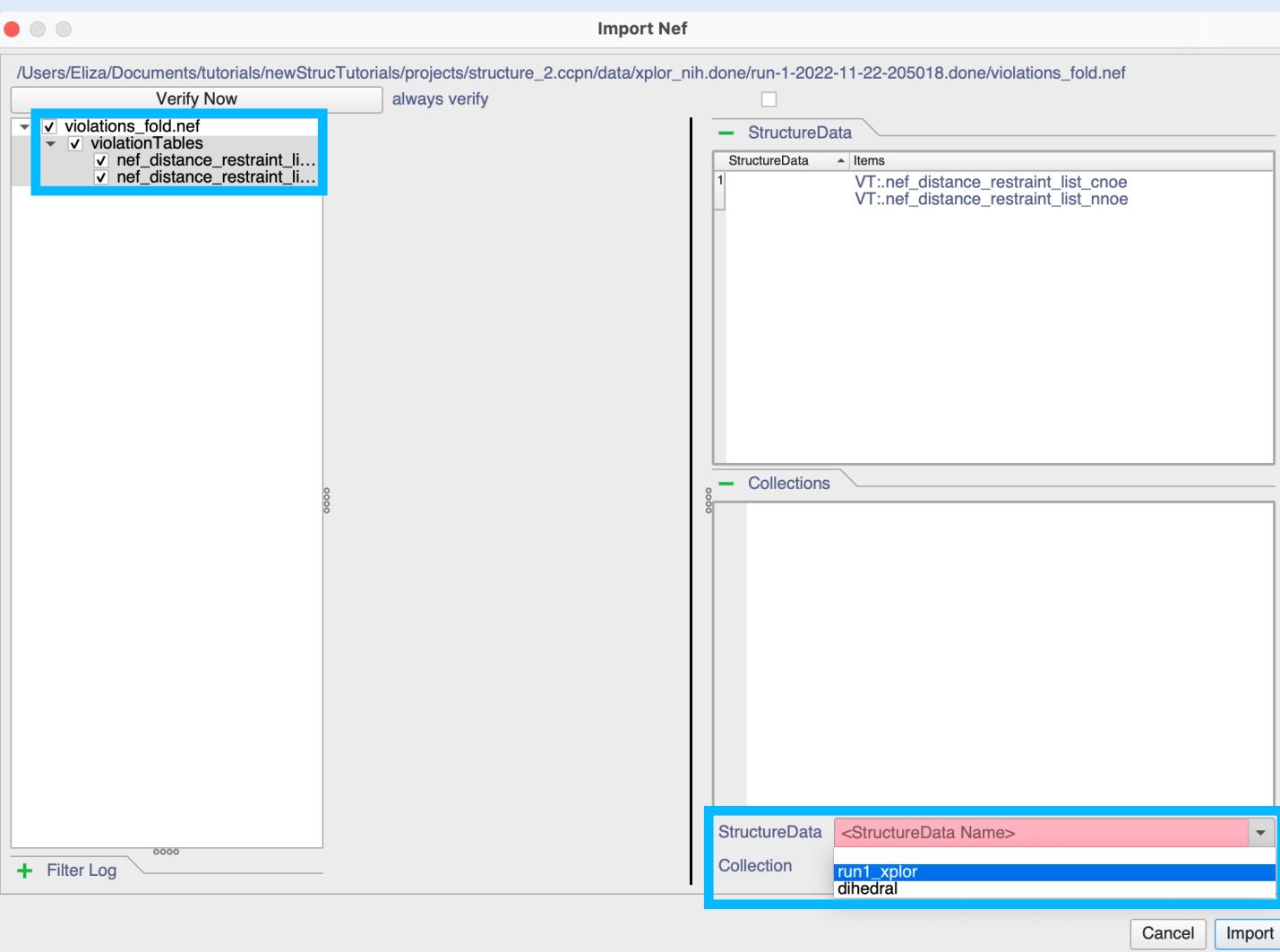
## 4D Split accepted and rejected peaks into separate peak lists

The list of restraints used for your final structure calculation will have been derived from only a subset of the peaks that you submitted to XPLOR-NIH. Peaks which were judged to be noise or other artefacts will have been **rejected** and only those judged to yield consistent restraints will have been **accepted**. Because the restraints imported from XPLOR-NIH are linked back to their original peaks in the NEF file, we can now partition our peaks into those which were accepted and those which were rejected.

This is by no means obligatory but can be a helpful way to manage your data as you proceed through further rounds of structure calculations.

- Go to Main Menu > Structure > Split Accepted and Rejected Peaks
- Select the PL:cnoe.1 (or 1 depending on your prior import) peak list  
This will probably take some time as the peaks will be analysed. Click on **Split peaks** to perform the partitioning.
- Repeat for the PL:nnoe.1 peak list.

