Biology and Medicine

Membrane Proteins: Insights from Computational Biology

Alejandro Giorgetti*

Applied Bioinformatics Group, Deptartment of Biotechnology, University of Verona, Verona, Italy

Cells are isolated form 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 glicerol 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 g-amino butyric acid type A receptors (GABAARs) and glycine receptors (GlyRs) modeling data were used to design mutagenesis experiments aimed at the characterization of glycosilation 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

*Corresponding author: Alejandro Giorgetti, Applied Bioinformatics Group, Deptartment of Biotechnology, University of Verona, strada Le grazie 15, 37134, Verona, Italy, E-mail: alejandro.giorgetti@univr.it

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

References

1. Almén MS, Nordström KJ, Fredriksson R, Schiöth HB (2009) Mapping the human membrane proteome: a majority of the human membrane proteins can be classified according to function and evolutionary origin. BMC Biol 7: 50.

2. Tramontano A, Cozzetto D, Giorgetti A, Raimondo D (2008) Methods in Molecular Biology, vol. 413: Protein Structure Prediction. (2ndedn), Humana Press Inc.: Totowa, New York.

3. Cozzetto D, Giorgetti A, Raimond D, Tramontano A (2008) The evaluation of protein structure prediction results. Mol Biotechnol 39: 1-8.

4. Chothia C, Lesk AM (1986) The relation between the divergence of sequence and structure in proteins. EMBO J 5: 823-826.

5. Piccoli S, Suk E, Garonzi M, Giorgetti A (2013) Genome-wide membrane protein structure prediction. Current Genomics 14: 324-329.

6. Mormon JP, Lehn P, Callebaut I (2009) Molecular models of the open and closed states of the whole human CFTR protein. Cell Mol. Life Sci 66: 3469-3486.

7. Shi Z, Tiwari AK, Shukla S, Robey RW, Singh S, et al. (2011) Sildenafil reverses ABCB1- and ABCG2- mediated chemotherapeutic drug resistance. Cancer Res 71: 3029-3041.

8. Yakata K, Tani K, Fujiyoshi Y (2011) Water permeability and characterization of aquaporin-11. J Struct Biol 174: 315-320.

9. Martins A.P, Marrone A, Ciancett A, Galán Cobo A, Echevarrí M (2012) Targeting aquaporin function: potent inhibition of aquaglyceroporin-3 by a gold-based compound. PloS One 7: e37435

10. Haeger, Kuzmin D, Detro-Dassen S, Lang N, Kilb M (2010) An intramembrane aromatic network determines pentameric assembly of Cys-loop receptors. Nat Struct Mol Biol 17: 90-98.

11. Castro PA, Figueroa M, Yevenes GE, San Martin LS, Aguayo LG (2012) The basicproperty of Lys385 is important for potentiation of the human a1 glycine receptor by ethanol. J Pharmacol Exp Ther 340: 339-349.

12. Gurba KN, Hernandez CC, Hu N, Macdonald RL (2012) GABRB3 mutation; G32R, associated with childhood Absence epilepsy αalters1β3γ2Lγ - aminobutyric acid type A (GABAA) receptor expression and channel gating. J Biol Chem 287: 12083-97.

13. Kulleperuma K, Smith SM, Morga D, Musset B, Holyoake J (2013) Construction and validation of a homology model of the human voltage-gated proton channel hHV1. J Gen Physiol 141: 445-465.

14. Durdagi S, Deshpan S, Duff HJ, Noskov SY (2012) Modeling of open; closed; and open-inactivated states of the hERG1 channel: structural mechanisms of the state-dependent drug binding. J Chem Inf Model 52: 2760-2774.

15. Depil K, Beyl S, Stary-Weinzinger A, Hohaus A,Timin E, et al. (2011)Timothy mutation disrupts the link between activation and inactivation in Ca(V)1.2 protein. J Biol Chem 286: 31557-31564.

16. Giorgetti A, Nair AV, Codega P, Torre V, Carloni P (2005) Structural basis of gating of CNG channels. FEBS Lett 579: 1968-1972.

17. Mazzolini M, Marchesi A, Giorgetti A, Torre V (2010) Gating in CNGA1 channels. Pflugers Arch 459: 547-555.

18. Giorgetti A, Carloni P (2003) Molecular modeling of ion channels: structural predictions. Curr Opin Chem Biol 7: 150-156.

19. Giorgetti A, Carloni P, Mistrik P, Torre V (2005) A homology model of the pore region of HCN channels. Biophys J 89: 932-944.

20. Griguoli M, Maul A, Nguyen C, Giorgetti A, Carloni P (2010) Nicotine blocks the hyperpolarization-activated current Ih and severely impairs the oscillatory behavior of orienslacunosum molecular interneurons. J Neurosci 30: 10773-10783.

21. Bisha I, Laio A, Magistrato A, Giorgetti A, Sgrignani JA (2013) Candidate Ion-Retaining State in the Inward-Facing Conformation of Sodium/Galactose Symporter: Clues from Atomistic Simulations. J Chem Theory Comput 9: 1240-1246.

