



Mestrelab Research

chemistry software solutions

**Mnova Software Tools for  
Fragment-Based Drug Discovery**

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## Agenda

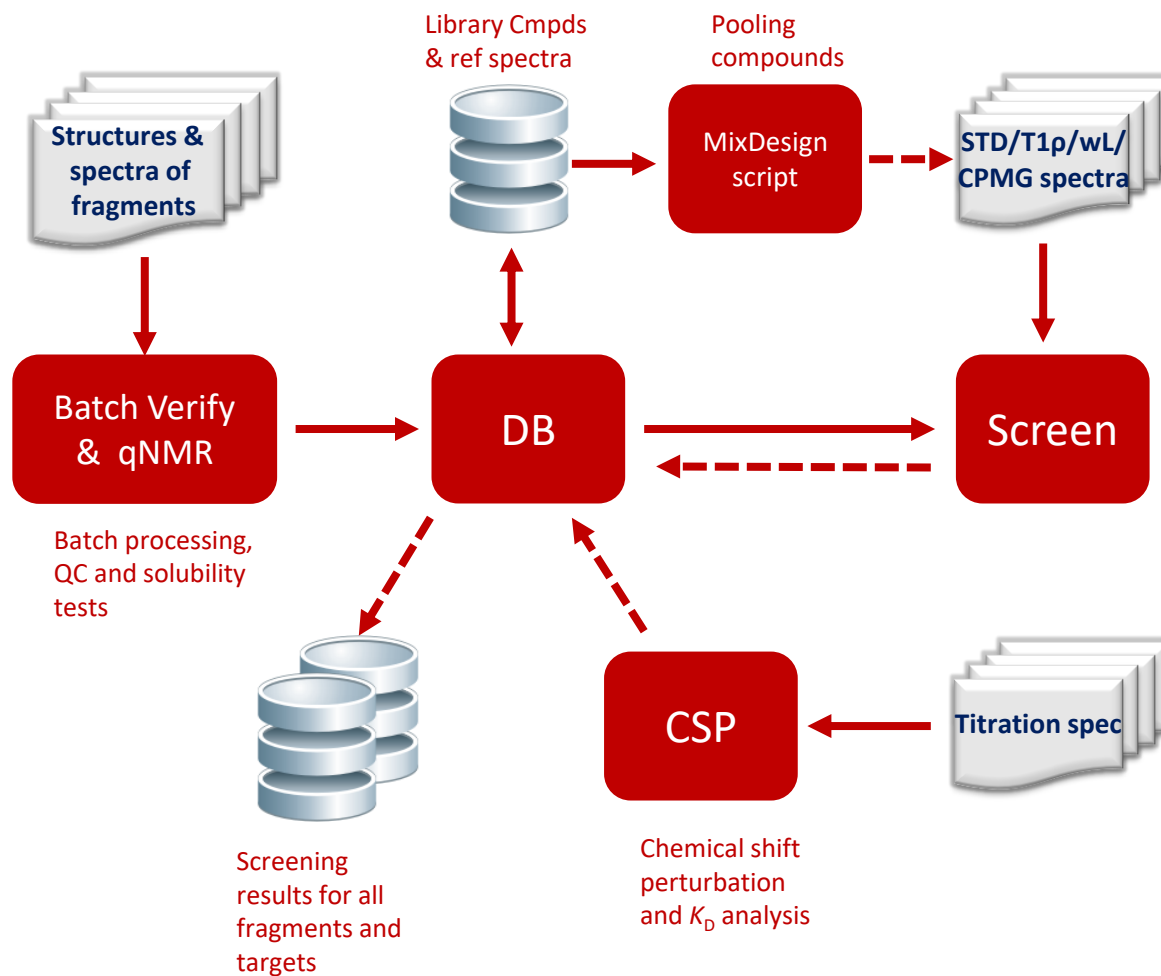
- Brief intro on fragment-based drug discovery (FBDD)
- The relevant Mnova software tools for
  - QC and solubility test of library compounds and building the reference spectra database.
  - Pooling of compounds with least peak overlap.
  - Batch analysis of 1D ligand-observed screening spectra.
  - Analysis of 2D chemical shift perturbation spectra.
- Demo
- Questions



 novating for our customers

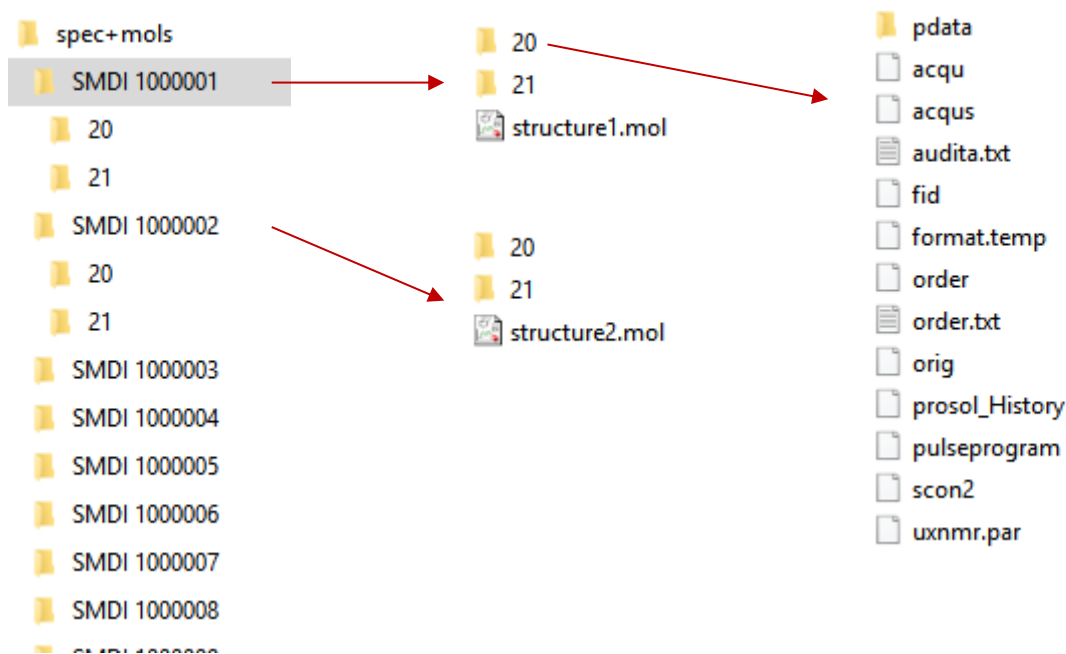
- ❑ NMR has been widely used for high-throughput or detailed hit finding and hit validation since mid-1990s
  - ❑ Ideally suited for detecting ligand-protein bindings with  $K_d$  in  $\mu\text{mol}$ - $\text{mmol}$  range.
  - ❑ “In-built” quality control: structure consistency check, concentration measurement, and binding assessment all from the same sample.
- ❑ Ligand-observed NMR binding spectra: commonly used for primary fragment screening, no labeling needed, no size restriction by receptor,  $^1\text{H}$  or  $^{19}\text{F}$ 
  - ❑ STD (Saturation transfer difference exp.)
  - ❑ T1 $\rho$  (Relaxation-edited exp.)
  - ❑ CPMG (Relaxation-edited exp.)
  - ❑ WaterLOGSY (Water-ligand observed via gradient spectroscopy)
- ❑ Protein-observed chemical shift perturbation spectra: Residue-specific info, mapping to binding site on protein,  $K_d$  measurement, SAR-by-NMR etc.
  - ❑  $^{15}\text{N}$  or  $^{13}\text{C}$  labeled HSQC spectra of protein.

- ❑ A typical mid-size compound library: 500-2000 compounds
- ❑  $^1\text{H}$  detected experiments:
  - ❑ Primary screening: 6-12 fragments with min. peak overlap per sample => 50-300 samples per library => primary hits in a few days.
  - ❑ Confirmation of hits: single compound samples.
- ❑  $^{19}\text{F}$  detected experiments: high sensitivity (low- $\mu\text{m}$  concen.), simple spectra, large  $\delta^{19}\text{F}$  range
  - ❑ Mixtures of 10-30 cmpds per sample => 20-50 samples per library.
- ❑  $^1\text{H}$ - $^{15}\text{N}$  or  $^1\text{H}$ - $^{13}\text{C}$  HSQC of target protein
  - ❑ One spectrum for each ligand to compare with the reference spectrum.
  - ❑ Or 6-10 titration points per ligand for titration analysis.
- ❑ **Hundreds or thousands of spectra to process and analyze: a bottleneck.**



## Quality control and databasing of library compounds

- The example here has a dataset organized shown below.  
Note your data does not have to be exactly like this.



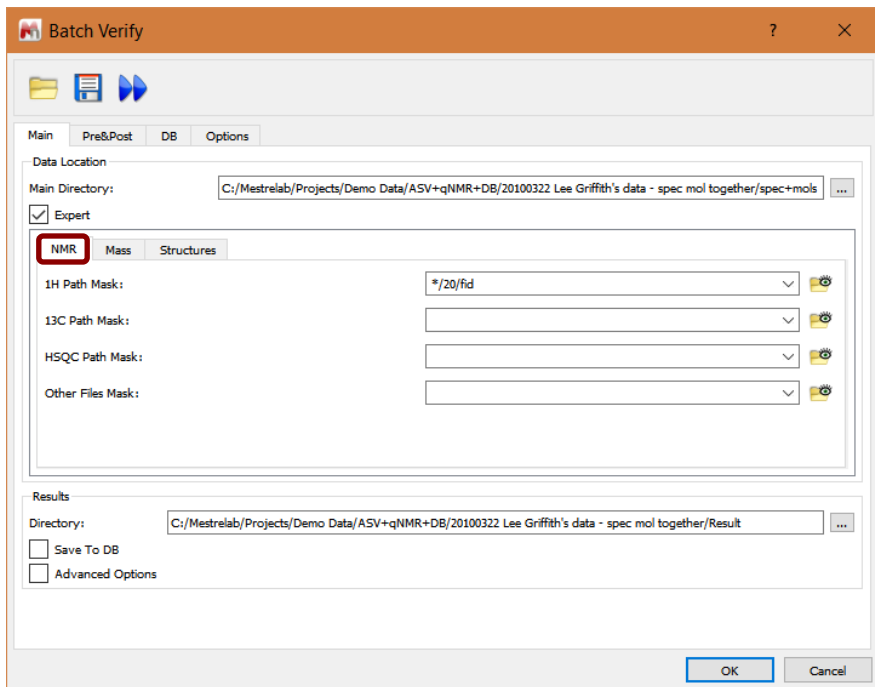
Multiple datasets located under a parent directory “spec+mols”

Each dataset has a H-1 (20), HSQC (21), and a molecule file .mol

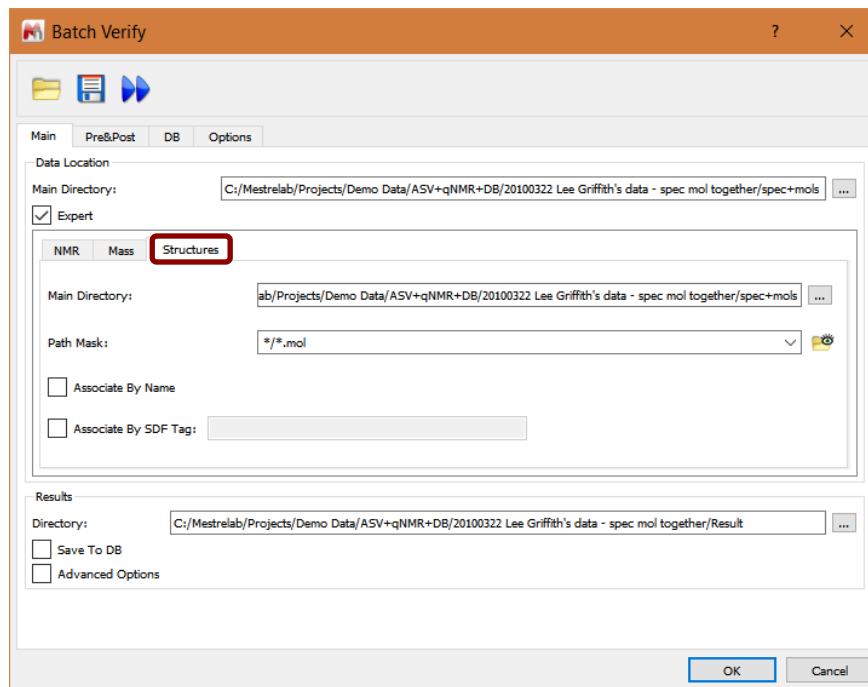
Each H-1 has the typical Bruker files. We will reprocess using the *fid* files

Once you are ready, start Batch Verify by choosing Analysis | Verification | Batch Verify. The sample data mentioned previously is used as an example here.

In the Main tab, setup the NMR and structure files to use and the Results folder etc.



