# TopSpin

## Fragment Based Screening Analysis Suite

User Manual

Version 001

Innovation with Integrity

NMR

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# Contents

# Contents

# 1 Introduction to Fragment Based Screening (FBS)

Ligand observed NMR is a powerful technique for the screening of fragment like small molecules to biomolecular targets in solution. In recent years methodological and technical advancements have enabled NMR based fragment screening to be performed in full automation and with significantly reduced consumption of unlabeled target protein and fragments. NMR based fragment screening has proven to produce high-quality hits, as QC of fragments can be performed from 1D $^{1}$H spectra of the screening mixture. The analysis of NMR based screening data, however, has proven to be cumbersome and a major pain point. In many cases, users have implemented homebuilt tools to facilitate the workflow.

*Here we present a novel software tool for interactive analysis of NMR based screening data that is embedded within the software suite from Bruker, which now supports the workflow from data acquisition and processing to data analysis and hit reporting.* The three most popular NMR experiments for fragment screening, Saturation Transfer Difference (STD), waterLOGSY, and relaxation based methods, are automatically identified. Reference 1D $^{1}$H spectra of fragments are recognized by unique identifiers of the employed molecules and presented to the user in multi-display mode together with the screening spectra. Hits are visually identified and selected by mouse click on the display. The results are stored in a project file that is automatically loaded on program launch. The tool allows the most flexible implementation in individual laboratory environments with few restrictions with regard to data storage and preparation; for instance, no databases need to be prepared. In addition, automation routines for NMR based screening experiments that are part of the latest TopSpin release are presented.

The goal of this documentation and our software developments are to enable the drug discovery community to setup NMR based screening experiments, facilitate data analysis and consequently reduce the hurdle for non-experts to use NMR for Fragment Based Lead Discovery (FBLD).

# Introduction to Fragment Based Screening (FBS)

# 2 Preparation of Data for Bruker FBS Module

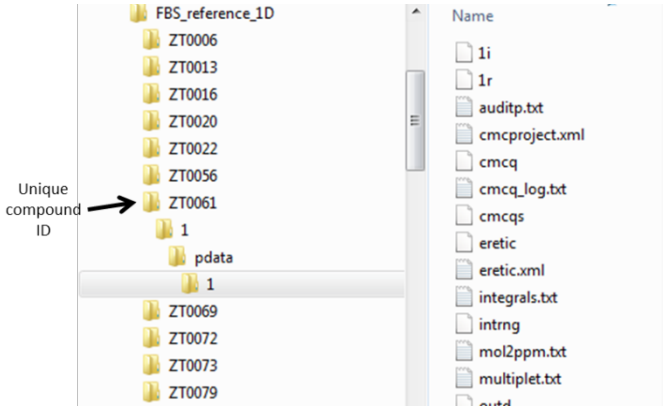
The Fragment-based Screening (FBS) tool in TopSpin displays fragment screening data and reference 1D spectra of compounds in a library in multi display mode for analysis and identification of hits. In most cases, compounds from a library are pooled in mixtures which are then screened against a target. The FBS tool in TopSpin requires the screening and reference data fulfill the following three requirements:

- Reference 1D spectra of each fragment in the library are stored in an individual data set and stored in one folder. The data set name should be the unique compound ID.
- The screening experiments for each mixture are stored in one data set. The name of this data set has to be unique within the screening campaign.
- Screening spectra that should be displayed in the FBS tool must have a SPECTYP set in the status processing parameters.

A mixture definition table links reference spectra of compounds used to make the mixtures for screening. In this chapter, the data organization and the interdependencies of the mixture definition table, reference spectra and screening spectra is described.

## 2.1 Preparation of Reference 1D Spectra of Individual Compounds

The FBS tool requires 1D $^{1}$H reference spectra of all individual compounds in the fragment library to be stored in individual data sets with the unique compound ID as data set name.




If reference 1D spectra are measured with CMCq, the required data structure is automatically generated.

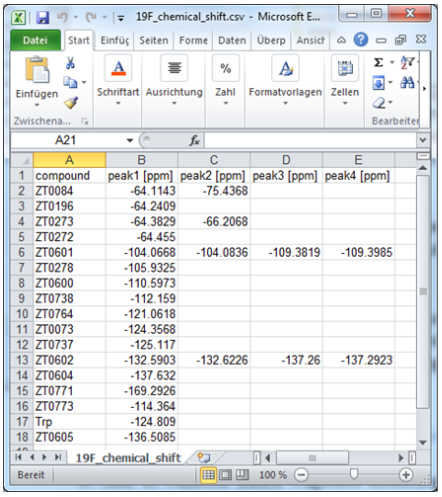
Bruker can provide a conversion tool that utilizes a table in Microsoft Excel format containing data set name, experiment number and unique compound ID and copies then the respective experiment for each fragment in the library into a new data set with the unique compound ID as the name and one 1D experiment of the compound. The original data remain untouched, but a copy in the format used for the FBS tool is created.

If the experiment folder of the reference 1D spectrum for a compound in the library contains a MOL file as description of the molecular structure, then it will be displayed in the FBS analysis tool.

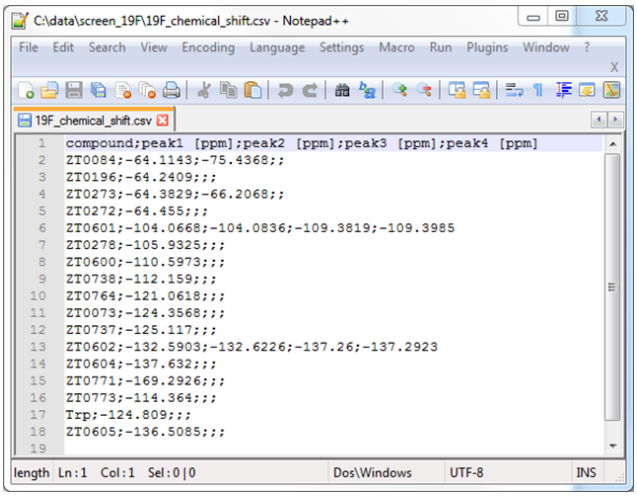
The installation of reference spectra in the required format has to be done only once and can then be used for all subsequent screening campaigns. New molecules can be added to the reference spectra folder by adding additional data sets named with the compound ID.

## 2.2 Preparation of 19F Chemical Shift Look Up Table

In case of fluorine based fragment screening, the FBS tool also allows use of a chemical shift look up table (peak list) for the compounds in the library that is used for screening. Peaks of the ligands in each mixture are then shown on top of the screening spectra. Alternatively, reference spectra and a peak list can be used. The peak list must be in semicolon separated csv file format. An example is shown below.




| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | compound | peak1 [ppm] | peak2 [ppm] | peak3 [ppm] | peak4 [ppm] |
| 2 | ZT0084 | -64.1143 | -75.4368 | | |
| 3 | ZT0196 | -64.2409 | | | |
| 4 | ZT0273 | -64.3829 | -66.2068 | | |
| 5 | ZT0272 | -64.455 | | | |
| 6 | ZT0601 | -104.0668 | -104.0836 | -109.3819 | -109.3985 |
| 7 | ZT0278 | -105.9325 | | | |
| 8 | ZT0600 | -110.5973 | | | |
| 9 | ZT0738 | -112.159 | | | |
| 10 | ZT0764 | -121.0618 | | | |
| 11 | ZT0073 | -124.3568 | | | |
| 12 | ZT0737 | -125.117 | | | |
| 13 | ZT0602 | -132.5903 | -132.6226 | -137.26 | -137.2923 |
| 14 | ZT0604 | -137.632 | | | |
| 15 | ZT0771 | -169.2926 | | | |
| 16 | ZT0773 | -114.364 | | | |
| 17 | Trp | -124.809 | | | |
| 18 | ZT0605 | -136.5085 | | | |



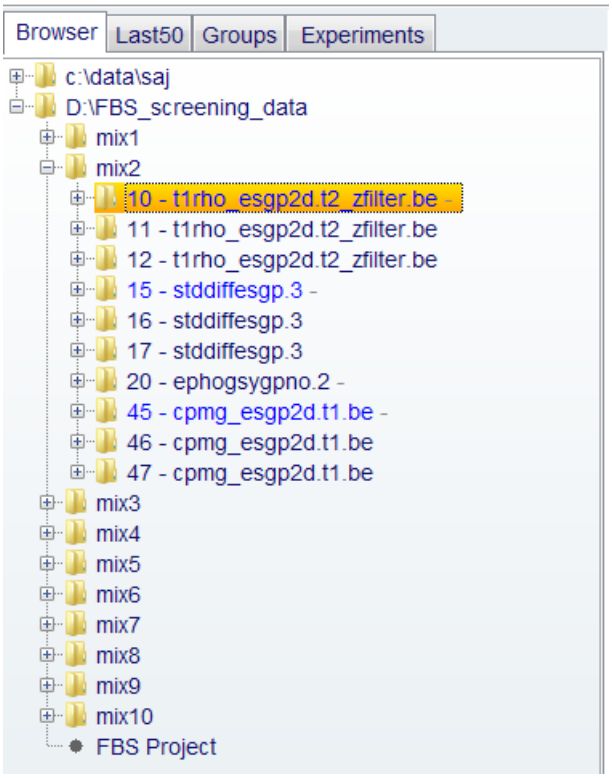

```
compound;peak1 [ppm];peak2 [ppm];peak3 [ppm];peak4 [ppm]
ZT0084;-64.1143;-75.4368;;
ZT0196;-64.2409;;;
ZT0273;-64.3829;-66.2068;;
ZT0272;-64.455;;;
ZT0601;-104.0668;-104.0836;-109.3819;-109.3985
ZT0278;-105.9325;;;
ZT0600;-110.5973;;;
ZT0738;-112.159;;;
ZT0764;-121.0618;;;
ZT0073;-124.3568;;;
ZT0737;-125.117;;;
ZT0602;-132.5903;-132.6226;-137.26;-137.2923
ZT0604;-137.632;;;
ZT0771;-169.2926;;;
ZT0773;-114.364;;;
Trp;-124.809;;;
ZT0605;-136.5085;;;
```

## 2.3 Preparation of Fragment Screening Data of Mixtures/Cocktails

The FBS analysis suite in TopSpin requires that spectra of each mixture are located in a separate directory/data set (e.g. mix1, mix2 …). In each data set, different experiments measured on the individual mixture are stored in separate experiment numbers. Experiments can be added at any time.

The type of experiment is recognized by the SPECTYP setting in the status processing parameters, which is demonstrated in *Setting the SPECTYP in Screening Experiments [▷ 9]* and *Psuedo 2D Experiments and SPECTYPs [▷ 10]*.

The exact experiment number is irrelevant. However, for experiments with accompanying reference spectra, like T1rho, after splitting the lower experiment number (expno) is expected to be the reference experiment (e.g. 10 ms) and the higher expno is expected to be the binding experiment (e.g. 200 ms).

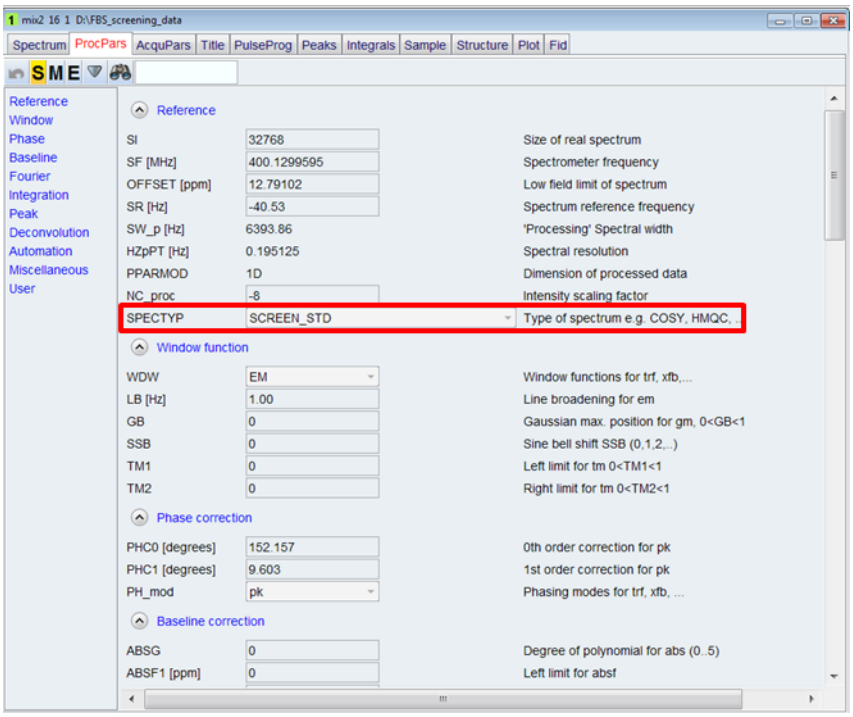



We recommend storing all data sets for the mixtures in a directory named with the project name, for instance the name of the target protein combined with the date.

