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Predicting functions of *cytochrome c oxidase subunit 1* from *Spinycheek crayfish* using computational methods

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Abstract

Understanding the cell functioning at molecular level is the goal of most of molecular biology researchers. Molecular biology involves macromolecules which are block of life, on research scene. Among others, proteins have a big range of functions and can only be clear if their structures are available. Assigning functions to all known sequences that are being generated in the public domain by different genomic projects, constitutes a big challenge. It is for that very reason the functions of cytochrome c oxidase subunit 1 from Spinycheek crayfish (Uniprot id: G3GHF6) were predicted using computational methods. Local sequence alignment was conducted to retrieve potential structural homologs having structures determined using experimental methods. Multiple sequence alignment has shown conserved motifs which could be of biological interest. Prediction of three-dimensional structure of cytochrome c oxidase subunit 1 through homology modeling followed by structure assessment and validation using ERRAT and PROCHECK, suggested the predicted model was of acceptable quality. Docking studies using HEX software demonstrated that this protein has affinity with heme ligand with eleven residues involved in these interactions. These interactions are similar to those observed when the heme ligand was docked onto the x-ray structure of the protein used as template for homology modeling exercise. This research shades lights on the function of cytochrome c oxidase subunit 1from Spinycheek crayfish.

Keywords: Protein interaction, cytochrome, modeling, multiple sequence alignment, Ramachandran Plot.

Introduction

Proteins constitute the most essential as well as multipurpose macromolecules of life. Understanding their functions is of importance in the elucidation of cell function at molecular level which may help in the understanding of ways to develop new and improved drugs, good quality crops, and even the artificial biochemicals[1]. *Cytochrome c oxidase* is a protein complex located in the inner membrane of mitochondria. Almost all respiratory chains of many bacteria are catalyzed by *Cytochrome c oxidase* complex. The energy harvesting reactions are also catalyzed by the *Cytochrome c oxidase*. This system has a catalytic activity to produce oxygen from cytochrome complex, belongs to the super family of heme-copper containing terminal oxidases[2]. The understanding of the *cytochrome c oxidase subunit 1*may be an accurate way to identify some species, since the gene encoding this subunit is assumed to be the barcode sequence for some organisms[3]. When viewed as mitochondrial DNA, the *cytochrome c oxidase subunit 1* has been extensively used as a molecular marker to study evolutional relationships of animals, due to simple structure of its genome[4]. Reza and his coworkers explained the use of this molecular marker in forensic investigations[5]. Also, the mitochondrial *Cytochrome c oxidase Subunit I* gene seems to be highly conserved in animals[6], reason why Hoeh et al. proposed that the *cytochrome c oxidase subunit 1* could be the best choice primer while analyzing evolutional relationships in metazoan invertebrates[7].

Protein three-dimensional (3D) structure prediction from primary sequence has important role in predicting protein function. As cited by Lawrence and his colleagues, over six million protein sequences have been deposited in biological databases and this number continue to grow rapidly[8]. This situation makes the prediction and the assignation of 3D structures and subsequent functions to respective sequences more urgent than ever as the relationship between structure and function constitute an indispensable component of modern biology[9]. For a better understanding of protein function, a good number of works have been conducted to predict protein structure and subsequent functions in order to meet the need to assign structure and function to the growing number of protein sequences[10,11]. Simultaneously, computational methods for protein structure-function prediction have been developed[12]. Pandey et al. noticed that early approaches for the prediction of protein function were focused on gene or expressed protein of interest, or a small group of natural proteins[1]. However, these approaches are qualified to be low throughput considering the big number of human efforts or experimental attempts essential for protein or single gene analysis[1]. It has been realized that even large-scale experimental attempts are inadequate to annotate an increasing number of proteins that is becoming available due modern biology where technology is applied[13].

This situation has shown the existence of a big gap between sequence and function of proteins newly discovered[14]. In the domain of genome function, many researches targeting the utilization of information related to genome for the prediction of relationship between protein structure and protein function, were conducted[15-18]. These data encourage the exploration of new gained genomic evidences for function prediction. The present work is aimed at predicting functions of the Cytochrome c oxidase subunit 1, by the use computational methods.

