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In Silico Analysis of the Structural and Biochemical Features of the Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF), Interleukin-3 (IL-3) and Interleukin-5 (IL-5) Receptors Subunit α

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Abstract Computational analysis has become an indispensable bioinformatics approaches for the characterization of proteins regarding the physicochemical properties, prediction of signal peptides and 3D structure. Additionally, computational studies of protein–ligand interactions provide a rational basis for the speedy identification of novel leads for drug. To date no any computational analysis evaluating such parameters for GM-CSF-Rα, IL-3-Rα and IL-5-Rα. Hence, the present work aimed at identifying the theoretical basis of the physicochemical, structural and functional proprieties for these proteins using online computational tools. In the present study, different bioinformatics tools were used to characterize the properties and structure of the GM-CSF-Rα, IL3Rα, and IL5Rα proteins. Firstly, the Physico-chemical characterization was computed by ExPasy's (ProtParam). Then Fingerprinting analysis was done with ScanProsite. Followed by the functional characterization of the transmembrane regions and phosphorylation sites using SOSUI server and NetPhos server respectively. Afterwards, secondary structure prediction and the protein-ligand binding site residues were predicted by PDBSUM, and the detected ligands and their interactions were visualized by LIGPLOT and Protein ligand interaction profiler (PILP) softwares. The residues in GM-CSF-Rα, IL-3Rα and IL-5Rα proteins that may undergo ubiquitination were detected by using the UbPred and BDM-PUB programs, the predicted peptides for sumoylation in GM-CSF-Rα, IL-3Rα and IL-5Rα proteins were detected by GPS-SUMO online service. Finally, the 3D structure of proteins was built by Chimera 1.8 program. In addition, the models were surveyed using ERRAT server; as a confirmation for the quality of the models. Our results revealed that GM-CSF-Rα is stable whereas the IL3Rα and IL5Rα are classified as unstable proteins. All proteins are membrane proteins, acidic and hydrophilic in nature, with serine being the most phosphorylated amino acid. Interestingly, fibronectin type-III (FN3) domain was detected among these proteins. Also, we detected the sequences belonging to the following families: HEMATOPO_REC_S_F2, ASN_GLYCOSYLATION, CK2_PHOSPHO_SITE, PKC_PHOSPHO_SITE, MYRISTYL, CAMP_PHOSPHO_SITE, and TYR_PHOSPHO. Moreover, we detected 9 kinases in GM-CSF-Rα, while 13 kinases in IL-3-Rα and 15 kinases in IL-5-Rα. In GM-CSF-Rα 3 binding sites were detected with two ligands (GOL and NAG), and 5 binding sites in IL-3-Rα and IL-5-Rα with 3ligands (NAG, FUL and BMA) and one ligand (BGC) respectively. Secondary structure prediction showed that Beta sheet dominated all the other conformations. Modeling the 3 D structure of proteins resulted in a quality of less than 90%. computational analysis of GM-CSF-Rα, IL-3-Rα and IL-5-Rα will give a deep insight and provide opportunities for understanding the function of these proteins, and developing novel therapeutics for treating certain leukemia and inflammatory diseases.

Keywords In silico analysis, Ligand binds site, Homology modeling and GM-CSF-Rα, IL3Rα, IL5Rα and α-subunit proteins

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1. Introduction

Experimental determination of protein structure and function is becoming increasingly important, as proteins

have attracted interest as drug targets, but it is labour intensive time consuming and expensive. Thus, the use of computational tools for appoints structure to a novel protein represents the most effective alternate to experimental methods [1]. In the last years, we have seen the emergence of computational methods that have been developed for predicting the primary, secondary and tertiary structures of proteins, as well as functional analyses, reducing the time needed to conduct experiments and allowing the more rapid acquisition of results. As far as physicochemical and structural characterizations of a protein, there is no doubt that in silico approaches help resolve these problems [2]. The receptors of hematopoietic cytokines: Granulocyte-macrophage colony-stimulating factor (GM-CSF), cytokines interleukin -3 (1L-3), and interleukin -5 (IL-5) are members of a family of proteins referred to as the "cytokine receptor family", which is characterized by the existence of a 200-residue ligand-binding module [3]. These high-affinity receptors consist of multiple subunits: α subunit which is specific for each ligand, and β subunit which is common for the three receptors. [3], [4], [5]. (GM-CSF) and the concerning (IL-3) and (IL-5) cytokines regulate the production and functional activation of hematopoietic cells. (GM-CSF) is a pleiotropic cytokine that monitors the production and function of blood cells, mainly monocyte /macrophages and all granulocytes. It is deregulated in clinical conditions such as rheumatoid arthritis and leukemia, likewise offers therapeutic value for other diseases [6]. GM-CSF also controls dendritic cell and T-cell function, so that linking innate and acquired immunity [7]. Interleukin 3 (IL-3) is a cytokine produced predominantly by antigen-activated T cells that links immunity to the hematopoietic system and plays a considerable role in leukemia as well as various immune pathologies [8]. By actions on several cell types, IL-3 participates to allergic inflammation, autoimmune diseases, and oncogenesis. Importantly, leukemic stem cells from patients with acute myeloid leukemia (AML) and chronic myeloid leukemia (CML) over express the IL-3 receptor α chain (IL3Rα), and this is associated with a poor prognosis in AML [8]. Interleukin 5 (IL-5) is a hematopoietic growth factor, primarily known as a T-cell-derived cytokine, has pleiotropic effects on different target cells, including eosinophils and B cells, and induces cell proliferation, survival and differentiation [9]. The capability of cytokines to impact the course of cell growth and differentiation uniquely rely on their recognition and binding by specific receptors; these cell surface molecules transducer the binding of cytokines into cytoplasmic signals that trigger developmental processes within the cell [10]. The subunits (GM-CSF-Rα, IL-3Rα and IL-5Rα) are cytokine-specific binding proteins, and each α subunit alone binds its specific ligand with low affinity. In contrast, the β subunit does not join any cytokine by itself, but forms high-affinity receptors with α subunits. Human GM-CSF, IL-3 and IL-5 receptors have only one type of β subunit (common β, or βc) which is participated by the three receptors [5]. The thorough structure of GM-CSF-Rα, IL-3Rα and IL-5Rα is identical: they are glycoproteins of - 60-80 kDa having the common motif of the cytokine receptor super family in the extracellular domain and they have a small cytoplasmic domain with a short stretch of an amino acid sequence which is conserved among these α subunits [5]. It has been speculated that the ligand-specific α subunits may have a role in transmitting ligand-specific signals, though the common β subunit plays a major role in signal transduction for proliferation [11]. The GM-CSF, IL-3 and IL-5 receptor α chains form a special subgroup and share features not found in other members of the cytokine receptor family, features which are suggested to be important for their interaction with the common beta chain and for their binding of the structurally-related ligands [5]. So, the main objective of this study is to fulfill a protein analysis of α Subunit of GM-CSF, IL-3, and IL-5 receptors using up-to-date bioinformatics tools, and to highlight the differences and similarities between these proteins. This will further reveal the complex nature of the mechanisms by which these receptors regulate signal transduction of hematopoietic stem cells. In addition, such understanding for these receptors provides opportunities for the development of new therapies to block the action of their cytokines in certain haematological malignancies. Nevertheless, the knowledge of these receptors is placed in context with advances in understanding of the structural biology of other members of the cytokine receptor family.

2. Materials and Methods

2.1. Extraction of Protein Sequences

The protein sequences of hematopoietic cytokines receptors were extracted from UniProt (http://www.uniprot.org/). The UniProt database is a substantial collection of protein sequences and their annotations. It has cross-references to over 150 databases and acts as a central axis to regulate protein information [12]. The protein sequences were retrieved in FASTA format, in order to be analyzed by computational methods.

2.2. Identification of Amino Acid Percentage Composition and Physico-Chemical Properties

The primary structure was predicted using the ProtParam server; it is free online tool (http://web.expasy.org/protparam/) in Expasy. The parameters computed by ProtParam include the molecular weight (M.Wt), isoelectric point (pI), amino acid composition, atomic composition, extinction coefficient (EC), estimated half-life, instability index(II), aliphatic index(AI) and grand average of hydropathicity (GRAVY). The amino acid and atomic compositions are self-explanatory. All the other parameters will be explained below [13].

Isoelectric Point (pI):

The calculated isoelectric point (pi) is useful since at this point the solubility is lost and the mobility in an electric field is zero. Isoelectric point is the pH at which the surface of the proteins is covered with the charge but a net charge of the protein is zero.

Extinction Coefficients (EC):

The extinction coefficient indicates how much light a protein absorbs at a certain wavelength. It is useful to have an estimation of this coefficient for analyzing a protein with a spectrophotometer when purifying it. [14]. It has been shown that it is possible to estimate the molar extinction coefficient of a protein from knowledge of its amino acid composition. For example, the molar extinction coefficient of Tyrosine, Tryptophan and Cystine (Tyrosine does not absorb appreciably at wavelengths >260 nm, while Cystine does) at a given wavelength.

Instability Index (II):

The instability index provides an estimate of the stability of a protein in a test tube. Statistical analysis of 12 unstable and 32 stable proteins has revealed that there are certain dipeptides, the occurrence of which is significantly different in the unstable proteins compared with those in the stable ones [15]. A protein whose instability index is smaller than 40 is predicted as stable, a value above 40 predicts that the protein may be unstable.

Aliphatic Index (AL):

The aliphatic index of a protein is defined as the relative volume occupied by aliphatic side chains (Alanine, Valine, Isoleucine, and leucine). It may be regarded as a positive factor for the increase of thermostability of globular [16].

Grand Average of Hydropathy (GRAVY):

The GRAVY value for a peptide or protein is calculated as the sum of hydropathy values of all the amino acids, divided by the number of residues in the sequence [17].

Estimated Half-Life:

The half-life is a prediction of the time required for half of a protein in a cell to degrade after its synthesis. ProtParam relies on the "N-end rule", which relates the half-life of a protein to the identity of its N-terminal residue; the prediction is given for three model organisms; human, yeast, and E. coli. The identity of the N terminal residue of a protein plays an important role in determining its stability in vivo. Proteins have strikingly different half-lives in vivo, from seconds to hours, depending on the nature of the amino acid at the N terminus and the different models.

2.3. Hydrophobicity Analysis

Percentages of hydrophobic and hydrophilic residues were calculated from the percentage of Amino Acid composition.

2.4. Fingerprinting Analysis

ScanProsite used for fingerprinting analysis, it is free online database and tool (http://prosite.expasy.org/scanprosite/). Also, it has a large collection of biologically meaningful signatures that are described as patterns (regular expressions), used for short motif detection, or generalized profiles (weight matrices) for sensitive detection of larger domains. Each signature is linked to detailed annotation that provides useful biological information on the protein family, domain, or functional sites identified by the signature [18]. PROSITE is copyright. It is produced by the SIB Swiss Institute Bioinformatics. There are no restrictions on its use by non-profit institutions as long as its content is in no way modified. Usage by and for commercial entities requires a license agreement.

2.5. Transmembrane Sequence Analysis

Transmembranase domains were predicted by using SOSUI server, which distinguishes between membrane and soluble proteins from amino acid sequences, and predicts the transmembrane helices for the former [19]. The system SOSUI is available through internet access (http://harrier.nagahama-i-bio.ac.jp/sosui/sosui_submit.html).

2.6. Prediction of Hydrophobic Residues

The hydrophobic residues were predicted by using pepwheel, it is available at (http://emboss.open-bio.org/wiki/Appdocs) on the EMBOSS Wiki. pepwheel program draws a helical wheel diagram for a protein sequence. This displays the sequence in a helical representation as if looking down the axis of the helix. It is useful for highlighting amphipathicity and other properties of residues around a helix. By default, aliphatic residues are marked with squares; hydrophilic residues are marked with diamonds, and positively charged residues with octagons, although this can be changed [20].

