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Original Article

Tumor necrosis factor alpha of teleosts: in silico characterization and homology modeling

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Abstract

Tumor necrosis factor alpha (TNF-α) is known to be crucial in many biological activities of organisms. In this study, physicochemical properties and modeling of TNF-α protein of fish was analyzed using *in silico* approach. TNF-α proteins selected from fish species, including grass carp (*Ctenopharyngodon idella*), zebra fish (*Danio rerio*), Nile tilapia (*Oreochromis niloticus*), goldfish (*Carassius auratus*), and rainbow trout (*Oncorhynchus mykiss*) were used in this study. Physicochemical characteristics with molecular weight, theoretical isoelectric point, extinction coefficient, aliphatic index, instability index, total number of negatively charged residues and positively charged residues, and grand average of hydropathicity were computed. All proteins were classified as transmembrane proteins. The "transmembrane region" and "TNF" domain were identified from protein sequences. The function prediction of proteins was also performed. Alpha helices and random coils were dominating in the secondary structure of the proteins. Three-dimensional structures were predicted and verified as good structures for the investigation of TNF-α of fish by online server validation.

Keywords: *in silico*, fish, TNF-α

1. Introduction

Tumor necrosis factor alpha (TNF-α) has been originally known as a monocyte product with antitumor activity which is a pleiotropic cytokine produced by various types of cells, including monocytes, macrophages, B-cells, T-cells and fibroblasts (Seckinger *et al.*, 1990; Vasanthi *et al.*, 2007; Maddahi *et al.*, 2011). TNF-α has been suggested to involve in cytotoxicity to tumor cell lines (Vasanthi *et al.*, 2007) and many biological activities such as inflammatory, blood-brain barrier, thrombogenic, and vascular changes that related to brain injury (Barone *et al.*, 1997; Maddahi *et al.*, 2011). It is also described as a strong immunomediator and proinflammatory cytokine involving in host defense against invasive pathogens (Liu *et al.*, 1994). At cellular level, it probably provokes the induction of cell survival, differentiation, proliferation, as well as both apoptotic and necrotic cell death upon stimulation (Goeddel *et al.*, 1986; Fiers, 1991; Wu and Hymowitz, 2009). The TNF-α effects are mediated through its two receptors, TNF-R1 (TNF receptor type 1; CD120a; p55/60) and TNF-R2 (TNF receptor type 2; CD120b; p75/80) (Lewis *et al.*, 1991; Wajant *et al.*, 2003), leading to promote the activation of the nuclear factor kappa B pathway, which in turn may inhibit TNF-α-induced cell death (Gesslein *et al.*, 2010).

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In fish, TNF-α is associated with ovarian function of brown trout (*Salmo trutta*) (Crespo *et al.*, 2010) and immune-related role in fish inflammatory responses after stimulation conditions of Japanese flounder (*Paralychthys olivaceus*) (Hirono *et al.*, 2000). Actually, TNF-α has been previously identified, cloned, and characterized in many fish species (Roca *et al.*, 2008; Liu *et al.*, 2015). Albeit these methods have been applied to analyze the physicochemical characterizations, molecular functions and structural features of proteins, comprising antifreeze (Hossain, 2012), mannose binding lectin homologue (Goel *et al.*, 2013), myeloid differentiation primary response 88 (Tuan and Wei-Min, 2015), and nuclear factor kappa B inhibitor alpha (Tuan *et al.*, 2015) from fish species, *in silico* analysis methods has not been performed in this protein (TNF-α) from fish species. In the current study, the TNF-α of five freshwater fish species, including grass carp (*Ctenopharyngodon idella*), zebra fish (*Danio rerio*), Nile tilapia (*Oreochromis niloticus*), goldfish (*Carassius auratus*), and rainbow trout (*Oncorhynchus mykiss*) were selected for investigation. The results of this study provide for the first time valuable information of the possible physiochemical properties, functions and structures of the protein in different fish species using *in silico* approach.

2. Materials and Methods

2.1 Protein sequence and physiochemical characterization

TNF-α proteins selected from 5 fish species, including grass carp (*Ct. idella*) (Accession number: ADY80577.1), zebra fish (*D. rerio*) (BAD98730.1), Nile tilapia (*O. niloticus*) (NP_001266462.1), goldfish (*C. auratus*) (ABU50127.1), and rainbow trout (*O. mykiss*) (CAB92316.1) were retrieved from the NCBI (National Center for Biotechnology Information) protein database (http://www.ncbi.nlm.nih.gov/) under the FASTA format for analysis. For the domain structures the Simple Molecular Architecture Research Tool (SMART) program (http://smart.embl-heidelberg.de/) was used. Multiple-sequence alignment was performed using the ClustalW2 server (http://www.ebi.ac.uk/Tools/msa/clustalw2/). Physiochemical properties of the proteins such as molecular weight (Mol. wt.), amino acid composition, theoretical isoelectric point (pI), total number of positive (Arg + Lys) and negative (Asp + Glu) residues (+R/-R), extinction coefficient (EC), instability index (II), aliphatic index (AI), and grand average of hydropathicity (GRAVY) were performed using Expasy's ProtParam prediction server (Gasteiger *et al.*, 2005).