The screenshot shows the 'Batch Verify' dialog box in the 'Main' tab. The 'Data Location' section has 'Main Directory' set to 'C:/Mestrelab/Projects/Demo Data/ASV+qNMR+DB/20100322 Lee Griffith's data - spec mol together/spec+mols'. The 'Expert' checkbox is checked. The 'NMR' tab is selected and highlighted with a red box. Below it, there are four path mask fields: '1H Path Mask' with the value '\*f20/fid', '13C Path Mask', 'HSQC Path Mask', and 'Other Files Mask'. The 'Results' section has 'Directory' set to 'C:/Mestrelab/Projects/Demo Data/ASV+qNMR+DB/20100322 Lee Griffith's data - spec mol together/Result'. There are 'OK' and 'Cancel' buttons at the bottom.

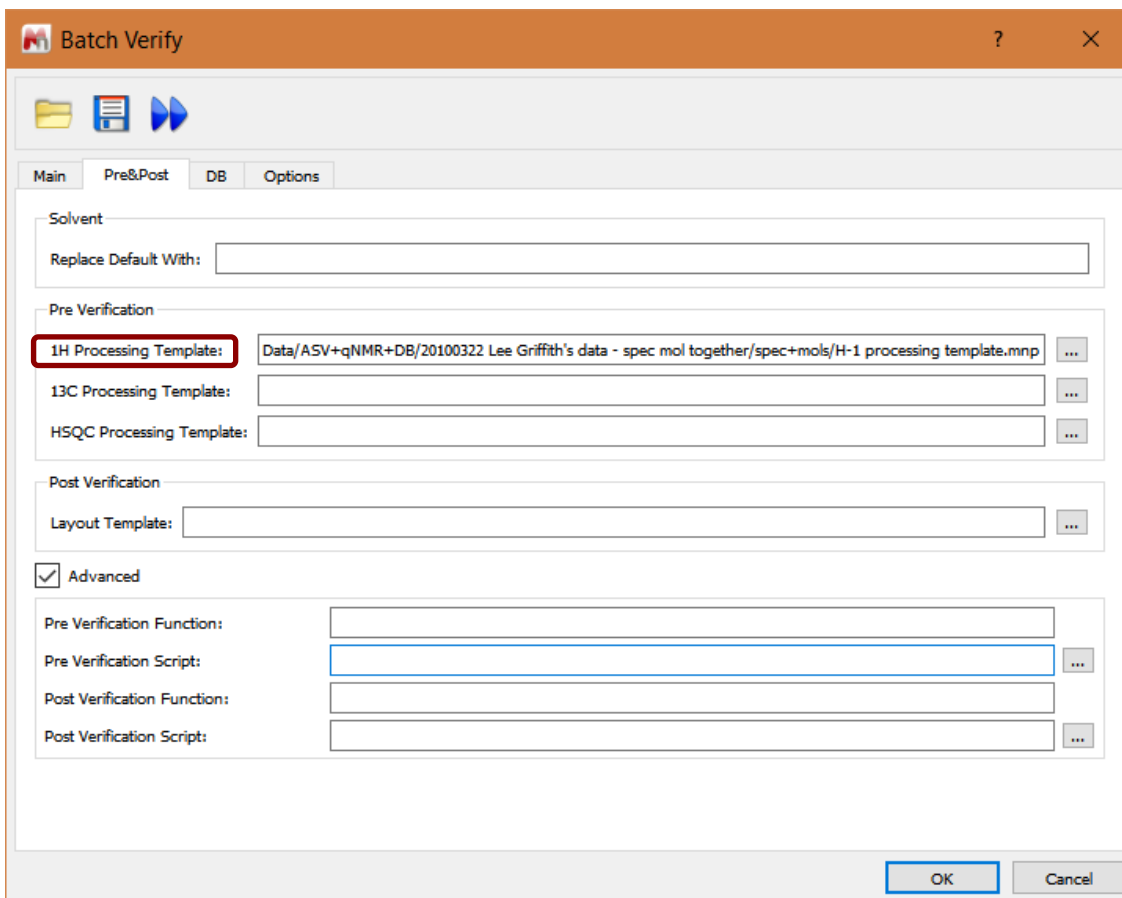


The screenshot shows the 'Batch Verify' dialog box in the 'Main' tab. The 'Data Location' section has 'Main Directory' set to 'C:/Mestrelab/Projects/Demo Data/ASV+qNMR+DB/20100322 Lee Griffith's data - spec mol together/spec+mols'. The 'Expert' checkbox is checked. The 'Structures' tab is selected and highlighted with a red box. Below it, there are two path mask fields: 'Main Directory' with the value 'ab/Projects/Demo Data/ASV+qNMR+DB/20100322 Lee Griffith's data - spec mol together/spec+mols' and 'Path Mask' with the value '\*/\*.mol'. There are also checkboxes for 'Associate By Name' and 'Associate By SDF Tag'. The 'Results' section has 'Directory' set to 'C:/Mestrelab/Projects/Demo Data/ASV+qNMR+DB/20100322 Lee Griffith's data - spec mol together/Result'. There are 'OK' and 'Cancel' buttons at the bottom.





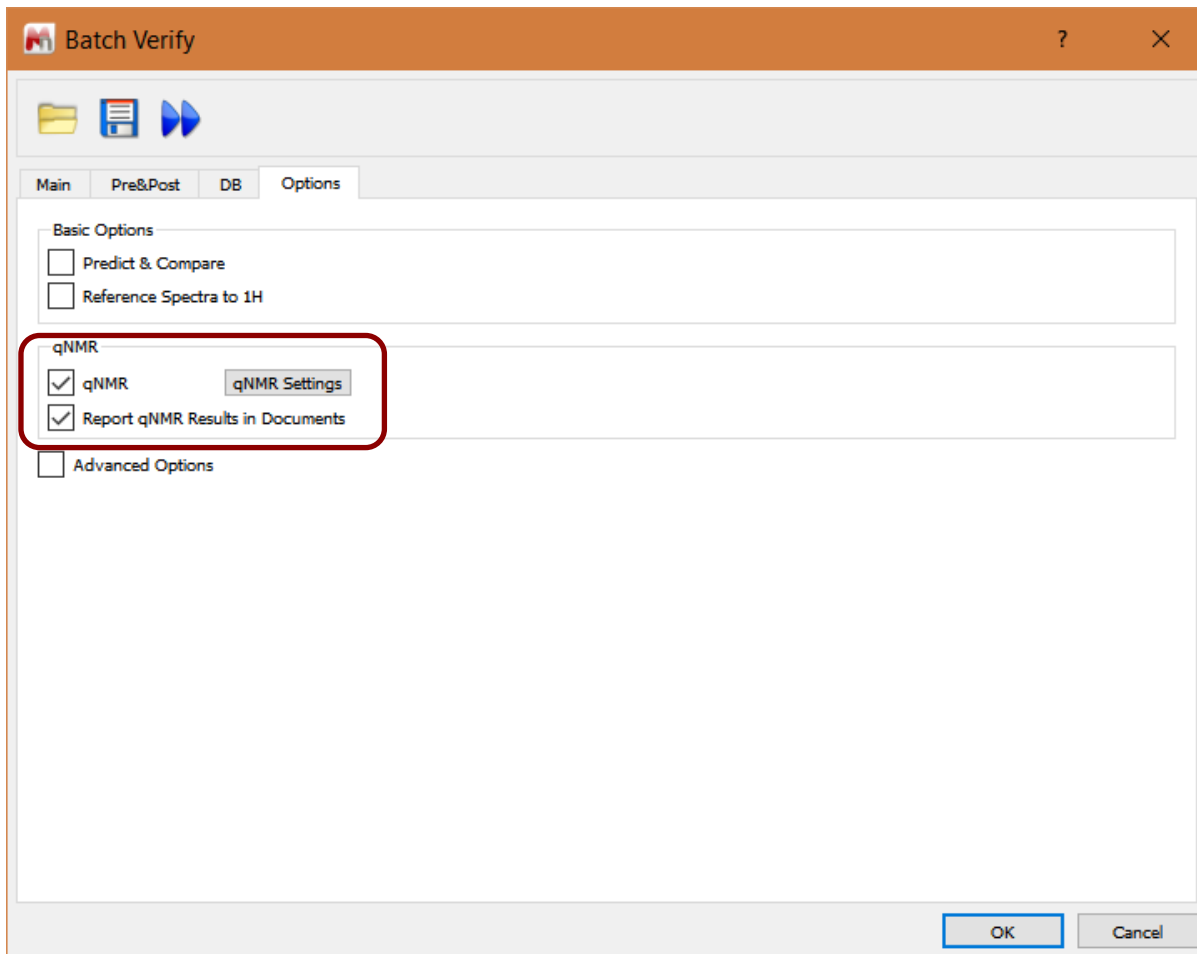
In the Pre&Post Tab, specify the processing template to use for all the H-1 NMR processing:



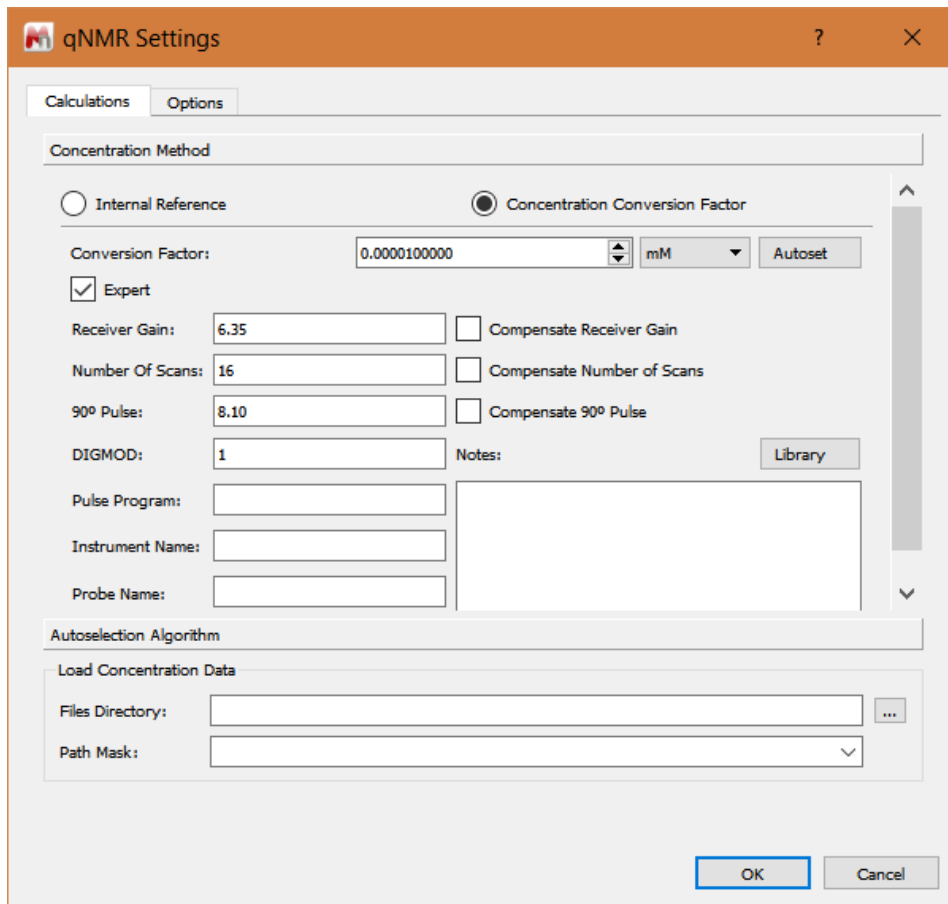
The screenshot shows the 'Batch Verify' dialog box with the 'Pre&Post' tab selected. The '1H Processing Template' field is highlighted with a red box and contains the path: 'Data/ASV+qNMR+DB/20100322 Lee Griffith's data - spec mol together/spec+mols/H-1 processing template.mnp'. Other fields include 'Solvent', 'Pre Verification' (13C and HSQC), 'Post Verification' (Layout Template), and 'Advanced' options (Pre/Post Verification Function and Script).

Field	Value
Solvent	Replace Default With: [Empty]
Pre Verification	
1H Processing Template	Data/ASV+qNMR+DB/20100322 Lee Griffith's data - spec mol together/spec+mols/H-1 processing template.mnp
13C Processing Template	[Empty]
HSQC Processing Template	[Empty]
Post Verification	
Layout Template	[Empty]
Advanced	
Pre Verification Function	[Empty]
Pre Verification Script	[Empty]
Post Verification Function	[Empty]
Post Verification Script	[Empty]

In the Options Tab, choose to do quantitation (determination of molar concentration using external reference info in this case).



In the Options Tab, click qNMR Settings button to define the details for molar concentration determination.