2.7. Prediction of Phosphorylation Sites

Phosphorylatiuonsites were predicted by using NetPhos 3.1 server, it is publicly available at the (http://www.cbs.dtu.dk/services/NetPhos/). The NetPhos 3.1 server produces neural network predictions for serine, threonine and tyrosine phosphorylation sites in eukaryotic proteins in eukaryotic proteins using ensembles of neural networks. Both generic and kinase specific predictions are performed. The kinase specific predictions are identical to the predictions by NetPhosK 1.0. Predictions are made for the following 17 kinases: ATM, CKI, CKII, CaM-II, DNAPK, EGFR, GSK3, INSR, PKA, PKB, PKC, PKG, RSK, SRC, cdc2, cdk5 and p38MAPK. [21].

2.8. Protein Ubiquitination Sites Prediction

UbPred and BDM-PUB programs were used to predict ubiquitylation sites [22]. In UbPred, lysine residues with a score of 0.62 were considered ubiquitylated. For BDM-PUB, the balanced cut-off option was selected. UbPred was developed by Predrag Radivojac (Indiana University, School of Informatics), Vladimir Vacic

(Columbia University) and Lilia Iakoucheva (University of California, San Diego). It is publicly available at the (http://www.ubpred.org/). BDM-PUB it is publicly available at the (http://bdmpub.biocuckoo.org/). Copyright © 2006-2009. The CUCKOO Workgroup, USTC.

2.9. Protein Sumoylation Sites Detection

The identification of small ubiquitin-like modifiers (SUMOs) sites was carried out with the help of GPS-SUMO web server (http://sumosp.biocuckoo.org/. It is a novel web server developed for the prediction of both sumoylation sites and SUMO-interaction motifs (SIMs) in proteins. Copyright © 2006-2014. The CUCKOO Workgroup. [23]. In addition, the primary structure of these peptides was drawn using pepdraw, a tool to draw peptide primary structure and calculate theoretical properties.

2.10. Signal Peptide Prediction (Predisi)

Prediction of signal peptides was performed using PrediSi (PREDIction of SIgnal peptides). It is new software for predicting signal peptide sequences and their cleavage positions in bacterial and eukaryotic proteins. Available at (http://www.predisi.de/) [24]. Coordinated by Karsten Hiller Institute for Microbiology, Technical University of Braunschweig.

2.11. Protein – Ligand Binding Sites Detection

The identification of specific ligand-binding site on the three proteins was performed by PDBsum, LIGPLOT and PLIP. PDBsum (http://www.ebi.ac.uk/pdbsum) is a pictorial database that provides an at-a-glance overview of the contents of each 3D structure deposited in the Protein Data Bank (PDB). It shows the molecule(s) that make up the structure (ie protein chains, DNA, ligands and metal ions) and schematic diagrams of the interactions between them [25]. Schematic 2-D representations of protein-ligand complexes from standard Protein Data Bank file input were automatically generated by the LIGPLOT (http://www.ebi.ac.uk/thornton-srv/software/LIGPLOT/program). The results of interactions shown are those mediated by hydrogen bonds and by hydrophobic contacts. Hydrogen bonds are indicated by dashed lines between the atoms involved, while hydrophobic contacts are represented by an arc with spokes radiating towards the ligand atoms they contact. The contacted atoms are shown with spokes radiating back [26]. Additionally, 3D structures of these protein –ligands complexes were presented by the protein–ligand interaction profiler (PLIP), a novel web service for fully automated detection and visualization of relevant non-covalent protein–ligand contacts in 3D structures, freely available at (http://plip.biotec.tu-dresden.de/plip-web/plip/index) [27].

2.12. 3D Structure of the Proteins

The 3-dimensional structure anticipation we applied CPH models 3.2 servers (http://www.cbs.dtu.dk/) to predict the PDB of proteins. It is a protein homology modeling server, where the template realization is based on profile-to profile arrangement, guided by secondary structure and presentation prognosis [28]. Visualization and characterization of the protein model were done by Chimera (version 1.8) Chimera (http://www.rbvi.ucsf.edu/chimera) is developed by the Resource for biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIGMS P41-GM103311) software [29].

2.13. Validation of 3D Models

Structural validation of proteins models was done by ERRAT; a program for verifying protein structures determined by crystallography. Error values are plotted as a function of the position of a sliding 9-residue window. The error function is based on the statistics of non-bonded atom-atom interactions in the reporting structure (compared to a database of reliable high-resolution structures) (http://services.mbi.ucla.edu/ERRAT/) [30].

3. Results

3.1. The Sequences Retrieve

The sequences for receptors of three hematopoietic cytokines: GM-CSF-Rα, IL3Rα, and IL5Rα proteins were retrieved from Uniprot database (www.uniprot.org), Homo sapiens database. The UniProt database is a substantial collection of protein sequences and their annotations. It has cross-references to over 150 databases and acts as a central axis to regulate protein information [12], using these sequences in FASTA format for further analysis. The description of proteins were analyzed in this study were shown in (Table 1).

Table 1. Proteins examined in this study

Accession number	Length [Amino Acid]	Description
P15509	400	Granulocyte-macrophage colony-stimulating factor receptor subunit alpha **(GM-CSF-Rα)**Homo sapiens
P26951	378	Interleukin-3 receptor subunit alpha **(IL3Rα)** Homo sapiens
Q01344	420	Interleukin-5 receptor subunit alpha **(IL5Rα)** Homo sapiens

3.2. Primary Structure Prediction

The parameters were computed by ProtParam. The Atom composition of proteins, the percentage of their hydrophobic and hydrophilic residue content, and their amino acid composition are shown in (Table 2), (Table 3) and (Table 4) respectively.

Table 2. Atoms composition and formulas for GM-CSF-Rα, IL-3Rα, and IL-5Rα proteins

Atomic composition	GM-CSF-Rα	IL-3Rα	IL-5Rα
Carbon (C)	2061	1936	2164
Hydrogen (H)	3210	3024	3362
Nitrogen (N)	574	536	554
Oxygen (O)	604	549	629
Sulfur (S)	16	23	15
Formula	C2061H3210N574O604S16	C1936H3024N536O549S23	C2164H3362N554O629S15
Total № of Atoms	6465	6068	6724

Table 3. Hydrophilic and hydrophobic residue content

Proteins	Percentage of Hydrophobic Residues	Percentage of Hydrophilic Residues	Net Hydrophobic Residues Content
GM-CSF-Rα	38.4%	61.8%	Low
IL-3Rα	44%	56.4%	Low
IL-5Rα	45.2%	52.9%	Low

Table 4. Amino acid composition (in %) of GM-CSF-Rα, IL-3Rα, and IL-5Rα proteins using ProtParam tool

Amino acid	IL-3Rα	GM-CSF-Rα	IL-5Rα
Ala (A)	6.6%	3.0%	6.0%
Arg (R)	6.9%	7.5%	3.6%
Asn (N)	4.8%	6.8%	4.5%
Asp (D)	4.8%	4.8%	4.8%
Cys (C)	3.7%	3.2%	2.6%
Gln (Q)	5.8%	3.8%	3.8%
Glu (E)	4.8%	6.8%	6.9%
Gly (G)	4.2%	5.0%	3.8%
His (H)	1.9%	2.2%	2.6%
Ile (I)	5.0%	5.2%	9.0%
Leu (L)	9.8%	10.8%	10.5%
Lys (K)	4.2%	4.5%	5.0%
Met (M)	2.4%	0.8%	1.0%
Phe (F)	4.5%	5.5%	3.6%
Pro (P)	5.3%	4.8%	5.5%
Ser (S)	5.8%	7.2%	7.6%
Thr (T)	6.6%	6.5%	6.7%
Trp (W)	2.6%	1.5%	2.6%
Tyr (Y)	2.9%	3.5%	3.6%
Val (V)	7.4%	6.8%	6.4%
Pyl (O)	0.0%	0.0%	0.0%
Sec (U)	0.0%	0.0%	0.0%

3.3. Physicochemical Analysis

The Physico-chemical characteristics involve the molecular weight, isoelectric point, total number of positive and negative residues, extinction coefficient, and grand average of hydropathicity are depicted in (Table 5).

Table 5. Physical and Chemical Characters of the Primary Structures of Predicted Proteins in Theory

Proteins	M.wt	-R	+R	EC(M-1cm-1)	GRAVY	pI
GM-CSF- Rα	46206.60	46	48	54610	-0.382	7.91
IL-3Rα	43329.95	36	42	72265	-0.202	8.60
IL-5Rα	47684.73	49	36	83475	-0.054	5.36

M.Wt. molecular weight; −R: number of negative residues (Arg + Lys); +R: number of positive residues (Asp + Glu); EC: extinction coefficient at 280 nm; GRAVY: grand average hydropathy, pI: isoelectric point

3.4. Half Lifetime, Stability and Solubility

The estimated half-life, instability index (II) and aliphatic index(AI) of proteins are shown in (Table 6).

Table 6. Estimated half-life of GM-CSF-Rα, IL-3Rα, and IL-5Rα proteins using ProtParam tool

Estimated half-life	GM-CSF- Rα	IL-3Rα	IL-5Rα
Mammalian reticulocytes, in vitro	30 hours	30 hours	30 hours
yeast, in vivo	>20 hours	>20 hours	>20 hours
Escherichia coli, in vivo).	>10 hours	>10 hours	>10 hours
N-terminal	M (Met)	M (Met)	M (Met)
Instability index(II)	38.88	44.49	43.11
Aliphatic index(AL)	84.97	85.87	100.74

3.5. Functional Site Predication

The potential domains as well as characteristic motifs and patterns contained in GM-CSF-Rα, IL-3Rα, and IL-5Rα proteins were investigated by ScanProsite. The results are shown in (Tables 7, 8, 9 and 10).

Table 7. Domains detected in GM-CSF-Rα, and IL5Rα proteins using ScanProsit

Proteins	Domain's name	Position in protein sequence
GM-CSF- Rα	Fibronectin type-III (FN3)	220 - 320
IL-3Rα	Fibronectin type-III (FN3)	32 - 123
IL-5Rα	Fibronectin type-III (FN3)	241 - 334

Table 8. GM-CSF- Rα protein expression profiles using ScanProsit

Signature	Predicted feature	Phosphorylation site	Position in protein sequence	Sequence
HEMATOPO_REC_S_F2	-	-	134 - 165	-
ASN_GLYCOSYLATION	CARBOHYD	*N-glycosylation*	46 - 49	NLSW
			54 - 57	NTTF
			99 - 102	NTSQ
			123 – 126	NFSC
			135 – 138	NCTW
			182 – 185	NLSG
			195 – 198	NGTS
			223 – 226	NVTV
			229 – 232	NTTH
			272 - 275	NVSG
			305 – 308	NWSS
PKC_PHOSPHO_SITE	MOD_RES	Phosphothreonine	63 – 65	TdK
		Phosphothreonine	82 – 84	TfR
		Phosphoserine	101 – 103	SqR
		Phosphoserine	114 – 116	SgR
		Phosphoserine	157 – 159	SkR
		Phosphothreonine	187 – 189	TsR
		Phosphothreonine	197 – 199	TsR
		Phosphothreonine	212 – 214	TkK
		Phosphothreonine	225 – 227	TvR
		Phosphoserine	293 – 295	SvK
		Phosphothreonine	395 – 397	TvK
CK2_PHOSPHO_SITE	MOD_RES	Phosphoserine	75 – 78	SnnE
		Phosphothreonine	82 - 85	TfrE
		Phosphoserine	114 - 117	SgrE
		Phosphothreonine	197 - 200	TsrE
		Phosphoserine	208 - 211	SllD
		Phosphoserine	247 - 250	SylD
		Phosphoserine	308 - 311	SwsE
		Phosphothreonine	382 - 385	TpeE
		Phosphothreonine	395 - 398	TvkE
MYRISTYL	-	*N-myristoylation*	173 - 178	GThvGC
	-		316 - 321	GSddGN
	-		320 - 325	GNlgSV
	-		334 - 339	GTlvCG
	-		339 - 344	GIvlGF
CAMP_PHOSPHO_SITE	-	*cAMP- and cGMP-dependent protein kinase phosphorylation*	259 - 262	RKnT

