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The multiple roles of computational chemistry in fragment-based drug design

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Abstract Fragment-based drug discovery (FBDD) represents a change in strategy from the screening of molecules with higher molecular weights and physical properties more akin to fully drug-like compounds, to the screening of smaller, less complex molecules. This is because it has been recognised that fragment hit molecules can be efficiently grown and optimised into leads, particularly after the binding mode to the target protein has been first determined by 3D structural elucidation, e.g. by NMR or X-ray crystallography. Several studies have shown that medicinal chemistry optimisation of an already drug-like hit or lead compound can result in a final compound with too high molecular weight and lipophilicity. The evolution of a lower molecular weight fragment hit therefore represents an attractive alternative approach to optimisation as it allows better control of compound properties. Computational chemistry can play an important role both prior to a fragment screen, in producing a target focussed fragment library, and post-screening in the evolution of a drug-like molecule from a fragment hit, both with and without the available fragment-target co-complex structure. We will review many of the current developments in the area and illustrate with some recent examples from successful FBDD discovery projects that we have conducted.

Keywords Fragment-based drug design · Fragment-assisted-drug design · Fragment library · Lead optimization · Computational chemistry · HSP90 · BCL-2 · PDE10a · BACE

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Introduction

Fragment-based Drug Design (FBDD) has emerged as an important technique for many drug discovery organizations. Computational Chemistry (CC) is useful both prior to the fragment screen, and afterwards in fragment-to-lead (F2L) and subsequent lead-optimization programmes. Computational methods for selection and creation of a focussed fragment library are an important first step to increasing the probability of success (enrichment) of the fragment screen. Following the gathering of hits from a fragment screen, prioritisation of fragment hits is conducted using a combination of CC and orthogonal NMR, and X-ray crystallography. CC methods can then be employed to evolve from the fragment hits towards more drug-like, small molecules with increased potency. This can be done iteratively and, as discussed later, enables F2L even without further structure determination. In fact, using CC for hit expansion via sub-structure and similarity search methods to access analogues for screening enables fast SAR expansion, even prior to structure determination. The flow-chart in Fig. 1 gives an overview of the FBDD process and this review will illustrate some of the common themes and methods applied, with specific examples from projects that we have conducted.

The explosion in the use of FBDD approaches in recent years has been extensively covered by a number of current reviews [4, 9, 20, 22, 29, 37, 42, 53, 58, 71, 78], with many of them concerned specifically with the roles of CC within the methodology [3, 48, 65, 74, 84, 92]. There are also three monographies covering the subject [51, 54, 91]. But

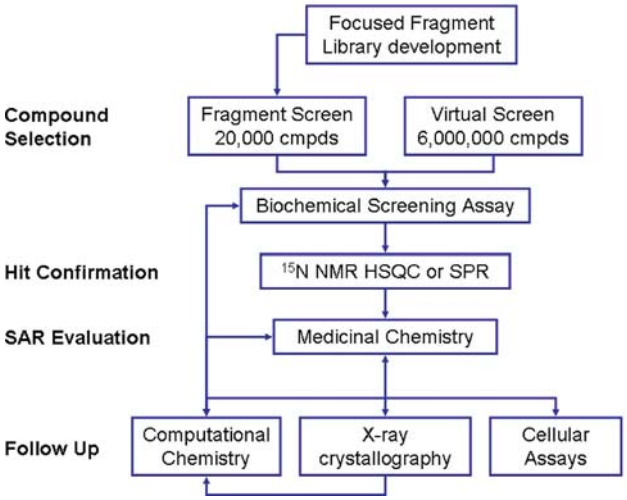

Fig. 1 This flow chart represents a generalized strategy for fragment-based drug design that incorporates computational chemistry at the beginning and follow-up stages of the process

FBDD has actually been around for longer than immediate reference searches might suggest—it was just being used as a technique, under a number of different names (e.g. needles, shapes, binding elements, or seed templates; [12], that was applied to tackling particularly challenging targets. After the seminal work on DNA gyrase by Boehm et al. [12], HIV-1 reverse transcriptase (RT) briefly became an important system for the demonstration and use of FBDD due to the need to stay ahead of the mutagenic capacity of the virus by finding new chemotypes and new allosteric binding sites to inhibit the enzyme. To follow Nevirapine, delavirdine and efavirenz, FBDD techniques were applied by many institutions to discover new non-nucleoside RT inhibitors. The work demonstrated the power of closely integrating screening, crystallography, chemistry and molecular modelling in a multidisciplinary effort [26, 52]. The fragment cocktail crystallography work at the University of Groningen is perhaps one of the first examples of a foray into FBDD [83]. This involved the use of X-ray crystallography whereby cocktails of fragments were soaked into preformed crystals. However, the use of crystallography to screen for active fragments limits the size of library that can be screened (albeit having the advantage of supplying immediate structural information for hits that are found [66]) and may miss many potential hits due to the dual requirement to satisfy the conditions for weak binding and crystal formation. In general then, other sensitive screening methods such as NMR [90], Surface Plasmon Resonance (SPR; [67], and higher-throughput, high-sensitivity biochemical screening methods such as fluorescence correlation spectroscopy (FCS; [43] are used to screen for bound fragments. FCS is one of a few methods with the sensitivity to screen for fragments, and the throughput to screen a reasonably sized library of >10,000 compounds. The combination of biochemical screening and NMR provides a particularly effective approach for discovering fragment hits [7, 42]. There has also been interest in the use of mass-spectrometry or isothermal titration calorimetry in FBDD screening [44, 82]. All of these techniques can be used as an orthogonal assay to confirm the hits from one of the screening methods. X-ray crystallography is usually a vital part of the F2L and lead-optimization process following the discovery of a fragment hit [55]. Companies like Abbott, Astex, Evotec, Plexxikon, SGX Pharmaceuticals and Sunesis have recently been pushing the boundaries of FBDD usage, particularly for more challenging targets.

An appealing advantage of FBDD strategies is that even in crowded intellectual property (IP) space for a particular target, new areas of useful chemical landscape can be found. This stands to reason since a considerably smaller number of fragment molecules are required to span chemical diversity space than is the case for lead-like molecules [31] therefore undertaking screening with a fragment set is more likely to find new chemotypes that will bind to a target. The additional, and sometimes most persuasive, benefit of FBDD methods is not only that they give an additional, and potentially more effective, approach to tackle a target, but that they can find chemotypes that bind to difficult targets, such as BACE (see below) which is not addressable via standard high-throughput screens (HTS). A target containing multiple small allosteric regulator sites, perhaps all of which are not well characterised and questionably druggable, again would lend itself very well to a FBDD approach. A fragment screen can in fact be used as effective measure of the druggability of a novel target class [35, 60].

