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Physicochemical and Structural Characterisation of Human Il-1α and Il-2 Proteins with Insilco Tools

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KEYWORDS

Interleukins, ExPAsy ProtParam, SOPMA, Swissport, Procheck

ABSTRACT:

Inflammation is a physiological reaction that occurs as a result of several types of injuries, including those caused by physical, chemical, or biological sources. Interleukins, which belong to the wider category of cytokines, modify cellular function in the context of inflammation. Interleukins are used in animal experiments to explore aspects linked to healthcare. The interleukin-1α and interleukin-2 families are particularly associated with harmful inflammatory processes, even more so than any other kind of cytokine. The objective of the current work was to analyze the physiochemical characteristics, secondary structures, and tertiary structures of IL-1α and IL-2 utilizing bioinformatic techniques. The amino acid sequences of IL-1α and IL-2 were obtained from the UniProt protein database. The physicochemical characterisation of IL-1α and IL-2 proteins showed that they are made up of 271 and 153 amino acids, with molecular weights of 30606.61 and 17627.1 Da, respectively. The predicted isoelectric (pI) values for the IL-1α and IL-2 peptides were determined to be 5.04 and 7.67, respectively. In their secondary structures, the IL-1α and IL-2 proteins display a higher proportion of extended strands and alpha helices respectively. The predicted 3D structure of IL-1α and IL-2 from humans demonstrates that P01583.1.A and P60568.1.A are the most appropriate templates. The QMEAN values for the predicted models of IL-1α and IL-2 were 0.51±0.05 and 0.73±0.07, respectively. The Ramachandran plot showed that most of the amino acid phi-psi distributions are in line with a right-handed helix. These results confirm that the expected model is stable and reliable.

Introduction

Inflammation is a physiological reaction that occurs in response to several types of damage, such as those caused by physical, chemical, or biological sources. The four fundamental indications of inflammation, as indicated, are redness (rubor) and swelling (tumour), accompanied by heat (calor) and pain (dolor). This inflammation is triggered by the production of cytokines, such as interleukins (Spencer, 1935). Cytokines can be classified into hematopoietins, which encompass interleukins and colony growth factors, interferons (INF), and the tumour necrosis factor (TNF) group of proteins. Interleukins are part of a larger category of cytokines that alter cell activity. Interleukins (IL) are a class of cytokines first believed to be exclusively secreted by leukocytes; however, further research has revealed their production by several different cells in the body. They play a critical role in the stimulation and specialization of immune cells, as well as growth, development, movement, and attachment. In addition, they possess both pro-inflammatory and anti-inflammatory characteristics. Interleukins primarily serve to regulate development, differentiation, and stimulation in the context of inflammatory and immune reactions. Interleukins are a diverse set of proteins that can induce various cellular and tissue responses by binding to receptors on cell surfaces with an elevated affinity. They possess both paracrine and autocrine functionality. Animal research also employs interleukins to investigate medical care aspects (Akdis et al., 2011). The IL-1 family of cytokines consists of 11 members, whereas the IL-1 family of receptors consists of 10 members. The interleukin-1α and

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interleukin-2 family members are particularly associated with harmful inflammation. However, they also play a role in enhancing the body's ability to fight off infections and establish an immunological response to foreign substances (Gay and Keith, 1991). IL-1 serves as a central controller of inflammation by regulating many innate immune mechanisms (Dinarello, 2009). IL-1 is secreted by macrophages, large granular lymphocytes, B cells, the endothelium, fibroblasts, and astrocytes. The main targets of IL-1 include tissue cells, endothelium, B cells, T cells, and macrophages. IL-1 activates lymphocytes, activates macrophages, improves the adhesion of leukocytes and endothelial cells, raises fever by activating the hypothalamus, and tells the liver to make acute-phase proteins. Additionally, it can induce programmed apoptosis in certain cell types and lead to cachexia (Dinarello, 2018). T lymphocytes secrete interleukin-2. The main effects of IL-2 include T-cell stimulation and differentiation, enhanced cytokines production, greater susceptibility to Fas-mediated cell death, and facilitation of the formation of regulatory T cells. It stimulates the rapid increase and activation of natural killer (NK) cells, as well as the growth and replication of B-cells and antibody production. In addition, it enhances the activation of cytotoxic lymphocytes and macrophages (Bachmann and Oxenius, 2007). Therefore, understanding the structure of IL-1α and IL-2 is important for studying the critical binding areas, proposing mutagenesis studies, and developing antagonists (Kroemer et al., 1996). The primary objective of this work was to analyse the physiochemical characteristics, secondary structures, and tertiary structures of IL-1α and IL-2 using bioinformatic tools.

