Fragment-Based Drug Design

By careful selection and experimental design, fragments can provide very useful starting points for medicinal chemistry-driven programmes, leading to tailor-made, selective molecules with highly favourable physico-chemical and ADMET properties

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Within the space of only a few years, fragment-based drug design (FBDD) has emerged as an efficient and productive route for *de novo* drug discovery. Using tailored sets of chemical fragments, FBDD is delivering high-quality drug leads against a multiplicity of new therapeutic targets in the pharmaceutical sector. FBDD is also a promising starting point for chemical probe design for rapid validation of molecular targets in drug discovery. Applying FBDD as a chemical biology tool is a powerful way for biotechnology companies to add a pharmaceutical dimension to their discovery programmes. In this overview of the FBDD approach, we review FBDD and its emerging role in design-led discovery biology.

THE FBDD APPROACH

Genome sequencing and expression analysis have enabled the identification of a plethora of new proteins and signalling pathways which drug discoverers are systematically triaging for tractable molecular targets. With validated targets in hand, chemists and structural biologists can collaborate to explore existing and novel areas of chemical space (1), in the search for potent and selective drug-like compounds.

Fragment-based drug design is a recent addition to the drug design armamentarium (2). Unlike *in silico* virtual screening, it is based upon the experimental determination of the mode of binding small chemical fragments at a binding site. Repeated visualisation of binding mode rationalises SAR, allowing very efficient lead optimisation through structure-informed design.

High-throughput screening (HTS) of pre-existing compounds in a variety of pharmacological assays is still the most widely used approach to hit discovery. However, experience suggests that half of all HTS screens fail to deliver appropriate chemical starting points for new discovery programmes. This is as much due to the nature of the chemical input as the discovery process – the non drug-like compounds present in many chemical archives, historically

assembled by random combinatorial chemistry approaches, often yield leads which cannot be optimised into development candidates (3). Re-engineering failing compounds can be time-consuming and unproductive. It is usually more efficient to start discovery from scratch, creating new target-focused libraries of 'lead-like' compounds which can be more readily optimised.

Moreover, not every target may be 'druggable' in the conventional sense (4). Within this wider context, structure-based drug design (SBDD) approaches have an important role to play, for which FBDD strategies have emerged as a powerful ally, capable of addressing both the routine (5) as well as more challenging targets, such as allosteric (6) and protein-protein interactions (7).

Access to a simple process for lead discovery, applicable across a range of biological targets, is an important way to capture commercial value intrinsic to new targets, especially for biotech companies and academic laboratories. FBDD delivers such a process.

FBDD deploys relatively small libraries of low molecular weight 'fragments', which are tested for binding affinity against the target of interest. Hit fragments are then either incrementally modified into more potent, selective molecules, or synthetically combined to develop SAR around new scaffolds. These two approaches to the FBDD process are shown in simplified form in Figure 1.

Higher hit rates are observed in fragment screening compared with HTS, since small fragments can sample 'recognition space' within targets more efficiently than larger, more complex molecules which may have restricted access to the same sites. Fragment hit rates can also provide a useful predictor of drug discovery success. FBDD hits also produce more predictable outcomes for optimisation than do HTS hits.

Small fragments can be elaborated in many ways to cover a huge area of chemical space, thereby favouring novelty in the resulting structures. Thus, in FBDD it is not necessary to screen the hundreds of thousands of structures often run through HTS assays to obtain an initial 'hit'. Instead, a typical fragment screen may explore less than 2,000 well-chosen fragments, although larger libraries may be needed to explore defined target spaces more comprehensively (8,9).

STEPS IN THE FBDD PROCESS

The FBDD process is shown schematically in Figure 2.

FBDD Library Provision

The first step in the FBDD process is the generation of a suitable Fragment Library. Libraries of fragments in use range from focused sets of 500 or so fragments, largely used for X-ray studies, to much more substantial generic screening sets of around 10,000-20,000, with a norm of 2,000 to 5,000 (see, for example, (10), largely used for biochemical and biophysical screening purposes. Fragments, being small entities, have low potency for most targets, and therefore have to be screened at high concentrations. It is therefore essential that they are soluble and free from inappropriate toxicities.

