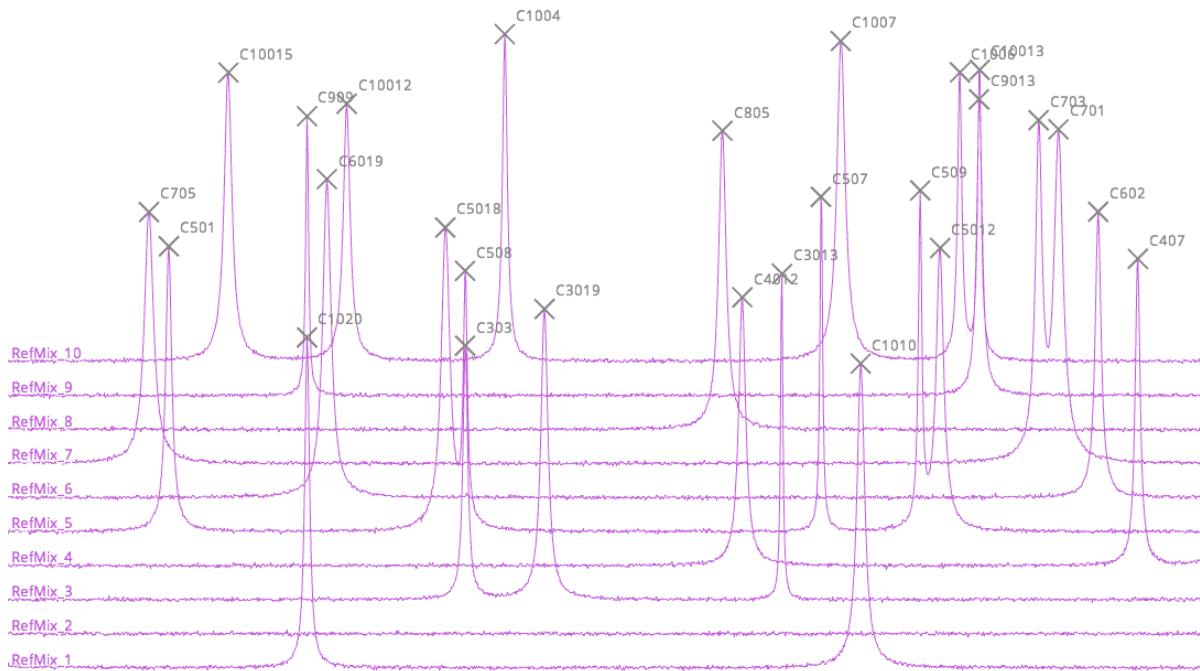


How To Import Reference Mixtures from NEF



Introduction

This *How To* will show you how to import Reference Mixture information from a NEF file in CcpNmr AnalysisScreen Version 3.1.

It is essentially a small subsection of our Screening Hit Analysis Tutorial. We provide artificial ^{19}F data for this tutorial, but the individual steps would be unchanged for ^1H data.

It is assumed that you have some basic familiarity with the program, e.g. from having completed our Beginners Tutorial.

You will need to use the data located in the **HowTo_ImportRefMixFromNEF** directory of the CcpNmr V3 NEF example data which you can download from the CCPN website <https://ccpn.ac.uk/support/tutorials/>.

Start CcpNmr Analysis V3

- Apple users by running Screen on the Launcher or using the terminal command: *bin/screen*
- Linux users by using the terminal command: *bin/screen*
- Windows users by double-clicking on the *screen.bat* file
- NMRbox users by using the terminal command *analysisscreen*

Disclaimer

Datasets used for this tutorial are randomly generated and don't have any biological significance. All spectra shown are synthetic and for demonstration purposes only. All compound names are randomly chosen and might have incorrect chemical properties or not be represented by the linked spectra.

Please note that the images shown are only representative and you may encounter minor differences in your setup.

Introduction

Getting started, basic operations

Sidebar

All data contained in a project, such as spectra and peak lists are located in the sidebar. **Double-clicking** on an item will open its properties popup.

