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Insilico Analysis of RHES Protein for Huntington's Disease

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Abstract

There are numerous subfields within tree science, including biotechnology, zoology, and botany. One of the newest developments in science is the discipline of bioinformatics, which is one of the fields that is booming right now. The multidisciplinary field of bioinformatics creates and enhances techniques for biological data storage, analysis, and interpretation. It solves biological problems, typically at the molecular level, by combining biology, computer science, microbiology, mathematics, statistics, and biochemistry. There are several uses for bioinformatics, including drug design, sequence alignment, gene expression detection, and gene discovery. Modelling is one of the main fields in which bioinformatics is applied. In the scientific field of computational biology, biological data is utilised to create algorithms. In order to better understand biology and the relationships between macromolecules, it also entails the development of mathematical modelling and computational simulation techniques. The mastermind behind the creation of numerous bioinformatics tools is the computational biologist. They created a number of algorithms for the development of software and tools. There are currently over 2,300,000 sequences available. However, its structure isn't accessible. With only 79000 structures now accessible, it is evident that predicting protein structures is a challenging task. A variety of techniques, including homology modelling, threading, and abinitio structure prediction, are available for predicting protein structures. However, determining which approach is more accurate for predicting structure is extremely challenging, even if there are several modelling tools available, like Phyre, Swiss model, and I-tasser. The goal of this study is to predict a novel protein structure and then use the server ProQ to assess the tool's quality.

Keywords**:** *Homology modelling, Abinitio, threading.*

1. INTRODUCTION

Recent research indicates that a key role in the pathogenesis of Huntington's disease is played by Rhes, a protein that expresses itself selectively in the striatum.A GTP-binding protein is called Rhes. The striatum is where most rhes is made. It is crucial for intrinsic GTPase activity. Additionally, it

controls the signalling channels. The aetiology of Huntington's disease is significantly influenced by the rhes protein.

A neurodegenerative condition that affects muscle contraction is Huntington's disease. It is an inherited illness that causes the brain's nerve cells to break. It will also impact one's capacity for thought and mobility. The aberrant expansion of CAG, a gene located on the fourth chromosome, is the cause of this disorder[1]. This can be found in the gene that produces the Huntington's disease-causing Huntingtin protein (Htt). The small GTPase Rhes significantly increases the cytotoxicity of mutant Htt. Rhes and Htt may be able to communicate. Next, it adds SUM01 protein to the mutant Htt, changing its striatum. This will lead to an increase in the poisonous soluble form of mutant Htt and a decrease in neuroprotective insoluble aggregates. The HD genes create the protein known as HTT[2]. In the central nervous system's neurons, it is highly expressed. Its roles include postsynaptic signalling, transcriptional control, and anti-apoptotic activity. It is also present in synaptic vesicles and mitochondria. The presence of an extended polyglutamine tail causes mutant Htt to arise, which will interfere with the proteins' ability to function normally[3].

Major Research Area

Comparative analysis on protein structure prediction (Protein structure prediction by using the methods: Threading and Ab initio structure prediction)