2.2 Functional analysis

The server SOSUI (Hirokawa *et al.*, 1998) was performed to identify the types of protein. The CYS_REC (http://linux1.softberry.com/) was used to predict the presence of disulphide bonds and their bonding patterns, which are crucial in defining the functional linkage and the stability of a protein. The *ab initio* predictions of protein function from sequences was performed by using the ProtFun 2.2 server (http://www.cbs.dtu.dk/services/ProtFun/).

2.3 Protein structure prediction

Secondary structures of TNF-α proteins of different fish species were predicted using Self-Optimized Prediction Method with Alignment (SOPMA) server with default parameters (window width: 17; similarity threshold: 8; number of states: 4) (Geourjon and Deleage, 1995). Homology modeling was constructed using SWISS-MODEL server (Schwede *et al.*, 2003; Arnold *et al.*, 2006). The modeled structures were selected on the basis of sequence identity with the Protein Data Bank (PDB) templates (Fiser, 2010). The stereochemical quality and accuracy of the predicted models were analyzed performing PROCHECK's Ramachandran plot analysis (Ramachandran *et al.*, 1963; Laskowski *et al.*, 1996), ProQ (Cristobal *et al.*, 2001) and ProSA (Sippl, 1993; Wiederstein and Sippl, 2007).

3. Results and Discussion

3.1 Physicochemical and functional characterization

All protein sequences were used as the templates for physicochemical characterization analyses (Table 1). The total number of amino acids was ranged from 228 to 247 and the molecular weight of proteins was from 25,370.5 to 27,490 Dal. The theoretical isoelectric point (pI) of all proteins was arranged from 5.23 to 7.65, indicating the protein of fish species are from acidic to base in characters. The pI value is the pH at which the positive and negative charges are

Table 1. Physiochemical characteristics computed using Expasy's ProtParam.

Species	No. of aa	Mol. wt.	pI	-R/+R	EC	II	AI	GRAVY
Grass carp	239	26012.7	7.65	23/24	20315/19940	27.37	89.41	0.005
Zebra fish	242	26765.1	5.96	25/21	37025/36900	31.42	80.91	-0.224
Nile tilapia	247	27490	5.42	30/23	54680/54430	30.36	81.38	-0.245
Goldfish	228	25370.5	5.35	29/21	28795/28420	45.68	86.40	-0.132
Rainbow trout	246	27112.5	5.23	27/18	50670/50420	27.10	81.34	-0.238

EC*—the first value is based on the assumption that all pairs of cysteine residues form cysteines and the second one that all cysteine residues are reduced.

balanced. The pI value is useful for the purification of proteins on a polyacrylamide gel by isoelectric focusing. The total number of negatively charged residues (Asp + Glu) and positively charged residues (Arg + Lys) was in ranging of 23 to 30 and 18 to 24, respectively. The extinction coefficient (EC) of proteins measured at 280 nm was from 20,315 to 54,680 $M^{-1}.cm^{-1}$ (assuming all pairs of cysteine residues form cysteine) and from 19,940 to 54,430 $M^{-1}.cm^{-1}$ (assuming all cysteine residues are reduced). The high value of EC indicates the high concentration of cysteine, tryptophan and tyrosine in the proteins. Methionine was considered as *N*-terminal of the fish TNF-α polypeptide chains. The estimated half-life of all proteins is 30 hours in mammalian reticulocytes (*in vitro*), > 20 hours in yeast (*in vivo*) and > 10 hours in *Escherichia coli* (*in vivo*). Instability index (II) value is a basic measure to evaluate the stability of proteins in a test tube, the II value of TNF-α of fish species was arranged from 27.10 to 45.68. The results indicate that except for the TNF-α protein of goldfish is probably not stable (II > 40) all are probably stable (II < 40) (Guruprasad *et al.*, 1990). The aliphatic index (AI) is a parameter for estimating thermalstability of a protein directly associating with the mole fraction of aliphatic side chains (alanine, isoleucine, leucine, and valine) in the protein (Ikai, 1980). In this study, high AI values of proteins (80.91-89.41) imply high thermostability of these proteins. Low grand average hydropathicity (GRAVY) values (from -0.245 to 0.005) of proteins from different fish species indicate they are hydrophilic in natural conditions. The amino acid composition in TNF-α computed using Expasy's ProtParam was showed in Table 2. The results revealed that leucine, alanine, valine, serine and glycine are relatively rich in all protein sequences of different fish species.

