Fragment-Based Drug Discovery

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Foreword

In 1995 Glaxo Wellcome acquired Affymax, the pioneering Combinatorial Chemistry company based in California, for ~$500m in an attempt to increase the productivity of its research. The 1990s was a decade in which many industry executives believed that in order to increase the output of their R&D groups 'industrialisation' of the whole process was required. As such, significant investment was made in infrastructure for high-throughput screening and combinatorial synthesis in most major pharmaceutical companies. The belief was that the ability to rapidly screen libraries of several 100,000 compounds against key disease targets would yield many new drugs.

Combinatorial chemistry was an approach to generate those huge compound libraries. As one of the Glaxo Wellcome scientists working at that time in research, I was able to get an early insight into this brave new world of drug discovery. Despite the initial promise and the huge increase in apparent productivity, we soon came to realise that many lead compounds discovered using these approaches did not have optimal physico-chemical properties that would allow them to be developed into high-quality drug candidates. The subsequent years have shown that our initial concerns were valid as this approach of industrialising drug discovery has largely failed. It is clear that drug discovery is a personal endeavour, not a process.

One of the goals of screening huge combinatorial chemistry libraries was an attempt to increase the area of chemical space that was being sampled. Compounds in these early libraries were typically large (MW > 350 Da) as they contained multiple functional groups to increase the chance of finding interactions with the protein target. An alternative, and at the time contrarian, approach to crack this same nut was to consider screening compounds that were much smaller and which contained perhaps only one functional group. The potential advantage of this approach was that the functional group would not be sterically hindered; as in the case where it was part of

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a larger molecule. However, the initial binding affinity against the target would be rather low, perhaps mM, which would pose significant challenges in detection. It turned out that there was an even bigger challenge; the cultural shift required in the minds of medicinal chemists to appreciate such a low-affinity starting point. There were many conversations in the early years during which this point was made; it was already difficult enough to optimise a μM hit from an HTS campaign, why would anyone consider starting with a mM fragment hit! Of course in those years many scientists continued to be seduced by ligand potency, not ligand efficiency.

As is now generally accepted, the fragment discovery approach is a deconvolution of the combinatorial methodology and should allow the same (or even more) chemical space to be sampled using significantly smaller numbers of compounds. Indeed, a library of 1000 fragments can be shown to represent a similar (or even greater) range of chemical space, when compared with a combinatorial library of 1 000 000 larger compounds. Therefore, the fragment approach is a more elegant and perhaps more intellectually satisfying approach which could explain why not only industrial groups but also academic groups have embraced it. A key challenge in fragment screening is the ability to detect the low affinity of the initial binding, which can be in the 5–10 mM range in the case of protein–protein systems, and then of course to develop those fragment hits into useful drug leads. However, many groups over the years have successfully overcome these challenges as highlighted by chapters in this book and also the many fragment-derived compounds that are now in clinical development. It is clear that the fragment approach is now established as one of the many technologies utilised by pharmaceutical companies to find new small-molecule drugs.

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Preface

Since the seminal work by Fesik *et al.* 'SAR by NMR' in 1996, fragment-based drug discovery (FBDD) has become an established technique within both the pharmaceutical industry and academia. The concept is simple; that is small molecules, or fragments (MW < 300 Da), are more likely to form a specific complex with a given protein, than are larger molecules. A consequence of this is that chemical space can be sampled much more efficiently using fragments than using molecules of greater complexity. Fragment-based screening, and successive optimisation of hits towards lead molecules, has been applied to many different protein targets across a variety of therapeutic areas. The first fragment-derived drug, vemurafenib, an inhibitor of mutant B-RAF (for treatment of melanoma), is now approved and being used to treat patients. At least 10 other fragment-derived drugs are in various stages of clinical trials.

This book aims to 'take stock' of the latest advances in the field of FBDD. In the following chapters, leading practitioners in the field from both industry and academia describe the latest techniques and applications. The authors lay out case studies, critical review and opinions which will give the reader a thorough appreciation of both the principles and best practice within FBDD.

