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In silico Homology Modeling and Docking Study of Translationally Controlled Tumor protein of *labeorohita*.

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Abstract— *Labeorohita, is a fish of the carp family Cyprinidea, found commonly in rivers and freshwater lakes in and around South Asia and South-East Asia. IT is used as a major diet all over India. Hence its culture is done at a heavy ratio accordance to other fishes. Translationally Controlled Tumor Protein found in Labeorohitahas a very similar polypeptide chain accordance with human. Human TCTP has a great effect on growth. Due to the unavailability of the Tertiary structure of TCTP of Labeorohita, clear functional activity is unable to detect. The following work is a try to build a tertiary structure and detecting its active sites by docking it with different active proteins.*

Keywords— TCTP, Protein Structure Prediction, Modeller.

I. INTRODUCTION

Labeorohita:-

BIOLOGICAL CLASSIFICATION:-

Kingdom:		Animalia
Phylum :		Chordata
Class	:	Actinopterygii
Order	:	Cypriniformes
Family	:	Cyprinidae
Genus	:	*Labeo*
Species :		*rohita*

The major carps of India fall under three genera, *Catla*, *Labeo* and *Cirrhinus*. Under the genus *Catla*, the species *C. catla*, under the genus *Labeo* fall the species *L. rohita*, *L. calbasu*, *L. fimbriatus*, *L. bata*, *L. gonius*, and under the genus *Cirrhinus* fall the species *Cirrhinusmrigala*, *C. reba*, *C. cirrhosa*. Interspecific hybridization work has been carried out between the species of the genus *Labeo* and intergeneric hybridization between the species of the above mention three genera (Reddy 1999).Due to their fast growing nature and taste, Indian major carps enjoy a prime position in the Indian aquaculture scenario.

Labeorohita, is a fish of the carp family Cyprinidea, found commonly in rivers and freshwater lakes in and around South Asia and South-East Asia. It is an omnivore. It is treated as a delicacy in Bangladesh, Nepal and the Indian states of Bihar, Odisha, Assam, West Bengal and Uttar Pradesh. The Maithil Brahmins and the Kayastha community of Bihar and Uttar Pradesh treat it as one of their most sacred foods: to be eaten on all auspicious occasions.

During the early stages of its lifecycle, it eats mainly zooplankton, but as it grows, it eats more and more phytoplankton, and as a juvenile or adult is an herbivorous column feeder, eating mainly phytoplankton and submerged vegetation. It has modified, thin hair-like gill rakers, suggesting that it feeds by sieving the water.

It is diurnal and generally solitary. It reaches sexual maturity between two and five years. In nature, it spawns in the marginal areas of flooded rivers.

PROTEIN STRUCTURE PREDICTION:-

Ever since the first protein structure is determined, computational biologists and computational chemists have attempted to develop software that could predict the protein structure.**Protein structure prediction** is the prediction of the three-dimensional structure of a protein from its amino acid sequence — that is, the prediction of its secondary, tertiary, and quaternary structure from its primary structure. Structure prediction is fundamentally different from the inverse problem of protein design. Protein structure prediction is one of the most important goals pursued by bioinformatics and theoretical chemistry; it is highly important in medicine (e.g., in drug design) and biotechnology (e.g., in the design of novel enzymes).

Secondary Structure Prediction:-

Secondary structure of protein refers to the interactions that occur between the CO and NH groups on amino acids in a polypeptide chain to form α helices, β sheets, turns, loops and other forms and that facilitate the folding into a three-dimensional structure. Physically, the driving force behind the formation of secondary structures is a complex combination of local and global forces. Locally, forces that act between residues or between the residue and the backbone of protein can affect the formation of secondary structures. These local effects include the repulsion between hydrophobic side chains of some amino acids

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and the hydrophilic backbone of the protein chain as well as the interaction between side chains and the surrounding solvent (Pauling *et al.*, 1951).

Secondary structure predictionof protein is a set of techniques in bioinformatics that aim to predict the local secondary structures of proteins based only on knowledge of their primary structure — amino acid. For proteins, a prediction consists of assigning regions of the amino acid sequence as likely alpha helices, beta strands (often noted as "extended" conformations) or turns. The prediction of secondary structure of a protein is the intermediate step of prediction of 3D- structure of protein.

Tertiary Structure Prediction:-

The **Tertiary structure** of protein is constituted by the spatial arrangement of secondary structures of protein. The tertiary structure is stabilized by the intermolecular H-bonds, Disulfide-bonds and the hydrophobic interactions (Pauling *et al.*, 1951). The tertiary structure of protein is the great assistance when planning experiments aimed at the understanding of protein function and during its binding process with other proteins and drugs.

Tertiary Structure Predictionof protein is applied to develop models of protein structure when the constraints from X-ray diffraction or NMR spectroscopy are not available. Tertiary structure predictionof protein is the bioinformatics approach that attempts to generation of new structure on the prior knowledge of protein structure.To predict or model the 3D structure of protein three different methods are use: **homology** (or **comparative**) **modelling**, **threading** and***ab initio*** **method**. The 3D structure of protein is necessary for:

- Enhance understanding of protein function and their interaction with other bio-molecules (proteins, enzymes, hormones, nucleotides etc.) or chemicals (Drugs).
- Explaining antigenic property of protein/protein complex.
- Understanding DNA-binding specificity.

Translationally Controlled TumorProtein:-

Translationally ControlledTumor Protein (TCTP) is a growth-associated protein ubiquitously present in wide verity of organisms from yeast to mammals (Bonnet *et al.*, 2000, MacDonald *et al.*, 2001, Bhisutthibhan*et al.*, 1998). In fact it is one of the 20 most abundantly expressed proteins in the cell. TCTP was initially identified in an Ehrlich ascites tumor cell lines, hence the name is (Bohm*et al.*, 1989). Subsequently, TCTP was demonstrated to be present in almost all normal cells (Sanchez *et al.*, 1997). TCTP is also variously known as IgE-dependent histamine-releasing factor (HRF) (MacDonald *et al.*, 1995), p23/p21 (Bohm*et al.*, 1989, Chitpatima*et al.*, 1988), and fortilin (Li *et al.*, 2001). TCTP is about 20-25 kDa in weight. The first structure of TCTP was solved by NMR spectroscopy in 2001 from *Schizosaccharomycespombe*.

TCTP was initially identified as a growth-related protein on the basis of its translationally-dependent regulation of expression (Chitpatima*et al.*, 1988).TCTP plays an important role in the process of tumorigenesis. TCTP not only upregulated in a number of tumor cell lines but also downregulated during tumor reversion (Tuynder*et al.*, 2002, Arcuri*et al.*, 2004). The expression of TCTP is upregulated by a variety of stress conditions such as oxidative stress, heat shock, and exposure to heavy metals(Sturzenbaum*et al.*, 1998, Bonnet *et al.*, 2000, Mak*et al.*, 2007).The function of TCTP is described as a heat stable, calcium binding (Gnanasekar*et al.*, 2002, Rao *et al.*, 2002), antioxidant protein (Gnanasekar*et al.*, 2007) that negatively regulates apoptosis (Li *et al.*, 2001, Gnanasekar*et al.*, 2009) and cause release of histamine from basophils (Gnanasekar*et al.*, 2002, Rao *et al.*, 2002, MacDonald *et al.*, 1995).

