

# TopSpin

Fragment Based Screening Analysis Suite
 User Manual
 Version 001

Innovation with Integrity

NMR

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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 <sup>1</sup>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 <sup>1</sup>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).

# 2 Preparation of Data for Bruker FBS Module

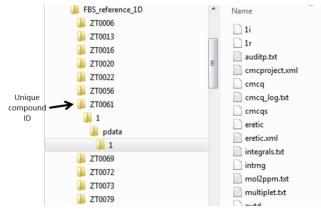
The Fragment-based Screening (FBS) tool in TopSpin displays fragment screening data and reference 1D spectra of compounds in a library in multi display mode for analysis and identification of hits. In most cases, compounds from a library are pooled in mixtures which are then screened against a target.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

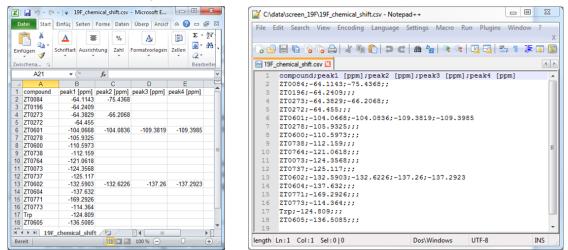
Bruker can provide a conversion tool that utilizes a table in Microsoft Excel format containing data set name, experiment number and unique compound ID and copies then the respective experiment for each fragment in the library into a new data set with the unique compound ID as the name and one 1D experiment 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.



# 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).

Browser Last50 Groups Experiments
⊕ <mark>]}</mark> c:\data\saj
□ D:\FBS_screening_data
🖶 🌗 mix1
🖨 🌗 mix2
🕀 🚹 10 - t1rho_esgp2d.t2_zfilter.be -
ia 📲 11 - t1rho_esgp2d.t2_zfilter.be
ie 📲 12 - t1rho_esgp2d.t2_zfilter.be
🕮 🌗 15 - stddiffesgp.3 -
🕸 🌗 16 - stddiffesgp.3
IT - stddiffesgp.3
🕮 🎍 20 - ephogsygpno.2 -
⊕ ↓ 45 - cpmg_esgp2d.t1.be -
46 - cpmg_esgp2d.t1.be
ie i 47 - cpmg_esgp2d.t1.be
🖶 🎍 mix3
🕀 🎍 mix4
⊕- <u>⊌</u> mix5 ⊕- <u>B</u> mix6
⊕ in mix7
⊕ Inix7 ⊕ Inix8
⊕ linxo
⊕ link9 ⊕ link10
FBS Project
+ i bo i rojou

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.

# **Preparation of Data for Bruker FBS Module**

Spectrum Proce	Pars AcquPars Title	PulseProg Peaks Integ	rals Sample Structure Plot Fid	
n <mark>S</mark> ME 🖤	<i>#</i> }			
Reference Nindow	Reference			
Phase	SI	32768	Size of real spectrum	
Baseline	SF [MHz]	400.1299595	Spectrometer frequency	
Fourier ntegration	OFFSET [ppm]	12.79102	Low field limit of spectrum	
Peak	SR [Hz]	-40.53	Spectrum reference frequency	
Deconvolution	SW_p [Hz]	6393.86	'Processing' Spectral width	
Automation	HZpPT [Hz]	0.195125	Spectral resolution	
Miscellaneous	PPARMOD	1D	Dimension of processed data	
Jser	NC_proc	-8	Intensity scaling factor	
	SPECTYP	SCREEN_STD	<ul> <li>Type of spectrum e.g. COSY, HMQC,</li> </ul>	
	<ul> <li>Window funct</li> </ul>	lion		
	WDW	EM ~	Window functions for trf, xfb,	
	LB [Hz]	1.00	Line broadening for em	
	GB	0	Gaussian max. position for gm, 0 <gb<1< td=""><td></td></gb<1<>	
	SSB	0	Sine bell shift SSB (0,1,2,)	
	TM1	0	Left limit for tm 0 <tm1<1< td=""><td></td></tm1<1<>	
	TM2	0	Right limit for tm 0 <tm2<1< td=""><td></td></tm2<1<>	
	Phase correct	tion		
	PHC0 [degrees]	152.157	Oth order correction for pk	
	PHC1 [degrees]	9.603	1st order correction for pk	
	PH_mod	pk ~	Phasing modes for trf, xfb,	
	Baseline corr	ection		
	ABSG	0	Degree of polynomial for abs (05)	
	ABSF1 [ppm]	0	Left limit for absf	
	•			

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.