To promote lead-like properties in the final leads derived from them, fragments usually comply with the 'Rule of 3' (Ro3, 11). To fulfil Ro3 requirements, fragments should have molecular weights of less than 300, cLogP less than three, and contain not more than three hydrogen bond donors and three acceptors. However, it is often more relevant to apply a limit on the heavy atom count in the fragments rather than a molecular weight limit, since some synthetic handles such as halides will dramatically increase the molecular weight of a fragment and these groups will not be present in derived lead compounds. An IOTA survey of lead-like, commercially available compounds (see Figure 3, page 20) shows that a relatively small proportion of available chemicals typically fulfil these requirements.

There is considerable debate about the optimal molecular weight for fragments within the perfect FBDD screening library. As fragments become smaller, their statistical likelihood of showing binding to features within any site becomes higher, although their binding efficiencies may diminish dramatically. The concept of 'ligand efficiency' has been introduced as an aid in fragment and lead prioritisation (12). Ligand efficiency (LE) can be regarded as the average binding energy per atom or per mass unit of the structure. Despite typically weak binding, fragments can be selected to show high LEs, both at the start of and during the FBDD process, in sharp contrast to their counterparts found in HTS collections. High-LE fragments are therefore favoured as starting points in lead discovery campaigns.

In the same way that HTS files can be enriched for specific entities by virtual screening (13), FBDD libraries

Figure 1: Schematic overview of two complementary FBDD approaches

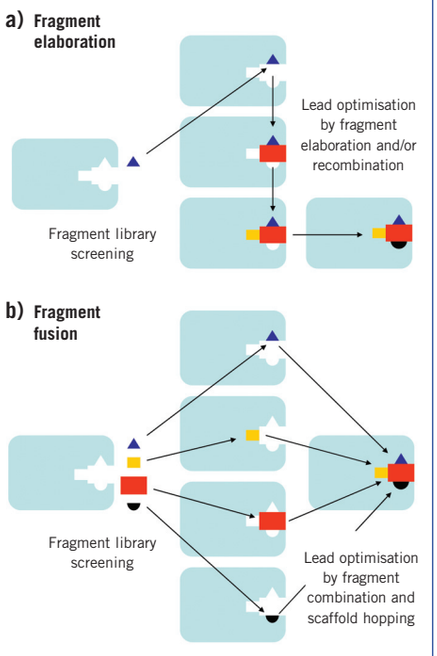
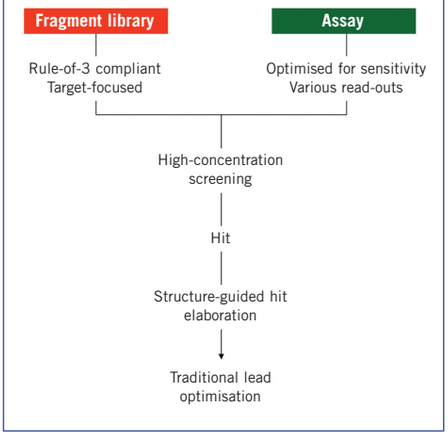


Figure 2: FBDD – the process



can be prioritised *in silico* with a specific target in mind. Some proposed binding sites – such as those in phosphatases, metalloproteases and plexstrin homology (PH) domain proteins – demonstrate quite unique, well-defined electrostatic characteristics which may restrict fragment binding. The concept of privileged structures within fragment libraries has been discussed (14). The usefulness of targeted fragments for site exploration within structural classes of targets is an area of active experimentation.

At IOTA, we use larger fragment screening sets of ~5,000 diverse fragments to survey targets. Within these sets are embedded a number of slightly larger fragments built upon more complex scaffolds. When screened alongside smaller Ro3 fragments also present in the library, these expanded fragment sets allow SAR to be established within fragment-led chemical series at an early stage of a project. More complex fragment sets are also useful for targets with larger, more amorphous binding sites, where LE is low. In such cases, larger fragments may be important to ensure a well-defined binding mode.

Fragment Screening Techniques

Small fragments will usually be weak binders to a target protein, and fragment screening assays must be tailored to deal with this. In most cases, fragments will be screened at high concentrations, typically 250-1,000µM, although with the advent of more sensitive pharmacological screening approaches, fragment screening can sometimes be performed at much lower concentrations (15).

