

Structural Biology and Drug Discovery of Difficult Targets: The Limits of Ligandability

Sachin Surade¹ and Tom L. Blundell^{1,*}

¹Department of Biochemistry, University of Cambridge, 80, Tennis Court Road, Cambridge CB2 1GA, United Kingdom

*Correspondence: tlb20@cam.ac.uk

DOI 10.1016/j.chembiol.2011.12.013

Over the past decade, researchers in the pharmaceutical industry and academia have made retrospective analyses of successful drug campaigns in order to establish “rules” to guide the selection of new target proteins. They have identified features that are considered undesirable and some that make targets “unligandable.” This review focuses on the factors that make targets difficult: featureless binding sites, the lack of hydrogen-bond donors and acceptors, the presence of metal ions, the need for adaptive changes in conformation, and the lipophilicity of residues at the protein-ligand interface. Protein-protein interfaces of multiprotein assemblies share many of these undesirable features, although those that involve concerted binding and folding in their assembly have better defined pockets or grooves, and these can provide opportunities for identifying hits and for lead optimization. In some protein-protein interfaces conformational changes—often involving rearrangement of large side chains such as those of tyrosine, tryptophan, or arginine—are required to configure an appropriate binding site, and this may require tethering of the ligands until higher affinity is achieved. In many enzymes, larger conformational rearrangements are required to form the binding site, and these can make fragment-based approaches particularly difficult.

What Makes a Difficult Target?

Two factors are usually considered to define a “druggable” protein target (Hopkins and Groom, 2002). The first is whether its modulation has a therapeutic effect. This depends on the analysis of the cellular pathways requiring a systems biological approach and is outside the scope of this review. The second concerns a more reductionist but equally important issue: the ability of a protein target to bind a small drug-like molecule. This is often known as “ligandability” (Edfeldt et al., 2011), it is defined by the chemistry and structure of the target binding site, and it is the focus of this review.

The ligandability of a protein is to some extent operationally defined; it depends on the nature of the screening and design approach. Conventional screening depends on a library of drug-like compounds, usually selected to be consistent with Lipinski’s “rule of 5” (Lipinski et al., 2001). In order to explore chemical space efficiently the library must be chemically diverse and large, usually comprising greater than 100,000 and often more than one million compounds. Affinities of “hits” should be micromolar or better, allowing roboticized bioassays of receptor, enzyme, or whole-cell activity. An alternative is structure-guided design of a ligand with appropriate drug-like properties; this can exploit structures of the receptor protein or ligand alone, or if available of the structure of a protein-ligand complex. Such approaches include protein interaction mimetics (Fletcher and Hamilton, 2005) and stapled helices (Stewart et al., 2010). Increasingly frequently in recent years fragment-based screening (Shuker et al., 1996; Blundell et al., 2002; Hajduk and Greer, 2007; Murray and Blundell, 2010) is exploited with small chemical libraries (Congreve et al., 2008; Hajduk and Greer, 2007), perhaps with as few as 1000 compounds of molecular weight less than 300 that are consistent with the “rule-of-three” (Congreve et al., 2003). Here, knowledge of the

structure of the protein target from X-ray or nuclear magnetic resonance (NMR) methods is very important. This strategy explores chemical space more efficiently, but because affinities are often millimolar, it most often exploits biophysical assays assessing binding to one partner of a functionally interacting pair, for example, enzyme-substrate, receptor-ligand or protein-protein, rather than disruption of an active protein complex in vitro or in a cellular assay.

The optimization of candidate leads depends optimally on binding sites that can match many pairs of atoms or groups in stable interactions; this has generally been achieved in evolution by creating concave grooves or pockets on the protein surface that will accommodate small chemical entities. The interactions need to compensate for loss of rotational and translational entropy of the small chemical entity and for loss of conformational entropy in both partners, as the flexibility will be restrained. However, water molecules are generally released and may compensate through a positive entropic contribution. Isothermal titration calorimetry can provide useful information on the nature of these interactions and can provide insights into the major determinants of the ligand-protein interactions. Fragments can also induce conformational change but this is kinetically and/or thermodynamically less likely than for drug-like molecules. Nevertheless, ligand efficiency of fragments is often high as all heavy atoms—C, N, O and S—can be more efficiently involved in productive interactions. Hann’s complexity rule says that efficient pairing of interacting atoms is less likely with larger drug-like molecules (Hann et al., 2001).

This review focuses on difficult targets, where many of these desirable features are absent and which would often be defined as “unligandable.” We discuss the challenges of designing ligands where binding sites are flat and featureless, and where flexibility and chemistry of binding sites are not optimal.

Binding-Site Ligandability and Selectivity

Ligandability

Binding-site ligandability can be assessed either computationally or experimentally. Currently available computational predictors for classical receptor and enzyme targets are often based upon the use of three-dimensional structural information of the target proteins to define the concavity of putative binding sites. These methods exploit geometrical scanning (PocketFinder, which is based on Ligsite; Hendlich et al., 1997), PocketDepth (Kalidas and Chandra, 2008), and Voronoi tessellation (Fpocket; Le Guilloux et al., 2009). Many approaches identify favorable interaction energies, often by using a van der Waals probe to explore the protein-binding site as in Q-SiteFinder (Laurie and Jackson, 2005) and i-SITE (Morita et al., 2008). Alternatively, they use random forest classifiers and residue-based properties (Site Predict; Bordner, 2009). A comparison of Finder, Fpocket, PocketFinder, and SiteMap using structures of an impressively large number (5,416) of protein-ligand complexes and the relevant uncomplexed forms demonstrated that on the average they identified 95% of the binding sites (Schmidtke et al., 2010). Thornton, Laskowski, and colleagues have carried out careful analyses of protein ligand-binding sites and combined evolutionary-sequence conservation and three-dimensional structure to predict protein-ligand-binding sites (Glaser et al., 2006; Kahraman et al., 2007).

Some experimental approaches used to indicate ligandability of a target measure hit rates, for example, in NMR-based or X-ray-based fragment screens (Hajduk et al., 2005). Indeed in an analysis of hit rates, best affinity and hit diversity, Edfeldt et al. (2011) showed that fragment-screening-based ligandability was an excellent predictor of success in high-throughput screening and hit-to-lead project progression.

Selectivity

It is well known that drug-like synthetic molecules tend to have fewer polar interactions and more lipophilic interactions with their protein targets than those with other proteins, small peptides and other natural molecules (Keserü and Makara, 2009). However, they also have larger numbers of unmatched nitrogen, oxygen, and sulfur atoms and show no correlation between lipophilicity and the proportion of polar contacts (Hann et al., 2001). These observations give some hope for optimizing interactions, especially those with difficult targets involving protein-protein interfaces of the kind discussed in the next section. Recent reviews (Pérot et al., 2010) and an open source repository for druggable and undruggable proteins (Schmidtke and Barril, 2010) have assisted in updating analyses of “druggability.” They show that that in addition to hydrophobicity, polar interactions play important roles.

A further and less discussed challenge in developing selective inhibitors arises with metalloproteins, where the metal ion is involved in catalytic mechanism and occurs in the binding site, often as a central part of the ligand binding. From a drug design perspective, the metal-binding site is the hot spot. Medicinal chemists have traditionally focused their efforts on chemical functions that directly interact with metals in the active site, leading to competitive inhibition (Fisher and Mobashery, 2006), sometimes giving specific inhibitors targeting Co (II) and Mn (II) (Ye et al., 2004). However, such strategies have usually resulted in broad-spectrum inhibitors as most of the metal-binding

groups are highly polar groups like hydroxymates and carboxylates, which are bound rather unselectively (Yiotakis and Dive, 2002), and can hamper their bioavailability (Overall and Kleifeld, 2006).