# Importing Results



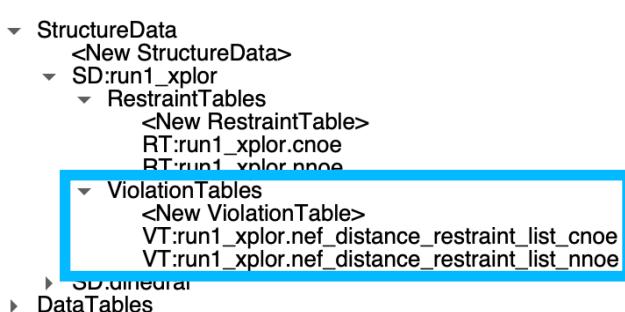
## 4E Importing violations from NEF

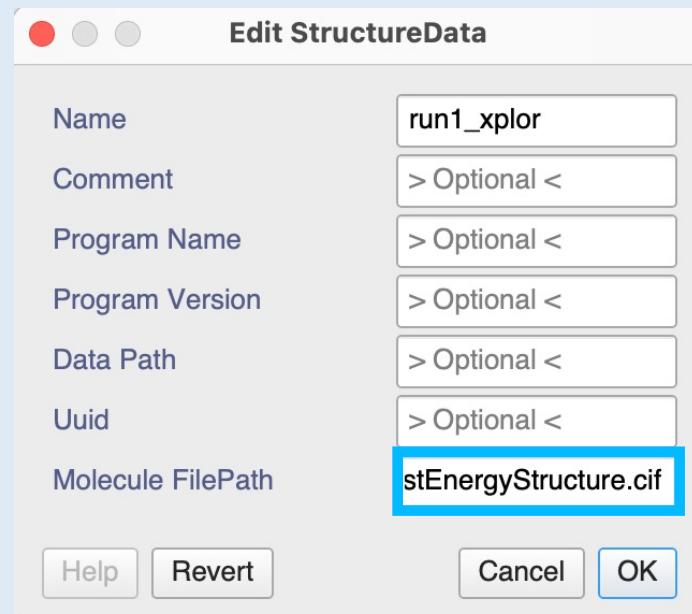
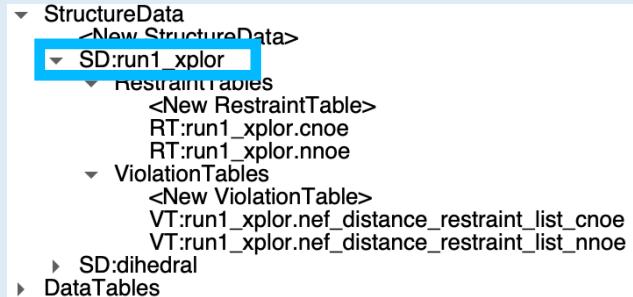
The **structure\_2 ccpn / data / xplor\_nih.done / run-1-2022-11-22-205018.done** directory contains a second NEF file called **violations\_fold.nef**. This contains the information about violated restraints from the final XPLOR-NIH structure calculation.

- Go to **File > Import > Nef File** and select the **structure\_2 ccpn / data / xplor\_nih.done / run-1-2022-11-22-205018.done / violations\_fold.nef** file or **drag** it onto the sidebar.
- When prompted select **Import**.
- Tick **violationTables** so that both **cnoe** and **nnoe** are ticked.
- Select **run1\_xplor** from the **StructureData** drop-down menu and click on **Import**.

You should now see two new **Violation Tables** in the **SD:run1\_xplor** **StructureData** section

in the sidebar:

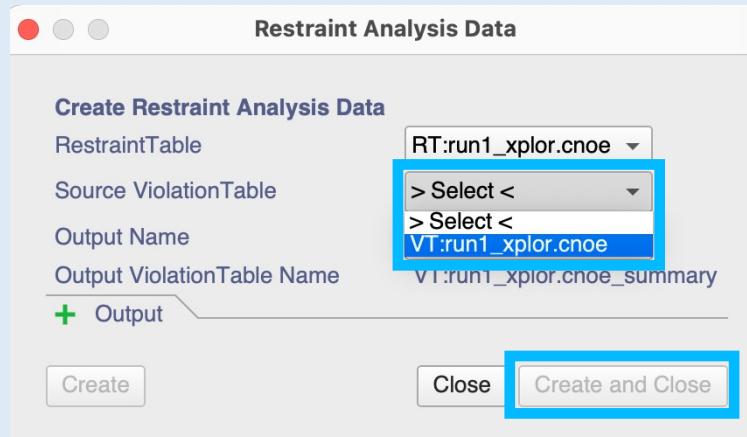
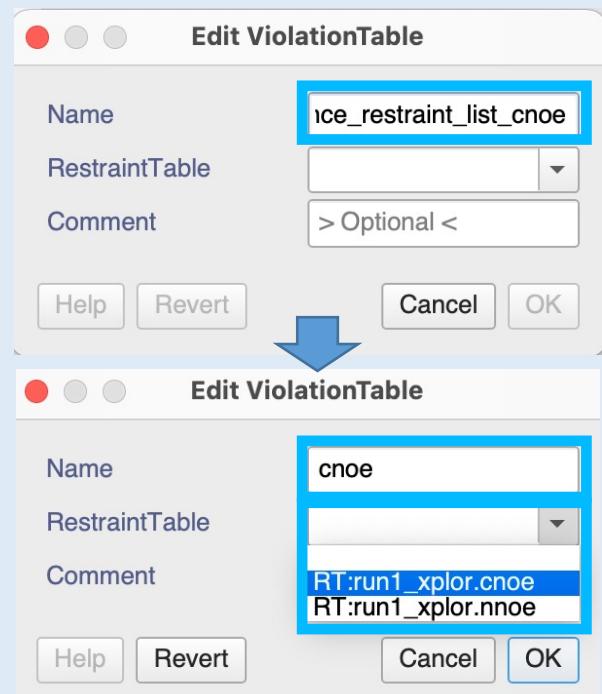
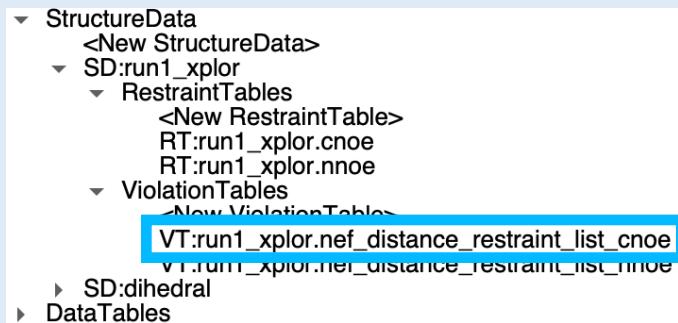




structure\_2 ccpn / data / xplor\_nih.done / run-1-2022-11-22-205018.done / fold / lowestEnergyStructure.cif

## 5A Set Molecule Path

- Double-click on SD:run1\_xplor in the sidebar.
- Set the **Molecule FilePath** to the full file path for the  
.../structure\_2 ccpn / data / xplor\_nih.done / run-1-2022-11-22-205018.done / fold / lowestEnergyStructure.cif file.
- Click **OK** to close the dialog box.