Material and methods

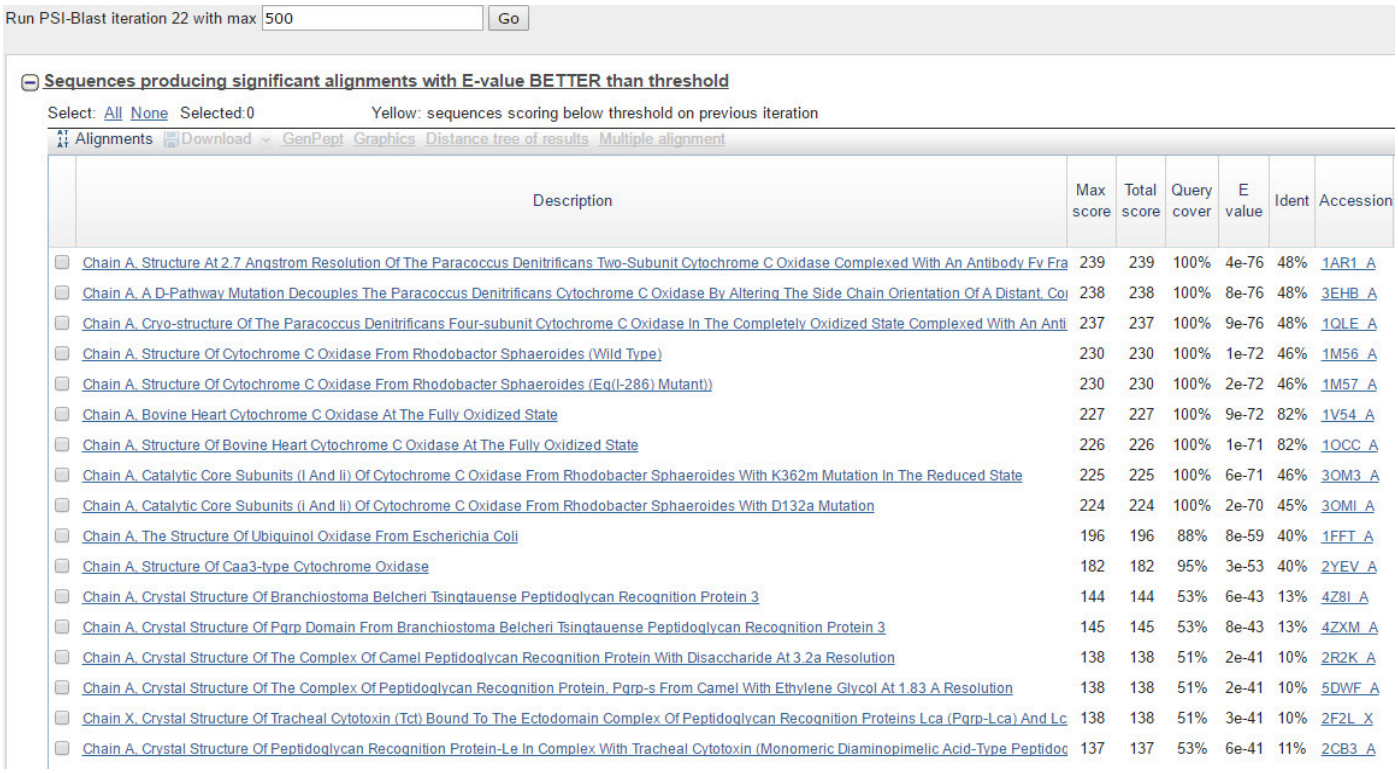
Primary sequence information (Accession number: G3GHF6) was downloaded in FASTA format from UniProt database[19]. Prediction of the distribution of transmembrane helices was performed by loading the FASTA sequence to TMHMM server[20]. Search for probable protein templates was performed by submitting the FASTA sequence of *Cytochrome c oxidase subunit 1* protein to BLAST program of NCBI, where PSI-BLAST option was activated with PDB as biological database[21]. Multiple sequence comparison was done by subjecting the first top five potential templates along with the query sequence, to CLUSTAL Omega server[22]. Biological functions of *Cytochrome c oxidase subunit 1* were predicted by submitting its sequence information to SIFTER (Statistical Inference of Function Through Through Evolutionary Relationships) Server[23]. This server explores statistically texted methods for predicting functions of various proteins function based on relationship tree of a big range of proteins families[24].

Three dimensional structure prediction of *Cytochrome c oxidase subunit 1* protein was done by accessing the alignment mode of SWISS MODEL workspace[25]. The target-template alignment was prepared using Deep View package and saved as project after exploring the iterative magic fit option of the package[26]. Coordinates of initial model obtained were subjected to quality control using ERRAT server[27]. Loop regions were further refined using MODLOOP webserver[28]. The energy of final refined 3D model of *Cytochrome c oxidase subunit 1* protein was minimized using Deep View software. The stereochemistry status of the energy minimized predicted 3D model was analyzed using PROCHECK webserver.

Molecular docking of heme ligand onto the predicted model of *Cytochrome c oxidase subunit 1* was done following the method detailed in our previous paper[29] using HEX 8.0 software[30]. The first complex of receptor-ligand resulted from docking exercise was subjected to PyMol[31] or Deep View packages for examination of interacting residues.

Results and discussion

Molecular modeling: The search for potential protein template from PDB databank was successfully completed by PSI-BLAST which was able to identify homologous proteins having more than 30% sequence identity (Figure-1). Many researchers have used PSI-BLAST to analyze sequence similarities among proteins[32,33].