The TCTP mRNA is expressed at constant levels in both growing and nongrowing cells, and the translation is regulated by its polypyrimidine-rich 5' untranslated region (Bohm*et al.*, 1991). TCTP localizes to microtubules from G_1 until metaphase and then detaches from the spindle at the metaphase-to-anaphase transition. Both *in vitro* tubulin binding by TCTP and sequence homology to the tubulin-binding domain of MAP-1B (Gachet*et al.*, 1999) support these localization data. In addition, TCTP levels in over expressing cells were correlated with microtubule stabilization and reduced growth rate *in vivo* (Gachet*et al.*, 1999).Dysregulation of TCTP has been shown to be associated with several disease conditions such as Cancer (Tuynder*et al.*, 2002), Alzheimer disease (DiLorenzo*et al.*, 2001), and Allergy (MacDonald *et al.*, 2001, Oikawa *et al.*, 2002) suggesting an important role for TCTP in the physiological homeostasis of cells. Despite the ubiquitous nature of TCTP, its exact cellular function is not clear and the true function of TCTP is still being debated.

Keeping the importance of TCTP in living cell, the present study was envisaged with the following objectives.

1. Analysis of 2-Dimensional structure of Translationally Controlled Tumor Protein (TCTP) by web base server.
2. Prediction of 3-Dimensional structure of Translationally Controlled Tumor Protein (TCTP) by homology modeling using modeller9.10.
3. Analysis of the 3-Dimensional structure.
4. Finding out the active sites by docking.

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II. MATERIALS AND METHODS

Resources used:

- **PC with internet facility**
  - Hardware configuration
    - RAM – 4GB
    - Hard disk – 500GB
    - Processor – Intel i3
- **Bioinformatics Databases and Tools used**
  - Databases
    - NCBI
    - PDB
  - Tools
    - BLAST
    - ClustalW
    - SOPMA
    - Modeller 9.10
    - SWISS PDB Viewer
    - Discovery Studio
    - SAVES
    - PatchDock
    - FireDock

TARGET PROTEIN:-

Table.1: FASTA sequence of GenBank of NCBI for Translationally Controlled Tumor Protein (TCTP) of Labeorohita.

>gi|13508433|gb|AAK27316.1| translationally controlled tumor protein [*Labeorohita*]
MIIYKDIITGDEMFSDIYKIKESENGMMIEVEGKMISRAEGEIDDALIGGNASAEVQDEGCESTTVSGVDIVLNHKLQ
ETSYDKKSYTAYIKDYMKAVKAKLQEVAPDRVDPFMANAPAEVKKILGNIKNFQFFTGESMNPDGMIGLLDFRED
GVTPYMLFFKDGLEIEKC

TEMPLATE SEUENCE:-

Table.2: FASTA sequence for Crystal Structure of Human Translationally Controlled Tumour Associated Protein.

>gi|109156944|pdb|1YZ1|A Chain A, Crystal Structure of Human Translationally Controlled Tumour Protein
EFMIIYRDLISHDEMFSDIYKIREIADGLCLEVEGKMVSRTEGNIDDSLIGGNASAEGPEGEGTESTVITGVDIVMNHH
LQETSFTKEAYKKYIKDYMKSIKGKLEEQRPERVKPFMTGAAEQIKHILANFKNYQFFIGENMNPDGMVALLDYRE
DGVTPYMIFFKDGLEMEKC

Template Search Assumptions:-

In the first phase of structure prediction for target protein sequence many hits were obtained in PDB-BLAST search. The template was chosen out of those hits by taking the best on the basis of following criteria:

- >25-45% sequence identity
- >35-55% sequence similarity
- <5% gaps

Alignment Tools:-

BLAST:-

The Basic Local Alignment Search Tool (BLAST) (Altschul*et al.,* 1991) finds regions of local similarity between sequences. The programme compares nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches. BLAST is a word based method by considering each word from the query sequence which is normally 3Amino Acids or 11Nucleotides. BLAST uses BLOSUM 62 as default scoring matrix. BLAST is developed and maintained by NCBI and GenBank.

ClustalW:-

ClustalW is a hierarchical multiple alignment program that combines a robust method for multiple sequence alignment with an easy-to-use interface. The program uses a series of different pair-score matrices, biases the location of gaps, and allows one to realign a set of aligned sequences to further refine the alignment. ClustalW is maintained by EBI.

STRUCTURE PREDICTION TOOLS:-

SOPMA:-

SOPMA (Self-Optimized Prediction Method with Alignment) is an improvement of SOPM (Self-Optimized Prediction Method) method. These methods are based on the **homologue method**. The improvement takes place in the fact that SOPMA takes into account information from an alignment of sequences belonging to the same family. If there are no homologous sequences the SOPMA prediction is the SOPM one.

Modeller9.10:-

Modeller is a program for predicting the three-dimensional structure of proteins using proteins whose structure is already known i.e., from experimental techniques (Eswar*et al.,* 2006, Marti-Renom*et al.,* 2000). Modeller is freely available for academic purposes. Modeller implements an automated approach to comparative protein structure modeling by satisfaction of spatial restraints (Sali and Blundell 1993, Fiser*et al.,* 2000). Briefly, the core modeling procedure begins with an alignment of the sequence to be modeled (target) with related known 3D structures (templates). This alignment is usually the input to the program. The output is a 3D model for the target sequence containing all main chain and side chain non-hydrogen atoms.

STRUCTURE VIEWING TOOLS:-

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Discovery Studio:-

Discovery Studio is a comprehensive software suite for analyzing and modeling molecular structures, sequences, and other data of relevance to life science researchers. The product includes functionality for viewing and editing data along with tools for performing basic data analysis.

The Discovery Studio Visualizer is a free viewer that can be used to open data generated by other software in the Discovery Studio product line. It is designed to offer an interactive environment for viewing and editing molecular structures, sequences, X-ray reflection data, scripts, and other data. It also provides a rich set of viewers for displaying plots and other graphical representations of data. The application runs on Windows and Linux and is a fully integrated desktop environment that provides access to standard operating system features such as the file system, clipboard, and printing services.

ENERGY MINIMIZING TOOL:-

Energy minimization is used to evaluate the energy of a structure as well as repair distorted geometries. Energy minimization is used when the molecule is manually distorted. Energy minimization is good to release local constraints, "make room" for a residue, but it will not pass through high energy barriers and stops in local minima. Energy minimization (energy optimization) methods are common techniques to compute the equilibrium configuration of molecules. The basic idea is that a stable state of a molecular system should correspond to a local minimum of their potential energy. This kind of calculation generally starts from an arbitrary state of molecules, and then the mathematical procedure of optimization allows us to move atoms (to vary variables) in a way to reduce the net forces (the gradients of potential energy) to nearly zero. Like molecular dynamics and Monte-Carlo approaches, periodic boundary conditions have been allowed in energy minimization methods, to make small systems. A well established algorithm of energy minimization can be an efficient tool for molecular structure optimization.

SWISS PDB Viewer:-

Swiss-PdbViewer (aka DeepView) is an application that provides a user friendly interface allowing to analyze several proteins at the same time. The proteins can be superimposed in order to deduce structural alignments and compare their active sites or any other relevant parts. Amino acid mutations, H-bonds, angles and distances between atoms are easy to obtain thanks to the intuitive graphic and menu interface.

Swiss-PdbViewer (aka DeepView) has been developed since 1994 by Nicolas Guex. It is tightly linked to SWISS-MODEL, an automated homology modeling server developed within the Swiss Institute of Bioinformatics (SIB) at the Structural Bioinformatics Group at the Biozentrum in Basel.

