Part I
The Concept of Fragment-based Drug Discovery

1
The Role of Fragment-based Discovery in Lead Finding

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1.1
Introduction

Fragment-based lead discovery (FBLD) is now firmly established as a mature collection of methods and approaches for the discovery of small molecules that bind to protein or nucleic acid targets. The approach is being successfully applied in the search for new drugs, with many compounds now in clinical trials (see summary in [1]) and with the first fragment-derived compound now treating patients [2]. The approach has also had a number of other impacts such as providing starting points for lead discovery for challenging, unconventional targets such as protein–protein interactions [3–5], increasing the use of biophysics to characterize compound binding and properties, and providing small groups, particularly in academia, with access to the tools to identify chemical probes of biological systems [6,7].

The other chapters in this book will discuss the details and new advances in the methods and provide examples of how fragments have been used in specific projects. In this chapter, I will draw on my own experiences and view of the literature to discuss three main areas. First, I will review current practice in FBLD, highlighting how and when fragments have an impact on the drug discovery process. Second, I will then review how the ideas have developed, with particular emphasis on the past 10 years. I will discuss how fragment methods and thinking have been extended and refined and how these developments have affected the lead discovery process in drug discovery. Finally, I will discuss some of the areas where we can see that improvements in fragment methods could have further impact on discovery.

The discussion will focus on fragment-based discovery against protein targets. Although there are a few examples of fragments being used against RNA [8–10] and DNA [11] targets, the majority of reported campaigns are against proteins. Two types of protein target will be considered. The first shall be called conventional targets. These are proteins such as kinases where although it is never straightforward to achieve the required selectivity and balance of physicochemical properties in the compound, the proteins usually behave in most of the

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experiments and assays. Crystal structures are usually readily obtained, large amounts of pure, homogeneous, and functional proteins can be generated for biophysical studies, and the activity assays are robust and well understood. The second class of target shall be called unconventional targets. There are two types here – the first are protein–protein interaction targets such as the proapoptotic Bcl-2 family or Ras, where experience over the years has eventually established reasonably robust assays and although crystal structures take some time to determine and the protein does not always behave in biophysical assays, it is possible to establish structure-based discovery. The main challenge here is the nature of the binding sites, with often large, hydrophobic, and sometimes flexible sites. The second type of unconventional targets are the results of recent advances in our understanding of mammalian disease biology and consist of new classes of enzymes (such as the ubiquitin processing machinery [12]), disrupting multiprotein complexes, and proteins that are intrinsically disordered in some way (such as the one described in [13]). Here, the primary challenges are often in producing sufficient, homogeneous, functional protein for study, knowing what the post-translational modification state or even which complex is the true target and establishing robust assays to report on activity or binding. This last issue is often not appreciated – it can take a long time to establish the assays on new classes of target, not only because there is intrinsic variability in the behavior of the system but also because there is often not a tool compound available with which to validate the assay.

1.2 What is FBLD?

There are two distinctive features of fragment-based discovery compared to other approaches to lead finding. The first is that the discovery process begins with screening a small (usually 1–2000 member) library of low molecular weight (typically less than 20 heavy atom) compounds for binding to a particular site on the target. Key is the molecular weight of the fragments – they are big enough to probe interactions in the protein but small enough to minimize chances of unfavorable interactions. The second distinctive feature lies in the approach to optimizing these hits to lead compounds, either through careful, usually structure-guided, growth of the fragment or through merging information from fragments and elsewhere to generate optimized hits.

In many ways, fragments can be viewed as a state of mind – an approach to use the fragments as chemical tools to dissect what the requirements are for the chemical matter that affects a particular target in the desired way (affinity, selectivity) and using a combination of rational, usually structure-guided, and often biophysics-based methods for generation of the optimized compounds. We can define a fragment approach as one of intent – and that intent affects the strategy, methods, and thinking that is applied during the early parts of a discovery project. Detection and characterization of such weakly binding compounds can be

problematic for some classes of target, with concerns over false positive and false negative hits, changes in binding mode, and so on. So, fragment methods engender a questioning, problem-solving approach to research. This is carried through into the usually structure-guided evolution of the initial fragment hits, which allows careful assembly of compounds that bind with high efficiency combined with suitable compound properties.

1.3 FBLD: Current Practice

Figure 1.1 and its legend summarize the contemporary approach to fragment-based discovery followed by most practitioners. There are five main components to a fragment platform: a fragment library, a method for finding which fragments bind, characterizing how the fragments bind by determining structure and biophysical measurements, exploring fragment SAR to identify the best fragment(s) to progress, and using the fragment(s) to generate lead compounds. Figure 1.1 also emphasizes how information about binding motifs is combined with information from HTS hits, literature compounds, or virtual screening hits. Other chapters in this book will provide detail on each of these different areas. In this chapter, I am focusing on the impact fragments have had on the lead discovery process. This is best done with some examples.

1.3.1 Using Fragments: Conventional Targets

Conventional targets are ones with well-defined active sites (such as most enzymes) where structural information is readily available. It is usual to get a large number of fragment hits for such targets – at Vernalis our experience has been 50–150 validated hits from screening a library of about 1500 fragments [14,15]. A lower hit rate can indicate there may be issues with progressing compounds against the target as discussed later. Modeling of the binding of these fragments can be helpful, but the most effective fragment to hit to lead optimization campaigns uses the detailed information available from experimental structures determined by X-ray crystallography (preferred) or if necessary by NMR. The main issue with NMR is the time it takes to generate structures. A suitable crystal form can generate many hundreds of crystal structures during the early months of a project, whereas it takes at best a few days for NMR methods to generate models for binding. In addition, NMR models rarely have the resolution to give confidence in some of the subtleties of binding mode necessary for design of selective compounds (such as for kinases).

The three main ways of using fragments are growing, merging, and linking. Figure 1.2a–c shows some representative examples that we can use to describe the essential features of each approach.

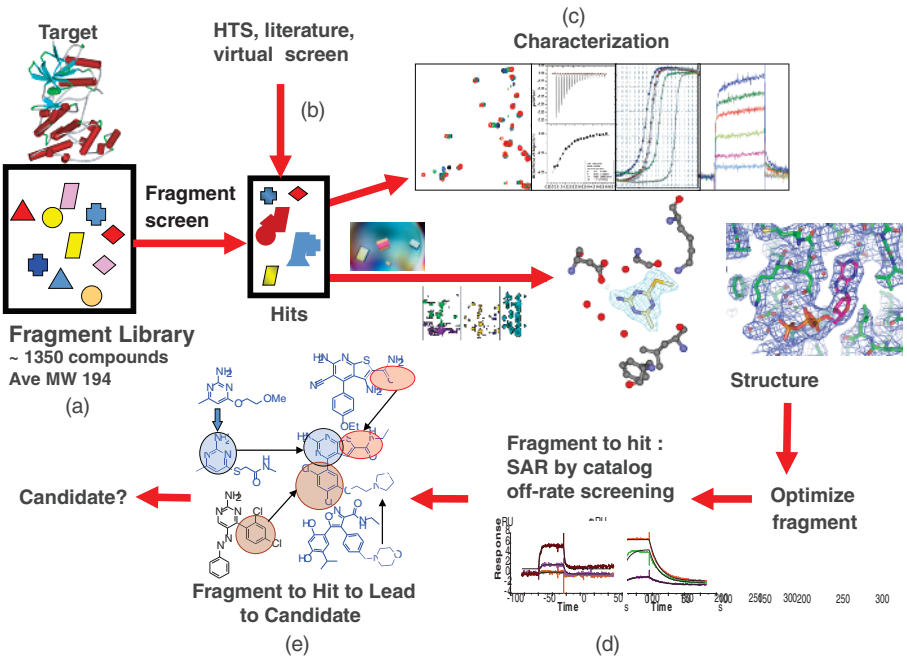

Figure 1.1 the FBLD process. There are five main components to a fragment platform. (a) Fragment library: there is an extensive literature on the design of fragment libraries [26,31,32,41]. The choice of compounds is constrained both by the demands of the screening methods (solubility, detection) and by the need to evolve the compounds (elaboration vectors, synthetic tractability) as well as avoiding reactive or toxic substructures. Key is the number of heavy atoms in the compounds. Analyses by Reymond [38,39] suggest that the number of possible lead-like compounds (chemical space) increases by around eightfold for each heavy atom. There are many approximations but this means that a fragment library of 1000 compounds of average MW 190 is equivalent to 10^8 compounds of MW 280 and 10^{18} compounds of MW 450. (b) Fragment screening: Table 1.1 summarizes the experiences at Vernalis over the years that are variously described elsewhere [15]. For all techniques, the main limitations are whether the protein target can be prepared in a suitable format for screening and whether the fragments are sufficiently soluble. The most robust method of screening is ligand-observed NMR, which has the dynamic range (typically from 5 mM to 100 nM) seen for fragment binding and particularly important for unconventional targets, as the integrity of the ligand and protein is checked at each experiment. (c) Characterizing fragment binding: for conventional targets, it is often possible to rapidly determine crystal structures of the fragment binding to the protein and, if the biochemical or binding assay is not suitable, use a biophysical method to validate and if possible quantify potency. For unconventional targets, this step is particularly important as the targets can have challenging binding sites, where conformational flexibility or large hydrophobic surfaces can challenge reliable detection of fragment binding. NMR methods can be used for unconventional targets, ranging from binding site localization (HSQC) to NMR-guided models (measuring NOE distances from ligand atoms to protein residues) and full structure determination. These are constrained by the size of the protein and requirement for isotope labeling. (d) Fragment SAR and optimization: there are two well-established methods – (1) SAR by catalog where features of the fragment are used to identify commercially available compounds for purchase and assay and

Table 1.1 A summary of the characteristics of the most widely used fragment screening methods.

