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Comparative Homology Modeling and Physicochemical Characterization of *Cyprinus carpio* Hsp70 Protein

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ABSTRACT

Background: Expression of the heat shock proteins (Hsp) is responsible for the protection from adverse climatic changes particularly heat stress in Common carp (*Cyprinus carpio)*. Although, with advancement of molecular techniques, Hsp70 protein has been isolated but this protein needs to be characterized by both physicochemically and structurally for the functional annotation of fish genome. So this current study was undertaken with aim of generation of various protein models and also for thorough physiochemical characterization of this protein

Methods: In this study, Hsp70 protein of common carp was characterized by both physiochemical and structurally through *insilco* platform and as the crystal structure of this protein is not available, protein models were created though homology modelling upon Modeller version 9.21, Phyre2 and Swiss-model and then the generated predicted models were evaluated through Rampage, Errat, Verify 3D, ProQ and ProSA analysis.

Result: Our investigation showed that this protein is very stable, hydrophilic with no disulphide bonds and the protein models which were generated from this study, are of good quality with z value of - 9.58, -9.48 and -10.93 and quality factor of 82.56, 59.43 and 95.27 respectively. So this study was concluded that the generated Hsp70 protein models would provide an avenue for the other researchers for development of high-throughput gene function assignment in fish.

Key words: *Cyprinus carpio*, Homology modeling, Hsp70, Physicochemical characteristics.

INTRODUCTION

In India, the fishing industry contributes the major economy employing over 14 million people with drastic increased in fish production *i. e* more than tenfold since 1947 and doubled between 1990 and 2010 [Food and Agriculture Organization (FAO) 2011]. The growth in the fish farming sector mainly comes from the freshwater aquaculture sector, as marine fish culture is hardly practiced on a large scale. It has been shown that about 12.8 percent of total animal protein consumed in India comes from freshwater fish (Roy, 2017). Different fishes such as *Labeo catla*, *Labeo rohita* and *Cirrhinus mrigala* has been considered as major Indian freshwater organisms since ancient time, but the common carp or European carp (*Cyprinus carpio)* has been domesticated and introduced into environments worldwide, and is often considered a most destructive invasive species in World (Zhou *et al*., 2003). Moreover, rearing of this common carp is challenging now a days, as these fish are often exposed to various environmental stress, such as sudden change in temperature, high stocking density, trauma, hypoxia, as well as viral and bacterial infections, which often results in huge economic loss to the farmers as well as to the nation (Portz *et al*., 2016). In respond this change in heat stimuli, various proteins are expressed in the fish among which heat shock proteins (HSPs) play an important role in protection from stress stimuli, metabolic insults and during wound healing or tissue remodeling in almost all organisms (Gupta *et al*., 2010). These are highly conserved proteins; perform chaperone-mediated autophagy not only during proper folding of the newly translated proteins but also during refolding of damaged proteins in fish (De Maio, 1999). In addition, it has been believed that HSP70 plays a critical role in formation of receptor complex and facilitates the nodal signaling in fish, by activating different receptors such as activin, nodal, transforming growth factor-β and bone morphogenetic protein (Michiaki *et al*., 2010). This protein also mediates the cellular homeostasis and protects the cells damaged from various environmental stress agents, such as heat shock, chemical exposure and UV or γ-irradiation, Padmini *et al*., 2008. Further, it has been shown that the lethal ammonia toxicity has been protected by endogenous expression of Hsp70 in *Cyprinus carpio* (Sung *et al*., 2012). Keeping in view the above importance, HSP70 has been

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identified and expressed in many fishes such as zebra fish (*Danio rerio*), mandarin fish (*Siniperca chuatsi*), rainbow trout (*Oncorhynchus mykiss*), Korean rockfish (Sebastes schlegeli) as well as Nile tilapia (*Oreochromis niloticus*) (Wang *et al*., 2014) however the structural characterization of fish Hsp70 has not been studied till yet. Although some physicochemical properties of this protein, such as molecular weight, isoelectric point, solubility (as hydrophilic property) and richness in B cells antigenic sites has been characterized, but there are still some questions regarding its tertiary structure. This prompts to undertake this study with aim of structural and functional characterization of existing Hsp70 protein through different bioinformatics tools. Now days the computational structural genomics is becoming an increasingly promising tool for fast and accurate prediction of protein structures, functions and their interactions (Radivojac *et al*., 2013). So this study utilized various computational tools for structural and functional characterization of Hsp70 which would provide a base for the researchers to get the detail scientific informations about the *Cyprinus carpio* Hsp70 protein.

