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Fragment based drug discovery

Fragments: past, present and future

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Fragment-based drug discovery has come a long way in a short period of time and is now being used throughout the biopharmaceutical industry. Here we review the origin of the approach, discuss how it is being applied and the prospects for future development. We illustrate this with examples from our own projects where we have found that information from fragments can inform the optimisation of hits identified by other means (e.g. HTS and/or virtual screening) and vice versa. We further discuss that fragment information can also be applied to the discovery of ligands for targets that are not readily amenable to structural analysis by experimentation such as GPCRs, particularly through the application of computational modelling methods.

Introduction

Fragment-based drug discovery (FBDD) has become an established technique for lead generation. The approach consists of initially screening a library of lower molecular weight molecules (or fragments) using a sensitive assay method. Structural insights are then obtained into the interaction of the identified weakly active fragment hits with the biological target to guide medicinal chemistry optimisation. There are many excellent reviews on FBDD in general which the reader is referred to (see [1–7]) and within this issue of Drug Discovery Today: Technologies there is a set of reviews on specific aspects of FBDD. In this article we review the origin, current status and future prospects of fragment-based drug discovery and the impact that this technique is making on the generation of new medicines from the perspective of the projects that we have run and the developments that we are seeking to make.

Past

It is difficult to precisely pinpoint the origin of FBDD. The idea that the activity of a drug molecule results from the sum of its parts originates with Ehrlich's 1909 definition of a pharmacophore that was updated sixty eight years later by Gund to be 'a set of structural features in a molecule that is recognized at a receptor site and is responsible for that molecule's activity' [8]. The concept of computational fragment codes for structural retrieval has been in use within the pharmaceutical industry since the 1950s [9] and the correlation of fragment sub-structural analysis with biological activity has been applied to drug design since the mid 1970s [10]. So, by the time of the 1990s the concept of drug fragments was familiar to medicinal chemists. Indeed, in his 1996 seminal article on the Hit-to-Lead process, Michne defined one of the key objectives of following-up high throughput screening hits 'is to find the minimum active fragment (pharmacophore) of interest in a complex active molecule' [11]. It can be argued that the concept of identifying smaller molecules by preparing analogues of screening hits in which chemical functionality is deleted in a systematic fashion has always been a corner-stone of SAR-driven medicinal chemistry. However, what was missing was the ability to screen

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directly for weakly active fragment compounds. This problem was solved by Fesik and co-workers at Abbott who first reported on the use of a screening method with high sensitivity to directly identify smaller fragment hits with weak activity [12]. In the Abbott SAR-by-NMR method the use of ^{15}N labelled protein enables the label free detection of binding of millimolar affinity ligands in a 2D-HSQC experiment.

In parallel with these developments the concept of analysing binding sites on protein surfaces to identify interaction hot spots was first explored computationally by Goodford [13] and subsequently experimentally by Mattos and Ringe [14]. In the GRID computational method the interaction energies between a small molecular probe and the protein are calculated at a set of defined grid points to identify regions of attraction between the probe and the protein. This approach was later extended by Karplus to generate functionality maps of binding sites by a multiple copy simultaneous search (MCSS) method [15]. In the multiple solvent crystal structure (MSCS) method several X-ray crystal structures, obtained by soaking preformed protein crystals with different organic solvents (e.g. acetonitrile, acetone, DMF, DMSO, ethanol, phenol, and dioxane) are superimposed in order to identify binding hot spots that can be used in the structure-based design of more potent ligands [14]. In the same era that these protocols were being developed Verlinde and co-workers developed the concept of linking fragments *in silico* and attempted the first studies of soaking preformed protein crystals with cocktails of fragments [16]. However, it was not until a few years later, once advances had been made in automation and rapid data processing, that structures could be reliably obtained in this manner [17–19]. Although, this has proven to be an effective method for identifying fragment binders, a separate assay system is still required to determine activity in order to generate SAR for driving the subsequent structure-based fragment optimisation. Furthermore, the procedure is limited to soaking of preformed crystals and to relatively small numbers of fragments. Therefore, an alternative approach of screening using a sensitive biophysical technique (e.g. NMR or SPR), and subsequently determining the ligand binding mode by X-ray crystallography, was quickly adopted, for example by Roche [20] and Vernalis [21].

Following on from these early advances, a definition for the characteristics of a fragment was developed and the concept of ligand efficiency was first proposed. From an analysis of a diverse set of fragment hits against a range of targets, Astex derived the 'rule-of-3' whereby a fragment is defined as a compound for which the MW is <300, the number of hydrogen bond donors is ≤ 3, the number of hydrogen bond acceptors is ≤ 3, and the $c\log P$ is ≤ 3 [22]. The concept of assessing the binding energy per non-hydrogen atom or ligand efficiency (LE) was proposed by Hopkins and co-workers as a means for selecting between hit compounds for optimisation [23]. Ligand efficiency is calculated as LE = $-RT \ln K_d$/number of non-hydrogen atoms but for comparison of hits assessed in the same assay K_d is often substituted by IC_{50}.

We and others have utilised from the outset high concentration biochemical assays for the screening of fragments [24,25]. The advantages of this approach are that functional activity can be obtained at an earlier stage and larger numbers of fragments can be readily screened enabling a wide coverage of fragment space [26]. However, those taking a purist approach to fragment-based drug discovery advocate screening of smaller fragments (100–250 Da) by biophysical methods and have concluded that 'in a typical high concentration biochemical assay the majority of the binding information will only come from the largest compounds in the screening library' [7]. This is contrary to our experience and other reports in the literature. Figure 1 shows that the molecular property profile of hits identified in multiple biochemical screening campaigns is very similar to the profile of the 20,000 member fragment library that we have screened. Furthermore, in a recent review Alex and Flocco present a summary of sixty eight fragment hits reported in the literature that have been progressed into lead compounds [27]. Twenty one of these fragment hits were discovered by bioassay, fifteen fragment hits were discovered by NMR screening and fourteen fragment hits were discovered by screening using X-ray crystallography. For those fragment hits where the reviewers were sufficiently confident in the reported activity we have calculated the average potency and average ligand efficiency (Table 1). From this one can see that there is little to choose between the three assay methods in terms of ability to identify weakly active compounds and the resulting ligand efficiencies.

