International Journal on Recent and Innovation Trends in Computing and Communication ISSN: 2321-8169
Volume: 2 Issue: 10 3208 – 3215

Bioinformatic Analysis, Structure Modeling and Active Site Prediction of Aquaporin Protein from Catfish Heteropneustes fossilis

Amisha Singh, Radha Chaube*
Zoology Department, Mahila Mahavidyalaya, Banaras Hindu University, Varanasi – 221 005, India.
*Corresponding author: chauberadha@rediffmail.com
Tel: 091-0542-2317546, Fax: 091-0542-2368174 (BHU)

Abstract- Aquaporins (AQPs) are membrane channel proteins which facilitate the rapid transport of water across cellular membrane and are of fundamental importance to the control of cell volume and transcellular water traffic. In the present study using bioinformatic tools an *in silico* modeling and analysis of aquaporin protein sequences of catfish *Heteropneustes fossilis* was conducted. Primary structure prediction and physiochemical characterization were performed by computing theoretical isoelectric point (pI), molecular weight, extinction coefficient, instability index and aliphatic index. Secondary structure assessment of aquaporin protein of Indian catfish using GOR IV reveals greater percentage of residues as alpha helix and random coils against the beta sheets. After performing homology modeling using MODELLER, a 3D structure of aquaporin of Indian catfish have been predicted from its amino acid sequence. After the prediction structure has been validated through various validation tools. This homology modeling based structure will provide an insite to its functional aspect and further studies which are based on tertiary structure of protein. Active site of aquaporin protein has been predicted using DoGSite scorer. Analysis of aquaporin active site will provide an insight to more précised functional characterization of this protein. In future, present study will allow the designing of mutant and inhibitors for the study of physiological role of aquaporin protein in *Heteropneustes fossilis.*

Keywords- *Aquaporin; catfish; physiochemical properties; structural comparison; RMSD; active site*

*****

I. INTRODUCTION

Aquaporins are trans-membrane intergral proteins which belongs to the superfamily of major intrinsic protein (MIPs) which facilitate bi-directional and passive transport of water across the lipid bilayer of cell membrane. Another member of MIPs which plays similar role to aquaporin is aquaglycerophorin which transports small molecules and glycerol across the lipid bilayer [1-2]. Over 200 aquaporin sequences have been found over wide range of organisms [3]. These aquaporin channel proteins are found both in prokaryotes and eukaryotes and plays a major role in maintaining fluid homeostatsis [4]. Approximately 30 years ago, aquaporins were discovered when a study was carried out regarding the system involved in the rapid movement of water across the biological membrane in Rh cell antigen. Aquaporins are located as homo-tetramer with each subunit having individually functional waterpore [5]. Early studies of aquaporin 1 pointed to an "Hour Glass Model" in which repeated sequences were responsible for forming the pore. These repeated sequences are NPA (Asn-Pro-Ala) motif which is characteristic and identifying feature of aquaporin protein [6]. When we move towards the classification of aquaporin, till now 13 aquaorins (0-12) has been discovered in mammals, but in case of lower vertebrates less information is available. Some work has been carried out in zebrafish (*Danio rerio*) and 18 sequences has been reported which are structurally reported to the four superfamilies of tetrapod aquaporin. Aquaporins (AQP0, AQP1 and AQP4) are water transporter, aquaglycerophorins (AQP3 and AQP7-10), urea transporter (AQP8) and two unorthodox aquaporins (AQP11 & AQP12) [7]. Aquaporin protein have six membrane spanning alpha helices and both C and N termini facing the cytosol. Two loops are found in aquaporin protein: cytosolic domain and extracytosolic domain. Cytosolic loop lies between second and third transmembrane domain (B) and extracytosolic domain lies between the fifth and sixth transmembrane domain (E). These two loops form short hydrophobic helices that clip halfway into the membrane from opposite sides. These two loops generally contain conserved NPA (Asn-Pro-Ala) motif. Asparagine residues are key to the formation of water selective filter [8].

In case of mammals, 13 isoforms of aquaporins have been identified and studied but still less information is available regarding the aquaporin in fishes [9-11]. In the current study our focus is on the full *in silico* analysis and modeling i.e homology modeling of Aquaporin-1b (ovarian aquaporin) of Indian catfish *Heteropneustes fossilis.* Aquaporin 1b of Indian catfish belongs to class I of aquaporin obtained from ovary [12]. Aquatic animals and fishes face various osmotic challenges in maintaining body fluid homeostasis. So, the study of aquaporins in these animals is of great importance. Experimental techniques (NMR and X-ray Crystallorgaphy) used for predicting the 3D structure of proteins are very much tedious and