STRUCTURE ANALYSING TOOLS:-

SAVES:-

Structural Analysis and Verification Server (SAVES) is maintained by NIH, MBI laboratory for structural genomics and proteomics. SAVES provides the following checking parameters to analyse a 3D structure of protein.

- **PROCHECK:** Checks the stereochemical quality of a protein structure by analyzing residue-by-residue geometry and overall structure geometry (Laskowski*et al.,* 1993).
- **WHAT - CHECK:** Derived from a subset of protein verification tools from the WHATIF program, this does extensive checking of many stereochemical parameters of the residues in the model.
- **ERRAT:**Analyzes the statistics of non-bonded interactions between different atom types and plots the value of the error function versus position of a 9-residue sliding window, calculated by a comparison with statistics from highly refined structures (Colovos C and Yeates T.O, 1993).
- **VERIFY - 3D:** Determines the compatibility of an atomic model (3D) with its own amino acid sequence (1D) by assigned a structural class based on its location and environment (alpha, beta, loop, polar, non-polar etc) and comparing the results to good structures (Bowie *et al.,* 1991).
- **PEOVE:** Calculates the volumes of atoms in macromolecules using an algorithm which treats the atoms like hard spheres and calculates a statistical Z-score deviation for the model from highly resolved (2.0 A^0 or better) and refined (R-factor of 0.2 or better) PDB-deposited structures.

DOCKING TOOLS:-

PatchDock:-

PatchDock algorithm (Schneidman-Duhovny*et al.,* 2005, Schneidman-Duhovny*et al.,* 2003) is inspired by object recognition and image segmentation techniques used in Computer Vision. Docking can be compared to assembling a jigsaw puzzle. When solving the puzzle we try to match two pieces by picking one piece and searching for the complementary one. We concentrate on the patterns that are unique for the puzzle element and look for the matching patterns in the rest of the pieces. PatchDock employs a similar technique. Given two molecules, their surfaces are divided into patches according to the surface shape. These patches correspond

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to patterns that visually distinguish between puzzle pieces. Once the patches are identified, they can be superimposed using shape matching algorithms. The algorithm has three major stages:

- Molecular Shape Representation - in this step we compute the molecular surface of the molecule. Next, we apply a segmentation algorithm for detection of geometric patches (concave, convex and flat surface pieces). The patches are filtered, so that only patches with 'hot spot' residues are retained.
- Surface Patch Matching - we apply a hybrid of the Geometric Hashing and Pose-Clustering matching techniques to match the patches detected in the previous step. Concave patches are matched with convex and flat patches with any type of patches.
- Filtering and Scoring - the candidate complexes from the previous step are examined. We discard all complexes with unacceptable penetrations of the atoms of the receptor to the atoms of the ligand. Finally, the remaining candidates are ranked according to a geometric shape complementarity score.

FireDock:-

The FireDock (Andrusier*et al.*, 2007) server addresses the refinement problem of protein-protein docking solutions. The method simultaneously targets the problem of flexibility and scoring of solutions produced by fast rigid-body docking algorithms. Given a set of up to 1000 potential docking candidates, FireDock refines and scores them according to an energy function, spending about 3.5 seconds per candidate solution. To the best of our knowledge, this is the first web server that allows performing large-scale flexible refinement and scoring of docking solutions online.

Transformations of docking candidates are generated by PatchDock (Schneidman-Duhovny*et al.*, 2005) and are given as an input to FireDock. First a coarse refinement is performed, using a restricted interface side-chain optimization with atomic radii scaling of 0.8, in order to allow a certain amount of steric clashes. The refined candidates are scored and ranked according to the energy function and are returned as an output. Then, FireDock is run again on the best 25 solutions for a final refinement. In this second run, a full interface side-chain optimization is performed with atomic radii scaling of 0.85, in order to reduce the amount of clashes.

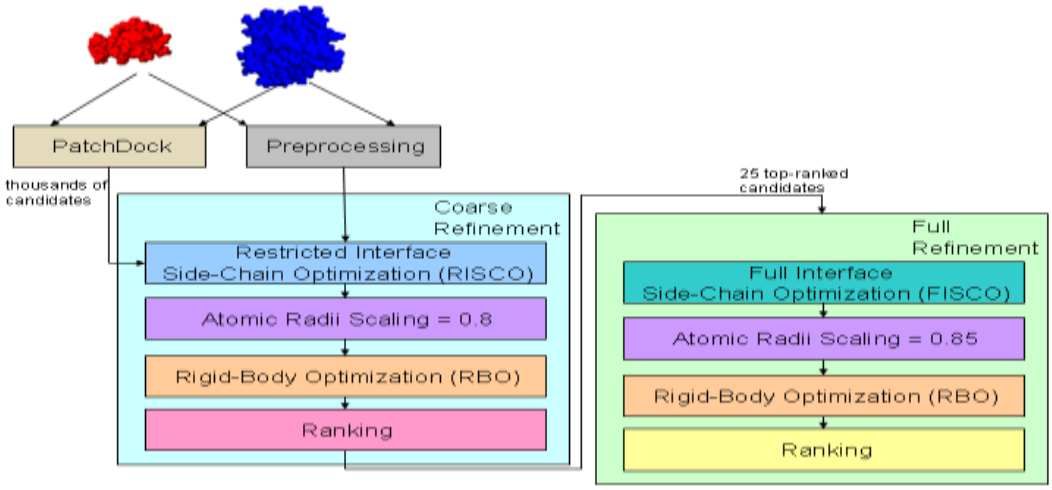

Fig.3: Working with FireDock

III. RESULT AND DISCUSSION

Result of Template Search:-

Table.3: Selected Template from BLAST search

Accession in PDB	Description	Max score	Total score	Query coverage	E-value	Method
1YZ1	Chain A, Crystal Structure of Human Translationally Controlled Tumour Protein >gi|109156944	251	640	100%	8e-85	Compositiona l matrix adjust

