Protein-Protein Complexes

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Abstract | Protein-protein complexes are prevalent in living systems and are central to the way biological processes function. Several of these complexes have been crystallized and their structures and functions characterized, while a much larger number of them (that may not crystallize) operate in the physiological system. Analyses of the known structures of protein-protein complexes reveal that they form through interactions between specific patches on the surface of the component proteins. These patches, called interacting domains, are made from mostly non-contiguous residues in the protein sequence. Not all, but only a few of these residues called "hot spots", contribute significantly to the binding free energy of these complexes. Interactions between the hot spot residues from two interacting domains control the thermodynamics and oligomeric states of the complexes. The hot spot residues have been targets for the design of small molecule agonists with medicinal applications. The interaction domains in protein-protein complexes vary in their contour, size, shape, complementarity and affinity. The number of interacting protomers in a complex can vary from 2 to >30. These complexes have been classified based on the 3-dimensional architecture, binding energies and lifetimes of these quaternary assemblies and the presence of symmetry in their structure. Protein-protein complexes have evolved to perform and regulate activities like signal transduction, metabolism, cell division and controlled cell death, in response to specific stimuli. These functions are outside the capacity of monomeric proteins. In this review, an overview of these features is discussed with an emphasis on the structures and intermolecular interactions in these complexes.

Keywords: Protein–Protein complexes, protein-protein interactions, interfaces, oligomers, protein surface, surface patches, hot spots, inhibitors, thermodynamics.

Proteases: are physiological enzymes that catalyze the hydrolysis of specific peptide bonds in proteins.

Molecular recognition: It is the selective interaction through non-covalent bonds between two or more molecules in a medium.

1. Introduction

Protein–protein interactions are central to most biological processes from cell multiplication to programmed cell death.[1,2] An important requirement of molecular biology is the discovery of all proteins and protein–protein interactions as well as their biochemical and biological functions in organisms.[3,4] An understanding of protein-protein complexes, their structural and functional characteristics[5–8] and the forces that drive their intermolecular interactions is essential for the elucidation of molecular recognition in cells and its effects on biological processes.[9–16]

Proteins are molecular machines of the cell. Several proteins function in their monomeric form with remarkably high specificity and rates. The bovine pancreatic chymotrypsin (chymotrypsin) (PDB: 7GCH) is a classic example of such an enzyme. It is a serine protease,[17] which catalyzes the specific hydrolysis of peptide bonds that are adjacent to the aromatic amino acid residues, Tyr, Phe and Trp. A vacant cavity (cleft) near the active site of chymotrypsin interacts with the phenyl ring of the substrate peptide during catalysis (Figure 1) and this interaction is crucial for its substrate

Figure 1: The crystal structure of γ-chymotrypsin complexed with the trifluoromethylketone inhibitor Ac-Leu-Phe-CF_3. The inset shows the interaction of the phenyl ring of Phe in the inhibitor with the cavity in chymotrypsin.

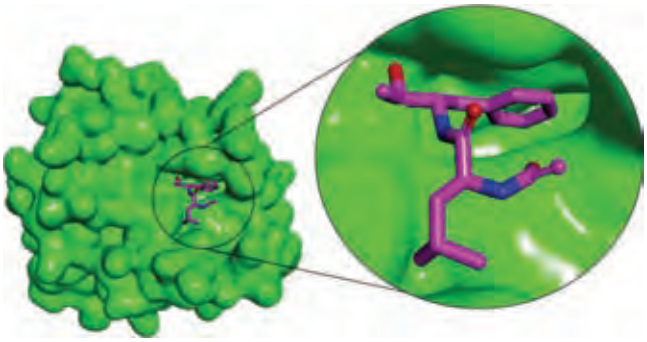
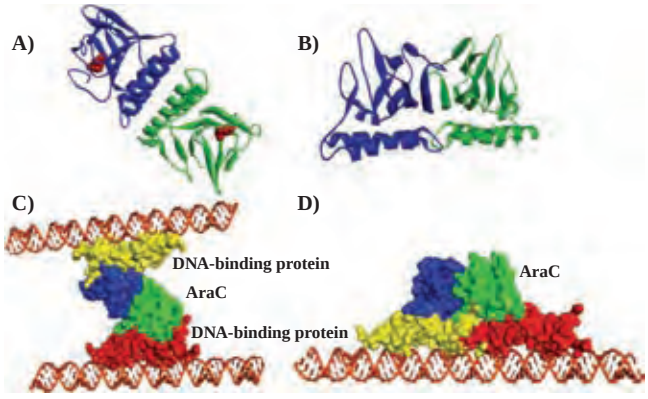


Figure 2: Allosteric regulation of the dimer conformation of the AraC protein from *E. coli*. A) A Ribbon diagram of the arabinose-bound AraC homodimer containing the α-helical interface. The arabinose molecule is shown in red. B) A Ribbon diagram of the AraC homodimer containing the non-contiguous α-sheet and turn interface. C) A Schematic representation of the arabinose-bound AraC dimer (the inactive form) complexed with DNA-binding proteins (yellow and red) from distant positions. The surface of the AraC dimer is shown (green and blue). D) A Schematic representation of the AraC dimer (active form) bound to adjacent DNA-binding proteins along the DNA. The surface of the AraC dimer is shown (green and blue).



specificity. The enzyme forms a transient covalently linked acyl-enzyme (ester) intermediate with the substrate, which enhances the rate of peptide bond hydrolysis[18] by a factor of at least 10^9.

A more fascinating aspect of proteins is that they can interact with each other and form stable multimeric assemblies, called protein-protein complexes.[19,20] Cells have evolved to build these large, dynamic complexes from several monomeric proteins. The assembly of these complexes occurs in a controlled fashion, unlike in an aggregation process.[21,22] Such protein-protein complexes can possess multiple active sites and hence have greater versatility of function in a single molecule, compared to monomeric proteins. Additionally, different oligomeric and conformational states of a protein-protein complex can each contribute to complementary or contradictory functions.[23] The stability of these states and the equilibrium between them are governed by their biophysical and biochemical environment in the cell. For example, AraC, a transcription regulator enzyme, forms two kinds of conformationally different complexes, depending on whether it is bound to arabinose or not (Figure 2). One of these complexes, the active form, promotes DNA transcription, and the other, the inactive form, represses it.[24] Owing to such functional versatility of protein-protein complexes, cells utilize them to not only catalyze complex reactions, but also to efficiently regulate them and do so selectively in response to stimuli or signal(s).

Various efforts over the past four decades have gone into the large scale analysis and understanding of the structures, functions and dynamism of protein-protein complexes, thus making possible the predictions of the properties of various novel proteins and their assemblies.[25–29] The identification of target-selective drugs and drug leads has also been facilitated. Progress in both these fields has been periodically reviewed individually. Aspects of the principles of interactions between proteins have also been discussed.[27–34] Here we present an overview of the structure-function relationships in protein-protein complexes along with the characteristics of the interaction domains between them.

A note about all the figures in this review is that, as in all other articles, structures from the Protein data Bank (PDB) are used to represent protein-protein complexes. Although biological macromolecules are dynamic, these structures provide good models for visualization. Most of the protein-protein complexes are shown in the molecular surface representation. Individual protomers are given different colors to clearly differentiate the boundaries of their surfaces and to highlight the interaction profile between the protomers. The molecular surface representation of protein complexes also helps to emphasize the fact that protein-protein interactions occur between the residues on the surface of the interacting protomers.

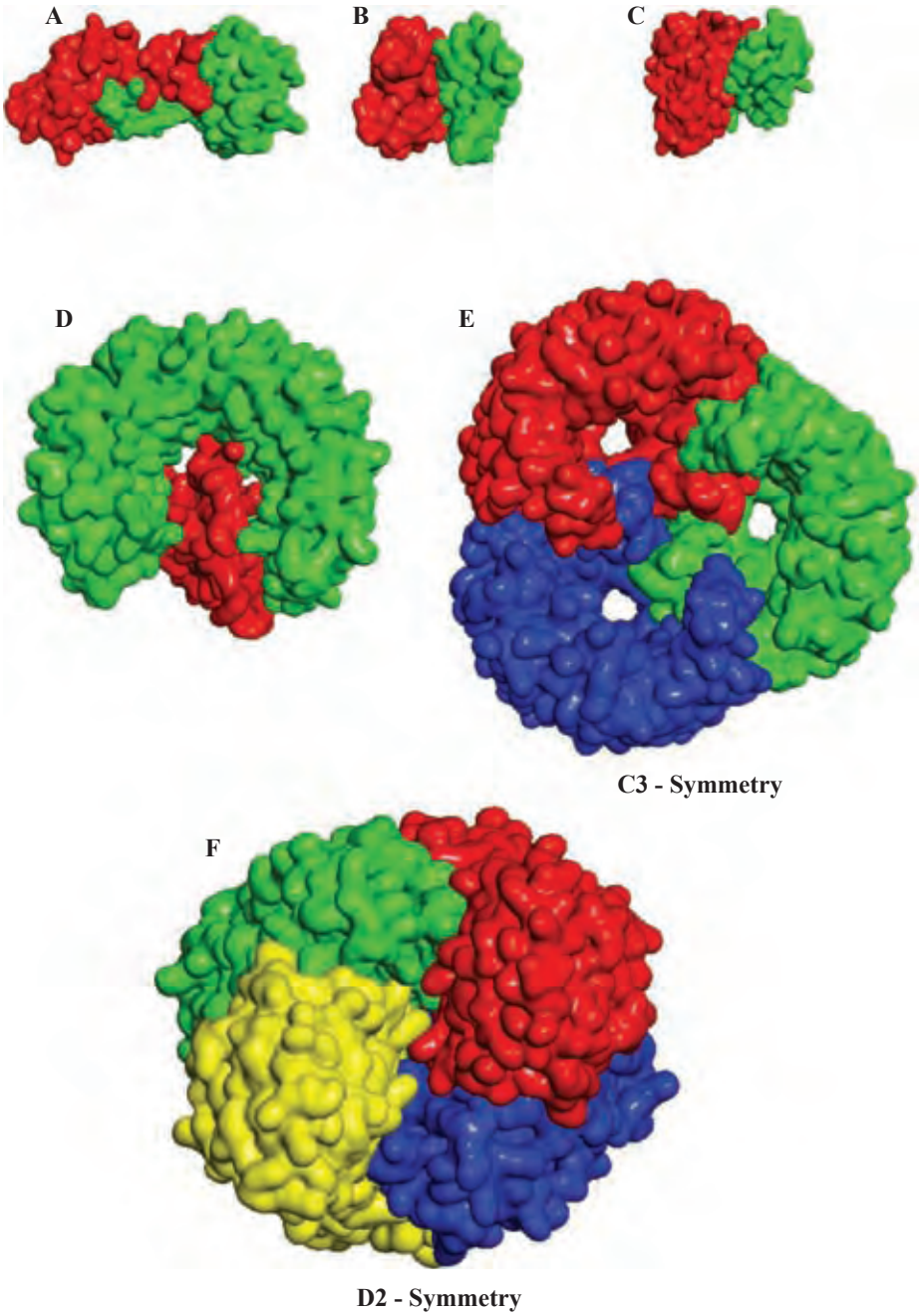
Protomer: is a single structural unit in an oligomeric protein-protein complex.

Residue: refers to an amino acid unit in a polypeptide.

2. Architecture of Protein-Protein Complexes

While most proteins fold into stable monomeric structures, a relatively large proportion of them also form highly ordered dynamic multimeric complexes with other proteins in the cell.[35] This is especially true among extracellular enzymes. Analysis of the PDB[36] reveals the distribution of the proteins found in a multimeric state (Figure 4).[37] Oligomeric protein complexes containing an

Figure 3: A) Rhodocetin (PDB: 1SB2) B) Cardiotoxin (PDB: 1CDT) C) Ferricytochrome c (PDB: 2CCY) D) Ribonuclease inhibitor (PDB: 1DFJ) E) Sucrose-specific porin (PDB: 1A0S) F) Phosphofructokinase (PDB: 6PFK) G) Mscl homolog from mycobacterium tuberculosis (PDB: 2OAR) H) Aminotransferase (PDB: 1A3G) I) N5-carboxyaminoimidazole Ribonucleotide synthetase (PDB: 3ORS) 8-protomers J) Bovine mitochondrial f1-atpase (PDB: 2W6I) 9-protomers K) Nucleocapsid-like particle (PDB: 3PTO) 10-protomers L) C-terminal domain of vesicular stomatitis virus (PDB: 3HHW) 20-protomers M) Proteasome inhibition (PDB: 3NZW) 30 protomers.



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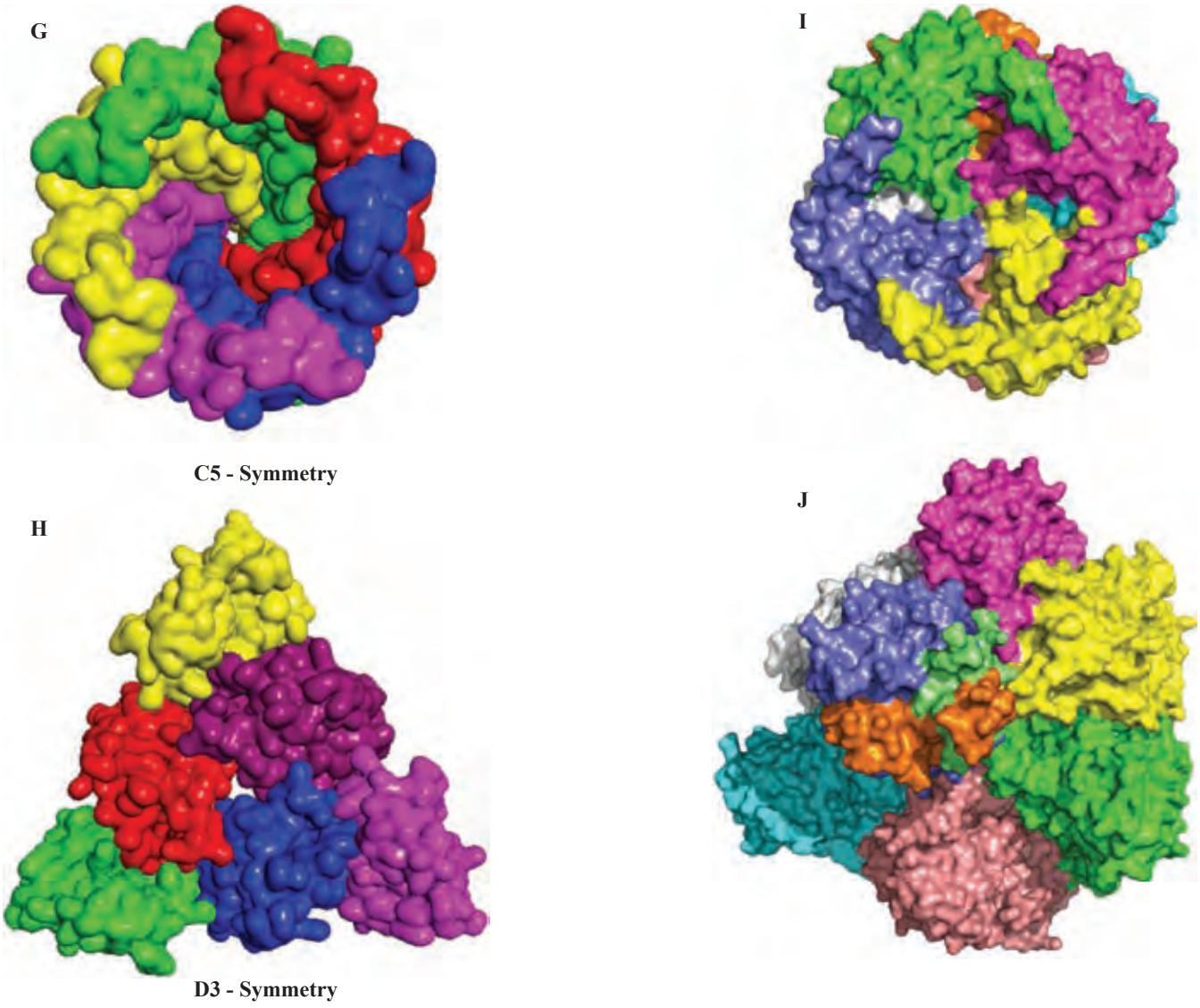


