Simplifying Fragment Screening in Drug Discovery with NMR

In this article, author presents the rationale for the rise of FBS and review key factors for the adoption of NMR - specifically highlighting how NMR methods can simplify FBS. A complete workflow solution for NMR-based fragment screening is discussed, which has proven effective in producing high-quality hits. Now, software, such as Topspin (Bruker), which includes a new FBS tool, looks set to greatly accelerate data analysis during NMR-based FBS by integrating all screening data into one place and automating much of the experimental process that is usually done manually.

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Fragment-based screening (FBS), as a viable and productive approach to drug discovery, came of age back in 2011 when Vemurafenib, the first compound to have been generated from a fragment-based drug discovery (FBDD) program, was approved by the US Food & Drug Administration (FDA). Many more have followed.

In parallel, analytical methodologies to support the FBS approach have been implemented and optimized, with NMR highlighted in early breakthrough publications and subsequently emerging as arguably the most appropriate technique.

Why FBS?

The principles of FBS have been known for around 35 years. In 1981, William Jenks wrote that the affinities of whole molecules could be understood as a function of the affinities of separate parts[1]. His paper stimulated significant academic curiosity but the practical difficulties of finding fragments and linking them proved too big a barrier for it to have any immediate impact on drug discovery. It remained an interesting theory.

This changed in 1996, with the breakthrough publication in Science magazine, in which Shuker et al, from Abbott Laboratories, gave the first real demonstration of fragment based drug discovery[2]. They coined the term 'SAR by NMR', meaning: structure-activity relationships that are obtained from NMR data.

The subsequent drive towards FBS should be seen as part of the search for more rational drug development strategies, and be set alongside the approaches of combinatorial chemistry and high throughput screening (HTS).

Three important advantages characterize FBS in comparison to HTS - FBS samples a broader chemical diversity; it produces a higher hit rate; and leads to a higher ligand efficiency because, rather than screening immense libraries of 'drug-sized' compounds for activity, FBS starts with a much smaller collection of very small chemical fragments that may only exhibit weak binding affinity. Fragments that show affinity are subsequently assembled to produce a fully-built ligand.

Figure 1 illustrates the key differences between HTS and FBS in terms of ligand efficiency[3].

Analytical tools and typical NMR screening methods

FBS places very specific analytical demands on the workflow. The detection of weak fragment binding (in the uM to mM range) by biochemical methods, that is spectrophotometric and fluorescence-based assays, is challenging, because the small change in signal above the baseline is difficult to observe.

In contrast, biophysical techniques such as NMR spectroscopy, isothermal titration calorimetry (ITC), thermal denaturation, surface plasmon resonance (SPR), and X-ray crystallography, among others, are more robust in detecting such weak interactions[4]. In particular, NMR spectroscopy is ideally suited for fragment-based screening

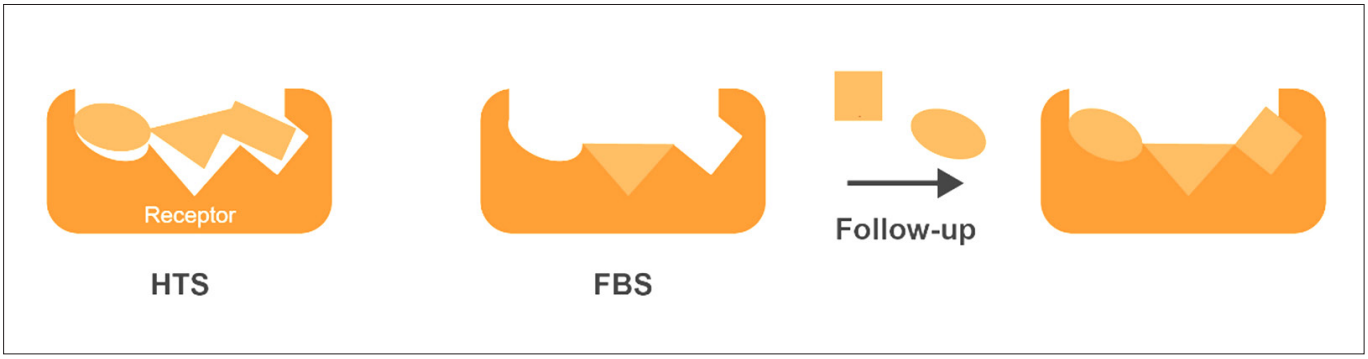

Figure 1: Potential drawback of HTS (left), and principle and advantages of FBS (right): In HTS, fully assembled, "drug-sized" ligands are identified, but with multiple compromised, non-optimal binding interactions. In FBS, ligands for individual subpockets are identified separately, and show few but good binding interactions. Follow-up strategies such as fragment elaboration or linking are used to increase ligand affinity.

because it can reliably detect binding up to single digit millimolar Kd values, often the only hits found for challenging targets[5,6]. Additionally, NMR can be used to quantify binding affinities in order to establish SARs.