Materials and Methods

Sequence retrieval and physicochemical characterisation

The amino acid sequences of proinflammatory cytokines such as IL-1α and IL-2 were obtained from UniProt, an open-access protein database available at www.uniprot.org. The physicochemical features of IL-1α and IL-2 were identified based on their main amino acid sequence. Utilising Expasy's ProtParam server, the physicochemical characteristics, including molecular weight, isoelectric point, number of positive and negative residues, extinction coefficient, instability index, and grand average hydropathy were determined (Gasteiger et al., 2005).

Secondary structure prediction

The SOPMA (Self-Optimised Prediction Method) was utilised to predict the secondary structure, employing neural network techniques. The amino acid sequence in FASTA format was entered into the SOPMA window and uploaded to the SOPMA server in order to predict the secondary structure. The SOPMA method starts by searching the SWISSPORT database and identifying the most comparable sequences using the FASTA program. Afterwards, the CLUSTAL programme was used to match the requested sequence with the established homologous protein sequences. Following that, SOPM was applied to each aligned sequence to predict the capacity of amino acids to display various secondary structural elements such as alpha helices, beta-sheets, random coils, and loops (Geourjon and Deleage, 1995).

Prediction of 3D model and quality assessment

The protein's three-dimensional (3D) structure plays a crucial role in regulating its interactions, activities, and localization. The current work utilised the ab-initio approach in conjunction with the Swiss model (Kelley et al., 2015) to predict a 3-dimensional structural model of IL-1α and IL-2. The Swiss model utilises a search algorithm to identify appropriate templates from the Swiss-model template library and Protein Data Bank (PDB). It then picks the templates with the most favourable crystal structure scores to construct a 3D model. The Swiss model uses ProMod3 (Arnold et al., 2006) to forecast the 3D structural model by aligning the target template thread alignments. After predicting the 3D structure, we assessed the model's quality using the QMEAN scoring tool.

3D model validation

The PROCHECK server was utilised to perform Ramachandran plot analysis in order to validate the predicted structural models of IL-1α and IL-2. The reference phi/psi plots for Gly, Pro, pre-Pro, and general amino acid residues were derived using a carefully selected set of high-quality protein structures from the Richardson's Group at Duke University. These plots were created by applying various filters, such as the B-factor cut-off and Vander Waals clashes. The plots were then divided into three regions such as favoured, allowed, and outlier.

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Results and Discussion

Physicochemical characterisation

The IL-1α and IL-2 proteins were subjected to physicochemical characterization. The IL-1α protein was found to be composed of 271 amino acids, and its molecular weight was estimated to be 30606.61 Da. The molecular weight of the IL-2 peptide, which consists of 153 amino acids, is 17627.1 Da. Table 1 displays the physicochemical properties of IL-1α and IL-2. The calculated isoelectric (pI) values for the IL-1α and IL-2 peptides were determined to be 5.04 and 7.67, respectively. The isoelectric point (pI) is the specific value at which a molecule becomes electrically neutral, with an equal balance of negative and positive charges. The protein's lack of mobility at pI, or isoelectric point, makes it a valuable tool for creating buffer systems used in isoelectric focusing separation. The extinction coefficient of the IL-1α protein is 26025 M^{-1} cm^{-1} when all pairs of Cys residues are converted into cystines. The IL-2 peptide has an extinction value of 11710 M^{-1} cm^{-1}, which means it absorbs light at a certain rate. If all the cystine residues are reduced, the extinction coefficient of IL-1α is 25900 M^{-1} cm^{-1}, and that of IL-2 is 11460 M^{-1} cm^{-1}. The greatest extinction coefficient indicates a significant concentration of Cys, Trp, and Tyr. The EC value quantifies the levels of tryptophan and tyrosine. The calculated equilibrium constants (ECs) are used to quantitatively analyse the relationships between proteins and ligands in a liquid medium.

