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Comparative Study of Homology Based Structure Prediction and Structure Validation Tools on Some Proteins from the bhlh family.

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ABSTRACT

Comparative homology modeling has become an efficient and easy method for predicting the unknown three dimensional structure of a protein based on sequence alignment. The steps involved are template alignment, loop assignment, model building and model refinement. However, it was noticed that though the basic steps for modeling the protein were same, the results produced by different tools that are available varied; possibly due to the efficiency of the algorithm and other factors. Here, five homology modelling tools were used to compare the results for some proteins of the bhlh family. It was also noticed that structure validation tools had different results. To compare the results ProcheckRamchandran plot from PDBSum Generate and Ramchandran plot from SPDBV were used. The differing results were compared using simple statistical approach and the inference was obtained as patterns for the tools.

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1. INTRODUCTION

Comparative or homology modeling performs as one of the best methods for the prediction of unknown protein structures. It helps to bridge the gap between the available sequence and structure information by providing reliable and accurate protein models. A protein template with known three dimensional structures and sufficiently high sequence identity to the target can be modeled with high accuracy models and also the proper alignment can be seen[1]. Homology models of proteins are of great interest for planning and analyzing biological experiments when no experimental three-dimensional structures are available. But, inspite of the progress in the field of NMR spectroscopy and X-ray- crystallography, 3-D structures of many therapeutically relevant proteins are still not available [2]. Many of these therapeutically relevant protein structures are required in the initial phases of drug designing and homology based modeling methods are used to analyze them. So, there is a need to predict the 3D structures of such proteins. In this study, we have considered five basic helix-loop-helix proteins to carry out the comparative homology modelling to predict their 3D structures along with comparing the software used for the same[6][8].

The basic helix-loop-helix (bHLH) proteins are a part of large superfamily of transcriptional regulators which are found in organisms from yeast to humans and function in critical developmental processes, including sex determination and the development of the nervous system and muscles.[10]Following are the proteins which we have considered in our study;

OLIG1: It is a closely related basic helix-loop-helix (bHLH) transcription factors that is expressed in myelinatingoligodendrocytes and their progenitor cells in the developing central nervous system (CNS). Olig2 is necessary for specification of oligodendrocytes, but the biological functions of Olig1 during oligodendrocyte lineage development are poorly understood, but, are assumed to be repairing the brain [5].

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NPAS1: This protein is a member of the basic helix-loop-helix (bHLH)-PAS family of transcription factors. Studies of a related mouse gene suggest that it functions in the neurons but the exact function is unclear. However, it is known that it may play protective or modulatory roles during late embryogenesis and postnatal development. [5]

MYOD1: It is involved in muscle differentiation (myogenic factor) and induces fibroblasts to differentiate into myoblasts. It activates muscle-specific promoters and interacts with, or, is inhibited by twist protein. This interaction probably involves the basic domains of both proteins. [5]

NPAS3: This protein is also a member of the basic helix-loop-helix and PAS domain-containing family of transcription factors. The protein is localized to the nucleus and may regulate genes involved in neurogenesis. Chromosomal abnormalities that affect the coding potential of this gene are associated with schizophrenia and mental retardation. [5]

NPAS4: It is a member of the basic helix-loop-helix-PER (bHLH-PAS) class of transcriptional regulators, which are involved in a wide range of physiologic and developmental events. [5]

Experimental techniques such as NMR and X-ray Crystallography are employed to predict the 3D structure of proteins. These techniques are very tedious and prolonged, not always succeeding in determining the structure of proteins. Therefore the homology modeling approach is considered to be a good choice of method for predicting the structure of protein for further functional analysis study. Another advantage of homology modeling approach is that it is very quick method for predicting the structure of protein. Homology modeling of a protein is a procedure in which constructing an atomic resolution model of a protein from its amino acid sequences ("target protein") using an experimental 3D structure of a related homologous protein ("template") is carried out. Homology modeling is based on the concept that the protein sequences among homologues and identity above 30 % in sequence is likely to give the similar structure. Homology modeling consists of 5 steps [13]

