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Homology modeling of apoprotein Opsin and covalent docking of 11-cis retinal and 11-cis 3, 4-didehyroretinal to obtain structures of Rhodopsin and Porphyropsin from Zebra danio, *Danio rerio* (Hamilton, 1822)

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Abstract

Opsin proteins are classically seven transmembrane receptor proteins which detect light. The present investigation describes the stereoscopic structure of the apoprotein Opsin by online structure determining tools using the crystallographic structure of Rhodopsin from *Bos taurus* as the template. The modeled structure was validated and checked through validated data tools for stereochemical quality of a protein structure, ProSa and the square root of the mean square deviation between the templates and the predicted structure was calculated using PyMol. It was found that the chromophores, 11-cis retinal and 11-cis 3, 4-didehydroretinal was covalently docked with apoprotein which gives the structures of Rhodopsin and Porphyropsin respectively. Due to its close homology to the human rhodopsin (accession P08100), zebra fish rhodopsin may serve as an excellent model for study of human diseases in future. This article represents the test of structure/function relationship of apoprotein opsin in this ornamental aquarium fish and provides a foundation for future work exploring cellular and molecular pathways of photoreception in retinal development and disease models.

Keywords: Zebra fish, Opsin, Rhodopsin, Porphyropsin, 11-cis retinal, 11-cis 3,4-didehyroretinal

1. Introduction

Studies have shown the use of visual pigments by representatives of almost all the vertebrates, where visual pigments are Rhodopsin and Porphyropsin. Both rod visual pigments contain the same apoprotein Opsin; however, they are covalently linked to different groups. Rhodopsin binds 11-cis-retinal, whereas Porphyropsin is linked to 11-cis 3, 4-didehydroretinal (La Franco *et al*., 2018; Ganong 2005). These pigments possess various spectral characteristics like maximum absorbance and absorbency index (Marschall, *et al.*, 2012). The absorption maxima of Rhodopsin are nearly 500 nm, whereas Porphyropsin is in the range of 520 – 535 nm. It has been found that the visual pigments may vary in response to light, temperature, and other environmental stimuli (Korenyak and Govardovskii, 2013).

Studies suggest that certain fish retinas comprise a visual purple pigment rather than the contemporary Rhodopsin of red - color which was confirmed spectrophotometrically (Enright *et al.*, 2015). Similar studies suggested that Porphyropsin a purple rod-pigment, is a characteristic of freshwater fishes, whereas the red rod visual pigment, Rhodopsin is characteristic of terrestrial craniate and most of the Sea fishes. It has also been found that many freshwater fishes have Porphyropsin and/or admixtures of Rhodopsin and Porphyrins (Corush., 2019; Toyama *et al.*, 2008, Ochuko et. al., 2014).

The organism *Danio rerio* was selected for the current investigation as its visual system shares high similarities with other vertebrates (Gestri *et al*., 2012; Golsmith and Harris 2003). Preliminary studies on *D. rerio* suggested that it possesses only Rhodopsin as visual pigment (Morrow and Chang 2015, Cameron 2002; Chinen *et.al.*, 2003), whereas in a later study it has been confirmed that they have a paired visual system involving both Rhodopsin and Porphyropsin (Allison *et.al.*, 2004).

In the present scenario, computational method such as homology modeling has been used to bridge the gap between sequence information and structures. The current investigation represents 3D models of Rhodopsin and Porphyropsin by homology modeling and docking methods for *D. rerio* which can be used to study the biochemical mechanism underlying the working of both the visual pigments.

2. Materials and Methods

2.1. Protein sequence analysis

Protein sequence analysis including, physicochemical parameters, molecular weight, sum of cationic and anionic residues, theoretical isoelectric point (pI), absorbency index (Porterfield and Zlotnick, 2010), aliphatic index

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(Sahay and Shakya, 2010), Grand Average hydropathy (GRAVY) (Wanyonyi *et.al.,* 2011), half-life (Sharma *et.al.,* 2014) and instability index (Gamage *et.al.,* 2019) was calculated using the Expasy's ProtParam prediction server (Gasteiger *et al.,* 2003).