When structural information from X-ray crystallography cannot be obtained for the target-fragment-hits co-complexes, CC can aid in several ways in the progression of the F2L process. A ligand-based CC follow-up involving a sub-structure shape and pharmacophore search (2D or 3D) of commercially available lead-like analogues can rapidly provide a set of prioritized compounds for a follow-up HTS assay. This second iteration has the potential to be a shortcut to lead-like compounds with good ligand efficiency from a standard HTS (fast SAR expansion). This sub-structure search approach can also be used to combine SAR data in cases where a fragment-based screen and a regular HTS are run in concert. This type of iteration has been used to good effect at Vertex using the NMR SHAPES method and following up with a 500 compound second screen this approach has led to the discovery of several micromolar hits [60]. Such searches can be conducted using the ROCS [40, 72] and SHAPS (based on CDL-libraries; [80]) search tools and we have routinely obtained several further experimental hits by this method—as discussed in more

detail later. If an intermediate case exists whereby a protein-fragment-hit co-complex is seemingly not solvable by X-ray crystallography, yet a protein structure is available containing a different lead-like/drug molecule, ligand-based shape fitting can be applied to assess if part of the bound drug molecule is similar to any/all of the fragments. In this case a docking or shape-based vHTS using the bound lead-like compound will likely already have been run, but the results of this screen can be rescored with the knowledge of the fragment hit structure. (The assumption here, of course, is that the bound lead and the fragments share part of the same binding site).

Often high molecular weight lead series molecules can become irretrievably hydrophobic and aggregate [52]. Many lead series compounds have failed due to lipophilicity issues and fragment-based approaches offer a way to avoid this dead-end for bioavailability. Assessment of ligand-lipophilicity efficiency (LLE) from the point of a fragment hit, and beyond, and scoring optimized sets based on a measurement of cLogP per heavy-atom, can help to drive the development of series that will avoid failure due to hydrophobicity [59]. It has been shown that an important path to optimization of a lead compound is an assessment of the normalized efficiency of the potency of a series of hit compounds [57]. Analysis of ligand efficiency (LE), has become an important factor in driving the F2L optimisation process. LE is defined as the binding potency of the ligand per non-hydrogen atom (NHA; [45], expressed as $LE = -\Delta G/NHA$ but often a LE approximation (LE_{ap}) is made through the use of IC_{50} data rather than K_d or K_i data (e.g. $LE_{ap} = -RT\ Ln(IC_{50})/NHA$). Even though fragment molecules bind with lower affinity than lead-like molecules they often bind with very good ligand efficiency—that is, even though they have fewer interactions with the protein, the interactions that they engage in are close to optimal for that segment of a binding site [38, 71]. The consequence of this is that building out from the bound fragment hit via rational design gives a higher likelihood of obtaining a sub-micromolar activity lead with good pharmaceutical properties, and focussing on LE has helped to successfully steer many FBDD projects [17, 36]. The other logical conclusion is that one can work backwards from a highly optimized lead compound to determine the minimal binding element and that prediction maps can be constructed that enable precise assessment of the lead optimization process [36]. This principle underlies the construction of focussed fragment libraries.

Construction of a fragment library

There are several different considerations to make during the construction of a fragment library—mostly depending on whether a concerted effort is being made to produce a library thought to be more specific for a particular target or target family (a focussed fragment library) or whether the library will be required to screen multiple targets. It is likely though that the underlying principles of what is required from the library, in terms of chemical descriptors and diversity, will be similar in either case. That is, the library must be representative of a wide expanse of chemical space [63], and satisfy certain physicochemical and druglikeness restraints [77]. Even though in certain cases, relatively larger, more lead-like molecules have been included in fragment screens, some consideration needs to be given to what actually constitutes a "fragment" and how this differs from the descriptions for lead-like and drug-like molecules. To this end a "rule of three" has been established (comparing to the Lipinski "rule of five" for druglikeness). The "rule of three" states that the molecular weight (MW) be <300 Da, the CLogP be $\leq$3, the number of H-bond donors be $\leq$3, with the additional limit that the number of rotatable bonds be $\leq$3 and that the polar surface area is restricted to $\leq$60 Å^2, can also be useful for a fragment library [21]. Several variations on these filters have been seen for different published fragment libraries [7]. In particular, in generating our fragment library we allowed the MW to rise to 350 Da for certain chemotypes, the number of rotatable bonds to be up to 5, and added the restrictions that the number of H-bond acceptors needed to be $\leq$4, and the mean polar surface area needed to be $\leq$70 Å^2. One of the current Evotec fragment screening libraries, constructed within the guidelines of these restrictions, is $\sim$20,000 compounds in size. As stated earlier, one major benefit of a fragment library over a standard HTS library is the ability to span chemical space with a smaller set of compounds and the fact that the likelihood of finding a binding event reduces dramatically with added complexity of the ligand [38]. Having said that, due to the binding orientation sensitivity of fragments, the likelihood is that the diversity grid is finer in fragment space than it is in lead-like space, but the size of the fragment library will be generally restricted by the throughput of the screening technique. It is likely that the fragment library will be used to screen multiple targets—many of which will be unknown at the point when the library is made. Computational methods can be used in the construction of a fragment library, either when deriving part of a focussed fragment library for a specific target, or when determining the chemical diversity and molecular attributes for a general fragment library. This can be done by both filtering currently available commercial databases and also proposing new compounds to synthesize that would appear to occupy an area of chemical space not covered by the available compounds and, perhaps, which are specific to a property required for a particular target.

The first thing that is straightforward to apply computationally are physical property and sub-structure filters, such as the "rule of three" and others mentioned earlier. An initial screen of a database of 2.2 million compounds yielded 68,000 "rule of three" compliant molecules (including the additional polar surface area and rotatable bond filters). Diversity algorithms can then be applied to assure a representative sampling of chemical space [79] together with detailed inspection of potential fragment library compounds by medicinal chemists.

Synthetic building blocks for drugs-like compounds would constitute ideal fragments but often are very reactive in their purchasable form. But the reactive species (e.g. an acid chloride) can be changed into something more amenable to screening (e.g. an amide; [76]. Fragment molecules for high throughput crystallography can benefit from the presence of bromine atoms; both due to its unique dispersion signal and the possibility of hit follow up with Suzuki cross coupling protocols [11]. Alternative approaches involve proposing a set of fragments (and their parent building blocks) based on the deconstruction of a set of larger bioactive compounds. This can be used to create a focussed fragment library for a particular target where known inhibitors are already available. The Retrosynthetic Combinatorial Analysis Procedure (RECAP; [61] and SHAPES [30] methods enable this process to produce a focussed fragment set. Of course if it then becomes suggestive to link compounds together, this can both be synthetically very difficult as well as requiring a linker which may negatively impact on the ligand efficiency that the process was aiming to maximize. Fragment deconstruction has not always proven to be the most fruitful pathway for a FBDD approach [6] even if fragment screening and expansion is proving to be generally successful.

A focussed fragment library can be produced either from a diverse library or produced separately and added to a diverse library later. Many of the tools used in virtual screening can be applied in fragment-screening, at this stage, to focus a library. For instance, Boehm et al. [12] used molecular docking in an in silico screening approach to identify a target focused set of 3,000 molecules from an initial set of 350 K compounds and researchers at Astex use a modified form of GOLD docking software to select focused sets of fragment molecules from a large database of commercially available compounds [39]. A pharmacophore search can also be used to identify a focussed set of fragments [10]. Fragments will be composed of drug-like chemical matter that will then be further filtered down to optimize physicochemical properties such as solubility, permeability (ClogP), molecular weight, and elimination of toxic and reactive groups.