		<u>S</u> tart	Process	A <u>n</u> alyse	P <u>u</u> blish	⊻iew	Manage	0	[1	ī
(	G <u>B</u> acl	<					Define <u>L</u> ist <del>√</del>	Define <u>C</u> ommand	nd e Execute -	

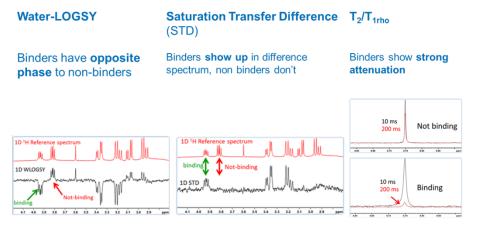
The serial processing function within TopSpin can be found under Process  $\Box$  Advanced  $\Box$  Process Dataset List (serial).

### 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).

	Browser	Last50	Groups	Experiments	
Pseudo 2D reference 1D screening 1D Pseudo 2D reference 1D screening 1D		ata\saj BS_scre nix1 nix2 11 - t1 12 - t1 15 - st 16 - st 16 - st 17 - st 16 - st 17 - st 45 - cc 45 - cc 45 - cc 47 - cc nix3 nix4 nix5 nix6 nix7 nix8	rho_esgp rho_esgp rho_esgp ddiffesgp ddiffesgp ddiffesgp ohogsygp	ta 2d t2_zhiter be 2d t2_zhiter be 2d t2_zhiter be 3 - 3 3 no.2 - 2d t1 be - 2d t1 be	
	⊕- <mark>⊯</mark> n ∟● F	nix10 ⁼BS Proj∉	ect		

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.