One consequence of screening smaller, simpler fragments is that their binding affinity is often relatively low (>1 mM). Developing assays of sufficient sensitivity, and fidelity, to identify fragments with low binding affinity has been a fundamental challenge of FBDD. As necessity is the mother of invention, this challenge has driven the development of highly sophisticated biophysical screening techniques based on X-ray protein crystallography, surface plasmon resonance (SPR) and nuclear magnetic resonance (NMR). SPR and NMR are covered in detail by Tony Giannetti and Isabelle Krimm in Chapters 2 and 3, respectively. X-ray protein crystallography features heavily in most chapters, both in a screening role and in support of structure-based fragment evolution. Comparing and contrasting the output from these

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biophysical screening techniques is an area of active debate. This is discussed by Ian Wall *et al.* in Chapter 4.

FBDD continues to evolve rapidly and has recently seen new applications in areas such as epigenetics, G protein-coupled receptors (GPCRs), protein–protein interactions, antibacterials and the identification of novel allosteric binding pockets. In this book, experts from each of these fields discuss lessons learned based on both their own experience and key examples from the literature. These chapters describe aspects of fragment library design, screening techniques and hit validation. Examples include strategies for optimisation of fragments towards lead compounds, and, ultimately, drug candidates.

We would like to thank all the contributors to the chapters in this book for their outstanding effort and commitment to this project. We would also like to thank the staff at the RSC for their support in bringing this book to completion. We hope that this book will provide a useful resource for scientists who are looking to understand the practice of FBDD.

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Personal Essay: Fragments in the Blogosphere

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1 Introduction

In July of 2008, Teddy Zartler, then at Merck, launched a blog called *Practical Fragments*. The mission statement was – and continues to be:

> *This blog is meant to allow Fragment-based Drug Design Practitioners to get together and discuss NON-CONFIDENTIAL issues regarding fragments.*

I had met Teddy just a few months before at Cambridge Healthtech Institute's annual Drug Discovery Chemistry conference in San Diego, and he invited me and a few other scientists to contribute posts to the new enterprise. Although I followed the literature closely, I hadn't spent much time reading blogs, so I was ambivalent. What purpose would a blog serve? In the spirit of experimentation, I decided to give it a try. Seven years and more than 450 posts later, *Practical Fragments* has left its small footprint on the web; the number of readers has grown steadily, and posts have even been cited in the primary literature. Still, the invitation to write for this book re-opened the original question of what purpose *Practical Fragments* serves. This chapter is an attempt to answer that question, and to touch on the broader question of what role social media can and should play in science.

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2 A Living Review

Prior to *Practical Fragments* I had written or co-authored a few reviews on fragment-based lead discovery (FBLD), including one of two early reviews that attempted to distill most of the literature up to early 2004.[1,2] In 2006, I published a "chemical update", which picked up where the previous review left off with 32 new examples that had come out in the past 2 years.[3] With the increasing growth of fragment examples in the literature it looked like reviews would be increasingly out of date by the time they were published, and so blogging seemed like a good way of highlighting papers more or less in real time. Indeed, one of the earliest posts highlighted a paper describing the discovery of AT7519, a clinical-stage cyclin-dependent kinase (CDK) inhibitor from Astex Therapeutics.[4]

Personally I find these types of success stories useful and even inspirational, and they continue to be a mainstay of *Practical Fragments*. One of the nice features of a blog is that referencing earlier posts provides context to new discoveries. For example, in 2012, researchers from AstraZeneca published an elegant example of fragment linking to generate a nM inhibitor of the anticancer target lactate dehydrogenase A (LDHA), which was covered on the blog.[5] Less than a year later researchers from Ariad published their work on the same target, which led to molecules with interesting similarities and differences.[6] A parallel situation arose when scientists at Genentech published their discovery of a novel fragment-binding site on Ras, perhaps the holy grail of oncology targets.[7] Shortly thereafter, researchers at Vanderbilt University published their own independent discovery of this binding site and associated fragments.[8] Being able to click from one post to another can be useful to someone new to the target or the field. Indeed, a figure from the more recent Ras blog post comparing structures from both papers was reprinted by other researchers in a *Journal of Medicinal Chemistry* review on the topic.[9]

As entries have accreted over the years, several of the molecules highlighted have entered the clinic, which has led to periodic summaries of clinical compounds derived from fragments. In early 2015, at least 30 molecules derived from fragments had entered clinical development, of which at least 16 were still active.[10] One drug, vemurafenib, was approved for sale in 2011.[11] At the suggestion of one commenter on the blog, this list was given a permanent link on the side-bar for easy reference.

