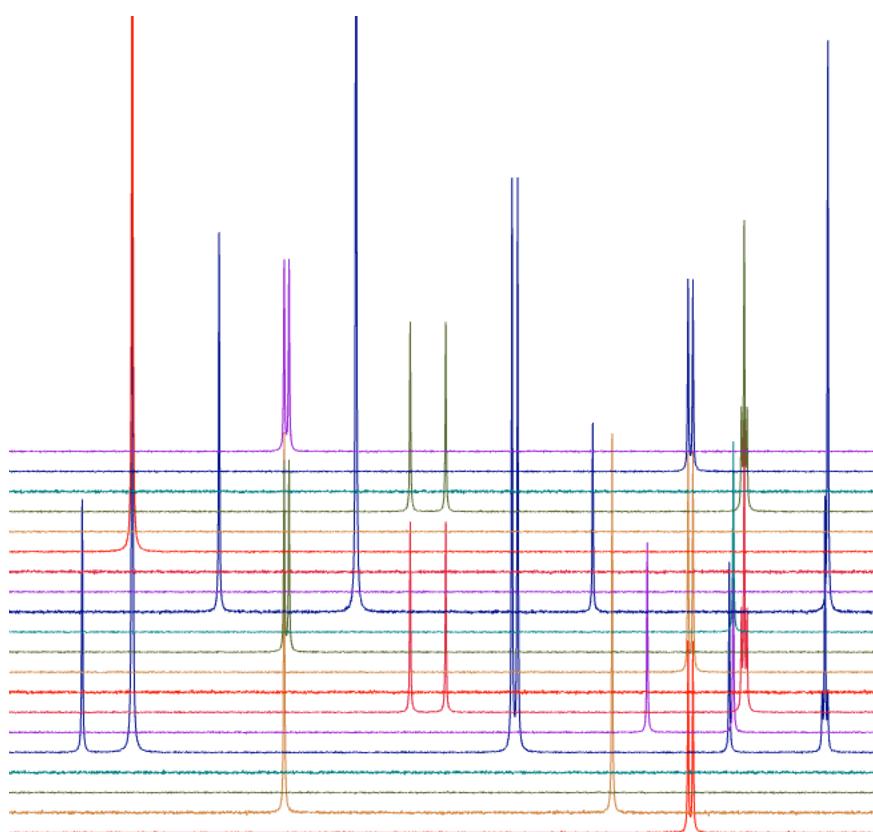


AnalysisScreen Tutorial



Introduction

This tutorial is designed to introduce you to CcpNmr AnalysisScreen 3.0.1. It begins by taking you through the basic concepts of SpectrumGroups, Samples and Substances before taking you through the other main functions of the programme.

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 `/data/ScreenTutorial` directory of the CCPN V3 examples data which you can download from <https://www.ccpn.ac.uk/v3-software/tutorials/tutorial-data-and-examples>. The data 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.

Contents:

1. Spectrum Groups
2. Samples and Substances
3. 1D Peak Picking
4. Pipelines
5. Picking Reference Peaks Pipeline
6. Peak Height Comparison Pipeline
7. Hit Analysis
8. Importing Data from an Excel Lookup File
9. Mixture Generation

Start CcpNmr Analysis V3

Apple users by double-clicking the *CcpNmrAnalysis* icon



Linux users by using the terminal command:
bin/screen

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

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.

Display

A display can contain multiple overlaid spectra which share the same axes. To show/hide a single spectrum, click on its 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 later on, 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**

Shortcuts

The program uses several shortcuts, for example **MK** for creating a mark at the current mouse position. You will need to 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.

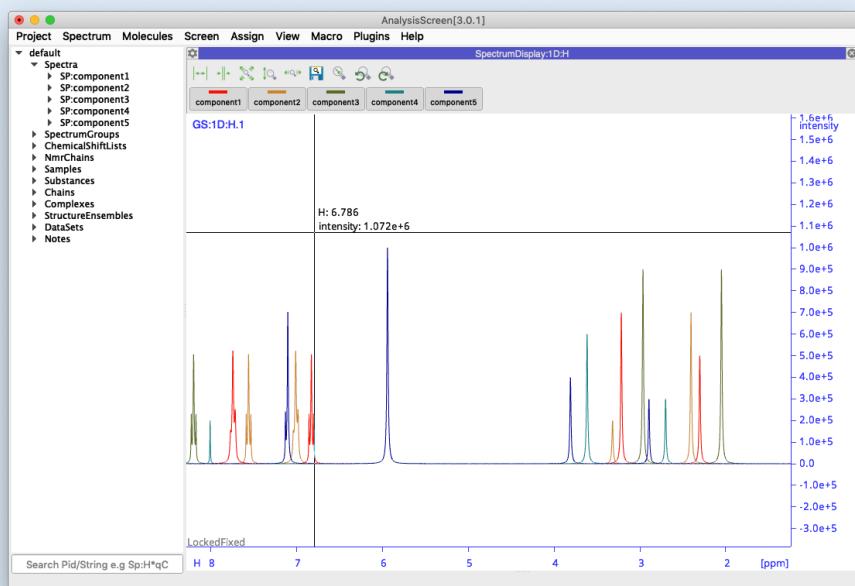
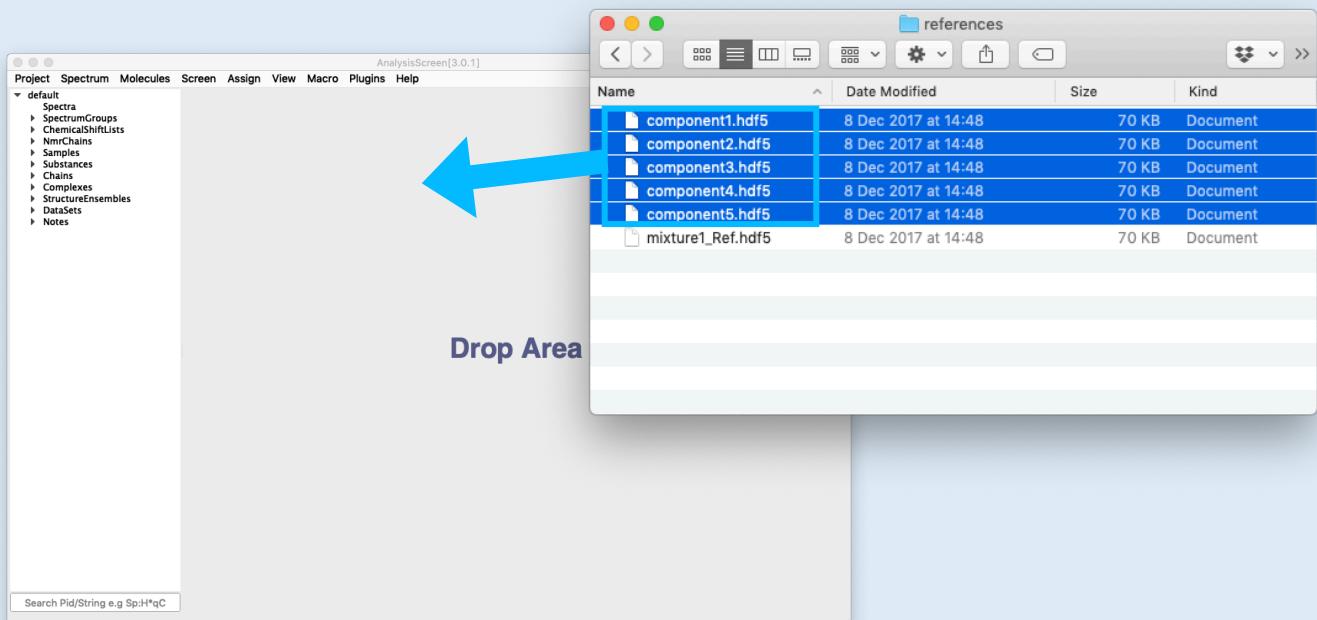
For more commands and operations

Main Menu → Help → Tutorials → Beginners Tutorial

OR

Main Menu → Help → Show Shortcuts

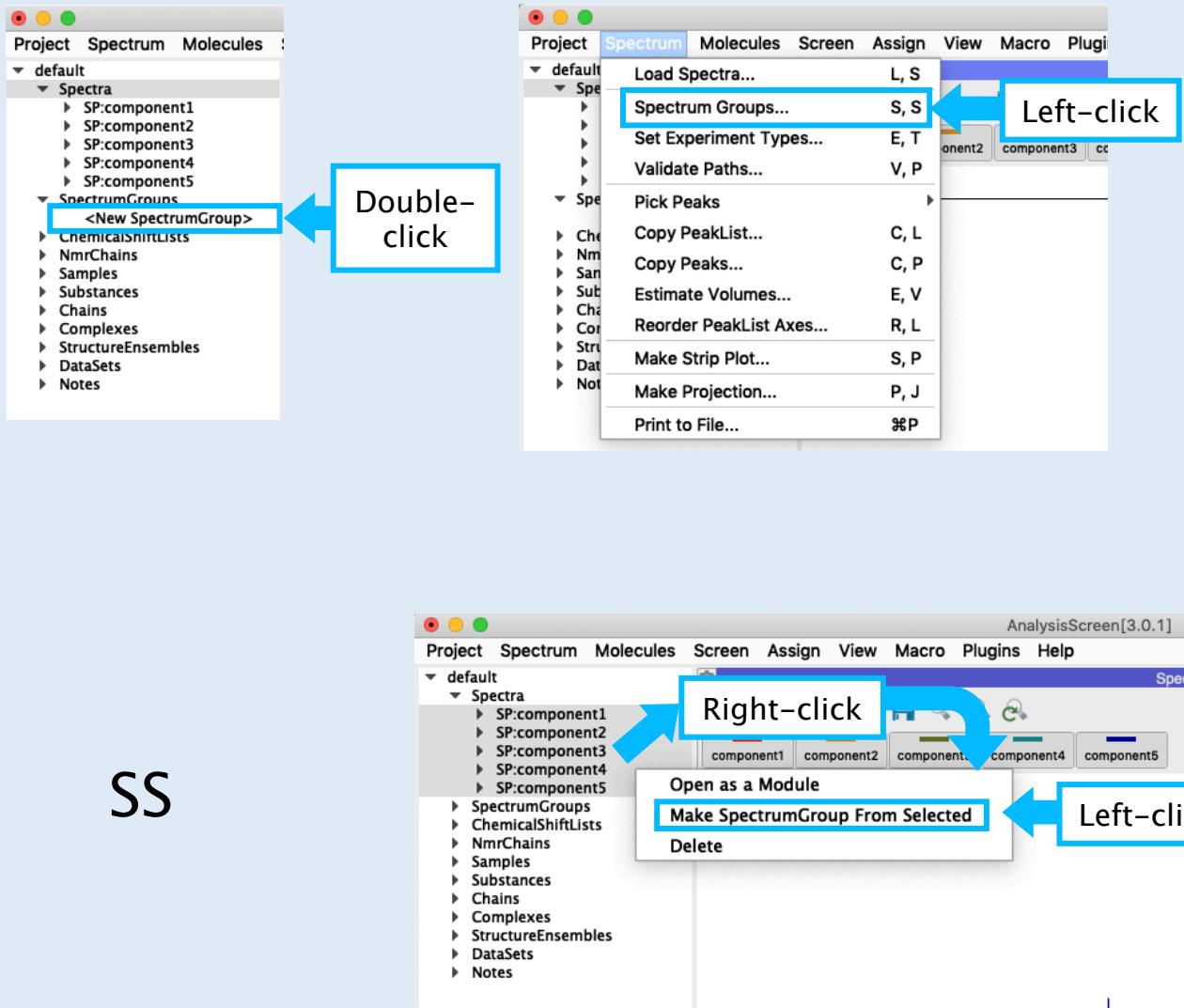
Spectrum Groups



1A • Drag & drop some spectra into the sidebar or drop area

Find some 1D spectra (e.g. in the **ScreenTutorial/spectra/1Ds** folder of the tutorial data folder) and drag them onto the sidebar or drop area.

Spectrum Groups



1B Open the Spectrum Group Dialog

- On the Sidebar, find the **SpectrumGroups** item, expand the branch and **double-click** on **<New SpectrumGroup>**

OR

- Use the shortcut **SS**

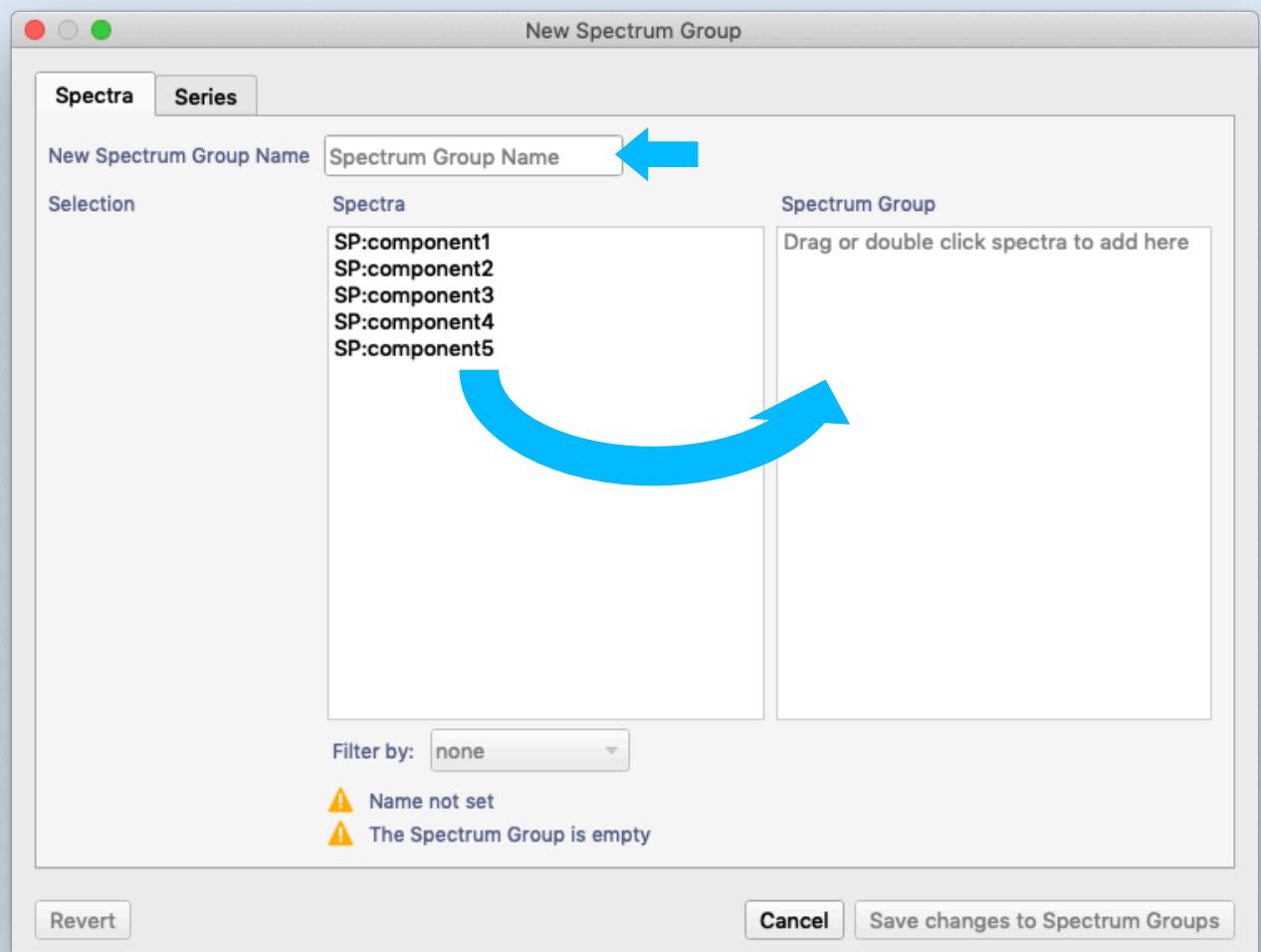
OR

- Go to **Main Menu → Spectrum → Spectrum Groups**

OR

- Select the spectra in the sidebar, **right-click** and select **Make SpectrumGroup from selected**

Spectrum Groups



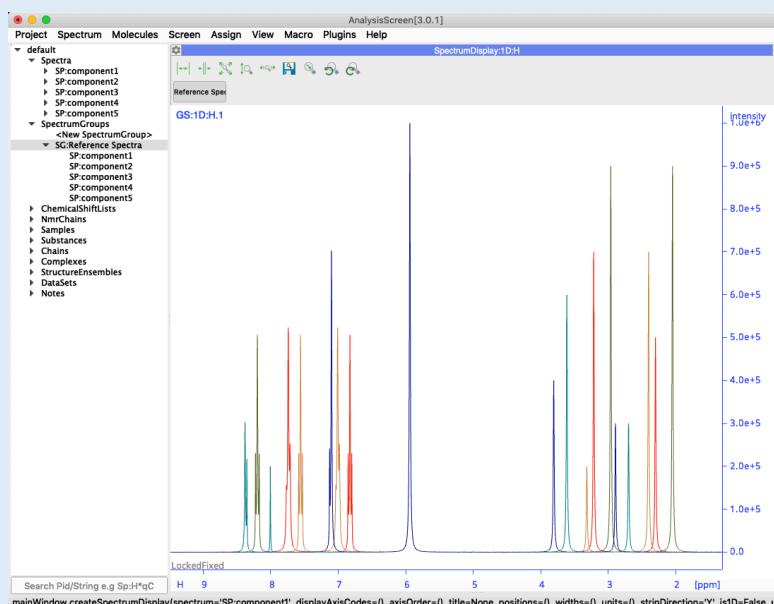
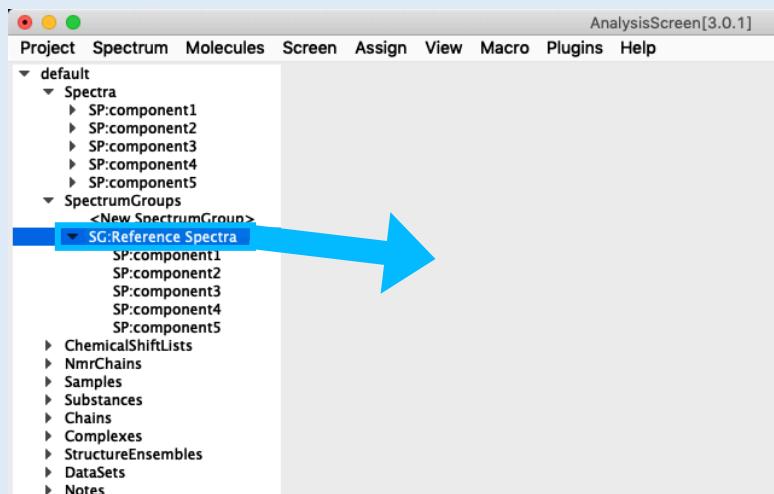
1c Set up Spectrum Group

- Enter a name for your Spectrum Group.
- In the left-hand list of spectra, choose the spectra you want to add to your Spectrum Group and **drag them into the right-hand box**.
If you wish, you can drag spectra back out of the Spectrum Group box, and you can drag spectra up and down to change their order within the Spectrum Group box.
- Click on **Save changes to Spectrum Groups**.

In the sidebar you will now see your new Spectrum Group and its constituent spectra.

<div style="border-left: 1px solid black; padding-left: 10px;"> ▼ default ▼ Spectra ▶ SP:component1 ▶ SP:component2 ▶ SP:component3 ▶ SP:component4 ▶ SP:component5 </div>	<div style="border-left: 1px solid black; padding-left: 10px;"> ▼ SpectrumGroups <New SpectrumGroup> ▼ SG:Reference Spectra SP:component1 SP:component2 SP:component3 SP:component4 SP:component5 </div>
	<div style="border-left: 1px solid black; padding-left: 10px;"> ▶ ChemicalShiftLists </div>

Spectrum Groups



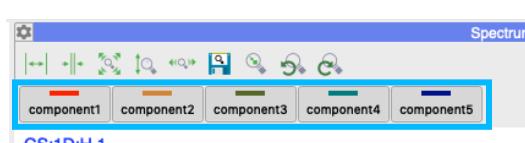
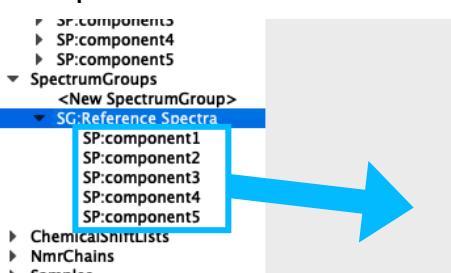
1D Display Spectrum Group

- Drag your Spectrum Group from the sidebar into the drop area to display the spectra.