	- 0 - X	]								
G v W (D:) → FBS_screening →	• • FBS_screeni P								C3 → U < CD:) → FBS_reference →	• 49 FBS_refere
Datei Bearbeiten Ansicht Extras ?		🕅 🔙 🔊 - (° -	-	cocktails.csv - 1	Aicrosoft Excel		_ O X		Datei Bearbeiten Ansicht Extras ?	
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> 🔶 Favoriten	Name	🖹 🕺	A Seite	a %			Σ · <sup>Δ</sup> Z <sup>7</sup> ·		Favoriten	Name
▷ 🥽 Bibliotheken ▲ 🕵 Computer (NBFAE01-C29C562)	⊯ mix2 ⊯ mix3 ⊯ mix4 ►		Schriftart Aus	srichtung Zahl	Formatvoria	gen Zellen			<ul> <li>Bibliotheken</li> <li>Scomputer (NBFAE01-C29C562)</li> </ul>	ZT0013 ZT0016 ZT0020
> 🏭 (C:) SYSTEM 4 👝 (D:) KINGSTON	initő mitő	Zwischenablage G	<b>-</b> (0	<i>f</i> ∗ mix			Bearbeiten	~	<ul> <li> <sup>6</sup> (C:) SYSTEM         <sup>6</sup> <sup>6</sup> <sup>6</sup> <sup>6</sup> <sup>6</sup> <sup>1</sup> <sup>6</sup> <sup>1</sup> <sup>1</sup> <sup>6</sup> <sup>1</sup> <sup>1</sup></li></ul>	JT0022
BS_reference_1D      BS_screening_data      G(h) StefanJehle (\\bruker.ch\DFS\home)	i mix7 i mix8	A A	B compound 1	C compound 2	D compound 3	E	F compound 5	-	FBS_reference_1D      FBS_screening_data      Get(H) StefanJehle (\\bruker.ch\DFS\home)	ZT0061 ZT0069 ZT0072
<ul> <li>▷ SteranJenie (\\bruker.cn\DrS\nome)</li> <li>▷ SteranJenie (\\bruker.cn\DrS\nome)</li> <li>▷ SteranJenie (\\bruker.cn\DrS\nome)</li> <li>▷ SteranJenie (\\bruker.cn\DrS\nome)</li> </ul>	) mis9 ) mis10 R cocktails.csv	2 mix1	ZT0006 ZT0013	ZT0016	ZT0022 ZT0191	ZT0061 ZT0412	ZT0411		<ul> <li>▷ Grie (n:) SteranJenie (\\Druker.cn\DFS\nome)</li> <li>▷ Grie (Q:) Billerica</li> <li>▷ Grie (W:) DFS-Share</li> </ul>	ZT0072 ZT0073 ZT0079
Netzwerk	FBDLock	4 mix3	ZT0083 ZT1669	ZT0072	ZT0162 ZT0219	ZT0209	ZT0413	=	> Surverk	ZT0080 ZT0083
	FragmentScreen.xml.bak	6 mix5	ZT0069	ZT0097	ZT0220	ZT0249	ZT0426			ZT0097 ZT0099 ZT0099
		8 mix7	ZT0073 ZT0079	ZT1673	ZT0287 ZT0275	ZT0292 ZT0280	ZT0417			ZT0111 ZT0112
		10 mix9	ZT0080 ZT0218	ZT0270	ZT0290					ZT0162 ZT0184
	< >	40	ZTOO56 iils 🖉	ZT0111	ZT0184	ZT0447	ZT0529	•	X	ZT0191
14 Elemente		Bereit		_		00 % 😑 🚽	•		42 Elemente	

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 <sup>19</sup>F screening spectrum, the setup dialogue from above is extended by another field that allows the user to load a <sup>19</sup>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 <sup>19</sup>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.

Fragment Based Screening Options	×
	BRUKER
Directories Spectra types Display layout Report layout Visible regions About	
Cocktail File	
C:\data\screen_19F\cocktails.csv	
Reference Ligand Spectra Folder	
Select F19 chemical shifts file	
C:\data\screen_19F\19F_chemical_shift.csv	
Reference Blank Experiment Folder	
OK Import settings	Cancel

#### 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).

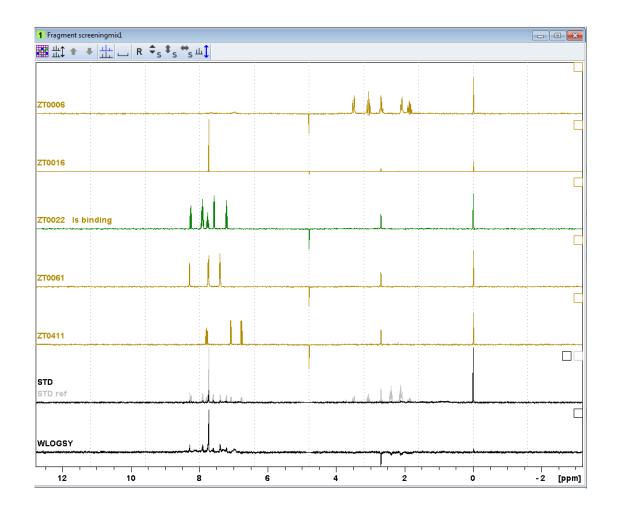
🖕 Fragment Ba	ased Screening Op	tions	Next Make		×
					BRUKER
Directories	Spectra types	Display layout	Report layout	Regions definition	About
SPECTYP			V	isible name	Has Reference
SCREEN_S	TD		S	TD	
SCREEN_V	VLOGSY		V	VLOGSY	
SCREEN_T	2		Т	2	
SCREEN_T	1R		Т	'1r	
SCREEN_S	TD_BLANK		1	D STD w/o protein	
Add your c	wn SPECTYP	]			
			0	K Import setting	gs Cancel

• 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.

