editorial

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Picking up the pieces with FBDD or FADD: invest early for future success

It is easier to achieve a desired result in short pieces. Gustav Mahler

Success in drug discovery is greatly enhanced through the generation of high quality lead molecules. Initiating lead optimisation with the very best lead series reduces attrition due to liabilities such as inadequate PK, competing IP and poor developability. Access to a variety of hit finding technologies in turn increases the prospects of generating the highest quality lead series. Now that FBDD (Fragment-Based Drug Discovery) has become an established technique for lead generation, as described in the review of de Esch and co-workers in this issue [1], an intriguing question to ask is whether the acronym FADD (Fragment-Assisted Drug Discovery) in fact reflects a more accurate picture of how fragment methods can benefit the biopharmaceutical industry. This is more than just a difference in semantics, but is, in fact, a broader question of when and how to apply fragment approaches to lead generation, either on their own or in concert with other hit finding techniques.

There are reports that clearly indicate that fragment approaches can be complimentary to other hit finding and optimisation methods and are often being used in parallel with them. In their seminal review from 2006 on fragment methods, Leach and co-workers describe 'Reduced Complexity Screening' at GSK whereby a set of fragment compounds is assayed by biochemical screening at high concentration (*ca.* 1 mM) alongside a full deck high throughput screen [2]. Also presented in the same paper is a workflow used at AstraZeneca for the application of high concentration screening (*ca.* 100 μM) of a set of 2000 fragments in parallel with every HTS. In a subsequent paper the AstraZeneca group describes this approach in more detail [3]. Notably, they applied fragment methods to both structurally tractable targets, as well as to GPCRs. In the latter case, informatics-driven mining for near neighbours of the fragment hits enabled the identification of more potent analogues by cherry picking and subsequent testing of higher molecular weight drug-like compounds from their HTS collection [3]. We also know from Abbott, pioneers of fragment methods, that frequently and target-dependently either HTS, fragment screening or synergistic combinations of both are used in the early hit discovery stage [4].

At Evotec we often apply fragment techniques as the sole route for lead generation; that is FBDD. A case in point is our recently published account of fragment hit identification and subsequent structure-led optimisation to provide a Hsp90 lead compound series [5]. It is our experience, however, that there is often great merit in following more than one hit finding strategy in concert with a fragment approach; that is FADD. For example, using our highly sensitive screening technique of fluorescence correlation spectroscopy we were able to directly identify multiple hit series, that included fragment inhibitors, from a full deck HTS campaign against prostaglandin D2 synthase. Again, structure-led optimisation of fragments provided an orally available lead series that exhibited *in vivo* efficacy [6].

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The key question is which hit finding approaches to apply to which targets? We conducted a uHTS campaign of *ca.* 200,000 compounds against the Alzheimer's target, BACE-1. For this aspartyl protease the hit rate was extremely low and no suitable starting points were found for medicinal chemistry optimisation. Subsequently however, a fragment screen of our 20,000 member fragment library using the same biochemical assay principle, but screening at a higher concentration (1 mM), followed by orthogonal testing using surface plasmon resonance (SPR), gave more than two dozen confirmed hits. Starting from this hit set, X-ray crystallography and medicinal chemistry optimisation have provided inhibitors with low micromolar activity suitable for further elaboration. This approach should be compared with a fragment screen we conducted of the same fragment library against the potential schizophrenia target PDE10a. In this instance we also applied a biochemical assay, that used the fluorescence correlation spectroscopy readout, but utilized protein NMR as an orthogonal assay. This approach furnished a high hit rate with multiple fragment–protein complex X-ray crystal structures being readily solved ahead of the start of medicinal chemistry optimisation. In parallel with this fragment screening campaign a virtual screen was conducted of commercially available compounds using molecular docking. One of the hits from the virtual screen exhibited submicromolar activity and represented a very attractive starting point for medicinal chemistry optimisation.

If we had known these outcomes ahead of undertaking the screens we might have conducted a fragment screen for BACE-1 at the outset and just a virtual screen for PDE10a. In the case of PDE10a, however, the X-ray structural information from the fragment hits is informing and assisting the medicinal chemistry optimisation of the virtual screening hits, as well as providing additional alternative compound series. In particular, analogues of the structures of the fragment hits have revealed the key target interactions available for optimising hits into leads through the overlay of information obtained from the initial fragment starting points.

Whilst the integration of hit finding methods can be achieved through parallel tracking of different approaches (fragment screening, HTS, virtual screening and/or fast-follower diversification from known ligands) there is also benefit in a sequential approach whereby a fragment screen is used to focus a subsequent hit finding strategy using additional methods. This is a key aspect of Vertex's SHAPES method whereby an initial screen of a small set of fragment compounds is conducted using protein NMR. The results are then used to direct the subsequent hit finding strategy through focussed or full deck HTS [7]. Novartis have coined the term 'Virtual Fragment Linking' (VFL) for a somewhat related process. In this case a fragment screen is first conducted and based on chemoinformatic analysis of the results, a subset of compounds from the full HTS library is selected for a second round of screening [8]. Good hit rate enrichments by the VFL method are reported for GPCR and kinase targets but not for less druggable targets. This highlights the need to employ all possible methods for hit finding for less druggable targets. Whereas, for targets of established druggability consideration should be made of the competitive environment and the prospects for accessing novel and patentable chemical equity when deciding how widely to screen, and by what methods.

Evotec has obtained productive outcomes when applying fragment approaches alone in a true FBDD approach. We consider, however, that where the target merits it (and resources are not limiting) that more than one hit finding approach should be used in parallel to provide the maximum information at the outset. It is our experience that this approach provides project teams with a greater diversity of starting points for medicinal chemistry optimisation [9]. One possible concern with this approach is that medicinal chemists may be tempted to focus on the more potent hits (these are often the HTS hits rather than the fragment hits) instead of considering which are the most ligand efficient starting points. Whilst it does not help that there is now a confusing proliferation of ligand efficiency metrics, a detailed analysis of hit sets using one of these metrics together with modern clustering techniques [10] can assist the medicinal chemist in the selection of starting points for optimisation.

In conclusion it should not be a question of FBDD or FADD but rather which hit finding techniques are most appropriate for the target, its druggability and resource constraints. Great success has been achieved for certain targets, particularly those amenable to X-ray crystallography, by using FBDD as the sole approach for lead generation [1]. For these and other targets, however, the fragment approach can also have an impact by providing a target druggability assessment through the initial screening of a fragment set [4,7,8]. The results from such a druggability assessment can then help further develop the hit finding strategy into FADD whether it be by focussed HTS, full deck HTS and/or virtual screening to provide the very best chemical starting points. It is this combination of hit finding technologies that needs to be at the heart of the most effective lead generation campaigns to ensure maximal success in drug discovery.

References

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