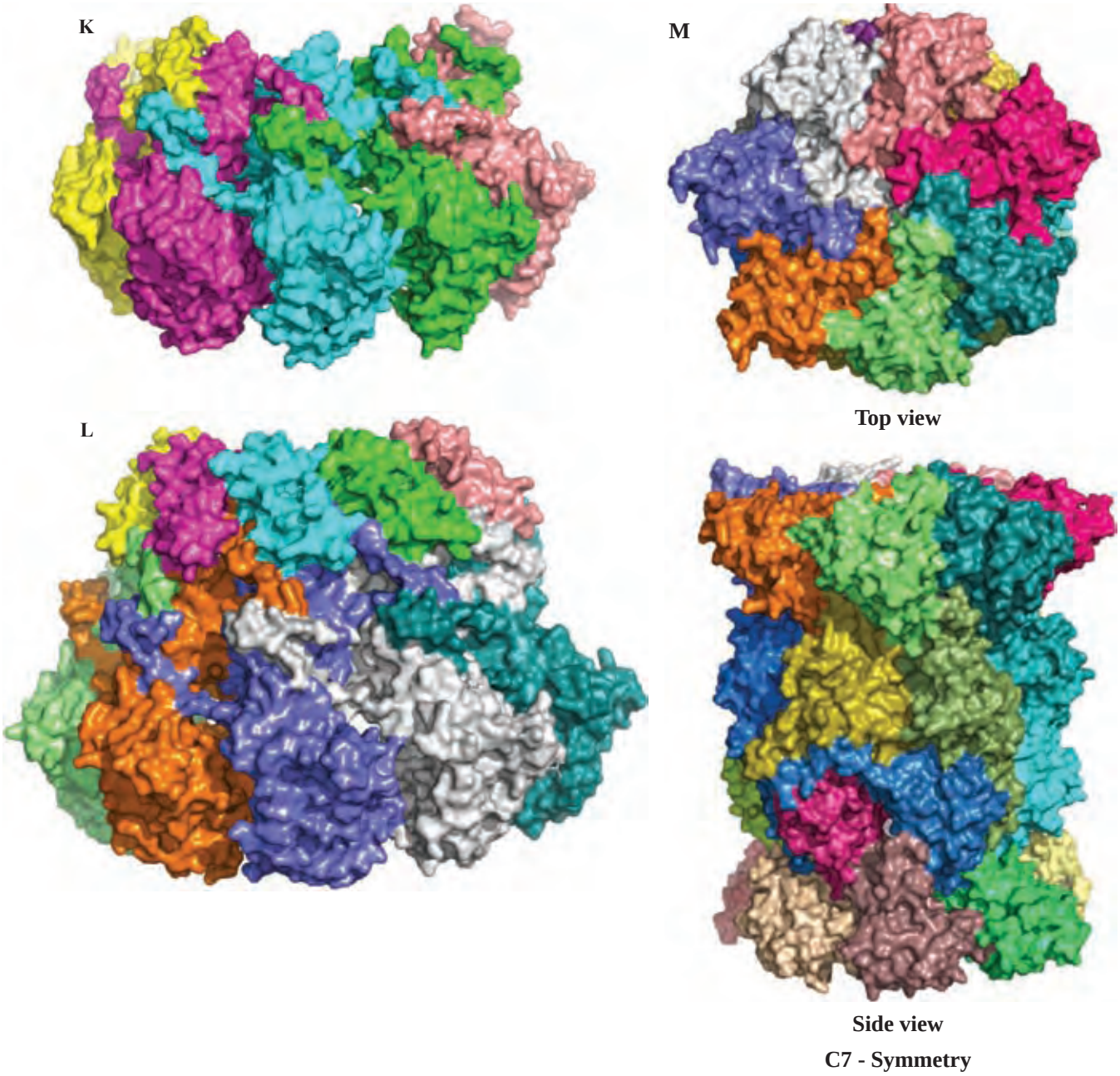
odd number of subunits (protomers) are less frequently observed than even-numbered ones. While protein-protein dimer complexes are most prevalent among oligomers found in the PDB, complexes containing more than 30 individual protomer subunits[17] have also been characterized. In *Escherichia coli*, an average oligomeric state of four has been estimated for the proteins.[38]

The quaternary architecture of these multimeric protein complexes represents one of the most intricate levels of molecular self-organization. Analyses of protein-protein complexes show that they exhibit large variations in their symmetry, size of the interacting domain and their molecular weight.[7,11,39–46] The assembly of protomers in most homooligomeric complexes is symmetric.[42–48] Most homodimers (complexes containing two molecules of the same subunit) are C_2 symmetric; homotrimers predominantly exhibit C_3 symmetry; and most tetramers and hexamers exhibit dihedral symmetry.[7,43] A few representative examples of lower and higher order oligomers containing 2 to 30 identical (homooligomer) or non-identical (heterooligomer) subunits in the complex are shown in Figure 3.

3. Activities of Protein-Protein Complexes

Protein-protein complexes have evolved from monomeric forms[49] in order to acquire new abilities and functions.[39,50] Unlike monomers, the functions of these complexes can be regulated and expressed

Figure 3: (Continued).



selectively in response to stimuli.[51–53] For example, adenosine-5′-monophosphate (AMP)-activated protein kinase (AMPK) is a heterotrimeric protein complex which has a key role in regulating cellular energy metabolism.[54] AMPK is an energy-sensing enzyme complex, which, in response to a fall in intracellular adenosine triphosphate (ATP) levels, activates energy-producing pathways and inhibits energy-consuming processes.[54] AMPK has been implicated in a number of diseases related to energy metabolism including type 2 diabetes, obesity and cancer.[55,56] AMPK remains inactive and is converted to a catalytically competent form only upon the phosphorylation of an activation loop within the kinase domain (Figure 5). The phosphorylation is, in turn, promoted by AMP binding to a c-regulatory domain. AMP binding also protects the enzyme against dephosphorylation.[57]

Some enzymes are functional only in their oligomeric form. Rhodocetin[58] is a unique heterodimer purified from the crude venom of the Malayan pit viper, Calloselasma rhodostoma. It

Figure 4: Multimeric states of proteins in the PDB as on July 2011.

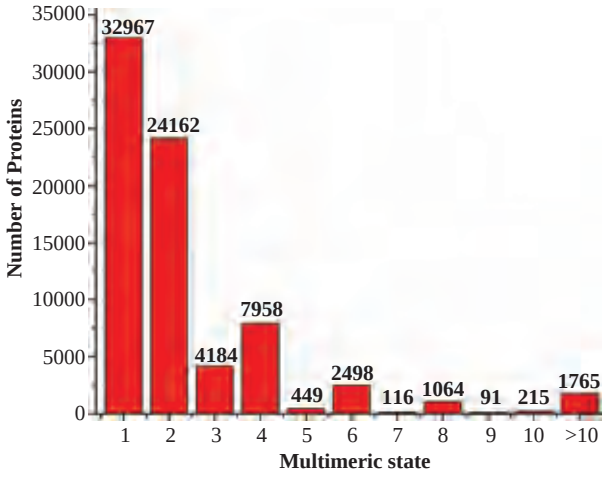
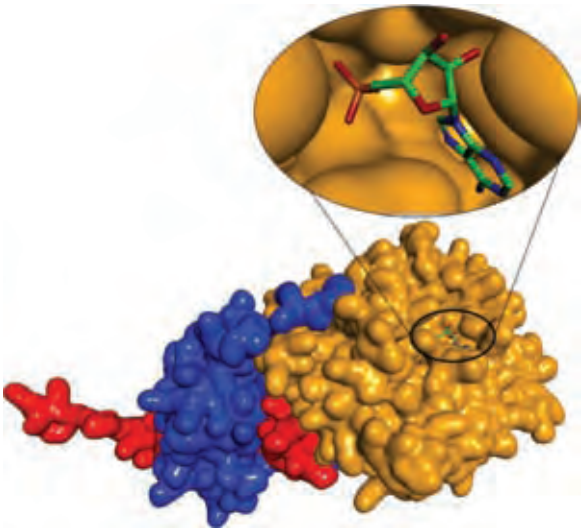


Figure 5: The crystal structure of AMP-activated protein kinase (AMPK) heterotrimer. The inset shows AMP bound to the AMP binding site in AMPK (PDB: 2Y8L).



consists of two protomers, the α- and β-subunits of 133 and 129 residues respectively. It inhibits collagen-induced aggregation and does so only when present as a dimer.

Another intelligent design is the multiple-active site complexes. The bovine trypsin-Bowman-Birk type inhibitor (BBI)[59] complex from soybean contains an assembly of cysteine-rich and highly cross-linked small proteins (Figure 6). They function as specific pseudo substrates for the digestive proteinases like trypsin and chymotrypsin and inhibit their activities.[59] BBI

Aggregation: is the process where molecules of the same kind (e.g. proteins) preferentially interact with each other rather than with the solvent.

Figure 6: Crystal structure of the complex formed by bovine trypsin and the Bowman-Birk type protease inhibitor (PDB: 1TAB). The inset shows the cysteine-rich and highly cross-linked Bowman-Birk inhibitor bound to the interacting domain of bovine trypsin.

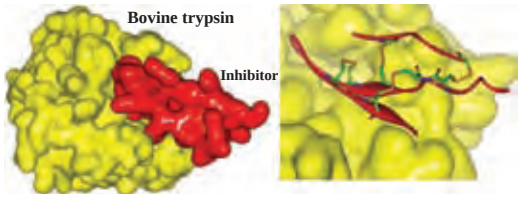
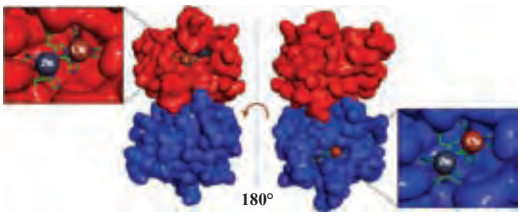


Figure 7: "Front" and "reverse" views of the crystal structure of superoxide dismutase homodimer enzyme complex (PDB: 2SOD), showing the active site on each of the two subunits. Inset show Zn and Cu coordinated at the active sites of the two protomers.

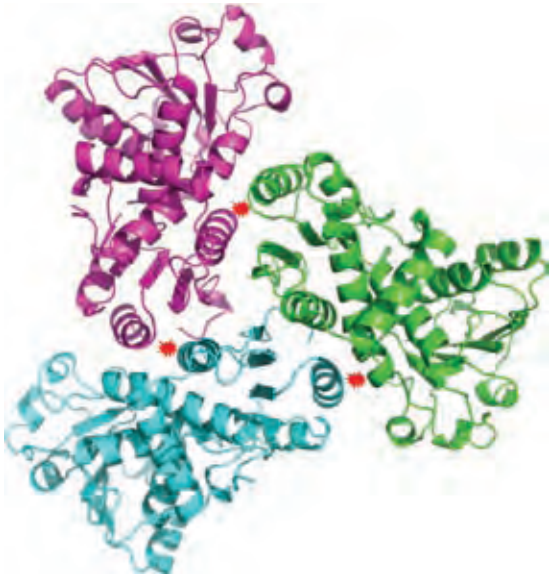


contains two independent inhibitory binding sites, one for trypsin and the other for chymotrypsin and binds each protease to form a 1:1 complex, which augments its inhibitory activity towards them. Superoxide dismutase,[60] a dimeric enzyme complex, has an active site on each of its two subunits, which increases the number of productive collisions between enzyme and substrate and hence the rate of substrate transformation (Figure 7).

The active site of the *E. coli* aspartate transcarbamoylase dimer complex[61] is formed at the junction of the two interacting subunits which renders it with improved specificity through substrate channeling and assisted catalysis through subtle inter subunit motions (Figure 8).

Another important complex biological process, where nature has evolved an elaborately designed set of protein complexes for regulated activity, is in signal transduction. G-protein-coupled-receptors (GPCRs) are a type of membrane-spanning receptor proteins which receive various physiological signals like hormones and environmental signals such as heat and light and transmit them from the extracellular environment to the cell interior.[62] The GPCR complex consists of a seven transmembrane

Figure 8: A Ribbon diagram of the crystal structure of the *E. coli* aspartate transcarbamoylase (ATCase C) trimer (PDB: 3CSU) viewed along the trimerization axis. The asterisks (red) represent the three inter-protomer active sites containing residues from adjacent chains.



helix (7TM) scaffold with an extracellular receptor domain and a cytoplasmic receptor surface (Figure 9).[63,64] The cytoplasmic receptor surface is bound to a heterotrimeric G protein complex ($G_{\alpha\beta\gamma}$) containing a guanosine diphosphate (GDP)-bound G_α subunit in its "silent" form. The binding of a signal, in the form of a hormone, to the extracellular receptor surface changes its conformation. This conformational change extends via the 7TM scaffold into the intracellular domain and catalyzes GDP→GTP (guanosine triphosphate) exchange in the G_α subunit.[65] The GTP-bound G protein then decouples from the receptor and dissociates into a G_α–GTP subunit and a $G_{\beta\gamma}$ dimer subunit. Both G_α–GTP and $G_{\beta\gamma}$ subunits can elicit cell-specific responses via particular effector proteins and the regulation of intracellular second messenger levels.[62] The hydrolysis of GTP to GDP within G_α results in the formation of G_α–GDP which re-associates with the $G_{\beta\gamma}$ dimer, both of which recouple with the cytoplasmic receptor in the 7TM scaffold, hence completing the G protein cycle.

Apart from performing regulated functional roles, protein complexes such as microtubules[66] also take care of the transient or permanent structural needs in cells. Microtubules are a network of protein filaments that spatially organize the cytoplasm of eukaryotic cells.[67] These are tube like dynamic polymers of tubulin, which is a heterodimer of α-tubulin and β-tubulin associated with GTP (Figure 10). GTP-bound tubulin tends to bind on to the so called (+) ends of tubulin and form protofilaments, which self-assemble into hollow microtubules. The hydrolysis of GTP to GDP through inter-domain contacts along the microtubule results in the destabilization and subsequent disassembly of the microtubule structure.[68] This dynamic behavior of microtubulin is coupled to chemical transitions in the adenosine triphosphatase (ATPase) cycle to effect the directed movement of proteins and other large molecular cargo along the microtubule surface, in and out of cells and organelles.[69] The microtubule self-assembly process also plays a major role in cell division.[70]

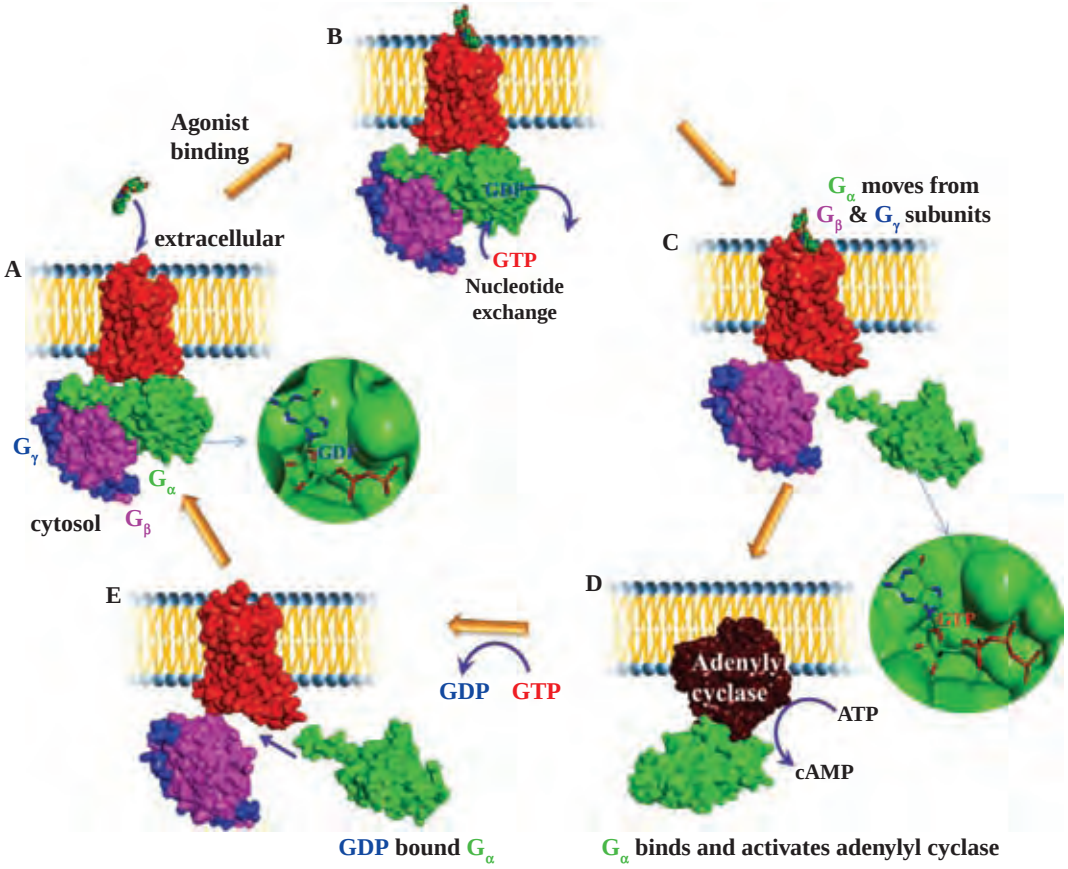
These preceding examples exemplify the remarkable structures and regulated dynamism inherent in oligomeric protein complexes and highlight their superior functional versatility over monomeric enzymes.