Run PSI-Blast iteration 22 with max 500 Go

Sequences producing significant alignments with E-value BETTER than threshold

Select: All None Selected:0 Yellow: sequences scoring below threshold on previous iteration

Alignments Download GenPept Graphics Distance tree of results Multiple alignment

Description	Max score	Total score	Query cover	E value	Ident	Accession
Chain A, Structure At 2.7 Angstrom Resolution Of The Paracoccus Denitrificans Two-Subunit Cytochrome C Oxidase Complexed With An Antibody Fv Fra	239	239	100%	4e-76	48%	1AR1_A
Chain A, A D-Pathway Mutation Decouples The Paracoccus Denitrificans Cytochrome C Oxidase By Altering The Side Chain Orientation Of A Distant, Cor	238	238	100%	8e-76	48%	3EHB_A
Chain A, Cryo-structure Of The Paracoccus Denitrificans Four-subunit Cytochrome C Oxidase In The Completely Oxidized State Complexed With An Anti	237	237	100%	9e-76	48%	1QLE_A
Chain A, Structure Of Cytochrome C Oxidase From Rhodobactor Sphaeroides (Wild Type)	230	230	100%	1e-72	46%	1M56_A
Chain A, Structure Of Cytochrome C Oxidase From Rhodobacter Sphaeroides (Eq(I-286) Mutant))	230	230	100%	2e-72	46%	1M57_A
Chain A, Bovine Heart Cytochrome C Oxidase At The Fully Oxidized State	227	227	100%	9e-72	82%	1V54_A
Chain A, Structure Of Bovine Heart Cytochrome C Oxidase At The Fully Oxidized State	226	226	100%	1e-71	82%	1OCC_A
Chain A, Catalytic Core Subunits (I And Ii) Of Cytochrome C Oxidase From Rhodobacter Sphaeroides With K362m Mutation In The Reduced State	225	225	100%	6e-71	46%	3OM3_A
Chain A, Catalytic Core Subunits (i And Ii) Of Cytochrome C Oxidase From Rhodobacter Sphaeroides With D132a Mutation	224	224	100%	2e-70	45%	3OMI_A
Chain A, The Structure Of Ubiquinol Oxidase From Escherichia Coli	196	196	88%	8e-59	40%	1FFT_A
Chain A, Structure Of Caa3-type Cytochrome Oxidase	182	182	95%	3e-53	40%	2YEV_A
Chain A, Crystal Structure Of Branchiostoma Belcheri Tsingtauense Peptidoglycan Recognition Protein 3	144	144	53%	6e-43	13%	4Z8I_A
Chain A, Crystal Structure Of Pgrp Domain From Branchiostoma Belcheri Tsingtauense Peptidoglycan Recognition Protein 3	145	145	53%	8e-43	13%	4ZXM_A
Chain A, Crystal Structure Of The Complex Of Camel Peptidoglycan Recognition Protein With Disaccharide At 3.2a Resolution	138	138	51%	2e-41	10%	2R2K_A
Chain A, Crystal Structure Of The Complex Of Peptidoglycan Recognition Protein, Pgrp-s From Camel With Ethylene Glycol At 1.83 A Resolution	138	138	51%	2e-41	10%	5DWF_A
Chain X, Crystal Structure Of Tracheal Cytotoxin (Tct) Bound To The Ectodomain Complex Of Peptidoglycan Recognition Proteins Lca (Pgrp-Lca) And Lc	138	138	51%	3e-41	10%	2F2L_X
Chain A, Crystal Structure Of Peptidoglycan Recognition Protein-Le In Complex With Tracheal Cytotoxin (Monomeric Diaminopimelic Acid-Type Peptidog	137	137	53%	6e-41	11%	2CB3_A

Figure-1: Top seventeen homologous proteins retrieved at the completion of 21st iteration by PSI-BLAST

TMHMM was able to detect four transmembrane helices, three extracellular loops and two intracellular loops (Figure-2). These results strongly suggest that *Cytochrome c oxidase subunit* 1 protein belongs to transmembrane protein family characterized by crossing the lipid bilayer several times[34,32]. In their book, Alberts and his coworkers, have explained that such proteins spanning the membrane several times may be effective in the transportation of ions and small water soluble molecules, across the membrane[34].

Multiple sequence alignment performed successfully by Clustal Omega was able to display some conserved residues between *Cytochrome c oxidase subunit 1* protein and top five homologous proteins retrieved from PDB databank. Among the conserved residues, tyrosine (Y) and proline (P) of the fully "*LYPPL*" conserved motif among the aligned proteins, were reported to be among residues that play a vital role in the electron transfer pathway[35].