MATERIALS AND METHODS

This experiment was conducted from July 2019 to February 2020 at the Department of Veterinary Biochemistry and Department of Bioinformatics, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha.

Retrieval of Hsp70 protein sequence

Amino acid sequences of the *Cyprinus carpio* Hsp70 (Gene Bank Accession number: AEO44578.1was retrieved from the NCBI protein database (http://www.ncbi.nlm.nih.gov/) in FASTA format as the target template and used for further analyses.

Physicochemical characteristics

The physiochemical properties of *Cyprinus carpio* Hsp70 protein including amino acids composition, molecular weight, theoretical pI, extinction coefficient, absorbance, half-life, instability index, aliphatic index, grand average of hydropathicity (GRAVY) was characterized under Prot Param tools on the expert protein analysis system (Expasy) server https://web.expasy.org/protparam/ (Gasteiger *et al*., 2005). The solubility and sub cellular localization of this protein was predicted by SOSUI (http://harrier.nagahama-i-bio.ac.jp/sosui/) and WoLF PSORT (https://www.genscript.com/wolf-psort.html) software respectively. Hydropathy-plot analysis was performed using the ProtScale server.

Secondary structural characterstics of Hsp70

The secondary structure of Hsp70 protein (α helix, β bridge, β turn, extended strand and random coil) was estimated by using GORIV secondary structure prediction method (Garnier *et al*., 1996). The PROSITE analysis was performed to find out the conserved domain present in *Cyprinus carpio* Hsp70 (Sigrist *et al*., 2012).

Model building of protein *Cyprinus carpio* Hsp70

Homology modeling was performed upon three types of *insilico* platform such as Modeller version 9.21, Phyre 2 and Swiss model. To build the tertiary structure of *Cyprinus carpio* Hsp70 protein, BLAST of the target protein was performed at the NCBI (https://blast.ncbi.nlm.nih.gov/ Blast.cgi? PROGRAM=blastp) by using BLASTp algorithm (Altschul *et al*., 1990) which showed 90% identity to *Bos taurus* (PDB ID: 1YUW) and was chosen as a template (based on its high resolution) to predict the three dimensional structure of *Cyprinus carpio* Hsp70. The modeling of the protein was performed with *Modeller version 9.21* with python script (Webb and Sali, 2016). The protein models generated by the MODELLER were ranked and scored using discrete optimized protein energy (DOPE) score. The best model out of 10 models with the lowest DOPE score was selected. The structural superimposition of refined model with the template (1YUW) *i.e. Bos taurus* Hsp70 was performed using Pymol version 2.3 graphical user interface (GUI). In addition, Phyre 2 web server was used to model the *Cyprinus carpio* Hsp70 protein upon template (PDB ID: 3CDF, chain A) with 100% confidence (Kelley *et al*., 2015). Further, SWISS Modeling was done via SWISS-MODEL server (ProMod3 3.0.0) that aligns the input Hsp70 target protein with pre existing template (PDB ID: 1yuw.1.A) to generate 3 D structure of our target protein (Waterhouse *et al*., 2018). Lastly, Structural analysis was performed and model figures generated by Pymol version 2.3 graphics viewer (DeLano, 2002).

Comparative energy minimization and quality verification of protein models

The comparative stereochemical quality and accuracy of the refined protein model generated by above three *insilco* tools were verified through RAMPAGE, ERRAT, Verify3D server [http://nih- server.mbi.ucla.edu] and the validation of the all model was performed by ProQ and ProSA server for selection of the best one.

RESULTS AND DISCUSSION

Cyprinus carpio Hsp70 protein is one important protein which not only helps in the protection from thermal stress but also act as marker in different malignant melanoma such as renal cell carcinoma (Ramp *et al*., 2007). So detail information regarding physiochemical and structural characteristics of Hsp70 is needed for the functional annotation and for the well management of common carp fish at the farmer level.