Protein Structure Prediction

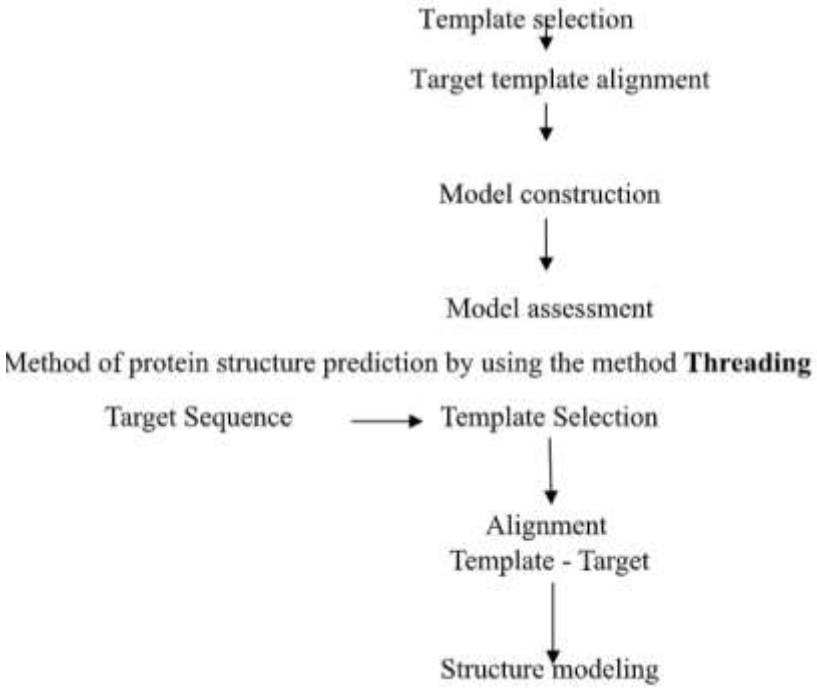
The main structure of proteins, which are represented by the amino acids found on DNA molecules, may be easily determined, but the secondary, tertiary, and quaternary structures of proteins are more challenging to ascertain. NMR and X-ray crystallography are employed as experimental techniques for this goal. As a result of its accuracy, X-ray crystallography is a method that is more commonly utilised. However, this experimental approach takes a lot of time. To circumvent this intricacy, computational biologists created multiple instruments for structural forecasting. Ab initio, Threading, and Comparative modelling are some of the techniques utilised to predict protein structures. The process of threading, also referred to as folding, involves breaking the target sequences into smaller segments and aligning each amino acid to a specific location in the template structure. This allows us to determine how well the target fits the template and, based on that fit, choose the best model for the structure prediction. Although homology modelling and threading are similar, their target protein structures differ [4].

Homology Modelling

Comparative modelling is another name for homology modelling. It entails creating a target protein using the homologous protein's experimental three-dimensional structure and amino acid sequence. Protein structure is more conserved than DNA sequence. The quality of the homologous protein's three-dimensional structure and amino acid sequence determine the calibre of the resulting model. The existence of alignment gaps makes this procedure much more complex. This indicates that the structural region is unique to the target and is absent from the template. As a result, the final model has inadequate resolution[5]. In addition, sequence identity is crucial for homology modelling. In

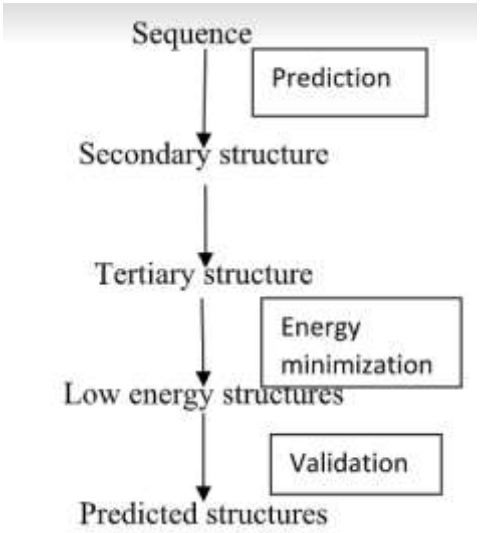
addition, sequence identity is crucial for homology modelling. A close relationship between the target and the template indicates that the newly modelled structure will be of good quality.

Steps in homology modeling



The basic idea of Abinitio method is to build empirical function that simulates real physical forces and potentials of chemical contacts. In Ab initio modeling we select the best structure on the basis of energy. Those having lowest energy that can be considered as the best structure, but this structure prediction method are very difficult and very useful.

Method of Protein structure prediction by using **Ab initio**



Existing System

The analysis part includes a detailed study of the existing system. Different types of protein structure prediction methods are available but their accuracy is entirely different. There is no specificity and more over the redundancy of data.

Limitations

Reliability
Accuracy
Lack of specificity

2. MATERIALS AND METHODOLOGY

Sequence retrieval

The sequence can be retrieved from the databases UniProt or Swiss Prot.UniProt and Swiss Prot are the protein sequence databases.the sequence of the Rhes protein is selected from the database UniProt.