The screenshot shows the 'qNMR Settings' dialog box with the 'Options' tab selected. The 'Concentration Method' section has 'Concentration Conversion Factor' selected. The 'Conversion Factor' is set to 0.0000100000 and the unit is mM. The 'Expert' checkbox is checked. The 'Receiver Gain' is 6.35, 'Number Of Scans' is 16, and '90° Pulse' is 8.10. The 'DIGMOD' is set to 1. The 'Autoselection Algorithm' section has 'Load Concentration Data' checked, with a 'Files Directory' field and a 'Path Mask' dropdown menu. The 'OK' and 'Cancel' buttons are at the bottom.

qNMR Settings

Calculations Options

Concentration Method

Internal Reference  Concentration Conversion Factor

Conversion Factor: 0.0000100000 mM Autoset

Expert

Receiver Gain: 6.35  Compensate Receiver Gain

Number Of Scans: 16  Compensate Number of Scans

90° Pulse: 8.10  Compensate 90° Pulse

DIGMOD: 1 Notes: Library

Pulse Program:

Instrument Name:

Probe Name:

Autoselection Algorithm

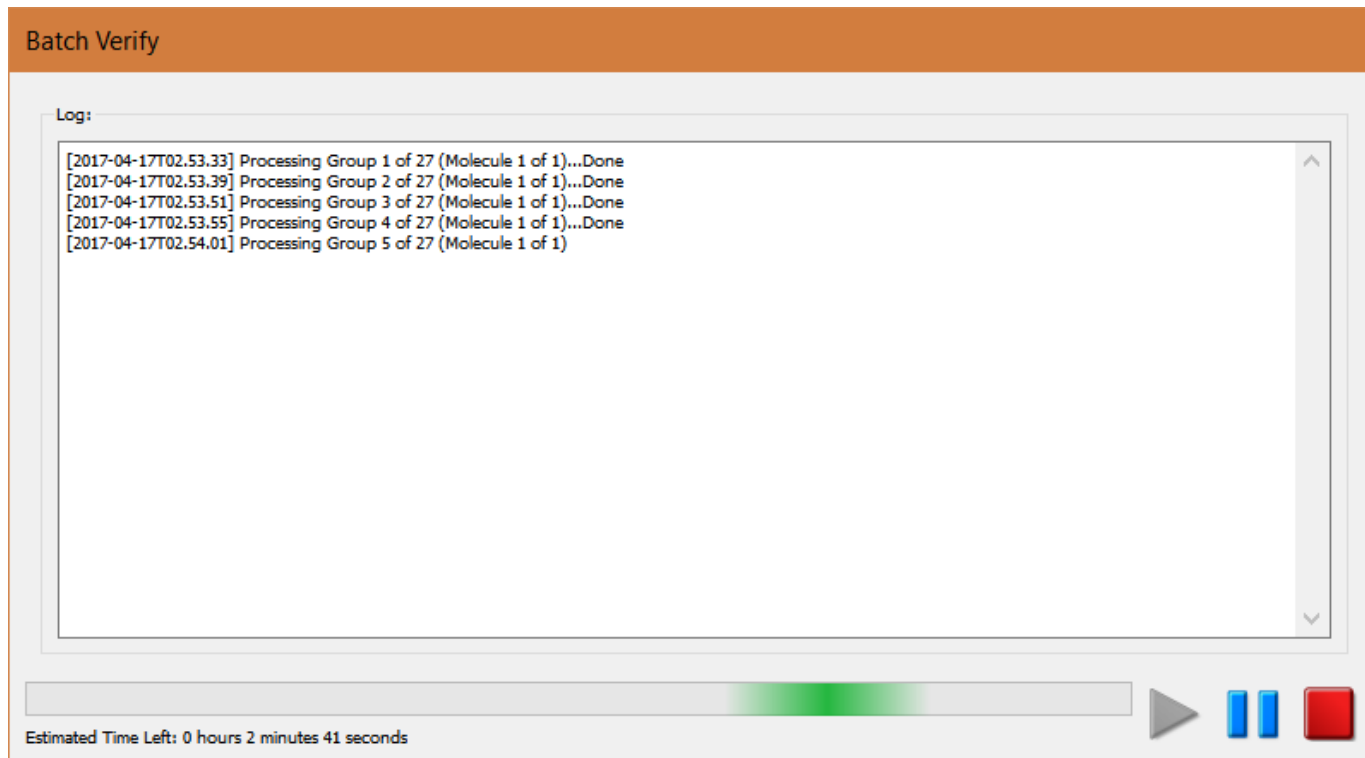
Load Concentration Data

Files Directory: ...

Path Mask: ▾

OK Cancel

Once the settings are done, click OK to start the batch processing. It process all the spectra, does structure verification and quantitation.



The screenshot displays the 'Batch Verify' software interface. At the top, there is an orange header bar with the text 'Batch Verify'. Below this is a large white log window with a vertical scrollbar on the right. The log contains the following text:

```
Log:  
[2017-04-17T02.53.33] Processing Group 1 of 27 (Molecule 1 of 1)...Done  
[2017-04-17T02.53.39] Processing Group 2 of 27 (Molecule 1 of 1)...Done  
[2017-04-17T02.53.51] Processing Group 3 of 27 (Molecule 1 of 1)...Done  
[2017-04-17T02.53.55] Processing Group 4 of 27 (Molecule 1 of 1)...Done  
[2017-04-17T02.54.01] Processing Group 5 of 27 (Molecule 1 of 1)
```

Below the log window is a progress bar with a green gradient. To the right of the progress bar are three buttons: a grey play button, two blue vertical bars, and a red stop button. At the bottom left of the interface, the text 'Estimated Time Left: 0 hours 2 minutes 41 seconds' is displayed.

Upon completion, the verification results are written to under the specified Results directory. Choose Analysis | Verification | Verify Viewer, and click the Load button to open the results.dat file. All the results are loaded for visualization:

Table View

Verify Viewer
✕

Load
Save
Clear
Settings
Report
Save to DB
Show Active

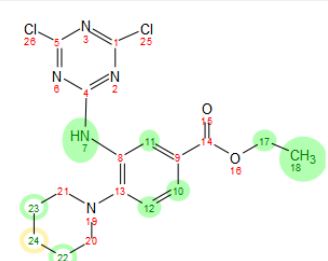
#	Title	Quality	Significance	Score	P	
<input checked="" type="checkbox"/>	12	LS00026291.10.fid	0.58	5.87	0.67(50%)	99
<input checked="" type="checkbox"/>	13	LS00027809.10.fid	0.61	6.07	0.71(50%)	99
<input checked="" type="checkbox"/>	14	LS00028324.10.fid	0.39	5.10	0.47(50%)	99
<input checked="" type="checkbox"/>	15	LS00028554.10.fid	0.41	6.12	0.47(50%)	90
<input checked="" type="checkbox"/>	16	LS00028600.10.fid	0.69	6.53	0.80(50%)	70
<input checked="" type="checkbox"/>	17	LS00022373.10.fid	0.23	4.77	0.28(50%)	99
<input checked="" type="checkbox"/>	18	LS00002059.10.fid	0.75	6.93	0.86(50%)	99
<input checked="" type="checkbox"/>	19	LS00013051.10.fid	0.79	7.31	0.90(50%)	99
<input checked="" type="checkbox"/>	20	LS00006172.10.fid	-0.38	6.30	-0.44(50%)	51

0.88	0.88	0.88	0.88	0.88
0.79	0.78	0.77	0.75	0.72
0.72	0.72	0.70	0.69	0.66
0.65	0.61	0.60	0.58	0.48
0.41	0.39	0.37	0.23	0.21
0.20	-0.38			

Individual Tests

Name	Quality	Score	Significance
1H Nuclides Count	0.02	0.06	0.69
1H Prediction Bounds Metric	0.02	0.04	1.21
1H Assignments	-0.49	-0.56	7.00

Plate View



qNMR

Concentration Average:	<input type="text" value="0.0814"/>	11(d)	1	8.31..8.35	7998.5180	0.0800	3179.6833
RMSD(%):	<input type="text" value="1.2034"/>	10(dd)	1	7.72..7.76	8193.6170	0.0819	2602.5795
		12(d)	1	7.31..7.35	8216.8616	0.0822	3095.7810

In the Verify Viewer, click on any items in the Table or Well-plate View to see the details of the spectrum/molecule. Pay attention to the ones with red/yellow flags. You can re-analyze the results (peak picking, multiplet analysis) and apply Verify or qNMR to revise the results for the current spectrum.

Verify Viewer

Load Save Clear Settings Report Save to DB Show Active

#	Title	Quality	Significance	Score	P
12	LS00026291.10.fid	0.58	5.87	0.67(50%)	99
13	LS00027809.10.fid	0.61	6.07	0.71(50%)	99
14	LS00028324.10.fid	0.39	5.10	0.47(50%)	99
15	LS00028554.10.fid	0.41	6.12	0.47(50%)	90
16	LS00028600.10.fid	0.69	6.53	0.80(50%)	70
17	LS00022373.10.fid	0.23	4.77	0.28(50%)	99
18	LS00002059.10.fid	0.75	6.93	0.86(50%)	99
19	LS00013051.10.fid	0.79	7.31	0.90(50%)	99
20	LS00006172.10.fid	-0.38	6.30	-0.44(50%)	51

Well Plate Showing: Verification Quality

Name	Quality	Score	Significance
1H Nuclides Count	0.02	0.06	0.69
1H Prediction Bounds Metric	0.02	0.04	1.21
1H Assignments	-0.49	-0.56	7.00

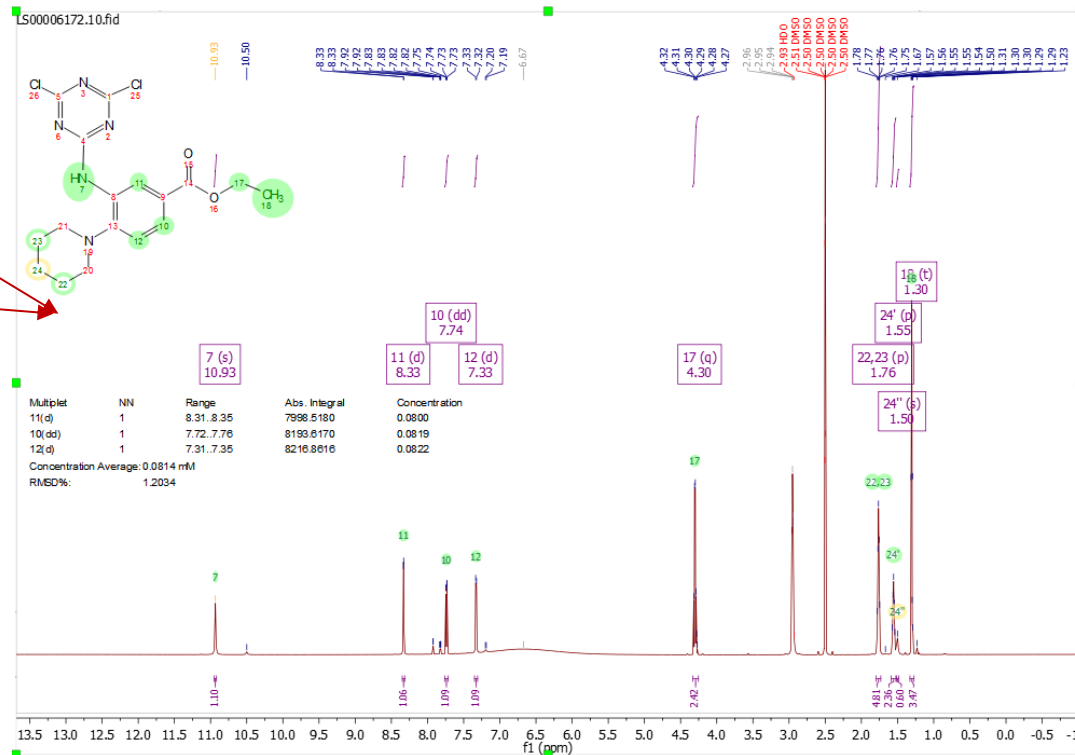
Individual Tests

Verify results

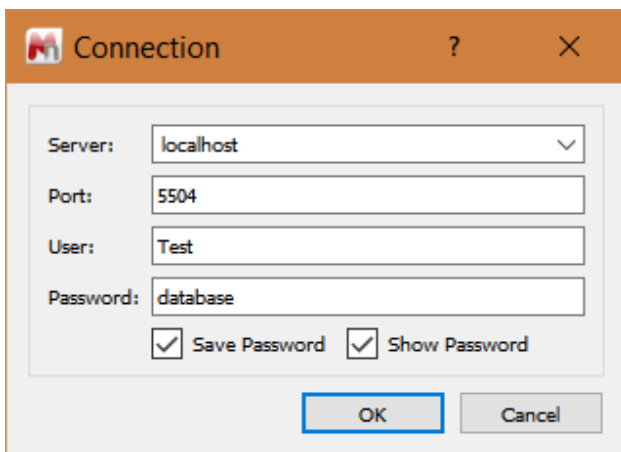
qNMR

Concentration Average:	11(d)	10(dd)	12(d)
0.0814	1	1	1
RMSD(%): 1.2034	8.31..8.35	7.72..7.76	7.31..7.35
	7998.5180	8193.6170	8216.8616
	0.0800	0.0819	0.0822
	3179.6833	2602.5795	3095.7810

Report



Once you are ready, create a new database to save the spectra and molecules.  
Choose Database | Connect to connect to the DB Server.



