Advances in Biochemistry & Applications in Medicine

Chapter 1

Protein-Protein Interactions as Potential Targets of Drug Designing

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Abstract

Protein–protein interactions (PPIs) control a large number of biological processes. An attempt to design novel drug molecules has led to an increasing interest in the protein surfaces. Studying interaction interfaces in PPIs has set the stage for drug discovery to identify therapeutic agents for a range of human ailments. Inhibiting or stabilizing PPIs with small molecule drugs controls cellular behaviours significantly affecting disease outcomes. On one hand inhibiting PPIs can modulate pathological conditions, while on the other hand stabilizing certain PPIs can potentially treat diseases. It is, therefore, the calculated targeting of specific PPIs, with either small molecule inhibitors or stabilizers, which will have significant pharmacological value. PPIs control regulatory pathways and have been widely studied to design novel chemotherapeutics. Examples of certain small molecule drugs targeting PPIs involved in different biological pathways are discussed here in.

Keywords: Protein–protein interactions; Drug designing; Small molecule inhibitors; Small molecule stabilizers

1. Introduction

The pathology of diseases often lies in a complex web of molecular interactions that needs to be understood both at clinical and molecular levels. Protein–protein interactions are of crucial importance in biological systems and have implications in the development of diseases. Recently, scientists have showed that certain PPIs could be targets of drugs with great significance in healthcare. The importance of the complex network of interactions between proteins and interactome is widely recognized in all biological systems. The human interactome is esti-

mated to involve approximately half a million PPIs, unravelling which, may provide answers to many baffling questions in biology. Moreover, PPIs provide a wealth of information for therapeutic intervention in a host of human diseases. Understanding the architectural interfaces of protein interactions has identified the fine structural features such as pockets, grooves, or clefts as potential docking sites for small drug molecules [1]. The structural intricacies of the interaction interfaces often poses a challenge as the binding sites of PPI surfaces are often formed by native high order protein conformations rather than the linear amino acid sequences, thus preventing the use of a linear peptide templates for designing new therapeutic agents [2]. Another impediment to alternative approach of drug design, is the lack of natural ligands which could mimic the molecular interactions. However, with the identification of "hot spots" in PPIs, it became possible to target a wider range of PPIs with small molecule drugs [3]. The small chemical mimics that modulate PPIs, can directly interfere with protein interactions at the interfaces causing disruption or stabilization of these interactions (**Figure 1**). Targeting PPIs for designing drugs has continued to be a daunting task. However, rapid advances in the understanding of disease processes and their underlying mechanisms highlight multifactorial strategies to identify and design effective drugs [45]. A large number of diseases, including Alzheimer's disease, Parkinson's disease, Huntington's disease and majority of cancers originate from aberrant interactions of specific PPIs. An increasing interest to investigate the unexplored PPIs for drug discovery is driven by the need to find novel therapeutic agents for a whole range of diseases with a high medical relevance. The disruption of PPI with small molecules for drug design has been significantly explored.

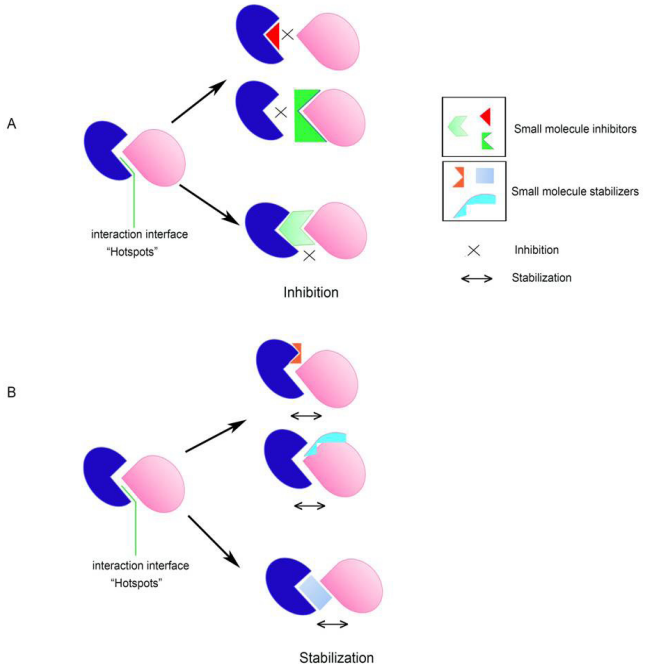

Figure 1: PPI modulators binding to interacting proteins and interaction interfaces. Small molecule inhibitors/stabilizers binds to the individual proteins or together to their binding interfaces there by preventing or reinforcing their interaction. (**A**) Inhibitors bind to interacting proteins either individually or together to cause the inhibition and modulate the interaction kinetics (**B**) Stabilizers bind to the individual interacting proteins or interaction interface together and modulate the interaction kinetics. These small molecule drugs induce a conformational change that inhibits or stabilizes the association with the proteins.

