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Structural and functional analysis of aquaporin protein of different fish species

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Abstract
The aquaporin (AQP) developed the most conserved class of protein families and plays a lead role in water transport and conservation in fish organ. This research was conducted basically focused on structural and functional analysis of aquaporin protein of 10 different fish sequence with Protparam, CFSSP, PSIPRED, SOPMA, Swiss model, Phyre2 etc. Through the physicochemical analysis it was proved that protein is unstable, quietly thermostable and Gravy of this protein shows hydrophilic property. And helix percentage range between 50.5-81.4%, sheets range 40.6-78 and turn range 7-15. Alpha helix and beta sheets are connected through the turns. Turns play a key role in folding by bringing together interactions between regular secondary structure elements. A turn helps to stabilize abrupt directional changes in the polypeptide chain. The tertiary structure of aquaporin in different fish was predicted by Swiss model, Phyre 2 and TM score servers and their similarity was verified by Verified 3D and ramachandran plot. For tertiary structure prediction '1j4n' (*Bos taurus*) was select as a model template. Phylogenetic tree was structured or constructed by MEGA 7 tools by neighbour joining method. According to the results, they derived from common ancestors. Protein-protein interaction was performed by String. After verification we get an Accession number through the PMDB. This obtained data provided a background for bioinformatics studies of structure and function also evolution of other organism.

Keywords: Aquaporin, motif, phylogeny, mega, PSIPRED, verified 3D, physicochemical and chou

Introduction
Aquaporin known as water channel which is a proteins, a larger family of major intrinsic proteins that form pores in the membrane of biological cells, mainly facilitating transport of water between cells [1]. Aquaporin also found in different cell membrane like bacteria, fungi, animal and plant cell through which water can flow more rapidly inside and outside of the cell by diffusing the phospholipids bilayer [2]. Genetic defects related with aquaporin genes, associated with several human diseases like Nephrogenic diabetes and neuromyelitis [5-9]. Agre reported the first high-resolution images of the three-dimensional structure of an aquaporin 1 [20]. Further using supercomputer simulations identified the pathway of water as it moved through the channel and demonstrate; how a pore can allow water to pass without the passage of small solutes [21]. Aquaporin is "the plumbing system for cells". Water passes through cells in an organized way, most rapidly aquaporin water channels in tissues [25]. Scientists assumed that water leaked through the cell membrane. Water molecules in and out of the cell through Aquaporin, where preventing the passage of ions and other solutes. Some aquaporin protein also known as aquaglyceroporin they transport other small uncharged dissolved molecules like; ammonia, CO_2, glycerol and urea.eg. The aquaporin 3 channels have a pore width of 8–10 Angstroms and allow the passage of hydrophilic molecules. The water pores totally block the ions like, proton which are essential to conserve membrane electron potential difference [26].Aquaporin proteins are composed of a bundle of six transmembrane α-helices, embedded in the cell membrane. Aquaporin form four clusters in the cell membrane, each of this four monomer act as a water channel [26]. Different aquaporin have different sized water channels. In mammals there are thirteen types of aquaporin are found and six of these are located at the kidney [33]. In Plant aquaporin are found in basically in the vacuoles membrane, transport of water across the plasma and vacuoles membrane which also called transcellular pathway [37]. Aquaporin are a diverse family of membrane proteins that are expressed predominantly in tissues in which edema and fluid imbalances are of major concern, Water movements across cell membranes is carried by osmotic and hydrostatic forces, this process influenced by the

Specific aquaporin water channels. Aquaporin-4 water channels play a central role in brain water regulation in neurologic disorders. The pharmacologic modulation and activity of various aquaporin potentially could provide novel treatments for a variety of disorders, including brain edema. Peter Agre at Johns Hopkins University Predicted that water pores must exist in very leaky cells and identified a specific transmembrane water pore that was later called aquaporin-1. For this accomplishment Agre shared the 2003 Nobel Prize in Chemistry with Rod MacKinnon for his work on the potassium channel [16]. Aquaporin are usually specific for water permeability and the passage of other solutes. All aquaporin are impermeable to charged solutes [17]. AQP3 has been identified in different teleost fish species. In zebra fish (*Danio rerio*), AQP3 gene is present as two duplicate isoforms resulting from a teleostean fish genome-wide duplication. In various teleost organs has the role of AQP3 in osmoregulatory processes. In teleost gill, AQP3 is expressed in 'chloride' cells, and in some species, in other epithelial cell types, where it may have different functions like prevention of dehydration. In eel oesophagus, immunohistochemistry shows that AQP3 is expressed in surface epithelial cells in the anterior oesophagus, but in mucus cells within the epithelium of the posterior oesophagus. In eel intestine, AQP3 is found in macrophage-like cells. In rectum, as in the posterior oesophagus AQP3 is expressed in mucus cells. In eel kidney, AQP3 is expressed in a subset of renal tubules, and localizes to the apical pole of tubule cells.

Materials and Methods

Obtained Sequence: The amino acids sequence of Aquaporin protein (Accession Number –KJ637327), (Accession number-NM_001135682), (Accession number -NM_001166121), (Accession number -AB083078), (Accession number -CP026264.1), (Accession number – CP026249), (Accession number -AY626941.1) (Accession number -AY363261.1) (Accession number -KJ815007.1), (Accession number -KX494981) was collected from NCBI database (http://www.ncbi.nlm.nih.gov).

Phylogenetic tree construction: Phylogenetic tree is a branching diagram, this helps to understand the evolutionary relationship among the biological species. MEGA 7 [33-36] was used to build the Phylogenetic trees. One tree is construct based on amino acid or nucleotide sequences of Aquaporin and another was constructed based on time tree of fish protein sequences.

Primary sequence analysis: Any amino acid sequence contains a message from transcription and translation of a gene. The physicochemical properties of amino acid sequences were analysed by Expasy'sprot param tool (http://web.expasy.org/protparam) [37]. The amino acid sequence contain various important information such as amino acid composition, physicochemical properties such as isoelectric point (pI), molecular weight(Mw), extinction co-efficient (EC- quantitative study of protein –protein and protein ligands interactions), instability index (II-stability of proteins), aliphatic index (AI- relative volume of protein occupied by aliphatic side chains), and Grand average of Hydropath cities (Gravy- sum of all hydropath city values of all amino acids divided by number of residues in a sequence). Then the amino acid composition of aquaporin protein of different fishes was analysed and in table 1 result details.

Table 1: Representation of Expasy prot param result for primary analysis of 10 different sequence of Aquaporin

Fishes	M. Wt.	PI	Total -ve residue	Total + ve residue	Atomic composition				Total no. Of atoms		Ext. coefficient	Instability index	Aliphatic index	Gravy
					C	H	N	O	S					
Alosa pseudoharengus	67478.96	5.39	3	2	2404	3978	822	989	219	8415	13625	48.95	21.09	0.742
Danio rerio aquaporin 1a	67870.66	5.08	0	0	2425	4042	810	1021	218	8516	13625	45.23	20.62	0.756
Oryzias latipes	45745.07	3.67	2	1						536	1490	52.45	20.86	0.752
Scophthalmus maximus	89855.62	5.00	0	0	3166	5242	1092	1330	311	11141	19375	50.23	18.04	0.758
Solea senegalensis	66712.08	5.09	0	0	2401	4003	801	1009	202	8416	12625	46.32	22.85	0.756
Sparus aurata	73233.32	5.06	0	0	2592	4289	897	1095	240	9113	15000	49.13	18.28	0.712
Squalus acanthias	63661.306	5.07	0	0	2243	3723	765	938	226	7895	14125	52.49	18.69	0.801
Danio rerio aquaporin 3b	74195.87	5.12	0	0	2722	4546	900	1169	175	9512	10875	42.18	23.44	0.590
Protopterus annectens	74010.28	5.11	0	0	2721	4556	888	1154	182	9501	11375	47.63	26.24	0.682
Scophthalmus maximus chromosome (22)	93690.22	6.35	1	1	0	0	0	0	0	0	19500	50.79	18.45	0.741

Secondary structure prediction: The secondary structure is related with protein folding. So, the helix, sheets, and turn of amino acid sequences of different fish species of aquaporin were predicted by PSI-blast based secondary structure prediction (PSIPRED) and Chou and Fasman secondary structure prediction (CFSSP) server (http://cho-fas,sourceforge.net /index. Php) [38].

