

Docking of human rhodopsin mutant (Gly90→Asp) with beta-arrestin and cyanidin 3-rutinoside to cure night blindness

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Abstract:

Motivation: Rhodopsin is a visual pigment present in rod cells of retina. It belongs to GPCR family and involves photoisomerization of 11-cis-retinal to all-trans-retinal isomers, conformational changes in rhodopsin and signal transduction cascade to generate a nerve impulse. This signaling pathway has been targeted to eliminate the effect of a mutation (Gly90→Asp) responsible for abnormal activation of G-protein without retinal conformations in the absence of light leading to congenital night blindness. A theoretical model of rhodopsin with induced mutation has been deliberated in order to find potential ligands which can offset this mutational effect. The binding interactions between the target mutated rhodopsin model and potential ligands have been predicted with the help of molecular docking. The results indicated strong functional benefits of ligands as an inhibitor and an agonist for mutated rhodopsin model. Therefore, we propose a new visual cascade model which can initiate the normal signaling of rhodopsin mutant with the help of proposed ligands and can provide a hope for vision in future.

Keywords: Molecular Docking, Arrestin, Rutinoside, Agonist, Cyanidin, Congenital Night Blindness, Rhodopsin

Background:

Rhodopsin is a visual pigment belonging to G-coupled Protein Receptor family (GPCR). It has seven transmembrane α -helix structure containing six loops and present in rod cells of retina. It has two building blocks; one is opsin protein called scotopsin and second is a cofactor known as Retinal [1]. The role of GPCR as extracellular ligand-binding proteins makes them attractive targets for drug design. GPCRs account for approximately 40% of all therapeutic intervention, and major GPCR research projects are found throughout the pharmaceutical industry [2, 3]. Molecular study of the protein progresses in the field of "Docking" which refers to the potential binding of macromolecules with each other in a favorable manner. The binding of agonist with GPCR proteins cause conformational changes in its structure. If a receptor in an active state encounters a G-protein, it may activate it. The binding of G proteins to receptor affect the receptor's affinity for ligands. By ISSN 0973-2063 (online) 0973-8894 (print) Bioinformation 8(3): 128-133 (2012)

binding suitable agonists, protein functionality can be enhanced [4]. Signal messages start by ligand binding to a GPCR's extracellular region which triggers changes in the protein's transmembrane region. This causes the release of guanosine diphosphate (GDP) and the uptake of guanosine triphosphate (GTP) from the G-protein (transducin), stimulating the activation of signaling pathway.

A mutational effect in human rhodopsin has been studied for night blindness i.e. Gly-90→Asp mutation on the second transmembrane helix, places an extra negative charge in the opsin pocket, which could contribute to partial deprotonation of the retinal Schiff base and increase photoreceptor noise. In vitro experiments suggest that transducin is activated by the Gly-90→Asp mutation in the absence of both the retinal chromophore and light, which is termed as "constitutive activity" [5]. There is no treatment for this abnormality yet;

therefore, it requires attention and need to explore possible remedies by applying drug designing techniques like docking and for this purpose, potential drug targets are to be discovered. One of the important classes of known inhibitors for rhodopsin is arrestin. Arrestins are a family of protein that is important for regulating signal transduction within cells. The phosphorylated receptor can be linked to arrestin molecules that prevent it from binding and activating G-protein, effectively switching it off for a short period of time. This mechanism is used to study rhodopsin in retina cells to compensate for exposure to light [6]. A model of interaction for the human Arrestin-Rhodopsin complex has been obtained by a protein docking approach, reinforced by experimental data and binding energy calculation [7]. The beta-arrestin molecules are found to have inhibitory action on G-protein and are involved in signaling/desensitization process. Several other companies including 7TM, a GPCR-focused drug company, and Perkin-Elmer have developed GPCR screening technologies that take advantage of the arrestin-receptor binding [8].