Table 9. IL-3Rα protein expression profiles using Scanprosit

Signature	Predicted feature	phosphorylation site	Position in protein sequence	Sequence
HEMATOPO_REC_S_F2	-	-	120 -151	-
CK2_PHOSPHO_SITE	MOD_RES	Phosphothreonine	19 - 22	TkeD
		Phosphothreonine	48 - 51	TdiE
		Phosphoserine	74 - 77	SlcE
		Phosphoserine	203 - 206	SqiE
ASN_GLYCOSYLATION	CARBOHYD	*N-glycosylation site*	46 - 49	NVTD
			64 - 67	NNSY
			80 - 83	NYTV
			109 - 112	NLTC
			212 - 215	NMTA
			218 - 221	NKTH
PKC_PHOSPHO_SITE	MOD_RES	Phosphothreonine	82 - 84	TvR
		Phosphoserine	100 - 102	SgK
		Phosphothreonine	196 - 198	TdK
		Phosphothreonine	214 - 216	TaK
TYR_PHOSPHO_SITE	-	*Tyrosine kinase phosphorylation*	146 – 154	RqqyEclhY
	-		273 - 279	Rar.Erv.Y
MYRISTYL	-	*N-myristoylation*	160 - 165	TriGC
	-		176 - 181	GSqsSH
	-		192 - 197	GIpcTD
	-		313 - 318	GTllAL
	-		363 - 368	GLeeCL

MOD_RES = (Modified residue). CARBOHYD = (Glycosylation)

Table 10. IL-5Rα protein expression profiles using Scan Prosit

Signature	Predicted feature	phosphorylation site	Position in protein sequence	Sequence
HEMATOPO_REC_S_F2	-	-	153-182	-
ASN_GLYCOSYLATION	CARBOHYD	N-glycosylation	35 - 38	NFTI
			131 - 134	NLTC
			137 - 140	NTTE
			142 - 145	NYSR
			216 - 219	NGSS
			244 - 247	NVTA
PKC_PHOSPHO_SITE	MOD_RES	Phosphothreonine	37 - 39	TiK
		Phosphoserine	97 - 99	SvR
		Phosphoserine	218 - 220	SsK
MYRISTYL	-	N-myristoylation	123 - 128	GSpgTS
	-		251 - 256	GTrlSI
	-		395 - 400	GSseTE
CK2_PHOSPHO_SITE	MOD_RES	Phosphothreonine	138 - 141	TteD
		Phosphoserine	177 - 180	SwtE
		Phosphoserine	296 - 299	SiiD
		Phosphoserine	302 - 305	SkyD
		Phosphothreonine	389 - 392	TnyE
		Phosphoserine	397 - 400	SetE
		Phosphothreonine	399 - 402	TeiE
		Phosphothreonine	414 - 417	TleD

MOD_RES = (Modified residue). CARBOHYD = (Glycosylation)

3.6. Prediction of the Transmembrane Site

The SOSUI server performed the identification of transmembrane region. The transmembrane regions and their length were classified in (Table 11).

Table 11. Transmembrane sequence analysis of SOSUI server

Proteins	N terminal	Transmembrane region	C terminal	type	length	Average of hydrophobicity
GM-CSF-Rα	1	MLLLVTSLLLCE	12	Signal Peptide	12	-0.381750
	9	LLCELPHPAFLLIPEKSDLRTVA	31	Secondary	23	
	325	VYIYVLLIVGTLVCGIVLGFLF	346	Primary	22	
IL-3Rα	1	MVLLWLTLLLIALPCLLQ	18	Signal Peptide (Primary)	18	-0.201587
	305	RTSLLIALGTLLALVCVFVICR	326	Primary	22	
	1	MIIVAHVLLILLGATE	16	Signal Peptide (Secondary)	16	
	29	SLLPPVNFTIKVTGLAQVLLQW	50	Secondary	22	
IL-5Rα	347	IMATICFILLILSLICKICHLWI	369	Primary	23	-0.054048

3.7. Helical Wheel Predicted by Pepwheel Program

The Hydrophilic residues for protein sequences were predicted by utilizing pepwheel program. By default, aliphatic residues are marked with squares; hydrophilic residues are marked with diamonds, and positively charged residues with octagons. The result summarizes in fig 1, 2 and 3.

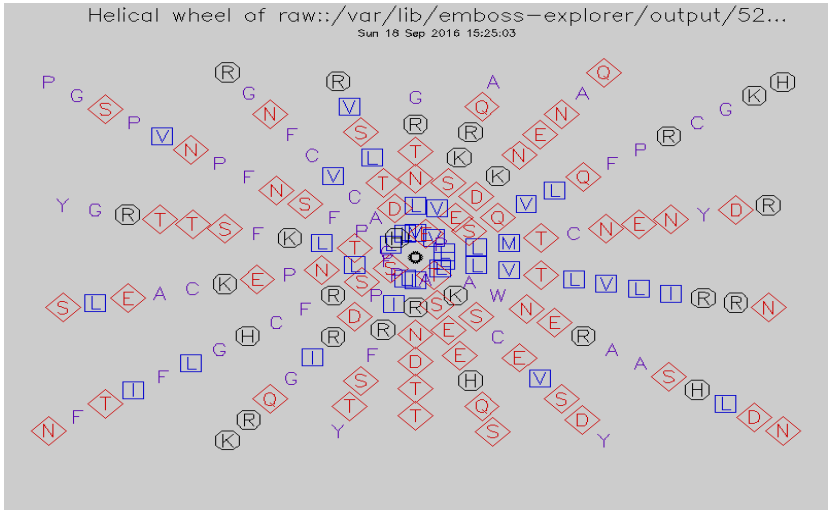


Figure 1. Helical wheel predicted by pepwheel for GM-CSF-Rα Protein

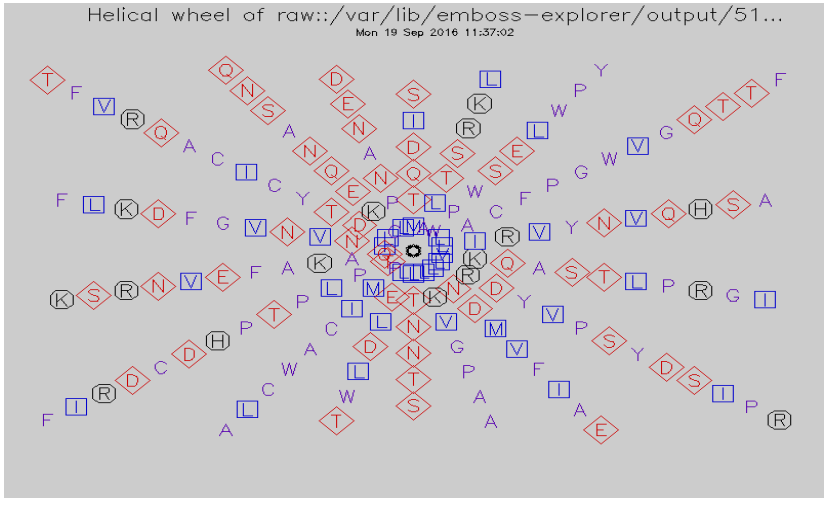


Figure 2. Helical wheel predicted by pepwheel for IL-3Rα Protein

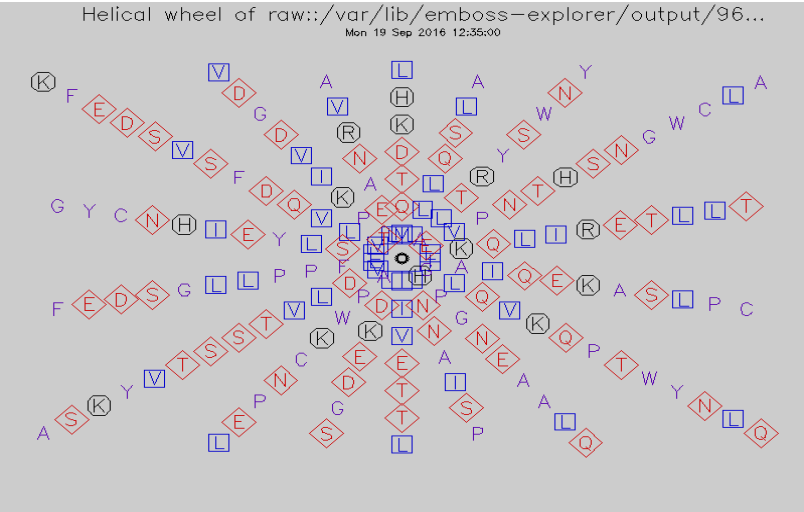


Figure 3. Helical wheel predicted by pepwheel for IL-5Rα Protein

Table 12. Phosphorylation and Kinase sites predicted in GM-CSF-Rα protein by using NetPhos3.1

Phosphorylation Site	No of serine, Threonine and Tyrosine	Position	Kinase
Serine	15	35	cdc2
		42	Unsp, PKA
		48	Unsp, CKII
		75	unsp
		101	PKC, DNAPK, ATM
		114	unsp
		157	Unsp, PKC
		208	Unsp, cdc2
		247	Unsp, PKC, cdc2
		274	CKII
		286	Unsp, PKC, cdc2
		293	Unsp, PKC, PKA, PKG, cdc2
		308	Unsp, CKII
		310	CKII
		317	CKII, cdc2
Threonine	13	29	PKC
		63	PKC
		82	PKC
		119	Unsp, PKG
		187	Unsp, PKC
		197	Unsp, PKC
		212	Unsp, PKC
		225	PKC
		230	Unsp
		242	PKC
		262	Unsp, DNAPK, PKA
		382	Unsp, CKI, CKI1
		395	Unsp, PKC, CKII
Tyrosine	4	111	unsp
		167	INSR
		168	unsp
		248	unsp

Table 13. Phosphorylation and Kinase sites predicted in IL-3Rα protein by using NetPhos3.1

Phosphorylation Site	No of serine, Threonine and Tyrosine	Position	Kinase
Serine	10	66	PKG, cdc2, PKA
		74	PKG,CKII
		174	Unsp, PKC
		175	Unsp, PKC, RSK
		177	Unsp, DNAPK, ATM
		188	PKA, PKG
		203	Unsp, ATM, CKI, PKG, DNAPK
		222	PKC
		259	PKA
		307	PKA, CKI
Threonine	9	48	CKII, PKG
		82	PKC,Unsp
		92	PKC
		196	PKC
		209	p38MAPK, GSK3
		214	PKC
		258	Unsp, PKA, PKG
		287	Unsp, cdk5
		314	PKC
Tyrosine	6	58	Unsp
		67	Unsp
		139	Unsp
		154	Unsp
		238	INSR
		279	Unsp

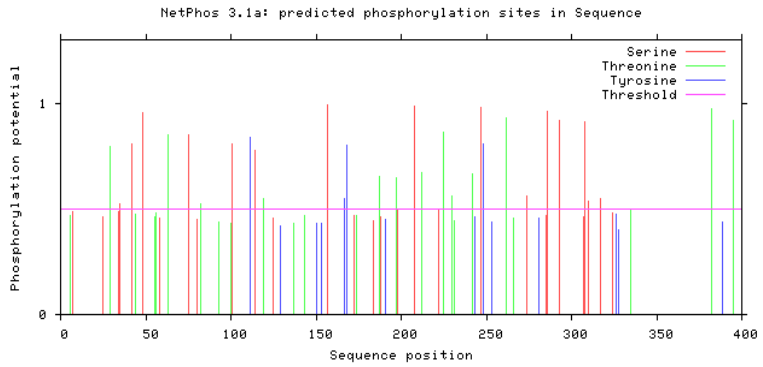


Figure 4. Predicted Phosphorylation sites in GM-CSF-Rα protein

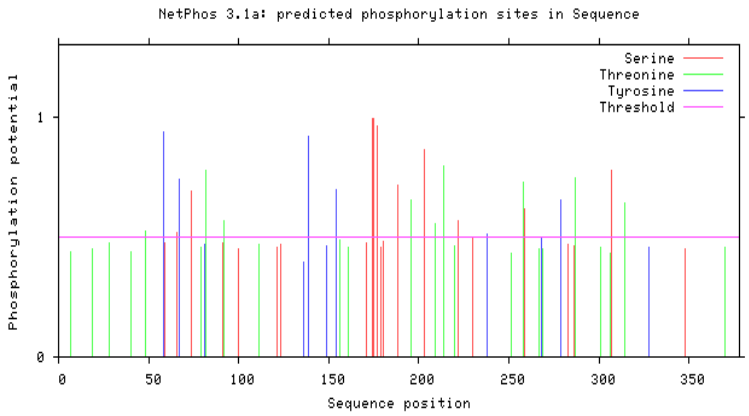


Figure 5. Predicted Phosphorylation sites in IL-3Rα protein

Table 14. Phosphorylation and Kinase site predicted in IL-5Rα protein by using NetPhos

