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REVIEW

Binding of small molecules at interface of protein–protein complex – A newer approach to rational drug design

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Abstract Protein–protein interaction is a vital process which drives many important physiological processes in the cell and has also been implicated in several diseases. Though the protein–protein interaction network is quite complex but understanding its interacting partners using both *in silico* as well as molecular biology techniques can provide better insights for targeting such interactions. Targeting protein–protein interaction with small molecules is a challenging task because of druggability issues. Nevertheless, several studies on the kinetics as well as thermodynamic properties of protein–protein interactions have immensely contributed toward better understanding of the affinity of these complexes. But, more recent studies on hot spots and interface residues have opened up new avenues in the drug discovery process. This approach has been used in the design of hot spot based modulators targeting protein–protein interaction with the objective of normalizing such interactions.

Contents

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1. Introduction

Protein–protein interaction is an important driving mechanism in many physiological processes in the cell and may also be involved in the pathogenesis of some diseases such as Alzheimer's cervical cancer, bacterial infection and prion diseases (Cohen and Prusiner, 1998; Selkoe, 1998; Loregian et al., 2002). Owing to the diversity of protein–protein interactions there is a need for careful investigation of the nature of the protein interface. The protein interface residues are a determinant of the specificity and stability of protein–protein interaction. The size of the protein interface decides whether the complex will be transient or obligatory. Protein–protein interaction is regulated by environmental conditions such as temperature, pH, ionic strength, etc. and also by cell mechanisms such as enzymes, covalent modification and non-covalent modification ligand binding etc (Furukawa et al., 2002; Eyster, 1998; Klemm et al., 1998; Markus and Benezra, 1999). Depending on their stability, protein complexes can be principally classified into two types: temporary and permanent stable complexes. The temporary complex interfaces have unique properties for each interacting pair of proteins whereas the permanent stable complex interfaces have similar properties on their surfaces as their formation is considered to be a continuation of protein folding (Dmitriev et al., 2002; Tsai et al., 1997).

Prediction of protein–protein interaction is crucial in drug discovery. Many physiological and pathological cellular processes depend on protein–protein interactions which can be influenced by external compounds. The modern drug discovery process involves three main steps-identification of prospective drug target, investigating its properties and designing of a corresponding ligand (Archakov et al., 2003). Therefore, knowledge of protein–protein interaction can be useful in designing modulators that can target the protein complex involved in various diseases. But a number of factors can contribute to the challenge of identifying small molecules that inhibit such interactions. These include the general lack of small-molecule starting points for drug design, the typical flatness of the interface, and the difficulty of distinguishing real from false binding, and the size and character of typical small-molecule libraries (Arkin and Wells, 2004). However, much of these problems have been solved through advancement of molecular biology and computational modeling techniques (Jin et al., 2013; Cheng et al., 2007; Huang and Jacobson, 2010).

2. Protein–protein interfaces: structure, composition and forces

Protein–protein interaction sites are formed by proteins with good shape and electrostatic complementarity (Janin, 1995; Jones and Thornton, 1996; Janin and Chothia, 1990). The standard size for the protein interfaces are 1200–2000 A^2 (Horton and Lewis, 1992). Small protein interfaces of size 1150–1200 A^2 are usually unstable and short-lived (Conte et al., 1999). Large protein interfaces are found in proteases, G-proteins and other proteins of the signal transduction pathways (Janin and Chothia, 1990; Horton and Lewis, 1992).

Protein–protein interfaces are mostly hydrophobic and consist of buried non polar surface area (Young et al., 1994). Thus hydrophobicity is the leading force in protein–protein interactions. The protein–protein complex is stabilized by a large gain in free energy change through increase in entropy, van der waals interactions and desolvation energy (Fernandez and Scheraga, 2003; Dill, 1990). Besides hydrophobic interactions, electrostatic forces also promote complex formation, which in turn defines the lifetime of protein complexes (Nicolini, 1999). It has been found that the average number of hydrogen bonds is proportional to the subunit area surfaces: one bond per 100–200 A° (Jones and Thornton, 1997a,b). Other hydrogen bonds are formed between protein contacts and surrounding water molecules (Laskowski et al., 1996; Vaughan et al., 1999).

It has been found that there is a highly uneven distribution of energetic contribution of individual protein residues across each subunit surface such that only a fraction of key residues contribute to the binding free energy of protein–protein complexes known as hot spots (Janin and Chothia, 1990; Conte et al., 1999). Hot spots have been defined as those sites where alanine mutations cause a significant increase in the binding free energy of at least 2.0 kcal/mol (Thorn and Bogan, 2001). In a protein–protein interface only a subset of the buried amino acids contribute most of the binding affinity which is determined by a change of free energy upon mutation of the residue to an alanine. These hot spots are not only helpful for the study of a single protein–protein dimer but also in the determination of probable binding sites for other binding partners (Thornton, 2001).

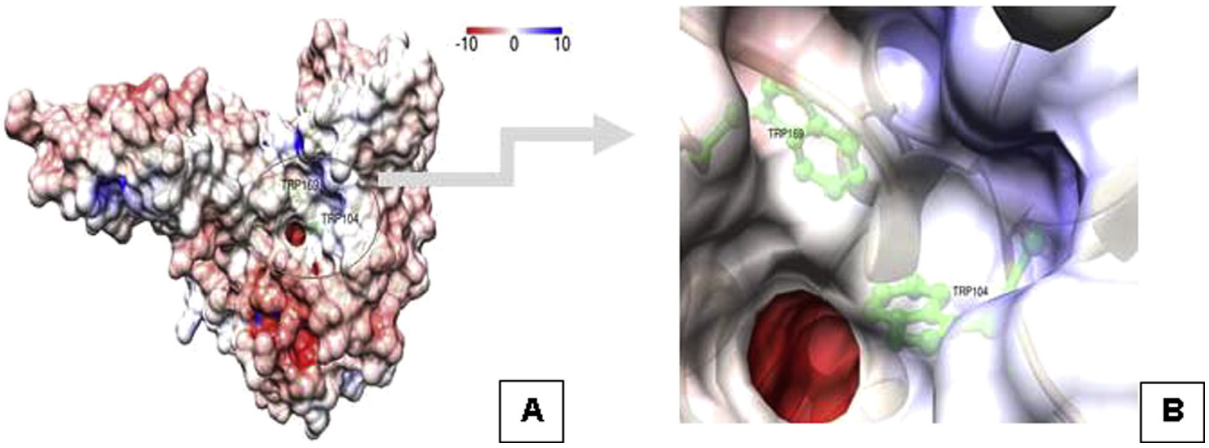

Figure 1 (A) The electrostatic potential surface of the protein complex formed between human growth hormone and growth hormone receptor [PDB ID: 1A22]. (B) Close up view of the two hot spot residues of the growth hormone receptor TRP 104 and TRP 169 depicted in ball and stick models (green) UCSF Chimera v 1.6.1 software was used to produce this picture.