UNIPROT

UniProt is a database of protein sequence and functional information. The UniProt Consortium comprises the European Bioinformatics Institute (EBI), the Swiss Institute of Bioinformatics (SIB) and the Protein Information Resource (PIR). The mission of UniProt is to support biological research by providing a freely accessible, stable, comprehensive, and accurately annotated protein sequence knowledgebase[6]. UniProt is comprised of four major components: the UniProt Archive, the UniProt Knowledgebase, the UniProt Reference Clusters and the UniProt Metagenomic and Environmental Sequence Database. The UniProt Knowledgebase (UniProtKB) is an expertly curated database, a central access point for integrated protein information with cross- sreferences to multiple sources. The UniProt Archive (UniParc) is a comprehensive sequence repository, reflecting the history of all protein sequences. UniProt Reference Clusters (UniRef) merge closely related sequences based on sequence identity to speed up searches while the UniProt Metagenomic and Environmental Sequences database (UniMES) was created to respond to the expanding area of metagenomic data[7].

SWISS PROT

SWISS-PROT is an annotated protein sequence database, which was created at the Department of Medical Biochemistry of the University of Geneva and has been a collaborative effort of the Department and the European Molecular Biology Laboratory (EMBL),. SWISS-PROT is a curated protein sequence database which provides a high level of annotation (such as the description of the function of a protein, its domains structure, post- translational modifications, variants, etc.), a minimal level of redundancy and high level of integration with other databases. Recent developments of the database include format and content enhancements, cross- references to additional databases, new documentation files and improvements to TrEMBL, a computer- annotated supplement to SWISS-PROT[8].

Template (Protein Structure) selection

PDB is the protein data bank; the protein structure can be downloaded from PDB.

PDB

The Protein Data Bank (PDB) is an archive of experimentally- determined three-dimensional structures of proteins, nucleic acids and other biological macromolecules. PDB was established at Brookhaven National Laboratory. Two forces converged to initiate the PDB. The primer force is a small but growing collection of sets of protein structure data determined by X-ray diffraction and the second force is the newly available molecular graphics display. The PDB is a key resource in the areas of structural biology, such as structural genomics. PDB helps to access information that can relate the biological functions of macromolecules to their three- dimensional structure[9].

Selected Protein

The selected protein for this case study is Rhes proteins.

RHES Protein

Rhes, the Ras Homolog Enriched in Striatum, is an intermediate-size GTP binding protein. Although its full functions are not yet known, Rhes is a GTP binding protein. Rhes is produced largely in the striatum. It is important for the activity of intrinsic GTPase. It also regulates the signaling pathways. Rhes protein plays a major role in Huntington’s disease pathogenesis[10].

Sequence of RHES Protein

>sp|Q96D21|RHES_HUMAN GTP-binding protein Rhes OS=Homo sapiens GN=RASD2 PE=1 SV=1MMKTLSSGNCTLSVPAKNSYRMVVLGASRVGKSSIVSRFLNGRFEDQYTPTIEDFHRK VYNIRGDMYQLDILDTSGNHPFPAMRRLSILTGDVFILVFSLDNRESFDEVKRLQKQILEVKSC LKNKTKEAAELPMVICGNKNDHGELCRQVPTTEAELLVSGDENCAYFEVSAKKNTNVDEMF YVLFSMAKLPHEMSPALHRKISVQYGDAFHPRPFCMRRVKEMDAYGMVSPFARRPSVNSDL KYIKAKVLREGQARERDKCTIQ

Protein Structure Prediction Tools

SWISS Model

Swiss model is an automated homology modeling server. The purpose of this server is to make protein modeling accessible to all people. It can be access by using the url http://www.expasy.org/swissmod/.This will help to justify experimental data (i.e. differences between unknown sequence and templates) and be useful to understand function.

Requirements for Swiss Model

- BLAST search P value $<10^{-5}$.
- >25% sequence identity with a template.
- Minimal projected model length of 25 amino acids.

I-TASSER

I-TASSER is a prediction method from Zhang's group. It is a Fully automated server, without any human intervention. It mainly focused on Template identification, Structure assembly, Atomic model construction and Model selection. The recent studies show that it is the most important tool for the structure prediction[11]. The major factor for this is the length of the sequence. It needs a sequence length of minimum 50 residues.