Attempts to gain selectivity of metalloprotease inhibitors have involved alternative hot spots and specific features of the metalloproteins other than the metal ion (Devel et al., 2010). HTS screens against matrix metalloproteinases have resulted in nonchelating, and often noncompetitive class of inhibitors, which offer better selectivity profiles (Chen et al., 2000; Johnson et al., 2007; Pochetti et al., 2009; Engel et al., 2005). A hybrid approach has been to attach a hydrophobic group specific for a nonmetallic site through a linker to the metal-binding group (Rouffet and Cohen, 2011). There remain major challenges in discovering and developing selective metalloprotein inhibitors, for example, in matrix metalloproteinases, where musculoskeletal toxicity thought to be arising from the target nondifferentiation (Renkiewicz et al., 2003).

Targeting Protein-Protein Interfaces

Protein-Protein Interfaces

As we have seen most current prediction methods depend on identification and scoring of pockets at the binding site for their likelihood to accommodate a small molecule. Protein-protein interfaces present special challenges in this respect as they have fewer well-defined concave binding sites than classical enzymes and receptors, and tend to be more lipophilic. However, they comprise a diverse set of protein-binding interfaces and an increased effort has been made over the past decade to predict druggable protein-protein interfaces (Wells and McClendon, 2007).

The current interest in targeting the protein-protein interfaces of multiprotein assemblies derives from their important and extensive roles in the regulation of most biochemical pathways involved in cell signaling, growth and survival (Venkatesan et al., 2009). They are now recognized as therapeutic targets that display greater structural and chemical diversity than protein kinases and proteases that have comprised the classical targets in these pathways. Although enzymes have led to the successful development of many new drugs, most enzymes tend have evolved as members of large superfamilies with common catalytic residues, for example, the Asp-Thr/Ser-Gly motifs of aspartic proteinases or common cofactor sites such as ATP sites of protein kinases. Protein-protein interactions are also important therapeutic targets in diseases where protein misfolding and aggregation occurs, such as Huntington's disease (Young, 2003) and Alzheimer's disease (Bonda et al., 2010).

Structural Classification of Protein-Protein Interfaces

Multiprotein systems may be classified as obligate interactions, where the subunits are not observed independently *in vivo*, or nonobligate interactions where they are. As the latter class is often dependent on location (Nooren and Thornton, 2003), it is also useful to further classify complexes as transient and non-transient, which define features of complexes once partners are recognized. In the former case components and complexes are in dynamic equilibrium, albeit with a range of binding affinities and on- and off-rates (Nooren and Thornton, 2003).

Further important descriptors define whether an interaction region comprises a discontinuous epitope, where strands of

polypeptide from different regions of the sequence contribute, or a continuous epitope in which a continuous region of polypeptide, often comprising a single secondary structure element (α helix or β strand), makes up the interaction interface. Schwyzer suggested this distinction for polypeptide hormones in the 1970s (Schwyzer et al., 1979). It was exemplified by glucagon where biophysical and X-ray analyses (Sasaki et al., 1975) together with NMR studies in a lipid-water interface (Braun et al., 1983) suggested a disorder-to-order transition of the polypeptide on receptor binding (Blundell, 1979; Blundell and Wood, 1982). Such concerted binding-and-folding interactions in proteins have been beautifully described by Wright and coworkers, who have demonstrated their important roles in intracellular pathways (Dyson and Wright, 2002). Transient protein-protein interfaces potentially offer additional distinct sub-sites for targeting using small molecule inhibitors (Blundell et al., 2006) as described below for the RAD51-BRCA2 interaction (Pellegrini et al., 2002).

Numerous computational resources describe protein-protein interactions in terms of their three-dimensional protein structures (Tuncbag et al., 2009). Useful databases include 3D Interaction Domains (3DID; Stein et al., 2011), DAPID (Domain Annotated Protein-protein Interaction Database; Chen et al., 2006), and PICCOLO (Bickerton, 2009; Bickerton et al., 2011); <http://www-cryst.bioc.cam.ac.uk/piccolo>. Such resources identify interfaces clustered to reflect interface groups sharing equivalent residue patches and relative geometries. Interactions are described at the level of interacting pairs of atoms, residues, and polypeptide chains. For example, PICCOLO includes 12 different interaction types, which can be used to analyze residue propensity, hydrophathy, polarity, contact preference, and sequence entropy.

Hot Spots

In 1995 Clackson and Wells showed that a small subset of residues contributes most to the binding free energy at the protein-protein interface on human growth hormone and its receptor (Clackson and Wells, 1995) and further alanine-scanning mutagenesis studies demonstrated that this was a general phenomenon (Bogan and Thorn, 1998). Hot spots in protein-protein interfaces can also be experimentally defined from fragment screening, where binding sites for multiple small molecules define the hot spot. Ciulli and co-workers have approached the problem by examining the contributions to binding affinity of the constituent fragments of a known ligand (Ciulli et al., 2006).

A very useful physical model (Robetta) has been described by Kortemme and Baker (Kortemme and Baker, 2002), which combines energetic, structural, and evolutionary information. More recent predictions are based upon support vector machine (SVM) methods (Lise et al., 2011) where the structure of a complex is used to predict hot spot residues.

Bogan and Thorn (1998) found that hot spots in protein-protein interfaces often include Tyr and Trp. These residues can contribute aromatic π -interactions with cations and CH-groups, establish weak hydrogen bonds through aromatic hydrogens with carbonyls and form hydrogen bonds through the indole nitrogen of tryptophan and the phenolic hydroxyl of tyrosine groups; they also have large hydrophobic surfaces that can protect hydrogen bonds from water (Chakrabarti and Bhattacharyya, 2007). Arginine is also important, as it can form a range of similar favorable interactions, in addition to ion pairs that force

it to the periphery of an interacting interface. Aspartate and asparagine with less side chain entropy are favored over glutamate and glutamine. Bogan and Thorn have proposed an O-ring theory where an energetically less important ring of residues surrounds the hot spots (Bogan and Thorn, 1998), and this has been further developed by Nussinov and coworkers in terms of "coupling" and "hot regions," where highly packed residues make it easier to remove water upon binding (Keskin et al., 2005).

Targeting Protein-Protein Interactions

Many thoughtful approaches have been developed in response to the need to target protein-protein interfaces in regulatory multiprotein assemblies. These include proteomimetics that use elegant chemistry to mimic protein surface structure (Fletcher and Hamilton, 2005). For α -helical peptide molecular recognition sites stapled peptides to crosslink adjacent residues in the helix have proved resistant to proteolysis and useful for several targets including BH3:Mcl-1 (Stewart et al., 2010), p53:MDM2/X (Bernal et al., 2010), and MAML-1:Notch (Moeller et al., 2009). Further innovations include foldamers, which are synthetic mimics of protein secondary structure elements (for review, see Wilson, 2009), peptide aptamers (Buerger and Groner, 2003), and antibody-like molecules (Traczewski and Rudnicka, 2011).