Protein 3D model prediction: Query sequence was taken amino acid sequence of aquaporin of different fishes. Comparative homology protein model of aquaporin was predicted through the Swiss model workspace and using phyre 2 by picking the most suited template [40].

Predicted protein model evaluation and submission: Predicted protein model was evaluated and verified from both Qmean and saves server (http://nihserver.mbi.ucla.edu/SAVES) Ramachandran plot, verified 3D, ERRAT [41] were evaluated from SAVES. The model in specified (PDB) format was submitted to protein Model Database (PMDB).

Functional analysis and protein-protein interaction study: To know the interaction of Aquaporin protein with other closely related proteins STRING v 10.0 servers [39] was used. Aquaporin of Escherichia coli K12 MG1655 (E. *coli* str. K-12 substr.MG1655,) and Another MBIC11017 was selected as a query sample and functional protein association network was generated. Moreover, the query sequence was also analysed to determine the family which the protein belongs.

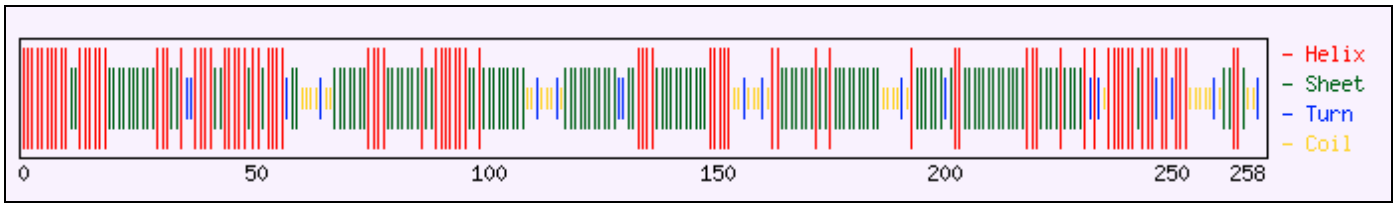
Conserved motif analysis: Conserved motif is a sequence pattern that occurs repeatedly in a group of related protein sequences. There are many tools for defining te existence or absence of the noticeable domains, but they are unable to recognise smaller individual motifs and more divergent patterns. Accordingly, the motifs of protein sequences were created using the program multiple Em for motif Elicitation (MEME; version 4.11.1) and Motif Alignment and search Tool (MAST; version 4.11.1) at website http://meme-suite.org/ (Bailey *et al*. 2009) to study the variation of aquaporin in fish species [42].

Primary sequence analysis: After performed physico chemical parameters analysis by the Expasy's prot param founded Instability Index range level is 42.18-52.45 of this aquaporin protein of all the species which are more than 40 so this protein is unstable, Aliphatic index side chain level 18.4-26.24 which indicate that the aquaporin is thermo stable and Gravy (0.590-0.801) of this protein indicate better interaction with water and shows hydrophilic property. pI (3.67-6.35) or isoelectric point of this protein indicate aquaporin is acidic. Minimum 45745.07 and maximum molecular weight 89855.62.

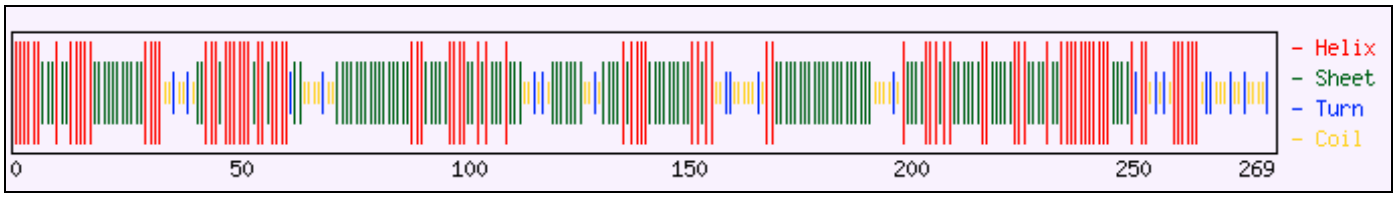
Results and Discussion

The aquaporin protein sequence belonging to different fish species were collected from NCBI database in fasta format. Among the fish sequences those with the range of amino acids were selected by NCBI filters. At first total 10 amino acid sequences of aquaporin protein of different fish species were collected. Through the blast tools all the sequence shows 87-90% similarity with query sequence.

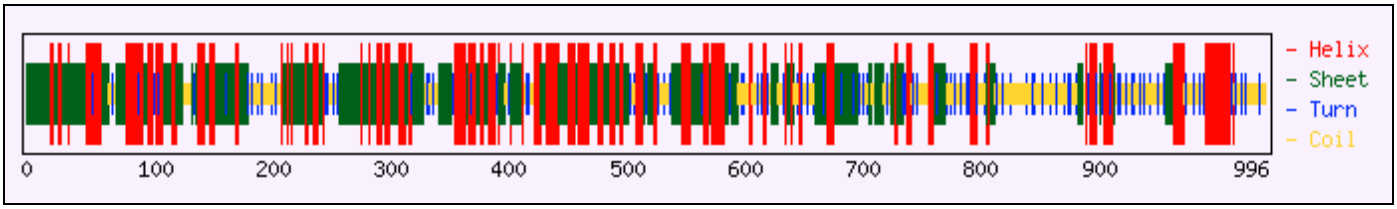
Secondary structure analysis: The secondary structure analysis of this protein was done by CFSSP method and determined total helixes, sheets, turns were representing by table 2 in below and figure. Helix percentage range 50.5-81.6, sheets range 40.6-74.8 and turn 7-15. Protein secondary structures are the alpha-helix and beta-sheet, percentage of these two secondary structures in protein influences protein nutritive value, quality and digestive behaviour.



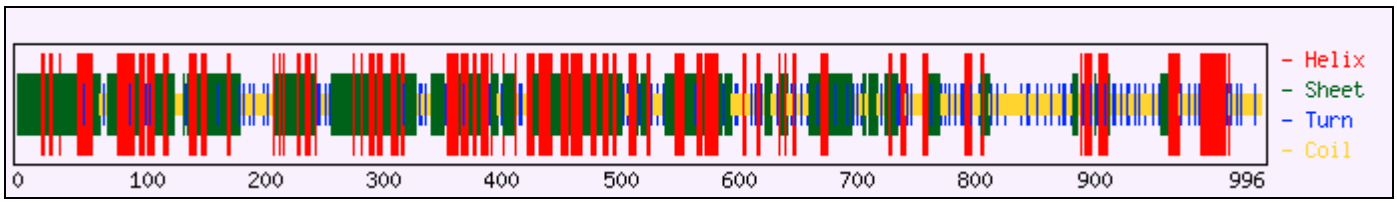
1. [Alosa pseudoharengus]



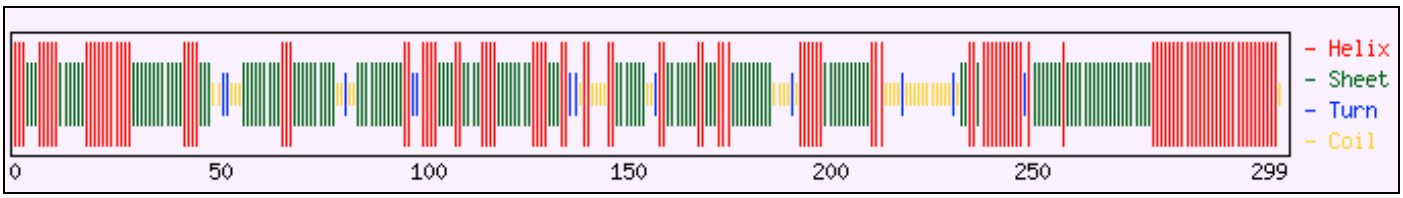
2. [Danio rerio]



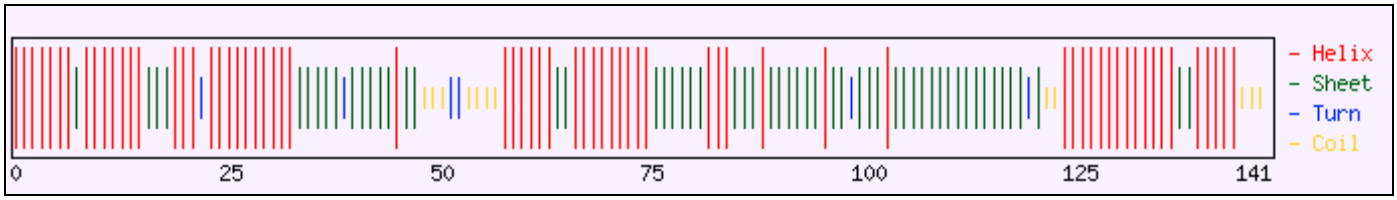
3. 1-like [Scophthalmus Maximus]