## 5B Creating Restraint Analysis Data

The violation tables imported directly from XPLOR show violations on a per model basis. A more helpful way to study violations is on a per restraint basis. We will therefore create a new set of violation tables which summarise the per model data per restraint.

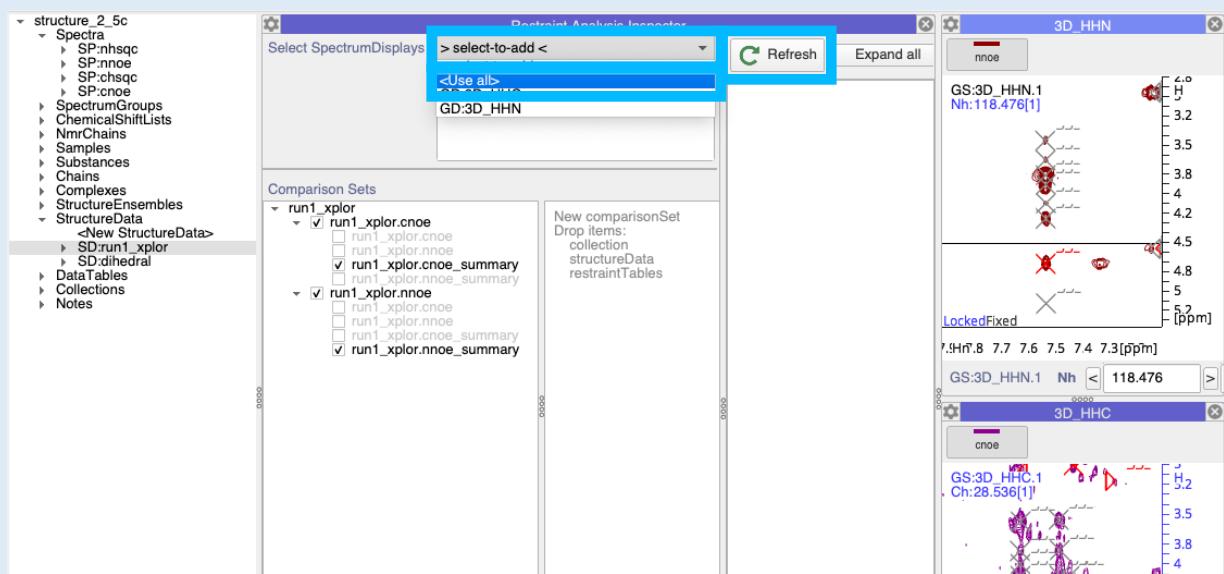
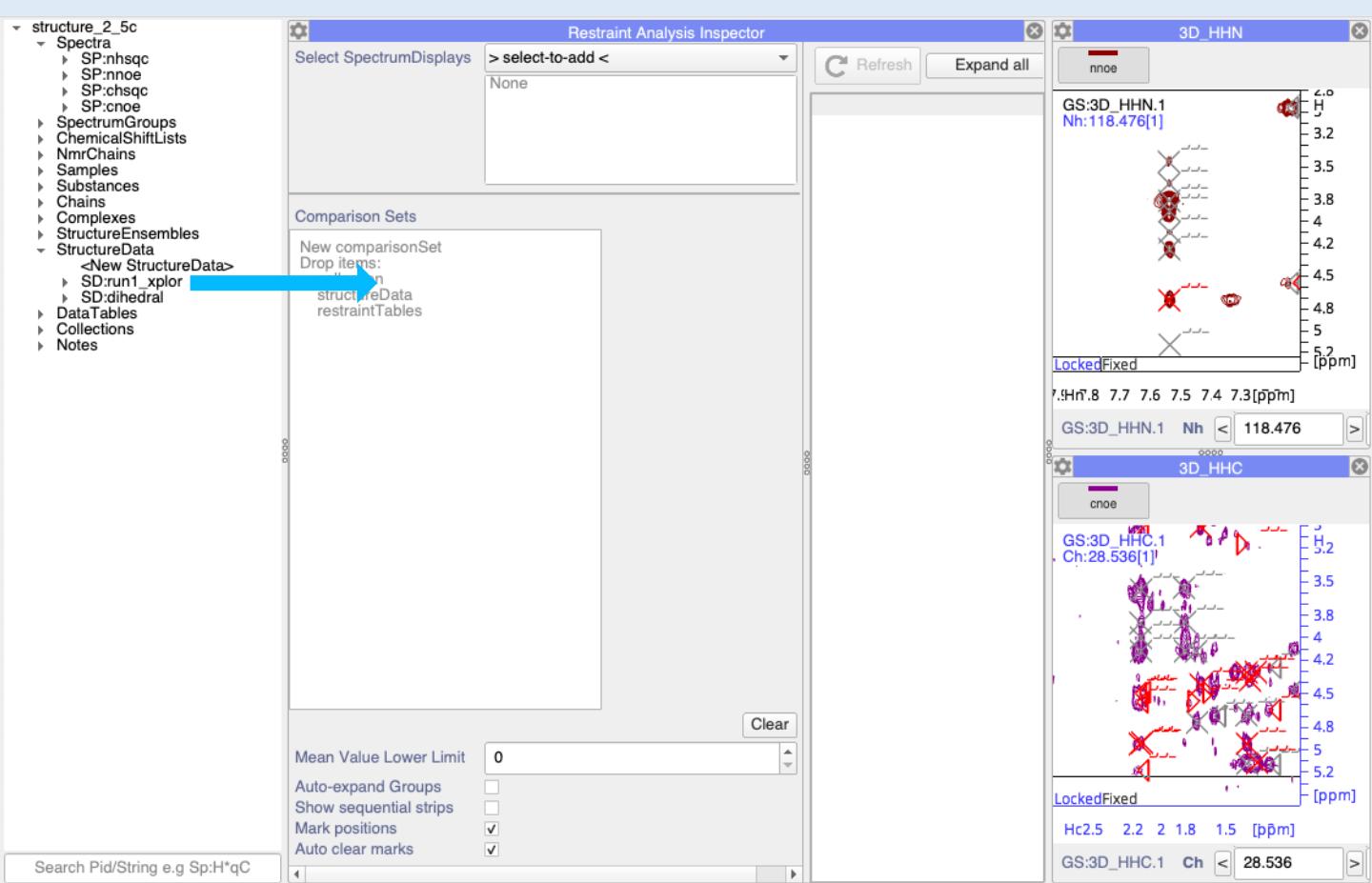
- Double-click on **VT:run1\_xplor.nef\_distance\_restraint\_list\_cnoe** in the sidebar. You can rename the object to **VT:run1\_xplor.cnoe**
- Select the **RT:run1\_xplor.cnoe** restraint table from the **RestraintTable** drop-down menu in order to add this to the violation table metadata. This will tell the program which restraint table the violation data is linked to.
- Go to **Main Menu > Structure > Create Restraint Analysis Data**.
- Select the **RT:run1\_xplor.cnoe** and **VT:run1\_xplor.cnoe** restraint and violation tables from the drop-down.
- Press **Create and Close**.

