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Structure prediction and analysis of Human Dopamine Receptor-1

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Abstract

Dopamine and its five different receptors play vital role in regulating the physiological functions of the human body. The receptors of dopamine neurotransmitter are crucial targets for pharmacological drugs for treatment of neurological disorders such as Schizophrenia, Parkinson's disease. The experimental 3-Dimensional (3D) structure of human dopamine receptor-1 (DRD1) has not been reported in PDB yet. Determining the 3D structure of human dopamine receptor-1 (DRD1) may provide in-depth knowledge about its functional analysis and would help in drug designing. Various servers and software based on ab-initio method, threading and homology modeling are employed to generate full length 3D model of human dopamine receptor DRD1. The structure validation was also performed to predict the best possible 3D model.

Keywords: Dopamine receptor-1 (DRD1), Structure prediction, Homology Modeling, Threading, Ab-initio method; Structure Validation.

Introduction

Dopamine is a metabolite of tyrosine [1]. This catecholamine neurotransmitter is involved in controlling various functions such as endocrine regulation, emotion, cognition and locomotory activity in the mammalian brain [2]. About 80% of the catecholamine content of the brain is formed by dopamine (figure 1). Dopamine shows its physiological effects after binding with membrane receptors, which belong to family of G-protein coupled receptors [3]. The presence of various dopamine receptors in the Central Nervous System was proved in 1972 from biochemical analysis which stated that dopamine can stimulate adenylyl cyclase.

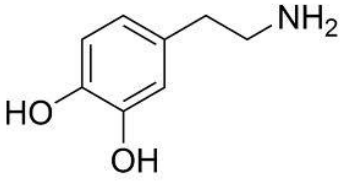

Figure 1: Structure of Dopamine

Dopamine receptors includes five different subtypes namely D1-D5, belongs to family of seven transmembrane domain G-protein coupled receptors [3]. Two different groups of receptors: D1 like and D2 like are characterized on their structural and functional aspects. D1 like are associated with stimulatory function and includes D1 and D5 receptors whereas D2 like are associated with inhibitory function, includes D2-D4 receptors. The D1 receptor is intronless and shares 80% homology in domain structure with D5 receptors. For stimulating cyclic AMP release, D1 like receptors have third intracellular (IC) loop which interacts with G-stimulatory protein (Gs) [4]. Dopamine receptors are present in striatum, limbic system, in pituitary gland, kidney, and adrenal gland and in cardiovascular system. The DRD1 gene encodes for the D1 class of dopamine receptor [5]. Since limited experimental structures of G-protein coupled receptors are present and only one GPCR's 3D structure has been reported yet-the bovine rhodopsin [6]. Various complications are noted in obtaining the ultimate 3D model of human dopamine receptor DRD1.Firstly, X-ray and

Nuclear magnetic Resonance (NMR) are labour intensive and time consuming process. Moreover, the similarity in binding sites of dopamine receptors and the issue of crystallizing membrane bound molecule creates problem in predicting the three dimensional model of human dopamine receptor DRD1. Hence, computational techniques which include homology modeling, threading and ab-initio are important tool for performing tertiary structure prediction of human dopamine receptor-1 (DRD1).

2. Tertiary Structure Prediction of Protein

The tertiary structure of protein refers to the unique three dimensional arrangements that globular proteins assume as a consequence of the interactions between the side chains in their primary structure. The basic unit of tertiary protein structure is the domain. The study of tertiary structure is important to study the folding pattern of protein. The tertiary structure can help in studying the interaction of protein with ligand molecules and in structure-based drug designing. The 3D structure of protein can be predicted computationally through homology modeling, threading and ab-initio methods.

Table 1: Comparison between different methods based on sequence similarity

Homology Modeling	Threading	Ab-initio method
Sequence similarity between template and target is greater than 35%	Sequence similarity between template and target is greater than 25%	When no suitable template is found

2.1 Homology Modeling

Homology Modeling is one of the alternatives to obtain 3D models of the target in absence of crystal structure. Comparative or homology modeling is a method to obtain protein 3D structure based on the idea that proteins having similar sequences have similar structures [7]. Pair wise alignment tools are implemented to search for best alignment between target-template sequences.

2.2 Threading

The threading method, sometimes referred as fold recognition method, works on the concept of identifying correct fold for the target sequence. Threading improvises the sequence alignment by providing structural information about the alignment. Threading works on different algorithms such as 3D profile method, Profile Hidden Markov Model. Since structure prediction has many approximations which may disturb the final alignment. Hence, threading often generates poor sequence-structure alignment [8].

2.3 *Ab-Initio* Method

The ab initio method basically aims at predicting the 3D structure of protein in its native state. It takes in account the physical and chemical properties of amino acids that make up the protein structure. The 3D models are built from basic scratch, i.e. ab-initio folding. This method of 3D structure prediction is quite slow and inaccurate [9].The de novo method of structure prediction is currently limited only for short amino acids sequence, preferably 200 residues.

3. Material and methods

3.1 Tools Used

For predicting the 3D structure of given protein, various computational tools were briefly studied. This included online web services and software which are mentioned in this section.

UniProtKB

UniProt Knowledgebase provides access to functional aspects about protein sequence and UniProtKB serves as an important hub of protein knowledge. UniProtKB provides access to a collection of protein sequences [10]. It can be accessed online for downloading a protein sequence at www.uniprot.org.

