NMR Solutions for drug discovery

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The principle of Fragment Based Screening
from efficient fragments to Drug candidates

**Fragment**
- high "ligand efficiency" (LE), medium IC$_{50}$

**Grow, optimize**

**Candidate**
- high LE, low IC$_{50}$

<table>
<thead>
<tr>
<th></th>
<th>Fragment</th>
<th>Grow</th>
<th>Candidate</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC$_{50}$</td>
<td>0.91μM</td>
<td>0.07μM</td>
<td>5.9nM</td>
</tr>
<tr>
<td>LE</td>
<td>0.59</td>
<td>0.55</td>
<td>0.49</td>
</tr>
</tbody>
</table>

**Advantages of FBS:**
- Fragment chemical space ($10^9$) << drug like chemical space ($10^{30}$)
  - Fragment space easier to explore
  - Smaller libraries with higher diversity (~ 1’000 – 5’000 compounds)
  - Higher hit rates (0.1% - 10%)
NMR in Fragment Based Screening

Summary:

- STD is the most used NMR experiment
- Protein detected NMR experiment is becoming more popular
- People use more than one experiment (e.g. STD, WaterLogsy, CPMG)
- 19F-ligand detection is becoming popular because of its sensitivity

More than one answer possible
### Fragment Based Screening

**NMR vs. SPR**

<table>
<thead>
<tr>
<th><strong>Throughput</strong></th>
<th>NMR Fragment Screening</th>
<th>SPR Fragment Screening</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 samples, 500-1000 compounds per day (19F 3000 compounds/day) (5-10 compounds per sample)</td>
<td>500 compounds per day, single point measurement (depends on instrument)</td>
</tr>
<tr>
<td><strong>Running costs</strong></td>
<td>Operational costs: ~45k per year (96 well format NMR tubes, cryogens and service contract)</td>
<td>Operational costs: 45-50k per year (chips for target immobilization, consumables, solvents and service)</td>
</tr>
<tr>
<td><strong>Data Quality</strong></td>
<td>QC of fragments possible as part of process, inherent concentration information aids hit validation</td>
<td>No QC of fragments possible during process, independent QC required</td>
</tr>
<tr>
<td><strong>Bad samples</strong></td>
<td>No issue, one tube per sample: bad sample does not stop the screen</td>
<td>Sticky compounds may dismantle the chip during screening</td>
</tr>
<tr>
<td><strong>Data Analysis</strong></td>
<td>Clumsy, home-built acquisition automation, data organization, no workflow support</td>
<td>Easy to use interface for operation, data analysis and export</td>
</tr>
</tbody>
</table>
NMR Fragment Based Screening

The concept

High ligand concentration, low protein concentration: **ideal for ligand observed NMR!**

**Excess of ligand**
(i.e. 0.25 mM)
small molecule, different NMR properties than protein

**Low protein concentration**
(i.e. 0.01 mM)
Large molecule with distinct NMR properties
Ligand in contact with protein (=binding) adopt it’s physical properties during residence time.

Excess of ligand (i.e. 0.25 mM) small molecule, different NMR properties than protein.

Low protein concentration (i.e. 0.01mM) Large molecule with distinct NMR properties.
NMR Fragment Based Screening

The concept

Binding ligand keeps properties when back in the pool, other ligands are unchanged
NMR Screening Methods

**Ligand observed methods** identify binders from mixtures

- NO isotopic labeling of the protein
- NO size limitation of the target
- Little amounts of protein needed (4-10 mgs)
- Little amounts of ligands needed (0.025 – 0.250 mM in each sample)

**Protein observed methods** identify binders and binding site on target

- Binding site on target can be identified
- Isotopic protein labeling required (\(^{13}\text{C}\) or \(^{15}\text{N}\))
  - Size limitation of target
- Amount of protein required depends on application
Three Basic $^1$H NMR Experiments in FBS