Another important class of known agonists for rhodopsin is cyanidin compounds. Cyanidin belongs to the group of anthocyanins (C6-C3-C6 structure) found in most red coloured berries such as bilberry, blackberry, and raspberry. The Visual acuity can be improved through administration of anthocyanin pigments to animal and humans as well to enhance the night vision [9, 10]. Three anthocyanins from black currant stimulated regeneration of rhodopsin and formation of regeneration intermediate was accelerated by cyanidin 3-rutinoside [10, 11]. These studies strongly reveal the fact that enhancement of rhodopsin regeneration is a mechanism by which anthocyanins enhance visual acuity. It has been studied and observed that the effects of different cyanidin compounds, like cyanidin 3-glycosides, glucoside and rutinoside, stimulate the regeneration of rhodopsin. The formation of regeneration intermediate was suggested to be accelerated by cyanidin 3-rutinoside [11]. The binding affinity of this important organic molecule with rhodopsin is yet to be predicted and analyzed through docking approach.

Methodology:

Bioinformatics approach and tools have been used to carry out this research as mentioned in the flow chart in (Figure 1).

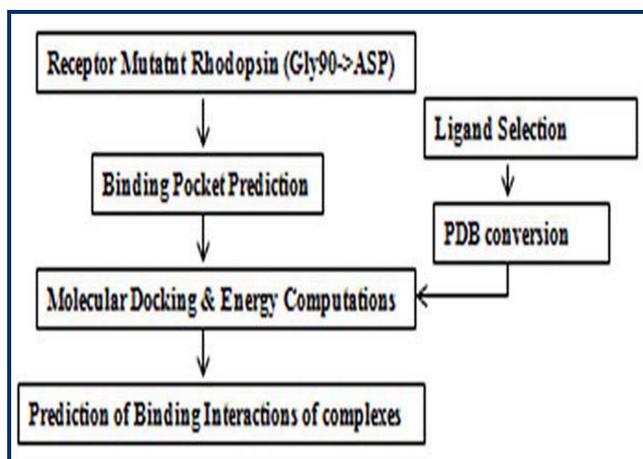


Figure 1: Flow chart of the methodology for molecular docking.

Target Mutant Receptor Macromolecule

A theoretical model of human rhodopsin protein has been obtained by homology modeling approach because it shows 93% similarity with bovine rhodopsin templates. This model has been refined and used further for docking as recommended [12]. A missense mutation; GLY-90 transformed ASP, is induced in the model with the help of Swiss PDB Viewer [13]. The structure of rhodopsin holds retinal molecule as its native ligand. Therefore, the theoretical model of rhodopsin along with its natural ligand was considered in order to conserve its conformational space during docking. The PDB structure of Cis-retinal was retrieved from online virtual chemistry laboratory [14].

Macromolecule Binding Pocket Prediction

The receptor macromolecule was first analyzed thoroughly for the possibility of existence of cavities and spaces which are necessary for the presence of native reactive groups and atoms involved in interactions with external ligands and facilitate their docking. For this purpose, the binding pockets of the mutant rhodopsin model were predicted by Pocket-Finder [15]. It searches and predicts top 10 potential binding sites for ligands and residues in close contact.

Ligand Structural Resources

Beta arrestin was chosen for its inhibitory property against rhodopsin signaling. The crystal structure of bovine beta-arrestin (PDB: 1G4R), comprising of 393 amino acids, at 1.9Å was taken from Protein Data Bank [16]. Cyanidin 3-rutinoside is another vital chemical ligand which is used for its agonistic property against rhodopsin. Its structure was sketched (Figure 2) in Accelrys Draw 4.0 [17] and converted to PDB format for docking by using UCSF Chimera [18].

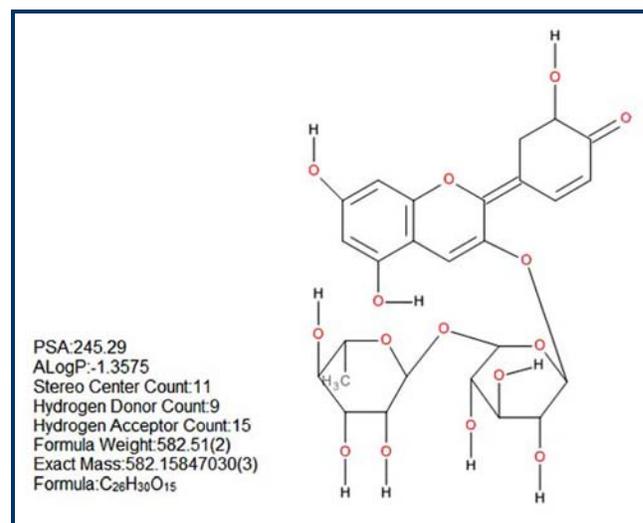


Figure 2: Structure of cyanidin 3-rutinoside sketched in Accelrys Draw 4.0.