Practical Fragments is not just about drugs and chemistry: many of the biophysical techniques used to identify and characterize fragments have been discussed too, including mainstays such as surface plasmon resonance (SPR),[12] isothermal titration calorimetry (ITC),[13] nuclear magnetic resonance (NMR), differential scanning fluorimetry (DSF),[14] and X-ray crystallography. Some of these are among the most popular posts.

Practical Fragments also highlights new or emerging methods, such as mass spectrometry (MS),[15] computational screening,[16] weak affinity chromatography (WAC),[17] target-immobilized NMR screening (TINS),[18] microscale

thermophoresis (MST),[19] capillary electrophoresis (CE),[20] inhibition in solution (ISA),[21] ultrafiltration,[22] and enthalpy arrays.[23] A nice feature of a blog is that when a new post refers to one of these techniques it is easy to hyperlink to the original post, which gives a newcomer to the field a full description, while not forcing an experienced researcher to read about SPR for the umpteenth time.

Finally, for reasons that remain obscure, fragment-based drug discovery attracts an inordinate number of reviews, special journal issues, and books. Many of these are covered as they are published, and the books (six counting this one!) are linked on the right side of the front page.

But *Practical Fragments* is about more than summarizing research findings. As described in the next section, a blog can be an excellent forum for discussing the limitations of experimental techniques. In particular, it can be a place where scientists offer each other tips, and warn against potential artifacts, anonymously if need be.

3 Warning Signs and Guideposts

What's worse than running a screen and coming up empty-handed? Ending up with false positives. To find low-affinity fragments researchers sometimes need to push techniques to their limits, which can lead to various types of artifacts. The problem is particularly acute given that fragment-based drug discovery is relatively new in many organizations, so people may not be aware of potential problems. If you're accustomed to screening compounds at 1 μM concentration, screening at 1 mM concentration could present unexpected challenges. Moreover, since fragment-based teams are often multidisciplinary, it can be easy for artifacts to creep through the cracks in expertise.

To arm the research community against such issues, *Practical Fragments* has highlighted a number of problems. Compound aggregation at high (and sometimes even low) μM concentration is a phenomenon that has led to numerous spurious reports in the literature and wasted efforts.[24] However, even gold-standard techniques like NMR and X-ray crystallography are subject to artifacts if one is not diligent.[25,26]

But it is not just a question of assays: some compounds are inherently reactive or likely to generate false positives, and if these compounds appear in a screening collection they will likely dominate any hits. Jonathan Baell has christened such molecules *pan-assay interference compounds*, or PAINS, and many of these are fragment-sized.[27,28] Some of the more insidious compounds can be reduced by common assay components such as dithiothreitol and then spontaneously re-oxidize in air to generate reactive hydrogen peroxide, which can confound many biochemical and cell-based assays. In response to a post on such molecules, one commenter wrote, "I learned something new today! Woot!".[29]

An enormous opportunity for blogs is to facilitate discussion among scientists more rapidly and less formally than in the traditional literature. A nice example of this was initiated by a post in 2013, entitled "Fragmenting

natural products – sometimes PAINfully".[30] This covered a prominent paper in which the researchers 'fragmented' natural products into individual components and used these to generate a fragment screening library. Unfortunately, the authors seemed to be unaware of PAINS, leading to the inclusion of many dubious compounds. The post attracted comments from the first author as well as from the *Nature Chemistry* editor who had handled the paper. If nothing else, the exchange made more people aware of problem compounds.