2.2. Prediction of the three-dimensional structure

The protein sequence of the apoprotein from the organism *D. rerio* was obtained from the UniProt database (accession number: P35359). The sequence was analyzed using the G protein coupled receptor- Sequence-structure-feature-extractor (GPCR –SSFE) database (Worth *et. al.,* 2011). Further, a protein BLAST was also carried out (Altschul *et.al.,* 1990). After obtaining the template, homology modeling was carried out using MODELLER 9v8 software. It makes use of a command interpreter for designing stereochemical structures of proteins along with their confounders by consummation of spatial restraints (Sali and Blundell 1993). The reliability of the model was analyzed by three (3) ways: (i) PROCHECK (Laskowski *et.al.,* 1993), (ii) by calculation of the RMSD (root mean square deviation) between the template and the predicted structure using PyMol (version 1.8, Schrödinger, Inc, USA), and (iii) using ProSa (Wiederstein and Sippl 2007).

2.3. Covalent docking

The receptor and the ligand structures were prepared with Glide and Prime. The Protein Preparation Wizard was used for preparing the receptor, and LigPrep was used for preparing the ligands (Reddy and Vanga 2017). A maximum of 10 poses per ligand are established and are subject to minimization of post-docking. The apoprotein Opsin is covalently bonded to 11-cis-retinal and 11-cis 3, 4-didehydroretinal to form structures of Rhodopsin and Porphyropsin respectively. Covalent docking to the nitrogen of Lysine 296 was performed using the Prime module of Schrodinger. The covalent docking facility allows the selection of the attachment point to the receptor and the possible attachment point on the ligand and then runs a prime loop prediction to find all feasible poses for the ligand.

3. Results and Discussion

In the current study, the sequence of Opsin from *D. rerio* has been repossessed from the UniProt database. The predicted physiochemical characteristic of Opsin showed that the protein has a molecular weight of 39.7064 kDa. Opsin has 20 (Arg + Lys) amino acid composition of positively charged and 22 (Asp + Glu) residues that are negatively charged. This also correlates with more number of negatively charged residues present in Opsin. The coefficient extinction of Opsin at 280 nm is 69175 M^{-1} cm^{-1} in lieu of the concentration of amino acids Phenylalanine, Trptophan and Tyrosine. It has been well established that for a protein is stable if the measured instability index is smaller than 40 and corresponding value above 40 foresees that the protein may be virtually unstable (Sahay and Shakya, 2010). The instability index of Opsin was found to be 46.09, which indicate that the protein is unstable in vitro. The aliphatic index for the protein sequence was found to be 85.90, which indicated thermo stability (Ashokan *et. al.,* 2010). The GRAVY value computed for carotenoid binding protein was 0.457. Molecular orientation using Ramachandran plot showed the modelled structure ha most of the residues (88.6%) in the fully allowed region, 11.1% in the additionally allowed region, 0.3% in the generously allowed region and nothing in the prohibited region.

3.1. Assessment of the three-dimensional structure

The sequence of the apoprotein from *D. rerio* was analyzed by the GPCR – SSFE database. Current result suggests the structure of Rhodopsin from organism *Bos taurus* (Protein data bank, PDB ID -1U19) as the template for the query sequence. The reasons for the selection of the template are presented in table 1.

Table 1. Reasons for the selection of the template

Helix	Template	Sequence Similarity	Reason for template choice
TMH1	1U19	89.7	Has Pro in same position
TMH2	1U19	100.0	Has Gly-Gly motif in same position
TMH3	1U19	91.4	No insertion or 2nd disulphide bridge and the highest sequence similarity to suggested template
TMH4	1U19	92.0	No second disulphide bridge between TMH3 and ECL2, no insertion and highest sequence similarity to suggested template
TMH5	1U19	96.3	No intra-ECL2 disulphide bridge, no disulphide bridge between ECL1-ECL2, no sequence similarity to the TMH5 extension of sRHO
TMH6	1U19	100.0	No disulphide bridge between TMH6 and ECL3, no sequence similarity to the TMH6 helix extension of sRHO and highest sequence similarity to suggested template
TMH7	1U19	95.8	Has insertion and highest sequence similarity to suggested template
Helix 8	1U19	100.0	Highest sequence similarity to suggested template