The binding sites of fragment hits and modes of binding can also be established from NMR experiments. In addition, as binding events are directly observed by NMR, the technique does not usually suffer from false-positive hit identification that can compromise other screening techniques. Importantly, the use of NMR for FBS does not require any prior knowledge of protein function or endogenous binding partners as would be needed in enzymatic or displacement-based assays.

So, NMR is ideally suited for detecting low affinity ligands in primary screens and, 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. Screening by NMR also allows the quality control of the screening library which means that NMR compares favorably to other methods: SPR or thermal shift, for example (Table 1).

The Remaining Challenge - Simplifying the Process

With the advantages described above, it

	NMR Fragment Screening	SPR Fragment Screening
Throughput	100 samples, 500-1000 compounds per day (**5-10 compounds per sample**) (^{19}F 3000 compounds/day, 30 cpd/sample)	500 compounds per day, single point measurement (depends on instrument)
Running costs	Operational costs: **~45k per year** (96 well format NMR tubes, cryogens and service contract)	Operational costs: **45-50k per year** (chips for target immobilization, 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 setup,** large dynamic range (mM-uM)	**Target must be immobilized in a functional form,** low dynamic range

Table 1: Fragment Based Screening NMR vs. SPR

NMR fragment-based screening allows the user to conduct a druggability assessment using a small ligandability library. If we consider that one HTS program will cost several hundred thousand US dollars, there is the potential to spend more than 1 million US dollars on a 6-month discovery campaign following up a target that proves to be not-druggable. When a small ligandability library is screened against the target, a ligandability score can be assigned according to the hit rate. The ligandability score correlates with the success rate in HTS, identifying not-druggable targets before they move into a discovery campaign.[7]

is not surprising to see that NMR-based FBS is now used in more than 50 per cent of fragment screening campaigns[8]. However, data handling and analysis has been a bottleneck as many 1D ^{1}H or 1D ^{19}F spectra must be analyzed in parallel by the operator. This can be a time consuming task.

Additional drawbacks include the sheer number of spectra generated, sometimes in the 100s or 1000s, all of which must be analysed manually by a trained operator - a time-consuming, labour-intensive activity. Moreover, current workflows can be cumbersome and typically involve manual data management, bookkeeping of experiment types, compound names and results. This has remained a major pain point. In many cases, users have implemented homebuilt tools to facilitate the workflow.

Now, specific FBS tools within NMR instrumentation software, such as Topspin (Bruker), streamlines this workflow, allowing the user to focus on information content and data interpretation. As is the case with most automated data handling, the likelihood of human error is reduced, and throughput is considerably improved.

Ideally, such an FBS tool should streamline the entire 'acquisition to analysis' workflow. All relevant data, experiment types, compound IDs, reference spectra and other information should be automatically recognized, collected and stored in a project file. Display should show the relevant data for interpretation.

Table 2 shows a complete workflow using the Topspin tool and Figure 2 highlights the three most popular NMR experiments for fragment screening: Saturation Transfer Difference (STD), waterLOGSY, and relaxation based methods, which are automatically identified.

Reference 1D 1H 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.