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Alignment with template:-

Table.4: BLAST alignment between Query and Template sequence

```
 Score =  251 bits (640),  Expect = 8e-85, Method: Compositional matrix adjust.
 Identities = 116/172 (67%), Positives = 144/172 (84%), Gaps = 1/172 (1%)

Query  1    MIIYKDIITGDEMFSDIYKIKESENGMMIEVEGKMISRAEGEIDDALIGGNASAE-VQDE  59
            MIIY+D+I+ DEMFSDIYKI+E  +G+ +EVEGKM+SR EG IDD+LIGGNASAE  + E
Sbjct  3    MIIYRDLISHDEMFSDIYKIREIADGLCLEVEGKMVSRTEGNIDDSLIGGNASAEGPEGE  62

Query  60   GCESTTVSGVDIVLNHKLQETSYDKKSYTAYIKDYMKAVKAKLQEVAPDRVDPFMANAPA  119
            G EST ++GVDIV+NH LQETS+ K++Y  YIKDYMK++K KL+E  P+RV PFM  A
Sbjct  63   GTESTVITGVDIVMNHHLQETSFTKEAYKKYIKDYMKSIKGKLEEQRPERVKPFMTGAAE  122

Query  120  EVKKILGNIKNFQFFTGESMNPDGMIGLLDFREDGVTPYMLFFKDGLEIEKC  171
            ++K IL N KN+QFF GE+MNPDGM+ LLD+REDGVTPYM+FFKDGLE+EKC
Sbjct  123  QIKHILANFKNYQFFIGENMNPDGMVALLDYREDGVTPYMIFFKDGLEMEKC  174
```

```
         10        20        30        40        50        60        70
          |         |         |         |         |         |         |
MIIYKDIITGDEMFSDIYKIKESENGMMIEVEGKMISRAEGEIDDALIGGNASAEVQDEGCESTTVSGVD
eeeehhecccchhhhtccccccctteeeeettceeecccccceeeecccchhcchhhhhhhhhhhhh
IVLNHKLQETSYDKKSYTAYIKDYMKAVKAKLQEVAPDRVDPFMANAPAEVKKILGNIKNFQFFTGESMN
eehhhhhccccchhhhhhhhhhhhhhhhhhhhttcchhhhhhhhhhhhhhhhhhhcctheeeetcccc
PDGMIGLLDFREDGVTPYMLFFKDGLEIEKC
ttceeeeeecctttccceeeeeettcccccc

Sequence length :   171

SOPMA :
   Alpha helix     (Hh) :    67 is  39.18%
   3_10 helix      (Gg) :     0 is   0.00%
   Pi helix        (Ii) :     0 is   0.00%
   Beta bridge     (Bb) :     0 is   0.00%
   Extended strand (Ee) :    35 is  20.47%
   Beta turn       (Tt) :    16 is   9.36%
   Bend region     (Ss) :     0 is   0.00%
   Random coil     (Cc) :    53 is  30.99%
   Ambigous states (?)  :     0 is   0.00%
   Other states         :     0 is   0.00%
```

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2D Structure Prediction:-

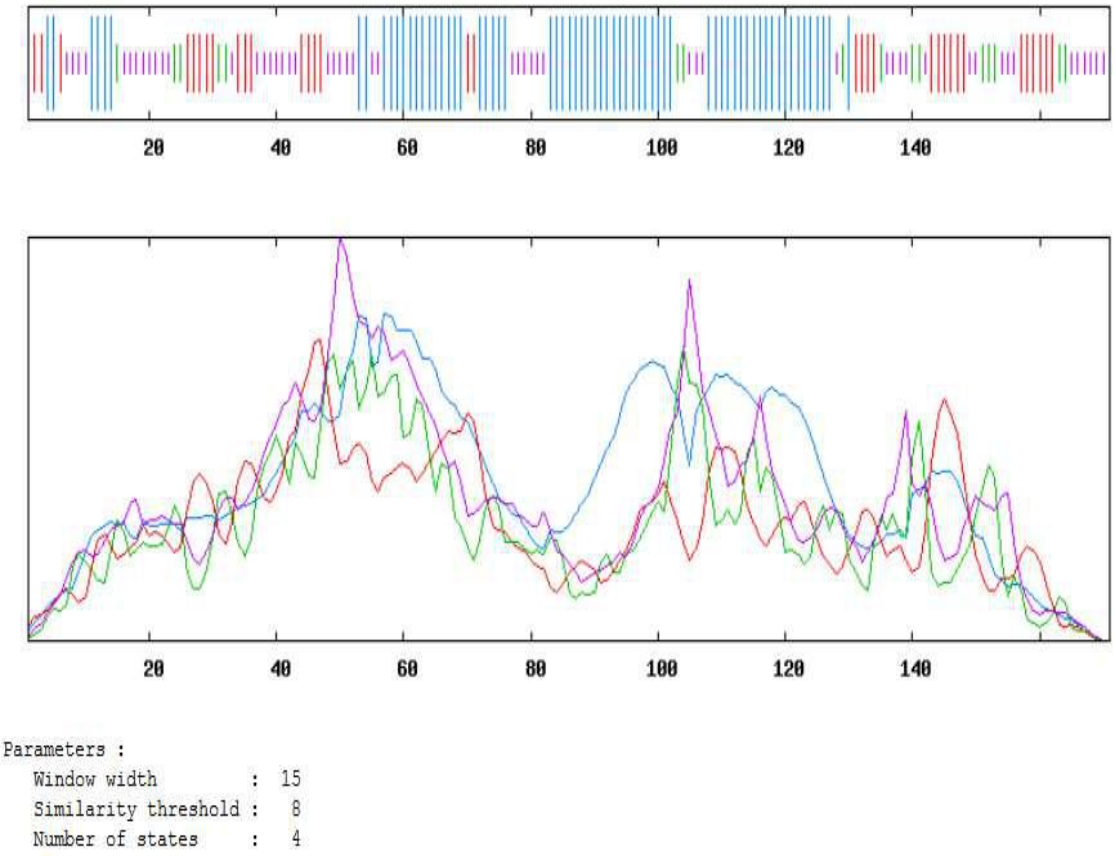

Fig.4: SOPMA result for TCTP (Labeorohita).

3D Structure Prediction:-

Table.5: Summary of successfully produced models by Modeller9.10

Filename	molpdf	DOPE score	GA341 score
tctp.B99990001.pdb	824.44885	-16549.04688	1.00000
tctp.B99990002.pdb	871.64148	-16372.80957	1.00000
tctp.B99990003.pdb	762.95618	-16747.47852	1.00000
tctp.B99990004.pdb	732.61566	-16452.86719	1.00000
tctp.B99990005.pdb	951.13629	-16677.70508	1.00000
tctp.B99990006.pdb	**761.53906**	**-16928.81836**	**1.00000**
tctp.B99990007.pdb	867.66919	-16628.26758	1.00000
tctp.B99990008.pdb	991.16754	-16495.96289	1.00000
tctp.B99990009.pdb	839.29321	-16376.09961	1.00000
tctp.B99990010.pdb	896.87634	-16572.61523	1.00000

MODEL ANALYSIS:-

The above predicted ten models were compared and found the model no-6 i.e. **tctp.B99990006.pdb** as best model depending on energy score of modeller 9.10 (lowest DOPE score). To analyse the structure the SAVES server is used and the following details are obtained.

The model taken is an appropriate model because the Ramachandran Plot shows that in the most favoured region there are about 94.7% residues are present and 5.3% residues are present in the additional allowed region. The Verify-3D shows the result of 65.70 which should above 80 for best model but it was overcome due to the Ramachandran Plot score. The ERRAT score also showed the model as a good model.