2. Targeting PPIs in Treatment of Cancers

Cancers have been extensively studied to explore PPIs involved in signalling pathways. Interaction of murine double minute 2 (MDM2) with p53 is widely studied PPIs in oncology (**Figure 2A-D**). p53 is a tumor suppressor that plays a critical role in cell cycle regulation, DNA repair and programmed cell death [6]. The mutations in p53, a transcription factor is the cause of almost 50% of human cancers [7] and in most other cancers, the function of p53 is disrupted by one of the several mechanisms. MDM2 is the inhibitor of p53 which directly binds to it and represses its activity by increasing its nuclear export and proteasomal degradation [8]. MDM2 and p53 interact with each other through hydrophobic residues in their N-terminal domains [9]. Scientists have tried to target these hydrophobic residues to identify small molecule drugs that can interrupt MDM2 and p53 interactions [10]. Such pursuits have resulted into designing of several MDM2-p53 interaction disruptors that are already in clinical phase.

Orally administrable imidazoline-based compounds that mimic p53 for binding to MDM2 have been designed. Yet another inhibitor of MDM2, RG7112 was identified by chemical screening and has found use in treatment of sarcoma, leukemia and neoplasms (Fig.2E) [11]. An chemical analogue of RG7112 is a pyrrolidine-containing compound RG7388, which is a more selective and potent p53-MDM2 inhibitor [12]. Many other small chemical molecules identified to selectively target MDM2/p53 interactions are MI-77301, AMG 232 (AM-8553), MK-8242 (SCH 900242), DS-3032b and CGM097 [13].

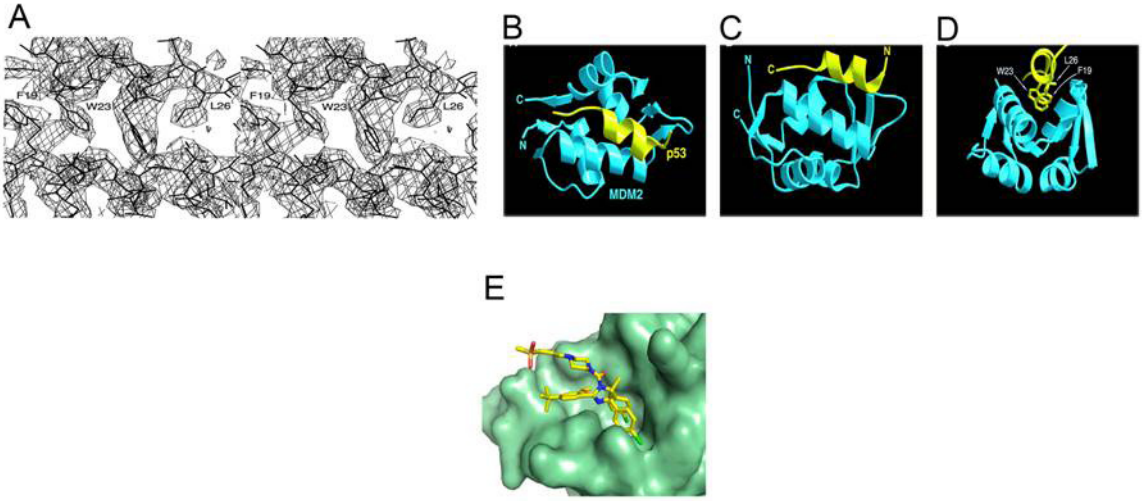

Figure 2: (**A**) Electron density map of the X. laevis MDM2-p53 interface at 3.0 Å resolution with 2.3 Å resolution represented in stick model. Stereo view shows the interactions of F19, W23, and L26 of p53 with the α2 helix of MDM2 10(**B**) The MDM2-p53 complex. MDM2 is in cyan, p53 peptide is in yellow (**C**) 90° rotation of the MDM2-p53 complex about the horizontal axis of (**B**) (**D**) The complex rotated approximately 90° about the vertical axis of (C), looking down the helix axis of p53 [10](**E**) Crystal structure of MDM2 bound to small molecule inhibitor RG7112 (carbon atoms in yellow, oxygen in red, nitrogen in blue, sulfur in orange and chlorine atoms in green) [11]. Figure 2(A-D) adapted from 10 and Figure 2(E) adapted from [12].