Evotec has two separate fragment libraries which were constructed independently and on the basis of different criteria. One has been built up for fragment screening by high concentration biochemical assays whilst the other was built up for fragment screening by NMR. Together these two collections stand at 30,000 diverse fragments. The techniques for fragment screening by NMR and high concentration biochemical assay are complementary. The process that was followed for the construction of the library for a biochemical fragment screening is summarized in Fig. 2. The process of building this library, as well as the similar process of building the NMR screening library, is reported in more detail by Brewer and co-workers [14] who describe many of the considerations and computational methods mentioned here. We have found that use of these two fragment libraries has favourably shifted lead discovery activities at Evotec towards lower Mw starting points, where chemical optimisation is faster and less resource consuming especially when advantage is taken of structural insights gained through detailed NMR analysis and/or X-ray crystallography of fragments in complex with target proteins. We have further adopted an approach whereby the diverse fragment collections are augmented by focussed, target-oriented sub-sets that are procured and screened

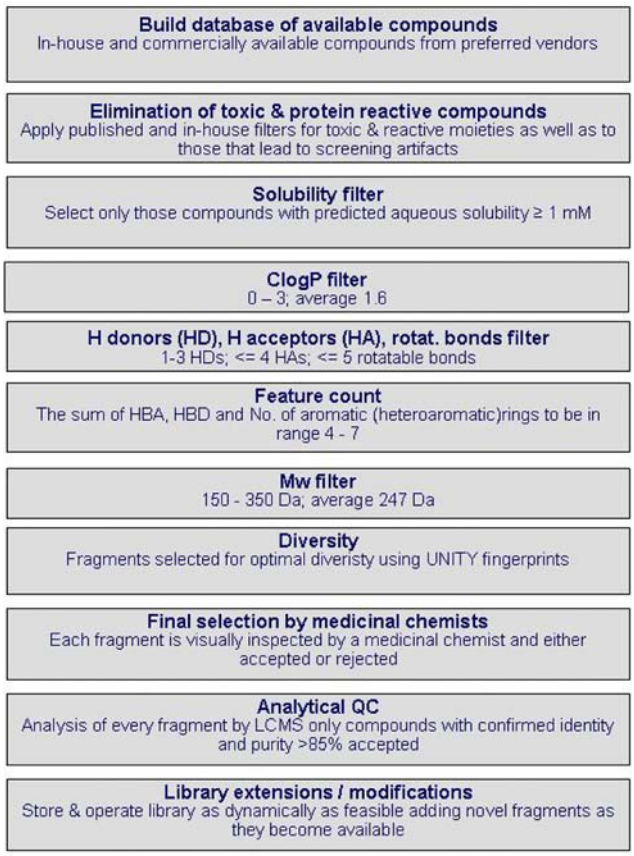

Fig. 2 Construction of a biochemical fragment screening library (after [14])

back-to-back with the diversity sets. We feel this combines the best of two screening worlds.

Optimization and expansion of fragment hits

FBDD has been growing in popularity over recent years and there are now many examples of successful FBDD projects. At least 17 clinical candidates have been produced by fragment-based discovery methods (Table 1). This list includes a Bcl-x_L targeting inhibitor, designed from a fragment start point, and representing the first non-natural drug candidate to target the protein–protein interaction of a cancer pathway [70]. An excellent review of 12 such FBDD investigations has been recently published [22]. CC has had an important role to play in expansion of fragment hits, using structural data obtained by X-ray crystallography of the co-complexes. Substructure searching and shape and pharmacophore searches can devise new and expanded compounds for a follow up screen. Software, such as LUDI [13] and HOOK [28] exists to help to automatically suggest how to grow fragments into a protein binding pocket. In a way, these methods were developed before the experimental techniques were really capable of taking advantage of them, and only now is that becoming possible. Methods such as "Virtual Fragment Linking" (VFL) exist to link positioned fragments together [23] and it is also possible to do this using combi-chem style generators within packages such as MOE. The use of the SeeDs and SHAPES methods as described at Vernalis and Vertex, respectively, is essentially underpinned by the idea of defragmenting drugs to make fragment libraries and, where possible, later linking fragments together during optimization [49]. Defragmentation of lead compounds to derive LE type information, and perhaps design focussed fragment libraries or guide further lead optimization, underpins the FragFCA method [62]. Defragmentation of a series of HTS hits is performed as SAR type data is extracted from a subsequent fragment screen. With consideration of potencies from both the HTS and fragment screen, an idea of which parts of the lead compounds are providing the most important interactions, is provided. The method of fragment screening has even been successfully tested on a number of GPCR targets [62].

Through the experience of multiple targets, we have observed that success in following up fragment hits, both in terms of CC and structural determination via X-ray crystallography will depend very much on the flexibility of the protein binding site. This, to some extent is true for any rational drug design regime but for FBDD it becomes a starker issue. A large flexible binding site means that the fragment can attain multiple orientations and in fact this orientation can change as the molecule is expanded into the remainder of the binding site, along with the structure of the binding site itself. Although we do not consider computational docking of fragments to be wholly reliable, we have had several docking results for fragment hits, for instance for Thrombin-like serine proteases and metalloenzymes, that predicted the position and orientation of multiple fragment hits with less than 1 Å deviation from the subsequently solved crystal structure. A more in depth discussion of fragment docking is provided in a publication

Table 1 Clinical candidates produced by fragment-based drug design protocols (after [22])

Drug candidate	Target	Indication	Phase	Company	Reference
ABT-263	Bcl2/Bcl-xl	Lung cancer/leukemia	I/IIa	Abbott/Genentech	[70]
ABT-869	VEGF & PDGFR	Cancer	II	Abbott	[25]
AT-9283	Aurora kinase	Cancer	I/IIa	Astex	[46]
LY-517717	Fxa	Blood clotting	II	Lilly/Protherics	[1]
NVP-AUY-922	Hsp90	Cancer	II	Vernalis/Novartis	[15]
PLX-204	PPAR	Diabetes	II	Plexxikon	[5]
SGX-523	cMet Y-kinase	Cancer	I	SGX	[33]
SGX-393	Bcr-AblT315I	Myeloid leukemia	I	SGX	[68]
ABT-518	MMP-2 & 9	Cancer	I	Abbott	[85]
AT-7519	CDK1, CDK2	Cancer	I	Astex	[88]
AT-13387	Hsp90	Cancer	I	Astex	[55]
IC-776	LFA-1	Psoriasis	I	Lilly/ICOS	–
PLX-4032	B-raf	Cancer	I	Plexxikon/Roche	[73]
PLX-5568	Kinase inhibitor	Pain/polycystic kidney disease	I	Plexxikon	–
SNS-314	Aurora kinase	Cancer	I	Sunesis	[69]
LP-261	Tubulin	Cancer	I	Locus	[64]
DG051	LFA4H	Myocardial infarction	I/II	deCODE	[41]

from the UCSF group [6]. Generally though, solving the structure of the protein-fragment co-complex is considered to be a fundamental step in the F2L optimization process. Below are several examples taken from our studies where fragment screening, aided by CC, has provided promising lead compounds.