To get more insights on biological functions of *Cytochrome c oxidase subunit 1 protein,* SIFTER server was used. This server was able to predict that cytochrome c oxidase sub unit 1 has a cytochrome-c oxidase activity with high confidence score (0.8). The high confidence score reflects the reliability of the biological function prediction[23,24]. This prediction seems to agree with results of sequence alignment, whereby top five homologous proteins retrieved from PDB databank all intervene in the cytochrome-c oxidase activity.

```
# tr|G3GHF6|G3GHF6_ORCLI Length: 213
# tr|G3GHF6|G3GHF6_ORCLI Number of predicted TMHs:  4
# tr|G3GHF6|G3GHF6_ORCLI Exp number of AAs in TMHs: 91.40454
# tr|G3GHF6|G3GHF6_ORCLI Exp number, first 60 AAs:  21.98773
# tr|G3GHF6|G3GHF6_ORCLI Total prob of N-in:        0.04640
# tr|G3GHF6|G3GHF6_ORCLI POSSIBLE N-term signal sequence
tr|G3GHF6|G3GHF6_ORCLI  TMHMM2.0        outside      1    38
tr|G3GHF6|G3GHF6_ORCLI  TMHMM2.0        TMhelix     39    61
tr|G3GHF6|G3GHF6_ORCLI  TMHMM2.0        inside      62    81
tr|G3GHF6|G3GHF6_ORCLI  TMHMM2.0        TMhelix     82   104
tr|G3GHF6|G3GHF6_ORCLI  TMHMM2.0        outside    105   118
tr|G3GHF6|G3GHF6_ORCLI  TMHMM2.0        TMhelix    119   141
tr|G3GHF6|G3GHF6_ORCLI  TMHMM2.0        inside     142   161