The instability indices of IL-1α and IL-2 were estimated to be 40.93 and 47.71, respectively. The protein instability index provides information on the stability of a protein when subjected to controlled laboratory conditions. According to Guruprasad et al., (1990) protein is considered stable if its instability index falls below 40, while a value above 40 indicates potential protein instability. Zaccaria et al., (1998) found that small elements positioned near the N-terminus of a protein have an influence on its stability, therefore altering how long it lasts. In a study conducted by Rogers et al., (1986) it was shown that proteins with a half-life of less than 5 hours had an instability index over 40, whereas proteins with a half-life beyond 16 hours displayed an instability index below 40. IL-1α has a half-life of about 30 hours in mammalian reticulocytes under in vitro conditions and a half-life exceeding 20 hours in yeast. When examined under laboratory circumstances, IL-2 has a half-life of around 30 hours in human reticulocytes and over 20 minutes in yeast. The aliphatic index of the protein sequences of IL-1α and IL-2 was calculated to be 84.94 and 108.37, respectively. The aliphatic index of these proteins indicates their ability to remain stable at various temperatures. The aliphatic index quantifies the percentage of a protein's volume occupied by aliphatic side chains, specifically those composed of amino acids A, V, I, and L. It is regarded as a beneficial element in enhancing the thermal stability of globular proteins. Protein sequences possessing a high aliphatic index are expected to exhibit stability across a broad spectrum of temperatures. The reduced thermal stability indicates a structure that is more flexible. The IL-1α and IL-2 indices in GRAVY were assessed as -0.338 and -0.007, respectively. The negative Grand Average of hydropathicity indicates that proteins have a polar and hydrophilic character, leading to increased interaction between the protein and water. The findings show that the presence of ionizable amino acids on the protein surface, which water can easily reach, primarily influences the isoelectric points (pIs) of proteins and protein complexes.

Table: 1. Physicochemical characteristics of human IL-1α and IL-2

Parameter		**IL-1α**	**IL-2**
Total no. of amino acids		271	153
Molecular weight		30606.61 Da	17627.1 Da
pI		5.04	7.67
Positively charged residues		27	16
Negatively charged residues		38	15
	Cys oxidised	26025 $M^{-1}cm^{-1}$	11710 $M^{-1}cm^{-1}$

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Extinction coefficient	Cys reduced	25900 $M^{-1}cm^{-1}$	11460 $M^{-1}cm^{-1}$
Instability index		84.94	108.37
Aliphatic index		40.93	47.71
GRAVY		-0.338	-0.007
Half-life	In mammalian reticulocytes	30 hours	30 hours
	In yeast	>20 hours	>20 hours
	In *E. coli*	>10 hours	>10 hours
Formula		$C_{1363}H_{2132}N_{350}O_{429}S_{10}$	$C_{790}H_{1285}N_{203}O_{230}S_{10}$

Table: 2. Amino acid composition of human IL-1α and IL-2

Amino acid	**IL-1α**		**IL-2**	
	No. of Residues	**% of Residues**	**No. of Residues**	**% of Residues**
Ala (A)	18	6.6	7	4.6
Arg (R)	6	2.2	5	3.3
Asn (N)	15	5.5	10	6.5
Asp (D)	19	7.0	3	2.0
Cys (C)	3	1.1	4	2.6
Gln (Q)	11	4.1	7	4.6
Glu (E)	19	7.0	12	7.8
Gly (G)	8	3.0	2	1.3
His (H)	6	2.2	3	2.0
Ile (I)	19	7.0	10	6.5
Leu (L)	25	9.2	27	17.6
Lys (K)	21	7.7	11	7.2
Met (M)	7	2.6	6	3.9
Phe (F)	13	4.8	6	3.9
Pro (P)	10	3.7	5	3.3
Ser (S)	28	10.3	11	7.2
Thr (T)	17	6.3	14	9.2
Trp (W)	2	0.7	1	0.7
Tyr (Y)	10	3.7	4	2.6
Val (V)	14	5.2	5	3.3
Pyl (O)	0	0.0	0	0.0
Sec (U)	0	0.0	0	0.0

Secondary structure

The secondary structure of IL-1α includes 22.14% alpha helices, 23.62% extended strands, and 54.24% random coils. The secondary structure of the IL-2 protein consists of 56.86% alpha helices, 11.11% extended strands, and 32.03% random coils. Table 3 displays the secondary structural components of IL-1α and IL-2. A protein's secondary structure reveals the shape of individual amino acids, indicating their positioning in a helix, strand, or coil. The results indicate that extended strands and alpha helices are the most common secondary structural components, with random coils in all sequences.

