# Homology Modeling and in-silico Characterization of Interphotoreceptor Retinoid Binding Protein from Peromyscus Keeni

**Anoushka Kotra[1], Akanksha Suman[2] and Neetu Jabalia[3]***

*[1,2,3]Amity Institute of Biotechnology Amity University Uttar Pradesh, Sec-125, Noida*
*E-mail: [1]nushibushi20@gmail.com, [2]akankshas206@gmail.com, [3]njabalia@amity.edu*

**Abstract—***The interphotoreceptor retinoid binding protein (IRBP) has a major role to play in the visual cycle. It is involved in the transportation of retinoid in the cone photoreceptors. Hence, it can be said that they function well in the cone specific area of the visual cycle. With the use of bioinformatics approach, we aim to determine various characteristics and structural information of IRBP from* Peromyscus keeni. *The protein sequences were retrieved from the NCBI in FASTA format. Using these sequences, physico-chemical properties were identified. Motif scan and Protscale were used for determining the functions and hydropathacity of the sequence respectively. Other studies include the secondary structure prediction which reveals about the helix, sheet and coil probability of the protein. 3-D model was generated using homology modeling approach. These analyses reveal the necessary information related to the structure of IRBP which may further be used for academic or other research purpose.*

**Keywords***: interphotoreceptor retinoid binding protein,* Peromyscus keeni, *hydropathicity*

## 1. INTRODUCTION

The vertebrates carry the retinoid and cone receptors that are responsible for the secretion of retinoid and a glycoprotein which is binded by the fatty acid. This is the interphotoreceptor binding protein(IRBP). It plays a vital role in the rhodopsin regeneration cycle.[1] The rod and cone photoreceptor of the cell comprises of an opsin pigment. This opsin pigment constitutes the retinaldehydechromophore that functions in the absorption of photon during light perception process. The RPE (Retinal Pigment Epithelium) are the cells that involve a multi enzyme pathway known as the visual cycle that is capable of restoration of light sensitivity due to chemical reisomerization of retinaldehyde. The absence of IRBP may lead to a delay in the process of transfer of some recently formed chromophore from RPE to photoreceptors.[2] The objective of our study is to characterize the protein sequence of *Peromyscus keeni* and analyze it structural information.

## 2. MATERIAL AND METHODS

**Sequence analysis-**Interophotoreceptor retinoid-binding protein sequence was retrieved from the database NCBI. The retrieved sequence was in FASTA format and used for further analysis[3].

**Functional characterization-** Motifs in the protein sequence was scanned using MotifScan[4]. For sequence hydropathicity and presence of transmembrane[5], ProtScale[6]was used.

**Physico-chemical characterization-** For physico-chemical characterization, isoelectric point(pI), molecular weight, total number of positive and negative residues, extinction coefficient[7], instability index[8], aliphatic index[9] and grand average hydropathicity were computed using ExpasyProtParam server[10].

**Secondary structure prediction-** The analysis of the secondary structure of protein sequence was based only on knowledge of their primary structure. The secondary structure feature of the protein sequence from *Peromyscuskeeni* was identified by GOR IV.

**Tertiary structure prediction-**In order to generate three dimensional model, homology modelling approach was applied. The modeling of 3D structure of the sequences was executed by Swiss-Modellerprogram[11].

**Sturcture Validation-** Followed by 3-D model the structure was further validated by Rampage. The energy of the structure was predicted by Swiss PDB viewer.

## 3. RESULT AND DISCUSSIONS

The amino acid sequence of interophotoreceptor retinoid-binding protein was retrieved from ProtParam database[10]. Amino acid composition in the interophotoreceptor retinoid-binding protein sequence from *Peromyscus keeni* is displayed(Table1).