Note that when you drag a Spectrum Group into the drop area, the spectra are displayed as a single entity and the Spectrum toolbar simply contains one button for the group.



If you select all the spectra in the group and then drag them into the drop area, the spectra will be shown separately, with each spectrum having its own button in the Spectrum Toolbar.



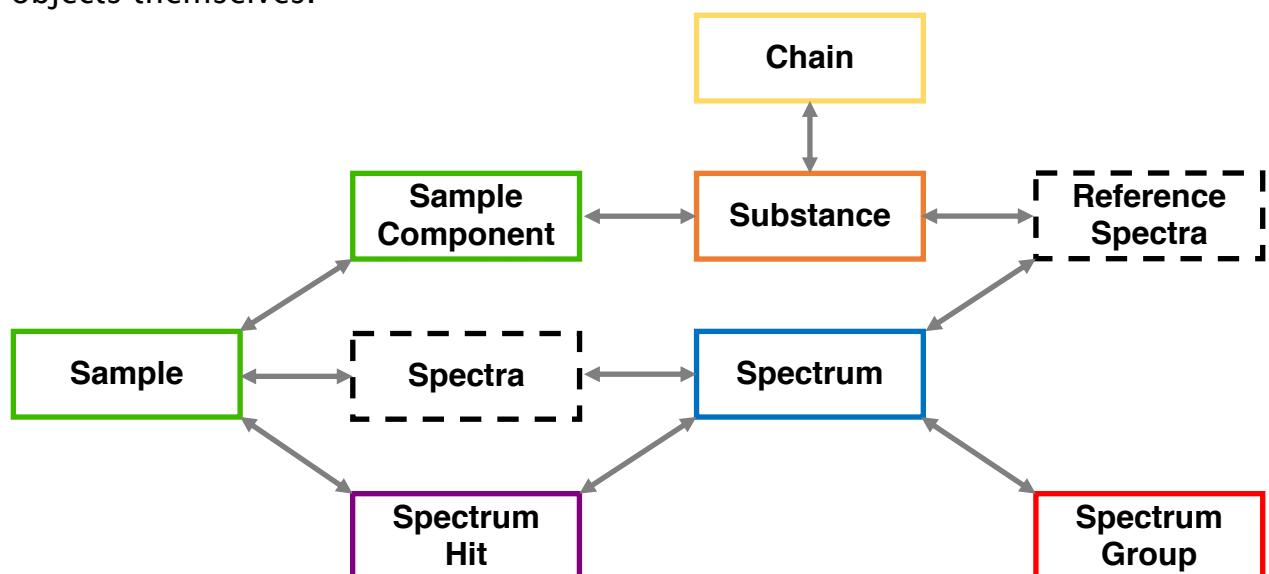
Substances and Samples

Substances, Samples and Sample Components in CcpNmr Analysis V3 (Explanation only)

Within Analysis V3, **Substances** are essentially any kind of chemical – they could be biological macromolecules, small molecules, salts or solvents. Imagine your list of **Substances** to be your chemical store cupboard. If a **Substance** is a polypeptide or polynucleotide, it may also have a **Chain** associated with it. And if you create a **Chain** (e.g. by importing a FASTA file), a corresponding **Substance** will automatically be created for the polypeptide/polynucleotide. As with a real chemical store cupboard, each **Substance** is associated with a particular type of isotopic labelling. You don't have to specify the labelling and (unlike in V2), the specification of the labelling is not formalised. You can either choose a predefined labelling scheme or enter your own. **Substances** can have reference spectra associated with them.

Samples correspond to actual samples that you run experiments on. They have properties such as amount (e.g. in ml), ionic strength and pH and may contain **Sample Components**. **Sample Components** are based on and linked to **Substances**. You can either create **Substances** in advance and then create your **Sample Components** from the **Substances**, or, as you create new **Sample Components** for your **Sample**, equivalent **Substances** will be created for you by the programme. **Samples** can be linked to **Spectra** which were run on that sample.

Schematic diagram showing how objects are linked in CcpNmr Analysis. **Sample Components** are nested inside the **Sample** branch in the sidebar. All other objects are accessed at the top level. **Spectra** and **Reference Spectra** are containers for other objects rather than objects themselves.



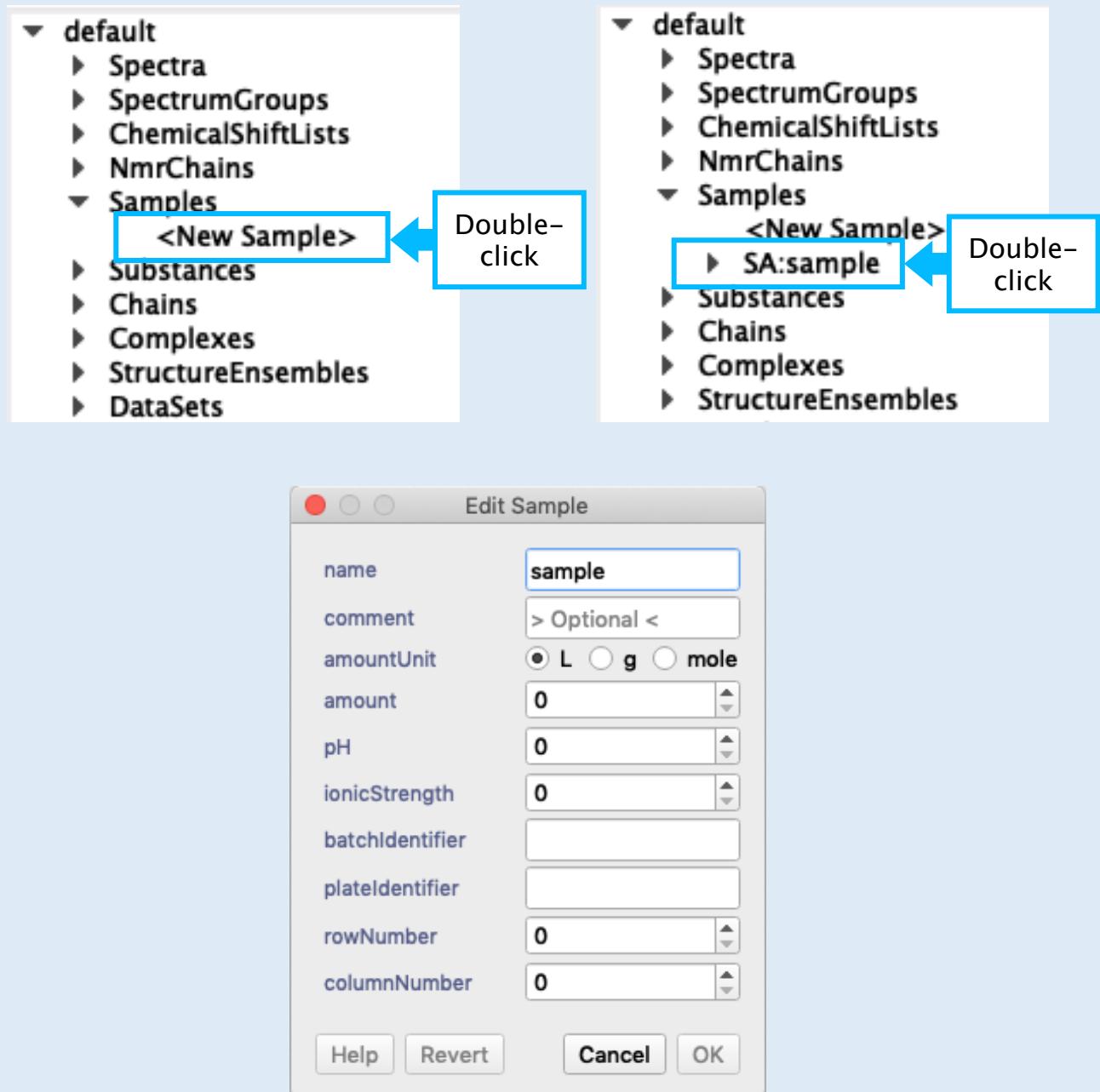
Substances and Samples

The screenshot shows the NMR-Console software interface. On the left, a sidebar tree view is expanded under the 'default' section, showing 'Spectra', 'SpectrumGroups', 'ChemicalShiftLists', 'NmrChains', 'Samples', and 'Substances'. Under 'Substances', a blue box highlights '<New Substance>' with a blue arrow pointing to it from the text 'Double-click'. To the right of the sidebar is a 'New Substance' dialog box. The 'Select source' section has 'New' selected. The 'Current substances' dropdown is set to '> Select <'. The 'name' field contains 'aspirin'. The 'labelling' field is set to 'None'. The 'comment' field is set to '> Optional <'. Below these fields are two sections: '+ More options' and '+ Compound view'. The '+ Compound view' section is expanded, showing a SMILES input field containing 'CC(=O)OC1=CC=CC=C1C(=O)O'. To the right of this input field is a 'Compound view' window displaying a 3D ball-and-stick model of the aspirin molecule. At the bottom of the dialog are 'Help', 'Revert', 'Cancel', and 'OK' buttons, with 'OK' highlighted by a blue box.

2A Create New Substance

- Expand the **Substances** branch in the sidebar and **double-click** on **<New Substance>**
- Enter a Substance **Name**, e.g. aspirin and, if you wish, also **Labelling**.
- By expanding the **More options** section you can view and edit many more properties such as empirical formula, molecular mass or atom and bond counts.
- Expanding **Compound view** will enable you to enter a small molecule SMILES, e.g. CC(=O)OC1=CC=CC=C1C(=O)O. Doing this will show the compound in the **Compound View** box.
- Click **Ok** to apply your changes and close the pop-up.

Substances and Samples



2B Create New Sample

- Expand the Samples branch in the sidebar and **double-click** on <New Sample>

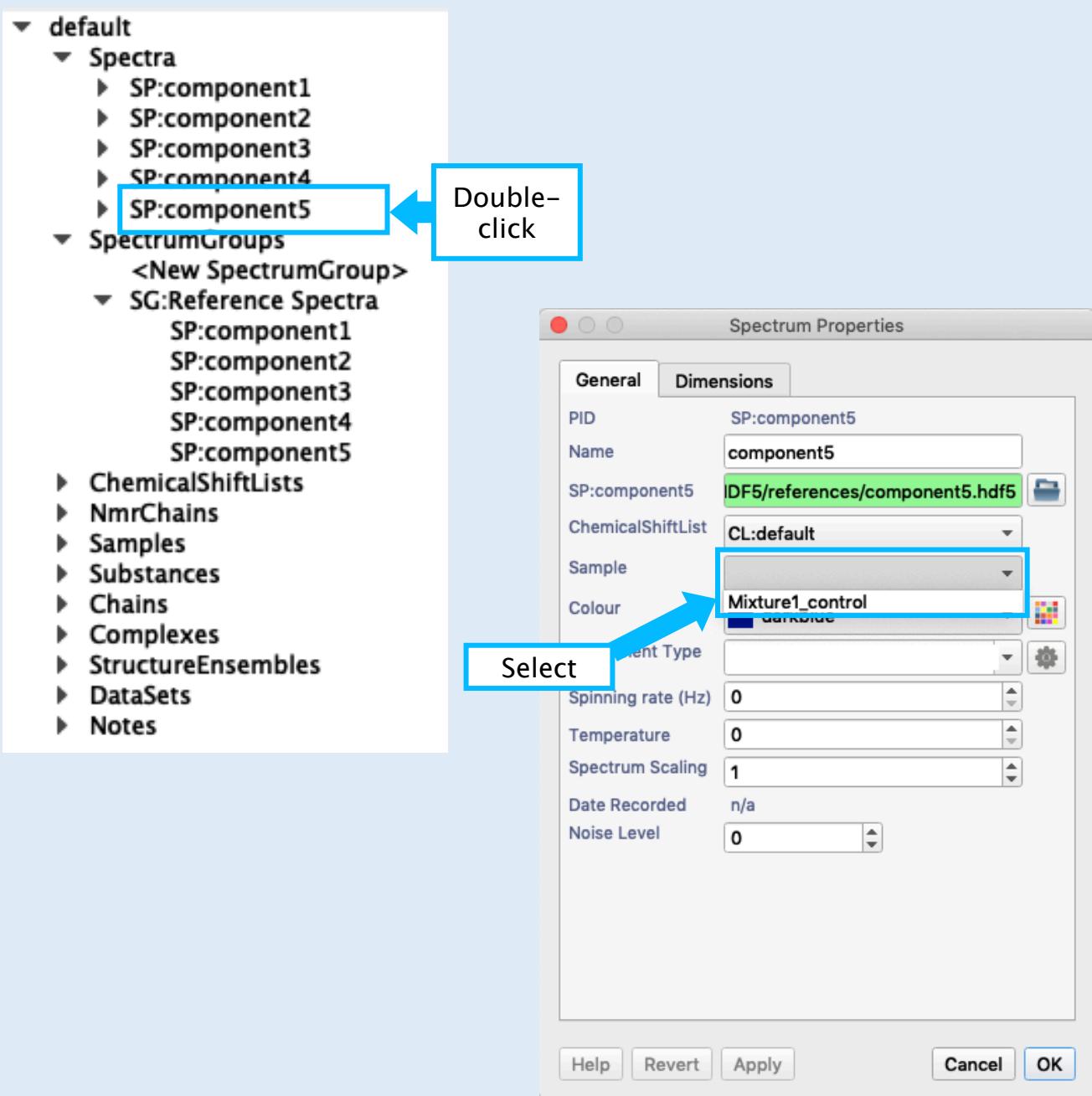
2C Open the Sample Properties Dialog

- Double-click** on SA:sample to open the Edit Sample dialog box.
- Enter a **Name** for the sample and any other properties such as **ionic strength** and **pH**.

The 'Edit Sample' dialog box is shown again, this time with a sample named 'Mixture1_control'. The properties entered are:

- name: Mixture1_control
- comment: > Optional <
- amountUnit: L (radio button selected)
- amount: 0.0006
- pH: 5.5
- ionicStrength: 0.00005

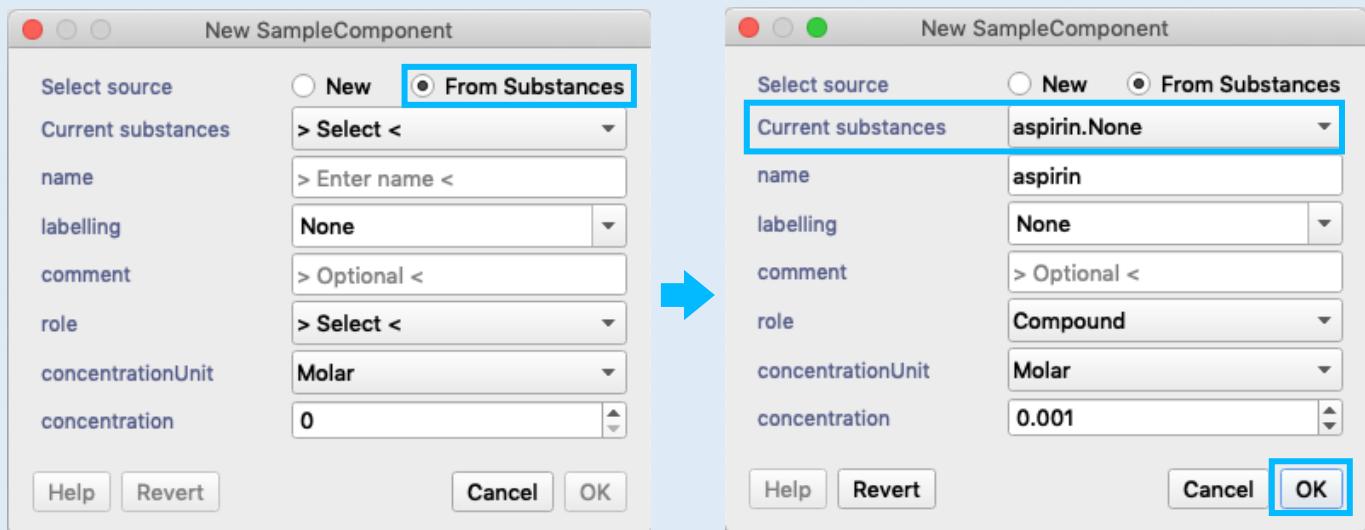
name	Mixture1_control
comment	> Optional <
amountUnit	<input checked="" type="radio"/> L <input type="radio"/> g <input type="radio"/> mole
amount	0.0006
pH	5.5
ionicStrength	0.00005



2D Link Spectra to Sample

- Find a Spectrum in the sidebar, either under **Spectra** or **Spectrum Groups**
- Double-click** the spectrum to bring up the **Spectrum Properties** pop-up
- Find the **Sample** pulldown entry and select your sample

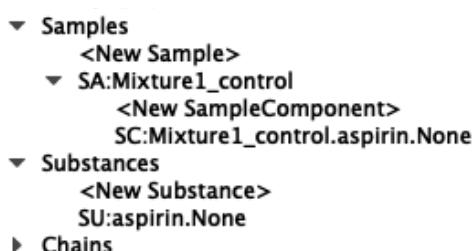
Substances and Samples



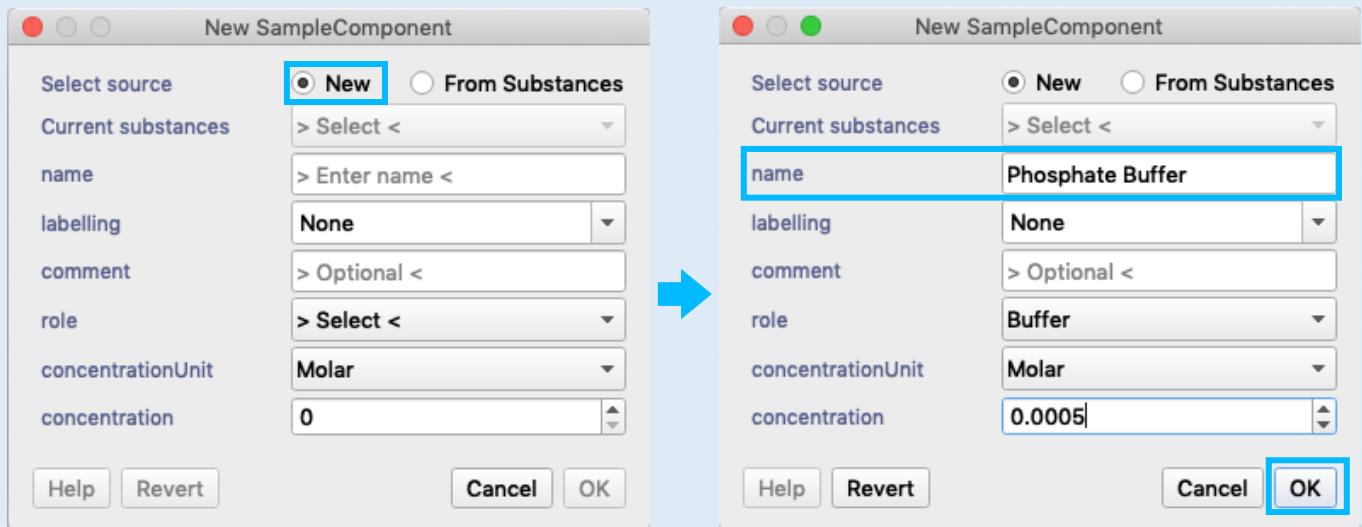
2E Create New Sample Component from existing Substance

- Expand your **Samples** branch in the sidebar further and **double-click** on **<New SampleComponent>**
- Select the **From Substances** radio button to use a **Substance** that you have previously already added to your project
- Select the **Substance** you want to use from the **drop-down menu** and enter any other parameters you wish to add, e.g. role, concentration etc.
- Click on **Ok**

Your new **Sample Component** will now be shown as part of your **Sample** in the sidebar.