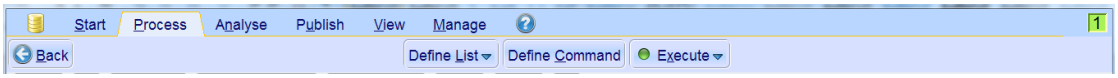
## 2.4 Setting the SPECTYP in Screening Experiments

The SPECTYP of each experiment is used to recognize and display specific experiment types. Standard SPECTYPs that come with TopSpin for fragment screening applications are SCREEN_STD, SCREEN_WLOGSY, SCREEN_T2 and SCREEN_T1R. User defined SPECTYPs can be added in the setup dialogue within the tool.

The SPECTYP must not only code for the NMR specific experiment type but can also code for chemistry related characteristics. For instance, an STD experiment that is measured on a mixture containing a competing ligand can be called SCREEN_STD_COMPETITION. This way, two STD experiments on the same mixture, one with competitor and one without can be displayed for a specific mixture.



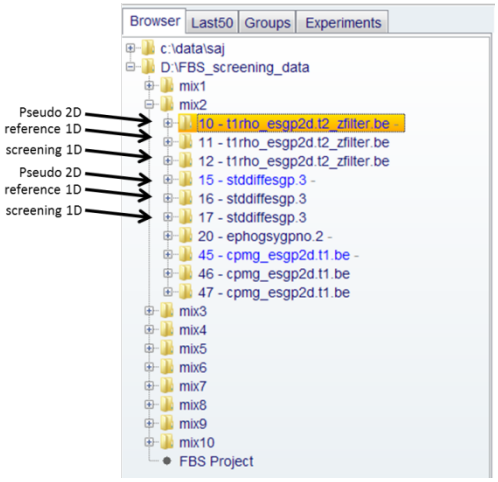

The SPECTYP can be changed retrospectively by typing **1s SPECTYP XXXX**, where XXXX is the SPECTYP that should be assigned to the respective experiment. For a list of experiments, the serial processing function in TopSpin can be used to first search data sets (Define List) for instance based on the same pulse program and then the command **1s SPECTYP XXXX** (Define Command) can be applied to the list of experiments.



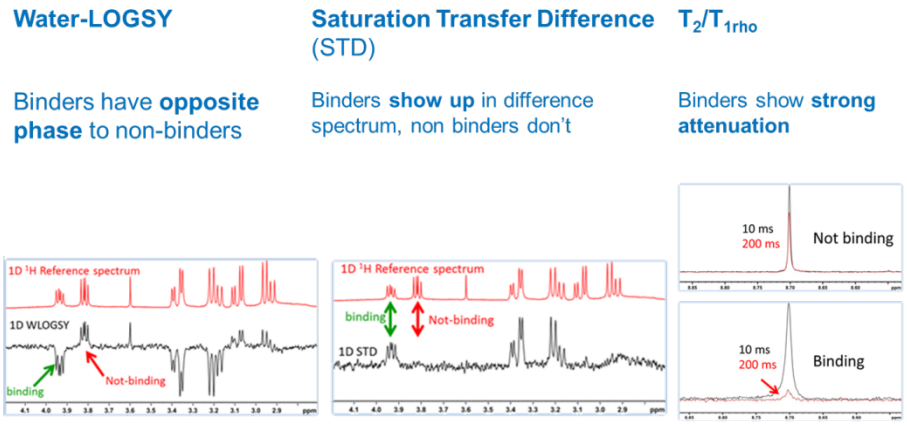

The serial processing function within TopSpin can be found under Process □ Advanced □ Process Dataset List (serial).

## 2.5 Psuedo 2D Experiments and SPECTYPs

Oftentimes, screening spectra are measured as pseudo 2D spectra. This applies for example to the parameter sets SCREEN_STD, SCREEN_T2 and SCREEN_T1R in the TopSpin suite. When standard processing parameters and automation programs are used, these pseudo 2D spectra are processed in 1D experiments following the experiment of the pseudo 2D. The SPECTYP of pseudo 2D spectra is automatically transferred to 1D spectra resulting from its processing. The FBS tool assumes that the resulting 1D spectra are one reference spectrum (i.e. off-resonance STD spectrum or relaxation based spectrum with short relaxation delay) and the screening spectrum with higher experiment number and same SPECTYP (i.e. STD difference spectrum or relaxation based spectrum with long relaxation delay).




The reference spectrum is usually overlaid and compared with the screening spectrum. Binding ligands can be distinguished from non-binders by observed effects.




## 2.6 Installation of Blank Spectra of Mixtures

It is often the case in fragment-based screening, that blank experiments are measured. These experiments are the same NMR experiments as for the screening against a protein target but without the protein present in the mixture. The second data folder is therefore indicated with **Reference Blank Experiment Folder**, but can also contain a second set of screening data.

In the setup dialogue a second data folder consisting of screening data sets on mixtures with no added protein can be entered which must be structured the same way as the screening data sets of mixtures with protein (*Preparation of Fragment Screening Data of Mixtures/ Cocktails [▶ 8]*. Optionally, this entry in the setup dialogue can be empty, if no second screening data folder is available or required.

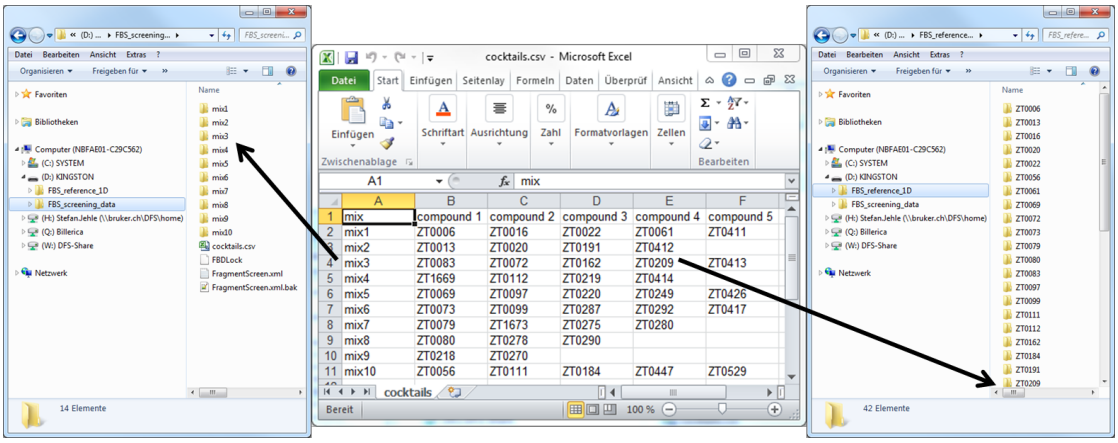
Similarly, the type of experiment is recognized by the SPECTYP setting in the status processing parameters, which is demonstrated *Setting the SPECTYP in Screening Experiments [▶ 9]*.

The exact experiment number is irrelevant. However, for experiments with accompanying reference spectra, like T1rho, after splitting the lower experiment number is expected to be the reference experiment (e.g. 10 ms) and the higher expno is expected to be the binding experiment (e.g 200 ms).

For a data set to be displayed in the FBS module, all spectra need to have a SPECTYP defined. Here for the blank experiments without protein we suggest to use the SPECTYPs SCREEN_T1R_BLANK, WLOGSY_BLANK etc.

## 2.7 Cocktail Definition Table

The cocktail definition table contains information on which compounds are pooled in a specific mixture. The naming of the mixtures and compound IDs must match the naming of the data sets in which the experiments are stored, for instance if **mix1** is used in the cocktail definition table, then the data set containing screening experiments for mix1 must have the same name. The same is true for the compound IDs used in the table, which must match the data set name in which the reference 1D spectrum is stored for a specific compound.




| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| 1 | mix | compound 1 | compound 2 | compound 3 | compound 4 | compound 5 |
| 2 | mix1 | ZT0006 | ZT0016 | ZT0022 | ZT0061 | ZT0411 |
| 3 | mix2 | ZT0013 | ZT0020 | ZT0191 | ZT0412 | |
| 4 | mix3 | ZT0083 | ZT0072 | ZT0162 | ZT0209 | ZT0413 |
| 5 | mix4 | ZT1669 | ZT0112 | ZT0219 | ZT0414 | |
| 6 | mix5 | ZT0069 | ZT0097 | ZT0220 | ZT0249 | ZT0426 |
| 7 | mix6 | ZT0073 | ZT0099 | ZT0287 | ZT0292 | ZT0417 |
| 8 | mix7 | ZT0079 | ZT1673 | ZT0275 | ZT0280 | |
| 9 | mix8 | ZT0080 | ZT0278 | ZT0290 | | |
| 10 | mix9 | ZT0218 | ZT0270 | | | |
| 11 | mix10 | ZT0056 | ZT0111 | ZT0184 | ZT0447 | ZT0529 |

The cocktail definition file must be in csv format (semicolon separated) which can be generated from Microsoft Excel. Once the FBS tool in topspin is launched, the cocktail definition file will be displayed as a guide to navigate through the screening data and reference spectra.

# 3 Configuration of FBS Analysis

This is only needed the first time you are working on a particular screening project.

## 3.1 Start TopSpin3.5-pl7

Start TopSpin 3.5 with patch level 7 which has the FBS analysis module included by default. There is no additional license needed for the FBS analysis suite.

## 3.2 Navigate to the FBS Data Set

The FBS analysis tool will only work on datasets in the folder format of mix1, mix2, mix3…

If it's not already mounted in your preferred directories do the following.

- Right click on the pane displaying the folder-tree in TopSpin.
- Choose **Add new directory**.
- Click **Browse** and navigate to your data folder.
- After double-clicking on it, it will appear in the navigation pane.
- Open a 1D screening spectrum in any of the mixture datasets in TopSpin.

## 3.3 Start the FBS Analysis Tool

- In TopSpin, open the **Analysis** tab.
- Open the **More**-menu to the right.
- Choose **Fragment based screening**.

Alternatively, just type **fbs** on the command line

## 3.4 Configuration of the Tool


If the FBS tool was not previously configured or if started the first time from a screening project, a pop up menu will appear that allows configuration of the tool.

The configuration setup can be accessed anytime through the **Open settings** dialog button next to the Bruker logo on the project table.

- Click on this icon next to the Bruker logo on the top right of the FBS window.

### 3.4.1 The Directories Tab

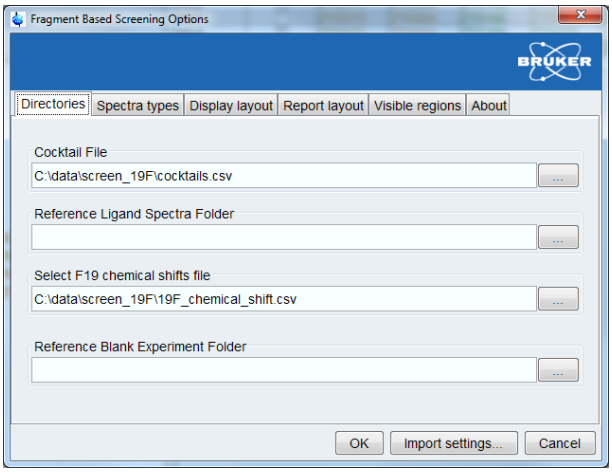
A new window with several tabs will appear. In the first tab **Directories** you can set the path to the files needed.

The **Cocktail File** describes the composition of the mixtures.

The **Reference Ligand Spectra Folder** contains spectra of all the individual ligands

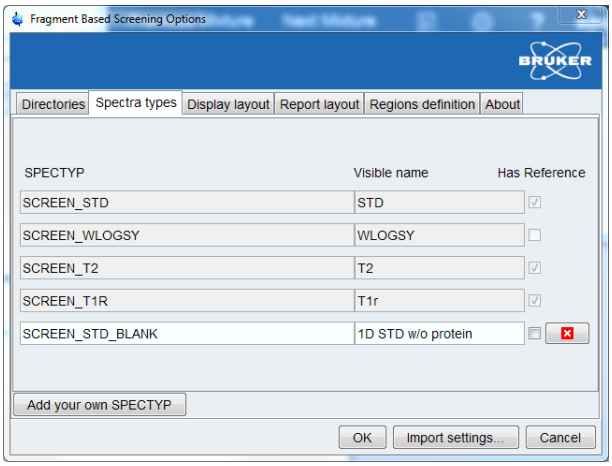
The **Reference Blank Experiment Folder** contains the spectra of the mixtures without protein or a second data folder with screening spectra. The field for the blank experiment folder can alternatively be left empty.

