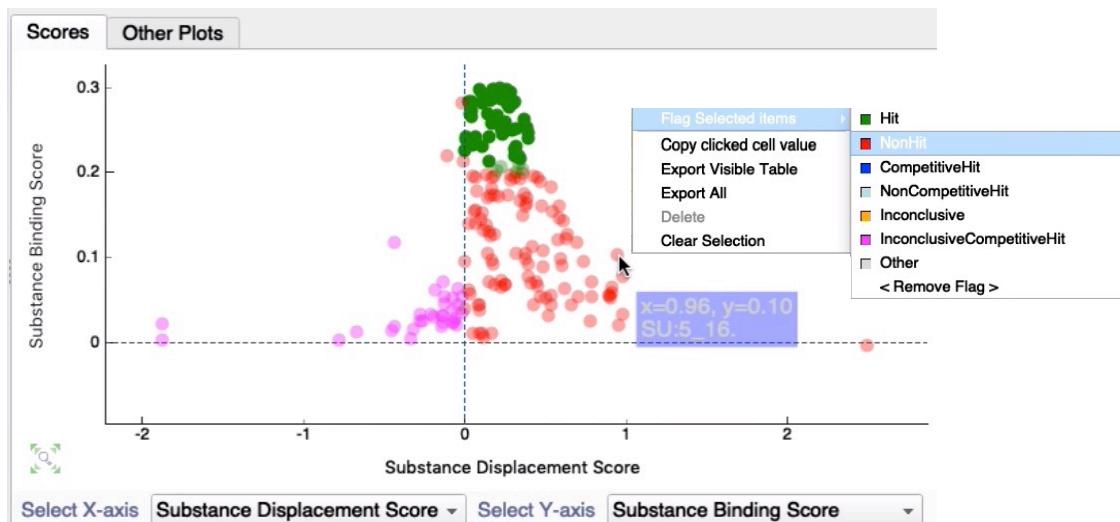


AnalysisScreen Introductory Tutorial



Introduction

This tutorial will introduce you to the basics of analysing fragment screening data in CcpNmr AnalysisScreen Version 3.2.

Further detailed information about AnalysisScreen is available online at <https://www.ccpn.ac.uk/manual/v3/index.html#screen>.

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 **CcpnScreeningTutorial** data available for download with this tutorial from the CCPN website (<https://ccpn.ac.uk/support/tutorials>).

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

Contents

1. Introduction and Summary
2. Import from Excel Files
3. Automated Peak Matching from Reference Singletons
4. Binding Scores and Calculation Engines
5. Plots and Filtering
6. Exporting Data
7. Appendix

Start CcpNmr Analysis V3

- Apple users by 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.

Reminder: basic operations in CcpNmr Analysis

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 **right-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 tutorial:

- | | |
|-----------|------------------------------------|
| HA | → Open the Hit Analysis GUI Module |
| PI | → Open the Pipeline GUI Module |

For more commands and operations

Main Menu → Help → Tutorials → Beginners Tutorial

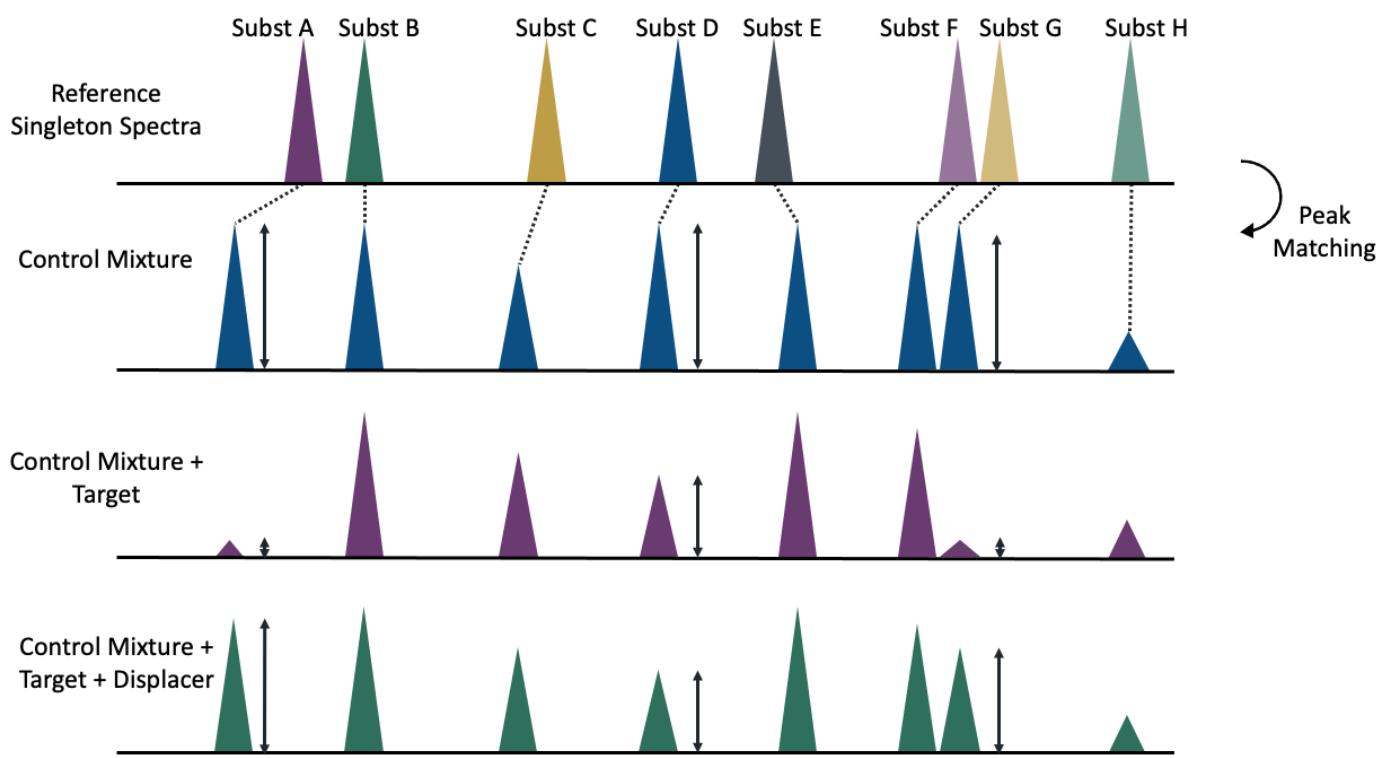
OR

Main Menu → Help → Show Shortcuts

Introduction

Schematic representation of a screening workflow

In this tutorial you will analyse several ^{19}F datasets but similar steps and tools in AnalysisScreen can also be applied to other experiment type analyses, such as ^1H relaxation-edited, WaterLOGSY and STD experiments.



We assume that you start with a set of individual spectra for each Substance (or compound) in your library. We refer to these as **Reference Singleton Spectra**.

Each screening experiment will start with the recording of a set of **Control Mixture Spectra**, a control spectrum of only the **Substance Mixtures**. Typically, some of the peaks in the Control Mixtures will move relative to the Reference Singleton Spectra. The first task, therefore, is to match the peaks from the Reference Singleton Spectra to those in the Control spectra so that we know which peak in the Control Mixtures belongs to which Substance. We will conduct this **Peak Matching** step automatically, though it is also possible to do this manually. If done automatically, a **Peak Matching Score** will help you find matches that might need to be checked/corrected manually before you proceed.

In addition to your Control Mixture Spectra, you will have spectra where you have added a **Target** molecule and perhaps also a **Displacer** (also referred to as a **Competitor**). These spectra are in fact also included in the Peak Matching step, so that you end up creating a **Screening Dataset** in which the peaks are matched across all spectra and to a particular Substance.

Now you can use the **Hit Analysis module** to calculate the **Peak Binding Score** from the Control and Target spectra. Then inspect, classify and flag your Hits and export them. Note that Binding Scores are also provided per Substance and per Sample.

Tutorial Summary

Main Steps

- Load data
 - Drag in **lookup_19F_tutorialdata.xlsx**
- Open modules
 - Open a SpectrumDisplay and Pipelines (**PI**)
- Run a pipeline on References SpectrumGroup:
 - Peak Picking Threshold (manual)
 - Peak Picker 1D
 - Annotate Peaks (select **Use linked Substance Name**)
- Set Peak Label Default
 - Under File > Preferences > Peaks, set Label to **Annotations**
- Run a pipeline on the Control, Target & Displacer SpectrumGroups:
 - Peak Picking Threshold (auto)
 - Propagate Peaks from References (propagate to **Controls**)
 - Copy PeakLists
 - SGs: **Control, N/A, Target and Displacer**, respectively
 - Set up Screening dataset
 - SGs: **Control, Control, Target and Displacer**, respectively;
 - Matching Engine: **Annotation Match**
 - select **Use Substance ReferenceSpectra**
- Open modules
 - Open a SpectrumDisplay and the HitAnalysis module (**HA**)
- Inspect data
 - View Modes: try different modes and navigate through tables
 - Calculation and Appearance Settings: try alternative options, refreshing if required 
 - Filters Settings: try filtering tables with 
 - Plotting area: try different parameter combinations
 - Flag Substances: right-click on selected items in table or plot
- Export Tables & Reports
 - right-click > **New from Selected** in table to extract to new dataset
 - right-click in table to export
 - right-click in Substances table to create PowerPoint report

2

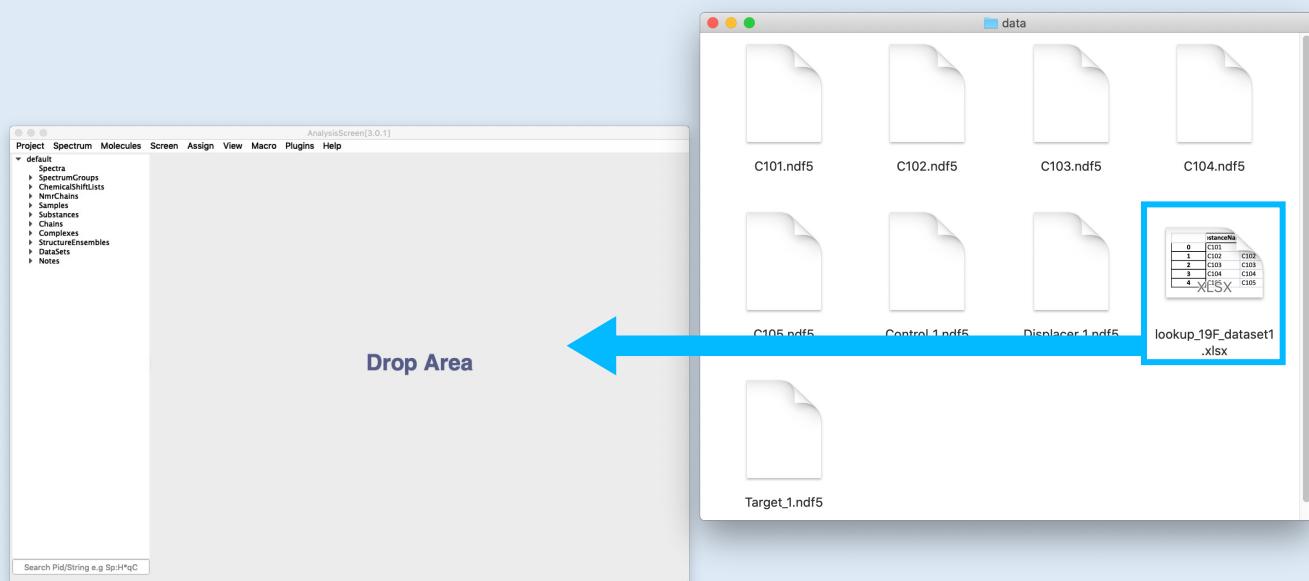
3

4

5

6

Import Data from Excel



2A Import Excel Lookup File into AnalysisScreen

- Drag & drop the `lookup_19F_tutorialdata.xls` file located in the `CcpnScreeningTutorial/data/` directory of the tutorial data from your file browser into the sidebar or drop area of AnalysisScreen.