The medicinal chemistry optimisation of fragment hits has been extensively reviewed elsewhere (see [1–5]). Most groups are following a process of computationally driven structure-based design that usually involves fragment expansion, fragment merging and/or fragment linking. Fragment expansion (or evolution) usually starts with searching and testing of commercially available analogues that feature the active fragment as a substructure and which are predicted to dock into the binding site. X-ray crystal structures of active fragment analogues in complex with the target protein are obtained routinely to check docking hypotheses and to guide further optimisation. This use of commercially available analogues can be quickly exploited and provides further information on which to design small, focussed, fragment expansion libraries that seek to target additional key interactions. Once again, molecular docking is usually conducted as a filter to select a subset of compounds for synthesis. The aim of this iterative structure-based design process is to improve potency to the desired level (usually single digit nanomolar) whilst not reducing the high level of ligand

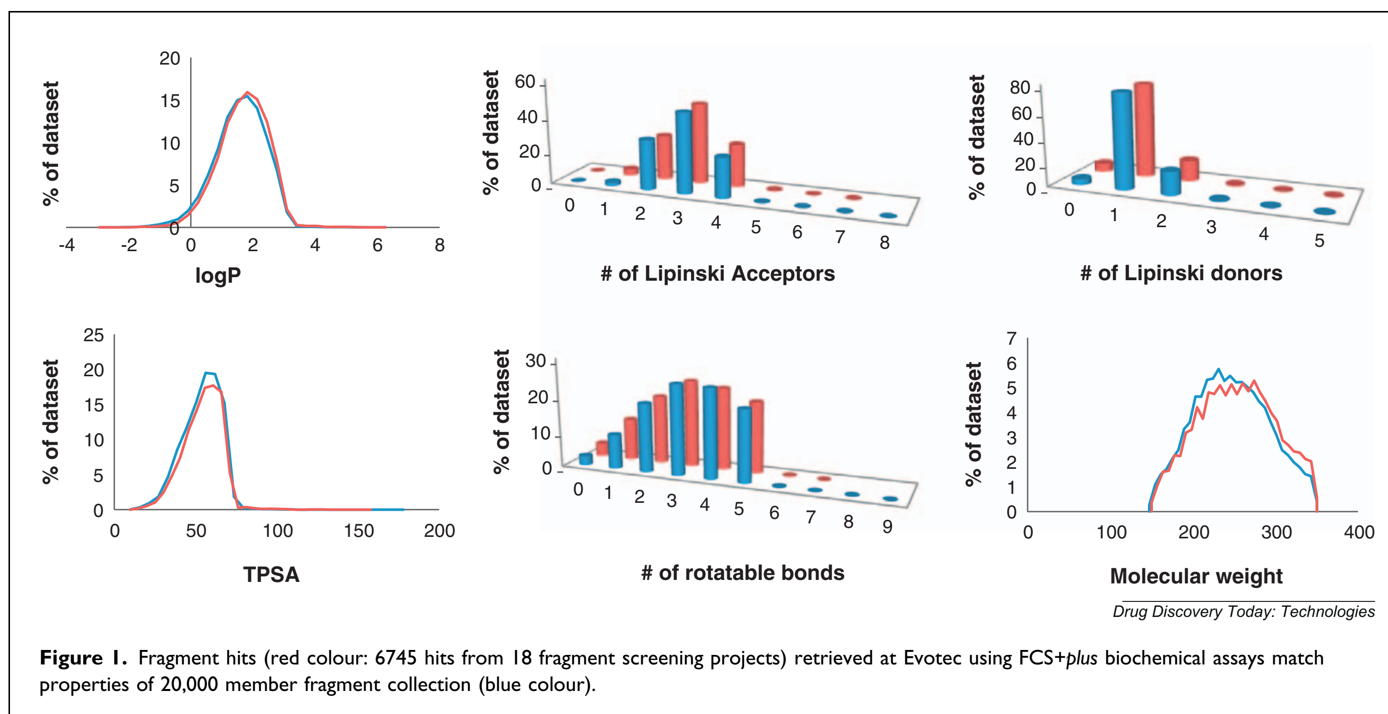

Figure 1. Fragment hits (red colour: 6745 hits from 18 fragment screening projects) retrieved at Evotec using FCS+*plus* biochemical assays match properties of 20,000 member fragment collection (blue colour).

efficiency of the starting fragment. We have applied this type of fragment expansion approach to good effect to discover a lead compound series of inhibitors against the cancer target heat shock protein 90 (Hsp90) [28]. The fragment merging process starts with the computational overlay of multiple fragment co-crystal structures with the target protein. This analysis can generate ideas for hybrid molecules that combine the features of two or more fragments to achieve several binding site interactions. An iterative structure-based design process is followed whereby X-ray crystal structures of the resultant hybrid molecules in complex with the target protein are obtained to verify hypotheses and aid the development of further fragment optimisation. In the very special case where there are two (or more) separate regions of a binding site to which fragments can bind the process of fragment linking can be contemplated. This is the most intellectually attractive method for fragment optimisation as it can result in rapid improvements in potencies. However, in practice this is difficult to achieve and is not a routine procedure for fragment optimisation. Firstly, few fragment screens result in the identification of fragments that bind to two different but adjacent locations within a binding site. Secondly, where they do, the design of a linker between the two fragments that maintains each part in its correct binding orientation is challenging. Nevertheless there are some notable examples of fragment linking particularly from the Abbott