HSP90

Heat shock protein 90 (Hsp90) is an ATP-dependent molecular chaperone that aids in the conformational maturation of numerous client proteins implicated in diverse cellular functions [56]. Hsp90 has been shown to play a key role in the stress response and protection of cells against the deleterious effects of mutation. Many of the Hsp90 client proteins are over-expressed and/or mutated in cancer (e.g. ERBB2, CDK4, C-Raf, B-Raf, c-Met, H-tert; [89], and inhibition of Hsp90 can bring about apoptosis of cancer cells. The functional protein is made up of three different domains. The N-terminal ATP binding domain is a particularly good target for structural biology approaches, including protein crystallography and for subsequent computationally guided structure-based drug design [27]. We have applied a FBDD approach to this target, as have a couple of other groups [16, 50, 81], in order to yield different chemical matter than that already identified [15, 18]. A biochemical screen against Hsp90 identified multiple weak-binding fragments. The most promising of these were submitted to crystal trials and a number of crystal structures showing fragment binding were obtained. Multiple conformations of the protein were observed, classified as open, closed and helical based on the conformation of a flexible loop that controls access to the ATP site and to a deeper, hydrophobic pocket only accessible in the helical form (Fig. 3). The majority of fragments were found to bind in the ATP site (Fig. 4a), but some were observed to bind in the helical pocket, forming no interactions with the ATP site. A further crystal structure was obtained which included this fragment bound in the helical pocket, and an additional fragment bound in the ATP site. It became apparent then that building/evolving out from the ATP-site fragments, into the helical pocket and, alternatively, the linking of the two adjacent fragment ligands, were both viable strategies that could yield new classes of Hsp90 binders.

In order to rapidly expand the known SAR around these compounds, substructure searches were performed against an in-silico library of 3.8 million commercially-available compounds to identify analogues for purchase. Knowledge of the fragment binding modes drawn from the crystal structures ensured that the substructure queries could be designed to select compounds that maintained

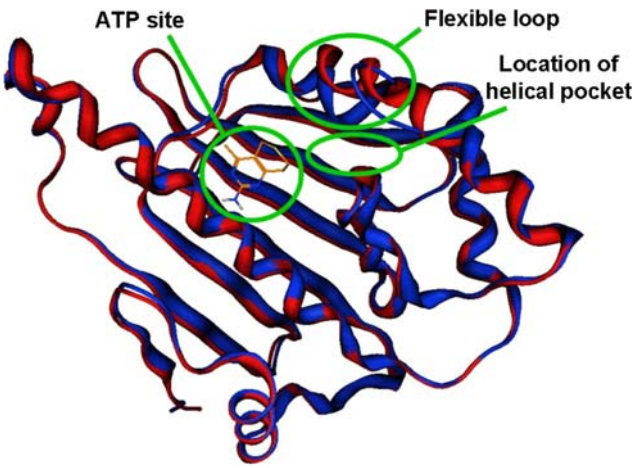

Fig. 3 Two forms of the Hsp90 protein structure are shown: Open (*blue*) and helical (*red*). The change in conformation from open to helical enables access to the helical pocket. Computational chemistry allowed for descriptions of how this pocket could be exploited for expansion and optimization of fragment hits

the key binding interactions. These hits were further filtered based on Lipinski rules and synthetic accessibility. The remaining hits were docked into two crystal structures of Hsp90, one in the open conformation and one in the helical conformation. Docking was performed with GOLD and, for analogues of fragments observed in the ATP site, a small scoring penalty was applied to poses that did not form a hydrogen bond with the important Asp93 residue. Docked poses were inspected visually to identify compounds that fit well in the pocket and maintained the key interactions of the original fragments, Asp93 and the conserved waters (Fig. 4a). Compounds that accessed the helical pocket, or provided an appropriate vector to access the helical pocket, were particularly favoured. Selected compounds were purchased for biochemical evaluation, leading to the discovery of new analogues that showed an increase in potency and were more amenable to further development than the original fragment hits (Fig. 4b).

The crystal structure including two bound fragments provided an opportunity to link these fragments in order to increase potency. Linking strategies were produced using CHARLIE, a part of the RACHEL de novo design suite in the Sybyl package, and QM calculations were performed to derive low energy conformations for the designed small molecules. Although not always applicable, fragment linking is a method that can be successful in a small number of cases [24]. Linker candidates were filtered based on the strain energy of the linked compound, the degree to which the linker distorted the original fragment binding mode and synthetic feasibility. Top-ranked candidates were synthesised and tested, resulting in the discovery of a

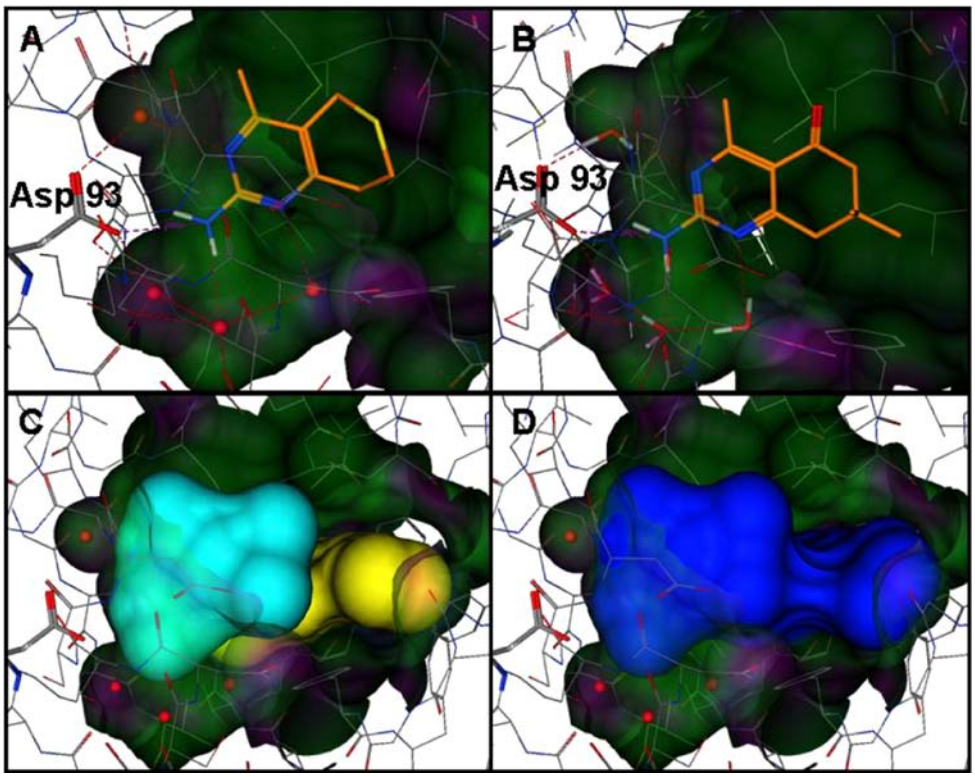

Fig. 4 **a** A fragment hit bound in the ATP site of Hsp90, showing the key interactions with conserved water molecules and Asp 93. **b** Docked pose of fragment analogue. Activity increased to <2 μM; the original hit was measured at 15 μM. (**c** and **d**) Shows two separate fragments (**c**) with activities ca. 1 mM were linked (**d**) to form a compound with 1.5 μM activity

linked compound that showed a 1,000-fold increase in activity over the original fragments (Figs. 4c, d).

