12

G-Protein Coupled Receptors: Experimental and Computational Approaches

Amirhossein Sakhteman, Hamid Nadri and Alireza Moradi
Faculty of Pharmacy, Shahid Sadoughi University of Medical Sciences, Yazd
Iran

1. Introduction

Guanine Nucleotide binding protein coupled receptors (GPCRs) are among the most important targets in the treatment of cancer, endocrine, neural and many other types of disorders. (Katritch, V. & Abagyan, R. 2011) It is believed that activation of some GPCRs is involved in conditions such as immunosupression and response to ischemia of the brain and heart. Therefore, antagonists and agonists of GPCRs are potential therapeutic agents in treatment of inflammatory and ischemic diseases. (Moro, S., Spalluto, G., & Jacobson, K. A. 2005)

The superfamily of GPCRs consists of about 800 receptors which can be divided into different families regarding the similarities in the protein sequence. (Marshall, F. H. & Foord, S. M. 2010)

1. Family A, including rhodopsin and adrenoreceptor
2. Family B, Secretin vasointestinal peptide (VIP), the members of this family bind to hormones and neuropeptides
3. Family C, which include at least eight subtypes of glutamate receptors, the major excitatory receptor in the CNS.
4. Family D, the fungal pheromone p family
5. Family E, the fungal pheromone A family
6. Family F, CAMP receptors of *Dictyostelium discoideum*

The family A receptors is the best studied family of GPCRs in terms of functional and structural viewpoints and is therefore the most important target of GPCRs in drug discovery. (Moro, S. et al. 2005) It was reported that about 30% of the market prescription drugs act on these targets. (Marshall, F. H. et al. 2010)

2. Features and functions of GPCRs

A common feature in class A GPCRs is a core consisting of seven transmembrane domains (TM) connected by three intracellular loops (IL1, IL2 and IL3) and three extracellular loops (EL1, EL2, EL3).(Fig 1) Another feature observed in this class is the two cysteine residues, one in TM3 and the other in EL2. These two cysteines form a disulfide bridge which is responsible for the packing and stabilization of a restricted number of conformations for the seven TM domains (Fig 1).

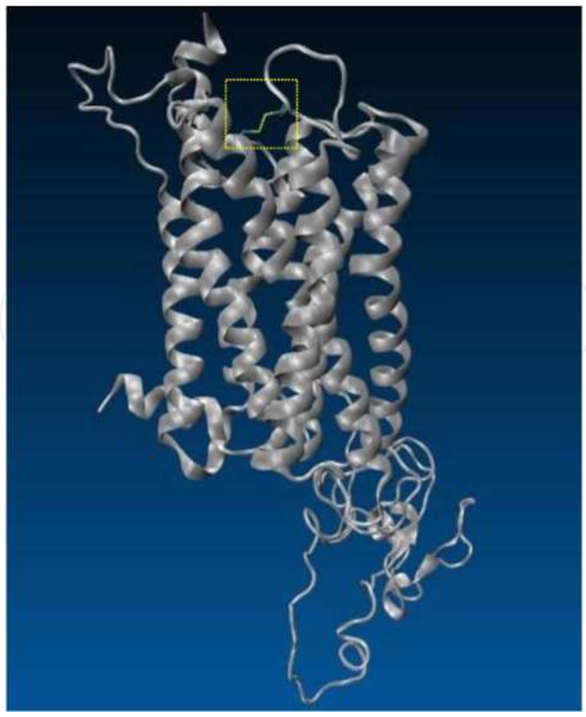

Fig. 1. The seven transmembrane structure of GPCRs. A disulfide bridge between TM3 and EL2 is conserved in most class A GPCRs.

