

# FBS

- Fragment-based Screening of Ligands by NMR

# Automated NMR Fragment-based Screening

Bruker's Fragment-based Screening (FBS) tool in TopSpin provides an integrated solution for the acquisition, analysis and reporting of ligand-detected screening data. Standard experiments run and are processed in automation. The hit identification is fast and straightforward using TopSpin's unique capabilities for multiple spectra display and scaling. FBS is a turnkey solution and yet allows user interaction at any point.

Fragment screening in the pharmaceutical industry has become a widely applied method for the discovery of lead molecules. Weak binding affinities in the  $\mu\text{M}$  to  $\text{mM}$  range are a hallmark of fragments and appropriate screening methods are needed for the detection of binding ligands. NMR-based Fragment Screening (FBS) is one of the most popular and reliable techniques and is used in more than 50% of screening campaigns. However, data handling and analysis has been a bottleneck as many 1D  $^1\text{H}$  or 1D  $^{19}\text{F}$  spectra must be analyzed in parallel by the operator which can be a time consuming task. Bruker now facilitates these tasks with a full TopSpin based workflow solution.

## Benefits

- Greatly simplifies NMR-based fragment screening
- Standard NMR screening experiments are included: Water-LOGSY, STD, T2/ T1 $\rho$ . They run in automation and are automatically recognized in the FBS software
- Customization of both acquisition and analysis is implemented at various steps
- Seamless workflow from acquisition to data to processing and analysis
- Automatic display of relevant data: screening experiment, reference spectra, compound IDs and cocktail composition
- Screening studies are organized and saved in projects
- On-the-fly automatic saving functionality of user's actions within a project
- Reporting in Microsoft Excel format

## Complete workflow solution in TopSpin 3.5 p17



### QC of library

- Integrity
- Concentration
- Purity
- Solubility
- Aggregators
- Fragment's peak list

### Make Mixtures

- Pool fragments in mixtures for higher throughput: 5-10 for  $^1\text{H}$  and 15-20 for  $^{19}\text{F}$  screening
- Reduce overlap of fragment peaks in mixtures

### Screen

Screen mixtures against a target molecule by using one or more NMR experiments. Typically two or more experiments are measured

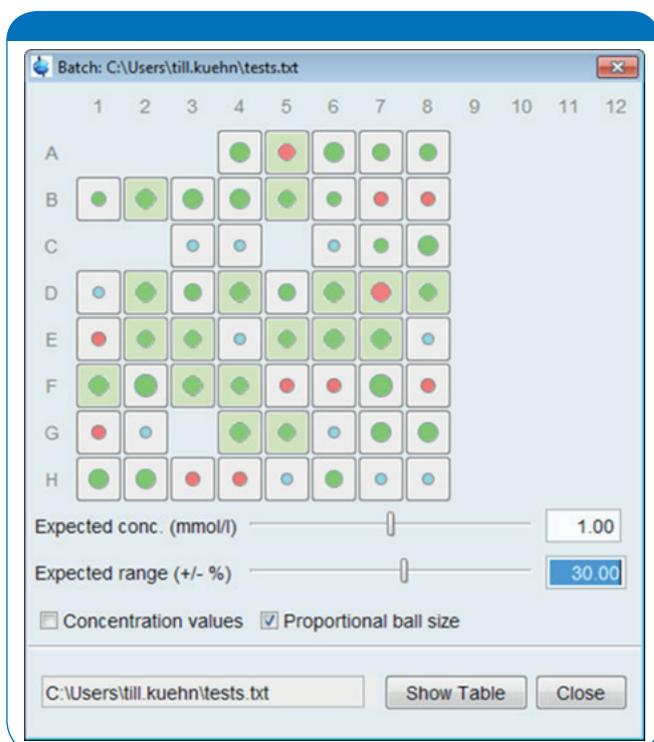
### Process

NMR data are automatically processed after the measurement when using standard parameter sets in TopSpin 3.5p17

### Analyze

Identify binders from non-binders in the mixture spectrum by qualitative comparison with non-binders and with reference 1D spectra

## ● Fragment Library Quality Control



The well plate display window: concentration and consistency decision for each sample in a 96-well plate. Each sample is represented by a circle whose size and color correspond to the calculated concentration and consistency decision.