Fragment Based Screening Opt	ions		X
		BRUKE	2 )
Directories Spectra types	Display layout Report layout	Regions definition About	
Spectrum 1	Spectrum 2	Show difference (S2-S1)	
SCREEN_STD	Reference Spectrum	• E	
SCREEN_WLOGSY	▼ Empty	•	
Empty	▼ Empty	- E	
Empty	✓ Empty	- E	
Empty	- Empty	- E	
Empty	- Empty	• E	
Empty	- Empty	•	
Reset to default	- Empt		
		DK Import settings Cance	el



- 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.

				1			Ś
Directories	Spectra types	Displa	y layout	Report layout	Reg	ions definition About	
	Spectrum 1			Spectrum 2		Show difference (S2-S1)	
SCR	EEN_STD	•	Referen	nce Spectrum	-		
SCR	EEN_WLOGSY	•	Empty		•		
SCR	EEN_T2	•	Referen	nce Spectrum	•		
Empt	y	•	Empty		•		
Empt	y	•	Empty		•		
Empt	y	•	Empty		•		
Empt	y	•	Empty		•		
Emni		_	Empty		_	[III]	

1 Fragment screeningmix1			
■ # # # # _ R	¢s ≉s ⇔s ш↓		
ZT0006		4 4 4 4 4 4	
ZT0016			
	. I .		
ZT0022 Is binding			
ZT0061			
210061			
ZT0411			
STD ref			
WLOGSY			
arige arisered a sheet and an elements a sheet a			
T2 ref - T2	- Mullulum	he Wimen	
T2			
T2 ref *1.15			
12 10	8 6	4 2 0	-2

- 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.

rectories Spectra types Display layout	Report la	ayout Regions definition About	
Column content		Column header	
Ligand_ID	- L	lgand ID	×
Ligand_Status	- E	Binding state	×
Ligand_User	- k	dentified by	×
Ligand_Time	- 1	lime of identification	×
Ligand_Structure	• 5	Structure of ligand	X
Ligand_Molecular_Formula	- N	Iolecular Formula of ligand	×
Ligand_Mass	- T	Volecular Mass of ligand [g/mol]	×
Ligand_Concentration	•	Concentration of ligand stock solution [mM]	×
Ligand_Comment	- ] [	Jser comment	X
Ligand_Path_SCREEN_STD	• S	STD Spectrum	X
Ligand_Status_SCREEN_STD	<b>▼</b> 5	STD State	X
Ligand_Comment_SCREEN_STD	- S	STD Comment	X
Ligand_Path_SCREEN_WLOGSY	- V	VLOGSY Spectrum	X
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Ligand_Path_SCREEN_T2	• T	72 Spectrum	×
Ligand_Status_SCREEN_T2	•]]	12 State	X
Ligand_Comment_SCREEN_T2	• 1	2 Comment	×
Ligand_Path_SCREEN_T1R	• 1	T1r Spectrum	X
Ligand_Status_SCREEN_T1R	•] [1	T1r State	X
Ligand_Comment_SCREEN_T1R	• 1	Tr Comment	×
Undefined	•		X
Undefined	•		×
Undefined	•		X

#### 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



🖕 Fragment Ba	ased Screening Op	tions			×
					BRUKER
Directories	Spectra types	Display layout	Report layout	Regions defined	nition About
	Visible region	s			
	Region name	5	Start	End	
	aliphatic		1.00	4.00	
	aromatic		5.50	9.00	
	Scaling regior	1			
	Region name	5	Start	End	
	noise		-1.00	-2.00	
		(	OK Imp	oort settings	Cancel

• 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.