PHYRE

Phyre (Protein Homology/AnalogY Recognition Engine) one of the tools used for the protein structure prediction. It is a web based server used the techniques of homology modeling.Phyre 2 is the advanced version of phyre that uses a fold library. This library is updated when the new structure is solved. Compared to Phyre, Phyre 2 has some additional features. The additional functionalities of the Phyre 2 include Batch processing (allows the users to submit more than one sequence), 3D ligand structure, multi template modeling and Trans membrane topology prediction[12].

Protein Quality Prediction

ProQ

ProQ is a server used to predict the quality of the structure. It detects the quality of the structure based on the neural network algorithm. The quality of the structure is calculated on the basis of LG score and MaxSub. LG score is a log of P value and MaxSub is a program to find the quality of the predicted structure and is a range from 0-1.0 is insignificant and 1 is very significant[13].

Visualization Tool

Pymol/Rasmol

Pymol or RasMol can be used to view the modeled protein. It provides coordinates for protein tertiary structure and manipulation. It will help to view the detailed structure of a protein; also we can rotate the coordinates. It will show the modeled protein in several models like Ribbon, Cartoon, and Ball and stick and so on.

3. RESULT AND DISCUSSION

I-TASSER Working

Structure prediction by using the tool I-Tasser mainly involves four steps. First step is the template identification by using LOMETS, and then the iterative structure assembly simulations can be done by using Monte Carlo Simulations.

Next step is the atomic level model construction and refinement. It can be done in two ways, first one is the construction of full atomic model by using REMO, and in the second step these models are refined by using FG-MD which helps to find out the bond length and torsion angles.

Function prediction is the final step. It helps to find out the gene ontology, enzyme commission number and the binding sites. The function prediction can be done by using cofactor approach.

I-TASSER Result

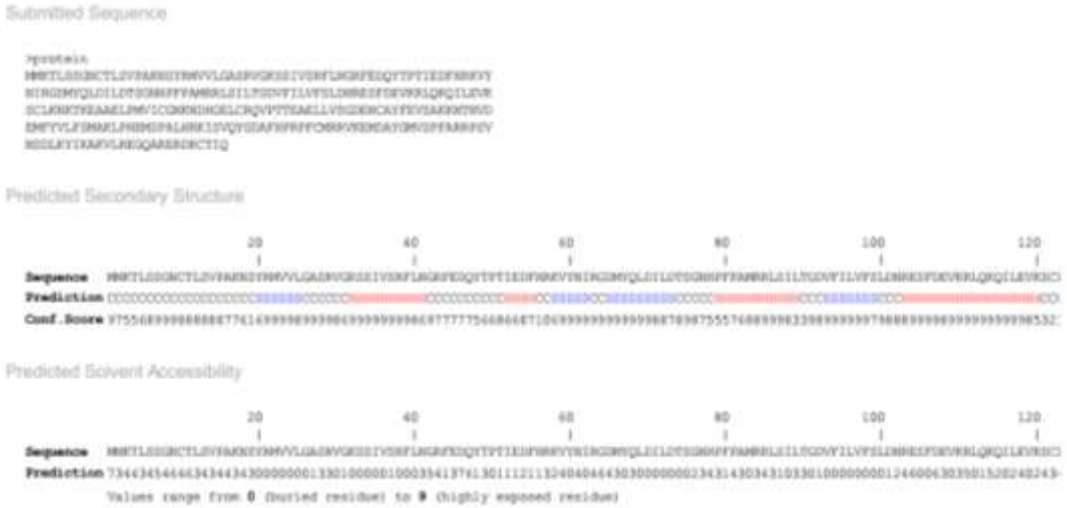


Fig: 1 - I-tasser result page containing information related to the input sequence in Fasta format, predicted secondary structure and score associated with them along with the secondary structure it contains the confidence scores, Predicted solvent accessibility value ranging from 0 to 9. From the solvent accessibility region, the hydration sites can be predicted.

