Fragment Screening by NMR in Drug Discovery

Dr. Stefan Jehle, Bruker BioSpin
Dr. Pavel Kessler, Bruker BioSpin
German Users Meeting, November 9th, 2016
The principle of Fragment Based Lead Discovery from efficient fragments to Drug candidates

Advantages of FBLD – start small and grow big:
- 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%)
- Cost efficient
NMR in Fragment based Screening

More than one answer possible

http://practicalfragments.blogspot.ch/2016/10/poll-results-affiliation-metrics-and.html
<table>
<thead>
<tr>
<th>Throughput</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></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>Running costs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Data Quality</td>
<td><strong>QC of fragments</strong> 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>Bad samples</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>Data Analysis</td>
<td><strong>Clumsy, home-built acquisition automation, data organization, no workflow support</strong></td>
<td>Easy to use interface for operation, data analysis and export</td>
</tr>
</tbody>
</table>

✔ New in Topspin: FBS Analysis Tool
Key Advantages of NMR screening: Solution Method and QC of Fragments

NMR based fragment binding assays feature four major advantages:

1. **QC of fragments, easy identification of degradation in spectra.**
2. NMR techniques do not require protein specific setup or knowledge about the protein’s function and can be applied to targets for which no bio-assay is available,
3. NMR techniques in solution are not compromised by crystal contacts or target immobilization,
4. binding from low uM to mM can be detected (fast and intermediate exchange)

※ Missing or broad peaks in 1D $^1$H spectra indicate compound degradation or aggregation
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 (13C or 15N)
- Size limitation of target
- Amount of protein required depends on application
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 (\(^{13}\)C or \(^{15}\)N)
- Size limitation of target
- Amount of protein required depends on application

➢ No crystal structure available?
➢ Binding site in crystal contact (often for PPI targets)?
### Affinity Ranges and NMR Detection Limits

**Equation:**

\[ K_D = \frac{k_{off}}{k_{on}} \]

<table>
<thead>
<tr>
<th>K(_D)</th>
<th>mM</th>
<th>μM</th>
<th>nM</th>
<th>pM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 mM</td>
<td>10 μM</td>
<td></td>
</tr>
<tr>
<td><em>k_{off}</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Fast exchange**
- **Intermediate exchange**
- **Slow exchange**

**Categories:**
- Fragments
- Ligand observed NMR
- Target observed NMR
- Approved drugs

**Residence time**
Chemical Exchange – NMR Timescale

Fast- and Intermediate Exchange

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

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

$K_D$ is fitted from a function of concentration and $\Delta \delta$
Chemical Exchange – NMR Timescale

**Slow Exchange**

- Peaks for free and bound state are both detected if binding site not saturated
- $K_D = \text{pM-nM}$
Chemical Exchange – NMR Timescale

Slow Exchange

- Peaks for free and bound state are both detected if binding site not saturated
- $K_D = \text{pM-nM}$
Chemical Exchange – NMR Timescale

Slow Exchange

- Peaks for free and bound state are both detected if binding site not saturated
- \( K_D = \text{pM-nM} \)
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&lt;sub&gt;2&lt;/sub&gt;/T&lt;sub&gt;P&lt;/sub&gt;</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>&lt;sup&gt;19&lt;/sup&gt;F-T&lt;sub&gt;2&lt;/sub&gt; (CF/CF&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>10  5  2  1</td>
<td>50</td>
</tr>
<tr>
<td>&lt;sup&gt;19&lt;/sup&gt;F-T&lt;sub&gt;2&lt;/sub&gt; (CF&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>10  5  2</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
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**
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.
Identification of “aggregators” by NMR

- Measure STD on cocktails
  - Identify aggregating small molecules
  - Design next generation library

- Aggregators can be identified in STD or relaxation based experiments from a sample that contains only the ligands and NOT the target.

1D STD of 100 μM inhibitor without target present. Inhibitor shows μM inhibition in enzymatic assay.

✅ Aggregators can be identified in STD or relaxation based experiments from a sample that contains only the ligands and NOT the target.
typical conditions:

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

600 MHz QCI-F CryoProbe

19F Fragment Screening: Special workshop 10:30, 19F Methods
You mix several ligands per sample with protein:

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

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

Data Analysis

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

Data acquisition

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
Data analysis and interpretation – up to now

For each sample, you
- Open the STD spectrum
- Open the STD reference
- Open the Water-LOGSY
- Open the T2 experiment
- Open the T2 reference
- Scale all of them for amplitude
- Search for, and open all single compound reference spectra for that mixture

Before you can start analyzing
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
The actual screening campaign

Data analysis and interpretation – TS3.5 next pl

✓ Customizable display layout

.... you start analyzing!
New FBS tool in Topspin

Data analysis and interpretation – TS3.5 next pl
New FBS tool in Topspin
Data analysis and interpretation – **TS3.5 next pl**
New FBS tool in Topspin

Data analysis and interpretation – **TS3.5 next pl**
New FBS tool in Topspin

Data analysis and interpretation – **TS3.5 next pl**
New FBS tool in Topspin

Data analysis and interpretation – **TS3.5 next pl**
New FBS tool in Topspin

Data analysis and interpretation – TS3.5 next pl

My comment
New FBS tool in Topspin
Data analysis and interpretation – **TS3.5 next pl**
New FBS tool in Topspin

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

Data analysis and interpretation – TS3.5 next pl

✓ Customizable report layout
Thank you for your attention

... and come see us for demos and discussions