4. Protein-Protein Interactions

In a typical protein complex, two or more protomers interact with each other through and along specific patches, called "interaction domains", on their surface.[71] A surface patch consists of a central surface accessible residue and its nearest surface accessible neighbors, comprising the C^α and other atoms of these residues. The residues at the protein-protein interaction domains predominantly contain apolar aliphatic or aromatic side chains. Both polar and apolar side chain containing residues including glycine (Gly) are found at the center of these domains.[72] So far as is known, protein-protein interactions in oligomeric protein complexes result from interactions between residues that are exposed at, rather than buried inside, the surface of the component proteins.[73] Thus the physical connection between protein surfaces in oligomeric complexes is like a hand-shake or a hand-in glove model, where the interaction is between two surfaces (Figure 12A), rather than like a fork-in-spaghetti model, where atoms on the surface of a protein are in contact with those at the core of the protein partner. This is true in all protein-protein complexes studied, including domain swapping complexes.

The surface patch of the hen egg white lysozyme (PDB: 1FDL), complexed with the monoclonal antilysozyme antibody D1.3, is centered around a glycine (Gly 20).[72] Residues 18–27 and 117–125 are also surface-exposed and comprise two discontiguous patches, through which the hen egg white lysozyme makes hydrogen bonds and van der Waals interactions with the antibody (Figure 11).

Figure 9: A Schematic representation of the G-protein-coupled-receptor (GPCR) cyclic pathway. A) Extracellular agonist binding to the β2AR of the 7-transmembrane helix (7TM) scaffold (red), bound to the G-protein, $G_{\alpha\beta\gamma}$ heterotrimer complex (PDB: 3SN6) at its cytosol end. The surface of G_α is shown in green, G_β in pink and G_γ in dark blue. The inset shows GDP bound to G_α a subunit of the G-protein heterotrimer complex. B) Receptor binding leads to conformational rearrangements at the cytoplasmic end of the 7TM segment and in the G-protein complex resulting in the exchange of GDP with GTP at the G_α subunit. C) the GTP-bound Gα protomer dissociates from the $G_{\beta\gamma}$ complex anchored to 7TM. The inset shows GTP bound to the G_α monomer. D) The GTP-bound G_α monomer binds to the adenyl cyclase complex (PDB: 1AZS) and catalyzes the synthesis of cyclic AMP. E) The Dephosphorylation of GTP leads to a GDP-bound G_α subunit which complexes with the free $G_{\beta\gamma}$ heterotrimer interface and completes the cycle.



A few water molecules at the lysozyme (antigen)-antibody interface also mediate contacts between the antigen and the antibody.[74]

Domain swapping complexes are an extreme possibility for protein-protein association where the interacting domain is made by the mutual exchange of an entire domain, like an α-helix or a β-sheet between each molecule of a dimer complex.[75] For example, in the dimer of bovine pancreatic ribonuclease (RNase A, PDB: 1A2W)[76] the N-terminal helix (residues 1–15) of each subunit is swapped into the major domain (residues 23–124) of the other subunit. The 3-stranded β-sheets of the two subunits are hydrogen bonded at their edges to form a continuous 6-stranded sheet across the dimer interface (Figure 13). There is no insertion of any residues from a protomer through the sheet surface of the other subunit. The two subunits of the RNase A dimer are related by a rotation of approximately 160 degrees.[76]

In the odorant-binding protein (OBP) homodimer complex from bovine nasal mucosa (PDB:1OBP),[77] the α-helix of each protomer stacks against the β-barrel of the other protomer (Figure 14).

The interacting domains in a protein-protein complex can also be appended through disulphide bonds. Bovine seminal ribonuclease[78] (PDB: 1BSR), is a dimeric enzyme in which the N-terminal segments of either monomer are interchanged so that each active site is formed by residues from both subunits. The two chains are related by a non-crystallographic two-fold symmetry and are covalently linked by two consecutive disulphide

Figure 10: Polymerization of tubulin heterodimers into microtubules. A) A GDP-bound tubulin heterodimer complex (PDB: 1TUB): α-subunit (green), β-subunit (red) and interdomain site (positive end) for GTP or GDP binding (blue) to the dimer. B) GTP-bound tubulin units bind on the positive end to form protofilaments (PDB: 1FFX). C) Protofilaments self-assemble into hollow microtubules (EMDB: 1131). Dephosphorylation of GTP leads to the disassembly of microtubules to the GDP-bound tubulin heterodimer complex.

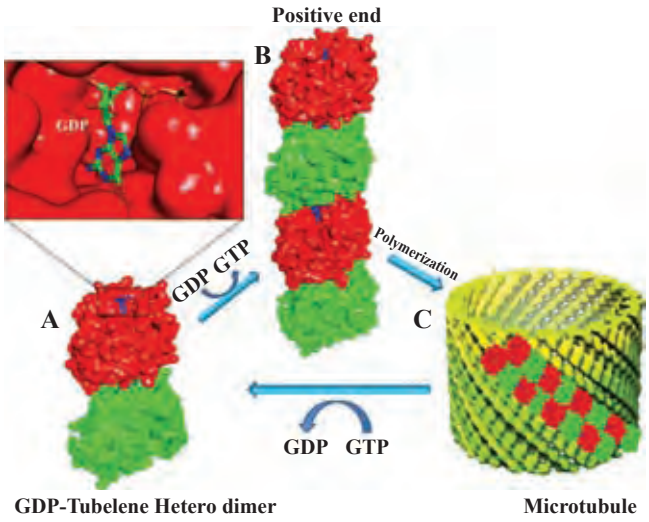
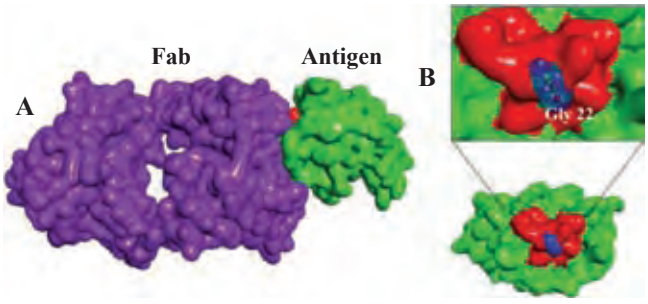


Figure 11: A) Complex of Fab, from the monoclonal Anti lysozyme antibody D1.3 (purple) and the antigen, hen egg white lysozyme (green)(PDB: 1FDL). B) The interacting domain on the surface of the hen egg white lysozyme comprising 9 residues (red patch) including the central Gly22 residue (blue). The inset shows Gly22 in a ball and stick representation (yellow) at the center of the interacting domain (red) on the surface of the hen egg white lysozyme (green).



bonds, which form an unusual sixteen-membered ring across the dimer interface (Figure 15).[79]

5. Formation of Protein-Protein Interfaces

An understanding of the physical nature of the interactions between protomers in protein-protein complexes is crucial to comprehending their physical characteristics, the origin, control and the consequences in biological processes.

Figure 12: Clip art showing interactions between surfaces in A) a handshake model and B) a fork-in-spaghetti model.

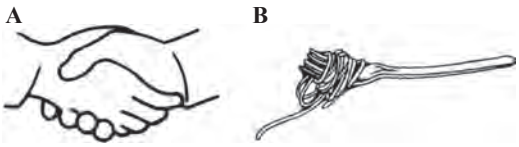
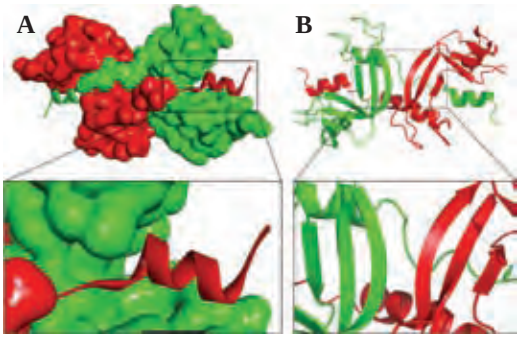


Figure 13: A) Crystal structure of the bovine pancreatic ribonuclease (RNase A) homodimer complex (PDB: 1A2W). The helical domains of each RNase A subunit are represented in a ribbon diagram. The inset shows the interaction between the helical domain of one protomer with the major binding domain of another. B) The ribbon diagram of the RNase A homodimer complex. The inset shows the edge-to-edge inter strand interactions between the two protomers.



An interacting domain on a protein surface may preexist in the monomeric form of a protomer, or may get freshly exposed during or as a result of the formation of the protein complex. When residues of an interacting domain are already exposed on the protomer surface, possibly anchored by intra-subunit bonding, complexation with the protomer partner is straight forward. However, most surface-exposed residues have few intramolecular contacts and are flexible.[21,80] In these cases, the residue conformations are not preformed or fixed until dimerization occurs.[39] Alternatively, the residues on an interaction domain may remain unavailable for interaction (buried inside the protein) and only get freshly exposed prior to binding with the protomer partner. For example, actin is a 372-residue long protein which is composed of four subdomains: subdomain I (residues 1–32, 70–144, and 338–372), subdomain II (residues 33–69), subdomain III (residues 145–180 and 270–337), and

Figure 14: Crystal structure of the odorant-binding protein homodimer complex. One subunit is shown in red and the other in green. The surface of the green monomer is shown in transparency. At the interacting domain, the helical domain of either monomer stacks against the β-barrel of the other.

Figure 15: A) The surface representation of the crystal structure of the Bovine seminal ribonuclease homodimer complex (PDB: 1BSR). B) A Ribbon diagram of 1BSR highlighting all the disulphide bonds in the dimer. The inset shows the two consecutive intermolecular disulphide bonds forming a 16-membered ring across the dimer interface. The disulphide bond is represented in a ball and stick model.

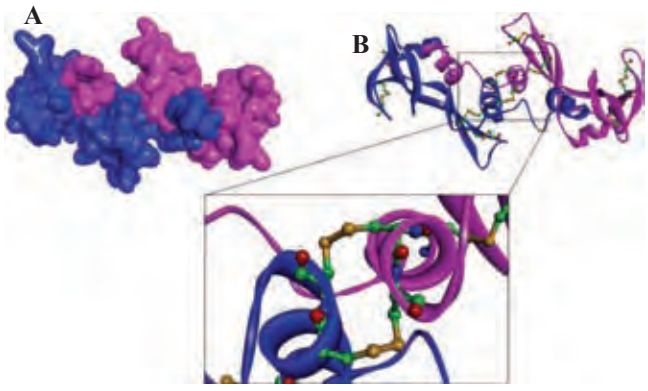
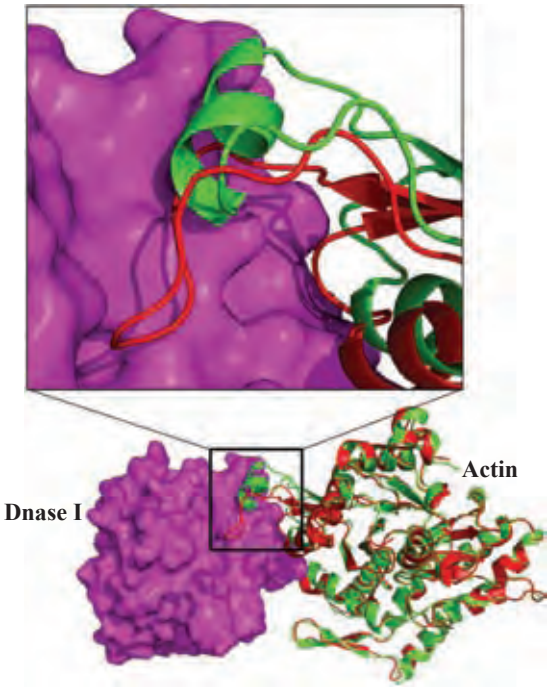


Figure 16: Crystal structure of the actin-DNase I complex (PDB: 1ATN). The surface of DNase I is represented in pink. The two conformational states of actin one bound with DNase I (red) and another unbound free monomer (green) are shown in the ribbon diagram and superimposed on each other. The inset shows the conformational change from helix (green) in the unbound form, to the turn (red) in the DNase I-bound form of the subdomain II of actin.



subdomain IV (residues 181–269).[81] It is the major component of the thin filaments in muscle cells and of the cytoskeleton in non-muscle cells.[82] The residues H40-G48 of the actin subdomain II bind to DNase I (PDB: 1ATN). They are folded into an α-helix in the unbound conformation and a β-turn in the bound conformation (Figure 16). This helix-to-turn conformational change in subdomain II of actin exposes new residues on its surface that form hydrogen bonds as an additional strand to the β-sheet in DNase I.[83]

Interacting domains may form as a result of large conformational changes in one or either of the protomers upon complexation.[83] The LIR-1 protein is an immune receptor protein expressed on the surface of lymphoid and myeloid cells (PDB: 1P7Q).[84] LIR-1 contains two domains named D1 and D2 which are positioned at an interdomain angle of ≈85° in the unbound form of LIR-1. This angle changes[85] to 100° when LIR-1 forms a dimer complex with the protein HLA-A2 (Figure 17).

Modifications in the presentation of the same interacting domains in a dimer can be facilitated by their binding with allosteric modulators.[23] An excellent example involves the arabinose promoted modification of the dimer conformation and function in AraC. AraC is a transcription regulator in *E. coli*, which controls genes involved in the uptake and catabolism of the sugar arabinose. In the monomeric form, it has two domains, an arabinose-binding domain and a DNA-binding domain. When it dimerizes, the dimer has two arabinose and two DNA-binding domains. The two DNA-binding

Figure 17: Crystal structure of LIR-1 bound to HLA-A2. LIR-1 is shown in a ribbon diagram. The surface representation of HLA-A2 is shown in pink. The crystal structure of HLA-A2 bound LIR-1 (green) is superimposed on the crystal structure of unbound LIR-1. The angular diagram shows the angles between the two β-sheet domains D1 and D2 in the bound (green) and the unbound (red) forms.

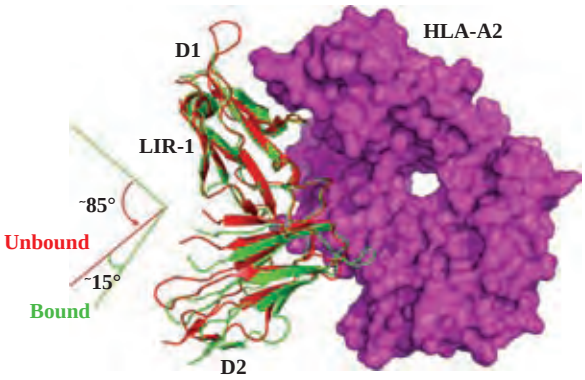
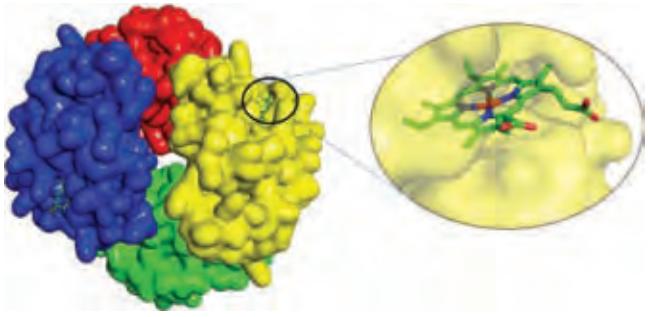


Figure 18: Surface representation of the crystal structure of human deoxyhaemoglobin (PDB: 2HHB). The inset shows a heme group attached to one of the monomers.