Swiss Model

An automated web based service has been designed in order to predict tertiary structure of protein. It incorporates programs and databases for performing homology modeling [11]. Protein models can be constructed easily on a computer having web connection. Target-template alignment is done after important template structures are selected from SWISS MODEL Template Library (SMTL) [11]. The SWISS-MODEL repository can be accessed at http://swissmodel.expasy.org/

I-TASSE

I-TASSER is an automated web server (as 'Zhang server') [12] which perform protein tertiary structure prediction. It is based on Iterative implementation of Threading Assembly Refinement (TASSER) program [13]. It was developed at KU Centre of Bioinformatics. I-Tasser modeling results depends on protein sequence size, server takes approximately 48hours to predict 3D structure of protein. I-TASSER includes a structure analysis parameter called C-Score [12]. C-Score correlates the real quality of final models. The I-TASSER can be accessed at http://zhanglab.ccmb.med.umich.edu/I-TASSER/

LOMETS

For predicting the 3D structure of protein, LOMETS a Local Meta Threading Server [13] has been developed. Using nine different servers such as FUGUE, HHSEARCH, PROSPECT2, SAM-TO2, SPARKS2, SP3, PAINT, PPA I and PPA II [13], consensus models are generated. Different algorithms are performed, template libraries are updated weekly and all the 9 servers are installed locally [13]. The LOMETS server can be accessed athttp://zhanglab.ccmb.med.umich.edu/LOMETS/

PHYRE-2

Protein Homology/Analogy Recognition Engine server performs scanning of known protein structures taken from Structural Classification of Proteins (SCOP) against non-redundant sequence databases and a protein fold profile is designed and stored in 'fold library'. Profile-Profile algorithm is performed on submitted amino acid sequence [15] and within 24hours, an email containing full length models of protein are sent to the user.The ROBETTA server can be accessed athttp://www.sbg.bio.ic.ac.uk/~phyre2/

Schrödinger

Schrödinger offers products from molecular modeling to an interactive, easy-to-use suite of drug design software. Maestro is the backbone of Schrödinger's computational programs. Maestro provides coordinated powerful interface for Schrödinger software. Molecular modeling, 3D visualization, enhanced 2D ligand interaction diagrams can be performed on Maestro. Schrödinger software is used to perform homology modeling for given protein sequence. Schrödinger Release 2014-2: Maestro version 9.8, Schrödinger, LLC, New York, 2014.

ROBETTA

ROBETTA is an online server which performs automated tertiary structure prediction of protein using ab initio fragment assembly. It was developed as part of ROSETTA software package. 'Ginzu' is a domain prediction method[16] used by ROBETTA. It takes around 4-6hours for performing structure prediction on a 150 amino acid residues [16]. The ROBETTA server can be accessed at http://robetta.bakerlab.org/

Structure Analysis and Verification Server

SAVES is a metaserver which runs six different programs for validating and checking protein structures after model refinement. It includes PROCHECK, WHAT_CHECK, VERIFY_3D, ERRAT, PROVE and Ramachandran Plot. PROCHECK performs analysis of residue-by-residue geometry of protein structure. 3D structure of protein can be uploaded in PDB format on SAVES [17]. It can be accessed at http://services.mbi.ucla.edu/SAVES/

3.2 Methodology

The protein sequence of 446 amino acids was retrieved in FASTA format from UniProtKB, using accession number-P21728 for human dopamine receptor DRD1. To perform homology modeling, SWISS-MODEL server and SCHRÖDINGER software were used. The basic method employed by SWISS-MODEL to predict 3D structure of protein is shown in figure 2

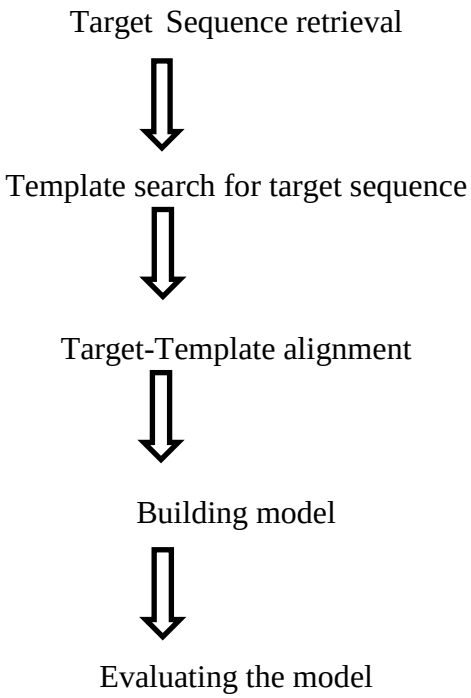

Figure 2: Process of 3D structure prediction using SWISS-MODEL

Schrödinger software was used to predict the 3D model of DRD1 protein. Protein BLAST was performed for the sequence of 446 amino acids. The parameters used were Expectation value=1.0, word size=3 and scoring matrix=BLOSUM 62.

Three online web servers namely I-TASSER, LOMETS, PHYRE2 were used for performing threading task to predict 3D structure of DRD1 protein. The amino acid sequence of 446 residues was submitted to these web servers online and predicted results were received via e-mail. The ab-initio method was also studied and the protein sequence of 446 amino acids was uploaded on ROBETTA server. This server generated a unique job id for the DRD1 protein and it took almost one week to generate best possible model for DRD1 sequence. QUARKS server failed to accept the protein sequence, as it is limited only to 200 amino acid sequences. SAVES server was used in order to perform structure assessment and validation of generated 3D model of DRD1 protein. All the predicted 3D models of DRD1 protein,

obtained using SWISS-MODEL, SCHRÖDINGER software, LOMETS, I-TASSER, PHYRE-2, ROBETTA server were uploaded in PDB format on SAVE server.

