Mestrelab Research

chemistry software solutions

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Using Mnova Screen to Process, Analyze and Report Ligand-Protein Binding Spectra for Fragment-based Lead Design

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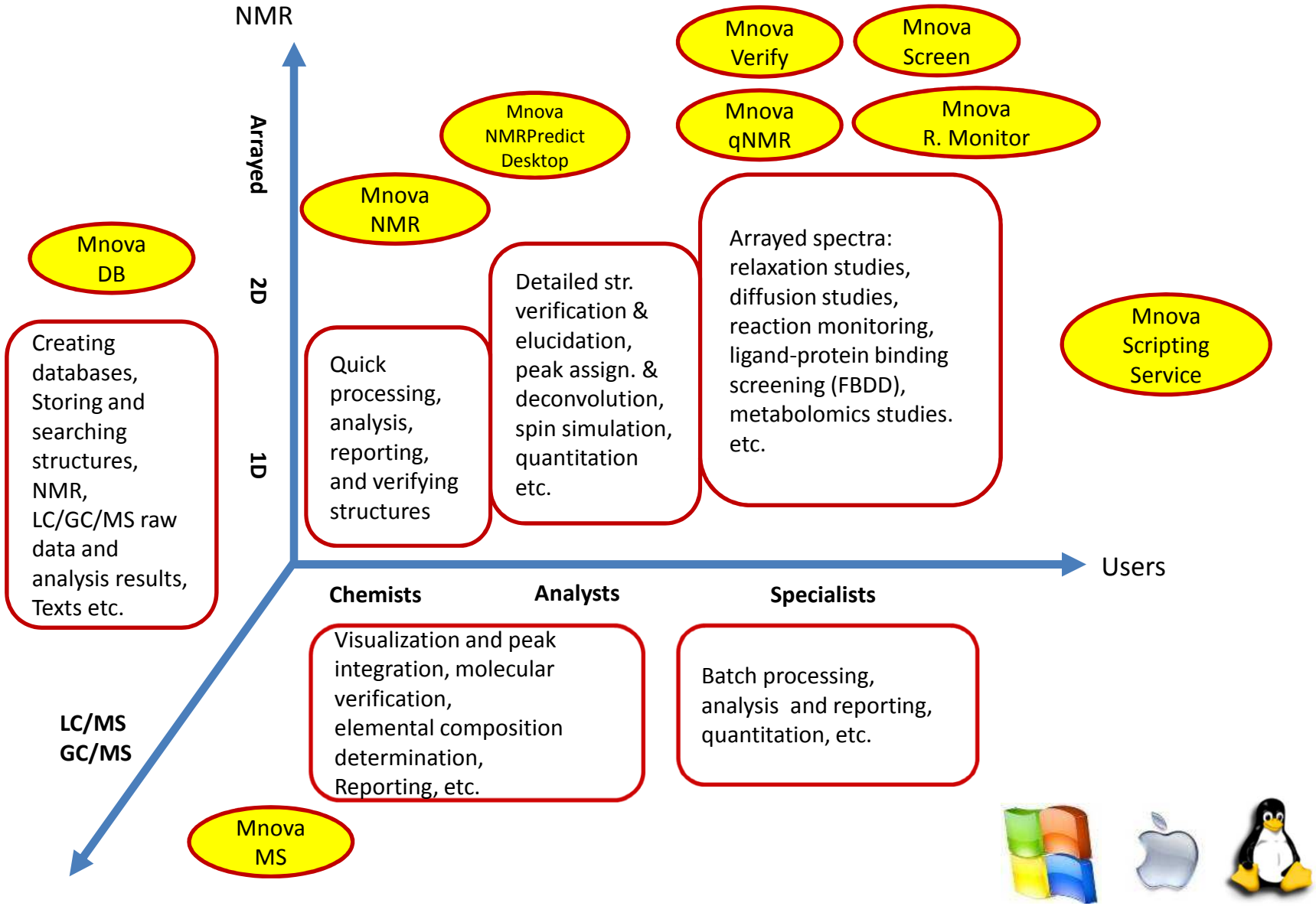
Mestrelab Research
chemistry software solutions

- 1996: A research project in University of Santiago de Compostela, Spain, developed free MestReC software for NMR processing
- 2004: Mestrelab Research incorporated in Santiago de Compostela
- 2004: New MestReNova (Mnova) platform and NMR plugin released
- 2006: NMRPredict Desktop for NMR prediction
- 2009: MS plugin for LC/GC/MS data analysis
- 2009: Global Spectral Deconvolution (GSD) algorithm released for NMR
- 2011: DB plugin for Database Management of NMR and MS
- 2012: Verify plugin for auto structure verification
- 2012: qNMR plugin for quantitative NMR analysis
- 2013: Reaction Monitor plugin for NMR-based reaction kinetics studies
- 2013: Screen plugin for high-throughput ligand-protein binding analysis
- An R&D company with >20 people and >80,000 registered users

Mnova products and applications



Fragment-based Drug Design Using NMR

- Lead generation from a compound library with weak binding (K_d ~uM – mM) in early stage of drug discovery
- Ligand-based screening: 1D ^{1}H or ^{19}F of mixtures of protein & ~8 ligands
  - Saturation transfer difference (STD)
  - Relaxation editing :
    - T1ρ
    - CPMG
  - WaterLOGSY
- Target-based screening: ^{1}H-^{15}N HSQC of target: Profiling, and titration using ligand ("SAR by NMR")
- Analysis is a bottleneck

S.B. Shuker, P.J, Hajduk, R. P. Meadows, S. W. Fesik, "Discovering high-affinity ligands for proteins: SAR by NMR.", *Science* **274** (5292): 1531-4 , 1996

J.Fejzo , C.A. Lepre, J.W. Peng, G.W. Bemis., M.A Ajay, J.M. Moore.; *Chem. Biol.* **6**(10):755-69, 1999

C. Dalvit, , P. Pevarello, M. Tato, M. Veronesi, A. Vulpetti, M. Sundstrom, *J. Biomol. NMR*, **18**(1), 65-68, 2000

M. Coles, M. Heller, H. Kessler, *Drug Discovery Today* **8**(17):803-810, 2003

The challenges of automated data analysis

- A lot of compounds and spectra: how to manage them?
- Batch processing and collating spectra: no consistent way of organizing data
- Peak picking: overlap, different S/N, a consistent threshold is impossible
- Peak misalignment: systematic and local
- How to detect the peak changes?
- How to report the results?
- How to facilitate the inspection of the results?

What Mnova can help you*

- ❑ Library compound: spectral processing, verification of structure, assignment of peaks
- ❑ Compound solubility/concentration determination
- ❑ Library compound/spectral management: databasing
- ❑ Plate design: auto choosing compounds with minimum peak overlap from a group of 1D spectra
- ❑ Batch processing and analysis of STD, waterLOGSY, T1ρ and similar spectra
- ❑ Inspection and editing results
- ❑ Batch processing and binning 2D HSQC

Mnova NMR

Mnova NMRPredict Desktop

Mnova Verify

Mnova qNMR

Mnova DB

Mnova Scripting

Mnova Screen

Mnova NMR

Mnova NMR

The common NMR experiments and what we do with them

Experiment	Sample contents	What to observe?	How to analyze?
Reference spectra	Single library compound	The reference peaks of the library compounds	Process the spectra, pick ref. peaks by GSD
Saturation transfer Detection (STD)	Mixture of *N* library compounds and protein. Blank (no protein) and/or competition (with protein and inhibitor) may be used for comparison	The on-resonance spectral peaks are expected to decrease if that compound is binding with the protein.	If the STD difference spectra are used, then the peaks are picked and matched to ref peaks. If the on/off-resonance spectral pairs are used, then the ref peaks are mapped to the STD pair. The change of peak intensities within the mapped regions are calculated and averaged. If available, blank and competition data will be used to detect false positive and non-specific binding if available.
T1ρ (and CPMG)	Mixture of *N* library compounds and protein. Blank (no protein) and/or competition (with protein and inhibitor) may be used for comparison	The peaks from the long spin-lock time spectrum are expected to decrease compared with the short spin-lock one, if that compound is binding with the protein. If inhibitor is used, the peaks are expected to restore to the original height if it is a specific binding	The ref peaks are mapped to the T1ρ pair. The change of peak intensities within the mapped regions are calculated and averaged. If available, blank and competition data will be used to detect false positive and non-specific binding if available.
WaterLOGSY	Mixture of *N* library compounds without protein (blank), and with protein. Competition sample (with protein and inhibitor) may be used for comparison	Negative are expected in the blank . Positive peaks are expected for a compound that binds with the protein. If inhibitor is used, such peaks are expected to restore to negative if it is a specific binding	The ref peaks are mapped to the wLogsy spectra. The change of peak intensities within the mapped regions are calculated and averaged. If available, blank and competition data will be used to detect false positive and non-specific binding if available.

What types of data can Mnova Screen handle?

- Mnova Screen handles any combination of the experimental types:
  - Reference : H-1 of the pure library compounds*
  - Scout: H-1 of the mixture, with or without protein
  - STD: without protein (blank), with protein, and with protein+inhibitor, in the form of difference spectra or on/off resonance pairs**
  - T1ρ (and CPMG): without protein (blank), with protein, and with protein+inhibitor, in the form of short/long spin-lock time pairs***
  - WaterLOGSY: without protein (blank), with protein, and with protein+inhibitor, all as single spectra
- ^{19}F data
- The spectral data can be raw data or processed data from various vendors (Bruker, Varian/Agilent, JEOL, JCAMP etc.)

*Note: *The use of ref spec is optional. If you don't use ref spec, you can use the Scout as the reference, and Mnova Screen will report the maximum and average peak intensity changes for all the Scout peaks.*
***The use of on/off resonance pairs is preferred, as their signal to noise ratio is higher than the difference spectra, and it is possible to calculate the percentage of intensity changes relative to the original spectra.*
**** Mnova Screen can also handle if only 3 experiments are used: Blank with short spin-lock time, Protein with long spin-lock time, and Protein+Inhibitor with long spin-lock time.*

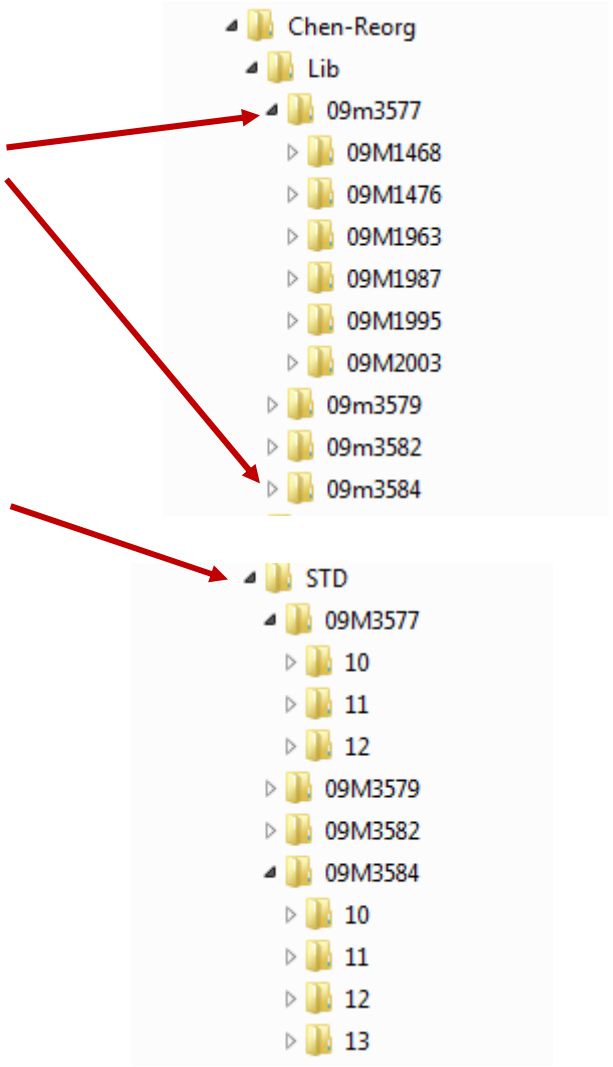
How to organize your dataset?

- There have been no standard ways to organize screening data. Mnova Screen was designed to accept as general ways of data organization as possible:
  - Mixture data and reference data are saved in separate folders with matching folder names
  - Mixture data and reference data are saved in the same folder for each sample, but with distinguished experimental names
  - Mixture data and reference data are matched by a definition file
- Several examples are illustrated in the subsequent slides
- If necessary, you can write a shell script to reorganize your data in the similar ways so that Mnova Screen can import them correctly

Data org. example I: Ref + STD

- In this example the ref. spectra for each mixture are saved under a folder "<mix_name>", where <mix_name> is the folder name of the corresponding mixture. The number of ref spectra can be different for each mixture.

- The mixture spectra (STD in this case) are saved under a separate folder. Each mixture, "<mix_name>", has several experiments (scouts and STD difference spectra, and some others - to be ignored here) :
  - 10: To be ignored
  - 11: STD difference spectra
  - 12: Scout
  - 13: To be ignored

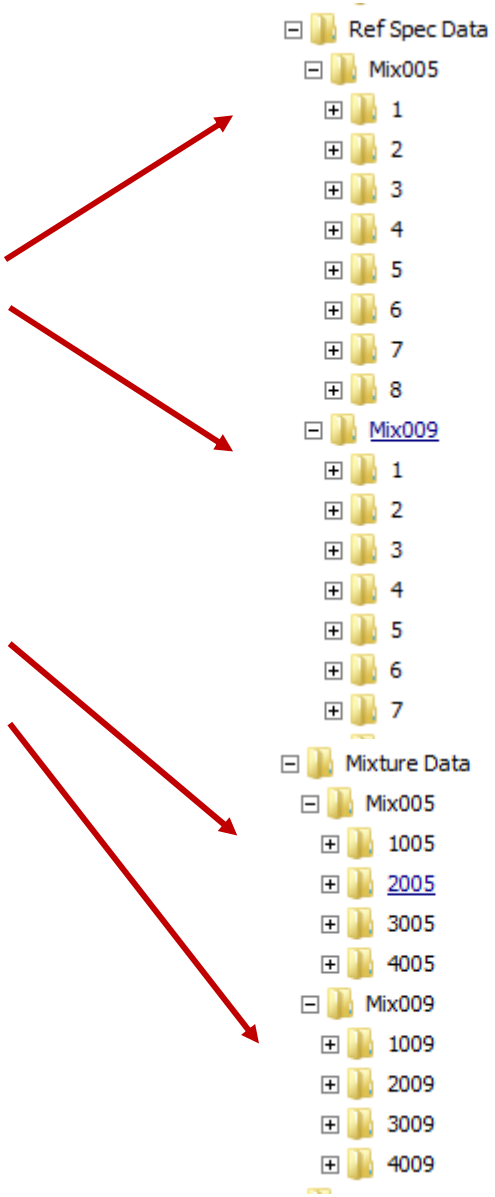


Note: This format is not limited to STD only. You can have other types of experiments for each mixture. You just need to name the experimental folders in a consistent way.

Data org. example II: Ref + STD + T1r

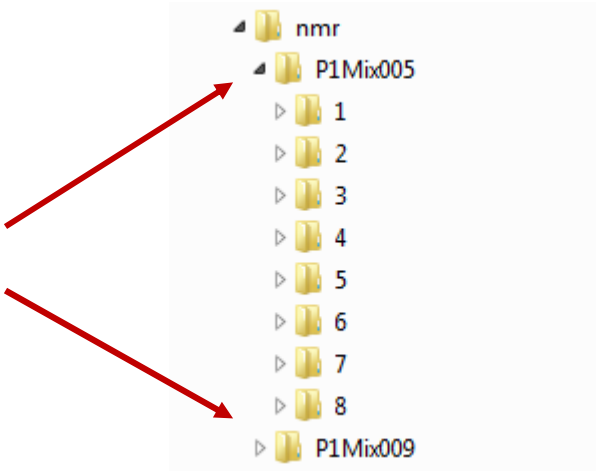
- In this example the ref spectra for each mixture are saved under a separate folders, such as “Mix005”, “Mix009”, etc.