Table 1: The *Cyprinus carpio* Hsp70 protein sequence retrieved from the Uni Prot.

Protein name	Uni. Id	Sequence length	Domain	Organism name	Compositional bias
Hsp70 protein	G3LU45	643	11-18 199-212 336-350	*Cyprinus carpio*	410-424

Cyprinus carpio Hsp70 protein sequence

The lengths of the retrieved Hsp70 protein (Uniport Id: G3LU45) showed 643 number of amino acid (Table 1), but there was no crystal structure available in the Uniport protein database, which can be predicted through homology modeling from its amino acids sequence. This protein showed three conserved domains, present in 11-18, 199-212 and 336-350 amino acid region but the status of the protein showed unreviewed (Zhang *et al.*, 2011). That means this protein needs to be physiochemically and structurally characterized thoroughly for further understanding of it functions at the molecular level.

Physicochemical characteristics

In this study, the physiochemical parameters were estimated upon ExPasy ProtParam tool and were shown in Table 2. The result suggested that the average molecular weight and the theoretical pI of *Cyprinus carpio* Hsp70 protein is 70kDa and 5.47 respectively which can be inferred that it is an acidic protein as acidic amino acids such as aspartic acid and glutamic acid are occurred 12% more abundance than the basic amino acids (Kozlowski, 2017). The instability index was found 35.24 which suggests this protein is more stable in nature which may be due presence of large number of various molecular interactions including hydrogen, hydrophobic, electrostatic as well as Vander wall force of attraction (Deller *et al.*, 2016). In this study, the aliphatic index was found 82.83 which indicate that this protein is thermally stable. This is due to large number of hydrophobic interactions which may be inferred that significant number of aliphatic side chain amino acids such as alanine, valine, isoleucine and leucine makes the internal core of this protein (Bischof and He, 2005). Further, the grand average hydropathy (GRAVY) was found -0.419 which indicates that the Hsp70 protein is more hydrophilic in nature which may be due presence of large number of hydrogen bonds in the protein. (Xi *et al.*, 2017). This study also suggested that it is a nuclear protein which might be induced in nucleus during environmental stress (Sampuda *et al.*, 2017). Moreover, no major peaks were found in the hydropathy plot during ProtoScale analysis shown in Fig 1 indicating the protein is more hydrophilic in nature (Kyte and Doolittle, 1982).

Structural analysis

The analysis of secondary structure of *Cyprinus carpio* Hsp70 predicted that there is more abundance of α helix (43.86%), extended strand (16.02%) and Random coil (40.12%) in the protein shown in Table 3 which can be inferred that this protein is very stable in nature. It might be due to extensive intra strand hydrogen bonding by the side chain of the amino acids (Fujiwara *et al.*, 2012) and the three conserved domains have been identified upon ProSite shown in Fig 2 which are at 11-18, 199-212, 336-350 amino acid length. It may be due to the protein has conserved and plays in similar function across species (Shennan *et al.*, 2020).

Table 2: Physicochemical characteristics of *Cyprinus carpio* Hsp70 protein.

Parameters	Value
Number of amino acids	643
Molecular weight	70461.70
Theoretical pI	5.47
Total number of negatively charged residues (Asp + Glu)	93
Total number of positively charged residues (Arg + Lys	81
Instability index	35.24
Aliphatic index	82.83
Grand average of hydropathicity (GRAVY)	-0.419
Solubility	Soluble
Hydrobhobicity	-0.418507
Sub cellular localization	Nuclear

Table 3: Secondary structure of *Cyprinus carpio* Hsp70 protein.

Types	Alpha helix	Extended strand	Random coil
Percentage	43.86%	16.02%	40.12%
Length	282	103	258

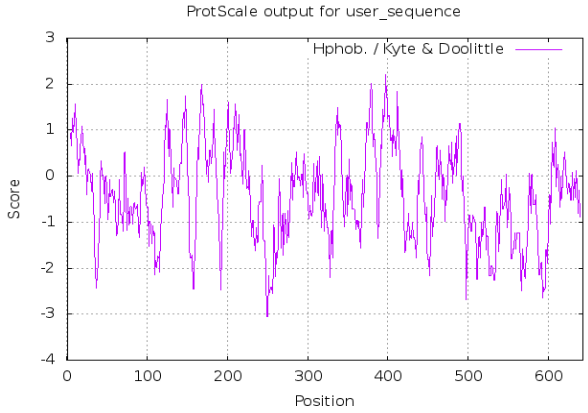


Fig 1: Showing hydropathy plot.