1. Fold assignment in which the similarity between the target sequence of interest and at least of one known protein structure (the template) is identified.
2. Target sequence and template sequence alignment.
3. The modeling done is based on the selected template.
4. Optimization of the model is performed.
5. Validation of the model

As the 3D structure of protein of bhlh family have yet not been build and in order to study the functional properties of protein more confidently, the objective of this work is to build the model of this protein using comparative modeling approach. It will provide insight into its structure and further some functional aspect. In this study we have analyzed and reviewed current approaches to carry out comparative homology modeling. As a case study, five tools for homology modelling and structure prediction were used to demonstrate the methodology and their structure prediction, but concentrating how the results from the tools differ.

2. SOFTWARE USED

For a given protein sequence, the comparative modelling procedure requires identification of homologous sequences with known structures, alignment of the query sequence to the selected template structure, 3D model construction, and refinement of the predicted model. The actual modeling process is of course much more complex, and the methods employed by various prediction servers to identify suitable templates and structures may widely differ. Following are the tools which we have utilised in our study. [2][7]

Phyre2

Protein Homology/analogy Recognition engine 2 (PHYRE2) is a free online homology modelling server. Phyre2 uses the alignment of hidden Markov models via HHsearch to significantly improve accuracy of alignment and detection rate. Phyre2 also incorporates a new ab-initio folding simulationcalled 'Poing' to model those regions of proteins in question which have no detectable homology to known structures. Poing is also used to combine multiple templates. Distance constraints from individual models are treated as linear elastic springs. Poing then synthesizes entire protein in the presence ofthese springs and at the same time models unconstrained regions using its physics simulation. [9]

MODELLER

It is a freely available offline tool used to model proteins. MODELLER builds a 3D structure that satisfies certain spatial restraints, including Cα-Cα bond length, main-chain and side-chain dihedral angles, and Van der Waals interactions. These restraints are expressed as probability density functions representing the probability of occurrence of a certain conformation and are calculated from the structures of homologous template sequences. Compilation of the probability density functions from individual template structures into a

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single representative probability density function, followed by optimization of this function, then describes the overall spatial restraints for the query protein. As a result, the predicted model represents the most probable structure of the protein in question, based on structural information derived from known homologues. [12]

ESypred 3D

ESyPred3D is an online tool which can be used to as an automated homology modelling program. This tool uses the advantage of increased alignment performances of a new alignment strategy. ESyPred3D uses the alignment obtained by weighing, combining and screening the results of various alignment programs. The final 3D structure of protein is modelled using the modelling program Modeller. [4]

Geno3D

Geno3D is a free and automatic web server for protein molecular modelling. For a query protein sequence, the homology modelling is performed by the server in six successive steps which are (i) identifying homologous proteins with known 3D structures using PSI-BLAST; (ii) providing the user all potential templates for target selection; (iii) performing the alignment of both query and subject sequences; (iv) extracting geometrical restraints (dihedral angles and distances) for corresponding atoms between the query and the template; (v) performing the 3D construction of the protein using a distance geometry approach and (vi) sending the results by e-mail to the user.[3]

3D-Jigsaw

This server again is a freely available online server which is used to build 3D models of proteins based on homologues of the known protein structures using Hidden Markov Model (HMM). The returned alignments are used to build the models. All models are preselected using populus energy. Gaps and missing residues are closed and filled using populous repair. Finally all models are recombined using the basic populus approach. In the populus approach, genetic algorithm (GA) is used as conformational space search engine. GAs is powerful search and optimization techniques, and has been used previously in a number of protein modelling efforts ranging from *ab initio* folding to model-building by homology. This method uses multiple initial models from a variety of modelling approaches.After 10 rounds or conversion; all models are ranked using a fine and coarse energy function weighted according to the highest SEQID found. The top five models are returned. [11]