Targeting PPIs in apoptosis or programmed cell resulting into activation of caspases can have implications in cancer research. Caspase-9 activates procaspase-3 and procaspase-7 by dimerization into a catalytically active form [14]. The inhibitor of apoptosis proteins (IAPs) which are constitutively active in tumor cells prevent programmed cell death. The XIAP (X-linked IAP) is a target of choice for drug development in cancers. It is by far the most potent caspase inhibitor among the IAP protein family. This protein interacts with initiator caspase-9 and inhibits its dimerization, which is required for its catalytic activity. Over the years, targeting XIAP–caspase interaction for the treatment of cancers has attracted a lot scientific attention. Since the identification of the SMAC protein as a natural inhibitor of XIAP, many SMAC mimics have been designed [15].

The B-cell lymphoma 2 (BCL2) family of proteins are involved in the intrinsic apoptotic pathway [16]. By nuclear magnetic resonance (NMR)-based screening scientists identified ABT-737, a potent small molecule drug that inhibits Bcl2, Bcl-XL, and Bcl2l2 (**Figure 3**) [17]. Further research led to the development of Navitoclax (ABT-263) as a more potent inhibitor of Bcl2 family, both in terms of pharmacokinetics and efficacy. However, suppression of Bcl-xL by ABT-63 led to the development of thrombocytopenia which was further circumvented by designing Bcl2-specific drug ABT-199 (RG7601). Mcl1 has been targeted by small drugs to treat cancers. Increased expression of Mcl-1 leads to sequestration of the proapoptotic proteins Bak, Bax, Bad, and Bim. Thus, targeting Mcl-1 has been effective to disrupt the interaction of Mcl-1 with proapoptotic factors and treat cancers [18]. Many indole-carboxylic acids bind to Mcl-1 particularly at low doses and effectively interrupt Mcl-1/Bim interactions.

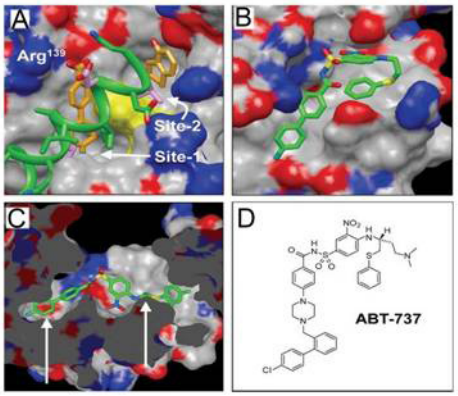

Figure 3: Generation of ABT-737. (**A**) Molecular surface of the complex of Bcl-XLwith a Bak- peptide (GQVGRQLAI-IGDDINR, green) overlaid on the ternary complex of Bcl-XL and NMR-based screening leads (orange). F97 is shown in yellow. Bak peptide residues (E83, L78, I85) critical for binding are shown in magenta. (**B**) Surface view of the NMR structure of Bcl-XL complexed with drug (**C**), Cutaway molecular surface for the NMR structure of domain III of human serum albumin bound to the thioethylamino-2,4-dimethylphenyl analogue of drug inhibitor. Arrows indicate proposed sites of modification. (**D**) Chemical structure of ABT-737. Fig 3 adapted from [17].

Targeting PPIs in the formation of complexes of transcription factors has also yielded significant results in treatment of cancers. Many researchers have effectively targeted bromodomains which mediate interaction of transcription factors to activate oncogenes [19]. These drugs have been efficient anti-cancer agents [20].

3. Drugs against PPIs Involved in Signal Transduction

Cells do not live in isolation and continuously coordinate and communicate with each other. A large variety of small molecules including peptides, proteins etc. act as signaling molecules in cell-to-cell communication. These signalling molecules called "ligands" interact with specific receptors at the surface of cells. Some signaling molecules especially steroids bind intracellular receptors. The binding of a ligand to its receptor leads to conformational changes in the receptor that eventually induces downstream signaling events to show cellular responses. Signalling proteins can act as adaptors or anchoring proteins, mediating PPIs. Owing to the cascade of signalling events, signalling proteins form complexes, either stable or transient with a number of proteins. However, these PPIs are specific and lead to precise downstream signals to affect a cellular response. Over 20 years a considerable progress in understanding of the mechanisms by which ligands bind to the receptors at the plasma membrane to transduce downstream signals has been made.