This may take a minute or so to run. Once it is finished you will see the new violation table **VT:run1\_xplor.cnoe\_summary** in the sidebar.

- Repeat for the nnoe data and then close the module.

# Analysing Results

Structure\_2



## 5C Using the Restraint Analysis Inspector

- Drag your **SP:c noe** and **SP:n noe** spectra into to the Drop Area to open them in two SpectrumDisplays. You will notice that there are some peaks marked red in both displays representing rejected peaks (Section 4D).
- Go to **Main Menu > View > Restraint Analysis Inspector**
- Open the Settings panel by clicking the gear icon
- Drag the **SD:run1\_xplor** StructureData from sidebar in the **Comparison Sets** box. You will see it populates and a new drop area opens.
- In the **Select SpectrumDisplays** pulldown click on **<Use all>**.
- Click the **Refresh** button to populate the table.
- Close the Settings panel with the gear icon

Restraint Analysis Inspector

| Row ▲ | Match      | run1_xplor                |         |       |             |  |
|-------|------------|---------------------------|---------|-------|-------------|--|
|       |            | Atoms                     | Mean    | STD   | Count > 0.3 |  |
| 1     | PK:....1.8 | A.10.GLY.H - A.11.ILE.H   | 0.000   | 0.000 | 0           |  |
| 2     | PK:....13  | + A.9.THR.H - A.11.ILE.H  | 0.000   | 0.000 | 0           |  |
| 4     | PK:....31  | A.57.LYS.H - A.58.ILE.H   | 0.000   | 0.000 | 0           |  |
| 5     | PK:....32  | A.75.THR.H - A.76.THR.H   | 0.000   | 0.000 | 0           |  |
| 6     | PK:....35  | + A.47.LEU.H - A.50.ALA.H | 0.000   | 0.000 | 0           |  |
| 8     | PK:....38  | A.37.ILE.H - A.75.THR.H   | 0.035   | 0.048 | 0           |  |
| 9     | PK:....41  | A.40.THR.H - A.41.ILE.H   | 0.000   | 0.000 | 0           |  |
| 10    | PK:....46  | A.31.THR.H - A.32.GLY.H   | -8...02 | 0.024 | 0           |  |
| 11    | PK:....49  | A.65.ALA.H - A.66.LYS.H   | 0.000   | 0.000 | 0           |  |
| 12    | PK:....51  | A.70.GLY.H - A.71.ASP.H   | -3...02 | 0.063 | 0           |  |
| 13    | PK:....52  | A.76.THR.H - A.82.GLY.H   | 0.010   | 0.026 | 0           |  |
| 14    | PK:....58  | + A.4.GLN.HE... A.7.LEU.H | 0.000   | 0.000 | 0           |  |

## 5D Using the Restraint Analysis Inspector Table (continued)

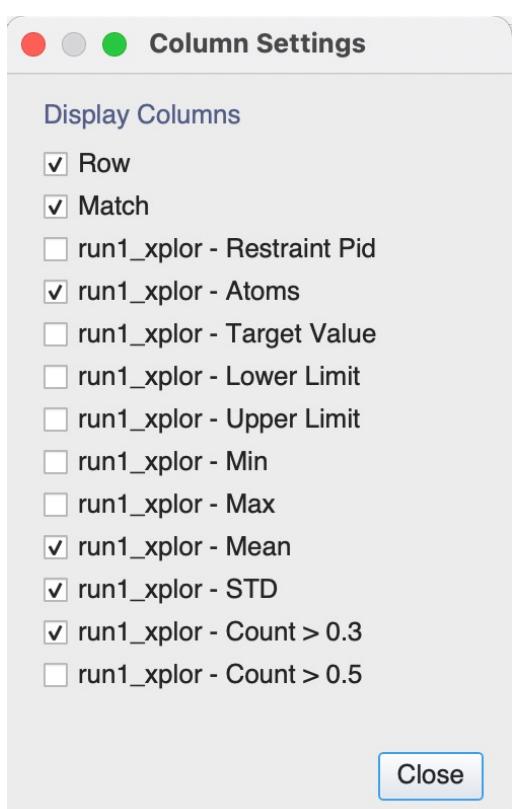
- Click on a column header to sort the table by that column.

