Reducing attrition early in discovery

Fragment based drug discovery is providing more efficient ways to identify and advance true drug leads. **Dr Paul Belcher**, Development Leader, Biacore*, GE Healthcare looks at recent developments and the benefits of surface plasmon resonance

Over the past decade fragment-based drug discovery (FBDD) has become firmly established and is now rivalling more traditional methods of hit identification, such as high throughput screening (HTS), for finding low molecular weight compounds that bind to protein targets. This widely adopted methodology delivered its first drug, Zelboraf (vemurafenib), to the market in 2011 and has generated a diverse pipeline of more than 26 clinical-stage compounds.

Unlike HTS, which screens larger, more drug-like compounds, FBDD aims to identify small chemical fragment hits, which are then combined or enlarged to produce a lead with higher affinity or tighter binding to the target. The success of FBDD in generating high-quality drug candidates results from the integration of structural biology, biophysical characterisation using techniques such as surface plasmon resonance (SPR), nuclear magnetic resonance (NMR) and X-ray, and rigorous medicinal chemistry.[1]

FBDD versus HTS

The basic concept of HTS involves screening large, chemically diverse libraries against a target and identifying compounds that bind. HTS libraries are typically designed to comply with the 'Lipinski rule of five' – i.e. they are approximately 250–500 Da in size but are often lipophilic, making them challenging to develop without reducing their 'drug-likeness'.[2] Additionally, poor absorption, distribution and metabolism characteristics, often due to high molecular weight and lipophilicity, are major causes for attrition in lead and optimisation in the clinic.

From a screening perspective, one of the challenges of HTS is that due to the compound size, chemical space is very large (estimated at 10^{63})[3], meaning that even the largest libraries sample a small percentage of diversity space resulting in low hit rates.

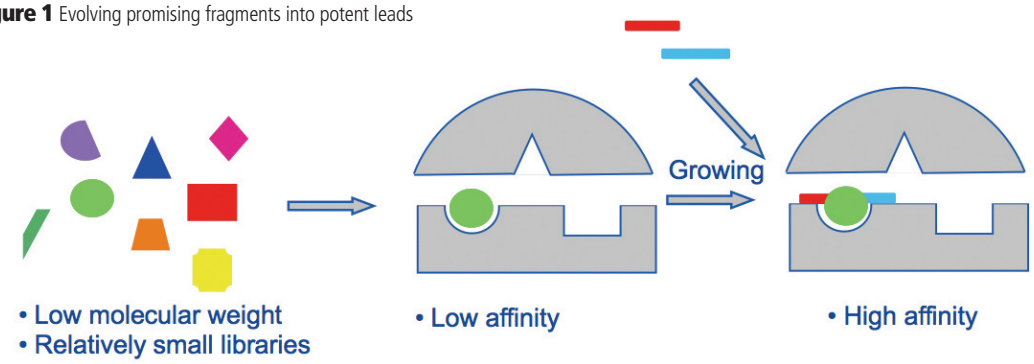
In contrast, FBDD works by screening regions or sections of the binding site with much smaller chemical entities (fragments) that are less complex and less structurally diverse than typical HTS compounds (see Figure 1). This enables a much greater proportion of chemical space to be explored using significantly smaller libraries. Therefore FBDD screens can be effective with libraries consisting of 500–2,000 molecules (vs hundreds of thousands with HTS) and can give a selection of hits with more diversity than would be seen with HTS consisting of more than a million compounds and often with more favourable physiochemical properties.[4]

A typical FBDD screen uses a combination of structural and functional binding information to identify fragments that bind to a target typically with lower affinity (KD values in the micromolar (μM) to millimolar (mM) range compared with larger 'drug-like' molecules that can form many more interactions (KD values in the nanomolar to μM range). Using meticulous, structurally guided medicinal chemistry, fragment hits can be optimised into more potent leads by synthesising larger compounds that incorporate additional target-ligand interactions, resulting in improved affinity, while maintaining 'drug-likeness.' A fragment approach provides the opportunity to identify novel starting points that can tackle new and challenging target classes using molecules with high ligand efficiency and ones that may fall outside a very crowded patent space.

Despite these advantages over HTS, FBDD does, however, present several challenges. The interaction of the fragment hit with the target binding site is normally very weak (0.1 to 10mM) and makes the reliable identification of interactions more challenging. The low affinities require high sample concentrations to obtain binding site occupancy, meaning that it is often necessary to work at the limits of solubility of the fragment library.