Further development of the hit compounds was performed in an iterative manner. Crystal structures and docked poses of hit compounds were inspected to identify substitution points that could be used to target additional interactions. An in-house MOE extension that placed a variety of probes—hydrophobic, positive polar and negative polar—at grid points around the binding site and calculated interaction energies between the probe and protein was used to facilitate this process (Fig. 5). Synthetically feasible analogues were proposed based on the strategies derived from this analysis, which were docked into both the open and closed forms of Hsp90. The docked poses were inspected visually to assess the fit of the compounds in the pocket and prioritised for synthesis accordingly.

Results from screening this next set of compounds were used to refine the selection procedure. As the quantity of activity data and the number of available crystal structures increased, binding mode descriptors were calculated using MOE and SILVER to assess the quality of docked poses of new compounds based on the presence or absence of key interactions, and the deviation of the core scaffold from it's crystallographically observed position. Further medicinal chemistry expansion yielded a highly active compound that was later shown to be active in a cellular assay. Further details on this program can be found in a recently published paper [8], and the co-crystal structure can be found at the

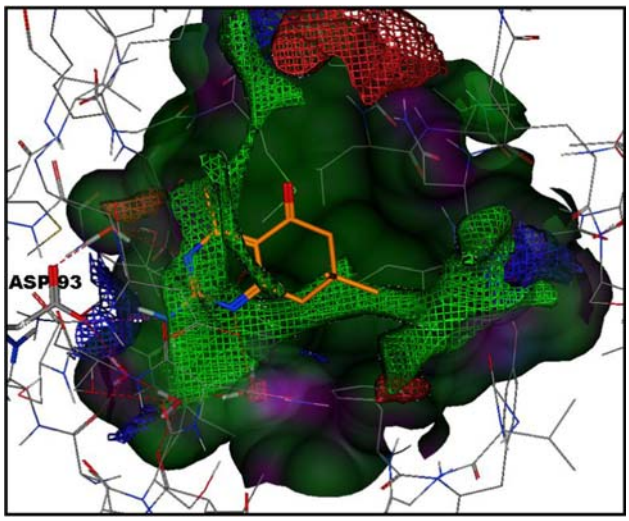

Fig. 5 Interaction energy plot for Hsp90 binding pocket shows favourable locations for hydrophobic (*green*), negative polar (*red*) and positive polar (*blue*) probes

PDB with accession code 3FT5. Figure 6 gives an overview of the processes involved for one particular fragment expansion.

PDE10a

Phosphodiesterase 10a (PDE10a) is another example of a target where we have had recent success in identifying a

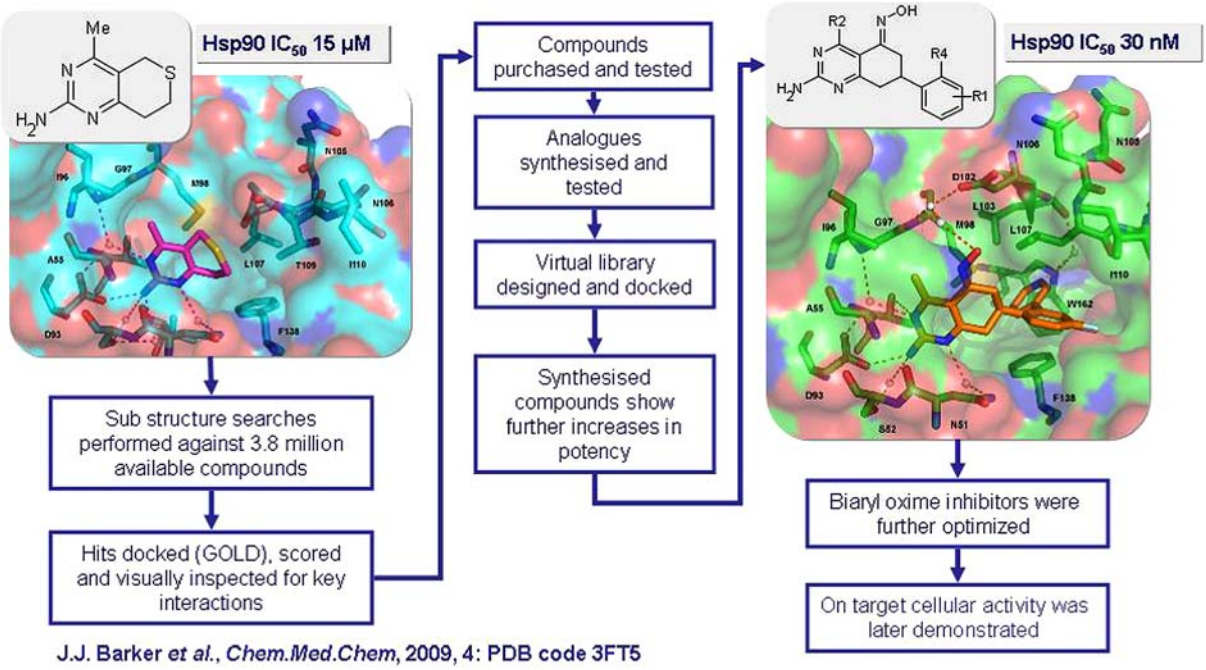

Fig. 6 This diagram indicates the multiple roles of computational chemistry, in combination with medicinal chemistry, in the expansion of a fragment hit to a potent lead-like compound that inhibits hsp90

potent lead compound from a FBDD program, heavily reliant on computational chemistry at both ends of the process. Members of the PDE superfamily differ in their tissue distributions, substrate and inhibitor specificities and regulatory mechanisms. They are divided into 11 families based on sequence homology within the catalytic domain. Of the 11 PDE families, PDE10a has the most restricted distribution. Phosphodiesterase 10a is expressed in the medium spiny projection neurons (MSNs) of the striatum; the principal input site of the basal ganglia. Dysfunction in the basal ganglia circuit has been implicated in various central nervous system (CNS) disorders, including Parkinson's disease, Huntington's disease, schizophrenia, obsessive–compulsive disorder and addiction. Available preclinical evidence demonstrates a role for PDE10a inhibitors in treating schizophrenia: PDE10a knock out mice show reduced spontaneous locomotor activity. Pfizer have recently disclosed a PDE10a inhibitor which is reported to be in Phase II trials for Schizophrenia [93]. Interestingly, PDE10a is also expressed in pancreatic islet cells and inhibitors have been shown to be insulin secretagogues. Pfizer (WO200512051) showed that PDE10a knock out mice fed a high-fat diet are resistant to developing obesity and hyperinsulinemia, compared to wild-type controls.