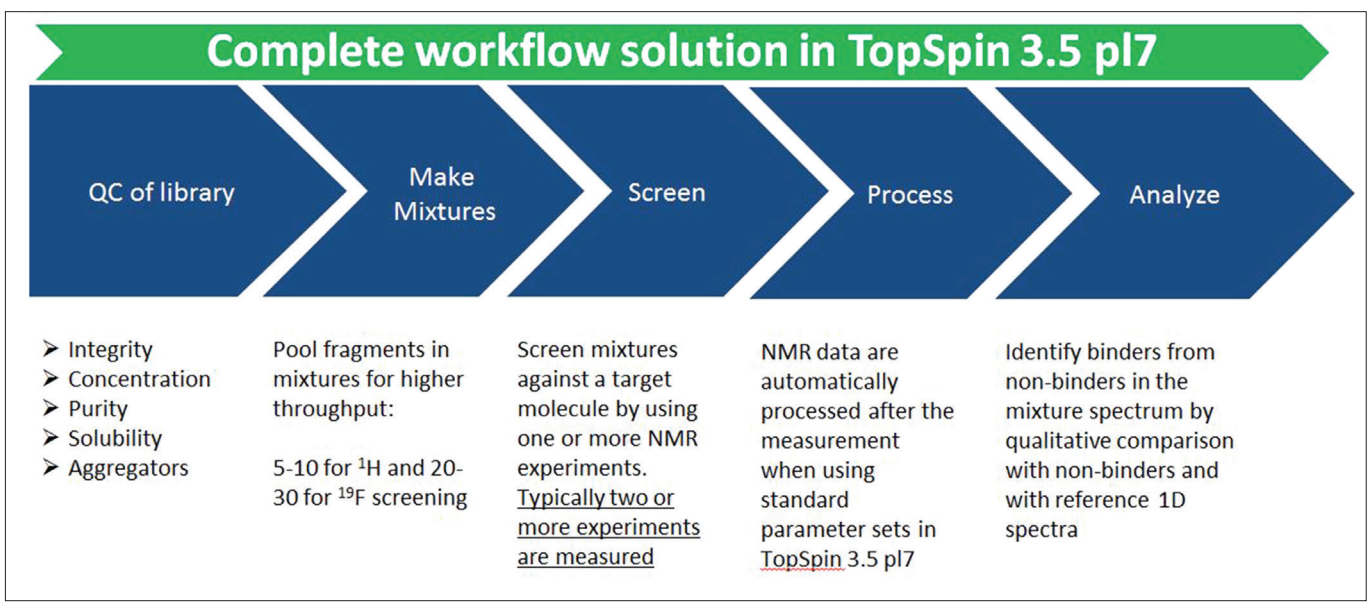

Table 2: Step-by-step Fragment Screening by NMR

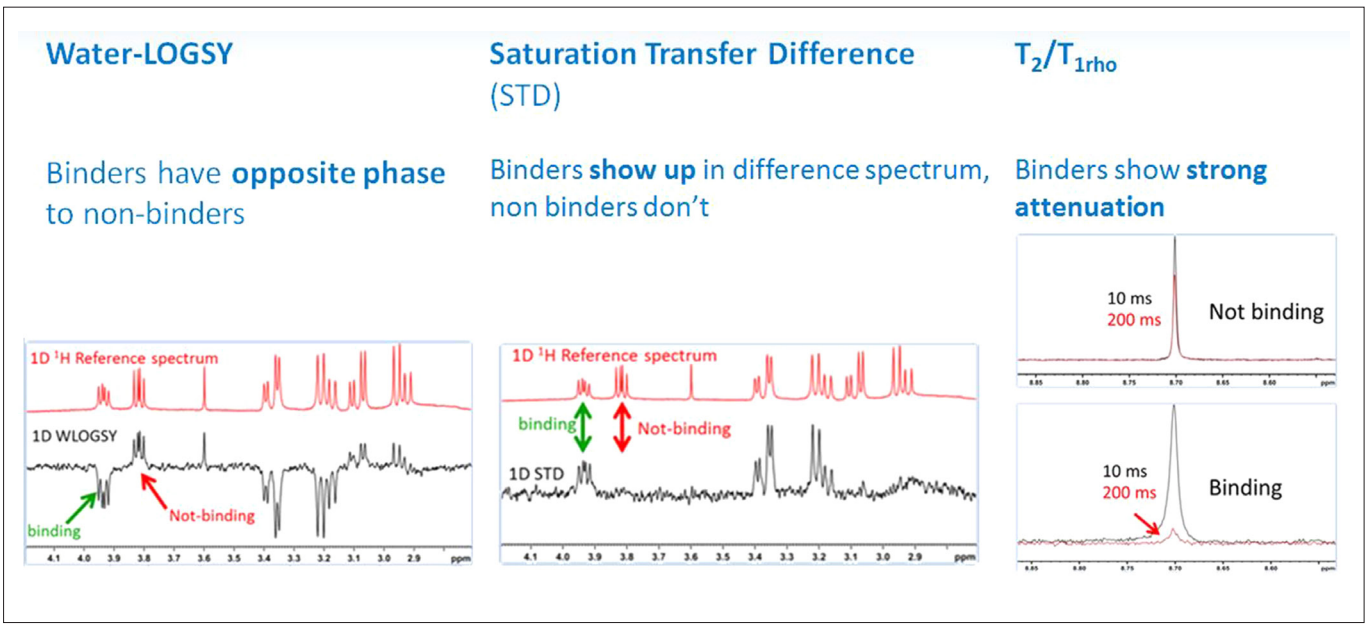

Figure 2: Three Basic 1H NMR Experiments in FBS

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 example, no databases need to be prepared. In addition, automation routines for NMR based screening experiments are presented.

Conclusion

The field of FBS has developed significantly since it was first described in 1996 and is now recognized for its important contribution to the drug discovery process. NMR-based fragment screening has proven to produce high-quality hits and now, enhanced workflow solutions that simplify methodology, particularly the data processing, are likely to further accelerate the success of the approach. These developments will impact on everyone working in drug discovery, whether it be in the pharmaceutical industry, contract research organizations or academia, including those with no prior experience in FBS. ■

References

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