prolonged, also not always succeed in determining structure of protein especially in case of membrane protein like our aquaporin protein. Thus, homology modeling approach via *in silico* method is a good option for predicting the structure of protein for further functional analysis study. Another importance of homology modeling approach is that it is very fast method for predicting the structure of protein as it is well known fact that there are far more sequences of protein as compared to their solved structures, so a fast approach is required for predicting its structure. Homology modeling will reduce this gap. Homology modeling of a protein is the method of constructing an atomic resolution model of a protein from its amino acid sequences("target protein") using an experimental 3D structure of a related homologous protein ("template"). Homology modeling is based on the concept that the protein sequences among homologues and identity above 30 % in sequence is likely to give the similar structure. Homology modeling consists of 5 steps :-(i)Fold assignment in which the similarity between the target sequence of interest and at least of one known protein structure (the template) is identified. (ii)Target sequence and template sequence alignment. (iii) Model building based on chosen template(s). (iv) Model optimization. (v)Model validation [13]. As the 3D structure of aquaporin protein of Indian catfish have yet not been build and in order to study the functional properties of protein more confidently, the objective of this work is to build the model of this protein using comparative modeling approach. It will provide insight into its structure and further some functional aspect.

II. METHODS

Sequence analysis of Aquaporin- 1b of *Heteropneustes fossilis*

Aquaporin-1b protein sequence of Indian catfish *Heteropneustes fossilis* (Accession no. ADK87346.1) was obtained from NCBI. The above obtained sequence was further used for complete protein sequence analysis (structural and functional annotation) and model building using comparative modeling approach. UsingExPasy's Protparam Server (http://expasy.org/cgi-bin/protparam) complete primary structure analysis of protein has been performed. GOR IV was used for secondary structure prediction of protein sequence of Aquaporin-1b.

Homology Modeling of Aquaporin-1b Protein

The modeling of the three dimensional structure of protein was performed by MODELLER automated modeling package. MODEL build by the MODELLER is based on the quality of the sequence alignment by BLAST and template used for the model building. To study the functional aspect of protein, 3D structure is more valuable rather than sequence. 3D structure of aquaporin-1b from Indian catfish has been modeled by homology or comparative modeling approach using MODELLER.

MODELLER is a computer based program used for producing homology model of proteins tertiary structure. It is based on a technique Spatial Restraints which is similar to Nuclear Magnetic Resonance. It uses a set of geometric criteria which creates a probability density function for the location of each atom in the protein [14]. After the building of the model, energy minimization was performed using $KOBa^{MIN}$(Knowledge Based Potential Refinement for Protein) [15]. Structure building itself is not sufficient to obtain the correct 3D structure unless the model has been evaluated for its accuracy. So in order to evaluate the model of the aquaporin structure obtained from MODELLER , RAMPAGE [16] and PROCHECK server [17] and ERRAT **(** http://nihserver.mbi.ucla.edu/ERRAT/) has been used.

Active site prediction of Aquaporin-1b protein of *Heteropneustes fossilis*

Protein interactions are important from the aspect of the cellular function and determining how these proteins interact with their ligands and other small molecules[18]. Predicting active site of the *in silico* modeled protein is further of great aspect as it provides more précised characterization of protein from functional point of view. Active site of aquaporin protein of *Heteropneustes fossilis* was predicted using DoGSite Scorer server based on grid based function prediction method [19].

III. RESULTS AND DISCUSSION

Primary Prediction and Physiochemical characterization of Protein

Using Protparam tool provided by Expasy primary structure have been performed and it has been found that it is of 263 amino acid long protein with its molecular weight of 27763.5 Da. Its isoelectric point pI is 5.77 which indicates its acidic property. pI is the pH at which the protein is very much compact and stable. At 280 nm its extinction coefficient has been calculated by the tool and is found to be 36950 with its unit. The computed extinction coefficient will provide help in quantitative study of protein-protein and protein-ligand interactions in solution.

Another important parameter called instability index has also been calculated and is found to be 30.22 which indicates that the protein is stable. Instability index provides the stability of protein in laboratory condition or in test tube. If the value of instability index is less than 40, then it is considered as stable protein [20].

Aliphatic index of protein was found to be 108.67 which indicate that this protein can survive at wide temperature range as the value is quiet high. GRAVY (Grand Average Hydropathy) index provides the interaction with water of a particular protein. Low value of GRAVY index indicates better interaction. Here it is 0.65 which indicates better interaction of aquaporin with water(Figure 1). Solvent accessibility of residues in protein has been calculated. While the prediction of solvent accessibility composition of aquaporin protein, it has been found that the residues exposed with more than 16 % of their surface have the composition of 63.12 % and others are 36.88 %.

Phosphorylation sites have been located in the sequence using NetPhosK 1.0 Server (http://www.cbs.dtu.dk/services/NetPhosK/) . Different kinases like CK II, DNA PK, DNA PK, cdc2, PKA have been found to be involved in the phosphorylation of protein. Highest score was predicted for the site 227 having serine residue (TABLE 1; Figure 2). After analysis, % composition of amino acids has been obtained and is shown in (TABLE 2).