GPCRs differ in the length and function of their N-terminal extracellular domains, C-terminal domain and intracellular loops. (Moro, S. et al. 2005) As an instance, glycoprotein hormone receptor (GPHR) family tether large amino terminal extracellular extensions which are responsible for the recognition and binding of dimeric agonists. Some studies in the area of GPCRs focused on interaction of ligands with GPCRs. There is strong evidence that in case of small molecules like the biogenic amines, the agonists of GPCRs interact directly with specific residues of TM helices of the receptor. On the other hand, for neuropeptides and small protein agonists like neurokinin the interactions involve both exoloops and amino portion of the receptor in association with the residues in TM helices. (Gilbert Vassart & Sabine Costagliola 2003)

2.1 Receptor activation in GPCRs

Many physiological procedures in the body are controlled by the GTPase including signal transduction, control of cellular growth, vesicle and protein transport and cytoskeletal assembly.(Smith, B., Hill, C., Godfrey, E. L., Rand, D., van den Berg, H., Thornton, S. et al. 2009). (Kobilka, B. K. 2007) Activation of a GPCR leads to nucleotide exchange on the Gα subunit and cause dissociation of the heterodimer and effector activation. GPCRs are mostly activated by diverse set of signals including small molecules, peptides and light. (Schneider, M., Wolf, S., Schlitter, J., & Gerwert, K. 2011) Members of GPCRs transduce signals by activation of at least one member of homologous hetrotrimeric G proteins. For example in FSH (Follicle Stimulating Hormone), receptor is activated by adrenaline which binds to the TM regions.

It should be noticed that in GPCRs, the ligand binds from the extracellular side and blocks the receptor. The activation or reduction in the basal activity of the heterotrimeric G-protein complex is dependent to the nature of the ligand such as agonists, antagonists and reverse agonists. The activation of G protein is in such a way that an exchange of guanosine diphosphate takes place in α subunit of G-protein. This exchange causes a conformational change in α subunit and leads to dissociation of α subunit from βγ subunits. (Fig 2) The two subunits introduce transduction systems in different ways. (Jaakola, V. P. & Ijzerman, A. P. 2010)

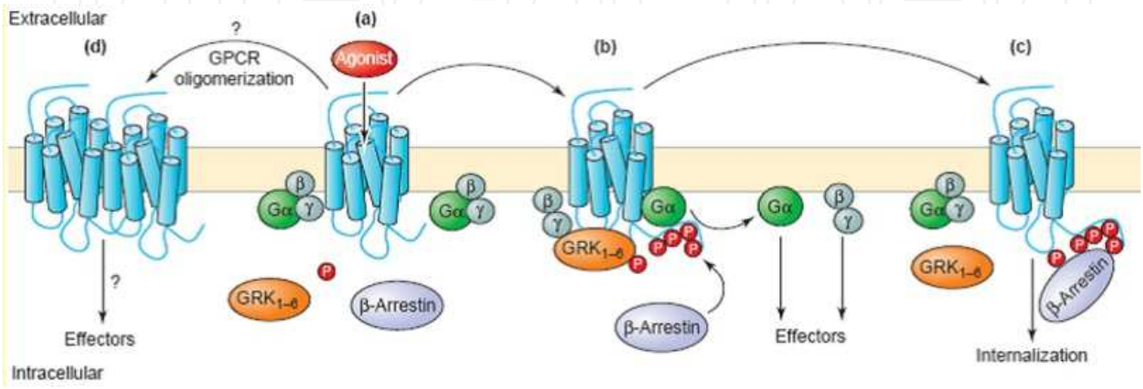

Fig. 2. a) Activation of GPCRs upon agonist binding b) conformational changes in hetrotrimeric G c) dissociation in G protein subunits proteins. d) A common feature observed in most GPCRs is the formation of dimers.