Substances and Samples

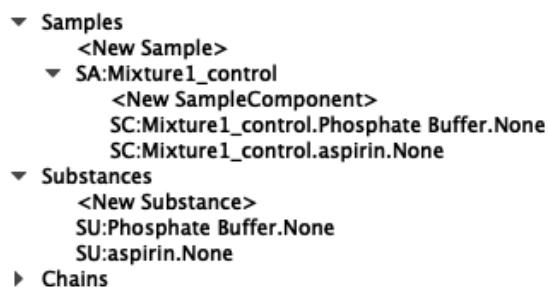


2F Create New Sample Component with new Substance

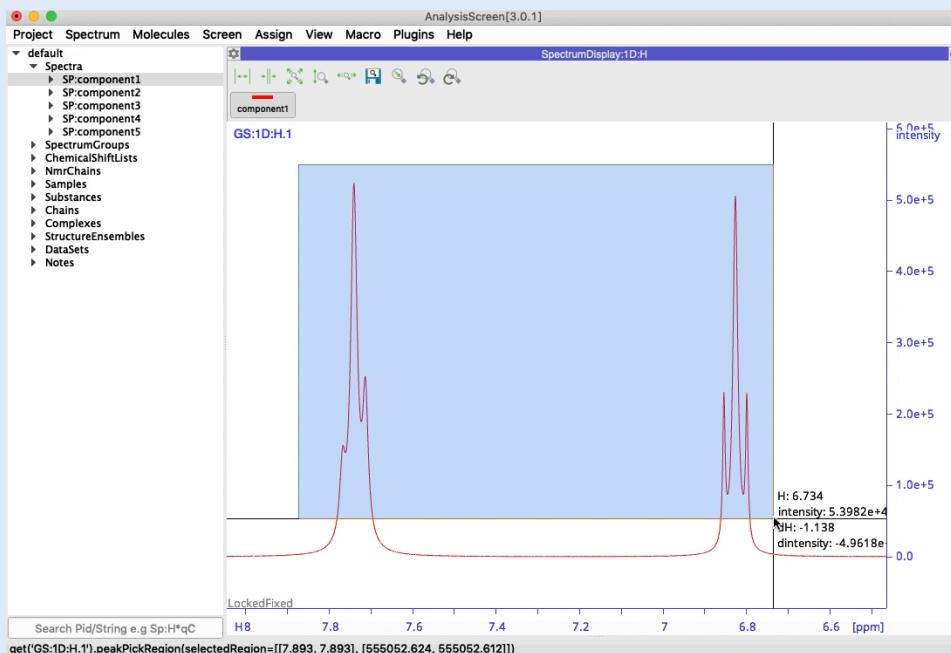
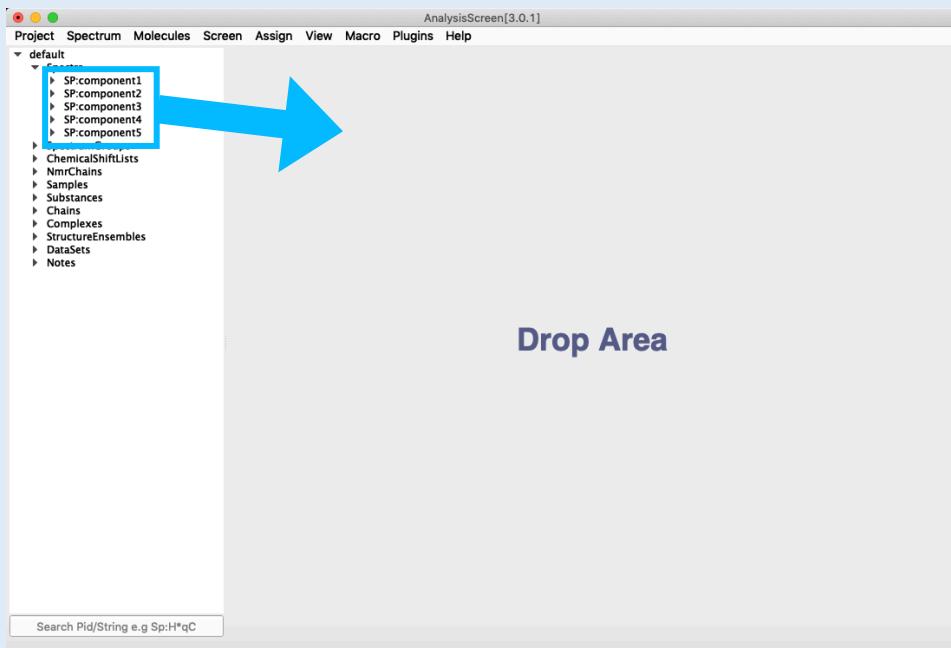
- In your **Samples** branch in the sidebar **double-click** on **<New SampleComponent>**
- Select the **New** radio button to add a new **Substance** to your sample that you don't already have in your project
- Enter the substance **Name** and any other parameters you wish to add, e.g. role, concentration etc.
- Click on **Ok**

Your new **Sample Component** will now be shown as part of your **Sample** in the sidebar.

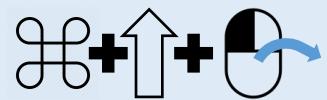
A new **Substance** is automatically created for you in the process which will be visible in the **Substances** branch.



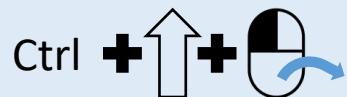
1D Peak Picking



Mac:



Linux/Windows:



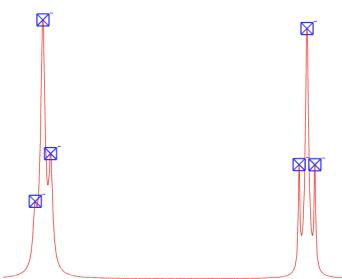
3A Drag Spectrum into Display

- Drag one or more 1D spectra from the sidebar into the drop area.

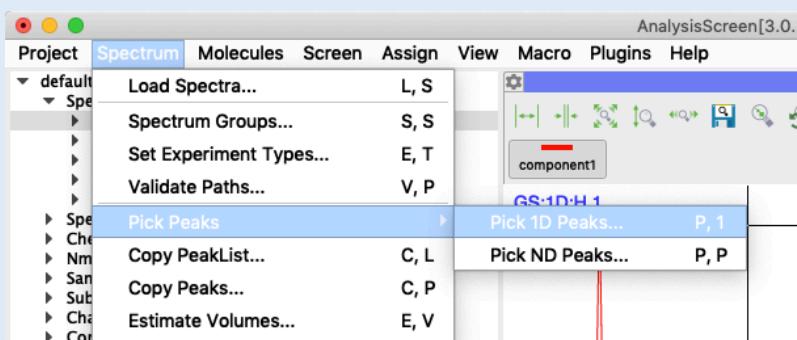
3B Pick Peak Manually

- Adjust the displayed region of the spectrum by changing the zoom or aspect ratio if necessary.
- Press **Shift + Ctrl (Cmd on Mac)** while **left-dragging** the mouse over the peaks you would like to pick. The region will be highlighted in blue.

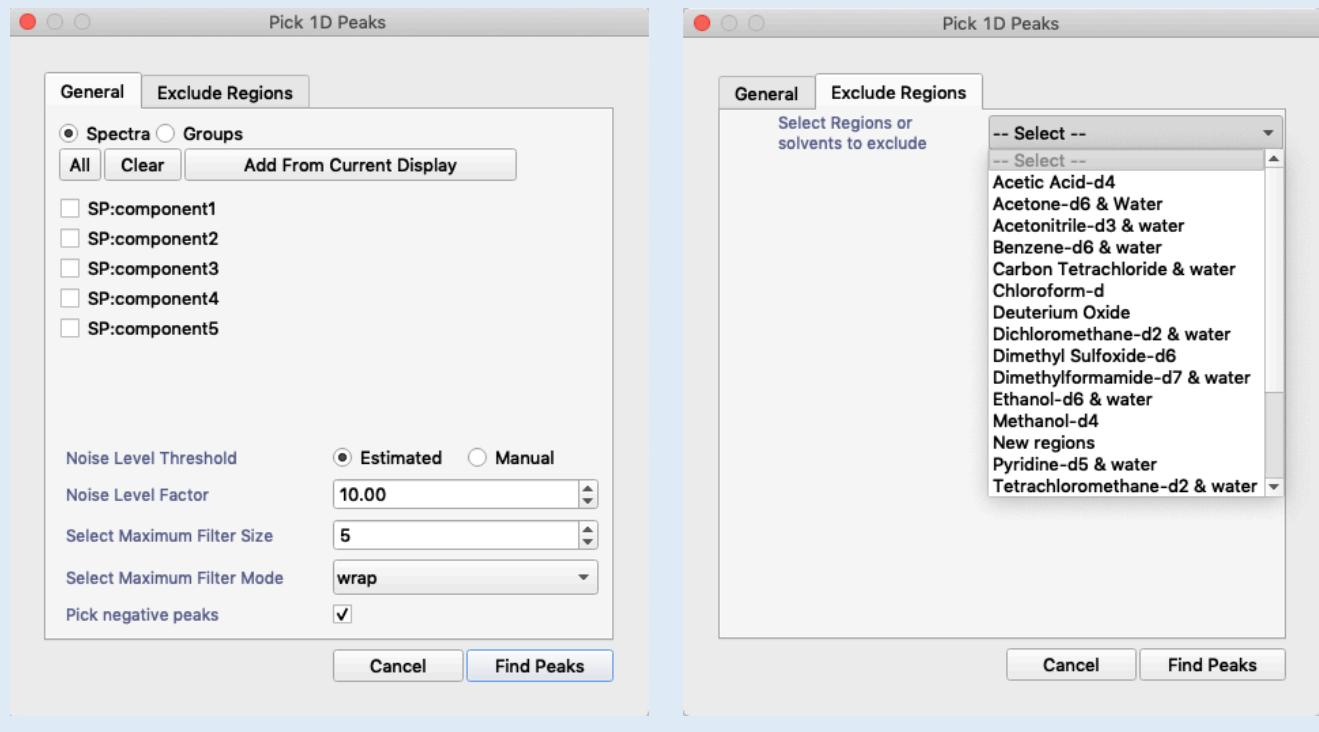
All picked peaks will be marked with a peak symbol:



1D Peak Picking



P1



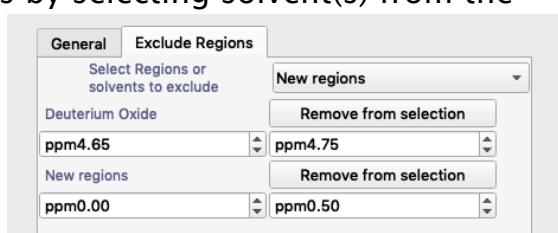
3C Automatic Peak Picking

- Go to Main Menu → Peak Picks → Pick 1D Peaks

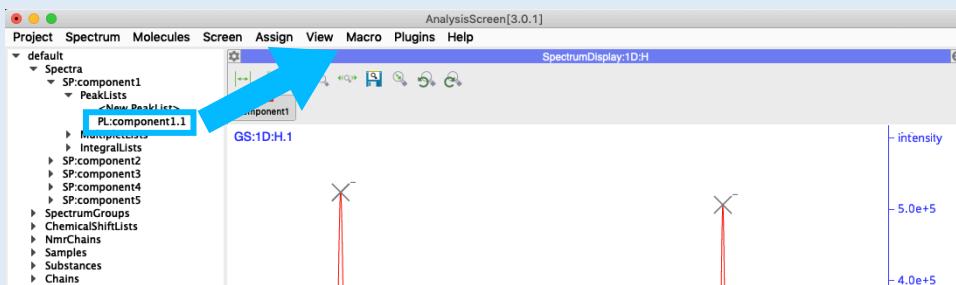
OR

- Use shortcut P1

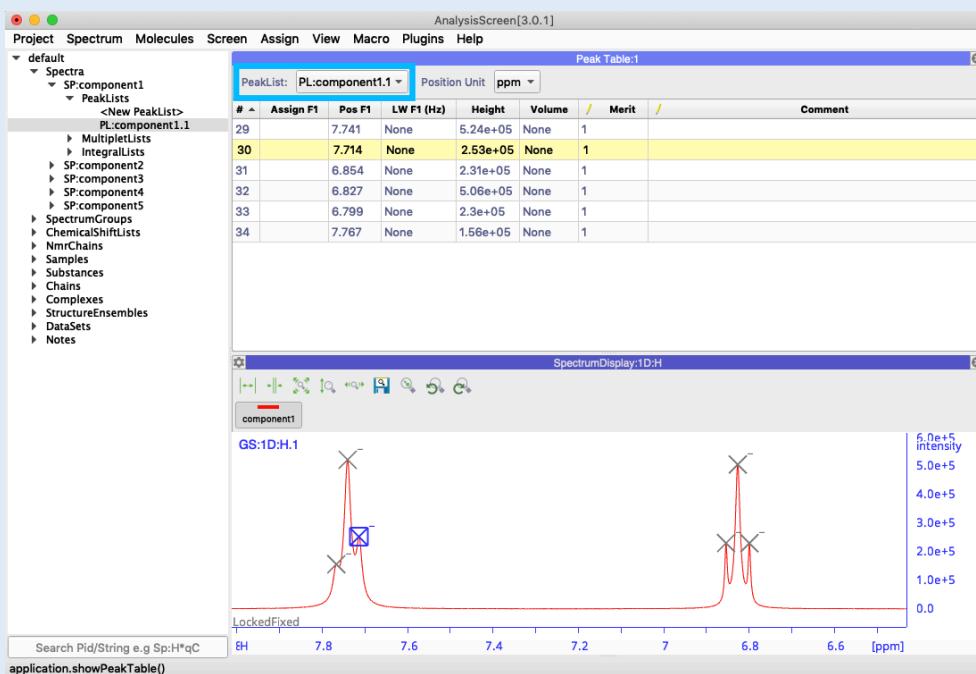
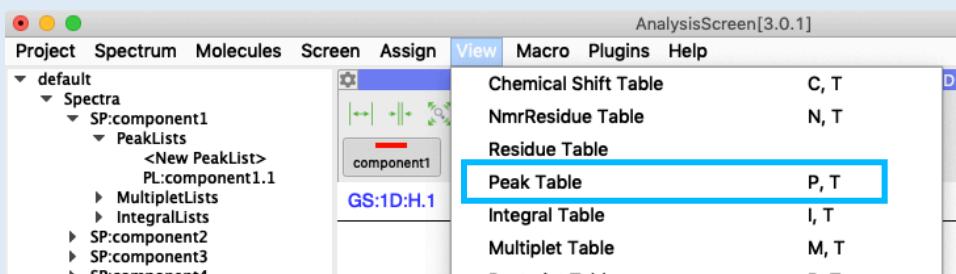
- Select the required **Spectra** or **SpectrumGroups**.
- Leave the **Noise Level Threshold** as **Estimated** if you have different types of noise across all the spectra you have selected.
- Increase the **Maximum Filter Mode** if you have very noisy spectra or spectra with a large number of “shoulder” peaks.
- In the **Exclude Regions** tab you can select regions of the spectrum where you don't want to pick peaks. Add regions by selecting solvent(s) from the list or add your own bespoke region by selecting **New regions**. Make multiple selections to add multiple regions.
- Click **Find Peaks** to pick the peaks.



1D Peak Picking



PT



3D Open Peak Table

- Expand the **Spectra** branch in the sidebar and then the **Peak Lists** branch.
- Drag a peak list into the drop-area.

OR

- Go to **Main Menu** → **View** → **Peak Table**

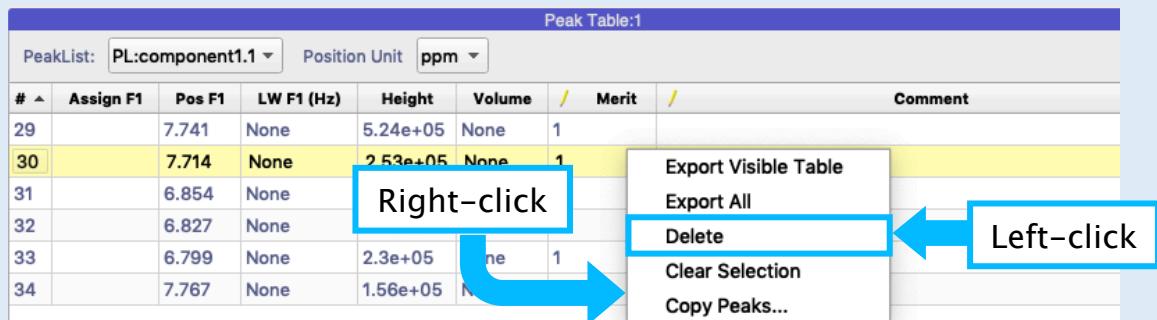
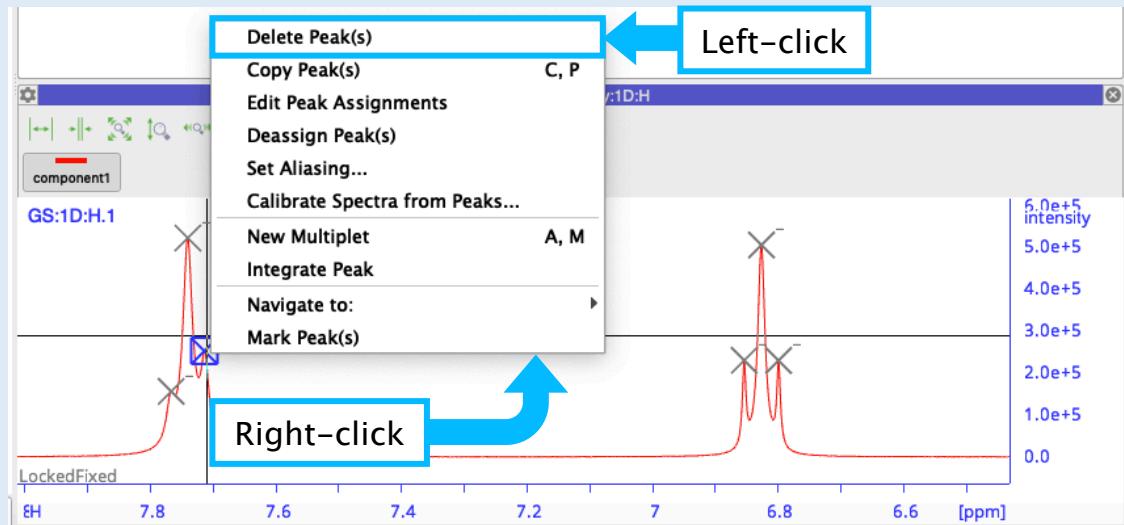
OR

- Use the shortcut **PT**

The peak table will open. You can use the drop-down menu to view a different peak table in that module.

Note that peaks selected in the table are also selected in the spectrum and *vice versa*.

1D Peak Picking



3E Deleting Peaks in the Spectrum Display

- Select a Peak by left-clicking on it. Press **Ctrl** (**Cmd** on a Mac) to select multiple peaks.
- Right-click on a selected peak and select **Delete Peak(s)**.

3F Deleting Peaks in the Peak Table

- Select the peak(s) you want to delete in the table. Use **Shift** or **Ctrl** (**Cmd** on Mac) to select multiple peaks, or **Ctrl/Cmd+A** to select all peaks in the table.
- Right-click and select **Delete**.