### Make mixture

To increase the throughput of the NMR screening, fragments are pooled into cocktails that are screened against the targets. The cocktails typically contain 5 to 10 fragments for  $^1\text{H}$  and 15 to 20 for  $^{19}\text{F}$  screening. The cocktail design is done once for all screening experiments and care is taken to reduce the overlap between the fragments' peaks in the  $^1\text{H}$  or  $^{19}\text{F}$  spectrum of the mixture. The fragment's peak list is readily obtained from the quality control and is used to design cocktails with optimally reduced peaks overlay.

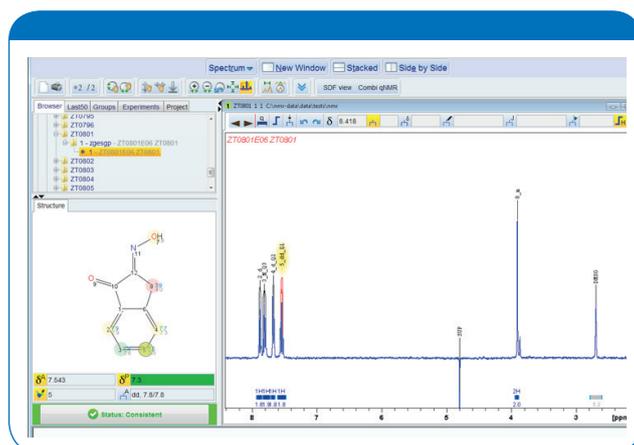


Designing a new fragment library or simply checking the quality of a commercial library involves hundreds of spectra to be measured, processed and validated. The outcome is a selection of fragments which are fully characterized and quantified. Those reference spectra are named by their compound ID and are ready for further use in hit identification in mixtures.

The library quality control is a complete workflow solution that facilitates automatic NMR based quality assurance in batches. A typical fragment library contains a few hundred compounds. Data acquisition is performed in a 96 well plate format with the SampleJet sample changer and using the software CMC-q and CMC-assist.

The quality assurance includes the check of:

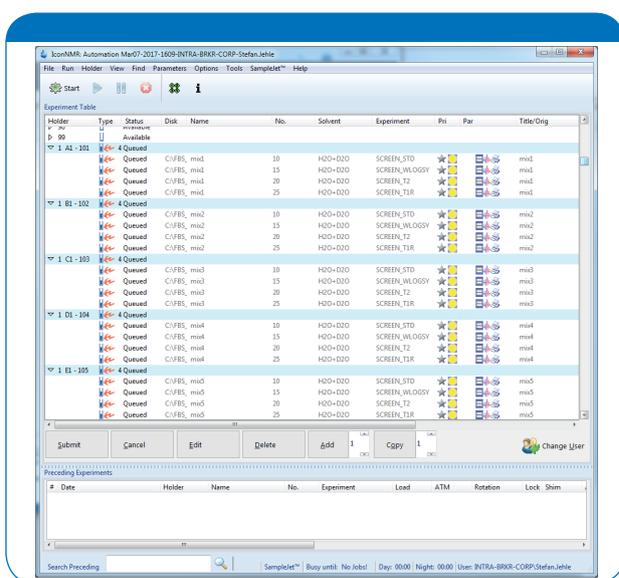
- Consistency check ensures compound integrity
- Concentration using a single reference (external) for all compounds
- Purity estimation by a 100% method
- Solubility by comparing the actual and expected concentration
- Aggregators as their NMR signal is drastically reduced.
- Spectra of fragments in buffer with automated solvent suppression



CMC-assist allows the user to review the details of the analysis: spectra interpretation, chemical shifts predictions, assignments of spectra feature to the molecular structure. The user can annotate and modify the automated analysis. The fragment's peaks are stored for further use in the mixture design.

## ● Screen & Analyze

The standard NMR experiments, Water-LOGSY, STD, T2 and T1p, are included in TopSpin 3.5p17 and are designed for ease of use and run in automation. TopSpin 2.1 and higher are also supported on request. Samples are queued and stored safely in a cooled 96 tube rack in the SampleJet. The measured data is stored and ready to be analysed with the FBS software.



In this example, IconNMR is running the four standard screening experiments for each of the 61 cocktails in presence of the target.