Peaks giving rise to ambiguous restraints are indicated by a green plus in the **Match** column and can be expanded to show all their constituent parts:

- Click on a green plus to expand or collapse an individual ambiguous restraint.
- Click on **Expand all** or **Collapse all** to expand/collapse all ambiguous restraints in one go.

You can reduce/change number of columns displayed in the table:

- Right-click on a column header and select **Column Settings...** to select your preferred columns.



The screenshot shows the Restriction Analysis Inspector window with a table of restraints and two 3D NMR spectrum displays.

**Table Data:**

| .46 | A.31.IHR.H - A.32.GLY.H | -8.02 | 0.024 | 0 |  |  |
|-----|-------------------------|-------|-------|---|--|--|
| .49 | A.65.ALA.H - A.66.LYS.H | -8.02 | 0.024 | 0 |  |  |
| .51 | A.70.GLY.H - A.71.LYS.H | -8.02 | 0.024 | 0 |  |  |
| .52 | A.76.THR.H - A.82.GLY.H | -8.02 | 0.024 | 0 |  |  |

**Filter Options:** Filter..., Copy clicked cell value, Delete Selection

**Restriction Analysis Inspector\_1:**

- Refresh, Expand all, Collapse all, Select < Select >
- Atoms: Mean, STD, Count > 0.3
- Row: 11, 26, 225, 230, 464, 467, 666, 677, 685, 741, 757
- Atoms: A.65.ALA.H - A.66.LYS.H, A.66.LYS.H - A.68.ASP.H, A.65.ALA.H - A.66.LYS.HE%, A.65.ALA.H - A.66.LYS.HD%, A.43.GLY.H - A.66.LYS.HE%, A.43.GLY.H - A.66.LYS.HG%, A.63.GLY.H - A.66.LYS.HG%, A.64.GLN.H - A.66.LYS.HE%, A.64.GLN.H - A.66.LYS.HG%, A.47.LEU.H - A.66.LYS.HG%, A.66.LYS.H - A.67.ASN.H
- Include: 66.LYS
- Filter in: ('run1\_xplor', 'Atoms')

**3D\_HHN Spectrum:**

- GS:3D\_HHN.1, Nh:118.476[1]
- Hδ: 8.3, 8.2, 8.1, 8 [ppm]
- LockedFixed

**3D\_HHC Spectrum:**

- GS:3D\_HHC.1, Ch:43.141[1]
- Hδ: 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4 [ppm]
- LockedFixed

**Table Data (Bottom):**

|      |                               |       |       |    |  |
|------|-------------------------------|-------|-------|----|--|
| 839  | A.66.LYS.H - A.67.ASN.HA      | 0.781 | 0.286 | 19 |  |
| 2758 | + A.47.LEU.HD% - A.66.LYS.HD% | 0.701 | 0.528 | 14 |  |
| 3338 | A.66.LYS.HA - A.66.LYS.HG%    | 0.643 | 0.094 | 20 |  |
| 1372 | A.20.THR.HG2% - A.66.LYS.HD%  | 0.513 | 0.344 | 16 |  |
| 846  | A.66.LYS.H - A.66.LYS.HB%     | 0.593 | 0.117 | 19 |  |
| 3811 | + A.46.CYS.HB% - A.66.LYS.HE% | 0.523 | 0.420 | 14 |  |
| 844  | A.66.LYS.H - A.66.LYS.HE%     | 0.512 | 0.190 | 18 |  |
| 3814 | + A.60.CYS.HB% - A.66.LYS.HE% | 0.474 | 0.302 | 15 |  |
| 3073 | A.66.LYS.HG% - A.69.LYS.H     | 0.452 | 0.367 | 11 |  |

**Filter Options:** Filter in: ('run1\_xplor', 'Count > 0.3'), Search, Reset, Close

**3D\_HHC Spectrum:**

- GS:3D\_HHC.1, Ch:42.235[1]
- Hδ: 3.2, 3.1, 3, 2.9 [ppm]
- LockedFixed

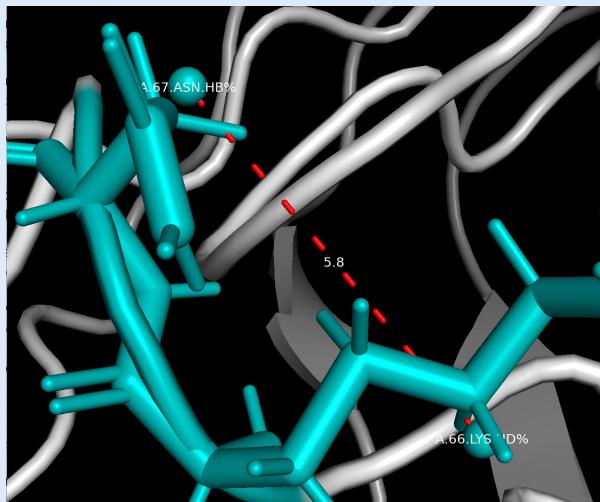
## 5 D Using the Restriction Analysis Table (continued)

- Right-click anywhere within the table and select **Filter...** or use shortcut **FT** to bring up the table filtering tool which will allow you to filter the table using several logicals including **Equals**, **Include**, **>**, **>=**, **<=** and **<** based either on the whole table or individual columns.
- You can use the filter panel to identify and examine restraints to a particular residues (in this case 66 LYS). You can apply another filter, for instance showing restraints violated in more than 10 models.
- Move through the table using arrow up/down keys, press **Return** to select a restraint and navigate to the corresponding linked peak in SpectrumDisplays and/or peak tables. (You can also double-click on a restraint to do this, but lots of double-clicking can become quite tedious!)