Phosphorylation Site	No of serine, Threonine and Tyrosine	Position	Kinase
Serine	23	29	PKA
		84	Unsp, PKA, PKC
		95	PKC
		97	PKC,cdc2
		107	cdc2
		112	Unsp, cdc2
		115	unsp
		124	GSK3,cdk5, p38MAPK, CKI
		128	PKA, unsp
		144	Unsp, cdc2
		148	Unsp, PKA
		177	unsp, PKA, RSK
		186	CKI
		205	PKC
		222	unsp
		255	unsp, PKA
		296	unsp
		302	unsp
		314	unsp
		326	DNAPK, ATM
		359	cdc2
		396	unsp, CKII
		397	Unsp, CKII, cdc2
Threonine	16	15	Cdc2
		37	PKC, unsp
		41	PKC
		71	CKII
		82	unsp
		88	PKC
		127	PKC
		138	unsp
		161	CKII
		167	unsp,DNAPK
		179	CKII
		189	PKC
		201	PKC, p38MAPK
		389	Unsp, CKII
		399	CKII, CKI
		414	unsp
Tyrosine	8	77	Unsp, INSR, EGFR
		143	unsp
		169	unsp, SRC, EGFR
		185	unsp, INSR, EGFR
		272	INSR
		330	unsp
		391	unsp
		406	EGFR

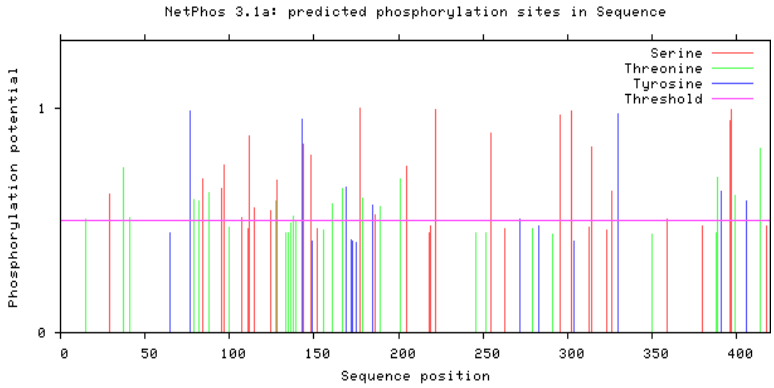


Figure 6. Predicted Phosphorylation sites in IL-5Rα protein

3.8. Prediction of Phosphorylation Sites

The NetPhos 3.1 server predicted Phosphorylation site (Serine, Threonine and Tyrosine) and Kinase site for GM-CSF-Rα, IL-3Rα proteins and IL-5Rα. The results showed that cdc2, unsp, PKA, PKC, CKII, CKI, ATM, INSR and DNAPK were common in all proteins. Whereas RSK, p38MAPK, cdk5 and GSK3 were placed in IL-3Rα and IL-5Rα, EGFR and SRC only found in IL-5Rα. The results shown in Table 12 and figure 4, 5 and 6.

3.9. Prediction of Protein Ubiquitination Sites

The residues in GM-CSF-Rα, IL-3Rα and IL-5Rα proteins that may undergo ubiquitylation were analyzed by using the UbPred and BDM-PUB programs. In the GM-CSF-Rα, 13 and 2 Ubiquitination sites were predicted by UbPred and BDM-PUB respectively. Only one site (K 387) was conformation by both tools. In IL-3Rα 6 and 3 Ubiquitination sites were predicted by UbPred and BDM-PUB respectively. Also, only one site (K 361) was conformation by both tools. In IL-5Rα 9 and 4 Ubiquitination sites were predicted by UbPred and BDM-PUB respectively. Only one site (K 393) was conformation by both tools. The result shown in tables (16), (17), (18), (19), (20), and (21).

Table 16. Predicted Ubiquitination sites in GM-CSF-Rα protein detected by Bayesian Discriminate Method (BDM)

Peptide	Position	Score	Threshold
AFLLIPEKSDLRTVA	24	1.09	0.3
QENTTFSKCFLTDKK	59	0.87	0.3
SKCFLTDKKNRVVEP	65	2.00	0.3
KCFLTDKKNRVVEPR	66	0.94	0.3
SQRGFQQKLLYPNSG	108	0.76	0.3
THCLVRWKQPRTYQK	238	0.37	0.3
KQPRTYQKLSYLDFQ	245	0.81	0.3
PSSEPRAKHSVKIRA	291	1.16	0.3
PRAKHSVKIRAADVR	295	2.51	0.3
FPPVPQIKDKLNDNH	363	0.70	0.3
PVPQIKDKLNDNHEV	365	0.85	0.3
EFTPEEGKGYREEVL	387	1.04	0.3
REEVLTVKEIT	397	1.92	0.3

Table 17. Predicted Ubiquitination sites in GM-CSF-Rα protein detected by UbPred server

Residue	Score	Ubiquitinated
260	0.77	Yes, Medium confidence
387	0.65	Yes, Low confidence

Low confidence $0.62 \leq s \leq 0.69$, Medium confidence $0.69 \leq s \leq 0.84$, High confidence $0.84 \leq s \leq 1.00$.

Table 18. Predicted Ubiquitination sites in IL-3Rα protein detected by Bayesian Discriminate Method (BDM)

Peptide	Position	Score	Threshold
LPCLLQTKEDPNPPI	20	0.34	0.3
PITNLRMKAKAQQLT	33	1.12	0.3
FGIPCTDKFVVFSQI	198	0.74	0.3
FPRIPHMKDPIGDSF	342	0.37	0.3
LVVWEAGKAGLEECL	361	0.76	0.3
TEVQVVQKT	377	2.96	0.3

Table 19. Predicted Ubiquitination sites in IL-3Rα protein detected by UbPred server

Residue	Score	Ubiquitinated
54	0.66	Yes, Low confidence
102	0.69	Yes, Medium confidence
361	0.63	Yes, Low confidence

Low confidence $0.62 \leq s \leq 0.69$, Medium confidence $0.69 \leq s \leq 0.84$, High confidence $0.84 \leq s \leq 1.00$.

Table 20. Predicted Ubiquitination sites in IL-5Rα protein detected by Bayesian Discriminate Method (BDM)

Peptide	Position	Score	Threshold
AQVLLQWKPNPDQEQ	51	0.49	0.3
QVKINAPKEDDYETR	73	0.51	0.3
KCVTILHKGFSASVR	92	1.32	0.3
EECQEYSKDTLGRNI	187	1.52	0.3
PRTFILSKGRDWLAV	206	0.89	0.3
VLVNGSSKHSAIRPF	220	0.65	0.3
FPPIPAPKSNIKDLF	379	2.50	0.3
PAPKSNIKDLFVTTN	383	1.16	0.3
FVTTNYEKAGSSETE	393	0.82	0.3

Table 21. Predicted Ubiquitination sites in IL-5Rα protein detected by UbPred server

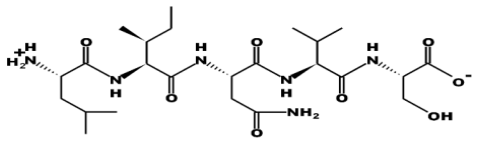
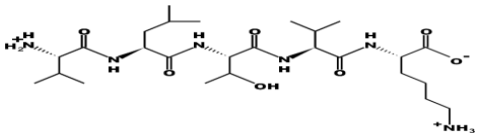
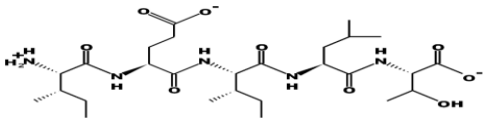
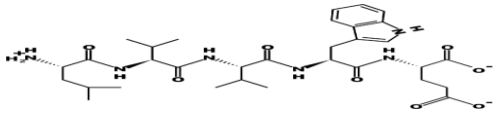
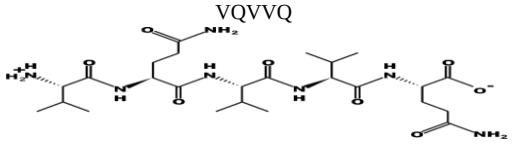
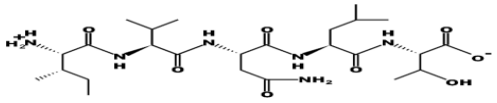
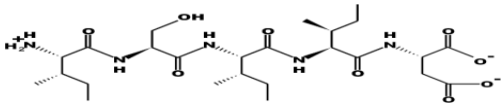
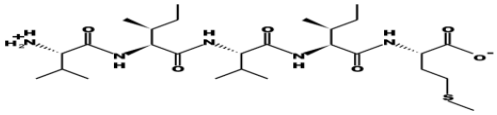
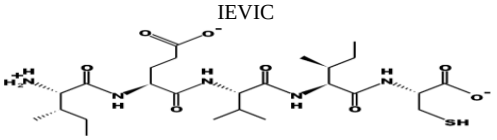
Residue	Score	Ubiquitinated
27	0.64	Yes, Low confidence
288	0.62	Yes, Low confidence
393	0.65	Yes, Low confidence
409	0.66	Yes, Low confidence

Low confidence $0.62 \leq s \leq 0.69$, Medium confidence $0.69 \leq s \leq 0.84$, High confidence $0.84 \leq s \leq 1.00$.

3.10. Protein Sumoylation Sites Detection

The predicted peptides for sumoylation in GM-CSF-Rα, IL-3Rα and IL- 5Rα proteins by using GPS-SUMO online service are displayed in (Table 22).