All proteins were classified as transmembrane proteins through SOSUI program. The transmembrane regions predicted from protein sequences were shown in Table 3. Structural analysis through the SMART revealed that all protein sequences had the "transmembrane region" at the N-terminus and a "tumor necrosis factor family (TNF)" domain.

Table 2. Amino acid composition in TNF-α computed using Expasy's ProtParam.

No.	Amino acid	Grass carp	Zebra fish	Nile tilapia	Goldfish	Rainbow trout
1	Alanine	10.88	7.02	8.91	9.65	9.35
2	Arginine	4.60	3.31	3.64	5.26	3.66
3	Asparagine	3.77	4.13	3.64	4.39	5.28
4	Aspartic acid	6.28	5.37	4.05	6.14	4.07
5	Cysteine	2.51	1.24	1.62	2.63	2.03
6	Glutamine	3.77	5.79	4.05	3.51	5.69
7	Glutamic acid	3.35	4.96	8.10	6.58	6.91
8	Glycine	6.28	8.26	7.29	5.70	10.16
9	Histidine	2.93	2.89	2.43	3.07	2.85
10	Isoleucine	4.18	4.13	4.45	3.95	4.88
11	Leucine	10.04	7.44	9.31	9.21	9.35
12	Lysine	5.44	5.37	5.67	3.95	3.66
13	Methionine	2.51	1.65	1.62	1.32	1.63
14	Phenylalanine	4.60	4.55	4.05	4.82	4.07
15	Proline	2.51	3.31	2.83	1.75	3.66
16	Serine	9.62	7.44	8.10	7.89	5.28
17	Threonine	5.44	7.44	7.69	6.58	5.69
18	Tryptophan	0.84	1.65	3.24	1.32	2.85
19	Tyrosine	2.51	4.13	2.83	3.51	3.25
20	Valine	7.95	9.92	6.48	8.77	5.69

Table 3. Types of protein and transmembrane region identified by using SOSUI.

Species	Type of protein	Length	Transmembrane region	N-C terminal
Grass carp	Transmembrane	22	WRVCGALLAVALCAAAAVCFTL	37-58
Zebra fish	Transmembrane	23	KTLAAVAFVGLCVVAAFFFTWHV	37-59
Nile tilapia	Transmembrane	23	AEWIWKVCAVLVVVALCLAGVLL	29-51
Goldfish	Transmembrane	22	WRVCGVLLAVALCAAAAVCFTF	26-47
Rainbow trout	Transmembrane	22	WRLCGVLLIAGLCAAAALLFAW	35-56

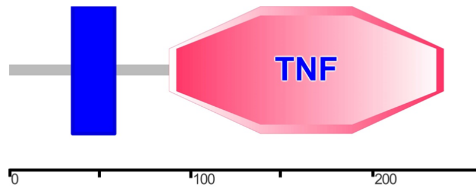

Figure 1. Architecture of the domain topology of TNF-α protein of grass carp, rendered by Simple Molecular Architecture Research Tool (SMART), showing the "transmembrane region" (blue box) and "tumor necrosis factor family (TNF)" domain (red box).

Figure 1 generated by using SMART analysis illustrates the structure features of TNF-α from grass carp, including "transmembrane region" and "TNF" domain as an example. The presence of conserved functional domains in TNF-α protein of fish species in this study may suggest that these proteins share the probably similar functions. The transmembrane domain inferred that these proteins might be released as membrane-anchored precursors to generate soluble homotrimeric cytokine (sTNF) forms by proteolytic cleaving of a metalloproteinase TNF-α converting enzyme (García-Castillo *et al.*, 2002; Wajant *et al.*, 2003). The TNF domain has been known as a monocyte-derived cytotoxin that involved in tumor regression, septic shock and cachexia (Fransen *et al.*, 1985; Kriegler *et al.*, 1988). This result is in accordance with previously reported results for gilthead seabream (*Sparus aurata*) (García-Castillo *et al.*, 2002), mandarin fish (*Siniperca chuatsi*) (Xiao *et al.*, 2007), and large yellow croaker (*Pseudosciaena crocea*) (Xie *et al.*, 2008), corroborating highly conserved functions across different species. The result of multiple sequence alignment generated by using ClustalW2 comparison among deduced amino acid of TNF-α indicates a high level of conservation of the TNF family signature in fishes (Figure 2). Additionally, CYS_REC was used to determine the cysteine residues and disulphide bonds, which are important in determining the thermostability of the proteins. The study results showed that cysteine residues were found in all proteins of fish species. However, only TNF-α protein of grass carp, goldfish and rainbow trout have the most probable pattern of cysteine residue in pairing (Table 4). The results indicated the probable presence of disulphide bonds in these proteins.