>gi|301641370|gb|ADK87346.1| aquaporin-1b [*Heteropneustes fossilis*]

Sequence: MKELQTLVFWRAVFAELIGTTMFVFYGVCAAVGNGNSSYPDHEVKVALAFGLAVAILSQS
LCHVSGAHLNPAVALAMLVSCQVSVCRALWYVVAQVTGAVIASGIVLGVRPSVVESLGPN
KLNGVSPGQGFGIEFLLTLQLVLCFLATMDKRRDMAGAAPFAIGLSVVMGHLAGISYTGC
GINPARSFGPALVSMEFEHHWVYWAGPLCGGVIAALLYDFILFPRGSDFLARLKVLCHGA
EALDAETEPLLEGGAPEAQWEKA

GRAVY: **0.65019011406844**

Figure 1. GRAVY index of Aquaporin 1b – of catfish *Heteropneustes fossilis.*

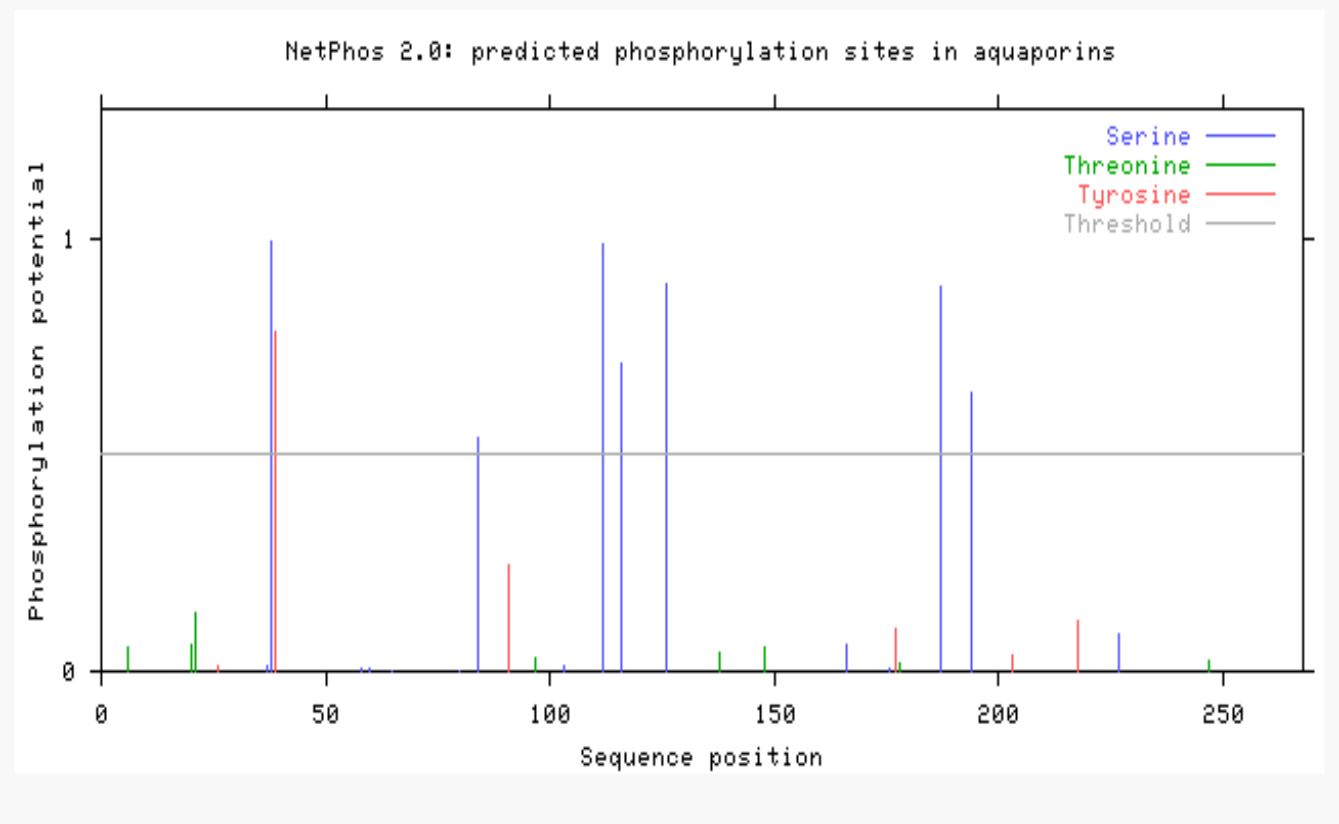

Figure 2. Phosphorylation Plot obtained from NetPhosK1.0 Server

TABLE 1. Phosphorylation site with different kinase

Amino Acid	Site	Kinase
Serine	38	CK II
Serine	38	DNAPK
Serine	58	DNAPK
Serine	60	Cdc2
Serine	103	PKA
Serine	112	PKA
Serine	126	PKA
Threonine	148	PKC
Serine	166	PKA
Serine	187	Cdc2
Serine	194	CK II
Serine	227	PKA
Threonine	247	CKII