Wells and co-workers describe interaction hot spots as ligand-efficient "footholds," with higher affinity achieved by through "cryptic" binding sites within the binding interface (Thanos et al., 2006; Wells and McClendon, 2007). This is encouraging for the use fragment-based drug discovery. However, although fragments allow novel areas of chemical space to be explored more efficiently, the initial hits have low affinity. Thus, in order to disrupt protein-protein interfaces, they usually require tethering (Erlanson et al., 2000) (see below), an approach that has been pioneered by Wells and co-workers, with some impressive successes (Wells and McClendon, 2007). An alternative fragment-based approach is to employ conventional fragment-based screening (Shuker et al., 1996; Blundell et al., 2002; Hajduk and Greer, 2007; Murray and Blundell, 2010). NMR, X-ray crystallography, surface plasmon resonance, differential scanning calorimetry, or isothermal calorimetry (ITC) can then be used to screen and validate fragment binding. However, for protein-protein interfaces this depends on whether the individual subunits are globular and whether they can be stabilized as monomers in solution. Fragment hits can subsequently be evolved into larger lead-like and drug-like molecules with higher affinity and potency.

There have been some useful efforts to classify small molecule binding to protein-protein interfaces. TIMBAL, a database of small molecules inhibiting multi-protein complexes (Higueruelo et al., 2009) that allows comparisons of these small molecules with drugs, chemical libraries and drug-like molecules in the PDB. The inhibitors of protein-protein interfaces are on the average bigger and more lipophilic and have fewer hydrogen bonding features. 2P2IDB (<http://2p2idb.cnrs-mrs.fr>) is also a hand-curated database of protein-protein complexes with small molecules (Bourgeas et al., 2010), which confirms the greater lipophilicity, and finds fewer charged residues and a greater number of nonpolar atoms than at interfaces with no known inhibitor. Interestingly the authors did not observe major

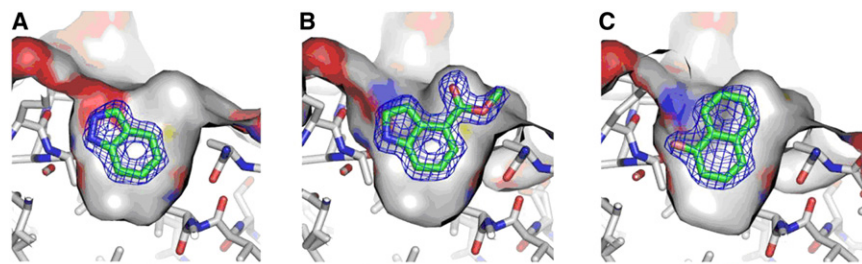


Figure 1. Fragment Binding at a Protein-Protein Interface of the Recombinase RAD51 and BRCA2

Mode of interaction of fragments binding to the phenylalanine-binding pocket of the RAD51 as observed using X-ray crystallography in the complex with the BRC4 peptide of BRCA2 (A) Indazole 5, (B) 4-methylester indole 4, (C) naphthalen-1-ol. (Figure by permission of D.S. Scott, M. Marsh, A. Coyne, C. Abell, and M. Hyvonen.)

conformational changes between protein in the complex bound to an inhibitor and the unliganded state.

We address the situation of conformational flexibility in protein-protein interfaces below. Here, we describe one example of the development of ligands to a protein-protein interface that involves concerted folding and binding of a peptide to a globular domain, very small pockets on a relatively featureless binding interface and little conformational change on ligand binding.

Targeting the Recombinase Rad51 Interface with BRCA2

Knowledge of the structures of RAD51 (Pellegrini et al., 2002) and its close orthologs in apo-form, in complexes of BRC repeats and other short peptides and in oligomers have been central to the development of fragment-based approaches targeting the protein-protein interaction between the human recombinase RAD51 and BRCA2. Fragment screening was initially carried out using thermal shift with hits validated using both ligand-observed NMR and X-ray crystallography (Figure 1). Different humanized proteins that are soluble and monomeric have been designed to be amenable to use with the different screening methods, most importantly to engineer crystal forms with unobstructed binding sites to be optimal for crystal soaking. ITC and X-ray crystallography have been used to explore the thermodynamics and structures of complexes with various peptides designed around the BRC4 repeat. NMR and fluorescence polarization detected competition assays have been exploited to verify binding at the expected sites. Chimeras of peptides and fragments with increased potency and molecules with high nanomolar potency have been obtained and work is now under way to target further local subsites to increase potency.

Targeting Transient and Flexible Binding Sites through Conformational Change

Conformational Flexibility in Binding Sites

Many binding sites are induced by the ligand or alternatively one conformer is stabilized from an ensemble on ligand recognition. For enzymes, this may correspond to the stabilization of a loop, which is brought transiently close to the ligand during catalysis, or alternatively a hinge-bending movement of a domain that naturally occurs on binding of cofactor or substrate. However, in many proteins the induced binding site does not have an obvious functional role in living organisms.

Exploiting conformational flexibility presents challenges for most structure-based methods, but particularly for fragment-based approaches where the binding energy of the first hit rarely stabilizes the necessary conformation. Thus, tethered fragments

are often exploited or the binding site stabilized in the absence of the ligand, for example, in a crystal lattice (Wells and McClendon, 2007). We now consider these challenging situations in turn.

Transient Active Sites

AroD is an essential enzyme from the shikimate pathway, which is involved in aromatic amino acid synthesis in some microorganisms. The absence of this pathway in humans makes the enzymes attractive targets for discovering anti-infectives (McConkey, 1999; Parish and Stoker, 2002). The active site of AroD is located in a cleft (Harris et al., 1993), but the catalytically important loop (Krell et al., 1995) is not seen in the electron density of the apo-form of the protein (Gourley et al., 1999). When the substrate is bound (Dias et al., 2011), the catalytic residues are brought into close proximity to the substrate. For this target fragment binding has proved unsuccessful. A partially formed AroD active site is required, which is induced only by designing substrate analogs (Dias et al., 2011; González-Bello and Castedo, 2007; Tizón et al., 2011). X-ray structures of successful competitive inhibitors bound to the aroD show that the residues from the loop region are ordered only in the case of high affinity ligands (Figure 2).

The attempts to design inhibitors of shikimate kinase, a further enzyme from the shikimate pathway, highlight the challenges when a natural ligand or cofactor must bind in order to bring about domain reorganization. Shikimate kinase belongs to the nucleoside monophosphate kinase family, which catalyze the phosphorylation of small molecules using ATP as a cofactor.

The several crystal structures of shikimate kinase (Dhaliwal et al., 2004; Cheng et al., 2005; Dias et al., 2007) represent various stages in the catalytic mechanism (Hartmann et al., 2006) and define conformational changes that reposition the ATP-binding domain, the shikimate-binding domain and the P loop (Krell et al., 2001) upon binding ATP and shikimate. Figure 3 shows that, in unliganded structure of the *Mycobacterium tuberculosis* shikimate kinase (PDB ID: 2IYT), the entire active site is open. Movements of the two domains relative to each other when the substrates bind create a more ligandable catalytically competent active site. Again, substrate analog or drug-like screening approaches are likely to be more effective than fragment-based approaches that have proved challenging in our hands.