**Water-LOGSY**
- Binders have **opposite phase** to non-binders

**Saturation Transfer Difference (STD)**
- Binders **show up** in difference spectrum, non binders don’t

**$T_2/T_1$rho**
- Binders show **strong attenuation**

![Image of NMR spectra showing differences between binders and non-binders](image-url)
Chemical Exchange – NMR Timescale

Intermediate Exchange:
- Peaks broaden if bound state is not saturated
- Reappear at saturation
- $K_D < 100 \, \mu M$

Fast exchange:
- $\Delta \delta$ is proportional to fraction bound
- Peaks move until saturation is reached
- $K_D > 100 \, \mu M$

$K_D$ is fitted from a function of concentration and $\Delta \delta$
Fluorine Fragment Screening

Spin-Echo pulse program for relaxation measurement

Two point measurement, binding fragments show faster relaxation when compared to non-binders
Fluorine Fragment Screening
Advantages and Opportunities

- Usually one peak per fragment
- No water suppression
  - No buffer signals
  - Up to 30 fragments per mixture
- Increased throughput
- Low compound and protein concentration
typical conditions:

- protein = 8 µM
- compound = 20 µM
- sample volume = 170 µL (3mm tube)
- measuring time = 8 min

600 MHz QCI-F CryoProbe

$^{19}$F-detected FBS

30 fragments
blue = free
red = protein added

Courtesy of Dr. M. Blommers, Novartis Pharma, Switzerland
19F Probe Options for FBS

- **CryoProbe QCIF**
  - 19F-observe
  - 1H-decouple
  - 19F S/N 6000:1 @ 600MHz

- **Smart probe BBFO**
  - 19F-observe,
  - 1H-decouple
  - 19F S/N 565:1 @ 600MHz

- **N₂ cooled CryoProbe Prodigy TCI**
  - 19F-observe
  - without 1H-decoupling
  - 19F S/N 2400:1 @ 600MHz
Experimental Parameters

Sample Concentration

<table>
<thead>
<tr>
<th>Method</th>
<th>[Protein] /µM</th>
<th>[Ligand] /µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligand-observed: ligand in excess</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T$_2$/T$_1$ρ</td>
<td>20</td>
<td>200</td>
</tr>
<tr>
<td>STD</td>
<td>10</td>
<td>200</td>
</tr>
<tr>
<td>WaterLOGSY</td>
<td>10</td>
<td>200</td>
</tr>
<tr>
<td>$^{19}$F-T$_2$ (CF/CF$_3$)</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>$^{19}$F-T$_2$ (CF$_3$)</td>
<td>10</td>
<td>25</td>
</tr>
</tbody>
</table>

- The protein concentration and fragment to protein ratio strongly depend on the molecular weight (MW) of the protein
- For targets with low sample availability the ratio can be as high as ~1:400

Test Samples

- Mixture of tryptophan and tyrosine (2 mM each) and 0.04 mM human serum albumin (HSA) (65 kDa) in 100 mM phosphate buffer pH 7.4
- Mixture of benzamidine and sucrose (2 mM each) and 0.04 mM trypsin (23.3 kDa) in 100 mM phosphate buffer pH 7.4
Screening preparation & library QC on-the-fly

It actually makes sense to check!

Our libraries had **ca. 30% bad samples!**

- 20% “no compound” → no compound in stock solution, or **not soluble in buffer**
- 10% decayed or wrong compound
- 50% concentration off by more than +/-30%
How about “aggregators”?