Table 1. Comparison of fragment screening methods and ligand efficiencies

Fragment screening method	Typical size of fragment library	Information provided	Activity ranges of hits (mean) μM*	LE ranges of hits (mean)*	Limitations with respect to diversity
X-ray crystallography	≤1000 as mixtures of 4–10 compounds	3D-structural information on fragment target interaction	10–2500 (450)	0.26–0.62 (0.41)	Small libraries only. Fragment hits may be missed by soaking of preformed crystals
Nuclear magnetic resonance	1000–10,000 as mixtures	Binding affinity together with binding site information through displacement of well characterised compound or HSQC data	2–17,000 (1850)	0.20–0.48 (0.32)	Small to medium sized libraries
Bioassay (HTFS)	20,000	Binding affinity at specific site or functional activity	0.1–41,000 (2220)	0.21–0.86 (0.50)	No limitation on library size only generic constraints for fragment solubility apply

*Data compiled from review by Alex and Flocco [27].

group [12] and we have had success in the case of the Hsp90 oncology target [29].

Present

Today fragment-based drug discovery has firmly entered the mainstream as a means of hit finding and optimisation (see [1–5] and the reviews within this issue of Drug Discovery Today: Technologies). In terms of screening, the most robust fragment hit finding techniques employ a combination of orthogonal assay formats for hit identification and confirmation, respectively. In such an approach, fragments are screened either by a biochemical screen with subsequent hit confirmation using a biophysical technique (e.g. NMR or SPR) or vice versa [24]. X-ray crystallography is the method of choice for gaining structural insights into the interaction of fragments with the biological target. However, there are several promising alternative approaches to fragment screening that are not currently in widespread use perhaps because the unique nature of their technology has precluded broad adoption. These specialist screening methods include Graffinity's SPR method using immobilised arrays of fragment libraries [30], ZoBio's Target Immobilised NMR Screening (TINS) approach [31] and Carmot Therapeutics' chemotype evolution process. The latter involves the generation of target specific ligands from the reaction of target specific baits (fragment like compounds that bind strongly to the protein and present additional reactive functionality) with a second set of companion fragments [32].

We have previously argued that the term fragment-assisted drug discovery (FADD) is more suitable than FBDD because in practice fragment approaches to drug discovery are now rarely being used in isolation but rather in concert with other drug discovery approaches. It is our view that access to a variety of hit finding technologies increases the prospects of generating the highest quality lead series during the course of a drug discovery programme. This in turn enables lead optimisation to be initiated with the very best lead series thus reducing attrition due to liabilities such as inadequate PK, competing IP and poor developability.

Whilst we do still on occasion apply fragment techniques as the sole route for lead generation (i.e. FBDD) increasingly we are employing fragment methods in combination with other hit finding methods following a FADD paradigm. An example of FADD is where we were able to identify multiple hit series that *included* fragment inhibitors from a full deck HTS campaign against prostaglandin D2 synthase using the highly sensitive screening technique of fluorescence correlation spectroscopy. Subsequent structure-led optimisation of the fragment hits ultimately furnished an orally bioavailable prostaglandin D2 synthase inhibitor that exhibited *in vivo* efficacy [24].

A key question that is often asked today is 'when to make use of fragment methods'? We recommend running a fragment screening campaign in parallel with other hit finding methods from the outset for the *majority* of discovery projects. This is illustrated for the following two case studies.

BACE-1

Beta-secretase (BACE1) is an aspartyl protease implicated in the pathogenesis of Alzheimer's disease for which it has been challenging to discover non-peptidic chemical starting points. We conducted a uHTS campaign of *ca.* 200,000 compounds against this target. The hit rate was extremely low and no suitable starting points were found for medicinal chemistry optimisation. Subsequently, a fragment screen of our 20,000 member fragment library using the same biochemical assay principle, but screening at a higher concentration (1 mM), followed by orthogonal testing using surface plasmon resonance (SPR), gave 26 confirmed fragment hits [33]. Starting from this hit set, X-ray crystallography and medicinal chemistry optimisation provided, in two iterations of design and synthesis, inhibitors with low micromolar activity suitable for further elaboration (Fig. 2) [34]. Thus for BACE1 the fragment screen 'unlocked' the target by providing suitable starting points for medicinal chemistry optimisation. Others have had similar experiences in hit finding for BACE1. For example, AstraZeneca have also reported that high throughput screening of BACE1 failed to provide suitable starting points whereas fragment screening provided good starting points for inhibitor generation and optimisation [35].

PDE10a

Phosphodiesterase 10a (PDE10a) is a potential schizophrenia target. In this instance we conducted both a virtual screen and a fragment screen (Fig. 3) in parallel. The virtual screen was conducted against our HTS library of lead- and

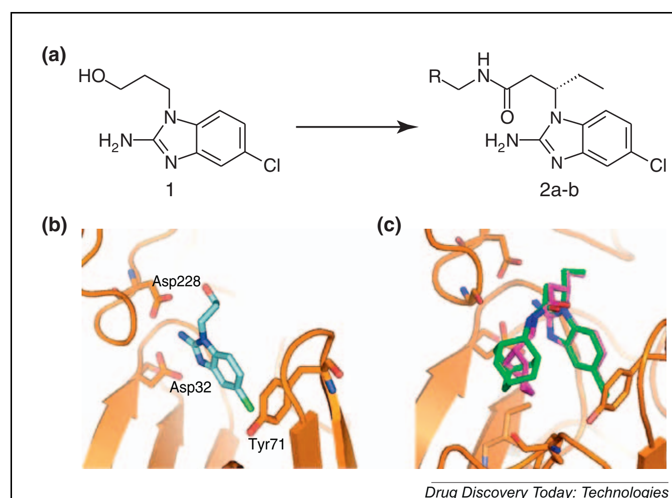

Figure 2. Rapid fragment optimisation for BACE1: **(a)** Fragment hit (compound 1; IC_{50} 770 μM) against BACE1 and more potent derivatives (compound 2a, R = cyclohexyl; IC_{50} 7 μM: compound 2b, R = adamantly; IC_{50} 9 μM); **(b)** crystal structure of compound 1 in complex with BACE1 (1.8 Å); **(c)** overlay of crystal structures of compounds 2a and 2b with BACE1 (2.6 Å and 2.4 Å respectively).