Fig 2: Showing conserved domain of the protein.

Homology modeling

As there is no avaibility of crystal structure for *Cyprinus carpio,* Hsp70, Modeller 9.21, Phyre 2 and Swiss model were used for prediction of protein models. Our study revealed that the *bos tautus* (PDB ID: 1YUW) has been solved to a resolution of 2.6 Å and chosen as the template for Modeller version 9.21. Further the alignment of the amino acid sequences of the Hsp70 protein from *Cyprinus carpio* (UniProtKB ID: G3LU45) and *bos taurus* (PDB ID: 1YUW) resulted identity of 89.87% which might be due presence of conserved amino acids which mediate common function in both of species (Jiang *et al*., 2005).

The Modeller 9.21 resulted ten protein models with different discrete optimized protein energy (DOPE) shown in Table 4. The model structure such as target. B99990002 .pdb shown in (Fig 3A) with the lowest DOPE score (- 59973. 63672 Kcal/mol) was assessed and subsequently used for further analyses as protein attains the lowest free energy during the native state (Rath *et al*., 2016). Further, the structural superimposition of, Hsp70 of *Cyprinus carpio* model with *bos tautus* (chain A) both before and after energy minimization (Fig 3B) revealed a root mean square deviation (RMSD) score of 0.108 Å indicating that the structures were closely related (Kufareva and Abagyan, 2012).

Phyre 2 was used to predict the *Cyprinus carpio* Hsp70 protein by single highest scoring template *i.e*. heat shock protein homolog sse1 (Chain C) (PDB ID: C3D2F) with 100% confidence and 88% identity shown in Fig 4A (Basyuni *et al*., 2018).

Table 4: Showing ten *Cyprinus carpio* Hsp70 protein models created upon modeller 9.21.

Models	molpdf	DOPE score	GA341 score
target.B99990001.pdb	4347.41113	-58783.83594	1.00000
target.B99990002.pdb	3990.09668	-59973.63672	1.00000
target.B99990003.pdb	4256.23828	-58263.72656	1.00000
target.B99990004.pdb	4090.98999	-59038.93750	1.00000
target.B99990005.pdb	4785.39844	-57954.79688	1.00000
target.B99990006.pdb	3984.65552	-59403.37500	1.00000
target.B99990007.pdb	4035.34033	-59396.20703	1.00000
target.B99990008.pdb	3982.48047	-59577.96484	1.00000
target.B99990009.pdb	3839.16968	-59038.03516	1.00000
target.B99990010.pdb	4155.04736	-59362.96875	1.00000

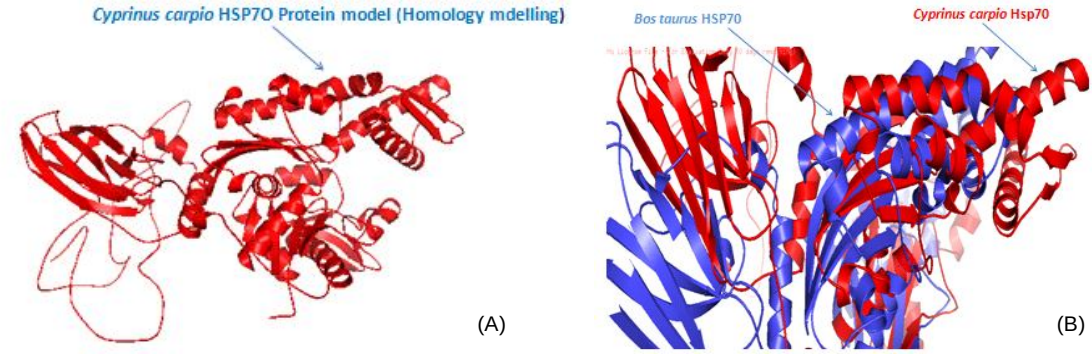


Fig 3: Protein model generated by modeller 9.21 presented in (A); The structural super imposition between Hsp70 (Red color) of *Cyprinus caprio* and template (PDB ID: 6AJ5, Chain A) (Blue color) of Bos taurus deciphered in (B).