Stimulation of receptors controls cellular physiology and disruption of ligand-receptor interaction leads to pathological conditions. Targeting ligand-receptors interactions by small drugs could, therefore be important for the treatment of a variety of diseases. Cushing's disease (CD) results from elevated levels of glucocorticoids leading to endogenous hypercortisolism. The glucocorticoids bind to glucocorticoid receptor (GR) to transduce downstream signalling. When unstimulated, GRs form a complex with chaperone HSP90 which prevents the misfolding of GR. HSP90 also facilitates glucocorticoid binding and translocation of the receptor to the nucleus [21]. Silibinin, an extract of milk thistle seeds has been used to treat prostate cancer and liver toxicity and also has therapeutic value in the treatment of CD. Silibinin binds to HSP90 and inhibits its interaction with GR [22]. The CD caused by misregulation of GR sensitivity can be pharmacologically treated with silibinin.

Cytokine interleukin-17A (IL-17A) plays an important role in several immune diseases including psoriasis, psoriatic arthritis, Crohn's disease and rheumatoid arthritis. IL-17A activates proinflammatory genes, increasing production of chemokines, cytokines and other antimicrobial peptides [23]. The interaction between IL receptor and IL-17 has been under investigation to treat inflammatory diseases. Certain IL-17A inhibitors have shown promising activity in clinical studies. A series of small molecule drugs (Ensemblins) that inhibit interaction of IL-17 with its receptor have been synthesized. NMR studies have helped understand the interaction of IL-2 with the α-subunit of its receptor (IL-2Rα). This approach has also helped in designing small molecule inhibitors of this interaction [24]. Tumor necrosis factor α (TNFα) is important for many inflammatory diseases, including inflammatory bowel disease and hepatitis. It can be therapeutically explored for clinical interventions. Chemical inhibitors for TNFα have been designed by computerized systems. (E)-4-(2-(4-chloro-3-nitrophenyl) binds to TNFα and inhibits the TNFα-induced signaling cascade [25]. Many drugs that prevent the

interaction of TNF with its receptor have also been designed and many are under preclinical trials. Another drug inhibiting PPIs in immune signalling is lifitegrast (SAR1118). It inhibits the interaction between LFA-1 and ICAM1 involved in T-cell activation [26].

4. Small Molecule Drugs for Neurological Diseases Treatment

PPIs between membrane-resident receptors and signaling molecules control neuronal function and provide targets for drug design in neuro-pharmacology. Aggregation of amyloid beta (Aβ) peptides in the brain leads to severe neuropathological conditions. Aβ peptides have been considered as a potential target for Alzheimer's disease (AD), as cerebral Aβ deposition plays a key role in the AD. Blocking the aggregation of Aβ peptide with small molecule drugs would, therefore, promise the development of novel therapeutics for AD. Elevated levels of Aβ (42) peptide formation causes early-onset familial AD due to mutations in Aβ precursor protein (AβPP). Lysis of AβPP by the β- and γ-secretases releases the N- and C-termini of the Aβ peptide. L-685 and L-458 have been identified as structurally novel inhibitors of AβPP γ-secretase interaction [27]. Alternatively, benzodiazepine-containing γ-secretase inhibitors have been designed that are potentially important for treatment of AD [28]. Structure-based studies have incorporated a substituted hydrocinnamide C-3 side chain to design highly potent inhibitors of γ-secretase [29].

Parkinson's disease (PD) is another serious neurodegenerative disease associated with protein misfolding and aggregation. Synucleins are small charged proteins primarily expressed in neuronal tissues. Misfolding and aggregation of α-synuclein (α-syn) results in the formation of self-associating β-pleats called “Lewy bodies”[30]. Dopamine inhibits fibrillization of α -syn generating spherical oligomers (**Figure 4**). Mutagenesis and competition assays with synthetic peptides identified 5 amino acid residues (125-129;YEMPS) to be important for the dopamine based inhibition of α-syn fibrillization. More importantly, the oxidation product of dopamine, dopaminochrome has been identified as a more specific inhibitor of α -syn oligomerization. Dopaminochrome inhibits α-syn fibrillization by inducing conformational changes through the interaction with the YEMPS region of α-syn [31]. A study which screened a library of small molecules to identify drugs to inhibit α-syn fibrilization, identified catecholamines as fibril inhibitors [32]. This provides an explanation for the role of α-syn in PD and has implications for chemopreventive and diagnostic applications in future.

