

# Homology Modelling of Cytochrome B from *Carangoides equula*

Nivetha Sarah Ebenezer<sup>1</sup>, Uma Maheswari M.<sup>2</sup>, Joyce Priyakumari C.<sup>3</sup>

<sup>1</sup>Research Assistant. Bioinformatics Infrastructure Facility (BIF), BTIS Net centre, Department of Zoology, Madras Christian College, Tambaram (East), Chennai-600059, Tamil Nadu, India

<sup>2</sup>Research Assistant. Bioinformatics Infrastructure Facility (BIF), BTIS Net centre, Department of Zoology, Madras Christian College, Tambaram (East), Chennai-600059, Tamil Nadu, India

<sup>3</sup>Assistant Professor. Bioinformatics Infrastructure Facility (BIF), BTIS Net centre, Department of Zoology, Madras Christian College, Tambaram (East), Chennai-600059, Tamil Nadu, India

**Abstract:** The *in silico* analysis of homology modelling and 3D structure prediction of Cytochrome B (CYTB) protein in *Carangoides equula* was carried out. Primary structure prediction / physicochemical characterization were performed by computing theoretical isoelectric point, molecular weight, extinction coefficient, instability index, aliphatic index and grand average hydropathy. Performing homology modelling, a high quality of protein 3D structure has been predicted for the amino acid sequence. Cytochrome B of *Carangoides equula* was compared to the 1BE3 structure of Cytochrome BC1 from Bovine, where the structure predicted through SWISS-MODEL was visualized in RASMOL. Hence, the Tertiary structure was predicted using homology modelling by using template to create a model from the target sequence. The model was validated using protein structure analysis tool, RAMPAGE. The assessment of secondary structure modelled using SOPMA revealed greater percentage of residues as alpha helix and random coils against the beta sheets. These structures will provide a good foundation for functional analysis of experimentally derived crystal structures.

**Keywords:** Carangidae, *Carangoides equula*, Cytochrome B, Homology modeling, Protein visualizer

## 1. Introduction

The protein **Cytochrome b** is found in the mitochondria of eukaryotic cells. It is a part of the electron transport chain and where it functions to be the key subunit of cytochrome bc1 and b6f complexes. In the mitochondrion of eukaryotes and in few prokaryotes, cytochrome b is a component of respiratory chain complex III which is also known as the bc1 complex (ubiquinol-cytochrome c reductase), plays a key role in cellular respiration [1]. These complexes are involved in electron transport, the propelling of protons to generate a proton-motive force, where the proton gradient is used for the ATP generation. The cytochrome b binds non-covalently to two hemes, known as b562, the high potential form and b566, the low potential form. Respiratory chain complex is composed of three subunits: cyt b, cyt c1, with one covalently bound heme c and a potential Rieske iron-sulphur protein comprising a single cluster [2]. Cytochrome b, a mitochondrial protein is also used to determine phylogenetic relationships between organisms, due to the variance of its sequence.

*Carangoides equula*, also known as the Whitefin trevally is a species of deep water offshore fish in the jack family Carangidae. They inhabit the tropical to temperate waters of the Indo-Pacific and central Pacific. They grow up to 37 cm in size. The body of the fish is rhomboid and compressed laterally. There are small cycloid scales present on the head and the body. Lateral line consists of two parts: beginning from postero-upper margin of operculum to the vertical of the 15th ray of dorsal fin and further it is straight up to the tail. Straight region of the lateral line consists of 31 scutes. Small cycloid scales are present on the body, breast, and head. Lengths of bases of second dorsal and anal fins are equal. It has a large pectoral fin, which is falciform, and a little longer

than the head. Ventral fins are short. Depth of third spine of dorsal fin is almost equal to the length of the largest ray of dorsal fin. The caudal fin is forked, where its upper and lower lobes are equal in length. There are two spines in the beginning of the anal fin base, which is followed by a spine and 24 rays [3].

Homology modelling is aimed to build 3D structure of a protein using templates which structures of related family members. When a target protein is given, the templates structures are searched in the library and suitable templates are identified. One of the most precise computational methods to generate consistent structural models is Homology modelling. This modelling is used to search the conformation space by minimally disturbing those existing experimentally solved structures. The technique of Homology modelling eases the strong requirement of force field and vast conformation searching, because it deals with the calculating the force field and replaces it in a great part, with the counting of sequence identities. The method concludes based on the statement that structural conformation of a protein is much more highly conserved than its amino acid sequence, and that small changes in sequence normally result in minute variation in the 3D structure [4].