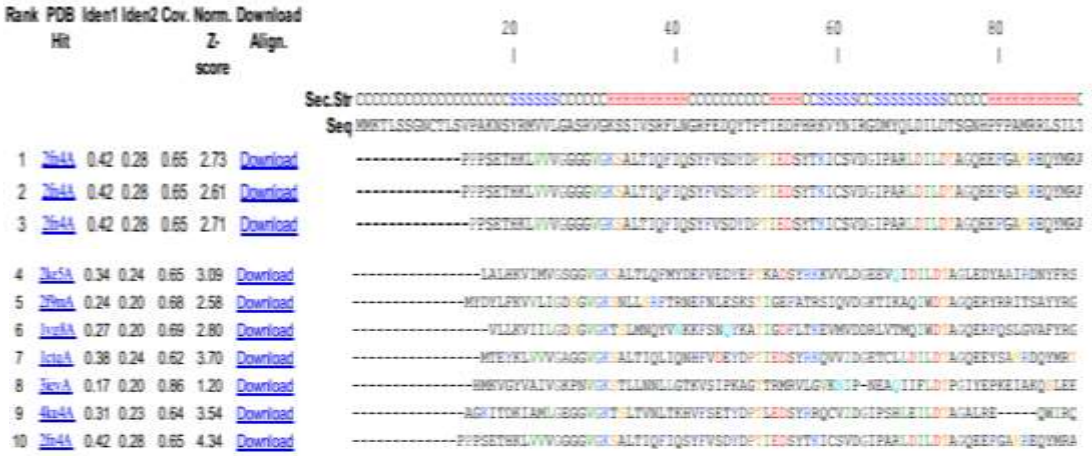


Fig: 2- Result page showing top ten templates used by I-Tasser. It also shows the corresponding alignment. It can be done by using LOMETS threading program. The quality of the threading alignment depends on the Z-score.

Top 10 Identified stuctural analogs in PDB

Click to view

Rank	PDB Hit	TM-score	RMSD[a]	IDEN[a]	Cov.	Download Alignment
1	3j38z	0.658	4.15	0.091	0.842	Download
2	3b82E	0.647	4.29	0.097	0.831	Download

	3	2bcgY	0.625	2.74	0.215	0.714	Download
	4	3dnyT	0.618	4.28	0.098	0.793	Download
	5	2fn4A	0.617	1.62	0.405	0.650	Download
	6	2f9mA	0.616	2.00	0.228	0.673	Download
	7	1vg8A	0.614	2.26	0.279	0.680	Download
	8	2wkpA	0.609	2.79	0.217	0.692	Download
	9	3bc1A	0.609	1.75	0.293	0.654	Download
	10	2rdo7	0.609	4.18	0.120	0.786	Download

Fig: 3- Result page showing top ten identified structural analogs in PDB.It depends on the TM-score.TM-score greater than 0.5 indicates similar topology.

Top 5 enzyme homologs in PDB

Click to view	Rank	$Cscore^{EC}$	PDB Hit	TM-score	$RMSD^a$	$IDEN^a$	Cov.	EC Number	Predicted Active Site Residues
	1	0.284	2bmeA	0.612	1.83	0.240	0.658	3.6.5.2	NA
	2	0.265	3bfkB	0.606	2.09	0.220	0.665	3.6.5.2	NA
	3	0.254	2f9mA	0.616	2.00	0.228	0.673	3.6.5.2	NA
	4	0.254	2e1rA	0.624	4.27	0.097	0.801	3.6.5.3	78
	5	0.240	1eloA	0.591	4.17	0.124	0.763	3.6.5.3	78

Fig: 4- Result page showing top five enzymes homologs in PDB.It can be calculated on the basis of EC-score. If EC-score is greater than 1.1 means there is a functional similarity between the query and the template.

