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Protein-protein interactions: From mechanism to protein design

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The complexity of life requires proteins to be able to transfer specific signals, build multi-unit machines, control the function of enzymes and regulate production and activities. Many of these tasks are performed through specific protein-protein interactions. This is feasible due to the almost unlimited potential for generating unique binding sites on proteins, characterized by their shape and surface chemistry. Despite the high degree of specificity, high-affinity protein-protein interactions are formed at rates close to the diffusion limit. We were intrigued to investigate the mechanism of protein-protein interactions, and to apply the gained knowledge towards protein design. Our research is conducted on a number of protein-protein interactions. Basic research into the mechanism of complex formation and stability is conducted on the interaction between TEM1-β-lactamase with its protein inhibitor BLIP. Structure-function studies are conducted on the interaction between different type I interferons and their receptors, using either the soluble extracellular domains of the receptors (for *in vitro* studies), or intact receptors (for in situ work). Protein-design is carried out on the interactions between growth-hormone and its receptor and between ras and its effectors. In addition we carry out bioinformatics work on a database of protein-protein interactions with known structures.

Studying the mechanism of protein-protein interactions

For studying the mechanism of protein-protein association, we introduced a thermodynamic framework for the association reaction, which enabled us to present a general formulation for the calculation of rates of association for mutant protein complexes. Moreover, we established a design protocol, which enables us to specifically alter rates of association, without affecting the rates of dissociation of a protein complex. This formulation was used for the successful design of a protein complex which binds 250 fold faster and tighter compared to the native proteins. Using computer simulations we demonstrated that the energy profile during association resembles in shape a funnel, with the final complex being at the energy minima. For faster associating protein complexes the energy funnel deepens and its volume increases. Currently, we are investigating the relevance of faster association in the biological environment both by mimicking the crowded environment and by relating faster binding with enhanced biological activity.

In parallel we investigated the forces which stabilize a protein complex once it is formed. The use of single mutations or even double-mutant cycles is not sufficient for this purpose, as the

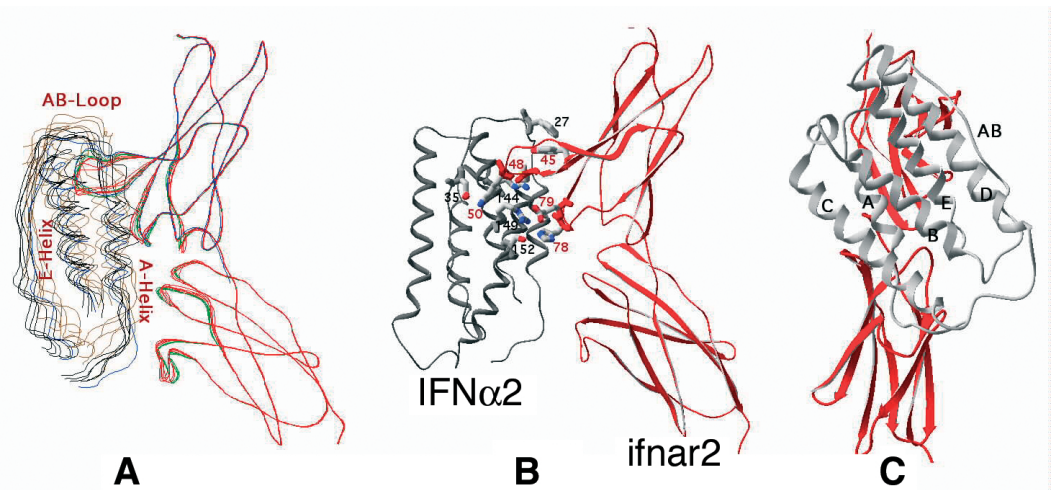

Fig. 1 *Structure of the interferon-receptor complex as determined from distance-constraint docking*

environmental effects are not taken specifically into account. Therefore, we implemented a modification of the multiple-mutant cycle method to investigate direct and cooperative contributions towards the strength of non-covalent interactions within a protein-complex interface. By analyzing all possible interactions within a binding unit, consisting of two buried salt-bridges and two H-bonds, we demonstrated experimentally that buried salt bridges do not have a net contribution to the free energy of binding, but that a network of those does. These experimental data were confirmed by theoretical calculations, and fit the previously suggested, but yet unproven theory.

The Interferon-receptor interaction as a paradigm for a restricted signal transduction network

In a second line of research, we aimed to decipher the relation between biophysical parameters of protein-protein interactions and biological activity. We were intrigued by the complex network of a large number of interferon subtypes binding the same two cell surface receptors, but seemingly causing differential activation. We assume that this differential activation is related to distinct modes of receptor binding, which can be investigated using purified proteins *in vitro*. Using Ala scanning, we identified the mutual binding sites on interferon and on one of its receptors, and demonstrated that interferon α binds the receptor differently than interferon β. While a simple relation between affinity and biological activity was established for the receptor, mutagenesis studies revealed a more complex nature of biological activity promoted by other binding surfaces of interferon. To get an insight into the structure of the interferon-receptor complex, we developed a docking algorithm which is based on using experimentally determined distance constraints (from double-mutant cycles) as the driving force for docking. The generated structure of the interferon-receptor complex gave us an interesting insight into the different modes of binding of α versus β interferons. The quality of this structure will be evaluated by an ongoing X-ray and NMR analysis of the structures.

Selected Publications

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