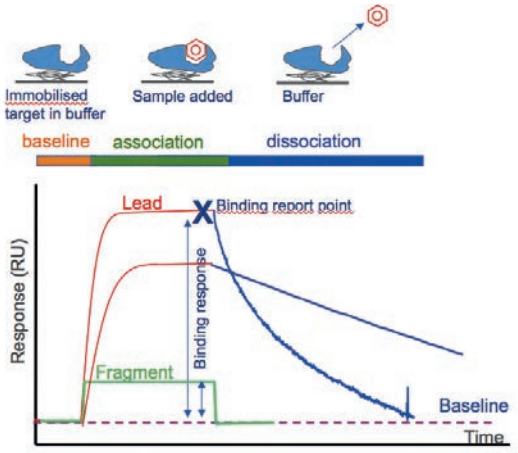
These challenges mean that evaluating hits from fragment screens requires different assay approaches from the functional biochemical assays most frequently used in HTS and demand that much more sensitive techniques be used. Consequently fragments are initially

Figure 1 Evolving promising fragments into potent leads



"A fragment approach gives the opportunity to identify novel starting points that can tackle new and challenging target classes using molecules with high ligand efficiency"

Figure 2 This 'sensorgram' traces the association and dissociation over the entire course of an interaction; the kinetics are revealed by the shape of the binding curve



k_a from association phase:
- Steeper slope = faster on-rate.
- Concentration dependent.

k_d from dissociation phase:
- Steeper slope = fast off-rate.
- Concentration independent.

$K_D = k_d/k_a$

screened at high concentration using sensitive biophysical techniques such as NMR, X-ray crystallography and SPR. No single method delivers all the required information to minimise false positives or negatives, so orthogonal methods should be employed to ensure reliable results.

SPR and screening advantages

Since the practice of FBDD was first reported in 1996 by Shuker *et al*[5], biophysical, structural methods such as NMR and X-ray crystallography have been used to identify the relative positions of and specific contacts between a fragment and target. These methods provide a high resolution of structural detail but they require large amounts of target protein, have limited throughput and typically do not provide information on the strength of binding.

In contrast, GE Healthcare's Biacore, an SPR-based biosensor technology, has evolved as a method of choice for screening fragment libraries and prioritising compounds prior to more detailed structural analysis. The advantages of SPR include low sample and target consumption, typically 10- to 100-fold less than other biophysical screening methods, i.e. 25–50μg protein and relatively high throughput.[6]

This allows the screening and selection of compounds based on binding characteristics, generating high information content for each fragment, such as binding strength/affinity (K_D), kinetics (k_{on}, k_{off}) or inhibition (IC_{50}), selectivity and specificity information, identification of promiscuous binders, as well as the assessment of target drugability.[7,8] These types of information are critical for the prioritisation of compounds for more detailed structural determinations.

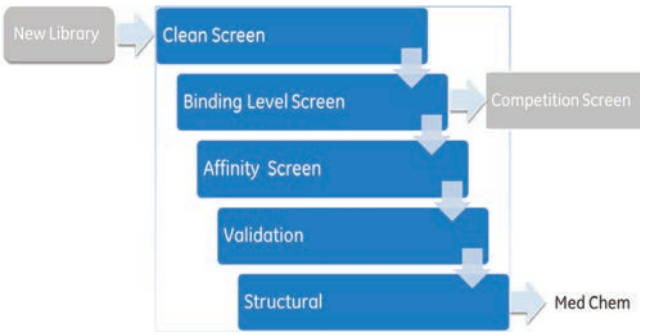
The SPR technology employed in Biacore systems monitor bimolecular interactions in real-time using a label-free detection system.[9,10] The fragment is injected over a sensor surface on which target proteins are immobilised, either singly in individual flow cells or as part of an array (for parallel screening against multiple targets). Interaction of the sample with the immobilised targets changes the refractive index, which is proportional to the change in mass at the surface, and data are presented as a profile of SPR response plotted against time. This 'sensorgram' traces the association and dissociation over the entire course of an interaction, and the kinetics are revealed by the shape of the binding curve (Figure 2).

The sensorgram provides real-time information about an entire interaction, with binding responses measured in resonance units (RU). Binding responses at specific times during the interaction can also be selected. Any remaining bound sample molecules may be removed in a regeneration step that prepares the surface for the next sample.