Connection

Server: localhost

Port: 5504

User: Test

Password: database

Save Password  Show Password

OK Cancel

Typically we add a custom field to hold the compound IDs of each spectrum.

Usually a short script is necessary to extract the compound IDs from the NMR filename, Title, or Comment fields and save them to the relevant field(s) in the database.

The screenshot shows the 'Database Info' window with the following configuration:

- Database Name:** TKD\_REF\_DB
- Server:** localhost:5504
- Description:** with corp\_id added
- Creator:** Test
- Server Version:** 1.7.0
- Fields:** 115
- Created:** 2017-01-30 07:39:44
- Client Version:** 11.1.0-18597
- Records:** 62

The 'Items' table lists the database items:

ID	Name	Type	Fields	Comment
1	Molecule	COMP	28	Molecules (mandatory).
2	NMR Spectrum	NMR	58	1D and 2D NMR spectra.

The 'Fields' table shows the definition of the custom field:

ID	Item	Page	Name	Type	Size	Content	Cor
2.82	NMR Spectrum	PageID		TEXT	100		
2.83	NMR Spectrum	Created		DATETIME	0	-CREATED-	
2.84	NMR Spectrum	Modified		DATETIME	0	-MODIFIED-	
2.85	NMR Spectrum	Mnova Version		TEXT	100		
2.115	NMR Spectrum		_REF_CORP_ID	TEXT	20	=Get RefID(item)	corp_id



Click the Save to DB button. This will save all the spectra/molecules to the database.

Verify Viewer

Load Save Clear Settings Report **Save to DB** Show Active

#	Title	Quality	Significance	Score	Purity
<input checked="" type="checkbox"/> 1		-0.58	6.95	-0.67(50%)	undefined
<input checked="" type="checkbox"/> 2		0.62	6.18	0.72(50%)	undefined
<input checked="" type="checkbox"/> 3		0.22	4.48	0.27(50%)	undefined
<input checked="" type="checkbox"/> 4		0.56	5.89	0.65(50%)	undefined
<input checked="" type="checkbox"/> 5		0.40	5.19	0.48(50%)	undefined
<input checked="" type="checkbox"/> 6		0.03	3.73	0.04(50%)	undefined
<input checked="" type="checkbox"/> 7		0.59	6.09	0.68(50%)	undefined
<input checked="" type="checkbox"/> 8	NMR fragment library compd	-0.35	7.19	-0.40(50%)	undefined
<input checked="" type="checkbox"/> 9		-0.25	5.07	-0.29(50%)	undefined
<input checked="" type="checkbox"/> 10		-0.03	3.53	-0.04(50%)	undefined
<input checked="" type="checkbox"/> 11		-0.35	7.24	-0.40(50%)	undefined
<input checked="" type="checkbox"/> 12		0.14	4.10	0.18(50%)	undefined

0.88	0.88	0.88	0.88	0.88
0.88	0.88	0.88	0.88	0.88
0.88	0.88	0.88	0.88	0.88
0.88	0.88	0.88	0.87	0.87
0.87	0.87	0.87	0.87	0.86
0.86	0.85	0.85	0.85	0.85
0.85	0.85	0.84	0.84	0.84
0.84	0.84	0.84	0.84	0.84
0.83	0.83	0.83	0.82	0.81
0.81	0.81	0.81	0.81	0.81
0.80	0.80	0.80	0.80	0.80
0.79	0.79	0.79	0.79	0.79
0.79	0.78	0.78	0.78	0.78
0.78	0.78	0.78	0.78	0.78
0.78	0.78	0.78	0.78	0.78
0.78	0.78	0.77	0.77	0.77
0.77	0.77	0.77	0.77	0.76

Well Plate Showing: Verification Quality

Individual Tests

Name	Quality	Score	Significance
1H Nuclides Count	-0.14	-0.30	0.88
1H Prediction Bounds Metric	-0.65	-1.00	1.84
1H Assignments	-0.59	-0.67	7.00

Record view of a record in the database:

Database - Record View

File View Configure

Molecule Preview

Button Navigator

mndb://Test@localhost:5504/...\_REF\_DB/3

Molecule

NMR Preview

Field	Content
3:0:8 Molecular Formula	C11H9FN2O
3:0:10 Monoisotopic Mass	204.06989099999998
3:1:34 Title	
3:1:38 Solvent	DMSO
3:1:45 Nucleus	1H
3:1:46 Acquisition Date	
3:1:55 Spectrometer Frequency	499.83945
3:1:57 Spectral Width	7997.60083063
3:1:58 Temperature	
3:1:65 Verification Quality	0.2197333985941979
3:1:67 Verification Significance	4.472947500201683
3:1:115 ..._REF_CORP_ID	S... Z

Table view of multiple records in the database:

Record	Molecule Molecular Formula	Molecule Monoisotopic Mas	Molecule Preview	NMR Spectrum Preview	NMR Spectrum Solvent	NMR Spectrum RP_ID	NMR Spectrum Verification Quality
1	C7H11N5O2	197.091275			DMSO		-0.5677405007238906
2	C8H6F3N3O	217.0462959999...			DMSO		0.561782572548758
3	C11H9FN2O	204.0698909999...			DMSO		0.2197333985941979
4	C8H7N3O	161.0589120000...			DMSO		0.5335363582986555
5	C6H5N5	147.054495			DMSO		0.5974437067849733
6	C7H4ClFN4	198.0108519999...			DMSO		0.03254973259570436
7	C11H10N2OS	218.0513839999...			DMSO		-0.399960292040904...
8	C8H8N2O2	164.058578			DMSO		-0.353878610277280...
9	C9H11NOS	181.0561349999...			DMSO		-0.234238736874625...
10	C7H11N5O2	197.091275			DMSO		-0.5677405007238906

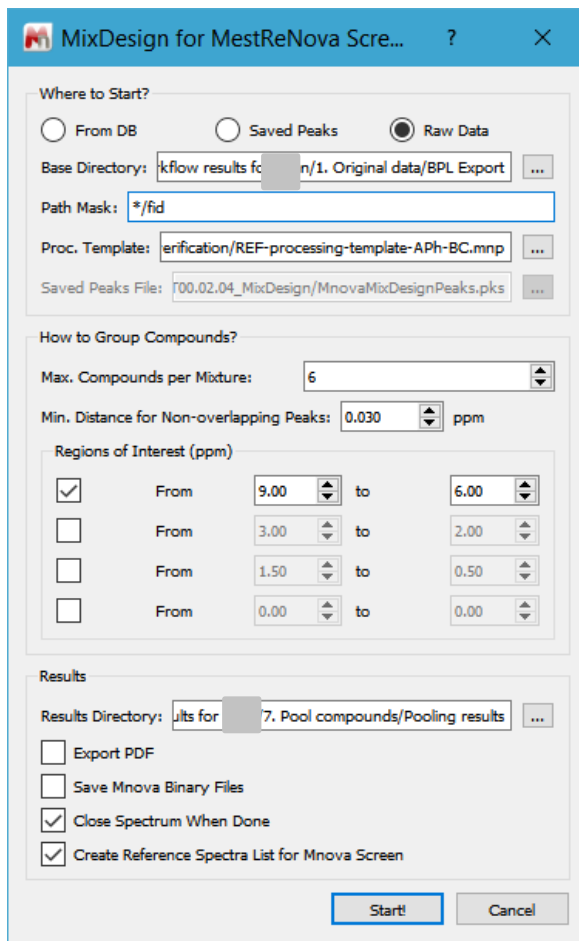
- We have done this for several libraries of 1-3K compounds.
- After some trial and error in setup, each run usually takes 3-4 hours to complete.
- The tools make it very convenient to browse through the results, focus on the ones with possible problems and make changes as necessary.
- There are typically ~10-20% of compounds with red flags and the problems are mostly real problems with the compound itself, low solubility, or low-quality spectra. Sometimes Mnova makes mistakes too.
- The database tools makes much more efficient to manage the data.

## MixDesign for pooling compounds



The MixDesign.qs script can pool compounds using the ref spec either saved in a DB or using the raw data. Choose Scripts | Run Script to run it.

If to start with the raw data, a processing template is used to process all the spectra:



The screenshot shows the 'MixDesign for MestReNova Screening' dialog box. It is divided into several sections:

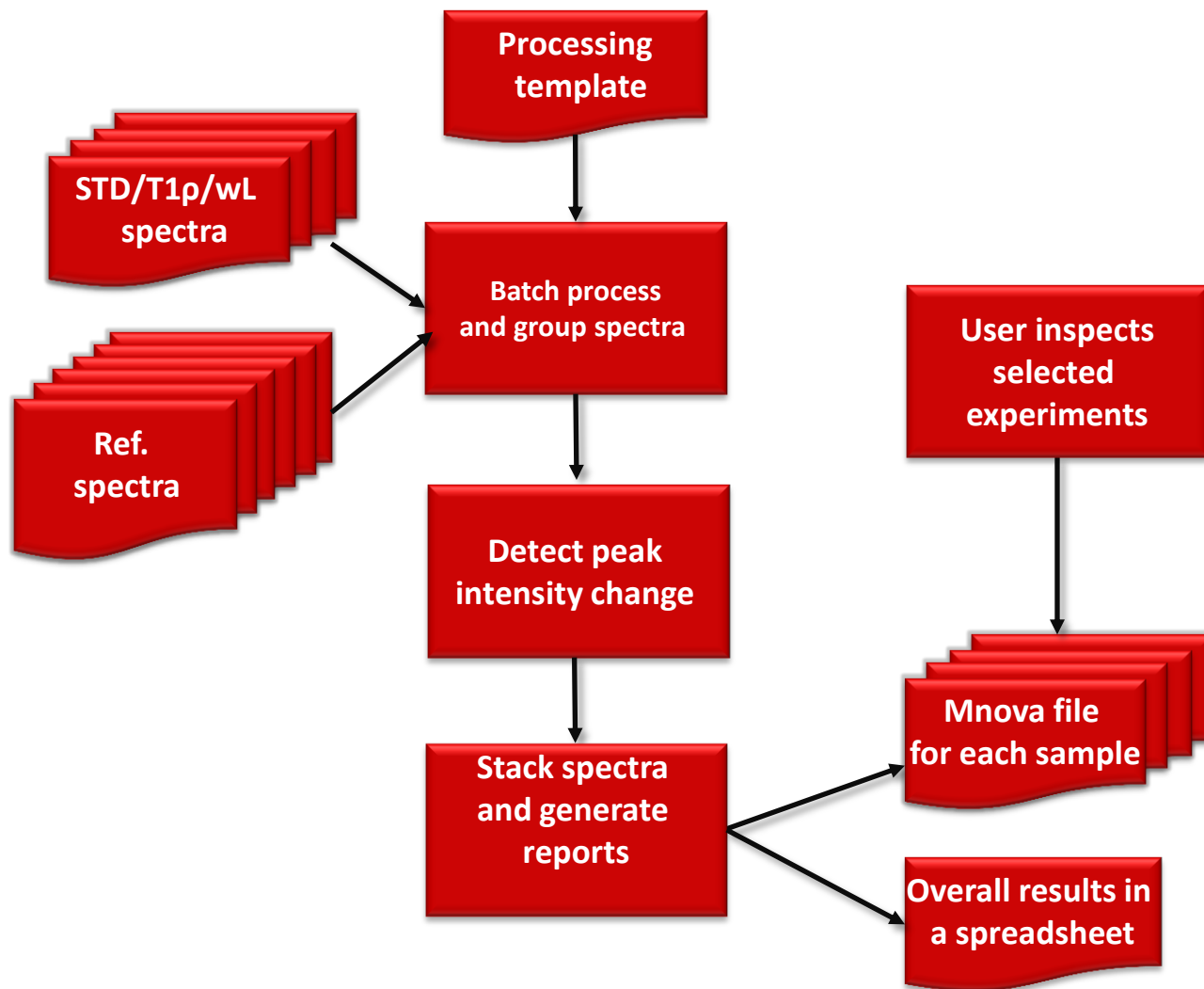
- Where to Start?**: Three radio buttons are present: 'From DB' (unselected), 'Saved Peaks' (unselected), and 'Raw Data' (selected).
- Base Directory**: A text field containing 'kflow results for [redacted]n/1. Original data/BPL Export' with a browse button '...'.  
**Path Mask**: A text field containing '\*/\*id'.
- Proc. Template**: A text field containing 'erification/REF-processing-template-APH-BC.mnp' with a browse button '...'.  
**Saved Peaks File**: A text field containing 'f00.02.04\_MixDesign/MnovaMixDesignPeaks.pks' with a browse button '...'.  
**How to Group Compounds?**:
  - Max. Compounds per Mixture**: A spinner box set to '6'.
  - Min. Distance for Non-overlapping Peaks**: A spinner box set to '0.030' ppm.
  - Regions of Interest (ppm)**: A list of four regions, each with a checkbox, 'From' and 'to' labels, and two spinner boxes:
    - Checked: From 9.00 to 6.00
    - Unchecked: From 3.00 to 2.00
    - Unchecked: From 1.50 to 0.50
    - Unchecked: From 0.00 to 0.00
- Results**:
  - Results Directory**: A text field containing 'uits for [redacted]7. Pool compounds/Pooling results' with a browse button '...'.  
 Export PDF  
 Save Mnova Binary Files  
 Close Spectrum When Done  
 Create Reference Spectra List for Mnova Screen

At the bottom, there are 'Start!' and 'Cancel' buttons.