Induced Binding Sites

Some of the most successful attempts to bind molecules at protein-protein interfaces have led to a reorganization of surface residues and creation of a ligand-binding cavity (Wells and McClendon, 2007). For fragment-based design these have often entailed tethering the fragment. An example is the 60 nM small

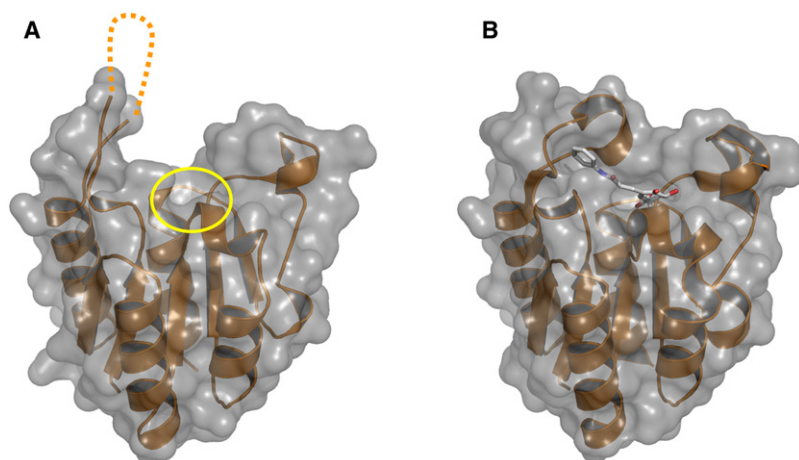


Figure 2. Conformational Flexibility in the Active Site of the Enzyme aroD

(A) Structure of aroD in unliganded form (PDB ID: 2DHQ), shown as the protein surface with secondary structure in red color. In unliganded aroD the catalytically important loop illustrated by dashed line, which forms the part of the active site, is disordered and cannot be seen in the electron density of the X-ray crystal structure, whereas the partially formed site is highlighted by yellow circle. (B) Structure of aroD in complex with a substrate-mimicking inhibitor (PDB ID: 3N86). Substrate analog inhibitors can bind the partially formed cavity, are capable of inducing conformational change in the loop, and can engage with loop residues productively.

molecule antagonist SP4206 of the cytokine/receptor interaction (interleukin-2 IL-2/IL2R α) (Braisted et al., 2003). The authors coupled selected fragments with a low micromolar hit to generate the high-affinity lead compound, employing a combination of tethering, structural biology, and computational analysis. The molecule stabilizes a conformation of IL-2 with a groove that accommodates the small molecule and is accompanied by repositioning the loop to embrace the furanoic acid moiety. The protein and small molecule bind in the same region—the adaptive hot spot—but trap very different conformations of IL-2 (Thanos et al., 2006).

Protein surface reorganization coupled with conformational changes was also exploited for designing novel ligands that bind BCL-X_L, a member of the BCL-2 family which inhibits apoptosis by binding α -helical regions of the proapoptotic molecules BAK and BAD (Oltersdorf et al., 2005; Petros et al., 2006; Bruncko et al., 2007). The molecular interaction interface between the helical peptide and the BCL-X_L lacks the large cavity characteristic of classical ligandable sites (PDB ID: 1R2D; Manion et al., 2004; Muchmore et al., 1996). An NMR screen of around 9000 fragments in the Abbott labs gave frag-

ment hits interacting with an initial hot spot, and these fragments were grown toward a second binding site. The compounds were then systematically optimized to give ABT-737, a potent inhibitor of BCL-X_L with selectivity toward BCL-X_L. A crystal structure of ABT-737 in complex with BCL-X_L (Lee et al., 2007) has subsequently shown that ABT-737 did not interact with the BCL-X_L in the same way as the proteins ligands, with few of the binding interactions characteristic of the protein domain and conformational changes affecting the protein interface pockets (Figure 4; Lee et al., 2007).

Another case where protein conformational flexibility was exploited for drug discovery was that of Imatinib inhibiting Bcr-Abl kinase. Imatinib was discovered using enzyme assays as the primary screen (Capdeville et al., 2002). Early characterization of Imatinib indicated a very high degree of selectivity toward Bcr-Abl compared to homologs, such as Src (Zimmermann et al., 1997; Schindler et al., 2000), an intriguing observation as these proteins had similar structures. Structural characterization of Imatinib-Bcr-Abl complex (Schindler et al., 2000; Nagar et al., 2002) revealed that Imatinib targeted a unique conformation of Bcr-ABI kinase. Compounds that were less sensitive to the conformation of the Bcr-Abl showed were less selective (Nagar et al., 2002; Figure 5).

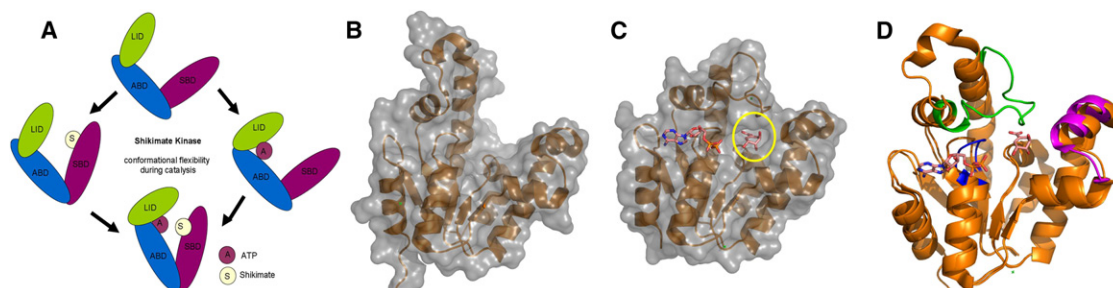


Figure 3. Conformational Flexibility of Shikimate Kinase in Response to Ligand Binding

(A) Shikimate kinase has three domains: the lid domain (LID), shikimate-binding domain (SBD), and ATP-binding domain (ABD), all of which undergo relative movements and conformational changes upon ligand binding. Depending on the sequence of ligand binding, shikimate kinase can adopt one of four conformational states. Adapted from Hartmann et al. (2006).

(B) Unliganded structure of the shikimate kinase (PDB ID: 2IYT) demonstrates the absence of a ligandable cavity, making it difficult to design ligands that bind this conformational state.

(C) Structure of the shikimate kinase in complex with ADP and shikimate (PDB ID: 2IYQ). After the ligand binds a new cavity is formed to accommodate the ligand, highlighted by yellow circle.

(D) Two structures representing different conformational states (PDB ID: 2IYT and 2IYQ) are superimposed. Upon shikimate binding, residues in magenta move. Residues from the lid domain (green) and some core residues (blue) are moved in response to ATP binding. These conformational changes create a ligandable site.

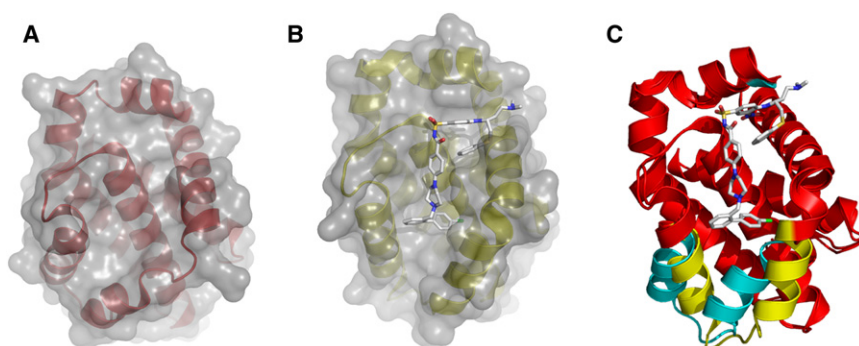


Figure 4. Surface Reorganization at a Protein-Protein Interface Mediated by Conformational Changes

(A) Unliganded structure of the oncogenic protein BCL-X_L (PDB ID: 1R2D).