Various screening methods have been employed to detect fragment binding, including nuclear magnetic resonance (NMR), X-ray crystallography, surface plasmon resonance (SPR) and mass spectrometry. NMR and X-ray crystallography provide additional advantages in that they also furnish significant structural information, which can establish the binding site and binding mode of the fragment, paving the way for detailed structure-based drug design approaches.

- *NMR* – Protein NMR was one of the first screening techniques applied to FBDD, known widely as 'SAR by NMR'. It originally required high concentrations of compound and large amounts of protein. It was also relatively slow. Recent improvements in methodology – such as the development of cryo-probes, miniaturisation of the NMR equipment and development of multiplexed nano-scale processes (16) – make NMR an increasingly attractive option in FBDD, with many researchers now routinely using NMR as a primary screen (10).
- *X-Ray Crystallography* – Of all the methods used to detect binding, crystallography provides the most useful information on the binding mode of a fragment. High-throughput crystallography (HTX, 2) has made it feasible to take crystallographic snapshots of bound fragments at each incremental step of their evolution into lead compounds. The advent of crystal structures for previously intractable membrane-bound targets – such as ion channels (17) and G-protein coupled receptors (18,19) – is beginning to extend this technology to an increasing number of valuable targets.

Surface Plasmon Resonance (SPR)

Other biophysical methodologies also have a place in FBDD screening, most notably Surface Plasmon Resonance (SPR), epitomised by Biacore-type technology but also including powerful on-chip applications. Understanding the thermodynamics of ligand-binding can be of considerable value during lead optimisation, reflecting as it does some of the intrinsic properties of a chemical series (20).

Biochemical Assays

Recently, reports of fragment-based screens using optimised biochemical assays have appeared, including automated fluorescence correlation spectroscopy (FCS, an industrial, miniaturised HTS screening format). Although automated FCS may be beyond the budgets of most academic labs, careful optimisation of 96-well MTP biochemical assays can often be used for FBDD, making fragment screening approaches more generally accessible.

Fragment Elaboration

Several strategies can be employed to elaborate fragment hits into valuable lead compounds (see Figure 1). If more than one fragment is found to bind in an active site, these fragments may be chemically linked to form a larger moiety. However, in practice, it is often difficult to achieve successful fusion, retaining the combined ligand efficiencies of the original fragments, since conformational restrictions will often apply to the resulting structure, forcing the

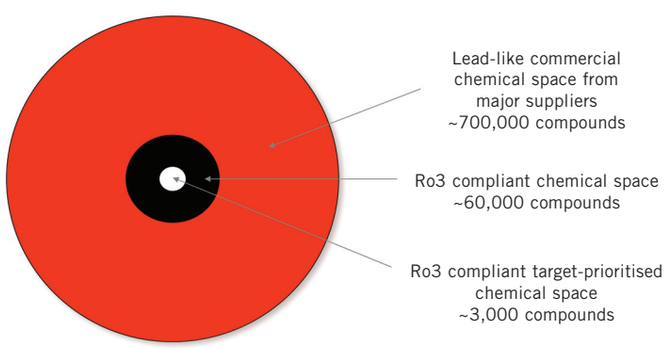


Figure 3: Ro3 compounds in commercial and virtual screening sets

individual fragments to adopt less favourable conformations in their interaction with the protein target.

Alternatively, a single bound fragment can be 'grown' into a target site with the assistance of SBDD techniques, which can identify suitable functionalities to attach to the original bound fragment. Fragment screening libraries backed by access to corresponding chemical intermediates with appropriate synthetic handles for vectorial site exploration can make FBDD a very efficient process.

The output from FBDD is often a design for a small focused library of fragment conglomerates which can then be synthesised and tested for activity, building up a picture of SAR data which can in turn be used to further develop optimal lead structures. Screening results can also be supplemented by the iterative use of pharmacophore modelling to generate further, related chemotypes. Lead optimisation from fragments can deliver increases in affinity of more than a 1,000-fold (10).