3. METHODOLOGY

3.1 Common Feature for Evaluation

The templates that were used to model the proteins mentioned, were kept common for each tool or server used for insilico modelling. Based on the alignment forming capacity of each server or tool, a template that was common to all the selected tools or servers was chosen for each of the five proteins. The PDB templates chosen were 3rty_a, 1mdy_a, 4f3l_b, 3rty_a, 1nkp_b for npas4, myod1, npas3, npas1, olig1 (each belonging to b-hlh class) respectively and had ~18-22% identity with ~100% query coverage. Ramchandran plots from SPDB Viewer and PDBSum Generate (procheck) were used to evaluate the validation of protein structures.

3.2 Homology based Modelling and Energy Minimization

The npas4, myod1, npas3, npas1, olig1 protein sequences were retrieved from NCBI-Protein database and sequence alignment followed by modelling was performed on each server (Jigsaw3D v3-Interactive, Easypred3D, Geno3D, Modeller v9.11, Phyre2-Normal) and it was found that the models obtained varied in appearance (visualized in Rasmol and SPDB Viewer). The physical parameters such as number of residues, bonds, helices, and sheets etc. that were modelled also varied. Hence, the differences were noted down in a tabular format as shown in Table 1 and the energy minimization values were computed by Swiss PDB Viewer.

Table 1. Fluctuating values produced by different modelling tools

		Structural Attributes							
Proteins	**Modelling Tool**	**No of Residues**	**No of Atoms**	**No of Bonds**	**Helices**	**Sheets**	**Glysine**	**Proline**	**Energy Minimization Value**
NPAS4	**3DJigsaw**	54	419	426	164	74	16	14	-237.35
	EasyPred3D	155	1191	1224	157	154	53	98	-2753.28
	Geno3D	86	677	688	102	61	16	21	-3401.53
	Modeller9.11	234	1797	1839	406	274	77	119	-6084.46
	Phyre2	152	1173	1219	232	434	48	98	-3785.14
MYOD1	**3DJigsaw**	95	750	763	388	0	16	28	-5154.21
	EasyPred3D*	0	0	0	0	0	0	0	0
	Geno3D	65	489	492	342	0	4	14	-4011.85
	Modeller9.11	320	2420	2483	419	51	80	259	-8222.58

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	Phyre2	68	562	565	372	0	4	14	-5047.02
	3DJigsaw*	0	0	0	0	0	0	0	0
	EasyPred3D	320	2567	2617	894	619	72	63	-10046.176
NPAS3	**Geno3D**	381	2914	2975	740	502	96	112	-15592.313
	Modeller9.11	933	7084	7236	750	470	328	448	-4265.259
	Phyre2	312	2508	2575	895	825	76	70	-1764.911
	3DJigsaw	256	1977	2018	514	585	108	77	18215.883
	EasyPred3D	315	2379	2441	584	456	124	182	-4281.933
NPAS1	**Geno3D**	264	1961	2002	366	443	97	42	-8838.838
	Modeller9.11	590	4425	4553	689	485	260	469	-5721.229
	Phyre2	233	1760	1823	394	586	92	119	7457.46
	3DJigsaw	83	643	650	460	0	24	28	-3686.884
	EasyPred3D	73	584	588	411	0	12	14	-4290.591
OLIG1	**Geno3D***	0	0	0	0	0	0	0	0
	Modeller9.11	271	1959	2002	410	0	112	217	-4525.964
	Phyre2	68	545	552	424	0	12	14	-2253.378

* No model was obtained as common template was not found.

3.3 Structure Validation

The structures of the modelled proteins belonging to the basic helix-loop-helix (npas4, myod1, npas3, npas1, olig1) were checked for validity using Ramchandran plots from SPDB Viewer and PDBSum Generate. Here, SPDB Viewer has its Ramchandran plot whereas PDBSum Generate produces Procheck-Ramchandran Plot; hence, the results that were obtained differed. The varying results obtained were noted in a tabular format (Table 2 and Table3 respectively).