Method	Sensitivity	Issues
Ligand-observed NMR – a number of NMR experiments (STD [82], Water-Logsy [83], and CPMG [84]) detect binding of a ligand to the protein	10 mM–100 nM	Requires large amounts of protein (many 10 s mgs) but the most robust method for detecting weak binding. Each experiment confirms that the ligand and protein maintain their integrity in solution; the use of a competitor ligand to displace the fragment can identify nonspecific binding. These features make the technique particularly suitable for weak binding to challenging targets. Requires careful design to identify allosteric or cryptic binding sites
Protein-observed NMR – HSQC experiment detects changes in the local environment of ^{15}N or ^{13}C nuclei as ligand added	5 mM–100 nM	Requires isotopic labeling of the protein; limited to proteins<35 kDa; can titrate ligand onto protein and determine K_D; pattern of changes in spectra can confirm the same binding site for different ligands and identify allosteric sites; assignment of spectrum allows localization of site
X-ray crystallography: either cocrystallization (crystals formed from the preformed protein–ligand complex) or soaking (high concentrations of ligand added to apo crystals)	All affinities	Cocrystallization can require different crystal conditions for each ligand. Soaking of apo crystals requires crystal form with accessible protein binding site; depending on crystal form can identify cryptic sites Crystal structure provides information-rich description of protein–ligand interactions ready for design
Surface plasmon resonance [47]; monitor molecular weight change as one component flows past the other attached to a surface	500 μM lower limit	Two modes – direct binding (protein attached, ligand flows) allows kinetics (k_{on} and k_{off}) to be measured; indirect, or affinity in solution, where tool compound attached and protein (in the presence of possible fragment) is flowed past. Main issue is immobilization and integrity of protein on surface

(*continued*)

(2) detailed design of bespoke compounds to optimize the fragment itself and explore potential vectors for elaboration. More recently, there have been new methods such as off-rate screening [16] that allow rapid profiling of compounds where substituents have been added to particular positions on the fragment, prospecting for suitable vectors for fragment evolution. This can be particularly important when limited structural information is available. (e) Fragment to candidate: medicinal chemistry optimization, supported where possible by rapid crystal structure determination, to bring together information from the portfolio of fragments, hits, HTS, literature, and so on to design and optimize lead compounds.

Table 1.1 (*Continued*)

Method	Sensitivity	Issues
Enzyme/binding assays	100 μM lower limit usually	The high concentrations of ligand interfere with most formats of assay preventing detection of mM binding fragments; effective for some assay formats and for well-defined active sites – for example, kinases
Isothermal titration calorimetry (ITC) [85]	1 mM–10 nM	Requires too much protein and ligand to be useful for screening, but the most robust method for measuring K_D as long as the interaction involves a change in ΔH
Mass spectrometry	100 μM	Requires protein/buffer system that "flies" in the mass spectrometer and an interaction that can survive in the gas phase. Effective for covalent interactions –too variable for weakly binding fragments
Weak-affinity chromatography [51] – immobilize the target on a silica column, then use LC–MS to identify retained ligands	1 μM upper limit	A cheap way of measuring weak interactions (using simple LC–MS equipment). As for SPR, the main limitation is attachment of protein to surface and behavior of the fragments on the surface
Thermal shift analysis (TSA) [86] – measure the melting temperature of the target by monitoring the increase in fluorescence as the target is heated up in the presence of a dye plus and minus the ligand	500 μM lower limit	A relatively reliable technique for detecting binding of ligands that bind better than 10 μM, but many false positives and negatives in detecting fragment binding – the change in melting temperature is too small to measure. Uses small amounts of material and inexpensive instrumentation

Fragment linking is a conceptually very attractive idea – find two fragments that bind in adjacent sites and achieve a high-affinity compound by linking them together.This was the basis of the initial SAR by NMR approach, but with a few exceptions, only the Abbott group (such as summarized in Figure 1.2a, see also Table 1.3), and the follow-on work by Fesik at Vanderbilt (see later), has

Figure 1.2 (a) Evolving fragments – linking. The SAR by NMR approach was developed by the Abbott group in the 1990s [22] (see also the reviews [63,64]). Protein-observed NMR screening of a library identifies the first site binder (screen 1) that can then be optimized (optimize 1). The second screen (screen 2) is then performed in the presence of an excess of the optimized first site binder to identify the second site binder that can also be optimized (optimize 2). NMR structure determination identifies appropriate vectors for linking the two fragments (link) to give a compound that can then be optimized. The first disclosed example was for FKBP [22]; the first drug discovery project was on stromelysin [65] and arguably the most successful was for the Bcl-2 family of proteins [66–69]. For stromelysin, compound **1** was not from screening but is a known metalloprotease binding motif. Screening in the presence of **1** identified compounds such as **2** that after

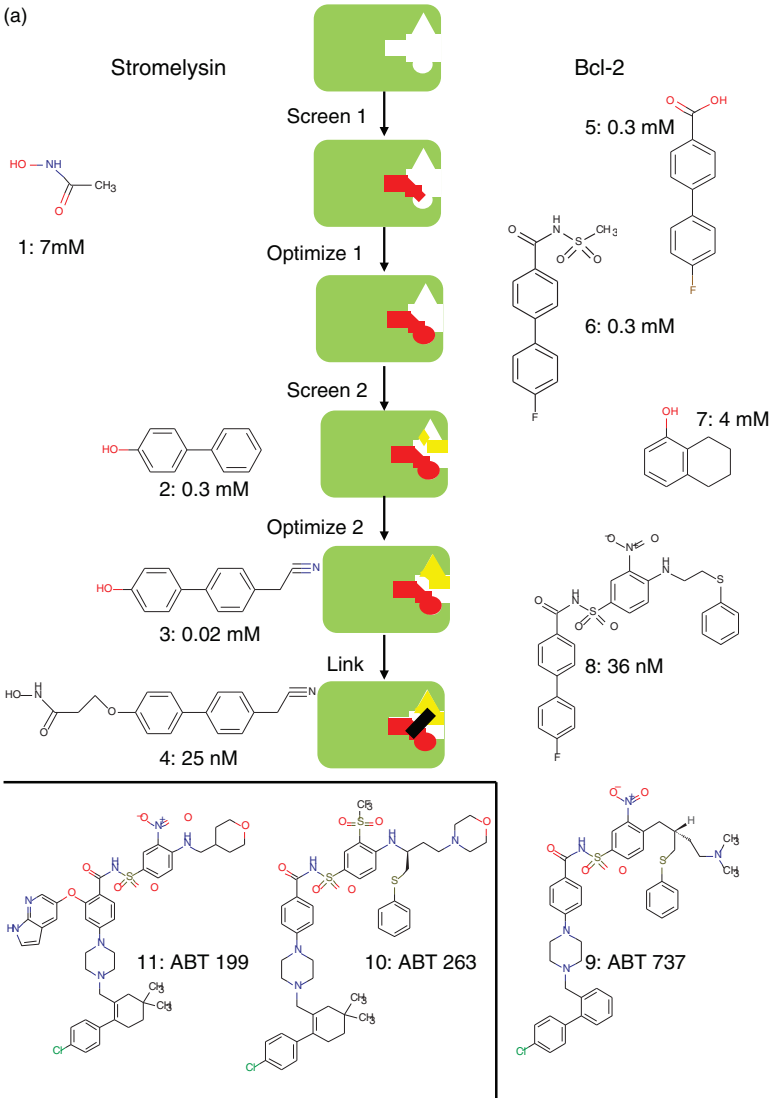

Figure 1.2 (a) (***Continued***) optimization gave **3**. Combining these in **4** very neatly demonstrates the power of the method – a large increase in potency, clearly retaining the two weakly binding fragments. For the Bcl-2 family, the evolution from the two site binding fragments **5** and **6** is less obvious in compound **7**, although the method did provide starting points for chemistry where conventional HTS failed. A considerable amount of medicinal chemistry optimization was needed to generate ABT-737 [66] that briefly entered clinical trials, followed by ABT-263 [70] with better drug-like properties though still with a dual Bcl-2/Bcl-x_L profile that can give undesired pharmacology. This has recently been succeeded in the clinic by the more Bcl-2 selective ABT-199 [71]. With few exceptions [72], the continued champion of the linking approach is Fesik, now at Vanderbilt (see Figure 1.2d). Most other practitioners find it difficult to identify such multiple sites and commit such dedicated chemistry resources to a linking strategy.