Protein interfaces have distinctive amino acid composition. The most important ones are tryptophan (21%), arginine (13.3%), and tyrosine (12.3%) (Moreira et al., 2007). In a protein complex formed between the human growth hormone and human growth hormone binding protein it is well illustrated that out of 29 interfacial residues only four residues with $\Delta G > 4.5$ kcal/mol were found to be hot spots and two of them were tryptophan (Moreira et al., 2007). Fig. 1A shows the electrostatic potential surface of the protein complex formed between human growth hormone and growth hormone receptor and Fig. 1B shows two hot spot residues TRP 104 and TRP 169 of the growth hormone receptor. The high propensity of tryptophan as hot spots is due to its large size and aromatic nature (Samanta et al., 2000). Further, the common residues which are disfavoured as hot spots are leucine, serine, threonine and valine because these residues determine distinctive protein structures (Bogan and Thorn, 1998).

Through detailed analysis of X-ray structures of 23 protein–protein complexes and binding free energy change upon alanine mutation, Bogan and Thorn suggested that the hot spot residues are surrounded by residues which are not important in binding but in shielding these hot spots from the solvent (Bogan and Thorn, 1998). This idea came to be known as O-ring theory as there structures that resemble the alphabet O. This theory is well supported by three observations. First, the hot spots in X-ray protein complex structures often have zero or low solvent accessible surface area. Second, many residues which are protected from solvent do not make significant contributions to the binding free energy Third, there are no residues with high solvent accessibility that make a large contribution to ΔG binding (Bogan and Thorn, 1998). Thus, inaccessibility to the solvent is defined to be one condition as a binding hot spot (Bogan and Thorn, 1998; Delano, 2002). The residues surrounding hot spots should be able to establish both hydrogen bonding and hydrophobic interactions in order to occlude the solvent. Such residues can be tryptophan, tyrosine or asparagine. Although, O-ring theory is the most acceptable hypothesis it does not have conclusive evidence. For example, the assumption that non hot spot residues do not contribute to binding is valid only if water or nearby side chains are not able to effectively substitute the eliminated atoms (Janin, 1999).

3. Thermodynamics and kinetics of protein–protein interactions

The formation of a protein–protein complex may be written as

$$A + B \underset{k_{off}}{\overset{k_{on}}{\leftrightarrow}} AB \tag{1}$$

where k_{on} is the second-order rate constant for the association reaction and k_{off} is the first order rate constant for the dissociation reaction. Their ratio is the equilibrium constant for association (K_a) or for dissociation (K_d) according to the law of mass action and can be expressed as

$$\frac{[A][B]}{[AB]} = K_d = \frac{1}{K_a} = \frac{k_{off}}{k_{on}} \tag{2}$$

The main thermodynamic parameters characterizing protein–protein complex- Gibb's free energy change (ΔG), entropy change (ΔS) and enthalpy change (ΔH) are inter- related by the following equations.

$$\Delta G = -RT \ln K_d \tag{3}$$

$$\Delta G = \Delta H - T\Delta S \tag{4}$$

where ΔG is the standard free energy change, R is a gas constant and T is absolute temperature and K_d is the equilibrium constant. So using Eq. (3) it is possible to estimate free energy change by determining K_d value. The K_d values for typical protein complexes lie in the range 10^{-4}–10^{-14} M which corresponds to a free energy change of 6–19 kcal/mol (kleanthous, 2000).Change of enthalpy ΔH depends on hydrogen bond formation, electrostatics and van der waals forces of interactions whereas change of entropy ΔS depends on the degrees of freedom of the system. When proteins form tight complexes the measurement of K_d value from kinetic methods is preferred over the equilibrium method. As a rule a point mutation in the protein interface reduces the affinity of the complex such that a mutation causes an increase in the dissociation rate

constant (k_{off}) and minor changes in the association rate constant (k_{on}) (Kleanthous, 2000; Schreiber et al., 1997).

4. Bioinformatics and proteomic methods for investigating protein–protein interaction

4.1. *In silico* prediction of protein–protein interactions

Some *in silico* methods in predicting protein–protein interactions are annotation by sequence similarity, phylogenetic profiling, metabolic pathway mapping, gene neighbor and domain fusion analyses. Annotation by sequence similarity involves finding homologous counterparts of the query protein in annotated databases using pairwise local sequence alignment (Berggård et al., 2007). This method predicts the functional partners of the query protein of the given organism with proteins involved in complex formation in other organisms. The basis of phylogenetic profiling of proteins involved in complex formation is that such proteins must be present or absent together in different organisms. A phylogenetic profile gives the occurrence of a certain protein in a set of organisms. If two or more proteins share a similar phylogenetic profile it is possible that these proteins are functionally linked (Eisenberg et al., 2000). Another method of predicting functional linkages between two proteins is gene neighborhood method. If two proteins are found in the neighborhood across different organisms in the genome then it is highly probable that these proteins could be functionally linked. This method could correctly identify functional links among eight enzymes of arginine biosynthesis of *Mycobacterium tuberculosis* (Eisenberg et al., 2000). Fused domain analysis method is based on the principle that two proteins are said to functionally linked in an organism if they correspond to different domains of a single protein in other organisms. A typical example of fused domain protein is P450BM-3. It consists of two domains P450 102 and NADPH-cytochrome P450 reductase domains, whereas these are usually two separate proteins which interact with each other (Ruettinger et al., 1989). Metabolic profiling also helps in predicting protein–protein interactions. It has been found that proteins involved in coupled enzymatic reaction form a temporary complex (Gomez and Rzetsky, 2002).

4.2. Protein–protein interaction databases

Several databases contain experimental information on protein–protein interactions. Because of variability in different experimental techniques the experimental information also varies on a wide range. The data submitted in the databases come from various sources such as high-throughput, data mining, small scale experiments etc. Some of the free access protein–protein interaction databases are listed in Table 1.

4.3. Proteomics methods for the detection and analysis of protein–protein interactions

4.3.1. Two hybrid system

The two hybrid system is a genetic method that measures protein–protein interactions on the basis of transcriptional activity. It relies on site specific transcriptional activators that consist of two domains- DNA binding (DB) domain and Transcriptional Activation (TA) domain (Hope and Struhl, 1986). The DB domain helps the activator to bind to the promoter of specific genes and the TA domain helps to recruit the transcriptional protein machinery to begin the transcription process. The two hybrid system method is based on the principle that for a gene expression the DB and TA domains of the activator have to be covalently linked or can be brought near to each other by protein–protein interaction. Fig. 2 shows the interaction between hypothetical proteins X and Y which bring the DNA binding domain (DBD) and Transcriptional Activation domain (TAD) in close proximity to each other and results in reporter gene expression. Any two proteins X and Y whose interaction is to be studied are tagged with the

Table 1 List of some freely accessed protein–protein interaction databases.