## 2. Materials and Methods

### 2.1. Retrieval of Sequence

The sequence of protein Cytochrome B from *Carangoides equula* was retrieved from the protein database UniProtKB (<http://www.uniprot.org/uniprot/A0A0U1XJR2>) in the FASTA format, a format which represents the protein sequence.

## 2.2 Primary Structure Prediction: Physico-Chemical Analysis

For physico-chemical characterization, molecular weight, isoelectric point, total number of positively and negatively charged residues, extinction coefficient(pI), half-life, instability index(II), aliphatic index(AI) and grand average hydropathy (GRAVY) were computed using ExPASy's Prot Paramserver (<http://us.expasy.org/tools/protparam.html>).

## 2.3 Homology modelling: Generation of 3-D Structure through using Swiss-model

The retrieved/targetFASTA sequence was pasted in the webpage of Swiss-model (<https://swissmodel.expasy.org/>). Templates sequences for the target sequence were searched. Using the searched template sequence, a new model was built. The 3-D crystal structure of Cytochrome BC1 protein was available at 3.00Å resolution (PDB:1BE3) and it was used as a template structure to generate the 3-D model of CYTB. The crystal 3-D structure of template was automatically searched from protein data bank (<http://www.rcsb.org/pdb/>). Homology modelling was used to generate the 3-D structure of CYTB with the help of Swiss Model and visualization of 3-D structure in RASMOL.

## 2.4 Evaluation and Validation of the 3D Structure

The minimum dope score and model score of the model and the template were used for the evaluation of the 3-D model and structure were validated with RAMPAGE. The program generates Ramachandran plot where amino acid residues in the allowed region and overall G- factor were considered.

## 2.5 Secondary Structure Prediction

The alpha helices and the beta sheets of the 3D structure of CYTB was predicted using a secondary structure prediction method called SOPMA ([https://npsa-prabi.ibcp.fr/cgi-bin/npsa\\_automat.pl?page=/NPSA/npsa\\_sopma.html](https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html)).

## 3. Results and Discussion

Cytochrome B receptor from *Carangoides equula* was A0A0U1XJR2 and the length of amino acids was 380, having size 42.4 kDa and 7.80pI value. The 3-D structural homology of Cytochrome B resembled with the crystal structure of Cytochrome BC1 receptor available at 3.00Å (PDB:1BE3).

The structural homology of CYTB amino acids was 75.99% identical with template 3-D structure, and the alignment of CYTB receptor with the template 1BE3 were shown (Fig. 2). The 3-D structure of CYTB receptor was shown in Fig. (3).

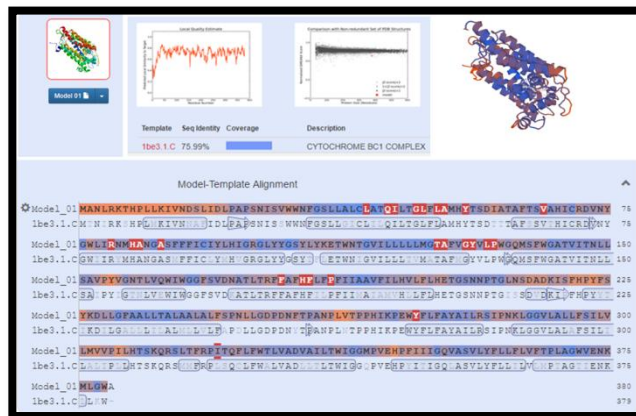


Figure 2: The model result showing the Model-Template alignment

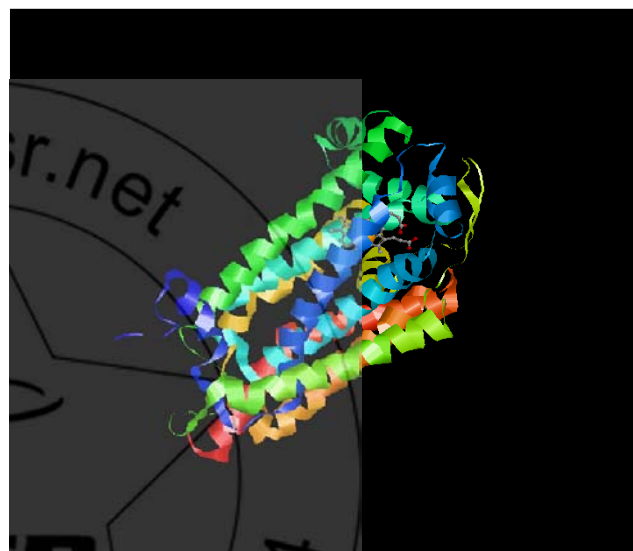


Figure 3: The three dimensional structure of Cytochrome B from *Carangoides equula*, viewed under RASMOL.