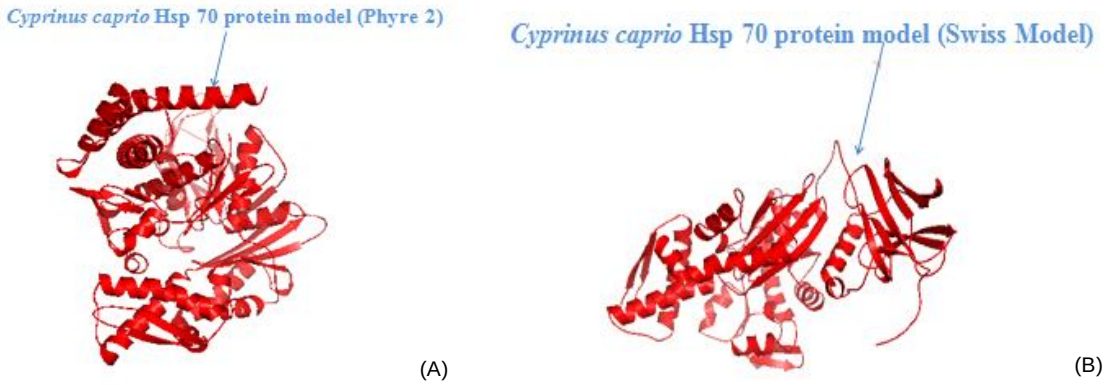


Fig 4: Protein model generated by Phyre 2 and Swiss modeling deciphered in (A) and (B) respectively.

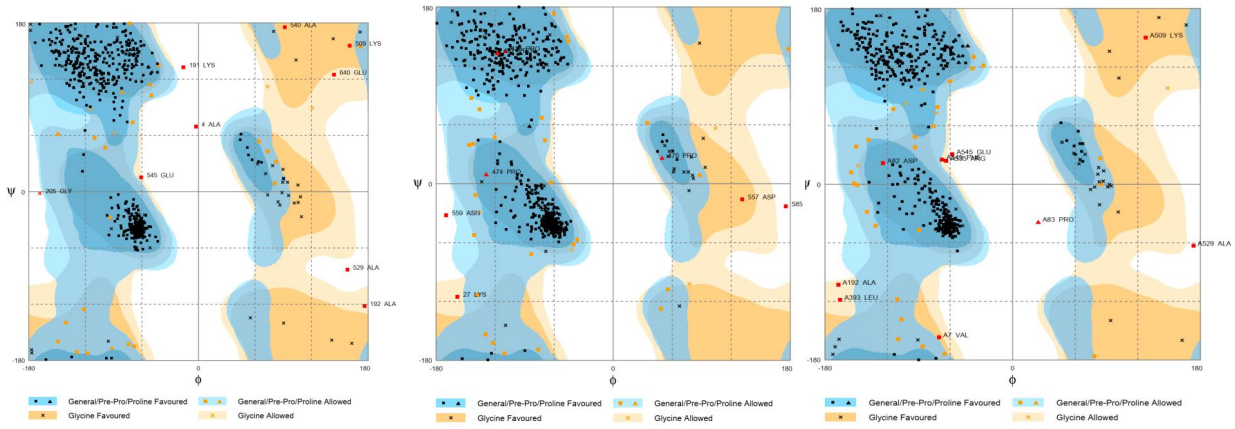
The result of Swiss modeling suggested that the generated model exist in oligomer state with no ligand showed in Fig 4B which may be due to presence of non enzymatic activity in the core of the protein (Schwede *et al*., 2003). The QMEAN and GMQE score was found -1.57 and 0.80 indicating that the model is accurate and showed good degree of nativeness (Sahoo *et al*., 2019). The BLAST result showed 89.87%, 57% of identity and similarity with template heat shock cognate 71 kDa protein (PDB ID: 1yuw.1.A) with resolution 2.60Å under X-ray. So it can be interfered that this two proteins are conserved across the species (Cheeseman *et al*., 2016).

