Magnifying Results

Nuclear magnetic resonance provides many benefits for pharma. Significantly, it makes fragment-based screening much easier in the drug and lead discovery fields

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Over the past 50 years, scientists have used nuclear magnetic resonance (NMR) to directly identify, quantify and characterise molecules, from small organic ones to large protein complexes. Highly valued for its unparalleled view into intact molecules, NMR reveals rich details of molecular dynamics and structural architecture, inaccessible by other analytical methods. NMR is a non-destructive technique, requiring minimal sample amounts, which allows the user to perform additional analyses.

Today's scientists often choose NMR as it offers various research and quality control (QC) methods in biology, chemistry, physics, medicine and materials science. The application range is broad, including materials, environmental and food science, medical and pharmaceutical research and more. Whether for routine analyses or ground-breaking research, now even small laboratories can easily adopt NMR in-house for definitive answers to molecular questions.

NMR methods can help to simplify fragment-based screening (FBS) in the lead discovery (FBLD) and drug discovery (FBDD) fields. A complete workflow solution for NMR-based fragment screening has proven effective in producing high-quality hits for FBLD. Certain software, together with a new FBS tool, can greatly accelerate data analysis during NMR-based FBS by incorporating all screening data into one place and automating many of the processes that are usually done manually.

An enhanced workflow solution to accelerate fragment-based drug discovery is beneficial to anyone working in that field, whether in the pharma industry, contract research organisations or academia – including those with no prior experience in FBS. A complete workflow solution that is user-friendly and easy to incorporate into existing workflows is advantageous, generating scope for people without expert data analysis knowledge to conduct this kind of research.

Screening Challenges

FBS is a widely applied method for lead molecule discovery in FBLD. FBS has emerged over the last 15 years as a popular alternative to high-throughput screening (HTS) for the lead compound identification in drug discovery. HTS is not always successful, with numerous drug discovery projects failing to deliver meaningful potential hits. HTS is in need of alternative approaches, and FBS has helped to address its limitations, requiring significantly fewer compounds to be screened and synthesised and resulting in a higher hit rate than traditional methods.

Furthermore, with the increasing impact of Alzheimer's disease and other age-related neurological disorders, designing molecules that not only bind to their target but also cross the blood brain barrier (BBB) becomes more important. For this purpose, the lead molecular footprint must be controlled while maintaining favourable physicochemical properties required for crossing the BBB, which can be achieved more effectively when using FBLD instead of HTS.

Fragment space is easier to explore (higher hit rates 0.1-10%), and a dramatically larger chemical structural space with fewer compounds can be investigated. However, a crucial step in the lead discovery process is the reliable initial fragment identification that, despite their high ligand efficiency, interacts weakly with the receptor due to their small size. Weak binding affinities in the uM to mM range is a hallmark of fragments, and appropriate ligand screening methods are needed for the detection of joining ligands, especially those with a low binding constant.

Numerous biophysical methods and techniques are used in the initial fragment screening phase, of which NMR

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Table 1: Fragment-based screening NMR versus SPR

	NMR fragment screening	SPR fragment screening
Throughput	100 samples, 500-1,000 compounds per day **(5-10 compounds per sample)** (^{19}F 3,000 compounds per day, 30 cpd per sample)	500 compounds per day, single point measurement (depends on instrument)
Running costs	Operational costs: approximately $45k per year (96 well-formatted NMR tubes, cryogens and service contract)	Operational costs: **$45-50k per year** (chips for target immobilisation, consumables, solvents and service)
Data quality	**QC of fragments** possible as part of process, inherent **concentration** information aids hit validation	No QC of fragments possible during process, independent QC required
Bad samples	No issue: one tube per sample – bad sample does not stop the screen	Sticky compounds may dismantle the chip during screening
Type of assay	**In solution, no protein-specific set-up, large dynamic range (mM-uM)**	**Target must be immobilised in a functional form, low dynamic range**

is the most popular and reliable methodology, followed by surface plasmon resonance (SPR) and fluorescence spectroscopy. NMR is ideally suited to detecting low affinity ligands in primary screens and also allows for screening library QC, which makes it superior to other methods like SPR or thermal shift (see Table 1).

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 with significantly reduced unlabelled target protein and fragment consumption.

NMR Screening Methods

Protein-observed methods identify binders and binding site on target:

- Isotopic protein labelling and assignment required (13C or 15N)
- Size limitation of target
- Amount of protein required depends on application

Ligand-observed methods identify binders from mixtures:

- No isotopic labelling of the protein
- No size limitation of the target
- Little amounts of protein needed (nM-uM per sample)
- Little amounts of ligands needed (0.025-0.250 mM per sample)

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 cumbersome. In many cases, users have implemented homebuilt tools to facilitate the workflow.