Predictions of protein function from sequences were *ab initio* performed by using ProtFun 2.2 server (Table 5). As shown in Table 5, the TNF-α of fish were classified as enzymes and assigned to three functional categories, including 'transport and binding' (TNF-α of grass carp and Nile tilapia), 'energy metabolism' (zebra fish and rainbow trout), and 'cell envelop' (goldfish). This suggests that these proteins may display different functions in different host cellular process. In gene ontology annotation, the proteins were assigned to two categories of 'immune response' (grass carp, Nile tilapia, goldfish, and rainbow trout) and 'Voltage-gated ion channel' (zebra fish). The analysis results indicated

Table 4. Probable pattern of pairs of disulphide bond computed using CYS_REC.

Species	CYS_REC
Grass carp	Cys40-Cys146 Cys55-Cys147
Goldfish	Cys38-Cys44 Cys90-Cys136
Rainbow trout	Cys47-Cys189 Cys57-Cys154

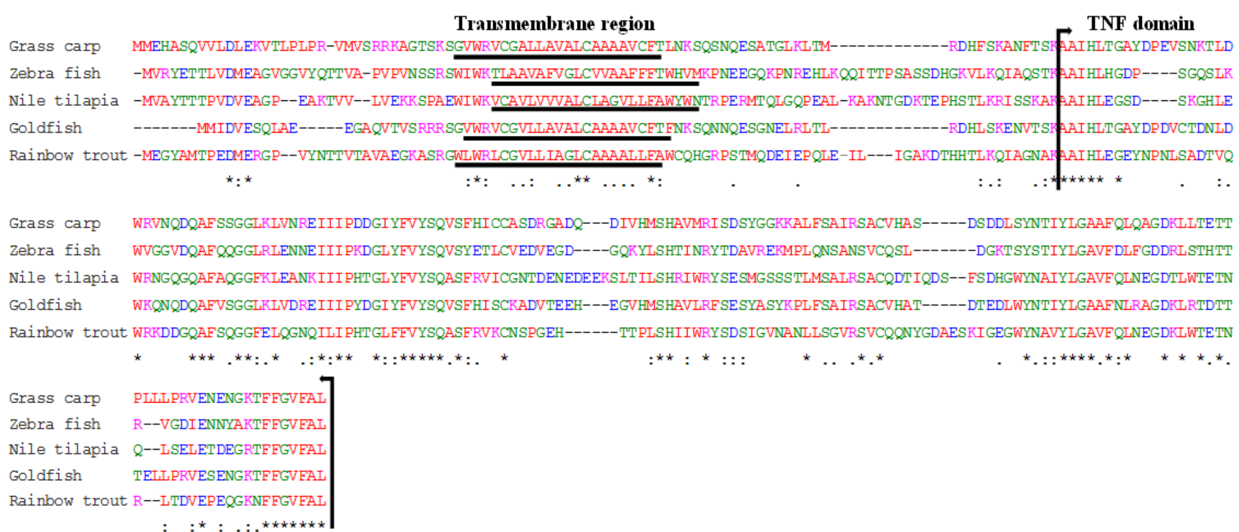

Figure 2. Multiple alignment of TNF-α protein of fish used in this study rendered by ClustalW2. Asterisk marks (*) indicate identical amino acids. Sequences are numbered on the right, conserved substitutions are indicated by (:), semi-conserved by (.) and deletions by dashes. Transmembrane region is indicated by lines. Tumor necrosis factor family domain is indicated by arrows.

that the TNF-α plays important roles in immune-related systems in fish species. Albeit, herein, TNF-α of zebra fish was classified into the category of 'voltage-gated ion channel' in gene ontology annotation with high Probs/Odds (0.146/6.629), similar to other fish species, it was identified as belonging to the category of 'immune response', where the odds value was higher than 1 (Probs/Odds: 0.089/1.041) (Jensen *et al.*, 2002; Jensen *et al.*, 2003). This suggests that TNF-α is also important in immune-associated functions in zebra fish.

3.2 Protein structure prediction and model validation

The secondary structure of TNF-α protein from fish species was predicted using SOPMA (Table 6). The results showed that except for TNF-α from grass carp all contain alpha helix as a predominant component among the secondary structure elements, followed by random coil, extended strand and beta turn. The rest of the structure elements were not predicted.