Table 22. Sumoylation sites in GM-CSF-Rα, IL-3Rα and IL-5Rα proteins detected by GPS-SUMO

Proteins	Peptide	position	Score	Cutoff	P-value
GM-CSF-Rα	LINVS 	**270 - 274**	**36.244**	**29.92**	**0.107**
	VLTVK 	**393 - 397**	**44.655**	**29.92**	**0.034**
IL3Rα	IEILT 	**205 - 209**	**35.11**	**29.92**	**0.089**
	LVVWE 	**354 - 358**	**30.343**	**29.92**	**0.126**
	VQVVQ 	**372 - 376**	**52.879**	**29.92**	**0.012**
IL5Rα	IVNLT 	**129-133**	**30.605**	**29.92**	**0.155**
	ISIID 	**295 - 299**	**36.72**	**29.92**	**0.114**
	VIVIM 	**344 - 348**	**31.936**	**29.92**	**0.105**
	IEVIC 	**401 - 405**	**41.15**	**29.92**	**0.051**

3.11. Ingle Peptide Prediction (Predisi)

Prediction of signal peptides was performed using PrediSi

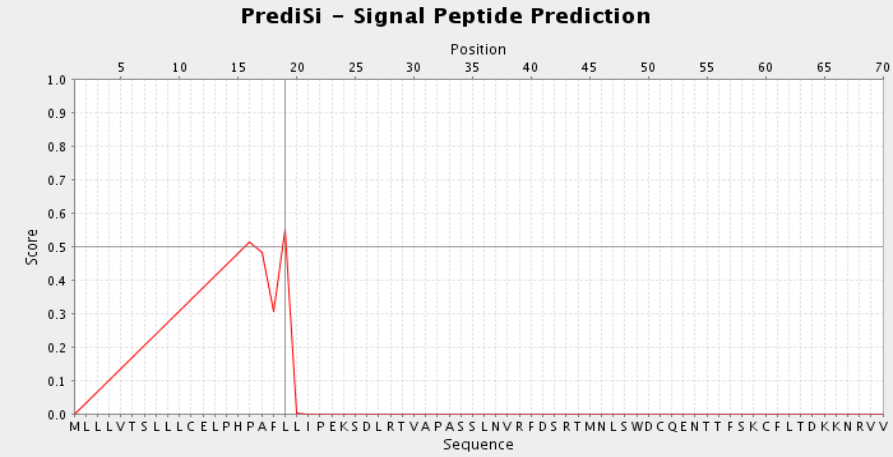


Figure 7. Signal peptide prediction for GM-CSF-Rα protein detected by Predisi tool

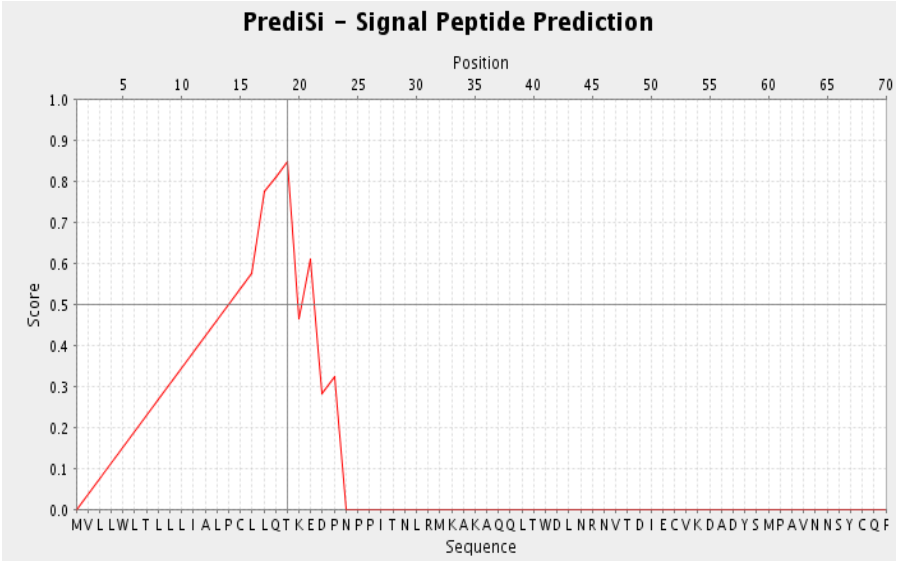


Figure 8. Signal peptide prediction for IL3Rα protein detected by Predisi tool

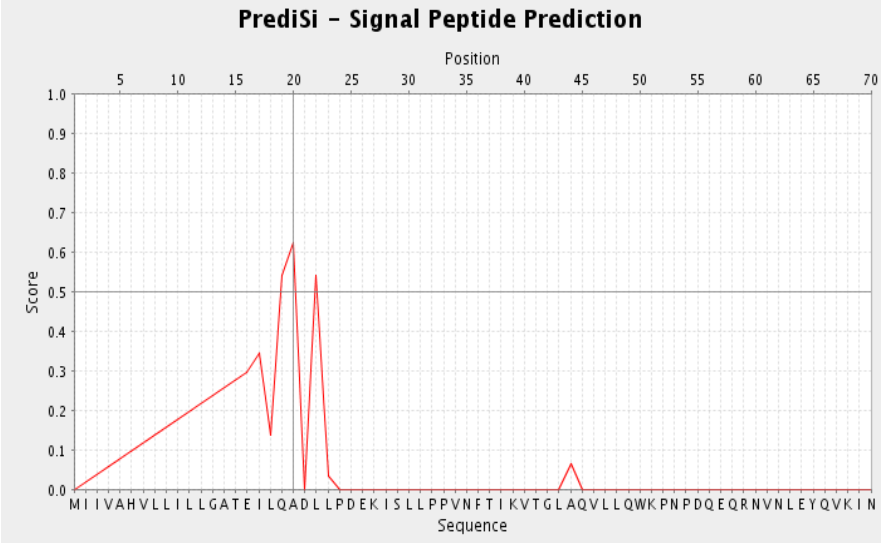


Figure 9. Signal peptide prediction for IL5Rα protein detected by Predisi tool

3.12. Protein–Ligand Binding Site Recognition

The predicted protein-ligand binding site residues by using PDB sum, and visualized by both LIGPLOT and PLIP softwares on the three proteins are presented in figures (10)-(24). An interaction diagram with interaction data is provided for each binding site.

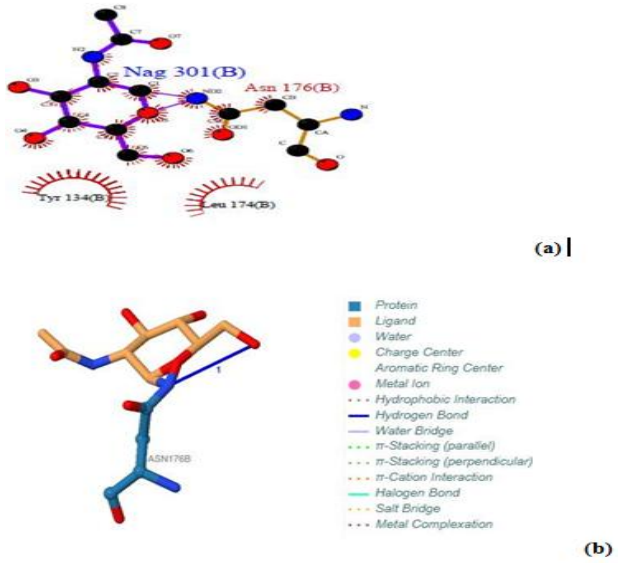


Figure 10. Prediction of binding site of GM- CSF-Rα proteins. (a) LIGPLOT diagram of Nag (B) 301 binding site, showing the interactions of the residue Asn 176(B) with the surrounding protein residues. (b) Nag (B) 301 binding site using protein-ligand interaction profiler

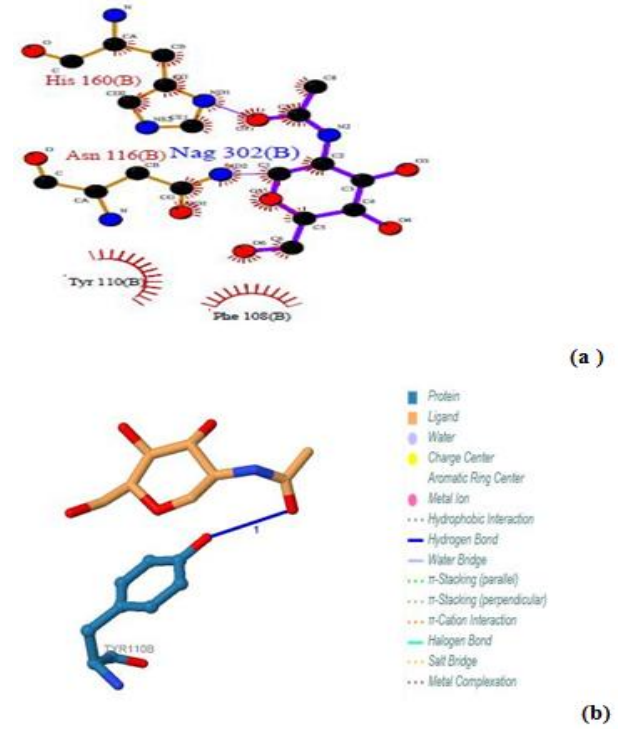


Figure 11. Prediction of binding site of GM- CSF-Rα proteins. (a) LIGPLOT diagram of Nag 302 (b) binding site, showing the interactions of the residues Asn 116(B) and His 160 (B) with the surrounding protein residues (B) Nag 302 (B) binding site using PILP

3.12.1. GM- CSF-Rα Protein

Three binding sites have been detected in position 301, 302 and 303. Tow ligands were found; GOL (glycerol) [Glycerin; propane-1, 2, 3-Triol]and NAG (Acetyl glucosamine) (N-Acetyl-D-Glucosamine), by the LIGPLOT NAG has interaction with Asn 176, and interaction with Asn 116 and His 160 residues. Also Gol has interaction with Ser 255. No ligands interactions found by PILP.

Figure 12. Prediction of binding site of GM- CSF-Rα proteins. (a) LIGPLOT diagram of Gol 303 (B) binding site, involving the interactions of the residue Ser 255 (B) with the surrounding protein residues

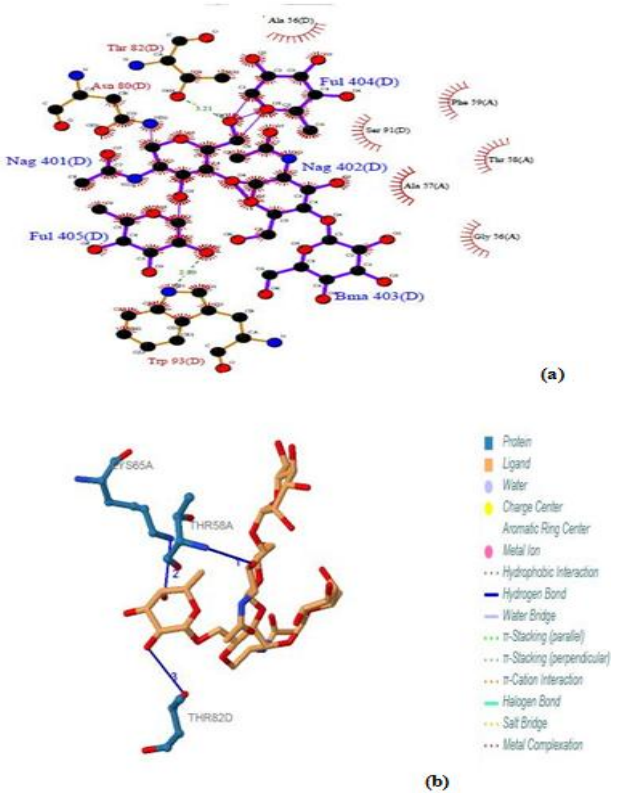


Figure 13. Prediction of binding site of IL-3Rα protein (a) LIGPLOT diagram of NAG 401(D) to FUL 405(D) binding sites (b) Composite ligand consists of NAG:D:401, NAG:D:402, BMA:D:403, FUL:D:404, FUL:D:405 visualized by PILP

3.12.2. IL-3Rα Protein

Five binding sites have been detected in position 401, 402, 403, 404 and 405 and visualized by PLIP & LIGPLOT tools. Three ligands were found; NAG (N-Acetyl-D-Glucosamine), FUL (Beta-L-Fucose) [6-Deoxy-Beta-L-Galactose] and BMA (Beta-D-Mannose, Alpha-D-Mannose). No interactions found by LIGPLOT and PILP.