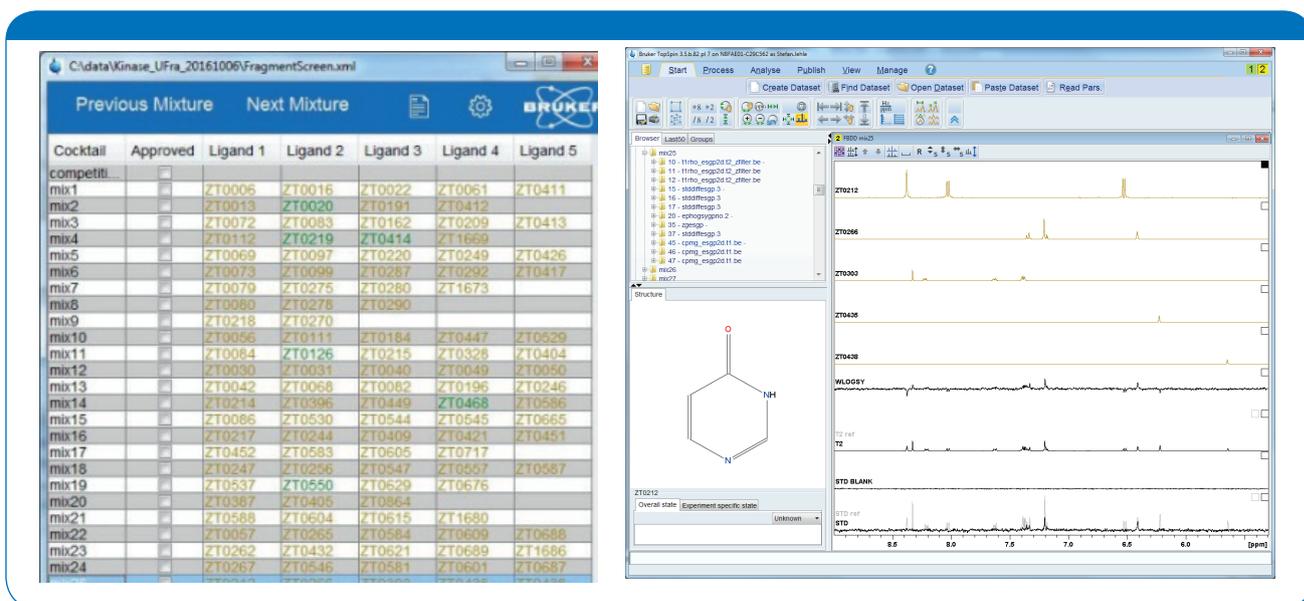
## Process

They are processed automatically and consistently for all screened mixtures:

- Water-LOGSY are processed with the same phase so binders show always with the same sign from one mixture to another.
- STD spectrum is phased using the phase of its reference spectrum which offers a much better signal to noise ratio.

## Analyze

In the FBS software, the screening data and the reference spectra are organized in the context of the cocktails. A single click in the mixture table allows you to visualize the data related to a given fragment or to a given mixture. Spectra are then shown in the multiple spectra display in TopSpin. The spectra can be scaled, translated up/down and left/right. The hit identification is quick and straightforward by a visual inspection of all relevant data in a single window. The user can identify and annotate hits which are turned into a green color in the mixture table. The analysis is exported to a spreadsheet report which contains all qualitative or quantitative user's annotations about binding.



In this example the mixture 24 was clicked and the corresponding display in TopSpin is obtained. The reference spectra of the 5 fragments of this cocktails are retrieved automatically and displayed. They are followed by the screening experiments WATER LOGSY, T2 and STD. A single fragment display is also available.

## ● Cooled SampleJet - The Versatile Sample Changer for High-Throughput

The SampleJet is the only robot for NMR automation which works with NMR tubes in 96-well format and hence compatible with standard liquid-handling lab-automation devices. The SampleJet allows a continuous automation workflow in the microwell-plate format from sample preparation through the NMR measurement and subsequent sample processing.

The SampleJet holds up to 480 NMR samples in five 96-well racks of which each can be set to an individual temperature between 4 and 44 °C for simultaneous measurement of chilled biological samples in aqueous buffer and compounds dissolved in DMSO.