OR

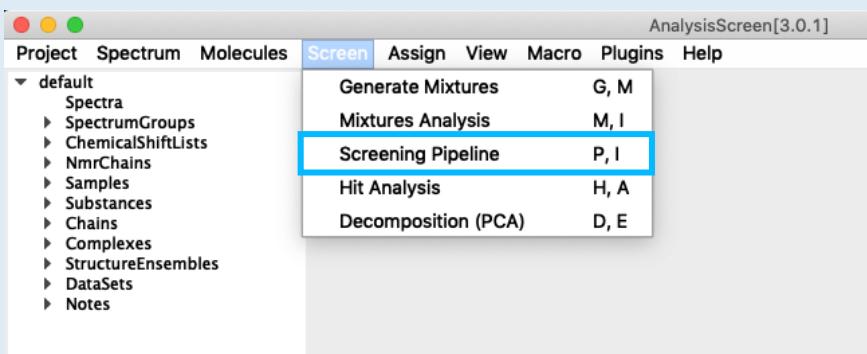
- Use the **Delete** button on your keyboard (**fn + Backspace** on Mac).

The Pipeline is the core module of AnalysisScreen. It allows you to apply several tasks or algorithms, called pipes, to single spectra or groups of spectra (SpectrumGroups).

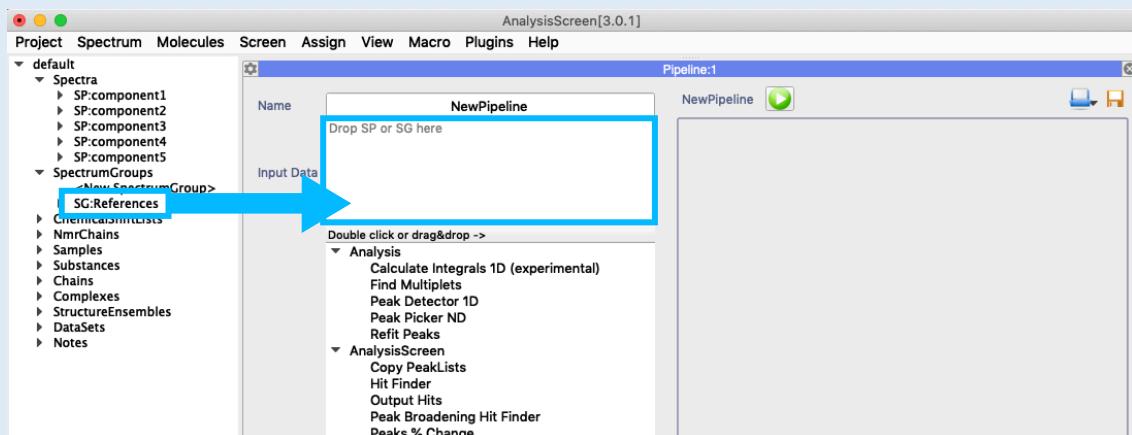
You can run single pipes or a queue of pipes, where the output from each pipe will be the new input for the next pipe.

The pipeline module has been built to be a flexible tool, and this tutorial will show you only a few of the features available for 1D spectra. We will be continually adding new pipes, including pipes for 2D spectra.

Information about pipes not shown here is available within the software.



PI

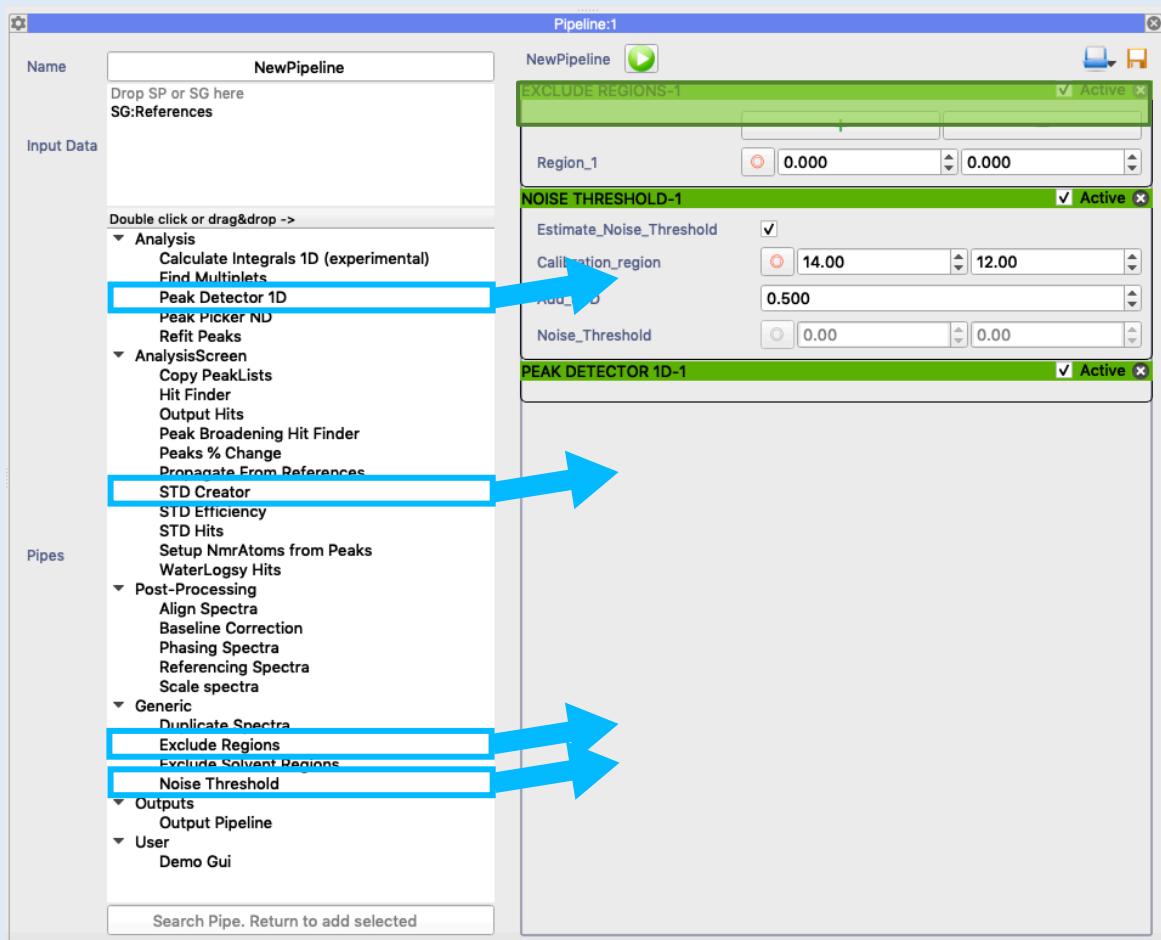


4A Open Pipeline Module

- Go to Main Menu → Screen → Pipelines
- OR
- Use the shortcut PI

4B Select Spectra to apply Pipeline to

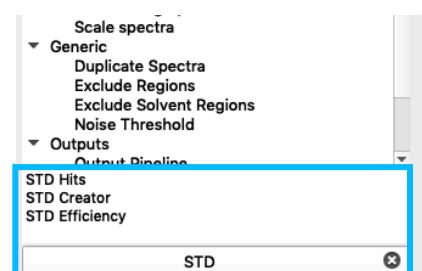
- Drag and Drop one or more Spectra or Spectrum Groups from the sidebar into the Input Data box.



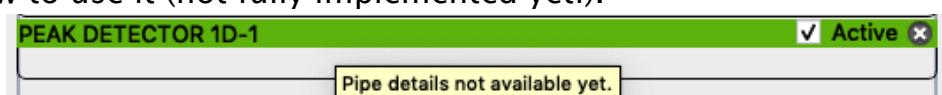
4C Add Pipes to Pipeline

- **Drag and Drop** pipes from the left-hand side of the module to the right (or **double-click** pipes on the left) in order to add pipes to your pipeline. Similar to when dragging modules, you will see a green box appear once you start to drag your pipe, showing you where your pipe will be dropped, e.g. above, below or in between other pipes that are already present.

You can search for pipes using the **Search Box** and **drag** pipes directly from the search results Box, or add a selected pipe by pressing **Enter**.



If you hover over the top bar of a pipe you will see information about what the pipe does and how to use it (not fully implemented yet!).



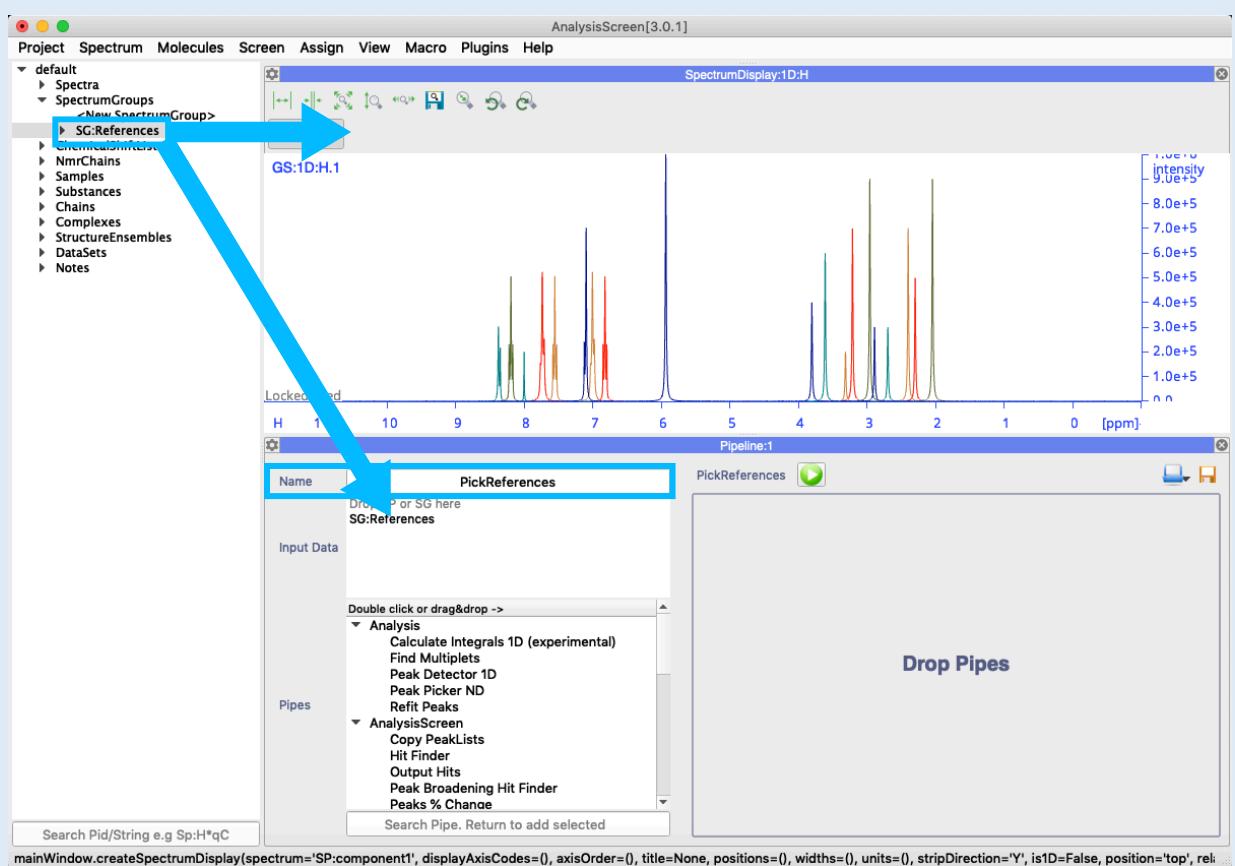
4D Saving and opening pipelines

You can save a pipeline as a .json file for later use, for use in another project or you can pass it on to a colleague to use.

- Click on the icon in the top right-hand corner to save your pipeline as a .json file.
- Click on the icon to open a saved pipeline.

5 Picking Reference Peaks Pipeline

This Pipeline will help you pick the peaks in all your reference spectra.



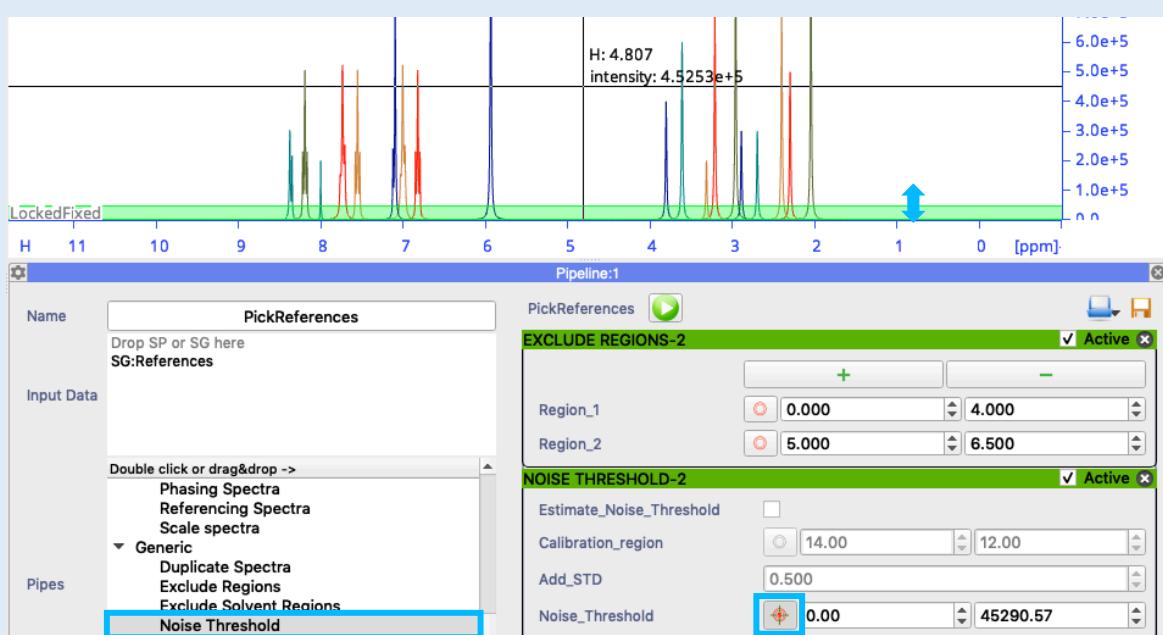
5A Open Project

- Drag the **ScreenTutorial/Projects/PickReferencePeaks ccpn** project into the Drop Area.
- Drag and drop the **References Spectrum Group** into the Drop Area.

5B Set up Pipeline

- Open the pipeline module with **PI** or **Main Menu → Screen → Pipelines** and place it below your spectra.
- Give your new Pipeline a name if you like.
- **Drag the References Spectrum Group** from the sidebar into the **Input box**.

5 Picking Reference Peaks Pipeline



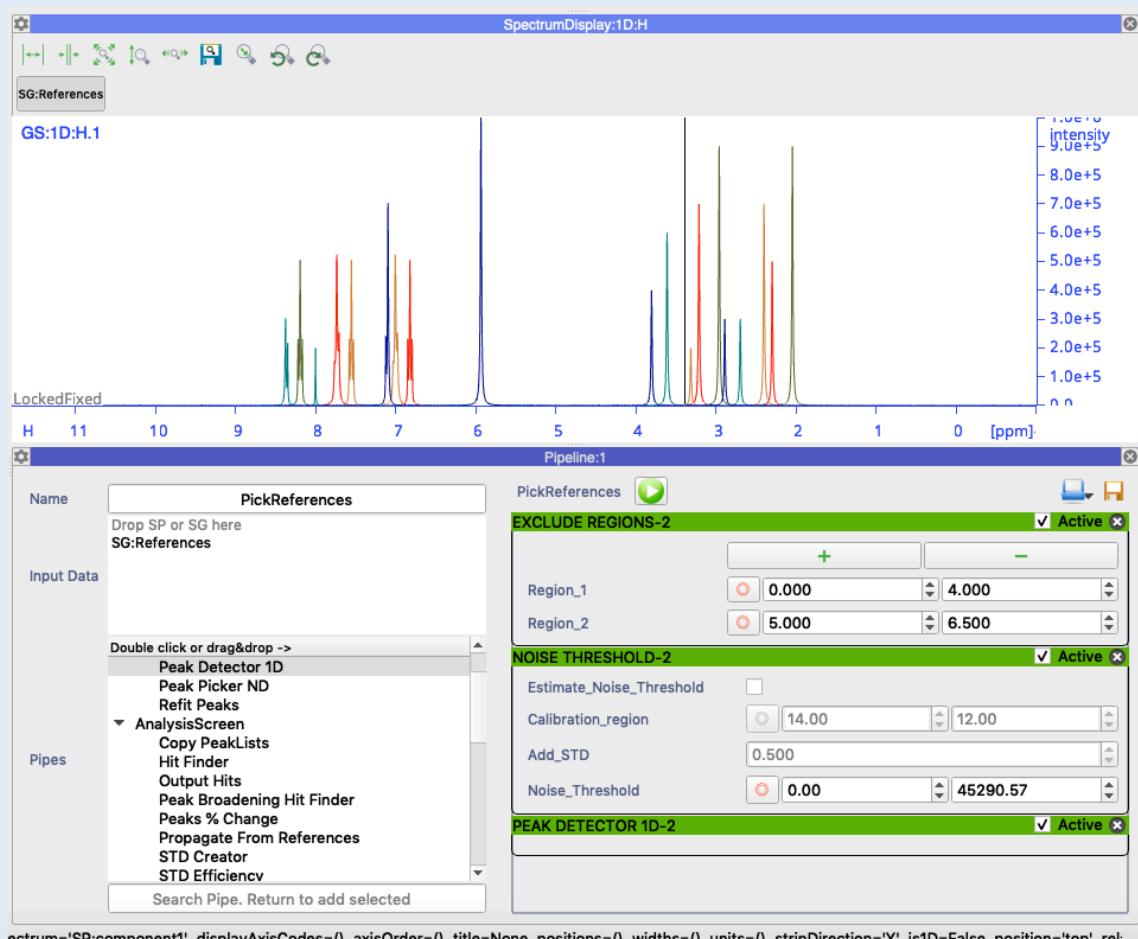
5C Add Exclude Regions Pipe

- Drag the **Exclude Regions** pipe into your pipeline.
- Set a region from 0–4ppm to be excluded from peak picking by entering the values into the boxes, or clicking on the icon and using the mouse to adjust the region in the Spectrum Display.
- Click on the + to add a second exclusion region at 5–6.5ppm

5D Add Noise Threshold Pipe

- Drag the **Noise Threshold** pipe into your pipeline.
- To use automatic Noise Estimation, leave **Estimation** ticked and select a region of the spectrum to be used for the estimation.
- To set a threshold manually, untick **Estimation** and set the noise level using the box or via the icon and the mouse in the display.

5 Picking Reference Peaks Pipeline

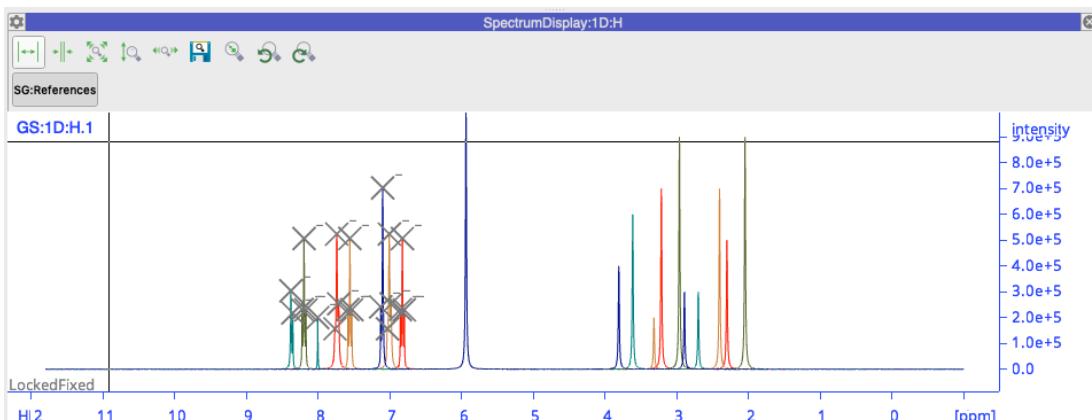


5E Add Peak Detector Pipe

- Drag the **Peak Detector** pipe into your pipeline. This works fully automatically with no manual adjustments required.