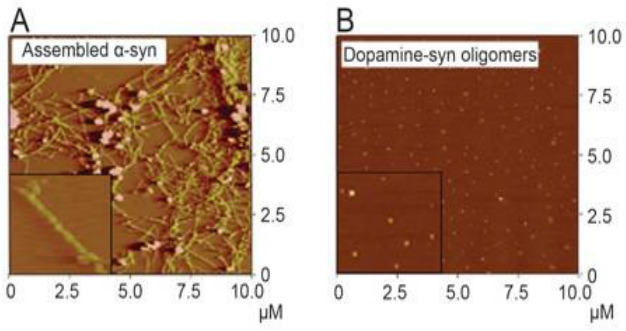

Figure 4: Dopamine inhibits α-syn fibril formation. (**A**) Atomic force microscopy (AFM) images showing the filamentous α-syn after assembly (***B***) AFM images showing spherical oligomers after α-syn is incubated with dopamine and purified (dopamine-syn oligomers). Figure 4 adapted from [31].

Table 1: List of small molecule inhibitors of PPIs discussed in the text. These molecules have been designed to inhibit specific PPIs that modulate biological processes.

PPIs	Small molecule inhibitors
MDM2/p53	RG7112
	MI-77301,
	AMG 232 (AM-8553),
	MK-8242 (SCH 900242),
	DS-3032b
	CGM097
Bcl2 family	ABT-737
	Navitoclax (ABT-263)
	ABT-199 (RG7601)
GR/HSP90	Silibinin
Amyloid beta peptides (AβP)	L-685 and L-458
	benzodiazepine-containing γ-secretase inhibitors
Synuclein	dopamine
	dopaminochrome
	catecholamines
IN/LEDGF	BI 224436
FtsZ/ZipA	indolo[2,3-a]quinolizin-7-one

5. PPIs and Immunity

Humans continually encounter microbes that are harmful and cause diseases. The pathology of these micro-organisms depends on both the pathogenicity of the organism and the robustness of the host immune system. The immune system is an interactive network of lymphoid tissues, circulating immune cells, antibodies, and cytokines. The essential function of the immune system is to recognize and effectively neutralize pathogens. The majority of viruses survive by overtaking the cellular machinery of hosts. PPIs between viral and host proteins or among viral proteins which are essential for their growth and progression in the host are important clinical targets. The retroviruses secrete the enzyme retroviral integrase (IN) which helps it to integrate the retroviral DNA into the DNA of the host. Human protein lens epithelium-derived growth factor (LEDGF) interacts with IN and helps the viral integration into the host genome, further preventing proteolytic degradation of IN. The interaction of LEDGF with IN has been targeted for effective anti-viral therapy. The small molecule inhibitors of this PPI called LEGDINs have been designed by analysing the structural interface of LEDGF-IN interactions. LEGDINs disrupt the LEDG-IN interactions and also act as the allosteric inhibitors of IN [35]. The most important LEDGINs are tert-Butoxy-(4-phenyl-quinolin-3-yl)-acetic acid

derivatives e.g BI 224436 which is an allosteric inhibitor of viral integrase of HIV-1[36,37]. (**Figure 5**).

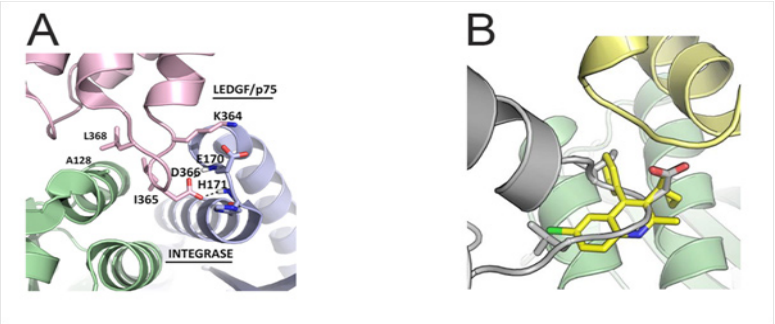

Figure 5: LEDGF/p75–IN interaction and its inhibition by LEDGINs (**A**) Representation of the LEDGF/p75-IN complex. IN molecules are shown in green and blue, whereas the LEDGF/p75 is shown in magenta. Residues critical for the interaction are represented as sticks and highlighted (**B**) Co-crystal structure of tert-Butoxy-(4-phenyl-quinolin-3-yl)-acetic acid derivative (yellow stick) bound in the LEDGF/p75 binding pocket of HIV-IN (green and yellow) . Figure 5 adapted from [35].