Simplifying with NMR

In pharma, this has become a widely applied method for lead molecule discovery, and NMR-based fragment screening is used in more than 50% of fragment screening campaigns (1). However, data handling and analysis has been a barrier as many 1D ^{1}H or 1D ^{19}F spectra must be analysed in parallel by the operator, which can be a time-consuming task. Additional drawbacks include the number of spectra generated, sometimes in the 100s or 1,000s, which must be analysed manually in parallel by a trained operator – it is a time-consuming, labour-intensive activity that creates a substantial obstacle in the process.

A software tool for the interactive analysis of NMR-based screening data offers a complete workflow solution from data acquisition and processing to data analysis and hit reporting. This, together with specific FBS tools, can help overcome many stumbling blocks by facilitating the workflow, improving efficiency and minimising human error.

Beneficial Workflows

Current workflows can be cumbersome and typically involve manual data management, bookkeeping of experiment types, compound names and results. A specific FBS tool within NMR instrumentation software streamlines this workflow, allowing the user to focus on information content and data analysis. This reduces human error and increases the efficiency considerably, making NMR-based FBS superior to orthogonal methods.

The FBS tool harnesses the power of NMR data by streamlining the entire acquisition to analysis workflow. All relevant data, experiment types, compound IDs, reference spectra and other information are automatically collected, stored in a project file and the display shows the relevant data for analysis.

Reference 1D ^{1}H spectra of fragments are recognised by

Water-ligand observed via gradient spectroscopy (WLOGSY)

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

Saturation Transfer Difference (STD)

Binders **show up** in difference spectrum, non-binders do not

T_2/T_{1rho}

Binders show **strong attenuation**

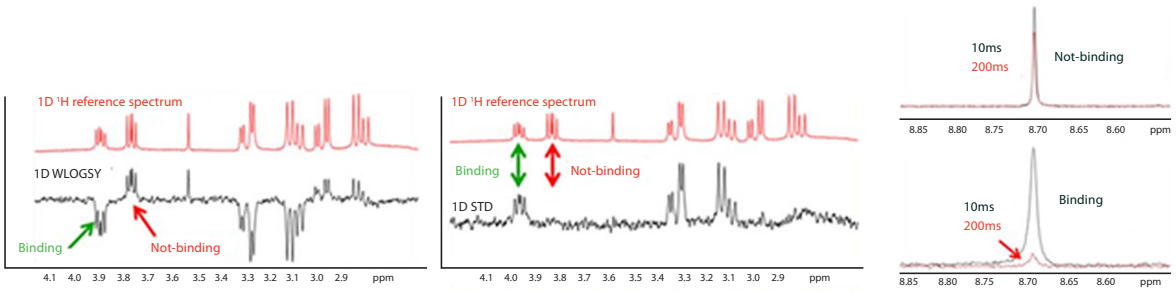

Figure 1: Three basic ^{1}H NMR experiments in FBS

unique identifiers of the employed molecules and presented to the user in multi-display mode together with the screening spectra. The tool allows the most flexible implementation in individual laboratory environments, with few restrictions in regard to data storage and preparation. For instance, no databases need to be prepared, and automation routines for NMR-based screening experiments are presented.

NMR FBS allows the user to conduct a druggability assessment using a small ligandability library. When one HTS costs several hundred thousand US dollars, spending more than one million is possible on a six-month discovery campaign on a nondruggable target. When a small ligandability library is screened against the target, a ligandability score can be assigned according to the hit rate, which correlates with the success rate in HTS, identifying nondruggable targets before the discovery campaign (2).

Conclusion

NMR-based fragment screening has proven to produce high-quality hits for FBLD. The workflow solution discussed helps to greatly accelerate data analysis during NMR-based

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FBS by incorporating all screening into one place and automating many of the processes that are usually done manually. The ability for all relevant data, experiment types, compound IDs, reference spectra and other information to be automatically collected and stored in a project file is extremely beneficial. During analysis, the ability to also automatically save each action, including any hits, ensures accurate records and frees up operators to focus on applying their chemical knowledge.

The field of FBDD has developed significantly over the past 15 years and is now recognised for its important contribution to the drug discovery process. NMR-based screening, despite its low intrinsic sensitivity, offers the largest dynamic range and is capable of capturing very weak interactions. These developments will enable the drug discovery community to set up NMR-based screening experiments, facilitate data analysis and consequently reduce the hurdles for non-experts using NMR for FBLD.

References

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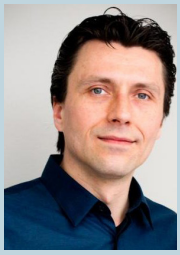

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