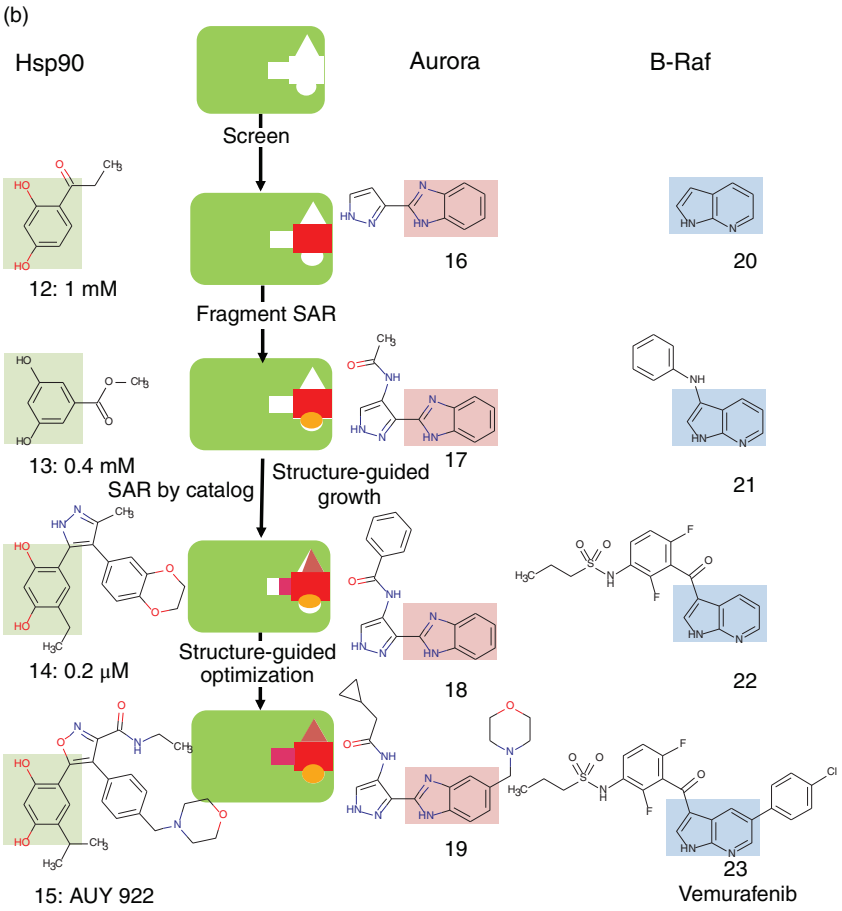

Figure 1.2 (b) Evolving fragments – growing. There are two widely used approaches for growing fragments. The first is SAR by catalog, where features of the bound fragment are used to search a database of accessible compounds that are then assayed. An example is the HSP90 program at Vernalis that led to **15**, AUY922, currently in multiple phase II clinical trials for various cancers. A ligand-observed screen identified the resorcinol **12** and near-neighbor fragments such as **13**. Search of available compounds for resorcinols that were subsequently triaged with pharmacophore-biased docking, identified compounds such as **14**, which show good affinity. Structure-guided optimization led to **15**. A summary of the HSP90 discovery project is available [73] as well as more details on AUY922 [74,75]. The second approach is growing by careful structure-guided ligand design. The Aurora example from Astex [76] is a particularly good example where fragment **16** was identified from a crystallographic screen of fragments against CDK2; exploration (**17**) identified good vectors for optimization, leading to the hit **18** that was subsequently optimized to the clinical candidate **19**. A similar chemogenomic approach (i.e., transferring knowledge about chemotypes that bind to a particular family) can be seen in the B-Raf kinase example from Plexxikon [2], where **20** was initially characterized binding to Pim-1 kinase, with the related **21** studied in FGFR1 kinase leading to **22** as a potent hit against
B-Raf that was optimized to Vemurafenib, **23**, the first fragment-derived compound on the market.

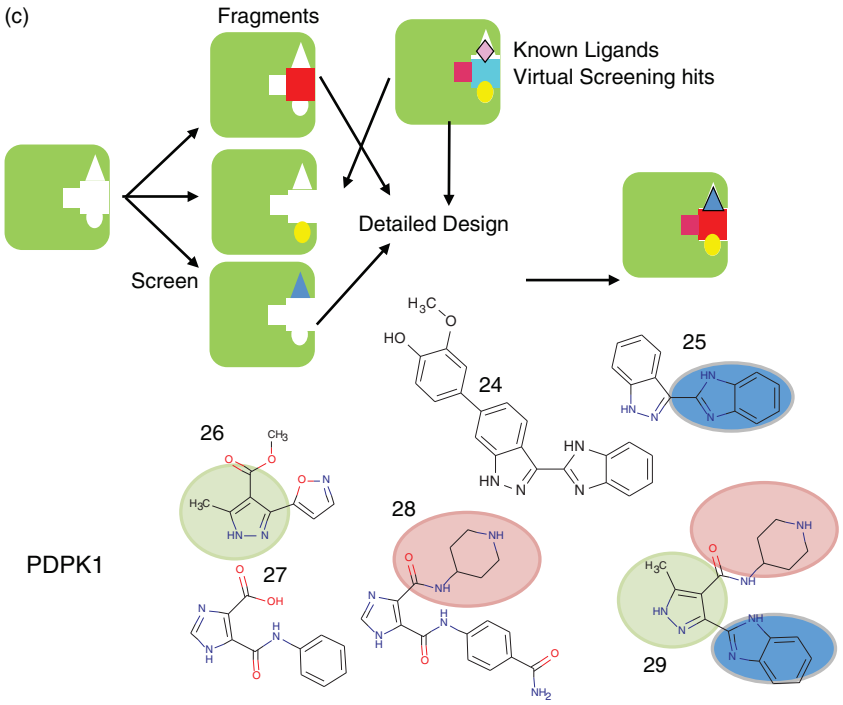

Figure 1.2 (c) Evolving fragments – merging. This is an approach where information from fragments, derived hits, literature, and HTS hits is all combined together to generate the new lead compound. The approach relies heavily on multiple crystal structures to identify the subtle opportunities provided by binding modes for novel scaffolds and achieving selectivity. The example shown is for PDPK1 [56]. Compound **24** is a known promiscuous kinase inhibitor from which the fragment **25** was derived and the crystal structure determined bound to PDPK1. A ligand-observed screen with staurosporine as competitor identified more than 80 fragment hits, for 50 of which crystal structures were determined. The crystal structures of fragments **26** and **27** showed distinctive binding modes. The crystal structure of an inhibitor from a published study on CDK2, bound to PDPK1, identified a hydrophobic region adjacent to the carboxylic acid of **27**. A search of the available chemicals identified a compound that with small optimization gave **28** with 1 μM affinity for PDPK1. Superposition of the structures of the hits suggested a number of combinations of scaffolds and features. One of these is taking the highlighted features from **25**, **26**, and **28** to generate **29** that showed low nM affinity with good selectivity against other kinases, but importantly affected *in vivo* PD markers in mouse xenograft models.

succeeded in making this work. Most practitioners have found that either the binding site of the target does not contain appropriate features or that they have found it difficult to either design or resource the chemistry required to link the two fragments and preserve the binding mode of the initial hits.