Template proteins with similar binding site:

Click to view	Rank	$Cscore^{LB}$	PDB Hit	TM-score	$RMSD^a$	$IDEN^a$	Cov.	BS-score	Lig. Name	Download Complex	Predicted binding site residues
	1	0.68	2fn4A	0.617	1.62	0.405	0.650	1.59	GDP	Download	29,30,31,32,33,34,46,140,141,143,144,172,173,174
	2	0.55	1nvxR	0.547	2.44	0.349	0.598	1.44	PO4	Download	28,29,30,31,32,33

3	0.36	1pljA	0.525	1.45	0.354	0.553	1.37	MG	Download	33,51,73
4	0.32	1aa9A	0.585	2.12	0.368	0.643	0.95	MG	Download	33,48,49,50
5	0.29	1xd2A	0.597	1.33	0.380	0.624	1.69	PO4	Download	28,32,48,50,51,75,76,77
6	0.21	3rs0A	0.587	1.61	0.380	0.624	1.65	YEG	Download	53,54,55,72,87
7	0.20	3rslA	0.559	1.39	0.385	0.587	1.61	RSF	Download	27,28,102,104,105
8	0.07	2uzi1	0.595	1.38	0.380	0.624	1.51	PEPTIDE	Download	33,41,43,45,49,50,52,53,54,55,56,80
9	0.07	3lbhA	0.597	1.34	0.380	0.624	1.48	ACT	Download	113,117,131,132,133,135
10	0.07	5p210	0.595	1.40	0.380	0.624	1.44	PEPTIDE	Download	63,64,153,157,161,164,166,168,169,170,181,188,192

Fig: 5 I-Tasser result page showing predicted binding site residues on the basis of cofactor algorithm.

Predicted Protein Structure

PHYRE Working

In the first step the submitted sequence is search against the large databases using PSI-Blast. Then it is analyzed by using neural network algorithm and predicts the disorders by using disopred. After that analyze the domain and then predict the structure on the basis of the template information.

PHYRE Result

Fig: 6- Phyre information related to secondary structure prediction and disorder prediction