- Example:
  - 1578  $^1\text{H}$  spectra/compounds.
  - 6 compounds per mixture.
  - ROI: 9-6 ppm.
  - 0.04 ppm as minimum distance for non-overlapping spectra.
  - Got 263 mixtures in ~40 minutes.
  - 13 of them have one spectrum completely overlapping.
  - The output spreadsheets can be used by Mnova Screen for associating mixture spectra and reference spectra.
- We continue to improve the script based on users' feedback

## Mnova Screen for ligand binding spectral analysis



- H-1 or F-19
- Single compounds or mixtures
- With or without reference spectra
- Single or multiple types of spectra (STD, T1rho, WaterLogsy, CPMG)
- Use of Blank, w/ Protein, & w/ Protein+ Inhibitors
- Mnova Screen can handle all of them

- ❑ 263 STD on/off resonance spectra for a total of 1,578 compounds.
- ❑ Each mixture has 6 compounds.
- ❑ A lookup table for Screen to find the reference spectra for each sample.

```
MScreen_DB_lookup.txt - Notepad
File Edit Format View Help
#USE_DB: true
#DB_SERVER: localhost
#DB_PORT: 5504
#DB_USER: Test
#DB_PASSWORD: database
#DB_DATABASE: REF_DB
#DB_ITEM: NMR Spectrum
#DB_FIELD: REF_CORP_ID
#MIXTURE: 001
S
Z
S
Z
S
Z
S
Z
S
Z
S
Z
#MIXTURE: 002
S
Z
S
Z
S
Z
S
Z
S
Z
#MIXTURE: 003
S
Z
S
Z
S
Z
S
Z
S
Z
```

Database related information. Enter info about your database server, login account, database name, item and field (where to find the compound IDs).

IDs of the fragments for each sample/mixture.

- ❑ The Processing and Analysis tab: setup for Reference spectra.

Mnova Screen 1H

Project Datasets Processing and Analysis

General Options

Minimum Matched Peaks to be Present:  % Optimise ?

Tolerance for Matching Peaks:  \* Ref Peak Width Optimise ?

Calculate Intensity Changes using Nearest GSD Peak instead of Sum Integration Change ?

Use Center of Matched Scout Peak instead of Reference Peak ?

Calculate Intensity Changes using Peak Height instead of Peak Area ?

Use Blank STD, T1rho and CPMG Peak Matching to Identify Missing References ?

Regions of Interest:

		From	To
1	<input checked="" type="checkbox"/>	6	9

Add Region Remove Region

Scout Reference T1rho STD wLogsy CPMG

Processing: *normalise largest peak (by area) in regions of interest to intensity 600*  
*Ignore peaks with height less than 10% of maximum peak* Edit ?

Save Parameters Load Parameters Reset Script Behaviour Summary OK Cancel

- ❑ The Processing and Analysis tab: setup for STD spectra.

Mnova Screen 1H

Project Datasets Processing and Analysis

General Options

Minimum Matched Peaks to be Present: 20 % Optimise ?

Tolerance for Matching Peaks: 2.0 \* Ref Peak Width Optimise ?

Calculate Intensity Changes using Nearest GSD Peak instead of Sum Integration Change ?

Use Center of Matched Scout Peak instead of Reference Peak ?

Calculate Intensity Changes using Peak Height instead of Peak Area ?

Use Blank STD, T1rho and CPMG Peak Matching to Identify Missing References ?

Regions of Interest:

	From	To
1 <input checked="" type="checkbox"/>	6	9

Add Region Remove Region

Scout Reference T1rho STD wLogsy CPMG

*don't normalise*  
*processing tmpl: C:/Users/Peng/Dropbox (Mestrelab)/MES [redacted] mplate-Ph-WTBC.mnp* Edit ?

Processing: *Off-resonance spectrum is the second one*  
*add difference spectrum*  
*Ignore peaks with height less than 20% of maximum peak*

Minimum average peak intensity change for hit: 10 % Optimise ?

Save Parameters Load Parameters Reset Script Behaviour Summary OK Cancel

☐ Batch processing finished in about 2 hours.

**Information**

263 experiments found:

001 - NOT BINDING (1 missing, 5 present, 0 specific hit, 0 non-selective hit)  
 002 - BINDING (0 missing, 5 present, 1 specific hit, 0 non-selective hit)  
 003 - BINDING (0 missing, 5 present, 1 specific hit, 0 non-selective hit)  
 004 - NOT BINDING (0 missing, 6 present, 0 specific hit, 0 non-selective hit)  
 005 - BINDING (0 missing, 5 present, 1 specific hit, 0 non-selective hit)  
 006 - NOT BINDING (0 missing, 6 present, 0 specific hit, 0 non-selective hit)  
 007 - BINDING (0 missing, 3 present, 3 specific hit, 0 non-selective hit)  
 008 - NOT BINDING (0 missing, 6 present, 0 specific hit, 0 non-selective hit)  
 009 - NOT BINDING (0 missing, 6 present, 0 specific hit, 0 non-selective hit)  
 010 - NOT BINDING (0 missing, 6 present, 0 specific hit, 0 non-selective hit)  
 011 - BINDING (0 missing, 5 present, 1 specific hit, 0 non-selective hit)  
 012 - BINDING (0 missing, 5 present, 1 specific hit, 0 non-selective hit)  
 013 - BINDING (0 missing, 4 present, 2 specific hit, 0 non-selective hit)  
 014 - NOT BINDING (0 missing, 6 present, 0 specific hit, 0 non-selective hit)  
 015 - NOT BINDING (0 missing, 6 present, 0 specific hit, 0 non-selective hit)  
 016 - BINDING (0 missing, 5 present, 1 specific hit, 0 non-selective hit)  
 017 - NOT BINDING (1 missing, 5 present, 0 specific hit, 0 non-selective hit)  
 018 - BINDING (0 missing, 5 present, 1 specific hit, 0 non-selective hit)  
 019 - BINDING (0 missing, 3 present, 3 specific hit, 0 non-selective hit)  
 020 - BINDING (0 missing, 4 present, 2 specific hit, 0 non-selective hit)  
 021 - BINDING (0 missing, 5 present, 1 specific hit, 0 non-selective hit)  
 022 - NOT BINDING (0 missing, 6 present, 0 specific hit, 0 non-selective hit)  
 023 - NOT BINDING (0 missing, 6 present, 0 specific hit, 0 non-selective hit)  
 024 - NOT BINDING (0 missing, 6 present, 0 specific hit, 0 non-selective hit)  
 025 - NOT BINDING (0 missing, 6 present, 0 specific hit, 0 non-selective hit)  
 026 - BINDING (0 missing, 3 present, 3 specific hit, 0 non-selective hit)  
 027 - BINDING (0 missing, 5 present, 1 specific hit, 0 non-selective hit)  
 028 - NOT BINDING (0 missing, 6 present, 0 specific hit, 0 non-selective hit)  
 029 - NOT BINDING (0 missing, 6 present, 0 specific hit, 0 non-selective hit)  
 030 - NOT BINDING (0 missing, 6 present, 0 specific hit, 0 non-selective hit)  
 031 - NOT BINDING (0 missing, 6 present, 0 specific hit, 0 non-selective hit)  
 032 - BINDING (0 missing, 5 present, 1 specific hit, 0 non-selective hit)  
 033 - BINDING (0 missing, 5 present, 1 specific hit, 0 non-selective hit)  
 034 - BINDING (1 missing, 4 present, 1 specific hit, 0 non-selective hit)  
 035 - NOT BINDING (0 missing, 6 present, 0 specific hit, 0 non-selective hit)  
 036 - BINDING (0 missing, 5 present, 1 specific hit, 0 non-selective hit)  
 037 - NOT BINDING (0 missing, 6 present, 0 specific hit, 0 non-selective hit)  
 038 - NOT BINDING (0 missing, 6 present, 0 specific hit, 0 non-selective hit)  
 039 - BINDING (0 missing, 5 present, 1 specific hit, 0 non-selective hit)  
 040 - BINDING (0 missing, 4 present, 2 specific hit, 0 non-selective hit)  
 041 - BINDING (0 missing, 5 present, 1 specific hit, 0 non-selective hit)  
 042 - NOT BINDING (0 missing, 6 present, 0 specific hit, 0 non-selective hit)  
 043 - NOT BINDING (0 missing, 6 present, 0 specific hit, 0 non-selective hit)  
 044 - BINDING (0 missing, 5 present, 1 specific hit, 0 non-selective hit)

**Mnova Screening Results Editor**

Experiment	Fragment 1	Fragment 2	Fragment 3	Fragment 4	Fragment 5	Fragment 6	Result	Comm
<input type="checkbox"/> 001	missing	present	present	present	present	present	NOT BINDING	Ref 001_
<input type="checkbox"/> 002	present	present	present	present	present	specific hit	BINDING	Ref 002_
<input type="checkbox"/> 003	present	present	specific hit	present	present	present	BINDING	Ref 003_
<input type="checkbox"/> 004	present	present	present	present	present	present	NOT BINDING	Ref 004_
<input type="checkbox"/> 005	present	present	present	specific hit	present	present	BINDING	Ref 005_
<input type="checkbox"/> 006	present	present	present	present	present	present	NOT BINDING	Ref 006_
<input type="checkbox"/> 007	specific hit	present	present	present	specific hit	specific hit	BINDING	Ref 007_
<input type="checkbox"/> 008	present	present	present	present	present	present	NOT BINDING	Ref 008_
<input type="checkbox"/> 009	present	present	present	present	present	present	NOT BINDING	Ref 009_
<input type="checkbox"/> 010	present	present	present	present	present	present	NOT BINDING	Ref 010_
<input type="checkbox"/> 011	present	present	present	present	present	specific hit	BINDING	Ref 011_
<input type="checkbox"/> 012	specific hit	present	present	present	present	present	BINDING	Ref 012_
<input type="checkbox"/> 013	present	specific hit	present	present	present	specific hit	BINDING	Ref 013_
<input type="checkbox"/> 014	present	present	present	present	present	present	NOT BINDING	Ref 014_
<input type="checkbox"/> 015	present	present	present	present	present	present	NOT BINDING	Ref 015_
<input type="checkbox"/> 016	present	present	present	present	specific hit	present	BINDING	Ref 016_
<input type="checkbox"/> 017	present	present	present	missing	present	present	NOT BINDING	Ref 0170
<input type="checkbox"/> 018	present	present	present	present	specific hit	present	BINDING	Ref 018_
<input type="checkbox"/> 019	specific hit	specific hit	specific hit	present	present	present	BINDING	Ref 019_
<input type="checkbox"/> 020	specific hit	specific hit	present	present	present	present	BINDING	Ref 020_
<input type="checkbox"/> 021	present	present	present	present	specific hit	present	BINDING	Ref 021_
<input type="checkbox"/> 022	present	present	present	present	present	present	NOT BINDING	Ref 022_
<input type="checkbox"/> 023	present	present	present	present	present	present	NOT BINDING	Ref 023_
<input type="checkbox"/> 024	present	present	present	present	present	present	NOT BINDING	Ref 024_