PPIs have also been targeted with small molecule drugs to inhibit the replication of human papilloma viruses (HPVs) in host cells. Interaction of E2 transcription factor and E1 initiation factor of human papillomaviruses (HPVs) is essential for the replication of viral DNA. A series of small molecule inhibitors that specifically bind to the N-terminal transactivation domain (TAD) of E2 (of HPV-11) and prevent its interaction with E1 have been described in [38].

In addition to viruses, bacterial PPIs have also been studied to design small molecule chemotherapeutic agents for bacterial infections. A tubulin homologue, FtsZ is involved in prokaryotic replication and is ubiquitous in eubacteria and archea. Like Tubulin, it assembles into protofilaments and mini-rings to form cytoskeletal structures. Recent advances in immunofluorescence and microscopy have showed that FtsZ is localized in septal rings in bacteria [39]. ZipA is a membrane anchored protein involved in the assembly of the septal ring to regulate cell division. X-ray crystallographic studies have showed that FtsZ and ZipA interact through C-termini and this interaction is essential for the bacterial replication [40]. Targeting the interaction of FtsZ and ZipA has become important for antibacterial therapy. A small molecule inhibitor, indolo[2,3-a]quinolizin-7-one targeting the key region of ZipA involved in binding to FtsZ has been synthesized. The crystal structure of this molecule bound to ZipA has also been solved [41]. Moreover, NMR screening coupled with structure-based analysis identified novel inhibitors of the ZipA/FtsZ complex and the X-ray crystal structures of these analogues with ZipA were also solved to gain insights into their structures for synthetic chemistry [42].

6. Stabilizing PPIs

In addition to inhibiting PPIs certain PPIs need to be stabilized for the desired molecular consequences of those interactions. Small molecule stabilizers of PPI either binds to one of the interaction partners or to the interaction interface and stabilize the interactions (**Figure 1B**).

Paclitaxel derived from Taxus brevifolia binds to β-tubulin, thereby stabilizing α-β tubulin polymerization [43,44] (**Figure 6**). This is one of the important anti-cancer drugs used clinically to stabilize monomer stabilization in microtubule assembly. Similarly other natural products like FK506 , rapamycin, fusicoccin, brefeldin and forskolin modulate disease conditions by stabilizing specific PPIs [45,46]. FK506 and rapamycin bind tightly to FKBP12, stabilizing FKBP12/calcineurin and FKBP12/mTOR interactions respectively. Recently a number of synthetic molecules have been reported to stabilize oligomeric interactions. Mizoribine (MIZ) an imidazole based nucleoside interacts with 14-3-3 proteins. 14-3-3 proteins interact with many signalling components, including the glucocorticoid receptor (GR). MIZ affects the conformation of 14-3-3 proteins and stabilizes its interaction with GR [47]. Many of the PPI stabilizers such cyclosporine, rapamycin, glycosides fusicoccin A and cotylenin A act as immunosuppressants and have been used as therapeutics for different diseases [48,49].

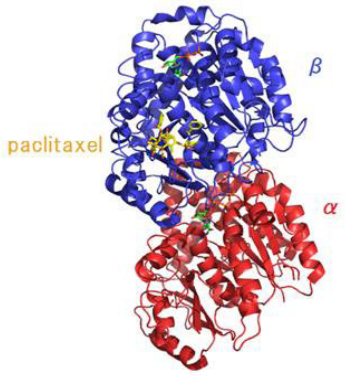

Figure 6: Binding of drug paclitaxel (yellow) to the complex of α- (red) and β-tubulin (blue) subunits. This file has been created from PDB file id= [2HXF http://www.rcsb.org/pdb/explore/explore.do?structureId= 2HXF] (Source: Takumasa)

PPIs	Small molecule stabilizers
Tubulin monomers	Paclitaxel
FKBP12/mTOR	Rapamycin
FKBP12/calcineurin	FK506
14-3-3/GR	Mizoribine
14-3-3	Cotylenin A

Table 2: List of certain small molecule stabilizers of PPIs discussed in the text. These molecules have been designed to stabilize specific PPIs to modulate cellular processes.