- Set the paths to the cocktail definition file, reference ligand spectra folder and, optionally, reference blank experiment folder (second data folder) in the first tab of the setup dialogue as indicated in the screenshot below.
- In case the FBS tool is launched from a 1D $^{19}$F screening spectrum, the setup dialogue from above is extended by another field that allows the user to load a $^{19}$F chemical shift list for the compounds in the fluorine library. The respective chemical shifts will then be displayed on top of the screening spectra for each compound in a mixture. In addition, reference $^{19}$F spectra can be loaded. Please note, that a MOL file for display of the chemical structure is only displayed if a reference ligand spectrum folder containing a MOL file is present.




### 3.4.2 The Spectra Types Tab

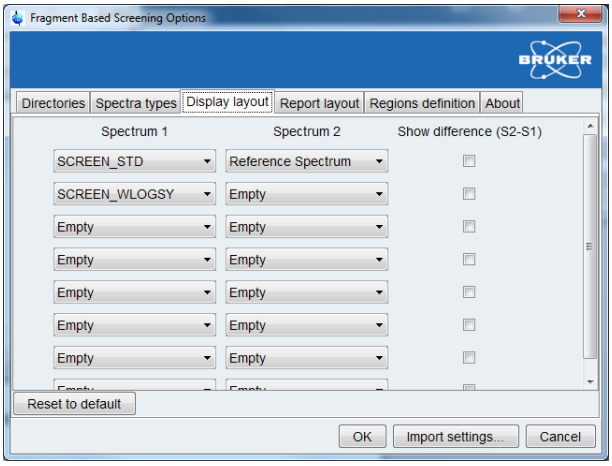
- Next, go to the **Spectra types** tab. Default spectra types are preset.
- Additional user defined spectra types can be added by clicking on **Add your own SPECTYP**. To display user defined spectra types, a SPECTYP defined in the status processing parameters of the experiment must be selected (see *Setting the SPECTYP in Screening Experiments [▶ 9]*) and on the right hand side a name that will be displayed in the FBS interface must be entered.
- If a reference spectrum is available, tick the check box **Has Reference**. The exact experiment number is irrelevant. However, for experiments with internal reference spectra, like T1rho or STD spectra, after splitting the lower experiment number is expected to be the reference experiment (e.g. 10 ms T1rho or STD off-resonance) and the higher expno is expected to be the binding experiment (e.g 200 ms or STD difference).

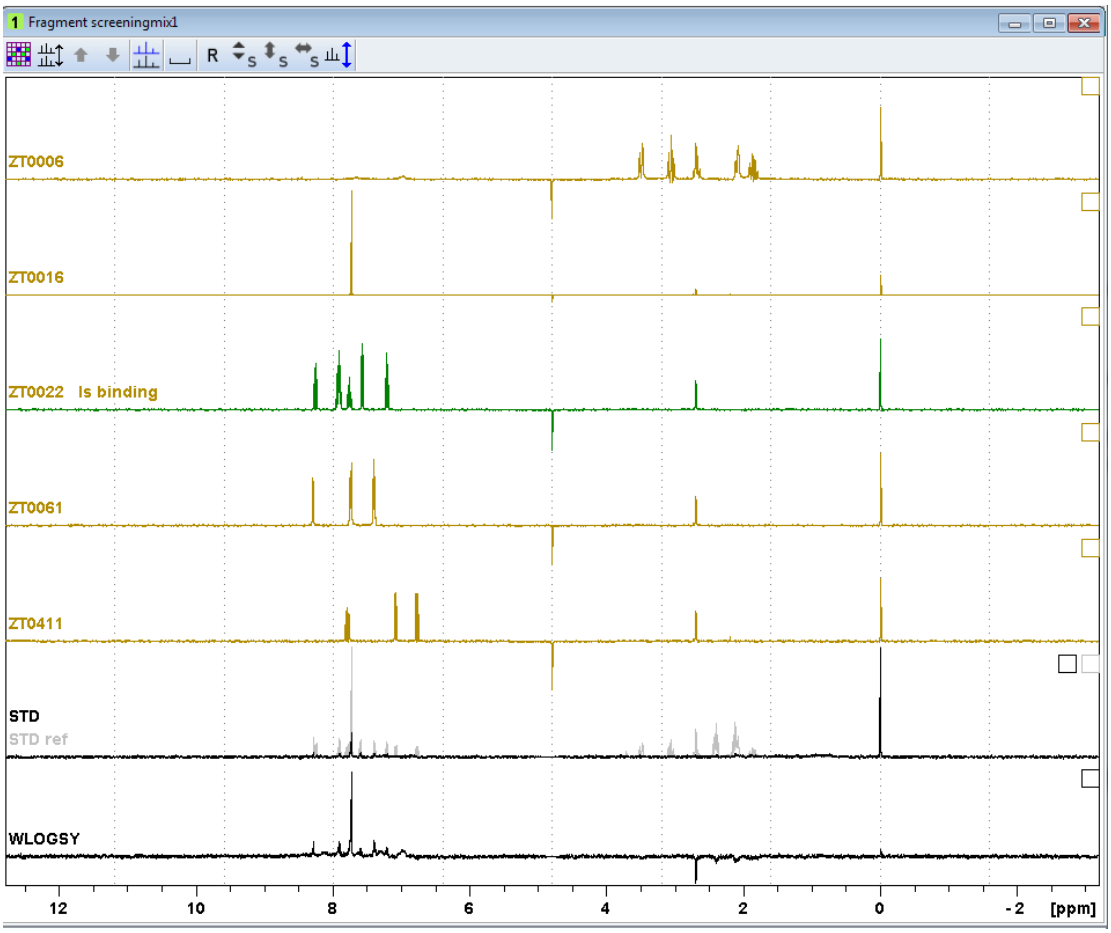



- User defined spectra types can be deleted by clicking on the button with the white cross in a red square, right of the user defined SPECTYP entry box

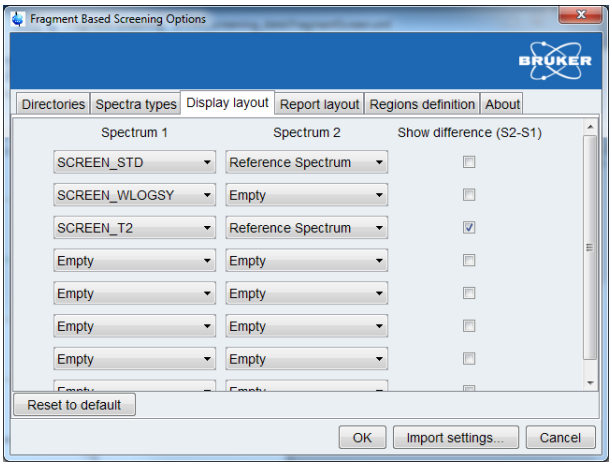
## 3.4.3 The Display Layout Tab

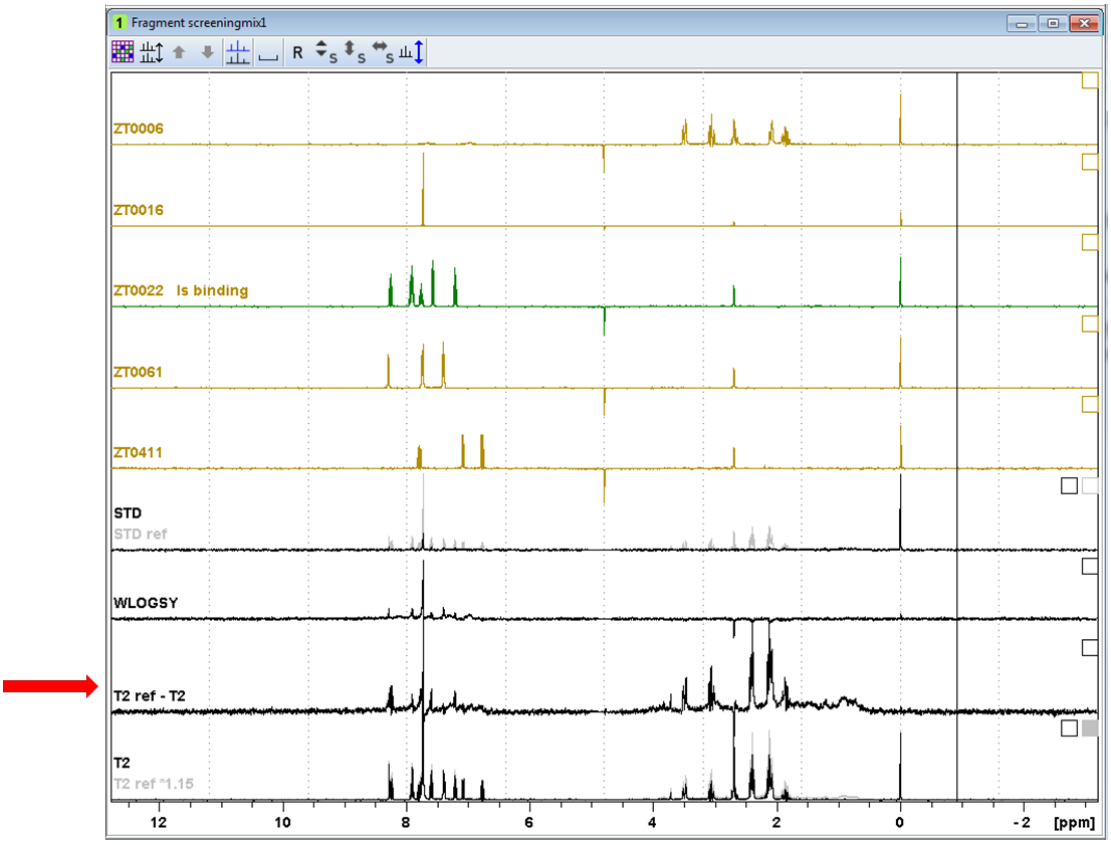
- Next, go to the **Display layout** tab. Here the sequence of spectra is defined (from bottom to top) which will be displayed in the spectrum window.
- In the screenshot below an example is shown in which SCREEN_WLOGSY is shown in one trace and SCREEN_STD and its reference spectrum are shown overlaid in another trace.
- The user can generate the most useful display layout for the individual screening project.






- On top, 1D spectra of the compounds in the mixture will be displayed.
- Screening spectrum 1 will be directly overlaid with spectrum 2 in one trace as shown here for the STD difference spectrum and STD reference spectrum (off-resonance spectrum).
- When using the Bruker provided parameter sets, the au-program proc_std automatically processes the pseudo2D STD spectrum, copies in expno+1 the off-resonance spectrum and in expno+2 the difference spectrum. The latter is generated by subtracting the on-resonance spectrum from the off-resonance spectrum on the time-domain.
- If the checkbox **Show difference (S2-S1)** is ticked, an additional trace will be shown on top with the difference Spectrum 1 subtracted from Spectrum 2. In case of relaxation spectra, the binding spectrum with long relaxation time (i.e. 200 ms) should be displayed as spectrum 1 which is then subtracted from the reference spectrum (i.e. 10 ms). See below for an example.



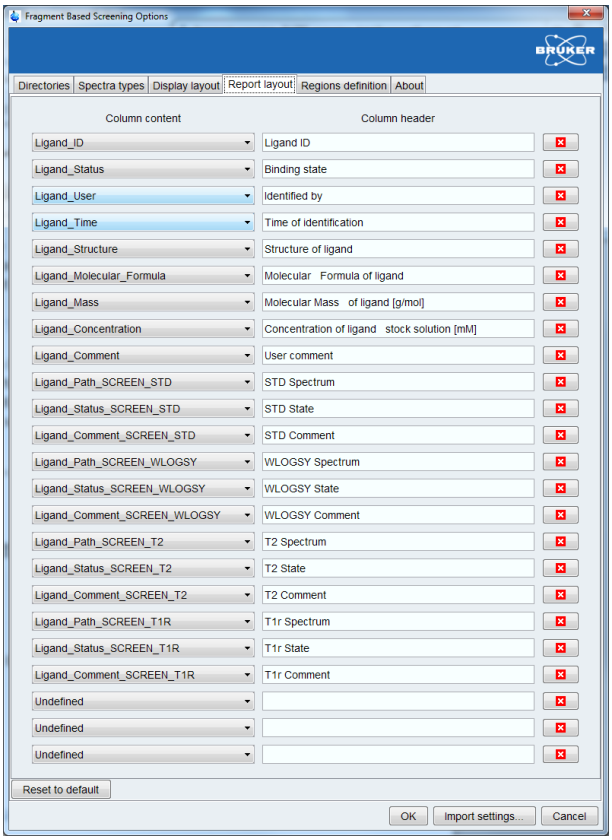

- If STD spectra should be displayed as difference of on- and off-resonance experiment, then the off resonance spectrum should be displayed in spectrum 2 and the on resonance experiment in spectrum 1. The difference will be displayed on top.
- When using the difference display of two spectra, the conventional difference display functions from TopSpin multi-display are available, i.e. spectra can be shifted with respect to each other by selecting one spectrum and moving it with the  button on top of the display.