- several nonspecific compounds form sub-micrometer aggregates
- aggregate species are responsible for the inhibition of many different enzymes
- Colloidal aggregation of organic molecules is the dominant mechanism for artifactual inhibition of proteins
- controls against it are widely deployed.
Tools for the practical work

New temperature control options for SampleJet & SampleCase

Individual temperature control for each of the 5 96 sample racks

- QC of DMSO stock solution
- QC and reference in buffer
- The actual protein screening

SampleCase: 24 positions. Individually temperature controlled and adjustable

- Heated up to 125° C
- Cooled down providing a fridge-cold sample storage platform
1. Preparation: run data for single compound reference

2. Mix compound cocktails, document in excel table

3. Run screening campaigns
   - Parameter sets to automatically setup and optimize screening experiments
   - Setup batch run in IconNMR
New in Topspin 3.5 pl6:

automation routines for acquisition and processing of FBS experiments
NMR FBS in practice

You mix several ligands per sample with protein:

- for higher sample throughput (ligands per sample) **AND**
- to identify binding by comparison to non-binder (qualitative aspect)

⇒ You need a **reference spectrum** of each individual compound to make the connection to the actual binding molecule

1. Before screening against any protein, you **run each compound of library**
2. Then make cocktails
3. Start screening campaigns
The actual screening campaign

Data analysis and interpretation – TS3.5 next pl

With TopSpin now, you

• Point to mixture table

• Indicate parent directory for single compound reference

• Indicate parent blank screening data (if applicable)
The actual screening campaign

Data analysis and interpretation – TS3.5 next pl

✓ Customizable Spectra Types by unique identifier
New FBS tool in Topspin

Data analysis and interpretation – TS3.5 next pl
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Data analysis and interpretation – **TS3.5 next**
The actual screening campaign
Data analysis and interpretation – TS3.5 next pl

✓ Customizable report layout
FBS installation on ts3.5pl6

1. Send me a e-mail to download the upgrade (matteo.pennestri@bruker.com)

2. Download the zipped classes folder from dropbox link

3. Backup you classes folder (e.g. classes_old)

4. Install the new classes for FBS tool
FBDD training course

NMR Applications in Drug Discovery

Introduction to NMR applications used in pharmaceutical industry for QC, screening and mixture analysis.

Topics:
- Theory of fragment binding to biomolecular targets
- Ligand observed 1H and 19F NMR fragment screening experiments
- HSQC chemical shift perturbation mapping
- Data analysis of NMR screening data
- Quality control of small-molecule libraries by CMCs
- Mixture analysis using ASSURENMR automation and robotics
- High-throughput acquisition software/Preparation of customized parameter sets
- Sample Preparation for NMR screening

Remarks:
In lectures and practical sessions the concepts of 1D and 2D NMR experiments like nOe, Dixon, Spectroscopy Transfer Difference and relaxation based methods to probe for binding of small-molecules to biomolecular targets are elaborated. The participants will learn practical aspects of data analysis in small groups. Further topics are the use of CMCs and ASSURE for quality control of fragment libraries and mixture analysis. In most cases, the above applications are run in high-throughput with appropriate automation routines and robotics which will be introduced to the participants during hands-on sessions. Lectures about the theory of fragment binding to biomolecular targets in the context of the NMR time scale complete the program.

The broad scope of the training course makes it a perfect introduction for scientists from academia and industry with a background in chemistry, biology or biochemistry with interest in using NMR for the above applications.

Prerequisite Qualification:
Open to all Bruker spectrometer users from academia and industry interested in the above topics

Overview: NMR Training Germany & Switzerland

Summary

New tools & experiments for FBS

- **CMC-q** to easily run and analyze single compounds in buffer for reference AND do QC on the fly (in buffer to test solubility!)
- New **Parameter sets** in TS3.5 pl6 to run optimized FBS experiments in full automation
- Use the **Experiment selector** in TopSpin to try out new stuff
- New **SampleJet & SampleCase temperature control option** to run DMSO samples in one plate, cooled protein samples in the other
- New tools in **IconNMR** to setup batches of screening samples quickly from customized Excel spread sheets
- **Brand new FBS tool** in TopSpin to help you quickly organize data, analyze it your way and keep track of your results reporting into configurable excel spread sheets
Thank you for your attention

... and come see us for demos and discussions