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Commentary

# Small molecule antagonists of proteins

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**Abstract**

The identification of small molecule antagonists of protein function is at the core of the pharmaceutical industry. Successful approaches to this problem, including screening and rational design, have been developed over the years to identify antagonists of enzymes and cellular receptors. These methods have been extended to the search for inhibitors of protein–protein interactions. While the very possibility of designing a small molecule inhibitor for such interactions was once doubted, there are examples of such inhibitors that are currently marketed products and many more inhibitors in various stages of research and development. Here we review the progress in identifying and designing small molecule protein inhibitors, with particular attention to those that block protein–protein interactions. We also discuss the physical character of protein–protein interfaces, and the resulting implications for small molecule lead discovery and design.



*Keywords:* Drug design; Protein–protein interactions; Inhibitor; Antagonist; Lead identification

## 1. Introduction

The use of small molecules as antagonists of protein function can be traced to the very origin of the pharmaceutical industry. The commercialization of aspirin at the end of the nineteenth century preceded our current understanding of its molecular mode of action in the acetylation of serine-530 and the resulting loss of cyclooxygenase activity from prostaglandin endoperoxide synthase-1 (cyclooxygenase-1) [1] by nearly a century. Progress in the disciplines of biology, pharmacology, biochemistry, and chemistry during the twentieth century has enabled a more reasoned approach to lead identification and disease intervention. Modern pharmaceutical research generally targets specific proteins whose aberrant or excessive expression and/or function has been linked to the cause of a human disease state. These protein targets fall into three classes that are amenable to antagonism by small molecules: enzymes, cellular receptors, and proteins involved in a protein–protein interaction. This review will attempt to summarize the progress in the identification of small molecule protein antagonists, with a particular focus on the protein–protein interaction case, and to comment on the future direction of these efforts over the next 5–10 years.

## 2. Enzymes

Prior to the advent of molecular biology with its ability to clone and express relatively large amounts of recombinant human proteins for lead discovery studies, most pharmaceutical research and discovery were pharmacologically driven. In this era, assays frequently utilized non-human proteins isolated from animal sources, cell and tissue homogenates, tissue samples, and animal models of human disease to study the pharmacological effect of lead molecules and develop an SAR enabling the identification of a clinical candidate. Lead molecules were routinely identified from natural product or company compound libraries. An example of an industry triumph from this era is the emergence of the class of NSAIDs including indomethacin, ibuprofen, and naproxen, which were identified and developed in the 1960s and 1970s [2]. These compounds improved upon the ability of aspirin to block prostaglandin biosynthesis, and have gained widespread acceptance as anti-inflammatory agents. With the

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*Abbreviations:* SAR, structure–activity relationship; NSAID, nonsteroidal anti-inflammatory drug; GPIIbIIIa, glycoprotein IIbIIIa; IL-2, interleukin-2; LFA-1, leukocyte functional antigen 1; RGD, arginine–glycine–aspartic acid; ICAM, intracellular adhesion molecule; VEGF, vascular endothelial growth factor; $\Delta G$, change in Gibbs free energy.

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advent of molecular biology in the late 1970s and 1980s and more current advances in structural biology, success in the identification of small molecule antagonists of enzymes, particularly proteases, has become almost routine today. These efforts have been aided by the increasingly more common appearance of protein structures through the 1980s and 1990s, the success of transition state analogs in the rapid identification of potent inhibitors [3], and the emergence of rational design as applied to ground state analogs of enzyme substrates and products [4]. The identification and development of aspartyl protease inhibitors provide a case study demonstrating the progress of current pharmaceutical research and the impact of these modern technological advances. The early success in the identification of potent renin inhibitors in the late 1970s and early 1980s [5] validated transition state and statine analogs as viable leads, but the ultimate commercial success of these combined approaches was achieved years later with the HIV protease inhibitors developed in the 1990s [6–8].