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Understanding a protein's secondary structure helps to analyse the hydrogen bonds present in the protein, which in turn provides valuable information on its structural and functional effectiveness. According to Buxbaum, (2007) random coils are important in proteins because they allow for flexibility and help with conformational changes. The prevalence of extremely flexible glycine and hydrophobic proline amino acids is responsible for the higher coil proportion. Proline has the unique ability to cause bends in polypeptide chains and disrupt an ordered secondary structure (Vidhya et al., 2013). According to Neelamathi et al., (2009) the presence of coiled sections indicates a high level of preservation and strength in the protein structure. When hydrophobic residues are added to a mixture, they form good bonds with the hydrophobic lipid bilayer (Ulmschneider and Sansom, 2001). Shelar et al., (2014) proposed a link between the structures of -helical proteins and their various activities. These functions encompass signal detection, receptor activation, ion and chemical transport across membranes, energy transfer, and preservation. The elongated conformation of α-helical proteins may also play a role in facilitating sliding motion and dynamic behaviour, both of which are critical for their optimal functioning.

Table: 3. Secondary structural elements of IL-1α and IL-2

Structural elements	IL-1α		IL-2	
	No. of residues	% of residues	No. of residues	% of residues
Alpha helices (h)	60	22.14	87	56.86
310 helix (g)	0	0.00	0	0.00
Pi helix (i)	0	0.00	0	0.00
β-bridges (b)	0	0.00	0	0.00
Extended strand (e)	64	23.62	17	11.11
β-turn (t)	0	0.00	0	0.00
Bend region (s)	0	0.00	0	0.00
Random coil (c)	147	54.24	49	32.03
Ambiguous states	0	0.00	0	0.00
Other states	0	0.00	0	0.00

3D structure

The 3D model of IL-1α and IL-2 from humans is predicted to exhibit the protein ID P01583.1 as a template that is very appropriate, having a sequence similarity of 100% and coverage of 100%. The highest ranked template in the PDB library is an alpha fold DB model of IL-1A in humans. Figure 1 displays the projected three-dimensional representation of human IL-1α. Whereas, the 3D model of IL-2 from humans exbibits P60568.1 as the most suitable template with a sequence similarity of 100% and coverage of 100%. The highest-ranked template in the PDB library is an alpha fold DB model of IL-2 in humans. Figure 2 depicts the projected three-dimensional representation of human IL-2. The confidence score is utilised to assess the accuracy and reliability of anticipated structural 3D models. It is determined by evaluating the threading alignments and convergence parameters of structure assembly simulations.

Life science research commonly uses homology modelling to generate structural models of proteins when actual structures are not available. Homology modeling is a widely used method for predicting structure in structural genomics and proteomics. The three-dimensional architecture of proteins provides precise insights into their interactions and localization in a stable conformation. The 3D structural characteristics of proteins are essential for comprehending their molecular

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functions. Biasini et al., (2014) stated that modeling and assessment approaches should provide an explanation for protein flexibility, as proteins may exist in structurally distinct functional states and are not static entities. The Swiss model was used to generate 3D structural models of target protein sequences. This approach was then employed to explore potential folds via threading, utilising template sequences from the PDB structure database as references.