**Table 1: Amino acid composition of Interphotoreceptor binding protein from *Peromyscus keeni***

| Name of amino acid | No. of amino acid | %Composition |
|---|---|---|
| Ala | 9 | 6.4 |
| Arg | 9 | 6.4 |
| Asn | 3 | 2.1 |
| Asp | 6 | 4.3 |
| Cys | 1 | 0.7 |
| Gln | 5 | 3.6 |
| Glu | 8 | 5.7 |
| Gly | 17 | 12.1 |
| His | 2 | 1.4 |
| Ile | 8 | 5.7 |
| Leu | 16 | 11.4 |
| Lys | 5 | 3.6 |
| Met | 2 | 1.4 |
| Phe | 2 | 1.4 |
| Pro | 7 | 5.0 |
| Ser | 8 | 5.7 |
| Thr | 13 | 9.3 |
| Trp | 2 | 1.4 |
| Tyr | 4 | 2.9 |
| Val | 13 | 9.3 |
| Pyl | 0 | 0.0 |
| Sec | 0 | 0.0 |
| (B) | 0 | 0.0 |
| (Z) | 0 | 0.0 |
| (X) | 0 | 0.0 |

A set of conserved amino acid residues located in the vicinity that provide clues to the functions is termed as motif. Motif was predicted using MotifScan(Table2).

**Table 2: Motifs predicted from the protein sequence of *Peromyscus keeni***

| Accession no. | Motif | Description | Start | End |
|---|---|---|---|---|
| AHL47335 | ASN_GLYCOSYLATION | N-glycosylation site | 22 | 25 |
| AHL47335 | CK2_PHOSPHO_SITE | Casein kinase II phosphorylation site | 15 | 18 |
| | | | 23 | 26 |
| | | | 124 | 127 |
| AHL47335 | MYRISTYL | N-myristoylation site | 108 | 113 |
| | | | 117 | 122 |
| AHL47335 | PKC_PHOSPHO_SITE | Protein kinase C phosphorylation site | 49 | 31 |

Isoelectric point (pI) is the pH at which the surface of protein is covered with charge but net charge of protein is 0. At pI proteins are stable and compact. The value of isoelectric point(pI) of the protein from *Peromyscuskeeni*was 6.94 which indicates that it is slightly neutral. The computed isoelectric point will be useful for developing buffer system when the enzyme is to be purified in solution by isoelectric focusing method. The molecular weight came out to be 15035.2. The Expasy'sProtparam[10] was used to determine the extinction coefficient of protein. Extinction coefficient of protein at 280nm is $16960M^{-1}cm^{-1}$, assuming all pairs of Cys residues form cystines and extinction coefficient even comes out to be same when all pairs of Cys residues are reduced. The computed protease concentrations and extinction coefficient will be useful in the quantitative study of protein-protein and protein-ligand interactions in solution.

The estimated half life of the protease is 20hours in mammalian reticulocytes, in vitro; 30mins in yeast, in vivo; >10hours in *E.coli,* in vivo. The instability index (I)[8] provides an estimate of the stability of protein in a test tube. A protein whose instability index is smaller than 40 is predicted as stable while the value above 40 predicts that the protein may be unstable. The instability index (I)[8] is computed to be 40.06. This classifies the protein as unstable. The total number of negatively charged residues(Asp+Glu) is 14 and the total number of positively charged residues(Arg+Lys) is also 14.

The aliphatic index(AI)[9], defined as the relative volume of a protein occupied by aliphatic side chains,is regarded as a positive factor for the increase of thermal stability of globular proteins. Aliphatic index for the protein from *Peromyscus keeni* was 100.21(Table3).

**Table 3: Physico chemical properties of Interphotoreceptor binding protein from *Peromyscus keeni***

| Properties | AHL47335 |
|---|---|
| No. of amino acids | 140 |
| Molecular weight | 15035.2 |
| pI | 6.94 |
| Positively charged residues | 14 |
| Negatively charged residues | |
| Extension coeffecients | 14 |
| Instability Index | |
| Aliphatic Index | 16960 |
| GRAVY | 40.06 |
| | 100.21 |
| | -0.031 |

The Grand Average hydropathy(GRAVY) value for a protein is calculated as the sum of hydropathy values of all the amino acids, divided by the total number of residues present in the interophotoreceptor retinoid-binding protein sequence. GRAVY index for the sequence -0.031. This low range of value indicated better interaction with water.

Secondary structural feature was predicted by GORIV. The conformational entropy associated with random coil significantly contributes to stabilization and protein folding. The random coiled structures were dominant followed by the alpha helix and extended parallel or anti parallel strands of beta sheets(Fig. 1).