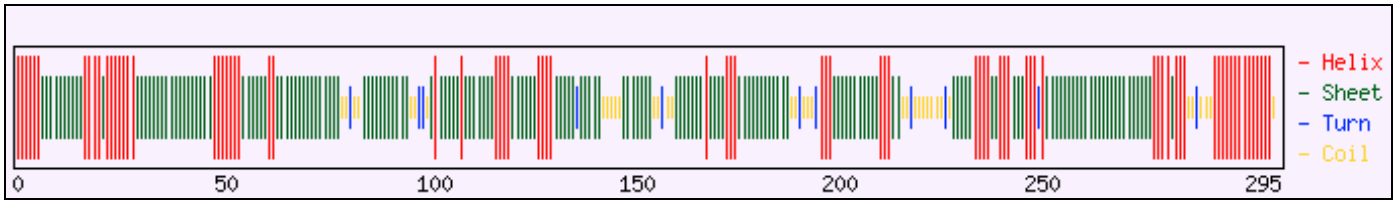
4. 2D -Scophthalmus Maximus



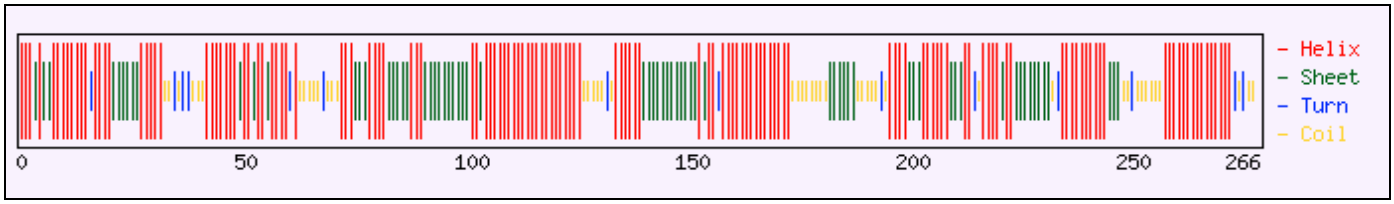
5. 3b -Danio rerio



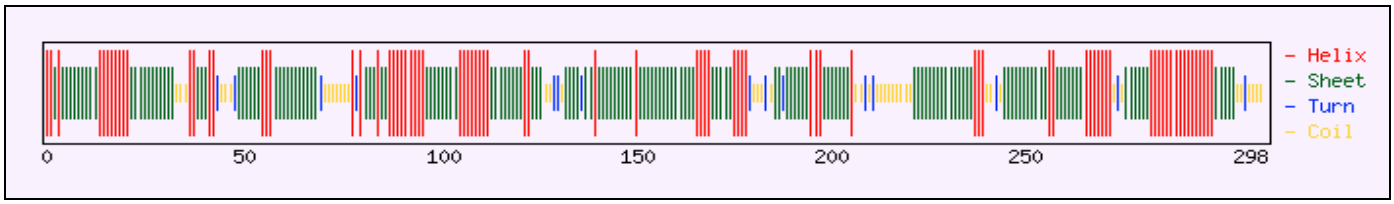
6. Oryzias latipes



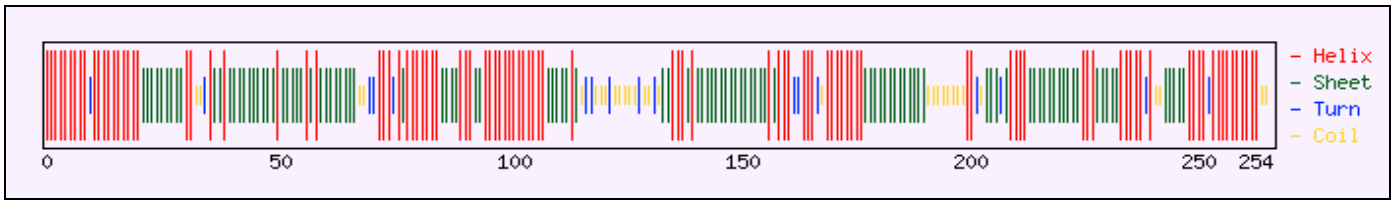
7. Protopterus annectens



8. Solea senegalensis



9. [Sparus aurata]



10. Squalus acanthias

Fig 1: Graphical representation of CFSSP method

Table 2: Representation of helix, sheets, Turn percentage and residues

	Species name	Residues			Percentage		
		Helix H	Sheets E	Turn T	Helix H	sheets E	Turn T
1	[Alosa pseudoharengus]	181	193	27	70.2	74.8	10.5
2	[Scophthalmus maximus]1	503	515	149	50.5	51.7	15.0
3	2D [Scophthalmus Maximus]	503	515	149	50.5	51.7	15.0
4	Oryzias latipes	105	89	13	74.5	63.1	9.2
5	Danio rerio 1a	188	186	30	69.9	69.1	11.2
6	3b [Danio rerio]	244	185	25	81.6	61.9	8.4
7	Protopterus annectens	212	230	22	71.9	78	7.5
8	Solea senegalensis	197	108	27	74.1	40.6	10.2
9	[Sparus aurata]	200	220	21	67.1	73.8	7.0
10	Squalus acanthias	199	156	25	78.3	61.4	9.8

Tertiary structure prediction: The tertiary structure of Aquaporin proteins was performed by the phyre2 server (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index) [47]. Web Lab Viewer Lite 4.2 was used for 3D structure visualization, Swiss model server used for 3D structure prediction [48, 49]. For 3D structure analysis Swiss model template library is use and highest identity showed template is taken for analysis and most suitable template 1j4n.1A is select for analysis which identity shows 63.28%. And then for the correctness of a protein model we verified by the verified 3D. An advantage of using verified 3D profiles for testing models is that profiles have not themselves been used in the determination of the structure.