For more details on how to set up your own Excel file, please see our online documentation at <https://www.ccpn.ac.uk/manual/v3/ScreenExcelFiles.html>.

We are also very happy to help create a script for you to import the data from your current system into your first AnalysisScreen compatible Excel file, simply contact us at support@ccpn.ac.uk for help.

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.

- Spectra
 - SP:C101
 - SP:C102
 - SP:C103
 - SP:C104
 - SP:C105
 - SP:C106
 - SP:C107
 - SP:C108
 - SP:C109
 - SP:C201
 - SP:C202
 - SP:C203
- SpectrumGroups
 - <New SpectrumGroup>
 - SG:References
 - SG:Control
 - SG:Target
 - SG:Displacer
- Samples
 - <New Sample>
 - SA:Control_1
 - <New SampleComponent>
 - SC:Control_1.C101.
 - SC:Control_1.C1010.
 - SC:Control_1.C1011.
 - SC:Control_1.C1012.
 - SC:Control_1.C1013.
- Substances
 - <New Substance>
 - SU:C1001.
 - SU:C10010.
 - SU:C10011.
 - SU:C10012.
 - SU:C10013.

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

Spectra

C101–C10020: reference spectra for Compounds/Substances C101–C10020

Control_1–10: compound mixture spectra containing 20 Substances each

Target_1–20: compound mixture spectra plus protein target

Displacer_1–20: compound mixture spectra plus protein target and a displacer compound (note that these spectra are optional when you do your own screen – they are simply included here for completeness)

SpectrumGroups

Collections of all the Reference, Control, Target and Displacer spectra.

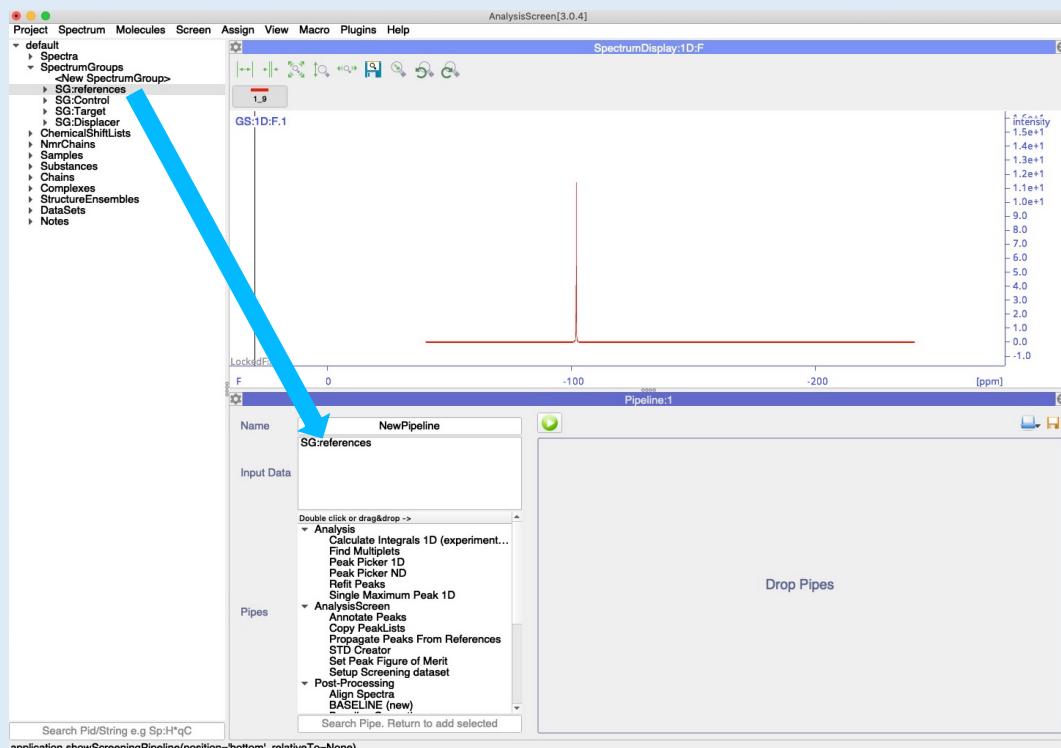
Substances

All the Substances for which there are reference spectra

Samples

Components included in each of the Control, Target and Displacer spectra.

Automatic Peak Matching from Reference Singletons



3A Open the first reference spectrum

- select the first spectrum in the Sidebar, drag and drop it onto the Drop Area.

3B Open the pipeline module

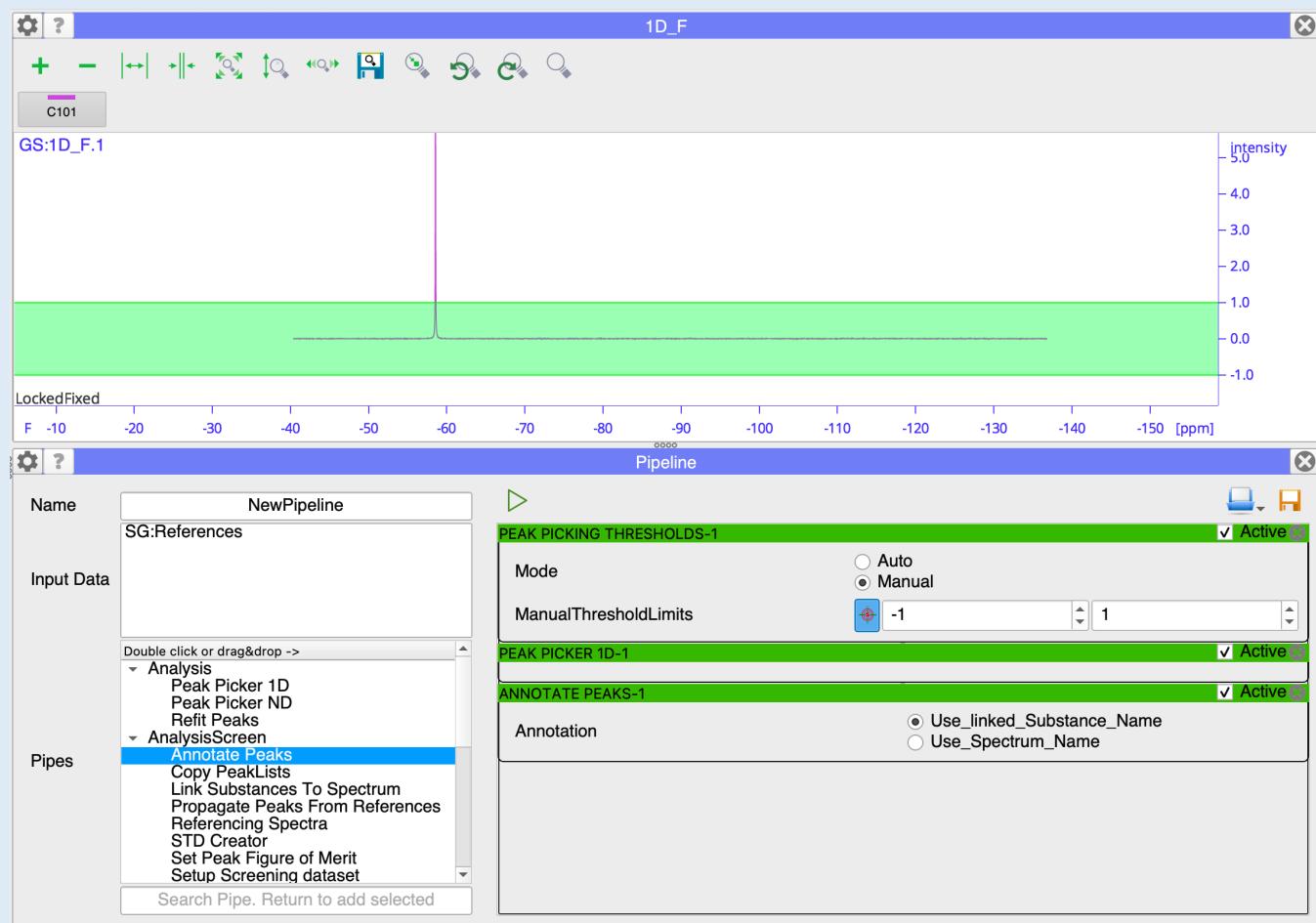
- Open the pipeline module from the main menu:

Menu → Screen → Screening Pipeline

or use the shortcut PI

- Expand the Sidebar branch for **SpectrumGroups**
- Select **SG:References**, drag & drop it into the **Input Data** of the Pipeline

Automatic Peak Matching from Reference Singletons



3c Picking and annotating peaks in the Reference Singleton spectra

- In the Pipes list widget search for and add the following pipes to the pipeline area by **double-clicking** the pipe name or via **drag & drop**:

1. Generic > Peak Picking Threshold

- Select **Manual**
- Click on the Target button
- Enter the **Peak Picking Threshold** values -1, 1 either by dragging the green lines that appear in the SpectrumDisplay or inserting the values in the entry boxes