## 3. Cellular receptors

Small molecule antagonists of cell surface receptors, particularly the class of G-protein coupled receptors, are another notable example of a pharmacologically driven lead discovery [2]. Many of these receptors bind small peptide hormones or small molecule natural products (e.g. angiotensin and histamine). Early work in identifying antagonists of these receptors utilized natural product and compound library screening against a labeled form of the natural ligand in competitive binding assays to identify lead structures. The initial leads were refined utilizing the cell- and tissue-based assays noted above. In general, functional receptors are relatively hard to overexpress and purify by recombinant means and have not been as amenable as enzymes to the rational design process. Some success has been had expressing the extracellular ligand binding domains of cell surface receptors as secreted proteins. These constructs can be used in competitive binding assays utilizing the cognate ligand of the receptor to identify small molecule receptor antagonists. In contrast to the enzymes discussed above, there have been far fewer receptor structures determined by crystallography, NMR, or other techniques. This is particularly true for the G-protein coupled receptors that have seven membrane spanning helices [9]. Consequently, it has been difficult to isolate, purify, and study these proteins in the absence of lipids whose presence confounds both NMR and X-ray methods [10]. Molecular modeling has been utilized to explore the structural features of membrane-bound receptors with some success, but structural uncertainties in these modeled receptor structures limit their utility in the rational design of antagonists. Rather than designing ligands to fit low resolution or computed structures of the membrane-bound receptors [11,12], workers in the field have turned toward mimicry of their native ligands. Many of these cognate ligands are peptides and small molecules, which are amenable to the rational design of analogs and mimetics (e.g. angiotensin [13,14] and histamine [15]). These analogs can exhibit both agonist and antagonist activities that can be difficult to separate. One particularly successful recent example can be found in the identification of Losartan, a potent, competitive, nonpeptide angiotensin II receptor antagonist and ligand mimetic [2,16].

## 4. Protein–protein interactions

Antagonists of protein–protein interactions have become a very important and somewhat controversial topic within the pharmaceutical industry. Two recent reviews have detailed currently marketed products and recent research successes in this class of protein antagonists [17,18]. Screening as a source of leads for this class of antagonists has not been as fruitful as it has been for enzymes and receptors, but as in the case of aspirin, it was successful in a pharmacologically driven lead discovery effort and, once again, success preceded know-how. The vinca alkaloids, which for nearly 50 years have been a frontline therapy in the treatment of cancer, were identified in a natural product screening program in the early 1950s [19,20]. However, it was not until 1996 that researchers determined the mechanism of action of vinblastine in binding to a site on β-tubulin that controls the polymerization of the α–β tubulin heterodimer and the formation of the mitotic spindle [21]. More recently, the vinca alkaloids have been labeled and used as probes in screens for other natural products and synthetic compounds that compete with the probe for the same binding site on β-tubulin. Compounds that compete for this site have been shown subsequently to have the same antimitotic effect as the vincas [22]. In a curious turn of events, more modern methods including rational design have been used to design mimics of vincristine and vinblastine that are in late-stage research or clinical evaluation as newer generation antimitotic agents [23].

Over the last 10 years, additional antagonists of protein–protein interactions have reached or are about to reach the market including a number of antibodies (e.g. Reopro, targeting GPIIbIIIa; Zenapax, targeting the IL-2 receptor; Raptiva™, targeting the CD11a subunit of LFA-1) [18]. Small molecule antagonists targeted to the same proteins as these antibodies have also been identified by rational design and/or the synthesis of analogs of the natural ligand of the targeted protein. Aggrestat and Integrilin, mimetics of fibrinogen's RGD adhesive epitope [18,24], have been approved for the treatment of patients undergoing the transluminal coronary angioplasty (PTCA) procedure used to restore flow to blocked coronary arteries. In the case of LFA-1 antagonists [25], lead molecules have been identified via three approaches: first, a traditional screening program [26];

second, a nontraditional combination of rational design and an NMR-based directed fragment screening process using a recombinantly expressed domain of the CD11a subunit of LFA-1 [27]; and third, via rational design based on the SAR of ICAM-1, the native ligand of LFA-1 [28].

## 5. Enzymes, receptors, and protein–protein interactions

The preceding discussion has provided specific cases demonstrating that small molecules can antagonize enzymes, cell-surface receptors, and protein–protein interactions. Numerous further examples of small molecule antagonists of enzymes and receptors can be found throughout Goodman and Gilman [2], as well as in the current scientific literature. In the case of protein–protein interactions, as recently as 5 years ago the existence of small molecule antagonists was controversial. However, the antagonism of protein–protein interactions by small molecules is now well recognized, and the issue focuses on how these antagonists may be efficiently identified. Where appropriate, the tools and strategies utilized to identify receptor antagonists seem to have worked (see Table 1 for a listing of protein–protein interactions that have been antagonized with small molecules). These very same strategies and tools (e.g. ligand SAR, ligand analogs, ligand mimetics, rational design, and screening for binders that compete with native ligand) have been utilized in special cases of receptor–ligand interactions where the ligand is a large protein rather than a peptide (e.g. GPIIbIIIa and LFA-1; see also Table 1). Consequently, small molecule antagonists of large protein–protein interactions can be identified via the use of the same techniques and tools that have been applied successfully to the identification of receptor/ligand antagonists over the last 50 years.