Fig 2: Template analysed score

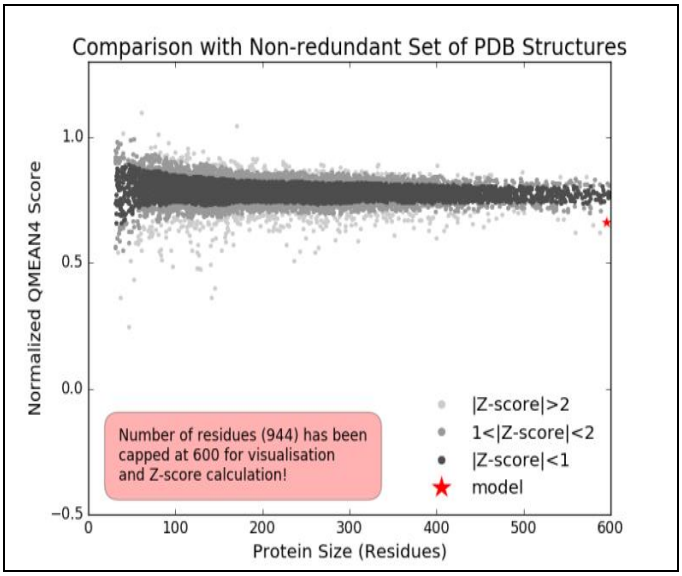


Fig 3: Qmean score

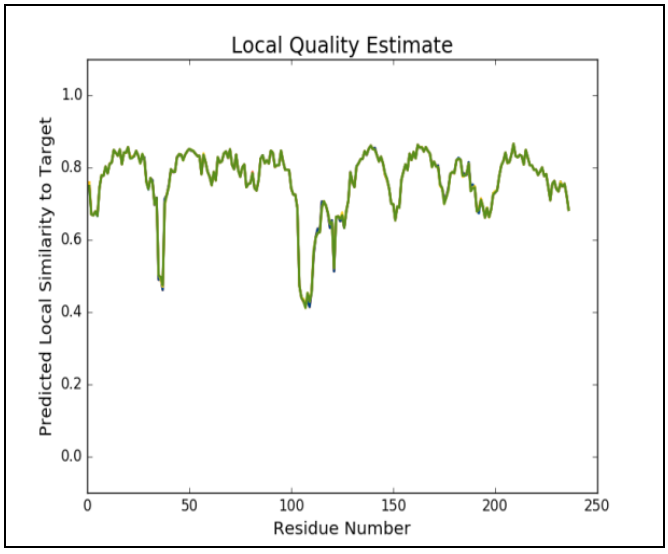


Fig 4: Estimated local similarity

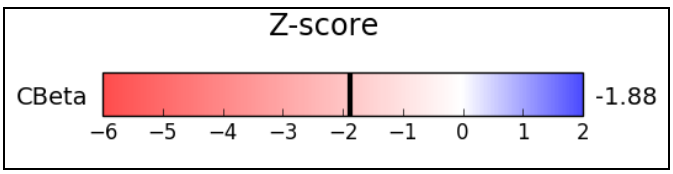


Fig 3.1: Z score determined

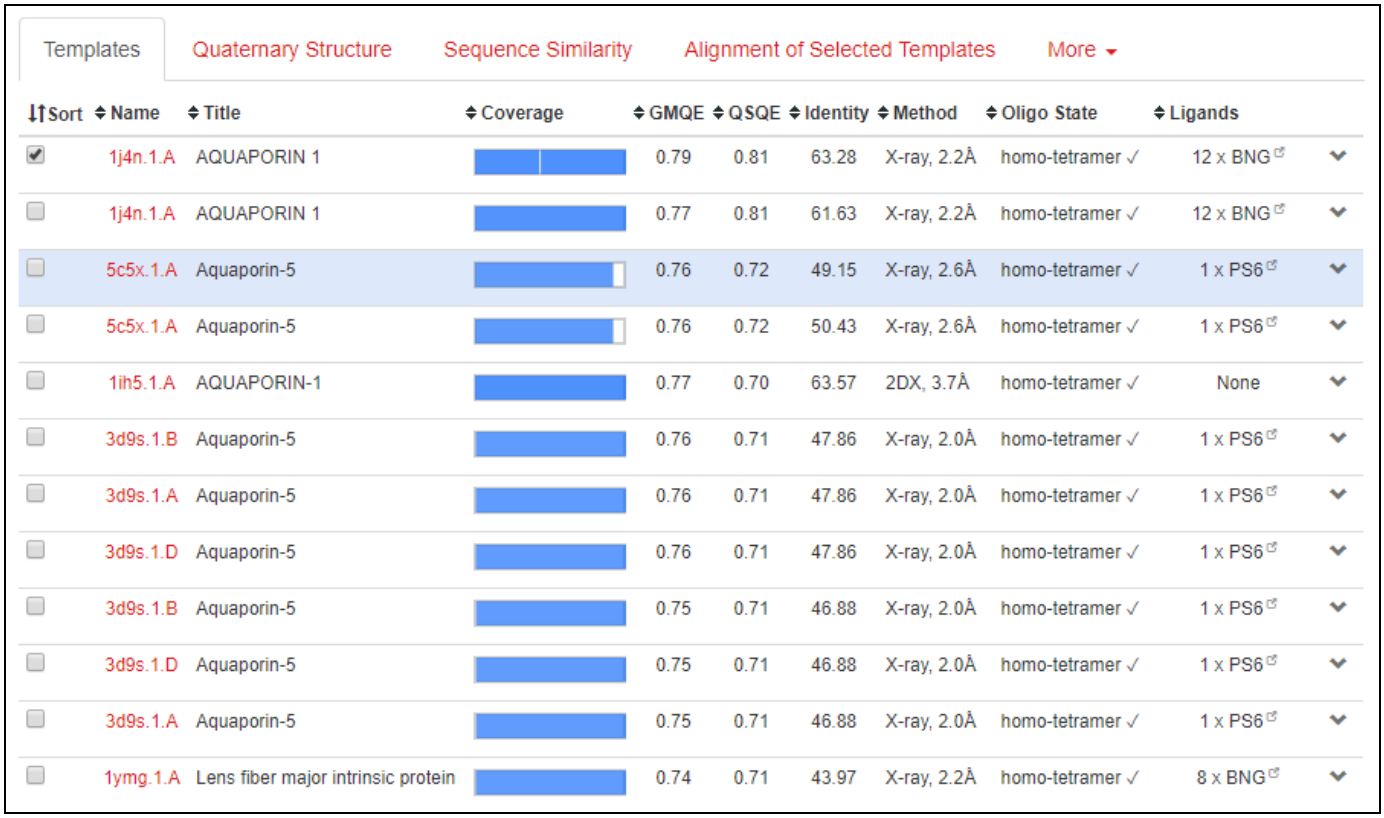


Fig 5: Swiss model template library and template identification

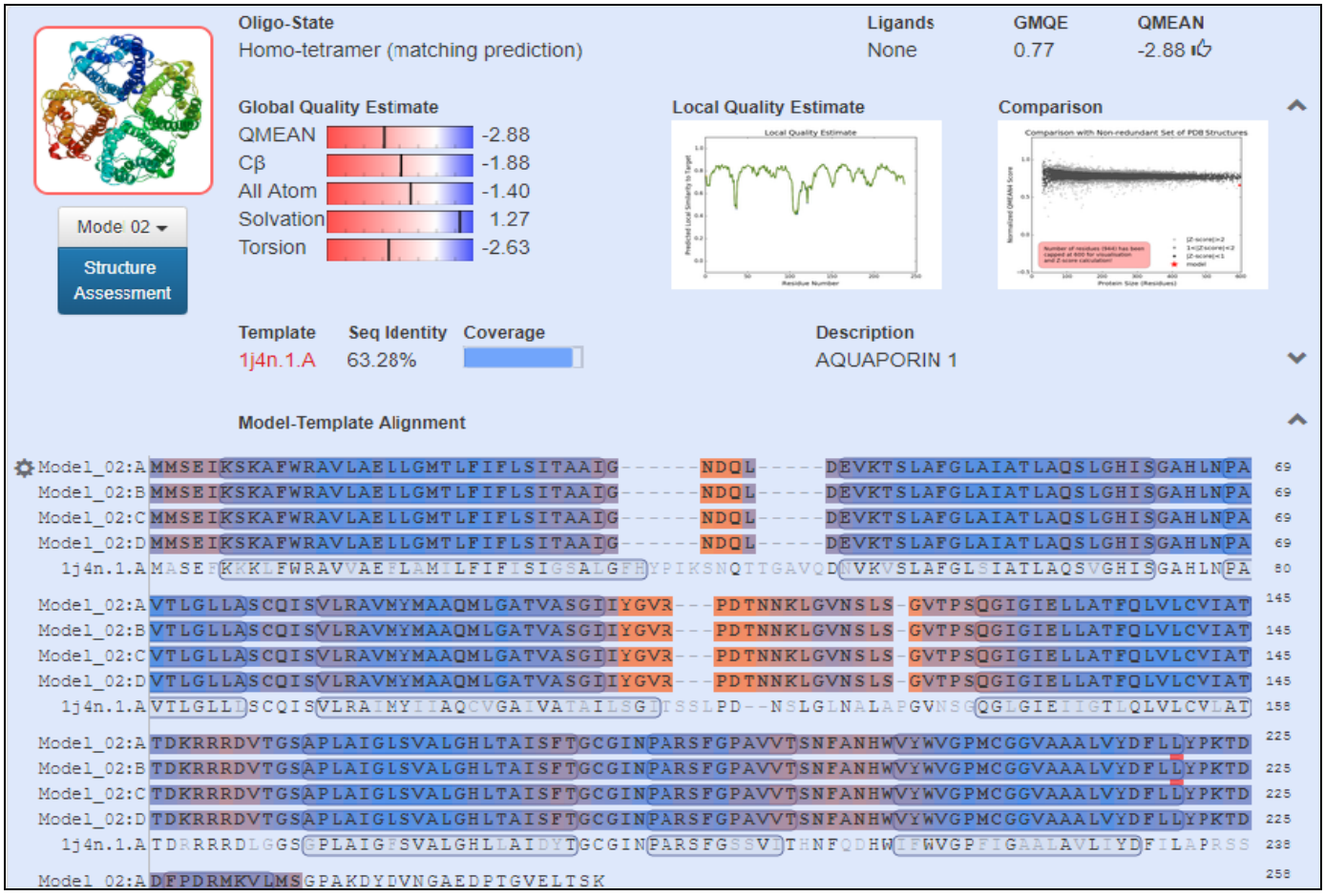


Fig 6: Template sequence identity coverage

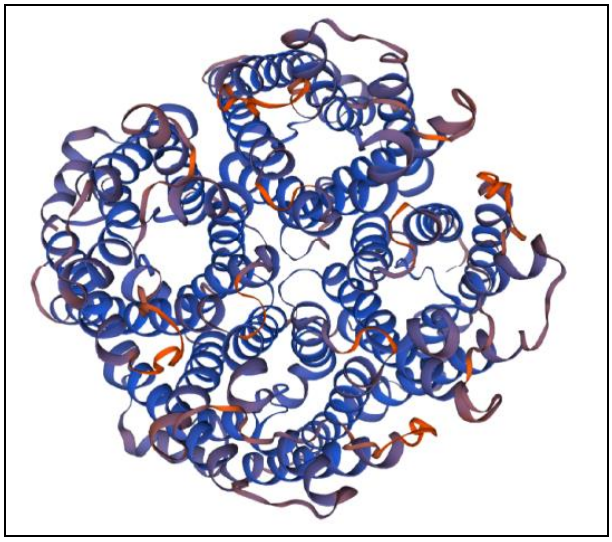


Fig 7: 3D structure of Aquaporin throgh the Swiss model server.