Some evidence verified that GPCRs exist as ensembles of conformations and two factors including binding of agonist and intracellular signaling proteins stabilize the active site of the receptor and accounts for the basal activity of GPCRs in the absence of agonists. The crystal structures of β2AR bound to an agonist and G-protein shows a large conformational change in the intracellular region of the receptor. It is likely that upon agonist binding reshuffling of short range intracellular contact cause large scale domain motions in the receptor. (Vaidehi, N. & Bhattacharya, S. 2011)

With the exception of rhodopsin, most family A GPCRs have considerable basal activity and both modeling and crystallographic data suggest that agonist dependent activation can vary between GPCRs. (Ahuja, S. & Smith, S. O. 2009;Deupi, X. & Kobilka, B. 2007;Katritch, V. et al. 2011) In contrast to rhodopsin where more information is present for the activation state of the molecule, in case of the other GPCRs much less is known about agonist induced conformational changes that occur during the activation of the receptor. (Wess, J., Han, S. J., Kim, S. K., Jacobson, K. A., & Li, J. H. 2008) The most similar parts for the GPCRs are the cytoplasmic ends of the TM segments adjacent to the second and third cytoplasmic domains which interact with G protein.

2.2 Dimerization of GPCRs

An important feature in GPCRs is formation of dimer which affects the receptors in terms of signal trafficking and pharmacology.(Marshall, F. H. et al. 2010). In many cases, the GPCR dimer can alter or regulate coupling or potency of other receptors. As an instance,

dimerization of κ-opioid receptor was shown to be in close relation with δ-opioid receptor dimerization. Another consequence of such dimerization is the augmented selectivity of some agonists such as 6-guanidinonal for the dimer with respect to any of the monomers. In addition, it was postulated that the binding of some drugs to more than one type of receptor is the result of dimerization. (Panetta, R. & Greenwood, M. T. 2008)

2.3 GPCRs and drug discovery

Most researches in the area of GPCRs focused on development of more selective or potent compounds of the orthostatic sites, which are apart from the binding sites of endogenous ligands. The allosteric modulators are also considered as promising therapeutic Agents. (Moro, S. et al. 2005)

While, the binding site for most small organic agonists is within TM segments, in case of peptide hormones and proteins the binding site is laid in the extracellular domain.(Kobilka, B. K. 2007) The intrinsic plasticity of GPCRs is a major problem in using their inactive state for agonist design in drug discovery. (Katritch, V. et al. 2011)

3. The role of experimental techniques in structural elucidation of GPCRs

The 3D structures of GPCRs have been identified using different techniques such as electron paramagnetic resonance spectroscopy (EPR), site directed mutagenesis, Fluorescence spectroscopy, cysteine cross-linking studies, Atomic Force Microscopy (AFM) and X-ray crystallography.

The first structure for GPCRs originated from cryoelectron microscopy of 2 dimensional crystals of bovine rhodopsin. Meanwhile, EPR has provided complementary evidence about photoactivation of rhodopsin including rotation and tilting of TM6 with respect to TM3.

It was clarified through electron paramagnetic resonance spectroscopy that photo activation of rhodopsin includes rotation and tilting of TM6 with respect to TM3.

By using some experimental techniques such as site directed mutagenesis data and cysteine scanning mutagenesis, it was possible to detect conformational changes in GPCRs.(Kobilka, B. K. 2007) As an instance, through site directed mutagenesis studies, it was proposed that the rotamer positions of the three residues including Cys 282, Trp 286 and Phe 290 of β2AR modulate the binding of TM6 around the highly conserved proline kink and lead to movement of cytoplasmic end of TM6 (Ahuja, S. et al. 2009;Deupi, X. et al. 2007) (Fig3).

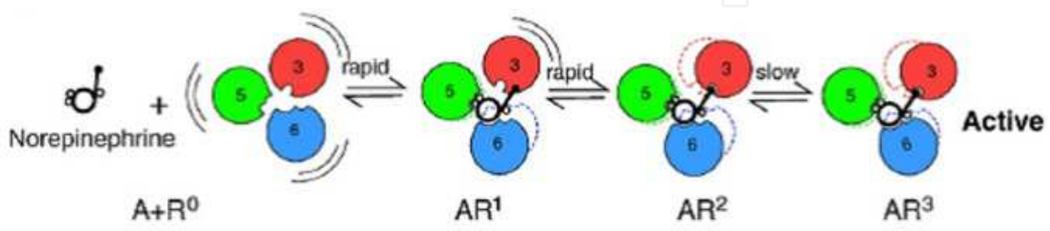

Fig. 3. Movement of TM6 (blue) in β2AR upon binding of an endogenous agonist (Norepinephrine) (Kobilka, B. K. 2007) with permission.