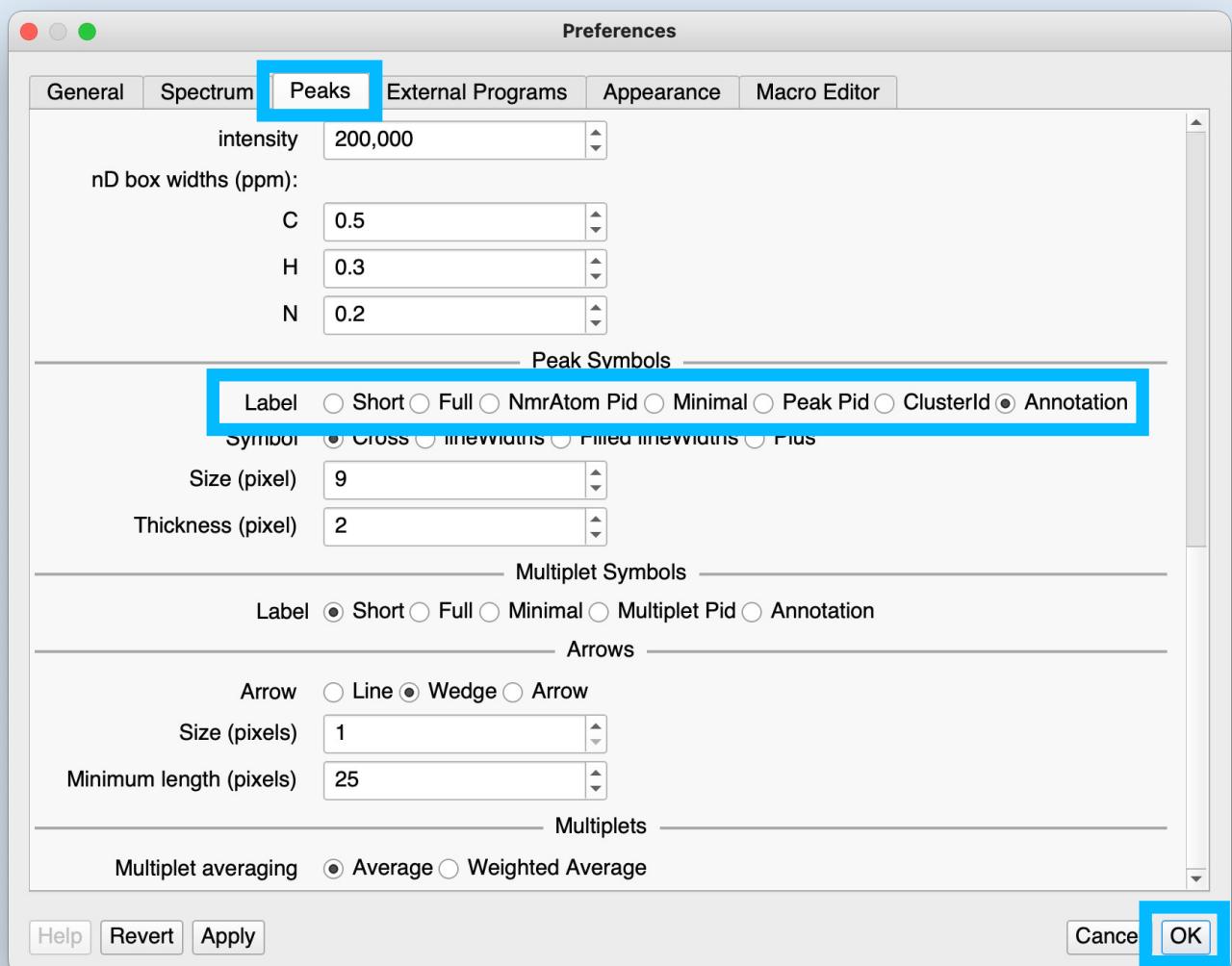
2. Analysis > Peak Picker 1D

3. AnalysisScreen > Annotate Peaks

- Select **Use linked Substance Name**

- Run the pipeline using the green play button (click once only!). A popup will appear when completed.
- If you wish, you can save this or any other pipeline you create by clicking on the icon. You can open a pipeline with – but check your parameters are set correctly, as not all are saved.

Automatic Peak Matching from Reference Singletons



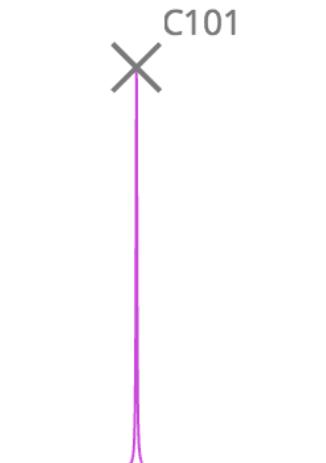
3D View Peak Labels as Annotations

AnalysisScreen uses Annotations to label peaks. However, by default the program will display NmrAtoms.

Switch to viewing Peak Labels as Annotations:

- Go to Main Menu -> File -> Preferences
- In the Peaks tab, go to the Peak Symbol section and set Label to Annotation.

You should now see that the Reference spectra have been peak picked and labelled with the compound associated with them.



Automatic Peak Matching from Reference Singletons

The screenshot shows the Bruker NMR Pipeline software interface with three active pipeline steps:

- PEAK PICKING THRESHOLDS-1**: Mode is set to Auto. ManualThresholdLimits is set to 0.
- PROPAGATE PEAKS FROM REFERENCES-1**: Propagate To is set to SG:Control. PeakList is set to Last. Snap Peaks is checked. Max snap distance (ppm) is set to 0.1.
- COPY PEAKLISTS-1**: Origin SpectrumGroup is set to SG:Control. Control SpectrumGroup is set to N/A. Target SpectrumGroup is set to SG:Target. Displacer SpectrumGroup is set to SG:Displacer. Destination PeakList is set to Last. Peak Properties is set to Snap_To_Extremum. Refit Peaks is unchecked. Skip Below Merit is set to 0.5. Optimise With Local Alignments is checked.

Propagate Peaks from References will copy peaks from several (Reference Singleton) peak lists and collate them into a smaller number of (Control mixture) peak lists.

Copy PeakLists will copy the peak lists one to one (from the Control mixtures to the Target and Displacer mixtures).

3E Setup screening pipeline

- Clear the input data (**right-click** → **Clear all**)
- On sidebar, multiselect **SG:Control**, **SG:Target** and **SG:Displacer**, and then drag & drop them into the pipeline **Input Data** area.
- Close all pipes (**right-click** on any pipe header → **Close All**)
- In the list of **Pipes** search for and add these pipes to the pipeline area:
 - 1. Generic > Peak Picking Threshold**
 - **Mode**: auto
(this allows for different samples having different signal to noise levels)
 - 2. AnalysisScreen > Propagate Peaks from References**
 - **Propagate to**: SG:Control
 - 3. AnalysisScreen > Copy PeakLists**
 - **Origin SpectrumGroup**: SG:Control
 - **Control SpectrumGroup**: N/A
 - **Target SpectrumGroup**: SG:Target
 - **Displacer SpectrumGroup**: SG:Displacer

continued....

Automatic Peak Matching from Reference Singletons

Optimise With Local Alignments	<input checked="" type="checkbox"/>
SETUP SCREENING DATASET-1	
Run Name	NewPipeline_25-03-06-11-51
Origin SpectrumGroup	SG:Control
Control SpectrumGroup	SG:Control
Target SpectrumGroup	SG:Target
Displacer SpectrumGroup	SG:Displacer
Matching Engine	Annotation Match
Experiment Type	19F
Use Substance referenceSpectra	<input type="checkbox"/>
AutoScale Control-Target Spectra	<input type="checkbox"/>

If you wish, you may choose to **AutoScale Control-Target Spectra**. This will automatically rescale your spectra to take account of sample dilution and other experimental effects (e.g. changes in shimming) that might affect the relative intensities of your spectra. Auto-scaling provides a more reproducible and robust way of accounting for differences in spectrum intensities than doing this manually.

...continued

5. AnalysisScreen > Setup Screening dataset

- **Run name:** 19F_dataset
- **Origin SpectrumGroup:** SG:Control
- **Control SpectrumGroup:** SG:Control
- **Target SpectrumGroup:** SG:Target
- **Displacer SpectrumGroup:** SG:Displacer
- **Matching Engine:** Annotation Match
- tick **Use Substance ReferenceSpectra**

(this option ensures the Reference Singleton spectra are used as the Reference Spectra)

- Run the pipeline using the green play button. 

The calculations should take less than a minute. When it is finished you should see a new entry under DataTables in your sidebar:

- **DataTables**
 - <New DataTable>
 - DT:19F_dataset
- **Collections**
- **Notes**

This is the new Screening Dataset that was set up in the final pipe. It is a DataTable containing all the peak positions, heights, volumes, binding scores, etc.

- Save the pipeline if you wish.

4 Binding Scores and Calculation Engines

The screenshot shows the CCPN software interface. At the top is the Main Menu with tabs: Screen (highlighted in blue), Macro, Plugins, and Help. Below the menu is a list of options:

Generate Mixtures...	G, M
Mixtures Analysis	M, I
Screening Pipeline	P, I
Hit Analysis	H, A
Compare Screens	D, C
Decomposition (PCA)	D, E

The "Hit Analysis" option is selected and highlighted in blue. Below the menu, the title bar reads "Hit Analysis (beta)". The main workspace contains a "DataTable" dropdown menu with options: "<>" (selected), "<> <New Item...>", and "# Sample Name". To the right of the dropdown is a "View" section with checkboxes for "Substances" (unchecked), "Samples" (checked), "Matches Only" (unchecked), and filter icons. Below the view is a table with columns: Sample Binding Score, Sample Displacement, Sample Matching, Substance Count. A scatter plot titled "Scores" is shown with axes from -0.01 to 0. A legend indicates "Select X-axis". At the bottom left is a table titled "Peak Binding Scores" with columns: Substance Name, Sample Name, Peak Binding Score, Peak Displacement, Peak Matching, Peak Lab, Ref-Targ Δ Shift, Ref-Targ |Δ| Shift, Cont-Targ |Δ| Shift.

4A Open data in program

You can now either continue with your project from **Section 3** or load a project which has already completed those steps:

- Drag and drop the **CcpnScreeningTutorial/19FScreening_completed ccpn** folder in the sidebar or Drop Area.
- Open a spectrum in a Spectrum Display module.

Open the Hit Analysis module:

- Go to **Main Menu** → **Screen** → **Hit Analysis** or use shortcut **HA**.
- From the **DataTable** drop-down menu select **19F_dataset** and wait a few moments for the tables to be updated.

4 Binding Scores and Calculation Engines

Hit Analysis Module Table Selections

The Hit Analysis module has multiple dynamic selections

Substance View: selecting a row on the **Substances** table will:

1. List all contributing peak matches in the **Peak Binding Scores** table
2. Display all the spectra associated with the substance
3. Select the relevant items in the Scores scatter plot on the right

Sample View: selecting a row on the **Samples** table will:

1. List all contributing peak matches in the **Peak Binding Scores** table for all the substances present in the sample
2. Display all the spectra associated with the sample, including all reference spectra

All Views: selecting a row on the **Peak Binding Scores** table will:

1. Select all peaks included in the match
2. Navigate to the Peak Position
3. Populate the **Match Editing** pop-up (click on **New/Edit Match...** to show this).

Double-clicks on tables will re-execute the single selection.