1 Fragment s	creeningmix1	<b>V</b>
■ 北1 🔹	₩ <u>11</u> _ R <b>‡</b>	, ‡ _ ↔ _ <u>ш</u> †
		Scale visible region
		Scale defined region
		Use uniform scale
		Edit regions
ZT0006	: :	

# 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.

🖕 Fragmen	t screening D	<pre>\FBS_screenin</pre>	g_data\Fragm	entScreen.xml					A1	▼ (0	$f_x$ mix				~
						C	$\sim$		A	В	С	D	E	F	
Previo	ous Mixtur	e Nex	t Mixture		ŝ	? ∎	RUKER	1	mix	compound 1	compound 2	compound 3	compound 4	compound 5	i f
					~~	· -7	$\sim$	2	mix1	ZT0006	ZT0016	ZT0022	ZT0061	ZT0411	
0				Line 10				3	mix2	ZT0013	ZT0020	ZT0191	ZT0412		
Cocktail	Approved	Ligand 1	Ligand 2	Ligand 3	Ligand 4	Ligand 5		4	mix3	ZT0083	ZT0072	ZT0162	ZT0209	ZT0413	
	(m)			ZT0022	ZT0061	ZT0411		5	mix4	ZT1669	ZT0112	ZT0219	ZT0414		Т
mix2		ZT0013	ZT0020	ZT0191	ZT0412			6	mix5	ZT0069	ZT0097	ZT0220	ZT0249	ZT0426	
mix3		ZT0072	ZT0083	ZT0162	ZT0209	ZT0413		7	mix6	ZT0073	ZT0099	ZT0287	ZT0292	ZT0417	
mix4		ZT0112	ZT0219	ZT0414	ZT1669			8	mix7	ZT0079	ZT1673	ZT0275	ZT0280		
mix5		ZT0069	ZT0097	ZT0220	ZT0249	ZT0426		ă	mix8	ZT0080	ZT0278	ZT0290	210200		
mix6		ZT0073	ZT0099	ZT0287	ZT0292	ZT0417		10	mix9	ZT0218	ZT0270	210230			
mix7		ZT0079	ZT0275	ZT0280	ZT1673							770404	770447	770500	
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mix10		ZT0056	ZT0111	ZT0184	ZT0447	ZT0529		Be	reit				100 % (	(	÷
								ļ						~ \	<u> </u>

# 4.1 Analysis of Mixtures

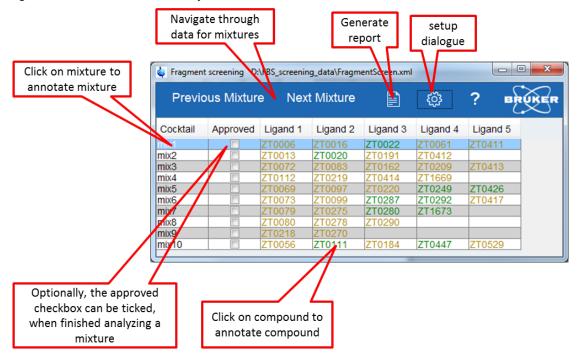
In this chapter, the various functions in the multi-display will be described and examples will be shown.

In the multi-display the 1D <sup>1</sup>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.

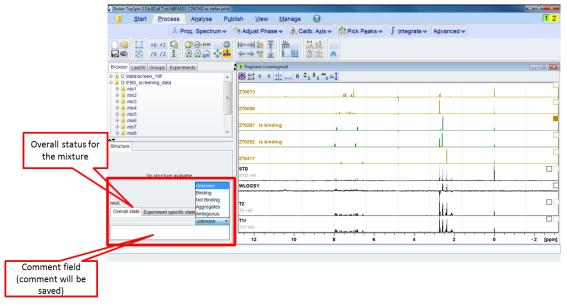
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A Pro <u>c</u> . Spectrum <del>▼</del>			grate V Advanced V	
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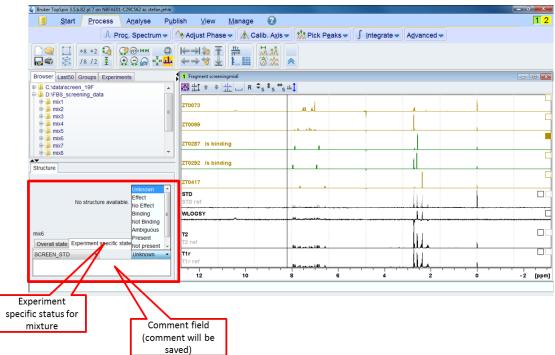
The project table allows the user to navigate through the screening data and open the setup dialogue. Compounds that have been manually identified as binding are colored green and ligands that have been manually identified as non-binders are colored red in the table.