The visualisation of compounds that look identical from a standard affinity perspective but clearly have completely different kinetic profiles can be used to illustrate that kinetics give an extra dimension to the characteristics of an interaction compared with affinity; this can be important to support on-going structure activity efforts in hit to lead development.

"Kinetics give an extra dimension to the characteristics of an interaction compared with affinity, which can be important to support further structure activity efforts in hit to lead development"

Figure 3 Typical SPR FBDD workflow



Binding affinity reflects the ratio of on- and off-rates (kinetics) and equal affinity interactions may have radically different kinetics. End point assays alone cannot resolve these crucial differences. Since SPR is a mass-dependent technique, the low molecular weights of fragments (Mw 80 to 300 Da) give low signals, therefore the use of SPR in FBDD requires sensitive, precision instrumentation such as Biacore 4000 and Biacore T200.

Development of a FBDD SPR workflow

A typical SPR workflow, as outlined in Figure 3, aims to decrease the number of fragments for assessing in greater detail, with the aim of identifying problematic compounds much earlier in the selection process.[8] A clean screen is often employed on new compound collections to identify and remove troublesome fragments, in particular problematic 'residual binders' that may affect the data quality in subsequent cycles. After this rapid

Figure 4a SPR fragment-based drug screening approach for: (a) Primary hit validation using SPR affinity screen and (b) Orthogonal hit confirmation: SPR, NMR and DSF

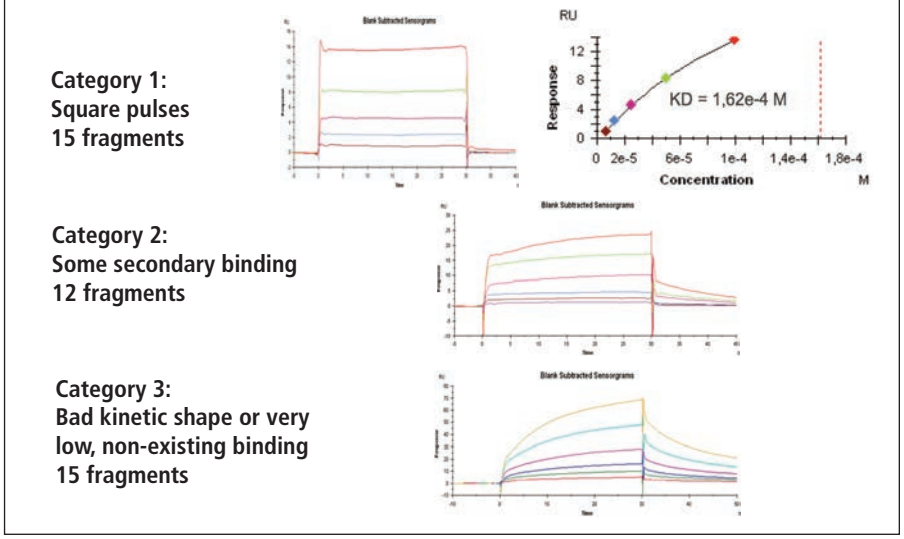
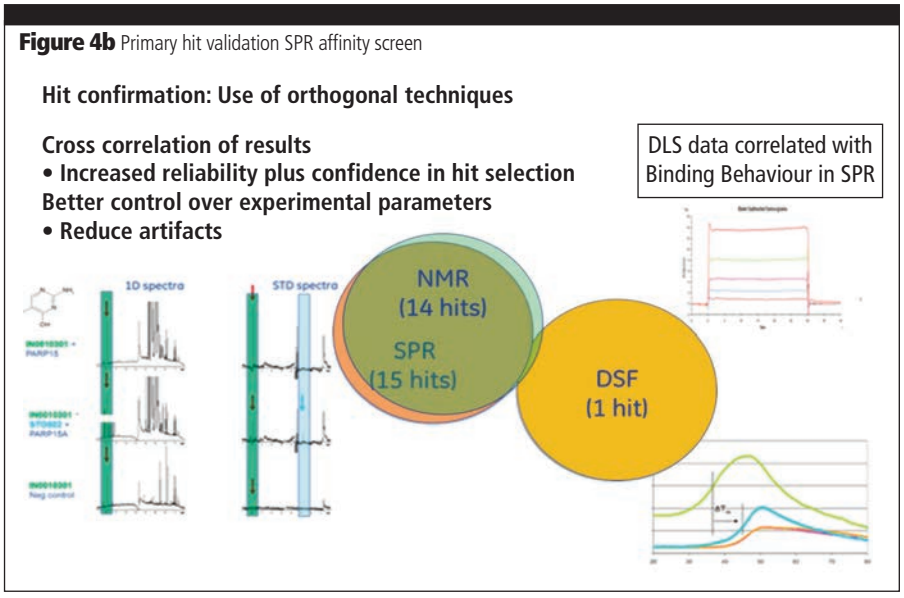