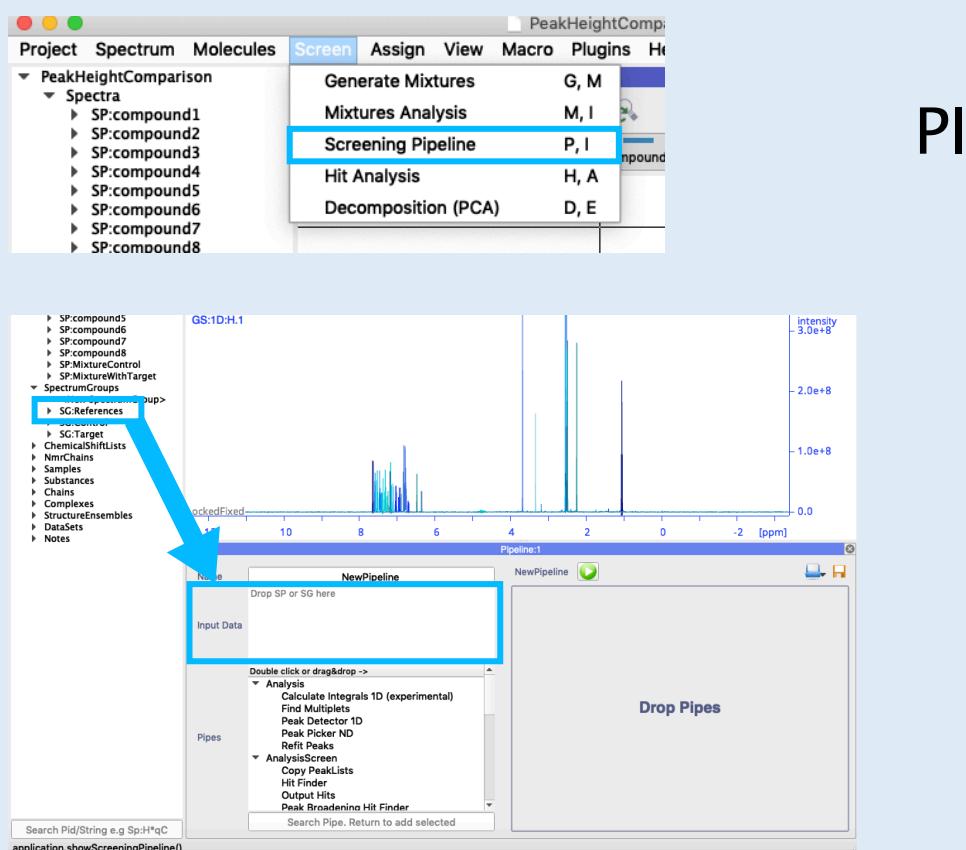
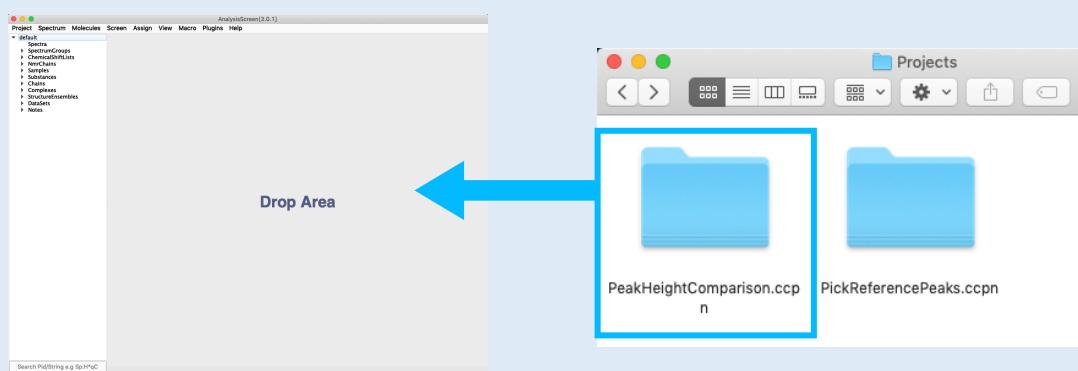
5F Run the Pipeline

- Click the green **Play button** . You should now see that the peaks in your reference spectra are picked (in the regions you did not exclude).



6 Peak Height Comparison Pipeline

This Pipeline will help you analyse the change in peak height between two spectra. It will take two spectra, re-reference and scale them relative to one another, pick the peaks in one, copy them to the other and then calculate the change in peak height.



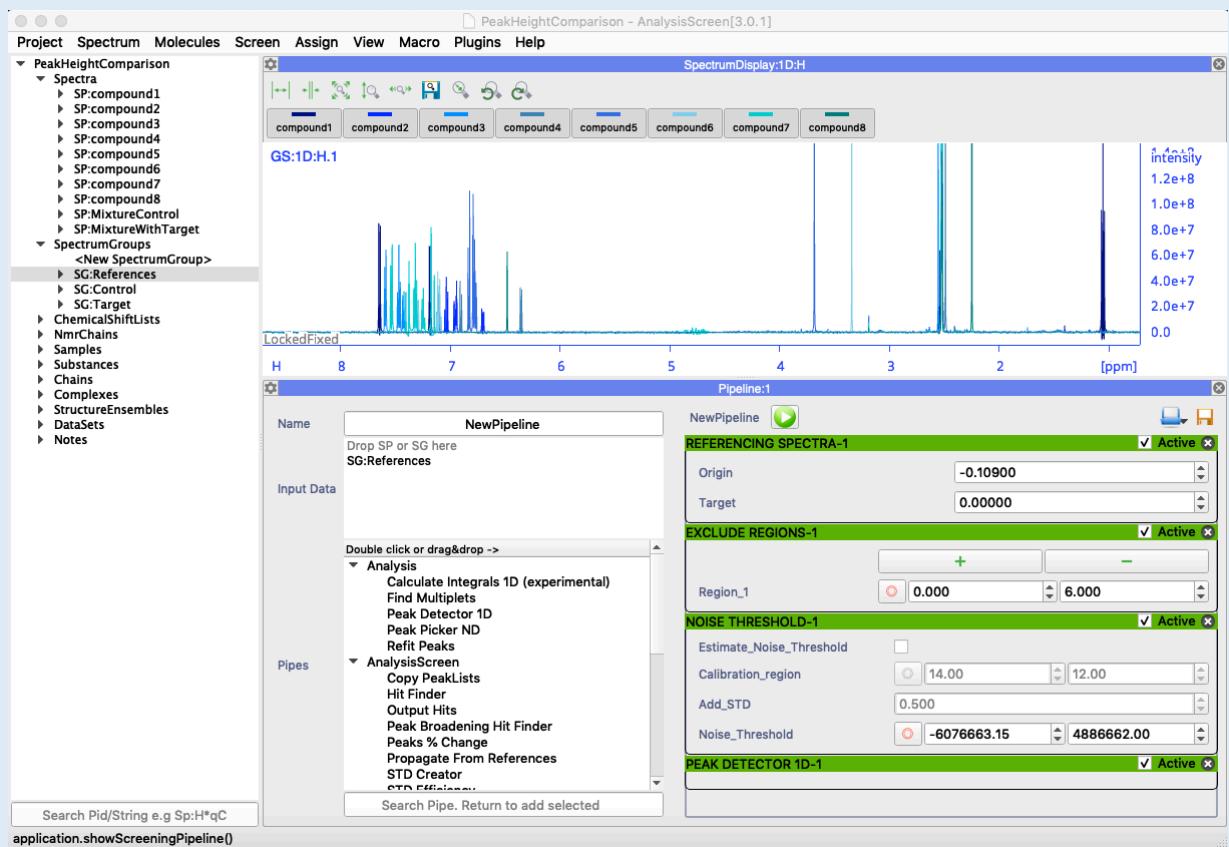
6A Open Project

- Drag the **ScreenTutorial/Projects/PeakHeightComparison ccpn** project into the Drop Area.

6B Set up Pipeline

- Open the pipeline module with **PI** or **Main Menu → Screen → Pipelines** and place it below your spectra.
- **Drag the References Spectrum Group** from the sidebar into the **Input Data** box.

6 Peak Height Comparison Pipeline

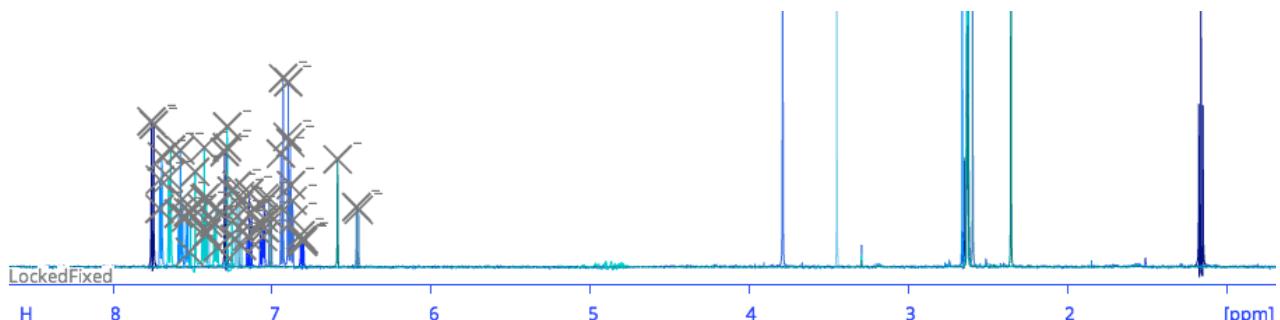


6C Add Pipes to pick reference peaks

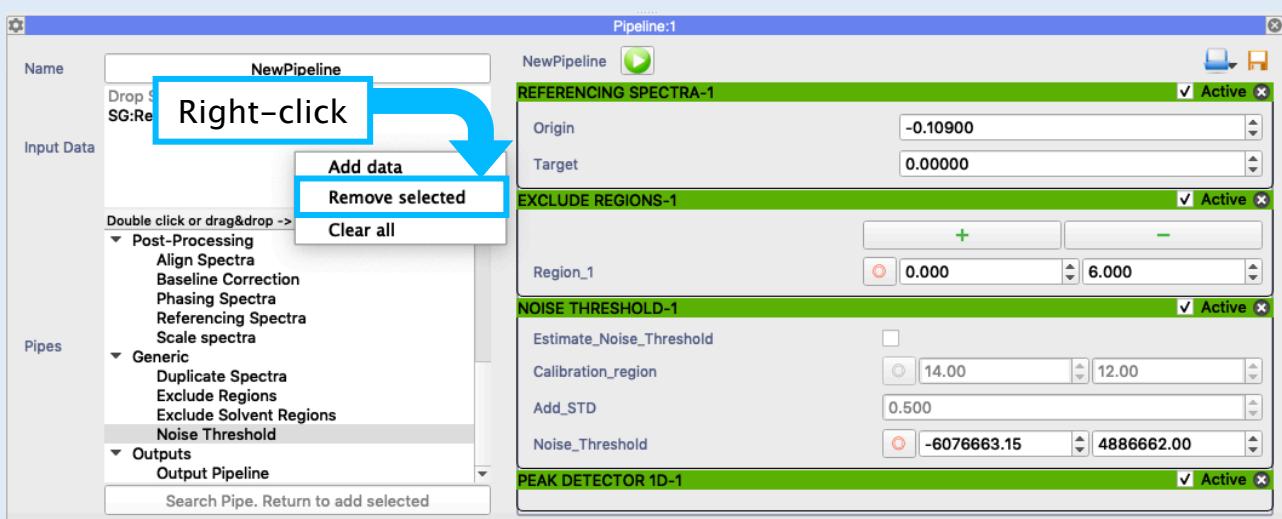
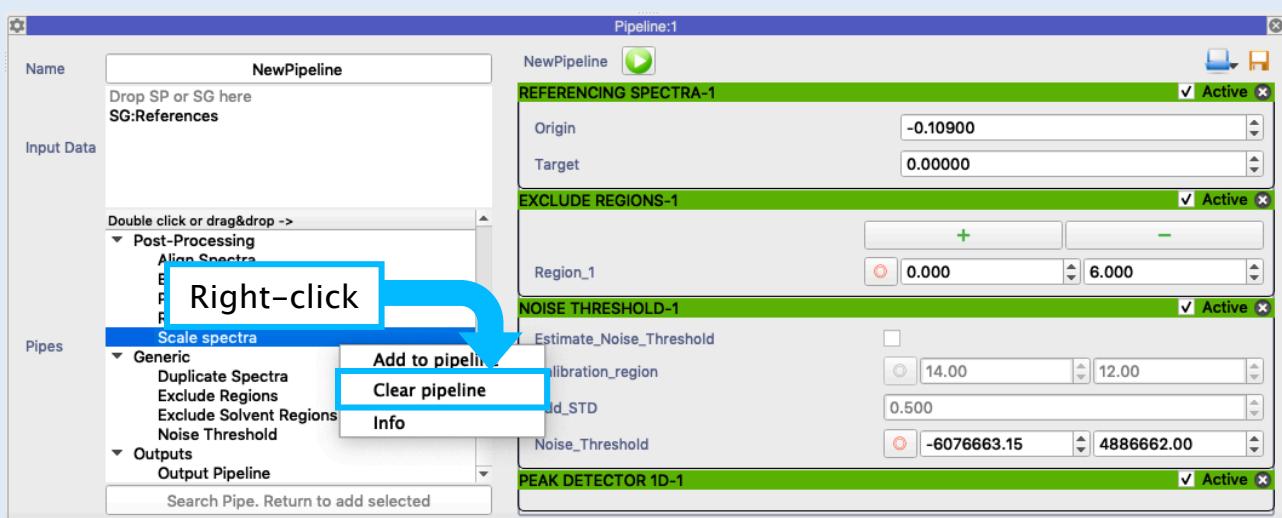
- Drag the **Re-reference** pipe into your pipeline and set **Origin** to -0.109 ppm
- Drag the **Exclude Regions** pipe into your pipeline and set the excluded region to 0–6 ppm.
- Drag the **Noise Threshold** pipe into your pipeline and set the noise threshold to an appropriate level.
- Drag the **Peak Detector** pipe into your pipeline.

6D Run Pipeline

- Run the pipeline by pressing on the green **Play** button:



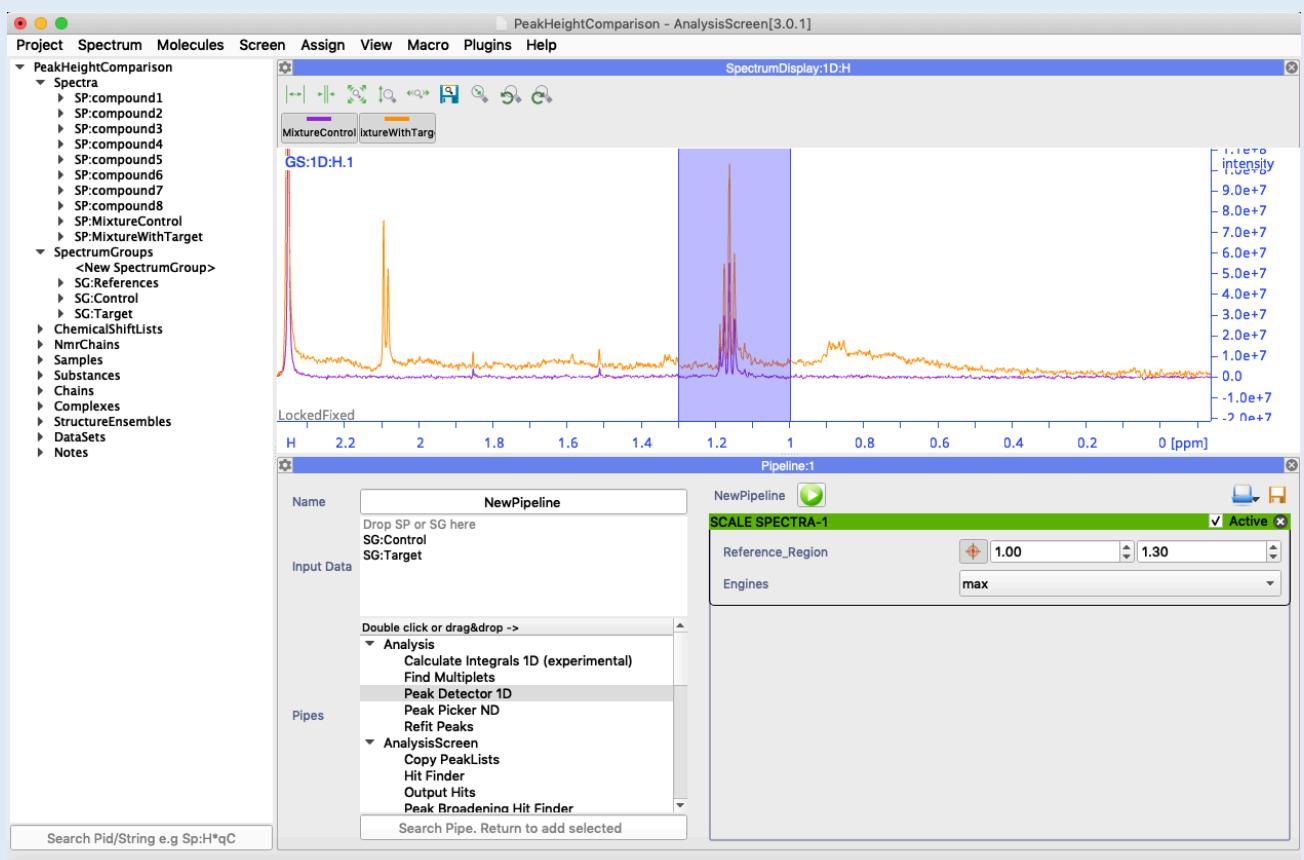
6 Peak Height Comparison Pipeline



6E Clear pipeline and input data

- Clear the pipeline by **right-clicking** in the **Pipes** box and selecting **Clear pipeline**.
- Clear the Input data by **right-clicking** in the **Input Data** box and selecting **Clear all**.

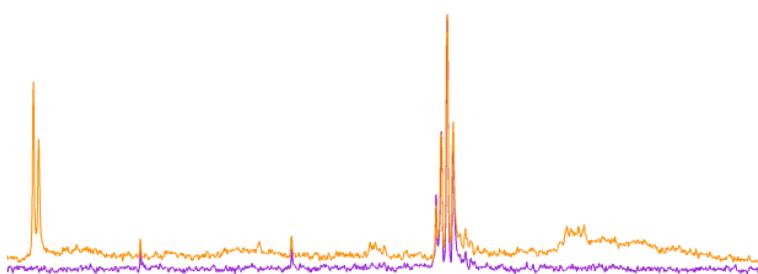
6 Peak Height Comparison Pipeline



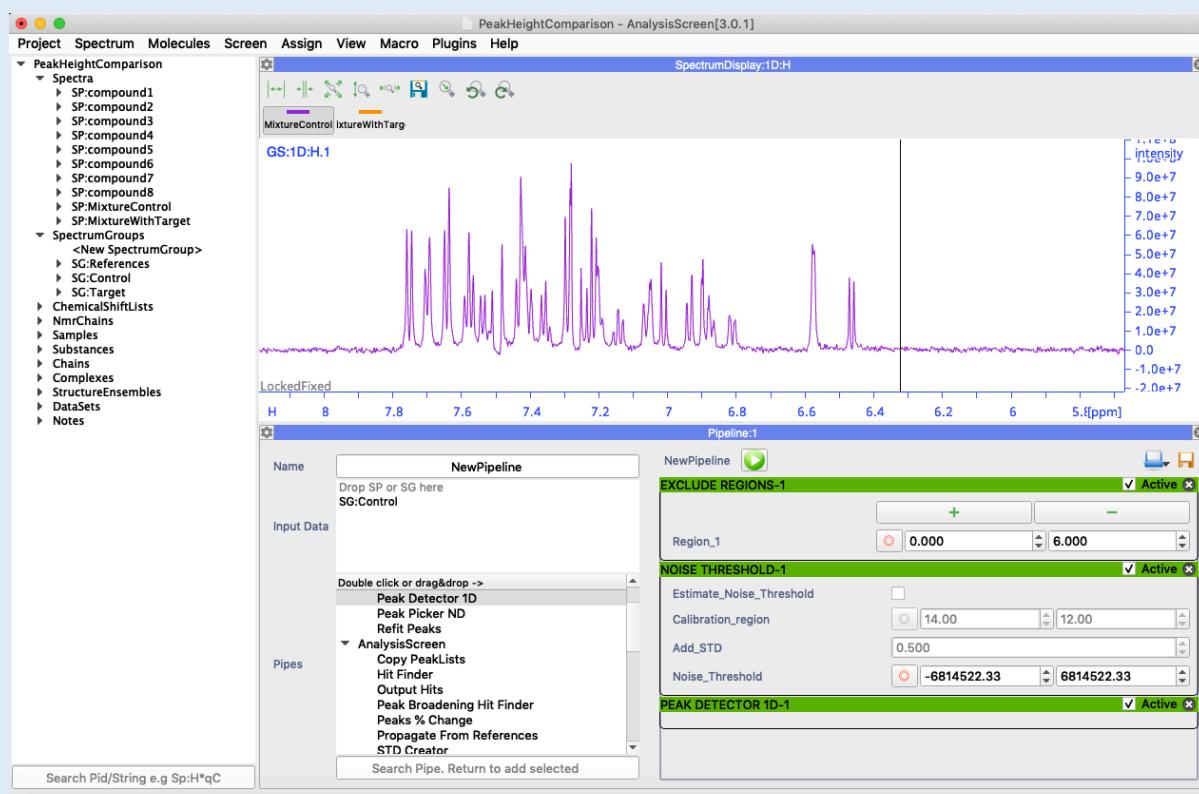
6F Scale Control and Target spectra

For this data set, the control and target spectra need to be scaled relative to one another.