Comparative quality evaluation of the generated protein models

In this study, the quality of the above generated *Cyprinus carpio* Hsp70 protein model by three above methods, were evaluated through different *Insilco* platforms and the parameters are given in Table 5. The Ramachandran plot gives the shifting of residues in the mutant structure towards the disallowed region due to mutation as compared to native counterpart (Pradhan *et al*., 2017). In this study, the analysis showed 94.2%, 93.8% and 92.7% of total amino acid residues lied in the favorable region of Modeller, Phyre 2

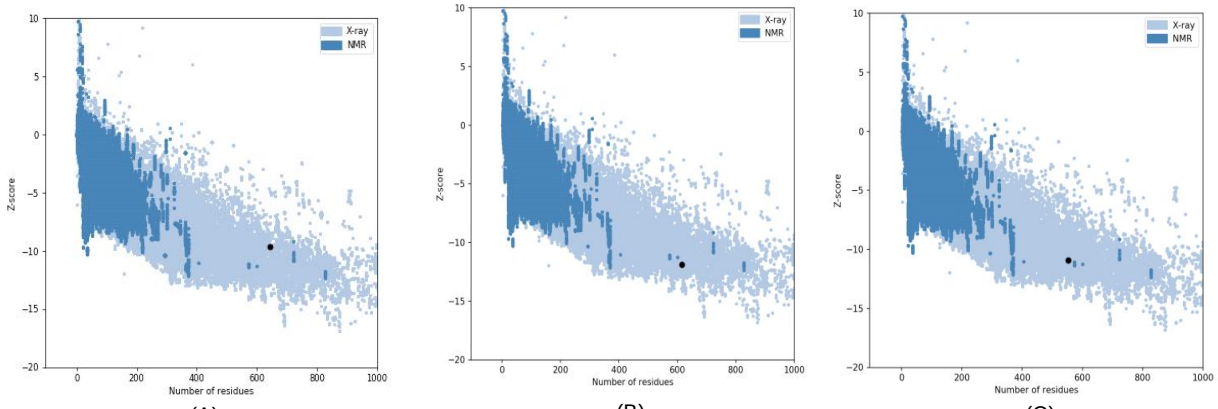
Table 5: Showing different quality evaluation parameters of *Cyprinus carpio* Hsp70 protein models generated by modeller 9.21, Phyre 2 and Swiss modeling.

Parameters	Modeller 9.21	Phyre 2	Swiss model
Number of residues in favored region (%)	94.2	93.8	92.7
Number of residues in allowed region (%)	4.4	4.9	5.4
Number of residues in outlier region (%)	1.4	1.3	1.8
Z value	-9.58	-9.48	-10.93
Predicted LGscore	3.71	4.72	4.972
Predicted MaxSub	0.35	0.435	0.419
Residues havingaveraged 3D-1D score >= 0.2	80.87%	83.93%	90.96%
Verify 3D (validation)	PASS	PASS	PASS
Quality factor	82.56	59.43	95.27



(A) (B) (C)

Fig 5: Showing Ramachandran Plot of protein model created by Modeller 9.21 (A), Phyre 2 (B) and Swiss model (C).



(A) (B) (C)

Fig 6: showing z plot of protein model created by Modeller 9.21 (A), Phyre 2 (B) and Swiss model (C) which describes the overall quality of model evaluated and deciphered.

and Swiss models respectively shown in Fig 5 suggests that the protein model predicted from Modeller is more stable than the other two which may be due to absence of the methylene group at Cβ position of the amino acid residue indicating more number of glycine residues in allowed region (Chakrabarti and Pal, 2001).

Further the ProSA-web analysis of the generated protein models revealed a Z score of -9.58, -9.48 and -10.93 (Fig 6) indicating Z value of all three falls in the middle of the graph suggesting all three models are of good quality and It may be due to the generated protein models have the conformations within the native experimental state (Wiederstein and Sippl, 2007).