The lines have been blurred between receptor–ligand interactions and protein–protein interactions. Some protein–protein interactions are receptor–ligand interactions and vice versa. Furthermore, the distinctions between enzymes, cell-surface receptors, and protein–protein interactions dissolve into a continuum with examples of receptors that have enzymatic activity (e.g. receptor tyrosine kinases), enzymes that are an assembly of subunits (e.g. the HIV protease dimer), enzyme–substrate complexes that are protein–protein interactions (e.g. whenever the substrate is a protein), and receptors that must homo- or heterodimerize in a protein–protein interaction to function [e.g. VEGF/VEGF receptor(s)] [29]. The VEGF/VEGF receptor case is particularly interesting at the mechanistic level because two VEGF receptor molecules are brought together by the binding of the VEGF dimer to the extracellular domain of the receptor [30,31]. This brings the cytosolic receptor kinase domain of one VEGF receptor in proximity to at least one of its substrates, the cytoplasmic domain of another VEGF receptor. In this mode, there are several defined points that can be antagonized to block angiogenesis: the dimerization of VEGF, the binding of VEGF and the receptor, the association of the VEGF receptor tyrosine kinase with its substrate(s), and the inhibition of the catalytic activity of the VEGF receptor kinase. An antagonist of a protein–protein interaction can exploit each of these points of intervention therapeutically. We have provided examples demonstrating that small molecules can bind to and antagonize a targeted member of either a protein–protein, receptor–ligand, or enzyme–substrate

Table 1
Examples of proteins involved in protein–protein interactions that have been antagonized by small molecules[a]

| Target protein | Protein partner | Leading reference |
|---|---|---|
| GPIIbIIIa | Fibrinogen | Expert Opin Emerg Drugs 1999;4:197–208 |
| $\alpha_V\beta_3$ | Vitronectin | Expert Opin Ther Pat 2000;10:1367–83 |
| $\alpha_V\beta_3$ | MMP2 | J Am Chem Soc 2001;123:1280–8 |
| VLA4 | VCAM | Curr Pharm Des 2002;8:1229–53 |
| LFA-1 | ICAM | Expert Opin Ther Pat 2001;11:1383–93 |
| IL-2 | IL-2 receptor α | J Am Chem Soc 1997;119:7589–90 |
| IL-1 receptor | IL-1 | Drug Des Discov 1998;15:191–8 |
| Trk A | NGF | J Pharmacol Exp Ther 1999;289:1271–6 |
| CCR5 | RANTES/HIV | Proc Natl Acad Sci USA 1999;96:5698–702 |
| Src SH2 domain | Phospho-tyrosine | Curr Opin Chem Biol 2001;5:409–15 |
| Grb2 SH2 | Phospho-tyrosine | Curr Opin Chem Biol 2001;5:409–15 |
| $p56^{lck}$ SH2 | Phospho-tyrosine | J Med Chem 2001;44:2421–31 |
| Bcl-2 family | Bak, BH3 domain | J Med Chem 2002;45:1543–58 |
| iNOS | Self-dimerization | Proc Natl Acad Sci USA 2000;97:1506–11 |
| HIV protease | Self-dimerization | Curr Opin Chem Biol 1998;2:62–6 |
| HIV protease | Substrate(s) | Annu Rep Med Chem 2001;36:247–56 |
| Calmodulin | ATPase | Neuropharmacology 1980;19:169–74 |
| Calmodulin | Myosin lt. chain kinase | J Am Chem Soc 2001;123:5382–3 |
| Tubulin | Tubulin | Med Res Rev 1998;18:259–96 |
| Hsp90 | p23 | Cell 1997;89:239–50 |

[a] Taken from Refs. [17,18].

interaction in an apparently similar manner. We will now explore the thermodynamics and kinetics of protein–protein interactions with the hope of understanding the challenges and opportunities in the identification of small molecule antagonists.

