THE 1988 OHOLO CONFERENCE

The conference titled *Computer-Assisted Modeling of Receptor-Ligand Interactions: Theoretical Aspects and Applications to Drug Design* was held in Eilat, Israel. Among the 100 or so participants were C. Levinthal, S. Lifson and H. Scheraga who count among the founding fathers of molecular mechanics and protein structure prediction. There were four main topics in the program: (1) computational methods used to model proteins; (2) physical methods, with examples from protein X-ray crystallography and NMR; (3) transmembrane signaling which was mainly centered on the acetylcholine receptor (AChR); and finally (4) receptor-directed drug design.

Over the past ten years there has been a flood of information available on the primary structure of transmembrane receptors due mainly to the cloning and sequencing techniques of genetic engineering. There are now amino acid sequences available for many of the known receptor families notably ion-gated channels like AChR, G-protein coupled receptors such as the adrenergic receptor, and various peptide hormone receptors, e.g., the insulin receptor. One major research goal is to be able to design drugs which will modulate receptor activity. This will only be achieved (rationally) when we have a high resolution picture of the molecular structure of the receptor and an understanding of its mechanism at a molecular level. This conference provided a useful overview of the area and also pointed out some of the major hurdles we can expect along the path to the grail of rational drug design.

The first challenge is, of course, the prediction of 3D protein structure from a primary sequence, and there were a number of contributions on this topic. Scheraga stressed that here the major problem to overcome was in finding the global energy minimum. Even with the aid of supercomputers, it is only possible to test the conformational space of a polypeptide some 20 amino acids in length using conventional sampling methods. One promising approach, which dramatically reduces computer time, is to use a build-up procedure in which allowed conformations of tetrapeptide units are combined to provide models of peptides of over 100 amino acids in length. Another major help in solving the multiple minimum problem comes from distance constraints provided by 2D NMR techniques. Even a few such experimentally determined constraints dramatically reduces the search problem and some examples were presented in which a combined experimental plus theoretical approach has been used (cyclosporin A from G. Marshall and T4-lysozyme and other small proteins from O. Jardetzky). One very reasonable approach to protein structure prediction is to use a homologous X-ray structure as the starting model. The crucial first step in this approach is to find the most suitable sequence alignment which should be better than 30%. An example of how well this template modeling can work if carefully applied was given by J. Greer who built a model of the anaphylatoxin C5a (77 amino acids) based on a crystal structure of the related C3a protein which shows 35% homology. This template modeling technique was also discussed by J. Moult who formulated some empirical 'rules' on how to choose possible conformations of modeled inserted loops based on favored electrostatic interactions and the amount of exposed hydrophobic surface.

Acetylcholine receptors have long held the limelight in receptor studies, not least because they

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are readily available from fish electric organ. The primary sequence of this five-subunit 270kDa transmembrane protein is known from cloning experiments and low resolution (>20Å) pictures of the structure which are available. Results presented by J. Lindstrom showed how many biochemical results, using antibody and toxin binding, could be interpreted in terms of receptor topology. He also drew attention to the various subtypes of AChRs found in brain and ganglion which are composed of only two types of subunites and, in contrast with muscle AChR, do not bind snake neurotoxins. An accumulation of this type of information will soon provide us with a useful molecular classification of the various transmembrane receptors into structural families. For example, it seems that the anion-channel receptors like GABA and glycine receptors may have a structural similarity to AChR found in brain and ganglion. A number of speculative 3D models of receptor structure were presented. Among the most detailed was a model of AChR from E. Kosower who managed to build up a picture of the ACh binding site and a molecular mechanism for ion flow across the transmembrane channel, both of which are consistent with the available biochemical data. One very interesting result of potential therapeutic value from J.M. Gershoni was that a synthetic 17 amino acid sequence from the alpha-AChR subunit binds strongly to neurotoxins. Preliminary results indicate that the sequence binds competitively with membrane-bound AChR in vivo and the polypeptide can be used as an antidote to snake toxin poisoning in mice. The theme of defining structural differences among receptor subtypes was continued by J. Ramachandran who discussed the four pharmacologically distinct muscarinic acetylcholine receptors (mAChR) which have been identified and sequenced. Hydropathy studies suggest structures with seven transmembrane domains similar to rhodopsin. There is a surprising homology between species with human M1 and M2 showing over 95% homology with porcine M1 and M2. Homology between the subtypes within a species is however less than 40%.

Even with little or no knowledge of receptor structure, it is still possible to optimize specific drug binding. One example of this was presented by G. Loew who managed to synthesize some high-affinity opioids which are selective for a particular receptor subtype. The design of these new molecules was based on a careful analysis of the pharmacological and structural properties of known drugs. A more geometric way of tackling this problem from G. Marshall showed how computer graphics with molecular mechanics can be used to determine the biologically relevant conformation of a drug. In this example, the 3D structures of a series of inhibitors to angiotensin-converting enzyme were compared and a model was developed which explained the measured affinity data.

A roundtable discussion at the end of the conference served to summarize the scope and limits of the theoretical methods used to examine receptor-ligand interactions. There was general agreement that 3D-receptor models were not reliable, but they serve to stimulate ideas and provide a working hypothesis for drug action. Indeed the reliability of any theoretical protein model is low. One (perhaps Freudian) quip from a panel member was: 'Anyone who believes his (own) model is making a serious mistake'. This was later rephrased but it does emphasize the scientific scepticism required when using model structures. In this context there was a call for far more groundwork to be carried out on known (X-ray and NMR) structures in order to firm up the theoretical methods. However, a model does not need to be absolutely accurate in every detail; its main purpose is to provide a 3D picture which fits the known experimental facts and can be used in the design of confirmatory experiments, or indeed help in the rational design of drugs. Perhaps the most optimistic note was struck by J. Ramachandran who pointed out that with cloning techniques it

is now possible to express recombinant receptors in milligram quantities. This means that we can expect, before very long, some new structural information (probably from X-ray crystallography) which will undoubtedly stimulate frantic activity within the modeling community.

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