- The mixture spectra are saved under separate folders with names matching those for the ref spectra. The experiment names have a consistent pattern defined as follows:
  - 1[nnn]: STD difference spectra
  - 2[nnn]: Scout
  - 3[nnn]: T1r with short spin-lock time
  - 4[nnn]: T1r with long spin-lock time



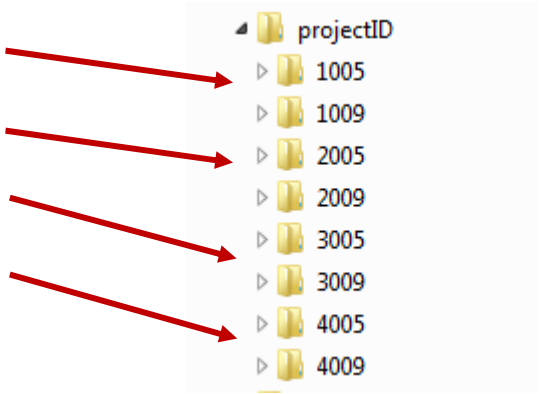
Data org. example III: Ref + STD + T1r

- This example is similar to Example II, but the mixture spectra are all saved under the same folder; hence there are rigorous requirements their experiment folder names match the ref folder names



- The ref spectra for each mixture are saved under a folder “P1Mix[nnn]”, where [nnn] corresponds to the mixture #.

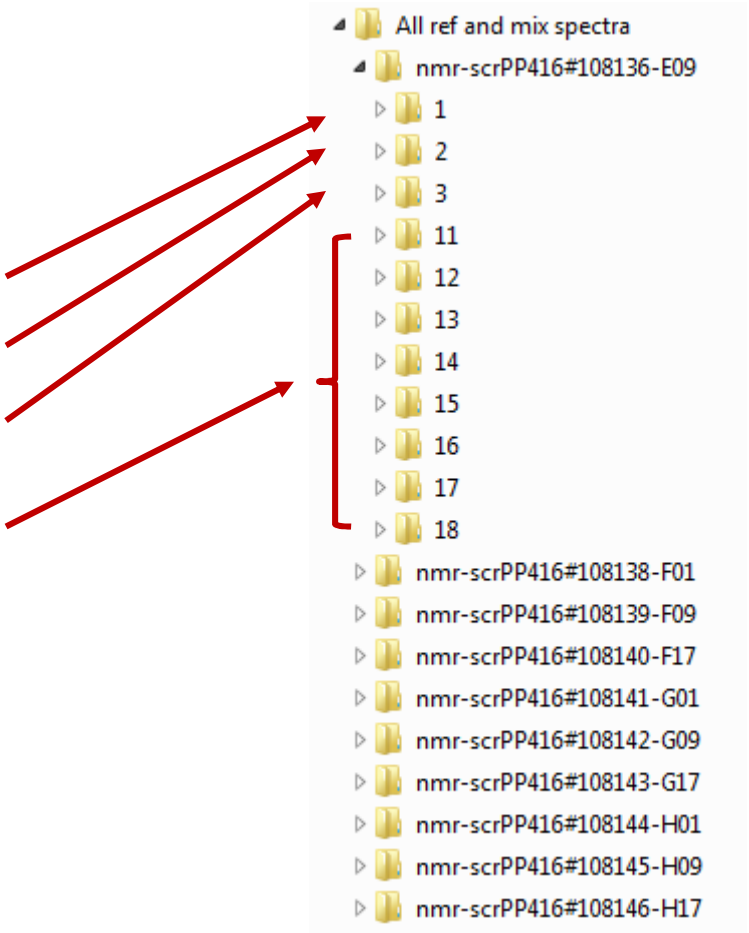
- The mixture spectra are all saved under one folder, with a naming convention of “[e][nnn]”, where [e] is the experimental type # and “nnn” matches the ref spectral folder names:



  - 1[nnn]: STD difference spectra
  - 2[nnn]: Scout
  - 3[nnn]: T1r with short spin-lock time
  - 4[nnn]: T1r with long spin-lock time

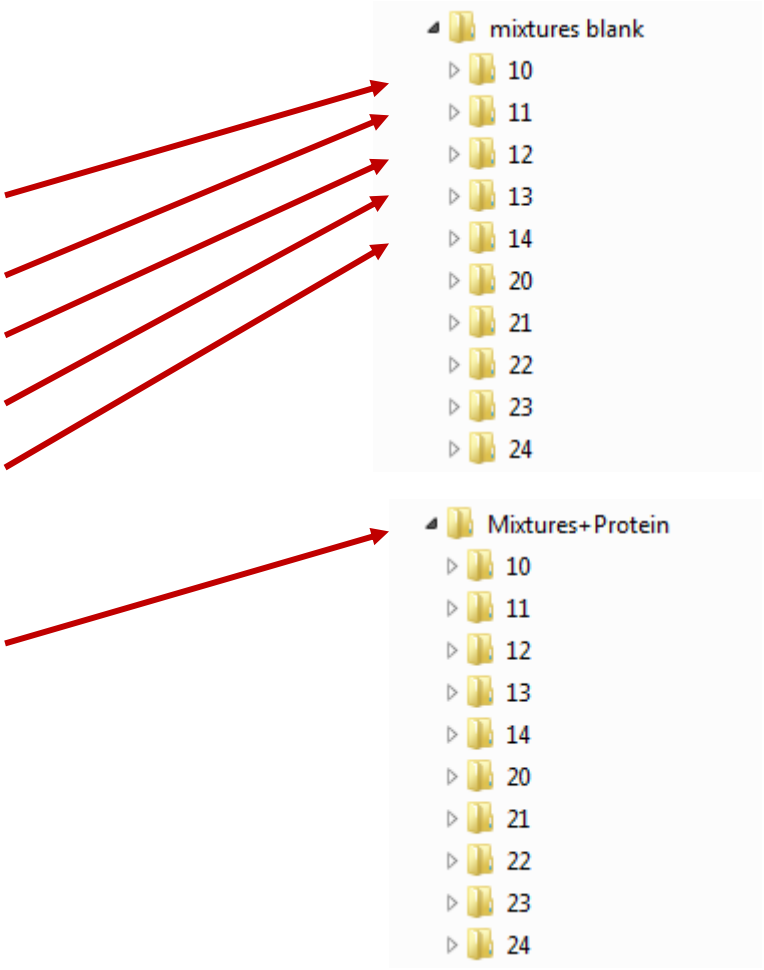
Data org. example IV: Ref + T1ρ

- In this example all the T1ρ and ref. spectra for the same mixture are saved under the same folder, with single digit names [m] for T1ρ, and double digit names [rr] for the 8 reference spectra.
- 3 T1ρ's are used for each mixture:
  - 1: T1ρ blank
  - 2: T1ρ with protein
  - 3: T1ρ with protein and inhibitor
- 8 ref. spectra are saved in the same folder (11-18)



Data org. example V: Ref + STD + T1r + wLogsy

- In this example all mixture blank spectra [m][e] are saved under one folder (e=0-4):
  - [m]0: Scout
  - [m]1: T1ρ with short spin-lock
  - [m]2: T1ρ with long spin-lock
  - [m]3: WaterLOGSY
  - [m]4 : STD pair (on/off resonance)
- All mixture spectra [m][e] with protein are saved under another folder with exactly the same names as for the blanks. *



**Note: The same number of folders with the exactly same names are expected in either folders. Each mixture must have exactly the same number (5) of experiments.*

Data org. example V: Ref + STD + T1r + wLogsy (continued)

- The names of the library compounds for each mixture are saved in the Comment parameter (read from the “title” file) of each experiment*

- The reference spectra are saved in other folders. The correspondence between the lib compound names and the reference spectra are defined in an Excel file**

	Parameter	Value
1	Data File Name	C:/Mestrelab/Projects/Demo Data/2D/Quinin
2	Title	Quinine 1H
3	Comment	A01 Lib-compound-1 Lib-compound-2 Lib-compound-3 Lib-compound-4 Lib-compound-5 Lib-compound-6 Lib-compound-7 Lib-compound-8

	A	B	C	D	E	F	G	H	I	J	K
1		Cpd ID	Mixture	data path					expno		
2		Lib-compound-1	1	C:/Mestrelab/Projects	spec-data	my_library	nmr	My_lib_rack_1	540	pdata	1
3		Lib-compound-2	1	C:/Mestrelab/Projects	spec-data	my_library	nmr	My_lib_rack_1	790	pdata	1
4		Lib-compound-3	1	C:/Mestrelab/Projects	spec-data	my_library	nmr	My_lib_rack_2	170	pdata	1
5		Lib-compound-4	1	C:/Mestrelab/Projects	spec-data	my_library	nmr	My_lib_rack_1	850	pdata	1
6		Lib-compound-5	1	C:/Mestrelab/Projects	spec-data	my_library	nmr	My_lib_rack_4	180	pdata	1
7		Lib-compound-6	1	C:/Mestrelab/Projects	spec-data	my_library	nmr	My_lib_rack_1	2460	pdata	1
8		Lib-compound-7	1	C:/Mestrelab/Projects	spec-data	my_library	nmr	My_lib_rack_1	870	pdata	1
9		Lib-compound-8	1	C:/Mestrelab/Projects	spec-data	my_library	nmr	My_lib_rack_1	320	pdata	1
10		Lib-compound-9	2	C:/Mestrelab/Projects	spec-data	my_library	nmr	My_lib_rack_1	10	pdata	1
11		Lib-compound-10	2	C:/Mestrelab/Projects	spec-data	my_library	nmr	My_lib_rack_1	630	pdata	1

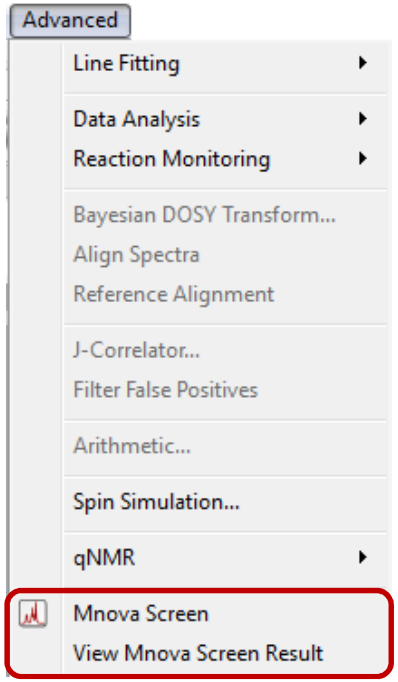
*Note: *The number of library compounds can be fewer than a maximum (8 in this case). If the spectrum of a lib compound cannot be found, it is ignored without interrupting the program.*
***Mnova Screen does not read Excel file, so the original Excel file should be exported to a .csv file.*

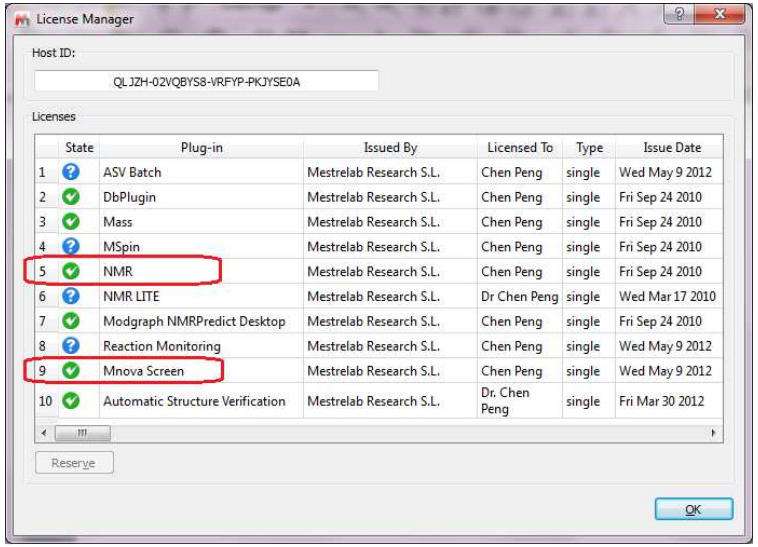
To install Mnova Screen

- Mnova Screen is a separately licensed plugin to the Mnova system. To run it, you need to install Mnova NMR and Screen, and have licenses to both plugins

- After installation of Mnova Screen you will see two commands on the Advanced menu:
  - **Mnova Screen**: for processing and analyzing FBS data
  - **View Mnova Screen Results**: for inspecting and refining the results from previous runs

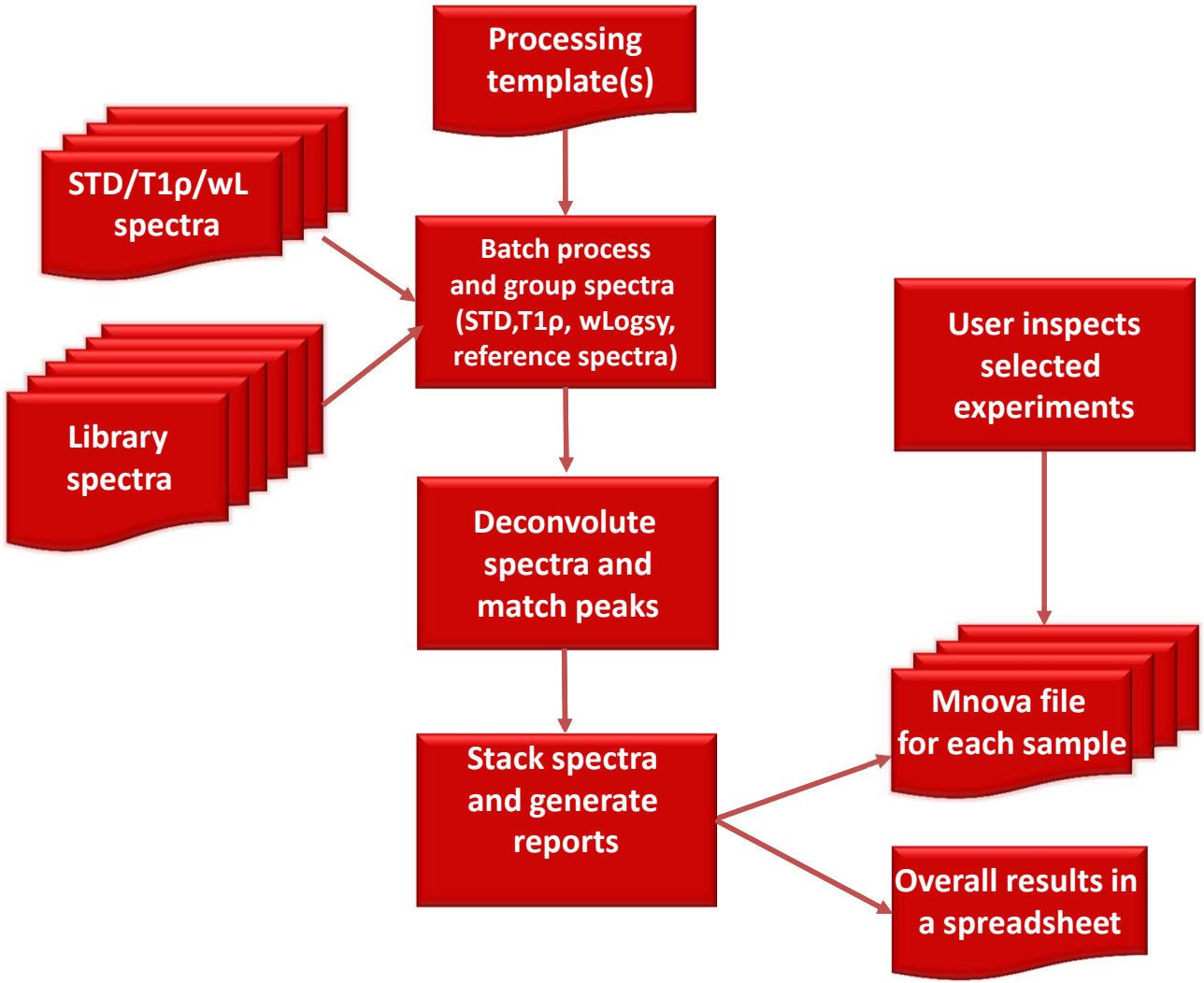
- Choose Help > License Manager and make sure you have properly activated both the NMR and Mnova Screen plugins**