The homology modelling was carried out and the result model was viewed using RASMOL, a tool for visualization of biological macromolecule structures. The validation of the structure was done on the basis of spatial arrangement amino acid residues in the most favoured region of Ramachandran plot. The torsion angles of 95.0% amino acid residues were found in the most favoured region, 4.8% amino acids were found in the allowed region and merely 0.3% amino acid residues were present in the outlier region. From the validation it can be inferred that the stability of the protein is high. The favoured Ramachandran plot for the CYTB receptor obtained by RAMPAGE was shown in Fig. (4).

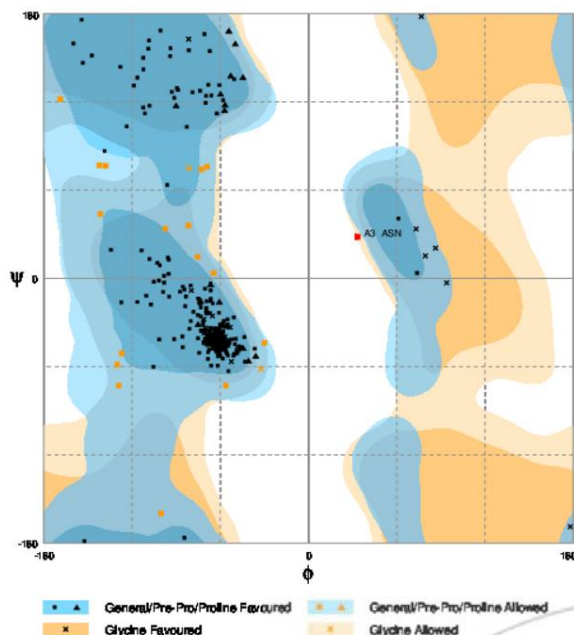


Figure 4: The spatial arrangement of amino acid residue of the modelled 3-D structure of Cytochrome B receptor in favoured region in the Ramachandran plot.

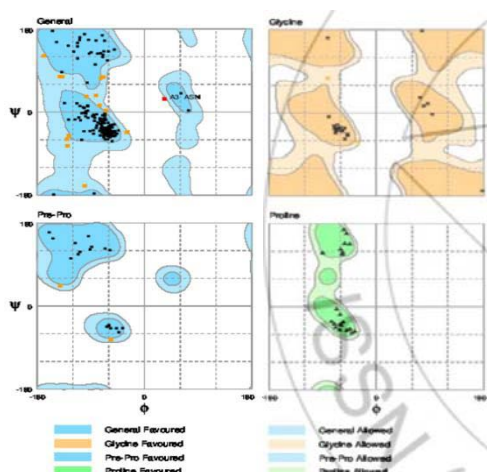


Figure 5: (a) The general favoured and allowed region.

#### Evaluation of residues

Residue [A 8 :HIS]	(-56.22, -72.93)	in Allowed region
Residue [A 19 :ILE]	(-128.97, -72.93)	in Allowed region
Residue [A 27 :ILE]	(-168.71, 121.92)	in Allowed region
Residue [A 30 :TRP]	(-64.55, 3.58)	in Allowed region
Residue [A 57 :SER]	(-97.38, 33.77)	in Allowed region
Residue [A 71 :ARG]	(-130.03, -58.67)	in Allowed region
Residue [A 109 :TYR]	(-68.95, 75.92)	in Allowed region
Residue [A 157 :GLY]	(-32.21, -61.42)	in Allowed region
Residue [A 171 :ASP]	(-100.21, -159.66)	in Allowed region
Residue [A 216 :ASP]	(-137.87, 76.53)	in Allowed region
Residue [A 246 :SER]	(-141.38, 43.70)	in Allowed region
Residue [A 251 :GLY]	(-81.27, 75.00)	in Allowed region
Residue [A 255 :ASN]	(-72.75, 74.10)	in Allowed region
Residue [A 256 :PHE]	(-81.49, 35.93)	in Allowed region
Residue [A 267 :HIS]	(-142.07, 76.92)	in Allowed region
Residue [A 272 :TRP]	(-29.84, -43.77)	in Allowed region
Residue [A 343 :VAL]	(-75.31, 14.60)	in Allowed region
Residue [A 364 :VAL]	(-126.91, -50.90)	in Allowed region
Residue [A 3 :ASN]	(33.54, 28.33)	in Outlier region
Number of residues in favoured region	(~98.0% expected)	: 358 ( 95.0%)
Number of residues in allowed region	(~2.0% expected)	: 18 ( 4.8%)
Number of residues in outlier region		: 1 ( 0.3%)