(B) Structure of BCL-X_L in complex with ABT-737 (PDB ID: 2YXJ) shows that the protein surface has undergone a significant reorganization in order to accommodate the inhibitor.

(C) Overview of conformational changes of BCL-X_L leading to the binding of ABT-737. The core residues that remain relatively unchanged are shown in red, whereas residues undergoing movements in response to ligand binding are yellow. Cyan residues are from the unliganded structure.

Conclusions

Over the past decade, researchers in the pharmaceutical and biotechnology industries and academia have made retrospective analyses of successful drug campaigns in order to establish “rules” to guide the selection of new target proteins. In this review, we have focused on difficult targets, many of which have been defined as “unligandable,” but which are not without hope of providing useful drugs. One group of difficult targets,

those that involve protein-protein interactions, has become of increasing interest in the search for selective intervention in regulatory pathways that involve multiple members of superfamilies such as protein kinases, phosphatases, and proteases. In fact, the characteristics of binding sites at protein-protein interfaces, particularly if they involve concerted binding and folding in the assembly of the targeted complexes, offer new opportunities. We have shown that, where small pockets occur at interfaces, small fragments may be helpful in exploring the limited chemical space.

Indeed, fragment-based approaches free the chemist from the restraints of the large chemical screening libraries that often reflect those compounds that have proved successful in campaigns against classical targets such as G protein-coupled receptors and protein kinases. They open up the possibility of finding new chemistries that will offer efficient lead optimization. Furthermore, fragment-based approaches make maximum use of new sensitive biophysical techniques such as surface plasmon resonance, isothermal calorimetry, nuclear magnetic resonance and X-ray crystallography that can provide high-throughput assessments of structures, kinetics, and thermodynamics of small-molecule binding to protein targets.

The discovery of Imatinib highlighted the importance of understanding the population of conformers available in order to achieve selectivity (Liu and Gray, 2006). The lesson is certainly to define 3D structures, kinetics, and thermodynamics at every stage of hit identification and lead optimization. However, the challenges and rewards of targets where conformational changes are required to configure an appropriate binding site seem evenly balanced.

Although conformations can be stabilized that are quite different from those that have been selected in evolution, they nevertheless provide excellent binding sites with high ligand efficiency. However, the desired conformations are sometimes difficult to induce experimentally by binding fragments. The challenge here is to stabilize the unusually conformers, perhaps by

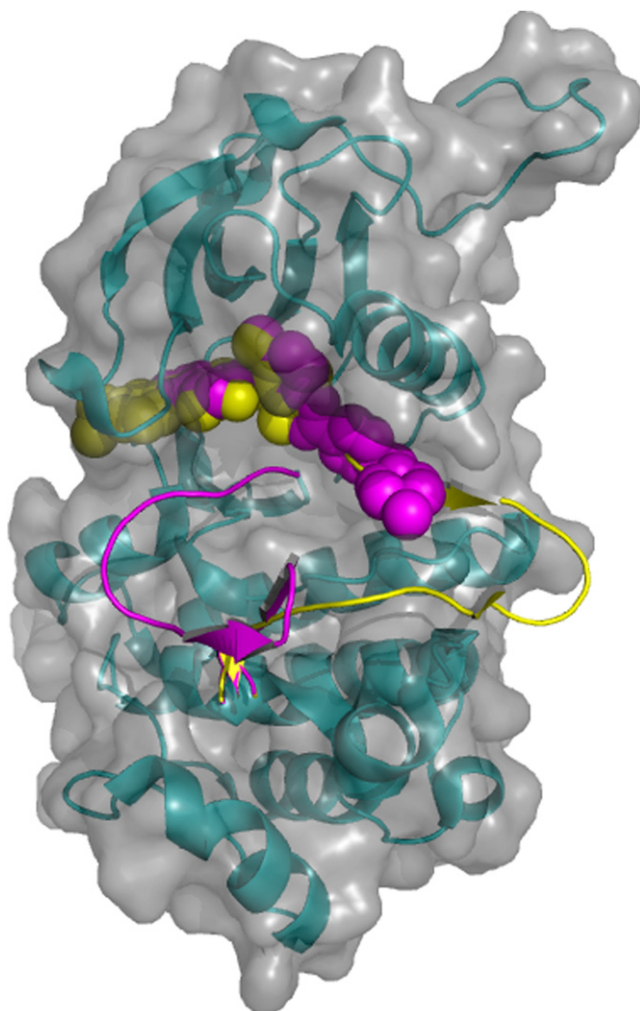


Figure 5. The Selectivity of Imatinib Depends on Targeting a Unique Conformation of Bcr-Abl

Two different activation loop conformations are shown in magenta and in yellow. The yellow loop represents the active conformation, which is targeted by the nonselective inhibitor (yellow spheres, PDB ID: 1M52), whereas the magenta loop is the inactive conformation targeted by Imatinib (magenta spheres, PDB ID: 1IEP) of the Bcr-abl kinase. The protein part that remains relatively unchanged after ligand binding is shown in cyan color and surface is shown.

protein engineering, by crosslinking or by incorporation in a crystal lattice.

The conformations frequently reflect relatively underpopulated members of ensembles that are difficult to predict, even with the full force of high-performance computing and efficient molecular dynamics. This is an area where more effort is required—and probably not just running molecular dynamics calculations for longer. However, once conformations are observed and rationalized, they can be exploited further and have much to offer in drug discovery.

ACKNOWLEDGMENTS

We are grateful to our many colleagues in the groups of Dr. Marko Hyvonen, Prof. Chris Abell, Dr. Duncan Scott, Dr. Anthony Coyne, Prof. Ashok Venkitaraman, as well as Dr. Will Pitt, Dr. Anja Winter, Anna Sigurdardottir, Dr. Leo Silvestre, Dr. May Marsh, Dr. Tim Sharpe, Dr. Michal Blaszczyk, Dr. Adrian Schreyer, Dr. Marcio Dias, and Alicia Higuieruelo in our group, for stimulating discussions. We thank the Wellcome Trust, the Bill and Melinda Gates Foundation, and the European Union for funding.