To download the installer of Mnova Suite (including NMR and NMRPredict Desktop): http://mestrelab.com/software/mnova-suite/download/.
To download the installer of Mnova Screen:
http://mestrelab.com/downloads/tmp/common/Mnova-Screen-1.0.0.1165.zip

Workflow of Mnova Screen



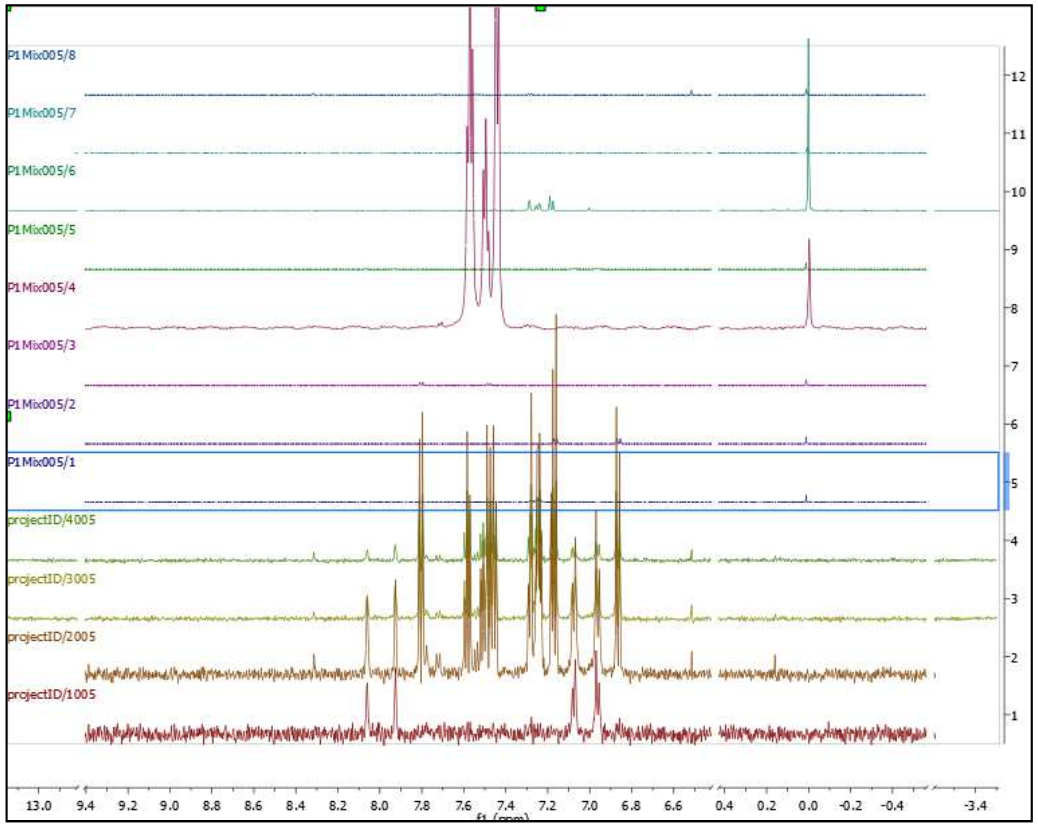
Before you start

- ❑ Make sure your data files are organized as described in the previous slides, and the incomplete experiments are moved away from the folders
- ❑ Open a few spectra data in Mnova and see how the auto processing works for your data. If not satisfactory, you choose Process > Processing Template, change the parameters until you are satisfied, and then save a processing template for later use.
- ❑ Stack a few sets of ref. spectra and mix. spectra together to see how well the peaks align. Find a peak (e.g. DSS) that can be used as reference and the chemical shift offset for aligning them.
- ❑ Also observe the local misalignment of individual peaks across the spectra, so that you have an idea about the tolerance for matching ref. peaks and mix. peaks.
- ❑ For T1ρ and WaterLOGSY, identify the peak (e.g. DMSO or DSS) that can be used as reference to normalize the spectral intensity, or make sure your experimental and acquisition conditions are identical
- ❑ Decide the spectral region(s) that you are interested in (ROI). Make sure solvent and buffer peaks are not included.
- ❑ The subsequent slides shows an example of how to figure out these settings.

Before you start: Check the processing of spectra

- ❑ Open all the mix. and ref. spectra of the first sample in Mnova, and stack them together. Use the Cut tool to show only the aromatic and DSS regions.

- ❑ In this case, the auto processing gives good phasing. So no processing template is needed.*



Ref. spectra

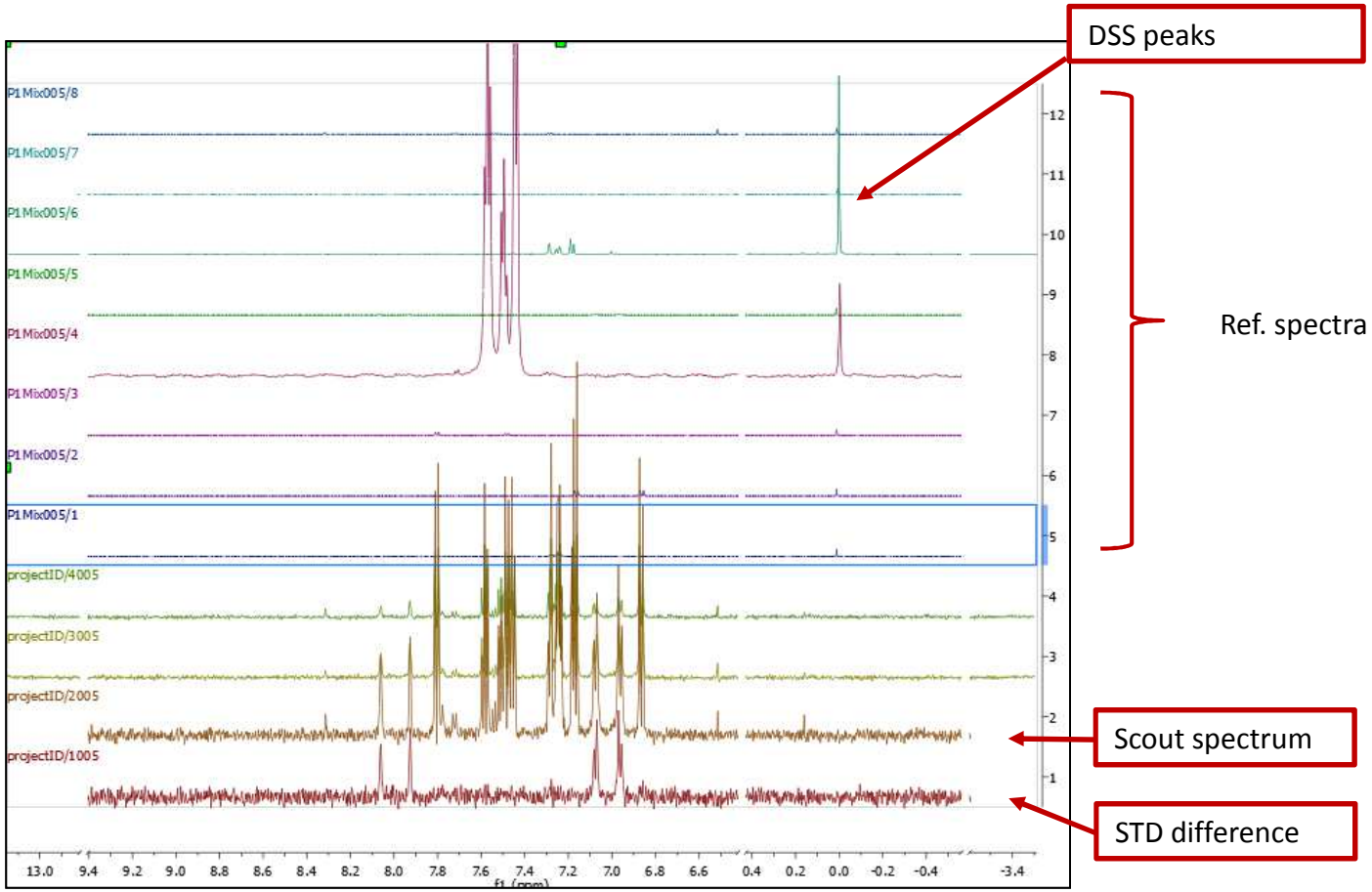
Mix. spectra

*Note: *Mnova Screen will automatically apply a baseline correction (3rd order Bernstein Polynomial) to a spectrum if no processing template is specified for it.*

Before you start: Check the Y-scales

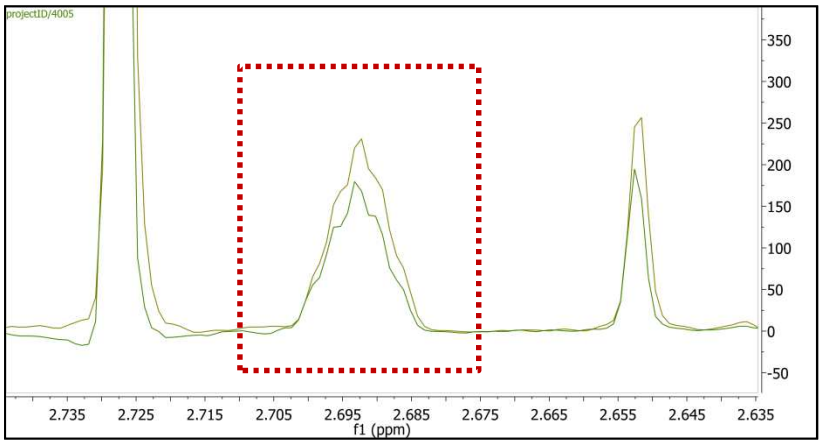
- It's observed that the spectra have very different Y scales so normalization is needed to make their peaks visible in the stack. The DSS peaks can be used for the ref spectra (e.g. setting their height to be 25,000)

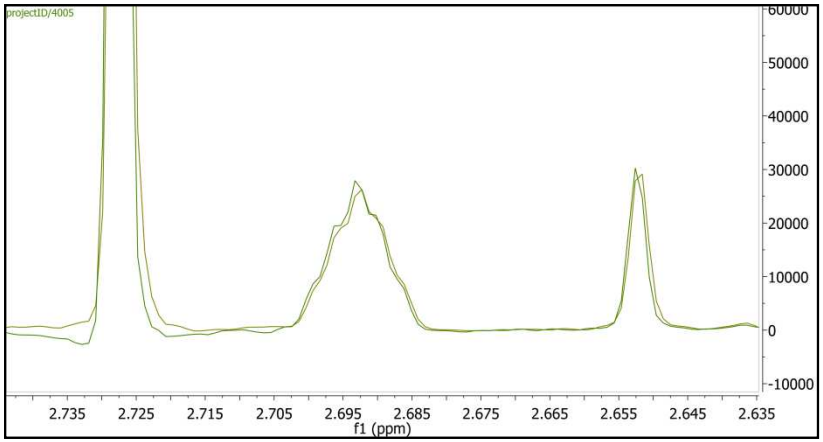
- The STD diff spectra does not have a visible DSS peak, so we can normalize them by setting the highest peak within the aromatic region to 25,000

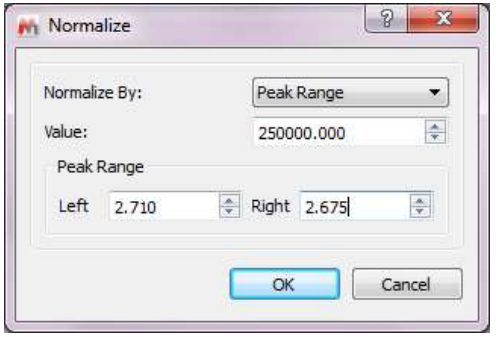


Before you start: Check the normalization

- The T1ρ pair can be normalized based on DMSO peak's areas within 2.71-2.675 ppm. By setting the integral to a proper number (e.g. 250,000), it will also make the Y scale of T1ρ similar to that of the other spectra, so that their peaks are visible in the stacked spectra.



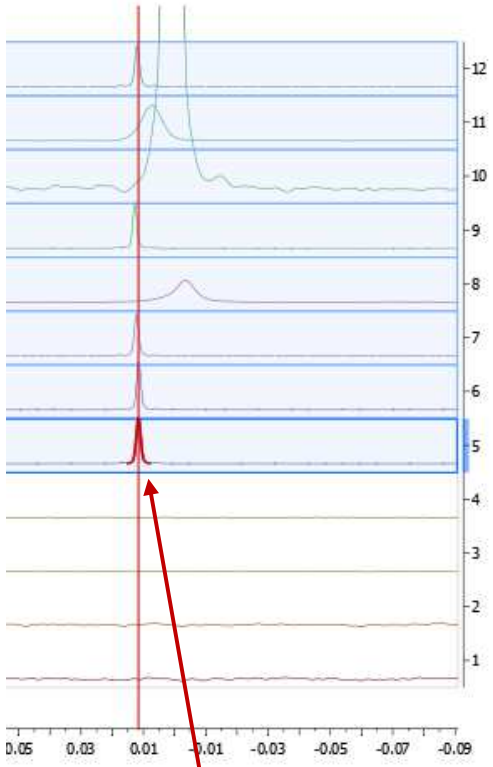


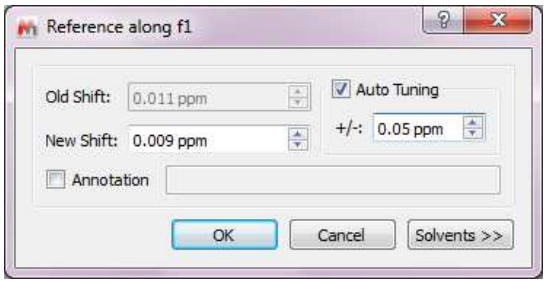


Choose Processing > Normalize, and set the parameters as above

Before you start: Check the spectral alignment

- In this case, the ref. spectra are not properly aligned, and they are not well-aligned with mix. spectra either. By trial and error, it's found that the misalignment can be offset by setting the DSS peaks of the ref spectra to 0.009 ppm





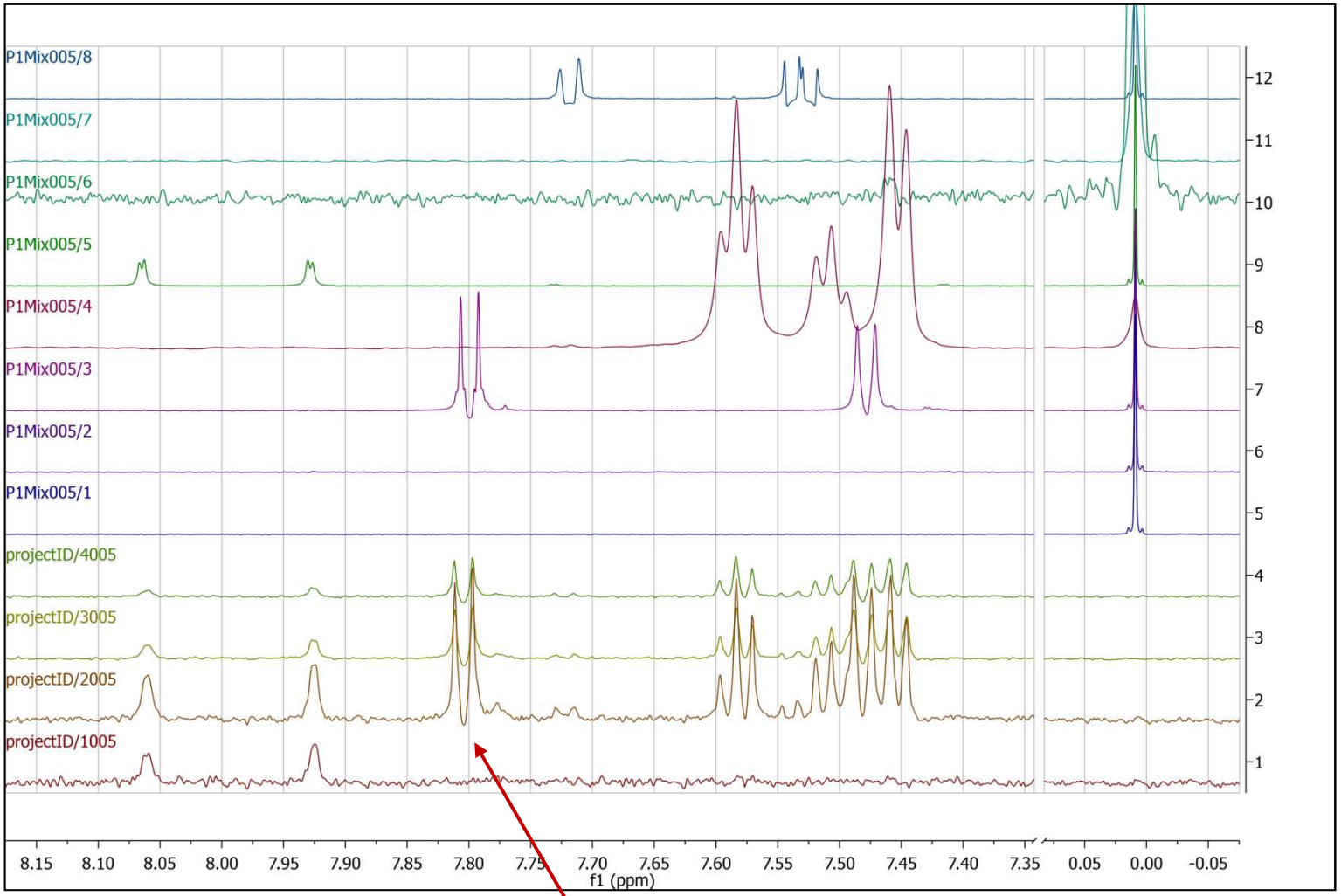
Check the ref. spectra in the Stacked Spectra table so only they will be changed.

