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Review

Systematic Targeting of Protein–Protein Interactions

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Over the past decade, protein–protein interactions (PPIs) have gone from being neglected as 'undruggable' to being considered attractive targets for the development of therapeutics. Recent advances in computational analysis, fragment-based screening, and molecular design have revealed promising strategies to address the basic molecular recognition challenge: how to target large protein surfaces with specificity. Several systematic and complementary workflows have been developed to yield successful inhibitors of PPIs. Here we review the major contemporary approaches utilized for the discovery of inhibitors and focus on a structure-based workflow, from the selection of a biological target to design.

Trends

Natural protein complexes are not always optimized for affinity. Computational approaches to locate underutilized and cryptic pockets are leading to new classes of potent inhibitors.

Traditional modulators of protein–protein interactions (PPIs) comprise orthosteric inhibitors; however, we are now seeing a rise in allosteric modulators as well as stabilizers of PPIs.

Protein secondary structure mimics have been well developed over the past decade and now there is a push for the development of protein tertiary and quaternary mimics.

Advances in proteomics are paving the way to identify direct protein targets of modulators.

Approaches to Targeting PPIs

Selective recognition of one protein by another–PPI–governs the three key dimensions of cellular life: growth, survival, and differentiation. Modulators of these interactions are critical both for understanding the cellular networks governing biological functions and for developing new therapeutics. Despite their fundamental role, PPIs are often considered unattractive targets for drug discovery, as illustrated by the fact that less than 0.01% of the PPIs constituting the interactome have been targeted with an inhibitor [1]. However, recent advances in proteomics, computational chemistry, and ligand design provide new road maps for manipulating these recalcitrant targets.

From this perspective, we focus on structure-guided approaches to developing PPI inhibitors. We begin with a brief discussion of workflows for phenotypic and target-guided screens as well as structure-based design. Within structure-based design, we highlight two complementary approaches rooted in fragment-based design and protein domain mimicry for the rational design of PPI inhibitors. These methods rely on mimicry of the interfacial residues that contribute most significantly to binding. Identification of these critical contacts, termed 'hot-spot' residues, is facilitated by computational assessment of protein–protein complexes.

Phenotypic Screens

Approaches to inhibitor design can be categorized into: (i) phenotypic screening; (ii) target-based screening; and (iii) structure-based design (Figure 1) [2,3]. In phenotypic screens, also referred to as 'forward chemical genetics', the goal is to find a hit from a collection of compounds that leads to a desired and specific biological result such as inhibition of mitosis, modulation of transcription of a particular gene, or inhibition of specific kinase signaling [4]. Phenotypic screens are often performed with libraries of drug-like molecules, and compounds that emerge from these screens become attractive leads for drug discovery [3]. A key benefit of phenotypic screens is that they provide impetus for finding new targets that drive the desired biological activity [4]. Several compounds that gave the field of chemical genetics its initial appeal have been discovered through phenotypic screens. Monastrol, an inhibitor of mitotic spindle formation, was found in a small-molecule library during a search for compounds that induced changes

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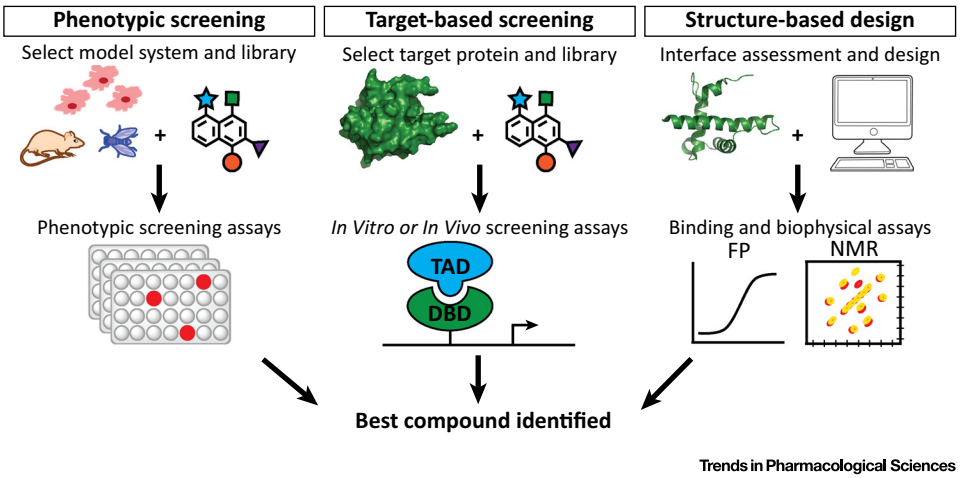

Figure 1. Approaches to Inhibitor Design Can Be Categorized into Phenotypic Screening, Target-Based Screening, and Structure-Based Design. Left: Phenotypic screening. A compound library is screened in a model system (i.e., cells, mice, flies) and analyzed for a specific phenotype. Center: Target-based screening. A library is screened against a particular protein target of interest in cell-free or cell-culture assays. Right: Structure-based design. A protein of interest is computationally assessed to design a modulator. Binding and biophysical assays are then performed on designed modulators to determine the best compound.

in spindle formation without perturbing tubulin polymerization [5,6]. The discovery of monastrol also led to the discovery of its target, motor protein Eg5, establishing the elegance and potential of forward chemical genetics [6]. Similarly, the anticancer drug lenalidomide, which has been approved by the US FDA, was discovered from a phenotypic screen, but its target, the E3 ligase protein cereblon, was not elucidated until years after its approval in 2012 [3,7]. Unsurprisingly, target identification and determination of the mechanism of action of advanced lead compounds remain significant bottlenecks [2]. Several high-throughput mass spectrometry strategies have been implemented to mitigate this challenge. They include, but are not limited to, classic affinity pull-down assays, activity-based protein profiling (ABPP), chemical capture compound assays, stable isotope labeling by amino acids in cell culture (SILAC), isotope-coded affinity tags (ICAT), isobaric tags for relative and absolute quantification (iTRAQ), drug affinity responsive target stability (DARTS), and stability of proteins from rates of oxidation (SPROX) [4,8,9].

Target-Based Screens

In target-based screening, also referred to as 'reverse chemical genetics', specific compounds are screened to modulate a particular target or protein of interest [4]. This approach requires a biologically validated target or pathway; however, a high-resolution structure of the target is not needed. Target-based drug discovery has gained prominence with the growing understanding of cellular networks and molecular targets [3,10]. Several methods, including ELISA-based screens, split luciferase, and yeast two-hybrid assays, are widely used to screen compounds against a desired protein of interest both *in vitro* and *in vivo* [11]. These approaches do not require an intimate knowledge of the molecular details of targeted protein interfaces. Nutlins, which are small-molecule ligands of Mdm2 and potent inhibitors of the p53–Mdm2 interaction, were discovered from a target-based high-throughput screen [12,13]. While high-throughput screening has become relatively low cost and efficient, replication of the PPI within the assay remains problematic. For example, only part of the protein target may be able to be expressed and amenable to an assay format, or multiprotein complexes and other cofactors may play a more substantial role *in vivo* than what is replicated in the assay [14]. Another general challenge of screening approaches for PPI targeting is that often the compound libraries are not structurally diverse enough to target large and diffuse interfaces [15]. To address this challenge,