REFERENCES

- Bernal, F., Wade, M., Godes, M., Davis, T.N., Whitehead, D.G., Kung, A.L., Wahl, G.M., and Walensky, L.D. (2010). A stapled p53 helix overcomes HDMX-mediated suppression of p53. *Cancer Cell* **18**, 411–422.
- Bickerton, G.R. (2009). Molecular characterization and evolutionary plasticity of protein-protein interfaces. PhD thesis, University of Cambridge, Cambridge, UK.
- Bickerton, G.R., Higuieruelo, A.P., and Blundell, T.L. (2011). Comprehensive, atomic-level characterization of structurally characterized protein-protein interactions: the PICCOLO database. *BMC Bioinformatics* **12**, 313–327.
- Blundell, T., and Wood, S. (1982). The conformation, flexibility, and dynamics of polypeptide hormones. *Annu. Rev. Biochem.* **51**, 123–154.
- Blundell, T.L. (1979). Conformation and molecular biology of polypeptide hormones II. Glucagon. *Trends Biochem. Sci.* **4**, 80–83.
- Blundell, T.L., Jhoti, H., and Abell, C. (2002). High-throughput crystallography for lead discovery in drug design. *Nat. Rev. Drug Discov.* **1**, 45–54.
- Blundell, T.L., Sibanda, B.L., Montalvão, R.W., Brewerton, S., Chelliah, V., Worth, C.L., Harmer, N.J., Davies, O., and Burke, D. (2006). Structural biology and bioinformatics in drug design: opportunities and challenges for target identification and lead discovery. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **361**, 413–423.
- Bogan, A.A., and Thorn, K.S. (1998). Anatomy of hot spots in protein interfaces. *J. Mol. Biol.* **280**, 1–9.
- Bonda, D.J., Lee, H.-P., Lee, H.G., Friedlich, A.L., Perry, G., Zhu, X., and Smith, M.A. (2010). Novel therapeutics for Alzheimer's disease: an update. *Curr. Opin. Drug Discov. Devel.* **13**, 235–246.
- Bordner, A.J. (2009). Predicting protein-protein binding sites in membrane proteins. *BMC Bioinformatics* **10**, 312.
- Bourgeas, R., Basse, M.-J., Morelli, X., and Roche, P. (2010). Atomic analysis of protein-protein interfaces with known inhibitors: the 2P2I database. *PLoS ONE* **5**, e9598.
- Braisted, A.C., Oslob, J.D., Delano, W.L., Hyde, J., McDowell, R.S., Waal, N., Yu, C., Arkin, M.R., and Raimundo, B.C. (2003). Discovery of a potent small molecule IL-2 inhibitor through fragment assembly. *J. Am. Chem. Soc.* **125**, 3714–3715.
- Braun, W., Wider, G., Lee, K.H., and Wüthrich, K. (1983). Conformation of glucagon in a lipid-water interphase by ¹H nuclear magnetic resonance. *J. Mol. Biol.* **169**, 921–948.
- Bruncko, M., Oost, T.K., Belli, B.A., Ding, H., Joseph, M.K., Kunzer, A., Martineau, D., McClellan, W.J., Mitten, M., Ng, S.-C., et al. (2007). Studies leading to potent, dual inhibitors of Bcl-2 and Bcl-xL. *J. Med. Chem.* **50**, 641–662.
- Buerger, C., and Groner, B. (2003). Bifunctional recombinant proteins in cancer therapy: cell penetrating peptide aptamers as inhibitors of growth factor signaling. *J. Cancer Res. Clin. Oncol.* **129**, 669–675.
- Capdeville, R., Buchdunger, E., Zimmermann, J., and Matter, A. (2002). Glivec (STI571, imatinib), a rationally developed, targeted anticancer drug. *Nat. Rev. Drug Discov.* **1**, 493–502.
- Chakrabarti, P., and Bhattacharyya, R. (2007). Geometry of nonbonded interactions involving planar groups in proteins. *Prog. Biophys. Mol. Biol.* **95**, 83–137.
- Chen, J.M., Nelson, F.C., Levin, J.I., Mobilio, D., Moy, F.J., Nilakantan, R., Zask, A., and Powers, R. (2000). Structure-based design of a novel, potent, and selective inhibitor for MMP-13 utilizing NMR spectroscopy and computer-aided molecular design. *J. Am. Chem. Soc.* **122**, 9648–9654.
- Chen, Y.-C., Chen, H.-C., and Yang, J.-M. (2006). DAPID: a 3D-domain annotated protein-protein interaction database. *Genome informatics. Genome Inform.* **17**, 206–215.
- Cheng, W.-C., Chang, Y.-N., and Wang, W.-C. (2005). Structural basis for shikimate-binding specificity of *Helicobacter pylori* shikimate kinase. *J. Bacteriol.* **187**, 8156–8163.
- Ciulli, A., Williams, G., Smith, A.G., Blundell, T.L., and Abell, C. (2006). Probing hot spots at protein-ligand binding sites: a fragment-based approach using biophysical methods. *J. Med. Chem.* **49**, 4992–5000.
- Clackson, T., and Wells, J.A. (1995). A hot spot of binding energy in a hormone-receptor interface. *Science* **267**, 383–386.
- Congreve, M., Carr, R., Murray, C., and Jhoti, H. (2003). A “rule of three” for fragment-based lead discovery? *Drug Discov. Today* **8**, 876–877.
- Congreve, M., Chessari, G., Tisi, D., and Woodhead, A.J. (2008). Recent developments in fragment-based drug discovery. *J. Med. Chem.* **51**, 3661–3680.
- Devel, L., Czarny, B., Beau, F., Georgiadis, D., Stura, E., and Dive, V. (2010). Third generation of matrix metalloprotease inhibitors: gain in selectivity by targeting the depth of the S1' cavity. *Biochimie* **92**, 1501–1508.
- Dhaliwal, B., Nichols, C.E., Ren, J., Lockyer, M., Charles, I., Hawkins, A.R., and Stammers, D.K. (2004). Crystallographic studies of shikimate binding and induced conformational changes in *Mycobacterium tuberculosis* shikimate kinase. *FEBS Lett.* **574**, 49–54.
- Dias, M.V.B., Faim, L.M., Vasconcelos, I.B., de Oliveira, J.S., Basso, L.A., Santos, D.S., and de Azevedo, W.F., Jr. (2007). Effects of the magnesium and chloride ions and shikimate on the structure of shikimate kinase from *Mycobacterium tuberculosis*. *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.* **63**, 1–6.
- Dias, M.V.B., Snee, W.C., Bromfield, K.M., Payne, R.J., Palaninathan, S.K., Ciulli, A., Howard, N.I., Abell, C., Sacchettini, J.C., and Blundell, T.L. (2011). Structural investigation of inhibitor designs targeting 3-dehydroquinate dehydratase from the shikimate pathway of *Mycobacterium tuberculosis*. *Biochem. J.* **436**, 729–739.
- Dyson, H.J., and Wright, P.E. (2002). Coupling of folding and binding for unstructured proteins. *Curr. Opin. Struct. Biol.* **12**, 54–60.
- Edfeldt, F.N.B., Folmer, R.H., and Breeze, A.L. (2011). Fragment screening to predict druggability (ligandability) and lead discovery success. *Drug Discov. Today* **16**, 284–287.
- Engel, C.K., Pirard, B., Schimanski, S., Kirsch, R., Habermann, J., Klingler, O., Schlotte, V., Weithmann, K.U., and Wendt, K.U. (2005). Structural basis for the highly selective inhibition of MMP-13. *Chem. Biol.* **12**, 181–189.
- Erlanson, D.A., Braisted, A.C., Raphael, D.R., Randal, M., Stroud, R.M., Gordon, E.M., and Wells, J.A. (2000). Site-directed ligand discovery. *Proc. Natl. Acad. Sci. USA* **97**, 9367–9372.
- Fisher, J.F., and Mobashery, S. (2006). Recent advances in MMP inhibitor design. *Cancer Metastasis Rev.* **25**, 115–136.
- Fletcher, S., and Hamilton, A.D. (2005). Protein surface recognition and proteomimetics: mimics of protein surface structure and function. *Curr. Opin. Chem. Biol.* **9**, 632–638.