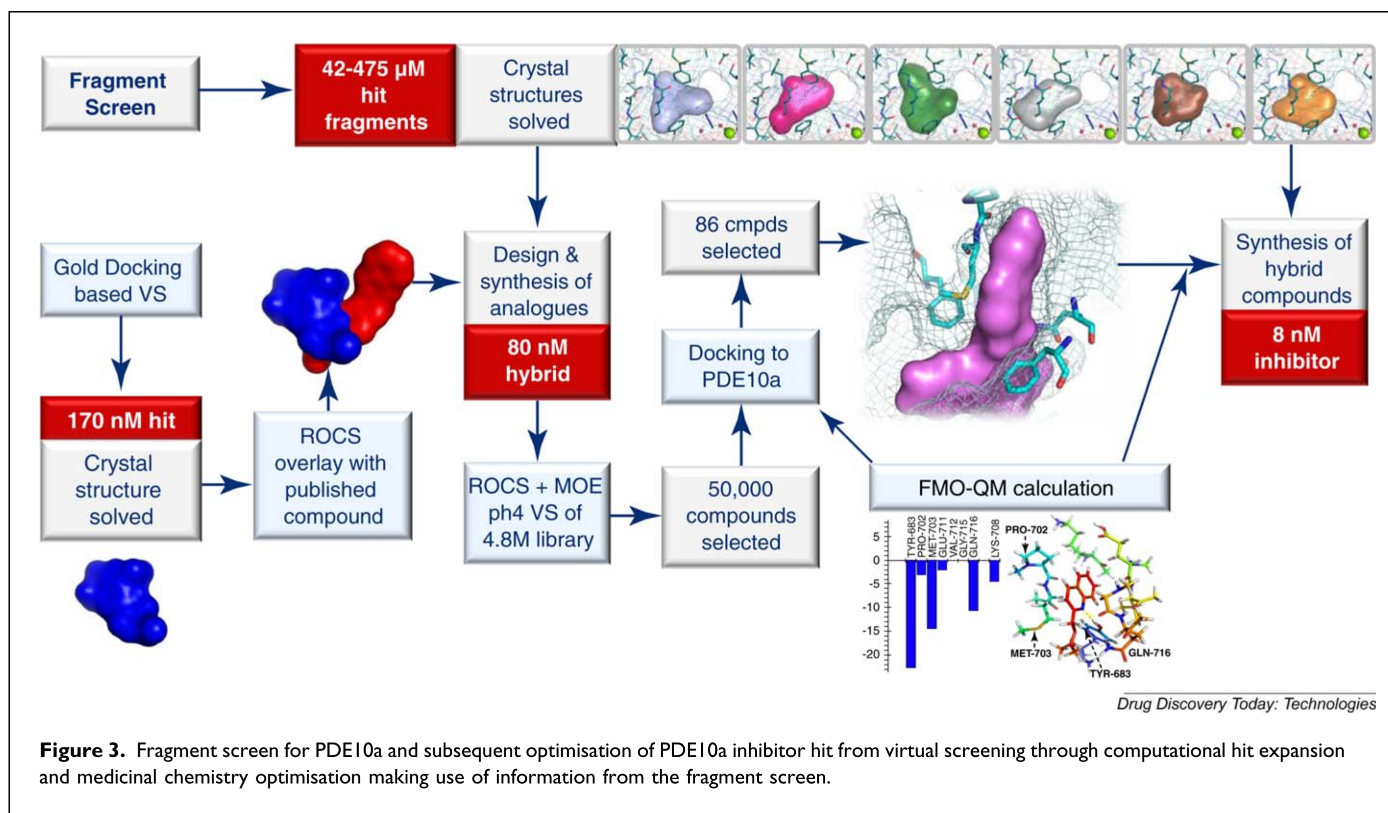

Figure 3. Fragment screen for PDE10a and subsequent optimisation of PDE10a inhibitor hit from virtual screening through computational hit expansion and medicinal chemistry optimisation making use of information from the fragment screen.

drug-like compounds using molecular docking whilst the fragment screen utilised a biochemical assay with a fluorescence correlation spectroscopy readout. This latter approach furnished a high hit rate (*ca*. 4% at a fragment screening concentration of 0.25 mM) and protein NMR was used as an orthogonal assay method for hit validation. Multiple fragment-protein complex X-ray crystal structures were then solved ahead of the start of medicinal chemistry optimisation. Whilst the fragment hits generally exhibited double-digit micromolar potency but with good ligand efficiencies (LE 0.29–0.48), one of the hits from the virtual screen exhibited sub-micromolar activity. This compound represented a very attractive starting point for rapid medicinal chemistry optimisation due to its high ligand efficiency (LE 0.42). The X-ray structural information obtained with the fragment hits has both informed and assisted the medicinal chemistry optimisation of the virtual screening hits, as well as providing additional alternative compound series. In particular, analogues of the fragment hits have revealed key target interactions enabling the optimisation of hits into leads through the overlay of information obtained from the initial fragment screening and virtual screening (VS) starting points.