Spectrum Display

A Spectrum Display can contain multiple overlaid spectra which share the same axes. To show/hide a single spectrum, click on its spectrum toolbar button. If you close a display, you can open a spectrum by **dragging and dropping** it into the drop area from the sidebar or by **right-clicking** on a sidebar item and selecting **Open as module**. You can also add additional spectra to a spectrum display module or drag several spectra into the drop area together to open them simultaneously.

Mouse

- Pan → **Left-drag** in display
- Zoom in/out → **Scroll wheel** in display
- Context menu → **Right-click**
- Select a peak → **Left-click** on a peak symbol “X”
- Move a peak → select first, then **middle-click and drag**

Two-Letter Shortcuts

Press the first letter on your keyboard e.g., **M**, followed by the second letter, e.g., **K** (case insensitive). Press **Esc** to cancel the first letter.

Common in this *HowTo*:

- VP** → Open the Validate Paths dialog box
- SK** → StacK Spectra
- SE** → Snap the selected peaks to Extremum
- RP** → Refit the selected Peaks

For more commands and operations

Main Menu → Help → Tutorials → Beginners Tutorial

OR

Main Menu → Help → Show Shortcuts

Introduction

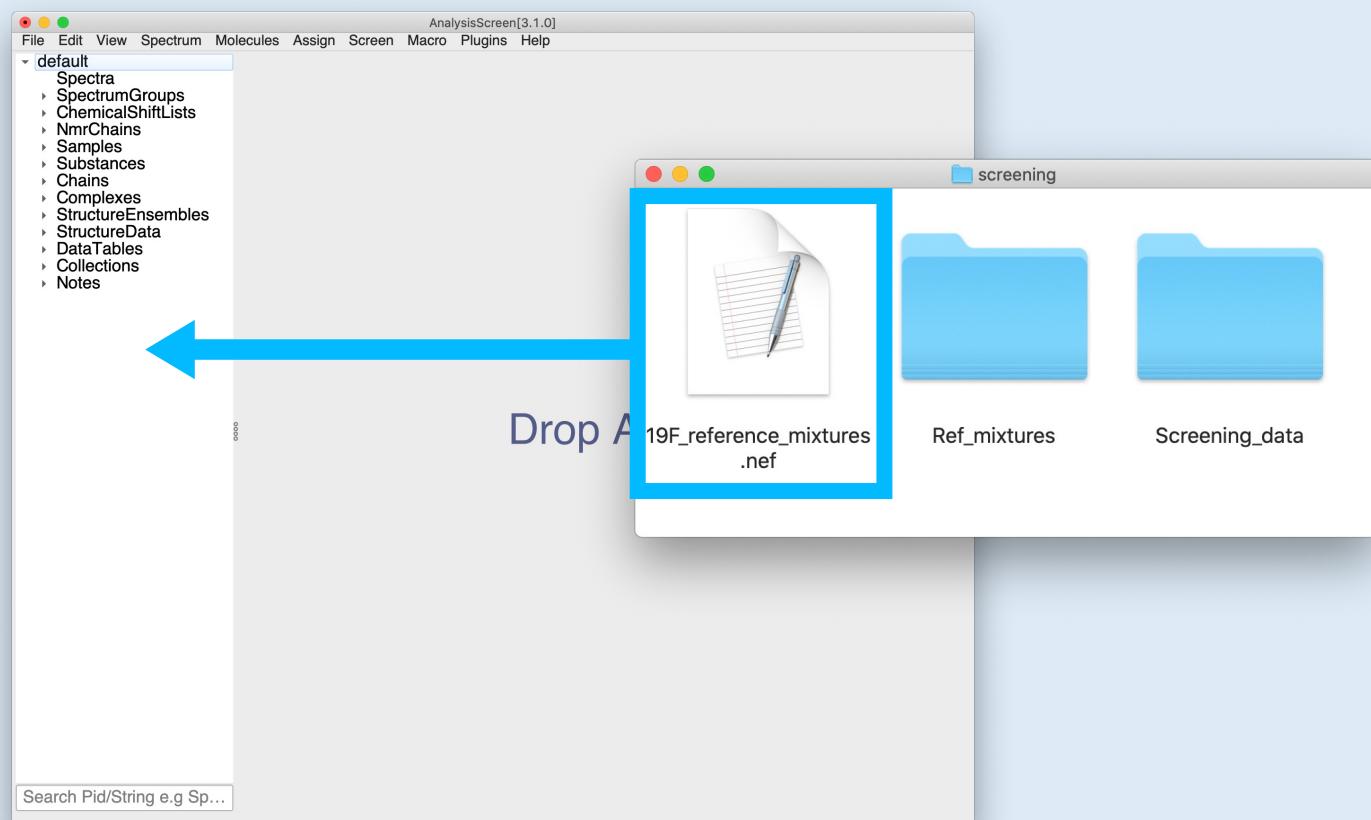
Why use NEF files in Screening?