The ProQ analysis of above three generated *Cyprinus carpio* Hsp70 models resulted Levitt-Gerstein (LG) and Maxsub score of (3.71, 0.35), (4.72, 0.435) and (4.972, 0.419) respectively, interfering that the protein model created by Modeler is of good and other two models generated by phyre 2 and Swiss modeling are of extremely good in quality. It might be due to correct optimization of the models to find their nativeness (Olejniczak and Storz, 2017).

In this study, Verify3D plot analysis of all three modelled protein (Fig 7) remarked as PASS and the 3D environment profile resulted 80.87%, 83.93% and 90.96% of the residues have averaged 3D-1D score ≥*0.2, which suggests the Swiss modelled protein is structurally more valid than other two, it may be due to more compatibility of the Swiss model in respect to their location and environment of α, β, loop, polar as well as non polar structure (Eisenberg *et al*., 1997).

Further the ERRAT analysis resulted that the overall quality values for all three Hsp70 models of *Cyprinus carpio* were 82.56, 59.43 and 95.27 (Fig 8), suggesting that the overall quality of the generated model from Swiss modeling is highest followed by model generated by modeller and the value was lowest in the model structure predicted by Phyre 2. It might be due less randomized distributions of the different atom types and non bonded atomic interactions in Swiss model than the other two models (Colovos and Yeates, 1993)

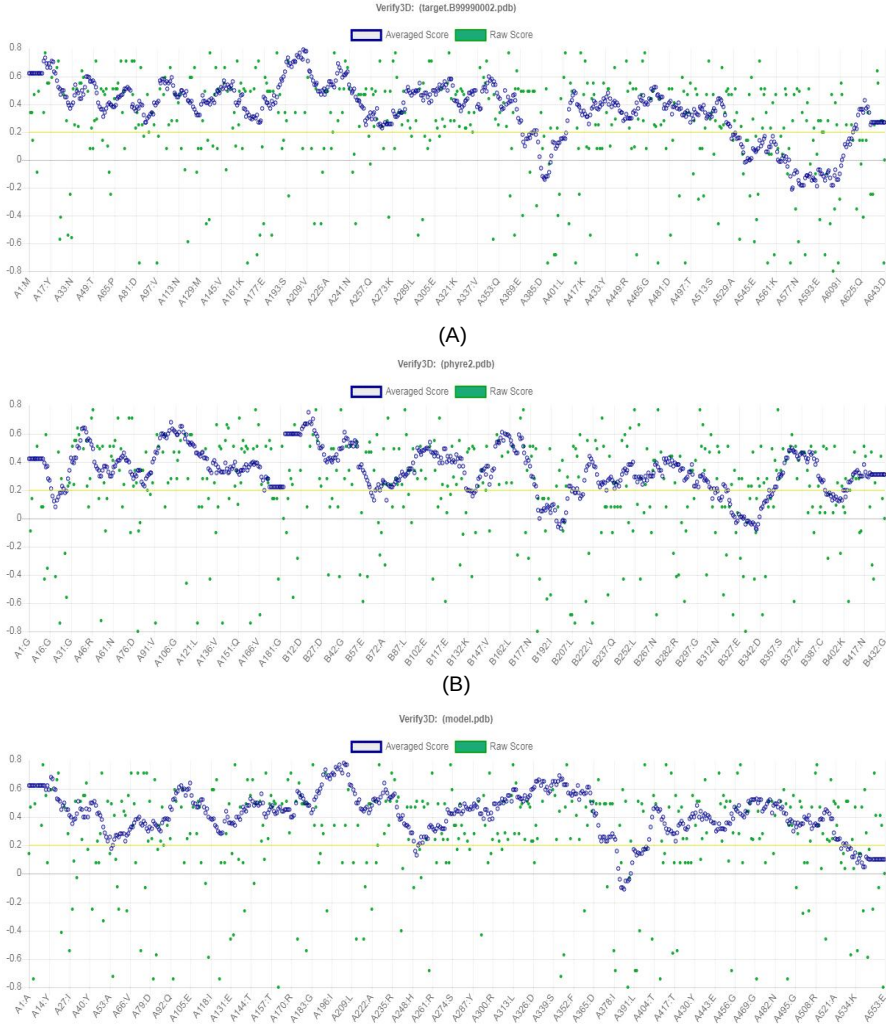


Fig 7: Showing verify 3 D plot of protein model created by modeller 9.21 (A), Phyre 2 (B) and Swiss model (C).