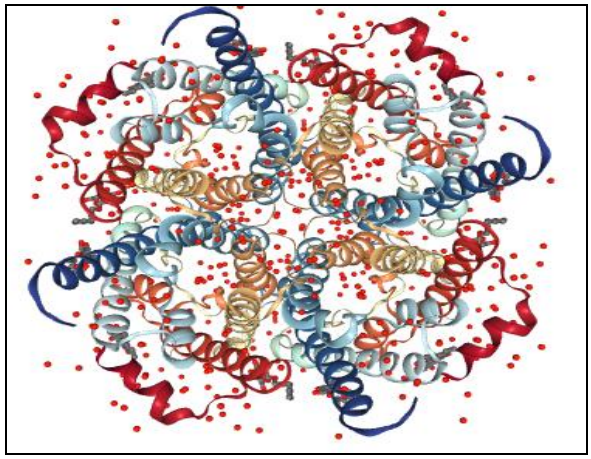


Fig 8: Varification through the varify 3d

Multiple sequence alignment and Phylogenetic analysis: The multiple sequence alignment of Aquaporin Protein was conducted through Mega and Phylogenetic Tree build.

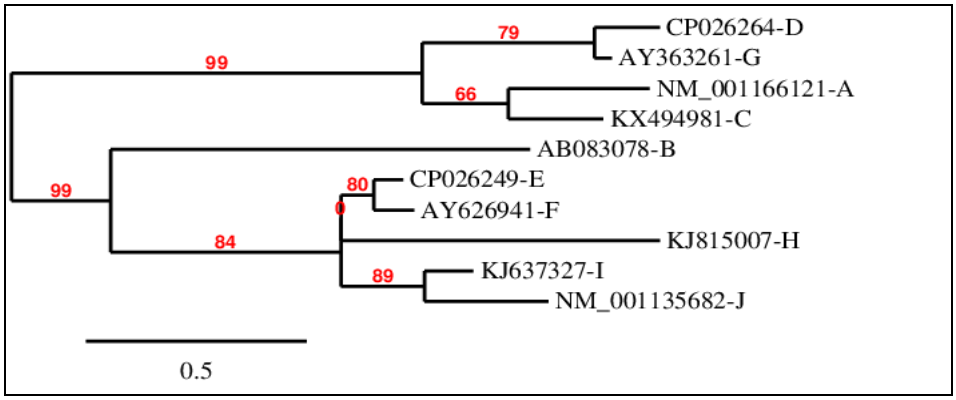


Fig 9: Phylogenetic tree analysis of amino acid sequence of aquaporin

The number of amino acid substitutions per site between sequences is shown. Analyses were conducted using the Poisson correction model [43]. The analysis involved 10 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 126 positions in the final dataset. Evolutionary analyses were conducted in MEGA 7 [44].

M6: Pairwise Distances (C:\Users\acer\AppData\Local\Temp\PhyloAnalysis.meg)

File Display Average Caption Help

	1	2	3	4	5	6	7	8	9	10
1. AIL02123 Alosa pseudoharengus										
2. NP 001129154 Danio rerio	0.272									
3. AWP03544 Scophthalmus maximus	1.469	1.469								
4. AWP21117 Scophthalmus maximus2	1.658	1.658	2.003							
5. NP 001159593 Danio rerio	1.199	1.173	2.064	1.540						
6. BAC20303 Oryzias latipes	1.402	1.402	2.197	2.271	1.792					
7. APG38013 Protopterus annectens	1.075	1.147	1.892	1.701	0.293	1.745				
8. AAV34612 Solea senegalensis	0.382	0.382	1.701	1.504	1.173	1.402	1.225			
9. AAR13054 Sparus aurata	1.199	1.147	1.745	1.892	0.574	1.946	0.588	1.225		
10. AJA30091 Squalus acanthias	0.693	0.725	1.841	1.701	1.225	1.402	1.030	0.793	1.075	

Fig 10: Score of pair wise distance alignment

After Phylogenetic tree analysis we get the highest value of pair wise distance contain between *Oryzias latipes* (BAC20303) and (AWP21117) *Scolpthalmus Maximus* 22.71% *And* Minimum value contain between *(AIL02123) Alosa Psedoharengus, Danio Rerio (NP_001159154) is* 2.72% and (AWP03544) *Scolpthalmus Maximus*. And overall distance all of the species is 1.333. Through the use of mega developed a phylogenetic tree by the NJ methods which are mentioned in table 3 result.

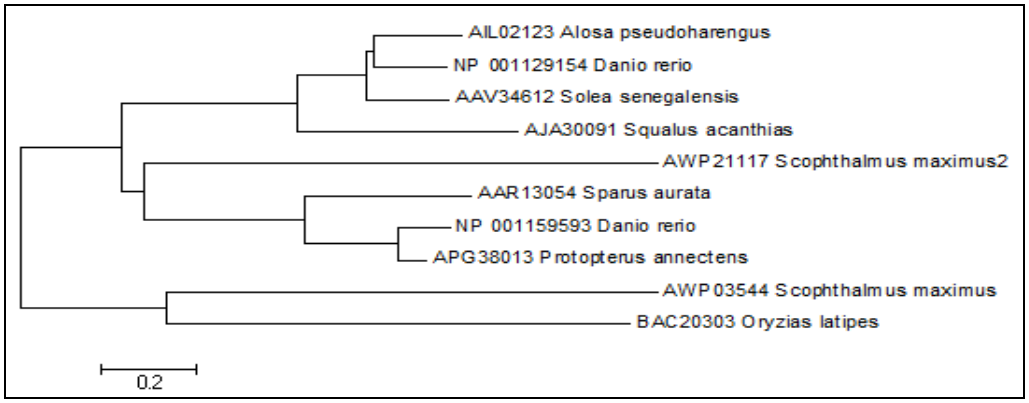


Fig 11: A - Phylogenetic tree

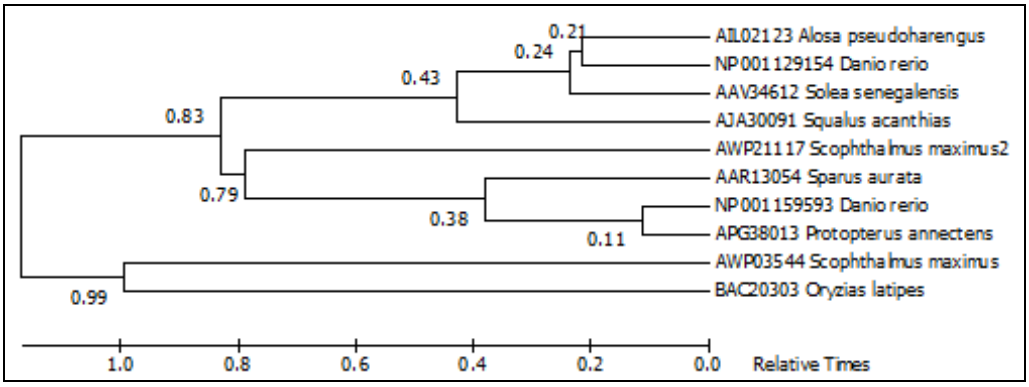


Fig 12: B Evolutionary relationships of taxa (time tree)

The Evolutionary relationship has been established among fishes belonging to different orders. *Alosa Psedoharengus* - *Danio rerio* Scophylus Maximus – *Oryzias Latipes and Danio rerio* -Protopterus annectens are also representing a homophylatic cladistic approach. The evolutionary history was determined through neighbour-joining method [43]. The optimal tree with the sum of branch length = 6.14782466. *Alosa pseudoharengus* and *Danio rerio* are connected by the sister node, and *solea senegalensis* and *Squalus acanthesis* are connected with them by the internal node. All these species maintained their evolutionary distances which are mentioned with build tree. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the Phylogenetic tree the evolutionary distances were computed using the Poisson correction method [44] and are in the units of the number of amino acid substitutions per site. In this tree total 131 positions in the final dataset [45].