Visualization Options

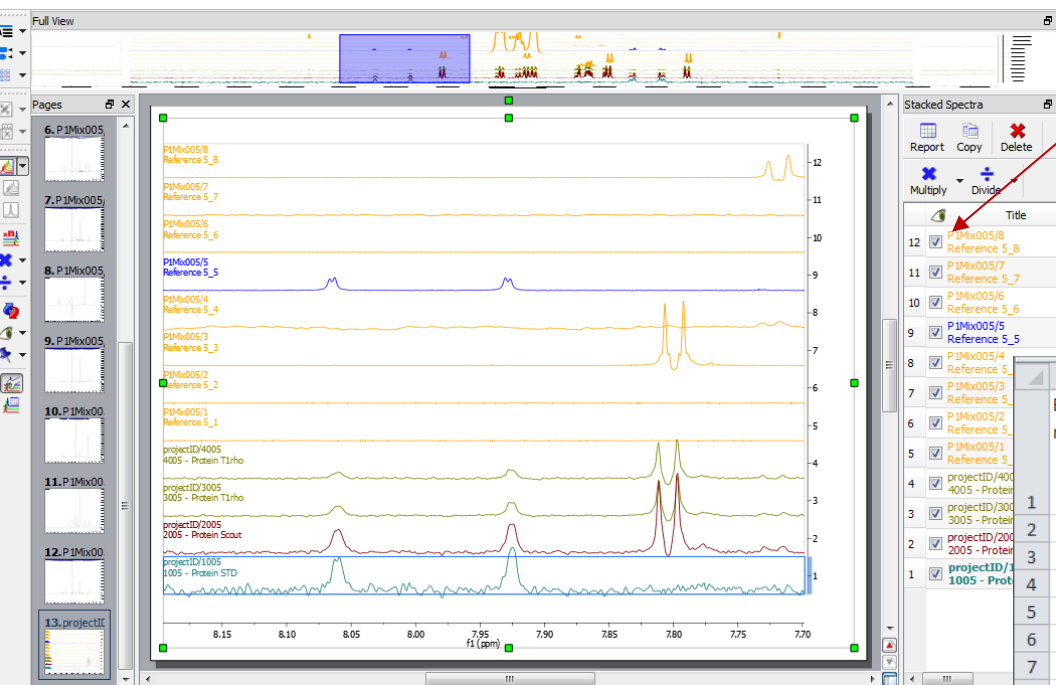
References:  Specific Hit  Non Selective Hit  Missing  Present

Experiments:  1 - STD Protein  2 - STD Protein  3 - STD Protein Difference

Reference Display Text: Status

Export Discard Save





Mnova Screening Results Editor

Experiment	Fragment 1	Fragment 2
005	present	present
009	missing	specific hit

Export Discard Save

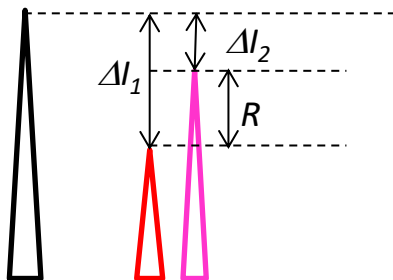
	A	B	C	D	E	F	G	H	I
	Experi	Refer	Status	Label	Ref	Average	STD Protein	T1rho Protein	T1rho Protein
	ment	ence			Peak	Scout/Blank	Match	Average	Max Intensity
					Count	Match	Proportion	Intensity	Change
						Proportion		Change	
1									
2	5	5_1	present	5_1	19	42.10%	0.00%	14.50%	43.40%
3	5	5_2	present	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%

- ❑ Two thresholds are defined by the user:
  - ❑  $T_1$ : minimum intensity decrease for a hit.
  - ❑  $T_2$ : minimum intensity recovery rate for a specific hit.
- ❑ If  $\Delta I_1 > T_1$  and  $R > T_2$  : specific hit (see Ex.1 below)
- ❑ If  $\Delta I_1 > T_1$  and  $R \leq T_2$  : non-specific hit (see Ex.1 below)

## Example 1

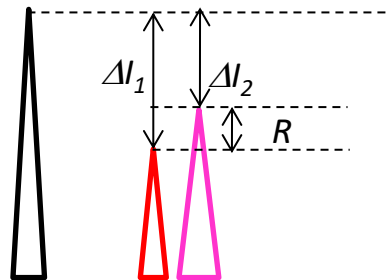
$T_2 = 5\%$

$\Delta I_1 \sim 60\%$      $\Delta I_2 \sim 25\%$ ,  $R \sim 35\%$



## Example 2

$\Delta I_1 \sim 60\%$      $\Delta I_2 \sim 58\%$   $R \sim 2\%$



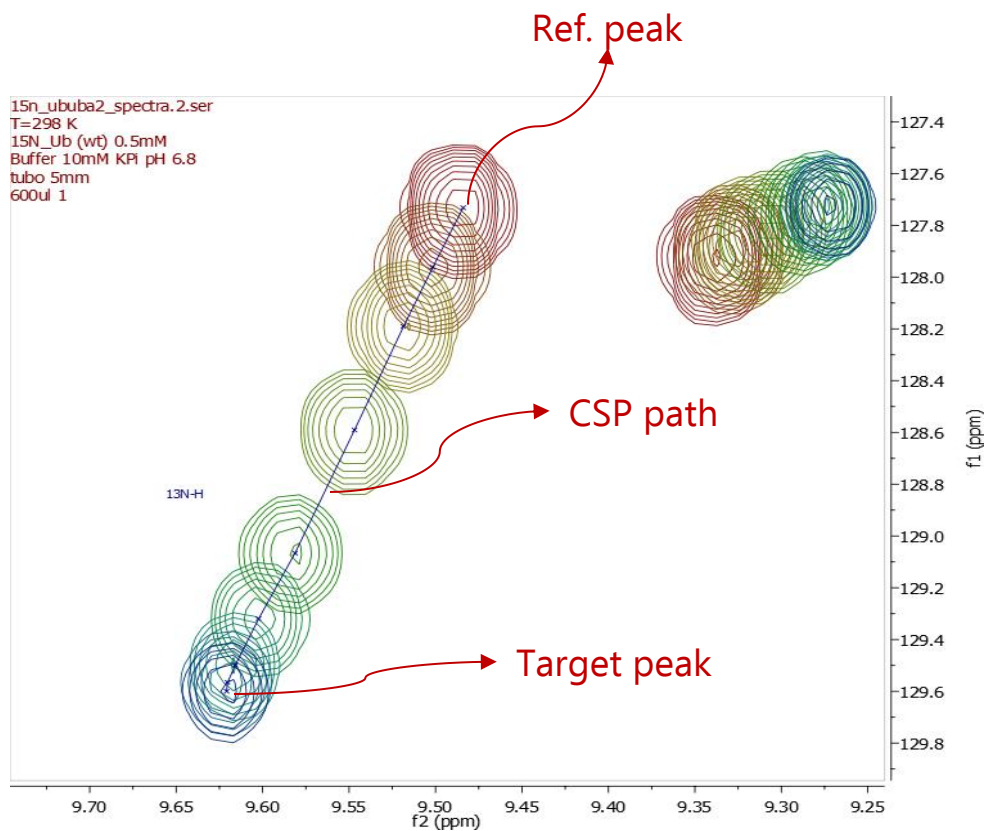
- Two mixtures with 8 compounds each.
- Using STD difference spectra, and T1rho (short/long-spin lock).

- Typically it takes about 3-4 hours to complete a screening batch of 2-300 mixtures.
- Using database of reference spectra is usually faster.
- The automated results are comparable with careful manual analysis results but much faster. See results comparison and discussion at C. Peng, A. Frommlet, M. Perez, C. Cobas, A. Blechschmidt, S. Dominguez, and A. Lingel., *J. Med. Chem.* 2016, **59**, 3303–3310.
- The tools that allow you to easily browse through the results, and verify and correct them manually is very convenient.
- Mnova Screen has been used routinely by > 10 companies.

## **Mnova CSP – Chemical shift perturbation analysis**

- Mnova CSP allows you to process and analyze a series CSP spectra fully automatically, or interactively, or both
- Full automatic processing and analysis starting from 2D raw data to  $K_D$ 's
  - Prepare 3 information files: Titration file, Ligand file, Peaks file; and enter them to CSP.
  - CSP processes all HSQC spectra, stacks them, tracks the peak movements, calculates the CSPs and  $K_D$  for each peak, and does statistics of all the  $K_D$ s.
- Manual analysis
  - You open and stack multiple HSQC spectra interactive.
  - You pick the peaks and let CSP monitor automatically track their shift path across all the spectra.
  - You manually correct the peak tracking as needed.
  - CSP calculates the CSPs and  $K_D$  for each peak, and does statistics of all the  $K_D$ s in real time.

- ❑ A reference peak, usually assigned to an amino acid residual in a protein, shifts its location in  $^1\text{H}/^{15}\text{N}$  (or  $^1\text{H}/^{13}\text{C}$ ) HSQC spectra as the ligand is added.
- ❑ [P]: concentration of protein
- ❑ [L]: concentration of ligand
- ❑ [L]/[P]: ratio of ligand/protein – Column “Lt/Pt” in CSP Panel



	Spectrum	Lt/Pt
1	15n_ububa2_spectra.2.ser	0
2	15n_ububa2_spectra.24.ser	0.25
3	15n_ububa2_spectra.26.ser	0.5
4	15n_ububa2_spectra.28.ser	1
5	15n_ububa2_spectra.30.ser	2
6	15n_ububa2_spectra.32.ser	3
7	15n_ububa2_spectra.34.ser	5
8	15n_ububa2_spectra.36.ser	7
9	15n_ububa2_spectra.38.ser	9

- The chemical shift changes along the path from the ref. peak to target peak is measured and normalized:

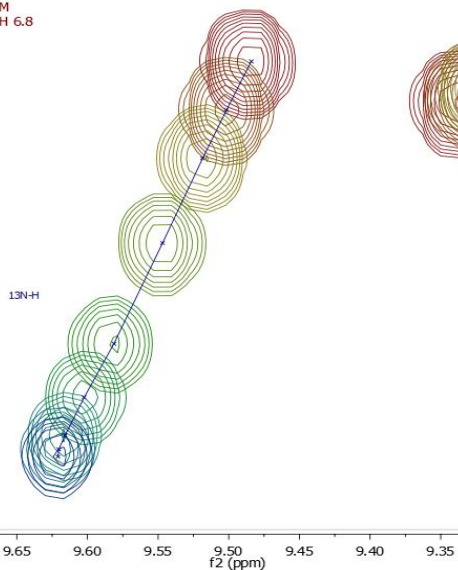
$$CSP \text{ (ppm)} = \sqrt{(F(H) \cdot \Delta\delta_H)^2 + (F(N) \cdot \Delta\delta_N)^2}$$

or

$$CSP \text{ (ppm)} = \sqrt{(F(H) \cdot \Delta\delta_H)^2 + (F(C) \cdot \Delta\delta_C)^2}$$

By default:  $F(H) = 1$ ;  $F(N) = 0.156$ ;  $F(C) = 0.185$ . You can change the values in Settings.

15n\_ububa2\_spectra.2.ser  
T=298 K  
15N\_Ub (wt) 0.5mM  
Buffer 10mM KPI pH 6.8  
tubo 5mm  
600ul 1

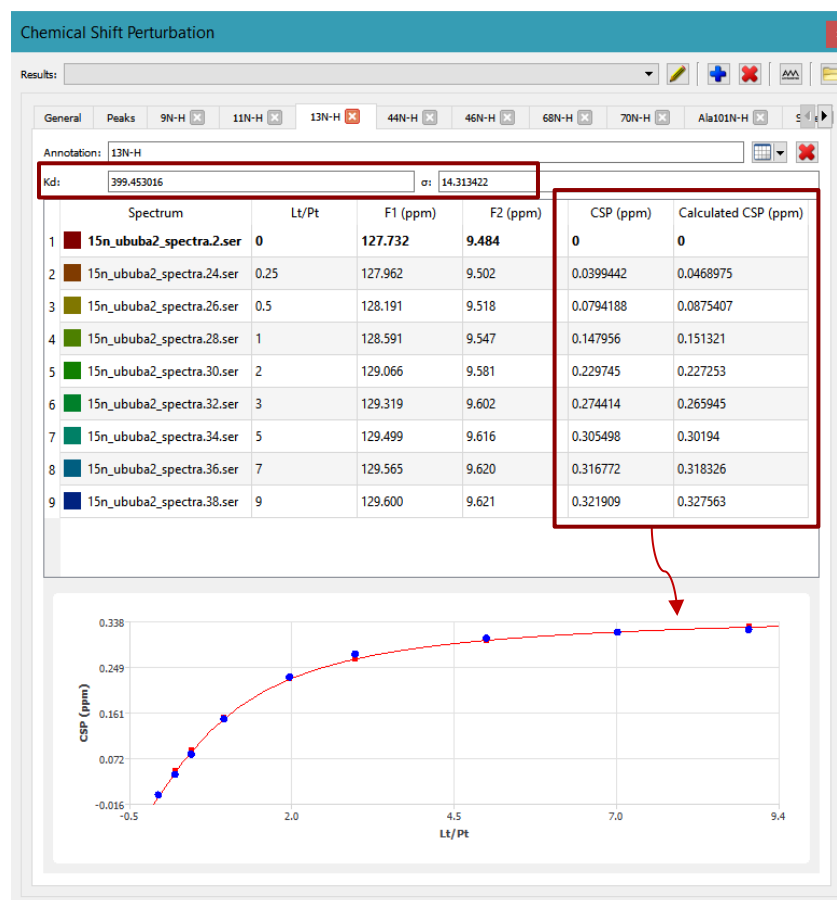
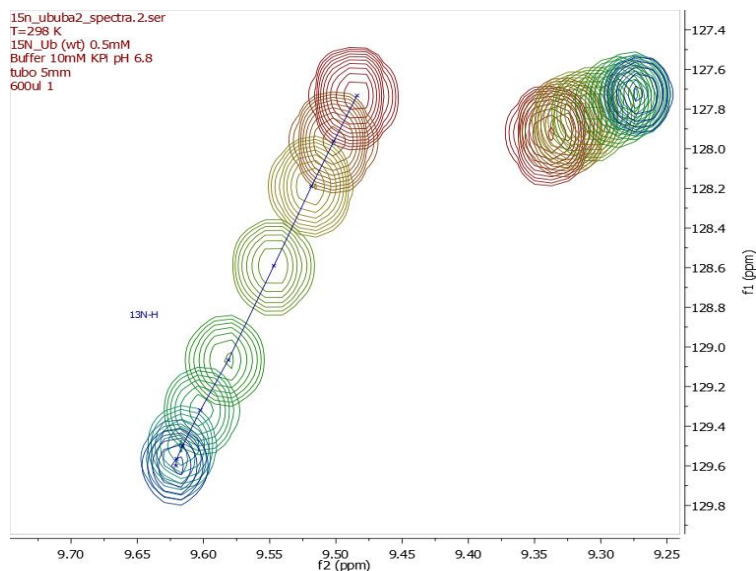