### 3.4.4 The Report Layout Tab

A report from the screening project can be generated. All relevant data, mixtures, ligand IDs, ligand states, experiment specific states, path where screening spectra are stored etc. can be exported in Microsoft Excel format.

In the Report layout tab, the user can select which information should be exported and the column of the Microsoft Excel file where it appears. This functionality should provide compatibility with most laboratory information systems.

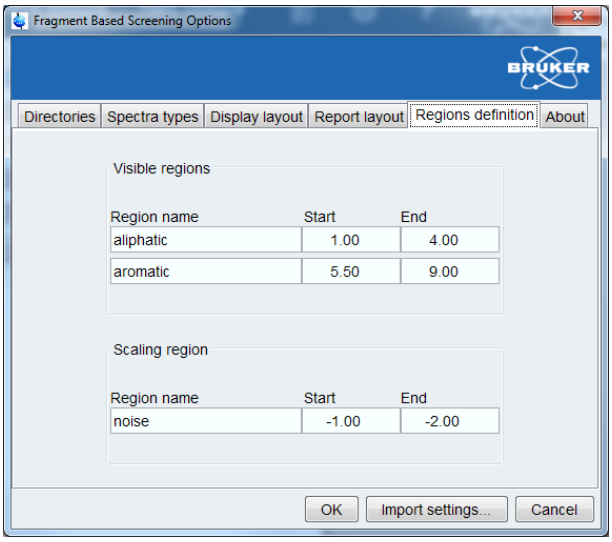
In the Report layout tab, each line represents the information content that will be exported in one column. The first line will be exported to column A, second line to column B etc. On the right a user defined column header can be selected. The button to the right of each line allows the user to clear contents of this line.



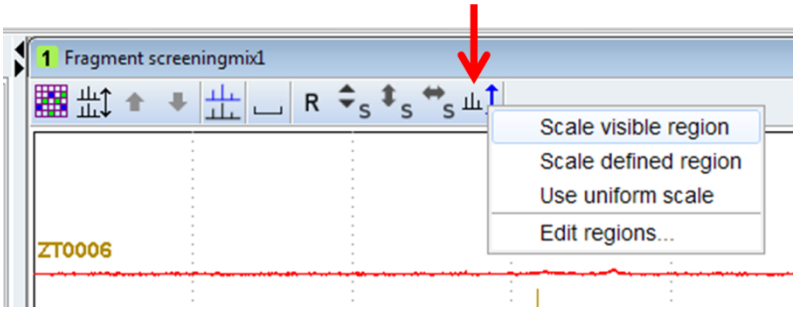

### 3.4.5 The Regions Definition Tab

In the **Regions definition** tab, the user can define spectral regions for quick display (Visible regions) and a Scaling region which is used for scaling multiple spectra.

- For the visible regions function, usually an aromatic and aliphatic region is selected. These two regions can be displayed one or the other by clicking on the respective button in the multi-display. 

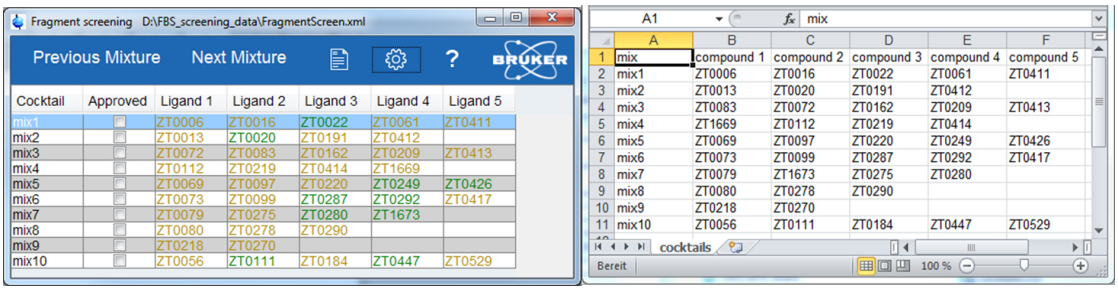



- In addition, a **Scaling region** can be selected. The scaling region can be for example a noise region or a signal region (TSP or competitor signal). The selected spectral region will be used to scale all visible spectra to the same level after clicking the scaling button in the multi-display.



# 4 Working with the FBS Analysis Tool

After clicking **OK** in the setup dialogue, the multi-display of the screening project will be generated and the project table, which represents the cocktail definition table, will be shown.

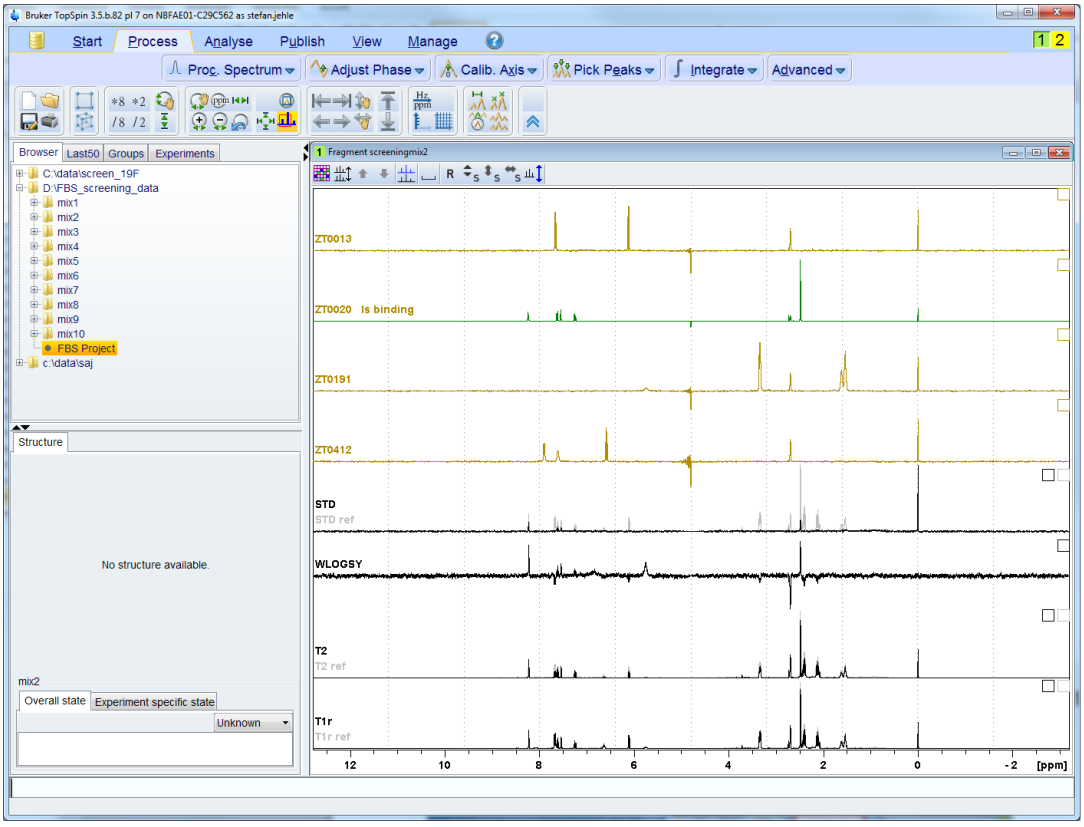



## 4.1 Analysis of Mixtures

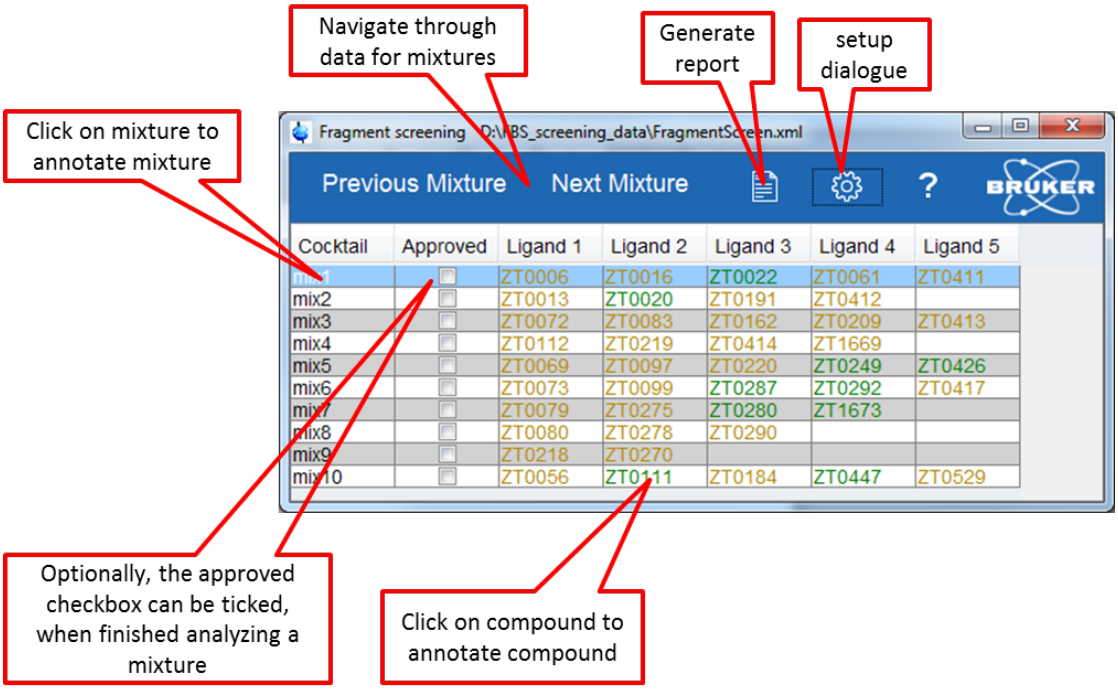
In this chapter, the various functions in the multi-display will be described and examples will be shown.

In the multi-display the 1D $^{1}$H spectra of the compounds in the mixture are shown on top and the screening spectra are displayed on the bottom as defined in the display layout.

If you have configured the tool previously (*Configuration of the Tool [▶ 13]* ), then a screen like the one below should appear. The buttons, navigation options and annotation possibilities are described in the screenshot.

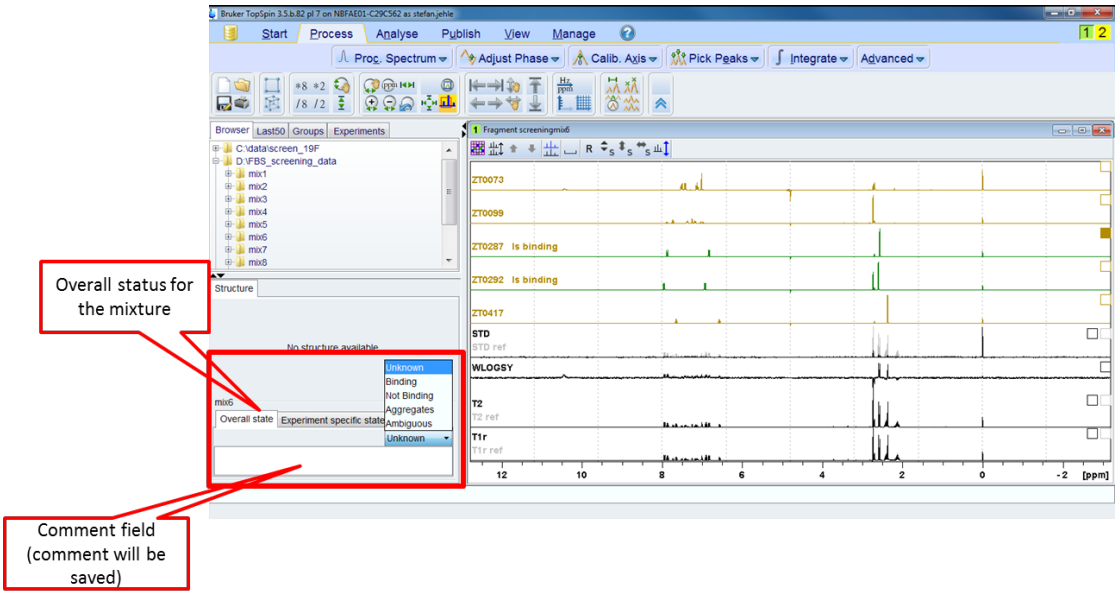


The project table allows the user to navigate through the screening data and open the setup dialogue. Compounds that have been manually identified as binding are colored green and ligands that have been manually identified as non-binders are colored red in the table.