Table 2. Values produced from SPDB Viewer Ramchandran Plot

		SPDB Viewer Ramchandran Plot					
Proteins	**Modelling Tool**	**Most Favoured Region**	**Most Favoured Region (%)- SPDBViewer Plot**	**Allowed Region**	**Allowed Region (%)**	**Disallowed Region**	**Disallowed Region (%)- SPDBViewer Plot**
	3DJigsaw	38	80.85	8	17.02	1	2.12
	EasyPred3D	118	85	13	9.35	8	5.7
NPAS4	**geno3D**	53	68.8	19	24.6	5	6.4
	Modeller9.11	191	88.8	19	8.8	5	2.3
	Phyre2	117	84.7	17	12.31	4	2.9
	3DJigsaw	65	74	18	20	4	4.5
	EasyPred3D*	0	0	0	0	0	0
MYOD1	**geno3D**	58	93.5	4	6.4	0	0
	Modeller9.11	180	92.3	11	5.6	4	2.05
	Phyre2	51	78.4	13	20	1	1.5
	3DJigsaw*	0	0	0	0	0	0
	EasyPred3D	288	91.7	19	6.05	7	2.2
NPAS3	**geno3D**	276	72.8	63	16.6	40	10.5
	Modeller9.11	751	80.6	113	12.13	67	7.19
	Phyre2	293	95.7	10	3.2	3	0.9
	3DJigsaw	209	83.9	24	9.6	16	6.4
	EasyPred3D	279	89.71	26	8.36	6	1.9
NPAS1	**geno3D**	184	70.2	59	22.5	19	7.2
	Modeller9.11	494	84.01	55	9.3	39	6.6
	Phyre2	204	89.08	18	7.8	7	3.05
	3DJigsaw	73	90.1	4	4.9	4	4.9
	EasyPred3D	66	92.9	3	4.22	2	2.8
OLIG1	**geno3D***	0	0	0	0	0	0
	Modeller9.11	245	91.07	18	6.6	6	2.2
	Phyre2	64	96.96	2	3.03	0	0

* No model was obtained as common template was not found.

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Table 3. Values produced from PDBSum Generate Procheck-Ramchandran Plot

		Procheck-Ramchandran Plot					
Proteins	**Modelling Tool**	**Most Favoured Region**	**Most Favoured Region (%)- SPDBViewer Plot**	**Allowed Region**	**Allowed Region (%)**	**Disallowed Region**	**Disallowed Region (%)- SPDBViewer Plot**
	3DJigsaw	38	80.85	8	17.02	1	2.12
	EasyPred3D	118	85	13	9.35	8	5.7
NPAS4	**geno3D**	53	68.8	19	24.6	5	6.4
	Modeller9.11	191	88.8	19	8.8	5	2.3
	Phyre2	117	84.7	17	12.31	4	2.9
	3DJigsaw	65	74	18	20	4	4.5
	EasyPred3D*	0	0	0	0	0	0
MYOD1	**geno3D**	58	93.5	4	6.4	0	0
	Modeller9.11	180	92.3	11	5.6	4	2.05
	Phyre2	51	78.4	13	20	1	1.5
	3DJigsaw*	0	0	0	0	0	0
	EasyPred3D	288	91.7	19	6.05	7	2.2
NPAS3	**geno3D**	276	72.8	63	16.6	40	10.5
	Modeller9.11	751	80.6	113	12.13	67	7.19
	Phyre2	293	95.7	10	3.2	3	0.9
	3DJigsaw	209	83.9	24	9.6	16	6.4
	EasyPred3D	279	89.71	26	8.36	6	1.9
NPAS1	**geno3D**	184	70.2	59	22.5	19	7.2
	Modeller9.11	494	84.01	55	9.3	39	6.6
	Phyre2	204	89.08	18	7.8	7	3.05
	3DJigsaw	73	90.1	4	4.9	4	4.9
	EasyPred3D	66	92.9	3	4.22	2	2.8
OLIG1	**geno3D***	0	0	0	0	0	0
	Modeller9.11	245	91.07	18	6.6	6	2.2
	Phyre2	64	96.96	2	3.03	0	0

* No model was obtained as common template was not found.