The strategy that we employed for the FBDD of PDE10a inhibitors involved the biochemical screening of our 20,000 fragment library supplemented by c.a. 200 additional fragments and lead-like compounds selected from an analysis of known PDE inhibitors. More than 250 fragments hits with PDE10a inhibition activity were identified from the primary screen. This hit set was reduced to 93 fragments for further progression based on interpretation of the concentration-response data, ligand efficiency, and medicinal chemistry knowledge for this specific target, and all bound with less than 1 mM potency. These were clustered into more than 30 structurally diverse chemical series. Ten co-crystal structures of fragments in complex with PDE10a were then obtained that enabled optimization of the fragments into lead-like molecules using CC (Fig. 7a). The modelling that followed was informed not only by the binding modes of fragments determined by X-ray crystallography but also by analysing the binding characteristics of known ligands for which crystal structures were available, such as 2O8H [19] and 2OUR (Fig. 7b; [86]). Based on this we were able to define important binding pocket shape and pharmacophore features that could be exploited to effectively expand and optimize the fragment hits. In parallel with the fragment screen a virtual screen was run that involved docking of 140,000 molecules, using GOLD, making use of structures 2O8H [19] and 2OUR (Fig. 7b; [86]). The top 3,600 compounds were refined using an MM-PBSA calculation using Amber8 (with GB solvation) to better rank the compounds. The chemical series obtained had novelty with respect to known PDE10a inhibitors, high ligand efficiency, and gave access to promising vectors for scaffold elaboration during optimisation. This structure-based virtual screen, and experimental testing by use of the

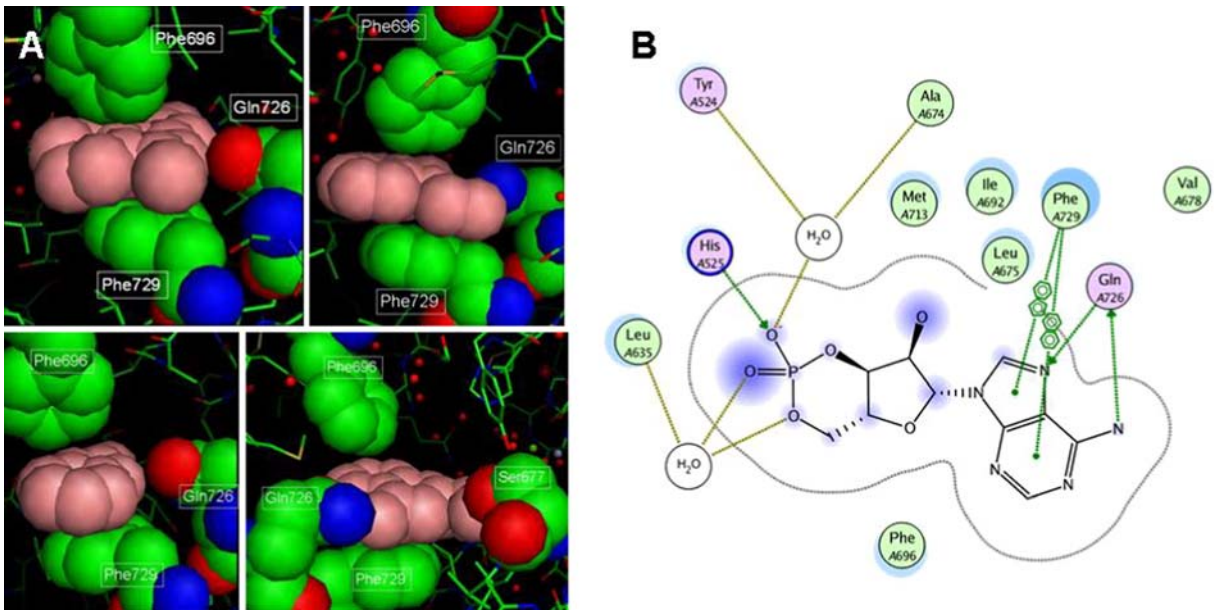

Fig. 7 This figure shows **a** co-crystal structures of fragment hits in the active site of PDE10a and **b** a diagram of the important interactions seen for the PDE10a with cAMP in the 2OUR structure [86]

same assay principle as used for the fragment screen, gave rise to a 170 nM active inhibitor of PDE10a, for which a crystal structure has been solved (Fig. 8). The combination of the information from this structure and that from the multiple X-ray crystal structures of the fragment hits is informing the application of CC to the subsequent successful medicinal chemistry optimisation process. We have found that this fragment-assisted-drug-design (FADD) approach to be a highly effective way of integrating CC and fragment methods.

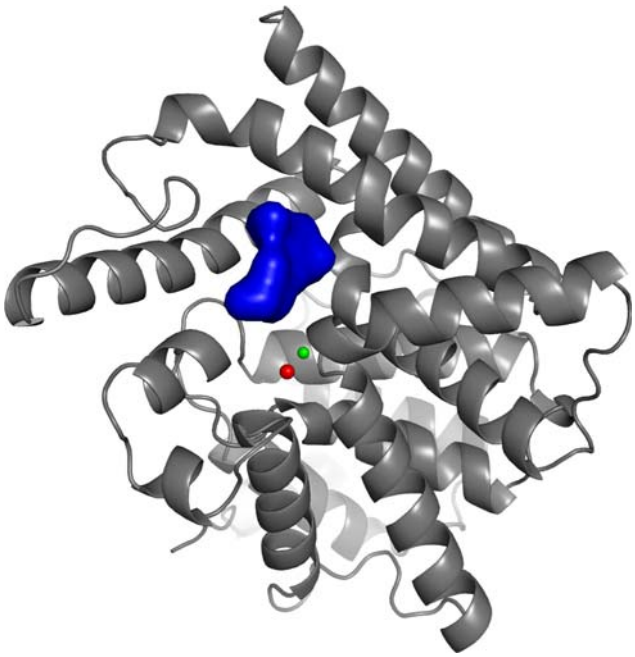

Fig. 8 *Ribbon* representation of the catalytic domain structure of human PDE10a with bound ligand (IC_{50} = 170 nM) shown as a *blue* surface. Magnesium and zinc atoms are shown as *red* and *green spheres*, respectively

BCL-2

Bcl-2, Bcl-x_L and Mcl-1 are anti-apoptotic proteins found at increased levels in many haematological malignancies and solid tumours [93]. Over-expression of this family of proteins, frequently in concert with other genetic lesions, shifts the normal apoptotic balance towards survival, resistance to chemotherapy and hence tumour growth. Several classes of Bcl-2 family inhibitors have been reported and both pan-Bcl-2 and target selective Bcl-2 inhibitors may have clinical utility (Fig. 9). The ABT-737 series [16] of Bcl-2/x_L inhibitors has provided preclinical target validation for cancer treatment and a member of this series, ABT-263 has recently been advanced into clinical trials. Using FBDD and SBDD (strategy as outlined in Fig. 1) we discovered by biochemical fragment and virtual screening, novel inhibitors of Bcl-2, Bcl-x_L and Mcl-1. This has afforded to date several series of multiple active drug-like Bcl-2 family inhibitors. A primary fragment screen was performed with our diverse 20,000 member fragment collection using confocal fluorescence-based equilibrium binding assays. The median percentage inhibition (% INH) values of the Bcl-2 and Bcl-x_L (Fig. 10a) and Bcl-2 and Mcl-1 (Fig. 10b) screens were correlated to highlight the selective and non-selective fragment hits and