By using site directed mutagenesis data, binding sites of many receptors such as melatonin have also been discovered. (Panetta, R. et al. 2008)

Fluorescence spectroscopy and cross linking studies of some receptors such as muscarine (M3) has also revealed this fact that upon agonist binding, rotation or tilting of cytoplasmic end of transmembrane domains can take place in GPCRs. In cysteine cross-linking studies of M3 muscarinic receptor, it was suggested that the movements of TM5 and TM6 occurs upon agonist binding. (Kobilka, B. K. 2007) Through fluorescence spectroscopy and by tagging fluorophore to the intracellular end of TM6 in β2AR, the receptor revealed a single population of fluoroscence life time. Although, in the presence of antagonists the peak was reinforced, agonists showed an additional peak in the fluorescence life time. (Vaidehi, N. et al. 2011). Documents for the movements of TM6 in rhodopsin have been also provided by chemical reactivity measurements and fluorescence spectroscopy and Zinc cross linking studies of histidines. (Kobilka, B. K. 2007)

The most direct evidence for the structure of a GPCR oligomer comes from AFM (Atomic Force Microscopy). An advantage of AFM is that it provides a 3D profile of the protein. (Simpson, L. M., Taddese, B., Wall, I. D., & Reynolds, C. A. 2010)

Despite the great applicability of the described methods, X-ray crystal structures are normally the starting points in the studies of GPCRs. Since 2000 the high resolution X-ray structures of GPCRs began to emerge among which bovine rhodopsin was the first case study. (Topiol, S. & Sabio, M. 2009)

Crystallography of GPCRs has encountered some limitations due to low level of expression and instability of the proteins outside the membrane. Other problems are also attributed to the folding of the protein and homogeneity during purification. Another limitation in crystallography of the proteins is using detergents for dissociation of the protein from the membrane which might lead to some modifications in 3D structure of the protein. During the procedure for the preparation of crystal structures, a cost effective and straight forward to use system of protein expression is bacteria. The most important problem in this issue is the post translational limitations in the bacteria such as glycosylation which is required for the correct folding of GPCRs. To solve this problem many researchers have focused on using yeasts for the expression of GPCRs. The main problem with using yeasts is the differences observed in yeasts membranes in comparison with those observed in human. The most efficient system for the expression of the GPCRs includes the baculovirus expression system in the insects' cells. This method has been successfully adopted for the receptors including the β-adrenoceptors, the adenosine A2A receptor and the chemokine receptor CXCR4. (Congreve, M., Langmead, C., & Marshall, F. H. 2011)

In crystallography of GPCRs a solution for obtaining the crystal structure in single conformation is addition of the ligand with the prefential affinity to one conformation of the receptor. (Congreve, M. et al. 2011)

Although by X-ray crystallographic data, it is possible to obtain static of the protein complex at near atomistic resolutions biophysical and structural studies are still needed to complete the data of crystallography. (Jaakola, V. P. et al. 2010)

4. Homology modeling studies of GPCRs

Regarding the limitations for using X-ray crystallography in case of GPCRs, molecular modeling techniques such as homology modeling and docking studies are needed to fill the gaps between the primary sequence and secondary structures for drug design studies. There are two major types of drug design including ligand based drug design and structure based drug design. In structure based drug design, the 3D structure of the target molecules is necessary for the study. (Ostopovici-Halip, L., Curpan, R., Mracec, M., & Bologa, C. G. 2011) An important goal of molecular modeling is to provide a microscopic details of the membrane proteins where there is no way to obtain enough information through experimental approaches. (Henin, J., Maigret, B., Tarek, M., Escrieut, C., Fourmy, D., & Chipot, C. 2006)

The crystal structures for seven GPCRs have been represented so far. Most GPCRs are representing the inactive state of the receptor and are therefore suitable for the discovery of antagonists and reverse agonists. (Schneider, M. et al. 2011)