4. Results & Discussions

Various servers and software predicted the 3D model of DRD1 protein. The templates selected for homology modeling by SWISS-MODEL server are discussed in Table 2

Table 2: Templates selected for homology modeling using SWISS-MODEL

Template	Similarity	Range	Coverage	Method	QMEAN
4iaq.1.A	0.39	17-348	0.70	X-ray	-6.66
3pbl.1.A	0.38	26-347	0.69	X-ray	-7.09
4bvn.1.A	0.41	21-347	0.66	X-ray	-5.84
3uon.1.A	0.35	20-346	0.74	X-ray	-9.52

Using SWISS-MODEL workspace, above mentioned different templates were selected on the basis of sequence similarity, coverage. The above mentioned templates show maximum coverage for protein sequence of human dopamine receptor (DRD1). These templates were generated on target-template alignment through SWISS-MODEL template library (SMTL) from Protein Data Bank (PDB).

All the 3D models were verified and structure assessment was done through PROCKECK method on SAVE server. Ramachandran plot is a potent tool to check the quality of predicted 3D models.

Ramachandran plot basically shows permitted value of and indicated on 2-D map. Bond angles resulting from the rotations at C_α for N-C_α bond are labeled as ϕ (phi) and C_α-C bond values are labeled as Ψ (psi). In figure [3], white area shows sterically disallowed regions in which any non-bonding interatomic distance is less than its corresponding van der Waals radii. These regions are disallowed for all amino acids except glycine as it lacks side chain. The red-shaded regions called most favoured region (also called low-energy regions) corresponds to conformations where there are no steric clashes between residues. The yellow-shaded region shows conformation having outer limit van der Waals distance i.e. the atoms are allowed to remain close together.

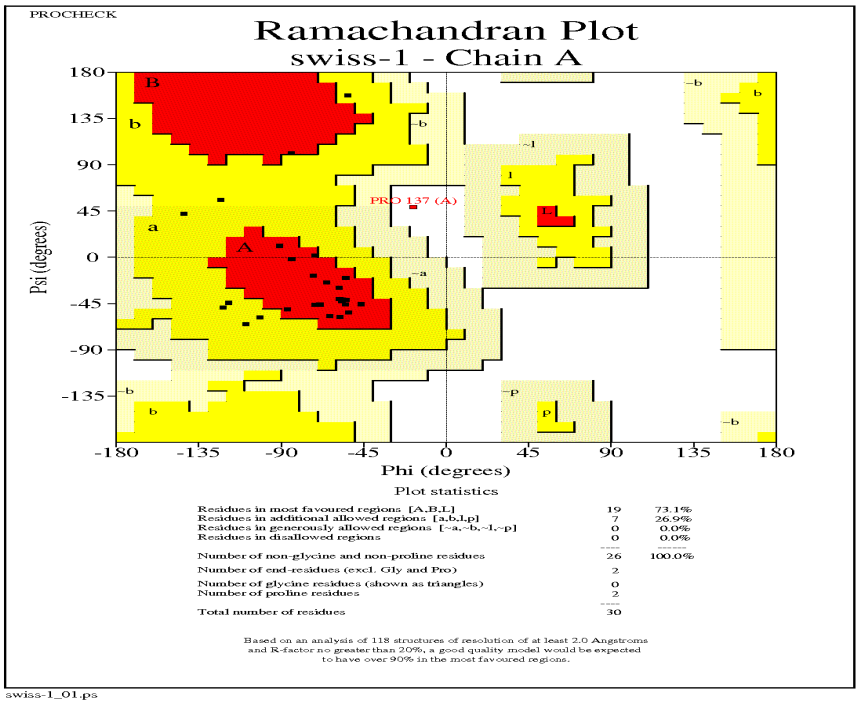

Figure 3: Ramachandran plot obtained for 3D model generated using SWISS-MODEL (01)

Ramachandran plot for 3D model generated using SWISS-MODEL server (Model#01): This 3D model covers only 30 residues out of which only 19 residues fall under most favoured region, 7 residues in additional allowed region and nil residues in disallowed region.