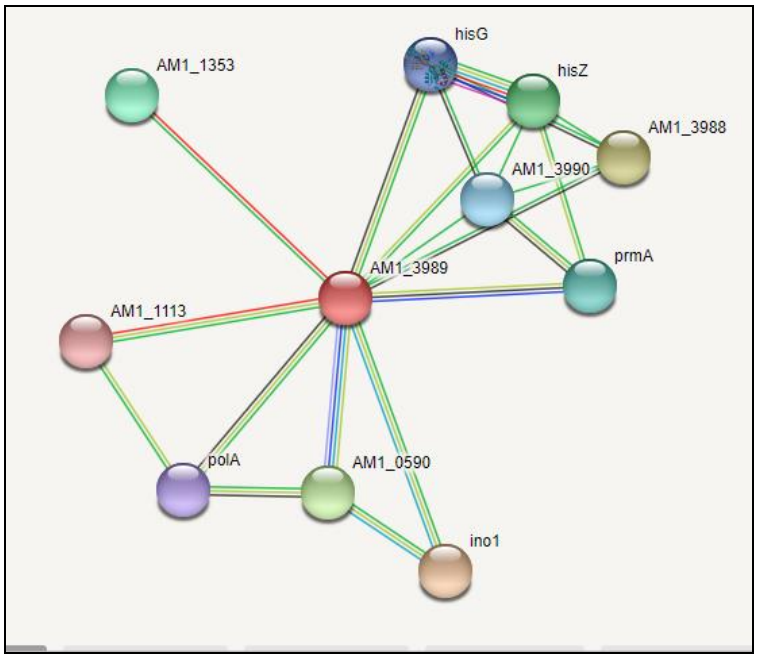


Fig 13: Protein- protein interaction map for the Aquaporin protein of *Acaryochloris marina or MBIC11017*

Your Input:

		Neighborhood	Gene Fusion	Cooccurence	Coexpression	Experiments	Databases	Textmining	[Homology]	Score
AM1_3989	annotation not available (277 aa)									

Predicted Functional Partners:

ino1	annotation not available (367 aa)						●	●		0.910
AM1_3988	annotation not available (121 aa)	●			●					0.883
AM1_0590	annotation not available (271 aa)			●			●	●	●	0.872
hisZ	ATP phosphoribosyltransferase regulatory subunit; Required for the first step of histidine biosynthesis. May allow the feedb...	●						●		0.845
AM1_1353	annotation not available (147 aa)		●							0.773
prmA	Ribosomal protein L11 methyltransferase; Methylates ribosomal protein L11 (312 aa)			●	●			●		0.604
AM1_3990	annotation not available (318 aa)	●								0.601
hisG	ATP phosphoribosyltransferase; Catalyzes the condensation of ATP and 5-phosphoribose 1- diphosphate to form N'-(5'-pho...	●			●			●		0.560
polA	DNA polymerase I; In addition to polymerase activity, this DNA polymerase exhibits 5'-3' exonuclease activity (957 aa)				●			●		0.552
AM1_1113	NAD kinase; Involved in the regulation of the intracellular balance of NAD and NADP, and is a key enzyme in the biosynthesis...		●							0.542

Your Current Organism:

Acaryochloris marina

NCBI taxonomy Id: 329726

Other names: A. marina MBIC11017, Acaryochloris marina, Acaryochloris marina MBIC11017

Fig 14: STRING servers of predicted interacting proteins with the query protein through the string.

Functional analysis: Functional analysis discovered ten potential interacting nodes of AM1_3989 in the protein interaction network resolved by STRING (Fig:-13, 14).The query protein AM1_3989, contain phosphomoestarase. Network nodes represent proteins splice isoforms or post-translational modifications are collapsed, i.e. each node represents all the proteins produced by a single, protein-coding gene locus. Edges represent protein-protein associations, proteins jointly contribute to a shared function; this does not necessarily mean they are physically binding each other.

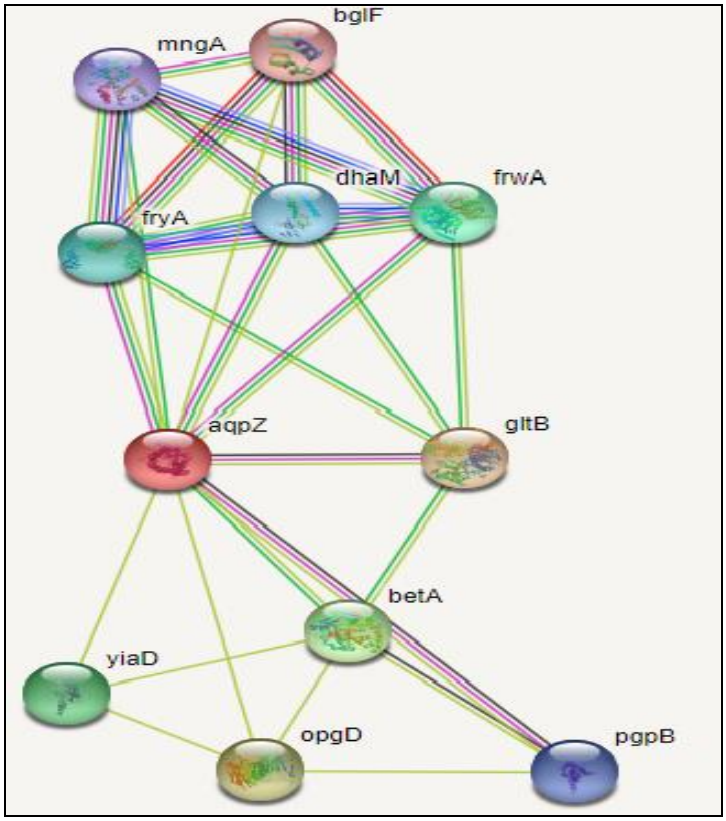


Fig 15: Protein-protein interaction

Aquaporin Z; permits osmotically driven movement of water in both directions, maintain cell turgor during volume expansion of cell. gltB, Catalyzes the conversion of L-glutamine and 2- oxoglutarate into two molecules of L-glutamate (1486 aa). opgD, Probably involved in the control of the structural glucose backbone of osmoregulated periplasmic glucans (OPGs) (551 aa). BetA, Involved in the biosynthesis of the osmoprotectant glycine betaine. yiaD, Suppresses temperature-sensitive mutations in BamB when overexpressed (219 aa). frwA, Multifunctional protein that includes general (non sugar-specific) and sugar-specific components of the phosphoenolpyruvate-dependent sugar

phosphotransferase system (sugar PTS). fryA, Multifunctional protein that includes general (non sugar-specific) and sugar-specific components of the phosphoenolpyruvate-dependent sugar phosphotransferase system. dhaM, Protein deacetylase that removes acetyl groups on specific lysine residues in target proteins. pgpB, Catalyzes the dephosphorylation of diacylglycerol diphosphate (DGPP) to phosphatidate. Mng A, PTS system 2-O-alpha-mannosyl-D-glycerate-specific EIIABC component. bglF, PTS system beta-glucoside-specific EIIBCA component.