Substances View

#	Substance Name	Sample Name	Substance Binding Score	Substance Displacement Score	Substance Matching Score	Flag	Relative S/N Ratio
1	C101	Target_1	0.142	0.286	5		67.85
12	C102	Target_1	0.202	0.162	5		81.07
14	C103	Target_1	0.124				
15	C104	Target_1	0.194				
16	C105	Target_1	0.267				
17	C106	Target_1	0.126				

Peak Binding Scores Table

Substance Name	Sample Name	Peak Binding Score	Peak Displacement	Peak Matching	Peak Lat Ref ΔS
C101	Target_1	0.142	0.286	5	0.0
C102	Target_1	0.202	0.162	5	0.0

Samples View

#	Sample Name	Sample Similarity Score	Sample Binding Score	Sample Displacement Score	Sample Matching Score	Substance Count
1	Target_1	0.995	2.69	13.2	5	20
2	Target_2	0.996	2.52	9.33	5	20
3	Target_3	0.995	2.56			
4	Target_4	0.996	2.67			
5	Target_5	0.995	3.42			
6	Target_6	0.995	3.23	5.15	5	20

Peak Binding Scores Table

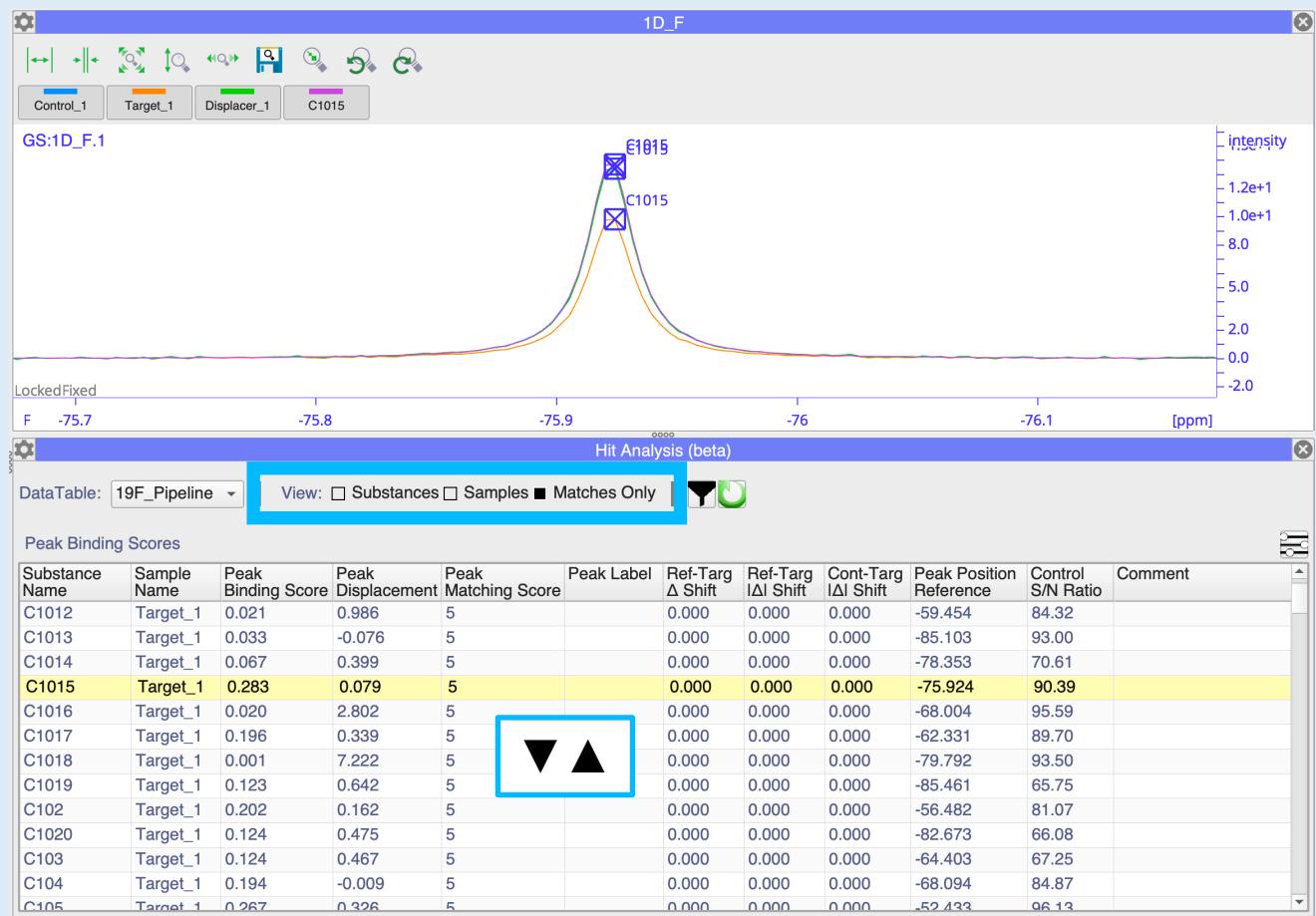
Substance Name	Sample Name	Peak Binding Score	Peak Displacement	Peak Matching	Ref-Targ Ref ΔShift	Ref-Targ IAI Shift
C101	Target_1	0.142	0.286	5	0.000	0.000
C102	Target_1	0.202	0.162	5	0.000	0.000

Matches Only View

Substance Name	Sample Name	Peak Binding Score	Peak Displacement	Peak Matching	Ref-Targ ΔShift	Ref-Targ IAI Shift	Cont-Targ IAI Shift	Peak Position Reference	Control S/N Ratio	Comment
C101	Target_1	0.142	0.286	5	0.000	0.000	0.000	58.554	67.85	
C1010	Target_1	0.052	0.912	5	0.000	0.000	0.000			
C1011	Target_1	0.191	0.003	5	0.000	0.000	0.000			
C1012	Target_1	0.021	0.986	5	0.000	0.000	0.000			

Peak Binding Scores Table

4 Binding Scores and Calculation Engines



4B Looking through peaks manually

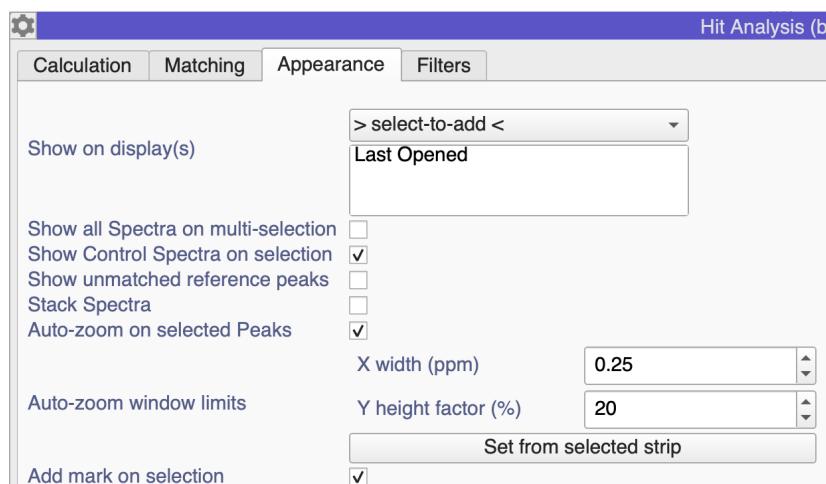
You can scan through all your peaks by hand fairly quickly to see if you spot anything interesting or unusual:

- Set the **View Mode** to **Matches Only**, so that you only see the **Peak Binding Scores** table.
- Use your **up/down arrow keys** to move down the list. Each time the Spectrum Display will automatically focus on the peak in question.

If you prefer to look at your peaks by Sample, simply:

- Set the **View Mode** to **Samples**
- Select the sample of interest in the upper table
- Use the **up/down arrow keys** in the **Peak Binding Scores Table** below to move through and see the peaks for that sample.

You can change the auto-zoom options in the **Appearance** tab of the Hit Analysis module **Settings** if you like.



4 Binding Scores and Calculation Engines

Scores and Scoring Engines

Peak Binding Score

This is a measure of how well a substance binds to the target. It is shown in the **Peak Binding Scores Table** and can be calculated using peak height, linewidth or volume, depending on the **Mode** set in the **Calculation** tab of the Hit Analysis module **Settings**. (Note that linewidths and volumes are not calculated by default when peaks are picked and have to be determined with **Spectrum → Estimate Volumes** or shortcut **EV**.)

The binding score is calculated according to the **Peak Binding Score Engine** specified in the **Calculation** tab of the Hit Analysis module **Settings**. When hovering over one of the options, a Tooltip will appear showing the actual equation.

You can also define your own equation in the free entry box:

use **V1** and **V2** to define the variables for the calculation matrix

- **V1** represents each Ligand signal in the presence of the target (Target)
- **V2** represents each Ligand signal in the absence of the target (Control)