When recording screening data you will typically record Control Spectra on your compound mixtures and Target Spectra on the mixtures with the target protein/macromolecule. However, you will always need to compare these back to a set of Reference Spectra, which contain information about which peak belongs to which substance. We recommend saving the information about the Reference Spectra and compounds in an NMR Exchange Format (NEF) file. You can then use this file as the basis for each of your screening projects. The advantage of using a NEF file rather than a template project, is that it is a single file rather than a whole folder, it is humanly readable, easily ammended (e.g. with Find/Replace), but nonetheless contains all the information you need. This includes the location of the spectra, spectrum colours, peaks, peak annotations, substances, samples etc.

For more information about how to set up such a NEF file, please see our Screening Hit Analysis Tutorial.

1

Import Reference Mixtures from NEF

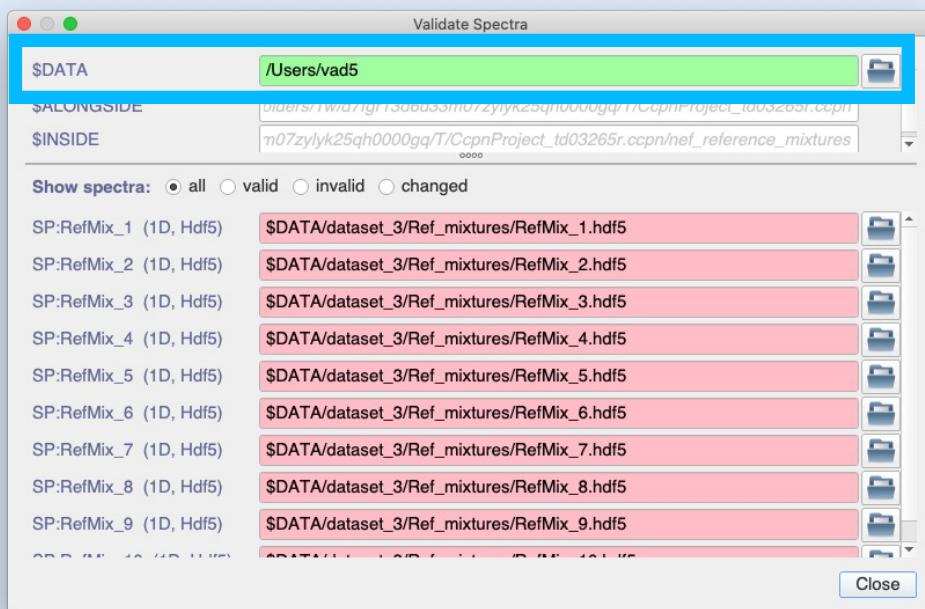


1A Import From NEF

The NEF file contains information about your Reference Mixtures, including PeakLists, Samples and Substances as well as some formatting.

- Select the **19F_reference_mixtures.nef** located in the **HowTo_ImportRefMixFromNEF/** directory and drag it onto the sidebar.
- When prompted, select to open as **New Project**.

