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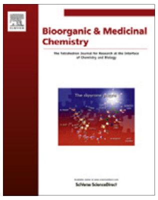

Plucking the high hanging fruit: A systematic approach for targeting protein–protein interactions

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ABSTRACT

Development of specific ligands for protein targets that help decode the complexities of protein–protein interaction networks is a key goal for the field of chemical biology. Despite the emergence of powerful in silico and experimental high-throughput screening strategies, the discovery of synthetic ligands that selectively modulate protein–protein interactions remains a challenge for bioorganic and medicinal chemists. This Perspective discusses emerging principles for the rational design of PPI inhibitors. Fundamentally, the approach seeks to adapt nature's protein recognition principles for the design of suitable secondary structure mimetics.

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1. Introduction

Protein–protein interactions (PPIs) are attractive targets for therapeutic intervention because of their fundamental roles in vital biological processes including gene expression, cell growth, proliferation, nutrient uptake, morphology, motility, intercellular communication and apoptosis. The past decade has seen emerging methods to inhibit these complexes, which have traditionally been termed 'undruggable'. Although it is early to say if inhibition of PPIs will become a routine strategy for drug design, preliminary success in the field provides guidelines for the types of interfaces that may be amenable to disruption by synthetic compounds.[1–4] Pharmaceutical chemists often gravitate toward design of enzyme inhibitors as drug candidates—enzymes as a class constitute roughly half of drug targets.[5] Enzymes are appealing targets for a list of reasons: (1) they serve as critical levers for biological functions, (2) nature offers small molecules that may be used as templates for further manipulation,[6] (3) mechanism-based inhibitors may be rationally designed,[7] and (4) enzyme pockets are often appropriately sized for small molecules.[8,9] The largely flat and pocket-less protein interfaces lack several of these features but are fascinating both because of basic challenges associated with molecular design and for the prospect of exploring relatively uncharted biology for therapeutic intervention.

2. A secondary structure-centric view of protein interfaces

The Protein Data Bank, with roughly 10,000 entries of multiprotein complexes, provides a treasure of illustrations to unravel the rules nature employs to bring protein partners together (Fig. 1). One approach for the design of synthetic inhibitors is based on mimicry of protein subdomains to interfere with these complex formations.[4,10–13] A second successful method utilizes computational and experimental high-throughput and fragment-based screening strategies to locate small molecule fragments that stick to protein surfaces.[14–19] Strategies that afford scaffolds for PPIs based on natural products and natural product like molecules,[20] peptide macrocycles[21] and phage display-based miniproteins[22–24] have also led to significant success. Although, PPIs cover large surface areas, often, a small subset of residues (termed 'hot spot residues') contributes significantly to the binding free energy.[25–28] An analysis of PPIs, which have been successfully inhibited by small molecules, suggests that a category of PPIs contains features that approximate enzyme active sites, that is, they contain an array of hot spot residues clustered within relatively small radii.[27–29] With this viewpoint, we and others have assessed the dataset in the PDB[30] to identify the subset of PPIs that can be potential targets for small molecules, and those that would require larger molecules.[4,31–34] Several computational strategies to define *pockets* on protein–protein interfaces for drug design have been outlined.[4,35–39] Our approach has centered on the role of secondary structures in mediating protein–protein interactions.[40] A key advantage of the protein secondary structure mimetic strategy is that the array of side chain residues along a conformationally defined backbone facilitates molecular design. A second advantage is that direct mimics of protein secondary structures provide medium-sized molecules, which may potentially target the chosen protein with high affinity and specificity.

A basic challenge associated with this secondary structure-centric approach involves dissection of the energetic contribution of the specific secondary structure to the protein–protein complex.

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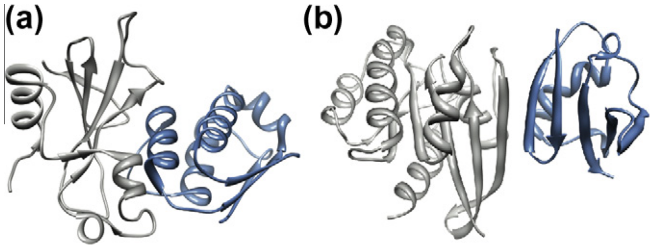

Figure 1. Protein–protein interactions are often mediated by secondary structures: (a) α-helical and (b) β-sheet interfaces from barnase_barstar (PDB code: 1BGS) and Raf-Rap (PDB code: 1GUA), respectively.

Three related questions include: (1) what is the minimum proportion of the free energy of binding that must reside on the secondary structure for its mimetic to retain inhibitory activity? (2) Are there interfaces that are naturally more suited for small mimics of target protein secondary structures and those that would require larger mimics? (3) How small can the mimetic be made to procure the ‘drug like’ properties linked to small molecules while retaining specificity associated with larger molecules? We have undertaken computational efforts to probe these questions and have begun to experimentally evaluate hypotheses emerging from these studies.