TABLE 2. Residues composition of Aquaporin- 1b protein of catfish *Heteropneustes fossilis*

%A: 12.9	**%C: 3.0**	**%D: 2.3**	**%E: 4.9**	**%F: 5.3**
%G: 10.7	**%H: 2.7**	**%I: 3.8**	**%K: 2.3**	**%L: 12.6**
%M: 2.7	**%N: 2.3**	**%P: 4.6**	**%Q: 2.7**	**%R: 3.0**
%S: 6.1	**%T: 3.0**	**%V: 11.0**	**%W: 1.9**	**%Y: 2.3**

Secondary Structure Prediction

Using GOR (tool for secondary structure prediction) regions of different secondary structures have been located in the entire protein sequence. After secondary structural annotation analysis it has been found that highest percentage is of helix (44.49 %), and then of random coil (43.35 %). Extended strand have been found to be 12.17 %. Other secondary structures like 3_{10} helix, bend bridge and bend region were totally absent. Higher percentage of helix composition indicates its helical chemistry (Figure 3; TABLE 3).

10 20 30 40 50 60 70

| | | | | | | |

MKELQTLVFWRAVFAELIGTTMFVFYGVCAAVGNGNSSYPDHEVKVALAFGLAVAILSQSLCHVSGAHLN
ccccchhhhhhhhhhhccceeeeeeeeeecccccccccchhhhhhhhhhhhhhhhhhcccccccccc
PAVALAMLVSCQVSVCRALWYVVAQVTGAVIASGIVLGVRPSVVESLGPNKLNGVSPGQGFGIEFLLTLQ
hhhhhhhhhccceeecccchhhhhhhhchhhhcceeecccccceecccccccccccccccchhhhhhhh
LVLCFLATMDKRRDMAGAAPFAIGLSVVMGHLAGISYTGCGINPARSFGPALVSMEFEHHWVYWAGPLCG
hhhhhhhhhhhhhhhhcccchhhhhhhhhcceeeeeecccccccccccccchhhhhhccceeeeecccccc
GVIAALLYDFILFPRGSDFLARLKVLCHGAEALDAETEPLLEGGAPEAQWEKA
cchhhhhhhhhhccccccchhhhhhhhhcccccchhhhhhhhccccccccceec

Figure 3. Secondary Structure Annotation of Aquaporin 1b of Indian catfish using GOR (h- Helix,e- extended strand, c-Random coil)

TABLE 3 Percentage composition of all the secondary structure present in Aquaporin 1b of catfish *Heteropneustes fossilis*

Alpha helix	(Hh) :	117 is 44.49%
3_{10} helix	(Gg) :	0 is 0.00%
Pi helix	(Ii) :	0 is 0.00%
Beta bridge	(Bb) :	0 is 0.00%
Extended strand	(Ee) :	32 is 12.17%
Beta turn	(Tt) :	0 is 0.00%
Bend region	(Ss) :	0 is 0.00%
Random coil	(Cc) :	114 is 43.35%
Ambigous states (?)	:	0 is 0.00%
Other states	:	0 is 0.00%

Modeling of aquaporin-1b protein of *Heteropneustes fossilis*

As described in the previous section, aquaporin model was obtained using the MODELLER program.

After modeling, MODELLER gives three best hits for the protein of which the best one was with the template**1j4nA** (Crystal structure of Aquaporin1 water channel). Other templates used were **2b6pA**(X-ray structure of lens Aquaporin 0 (AQP 0) (lens mip in an open pore state), **3qd8A** (Crystal structure of Human Aquaporin at 1.8 and its mechanism of conductance).

The region of 3-235 amino acid residues was aligned. The best model was selected on the basis of sequence identity and DOPE score. The DOPE score with the template 1j4n:A was -0.32.

This main model was used for further analysis like energy minimization and model evaluation.

Energy minimized structure of the model provide an insight to the stable structure of the protein with all the residues and the side chain in its minimum energy state which is the stable state. After the energy minimization we get end point whose potential energy is lower than before. Chances are that the structure obtained after the energy minimization is very much similar to its native structure.

After energy minimization with KOBa^{MIN}SERVER , following data have been obtained which indicates the stability and native state of protein:gdt value is 0.9; rms value is 0.51. Here the gdt represents Global Distance Test which is the measure of similarity between two protein structure with identical amino acid sequence and different tertiary structure. RMS is Root Mean square value which represents the average distance between the atoms (usually backbone atoms) of superimposed protein structure.In our case initial protein and reference protein both are the same.