The three-dimensional structures of fish TNF-α protein were modeled based on the sequence and structural similarity to different available protein structure templates from the PDB (Table 7). The final structure of the models represented with the Swiss PDB Viewer was shown in Figure 3a. Validation of predicted models performing PROCHECK's Ramachandran plots, ProQ, and ProSA were presented in Table 7. Results showed that 63.9 to 84.1% of residues found in the most favored regions, 12.9 to 29.3% of residues in the additional allowed regions, 1.4 to 4.2% of residues in the generously allowed regions, and 0 to 5.3% of residues in the disallowed regions of the proteins. All protein models contained the lower than 90% fell of residues in the most favored regions, but a higher residue number were found in the additional allowed regions, indicating that they may be near to be good quality models. The overall average G-factor of dihedral angles and main-chain covalent forces of protein's models was ranged from -0.51 to 0.28. The results implied that the models were accepted, as they were greater than the acceptable value (> -0.50), excepting the model for grass carp TNF-α (when its G-factor was -0.51) (Ramachandran *et al.*, 1963). The LGscore value indicates "extremely good" quality (> 4.0) for the models of TNF-α of all fish species. MaxSub validation also implies "very good" models (> 0.5) of the TNF-α for grass carp Nile tilapia and goldfish, and "correct" models (> 0.1) of the remaining models (Cristobal *et al.*, 2001). The Z-scores in ProSA of all models were ranged from -5.05 to -3.3, which are within the range of scores typically

Table 5. Function prediction using ProtFun 2.2 Server.

Item	Grass carp	Zebra fish	Nile tilapia	Goldfish	Rainbow trout
Functional category	Transport and binding (0.746/1.820)*	Energy metabolism (0.256/2.841)	Transport and binding (0.616/1.502)	Cell envelope (0.303/4.966)	Energy metabolism (0.280/3.116)
Enzyme/ nonenzyme	Enzyme (0.288/1.006)	Enzyme (0.425/1.485)	Enzyme (0.542/1.893)	Enzyme (0.293/1.023)	Enzyme (0.568/1.983)
Gene Ontology category	Immune response (0.295/3.473)	Voltage-gated ion channel (0.146/6.629)	Immune response (0.256/3.011)	Immune response (0.346/4.075)	Immune response (0.101/1.185)

(Prob/Odds)*—The first number is the estimated probability that the entry belongs to the class in question and the second number represents the odds that the sequence belongs to that class/category.

Table 6. Secondary structure elements (in %) of TNF-α from freshwater fish species.

Element	Grass carp	Zebra fish	Nile tilapia	Goldfish	Rainbow trout
Alpha helix	28.03	37.6	34.82	41.23	32.11
3_{10} helix	0	0	0	0	0
Pi helix	0	0	0	0	0
Beta bridge	0	0	0	0	0
Extended strand	28.03	24.38	26.32	23.68	25.61
Beta turn	10.46	10.74	9.31	7.46	11.38
Bend region	0	0	0	0	0
Random coil	33.47	27.27	29.55	27.63	30.89
Ambiguous states	0	0	0	0	0
Other states	0	0	0	0	0

Table 7. Validation analysis for proposed models of the fish TNF-á protein.

Index	Grass carp	Zebra fish	Nile tilapia	Goldfish	Rainbow trout
Template (PDB ID)	2re9.1.B	2zpx.1.C	2tnf.1.B	1a8m.1C	1a8m.1.A
Residue range	83 - 239	96 - 242	93 - 247	71 - 228	90 - 246
Resolution (Å)	2.1	2.83	1.4	2.3	2.3
Sequence identity (%)	29.81	31.69	34.69	29.93	33.56
PROCHECK					
Total number of residues	157	147	155	158	157
Most favored regions (%)	82.1	83.6	84.1	75.5	63.9
Additional allowed regions (%)	12.9	14.8	13.8	17.5	29.3
Generously allowed regions (%)	3.6	1.6	1.4	4.2	1.5
Disallowed regions (%)	1.4	0	0.7	2.8	5.3
Non-glycine and non-proline residues	140	128	138	143	133
End-residues	2	2	2	2	2
Glycine residues	11	14	14	9	17
Proline residues	4	3	1	4	5
G-factors	-0.51	-0.35	0.28	-0.45	-0.32
ProQ					
Lgscore	5.872	5.037	5.236	4.894	4.118
MaxSub	0.603	0.481	0.576	0.544	0.442
ProSA					
Z-Score	-5.05	-4.6	-4.53	-3.3	-4.43

found for native proteins of similar size (Figure 3b), while plots of single residue energies revealed predominantly negative values, also indicating good quality of the proposed models (Wiederstein and Sippl, 2007) (Figure 3c). Overall, all of these validation results suggest that the proposed models of TNF-α for different fish species can be accepted as relatively accurate. This study provides basic information on the TNF-α protein from fish species, which can be used for molecular docking studies which are helpful in providing potential ligand molecules against pathogen infections.

4. Conclusions

The current study is the first time performing homology modeling of the TNF-α protein from fish species by using *in silico* methods. The physicochemical properties of the proteins were profoundly investigated. All proteins were classified as transmembrane proteins. Cysteine residues were found in all proteins, while high probabilities of cysteine in pairs were found in protein sequences of grass carp, goldfish and rainbow trout. An approximately number of alpha helices and random coils were computed to be dominating, followed by extended strands in the secondary structure of all proteins. The three-dimensional models of proteins was predicted and validated as good structures for investigation of TNF-α of fish. This study provides a better comprehensive on the basic information of TNF-α protein from fish species.