- Open the **MixtureControl** and **MixtureWithTarget** spectra in a Spectrum Display.
- Drag the **Control** and **Target** Spectrum Groups into the **Input Data** box.
- Drag the **Scale Spectra** pipe into the pipeline.
- Select the region around the multiplet at 1.16 ppm as your **Reference Region**.
- Choose **max** from the **Engine** drop-down.
- Run the pipeline by pressing the **Play** button:

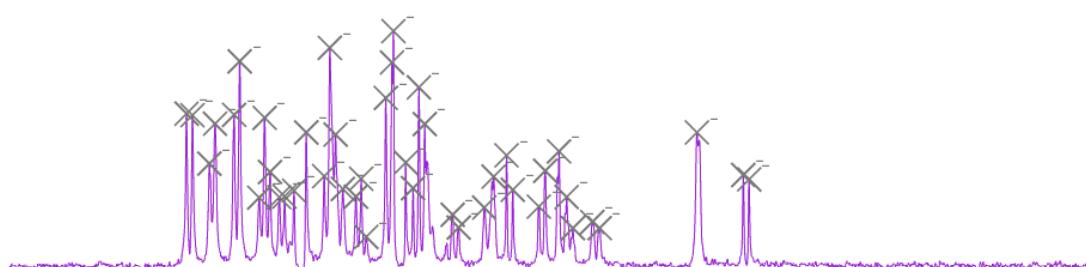


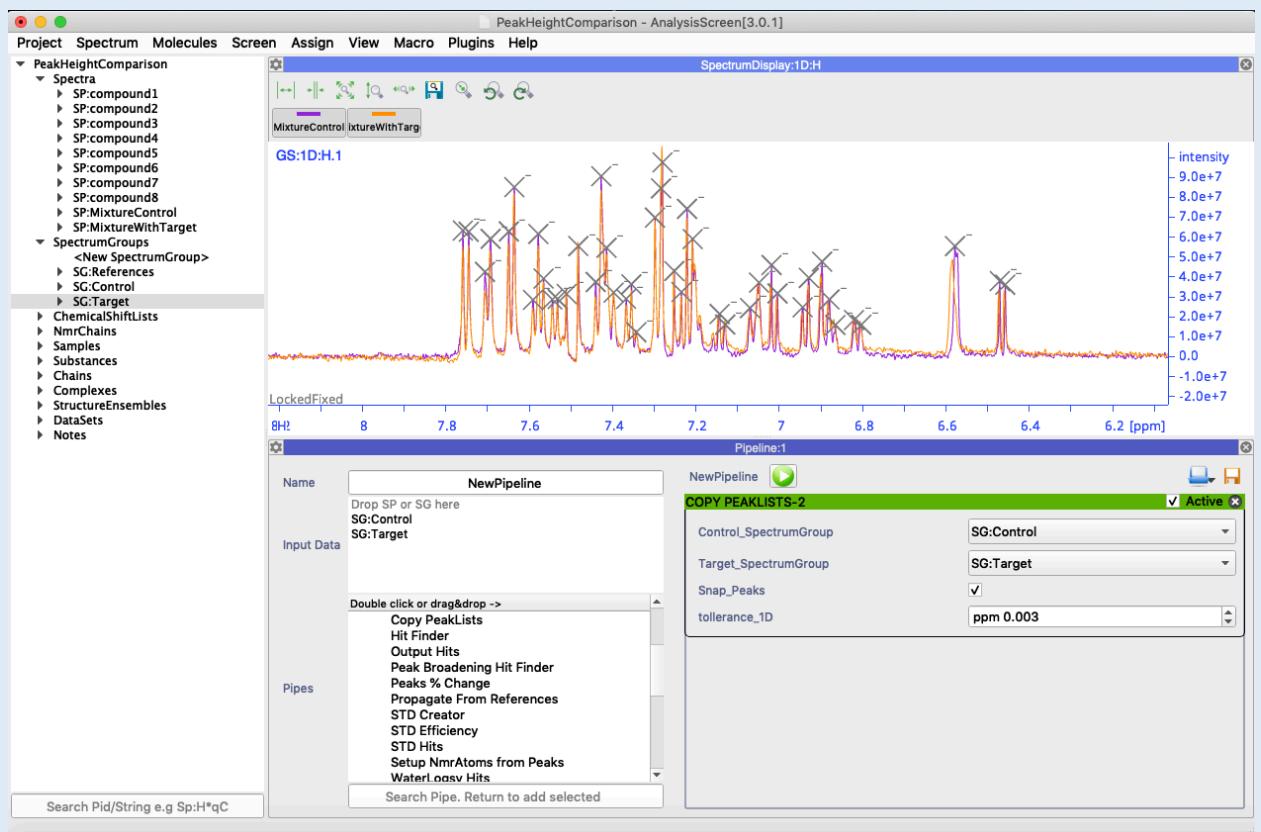
6 Peak Height Comparison Pipeline



6G Pick peaks in control spectrum

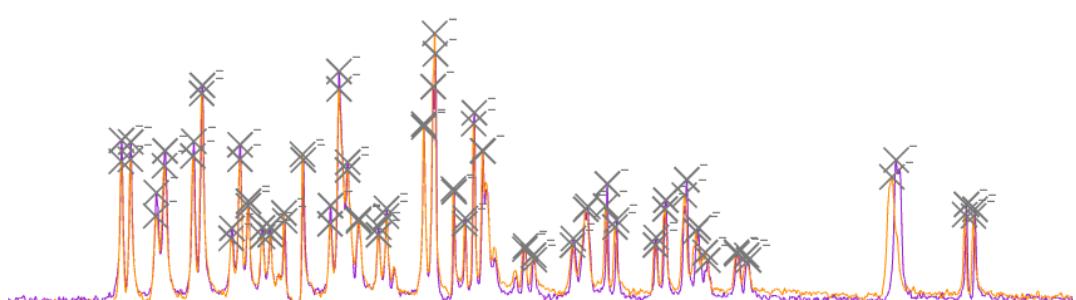
- Clear the pipeline and remove the **Target Spectrum Group** from the Pipeline **Input Data** box.
- Drag the **Exclude Regions** pipe into your pipeline and set the excluded region to 0–6 ppm.
- Drag the **Noise Threshold** pipe into your pipeline and set the noise threshold to an appropriate level.
- Drag the **Peak Detector** pipe into your pipeline.
- Run the pipeline by pressing the **Play** button



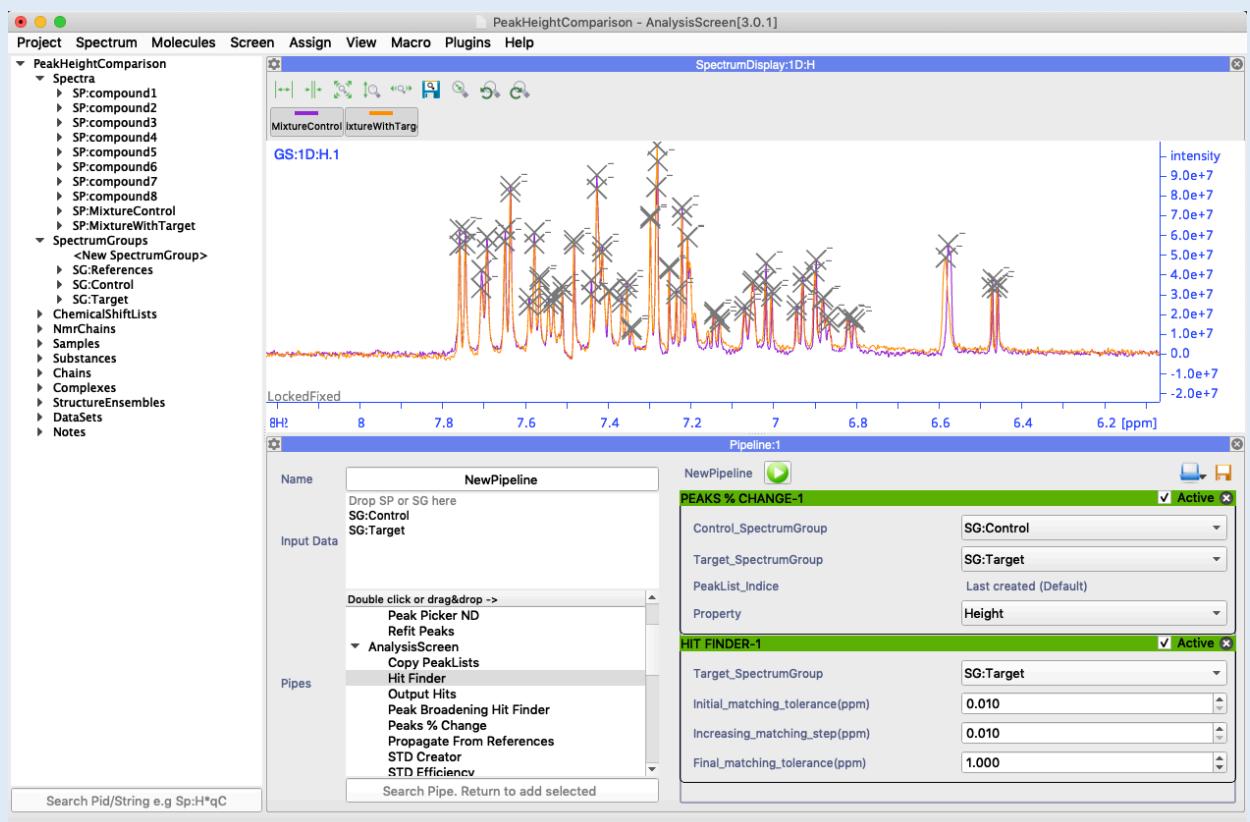


6H Copy Peak List from Control to Target

- Clear the pipeline
- Add the **Target** Spectrum Group from the Pipeline **Input Data** box.
- Drag the **Copy Peak Lists** pipe into your pipeline.
- Make sure the **Target** and **Control Spectrum Groups** are correctly selected from the drop-down menus.
- Run the pipeline by pressing the **Play** button.



6 Peak Height Comparison Pipeline



Peak Table:1						
PeakList: PL:MixtureWithTarget.2		Position Unit ppm				
#	Assign F1	Pos F1	LW F1 (Hz)	Height	Volume	Merit
1		7.759	None	5.5e+07	None	0.141
2		7.746	None	5.58e+07	None	0.115
3		7.707	None	3.33e+07	None	0.244
4		7.694	None	5.32e+07	None	0.106
5		7.650	None	5.71e+07	None	0.0965
6		7.637	None	8.19e+07	None	0.0382
7		7.592	None	2.45e+07	None	0.151
8		7.578	None	5.58e+07	None	0.0996

6| Determine % change in peak heights and find Hits

- Clear the pipeline
- Drag the **Peaks % Change** pipe into your pipeline and make sure the **Control** and **Target Spectrum Groups** are appropriately selected.

Make sure that **Height** is selected as the **Property**.

The results will be stored in the **Merit** column of the **Target** spectrum peak list.

- Drag the **Hit Finder** pipe into your pipeline.

The **Target Spectrum Group** drop-down selects the peak list to use for the analysis.

- Run the pipeline by pressing the **Play** button.

The results are stored in a new **Dataset** which can be analysed using the **Hit Analysis** module.

Hit Analysis Module

This module will allow you to inspect the Spectrum Hits obtained after using the HitFinder pipe, e.g. as shown in section 6.

The screenshot shows the NMR-Console software interface. At the top, there's a menu bar with 'Project', 'Spectrum', 'Molecules', 'Screen', 'Assign', 'View', 'Macro', 'Plugins', and 'Help'. The 'Screen' menu is currently selected. A sub-menu for 'Screen' is open, showing several options: 'Generate Mixtures' (G, M), 'Mixtures Analysis' (M, I), 'Screening Pipeline' (P, I), 'Hit Analysis' (H, A), and 'Decomposition (PCA)' (D, E). The 'Hit Analysis' option is highlighted with a blue box. To the right of the menu, the letters 'HA' are displayed. Below the menu, there's a tree view of project files under 'default'. The main workspace shows a table titled 'Hit Analysis:1' with data for various compounds. One row is selected, showing details like Reference Peak Positions, Matched Peak Positions, and Peak Merits. Below the table is a 'SpectrumDisplay:1:D:H' window showing a 1D spectrum for 'compound1'. The x-axis is labeled '[ppm]' and ranges from 7.6 to 7.9. The y-axis is labeled 'Intensity' and ranges from 0.0e+0 to 1.4e+9. The spectrum shows several peaks, some with blue markers and others with orange markers. At the bottom left, there's a search bar with 'Search Pid/String e.g Sp:H*qc' and a dropdown menu with 'LockedFixed'. At the bottom, there's a small window titled 'Display(s): > select-to-add <' with a dropdown menu showing 'Last Opened'.

7A Open the Hit Analysis Module

- Go to Main Menu → Screen → Hit Analysis

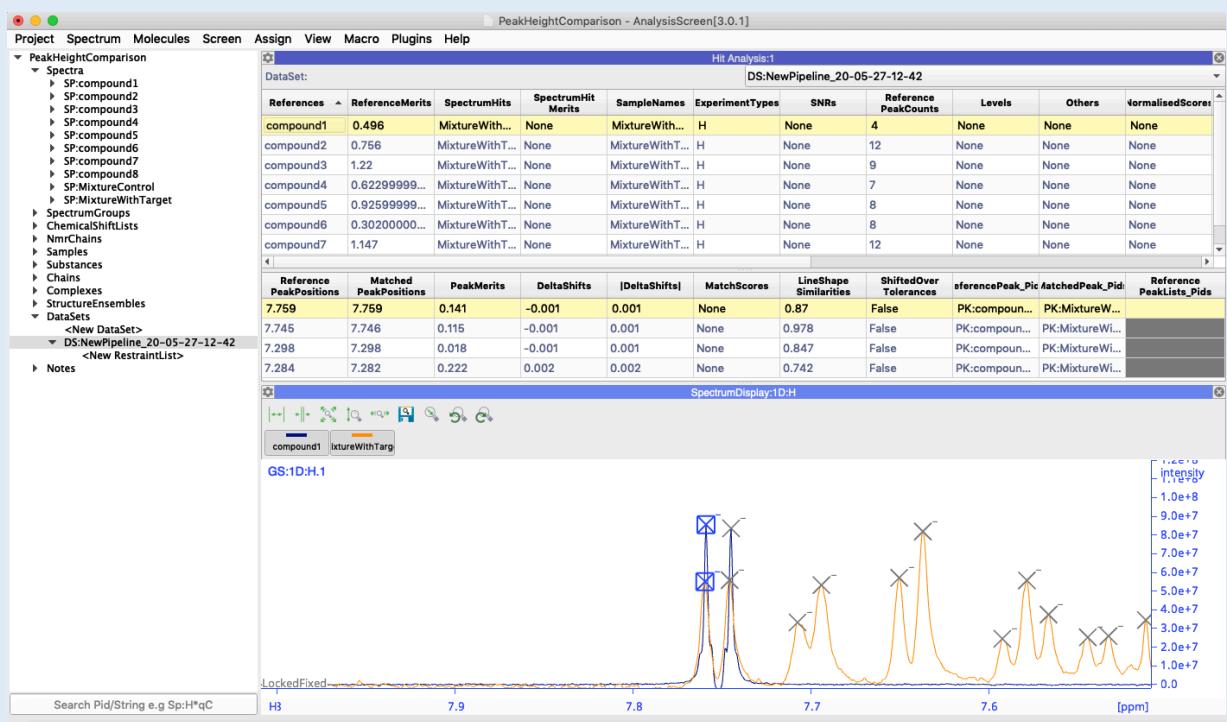
OR

- Use the shortcut **HA**

Make sure you have at least one **Spectrum Display** module open. As you click on rows in the **Hit Analysis** module, you should see corresponding spectra being shown in the **Spectrum Display**. If want to choose manually which **Spectrum Display** to use, then:

- Click on the **Hit Analysis** module **Settings** gear icon:
- Select the **Spectrum Display** of your choice.

Hit Analysis Module



Although the reference spectra were not explicitly selected in the HitFinder pipe, there is an implicit link to them, because the MixtureControl and MixtureWithTarget spectra are linked to samples which contain components whose substances are in turn linked to the reference spectra (see figure on page 7). Such links are easily established by inputting your data via an Excel spreadsheet (see section 8).

7B Investigate your results

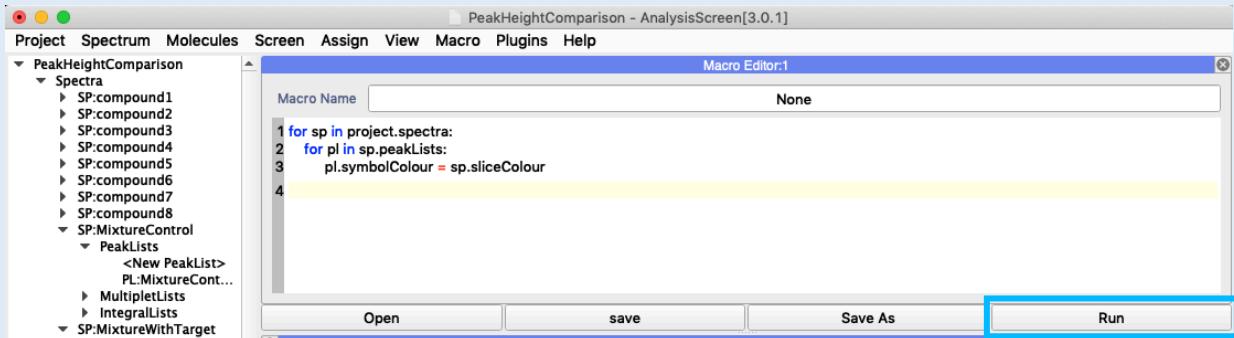
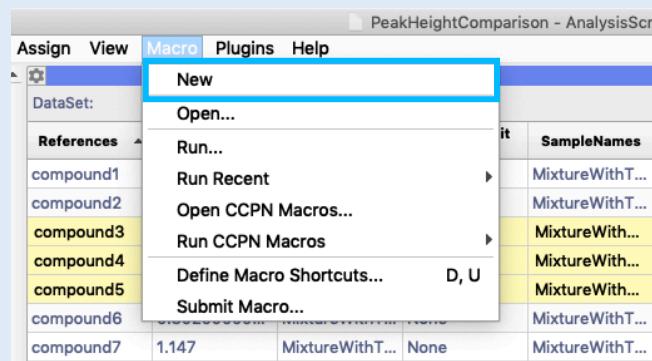
The **Hit Analysis** module contains two tables. The top table contains a list of the reference spectra and the lower table contains a list of all the peaks in the reference spectra selected above. The **Spectrum Display** will update to show only your target spectrum and the reference spectra selected above. Selecting a peak in the table will navigate to it in the **Spectrum Display** and select it and its corresponding target spectrum peak. Selecting a target peak in the **Spectrum Display** will highlight all reference peaks linked to it and their samples in the **Hit Analysis** module.