Restraint Analysis Inspector\_1

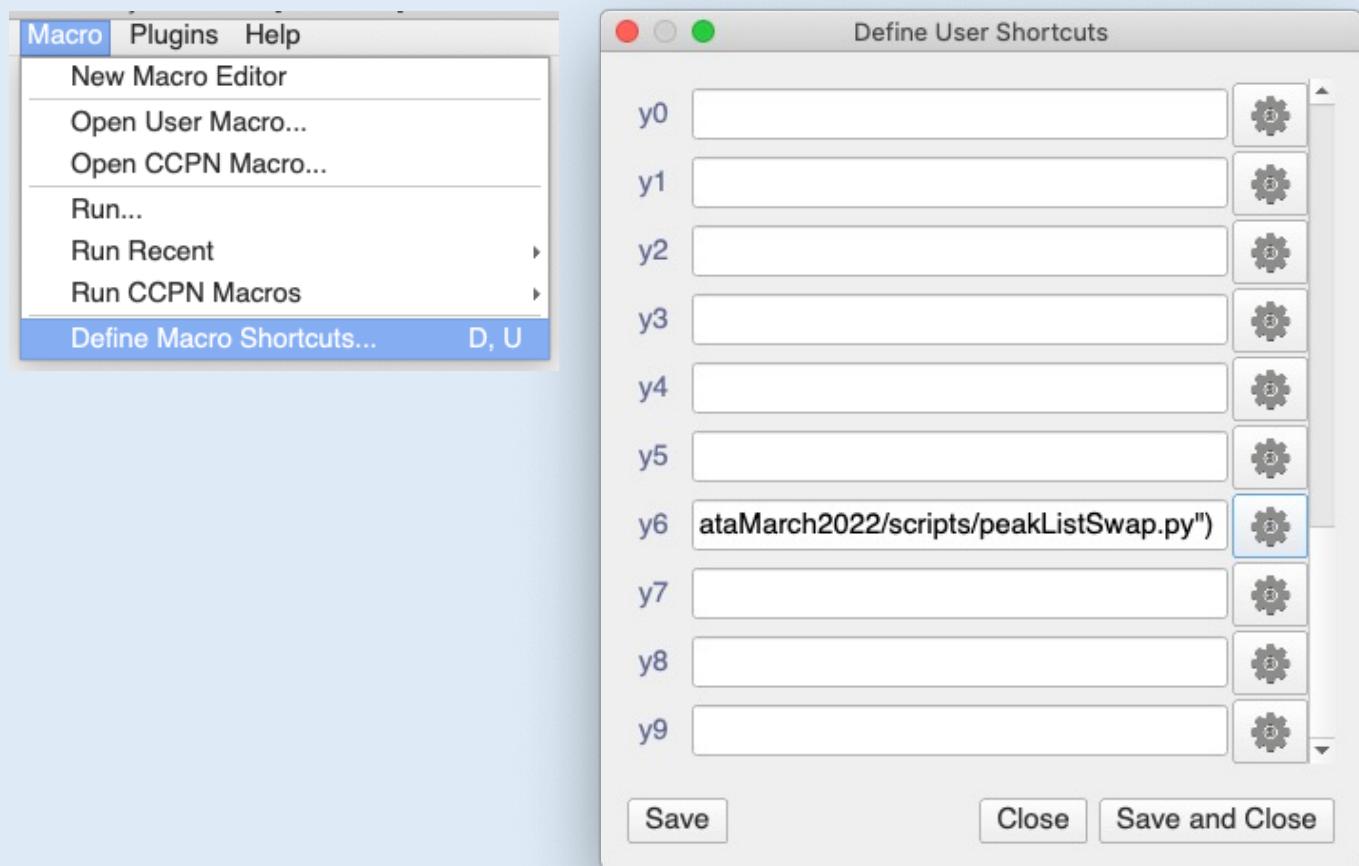
The screenshot shows the 'Restraint Analysis Inspector\_1' window with a table of restraint data. The table has columns for Row, Atoms, Mean, SD, and Count. A blue box highlights the 'SD:run1\_xplor' dropdown menu and the 'Select' button. Below the window is a Pymol interface showing a protein structure with cyan sticks representing a selected restraint.

| Row  | Atoms                       | Mean  | SD    | Count |
|------|-----------------------------|-------|-------|-------|
| 2005 | A.16.GLY.HA% - A.66.LYS.HA  | 4.552 | 1.656 | 19    |
| 3337 | A.41.ILE.HA - A.66.LYS.HG%  | 2.201 | 0.302 | 20    |
| 4213 | A.63.GLY.HA% - A.66.LYS.HB% | 2.156 | 0.418 | 20    |
| 3813 | A.66.LYS.HE% - A.67.ASN.HB% | 1.304 | 1.144 | 13    |
| 2523 | A.66.LYS.HD% - A.67.ASN.HB% | 1.296 | 0.747 | 16    |



## 5D Using the Restraint Analysis Inspector Table (*continued*)

- You can view the distance on the structure associated with the restraint table specified in section 5A.
- Select the structure data in the pulldown menu and press to open new instance of Pymol.