Molecular Docking and Energy Computations

Molecular docking of the structures of receptor macromolecule and its potential ligands was done by Hex 4.5 [19] and AutoDockTools [20]. Hex 4.5 performs macromolecular docking by using Spherical Polar Fourier (SPF) correlations in order to calculate total energy values (Etotal) of the docked complexes. It

provides user friendly graphical environment to visualize the docked complexes and animated form of total poses performed during docking.

Prediction of Binding Interactions of Complexes

The binding interactions of the complexes were predicted by AutoDockTools [20]. Autodock 4.0 is considered to be one of the best known structure based rational drug design program which predicts protein-ligand interactions and protein-protein interactions. It operates two major tools during the execution of docking. One is AutoGrid that computes atomic affinity grid maps and other is AutoDock that generates docking complexes. Complexes were further analyzed in Python Molecular Viewer interface PMV 1.5.2 [20] in order to reveal the binding interactions i.e., hydrogen bonds, vander waals forces, hydrophobic interactions and energy calculations.

Results:

The mutant version (GLY90→ASP) of homology model of Human rhodopsin was thoroughly analyzed to find all possible binding pockets and close contact residues in the receptor's vicinity with the help of Pocket-Finder Server [15]. It predicted top ten binding sites in the structure of rhodopsin, out of which, two binding sites have been selected and highlighted with maximum cavity and large number of residues in receptor's vicinity (Figure 3). Site 1 has 541 Cubic Angstroms volume and contains 29 residues (104 atoms) in binding pocket. It is surrounded by close contact residues indicated by ball and stick representation in blue color (Figure, 3). Site 2 has 386 Cubic Angstroms volume and contains 23 residues (92 atoms) in binding pocket. It is surrounded by close contact residues indicated by ball and stick representation in purple color (Figure 3). Rests of the sites are indicated by gray mesh and occupy smaller volume. Therefore, the binding sites 1 and 2 were considered favorable for the docking of potential ligands.

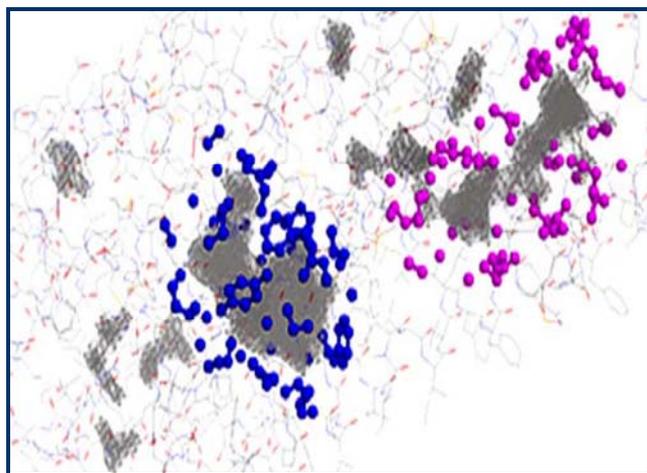


Figure 3: Structure of human rhodopsin protein with ten binding pockets (indicated in gray mesh) as predicted by Pocket-Finder [15] and visualized in RASMOL 2.7.3.1v [21].