4. RESULTS AND DISCUSSION

4.1 Data Interpretation of Table 1

Table1 displays the fluctuating structural attributes from different tools used. Standard deviations (σ) of each protein from the different tools were obtained to understand variation from the average of Most Favoured Regions (MFR) from PDBSum Generate Procheck-Ramchandran Plot. Similarly, to analyse if there is pattern similarity, standard deviation (σ) of Number of Residues modelled for each protein by the tools were also obtained (Table 4).

Table 4. Standard Deviation (σ) of MFR and No of Residues from each tool for the Proteins

Proteins	**Modelling Tool**	**Most Favoured Region (PDBSum Generate)**	**σ (MFR)**	**No of Residues**	**σ (No of Residues)**
NPAS4	**3DJigsaw**	39	52.18429	54	69.72231
	EasyPred3D	105		155	
	Geno3D	49		86	
	Modeller9.11	169		234	
	Phyre2	104		152	
MYOD1	**3DJigsaw**	61	61.75961	95	122.7436
	EasyPred3D*	0		0	
	Geno3D	61		65	
	Modeller9.11	180		320	
	Phyre2	49		68	
NPAS3	**3DJigsaw***	0	174.2804	0	338.3825
	EasyPred3D	258		320	
	Geno3D	227		381	
	Modeller9.11	595		933	

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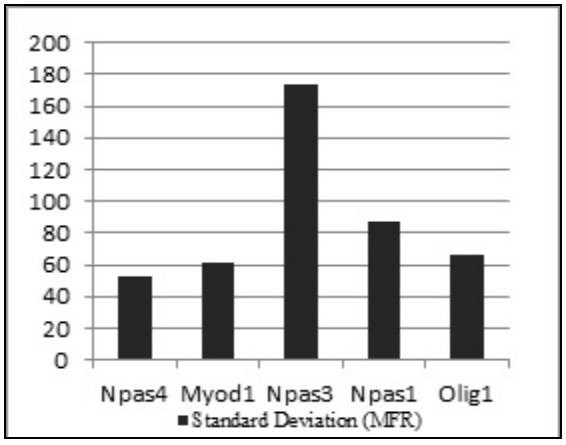
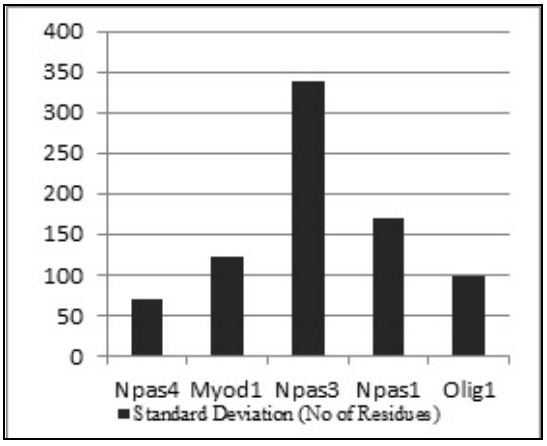
	Phyre2	258		312	
NPAS1	**3DJigsaw**	174	87.00287	256	170.0086
	EasyPred3D	227		315	
	Geno3D	147		264	
	Modeller9.11	360		590	
	Phyre2	162		233	
OLIG1	**3DJigsaw**	56	66.21367	83	98.36454
	EasyPred3D	60		73	
	Geno3D*	0		0	
	Modeller9.11	189		271	
	Phyre2	54		68	

The standard deviations obtained were plotted on a graph (Graph 1 and Graph 2) and a pattern was observed. This shows that there is constant variation in the models produced by the tools (Jigsaw3D v3-Interactive, Easypred3D, Geno3D, Modeller v9.11, Phyre2-Normal)