A quiet notable energy change have been found in both states i.e initial and refined. Initial energy of structure was -3116.300 and after refinement it was -7552.8571 kcal/mol. A change of -4436.8271 kcal/mol was observed. After the validation of the model using RAMPAGE server, ERRAT server and PROCHECK assessment the model can be considered as a good quality model as 94.4 % of its residues are in favored region (excluding glycines,prolines and terminal residues). PROCHECK server gives 89.9 % of its residues in allowed region. The ERRAT result gives the overall quality factor of the generated model to be 96.889 % (Figure 4).

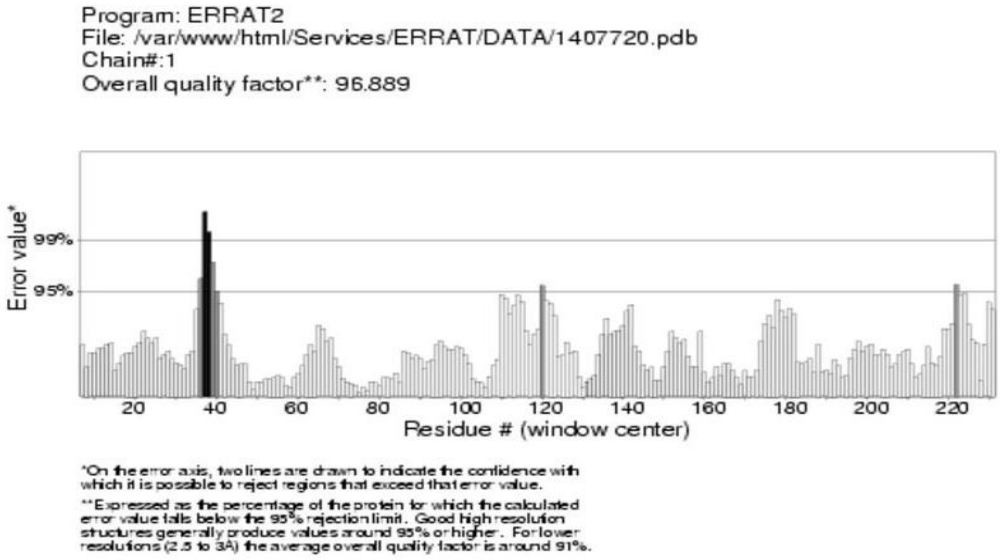

Figure 4. The figure shows the quality of the modeled protein : 96.889 %.

```
gi|301641370|gb|ADK87346.1|     -MKELQTLVFWRAVFAELIGTTMFVFYGVCAAVG-------NGNSSYPDH 42
1J4N_A|PDBID|CHAIN|SEQUENCE     MASEFKKKLFWRAVVAEFLAMILFIFISIGSALGFHYPIKSNQTTGAVQD 50
                                  .*::. :*****.**::.  :*:* .: :*:*       * .:.  :.

gi|301641370|gb|ADK87346.1|     EVKVALAFGLAVAILSQSLCHVSGAHLNPAVALAMLVSCQVSVCRALWYV 92
1J4N_A|PDBID|CHAIN|SEQUENCE     NVKVSLAFGLSIATLAQSVGHISGAHLNPAVTLGLLLSCQISVLRAIMYI 100
                                :***:*****::* *:**: *:**********:*.:*:***:** **: *:

gi|301641370|gb|ADK87346.1|     VAQVTGAVIASGIVLGVRPSVVES-LGPNKLN-GVSPGQGFGIEFLLTLQ 140
1J4N_A|PDBID|CHAIN|SEQUENCE     IAQCVGAIVATAILSGITSSLPDNSLGLNALAPGVNSGQGLGIEIIGTLQ 150
                                :** .**::*:.*: *: .*: :. ** * *  **..***:***:: ***

gi|301641370|gb|ADK87346.1|     LVLCFLATMDKRR-DMAGAAPFAIGLSVVMGHLAGISYTGCGINPARSFG 189
1J4N_A|PDBID|CHAIN|SEQUENCE     LVLCVLATTDRRRRDLGGSGPLAIGFSVALGHLLAIDYTGCGINPARSFG 200
                                ****.*** *:** *:.*:.*:***:**.:*** .*.************

gi|301641370|gb|ADK87346.1|     PALVSMEFEHHWVYWAGPLCGGVIAALLYDFILFPRGSDFLARLKVLCHG 239
1J4N_A|PDBID|CHAIN|SEQUENCE     SSVITHNFQDHWIFWVGPFIGAALAVLIYDFILAPRSSDLTDRVKVWTSG 250
                                .:::: :*:.**::*.**: *..:*.*:***** **.**:  *:**   *

gi|301641370|gb|ADK87346.1|     -AEALDAETEPLLEGGAPEAQWEKA 263
1J4N_A|PDBID|CHAIN|SEQUENCE     QVEEYDLDADDINSRVEMKPK---- 271
                                 .*  * ::: : .    :.:
```

Figure 5. Sequence Alignment of Aquaporin 1b- *Heteropneustes fossilis* with its template 1jn4: A (Human aquaporin) (Shows 54 % sequence identity).