Our initial analysis has focused on PPIs that feature helices at interfaces, although the approach can be extended to other motifs. Targeting of α-helical interfaces offers several basic advantages: α-helices constitute the largest class of protein secondary structures—roughly 60% of protein–protein interactions in the current PDB contain helices at interfaces.[31] And, helices are often easier to mimic than other secondary structures such as β-strands, which tend to aggregate; although, there has been significant progress in the design of β-strand mimics.[41–48] Importantly, stable mimics of interfacial helices have been shown to be useful as potential leads for drug design.[11,49–55]

In this Perspective, we discuss structural attributes of PPIs that our group uses to initiate design of either small molecule helix mimetics[56–60] or stabilized peptide helices.[10,13,61–63] Some interfaces may be targeted by either strategy. Stabilized peptide helices utilize constraints to order the peptide backbone while small molecule or nonpeptidic helix mimetics array the critical peptide side chain functional groups on a synthetic scaffold.[13] On the basis of spatial arrangement of hotspot residues at the interface, revealed by alanine scanning mutagenesis data,[64,65] we classified PPIs as *binding clefts* or *extended interfaces* (Fig. 2).[31] Receptors with clefts are targeted by helices with two or more hot spot residues within a 7 Å radius, while the extended interfaces category features a distribution of hot spot residues over a larger distance of 7–30 Å. Camacho and coworkers recently suggested that a combination of computed change in solvent accessible surface areas (ΔSASA) and energy scores may be a better gauge of hot spot residues than alanine scans alone.[33] For alanine mutagenesis scans, the $\Delta\Delta G$ value refers to the change in free energy when a residue is mutated to alanine, thus a positive value indicates that mutation to alanine decreases the affinity of PPI and wild type residue contributes to binding. For interfacial residues, the ΔSASA of a residue is calculated by subtracting the SASA of the residue in the PPI complex from the SASA of the individual residue without any partner protein chains, and a positive value indicates that the residue is buried in the PPI complex and less accessible to solvent. Rosetta[29,65] and PocketQuery[66] offer easily accessible resources for such computational analyses.

We hypothesized that a single turn of the α-helix approximates the distance traversed by typical small drug candidates,[39] that is, Lipinski’s ‘rules of five’ compliant molecules.[67,68] Indeed, the classical small molecule inhibitors of PPIs—notably the nutlin family compounds developed by Roche to target the p53/Hdm2 complex—are mimics of residues that span one helical turn.[69,70] The high density of hot spot residues in *binding clefts* evoke array of functionality in enzymatic pockets. Based on this analysis, we postulate that *binding clefts* may be targetable by small molecule or nonpeptidic helix mimetics; however, *extended interfaces* that feature hot spot residues spanning a much larger number of helical turns will likely require medium to large sized molecules such as stabilized peptide helices for specific inhibition.[31] Although, these larger inhibitors will not fit the mold of canonical ‘drug-like’ molecules, emerging evidence suggests that macrocycles and other discretely folded molecules may translocate to target intracellular interactions.[71–73]

We further classified interfacial interactions as potentially capable of leading to ‘high affinity’ inhibitors if the average experimental/computed $\Delta\Delta G_{avg}$ for alanine mutagenesis of 2–4 hot spot

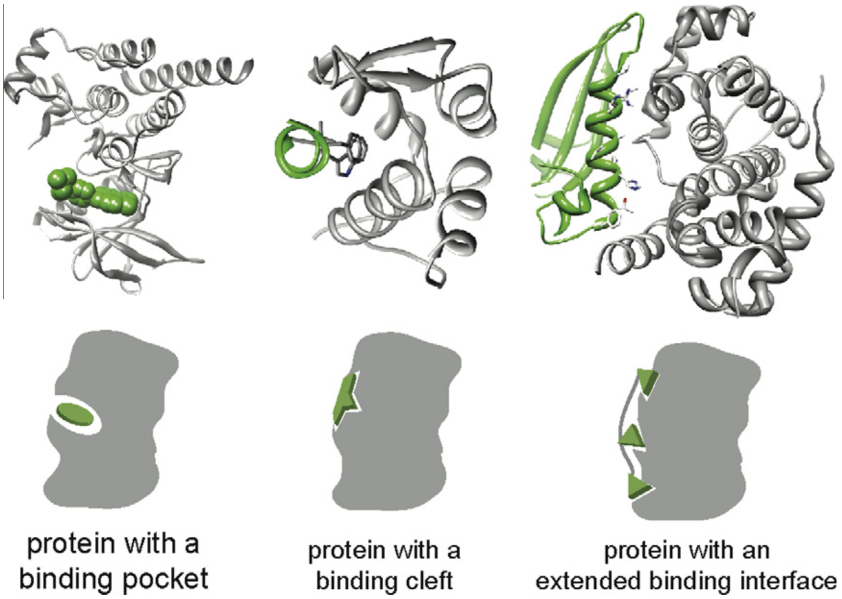
protein with a binding pocket
protein with a binding cleft
protein with an extended binding interface

Figure 2. Helical interfaces can be divided between those that feature *clefts* for binding and those with *extended interfaces*. The cleft interfaces may be compared to enzyme pockets in that a high density of important contacts is concentrated in a small region. The Gleevek/tyrosine kinase (PDB code: 1XBB), p53/MDM2 (PDB code: 1YCR) and cyclin-dependent kinase6/D-type viral cyclin (PDB code: 1G3N) complexes are representative examples of enzyme pockets, binding cleft and extended interfaces, respectively.

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