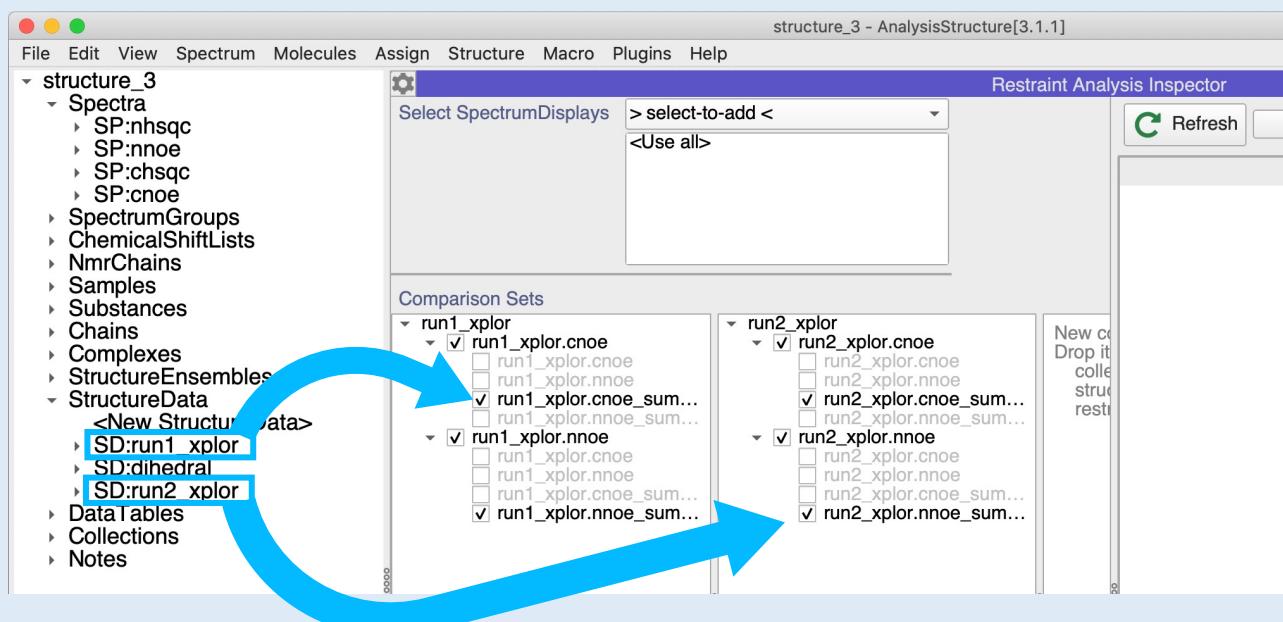


## 5E Move peaks between lists

If you identify peaks you want to move from peak list 1 (accepted peaks) to peak list 2 (rejected peaks) or the other way round, a convenient way of doing this is to have a keyboard shortcut. If you only have two peak lists associated with your spectra, one for accepted peaks, and one for rejected ones, you can use the `.../structure_workshop/scripts/peakListSwap.py` macro and associate it with a keyboard shortcut.

- Go to **Macro > Define Macro Shortcuts...**
- Click on the gear box button next to your chosen shortcut (e.g. **y6** which is an easy combination on the keyboard) and navigate to the `.../structure_workshop/scripts/peakListSwap.py` file on your computer.
- Click Save and Close

To use the macro, simply select one or more peaks which you want to move from one peak list to another and press the key combination **y6**.



## 5F Comparing data from multiple runs

One of the strengths of the Restriction Analysis Inspector is that it can be used to compare restraints and violations from multiple runs (either consecutive runs or runs using different parameters or structure calculation software).

Either:

- Import the NOESY restraints and violation data from a second run in the **/structure\_2 ccpn /data/xplor\_nih.done /run-2-2022-11-23-201806.done** folder following the steps in 4C and 4E.
- Create new violation tables as shown in step 5C.

Or:

- Open the **structure\_3 ccpn** project
- In a **Restriction Analysis Inspector** module open the Settings panel.
- Drag the **SD:run1\_xplor** StructureData into the Comparison Sets box.
- Drag the **SD:run2\_xplor** StructureData into the new Comparison Sets drop area.
- Ensure that in both cases the both the cnoe and nnoe summary violations tables have been selected.

You will be able to see that some peaks were not included in the second run and that violations and even assignments vary between runs.

Assignments vary:

|     | run1_xplor                   |       |       |           | run2_xplor                 |            |       |             |
|-----|------------------------------|-------|-------|-----------|----------------------------|------------|-------|-------------|
|     | Atoms                        | Mean  | STD   | Count ... | Atoms                      | Mean       | STD   | Count > 0.3 |
| ... | + A.20.THR.HG2% - A.23.THR.H | 1.311 | 0.232 | 20        | A.11.ILE.HG1% - A.23.THR.H | 0.000      | 0.000 | 0           |
| ... | A.11.ILE.HG1% - A.12.SER.H   | 0.700 | 0.312 | 20        | A.11.ILE.HG1% - A.12.SER.H | -8.700e-03 | 0.016 | 0           |
| ... | A.11.ILE.HB - A.12.SER.H     | 0.842 | 0.132 | 20        | A.11.ILE.HB - A.12.SER.H   | 0.000      | 0.000 | 0           |
| ... | A.20.THR.HB - A.62.VAL.H     | 0.647 | 0.202 | 20        | A.20.THR.HB - A.62.VAL.H   | 0.102      | 0.072 | 0           |
| ... | A.41.ILE.H - A.41.ILE.HG1%   | 1.378 | 0.310 | 20        | A.41.ILE.H - A.41.ILE.HG1% | 1.173      | 0.322 | 20          |
| ... | A.84.SER.HB% - A.86.VAL.H    | 0.643 | 0.107 | 20        | A.84.SER.HB% - A.86.VAL.H  | 0.026      | 0.053 | 0           |
| ... | A.18.PRO.HA - A.19.TRP.H     | 0.654 | 0.058 | 20        | A.18.PRO.HA - A.19.TRP.H   | 0.641      | 0.011 | 20          |
| ... | A.4.GLN.HE2% - A.80.GLY.H    | 0.766 | 0.143 | 20        | -                          | -          | -     | -           |
| ... | A.17.ILE.HD1% - A.22.VAL.H   | 1.434 | 0.279 | 20        | -                          | -          | -     | -           |
| ... | A.22.VAL.HG% - A.45.ASN.H    | 2.993 | 1.285 | 20        | -                          | -          | -     | -           |
| ... | A.37.ILE.H - A.76.THR.HB     | 1.198 | 0.287 | 20        | -                          | -          | -     | -           |
| ... | + A.39.LEU.HB% - A.40.THR.H  | 0.590 | 0.078 | 20        | A.36.LEU.HD% - A.40.THR.H  | 0.000      | 0.000 | 0           |
| ... | A.27.GLU.HA - A.29.LEU.H     | 1.144 | 0.129 | 20        | A.27.GLU.HA - A.29.LEU.H   | 0.530      | 0.052 | 20          |
| ... | A.28.ASN.HA - A.31.THR.H     | 0.911 | 0.166 | 20        | -                          | -          | -     | -           |