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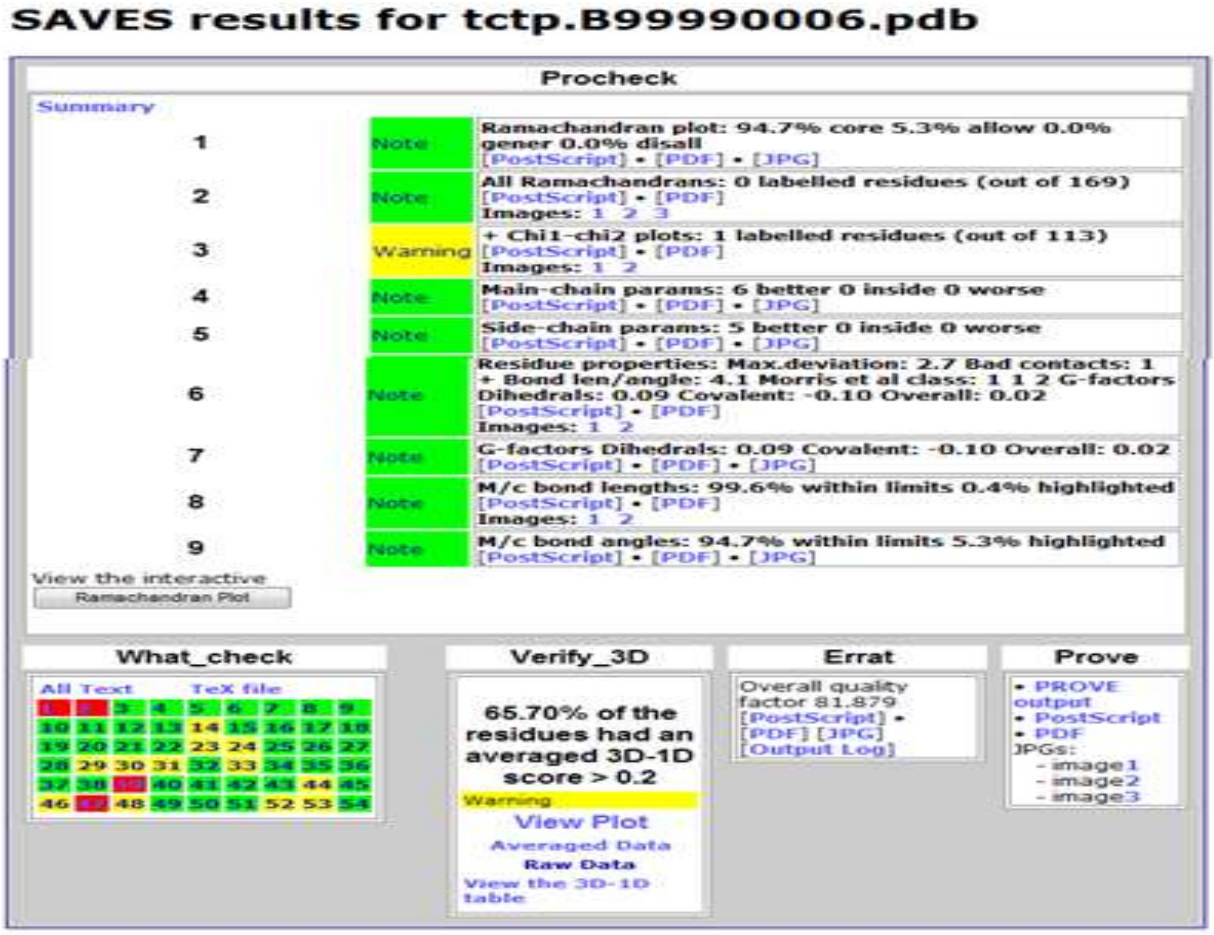

Fig.5: SAVES result for modeled TCTP.

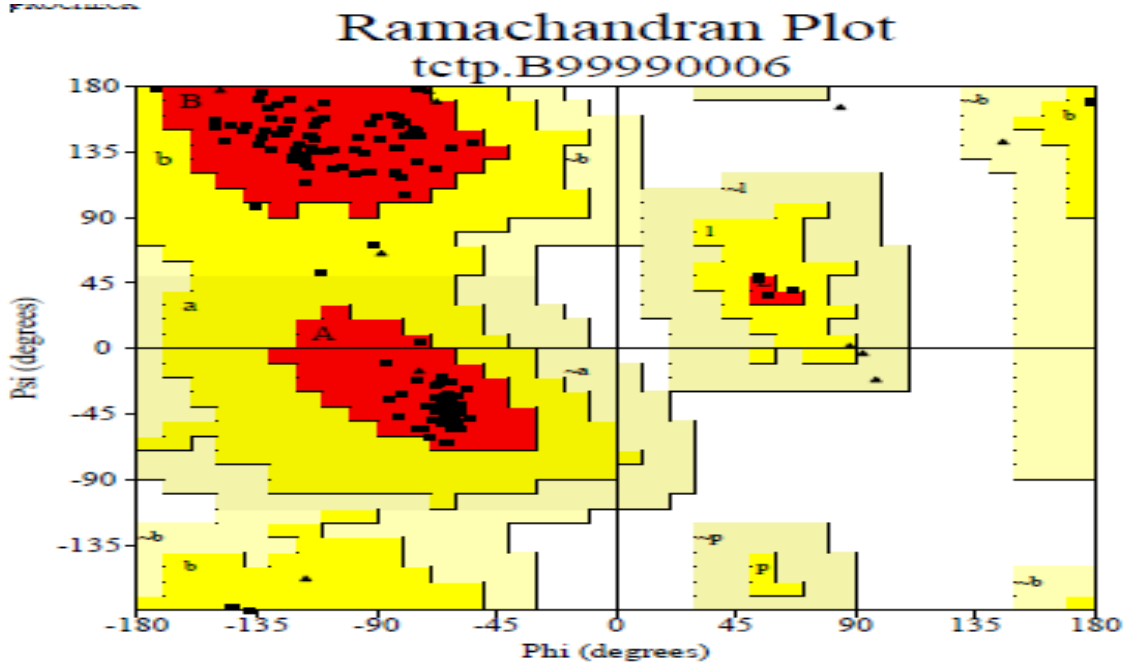

Fig.6: Ramachandran Plot for the modeled TCTP.

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Plot statistics:-

Table.6: Ramachandran plot statistics.

Residues in most favoured regions (A,B,I)	142	94.7%	
Residues in additional allowed regions (a,b,l,p)	8	5.3%	
Residues in generically allowed region (~a/,~b/,~l/,~p)	0	0.0%	
Residues in disallowed regions	0	0.0%	
		-----	--------
Number of non-glycine non-proline residues	150	100.0%	
Number of end residues (excl. Gly and Pro)	2		
Number of glycine residues (shown as triangle)	14		
Number of proline residues		5	