Although the potential for discussion is inherent in the blog format, it is a rare post that receives more than one or two comments. This is in contrast to some other chemistry blogs such as Derek Lowe's excellent *In the Pipeline*,[31] which routinely receives dozens of comments for each post. Some of this is just a matter of scale: fragment-based drug discovery is a rather small niche, and *In the Pipeline* can receive more page views in a month than *Practical Fragments* has in its history. That said, Teddy did ask why people didn't comment more. Several of the responses were along the lines of, "I don't really have much to add. I mostly read the blog to keep current on what's going on in FBDD (something that your posts excel at), and it seems silly just to chime in to a post and say 'cool'." On a more charming note, one commenter wrote that "comments tend to come from controversy and I think the current FBDD community is quite a contented bunch".[32]

4 A Mirror for the Community

In addition to commenting, another useful feature of blogs is the ability to run polls to gather information of interest to the community. Of course, like polls everywhere, these can be subject to low turnout and self-selection among participants. There is another unfortunate (and unexpected) similarity to some political polls: it turns out that the *Blogger* platform on which *Practical Fragments* is housed has a nasty habit of 'losing' votes – a problem that accelerates over time – which means that poll results need to be captured and archived before degrading. All those caveats aside, *Practical Fragments* has been able to capture some interesting data, starting with the question of readership (Figure 1, left panel), which in 2010 was split roughly evenly between academia and industry and between practitioners and aficionados of fragment-based lead discovery.[33] A repeat poll in 2013 showed a similar distribution, though with a slight shift towards industry (Figure 1, right panel).[34]

Polls can also reveal which techniques researchers in the community use to find fragments. In 2011 *Practical Fragments* asked this question, resulting in the data in Figure 2 (blue bars). Respondents could select multiple techniques, and one striking finding was that the average respondent used between two and three different techniques, presumably to help weed out artifacts, confirm true positives, and obtain more detailed structural information.[35] A repeat of this poll in 2013 revealed similar findings, though with an increase in the extent to which crystallography was used as well as an increase in the number of individual techniques used (Figure 2, red bars).[34]

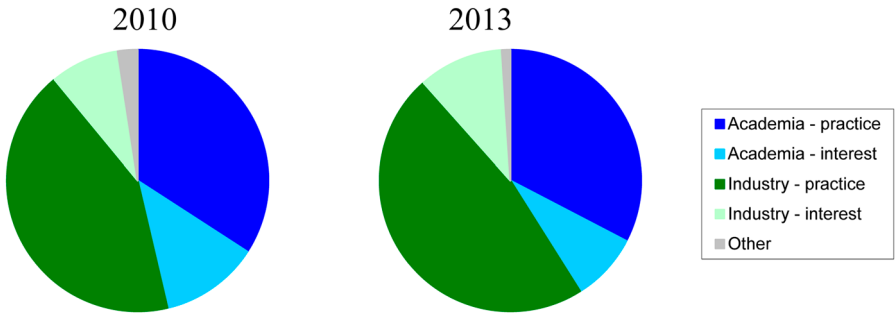

Figure 1 Demographics of readership. Left: Poll ran May–June 2010 and received 82 responses. Right: Poll ran December 2013 and received 95 responses.

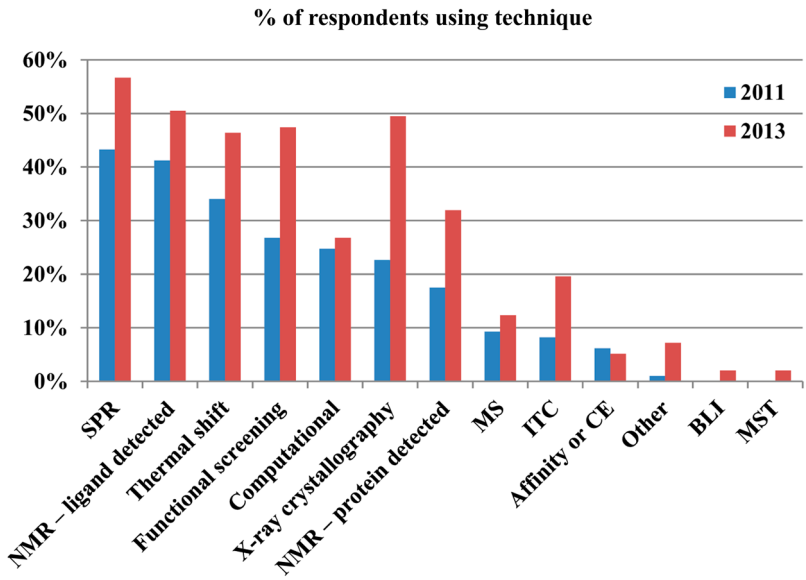

Figure 2 Fragment screening methods; respondents could choose multiple metrics. The 2011 poll ran September 2011 and received 97 responses (blue). The 2013 poll ran December 2013 and received about 96 responses (red). BLI = biolayer interferometry; other abbreviations as noted in text.