Figure 4b Primary hit validation SPR affinity screen



library clean-up step, fragments can be prioritised for further analysis using a Binding Level Screen, whereby fragments are screened against the targets at a single concentration and well behaved binders (based upon binding characteristics of the sensorgram) selected. This approach provides a rapid overview of library content and in addition to identifying binders to the target it allows the early identification of compounds that exhibit undesirable behaviours, such as non-specific or multi-site binding, secondary interactions, covalent modifiers and even aggregation. The prioritised fragments are then run in a dose response affinity screen to verify binding and determine the affinity (K_D) for ranking based on affinity and ligand efficiency. These confirmed hits are then validated in further orthogonal biophysical techniques before entering the iterative cycle of medicinal chemistry driven optimisation.

An SPR-based fragment-based screening approach was chosen by scientists at iNovocia and the Karolinska Institute to identify possible starting points for the development of chemical probes to the less known Poly-ADP-ribose)-polymerase) (PARP) family members such as PARP 15. From an initial screen of a small 987 fragment library at 200μM, 42 hits were identified based on the binding response and sensorgram 'shape'. The binding of these hits to the PARP 15 target was then characterised in a dose response using a Biacore affinity screen. Based on the displayed concentration dependence of binding the fragments were divided into three categories: 1. square pulses; 2. some secondary binding and category; 3. bad kinetic shape or very low/non-existing binding. The primary hits identified by the Biacore screen (category 1) were then submitted for hit conformation and validation using NMR and differential scanning fluorescence (DSF). While DSF gave poor correlation of results, NMR and SPR gave good cross-correlation, thereby increasing reliability and confidence in hit selection (see Figures 4a and 4b).

In conclusion, SPR-based biosensors are becoming powerful tools in FBDD and lead finding due to the sensitivity requirements to observe binding interactions of low-affinity and low-molecular weight compounds to target proteins. This approach offers not only novel ways of finding hits, but also more efficient ways to identify and advance true hits for chemistry and later stage biology by providing quantitative binding information for ranking fragments by affinity and ligand efficiency.

This information is crucial to support ongoing structure–activity efforts during fragment hit-to-lead development. While SPR can be used as a standalone technique, more importantly it is used in tandem with other orthogonal biophysical techniques such as NMR and X-ray crystallography to increase reliability and confidence in hit selection.

REFERENCES

1. Congreve, M., *et al, J. Med. Chem.* **56** (3): p1160–1170 (2013).
2. Keserü, G.M. & Makara, G.M. *Nature Rev. Drug Discov.* **8**: p203–212 (2009).
3. Bohacek R.S, McMartin C, & Guida W.C., *Med. Res. Rev.* **16**: 3–50 (1996).
4. Hopkins, A. L., Keserü, G.M., Leeson, P.D, *et al, Nature Rev. Drug Discov.* **13**: p105–121 (2014).
5. Shuker S.B., Hajduk P.J. & Meadows R.P., *et al., Science.* **274**: p1531–1534 (1996).
6. Navratilova, I. &Hopkins, A. L., *ACS Med. Chem. Lett.* **1**: p44–48 (2010).
7. Giannetti, A.M., Koch, B.D. and Browner M.F., *J. Med. Chem.* **51**(3): p574–580 (2008).
8. Grädler, U *et al. Bioorg. Med. Chem. Lett.* Oct 1;23(19): p5401–5409 (2013).
9. Malmqvist, M., *Nature* **361**, p186–187 (1993).
10. Fagerstam, L. G.; Frostell-Karlsson, A.; Karlsson, R *et al, I. J. Chromatogr.* **597**: p397–410 (1992).

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CONTACT

Dr Paul Belcher
GE Healthcare Bio-sciences Corp
800 Centennial Ave., Piscataway,
NJ 08854, US
Tel +1 732 457 8000
www.gelifesciences.com