The following arithmetic operations are supported:

``` + ```, ``` - ```, ``` * ```, ``` / ```, ``` ** ```, ``` % ```, ``` || ```

The screenshot shows the 'Hit Analysis (beta)' software interface. On the left, there's a navigation bar with icons for Settings, File, Edit, View, Tools, Help, and a magnifying glass. Below it is a toolbar with icons for Open, Save, Print, and others. The main window has tabs for Calculation, Matching, Appearance, and Filters. Under Calculation, the Mode is set to Height (radio button selected). The Engine dropdown is set to AbsoluteRelativeChange. A tooltip is displayed over this dropdown, containing the formula  $\frac{|V2| - |V1|}{|V2|}$  and a description: 'V1 : +Target. Ligand signal in the presence of the target. V2 : -Target. Ligand signal in the absence of the target (Control). A higher value indicates a greater value change. E.g. a larger Intensity drop.' To the right, there's a 'DataTable' section with a dropdown menu set to '19F\_Pipeline'. A table shows data for substances C703, C704, and C705, all labeled 'Target\_7'. At the bottom, there's another table for Displacement Engine settings, with the DisplacementFraction option selected and the formula  $v1/(V2+V3)$ .

### Peak Displacement Score

This is a measure of how well a substance binds the target in the presence of a Displacer/Competitor (which is usually expected to bind more strongly than the fragments used in the screen).

It is calculated using the equation selected in **Displacement Engine**. Again, it is possible to enter your own equation, defining the Displacer signal with the variable **V3**.

**continued...**

# 4 Binding Scores and Calculation Engines

## ... Scores and Scoring Engines continued

### Substance and Sample Binding Scores

These scores are shown in the **Substances** and **Samples Tables**, respectively, and are derived from all the peaks matched to that Substance or Sample. Therefore, they can be used to assess the Substance or Sample binding quality. Note that in the case of  $^{19}\text{F}$  screens (and this demo set), most Substances only have one peak, so the Substance Binding Score will usually be the same as the Peak Binding Score.

The Substance and Sample Binding Scores are given by the **Total Substance/Sample Binding Score** selected in the Calculation tab of the Hit Analysis Settings:

**min**: the minimum peak binding score in that substance/sample

**max**: the maximum peak binding score in that substance/sample

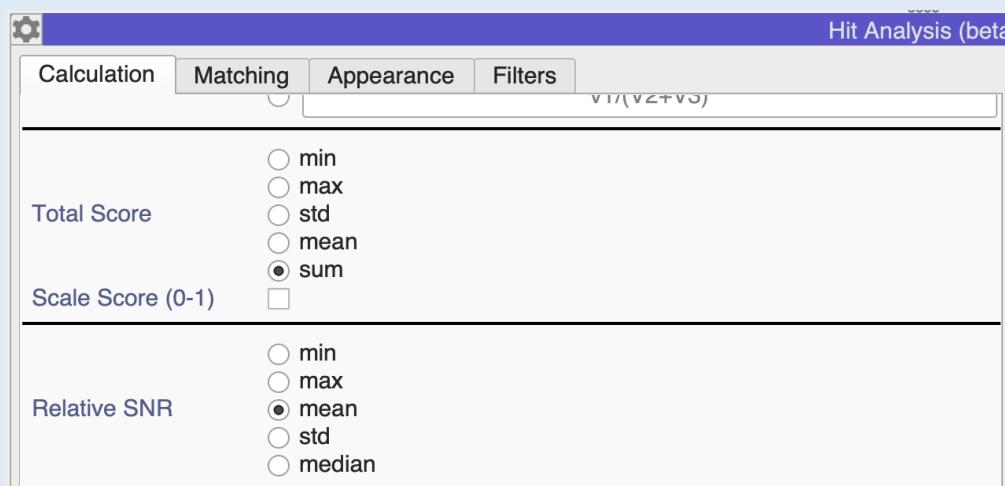
**stDev**: the standard deviation of all peak binding scores in that substance/sample

**mean**: the mean peak binding score for that substance/sample

**sum**: the sum of all peak binding scores for that substance/sample

If you wish, you can select **Scale Scores (0-1)** to get a quick sense of their relative size.

You can select similar options for the **Relative Signal to Noise Ratio (SNR)** shown in the tables.



### Peak Matching Scores

When matching peaks automatically (see Section 9) the Peak Matching Scores give an indication for how reliably the peaks were matched: 0 is unreliable, 5 is most reliable.

# 4 Binding Scores and Calculation Engines

The screenshot shows the 'Hit Analysis (beta)' settings panel with the 'Calculation' tab selected. The interface includes tabs for 'Calculation', 'Matching', 'Appearance', and 'Filters'. In the 'Calculation' tab, there are several configuration options:

- Show on display(s):** A dropdown menu set to '> select-to-add <' with an option for 'Last Opened'.
- Show all Spectra on multi-selection:** An unchecked checkbox.
- Show Control Spectra on selection:** A checked checkbox.
- Show unmatched reference peaks:** An unchecked checkbox.
- Stack Spectra:** An unchecked checkbox.
- Auto-zoom on selected Peaks:** A checked checkbox.
- X width (ppm):** A numeric input field set to 0.25.
- Y height factor (%):** A numeric input field set to 20.
- Set from selected strip:** A button.
- Add mark on selection:** A checked checkbox.
- Auto-Update on setting(s) changed:** A checked checkbox.
- Auto-Update on peak(s) changed:** An unchecked checkbox.
- Auto-Update matching score:** A checked checkbox.

## 4C Updating the tables

- Open the Hit Analysis **Settings** panel with the gear icon
- In the **Calculation** tab, try changing the **Peak Binding Score Engine** or **Total Score** calculation method.

As soon as you change any settings or modify / re-fit peaks, the whole module will update and recalculate all scores automatically. For larger datasets this can be time-expensive. You can disable this feature:

- In **Settings → Appearance tab**
- untick **Auto-Updates on setting(s) changed**
- untick **Auto-Updates on peak(s) changed**

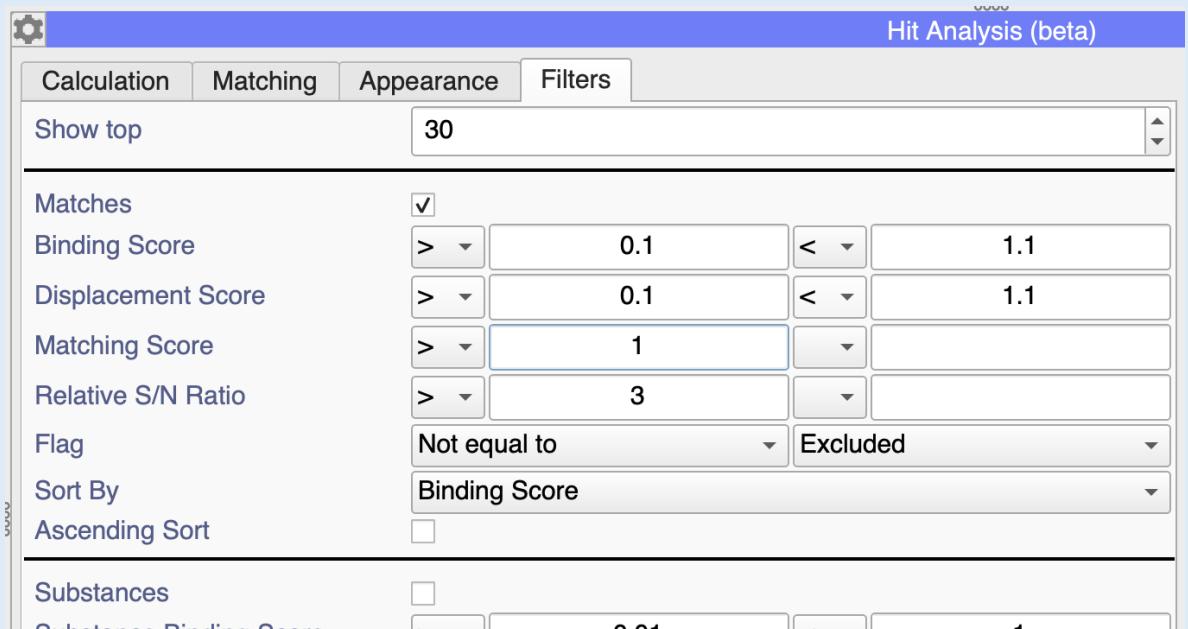
The refresh button will turn orange whenever changes are detected while working on the dataset. Click the refresh button to update all scores, after which it will turn green.