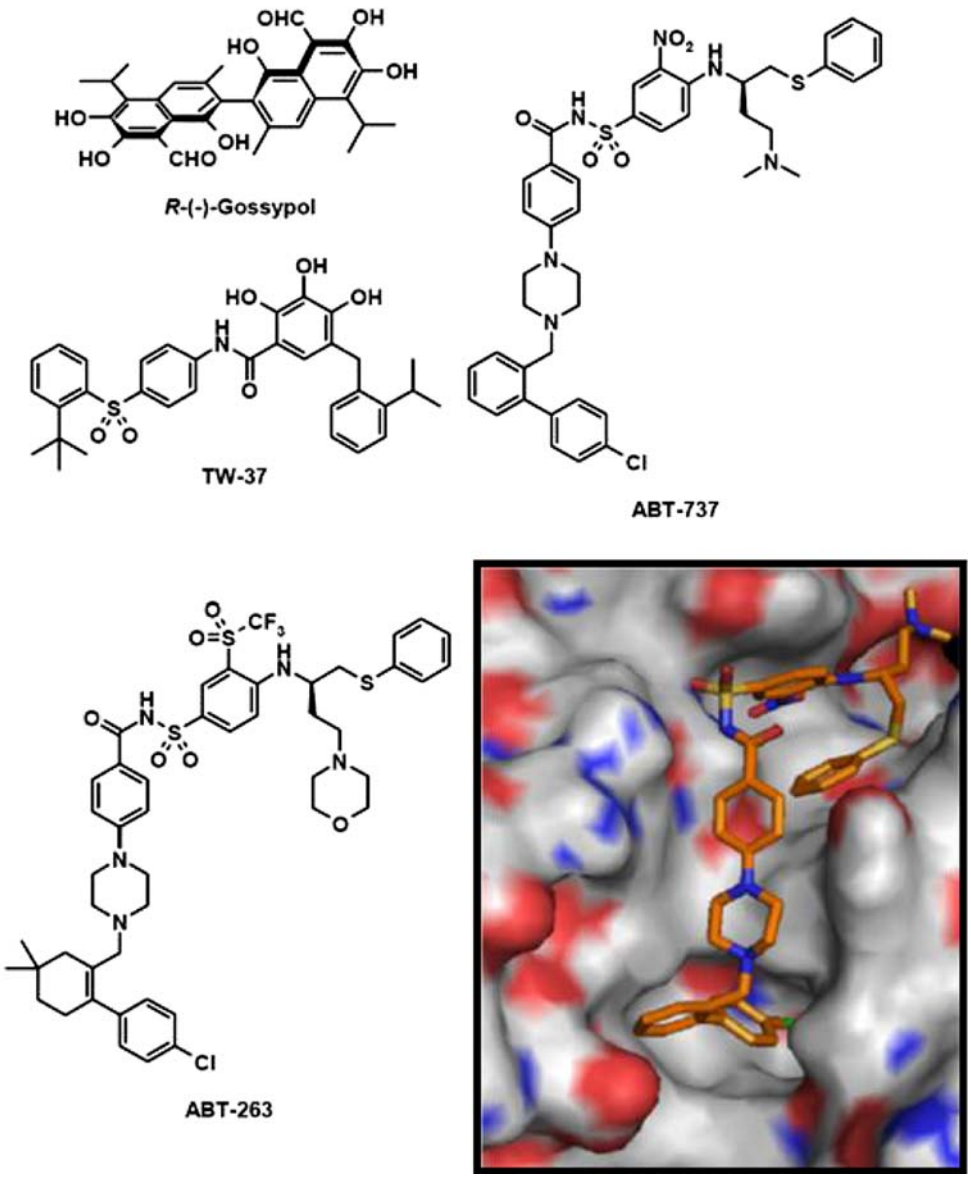

Fig. 9 **a** A series of known BCL-2 family inhibitors. **b** Shows the ABT-737 NMR-based structural model derived by the Abbott group which was utilised to define the important pharmacophore points

dual Bcl-2 and Bcl-x_L fragments were prioritised for further investigation. We confirmed target binding by protein observed NMR and this led to multiple fragment hits with plausible binding hypotheses and reasonable ligand efficiencies. In parallel with the fragment screen a docking based virtual screen was conducted to select a set of compounds for testing from our HTS screening set of 250,000 compounds. A pharmacophore directed scoring regimen was used and based on the positive results of this first virtual screen together with information from the fragment screen the docking and scoring procedure was refined and the virtual screen repeated on a larger set of commercially available compounds which provided further hits. In order to fully exploit this FADD approach we established crystallographic systems to investigate the fragment binding mode and guide the subsequent lead generation by CC and further chemistry. Hits were followed up using an analogue-by-catalogue approach and the most promising hit series were advanced into medicinal chemistry. By merging the SAR of the fragment and virtual screening series we have identified compounds with sub-μM activity against Bcl-2 family members. Evaluation in cellular assay systems is ongoing.

BACE

Beta secretase-1 (BACE1) is a classic example of a seemingly unsuitable target for traditional HTS methods but one that we and others [3, 34] have found to be amenable to FBDD methods. BACE is considered an attractive therapeutic target because it catalyzes the rate limiting step