Database	Type of information	URL	References
DIP (Database of Interacting Proteins)	Interactions (direct binding) between proteins	http://dip.doe-mbi.ucla.edu	Salwinski et al. (2004)
IntAct	Interactions (direct binding) between proteins	http://www.ebi.ac.uk/intact	Hermjakob et al. (2004)
BIND (Biomolecular Interaction Network Database)	Interactions (binding) between biomolecules	http://www.bind.ca/	Bader et al. (2003)
MINT (Molecular INTeraction database)	Interactions (both direct and indirect relationships) between proteins	http://mint.bio.uniroma2.it/mint/	Zanzoni et al. (2002)
BRITE (Biomolecular Relations in Information Transmission and Expression)	Generalized interactions between proteins (including direct binding) [part of KEGG]	http://www.genome.jp/brite/	Kanehisa et al. (2004)
InterDom	Integrative database of putative protein domain interactions	http://interdom.lit.org.sg	Ng et al. (2003)
BID (Binding Interface Database)	Detailed data on protein interfaces	http://tsailab.org/BID/	Fischer et al. (2003)
ASEdd (Alanine Scanning Energetics database)	Energetics of side–chain interactions of heterodimeric interfaces, from alanine scanning mutagenesis	http://www.asedb.org	Thorn and Bogan (2001)
KDBI (kinetic Data of Biomolecular Interactions)	Kinetic parameters of protein–protein and other interactions	kdbi/kdbi.asp	Ji et al. (2003)
PDB (Protein Data Bank)	Atomic structures of proteins, including those of protein complexes	http://www.rcsb.org/pdb/	Berman et al. (2000)

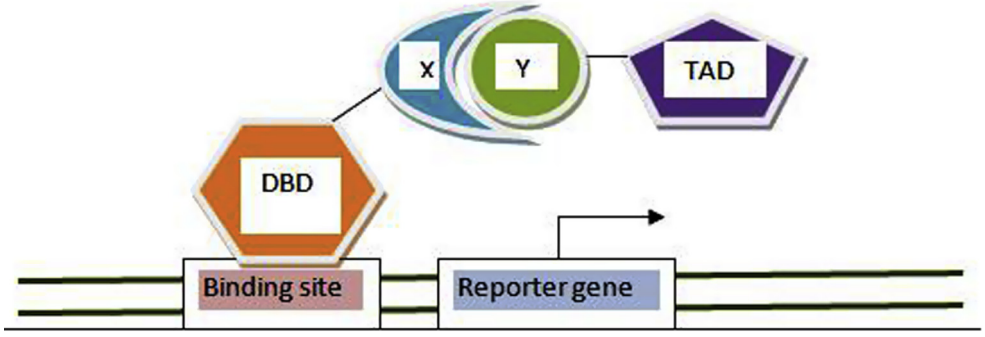

Figure 2 The two hybrid system: the interaction between proteins X and Y bring the DNA binding domain (DBD) and Transcriptional Activation domain (TAD) in close proximity to each other and results in reporter gene expression.

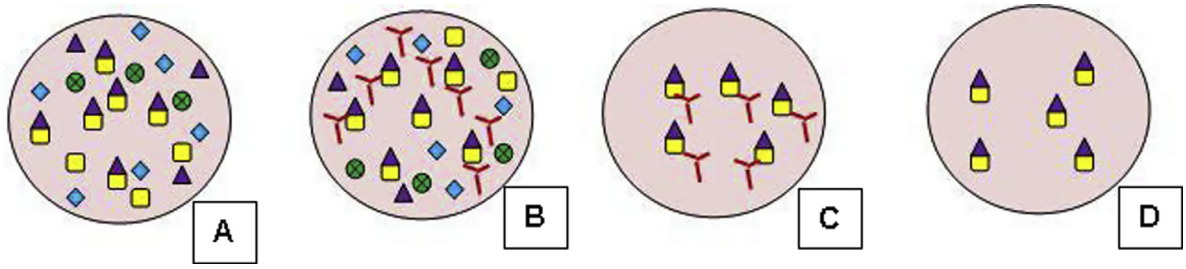

Figure 3 (A–D) Sequential steps involved in co-immunoprecipitation for the detection of protein–protein complexes in a protein mixture.

DB and TA domains separately and expressed in a cell containing reporter genes. If the two proteins interact with each other it brings DB and TA domains of the activator in close proximity and this functionally active transcriptional activator in turn switches on the reporter gene expression. Various versions of the two hybrid system are available in which the DB domains can be derived from yeast Gal4 protein (Chien et al., 1991) or *Escherichia coli* Lex A protein (Vojtek et al., 1993) and the TA domains can be derived from Gal4 protein (Chien et al., 1991) or herpes simplex virus VP16 protein (Dalton and Treisman, 1992). The reporter genes include *E. coli* lacZ gene (Fields and Song, 1989) and selectable yeast genes such HIS3 (Durfee et al., 1993) and LEU2 (Zervos et al., 1993).

4.3.2. Co-immunoprecipitation

Co immunoprecipitation (Co-IP) is a commonly used technique for the determination of protein–protein interaction (Berggård et al., 2007). In this method, protein–protein complexes from a cell lysate or protein mixture are captured using a specific antibody. Then, the antibody is immobilized using protein A or protein G covalently attached to Sepharose beads. After washing of the beads, the antibody and protein–protein complexes are eluted (*e.g.*, by boiling). The bound proteins can then be identified by MS or by immunoblotting. Fig. 3 shows sequential steps (A–D) involved in co-immunoprecipitation for the detection of protein–protein complexes in a protein mixture. There are two ways of performing Co-IP- (i) Co-IP from cell-lines or tissues expressing their endogenous proteins. The advantage of this approach is that endogenous protein complexes can also be studied. The disadvantage is that highly specific antibodies are required. (ii) The second approach is by using cells transfected with a plasmid encoding a tagged bait protein (Masters, 2004). The advantage of this approach is that there are minimum chances of cross reactivity with other proteins as it is expected that the antibody directed against the tag is highly specific.

4.3.3. Confocal microscopy

The basis of the confocal microscopy method is that if two or more proteins are co-localized in a cell then it is highly probable that they also interact with each other *in vivo* (Miyashita, 2004). In this method cells are transfected using plasmids that encode first protein fused to a tag and second protein fused to a different tag. Next, the sample is incubated with secondary antibodies labeled with different fluorophores (*e.g.*, Cy2 and Cy3). Because the two fluorophores will display different emission maxima, the intracellular localization of the proteins can be monitored (Berggård et al., 2007). If the two proteins are colocalized, the fluorescent probes will also be colocalized.