Homology modeling is a knowledge based approach relying upon the crystallographic structure of related receptor and experimental information. This method has limitations when targeting active receptors. (Henin, J. et al. 2006)

Homology modeling made it possible to align the protein of interest with the homologous structure and to subsequently evaluate the model with different scoring functions. Since the most important part of a homology modeling study is the alignment procedure, errors in predicting the structure protein with low homology are normally common. The alignment procedure is usually done by different methods such as cluatalW or T-cofee servers. In order to refine homology based models energy minimization or limited conformational sampling using molecular dynamic simulations are used. (Harterich, S., Koschatzky, S., Einsiedel, J., & Gmeiner, P. 2008)

Due to diversity in loop area of the proteins, a loop refinement is usually necessary in most homology modeling studies. The available loop modeling algorithms are limited to up to 13 residues long. Therefore in loop refinement step considerations must be taken with loops of the long size. (Ostopovici-Halip, L. et al. 2011)

By the emergence of 3D structure for bovine rhodopsin, this receptor was widely used as template structure homology modeling studies of GPCRs. For example, 3D structure of neurotensin, a neuropeptide distributed in the CNS was modeled based on Rhodopsin as template.(Harterich, S. et al. 2008) Two other GPCRs namely, the principal canabinoid receptors CB1 and CB2 are the components of endogenous endocanabinoid systems. The 3D structures of CB1 and CB2 have also been modeled using Rhodopsin as template. (Pei, Y., Mercier, R. W., Anday, J. K., Thakur, G. A., Zvonok, A. M., Hurst, D. et al. 2008)

Another GPCR which has been characterized by homology modeling techniques was melatonin receptor. This receptor is responsible for the effects of melatonin, a compound taking part in resynchronization of biological rhythms such as sleep. In case of MT1 and MT2 receptors, the helices of the receptor were supposed to be superposable with the experimentally known helices of bovine rhodopsin. It was also reported that the identity of MT1 towards rhodopsin is more in respect to MT2 (23% vs 19%).

In other studies the active state of opsin has been used as template to model active structures of β2-adrenergic receptor. Interaction fingerprint studies have been used for dynamic ligand binding study of the interaction of ligands in the active and inactive states. It was concluded that the active structure of opsin is suitable for modeling GPCR agonists. (Schneider, M. et al. 2011)

In spite of the many reports for the usefulness of rhodopsin in homology modeling studies, rhodopsin is merely suitable for antagonist design. The reason can be explained by the fact that rhodopsin is merely crystallized in its inactive state. Therefore, in order to design agonists of GPCRs, it is important to obtain information about the active states of the receptor.(Topiol, S. et al. 2009). There are some other limitations for using rhodopsin as template in homology modeling of GPCRs. One is that it has low homology (less than 25%) with family A GPCRs and no homology with other families of GPCRs such as secretin, adhesion and metabotropic receptors. The other limitation is the very complicated mechanism of activation in rhodopsin in comparison with the other GPCRs. The binding of the ligand to rhodopsin is covalent and signaling is conducted through activation of the ligand by photoisomerism. (Congreve, M. et al. 2011)

Another problem with using rhodopsin is that the binding domains are arranged clockwise in this receptor while sequentially oriented anticlockwise in case of others.(Claude Nofre 2001) Therefore, it is very difficult to obtain a reasonable overview for the activation of the GPCRs from rhodopsin antagonist binding.(Congreve, M. et al. 2011) Based on the findings it was claimed that rhodopsin might not be a suitable template for some GPCRs such as the cholecystokinin CCK1 receptor. (Kobilka, B. K. 2007) A revolution in the field of GPCRs has occurred after publication of the crystal structure of β1 and β2- adrenoceptor.(Congreve, M. et al. 2011) Afterwards, the crystal structures of adenosine A_2A (A_2AR), Chemokine CXCR4, dopamine D3 and histamine H1 in complex with antagonists have been reported which made a reasonable framework for the studies of GPCR functions and drug discovery. (Katritch, V. et al. 2011)