After trying out the effect of changes make sure you select

- Engine: **AbsoluteRelativeChange**

in the **Calculation** tab.

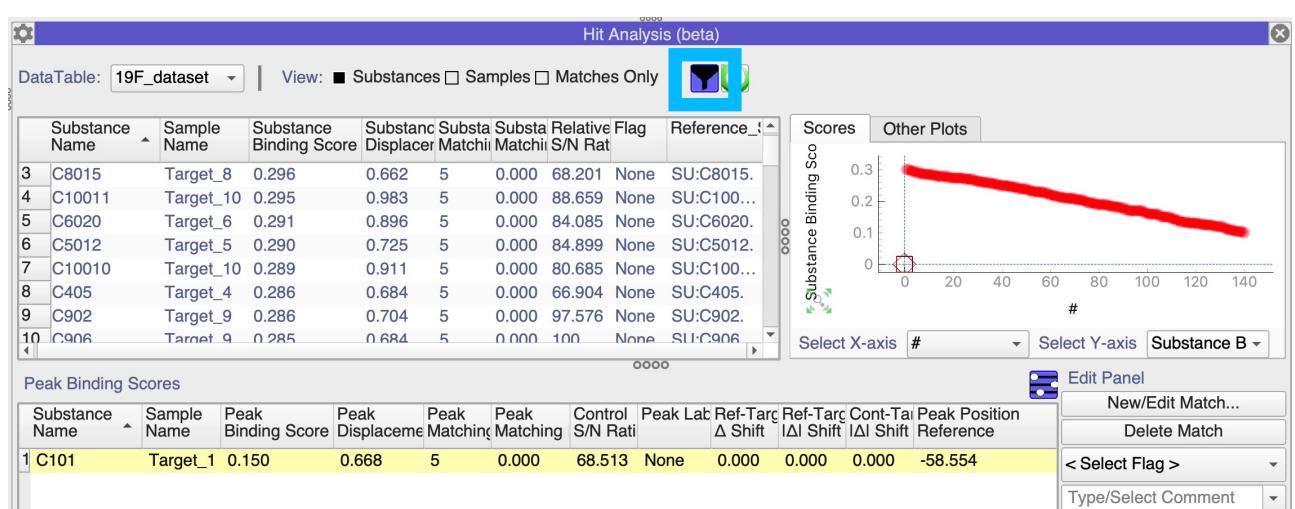
# Filtering and Plots

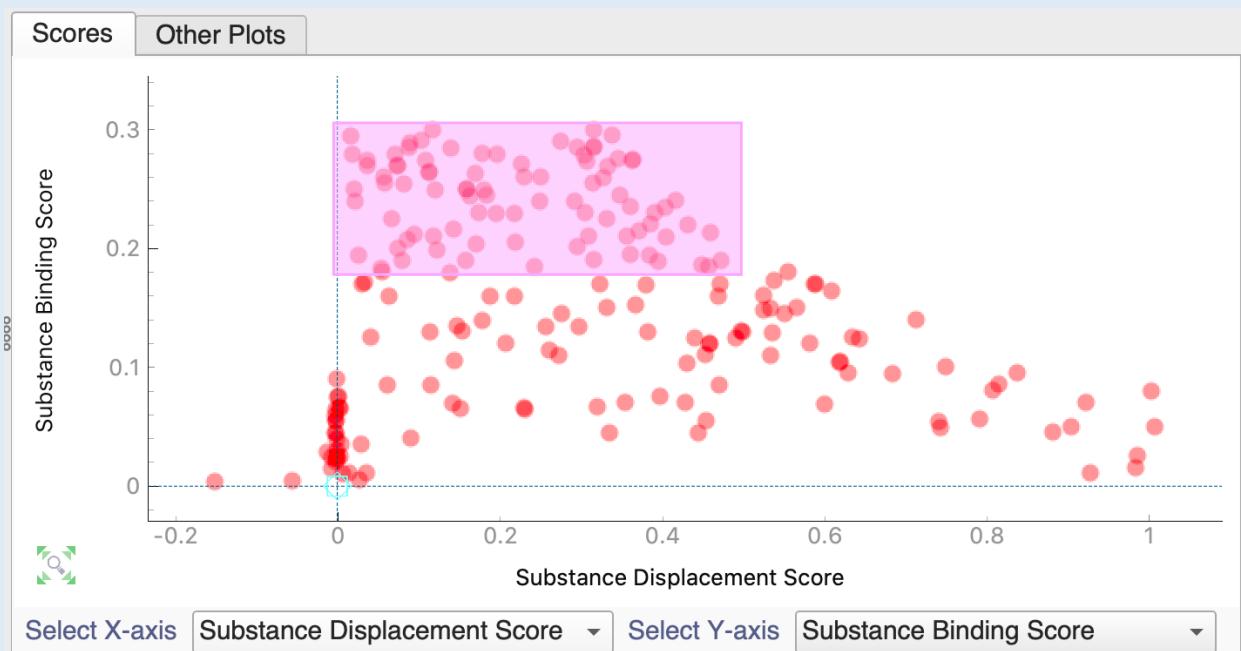


## 5A Filtering

Filters are a useful way to find to find Hits quickly and they also reduce the size of the tables which can speed up the program.

- Select the **Substances** View mode
  - Go to Hit Analysis Settings Panel → Filters
  - Select the **Matches** filters and set them up as shown in the image above
  - Close the Settings and click on the filtering icon to activate the filters.
- The filtering icon will go blue and the Substances table and Scores Plot will update based on the filtering criteria.





## 5B Plots

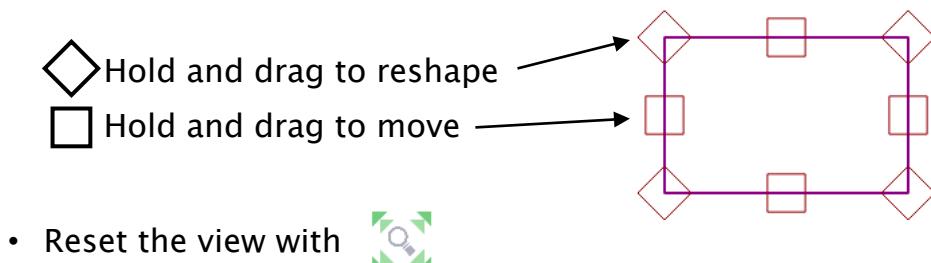
The Hit Analysis module has a Plots window in which you can plot any two variables of your choice against one another. If you have done automatic peak matching, you could, for example, plot the **Substance Matching Score** along the y-axis in order to find outliers quickly.

- Selecting an item in a table will select it in the plot and vice versa.

You can manipulate the plot and its points like a spectrum and peaks:

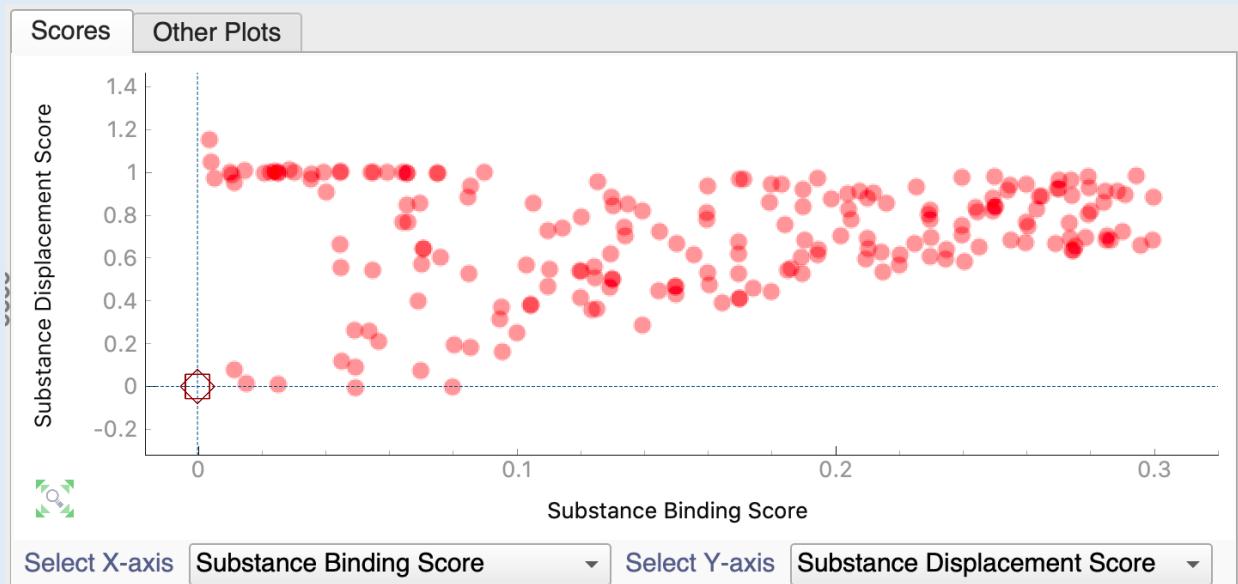
- Zoom with the **mouse wheel**, either on the plot or selectively on a single axis
- Move the plot around with **left-drag**
- (Multi-)select items with **Ctrl/Cmd + left-click**
- Select items in an area with **Ctrl/Cmd + left-drag**

This will draw a Region of Interest (ROI) box which you can change as follows:



Additional options are available in the **Appearance** tab of the **Settings**:

Roi Limits	xMin 0	xMax 0	yMin 0	yMax 0
<input checked="" type="checkbox"/> Link Roi with selection box				
ROI line colour <input checked="" type="color"/> purple				
Scatter point colour	<input checked="" type="color"/> red			
Scatter point size	10			
Scatter point Symbol	<input checked="" type="radio"/> circle			



## 5c Useful Plots for analysing Screening Data

### # vs Substance Matching Score

This can be a quick way to assess how many matches may need manual checking. Sorting the table by the Matching score will enable you to move through these quickly and assess them, making changes if necessary.

### Substance Binding Score vs. Relative Signal to Noise

This can be a helpful way to choose thresholds for the Signal to Noise and Binding Scores.

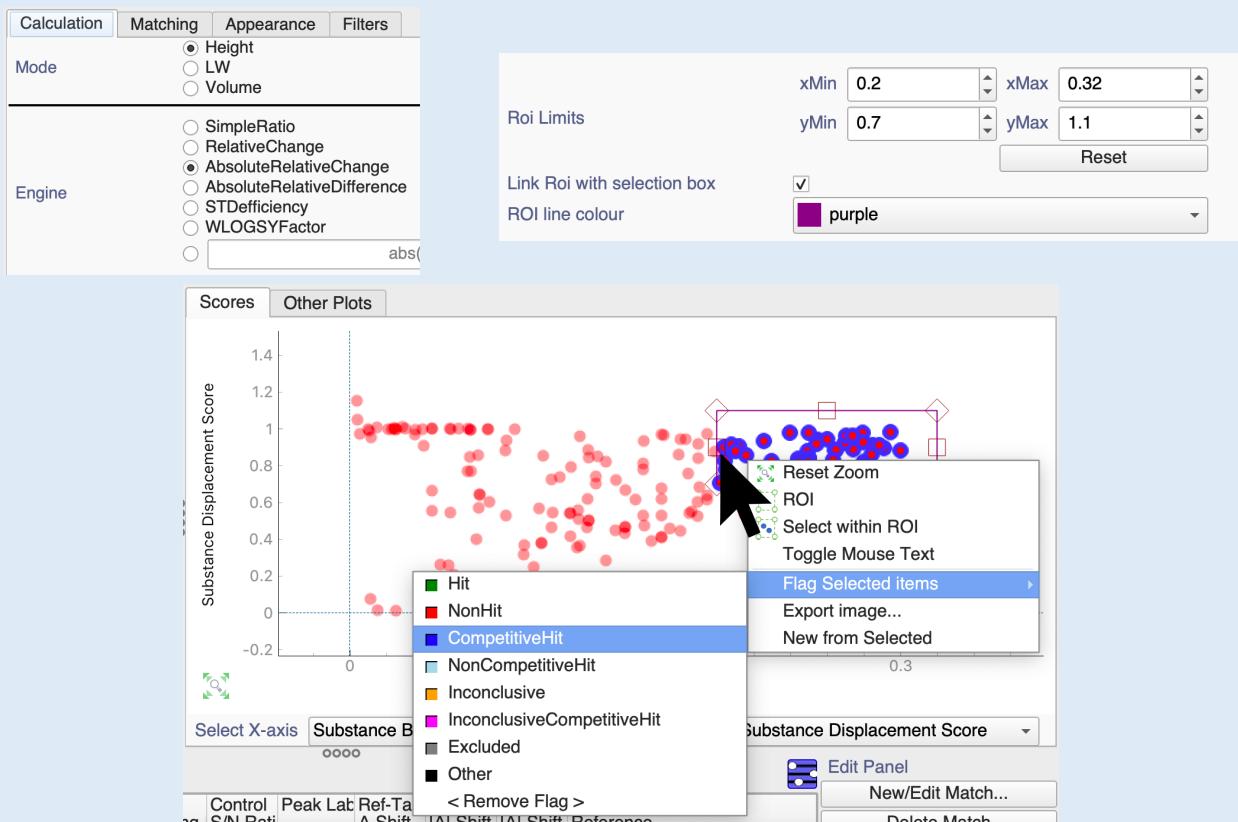
### # vs Substance Binding Score

When filtering with **Sort By Binding Score**, this can be a helpful way to choose a suitable threshold Binding Score above which to mark Hits.

### Substance Binding Score vs Substance Displacement Score

If using a Displacer, this is a useful plot on which to identify Competitive Hits.

Further types of plots, including the molecular structure of the substances (if SMILES were entered into the project), are available in the **Other Plots** tab. More information on these is available from our online documentation on the Hit Analysis Plots at <https://www.ccpn.ac.uk/manual/v3/ScreenHAPlots.html>.



Before flagging Substances, always inspect the matches with the table selections. Snap peaks with the shortcut **SE**, or correct matches using the **New/Edit Match...** button in the **Edit Panel**.

## 5D Setting Flags in the Scatter Plot

- View mode: **Substances**
- Scatter Plot: X-axis: **Substance Binding Score**  
Y-axis: **Substance Displacement Score**

Open Settings:

- **Calculation** tab
  - Engine: **AbsoluteRelativeChange**
- **Appearance** tab
  - **Roi Limits**: click **Reset**
  - xMin: 0.2      xMax: 0.32
  - yMin: 0.7      yMax: 1.1

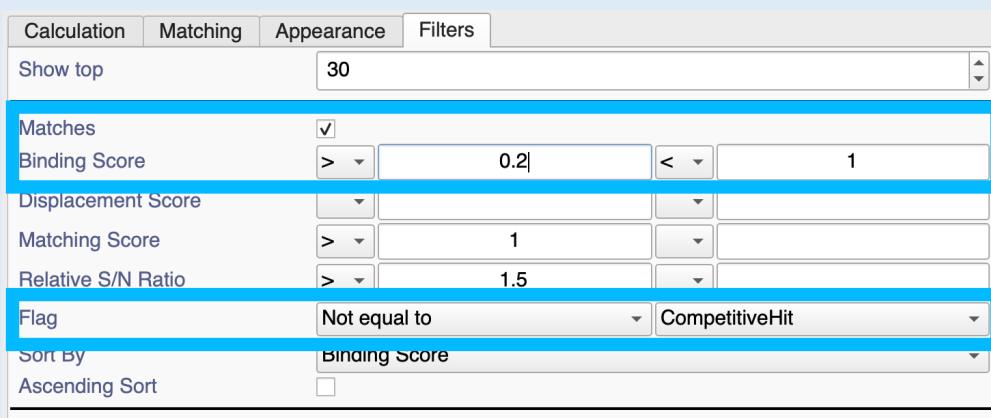
This will create a **Region of Interest** on the scatter plot.

On the scatter plot:

- **right-click** on an item in the plot → **Select within ROI**
- **right-click** on an item in the plot → **Flag Selected Items** → **CompetitiveHit**

Note: when using Reference Mixtures imported from a NEF file, any peaks that have a **Merit** value of 0, have their matches automatically flagged as **Excluded**. Matches with this flag are not included in aggregated (total) Substance/Sample scores.

# Filtering and Plots



Substance Name	Sample Name	Peak Binding Sc	Peak Displaceme	Peak Matching	Peak Matching	Control S/N Ratic	Peak Lab	Ref-Targ	Ref-Targ	Cont-Tai	Peak Position
1 C701	Target_7	0.300	0.684	5	0	81.380	nan	0.000	0.000	0.000	-59.544
2 C8015	Target_8	0.296	0.662	5	0	68.201	nan	0.000	0.000	0.000	-86.813
3 C405	Target_4	0.286	0.684	5	0	66.904	nan	0.000	0.000	0.000	-73.491
4 C906	Target_9	0.285	0.684	5	0	100.828	nan	0.000	0.000	0.000	-86.632
5 C8011	Target_8	0.279	0.695	5	0	65.658	nan	0.000	0.000	0.000	-71.514
6 C702	Target_7	0.275	0.654	5	0	100.464	nan	0.000	0.000	0.000	-77.453
7 C905	Target_9	0.275	0.636	5	0	77.641	nan	0.000	0.000	0.000	-89.061
8 C408	Target_4	0.274	0.637	5	0	70.055	nan	0.000	0.000	0.000	-53.782
9 C606	Target_6	0.274	0.692	5	0	97.808	nan	0.000	0.000	0.000	-73.133