Further the sequence of amino acid for the apoprotein was also compared with the known structured sequence using PDB BLAST (Altschul *et.al.,* 1990). The result signifies that the co-crystallized structure of Rhodopsin from organism *Bos taurus* (PDB ID-1U19) had the best sequence identity (83%) and had no gaps. After many rounds of loop refinement, an optimum structure was obtained (Fig 1). The arrangement of the phi and psi angles for the amino acid residues was presented in Fig. 2 A (Table 2). The percentage of phi and psi angles in the favorably allowed region was 88.6% and none of the residues was located in the prohibited region. The considered mean square root deviation among the intended model and template structure was found to be 0.213Å (Fig. 2 D). The Z-scores of the model and the template were -3.5 and -4.11 validated the quality of the model. Comparative study of the model and the guided structure shows similar profiles, as seen in Fig. 2B, C.

Table 2 . Ramachandran plot statistics for the 3D model of rhodopsin, calculated using PROCHECK

Residues in most favoured regions	88.6%
Residues in additional allowed regions	11.1%
Residues in generously allowed regions	0.3%
Residues in disallowed regions	0.0%

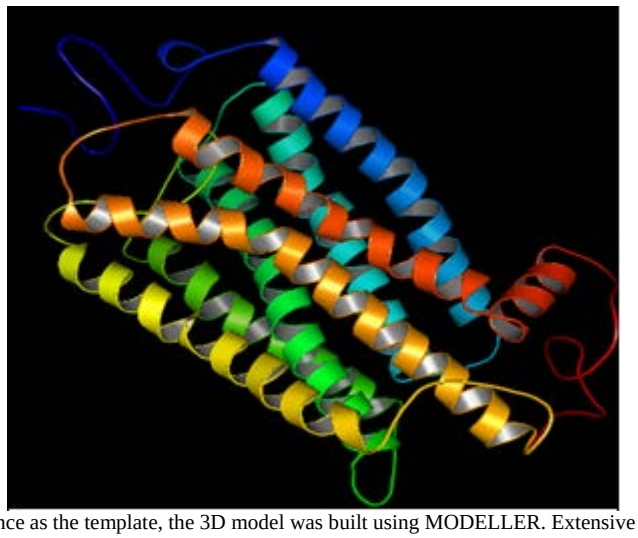

Figure 1. Using *Bos taurus* sequence as the template, the 3D model was built using MODELLER. Extensive loop refinements were carried out as it was found through the Ramachandran plot that residues in the loops were present in the disallowed region. Thus, after many rounds of loop refinement an optimum structure was obtained. In the image, blue represents the template and orange represents the structure of rhodopsin.