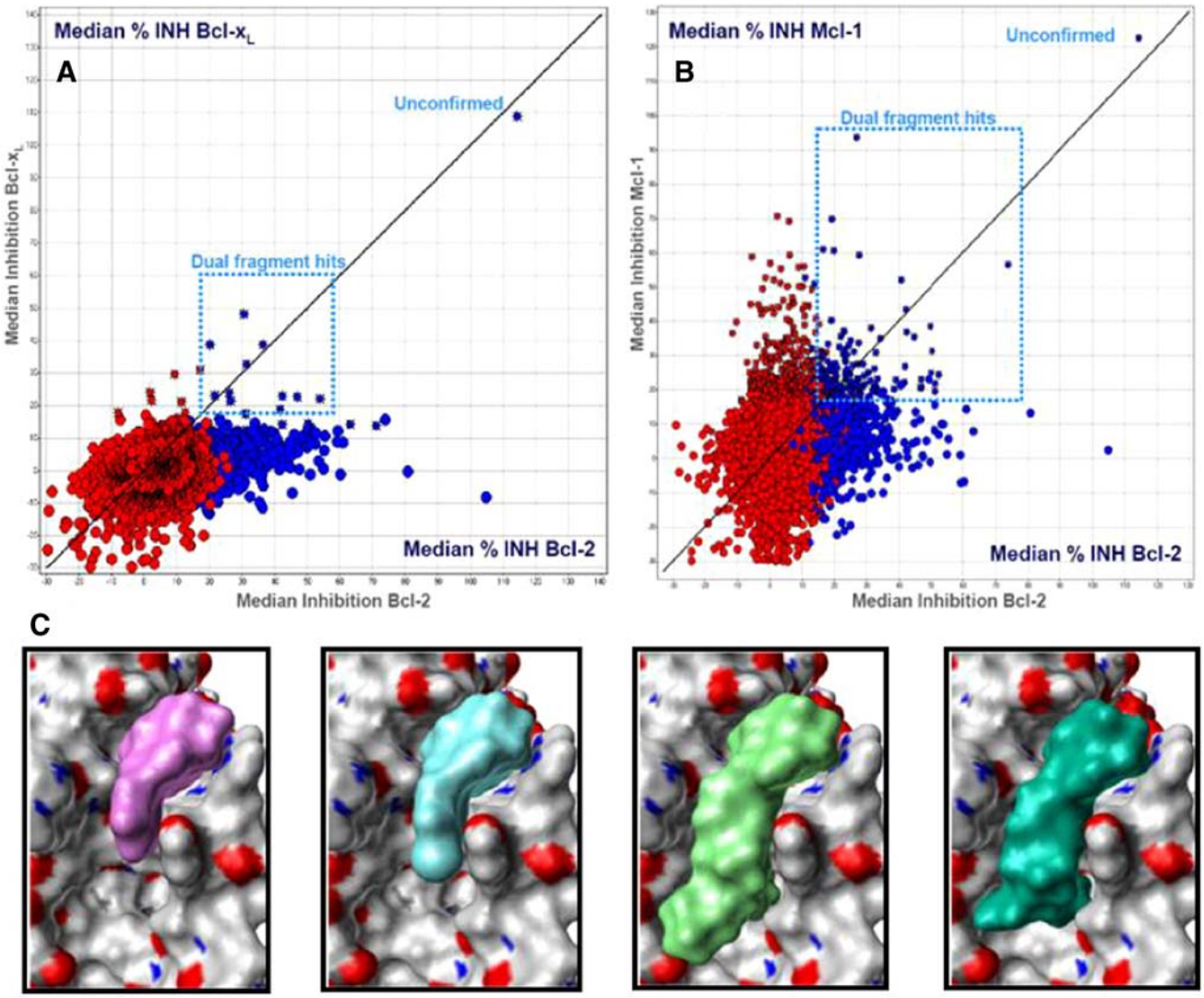

Fig. 10 **a** Median % inhibition values for Bcl-2 and Bcl-x_L and **b** for Bcl-2 and Mcl-1. For each, *Y*-axis hit: *red star*; *X*-axis hit: *blue circle*; *X*- and *Y*-axis hit: *blue star*; no hit: *red circles*, and below in (**c**) are shown proposed binding hypotheses, from docking into Bcl-2, four potent inhibitors of BCL family proteins

of amyloid plaque formation, one of the pathological hallmarks of Alzheimer's disease (AD). Many companies continue to work on β-secretase as a target for AD but the technical difficulties associated with producing potent small molecule inhibitors that are both orally bioavailable and are able to cross the blood–brain barrier (BBB) has limited the identification of development candidates. Evotec has employed a FBDD approach with the goal of identifying orally active, potent and novel BACE1 inhibitors. We believe that this approach is more likely to lead to small molecule, drug-like inhibitors with appropriate pharmacokinetic and pharmacodynamic properties. The initial screen was of 20,000 fragments using a biochemical confocal fluorescence based assay. The screen produced 60 fragment hits. For this project, rather than using NMR for the hit confirmation, an indirect surface-plasmon resonance (SPR) assay was used. This reduced the hits to 30 which were then filtered and clustered down to 7 active scaffolds. Analogues of these scaffolds were then docked and tested and this allowed the determination of several highly potent compounds. Four co-crystal structures for the fragment hits were solved (Fig. 11). Re-modelling and optimization of the bound fragments, expanding into the S1 and S3 pockets to pick up further interactions, has formed the basis for an on-going structure-guided medicinal chemistry program.

Conclusions and discussion

Herein we have provided a brief overview of some recent trends in FBDD and how the technology has benefitted from computational chemistry. In short, using CC to guide

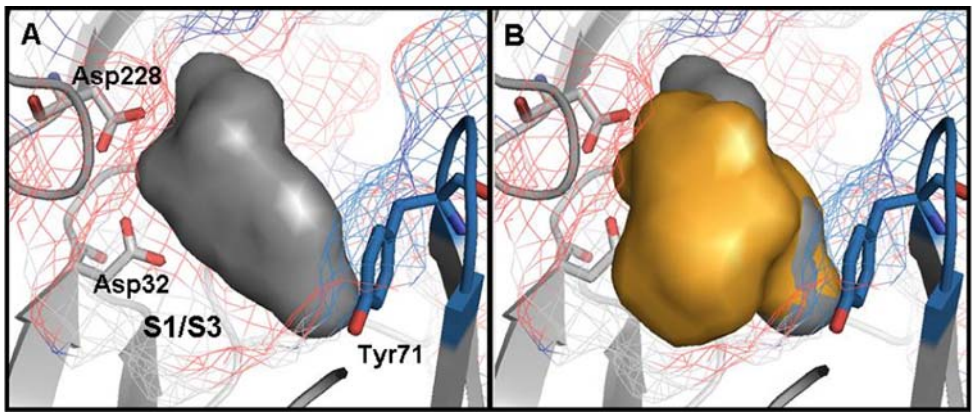

Fig. 11 Co-crystal structure of a hit fragment (*grey*) seen in the active site of BACE1 @1.8 Å resolution. Asp32 and Asp228 are the catalytic aspartates, essential for the function of BACE1. Tyr71 is the so called gatekeeper tyrosine, proposed to play a critical role in closing the flap of the protein. Tractable vectors have been identified for accessing the S1 and S3 pockets, increasing the potency 50× from an 800 μM fragment to a 16 μM inhibitor (*orange*). The co-crystal structure of this inhibitor was solved @2.1 Å resolution allowing further optimizations and potency increase guided by computational chemistry

both the construction of a fragment-screening library and the optimization and expansion of the hits can enable the design of highly efficient lead compounds for difficult-to-drug targets. FBDD is becoming a more established tool, alongside other HTS and computational methods, for rational structure-based drug design. Perhaps its power will not be completely acknowledged until a drug designed by FBDD actually makes it to market. There are three main challenges in the FBDD field. The first, which we have addressed in some detail here, is how to construct the right fragment library for screening in the first place. The second challenge is how to decide which fragments to progress, and generally this is solved by the use of orthogonal confirmatory screening techniques such as biochemical assays, NMR or SPR, followed by X-ray crystallography to determine the structure of the co-complex. The third challenge is how to expand the fragment hits, something which CC, with the aid of a structure, is well established to act as a guide. Clearly medicinal chemistry must still seek to actively drive the inclusion of drug-like properties into the lead evolution process regardless of the nature of the hit and CC and structural biology can help with this. Where protocols are less well established is for optimization of fragment hits for which no X-ray crystal structure of the co-complex can be solved. For example, AstraZeneca employ fragment screening for GPCR targets [3]. The use of substructure, shape and pharmacophore searches, along with optimized homology modelling, may enable CC to have an important impact in this area. Docking scoring functions are still not reliable enough to predict both the location and the orientation of a small bound fragment to a target protein structure [75, 87], although if the binding site is at least known, and the binding site is small, docking of the fragment may prove to correctly predict the orientation. There are many more rigorous scoring function methods, including Poisson-Boltzman based methods, free-energy perturbation calculations, and even semi-empirical/quantum methods. High-performance parallel computing needs to be more widely accessible before these complex methods will really be useful in virtual screening, but they are already capable of optimizing fragment-hit binding orientations, as well as suggesting directions for molecular expansion [32, 47]. It is perhaps this area where CC can have the most impact on the success of FBDD programs in the future.

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