DNA: is deoxyribonucleic acid.

domains each bind to a different DNA site. With arabinose bound (PDB: 2ARC), AraC dimerizes such that the two DNA sites to which the protein attaches itself are distantly separated, creating a 210-base pair loop in the DNA and repressing the transcription of the promoters pBAD (the promoter for the arabinose operon) and pC. If the effector (arabinose) molecule is absent (PDB: 2ARA), the protein dimerizes along a completely different dimerization interface (interacting domain) and places the two DNA-binding domains close together (Figure 2). Now the dimer attaches itself to two adjacent DNA sites and promotes pBAD transcription instead of repressing it.[24]

The interacting domains within the subunits of oligomers can undergo repeated modification by allosteric modulation of individual protomer subunits in proteins such as hemoglobin that exhibit positive cooperativity. The hemoglobin tetrameric complex has multiple substrate binding sites (Figure 18). The quaternary structure of the oligomeric protein is such that it has an initial low affinity for the substrate.[86] However, as the ligand binds to one subunit of the oligomer, it introduces small changes in its local tertiary structure. As a result, the structural constraints of the quaternary oligomer complex relax, large changes occur in the quaternary structure, and the unliganded binding sites (active sites) exhibit increased ligand affinity.[87]

On the other hand, simple changes in specific biophysical and biochemical conditions like the change in concentration of a modulator (like Ca^{2+} Mg^{2+} and Na^{+})[88–90] can also prompt changes in dimerization interface and function. Evolution has utilized the unique ability of polypeptide chains to isomerize and adopt thermodynamically stable environment-dependent conformations to modify their interacting domains and regulate their recognitions and interactions with other cellular proteins.

6. Why Large Oligomeric Protein-Protein Complexes?

It is apparent from these examples that in various contexts, large protein-protein complexes are more beneficial to cells than are small proteins. The former can present a greater number of interacting domains (patches) on their surface than the latter. However, it is notable that evolution has chosen to build these large structures through multimeric associations of several small subunits instead of using single long high-molecular weight polypeptides. It has been proposed that this is because 1) assuming a constant probability of errors during protein expression (synthesis), a longer chain will have a greater chance of containing a deleterious error than a shorter one; 2) it is easier to selectively eliminate faulty subunits from multimeric complexes after several cycles of use; 3) the functional versatility of a protomer can be enhanced if it can function both as a monomer and as part of various multimeric complexes.[91]

7. Thermodynamics of Protein-Protein Interactions

The association of molecules to form higher ordered oligomers is in many respects, analogous to the block condensation model for protein folding where pre-folded subunits associate

to form higher order structures.[92] Given the multitude of molecules that float around in the cell, however, the specific molecular recognition and association between folded monomers or their conformational isomers seems like a random event. Then how do biological processes such as signaling and inhibition which require error proof, and highly specific associations between protein molecules occur, even at extremely low physiological concentrations? This question can be addressed by understanding the thermodynamics of the protein-protein association reaction.

The Gibbs free energy ($\Delta G_{observed}$) of the association reaction between two monomeric proteins, in their stable solution conformations or in their isomerized forms, is dependent on their observed association constant K_a at temperature T, as in the equation:

$$\Delta G_{observed} = -RT \ln K_a$$

The kinetics of assembly of several proteins is analogous to bringing together two rigid objects. This means that in these cases, no intermediates are involved during the association of the subunits into a protein complex. However, as we saw in the examples of the preceding section, most association events are initiated by the change in conformation (i.e. the isomerization) of one or more of the interacting protomers. If the association process involves both the isomerization of the monomers and the formation of an intermediate structure before realizing the stable macromolecular quaternary complex, the theoretical association energy (ΔG) will have contributions from three terms:

$$\Delta G = \Delta G_{interaction} + \Delta G_{isomerization} + \Delta G_{rot,trans}$$

where $\Delta G_{interaction}$ represents the interaction energy, $\Delta G_{isomerization}$ the isomerization energy which accounts for the energy lost if the conformations of the free monomers are altered during complex formation, and $\Delta G_{rot,trans}$ the energy lost in fixing one molecule relative to the other (Figure 19). In more accurate expressions, the energy required to solvate each of these forms, and to transfer the atoms from one form of each of the protomers to another, may also be included.

The interaction energy $\Delta G_{interaction}$ results from the non-bonded interactions of both polar and apolar atoms at the protein-protein interface and is the driving force for the association process. This term has an enthalpic component due to van der Waals interactions, hydrogen bonds, and charged electrostatic interactions as well as an entropic component that results from the liberation of bound water molecules from the interface (the hydrophobic interactions). For molecules to associate and form a stable complex, the interaction energy ($\Delta G_{interaction}$) must be sufficiently large to overcome the two opposing energies ($\Delta G_{isomerization}$ and $\Delta G_{rot,trans}$).[52]

An important consequence of this feature is that not all proteins can associate with each other, because it is not always thermodynamically favorable. The interaction domains of proteins in complexes have evolved to have the optimum shape, size and topology such that their net energy of association is favorable only with their designated partners under suitable physiological conditions. Proteins that do not engage in protein-protein interactions have surfaces that prevent them from accidently interacting with other proteins. Because of this structural feature, these proteins can exist at relatively high levels inside the cell. It may, however, be energetically feasible for an interacting domain to associate with more than one protein partner. Generally, the selectivity of interactions in such proteins is governed by the control of cellular expression of one of the protein partners. In complexes whose oligomeric states are regulated by binding with allosteric modulators, the binding event switches the protein between two conformational states, only one of which contains the interaction domain suitable for oligomerization. One may recall that in GPCRs a cascade of such conformational changes enable the

Figure 19: A Simplified version of the association pathway for a homodimer between two monomers (a). Initially conformational changes occur in both monomers (a→a′) before they overcome the diffusion barrier and fix themselves into position (aa) with respect to each other.

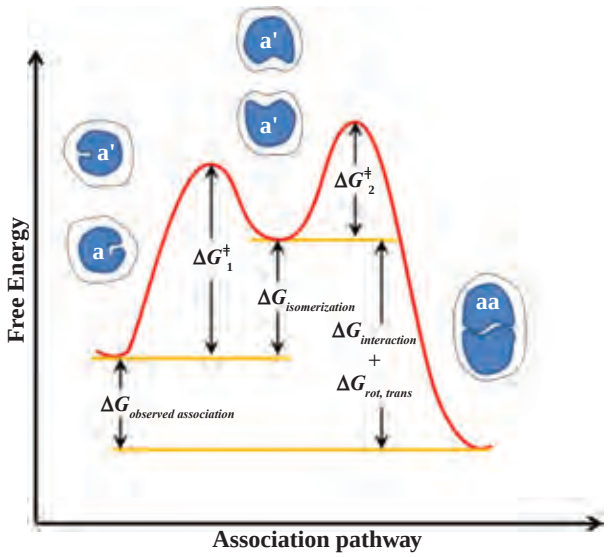
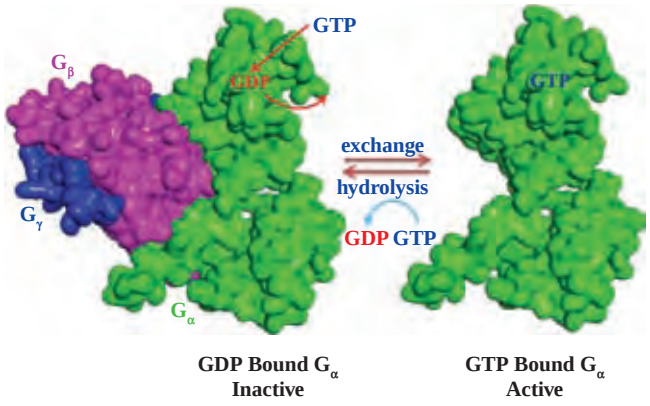


Figure 20: The equilibrium between the inactive $G_{\alpha\beta\gamma}$ G-protein heterotrimer complex containing the GDP-bound G_α protomer and the catalytically active GTP-bound G_α protomer. The forward reaction occurs when GTP exchanges with GDP at the binding site of the G_α subunit. The reverse reaction occurs when GTP undergoes dephosphorylation to give a GDP-bound G_α subunit.



cyclic activation and inactivation of the G_α subunit in the G-protein complex (Figure 19). While the G_α subunit is active in the GTP bound form, it becomes inactive upon the dephosphorylation of GTP to GDP (Figure 20).

Å: is the symbol for angstrom, a unit of length. It refers to one tenth of a nanometer.

The thermodynamic picture discussed above is a simplification of the structural and biophysical determinants that lead to the formation of a protein-protein complex. The characterization of the complete set of determinants is more complex and will enable the elucidation of the intriguing processes that they influence and their evolutionary mechanisms in biological systems. Such understanding has already improved our ability to predict the functions of novel proteins and their reaction pathways in cells, and to design potential drugs.[93–96]

8. Protein-Protein Interaction Interfaces

Understanding the characteristics and function of a protein-protein complex requires a molecular analysis of the interacting domains of the proteins. These domains are termed as interfaces. Interfaces are optimal tight fitting[7,52,97] regions characterized by complementary pockets scattered throughout, with the central region being enriched in structurally conserved residues.[11,98–102] Interfaces are defined based on the change in their solvent-accessible surface area (Δ ASA), calculated from a knowledge of the protomer structure[103] when going from a monomeric to an oligomeric state. The interface atoms are defined[104–115] as those whose ASAs decrease by > 1 Å^2 upon complexation. Since proteins interact through these interfaces, a number of their physico-chemical attributes like frequencies of atom-types, functional groups, amino acids,[53,116] salt bridges,[117] hydrogen bonds[39] and hydrophobicity,[39] have been analyzed as possible determinants of the oligomer characteristics.[5,7] Owing to the statistical nature of several of these analyses,[2,5,40] and due to the ambiguities inherent in the interpretations of the experimental methods[22,118–124] employed, it is commonplace to generalize these characteristics. Such generalizations have served well to design algorithms for prediction[96,125–133] of protein-protein interaction interfaces on protein surfaces. However, due to the complexity and uniqueness of each protein-protein interaction interface, inferences made from such analyses must be treated with caution.

Protein–protein interaction interfaces are complex and can be characterized by their size, shape, electrostatic and hydrophobic interactions,[15,116] surface complementarity[11] and flexibility of the protomers. Several reviews[5,7,39,46,114,134,135] have discussed these features and their significances in detail. The chief among these features is discussed here. The size of the protein interface is usually measured in terms of the total Δ ASA for both molecules involved in the complexation event (going from a monomeric state to a dimeric state), and is expressed in area (Å^2) units. Since both the interacting protomer surfaces would contribute to approximately equal areas at the interface, the interface size is defined as half the total Δ ASA.[11] In general the size of the interface area depends on the molecular weight of the interacting protomers and can vary from just a few hundred Å^2 (for example, the human TβR2 ectodomain-TGF-β3 complex,[136] PDB: 1KTZ, interface area of only 493 Å^2) to >5000 Å^2. Analyses of the protein-protein complexes in the PDB have revealed a standard size of 1200–2000 Å^2 for the interfaces[52,114](for example, the actin-deoxyribonuclease 1 complex,[81] PDB:1ATN—Figure 21). The size and the buried hydrophobic surface area have a direct correlation with the energy of the protein-protein interaction.[25] Smaller interfaces (1150–1200 Å^2) normally constitute short-lived and low-stability complexes[21] (dissociation constant,$K_d > 10^{-7}$ M)(for example, IgG1 E8 Fab fragment-cytochrome C complex,[137] PDB:1WEJ). The larger ones (2000–4660 Å^2) are usually observed in inhibitor complexes[51] (for example, Enoylreductase complex,[138] PDB:1DFG) which require strong and tight binding ($K_d \leq 10^{-8}$ M). However, the interface area does not always correlate with the stability or the dissociation constant of the complex. For example, the TDPI-Trypsin complex (PDB: 1UUY)[139] which is a permanent complex (K_d = 5.6 nM), has an interface area of only 640 Å^2.

Figure 21: The surface representations of the crystal structures of three dimer complexes with different protein interface sizes. A) A heterodimer complex between the IgG1 E8 Fab fragment and cytochrome C (PDB:1WEJ) Interface area: 1180 Å^2. B) Actin-deoxyribonuclease 1 heterodimer complex (PDB: 1ATN) Interface area: 1770 Å^2. C) Enoylreductase homodimer complex (PDB: 1DFG) Interface area: 2580 Å^2.

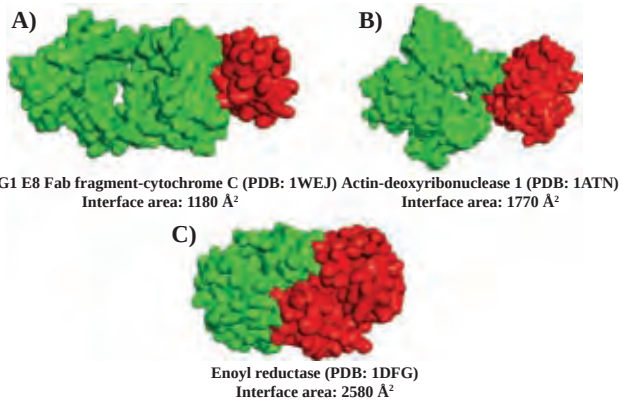


Figure 22: A surface representation of the interface between streptococcal protein G and the human immunoglobulin IgG (PDB:1FCC). The hot spot residues are shown in a stick representation at the interface. The inset shows the details of the hydrogen bonding interactions between the hot spot residue Gly27 of the C2 fragment of streptococcal protein G (light pink) and the warm spots Ser254 and Ile253 of the human immunoglobulin IgG (yellow). Protein G (yellow) and IgG (pink) are shown in a ribbon representation in the inset.

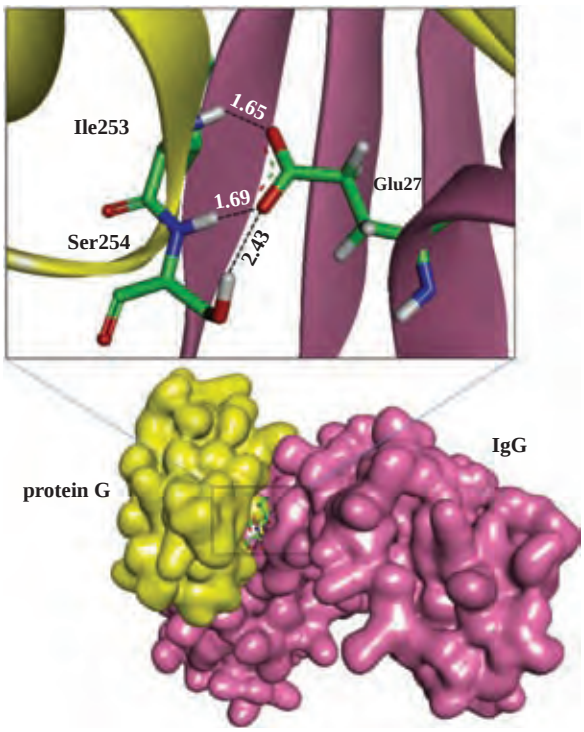
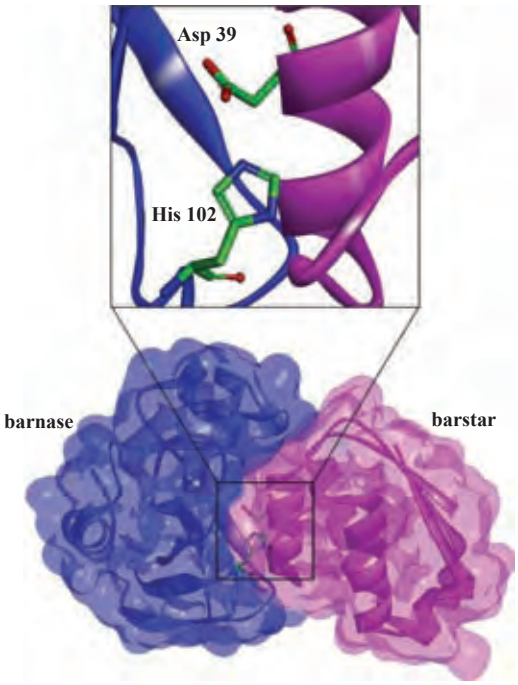


Figure 23: A surface representation of the barnase-barstar heterodimer interface (PDB: 1BSR). The single pair of hot spots at the interface between His102 of barnase and the Asp39 residue of barstar is shown in a stick representation. The inset shows the ribbon representation of the relative orientations of His102 and Asp39 (shown as in stick form) at the barnase-barstar interface.