Choose the Reference tool , and click on the DSS peak of the active spectrum

Turn on the Auto Tuning option and enter the values as shown above.

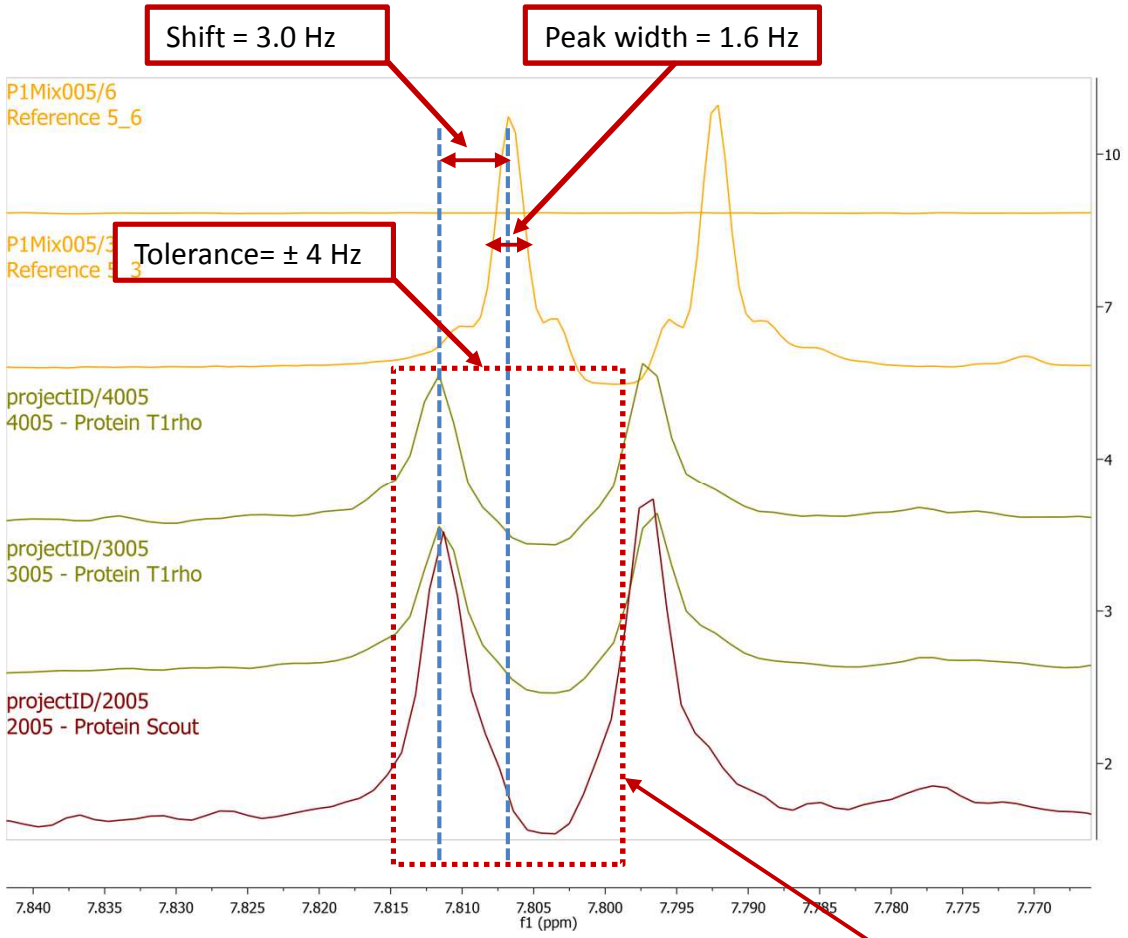
Before you start: Check the spectral alignment

- Most of the peaks are well-aligned now, though local shift of some peaks is inevitable.



A typical local peak mis-alignment that cannot be offset by changing chemical shift reference. Hence a tolerance for peak matching is always needed. See next page for details

Before you start: Estimate peak match tolerance



*Tip: To measure the distance between two peaks, click **C** key for Cross-hair Mode. Next click on a peak top and drag to another one. The distance will be reported in an Info View dialog.*

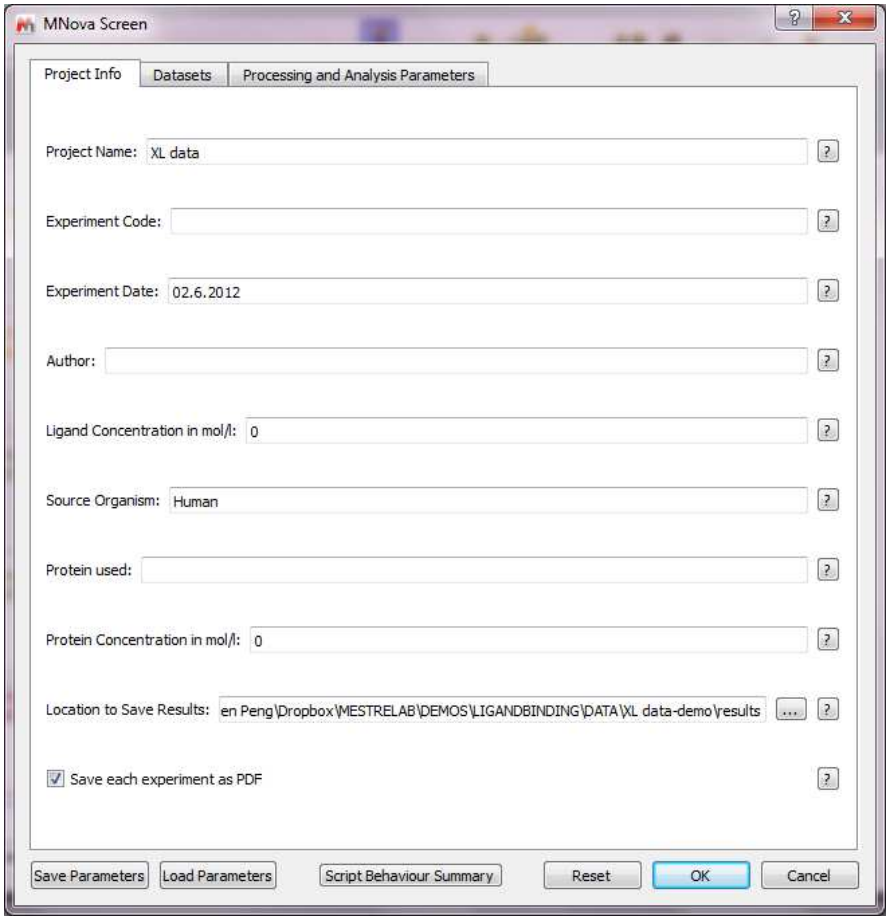
A tolerance of ±2.5 x peak width (=8 Hz for this peak) will be used for matching peaks, or integrating peaks for measuring the change of peak intensity

Setup parameters for Mnova Screen

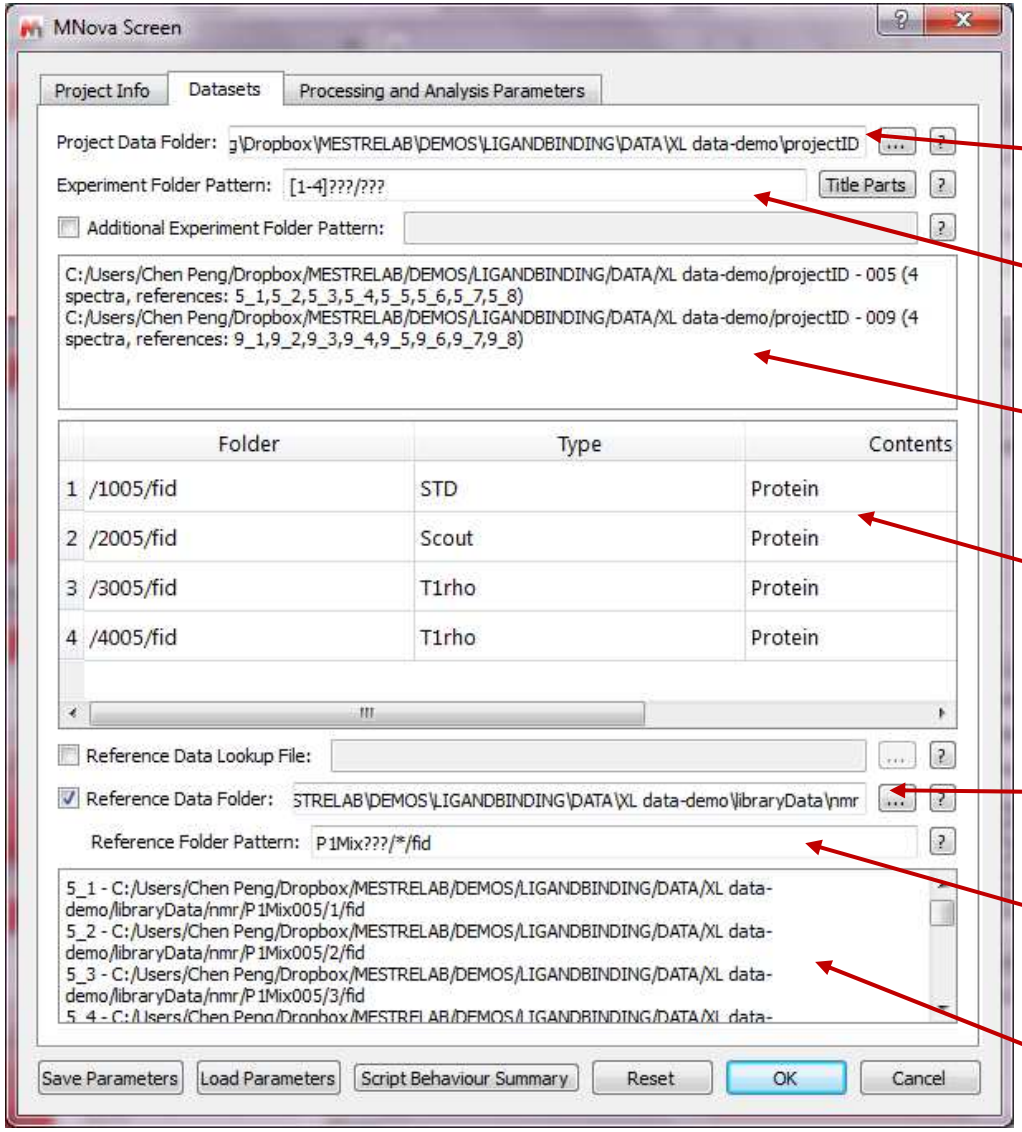
- Mnova Screen allows you to run against a specific mixture, a group of mixtures, or all of your mixtures. It is better to start with one or a few mixtures to optimize your parameters before you start to process all mixtures

- Choose Scripts > Mnova Screen, and fill in the relevant info in the Project Info tab:

Tip: Click the question buttons to see specific instructions



Define your datasets



Parent folder for the mixture data

This will read all folders whose name starts with 1, 2, 3 or 4, and ends with 3 other characters.* The file name is limited to 3 characters (“fid” or “ser”)

The matched mix. data (and ref. data when defined) are summarized here

The matched data files for the first mixture is listed here. You click on the Type cells to define their experimental Types and sample Contents**

Parent folder for the reference data

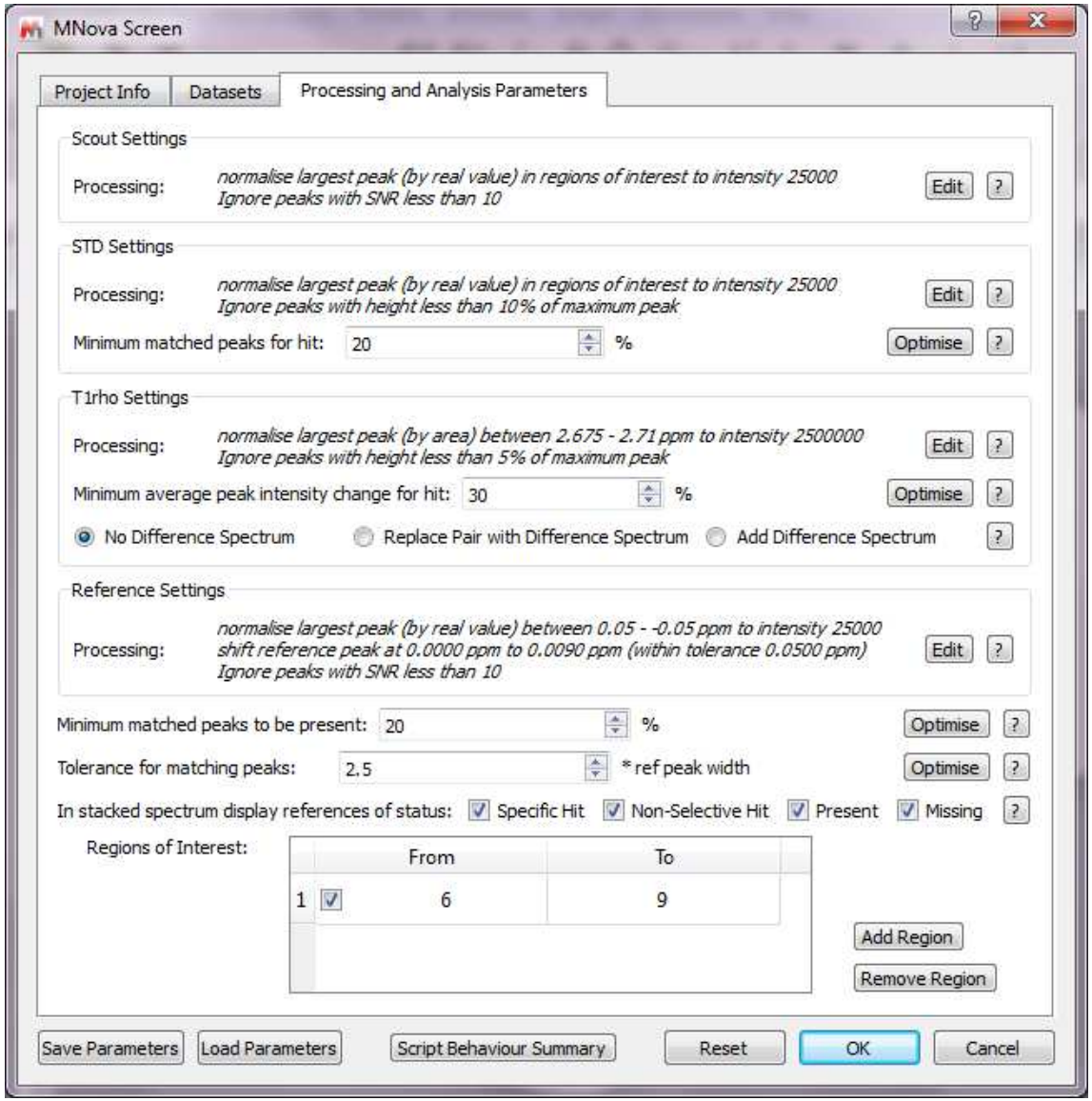
This will read all folders under each folder that that starts with “P1Mix” and ends with a matching number with the mix. folder. “fid” file is searched.