4.3.4. Surface plasmon resonance (SPR)

SPR is another widely used technique for the study of protein–protein interactions (Szabo et al., 1995; Huber and Mueller, 2006). The two important advantages of this method are firstly very little sample is required (mg) and secondly there is no need for labeling samples. This method provides information not only on affinities but also on the rates of association and dissociation between protein–protein complexes in regulatory pathways. This method can be applied for all kinds of proteins, because it relies on the phenomenon of SPR attributed to thin metal films (eg., gold or silver) and the signal recorded (the angle of minimum reflected light) depends on the refractive index close to the surface (Berggård et al., 2007).

4.3.5. Isothermal titration calorimetry (ITC)

ITC has been used extensively in studying protein–ligand and protein–protein interactions (Freire et al., 1990; Doyle, 1997; Jelessarov and Bosshard, 1999). Protein–protein interaction brings changes in the thermodynamic parameters such as ΔG, ΔH and ΔS, which can be measured by the highly sensitive calorimetry technique. Compared to SPR which needs the protein to be bound to the surface and which ultimately interferes

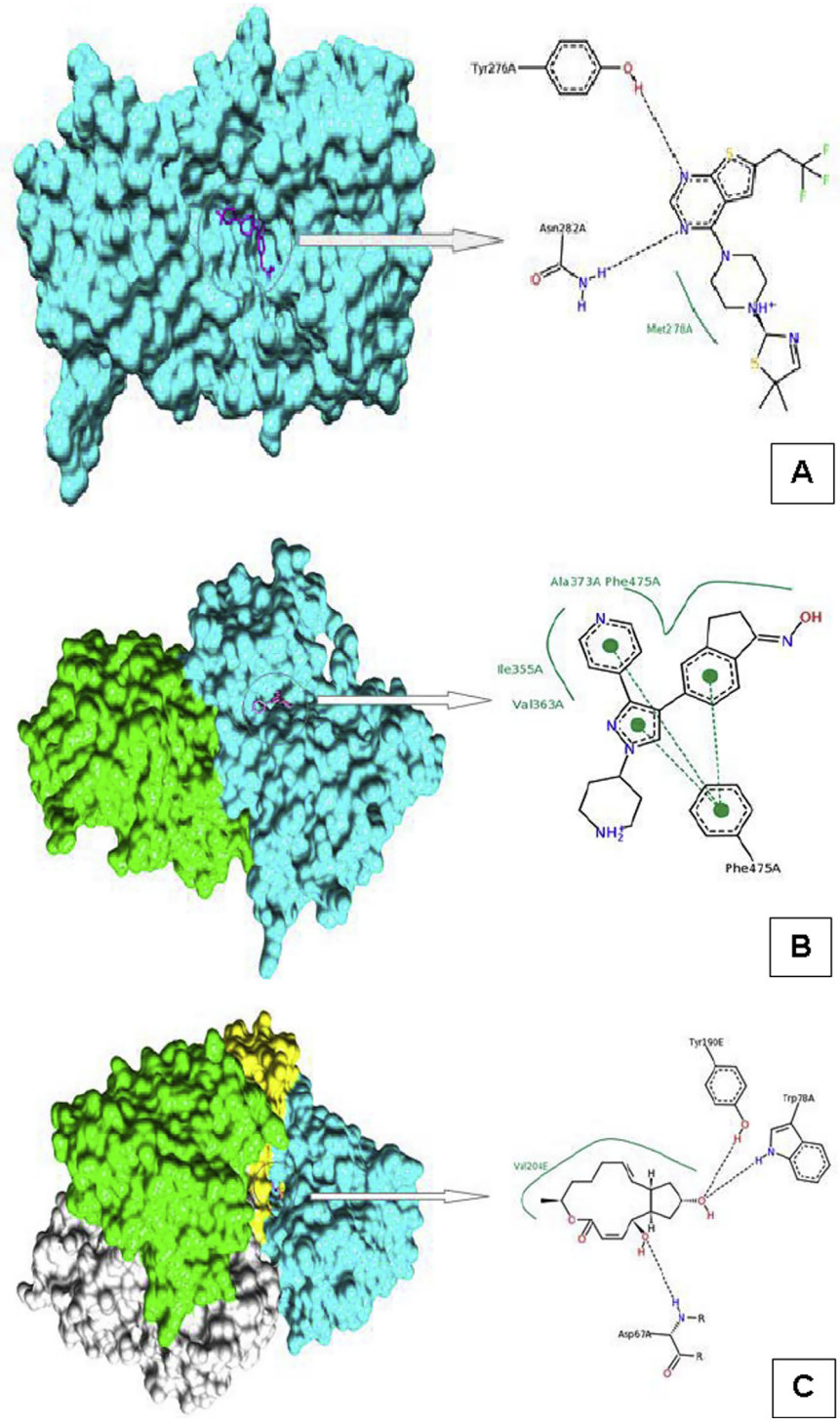

Figure 4 Three different classes of PPI modulators: (A) binding pose and molecular interaction of orthosteric inhibitor MI-2-2 bound to Menin [Protein Data Bank (PDB) code: 4GQ4]. (B) Binding pose and molecular interaction of allosteric Inhibitor PLX4032 bound to BRaf [PDB code: 3OMV]. (C) Binding pose and molecular interaction of interfacial binding inhibitor BFA bound to ARF1–Sec7 domain complex [PDB code: 1R8Q] the binding site of the ligand is indicated by orange rectangular box. The pictures were taken using PYMOL software v1.1.

with the binding or prolongs the duration of experiment, ITC is much simpler and doesn't need the protein to be adhered to the surface (Velazquez-Campoy et al., 2004). Compared to the other techniques which singly measure the binding affinity, ITC measures both enthalpy as well as entropic components of binding affinity (Velazquez-Campoy et al., 2004).

5. Protein–protein interaction as drug targets

Druggability is defined as modulating the function of a target protein by small-molecules with high affinity (Hopkins and Groom, 2002). Most of the proteins involved in disease pathways exert their functions through interactions with other proteins, and they lack obvious druggable pockets for small molecules. Although protein–protein interactions (PPIs) are important in many cellular functions and disease pathways, targeting protein–protein interaction with small molecule is a challenging task. The main reasons are that protein–protein interface hot spots are unevenly distributed across the protein surfaces and secondly the shape of protein–protein recognition sites which are mostly flat unlike protein–ligand recognition sites which have deeper cleft (Jin et al., 2013). The druggability issue with protein–protein contacts has greatly been solved with the growing computational modeling methods (Cheng et al., 2007; Huang and Jacobson, 2010), X-ray crystallography techniques and the advancement of fragment based drug discovery(Huang and Jacobson, 2010; Braisted et al., 2003).

