

FRAGMENT-BASED DRUG DESIGN: WHY IT'S SO IMPORTANT

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 (see www.iotapharma.com/libraries) FBDD is delivering high-quality drug leads against a multiplicity of new therapeutic targets in the pharmaceutical sector.

FBDD also offers the prospect of rapid hypothesis testing and validation of recently discovered molecular targets for use in drug discovery, of particular relevance for start-up biotechnology companies eager to add a new dimension to their discovery offerings.

In this overview of the FBDD approach, IOTA founders Dr Bailey and Dr Boyd review FBDD and its role in design-led discovery biology.

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Dr Bailey is a Cambridge-based serial entrepreneur. Co-founder and CEO of IOTA Pharmaceuticals Ltd, he was previously the founding CEO of two Cambridge-based start-ups, De Novo Pharmaceuticals Ltd and Purely Proteins Ltd. David headed up the Molecular Sciences Department at Pfizer in Sandwich for 8 years, before becoming Vice President at the Californian biotech company Incyte Genomics. He is a Board Director of the Babraham Institute in Cambridge, and holds a PhD in Biochemistry from Cambridge University.



Dr Boyd is an expert in computational chemistry. Co-founder and CSO of IOTA Pharmaceuticals Ltd, she is an organic chemist by training, with experience in the application of chemoinformatics and molecular design tools to accelerate the drug discovery process, both for affinity and ADMET property prediction. Susan has worked in the computational chemistry or chemoinformatics departments of Pfizer (Sandwich), Celltech (Cambridge), Scynexis (Ongar, Essex), and Molecular Simulations Inc (now Accelrys). Dr Boyd has a PhD in Computational Chemistry.

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Other IOTA resources:

See <http://www.iotapharma.com/literature> for a comprehensive review of the FBDD literature.

See <http://www.iotapharma.com/academia> for a list of selected academic laboratories working in the FBDD area.

See <http://www.iotapharma.com/targets> for a list of molecular targets against which FBDD has been used.

BACKGROUND

Gene sequencing and expression analysis have enabled the identification of a plethora of new proteins and signalling pathways¹, which structural biologists and population geneticists are assiduously triaging for tractable therapeutic targets². In parallel, chemists and biologists are using these targets to search for pharmaceuticals and other chemical tools with which to dissect biological function.

The widespread use of enabling technologies such as combinatorial chemistry and structure-based computational design has allowed access to new and useful areas of chemical space³⁻⁵, while high-throughput screening (HTS) has efficiently coupled specific targets to compound screening files. By industrialising the screening process, HTS gives rapid access to a multitude of new data on selected targets.

THE IMPORTANCE OF LIBRARIES OF DRUG-LIKE COMPOUNDS

However, despite early hopes, HTS using pre-existing compound sets has proven an inefficient starting point for successful drug discovery^{6,7}. This is as much due to the nature of the chemical input as the discovery process - the complex, non drug-like compounds present in chemical archives, often assembled by combinatorial chemistry, produce ADMET problems which cannot easily be overcome during the lead optimisation process.

Re-engineering failing compounds can be time-consuming and is unproductive. It is often more efficient to start discovery from scratch, using optimised strategies built on the alignment of computational and experimental approaches, keeping drug-like properties, epitomised in Lipinski's "Rule of 5", constantly in mind^{8,9}.

Delivery problems are often exacerbated by target-specific issues: not every target may be "druggable" in a conventional sense¹⁰, forcing exploration of other less traditional routes of intervention (such as targeting protein-protein and allosteric interactions).