The prediction of the binding sites facilitates molecular docking. The mutant rhodopsin was first docked with its native ligand "cis-retinal" so that its native binding pocket can be conserved and other candidate ligands can be docked in suitable orientation in the predicted binding sites. Cis-retinal was

docked in binding site 1 (29 residues in close contact) because it resides in the vicinity of Helix-VII which is the native binding pocket for retinal conformations. The binding interactions of the rhodopsin with cis-retinal indicate one hydrogen bond and presence of hydrophobic and vander waals interactions with rhodopsin (Figure 6) which is a clear evidence for its native affinity with it. This complex was further docked with beta-arrestin. The results of the interactions showed two hydrogen bonds, hydrophobic and vander waals interactions with receptor (Figure 7) which shows strong affinity between beta arrestin and rhodopsin being similar in nature. Later, rhodopsin was docked with cyanidin 3-rutinoside and showed hydrophobic and vander waals interactions with rhodopsin (Figure 8). The energy computations of the complexes as predicted by Hex and ADT have been recorded Table 1 (see supplementary material). It has been noticed from Hex results that beta-arrestin has least energy (E_{total}) than organic molecules because beta-arrestin is a protein molecule therefore; it has more binding affinity with rhodopsin due to similar protein nature. However, binding energy of cyanidin, as computed by Hex and ADT, is more or less identical to that of retinal. These results lead to successful hypothesis that these complexes can work for rhodopsin activation and regeneration to initiate the visual process normally.

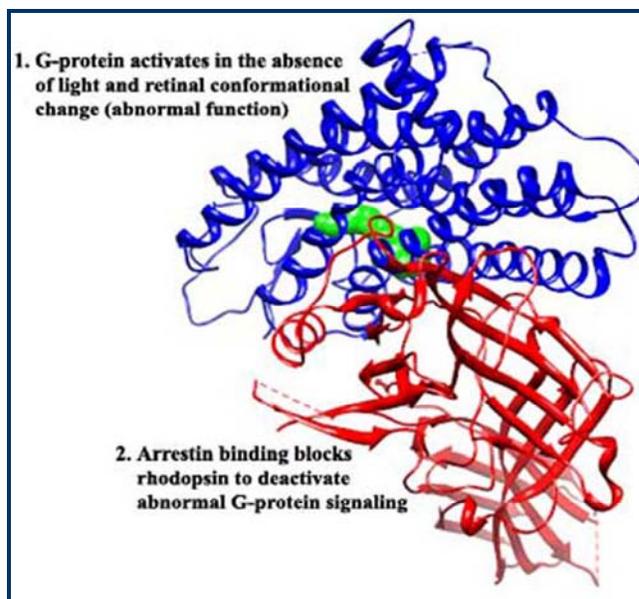


Figure 4: Docked model of mutant Rhodopsin (Blue) bound with Cis-retinal (green) and beta-arrestin (red). Image produced by using UCSF Chimera 1.5.3 [18].

Discussion:

GPCRs undergo multiple conformational changes upon agonist binding so new agonist exploration and development is in progress possibly through docking techniques [4]. In this study, a human rhodopsin mutation (Gly90→Asp) [5] was induced and modeled and docked with its potential ligands successively in order to propose a cure for the congenital night-blindness. The docking interactions of mutant rhodopsin model and beta-arrestin show close affinity with each other which conforms to previous studies conducted on the inhibitory effect of beta arrestin on rhodopsin [6, 8]. This is a clear evidence of this effective complex that arrestin can work as an inhibitor to

switch off the abnormal rhodopsin (**Figure 4**). However, the docking interactions of cyanidin 3-rutinoside with rhodopsin are also found effective and favorable to work as agonist for its regeneration and activation (**Figure 5**) which conforms to the previous experimental studies conducted on cyanidin compounds in order to improve night vision [10, 11]. Therefore, both of these ligands prove to have effective role in the improvement of night vision.