			$\Delta\delta_N$	$\Delta\delta_H$	CSP
	Spectrum	Lt/Pt	F1 (ppm)	F2 (ppm)	CSP (ppm)
1	15n_ububa2_spectra.2.ser	0	127.732	9.484	0
2	15n_ububa2_spectra.24.ser	0.25	127.962	9.502	0.0399442
3	15n_ububa2_spectra.26.ser	0.5	128.191	9.518	0.0794188
4	15n_ububa2_spectra.28.ser	1	128.591	9.547	0.147956
5	15n_ububa2_spectra.30.ser	2	129.066	9.581	0.229745
6	15n_ububa2_spectra.32.ser	3	129.319	9.602	0.274414
7	15n_ububa2_spectra.34.ser	5	129.499	9.616	0.305498
8	15n_ububa2_spectra.36.ser	7	129.565	9.620	0.316772
9	15n_ububa2_spectra.38.ser	9	129.600	9.621	0.321909

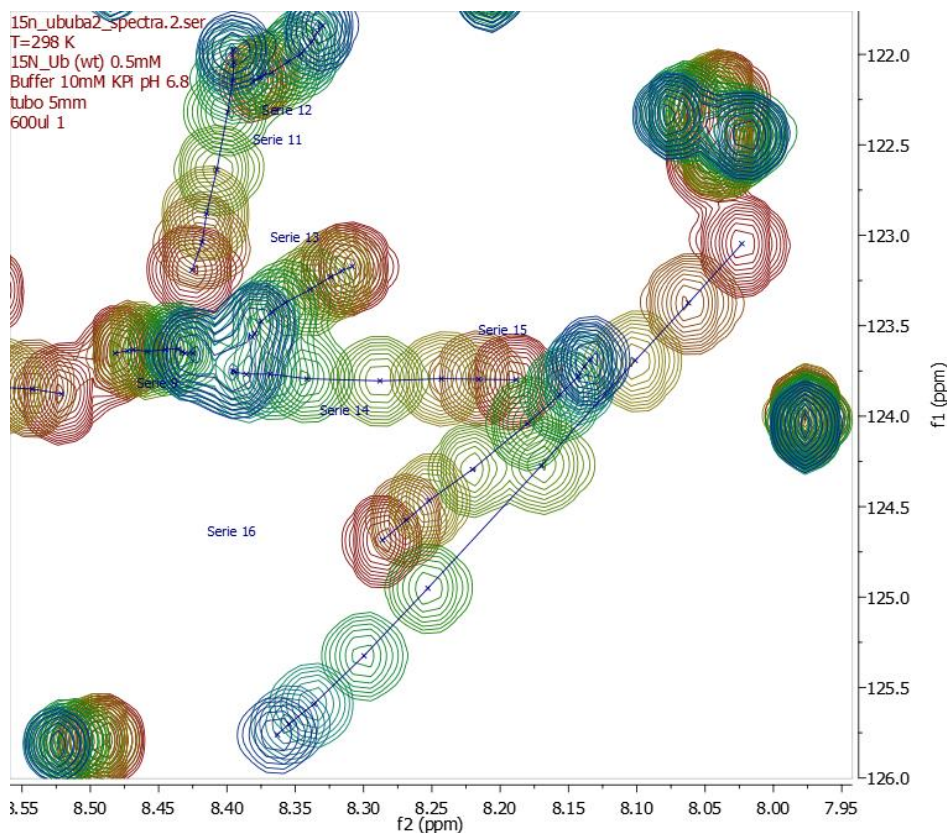


- The CPS values are plotted against the ratios of ligand/protein concentrations and fit to a titration curve to determine the dissociation constant,  $K_d$  and the fitting error ( $\sigma$ ) according to

$$CSP = \frac{\Delta CSP_{max}}{2} \left\{ \left( 1 + \left( \frac{L_T}{P_T} \right) + \frac{K_d}{P_T} \right) - \sqrt{\left( 1 + \left( \frac{L_T}{P_T} \right) + \frac{K_d}{P_T} \right)^2 - 4 \left( \frac{L_T}{P_T} \right)} \right\}$$



- ❑ Multiple reference peaks can be tracked and  $K_d$  calculated for each of them.
- ❑ The average  $K_d$  and standard deviation are automatically computed for them.



Tip: Un-check the peaks that you don't want to be used for the statistics analysis. The results will be automatically updated.

Chemical Shift Perturbation

Results:

General Peaks 9N-H 11N-H 13N-H 44N-H 46N-H

	Peak	Kd	$\sigma(Kd)$	CSP Max	$\sigma(CSP Max)$
2	<input checked="" type="checkbox"/> 11N-H	441.764	18.0262	0.168528	0.00171815
3	<input checked="" type="checkbox"/> 13N-H	399.453	14.3134	0.359911	0.00278215
4	<input checked="" type="checkbox"/> 44N-H	357.353	15.8369	0.177349	0.00174786
5	<input checked="" type="checkbox"/> 46N-H	327.974	12.1341	0.139591	0.000704416
6	<input checked="" type="checkbox"/> 68N-H	364.523	14.2341	0.219173	0.00146839
7	<input checked="" type="checkbox"/> 70N-H	420.419	14.0237	0.307951	0.00210848
1	<input checked="" type="checkbox"/> 9N-H	383.71	11.9505	0.181504	0.000878667
8	<input checked="" type="checkbox"/> Ala101N-H	219.705	5.12601	0.116205	0.000387167
10	<input checked="" type="checkbox"/> Serie 10	340.146	19.9291	0.194521	0.00244017
11	<input checked="" type="checkbox"/> Serie 11	371.154	14.1002	0.213022	0.00140321
12	<input checked="" type="checkbox"/> Serie 12	2038.32	76.4204	0.104121	0.0014684
13	<input checked="" type="checkbox"/> Serie 13	718.146	16.9375	0.112043	0.000638918
14	<input checked="" type="checkbox"/> Serie 14	346.897	12.6531	0.229072	0.00167247
15	<input checked="" type="checkbox"/> Serie 15	508.714	21.0739	0.247936	0.00196028
16	<input checked="" type="checkbox"/> Serie 16	447.74	13.1083	0.610116	0.00368117
9	<input checked="" type="checkbox"/> Serie 9	446.069	29.4601	0.0611427	0.00113123

Average Kd: 337.880037  $\sigma$ : 3.154530

- ❑ Choose Advanced > Chemical Shift Perturbation to open the CSP Panel.
- ❑ Click Open and enter the relevant info files and Base directory (where the 2D HSQC spectra are located). Click OK to start the auto processing.

The screenshot shows the 'CSP Open' dialog box with the following fields:

- Titration file: C:/Mestrelab/Projects/Demo Data/CSP/CSP-UNIVR/titration.bt
- Ligands file: C:/Mestrelab/Projects/Demo Data/CSP/CSP-UNIVR/titration.bt
- Peaks file: C:/Mestrelab/Projects/Demo Data/CSP/CSP-UNIVR/peaklist.bt
- Base directory: s/Demo Data/CSP/CSP-UNIVR/ubauba2/15n\_ububa2\_spectra

Callouts point to the following files and their contents:

- Titration file:**

```
[P] uM      500
EXPNO      [L]/[P]
REF
2           0.00

L1
4           125
6           250
8           500
10          1000
12          1500
14          2500
16          3500
18          4500

L2
24          125
26          250
28          500
30          1000
32          1500
34          2500
36          3500
38          4500
```
- Ligand file:**

```
L1 ububa
L2 ububa2
|
```
- Peaks file:**

Assignment	w1	w2
9N-H	105.941	7.562
11N-H	121.982	7.190
13N-H	127.733	9.484
44N-H	122.410	9.051
46N-H	133.093	8.945
68N-H	119.736	9.148
70N-H	126.581	9.097

There are 17 2D <sup>1</sup>H/<sup>15</sup>N HSQC spectra in the Base directory. They are used based on the info in the Titration file

- ❑ If you put multiple ligands in the Titration file, then they will be processed and saved as multiple Mnova documents.
- ❑ Use the Document Menu to switch between the documents for details.

## Titration file

[P]uM	500
EXPNO	[L]/[P]
REF	
2	0.00
L1	
4	125
6	250
8	500
10	1000
12	1500
14	2500
16	3500
18	4500
L2	
24	125
26	250
28	500
30	1000
32	1500
34	2500
36	3500
38	4500

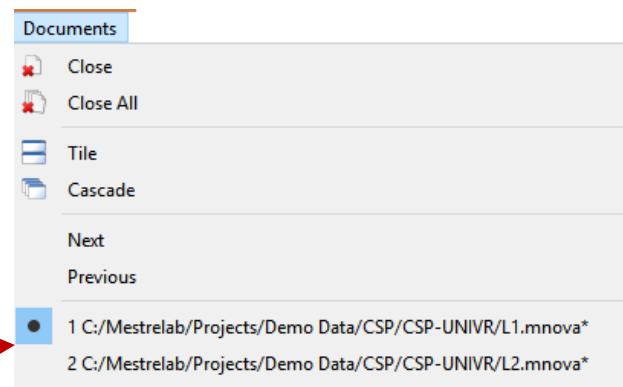
Spectra and concentrations for Ligand #1