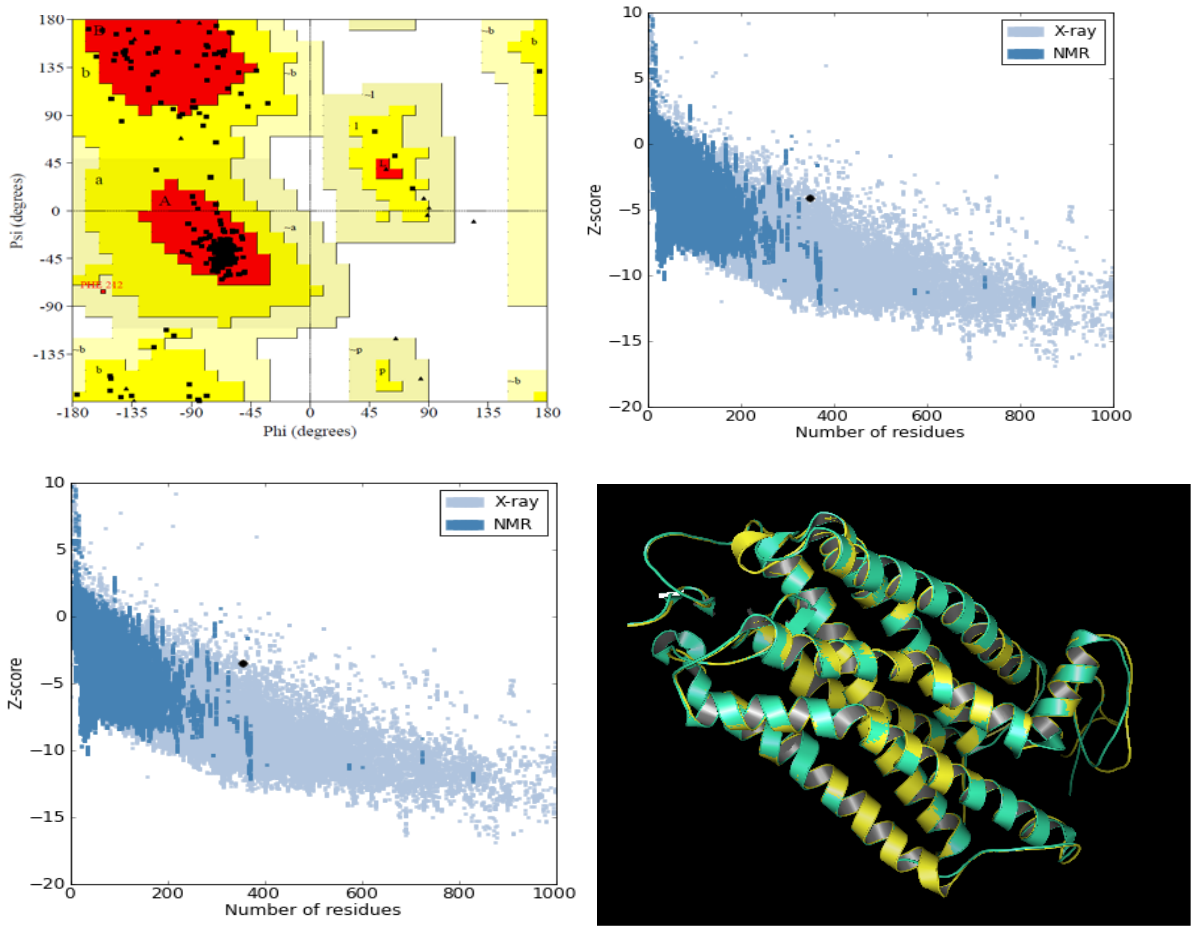

Plate 2. A. Ramachandran plot for the modeled structure of apoprotein from organism *D. rerio*. Red color represents most favored region. Additional allowed, generously allowed and disallowed regions are represented by yellow, light yellow and white colors respectively.
B. and C: The plot contains the Z-scores of all experimentally determined protein chains in the current PDB that have been solved by either X-ray diffraction or NMR. The plot is used to check whether the Z-score of the 3D structure is within the range of scores typically found for native proteins of a similar size. The Z-score of −4.11 and -3.5 (fig: 2 B and 2 C respectively) represents the overall quality of the template structure and the target respectively.

3.2. Covalent docking

Covalent docking was performed using the prime Schrodinger. Apoprotein opsin is covalently attached to the chomatophore 11 cis retinal to obtain the structure of rhodhopsin and in porphyrosin, 11 cis 3,4, di-dehydroretinol is the chromatophore that is attached to the apoprotein. In Rhodopsin and Porphyropsin, the chromophores are covalently attached to opsin through the nitrogen of Lysine 296 (Fig 3 A and Fig 3 B). On comparing the residues that are involved in the binding of

11-cis-retinal and 11-cis 3, 4-didehydroretinal, residues like Glutamic acid 113, Alanine 117, Threonine 118, Glycine 121, Glutamic acid 122, Serine 186, Cysteine 187, Tyrosine 191, Methionine 207, Histidine 211, Phenylalanine 212, Phenylalanine 261, Trptophan 265, Tyrosine 268, Alanine 269, Alanine 292, Lysine 296 were found to be common (Fig. 3 C, D). Out of all these residues, lysine 296 is covalently attached and rests are encompassed in hydrophobic interactions with both the chromophores. The alignment of the template and the covalently docked structures (Rhodopsin and Porphyropsin) were checked and confirmed. It was found that the chromophores were bound in a similar fashion as that in the template (Fig 3 E, F). The considered RMSD between Rhodopsin and the template was found to be 0.299 Å and the root mean square deviation between Porphyropsin and the model template was 0.302 Å. The backbone atoms were considered for these calculations. These results indicate great similarity of the structure with that of the template and can be used for further analysis. It is also important to mention that zebra fish rhodopsin shares ~80.4% homology to the human rhodopsin (accession P08100). Therefore, it may serve as an excellent model for the study of human diseases.