References

Arnold, K., Bordoli, L., Kopp, J., and Schwede, T. 2006. The SWISS-MODEL workspace: A web-based environment for protein structure homology modelling. Bioinformatics. 22(2), 195-201.

Barone, F., Arvin, B., White, R., Miller, A., Webb, C., Willette, R., Lysko, P., and Feuerstein, G. 1997. Tumor necrosis factor-α a mediator of focal ischemic brain injury. Stroke. 28(6), 1233-1244.

Crespo, D., Bonnet, E., Roher, N., MacKenzie, S.A., Krasnov, A., Goetz, F.W., Bobe, J., and Planas, J.V. 2010. Cellular and molecular evidence for a role of tumor necrosis factor alpha in the ovulatory mechanism of trout. Reproductive Biology and Endocrinology. 8(1), 34.

Cristobal, S., Zemla, A., Fischer, D., Rychlewski, L., and Elofsson, A. 2001. A study of quality measures for protein threading models. BMC Bioinformatics. 2(1), 5.

Fiers, W. 1991. Tumor necrosis factor characterization at the molecular, cellular and *in vivo* level. FEBS Letters. 285(2), 199-212.

Fiser, A. 2010. Template-based protein structure modeling. In Computational Biology, editor, Springer, Germany, pp. 73-94.

Fransen, L., Mûller, R., Marmenout, A., Tavernier, J., Van der Heyden, J., Kawashima, E., Chollet, A., Tizard, R., Van