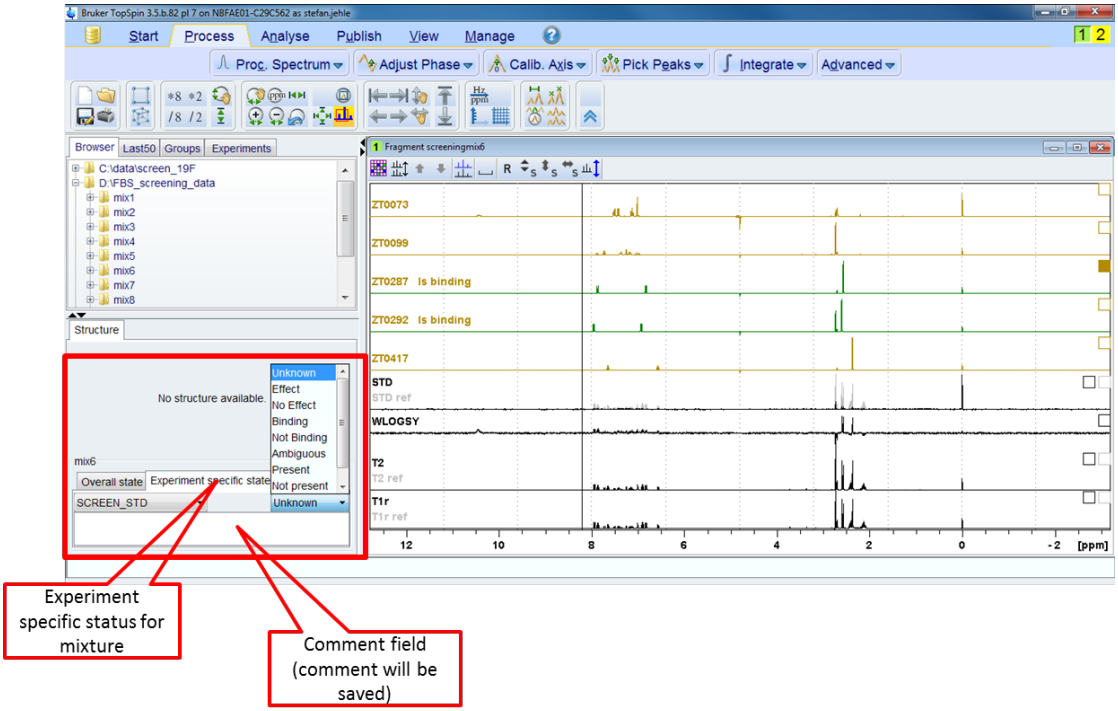



Double-click on a compound in the table will automatically change the ligand-status to binding. If a binding ligand is double-clicked, its status will change to unknown (default).

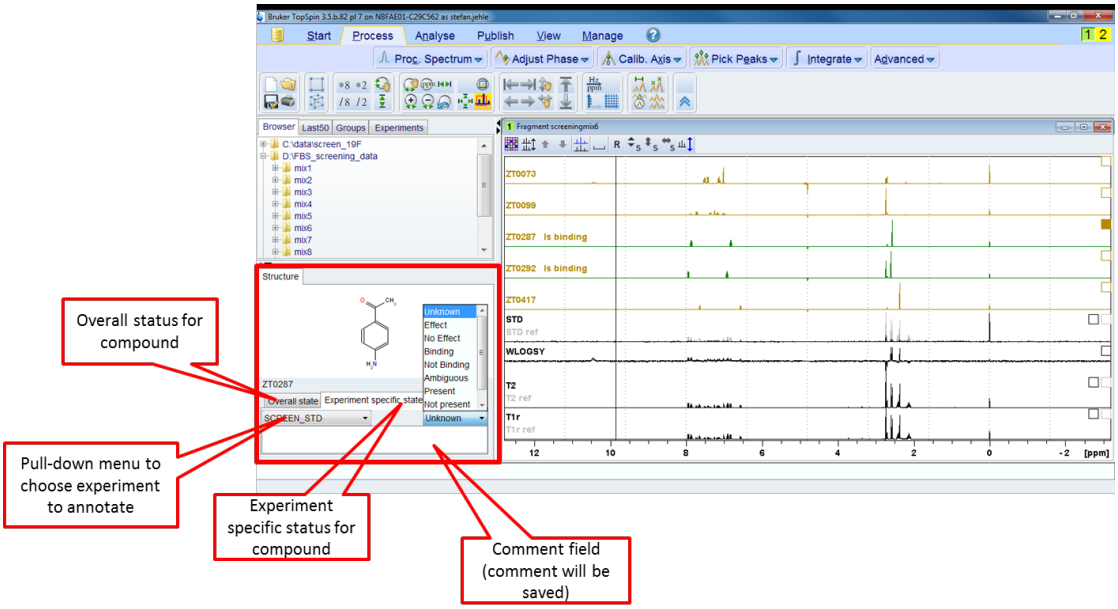
When the mixture is selected in the table, then the user can annotate the data connected with this mixture:



When the tab for experiment specific status is clicked, the individual screening experiments measured on this mixture can be annotated.

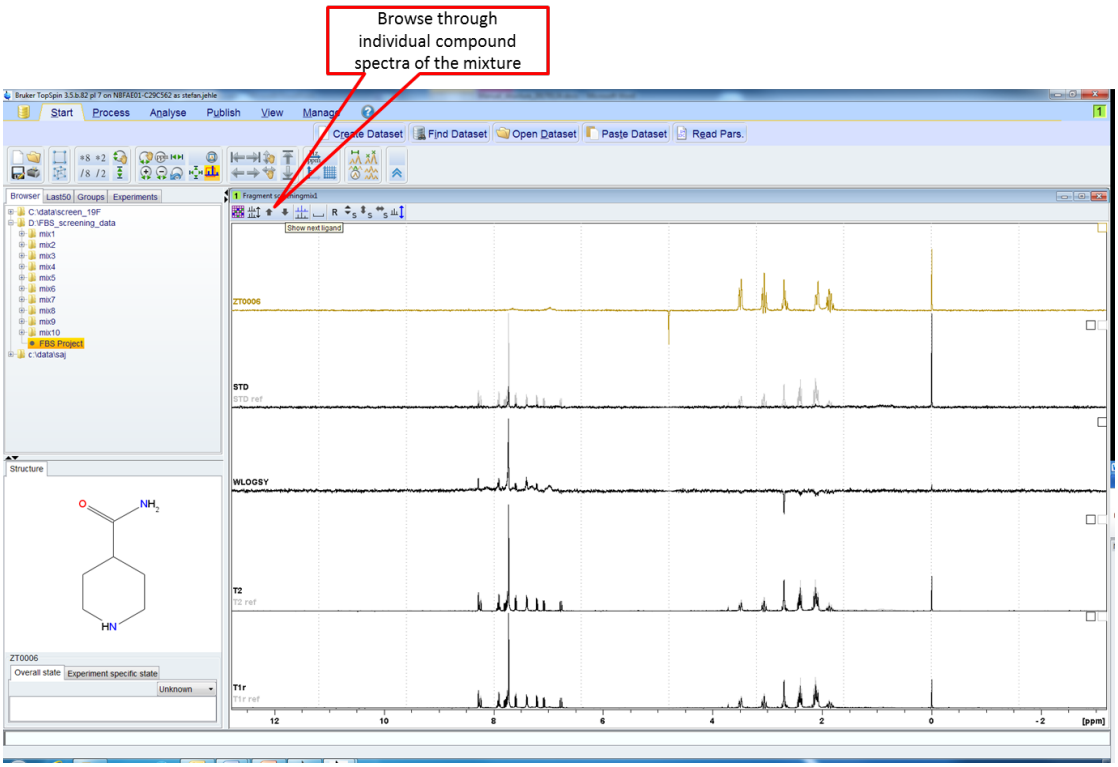



When a compound of the mixture is selected in the project table, then the molecular structure of this compound will be displayed (only if stored with 1D $^{1}H$ spectrum in reference ligand spectra folder) and the user can annotate the data connected with this compound. Also here annotation of an overall status or an experiment specific status is possible as for the mixture. The individual experiments measured on the mixture with this compound can be selected from the pull-down menu. Below a screenshot for the SCREEN_STD experiment specific status is shown:

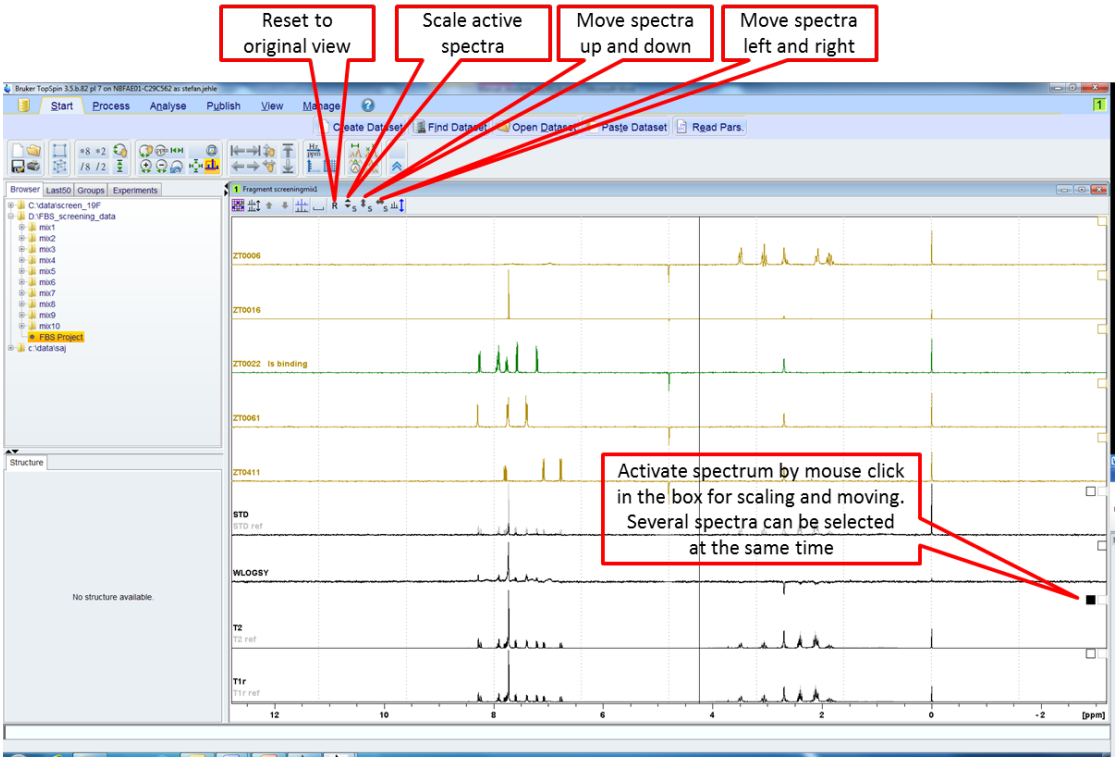


Each ligand can have only one defined status within one project. A ligand cannot have the status binding in one mixture and not binding or unknown in another. The most recent status assigned to a ligand will always overwrite a previously assigned status or its default status.