By conducting a virtual screen and a fragment screen in parallel, the elements of a computationally focused set along with the fundamentally diverse fragment library are combined to maximize the likelihood of finding good hits. In addition, the information from the two sets of hits can then be combined, via crystal structures, docking, or 3D ligand overlay, to determine the optimum number, and nature, of possible interactions. In the case of the PDE10a example shown in Fig. 3, the virtual screening hit capitalized on the majority of the interactions available for that pocket and the fragments were overlaid with the hit to make sure that ALL confirmed interactions were being picked up during design of a virtual library. This information was combined with the addition of a known, highly selective PDE10a compound (from Pfizer), which was overlaid with the vHTS hit using the OpenEye software for rapid overlay of chemical structures (ROCS). The path of evolution of the vHTS hit is described in Fig. 3. It used a combination of ligand and structure-based computational methods to create and score libraries of hybrid compounds and with the synthesis of only ~30 compounds, rapidly progressed to a set of single digit nanomolar and selective compounds.

The above examples illustrate the power of fragment approaches and support the premise of performing fragment screening at the outset in conjunction with other hit finding methods. There have been several recent reports on the use of virtual screening to select a subset of fragment compounds for testing and also where both a fragment screen and a virtual screen have been conducted in parallel as we have done for PDE10a. For example Sanofi-Aventis have disclosed inhibitors of MIF tautomerase discovered from virtual and fragment screening [36,37]. Other researchers have also reported on the complementary use of fragment approaches with other hit finding and optimisation methods: Abbott, pioneers of fragment methods, often employ a synergistic combination of

HTS and fragment screening in the early hit discovery stage [38]; Workers at GSK advocate 'Reduced Complexity Screening' whereby a set of fragment compounds is assayed by biochemical screening at high concentration (*ca.* 1 mM) alongside a full deck high throughput screen [6]; AstraZeneca have adopted a process whereby high concentration screening (*ca.* 100 μM) of a set of 2000 fragments is conducted in parallel with every HTS [35].

Future

What will be the future developments in fragment-based (or assisted) drug discovery? Developments can be expected in library design (see reviews in this issue of Drug Discovery Today: Technologies) and computational methods. With regard to the latter, advances in the application of detailed computational analysis of fragment co-crystal structures can be expected. For example we have found that the *ab initio* fragment molecular orbital (FMO) method [39] can provide detailed insights into the interactions that drive binding of fragments to target proteins (see Box 1). We also expect to see an analysis of the thermodynamic signature of fragment binding being used to triage which fragments to take forward into optimisation. This follows the suggestion that 'best in class' drugs may be distinguished from 'first in class' drugs on the basis of a higher enthalpic contribution to binding, in comparison to the entropic contribution, leading to greater selectivity and fewer side effects [40].

The current major limitation of the fragment method is generally considered to be the need to obtain high resolution X-ray crystal structures of the fragment compounds in complex with the target protein to make the most of the information derived from fragments. We and others are seeking to overcome this limitation and have been undertaking fragment screens of targets that are not structure enabled. For example, AstraZeneca are applying fragment approaches to GPCRs whereby informatics-driven mining for near neighbours of the fragment hits enables identification of more potent analogues by cherry picking and subsequent testing of higher molecular weight drug-like compounds from the AZ HTS collection [35]. Novartis have coined the term 'Virtual Fragment Linking' (VFL) for a somewhat related process. In this case a fragment screen is first conducted and based on chemoinformatic analysis of the results, a subset of compounds from the full HTS library are selected for a second round of screening [41]. Good hit rate enrichments by the VFL method are reported for GPCR targets but not for less druggable targets.

It should be remembered that many known drugs are relatively small as are many natural receptor ligands and enzyme substrates. We calculate that 18% of FDA approved drugs in the DrugBank database conform to the rule of three definition of a fragment. An example of a natural ligand of small size is histamine and recently there have been notable reports of the discovery of fragment-like ligands for the H4 receptor through receptor modelling and virtual screening [42] and fragment-based design of H4 receptor ligands [43].

We are also seeking to apply fragment methods to GPCRs and have developed sophisticated *in silico* methods for generating high quality, chemistry optimized, GPCR models. These have allowed us to rapidly enhance the potency and selectivity of initial fragment hits obtained against GPCRs. For example we screened, using a functional Ca^{2+} mobilization cellular assay, a subset (1700 compounds) of our fragment collection against three histamine receptors (H1, H3 and H4) at 20 μM (Fig. 4). Over 100 primary antagonist hits ($IC_{50} > 1$ μM) were obtained and encouraging selectivity was observed for many of these fragments. *In silico* hit expansion, making use of our GPCR binding site modelling, was used to