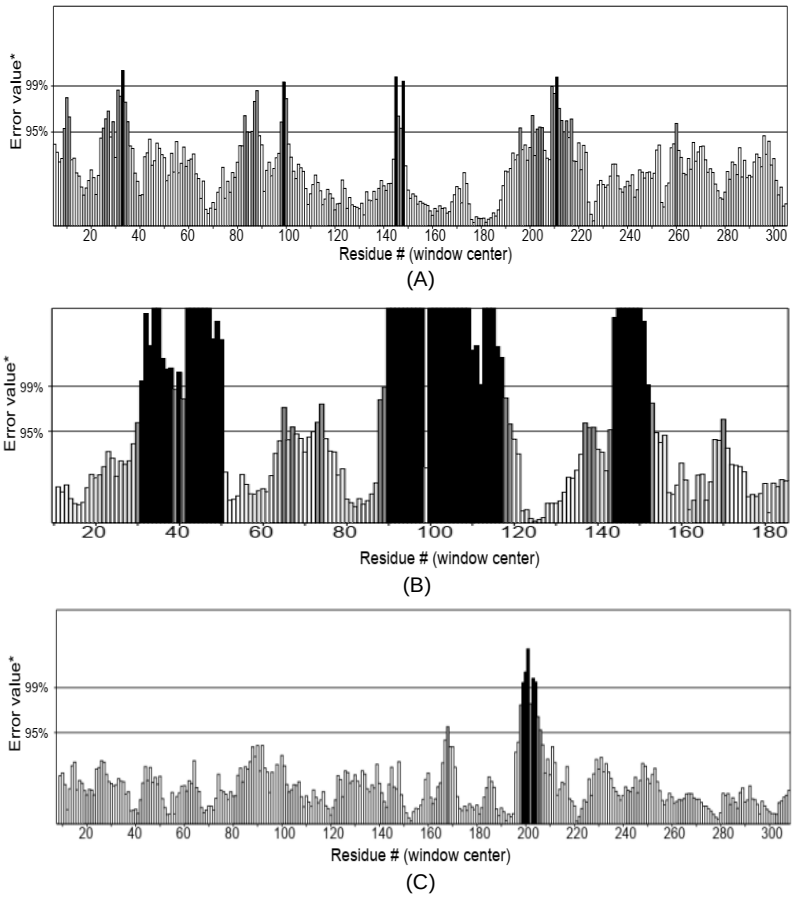


Fig 8: Showing verify 3 D plot of protein model created by modeller 9.21 (A), Phyre 2 (B) and Swiss model (C) which describes the quality of model.

CONCLUSION

This study may be concluded that computational biology provides a suitable platform to accomplish physiochemical and structural characteristics of any biological active protein. *Cyprinus carpio* is widely domesticated fresh water fish not only cultivated in European region but also seen in mostly in Asia and American countries. Hsp70 protein, a molecular chaperone implicated in a wide variety of cellular processes, including protection of the proteome from stress, folding and transport of newly synthesized polypeptides, activation of proteolysis of misfolded proteins and the formation and dissociation of protein complexes, has vital role in protection mechanism of fish from environmental stress. To help better understanding of Hsp70 protein, this study was carried out to analyse the physicochemical properties as well as to generate valid protein models through different *insilico* approaches which would provide good platform to understand the functional biology of this protein. The physiochemical and secondary structural characterization revealed that this protein is very stable, acidic, nuclear, and hydrophilic in nature and constituted with more alpha helices in the secondary structure. Further, three proteins models are predicted though homology modeling upon Modeller, Phyre 2 and Swiss model and it can be inferred the model derived from Swiss model is very good in quality though evaluation of the models on various *insilico* analysis. Lastly it may be concluded that all the three generated models of Hsp70 are streiochemically stable and can be further utilized as scientific resources for functional annotation of this protein.

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Conflict of interest

The author(s) certify that there is no conflict of interest with any financial/research/academic organization, with regards to the research work discussed in the manuscript

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