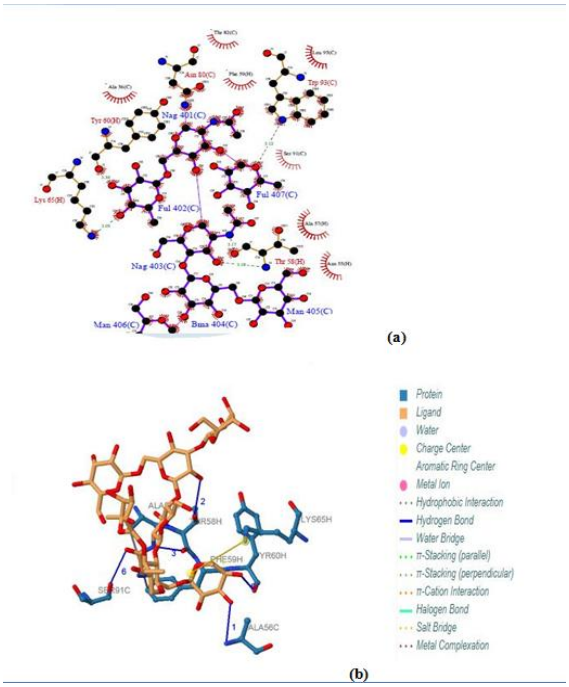


Figure 14. Prediction of binding site of IL-3Rα protein. (a) LIGPLOT diagram of NAG 401(C) to FUL 407(C) binding sites (b) Composite ligand consists of NAG:C:401, FUL:C:402, NAG:C:403, BMA:C:404, MAN:C:405, MAN:C:406, FUL:C:407 by PILP

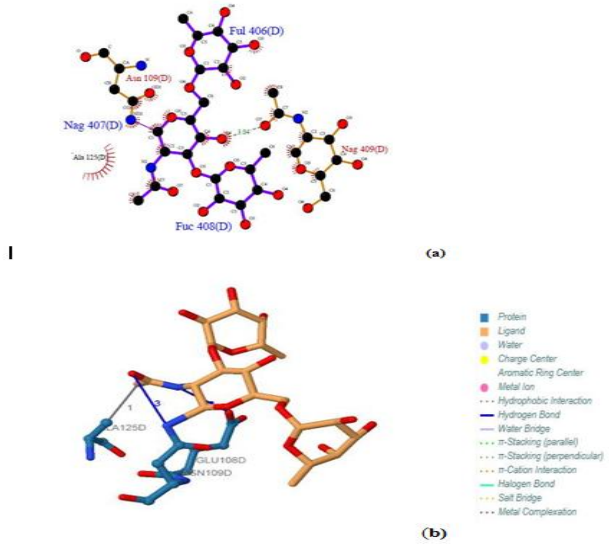


Figure 15. Prediction of binding site of IL-3Rα protein. (a) LIGPLOT diagram of FUL: D:406, NAG: D:407, FUC: D:408 binding sites (b) Composite ligand consists of FUL: D:406, NAG: D:407, FUC: D:408 by PILP

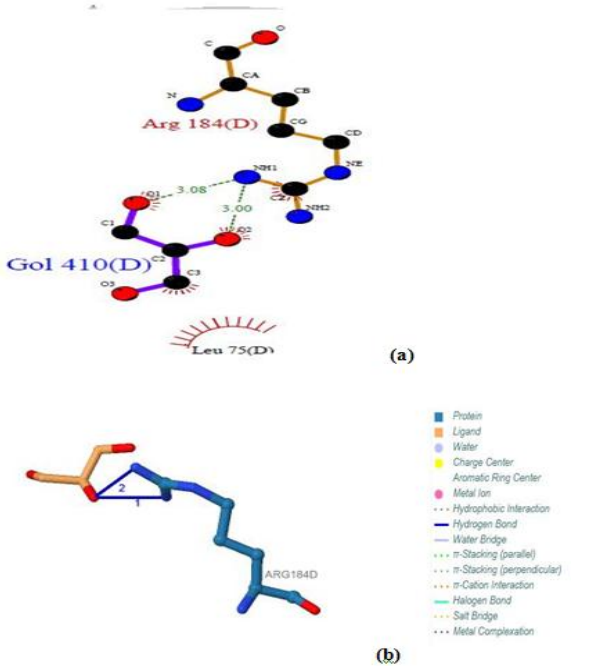


Figure 16. Prediction of binding site of IL-3Rα protein. (a) LIGPLOT diagram of GOL 410 (D) binding site (b) GOL 410 (D) binding site by PILP

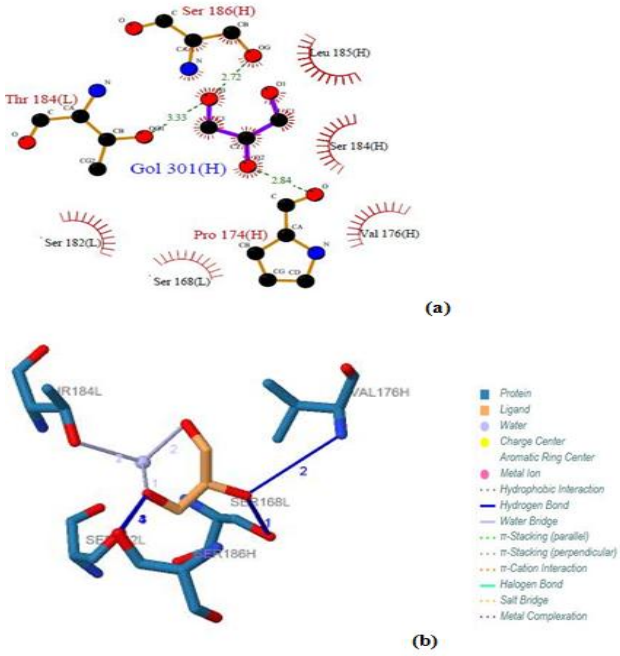


Figure 17. Prediction of binding site of IL-3Rα protein. (a) LIGPLOT diagram of GOL 301 (H) binding site (b) GOL 301 (H) by PILP

3.12.3. IL-5 Rα Protein

Five binding sites have been detected in position 316, 317, 318 and 319, and visualized by PLIP & LIGPLOT tools. One ligand was detected; BGC - Beta- D- Glucose. No interactions found by LIGPLOT and PILP.

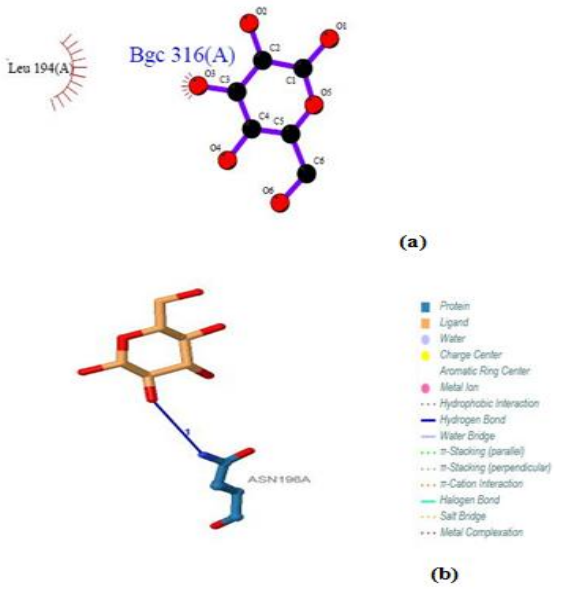


Figure 18. Prediction of binding site of IL-5 Rα protein. (a) LIGPLOT diagram of 316 (A) binding site (b) 316 (A) binding site by PILP

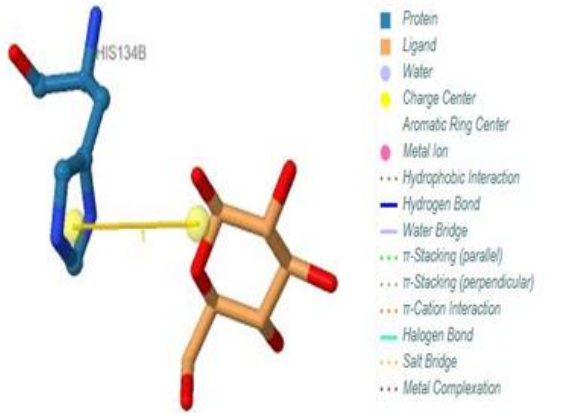


Figure 19. Prediction of binding site of IL-5 Rα protein. 316 (B) binding site by PILP. NO result found by using LIGPLOT server for this site

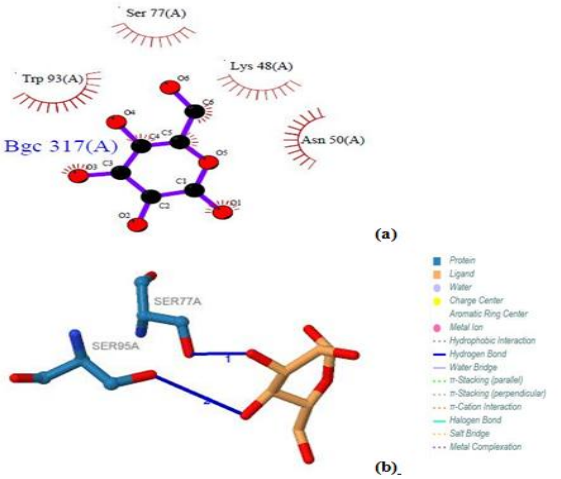


Figure 20. Prediction of binding site of IL-5 Rα protein. (a) LIGPLOT diagram of 317 (A) binding site (b) 317 (A) binding site by PILP

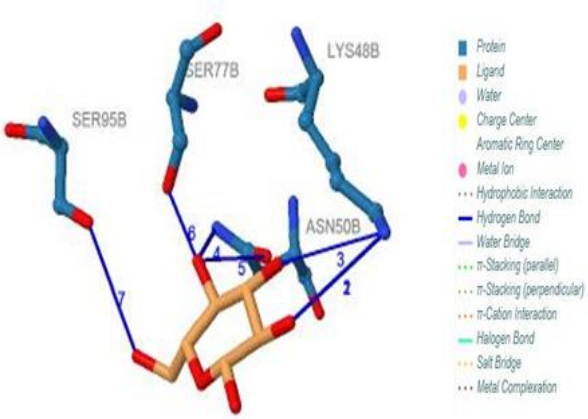


Figure 21. Prediction of binding site of IL-5 Rα protein. 317 (B) binding site by PILP. NO result found by using LIGPLOT server for this site

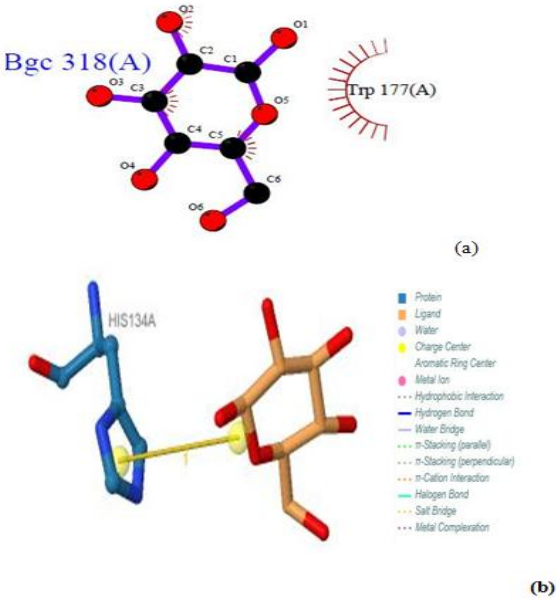


Figure 22. Prediction of binding site of IL-5 Rα protein. (a) LIGPLOT diagram of 318 (A) binding site (b) 318 (A) binding site by PILP

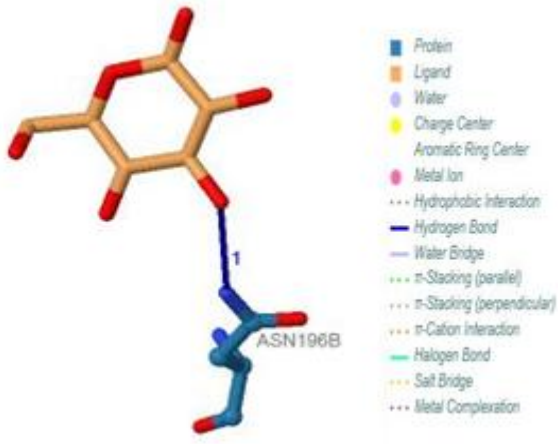


Figure 23. Prediction of binding site of IL-5 Rα protein. 318 (B) binding site by PILP. No result found by using LIGPLOT server for this ligand

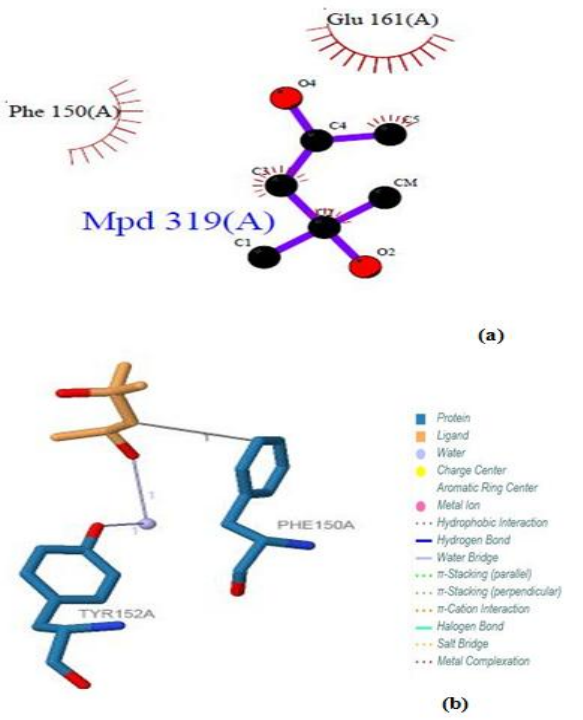


Figure 24. Prediction of binding site of IL-5 Rα protein. (a) LIGPLOT diagram of 319(A) binding site (b) 319 (A) binding site by PILP

3.13. Protein Secondary Structure Prediction

The secondary structure prediction was carried out with the help of PDBsum software. The results are shown in figure 25, 26 and 27.