The β2AR is almost a good model for the studies of agonist binding since much information is obtained about the site of interaction between the receptor and catechol amine ligands. (Kobilka, B. K. 2007)

The crystal structures of ß1 and ß2 have been used for homology modeling of 5-HT_2C receptor. They showed similar homology rates of 41% and 62% with the regions of 5-HT_2C. (Renault, N., Gohier, A., Chavatte, P., & Farce, A. 2010)

Another successful example of homology modeling studies using β2-AR as template was in Alpha 2 adrenoreceptor (α_2ARs) receptors. These receptors with wide distributions are responsible for many activities such as the control of nervous system and cardiovascular systems. In this study, the resulted models have been minimized using the OPLS2005 force field implemented in schrodinger package. (Ostopovici-Halip, L. et al. 2011)

In many cases of homology modeling, the validity of the structures was verified by ramachandran plot. A common method for docking the homology based models is WHATIF algorithm which generates ramachandran plots to identify outliers in terms of torsion angles and also compares the quality of the model with reliable structures presented in the form of Z-scores. It is also possible to get consensus votes through WHATIF to select

between homologous structures. (Abu-Hammad, A., Zalloum, W. A., Zalloum, H., Abu-Sheikha, G., & Taha, M. O. 2009)

Another tool to assess the structural validity of the models is to use hydrophobic moments of the helices. By this method, it is possible to obtain the orientation of hydrophobic moment in transmembrane domains. (Panetta, R. et al. 2008)

5. Simulation studies of GPCRs

In modeling studies for keeping the receptors electrochemically sealed the interaction of the lipids and proteins are needed. (Escriba, P. V., Wedegaertner, P. B., Goni, F. M., & Vogler, O. 2007)

Different molecular dynamic (MD) simulation methods have been used to study GPCRs. While all atom MD simulations in lipid bilayer and water is used to study the dynamics of the membrane proteins, By using targeted MD simulation such as metadynamics, it was possible to study the process of activation in the receptors. In metadynamics a Gaussian term is added to the free energy which disallows the system from returning to previous state. A pitfall of this method is the bias used for forcing the system change its state. The requirement of this method is the primary knowledge needed about the active and inactive states of the receptor. By using this method it is possible to obtain the intermediates in the activation process of the receptor. (Vaidehi, N. et al. 2011)

Since the ligand induced conformational change in GPCRs happen in the range of microseconds, all atom MD simulations are not able to predict large scale simulations such as conformational change in GPCRs. (Vaidehi, N. et al. 2011)

Another simulation method is elastic network model (ENM) in which the protein is represented as a collection of beads connected by springs, where beads refer to protein residues and springs refer to connections. By using this method it is possible to study the micro second simulations. (Vaidehi, N. et al. 2011)

LITicon is a method in which the receptor conformations are permitted to have coarse grain degree of freedom to avoid the built in bias observed in targeted MD simulations. In this method the TMs are considered as rigid bodies connected to each other by flexible loops. The TM helices are rotated in a desired range of rotation angles and the side chain conformations are optimized for each backbone conformation using a rotamer library. Subsequently, the potential energy is minimized using all atom force field function. By this method it is possible to obtain an energy landscape for the GPCRs in the rotational span of the TM helices. After identifying the local minima in the landscape, the global minima state of energy landscape is chosen on the most stable state of the protein. In LITicon, the coarse grain simulation is used to forecast the ensemble of active and inactive states from the inactive crystal structure of the protein. A problem with coarse grain method is some significant barriers which might be missing during the activation pathway.