And just as there are many ways to find fragments, there are lots of ways to evaluate them too. Ligand efficiency is the simplest,[36] but a whole cottage industry has arisen to supply new metrics that incorporate more data.[37] Do people use these other metrics? A poll in 2011 found that ligand efficiency (LE) was the overwhelming favorite, trailed by ligand lipophilic efficiency (LLE, Figure 3).[38] Of course, these findings could change over time; the third most common metric, LLE_{AT}, had been published online only a month before our poll,[39] so perhaps we will revisit this question, particularly in light of some of the recent controversies around the appropriateness of LE.[40]

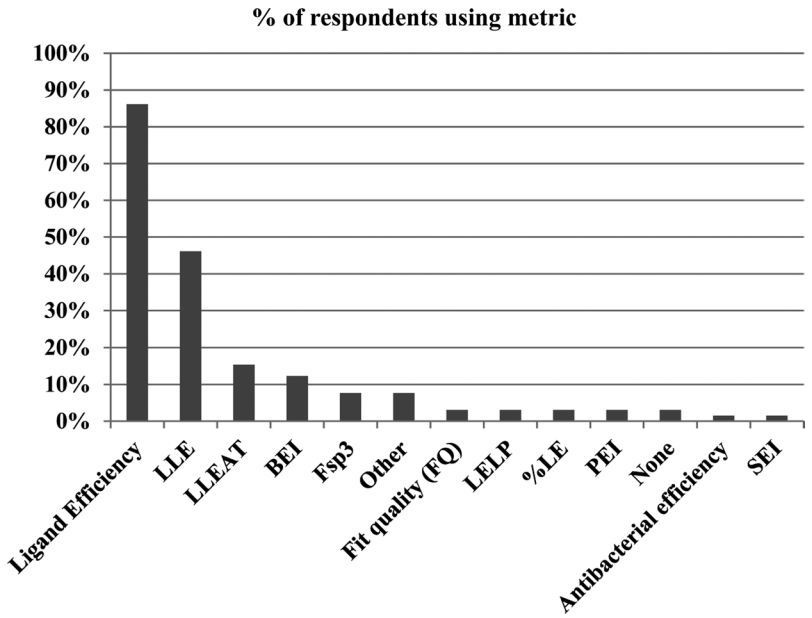

Figure 3 Metrics used to evaluate fragments. Poll ran July 2011 and received 65 responses; respondents could choose multiple metrics. LLE = ligand lipophilic efficiency; BEI = binding efficiency index; Fsp3 = fraction of sp3-hybridized carbons; LELP = ligand efficiency dependent lipophilicity; %LE = % ligand efficiency; PEI = percentage efficiency index; SEI = surface-binding efficiency index. See ref. 38 for links to full definitions.

LE is defined as the binding energy divided by the number of heavy atoms,[36] but some metrics use molecular weight instead of heavy atoms; the idea is that larger atoms such as bromine should suffer a penalty compared with smaller atoms such as fluorine. That said, in a 2013 poll, 27 out of 38 respondents used only heavy atoms, 1 used both heavy atoms and molecular weight, and only 2 used molecular weight alone (with the remaining ignoring size altogether).[41]

Several polls have explored fragment screening libraries. One of the selling points of fragment methods is that you can cover chemical space more effectively with a smaller library,[42] but what is the optimal library size? A poll in 2013 found a median of 1000–2000 fragments (Figure 4).[34] This is consistent with a 2013 analysis of published libraries, which found a median of 1300 fragments among the 22 libraries summarized.[43]