10 C105	Target_1	0.269	0.666	5	0	96.426	nan	0.000	0.000	0.000	-52.433
11 C2013	Target_2	0.260	0.672	5	0	73.714	nan	0.000	0.000	0.000	-85.551
12 C7017	Target_7	0.255	0.685	5	0	83.418	nan	0.000	0.000	0.000	-57.563
13 C805	Target_8	0.245	0.652	5	0	61.124	nan	0.000	0.000	0.000	-54.772

## 5E Setting Flags in the Peak Binding Scores Table

We will now mark all the non-competitive Hits:

- View mode: **Matches Only**

Open Settings:

- Filters tab
  - Matches: **ticked**
  - Binding Score: **> 0.2**      **< 1**
  - Flag: **Not equal to**      **CompetitiveHit**

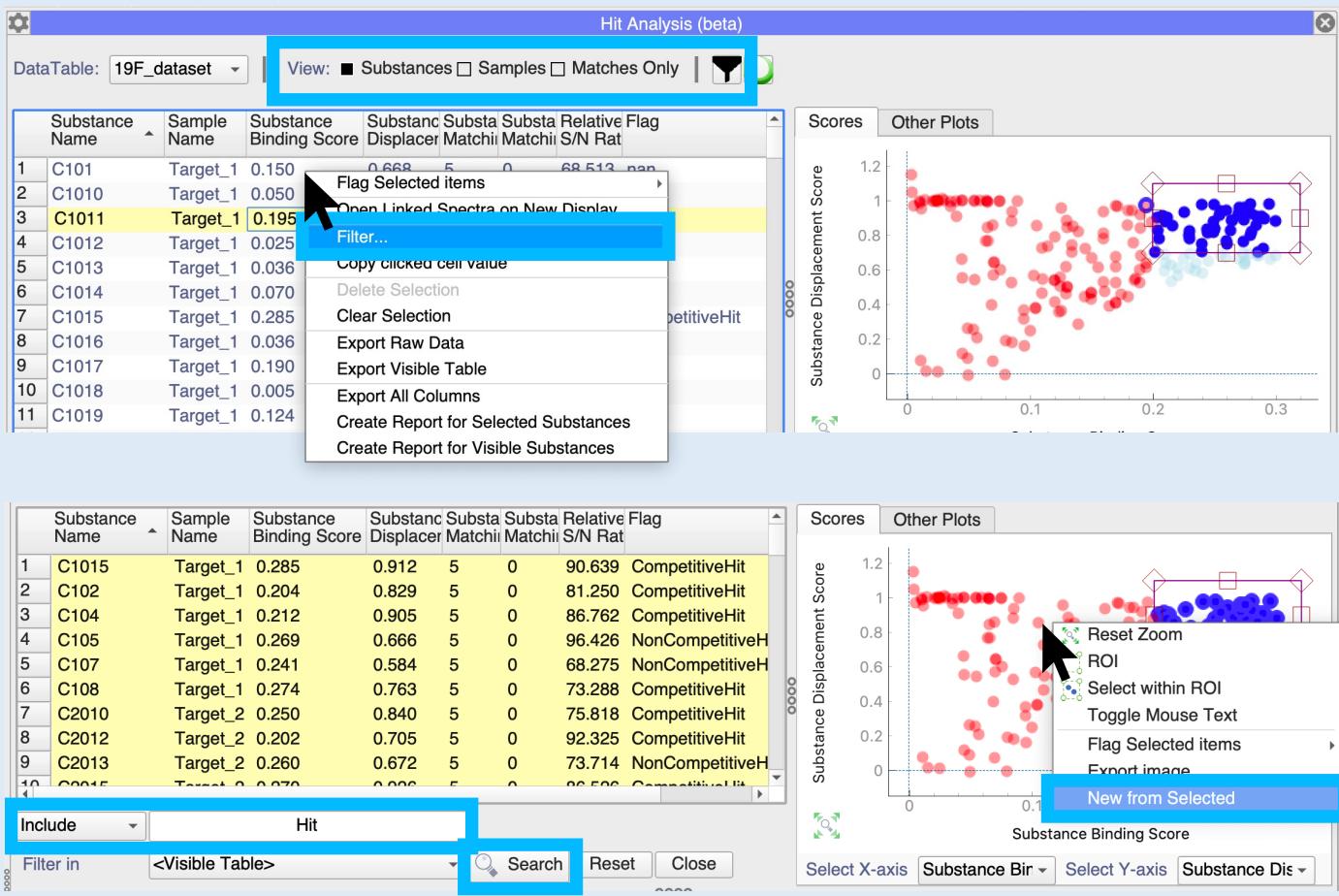
In the main Hit Analysis Module:

- Make sure Filtering is on ( ) and you click the Update button if orange
- Type **Cmd/Ctrl+A** to select all rows in the table

In the Edit Panel:

- click on **<Select Flag>**
- select **NonCompetitiveHit**

# Exporting Data



## 6A Extracting data

- Switch the **Filtering** off →
- Set the View mode to **Substances**

Use the normal Table Filtering function:

- **right-click** and select **Filter**
  - Select **Include** from the drop-down menu
  - Type **Hit** into the search
  - Press **Enter** or the **Search** button
- Select all the remaining rows with **Ctrl/Cmd+A**

Move to the scatter plot:

- **right-click** → **New from Selected**

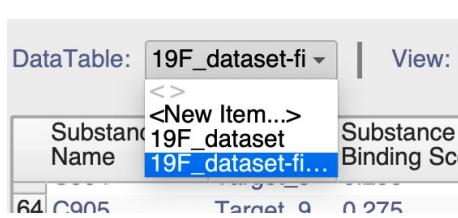
This will create a new dataset containing only this subset of Substances. You can rename the dataset by double-clicking on it in the sidebar under

**DataTables**.

▼ **DataTables**  
 <New DataTable>  
 DT:19F\_dataset  
 DT:19F\_dataset-filtered

- Select the newly created dataset on the Hit Analysis module, by selecting it from the **DataTable** dropdown menu.

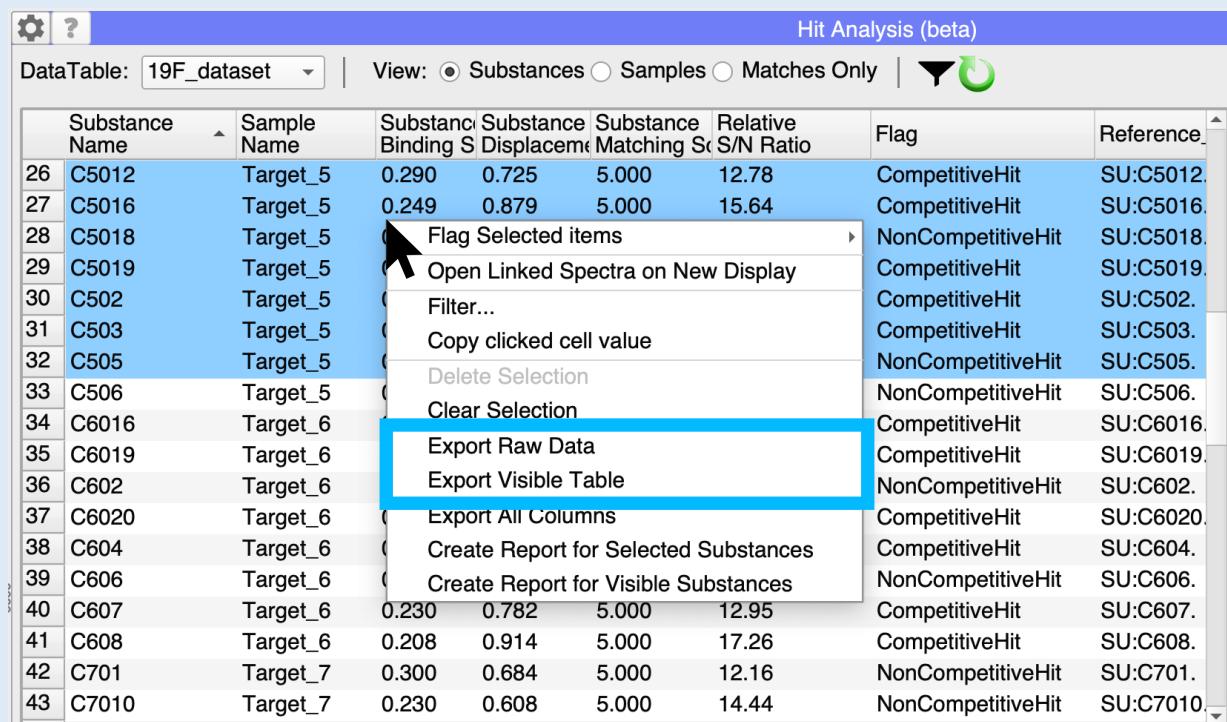
Either continue to inspect the data as shown in Sections 4 and 5 or export the table as shown in Section 6B.



There are two options for exporting data from a Hit Analysis module table:

**Export Visible Table** only exports those columns and rows currently in the table (e.g. after any Table Filtering has been applied or columns have been added / removed after right-clicking on the column heading)

**Export Raw Data** will export the underlying DataTable (accessible from **DataTables** in the sidebar) which is used to build the Hit Analysis module. This includes peak metadata, peak ppm positions, heights etc.



The screenshot shows a 'Hit Analysis (beta)' interface with a substance table. The table has columns: Substance Name, Sample Name, Substance Binding, Substance Displacement, Substance Matching Score, Relative S/N Ratio, Flag, and Reference. A context menu is open over the first few rows, with 'Export Raw Data' highlighted. The menu options are: Flag Selected items, Open Linked Spectra on New Display, Filter..., Copy clicked cell value, Delete Selection, Clear Selection, Export Raw Data, Export Visible Table, Export All Columns, Create Report for Selected Substances, and Create Report for Visible Substances.

Substance Name	Sample Name	Substance Binding	Substance Displacement	Substance Matching Score	Relative S/N Ratio	Flag	Reference
26 C5012	Target_5	0.290	0.725	5.000	12.78	CompetitiveHit	SU:C5012.
27 C5016	Target_5	0.249	0.879	5.000	15.64	CompetitiveHit	SU:C5016.
28 C5018	Target_5					NonCompetitiveHit	SU:C5018.
29 C5019	Target_5					CompetitiveHit	SU:C5019.
30 C502	Target_5					CompetitiveHit	SU:C502.
31 C503	Target_5					CompetitiveHit	SU:C503.
32 C505	Target_5					NonCompetitiveHit	SU:C505.
33 C506	Target_5					NonCompetitiveHit	SU:C506.
34 C6016	Target_6					CompetitiveHit	SU:C6016.
35 C6019	Target_6					CompetitiveHit	SU:C6019.
36 C602	Target_6					NonCompetitiveHit	SU:C602.
37 C6020	Target_6					CompetitiveHit	SU:C6020.
38 C604	Target_6					CompetitiveHit	SU:C604.
39 C606	Target_6					NonCompetitiveHit	SU:C606.
40 C607	Target_6	0.230	0.782	5.000	12.95	CompetitiveHit	SU:C607.
41 C608	Target_6	0.208	0.914	5.000	17.26	CompetitiveHit	SU:C608.
42 C701	Target_7	0.300	0.684	5.000	12.16	NonCompetitiveHit	SU:C701.
43 C7010	Target_7	0.230	0.608	5.000	14.44	NonCompetitiveHit	SU:C7010.

## 6B Export data

- Right-click on a Substance Table row and select one of these options:
  - Export Raw Data**
  - Export Visible Data**

In the **Save Table** file dialog:

- Type a name plus the extension OR select the extension from the dropdown menu.

Possible file types and their extensions are:

Excel	.xlsx
Comma-separated	.csv
Tab-separated	.tsv
JSON	.json

# Exporting Data

Hit Analysis (beta)

DataTable: 19F\_dataset | View:  Substances  Samples  Matches Only |

	Substance Name	Sample Name	Substance Binding Score	Substance Displacement Score	Substance Matching Score	Relative S/N Ratio	Flag	Reference
26	C5012	Target_5	0.290	0.725	5.000	12.78	CompetitiveHit	SU:C5012.
27	C5016	Target_5	0.249	0.879	5.000	15.64	CompetitiveHit	SU:C5016.
28	C5018	Target_5					NonCompetitiveHit	SU:C5018.
29	C5019	Target_5					CompetitiveHit	SU:C5019.
30	C502	Target_5					CompetitiveHit	SU:C502.