The growing approach is summarized in Figure 1.2b. This has been the most widely used with a number of variants. The first is to use the idea of SAR by

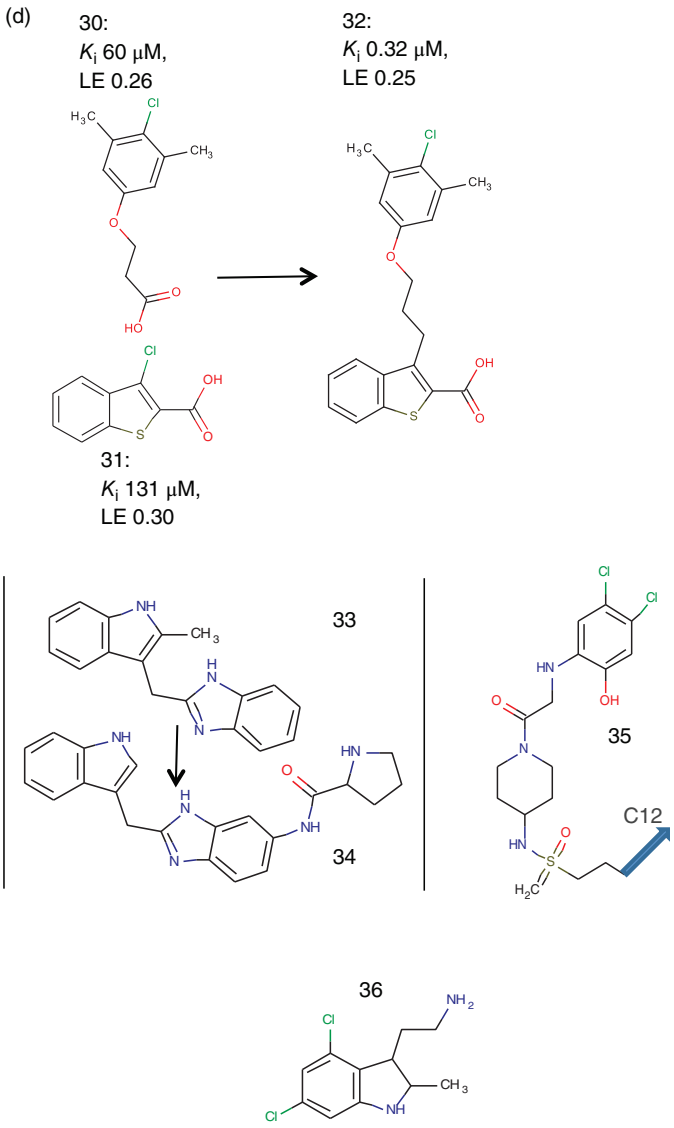

Figure 1.2 (d) Recent protein–protein interaction FBLD projects. A number of studies have been published recently on the use of fragments for therapeutically important protein–protein interaction targets that have been studied extensively but with little published success through conventional drug discovery efforts. The Bcl-2 family member Mcl-1 is such an example. The Fesik group at Vanderbilt used SAR by NMR to identify **30** and **31** binding in adjacent sites that when linked (**32**) gave a useful starting lead compound for further evolution [77]. The same group used a similar approach to identify **33** that when grown gave **34** as an inhibitor of Ras [4], a key oncogene. Other recently published Ras projects are tethering through the G12C mutant as in **35** from a group at UCSF [5] and identification of another binding pocket for fragment **36** at Genentech [3].

catalog – that is, where the fragment hit provides a central scaffold with which to search for near-neighbor compounds from available compounds (either the corporate collection or that can be purchased). The second is to generate limited libraries of compounds based on the fragment to explore vectors for affinity or selectivity. Here, a recent innovation is to look at changes in the off-rate of binding as a marker for improvements in binding (the so-called off-rate screening [16]). For both of these approaches, the key value is in characterizing possible vectors for elaboration and the types of functional groups that could be used – developing SAR around the hit fragment. It is important to characterize the binding as it is probable that the evolved compound will include additional atoms that are not optimal – and these should be removed before further optimization is attempted. The final and most widely used method for growing fragments is to use detailed structure-based design to iteratively grow the fragment a few atoms at a time to pick up specific interactions with the binding site.

In the merging approach, insights from the binding mode of the fragments are combined with information from literature, virtual screening, or HTS hits to design new scaffolds. The PDPK1 in Figure 1.2c is one example; others include some of the series developed at Pfizer against biotin carboxylase [17] and the oral compound BEP800 [18] against HSP90 designed at Vernalis. For this type of approach, the main requirements are multiple crystal structures and confidence from the medicinal chemists to embark on such radical compound redesign. This is one of the approaches that is being used more frequently as fragment-based approaches are being embraced by large pharmaceutical companies and integrated with HTS. It is fascinating to see how a medicinal chemist with experience of fragment-based discovery approaches HTS hits – their first instinct is to dissect the compound down to the core binding motifs, and once that has been identified at the fragment level and then optimized, the functionalization from the HTS hit can be reassembled.

1.3.2
Using Fragments: Unconventional Targets

One of the striking advantages of FBLD is that fragment hits can be found for nearly all targets (see Figure 1.5 later). This is particularly valuable for more challenging unconventional targets such as protein–protein interactions, multiprotein complexes, intrinsically disordered proteins, and new classes of targets (such as ubiquitin-specific proteases [12]) where HTS screening of conventional libraries fails to identify tractable hits. The early, high-profile example of this was the discovery of ABT-737 (and subsequently, ABT-263 and ABT-199) as seen in Figure 1.2a; additional examples are shown in Figure 1.2d such as the work on Mcl-1 by the Vanderbilt group and that at Genentech and UCSF in identifying hit compounds that affect the activity of K-Ras (referenced in the figure legends). These proteins are often difficult to work with in terms of solubility, homogeneity, and folding. In addition, the binding sites are often quite shallow or diffuse,

which means that binding affinity (and thus ligand efficiency, see Table 1.3) is low. The main published successes have, therefore, not surprisingly, used NMR methods to detect and characterize binding, a technique that not only has the necessary sensitivity but also importantly has the in-built quality control to assess the protein state for each binding experiment.

1.4 What do Fragments Bring to Lead Discovery?

Later in this chapter, I will discuss some of the details of how our ideas and practice of FBLD has evolved over the past 10 years. Here, the major features will be summarized of how FBLD is used and has had an impact on drug discovery, with a somewhat arbitrary separation of comments against both conventional and unconventional classes of targets.

For conventional targets:

- Fragments can sample the chemical space of what will bind to a binding site. There are still not many examples where this has been analyzed in detail (see [19] for an analysis on HSP90 compounds), but fragments usually recapitulate the key binding features seen in compounds derived by other methods (such as HTS or natural product derivatives).
- Fragments can show selectivity even for closely related proteins and even when a fragment binds to many similar targets, it can adopt different binding modes (– see the example for kinases in Table 1.4 and Figure 1.7 discussed later).
- Where crystal structures are available, the important first step in assimilating the set of fragment hits is to categorize the fragments on binding mode.
- The selection of which fragments to take forward for evolution is as much about opportunity (such as IP, selectivity, and chemical tractability) as the current affinity of a specific fragment. Often, there will be regions of a fragment that are not optimal or required for binding. For this reason, it is important to explore the SAR of the initial fragment(s) before optimization, identifying which binding modes and potential vectors offer the opportunity to gain selectivity and affinity.
- It is usually the case that the central core of the fragment does not change the binding mode as the fragment is evolved. If it does, then it can be a sign that the initial fragment was not optimal or that the additional atoms added to the fragment have challenged the binding efficiency – as seen in the evolution of the same fragment in three different kinases [20].
- A concern voiced by some is how it is possible to achieve novelty when most are screening very similar fragment libraries against the same targets. As discussed later (and shown in Figure 1.8), even where the same fragments are found, the medicinal chemists will optimize differently.

For unconventional targets:

- Fragments provide the opportunity to assess challenging targets for chemical matter that binds; the hit rate can be an indication of how difficult it is going to be to progress compounds against a target (see Figure 1.5 later).
- It is usually not an issue in identifying fragments that bind to such targets.
- The major challenge is establishing robust, validated assays – both for establishing binding and for activity. An issue is that, often, there are not validated tool compounds available and so it can take some time (and iteration with evolving fragments) to establish an assay that can be relied on.
- The use of multiple (sometimes called orthogonal) binding experiments can be crucial to success – helping to validate the binding and binding mode.
- A major issue is the time it takes to generate lead compounds – it takes commitment to the long haul. A project can spend many years in the exploratory phase, establishing robust assays and validated starting points before a drug discovery program can begin. This time in fragment and early hit space does not necessarily require large resources, but it can be long. The key to continuation of the project is maintaining confidence in the target and hits and that the next steps for the project are clear.