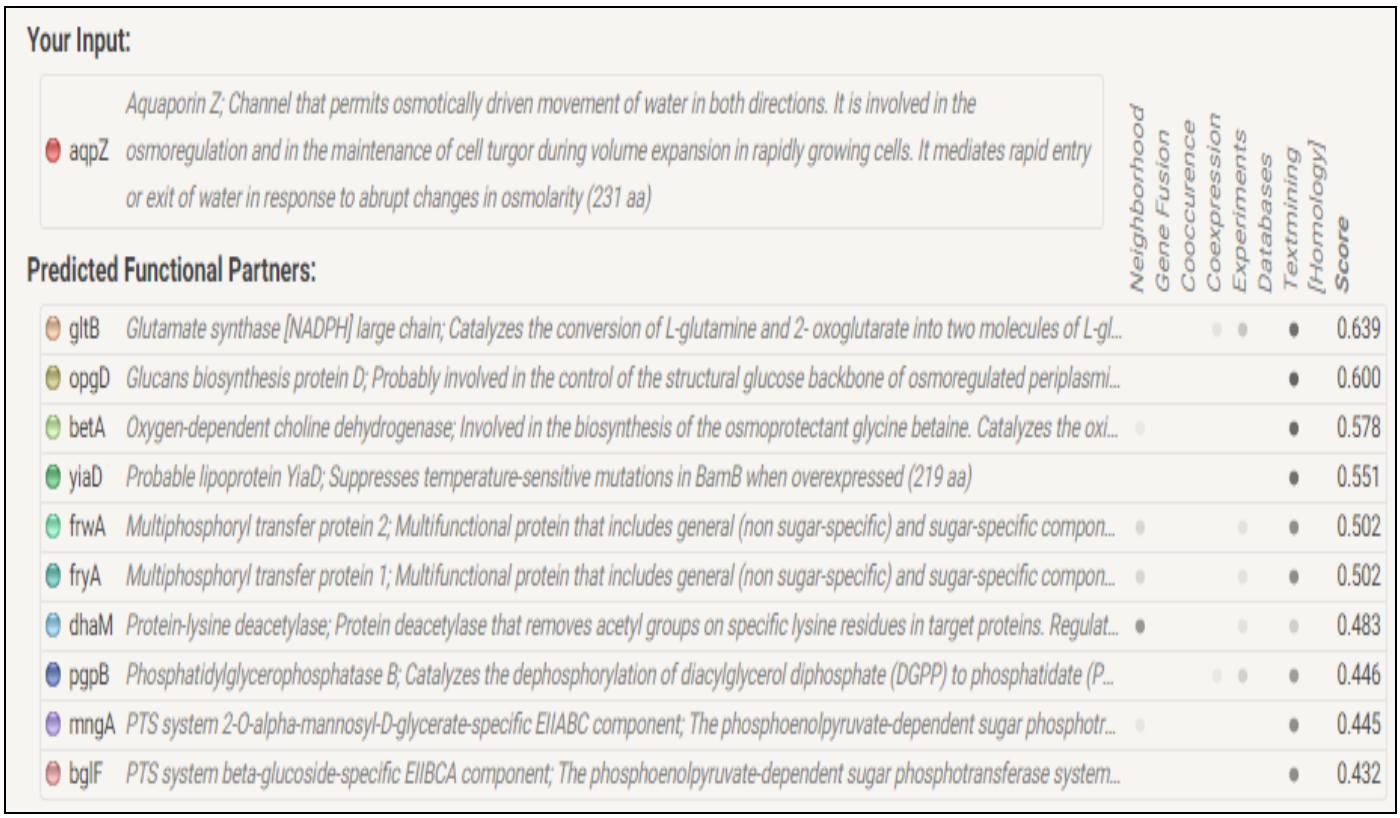


Fig 16: String Analysis for aquaporin

Functional analysis revealed ten potential interacting partners of aqpZ in the protein interaction networks as resolved by STRING. The closest interacting protein having the shortest node was found gltB with score 0.639, while the distant interacting protein having the short node was found bglF with 0.432 and between them opgD 0.600, beta with 0.578, yiaD 0.551, frwA 0.502, dhaM 0.483, pgpB0.446 and mngA 0.445 are present.

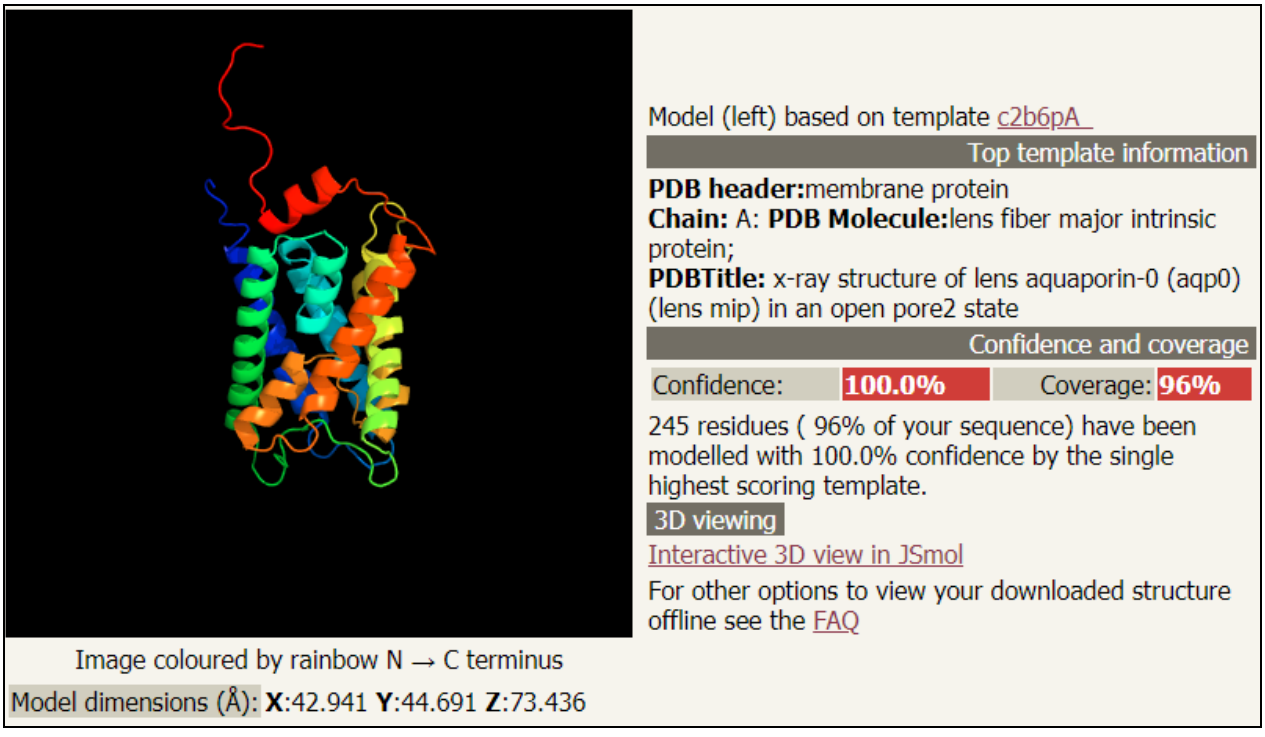


Fig 17: 3D model through the Phyre2 server for *Squalus acanthias* of aquaporin

3D protein modelling, analysis and submission: previously well known template sequence is needed with significant similarly with query sequence to predict 3d dimensional structure. Here the select template sequence is c2b6pA. The sequence identity of the template sequence with query sequence was 96%.

Conserved motif analysis: Conserved motif analyses were done by the Multiple Em for Motif Elicitation (MEME; version 4.9.1) [11] and Motif Alignment and Search Tool (MAST; version 4.9.1) [12]. MEME analyses were applied as follows: Motif Site Distribution - ZOOPS: Zero or one site per distribution, this alphabet has only one strand, Maximum Number of Motifs 3, minimum motif Width 6, maximum width 50, minimum sites per motif-2, and maximum sites per motif 10.