You can sort the tables by any column by **clicking on the column heading**. Click on the **column heading** a second time to reverse the sort order.

To show/hide columns in the tables:

- **Right-click** on the table header and select **Columns Settings...**.
- Check/Uncheck the columns as required.

Hit Analysis Module



7C Change peak symbol colours

You may find it easier to examine your data if the peak symbols have the same colour as your spectra. You can easily change the peak symbol colour using a short Macro:

- Go to Main Menu → Macro → New
- Copy and Paste the following text into the Macro Editor window:

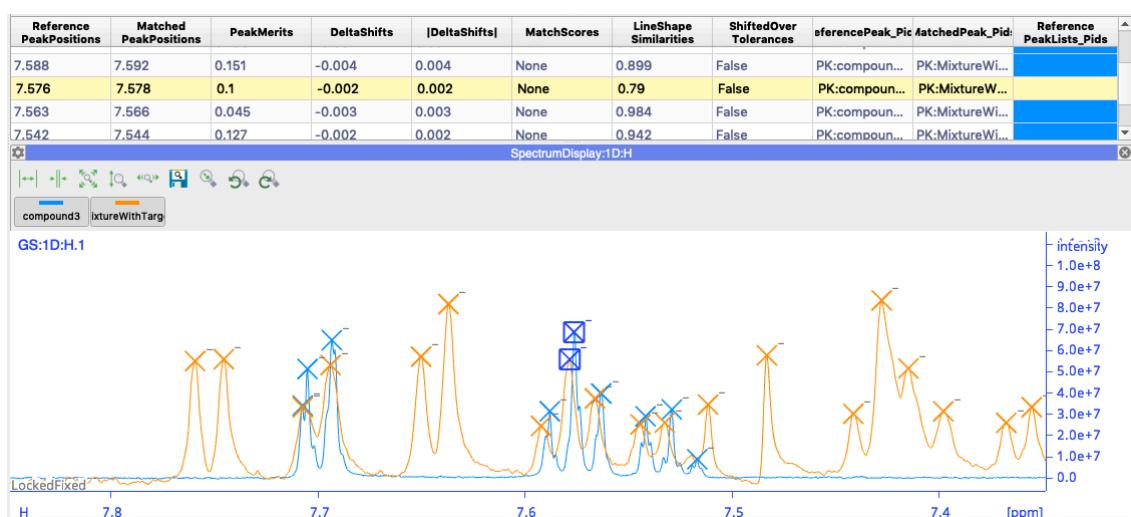
```

for sp in project.spectra:
    for pl in sp.peakLists:
        pl.symbolColour = sp.sliceColour

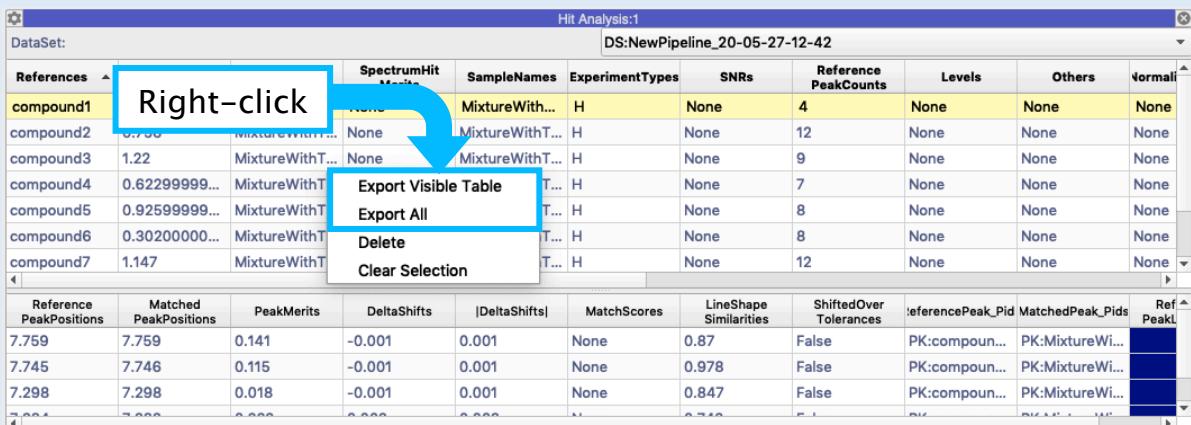
```

making sure that the indentations are retained correctly.

- Click on the Run button. Your Hit Analysis and Spectrum Display modules should update with the new peak list colours:



- Now close the Macro Editor module again, saving the macro if you wish.



A screenshot of the Hit Analysis Module interface. At the top, it says "Hit Analysis:1" and "DS>NewPipeline_20-05-27-12-42". Below this is a table titled "DataSet". A blue box highlights the first row of the table, which contains the compound names: "compound1", "compound2", "compound3", "compound4", "compound5", "compound6", and "compound7". A blue arrow points from the text "Right-click" to the context menu that appears when right-clicking on the "compound1" row. This menu has four options: "Export Visible Table", "Export All", "Delete", and "Clear Selection". The "Export Visible Table" option is highlighted with a blue box.

References	SpectrumHit	SampleNames	ExperimentTypes	SNRs	Reference PeakCounts	Levels	Others	Normal
compound1	None	MixtureWith...	H	None	4	None	None	None
compound2	0.750	MixtureWithT...	None	MixtureWithT...	H	None	12	None
compound3	1.22	MixtureWithT...	None	MixtureWithT...	H	None	9	None
compound4	0.62299999...	MixtureWithT...	T...	H	None	7	None	None
compound5	0.92599999...	MixtureWithT...	T...	H	None	8	None	None
compound6	0.30200000...	MixtureWithT...	T...	H	None	8	None	None
compound7	1.147	MixtureWithT...	T...	H	None	12	None	None
Reference PeakPositions	Matched PeakPositions	PeakMerits	DeltaShifts	DeltaShifts	MatchScores	LineShape Similarities	ShiftedOver Tolerances	ReferencePeak_Pid MatchedPeak_Pids Ref PeakL
7.759	7.759	0.141	-0.001	0.001	None	0.87	False	PK:compoun...
7.745	7.746	0.115	-0.001	0.001	None	0.978	False	PK:compoun...
7.298	7.298	0.018	-0.001	0.001	None	0.847	False	PK:compoun...
7.004	7.000	0.000	0.000	0.000	None	0.740	False	PK:MixtureWi...

7D Export Spectrum Hits

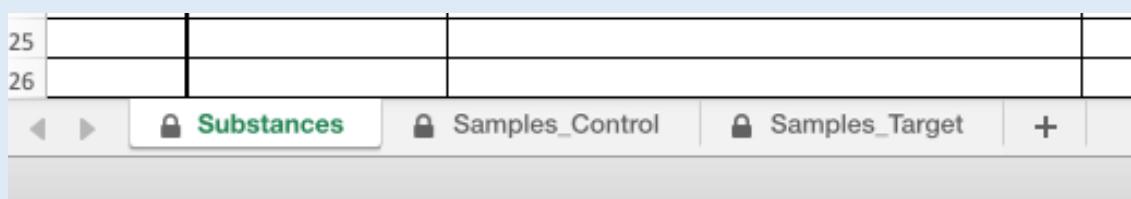
- Right-click on the table and select **Export Visible Table** to export the columns and rows currently shown or **Export All** to export all columns whether hidden or shown and without any rounding.

When you save the file, you can choose to do so in Excel, .csv or .tsv format.

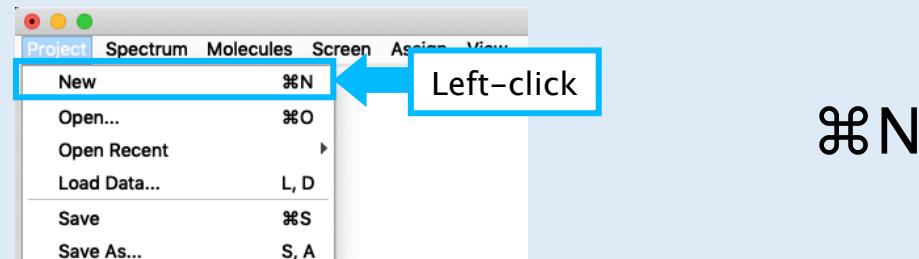
In this section you will learn how to create an Excel file to load all your spectra and metadata into the program automatically.

Using a lookup file you can easily create CcpNmr objects which, once loaded, will be immediately available in the sidebar.

The program can read .xls or .xlxs files with multiple sheets that include the words **Sample** or **Substance** in the sheet name.



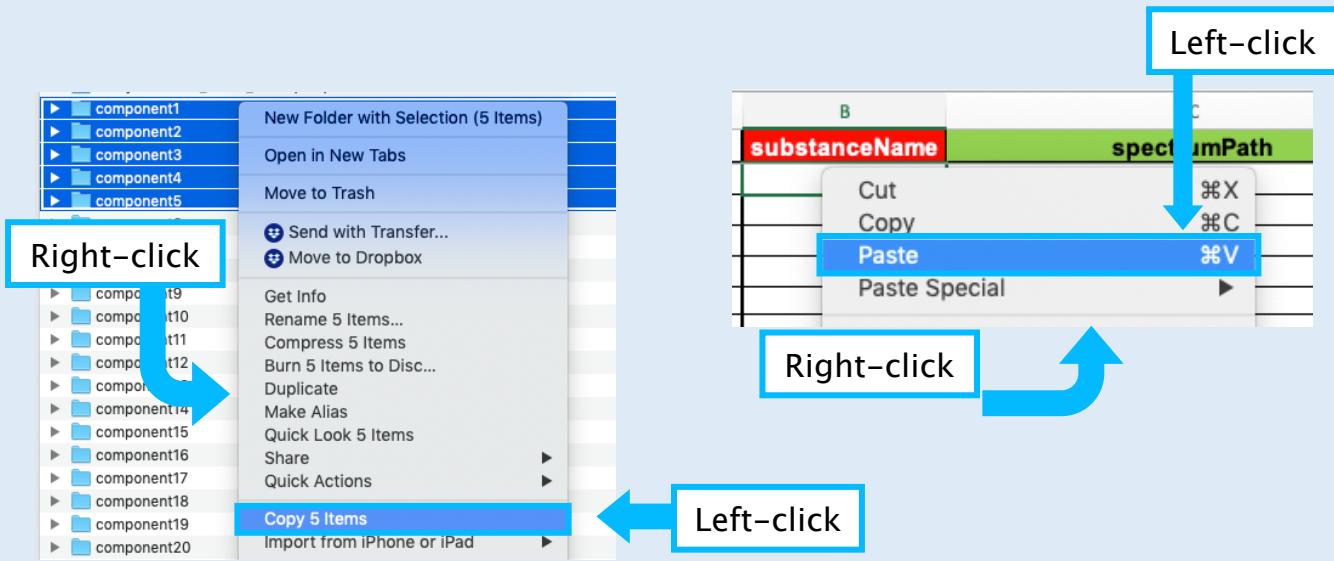
You can create files that contain either the Substance or Sample page or both.



8A Create a New Project

- Go to **Main Menu → Project → New**
- OR
- Use the shortcut **⌘N**

N	substanceName	spectrumPath	spectrumGroupName	experimentType	comment	smiles	synonyms	...
1	component1	component1	References	H	c.uk/chebi/searchId.do?chebi	C1=CC#C=C1	1,2-didehydrobenzene	
2	component2	component2	References	H	c.uk/chebi/searchId.do?chebi	COC1=CC=CC#C1	1-methoxycyclohexa-1,3-dien-5-yne	
3	component3	component3	References	H	c.uk/chebi/searchId.do?chebi	CC[NH3+]	ethylaminium	
4	component4	component4	References	H	mid=64B6AB2667E275BC3E6	CC(O)C(O)=O	3-hydroxybutyric acid	
5	component5	component5	References	H	c.uk/chebi/searchId.do?chebi	CC[NH3+]	mevalonic acid	



8B Create the Substance Sheet

The first sheet, **Substance**, can contain metadata associated with small molecules whose spectra have been used as references in a screen.

- **Copy** the Lookup file template from the **ScreenTutorial** directory to the directory containing your spectra (**ScreenTutorial/spectra/LookupData** if using the tutorial data).
- Open the template and fill in the **substanceName** column. This is the only mandatory column to fill in. A quick way to fill the table on a Mac is to multi-select the directory or spectra files you want to include, then **copy and paste** them into the Excel cell under **substanceName**. Select the first five components.

OR

copy and paste the following:

component1

component2

component3

component4

component5

	A	B	C	D	E
1	N	substanceName	spectrumPath	spectrumGroupName	experimentType
2	1	component1	component1	References	H
3	2	component2	component2	References	H
4	3	component3	component3	References	H
5	4	component4	component4	References	H
6	5	component5	component5	References	H

8C Add reference spectrum information

To include the Substance reference spectra, you need to insert the **spectrumPath** (AnalayisScreen will recognise any spectrum format).

You have three options:

1. If all the spectra files are located in the same directory as the lookup file, insert only the file names as above.
 2. If the spectra are located in a subdirectory, insert the directory name first followed by a slash and the filename (the relative path starting from the Excel file), e.g. references/component1
 3. If the spectra files are located in a completely different location, insert the full path, e.g. /Users/username/Desktop/data3/MySpectra/component1
For clarity, we recommend keeping all the files in the same directory together with the Excel lookup file.
- Insert the **spectrumGroupName**; e.g. References. This will create a **Spectrum Group** with that name and place the spectra into it.
 - Insert the **experimentType**. The list of experiment types, their nomenclatures and more information, can be found at <https://www CCPN.ac.uk/v3-software/documentation/v3-experiment-types/view>.
For these 1-dimensional spectra, simply type **H** into the cell.

	F	G	H	I	J	K	L	M
1	comment	smiles	synonyms	molecularMass	empiricalFormula	atomCount	hBondAcceptorCount	hBondDonorCount
2	c.uk/chebi/searchId.do?chebil	C1=CC#CC=C1	1,2-didehydrobenzene	185		1	3	
3	c.uk/chebi/searchId.do?chebil	COCC1=CC=CC#C1	1-methoxycyclohexa-1,3-dien-5-yne	190		1	2	
4	c.uk/chebi/searchId.do?chebil	CC[NH3+]	ethylaminium	144		2	1	
5	mid=64B6AB2667E275BC3E6	CC(O)CC(O)=O	3-hydroxybutyric acid	190		3	1	
6	c.uk/chebi/searchId.do?chebil	CC(O)(CCO)CC(O)=O	mevalonic acid	185		1	1	

8D Add Substance metadata

- The **comment** column will store any textual information about the substance.
- If you enter the **smiles** for your substances, the programme will automatically generate the structures inside the software. For the tutorial, copy and paste these SMILES:

C1=CC#CC=C1

COCC1=CC=CC#C1

CC[NH3+]

CC(O)CC(O)=O

CC(O)(CCO)CC(O)=O

In Excel, click on the small folder on the bottom right of the pasted items and select **Match Destination formatting**

- In the **synonyms** column insert the chemical name of the substance and again select **Match Destination formatting**, e.g.
 - 1,2-didehydrobenzene
 - 1-methoxycyclohexa-1,3-dien-5-yne
 - ethylaminium
 - 3-hydroxybutyric acid
 - mevalonic acid
- All the following columns contain the substance chemical properties. Fill them if you want to display them within the software.
- Save the file.

A fully completed lookup is provided at ScreenTutorial/spectra/LookupData/LookupExample.xls. This file can be opened in AnalysisScreen by **dragging and dropping** it into the sidebar or drop area.

N	sampleName	spectrumGroupName	spectrumPath	experimentType	sampleComponents	pH	ionicStrength	amount
1	Sample1	Control		STD.H	component1,component2,component3,component4,component5	5.5		
2	Sample1	Displacer		STD.H				
3	Sample1	OffResonance	STDs/sample1OffReson	STD.H				
4	Sample1	OnResonance	STDs/sample1OnReson	STD.H				
5	Sample1	STDTarget		STD.H				
6	Sample2	Control		STD.H	component6,component7,component8,component9,component10	5.5		
7	Sample2	Displacer		STD.H				
8	Sample2	OffResonance	STDs/sample1OffReson	STD.H				
9	Sample2	OnResonance	STDs/sample1OnReson	STD.H				
10	Sample2	STDTarget		STD.H				
11	Sample3	Control		STD.H	component11,component12,component13,component14,component15	5.5		
12	Sample3	Displacer		STD.H				

8E Create the Samples Sheet

The next sheet in the template is **Samples**. This can contain metadata associated with particular samples, e.g. in a screening trial the sample could contain lots of spectra recorded with different experimental conditions.

The only mandatory column is the **sampleName** column.

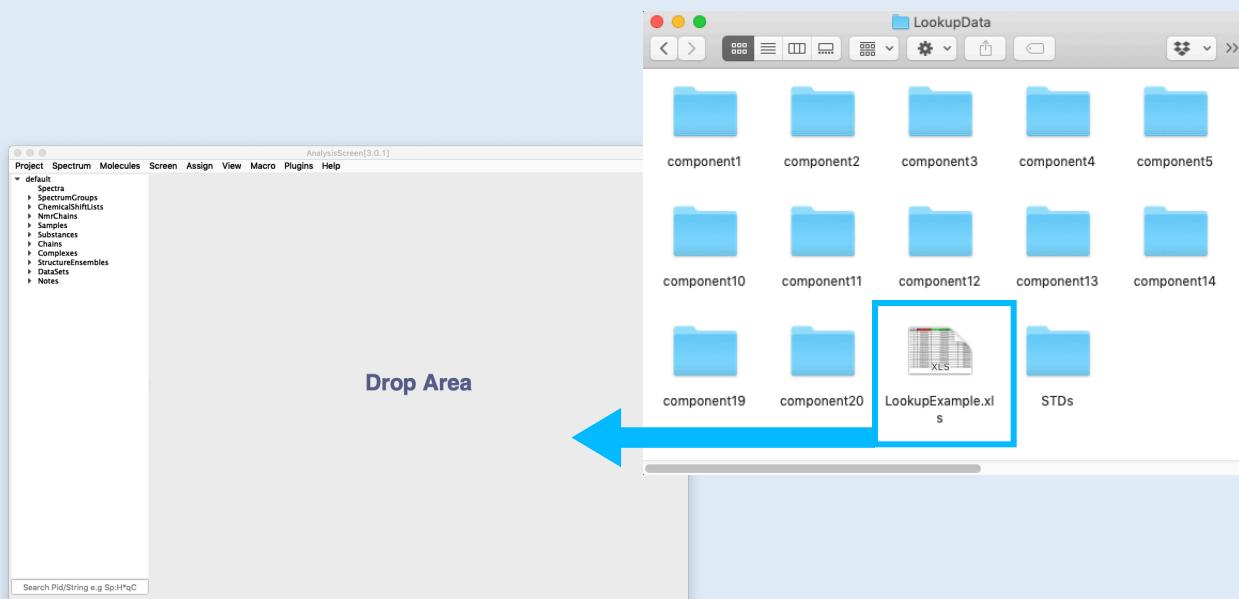
- Insert the **sampleName** in the first column, e.g. **Sample1**

The next three columns are specific to the spectra recorded for this sample:

- Insert the **spectrumGroupName**, e.g. **STD_Target**, if you want the spectrum to be included in a Spectrum Group
- Insert the **spectrumPath**, e.g. **STDs/Sample1_Std** (see the section 9C for how to insert the spectrum path)
- Insert the spectrum: **experimentType**, e.g. **STD.H** (see the [documentation](#) for information on the **Experiment Type** nomenclature)
- Fill in the **sampleComponents** column. Insert the names of the components that are present in the sample. In the case of a mixture containing components 1 to 5, insert as a comma-separated list without spaces:
component1,component2,component3,component4,component5
- The other columns record a sample's chemical properties and other information. Fill them in if you want to display them within the software.