22. Pietra F (2009) Docking and MD simulations of the interaction of the tarantula peptide psalmotoxin-1 with ASIC1a channels using a homology model. J Chem Inf Model 49: 972-977.

23. Kranjc A, Grillo FW, Rievaj J, Boccaccio A, Pietrucci F (2009) Regulation of bestrophins by Ca2+: a theoretical and experimental study. PLoS One 4: e4672.

24. Govindaraj RG, Manavalan B, Basith S, Choi S (2011) Comparative analysis ofspecies-specific ligand recognition in Toll-like receptor 8 signaling: a hypothesis. PloS One 6: e25118.

25. Senderowitz H, Marantz Y (2009) G Protein-coupled receptors: target-based in silico screening. Curr Pharm Des 15: 4049-4068.

26. de Graaf C, Rognan D (2009) Customizing G Protein-coupled receptor models for structure-based virtual screening. Curr Pharm Des 15: 4026-4048.

27. Cui M, Jiang P, Maillet E, Max M, Margolskee RF, et al (2006) The heterodimeric sweet taste receptor has multiple potential ligand binding sites. Curr Pharm Des 12: 4591-4600.

28. Brockhoff A, Behrens M, Niv MY, Meyerhof W (2010) Structural requirements of bitter taste receptor activation. Proc Natl Acad Sci 107: 11110-11115.

29. Reisert J, Restrepo D (2009) Molecular tuning of odorant receptors and its implication for odor signal processing. Chem Senses 34: 535-545.

30. Petrel C, Kessler A, Dauban P, Dodd RH, Rognan D (2004) Positive and negative allosteric modulators of the Ca²⁺-sensing receptor interact within overlapping but not identical binding sites in the transmembrane domain. J Biol Chem 279: 18990-18997.

31. Bhattacharya S, Subramanian G, Hall S, Lin J, Laoui A (2010) Allosteric antagonist binding sites in class B GPCRs: corticotropin receptor 1. J Comput Aided Mol Des 24: 659-674.

32. Niv MY, Skrabanek L, Filizola M, Weinstein H (2006) Modeling activated states of GPCRs: the rhodopsin template. J Comput Aided Mol Des 20: 437-448.

33. Niv MY, Filizola M (2008) Influence of oligomerization on the dynamics of G-protein-coupled receptors as assessed by normal mode analysis. Proteins 71: 575-586.

34. Ivanov AA, Barak D, Jacobson KA (2009) Evaluation of homology modeling of G-protein-coupled receptors in light of the A (2A) adenosine receptor crystallographic structure. J Med Chem 52: 3284-3292.

35. Slack JP, Brockhoff A, Batram C, Menzel S, Sonnabend C (2010) Modulation of bitter taste perception by a small molecule hTAS2R antagonist. Curr Biol 20: 1104-1109.

36. Biarnés X, Marchiori A, Giorgetti A, Lanzara C, Gasparini P, et al. (2010) Insights into the Binding of Phenyltiocarbamide (PTC) Agonist to Its Target Human TAS2R38 Bitter Receptor. PLoS One 5: e12394.

37. Leguèbe M, Nguyen C, Capece L, Hoang Z, Giorgetti A, et al. (2012) Hybrid Molecular Mechanics/Coarse-Grained Simulations for Structural Prediction of G-Protein Coupled Receptor/Ligand Complexes. PLoS One 7: e47332.

38. Marchiori A, Capece L, Giorgetti A, Gasparini P, Behrens M, et al. (2013) Coarse-Grained/Molecular Mechanics of the TAS2R38 Bitter Taste Receptor: Experimentally-Validated Detailed Structural Prediction of Agonist Binding. PLoS One 8: e64675.

39. Lupieri P, Nguyen CHH, Bafghi ZG, Giorgetti A, Carloni P (2009) Computational molecular biology approaches to ligand-target interactions. HFSP J 3: 228-239.

40. Vaidehi N, Pease JE, Horuk R (2009) Modeling small molecule-compound binding to G-protein-coupled receptors. Methods Enzymol 460: 263-288.

41. Simms J, Hall NE, Lam PH, Miller LJ, Christopoulos A, et al. (2009) Homology modeling of GPCRs. Methods Mol. Biol 552: 97-113.

42. Levit A, Barak D, Behrens M, Meyerhof W, Niv MY (2012) Homology Model-Assisted Elucidation of Binding Sites in GPCRs In: Membrane Protein Structure and Dynamics: Methods and Protocols (Methods in Molecular Biology), 1stedn; Vaidehi, N.; Klein-Seetharaman, J.; Eds.; Springer Science Business Media: London, pp. 179-205.

43. Sandal M, Duy TP, Cona M, Zung H, Carloni P, et al. (2013) GOMoDo: A GPCRs Online Modeling and Docking Webserver. PLoS One 8: e74092.