## 6. Physical nature of protein–protein interactions

The forces that contribute to protein–protein interactions include van der Waals attraction and repulsion, electrostatics, hydrogen bonds, and the hydrophobic effect. As expected, in order for two or more proteins to recognize each other and bind in solution, the protein surfaces involved in protein–protein interactions show a great deal of complementarity, both in shape and in the juxtaposition of hydrophobic, polar, and charged amino acids. These complementary surfaces are largely comprised of the side chains of each protein's non-polar and polar surface residues, although interactions with the main chain are also observed. At the interface of two proteins, non-polar regions of each protein are juxtaposed, as are the polar regions. The positive electronic character of one protein is countered by a negative feature on the partner and vice versa. When a small molecule binds to a protein in a region crucial to the formation of a protein–protein interaction, it forms a protein–small molecule complex that presents an altered surface shape and electronic profile to that protein's native protein partner (Fig. 1). Consequently, the binding of such a small molecule disrupts the complementarity and antagonizes the association kinetics and thermodynamics of a protein–protein complex. Obviously, knowledge of the physical nature of the protein–protein interaction surface is valuable to the design of small molecules.

In the discussion of protein–protein interactions, some distinctions must be made between homodimers, in which protein–protein interactions are often coupled with the protein's folding during its expression, and heterodimers, for which a rigid model of protein association is often valid. Most homodimers tend to be tightly bound, and the interfacial regions are more strongly hydrophobic than the rest of the exterior. The monomeric proteins are normally unstable and denatured in solution. In contrast, heterodimers are less strongly bound, and the interaction surfaces are more hydrophilic, reflecting the fact that the monomeric proteins remain stably folded in solution. Homodimers will likely be so strongly bound that there will be little or no free monomer for an antagonist to bind to. Thus, they will not offer a tractable small molecule target. The weaker and dynamic binding of heterodimers may allow small molecule intervention. Unfortunately, heterodimer interfaces are often flatter, providing less of a defined binding pocket in which a small molecule would form a stable small molecule–protein complex.

The physical nature of protein surfaces involved in protein–protein interactions has been studied by many groups [33,34]. Some of the aspects with potential relevance to small molecule design include the size (surface area) of the protein–protein interface, the amino acid composition of the interface, particularly the existence of binding energy ''hot spots'', the effect of long-range electrostatic interactions on protein–protein recognition and kinetics, and the characteristics of the interface that may allow the binding region to be located without direct experimental knowledge of the complex geometry. Below we review recent work in those areas.

## 7. Size of the interface

Lo Conte *et al.* [35] analyzed 75 protein–protein complexes for which a three-dimensional structure was available. They identified 52 complexes that did not undergo significant conformational change upon complexation. The interfaces in these systems involved surface areas of $1600 \pm 400$ Å$^2$. None of the complexes buried less than 1100 Å$^2$. Twenty complexes buried an even larger amount of surface area (2000–4600 Å$^2$) and also exhibited large changes in conformation upon binding. One of the smallest interfaces (the 434 repressor homodimer) so far considered has a buried surface area of 368 Å$^2$ [33]. In contrast, a sample of 20 marketed drugs have solvent-accessible surface areas that range from approximately 150 to 500 Å$^2$. There is no doubt that protein–protein interactions are much larger targets than enzyme active sites. Inhibitors of a size commensurate with that of most protein–protein interactions would no longer be considered ''small'' molecules, and would likely suffer from problems with bioavailability, and possibly antigenicity. More optimistically, a successful inhibitor does not, in principle, need to cover the whole interface; it must just disrupt binding to the extent that the desired therapeutic effect is achieved. Still, the shear size of protein–protein interfaces poses a challenge to small molecule design.

## 8. Amino acid composition of the interface

Lo Conte *et al.* [35] found that the surfaces involved in protein–protein interactions were grossly similar in amino acid composition to those of the rest of the proteins. Whereas the solvent-accessible surface area of small globular proteins is 57% non-polar, 24% polar, and 19% charged [36], Lo Conte *et al.* [35] found the interfaces to be 56% non-polar, 29% polar, and 15% charged, although with large variation. This is not good news for small molecule design, as it implies there is no special character to the interfaces we wish to target. However, closer analysis by Bogan and Thorn [37], based on a variety of alanine scanning mutagenesis data [38], suggests that protein–protein interfaces have a small region in which most of the binding energy is localized. This is comprised of a