To add extra spectra for the same sample, repeat points 1 to 3 as shown in the figure. There is no need to duplicate the samples properties (yellow columns) as long as the sample name is the same. If you add the same information twice, only the first entry will be considered.

To add an extra sample, simply fill in further rows.



8F Import Excel Lookup File into AnalysisScreen

- Drag and Drop the **LookupExample.xls** file located in the **ScreenTutorial/spectra/LookupData** directory of the tutorial data from your file browser into the **sidebar** or **drop area** of AnalysisScreen.

You will now be able to see all the imported data in the sidebar.

▼ default	▼ SpectrumGroups -> New SpectrumGroup>
▼ Spectra	▶ SG:References
▶ SP:component1-1	▶ SG:OnResonance
▶ SP:component2-1	▶ SG:Control
▶ SP:component3-1	▶ SG:STDTarget
▶ SP:component4-1	▶ SG:OffResonance
▶ SP:component5-1	▶ SG:Displacer
▶ SP:component6-1	▶ ChemicalShiftLists
▶ SP:component7-1	▶ NmrChains
▶ SP:component8-1	▼ Samples -> New Sample>
▶ SP:component9-1	▶ SA:Sample2
▶ SP:component10-1	▶ SA:Sample3
▶ SP:component11-1	▶ SA:Sample1
▶ SP:component12-1	▶ SA:Sample4
▶ SP:component13-1	▼ Substances -> New Substance>
▶ SP:component14-1	SU:component1.
▶ SP:component15-1	SU:component10.
▶ SP:component16-1	SU:component11.
▶ SP:component17-1	SU:component12.
▶ SP:component18-1	SU:component13.
▶ SP:component19-1	SU:component14.
▶ SP:component20-1	SU:component15.
▶ SP:sample1OffResonance-1	SU:component16.
▶ SP:sample1OnResonance-1	SU:component17.
▶ SP:sample1OffResonance-1_1	SU:component18.
▶ SP:sample1OnResonance-1_1	SU:component19.
▶ SP:sample1OffResonance-1_2	SU:component2.
▶ SP:sample1OnResonance-1_2	SU:component20.
▶ SP:sample1OffResonance-1_3	SU:component3.
▶ SP:sample1OnResonance-1_3	

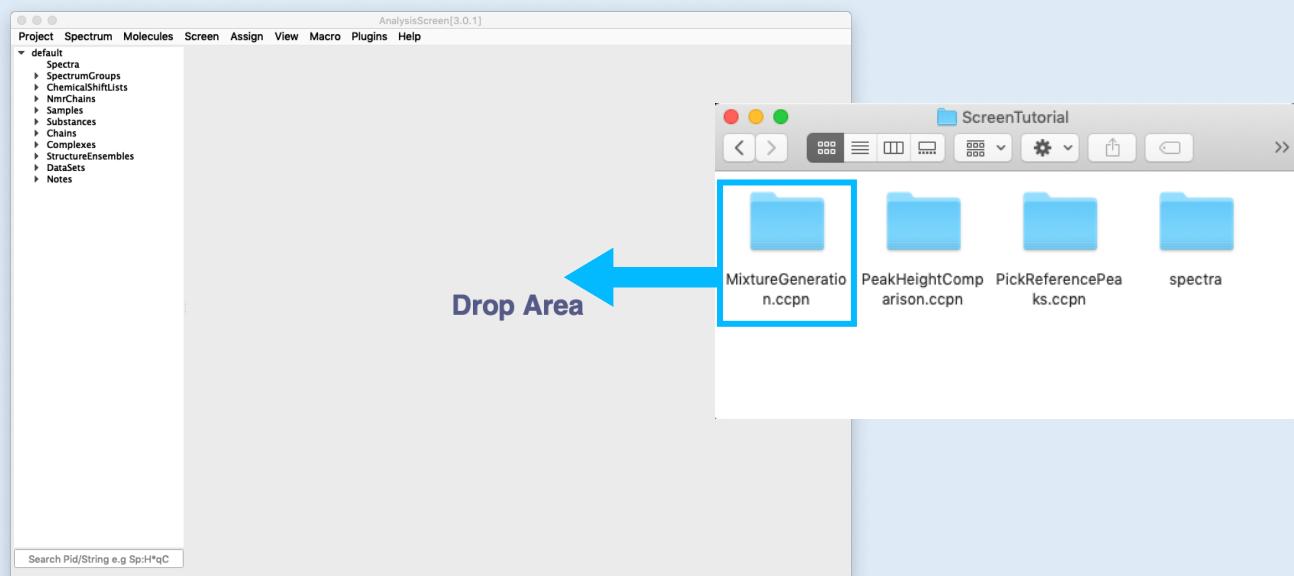
Please note that you cannot drop the same lookup file containing the same values into the same project twice. This is because the project cannot create new objects with pre-existing names. When dropping the same file onto a project twice, only the first entries will be used.

Mixture Generation

In order to decrease the experimental resources in NMR screening, a common approach is to analyse several compounds against a target at the same time. This can translate into a very crowded spectrum that leads to difficult, error prone and time-consuming interpretation of spectra.

In this section you will learn how to create cocktails of one dimensional reference spectra with minimal peak overlap. The new mixtures can be manually analysed and exported to Excel, ready to be printed and prepared in the lab.

The tutorial uses 20 synthetic spectra, representing the reference spectra of 20 small molecules.



9A Open Project

Open the **MixtureGeneration ccpn** project in the **ScreenTutorial** directory of the tutorial sample data. You can do this in one of the following ways:

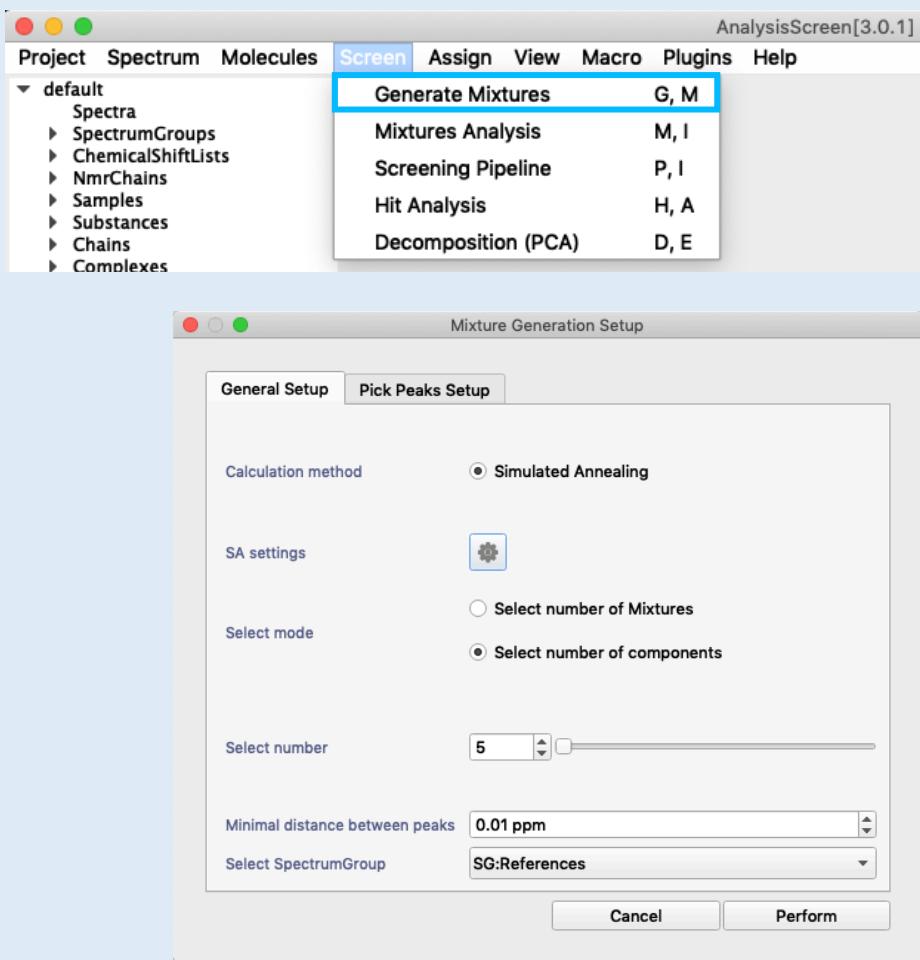
- Drag the project from your file browser into the sidebar or drop area

OR

- Go to **Main Menu → Project → Open...** and select the project

OR

- Use the shortcut **Ctrl+O** (or **Cmd+O** on a Mac) and select the project



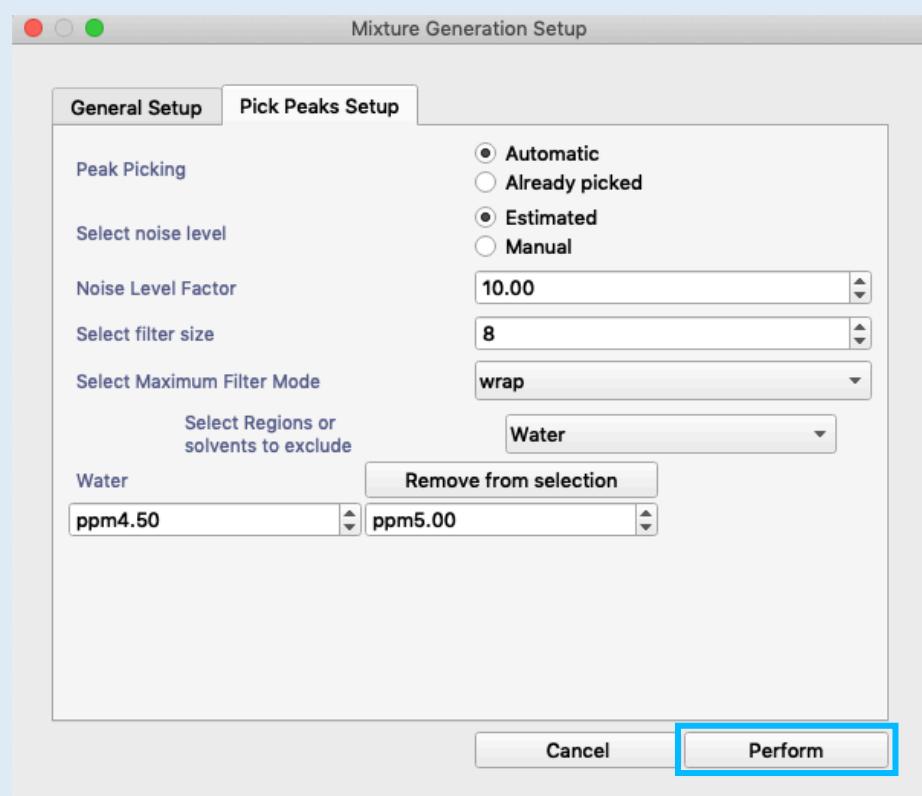
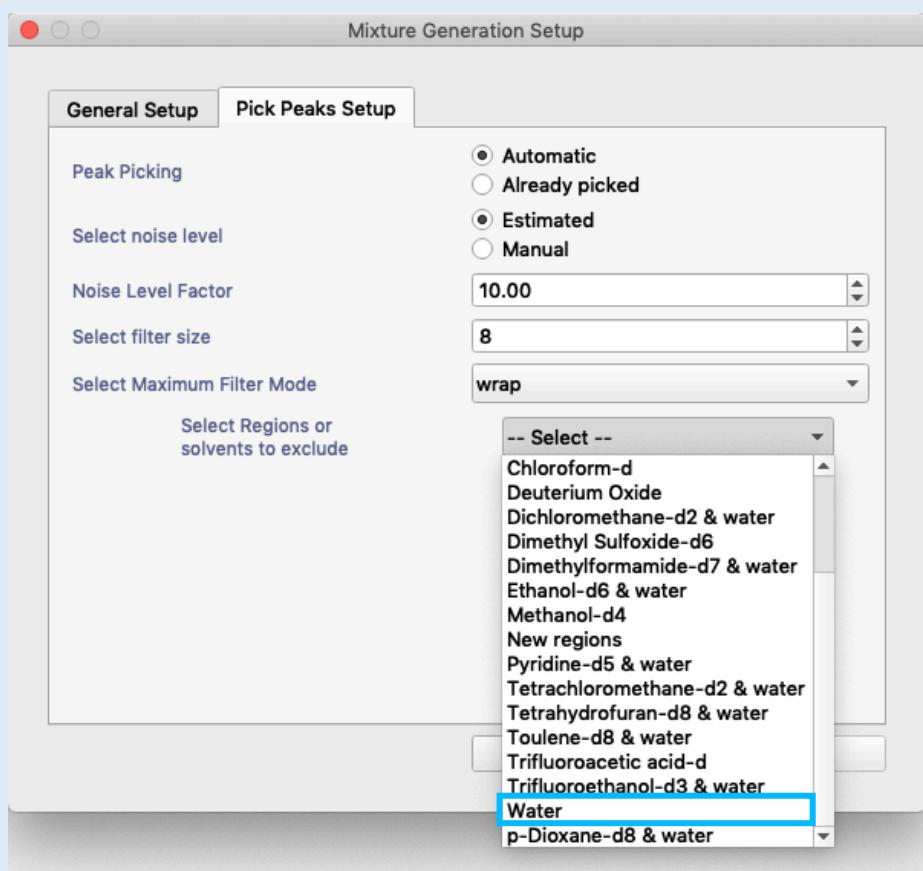
9B Set up Mixture Generation

- Go to Main Menu → Screen → Generate Mixtures or use shortcut GM

The calculation will be conducted by simulated annealing (SA). You can change the SA parameters by clicking on the gear icon. For the tutorial we will keep the default parameters.

- Select the **Number of Components** selection method to choose how many components you want in each mixture. (**Number of Mixtures** allows the creation of a set number of mixtures with components equally distributed among them.) The project contains 20 reference spectra, therefore select any value between 2 and 10 in the option number of Mixtures or between 2 and 20 for number of Components on the slider or in the spin box. Five components per mixture is a common choice.
- Leave the **minimal distance between peaks** at 0.01ppm. The algorithm will try to return mixtures where two adjacent peaks from separate spectra are separated by at least the selected value.
- Select the **Spectrum Group** you want to use to create the mixture, in this case: **SG:References**

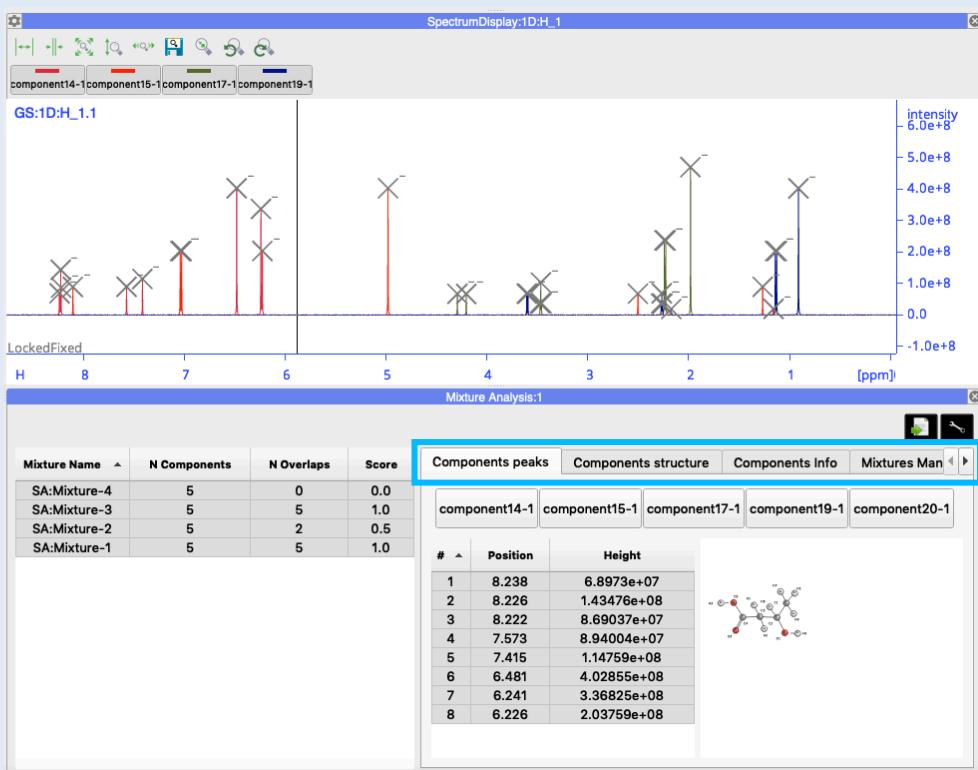
Mixture Generation



9C Set up Peak Picking

- Go to the 'Pick Peaks Setup' tab. Here you can select whether or not to pick the peaks first or use previously picked peaks. See **Section 3** above on **1D Peak Picking** for more details
- Select **Water** from the drop-down as a **solvent region to exclude**.
- Click **Perform** to start the calculation.

Mixture Generation



9D Mixtures Analysis

The module mixture analysis will be opened automatically. If you close it, you can reopen it with **Main Menu → Screen → Mixture Analysis** or shortcut **MI**.

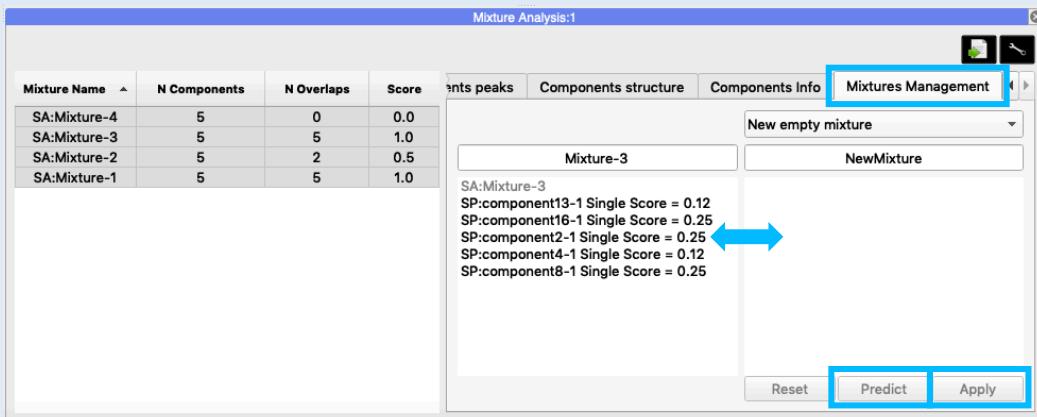
The table on the left-hand side of the module lists all the mixtures with scores. The scores are derived from placing a penalty on the distance between the best-resolved peak and its closest neighbour. The more overlapped the peaks, the higher the score. A score of 0.0 means there are no overlapped peaks in the mixture.

The table also lists the number of components and overlapped peaks present in each mixture. It can be sorted into ascending or descending order by clicking on the table header column.

Use the four tabs on the right to do a full inspection of the mixture selected in the table on the left:

The **Component Peaks** tab has a toolbar containing the spectrum component of each mixture.

- Click a button to show the PeakList. If a substance is linked to the spectrum, the molecular structure will be shown on the right.
 - Use the **Components Structure** and **Components Info** tabs to visualise additional mixture properties.



9_E Mixtures Management

The **Mixture Management** tab will allow you to move components between mixtures by hand to try to improve scores or create new mixtures.

- Select an option from the pulldown menu on the right, e.g. **New empty mixture** or any other mixture. **Drag and drop** the components between the windows and click the **Predict** button to recalculate the scores, then **Apply** to get the new mixtures.

Once you are happy with your mixtures you can export them to an Excel file using the export button  in the top right corner.

Contact Us

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<https://www ccpn ac uk forums>

Cite Us

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

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)

Tutorial Version History:

Beta (LGM): First version

3.0 (VAH): Re-worked and re-designed