Figure 5: (b) Evaluation of the residues

The parameters, molecular weight, isoelectric point, total number of positively and negatively charged residues, extinction coefficient, half-life, instability index, aliphatic index and grand average hydropathy were computed using the ExPASy's ProtParam tool. The isoelectric point value for CYTB revealed its basic behavior. Extinction coefficient

values for protein at 280 nm showing 92360-92485 M-1cm-1. Cys residue was very low in concentration. This indicates that these proteins cannot be analyzed using UV spectral methods. From value of the instability index which is computed to be 31.61, the protein can be classified as stable. The aliphatic index of the protein shows a high value which infers that the protein may be stable for a broad range of temperature. The GRAVY index showing a low value indicated that the protein could result in a better interaction with water.

Table 1: The Physico-chemical analysis of Cytochrome B using ProtParam

Number of amino acids: 380	Atomic composition:
Molecular weight: 42449.07	Carbon C 2032
Theoretical pI: 7.80	Hydrogen H 3045
	Nitrogen N 475
	Oxygen O 498
	Sulfur S 11
	Formula: C <sub>2032</sub> H <sub>3045</sub> N <sub>475</sub> O <sub>498</sub> S <sub>11</sub>
	Total number of atoms: 6061
Amino acid composition:	Extinction coefficients:
Ala (A) 33 8.7%	Extinction coefficients are in units of M <sup>-1</sup> cm <sup>2</sup> , at 280 nm measured in water.
Arg (R) 8 2.1%	Ext. coefficient: 92485
Asn (N) 19 5.0%	Abs 0.1% (1 g/l) 2.179, assuming all pairs of Cys residues form cystines
Asp (D) 11 2.9%	Ext. coefficient: 92360
Cys (C) 3 0.8%	Abs 0.1% (1 g/l) 2.176, assuming all Cys residues are reduced
Gln (Q) 6 1.6%	Estimated half-life:
Glu (E) 5 1.3%	The N-terminal of the sequence considered is N (Met).
Gly (G) 26 6.8%	The estimated half-life is: 30 hours (mammalian reticulocytes, in vitro).
His (H) 12 3.2%	>20 hours (yeast, in vivo).
Ile (I) 28 7.4%	>10 hours (Escherichia coli, in vivo).
Leu (L) 60 15.8%	Instability index:
Lys (K) 9 2.3%	The instability index (II) is computed to be 31.61
Met (M) 8 2.1%	This classifies the protein as stable.
Phe (F) 32 8.4%	Aliphatic index: 33.84
Pro (P) 21 5.5%	Grand average of hydropathicity (GRAVY): 0.720
Ser (S) 21 5.5%	
Thr (T) 25 6.6%	
Trp (W) 13 3.4%	
Tyr (Y) 14 3.7%	
Val (V) 26 6.8%	
Pro (P) 0 0.0%	
Sec (U) 0 0.0%	
(R) 0 0.0%	
(Z) 0 0.0%	
(X) 0 0.0%	
Total number of negatively charged residues (Asp + Glu): 16	
Total number of positively charged residues (Arg + Lys): 17	

The secondary structure prediction of Cytochrome B from *Carangoides equula* was carried out by an online tool called SOPMA. The length of CYTB sequence of *C. equula* is 380 AA.



The 'h' represents the helices, 'c' represents the coils, 'e' represents the extended beta sheets and the 't' represents the turns. The CYTB structure of *C. equula* has 42.11% (160 residues) of alpha helix, 20.00% (76 residues) of Beta sheets, 29.21% of coils and 8.68% of turns.

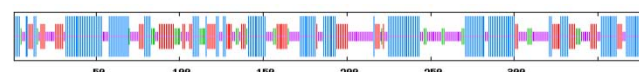


Figure 6: The SOPMA result showing secondary structure of the protein CYTB



In the secondary structure prediction, alpha helices, beta sheets, turns and coils of the predicted structure were found. In this study, it is revealed that evolution is the major factor playing a role in this organism. The phenotype of an individual's organism results from its genotype and the environmental influence. Therefore we may predict that the CYTB in *C.equula* had the fine number residues.

#### 4. Conclusion

In this study Cytochrome B protein of *Carangoides equula* (Whitefin trevally) was selected. Primary structure prediction/physicochemical characterization were performed by computing molecular weight, isoelectric point, total number of positively and negatively charged residues, extinction coefficient, half-life, instability index, aliphatic index and grand average hydropathy. Structural analysis was performed and figures representations were generated with RASMOL. The modelling of the three dimensional structure of the CYTB protein was performed by SWISS-MODEL homology modelling program. The models were validated using protein structure checking tools RAMPAGE. These structures will provide a good basis for functional analysis of experimentally derived structures.