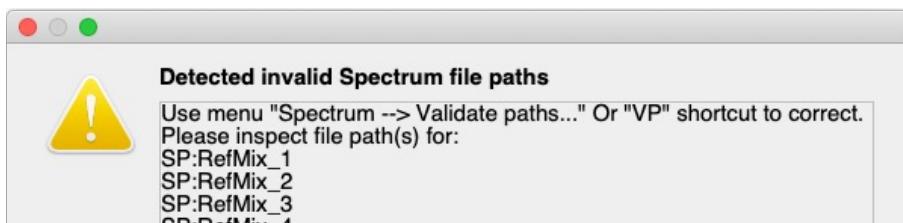
1 Import Reference Mixtures from NEF



Shortcut
VP

1B Correct Spectrum file paths

You will probably find that the program has told you that it can't find the spectrum file paths.



This is because the spectrum files will be in a different directory on your computer compared to one on which the NEF file was created. Open the **Validate Paths** pop-up with

- Main Menu → Spectrum → Validate paths...

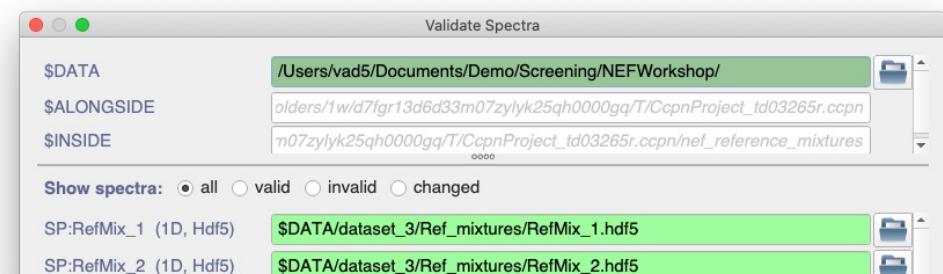
OR

- type shortcut **VP**

A pink background to your file paths shows that the spectra can't be found.

Change your **\$DATA** path at the top to the full path of the directory that your **HowTo_ImportRefMixFromNEF/** folder is in, either by typing it, or by clicking on the folder icon and selecting the directory interactively.

Once entered, the path boxes should turn green.