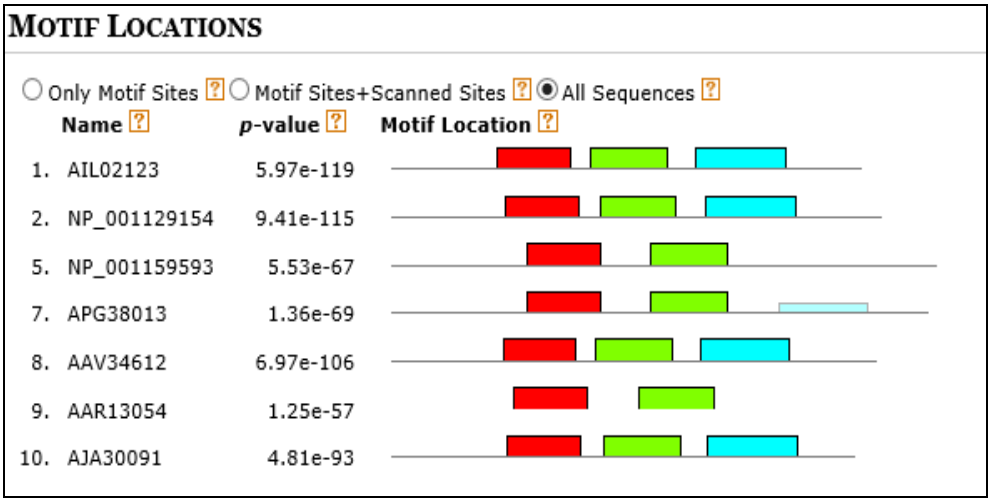


Fig 18: Conserved motif location

Result

Motif analysis of aquaporin protein was performed for finding patterns of conserved motifs and motifs sequences by MEME. The MEME represents motifs as position-dependent letter-probability matrices. It designates the probability of each possible letter at each position in the pattern, where as motifs in MAST are represented as position-dependent scoring matrices, which describe the score of each possible letter at each position in the pattern [21]. 7 conserved motifs site of aquaporin from this 10 fish species sequences protein were determined by MEME (Fig 1), which are listed in Table 2. These result also determined that six motifs were shared by all fishes (Fig 2). In present study, the investigation of Aquaporin protein for 10 species of freshwater species was done by the use of bioinformatics tools. MEME and MAST analyses of proteins were performed in order to find patterns of conserved motifs. Analysis among 10 conserved motifs in 10 different species (Table 2) and (Fig1, 2). Motif 1 (50 aa, E-value=1.0e-1052), motif 2 (50 aa, E-value=5.2e-1013), motif 3 (50 aa, E-value=2.1e-978), motif 4 (11 aa, E-value=5.0e-134), motif 5 (6 aa, E-value=1.8e-039), motif 6 (6 aa, E-value=4.3e-039) were common in 23 species. The motif site result described in table 4 and 5. And amino acids related data those are present in motif site are briefly described in table 6.

Table 4: Conserved motif analysis

Sl. no		
1	Motif Site Distribution	ZOOPS: Zero or one site per sequence
2	Objective Function	E-value of product of p-values
3	Starting Point Function	E-value of product of p-values
4	Site Strand Handling	This alphabet only has one strand
5	Maximum Number of Motifs	3
6	Motif E-value Threshold	no limit
7	Minimum Motif Width	6
8	Maximum Motif Width	50
9	Minimum Sites per Motif	2
10	Maximum Sites per Motif	10

Table 5: Motif site location

1.	AIL02123
2.	NP_001129154
5.	NP_001159593
7.	APG38013
8.	AAV34612
9.	AAR13054
10.	AJA30091

Table 6: Amino acid List for conserve motif Amino acid sequence list of conserved motif

	Name	Freq.	Bg.
A	Alanine	0.0914	0.0914
C	Cysteine	0.0214	0.0214
D	Aspartic acid	0.0384	0.0384
E	Glutamic acid	0.0438	0.0438
F	Phenylalanine	0.0476	0.0476
G	Glycine	0.0983	0.0983
H	Histidine	0.02	0.0199
I	Isoleucine	0.0542	0.0542
K	Lysine	0.0375	0.0375
L	Leucine	0.106	0.106
M	Methionine	0.0286	0.0286
N	Asparagine	0.0304	0.0304
P	Proline	0.0515	0.0515
Q	Glutamine	0.0387	0.0387
R	Arginine	0.0539	0.0539
S	Serine	0.0747	0.0747
T	Threonine	0.0527	0.0527
V	Valine	0.0721	0.0721
W	Tryptophan	0.0155	0.0155
Y	Tyrosine	0.0235	0.0235

After verification we get a PMDB ID PM0082159 through the Protein model database.

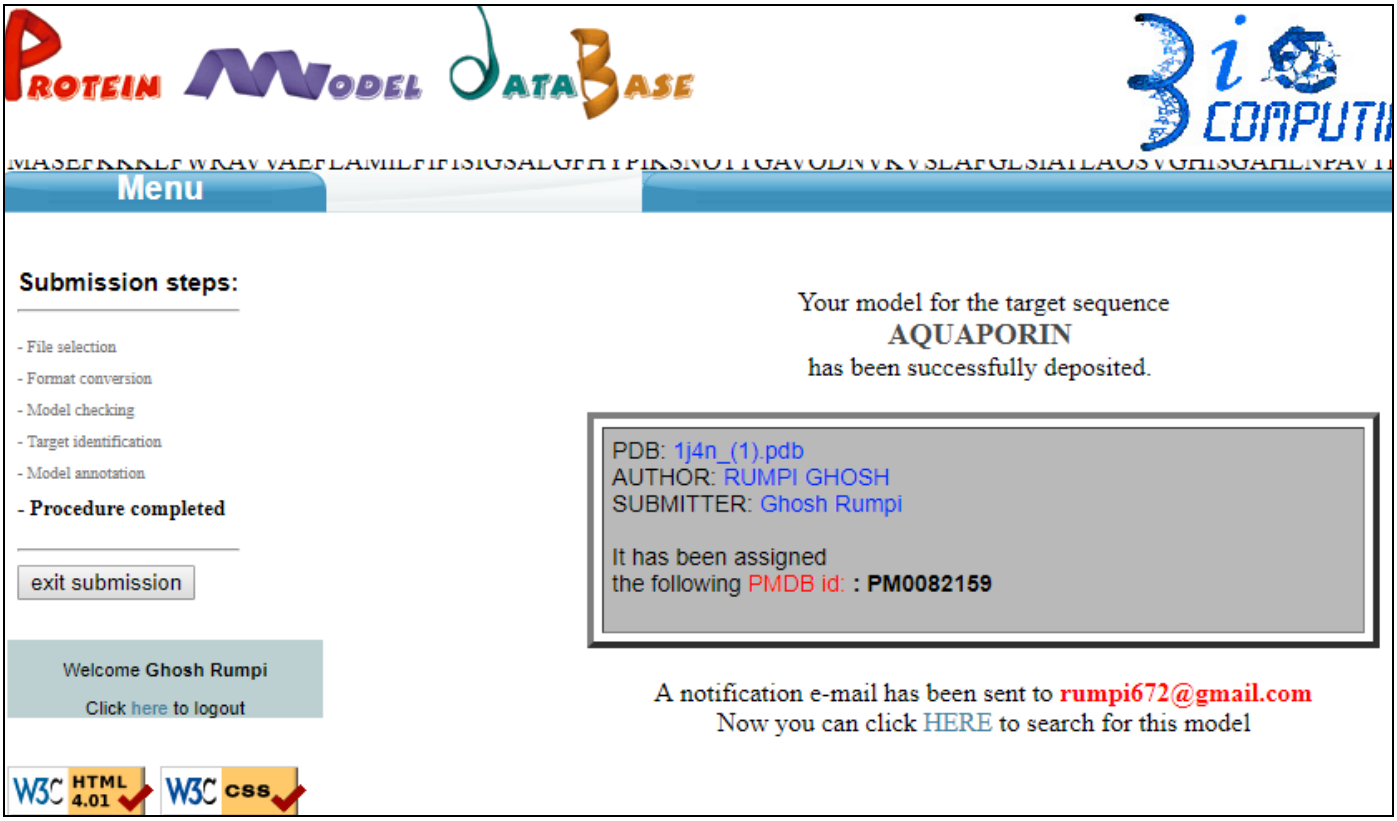


Fig 19: protein model database

Conclusions

Aquaporin, involved in many physiological dysfunctions in sepsis and their expressions are differently regulated. Membrane integral protein Aquaporin responsible for transport of water and other small neutral molecules. Growing evidence points to the involvement of plant aquaporins in CO_2 delivery for photosynthesis. The role of these channel proteins in the transport of O_2 and other gases. Prediction of 3D model of protein by insilico analysis is highly challenging aspect to corroborate the data obtained from the NMR or X-ray crystallographic based methods. Therefore, insilico analysis of protein structure is one of the very useful method for study the structure and function aspects to corroborate where the structural data sometimes not readily available because of the non availability of crystal structures. In this study of Aquaporin protein of this 10 fish sequence pI is 5-6.5 that showing acidic property, Instability index is more than 40 and protein is unstable, Aliphatic Index indicate this protein is quietly thermo stable and GRAVY of this protein indicate better interaction with water and hydrophilic property of this protein. Through the 2D structure analysis we determined the Helix, sheets, and turn. Phylogenetic analysis of aquaporin protein After Phylogenetic tree analysis we get the highest value 22.71% *And* Minimum value *is* 2.72% and overall

distance all of the species is 1.333. And finally correctness of this analysis verified through PMDB and PMDB provide ID PM0082159.

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