31	C503	Target_5					CompetitiveHit	SU:C503.
32	C505	Target_5					NonCompetitiveHit	SU:C505.
33	C506	Target_5					NonCompetitiveHit	SU:C506.
34	C6016	Target_6					CompetitiveHit	SU:C6016.
35	C6019	Target_6					CompetitiveHit	SU:C6019.
36	C602	Target_6					NonCompetitiveHit	SU:C602.
37	C6020	Target_6					CompetitiveHit	SU:C6020.
38	C604	Target_6					CompetitiveHit	SU:C604.
39	C606	Target_6					NonCompetitiveHit	SU:C606.
40	C607	Target_6	0.250	0.752	5.000	12.55	CompetitiveHit	SU:C607.
41	C608	Target_6	0.208	0.914	5.000	17.26	CompetitiveHit	SU:C608.

Right-click context menu options:

- Flag Selected items
- Open Linked Spectra on New Display
- Filter...
- Copy clicked cell value
- Delete Selection
- Clear Selection
- Export Raw Data
- Export Visible Table
- Export All Columns
- Create Report for Selected Substances
- Create Report for Visible Substances

## 6C Creating PowerPoint report slides

- Select your Substances of interest in the Substance Table
- Right-click and select one of:
  - Create Report for Selected Substances
  - Create Report for Visible Substances

In the Save Table file dialog:

- Select your file name and directory to save the .pptx file

Your PowerPoint file will include a report slide with details of the pipeline and parameters used, a substances summary table and then individual report slides for each substance.

Find out how to make changes to the templates in our online documentation at  
<https://www CCPN.ac.uk/manual/v3/ScreenReports.html>

**CcpNmr Screening Report**

Operator: Vicky Higman  
 Date/Time: 06/03/25 12:21:29  
 CcpNmr Version: 3.3.1

Hit Analysis Calculation Settings:  
 - Calculation Mode: Height  
 - Calculation Engine: Absolute Relative C  
 - Displacement Engine: Displacement Fra  
 - Total Score Engine: Sum  
 - Relative S/N Engine:  
 - Scale Delta Score: No

Data Paths:  
 - Control: /Users/vad5/D...  
 - Target: /Users/vad5/D...  
 - Displacer: /Users/vad5/

**Substances Summary**

Index	Sample Name	Substance Name	Binding Score	Displacement Score	Matching Score	Control S/N	Flag
1	Target_5	C5012	0.29	0.72	5	12.78	CompetitiveHit
2	Target_5	C5016	0.25	0.88	5	15.64	CompetitiveHit
3	Target_5	C5018	0.23	0.69	5	14.6	NonCompetitiveHit
4	Target_5	C5019	0.24	0.84	5	14.75	CompetitiveHit
5	Target_5	C502	0.23	0.83	5	14.35	CompetitiveHit

**C5012 (Target\_5)**

1) Binding Score: 0.29 – Displacement Score: 0.72 – Matching Score: 5

Chemical structure: CN1C=NC2=C1C(=O)N(CCN2C)C

Peak list:

- C5012.1.1
- Control\_5.1.4
- Target\_5.1.4
- Displacer\_5.1.4

Reference Peak Pid: PK:C5012.1.1

Binding Score	0.29
Matching Score	5
Displacement Score	0.72
Control S/N	12.78
Reference Position (ppm)	-86.971
Label	CompetitiveHit
Comment	

[ppm]

# Appendix

## CcpNmr AnalysisScreen Nomenclature

### Sample

A CcpNmr object containing information about the physical NMR sample, e.g., pH, ionic strength etc.  
CcpNmr links: Sample component, Spectrum (e.g.: the spectrum Control, Target...)

### SampleComponent

A CcpNmr object containing information about the Substance in the physical NMR sample (e.g., concentration).  
CcpNmr links: Substance

### Substance

A CcpNmr object containing information about a molecule, (e.g., a small molecule and its general properties such as: SMILES, MW etc.).  
CcpNmr links: Sample component, Spectrum (e.g.: the Singleton Spectrum)

### SpectrumGroup

A CcpNmr object containing a collection of spectra.  
CcpNmr links: Spectra

### Control (spectrum)

The spectrum recorded at time X for a sample containing one or multiple substances prior the addition of a biological target.  
CcpNmr links: Sample

### Target (spectrum)

The spectrum recorded at time X for a sample containing one or multiple substances plus a biological target.  
CcpNmr links: Sample

### Displacer (spectrum)

The spectrum recorded at time X for a sample containing one or multiple substances plus a biological target and a known binder. Also referred to as a “displacer”.  
CcpNmr links: Sample

### Reference Mixture

The spectrum recorded for a sample containing multiple substances. Its peaks and their annotations are used as a template and to identify substances in future screening analyses.  
CcpNmr links: Substances

### Reference Singleton

The spectrum recorded for only one substance.  
CcpNmr links: Substance

### Binding Substance

The substance linked to a spectrum (reference) whose peaks have been matched to the spectral peaks (Control – Target) denoting a binding event.

### Peak Match

The virtual linkage between a Reference – Control – Target ( – Displacer) peak in a Screening Dataset.

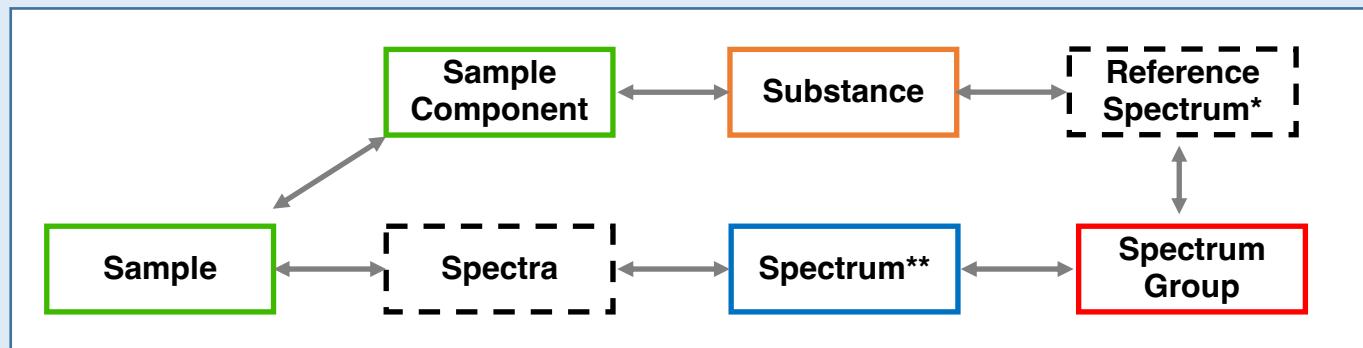


Figure showing CcpNmr AnalysisScreen object links

The schematic diagram shows how objects are linked in CcpNmr AnalysisScreen.

\*Reference Singleton; \*\*Control, Target, Displacer, Reference Mixture

## 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 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)