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).

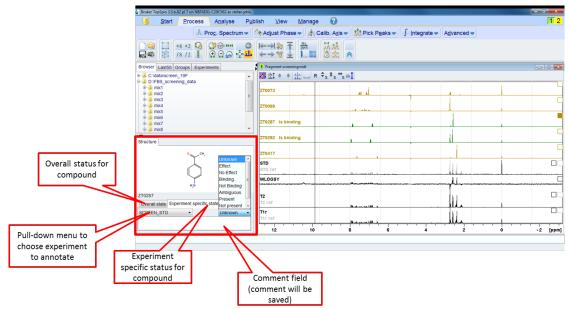
When the mixture is selected in the table, then the user can annotate the data connected with this mixture:





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 <sup>1</sup>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 <sup>1</sup>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:

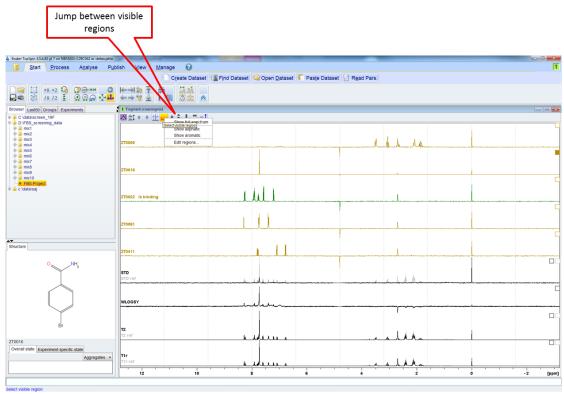
	individu	ise through Ial compound of the mixture		
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L	12 10	8 6	4 2 Ó	-2 [ppm]

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.

# Working with the FBS Analysis Tool

	Reset to original view	Scale active spectra	Move spectra up and down	Move spectra left and right		
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Bruker TopSpin 3.5.82 pl 7 on N8FA601-C29C562 as stelan jehle     Start Process Analyse Pul	blish ⊻lew <u>Manage</u> Ceate Date	set Find Data of Open Datas	Paste Dataset	i Pars.		<u> </u>
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lei <mark>is</mark> c∶idataisaj	ZT0022 is binding	L				
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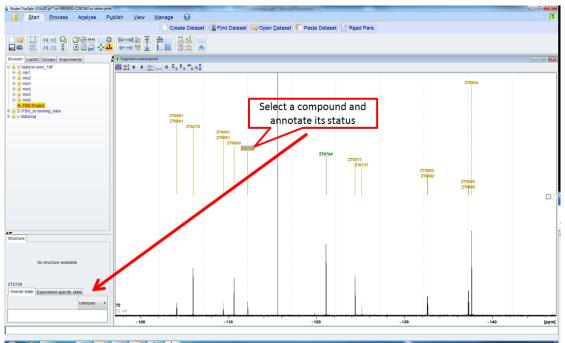
Additional functionalities in the multi-display are the **Select visible regions** button, that allows jumping between the two regions which can be defined in the configuration menu.