The actual file paths for the ref. spectra are listed here.

*Note: * You can limit the samples you want to run, e.g. use “[1-4]005/???” for one sample, or “[1-4]??5/???” for all samples ending with 5, and so on.*
*** Click on a Type cell to define it as Scout, STD, T1ρ, or wLogsy. Click on a Contents cell to define it as Blank, Protein, or Protein+Inhibitor.*

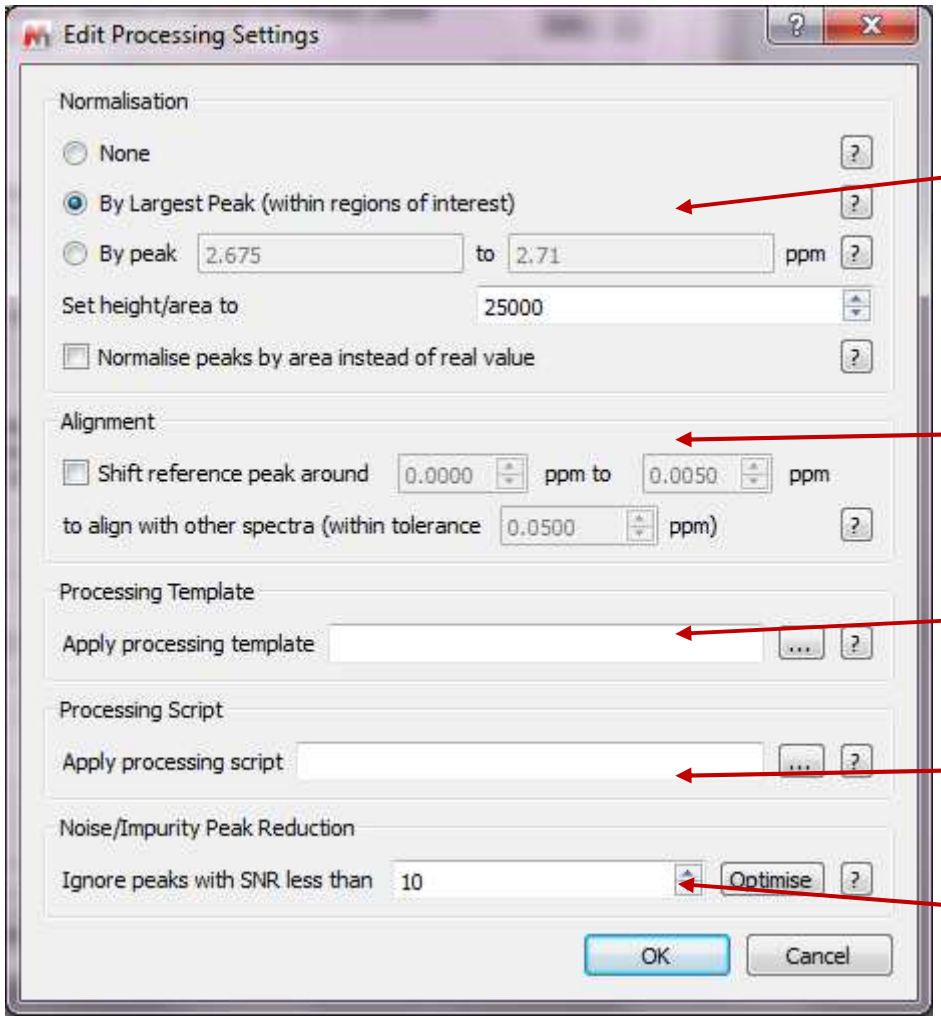
Define processing and analysis parameters

- These parameters are defined based on the previous inspection of the sample spectra.
- See details in the subsequent slides



Processing settings for Scout spectra

- In this case, the scout spectra are used to identify whether a ref compound presents in the mixture or not. Click the Edit button next to open it:



Scout spectra are normalized by setting the highest peak within ROIs to 25,000. This is for visualization only.

No alignment is done for scouts

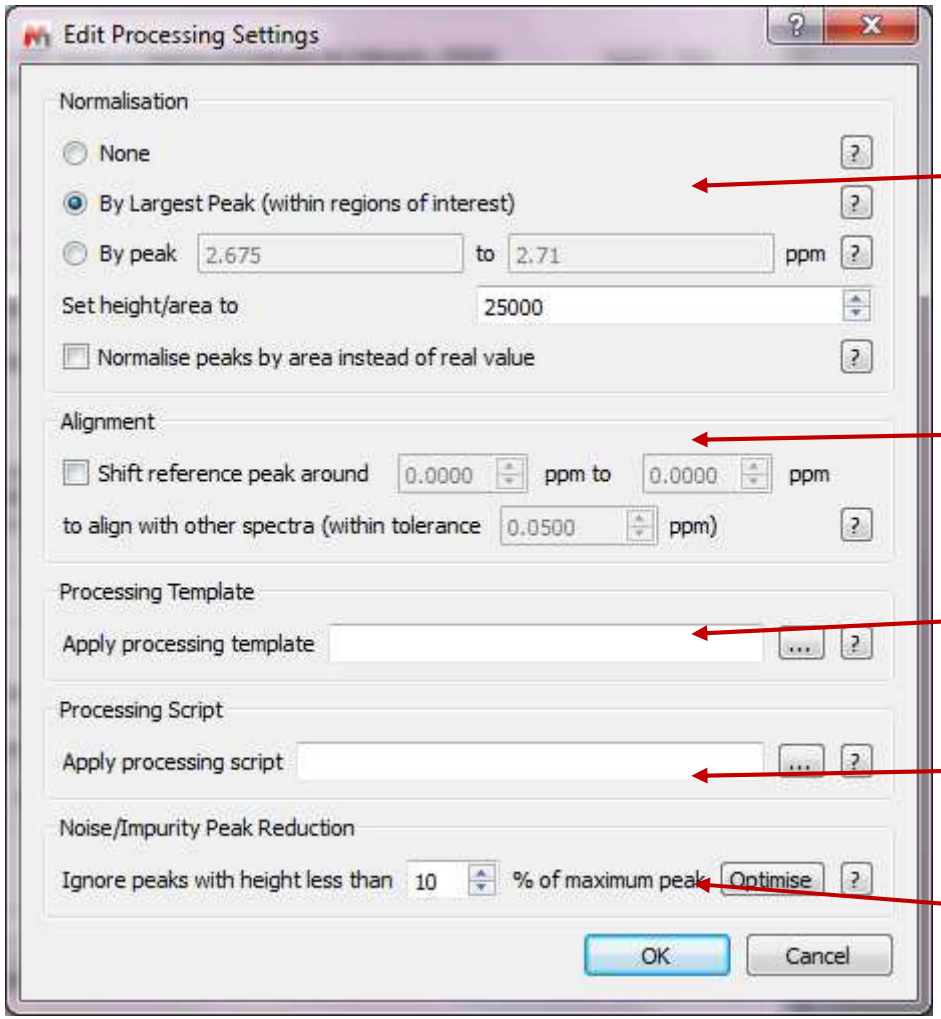
Scout are processed using the original processing parameters, except zero-filling to 64K, and applying Bernstein trinomial baseline correction

No extra processing script is applied

Peaks with S/N Ratio less than 10 will be discarded to reduce noise/impurity peaks.

Processing settings for STD diff spectra

- In this case, STD diff spectra are used to identify primary hits – Screen matches ref peaks with the STD diff peaks and identify primary hits based on the percentage of matched ref peaks



STD diff spectra are normalized by setting the highest peak within ROIs to 25,000 (This is for visualization only)

No alignment is done for STD

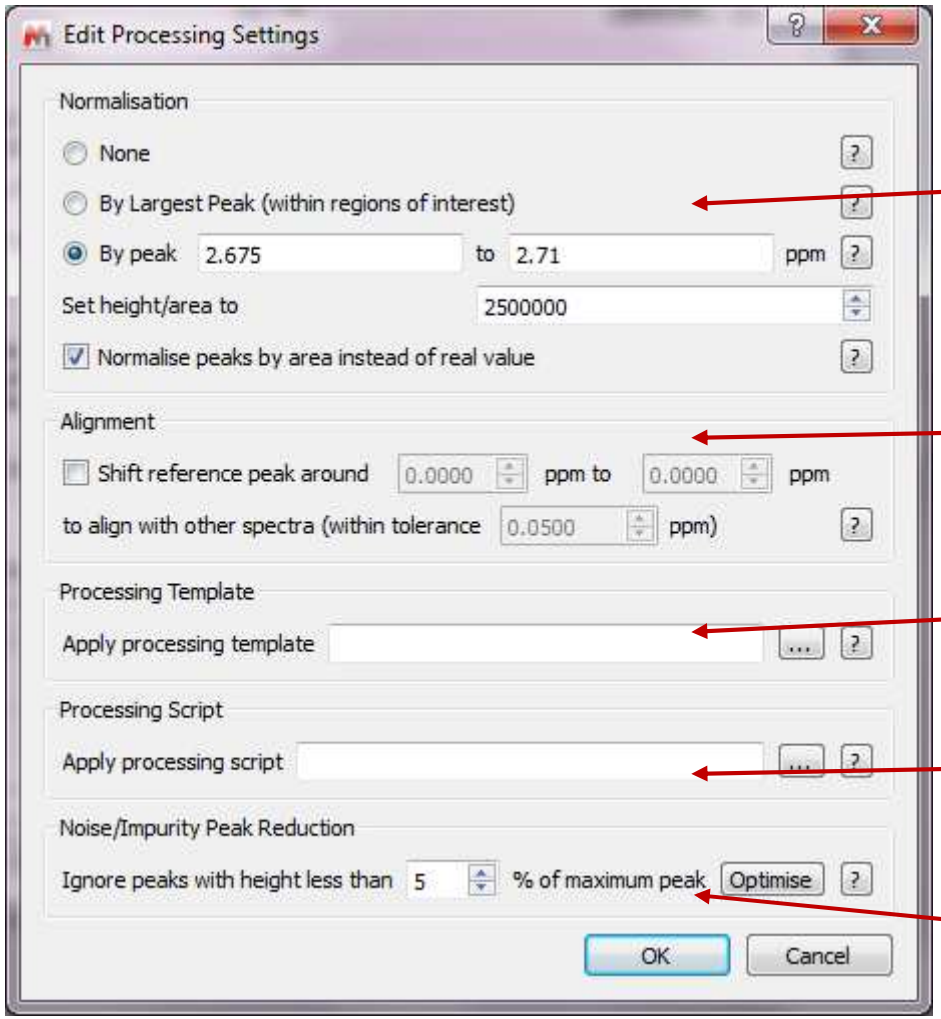
STD are processed using the original processing parameters, except zero-filling to 64K, and applying Bernstein trinomial baseline correction

No extra processing script is applied

STD peaks with height less than 10% of the max. peak in the ROIs are discarded to reduce noise/impurity peaks.

Processing settings for T1ρ spectra

- In this case, T1ρ spectral pairs are used to identify primary hits: Ref peaks are mapped to the T1ρ spectra and the decrease of peak intensity from the short spin-lock T1ρ to the long spin-lock T1ρ is used to identify primary hits



T1ρ spectra are normalized by setting the spectral area within 2.675-2.71 ppm to 2500,000. Note this affects both the analysis results and visualization.

No alignment is done for STD

T1ρ are processed using the original processing parameters, except zero-filling to 64K, and applying Bernstein trinomial baseline correction

No extra processing script is applied

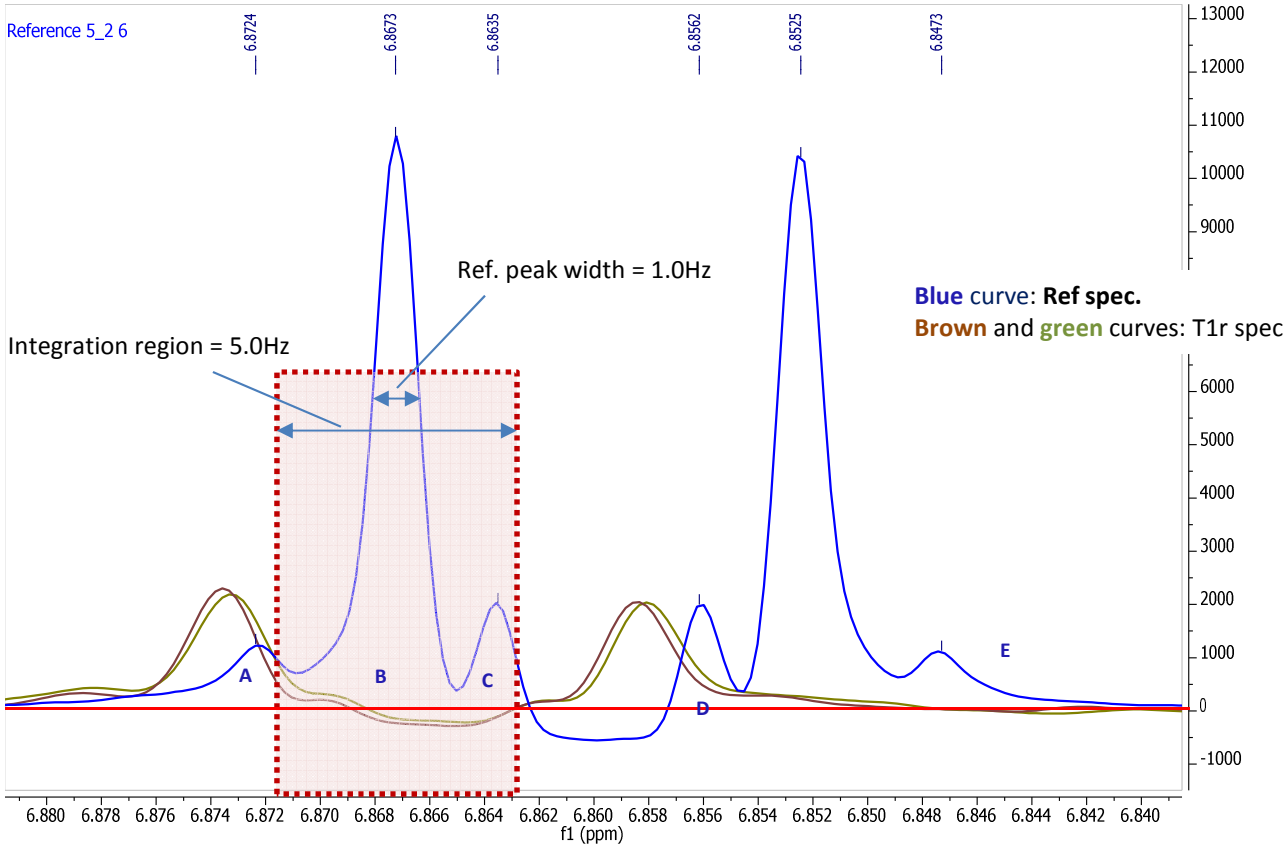
This threshold is used to avoid false positives caused by comparing noise and impurity peaks . See details in the next slide

To avoid false positives caused by local peak shift

- In this case, local peak shift makes Ref Peak B map to a region that contains mostly base points in either T1ρ spectrum (shown as the pink box, with +/- 2.5* ref. peak width). The resulting intensity change can be random and easily causes a false positive.