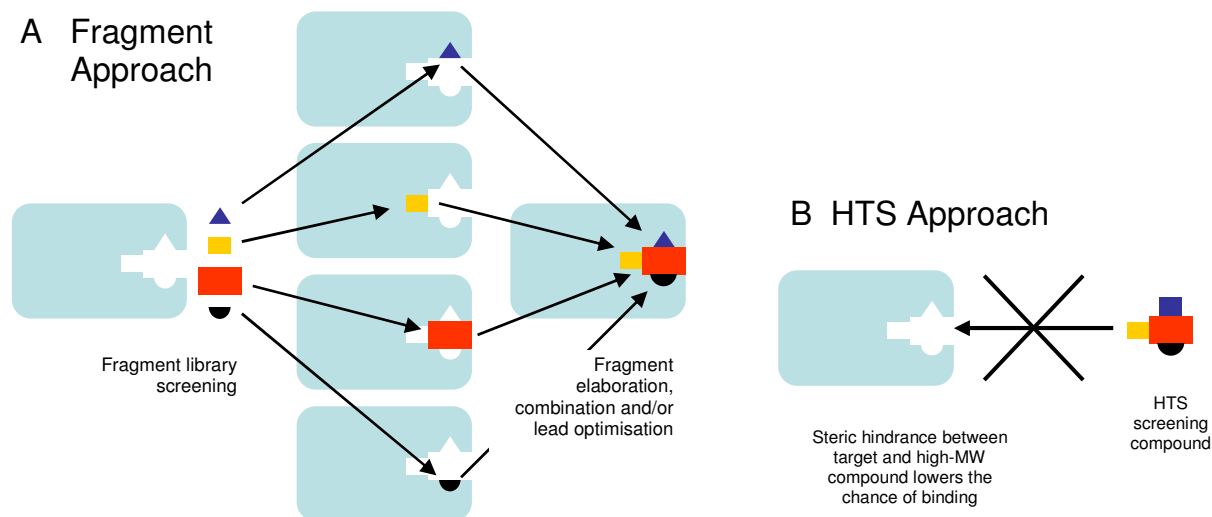
Within this context, design-led approaches play an important role¹¹⁻¹³, and over the past decade, fragment-based drug design (FBDD) has emerged as a powerful adjunct to drug discovery¹⁴.

FRAGMENT-BASED DRUG DESIGN - FBDD - THE APPROACH

FBDD deploys relatively small libraries of low molecular weight "fragments" which are tested for binding affinity, then incrementally modified into more potent, selective molecules against the target(s) of interest. The FBDD process is shown in Figure 1.

FIGURE 1

Diagrammatic comparison of FBDD and HTS



FBDD PROVIDES MORE QUALITY HITS THAN HTS

Higher "hit" rates are observed in fragment screening compared to HTS¹⁵, since small fragments can sample "recognition space" within targets more efficiently than larger, more complex molecules which may have restricted access to the same sites¹⁶, even when the compounds are "drug-like".

Because of this, and because small fragments can be assembled in many ways to cover a huge area of chemical space, thereby favouring novelty in the resulting structures, it is not necessary to screen the hundreds of thousands of structures often run through HTS assays to obtain a "hit". Instead, a typical fragment screen may explore less than 2000 well-chosen fragments, although larger libraries may be needed to explore target space more comprehensively¹⁷.

COMMERCIAL DRIVERS FOR BIOTECH COMPANIES

There is a commercial driver here. When a small biotech's only access to drugs lay in the large compound collections of their more established pharma cousins, then much of the value residing in their biology could only be crystallized by partnering. A biotech company's own value proposition (and that of its investors) can be severely compromised with NCE discovery in the hands of others.

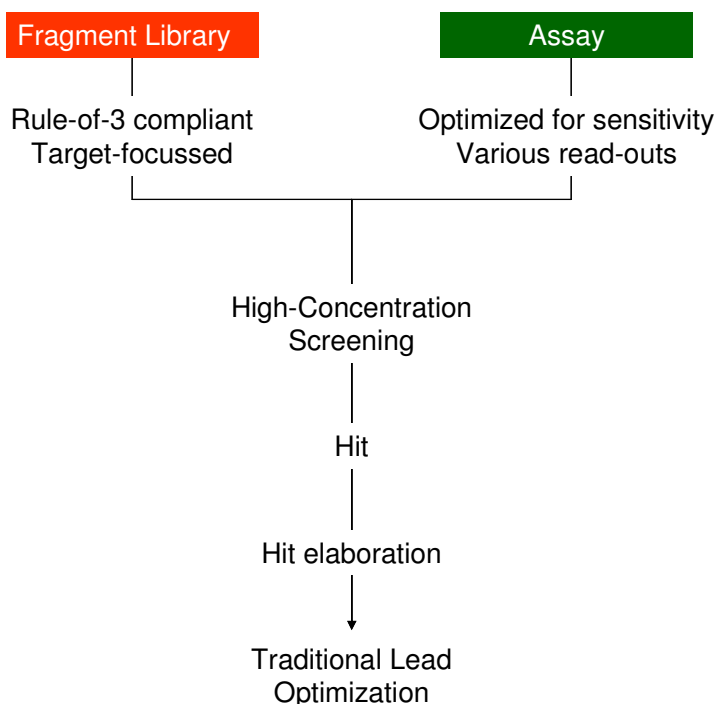
Access to a simple process for lead discovery, applicable across a range of biological targets, is an important way of capturing this intrinsic value. Fragment-based drug discovery delivers such a process.