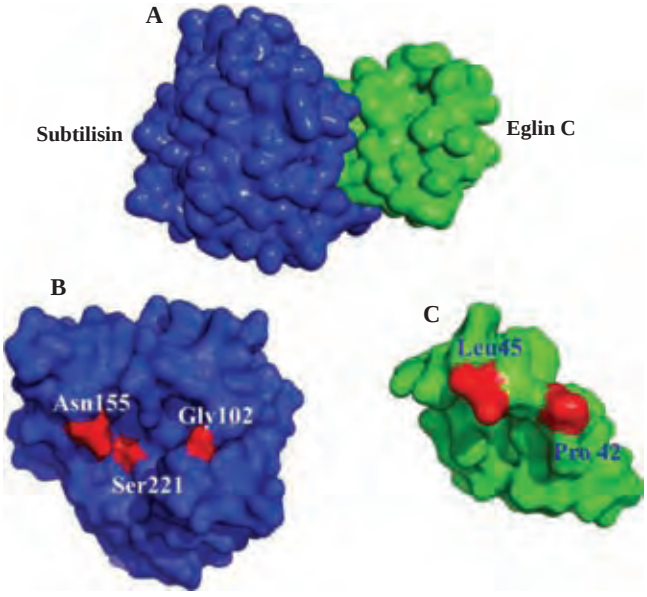


Approximately 2/3rd of the interface surface comprises non-polar atoms.[39] The few conserved polar atoms at the binding interface form hydrogen bonds and counter the entropic cost on binding.[73] The surrounding residues generally provide a flexible cushion. On an average, there is about one hydrogen bond per 100–200 Å^2 of the interface surface.[39] The hydrogen bonds formed between side chain COOH of Glu27 of the C2 fragment of streptococcal protein G and the hydrogen bond donor atoms in Ser254 and Ile253 of the human immunoglobulin IgG (PDB: 1FCC)[114] are shown in Figure 22. The electrostatic complementarity of interacting surfaces—in other words, the complementarity in shape and juxtaposition of the electrostatic surfaces—contributes to strong binding, rather than the complementary positioning of the hydrogen bonding groups or charges. This is important, since, unlike in the intramolecular backbone hydrogen bonds in proteins, the interface hydrogen bonding atoms are generally in non-ideal geometry, and hence the hydrogen bonds are usually weak in terms of

energy.[117] However, hydrogen bonds and salt bridges directly influence the binding specificity, kinetics, lifetime and binding strength of complexes.[140] The His102 of barnase forms a strong electrostatic interaction with Asp39 of barstar and steers and rapid association (rate constant is 10^8 s^{-1} M^{-1})[141] between the two proteins (Figure 23).[142] It is to be noted that protein affinity and specificity are not necessarily coupled.

By combining X-ray crystallography with site-directed mutagenesis, researchers have analyzed how protein–protein interfaces function. Through alanine scanning mutagenesis of proteins (where subsets of the protein surface are systematically mutated to alanine and the change in the binding free energy of the complex is studied) it is now understood that only a few of the residues at the interacting domain, termed "hot spots", make a significant contribution to the binding free energy of protein-protein complexes.[141,143,144] A residue is defined as a hot spot if there is a significant binding free energy change ($\Delta\Delta G \geq 4$ kcal/mol) when it is mutated to alanine.[12,114,145] However, in order to map all the residues that contribute to the binding free energy of a protein-protein complex, a lower cut off of $\Delta\Delta G \geq 2$ kcal/mol is generally used during the analysis and identification[128,143,146] of potential hot spot residues in protein complexes.

Figure 24: A) Surface representation of the complex between subtilisin Carlsberg (blue) and eglin C (green) (PDB: 1CSE). B) Front view of the interface on subtilisin Carlsberg showing three hot spot residues (Gly102, Asn155 and Ser221) (red). C) Front view of the interface on eglin C showing two hot spot residues (Pro42 and Leu45) (red).



Hot spots are found on either side of the interacting interface. Usually, hot spot residues from two interacting domains form tightly packed pairs, and can be involved in intermolecular hydrogen bonds (Figure 22).

Systematic analyses of hot spots have shown that tryptophan, arginine and tyrosine (in that order) are the fundamental (most conserved) hot spot amino acids.[147] The aromatic residues are understood to contribute through hydrogen bonding (tryptophan is a donor and tyrosine can be both a donor and an acceptor), and the protection of fragile hydrogen bonds from water and π-π interactions. Tryptophan is the most frequently observed hot spot residue due to its large flat aromatic surface. In the growth hormone-growth hormone binding protein complex, there are 29 interfacial residues, out of which four are hot spots.[12,148] Two of these hot spots are tryptophan ($\Delta\Delta G = 4.5$ kcal/mol). Tyrosine is a more frequently observed hot spot residue than phenylalanine due to its hydrogen-bonding ability. Arginine can participate in a hydrogen-bonding network with upto five hydrogen bond donors and a salt bridge through the positive charge on the guanidinium group. Aspartate and aspargine are favored over glutamate and glutamine, presumably due to differences in side-chain conformational entropy. Isoleucine is more than 10 times as frequently seen as a hot spot as leucine. Serine, leucine (not isoleucine) and valine seldom function as hot spots.[12] Owing to the large impact of the hot spot residues on the binding free energy of complexes, they are protected from the bulk solvent by the surrounding residues. A Few common characteristics are discerned for these surrounding residues (See Figures 24 and 25 for examples of hot spots). Their hydrophobicity, shape, charge and interfacial residue type inadequately explain or predict the high energy hot spots.[149]

Most protein-protein interfaces, especially those in homodimers, are complementary to each other. Complementarity is conferred by the careful structuring of interfaces between interacting domains that allows strong interactions between protein pairs (like hot spots) while minimizing the strength of unwanted interactions.[125] Either both the interacting surfaces are found to be planar (Figure 26) as in the serratia endonuclease dimer (PDB: 1SMN) with 4 salt bridges and several hydrogen bonds and nonpolar interactions in the interface,[150] or one of them contains a crevice or a pocket into which a residue or a whole secondary structural domain from the other protomer protrudes.[151] All types of secondary structures (helices, α-sheets, turns and random coil) have been found in the contact areas of

Figure 25: A surface representation of the crystal structure of antigen-complexed Fabs from the mouse monoclonal anti hen egg white lysozyme antibody D44.1 (Center). The front views of the interface for each of the protomers constituting the heterotrimer, with their hot spot residues (red), are shown along the periphery of the trimer complex.

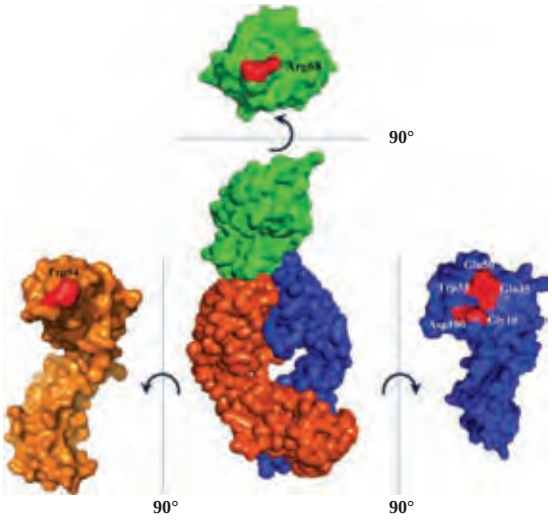


Figure 26: A surface representation highlighting the interface residues (blue and red) along the flat interface of the serratia endonuclease homodimer complex (PDB: 1SMN). The inset shows the stick model of 4 salt bridges between hot spot residues (Arg136-Asp225; Glu239-Lys223; Lys 223-Glu 239; Asp225-Arg136) at the interface.

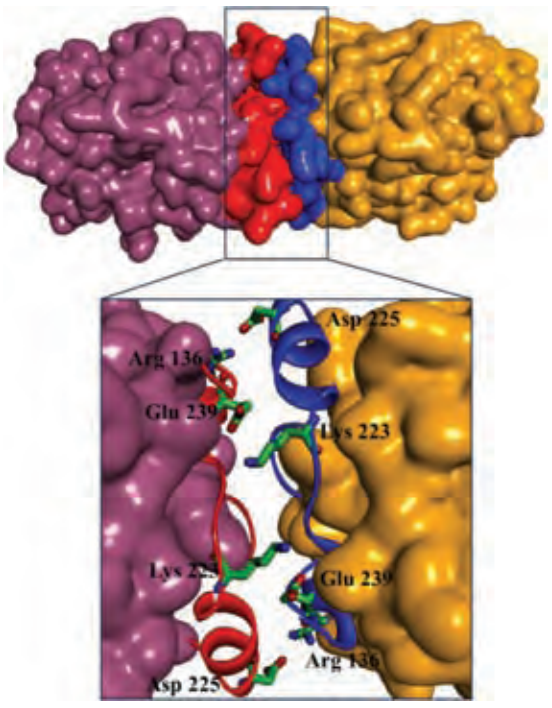


Figure 27: A) The ribbon diagram of the solution structure of the interleukin-8 (IL-8) (PDB: 1IL8) homodimer complex showing a flat β-sheet interface. B) The structure of the ferricytochrome c–*Rhodospirillum molischianum* (PDB: 2CCY) heterodimer showing a flat α-helix at the interface. C) Structure of the complex between bovine pancreatic α-chymotrypsin and the third domain of the Kazal-type ovomucoid from Turkey (PDB: 1CHO) showing the loop (protrusion) at the interface.

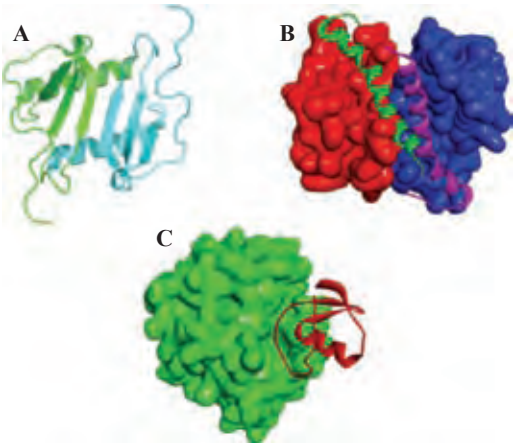


Figure 28: Structure of the heterodimer complex between subtilisin Carlsberg (green, surface representation) and eglin C (ribbon), an elastase inhibitor (PDB: 1CSE) complex. The protrusion interface which forms a loop (residues Gly40-Arg48) is shown in a ribbon + stick representation.

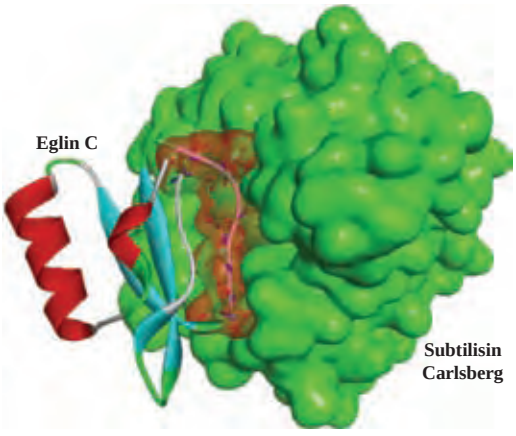


Figure 29: A surface representation of the heterodimer complex between trypsin and serpin showing the 20 residue loop protrusion (ribbon + stick) from serpin at the interaction interface of the complex. The inset shows a magnified image of the 20 residue loop (red ribbon) from serpin protruding into a complementary cavity on the trypsin surface.

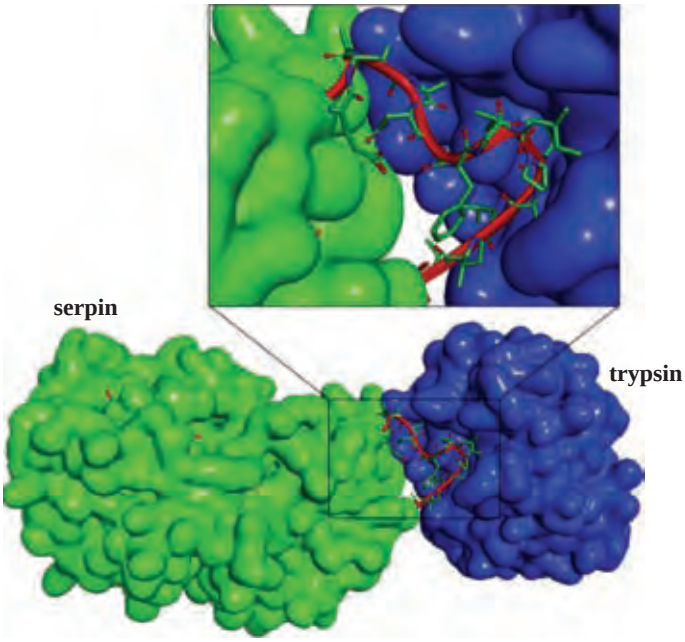
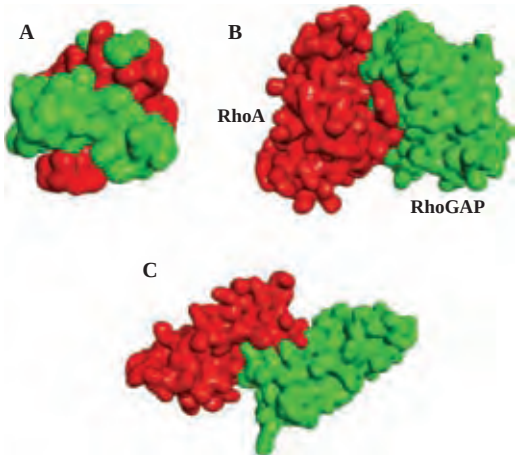


Figure 30: A surface representation of three types of dimers: A) The C2-symmetric Arc repressor obligate homodimer complex (PDB: 1MYK). B) The non-obligate heterodimer Rho A-Rho GAP complex (PDB: 1TX4). C) The isologous lysine homodimer complex (PDB: 1LYN).



the interacting proteins. Representative examples of dimers containing antiparallel β-sheet (interleukin-8 (IL-8), PDB:1IL8),[152] α-helices (ferricytochrome c–*Rhodospirillum molischianum*, PDB: 2CCY)[153] and loop (bovine pancreatic α-chymotrypsin and the third domain of the Kazal-type ovomucoid from Turkey (OMTKY3), PDB: 1CHO),[101] at the interfaces are shown in Figure 27. In the subtilisin Carlsberg-eglin C complex (1 CSE),[154] an elastase inhibitor with a protrusion interface, most of the intermolecular contacts are contributed by nine residues (Gly40-Arg48) in a loop (Figure 28). The complex (PDB: 1I99) between the serine protease inhibitor (Michaelisserpin) and trypsin shows a 20 residue loop that protrudes from the serpin molecule and interacts with a rather large concave region in trypsin (Figure 29).[155]

9. Classification

Protein complexes have been so termed based on the number and identities of the protomers and the interfaces that form the interaction. Oligomers of the same protomer are called homooligomers. Oligomers containing non-identical polypeptide chains are called heterooligomers.[156] When a protomer of an oligomeric complex is not found as a stable structure on its own in vivo, the complex is called obligate. When a protomer is active only in its oligomeric form, then it is said to be functionally obligate. The Arc repressor is an obligate dimer (Figure 2) whose dimeric structure is essential for DNA-binding.[157] When the protomers of an oligomeric complex can exist as stable structures, the complex is called non-obligate. The RNase A dimer is a homodimeric non-obligate protein complex[158] (Figure 13). Many known hetero-oligomeric protein complexes, like the RhoA-RhoGAP dimer,[159] (Figure 30) involve non-obligate interactions of protomers that exist independently.[2]

When the association of monomers in an oligomeric complex involves an association between the same interaction domains on each subunit, it is called an isologous association. Most isologous oligomers exhibit circular symmetry. Arc repressor and lysin dimer complexes are isologous and are complexed in a C_2 symmetric fashion. When different interfaces are involved in the individual monomers of a complex, it is called a heterologous association. Protein-protein interactions can also be vaguely distinguished based on the lifetime of the complex. A permanent complex is stable and exists primarily in the complexed form. A transient complex associates and dissociates in vivo.[5] The dynamic equilibrium of transient complexes is controlled by molecular or biophysical triggers. Transient complexes play a key role in almost all regulatory activities, signal transductions and metabolic processes.