- Glaser, F., Morris, R.J., Najmanovich, R.J., Laskowski, R.A., and Thornton, J.M. (2006). A method for localizing ligand binding pockets in protein structures. *Proteins* 62, 479–488.
- González-Bello, C., and Castedo, L. (2007). Progress in type II dehydroquinase inhibitors: from concept to practice. *Med. Res. Rev.* 27, 177–208.
- Gourley, D.G., Shrive, A.K., Polikarpov, I., Krell, T., Coggins, J.R., Hawkins, A.R., Isaacs, N.W., and Sawyer, L. (1999). The two types of 3-dehydroquinase have distinct structures but catalyze the same overall reaction. *Nat. Struct. Biol.* 6, 521–525.
- Le Guilloux, V., Schmidtke, P., and Tuffery, P. (2009). Fpocket: an open source platform for ligand pocket detection. *BMC Bioinformatics* 10, 168.
- 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.
- Hajduk, P.J., Huth, J.R., and Fesik, S.W. (2005). Druggability indices for protein targets derived from NMR-based screening data. *J. Med. Chem.* 48, 2518–2525.
- Hann, M.M., Leach, A.R., and Harper, G. (2001). Molecular complexity and its impact on the probability of finding leads for drug discovery. *J. Chem. Inf. Comput. Sci.* 41, 856–864.
- Harris, J., Kleanthous, C., Coggins, J.R., Hawkins, A.R., and Abell, C. (1993). Different mechanistic and stereochemical courses for the reactions catalysed by type I and type II dehydroquinases. *J. Chem. Soc. Chem. Commun.* 1080.
- Hartmann, M.D., Bourenkov, G.P., Oberschall, A., Strizhov, N., and Bartunik, H.D. (2006). Mechanism of phosphoryl transfer catalyzed by shikimate kinase from *Mycobacterium tuberculosis*. *J. Mol. Biol.* 364, 411–423.
- Hendlich, M., Rippmann, F., and Barnickel, G. (1997). LIGSITE: automatic and efficient detection of potential small molecule-binding sites in proteins. *J. Mol. Graph. Model.* 15, 359–363, 389.
- Higueruelo, A.P., Schreyer, A., Bickerton, G.R.J., Pitt, W.R., Groom, C.R., and Blundell, T.L. (2009). Atomic interactions and profile of small molecules disrupting protein-protein interfaces: the TIMBAL database. *Chem. Biol. Drug Des.* 74, 457–467.
- Hopkins, A.L., and Groom, C.R. (2002). The druggable genome. *Nat. Rev. Drug Discov.* 1, 727–730.
- Johnson, A.R., Pavlovsky, A.G., Ortwine, D.F., Prior, F., Man, C.-F., Bornemeier, D.A., Banotai, C.A., Mueller, W.T., McConnell, P., Yan, C., et al. (2007). Discovery and characterization of a novel inhibitor of matrix metalloproteinase-13 that reduces cartilage damage in vivo without joint fibroplasia side effects. *J. Biol. Chem.* 282, 27781–27791.
- Kahraman, A., Morris, R.J., Laskowski, R.A., and Thornton, J.M. (2007). Shape variation in protein binding pockets and their ligands. *J. Mol. Biol.* 368, 283–301.
- Kalidas, Y., and Chandra, N. (2008). PocketDepth: a new depth based algorithm for identification of ligand binding sites in proteins. *J. Struct. Biol.* 161, 31–42.
- Keserü, G.M., and Makara, G.M. (2009). The influence of lead discovery strategies on the properties of drug candidates. *Nat. Rev. Drug Discov.* 8, 203–212.
- Keskin, O., Ma, B., Rogale, K., Gunasekaran, K., and Nussinov, R. (2005). Protein-protein interactions: organization, cooperativity and mapping in a bottom-up Systems Biology approach. *Phys. Biol.* 2, S24–S35.
- Kortemme, T., and Baker, D. (2002). A simple physical model for binding energy hot spots in protein-protein complexes. *Proc. Natl. Acad. Sci. USA* 99, 14116–14121.
- Krell, T., Pitt, A.R., and Coggins, J.R. (1995). The use of electrospray mass spectrometry to identify an essential arginine residue in type II dehydroquinases. *FEBS Lett.* 360, 93–96.
- Krell, T., Maclean, J., Boam, D.J., Cooper, A., Resmini, M., Brocklehurst, K., Kelly, S.M., Price, N.C., Laphorn, A.J., and Coggins, J.R. (2001). Biochemical and X-ray crystallographic studies on shikimate kinase: the important structural role of the P-loop lysine. *Protein Sci.* 10, 1137–1149.
- Laurie, A.T.R., and Jackson, R.M. (2005). Q-SiteFinder: an energy-based method for the prediction of protein-ligand binding sites. *Bioinformatics* 21, 1908–1916.
- Lee, E.F., Czabotar, P.E., Smith, B.J., Deshayes, K., Zobel, K., Colman, P.M., and Fairlie, W.D. (2007). Crystal structure of ABT-737 complexed with Bcl-xL: implications for selectivity of antagonists of the Bcl-2 family. *Cell Death Differ.* 14, 1711–1713.
- Lipinski, C.A., Lombardo, F., Dominy, B.W., and Feeney, P.J. (2001). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* 46, 3–26.
- Lise, S., Buchan, D., Pontil, M., and Jones, D.T. (2011). Predictions of hot spot residues at protein-protein interfaces using support vector machines. *PLoS ONE* 6, e16774.
- Liu, Y., and Gray, N.S. (2006). Rational design of inhibitors that bind to inactive kinase conformations. *Nat. Chem. Biol.* 2, 358–364.
- Manion, M.K., O'Neill, J.W., Giedt, C.D., Kim, K.M., Zhang, K.Y.Z., and Hockenbery, D.M. (2004). Bcl-XL mutations suppress cellular sensitivity to antimycin A. *J. Biol. Chem.* 279, 2159–2165.
- McConkey, G.A. (1999). Targeting the shikimate pathway in the malaria parasite *Plasmodium falciparum*. *Antimicrob. Agents Chemother.* 43, 175–177.
- Moellering, R.E., Cornejo, M., Davis, T.N., Del Bianco, C., Aster, J.C., Blacklow, S.C., Kung, A.L., Gilliland, D.G., Verdine, G.L., and Bradner, J.E. (2009). Direct inhibition of the NOTCH transcription factor complex. *Nature* 462, 182–188.
- Morita, M., Nakamura, S., and Shimizu, K. (2008). Highly accurate method for ligand-binding site prediction in unbound state (apo) protein structures. *Proteins* 73, 468–479.
- Muchmore, S.W., Sattler, M., Liang, H., Meadows, R.P., Harlan, J.E., Yoon, H.S., Nettesheim, D., Chang, B.S., Thompson, C.B., Wong, S.L., et al. (1996). X-ray and NMR structure of human Bcl-xL, an inhibitor of programmed cell death. *Nature* 381, 335–341.
- Murray, C.W., and Blundell, T.L. (2010). Structural biology in fragment-based drug design. *Curr. Opin. Struct. Biol.* 20, 497–507.
- Nagar, B., Bornmann, W.G., Pellicena, P., Schindler, T., Veach, D.R., Miller, W.T., Clarkson, B., and Kuriyan, J. (2002). Crystal structures of the kinase domain of c-Abl in complex with the small molecule inhibitors PD173955 and imatinib (STI-571). *Cancer Res.* 62, 4236–4243.
- Nooren, I.M.A., and Thornton, J.M. (2003). Structural characterisation and functional significance of transient protein-protein interactions. *J. Mol. Biol.* 325, 991–1018.
- Overall, C.M., and Kleifeld, O. (2006). Towards third generation matrix metalloproteinase inhibitors for cancer therapy. *Br. J. Cancer* 94, 941–946.
- Oltersdorf, T., Elmore, S.W., Shoemaker, A.R., Armstrong, R.C., Augeri, D.J., Belli, B.A., Bruncko, M., Deckwerth, T.L., Dinges, J., Hajduk, P.J., et al. (2005). An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature* 435, 677–681.
- Parish, T., and Stoker, N.G. (2002). The common aromatic amino acid biosynthesis pathway is essential in *Mycobacterium tuberculosis*. *Microbiology* 148, 3069–3077.
- Pellegrini, L., Yu, D.S., Lo, T., Anand, S., Lee, M., Blundell, T.L., and Venkitaraman, A.R. (2002). Insights into DNA recombination from the structure of a RAD51-BRCA2 complex. *Nature* 420, 287–293.
- Petros, A.M., Dinges, J., Augeri, D.J., Baumeister, S.A., Betebenner, D.A., Bures, M.G., Elmore, S.W., Hajduk, P.J., Joseph, M.K., Landis, S.K., et al. (2006). Discovery of a potent inhibitor of the antiapoptotic protein Bcl-xL from NMR and parallel synthesis. *J. Med. Chem.* 49, 656–663.
- Pochetti, G., Montanari, R., Gege, C., Chevrier, C., Taveras, A.G., and Mazza, F. (2009). Extra binding region induced by non-zinc chelating inhibitors into the S1' subsite of matrix metalloproteinase 8 (MMP-8). *J. Med. Chem.* 52, 1040–1049.
- Pérot, S., Sperandio, O., Miteva, M.A., Camproux, A.-C., and Villoutreix, B.O. (2010). Druggable pockets and binding site centric chemical space: a paradigm shift in drug discovery. *Drug Discov. Today* 15, 656–667.