FRAGMENT-BASED DRUG DISCOVERY - THE PROCESS

The FBDD process is shown diagrammatically in Figure 2.

FIGURE 2

FBDD - The Process



1. 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 focussed sets of 100 or so fragments, to much more substantial generic screening sets of around 10,000 - 20,000¹⁸.

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 ensure lead-like properties in the final leads derived from them, fragments are designed to comply with the "Rule of 3"¹⁹. They should have molecular weights of less than 300, cLogP less than 3, and contain not more than 3 hydrogen bond donors and three acceptors.

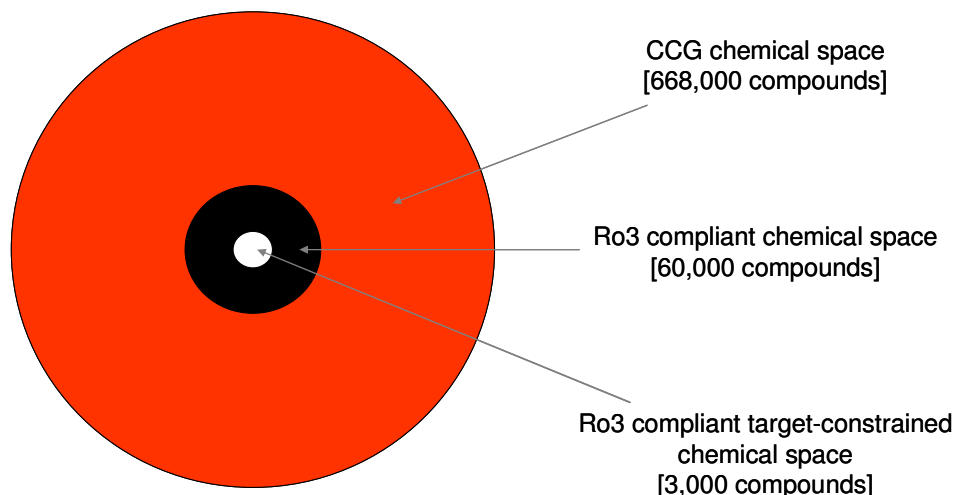
The concept of "ligand efficiency" has also been introduced as an aid in hit and lead prioritisation²⁰. Ligand efficiency can be regarded as the average binding energy per atom or per mass unit of the structure. Despite typically weak binding, fragments can be selected to show high ligand efficiencies, both at the start and during the FBDD process, especially when compared to their fully-grown counterparts found in HTS collections, making them favoured as starting points in lead discovery campaigns²¹.

In the same way that HTS files can be enriched for specific entities by virtual screening²², FBDD libraries can be prioritised *in silico* with a specific target in mind.

An IOTA survey of commercially available compounds (Figure 3) shows a relatively small proportion of available chemicals to fulfil these requirements.

FIGURE 3

Ro3 compounds in CCG and HTvS sets.



IOTA's FAMILY FRAGMENTS

IOTA offers a range of FBDD libraries, tailored to both general and focussed design applications (www.iotapharma.com/libraries).

Our Family-Focussed Fragments are pre-screened *in silico* against a range of specific gene families, providing an optimised set of fragments tailored to the target of interest. Each library has been selected for optimal elaboration with an accompanying chemical strategy which maximizes ligand efficiency.

Family Fragments available "off-the-shelf" include those for kinase, protease, phosphodiesterase, and phosphatase protein families. Family Fragments targeting other target classes, or even individual targets, can be provided on request.

Contact us with your requirements: <mailto:info@iotapharma.com>.

We also provide a fragment screening set containing slightly larger fragments than those dictated by the Rule-of-3. This set is particularly useful for targets with larger binding sites, where smaller fragments might bind promiscuously and lead to less reliable data²³.

2. FRAGMENT SCREENING TECHNIQUES

Small fragments will be, by definition, weak binders to any target protein, hence fragment screening assays must be tailored to deal with this. Compounds are usually screened at high concentrations, typically 250-1000 μ M²⁴.