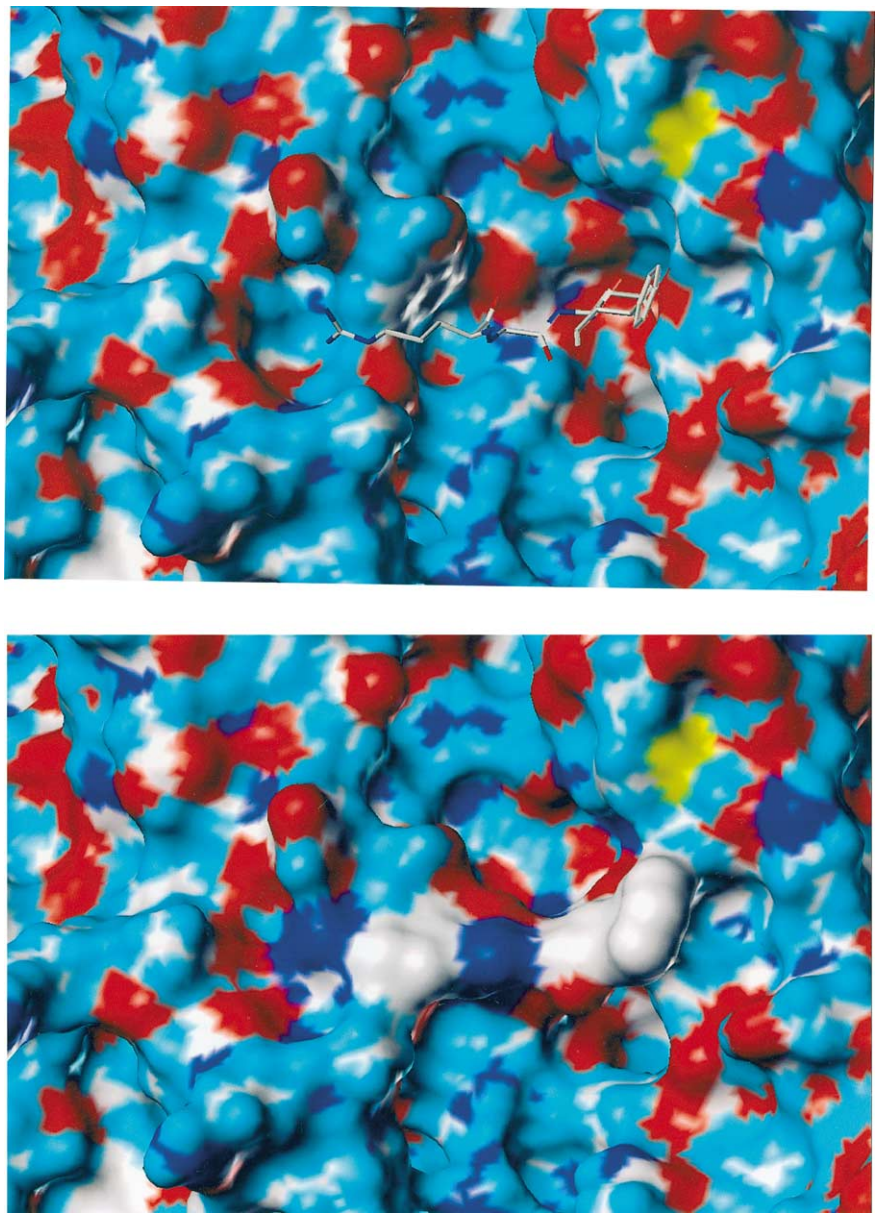

Fig. 1. (Upper panel) The Connolly molecular surface of the extracellular segment of integrin $\alpha_V\beta_3$ shown with a stick representation of a bound RGD peptide antagonist superimposed. Data from Arnaout and coworkers [32]. (Lower panel) Combined molecular surface of the RGD peptide and $\alpha_V\beta_3$, showing how the surface is modified by the presence of the peptide antagonist.

relatively polar core surrounded by a ring of hydrophobic residues, which may act to exclude water from the binding site and stabilize the interaction. Some researchers suggest that only three residues (Trp, Arg, and Tyr, in this order) contribute most of the binding energy, with Ile, Asp, and His contributing somewhat less. A similar study of antibody–antigen interactions [39], although relying strictly on computational energy calculations, also indicated Tyr, Trp, and Asp as important residues, but predicted significant contributions from Asn, Ser, and Glu. Jones and Thorton [33] found that His, Tyr, and Phe were common at interfaces. Other studies have also indicated the presence of compact protein epitopes, and that particular residues may be favored for binding, although there is obviously no agreement on the residues involved. Whereas the amount of data available on protein–protein interactions is still quite limited, all of these characterizations will likely require some revision. While it is interesting to consider that protein–protein interactions are dominated by particular residues, it is more important to small molecule inhibitor design that the binding energy is localized, rather than spread over the entire interface.