For all targets:

- A fragment screen is a rapid way of assessing how difficult it will be to find new chemical matter that will bind to a particular target – a low fragment hit rate does not necessarily mean the target is undruggable, but it can indicate that finding high affinity, selective compounds could be a challenge.
- Start the fragment campaign early enough in the project cycle
  - Many large companies have found they needed to establish dedicated teams that promoted the fragment approaches – this required top-down implementation by management to ensure the resources were applied (and staff objectives suitably adjusted).
  - The main issues are cultural – most organizations have the different technologies/capabilities in place to perform fragment screening. Key is integration alongside more conventional HTS type of methods and building a culture of seeing the techniques as complementary and not a competition.
- The focus (and required assays) is on binding rather than activity in the early part of lead identification.
- An important feature of fragments is that the optimization process starts with a core that is small. Careful optimization can maximize the ligand efficiency of evolving compounds and ensure incorporation of the optimal properties. A drop in ligand efficiency on optimization should always be questioned. In addition, maintaining a high ligand efficiency during early to mid lead optimization allows that efficiency to be spent in fine-tuning

the physicochemical properties and efficacy of the compound in the later stages. Overall, fragments provide the opportunities and scope for the medicinal chemist to develop compounds with optimal properties.

- For most targets, fragments provide choice – this gives potential for many different chemical series as starting points, providing backups, ideas on key interactions and binding modes that can be exploited in optimization or the potential for scaffold hopping if issues appear during optimization (such as physicochemical properties, CYP inhibition, or hERG).

In addition to explicit FBLD campaigns, the fragment ideas have permeated conventional lead optimization. The most recent example I have come across is the work at Kaken Pharmaceuticals on PDE7 [21], where a novel HTS hit was dissected back to a fragment and then evolved – there are many other examples emerging in the literature that show how fragment thinking has infected many areas of medicinal chemistry.

1.5 How did We Get Here?

1.5.1 Evolution of the Early Ideas and History

It is now nearly 20 years since the first publication described a fragment-based approach to discover potent lead compounds [22] and almost 10 years since the publication of the first edition of this book [23]. Table 1.2 provides a summary of the ideas and methods that made an important contribution to the development of the first use of fragments in ligand discovery. Table 1.3 summarizes the early developments in the field that led to the publication of the first edition of this book in 2006.

1.5.2 What has Changed Since the First Book was Published in 2006?

In 2006, the publication of the first edition of this book announced the arrival of fragment-based discovery as a thriving area of method development and application in the pharmaceutical industry.

The book contained 16 chapters which can be summarized as follows:

1) Introduction with a brief sketch of the field
2) The idea of multivalency
3) Ideas in entropy of binding and combination in fragment binding
4) Multiple solvent crystal soaking (MSCS) as a method for experimental solvent mapping
5) Cheminformatics analysis of fragments in literature ligands

Table 1.2 The ideas and concepts that underpinned the emergence of the first demonstration of fragment-based lead discovery in 1996.

Double the ΔG; square the K_D: papers by Jencks [87] from the early 1970s remind the community that $\Delta G = -RT\ln K$ – so combining two weak interactions gives a strong association. Also that the first ligand binding overcomes rotational and translational entropy, so additional interactions are stronger.
High-throughput crystallography: Perutz and coworkers [88] demonstrated in the early 1980s the benefit of multiple crystal structures in analyzing protein–drug interactions
Functional group efficiency: Andrews, Craik, and Martin [89] developed the idea in the early 1980s that particular functional groups make a distinct average contribution to binding
Functional group binding – computational: Goodford [90] developed GRID in the early 1980s to map the predicted binding of single-point probes to an active site with impact on drug discovery such as Relenza [91]. The MCSS [92] approach extended this to larger functional groups and LUDI [93] derived interactions from crystal structures
Functional group binding – experimental: the first experimental mapping of a binding site was by crystallography, with the MCSC approach pioneered by Ringe and coworkers [94] in the early 1990s and developed by others [95,96]
Fragment linking – computational: Approaches such as HOOK [97], Caveat [98], and LUDI [99] were developed in the early 1990s to link functional groups; the main challenges were predicting binding affinity and design of synthetically tractable molecules

6) Analysis of fragments in marketed drugs
7) Multiple copy simultaneous search (MCSS) for computational solvent mapping
8) Using NMR both to identify fragments that bind and to guide fragment assembly
9) SAR by NMR from the Abbott group
10) The Astex platform with a focus on high-throughput crystallography and library design
11) The platform developed at SGX using high-throughput crystallography with some examples and discussion of fragment evolution strategies
12) The use of NMR and X-ray crystallography for some particular examples of using fragments at Abbott
13) The use of mass spectrometry
14) Tethering
15) Click chemistry
16) Dynamic combinatorial libraries

It is fascinating to look critically at these chapters from this distance of 10 years and how it represented the state of FBLD at that time. Many of the methods described in the chapters on click chemistry, dynamic combinatorial libraries, MCSS, and multiple solvent crystal structure (MCSC) have found little application in practical FBLD. However, quite significant parts of Chapters 8–10 on the central methods and Chapter 11 on a crystallographic platform and fragment

Table 1.3 The developments in FBLD between 1996 and 2005.

SAR by NMR: the phrase "SAR by NMR" was coined to describe the approach developed at Abbott that saw the first publication on fragment-based drug discovery [22]. Protein-observed NMR (^{15}N-^{1}H HSQC) spectroscopy is used to screen for binding of a fragment that is then optimized. A second screen is then performed in the presence of the optimized fragment to identify second site binders, which are then linked together. The optimization and linking of fragments is aided by structure determination by NMR. The first paper demonstrated proof of concept in identifying nanomolar inhibitors of the FK506 binding protein [22]; subsequent early disclosures were on stromelysin [65], E2 from papilloma virus [100], urokinase [101], and phosphatases [29]
Combinatorics: an early idea to emphasize the power of the method is as follows [65]: screening of a 1000 fragment library for binding to each of two sites and then trying 10 different linkers in a total of 2010 experiments, which samples the $1000 \times 1000 \times 10$ (10 million) possible compounds
Detecting weak binding: techniques established and widely used by 2005 were protein-observed NMR [22] (^{15}N-^{1}H HSQC), ligand-observed NMR [26] (STD, Water-Logsy, CPMG), X-ray structure of fragment mixtures soaked into apo crystals. Enzyme/binding assays dismissed by most (Plexikkon scaffolds were the exception) [102]
High-throughput crystallography: Nienaber at Abbott was the first to publish screening by crystallography [103]; there was considerable development and promotion by companies such as Syrrx [104], SGX [105], and Astex [106]
Fragment evolution: two main approaches had been established by the mid-2000s – linking and growing [107]. Using the target protein as a template for self-assembly is an attractive idea that was discussed frequently, such as a chapter in the first edition of this book and mentioned in this review [107]. Also of great promise was the idea of tethering [107–110] from Sunesis and colleagues
Hann complexity [111]; this is a concept whose significance has grown over the years. This was a theoretical analysis arguing that the chance of finding a hit is a balance between enough complexity in the molecule to bind with sufficient affinity to register in an assay and being too complex that the compound no longer will fit into the binding site
Ligand efficiency [112]: this concept (that the efficiency of binding is the amount of free energy of binding for each heavy atom) built on earlier ideas about the maximum affinity obtainable by ligands [113]. The metric was rapidly adopted by the fragment community, with slower uptake by more conventional medicinal chemists. It is a persuasive way of demonstrating that a small (10 heavy atom) compound with a K_D of 1 mM is equivalent in "quality of binding" as a drug-like compound (30 heavy atom) with a K_D of 1 nM.
Fragment library design: the essential details were established of the approach to identifying fragments with defined criteria for exclusion (toxicity/reactivity) and with desired properties and opportunities for evolution, together with the rigorous QC for library curation [26]. The so-called "rule of 3" was identified by Astex [114] and perhaps overused in the community (people find security in rules – it saves thinking)

This is a somewhat selective precis of the origin of the underlying principles and early applications of fragment-based methods (see also [81]).

evolution would not require much updating to be included in this book. The chapter on tethering is relatively up to date; the approach remains intellectually appealing, and although there have been some high-profile applications, the approach [5,24] has not been widely used. Apart from these chapters, the book lacked in real examples of what we would today call FBLD in practice. There is some screening data presented and linking portions of molecules; however, most of these were small compounds identified by HTS. At first consideration, this is a striking omission. However, looking back over the publication timelines, the first examples of success at companies such as Astex and Vernalis were being presented at conferences but had not been published at the time the book was assembled.

The book did not contain a comprehensive summary of the developments across the FBLD field – it carried individual reports from particular practitioners where they tend to stress their own platforms. Combining the book with two representative papers from Astex [25] and Vernalis [26] allows a summary of the contemporary state of FBLD in the mid-2000s from which to begin our discussion of how the methods and approach have changed.