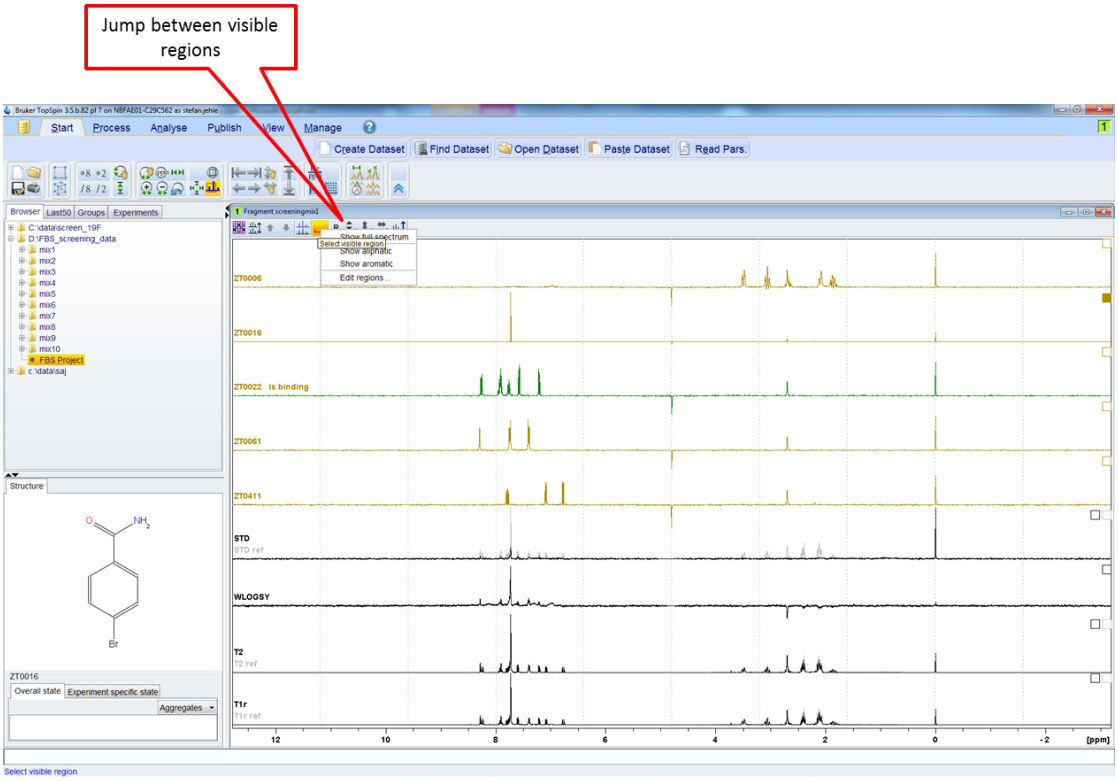
The project table button at the top left in the multi-display  will bring the project table to the foreground. By default, all 1D $^{1}H$ spectra of the compounds present in the mixture are shown. By clicking on the button right from the project table button only one compound spectrum is shown. When this option is selected, the two arrows next will be active and allow browsing through the compound spectra of the mixture:

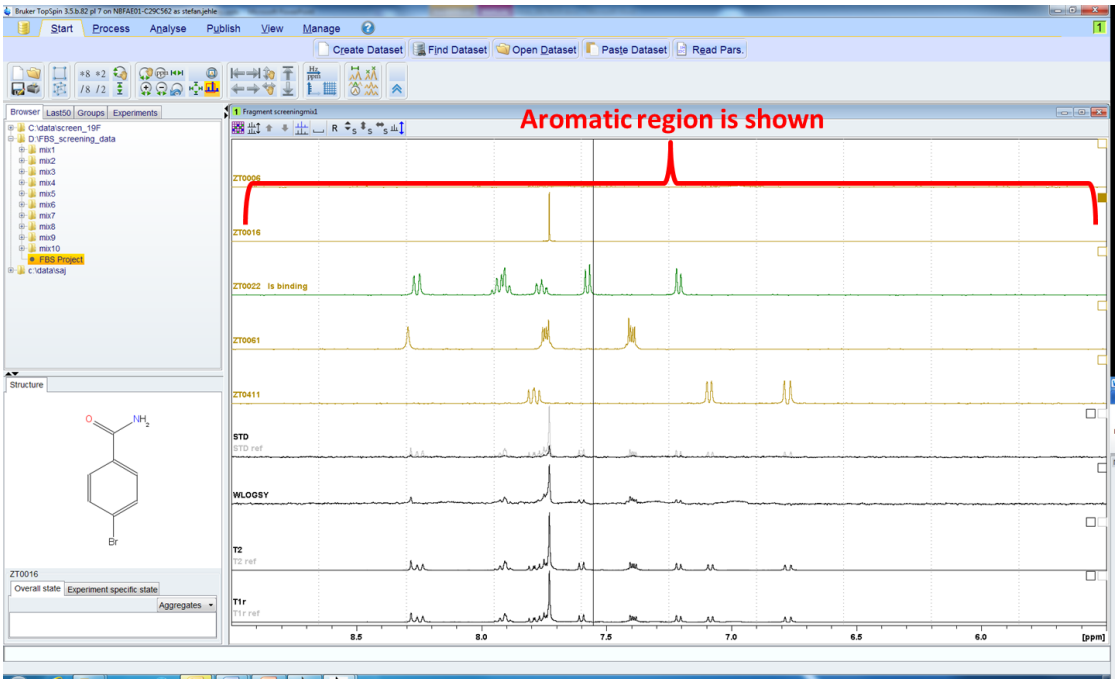



In the multi-display mode the classical operations available in TopSpin are available like moving and scaling spectra. Spectra for operations can be activated by ticking the check box on the right hand side. For spectra in overlay, two check boxes are available that can be selected individually. Also several spectra can be selected and moved or scaled simultaneously.



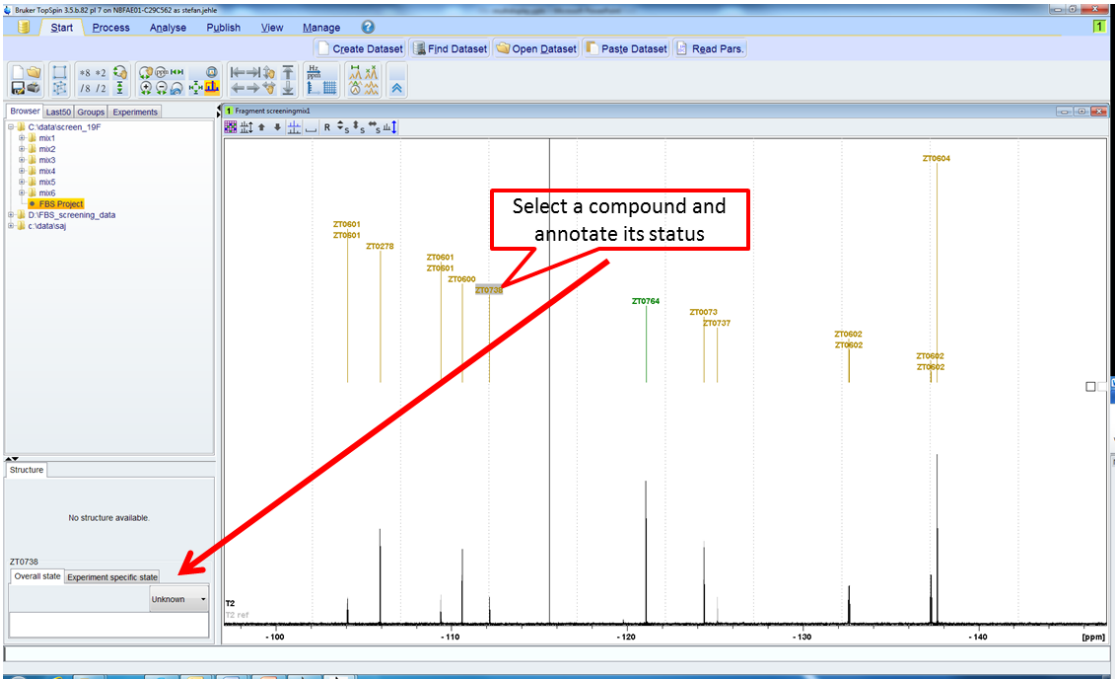

Additional functionalities in the multi-display are the **Select visible regions** button, that allows jumping between the two regions which can be defined in the configuration menu.



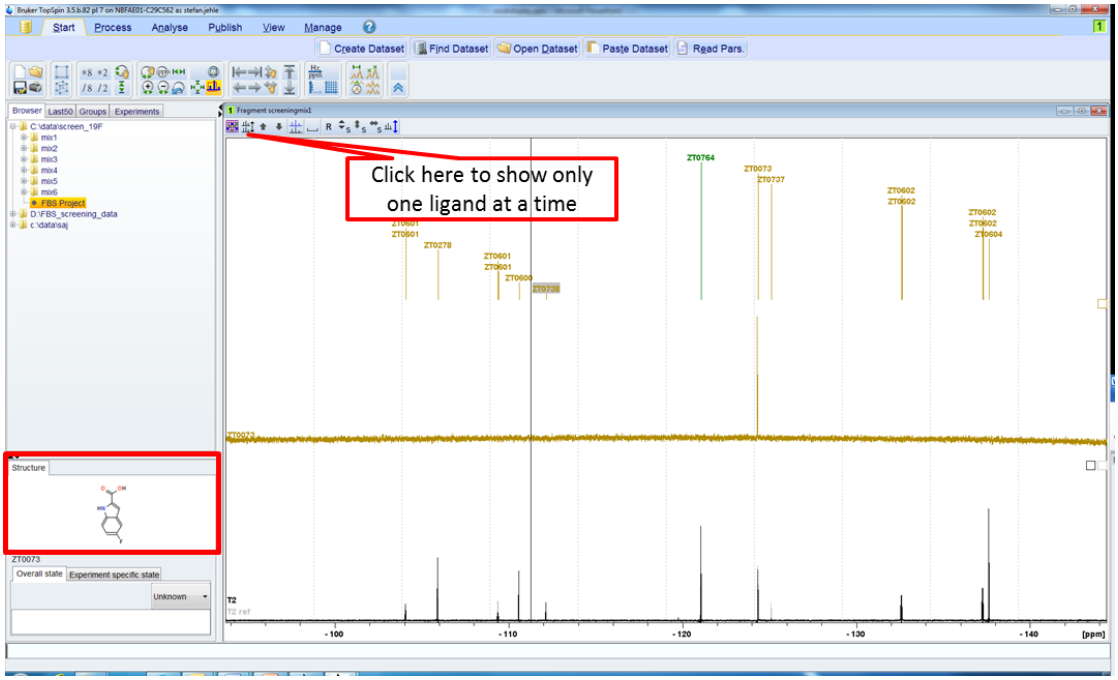



## 4.2 Analysis of Fluorine Screening Spectra

When analyzing fluorine screening data, there is the option to only show peaks of the compounds in the mixture on top of the screening spectrum instead of showing the reference ligand spectra. All functionalities are the same as for the 1H screening, like annotating ligand status and experiment specific status. Ligands are selected directly by clicking on the ligand ID that appears at the chemical shift where the peak would be expected for this individual ligand:



Molecular structures of ligands are only displayed if reference ligand spectra containing MOL files are available and have been set up during the configuration (*The Directories Tab [▶ 14]*):

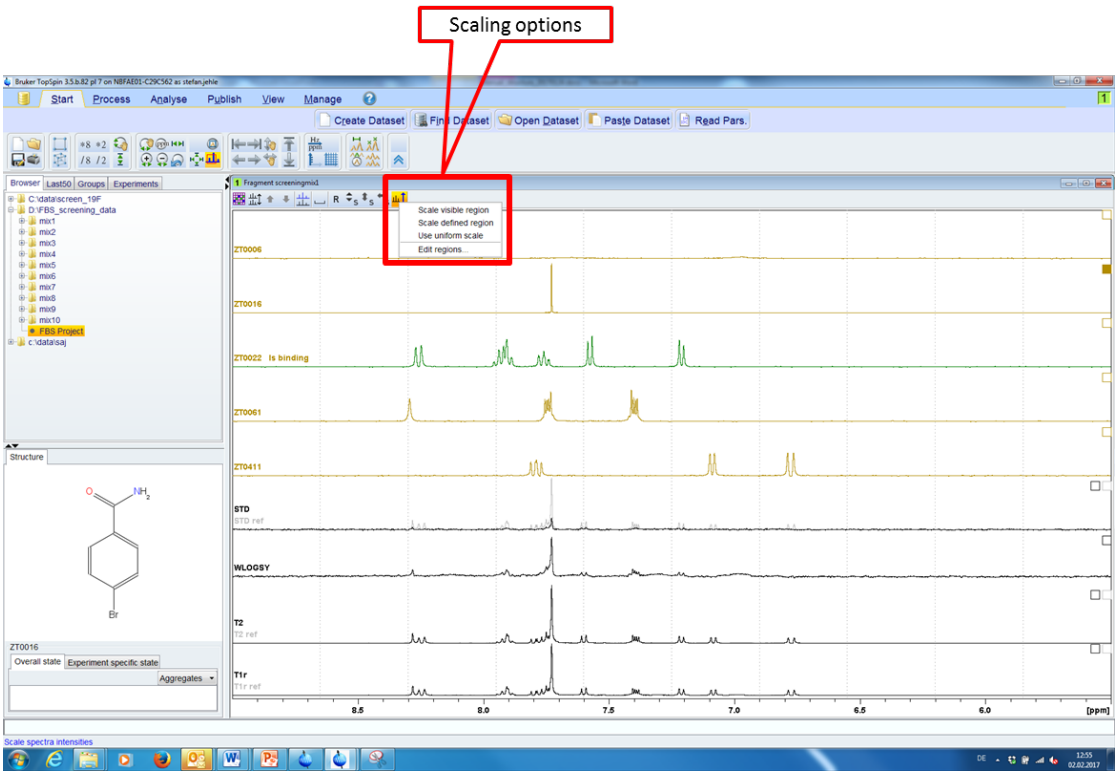



## 4.3 Scaling of Spectra

Spectra in the FBS tool are automatically scaled to make best use of the visible region. This is accomplished by scaling the most intense peak of a trace filling the available horizontal space. In STD spectra for instance, the difference spectrum has a different scale than the reference spectrum.