FBDD elaboration using crystallographic information has been widely reported, but FBDD is not limited to targets that can be crystallised. The increasing use of NMR, SPR and biochemical screening methods is making the approach more widely applicable to a range of targets, including those for which no crystal structures have yet been solved.

FBDD IN ACTION

By careful selection and experimental design, fragments can thus prove to be very useful starting points for medicinal chemistry-driven programmes, leading to tailor-made, selective molecules with highly favourable physico-chemical and ADMET properties.

One apparent disadvantage of the FBDD approach – when small, generic fragments are used as design 'seeds' – lies in the uniformity of the process. Two laboratories using the same fragment in identical modes might reasonably be expected to 'discover' similar chemical series. Perhaps the real challenge and opportunity of FBDD is that it should enable us to more efficiently navigate chemical space towards areas that are currently unexplored (and which therefore have high intellectual property potential), and which are safe (in terms of increased selectivity and appropriate ADMET characteristics). The use of differentiated fragment collections containing new, diverse scaffold sets may address these opportunities, incorporating novelty into a series and enabling 'scaffold hopping'.

FBDD was first described as a technological approach more than a decade ago (reviewed in 5). Since then, fragment screening has produced potent, selective lead compounds for a wide variety of targets, including many kinases, ATP-ases and proteases. A comprehensive literature review of FBDD and the targets against which it has been deployed can be found at www.iotapharma.com. In some of these studies, synthesis of a very small number of compounds has proven necessary in the journey from the initial fragment hit to a successful lead molecule (21).

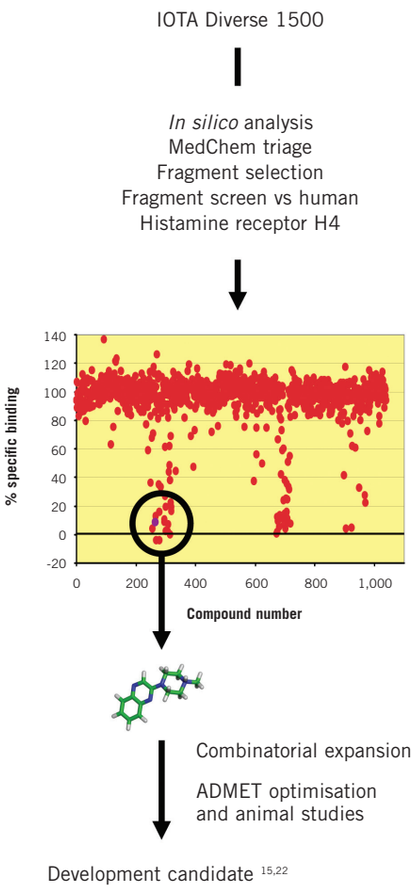


Figure 4: Discovery of novel human histamine H4 antagonists by FBDD

A worked example of the use of FBDD in a discovery programme to identify a GPCR antagonist is shown in Figure 4. This programme used IOTA's diverse fragment library to survey scaffold space, from which a number of new classes of ligand with activity against the human H4 receptor were identified, some of which have been subsequently developed into candidate drugs (15, 22).

THE FUTURE

FBDD has developed over the last decade to take its place in the standard armoury of pharmaceutical research. It has yielded numerous, well-documented successes, and has proven to be of particular value for targets where there is much structural information, and which possess a well-defined, reasonably small binding site. Developments in structural biology and high-sensitivity screening should now pave the way for the technique to be applied to a much wider range of targets than was previously possible.

Careful design and selection of fragment collections should increase the overall efficiency of the process, as will the close coupling of FBDD to SBDD strategies for lead optimisation. The availability of bespoke fragment collections designed against individual targets or target families should facilitate the successful application of FBDD to an expanding range of protein targets, including more challenging targets such as protein-protein interactions and allosteric sites.

Fragment-based design represents a paradigm shift in drug discovery. In recent years we have seen fewer approvals of new drugs, increases in development costs and high-profile drug withdrawals – despite advancements in genetics, chemistry and protein engineering. The development of rapid, efficient and cost-effective platforms for multiplexing drug discovery has never been more urgently required.

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A more comprehensive set of literature references and a review of the laboratories using FBDD and SBDD can be found at www.iotapharma.com and www.drugdesignresource.com respectively.