In terms of fragment library design, a frequent question is how large fragments can be. Astex's *Rule of 3* suggests an upper cut-off of 300 Da,[44] and a poll in 2012 found that most people put an upper limit of 20 atoms,[45] which translates to roughly 260 Da according to a Pfizer analysis that found the average non-hydrogen atom in their corporate collection had a mass of 13.286 Da (Figure 5).[36]

Perhaps just as interesting is the question of how small a fragment people would put in their library, the subject of a poll in 2013 (Figure 6).[46]

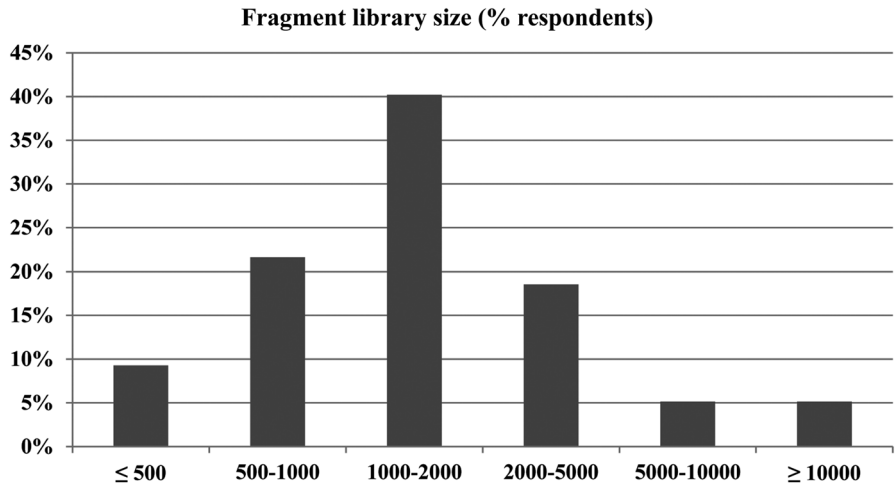

Figure 4 Fragment library size. Poll ran December 2013 and received 97 responses.

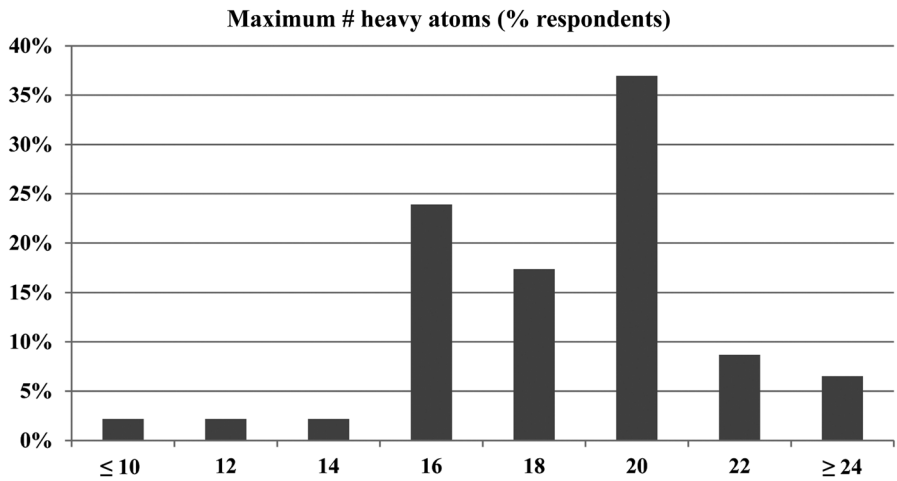

Figure 5 Maximum size of fragments allowed in a library. Poll ran May 2012 and received 46 responses.

Obviously you want your fragment to be as small as possible, but make it too small and you run the risk of having fragments that are so weak that you cannot detect them. As it happens, the median bottom bound is 7 or 8 heavy atoms, basically the size of 4-aminopyridine (marketed as fampridine) or 1,2-benzoquinone (if you didn't screen out your PAINS).

Finally, *Practical Fragments* is an ideal forum to publicize, summarize and discuss conferences and fragment-related events. One of the *Links of Utility* on the right side of the blog is to *Upcoming events*, which is updated frequently.