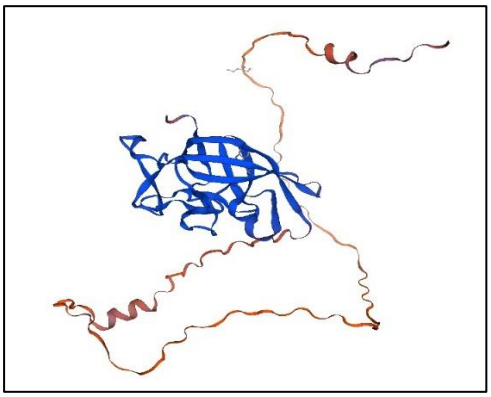

Figure: 1. Predicted 3D model of human IL-1α

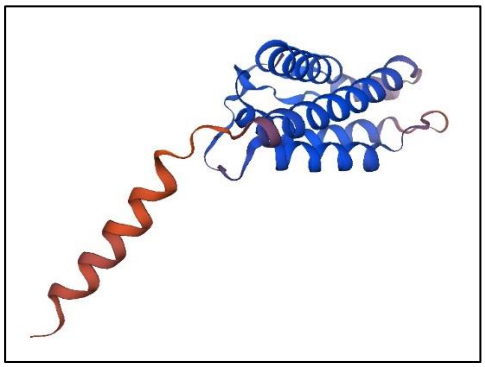

Figure: 2. Predicted 3D model of human IL-2

Quality assessment of predicted 3D models

In the present study, the QMEAN values for the predicted models of IL-1α and IL-2 were 0.51±0.05 and 0.73±0.07, respectively. The density map of the QMEAN score indicates that the expected consistency of the model falls within the range of 0 to 1. The projected model's QMEAN score closely approached zero, indicating a good model quality. Figure 3 and Figure 4 display the density plots generated by the QMEAN server for the projected 3D models of IL-1 and IL-2, respectively.

The QMEAN server was used to assess the accuracy of the projected 3D model of pectinase. QMEAN is a comprehensive index that combines statistical probabilities of average energy and model reliability with projected structural properties derived from the target protein sequence (Sippl, 1993). According to Benkert et al., (2011) the effectiveness of the projected model relies on the QMEAN score, which was standardised based on the number of interactions.

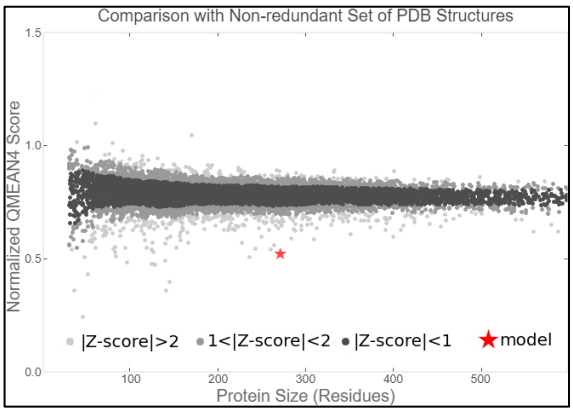

Figure: 3. The plot showing the QMEAN and Z-score of the predicted 3D model of IL-1α.

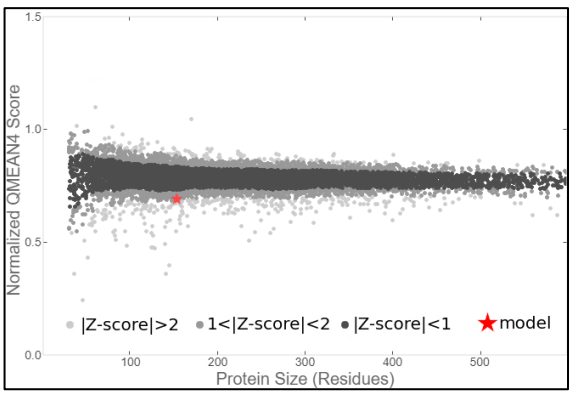

Figure: 4. The plot showing the QMEAN and Z-score of the predicted 3D model of IL-2.