tr|G3GHF6|G3GHF6_ORCLI  TMHMM2.0        TMhelix    162   184
tr|G3GHF6|G3GHF6_ORCLI  TMHMM2.0        outside    185   213
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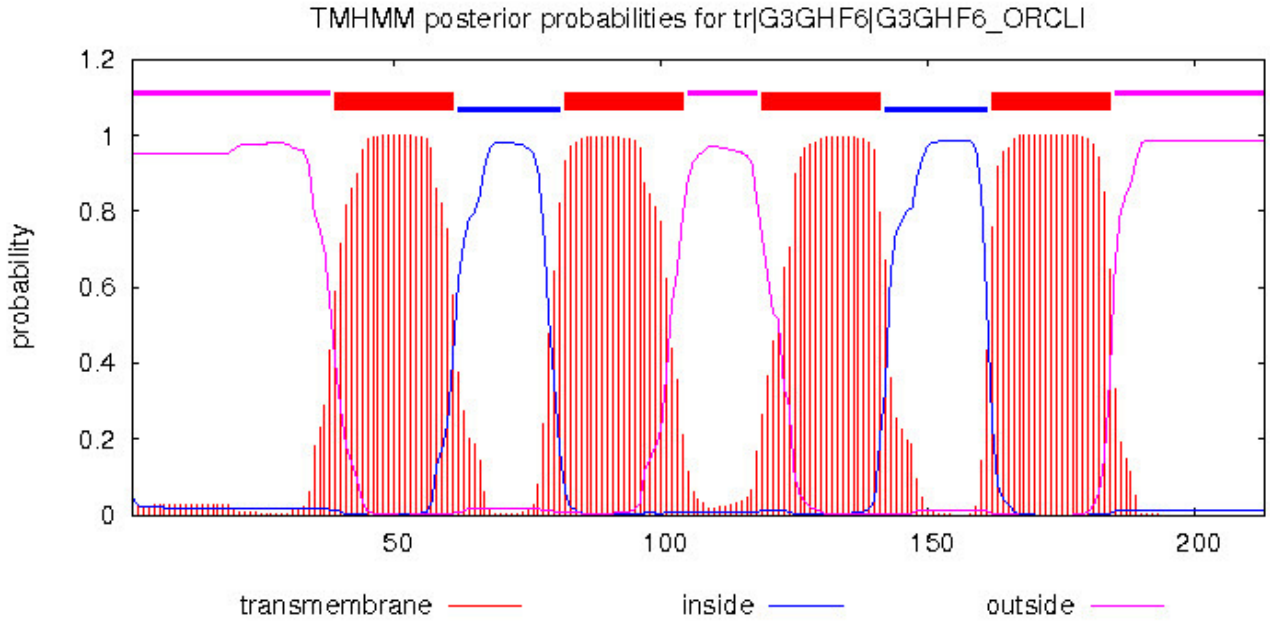


Figure-2: Prediction of the orientation of *Cytochrome c oxidase subunit 1* protein in the membrane. Transmembrane helices are displayed in red, intracellular loops in blue and extracellular loops in magenta. The number of residues for each helix and loop is displayed above the graphical representation.

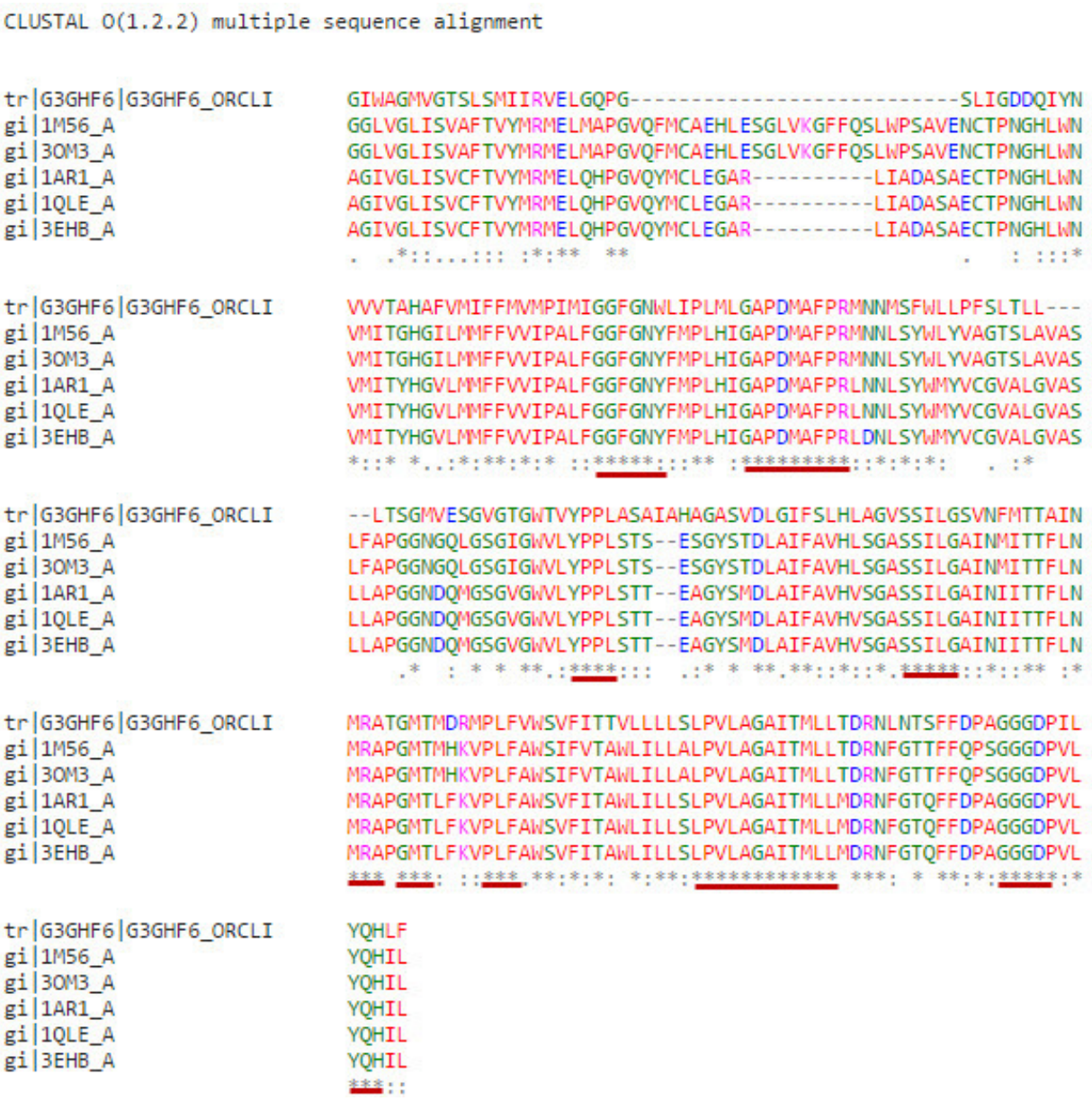
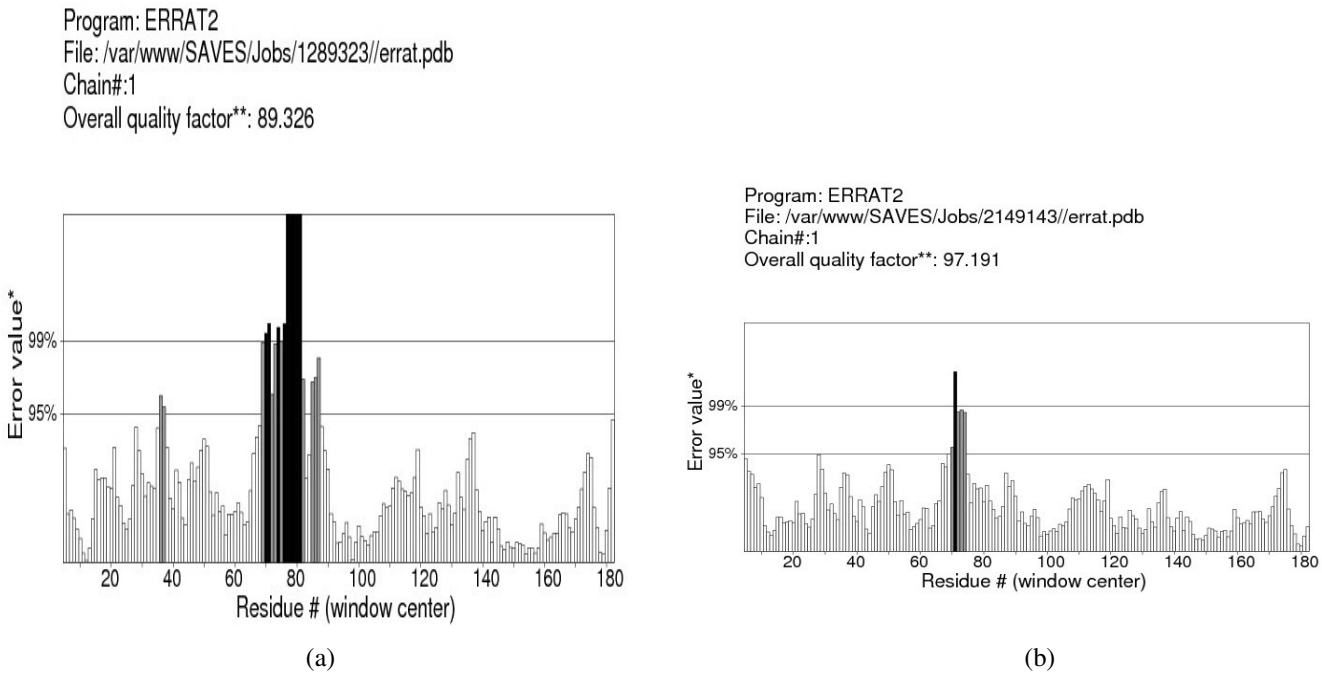


Figure-3: Multiple sequence alignment of five top homologous proteins retrieved from PDB databank, fully conserved motifs are underlined in red

Structure prediction: The alignment mode of SWISS MODEL was able to fold initial sequence of cytochrome c oxidase sub unit 1 protein into a 3D model. This was done using homology modelling approach where by the X-ray determined structure of Paracoccusdenitrificans (PDB id: 1AR1 chain A) was used as the template suggested by PSI-BLAST. Homology modelling approach has been extensively used to understand protein structure prediction and the relationship between the structure and function of those proteins, by various researchers[36,37]. Coordinates of the initial model predicted was subjected to quality control using ERRAT web server. The quality factor of the predicted model was a bit lower (89.3) suggesting the presence of loops to be refined (Figure-4). MODLOOP server was successful on refining loop regions, thence increase the quality factor of the predicted model. At the completion of loop region refinement, the quality of the predicted model increased to 97.1 while the energy reduced from -5280.5KJMol^{-1} before loop refinement to -5740.9 KJMol^{-1} after this exercise. The high-quality factor of the predicted model suggests that the model is of acceptable range and can be used for further analyses. The energy minimized model of cytochrome c oxidase sub unit 1 from Spinycheek crayfish was further validated by PROCHECK server which revealed how residues are distributed in regions of Ramachandran plot (Figure-5). The overall factor was of the acceptable range, suggesting the predicted model to be of high quality[38].



(a) (b)

Figure-4: Quality factor of the predicted model, displayed by ERRAT server before (a) and after (b) loop refinement showing change in the quality factor of the predicted model of cytochrome c oxidase subunit 1 from *Spinycheek crayfish*

Procheck: [+/-]