10. Protein-Protein Interaction Inhibitors

Protein-protein complexes are ubiquitous and are central to the activities of biological systems. It has long been realized that small molecule antagonists that disrupt complex formation can have potential as drugs.[160] Small molecules that competitively bind directly and strongly to hot spots of protein-protein interfaces constitute a class of drugs with potential for high selectivity. These molecules are generally flat, owing to the flat nature of their target interface. Several highly selective human therapeutics have been designed. For example, the flat molecule ligands **1** and **2** (Figure 31) bind at the hot spot of B7-1, an important T-cell activation modulator and efficiently inhibit its binding with the protein CD28.[161,162] Natural small molecule substrates of complex enzymes have traditionally served as lead templates for designing such antagonists. These molecules map the epitope of the binding site. The design of the small-molecule inhibitors, **3** and **4**,[163–165] of interleukin-2 comes under this category. Alternatively, peptide based epitope mimics are also known, such as the constrained peptidomimic that reproduces the bioactive helical face of the 16 residue helical domain of the BAK regulator protein that binds to the interaction domain of BCL-xL, an antiapoptotic protein (Figure 32).[166]

Epitope: is the surface patch on an antigen molecule, which is recognized for binding by molecules of the immune system, like antibodies and T cells.

Molecules that bind allosteric sites on protein-protein complexes or their protomers form another class of potential drugs. These drugs are effective in inhibiting large conformational changes that are at the origin of protein-protein complex formation. LFA1 is an extracellular protein (integrin family) whose activity is regulated by metal ions outside the cell and signaling pathways inside the cell. By binding other cell-surface molecules called cell-adhesion molecules, LFA1 mediates cell-cell adhesion, extravasation and T-cell activation. Small molecule **5**[167,168] binds in the hydrophobic pocket in the I-domain of LFA1 (Figure 33) and allosterically disorients its interface topology, hence inhibiting its activity.

Figure 31: Chemdraw structures of small molecule inhibitors 1 and 2, of interleukin-2 and 3, 4, 5, of the T-cell activation modulator B7-1 with flat interfaces. The inhibitory activities (IC_{50}) of the molecules are shown.

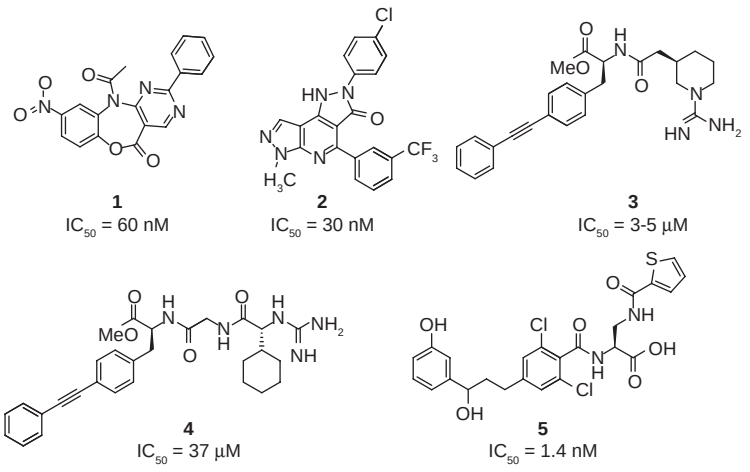


Figure 32: Structure of the antiapoptotic protein BCL-XL (surface representation) bound to a sixteen-residue peptide inhibitor derived from the BAK regulator protein (ribbon representation, green). The hotspot contacts at the interface are labeled.

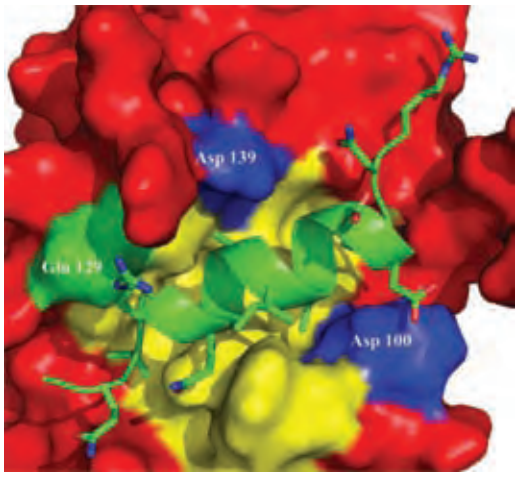
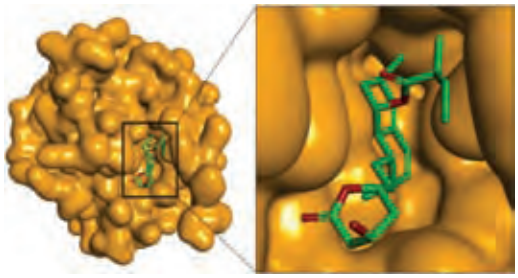


Figure 33: Structure of lovastatin (stick model) bound to the I-domain of LFA-1 (PDB: 1CQP) (surface representation). The inset shows a magnified image of lovastatin protruding into the interface pocket of LFA01.



11. Conclusions

The number of protein-protein complexes in biological systems is much larger than what has been characterized. Patterns have, however, emerged from structural, biophysical and statistical analyses of the known complexes. These complexes have evolved to perform multifarious activities that are not possible by monomeric proteins. Proteins interact along their surfaces to form protein-protein complexes. The interacting domains vary in their contour, complementarity and affinity. A few key residues on small patches on the surface of interacting proteins seem to greatly influence the thermodynamics and the interaction characteristics of protein-protein complexes. These residues have been prime targets for drug design and therapeutics. Advances in genomics and proteomics will further refine our understanding of protein-protein complexes and their biological functions.

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References

1. Huang, Z.: 'The Chemical Biology of Apoptosis: Exploring Protein-Protein Interactions and the Life and Death of Cells with Small Molecules'. *Chem. Biol.* 9, 10, pp. 1059–1072, (2002).
2. Nooren, I.M.A.; Thornton, J.M.: 'Diversity of protein-protein interactions'. *EMBO J.* 22, 14, pp. 3486–3492, (2003).
3. Ryan, D.P.; Matthews, J.M.: 'Protein-protein interactions in human disease'. *Curr. Opin. Struct. Biol.* 15, 4, pp. 441–446, (2005).
4. Archakov, A.I.; Govorun, V.M.; Dubanov, A.V.; Ivanov, Y.D.; Veselovsky, A.V.; Lewi, P.; Janssen, P.: 'Protein-protein interactions as a target for drugs in proteomics'. *Proteomics.* 3, 4, pp. 380–391, (2003).
5. Nooren, I.M.A.; Thornton, J.M.: 'Structural Characterisation and Functional Significance of Transient Protein-Protein Interactions'. *J. Mol. Biol.* 325, 5, pp. 991–1018, (2003).
6. Schmitt, S.; Kuhn, D.; Klebe, G.: 'A New Method to Detect Related Function Among Proteins Independent of Sequence and Fold Homology'. *J. Mol. Biol.* 323, 2, pp. 387–406, (2002).
7. Ponstingl, H.; Thomas, K.; Gorse, D.; Thornton, J.M.: 'Morphological aspects of oligomeric protein structures'. *Prog. Biophys. Mol. Biol.* 89, 1, pp. 9–35, (2005).
8. Marcotte, E.M.; Pellegrini, M.; Ng, H.-L.; Rice, D.W.; Yeates, T.O.; Eisenberg, D.: 'Detecting Protein Function and Protein-Protein Interactions from Genome Sequences'. *Science*. 285, 5428, pp. 751–753, (1999).
9. Janin, J.: 'Elusive affinities'. *Proteins: Struct., Funct., Bioinf.* 21, 1, pp. 30–39, (1995).
10. Janin, J.: 'Protein-protein recognition'. *Prog. Biophys. Mol. Biol.* 64, 2–3, pp. 145–166, (1995).
11. Jones, S.; Thornton, J.M.: 'Principles of protein-protein interactions'. *Proc. Natl. Acad. Sci. U. S. A.* 93, 1, pp. 13–20, (1996).
12. Bogan, A.A.; Thorn, K.S.: 'Anatomy of hot spots in protein interfaces'. *J. Mol. Biol.* 280, 1, pp. 1–9, (1998).
13. Ma, B.; Kumar, S.; Tsai, C.-J.; Nussinov, R.: 'Folding funnels and binding mechanisms'. *Protein Eng.* 12, 9, pp. 713–720, (1999).
14. Tsai, C.-J.; Lin, S.L.; Wolfson, H.J.; Nussinov, R.: 'A Dataset of Protein-Protein Interfaces Generated with a Sequence-order-independent Comparison Technique'. *J. Mol. Biol.* 260, 4, pp. 604–620, (1996).
15. Tsai, C.-J.; Nussinov, R.: 'Hydrophobic folding units at protein-protein interfaces: Implications to protein folding and to protein-protein association'. *Protein Sci.* 6, 7, pp. 1426–1437, (1997).
16. Tsai, C.-J.; Kumar, S.; Ma, B.; Nussinov, R.: 'Folding funnels, binding funnels, and protein function'. *Protein Sci.* 8, 6, pp. 1181–1190, (1999).
17. Groll, M.; Gallastegui, N.; Maréchal, X.; Le Ravalec, V.; Basse, N.; Richy, N.; Genin, E.; Huber, R.; Moroder, L.; Vidal, J.; Reboud-Ravaux, M.: '20S Proteasome Inhibition: Designing Noncovalent Linear Peptide Mimics of the Natural Product TMC-95A'. *ChemMedChem* 5, 10, pp. 1701–1705, (2010).
18. Lehninger, A.; Nelson, D.; Cox, M.: *Lehninger Principles of Biochemistry*, W.H. Freeman, (2008).
19. Brooijmans, N.; Sharp, K.A.; Kuntz, I.D.: 'Stability of macromolecular complexes'. *Proteins: Struct., Funct., Bioinf.* 48, 4, pp. 645–653, (2002).
20. Janin, J.: 'Principles of protein-protein recognition from structure to thermodynamics'. *Biochimie* 77, 7–8, pp. 497–505, (1995).
21. Conte, L.L.; Chothia, C.; Janin, J.: 'The atomic structure of protein-protein recognition sites'. *J. Mol. Biol.* 285, 5, pp. 2177–2198, (1999).
22. Russell, R.B.; Alber, F.; Aloy, P.; Davis, F.P.; Korkin, D.; Pichaud, M.; Topf, M.; Sali, A.: 'A structural perspective on protein-protein interactions'. *Curr. Opin. Struct. Biol.* 14, 3, pp. 313–324, (2004).
23. Laskowski, R.A.; Gerick, F.; Thornton, J.M.: 'The structural basis of allosteric regulation in proteins'. *FEBS Lett.* 583, 11, pp. 1692–1698, (2009).
24. Soisson, S.M.; MacDougall-Shackleton, B.; Schleif, R.; Wolberger, C.: 'Structural Basis for Ligand-Regulated Oligomerization of AraC'. *Science*. 276, 5311, pp. 421–425, (1997).
25. Miller, S.; Lesk, A.M.; Janin, J.; Chothia, C.: 'The accessible surface area and stability of oligomeric proteins'. *Nature*. 328, 6133, pp. 834–836, (1987).
26. Ali, M.H.; Imperiali, B.: 'Protein oligomerization: How and why'. *Biorg. Med. Chem.* 13, 17, pp. 5013–5020, (2005).
27. Lee, S.-G.; Chmielewski, J.: 'Cross-linked Peptoid-Based Dimerization Inhibitors of HIV-1 Protease'. *ChemBioChem* 11, 11, pp. 1513–1516, (2010).
28. Fasan, R.; Dias, R.L.A.; Moehle, K.; Zerbe, O.; Obrecht, D.; Mittl, P.R.E.; Grütter, M.G.; Robinson, J.A.: 'Structure–Activity Studies in a Family of β-Hairpin Protein Epitope Mimetic Inhibitors of the p53–HDM2 Protein–Protein Interaction'. *ChemBioChem* 7, 3, pp. 515–526, (2006).
29. Vicari, D.; Foy, K.C.; Liotta, E.M.; Kaumaya, P.T.P.: 'Engineered Conformation-dependent VEGF Peptide Mimics Are Effective in Inhibiting VEGF Signaling Pathways'. *J. Biol. Chem.* 286, 15, pp. 13612–13625, (2011).
30. Athanassiou, Z.; Patora, K.; Dias, R.L.A.; Moehle, K.; Robinson, J.A.; Varani, G.: 'Structure-Guided Peptidomimetic Design Leads to Nanomolar β-Hairpin Inhibitors of the Tat–TAR Interaction of Bovine Immunodeficiency Virus†,‡'. *Biochemistry* 46, 3, pp. 741–751, (2007).
31. Tomoda, K.; Takahashi, N.; Hibi, Y.; Asamitsu, K.; Ishida, H.; Kondo, T.; Fujii, Y.; Okamoto, T.: 'Molecular docking analysis of the protein–protein interaction between RelA-associated inhibitor and tumor suppressor protein p53 and its inhibitory effect on p53 action'. *Cancer Sci.* 99, 3, pp. 615–622, (2008).