Monte Carlo (MC) simulations have been also used to calculate the pathway for the activation of some receptors. By MC simulation, it was possible to search the minimum energy from the inactive state towards the ligand stabilized states. An important note to be considered in the computational studies of GPCRs, is the role of water molecules in the

activation procedure which has been proposed by many researches to take role in conformational changes of GPCRs. It must be denoted that some water molecules in the crystal structure of the GPCRs might be either absent or not well resolved. It is known that multiscale methods with a combination of coarse grain and fine grain all atom methods are required for understanding the conformational changes of GPCRs. (Vaidehi, N. et al. 2011)

Although some studies have reported the usefulness of MD simulations in studying the changes during dimerization, it was normally difficult to study the GPCRs in dimer form by molecular dynamic simulation methods. (Simpson, L. M. et al. 2010) Recent developments in protein-protein docking made it possible to perform studies on dimer formation.

As an instance, the 5HT4 receptor was subjected to docking approach using GRAMM wherein the interface for dimerization was TM2.4- TM2.4. (Simpson, L. M. et al. 2010)

Docking simulation studies have also been taken to predict the binding mode in GPCRs and estimate the ligand- receptor affinities in case of many receptors such as cholecystokinin (CCK). (Henin, J. et al. 2006)

A further step in modeling based discovery of drugs for GPCRs is to identify potential binding sites in the receptors. The binding site for some GPCRs such as human sweet receptor (HSR) was modeled using ligand based approach. (Claude Nofre 2001)

A successful example for using computational methods was in melanin concentrating hormone (MCH1R). The 3D structure of melanin concentrating hormone which belongs to rhodopsin superfamily was predicted using homology based modeling studies. During the procedure the models were built by the web based model suit SWISS MODEL and scanned for the ligands binding site. The model was then subjected to docking studies of ligands with known activities. The result of the docking step was used for making comparative molecular force field analysis. The combinations of docking/scoring/COMFA were previously reported to be successful in predicting docked conformer/pose closed to that of cocrystalized ligand. In these types of studies, the validity of COMFA models can be verified using ligand based approaches. (Abu-Hammad, A., Zalloum, W. A., Zalloum, H., Abu-Sheikha, G., & Taha, M. O. 2009)

6. Conclusion

Different experimental and computational approaches proposed the role of molecular switches on structural and conformational changes of GPCRs.

By using experimental techniques such as site directed spin labeling, it was observed that in case of receptors such as rhodopsin, a well conserved salt bridge between TM3 and TM6 known as ionic lock is broken during activation. This cleavage leads to flexibility of TM 6 and its movement towards TM3.

Based on molecular modeling studies, it was suggested that in case of some receptors such as MCH, the binding site is a cleft inside the helical domain of the receptor including three hydrophobic regions and a hydrogen bonding polar region.(Abu-Hammad, A. et al. 2009) Other studies revealed that polar interactions of serines with agonists and the movement of TM5 in B_2AR pocket is resulted by shift of TM7 towards TM3 upon agonist binding. An

optimal confrontation for this was made based on virtual ligand screening of known ligands. (Katritch, V. et al. 2011)

Biochemical and mutagenesis of B2AR established two major interactions for full agonists in which the amine group forms a salt bridge in Aspll3 while the hydrogen groups of catechol interact with serine in TM5. Analysis of B2AR demonstrated that an inward shift (~2 A) of TM5 is needed for binding of full agonists. The same results have been observed in induced fit docking studies with flexible TM helices. The TM5 shift was caused by conformational freedom in this domain and strong H-binding between catechol OH and Ser 207. The modeling studies based on Adenosine A2A receptor was another example for modeling of agonists. In this case some interactions were revealed to be common for agonists and antagonists such as aromatic ring and amine core contacts. It was seen that adjustment of ligand in an optimal position and engagement of all polar interactions is needed for the shift of the conserved Trp 6.48. (Katritch, V. et al. 2011)

In case of M_3 receptor it was predicted that the binding of Ach to M_3 triggers conformational changes within the TM receptor core. Agonist binding causes the disruption of the existing interhelical interactions and promotes a set of interactions that leads to a new favorable conformational state for the receptor. (Wess, J. et al. 2008)

