

Membrane Proteins: Insights from Computational Biology

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Cells are isolated from the external world by a lipidic membrane. This barrier has also an important role in cellular communication because it is the target of all the extracellular stimuli acting on the cell. Several proteins, i.e. integral membrane proteins, are thus specialized in detecting extra cellular signals and translating the information to the cell, allowing a response. It has been suggested that around the 20% of proteins encoded in the human genome, code for membrane proteins [1]. Unfortunately, due to the difficulties in expression, just few of them have been deeply characterized at the structural level, i.e. about 40 of the just 400 unique membrane proteins solved by X-ray crystallography are human proteins. Encouragingly, in the last decade there was an exponential increase in the number of solved crystal structures of membrane proteins. This make us confident that in the forthcoming years, most of the membrane protein families will count with at least one member for which the structure is known. These numbers still represent a small portion of the entire human membrane proteome.

Membrane proteins are the principal players in a variety of signaling pathways, thus attracting a huge interest in therapeutic intervention, as the majority pharmaceutical compounds target membrane proteins, i.e. 30 % of the FDA approved drugs. This huge number implies that, a gain of knowledge in the structure/function relationship is key in any rational drug design process. Unfortunately, the paucity of structural information limits extremely the use of structure-based drug design approaches. Thus computational biology tools, like homology modeling techniques have extensively been used to overcome these difficulties [2-4]. Indeed, recent calculations using different techniques, showed that, as of today, around 1/3 of the human membrane proteome could be reliable modeled using homology modeling [5].

Once the structure of the protein is solved (or modeled), virtual molecular docking experiments should be carried out in order to characterize the binding cavities. Particularly challenging is to reach a correct orientation of the side chains in the binding site: for an accurate molecular docking this orientation is crucial. Unfortunately, in most of the cases, the low resolution of homology models cannot overcome this problem. The need of extensive membrane protein characterization, thus calls for alternative innovative approaches. One of the most popular approaches undertaken by the scientific community consists in using an extensive combination of computational biology techniques with molecular biology validating experiments. Indeed, analyzing the literature of the last few years, a careful reader can find more than 400 research articles, in which combined approaches have been successfully used for the structure-function relationship characterization on membrane proteins.

Here I will briefly list the most relevant ones, at my advice, so the reader can have an overview on the variety of systems that were characterized, at different levels, using combined experimental/computational approaches. Although not all of them were pure rational structure based drug design, the contributions point to a gain of insights into the structural determinants underlying the functioning of membrane proteins, a fundamental step needed for modern drug-design approaches.

Homology modeling approaches were used to study the conformational changes between the holo and apo physiological states

of the ATP-binding cassette (ABC) superfamily of proteins [6,7] and for characterizing the water and glycerol permeability and response to drug inhibitors of aquaporins [8,9], an argument clearly related to drug design. In order to study ligand gated ion channels like γ -amino butyric acid type A receptors (GABAARs) and glycine receptors (GlyRs) modeling data were used to design mutagenesis experiments aimed at the characterization of glycosylation sites, found to be altered in disease states [10-12]. Also here, computational biology was used as a bridge between basic biology and medicine. In other interesting cases, homology models combined with electrophysiology and site-directed mutagenesis experiments were used to characterize the open conformation and accessibilities of an important variety of voltage-gated ion channels, characterizing their different activation states [13-15]. Similar approaches were also used to characterize the activation mechanisms in cyclic nucleotide channels [16-20]. Using homology models in combination with other computational biology techniques, i.e. molecular dynamic and metadynamics (MTD), an alternative Na^+ binding site of Sodium-Galactose Transporter (SGLT) symporter protein was predicted [21]. In the case of Acid-sensing ASIC channels [22] and calcium-activated anion channel bestrophin, homology models combined with mutagenesis experiments were used to characterize the interactions with toxins in the former, and to evaluate how specific mutations affect its capacity to bind calcium ions [23] for the latter. Another examples include membrane receptors, i.e. proteins that allow the cell to communicate with the external world: TLR8, a member of the Toll-like receptors (TLRs) family, were studied with the main aim of unraveling the interactions of the receptors with an antiviral compound, R848, involved in the activation of the full TLR8 pathway [24]. Several groups have also successfully applied homology-based structure modeling approaches of G-Protein couple receptors (GPCRs) to ligand-binding elucidation [22-43].

Summarizing, membrane proteins are of the utmost importance for the survival of any living being, thus a deep insight into the molecular mechanisms underlying their function is needed for a complete characterization of the way our cells exchange information with the environment. In the case of drug design protocols, the availability of membrane protein structures or, as we saw before, the possibility of gaining structural information by homology modeling combined with experiments, will allow a shift paradigm from ligand-based to target-based drug design. The great gain of the structure-based methods over ligand-based methods, resides in the fact that the possibility of a detailed structural analysis may pave the way, not only, to the development of

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'classical' orthosteric inhibitors, but will also open the door to novel solutions like the development of allosteric modulators.

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