1 Import Reference Mixtures from NEF

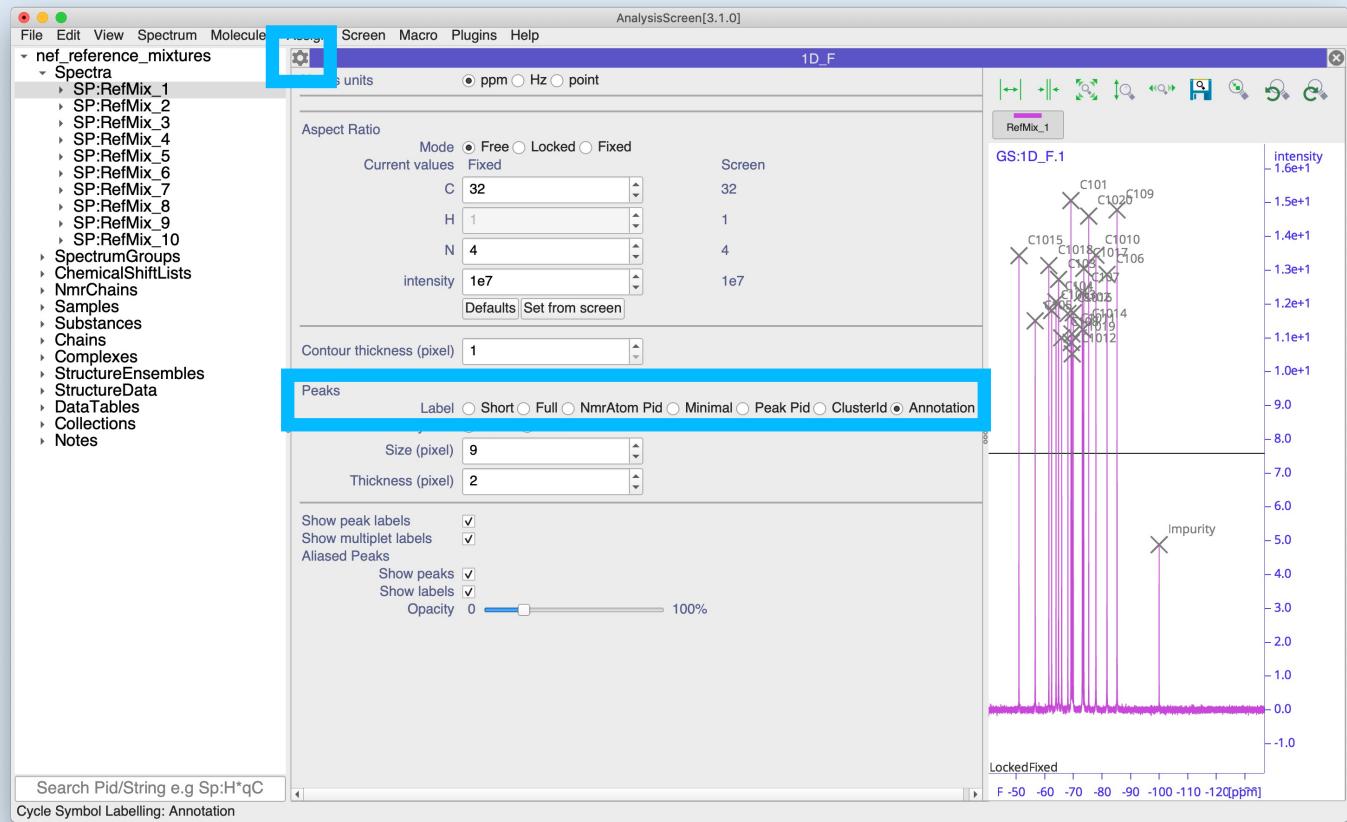
↳ SA:RefMix_3
↳ SA:RefMix_4
↳ SA:RefMix_5
↳ SA:RefMix_6
↳ SA:RefMix_7
↳ SA:RefMix_8
↳ SA:RefMix_9
↳ SA:RefMix_10
↳ Substances
<New Substance>
SU:C1001.
SU:C10010.
SU:C10011.
SU:C10012.
SU:C10013.
SU:C10014.
SU:C10015.
SU:C10016.
SU:C10017.
SU:C10018.
SU:C10019.
SU:C1002.
SU:C10020.

1c Check Data

In the sidebar you should now be able to see that your project contains your ten Reference Mixture Spectra, a **RefMix** SpectrumGroup, ten Samples and 200 Substances. The **RefMix** SpectrumGroup contains all ten spectra, and the Samples each contain 20 Substances:

↳ SpectrumGroups
<New SpectrumGroup>
↳ SG:RefMix
SP:RefMix_1
SP:RefMix_2
SP:RefMix_3
SP:RefMix_4
SP:RefMix_5
SP:RefMix_6
SP:RefMix_7
SP:RefMix_8
SP:RefMix_9
SP:RefMix_10
↳ Samples
<New Sample>
↳ SA:RefMix_1
↳ SA:RefMix_2
↳ SA:RefMix_3
↳ SA:RefMix_4
↳ Samples
<New Sample>
↳ SA:RefMix_1
SC:RefMix_1.C101.
SC:RefMix_1.C1010.
SC:RefMix_1.C1011.
SC:RefMix_1.C1012.
SC:RefMix_1.C1013.
SC:RefMix_1.C1014.
SC:RefMix_1.C1015.
SC:RefMix_1.C1016.
SC:RefMix_1.C1017.
SC:RefMix_1.C1018.
SC:RefMix_1.C1019.
SC:RefMix_1.C102.
SC:RefMix_1.C1020.
SC:RefMix_1.C103.
SC:RefMix_1.C104.
SC:RefMix_1.C105.
SC:RefMix_1.C106.
SC:RefMix_1.C107.
SC:RefMix_1.C108.
SC:RefMix_1.C109.

1 Import Reference Mixtures from NEF



1D Inspect Spectra and Peaks

- Drag the first Spectrum (**SP:RefMix_1**) from the sidebar into the **Drop Area**.
 - Open the Settings panel by clicking on the Gear Icon  in the top left corner of the SpectrumDisplay.
 - In the **Peaks** section, choose **Annotation** as the **Label**.
 - Close the Settings panel by clicking on the Gear Icon again.