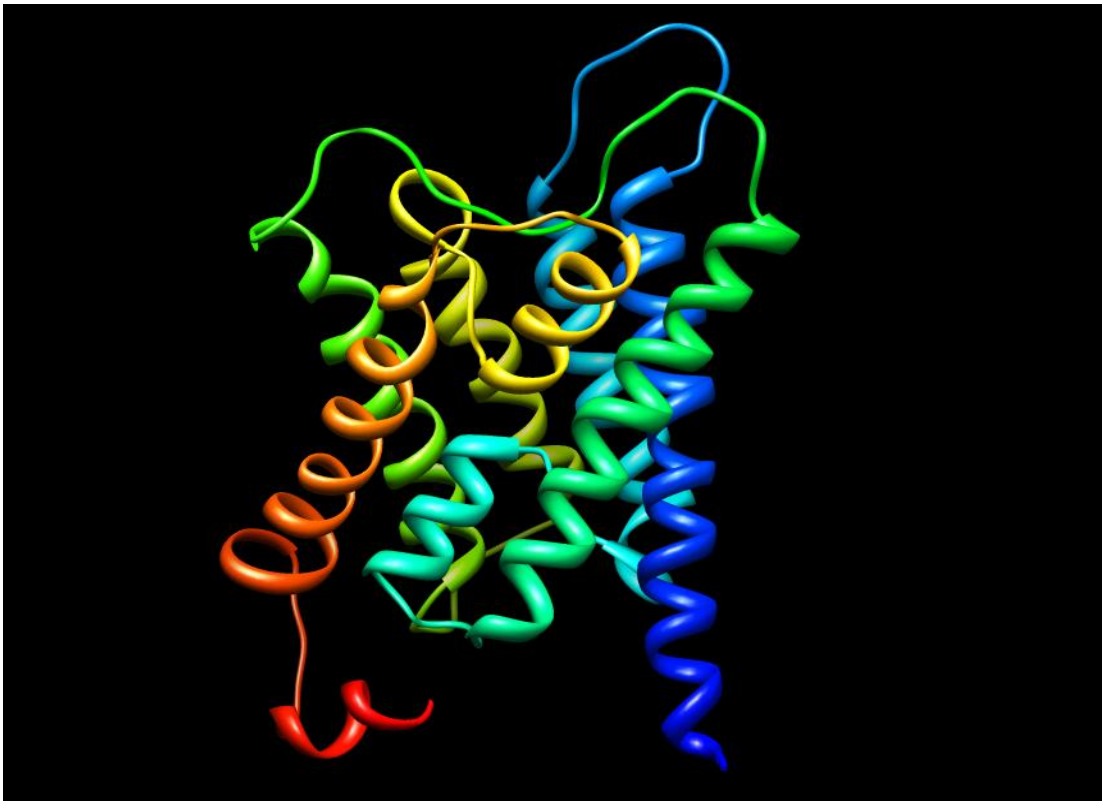

Figure 6. 3D structure of the *Heteropneustes fossilis* Aquaporin 1b using 1j4n :A(Human Aquaporin as template) and energy minimized by KoBaMin Server.

Active site prediction of Aquaporin protein of *Heteropneustes fossilis*

The potential binding pockets and sub-pockets are detected in the predicted structure model of aquaporin- 1b of *Heteropneustes fossilis*. These pocketes are further analysed in tems of their geometrical as well as physicochemical properties. The DoGSite Scorer Server predicts 7 pockets (listed in TABLE 4). The ligand and other small molecules usually interacts with the binding site having largest interacting cavity; in our predicted active sites, active pocket P0 is selected as a potential active site or binding pocket. Descriptions of amino acid composition of this active site are calculated in terms of their apolar amino acid ratio, polar amino acid ratio, positive amino acid ratio. Their respective values are 0.54, 0.29, 0.12. In most favourable binding site amino acids contain high conservation residue score amino acids : Ala, Arg, Asp, Cys, Gln, Gly, His, Ile, Leu, Lys, Met, Pro, Ser, Val, Phe, Thr, Trp and Tyr.