## 9. Electrostatics and kinetics of binding

Long-range electrostatic interactions play a large role in molecular recognition between proteins in solution [40].

For example, the kinetics of binding of human growth hormone (hGH) to its receptor is increased by mutations that change the total charge of one of the proteins, through an increase in Coulombic attraction [41]. There is evidence that such electrostatic mutations increase the rates of association ($k_{on}$), without affecting the off rates, indicating that these electrostatic interactions help steer the proteins to fruitful collisions in solution, but do not contribute to keeping the complex bound. The concept of electrostatic mutations has been placed on a more theoretical framework by Schreiber, Fersht, and coworkers [42–46]. For several protein complexes (e.g. hirudin–thrombin, acetylcholinesterase–fasciculin, barnase–barstar, and others), calculations of electrostatic interactions using a Debye–Huckel model accurately predict changes in association rates. More computationally demanding Poisson–Boltzmann and Brownian dynamics [47] methods have also been used to study binding, although they may not be necessary for simply assessing the kinetic effects of electrostatic mutations. While it is now clear that such information may be useful for the design of protein therapeutics, it is not clear if such knowledge can aid in the design of small molecule inhibitors. The electrostatic mutations made in proteins are generally outside of the binding site, and thus have little or no effect on the direct binding interaction. In contrast, in a typical drug molecule, any charge alteration would almost certainly change the molecule binding interactions. In addition, whereas proteins in solution can tolerate a large total charge, and thus several electrostatic mutations, the addition of a single charged group to a small molecule to enhance binding can have a large adverse effect on adsorption and, thus, bioavailability.

## 10. Determination of the interfacial region

As an increasing number of protein targets become available from structural genomics, there will be a need to determine protein function, and thus possible protein–protein interaction sites, with greater speed. In some cases, we may have a crystal structure of the protein complex prior to the design of a small molecule inhibitor. More likely, such information will be lacking and the location of the interface, the determination of which of the interacting proteins to mimic, and the determination of the particular amino acids that are important to binding will need to be done by some form of random or targeted mutagenesis in order to generate a protein SAR. Computational techniques, such as building a model of the complex from a homologous protein, or by docking the individual proteins together, may also be used. In addition, the physical properties of the protein may give indications. For example, the analysis of Jones and Thorton [33] suggests that it may be possible to distinguish protein–protein interaction sites from the rest of the protein surface in the absence of a complex structure. They based their analysis on the propensity of amino acids, the accessible surface area, a protrusion index, the planarity of the interfacial region, and hydrophobicity [33]. While none of these metrics was by itself able to reliably predict an interaction, together they could predict the interfacial regions of homodimers with >70% accuracy.

## 11. Computational tools for the design of small molecule protein–protein antagonists

A wide variety of theoretical methods have been used traditionally in the design of small molecule inhibitors. Computationally, potential key interactions can be discerned by a variety of means. It is trivial to look at the electrostatic potential at the interface, either mapped onto the van der Waals surface or the electron density isosurface, or as contour surfaces in two- or three-dimensional projections, to identify the points of the strongest negative or positive charge. Accurate representations of electrostatics can be obtained by methods that solve the Possion–Boltzmann equation. These methods were pioneered by several groups, and a number of academic and commercial computer codes are now readily available. An alternate prediction of possible binding interactions can be obtained with the program GRID, developed by Peter Goodford and his group [48]. In this case, the propensity of a variety of functional groups to occupy particular places on the surface can be predicted. These groups include positive and negative charges, but extend to more specific functionality, such as carbonyl, amine, and methyl groups. In addition to these rather static pictures of the protein–protein interface, we may also consider the dynamic nature of the interface by running molecular dynamics simulations of the complex or the individual proteins. Obviously, regions of the interface that are less mobile will likely make better targets for small molecule inhibitors than regions that undergo large conformational changes.