Box 1. Fragment Molecular Orbital (FMO) method

The application of computational chemistry to guide medicinal chemistry, within a drug discovery program (e.g. one involving fragment hit data), is limited by two major factors: the accuracy of most scoring functions and the applicability of the more accurate methods (e.g. quantum mechanical (QM)), to the system size, number of systems, available computational resources and results turn-around time required to be useful. *Ab initio* QM methods, for instance, calculated with the electron correlation Møller–Plesset (MP2) method with the 6-31G** basis set, give one of the best possible descriptions of atomic and electronic interactions. It is desirable to score and analyze ligand binding interactions at this level of accuracy but this is often too expensive computationally to be applicable to a medicinal chemistry environment. The fragment molecular orbital (FMO) protocol, initially developed by Kitaura's group [39,45,46] enables QM-level examination of interactions between proteins and peptides, metal ions, DNA, and small molecules [47]. It is possible to run dozens of calculations per day, on a relatively small cluster, at the theory level to achieve the required accuracy, for example, MP2 with a 6-31G** basis set. MP2 includes the ability to observe the dispersion type interactions of aromatic and alkyl chains often seen in protein–ligand interactions and also interactions, such as halogen-pi which are difficult to capture with molecular mechanics (MM) force-fields alone [48]. The FMO methodology, implemented in GAMESS (General Atomic and Molecular Electronic Structure System) or some other QM packages such as ABINIT-MP, enables QM examination of small molecule structure–activity relationships (SAR) within a fast moving medicinal chemistry program. Initial start-points for the calculations are usually a crystal structure, but the method can then be used in a combinatorial fashion to drive expansion of fragment/lead-like hits via multiple calculations, or a small library of ligands, based around a single crystal structure. FMO calculations involve the fragmentation of the system into small manageable fragments of atoms, typically amino-acid residues and the ligand, for which the monomer and dimer energies are calculated. The pairwise interaction energies are then calculated and the total energy of the system can be obtained by summing the energies of the monomers and the pair interaction energies. For ligand–protein interactions, particularly in the context of understanding SAR for guidance of medicinal chemistry, the most important pairwise fragment interactions are those between the ligand and the protein fragments. Although solvation and entropic terms can be added, it is clear that FMO/QM calculations used in this simple manner, are optimizing primarily for enthalpy, and considerations of entropy are largely being ignored. Therefore experimental techniques that determine the enthalpic and entropic contribution of some ligands, particularly of fragment start-points, are helpful in guiding when to apply FMO and which fragments to evolve.

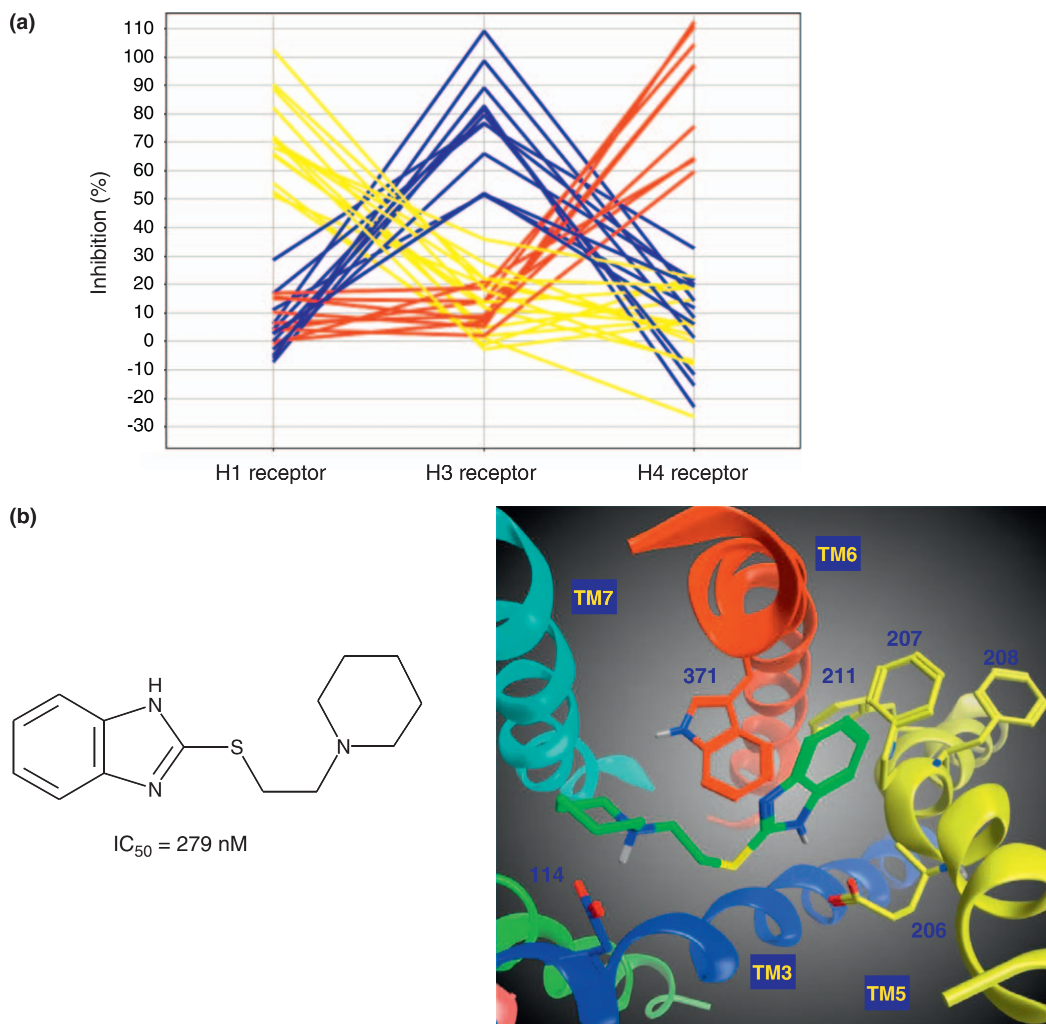

Figure 4. (a) Plot of % inhibition at 20 μM for H1, H3 and H4 receptors displaying H1 selective antagonists (yellow), H3 selective antagonists (blue) and H4 selective antagonists (red). **(b)** The structure of an H3 antagonist from the GPCR model based fragment hit expansion, and an image of the compound docked into an H3 receptor model.

find a set of compounds that would constitute the first stage in the evolution of these fragment hits. This involved the use of the target selective fragment hits in optimizing the ligand binding pockets of both the H3 and H4 receptor models. A combination of ligand and structure-based virtual screening was then used to select ~170 analogues, which were purchased. Over 70% of these analogues were active against either H3 or H4, with several found to be an order of magnitude more potent than the fragment hits upon testing (i.e. sub-micromolar).

