Protein-protein interactions: biophysics, design and biological applications

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Specific protein-protein interactions form a major part of the basic organization of living cells. As such, macromolecular recognition is fundamental for biological regulation. The structure of a protein complex holds information about the relative mutual organization of two proteins in a frozen state, but not about the kinetic or thermodynamic parameters that are central to their function. For the last 15 years I have been fascinated with the question of how the structure of protein-protein interactions is linked to their activity. To address this question, I have adopted a multidisciplinary approach including: bioinformatics and algorithm development, wet biophysical bench work, protein-design and engineering and applied biology. My group members, coming from various backgrounds that include mathematics, computer science, biophysics and biology enabled this multidisciplinary research plan. The different disciplines are integrated within the different research projects.

Biophysics of protein-protein interactions

Protein complex formation is a kinetic process, which has structural and functional implications. Both the kinetic and the structure/function aspects of protein-protein interactions have been investigated in detail in my laboratory.

Association of protein-complexes: In earlier studies we described the pathway of protein-protein association, and developed computer-based tools to design faster associating protein complexes. These tools are accessible through the web at www.weizmann.ac.il/-home/bcges/PARE.html and http://bip.weizmann.ac.il/hypareb-/main. More recently, we extended our understanding on the nature of the transition state for binding, and investigated how molecular crowding affects binding. We provided experimental evidence that, indeed, electrostatic forces stabilize specifically the encounter complex for association and thereby increase the overall rate of association, however without an effect on the rate of final docking. Combining experimental and theoretical work, we showed that the transition state for association may be either specific or diffusive and went on to show, using computer based protein-design that it was possible to move between the two. In addition, we initiated a study on the mechanism of association in crowded environments (mimicking the cellular environment). Surprisingly, we found that both association and dissociation in a crowded environment is almost as rapid as in water, and that basic principles of polymer chemistry can be used to explain this phenomenon.

The molecular architecture of protein-protein interfaces: For many years there has been an interest to decipher the physico-chemical nature of protein binding site. We provided a partial solution to this question by drawing the interface as a connected graph (network), with the amino-acids being the nodes, and the bonds being the edges. The graph showed that protein-protein interfaces are made of an aggregate of independent modules, with each module comprising of a number of cooperatively interacting residues. The space between modules is occupied by interface water molecules, which we showed experimentally to be neutral in their contribution to binding. Experimental studies using a large number of mutants, as well as x-ray crystallography confirmed this interface architecture. In two recent publications

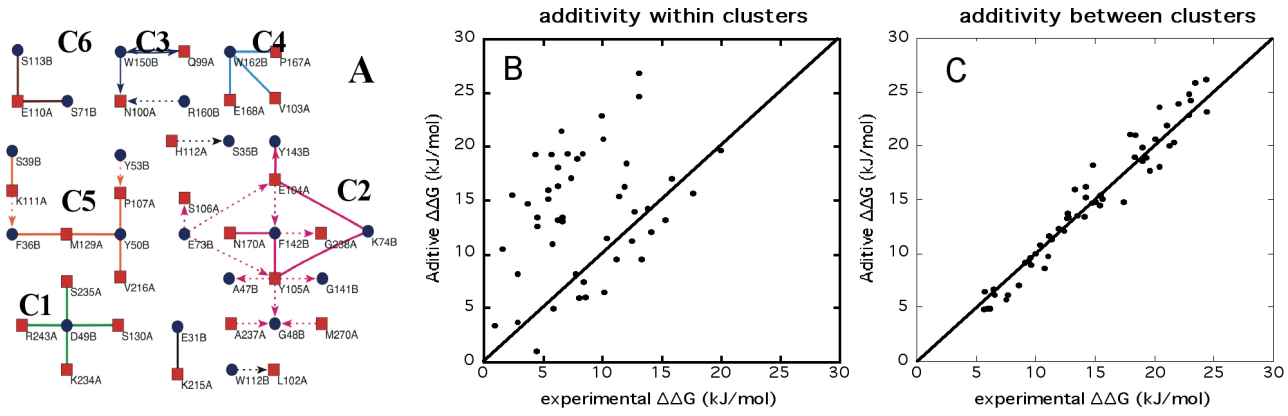

Fig. 1. *Mapping the protein-protein interaction between TEM1 and its inhibitor BLIP. In (A) a connectivity map was derived for this complex with TEM1 and BLIP residues being nodes in the graph (squares and circles, respectively). Three interaction types are shown in the map: side-chain side-chain (solid lines), backbone side-chain (dotted lines) and interactions of both side-chain side-chain and backbone side-chain between the same pair of residues (arrows point to the backbone donor atom). (B) and (C) show the degree of additivity of free energy of binding between mutations on TEM1 and BLIP within or between the six clusters shown in (A). Additive ΔΔG is defined as* $\Delta\Delta G_{mut1} + \Delta\Delta G_{mut2}$, *plotted versus the experimentally determined values of the double-mutant* ($\Delta\Delta G_{mut1,mut2}$).

we also showed that modules are evolutionarily more stable than their residue composition, and that viewing proteins as graphs is also very useful in understanding protein-structure. To facilitate the analysis of protein-interactions using connected graphs, we established a web server named AquaProt (http://bioinfo.weizmann.ac.il/aquaprot).

An intriguing question is whether the location of binding sites are imprinted in the unbound structure of the proteins, irrespective of their partner, and whether this information is transferred to the surrounding water. In other words, do binding sites possess unique features, which can be exploited to predict their location without knowledge of their partner. Bioinformatic studies of interfaces have indeed shown this to be true. From this information we successfully developed computer algorithms to predict the location of protein-binding sites and implemented it for docking (http://bioportal.weizmann.ac.il/promate).

Different ligand-receptor interactions drive the unique activities of type I interferons

In studying interferons we make use of our strength in biophysics and bioinformatics to quantitatively investigate this biological system. Interferons α and β are multifunctional cytokines that exhibit different activities, such as their antiviral and anti-proliferative action, through binding a common receptor composed of two transmembrane proteins, IFNAR1 and IFNAR2. Initially, we assumed that the differential activities are related to differences in the mode of binding of these different interferon ligands with their receptors. We, therefore, performed a series of structure/function studies to pinpoint whether these two interferons bind differently to the receptor. From these studies we now understand that the difference between the activities of the different interferons is related to the integral life-time of the ternary receptor complex. To prove this point, we engineered an α-interferon to gain the biological activities associated with interferon-β. Further increase in the binding affinity of IFNα2 to IFNAR1 resulted in a "super" active interferon, which has anti-proliferative activity greater than any other known interferon while its antiviral potency was only slightly increased. Tighter binding stimulates a longer lasting response, which in turn activates additional signal transduction cascades. The detailed biophysical knowledge gained from the interferon/receptor interaction enabled us to construct a series of engineered interferons with altered biological activities.

Selected publication

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