Various screening methods have been employed to detect fragment binding, including nuclear magnetic resonance (NMR), X-ray crystallography 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 perhaps even the binding mode of the fragment, paving the way for structure-based drug design approaches.

- **NMR**

Protein NMR was one of the first screening techniques applied to FBDD, known widely as "SAR by NMR"²⁵. It originally required high concentrations of compound and large amounts of protein. It was also relatively slow. Recent improvements in methodology (the development of cryo-probes, miniaturisation of the NMR equipment and development of multiplexed nano-scale processes²⁶) make NMR an increasingly attractive option in FBDD.

- **X-Ray Crystallography**

Of all the methods used to detect binding, crystallography provides the most information on the binding mode of the fragment. High-throughput crystallography^{27,28} has made it feasible to take crystallographic snapshots of bound fragments at each incremental step of their evolution into lead compounds, and many leading players in fragment screening use high-throughput crystallography as a routine component of their FBDD programmes.

- **SPR**

Biophysical methodologies also have a place in FBDD screening. One of the most valuable of these is Surface Plasmon Resonance (SPR) epitomised by Biacore-type technology²⁹.

- **Biochemical Assays**

Recently, reports of fragment-based screens using optimised biochemical assays have appeared³⁰. Automated fluorescence correlation spectroscopy (an industrial, miniaturised HTS screening format), may be beyond most academic lab budgets, but carefully optimised 96-well biochemical assays make the FBDD approach accessible to everyone.

IOTA will be happy to discuss ways in which you can optimize fragment assays for your target. Contact us with your requirements: <mailto:info@iotapharma.com>.

3. FRAGMENT ELABORATION - TRADITIONAL MEDICINAL CHEMISTRY

Several strategies can be employed to elaborate fragment hits into valuable lead compounds. If more than one fragment is found to bind in an active site, these fragments may be chemically linked to form a larger moiety. In practice, it is often difficult to achieve successful linkage which retains the combined activity of the original fragments since conformational restrictions will play a part in the resulting structure, often forcing the individual fragments to adopt less favourable conformations in their interaction with the protein.

Alternatively, a single bound fragment can be "grown" with the assistance of structure-based drug design (SBDD) techniques, which can identify suitable fragments to attach to the original bound moiety. The output from SBDD is often a design for a small focussed library of fragment conglomerates which can then be synthesised and tested for activity, building up structure-activity relationship data which can in turn be used to further develop the fragment structure.

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 HCS methodologies is making the approach more widely applicable to a range of targets, including those for which no crystal structures have yet been solved.

By careful selection and design, fragments automatically provide better starting points for chemical elaboration into lead compounds than do typical high-throughput screening collections, as they will be lower molecular weight, more soluble, comparatively non-lipophilic and simpler in structure. As such, they enjoy considerable advantages for development into pharmacokinetically favourable leads.

Also, hits from FBDD programmes are typically more selective for their target than are HTS hits. The incremental approach of FBDD also has benefits for design of ligands for the traditionally more difficult targets.

FBDD-LED CAMPAIGNS AGAINST INDIVIDUAL TARGETS

FBDD was first described as a technological approach a decade ago^{25,14}. Since then, fragment screening has produced potent, selective lead compounds for a variety of targets, including kinases, ATP-ases and proteases, as detailed in Table 1.