Some examples of PPI modulators include molecules that inhibit the recognition of IL-2 by its receptor IL2Rα (Braisted et al., 2003), the binding of tumor suppressor p53 to its E3 ligase MDM2 (Vassilev, 2004) and the binding of Bcl2/Bcl-XL to the proapoptotic molecule BAK (Oltersdorf et al., 2005). There are three different mechanisms by which small molecules inhibit PPI- orthosteric inhibition, allosteric regulation, and interfacial binding/stabilization. The first mechanism, orthosteric inhibition, involves direct competition against the interacting partners. The orthosteric inhibitors bind to the target proteins at sites that overlap with the areas used for interacting with the partner proteins, thus directly inhibiting the formation of macromolecular complexes. Some examples in this category include the inhibitors against the Menin-MLL complex, the bromodomain-acetylated histone complex, the Ras-SOS1 complex, and the xIAP-caspase interaction (Flygare et al., 2012). Fig. 4A shows orthosteric inhibitor MI-2-2 bound to Menin. The second mechanism, allosteric regulation involves binding of small molecules to target proteins at sites distinct from the macromolecular interface. Binding of the ligand induces changes in conformation and prevents the macromolecular interaction in an allosteric manner. Some examples of allosteric inhibitors include the PLX series of BRaf inhibitors that block BRaf-CRaf heterodimerization and activation (Hatzivassiliou et al., 2010). Fig. 4B shows allosteric Inhibitor PLX4032 bound to BRaf. The third category includes interfacial binders, whereby the ligand and proteins form a ternary complex. An interfacial inhibitor binds to a pocket at the macromolecular interface and locks the complex into a nonfunctional conformation. A representative example in this category is brefeldin A (BFA). Fig. 4C shows interfacial binding inhibitor BFA bound to ARF1–Sec7 domain complex. BFA is a natural compound that traps ADP-ribosylation factor (ARF) and Sec7-domain-containing ARF exchange factors in a dead-end complex and inhibits the nucleotide exchange reaction for ARF (Mossessova et al., 2003).

There are several examples of hot spot based drug design. This includes a series of spirooxindole containing inhibitors such as MI-63 and MI-888 for murine double minute 2 (MDM2)/tumor suppressor p53 interactions can inhibit the growth of cancers with wild type p53 (Guo et al., 2014). Fig. 5A and B shows the chemical structure of spirooxindole core containing MDM2/p53 PPI inhibitors MI-63 MI-888 respectively. The oxindole moiety of the spirooxindole core was designed to mimic the binding mode of the side chain of p53 TRP23 (Guo et al., 2014). The MI-63 and MI-888 inhibitors were designed using hot spot based approach and exhibits Ki value of $0.003 \pm 0.0015\ \mu M$ and $0.00044 \pm 0.00022\ \mu M$ respectively (Ding et al., 2006; Zhao et al., 2013).

Another example of hot spot based PPI inhibitor design include inhibitors for von Hippel–Lindau (VHL)/hypoxia-inducible factor 1a (HIF1a) interactions (Buckley et al., 2012). The formation of the VHL/HIF1 protein complex promotes the ubiquitination and degradation of HIF1a by the

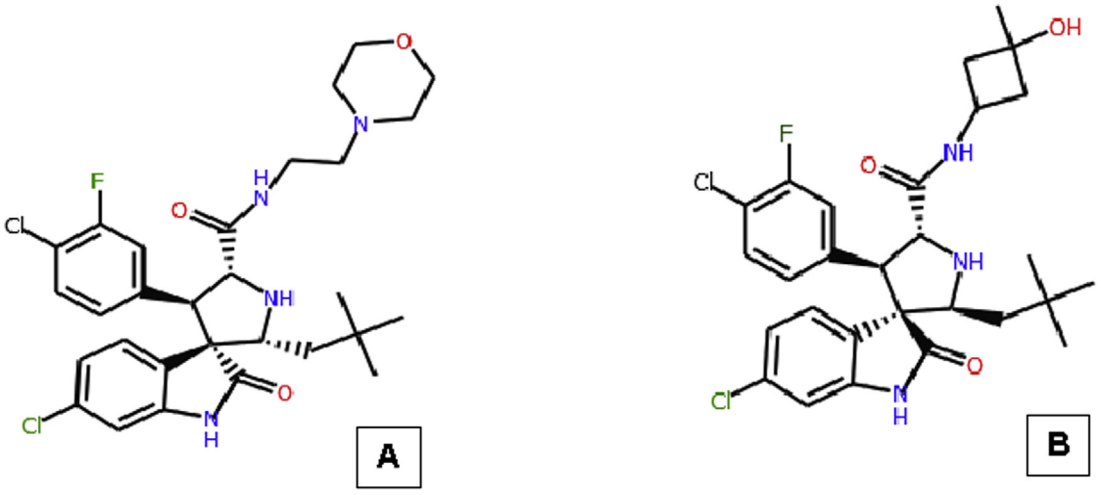

Figure 5 Structures of hot spot-based design of spirooxindole core containing MDM2/p53 PPI inhibitors (A) MI-63 (B) MI-888.

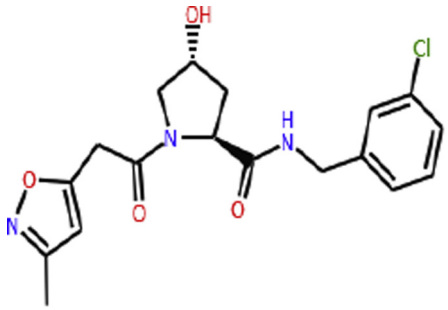

Figure 6 Structure of hot spot-based design of hydroxyproline-containing VHL/HIF1a PPI inhibitor.

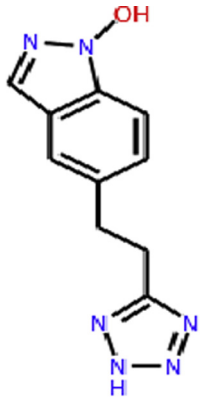

Figure 7 Structure of hot spot-based design of b-catenin/T-cell factor PPI inhibitor.

proteasome and its abnormal activity has also been implicated in chronic anemia (Ziello et al., 2007). It was found that 3-Hydroxyl-L-proline (Hyp) 564 of HIF1a is an important hot spot for interacting with VHL (Guo et al., 2014). This residue was used as a starting point to design a new inhibitor which exhibited an IC50 value of 117 ± 10 l M (Guo et al., 2014). Fig. 6 shows the chemical structure of hydroxyproline-containing VHL/HIF1 a PPI inhibitor.