From a purely theoretical point of view, the size of the interface should be inconsequential. This is almost certainly the case for Poisson–Boltzmann methods that depend primarily on the atomic partial charges of the protein, which can be reasonably approximated in all cases. The general applicability of methods such as GRID to all cases of protein–protein interactions is slightly less clear. Whereas the method has been validated in the case of traditional enzyme–substrate systems with their well-defined binding pockets, it remains untested with regard to the more open surfaces of other protein–protein interactions.

Although the above techniques help us to understand the binding requirements of the interface, they do not in themselves give us small molecule leads. One of the more widely used and successful methods for lead discovery with traditional enzyme sites is *docking* [49]. In its crudest form, docking involves fitting a rigid molecule into a rigid

receptor. It has evolved to consider the conformational flexibility of both ligand and receptor. Part of the success of docking has surely been due to the steric restraints imposed by a well-defined enzyme–substrate pocket; many molecules simply cannot fit into a given pocket, and can thus be quickly and reliably discarded. Such steric and topographical constraints are greatly reduced at many protein–protein interfaces, and are absent in the limit of a completely flat surface. In such cases, the ability of the methods to identify leads depends almost entirely upon the accuracy of the potential or scoring function. Further complications arise from the fact that molecules interfering with a protein–protein interaction will still likely be substantially solvated while the methods are currently reparameterized for 'traditional' enzyme–substrate sites in which the ligand is largely desolvated. In addition to the enthalpic penalty for this desolvation, there is likely less loss of entropy when a molecule binds to a flatter protein–protein interface than into a traditional binding pocket.

All of these limitations can, in principle, be mitigated in the short term by careful reparameterization of the methods, and, as computer power becomes greater, by simply treating the problem more rigorously. For example, molecular dynamics simulations with explicit or implicit solvation could be used to explore the conformation space of a potential ligand binding to an interface, with binding energies calculated by free energy perturbation or less computationally expensive means. At present, such calculations are generally too costly to do on the scale needed to screen a data base. Ultimately, we would like to be able to calculate accurately (within 1 kcal/mol) the $\Delta G$ of binding for any given small molecule–protein complex. While such accuracy has indeed been achieved in an increasing number of cases, such calculations are still far from routine. While we expect the huge advances in computer power and algorithm design to one day make such predictions common, we currently must take advantage of computational approximations in order to make the work tractable.

## 12. Summary

From the preceding discussion, it is clear that small molecule antagonists of proteins can be identified and developed as human pharmaceuticals. The most critical challenge in this process is the choice of the protein target. Considerations should include the validity and technical feasibility of the specific protein target. We have provided examples of small molecule inhibitors of enzymes, cellular receptors, and proteins involved in a protein–protein interaction. While the latter is recognized as the most challenging of these systems, the tools used to identify antagonists of enzymes and receptors have been successfully adapted and applied to protein–protein interactions.

Given the size discrepancy between small molecules and the large protein–protein interactions that they are asked to antagonize (e.g. the molecular weight of fibrinogen is nearly 1000 times that of the small molecule antagonist Aggrestat), many researchers perceive the task as impossible. Indeed, it is unrealistic to expect a small molecule to insert itself into a protein–protein complex and pry it apart. However, with the selection of a target where there is a dynamic equilibrium between the monomeric proteins and the complex, a well-engineered small molecule can bind to one of the protein monomers, and form a small molecule–protein complex with altered topographical and electrostatic properties (Fig. 1) that is incapable of supporting the binding of the protein's cognate ligand.

Major challenges exist in the selection of protein–protein interactions that are amenable to small molecule antagonism. Those targets where one of the proteins has a relatively compact epitope have been successfully inhibited with high-affinity small molecules [17,18]. Many of these opportunities have been identified through protein structural and/or mutagenesis studies to have a number of critical amino acid side chains on the protein surface that are clustered within the dimensions of a small molecule framework. One can speculate that these epitopes have evolved over time for a specific purpose and that compounds which mimic them may have an advantage as pharmaceuticals in their safety profiles. As our experience in the antagonism of protein–protein interactions increases, our confidence and recognition of additional factors contributing to their success will certainly expand. Our ability to recognize and target these systems will be challenged over the next 5–10 years by the enormity of the number of protein targets that have been identified as part of the human genome. As noted for the case of VEGF and its receptor(s), a single target can provide multiple points of intervention for an antagonist of a protein–protein interaction. Computational methods combined with physical studies of protein structure and function (SAR) will be crucial to increased success in the identification of antagonists of protein–protein interactions.

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