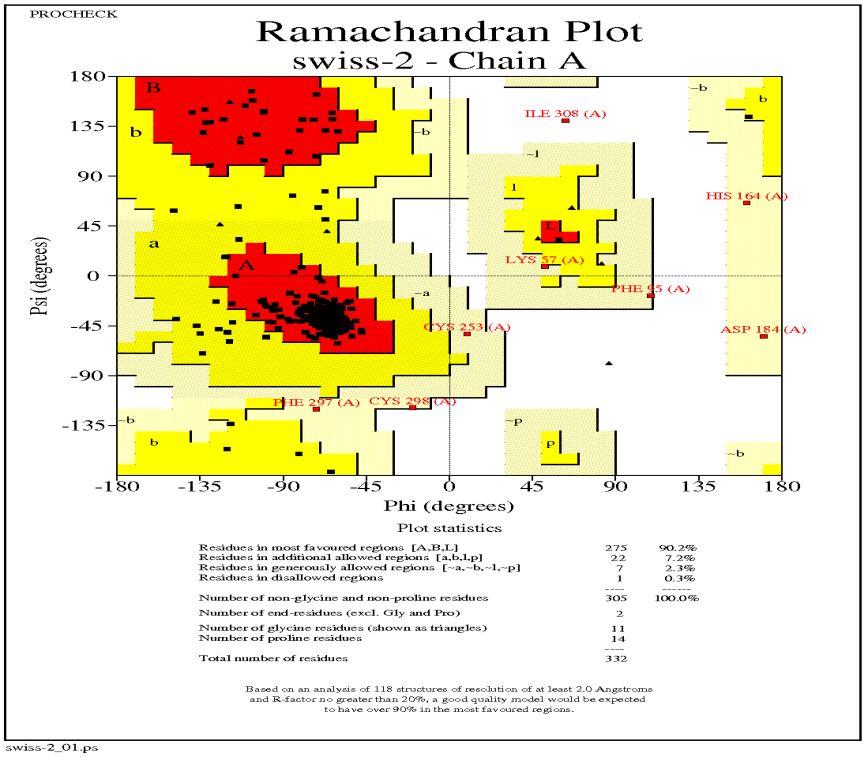

Figure 4: Ramachandran plot obtained for 3D model generated using SWISS-MODEL (02)

Ramachandran plot for 3D model generated using SWISS-MODEL server (Model#02): This 3D model covers only 332 residues out of which only 275 residues fall under most favoured regions, 22 residues in additional allowed regions, 7 residues in generously allowed regions and 1 residue in disallowed region.

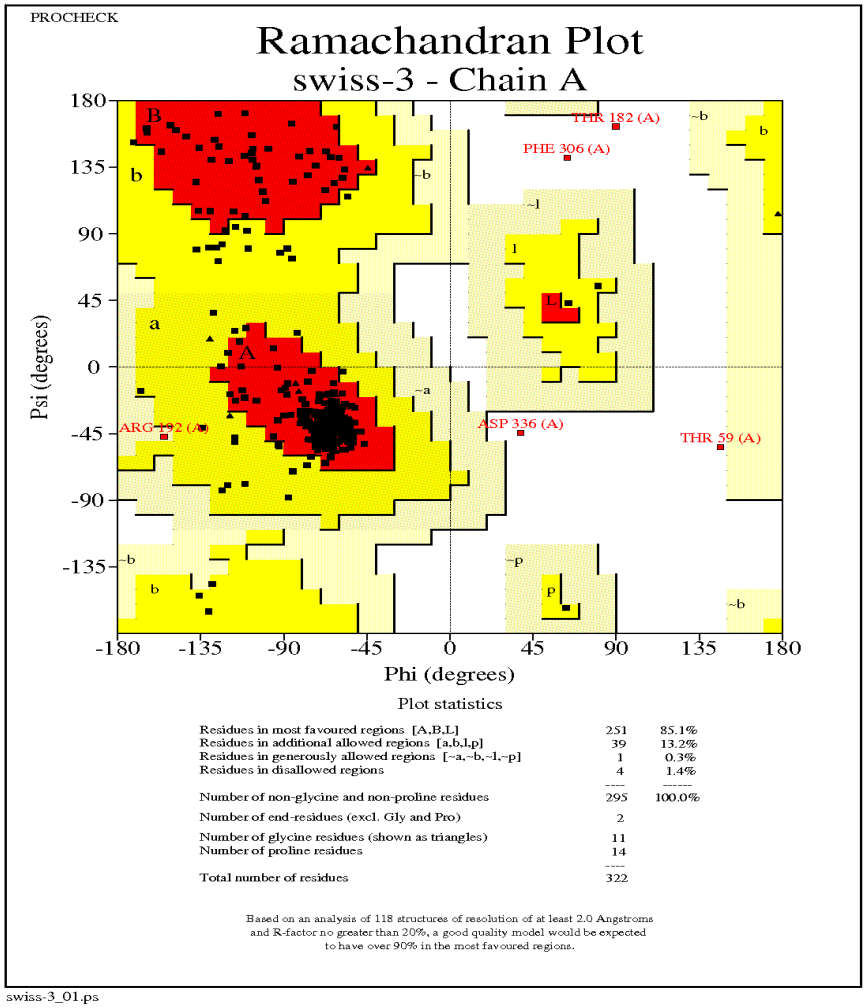

Figure 5: Ramachandran plot obtained for 3D model generated using SWISS-MODEL (03)

Ramachandran plot for 3D model generated using SWISS-MODEL server (Model#03): This 3D model covers only 322 residues out of which only 251 residues fall under most favoured regions, 39 residues in additional allowed regions, 1 residue in generously allowed regions and 4 residues in disallowed regions.