An important molecular switch is the ionic lock bottom highly conserved D/E motif found in all class A GPCRs. This ionic interaction holds together the cytoplasmic ends of TM3 and TM6 in many amine receptors. Another example for the role of ionic lock is in Angiotensin 1 receptors. The evidence shows that Asn111 interacts with Asn295 in TM7 to stabilize the inactive state of the receptor. (Ahuja, S. et al. 2009;Deupi, X. et al. 2007) In another study it was postulated that reduction of conserved disulfide bridge might be a molecular switch for the activation of the receptor. This study was based on molecular dynamic simulation and virtual screening of dopamine D_2 receptor. It was observed that a predictive model for the catechol binding cavity of D_2 had reduced disulfide bridge. The movement of TM6 towards TM5 was supposed to be the result of cleavage in the conserved disulfide bridge (Fig 4) (Sakhteman, A., Lahtela-Kakkonen, M., & Poso, A. 2011)

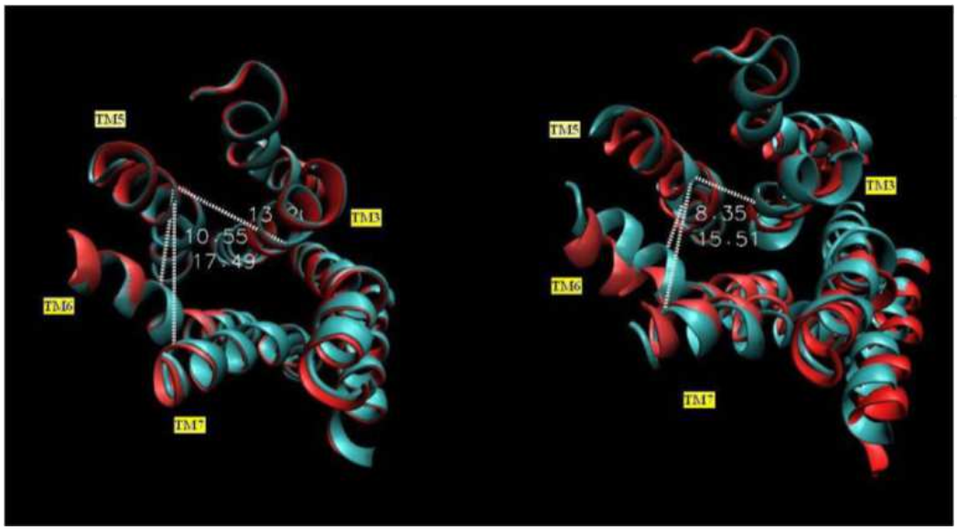

Fig. 4. Movement of TM6 towards TM5 in D_2 model with reduced disulfide bridge.

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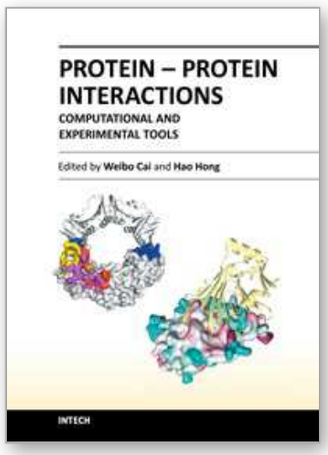

Protein-Protein Interactions - Computational and Experimental Tools

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Proteins are indispensable players in virtually all biological events. The functions of proteins are coordinated through intricate regulatory networks of transient protein-protein interactions (PPIs). To predict and/or study PPIs, a wide variety of techniques have been developed over the last several decades. Many in vitro and in vivo assays have been implemented to explore the mechanism of these ubiquitous interactions. However, despite significant advances in these experimental approaches, many limitations exist such as false-positives/false-negatives, difficulty in obtaining crystal structures of proteins, challenges in the detection of transient PPI, among others. To overcome these limitations, many computational approaches have been developed which are becoming increasingly widely used to facilitate the investigation of PPIs. This book has gathered an ensemble of experts in the field, in 22 chapters, which have been broadly categorized into Computational Approaches, Experimental Approaches, and Others.

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University Campus STeP Ri
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Phone: +385 (51) 770 447
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InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
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