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# 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]):

Bruker TopSpin 3.5.b.82 pl 7 on NBFAE01-C29C562 as stefan.jehle		
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	ZT0278	
	270601	
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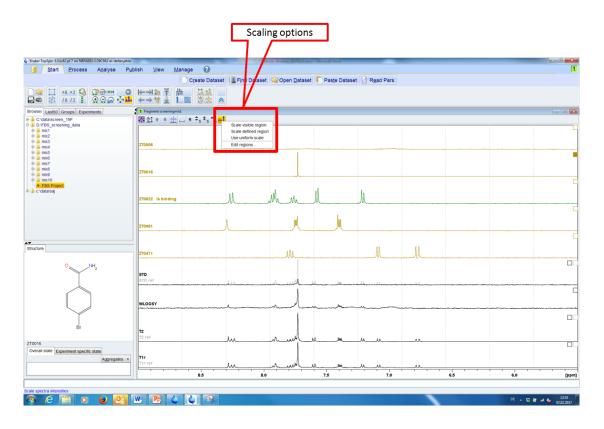
# 4.3 Scaling of Spectra

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.

# Working with the FBS Analysis Tool



# 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]).

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	ous Mixture		t Mixture		ø	? ∎₹
Cocktail	Approved	Ligand 1	Ligand 2	Ligand 3	Ligand 4	Ligand 5
mix1		ZT0006	ZT0016	ZT0022	ZT0061	ZT0411
mix2		ZT0013	ZT0020	ZT0191	ZT0412	
mix3						
mix4		ZT0112	ZT0219	ZT0414	ZT1669	
mix5					ZT0249	ZT0426
mix6		ZT0073	ZT0099	ZT0287	ZT0292	ZT0417
mix7				ZT0280	ZT1673	
mix8		ZT0080	ZT0278	ZT0290		
mix9						
mix10		ZT0056	ZT0111	ZT0184	ZT0447	ZT0529

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æ	🔰 mix1	Select ligands included in
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	🗼 mix4	Not Binding Ligands Only
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	🍌 mix10	
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Computer		
6	File name:	ОК
	File hanie.	OK .

# 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.

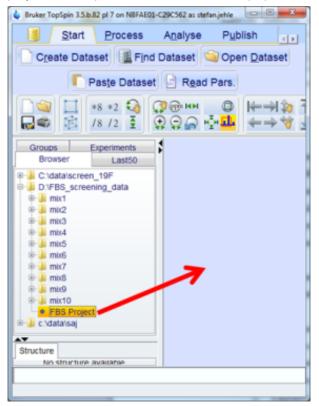
All user actions are logged in the backend project file, like changing the status of a ligand from unknown to binding. The user action is saved with a time stamp and can be exported in the report.

### 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:

🖕 Fragment Based Screening Options	📕 🦉 Si	elect FBS proje	ct file			×
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		9	🎍 mix1			
Directories Spectra types Display layout Report layout Regions definition About	7.	uletzt ve	imix2			
	20	uleizi ve	mix3 mix4			
Cocktail File			mix5			
D:\FBS_screening_data\cocktails.csv		Desktop	imix6			
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			FragmentS	creen.xml		
Reference Blank Experiment Folder	Co	omputer				
		0	File name:	1		
						ОК
OK Import settings Cancel	1	Netzwerk	Files of type:	FBS project file	-	Cancel
Click import settin configuration fr project						

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

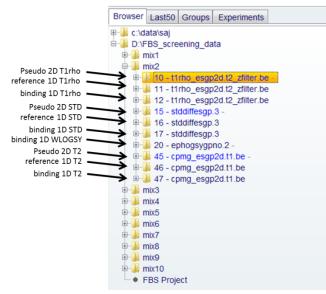
SCREEN\_WLOGSY will create a 1D 1H spectrum. SCREEN\_STD, SCREEN\_T2 and SCREEN\_T1R will create a 2D data set in which two 1D 1H spectra are stored. For the STD experiment, one off- and one on-resonance spectrum will be stored, for the two relaxation based experiments T2 and T1R two 1D 1H spectra, one 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:

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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
22 II 4 ► M Tabelle1 / 2				14			▼
Bereit							