What is striking is that most of the essential ideas in fragment-based discovery had made the transition from the SAR-by-NMR approach to be essentially the same approach as followed today. The major difference has been in the extent of application and demonstration of success. Other articles in this book will describe those successes. What I want to focus on in the next section are the small differences in emphasis and demonstration of ideas and principles that emphasize the strength and opportunity given by the approach.

1.6
Evolution of the Methods and Their Application Since 2005

Most new technologies follow the same cycle illustrated in Figure 1.3. When a new technology arrives (the technology trigger), there is great expectation of the impact this technology will have. These expectations are usually overhyped (perhaps they have to be to realize the investment for developing and implementing the methods). The methods are then often implemented and applied inexpertly or to the wrong problems. This leads to disillusionment – until the practitioners become more experienced with the methods and how and when to apply them. The methods then contribute to the general productivity of the area. There have been many such hype cycles in drug discovery – molecular modeling, combinatorial chemistry, genomics, proteomics, and so on. Fragment technologies have to some extent gone through such a cycle – the early introduction of SAR by NMR led to a number of large companies attempting to replicate the success of the Abbott group and failing. The advantage fragments have had is that the methods do not require very large investments and so small, structure-based companies such as Astex, Vernalis, Plexikkon, SGX, and others were able to develop, implement, and refine the methods with a focus that comes from within small companies.

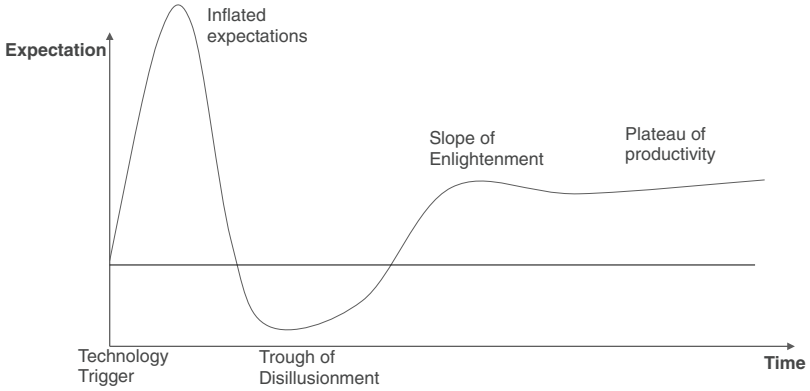

Figure 1.3 The technology hype cycle. The first reference I can find to the use of this was in an editorial introducing fuzzy systems by Bezdek [78]; it has subsequently been branded by Gartner management consultants and can be applied to most new technologies; the first use I saw for drug discovery was by Mark Murcko.

The successes mean that all accept FBLD has reached that plateau of productivity, at least for conventional targets. This is evident in the rise in the number of publications describing projects where FBLD was the main hit-finding approach. Figure 1.4 shows an analysis by Derek Lowe (personal communication) of the number of papers in the *Journal of Medicinal Chemistry* each year. Between 1996 and 2004, there was 1 review [27] and 3 projects (IL2 [28],

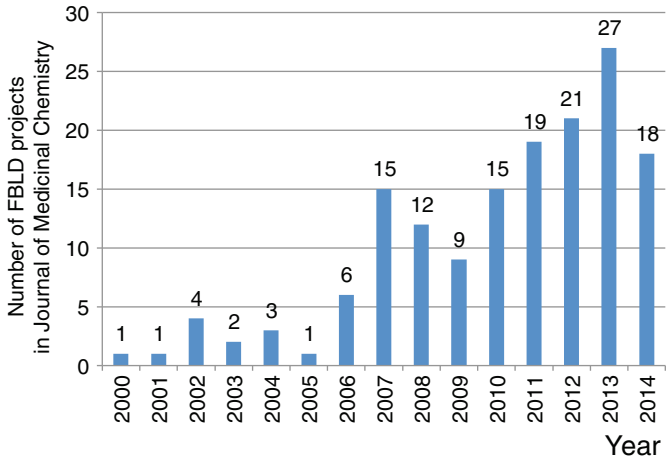

Figure 1.4 Publications on FBLD. This plot uses data provided by Derek Lowe from Vertex who counted the number of publications on fragment-based discovery that appeared in different years in the *Journal of Medicinal Chemistry* (up to August 2014). The method was to search for fragments as a keyword and then check from the abstract that they were relevant.

PTP1B [29], and Src SH2 [27,30]), whereas in the first 6 months of 2014, there were 13 relevant papers.

So, what are the main differences between 2005 and now? The following is a rather personal and anecdotal summary of the emergence and demonstration of some of the key ideas and methods that underpin and inform current practice in FBLD. The approach has been to go through the various talks I have given at fragment-based discovery meetings over the past 10 years and summarize the main results that I was presenting under the general title of fragmentology (a rather obvious pun on the tendency of many in the United States to corrupt my first name). The timing of these disclosures was sometimes limited by confidentiality issues, but it is a useful reflection of the emerging ideas and practices.

1.6.1 Developments in Fragment Libraries

The essential principles of how to design a fragment library were established in the mid-2000s and there have been a succession of papers describing only small variations in this basic approach from various organizations [31–33], including one personal example of the design of a library that maximally represents available compounds to aid in SAR by catalog [34]. Perhaps, a distinctive lesson to be learnt for a large organization is to ensure the whole team who need to use the fragments are involved in the design of the library and thus have ownership of its use [31]. One recent development is to have a library of fragments containing fluorine to exploit the sensitivity of ^{19}F NMR [35]. There is an issue of the limitations of which compounds can be synthesized or are available containing such an atom. Another way to exploit ^{19}F is to screen a generic fragment library for displacement of a ^{19}F-labeled probe molecule [36].

The notion that a fragment potentially samples a huge chemical space has been around from the early days of fragment library design. From the early days of FBLD, the chemical universe was cited as containing up to 10^{60} compounds with less than 30 heavy atoms [37]. An exhaustive analysis by Reymond showed that using known chemistries, the number of compounds of up to 11 heavy atoms increases by about eightfold per heavy atom [38,39]. This suggests that a library of 1000 compounds of MW 190 (13 heavy atoms) is equivalent to 10^9 compounds of MW 280 (20 HA) and 10^{20} compounds of MW 450 (32 HA). More recent analyses [39] from this group were less exhaustive and gave a smaller average rate of growth (about fivefold per HA), but this is still a powerful demonstration for fragments being as small as possible and the size of compounds in the libraries of most practitioners have reflected this. The Vernalis library is currently average MW 190 Da; that for Astex is anecdotally closer to MW 170 Da. However, as shown recently, it is still likely that screening a library of a few thousand fragments will leave gaps in chemical space coverage, and perhaps virtual screening can help to identify these [40].

There has been some analysis of the properties of the fragments that are hits versus not hits against any targets [14,41,42], which have concluded there are

no distinctive features or reasons to exclude a particular compound from the library – in some senses, the nonhits are fragments waiting for the right target. In general, the hit rates from larger compounds (i.e., above 20 or so heavy atoms) is seen to be lower, for the obvious reasons of complexity. One of the few trends is that fragments that are hits against protein–protein interaction targets are slightly larger than those for conventional targets and have a slightly higher lipophilicity. This is not surprising, given the nature of those binding sites and that the library compounds need to be highly soluble for screening and thus have many polar groups. During the late 2000s, a theme emerging from analysis of developability of drugs was that an increase in 3D character is useful [43] and there has been some discussion of generating 3D fragment libraries. To date, this has remained a hypothesis – there are not yet the compounds available to test if having more 3D fragments (usually requiring more sp^3 character in the core scaffold) has any advantage.