Peak not assigned in second run

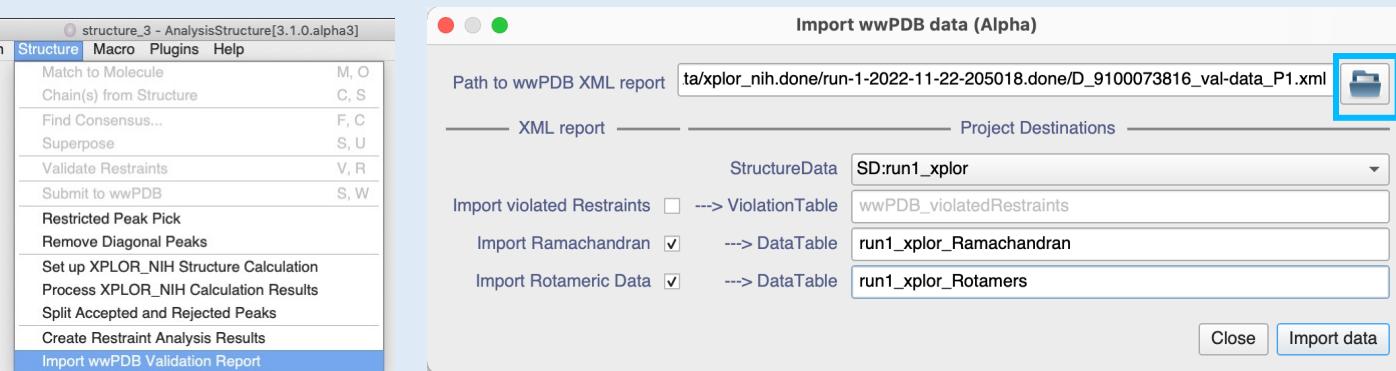
Violation count varies

## 5G Comparing restraints from consecutive runs

In your example data, you will be able to see that some peaks were not included in the second run and that violations and even assignments may vary between runs.

# 6 Importing wwPDB Reports

structure\_2



structure\_2 ccpn / data / xplor\_nih.done / run-1-2022-11-22-205018.done / D\_9100073816\_val-data\_P1.xml

DataTables  
<New DataTable>  
DT:run1\_xplor\_Rama  
DT:run1\_xplor\_Rama\_short  
DT:run1\_xplor\_Rotamers\_short

## 6A Import of wwPDB validation report

wwPDB validation report can be generated on

<https://validate.rcsb-1.wwpdb.org/> website using

/structure\_2 ccpn / data / xplor\_nih.done / run-1-2022-11-22-205018.done / fold / ensemble.pdb and /run-1-2022-11-22-205018.done / out.nef.

- Go to **Main Menu > Structure > Import wwPDB Validation Report**
- Click on the file selection icon to select the **structure\_2 ccpn / data / xplor\_nih.done / run-1-2022-11-22-205018.done / D\_9100073816\_val-data\_P1.xml** file
- Tick **Import** for the Ramachandran and Rotameric Data, optionally also the Violated Restraints.
- Change any names under **Project Destinations** if desired.
- Click **Import data**.

The data will be placed in the **DataTables** section of the sidebar

## 6B Inspect Ramachandran data

- Drag the **DT:wwPDB\_Ramachdran\_short** data table into the drop area. This table shows you a per-residue summary of the number of models in the favoured, allowed and outlier regions of the Ramachandran plot.

| resnum | resname | favored | allowed | outlier |
|--------|---------|---------|---------|---------|
| 66     | LYS     | 0       | 0       | 20      |
| 42     | CYS     | 2       | 5       | 13      |
| 63     | GLY     | 2       | 6       | 12      |
| 12     | SER     | 1       | 7       | 12      |
| 68     | ASP     | 8       | 3       | 9       |

## Contact Us

**Website:**

[www ccpn ac uk](http://www ccpn ac uk)

**Suggestions and comments:**

[support@ccpn.ac.uk](mailto:support@ccpn.ac.uk)

**Issues and bug report:**

<https://forum.ccpn.ac.uk/>

## Cite Us

Skinner, S. P. et al. CcpNmr AnalysisAssign: a flexible platform for integrated NMR analysis. *J. Biomol. NMR* 66 (2016).

## Cite NEF

Gutmanas, A. et al. NMR Exchange Format: A unified and open standard for representation of NMR restraint data. *Nature Structural and Molecular Biology* 22, 433–434 (2015).