Total number of residues	171		

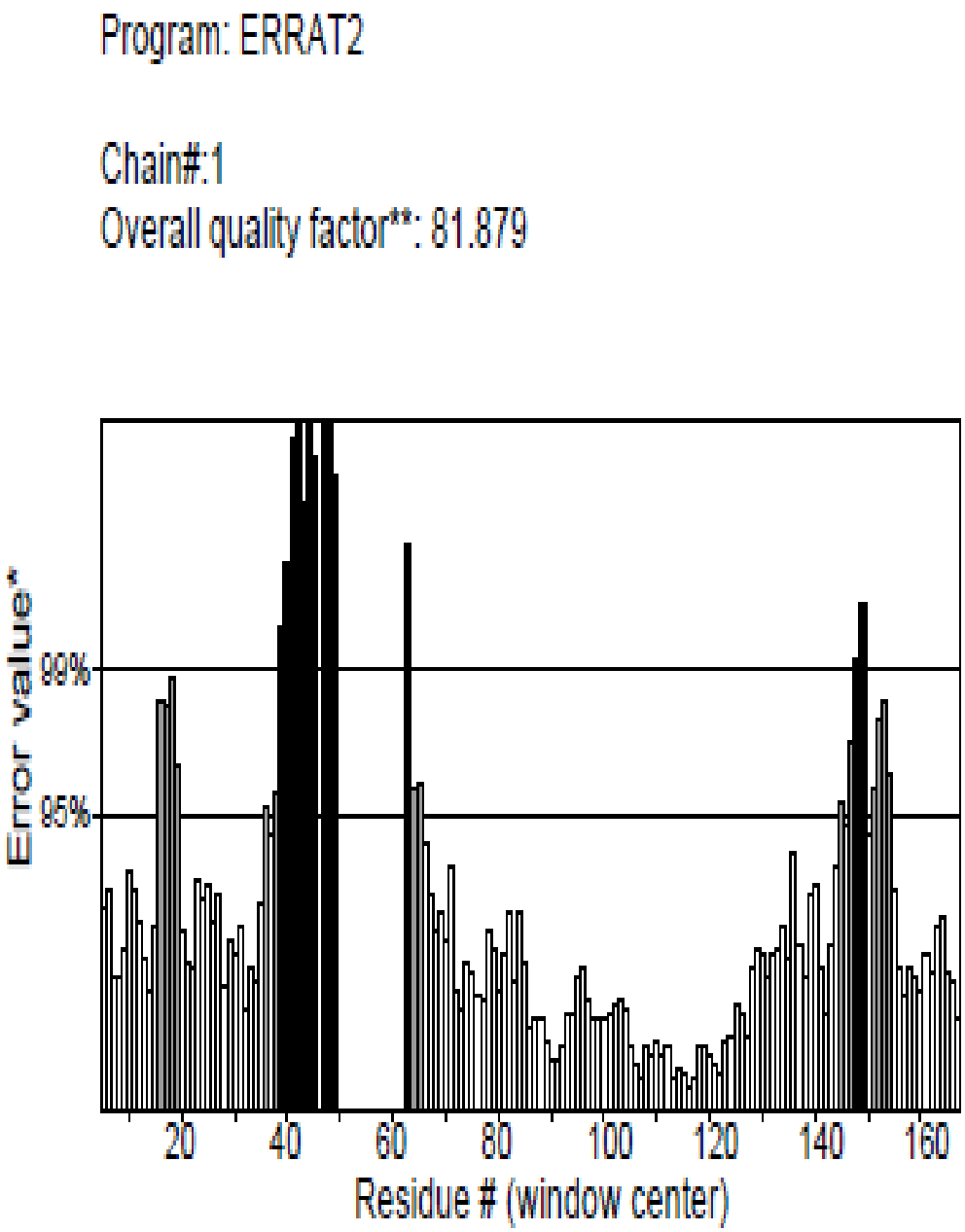

Fig.7: ERRAT graph for modeled TCTP.

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VISUALIZING 3D STRUCTURE OF TARGET PROTEIN (TCTP):-

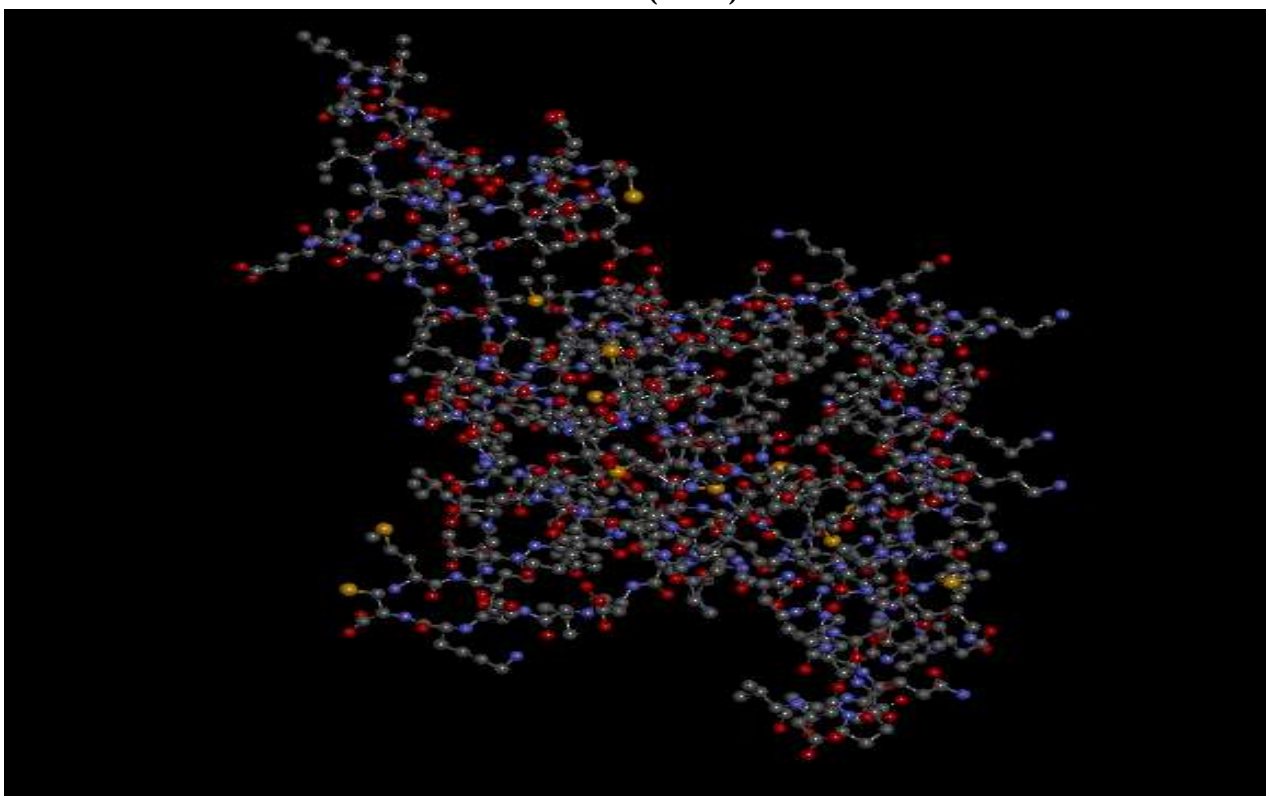

Fig.8: Wireframe structure of TCTP of L. rohitain Discovery Studio.

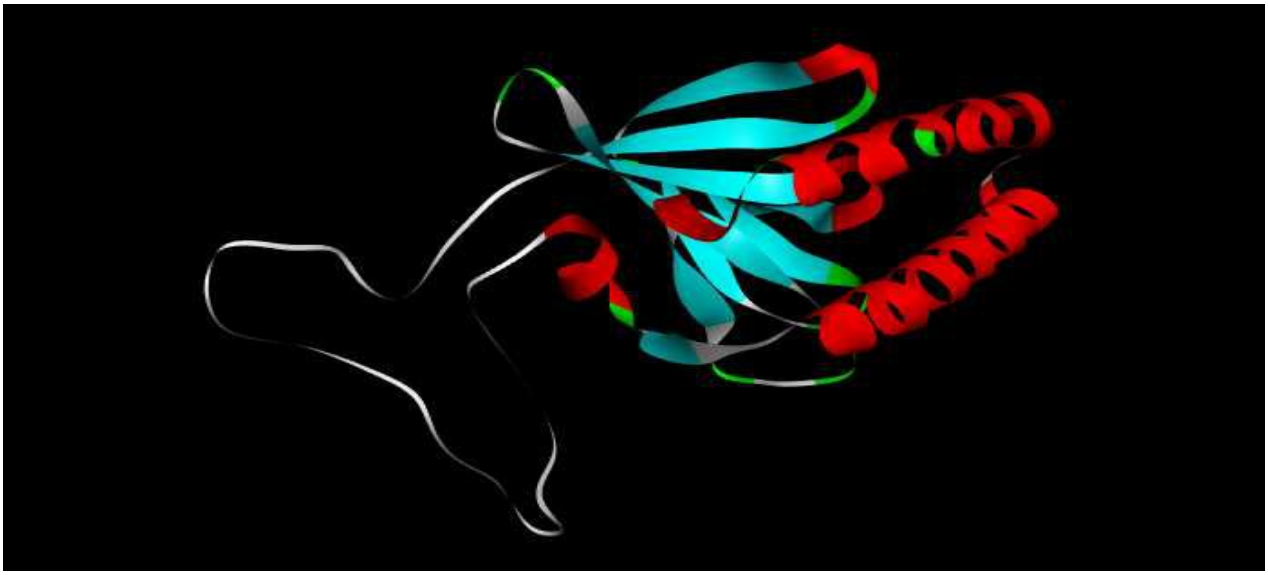

Fig.9: Ribbon structure of TCTP of L. rohitain Discovery Studio.