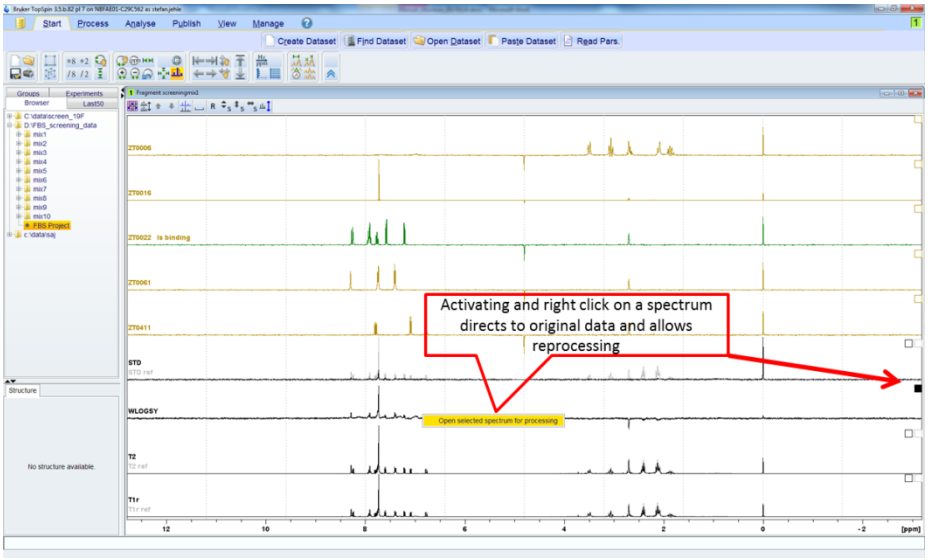
Alternative scaling options are available, for example, a scaling region can be selected in the configuration menu. The user can specify a region, which can be for instance noise or the signal of a competitor that is always scaled to the same level in all visible spectra. **If this option is selected, it is important to show the STD spectra not overlaid with the reference spectrum in order to show proper scaling.**

Other options are uniform scaling, which is necessary for comparing quantitatively signal intensities and scaling of the visible region to make best use of the available space in each individual trace.



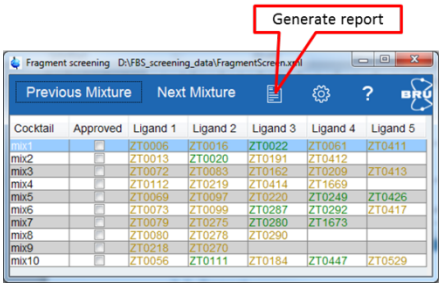

## 4.4 Data Processing and Linking

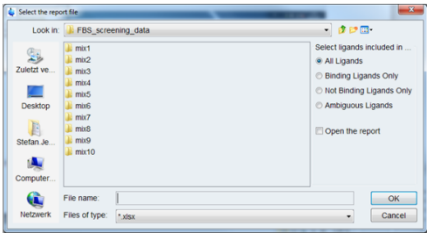
The FBS tool is implemented in TopSpin and original screening data can be accessed and processed at any time in a second TopSpin tab. When a spectrum is activated, right clicking on the spectrum will open a link to the original data. Clicking on the pop-up will open the spectrum and it can be processed. Going back to the screening project can be achieved through the project table that remains open. After reloading a mixture to refresh the screen, the processed data are refreshed as well and displayed.



## 4.5 Report

A report can be generated by clicking on the report button in the project table. A new pop-up window will appear in which the user can select which information will be exported, all ligands, binding ligands, not binding ligands or ambiguous ligands. The report layout can be defined in the configuration menu (*The Report Layout Tab [▶ 18]*).



# 5 Project Management

## 5.1 Data Saving

All project information, spectra storage locations, ligand information and other annotations etc. are saved on the fly in a project file.

A ligand status if binding or not etc. is saved only once with the ligand and a ligand cannot be annotated as binding and not binding in the same project.

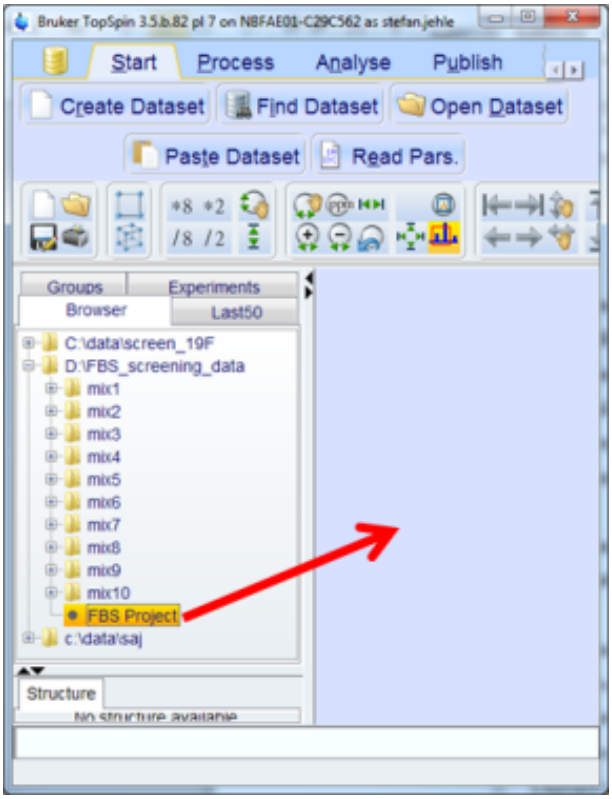
All user actions are logged in the backend project file, like changing the status of a ligand from unknown to binding. The user action is saved with a time stamp and can be exported in the report.

## 5.2 Reloading a Project

A screening project can be closed by closing the project table at any time. The last status at the time of closing will be reloaded when the project is opened again.

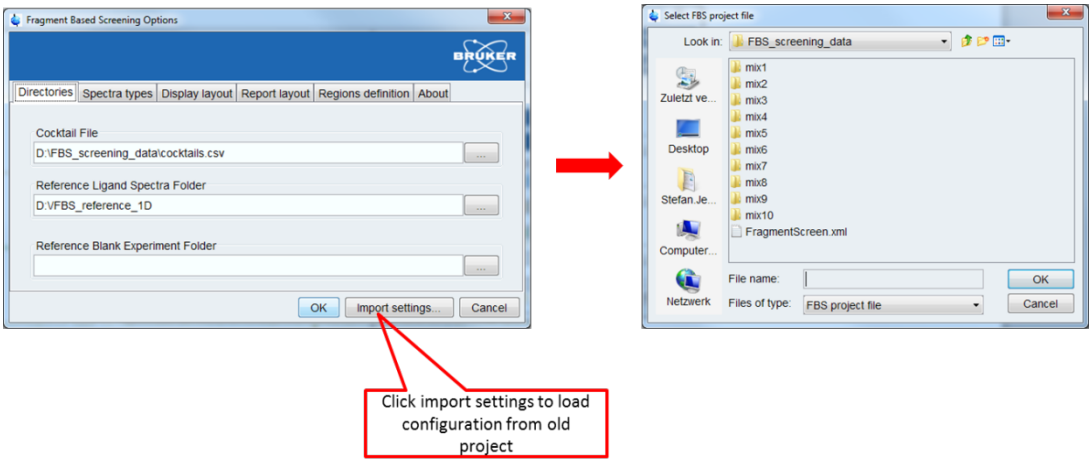
Once a project file has been automatically generated (after initial configuration as described in *Configuration of FBS Analysis [▷ 13]*) the project will be reloaded after typing **fbs** in the command line from any of the screening experiments within a project (or **fbs** command in analysis menu in TopSpin).

The project is also shown in the TopSpin data browser and can be loaded by dragging the project in the spectrum window within TopSpin.



## 5.3 Import Settings

In many cases, customized SPECTYPs and the display layout need to be imported in new screening projects. In order to facilitate this process, the user can select to import settings from an old screening project in the configuration menu:




A pop-up window will appear with a file browser, browse to the project folder from which you want to import the settings, select the file FragmentScreen.xml and click **OK**. Then all settings are imported, but not states of ligands that are related with hit identification.

## 5.4 Read Only Mode

It is possible, that two users open the same screening project at the same time. This is the case if the project is saved on a server from which, for example, a scientist and a supervisor can access the data. The first user who accessed the project will always have write rights and the second user will be able to see and navigate through the data, but will not be able to change (write) the project.

# 6 Parameter Sets for Fragment-Based Screening in TopSpin 3.57

There are four parameter sets available in TopSpin 3.57 that will produce data already in a format that will be recognized by the FBS analysis suite.

The parameter sets are:

- SCREEN_WLOGSY
- SCREEN_STD
- SCREEN_T2
- SCREEN_T1R

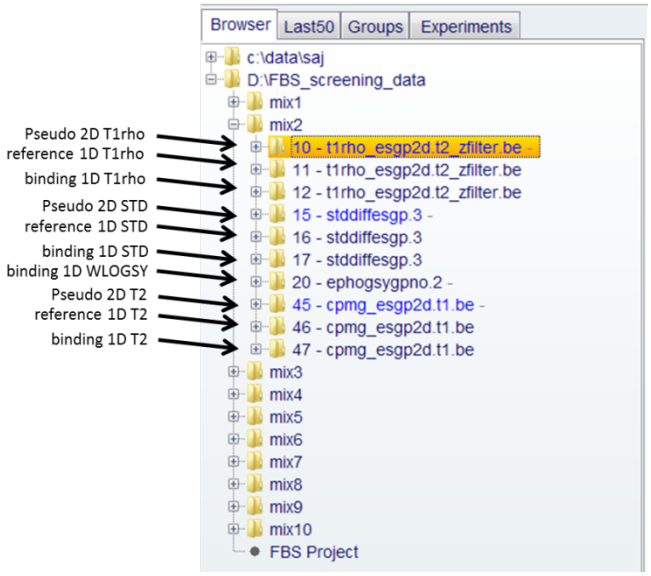
SCREEN_WLOGSY will create a 1D 1H spectrum. SCREEN_STD, SCREEN_T2 and SCREEN_T1R will create a 2D data set in which two 1D 1H spectra are stored. For the STD experiment, one off- and one on-resonance spectrum will be stored, for the two relaxation based experiments T2 and T1R two 1D 1H spectra, one with short relaxation delay of 10 ms (reference experiment) and one with long relaxation delay of 200 ms (binding experiment) will be stored. The au program for processing that is implemented in the parameter sets of the 2D screening experiments will automatically process the reference experiment and the binding experiment in consecutive experiment numbers following the 2D experiment. In the case of the STD spectrum, the reference experiment is the off resonance spectrum and the binding experiment will be the difference spectrum.

IMPORTANT: Always leave two (or more) experiment numbers empty after the 2D screening experiments SCREEN_STD, SCREEN_T2 and SCREEN_T1R in order to leave room for the processing of resulting 1D screening experiments for analysis.

The parameter sets also contain au-programs for automatic NMR parameter calibration for acquisition.