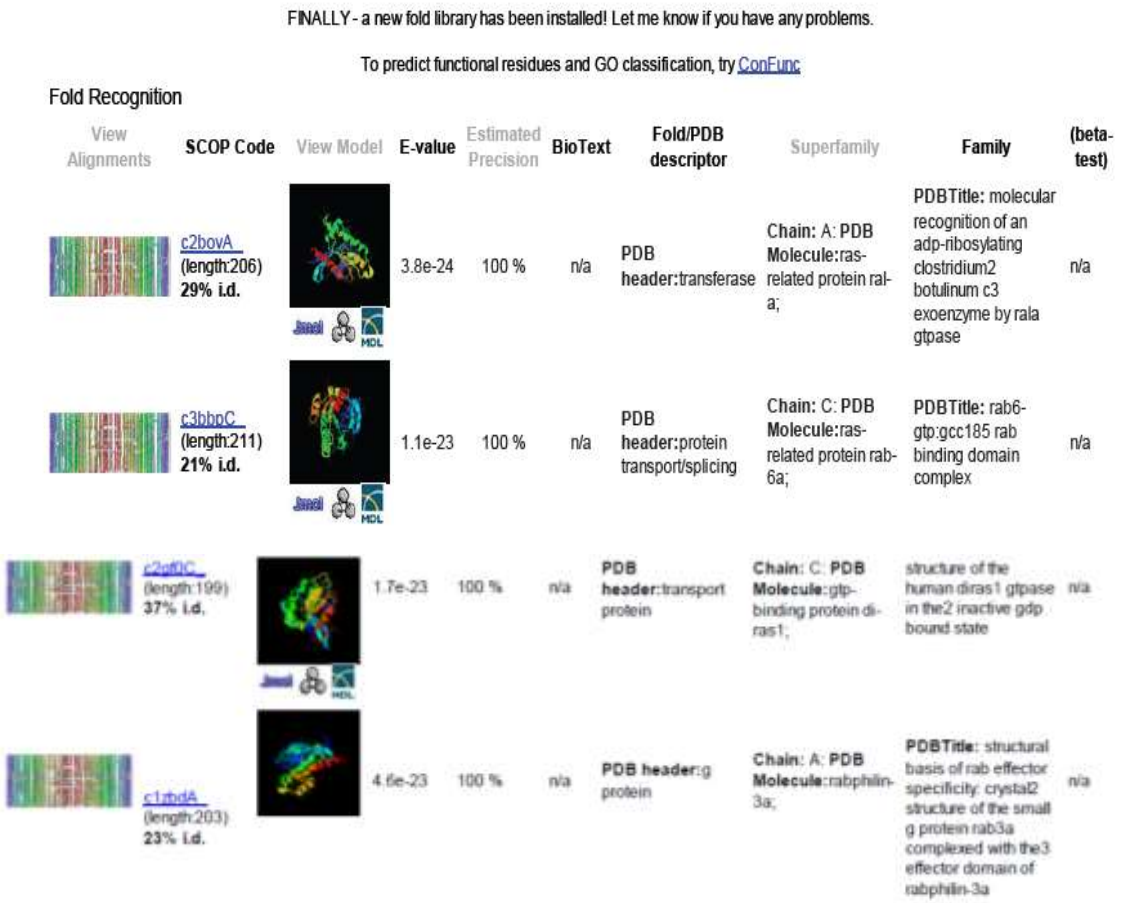


Fig: 7- Detailed Template Information

It contains the information related to the predicted 3D structure and their images. The information is selected from the SCOP or PDB. It depends the template. It also contains the alignment view. It helps the user to find the individual aligned residues. Jmol is used to permit the 3D viewing model of the protein model.

Predicted structure by using Phyre

SWISS Model Working

Modeling a new protein by using Swiss Model mainly involves four steps. First step is the identification of templates, in this Blast is used to find out the homologous sequence. HHsearch also used for this. Then the template is derived from the PDB and stored it in the Swiss Model Template Library. Finally the model is built by using rigid fragment assembly method.

SWISS Model Result

QMEAN4 global scores:

The QMEAN4 score is a composite score consisting of a linear combination of 4 statistical potential terms (estimated model reliability between 0-1). The pseudo-energies of the contributing terms are given below together with their Z-scores with respect to scores obtained for high-resolution experimental structures of similar size solved by X-ray crystallography:

Scoring function term	Raw score	Z-score
C_beta interaction energy	-94.81	-0.42
All-atom pairwise energy	-5155.17	-0.52
Solvation energy	-15.30	-0.28
Torsion angle energy	-28.53	-1.51
QMEAN4 score	0.670	-1.50

Fig: 8- Swiss Model results showing the scoring functions

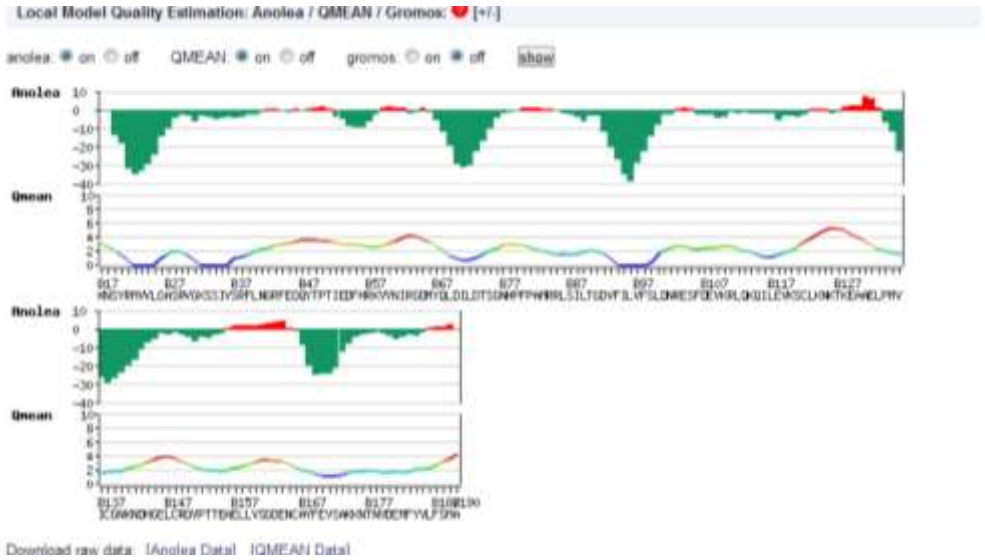
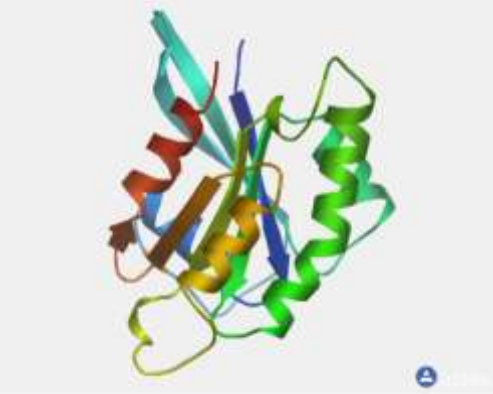