DOCKING RESULT FOR TCTP:-

TCTP vs. Na^+, K^+-ATPase:-

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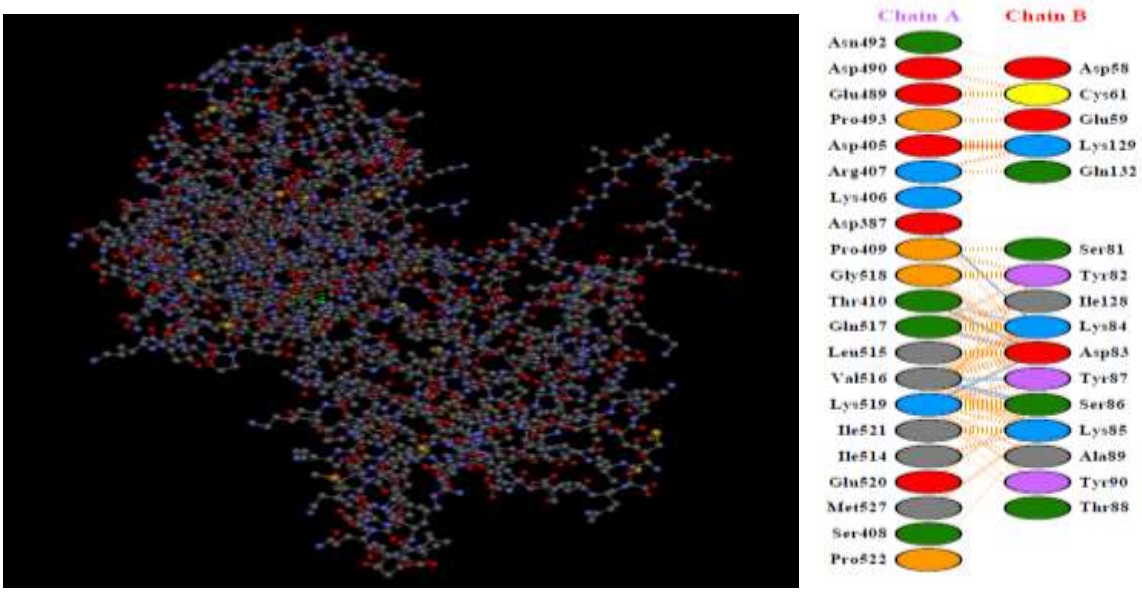

A **B**

Fig.10: (A) Shows the docked complex between TCTP(chain-B) and Na^+, K^+-ATPase(chain-A) along with the H-bonds formed between them(In green colour) and (B) shows the amino acid residues of both protein chains forming H-bond (blue colour), Salt bridge(red colour) and hydrophobic interactions(yellow colour).

Table.7: List of atom-atom interactions across protein-protein interface Hydrogen bonds (H) and Salt bridges(S) between Na^+, K^+ ATPase.

Sl. No	Na^+,K^+ ATPase						TCTP					Chain Distance (Bond Type)
	Atom No	Atom Name	Res Name	Res No	Chain	↔	Atom No	Atom Name	Res Name	Res No	Chain	
1	2260	N	LYS	519	A	↔	629	O	GLU	83	B	**2.06(H)**
2	1559	OG1	THR	410	A	↔	632	OD1	GLU	83	B	**1.29(H)**
3	2247	N	GLN	517	A	↔	632	OD1	SER	83	B	**2.63(H)**
4	1422	OD1	ASP	387	A	↔	634	N	SER	84	B	**2.89(H)**
5	1559	OG1	THR	410	A	↔	634	N	ALA	84	B	**3.19(H)**
6	2243	O	VAL	516	A	↔	652	N	HIS	86	B	**2.41(H)**
7	2243	O	VAL	516	A	↔	657	OG	GLN	86	B	**3.27(H)**
8	2243	O	VAL	516	A	↔	658	N	CYS	87	B	**2.85(H)**
9	1520	OD2	ASP	405	A	↔	996	NZ	LYS	129	B	**2.25(S)**

TCTP vs. MCL1:-

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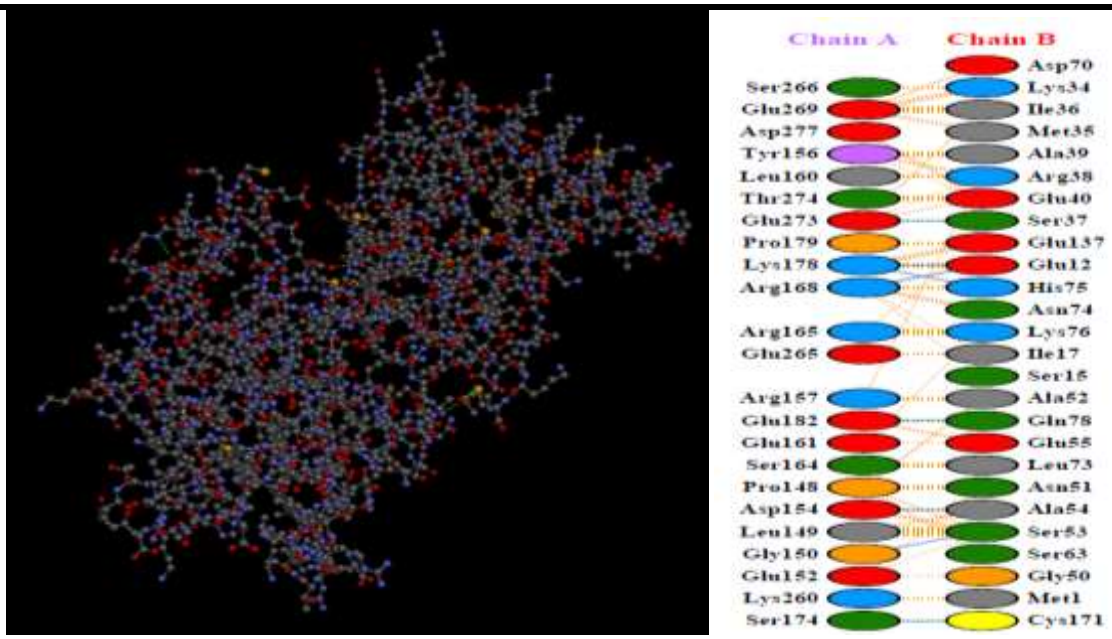

A **B**

Fig.11: (A) Shows the docked complex between TCTP(chain-B) and MCL1(chain-A) along with the H-bonds formed between them(In green colour) and (B) shows the amino acid residues of both protein chains forming H-bond (blue colour), Salt bridge(red colour) and hydrophobic interactions(yellow colour).