Spectra and concentrations for Ligand #2

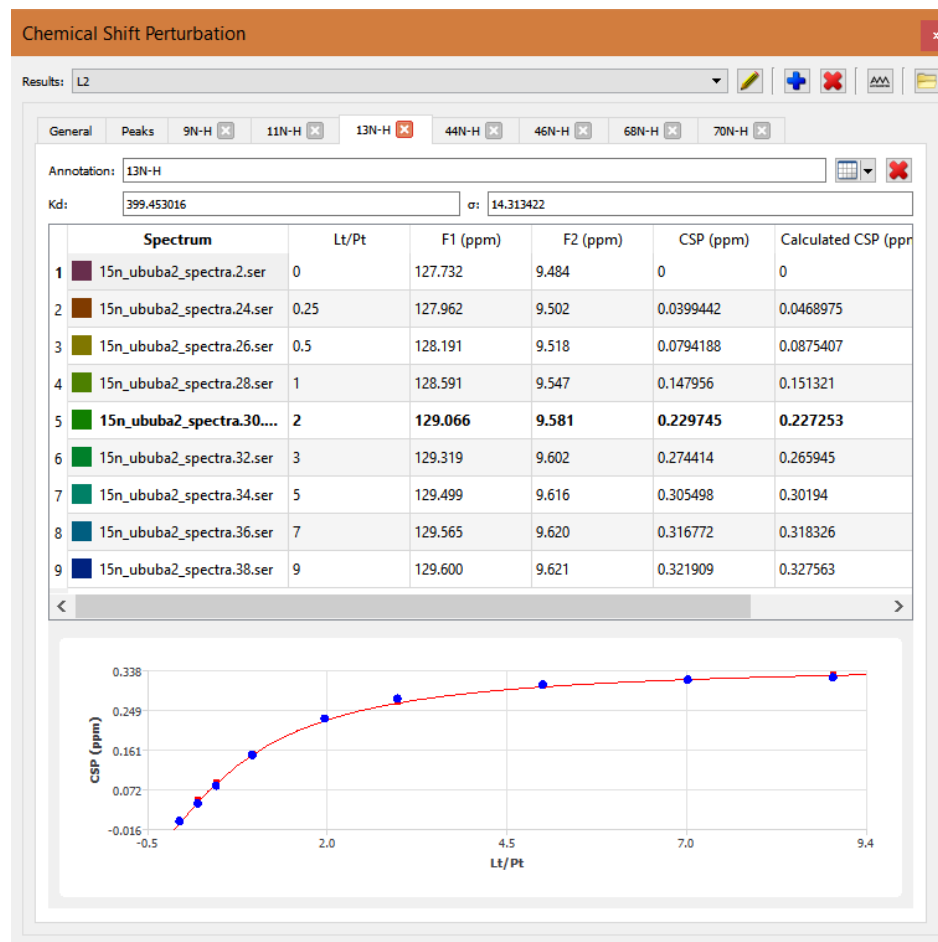
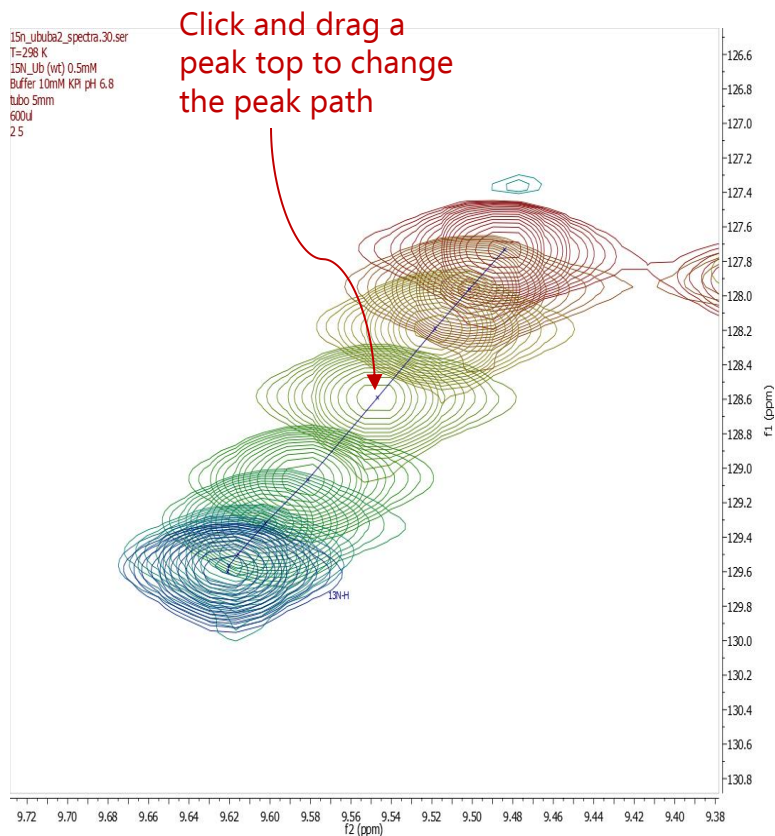
## Ligand file

```
L1 ububa
L2 ububa2
|
```

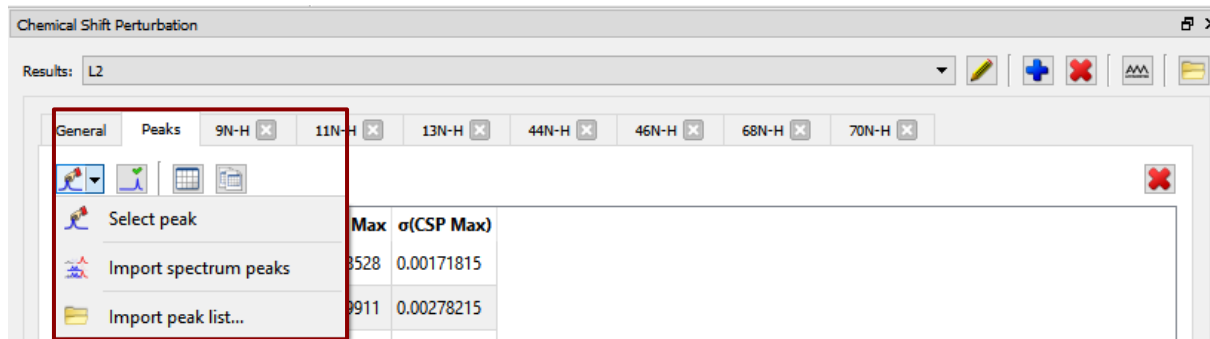
Labels for all ligands



- ❑ From the Peaks Tab in CSP Panel, double click on a row to switch to display its details and zoom to that peak path in the spectra.
- ❑ Click on any peak top and drag to change the peak path. The CSP and Kd results are updated automatically.

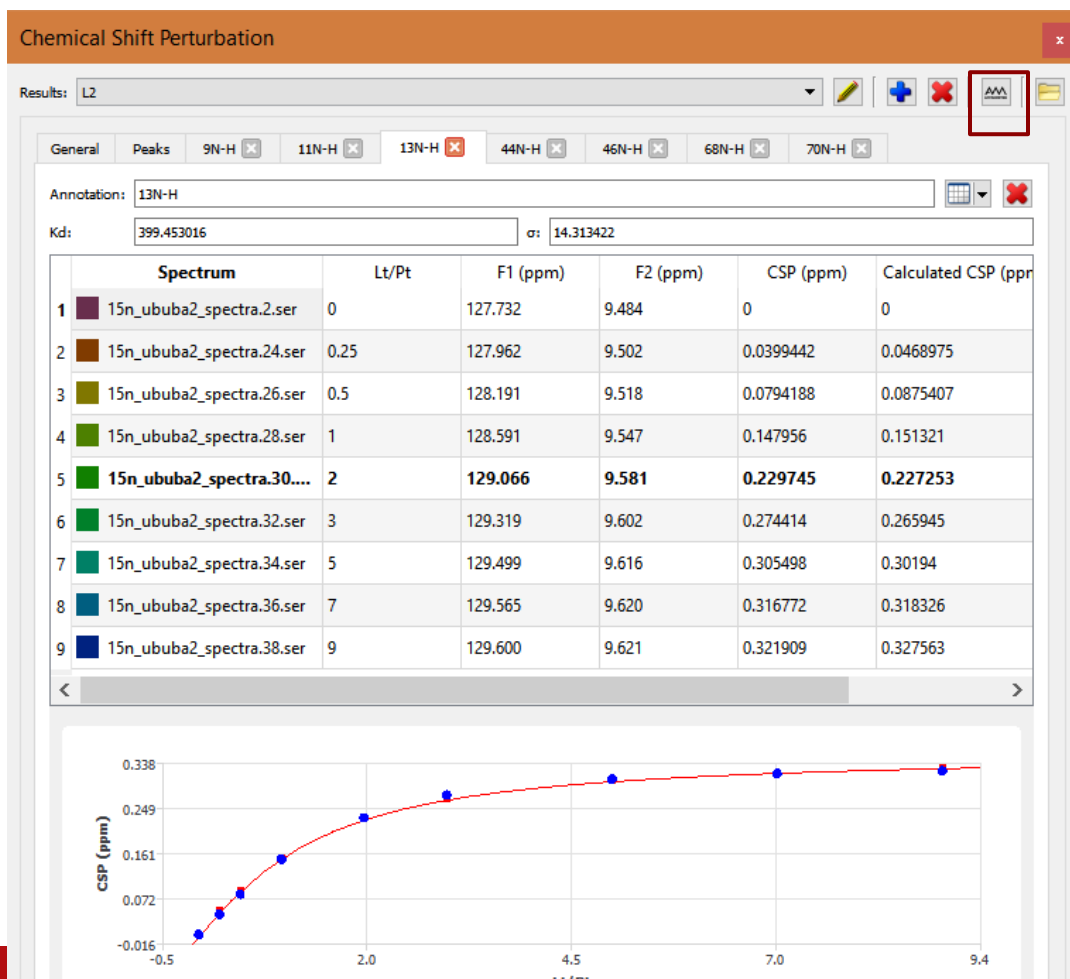


- ❑ You can enter the peaks in 3 ways:
  - ❑ **Select peak:** click to select reference peaks in the stacked plot.
  - ❑ **Import spectrum peaks:** do auto or manual peak picking in the reference spectrum first, and use those as the reference peaks.
  - ❑ **Import peak list:** Use peaks in a peak assignment table as reference peaks.
- ❑ Mnova CSP automatically track peaks across the titration spectra, and you can manual correct the peak paths if necessary.



	Max	$\sigma(\text{CSP Max})$
	3528	0.00171815
	9911	0.00278215

- We collaborate with AffiniMeter Inc. for ligand-protein binding studies.
- The CSP results can be sent to the AffiniMeter for further analysis.
- See <https://www.affinimeter.com/> for more details.



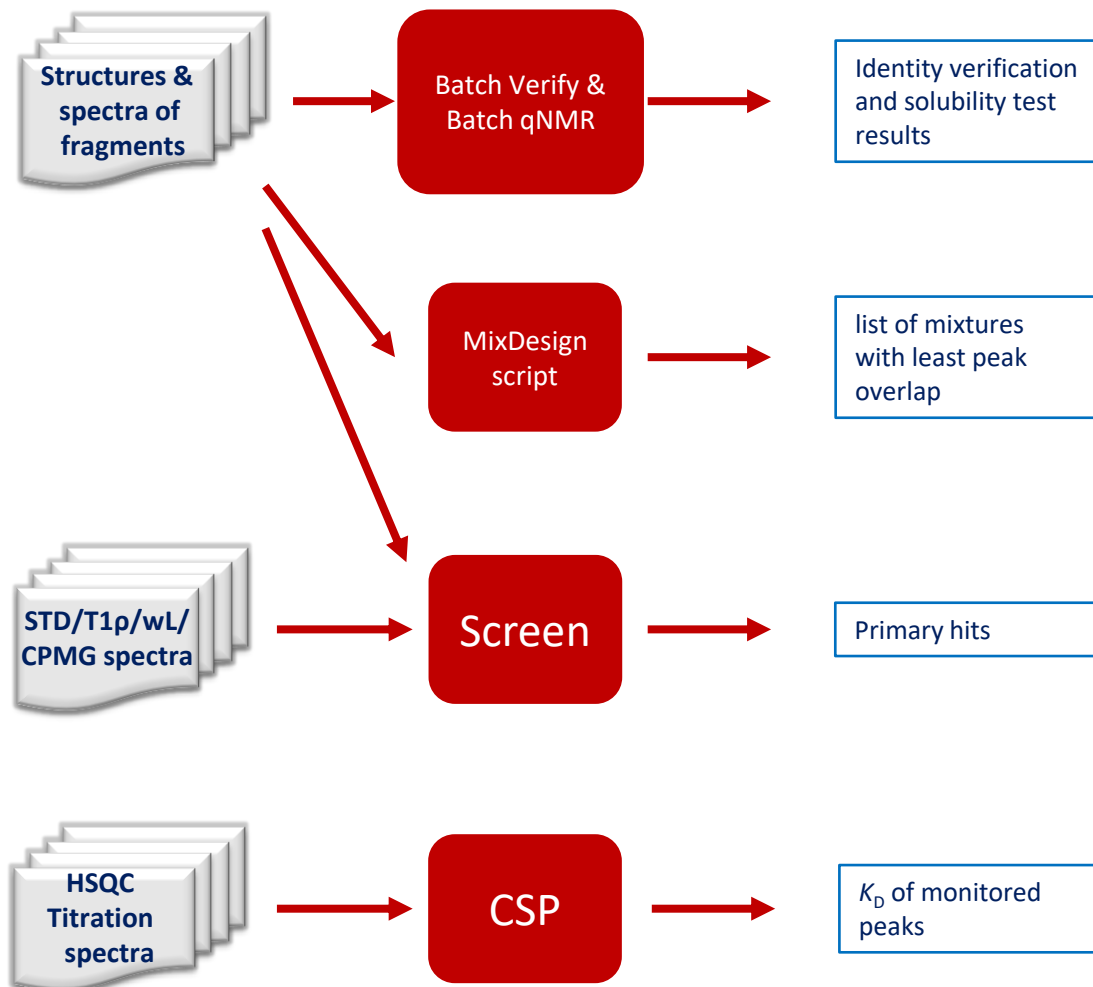
- H-N HSQC titration spectra, 8 points for each ligand.
- Two ligands.



## Conclusions



They can also work independently  
& without a database






- Flexible: use tools when you need.
- Processing and peak picking reference spectra at each run - Not an efficient way to manage and reuse your reference spectra.
- Results saved as flat files - not efficient for info management and data mining.

## Our collaborators

-  Pfizer (La Jolla): Jiangli Yan and Wei Wang
-  Novartis (Cambridge): Xiaolu Zhang and Jasna Fejzo
-  Novartis (Emeryville): Andreas Lingel and Alexandra Frommlet
-  Abbvie: Andrew Petros and Andrew Namanja

## Developers and product managers at Mestrelab Research

-  Chen Peng, Manuel Perez, and Silvia Mari
-  Agustin Barba and Jose Garcia
-  Carlos Cobas, Stan Sykora, Santi Dominguez

 Thank you for attending the webinar!  
Questions?