This example illustrates that the utilisation of information derived from fragment hits is not limited by access to X-ray crystal structures if the hypothesis for hit expansion can be generated through the utilisation of high quality computational models. We expect to see many more examples of this type of approach being applied within drug discovery in the future.

Conclusions

The birth of fragment-based drug discovery (FBDD) resulted from the coupling of theoretical and computational understanding of drug discovery and experimental advancements in structural analysis and biophysical methods to detect weak ligand–protein interactions. The concepts of ligand efficiency allied to the early successes in fragment optimisation have made it easier for the drug discovery community to broadly embrace the approach. However, rather than taking a purist's view of using FBDD on its own many groups, including ourselves, are taking a fragment-assisted drug discovery (FADD) strategy whereby fragments are incorporated alongside and used to inform a comprehensive approach to hit finding and optimisation. Whilst productive outcomes can be obtained when applying fragment methods alone (in a true FBDD approach) maximum benefit is obtained when employing fragment approaches in parallel with other hit

finding methods. If a robust and sensitive high throughput assay method is developed at the outset then there is little incremental cost in performing a high concentration screen of fragments alongside every HTS campaign. During the course of assay development a virtual screen can also be conducted (based on known ligands and/or target structural information) to select additional compounds from third party vendors that can be then screened at the same time as the HTS and fragment screens. This holistic approach to hit generation gives medicinal chemistry teams the maximum amount of information ahead of commencing hit-to-lead optimisation and so provides the basis for developing the very best lead series for each target [44]. The key to this strategy is for the medicinal chemists not to be seduced by the most potent hit compounds but to make best use of *in silico* methods to assess the various hit options in terms of ligand efficiency, potential liabilities and optimal vectors for substituting the hit scaffold for enhanced target engagement. A further benefit of this approach is that the greater diversity of starting points more readily allows circumvention of competitor IP for well precedented targets and for less druggable targets this screening-wide approach increases the likelihood of obtaining suitable starting points on which to base a drug discovery program.

The use of computational methods and tools is at the heart of maximising the benefit of the information provided by fragment hits. In the past there has been a high reliance on first obtaining high resolution X-ray crystal structure data of fragments in complex with the protein target. However, computational chemists are now extending the scope of fragment methodology through the development of high quality structural models for proteins, such as GPCRs, that are currently not readily amenable to structure determination.

Thus, the use of fragments, when employed in conjunction with other hit finding methods and the very best computational methods, can assist drug discovery irrespective of the target and access to structural information. As such the use of fragments should not necessarily be considered as a separate technique but rather as a method to integrate with other approaches.

Disclosure statement

MW, OI, RJL, TH and DH are employees of Evotec which is a drug discovery services company that applies fragment methods within collaborative research projects with biotech and pharmaceutical companies.

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References

1 Chessari, G. and Woodhead, A.J. (2009) From fragment to clinical candidate – a historical perspective. *Drug Discov. Today* 14, 668–675
2 Coyne, A.G. *et al.* (2010) Drugging challenging targets using fragment-based approaches. *Curr. Opin. Chem. Biol.* 14, 299–307
3 de Kloe, G.E. *et al.* (2009) Transforming fragments into candidates: small becomes big in medicinal chemistry. *Drug Discov. Today* 14, 630–646
4 Hadjuk, P.J. and Greer, J. (2007) A decade of fragment-based drug design: strategic advances and lessons learned. *Nat. Rev. Drug Discov.* 6, 211–219
5 Law, R. *et al.* (2009) The multiple roles of computational chemistry in fragment-based drug design. *J. Comput. Aided Mol. Des.* 23, 459–473
6 Leach, A.R. *et al.* (2006) Fragment screening: an introduction. *Mol. Biosyst.* 2, 429–446
7 Congreve, M. *et al.* (2008) Recent developments in fragment-based drug discovery. *J. Med. Chem.* 51, 3661–3680
8 Gund, P. (1977) Three-dimensional pharmacophoric pattern searching. *Prog. Mol. Subcell. Biol.* 5, 117–143
9 Craig, P.N. and Ebert, H.M. (1969) Eleven years of structure retrieval using the SK&F fragment codes. *J. Chem. Doc.* 9, 141–146
10 Cramer, R.D., III *et al.* (1974) Substructural analysis. A novel approach to the problem of drug design. *J. Med. Chem.* 17, 533–535
11 Michne, W.F. (1996) Hit-to-lead chemistry: a key element in new lead generation. *Pharmaceut. News* 3, 19–21
12 Shuker, S.B. *et al.* (1996) Discovering high-affinity ligands for proteins: SAR by NMR. *Science* 274, 1531–1534
13 Goodford, P.J. (1985) A computational procedure for determining energetically favourable binding sites on biologically important macromolecules. *J. Med. Chem.* 28, 849–857
14 Mattos, C. and Ringe, D. (1996) Locating and characterizing binding sites on proteins. *Nat. Biotechnol.* 14, 595–599
15 Miranker, A. and Karplus, A. (1991) Functionality maps of binding sites: a multiple copy simultaneous search method. *Proteins* 11, 29–34
16 Verlinde, C.L.M.J. *et al.* (2009) Fragment-based cocktail crystallography by the medical structural genomics of pathogenic protozoa consortium. *Curr. Top. Med. Chem.* 9, 1678–1687
17 Nienaber, V.L. *et al.* (2000) Discovering novel ligands for macromolecules using X-ray crystallographic screening. *Nat. Biotechnol.* 18, 1105–1108
18 Blaney, J. *et al.* (2006) Fragment-based lead discovery and optimization using X-ray crystallography, computational chemistry and high-throughput organic synthesis. In *Fragment Based Approaches in Drug Discovery* (Jahnke, W. and Erlanson, D.A., eds), pp. 55–66, Wiley-VCH
19 Hartshorn, M.J. *et al.* (2005) Fragment-based lead discovery using X-ray crystallography. *J. Med. Chem.* 48, 403–413
20 Boehm, H.J. *et al.* (2000) Novel inhibitors of DNA gyrase: 3D structure based biased needle screening, hit validation by biophysical methods, and 3D guided optimisation. A promising alternative to random screening. *J. Med. Chem.* 43, 2664–2674
21 Hubbard, R.E. *et al.* (2007) The SeeDs approach: integrating fragments into drug discovery. *Curr. Top. Med. Chem.* 7, 1568–1581
22 Congreve, M. *et al.* (2003) A 'rule of three' for fragment-based lead discovery? *Drug Discov.Today* 8, 876–877
23 Hopkins, A.L. *et al.* (2004) Ligand efficiency: a useful metric for lead selection. *Drug Discov. Today* 9, 430–431
24 Hesterkamp, T. *et al.* (2007) Fragment based drug discovery using fluorescence correlation spectroscopy techniques: challenges and solutions. *Curr. Top. Med. Chem.* 7, 1582–1591
25 Card, G.L. *et al.* (2005) A family of phosphodiesterase inhibitors discovered by cocrystallography and scaffold-based drug design. *Nat. Biotechnol.* 23, 201–207
26 Hesterkamp, T. and Whittaker, M. (2008) Fragment-based activity space: smaller is better. *Curr. Opin. Chem. Biol.* 12, 260–268
27 Alex, A.A. and Flocco, M.M. (2007) Fragment-based drug discovery: what has it achieved so far? *Curr. Top. Med. Chem.* 7, 1544–1567
28 Barker, J.J. *et al.* (2009) Fragment-based identification of Hsp90 inhibitors. *ChemMedChem* 4, 963–966
29 Barker, J.J. *et al.* (2010) Discovery of a novel Hsp90 inhibitor by fragment linking. *ChemMedChem* 5, 1697–1700