TABLE 1

Published FBDD Studies

Abl kinase	[14]
Adipocyte lipid-binding protein-2 (aP2)	[42]
Anthrax lethal factor	[43]
v-akt murine thymoma viral oncogene homolog-1 (AKT-1)	[14]
Aurora kinases	[44]
BACE-1 (beta-site APP-cleaving enzyme-1; beta-secretase)	[40] [41]
B-cell CLL/lymphoma 2 (BCL-2), BCL-2-like 1 (BCL-XL)	[45]
Carbonic anhydrase	[46]
Casein kinase-2 (CK2)	[14]
Caspase 1	[47]
Caspase 3	[48]
Cathepsin S	[49]
CDKs	[21] [27]
Checkpoint kinase-1 (CHK1)	[14]
Dihydrofolate reductase	[50]
Dihydroneopterin aldolase (DHNA)	[51]
DNA Gyrase	[52]
Factor VIIa	[53]
FK506-binding protein (FKBP)	[25]
FMS/KIT	[14]
Galactosyltransferase	[55]
Hepatitis C virus (HCV) polymerase	[14]
Heat shock protein-90 (HSP90)	[14]
HepC NS3 protease	[56]
HIV-1 reverse transcriptase	[57]
3 α -Hydroxysteroid dehydrogenase (3 α -HSD)	[14]
Interleukin-2	[58]
JAK-2 kinase	[14]
Kinase insert domain receptor (KDR)	[14]
c-Jun N-terminal kinase-2 (JNK2)	[14]
Jun kinase-3 (JNK3)	[42]
Leukocyte function-associated antigen-1 (LFA)	[59]
Malic enzyme	[60]
Mouse double minute-2 (MDM2)	[14]
Metalloproteinases	[61] [62]
p38alpha MAP kinase	[27] [63]
Methionine aminopeptidase-2 (MetAP2)	[14]
Phosphoinositide-dependent protein kinase-1 (PDK1)	[14]
Peroxisome proliferator-activated receptor (PPAR)	[14]
Phosphodiesterases	[64]
Poly (ADP-ribose) polymerase (PARP)	[14]
Protein kinase B	[37] [38]
Protein-protein interactions	[65]
Protein tyrosine phosphatase-1B (PTP-1B)	[27] [66] [67]
Oncogenic v-raf murine sarcoma viral oncogene homolog B1 (B-Raf)	[14]
Regulatory erythroid kinase (REDK)	[14]
Ribonuclease A	[27]
Survivin	[14]
Thrombin	[68] [27]
SH2 domain of (pp60)Src	[69] [70]
v-Src	[71]
SYK	[14]
ZipA/FtsZ complex	[72]

In some of these studies, synthesis of a very small number of compounds⁶⁴ has proven necessary in the journey from the initial fragment hit into its final incarnation as a successful lead molecule. This is in contrast to HTS hits, which often require hundreds of analogues to be subsequently synthesised and tested before lead criteria are achieved.

PATHWAY COMPLEXITY, STRUCTURAL HOMOLOGY & MULTI-TARGET DESIGN - TODAY'S CHALLENGES FOR STRUCTURE-BASED DRUG DESIGN

Single-target discovery projects can be successful in relatively simple biological systems (for example, targeting HIV-1 protease in the antiviral area). However, the multifactorial nature of diseases such as diabetes, atherosclerosis and cancer, uncovered by gene expression microarray⁶⁵ and genetic studies², poses a higher-order problem for structure-based drug design. The redundancy evident in most biochemical pathways, at the regulatory³⁰ as well as structural³³ levels, poses additional challenges.

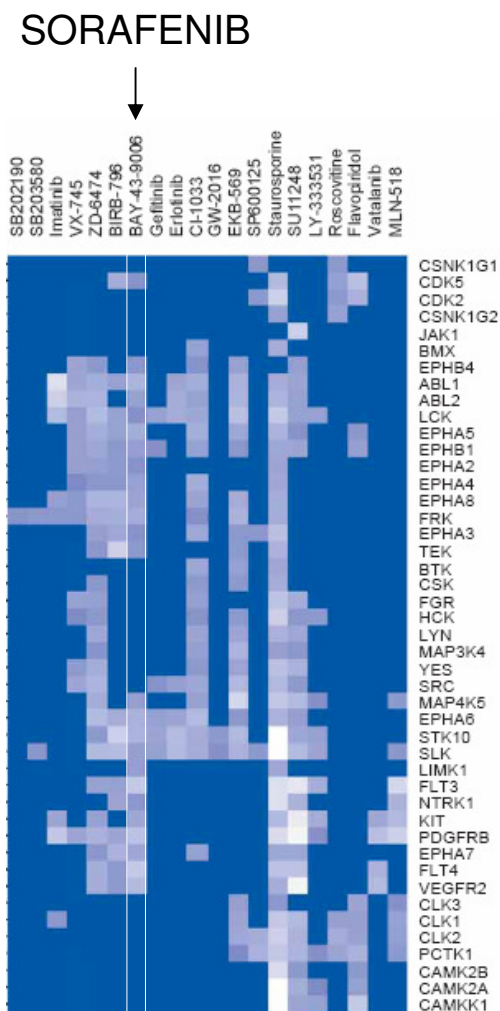
However, FBDD not only provides a logical route to building selective and potent NCEs - it also allows the creation of a flexible platform for fine-tuning these parameters within and between gene families, offering the prospect of intra-family⁶⁴ and poly-target^{31,32} chemical design.