Ji and coworkers used the fragment hopping approach for the design of potent and selective PPI inhibitors (Ji et al., 2008). Fragment hopping method is a new fragment based approach of drug design that requires the extraction of key binding elements based on the binding mode between the projecting hot spots and the concave hot spot pocket (Guo et al., 2014). This approach was employed to design effective as well as selective inhibitors for b-catenin/T-cell factor (Tcf) interactions (Yu et al., 2013).The aberrant formation of b-catenin/Tcf protein–protein complex is known to hyper activate Wnt target genes that eventually cause the initiation and progression of many cancers and fibroses (Van de Wetering et al., 2002). UU-T01 inhibitor was designed which mimics the binding mode of side chain carboxylic acids of Tcf4 D16 and E17 and exhibit Ki values 3.14 ± 0.48 l M (Guo et al., 2014). Fig. 7 shows the chemical structure of b-catenin/T-cell factor PPI inhibitor UU-T01. This compound completely disrupts b-catenin/Tcf interactions and is two orders of magnitude more potent than known dipeptides (Guo et al., 2014).

6. Conclusions

Protein–protein interaction is an essential process in the cell but its aberrant activities such as alteration of downstream target genes may lead to many disease conditions. Though targeting protein–protein interactions with small molecules theoretically seems feasible it is itself a challenging task. Some of the bottlenecks are lack of a deeper cleft in the binding site, difficulty in identification of hot spot residues, diversity in protein–protein interactions etc. But with the rapid advancement in computational methods and molecular biology techniques, it has been possible to design modulators which can either destabilize protein–protein interaction or render the protein complexes inactive by locking the complexes in a nonfunctional state. Such approaches will have a great impact in pharmaceutical sciences in developing modulators which can restore the normal functions of protein–protein interactions in disease pathways.

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References

Archakov, A.I., Govorun, V.M., Dubanov, A.V., Ivanov, Y.D., Veselovsky, A.V., Lewi, P., Janssen, P., 2003. Protein–protein interactions as a target for drugs in proteomics. Proteomics 3 (4), 380–391.

Arkin, M.R., Wells, J.A., 2004. Small-molecule inhibitors of protein–protein interactions: progressing towards the dream. Nat. Rev. Drug Discov. 3 (4), 301–317.

Bader, G.D., Betel, D., Hogue, C.W., 2003. BIND: the biomolecular interaction network database. Nucleic Acids Res. 31 (1), 248–250.

Berggård, T., Linse, S., James, P., 2007. Methods for the detection and analysis of protein–protein interactions. Proteomics 7 (16), 2833–2842.

Berman, H.M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T.N., Weissig, H., Shindyalov, I.N., Bourne, P.E., 2000. The protein data bank. Nucleic Acids Res. 28 (1), 235–242.

Bogan, A.A., Thorn, K.S., 1998. Anatomy of hot spots in protein interfaces. J. Mol. Biol. 280 (1), 1–9.

Braisted, A.C., Oslob, J.D., Delano, W.L., Hyde, J., McDowell, R.S., Waal, N., Yu, C., Arkin, M.R., Raimundo, B.C., 2003. Discovery of a potent small molecule IL-2 inhibitor through fragment assembly. J. Am. Chem. Soc. 125 (13), 3714–3715.

Buckley, D.L., Van Molle, I., Gareiss, P.C., Tae, H.S., Michel, J., Noblin, D.J., Jorgensen, W.L., Ciulli, A., Crews, C.M., 2012. Targeting the von Hippel-Lindau E3 ubiquitin ligase using small molecules to disrupt the VHL/HIF-1α interaction. J. Am. Chem. Soc. 134 (10), 4465–4468.

Cheng, A.C., Coleman, R.G., Smyth, K.T., Cao, Q., Soulard, P., Caffrey, D.R., Salzberg, A.C., Huang, E.S., 2007. Structure-based maximal affinity model predicts small-molecule druggability. Nat. Biotechnol. 25 (1), 71–75.

Chien, C.T., Bartel, P.L., Sternglanz, R., Fields, S., 1991. The two hybrid system: a method to identify and clone genes for proteins that interact with a protein of interest. Proc. Natl. Acad. Sci. USA 88 (21), 9578–9582.

Cohen, F.E., Prusiner, S.B., 1998. Pathologic conformations of prion proteins. Annu. Rev. Biochem. 67, 793–819.

Conte, L.L., Chothia, C., Janin, J., 1999. The atomic structure of protein–protein recognition sites. J. Mol. Biol. 285 (5), 2177–2198.