```
+----------<<<  P  R  O  C  H  E  C  K     S  U  M  M  A  R  Y  >>>----------+
|                                                                             |
| input_atom_only.pdb   2.5                                    186 residues   |
|                                                                             |
*| Ramachandran plot:   86.5% core   11.5% allow    0.6% gener    1.3% disall  |
|                                                                             |
*| All Ramachandrans:    9 labelled residues (out of 184)                      |
+| Chi1-chi2 plots:      1 labelled residues (out of 101)                      |
 | Main-chain params:    6 better     0 inside      0 worse                    |
 | Side-chain params:    5 better     0 inside      0 worse                    |
 |                                                                             |
*| Residue properties: Max.deviation:     8.0            Bad contacts:    6   |
*|                     Bond len/angle:   10.5    Morris et al class:  1  1  2  |
 |                                                                             |
 | G-factors           Dihedrals:  -0.02  Covalent:   0.16    Overall:   0.06  |
 |                                                                             |
 | M/c bond lengths: 99.8% within limits   0.2% highlighted                    |
*| M/c bond angles:  96.2% within limits   3.8% highlighted       1 off graph  |
*| Planar groups:    75.0% within limits  25.0% highlighted       2 off graph  |
 |                                                                             |
+-----------------------------------------------------------------------------+
  + May be worth investigating further.  * Worth investigating further.
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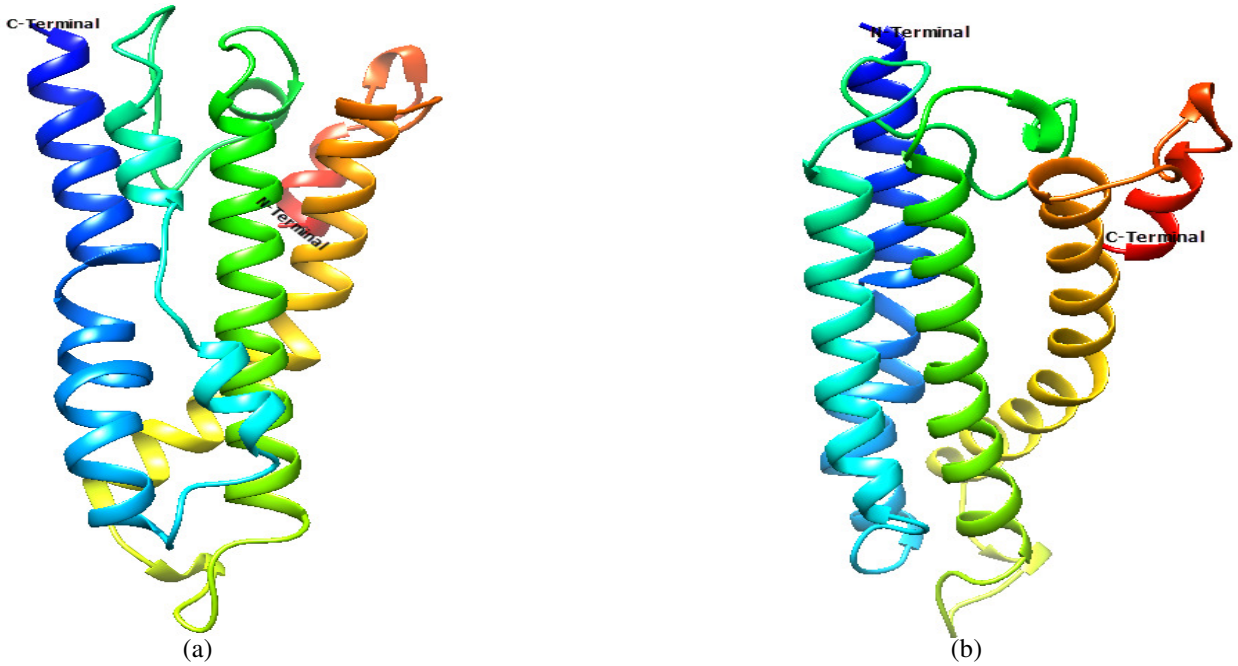
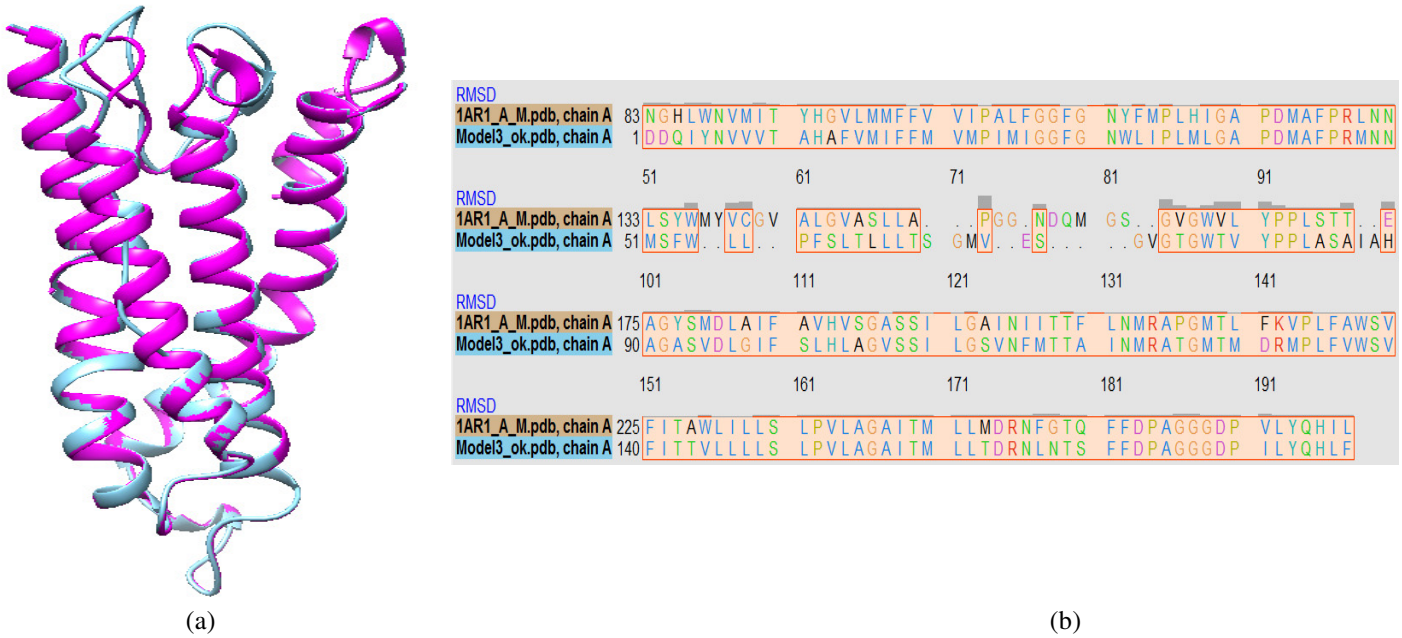
Figure-5: Ramachandran plot showing the distribution of residues of the predicted model of cytochrome c oxidase subunit 1 from *Spinycheek crayfish*