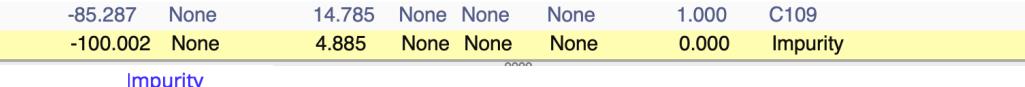
You should now see each of the peaks labelled with the Substance name that it belongs to.

- In the sidebar, expand the **RefMix_1** spectrum its **PeakLists**
 - Drag the **RefMix_1.1** PeakList into the **Drop Area** (or right-click and select **Open as a Module**)
 - In the SpectrumDisplay, select the peak at -100 ppm marked **Impurity**.

You should see that that this peak has a Merit value of 0.0 compared to 1.0 for all the other peaks. This information comes from the NEF file and ensures this peak will be automatically excluded from the screening analysis.

Peak Table

PeakList	PL:RefMix_1.1	Position Unit	ppm	#	Assign F1	Pos F1	LW F1 (Hz)	Height	S/N	Volume	/ ClusterId	/ Merit	/ Annotation	/ Comment
17		-81.777	None	12.880	None	None	None	1.000			C106			
18		-73.137	None	12.330	None	None	None	1.000			C107			
19		-65.936	None	11.002	None	None	None	1.000			C108			
20		-85.287	None	14.785	None	None	None	1.000			C109			
21		-100.002	None	4.885	None	None	None	0.000			Impurity			



The figure shows a 1D NMR spectrum with the x-axis ranging from -90 to -110 ppm. A single, very sharp peak is visible at approximately -100.002 ppm. This peak is highlighted with a red crosshair and labeled "Impurity" in blue text above the plot area.

Import Screening Data from Excel

	A	B	C	D	E	F	G	H	I	J	K
1	sampleName	numGroup	spectrumP	spectrumNan	comment	pH	ionicStrength	amount	amountUnit	hazardou	
2	0	Control_1	Control	Control_1	Control_1	8	8.5	9.133	g		
3	1	Control_2	Control	Control_2	Control_2	6	8.5	7.571	g		
4	2	Control_3	Control	Control_3	Control_3	7	7	8.078	g		
5	3	Control_4	Control	Control_4	Control_4	7	7.5	8.878	g		
6	4	Control_5	Control	Control_5	Control_5	9	8.5	9.731	g		
7	5	Control_6	Control	Control_6	Control_6	6	7	8.176	g		
8	6	Control_7	Control	Control_7	Control_7	7	7.5	9.475	g		
9	7	Control_8	Control	Control_8	Control_8	7	9	9.955	g		
10	8	Control_9	Control	Control_9	Control_9	7.5	7	7.603	g		
11	9	Control_10	Control	Control_10	Control_10	9	8	7.964	g		
12											