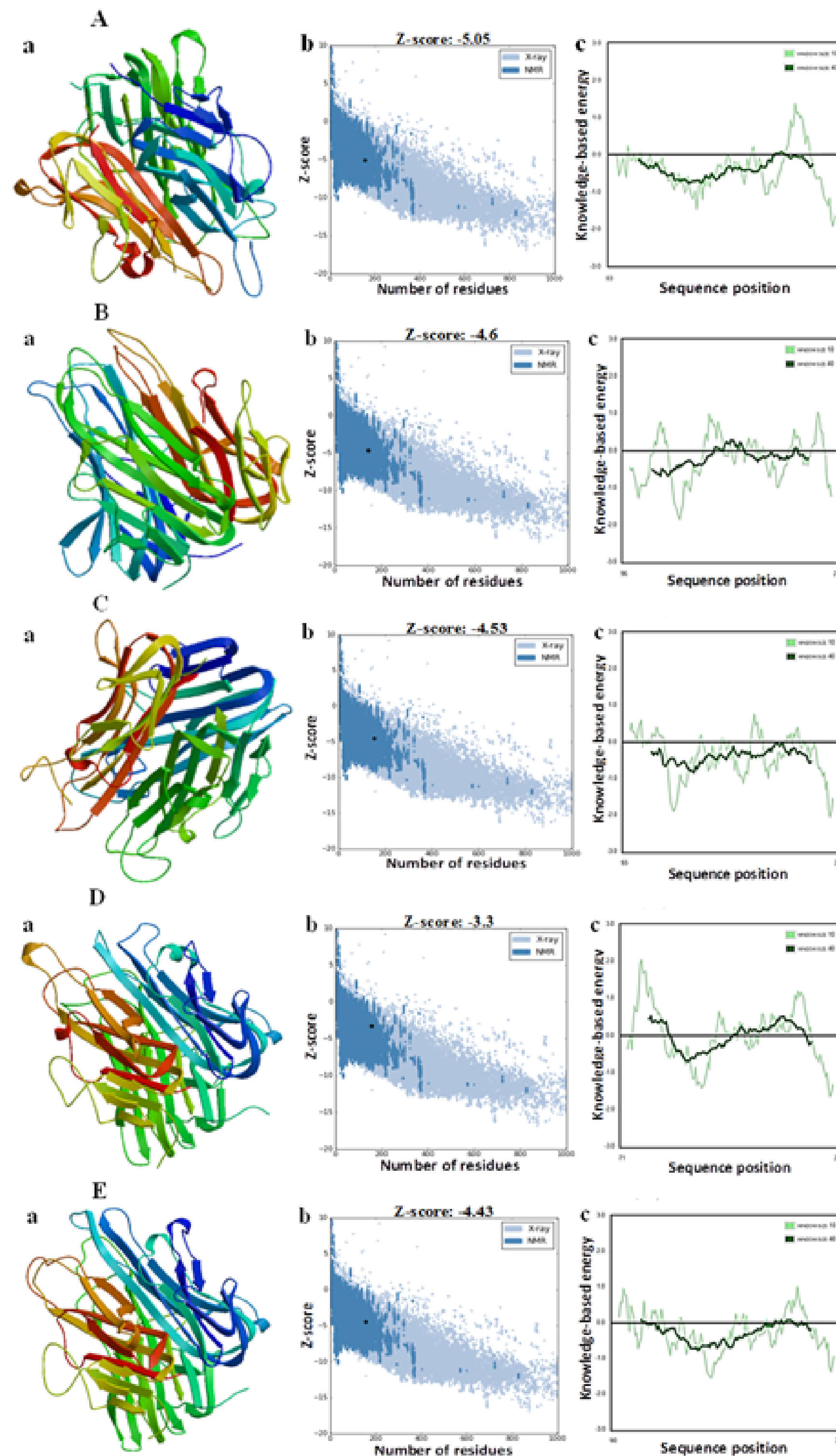

Figure 3. TNF-α from (A): grass carp; (B): zebra fish; (C): Nile tilapia; (D): goldfish and (E): rainbow trout with (a) Three-dimensional structures rendered by using SWISS-MODEL and (b) and (c) ProSA-web validation. (b) Z-scores of protein chain in PDB determined by X-ray crystallography or NMR spectroscopy with respect to their length, the z-scores of proteins are highlighted as large dots, which are within the range of scores typically found for native proteins of similar size. (c) A Plots of single residue energies for each of the models, which show the model quality by plotting energies as a function of amino acid sequence position *i*. A plot of single residue energies is smoothed by calculating the average energy over each 40-residue fragments (i,i+39), which is then assigned to the 'central' residue of the fragment at position i+19 (window size 40; dark-green line). A smaller window size of 10 residues is shown in the background of the plot (light-green line). Negative values indicate good models.

Heuverswyn, H., and Van Vliet, A. 1985. Molecular cloning of mouse tumour necrosis factor cDNA and its eukaryotic expression. Nucleic Acids Research. 13(12), 4417-4429.

García-Castillo, J., Pelegrín, P., Mulero, V., and Meseguer, J. 2002. Molecular cloning and expression analysis of tumor necrosis factor á from a marine fish reveal its constitutive expression and ubiquitous nature. Immunogenetics. 54(3), 200-207.

Gasteiger, E., Hoogland, C., Gattiker, A., Wilkins, M.R., Appel, R.D., and Bairoch, A. 2005. Protein identification and analysis tools on the ExPASy server, Springer, Germany.

Geourjon, C. and Deleage, G. 1995. SOPMA: Significant improvements in protein secondary structure prediction by consensus prediction from multiple alignments. Computer Applications in Bioscience. 11(6), 681-684.

Gesslein, B., Håkansson, G., Gustafsson, L., Ekström, P., and Malmsjö, M. 2010. Tumor necrosis factor and its receptors in the neuroretina and retinal vasculature after ischemia-reperfusion injury in the pig retina. Molecular Vision. 162317.

Goeddel, D., Aggarwal, B., Gray, P., Leung, D., Nedwin, G., Palladino, M., Patton, J., Pennica, D., Shepard, H., and Sugarman, B. 1986. Tumor necrosis factors: gene structure and biological activities. Cold Spring Harbor Symposia on Quantitative Biology, 597-609.

Goel, C., Barat, A., Pande, V., and Sahoo, P. 2013. Comparative *in silico* analysis of Mbl homologues of teleosts. World Journal of Fish and Marine Sciences. 5(4), 426-429.

Guruprasad, K., Reddy, B.B., and Pandit, M.W. 1990. 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. Protein Engineering, Design and Selection. 4(2), 155-161.

Hirokawa, T., Boon-Chieng, S., and Mitaku, S. 1998. SOSUI: Classification and secondary structure prediction system for membrane proteins. Bioinformatics. 14(4), 378-379.

Hirono, I., Nam, B.-H., Kurobe, T., and Aoki, T. 2000. Molecular Cloning, Characterization, and Expression of TNF cDNA and Gene from Japanese Flounder *Paralychthys olivaceus*. The Journal of Immunology. 165(8), 4423-4427.

Hossain, M. 2012. Fish antifreeze proteins: Computational analysis and physicochemical characterization. International Current Pharmaceutical Journal. 1(2), 18-26.

Ikai, A. 1980. Thermostability and aliphatic index of globular proteins. Journal of Biochemistry. 88(6), 1895-1898.

Jensen, L.J., Gupta, R., Blom, N., Devos, D., Tamames, J., Kesmir, C., Nielsen, H., Stærfeldt, H.H., Rapacki, K., and Workman, C. 2002. Prediction of human protein function from post-translational modifications and localization features. Journal of Molecular Biology. 319(5), 1257-1265.

Jensen, L.J., Gupta, R., Staerfeldt, H.-H., and Brunak, S. 2003. Prediction of human protein function according to Gene Ontology categories. Bioinformatics. 19(5), 635-642.