#### 5. Acknowledgments

I thank Biotechnology information system network (BTISNET) centre sponsored by Department of Biotechnology, Ministry of Science and Technology and Government of India for supporting my work.

#### References

- [1] Berry E.A, Guergova-Kuras M, Huang L.S, Crofts AR (2000). Structure and function of cytochrome bc complexes. Annual Review of Biochemistry. Vol. 69: 1005-1075. DOI:10.1146/annurev.biochem.69.1.1005.
- [2] Mahender Singh\*, Ashish Gupta and Lakra W.S (2012). In silico 3-D structure prediction of cytochrome b protein of sisorid catfish *Glyptothorax ngapang*. Indian Journal of Biotechnology. Vol 11, April 2012, pp 156-162.
- [3] Balanov A.A and Markevich A.I (2011). First Occurrence of *Carangoides equula* (Temminck et Schlegel, 1844) (Carangidae) in Russian Waters. Journal of Ichthyology. Vol. 51, No. 8, pp. 666–669. DOI: 10.1134/S0032945211040023.
- [4] Vyas V.K, Ukawala R.D, Ghate M, and Chintha C (2012). Homology Modelling a Fast Tool for Drug Discovery: Current Perspectives. Indian J Pharm Sci. 2012 Jan;74(1):1-17. DOI: 10.4103/0251-474X.102537.
- [5] Ashok Kumar K\*, Geetha N, Prabhavathi M., Saradha Devi K.M (2011). Homology modelling and structure prediction of thioredoxin (TRX) protein in wheat (*Triticum aestivum* L.). International Journal of Biosciences (IJB). Vol. 1, No. 1, p. 20-32.
- [6] Asif S. Tamboli, Niraj R. Rane, Swapnil M. Patil, Shivtej P. Biradar, Pankaj K. Pawar, Sanjay P. Govindwar (2015). Physicochemical characterization, structural analysis and homology modelling of bacterial

and fungal laccases using in silico methods. *New Model Anal Health Inform Bioinforma*. DOI: 10.1177/s13721-015-0089-y.

- [7] Jose Castesana (2001). Cytochrome *b* Phylogeny and the Taxonomy of Great Apes and Mammals. *Mol. Biol. Evol.* 18(4):465–471.
- [8] Lorenza Bordoli, Florian Kiefer, Konstantin Arnold, Pascal Benkert, James Battey & Torsten Schwede (2008). Protein structure homology modelling using SWISS-MODEL workspace. *Nature Protocols*. DOI:10.1038/nprot.2008.197.
- [9] Sabeena M, Kaiser Jamil (2014). Functional Characterization and Structure Prediction of HDAC-10: A Bioinformatics Approach. *International Journal of Science and Research (IJSR)*. Volume 3 Issue 6, June 2014.
- [10] Sahay A, Shakya M (2010). In silico Analysis and Homology Modelling of Antioxidant Proteins of Spinach. *J Proteomics Bioinform*. 3:148-154. DOI:10.4172/jpb.1000134.
- [11] Sudeep Roy\*, Nidhi Maheshwari, Rashi Chauhan, Naresh Kumar Sen, Ashok Sharma (2011). Structure prediction and functional characterization of secondary metabolite proteins of Ocimum. *Bioinformation*. 6(8): 315-319.
- [12] Vijai Singh and Pallavi Somvanshi\* (2009). Homology Modelling of Adenosine A2A Receptor and Molecular Docking for Exploration of Appropriate Potent Antagonists for Treatment of Parkinson's Disease. *Current Aging Science*. DOI: 10.2174/1874609810902020127.

#### Author Profile



**Nivetha Sarah Ebenezer** completed her B.Sc. degree in Advanced Zoology and Biotechnology from Stella Maris College (2014) and M.Sc. degree in Zoology from Madras Christian College in (2016). Currently she is working as a Research Assistant in Bioinformatics Infrastructure Facility (BIF), of BTIS Net centre at Madras Christian College.



**Uma Maheswari. M** completed her B.Tech Biotechnology in 2013. She has also completed a project in genetic engineering. Currently she is working as Research assistant in Bioinformatics Infrastructure Facility (BIF) of BTIS Net centre at Madras Christian College, Chennai.



**Joyce Priyakumari C.** is the co-coordinator of Bioinformatics Infrastructure Facility (BIF) of BTIS Net centre at Madras Christian College. She holds a PhD from Madras University and currently teaches Molecular biology, Biotechnology and Bioinformatics at Madras Christian College, Chennai.