Graph 1. Standard deviations of MFR of models from each tool for the proteins



Graph 2. Standard deviations of number of residues of models from each tool for the tool

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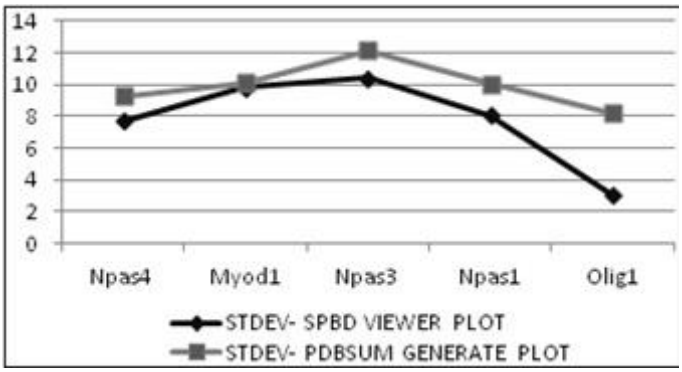
The models obtained were checked for structure validation using Ramchandran Plots from SPDB Viewer and PDBSum Generate and it was observed that the results from Plots also varied. To understand the variations in the results for structure validation tools, standard deviation of the MFR produced by SPDB Viewer-Ramchandran Plot and PDBSum Generate-Ramchandran Plot were obtained and graphs were plotted (Table 5, Graph 3).

Table 5. Standard deviations (σ) of MFR from SPDBV and PDBSum Generate Plots

Proteins	Modelling Tool	Most Favoured Region (SPDB Viewer-Ramchandran Plot)	σ	Most Favoured Region (PDBSum Generate-Ramchandran Plot)	σ
NPAS4	3DJigsaw	38	7.704024922	39	9.262397098
	EasyPred3D	118		105	
	geno3D	53		49	
	Modeller9.11	191		169	
	Phyre2	117		104	
MYOD1	3DJigsaw	65	9.8198778	61	10.14379942
	EasyPred3D*	0		0	
	geno3D	58		54	
	Modeller9.11	180		180	
	Phyre2	51		49	
NPAS3	3DJigsaw*	0	10.44700914	0	12.18664296
	EasyPred3D	288		258	
	geno3D	276		227	
	Modeller9.11	751		595	
	Phyre2	293		258	
NPAS1	3DJigsaw	209	8.100462435	174	10.05524739
	EasyPred3D	279		227	
	geno3D	184		147	
	Modeller9.11	494		360	
	Phyre2	204		162	
OLIG1	3DJigsaw	73	3.032670715	56	8.224506064
	EasyPred3D	66		60	
	geno3D*	0		0	
	Modeller9.11	245		189	
	Phyre2	64		54	

'*'-No model was obtained as common template was not found. For such cases the values are considered to be zero and were not calculated while computing Standard Deviations.

For better understanding, the standard deviations were plotted on a graph and the difference was observed



Graph 3. Standard deviations of MFR from Ramchandran Plots of SPDB Viewer and PDBSum Generate.

Hence using these tools homology model of these five bhlh proteins was generated. Using the swiss model software the models of these five proteins generated from the five software were superimposed on each other and a single image was obtained from it. Fig 1 illustrates the same.