In addition to the operation in batch mode, the SampleJet is also designed for sequential single tube submission for open-access routine NMR. There are 96 open shop positions for 4 and 7 inch tubes without spinner and three positions with spinner for 7 inch tubes. This allows a total of 579 samples to be loaded in the robot.

A variety of tube sizes are available, the most common ones for fragment screening are 3 mm and 1.7 mm for sample volumes of 170 µl and 37 µl, respectively. The low sample volumes enables the screening of mass limited targets.

### Features

- Five positions for NMR tube racks in 96-well format allow the handling of batches with up to 480 sample tubes
- Spinner-free open access applications: SampleJet provides 96 positions for tubes without requiring spinners (plus 3 positions for tubes with spinners)
- The SampleJet can handle the most common sample tube diameters such as 5 mm (550 µl), 3 mm (170 µl), 1.7 mm (37 µl) and 1 mm (8 µl)
- Tube diameters are automatically recognized and the appropriate spinner is chosen
- Automatic tube and rack bar code identification
- Compatible with most Bruker BioSpin magnets and spectrometer lines
- Control software TopSpin and IconNMR™
- Individual temperature control between 4 and 44 °C for each 96-well rack allowing many different applications in parallel



The SampleJet's 96 tube rack is compatible with sample preparation using standard liquid handling equipment.

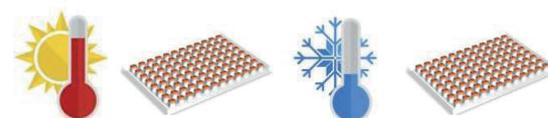
### SampleJet and new temperature control option

- Holds 5 racks with 96 tubes each
- Tube diameter automatically recognized
- Standard 96 well format
- No spinner needed

#### NMR tube diameters:

1.0, 1.7, 3 and 5 mm with sample volumes of 8, 37, 170 and 550 µl

### Individual temperature control for each of the five 96 sample racks



- QC of DMSO stock solution
- QC and reference 1D in buffer
- Protein containing screening samples

## ● CryoProbes

All our CryoProbes are designed with a cryogenically cooled preamplifier for the deuterium channel which guarantees highest sensitivity for the lock channel, resulting in excellent stability of the spectrometer.

### 600 MHz or 500 MHz

All probe options are available for 500 and 600 MHz NMR systems. The sample throughput listed in the table 1 is for a 600 MHz system. The throughput on a 500 MHz system is at least 50% lower.

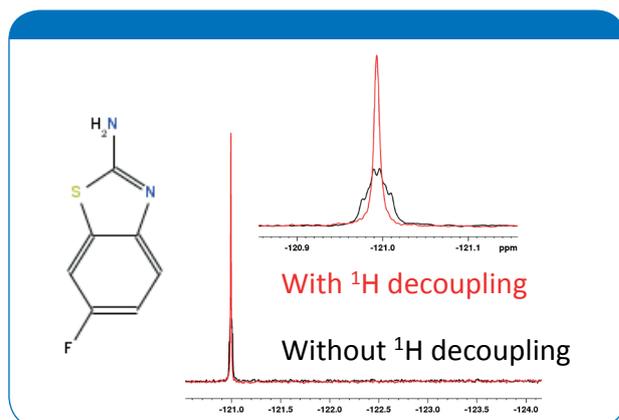
### TCI Helium Cooled CryoProbe

The TCI CryoProbe is a proton-optimized triple resonance NMR 'inverse' probe, featuring three fully independent channels (plus lock channel).

The TCI CryoProbe has the highest sensitivity for  $^1\text{H}$  detection and is therefore ideally suited for  $^1\text{H}$  observed fragment screening. Furthermore,  $^{19}\text{F}$  observed screening is possible but without simultaneous  $^1\text{H}$  decoupling.

### QCIF Helium Cooled CryoProbe

The QCIF CryoProbe is the most versatile fluorine optimized quadruple resonance NMR  $^1\text{H}$  optimized probe, featuring four fully independent channels (plus lock channel). This enables  $^1\text{H}$  decoupled  $^{19}\text{F}$  detection which is ideal for screening of  $^{19}\text{F}$  fragment libraries (3-FABS, FAXS, etc.). The probe also permits simultaneous decoupling on multiple nuclei such as  $^{13}\text{C}$ ,  $^{19}\text{F}$  and  $^{15}\text{N}$ .