1.6.2 Fragment Hit Rate and Druggability

There have been a number of papers suggesting that the hit rate from a fragment screen reflects how difficult it will be to generate high-affinity ligands against a target. The first publications [44,45] were followed by others [14,41]; a more recent analysis of the Vernalis experiences (see Figure 1.5) [15] suggests that the

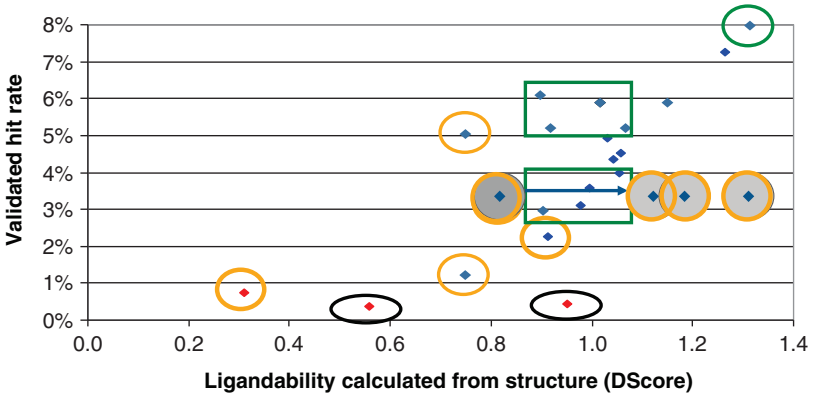

Figure 1.5 Druggability and conformational change. This plot is an update of an analysis published in 2009 [14] on the number of validated hits identified binding to different targets by ligand-observed NMR screening of the Vernalis fragment library. Each point represents a different target with kinases highlighted with a green box and protein–protein interaction targets with a yellow circle; the *x*-axis is the calculated druggability for a representative structure calculated with the program Sitemap. The red points are targets for which it proved difficult to progress in lead optimization (from left to right, β-catenin, Pin1, and Hsp70). The blue arrow and shaded yellow circles reflect how the shape of the binding site changed as leads were optimized for Bcl-2.

initial hit rate from fragment screening gives an indication of the "ligandability," with two main additional observations. The first is that for some targets (such as protein–protein interaction targets), the binding surface is quite malleable and that the right ligands can induce or select a conformation of the protein that is able to bind a ligand more strongly; this is a demonstration of a principle discussed by others [46]. The second is that a fragment screen hit rate that is lower than that expected on the basis of the known structure of the target can indicate possible issues in evolving ligands against that target.

1.6.3 Developments in Fragment Screening

This issue will be discussed at length in other chapters in this book. The most striking change since 2005 is the widespread use of surface plasmon resonance (SPR). This is primarily because there is a better understanding and wider expertise in how to use the method [47,48] as well as improvements in the sensitivity of the hardware. There continue to be proposals for new methods. Some are variations on immobilization of the protein to allow detection of ligand binding by optical [49], NMR [50], or mass spectrometry methods [51], others are monitoring changes on a fluorescent labeled protein [52] or exploiting the sensitivity of ^{19}F in NMR spectroscopy [53].

1.6.4 Ways of Evolving Fragments

Again, there will be many examples in this book of fragment growing, merging, and perhaps linking. One new idea for exploring the potential for optimization of a fragment is to exploit SPR to screen rapidly (and cheaply) small libraries of compounds that exploit a particular vector on a fragment. The essential idea is that the increase in binding affinity typically seen on evolving a fragment from 100 s to single-digit μM is a change in the off-rate of binding [16].

1.6.5 Integrating Fragments Alongside Other Lead-Finding Strategies

All of these successes have led to increased investment in the methods within most pharmaceutical companies. There has been a tendency for FBLD to be the method of last resort for unconventional targets that fail in HTS. For these targets, the main issue is to allow the flexible, multidisciplinary and problem-solving approach the time necessary to get FBLD methods to work. Where there is still a real challenge in some companies is how to organize project teams and processes to effectively use fragment methods for more conventional targets such as kinases. There are real opportunities in large companies to combine the insights that can be given by fragments with information provided by HTS hits, either from a fragment screen or by designing fragments from the HTS hit. However, a fragment

optimization campaign can spend much longer working with relatively low-affinity compounds (typically in the hundreds of μM range) compared to HTS hits (which often begin with tens of nM affinity). If affinity is the metric used to drive the project, then the HTS hit will displace the fragment hit. However, the fragments can often give hits with better properties – better not necessarily faster.

1.6.6 Fragments Can be Selective

An early concern was that the same fragments would emerge as hits for multiple examples of the same protein family. The protein kinases represent a major area of activity for the past 10 years; a surprising degree of selectivity is seen in the fragment hits found against such targets. Two main points emerge from an unpublished Vernalis analysis of screening the same library against eight different protein kinases. The first is that the number of fragments that are just a hit for a single kinase (the leading diagonal of Table 1.4) is quite high; the pattern of selectivity (the off-diagonal numbers) is also quite marked. Second, there are remarkably few fragments that are hits against more than two kinases (the numbers are 41, 26, 14, 14, 8, and 2 fragments for 3, 4, 5, 6, 7, and 8 kinases, respectively). One of the fragments always seen binding is compound **37** but as shown in Figure 1.6, it has the capability of binding in many different ways. It is not like a conventional "frequent hitter" – the promiscuity is real and not an assay artifact.

Table 1.4 Fragments are selective.

Total number of hits	**28**	**102**	**55**	**37**	**51**	**42**	**32**	**42**
	CDK2	**DYRK1A**	**Kin1**	**Jnk3**	**Pak1**	**Pak4**	**PDPK1**	**Stk33**
CDK2	6	14	11	9	8	5	8	13
DYRK1A		30	35	19	18	21	20	29
Kin1			9	18	20	12	14	12
Jnk3				7	13	9	12	10
Pak1					20	12	7	7
Pak4						13	8	7
PDPK1							4	12
Stk33								8

The table summarizes the results at Vernalis for screening a series of kinase targets by ligand-observed NMR. The experiments were performed over a 10 year period when the fragment library consisted of between 1100 and 1500 fragments. As the library has been updated and has evolved over the years [14], only 565 fragments were screened against all 8 kinase targets. Of these, 297 were never a hit against any kinase. Ninety-six of the fragments hit a single kinase; this is not a function of the threshold for binding. In general, kinases bind fragments with relatively high affinity (up to 100 nM) so the fragments are showing considerable selectivity. The table summarizes how many of the 565 fragments were hits against each of the kinases and how many were hits against two kinases. For example, the highlighted boxes show that although 12 fragments were hits for both Pak1 and Pak4, there were 20 and 13 fragments out of the 565 that were hits for Pak1 and Pak4, respectively, and not for any of the other kinases.

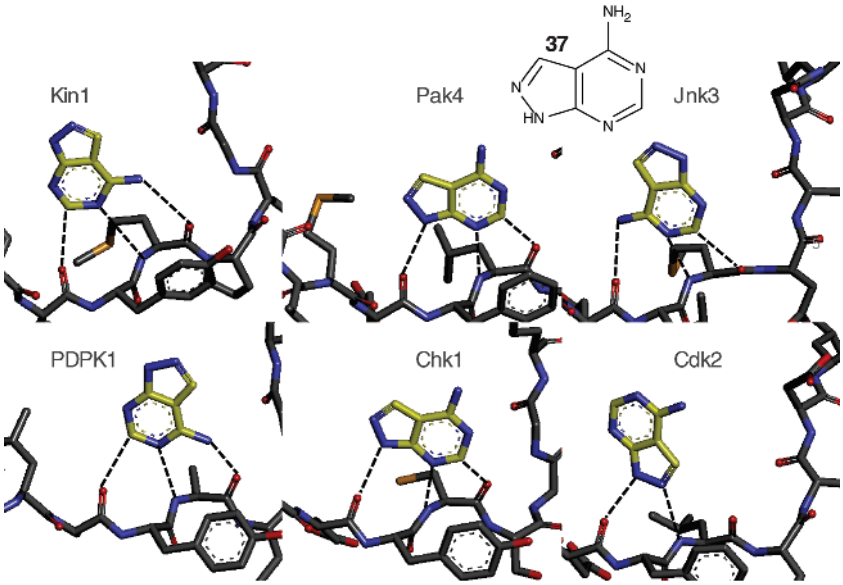

Figure 1.6 Binding modes of fragments. Details of the crystal structures of **37** bound to six different kinases. The hinge binding region of the ATP binding site of each kinase is shown in stick with carbon atoms in gray, oxygen in red, nitrogen in blue, and sulfur in yellow; the carbon atoms of **37** are shown in yellow with hydrogen bonds to the hinge backbone shown as dashed lines.

1.6.7
Fragment Binding Modes

As fragments became established, there was a flurry of activity disassembling known compounds. Not surprisingly, the fragments sometimes adopted the same binding mode as the parent ligand, sometimes not; it depends on the

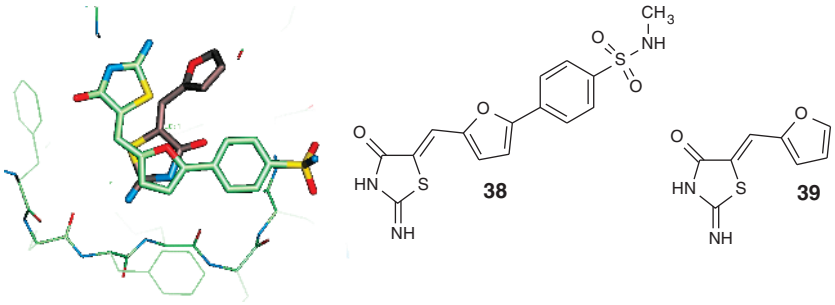

Figure 1.7 Deconstruction of hits to fragments. Overlay of the crystal structure of compounds in stick **38** (light green carbons) and **39** (dark brown carbons) bound to the hinge region of CDK2 (lines).