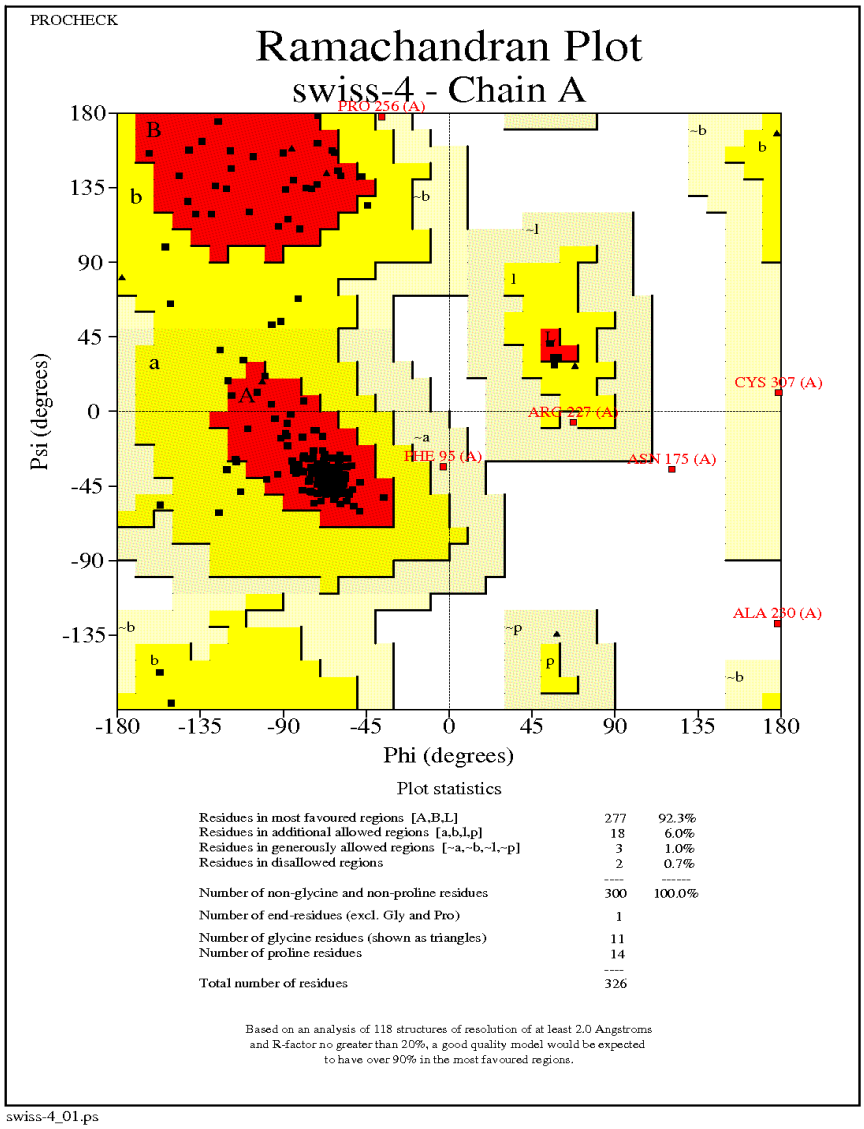

Figure 6: Ramachandran plot obtained for 3D model generated using SWISS-MODEL (04)

Ramachandran plot for 3D model generated using SWISS-MODEL server (Model#04): This 3D model covers only 326 residues out of which only 277 residues fall under most favoured region, 18 residues in additional allowed regions, 3 residues in generously allowed regions and 2 residues in disallowed regions.

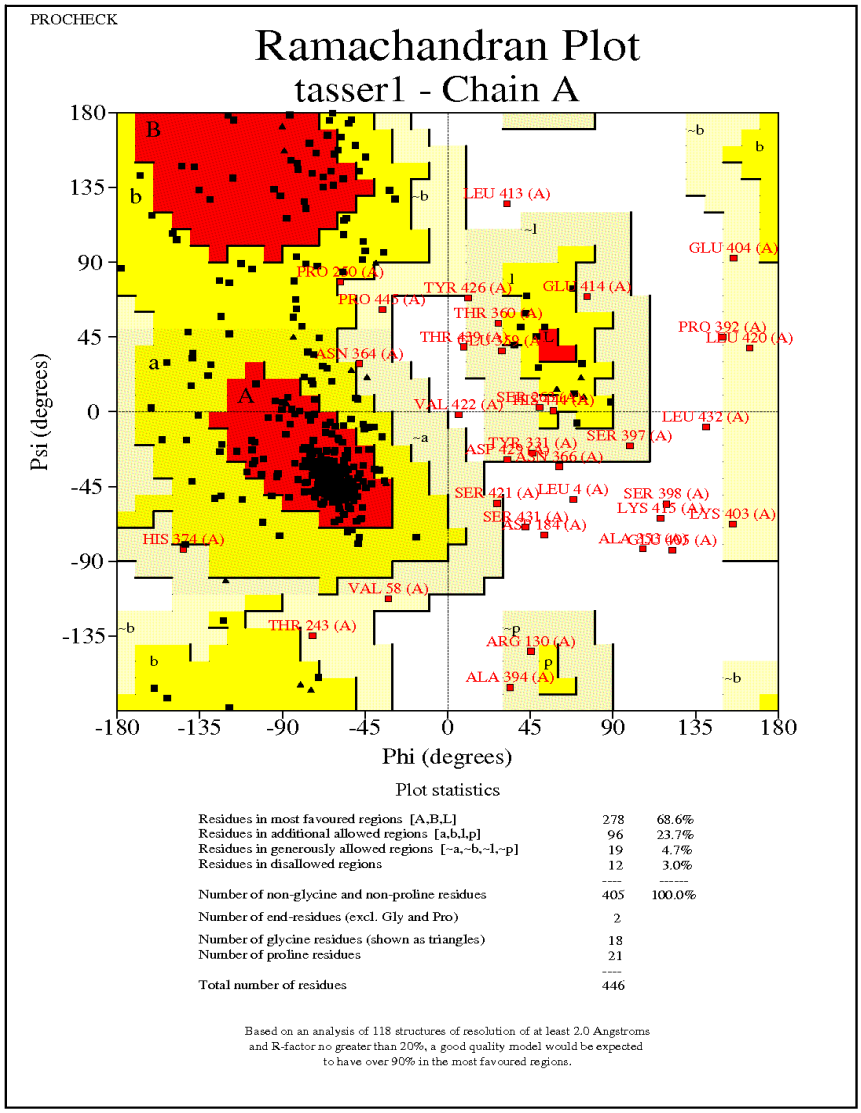

Figure 7: Ramachandran plot obtained for 3D model generated using I-TASSER server (01)

Ramachandran plot for 3D model generated using I-Tasser server (I-tasser#01): This 3D model covers all 446 residues out of which only 278 residues fall under most favoured region, 96 residues in additional allowed regions, 19 residues in generously allowed region and 12 residues in disallowed regions.