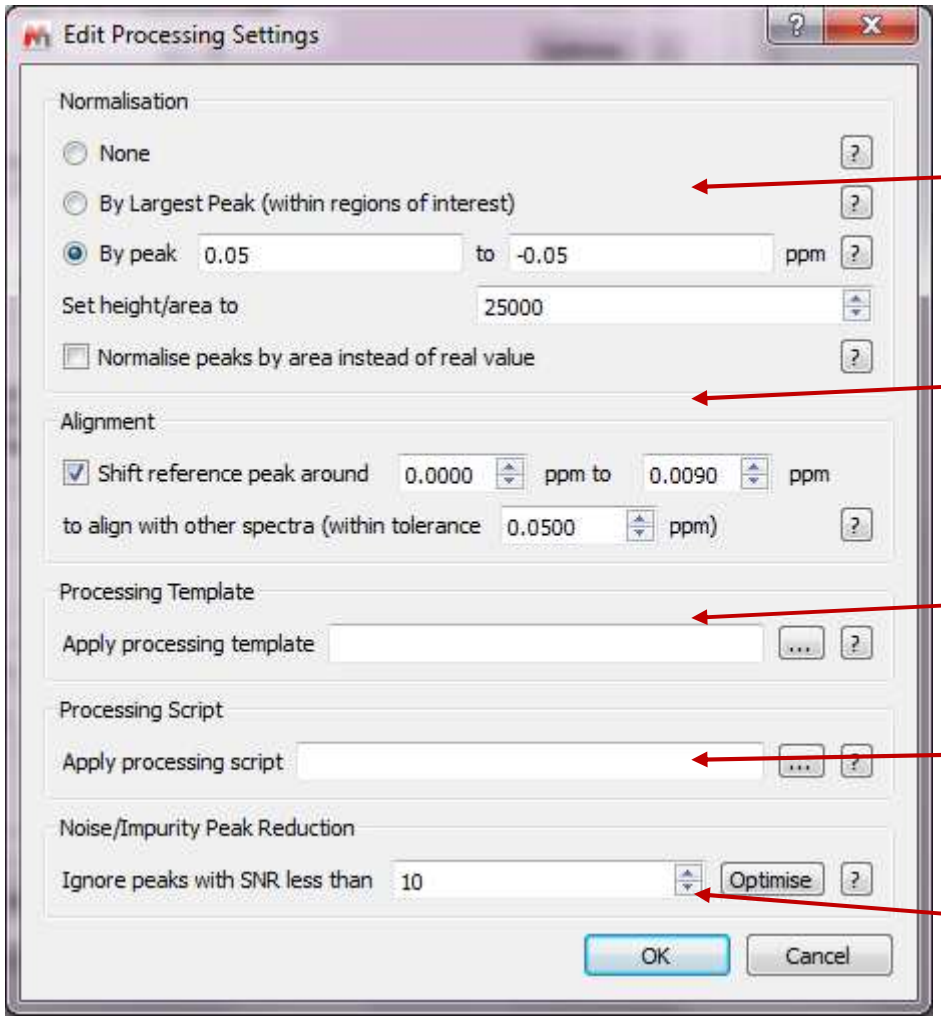
- Our solution is to get the highest intensity within the region for each T1r. Next compare it with the highest intensity within the ROIs. The ratio from at least one T1r must exceed the threshold (e.g. 5%). Otherwise we ignore this ref peak. Note that the T1r with long spinlock may have a peak totally disappears, so we can only require one of them to meet this category.

- The absolute values are used above to cover wLogsy, as peaks can be positive and negative in it.



Processing settings for reference spectra

- It is critical to align ref spectra with mixture ones, as the ref peaks (picked using GSD) are used to match mixture peaks.



Ref spectra are normalized by setting the spectral area within 0.05- -0.05 ppm to 25,000. This is only for visualization.

Ref spectra are aligned with the other mixture spectra by setting their highest (TSS) peak within 0 +/- 0.05 ppm to 0.009 ppm

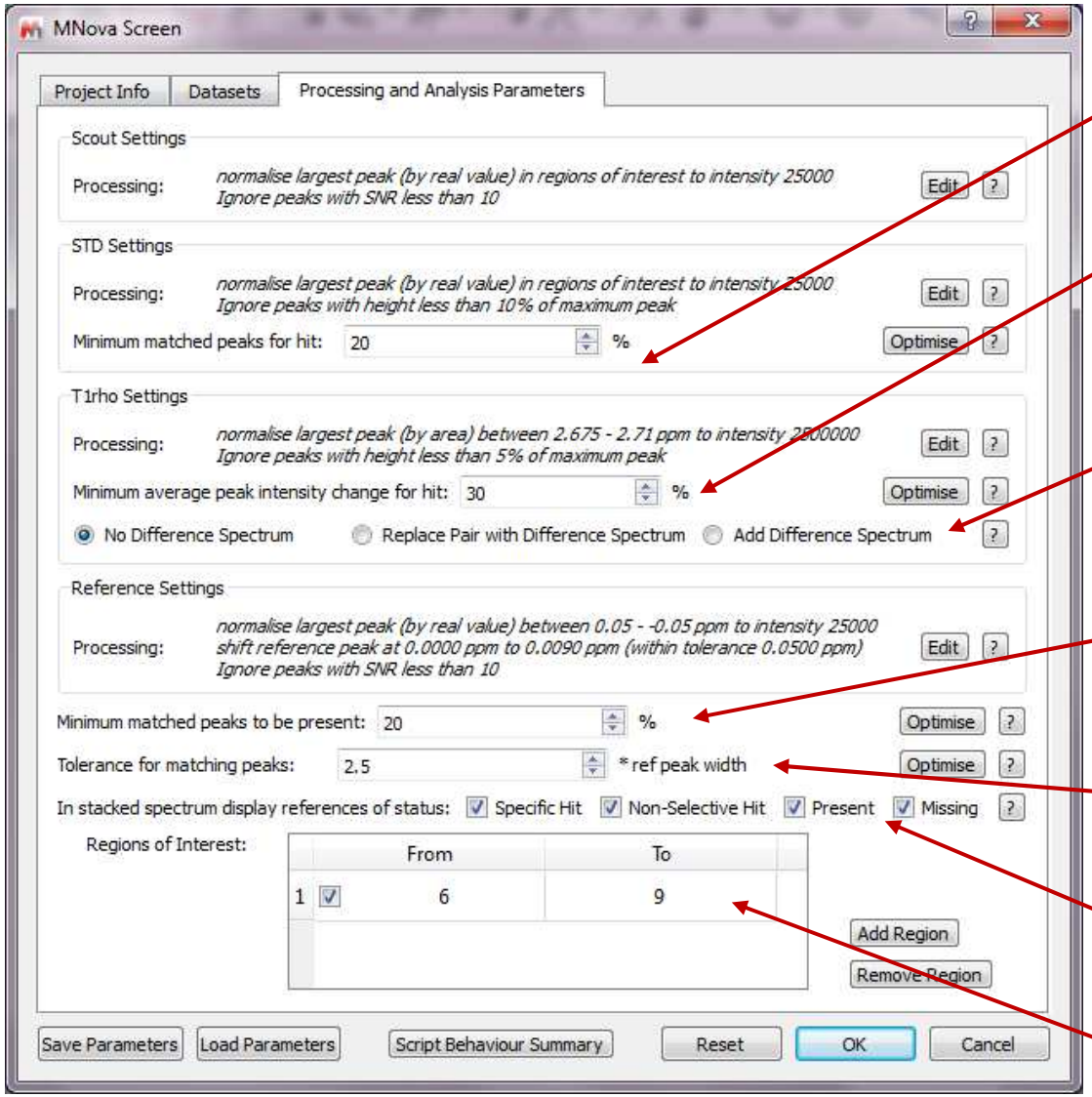
Ref spec are processed using the original processing parameters, except zero-filling to 64K, and applying Bernstein trinomial baseline correction

No extra processing script is applied

Ref peaks with S/N Ratio less than 10 will be discarded to reduce noise/impurity peaks.

Other settings for analysis

- It is critical to align ref spectra with mixture ones, as the ref peaks (picked using GSD) are used to match mixture peaks.



A fragment is considered a primary hit if at least 20% of its ref peaks match STD diff peaks

A fragment is considered a primary hit if the average T1ρ intensity decrease is 30% or more

Options to display difference spectra for T1ρ spectral pairs

A fragment is considered present (in the mixture) if at least 20% of the ref peaks match Scout peaks

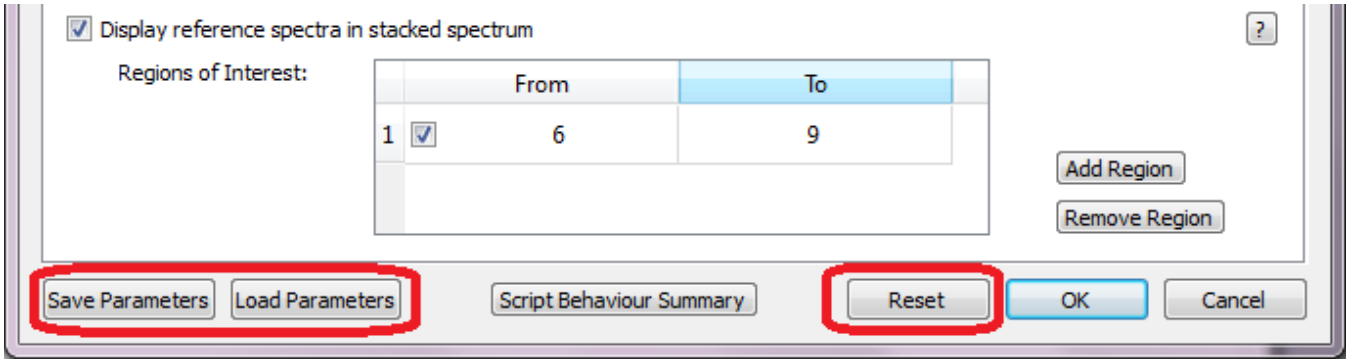
Tolerance for match a ref peak with STD diff peak or T1ρ integration region: +/- 2.5xRef peak width

Which ref spectra to display in Stacked Spectral Table

Region of Interets (ROIs) is set to 6-9 ppm

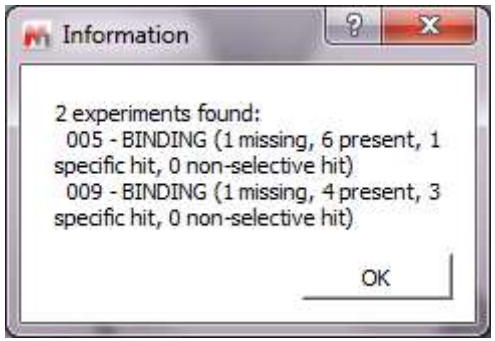
Save, load, or reset your parameters

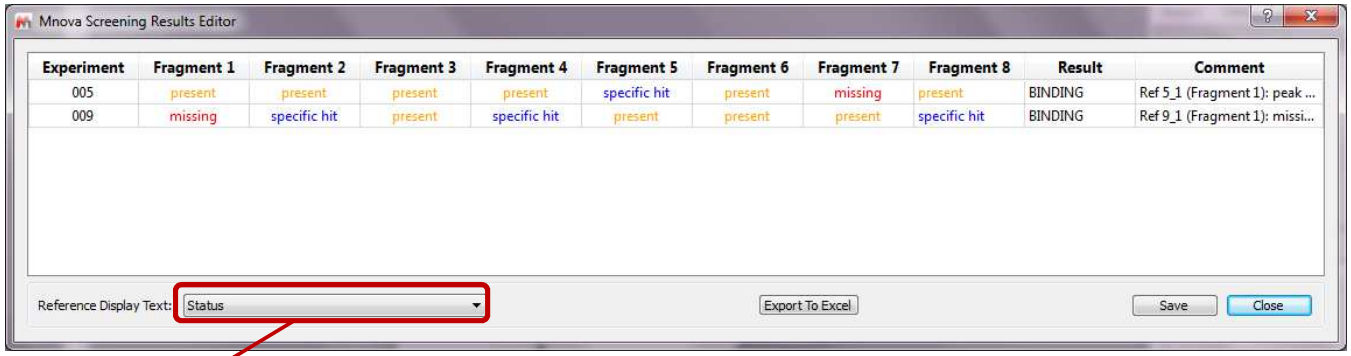
- All the parameters can be saved into a .txt file for later use
- You can load a parameters file to reuse the previous settings
- Some times Mnova Screen gets confused during the parameters setup. Click Reset to remove all the settings.

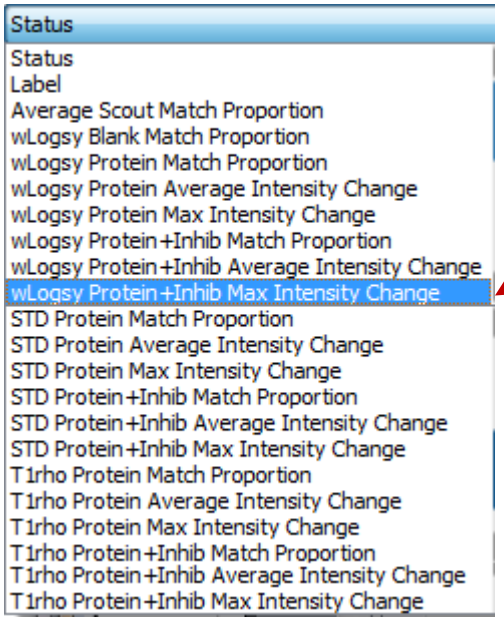


Results from Mnova Screen

- After the completion of a run, Mnova Screen summarizes the results in a dialog
- Upon clicking OK, Screen displays a spreadsheet showing an overview of the results
- Use the Reference Display Text box to choose the details for each type of data







Missing: not enough ref peaks matched the scout peaks, hence the compound is considered missing from the mixture.

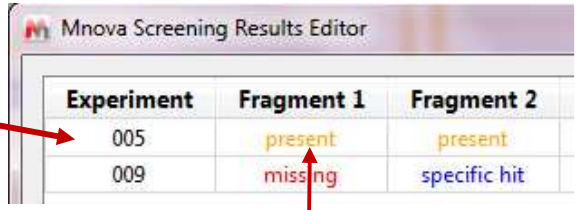
Present: The compound presents, but none of the intensity changes meets the criteria

Non-selective hit: At least one of the intensity changes meets the criteria , but has not passed the competition test (if Protein+Inhibitor spec are used).

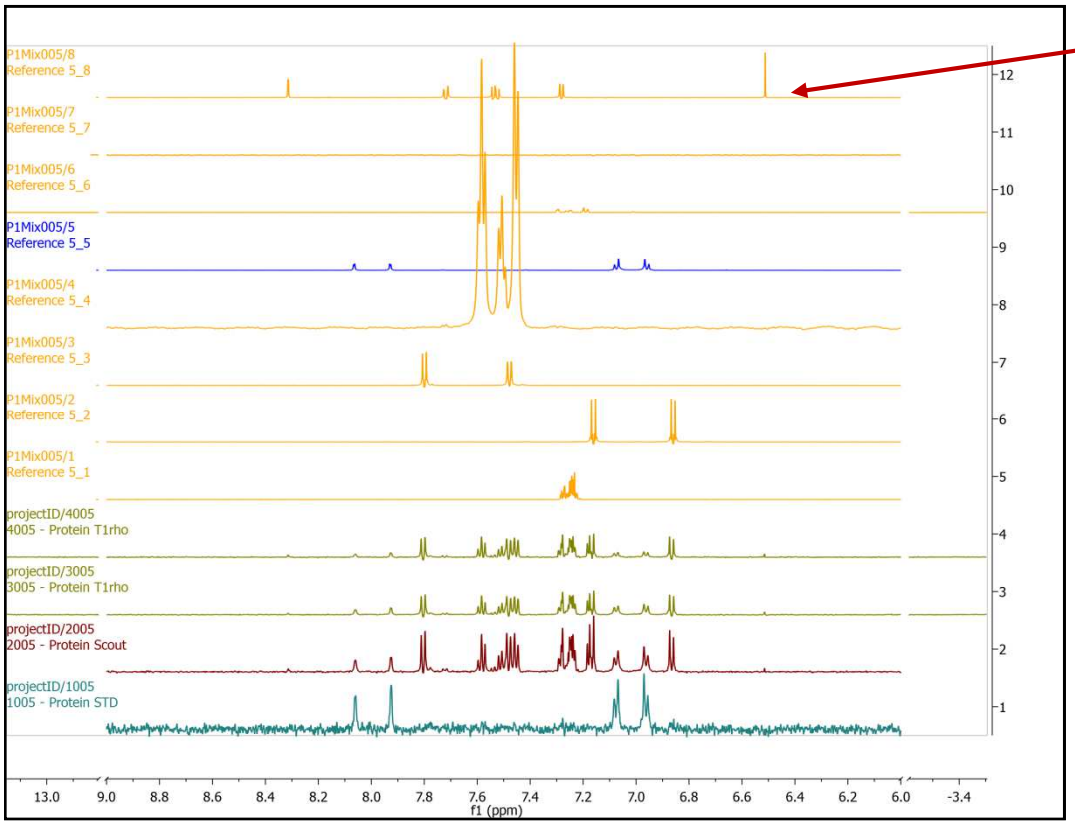
Specific hit: At least one of the intensity changes meets the criteria , and also passed the competition test, if any.