30 Neumann, T. *et al*. (2007) SPR-based fragment screening: advantages and applications. *Curr. Top. Med. Chem.* 7, 1630–1642

31 Vanwetswinkel, S. *et al*. (2005) TINS, target immobilized NMR screening: an efficient and sensitive method for ligand discovery. *Chem. Biol.* 12, 207–216

32 Cancilla, M.T. *et al*. (2008) Discovery of an Aurora kinase inhibitor through site-specific dynamic combinatorial chemistry. *Bioorg. Med. Chem. Lett.* 18, 3978–3981

33 Godemann, R. *et al*. (2009) Fragment-based discovery of BACE1 inhibitors using functional assays. *Biochemistry* 48, 10743–10751

34 Madden, J. *et al*. (2010) Fragment based discovery and optimisation of BACE1 inhibitors. *Bioorg. Med. Chem. Lett.* 20, 5329–5333

35 Albert, J.S. *et al*. (2007) An integrated approach to fragment-based lead generation: philosophy, strategy and case studies from AstraZeneca's drug discovery programmes. *Curr. Top. Med. Chem.* 7, 1600–1629

36 McLean, L.R. *et al*. (2009) Discovery of covalent inhibitors for MIF tautomerase via cocrystal structures with phantom hits from virtual screening. *Bioorg. Med. Chem. Lett.* 19, 6717–6720

37 McLean, L.R. *et al*. (2010) Fragment screening of inhibitors for MIF tautomerase reveals a cryptic surface binding site. *Bioorg. Med. Chem. Lett.* 20, 1821–1824

38 Hajduk, P.J. and Greer, J. (2007) A decade of fragment-based drug design: strategic advances and lessons learned. *Nat. Rev. Drug Discov.* 6, 211–219

39 Kitaura, K. *et al*. (1999) Fragment molecular orbital method: an approximate computational method for large molecules. *Chem. Phys. Lett.* 313, 701–706

40 Freire, E. (2008) Do enthalpy and entropy distinguish first in class from best in class? *Drug Discov. Today* 13, 869–874

41 Crisman, T.J. *et al*. (2008) 'Virtual Fragment Linking': an approach to identify potent binders from low affinity fragment hits. *J. Med. Chem.* 51, 2481–2491

42 Tanrikulu, Y. *et al*. (2009) Homology model adjustment and ligand screening with a pseudoreceptor of the human histamine H4 receptor. *ChemMedChem* 4, 820–827

43 Smits, R.A. *et al*. (2008) Fragment based design of new H4 receptor–ligands with anti-inflammatory properties in vivo. *J. Med. Chem.* 51, 2457–2467

44 Barker, J. *et al*. (2008) Integrating HTS and fragment-based drug discovery. *Drug Discov. World* Summer, 67–73

45 Fukuzawa, K. *et al*. (2005) Ab initio quantum mechanical study of the binding energies of human estrogen receptor alpha with its ligands: an application of fragment molecular orbital method. *J. Comp. Chem.* 26, 1–10

46 Fedorov, D.G. and Kitaura, K. (2007) Extending the power of quantum chemistry to large systems with the fragment molecular orbital method. *J. Phys. Chem. A* 111, 6904–6914

47 Ito, M. *et al*. (2008) Ab initio fragment molecular orbital study of molecular interactions in liganded retinoid X receptor: specification of residues associated with ligand inducible information transmission. *J. Phys. Chem. B* 112, 12081–12094

48 Imai, Y.N. *et al*. (2008) Cl–π interactions in protein–ligand complexes. *Protein Sci.* 17, 1129–1137