Kriegler, M., Perez, C., DeFay, K., Albert, I., and Lu, S. 1988. A novel form of TNF/cachectin is a cell surface cytotoxic transmembrane protein: ramifications for the complex physiology of TNF. Cell. 53(1), 45-53.

Laskowski, R.A., Rullmann, J.A.C., MacArthur, M.W., Kaptein, R., and Thornton, J.M. 1996. AQUA and PROCHECK-NMR: Programs for checking the quality of protein structures solved by NMR. Journal of Biomolecular NMR. 8(4), 477-486.

Lewis, M., Tartaglia, L.A., Lee, A., Bennett, G.L., Rice, G.C., Wong, G., Chen, E.Y., and Goeddel, D.V. 1991. Cloning and expression of cDNAs for two distinct murine tumor necrosis factor receptors demonstrate one receptor is species specific. Proceedings of the National Academy of Sciences. 88(7), 2830-2834.

Liu, D., Mai, K., and Ai, Q. 2015. Tumor necrosis factor alpha is a potent regulator in fish adipose tissue. Aquaculture. 43665-71.

Liu, T., Clark, R., McDonnell, P., Young, P., White, R., Barone, F., and Feuerstein, G. 1994. Tumor necrosis factor-alpha expression in ischemic neurons. Stroke. 25(7), 1481-1488.

Maddahi, A., Kruse, L., Chen, Q.-W., and Edvinsson, L. 2011. The role of tumor necrosis factor-alpha and TNF-alpha receptors in cerebral arteries following cerebral ischemia in rat. Journal of Neuroinflammation. 8(1), 107.

Ramachandran, G., Ramakrishnan, C., and Sasisekhran, V. 1963. Stereochemistry of polypeptide chain configuarations. Journal of Molecular Biology. 795-99.

Roca, F.J., Mulero, I., López-Muñoz, A., Sepulcre, M.P., Renshaw, S.A., Meseguer, J., and Mulero, V. 2008. Evolution of the inflammatory response in vertebrates: Fish TNF-α is a powerful activator of endothelial cells but hardly activates phagocytes. The Journal of Immunology. 181(7), 5071-5081.

Schwede, T., Kopp, J., Guex, N., and Peitsch, M.C. 2003. SWISS-MODEL: An automated protein homology-modeling server. Nucleic Acids Research. 31(13), 3381-3385.

Seckinger, P., Zhang, J.H., Hauptmann, B., and Dayer, J.M. 1990. Characterization of a tumor necrosis factor alpha (TNF-alpha) inhibitor: evidence of immunological cross-reactivity with the TNF receptor. Proceedings of the National Academy of Sciences of the United States of America. 87(13), 5188-5192.

Sippl, M.J. 1993. Recognition of errors in three-dimensional structures of proteins. Proteins: Structure, Function, and Genetics. 17(4), 355-362.

Tuan, T.N. and Wei-Min, W. 2015. Freshwater fish myeloid differentiation primary response protein MyD88: Characterisation and homology modelling by using

computational tools. Asian Fisheries Science. 28(1), 1-14.

Tuan, T.N., Wei-Min, W., and Duc, P.M. 2015. Characterisation and homology modelling of finfish NF-Kappa B inhibitor alpha using *in silico* analysis. Journal of Science and Development. 13(2), 216-225.

Vasanthi, P., Nalini, G., and Rajasekhar, G. 2007. Role of tumor necrosis factor-alpha in rheumatoid arthritis: A review. APLAR Journal of Rheumatology. 10(4), 270-274.

Wajant, H., Pfizenmaier, K., and Scheurich, P. 2003. Tumor necrosis factor signaling. Cell Death and Differentiation. 10(1), 45-65.

Wiederstein, M. and Sippl, M.J. 2007. ProSA-web: Interactive web service for the recognition of errors in three-dimensional structures of proteins. Nucleic Acids Research. 35(Supplement 2), W407-W410.

Wu, H. and Hymowitz, S.G. 2009. Structure and function of tumor necrosis factor (TNF) at the cell surface. In Handbook of Cell Signaling, editor, Oxford: Academic Press, U.K., pp. 265-275.

Xiao, J., Zhou, Z.-C., Chen, C., Huo, W.-L., Yin, Z.-X., Weng, S.-P., Chan, S.-M., Yu, X.-Q., and He, J.-G. 2007. Tumor necrosis factor-alpha gene from mandarin fish, *Siniperca chuatsi*: molecular cloning, cytotoxicity analysis and expression profile. Molecular Immunology. 44(14), 3615-3622.

Xie, F.J., Zhang, Z.P., Lin, P., Wang, S.H., Zou, Z.H., and Wang, Y.L. 2008. Cloning and infection response of tumour-necrosis factor alpha in large yellow croaker *Pseudosciaena crocea* (Richardson). Journal of Fish Biology. 73(5), 1149-1160.