TABLE 4 Pockets and descriptors calculation for aquaporin-1b of *Heteropneustes fossilis.*

Pocket Name	Descriptors				
	Volume ($Å^3$)	Surface ($Å^2$)	Lipo Surface ($Å^2$)	Depth (Å)	Simple Score (Å)
P0	662.98	984.31	754.80	18.39	0.46
P1	429.89	778.97	490.44	18.87	0.25
P2	358.56	530.55	330.28	18.39	0.14
P3	338.56	247.40	204.13	18.97	0.05
P4	200.70	395.43	290.95	10.15	0.10
P5	151.04	428.15	278.43	8.59	0.02
P6	106.64	305.04	294.80	7.17	0.08

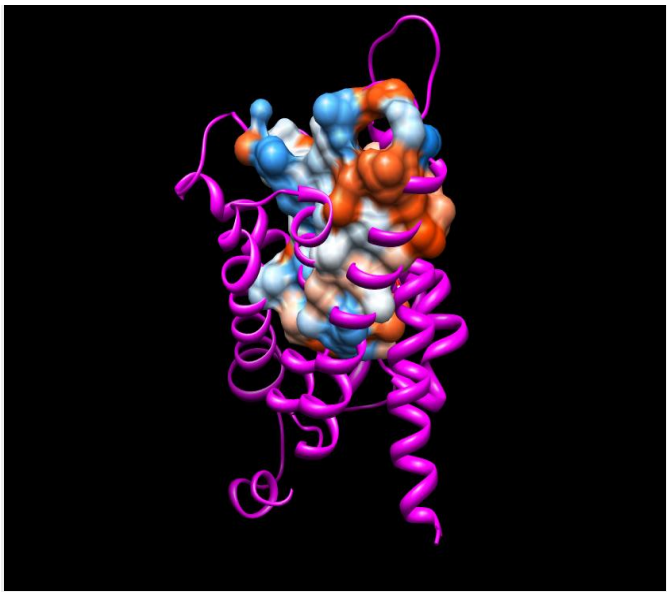

Figure 7. Aquaporin 1b- *Heteropneustes fossilis* with its main active pocket.

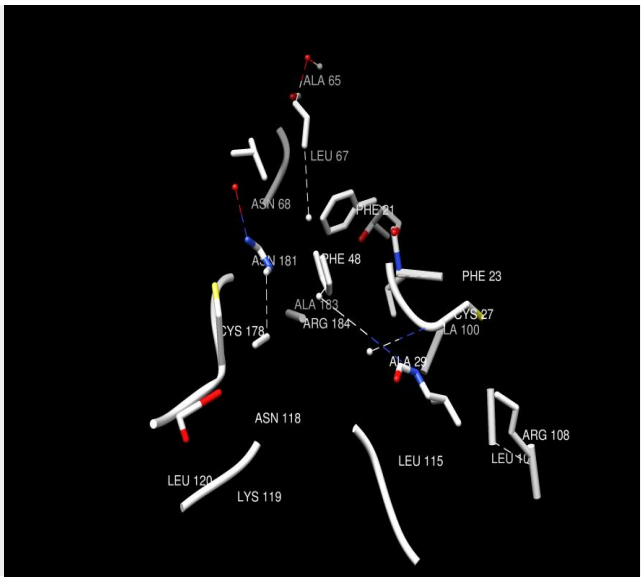

Figure 8. Active site P0 with its amino acid residues of Aquaporin-1b *Heteropneustes fossilis.*

IV. CONCLUSIONS

Aquaporin protein is one of the important protein which plays a crucial role in water and solute transport across the lipid bilayer of the plasma membrane. In this study, aquaporin protein of Indian catfish *Heteropneustes fossilis* was selected and its complete *in silico* study was performed. Primary structure analysis and physiochemical characterization was performed by calculating various indices, GRAVY, molecular weight and pI. Structural analysis was performed after building the model using

MODELLER provided by MODWEB server. The model was energy minimized using KOBa^{MIN} server and validated through ERRAT, PROCHECK and RAMPAGE. This structure will provide the good foundation for functional study of protein as the crystal structure of this protein using X-ray crystallography and NMR have yet not provided. Further, for more precise functional characterization of the protein its active site has been predicted using DoGsite score Server. Active site prediction will provide an insight for the study of ligand or small molecule binding through docking studies. The model obtained here may be used as a starting point for designing various drugs associated with aquaporin dysfunction.The model designed may further be used for the study of physiological role of aquaporin by changing some of the amino acid residues i.e. by some mutations in the structure which effects the functional role of the protein.

Conflict of Interest statement

None declared

V. REFERENCES

[1] A. Bansal, S . Ramakrishnan, "Homology modeling of major intrinsic proteins in rice, maize and *Arabidopsis* : Comparative analysis of transmembrane helix association and aromatic/arginine selectivity filters," BMC Struc Biol , vol.7, pp. 27 , 2007.