- Renkiewicz, R., Qiu, L., Lesch, C., Sun, X., Devalaraja, R., Cody, T., Kaldjian, E., Welgus, H., and Baragi, V. (2003). Broad-spectrum matrix metalloproteinase inhibitor marimastat-induced musculoskeletal side effects in rats. *Arthritis Rheum.* *48*, 1742–1749.
- Rouffet, M., and Cohen, S.M. (2011). Emerging trends in metalloprotein inhibition. *Dalton Trans.* *40*, 3445–3454.
- Sasaki, K., Dockerill, S., Adamiak, D.A., Tickle, I.J., and Blundell, T. (1975). X-ray analysis of glucagon and its relationship to receptor binding. *Nature* *257*, 751–757.
- Schindler, T., Bormann, W., Pellicena, P., Miller, W.T., Clarkson, B., and Kuriyan, J. (2000). Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. *Science* *289*, 1938–1942.
- Schmidtke, P., and Barril, X. (2010). Understanding and predicting druggability. A high-throughput method for detection of drug binding sites. *J. Med. Chem.* *53*, 5858–5867.
- Schmidtke, P., Souaille, C., Estienne, F., Baurin, N., and Kroemer, R.T. (2010). Large-scale comparison of four binding site detection algorithms. *J. Chem. Inf. Model.* *50*, 2191–2200.
- Schwyzler, R., Kriwaczek, V.M., Baumann, K., Haller, H.-R., Wider, G., and Wiltzius, A.P. (1979). Hormone-receptor interactions: a study of the binding of hormone-substituted tobacco mosaic virus to membrane vesicles by dynamic light scattering and by transient electric bi-refringence. *Pure Appl. Chem.* *51*, 831–835.
- Shuker, S.B., Hajduk, P.J., Meadows, R.P., and Fesik, S.W. (1996). Discovering high-affinity ligands for proteins: SAR by NMR. *Science* *274*, 1531–1534.
- Stein, A., Céol, A., and Aloy, P. (2011). 3did: identification and classification of domain-based interactions of known three-dimensional structure. *Nucleic Acids Res.* *39* (Database issue), D718–D723.
- Stewart, M.L., Fire, E., Keating, A.E., and Walensky, L.D. (2010). The MCL-1 BH3 helix is an exclusive MCL-1 inhibitor and apoptosis sensitizer. *Nat. Chem. Biol.* *6*, 595–601.
- Thanos, C.D., DeLano, W.L., and Wells, J.A. (2006). Hot-spot mimicry of a cytokine receptor by a small molecule. *Proc. Natl. Acad. Sci. USA* *103*, 15422–15427.
- Tizón, L., Otero, J.M., Prazeres, V.F.V., Llamas-Saiz, A.L., Fox, G.C., van Raaij, M.J., Lamb, H., Hawkins, A.R., Ainsa, J.A., Castedo, L., and González-Bello, C. (2011). A prodrug approach for improving antituberculosis activity of potent *Mycobacterium tuberculosis* type II dehydroquinase inhibitors. *J. Med. Chem.* *54*, 6063–6084.
- Traczewski, P., and Rudnicka, L. (2011). Treatment of systemic lupus erythematosus with epratuzumab. *Br. J. Clin. Pharmacol.* *71*, 175–182.
- Tuncbag, N., Kar, G., Keskin, O., Gursoy, A., and Nussinov, R. (2009). A survey of available tools and web servers for analysis of protein-protein interactions and interfaces. *Brief. Bioinform.* *10*, 217–232.
- Venkatesan, K., Rual, J.-F., Vazquez, A., Stelzl, U., Lemmens, I., Hirozane-Kishikawa, T., Hao, T., Zenkner, M., Xin, X., Goh, K.-I., et al. (2009). An empirical framework for binary interactome mapping. *Nat. Methods* *6*, 83–90.
- Wells, J.A., and McClendon, C.L. (2007). Reaching for high-hanging fruit in drug discovery at protein-protein interfaces. *Nature* *450*, 1001–1009.
- Wilson, A.J. (2009). Inhibition of protein-protein interactions using designed molecules. *Chem. Soc. Rev.* *38*, 3289–3300.
- Ye, Q.-Z., Xie, S.-X., Huang, M., Huang, W.-J., Lu, J.-P., and Ma, Z.-Q. (2004). Metalloform-selective inhibitors of escherichia coli methionine aminopeptidase and X-ray structure of a Mn(II)-form enzyme complexed with an inhibitor. *J. Am. Chem. Soc.* *126*, 13940–13941.
- Yiotakis, A., and Dive, V. (2002). Third-generation MMP inhibitors: recent advances in the development of highly selective inhibitors. In *The Cancer Degradome*, D. Edwards, G. Hoyer-Hansen, F. Blasi, and B.F. Sloane, eds. (Springer), pp. 811–822.
- Young, A.B. (2003). Huntingtin in health and disease. *J. Clin. Invest.* *111*, 299–302.
- Zimmermann, J., Buchdunger, E., Mett, H., Meyer, T., and Lydon, N.B. (1997). Potent and selective inhibitors of the Abl-kinase: phenylamino-pyrimidine (PAP) derivatives. *Bioorg. Med. Chem. Lett.* *7*, 187–192.