Fig 9: - Local model quality estimation by using Gromos, Qmean or Anolea. In this the quality of the model is estimated using Anolea(The atomic empirical mean force potential, It helps to detect the packing quality of the model. It is also suitable for the calculation of energy.

Predicted Model by Using Swiss Model



Protein Quality Prediction

Several tools and methods are available for the structure prediction. But it is very difficult to predict which one is the best model. The quality of the protein can be detected by using the tool ProQ.

ProQ

ProQ is a server used to predict the quality of the structure. It detects the quality of the structure based on the neural network algorithm. The quality of the structure is calculated on the basis of LG score and MaxSub.LG score is a log of P value and MaxSub is a program to find the quality of the predicted structure and is ranges from 0-1.0 is insignificant and 1 is very significant.

ProQ Results

Quality prediction of the modeled protein obtained from I-Tasser.
Prediction not using predicted secondary structure
(OBS: By using predicted secondary structure the prediction will be more reliable)
==========
Predicted LGscore : **3.096**
Predicted MaxSub : **0.354**
==========

Different ranges of quality:
LGscore>1.5 fairly good model
LGscore>2.5 very good model
LGscore>4 extremly good model

Quality prediction of the modeled protein obtained from Phyre.
Prediction not using predicted secondary structure
(OBS: By using predicted secondary structure the prediction will be more reliable)
==========
Predicted LGscore : **3.878**
Predicted MaxSub : **0.455**
==========

Different ranges of quality:
LGscore>1.5 fairly good model
LGscore>2.5 very good model
LGscore>4 extremly good model

MaxSub>0.1 fairly good model

MaxSub>0.5 very good model
MaxSub>0.8 extremly good model

Quality prediction of the modeled protein obtained from Swiss model
Prediction not using predicted secondary structure
(OBS: By using predicted secondary structure the prediction will be more reliable)
==========
Predicted LGscore : **4.567**
Predicted MaxSub : **0.466**
==========

Different ranges of quality:
LGscore>1.5 fairly good model
LGscore>2.5 very good model
LGscore>4 extremely good model

MaxSub>0.1 fairly good model
MaxSub>0.5 very good model
MaxSub>0.8 extremely good model

ProQ Result Interpretation
The quality of the protein increased on the basis of LG score and Maxsub. If the LG score is greater than 4 means it can be considered as extremely good model.In this case study the protein modeled by using Swiss model shows a LG score of 4.567 hence it can be considered as extremely good model, and other two are very good model(Quality prediction of the modeled protein obtained from Phyre and I-Tasser).

3. CONCLUSION

One of the main uses of bioinformatics is the prediction of protein structures. It is more beneficial for the creation of novel drugs. All of the methods—homology modelling, threading, and Abinitio structure prediction—are useful for predicting novel models. The I-Tasser, Swiss Model, and Phyre resulting structures are nearly identical; the variations arise from the use of various parameters. The tools operate using certain algorithms (e.g., cofactor method, neural network, etc.). Certain factors were used by these algorithms to determine how they should operate (some taking into account the loop area, helices, and binding site, while others evaluating hydrophobicity, confirmations, and stabilities). This also caused a change in the structure's quality. We can infer from this case study that the Swiss Model yielded a higher-quality model than Phyre and I-Tasser. Therefore, employing threading to predict protein structure yields more accurate results than ab initio structure prediction. We are also able to determine the function from the anticipated structure. The modeled structure of the Rhes can be used for the further analysis of Huntington disease. According to this Swiss Model is the most reliable tool for the structure prediction.

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