Table.8: List of atom-atom interactions across protein-protein interface Hydrogen bond between TCTP and MCL1.

Sl. No	MCL1						TCTP					Chain Distance
	Atom No	Atom Name	Res Name	Res No	Chain		Atom No	Atom Name	Res Name	Res No	Chain	
1	1590	NZ	LYS	178	A	↔	92	O	GLU	12	B	**2.52**
2	1517	NH1	ARG	168	A	↔	97	OE2	GLU	12	B	**2.86**
3	2329	OE1	GLU	273	A	↔	291	N	SER	37	B	**3.13**
4	1357	N	GLY	150	A	↔	406	OG	SER	53	B	**3.18**
5	1390	OD1	ASP	154	A	↔	407	N	ALA	54	B	**2.82**
6	1590	NZ	LYS	178	A	↔	559	O	HIS	75	B	**2.63**
7	1617	OE1	GLU	182	A	↔	591	NE2	GLN	78	B	**2.57**
8	1558	OG	SER	174	A	↔	1336	SG	CYS	171	B	**3.08**

TCTP vs. POLOKINASE:-

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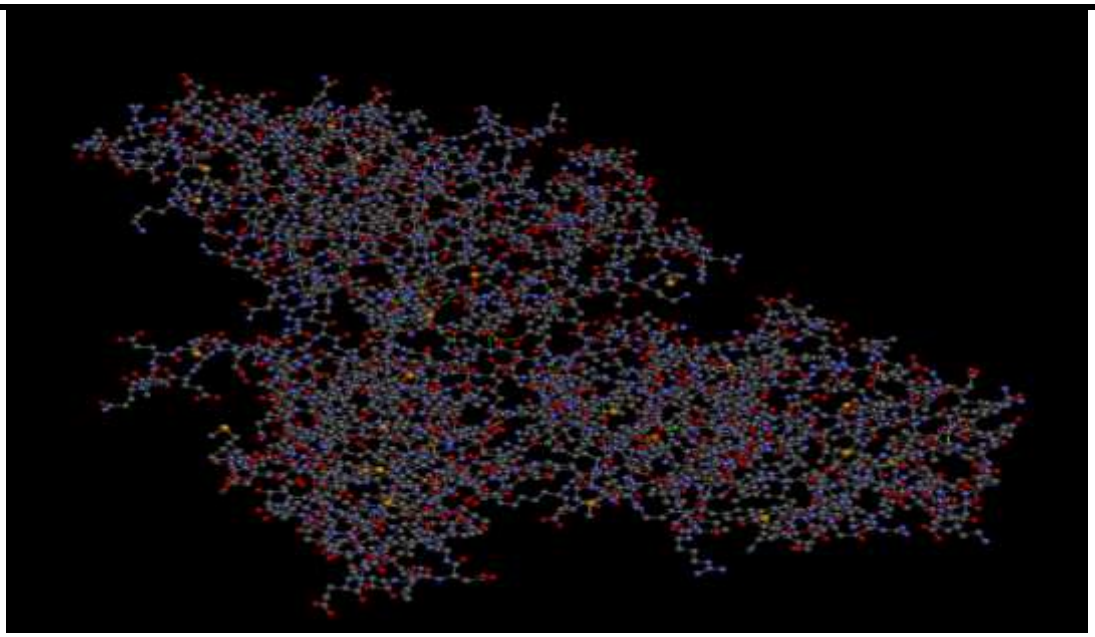
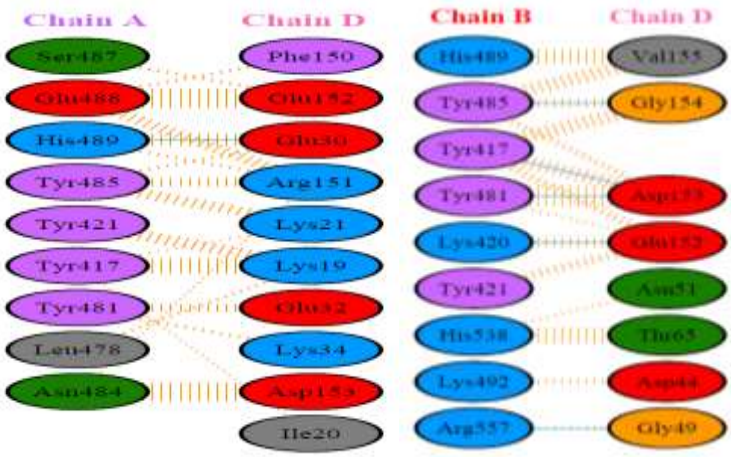


A



B **C**

Fig.12: (A): Shows the docked complex between TCTP (chain-D) and Polokinase (chain-A and B) along with the H-bonds formed between them (In green colour).
(B): shows the amino acid residues of protein chains (A and D) forming H-bond (blue colour), Salt bridge (red colour) and hydrophobic interactions (yellow colour).
(C): shows the amino acid residues of protein chains (B and D) forming H-bond (blue colour), Salt bridge (red colour) and hydrophobic interactions (yellow colour).

Table.9: List of atom-atom interactions across protein-protein interface Hydrogen bonds between TCTP and POLOKINASE.

Sl. No	POLOKINASE						TCTP					Chain Distance
	Atom No	Atom Name	Res Name	Res No	Chain	↔	Atom No	Atom Name	Res Name	Res No	Chain	
1	2281	NE1	HIS	489	A	↔	245	OE2	GLU	30	D	**2.52**
2	4630	NH2	ARG	557	B	↔	383	O	GLY	49	D	**3.05**
3	3520	NZ	LYS	420	B	↔	1181	OE1	GLU	152	D	**3.28**
4	3497	OH	TYR	417	B	↔	1186	O	ASP	153	D	**2.12**
5	4006	OH	TYR	481	B	↔	1186	O	ASP	153	D	**2.08**
6	4048	OH	TYR	485	B	↔	1194	O	GLY	154	D	**2.93**

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DOCKING ANALYSIS:-

From the above DOCKING results with various proteins shows the active sites of TCTP. The most active site of the prepared structure is ASP (83 and 153), LYS (84) and GLU (30 and 152).

IV. DISCUSSION

TCTP is a highly conserved protein in the course of evolution. According to its conservancy and frequent occurrence it should have a huge function. Though many functions are discovered the main function of TCTP is still unknown. The above in silico study shows the prediction of 2D and 3D structure of TCTP of *Labeorohita*. The prediction of 2D structure is an essential step towards the prediction of 3D structure. From the above study it can be concluded that the 2D structure of TCTP has 39.18% of Alpha helix, 29.83% of Beta sheets and 30.99% of Random coils in its Amino Acid chain.

The 3D structure of TCTP was prepared in Modeller9.10 and out of the ten structures the best structure was chosen on the basis of lowest DOPE scores then the structure was analysed under SAVES server. The SAVES result showed that about 94.7% residues are present in the most favoured region in Ramachandran plot and <5.3% residues are present in additional allowed region and the ERRAT showed 81.879 which conforms the good quality of the structure. The best model was visualized in Discovery Studio. The Docking study was done in PatchDock server and refined in FireDock showed that the TCTP was mostly active at residues between 82-85 and 151-155. The work can be extended further to predict its functions and other expects.

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