Display stacked spectra in Mnova

- Double click on the row header to open the corresponding .mnova document in Mnova
- You can double click on other cells to change the status, or edit the comments
- On the stacked spectra in Mnova, you can also double click on a ref. spectrum to change its status



Double click on a cell or on the spectrum here to change its status manually

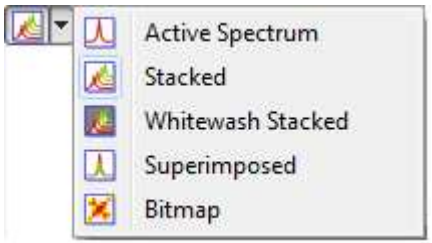


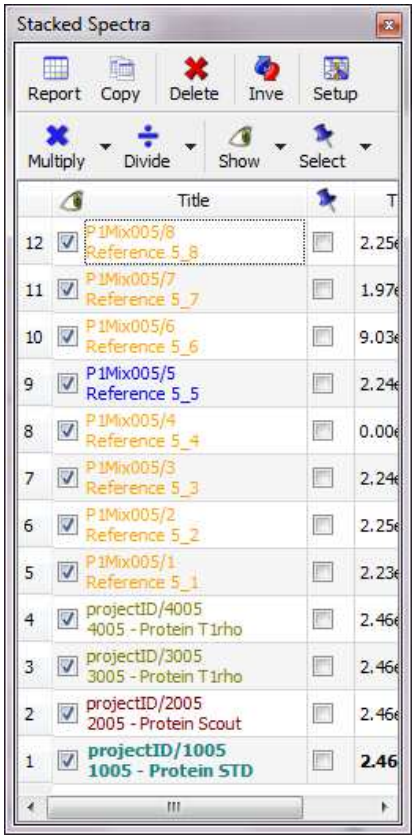
Ref. spectra

Mix. spectra

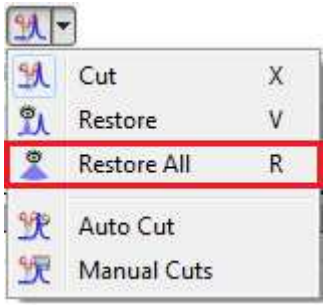
Tools for inspecting the results

- Mnova provides many tools for visualizing stacked spectra conveniently
- Click  to open and dock the Stacked Spectra Table to the right of the Mnova window. Use it to choose which ones to display
- Use the Stacked Tool to switch to Stacked, Superimposed or Active Only mode to compare spectra in different ways



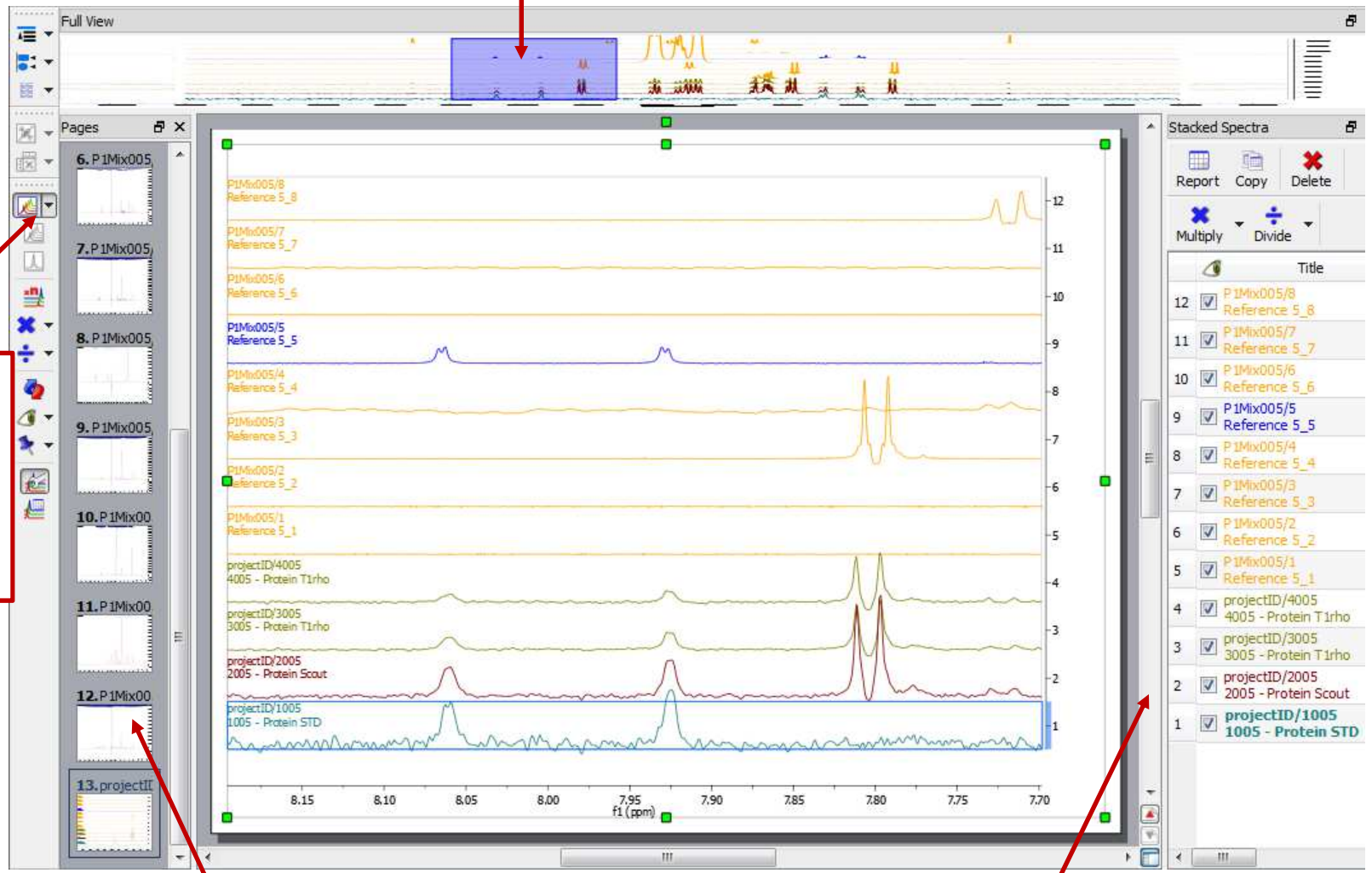


- The log.txt file generated in the Results folder logs many details of the processing and analysis for every spectra.



Tip: Mnova Screen "cuts" the display of the stacked spectra so that only the region of interest is visible. To restore the display of the "cut" regions, click "Restore All" in the Cut options.

Full View: The whole spectrum and zoom-in region. Drag the blue box to see other parts of the spectra. (Choose **View | Full View** to open Full View if not yet)



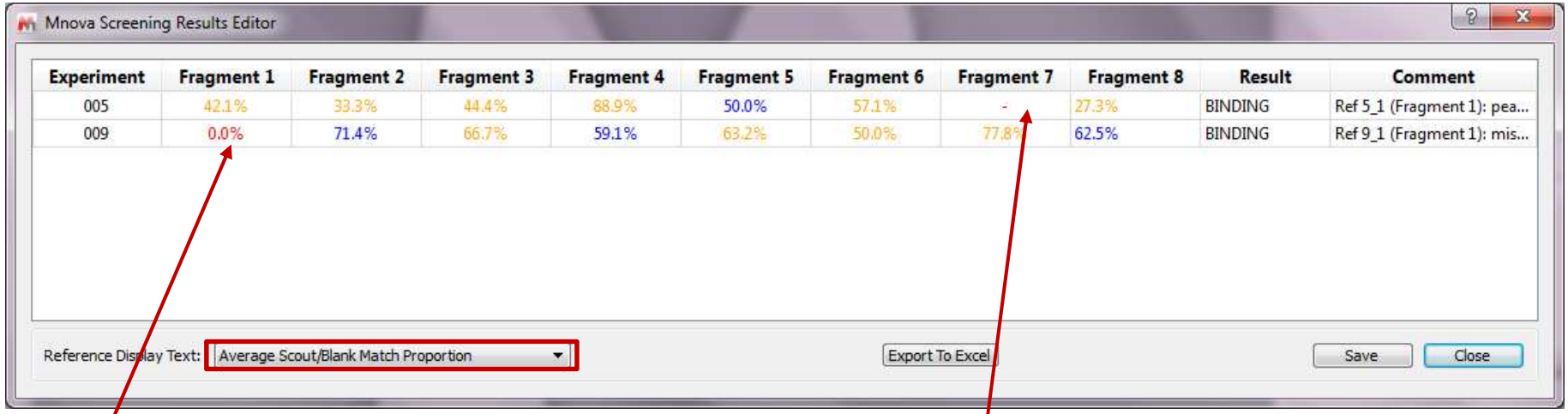
Stacked Mode: choose to display the spectra as Stacked/Superimposed/Active Only

Pages View: scroll to different pages to see the processing and pick picking results for each spectrum

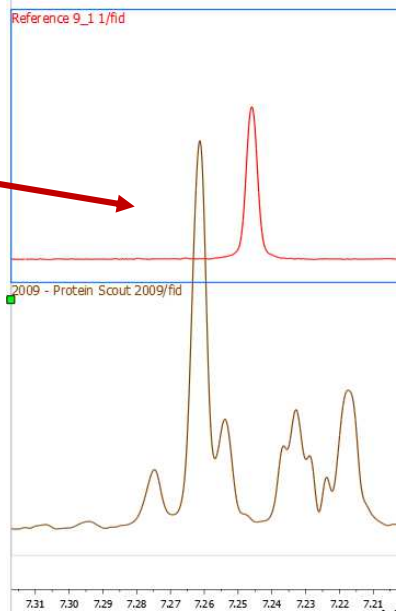
Stacked Spectra Table: Check/uncheck the boxes here to choose which spectra to display

Details of the results: The missing ones

- Choose to display the “Average Scout/Blank Match Proportion” results to see why one of them is taken as missing (red):



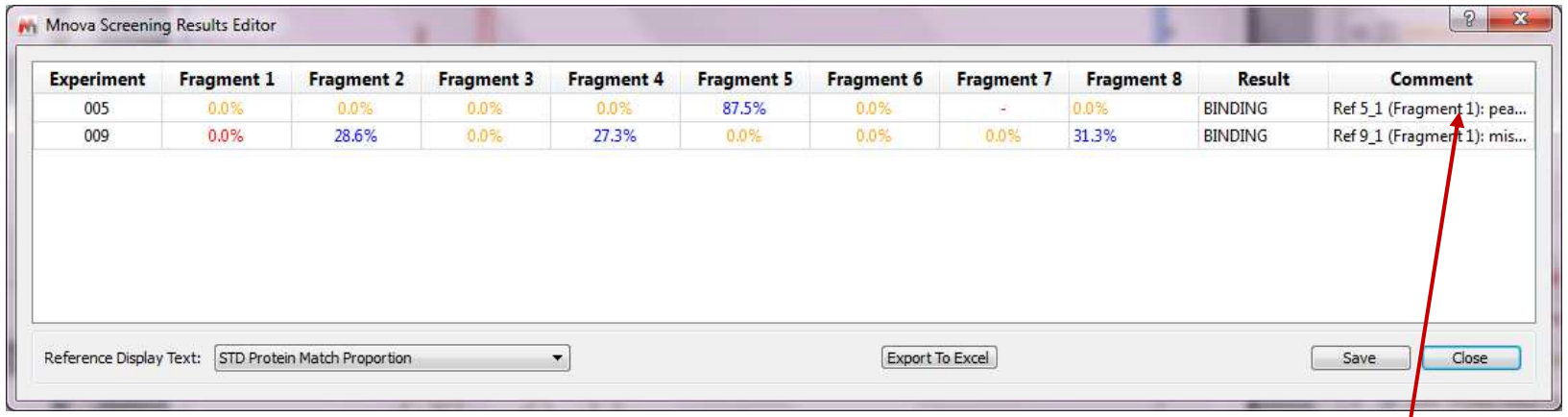
This one is missing as the only peak within ROI does not matches any Scout peaks

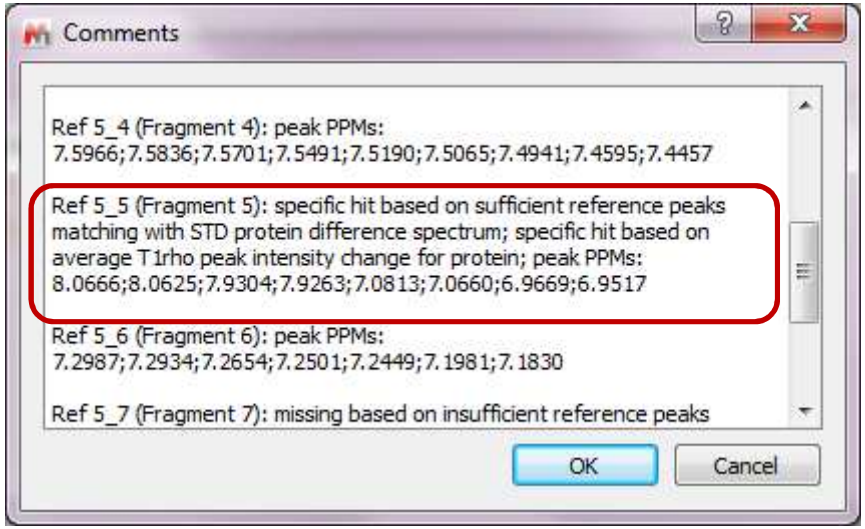


This one is missing as there is no ref peaks within the ROI

Details of the results: Hits from STD

- Choose to display the “STD Protein Match Proportion” results. Fragment 5 of Experiment 005 are taken as a hit because 87.5% of its ref peaks matched with the STD difference peaks (> the threshold of 20%):

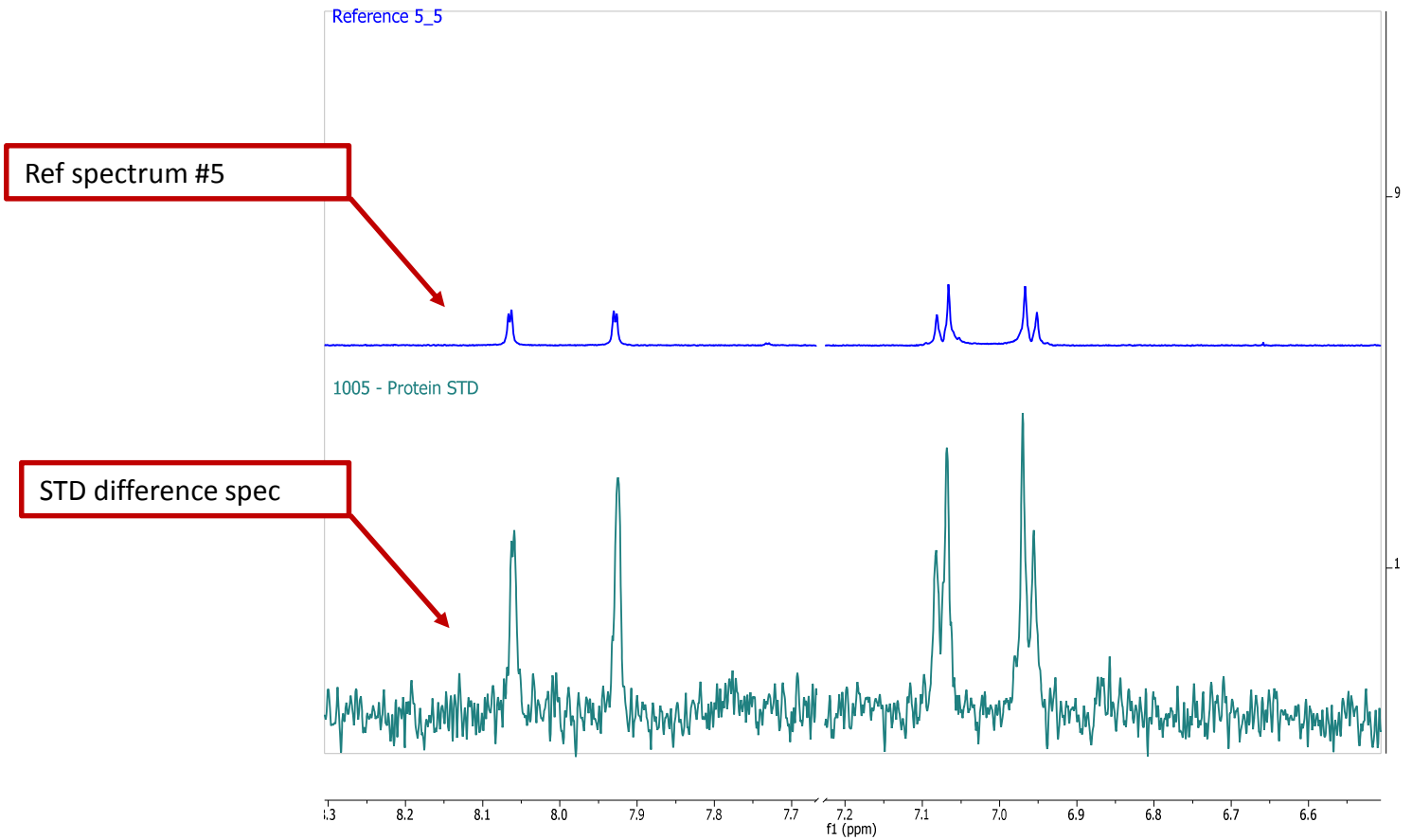




Double click on Comment cell to open the Comments dialog, and you can manually edit the texts here