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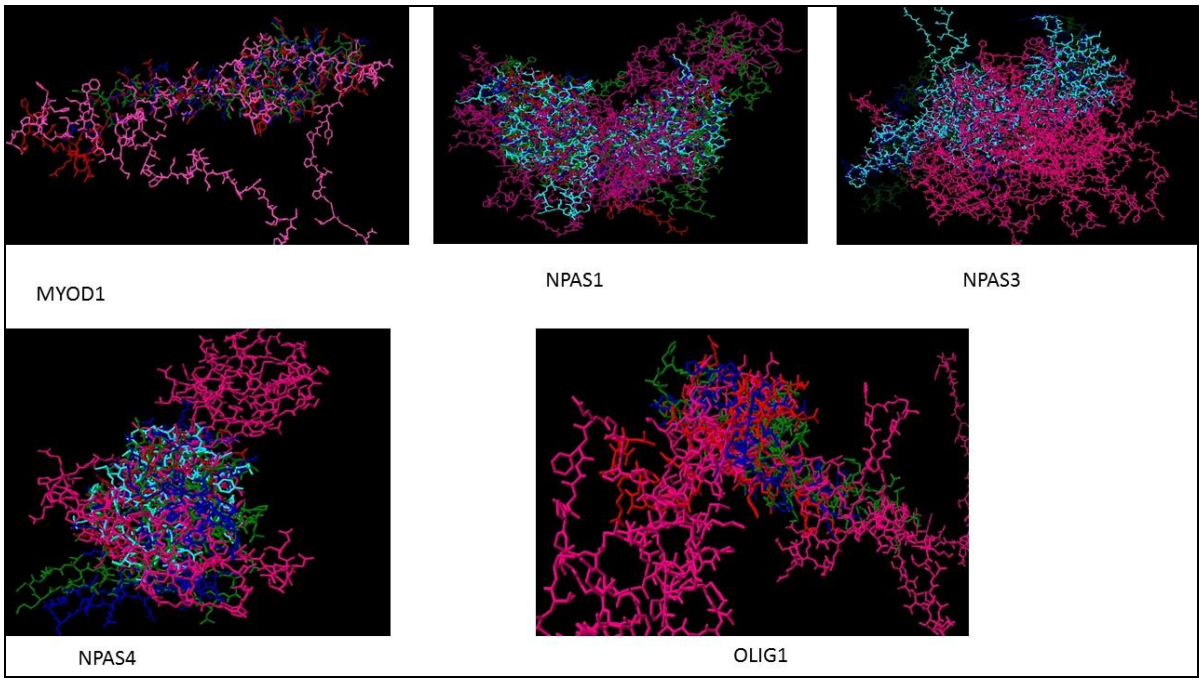

Fig 1: This figure shows the homology models generated for MYOD1, NPAS1, NPAS3, NPAS4 and OLIG1.

From the tables (Table 2 and Table 3) and Graph 3, it can be analysed that though the number of residues were the same for the Ramchandran Plots, both 'SPDB Viewer' and 'PDBSum Generate' produced varying results. It is evident from the graph that the values obtained for MFR (Most Favoured Region) had more residues from SPDB Viewer when compared to MFR from PDBSum Generate as the Standard deviation of MFR-PDBSum Generate was more. Also, Ramchandran plots from SPDBV had Most Favoured Region, Allowed Regions and Disallowed Regions whereas that from PDBSum Generate had Most Favoured Regions, Additionally Allowed Regions, Generously Allowed Region and Disallowed Regions. Here, 'Additionally Allowed' and 'Generously Allowed Regions' were combined as 'Allowed Region' for convenience.

5. CONCLUSION

High-resolution crystallographic structures becoming available for all human and pathogen proteins and complexes are a very unlikely scenario. The only practical manner of exploring ligand – protein interactions for most systems is to use comparative protein structure models. It is now clear that comparative models, based on as little as 30% sequence identity to known template structures, can be useful. It can be concluded that different homology modelling tools (Jigsaw3D v3-Interactive, Easypred3D, Geno3D, Modeller v9.11, Phyre2-Normal) produced varying results although common templates were used and the difference was observed to be varying in terms of the number of residues modelled. It was also observed that Ramchandran plots from structure validation tools (SPDB Viewer and PDBSum Generate) also varied. It was inferred that Ramchandran plot from SPDB Viewer produced more residues in Most Favoured Regions than Procheck Ramchandran plot from PDBSum Generate. However, to establish a much clearer picture, more proteins need to be considered for the study and further validation has to be performed.

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