Analyzing the relationship between structure and function of cytochrome c oxidase subunit 1 from *Spinycheek crayfish through structural superimposition:* The predicted model of cytochrome c subunit 1 from *Spinycheek crayfish* (Figure-6 (a)) shows structural similarities when compared to the x-ray template structure used for homology modeling purpose (1AR1) (Figure-6(b). The superimposition of both structures shows that the Cα element of 178 residues got superimposed with general RMSD of 0.5Å (Figure-7(a)) suggesting a high similarity level between both structures. The structural alignment shows that many regions of both proteins got superimposed (Figure-7(b)).



(a) (b)

Figure-6: (a) 3D structure of the predicted model and (b) 3D structure of the X-ray structure of the template protein used for homology modeling (only the aligning region was displayed). The images were produced using CHIMERA package.



(a) (b)

Figure-7: (a) superimposition of template structure (colored in magenta) onto the predicted model of cytochrome c oxidase subunit 1 (colored in cyan) and structural alignment of both protein (b). Images were generated using CHIMERA package

Docking studies: Docking studies of the hemeligand onto the receptor which is the predicted 3D model of cytochrome c oxidase subunit 1 from Spinycheek crayfish was successfully completed using HEX software. Hex was able to generate ten docked conformations. Among all the conformations, the first pose having lower docking energy (-994.21 kcal mol^{-1}) was selected for analyzing interacting residue between cytochrome c oxidase subunit 1 and heme ligand.

Eleven residues were interacting with the ligand, when displayed using Deep View package. These are: asp^{2}, tyr^{5}, asn^{6}, val^{73}, gly^{74}, thr^{75}, tyr^{80}, pro^{82}, leu^{83}, pro^{173}, gln^{183} (Figure-9 (a)).

Majority of the interacting residues being nonpolar (5) valine, glycine, proline and leucine, followed by polar (4): tyrosine, asparagine, threonine and glutamine. Aspartate is the only acidic residue involved in the interaction with the ligand. Interacting residues between the template structure used while modeling the 3D model structure of cytochrome c oxidase subunit 1, and the ligand were also analyzed for comparison.

While 11 residues are involved in the interaction between ligand-predicted model, ten amino acids were displayed when the ligand was docked to native template structure (docking energy: -1019.6 kcal mol^{-1}) of the protein used for homology modeling (1AR1), these are gly^{84}, asn^{88}, tyr^{167}, pro^{168}, pro^{169}, leu^{170}, pro^{258}, pro^{264}, val^{265}, gln^{268} (Figure-9(b)).

Majority of these interacting residues are non-polar: valine, glycine, proline and leucine, followed by polar residues: asparagine, tyrosine and glycine. Docking results strongly agree with published data explaining that tyrosine and proline are involved in the electron transfer pathway[39]. Docking has been used by various researchers with the intention to explain the protein-protein or protein-ligand interactions at molecular level[40-42].

Conclusion

Studies undertaken in this research shows that cytochrome c oxidase subunit 1 from Spinycheek crayfish which demonstrate characteristics of transmembrane proteins, that this protein plays a role in the electron transfer pathway. This was confirmed by its affinity with heme ligand; docking studies undertaken after predicting the 3D model of cytochrome c oxidase subunit 1 from Spinycheek crayfish indicates that the predicted model interacts with the ligand with more than ten residues.

This number of interacting residues could be increased with molecular dynamics simulation which would refine the complex thence increase the interaction between involved molecules. Even though molecular dynamics simulation was not undertaken due to lack of facilities, we hope that this research lights on the function of cytochrome c oxidase subunit 1 from Spinycheek crayfish.

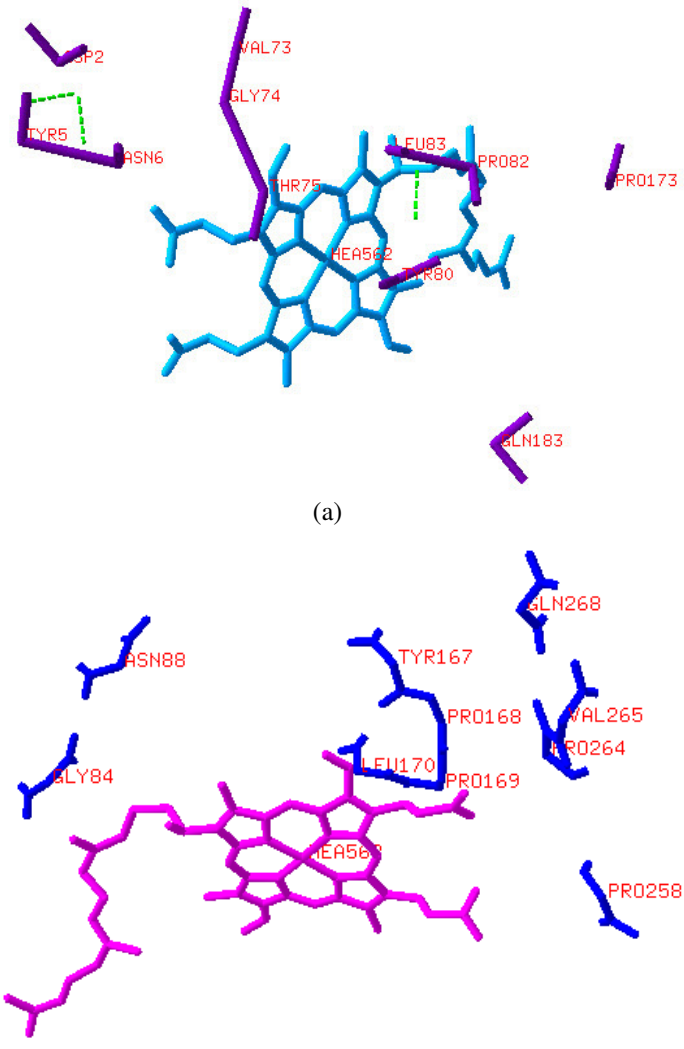


Figure-9: Interacting residues between (a) heme ligand and the predicted model of cytochrome c oxidase subunit 1 from ***Spinycheek crayfish***. and the (b) the x-ray determined structure of 1AR1 used as template for homology modeling, and heme ligand. Images were prepared using Deep View package.

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