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 Spreads	sheet (.xls(x)/.csv	) file	X				
Load	from Spreadshee	t .xls(x)/.csv File					
Data Set							
Disk	[Disk]		-				
Sample Name	[SampleID]		•				
Expno	[Expno]						
-Solvent / Experi	ment						
Solvent	[Solvent]		-				
Experiment	[Experiment]		-				
Parameters	[Par]		-				
	stency Check/Sc	reening					
Mol File			•				
-Spread Sheet Ex	traction						
Start at/Use Sar	mple Position	[Holder]	•				
Begin at CSV Fi	Begin at CSV File Row						
Stop at CSV File	Stop at CSV File Row						
Include the foll in title/originat	owing columns or information	[Title]	•				
Load into Set	up Window	Close					

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

Run		17-0949-INTRA-8R															•
-		Parameters Opti	uns Tools S	ampleJet Help													
Start 📄	• 88 🗳	<b>#</b> i															
iment Table																	
77 78 99	Type Stat Avail Avail Avail	able able able		Disk		Name	No.	Solvent	Experiment	Pri	Analysis	Par	Title/Orig	Time	User	Start Time	
	Ker Que	ied		D:\FBS_screening	_data\	mid	10	H2O+D2O	SCREEN_STD	*	0	<b>=</b> 4\$	Title mid.	00:06:4	42 INTRA	BR 09:49 Mon Feb 13 2017	
81 - 102	Quei			D:\FBS_screening,	_data\	mid	15	H2O+D2O	SCREEN_WLOGSY	*	۵.	<b>=\$</b> \$	Title mid.	00:10:1	LI INTRA	BR 09:59 Mon Feb 13 2017	
	Lee Que			D:\FBS_screening	_data\	mit2	10	H20+D20	SCREEN_STD	* 📃	<u>A</u>	<b>=4\$</b>	Title mix2	00:06:	12 INTRA	BR 10:10 Mon Feb 13 2017	
	Cuer Cuer			D:\FBS_screening	_data\	mb/2	15	H2O+D2O	SCREEN_WLOGSY	*		<b>=\$</b> \$	Title mix2	00:10:1	LI INTRA	BR 10:20 Mon Feb 13 2017	
	ter Que			D:\FBS_screening	_data\	mið	10	H2O+D2O	SCREEN_STD	*	۵.	<b>=\$</b> \$	Title mix3	00:06:4	12 INTRA	BR 10:30 Mon Feb 13 2017	
	Cuer			D:\FBS_screening	_data\	miß	15	H20+D20	SCREEN_WLOGSY	*	Q	<b>=</b> \$\$	Title mis3	00:10:1	LI INTRA	BR 10:41 Mon Feb 13 2017	
	Quer	ied		D:\FBS_screening	_data\	mit#	10	H20+D20	SCREEN_STD	*	<u>.</u>	<b>=</b> 4\$	Title mix4	00:06:4	42 INTRA	BR 10:51 Mon Feb 13 2017	
E1 - 105	Che Que			D:\FBS_screening	_data\	mie4	15	H20+D20	SCREEN_WLOGSY	*	Ø.	<b>=\$</b> \$	Title mix4	00:10:1	11 INTRA	BR 11:02 Mon Feb 13 2017	
	Ke Que			D:\FBS_screening	_data\	mið	10	H2O+D2O	SCREEN_STD	*	4	<b>=</b> 4%	Title mid5	00:06:4	12 INTRA	BR 11:12 Mon Feb 13 2017	
	Cuer Cuer Cuer Cuer			D:\FBS_screening	_data\	mið	15	H2O+D2O	SCREEN_WLOGSY	*	Ð.	<b>=\$</b> \$	Title mid5	00:10:3	LI INTRA	BR 11:22 Mon Feb 13 2017	
	Ke Que			D:\FBS_screening	_data\	mido	10	H20+D20	SCREEN_STD	*	Q	<b>=\$</b> \$	Title mid	00:06×	12 INTRA	BR 11:32 Mon Feb 13 2017	
bmit	<u>C</u> ancel	Edit		<u>D</u> elete	≜dd 1	С <u>о</u> ру 1										🎒 Ch	ange
ing Experime	ients																
			Name	No.	Experiment	Load ATM	Rotation	Lock Shim	Acg Proc	Consiste		Disk 1	itle/Orig Re	marks			

# 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

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