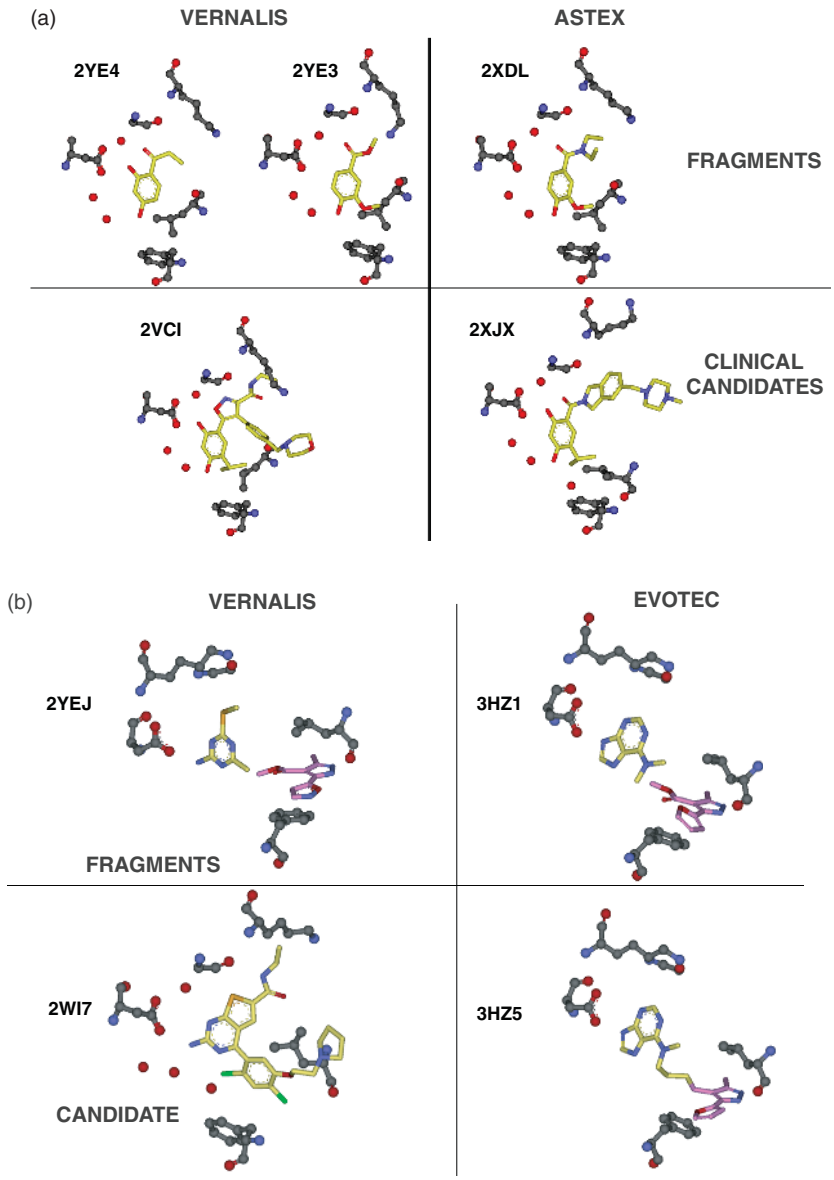

Figure 1.8 Chemical space and novelty. Crystal structures (PDB codes shown) of various compounds (stick with yellow or purple carbons) bound to HSP90 (ball and stick, key residues shown). The top panels of (a) show fragments discovered at Vernalis (left) [73,74] and Astex (right) [79]; clinical candidates derived from the fragments at each company are shown in the bottom panels. The top panels in (b) show a pair of fragments discovered at Vernalis (left) [18,73] and Evotec [72,80] (right) with the resulting optimized compounds from each company in the bottom panels.

efficiency of binding and the compromises required to bind the full ligand [54,55]. An example that reinforces this is from a Vernalis screen against CDK2 where there is very different binding mode (Figure 1.7) for the initial virtual screening hit **38** with that seen for the derived fragment **39**.

1.6.8
Fragments, Chemical Space, and Novelty

Another issue that is often raised is that there are similarities in the fragment libraries in different organizations and so similar, if not the same, fragments are likely to emerge from screening of the same target. As Figure 1.8 demonstrates for HSP90, that can be the case, but the fragment evolution depends on the medicinal and structural chemistry involved in optimization. Figure 1.8a summarizes the initial phenol/resorcinol fragments found at Vernalis and subsequently at Astex; there are some similarities in the final clinical candidates but they reside in sufficiently different IP space. By contrast, the dual fragment binding seen at Vernalis and then at Evotec (Figure 1.8b) resulted in very different candidates being developed. At Vernalis, features were merged to give the final compound that entered preclinical trials, whereas Evotec attempted a linking strategy to give a lead compound that was not pursued.

1.7
Current Application and Impact

In addition to the impact on drug discovery, I would like to emphasize three other significant impacts of FBLD methods.

The first is the ability to rapidly develop tool compounds that are potent and selective enough to ask questions about targets. This is very important for assessing targets in pharmaceutical discovery, such as the work at Vernalis on PDPK1 [56]. It is also proving extremely valuable in academia and there are an increasing number of examples emerging from such laboratories on protein–protein interactions [5,57], exploring potential new antibiotic targets [6] and the recent discovery of enzyme activators with potential for improving industrial enzymes [7].

Second, FBLD has helped to promote (and required) the development of biophysical methods in drug discovery. The rational approach to FBLD has increased the focus on compound properties and provides routes to resolving any issues. This has allowed some of the lessons learnt from analysis of the past decade or so of drug discovery to be considered during optimization.

Finally, the methods have emphasized the advantage of a nonindustrialized approach to drug discovery. FBLD campaigns against conventional targets such as kinases require some modification of the metrics usually applied – the project can spend considerably longer working on lower affinity compounds while suitable fragments are identified and suitable SAR developed to allow

incorporation of selectivity and affinity. But for many such projects, the eventual time taken to achieve an advanced lead series is not dissimilar from starting with an HTS and then trying to fix the problems in the HTS hit. Where the strain really appears in large companies is setting the expectation that to be successful on an unconventional target can take many years – some of the recent projects such as the BH3 mimetics have taken 5–7 years to identify clinical candidates.

1.8 Future Opportunities

So, what are the areas of FBLD where we can expect some new developments in the next 10 years? What additional impacts will fragment-based methods have on lead discovery? There will continue to be improvements in the methods: for identifying fragment hits (reduced cost in terms of sample requirements and instrument as well as methods for robust characterization of very weak, mM small fragments [51,58]); for characterizing the kinetics and thermodynamics of binding (wider range of faster, cheaper approaches); and in additional ideas such as off-rate screening by SPR [16] to allow rapid elaboration of fragments. In terms of application, we will see an increased deployment and expertise base in the academic community, able to use the methods to not only identify chemical tools to probe biology but also bring about further integration within the pharmaceutical industry, realizing the full potential synergy between FBLD, HTS, and other hit- and lead-finding technologies (DNA-encoded libraries, tethering, etc.). Alongside this, there will be improvements in the methods, perhaps most striking will be the engagement with academic synthetic organic chemists, devising novel chemistries, such as seen recently [5,59].

An area that has not developed as rapidly as (I) expected is the use of computational methods in support of the process. There are some attempts at docking and simulation methods to augment experimental screening [60–62], but as yet there is no substantial body of research that exploits the vast amounts of detailed structural information available on protein–fragment complexes – be that analyzing the origins of binding thermodynamics and kinetics or devising ways of merging fragment information together to design new compounds.

There are still issues with effective integration of the methods in some organizations: fragments are sometimes deployed only when HTS or other hit-finding methods have failed, the fragment screening is not initiated early enough for the fragment hits to have an impact on the choice of lead for progression, or the metrics applied in the early hit-finding phase do not give time for the fragments to be properly considered. For example, some organizations have defined schedules for progression from hit to lead or have a definition of a hit that requires biological activity. Such rigid criteria are perhaps needed to prioritize resources across many competing projects – such an industrialized approach allows managers to manage. But research, even

for a conventional target, needs to respond to the data to ensure the best possible compounds are taken forward.

In conclusion, although the essential features of FBLD methods were established in the early 2000s, the surge in application of the methods and demonstration of success has happened only since the publication of the first edition of this book. The methods and their impact on lead discovery have continued to develop; it will be fascinating to see if another edition of this book is justified in a space of a decade or if, as we are beginning to see, the methods become sufficiently integrated into the general lexicon of drug discovery that it is no longer seen as a separate field.

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