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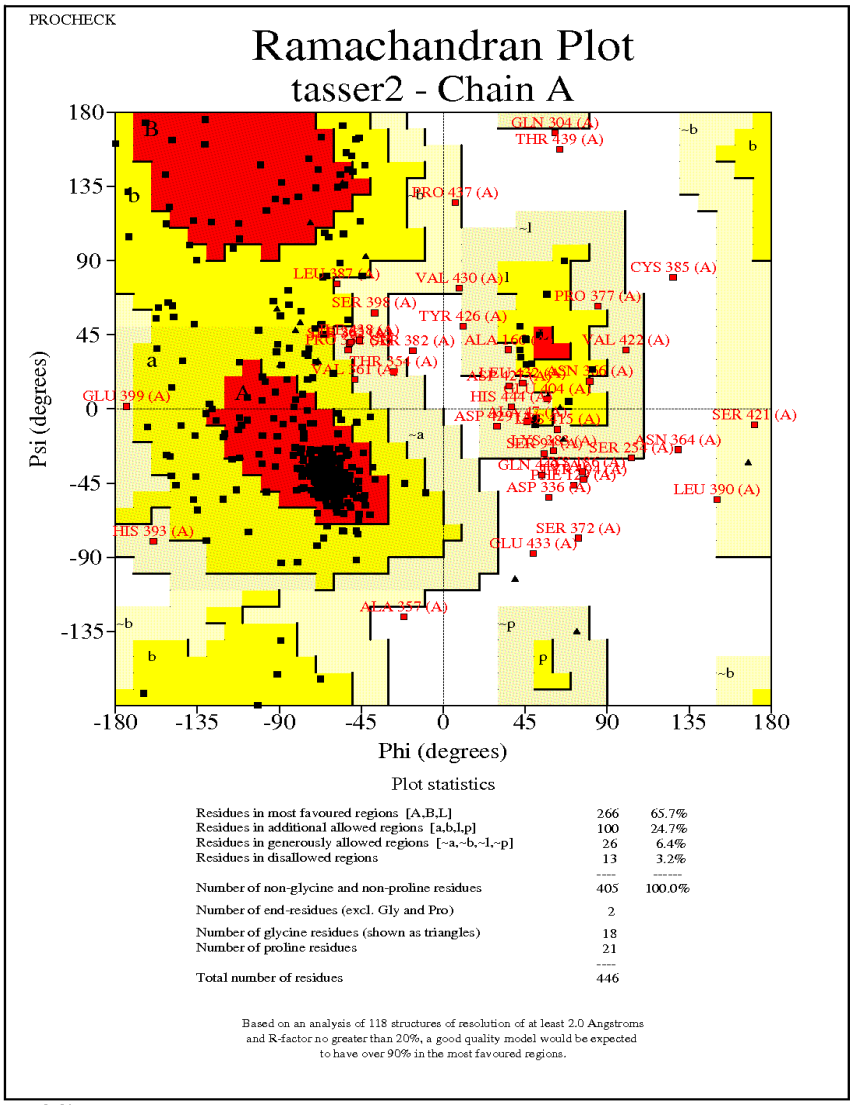

Figure 8: Ramachandran plot obtained for 3D model generated using I-TASSER (02)

Ramachandran plot for 3D model generated using I-Tasser server (I-tasser#02): This 3D model covers all 446 residues out of which only 266 residues fall under most favoured region, 100 residues in additional allowed regions, 26 residues in generously allowed regions and 13 residues in disallowed regions.

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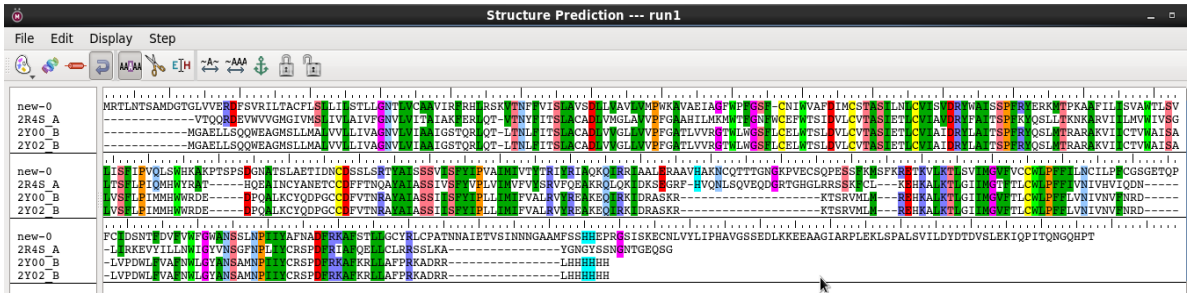