32. Mukherjee, G.; Patra, N.; Barua, P.; Jayaram, B.: 'A fast empirical GAFF compatible partial atomic charge assignment scheme for modeling interactions of small molecules with biomolecular targets'. *J. Comput. Chem*. 32, 5, pp. 893–907, (2011).
33. Simmen, K.; Newell, A.; Robinson, M.; Mills, J.; Canning, G.; Handa, R.; Parkes, K.; Borkakoti, N.; Jupp, R.: 'Protein interactions in the herpes simplex virus type 1 VP16-induced complex: VP16 peptide inhibition and mutational analysis of host cell factor requirements'. *J. Virol*. 71, 5, pp. 3886–3894, (1997).
34. Bhakoo, A.; Raynes, J.G.; Heal, J.R.; Keller, M.; Miller, A.D.: 'De-novo design of complementary (antisense) peptide mini-receptor inhibitor of interleukin 18 (IL-18)'. *Mol. Immunol*. 41, 12, pp. 1217–1224, (2004).
35. Keskin, O.; Nussinov, R.: 'Similar Binding Sites and Different Partners: Implications to Shared Proteins in Cellular Pathways'. *Structure*. 15, 3, pp. 341–354, (2007).
36. The statistical data for various multimeric states were obtained from the PDB website (http://www.rcsb.org/pdb/search/advsearch.do). The number of complexes in a multimeric form was obtained by using the "Oligomeric State" feature under the advanced search option in the PDB website (http://www.rcsb.org/pdb/home/home.do). The results were refined to obtain only protein-protein interactions (PPIs).
37. Bernstein, F.C.; Koetzle, T.F.; Williams, G.J.B.; Meyer, J.E.F.; Brice, M.D.; Rodgers, J.R.; Kennard, O.; Shimanouchi, T.; Tasumi, M.: 'The protein data bank: A computer-based archival file for macromolecular structures'. *J. Mol. Biol*. 112, 3, pp. 535–542, (1977).
38. Goodsell, D.S.: 'Inside a living cell'. *Trends Biochem. Sci*. 16, pp. 203–206, (1991).
39. Jones, S.; Thornton, J.M.: 'Protein-protein interactions: A review of protein dimer structures'. *Prog. Biophys. Mol. Biol*. 63, 1, pp. 31–59, (1995).
40. Chakrabarti, P.; Janin, J.: 'Dissecting protein–protein recognition sites'. *Proteins: Struct., Funct., Bioinf*. 47, 3, pp. 334–343, (2002).
41. Prasad Bahadur, R.; Chakrabarti, P.; Rodier, F.; Janin, J.: 'A Dissection of Specific and Non-specific Protein-Protein Interfaces'. *J. Mol. Biol*. 336, 4, pp. 943–955, (2004).
42. Bordner, A.J.; Abagyan, R.: 'Statistical analysis and prediction of protein–protein interfaces'. *Proteins: Struct., Funct., Bioinf*. 60, 3, pp. 353–366, (2005).
43. Dayhoff, J.E.; Shoemaker, B.A.; Bryant, S.H.; Panchenko, A.R.: 'Evolution of Protein Binding Modes in Homooligomers'. *J. Mol. Biol*. 395, 4, pp. 860–870, (2010).
44. Xu, Q.; Canutescu, A.A.; Wang, G.; Shapovalov, M.; Obradovic, Z.; Dunbrack Jr, R. L.: 'Statistical Analysis of Interface Similarity in Crystals of Homologous Proteins'. *J. Mol. Biol*. 381, 2, pp. 487–507, (2008).
45. Ansari, S.; Helms, V.: 'Statistical analysis of predominantly transient protein–protein interfaces'. *Proteins: Struct., Funct., Bioinf*. 61, 2, pp. 344–355, (2005).
46. Ofran, Y.; Rost, B.: 'Analysing Six Types of Protein-Protein Interfaces'. *J. Mol. Biol*. 325, 2, pp. 377–387, (2003).
47. Cornish-Bowden, A.J.; Koshland, D.E.: 'The Quaternary Structure of Proteins Composed of Identical Subunits'. *J. Biol. Chem*. 246, 10, pp. 3092–3102, (1971).
48. Blundell, T.L.; Srinivasan, N.: 'Symmetry, stability, and dynamics of multidomain and multicomponent protein systems'. *Proc. Natl. Acad. Sci. U.S.A*. 93, 25, pp. 14243–14248, (1996).
49. Canals, A.; Pous, J.; Guasch, A.; Benito, A.; Ribó, M.; Vilanova, M.; Coll, M.: 'The Structure of an Engineered Domain-Swapped Ribonuclease Dimer and Its Implications for the Evolution of Proteins toward Oligomerization'. *Structure*. 9, 10, pp. 967–976, (2001).
50. Luo, Y.; Yang, C.; Lu, W.; Xie, R.; Jin, C.; Huang, P.; Wang, F.; McKeehan, W.L.: 'Metabolic Regulator βKlotho Interacts with Fibroblast Growth Factor Receptor 4 (FGFR4) to Induce Apoptosis and Inhibit Tumor Cell Proliferation'. *J. Biol. Chem*. 285, 39, pp. 30069–30078, (2010).
51. Janin, J.; Chothia, C.: 'The structure of protein-protein recognition sites'. *J. Biol. Chem*. 265, 27, pp. 16027–16030, (1990).
52. Horton, N.; Lewis, M.: 'Calculation of the free energy of association for protein complexes'. *Protein Sci*. 1, 1, pp. 169–181, (1992).
53. Veselovsky, A.V.; Ivanov, Y.D.; Ivanov, A.S.; Archakov, A.I.; Lewi, P.; Janssen, P.: 'Protein–protein interactions: mechanisms and modification by drugs'. *J. Mol. Recognit*. 15, 6, pp. 405–422, (2002).
54. Carling, D.: 'The AMP-activated protein kinase cascade - a unifying system for energy control'. *Trends Biochem. Sci*. 29, 1, pp. 18–24, (2004).
55. Kahn, B.B.; Alquier, T.; Carling, D.; Hardie, D.G.: 'AMP-activated protein kinase: Ancient energy gauge provides clues to modern understanding of metabolism'. *Cell Metab*. 1, 1, pp. 15–25, (2005).
56. Shackelford, D.B.; Shaw, R.J.: 'The LKB1-AMPK pathway: metabolism and growth control in tumour suppression'. *Nat. Rev. Cancer*. 9, 8, pp. 563–575, (2009).
57. Xiao, B.; Sanders, M.J.; Underwood, E.; Heath, R.; Mayer, F.V.; Carmena, D.; Jing, C.; Walker, P.A.; Eccleston, J.F.; Haire, L.F.; Saiu, P.; Howell, S.A.; Aasland, R.; Martin, S.R.; Carling, D.; Gamblin, S.J.: 'Structure of mammalian AMPK and its regulation by ADP'. *Nature*. 472, 7342, pp. 230–233, (2011).
58. Paaventhan, P.; Kong, C.; Joseph, J.S.; Chung, M.C.M.; Kolatkar, P.R.: 'Structure of rhodocetin reveals noncovalently bound heterodimer interface'. *Protein Sci*. 14, 1, pp. 169–175, (2005).
59. Tsunogae, Y.; Tanaka, I.; Yamane, T.; Kikkawa, J.-i.; Ashida, T.; Ishikawa C.; Watanabe, K.; Nakamura, S.; Takahashi, K.: 'Structure of the Trypsin-Binding Domain of Bowman-Birk Type Protease Inhibitor and Its Interaction with Trypsin'. *J. Biochem*. 100, 6, pp. 1637–1646, (1986).
60. Tainer, J.A.; Getzoff, E.D.; Beem, K.M.; Richardson, J.S.; Richardson, D.C.: 'Determination and analysis of the 2 Å structure of copper, zinc superoxide dismutase'. *J. Mol. Biol*. 160, 2, pp. 181–217, (1982).
61. Iversen, L.: 'High times for cannabis research'. *Proc. Natl. Acad. Sci. U. S. A*. 96, 10, pp. 5338–5339, (1999).
62. Hofmann, K.P.; Scheerer, P.; Hildebrand, P.W.; Choe, H.-W.; Park, J.H.; Heck, M.; Ernst, O.P.: 'A G protein-coupled receptor at work: the rhodopsin model'. *Trends Biochem. Sci*. 34, 11, pp. 540–552, (2009).
63. Swaminath, G.; Xiang, Y.; Lee, T.W.; Steenhuis, J.; Parnot, C.; Kobilka, B.K.: 'Sequential Binding of Agonists to the β2 Adrenoceptor'. *J. Biol. Chem*. 279, 1, pp. 686–691, (2004).
64. Nygaard, R.; Frimurer, T.M.; Holst, B.; Rosenkilde, M.M.; Schwartz, T.W.: 'Ligand binding and micro-switches in 7TM receptor structures'. *Trends Pharmacol. Sci*. 30, 5, pp. 249–259, (2009).
65. Rosenbaum, D.M.; Rasmussen, S.G.F.; Kobilka, B.K.: 'The structure and function of G-protein-coupled receptors'. *Nature*. 459, 7245, pp. 356–363, (2009).
66. Nogales, E.; Wolf, S.G.; Downing, K.H.: 'Structure of the [α][β] tubulin dimer by electron crystallography'. *Nature*. 391, 6663, pp. 199–203, (1998).
67. Song, Y.H.; Marx, A.; Muller, J.; Woehlke, G.; Schliwa, M.; Krebs, A.; Hoenger, A.; Mandelkow, E.: 'Structure

of a fast kinesin: implications for ATPase mechanism and interactions with microtubules'. *EMBO J*. 20, 22, pp. 6213–6225, (2001).
68. Carter, A.P.; Cho, C.; Jin, L.; Vale, R.D.: 'Crystal Structure of the Dynein Motor Domain'. *Science*. 331, 6021, pp. 1159–1165, (2011).
69. Wade, R.: 'On and Around Microtubules: An Overview'. *Mol. Biotechnol*. 43, 2, pp. 177–191, (2009).
70. Kaltschmidt, J.A.; Brand, A.H.: 'Asymmetric cell division: microtubule dynamics and spindle asymmetry'. *J. Cell Sci*. 115, 11, pp. 2257–2264, (2002).
71. Jones, S.; Thornton, J.M.: 'Analysis of protein-protein interaction sites using surface patches'. *J. Mol. Biol*. 272, 1, pp. 121–132, (1997).
72. Jones, S.; Thornton, J.M.: 'Prediction of protein-protein interaction sites using patch analysis'. *J. Mol. Biol*. 272, 1, pp. 133–143, (1997).
73. Ma, B.; Elkayam, T.; Wolfson, H.; Nussinov, R.: 'Protein–protein interactions: Structurally conserved residues distinguish between binding sites and exposed protein surfaces'. *Proc. Natl. Acad. Sci. U. S. A*. 100, 10, pp. 5772–5777, (2003).
74. Fischmann, T.O.; Bentley, G.A.; Bhat, T.N.; Boulot, G.; Mariuzza, R.A.; Phillips, S.E.; Tello, D.; Poljak, R.J.: 'Crystallographic refinement of the three-dimensional structure of the FabD1.3-lysozyme complex at 2.5-A resolution'. *J. Biol. Chem*. 266, 20, pp. 12915–12920, (1991).
75. Rousseau, F.; Schymkowitz, J.W.H.; Itzhaki, L.S.: 'The Unfolding Story of Three-Dimensional Domain Swapping'. *Structure*. 11, 3, pp. 243–251, (2003).
76. Liu, Y.; Hart, P.J.; Schlunegger, M.P.; Eisenberg, D.: 'The crystal structure of a 3D domain-swapped dimer of RNase A at a 2.1-Å resolution'. *Proc. Natl. Acad. Sci. U. S. A*. 95, 7, pp. 3437–3442, (1998).
77. Tegoni, M.; Ramoni, R.; Bignetti, E.; Spinelli, S.; Cambillau, C.: 'Domain swapping creates a third putative combining site in bovine odorant binding protein dimer'. *Nat. Struct. Mol. Biol*. 3, 10, pp. 863–867, (1996).
78. Lee, J.E.; Raines, R.T.: 'Cytotoxicity of Bovine Seminal Ribonuclease: Monomer versus Dimer†'. *Biochemistry* 44, 48, pp. 15760–15767, (2005).
79. Mazzarella, L.; Capasso, S.; Demasi, D.; Di Lorenzo, G.; Mattia, C.A.; Zagari, A.: 'Bovine seminal ribonuclease: structure at 1.9 Å resolution'. *Acta Crystallogr. Sect. D. Biol. Crystallogr*. 49, 4, pp. 389–402, (1993).
80. Sheinerman, F.B.; Norel, R.; Honig, B.: 'Electrostatic aspects of protein-protein interactions'. *Curr. Opin. Struct. Biol*. 10, 2, pp. 153–159, (2000).
81. Kabsch, W.; Mannherz, H.G.; Suck, D.; Pai, E.F.; Holmes, K.C.: 'Atomic structure of the actin: DNase I complex'. *Nature*. 347, 6288, pp. 37–44, (1990).
82. Pollard, T.D.; Blanchoin, L.; Mullins, R.D.: 'Molecular Mechanisms Controlling Actin Filament Dynamics In Nonmuscle Cells'. *Annu. Rev. Biophys. Biomol. Struct*. 29, 1, pp. 545–576, (2000).
83. Tobi, D.; Bahar, I.: 'Structural changes involved in protein binding correlate with intrinsic motions of proteins in the unbound state'. *Proc. Natl. Acad. Sci. U. S. A*. 102, 52, pp. 18908–18913, (2005).
84. Borges, L.; Cosman, D.: 'LIRs/ILTs/MIRs, inhibitory and stimulatory Ig-superfamily receptors expressed in myeloid and lymphoid cells'. *Cytokine Growth Factor Rev*. 11, 3, pp. 209–217, (2000).
85. Willcox, B.E.; Thomas, L.M.; Bjorkman, P.J.: 'Crystal structure of HLA-A2 bound to LIR-1, a host and viral major histocompatibility complex receptor'. *Nat. Immunol*. 4, 9, pp. 913–919, (2003).
86. Mills, F.C.; Johnson, M.L.; Ackers, G.K.: 'Oxygenation-linked subunit interactions in human hemoglobin: experimental studies on the concentration dependence of oxygenation curves'. *Biochemistry* 15, 24, pp. 5350–5362, (1976).
87. Kavanaugh, J.S.; Rogers, P.H.; Arnone, A.: 'Crystallographic Evidence for a New Ensemble of Ligand-Induced Allosteric Transitions in Hemoglobin: The T-to-THigh Quaternary Transitions'. *Biochemistry* 44, 16, pp. 6101–6121, (2005).
88. Chuderland, D.; Seger, R.: 'Calcium regulates ERK signaling by modulating its protein-protein interactions'. *Communicative & Integrative Biology*. 1, 1, pp. 4–5, (2008).
89. Leone, F.A.; Rezende, L.A.; Ciancaglini, P.; Pizauro, J.M.: 'Allosteric modulation of pyrophosphatase activity of rat osseous plate alkaline phosphatase by magnesium ions'. *Int. J. Biochem. Cell Biol*. 30, 1, pp. 89–97, (1998).
90. Selent, J.; Sanz, F.; Pastor, M.; De Fabritiis, G.: 'Induced Effects of Sodium Ions on Dopaminergic G-Protein Coupled Receptors'. *PLoS Comput. Biol*. 6, 8, pp. e1000884, (2010).
91. D'Alessio, G.: 'The evolutionary transition from monomeric to oligomeric proteins: tools, the environment, hypotheses'. *Prog. Biophys. Mol. Biol*. 72, 3, pp. 271–298, (1999).
92. Richmond, T.J.; Richards, F.M.: 'Packing of [α.]-helices: Geometrical constraints and contact areas'. *J. Mol. Biol*. 119, 4, pp. 537–555, (1978).
93. Smith, G.R.; Sternberg, M.J.E.; Bates, P.A.: 'The Relationship between the Flexibility of Proteins and their Conformational States on Forming Protein-Protein Complexes with an Application to Protein-Protein Docking'. *J. Mol. Biol*. 347, 5, pp. 1077–1101, (2005).
94. Gray, J.J.; Moughon, S.; Wang, C.; Schueler-Furman, O.; Kuhlman, B.; Rohl, C.A.; Baker, D.: 'Protein-Protein Docking with Simultaneous Optimization of Rigid-body Displacement and Side-chain Conformations'. *J. Mol. Biol*. 331, 1, pp. 281–299, (2003).
95. Hirose, S.; Yokota, K.; Kuroda, Y.; Wako, H.; Endo, S.; Kanai, S.; Noguchi, T.: 'Prediction of protein motions from amino acid sequence and its application to protein-protein interaction'. *BMC Structural Biology*. 10, 1, pp. 20, (2010).
96. Chang, D.; Syu, Y.-T.; Lin, P.-C.: 'Predicting the protein-protein interactions using primary structures with predicted protein surface'. *BMC Bioinformatics* 11, Suppl 1, pp. S3, (2010).
97. Korn, A.P.; Burnett, R.M.: 'Distribution and complementarity of hydropathy in mutisunit proteins'. *Proteins: Struct., Funct., Bioinf*. 9, 1, pp. 37–55, (1991).
98. Johnson, J.E.: 'Functional implications of protein-protein interactions in icosahedral viruses'. *Proc. Natl. Acad. Sci. U. S. A*. 93, 1, pp. 27–33, (1996).
99. Chothia, C.; Janin, J.: 'Principles of protein-protein recognition'. *Nature*. 256, 5520, pp. 705–708, (1975).
100. Morgan, R.S.; Miller, S.L.; McAdon, J.M.: 'The symmetry of self-complementary surfaces'. *J. Mol. Biol*. 127, 1, pp. 31–38, (1979).
101. Fujinaga, M.; Sielecki, A.R.; Read, R.J.; Ardelt, W.; Laskowski, M.; James, M.N.G.: 'Crystal and molecular structures of the complex of [α]-chymotrypsin with its inhibitor Turkey ovomucoid third domain at 1.8 Å resolution'. *J. Mol. Biol*. 195, 2, pp. 397–418, (1987).
102. Lawrence, M.C.; Colman, P.M.: 'Shape Complementarity at Protein/Protein Interfaces'. *J. Mol. Biol*. 234, 4, pp. 946–950, (1993).
103. Lee, B.; Richards, F.M.: 'The interpretation of protein structures: Estimation of static accessibility'. *J. Mol. Biol*. 55, 3, pp. 379–400, IN373-IN374, (1971).