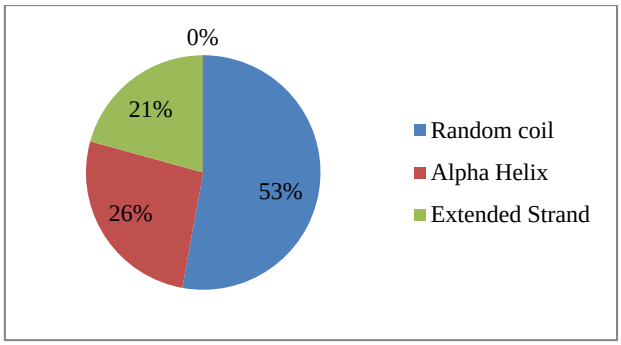


**Fig. 1: Graphical Representation of Secondary Structure composition of Interphotoreceptor binding protein**

After selection of potential template for the enzymes, 3D models were generated by the use of Swiss-modeler program(Table4)[11].

**Table 4: Tertiary structure prediction of Interphotoreceptor Binding Protein from *Peromyscus keeni***

| **Accession no.** | **AHL47335** |
|---|---|
| Modeled residue range | 1-140 Amino Acids |
| Template | 4lur.1.A |
| Sequence identity | 44.20% |
| Total energy | -5425.536 KJ/Mol |

Fig. 3 showed the 3-D modelled structure observed by using Swissmodellar.

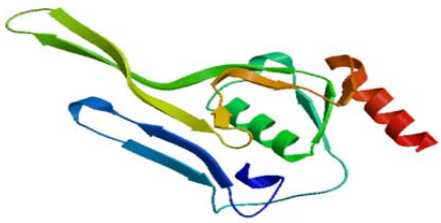

**Fig. 3: The ribbon representation of Interphotoreceptor retinoid bining protein in *Peromyscus keeni***

Energy of the 3-D model was minimized by Swiss PDB Viewer shown in Fig. 4.

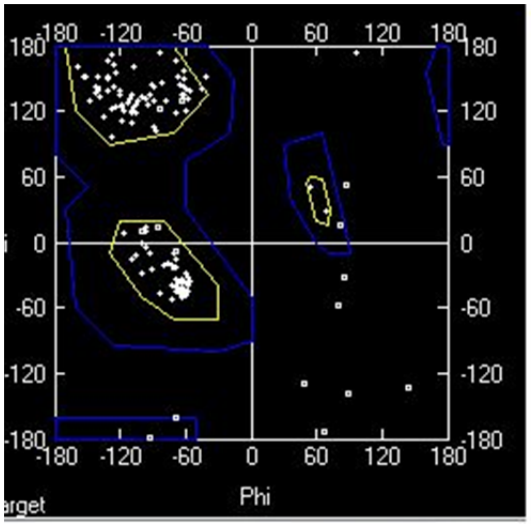


**Fig. 4: Ramachandran plot of Interphotoreceptor retinoid bining protein in *Peromyscus keeni***

The structure interphotoreceptor Binding protein was validated by using Rampage server. It revealed that around 98.6% residues were in favoured regions. (Table5).

**Table 5: Rampage evaluation results of Interphotoreceptor Binding Protein showing accuracy of model**

| **S.no.** | **Acc.no.** | **Number of Residues** | | |
|---|---|---|---|---|
| | | **Favored region** | **Allowed Region** | **Outlier Reg.** |
| 1. | **AHL47335** | ~98.6% expected :136(95.2%) | ( ~ 2.0 % expected ) : 1:(0.7%) | 1:(0.7%) |

## 4. CONCLUSION

The analysis of amino acid sequence of Interphotoreceptor binding protein from *Peromyscus keeni* was performed in order to understand its various characteristics, physic chemical properties and its 3D structure using the *in-silico* analysis technique. The primary structure determinesthat the protein is hydrophillic, however it may be unstable over a wide range of temperature due to its instability index.The study of secondary structure reveals that random coil structures were more abundant followed by the alpha helix and extended strand of Beta sheet that may be parallel or anti parallel in conformation.The modeled structural data may further aid its use in academic and industrial research.

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