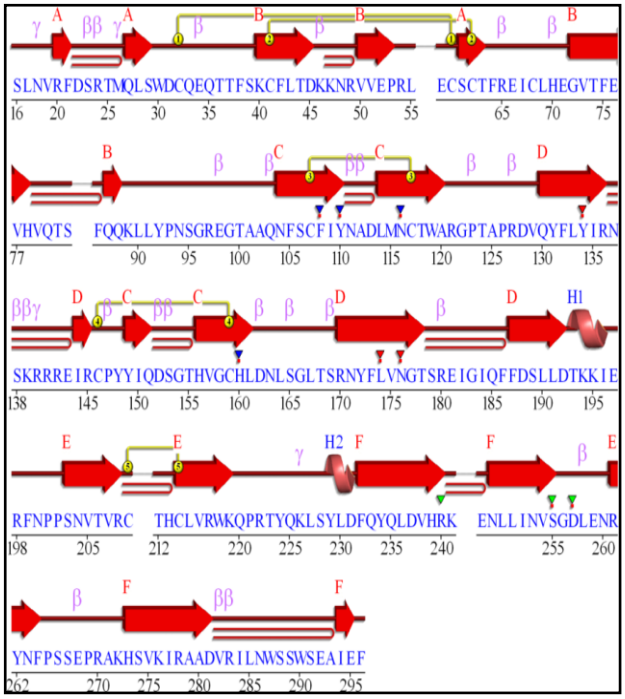


Figure 25. GM-CSF-Rα secondary protein structure

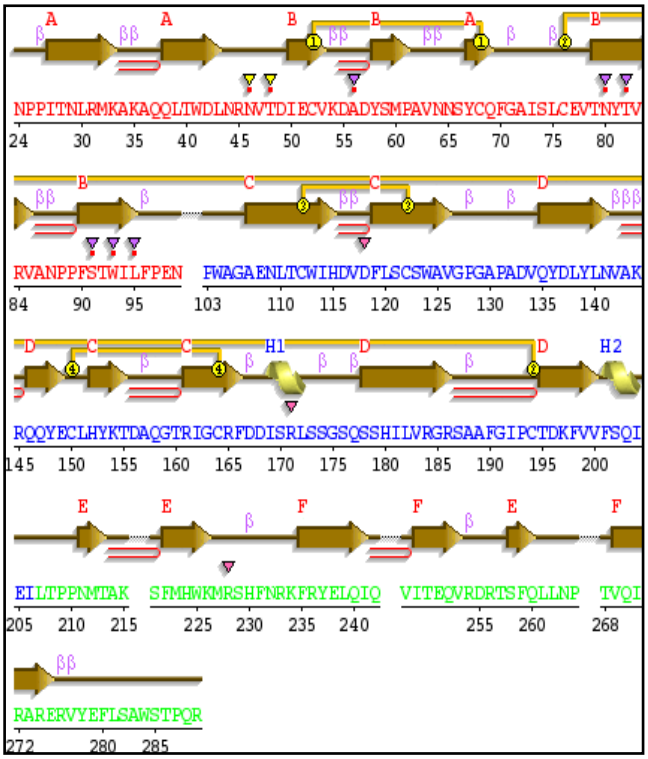


Figure 26. IL-3Rα secondary protein structure

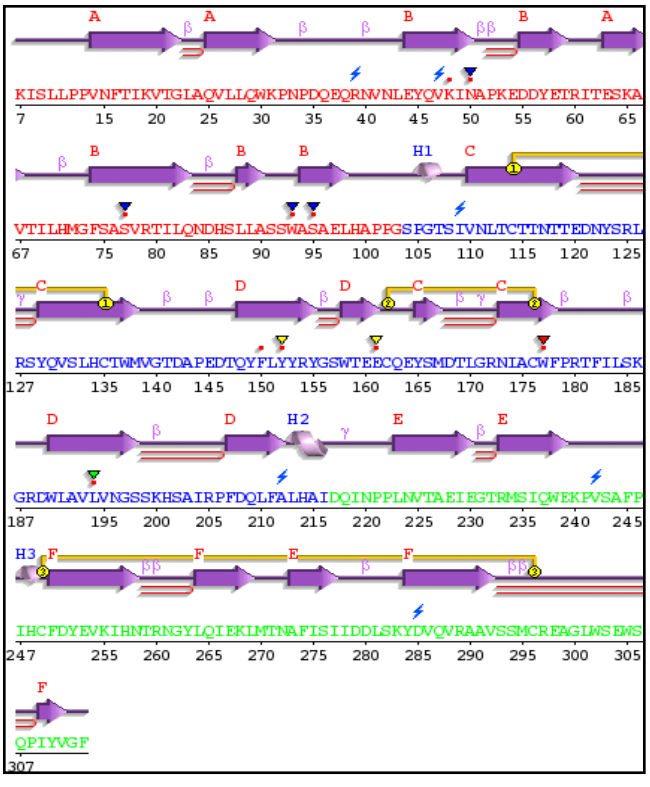


Figure 27. IL-5Rα secondary protein structure

Sec. struc: Helices labelled H1, H2, ... and strands by their sheets A, B, ...

Helix Strand

Motifs: β beta turn beta hairpin

Disulphides: 1 1 disulphide bond

Residue contacts: to ligand

PDB SITE records: AC1 AC2 AC3 AC4 AC5 AC6 AC7

3.14. 3D Structure of the Proteins

3D structure of proteins was determined by homology modeling, using CPH models 3.2 server. Visualization of the proteins model was done by Chimera (version 1.8) program. The results are shown in figures 28, 29 and 30.

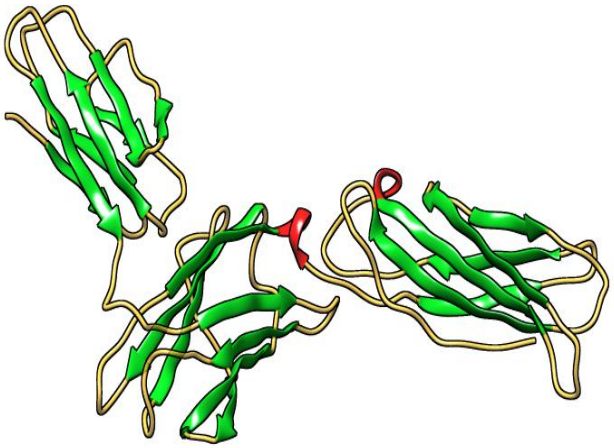


Figure 28. Three dimensional structure of GM-CSF-Rα protein [PDB 4RS1]

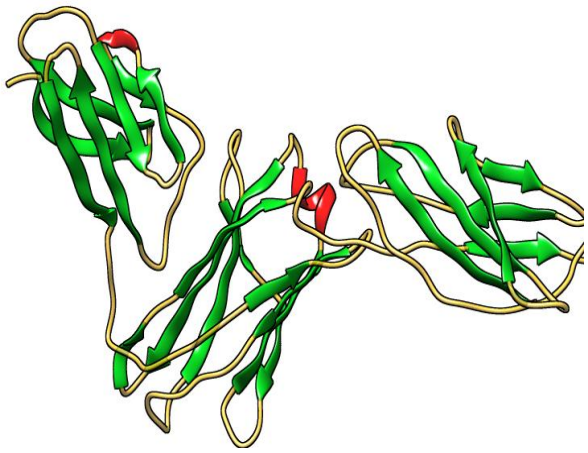


Figure 29. Three dimensional structure of IL-3Rα protein [PDB 4jzj]

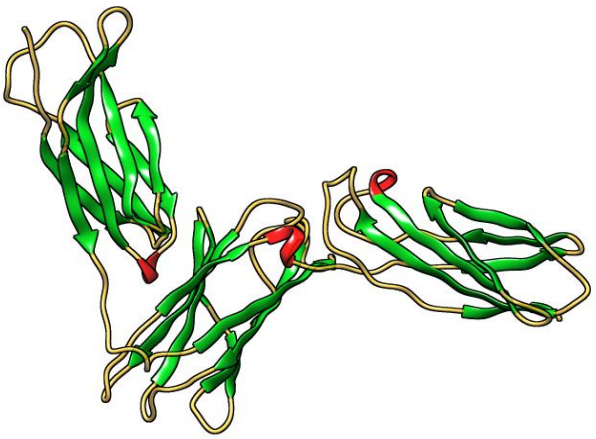


Figure 30. Three dimensional structure of IL-5Rα protein [PDB 3va2]

3.15. Validation of Proteins

The validation of the modeled structure was carried out using ERRAT. The results are shown in figure 31, 32 and 33.