104. Tan, K.P.; Varadarajan, R.; Madhusudhan, M.S.: 'DEPTH: a web server to compute depth and predict small-molecule binding cavities in proteins'. *Nucleic Acids Res*. 39, suppl 2, pp. W242-W248, (2011).
105. Tina, K.G.; Bhadra, R.; Srinivasan, N.: 'PIC: Protein Interactions Calculator'. *Nucleic Acids Res*. 35, suppl 2, pp. W473-W476, (2007).
106. Gadkari, R.A.; Varughese, D.; Srinivasan, N.: 'Recognition of Interaction Interface Residues in Low-Resolution Structures of Protein Assemblies Solely from the Positions of Cα Atoms'. *PLoS ONE* 4, 2, pp. e4476, (2009).
107. Sonavane, S.; Chakrabarti, P.: 'Cavities and Atomic Packing in Protein Structures and Interfaces'. *PLoS Comput. Biol*. 4, 9, pp. e1000188, (2008).
108. Chen, R.; Weng, Z.: 'Docking unbound proteins using shape complementarity, desolvation, and electrostatics'. *Proteins: Struct., Funct., Bioinf*. 47, 3, pp. 281–294, (2002).
109. Chakravarty, S.; Varadarajan, R.: 'Residue depth: a novel parameter for the analysis of protein structure and stability'. *Structure (London, England : 1993)* 7, 7, pp. 723–732, (1999).
110. Bhinge, A.; Chakrabarti, P.; Uthanumallian, K.; Bajaj, K.; Chakraborty, K.; Varadarajan, R.: 'Accurate Detection of Protein:Ligand Binding Sites Using Molecular Dynamics Simulations'. *Structure*. 12, 11, pp. 1989–1999, (2004).
111. Guharoy, M.; Chakrabarti, P.: 'Conserved residue clusters at protein-protein interfaces and their use in binding site identification'. *BMC Bioinformatics* 11, 1, pp. 286, (2010).
112. Bogan, A.; Thorn, K.: 'Anatomy of hot spots in protein interfaces'. *J. Mol. Biol*. 280, 1, pp. 1–9, (1998).
113. Ofran, Y.; Rost, B.: 'Protein–Protein Interaction Hotspots Carved into Sequences'. *PLoS Comput. Biol*. 3, 7, pp. e119, (2007).
114. Moreira, I.S.; Fernandes, P. A.; Ramos, M. J.: 'Hot spots—A review of the protein–protein interface determinant amino-acid residues'. *Proteins: Struct., Funct., Bioinf*. 68, 4, pp. 803–812, (2007).
115. Chen, R.; Mintseris, J.; Janin, J.; Weng, Z.: 'A protein–protein docking benchmark'. *Proteins: Struct., Funct., Bioinf*. 52, 1, pp. 88–91, (2003).
116. Fernández, A.; Scheraga, H.A.: 'Insufficiently dehydrated hydrogen bonds as determinants of protein interactions'. *Proc. Natl. Acad. Sci. U. S. A*. 100, 1, pp. 113–118, (2003).
117. Xu, D.; Tsai, C.J.; Nussinov, R.: 'Hydrogen bonds and salt bridges across protein-protein interfaces'. *Protein Eng*. 10, 9, pp. 999–1012, (1997).
118. Symmons, M.F.; Jones, G.H.; Luisi, B.F.: 'A Duplicated Fold Is the Structural Basis for Polynucleotide Phosphorylase Catalytic Activity, Processivity, and Regulation'. *Structure*. 8, 11, pp. 1215–1226, (2000).
119. Selenko, P.; Sprangers, R.; Stier, G.; Buhler, D.; Fischer, U.; Sattler, M.: 'SMN Tudor domain structure and its interaction with the Sm proteins'. *Nat. Struct. Mol. Biol*. 8, 1, pp. 27–31, (2001).
120. Ito, T.; Chiba, T.; Ozawa, R.; Yoshida, M.; Hattori, M.; Sakaki, Y.: 'A comprehensive two-hybrid analysis to explore the yeast protein interactome'. *Proc. Natl. Acad. Sci. U. S. A*. 98, 8, pp. 4569–4574, (2001).
121. Baumeister, W.; Grimm, R.; Walz, J.: 'Electron tomography of molecules and cells'. *Trends. cell biol*. 9, 2, pp. 81–85, (1999).
122. Ranish, J.A.; Yi, E.C.; Leslie, D.M.; Purvine, S.O.; Goodlett, D.R.; Eng, J.; Aebersold, R.: 'The study of macromolecular complexes by quantitative proteomics'. *Nat. Genet*. 33, 3, pp. 349–355, (2003).
123. Droit, A.; Poirier, G.G.; Hunter, J.M.: 'Experimental and bioinformatic approaches for interrogating protein–protein interactions to determine protein function'. *J. Mol. Endocrinol*. 34, 2, pp. 263–280, (2005).
124. Loaiza, O.A.; Lamas-Ardisana, P.J.; Jubete, E.; Ochoteco, E.; Loinaz, I.; Cabañero, G. n.; García, I.; Penadés, S.: 'Nanostructured Disposable Impedimetric Sensors as Tools for Specific Biomolecular Interactions: Sensitive Recognition of Concanavalin A'. *Anal. Chem*. 83, 8, pp. 2987–2995, (2011).
125. Humphris, E.L.; Kortemme, T.: 'Prediction of Protein-Protein Interface Sequence Diversity Using Flexible Backbone Computational Protein Design'. *Structure*. 16, 12, pp. 1777–1788, (2008).
126. Burgoyne, N.J.; Jackson, R.M.: 'Predicting protein interaction sites: binding hot-spots in protein–protein and protein–ligand interfaces'. *Bioinformatics* 22, 11, pp. 1335–1342, (2006).
127. Bradshaw, R.T.; Patel, B.H.; Tate, E.W.; Leatherbarrow, R.J.; Gould, I.R.: 'Comparing experimental and computational alanine scanning techniques for probing a prototypical protein–protein interaction'. *Protein Eng., Des. Sel*. 24, 1–2, pp. 197–207, (2011).
128. Verkhivker, G.M.; Bouzida, D.; Gehlhaar, D.K.; Rejto, P.A.; Freer, S. T.; Rose, P. W.: 'Computational detection of the binding-site hot spot at the remodeled human growth hormone–receptor interface'. *Proteins: Struct., Funct., Bioinf*. 53, 2, pp. 201–219, (2003).
129. Dell'Orco, D.: 'Fast predictions of thermodynamics and kinetics of protein-protein recognition from structures: from molecular design to systems biology'. *Molecular BioSystems* 5, 4, pp. 323–334, (2009).
130. Aytuna, A.S.; Gursoy, A.; Keskin, O.: 'Prediction of protein–protein interactions by combining structure and sequence conservation in protein interfaces'. *Bioinformatics* 21, 12, pp. 2850–2855.
131. Bradford, J.R.; Needham, C.J.; Bulpitt, A.J.; Westhead, D.R.: 'Insights into Protein-Protein Interfaces using a Bayesian Network Prediction Method'. *J. Mol. Biol*. 362, 2, pp. 365–386, (2006).
132. Hoskins, J.; Lovell, S.; Blundell, T.L.: 'An algorithm for predicting protein–protein interaction sites: Abnormally exposed amino acid residues and secondary structure elements'. *Protein Sci*. 15, 5, pp. 1017–1029, (2006).
133. Block, P.; Paern, J.; Hüllermeier, E.; Sanschagrin, P.; Sotriffer, C.A.; Klebe, G.: 'Physicochemical descriptors to discriminate protein–protein interactions in permanent and transient complexes selected by means of machine learning algorithms'. *Proteins: Struct., Funct., Bioinf*. 65, 3, pp. 607–622, (2006).
134. Keskin, O.; Gursoy, A.; Ma, B.; Nussinov, R.: 'Principles of Protein–Protein Interactions: What are the Preferred Ways For Proteins To Interact?'. *Chem. Rev*. 108, 4, pp. 1225–1244, (2008).
135. Acuner Ozbabacan, S.E.; Engin, H.B.; Gursoy, A.; Keskin, O.: 'Transient protein–protein interactions'. *Protein Eng., Des. Sel*. pp. (2011).
136. De Crescenzo, G.; Hinck, C.S.; Shu, Z.; Zúñiga, J.; Yang, J.; Tang, Y.; Baardsnes, J.; Mendoza, V.; Sun, L.; López-Casillas, F.; O'Connor-McCourt, M.; Hinck, A.P.: 'Three Key Residues Underlie the Differential Affinity of the TGFβ Isoforms for the TGFβ Type II Receptor'. *J. Mol. Biol*. 355, 1, pp. 47–62, (2006).
137. Mylvaganam, S.E.; Paterson, Y.; Getzoff, E.D.: 'Structural basis for the binding of an anti-cytochrome c antibody to its antigen: crystal structures of FabE8-cytochrome c complex to 1.8 Å resolution and FabE8 to 2.26 Å resolution'. *J. Mol. Biol*. 281, 2, pp. 301–322, (1998).
138. Baldock, C.; Rafferty, J.B.; Sedelnikova, S.E.; Baker, P.J.; Stuitje, A.R.; Slabas, A.R.; Hawkes, T.R.; Rice, D.W.: 'A Mechanism of Drug Action Revealed by Structural

Studies of Enoyl Reductase'. *Science*. 274, 5295, pp. 2107–2110, (1996).
139. Paesen, G.C.; Siebold, C.; Harlos, K.; Peacey, M.F.; Nuttall, P.A.; Stuart, D.I.: 'A Tick Protein with a Modified Kunitz Fold Inhibits Human Tryptase'. *J. Mol. Biol*. 368, 4, pp. 1172–1186, (2007).
140. Camacho, C.J.; Weng, Z.; Vajda, S.; DeLisi, C.: 'Free Energy Landscapes of Encounter Complexes in Protein-Protein Association'. *Biophys. J*. 76, 3, pp. 1166–1178, (1999).
141. Buckle, A.M.; Schreiber, G.; Fersht, A.R.: 'Protein-protein recognition: Crystal structural analysis of a barnase-barstar complex at 2.0-.ANG. resolution'. *Biochemistry* 33, 30, pp. 8878–8889, (1994).
142. Vijayakumar, M.; Wong, K.-Y.; Schreiber, G.; Fersht, A.R.; Szabo, A.; Zhou, H.-X.: 'Electrostatic enhancement of diffusion-controlled protein-protein association: comparison of theory and experiment on barnase and barstar'. *J. Mol. Biol*. 278, 5, pp. 1015–1024, (1998).
143. DeLano, W.L.: 'Unraveling hot spots in binding interfaces: progress and challenges'. *Curr. Opin. Struct. Biol*. 12, 1, pp. 14–20, (2002).
144. Clackson, T.; Wells, J.: 'A hot spot of binding energy in a hormone-receptor interface'. *Science*. 267, 5196, pp. 383–386, (1995).
145. Thorn, K.S.; Bogan, A.A.: 'ASEdb: a database of alanine mutations and their effects on the free energy of binding in protein interactions'. *Bioinformatics* 17, 3, pp. 284–285, (2001).
146. Åqvist, J.; Luzhkov, V.B.; Brandsdal, B.O.: 'Ligand Binding Affinities from MD Simulations'. *Acc. Chem. Res*. 35, 6, pp. 358–365, (2002).
147. Hu, Z.; Ma, B.; Wolfson, H.; Nussinov, R.: 'Conservation of polar residues as hot spots at protein interfaces'. *Proteins: Struct., Funct., Bioinf*. 39, 4, pp. 331–342, (2000).
148. Lichtarge, O.; Bourne, H.R.; Cohen, F.E.: 'An Evolutionary Trace Method Defines Binding Surfaces Common to Protein Families'. *J. Mol. Biol*. 257, 2, pp. 342–358, (1996).
149. Keskin, O.; Ma, B.; Nussinov, R.: 'Hot Regions in Protein-Protein Interactions: The Organization and Contribution of Structurally Conserved Hot Spot Residues'. *J. Mol. Biol*. 345, 5, pp. 1281–1294, (2005).
150. Miller, M.D.; Krause, K.L.: 'Identification of the Serratia endonuclease dimer: Structural basis and implications for catalysis'. *Protein Sci*. 5, 1, pp. 24–33, (1996).
151. Hurley, J.H.; Thorsness, P.E.; Ramalingam, V.; Helmers, N.H.; Koshland, D. E.; Stroud, R. M.: 'Structure of a bacterial enzyme regulated by phosphorylation, isocitrate dehydrogenase'. *Proc. Natl. Acad. Sci. U. S. A*. 86, 22, pp. 8635–8639, (1989).
152. Clore, G.M.; Appella, E.; Yamada, M.; Matsushima, K.; Gronenborn, A.M.: 'Three-dimensional structure of interleukin 8 in solution'. *Biochemistry* 29, 7, pp. 1689–1696, (1990).
153. Finzel, B.C.; Weber, P.C.; Hardman, K.D.; Salemme, F.R.: 'Structure of ferricytochrome c' from Rhodospirillum molischianum at 1.67 Å resolution'. *J. Mol. Biol*. 186, 3, pp. 627–643, (1985).
154. Bode, W.; Papamokos, E.; Musil, D.: 'The high-resolution X-ray crystal structure of the complex formed between subtilisin Carlsberg and eglin c, an elastase inhibitor from the leech Hirudo medicinalis'. *Eur. J. Biochem*. 166, 3, pp. 673–692, (1987).
155. Ye, S.; Cech, A.L.; Belmares, R.; Bergstrom, R.C.; Tong, Y.; Corey, D.R.; Kanost, M.R.; Goldsmith, E.J.: 'The structure of a Michaelis serpin-protease complex'. *Nat. Struct. Mol. Biol*. 8, 11, pp. 979–983, (2001).
156. Monod, J.; Wyman, J.; Changeux, J.-P.: 'On the nature of allosteric transitions: A plausible model'. *J. Mol. Biol*. 12, 1, pp. 88–118, (1965).
157. Schildbach, J.F.; Milla, M.E.; Jeffrey, P.D.; Raumann, B.E.; Sauer, R.T.: 'Crystal structure, folding, and operator binding of the hyperstable Arc repressor mutant PL8'. *Biochemistry* 34, 4, pp. 1405–1412, (1995).
158. Liu, Y.; Gotte, G.; Libonati, M.; Eisenberg, D.: 'A domain-swapped RNase A dimer with implications for amyloid formation'. *Nat. Struct. Mol. Biol*. 8, 3, pp. 211–214, (2001).
159. Rittinger, K.; Walker, P.A.; Eccleston, J.F.; Smerdon, S.J.; Gamblin, S.J.: 'Structure at 1.65[thinsp]A of RhoA and its GTPase-activating protein in complex with a transition-state analogue'. *Nature*. 389, 6652, pp. 758–762, (1997).
160. Arkin, M.R.; Wells, J.A.: 'Small-molecule inhibitors of protein-protein interactions: progressing towards the dream'. *Nat Rev Drug Discov*. 3, 4, pp. 301–317, (2004).
161. Erbe, D.V.; Wang, S.; Xing, Y.; Tobin, J.F.: 'Small Molecule Ligands Define a Binding Site on the Immune Regulatory Protein B7.1'. *J. Biol. Chem*. 277, 9, pp. 7363–7368, (2002).
162. Green, N.J.; Xiang, J.; Chen, J.; Chen, L.; Davies, A.M.; Erbe, D.; Tam, S.; Tobin, J.F.: 'Structure-activity studies of a series of dipyrazolo[3,4-b:3',4'-d]pyridin-3-ones binding to the immune regulatory protein B7.1'. *Biorg. Med. Chem*. 11, 13, pp. 2991–3013, (2003).
163. Emerson, S.D.; Palermo, R.; Liu, C.-M.; Tilley, J.W.; Chen, L.; Danho, W.; Madison, V.S.; Greeley, D.N.; Ju, G.; Fry, D.C.: 'NMR characterization of interleukin-2 in complexes with the IL-2Rα receptor component, and with low molecular weight compounds that inhibit the IL-2/IL-Rα interaction'. *Protein Sci*. 12, 4, pp. 811–822, (2003).
164. Tilley, J. W.; Chen, L.; Fry, D.C.; Emerson, S.D.; Powers, G.D.; Biondi, D.; Varnell, T.; Trilles, R.; Guthrie, R.; Mennona, F.; Kaplan, G.; LeMahieu, R.A.; Carson, M.; Han, R.-J.; Liu, C.M.; Palermo, R.; Ju, G.: 'Identification of a Small Molecule Inhibitor of the IL-2/IL-2Rα Receptor Interaction Which Binds to IL-2'. *J. Am. Chem. Soc*. 119, 32, pp. 7589–7590, (1997).
165. Hyde, J.; Braisted, A.C.; Randal, M.; Arkin, M.R.: 'Discovery and Characterization of Cooperative Ligand Binding in the Adaptive Region of Interleukin-2'. *Biochemistry* 42, 21, pp. 6475–6483, (2003).
166. Sattler, M.; Liang, H.; Nettesheim, D.; Meadows, R.P.; Harlan, J.E.; Eberstadt, M.; Yoon, H.S.; Shuker, S.B.; Chang, B.S.; Minn, A.J.; Thompson, C.B.; Fesik, S.W.: 'Structure of Bcl-xL-Bak Peptide Complex: Recognition Between Regulators of Apoptosis'. *Science*. 275, 5302, pp. 983–986, (1997).
167. Gadek, T.R.; Burdick, D.J.; McDowell, R.S.; Stanley, M.S.; Marsters, J.C.; Paris, K.J.; Oare, D.A.; Reynolds, M.E.; Ladner, C.; Zioncheck, K.A.; Lee, W.P.; Gribling, P.; Dennis, M.S.; Skelton, N.J.; Tumas, D.B.; Clark, K.R.; Keating, S.M.; Beresini, M.H.; Tilley, J.W.; Presta, L.G.; Bodary, S.C.: 'Generation of an LFA-1 Antagonist by the Transfer of the ICAM-1 Immunoregulatory Epitope to a Small Molecule'. *Science*. 295, 5557, pp. 1086–1089, (2002).
168. Shimaoka, M.; Salas, A.; Yang, W.; Weitz-Schmidt, G.; Springer, T. A.: 'Small Molecule Integrin Antagonists that Bind to the [β]2 Subunit I-like Domain and Activate Signals in One Direction and Block Them in the Other'. *Immunity* 19, 3, pp. 391–402, (2003).

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