Model validation

The PROCHECK server was used to carry out a Ramachandran plot assessment in order to assess the structural integrity of the predicted IL-1α and IL-2

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models, specifically focusing on the geometric aspects of the backbone conformations. The Ramachandran plot of the IL-1α 3D model shows that 80.5% of residues are located in the most favoured region, 12.0% of residues are located in the additionally allowed region, 3.6% of residues are located in the generously allowed regions, and 4.0% of residues are located in the disallowed region. Figure 5 displays the Ramachandran plot of the predicted 3D model of IL-1α, while Table 4 presents the corresponding data. The Ramachandran plot of the IL-2 3D model reveals that 95.1% of residues are located in the most favourable region, whereas 4.9% of residues are located in the additionally allowed region. Figure 6 displays the Ramachandran plot of the anticipated IL-2 3D model, while Table 6 presents the corresponding data. These results show that most of the amino acid phi-psi conformations are in line with a right-handed helix. This means that the predicted model is reliable and stable. In this study, the threading technique was employed to build the 3D structure of the protein. A threading approach improves the accuracy of sequence alignment by adding structural information about the secondary and tertiary structures of proteins to the process of alignment. The ab initio approach explores the most favourable structure that leads to the natural conformations of protein molecules, given their vast range of possible shapes. These techniques are based on the principle that a protein molecule's native structure has the lowest free energy among all its possible conformations. Arshad et al., (2013) utilised PROCHECK to validate the three-dimensional structure of silicon transporters.

Table: 4. Ramachandran plot statistics of predicted IL-1α and IL-2 models by PROCHECK.

Residues	IL-1α		IL-2	
	No. of Residues	% of Residues	No. of Residues	% of Residues
Most favoured regions	202	80.5	137	95.1
Additionally allowed regions	30	12.0	7	4.9
Generously allowed regions	9	3.6	0	0.0
Disallowed regions	10	4.0	0	0.0
Non-Glycine and Non-Proline	251	100	144	100
End Residues	2	-	2	---
Glycine	8	-	2	---
Proline	10	-	2	---

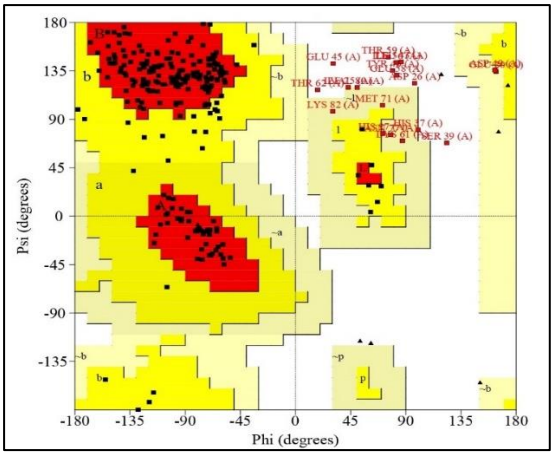

Figure: 5. Ramachandran plot statistics of predicted IL-1α structural model by PROCHECK.

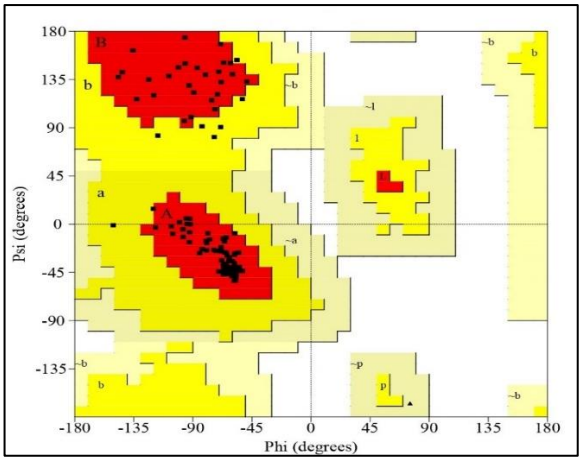

Figure: 6. Ramachandran plot statistics of predicted IL-2 structural model by PROCHECK.

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Conclusion

This study revealed that the IL-1α and IL-2 proteins in humans had acidic and basic characteristics, with pI values of 5.04 and 7.67, respectively. The analysis of IL-1α and IL-2's secondary structure demonstrated that IL-1α has a higher proportion of extended strands, whereas IL-2 has a greater number of alpha helices. In addition, the homology modelling of IL-1α and IL-2 from humans identified P01583.1.A and P60568.1.A as the most appropriate templates. The QMEAN values for the projected models of IL-1α and IL-2 were 0.51±0.05 and 0.73±0.07, respectively. The current analysis demonstrated that the anticipated structural models of IL-1α and IL-2 were dependable and consistent.

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Conflict of interest

The authors declare that there is no conflict of interest.

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