Details of the results: Hits from STD

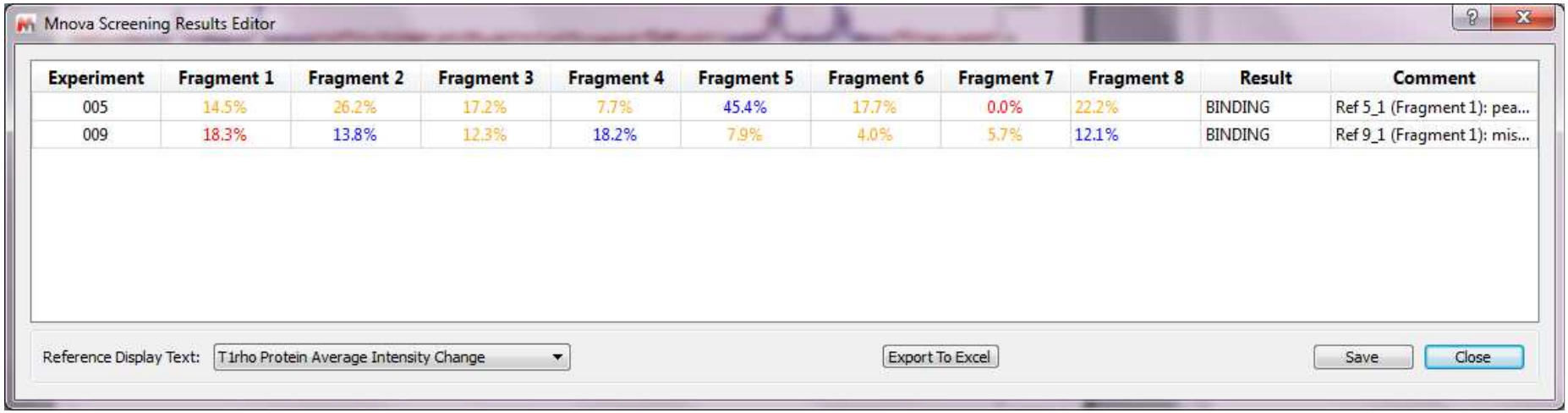
- Use the display tools to check the STD difference and the ref spec #5



Tip: Scroll to the pages containing the single ref. or STD diff spectra to see the GSD peak picking results. In the Superimposed or Active Only mode, you can also see the peak labels of the active spectrum in the stacked spectra. The details of peak matching analysis can be found in the log.txt file in the result folder.

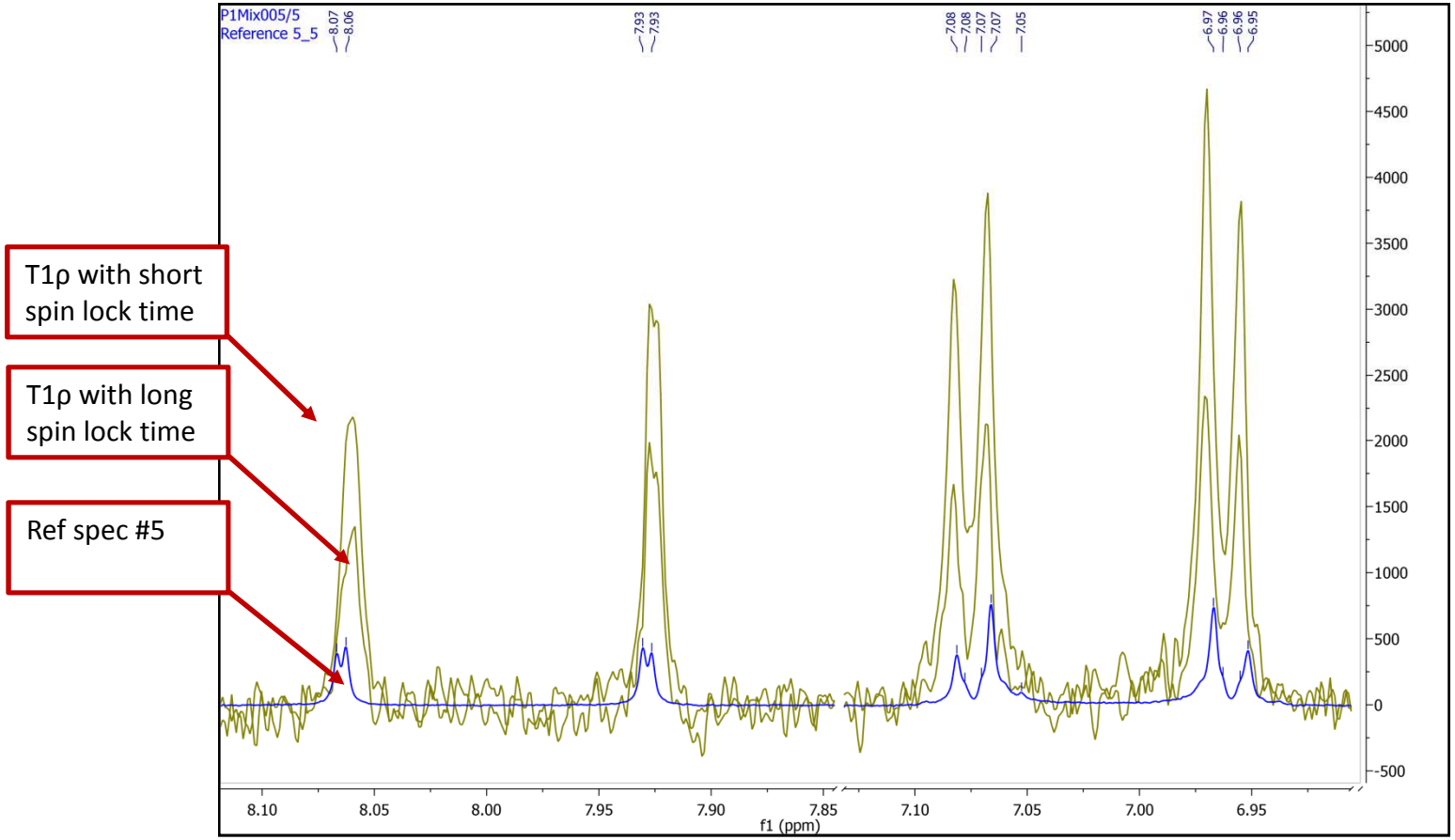
Details of the results: Hits from T1ρ

- Choose to display the “T1rho Protein Average Intensity Change” results. Fragment 5 of Experiment 005 are taken as a hit because its peaks have an average intensity changes of 45.4% (> threshold of 30%):



Details of the results: Hits from T1ρ

- Use the display tools to check the T1ρ pair and the Ref #5



Tip: In the Superimposed or Active Only mode, you can also see the peak labels of the active spectrum (as shown above). The details of peak matching analysis can be found in the log.txt file in the result folder.

Log.txt: Details of the analysis

- The log.txt file logs all the details of spectral processing and analysis. Use a text editor such as Notepad++ to view it.

```
297 Processing reference 5_5 (Fragment 5)..
298 8 peaks found over threshold snr 10 (not including negative peaks) for Reference 5_5 - [
299     8.07ppm:intensity=2439.59,realVal=2571.49,
300     8.06ppm:intensity=2723.38,realVal=2831.62,
301     7.93ppm:intensity=2759.97,realVal=2830.31,
302     7.93ppm:intensity=2393.38,realVal=2530.43,
303     7.08ppm:intensity=2471.07,realVal=2488.81,
304     7.07ppm:intensity=5102.49,realVal=4972.08,
305     6.97ppm:intensity=4834.10,realVal=4854.85,
306     6.95ppm:intensity=2619.42,realVal=2679.31]
307 4 peaks in 5_5 matched out of 8 against scout[Protein]:
308     source ppm=7.93-->target ppm=7.93
309     source ppm=7.07-->target ppm=7.07
310     source ppm=6.97-->target ppm=6.97
311     source ppm=6.95-->target ppm=6.96
312 7 peaks in 5_5 matched out of 8 against STD Protein (re-using target peaks):
313     source ppm=8.07-->target ppm=8.06
314     source ppm=8.06-->target ppm=8.06
315     source ppm=7.93-->target ppm=7.93
316     source ppm=7.93-->target ppm=7.93
317     source ppm=7.07-->target ppm=7.07
318     source ppm=6.97-->target ppm=6.97
319     source ppm=6.95-->target ppm=6.96
320 use threshold peak height: 10% of 24980.744140625 from STD Protein = 2498.0744140625
321 Calculating relative intensities of peak sets for Protein T1rho
322 use threshold peak height: 5% of 26911.482421875 from T1rho Protein = 1345.57412109375
323 Relative intensities of peak sets using standard SUM method: [
324     ref PPM=8.0666, range=8.0578-8.0754, I=0.40765
325     ref PPM=8.0625, range=8.0553-8.0697, I=0.42468
326     ref PPM=7.9304, range=7.9219-7.9389, I=0.39886
327     ref PPM=7.9263, range=7.9192-7.9333, I=0.38482
328     ref PPM=7.0813, range=7.0707-7.0918, I=0.51242
329     ref PPM=7.0660, range=7.0567-7.0754, I=0.50108
330     ref PPM=6.9669, range=6.9564-6.9774, I=0.48468
331     ref PPM=6.9517, range=6.9409-6.9626, I=0.51915]
332 Average intensity change % for 5_5 and T1rho spectra = 45.4%
333 Maximum intensity change % for 5_5 and T1rho spectra = 51.9%
334 Status of ref 5_5 (Fragment 5) = specific hit
```

Details of the GSD peaks from the ref spec 5_5

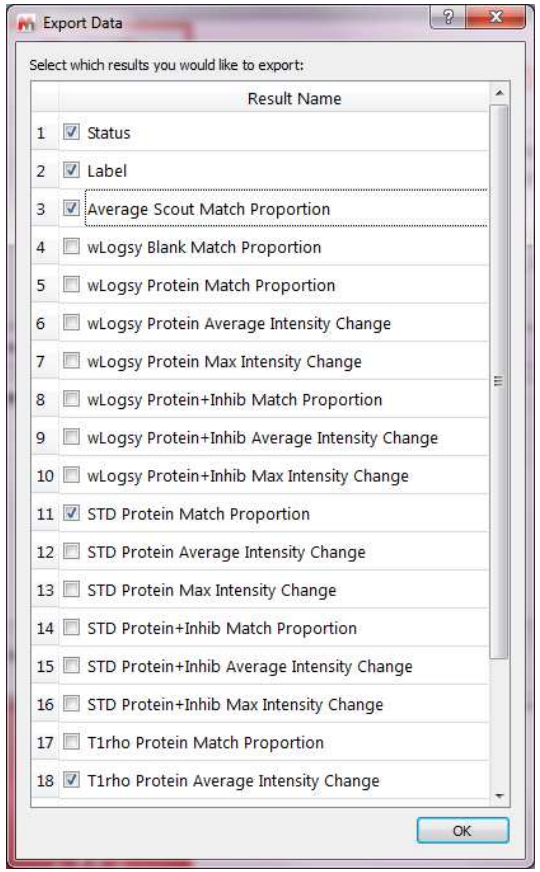
Details of the match of ref peaks with the scout peaks

Details of the match of the ref peaks with the STD diff peaks

Details of the calculation of peak intensity changes in T1ρ. For example, for ref peak at 8.0666 ppm, sum-integration is done within 8.0576-8.0754 ppm, and the decrease of spectral integration is 40.765%

Export results to a spreadsheet

- After examining and revising the results, you can export the results to a spreadsheet by clicking the Export To Excel button, and then choosing the results that you want to export:

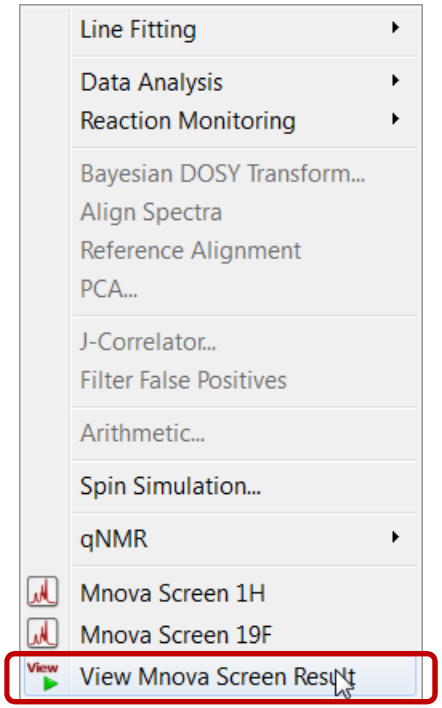


	A	B	C	D	E	F	G	H	I
1	Experiment	Reference	Status	Label	Ref Peak Count	Average Scout/Blank Match Proportion	STD Protein Match Proportion	T1rho Protein Average Intensity Change	T1rho Protein Max Intensity Change
2	5	5_1	present	5_1	19	42.10%	0.00%	14.50%	43.40%
3	5	5_2	present	5_2	12	33.30%	0.00%	26.20%	81.80%
4	5	5_3	present	5_3	9	44.40%	0.00%	17.20%	33.60%
5	5	5_4	present	5_4	9	88.90%	0.00%	7.70%	19.50%
6	5	5_5	specific hit	5_5	8	50.00%	87.50%	45.40%	51.90%
7	5	5_6	present	5_6	7	57.10%	0.00%	17.70%	45.10%
8	5	5_7	missing	5_7	0	-	-	0.00%	0.00%
9	5	5_8	present	5_8	11	27.30%	0.00%	22.20%	52.20%
10	9	9_1	missing	9_1	1	0.00%	0.00%	18.30%	18.30%
11	9	9_2	specific hit	9_2	7	71.40%	28.60%	13.80%	24.90%
12	9	9_3	present	9_3	12	66.70%	0.00%	12.30%	31.80%
13	9	9_4	specific hit	9_4	22	59.10%	27.30%	18.20%	28.40%
14	9	9_5	present	9_5	19	63.20%	0.00%	7.90%	30.90%
15	9	9_6	present	9_6	8	50.00%	0.00%	4.00%	6.60%
16	9	9_7	present	9_7	9	77.80%	0.00%	5.70%	22.40%
17	9	9_8	specific hit	9_8	32	62.50%	31.30%	12.10%	43.10%

Save and reload the results

- After examining and revising the results, you can click the Save button to save the results before closing the Editor.

- If you want to edit the previous results again, choose Advanced > View Mnova Screen Results, and open the editor.csv file saved in the Results folder.



Thank you for your time!