[2] S.I Wallace , D. M Roberts, "Homology Modeling of Representative Subfamilies of Arabidopsis major Intrinsic Proteins.Classificaion based on Aromatic/arginine Selectivity Filters," Plant Physiol., Vol. 135, pp. 1059- 1068, 2004.

[3] S . Hohmann, R.M Bill, G .Kayingo , B.A Prior, "Microbial MIP Channels Trends,"Microbiol, vol. 8**,** pp. 33-38, 2000.

[4] E .Migliati , N .Meurice, P . DuBois, J.S Fang , S .Somasekharan , E .Beckett , G . Flynn , J. A Yool, "Inhibition of Aquaporin 1 and Aquaporin 4 water permeability by a derivative of a Loop Diuretic Bumetanide Acting at an internal Pore Occluding Binding Site," Mol. Pharmacol., vol. 76, pp.105-112, 2009.

[5] A.T Sequeira, F . Chauvigne, M .Fabra , J. Lazano, D .Raldua, J . Cerda, "Structural and functional divergence of two fish aquaporin 1 water channels following teleost specific gene duplication," BMC Evo. Biol., vol.8, pp.259, 2008.

[6] J. S Jung, R.V Bhat, G.M Preston, W . B Guggino, J.M Baraban, P .Agne, "Molecular characterization of an aquaporin cDNA from brain: candidate osmoreceptor and regulator of water balance," Proc NaH Acad Sci, vol.91, pp.13052-60, 1994.

[7] A.T Sequeira, M .Calusinska, R.N Finn, F .Chauvigne, J. Lozano, J .Cerda, "The zebrafish genome encodes the largest vertebrate repertoire of functional aquaporins with dual paralogy and substrate specificities similar to mammals," BMC Evol Biol, vol. 10, pp.38, 2010.

[8] C.M Krane, B.K Kishore, "Aquaporins: the membrane water channels of biological world," Biologist, vol. 50(2), pp. 81-86, 2003.

[9] K. Ishibashi , M . Kuwahara , S . Sasaki, **"**Molecular Biology of Aquaporins," Rev Physiol Biochem Pharmaco., vol.141, pp.1-32, 2000.

[10] M.G Presto, "Cloning of gene family members using the polymerase chain reaction with degenerate oligonucleotide primer methods," Mol Bio., vol.69, pp.97-113, 1997.

[11] K .Takata, T . Matsuzaki, Y .Tajika, "Aquaporins: water channel proteins of thecell membrane," Prog. Histochem. Cytochem., vol. 39**,** pp.1-83, 2004.

[12] R .Chaube , F .Chauvigne, A.T Sequeira , K.P Joy, A. Acharjee , V. Singh , J .Cereda, "Molecular and functional characterization of catfish(*Heteropneusteus fossilis*) aquaporin 1b: Changes in expression during ovarian development and hormone induced follicular maturation*,"* Gen and Comp Endocrino., vol.170, pp.162-171, 2010.

[13] E. Krieger, B.S Nabuurs , G. Vriend,"Homology Modeling," Struct Bioinfo vol., 25, pp. 507-521, 2003.

[14] N . Eswar , W . Ben , A.M.R Marc, M. S Madhusudhan, D .Eramian, M. Y Shen, U .Pieper,. A . Sali,"Comparative Protein Structure Modeling using Modeller," Curr Prot Bioinfo. Vol. 5(6), pp. 1-30, 2006.

[15] P. G Rodrigue, L. M Joao, M .Levitt, G .Chopra, "KoBaMIN : a knowledge based minimization web server for protein structure refinement," Nucleic Acids Res., vol.40, pp. 23-328, 2012.

[16] S.C Lovell, I.A.S III Davis, P.I.W de Bakker , J.M Word, G.M Prisant, J.S Richardson, D.C Richardson, "Structure validation by Calpha geometry:phi,psi and Cbeta deviation,"Proteins Structure,Function and Genetics ,vol. 50, pp. 437-450, 1990.

[17] R.A Laskowski., M.W Macarthur, D.S Moss, J.M Thornton, "PROCHECK : a program to check the stereochemical quality of protein structures," J Appl Cryst. Vol.26(2), pp. 283-291, 1993.

[18] N.J Burgoyne, R.M Jackson, "Predicting protein interaction sites: binding hot – spots in Protein- protein and protein-ligand interfaces," Bioinfo vol. 22, pp. 1335-1342, 2006 .

[19] A .Volkamer, D .Kuhn, F .Rippmann, M .Rarey,"DoGSiteScorer : A web-server for automatic binding site prediction, analysis, and druggability assessment," Bioinfo 2012.

[20] K .Guruprasad, B.V.P Reddy, M.W Pandit, "Correlation between stability of a protein and its dipeptide composition: a novel approach for predicting in vivo stability of a protein from its primary sequence," Prot Eng vol. 4**,** pp. 155-164, 1990.