SampleControl SampleTarget SampleDisplacer +

Ready 180%

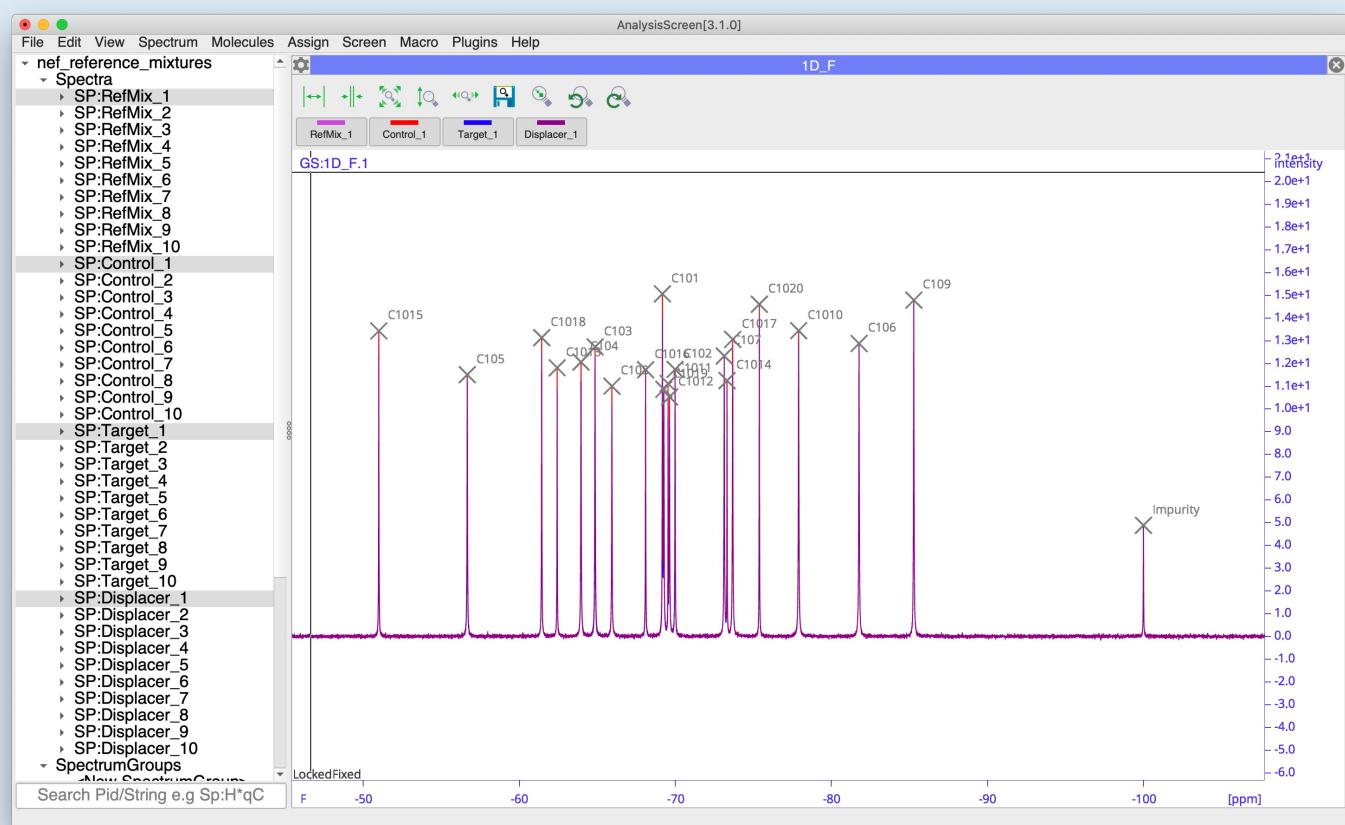
2A Import Screening Data from Excel

Import a set of screening data from an Excel file

- Load the **lookup_19F_ScreeningData.xlsx** Excel file from the **HowTo_ImportRefMixFromNEF/Screening_data/** folder by dragging it onto the sidebar.

This lookup file only contains the **Sample** sheets for the Control, Target and Displacer data without the **SampleComponents** field and the **Substances** sheet. This information has already been imported into the project from the NEF file.

Import Screening Data from Excel



2B Check and Display Data

If you wish, check the additional Spectra, SpectrumGroups and Samples that have been created in your project and take a look at your spectra. For example,

- Drag the **RefMix1**, **Control1**, **Target1** and **Displacer1** spectra into the **Drop Area**
- Check that the peaks overlap.

2C Save Project

Remember to save your project:

- Go to Main Menu → File → Save As... and choose a new name for your project.

You are now ready to analyse your screening data with CcpNmr AnalysisScreen's automated peak matching and Hit Analysis tools.

Contact Us

Website:

www ccpn ac uk

Suggestions and comments:

support@ccpn.ac.uk

Issues and bug reports:

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

Cite Us

Mureddu, L. et al. CcpNmr AnalysisScreen, a new software programme with dedicated automated analysis tools for fragment-based drug discovery by NMR. *J. Biomol. NMR* (2020)

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)