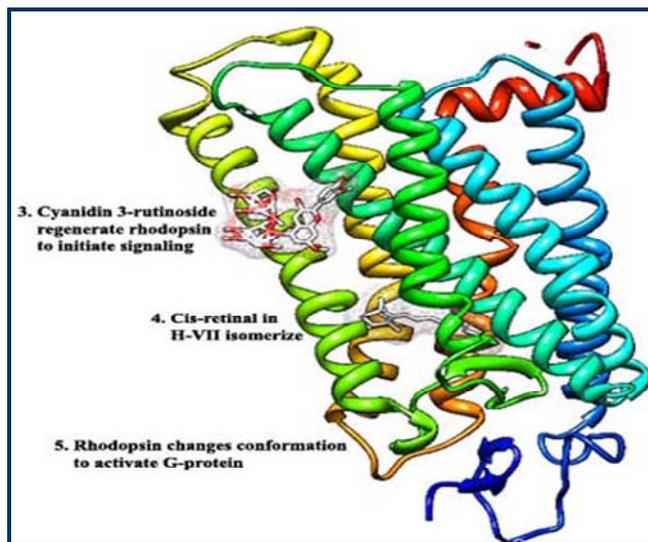


Figure 5: Docked model of mutant Rhodopsin bound with Cis-retinal (Sticks) and cyanidin 3-rutinoside (Sticks). Image produced by using UCSF Chimera 1.5.3 [18].

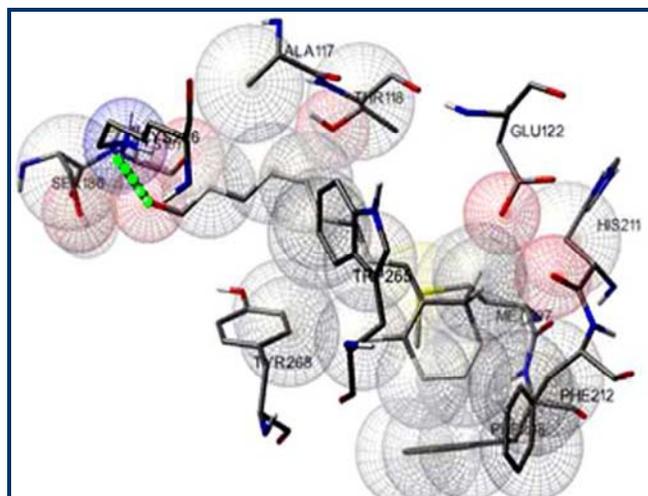


Figure 6: Detailed view of binding interactions between the docked complexes of mutant rhodopsin model with cis-retinal, visualized in PMV 1.5.2 [20]. Hydrogen bond between LYS296:HZ1 of rhodopsin with 1: O of Cis-retinal is indicated in green and hydrophobic and Vander Waals interactions indicated in red and gray mesh circles among the receptor protein residues in close contact i.e., SER186, LYS296, ALA117, THR118, GLU122, HIS211, TYR265, TRP 268, MET107, PHE218 and PHE212.

Depending on the structural and functional evidences of beta arrestin and cyanidin 3-rutinoside, the malfunctioning in the ISSN 0973-2063 (online) 0973-8894 (print) Bioinformation 8(3):128-133 (2012)

rhodopsin visual cascade can be mended. The proposed and corrected signaling pathway is described with the help of figures. In **Figure 4**, beta-arrestin molecule binds to the mutated rhodopsin and deactivates G-protein by blocking rhodopsin receptor site. Arrestin will work for a short period of time and deattaches after deactivating G-protein. In **Figure 5**, cyanidin will bind to rhodopsin just near to cis-retinal in Helix-VII and help in rhodopsin regeneration in order to enhance signaling. In this way retinal will isomerize due to which rhodopsin can change conformation to carry out signal transduction cascade normally.