7. Use of Computational Methods to Design Drugs for Specific PPIs.

New approaches, driven by improved computational methodologies, have allowed the use of computer-assisted design of structure-based drugs. Novel computational tools have been used to understand PPIs and design small molecule drugs. Studying a PPI as a potential drug target requires identification and characterization of the binding interface. A number of computational methods have been used to identify PPI interfaces [50]. With faster high-throughput screening, thousands of molecules can be assayed with robotic automation. The serious drawback of using computations for analysing PPIs is that it is expensive and requires

huge resources. Computational methods often rely on the protein structure and also become useful in the cases where the structure cannot be determined by experimental methods. Several computational methods have been used for protein structure prediction including homology modeling [52] threading approaches [53], and ab initio folding [54]. Using computational methods for studying PPIs can aid in;

7.1. Predicting PPI interfaces

Studying a PPI as a potential therapeutic target requires identification and characterization of the binding interfaces. A number of computational methods have been used to identify PPI interfaces from protein structures [55,56]. Methods based on the similarity of interface regions have been successfully used to predict the topography of binding interfaces, but require a reference template [57]. However, due to protein flexibility, it is easier to identify a binding interface from an isolated PPI complex. In addition, computational methods could be very useful to identify potential allosteric binding sites modulating PPIs. Although efforts have been made in this direction, it still remains an area with the scope of significant improvement [58].

7.2. Identifying binding hotspots

Hotspots at protein surfaces can be identified by a number of methods 59 such as the alanine scanning energetics database [60], the binding interface database [61] and the HotSprint database [62]. Softwares have been generated that accurately predict the protein surface hotspots, such as the HotPoint and the ligand binding hotspots, such as SiteMap [63,64].

7.3. Modeling molecular flexibility

Protein flexibility is a very important feature of recognition kinetics in PPIs. This is also true for the interactions between proteins and small-molecule drugs. Thus conformational flexibility must be considered for the efficiency of computational methods in modeling a broad range of PPIs. Using predefined structural ensembles, where an ensemble of multiple protein structures is used for analysis rather than a single protein, has proved useful in molecular docking. For selecting the ensemble, protein structures can be determined by NMR or X-ray crystallography or computationally by molecular dynamics [65]. Ensemble selection is a critical determinant for performing the molecular docking, assessing druggbility and hotspot identification. However, this computational area needs improvement to predict the PPIs.

7.4. Virtual screening

It is used to enrich libraries for small molecules with an increased likelihood of hitting a particular target. There are, however, a number of pitfalls associated virtual screening that should be understood before using it. It might be expected that virtual screening has greater utility in identifying small molecule targets of PPIs. However, the majority of these methods

have been optimized for hidden active sites, and it is not yet clear whether these will translate to making calculations at protein surfaces.

8. Future Perspectives

Drug discovery for identifying effective therapeutic agents is target driven and PPIs have emerged as attractive molecular targets for drug designing. PPIs regulate almost all biological processes, including cell growth and development, pathological conditions and signaling pathways. The study of protein networks has provided mechanistic insights into cellular processes governed by signalling molecules. Understanding how proteins bind and communicate to initiate cellular events has revealed the molecular basis of different diseases especially cancer. Classic small molecule drugs consist mainly of planar molecules where as successful disruption of interaction requires interaction in 3D. Improved design of small drug molecules needs to consider conformational changes for the binding kinetics of interactions. Additionally, peptides that could specifically target PPIs with improved efficiency need to be designed.

Traditional drugs have mainly targeted distinct binding clepts. However, with the advent of new technologies and improved design, finer architectural features of protein-protein interfaces have been understood. The recent knowledge of how small drug molecules bind to protein interfaces has opened up new vistas for drug development. Targeting PPIs for treatment of diseases constitutes an active area of research and structural aspects of protein binding need to be studied to design novel therapeutics with clinical advantages.

As no major breakthrough has been achieved by using computational methods for optimum screening conditions and designing desirable architectural patterns of drug molecules for PPI inhibition or stabilization. Therefore developing new methodologies, for both experimental and computational strategies, to target PPIs is needed as it would lead to the design of molecules capable of modulating new, more specific and previously undruggable targets.

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