Dalton, S., Treisman, R., 1992. Characterization of SAP-1, a protein recruited by serum response factor to the c-fos serum response element. Cell 68 (3), 597–612.
DeLano, W.L., 2002. Unraveling hot spots in binding interfaces: progress and challenges. Curr. Opin. Struct. Biol. 12 (1), 14–20.
Dill, K.A., 1990. Dominant forces in protein folding. Biochemistry 29 (3), 7133–7155.
Ding, K., Lu, Y., Nikolovska-Coleska, Z., Wang, G., Qiu, S., Shangary, S., Gao, W., Qin, D., Stuckey, J., Krajewski, K., Roller, P.P., Wang, S., 2006. Structure-based design of spiro-oxindoles as potent, specific small-molecule inhibitors of the MDM2-p53 interaction. J. Med. Chem. 49 (12), 3432–3435.
Dmitriev, D.A., Massino, Y.S., Segal, O.L., Smirnova, M.B., Pavlova, E.V., Gurevich, K.G., Gnedenko, O.V., Ivanov, Y.D., Kolyaskina, G.I., Archakov, A.I., Osipov, A.P., Dmitriev, A.D., Egorov, A.M., 2002. Analysis of the binding of bispecific monoclonal antibodies with immobilized antigens (human IgG and horseradish peroxidase) using a resonant mirror biosensor. J. Immunol. Methods 261 (1–2), 103–118.
Doyle, M.L., 1997. Characterization of binding interactions by isothermal titration. Curr. Opin. Biotechnol. 8 (1), 31–35.
Durfee, T., Becherer, K., Chen, P.L., Yeh, S.H., Yang, Y., Kilburn, A. E., Lee, W.H., Elledge, S.J., 1993. The retinoblastoma protein associates with the protein phosphatase type 1 catalytic subunit. Genes Dev. 7 (4), 555–569.
Eisenberg, D., Marcotte, E.M., Xenarios, I., Yeates, T.O., 2000. Protein function in the post-genomic era. Nature 405 (6788), 823–826.
Eyster, K., 1998. Introduction to signal transduction: a primer for untangling the web of intracellular messengers. Biochem. Pharmacol. 55 (12), 1927–1938.
Fernandez, A., Scheraga, H.A., 2003. Insufficiently dehydrated hydrogen bonds as determinants of protein interactions. Proc. Natl. Acad. Sci. USA 100 (1), 113–118.
Fields, S., Song, O., 1989. A novel genetic system to detect protein protein interactions. Nature 340 (6230), 245–246.
Fischer, T.B., Arunachalam, K.V., Bailey, D., Mangual, V., Bakhru, S., Russo, R., Huang, D., Paczkowski, M., Lalchandani, V., Ramachandra, C., Ellison, B., Galer, S., Shapley, J., Fuentes, E., Tsai, J., 2003. The binding interface database (BID): a compilation of amino acid hot spots in protein interfaces. Bioinformatics 19 (11), 1453–1454.
Flygare, J.A., Beresini, M., Budha, N., Chan, H., Chan, I.T., Cheeti, S., Cohen, F., Deshayes, K., Doerner, K., Eckhardt, S.G., Elliott, L.O., Feng, B., Franklin, M.C., Reisner, S.F., Gazzard, L., Halladay, J., Hymowitz, S.G., La, H., LoRusso, P., Maurer, B., Murray, L., Plise, E., Quan, C., Stephan, J.P., Young, S.G., Tom, J., Tsui, V., Um, J., Varfolomeev, E., Vucic, D., Wagner, A.J., Wallweber, H.J., Wang, L., Ware, J., Wen, Z., Wong, H., Wong, J. M., Wong, M., Wong, S., Yu, R., Zobel, K., Fairbrother, W.J., 2012. Discovery of a potent small-molecule antagonist of inhibitor of apoptosis (IAP) proteins and clinical candidate for the treatment of cancer (GDC-0152). J. Med. Chem. 55 (9), 4101–4113.
Freire, E., Mayorga, O.L., Straume, M., 1990. Isothermal titration calorimetry. Anal. Chem. 62, 950–959.
Furukawa, Y., Ishimory, K., Morishima, I., 2002. Oxidation-state-dependent protein docking between cytochrome c and cytochrome b(5): high-pressure laser flash photolysis study. Biochemistry 41 (31), 9824–9832.
Gomez, S.M., Rzetsky, A., 2002. Towards the prediction of complete protein–protein interaction networks. Pac. Symp. Biocomput. 7, 413–424.
Guo, W., Wisniewski, J.A., Ji, H., 2014. Hot spot-based design of small-molecule inhibitors for protein–protein interactions. Bioorg. Med. Chem. Lett. 24 (11), 2546–2554.
Hatzivassiliou, G., Song, K., Yen, I., Brandhuber, B.J., Anderson, D. J., Alvarado, R., Ludlam, M.J., Stokoe, D., Gloor, S.L., Vigers, G., Morales, T., Aliagas, I., Liu, B., Sideris, S., Hoeflich, K.P., Jaiswal, B.S., Seshagiri, S., Koeppen, H., Belvin, M., Friedman, L. S., Malek, S., 2010. RAF inhibitors prime wildtype RAF to activate the MAPK pathway and enhance growth. Nature 464 (7287), 431–435.
Hermjakob, H., Montecchi-Palazzi, L., Lewington, C., Mudali, S., Kerrien, S., Orchard, S., Vingron, M., Roechert, B., Roepstorff, P., Valencia, A., Margalit, H., Armstrong, J., Bairoch, A., Cesareni, G., Sherman, D., Apweiler, R., 2004. IntAct: an open source molecular interaction database. Nucleic Acids Res. 32, D452–D455.
Hope, I.A., Struhl, K., 1986. Functional dissection of a eukaryotic transcriptional activator protein, GCN4 of yeast. Cell 46 (6), 885–894.
Hopkins, A.L., Groom, C.R., 2002. The druggable genome. Nat. Rev. Drug Discov. 1 (9), 727–730.
Horton, N., Lewis, M., 1992. Calculation of the free energy of association for protein complexes. Protein Sci. 1 (1), 169–181.
Huang, N., Jacobson, M.P., 2010. Binding-site assessment by virtual fragment screening. PLoS ONE 5 (4), e10109.
Huber, W., Mueller, F., 2006. Biomolecular interaction analysis in drug discovery using surface plasmon resonance technology. Curr. Pharm. Des. 12 (31), 3999–4021.
Janin, J., 1995. Protein–protein recognition. Prog. Biophys. Mol. Biol. 64 (2–3), 145–166.
Janin, J., 1999. Wet and dry interfaces: the role of solvent in protein–protein and protein–DNA recognition. Structure 7 (12), R277–R279.
Janin, J., Chothia, C., 1990. The structure of protein–protein recognition sites. J. Biol. Chem. 265 (27), 16027–16030.
Jelessarov, I., Bosshard, H.R., 1999. Isothermal titration calorimetry and differential scanning calorimetry as complementary tools to investigate the energetics of biomolecular recognition. J. Mol. Recogn. 12 (1), 3–18.
Ji, Z.L., Chen, X., Zhen, C.J., Yao, L.X., Han, L.Y., Yeo, W.K., Chung, P.C., Puy, H.S., Tay, Y.T., Muhammad, A., Chen, Y.Z., 2003. KDBI: kinetic data of bio-molecular interactions database. Nucleic Acids Res. 31 (1), 255–257.
Ji, H., Stanton, B.Z., Igarashi, J., Li, H., Martasek, P., Roman, L.J., Poulos, T.L., Silverman, R.B., 2008. Minimal pharmacophoric elements and fragment hopping, an approach directed at molecular diversity and isozyme selectivity. Design of selective neuronal nitric oxide synthase inhibitors. J. Am. Chem. Soc. 130 (12), 3900–3914.
Jin, L., Wang, W., Fang, G., 2013. Targeting protein–protein interaction by small molecules. Annu. Rev. Pharmacol. Toxicol. 54, 435–456.
Jones, S., Thornton, J.M., 1996. Principles of protein–protein interactions. Proc. Natl. Acad. Sci. 93 (1), 13–20.
Jones, S., Thornton, J.M., 1997a. Analysis of protein–protein interaction sites using surface patches. J. Mol. Biol. 272 (1), 121–132.
Jones, S., Thornton, J.M., 1997b. Prediction of protein–protein interaction sites using patch analysis. J. Mol. Biol. 272 (1), 133–143.
Kanehisa, M., Goto, S., Kawashima, S., Okuno, Y., Hattori, M., 2004. The KEGG resource for deciphering the genome. Nucleic Acids Res. 32, D277–D280.
Kleanthous, C., 2000. Protein–protein Recognition, first ed. Oxford University Press, New York.
Klemm, J.D., Schreiber, S.L., Crabtree, G.R., 1998. Dimerization as a regulatory mechanism in signal transduction. Annu. Rev. Immunol. 16, 569–592.
Laskowski, R.A., Luscombe, N.M., Swindells, M.B., Thornton, J.M., 1996. Protein clefts in molecular recognition and function. Protein Sci. 5 (12), 2438–2452.
Loregian, A., Mardsen, H.S., Palu, G., 2002. Protein–protein interactions as targets for antiviral chemotherapy. Rev. Med. Virol. 12 (4), 239–262.
Markus, M., Benezra, R., 1999. Two isoforms of protein disulfide isomerase alter the dimerization status of E2A proteins by a redox mechanism. J. Biol. Chem. 274, 1040–1049.