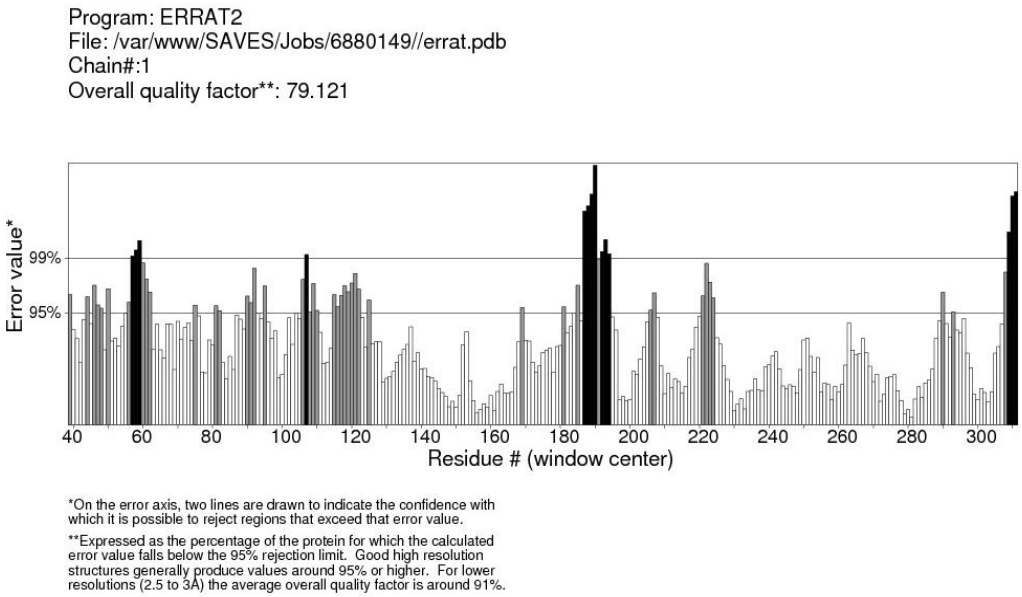


Figure 31. Validation ofGM-CSF-Rα protein by ERRAT server

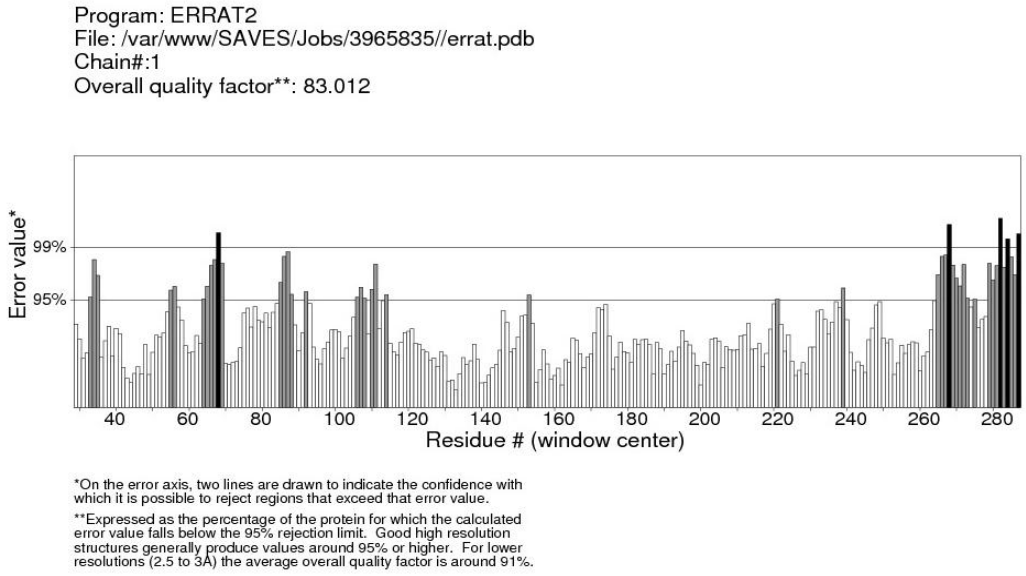


Figure 32. Validation of IL-3Rα protein by ERRAT server

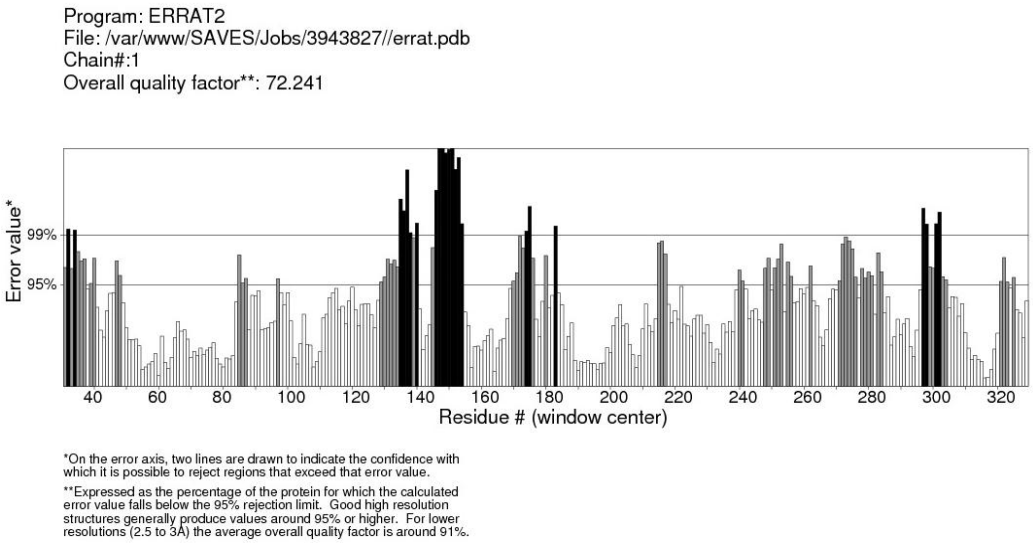


Figure 33. Validation of IL-5Rα protein by ERRAT server

4. Discussion

Computational analysis of protein sequences has become a highly rich scope of renewed science and a highly interdisciplinary area, where statistical and algorithmic procedures have a significant role. The present study was to perform sequence and structure analysis of three proteins, GM-CSF-Rα, IL3Rα, and IL5Rα. ProtParam software was used to find out the physiochemical properties for the proteins from their sequences, which are essential for understanding proteins function. Leucine (Leu) amino acid was found in rich amounts in these proteins, while Pyrrolysine (Pyl) and Selenocysteine (Sec) were absent. This may explain the high aliphatic index (AL) of these proteins, indicating that they are stable for a wide range of temperature. An isoelectric point above 7 (7.91 for GM-CSF-Rα and 8.60 for IL-3Rα) as well as a higher number of positive residues (+R) indicates that these proteins has a positive charge, whereas IL5Rα protein which is below 7 (5.36) and a higher negative residues (-R) has a negative charge. This value, computed isoelectric point, has an advantage in developing buffer system for purification by isoelectric focusing method [31]. The Instability index (II) less than 40 in GM-CSF-Rα indicates that it may be stable for a wide range of temperatures whereas the IL3Rα, and IL5Rα classified as an unstable proteins. The lower value of (GRAVY) in all proteins may be a signal for the possibility of better interaction with water like a protein of hydrophilic nature. The N-terminal of these proteins sequences considered is M (Met). Therefore estimated half-life is 30 hours (mammalian reticulocytes, in vitro), >20 hours (yeast, in vivo) and >10 hours min (*Escherichia coli*, in vivo) [31]. Another parameter, extinction coefficient (EC) at 280 nm. EC, which is important in the quantitative study of

protein–protein and protein–ligand interactions in solution, is calculated from amino acids composition and found to be higher among these proteins.

Fingerprinting analysis was performed by ScanProsite detecting Fibronectin type III (FN3) domain in GM-CSF-Rα and IL-5Rα proteins. These domains are found in many different proteins including cell surface receptors and cell adhesion molecules [32]. Koide A. and coworkers revealed that it is a small independent folding unit which occurs in many animal proteins involving in ligand binding. The beta-sandwich structure of FN3 exceedingly look alike that of immunoglobulin domains [33].

Protein signatures are dynamic mining tool eligible to identify protein sequences having the same functional residues, belonging to the same class of proteins from the numerous sequences in the non-redundant databases [14]. Among these three proteins, sequences belonging to the following families were detected: HEMATOPO_REC_S_F2, ASN_GLYCOSYLATION, CK2_PHOSPHO_SITE, PKC_PHOSPHO_SITE, MYRISTYL, CAMP_PHOSPHO_SITE, and TYR_PHOSPHO_ .SOSUI server classified all proteins as membrane proteins, primary and secondary in nature. And the transmembrane region of all proteins is rich in hydrophobic amino acids. The helix of proteins is visualized using PepWheel. Another substantial aspect of the protein analysis concerns post-translational modifications (PTMs). They are known to be essential mechanisms in the eukaryotic cells associated with protein functions and signaling networks. A growing body of evidences suggested that the complex signaling networks involved in the regulation of cellular pluripotency are strictly controlled by multiple mechanisms, including post-translational modifications (PTMs) [34]. Therefore, it is important to use bioinformatics tools to predict the sites for post translational modifications in proteins analysis. Protein phosphorylation is one of the most abundant post-translational modifications. It is implicated in the regulation of many cellular processes and states. Many signaling pathways involved in the embryonic development and the modulation of gene expression for cellular pluripotency and differentiation are starting from the activation of growth factor receptors that are recognized receptor tyrosine kinases (RTKs; e.g., FGFR and IGF1R) or receptor serine/threonine kinases (e.g., TGFβR and BMPR1/2). [34]. Phosphorylation is the most common and important mechanism of acute and reversible regulation of protein function. Protein phosphorylation has a significant role in essentially all aspects of cell biology. Most polypeptide growth factors and cytokines stimulate phosphorylation upon binding to their receptors [35].The phosphorylation site prediction showed that serine is the most phosphorylated amino acid among these proteins, with different kinases for each protein. cdc2, Unsp, PKA, PKC, DNAPK, ATM, CKII and PKG kinases acting on GM-CSF-Rα protein, while PKG, cdc2, PKA, CKII, Unsp, PKC, PKC, RSK, DNAPK, ATM, CKI and PKA, Unsp, PKC, cdc2, GSK3, cdk5, p38MAPK, CKI, RSK, DNAPK, ATM, CKII acting on IL-3Rα and IL-5Rα proteins respectively. Ubiquitination is an important and popular protein posttranslational modification than earlier expected. Regulation of transcription factor activity, budding of retroviral virions, receptor endocytosis and lysosomal trafficking, control of insulin6 and TGF-β signaling pathways are examples of just a few processes that depend on ubiquitination. [22]. UbPred predicted that 2 lysine residues in GM-CSF-Rα undergo ubiquitination. In contrast, BDM-PUB predicted that 13 lysine residues undergo ubiquitination. Both UbPred and BDM-PUB predicted that residue (K 387) undergo ubiquitination. Similarly, UbPred predicted that 3 lysine residues in IL3Rα undergo ubiquitination, BDM-PUB predicted 6, and both predicted (K361). For IL5Rα, UbPred predicted 4, BDM-PUB predicted 9, and (K393) predicted by both of them. Small ubiquitin-like modifiers (SUMOs) play an essential role in the regulation of a variety of biological processes such as cellular signaling by modifying specific lysine residues in protein substrates. There are numerous clues that the aberrance of SUMO regulation is extremely associated with various diseases, such as cancers. Thus, the identification of SUMO modification sites in proteins is essential for understanding the biological functions and regulatory mechanisms of SUMOs, and provides possible targets for further diagnostic and therapeutic considerations. The process by which proteins being covalently modified by SUMOs is named sumoylation, which is one of the most significant and ubiquitous post-translational modifications (PTMs) of proteins [23]. In the present study; 2 residues in GM-CSF-Rα, 3 in IL-3Rα and 4 in IL-5Rα proteins are possible sumoylation sites detected by GPS-SUMO online service. The prediction of signal peptides has become a substantial application of genomics and proteomics studies. After translocation of the protein across the cell membrane, the N-terminal signal peptide is usually cleaved off by an extracellular signal peptidase. The cleavage site for the signal peptidase is located in the c-region. However, the degree of signal sequence conservation and length, as well as the cleavage site position, differs significantly between different proteins. Furthermore, main variations were observed between eukaryotic and bacterial signal sequences. So, for different objectives, it is advisable to recognize signal peptides and their corresponding cleavage positions [24]. According to Predisi software, the three proteins examined here were predicted for secretion, with different scores and cleavage sites. Detection of protein–ligand binding sites is important to protein function annotation and drug designing. Xing Du et al defined “ligand” as any molecule capable of binding to a protein with a high specificity and affinity [36]. It is important to understand thoroughly the protein-ligand interactions in order to give a deep insight into the protein function. In addition, such understanding can facilitate the discovery, design, and development of drugs [36]. For GM- CSF-Rα; 3 binding sites have been detected and visualized by PLIP &

LIGPLOT. Likewise, 5 binding sites have been detected for IL-3- Rα and IL-5- Rα. Protein secondary structures are steady local conformations of a polypeptide chain. They are significant in preserving a protein three-dimensional structure. Secondary structure prediction for these proteins showed that Beta sheet dominated all the other conformations. Protein 3D structure is very important in understanding the protein interactions, functions and their localization. Homology modeling approach is the most common structure prediction method. CPH server for Homology modeling and Chimera for visualization of these models were utilized in this study. Reliability of these models were further checked by ERRAT, where a model having more than 90% residues in suitable region is considered as good quality model. Results from ERRAT showed low quality of these sequences (less than 90%). It remains to be seen whether the lessons learned from this study can be applied to other members of this cytokine receptor super family.

5. Conclusions

Computer-assisted description of the features of various proteins is an important mission in the search for proteomes knowledge. The structural and functional analysis of the α subunits of these receptors provides an insight into their mechanism of activation and for the development of therapeutics. Further work is now needed to extend these observations in order to support advances in therapeutic options. Also, comparison In silico analysis between α and β subunits is required to help understanding the role of α subunits in the overall function of these receptors.

ACKNOWLEDGEMENTS

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