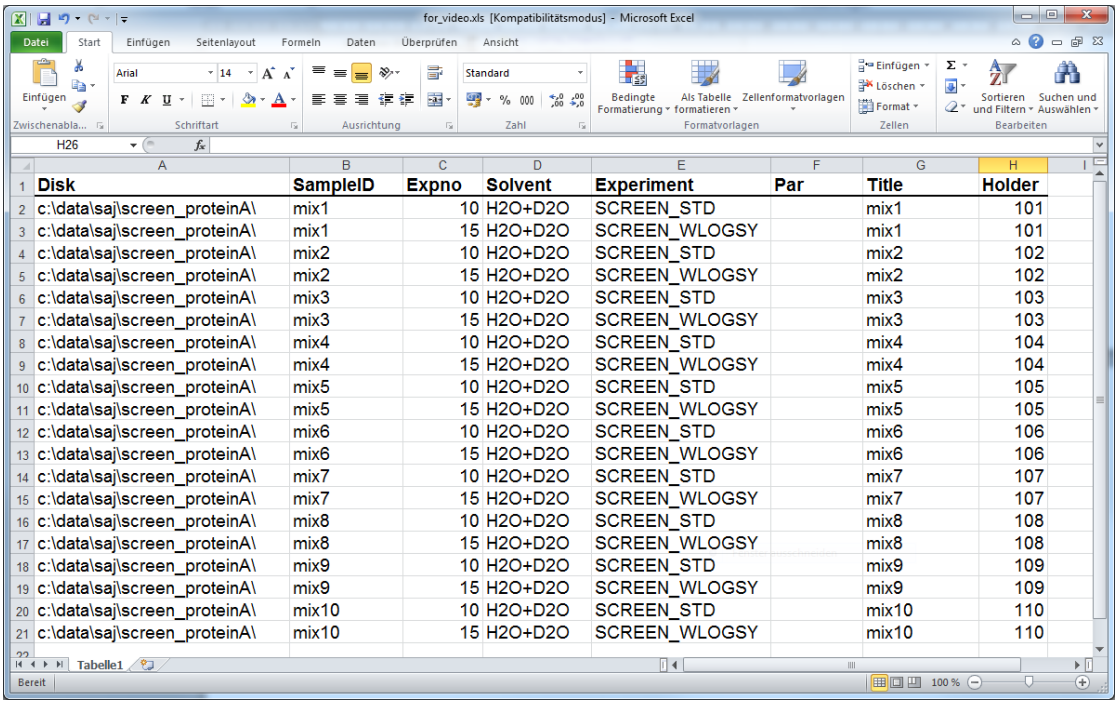
The FBS analysis tool in TopSpin will read the SPECTYP of the experiments available in a screening project and load them for display, the experiment number can be variable.

In the following screenshot, a typical data organization for a screening project organized in mixtures is shown:



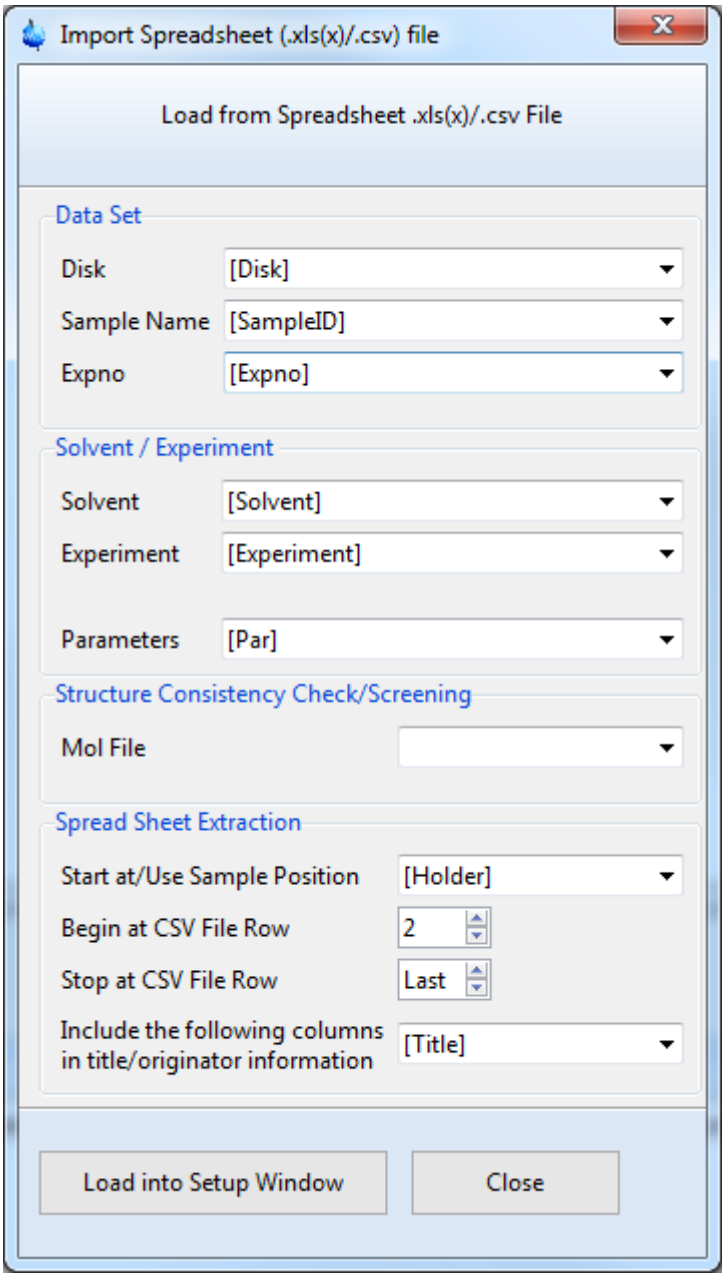
# 7 New Tools in IconNMR for Fragment Screening

Starting a screening campaign is now much easier in IconNMR. The screen can be loaded from an Excel spreadsheet as shown below:

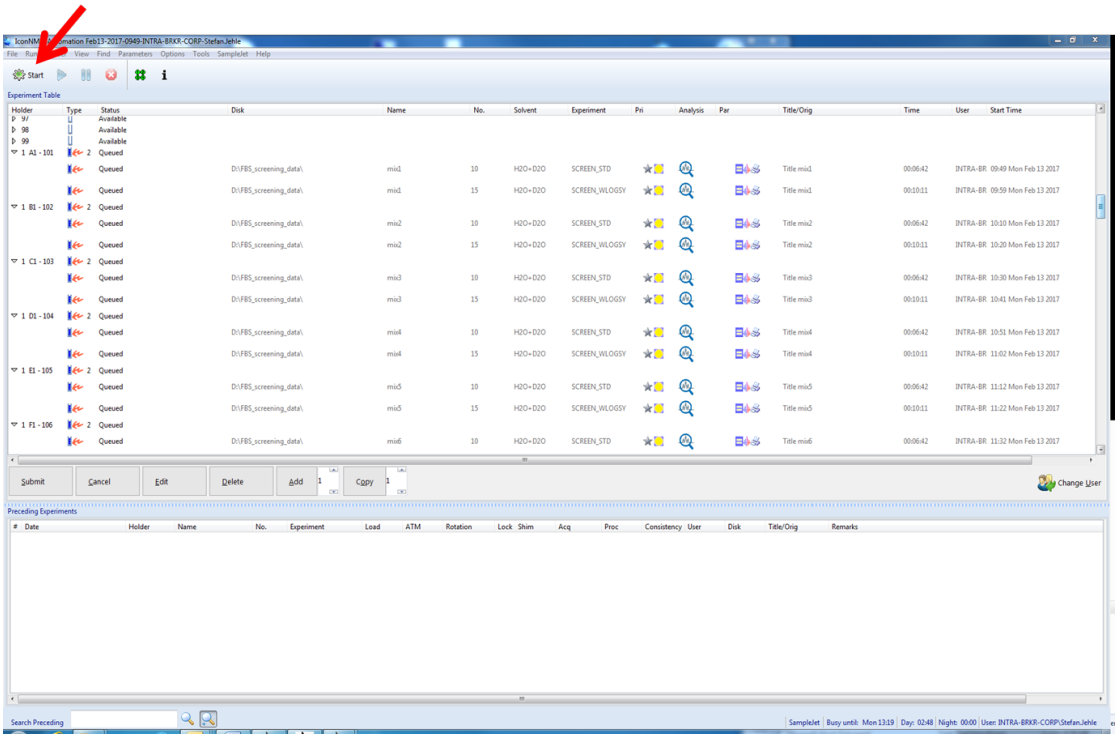



| | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| 1 | **Disk** | **SampleID** | **Expno** | **Solvent** | **Experiment** | **Par** | **Title** | **Holder** |
| 2 | c:\data\saj\screen_proteinA\ | mix1 | 10 | H2O+D2O | SCREEN_STD | | mix1 | 101 |
| 3 | c:\data\saj\screen_proteinA\ | mix1 | 15 | H2O+D2O | SCREEN_WLOGSY | | mix1 | 101 |
| 4 | c:\data\saj\screen_proteinA\ | mix2 | 10 | H2O+D2O | SCREEN_STD | | mix2 | 102 |
| 5 | c:\data\saj\screen_proteinA\ | mix2 | 15 | H2O+D2O | SCREEN_WLOGSY | | mix2 | 102 |
| 6 | c:\data\saj\screen_proteinA\ | mix3 | 10 | H2O+D2O | SCREEN_STD | | mix3 | 103 |
| 7 | c:\data\saj\screen_proteinA\ | mix3 | 15 | H2O+D2O | SCREEN_WLOGSY | | mix3 | 103 |
| 8 | c:\data\saj\screen_proteinA\ | mix4 | 10 | H2O+D2O | SCREEN_STD | | mix4 | 104 |
| 9 | c:\data\saj\screen_proteinA\ | mix4 | 15 | H2O+D2O | SCREEN_WLOGSY | | mix4 | 104 |
| 10 | c:\data\saj\screen_proteinA\ | mix5 | 10 | H2O+D2O | SCREEN_STD | | mix5 | 105 |
| 11 | c:\data\saj\screen_proteinA\ | mix5 | 15 | H2O+D2O | SCREEN_WLOGSY | | mix5 | 105 |
| 12 | c:\data\saj\screen_proteinA\ | mix6 | 10 | H2O+D2O | SCREEN_STD | | mix6 | 106 |
| 13 | c:\data\saj\screen_proteinA\ | mix6 | 15 | H2O+D2O | SCREEN_WLOGSY | | mix6 | 106 |
| 14 | c:\data\saj\screen_proteinA\ | mix7 | 10 | H2O+D2O | SCREEN_STD | | mix7 | 107 |
| 15 | c:\data\saj\screen_proteinA\ | mix7 | 15 | H2O+D2O | SCREEN_WLOGSY | | mix7 | 107 |
| 16 | c:\data\saj\screen_proteinA\ | mix8 | 10 | H2O+D2O | SCREEN_STD | | mix8 | 108 |
| 17 | c:\data\saj\screen_proteinA\ | mix8 | 15 | H2O+D2O | SCREEN_WLOGSY | | mix8 | 108 |
| 18 | c:\data\saj\screen_proteinA\ | mix9 | 10 | H2O+D2O | SCREEN_STD | | mix9 | 109 |
| 19 | c:\data\saj\screen_proteinA\ | mix9 | 15 | H2O+D2O | SCREEN_WLOGSY | | mix9 | 109 |
| 20 | c:\data\saj\screen_proteinA\ | mix10 | 10 | H2O+D2O | SCREEN_STD | | mix10 | 110 |
| 21 | c:\data\saj\screen_proteinA\ | mix10 | 15 | H2O+D2O | SCREEN_WLOGSY | | mix10 | 110 |

Header Information is automatically recognized in IconNMR when using the option to load an Excel table. It is now possible to load more than one experiment per sample into iconNMR and also the holder or barcode information can be imported with the spreadsheet. The experiment will be automatically assigned to the holder or barcode, even if the samples are not sequentially entered in the table:


Import Spreadsheet (.xls(x)/.csv) file
Load from Spreadsheet .xls(x)/.csv File
Data Set
Disk
[Disk]
Sample Name
[SampleID]
Expno
[Expno]
Solvent / Experiment
Solvent
[Solvent]
Experiment
[Experiment]
Parameters
[Par]
Structure Consistency Check/Screening
Mol File
Spread Sheet Extraction
Start at/Use Sample Position
[Holder]
Begin at CSV File Row
2
Stop at CSV File Row
Last
Include the following columns in title/originator information
[Title]
Load into Setup Window
Close

After clicking on **Load into Setup Window** the following screen will appear, from which the IconNMR run can be started.



# 8 Contact

**Manufacturer**

Bruker BioSpin GmbH
Silberstreifen 4
D-76287 Rheinstetten
Germany
*http://www.bruker.com*

WEEE DE43181702

**Fragment Based Screening Contact**

E-mail: *fbs@bruker.com*

**NMR Hotlines**

Contact our NMR service centers.

Bruker BioSpin NMR provides dedicated hotlines and service centers, so that our specialists can respond as quickly as possible to all your service requests, applications questions, software or technical needs.

Please select the NMR service center or hotline you wish to contact from our list available at:

*https://www.bruker.com/service/information-communication/helpdesk.html*

Phone: +49 721-5161-6155
E-mail: *nmr-support@bruker.com*