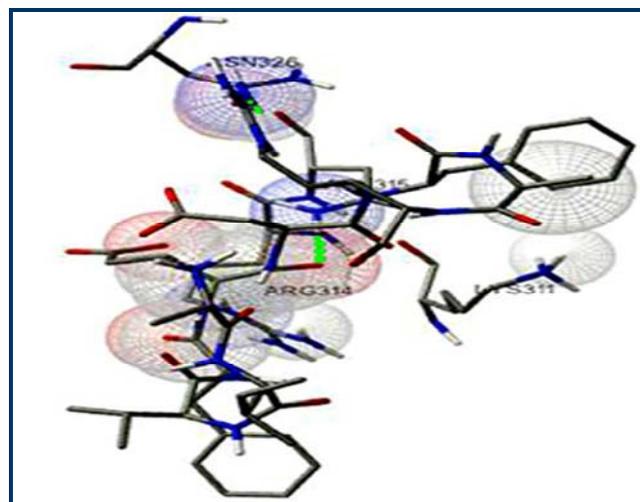


Figure 7: Detailed view of binding interactions between the docked complexes of mutant rhodopsin model with beta -arrestin, visualized in PMV 1.5.2 [20]. Two hydrogen bonds are found between ASN315:HD22 of rhodopsin with ARG393:HH22 of beta-arrestin and ASN326:OD1 of rhodopsin with GLU389:O of beta-arrestin as indicated in green while hydrophobic and Vander Waals interactions indicated in red and gray mesh circles among the receptor protein residues in close contact i.e., ASN315, ARG314, LYS311 and ASN326.

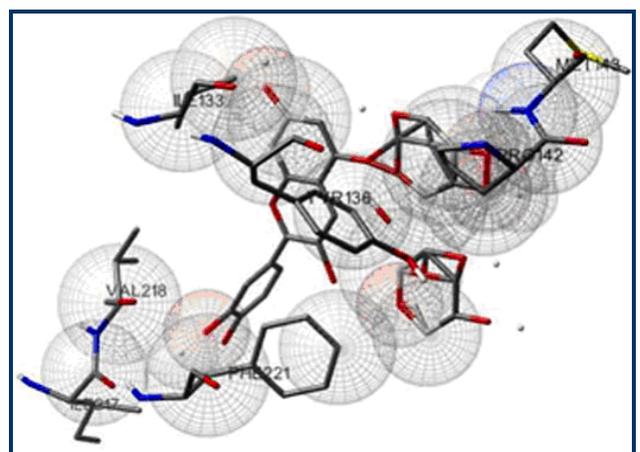


Figure 8: Detailed view of binding interactions between the docked complexes of mutant rhodopsin model with cyanidin 3-rutinoside, visualized in PMV 1.5.2 [20]. Hydrophobic and Vander Waals interactions are indicated in red and gray mesh circles among the receptor protein residues in close contact i.e.,

ILE133, MET143, PRO142, TYR 136, VAL218, PHE221 and ILE217.

Conclusion:

In this research work, the malfunctioning of visual transduction cascade of rhodopsin as a result of a missense mutation has been studied. The rhodopsin mutant model was docked with two potential ligands. One is beta arrestin molecules which bind to rhodopsin to offset the effect of abnormal rhodopsin signaling without light and retinal isomerization. After that, subsequent docking of cyanidin 3-rutinoside with mutant rhodopsin initiates its activation and regeneration to trigger normal visual transduction cascade. This research work will help in the structural studies of agonist activation and protein inhibition and future drug discovery process including prediction of ADME/T (Administration, Distribution, Metabolism, and Elimination/Toxicity) of these compounds with the help of commercial ADME/T tools and its in vitro testing in the molecular laboratories to measure their effects on model organisms and drug development for congenital night blindness.

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Supplementary material:

Table 1: Energy calculations of docking complexes of mutant rhodopsin model with Cis retinal, beta-arrestin and cyaniding 3-rutinoside.

Docking Complex	Hex				ADT							
	ETV	ESV	EFV	VSV	BE	LE	IE	VDW	EE	TE	ToE	Hb
mRHO+ Cis Retinal	-140.21	-97.05	-43.16	118.31	-6.95	-0.37	-8.6	-8.46	-0.14	0.0	1.65	1
mRHO+ Arrestin	-535.61	-624.38	88.77	521.44	-8	370.58	4.94	-3.47	-2.71	-8.19	382.24	2
mRHO+ Cyanidin 3-rutinoside	-99.60	-75.17	-24.42	103.65	-8.54	-0.21	-9.09	-8.49	-0.15	0.02	0.55	No

*mRHO= Mutant Rhodopsin, *ETV = Etotal Value, *ESV= EShape Value, *EFV= Eforce Value, *VSV= Vshape Value, *BE= Binding Energy, *LE= Ligand Efficiency, *IE= Intermolecular Energy, *VDW= Vander waals and Hbond desolvation energy, *EE= Electrostatic Energy, *TE= Total Energy, *ToE=Torsion Energy, *Hb= Hydrogen Bonds