$^1\text{H}$  decoupling significantly improves signal to noise in  $^{19}\text{F}$  spectra

### TCI Helium Cooled MicroCryoProbe™ for Small Volume Applications

Bruker Biospin's 1.7 mm TCI MicroCryoProbe™ is the ultimate tool commercially available for unparalleled mass sensitivity. It allows for highest  $^1\text{H}$  sensitivity on a sample volume of only 37  $\mu\text{l}$ .

This extreme sensitivity jump makes the 1.7 mm MicroCryoProbe an ideal tool for any NMR analysis with limited sample amounts, e.g. mass limited proteins, natural products etc.

For many "real life" samples the use of this probe has been the only way to obtain spectra that allowed solving difficult problems, for instance where the screening of thousands of compounds benefits from reduced target and compound quantities or biological samples where the expression does not yield large quantities. All these applications have received a significant boost from the use of the 1.7 mm TCI MicroCryoProbe.

### TCI CryoProbe™ Prodigy

CryoProbe™ Prodigy delivers tremendous boosts in sensitivity at moderate investment costs when compared to room-temperature probes. It costs significantly less than conventional helium cooled CryoProbes; the TCI CryoProbe Prodigy uses nitrogen-cooled RF coils and preamplifiers to deliver a sensitivity enhancement over room temperature (RT) probes of a factor of 2 for  $^1\text{H}$ .

Siting is easy, as the Prodigy package comprises a control unit and a liquid nitrogen vessel in addition to the probe. The Prodigy has the potential to become the probe of choice for routine industrial and academic labs.

Table 1	TCI	QCIF	TCI micro	TCI Prodigy
Observable nuclei	<sup>1</sup> H, <sup>19</sup> F, <sup>13</sup> C, <sup>15</sup> N	<sup>1</sup> H, <sup>19</sup> F, <sup>13</sup> C, <sup>15</sup> N	<sup>1</sup> H, <sup>13</sup> C, <sup>15</sup> N	<sup>1</sup> H, <sup>19</sup> F, <sup>13</sup> C, <sup>15</sup> N
<sup>1</sup> H decoupling during <sup>19</sup> F acquisition	No	Yes	-	No
Protein observed (HSQC)	Yes	Yes	Yes	Yes
Typical throughput <sup>1</sup> H (samples/compounds per day at <b>600 MHz</b> )*	100/500-1000	70/350-700	25/125-250	50/250-500
Throughput <sup>19</sup> F (samples/compounds per day at <b>600 MHz</b> )* **	60/1200-1800***	90/1800-2700	-	35/700-1050***
Recommended tube sizes		3 mm (170 µl) 5 mm (550 µl)	1.7 mm (37 µl)	3 mm (170 µl) 5 mm (550 µl)

\* at 0.2 mM compound concentration per sample and mixtures of 5-10 compounds in 3 mm tubes (170 µl) except TCI micro for which 1.7 mm tubes (37 µl) are assumed

\*\* at 0.02 mM compound concentration per sample and mixtures of 20-30 compounds in 3 mm tubes (170 µl)

\*\*\* <sup>19</sup>F spectra without <sup>1</sup>H decoupling will show multiplets where <sup>19</sup>F is coupled to <sup>1</sup>H; this decreases the sensitivity and may result in line broadening

### What Configuration Works for You?

Contact us at [fbs@bruker.com](mailto:fbs@bruker.com) and our applications specialist will help you configure the system that's right for you. (for a quote contact us at [fbs@bruker.com](mailto:fbs@bruker.com))

	500 MHz with Sample Jet	600 MHz with SampleJet
TCI N2-cooled Prodigy CryoProbe		
TCI He-cooled CryoProbe		
QCIF He-cooled CryoProbe		
TCI He-cooled Micro-CryoProbe		

### Accessories

SampleJet	
SampleProTube liquid handler	
TCI N2-cooled Prodigy CryoProbe	
TCI He-cooled CryoProbe	
QCIF He-cooled CryoProbe	
TCI He-cooled Micro-CryoProbe	



[fbs@bruker.com](mailto:fbs@bruker.com)  
[www.bruker.com](http://www.bruker.com)