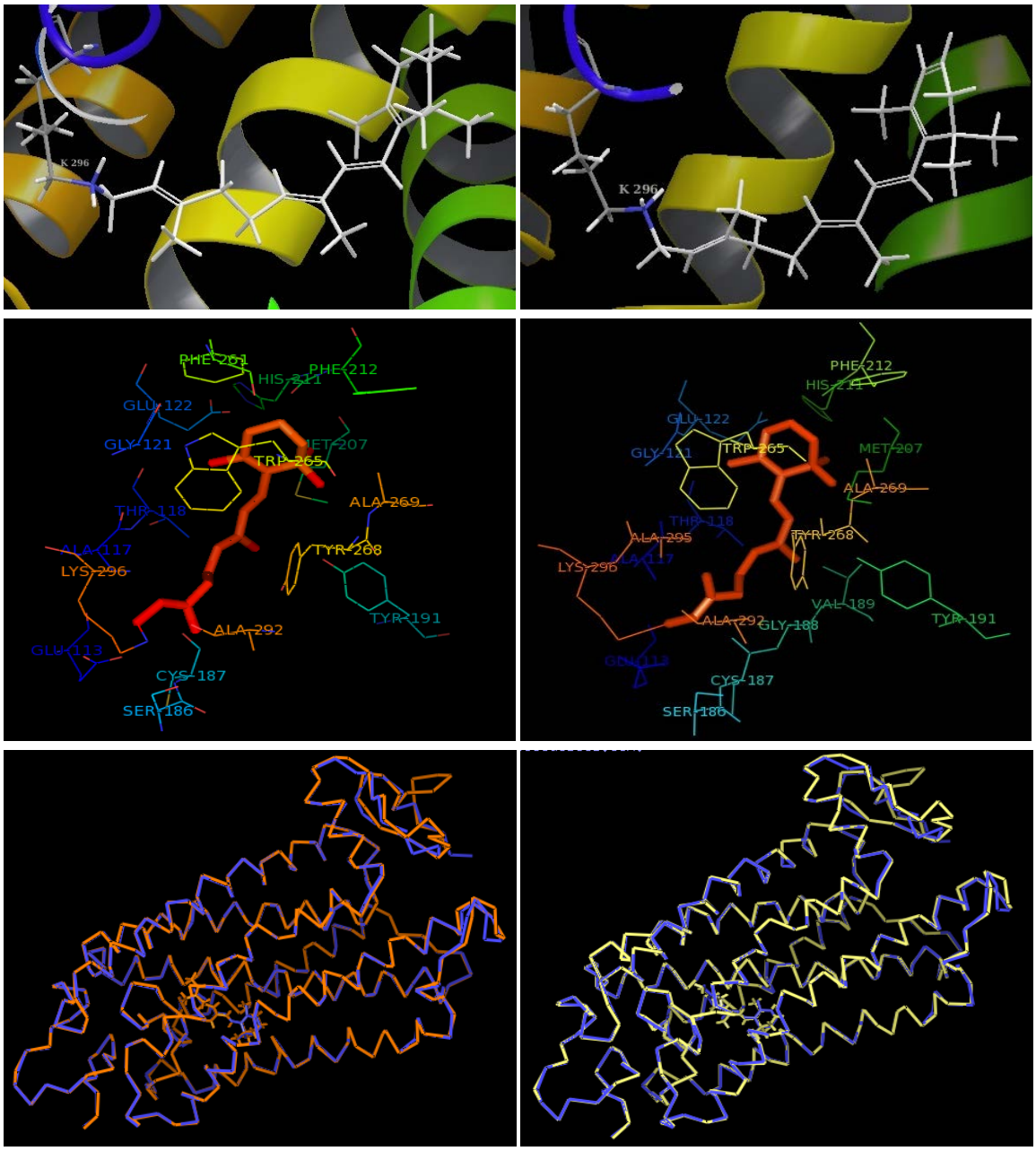

Figure 3. A, B Covalent binding of the chromophore 11-cis-retinal and 11-cis 3, 4-didehydroretinal to apoprotein Opsin through nitrogen of lysine 296 to obtain structures of rhodopsin and porphyropsin respectively. **C and D** show the amino acids that are involved in the binding of the chromophores, 11-cis-retinal and 11-cis 3, 4-didehydroretinal in rhodopsin and porphyropsin respectively. **E** The image of superimposed template and rhodopsin generated using PyMol. In the image, blue represents the template and orange represents the structure of rhodopsin. It can be observed that the 11-cis-retinal aligns in a similar fashion as in the template. **F** The image of superimposed template and porphyropsin generated using PyMol. In the image, blue represents the template and yellow represents the structure of porphyropsin. It can be observed that the 11-cis 3, 4-didehydroretinal aligns in a similar fashion as that of retinal in the template.

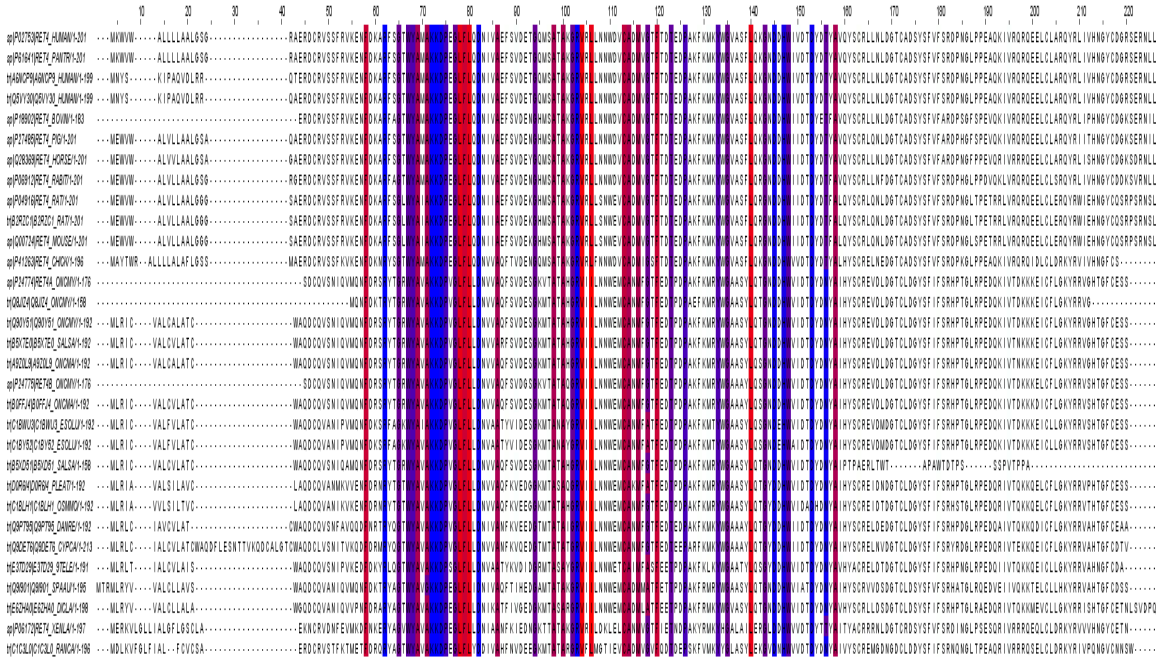

Figure 4. Sequence alignment of retinol binding proteins belonging to Lipocalin family. The residues showing 100% conservation are highlighted.

4. Conclusion

The physiochemical characteristics provided in depth perception about the nature of the apoprotein. Validation and evaluation of 3-D structure of the apoprotein show that the projected model is reliable and is reasonable at the present level of theory. Covalent docking of chromophores 11-cis-retinal and 11-cis 3, 4-didehydroretinal to lysine 296 resulted in the structures of Rhodopsin and Porphyropsin respectively. The predicted 3D structures and the residues involved in the binding of ligand can be used for guiding structural site-directed mutagenesis investigation. The structures may be used further to predict the key residues in the active site of the enzyme. Further, it can be used in understanding the structure – function relationships, and subsequently gain insight into its catalytic mechanism.

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