One particularly relevant target family is that of the protein kinases. Despite the highly-conserved nature of the kinase active site⁷², this family has been the subject of much design work, including the use of FBDD⁷³. Their ATP-binding mechanism also poses toxicity constraints due to an inhibitor's potential non-kinase interactions⁷⁴.

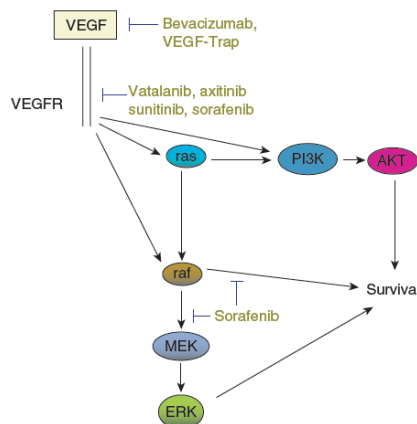
The challenge facing drug design in this area is amply illustrated in Figure 4, which shows the kinase-interaction map for clinical kinase inhibitors⁷⁵. Defining therapeutically relevant selectivity will be key to clinical success in these programmes.

FIGURE 4

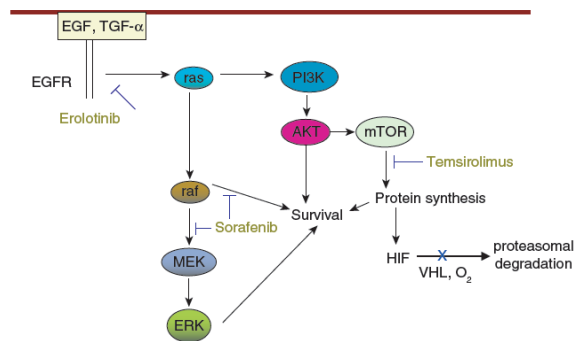
A Specificity of clinical kinase inhibitors



B Endothelial proliferative pathways



C Renal cancer proliferative pathways



A series of therapeutically effective multi-kinase inhibitors have emerged over the last 5 years. The first of these was the Novartis Abl tyrosine kinase inhibitor Gleevec. Gleevec (imatinib) specifically and potently inhibits a narrow spectrum of tyrosine kinases including c-Fms, c-Kit, and PDGFR α/β , as well as Abl, and has therapeutic activity in CML and GIST⁷⁶. Some cancer patients become resistant to the drug and the search for more effective agents continues^{77,78}. Importantly, discovery continues with this class of compound in the clinic: imatinib has recently been proposed as a powerful approach to treat RA and other inflammatory diseases⁷⁹.

A new series of multi-kinase inhibitors is now in development for cancer. Onyx's Nexavar (sorafenib), developed with Bayer Pharmaceuticals, targets two pathways: the RAF/MEK/ERK pathway (involved in cell proliferation) and the VEGFR-2/PDGFR- β signaling cascade (involved in angiogenesis)^{80,81}. Onyx originally chose this drug because it hit RAF – a central kinase in the RAS pathway, illustrated in Figure 4. Only later did it become apparent that sorafenib had quite broad specificity.

Thus, our increasing understanding of the molecular components of cell- and tissue-specific signalling has already led to a new generation of small-molecule effectors, largely by serendipitous discovery rather than precise design. Fine-tuning the pharmaceutical armamentarium to address the emerging opportunity of gene family-selectivity by design-led discovery promises to be an exciting if challenging opportunity⁸².

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 engineering⁸³. The development of rapid, efficient and cost-effective platforms for drug discovery has never been more urgently required. Fragment-based design may represent the paradigm shift in drug discovery required to address this requirement.

THE FUTURE FOR FBDD

FBDD has developed over the last decade to take its place in the standard armoury of the pharmaceutical industry. It has yielded numerous, well-documented successes, and has proven to be the tool of choice for targets where much structural information is forthcoming, and which possess a well-defined, reasonably small binding site. Developments in 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 SBDD techniques to direct the selection of fragments to screen, and subsequent fragment conglomerates to be created. The availability of bespoke fragment collections designed against individual targets or target families should facilitate the successful application of FBDD to this expanding range of protein targets.

If you are interested in deploying FBDD against your target, contact us with your requirements, at info@iotapharma.com

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A comprehensive set of references covering the FBDD area, including those used in this overview, can be found at www.iotapharma.com/literature

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