Figure 9: Alignment generated by Schrödinger

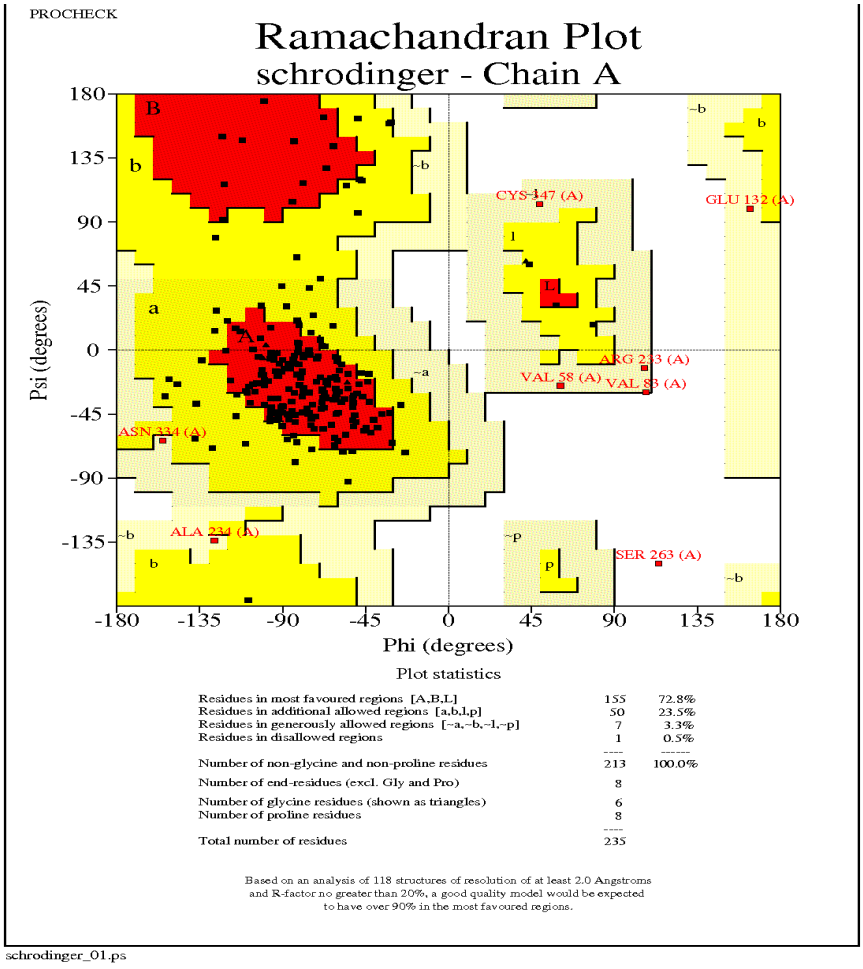

Figure 10: Ramachandran plot obtained for 3D model generated using Schrödinger

Ramachandran plot for 3D model generated using Schrödinger software (Schrödinger #01): This 3D model covers only 235 residues out of which only 155 residues fall under most favoured regions, 50 residues in additional allowed regions, 7 residues in generously allowed regions and 1 residue in disallowed regions. Ramachandran plot values as obtained after structure verification using SAVE server.

Table 3: Values obtained for 3D models generated by SWISS-MODEL

Regions	Swiss-Model#01	Swiss-Model#02	Swiss-Model#03	Swiss-Model#04
Most favoured	73.1%	90.2%	85.1%	92.3%
Add. Allowed	26.9%	7.2%	13.2%	6.0%
Gen. allowed	0.0%	2.3%	0.3%	1.0%
Disallowed	0.0%	0.3%	1.4%	0.7%

Table 4: Values obtained for 3D models generated by I-Tasser & Schrödinger

Regions	I-Tasser#01	I-Tasser#02	Schrödinger #01
Most favoured	68.6%	65.7%	72.8%
Add. Allowed	23.7%	24.7%	23.5%
Gen. allowed	4.7%	6.4%	3.5%
Disallowed	3.0%	3.2%	0.5%

Comparing all the 3D structures predicted, the model generated through I-Tasser could be considered an appropriate model.

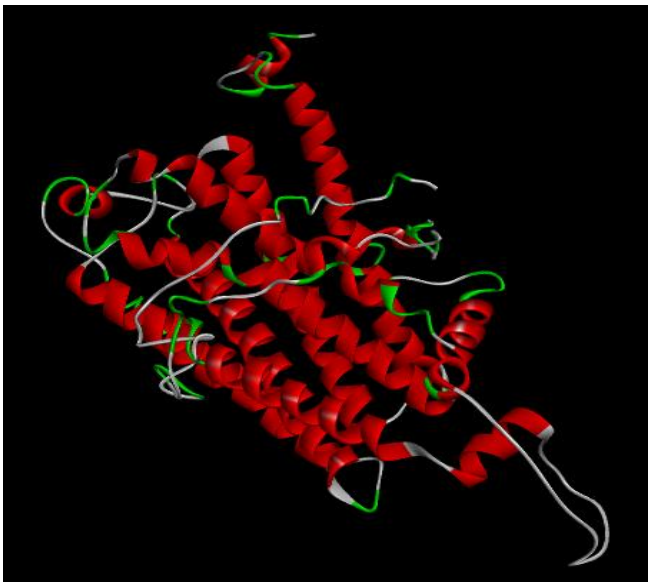

Figure 11: The 3D structure of human dopamine receptor-1 (DRD1) generated through I-Tasser server. This structure reveals helical arrangement of protein.