Masters, S.C., 2004. Co-immunoprecipitation from transfected cells. Methods Mol. Biol. 261, 337–350.
Miyashita, T., 2004. Confocal microscopy for intracellular co-localization of proteins. Methods Mol. Biol. 261, 399–410.
Moreira, I.S., Fernandes, P.A., Ramos, M.J., 2007. Hot spots–a review of the protein–protein interface determinant amino-acid residues. Proteins 68 (4), 803–812.
Mossessova, E., Corpina, R.A., Goldberg, J., 2003. Crystal structure of ARF1·Sec7 complexed with Brefeldin A and its implications for the guanine nucleotide exchange mechanism. Mol. Cell 12 (6), 1403–1411.
Ng, S.K., Zhang, Z., Tan, S.H., Lin, K., 2003. InterDom: a database of putative interacting protein domains for validating predicted protein interactions and complexes. Nucleic Acids Res. 31 (1), 251–254.
Nicolini, C., 1999. Biophysics of Electron Transfer and Molecular Bioelectronics, first ed. Springer, US.
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., Joseph, M.K., Kitada, S., Korsmeyer, S.J., Kunzer, A.R., Letai, A., Li, C., Mitten, M.J., Nettesheim, D.G., Ng, S., Nimmer, P.M., O'Connor, J.M., Oleksijew, A., Petros, A.M., Reed, J.C., Shen, W., Tahir, S.K., Thompson, C.B., Tomaselli, K. J., Wang, B., Wendt, M.D., Zhang, H., Fesik, S.W., Rosenberg, S. H., 2005. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature 435 (7042), 677–681.
Ruettinger, R.T., Wen, L.P., Fulco, A.J., 1989. Coding nucleotide, 5' regulatory, and deduced amino acid sequences of P-450BM-3, a single peptide cytochrome P-450:NADPH-P-450 reductase from Bacillus megaterium. J. Biol. Chem. 264 (19), 10987–10995.
Salwinski, L., Miller, C.S., Smith, A.J., Pettit, F.K., Bowie, J.U., Eisenberg, D., 2004. The database of interacting proteins: 2004 update. Nucleic Acids Res. 32, D449–D451.
Samanta, U., Pal, D., Chakrabarti, P., 2000. Environment of tryptophan side chains in proteins. Proteins 38 (3), 288–300.
Schreiber, G., Frisch, C., Fersht, A.R., 1997. The role of Glu73 of barnase in catalysis and the binding of barstar. J. Mol. Biol. 270 (1), 111–122.
Selkoe, D.J., 1998. The cell biology of beta-amyloid precursor protein and presenilin in Alzheimer's disease. Trends Cell Biol. 8 (11), 443–457.
Szabo, A., Stolz, L., Granzow, R., 1995. Surface plasmon resonance and its use in biomolecular interaction analysis (BIA). Curr. Opin. Struct. Biol. 5 (5), 699–705.
Thorn, K.S., Bogan, A.A., 2001. ASEdb: a database of alanine mutations and their effects on the free energy of binding in protein interactions. Bioinformatics 17 (3), 284–285.
Thornton, J.M., 2001. The Hans Neurath Award lecture of The Protein Society: proteins—a testament to physics, chemistry, and evolution. Protein Sci. 10 (1), 3–11.
Tsai, C.J., Xu, D., Nussinov, R., 1997. Structural motifs at protein–protein interfaces: protein cores versus two-state and three-state model complexes. Protein Sci. 6 (9), 1793–1805.
Van de Wetering, M., Sancho, E., Verweij, C., de Lau, W., Oving, I., Hurlstone, A., van der Horn, K., Batlle, E., Coudreuse, D., Haramis, A.P., Tjon-Pon-Fong, M., Moerer, P., van den Born, M., Soete, G., Pals, S., Eilers, M., Medema, R., Clevers, H., 2002. The beta-catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. Cell 111 (2), 241–250.
Vassilev, L.T., 2004. Small-molecule antagonists of p53-MDM2 binding: research tools and potential therapeutics. Cell Cycle 3 (4), 419–421.
Vaughan, C.K., Buckle, A.M., Fersht, A.R., 1999. Structural response to mutation at a protein–protein interface. J. Mol. Biol. 286 (5), 1487–1506.
Velazquez-Campoy, A., Leavitt, S.A., Freire, E., 2004. Characterization of protein–protein interactions by isothermal titration calorimetry. Methods Mol. Biol. 261, 35–54.
Vojtek, A.B., Hollenberg, S.M., Cooper, J.A., 1993. Mammalian Ras interacts directly with the serine/threonine kinase Raf. Cell 74 (1), 205–214.
Young, L., Jernigan, R.L., Covell, D.G., 1994. A role for surface hydrophobicity in protein–protein recognition. Protein Sci. 3 (5), 717–729.
Yu, B., Huang, Z., Zhang, M., Dillard, D.R., Ji, H., 2013. Rational design of small-molecule inhibitors for β-catenin/T-cell factor protein–protein interactions by bioisostere replacement. ACS Chem. Biol. 8 (3), 524–529.
Zanzoni, A., Montecchi-Palazzi, L., Quondam, M., Ausiello, G., Helmer-Citterich, M., Cesareni, G., 2002. MINT: a molecular INTeraction database. FEBS Lett. 513 (1), 135–140.
Zervos, A.S., Gyuris, J., Brent, R., 1993. Mxi1, a protein that specifically interacts with Max to bind Myc-Max recognition sites. Cell 72 (2), 223–232.
Zhao, Y., Yu, S., Sun, W., Liu, L., Lu, J., McEachern, D., Shargary, S., Bernard, D., Li, X., Zhao, T., Zou, P., Sun, D., Wang, S., 2013. Potent small-molecule inhibitor of the MDM2-p53 interaction (MI-888) achieved complete and durable tumor regression in mice. J. Med. Chem. 56 (13), 5553–5561.
Ziello, J.E., Jovina, I.S., Huanga, Y., 2007. Hypoxia-Inducible Factor (HIF)-1 regulatory pathway and its potential for therapeutic intervention in malignancy and ischemia. Yale J. Biol. Med. 80 (2), 51–60.