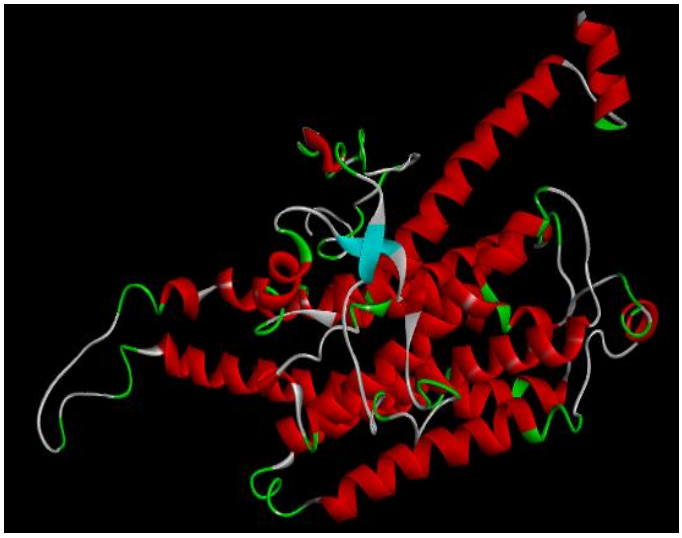

Figure 12: The 3D structure of human dopamine receptor-1 (DRD1) generated through I-Tasser server. This structure reveals helical and beta sheets arrangement of protein.`

5. Conclusion

The major objective of this study was to predict the full length 3D model of human dopamine receptor-1 (DRD1). The SWISS-MODEL server and Schrödinger software which were used to perform homology modeling for 3D structure prediction of human dopamine receptor-1 (DRD1) protein, predicted structure for approximately 322 residues only. I-Tasser server which performs threading for 3D structure prediction has generated 3D model for 446 residues. Hence, the 3D model generated through I-Tasser server could be considered an appropriate 3D model of human dopamine receptor-1 (DRD1). This thorough study using different tools and techniques provided an in-depth understanding of homology modeling, threading and *ab-initio* methods for predicting the tertiary structure of protein.

5. REFERENCES

[1] Carlsson A, Lindqvist M, and Magnusson T (1957) 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. *Nature*; **180:**1200.

[2] Missale C, Nash SR, Robinson SW, Jaber M and Caron MG (1998) Dopamine receptors: from structure to function. *Physiological Reviews*; **78**: 189-225

[3] Vallone D, Picetti R and Borrelli E (2000) Structure and function of dopamine receptors. *Neuroscience and Behavioural Reviews*; **24**: 125-132

[4] Pivonello R et al. (2007) Novel insights in dopamine receptor physiology. *European Journal of Endocrinology*; **156**: S13-S21

[5] DRD1 dopamine receptor D1 [Homo sapiens (human)] Gene ID: 1812 retrieved from the World Wide Web http://www.ncbi.nlm.nih.gov/gene/1812

[6] Goddard III WA and Abrol R (2007) 3-Dimensional Structures of G-protein coupled receptors and Binding sites of Agonists and Antagonists. *The Journal of Nutrition*

[7] Cavasotto CN and Pathak SS (2009) Homology Modeling in Drug Discovery: current trends and applications. *Drug Discovery Today*; **14**: 676-683

[8] Zhang H (2002) Protein Tertiary Structures: Prediction from amino acid sequences. *Encyclopedia of Life Sciences*

[9] Rokde CN, Kshirsagar M (2013) A Comparative study of protein tertiary structure prediction methods. *International Journal of Computer Science and Informatics*; **3**: 31-34

[10] Magrane M (2011) UniProt Knowledgebase: a hub of integrated protein data. *Database*

[11] Arnold K, Bordoli L, Kopp J, Schwede T (2006) The SWISS-Model workspace: a web based environment for protein structure homology modeling. *Bioinformatics*; **22**: 195-201

[12] Zhang Y (2008) I-Tasser server for protein 3D structure prediction. *BMC Bioinformatics*; **40**: 1471-2105

[13] Zhang Y, Skolnick J: Automated structure prediction of weakly homologous proteins on a genomic scale.*Proceedings of theNational Academy of Sciences of the United States of America* 2004, **101:**7594-7599.

[14] Wu S and Zhang Y (2007) Lomets: A Local meta-threading-server for protein structure prediction. *Nucleic Acid Research*; **35**: 3375-3382

[15] Kelley LA and Sternberg MJE (2009) Protein structure prediction on the web: a case study using the Phyre server. *Nature Protocols*; **4**: 363-371

[16] Kim DE, Chivian D and Baker D (2004) Protein structure prediction and analysis using the Robetta server. *Nucleic Acids Research*; **32**: W526-W531

[17] Colovos C, Yeates TO (1993) Verification of protein structures:patterns of nonbonded atomic interactions. *Protein science: a publication of the Protein Society*; **2**: 1511-1519

APPENDIX

Human dopamine receptor DRD1 protein sequence retrieved from UniProt (accession no. – P21728)
>sp|P21728|DRD1_HUMAN D(1A) dopamine receptor OS=Homo sapiens GN=DRD1 PE=1 SV=1
MRTLNTSAMDGTGLVVERDFSVRILTACFLSLLILSTLLGNTLVCAAVIRFRHLRSKVTN
FFVISLAVSDLLVAVLVMPWKAVAEIAGFWPFGSFCNIWVAFDIMCSTASILNLCVISVD
RYWAISSPFRYERKMTPKAAFILISVAWTLSVLISFIPVQLSWHKAKPTSPSDGNATSLA
ETIDNCDSSLSRTYAISSSVISFYIPVAIMIVTYTRIYRIAQKQIRRIAALERAAVHAKN
CQTTTGNGKPVECSQPESSFKMSFKRETKVLKTLSVIMGVFVCCWLPFFILNCILPFCGS
GETQPFCIDSNTFDVFVWFGWANSSLNPIIYAFNADFRKAFSTLLGCYRLCPATNNAIET
VSINNNGAAMFSSHHEPRGSISKECNLVYLIPHAVGSSEDLKKEEAAGIARPLEKLSPAL
SVILDYDTDVSLEKIQPITQNGQHPT